Synthesis and Evaluation of Novel Prodrugs of Foscarnet and Dideoxycytidine with a Universal Carrier Compound Comprising a Chemiluminescent and a Photochromic Conjugate

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ABSTRACT: To facilitate intracellular delivery of hydrophilic drugs, a general lipophilic carrier molecule was designed and synthesized. The carrier comprised a chemiluminescent-photochromic conjugate that potentiates diffusion across cell membranes to enhance intracellular uptake of the drug. The designed mechanism involves activation of the chemiluminescent moiety by intracellular oxygen free radicals and intermolecular energy transfer of the excited state energy to the photochromic moiety to result in release of the drug to allow the desired pharmacological effect to occur. Prodrugs of foscarnet and dideoxycytidine with several carriers caused suppression of a human immunodeficiency virus infection in human cultured macrophages that was up to five times more effective than the drug alone. Successful *in vivo* efficacy testing of prodrug has been accomplished by demonstrating the suppression of a retroviral infection of Friend leukemia virus in mice. Acute toxicity studies of the carrier indicated that it was nontoxic. © 2004 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 93:1320–1336, 2004

Keywords: prodrug; anti-HIV; foscarnet; dideoxycytidine; oxygen free radicals; chemiluminescence; intermolecular energy transfer; photochromism

INTRODUCTION

Drug Discovery and Drug Delivery: Typical Approach

The principle initial step to obtain new drugs comprises drug discovery, which typically involves *in vitro* enzymatic kinetics assays. Promising compounds that are discovered by *in vitro* screening assays are subsequently tested *in vivo*. However, only a small percentage of discovered compounds produce the desired effect *in vivo* or have a high therapeutic ratio because of one or more factors, for example:

They cannot obtain access to their target receptor or site of action because they are impermeant to cells or biological barriers, such as the blood–brain barrier, due to unfavorable energetics.
They are toxic in their free form.
They are toxic as a consequence of being nonselective with regard to their access to and action with one biological environment or compartment relative to another.
They are rapidly inactivated or excreted.

Most biologically active agents are impermeant because of the possession of polar or charge groups. They are typically salts with a low lipid membrane partition capability. In these cases, compounds that demonstrate *in vitro* efficacy are ineffective therapeutics. Consequently, the drug chemists alter the drug structure, and the

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biologists test the altered compounds in a repeating process until a suitable compromise between drug activity, bioavailability, and toxicity is achieved.

Past attempts to increase the bioavailability of drugs include bulk delivery strategies, including the use of liposomes, and drug delivery strategies involving the formation of derivatives of drugs such as ester derivatives. The major limitations in the case of liposomes are the inability to direct the bulk release to a specific tissue for the most part, the lack of a mechanism to increase the permeability of the drug, and the clearing of the liposomes by the reticuloendothelial system (liver). The major weakness of the esterified drugs strategy is that the mechanism of free drug release depends on the existence of an enzyme of the organism to cleave the bond between the ester and the drug. Such enzymes are typically not present or have little activity in the target cells or biological compartment on the prodrug.

Many potent anti–human immunodeficiency virus (HIV) drugs comprise nucleoside or nucleotide analogs that are effective reverse transcriptase or polymerase inhibitors, but have poor bioavailability because of low lipophilicity with poor diffusion capability across cell membranes. In our prodrug studies, the drug comprised a reverse transcriptase inhibitor, either phosphonoformate (foscarnet) or dideoxycytidine (ddc). Foscarnet showed great promise as an anti-HIV drug as indicated by *in vivo* screening assays and in clinical trials. In the latter case, there was significant HIV suppression over 2 weeks at mean serum concentrations of 261 μmol/L. However, the treatment was interrupted because of renal function impairment.[1] In an attempt to improve the bioavailability of this very lipophobic drug and to improve the therapeutic ratio, many prodrug schemes have developed such as foscarnet encapsulated in liposomes[2,3] or linked to alkylalcohols,[4,5] covalent lipid conjugates,[6,7] steroid derivatives,[8] and glycerophospholipid derivatives.[9] In addition, more lipophilic analogs such as those containing sulfur,[10] 2-hydroxy-1,4,2-dioxaphosphorinane-2,3-dioxide derivatives,[11] glucosyl esters,[12] and ester derivatives[13] have been synthesized. Limitations of these strategies are the requirement of a means to cause selective intracellular cleavage of the modified group to recover the free drug and a reduction of the potency of the modified compounds compared with that of foscarnet alone. Furthermore, anti-HIV prodrugs of nucleotides such as carbonates of zidovudine (AZT) have been synthesized and evaluated wherein cyclic intramolecular rearrangement recovers the original drug.[14]

Drug Delivery: A New Approach

The goal of our research was to develop a cellular permeant prodrug in which intracellular drug release occurs when the prodrug reacts with cellular free radicals via a mechanism involving chemiluminescence, photochromism, and intramolecular energy transfer. Thus, each prodrug comprised a three-part molecule, A-B-D, in which each part was a functionality with a defined purpose. A represents a chemiluminescent functionality that undergoes a reaction with oxygen free radicals to produce an excited electronic state in A. Then A undergoes intramolecular energy transfer from its own excited state to the photochromic B functionality which is an energy acceptor. Upon receiving energy from A, B achieves a high energy state which relaxes through heterolytic cleavage of the covalent bond of B with drug D to achieve intracellular release. The free drug, D, effects a therapeutic functional change by a mechanism that comprises receptor-mediated mechanisms including reversible and irreversible competitive agonism or antagonism, including a molecule known as a suicide substrate, or a transition state analog, or a noncompetitive or uncompetitive agonism or antagonism, or the action is by a nonreceptor-mediated mechanism including a "counterfeit incorporation mechanism." The reaction effects the release of a drug moiety into the desired compartment in active form to achieve a greater therapeutic effect or therapeutic ratio relative to the free drug alone as a consequence of altered pharmacokinetics or pharmacodynamics such as a desirable kinetics of release, a resistance to inactivation or excretion, greater solubility, enhanced absorption, a diminished toxicity, or greater access to the cellular or biological compartment which is the site of action of the drug.

In our approach, drug release involved drug-conjugate bond cleavage by a photochemical reaction of a photochromic moiety. Photochromic systems undergo a color change upon absorption of electromagnetic radiation by mechanisms such as conformational changes or photodissociation. Salt isomerism photochromic systems belong to the latter class and typically involve the breaking of the bond between a bleaching agent and a positive center of a dye. The color of a salt isomerism dye is due to the presence of a long

conjugated chain of alternating single and double bonds. The resonance of the positive center through the conjugated chain appears to be essential for phototropy. A nucleophilic group reacts with the positive charge as it crosses through the conjugated chain and forms the leuco (colorless) compound which thus inhibits color resonance by preventing conjugation. The conjugated chain is regenerated by the action of ultraviolet light restoring the color resonance. The quantum yield of this reaction may approach one.[15] This is illustrated in the example shown in Figure 1. In the present case, it is this photodissociation reaction that is important to the mechanism of the carrier molecules of our research. Bleaching agents (molecules that covalently bond to B, the photochromic functionality of the carrier) include essentially any nucleophilic groups of a drug including phosphate, sulfide, sulfite, sulfate, carboxylate, hydroxyl, or amine. Several carrier molecules were synthesized. Each carrier molecule has one chemiluminescent moiety (A) and one photochromic moiety (B). Thus, the carrier molecule is represented as A-B. Prodrugs were synthesized by attaching a drug moiety, D, to each carrier, either foscarnet or anti-HIV nucleotide ddc shown in Figure 2A,B, respectively.

Our release scheme also depends on chemiluminescence to provide an energy source to cause the cleavage of the drug-carrier bond. Chemiluminescence involves the relaxation of the energy from a chemical reaction by release of a photon rather than the relaxation by thermalization in vibration, rotational, and translational modes. Typically the mechanism of chemiluminescence involves the achievement of a metastable electronic energy state.

Molecules newly formed in chemical reactions usually release their excess energy by colliding with solvent molecules, vessel walls, etc. Occasionally, however, excess energy is lost by radiation. Energetic reactions in gases, or shock waves generated mechanically, can often give rise to such high temperatures of the products that thermal radiation occurs (flames and explosions), but

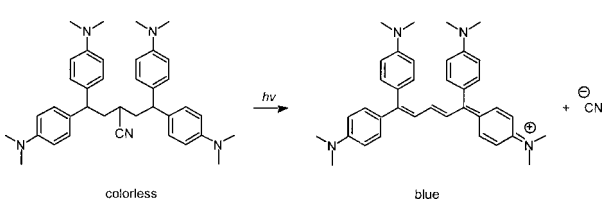

Figure 1. Photochromic reaction of a leuco dye.

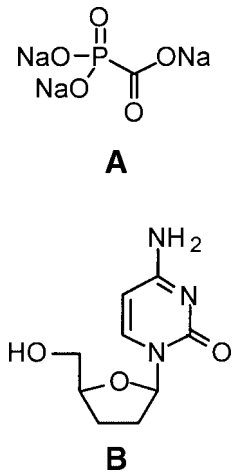

Figure 2. Structures of drugs. (A) foscarnet (phosphonoformate); (B) dideoxycytidine (ddc).

sometimes true chemiluminescence is observed, characterized by a much stronger emission at a selective region of the spectrum than an ideally perfect thermal radiator would emit at the same temperature. In fact, here the concept of temperature becomes somewhat blurred because molecules of very high energy are being produced which radiate their characteristic spectra before they have properly equilibrated to the bulk temperature energy of the system. Ordinary reactions in solution never get hot enough to emit thermally; any visible light observed must be chemiluminescence. The actual emission process must follow basic quantum principles, that is, molecules must be formed in an energetic quantum state of definite "mean-life" (fixed by the degree to which transitions between levels are "allowed"), and must resist de-activation and equilibration for a sufficient time to allow radiation to take place. For emission in the visible region, individual molecules must be formed with energies of 40 (red) to 70 (blue) kcal/mole. The nature of the excitation must be electronic, and if the radiational transition to the ground state is "allowed," the radiational lifetime is of the order of 10^{-8} s. Less-allowed transitions have longer lifetimes, up to a thousand million times or so longer for so-called "forbidden" transitions. The length of time between collisions in liquids is about 10^{-12} s, so that radiation is possible only if the electronically excited molecules are stable over many thousands of collisions. The kind of molecule with this property forms an electronically excited metastable fluorescent molecule typically having allowed transitions such as singlet → singlet. The radiational lifetimes of forbidden transitions, such as triplet → singlet, are so

long that energy robbing (quenching) by traces of dissolved oxygen or other molecules present competes very effectively with light emission.

The best-known chemiluminescent reaction, because the substance involved is commercially available, is the oxidation of 3-amino-phthalhydrazide, often called "luminol." Its solution in 0.03 N NaOH, containing some hydrogen peroxide, gives a bright light emission when ferricyanide or hypochlorite solution is added. The emission may be green or blue depending on the degree of ionization and solvation of the product ion. Measurements of the light-yield (light quanta emitted/molecules oxidized) combined with the figure for the quantum yield of fluorescence of the product indicate that a large fraction of the product molecules are formed in their electronically excited state. The overall reaction may be represented as shown in Figure 3.

Chemiluminescent molecules such as isoluminol have been used as "reporters" of intracellular free radicals.[16] This is the basis of selective drug release in our scheme. Electron transferring and transporting elements are ubiquitous and are necessary for life. All eukaryotic and prokaryotic organisms depend on electron transferring and transporting elements which include metal containing hemes and nonmetal containing molecules such as flavins to convert the energy stored in the chemical bonds of foodstuffs into a form utilizable for the maintenance of the highly negative entropic state of life. The chemical energy conversion process generally involves a coupled series of electron carriers which is called an electron transport chain. Free radicals of oxygen are produced during aerobic respiration in mitochondria as electrons are carried by electron carriers of the electron transport chain to the ultimate electron acceptor, oxygen, and superoxide and peroxide, partial reduction products of oxygen, are continuously produced during cytosolic hydroxylation and oxygenation reactions as well as during other reactions that involve enzymatic reduction of oxygen.

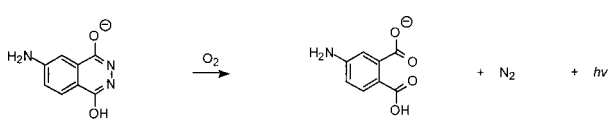

Figure 3. Chemiluminescence is the production of light by chemical reactions. Electronically excited states are formed in the chemical reaction, and light emission is the means by which relaxation occurs. Isoluminol [5-amino-2,3-dihydrophthalazine-1,4-dione] and analogs are chemiluminescent by oxidation reactions.

The cytosol as well as mitochondria of aerobic cells contain high concentrations of the enzyme superoxide dismutase which converts superoxide into hydrogen peroxide and molecular oxygen. Oxygen radicals which include hydrogen peroxide and superoxide are found in greater concentration in the mitochondria relative to the cytosol because reduction of oxygen occurs to a greater extent in the former compartment; however, appreciable concentrations are found in both compartments.

The prodrugs were designed to permeate to white blood cells and undergo an oxidation reduction reaction with the target cell's electron carriers or react with free radicals produced as a consequence of electron transport. Radicals are naturally produced during respiration where oxygen is the acceptor of electron transport. Approximately 6% of breathed oxygen is converted to such radicals inside of cells as part of the respiratory process.[17]

A representative prodrug is the product of the covalent linkage of the polymethine dye with a bleaching drug such as foscarnet and with a chemiluminescent reactive molecule such as isoluminol. This conjugate represents a molecule that releases foscarnet in the presence of oxygen free radicals. In our drug-release scheme, the energy of the reaction of the chemiluminescent moiety such as luminol with oxygen radicals undergoes intramolecular electronic energy transfer by radiative and nonradiative mechanisms. The latter dominate and include coulombic interactions, dipole–dipole resonance, and exchange interaction. These processes increase the quantum yield for drug release above that which would be produced by luminescence transfer alone. For example, Förster, in a quantum mechanical treatment of resonance transfer, in the region of spectral overlap involving allowed transitions of two well separated molecules has only considered dipole–dipole interactions in deriving an experimentally verified formula that predicts a distance of 5–10 nm as the distance at which transfer and spontaneous decay of the excited donor are equally probable.[18] The formula predicts that the transfer probability is inversely proportional to the separation distance raised to the sixth power. However, in our case, the donor and acceptor functionalities of a conjugate are covalently linked. Because the separation distance is of the order of angstroms, the transfer probability is great. In fact, the efficiency of transfer has been studied in certain molecules that consist of two independent

chromophores separated by one or more saturated bonds. In such cases, energy transfer over large distances has been observed to be in agreement with predictions from Förster's theory.[18–20]

Energy transfer has been shown to occur in the chemiluminescence of hydrazides such as isoluminol which are linked to some highly fluorescent groups such as an acridone moiety. Oxidation of the hydrazide portion of the conjugate results in emission of the acridone moiety due to rapid intramolecular singlet–singlet energy transfer from the electronically excited phthalate ion to the acridone portion of the molecule.[21]

An exemplary carrier molecule, MTLJ-1, 1,5-di-(*p*-*N*-2-(*N*-(4-aminobutyl)-*N*-ethyliso-luminol)-*N*-ethylaminophenyl)-1,5-bis-(*p*-*N*,*N*-dimethylaniline)-1,3-pentadiene, comprising two molecules of an isoluminol derivative (A functionality) bound to a polymethine dye derivative (B functionality) is shown in Figure 4A. The release of a prodrug by the mechanism given in Figure 1 was tested by reaction of a prodrug comprising deferoxamine, an iron chelating agent, and the carrier, MTLJ-1. Because deferoxamine changes color upon complexing with iron with a maximum absorbance at 425 nm, its release from the prodrug was determined by measuring the absorbance of the iron-deferoxamine complex (425 nm) which formed upon release of deferoxamine. MTLJ-1 and four other carrier molecules, YY99811-1, **6a**, GZW2-33-1, and GZW1-98-2, shown in Figure 5A–E, respectively, were tested alone and as prodrugs with foscarnet and ddc. The *in vivo* and *in vitro* results of the efficacy against retroviruses were compared with those of each drug alone.

RESULTS AND DISCUSSION

The results of the release of deferoxamine from the prodrug Des-MTLJ-1 by reaction with a source of free radicals is shown in Table 1. The absorbance at 425 nm doubled upon addition of hydrogen peroxide to Des-MTLJ-1; whereas, no change in absorbance was observed in the case of the control, Des-dye, of the same structure as the prodrug, except that the chemiluminescent moiety, isoluminol, was absent. These results support the mechanism shown in Figure 1. The drugs, foscarnet and ddc, shown in Figure 2A and B are highly polar and are highly soluble in polar solvents. In contrast, the carriers used in these studies shown in Figure 4A–E are soluble in dimethylformamide, dimethyl sulfoxide (DMSO), tetrahydrofuran (THF)/H_2O (2:1), CH_3CN/H_2O (2:1), and insoluble in H_2O. The solubility in nonpolar solvents favors cell permeability. The enhanced permeability due to the lipophilicity of the carriers is evidenced by the lack of activity of the carrier alone, but an enhanced effect as a prodrug as presented next.

Prodrugs were tested in cultured macrophages at Southern Research Institute, Frederick, MD, with further testing performed under contract with the National Institutes of Health (NIH), and prodrugs were tested in a murine model at Luminide Pharmaceutical Corporation (LPC).

Southern Research Institute Evaluation of Prodrug Anti-HIV-1 Potency in Macrophage Assay

A negative control prodrug YY99811-1-foscarnet versus that of **6a**-foscarnet and **6a**-ddc were tested for the suppression of an HIV infection in human cultured macrophages at Southern Research Institute. The results of the tests are given in Tables 2–4. The structures of the drug compounds, foscarnet and ddc, are given in Figure 2A,B, respectively. The structure of YY99811-1 versus that of **6a** are shown in Figure 4B and C, respectively. The substitution of an oxygen for a nitrogen atom on structure YY99811-1 versus **6a** results in a decreased ability for a drug to be released from the corresponding prodrug because the conjugation of the photochromic moiety is greatly diminished. The results of the tests of the negative control carrier and the corresponding Foscavir prodrug are given in Table 2. The data given in Table 2 indicate that YY99811-1 was nontoxic. The corresponding prodrug had no effect as anticipated. The Foscavir-YY99811-1 conjugate served as a negative control for prodrugs with the potential for release of the drug.

The results of ddc and foscarnet prodrugs of carrier **6a** are given in Tables 3 and 4, respectively. In each case, the carrier was found to be nontoxic and the corresponding prodrug to be as efficacious as the free drug alone. This indicates that the prodrug was highly effective at drug release in the presence of HIV-1-infected human monocytes.

NIH Contracted Southern Research Institute Evaluation of Prodrug Anti-HIV-1 Potency in Macrophage Assay

Southern Research Institute, under contract with NIH, advanced the tests described in the

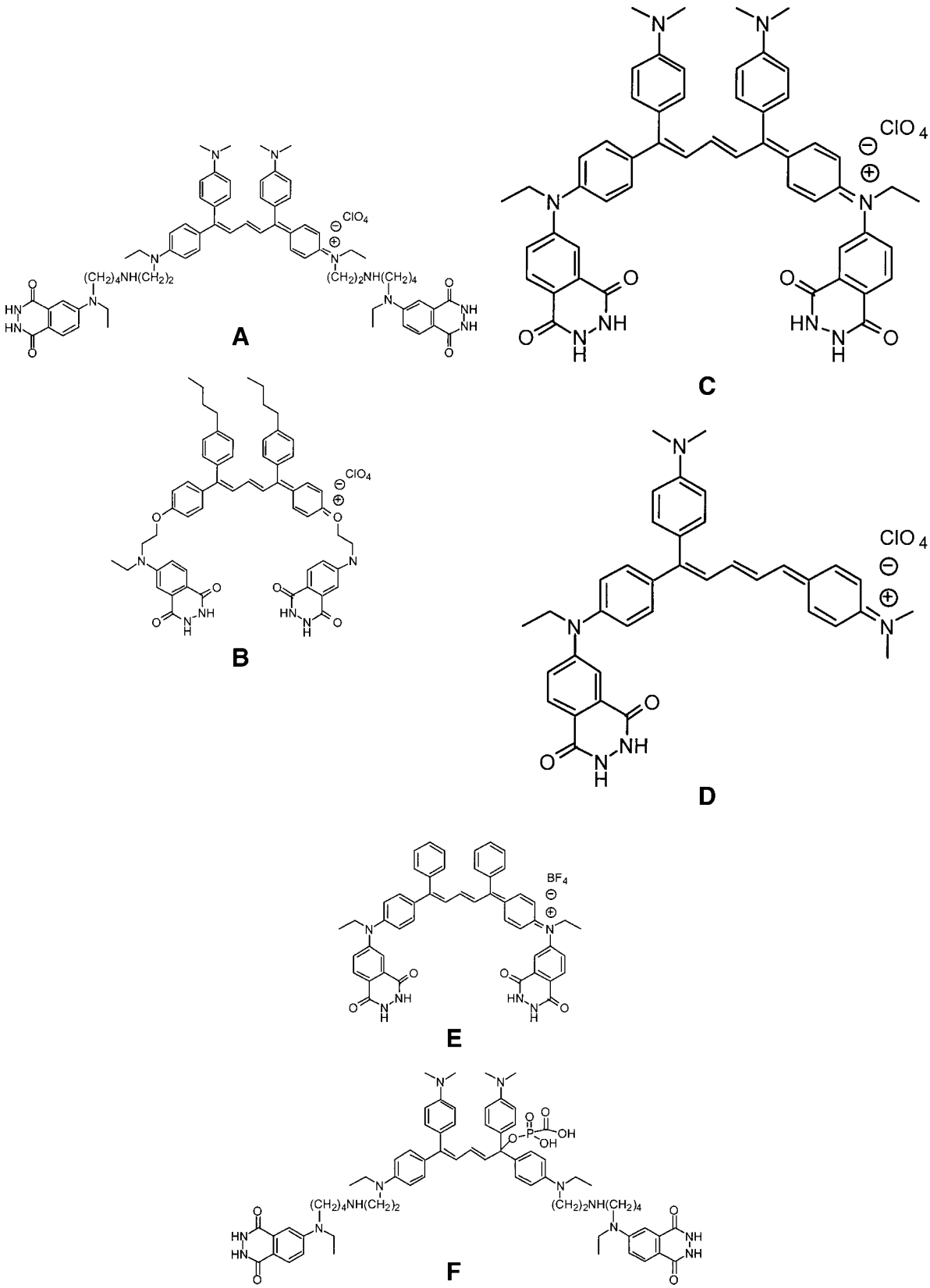

Figure 4. Structures of carriers and prodrug. (A) carrier MTLJ-1, (B) carrier YY99811-1, (C) carrier **6a**, (D) carrier GZW2-33-1, (E) carrier GZW1-98-2, and (F) prodrug MTLJ-1-foscarnet.

"Southern Research Institute Evaluation of Prodrug Anti-HIV-1 Potency in Macrophage Assay" section by retesting the **6a** carrier as well as two additional carriers, GZW2-33-1 and GZW1-98-2. The structure of the test compound ddc, and carriers **6a**, GZW2-33-1, and GZW1-98-2 are given in Figures 2B and 4C–E, respectively. The results of the NIH-sponsored tests are given in Table 5. The prodrugs caused suppression of an HIV infection in human cultured macrophages that was up to five times more effective than the very potent drug ddc alone. NIH rated the prodrugs "highly active" and determined that the prodrug was efficacious in the potentiation of ddc. The prodrugs were further demonstrated to be nontoxic.

LPC Evaluation of Prodrug Antiretroviral Potency in a Murine Model

LPC performed *in vivo* testing of the prodrug MTLJ-1-foscarnet for the suppression of Friend leukemia virus (FLV) infection in mice as compared with foscarnet alone. The results are given in Table 6. The structures of the foscarnet, carrier MTLJ-1, and prodrug, MTLJ-1-foscarnet are given in Figures 2A, 4A, and 4F, respectively.

These results indicate that MTLJ-1-foscarnet was highly effective as demonstrated by the absence of splenomegaly in the animals that were administered this compound. The spleen weights of the virus + MTLJ-1-foscarnet group are the same as those of the no virus, no treatment group; whereas, the spleen weights of the virus + foscarnet group are the same as those of the virus alone group.

The data indicate that the prodrug MTLJ-1-foscarnet is effective and that foscarnet is ineffective at a significance level of 0.01 as determined by the Student's t test. The results of the toxicity testing of the prodrug were $LD_{50} > 1250$ mg/kg intraperitoneally (IP) which indicates that it was nontoxic. The data further indicated that MTLJ-1-foscarnet was nontoxic by the weight gain of 2.5 g by the virus + MTLJ-1-foscarnet group as compared with the 1.5-g weight gain by the virus + foscarnet group.

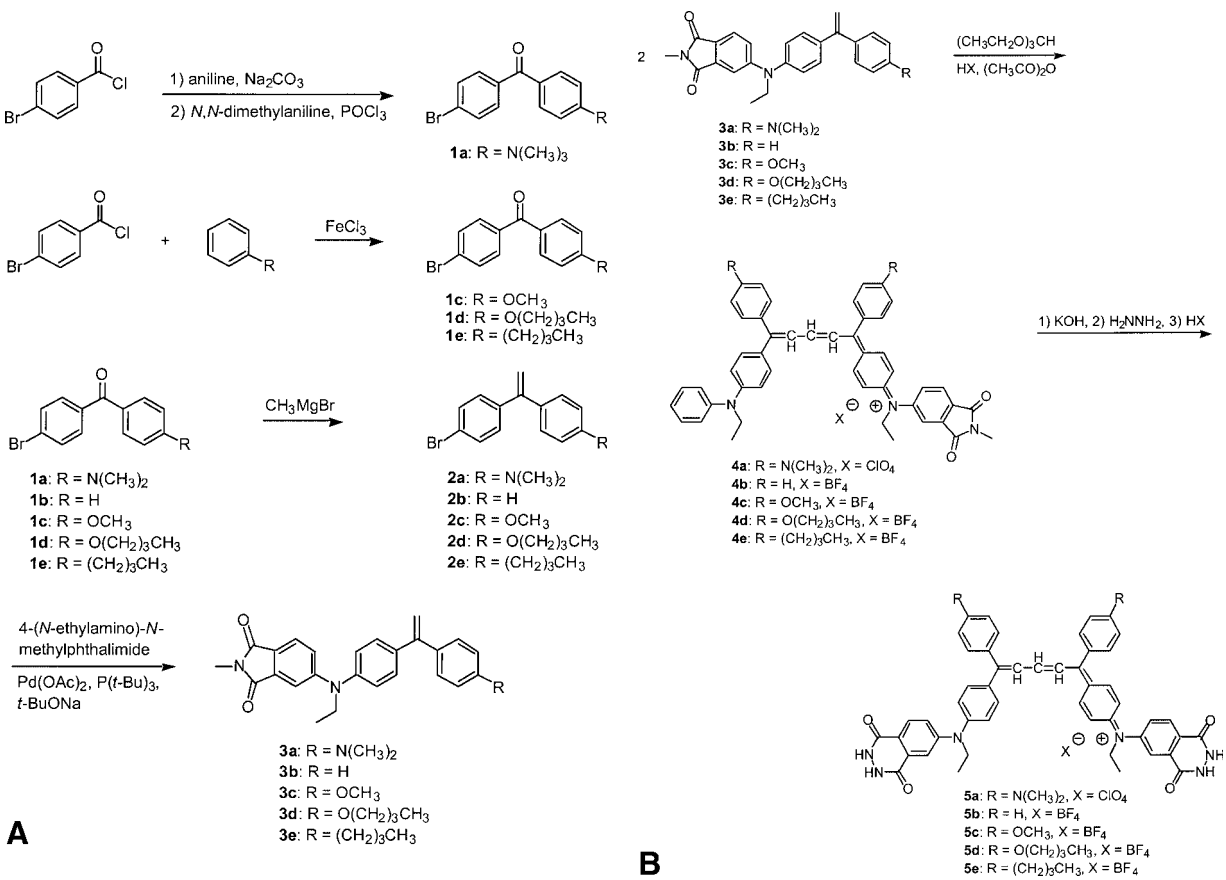

Figure 5. Schematic for the synthesis of carriers and prodrugs.

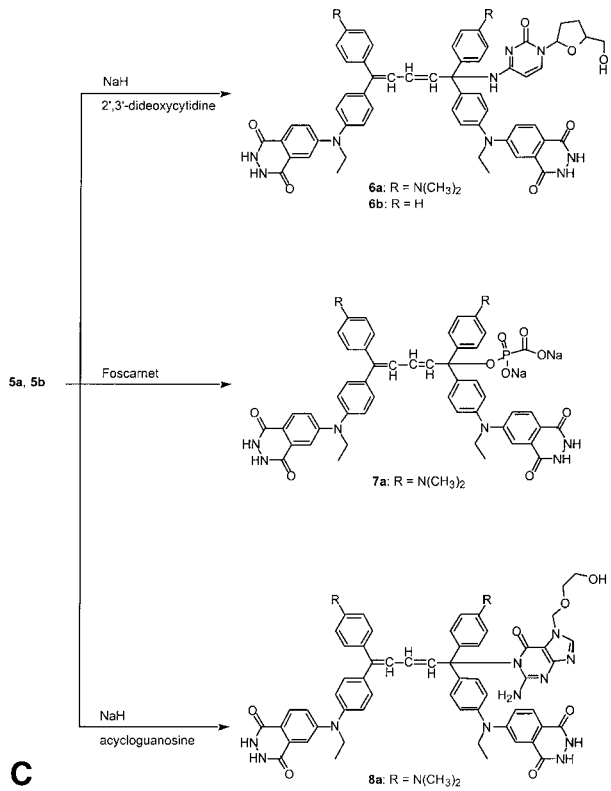

Figure 5. (*Continued*)

CONCLUSIONS

A chemiluminescent-photochromic conjugate carrier molecule was designed and synthesized to enhance the cellular uptake of anti-HIV drugs. An increase in the activity of foscarnet and ddc was observed in cultured macrophages infected with HIV and in mice infected with the retrovirus FLV.

Table 1. Release of Deferoxamine from the Prodrug Des-MTLJ-1 by Reaction with a Source of Free Radicals, H_2O_2, Determined by Recording the Change in Absorbance of Deferoxamine at 425 nm

Experiment Number	Absorbance at 425 nm	
	$+H_2O_2$	$-H_2O_2$
1	0.297	0.135
2	0.280	0.155
3	0.275	0.149
4	0.297	0.140
5	0.279	0.125
6	0.260	0.136

Because the carrier is independent of the drug, biologically active agents having the optimal structure to achieve the highest therapeutic ratio may be attached to such a carrier which is modified to achieve optimal bioavailability for a given drug. Thus, the physical-chemical properties of a prodrug which change its bioavailability, can be manipulated without altering the optimal drug structure. Thousands of existing promising drugs can be salvaged, and existing drugs can be made more potent with a higher therapeutic ratio. The discovery of a promising drug by conventional means costs the pharmaceutical industry, by one rule of thumb, approximately 10 million dollars per application.[22,23] Because our prodrugs may be able to utilize and potentiate known biologically active compounds, tremendous potential savings in drug product development may be realized.

EXPERIMENTAL

General Instrumentation and Materials in the Synthesis of the Carriers and Prodrugs

A schematic for the synthesis of carriers and prodrugs is shown in Figure 5. Unless otherwise specified, all organic and inorganic reagents and solvents were purchased from commercial suppliers and were used directly without further purification. Elemental analyses (Anal.) were performed at Atlantic Microlab, Inc., Norcross, Georgia. Fast atom bombardment (FAB) mass spectroscopy was performed on a VG Analytical ZAB 2-SE high field mass spectrometer at M-Scan, Inc., West Chester, PA. Melting points (m.p.) were obtained using an IA9100 electrothermal digital melting point apparatus. The majority of the proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Varian Unity Inova 400-MHz spectrometer at Spectral Data Services, Inc. at Champaign, IL. Chemical shifts (δ) are reported in parts per million relative to tetramethylsilane used as internal standard in deuterated DMSO-d_6. Thin layer Chromatography (R*f*) was performed on Baker Si250F silica gel TLC plates.

4-Bromo-4′-(*N*,*N*-dimethylamino) benzophenone (1a)

A solution of aniline (19.1 g, 0.21 mol) and anhydrous sodium carbonate (42 g, 0.4 mol) in anhydrous tetrahydrofuran (250 mL) was brought

Table 2. Southern Research Institute Test Results of a Negative Control Carrier and the Corresponding Negative Control Foscarnet Prodrug

Compound	IC_{50} (μM)[a]	TC_{50} (μM)[b]	TI (TC_{50}/IC_{50})[c]
Foscarnet	0.55	>10	>18
YY99811-1 (carrier)	>10	>10	NA
YY99811-1-foscarnet	>10	>10	NA

NA, not applicable.
[a]50% inhibition of virus replication.
[b]50% cytotoxicity.
[c]Therapeutic index.

to reflux under nitrogen. Upon stirring, 4-bromobenzoyl chloride (50 g, 0.23 mol) was added in portions over a period of 1 h. The resulting mixture was refluxed for 4 h and the solvent was then removed under reduced pressure using a rotary evaporator. The crude product deposited was stirred in cold water and collected by filtration. The washing procedure was repeated and the white powder (57.6 g, m.p. 202°–203°C) obtained was air-dried and used for the following reaction without further purification. A mixture of the white powder (30 g), *N*,*N*-dimethylaniline (43 g, 0.35 mol), and phosphorous oxychloride (25 g, 0.16 mol) was heated in an oil bath at 112°C. The exothermic displacement reaction that occurred in the mixture was indicated by the color change from green to brown as well as by the rapid increase of the temperature to 140°C. The oil bath was removed and the mixture was cooled to 110°C in an ice-water bath and then continuously stirred at 100°–105°C for 3 h. The mixture was cooled to 60°C and poured into an aqueous HCl solution (1.6 N, 220 mL) and then stirred at room temperature overnight. The crude product deposited was collected by filtration and washed with cold water three times and then air-dried to give 39 g of green solids. The filtrate was diluted with 1 L of water and the precipitate was collected by filtration and washed with cold water three times and then air-dried to give 3 g of green solids. The green solids were combined and recrystallized from ethanol to give the desired product **1a** (23.3 g, 0.076 mol, 69% yield) as green sandy crystal: m.p. 126.4°–127.4°C; MS (FAB, MH^+, $C_{15}H_{14}ONBr$) calcd. 304, found 304; ^1H NMR (DMSO-d_6) δ 3.04 (s, 6H), 6.77 (d, $J = 9.0$ Hz, 2H), 7.57 (d, $J = 8.2$ Hz, 2H), 7.64 (d, $J = 8.9$ Hz, 2H), 7.72 (d, $J = 8.2$ Hz, 2H); Anal. $C_{15}H_{14}ONBr$, calcd. C 59.23, H 4.64, N 4.61, Br 26.27, found C 59.10, H 4.67, N 4.53, Br 26.09.

4-Bromo-4′-methoxybenzophenone (1c)[24]

A mixture of anisole (30 mL, 276 mmol), 4-bromobenzoyl chloride (12.4 g, 56.5 mmol), and ferric chloride (0.3 g, 1.85 mmol) was heated under argon atmosphere in an oil bath at 144°C for 2 h. The mixture was stirred at room temperature for 1 h followed by refluxing the mixture in 50 mL of 10% KOH for 30 min. After cooling to room temperature, toluene (100 mL) was added to the mixture and the resulting

Table 3. Southern Research Institute Test Results of the **6a** Carrier and the Corresponding ddc Prodrug

Compound	IC_{50} (μM)[a]	TC_{50} (μM)[b]	TI (TC_{50}/IC_{50})[c]
ddc	0.07	>473.00	>6757.14
6a (carrier)	>100.00	>100.00	NA
6a-ddc	0.03	>100.00	>3333.33

NA, not applicable.
[a]50% inhibition of virus replication.
[b]50% cytotoxicity.
[c]Therapeutic index.

Table 4. Southern Research Institute Test Results of the **6a** Carrier and the Corresponding Foscarnet Prodrug

Compound	IC_{50} (μM)[a]	TC_{50} (μM)[b]	TI (TC_{50}/IC_{50})[c]
Foscavir	1.83	>333.20	>182.08
6a (carrier)	23.14	>103.79	>4.48
6a-foscarnet	2.12	62.88	29.66

[a]50% inhibition of virus replication.
[b]50% cytotoxicity.
[c]Therapeutic index.

Table 5. Southern Research Institute Test Results of ddc Prodrugs of **6a**, GZW1-98-2, and GZW2-33-1 Carriers Performed under NIH Contract

Compound	IC_{50} (μM)[a]	TC_{50} (μM)[b]	TI (TC_{50}/IC_{50})[c]
ddc	0.040	>100	>2500
6a (carrier)	2.62	>100	>38.2
6a-ddc	0.008	>100	>12,500
GZW1-98-2 (carrier)	23.1	>100	>4.3
GZW1-98-2-ddc	0.019	>100	>5263
GZW2-33-1	18.9	>100	>5.3
GZW2-33-1-ddc	0.021	>100	>4762

[a]50% inhibition of virus replication.
[b]50% cytotoxicity.
[c]Therapeutic index.

solution was filtered. The organic layer was separated from the filtrate and washed with water (300 mL) twice. Toluene was removed from the solution under reduced pressure. The crude product deposited was recrystallized from toluene to give the product **1c** (9.25 g, 31.77 mmol, 56% yield) as white sandy solids: m.p. 156.0°–157.6°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.89 (s, 3H), 6.97 (d, $J = 9.0$ Hz, 2H), 7.62 (s, 4H), 7.79 (d, $J = 8.7$ Hz, 2H); Anal. $C_{14}H_{11}O_2Br$, calcd. C 57.76, H 3.81, found C 57.79, H 3.72.

4-Bromo-4′-*n*-butoxybenzophenone (1d)[24]

A mixture of *n*-butyl phenyl ether (30 g, 200 mmol), 4-bromobenzoyl chloride (11.4 g, 51.9 mmol), and ferric chloride (0.3 g, 1.85 mmol) was heated under argon atmosphere in an oil bath at 144°C for 3 h. The mixture was cooled to 60°C followed by the addition of water (30 mL) and toluene (100 mL). The organic layer was separated from the resulting mixture and washed with 1 N HCl (30 mL × 3) and water (30 mL × 3). Toluene was removed from the resulting solution via a rotary evaporator under reduced pressure. The crude product deposited was recrystallized from ethanol to give the product **1d** (14.27 g, 42.8 mmol, 82% yield) as white sandy solids: m.p. 115.4°–117.2°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.99 (t, $J = 7.3$ Hz, 3H), 1.51 (m, 2H), 1.80 (m, 2H), 4.04 (t, $J = 6.4$, 2H), 6.95 (d, $J = 8.7$ Hz, 2H), 7.62 (s, 4H), 7.79 (d, $J = 8.7$ Hz, 2H); Anal. $C_{17}H_{17}O_2Br$, calcd. C 61.28, H 5.14, found C 61.35, H 5.20.

4-Bromo-4′-*n*-butylbenzophenone (1e)[24]

A mixture of *n*-butylbenzene (25 mL, 160 mmol), 4-bromobenzoyl chloride (12.3 g, 56 mmol), and ferric chloride (0.47 g, 2.8 mmol) was heated under argon atmosphere in an oil bath at 144°C for 6 h. After the mixture was cooled to 60°C, water was added (20 mL) and the resulting mixture was extracted by toluene (200 mL). Toluene was removed from the extract via a rotary evaporator under reduced pressure. The crude product deposited was recrystallized from 90% ethanol solution to give 12.5 g of solids.

1-(4-Bromophenyl)-1-[4-(*N,N*-dimethylamino)phenyl]ethene (2a)

Upon stirring a benzene (35 mL) solution of **1a** (3.3 g, 10.8 mmol) under a N_2 atmosphere, an ethereal solution of methylmagnesium bromide (3 M, 6.5 mL, 20 mmol) was added dropwise. The resulting solution was refluxed under N_2 for 4 h and then allowed to cool to room temperature. Upon stirring the resulting solution, a saturated aqueous solution of NH_4Cl (5 mL) was added and the final mixture was filtered through a filter paper. The precipitate collected was stirred in hot benzene (30 mL) and then filtered. The filtrates

Table 6. Effect of Prodrug MTLJ-1-Foscarnet on 4-Week-Old Swiss Mice Infected with FLV

	No Virus, no Treatment	Virus Alone	Virus + Foscarnet	Virus + Carrier Foscarnet	Virus + MTLJ-1-Foscarnet
Average final weight (g)	20.8	20.3	18.9	21.7	22.4
Average weight change (g)	+1.6	+0.1	+1.5	+0.4	+2.5
Average spleen weight (g)	0.0907	0.1647	0.1727	0.1272	0.0914[a]
Standard deviation of spleen weights (g)	0.02	0.07	0.1	0.03	0.02

[a]$p < 0.01$ (MTLJ-1-foscarnet).

were combined and azeotropically removed the water by refluxing the solution in the presence of *p*-toluenesulfonic acid monohydrate (0.15 g, 0.8 mmol) for 1 h. After cooling to room temperature, potassium bicarbonate (1 g, 10 mmol) was added and the mixture was stirred for 30 min and then filtered through a filter paper. Solvent was removed from the filtrate under reduced pressure using a rotary evaporator and the crude product deposited was recrystallized from ethanol to give the desired product **2a** (2.4 g, 7.9 mmol, 74% yield) as white crystal: m.p. 126.0°–126.8°C; MS (FAB, MH^+) 303; ^{1}H NMR (DMSO-d_6) δ 3.91 (s, 6H), 5.24 (s, 1H), 5.38 (s, 1H), 6.70 (d, $J = 8.9$ Hz, 2H), 7.11 (d, $J = 8.7$ Hz, 2H), 7.24 (d, $J = 8.5$ Hz, 2H), 7.55 (d, $J = 8.5$ Hz, 2H); Anal. $C_{16}H_{16}NBr$, calcd. C 63.59, H 5.34, N 4.64, Br 26.44, found C 63.61, H 5.35, N 4.47, Br 26.63.

1-(4-Bromophenyl)-1-phenylethene (2b)

The same procedure described for the synthesis of **2a** was used except that 4-bromobenzophenone (**1b**, 10 g, 38.3 mmol) and 1.2 equivalent of methylmagnesium bromide were used to generate the crude product **2b** (10 g) as light yellow oil. The crude product was used for the preparation of **3b** without further purification: ^{1}H NMR (DMSO-d_6) δ 5.52 (s, 1H), 5.54 (s, 1H), 7.25 (d $J = 8.3$ Hz, 2H), 7.29 (m, 2H), 7.37 (m, 3H), 7.57 (d, $J = 8.3$ Hz, 2H).

1-(4-Bromophenyl)-1-(4-methoxyphenyl) ethene (2c)

The same procedure described for the synthesis of **2a** was used except that 4-bromo-4′-methoxybenzophenone (**1c**, 7.0 g, 24.0 mmol) and 2.0 equivalent of methylmagnesium bromide were used to generate the crude product followed by recrystallization from ethanol to give **2c** (4.88 g, 16.9 mmol, 70% yield): m.p. 91.2°–92.6°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.83 (s, 3H), 5.34 (s, 1H), 5.40 (s, 1H), 6.87 (d, $J = 9.0$ Hz, 2H), 7.23 (m, 4H), 7.44 (d, $J = 8.7$ Hz, 2H); Anal. $C_{15}H_{13}OBr$, calcd. C 62.30, H 5.34, N 4.53, found C 62.27, H 4.53.

1-(4-Bromophenyl)-1-(4-*n*-butoxyphenyl) ethene (2d)

The same procedure described for the synthesis of **2a** was used except that 4-bromo-4′-*n*-butoxybenzophenone (**1d**, 8.0 g, 24.0 mmol) and 1.5 equivalent of methylmagnesium bromide were used to generate the crude product **2d** (7.85 g, 23.7 mmol, 98% yield) and it was used for the preparation of **3d**. The crude product could be purified by recrystallization from ethanol to give white crystal: m.p. 71.8°–73.0°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98 (t, $J = 7.2$ Hz, 3H), 1.50 (m, 2H), 1.77 (m, 2H), 3.97 (t, $J = 6.6$ Hz, 2H), 5.32 (s, 1H), 5.39 (s, 1H), 6.85 (d, $J = 8.7$ Hz, 2H), 7.22 (m, 4H), 7.45 (d, $J = 8.4$ Hz, 2H); Anal. $C_{18}H_{19}OBr$, calcd. C 65.27, H 5.78, found C 65.27, H 5.76.

1-(4-Bromophenyl)-1-(4-*n*-butylphenyl)ethene (2e)

The same procedure described for the synthesis of **2a** was used except that 4-bromo-4′-*n*-butylbenzophenone (**1e**, 9.0 g) and 18.9 mL of the 3 M ethereal solution of methylmagnesium bromide were used to generate the crude product followed by recrystallization from ethanol to give 4.68 g of solids.

1-(4-*N*,*N*-Dimethylaminophenyl)-1-[4-*N*-ethyl-*N*-(*N*-methylphthalimid-4-yl)-aminophenyl]ethene (3a)[25]

An anhydrous toluene solution (5 mL) containing palladium acetate (18 mg, 0.08 mmol) and tri-*tert*-butylphosphine (66.6 mg, 0.30 mmol) was added to a suspension of **2a** (1.0 g, 3.3 mmol), 4-(*N*-ethylamino)-*N*-methylphthalimide[26] (0.67 g, 3.3 mmol), and sodium *tert*-butoxide (0.40 g, 4.0 mmol) in anhydrous toluene (10 mL). The resulting red mixture was stirred at 100°C under N_2 for 3 h. After cooling to room temperature, water was added to the mixture and extracted with ethyl acetate (50 mL) three times. The organic extracts were combined and washed with saturated aqueous NaCl and then dried over $MgSO_4$ and filtered. The filtrate was concentrated to a syrup and then dissolved in a mixture of ethyl acetate and hexane (4:1, 100 mL). After allowing the mixture to stand at 4°C overnight, the yellow crystals formed were collected by decanting the supernatant and dried under reduced pressure to give the desired product **3a** (0.90 g, 2.1 mmol, 64% yield): m.p. 136.1°–138.0°C; MS (FAB, MH^+) 425; ^{1}H NMR (DMSO-d_6) δ 1.19 (t, $J = 7.1$ Hz, 3H), 2.92 (s, 6H), 2.97 (s, 3H), 3.88 (q, $J = 7.1$ Hz, 2H), 5.31 (s, 1H), 5.37 (s, 1H), 6.73 (d, $J = 9.0$ Hz, 2H), 6.97–7.00 (m, 2H), 7.18 (d, $J = 8.7$ Hz, 2H), 7.26 (d, $J = 8.3$ Hz, 2H), 7.42 (d, $J = 8.2$ Hz, 2H), 7.62 (d, $J = 8.3$ Hz, 1H); Anal. $C_{27}H_{27}N_3O_2$, calcd. C 76.21, H 6.40, N 9.87, found C 75.97, H 6.50, N 9.62.

1-[4-*N*-Ethyl-*N*-(*N*-methylphthalimid-4-yl)-aminophenyl]-1-phenylethene (3b)[25]

Using a micro syringe, $P(^tBu)_3$ (432 μL, 1.56 mmol) was added to a mixture of crude **2b** (2.0 g), 4-(*N*-ethylamino)-*N*-methylphthalimide[26] (1.35 g, 6.61 mmol), $Pd(OAc)_2$ (88 mg, 0.39 mmol), and NaO^tBu (0.89 g, 9.26 mmol) in anhydrous toluene (20 mL). The red mixture was stirred at 100°C under argon for 4 h. After cooling to room temperature, water (100 mL) was added to the mixture and extracted with ethyl acetate (100 mL) twice. The organic extracts were combined and washed with saturated aqueous NaCl and then dried over $MgSO_4$ and filtered. The filtrate was concentrated to a syrup and flash chromatographed on a silica gel (32–63 μm, 60 Å) column using 30% ethyl acetate in hexane as the eluent to give the desired product **3b** as a yellow syrup (1.51 g): R*f* = 0.59; ^{1}H NMR (DMSO-d_6) δ 1.19 (t, J = 7.1 Hz, 3H), 2.97 (s, 3H), 3.88 (q, J = 7.0 Hz, 2H), 5.50 (s, 1H), 5.59 (s, 1H), 6.98–7.02 (m, 2H), 7.28 (d, J = 8.6 Hz, 2H), 7.34–7.44 (m, 7H), 7.62 (d, J = 8.3 Hz, 1H).

1-(4-Methoxyphenyl)-1-[4-*N*-ethyl-*N*-(*N*-methylphthalimid-4-yl)-aminophenyl]ethene (3c)[25]

The same procedure described for the synthesis of **3a** was used except that 1-(4-bromophenyl)-1-(4-methoxyphenyl)ethene (**2c**, 1.0 g, 3.46 mmol), 4-(*N*-ethylamino)-*N*-methylphthalimide[26] (0.7 g, 3.47 mmol), $Pd(OAc)_2$ (40 mg, 0.18 mmol), tri-*tert*-butylphosphine (192 μL, 0.69 mmol), and NaO^tBu (0.4 g, 4.16 mmol) in anhydrous toluene (15 mL) were used to generate the crude product. The crude product was flash chromatographed on a silica gel (32–63 μm, 60 Å) column using 20% ethyl acetate in hexane as the eluent and the isolate obtained was recrystallized from a mixture of ethyl acetate and hexane to give the desired product **3c** (279 mg, 0.67 mmol, 19% yield) as yellow sandy crystal: m.p. 122.1°–122.7°C; ^{1}H NMR (DMSO-d_6) δ 1.19 (t, J = 7.0 Hz, 3H), 2.97 (s, 3H), 3.79 (s, 3H), 3.88 (q, J = 7.0 Hz, 2H), 5.43 (s, 1H), 5.46 (s, 1H), 6.95–7.00 (m, 4H), 7.26–7.30 (m, 4H), 7.41 (d, J = 8.6 Hz, 2H), 7.62 (d, J = 8.3 Hz, 1H); Anal. $C_{26}H_{24}N_2O_3$, calcd. C 75.71, H 5.86, N 6.79, found C 75.97, H 5.97, N 6.84.

1-(4-*n*-Butoxyphenyl)-1-[4-*N*-ethyl-*N*-(*N*-methylphthalimid-4-yl)-aminophenyl]ethene (3d)[25]

The same procedure described for the synthesis of **3a** was used except that 1-(4-bromophenyl)-1-(4-*n*-butoxyphenyl)ethene (**2d**, 1.0 g, 3.02 mmol), 4-(*N*-ethylamino)-*N*-methylphthalimide[26] (0.62 g, 3.02 mmol), $Pd(OAc)_2$ (34 mg, 0.15 mmol), tri-*tert*-butylphosphine (166 μL, 0.60 mmol), and NaO^tBu (0.35 g, 3.60 mmol) in anhydrous toluene (15 mL) were used to generate the crude product. The crude product was flash chromatographed on a silica gel (32–63 μm, 60 Å) column using 20% ethyl acetate in hexane as the eluent and the isolate obtained was recrystallized from a mixture of ethyl acetate and hexane to give the desired product **3d** (603 mg, 1.33 mmol, 44% yield) as yellow crystal: m.p. 120.7°–122.2°C; ^{1}H NMR (DMSO-d_6) δ 0.94 (t, J = 7.4 Hz, 3H), 1.19 (t, J = 7.1 Hz, 3H), 1.44 (m, 2H), 1.72 (m, 2H), 2.97 (s, 3H), 3.88 (q, J = 7.0 Hz, 2H), 3.99 (t, J = 6.4 Hz, 2H), 5.42 (s, 1H), 5.45 (s, 1H), 6.93–7.01 (m, 4H), 7.24–7.29 (m, 4H), 7.41 (d, J = 8.4 Hz, 2H), 7.62 (d, J = 8.3 Hz, 1H); Anal. $C_{29}H_{30}N_2O_3$, calcd. C 76.63, H 6.65, N 6.16, found C 76.67, H 6.74, N 6.05.

1-(4-*n*-Butylphenyl)-1-[4-*N*-ethyl-*N*-(*N*-methylphthalimid-4-yl)-aminophenyl]ethene (3e)[25]

The same procedure described for the synthesis of **3a** was used except that 1-(4-bromophenyl)-1-(4-*n*-butylphenyl)ethene (**2e**, 1.0 g), 4-(*N*-ethylamino)-*N*-methylphthalimide[26] (0.66 g, 3.2 mmol), $Pd(OAc)_2$ (37 mg, 0.16 mmol), tri-*tert*-butylphosphine (182 μL, 0.66 mmol), and NaO^tBu (0.38 g, 3.95 mmol) in anhydrous toluene (15 mL) were used to generate the crude product. The crude product was flash chromatographed on a silica gel (32–63 μm, 60 Å) column using methylene chloride as the eluent to give **3e** (522 mg) of yellow solids: m.p. 112.8°–114.0°C.

N-Ethyl-*N*-(*N*-methylphthalimid-4-yl)-{4-[1,5-bis(4-*N*,*N*-dimethylaminophenyl)-5-(4-*N*-ethyl-*N*-(*N*-methylphthalimid-4-yl)aminophenyl)-2,4-pentadienylidene]-2,5-cyclohexadien-1-ylidene}ammonium perchlorate (4a)

Upon stirring a mixture of **3a** (0.85 g, 2.00 mmol) and triethyl orthoformate (0.355 g, 2.40 mmol) in acetic anhydride (4 mL) under an argon atmosphere at room temperature, 70% perchloric acid (0.172 g, 1.20 mmol) was added dropwise. The blue solution was refluxed for 90 min and cooled to room temperature. The product **4a** was obtained from the resulting mixture by filtration and washed with tetrahydrofuran twice and air-dried to give a dark blue powder (0.84 g, 0.87 mmol, 87%

yield): m.p. 197.9°–200.3°C (decomposed); HRMS (FAB, M^+, $C_{55}H_{53}N_6O_4^+$) calcd. 861.4128, found 861.4163; Anal. $C_{55}H_{53}N_6O_8Cl \cdot 0.29\ HClO_4 \cdot 0.50\ H_2O$, calcd. C 66.10, H 5.48, N 8.41, Cl 4.57, found C 66.07, H 5.47, N 8.20, Cl 4.57.

N-Ethyl-*N*-(*N*-methylphthalimid-4-yl)-{4-[1,5-diphenyl-5-(4-*N*-ethyl-*N*-(*N*-methylphthalimid-4-yl)aminophenyl)-2,4-pentadienylidene]-2,5-cyclohexadien-1-ylidene}ammonium tetrafluoroborate (4b)

Upon stirring a light yellow solution of **3b** (0.54 g, 1.41 mmol) and triethyl orthoformate (283 μL, 1.72 mmol) in acetic anhydride (4 mL) at room temperature under an argon atmosphere, an ethereal solution of tetrafluoroboric acid (54 wt %, 99 μL, 0.705 mmol) was added with a micro syringe. The solution immediately changed its color to red followed by an intense blue color after being stirred for 30 min. The dark blue solution was heated at 90°C for 2 h and allowed to cool to room temperature. Diethyl ether was added to the solution and the precipitate was collected by filtration and air-dried to give **4b** as dark blue solids (0.35 g, 0.406 mmol, 57% yield): HRMS (FAB, M^+ $C_{51}H_{43}N_4O_4^+$) calcd. 775.3284, found 775.3333.

N-Ethyl-*N*-(*N*-methylphthalimid-4-yl)-{4-[1,5-bis(4-methoxyphenyl)-5-(4-*N*-ethyl-*N*-(*N*-methylphthalimid-4-yl)aminophenyl)-2,4-pentadienylidene]-2,5-cyclohexadien-1-ylidene}ammonium tetrafluoroborate (4c)

A solution of **3c** (150 mg, 0.364 mmol) in acetic anhydride (3 mL) was warmed in a water bath under an argon atmosphere until the solids had dissolved. Upon stirring and cooling the mixture to room temperature, triethyl orthoformate (90 μL, 0.55 mmol) was added followed by the addition of an ethereal solution of tetrafluoroboric acid (54 wt %, 30 μL, 0.22 mmol) through a micro syringe. The resulting dark red solution was heated at 80°C under an argon atmosphere for 30 min and allowed to cool to room temperature. Diethyl ether was added to the resulting dark blue mixture and the mixture was stood overnight to precipitate the crude product. The dark blue precipitate was collected by filtration and washed with diethyl ether. Recrystallization of the solids from methylene chloride and diethyl ether gave **4c** (126 mg) as black crystal: MS (FAB, M^+, $C_{53}H_{47}N_4O_6^+$) calcd. 836, found 836.

N-Ethyl-*N*-(*N*-methylphthalimid-4-yl)-{4-[1,5-bis(4-*n*-butoxyphenyl)-5-(4-*N*-ethyl-*N*-(*N*-methylphthalimid-4-yl)aminophenyl)-2,4-pentadienylidene]-2,5-cyclohexadien-1-ylidene}ammonium tetrafluoroborate (4d)

A solution of **3d** (139 mg, 0.306 mmol) in acetic anhydride (3 mL) was warmed in a water bath under an argon atmosphere until the solids had dissolved. Upon stirring and cooling the mixture to room temperature, triethyl orthoformate (75 μL, 0.46 mmol) was added followed by the addition of an ethereal solution of tetrafluoroboric acid (54 wt %, 25 μL, 0.184 mmol) through a micro syringe. The resulting dark red solution was heated at 80°C under an argon atmosphere for 30 min and allowed to cool to room temperature. Diethyl ether was added to the resulting dark blue mixture and the mixture was stood overnight to precipitate the crude product. The dark blue precipitate was collected by filtration and washed with diethyl ether. Recrystallization of the solids from methylene chloride and diethyl ether gave **4d** (129 mg) as black crystal: MS (FAB, M^+, $C_{59}H_{59}N_4O_6^+$) calcd. 919, found 919.

N-Ethyl-*N*-(*N*-methylphthalimid-4-yl)-{4-[1,5-bis(4-*n*-butylphenyl)-5-(4-*N*-ethyl-*N*-(*N*-methylphthalimid-4-yl)aminophenyl)-2,4-pentadienylidene]-2,5-cyclohexadien-1-ylidene}ammonium tetrafluoroborate (4e)

Upon stirring a solution of **3e** (80 mg, 0.18 mmol) and triethyl orthoformate (36.7 μL, 0.22 mmol) in acetic anhydride (1 mL) at room temperature under an argon atmosphere, an ethereal solution of tetrafluoroboric acid (54 wt %, 12.8 μL, 0.09 mmol) was added with a micro syringe. The stirring was continued for 30 min. The solution was then heated at 110°C for 1 h and allowed to cool to room temperature. Diethyl ether (100 mL) was added to the solution and the precipitate was collected by filtration and air-dried to give **4e** as dark blue solids (47 mg): MS (FAB, M^+ $C_{59}H_{59}N_4O_4^+$) calcd. 887, found 887.

N-Ethyl-*N*-(2,3-dihydro-1,4-phthalazinedion-6-yl)-{4-[1,5-bis(4-*N*,*N*-dimethylaminophenyl)-5-(4-*N*-ethyl-*N*-(2,3-dihydro-1,4-phthalazinedion-6-yl)aminophenyl)-2,4-pentadienylidene]-2,5-cyclohexadien-1-ylidene}ammonium perchlorate (5a)

A solution of **4a** (520 mg, 0.54 mmol) and potassium hydroxide (56 mg, 1.00 mmol) in

methanol (120 mL) was refluxed for 1 h and then cooled to room temperature. Hydrazine (700 μL, 22 mmol) was added and oxygen was removed from the mixture via freeze-pump-thaw cycle three times followed by reflux under argon for 2 h. Methanol was removed from the mixture by distillation and the yellow solids deposited were dried under vacuum. The solids were dissolved in a mixture of THF and water and the solution was adjusted to pH 2 with 70% $HClO_4$. The precipitate was collected by filtration, rinsed with water, and dried under vacuum to give **5a** (516 mg, 0.53 mmol, 98% yield) as a blue powder: HRMS (FAB, M^+, $C_{53}H_{51}N_8O_4^+$) calcd. 863.4033, found 863.3989.

N-Ethyl-*N*-(2,3-dihydro-1,4-phthalazinedion-6-yl)-{4-[1,5-diphenyl-5-(4-*N*-ethyl-*N*-(2,3-dihydro-1,4-phthalazinedion-6-yl)aminophenyl)-2,4-pentadienylidene]-2,5-cyclohexadien-1-ylidene}ammonium tetrafluoroborate (5b)

A solution of **4b** (250 mg, 0.29 mmol) and potassium hydroxide (50 mg, 0.89 mmol) in methanol (100 mL) was refluxed for 2 h and a gray precipitate was formed. The mixture was cooled to room temperature and hydrazine (370 μL, 11.6 mmol) was added. Oxygen was removed from the mixture via freeze-pump-thaw cycle three times and the mixture was refluxed under argon for 2 h. Methanol was removed from the mixture by distillation and the residue was dried under vacuum. The solids obtained were stirred in a dimethylformamide solution (5 mL) containing HBF_4 ethereal solution (54 wt %, 100 μL) and silica gel (3 g, 32–63 μm, 60 Å) for 15 min. The solvent was removed at 50°C under reduced pressure and the resulting silica gel was subjected to column chromatography using an eluent of methylene chloride/ethanol/acetic acid (90:10:1, v/v). The desired product **5b** was obtained as a brown powder: MS (FAB, M^+, $C_{49}H_{41}N_6O_4^+$) calcd. 777, found 777.

N-Ethyl-*N*-(2,3-dihydro-1,4-phthalazinedion-6-yl)-{4-[1,5-bis(4-methoxyphenyl)-5-(4-*N*-ethyl-*N*-(2,3-dihydro-1,4-phthalazinedion-6-yl)aminophenyl)-2,4-pentadienylidene]-2,5-cyclohexadien-1-ylidene}ammonium tetrafluoroborate (5c)

A solution of **4c** (120 mg, 0.128 mmol) and potassium hydroxide (15 mg, 0.26 mmol) in anhydrous ethanol (30 mL) was refluxed for 1 h and then cooled to room temperature. Hydrazine (200 μL, 6.4 mmol) was added and oxygen was removed from the mixture via freeze-pump-thaw cycle three times followed by reflux under argon for 2 h. Ethanol was removed from the mixture by distillation and the yellow solids deposited were dried under vacuum. The solids were dissolved in a mixture of THF and water and the solution was adjusted to pH 2 with tetrafluoroboric acid (54 wt % in diethyl ether). The precipitate was collected by filtration, rinsed with water, and dried under vacuum. The product was stirred with anhydrous THF (20 mL) for 5 h, filtered, and dried under vacuum to give **5c** (107 mg) as blue solids: MS (FAB, M^+, $C_{51}H_{45}N_6O_6^+$) calcd. 837, found 837.

N-Ethyl-*N*-(2,3-dihydro-1,4-phthalazinedion-6-yl)-{4-[1,5-bis(4-*n*-butoxyphenyl)-5-(4-*N*-ethyl-*N*-(2,3-dihydro-1,4-phthalazinedion-6-yl)aminophenyl)-2,4-pentadienylidene]-2,5-cyclohexadien-1-ylidene}ammonium tetrafluoroborate (5d)

The same procedure described for the synthesis of **5c** was used except for starting with 133 mg (0.130 mmol) of **4d** to generate 74 mg of **5d** as light blue solids: MS (FAB, M^+, $C_{57}H_{57}N_6O_6^+$) calcd. 921, found 921.

N-Ethyl-*N*-(2,3-dihydro-1,4-phthalazinedion-6-yl)-{4-[1,5-bis(4-*n*-butylphenyl)-5-(4-*N*-ethyl-*N*-(2,3-dihydro-1,4-phthalazinedion-6-yl)aminophenyl)-2,4-pentadienylidene]-2,5-cyclohexadien-1-ylidene}ammonium tetrafluoroborate (5e)

To a solution of **4e** (100 mg, 0.103 mmol) in 25 mL of anhydrous ethanol, hydrazine (131 μL, 4.1 mmol) was added and oxygen was removed from the mixture via freeze-pump-thaw cycle three times followed by reflux under argon atmosphere for 2 h. Solvent was removed from the mixture under reduced pressure and the solids deposited were redissolved in 30 mL of anhydrous THF. Upon stirring the solution at room temperature, an ethereal solution of tetrafluoroboric acid (54 wt %, 150 μL) was added and the stirring was continued at room temperature for 6 h. Diethyl ether (200 mL) was added to the solution and the precipitate was collected by filtration and air-dried to give **5e** as dark blue solids (70 mg): MS (FAB, M^+ $C_{57}H_{57}N_6O_4^+$) calcd. 889, found 889.

Preparation of Prodrug 6a

A mixture of 2′,3′-dideoxycytidine (19.2 mg, 0.091 mmol), sodium hydride (60% in mineral

oil, 3.7 mg, 0.093 mmol), and freshly distilled DMSO (4.5 mL) were stirred under argon at room temperature for 2 h. An aliquot (1.84 mL) of this resulting clear solution was added via a syringe to a solution containing 29.8 mg (0.0308 mmol) of **5a** and 0.35 mL of freshly distilled DMSO in an argon atmosphere. The resulting mixture was stirred under the same atmosphere at room temperature for 2 h yielding a dark green solution of **6a** (0.0141 M). This solution was used for the *in vitro* test against HIV without further purification.

Preparation of Prodrug 6b

The same procedure described for the preparation of **6a** was used to prepare a 2 mL of 0.0103 M DMSO solution of **6b**. This solution was used for the *in vitro* test against HIV without further purification.

Preparation of Prodrug 7a

A mixture of **5a** (20.9 mg, 0.0217 mmol) and phosphonoformic acid trisodium salt hexahydrate (6.6 mg, 0.022 mmol) was stirred at room temperature under argon overnight in 2.0 mL of freshly distilled DMSO to give a light yellow solution of **7a** (0.0109 M). This solution was used for the *in vitro* test against HIV without further purification.

Preparation of Prodrug 8a

A mixture of acycloguanosine (40.7 mg, 0.181 mmol) and sodium hydride (60% in mineral oil, 10.5 mg, 0.263 mmol) was stirred for 3 h at room temperature under argon in 4.5 mL of freshly distilled DMSO. An aliquot (1.126 mL) of this resulting clear solution was added via a syringe to a blue solution containing 49.1 mg (0.0510 mmol) of **5a** and 0.874 mL of freshly distilled DMSO in an argon atmosphere. The resulting mixture was stirred under the same atmosphere at room temperature for 2 h yielding a light yellow solution of **8a** (0.0255 M). This solution was used for the *in vitro* test against herpes simplex virus without further purification. (Report of results in progress.)

Evaluation of Release of Deferoxamine from Prodrug Des-MTLJ-1

Fifteen milligrams of 5-deferoxamine-1,5-di-(*N*-2-(4-aminobutyl)-*N*-ethylisoluminol)-*N*-ethylaminophenyl)-1,5-bis-(*p*-*N*,*N*-dimethylaniline)-1,3-pentadiene (Des-MTLJ-1) was prepared by reacting 5 mg of deferoxamine hydrochloride with 1 mg of $NaHCO_3$ and 10 mg of 1,5-di-(*p*-*N*-2-(*N*-(4-aminobutyl)-*N*-ethylisoluminol)-*N*-ethylaminophenyl)-1,5-bis-(*p*,*N*,*N*-dimethylaniline)-1,3-pentadiene in 86% ethanol/14% H_2O. The solution was made to a volume of 24 mL by the addition of 86% ethanol/14% H_2O and divided into 12 equal volume aliquots each of 2 mL. The aliquots were divided into six pairs. The first member of each pair was reacted with 0.05 mL of NaOH, 0.25 mL of 5 mM $FeCl_3$, and 0.05 mL of 3% H_2O_2. The absorbance at 425 nm was recorded at 60 s after the mixing of reagents and 86% ethanol/14% H_2O was used as the blank.

The control was a conjugate comprising deferoxamine bound to the corresponding photochromic moiety, 5-deferoxamine-1,5-di-(*p*-*N*-2-chloroethylaminophenyl)-1,5-bis-(*p*-*N*,*N*-dimethylaniline)-1,3-pentadiene (Des-dye). Thirty-four milligrams of the conjugate was prepared by reacting 14 mg of deferoxamine hydrochloride with 2 mg of $NaHCO_3$ and 20 mg of 1,5-di-(*p*-*N*-2-chloroethylaminophenyl)-1,5-bis-(*p*-*N*,*N*-dimethylaniline)-1,3-pentadiene in 86% ethanol/14% H_2O. The solution was made to a volume of 24 mL by addition of 86% ethanol/14% H_2O and divided into 12 equal volume aliquots each of 2 mL. The aliquots were divided into six pairs. The first member of each pair was reacted with 0.05 mL of NaOH, 0.2 mL of 10 mM $FeCl_3$. The second member of each pair was reacted with 0.05 mL of NaOH, 0.25 mL of 5 mM $FeCl_3$, and 0.05 mL of 3% H_2O_2. The absorbance at 425 nm was recorded at 60 s after the mixing of reagents and 86% ethanol/14% H_2O was used as the blank.

Evaluation of Prodrug Anti-HIV-1 Potency in Macrophage Assay

The anti-HIV-1 evaluation of the carriers and their conjugates was performed in 6-day-old monocyte/macrophages at Southern Research Institute. Briefly, peripheral blood monocytes were isolated from normal HIV-1–negative donors by plastic adherence following Ficoll Hypaque purification of the buffy coats. The monocytes were then cultured for 6 days to a macrophage-like phenotype. The test compounds were serially diluted and added to the cultures followed by the addition of a pretitered amount of the Ba-L strain of HIV-1 obtained from the National Institute of Allergy and Infectious

Diseases AIDS Research and Reference Reagent Repository. Cultures were washed by media removal 24 h postinfection, fresh compound was added, and the cultures were continued for an additional 6 days. HIV p24 antigen content to assess virus replication was measured by p24 enzyme-linked immunosorbent assay. AZT and ddc were used as positive control compounds and run in parallel with each determination. Toxicity of the test materials was measured on replicate plates that did not receive virus, but were treated and set up identically to those receiving virus. At assay termination, the assay plates were stained with the tetrazolium-based dye MTS to determine cell viability and quantify compound toxicity. Using a computer program at Southern Research Institute, IC_{50} (50% inhibition of virus replication), TC_{50} (50% cytotoxicity), and a therapeutic index (TI, TC_{50}/IC_{50}) were obtained.

Evaluation of Foscarnet Prodrug Antiretroviral Potency in a Murine Model

The effect of prodrug MTLJ-1-foscarnet on 4-week-old Swiss mice infected with FLV was tested by the LPC. The mice were infected with FLV by an IP injection with 0.5 mL viral solution prepared by resuspending F4-6 cells (1×10^6 cells/mL) in fresh media and harvesting that media 24 h later and filtering through a 0.22-mm filter. The foscarnet group received 300 nM foscarnet on days 5–9. The carrier group received 300 nM carrier on days 5–9. The MTLJ-1-foscarnet group received 300 nM MTLJ-1-foscarnet on days 5–9. All mice were sacrificed on day 12. The spleens were removed and weighed. The toxicity of the carrier MTLJ-1 was also evaluated by the determination of its LD_{50}.

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