Anti-AIDS Agents. 52.† Synthesis and Anti-HIV Activity of Hydroxymethyl (3′*R*,4′*R*)-3′,4′-Di-*O*-(*S*)-camphanoyl-(+)-*cis*-khellactone Derivatives

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Received August 28, 2003

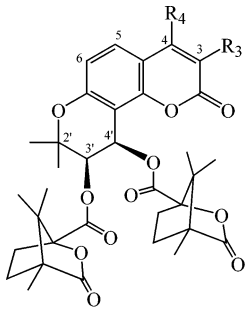
To enhance the water solubility and oral bioavailability of DCK analogues, 12 new mono- and disubstituted (3′*R*,4′*R*)-3′,4′-di-*O*-(*S*)-camphanoyl-(+)-*cis*-khellactone (DCK) analogues were synthesized and evaluated for inhibition of HIV-1 replication in H9 lymphocytes. 3-Hydroxymethyl-4-methyl-DCK (**4c**) exhibited significant anti-HIV activity in H9 lymphocytes and primary peripheral blood mononuclear cells with EC_{50} values of 0.004 and 0.024 μM, respectively. Although this compound was not as potent as 4-methyl-DCK (**2**) and 3-bromomethyl-4-methyl-DCK (**4a**), it provides increased water solubility and possible linkage to other moieties. Of particular note, **4c** exhibits moderate oral bioavailability (15%) when administered as a carboxymethylcellulose suspension to rats, whereas **2** is not orally bioavailable in the same formulation. Further studies on mechanism of action suggest that **4c** inhibits the production of double-stranded viral DNA from the single-stranded DNA intermediate. In addition, **4a** is the most potent compound in this series of new analogues, with EC_{50} and TI values of 0.00011 μM and 189,600, respectively. Thus, further modification at the 3-position of the coumarin ring can improve the potency of new DCK analogues.

Introduction

Since its first report in the 1980s, acquired immunodeficiency syndrome (AIDS), which is caused by the human immunodeficiency virus (HIV) and results in life-threatening opportunistic infections and malignancies, has rapidly spread through the human population and become a major worldwide pandemic.[1,2] Millions of people have died from AIDS and 42 million people were living with HIV or AIDS at the end of 2002.[3]

In our previous research, 3′,4′-di-*O*-(*S*)-camphanoyl-(+)-*cis*-khellactone (DCK, **1**) was identified as a potent anti-HIV agent with a remarkable EC_{50} value of 2.56 $\times$ 10^{-4} μM and a therapeutic index (TI) of 1.37 $\times$ 10^5 against HIV-1_{IIIB} replication in H9 lymphocytes.[4,5] It was more potent than AZT when tested in parallel in the same assay.

Subsequently, DCK was modified to determine the pharmacophore(s) responsible for anti-HIV activity. Several series of (+)-*cis*-khellactone derivatives, including mono- and disubstituted DCK analogues, were synthesized and evaluated for anti-HIV activity.[6,7] The SAR study results indicated that 3′*R*,4′*R* configurations and planarity of the coumarin ring system are essential structural features for anti-HIV activity; alkyl/*O*-alkyl substituents at the 3-, 4-, and 5-positions on the coumarin are favorable for enhanced anti-HIV activity and decreased toxicity of DCK;[8,9] and a methyl group on the coumarin and two bulky (*S*)-camphanoyl groups at the 3′- and 4′-positions are preferred to other substituents. Among more than 100 synthesized khellactone derivatives,[10] the most promising compound was **2**. It was significantly more potent than DCK and AZT with an EC_{50} value of 1.83 $\times$ 10^{-6} μM and a TI of >6.89 $\times$ 10^7. In addition, more than 20 DCK analogues exhibited more potent anti-HIV activity than AZT, even though some of them were less active than DCK. Based on these results, we conclude that the (+)-*cis*-khellactone derivatives are a family of potent anti-HIV agents. Preliminary mechanism of action studies showed that DCK and its active analogues block viral replication after viral entry but prior to integration of proviral DNA; therefore, they have a novel mechanism of action compared to current anti-HIV/AIDS drugs. Because compound **2** exhibits high potency and is readily synthesized,[8] it was chosen as a drug candidate for preclinical studies.



DCK (**1**) $R_3 = R_4 = H$
4-Methyl DCK (**2**) $R_3 = H$, $R_4 = CH_3$

† Anti-AIDS Agents 52. For part 51, see: Yu, D.; Suzuki, M.; Xie, L.; Morris-Natschke, S. L.; Lee, K. H. *Med. Res. Rev.* **2003**, *23*, 322−345.

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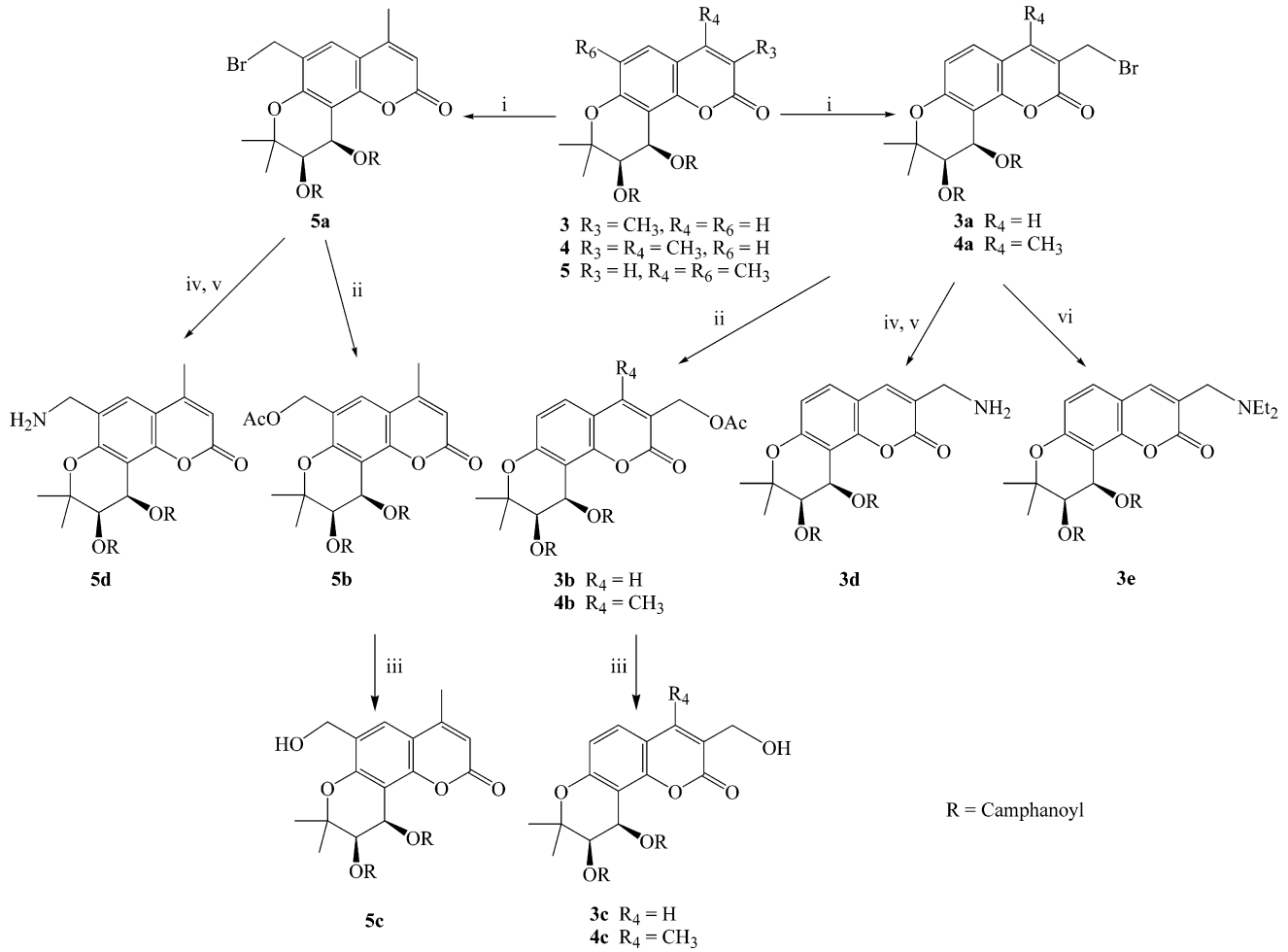
10.1021/jm030416y CCC: $27.50
Published on Web 12/23/2003

Scheme 1



Reagents and conditions: (i) *N*-bromosuccinimide in benzene, reflux; (ii) acetic anhydride, NaOAc, reflux; (iii) HCl (2 N) in EtOH, reflux; (iv) diethylamine in toluene, reflux; (v) hexanemethylenetetramine in $CHCl_3$, reflux; (vi) HCl (2 N), EtOH, 100 °C.

However, the low solubility and poor bioavailability of **2** has limited its further development.

In the current study, we synthesized 12 new DCK analogues in order to identify new drug candidates with potent anti-HIV activity and acceptable pharmaceutical properties. This paper reports their synthesis and bioassay data. The results of additional mechanism of action studies are also summarized.

Design

To improve solubility and bioavailability, we focused on introducing polar functional groups, such as hydroxyl or amine, into our active DCK analogues. Both hydroxyl and amine moieties can serve as hydrogen-bond donors and acceptors, resulting in enhanced aqueous solubility and potentially improving bioavailability. The introduction of a polar group also can serve as a linker with other polar moieties to form prodrugs that might improve the pharmaceutical properties of DCK analogues. Accordingly, we targeted DCK analogues with a hydroxymethyl or aminomethyl introduced at the 3- or 6-position. The log *P* values for a subset of these compounds were calculated by using ACD log *P* software. The calculated log *P* values of **3c**, **4c**, and **5c** are 3.04, 3.65, and 3.06, respectively, while our lead compound **2** has a calculated log *P* value of 4.82. Accordingly, compounds **3c**, **4c**, and **5c** and related compounds were synthesized and evaluated in vitro against HIV-1 replication in H9 lymphocytes.

Chemistry

The syntheses of **3a**−**3e** were previously described without full experimental details[11] and, thus, are presented herein. According to Scheme 1, our syntheses began with methylated DCKs (**3**, **4**, and **5**), which were synthesized from corresponding methylated 7-hydroxycoumarins.[6,7] Compounds **3**, **4**, and **5** were treated with *N*-bromosuccinimide to produce 3-bromomethyl (**3a** and **4a**) and 6-bromomethyl (**5a**) substituted DCKs with yields of 67−77%. Minor amounts of 3- or 6-dibromomethyl compounds were formed, depending on the amount of bromosuccinimide. The 4-methyl group was unlikely to be brominated. The bromomethyl-DCK derivatives (**3a**, **4a**, and **5a**) were reacted with acetic anhydride in the presence of sodium acetate to provide the corresponding acetoxymethyl-DCK derivatives (**3b**, **4b**, and **5b**) with yields of 79−84%. Acidic hydrolysis produced the corresponding hydroxymethyl-DCKs (**3c**, **4c**, and **5c**) in greater than 85% yields. Compounds **3a** and **5a** were treated with hexanemethylenetetramine, followed by hydrolysis, to yield the corresponding aminomethyl-DCKs (**3d** and **5d**). In addition, **3a** was reacted with

Table 1. Anti-HIV Activities of DCK Analogs in Acutely Infected H9 Lymphocytes[a]

compd	IC_{50} (μM)[b]	EC_{50} (μM)[c]	TI[d]
3a	>11.1	0.059	>186
3b	11.6	0.017	676
3c	23.0	0.029	806
3d	>15.4	0.677	>23
3e	>14.1	3.67	>4
4a	>20.9	0.00011	>189600
4b	14.8	0.026	567
4c	24.9	0.004	5,800
5a	>13.7	0.156	>88
5b	>14.1	0.544	>26
5c	>15.0	0.111	>135
5d	>15.0	0.148	>102
DCK (**1**)[e]	>16.1	0.049	>328
4-methyl-DCK (**2**)[f]	23.6	0.0015	16000
AZT	1872	0.187	10000

[a] All data presented are averages of at least two separate experiments performed by Panacos Pharmaceutical Inc. [b] Concentration that inhibits uninfected H9 cell growth by 50%. [c] Concentration that inhibits viral replication by 50%. [d] Therapeutic index (TI) = IC_{50}/EC_{50}. [e] $EC_{50} = 2.56 \times 10^{-4}$ and TI = 1.37×10^5 in previous screenings and publications.[4,5] [f] $EC_{50} = 1.83 \times 10^{-6}$ and TI = 6.89×10^7 in previous screenings and publication.[7]

diethylamine to afford 3-diethylaminomethyl DCK (**3e**) in 71% yield.[12]

Results and Discussion

All newly synthesized DCK analogues (**3a**−**3e**, **4a**−**4c**, and **5a**−**5d**) were tested in parallel with DCK (**1**), 4-methyl-DCK (**2**), and AZT against HIV-1 replication in acutely infected H9 lymphocytes, and the data are listed in Table 1.

With EC_{50} values of 0.004 and 0.029 μM, compounds **4c** and **3c** were more potent than **1** and AZT, but somewhat less active than **2**. The 3-acetoxymethyl-DCKs (**3b** and **4b**) and 3-bromomethyl-DCK (**3a**) also showed EC_{50} values similar to that of **1** and better than that of AZT. Interestingly, **4a** exhibited extremely potent anti-HIV activity with an EC_{50} value of 0.00011 μM and a TI value of 189,600 and, thus, was significantly more potent than **2**. All analogues incorporating bromomethyl, hydroxymethyl, or acetoxymethyl groups at the 3-position retained activity similar to or better than **1** and AZT. Space-filling models would also predict that position-3 could accommodate bulky substituents, as seen with **4a**. On the other hand, both **3d** and **3e**, which contain amino moieties, were less potent than AZT, thus suggesting that an aminomethyl or diethylaminomethyl group in the 3-position is not favored for anti-HIV activity.

In contrast with the 3-substituted compounds, the 6-bromomethyl and 6-hydroxymethyl as well as 6-aminomethyl analogues (**5a**, **5c**, and **5d**) showed only moderate antiviral activity. They were more potent than AZT, but much less potent than **1** and **2** in the HIV_{IIIB}/H9 assay. Unlike the 3-acetoxymethyl DCKs (**3b** and **4b**), 6-acetoxymethyl DCK (**5b**) was less active than AZT. These results are consistent with our previous studies that the 3-position of DCK is favorable for modification, but the 6-position is not.

While compounds **2** and **4c** have similar potencies, **4c** should be more water-soluble and could be easily converted to a prodrug. Thus, **4c** was chosen as a candidate for preclinical studies, even though it is less active than **4a**. More studies were performed to examine potency, oral bioavailability, and mechanism of action of **4c** with comparative pharmacokinetics in rats evaluated for **2** and **5c**. Additional in vitro studies demonstrated that **4c** exhibits significant activity against a panel of primary HIV-1 isolates propagated in primary peripheral blood mononuclear cells. The median EC_{50} against these viruses was 0.024 μM, similar to those of the approved drugs AZT, nevirapine, and indinavir, which were tested in parallel (data not shown).

Table 2. Pharmacokinetic Parameters for DCK Analogs in Male Sprague−Dawley Rats following an Intravenous Bolus Dose at 10−20 mg/kg

compd	CL (L/min/kg)	Vd (L/kg)	Vss (L/kg)	$t_{1/2}$ (min)	F (%)
2	3.51 ± 1.08[a]	12.0 ± 1.32	10.1 ± 0.74	145 ± 47	ND[b]
4c	4.74 ± 2.40	22.9 ± 17.3	18.5 ± 17.3	187 ± 126	15
5c	4.74 ± 0.18	5.90 ± 0.97	3.89 ± 0.32	52 ± 8	ND

[a] $N = 3$ rats for compounds **2** and **5c**, and $N = 4$ for compound **4c**. [b] ND = no bioavailability detected within the assay detection limits employed.

Compound **4c** exhibited moderate oral bioavailability (F = 15%) when administered orally to rats as a suspension in carboxymethylcellulose. In contrast, compounds **2** and **5c** had undetectable oral bioavailability under the same conditions (Table 2). These results suggest that the addition of hydroxy moieties provides additional solubility, subsequently enhancing oral bioavailability. However, solubility alone cannot explain the differences in bioavailability observed for the three compounds studied. All three compounds had high systemic clearances, suggesting that hepatic first-pass metabolism is likely a major factor in limiting oral bioavailability of this series. Preliminary studies (data not shown) have indicated that all of the compounds are subject to rapid oxidative metabolism in human and rat liver microsomes and that P450 3A4 is the primary isoform that metabolizes **4c**. All three compounds had relatively large volumes of distribution, reflecting their fairly high lipophilicity.

To further elucidate the mechanism of action of this compound, a time-of-addition study was performed, where the point in viral replication inhibited by **4c** was compared to the inhibitors acting at attachment (Chicago Sky Blue) or reverse transcription (AZT and nevirapine). This study indicated that **4c** acts at a point in the virus life cycle immediately following the target for AZT and nevirapine, which are reverse transcriptase (RT) inhibitors. Additional mechanism of action studies are ongoing and preliminary data suggest that **4c** inhibits the production of double-stranded viral DNA from the single-stranded DNA intermediate, in contrast to traditional RT inhibitors that block the generation of single stranded DNA from the RNA template.

In conclusion, the 3-position of the DCK series can tolerate hydrophobic groups, such bromo, alkyl, and acyl. A polar hydroxyl group can also be tolerated, but a charged amino group is not favored at this position. The overall activities of 6-substituted analogues are lower than those of the 3-substituted DCKs. The 3-hydroxymethyl-DCK analogue retains anti-HIV activity, has increased water solubility, is orally bioavailable, and

could potentially be linked with other functional moieties to form prodrugs.

Experimental Section

Chemistry. Melting points were measured with a Fisher Johns melting apparatus without correction. ^{1}H NMR spectra were measured on a 300-MHz Varian Gemini 2000 spectrometer using TMS as internal standard. The solvent used was $CDCl_3$. Mass spectra were measured on a PE-SCIEX API-3000 with turbo ion spray source. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. All target compounds were analyzed for C and H and gave values within ±0.4% of the theoretical values. Optical rotations were measured with a Jasco Dip-1000 digital polarimeter at 25 °C at the sodium D line. The diastereoisomeric excess percentages were determined from the intensity of protons at the 3′-position in the ^{1}H NMR spectra. TLC and PTLC were performed on precoated silica gel GF plates purchased from Analtech, Inc. The Flash+ system was used for medium-pressure column chromatography. Silica gel (200−400 mesh) from Aldrich, Inc., was used for column chromatography. All other chemicals were obtained from Aldrich, Inc.

Synthesis of Bromomethyl-3′,4′-Di-*O*-(*S*)-camphanoyl-(+)-*cis*-khellactones A mixture of methylated DCK (0.26 mmol) and *N*-bromosuccinimide (NBS, 46 mg, 0.26 mmol) in 5−10 mL of anhydrous benzene was heated under reflux for 2−6 h until the reaction was completed as monitored by TLC. The mixture was filtered and solvent was removed in vacuo. The residue was separated by PTLC (hexanes−EtOAc 6:4) to give pure bromomethyl-DCKs.

(3′*R*,4′*R*)-3′,4′-Di-*O*-(*S*)-camphanoyl-3-bromomethyl-(+)-*cis*-khellactone (3a): 77% yield (starting with 380 mg of **3**); white solid; mp 153−155 °C; MS (ESI+) *m*/*z* (%) 736 (M^+ + Na − 1, 90), 737 (M^+ + Na, 45), 738 (M^+ + 2 + Na − 1, 100), 739 (M^+ + 2 + Na, 30); ^{1}H NMR δ 0.98−1.11 (each 3H, s, 6×CH_3 in camphanoyl group), 1.45 and 1.49 (each 3H, s, 2×CH_3-2′), 1.68, 1.91, 2.24, and 2.51 (each 2H, m, CH_2 in camphanoyl group), 4.38 (2H, s, CH_2-3), 5.39 (1H, d, *J* = 4.8 Hz, H-3′), 6.64 (1H, d, *J* = 4.8 Hz, H-4′), 6.84 (1H, d, *J* = 8.7 Hz, H-6), 7.42 (1H, d, *J* = 8.7 Hz, H-5), 7.76 (1H, s, H-4); 85% de; $[\alpha]_D$ +98.79° (*c* 0.33, $CHCl_3$). Anal. ($C_{35}H_{39}BrO_{11}$) C, H.

(3′*R*,4′*R*)-3′,4′-Di-*O*-(*S*)-camphanoyl-3-bromomethyl-4-methyl-(+)-*cis*-khellactone (4a): yield 75% (starting with 170 mg of **4**); white solid; mp 175−177 °C; MS (ESI+) *m*/*z* (%) 751 (M^+ + Na, 98) 753 (M^+ + 2 + Na, 100); ^{1}H NMR δ 0.94−1.11 (each 3H, s, 6×CH_3 in camphanoyl group), 1.45 and 1.49 (each 3H, s, 2×CH_3-2′), 1.68, 1.92, 2.20, and 2.52 (each 2H, m, CH_2 in camphanoyl group), 2.41 (3H, s, CH_3-4), 5.39 (1H, d, *J* = 4.8 Hz, H-3′), 6.65 (1H, d, *J* = 4.8 Hz, H-4′), 6.80 (1H, d, *J* = 8.8 Hz, H-6), 7.61 (1H, d, *J* = 8.8 Hz, H-5); 83% de; $[\alpha]_D$ +14.6° (*c* 0.73, $CHCl_3$). Anal. ($C_{36}H_{41}BrO_{11}$·2.5H_2O) C, H.

(3′*R*,4′*R*)-3′,4′-Di-*O*-(*S*)-camphanoyl-4-methyl-6-bromomethyl-(+)-*cis*-khellactone (5a): yield 67% (starting with 200 mg of **5**); white solid, mp 252−254 °C; MS (ESI+) *m*/*z* (%) 751 (M^+ + Na, 70), 753 (M^+ + 2 + Na, 80); ^{1}H NMR δ 0.99−1.12 (each 3H, s, 6·CH_3 in camphanoyl group), 1.52 and 1.53 (each 3H, s, 2×CH_3-2′), 1.65, 1.95, 2.20, and 2.50 (each 2H, m, CH_2 in camphanoyl group), 2.40 (3H, s, CH_3-4), 4.53, 4.56 (each 1H, t, *J* = 9.9 Hz, CH_2-6), 5.43 (1H, d, *J* = 4.8 Hz, H-3′), 6.13 (1H, s, H-3), 6.65 (1H, d, *J* = 4.8 Hz, H-4′), 7.59 (1H, s, H-5); 82% de; $[\alpha]_D$ −2.8° (*c* 0.71, $CHCl_3$). Anal. ($C_{36}H_{41}BrO_{11}$) C, H.

Synthesis of Acetoxymethyl-3′,4′-di-*O*-(*S*)-camphanoyl-(+)-*cis*-khellactones. A mixture of bromomethyl-DCK (0.07 mmol) and excess NaOAc in Ac_2O (3 mL) was heated to reflux for 3−4 h. The reaction mixture was poured into ice water and let stand overnight. The precipitated solid was filtered, washed with water, and dried to obtain the target products.

(3′*R*,4′*R*)-3′,4′-Di-*O*-(*S*)-camphanoyl-3-acetoxymethyl-(+)-*cis*-khellactone (3b): yield 79% (starting with 115 mg of **3a**), white solid; mp 138−140 °C; MS (ESI+) *m*/*z* (%) 717 (M^+ + Na, 100); ^{1}H NMR δ 0.95−1.12 (each 3H, s, 6×CH_3 in camphanoyl group), 1.45 and 1.49 (each 3H, s, 2·CH_3-2′), 1.62, 1.90, 2.24, and 2.49 (each 2H, m, CH_2 in camphanoyl group), 2.19 (3H, s, CH_3CO-3), 5.00 (2H, m, CH_2-3), 5.40 (1H, d, *J* = 4.8 Hz, H-3′), 6.64 (1H, d, *J* = 4.8 Hz, H-4′), 6.83 (1H, d, *J* = 8.7 Hz, H-6), 7.42 (1H, d, *J* = 8.7 Hz, H-5), 7.69 (1H, s, H-4); 76% de; $[\alpha]_D$ +11.0° (*c* 0.21, $CHCl_3$). Anal. ($C_{37}H_{42}O_{13}$) C, H.

(3′*R*,4′*R*)-3′,4′-Di-*O*-(*S*)-camphanoyl-3-acetoxymethyl-4-methyl-(+)-*cis*-khellactone (4b): yield 86% (starting with 500 mg of **4a**), brownish solid; mp 148−149 °C; MS (ESI+) *m*/*z* (%) 731 (M^+ + Na, 100); ^{1}H NMR δ 0.95−1.11 (each 3H, s, 6×CH_3, in camphanoyl group), 1.45 and 1.49 (each 3H, s, 2×CH_3-2′), 1.60, 1.90, 2.21, and 2.52 (each 2H, m, CH_2 in camphanoyl group), 2.06 (3H, s, CH_3CO-3), 2.48 (3H, s, CH_3-4), 5.14 (2H, s, OCH_2-3), 5.40 (1H, d, *J* = 4.5 Hz, H-3′), 6.64 (1H, d, *J* = 4.5 Hz, H-4′), 6.82 (1H, d, *J* = 8.8 Hz, H-6), 7.61 (1H, d, *J* = 8.8 Hz, H-5); 79% de; $[\alpha]_D$ +10.6° (*c* 0.155, $CHCl_3$). Anal. ($C_{38}H_{44}O_{13}$·0.75H_2O) C, H.

(3′*R*,4′*R*)-3′,4′-Di-*O*-(*S*)-camphanoyl-4-methyl-6-acetoxymethyl-(+)-*cis*-khellactone (5b): yield 84% (starting with 50 mg of **5a**), white solid, mp 138−140 °C; MS (ESI-) *m*/*z* (%) 707 (100, M^+ − 1); ^{1}H NMR δ 0.92−1.12 (each 3H, s, 6×CH_3 in camphanoyl group), 1.48 and 1.51 (each 3H, s, 2×CH_3-2′), 1.70, 1.90, 2.20, and 2.48 (each 2H, m, CH_2 in camphanoyl group), 2.12 (3H, s, CH_3CO-6), 2.41 (3H, s, CH_3-4), 5.16 (2H, s, OCH_2-6), 5.41 (1H, d, *J* = 4.8 Hz, H-3′), 6.13 (1H, s, H-3), 6.65 (1H, d, *J* = 4.8 Hz, H-4′), 7.60 (1H, s, H-5); 96% de; $[\alpha]_D$ −10.0° (*c* 0.42, $CHCl_3$). Anal. ($C_{38}H_{44}O_{13}$·1.5H_2O) C, H.

Synthesis of Hydroxymethyl-3′,4′-di-*O*-(*S*)-camphanoyl-(+)-*cis*-khellactones. A mixture of acetoxymethyl-DCK (0.04 mmol) and aqueous HCl (2 N, 0.5 mL) was heated to reflux in 3 mL of EtOH for 1 h. The mixture was poured into ice water and let stand overnight. The white solid was filtered and washed with water three times.

(3′*R*,4′*R*)-3′,4′-Di-*O*-(*S*)-camphanoyl-3-hydroxymethyl-(+)-*cis*-khellactone (3c): yield 88% (starting with 30 mg of **3b**), white solid; mp 155−156 °C; MS (ESI+) *m*/*z* (%) 675 (M^+ + Na, 55), 674 (M^+ + Na − 1, 100); ^{1}H NMR δ 0.98−1.11 (each 3H, s, 6×CH_3 in camphanoyl group), 1.45 and 1.49 (each 3H, s, 2×CH_3-2′), 1.66, 1.92, 2.22, and 2.49 (each 2H, m, CH_2 in camphanoyl group), 4.56 (2H, m, CH_2-3), 5.39 (1H, d, *J* = 4.8 Hz, H-3′), 6.65 (1H, d, *J* = 4.8 Hz, H-4′), 6.83 (1H, d, *J* = 8.7 Hz, H-6), 7.43 (1H, d, *J* = 8.7 Hz, H-5), 7.67 (1H, s, H-4); 77% de; $[\alpha]_D$ +36.1° (*c* 0.89, $CHCl_3$). Anal. ($C_{35}H_{40}O_{12}$·0.5H_2O) C, H.

(3′*R*,4′*R*)-3′,4′-Di-*O*-(S)-camphanoyl-3-hydroxymethyl-4-methyl-(+)-*cis*-khellactone (4c): yield 85% (starting with 150 mg of **4b**), yellowish solid, mp 164−165 °C; MS (ESI+) *m*/*z* (%) 689 (M^+ + Na, 100); ^{1}H NMR δ 0.96−1.10 (each 3H, s, 6×CH_3), 1.43 and 1.47 (each 3H, s, 2×CH_3-2′), 1.66, 1.90, 2.20, and 2.47 (each 2H, m, CH_2 in camphanoyl group), 2.46 (3H, s, CH_3-4), 4.63 (2H, s, CH_2-3), 5.37 (1H, d, *J* = 4.5 Hz, H-3′), 6.85 (1H, d, *J* = 4.5 Hz, H-4′), 6.10 (1H, d, *J* = 8.8 Hz, H-6), 6.85 (1H, d, *J* = 8.8 Hz, H-5); 90% de; $[\alpha]_D$ +18.5° (*c* 1.20, $CHCl_3$). Anal. ($C_{36}H_{42}O_{12}$·0.5H_2O) C, H.

(3′*R*,4′*R*)-3′,4′-Di-*O*-(*S*)-camphanoyl-4-methyl-6-hydroxymethyl-(+)-*cis*-khellactone (5c): yield 87% (starting with 168 mg of **5b**), white solid, mp 272−274 °C; MS (ESI-) *m*/*z* (%) 665 (M^+ − 1, 100); ^{1}H NMR δ 0.92−1.11 (each 3H, s, 6×CH_3), 1.48 and 1.51 (each 3H, s, 2×CH_3-2′), 1.60, 1.92, 2.20, and 2.48 (each 2H, m, CH_2 in camphanoyl group), 2.48 (3H, s, CH_3-4), 4.70 (2H, m, CH_2-6), 5.41 (1H, d, *J* = 4.8 Hz, H-3′), 6.13 (1H, s, H-3), 6.66 (1H, d, *J* = 4.8 Hz, H-4′), 7.63 (1H, s, H-5); 89% de; $[\alpha]_D$ −5.9° (*c* 0.51, $CHCl_3$). Anal. ($C_{36}H_{42}O_{12}$) C, H.

Synthesis of Aminomethyl-3′,4′-di-*O*-(*S*)-camphanoyl-(+)-*cis*-khellactones. A mixture of corresponding bromomethyl-DCK (0.1 mmol) and hexanemethylenetetramine (34 mg, 0.2 mmol) was refluxed in $CHCl_3$ for 3−6 h. The solvent was removed to obtain a yellow solid. The residue was refluxed in 3 mL of EtOH and 1.0 mL of concentrated HCl for 15 min. $CHCl_3$ was added after the mixture had cooled. The crude product was filtered and then recrystallized in a mixed solvent of MeOH, dichloromethane, and hexane to produce yellow crystals of pure target compound.

(3′*R*,4′*R*)-3′,4′-Di-*O*-(*S*)-camphanoyl-3-aminomethyl-(+)-*cis*-khellactone (3d): yield 76% (starting with 73 mg of **3a**),

brown solid; mp 138−140 °C; MS (ESI-) *m/z* (%): 652 (M^+ + 1, 55), 651 (M^+, 100); ^{1}H NMR δ 0.98−1.11 (each 3H, s, 6×CH_3), 1.45 and 1.49 (each 3H, s, 2×CH_3-2′), 1.69, 1.90, 2.23, and 2.50 (each 2H, m, CH_2 in camphanoyl group), 2.60 (2H, br s, NH_2), 3.79 (2H, s, CH_2-3), 5.39 (1H, d, J = 4.8 Hz, H-3′), 6.65 (1H, d, J = 4.8 Hz, H-4′), 6.82 (1H, d, J = 8.7 Hz, H-6), 7.42 (1H, d, J = 8.7 Hz, H-5), 7.67 (1H, s, H-4); 85% de; $[\alpha]_D$ −80.0° (c 0.08, $CHCl_3$). Anal. ($C_{35}H_{41}NO_{11}$·1.25H_2O) C, H, N.

(3′*R*,4′*R*)-3′,4′-Di-*O*-(*S*)-camphanoyl-4-methyl-6-aminomethyl-(+)-*cis*-khellactone (5d): yield 57% (starting with 73 mg of **5a**), yellow solid; mp 140−142 °C; MS (ESI+) *m/z* (%) 666 (100, M^+ + 1): ^{1}H NMR δ 0.97−1.12 (each 3H, s, 6×CH_3), 1.48 and 1.51 (each 3H, s, 2×CH_3-2′), 1.65, 1.95, 2.20, and 2.50 (each 2H, m, CH_2 in camphanoyl group), 2.40 (3H, s, CH_3-4), 3.88 (2H, s, CH_2-6), 5.41 (1H, d, J = 4.8 Hz, H-3′), 6.11 (1H, s, H-3), 6.66 (1H, d, J = 4.8 Hz, H-4′), 7.55 (1H, s, H-5); 86% de; $[\alpha]_D$ −10.2° (*c* 0.69, $CHCl_3$). Anal. ($C_{36}H_{43}NO_{11}$·H_2O) C, H, N.

(3′*R*,4′*R*)-3′,4′-Di-*O*-(*S*)-camphanoyl-3-diethylaminomethyl-(+)-*cis*-khellactone (3e). A mixture of 3-bromomethyl-DCK (**3a**, 40 mg, 0.056 mmol) and diethylamine (0.06 mL, 0.6 mmol) in 6 mL of anhydrous toluene was heated under reflux for 10 h to afford 3-diethylaminomethyl-DCK (**3e**): yield 71%, yellow solid; mp 123−125 °C; MS (ESI+) *m/z* (%) 708 (M^+ + 1, 50), 707 (M^+, 100); ^{1}H NMR δ 0.98−1.11 (each 3H, s, 6×CH_3), 1.45 and 1.49 (each 3H, s, 2×CH_3-2′), 1.07 (6H, t, NCH_2CH_3-3), 1.69, 1.90, 2.22, and 2.50 (each 2H, m, CH_2 in camphanoyl group), 2.60 (4H, q, NCH_2CH_3-3), 3.47 (2H, s, CH_2-3), 5.39 (1H, d, J = 4.8 Hz, H-3′), 6.65 (1H, d, J = 4.8 Hz, H-4′), 6.81 (1H, d, J = 8.7 Hz, H-6), 7.43 (1H, d, J = 8.7 Hz, H-5), 7.81 (1H, s, H-4); 90% de; $[\alpha]_D$ +23.8 (*c* 0.21, $CHCl_3$). Anal. ($C_{39}H_{49}NO_{11}$) C, H, N.

HIV Growth Inhibition Assay in H9 Lymphocytes. The evaluation of HIV-1 inhibition was carried out as follows using H9 lymphocytes. The human T-cell line, H9, was maintained in continuous culture with complete medium (RPMI 1640 with 10% fetal calf serum supplemented with L-glutamine at 5% CO_2 and 37 °C. Test samples were prepared as described previously[6] and to each sample well was added 90 μL of media containing H9 cells at 3 × 10^5 cells/mL and 45 μL of virus inoculum (HIV-1 IIIIB isolate) containing 125 $TCID_{50}$. Control wells containing virus and cells only (no drug) and cells only (no virus or drug) were also prepared. A second set of samples was prepared identical to the first and were added to cells under identical conditions without virus (mock infection) for toxicity determinations (IC_{50} defined below). In addition, AZT was also assayed during each experiment as a positive drug control. On days 1 and 4 postinfection (PI), spent media was removed from each well and replaced with fresh media. On day 6 PI, the assay was terminated and culture supernatants were harvested for analysis of virus replication by p24 antigen capture. The compound toxicity was determined by XTT using the mock-infected sample wells. If a test sample inhibited virus replication and was not toxic, its effects were reported in the following terms: IC_{50}, the concentration of test sample that was toxic to 50% of the mock-infected cells; EC_{50}, the concentration of the test sample that was able to suppress HIV replication by 50%; and the therapeutic index (TI), the ratio of the IC_{50} to EC_{50}.

Time of Addition Study. This is a single cycle replication assay involving addition of titered $HIV\text{-}1_{IIIB}$ to HeLa CD4 LTR-*b*-gal cells. Compounds **4c**, AZT (zidovudine), nevirapine, or CSB (Chicago Sky Blue) were added at various time intervals after adding virus (−1, 0, 2, 4, 8, 12, and 24 h). At 2 h after virus addition, the cells were washed three times to remove unbound virus, and fresh compound was added back where appropriate. At 48 h postinfection, the level of β-galactosidase enzyme expression was determined by chemiluminescence using the Tropix Gal-screen (Bedford, Mass).

Disposition/Pharmacokinetic Study. Compounds **2**, **4c**, and **5c** were administered via the intravenous (iv, 10−20 mg/kg) and oral (po suspension, 25 mg/kg) routes to adult male Sprague−Dawley rats (approximately 250 g). Nine blood samples (0.4 mL) were collected sequentially over an 8-h period. Concentrations of the respective compounds were detected using both ultraviolet and fluorescence detection. Reversed phase HPLC was conducted with an ODS, 5 μM particle size, 150 × 4.6 mm Axxiom column (Thompson Instruments, Chantilly, VA) using 55% acetonitrile and water as the mobile phase with 25 mM acetic acid buffer at 1.5 mL/min. Detection was carried out at 320 nm (λ_{max}) for UV detection and at an excitation wavelength of 320 nm and emission wavelength of 380 nm for fluorescence detection. HPLC employed an HP1100 with UV and quantitative analysis using the HP Chemstation. For fluorescence detection, an RF 551 detector from Shimadzu was employed with data recorded on an HP 3396 integrator. The detection limit for the assay for all three compounds examined in rats was approximately 50 ng/mL by UV and 10 ng/mL by fluorescence using 0.10 mL of plasma. Pharmacokinetic parameters were calculated by noncompartmental analysis (WinNonlin) and the half-life reported is the terminal log-linear phase of the plasma concentration vs time profile.

Acknowledgment. This investigation was supported by Grant AI-33066 from the National Institute of Allergy and Infectious Diseases (NIAID) awarded to K.H.L. and Grant R44 AI46115 from NIAID awarded to G.P.A.

References

(1) Gottlieb, M. S.; Schroff, R.; Schanker, H. M.; Weisman, J. D.; Fan, P. T.; Wolf, R. A.; Saxon, A. Pneumocystis Carinii Pneumonia and Mucosal Candidiasis in Previously Healthy Homosexual Men. *N. Engl. J. Med.* **1981**, *305*, 1425−1431.

(2) Barre-Sinoussi, F.; Chermann, J. C.; Rey, F.; Nugeyre, M. T.; Chamaret, S.; Gruest, J.; Dauguet, C.; Axler-Blin, C.; Vezinet-Brun, F.; Rouzioux, C.; Rozenbaum, W.; Montagnier, L. Isolation of a T-Lymphotropic Retrovirus from a Patient at Risk for Acquired Immune Deficiency Syndrome (AIDS). *Science* **1983**, *220*, 868−871.

(3) UNAIDS and WHO. AIDS Epidemic Update. *Trop. Doc.* **2001**, *32*, 189.

(4) Huang, L.; Kashiwada, Y.; Cosentino, L. M.; Fan, S.; Lee, K. H. 3′,4′-Di-*O*-(−)-Camphanoyl-(+)-*cis*-Khellactone and Related Compounds: A New Class of Potent Anti-HIV Agents. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 593−598.

(5) Huang, L.; Kashiwada, Y.; Cosentino, L. M.; Fan, S.; Chen, C. H.; McPhail, A. T.; Fujioka, T.; Mihashi, K.; Lee, K. H. Anti-AIDS Agents. 15. Synthesis and Anti-HIV Activity of Dihydroseselins and Related Analogues. *J. Med. Chem.* **1994**, *37*, 3947−3955.

(6) Xie, L.; Takeuchi, Y.; Cosentino, L. M.; McPhail, A. T.; Lee, K. H. Anti-AIDS Agents. 42. Synthesis and Anti-HIV Activity of Disubstituted (3′R,4′R)-3′,4′-di-*O*-(S)-Camphanoyl-(+)-*cis*-Khellactone Analogues. *J. Med. Chem.* **2001**, *44*, 664−671.

(7) Xie, L.; Takeuchi, Y.; Cosentino, L. M.; Lee, K. H. Anti-AIDS Agents. 37. Synthesis and Structure−Activity Relationships of (3′R,4′R)−(+)-*cis*-Khellactone Derivatives as Novel Potent Anti-HIV Agents. *J. Med. Chem.* **1999**, *42*, 2662−2672.

(8) Xie, L.; Takeuchi, Y.; Cosentino, L. M.; Lee, K. H. Anti-AIDS Agents 33 Synthesis and Anti-HIV Activity of Monomethyl Substituted 3′,4′-di-*O*-(−)-Camphanoyl-(+)-*cis*-Khellactone (DCK) Analogues. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2151−2156.

(9) Takeuchi, Y.; Xie, L.; Cosentino, L. M.; Lee, K. H. Anti-AIDS agents−XXVIII. Synthesis and Anti-HIV Activity of Methoxy Substituted 3′,4′-di-*O*-(−)-Camphanoyl-(+)-*cis*-Khellactone (DCK) Analogues. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2573−2578.

(10) Yu, D.; Suzuki, M.; Xie, L.; Morris-Natschke, S. L.; Lee, K. H. Recent Progress in the Development of Coumarin Derivatives as Potent Anti-HIV Agents. *Med. Res. Rev.* **2003**, *23*, 322−345.

(11) Xie, L.; Allaway, G.; Wild, C.; Kilgore, N.; Lee, K. H. Anti-AIDS Agents Part 47. Synthesis and Anti-HIV Activity of 3-Substituted 3′,4′-di-*O*-(S)-Camphanoyl-(3′R,4′R)-(+)-*cis*-Khellactone Derivatives. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2291−2293.

(12) Chilin, A.; Marzano, C.; Guiotto, A.; Manzini, P.; Baccichetti, F.; Carlassare, F.; Bordin, F. Synthesis and Biological Activity of (Hydroxymethyl)- and (Diethylaminomethyl)benzopsoralens. *J. Med. Chem.* **1999**, *42*, 2936−2945.

JM030416Y