Articles

Anti-AIDS Agents. 42. Synthesis and Anti-HIV Activity of Disubstituted (3′*R*,4′*R*)-3′,4′-Di-*O*-(*S*)-camphanoyl-(+)-*cis*-khellactone Analogues

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A series of disubstituted 3′,4′-di-*O*-(*S*)-camphanoyl-(+)-*cis*-khellactone (DCK) analogues (**1**−**10**) were synthesized and evaluated for inhibition of HIV-1 replication in H9 lymphocytes. 5-Methoxy-4-methyl DCK (**8**) was the most promising compound with an EC_{50} value of 7.21×10^{-6} μM and a therapeutic index of $>2.08 \times 10,^{7}$ which were much better than those of lead compound DCK in the same assay. Another six disubstituted DCK analogues (**1**−**5** and **7**) were more potent than AZT but less active than DCK. Conformational analysis suggested that resonance of the coumarin system is an essential structural feature for potent anti-HIV activity. Steric compression of C(4) and C(5) substituents of the coumarin moiety can reduce the overall planarity and thus resonance of the coumarin nucleus, resulting in a decrease or lack of anti-HIV activity.

Introduction

Since the early 1980s, acquired immune-deficiency syndrome (AIDS) has spread rapidly across the world and has become a serious global threat to human health and life. Millions of people have been killed by the AIDS epidemic, and the total number of people living with the virus has risen to more than 33.4 million worldwide. As of now, the FDA has approved 14 anti-HIV agents as drugs for clinical use, including 6 nucleoside reverse transcriptase inhibitors (NRTIs), 3 nonnucleoside reverse transcriptase inhibitors (NNRTIs), and 5 protease inhibitors (PIs). All these drugs are currently used alone or as a part of a combination regimen to treat HIV infection/AIDS disease. These available drugs have made a marked impact on suppression of HIV infection but still have limited or transient clinical benefits in HIV-infected individuals due to rapid development of HIV resistance, side effects, and/or toxicity. Drug resistance is a large problem for all anti-HIV drugs, because HIV has the potential to constantly mutate and eventually is no longer sensitive to chemotherapy. Thus, HIV continues its life cycle as before drug treatment and damages the immune system. Consequently, these limitations still necessitate development of novel anti-HIV agents with new mechanism(s) of action.

In our previous studies, (3′*R*,4′*R*)-3′,4′-di-*O*-(*S*)-camphanoyl-(+)-*cis*-khellactone (DCK) was discovered as a potent anti-HIV agent with an EC_{50} value of 2.56×10^{-4} μM and a therapeutic index (TI) of 1.37×10^{5}.[2] Subsequently, 24 (3′*R*,4′*R*)-(+)-*cis*-khellactone derivatives were designed, synthesized, and evaluated in an in vitro anti-HIV assay.[3] Among them, 3-methyl DCK, 4-methyl DCK, and 5-methyl DCK were much more potent than DCK and AZT in the same assay with EC_{50} and TI values ranging from 5.25×10^{-5} to 2.39×10^{-7} μM and 2.15×10^{6} to 3.97×10^{8}, respectively. Thus, these first structure−activity relationship (SAR) studies indicated that alkyl/*O*-alkyl substituents at the 3-, 4-, and 5-positions on the coumarin nucleus are favorable for enhanced anti-HIV activity and decreased toxicity of DCK and a methyl group is preferred to other substituents. Even though the mechanism of action of DCK and its analogues is still unknown, current mechanistic studies indicate that they do not inhibit RT, integrase, or protease in the HIV life cycle but are strongly synergistic with approved drugs such as AZT. Therefore, DCK and its analogues might inhibit HIV-1 replication by a novel mechanism. These exciting results prompted us to further modify DCK in order to ascertain the pharmacophore(s) for this compound type as potent anti-HIV agents. In this paper, we report the synthesis and the anti-HIV bioassay results of 10 disubstituted DCK analogues (**1**−**10**).

Chemistry

On the basis of previous results with monomethylated DCK analogues, we first wanted to explore the effects of two methyl substituents on anti-HIV activity. Accordingly, 3,4-dimethyl (**1**), 4,5-dimethyl (**2**), 3,5-dimethyl (**3**), and 4,6-dimethyl (**4**) DCK analogues were synthesized. After reviewing their bioassay data, other 3,4-disubstituted (**5**−**7**) and 4,5-disubstituted (**8**−**10**) DCK analogues were investigated. Each of these designed target compounds, except **7**, contains at least one

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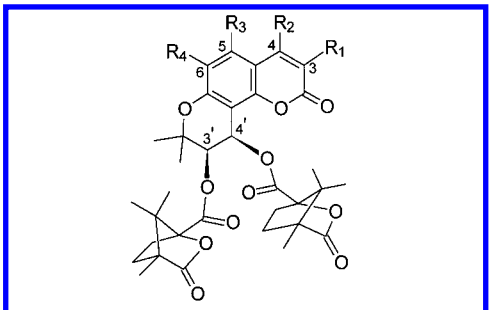
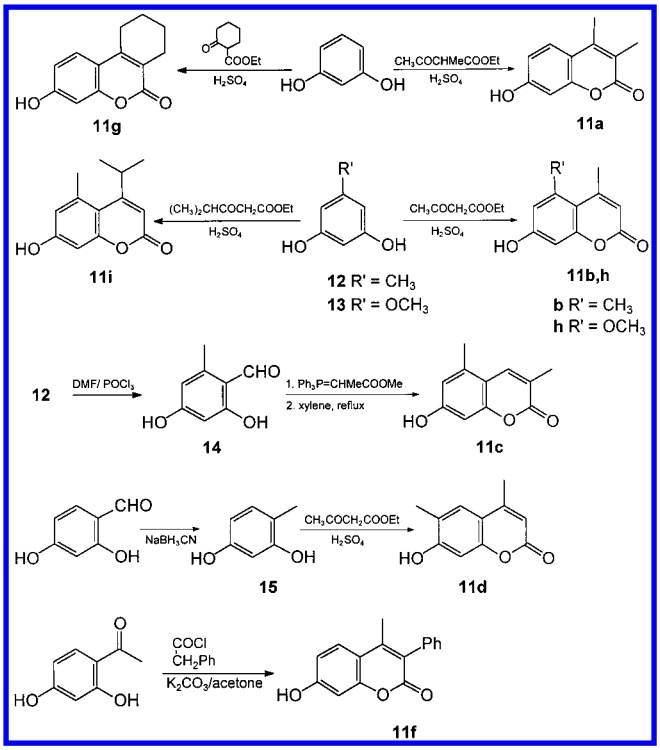


	R_1	R_2	R_3	R_4
DCK	H	H	H	H
4-Me-DCK	H	CH_3	H	H
1	CH_3	CH_3	H	H
2	H	CH_3	CH_3	H
3	CH_3	H	CH_3	H
4	H	CH_3	H	CH_3
5	Cl	CH_3	H	H
6	Ph	CH_3	H	H
7		$-(CH_2)_4-$	H	H
8	H	CH_3	OCH_3	H
9	H	$CH(CH_3)_2$	CH_3	H
10	H	CH_3	$OCH_2C_6H_5$	H

Figure 1. DCK, 4-methyl DCK, and disubstituted DCK analogues.

Scheme 1. Syntheses of Disubstituted 7-Hydroxycoumarins **11a−d,f−i**



methyl group on the coumarin nucleus (Figure 1) in order to maintain potent anti-HIV activity.

As shown in Scheme 1, disubstituted 7-hydroxycoumarins **11a,b,d,g−i** were synthesized from different 1,3-dihydroxybenzene derivatives using a Pechmann reaction with various β-keto esters in the presence of sulfuric acid at room temperature.[4] 2,4-Dihydroxy-6-methylbenzaldehyde (**14**), prepared by reacting DMF/$POCl_3$[5] with 3,5-dihydroxytoluene (**12**), underwent a Wittig reaction followed by an intramolecular cyclization to afford 3,5-dimethyl-7-hydroxycoumarin (**11c**).[6,7] 3-Chloro-4-methyl-7-hydroxycoumarin (**11e**) was commercially available. 7-Hydroxy-4-methyl-3-phenylcoumarin (**11f**) was prepared using a base-catalyzed Kostanecki condensation[8] between 2,4-dihydroxyacetophenone and phenylacetyl chloride with anhydrous potassium carbonate in acetone.

These nine disubstituted 7-hydroxycoumarin intermediates **11a−i** were then converted to the corresponding seselin derivatives **16a−i** by alkylation with 3-chloro-3-methyl-1-butyne in dimethylformamide (DMF) at 70−80 °C in the presence of potassium iodide and potassium carbonate followed by cyclization in *N*,*N*-diethylaniline at reflux temperature (Scheme 2). Finally, asymmetric dihydroxylation[9] followed by acylation gave disubstituted DCK analogues **1−9**, respectively.

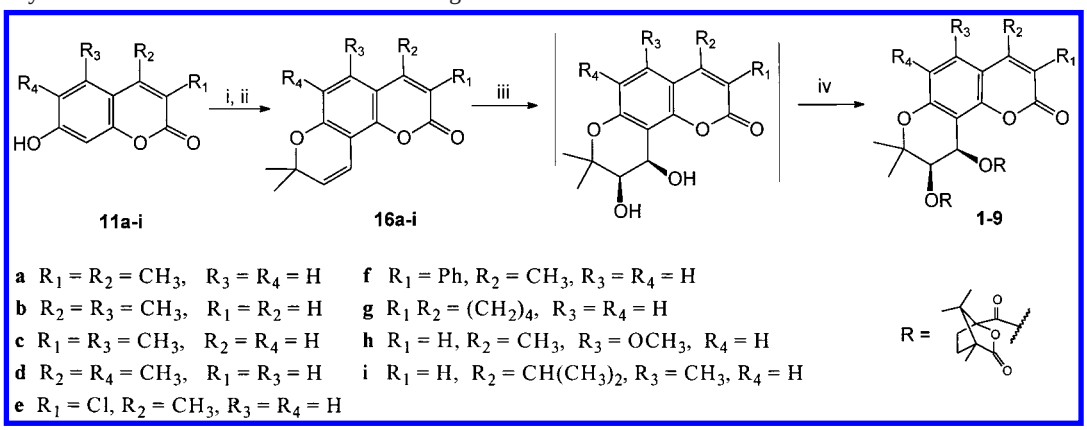
The asymmetric dihydroxylation (AD) afforded most target compounds with >76% diastereomeric excess (d.e.). However, under the same conditions, 4-isopropyl-5-methyl DCK (**9**) was obtained in a 1:1 diastereomeric mixture, as per ^{1}H NMR. The isomers were separated by crystallization from ethanol. Compound **9** was soluble in ethanol, while the (3′*S*,4′*S*)-isomer crystallized. Both diastereomers were obtained in >95% d.e.

A different synthetic route was explored for the synthesis of **10** and **8** as shown in Scheme 3. Friedel−Crafts acylation of 1,3,5-trihydroxybenzene with 3,3-dimethylacrylic acid[10] in the presence of boron trifluoride diethyl etherate gave 5,7-dihydroxy-2,2-dimethyl-4-chromanone (**17**). The 7-phenolic group in **17** was selectively benzylated using benzyl bromide (1:1 molar ratio) in acetone in the presence of K_2CO_3 to afford 7-benzyloxy-2,2-dimethyl-5-hydroxy-4-chromanone (**18**). Next, the 4-carbonyl group in **18** was reduced readily to a methylene with $NaBH_4$ in refluxing THF solution under basic conditions. Then the lactone ring was formed by a Pechmann reaction to afford 5-benzyloxy-4-methyl-3′,4′-dihydroseselin (**20**). Satisfactory yields were obtained using boron trifluoride diethyl etherate, rather than sulfuric acid, as a catalyst. Compound **20** was dehydrogenated with DDQ in dioxane at reflux to afford 5-benzyloxy-4-methylseselin (**22**) in 70% yield.[11] Compound **22** was then converted to 5-benzyloxy-4-methyl DCK (**10**) by asymmetric dihydroxylation and acylation in the same manner as described above. Using the same synthetic route, 5-methoxy-4-methyl DCK (**8**) was also synthesized successfully.

Bioassay Results

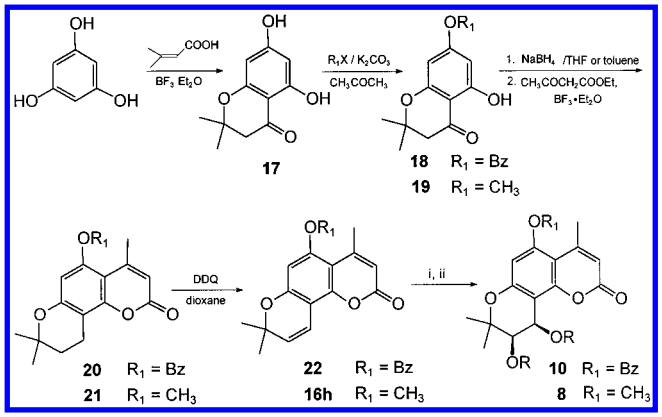
Disubstituted DCK analogues **1−10** were screened for inhibition of HIV replication in H9 lymphocytes, and their bioassay data are shown in Table 1. 5-Methoxy-4-methyl DCK (**8**) was the most promising compound. It exhibited extremely potent inhibitory activity against HIV-1 replication with an EC_{50} value of 7.21×10^{-6} μM and a TI of $>2.08 \times 10^7$, which were much better than those of DCK and AZT and similar to those of 4-methyl DCK (EC_{50} 1.83×10^{-6} μM, TI $>6.89 \times 10^7$) in the same assay. Dimethyl DCK analogues **1−4** were more potent than AZT but less active than DCK, although the previously prepared monomethyl DCK analogues were more active than DCK.[3] 3-Chloro-4-methyl DCK (**5**) and 3,4-cyclohexano DCK (**7**) also showed potent anti-HIV activity, comparable to those of the dimethyl DCK compounds. These results indicated that anti-HIV activity could be maintained when two methyl or other aliphatic substituent(s) were placed on the DCK cou-

Scheme 2. Syntheses of Disubstituted DCK Analogues **1**−**9**[a]



[a] (i) 3-Chloro-3-methyl-1-butyne, KI, K_2CO_3 in DMF or acetone, 70−80 °C; (ii) *N*,*N*-diethylaniline, reflux; (iii) $K_2Os_2(OH)_4$, K_2CO_3, $K_3Fe(CN)_6$, $(DHQ)_2$−PYR in *t*-BuOH/H_2O (v/v = 1:1), ice bath; (iv) (*S*)-(−)-camphanoyl chloride, pyridine in CH_2Cl_2.

Scheme 3. Syntheses of 4,5-Disubstituted DCK Analogues **10** and **8**[a]



[a] (i) $K_2Os_2(OH)_4$, K_2CO_3, $K_3Fe(CN)_6$, $(DHQ)_2$−PYR in *t*-BuOH/H_2O (v/v = 1:1), ice bath; (ii) (*S*)-(−)-camphanic chloride, pyridine in CH_2Cl_2.

Table 1. Anti-HIV Activity of Disubstituted DCK Analogues **1**−**10**[a] in H9 Lymphocytes[b]

compd	IC_{50} (μM)	EC_{50} (μM)	TI
1	>154	1.92×10^{-3}	$>8.02 \times 10^4$
2	>154	4.19×10^{-3}	$>3.68 \times 10^4$
3	>154	9.10×10^{-3}	$>1.69 \times 10^4$
4	3.75	4.69×10^{-3}	1.25×10^3
5	104	2.01×10^{-3}	5.17×10^4
6	>140	43.7	>3.20
7	>148	2.12×10^{-3}	$>6.98 \times 10^4$
8	>150	7.21×10^{-6}	$>2.08 \times 10^7$
9	>147	no suppression	
10	135	1.54	87.7
DCK	35	2.56×10^{-4}	1.37×10^5
AZT	1875	4.50×10^{-2}	4.17×10^4

[a] All data presented are averages of at least two separate experiments. [b] Assay in H9 lymphocytes was performed by BBI-Biotech Research Laboratories, Inc., Gaithersburg, MD.

marin nucleus. However, 4-methyl-3-phenyl DCK (**6**) and 5-benzyloxy-4-methyl DCK (**10**) suppressed HIV-1 replication only slightly, suggesting that an aromatic substituent on the coumarin nucleus is not favorable for anti-HIV activity. Furthermore, 4-isopropyl-5-methyl DCK (**9**) did not suppress HIV-1 replication in this assay.

Discussion

Although monomethyl DCK analogues (3-, 4-, and 5-methyl DCK) previously exhibited extremely potent anti-HIV activity and were more active than DCK, dimethyl DCK analogues **1**−**4** all showed lower anti-HIV activity than DCK in H9 lymphocytes. However, 5-methoxy-4-methyl DCK (**8**) was more active than DCK, AZT, and 4,5-dimethyl DCK (**2**) and was comparable to 4-methyl DCK. In contrast, 4-isopropyl-5-methyl DCK (**9**) was completely inactive in the same assay. These results prompted us to consider whether steric compression between adjacent substituents could alter the molecular three-dimensional orientation, thereby directly affecting the molecular affinity with target receptor/enzyme and resulting in different anti-HIV activity.

To obtain a better understanding of the SARs of disubstituted DCK analogues, we performed conformational searches for 4-methyl DCK, **2**, **8**, and **9** using Sybyl software. The resulting stable three-dimensional structures and torsional angles (Figure 2) suggested that the steric compression between two bulky substituents at the 4- and 5-positions caused deformation of the coumarin system. The 5-methyl groups in the structures of **9** and **2** were forced out of the resonance plane with a torsional angle [C(4)−C(4a)−C(5)−C(Me-5)] of 42.9° in **9** and 29.5° in **2**. Comparatively, the

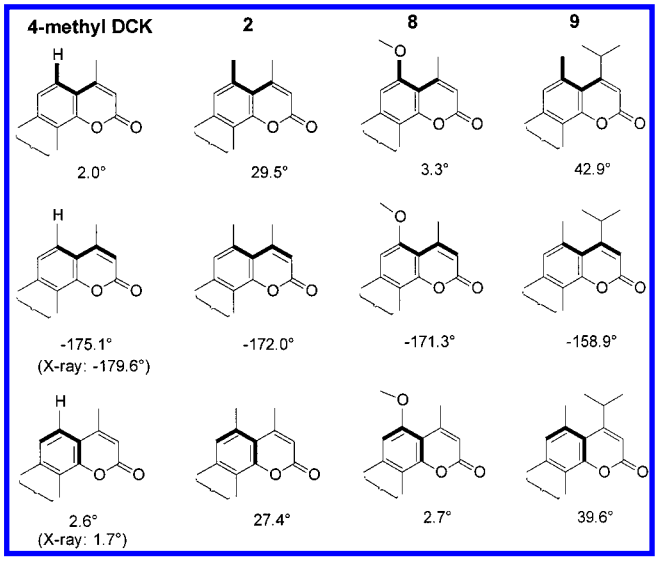


Figure 2. Torsional angles of 4-methyl DCK, **2**, **8**, and **9**. The torsion angle A−B−C−D is defined as positive if, when viewed along the B−C bond, atom A must be rotated clockwise to eclipse atom D.

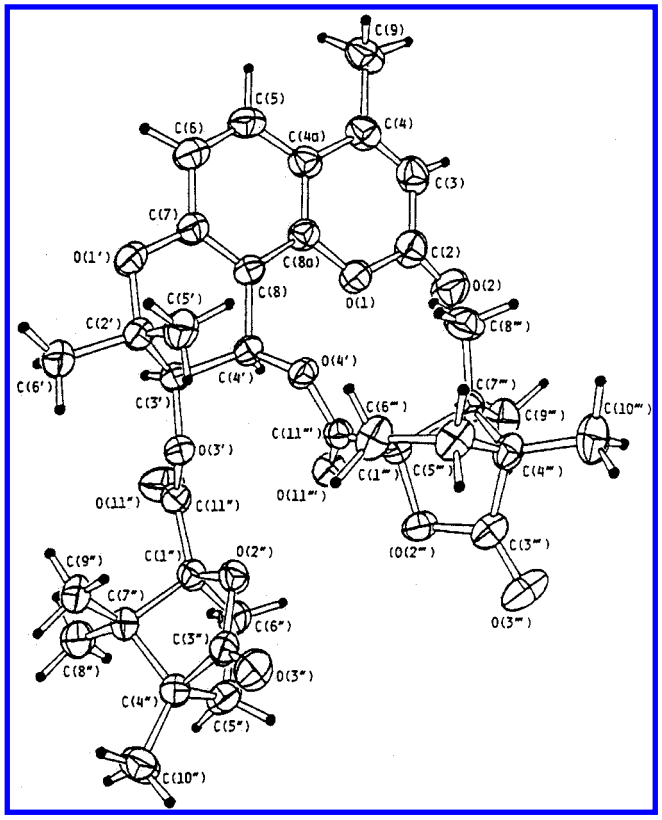


Figure 3. X-ray structure of 4-methyl DCK. ORTEP diagram (40% probability ellipsoids) showing the crystallographic atom-numbering scheme and solid-state conformation; small filled circles represent hydrogen atoms.

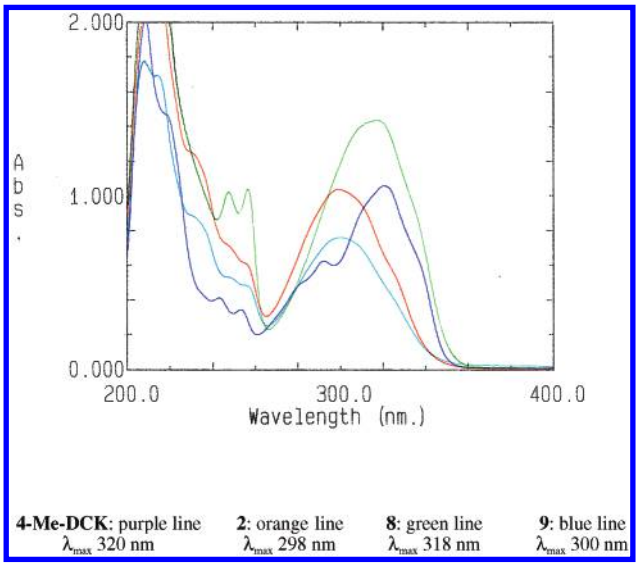


Figure 4. UV spectra of 4-methyl DCK, **2**, **8**, and **9**.

corresponding torsional angles in **8** and 4-methyl DCK were 3.3° and 2.0°, respectively. Other torsional angles in **2** and **9** also show obvious deviations from planarity, unlike those in 4-methyl DCK and **8**, which are close to 0° or 180°. Furthermore, the solid-state conformation and torsion angle data for 4-methyl DCK from an X-ray diffraction study (see Figure 3) were similar to the computational data.

The UV spectral data of 4-methyl DCK, **2**, **8**, and **9** (Figure 4) supported their calculated conformations. The longest wavelength absorption band for 4-methyl DCK was at 320 nm, indicating full resonance in the coumarin system. With **2** and **9,** the corresponding absorption bands shifted to 298 and 300 nm, respectively. This blue shift of the absorption band indicates a loss of coumarin resonance because of steric compression between the two substitutents at the 4- and 5-positions. In contrast, **8** possessed an absorption band at 318 nm, very similar to that found in 4-methyl DCK.

The foregoing studies indicated that the presence of substituents at both the 4- and 5-positions results in overcrowding, which is relieved in part by out-of-plane displacements thereby disturbing the overall planarity of the coumarin system. Accordingly, these steric effects and consequent skeletal deformation might be responsible for the lack of anti-HIV activity in **9** and obvious decrease in **2**. On the other hand, with 5-methoxy-4-methyl DCK (**8**), the unfavorable steric interaction of the methoxy group with the 4-methyl group is minimized by (i) rotation of the C(5)−O bond and (ii) the C(4)−CH_3 bond. Therefore, the coumarin moiety in compound **8** maintains maximum resonance and is significantly more active than DCK as an anti-HIV agent.

In summary, these studies indicated the following conclusions: (1) A planar coumarin system is probably an essential structural feature for potent anti-HIV activity. (2) Steric compression of C(4) and C(5) substituents of the coumarin moiety can reduce the overall planarity and thus resonance of the coumarin system, resulting in decreased or completely lost anti-HIV activity. (3) Methyl or other aliphatic substitutions on the coumarin nucleus are favorable for anti-HIV activity, whereas aromatic substituents are not.

Experimental Section

Chemistry. Melting points were measured with a Fisher Johns melting apparatus without correction. ^{1}H NMR spectra were measured on a Bruker AC-300 MHz spectrometer using TMS as internal standard. The solvent used was $CDCl_3$ unless indicated. UV spectra were measured on a Shimadzu UV-2101PC, UV−vis scanning spectrophotometer using EtOH as solvent. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. All target compounds were analyzed for C, 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 intensity of protons at the 3′-position in the ^{1}H NMR spectra. TLC was performed on a precoated silica gel GF plate purchased from Analtech, Inc. Silica gel (200−400 mesh) from Aldrich, Inc., was used for column chromatography. All other chemicals were obtained from Aldrich, Inc.

General Procedure for Synthesizing Disubstituted 7-Hydroxycoumarins 11. To a mixture of an appropriate phenol and a β-keto ester in a 1:1.5 molar ratio was slowly added excess H_2SO_4 (98%) with stirring at 0 °C within 1 h. Then, the mixture was stirred at room temperature until the reaction was complete as monitored by TLC. The mixture was poured into ice−water and allowed to stand overnight. The precipitated solid was filtered, washed with water until neutral, and dried in vacuo to afford the product.

3,4-Dimethyl-7-hydroxycoumarin (11a): 87% yield (starting with 8 mmol of 1,3-dihydroxybenzene and 12 mmol of ethyl 2-methylacetoacetate for 16 h); colorless crystals from EtOH; mp 220 °C dec; ^{1}H NMR (CD_3OD) δ 2.11 (3H, s, CH_3-3), 2.38 (3H, s, CH_3-4), 6.65 (1H, d, J = 2.4 Hz, H-8), 6.77 (1H, dd, J = 2.4 and 8.8 Hz, H-6), 7.57 (1H, d, J = 8.8 Hz, H-5).

4,5-Dimethyl-7-hydroxycoumarin (11b): 77% yield (starting with 4 mmol of 3,5-dihydroxytoluene and 6 mmol of ethyl acetoacetate for 4 h); yellow solid; mp 235 °C dec; ^{1}H NMR

(CD_3OD) δ 2.31 (3H, s, CH_3-5), 2.60 (3H, s, CH_3-4), 5.98 (1H, s, H-3), 6.54 (1H, s, H-8), 6.59 (1H, s, H-6).

7-Hydroxy-4,6-dimethylcoumarin (11d): 92% yield (starting with 4 mmol of 2,4-dihydroxytoluene **15** and 6 mmol of ethyl acetoacetate for 24 h); yellow solid; mp 268 °C dec; ^{1}H NMR (CD_3OD) δ 2.39 (3H, s, CH_3-6), 2.57 (3H, s, CH_3-4), 6.21 (1H, s, H-3), 6.58 (1H, s, H-8), 7.61 (1H, s, H-5).

7-Hydroxy-3,4-tetrahydrobenzocoumarin (11g): 98% yield (starting with 8 mmol of 1,3-dihydroxybenzene and 12 mmol of ethyl 2-cyclohexanonecarboxylate for 24 h); yellow solid; mp 225−7 °C; ^{1}H NMR (DMSO) δ 1.73 (4H, m, 2 × CH_2), 2.39 (2H, t, J = 5.7 Hz, CH_2-4), 2.73 (2H, t, J = 5.7 Hz, CH_2-3), 6.69 (1H, d, J = 2.4 Hz, H-8), 6.77 (1H, dd, J = 2.4 and 8.8 Hz, H-6), 7.54 (1H, d, J = 8.8 Hz, H-5), 10.35 (1H, br, OH-7).

7-Hydroxy-5-methoxy-4-methylcoumarin (11h): 72% yield (starting with 4 mmol of 5-methoxyresorcinol and 6 mmol of ethyl acetoacetate for 4 h); yellow solid; mp 223−6 °C; ^{1}H NMR δ 2.46 (3H, s, CH_3-4), 3.77 (3H, s, CH_3O-5), 5.93 (1H, s, H-3), 6.31 (1H, d, J = 2.4 Hz, H-8), 6.42 (1H, d, J = 2.4 Hz, H-6).

7-Hydroxy-4-isopropyl-5-methylcoumarin (11i): 33% yield (starting with 4 mmol of 3,5-dihydroxytoluene and 6 mmol of ethyl isobutyryl acetate for 16 h); white solid; mp 173−4 °C; ^{1}H NMR δ 1.22 (6H, d, J = 6.9 Hz, $CH(CH_3)_2$-4), 2.30 (3H, s, CH_3-5), 4.11 (1H, m, J = 6.9 Hz, $CH(CH_3)_2$-4), 4.59 (br, OH-7), 6.10 (1H, s, H-3), 6.55 (1H, s, H-8), 6.63 (1H, H-6).

7-Hydroxy-3,5-dimethylcoumarin (11c). To a solution of **14** (273 mg, 1.8 mmol) in 5 mL of anhydrous DMF under N_2 was added $Ph_3P{=}CCH_3COOMe$ (2.0 mmol). The mixture was stirred and heated to reflux for 6 h. After cooling to room temperature, the mixture was diluted with EtOAc and extracted three times with 10% aq KOH. The combined water layer was acidified with 10% aq HCl to pH 3. Then, the precipitated solid was filtered and solution was extracted again with EtOAc three times. The organic phase was washed with water until neutral, then dried over Na_2SO_4. Finally, removal of the solvent furnished 277 mg of pure **11c:** 81% yield; mp 147 °C dec; ^{1}H NMR δ 2.12 (3H, s, CH_3-3), 2.39 (3H, s, CH_3-5), 6.57 (2H, s, H-6 & H-8), 7.61 (1H, s, H-4).

7-Hydroxy-4-methyl-3-phenylcoumarin (11f). A mixture of 2,4-dihydroxyacetophenone (1.52 g, 10 mmol) and phenylacetyl chloride (4,4 mL, 30 mmol) in acetone in the presence of K_2CO_3 was heated to reflux for 30 h. K_2CO_3 was filtered and solvent was removed. The residue was crystallized from EtOH to produce needle crystals of 1.2 g of **11f:** 48% yield; mp 225−8 °C dec; ^{1}H NMR (CD_3Cl + CD_3OD) δ 2.23 (3H, s, CH_3-4), 6.78 (1H, d, J = 2.4 Hz, H-8), 6.80 (1H, dd, J = 2.4 and 8.8 Hz, H-6), 7.23−7.41 (5H, m, ArH), 7.50 (1H, d, J = 8.8 Hz, H-5).

2,4-Dihydroxy-6-methylbenzaldehyde (14). 1 mL of phosphorus oxychloride ($POCl_3$) was added dropwise into 5 mL of DMF below 10 °C with rapid stirring over 0.5 h. 3,5-Dihydroxytoluene (**12**) (1.10 g, 8.88 mmol) in 5 mL of DMF was added slowly keeping the temperature below 10 °C. The mixture was warmed to room temperature and stirred for 1 h. Ice and 10% aq NaOH were then added successively until the pH was 9−10 and solid appeared. The mixture was heated to boiling for 10 min, then adjusted to pH 3 with 10% aq HCl after cooling to room temperature. The solid product was collected, washed with water until neutral, and dried to give **14**: 1.25 g, 93% yield; mp 152−4 °C; ^{1}H NMR δ 2.55 (3H, s, CH_3), 6.20 and 6.22 (each 1H, s, ArH), 10.20 (1H, CHO), 5.50 and 12.40 (OH-4 and OH-2, disappeared in D_2O).

2,4-Dihydroxytoluene (15). To a solution of 2,4-dihydroxybenzaldehyde (690 mg, 5 mmol) and sodium cyanoborohydride (1.0 g) in 30 mL of THF was added methyl orange as an indictor, giving the solution a yellow color; aq HCl solution (15 mL, 1 N in water) was slowly added to the reaction system, keeping the solution orange. The mixture was stirred for 3 h at room temperature. Water was added, and the mixture was extracted with Et_2O three times. After removal of solvent, product **15** (611 mg) was obtained: 98.6% yield; mp 105−6 °C; ^{1}H NMR (CD_3OD) δ 2.06 (3H, s, CH_3), 6.20 (1H, dd, J = 2.0 and 8.8 Hz, H-5), 6.28 (1H, J = 2.0 Hz, H-3), 6.84 (1H, d, J = 8.8 Hz, H-6).

General Procedure for Synthesizing Disubstituted Seselins 16. A mixture of disubstituted 7-hydroxycoumarin **11** (5 mmol), K_2CO_3 (12.5 mmol), KI (5 mmol), and 3-chloro-3-methyl-1-butyne (1−2 mL, excess) in DMF (10 mL) was heated to 70−80 °C with stirring until the reaction was complete as monitored by TLC. Solid K_2CO_3 was filtered. The filtrate was concentrated in vacuo. The residue, without purification, was directly heated to reflux in 10 mL of *N*,*N*-diethylaniline for 4−6 h. The reaction mixture was cooled to room temperature, diluted with EtOAc and washed with 10% aq HCl, water and brine. The organic layer was separated and solvent was removed in vacuo. The residue was purified by column chromatography or TLC with an eluant of hexane: EtOAc = 7:3 to afford the substituted seselin **16**.

3,4-Dimethylseselin (16a): yield 38% (starting with 950 mg of **11a**); mp 121−2 °C; ^{1}H NMR δ 1.48 (6H, s, 2 × CH_3-2′), 2.20 (3H, s, CH_3-3), 2.36 (3H, s, CH_3-4), 5.72 (1H, d, J = 10.2 Hz, H-3′), 6.74 (1H, d, J = 8.8 Hz, H-6), 6.94 (1H, d, J = 10.2 Hz, H-4′), 7.36 (1H, d, J = 8.8 Hz, H-5).

4,5-Dimethylseselin (16b): yield 38% (starting with 440 mg of **11b**); mp 95−6 °C; ^{1}H NMR δ 1.49 (6H, s, 2 × CH_3-2′), 2.35 (3H, s, CH_3-4), 2.60 (3H, s, CH_3-5), 5.64 (1H, d, J = 10.2 Hz, H-3′), 6.04 (1H, s, H-3), 6.48 (1H, d, J = 10.2 Hz, H-4′), 6.69 (1H, s, H-6).

3,5-Dimethylseselin (16c): yield 40% (starting with 250 mg of **11c**); mp 184−7 °C; ^{1}H NMR δ 1.46 (6H, s, 2 × CH_3-2′), 2.20 (3H, s, CH_3-3), 2.45 (3H, s, CH_3-5), 5.66 (1H, d, J = 10.2 Hz, H-3′), 6.58 (1H, s, H-6), 6.88 (1H, d, J = 10.2 Hz, H-4′), 7.62 (1H, s, H-4).

4,6-Dimethylseselin (16d): yield 43% (starting with 700 mg of **11d**); mp 153−5 °C; ^{1}H NMR δ 1.46 (6H, s, 2 × CH_3-2′), 2.22 (3H, s, CH_3-6), 2.36 (3H, s, CH_3-4), 5.70 (1H, d, J = 10.2 Hz, H-3′), 6.09 (1H, s, H-3), 6.90 (1H, d, J = 10.2 Hz, H-4′), 7.19 (1H, s, H-5).

3-Chloro-4-methylseselin (16e): yield 26% (starting with 378 mg of **11e**); mp 154−5 °C; ^{1}H NMR δ 1.55 (6H, s, 2 × CH_3-2′), 2.51 (3H, s, CH_3-4), 5.72 (1H, d, J = 10.2 Hz, H-3′), 6.76 (1H, d, J = 8.8 Hz, H-6), 6.86 (1H, d, J = 10.2 Hz, H-4′), 7.35 (1H, d, J = 8.8 Hz, H-5).

4-Methyl-3-phenylseselin (16f): yield 38% (starting with 504 mg of **11f**); light yellow plate; mp 97−9 °C; ^{1}H NMR δ 1.50 (6H, s, 2 × CH_3-2′), 2.26 (3H, s, CH_3-4), 5.75 (1H, d, J = 10.2 Hz, H-3′), 6.78 (1H, J = 8.8 Hz, H-6), 7.00 (1H, d, J = 10.2 Hz, H-4′), 7.30 (1H, J = 8.8 Hz, H-5), 7.45 (5H, m, ArH-3).

3,4-Tetrahydrobenzoseselin (16g): yield 33% (starting with 648 mg of **11g**); mp 152−4 °C; ^{1}H NMR δ 1.49 (6H, s, 2 × CH_3-2′), 1.82 (4H, m, 2 × CH_2), 2.57 (2H, m, CH_2-4), 2.73 (2H, m, CH_2-3), 5.72 (1H, d, J = 10.2 Hz, H-3′), 6.73 (1H, J = 8.8 Hz, H-6), 6.94 (1H, d, J = 10.2 Hz, H-4′), 7.32 (1H, J = 8.8 Hz, H-5).

5-Methoxy-4-methylseselin (16h): yield 40% (starting with 500 mg of **11h**); mp 174−5 °C; ^{1}H NMR δ 1.45 (6H, s, 2 × CH_3-2′), 2.53 (3H, s, CH_3-4), 3.87 (3H, s, CH_3O-5), 5.58 (1H, d, J = 10.2 Hz, H-3′), 5.94 (1H, s, H-3), 6.26 (1H, s, H-6), 6.84 (1H, d, J = 10.2 Hz, H-4′).

4-Isopropyl-5-methylseselin (16i): yield 22% (starting with 218 mg of **11i**); mp 65−8 °C; ^{1}H NMR δ 1.27 (6H, d, J = 6.9 Hz, $CH(CH_3)_2$-4), 1.50 (6H, s, 2 × CH_3-2′), 2.35 (3H, s, CH_3-5), 4.00 (1H, m, J = 6.9 Hz, $CH(CH_3)_2$-4), 5.65 (1H, d, J = 10.2 Hz, H-3′), 6.22 (1H, s, H-3), 6.50 (1H, d, J = 10.2 Hz, H-4′), 6.72 (1H, s, H-6).

General Procedure of Asymmetric Dihydroxylation and Acylation for Synthesizing Substituted (3′*R*,4′*R*)-Di-*O*-(−)-camphanoyl-(+)-*cis*-khellactones. A mixture of $K_3Fe(CN)_6$ (150 mg, 0.75 mmol), K_2CO_3 (105 mg, 0.75 mmol), 2,5-diphenyl-4,6-bis(9-*O*-dihydroquinyl)pyrimidine [$(DHQ)_2$-PYR] (4.4 mg, 0.005 mmol), and $K_2OsO_2(OH)_4$ (1.8 mg, 0.005 mmol) was solubilized in 5 mL of *t*-BuOH/H_2O (v/v, 1:1) at room temperature. Then, the solution was cooled to 0 °C and methanesulfonamide (0.25 mmol) added under stirring. When the solution turned from a light yellow to an orange color, the

substituted seselin compound (0.25 mmol) was added. The mixture was stirred at 0 °C for 2−4 days. $Na_2S_2O_5$ (excess), water, and $CHCl_3$ were added. After stirring for 0.5 h at room temperature, the mixture was extracted with $CHCl_3$ three times. The combined organic layer was dried over $MgSO_4$, then solvent was removed. The residue was separated by TLC to obtain the pure substituted (+)-*cis*-khellactone. However, the substituted (+)-*cis*-khellactone could be directly acylated, without further purification, with (*S*)-(−)-camphanic chloride (excess) in Py/CH_2Cl_2 for 1−2 days at room temperature. The mixture was diluted with EtOAc and washed with 10% aq HCl, water and brine, successively. The organic phase was dried over anhydrous $MgSO_4$, filtered, and concentrated. The residue was separated by TLC (eluant: hexane/EtOAc = 7:3) and afforded the appropriately substituted 3′,4′-di-*O*-(*S*)-camphanoyl-(+)-*cis*-khellactone derivative.

(3′*R*,4′*R*)-3,4-Dimethyl-3′,4′-di-*O*-(*S*)-camphanoyl-(+)-*cis*-khellactone (1): yield 64% (starting with 256 mg of **16a**); white solid; mp 118−20 °C; ^{1}H NMR δ 0.93−1.12 (15H, ms, 5 × CH_3), 1.27, 1.49, and 1.55 (each 3H, s, CH_3), 1.73, 1.92, 2.20, and 2.48 (each 2H, m, CH_2 in camphanoyl group), 2.13 (3H, s, CH_3-3), 2.38 (3H, s, CH_3-4), 5.40 (1H, d, J = 4.8 Hz, H-3′), 6.66 (1H, d, J = 4.8 Hz, H-4′), 6.63 (1H, d, J = 8.8 Hz, H-6), and 7.54 (1H, d, J = 8.8 Hz, H-5); 88% d.e.; $[\alpha]_D$ +6.14° (*c* 2.15, $CHCl_3$). Anal. ($C_{36}H_{42}O_{11}\cdot 2H_2O$) C, H.

(3′*R*,4′*R*)-4,5-Dimethyl-3′,4′-di-*O*-(*S*)-camphanoyl-(+)-*cis*-khellactone (2): yield 70% (starting with 256 mg of **16b**); white solid; mp 137−8 °C; UV λ_{max} nm (ϵ) 298 (6141), 215 (12782); ^{1}H NMR δ 0.96−1.15 (18H, ms, 6 × CH_3), 1.50 and 1.56 (each 3H, s, CH_3), 1.73, 1.93, 2.22, and 2.46 (each 2H, m, CH_2 in camphanoyl group), 2.24 (3H, s, CH_3-4), 2.58 (3H, s, CH_3-5), 5.38 (1H, d, J = 4.8 Hz, H-3′), 6.12 (1H, s, H-3), 6.41 (1H, d, J = 4.8 Hz, H-4′), 6.83 (1H, s, H-6); 72% d.e.; $[\alpha]_D$ −70.77° (*c* 0.38, $CHCl_3$). Anal. ($C_{36}H_{42}O_{11}\cdot H_2O$) C, H.

(3′*R*,4′*R*)-3,5-Dimethyl-3′,4′-di-*O*-(*S*)-camphanoyl-(+)-*cis*-khellactone (3): yield 82% (starting with 70 mg of **16c**); white solid; mp 98−100 °C; ^{1}H NMR δ 0.93−1.12 (15H, ms, 5 × CH_3), 1.27, 1.42, and 1.46 (each 3H, s, CH_3), 1.69, 1.89, 2.20, and 2.50 (each 2H, m, CH_2 in camphanoyl group), 2.16 (3H, s, CH_3-3), 2.46 (3H, s, CH_3-5), 5.36 (1H, d, J = 4.8 Hz, H-3′), 6.61 (1H, d, J = 4.8 Hz, H-4′), 6.65 (1H, s, H-6), and 7.61 (1H, s, H-4); 80% d.e.; $[\alpha]_D$ −4.47° (*c* 0.76, $CHCl_3$). Anal. ($C_{36}H_{42}O_{11}\cdot {}^1/_2H_2O$) C, H.

(3′*R*,4′*R*)-4,6-Dimethyl-3′,4′-di-*O*-(*S*)-camphanoyl-(+)-*cis*-khellactone (4): yield 68% (starting with 400 mg of **16d**); white solid; mp 250 °C dec; ^{1}H NMR δ 0.99−1.11 (18H, ms, 6 × CH_3), 1.47, and 1.49 (each 3H, s, CH_3), 1.65, 1.92, 2.20, and 2.45 (each 2H, m, CH_2 in camphanoyl group), 2.25 (3H, s, CH_3-6), 2.98 (3H, s, CH_3-4), 5.39 (1H, d, J = 4.8 Hz, H-3′), 6.09 (1H, s, H-3), 6.65 (1H, d, J = 4.8 Hz, H-4′), and 7.37 (1H, s, H-5); 86% d.e.; $[\alpha]_D$ −2.00° (*c* 0.50, $CHCl_3$). Anal. ($C_{36}H_{42}O_{11}\cdot H_2O$) C, H.

(3′*R*,4′*R*)-3-Chloro-3′,4′-di-*O*-(*S*)-camphanoyl-4-methyl-(+)-*cis*-khellactone (5): yield 31% (starting with 69 mg of **16e**); white solid; mp 203−4 °C; MS *m*/*z* (%) 670 (M^+, 10), 672 (M + 2, 3); ^{1}H NMR δ 0.99−1.13 (15H, ms, 5 × CH_3), 1.28, 1.46, and 1.49 (each 3H, s, CH_3), 1.72, 1.94, 2.23, and 2.52 (each 2H, m, CH_2 in camphanoyl group), 2.64 (3H, s, CH_3-4), 5.38 (1H, d, J = 4.8 Hz, H-3′), 6.60 (1H, d, J = 4.8 Hz, H-4′), 6.85 (1H, d, J = 8.8 Hz, H-6), and 7.56 (1H, d, J = 8.8 Hz, H-5); 76% d.e.; $[\alpha]_D$ −343.2° (*c* 0.50, $CHCl_3$). Anal. ($C_{35}H_{39}O_{11}Cl$).

(3′*R*,4′*R*)-3′,4′-Di-*O*-(*S*)-camphanoyl-4-methyl-3-phenyl-(+)-*cis*-khellactone (6): yield 61% (starting with 140 mg of **16f**); white solid; mp 157−9 °C; ^{1}H NMR δ 0.96−1.15 (15H, ms, 5 × CH_3), 1.27, 1.47, and 1.52 (each 3H, s, CH_3), 1.71, 1.92, 2.20, and 2.52 (each 2H, m, CH_2 in camphanoyl group), 2.29 (3H, s, CH_3-4), 5.44 (1H, d, J = 4.8 Hz, H-3′), 6.70 (1H, d, J = 4.8 Hz, H-4′), 6.88 (1H, d, J = 8.8 Hz, H-6), 7.44 (5H, m, ArH-3) and 7.62 (1H, d, J = 8.8 Hz, H-5); 87% d.e.; $[\alpha]_D$ +20.08° (*c* 2.58, $CHCl_3$). Anal. ($C_{41}H_{44}O_{11}\cdot {}^1/_2H_2O$) C, H.

(3′*R*,4′*R*)-3′,4′-Di-*O*-(*S*)-camphanoyl-3,4-tetrahydrobenzo-(+)-*cis*-khellactone (7): yield 37% (starting with 141 mg of **16g**); white solid; mp 160−3 °C; ^{1}H NMR δ 0.96−1.12 (15H, ms, 5 × CH_3), 1.29, 1.44, and 1.48 (each 3H, s, CH_3), 1.70, 1.92, 2.24, and 2.52 (each 2H, m, CH_2 in camphanoyl group), 1.80 (4H, m, 2 × CH_2), 2.54 (2H, m, CH_2), 2.72 (2H, m, CH_2), 5.39 (1H, d, J = 4.8 Hz, H-3′), 6.64 (1H, d, J = 4.8 Hz, H-4′), 6.82 (1H, d, J = 8.8 Hz, H-6), and 7.50 (1H, d, J = 8.8 Hz, H-5); 75% d.e.; $[\alpha]_D$ +9.00° (*c* 2.29, $CHCl_3$). Anal. ($C_{38}H_{44}O_{11}\cdot {}^1/_2H_2O$) C, H.

(3′*R*,4′*R*)-3′,4′-Di-*O*-(*S*)-camphanoyl-5-methoxy-4-methyl-(+)-*cis*-khellactone (8): yield 44% (starting with 125 mg of **16h**); mp 144−6 °C; UV λ_{max} nm (ϵ) 318 (7215), 257 (5225), 248 (5135), 215 (13440); ^{1}H NMR δ 0.98−1.14 (15H, ms, 5 × CH_3), 1.44, 1.57 and 2.14 (each 3H, s, CH_3), 1.69, 1.97, 2.17, and 2.52 (each 2H, m, CH_2 in camphanoyl group), 2.64 (3H, s, CH_3-4), 3.89 (3H, s, CH_3O-5), 5.36 (1H, d, J = 4.8 Hz, H-3′), 5.95 (1H, s, H-3), 6.27 (1H, s, H-6), 6.58 (1H, d, J = 4.8 Hz, H-4′); 90% d.e.; $[\alpha]_D$ −9.62° (*c* 0.52, $CHCl_3$). Anal. ($C_{36}H_{42}O_{12}\cdot {}^1/_2H_2O$) C, H.

(3′*R*,4′*R*)-3′,4′-Di-*O*-(*S*)-camphanoyl-4-isopropyl-5-methyl-(+)-*cis*-khellactone (9): yield 36% (starting with 110 mg of **16i**); white solid; mp 218−20 °C; UV λ_{max} nm (ϵ) 300 (6392), 208 (14875); ^{1}H NMR δ 0.96−1.15 and 1.27−1.30 (18H, ms, 6 × CH_3), 1.50 and 1.57 (each 3H, s, CH_3), 1.69, 1.94, 2.19, and 2.49 (each 2H, m, CH_2 in camphanoyl group), 1.12 and 1.13 (each 3H, d, J = 6.6 Hz, $CH(CH_3)_2$-4), 2.24 (3H, s, CH_3-5), 3.12 (1H, m, J = 6.6 Hz, $CH(CH_3)_2$-4), 5.39 (1H, d, J = 4.8 Hz, H-3′), 6.28 (1H, s, H-3), 6.40 (1H, d, J = 4.8 Hz, H-4′), 6.86 (1H, s, H-6); 95% d.e.; $[\alpha]_D$ −54.74° (*c* 0.38, $CHCl_3$). Anal. ($C_{38}H_{46}O_{11}$) C, H.

5,7-Dihydroxy-2,2-dimethyl-4-chromanone (17). To a mixture of 1,3,5-benzenetriol dihydrate (6.48 g, 40 mmol) and 3,3-dimethylacrylic acid (4.80 g, 48 mmol) was added 20 mL of $BF_3\cdot Et_2O$ at room temperature, and the mixture was heated to 70 °C for 2.5 h, when the solution color changed to orange from light yellow. After cooling to room temperature, the reaction mixture was poured into ice−water and 10% aq KOH added to pH 10. The solution was washed with EtOAc three times. Then the aqueous solution was acidified by 10% aq HCl and stirred for 10 min. The resulting precipitate was collected by filtration, and washed with water until neutral. After drying, 6.90 g of **17** was obtained: 83% yield; mp 189−90 °C; ^{1}H NMR δ 1.46 (6H, s, 2 × CH_3-2), 2.70 (2H, s, CH_2-3), 5.37 (1H, br, OH-7), 5.88 and 5.94 (each 1H, d, J = 2.1 Hz, ArH-6 and ArH-8), 12.04 (1H, s, OH-5, chelating with carbonyl at C-4).

7-Benzyloxy-2,2-dimethyl-5-hydroxy-4-chromanone (18). A mixture of compound **17** (624 mg, 3 mmol), benzyl bromide (0.37 mL, 3 mmol), and K_2CO_3 (828 mg, 6 mmol) was refluxed in acetone (20 mL) for 2 h. The solid was filtered, solvent was removed, and the residue was recrystallized from 90% EtOH to give white needle crystals of **18:** 644 mg, 72%; mp 131−2 °C; ^{1}H NMR δ 1.47 (6H, s, 2 × CH_3-2), 2.70 (2H, s, CH_2-3), 5.07 (2H, s, CH_2Ph-7), 6.02 and 6.10 (each 1H, d, J = 2.1 Hz, ArH-6 and ArH-8), 7.39 (5H, m, PhH-7), 12.02 (1H, s, OH-5, chelating with carbonyl at C-4).

2,2-Dimethyl-5-hydroxy-7-methoxy-4-chromanone (19). A mixture of **17** (5.20 g, 25.0 mmol), K_2CO_3 (16.91 g, 50.0 mmol), and MeI (2.34 mL, 35 mmol) in acetone (50 mL) was refluxed for 1 h. The reaction mixture was filtered and solvent was removed in vacuo. The residue was extracted with hexane several times. The hexane was removed to give **19**: 4.11 g, 74%; mp 71−3 °C; ^{1}H NMR δ 1.47 (6H, s, 2 × CH_3-2), 2.70 (2H, s, CH_2-3), 3.82 (3H, s, CH_3O), 5.95 and 6.02 (each 1H, d, J = 2.1 Hz, ArH-6 and ArH-8), 12.03 (1H, s, OH-5, chelating with carbonyl at C-4).

5-Benzyloxy-4-methyl-3′,4′-dihydroseselin (20). Compound **18** (298 mg, 1 mmol) in THF (15 mL) was added dropwise to $NaBH_4$ (140 mg, 5 mmol) in THF (5 mL), then 1 mL of 10% aq KOH was added to completely solubilize the $NaBH_4$. The mixture was refluxed for 24 h. After cooling to room temperature, the reaction mixture was poured into ice−water, acidified with 10% aq HCl to neutral, and extracted with Et_2O three times. The organic phase was washed with brine, and dried over anhydrous Na_2SO_4. After removal of the solvent, crude product was dissolved in anhydrous CH_2Cl_2 (8 mL), and ethyl acetoacetate (0.8 mL, 0.67 mmol) and $BF_3\cdot Et_2O$

were added under N_2 protection with stirring at room temperature. After 24 h, the reaction was complete as monitored by TLC. The mixture was poured into ice−water and extracted with CH_2Cl_2 three times. The organic phase was washed with water, brine, and dried over anhydrous Na_2SO_4. The solvent was removed and the residue was purified by TLC to give **20:** 193 mg, 55%; mp 137−9 °C; ^{1}H NMR δ 1.37 (6H, s, 2 × CH_3-2′), 1.84 (2H, t, J = 6.6 Hz, CH_2-3′), 2.49 (3H, s, CH_3-4), 2.83 (2H, t, J = 6.6 Hz, CH_2-4′), 5.05 (2H, s, OCH_2-7), 5.94 (1H, s, H-3), 6.33 (1H, s, H-6), 7.39 (5H, m, ArH-7).

5-Methoxy-4-methyl-3′,4′-dihydroseselin (21). To a mixture of **19** (4.44 g, 20.0 mmol) in 10% aq KOH (100 mL) and toluene (50 mL) was added $NaBH_4$ (3.78 g, 20.0 mmol) and the solution refluxed for 4 h. The subsequent procedure was the same as in the preparation of **20. 21**: 2.62 g, 48%; mp 174−5 °C; ^{1}H NMR δ 1.38 (6H, s, 2 × CH_3-2′), 1.85 (2H, t, J = 6.6 Hz, CH_2-3′), 2.55 (3H, s, CH_3-4), 2.83 (2H, t, J = 6.6 Hz, CH_2-4′), 3.83 (3H, s, OCH_3), 5.94 (1H, s, H-3), 6.23 (1H, s, H-6).

5-Benzyloxy-4-methylseselin (22). A solution of **20** (100 mg, 0.30 mmol) in 1,4-dioxane (12 mL) was added to a solution of DDQ (160 mg, 0.7 mmol) in anhydrous 1,4-dioxane (12 mL) over 1 h under a nitrogen atmosphere at room temperature. The darkened reaction mixture was then heated to reflux for 24 h and solvent removed in vacuo. The residue was dissolved in CH_2Cl_2 and passed through a short column of Florisil (60−100 mesh), then washed with CH_2Cl_2 to obtain **22:** 70 mg, 67%; mp 153−4 °C; ^{1}H NMR δ 1.47 (6H, s, 2 × CH_3-2′), 2.47 (3H, s, CH_3-4), 5.09 (2H, s, OCH_2-7), 5.58 (1H, d, J = 10 Hz, H-3′), 5.93 (1H, s, H-3), 6.36 (1H, s, H-6), 6.85 (1H, d, J = 10 Hz, H-4′), 7.42 (5H, m, ArH-7).

5-Methoxy-4-methylseselin (16h). The procedure was identical to that used for the preparation of **22:** yield 48% (starting with 4.45 g of **21**); mp 174−5 °C.

(3′*R*,4′*R*)-5-Benzyloxy-3′,4′-di-*O*-(*S*)-camphanoyl-4-methyl-(+)-*cis*-khellactone (10). The procedure was the same as for general preparation of other DCK analogues: yield 56% (starting with 76 mg of **22**); mp 111−3 °C; ^{1}H NMR δ 0.98−1.16 (15H, ms, 5 × CH_3), 1.27, 1.44 and 1.50 (each 3H, s, CH_3), 1.71, 1.94, 2.17, and 2.48 (each 2H, m, CH_2 in camphanoyl group), 2.47 (3H, s, CH_3-4), 5.11 (2H, s, CH_2O-5), 5.36 (1H, d, J = 4.8 Hz, H-3′), 5.94 (1H, s, H-3), 6.36 (1H, s, H-6), 6.59 (1H, d, J = 4.8 Hz, H-4′), 7.42 (5H, m, ArH-5); 78% d.e.; $[\alpha]_D$ −18.13° (*c* 0.75, $CHCl_3$). Anal. ($C_{42}H_{46}O_{12}\cdot{}^1/_2H_2O$) C, H.

HIV Growth Inhibition Assay in H9 Lymphocytes. The T cell line, H9, was maintained in continuous culture with complete medium (RPMI 1640 with 10% fetal calf serum (FCS) supplemented with L-glutamine) at 5% CO_2 and 37 °C. Aliquots of this cell line were used in experiments only when in log-phase of growth. Test samples were first dissolved in dimethyl sulfoxide (DMSO). The following were the final drug concentrations routinely used for screening: 100, 20, 4 and 0.8 μg/mL, but for active agents additional dilutions were prepared for subsequent testing so that an accurate EC_{50} value could be achieved. As the test samples were being prepared, an aliquot of the T cell line, H9, was infected with HIV-1 (IIIB isolate) while another aliquot was mock-infected with complete medium. The mock-infected was used for toxicity determinations (IC_{50}). The stock virus used for these studies typically had a $TCID_{50}$ value of 10^4 infectious units/mL. The appropriate amount of virus for a multiplicity of infection (moi) between 0.1 and 0.01 infectious units/cell was added to the first aliquot of H9 cells. The other aliquot of H9 cells only received culture medium and then was incubated under identical conditions as the HIV-infected H9 cells. After a 4-h incubation at 37 °C and 5% CO_2, both cell populations were washed 3 times with fresh medium and then added to the appropriate wells of a 24-well plate containing the various concentrations of the test drug or culture medium (positive infected control/negative drug control). In addition, AZT was also assayed during each experiment as a positive drug control. The plates were incubated at 37 °C and 5% CO_2 for 4 days. Cell-free supernatants were collected on day 4 for use in our in-house p24 antigen ELISA assay. p24 antigen is a core protein of HIV and therefore is an indirect measure of virus present in the supernatants. Toxicity was determined by performing cell counts on a Coulter counter on the mock-infected H9 lymphocytes, which had either received culture medium (no toxicity) or test sample or AZT.

X-ray Crystal Structure Analysis of 4-Methyl-(3′*R*,4′*R*)-3′,4′-di-*O*-(*S*)-camphanoyl-(+)-*cis*-khellactone. Crystal data: $C_{35}H_{40}O_{11}$, mol wt = 636.70, hexagonal, space group $P6_5$-(C_6^3) No. 170, $a = b = 13.748(1)$ Å, $c = 29.990(4)$ Å, $\alpha = 90.0(-)°$, $\beta = 90.0(-)°$, $\gamma = 120.0(-)°$, $V = 4909(1)$ Å^3, $Z = 6$, $D_{calcd} = 1.292$ g cm^{-3}, μ (Cu Kα radiation, $\lambda = 1.5418$ Å) = 7.6 cm^{-1}, crystal dimensions = 0.40 × 0.40 × 0.60 mm.

All measurements were made at 298 K on an Enraf-Nonius CAD-4 diffractometer (Cu Kα radiation, graphite monochromator). Laue symmetry and systematic absences (00*l* when $l \neq 6n$) indicated that the space group was $P6_1$ or $P6_5$; the latter alternative was shown during the course of the analysis to be the correct choice (vide infra). Refined unit cell parameters were calculated from the diffractometer setting angles for 25 reflections ($35° < \theta < 40°$) widely separated in reciprocal space. Intensity data (3421 nonequivalent $+h,+k,-l$ reflections), recorded by means of $\theta-2\theta$ scans (scan width $(0.80 + 0.14 \tan \theta)°$; $\theta_{max} = 75°$), yielded 2897 reflections with $I > 2.0\sigma(I)$ for use in the structure analysis and parameter refinement. The intensities of four reference reflections, monitored every 2 h during data collection, showed no significant variation (<1% overall). The data were corrected for the usual Lorentz and polarization effects.

The crystal structure was solved by direct methods. Approximate coordinates for all non-hydrogen atoms were obtained from an *E*-map. Of the two alternative space goup choices, $P6_5$ gave the enantiomer consistent with the known absolute stereochemistry of the camphanoyl moiety. Positional and temperature factor parameters (first isotropic and then anisotropic) were adjusted by means of several rounds of full-matrix least-squares calculations during which $\Sigma w\Delta^2$ [$w = 1/\sigma^2|F_o|$, $\Delta = (|F_o| - |F_c|)$] was minimized. Hydrogen atom positional and isotropic thermal parameteres were also refined during the later least-squares iterations and an extinction correction (g) was included as a variable. The parameter refinement converged (maximum shift esd = 0.03) at $R = \Sigma(||F_o| - |F_c||/|F_o|) = 0.030$, $R_w = [\Sigma w(|F_o| - |F_c|)^2/\Sigma w|F_o|^2]^{1/2} = 0.040$, GOF = $[\Sigma w(|F_o| - |F_c|)^2/(N_{observations} - N_{parameters})]^{1/2} = 1.27$. A final difference Fourier synthesis contained no unusual features [$\Delta\rho$ (e/Å^3) = 0.14 (maximum), −0.12 (minimum)].

Crystallographic calculations were performed by use of the Enraf-Nonius Structure Determination Package (SDP 3.0). For all structure factor calculations, neutral atom scattering factors and their anomalous scattering corrections were taken from *International Tables*.[12]

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Supporting Information Available: Tables of fractional atomic coordinates and temperature factor parameters, bond lengths, bond angles, and torsion angles for 4-methyl-(3′*R*,4′*R*)-3′,4′-di-*O*-(*S*)-camphanoyl-(+)-*cis*-khellactone (4-methyl DCK). This material is available free of charge via the Internet at http://pubs.acs.org.

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