Anti-AIDS Agents. 37.[1] Synthesis and Structure−Activity Relationships of (3′*R*,4′*R*)-(+)-*cis*-Khellactone Derivatives as Novel Potent Anti-HIV Agents

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To explore the structural requirements of (+)-*cis*-khellactone derivatives as novel anti-HIV agents, 24 monosubstituted 3′,4′-di-*O*-(*S*)-camphanoyl-(+)-*cis*-khellactone (DCK) derivatives were synthesized asymmetrically. These compounds included 4 isomeric monomethoxy analogues (**3**−**6**), 4 isomeric monomethyl analogues (**7**−**10**), 4 4-alkyl/aryl-substituted analogues (**11**−**14**), and 12 4-methyl-(+)-*cis*-khellactone derivatives (**15**−**26**) with varying 3′,4′-substituents. These (+)-*cis*-khellactone derivatives were screened against HIV-1 replication in acutely infected H9 lymphocytes. The results demonstrated that the (3′*R*,4′*R*)-(+)-*cis*-khellactone skeleton, two (*S*)-(−)-camphanoyl groups at the 3′- and 4′-positions, and a methyl group on the coumarin ring, except at the 6-position, were optimal structural moieties for anti-HIV activity. 3-Methyl- (**7**), 4-methyl- (**8**), and 5-methyl- (**9**) 3′,4′-di-*O*-(*S*)-camphanoyl-(3′*R*,4′*R*)-(+)-*cis*-khellactone showed EC_{50} and therapeutic index values of $<5.25 \times 10^{-5}$ μM and $>2.15 \times 10^{6}$, respectively, in H9 lymphocytes, which are much better than those of DCK and AZT in the same assay. Furthermore, **8** and **9** also showed potent inhibitory activity against HIV-1 replication in the CEM-SS cell line, and most monosubstituted DCK analogues were less toxic than DCK in both assays.

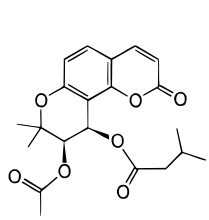
Introduction

Acquired immunodeficiency syndrome (AIDS), which is caused by the human immunodeficiency virus (HIV),[2,3] is now spreading rapidly among many populations and has become a serious global threat to human health and life. Progress in HIV biology has provided detailed knowledge of molecular events in the replication cycle of HIV-1. Such knowledge is required to develop effective antiviral agents and strategies aimed at eliminating HIV replication.[4] Current understanding of molecular events in the HIV life cycle proposes seven steps: viral entry, reverse transcription, integration, gene expression, assembly, budding, and maturation. In theory, every stage in the viral life cycle could serve as a potential target for designing anti-HIV agents and therapies.[5,6]

Thirteen inhibitors of reverse transcriptase and protease have been approved by the FDA[7] and are currently used alone or as a part of a combination regimen to treat HIV infection in AIDS patients. These drugs can effectively suppress HIV replication, thus delaying disease progress and prolonging patients' survival. However, all these drugs have limited or transient clinical benefits in HIV-infected individuals due to rapid development of HIV resistance,[8−13] adverse side effects, and/or toxicity. Therefore, many research approaches are still underway to discover diverse anti-HIV agents with novel structures or mechanism(s) of action.

Our research approach is to discover novel plant-derived natural products as new lead compounds for potential anti-HIV agents, and to modify these compounds to find still more potent anti-HIV agents. In our previous bioactivity-directed search for plant-derived naturally occurring compounds, suksdorfin (**1**)[14−17] (Chart 1) was isolated from the fruit of *Lomatium suksdorfii* and identified as (3′*R*,4′*R*)-3′-acetoxy-4′-isovaleryloxy-(+)-*cis*-khellactone. Suksdorfin inhibited HIV-1 replication in H9 lymphocytes with an in vitro EC_{50} value of 1.3 μM and a therapeutic index value of >40. The discovery of suksdorfin (**1**) led to the syntheses of 42 khellactone derivatives by modification of **1**.[18] Among these synthetic compounds, the most promising lead compound was 3′,4′-di-*O*-(*S*)-(−)-camphanoyl-(3′*R*,4′*R*)-(+)-*cis*-khellactone (DCK) (**2**), which showed extremely potent inhibitory activity ($EC_{50} = 2.56 \times 10^{-4}$ μM) against HIV-1 replication in the H9 cell line and had a remarkable therapeutic index (TI = 136719). In comparison, the values of AZT in the same assay were 0.045 μM and 41667. However, three diastereoisomers of **2** with (3′*S*,4′*S*), (3′*R*,4′*S*), and (3′*S*,4′*R*) configurations were at least 10000 times less active.[19] Moreover, other synthetic racemic khellactone derivatives with different *O*-acyl or *O*-alkyl groups at the 3′- and 4′-positions were inactive or were active only at much higher concentra-

Chart 1. Suksdorfin (**1**) and DCK (**2**)



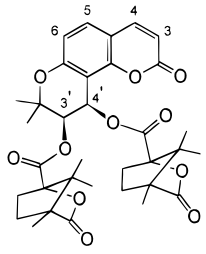


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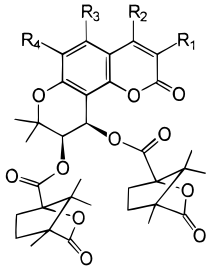
Chart 2. DCK Analogues **3**−**14**



3 $R_1 = OCH_3$ $R_2 = R_3 = R_4 = H$
4 $R_2 = OCH_3$ $R_1 = R_3 = R_4 = H$
5 $R_3 = OCH_3$ $R_1 = R_2 = R_4 = H$
6 $R_4 = OCH_3$ $R_1 = R_2 = R_3 = H$
7 $R_1 = CH_3$ $R_2 = R_3 = R_4 = H$
8 $R_2 = CH_3$ $R_1 = R_3 = R_4 = H$
9 $R_3 = CH_3$ $R_1 = R_2 = R_4 = H$
10 $R_4 = CH_3$ $R_1 = R_2 = R_3 = H$
11 $R_2 = CH_2CH_2CH_3$ $R_1 = R_3 = R_4 = H$
12 $R_2 = CH(CH_3)_2$ $R_1 = R_3 = R_4 = H$
13 $R_2 = C_6H_5$ $R_1 = R_3 = R_4 = H$
14 $R_2 = CF_3$ $R_1 = R_3 = R_4 = H$

tions in the same assay. The anti-HIV activity of DCK was further confirmed by assays in the U937 cell line and PHA-stimulated peripheral blood mononuclear cells (PBMCs). In addition, DCK specifically inhibited the replication of the HIV-1 strain, not the HIV-2 strain. Furthermore, the preliminary mechanism studies showed that DCK did not inhibit reverse transcriptase, protease, or the fusion process. Therefore, DCK might have a novel mechanism of action, and its anti-HIV activity is highly stereospecific. These previous results strongly encouraged us to prepare additional derivatives of DCK by using asymmetric synthesis techniques and study the structure−activity relationships for this compound type in order to find a more active and less toxic anti-HIV agent.

Design

Based on the structure of DCK (**2**) (Chart 1), we know that the khellactone nucleus and *R* absolute configurations of the 3′- and 4′-carbons are essential for the anti-HIV activity of DCK, while bulky substitutions at these two positions may also be key contributors. These structural motifs determine the three-dimensional orientation of DCK and the basic size of the molecule. However, in principle, various substituents located on the boundary of the molecular skeleton should directly influence molecular biological activity. They modify molecular size, shape, and active surface sites and can change some pharmacological properties, such as solubility, stability, toxicity, and affinity with target receptor/enzyme. Thus, the modification of DCK reported in this paper focuses on introducing additional substituents and/or changing boundary substituents on the (+)-*cis*-khellactone skeleton of DCK.

We previously explored the substitution position on the khellactone nucleus by preparing four monomethoxy DCK analogues, with the methoxy at the 3-, 4-, 5-, or 6-position on the coumarin ring (**3**−**6**),[20] respectively. Then, 3-methyl (**7**), 4-methyl (**8**), 5-methyl (**9**), and 6-methyl (**10**) DCK analogues were also synthesized[21] to investigate the impact of a different substituent at these positions (Chart 2). After reviewing bioassay results, we have now introduced different alkyl groups, i.e., propyl, isopropyl, phenyl, and trifluoromethyl, at the 4-position of the coumarin nucleus (a beneficial position) to give 4-substituted DCK analogues (**11**−**14**). Finally, we designed a series of 4-methyl-(+)-*cis*-khellactone derivatives (**15**−**26**) (Chart 3). Acyl groups with various volumes (bulky/small) replaced the two (*S*)-(−)-camphanoyl groups at the 3′- and 4′-positions in order to confirm the uniqueness of these groups for anti-HIV activity.

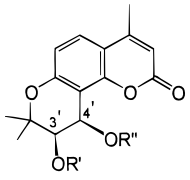
For all designed target compounds (**3**−**26**), our synthetic strategy was to (a) prepare variously substituted 7-hydroxy coumarins (**I**) from 1,3-dihydroxybenzene and its substituted derivatives, (b) produce substituted seselins (**II**), (c) asymmetrically synthesize substituted (+)-*cis*-khellactones with required 3′*R*,4′*R* configurations (**III**), and (d) finally, produce several monosubstituted (+)-*cis*-khellactone derivatives (Scheme 1). All target compounds were tested against HIV-1 replication in acutely infected H9 lymphocytes. At the same time, these synthetic compounds were also assayed at NCI against HIV-1 replication in the CEM-SS cell line.

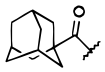
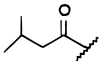
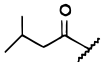
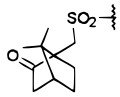
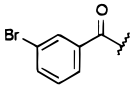
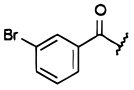
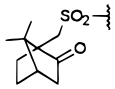
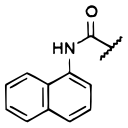
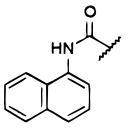
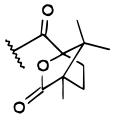
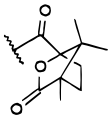
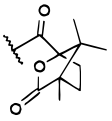
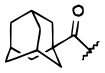
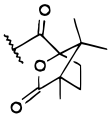
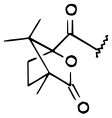
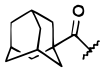
Chemistry

The syntheses of **3**−**10** were previously described, but without the full experimental details presented herein. Scheme 2 shows the syntheses of monomethoxylated 7-hydroxycoumarins (**27**−**29**). Commercially available 2,4-dihydroxybenzaldehyde (**51**) was treated with methoxyacetyl chloride in dimethylformamide (DMF) in the presence of sodium methoxyacetate to produce 7-hydroxy-3-methoxycoumarin (**27**). 7-Hydroxy-4-methoxycoumarin (**28**) was synthesized in three steps from 1,3-dihydroxybenzene (**52**). Compound **52** was condensed with cyanoacetic acid in the presence of $ZnCl_2$/HCl to afford 4-amino-7-hydroxycoumarin (**53**) in a 50% yield. Subsequent hydrolysis of **53** in 50% H_2SO_4 then gave 4,7-dihydroxycoumarin (**54**) in a 68% yield.[22] Compound **54** was also synthesized directly by treating **52** with malonic acid in $BF_3 \cdot Et_2O$; however, the 28% yield was lower than that of the former two-step route. Subsequently, **54** was methylated ($MeOH/H_2SO_4$) selectively at the 4-hydroxyl group[23] to give **28**. 7-Hydroxy-5-methoxycoumarin (**29**) was prepared from 2,4,6-trihydroxybenzaldehyde (**55**) via a multistep sequence. Reacting **55** with acetic anhydride in pyridine and CH_2Cl_2 for 30 min produced the expected 2,4-diacetoxy-6-hydroxybenzaldehyde (**56**) because chelation with the carbonyl group hindered reaction of the *ortho*-hydroxyl group. However, if the reaction time was extended to 1 h or more, triacetoxybenzaldehyde was obtained in an 81% yield and could be conveniently converted to **56** by heating in MeOH/Py. Compound **56** then successively underwent a Wittig reaction with $Ph_3P{=}CHCOOMe$ (**57**), methylation of the 6-hydroxyl group with MeI in DMF in the presence of K_2CO_3, cleavage of the 2,4-diacetyl groups, and intramolecular thermal cyclization in xylene to afford 7-hydroxy-5-methoxycoumarin (**29**) in an overall yield of 45%.

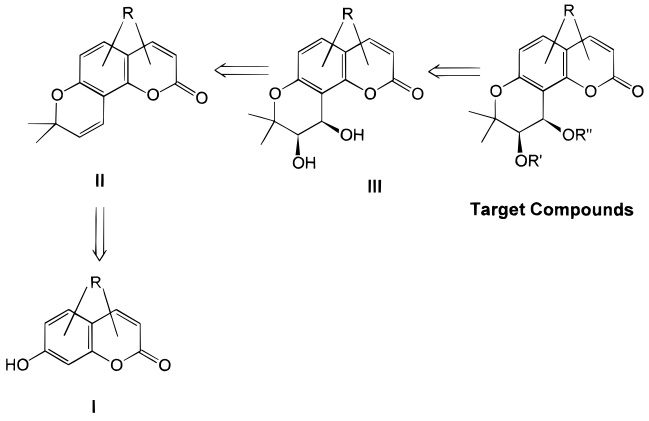
For syntheses of methylated 7-hydroxycoumarins (**31, 33**, and **34**) (Scheme 3), a Wittig reaction and an intramolecular cyclization were used to build the coumarin nucleus from substituted salicylaldehydes (**51, 60,** and **62**).[24,25] Compound **51** was reacted with $Ph_3P{=}CCH_3$-COOMe (**58**) in CH_2Cl_2 at room temperature and then, without purification, heated in xylene to reflux to produce 7-hydroxy-3-methylcoumarin (**31**). 3,5-Dihydroxytoluene (**59**) was reacted with phosphorus oxychloride in excess DMF[26,27] to afford an excellent yield

Chart 3. 4-Methyl-(+)-*cis*-khellactone Derivatives **15**−**26**



	R'	R"		R'	R"
15	Ac	Ac	**21**	H	
16			**22**		H
17			**23**		H
18			**24**	Ac	
19			**25**	Ac	
20	H		**26**		

Scheme 1. Synthetic Strategy to Monosubstituted (+)-*cis*-Khellactone Derivatives



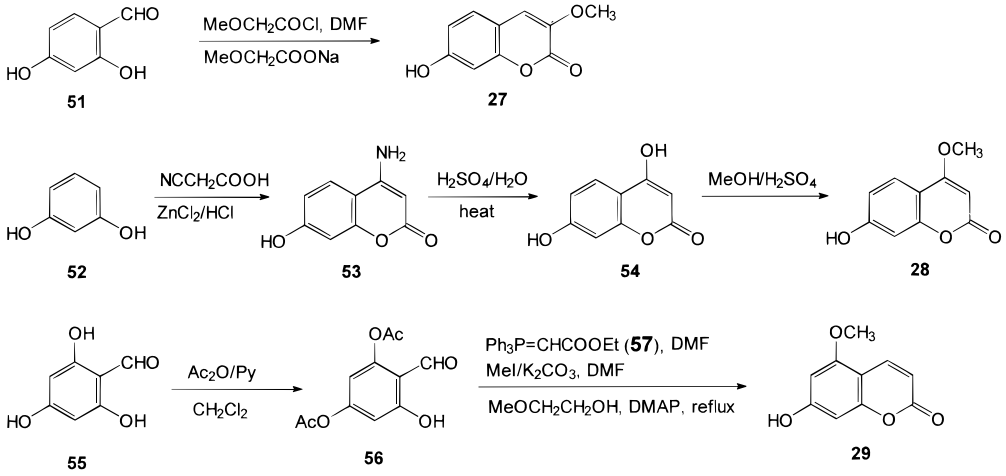
of 2,4-dihydroxy-6-methylbenzaldehyde (**60**). Reaction of DMF with phosphorus oxychloride forms a complex that reacts with nucleophilic aromatic substrates to afford, after hydrolysis of the initial products, the aldehyde. In the same manner, 2,4-dihydroxy-5-methylbenzaldehyde (**62**) was produced from 2,4-dihydroxytoluene (**61**), which was obtained in a 98% yield by reducing **51** with $NaBH_3CN$.[28] Similarly, treating **60** and **62** with $Ph_3P{=}CHCOOMe$ (**57**) followed by thermal cyclization as described above afforded **33** and **34,** respectively.

Scheme 4 shows the syntheses of 4-substituted 7-hydroxycoumarins. Compound **52** was condensed with appropriate β-keto esters in the presence of H_2SO_4[29] to produce **35**−**37**. 7-Hydroxy-6-methoxycoumarin (**30**), 7-hydroxy-4-methylcoumarin (**32**), and 7-hydroxy-4-(trifluoromethyl)-coumarin (**38**) are commercially available.

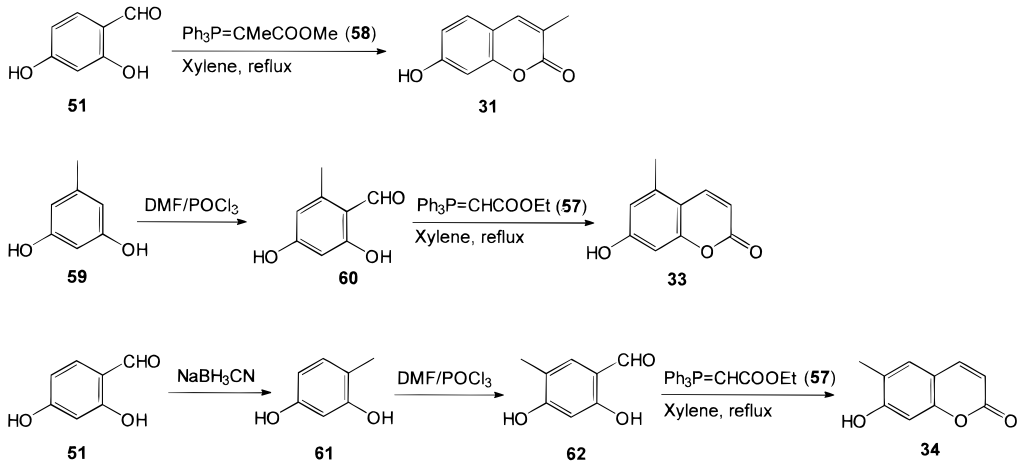
As shown in Scheme 5, substituted 7-hydroxycoumarin derivatives (**27**−**37**) were reacted with 3-chloro-3-methyl-1-butyne in DMF in the presence of anhydrous potassium carbonate and potassium iodide to produce the corresponding aryl α,α-dimethylpropargyl ethers following literature methods.[30,31] However, **38** reacted with 3-chloro-3-methyl-1-butyne only in boiling acetone rather than in DMF. In addition, 4-aminoseselin and 4-hydroxyseselin could not be synthesized from compounds **53** and **54**, respectively, under the same conditions in either DMF or acetone. Thermal rearrangement of the ether derivatives of **27**−**38** occurred in boiling diethylaniline to form seselin derivatives **39**−**50**.

As in the asymmetric synthesis of DCK,[32] substituted (+)-*cis*-khellactones were successfully synthesized by using osmium-catalyzed asymmetric dihydroxylation[33−36] of appropriately substituted seselins **39**−**50** in the presence of an enantioselective ligand: hydroquinine 2,5-diphenyl-4,6-pyrimidinediyl diether, $(DHQ)_2$-PYR. Without further purification, the substituted (+)-*cis*-khellactones were acylated with (*S*)-(−)-camphanic chloride in the presence of pyridine in CH_2Cl_2 to produce target compounds **3**−**14**. Most reactions were highly stereoselective (>88%). However, **13** was obtained with

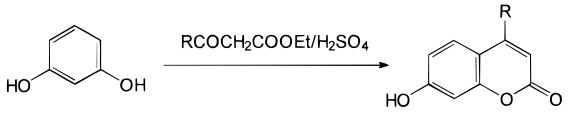
Scheme 2. Syntheses of Methoxy Substituted 7-Hydroxycoumarins (**27**−**29**)



Scheme 3. Syntheses of Methylated 7-Hydroxycoumarins (**31**, **33**, and **34**)



Scheme 4. Syntheses of 4-Substituted 7-Hydroxycoumarins (**35**−**37**)



35 R = $CH_2CH_2CH_3$
36 R = $CH(CH_3)_2$
37 R = C_6H_5

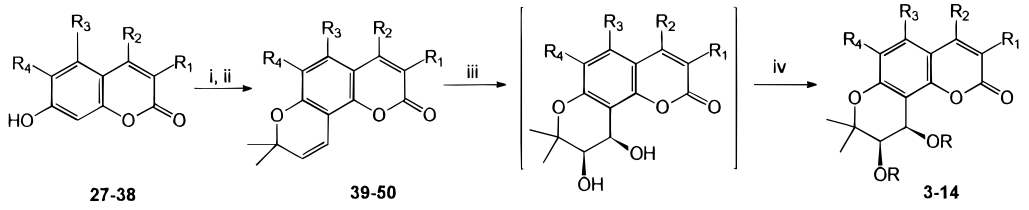
only 65% d.e.; the pair of diastereoisomers was separated by TLC eluted with 40:2 (v/v) CH_2Cl_2/acetone.

4-Methyl-(+)-*cis*-khellactone derivatives (**15**−**26**) were synthesized from 4-methyl-(+)-*cis*-khellactone (**63**) and various acyl chlorides using standard procedures (Scheme 6). When 1-adamantanecarbonyl chloride was the acylating agent, only the monoester **21** was obtained, probably due to the steric hindrance of the bulky substituent *ortho* to a hydroxyl group. The proton NMR spectra of **21** indicated that the adamantanecarbonyl group was at the 4′-position of the (+)-*cis*-khellactone skeleton. Similarly, treating **63** with (1*S*)-(+)- or (1*R*)-(−)-10-camphorsulfonyl chloride gave monoester compounds **22** and **23**, respectively, but both were the 3′-acyl-4′-hydroxy-(+)-*cis*-khellactone derivatives. Subsequent acylation of **21** and **20** with other acylating agents produced **24**−**26**, which have different acyl groups at the 3′- and 4′-positions. In addition, compound **19** with two (*R*)-(+)-camphanoyl groups was also synthesized to investigate how the stereochemistry of the acyl group affects anti-HIV activity.

Results and Discussion

All target compounds **3**−**26** were tested against HIV-1 replication in acutely infected H9 lymphocytes. The data for monosubstituted DCK analogues (**3**−**14**) and 4-methyl-(+)-*cis*-khellactone derivatives (**15**−**26**) are shown in Tables 1 and 2, respectively. 5-Methyl and 4-methyl DCK (**9** and **8**) exhibited extremely high anti-HIV activity in this assay. They had EC_{50} values of 2.39×10^{-7} μM and 1.83×10^{-6} μM and remarkable therapeutic indexes (TI) of $>3.97 \times 10^{8}$ and $>6.89 \times 10^{7}$, respectively. 3-Methyl DCK (**7**) also showed very potent anti-HIV activity with an EC_{50} value of 5.25×10^{-5} μM and a TI value of $>2.15 \times 10^{6}$. The EC_{50} and TI values of **7**, **8**, and **9** were much better than those of DCK and AZT in the same assay. However, 6-methyl DCK (**10**) was much less active ($EC_{50} = 0.151$ μM) and had a lower therapeutic index (218) than DCK. Similar results were observed with the four monomethoxy-substituted DCK isomers (**3**−**6**). 5-Methoxy DCK (**5**) exhibited potent anti-HIV activity with an EC_{50} value of 1.92×10^{-4} μM and a TI value of 7.97×10^{5}, which were slightly better than those of DCK. 3-Methoxy- and 4-methoxy-DCK analogues (**3** and **4**) were also active ($EC_{50} = 2.38 \times 10^{-3}$ and 2.99×10^{-3} μM, TI $> 6.43 \times 10^{4}$ and $> 5.12 \times 10^{4}$).

Scheme 5. Syntheses of DCK Analogues **3**−**14**



27, 39 $R_1 = OCH_3$ $R_2 = R_3 = R_4 = H$
28, 40 $R_2 = OCH_3$ $R_1 = R_3 = R_4 = H$
29, 41 $R_3 = OCH_3$ $R_1 = R_2 = R_4 = H$
30, 42 $R_4 = OCH_3$ $R_1 = R_2 = R_3 = H$
31, 43 $R_1 = CH_3$ $R_2 = R_3 = R_4 = H$
32, 44 $R_2 = CH_3$ $R_1 = R_3 = R_4 = H$
33, 45 $R_3 = CH_3$ $R_1 = R_2 = R_4 = H$
34, 46 $R_4 = CH_3$ $R_1 = R_2 = R_3 = H$
35, 47 $R_2 = CH_2CH_2CH_3$ $R_1 = R_3 = R_4 = H$
36, 48 $R_2 = CH(CH_3)_2$ $R_1 = R_3 = R_4 = H$
37, 49 $R_2 = C_6H_5$ $R_1 = R_3 = R_4 = H$
38, 50 $R_2 = CF_3$ $R_1 = R_3 = R_4 = H$

i. 3-Chloro-3-methyl-1-butyne, KI, K_2CO_3 in DMF or acetone, 70-80 °C
ii. N,N-Diethylaniline, reflux
iii. $K_2OsO_2(OH)_4$, K_2CO_3, $K_3Fe(CN)_6$, $(DHQ)_2$-PYR in *t*-BuOH/H_2O (v/v = 1:1), ice-bath
iv. (*S*)-(-)-Camphanic chloride, pyridine in CH_2Cl_2

R =

Scheme 6. Syntheses of 4-Methyl-(+)-*cis*-khellactone Derivatives **15**−**26**

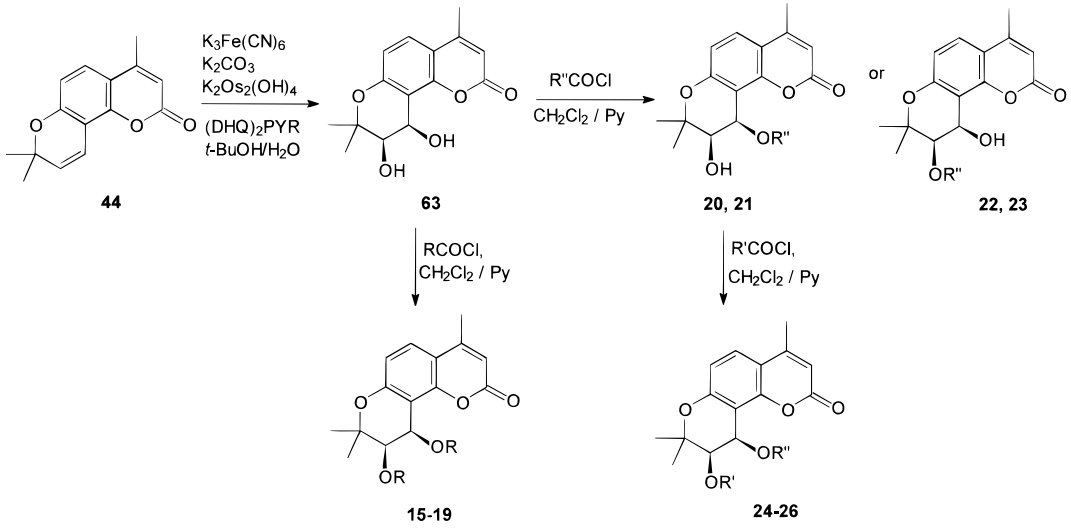


Table 1. Anti-HIV Activity of Monosubstituted DCK Analogues (**3**−**14**)[a]

	H9 lymphocytes[b]			CEM-SS cell line[c]		
compd	IC_{50} (μM)	EC_{50} (μM)	therapeutic index	IC_{50} (μM)	EC_{50} (μM)	therapeutic index
3	>153	2.38×10^{-3}	$>6.43 \times 10^{4}$	16.8	0.494	34
4	>153	2.99×10^{-3}	$>5.12 \times 10^{4}$	>200	0.125	>1600
5	>153	1.92×10^{-4}	$>7.97 \times 10^{5}$	>200	0.128	>1560
6	>153	15.8	>9.68	>200	inactive	
7	>113	5.25×10^{-5}	$>2.15 \times 10^{6}$	>12.5	0.213	>58.7
8	>126	1.83×10^{-6}	$>6.89 \times 10^{7}$	>200	0.0635	>3150
9	>95	2.39×10^{-7}	$>3.97 \times 10^{8}$	>200	0.0635	>3150
10	33	0.151	218	>200	0.376	>532
11	>151	1.75×10^{-2}	$>8.63 \times 10^{3}$	>200	0.763	>262
12	>151	3.15×10^{-2}	$>4.79 \times 10^{3}$	>128	0.584	>219
13	>143	0.12	$>1.19 \times 10^{3}$			
14	>145	1.81	>80.1			
DCK	35	2.56×10^{-4}	1.37×10^{5}	14−26	0.14−0.26	100
AZT	1875	4.50×10^{-2}	4.17×10^{4}	>1	3.8×10^{-3}	>263

[a] All data presented in this table are averages of at least two separate experiments. [b] Assay in H9 lymphocytes was performed by BBI−Biotech Research Laboratories, Inc., Gaithersburg, MD. [c] Assay in the CEM-SS cell line was performed by the National Cancer Institute, Bethesda, MD.

They were more active and more selective than AZT, but less than DCK. Similar to **10**, 6-methoxy-DCK (**6**) was less active with an EC_{50} value of 15.8 μM and a TI value of >9.68. These data demonstrated that introducing a substituent at the 3-, 4-, or 5-position on the coumarin nucleus could greatly enhance the anti-HIV activity of DCK, whereas substitution at the 6-position significantly decreased activity.

Further comparison of **4**, **8**, and **11**−**14** showed that different substituents at the 4-position, a position

Table 2. Anti-HIV Activity of 4-Methyl-(+)-*cis*-khellactone Derivatives (**15**−**26**) in Acutely Infected H9 Lymphocytes[a]

compd	IC_{50} (μM)	EC_{50} (μM)	therapeutic index
15	171	83.06	2.06
16	42	no suppression	
17	>91.6	no suppression	
18	98	no suppression	
19	>157	6.60×10^{-3}	$>2.38 \times 10^4$
20	>219	no suppression	
21	43.2	no suppression	
22	42	no suppression	
23	45	no suppression	
24	123	5.52×10^{-2}	2.23×10^3
25	19	1.14	16.67
26	21.3	0.60	35.5
DCK	35	2.56×10^{-4}	1.37×10^5
AZT	1875	4.50×10^{-2}	4.17×10^4

[a] All data presented in this table are averages of at least two separate experiments.This assay was performed by BBI−Biotech Research Laboratories, Inc., Gaithersburg, MD.

beneficial to activity, resulted in obvious differences in anti-HIV activity. 4-Propyl-DCK (**11**) and 4-isopropyl-DCK (**12**) exhibited potent anti-HIV activity with EC_{50} values of 1.75×10^{-2} and 3.15×10^{-2} μM, respectively, which were slightly better than that of AZT. However, their therapeutic index values were lower than that of AZT. Moreover, 4-phenyl-DCK (**13**) and 4-trifluoromethyl-DCK (**14**) were less active than AZT and DCK. The extremely high anti-HIV activity of **8** indicated that a methyl group on the coumarin ring probably fits well into a hydrophobic cleft on the active surface of its target, greatly increasing the affinity of the agent and the desired pharmacological response. Comparatively, the propyl group has a longer chain than the methyl; the isopropyl group has a branched chain with larger volume, which could interfere with receptor binding; and the phenyl group has an aromatic planar shape quite different from that of the methyl. The low activity of **14** indicated that the trifluoromethyl group is an unsuitable substituent to fit into hydrophobic region, even though the trifluoromethyl group is similar to the methyl group in size and shape. The strong electronegativity of the fluorine atom can make the trifluoromethyl group interact primarily with polar functional groups as a hydrogen bond acceptor. These results demonstrated that the shape and size of the boundary substituents were directly related to molecular activity and that a methyl group was obviously preferable to other substituents.

The bioassay results of 4-methyl-(+)-*cis*-khellactone derivatives (**15**−**26**) (Table 2) provided more information about the anti-HIV structure−activity relationships for this compound type. When the two (−)-camphanoyl groups in DCK were replaced with aromatic acyl groups (see **17** and **18**), the anti-HIV activity was abolished. Compounds **15** and **16** contain two acyl groups with small volumes at the 3′- and 4′-position and were also inactive. Moreover, the 4-methyl-mono-acyl-(+)-*cis*-khellactone derivatives **20**−**23** did not suppress HIV-1 replication in H9 lymphocytes, even though all contain one bulky acyl group. More interestingly, when the inactive monoester compounds **20** and **21** were further acylated, the corresponding diester compounds **24**−**26**, each with a pair of different acyl groups, did exhibit anti-HIV activity with EC_{50} values of 0.0552, 1.14, and 0.60 μM, respectively. Compound **19**, which contains two 3′,4′-di-*O*-(*R*)-camphanoyl substitutions, still showed potent anti-HIV activity with an EC_{50} value of 6.60×10^{-3} μM and a therapeutic index value of $> 2.38 \times 10^4$, which are better than those of AZT but slightly less than those of DCK. This fact indicated that the volume, size, and shape of the camphanoyl group are more important than the absolute configuration of its chiral carbon.

Furthermore, compounds **3**−**12** were also tested against HIV-1 replication in the CEM-SS cell lines[37,38] and most exhibited potent anti-HIV activity, except for **6**, and low toxicity, except for **3** and **7** (Table 1). These results were basically consistent with those in the H9 cell line. In the CEM-SS assay, **8** and **9** were more active (both had EC_{50} values of 0.0635 μM and TI values of >3150) than DCK (EC_{50} = 0.14−0.26 μM, TI 100) and much more selective than AZT ($EC_{50} = 3.8 \times 10^{-3}$ μM, TI > 263). Compounds **4** and **5** had TI values of >1600 and >1560, respectively, which were higher than that of AZT.

In conclusion, this study provided the following results. (1) The 3-, 4-, and 5-positions on the coumain nucleus are favorable for modification by introducing an appropriate alkyl or *O*-alkyl substituent. (2) Different substituents at the same favorable position widely affect anti-HIV activity. (3) A methyl substituent is preferable to other alkyl groups. (4) Two (*S*)-(−)-camphanoyl groups at the 3′- and 4′-positions are functionally and structurally optimal for anti-HIV activity of DCK and its analogues. (5) Most monosubstituted DCK analogues, except **3**, **7**, and **10**, showed decreased cellular toxicity, compared with DCK in both assays.

Among our target compounds, 4-methyl DCK (**8**) and 5-methyl DCK (**9**) were the most promising compounds in both assays, but **5** and **7** were also favorable in acutely infected H9 lymphocytes. These initial results indicate that (+)-*cis*-khellactone diester derivatives are novel potent anti-HIV agents. Currently, active compounds are tested in additional in vitro assays using different HIV-1 virus strains and cell lines. Further modification of DCK and studies on the mechanism of action for this compound type are under investigation.

Experimental Section

Chemistry. Melting points were measured with a Fisher Johns melting apparatus without correction. The proton nuclear magnetic resonance (^{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. Elementary analysis was 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 intensity of protons at the 3′-position in the ^{1}H NMR spectra. Thin-layer chromatography (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.

7-Hydroxy-3-methoxycoumarin (27). Sodium methoxyacetate (4.48 g, 40.0 mmol), which was prepared from methoxy acetic acid and NaOH in EtOH, was dissolved in 20 mL of DMF. Then, methoxyacetyl chloride (2.20 mL, 24.0 mmol) was added dropwise to the solution at 0 °C. The mixture was stirred at room temperature for 10 min, 1.11 g (8.0 mmol) of 2,4-dihydroxybenzaldehyde was added, and the solution refluxed for 3 h. After cooling to room temperature, the reaction mixture was extracted with EtOAc. The organic phase was washed

with 10% aqueous HCl, water, and brine, and dried over anhydrous $MgSO_4$. The solvent was removed in vacuo, and the residue was purified by column chromatography (SiO_2, EtOAc/hexane = 2:1) to give **27** (0.67 g, 44%) as yellow needles: mp 256−8 °C (lit. 257−8 °C); ^{1}H NMR (DMSO-d_6) δ 3.39 (3H, s, CH_3O-3), 6.70 (1H, d, J = 2.2 Hz, H-8), 6.77 (1H, dd, J = 2.2 and 9.6 Hz, H-6), 7.27 (1H, s, H-4), 7.42 (1H, d, J = 9.6 Hz, H-5).

4-Amino-7-hydroxycoumarin (53). To a solution of resorcinol (**52**) (1.10 g, 10 mmol) and cyanoacetic acid (850 mg, 10 mmol) in 10 mL of anhydrous Et_2O was added powdered zinc chloride (500 mg) under N_2. A very rapid stream of dry HCl was immediately passed through the mixture for 2 h. A white solid formed, water was added to the residue, and the mixture was left to stand overnight. The solid was filtered, washed with water, and dried to obtain 982 mg of **53** in a 66% yield: mp 340 °C (lit.); ^{1}H NMR (DMSO-d_6) δ 5.01 (1H, s, H-3), 6.59 (1H, d, J = 2.4 Hz, H-8), 6.70 (1H, dd, J = 8.4 and 2.4 Hz, H-6), 7.20 (2H, br, NH_2, disappeared in D_2O), 7.79 (1H, d, J = 8.4 Hz, H-5), 10.37 (1H, s, OH-7, disappeared in D_2O).

4,7-Dihydroxycoumarin (54). Method 1. Compound **53** (825 mg, 4.66 mmol) in 20 mL of 50% aqueous H_2SO_4 was hydrolyzed by heating (water bath) for 4 h. First, the solution cleared, then solid appeared. The solid was filtered, washed with water until neutral, and dried to obtain 562 mg of 4,7-dihydroxycoumarin (**54**) in a 68% yield. Recrystallization from hot water or EtOH/H_2O (1:2) gave white long needle crystals: mp 260−2 °C (lit. 260 °C); ^{1}H NMR (DMSO-d_6) δ 5.39 (1H, s, H-3), 6.67 (1H, d, J = 2.4 Hz, H-8), 6.77 (1H, dd, J = 8.4 and 2.4 Hz, H-6), 7.77 (1H, d, J = 8.4 Hz, H-5), 10.53 (1H, s, OH-7, disappeared in D_2O), and 12.24 (1H, s, OH-7, disappeared in D_2O).

Method 2. A mixture of 1,3-dihydroxybenzene (**52**) (4.40 g, 40.0 mmol), malonic acid (12.48 g, 120 mmol), and BF_3·Et_2O (20 mL) was heated at 90 °C for 24 h and then was poured into ice water. The resulting precipitate was filtered and washed with Et_2O to afford **54** (1.96 g, 28% yield).

7-Hydroxy-4-methoxycoumarin (28). A mixture of **54** (1.25 g, 7.0 mmol), MeOH (70 mL), and H_2SO_4 (7 mL) was heated to reflux for 1.5 h. After cooling to room temperature, the resulting crystals were filtered and washed with Et_2O to obtain pure compound **28** (0.81 g, 60%) as colorless needles from MeOH: mp 272−3 °C (lit. 275−6 °C); ^{1}H NMR (DMSO-d_6) δ 3.97 (3H, s, CH_3O-4), 5.68 (1H, s, H-3), 6.70 (1H, d, J = 2.1 Hz, H-8), 6.78 (1H, dd, J = 8.8 and 2.1 Hz, H-6), 7.61 (1H, d, J = 8.8 Hz, H-5), 10.58 (1H, s, OH-7, disappeared in D_2O).

2,4-Diacetoxyl-6-hydroxybenzaldehyde (56). Method 1. To a mixture of 2,4,6-trihydroxybenzaldehyde (**55**) (2.44 g, 1.58 mmol), pyridine (5 mL), and CH_2Cl_2 (50 mL) was added dropwise Ac_2O (3 mL, 3.18 mmol) at 0 °C. The mixture was stirred for 30 min at room temperature, washed with 10% aqueous HCl, water, and brine, successively, and dried over $MgSO_4$. The solvent was removed in vacuo. The residue was chromatographed (SiO_2, EtOAc/hexane = 1:7), and the product was crystallized from a mixture of EtOAc and hexane to give **56** (1.87 g) in a 50% yield: mp 103−4 °C (lit. 102−3 °C); ^{1}H NMR δ 2.30 and 2.38 (each 3H, s, 2 × CH_3), 6.61 and 6.66 (each 1H, d, J = 2.2 Hz, ArH), 10.05 (1H, s, CHO), 11.77 (1H, s, OH).

Method 2. To a solution of **55** (3.08 g, 20 mmol) in pyridine (20 mL) at 0 °C was added acetic anhydride (7.6 mL, 80.5 mmol) over 5 min. Then the mixture was stirred for 1 h at room temperature. Cold 10% HCl aqueous solution was added and the solution extracted with EtOAc. The organic layer was washed with brine and dried over anhydrous $MgSO_4$. After the solvent was removed, the residue was crystallized from a mixture of EtOAc and EtOH to give 2,4,6-triacetoxybenzaldehyde (4.52 g, yield 81%), mp 94−6 °C (lit. 101 °C). 2,4,6-Triacetoxybenzaldehyde in MeOH in the presence of pyridine was stirred for 3 h at room temperature to produce **56** in a yield of 73%.

7-Hydroxy-5-methoxycoumarin (29). To a solution of **56** (0.48 g, 2.0 mmol) in DMF (5 mL) was added Ph_3P=CHCOOMe (**57**) (0.74 g, 2.2 mmol), and stirring continued for 10 min at room temperature. MeI (0.18 mL, 2.9 mmol) and K_2CO_3 (0.41 g, 3.0 mmol) were added, and stirring continued for 1.5 h at room temperature. After EtOAc and water were added, the organic layer was separated, washed with water and brine, successively, and dried over $MgSO_4$. The solvent was removed in vacuo. The residue was mixed with MeOH (10 mL) and Et_3N (0.5 mL) and refluxed for 3.5 h to hydrolyze the acetyl groups. After MeOH and Et_3N were removed, the residue was heated to reflux in xylene (2 mL) for another 2.5 h. Finally, the mixture was chromatographed (SiO_2, EtOAc/hexane = 1:3) to give the desired product **29** (0.18 g, 46% yield): mp 257−8 °C (lit. 243−5 °C); ^{1}H NMR δ 3.88 (3H, s, OCH_3-5), 6.07 (1H, d, J = 9.6 Hz, H-3), 6.28 (1H, d, J = 2.0 Hz, H-8), 6.42 (1H, d, J = 2.0 Hz, H-6), 7.96 (1H, d, J = 9.6 Hz, H-4).

Ph_3P=CMeCOOMe (58). A mixture of **57** (2.0 g, 6.0 mmol), MeI (0.4 mL, 6.4 mmol), and 10 mL of CH_2Cl_2 (anhydrous) was heated to reflux for 1 h. The solvent was removed in vacuo to give a white solid. To the residue was added 10 mL of CH_2Cl_2 (anhydrous) under N_2 protection. *t*-BuOK (6 mL, 1.0 M in THF) was added at room temperature and stirred for 30 min. The brown reaction mixture was poured into water and extracted with EtOAc. The organic layer was washed with brine and dried over anhydrous $MgSO_4$. The solvent was removed, and the residue was crystallized from Et_2O to afford **58** (740 mg, 81% yield): mp 147−9 °C (lit. 145 °C).

7-Hydroxy-3-methylcoumarin (31). A mixture of **51** (0.28 g, 2.0 mmol) and **58** (0.84 g, 2.4 mmol) in 5 mL of DMF was heated to reflux for 7 h. EtOAc was added to the mixture, and the organic layer was washed with water. The organic layer was then extracted with 10% aqueous KOH three times. The combined basic water layer was washed with EtOAc, then acidified with 10% aqueous HCl, and extracted with EtOAc again. Finally, the organic phase was washed with water and brine and dried over $MgSO_4$. After the solvent was removed, compound **31** (0.27 g) was obtained in a yield of 77%: mp 218−220 °C (lit. 217−9 °C); ^{1}H NMR δ 2.18 (3H, s, CH_3-3), 6.77 (1H, dd, J = 2.2 and 9.6 Hz, H-6), 6.84 (1H, d, J = 2.2 Hz, H-8), 7.30 (1H, d, J = 9.6 Hz, H-5), 7.46 (1H, s, H-4).

2,4-Dihydroxy-6-methylbenzaldehyde (60). One milliliter of phosphorus chloride was dropped into 5 mL of DMF below 10 °C with rapid stirring over 0.5 h. 3,5-Dihydroxytoluene (1.42 g, 13 mmol) in 5 mL of DMF was slowly added, keeping the temperature below 10 °C. The mixture was warmed to room temperature and stirred for 1 h. Then, ice and 10% aqueous NaOH were added successively until pH was 9−10 and solid appeared. The mixture was heated to boiling for 10 min and then adjusted to pH 3 with 10% aqueous HCl after cooling to room temperature. The solid product was collected, washed with water until neutral, and dried to give **60** (1.25 g, 82% yield): mp 152−4 °C (lit. 178 °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).

7-Hydroxy-5-methylcoumarin (33). To a solution of **60** (880 mg, 5.79 mmol) in 15 mL of MeOH under N_2 was added Ph_3P=CHCOOEt (**57**) (2.3 g, mmol) in 10 mL of CH_2Cl_2. The mixture was stirred for 1.5 h at room temperature. After removal of solvent in vacuo, the residue was dissolved in 10 mL of xylene and refluxed for 2 days. The mixture was diluted with EtOAc and extracted three times with 10% aqueous KOH. The combined water layer was acidified with 10% aqueous HCl to pH 3. The precipitated solid was filtered, washed with water until neutral, and dried to obtain 553 mg of pure **33** in a 54% yield: mp 228 °C (dec) (lit. 248−50 °C); ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 2.42 (3H, s, CH_3-5), 6.18 (1H, d, J = 9.6 Hz, H-3), 6.60 (2H, s, H-6 and H-8), 7.82 (1H, d, J = 9.6 Hz, H-4).

2,4-Dihydroxytoluene (61). To a solution of 2,4-dihydroxybenzaldehyde **51** (690 mg, 5 mmol) and sodium cyanoborohydride (1.0 g, 15 mmol) in 30 mL of THF was added methyl orange as an indictor, giving the solution a yellow color. HCl aqueous solution (15 mL, 1 N in water) was slowly added to the reaction system, keeping the solution red. 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 **61** (680 mg) was obtained in a yield

of 98.6% as a light pink solid: mp 105−6 °C (lit. 104−6 °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 = 2.1 Hz, H-3), 6.84 (1H, d, J = 8.8 Hz, H-6).

2,4-Dihydroxy-5-methylbenzaldehyde (62). The procedure was the same as that for the preparation of **60**: yield 60% from **61**; mp 125−9 °C (lit. 142−4 °C); ^{1}H NMR δ 2.17 (3H, s, CH_3), 6.30 (1H, s, H-3), 7.22 (1H, s, H-6), 9.63 (1H, s, CHO).

7-Hydroxy-6-methylcoumarin (34). The procedure was the same as that for the preparation of **33**: yield 60% from **62**, light yellow solid; mp 255−6 °C (dec) (lit. 147−8 °C); ^{1}H NMR (CD_3OD) δ 2.18 (3H, s, CH_3-6), 6.13 (1H, d, J = 9.6 Hz, H-3), 6.66 (1H, s, H-8), 7.29 (1H, s, H-5), 7.79 (1H, d, J = 9.6 Hz, H-4).

General Procedure for Synthesizing 4-Alkyl-7-hydroxycoumarins (35−37). To a mixture of 1,3-dihydroxybenzene (**40**) and the appropriate β-keto ester (ratio 1:1.5) 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 left to stand overnight. The precipitated solid was filtered, washed with water until neutral, and dried in vacuo to afford the product.

7-Hydroxy-4-propylcoumarin (35): 88% yield (starting with 8 mmol of **40** and 12 mmol of ethyl butyryl acetate); mp 127−8 °C (lit. 130 °C); ^{1}H NMR δ 1.06 (3H, t, J = 7.5 Hz, CH_3 in propyl group at 4-position), 1.73 (2H, six, J = 7.5 Hz, CH_2 in propyl group at the 4-position), 2.73 (2H, t, J = 7.5 Hz, CH_2 in propyl group at the 4-position), 6.15 (1H, s, H-3), 6.89 (1H, dd, J = 2.4 and 8.8 Hz, H-6), 7.88 (1H, d, J = 2.4 Hz, H-8), 7.54 (1H, d, J = 8.8 Hz, H-5).

7-Hydroxy-4-isopropylcoumarin (36): 74% yield (starting with 8 mmol of **40** and 12 mmol of ethyl isobutyryl acetate); mp 123−4 °C (lit. 62−4 °C); ^{1}H NMR (DMSO-d_6) δ 1.20 (6H, d, J = 7.2 Hz, 2 × CH_3 in isopropyl group at the 4-position), 3.28 (1H, m, J = 7.2 Hz, CH in isopropyl group at the 4-position), 6.05 (1H, s, H-3), 6.72 (1H, d, J = 2.1 Hz, H-8), 6.80 (1H, dd, J = 2.1 and 8.8 Hz, H-6), 7.69 (1H, d, J = 8.8 Hz, H-5).

7-Hydroxy-4-phenylcoumarin (37): 69% yield (starting with 2 mmol of **40** and 3 mmol of ethyl benzoyl acetate); mp 247−9 °C (lit. 247−8 °C); ^{1}H NMR (DMSO) δ 6.12 (1H, s, H-3), 6.75 (1H, d, J = 2.4 Hz, H-8), 6.78 (1H, dd, J = 2.4 and 8.8 Hz, H-6), 7.27 (1H, d, J = 8.8 Hz, H-5), 7.46−7.55 (5H, m, ArH at the 4-position).

General Procedure for Synthesizing Substituted Seselins (39−50) from Substituted 7-Hydroxycoumarin Derivatives. A mixture of substituted 7-hydroxycoumarin (5 mmol), K_2CO_3 (12.5 mmol), KI (5 mmol), and excess (1−2 mL) of 3-chloro-3-methyl-1-butyne in 10 mL of DMF 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% aqueous 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.

3-Methoxyseselin (39): yield 46% (starting with 1.34 g of **27**), colorless needles; mp 158−161 °C; ^{1}H NMR δ 1.46 (6H, s, 2 × CH_3), 3.88 (3H, s, CH_3O-3), 5.73 (1H, d, J = 10.2 Hz, H-3′), 6.73 (1H, d, J = 8.4 Hz, H-6), 6.78 (1H, s, H-4), 6.86 (1H, d, J = 10.2 Hz, H-4′), 7.13 (1H, d, J = 8.4 Hz, H-5). Anal. ($C_{15}H_{14}O_4 \cdot {}^1/_2H_2O$) C, H.

4-Methoxyseselin (40): yield 50% (starting with 960 mg of **28**), colorless needles; mp 167−8 °C; ^{1}H NMR δ 1.47 (6H, s, 2 × CH_3), 3.96 (3H, s, CH_3O-4), 5.55 (1H, s, H-3), 5.70 (1H, d, J = 10.2 Hz, H-3′), 6.71 (1H, d, J = 8.8 Hz, H-6), 6.88 (1H, d, J = 10.2 Hz, H-4′), 7.55 (1H, d, J = 8.8 Hz, H-5). Anal. ($C_{15}H_{14}O_4$) C, H.

5-Methoxyseselin (41): yield 46% (starting with 260 mg of **29**); mp 154−6 °C; ^{1}H NMR δ 1.47 (6H, s, 2 × CH_3), 3.88 (3H, s, CH_3O-5), 5.58 (1H, d, J = 10.2 Hz, H-3′), 6.13 (1H, d, J = 9.9 Hz, H-3), 6.24 (1H, s, H-6), 6.79 (1H, d, J = 10.2 Hz, H-4′), 7.96 (1H, d, J = 9.9 Hz, H-4). Anal. ($C_{15}H_{14}O_4$) C, H.

6-Methoxyseselin (42): yield 62% (starting with 260 mg of **30**), as yellow solid; mp 133−5 °C; ^{1}H NMR δ 1.67 (6H, s, 2 × CH_3), 4.03 (3H, s, OCH_3), 5.88 (1H, d, J = 10.2 Hz, H-3′), 6.39 (1H, d, J = 9.6 Hz, H-3), 6.91 (1H, s, H-5), 7.02 (1H, d, J = 10.2 Hz, H-4′), 7.72 (1H, d, J = 9.6 Hz, H-4). Anal. ($C_{15}H_{14}O_4$) C, H.

3-Methylseselin (43): yield 43% (starting with 1.06 g of **31**), colorless needles; mp 93−96 °C; ^{1}H NMR δ 1.46 (6H, s, 2 × CH_3), 2.17 (3H, s, CH_3-3), 5.71 (1H, d, J = 10.2 Hz, H-3′), 6.70 (1H, d, J = 8.4 Hz, H-6), 6.89 (1H, d, J = 10.2 Hz, H-4′), 7.14 (1H, d, J = 8.4 Hz, H-5), 7.41 (1H, s, H-4). Anal. ($C_{15}H_{14}O_3 \cdot {}^1/_2H_2O$) C, H.

4-Methylseselin (44): yield 30% (starting with 880 mg of **32**), white solid crystallized in MeOH; mp 141−3 °C; ^{1}H NMR δ 1.42 (6H, s, 2 × CH_3), 2.38 (3H, s, CH_3-4), 5.72 (1H, d, J = 10.0 Hz, H-3′), 6.10 (1H, s, H-3), 6.76 (1H, d, J = 8.8 Hz, H-6), 6.88 (1H, d, J = 10.0 Hz, H-4′), 7.36 (1H, d, J = 8.8 Hz, H-5). Anal. ($C_{15}H_{14}O_3$) C, H.

5-Methylseselin (45): yield 17% (starting with 513 mg of **33**), white solid; mp 143−4 °C; ^{1}H NMR δ 1.47 (3H, s, CH_3-2′), 1.60 (3H, s, CH_3-2′), 2.45 (3H, s, CH_3-5), 5.68 (1H, d, J = 10.2 Hz, H-3′), 6.24 (1H, d, J = 9.6 Hz, H-3), 6.60 (1H, s, H-6), 6.87 (1H, d, J = 10.2 Hz, H-4′), 7.82 (1H, d, J = 9.6 Hz, H-4). Anal. ($C_{15}H_{14}O_3$) C, H.

6-Methylseselin (46): yield 50% (starting with 176 mg of **34**), white solid; mp 78−80 °C; ^{1}H NMR δ 1.49 (6H, s, 2 × CH_3-2′), 2.21 (3H, s, CH_3-6), 5.73 (1H, d, J = 10.2 Hz, H-3′), 6.22 (1H, d, J = 9.6 Hz, H-3), 6.89 (1H, d, J = 10.2 Hz, H-4′), 7.08 (1H, s, H-5), 7.57 (1H, d, J = 9.6 Hz, H-4). Anal. ($C_{15}H_{14}O_3$) C, H.

4-Propylseselin (47): yield 33% (starting with 408 mg of **35**), yellow crystals from Et_2O; mp 110−1 °C; ^{1}H NMR δ 1.06 (3H, t, J = 7.5 Hz, CH_3-4), 1.48 (6H, s, 2 × CH_3), 1.70 (2H, m, J = 7.5 Hz, CH_2), 2.69 (2H, t, J = 7.5 Hz, CH_2), 5.72 (1H, d, J = 10.2 Hz, H-3′), 6.12 (1H, s, H-3), 6.74 (1H, d, J = 8.8 Hz, H-6), 6.92 (1H, d, J = 10.2 Hz, H-4′), 7.38 (1H, d, J = 8.8 Hz, H-5). Anal. ($C_{17}H_{18}O_3$) C, H.

4-Isopropylseselin (48): yield 38% (starting with 408 mg of **36**); mp 141−3 °C; ^{1}H NMR δ 1.31 (6H, d, J = 7.5 Hz, 2 × CH_3-4), 1.48 (6H, s, 2 × CH_3), 3.24 (1H, m, J = 7.5 Hz, CH-4), 5.72 (1H, d, J = 10.2 Hz, H-3′), 6.16 (1H, s, H-3), 6.76 (1H, d, J = 8.8 Hz, H-6), 6.92 (1H, d, J = 10.2 Hz, H-4′), 7.44 (1H, d, J = 8.8 Hz, H-5). Anal. ($C_{17}H_{18}O_3 \cdot 1/4H_2O$) C, H.

4-Phenylseselin (49): yield 46% (starting with 238 mg of **37**); mp 120−2 °C; ^{1}H NMR δ 1.50 (6H, s, 2 × CH_3), 5.76 (1H, d, J = 10.2 Hz, H-3′), 6.21 (1H, s, H-3), 6.69 (1H, d, J = 8.8 Hz, H-6), 6.97 (1H, d, J = 10.2 Hz, H-4′), 7.22 (1H, d, J = 8.8 Hz, H-5), 7.42−7.53 (5H, m, ArH). Anal. ($C_{20}H_{16}O_3 \cdot {}^1/_4H_2O$) C, H.

4-(Trifluoromethyl)seselin (50): yield 29% (starting with 230 mg of **38** in acetone contained 2% water); mp 150−1 °C; ^{1}H NMR δ 1.50 (6H, s, 2 × CH_3), 5.77 (1H, d, J = 10.2 Hz, H-3′), 6.61 (1H, s, H-3), 6.81 (1H, d, J = 8.8 Hz, H-6), 6.88 (1H, d, J = 10.2 Hz, H-4′), 7.47 (1H, d, J = 8.8 Hz, H-5). Anal. ($C_{15}H_{11}O_3F_3 \cdot {}^1/_4H_2O$) C, H.

General Procedure of Asymmetric Dihydroxylation and Esterification for Synthesizing Substituted 3′,4′-Di-*O*-(−)-camphanoyl-(+)-*cis*-khellactones (3−14). A mixture of $K_3Fe(CN)_6$ (150 mg, 0.75 mmol), K_2CO_3 (105 mg, 0.75 mmol), and 2,5-diphenyl-4,6-bis(9-*O*-dihydroquinyl)-pyrimidine [$(DHQ)_2$-PYR] (4.4 mg, 0.005 mmol), $K_2OsO_2(OH)_4$ (0.005 mmol), was dissolved 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. Reaction was monitored using TLC, and at completion, $Na_2S_2O_5$ (excess), water, and $CHCl_3$ were added. After being stirred for 0.5 h at

room temperature, the mixture was extracted with $CHCl_3$ three times. The combined organic layer was dried over $MgSO_4$, and 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 (greater than 0.5 mmol) in Py/CH_2Cl_2 for 1−2 days at room temperature. The mixture was diluted with EtOAc and washed with 10% aqueous 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*-(−)-camphanoyl-(+)-*cis*-khellactone derivative.

3′,4′-Di-*O*-(*S*)-(−)-camphanoyl-3-methoxy-(+)-*cis*-khellactone (3): yield 47% (starting with 103 mg of **39**), white solid, mp 149−50 °C; MS (Cl−NH_3) *m*/*z* (%) 670 (M + NH_4^+, 100); ^{1}H NMR δ 0.93−1.12 (15H, m.s., 5 × CH_3), 1.43, 1.47, and 1.55 (each 3H, s, CH_3), 1.69, 1.92, 2.22, and 2.48 (each 2H, m, CH_2 in camphanoyl group), 3.88 (3H, s, CH_3O-3), 5.39 (1H, d, *J* = 4.8 Hz, H-3′), 6.64 (1H, d, *J* = 4.8 Hz, H-4′), 6.78 (1H, s, H-4), 6.83 (1H, d, *J* = 8.8 Hz, H-6), and 7.34 (1H, d, *J* = 8.8 Hz, H-5); 80% d.e.; $[\alpha]_D$ +12.87° (*c* 0.72, $CHCl_3$). Anal. ($C_{35}H_{40}O_{12}\cdot{}^1/_2H_2O$) C, H.

3′,4′-Di-*O*-(*S*)-(−)-camphanoyl-4-methoxy-(+)-*cis*-khellactone (4): yield 60% (starting with 103 mg of **40**), white solid; mp 174−6 °C; MS (Cl−NH_3) *m*/*z* (%) 670 (M + NH_4^+, 75); ^{1}H NMR δ 0.93−1.14 (18H, m.s., 6 × CH_3), 1.45, and 1.49 (each 3H, s, CH_3), 1.68, 1.92, 2.24, and 2.48 (each 2H, m, CH_2 in camphanoyl group), 3.97 (3H, s, CH_3O-4), 5.38 (1H, d, *J* = 4.8 Hz, H-3′), 5.53 (1H, s, H-3), 6.64 (1H, d, *J* = 4.8 Hz, H-4′), 6.81 (1H, d, *J* = 8.8 Hz, H-6), and 7.74 (1H, d, *J* = 8.8 Hz, H-5); 73% d.e.; $[\alpha]_D$ +2.34° (*c* 0.69, $CHCl_3$). Anal. ($C_{35}H_{40}O_{12}\cdot{}^1/_2H_2O$) C, H.

3′,4′-Di-*O*-(*S*)-(−)-camphanoyl-5-methoxy-(+)-*cis*-khellactone (5): yield 50% (starting with 103 mg of **41**), white solid; mp 168−70 °C; MS (Cl−NH_3) *m*/*z* (%) 670 (M + NH_4^+, 60), 652 (M^+, 7); ^{1}H NMR δ 0.98−1.14 (15H, m, 5 × CH_3), 1.44, 1.50, and 1.55 (each 3H, s, CH_3), 1.71, 1.90, 2.22, and 2.49 (each 2H, m, CH_2 in camphanoyl group), 3.90 (3H, s, CH_3O-5), 5.40 (1H, d, *J* = 4.8 Hz, H-3′), 6.27 (1H, d, *J* = 9.8 Hz, H-3), 6.65 (1H, d, *J* = 4.8 Hz, H-4′), 6.25 (1H, s, H-6), and 7.97 (1H, d, *J* = 9.8 Hz, H-4); 86% d.e.; $[\alpha]_D$ −4.44° (*c* 0.45, $CHCl_3$). Anal. ($C_{35}H_{40}O_{12}\cdot{}^1/_2H_2O$) C, H.

3′,4′-Di-*O*-(*S*)-(−)-camphanoyl-6-methoxy-(+)-*cis*-khellactone (6): yield 61% (starting with 65 mg of **42**), white solid; mp 262−4 °C; MS (EI) *m*/*z* (%) 652 (M^+, 20); ^{1}H NMR δ 0.98−1.14 (15H, m.s., 7 × CH_3), 1.52 (3H, s, CH_3), 1.72, 1.97, 2.10, and 2.48 (each 2H, m, CH_2 in camphanoyl group), 3.92 (3H, s, CH_3O-6), 5.40 (1H, d, *J* = 4.8 Hz, H-3′), 6.27 (1H, d, *J* = 9.8 Hz, H-3), 6.65 (1H, d, *J* = 4.8 Hz, H-4′), 6.90 (1H, s, H-5), and 7.60 (1H, d, *J* = 9.8 Hz, H-4); >90% d.e.; $[\alpha]_D$ −18.26° (*c* 0.46, $CHCl_3$). Anal. ($C_{35}H_{40}O_{12}\cdot 2{}^1/_2H_2O$) C, H.

3′,4′-Di-*O*-(*S*)-(−)-camphanoyl-3-methyl-(+)-*cis*-khellactone (7): yield 47% (starting with 113 mg of **43**), white solid; mp 143−5 °C; ^{1}H NMR δ 0.96−1.15 (15H, m.s., 5 × CH_3), 1.45, 1.49, and 2.19 (each 3H, s, CH_3), 1.69, 1.92, 2.25, and 2.48 (each 2H, m, CH_2 in camphanoyl group), 2.16 (3H, s, CH_3-3), 5.40 (1H, d, *J* = 4.8 Hz, H-3′), 6.66 (1H, d, *J* = 4.8 Hz, H-4′), 6.80 (1H, d, *J* = 8.8 Hz, H-6), 7.35 (1H, d, *J* = 8.8 Hz, H-5) and 7.43(1H, s, H-4); >90% d.e.; $[\alpha]_D$ +21.08° (*c* 0.37, $CHCl_3$). Anal. ($C_{35}H_{40}O_{11}\cdot H_2O$) C, H.

3′,4′-Di-*O*-(*S*)-(−)-camphanoyl-4-methyl-(+)-*cis*-khellactone (8): yield 50% (starting with 60 mg of **44**), white solid; mp 264−7 °C; MS *m*/*z* (%) 636 (M^+, 7), 423 (100), and 227 (82); ^{1}H NMR δ 0.98−1.14 (12H, m.s., 4 × CH_3), 1.27, 1.50, 1.69, and 2.41 (each 3H, s, CH_3), 1.71, 2.04, 2.21, and 2.46 (each 2H, m, CH_2 in camphanoyl group), 2.41 (3H, s, CH_3-4), 5.40 (1H, d, *J* = 4.8 Hz, H-3′), 6.13 (1H, s, H-3), 6.67 (1H, d, *J* = 4.8 Hz, H-4′), 6.85 (1H, d, *J* = 8.8 Hz, H-6), and 7.54 (1H, d, *J* = 8.8 Hz, H-5); 84% d.e.; $[\alpha]_D$ +8.4° (*c* 0.50, $CHCl_3$). Anal. ($C_{35}H_{40}O_{11}\cdot 2{}^1/_2H_2O$) C, H.

3′,4′-Di-*O*-(*S*)-(−)-camphanoyl-5-methyl-(+)-*cis*-khellactone (9): yield 66.5% (starting with 60 mg of **45**), white solid; mp 163−4 °C; ^{1}H NMR δ 0.97−1.13 (12H, m.s., 4 × CH_3), 1.27, 1.50, 1.69, and 2.41 (each 3H, s, CH_3), 1.71, 1.91, 2.22, and 2.48 (each 2H, m, CH_2 in camphanoyl group), 2.43 (3H, s, CH_3-5), 5.36 (1H, d, *J* = 4.8 Hz, H-3′), 6.23 (1H, d, *J* = 9.8 Hz, H-3), 6.62 (1H, d, *J* = 4.8 Hz, H-4′), 6.67 (1H, s, H-6), and 7.80 (1H, d, *J* = 9.8 Hz, H-4); 81% d.e.; $[\alpha]_D$ +18.92° (*c* 0.37, $CHCl_3$). Anal. ($C_{35}H_{40}O_{11}\cdot 1{}^1/_2H_2O$) C, H.

3′,4′-Di-*O*-(*S*)-(−)-camphanoyl-6-methyl-(+)-*cis*-khellactone (10): yield 28% (starting with 80 mg of **46**), white solid; mp 206−7 °C; ^{1}H NMR δ 0.92−1.12 (15H, m.s., 5 × CH_3), 1.47, 1.49, and 2.05 (each 3H, s, CH_3), 1.66, 1.92, 2.23, and 2.48 (each 2H, m, CH_2 in camphanoyl group), 2.23 (3H, s, CH_3-6), 5.39 (1H, d, *J* = 4.8 Hz, H-3′), 6.22 (1H, d, *J* = 9.8 Hz, H-3), 6.66 (1H, d, *J* = 4.8 Hz, H-4′), 7.26 (1H, s, H-5), and 7.58 (1H, d, *J* = 9.8 Hz, H-4); 87% d.e.; $[\alpha]_D$ +8.45° (*c* 0.97, $CHCl_3$). Anal. ($C_{35}H_{40}O_{11}\cdot H_2O$) C, H.

3′,4′-Di-*O*-(*S*)-(−)-camphanoyl-4-propyl-(+)-*cis*-khellactone (11): yield 26% (starting with 70 mg of **47**), white crystals from EtOH; mp 270−1 °C; ^{1}H NMR δ 0.99−1.12 (15H, m.s., 5 × CH_3), 1.46, 1.50, and 1.56 (each 3H, s, CH_3), 1.65, 1.91, 2.24, and 2.51 (each 2H, m, CH_2 in camphanoyl group), 1.06 (3H, t, *J* = 7.5 Hz, CH_3 of propyl group at 4-position), 1.73 (2H, six, *J* = 7.5 Hz, CH_2 of propyl group at 4-position), 2.70 (2H, t, *J* = 5 Hz, CH_2 of propyl group at 4-position), 5.40 (1H, d, *J* = 4.8 Hz, H-3′), 6.11 (1H, s, H-3), 6.66 (1H, d, *J* = 4.8 Hz, H-4′), 6.84 (1H, d, *J* = 8.8 Hz, H-6), and 7.57 (1H, d, *J* = 8.8 Hz, H-5); 100% d.e.; $[\alpha]_D$ +17.95° (*c* 0.39, $CHCl_3$). Anal. ($C_{37}H_{44}O_{11}$) C, H.

3′,4′-Di-*O*-(*S*)-(−)-camphanoyl-4-isopropyl-(+)-*cis*-khellactone (12): yield 41% (starting with 110 mg of **48**), white crystals from EtOH; mp 260−1 °C; ^{1}H NMR δ 0.97−1.11 (15H, m.s., 5 × CH_3), 1.33, 1.45, and 1.49 (each 3H, s, CH_3), 1.65, 1.91, 2.20, and 2.50 (each 2H, m, CH_2 in camphanoyl group), 1.30 (6H, d, *J* = 6.6 Hz, 2 × CH_3 at 4-position), 3.32 (1H, five, *J* = 6.6 Hz, at 4-position), 5.38 (1H, d, *J* = 4.8 Hz, H-3′), 6.14 (1H, s, H-3), 6.64 (1H, d, *J* = 4.8 Hz, H-4′), 6.84 (1H, d, *J* = 8.8 Hz, H-6), and 7.61 (1H, d, *J* = 8.8 Hz, H-5); 100% d.e.; $[\alpha]_D$ +19.00° (*c* 0.60, $CHCl_3$). Anal. ($C_{37}H_{44}O_{11}$) C, H.

3′,4′-Di-*O*-(*S*)-(−)-camphanoyl-4-phenyl-(+)-*cis*-khellactone (13): yield 52% (starting with 120 mg of **49**), isomer mixture with a ratio of 3′*R*,4′*R*:3′*S*,4′*S* ≈ 50:50. The pair of diastereoisomers was separated by TLC with an eluant of 40:2 CH_2Cl_2/acetone to obtain the product with lower R_f value as a white solid with mp 148−50 °C: ^{1}H NMR δ 1.00−1.15 (12H, m.s., 4 × CH_3), 1.27, 1.48, 1.52, and 1.56 (each 3H, s, CH_3), 1.72, 1.95, 2.24, and 2.54 (each 2H, m, CH_2 in camphanoyl group), 5.43 (1H, d, *J* = 4.8 Hz, H-3′), 6.21 (1H, s, H-3), 6.71 (1H, d, *J* = 4.8 Hz, H-4′), 6.78 (1H, d, *J* = 8.8 Hz, H-6), 7.44 (1H, d, *J* = 8.8 Hz, H-5), 7.42 and 7.54 (5H, d.m. ArH at 4-position); 65% d.e.; $[\alpha]_D$ +4.36° (*c* 0.55,$CHCl_3$). Anal. ($C_{40}H_{42}O_{11}\cdot H_2O$) C, H.

3′,4′-Di-*O*-(*S*)-(−)-camphanoyl-4-trifluoromethyl-(+)-*cis*-khellactone (14): yield 29% (starting with 37 mg of **50**), white solid; mp 232−4 °C; MS (EI) *m*/*z* (%) 690 (M^+, 1), 477 (30), 281 (80); ^{1}H NMR δ 0.98−1.13 (12H, m.s., 4 × CH_3), 1.28, 1.48, 1.51, and 2.16 (each 3H, s, CH_3), 1.72, 1.94, 2.21, and 2.51 (each 2H, m, CH_2 in camphanoyl group), 2.64 (s, CF_3), 5.40 (1H, d, *J* = 4.8 Hz, H-3′), 6.63 (1H, s, H-3), 6.64 (1H, d, *J* = 4.8 Hz, H-4′), 6.92 (1H, d, *J* = 9.0 Hz, H-6), and 7.67 (1H, d, *J* = 9.0 Hz, H-5); >86% d.e.; $[\alpha]_D$ +17.00° (*c* 0.33, $CHCl_3$).

4-Methyl-(+)-*cis*-khellactone (63): yield 62% (starting with 890 mg of **44**), white solid; mp 196−7 °C; ^{1}H NMR δ 1.42 and 1.47 (each 3H, s, 2′-CH_3 × 2), 2.42 (3H, s, 4-CH_3), 3.27 and 4.15 (each 1H, br, OH × 2), 3.87 (1H, d, *J* = 4.8 Hz, 3′-CH), 5.21 (1H, d, *J* = 4.8 Hz, 4′-CH), 6.16 (1H, s, 3-H), 6.83 (1H, d, *J* = 8.8 Hz, 6-H), 7.48 (1H, d, *J* = 8.8 Hz, 5-H); 73% e.e., with was determined by ^{1}H NMR spectrum of **7**; $[\alpha]_D$ +46.86° (*c* 0.70, $CHCl_3$). Anal. ($C_{15}H_{16}O_5$) C, H.

General Procedure of Acylation for Synthesizing 4-Methyl-(+)-*cis*-khellactones Derivatives (15−26). To a solution of **63** (50 mg, 0.18 mmol) in CH_2Cl_2 (2 mL) and pyridine (1 mL) was added excess acylating agent at 0 °C. The

mixture was stirred at room temperature until complete as monitored by TLC. After the usual workup, the residue was separated using TLC to obtain product.

3′,4′-Di-*O*-acetyl-4-methyl-(+)-*cis*-khellactone (15): 71% yield from excess acetyl chloride as a white solid; mp 134−5 °C; ^{1}H NMR δ 1.42 and 1.46 (each 3H, s, 2′-CH_3 × 2), 2.11 and 2.14 (each 3H, s, $COCH_3$ × 2), 2.40 (3H, s, 4-CH_3), 5.32 (1H, d, J = 4.8 Hz, 3′-CH), 6.14 (1H, s, 3-H), 6.55 (1H, d, J = 4.8 Hz, 4′-CH), 6.84 (1H, d, J = 8.8 Hz, 6-H), 7.51 (1H, d, J = 8.8 Hz, 5-H); $[\alpha]_D$ −22.75° (*c* 0.615, $CHCl_3$). Anal. ($C_{19}H_{20}O_7$) C, H.

3′,4′-Di-*O*-isovaleryl-4-methyl-(+)-*cis*-khellactone (16): yield 77.5% from excess isovaleryl chloride as a light yellow oil; ^{1}H NMR δ 1.00 (12H, m, CH_3 × 4), 1.42 and 1.45 (each 3H, s, 2′-CH_3), 2.15 and 2.28 (each 2H, m, $COCH_2$), 2.21 (2H, m, CH × 2), 2.39 (3H, s, 4-CH_3), 5.33 (1H, d, J = 4.8 Hz, 3′-CH), 6.12 (1H, s, 3-H), 6.57 (1H, d, J = 4.8 Hz, 4′-CH), 6.82 (1H, d, J = 8.8 Hz, 6-H), 7.51 (1H, d, J = 8.8 Hz, 5-H); $[\alpha]_D$ −3.93° (*c* 0.56, $CHCl_3$). Anal. ($C_{25}H_{32}O_7 \cdot {}^1/_4H_2O$) C, H.

3′,4′-Di-*O*-*m*-bromobenzoyl-4-methyl-(+)-*cis*-khellactone (17): yield 84% from excess *m*-bromobenzoyl chloride as a white solid; mp 205−8 °C; ^{1}H NMR δ 1.52 and 1.63 (each 3H, s, 2′-CH_3), 2.40 (3H, s, 4-CH_3), 5.67 (1H, d, J = 4.8 Hz, 3′-CH), 6.10 (1H, s, 3-H), 6.93 (1H, d, J = 4.8 Hz, 4′-CH), 6.95 (1H, d, J = 8.8 Hz, 6-H), 7.58 (1H, d, J = 8.8 Hz, 5-H), 7.24−7.29 (2H, m, ArH), 7.63−7.96 (6H, m, ArH); $[\alpha]_D$ +47.0° (*c* 0.865, dioxane). Anal. ($C_{29}H_{22}O_7Br_2$) C, H.

3′,4′-Di-*O*-[*N*-(1-naphthyl)carbamoyl]-4-methyl-(+)-*cis*-khellactone (18): yield 25% from excess 1-naphthyl isocyanate as a white solid; mp 168−70 °C; ^{1}H NMR δ 1.26 and 2.19 (each 3H, s, 2′-CH_3), 2.41 (3H, s, 4-CH_3), 5.50 (1H, d, J = 4.8 Hz, 3′-CH), 6.17 (1H, s, 3-H), 6.71 (1H, d, J = 4.8 Hz, 4′-CH), 6.85−7.88 (16H, m, ArH); $[\alpha]_D$ +88.00° (*c* 0.35, $CHCl_3$); Anal. ($C_{37}H_{30}O_7N_2 \cdot {}^1/_2H_2O$) C, H, N.

3′,4′-Di-*O*-(+)-camphanoyl-4-methyl-(+)-*cis*-khellactone (19) and 4′-*O*-(+)-Camphanoyl-3′-hydroxy-4-methyl-(+)-*cis*-khellactone (20). A mixture of **19** and **20** was produced from **63** (46 mg) treated with excess (*R*)-(+)-camphanic chloride.

19: yield 15%; mp 145−7 °C; ^{1}H NMR δ 0.98−1.14 (18H, m.s., 6 × CH_3), 1.46 and 1.67 (each 3H, s, CH_3), 1.72, 1.92, 2.12 and 2.40 (each 2H, m, CH_2 in camphanoyl group), 2.54 (s, 4-CH_3), 5.48 (1H, d, J = 4.8 Hz, H-3′), 6.11 (1H, s, 3-H), 6.76 (1H, d, J = 4.8 Hz, 4′-H), 6.84 (1H, d, J = 9.0 Hz, H-6), and 7.54 (1H, d, J = 9.0 Hz, H-5); $[\alpha]_D$ +27.0° (*c* 0.90, $CHCl_3$). Anal. ($C_{35}H_{40}O_{11} \cdot {}^1/_2H_2O$) C, H.

20: yield 48%; mp 117−20 °C; ^{1}H NMR δ 1.00, 1.09, 1.12, 1.41 and 1.50 (each 3H, s, CH_3), 1.68, 1.92, 2.06, and 2.50 (each 1H, m, CH_2 in camphanoyl group), 2.41 (s, 4-CH_3), 4.13 (1H, m 3′-H), 6.12 (1H, s, 3-H), 6.55 (1H, d, J = 4.8 Hz, 4′-H), 6.84 (1H, d, J = 9.0 Hz, H-6), and 7.54 (1H, d, J = 9.0 Hz, H-5); $[\alpha]_D$ +82.0° (*c* 1.61, $CHCl_3$). Anal. ($C_{25}H_{28}O_8 \cdot {}^3/_4H_2O$) C, H.

4′-*O*-Adamantanecarbonyl-3′-hydroxy-4-methyl-(+)-*cis*-khellactone (21): yield 64% from excess adamantanecarbonyl chloride as a white solid; mp 115−7 °C; ^{1}H NMR δ 1.41 and 1.50 (each 3H, s, 2′-CH_3), 1.71 (12H, m, CH_2 × 6), 2.01 (3H, m, CH × 3), 4.06 (1H, d, J = 4.8 Hz, 3′-H), 6.13 (1H, s, 3-H), 6.36 (1H, d, J = 4.8 Hz, 4′-H), 6.83 (1H, d, J = 9.0 Hz, 6-H), 7.52 (1H, d, J = 9.0 Hz, 5-H); $[\alpha]_D$ +62.6° (*c* 1.53, $CHCl_3$). Anal. ($C_{26}H_{30}O_6$) C, H.

3′-*O*-(1*S*)-(+)-10-Camphorsulfonyl-4′-hydroxy-4-methyl-(+)-*cis*-khellactone (22): yield 52% from excess (1*S*)-(+)-camphorsulfonyl chloride as a white solid; mp 100−2 °C; ^{1}H NMR δ 0.89, 1.10, 1.51, and 1.68 (each 3H, s, CH_3), 1.44 (2H, m, CH_2), 2.05 (2H, m, CH_2), 2.11 (1H, m, CH), 2.36 (2H, m, $COCH_2$), 2.42 (3H, s, 4-CH_3), 3.19 and 3.73 (each 1H, d, J = 1.5 Hz, SCH_2), 5.19 (1H, d, J = 4.8 Hz, 3′-H), 5.54 (1H, d, J = 4.8 Hz, 4′-H), 6.21 (1H, s, 3-H), 6.85 (1H, d, J = 9.0 Hz, 6-H), 7.55 (1H, d, J = 9.0 Hz, 5-H); $[\alpha]_D$ −7.40° (*c* 0.46, $CHCl_3$); Anal. ($C_{25}H_{30}O_8$ S·H_2O) C, H.

3′-*O*-(1*R*)-(−)-10-Camphorsulfonyl-4′-hydroxy-4-methyl-(+)-*cis*-khellactone (23): yield 45% from excess (1*R*)-(−)-camphorsulfonyl chloride as a white solid, mp 96−7 °C; ^{1}H NMR δ 0.89, 1.10, 1.51 and 1.66 (each 3H, s, CH_3), 1.44 (2H, m, CH_2), 2.05 (2H, m, CH_2), 2.13 (1H, m, CH), 2.36 (2H, m, $COCH_2$), 2.43 (3H, s, 4-CH_3), 3.15 and 3.73 (each 1H, d, J = 1.5 Hz, SCH_2), 5.19 (1H, d, J = 4.8 Hz, 3′-H), 5.52 (1H, d, J = 4.8 Hz, 4′-H), 6.21 (1H, s, 3-H), 6.85 (1H, d, J = 9.0 Hz, 6-H), 7.55 (1H, d, J = 9.0 Hz, 5-H); $[\alpha]_D$ −48.95° (*c* 0.38, $CHCl_3$). Anal. ($C_{25}H_{30}O_8$ S·$1^1/_2H_2O$) C, H.

3′-*O*-Acetyl-4′-*O*-(+)-(1*R*)-camphanoyl-4-methyl-(+)-*cis*-khellactone (24): yield 56% from **20** treated with excess acetic anhydride as a white solid; mp 114−5 °C; ^{1}H NMR δ 0.96, 1.09, 1.13, 1.44 and 1.50 (each 3H, s, CH_3), 1.68, 1.92, 2.06, and 2.50 (each 1H, m, CH_2 in camphanoyl group), 2.11 (3H, s, $COCH_3$), 2.40 (s, 4-CH_3), 5.32 (1H, d, J = 4.8 Hz, 3′-H), 6.10 (1H, s, 3-H), 6.67 (1H, d, J = 4.8 Hz, 4′-H), 6.84 (1H, d, J = 9.0 Hz, H-6), and 7.54 (1H, d, J = 9.0 Hz, H-5); $[\alpha]_D$ +19.1° (*c* 0.67, $CHCl_3$). Anal. ($C_{27}H_{30}O_9 \cdot 2H_2O$) C, H.

3′-*O*-Acetyl-4′-*O*-adamantanecarbonyl-4-methyl-(+)-*cis*-khellactone (25): yield 70% from **21** treated from excess acetic anhydride; mp 127−9 °C; ^{1}H NMR δ 1.42 and 1.46 (each 3H, s, 2′-CH_3), 1.64 and 1.94 (each 6H, s br, CH_2 × 6), 1.99 (2H, m, CH × 2), 2.10 (3H, s, $COCH_3$), 2.19 (1H, s, CH), 5.32 (1H, d, J = 4.8 Hz, 3′-H), 6.12 (1H, s, 3-H), 6.52 (1H, d, J = 4.8 Hz, 4′-H), 6.83 (1H, d, J = 9.0 Hz, 6-H), 7.51 (1H, d, J = 9.0 Hz, 5-H); $[\alpha]_D$ +20.3° (*c* 0.58, $CHCl_3$). Anal. ($C_{28}H_{32}O_7 \cdot 1^1/_2H_2O$) C, H.

4′-*O*-Adamantanecarbonyl-3′-*O*-(−)-camphanoyl-4-methyl-(+)-*cis*-khellactone (26): yield 78% from **21** treated with excess (−)-champhanic chloride; mp 256−7 °C; ^{1}H NMR δ 0.99, 1.06, 1.13, 1.42 and 1.51 (each 3H, s, CH_3 × 5), 1.68 and 1.90 (each 6H, s br, CH_2 × 6), 1.93 (2H, m, CH × 2), 2.10 (3H, s, $COCH_3$), 2.19 (1H, s, CH), 1.74, 1.90, 2.21 and 2.49 (each 1H, m, 2 × CH_2 in camphanoyl group), 5.34 (1H, d, J = 4.8 Hz, 3′-H), 6.12 (1H, s, 3-H), 6.58 (1H, d, J = 4.8 Hz, 4′-H), 6.84 (1H, d, J = 9.0 Hz, 6-H), 7.53 (1H, d, J = 9.0 Hz, 5-H); 95% d.e.; $[\alpha]_D$ +13.23° (*c* 0.65, $CHCl_3$). Anal. ($C_{36}H_{42}O_9 \cdot {}^1/_8H_2O$) C, H.

HIV Growth Inhibition Assay in H9 Lymphocytes. This assay was performed by Biotech Research Laboratories, Boston Biomedica, Inc. The general procedure was described in refs 18, 20, and 21.

HIV Growth Inhibition Assay in the CEM-SS Cell Line.[37,38] The promising active compounds were sent to NCI for further anti-HIV testing in CEM lymphocytes. The procedure follows below. (1) The candidate agent is dissolved in DMSO and then diluted 1:100 in cell culture medium before preparing serial half-log 10 dilutions. T4 lymphocytes (CEM cell line) are added, and after a brief interval, HIV-1 is added, resulting in a 1:200 final dilution of the compound. Uninfected cells with the compound serve as a toxicity control, and infected and uninfected cells without the compound serve as basic controls. (2) Cultures are incubated at 37 °C in a 5% carbon dioxide atmosphere for 6 days. (3) The tetrazolium salt, XTT, is added to all wells, and cultures are incubated to allow formazan color development by viable cells. (4) Individual wells are analyzed spectrophotometrically to quantitate formazan production and, in addition, are viewed microscopically for detection of viable cells and confirmation of protective activity. (5) Drug-treated virus-infected cells are compared with drug-treated noninfected cells and with other appropriate controls (untreated infected and untreated noninfected cells, drug-containing wells without cells, etc.) on the same plate. (6) Data are reviewed in comparison with other tests done at the same time, and a determination about activity is made.

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