Anti-AIDS Agents. 15. Synthesis and Anti-HIV Activity of Dihydroseselins and Related Analogs[1]

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Forty-two dihydroseselins based on the structure of suksdorfin (**1**) were synthesized in order to evaluate their anti-HIV activity. These synthetic derivatives include 3′,4′-di-*O*-acyl- and 3′- or 4′-*O*-acyl-*cis*-dihydroseselins (**8**–**21**) and 3′,4′-*trans*-dihydroseselins with *O*-acyl and/or *O*-alkyl groups at the 3′ and 4′ positions (**6**, **22**–**43**). Two 4′-azido (**44**, **45**) and three 4′-alkylamido (**46**, **48**, **49**) derivatives were also prepared. By using optically pure reagents, three pairs of diastereoisomers were synthesized and separated as optically pure compounds (**14**, **15**; **16**, **17**; **38**, **39**). Together with the above synthetic derivatives, seselin (**3**) and (±)-*cis*- (**4**), (+)-*cis*- (**5**), and (±)-*trans*-dihydroseselin-3′,4′-diol (**7**) were also tested for their *in vitro* anti-HIV activity. An optically pure compound, 3′,4′-di-*O*-(−)-camphanoyl-(+)-*cis*-khellactone (**16**), showed potent inhibitory activity and remarkable selectivity against HIV replication. The EC_{50} value and *in vitro* therapeutic index (TI) of **16** are 4×10^{-4} μM and 136 719, respectively, which are better than those shown by AZT in the same assay. In addition, compound **16** is also active against HIV replication in a monocytic cell line and in peripheral blood mononuclear cells (PBMCs). Our *in vitro* assay indicated that, like compound **1**, compound **16** is not an inhibitor of HIV-1 reverse transcriptase. Moreover, the anti-HIV activity of **16** is stereoselective as its three diastereoisomers (**17**, **38**, **39**) are at least 10 000 times less active. Since other synthetic dihydroseselin derivatives with different substituents or without any substituents are inactive or are active only at much higher concentrations, the antiviral potency of **16** could be associated with the camphanoyl moieties of its structure. Therefore, compound **16** represents a unique coumarin structure with promising anti-HIV activity.

Introduction

Acquired immunodeficiency syndrome (AIDS), the disease caused by the human immunodeficiency virus (HIV), has become a serious threat to public health due to its rapid spread, high mortality rate, and incurability. Although the first generation anti-HIV compounds, such as AZT and other HIV reverse transcriptase (RT) inhibitors, are very effective against viral replication in laboratory tests, they have limited or transient clinical benefit due to their toxicities and to the development of drug resistant virus.[2,3] Currently, the development of new anti-HIV agents is focused on discovering compounds either with novel structures or active through new mechanism(s) of action.

Recently, in our continuing bioactivity-directed isolation and characterization of new plant anti-HIV agents, suksdorfin (**1**),[4–6] isolated from *Lomatium suksdorfii*, was found to have anti-HIV activity with an *in vitro* EC_{50} value of 2.6 ± 2.1 μM and a therapeutic index (TI) 30.6 ± 22.4.[7] Combinations of **1** and the anti-HIV nucleosides ddI and ddC demonstrated statiscal synergy in inhibition HIV-1 replication (ddC > ddI). However, the viral inhibition mediated by combining **1** with AZT was not statistically synergistic. Furthermore, the presence of suksdorfin did not antagonize the suppression mediated by three nucleoside reverse transcriptase inhibitors. These results suggested that **1** may exert its anti-HIV activity through mechanism(s) other than inhibition of HIV RT and has potential for use in drug combination with RT inhibitors for AIDS therapy. Despite the potential clinical and research applications, **1** has a relatively low therapeutic index, which might limit its usefulness. In attempts to discover compounds with more potent and selective anti-HIV activity, our further efforts have been directed to modification of dihydroseselin analogs related to **1**. The syntheses of these analogs and their bioassay results are described in this paper.

Chemistry

Suksdorfin [(3′*R*,4′*R*)-3′-acetoxy-4′-(isovaleryloxy)-3′,4′-dihydroseselin] (**1**)[5,6] is a pyranocoumarin derivative, which has two *cis*-oriented acyl groups at the 3′ and 4′ positions (Figure 1). Our early study of coumarin derivatives, including **1** and pteryxin (**2**, Figure 1), suggested that changing the acyl group at the 3′ and 4′

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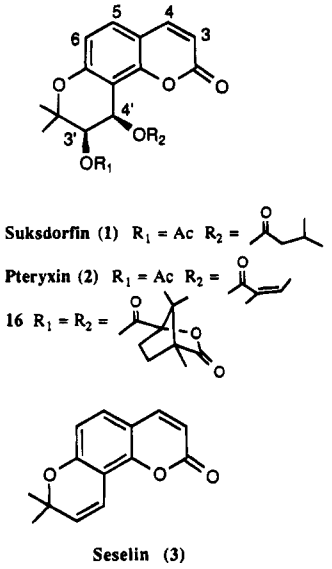


Figure 1. Structures of suksdorfin (**1**), pterysin (**2**), seselin (**3**), and 3′,4′-di-*O*-(−)-camphanoyl-(+)-*cis*-khellactone (**16**).

positions of the pyran ring affected the biological activities.[7] This finding led us to focus on chemical modification at the 3′ and 4′ positions of the dihydroseselin skeleton. By introducing different chemical moieties as well as changing the configurations at the 3′ and 4′ positions, two groups of dihydroseselin derivatives were synthesized and classified according to their 3′ and 4′ configurations: one with a *cis* configuration as found in compound **1**, and the other with a *trans* configuration.

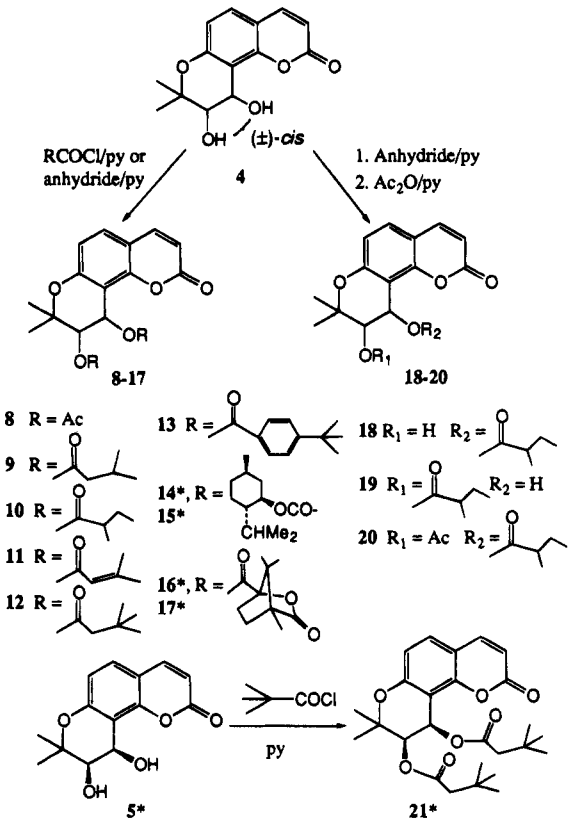
Since the syntheses began with a corresponding racemic material (**4**, **6**, **7**), most of the *cis* and *trans* products are (±)-mixtures except for **21**, which was synthesized from (+)-*cis*-khellactone (**5**). Optically pure compounds **14**−**17**, **38**, and **39** were obtained by introducing an optically active acyl group, which generally gave separable diastereoisomers.

Scheme 1 shows the syntheses of the compounds with a *cis* configuration. The (±)-3′,4′-di-*O*-acyl-*cis*-khellactone derivatives with two identical acyl groups at the 3′ and 4′ positions (**8**−**17**) were synthesized from (±)-*cis*-khellactone (**4**) using standard procedures. When using (*S*)-(+)-α-methylbutyric anhydride, monoesterified compounds (**18**, **19**) were produced. Subsequent acetylation of **18** with Ac_2O/pyridine gave compound **20** with different ester groups at C3′ and C4′. Although **18**−**20** are mixtures of diastereoisomers, separation by column chromatography was not successful. Compound **21**, one optically pure component of racemic **12**, was prepared from (+)-*cis*-khellactone (**5**), which was obtained by hydrolysis of **1**.

For syntheses of compounds with a *trans* configuration at the 3′ and 4′ carbons (which is different from the configuration of **1**), we introduced various esters at the 3′ and/or 4′ positions of the *trans*-khellactone molecule. In addition to the usual ester derivatives, compounds with azido (**44**, **45**), amide (**46**, **48**, **49**), and ether (**24**−**34**, **43**) substituent(s) were also prepared.

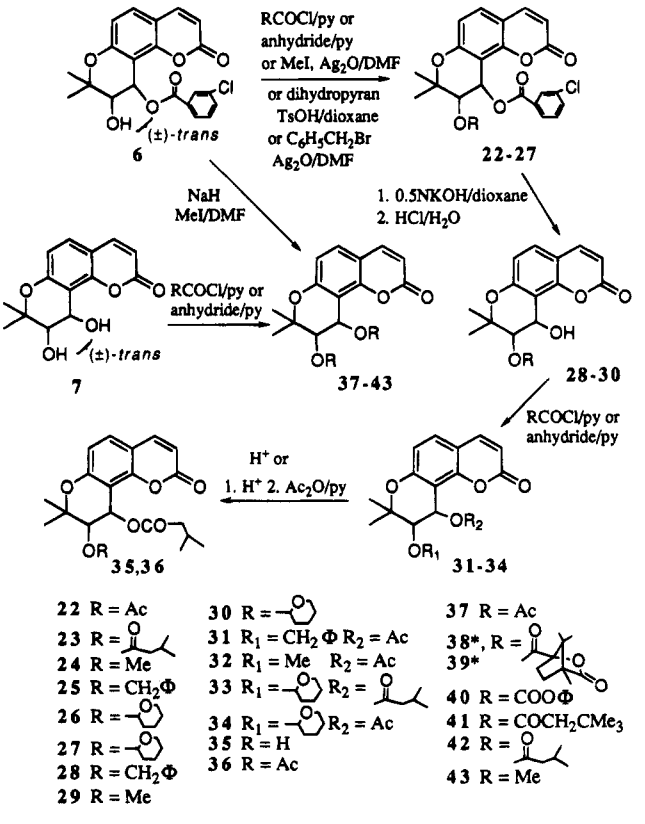
Scheme 2 summarizes the general synthetic procedures for the *trans* compounds. Compounds **37**−**42** with two identical ester groups were synthesized directly from **7**, while **43** was produced by treatment of **6** with NaH and MeI. Compounds **22**−**27**, which have two different substituents at C3′ and C4′, were obtained by

Scheme 1. Syntheses of *cis*-Khellactone Derivatives



* Optically pure compounds

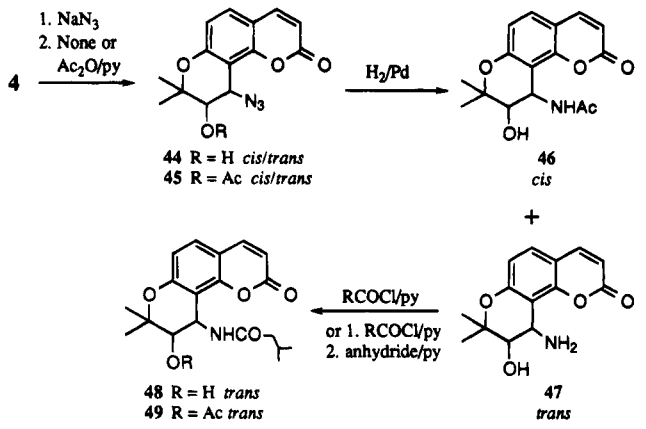
Scheme 2. Syntheses of *trans*-Khellactone Derivatives



* Optically pure compounds

treatment of the monoester **6** with acylating or alkylating reagents. By using 3,4-dihydro-2*H*-pyran, two isomers (**26**, **27**) were generated as a result of equal

Scheme 3. Syntheses of Azido- and Amidokhellactone Derivatives



addition of the hydroxyl oxygen to the pyran double bond from two opposite directions. These isomers with either *R* or *S* configuration at the acetal carbon were separated as their own 3′,4′-*trans* racemic mixtures. Subsequent treatment of the 4′-*O*-alkyl compounds **24**–**26** with base gave the hydrolysis products **28**–**30**. Acylation of **28**–**30** yielded a series of 3′-*O*-alkyl-4′-*O*-acylkhellactones (**31**–**34**). Finally, compound **36**, which is the *trans* isomer of **1**, was synthesized by acidic removal of the tetrahydropyranyl moiety of **33** to give **35**, followed by acetylation of **35** with Ac_2O/pyridine.

Two 4′-azido- (**44**, **45**) and three 4′-(alkylamido)-dihydroseselin derivatives (**46**, **48**, **49**) were synthesized from *cis*-khellactone (**4**) (Scheme 3). Treatment of **4** with sodium azide and trifluoroacetic acid gave selective substitution at the 4′-position and produced a mixture of *cis* and *trans* azido isomers (**44**). After acetylation of **44** with Ac_2O/pyridine, the product (**45**) was further reduced by hydrogenation on palladium/carbon. The expected amine derivative (**47**) was obtained togther with an acetamido derivative (**46**). This amide derivative (**46**) was a product of intramolecular migration of the 3′-acetyl group to the 4′-amine. This migration probably occurred in the *cis* isomer, since a similar migration of an acetyl group has been observed in a *cis* dihydroseselin.[8] The amine (**47**) was acylated to give **48**. Acetylation of **48** yielded compound **49**, which is a *trans* amido analog of **1**.

Stereochemistry of Compounds 16, 17, 38, 39. With the optically pure reagent (−)-camphanic chloride, compounds **16** and **17**, **38** and **39** were obtained as a mixture of diastereoisomers, which were separated by repeated column chromatography. On the other hand, hydrolysis with suksudorfin (**1**) with KOH in dioxane yielded (+)-*cis*-kehllactone [3′(*R*),4′(*R*)-configurations] as well as (−)-*trans*-kehllactone [3′(*R*),4′(*S*)-confuguarations].[6,9] Treatment of (+)-*cis*-kehllactone with (−)-camphanoyl chloride gave 3′,4′-di-*O*-(−)-camphanoyl-(+)-*cis*-kehllactone, which was shown to be identical with **16**. Therefore, the configurations at C-3′ and C-4′ in compound **17**, the diastereoisomer of **16**, were assigned as *S* and *S*, respectively. In contrast, (−)-*trans*-kehllactone was treated with (−)-camphanoyl chloride, furnishing 3′,4′-di-*O*-(−)-camphanoyl-(−)-*trans*-kehllactone, which was identical with **38**, thus confirming its stereostructure. Accordingly, the structure of **39** was assigned as 3′,4′-di-*O*-(−)-camphanoyl-(+)-*trans*-kehllactone.

Table 1. Anti-HIV Activities of Dihydroseselin Analogs in Acutely Infected H9 Lymphocytes

compd	EC_{50} (μM)	TI[a]	compd	EC_{50} (μM)	TI[a]
3	3.5	5	**24**	241	0.34
4	–[b]	–	**25**	3.1	5
5	–	–	**26**	–	–
6	4.2	2.4	**27**	8.3	10
7	–	–	**28**	43	2.7
8	69.4	0.4	**29**	–	–
9	7	2	**30**	–	–
10	7.0	14.4	**31**	38	2.3
11	4.7	3.5	**32**	–	–
12	–	–	**33**	–	–
13	<1.4	>2.2	**34**	–	–
14	–	–	**35**	–	–
15	11.4	2.9	**36**	–	–
16	4×10^{-4}	136,719	**37**	43	3
17	144.7	1.1	**38**	24.1	3
18	–	–	**39**	32	>5
19	–	–	**40**	–	–
20	–	–	**41**	–	–
21	21.8	2	**42**	20.9	3
22	–	–	**43**	–	–
23	9.3	1.4	**44**	115.5	2.9
1	2.6 ± 2.1	30.6 ± 22.4	**45**	115.9	1
2	4.6	4.5	**46**	–	–
			48	–	–
AZT	0.15	12,500	**49**	–	–

[a] *In vitro* therapeutic index, ratio of IC_{50}:EC_{50}. [b] –: no suppression.

Compounds **14** and **15** are another pair of diastereoisomers for which the absolute configurations still remain to be established.

Results and Discussion

The anti-HIV assay indicates that compound **16**, 3′,4′-di-*O*-(−)-camphanoyl-(+)-*cis*-khellactone (Figure 1) has potent anti-HIV activity in acutely infected H9 lymphocytes ($EC_{50} = 4 \times 10^{-4}$ μM) and a remarkable therapeutic index (136 719), which are better than those of AZT and compound **1** in the same assay (Table 1).

Two *cis* compounds (**10** and **11**) also show moderate anti-HIV activities with EC_{50} values of 7.0 and 4.7 μM, and TIs 14.4 and 3.5, respectively. Three *trans* compounds with a 4′-*m*-chlorobenzoyl group (**23**, **25**, and **27**) are slightly active with EC_{50} values ranging from 3.1 to 8.3 μM, and TIs from 1.4 to 10. However, none of them has biological profiles better than compound **1**. Other dihydroseselin derivatives in Table 1, either with various 3′ and 4′ substituents or without any substituents, such as seselin (**3**, Figure 1) or khellactones (**4**, **5**, and **7**), are inactive or toxic in the assay.

Moreover, our study indicates that the anti-HIV activity of compound **16**, the (+)-*cis*-khellactone derivative, is highly stereospecific as the (−)-*cis* (**17**), (−)-*trans* (**38**), and (+)-*trans* (**39**) isomers of **16** are at least ten thousand times less active. In addition, the data suggest that the stereochemistry of **1** may also play a role in its anti-HIV activity, as its (±)-*trans* isomer (**36**) and amido (±)-*trans* analog (**49**) are both inactive in the same assay. Since the stereochemistry in compounds **1** and **16** is identical at the 3′ and 4′ positions, the 3′ and 4′ configurations of the khellactone derivatives might be very important for the anti-HIV activity in this type of compound.

Therefore, a possible explanation for the low anti-HIV activity observed in most of the synthetic dihydroseselin analogs is that they are racemic mixtures. Since the anti-HIV activity of this type of compound is stereospe-

Table 2. Anti-HIV Activity of **16** in Different *in Vitro* Assays

		H9 lymphocytes	PBMCs	U937
16	EC_{50} (μM)	4×10^{-4}	2.9×10^{-2}	2.1×10^{-3}
	TI[a]	136 719	472	11,719

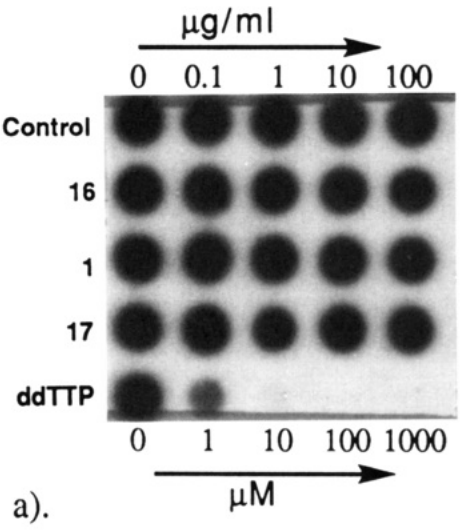
[a] *In vitro* therapeutic index, ratio of IC_{50}:EC_{50}.

cific as evidenced in **1** and **16**, an optically pure compound with the preferred configuration would display a lower EC_{50} value than its corresponding isomeric mixture.

Further comparison of the 4′ acyl groups of **1** and **16** suggested a structural similarity between the isovaleryl group in **1** and the (−)-camphanoyl group in **16**. Compound **16** also contains an isovaleryl domain. However, the isovaleryl domain in **16** is conformationally more rigid, since it is within the frame of 4′-camphanoyl group neighboring another bulky camphanoyl substituent. On the other hand, the isovaleryl in **1** is more conformationally flexible, in which its terminal carbon atoms are disordered over two orientations in a X-ray crystallographic analysis (data not shown). Therefore, it seems the conformational feature of the isovaleryl domains at C4′ could play a role in the anti-HIV activity of **1** and **16**. However, a general comparison of the substitution pattern between **16** and **1**, as well as other related compounds (**9**−**12**, **21**), indicated that the dramatically increased potency in **16** should be related to the presence of the unique camphanoyl groups, which appear to be structurally required for high anti-HIV activity and remarkable selectivity in the dihydroseselin type molecule.

Compound **16** also demonstrated potent inhibition of viral replication in further assays using HIV-1 infected PHA-stimulated PBMCs and a promonocytic cell line, U937 (Table 2). In an attempt to determine the mechanism of anti-HIV activity, compounds **16** and **1** were tested for anti-HIV-RT activity. However, in an *in vitro* RT assay using the poly(A) as the template, both compounds showed no activity up to 100 μg/mL, while dideoxythymidine triphosphate (ddTTP) displayed a dose-dependent inhibition of RT activity (Figure 2).

Recently, four coumarin derivatives, calanolides A and B and inophyllums B and P, isolated from *Calophyllum lanigerum* and *Calophyllum inophyllum* Linn, respectively, were reported as potent anti-HIV agents.[10,11] Their anti-HIV activity is due to inhibition of the HIV-1 reverse transcriptase. Structurally, these coumarin derivatives possess the same tricyclic pyranocoumarin subunit found in compounds **1** and **16**; however, they have an additional 5,6-ring system and a different substituent pattern at the C-4, C-2′, C-3′, and C-4′ positions (Figure 3). Moreover, the anti-HIV mechanisms of these tetracyclic coumarins and of the tricyclic compounds **1** and **16** are different. The anti-HIV activity of compound **16** is also about 2 orders of magnitude greater than that reported for the tetracyclic coumarins. Consequently, compounds **16** and **1**, especially **16**, are functionally and structurally unique anti-HIV coumarins that might exert their antiviral activities by a mechanism other than inhibition of HIV-RT. Their mechanisms of action are currently under investigation.



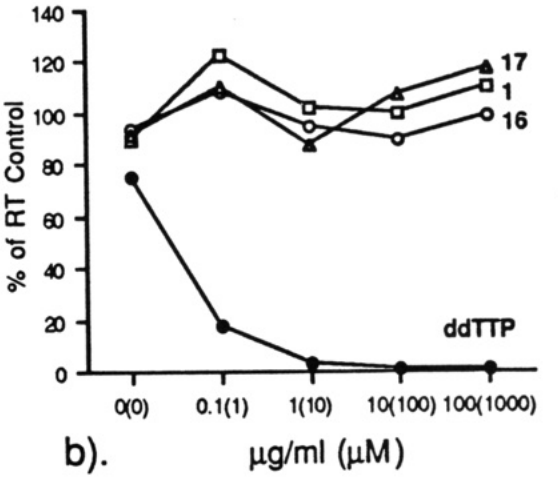
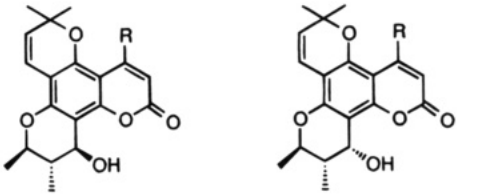


Figure 2. Assay of compounds **1**, **16**, and **17** on HIV-1 RT.[15,16] (a) Autoradiography of RT assay products. The intensity of the dots is proportional to the amount of catalytic product of HIV-1 reverse transcriptase. Control: samples are the reaction mixtures in the absence of drug. The concentrations of compounds **1**, **16**, and **17** are indicated on the top of the figure, while the concentrations of ddTTP are at the bottom. (b) Effect of compounds on RT activity as percent of control. For **1**, **16**, and **17**, each point represents the mean of two experiments.



Calanolide A R = propyl
Inophyllum B R = phenyl

Calanolide B R = propyl
Inophyllum P R = phenyl

Figure 3. Structures of calanolides and inophyllums.

Experimental Section

Chemistry. Melting points were measured with a Fisher-Johns melting point apparatus without correction. Optical rotations were determined with a Rudolph Research Autopol III polarimeter. IR spectra were recorded on a Perkin-Elmer Model 1320 spectrometer. The proton nuclear magnetic resonance (^{1}H NMR) spectra were measured on a Bruker AC-300 spectrometer with Me_4Si (TMS) as the internal reference with $CDCl_3$ as solvent. Elemental analyses were determined

by Atlantic Microlab, Inc., Norcross, GA. HR-FAB MS and positive FAB MS were recorded on a HX-110 JEOL spectrometer. Thin-layer chromatography (TLC) silica gel plates were purchased from Analtech, Inc. Silica gel (230–400 mesh) from Aldrich, Inc., was used for column chromatography.

Seselin (3). Seselin (**3**) was prepared according to the procedure reported by Hlubuek.[12] A mixture of 7-hydroxycoumarin (Aldrich Chemical Co.) (8 g, 49 mmol), K_2CO_3 (8 g, 58 mmol), and KI (1.2 g, 7.2 mmol) in 98% aqueous acetone (200 mL) was stirred at room temperature for 1 h, and then 3-chloro-3-methylbut-1-yne (8 g, 49 mmol) was added. The whole mixture was refluxed for 24 h with stirring. After addition of additional 3-chloro-3-methylbut-1-yne (8 g, 49 mmol) and K_2CO_3 (8 g, 58 mmol), the mixture was further refluxed for 24 h. After cooling, the mixture was filtered and concentrated to dryness. The residue was dissolved in Et_2O, washed successively with H_2O and brine, dried over Na_2SO_4, and concentrated. The residue was then dissolved in *N,N*-diethylaniline (100 mL), and the solution was refluxed for 2 h. After cooling, the solution was diluted with Et_2O, washed successively with 2 N H_2SO_4, water, and brine, dried over Na_2SO_4, and concentrated. The product was purified by silica gel chromatography [hexane–EtOAc (4:1 → 3:1)] to yield **3** (7.11 g, 63.6% yield) as pale yellow prisms (from MeOH): mp 119–120 °C; IR (KBr) 1715 (CO), 1620 (C=C) cm^{-1}; ^1H NMR δ 1.47 (s, 6H, 2′-$(CH_3)_2$), 5.73 (d, 1H, J =10 Hz, H-3′), 6.23 (d, 1H, J = 9.5 Hz, H-3), 6.72 (d, 1H, J = 8.5 Hz, H-6), 6.88 (d, 1H, J = 10 Hz, H-4′), 7.21 (d, 1H, J = 8.5 Hz, H-5), 7.60 (d, 1H, J = 9.5 Hz, H-4).

(±)-*cis*-Kehllactone (4). To a solution of seselin (**3**) (500 mg, 2.2 mmol) and *N*-methylmorpholine *N*-oxide (505 mg, 4.4 mmol) in 80% aqueous acetone (11 mL) was added 3% OsO_4 aqueous solution (1 mL), and the whole mixture was stirred at room temperature for 18 h. The reaction mixture was diluted with H_2O and concentrated to give an aqueous solution, which was extracted with EtOAc. The EtOAc layer was dried over Na_2SO_4 and concentrated to give a residue, which was crystallized from benzene, yielding **4** (497 mg, 86% yield) as colorless needles; mp 157–158 °C; IR (KBr) 3400 (OH), 1730 (CO), 1690, 1610 (C=C) cm^{-1}; ^1H NMR δ 1.41, 1.47 (each s, 3H, 2′-$(CH_3)_2$), 3.26 (d, 1H, J = 5 Hz, 3′-OH), 3.87 (t, 1H, J = 5 Hz, H-3′), 4.08 (br s, 1H, 4′-OH), 5.21 (d, 1H, J = 5 Hz, H-4′), 6.25 (d, 1H, J = 9.5 Hz, H-3), 6.80 (d, 1H, J = 8.5 Hz, H-6), 7.32 (d, 1H, J = 8.5 Hz, H-5), 7.65 (d, 1H, J = 9.5 Hz, H-4), which were identified as (±)-*cis*-kehllactone by comparison of the physical and spectral data with those described in the literature.[8]

4′-*O*-(*m*-Chlorobenzoyl)-(±)-*trans*-kehllactone (6). A mixture of seselin (**3**) (850 mg, 3.7 mmol) and *m*-chloroperbenzoic acid (800 mg, 5.6 mmol) in $CHCl_3$ (25 mL) was kept at room temperature for 2 days. The reaction mixture was diluted with ether, washed successively with saturated $NaHCO_3$ aqueous solution, water, and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was chromatographed over silica gel column [hexane–EtOAc (3:1)] to give **6** as colorless prisms (MeOH) (770 mg, 51% yield): mp 223–224 °C; IR (KBr) 3440 (OH), 1720 (CO), 1605 (C=C) cm^{-1}; ^1H NMR δ 1.48, 1.52 (each s, 3H, 2′-$(CH_3)_2$), 4.06 (d, 1H, J = 4.5 Hz, H-3′), 6.21 (d, 1H, J = 9.5 Hz, H-3), 6.39 (d, 1H, J = 8.5 Hz, H-6), 7.36 (t, 1H, J = 8 Hz, H-5″), 7.41 (d, 1H, J = 8.5 Hz, H-5), 7.52 (dd, 1H, J = 2, 8 Hz, H-4″), 7.62 (d, 1H, J = 9.5 Hz, H-4), 7.87–7.98 (m, 3H in total, H-2″ and 6″). Anal. ($C_{21}H_{17}ClO_6$) C, H, Cl.

(±)-*trans*-Kehllactone (7). A solution of **6** (750 mg, 1.88 mmol) in dioxane (12 mL) and 0.5 N KOH (18 mL) was stirred at room temperature for 30 min. The mixture was acidified with 20% HCl and stirred further 1 h at room temperature. The mixture was concentrated under reduced pressure to give an aqueous solution, which was extracted by EtOAc. The EtOAc layer was dried over Na_2SO_4 and concentrated. The product was purified by preparative TLC [hexane–EtOAc (1:2)] to afford **7**[13] (176 mg, 35.8% yield) as colorless needles (MeOH); mp 185–186 °C; IR (KBr) 3380 and 3425 (OH), 1700 (CO), 1600 (C=C) cm^{-1}; ^1H NMR δ 1.31, 1.54 (each s, 3H, 2′-$(CH_3)_2$), 2.78 (d, 1H, J = 4, 7 Hz, 3′-OH), 3.85 (dd, 1H, J = 4, 7 Hz, H-3′), 3.98 (d, 1H, J = 3.5 Hz, 4′-OH), 5.00 (dd, 1H, J = 3.5, 7 Hz, H-4′), 6.25 (d, 1H, J = 9.5 Hz, H-3), 6.79 (d, 1H, J = 8.5 Hz, H-6), 7.32 (d, 1H, J = 8.5 Hz, H-5), 7.66 (d, 1H, J = 9.5 Hz, H-4).

Preparation of (+)-*cis*-Kehllactone (5) and (−)-*trans*-Kehllactone. A solution of suksdorfin (**1**) (750 mg, 1.93 mmol) in dioxane (12 mL) and 0.5 N KOH (18 mL) was stirred at room temperature for 30 min. The reaction mixture was acidified with 20% HCl and stirred further 1 h at room temperature. The mixture was concentrated under reduced pressure to give an aqueous solution, which was extracted by EtOAc. The EtOAc layer was dried over Na_2SO_4 and concentrated to give a residue, which was subjected to silica gel chromatography. Elution with $CHCl_3$–MeOH–H_2O (10:1:0.1) afforded (+)-*cis*-kehllactone (30 mg) as colorless needles (benzene): mp 174–175 °C; $[\alpha]^{20}_D$ +87° (c = 0.5, $CHCl_3$); IR and ^1H NMR were identical with **4**. Further elution with same solvent system furnished (−)-*trans*-kehllactone (12 mg) as colorless needles (benzene); mp 182–185 °C; $[\alpha]^{20}_D$ −20.2° (c = 0.5, $CHCl_3$); IR and ^1H NMR were identical with **7**. The structure comfirmation of these products was obtained by comparison of the physical and spectral data with those described in the literature.[9,14]

General Procedure for Synthesizing *O*-Acylkhellactones (8, 9, 11–17, 20–23, 31–34, 36–42) and (±)-*trans*-3′-(Acyloxy)-4′-azido- (45) and (±)-*trans*-3′-(Acyloxy)-4′-(alkylamido)dihydroseselins (49). To a solution of a corresponding alcohol (**4–7**, **19**, **28–30**, **35**, **44**, and **48**) or amine (**47**) in anhydrous CH_2Cl_2 containing 20% dry pyridine was added an appropriate acyl chloride or anhydride (2.5–3 equiv) at 0 °C or at room temperature, respectively. The mixture was allowed to stand overnight, and the volatiles were evaporated *in vacuo*. Alternatively, the mixture was poured into water and extracted with $CHCl_3$. The organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue was chromatographed on preparative TLC plates or silica gel columns to afford the product.

3′,4′-Di-*O*-acetyl-*cis*-khellactone (8): yield 83% (starting with 20.0 mg of **4**); crystallization from acetone gave colorless needles; mp 164–165 ° C; HR-FAB MS calcd for $C_{18}H_{18}O_7$ 346.1053, found m/z 346.1054; positive FAB MS m/z 715 (2M + Na)$^+$, 369 (M + Na)$^+$; IR (KBr) 1750 (CO, lactone), 1720 (CO, ester), 1612 (C=C) cm^{-1}; ^1H NMR δ 1.41, 1.45 (each s, 3H, 2′-$(CH_3)_2$), 2.14, 2.10 (each s, 3H, $OCOCH_3$-3′,4′), 5.30 (d, J = 5.0 Hz, 1H, H-3′), 6.24 (d, J = 9.5 Hz, 1H, H-3), 6.54 (d, J = 5.0 Hz, 1H, H-4′), 6.80 (d, J = 8.5 Hz, 1H, H-6), 7.35 (d, J = 8.5 Hz, 1H, H-5), 7.60 (d, J = 9.5 Hz, 1H, H-4). Anal. ($C_{18}H_{18}O_7$·$^1/_4H_2O$) C, H.

3′,4′-Di-*O*-isovaleryl-*cis*-khellactone (9): yield 62% (starting with 25.5 mg of **4**); amorphous; mp 126–128 °C; HR-FAB MS calcd for $C_{24}H_{30}O_7Na$ 453.1889, found m/z 453.1889 (M + Na)$^+$; positive FAB MS m/z 883 (2M + Na)$^+$, 453 (M + Na)$^+$; IR (KBr) 1745 (CO, lactone), 1730 (CO, ester), 1608 (C=C) cm^{-1}; ^1H NMR δ 0.95–1.05 (m, 12H, 2 x isovaleryl $(CH_3)_2$), 1.41, 1.44 (each s, 3H, 2′-$(CH_3)_2$), 2.06–2.35 (m, 2H, 2 x CH), 2.23 (m, 4H, 2 x CH_2), 5.32 (d, J = 5.0 Hz, 1H, H-3′), 6.23 (d, J = 9.5 Hz, 1H, H-3), 6.55 (d, J = 5.0 Hz, 1H, H-4′), 6.79 (d, J = 8.5 Hz, 1H, H-6), 7.35 (d, J = 8.5 Hz, 1H, H-5), 7.58 (d, J = 9.5 Hz, 1H, H-4). Anal. ($C_{24}H_{30}O_7$·$^1/_4H_2O$) C, H.

3′,4′-Di-*O*-senecioyl-*cis*-khellactone (11): yield 30% (starting with 25.0 mg of **4**); amorphous; mp 127–129 °C; IR (KBr) 1760 (CO, lactone), 1740 (CO, ester), 1650 (C=C, senecioyl), and 1615 (C=C) cm^{-1}; ^1H NMR δ 1.43, 1.47 (each s, 3H, 2′-$(CH_3)_2$), 1.88, 1.89, 2.15, 2.10 (each s, 3H, 2 x senecioyl $(CH_3)_2$), 5.36 (d, J = 5.0 Hz, 1H, H-3′), 5.65 (each s, 1H, 2 x senecioyl CH), 6.21 (d, J = 9.5 Hz, 1H, H-3), 6.63 (d, J = 5.0 Hz, 1H, H-4′), 6.80 (d, J = 8.5 Hz, 1H, H-6), 7.34 (d, J = 8.5 Hz, 1H, H-5), 7.58 (d, J = 9.5 Hz, 1H, H-4). Anal. ($C_{24}H_{26}O_7$) C, H.

3′,4′-Bis-*O*-(*tert*-butylacetyl)-*cis*-khellactone (12): yield 34% (starting with 53.0 mg of **4**); crystallization from EtOH gave colorless needles; mp 133–134 ° C; IR (KBr) 1745 (CO), 1605 (C=C) cm^{-1}; ^1H NMR δ 1.05, 1.60 (each s, 9H, 2 x butyl $(CH_3)_3$), 1.43, 1.45 (each s, 3H, 2′-$(CH_3)_2$), 2.20–2.37 (m, 4H, 2 x CH_2), 5.31 (d, J = 4.5 Hz, 1H, H-3′), 6.22 (d, J = 9.5 Hz, 1H, H-3), 6.55 (d, J = 4.5 Hz, 1H, H-4′), 6.78 (d, J = 8.5 Hz, 1H,

H-6), 7.34 (d, J = 8.5 Hz, 1H, H-5), 7.58 (d, J = 9.5 Hz, 1H, H-4). Anal. ($C_{26}H_{34}O_7$) C, H.

3′,4′-Bis-*O*-(4-*tert*-butylbenzoyl)-*cis*-khellactone (13): yield 81% (starting with 48.0 mg of **4**); amorphous; mp 255–256 ° C; HR-FAB MS calcd for $C_{36}H_{38}O_7Na$ 805.2516, found m/z 805.2518 (M + Na)$^+$; positive FAB MS m/z 805 (M + Na)$^+$; IR (KBr) 1740 (CO), 1615 (C=C) cm^{-1}; ^{1}H NMR δ 1.30 (s, 18H, 2 x butyl (CH_3)$_3$), 1.50, 1.64 (each s, 3H, 2′-(CH_3)$_2$), 5.65 (d, J = 5.0 Hz, 1H, H-3′), 6.18 (d, J = 9.5 Hz, 1H, H-3), 6.88 (d, J = 8.5 Hz, 1H, H-6), 6.94 (d, J = 5.0 Hz, 1H, H-4′), 7.40 (d, J = 8.5 Hz, 1H, H-5), 7.58 (d, J = 9.5 Hz, 1H, H-4), 7.33–7.35 and 7.79–7.83 (m, 8H, benzoyl H). Anal. ($C_{36}H_{38}O_7$·$^1/_2H_2O$) C, H.

3′,4′-Bis-*O*-((−)-3-menthyloxycarbonyl)-*cis*-khellactone (14): yield 50% (starting with 93.0 mg of **4**); gum; HR-FAB MS calcd for $C_{36}H_{50}O_9Na$ 649.3352, found m/z 649.3350 (M + Na)$^+$; positive FAB MS m/z 649 (M + Na)$^+$; IR (KBr) 1750 (CO), 1610 (C=C) cm^{-1}; $[\alpha]^{20}_D$ −53.2° (c = 0.5, $CHCl_3$); ^{1}H NMR δ 0.79–0.97 (m, 22H, 6 x menthyl CH_3 and 2 x menthyl CH_2), 0.99–1.14 (m, 4H, 2 x menthyl CH_2), 1.38–1.42 (m, 2H, 2 x menthyl CH), 1.45, 1.47 (each s, 3H, 2′-(CH_3)$_2$), 1.65–1.69 (m, 4H, 2 x menthyl CH_2), 1.91–2.35 (m, 4H, 4 x menthyl CH), 4.58–4.63 (m, 2H, 2 x menthyl OCOCH), 5.10 (d, J = 5.0 Hz, 1H, H-3′), 6.22 (d, J = 9.5 Hz, 1H, H-3), 6.45 (d, J = 5.0 Hz, 1H, H-4′), 6.76 (d, J = 8.5 Hz, 1H, H-6), 7.32 (d, J = 8.5 Hz, 1H, H-5), 7.56 (d, J = 9.5 Hz, 1H, H-4).

3′,4′-Bis-*O*-((−)-3-menthyloxycarbonyl)-*cis*-khellactone (15): yield 37% (starting with 93.0 mg of **4**); gum; HR-FAB MS calcd for $C_{36}H_{50}O_9Na$ 649.3352, found m/z 649.3353 (M + Na)$^+$; positive FAB MS m/z 649 (M + Na)$^+$; IR (KBr) 1760 (CO), 1613 (C=C) cm^{-1}; $[\alpha]^{20}_D$ −43.6° (c = 0.5, $CHCl_3$); ^{1}H NMR δ 0.81–0.97 (m, 20H, 6 x menthyl CH_3, menthyl CH_2), 1.01–1.14 (m, 4H, 2 x menthyl CH_2), 1.33–1.49 (m, 4H, menthyl CH_2 and 2 x menthyl CH), 1.66–1.71 (m, 4H, 2 x menthyl CH_2), 1.98–2.17 (m, 4H, 4 x menthyl CH), 4.53–4.72 (m, 2H, 2 x menthyl OCOCH), 1.47, 1.48 (each s, 3H, 2′-(CH_3)$_2$), 5.10 (d, J = 5.0 Hz, 1H, H-3′), 6.22 (d, J = 9.5 Hz, 1H, H-3), 6.41 (d, J = 5.0 Hz, 1H, H-4′), 6.76 (d, J = 8.5 Hz, 1H, H-6), 7.34 (d, J = 8.5 Hz, 1H, H-5), 7.57 (d, J = 9.5 Hz, 1H, H-4). Anal. ($C_{36}H_{50}O_9$·$^1/_2H_2O$) C, H.

3′,4′-Di-*O*-(−)-camphanoyl-(+)-*cis*-khellactone (16): yield 27% (starting with 200.0 mg of **4**); colorless needles (from EtOH); mp 200–202 ° C; $[\alpha]^{20}_D$ +31.1° (c = 0.5, $CHCl_3$); positive FAB MS m/z 623 (M + H)$^+$, 425 (M − camphanic acid)$^+$, 227 (M − 2 x camphanic acid)$^+$; IR (KBr) 1790, 1745 (COO), 1605 (C=C) cm^{-1}; ^{1}H NMR δ 0.98, 1.01, 1.08, 1.10, 1.11, 1.12 (each s, 3H, camphanoyl CH_3), 1.45, 1.50 (each s, 3H, 2′-CH_3), 1.70, 1.94, 2.23, 2.50 (each m, 2H, camphanoyl CH_2), 5.39 (d, 1H, J = 5 Hz, H-3′), 6.24 (d, 1H, J = 9.5 Hz, H-3), 6.66 (d, 1H, J = 5 Hz, H-4′), 6.82 (d, 1H, J = 8.5 Hz, H-6), 7.41 (d, 1H, J = 8.5 Hz, H-5), 7.62 (d, 1H, J = 9.5 Hz, H-4). Anal. ($C_{34}H_{38}O_{11}$) C, H.

3′,4′-Di-*O*-(−)-camphanoyl-(−)-*cis*-khellactone (17): yield 80% (starting with 200.0 mg of **4**); colorless needles (from EtOH); mp 242–244 ° C; $[\alpha]^{20}_D$ −67.7° (c = 0.5, $CHCl_3$); positive FAB MS m/z 623 (M + H)$^+$, 425 (M − camphanic acid)$^+$, 227 (M − 2 x camphanic acid)$^+$; IR (KBr) 1780, 1750 (COO), 1605 (C=C) cm^{-1}; ^{1}H NMR δ 0.94, 1.04, 1.06, 1.12, 1.13, (each s, 18H in total, camphanoyl CH_3), 1.45, 1.56 (each s, 3H, 2′-CH_3), 1.70, 1.93, 2.10, 2.34, 2.55 (each m, 8H in total, camphanoyl CH_2), 5.47 (d, 1H, J = 4.5 Hz, H-3′), 6.22 (d, 1H, J = 9.5 Hz, H-3), 6.74 (d, 1H, J = 4.5 Hz, H-4′), 6.82 (d, 1H, J = 8.5 Hz, H-6), 7.40 (d, 1H, J = 8.5 Hz, H-5), 7.61 (d, 1H, J = 9.5 Hz, H-4). Anal. ($C_{34}H_{38}O_{11}$) C, H.

3′-*O*-Acetyl-4′-(α-methylbutyryl)-*cis*-khellactone (20): yield 54% (starting with 9.0 mg of **19**); gum; HR-FAB MS calcd for $C_{21}H_{24}O_7Na$ 411.1420, found m/z 411.1423 (M + Na)$^+$; positive FAB MS m/z 411 (M + Na)$^+$; IR (KBr) 1740 (CO), 1610 (C=C) cm^{-1}; ^{1}H NMR δ 0.94, 0.96 (each t, J = 7.5 Hz, $^3/_2$H, CH_2CH_3), 1.17, 1.22 (each d, J = 7.0 Hz, $^3/_2$H, $CHCH_3$), 1.42, 1.45 (each s, 3H, 2′-(CH_3)$_2$), 1.45–1.80 (m, 2H, CH_2), 2.09 (s, 3H, $COCH_3$), 2.35–2.46 (m, 1H, CH), 5.30, 5.32 (each d, J = 5.0 Hz, $^1/_2$H, H-3′), 6.23 (d, J = 9.5 Hz, 1H, H-3), 6.53, 6.54 (each d, J = 5.0 Hz, $^1/_2$H, H-4′), 6.80 (d, J = 8.5 Hz, 1H, H-6), 7.36 (d, J = 8.5 Hz, 1H, H-5), 7.59 (d, J = 9.5 Hz, 1H, H-4).

3′,4′-Bis-*O*-(*tert*-butylacetyl)-(+)-*cis*-khellactone (21): yield 58% (starting with 15.0 mg of **5**); mp, IR, and ^{1}H NMR are the same as **12**, $[\alpha]^{20}_D$ −13° (c = 0.5, $CHCl_3$).

3′-*O*-Acetyl-4′-*O*-(*m*-chlorobenzoyl)-*trans*-khellactone (22): yield 65% (starting with 46.0 mg of **6**); crystallization from acetone gave colorless prisms; mp 205–206 ° C; IR (KBr) 1740 (CO, lactone), 1720 (CO, ester), 1605 (C=C) cm^{-1}; ^{1}H NMR δ 1.41, 1.52 (each s, 3H, 2′-(CH_3)$_2$), 2.11 (s, 3H, $COCH_3$), 5.41 (d, J = 3.5 Hz, 1H, H-3′), 6.20 (d, J = 9.5 Hz, 1H, H-3), 6.43 (d, J = 3.5 Hz, 1H, H-4′), 6.87 (d, J = 8.5 Hz, 1H, H-6), 7.33–7.38 (m, 1H, benzoyl H-5″), 7.42 (d, J = 8.5 Hz, 1H, H-5), 7.51 (d, J = 8.0 Hz, 1H, benzoyl H-4″), 7.60 (d, J = 9.5 Hz, 1H, H-4), 7.90–7.95 (m, 2H, benzoyl H-2″,6″). Anal. ($C_{23}H_{19}ClO_7$) C, H, Cl.

3′-*O*-Isovaleryl-4′-*O*-(*m*-chlorobenzoyl)-*trans*-khellactone (23): yield 56% (starting with 50.0 mg of **6**); amorphous; mp 144–146 ° C; IR (KBr) 1730 (CO), 1610 (C=C) cm^{-1}; ^{1}H NMR δ 0.93 (d, J = 6.5 Hz, 6H, isovaleryl (CH_3)$_2$), 1.40, 1.51 (each s, 3H, 2′-(CH_3)$_2$), 2.04–2.11 (m, 1H, CH), 2.23–2.26 (m, 2H, CH_2), 5.42 (d, J = 4.0 Hz, 1H, H-3′), 6.20 (d, J = 9.5 Hz, 1H, H-3), 6.43 (d, J = 4.0 Hz, 1H, H-4′), 6.86 (d, J = 8.5 Hz, 1H, H-6), 7.32–7.37 (m, 1H, benzoyl H-5″), 7.40 (d, J = 8.5 Hz, 1H, H-5), 7.50 (d, J = 8.0 Hz, 1H, benzoyl H-4″), 7.60 (d, J = 9.5 Hz, 1H, H-4), 7.90–7.95 (m, 2H, benzoyl H-2″,6″). Anal. ($C_{26}H_{25}ClO_7$) C, H, Cl.

3′-*O*-Benzyl-4′-*O*-acetyl-*trans*-khellactone (31): yield 80% (starting with 70.0 mg of **28**); amorphous; mp 153–155 °C; HR-FAB MS calcd for $C_{23}H_{22}O_6$ 394.1416, found m/z 394.1413; positive FAB MS m/z 811 (2M + Na)$^+$, 417 (M + Na)$^+$; IR (KBr) 1730 (CO, lactone), 1710 (CO, ester), 1610 (C=C) cm^{-1}; ^{1}H NMR δ 1.33, 1.38 (each s, 3H, 2′-(CH_3)$_2$), 2.13 (s, 3H, $COCH_3$), 3.58 (d, J = 2.5 Hz, 1H, H-3′), 4.75, 4.89 (each d, J = 12.0 Hz, 1H, $ArCH_2$), 6.24 (d, J = 9.5 Hz, 1H, H-3), 6.32 (d, J = 2.5 Hz, 1H, H-4′), 6.80 (d, J = 8.5 Hz, 1H, H-6), 7.28–7.41 (m, 6H, H-5 and benzyl ArH-2′,3′,4′,5′,6′), 7.61 (d, J = 9.5 Hz, 1H, H-4). Anal. ($C_{23}H_{22}O_6$·$^1/_4H_2O$) C, H.

3′-*O*-Methyl-4′-*O*-acetyl-*trans*-khellactone (32): yield 64% (starting with 12.2 mg of **29**); amorphous; mp 148–150 ° C; IR (KBr) 1735 (CO), 1608 (C=C) cm^{-1}; ^{1}H NMR δ 1.39, 1.49 (each s, 3H, 2′-(CH_3)$_2$), 2.14 (s, 3H, $COCH_3$), 3.40 (d, J = 3.0 Hz, 1H, H-3′), 3.62 (s, 3H, OMe), 6.24 (d, J = 9.5 Hz, 1H, H-3), 6.25 (d, J = 3.0 Hz, 1H, H-4′), 6.80 (d, J = 8.5 Hz, 1H, H-6), 7.35 (d, J = 8.5 Hz, 1H, H-5), 7.62 (d, J = 9.5 Hz, 1H, H-4). Anal. ($C_{17}H_{18}O_6$) C, H.

3′-*O*-(2″-Tetrahydropyranyl)-4′-*O*-isovaleryl-*trans*-khellactone (33): yield 67% (starting with 57.0 mg of **30**); yellow oil; HR-FAB MS calcd for $C_{24}H_{30}O_7Na$ 453.1889, found m/z 453.1891 (M + Na)$^+$; positive FAB MS m/z 453 (M + Na)$^+$; IR (KBr) 1740 (CO), 1610 (C=C) cm^{-1}; ^{1}H NMR δ 0.96–1.00 (m, 6H, isovaleryl (CH_3)$_2$), 1.39, 1.47 (each s, 3H, 2′-(CH_3)$_2$), 142–1.52, 1.69–1.78 (m, 6H, tetrahydropyranyl CH_2-3″,4″,5″), 2.08–2.17 (m, 1H, CH), 2.21–2.25 (m, 2H, isovaleryl CH_2), 3.55–3.62, 3.87–3.99 (m, 2H, CH_2-6″), 3.84 (d, J = 2.5 Hz, 1H, H-3′), 4.84–4.86 (m, 1H, CH-1″), 6.21 (d, J = 9.5 Hz, 1H, H-3), 6.46 (d, J = 2.5 Hz, 1H, H-4′), 6.80 (d, J = 8.5 Hz, 1H, H-6), 7.33 (d, J = 8.5 Hz, 1H, H-5), 7.59 (d, J = 9.5 Hz, 1H, H-4).

3′-*O*-(2″-Tetrahydropyranyl)-4′-*O*-acetyl-*trans*-khellactone (34): yield 16% (starting with 190.0 mg of **30**); crystallization from acetone gave colorless needles; mp 169–170 °C; IR (KBr) 1740 (CO) and 1605 (C=C) cm^{-1}; ^{1}H NMR δ 1.41, 1.60 (each s, 3H, 2′-(CH_3)$_2$), 2.13 (s, 3H, $COCH_3$), 144–1.58, 1.67–1.77 (m, 6H, tetrahydropyranyl CH_2-3″,4″,5″), 3.55–3.63, 3.93–3.96 (m, 2H, CH_2-6″), 3.98 (d, J = 2.5 Hz, 1H, H-3′), 5.06–5.08 (m, 1H, CH-1″), 6.15 (d, J = 2.5 Hz, 1H, H-4′), 6.25 (d, J = 9.5 Hz, 1H, H-3), 6.83 (d, J = 8.5 Hz, 1H, H-6), 7.36 (d, J = 8.5 Hz, 1H, H-5), 7.62 (d, J = 9.5 Hz, 1H, H-4). Anal. ($C_{21}H_{24}O_7$) C, H.

3′-*O*-Acetyl-4′-*O*-isovaleryl-*trans*-khellactone (36): yield 50% (starting with 30.0 mg of **35**); crystallization from acetone gave colorless needles; mp 175–176 ° C; IR (KBr) 1748 (CO, lactone), 1730 (CO, ester), 1610 (C=C) cm^{-1}; ^{1}H NMR δ 0.98, 0.99 (each d, J = 6.5 Hz, 3H, isovaleryl (CH_3)$_2$), 1.38, 1.45 (each s, 3H, 2′-(CH_3)$_2$), 2.10 (s, 3H, $COCH_3$), 2.11–2.21 (m, 1H, CH), 2.24–2.35 (m, 2H, CH_2), 5.29 (d, J = 4.0 Hz, 1H, H-3′), 6.22 (d, J = 4.0 Hz, 1H, H-4′), 6.24 (d, J = 9.5 Hz, 1H, H-3), 6.83

(d, J = 8.5 Hz, 1H, H-6), 7.38 (d, J = 8.5 Hz, 1H, H-5), 7.62 (d, J = 9.5 Hz, 1H, H-4). Anal. ($C_{21}H_{24}O_7$) C, H.

3′,4′-Di-*O*-acetyl-*trans*-khellactone (37): yield 68% (starting with 20.0 mg of **7**); crystallization from acetone gave colorless needles; mp >260 °C; HR-FAB MS calcd for $C_{18}H_{18}O_7Na$ 369.0950, found m/z 369.0951 $(M + Na)^+$; positive FAB MS m/z 369 $(M + Na)^+$; IR (KBr) 1758 (CO, lactone), 1735 (CO, ester), 1612 (C=C) cm^{-1}; ^1H NMR δ 1.37, 1.45 (each s, 3H, 2′-$(CH_3)_2$), 2.11, 2.14 (each s, 3H, $OCOCH_3$-3′,4′), 5.29 (d, J = 4.5 Hz, 1H, H-3′), 6.22 (d, J = 4.5 Hz, 1H, H-4′), 6.25 (d, J = 9.5 Hz, 1H, H-3), 6.82 (d, J = 8.5 Hz, 1H, H-6), 7.37 (d, J = 8.5 Hz, 1H, H-5), 7.61 (d, J = 9.5 Hz, 1H, H-4). Anal. ($C_{18}H_{18}O_7{}^3/_4H_2O$) C, H.

3′,4′-Di-*O*-(−)-camphanoyl-(−)-*trans*-khellactone (38): yield 51% (starting with 100.0 mg of **7**); colorless needles (from EtOH); mp 249−251 °C; $[\alpha]^{20}_D$ +18.4° (c = 0.5, $CHCl_3$); positive FAB MS m/z 623 $(M + H)^+$, 425 (M − camphanic acid)$^+$, 227 (M − 2 x camphanic acid)$^+$; IR (KBr) 1790, 1770, 1750 (COO), 1610 (C=C) cm^{-1}; ^1H NMR δ 0.97, 0.98, 1.00, 1.08, 1.09, 1.12 (each s, 3H, camphanoyl CH_3), 1.41, 1.50 (each s, 3H, 2′-CH_3), 1.66, 1.93, 2.07, 2.46, 2.50 (each m, 8H in total, camphanoyl CH_2), 5.39 (d, 1H, J = 3.5 Hz, H-3′), 6.24 (d, 1H, J = 9.5 Hz, H-3), 6.30 (d, 1H, J = 3.5 Hz, H-4′), 6.86 (d, 1H, J = 8.5 Hz, H-6), 7.42 (d, 1H, J = 8.5 Hz, H-5), 7.63 (d, 1H, J = 9.5 Hz, H-4). Anal. ($C_{34}H_{38}O_{11}$) C, H.

3′,4′-Di-*O*-(−)-camphanoyl-(+)-*trans*-khellactone (39): yield 19% (starting with 100.0 mg of **7**); colorless needles (from EtOH); mp 253−254 °C; $[\alpha]^{20}_D$ −42.0° (c = 0.5, $CHCl_3$); positive FAB MS m/z 623 $(M + H)^+$, 425 (M − camphanic acid)$^+$, 227 (M − 2 x camphanic acid)$^+$; IR (KBr) 1800, 1750, 1735 (COO), 1605 (C=C) cm^{-1}; ^1H NMR δ 0.99, 1.06, 1.07, 1.09, 1.10 (each s, 18H in total, camphanoyl CH_3), 1.41, 1.50 (each s, 3H, 2′-CH_3), 1.68, 1.92, 2.12, 2.49 (each m, 2H, camphanoyl CH_2), 5.40 (d, 1H, J = 3.5 Hz, H-3′), 6.26 (d, 1H, J = 9.5 Hz, H-3), 6.29 (1H, d, J = 3.5 Hz, H-4′), 6.84 (1H, d, J = 8.5 Hz, H-6), 7.41 (1H, d, J = 8.5 Hz, H-5), 7.64 (1H, d, J = 9.5 Hz, H-4). Anal. ($C_{34}H_{38}O_{11}$) C, H.

3′,4′-Bis-*O*-(phenyloxycarbonyl)-*trans*-khellactone (40): yield 38% (starting with 54.0 mg of **7**); amorphous; mp 134−135 °C; HR-FAB MS calcd for $C_{28}H_{22}O_9Na$ 525.1162, found m/z 525.1163 $(M + Na)^+$; positive FAB MS m/z 1027 $(2M + Na)^+$, 525 $(M + Na)^+$; IR (KBr) 1775 (CO, lactone), 1740 (CO, ester), 1610 (C=C) cm^{-1}; ^1H NMR δ 1.51, 1.59 (each s, 3H, 2′-$(CH_3)_2$), 5.33 (d, J = 4.5 Hz, 1H, H-3′), 6.30 (d, J = 9.5 Hz, 1H, H-3), 6.38 (d, J = 4.5 Hz, 1H, H-4′), 6.86 (d, J = 8.5 Hz, 1H, H-6), 7.15−7.44 (m, 11H, H-5 and 2 x phenyl Hs), 7.64 (d, J = 9.5 Hz, 1H, H-4). Anal. ($C_{28}H_{22}O_9{}^1/_2H_2O$) C, H.

3′,4′-Bis-*O*-(*tert*-butylacetyl)-*trans*-khellactone (41): yield 59% (starting with 170.0 mg of **7**); crystallization from MeOH gave colorless needles; mp 118−120 °C; IR (KBr) 1745 (CO, lactone), 1730 (CO, ester), 1605 (C=C) cm^{-1}; ^1H NMR δ 1.02, 1.05 (each s, 9H, 2 x butyl $(CH_3)_3$), 1.38, 1.45 (each s, 3H, 2′-$(CH_3)_2$), 2.22−2.33 (m, 4H, 2 x CH_2), 5.28 (d, J = 3.5 Hz, 1H, H-3′), 6.18 (d, J = 3.5 Hz, 1H, H-4′), 6.23 (d, J = 9.5 Hz, 1H, H-3), 6.82 (d, J = 8.5 Hz, 1H, H-6), 7.36 (d, J = 8.5 Hz, 1H, H-5), 7.60 (d, J = 9.5 Hz, 1H, H-4). Anal. ($C_{26}H_{34}O_7$) C, H.

3′,4′-Di-*O*-isovaleryl-*trans*-khellactone (42): yield 37% (starting with 98.0 mg of **7**); crystallization from acetone gave colorless needles; mp 110−111 °C; HR-FAB MS calcd for $C_{24}H_{30}O_7Na$ 453.1889, found m/z 453.1889 $(M + Na)^+$; positive FAB MS m/z 453 $(M + Na)^+$; IR (KBr) 1750 (CO, lactone), 1730 (CO, ester), 1608 (C=C) cm^{-1}; ^1H NMR δ 0.89−0.99 (m, 12H, 2 x isovaleryl $(CH_3)_2$), 1.37, 1.44 (each s, 3H, 2′-$(CH_3)_2$), 2.10−2.30 (m, 6H, 2 x isovaleryl CH_2 and CH), 5.28 (d, J = 4.0 Hz, 1H, H-3′), 6.19 (d, J = 4.0 Hz, 1H, H-4′), 6.24 (d, J = 9.5 Hz, 1H, H-3), 6.82 (d, J = 8.5 Hz, 1H, H-6), 7.37 (d, J = 8.5 Hz, 1H, H-5), 7.61 (d, J = 9.5 Hz, 1H, H-4). Anal. ($C_{24}H_{30}O_7{\cdot}H_2O$) C, H.

Mixtures of (±)-*trans*- and (±)-*cis*-3′-Acetoxy-4′-azidodihydroseselins (45): yield 88% (starting with 61.0 mg of **44**); amorphous; mp 126−127 °C; IR (KBr) 2120 (N_3), 1740 (CO), 1610 (C=C) cm^{-1}; ^1H NMR δ 1.39, 1.43, 1.45, 1.46 (each s, $^3/_2$H, 2′-$(CH_3)_2$), 2.10, 2.23 (each s, $^3/_2$H, $COCH_3$), 4.91 (d, J = 3.0 Hz, $^1/_2$H, *trans*-H-3′), 5.16 (d, J = 5.0 Hz, $^1/_2$H, *cis*-H-3′), 5.18 (d, J = 3.0 Hz, $^1/_2$H, *trans*-H-4′), 5.28 (d, J = 5.0 Hz, $^1/_2$H, *cis*-H4′), 6.28 (d, J = 9.5 Hz, $^1/_2$H, *cis*-H-3), 6.30 (d, J = 9.5 Hz, $^1/_2$H, *trans*-H-3), 6.78 (d, J = 8.5 Hz, $^1/_2$H, *cis*-H-6), 6.82 (d, J = 8.5 Hz, $^1/_2$H, *trans*-H-6), 7.36, 7.38 (each d, J = 8.5 Hz, $^1/_2$H, H-5), 7.63, 7.64 (each d, J = 9.5 Hz, $^1/_2$H, H-4). Anal. ($C_{16}H_{15}N_3O_5$) C, H, N.

(±)-*trans*-3′-Acetoxy-4′-isovaleramidodihydroseselin (49): yield 58% (starting with 20.0 mg of **48**); crystallization from methanol gave prisms; mp 244−245 ° C; HR-FAB MS calcd for $C_{21}H_{25}NO_6Na$ 410.1579, found m/z 410.1577 $(M + Na)^+$; positive FAB MS m/z 410 $(M + Na)^+$; IR (KBr) 1750 (CO, lactone), 1735 (CO, ester), 1638 (CO, amide), 1608 (C=C) cm^{-1}; ^1H NMR δ 0.90, 0.95 (each d, J = 6.5 Hz, 3H, isovaleryl $(CH_3)_2$), 1.32, 1.42 (each s, 3H, 2′-$(CH_3)_2$), 1.75−2.09 (m, 1H, isovaleryl CH), 2.12 (s, 3H, $OCOCH_3$), 2.13−2.25 (m, 2H, CH_2), 5.29 (t, J = 7.5 Hz, 1H, H-4′), 5.40 (d, J = 7.5 Hz, 1H, H-3′), 5.60 (d, J = 7.5 Hz, 1H, NH), 6.23 (d, J = 9.5 Hz, 1H, H-3), 6.80 (d, J = 8.5 Hz, 1H, H-6), 7.32 (d, J = 8.5 Hz, 1H, H-5), 7.59 (d, J = 9.5 Hz, 1H, H-4).

Procedure for Synthesizing 10, 18, 19. To a solution of **4** (150.0 mg, 0.57 mmol) and dry pyridine (1 mL) in anhydrous CH_2Cl_2 (5 mL) was added (*S*)-(+)-α-methylbutyric anhydride (1 mL, 5.02 mmol) at room temperature. The reaction mixture was stirred overnight and was then concentrated *in vacuo*. The residue was column chromatographed on silica gel to give **18** (12.0 mg), **19** (27.0 mg), and **10** (135.0 mg).

3′,4′-Bis-*O*-((+)-α-methylbutyryl)-*cis*-khellactone (10): yield 55% (starting with 150.0 mg of **4**); crystallization from acetone gave colorless prisms; mp 121−123 ° C; HR-FAB MS calcd for $C_{24}H_{30}O_7Na$ 453.1889, found m/z 453.1889 $(M + Na)^+$; positive FAB MS m/z 453 $(M + Na)^+$; IR (KBr) 1755 (CO, lactone), 1730 (CO, ester), 1605 (C=C) cm^{-1}; ^1H NMR δ 0.90−0.96 (m, 6H, 2 x CH_2CH_3), 1.16−1.23 (m, 6H, 2 x $CHCH_3$), 1.41, 1.41, 1.45, 1.46 (each s, $^3/_2$H, 2′-$(CH_3)_2$), 1.51−1.78 (m, 4H, 2 x CH_2), 2.34−2.43 (m, 2H, 2 x CH), 5.33 (t, J = 5.0 Hz, 1H, H-3′), 6.22 (d, J = 9.5 Hz, 1H, H-3), 6.57 (t, J = 5.0 Hz, 1H, H-4′), 6.80 (d, J = 8.5 Hz, 1H, H-6), 7.36 (d, J = 8.5 Hz, 1H, H-5), 7.58 (d, J = 9.5 Hz, 1H, H-4). Anal. ($C_{24}H_{30}O_7H_2O$) C, H.

4′-*O*-((+)-α-Methylbutyryl)-*cis*-khellactone (18): yield 6% (starting with 150.0 mg of **4**); amorphous; mp 168−169 ° C; IR (KBr) 3520 (OH) 1710 (CO), 1610 (C=C) cm^{-1}; ^1H NMR δ 0.92−0.99 (m, 3H, CH_2CH_3), 1.20, 1.25 (each d, J = 7.0 Hz, $^3/_2$H, $CHCH_3$), 1.41−1.49 (m, 6H, 2′-$(CH_3)_2$), 1.50−1.81 (m, 4H, 2 x CH_2), 2.46, 2.51 (each d, J = 7.0 Hz, $^1/_2$H, CH), 2.80 (t, J = 5.0 Hz, OH, D_2O exchangeable), 4.05 (m, 1H, H-3′), 6.23 (d, J = 9.5 Hz, 1H, H-3), 6.38, 6.39 (each d, J = 4.5 Hz, $^1/_2$H, H-4′), 6.79 (d, J = 8.5 Hz, 1H, H-6), 7.35 (d, J = 8.5 Hz, 1H, H-5), 7.60 (d, J = 9.5 Hz, 1H, H-4). Anal. ($C_{19}H_{22}O_6$) C, H.

3′-*O*-((+)-α-Methylbutyryl)-*cis*-khellactone (19): yield 14% (starting with 150.0 mg of **4**); gum; HR-FAB MS calcd for $C_{19}H_{22}O_6Na$ 369.1314, found m/z 369.1314 $(M + Na)^+$; positive FAB MS m/z 369 $(M + Na)^+$; IR (KBr) 3480 (OH), 1730 (CO), 1608 (C=C) cm^{-1}; ^1H NMR δ 0.93−0.98 (m, 3H, CH_2CH_3), 1.21 (d, J = 7.0 Hz, 3H, $CHCH_3$), 1.40, 1.49 (each s, 3H, 2′-$(CH_3)_2$), 1.51−1.81 (m, 2H, CH_2), 2.46−2.55 (m, 1H, CH), 2.98−2.31 (m, 1H, OH, D_2O exchangeable), 5.14 (d, J = 5.0 Hz, 1H, H-3′), 5.42 (s, br, 1H, H-4′), 6.26 (d, J = 9.5 Hz, 1H, H-3), 6.78 (d, J = 8.5 Hz, 1H, H-6), 7.34 (d, J = 8.5 Hz, 1H, H-5), 7.64 (d, J = 9.5 Hz, 1H, H-4).

General Procedure for Synthesizing 3′-*O*-Alkyl-4′-*O*-acyl-*trans*-khellactones (24, 25). To a mixture of **6** and Ag_2O (catalytic amount) in DMF was added a corresponding alkyl halide at 10−12 °C. After stirring for 2.5−4 h, the reaction mixture was kept in a refrigerator overnight. The catalyst was filtered, and the filtrate was washed with water and brine and then concentrated *in vacuo*. The residue was purified by column chromatography on silica gel to give the product.

3′-*O*-Methyl-4′-*O*-(*m*-chlorobenzoyl)-*trans*-khellactone (24): yield 94% (starting with 1.36 g of **6**); amorphous; mp 173−175 ° C; IR (KBr) 1730 (CO), 1608 (C=C) cm^{-1}; ^1H NMR δ 1.45, 1.53 (each s, 3H, 2′-$(CH_3)_2$), 3.50 (d, J = 2.5 Hz, 1H, H-3′), 3.67 (s, 3H, OMe), 6.20 (d, J = 9.5 Hz, 1H, H-3), 6.50 (d, J = 2.5 Hz, 1H, H-4′), 6.85 (d, J = 8.5 Hz, 1H, H-6), 7.33−7.40 (m, 2H, H-5 and benzoyl H-5″), 7.51−7.54 (m, 1H, H-4″), 7.61 (d, J = 9.5 Hz, 1H, H-4), 7.90−7.95 (m, 2H, H-2″,6″). Anal. ($C_{22}H_{19}ClO_6$) C, H, Cl.

3′-*O*-Benzyl-4′-*O*-(*m*-chlorobenzoyl)-*trans*-khellactone (25): yield 56% (starting with 136.0 mg of **6**); crystallization from acetone gave colorless needles; mp 168–170 °C; IR (KBr) 1730 (CO, lactone), 1710 (CO, ester), 1608 (C=C) cm^{-1}; ^1H NMR δ 1.42 (s, 6H, 2′-$(CH_3)_2$), 3.70 (d, J = 2.5 Hz, 1H, H-3′), 4.83, 4.97 (each d, J = 12.0 Hz, 1H, $ArCH_2$), 6.23 (d, J = 9.5 Hz, 1H, H-3), 6.60 (d, J = 2.5 Hz, 1H, H-4′), 6.87 (d, J = 8.5 Hz, 1H, H-6), 7.30–7.44 (m, 2H, H-5 and benzoyl H-5″), 7.52–7.55 (m, 1H, H-4″), 7.62 (d, J = 9.5 Hz, 1H, H-4), 7.91–7.97 (m, 2H, H-2″,6″). Anal. ($C_{28}H_{23}ClO_6$) C, H, Cl.

3′-*O*-(2″-Tetrahydropyranyl)-4′-*O*-(*m*-chlorobenzoyl)-*trans*-khellactones (26, 27). A solution of **6** (100.0 mg, 0.25 mmol) and tosic acid monohydrate (20.0 mg, 0.018 mmol) in 3,4-dihydro-2*H*-pyran (1 mL, 10.96 mmol) was stirred at room temperature for 30 min. The reaction mixture was neutralized by methanolic NaOMe and concentrated *in vacuo*. The residue was separated by preparative TLC to give **26** (56.0 mg) and **27** (66.0 mg) [R_f 0.17 and 0.22, respectively; hexane–ethyl acetate (3:1)].

26: yield 46% (starting from 100.0 mg of **6**); crystallization from acetone gave colorless needles; mp 216–217 °C; IR (KBr) 1730 (CO), 1608 (C=C) cm^{-1}; ^1H NMR δ 1.47, 1.48 (each s, 3H, 2′-$(CH_3)_2$), 1.56–1.76 (m, 6H, tetrahydropyranyl CH_2-3″,4″,5″), 3.52–3.58, 3.83–3.90 (m, 2H, CH_2-6″), 3.98 (d, J = 3.0 Hz, 1H, H-3′), 4.86–4.88 (m, 1H, CH-1″), 6.18 (d, J = 9.5 Hz, 1H, H-3), 6.71 (d, J = 3.0 Hz, 1H, H-4′), 6.83 (d, J = 8.5 Hz, 1H, H-6), 7.31–7.50 (m, 3H, H-5 and benzoyl H-4‴,5‴), 7.58 (d, J = 9.5 Hz, 1H, H-4), 7.90–7.95 (m, 2H, H-2‴,6‴). Anal. ($C_{26}H_{25}ClO_7$) C, H, Cl.

27: yield 54.5% (starting from 100.0 mg of **6**); crystallization from acetone gave colorless needles; mp 191–194 °C; IR (KBr) 1715 (CO), 1608 (C=C) cm^{-1}; ^1H NMR δ 1.47, 1.56 (each s, 3H, 2′-$(CH_3)_2$), 1.54–1.78 (m, 6H, tetrahydropyranyl CH_2-2″,3″,4″), 3.58–3.62, 3.92–3.98 (m, 2H, CH_2-6″), 4.08 (d, J = 2.0 Hz, 1H, H-3′), 5.17 (d, J = 5.5 Hz, 1H, CH-1″), 6.21 (d, J = 9.5 Hz, 1H, H-3), 6.39 (d, J = 2.0 Hz, 1H, H-4′), 6.86 (d, J = 8.5 Hz, 1H, H-6), 7.33–7.53 (m, 3H, H-5, benzoyl H-4‴,5‴), 7.61 (d, J = 9.5 Hz, 1H, H-4), 7.88–7.94 (m, 2H, benzoyl H-2‴,6‴). Anal. ($C_{26}H_{25}ClO_7$) C, H, Cl.

General Procedure for Synthesizing 3′-*O*-Alkyl-*trans*-khellactones (28–30). To a solution of a corresponding ester (**24**–**27**) in dioxane (6–15 mL) was added KOH (0.5 N, 7–20 mL) dropwise. After stirring for 15 min to 1 h, the reaction mixture was acidified with dilute HCl and extracted with $CHCl_3$ or ethyl acetate. The organic fraction was worked up as described above (in the synthesis of **8**, **9**) to afford the corresponding product.

3′-*O*-Benzyl-*trans*-khellactone (28): yield 78% (starting with 117.0 mg of 25); gum; HR-FAB MS calcd for $C_{21}H_{21}O_5$ 353.1389, found m/z 353.1389 $(M + H)^+$; positive FAB MS m/z 727 $(2M + Na)^+$, 375 $(M + Na)^+$; IR (KBr) 3440 (OH), 1740 (CO), 1605 (C=C) cm^{-1}; ^1H NMR δ 1.33, 1.44 (each s, 3H, 2′-$(CH_3)_2$), 3.64 (d, J = 5.0 Hz, 1H, H-3′), 4.71, 4.95 (each d, J = 11.5 Hz, 1H, $ArCH_2$), 5.21 (d, J = 5.0 Hz, 1H, H-4′), 6.25 (d, J = 9.5 Hz, 1H, H-3), 6.78 (d, J = 8.5 Hz, 1H, H-6), 7.28–7.39 (m, 6H, H-5 and benzyl ArH-2″,3″,4″,5″,6″), 7.65 (d, J = 9.5 Hz, 1H, H-4).

3′-*O*-Methyl-*trans*-khellactone (29): yield 36% (starting with 100.0 mg of **24**); amorphous; mp 184 °C sublimed; HR-FAB MS calcd for $C_{15}H_{17}O_5$ 277.1076, found m/z 277.1078; $(M + H)^+$ Positive FAB MS m/z 575 $(2M + Na)^+$, 299 $(M + Na)^+$; IR (KBr) 3390 (OH), 1700 (CO), 1608 (C=C) cm^{-1}; ^1H NMR δ 1.37, 1.46 (each s, 3H, 2′-$(CH_3)_2$), 3.39 (d, J = 4.5 Hz, 1H, H-3′), 3.60 (s, 3H, OMe), 5.12 (d, J = 4.5 Hz, 1H, H-4′), 6.25 (d, J = 9.5 Hz, 1H, H-3), 6.78 (d, J = 8.5 Hz, 1H, H-6), 7.31 (d, J = 8.5, 1H, H-5), 7.65 (d, J = 9.5 Hz, 1H, H-4). Anal. ($C_{15}H_{16}O_5 \cdot 1/4H_2O$) C, H.

3′-*O*-(2″-Tetrahydropyranyl)-*trans*-khellactone (30): yield 41% (starting with 850.0 mg of mixture of **26**); amorphous; mp 126–130 °C; HR-FAB MS calcd for $C_{19}H_{22}O_6Na$ 369.1314, found m/z 369.1315 $(M + Na)^+$; positive FAB MS m/z 369 $(M + Na)^+$; IR (KBr) 3420 (OH), 1728 (CO), 1610 (C=C) cm^{-1}; ^1H NMR δ 1.29, 1.47 (each s, 3H, 2′-$(CH_3)_2$), 1.50–1.59, 1.81–1.85 (m, 6H, tetrahydropyranyl CH_2-2″,3″,4″), 3.59–3.62, 4.02–4.06 (m, 2H, CH_2-6″), 3.73 (d, J = 5.5 Hz, 1H, H-3′), 4.67 (d, J = 4.5 Hz, 1H, CH-1″), 5.10 (d, J = 5.5 Hz, 1H, H-4′), 6.55 (d, J = 9.5 Hz, 1H, H-3), 6.77 (d, J = 8.5 Hz, 1H, H-6), 7.31 (d, J = 8.5 Hz, 1H, H-5), 7.63 (d, J = 9.5 Hz, 1H, H-4). Anal. ($C_{19}H_{22}O_6 \cdot 1/4H_2O$) C, H.

4′-*O*-Isovaleryl-*trans*-khellactone (35). A solution of **33** (48.0 mg, 0.11 mmol) in AcOH (20%, aqueous) was refluxed for 30 min. The reaction mixture was cooled and extracted with $CHCl_3$. The organic layer was worked up as described above (in the synthesis of **8**, **9**) to afford **35**: yield 46%; crystallization from methanol gave colorless prisms; mp 185–187 °C; IR (KBr) 3420 (OH), 1740 (CO, lactone), 1700 (CO, ester), 1605 (C=C) cm^{-1}; ^1H NMR δ 0.98, 1.00 (each d, J = 6.5 Hz, 3H, isovaleryl $(CH_3)_2$), 1.39, 1.46 (each s, 3H, 2′-$(CH_3)_2$), 2.11–2.25 (m, 1H, CH), 2.27–2.40 (m, 2H, CH_2), 3.13 (d, J = 4.5 Hz, 1H, OH), 3.91 (t, J = 4.5 Hz, 1H, H-3′), 6.09 (d, J = 4.5 Hz, 1H, H-4′), 6.23 (d, J = 9.5 Hz, 1H, H-3), 6.81 (d, J = 8.5 Hz, 1H, H-6), 7.35 (d, J = 8.5 Hz, 1H, H-5), 7.61 (d, J = 9.5 Hz, 1H, H-4). Anal. ($C_{19}H_{22}O_6$) C, H.

3′,4′-Di-*O*-methyl-*trans*-khellactone (43). To a mixture of **6** (32.0 mg, 0.08 mmol) and NaH (12 mg, 0.5 mmol) in DMF (1 mL) was added MeI (0.5 mL, 8 mmol) at 0 °C. After the mixture was stirred for 5 h, the temperature was raised to room temperature. The reaction mixture was then neutralized with dilute HCl and extracted with ethyl acetate. The organic fraction was worked up as described above (in the synthesis of **8**, **9**) to afford **43**: yield 43%; crystallization from acetone gave colorless prisms; mp 164–166 °C; IR (KBr) 1720 (CO), 1605 (C=C) cm^{-1}; ^1H NMR δ 1.41, 1.49 (each s, 3H, 2′-$(CH_3)_2$), 3.35 (d, J = 2.5 Hz, 1H, H-3′), 3.53 (s, 3H, OMe-3′), 3.73 (s, 3H, OMe-4′), 4.57 (d, J = 2.5 Hz, 1H, H-4′), 6.24 (d, J = 9.5 Hz, 1H, H-3), 6.76 (d, J = 8.5 Hz, 1H, H-6), 7.29 (d J = 8.5 Hz, H-5), 7.61 (d, J = 9.5 Hz, 1H, H-4). Anal. ($C_{16}H_{18}O_5$) C, H.

Mixture of (±)-*trans*- and (±)-*cis*-3′-Hydroxy-4′-azidodihydroseselins (44). To a solution of **4** (100.0 mg, 0.38 mmol) and NaN_3 (50.0 mg, 0.76 mmol) in $CHCl_3$ (6 mL) was added trifluoroacetic acid (3 mL) dropwise at room temperature. After the mixture was stirred overnight, additional NaN_3 (25 mg, 0.38 mmol) was added, and the reaction was continued for another night. The reaction mixture was washed with water and brine, dried over Na_2SO_4, and concentrated on a rotary evaporator. The residue was purified by preparative TLC to give **44**: yield 92%; amorphous; 162 °C dec; HR-FAB MS calcd for $C_{14}H_{14}N_3O_4$ 288.0984, found m/z 288.0984 $(M + Na)^+$; positive FAB MS m/z 597 $(2M + Na)^+$, 310 $(M + Na)^+$; IR (KBr) 3540 (OH), 2100 (N_3), 1720 (CO), 1608 (C=C) cm^{-1}; ^1H NMR δ 1.37, 1.48 (each s, 3H, 2′-$(CH_3)_2$), 2.31 (d, J = 5.5 Hz, 3/4H, *trans*-OH, D_2O exchangeable), 2.57 (d, J = 8.9 Hz, 1/4H, *cis*-OH, D_2O exchangeable), 3.82 (t, J = 5.5 Hz, 3/4H, *trans*-H-3′), 3.94 (dd, J = 8.9 Hz, J = 5.5 Hz, 1/4H, H-3′), 4.82 (d, J = 5.5 Hz, 1H, *trans*-H-4′), 5.17 (d, J = 5.5 Hz, 1/4H, *cis*-H-4′), 6.28 (d, J = 9.5 Hz, 1/4H, *cis*-H-3), 6.30 (d, J = 9.5 Hz, 3/4H, *trans*-H-3), 6.78 (d, J = 8.5 Hz, 1/4H, *cis*-H-6), 6.79 (d, J = 8.5 Hz, 3/4H, *trans*-H-6), 7.35 (d, J = 8.5 Hz, 3/4H, *trans*-H-5), 7.35 (d, J = 8.5 Hz, 1/4H, *cis*-H-5), 7.63 (d, J = 9.5 Hz, 1H, H-4). Anal. ($C_{14}H_{13}N_3O_4 \cdot 1/8H_2O$) C, H, N.

(±)-*cis*-3′-Hydroxy-4′-acetamidodihydroseselin (46) and (±)-*trans*-3′-Hydroxy-4′-isovaleramidodihydroseselin (48). A mixture of **45** (140.0 mg, 0.48 mmol) and $Pd/CaCO_3$ (90.0 mg, poisoned with Pb) in EtOH (5 mL) was stirred under an H_2 atmosphere for 2 days. The catalyst was filtered, and the filtrate was concentrated and dried *in vacuo*. The residue was dissolved in pyridine (0.1 mL) and CH_2Cl_2 (5 mL), and then isovaleryl chloride (0.1 mL, 0.8 mmol) was added. After 16 h, the reaction mixture was extracted with $CHCl_3$, and the organic fraction was worked up as described above (in the synthesis of **8**, **9**) to afford **46** (46.0 mg) and **48** (34.0 mg).

46: yield 31%; crystallization from EtOH gave prisms; mp 260–262 °C; HR-FAB MS calcd for $C_{16}H_{17}NO_5Na$ 326.1004, found m/z 326.1011 $(M + Na)^+$; positive FAB MS m/z 326 $(M + Na)^+$; IR (KBr) 3360 (OH), 3300 (NH), 1730 (CO), 1650 (CO, amide), 1615 (C=C) cm^{-1}; ^1H NMR δ 1.39, 1.47 (each s, 3H, 2′-$(CH_3)_2$), 2.15 (s, 3H, $COCH_3$), 3.88 (d, J = 4.5 Hz, 1H, H-3′), 5.57 (d, J = 4.5 Hz, 1H, H-4′), 5.90 (d, J = 7.5 Hz, 1H, NH), 6.24 (d, J = 9.5 Hz, 1H, H-3), 6.80 (d, J = 8.5 Hz, 1H, H-6), 7.32 (d, J = 8.5 Hz, 1H, H-5), 7.60 (d, J = 9.5 Hz, 1H, H-4).

48: yield 34%; crystallization from EtOH gave prisms; mp

232–234 ° C; IR (KBr) 3520 (OH), 3270 (NH), 1720 (CO), 1655 (CO, amide), 1608 (C=C) cm^{-1}; ^1H NMR δ 0.95–1.03 (m, 6H, isovaleryl $(CH_3)_2$), 1.26, 1.52 (each s, 3H, 2′-$(CH_3)_2$), 2.06–2.30 (m, 3H, isovaleryl CH_2 and CH), 3.99 (d, J = 7.0 Hz, 1H, H-3′), 5.08 (dd, J = 7.0 Hz, J = 5.0 Hz, 1H, H-4′), 5.31 (s, 1H, OH, D_2O exchangeable), 5.91 (d, J = 5.0 Hz, 1H, NH, D_2O exchangeable), 6.25 (d, J = 9.5 Hz, 1H, H-3), 6.81 (d, J = 8.5 Hz, 1H, H-6), 7.34 (d, J = 8.5 Hz, 1H, H-5), 7.62 (d, J = 9.5 Hz, 1H, H-4). Anal. ($C_{19}H_{23}NO_5$) C, H, N.

Anti-HIV Assay. The T cell line, H9, and the promonocytic cell line, U937, were maintained separately in continuous culture with complete medium (RPMI 1640 with 10% fetal calf serum [FCS]) at 5% CO_2 and 37 ° C. Cell lines were used in experiments only when in log phase of growth, whereas uninfected peripheral blood mononuclear cells (PBMCs) were first stimulated with PHA (1 μg/mL) for 3 days. All cell targets were incubated with HIV-1 (IIIB isolated, $TCID_{50}$ 10^4 IU/mL, at a multiplicity of infection of 0.1–0.01 IU/cell) for 1 h at 37 ° C and 5% CO_2. The cell lines and PBMCs were washed thoroughly to remove unadsorbed virions and resuspended at 4×10^5 cells/mL in complete medium or complete medium with 10% v/v interleukin 2 (IL-2), respectively. Aliquots (1 mL) were placed in wells of 24-well culture plates containing an equal volume of test compound (diluted in the appropriate culture medium). Each test compound had its toxicity assessed by determining the number of compound-exposed uninfected cells that remained after 4 days at 37 °C and 5% CO_2. A p24 antigen ELISA assay was used to determine the level of virus released in the medium of the HIV-infected cultures. The p24 antigen assay uses a HIV-1 anti-p24 specific monoclonal antibody as the capture antibody coated on 96-well plates. Following a sample incubation period, rabbit serum containing antibodies for HIV-1 p24 is used to tag any p24 "captured" onto the microtiter well surface. Peroxidase conjugated goat anti-rabbit serum is then used to tag HIV-1 p24 specific rabbit antibodies that have complexed with captured p24. The presence of p24 in test samples is then revealed by addition of substrate. The cut-off for the p24 ELISA assay is 12.5 pg/mL. P24 in the culture medium was quantitated against a standard curve containing known amounts of p24. The effective (EC_{50}) and inhibitory (IC_{50}) concentrations for anti-HIV activity and cytotoxicity, respectively, were determined.

HIV-1 Reverse Transcriptase Assay. HIV-1 reverse transcriptase microassay was adapted from refs 15 and 16. Briefly, 10 mL of virion associated HIV-1 IIIB reverse transcriptase in 1% Triton X-100 was mixed with 50 mL of a reaction cocktail containing 50 mM Tri-HCl (pH 7.8), 75 mM KCl, 2 mM dithiothreitol, 5 mM $MgCl_2$, poly(A) (5 mg/mL; Pharmacia), oligo(dT) (0.25 unit/mL; Pharmacia), 0.05% Nonidet P40, and [^{32}P]dTTP (10 mCi/mL) in the presence of various concentrations of test compounds. After incubating 1 h at 37 °C, 40 mL of the reaction mixture was applied to a Schleicher & Schuell NA 45 membrane saturated with 2 x SSC (0.3 M NaCl, 30 mM sodium citrate, pH 7.0) in a Schleicher & Schuell Minifold over one sheet of GB003 filter paper. Each well of the minifold was washed four times with 2 x SSC. Autoradiography was performed, and radioactivity was quantified with a Packard Matrix (Meriden, CT) 9600 direct β counter.

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