Chemistry and Biology of Cylindrols: Novel Inhibitors of Ras Farnesyl-Protein Transferase from *Cylindrocarpon lucidum*

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Farnesyl-protein transferase (FPTase) is an enzyme responsible for the farnesylation of Ras protein. Farnesylation is required for cell-transforming activity in several tumor-types, and therefore, inhibition of FPTase activity may be a potential target for anticancer drugs. Our continued search for novel inhibitors led to the isolation of a number of bicyclic resorcinaldehyde cyclohexanone derivatives named here cylindrols A_1 to A_4, cylindrols B and B_1, and a number of known compounds, from *Cylindrocarpon lucidum*. The compounds were isolated by bioassay-guided separation using Sephadex LH-20, silica gel, and reverse phase HPLC. Structures were elucidated by extensive application of 2D NMR and X-ray crystallography. The determination of absolute stereochemistry was accomplished by CD measurements. Chemical transformations of the most abundant compound resulted in a number of key derivatives which were critical for the evaluation of structure activity relationship. These compounds are members of ascochlorin family and showed a wide range of inhibitory activity (0.7 μM to >140 μM) against FPTase. The FPTase activity was noncompetitive with respect to both substrates. Isolation, structures, chemical transformations, and FPTase activity are discussed in detail.

Ras (p21) protein, a guanine nucleotide (GTP) binding protein, is a product of the *ras* oncogene and plays an important role in signal transduction and regulation of cell proliferation. It also helps link cell-surface growth factor receptors to intracellular pathways which are responsible for regulating cell growth.[1] Mutated forms of the Ras protein can lead to unregulated cell transformation and are associated with a number of human cancers.[2] Association of the Ras protein to the inner surface of the cell membrane is a key requirement for both regulated and unregulated cell transformation. A series of post-translational modifications are essential for the cell membrane association of Ras protein. One key modification is farnesylation of Ras by farnesyl-protein transferase (FPTase) on the C-terminus cysteine residue of the CAAX motif.[3] Subsequent events include proteolytic cleavage of the AAX tripeptide and esterification of the newly freed cysteine carboxy group. Selective inhibition of FPTase represents an indirect (by the way of blocking the association of mutated Ras to the cell membrane) but attractive target toward the development of chemotherapeutic agents for pancreatic and colon carcinomas where mutated forms of *ras* are common.[4] FPTase inhibitors have been shown to block the growth of tumors in a nude mouse explant model and this has validated the approach.[5]

The search for inhibitors of FPTase, based on either natural products or the CAAX tetrapeptide, has intensified. The natural product inhibitors reported to date fall into three main classes: (a) Inhibitors that are competitive with farnesyl diphosphate (FDP), including chaetomellic acids,[6] actinoplanic acids,[7] and manumycin analogs;[8] (b) inhibitors that are competitive with Ras-peptide such as the pepticinnamins;[9] and (c) those

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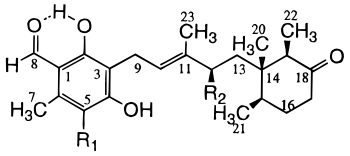
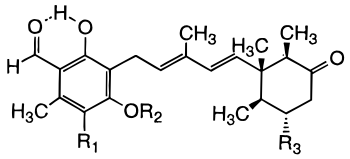
inhibitors that are either not competitive with either of the FPTase substrates or their mechanism of inhibition is not yet known; this class of inhibitors includes fusidienol,[10] preussomerins,[11] gliotoxin,[12] and 10′-desmethoxystreptonigrin.[13] We have continued screening for novel inhibitors of FPTase and recently reported[14] yet another class of novel inhibitor, cylindrol A (**1a**), from *Cylindrocarpon lucidum* (Ascomycotina, Hypocreales). Cylindrol A is a novel bicyclic compound with a resorcinaldehyde group linked to a cyclohexanone unit by a five-carbon chain. We wish to describe bioassay-guided (bovine FPTase[15]) isolation,[16] structure elucidation including determination of absolute stereochemistry, chemical modification, and structure−activity relationship of several novel cylindrols (**1a**−**e, 2a,b**) and other related (**1f**, **2**−**12**) compounds. These compounds are members of ascochlorin family.



1a: R_1 = H, R_2 = $OCOCH_2CH_3$ (Cylindrol A)
1b: R_1 = H, R_2 = $OCOCH_3$ (Cylindrol A_1)
1c: R_1 = H, R_2 = $OCOCH_2CH_2CH_3$ (Cylindrol A_2)
1d: R_1 = H, R_2 = $OCOCH_2CH(CH_3)_2$ (Cylindrol A_3)
1e: R_1 = Cl, R_2 = $OCOCH_2CH(CH_3)_2$ (Cylindrol A_4)
1f: R_1 = H, R_2 = H



2a: R_1 = R_2 = R_3 = H (Cylindrol B)
2b: R_1 = R_2 = H, R_3 = $OCOCH_3$ (Cylindrol B_1)
2c: R_1 = Cl, R_2 = H, R_3 = $OCOCH_3$
2d: R_1 = H, R_2 = CH_3, R_3 = H

Isolation

The fermentation broth of *C. lucidum* (MF 5710, ATCC 74261) was extracted with methyl ethyl ketone and chromatographed on Sephadex LH-20 in size exclusion mode using methanol as the eluent. The FPTase active components were eluted after one half column volume and were combined to give six successive fractions A−F. Cylindrols A−A_4 and B, B_1 (**1a**−**e**, **2a**,**b**), compounds **1f, 2**−**4** and cylindrocarpol **5** were isolated from these fractions by crystallization, reverse phase HPLC, and normal phase silica gel chromatography or combinations of both (see Experimental Section for details). These compounds were present in widely differing amounts: the most abundant compound was **1f** (∼300 mg/L) while compound **3** was found at less than 1 mg/L. Amounts of cylindrols A−A_4 and B, B_1 (**1a**−**e**, **2a**,**b**) ranged from 2 mg to 80 mg/L of broth.

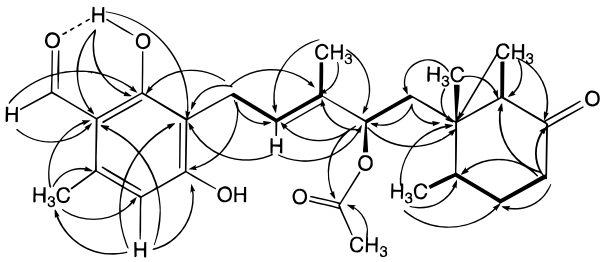


Figure 1. COSY (—) and HMBC ($^nJ_{CH}$ = 7 Hz) correlations of Cylindrol A_1 (**1b**).

Structure Elucidation

Cylindrol A_1 (1b). Cylindrol A_1 is structurally very closely related to cylindrol A (**1a**) whose structure has been determined[14] by a single crystal X-ray crystallography. Electron impact (EI) mass spectral analysis of cylindrol A_1 gave a molecular ion at *m/z* 430 which upon high resolution measurement (430.2470) gave a molecular formula $C_{25}H_{34}O_6$, and this was supported by the ^{13}C NMR spectrum of cylindrol A_1. The molecular formula of **1b** suggested that it has nine degrees of unsaturation. The infrared spectrum of **1b** displayed absorption bands for hydroxy (3274 cm^{-1}), ester (1731 cm^{-1}), cyclic ketone (1708 cm^{-1}), and conjugated carbonyl (1628 cm^{-1}) groups. The absorption bands for ester and ketones were partially fused. The UV spectrum gave absorption bands at 221 and 295 nm indicating a high degree of conjugation. ^{13}C NMR analysis (Table 1) of cylindrol A_1 in $CDCl_3$ displayed 25 carbons. The APT spectrum revealed the presence of the following types of carbon: six methyls, four methylenes, an oxygenated methine, two aliphatic methines, an aliphatic quaternary, two olefinic/aromatic methines, six olefinic/aromatic quaternaries, an ester carbonyl, an aldehyde, and a cyclic ketone. The ^{13}C NMR shifts were assigned using an HMQC experiment.

The ^1H NMR (400 MHz) analysis (Table 2) of **1b** in $CDCl_3$ exhibited two methyl doublets (δ 0.83 and 0.97) with J = 6.6 Hz each connected to two methines (δ 2.56 and 1.94) and an angular methyl singlet. The remaining three methyl groups were singlets and were sufficiently deshielded to be either aromatic, olefinic, or acetate. One of the two olefinic protons appeared as a singlet while the second appeared as a triplet. The most deshielded proton appeared at δ 12.67 that could be exchanged, *albeit* slowly, with deuterium upon shaking with D_2O, indicating a strong chelation possibly with a *peri*-carbonyl. Analysis of a 2D $^1H-^1H$ COSY spectrum of cylindrol A_1 gave four partial fragments, as shown by bold lines (Figure 1), establishing structural fragments C9−C11−C23, C12−C13, C21−C15−C16−C17, and C19−C22. An HMBC experiment was used to assemble these fragments into cylindrol A_1 and correlations are illustrated by arrows in Figure 1. The aldehyde proton (H-8, δ 10.05) gave strong two and three bond HMBC correlations to C-1 (δ 113.24) and C-2 (δ 163.77). The chelated hydroxy proton (C2-OH, δ 12.67) produced correlations to C-1, C-2, and C-3 (δ 111.43). The aromatic methyl group (H-7)

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Table 1. ^{13}C NMR Assignment of Cylindrols in $CDCl_3$[a]

position	1b	1d	1e	1f	2a	2b	5
1	113.24	113.29	113.14*	113.25	113.39	113.46	113.10
2	163.77	163.75	162.25	163.71	163.76	163.77	163.65
3	111.43	111.36	113.32*	111.92	111.87	111.81	112.35
4	161.74	161.60	156.25	162.26	161.59	161.34	162.58
5	110.52	110.57	113.51*	110.66	110.48	110.41	110.69
6	142.03	142.01	137.87	141.93	142.03	142.09	141.67
7	17.94	17.92	14.41	17.97	17.96	17.96	17.95
8	192.93	192.93	193.20	192.94	192.97	192.99	192.33
9	20.74	20.72	21.53	21.24	21.32	21.32	21.05
10	125.49	125.17	124.86	121.31	127.51	128.24	122.08
11	136.20	136.63	135.38	138.49	135.15	134.74	137.45
12	76.27	75.90	75.54	32.67	132.86	133.84	39.24
13	39.53	39.55	39.58	35.64	136.17	134.76	25.55
14	44.08	44.08	44.04	43.56	48.51	47.19	124.70
15	36.67	36.70	36.59	36.11	40.82	45.32	135.02
16	31.12	31.10	31.14	30.97	31.12	73.62	35.54
17	41.44	41.46	41.44	41.55	41.56	45.59	29.33
18	213.82	213.25	213.25	214.48	211.50	207.80	78.26
19	50.45	50.40	50.40	50.52	53.57	53.80	73.43
20	15.42	15.37	15.37	15.33	10.34	11.50	26.39*
21	15.50	15.57	15.57	15.63	16.29	12.71	23.37*
22	8.02	7.92	7.92	7.57	8.89	8.84	15.95**
23	11.80	11.87	11.87	16.48	12.71	12.43	15.99**
1′	170.51	172.55	172.17	–	–	170.09	–
2′	21.33	43.81	43.80	–	–	21.02	–
3′	–	25.70	25.69	–	–	–	–
4′	–	22.32	22.25	–	–	–	–
5′	–	22.37	22.31	–	–	–	–

[a] * and ** chemical shifts in same column may be interchanged.

Table 2. ^{1}H-NMR Assignment (δ H, mult, J) of Cylindrols in $CDCl_3$

no.	1b	1c	1d	1e	1f	2a	2b	5
5	6.18, s	6.18, s	6.17, s	–	6.23, s	6.21, s	6.20, s	6.20, s
7	2.46, s	2.47, s	2.47, s	2.56, s	2.48, d, 0.6	2.49, s	2.50, s	2.47, s
8	10.05, s	10.06, s	10.06, s	10.13, s	10.07, s	10.08, s	10.09, s	10.04, s
9	3.35, dd, 14.7, 6.9 3.41, dd, 14.7, 6.9	3.32, dd, 15.6, 7.2 3.39, dd, 15.6, 7.2	3.32, dd, 15, 3.9 3.41, dd, 15, 7.2	3.34, dd, 14,7.2 3.38, dd, 14.4, 7.6	3.38, d, 6.9	3.50, t, 7.2	3.50, d, 7.5	3.35, dd, 6.4, 2.4
10	5.60, t, 6.9	5.59, brt, 6.9	5.59, t, 7.2	5.54, t, 7.2	5.28, qt, 6.9, 1.2	5.52, t, 6.9	5.54, t, 7.5	5.18, qt, 1.6, 7.0
12	5.34, dd, 7.5, 3.9	5.36, dd, 8, 4	5.34, dd, 7.8, 3.6	5.35, dd, 7.6, 4	2.00, m, 1.83, m	5.92, d, 16.2	5.94, d, 15.9	2.09, m
13	1.56, dd, 15.6, 4.5 1.84, dd, 16, 7.5	1.56, dd, 15.6, 4.5 1.85, dd, 15.6, 7.2	1.54, dd, 15.6, 3.6 1.85, dd, 15.6, 7.5	1.50, dd, 15.9, 4.2 1.81, dd, 15.6, 7.8	1.40, m 1.40, m	5.41, d, 16.2	5.36, d, 16.2	2.10, m
14	–	–	–	–	–	–	–	5.13, qt, 1.2, 6.8
15	1.94, m	1.95, m	1.95, m	1.94, m	1.98, m	1.80, m	2.00, m	–
16	1.55, m 1.79, m	1.53, m 1.78, m	1.54, m 1.79, m	1.53, m 1.80, m	1.60, m 1.80, m	1.63, m	4.90, dt, 11, 5.7	2.12, m; 2.07, m
17	2.18, dd, 13.6, 7.2 2.27, ddd, 13.2,5,2	2.20, m 2.30, m	2.18, m 2.25, m	2.19, m 2.25, m	2.34, m	2.40, m	2.41, dd, 13, 6.7 2.88, dd, 13.5, 5.7	1.45, m 1.62, m
18	–	–	–	–	–	–	–	3.39, dd, 10.4, 2.0
19	2.56, q, 6.3	2.56, q, 7.2	2.56, q, 6.3	2.52, q, 6.6	2.45, q, 6.5	2.40, q, 6.5	2.47, q, 6.6	–
20	0.56, s	0.57, s	0.56, s	0.52, s	0.57, s	0.71, s	0.74,s	1.18*, s
21	0.97, d, 6.6	0.97, d, 6.9	0.97, d, 6.6	0.94, d, 6.9	0.88, d, 6.8	0.84, d, 6.6	0.87, d, 6.9	1.22*, s
22	0.83, d, 6.6	0.84, d, 6.3	0.84, d, 6.6	0.79, d, 6.9	0.91, d, 6.6	0.82, d, 6.9	0.87, d, 6.9	1.58, d, 1.2
23	1.83, s	1.81, s	1.81, s	1.79, s	1.83, d, 1.2	1.93, s	1.93, s	1.75, d, 1.2
2′	2.05, s	2.22, m	2.12, d, 6.3	2.13, d, 6.6	–	–	2.07, s	–
3′	–	1.64, m	2.10, m	2.08, m	–	–	–	–
4′	–	0.96, t, 6.9	0.93, d, 6.3	0.91, d, 6.3	–	–	–	–
5′	–	–	0.93, d, 6.3	0.91, d, 6.3	–	–	–	–
2-OH	12.67, s	12.69, s	12.68, s	12.66, s	11.71, s	12.71, s	12.71, s	12.71, s
4-OH	6.51, s	6.06, brs	6.07, brs	6.30, brs	6.54, brs	6.06, brs	5.80, brs	7.38, brs

showed correlations to three carbons C-6 (δ 142.03), C-1, and methine carbon C-5 (δ 110.52). The aromatic proton (H-5) furnished strong correlations through three bonds to the aromatic methyl group (δ 17.94), C-1, and C-3, and a weak correlation *via* two bonds to C-4 (δ 161.74). The latter two carbons and C-2 were strongly correlated to both benzylic protons (H-9). These correlations fully established the substitution pattern around the aromatic ring and indicated that the side chain was attached, at C3, between *m*-diphenol.

The substitution pattern in the cyclohexanone ring of cylindrol A_1 was similarly established using HMBC correlations from the protons of three methyl groups (H-20, H-21, and H-22) and from H-17 to the respective carbons. The ketone group was placed at C-18 based on the deshielding of H-17 and H-19 and the correlation of C-22 methyl protons to the carbonyl carbon (δ 213.82).

The connectivity of the aromatic and the cyclohexanone ring through a five-carbon chain and acetate substitution in the chain was accomplished from HMBC correlations as follows: the correlations from benzylic protons to C-10 and C-11; H-23 to oxygenated methine carbon (C-12); angular methyl protons to the C-13; and H-13 to C-12 and C-14 (fragment previously derived from COSY)

established the chain connections at C3 and C14. Additionally, the HMBC correlations of H-12 to the acetate carbonyl at δ 170.51 confirmed the presence of the acetate group at C12. This structural assignment was supported by mass spectral fragmentation of cylindrol A. Mass spectral fragmentation and absolute stereochemical assignments have been grouped together and are discussed under a separate heading (*vide infra*).

Cylindrol A_2 (1c). EIMS analysis of **1c** gave a molecular ion at *m*/*z* 458.2668 which corresponded to a molecular formula of $C_{27}H_{38}O_6$, i.e. up by one methylene unit from cylindrol A and two methylene units from A_1. Comparison of the ^{1}H NMR spectrum of **1c** with that of **1a** and **1b** suggested that the extra methylene group was present in the C12 side chain. This observation was confirmed by 2D ^{1}H−^{1}H COSY and TOCSY spectrum of cylindrol A_2. The EI mass spectral fragmentation was identical to that of other non chlorinated cylindrols and is discussed later.

Cylindrol A_3 (1d). The high resolution EI mass spectral analysis of cylindrol A_3 gave a molecular formula of $C_{28}H_{40}O_6$ (472.2824, M^+), suggesting the presence of two additional methylene units. ^{1}H NMR spectrum (Table 1) and ^{13}C NMR (Table 2) of cylindrol A_3 was generally similar to that of cylindrols A and A_1 except for the presence of a two methyl doublet at δ 0.93 and the absence of the acetate methyl (**1b**) and the methyl triplet (**1a**). 2D ^{1}H-^{1}H COSY and TOCSY spectral analysis of cylindrol A_3 revealed that the two new methyl groups were connected to a methine (δ 2.10, m), connected in turn to a methylene (d, δ 2.12) linked to a carbonyl group. This connectivity established a isopentanoyl side chain at C12.

Cylindrol A_4 (1e). Mass spectral analysis of cylindrol A_4, like other cylindrols, gave a molecular formula of $C_{28}H_{39}ClO_6$ (506.2463, M^+). That A_4 is a C-5 chloro-A_3 was established by comparison of the molecular formula and ^{1}H and ^{13}C NMR spectra (Table 1 and 2) of cylindrol A_3 and A_4. Chlorine-induced shifts of >3 ppm were observed for several of the aromatic carbons (C-4, C-5, and C-6 and the aromatic CH_3) in the ^{13}C NMR spectrum of **1e**. This was further substantiated by the comparison of mass spectral fragmentation (*vide infra*) and the presence of a chloride band (732 and 713 cm^{-1}) in the infrared spectrum.

Cylindrol B (2a). Molecular formula of $C_{23}H_{30}O_4$ (MW 370) was derived from the high resolution mass spectral analysis of cylindrol B and this was supported by ^{13}C NMR spectrum (Table 1). Comparison of the NMR spectra of the previously described cylindrols with that of **2a** suggested the absence of the acyl group at C12 and presence of an additional olefin at C12−C13. The structure of cylindrol B (**2a**) was verified by 2D NMR using the methods described for **1b,** and the ^{13}C and ^{1}H NMR assignments are listed in Tables 1 and 2. The chemical shift of C-14 methyl group was influenced by $\Delta^{12,13}$ and was shifted upfield in the ^{13}C NMR spectrum. The large scalar coupling between H12−H13 (J = 16 Hz) revealed a *E* olefin geometry. Deacylation of series A cylindrols could easily produce cylindrol B.

Cylindrol B_1 (2b). Mass and ^{13}C NMR spectral analysis of cylindrol B_1 gave a molecular formula $C_{25}H_{32}O_6$. Most of the ^{13}C and ^{1}H NMR spectrum (Table 1) of **2b** was identical to the spectrum of **2a** except for the presence of an ester carbonyl (δ 170.09), an extra methyl group (δ 21.02; 2.07), and replacement of one of the methylene groups with a oxymethine carbon (δ 73.62;

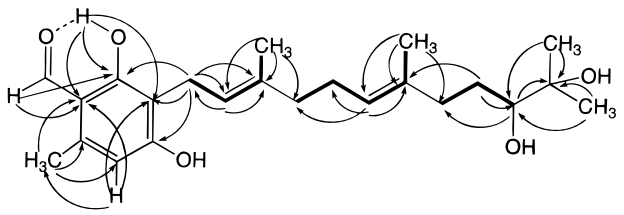
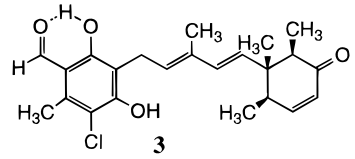
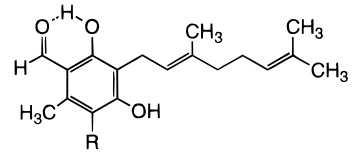


Figure 2. COSY (—) and selected HMBC ($^nJ_{XH}$ = 7 Hz) correlations of Cylindrocarpol (**5**).

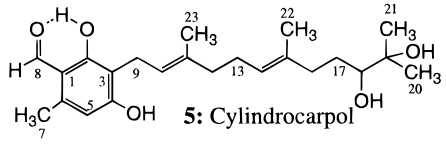
4.90). This obvious acetate group (IR: 1740 cm^{-1}) was placed in the cyclohexanone ring at C-16 by analysis of 2D COSY spectrum. The C-16 proton appeared as a doublet of a triplets (J = 11 Hz, 5.7 Hz) at δ 4.90. The presence of the triplet with a large coupling constant (J = 11) is a clear indication that H-16 is axially oriented and that the coupling is due to the H-15 and H-17 axial protons. Therefore, the acetate group must be equatorial, thus establishing the α stereochemistry at C-16 (see later for the β isomer).



3



4a: R = H
4b: R = Cl



5: Cylindrocarpol

A number of bicyclic compounds (**1f**, **2c, 3**) related to cylindrols and monocyclic compounds (**4**, **5**) have also been isolated from extracts of *C. lucidum*. Cylindrocarpol (**5**), a novel acyclic sesquiterpenoid resorcinaldehyde, was isolated from the polar fractions. HREIMS of **5** revealed a molecular formula $C_{23}H_{34}O_5$. The structure of cylindrocarpol was elucidated by 2D NMR spectra including HMBC experiment (Figure 2). The ^{1}H and ^{13}C NMR assignments are summarized in Table 1 and 2.

Compounds **1f** (LL-Z1272ϵ) and **2c** (LL-Z1272ζ) were originally isolated from a *Fusarium* species by Lederle group[17] and later from *Nectria coccinea*[18] and *Verticillium* species.[19] Compound **3** was prepared[17] from **2c** and was recently reported from *Verticillium*[19] as a natural product. The monocyclic compounds **4a** (colletorin B) and **4b** (5-chlorocolletorin B) have been reported from *Cephalosporium diospyr*[20] and *Colletotrichum nicotianae*,[21] respectively. The identity of these compounds were

(17) Ellestad, G. A.; Evans, R. H., Jr.; Kunstmann, M. P. *Tetrahedron* **1969**, *25*, 1323.
(18) Aldridge, D. C.; Borrow, A.; Foster, R. G.; Large, M. S.; Spencer, H.; Turner, W. B. *J. Chem. Soc., Perkin Trans. 1* **1972**, 2136.
(19) Takamatsu, S.; Rho, M.-C.; Masuma, R.; Hayashi, M.; Komiyama, K.; Tanaka, H.; Omura, S. *Chem. Pharm. Bull.* **1994**, *42*, 953.
(20) Kawagishi, H.; Sato, H.; Sakamura, S.; Kobayashi, K.; Ui, T. *Agric. Biol. Chem.* **1984**, *48*, 1903.

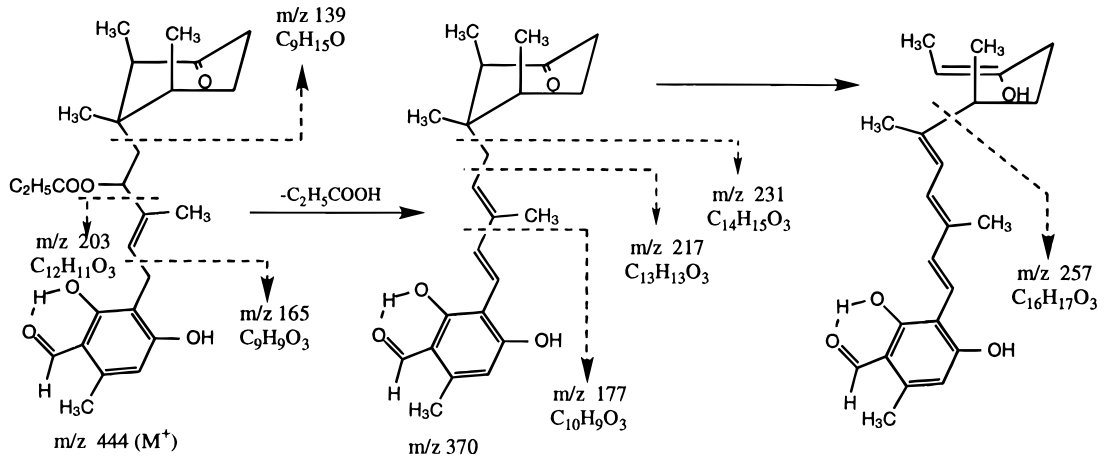


Figure 3. Mass spectral fragmentations of cylindrols as exemplified by fragmentation of Cylindrol A.

established by comparison of spectroscopic data with the literature data. Whenever good literature spectroscopic data was not available, the structure was verified by extensive 2D NMR spectroscopic analysis. These compounds contributed significantly toward the evaluation of the structure−activity relationship.

Mass Spectral Studies of Cylindrols. Due to an identical mass spectral fragmentation pattern for all the series A cylindrols, they can be discussed together and are exemplified by the fragmentation of cylindrol A. The cleavage of bonds C13-C14 and C9-C10 produced two major and characteristic fragment ions at *m/z* 139 ($C_9H_{15}O$) and 165 ($C_9H_9O_3$) in the EI mass spectrum. The other major fragment arose from acyl elimination followed by rearrangement to give a ion at *m/z* 370 ($C_{23}H_{30}O_4$). Most of the further fragmentation is derived from this fragment as illustrated in Figure 3. Cylindrols A−A_3 (**1a**−**d**) produced common fragment ions except for their molecular ion. Chlorine-containing cylindrol A_4 (**1e**) differs in its fragmentation only to the extent that all the fragment ions containing an aromatic ring were shifted by 34 mmu. The mass spectral fragmentations of the compounds **1f**, **2**, and **3** were significantly different than cylindrols.[17]

Relative and Absolute Stereochemistry of Cylindrols 1a−e, 2a,b. Using single-crystal X-ray diffraction the relative stereochemistries of cylindrol A (**1a**)[14,22b] and compound **1f**[22ab] have been established. The crystals used for the X-ray analysis were obtained as needles from acetone−hexane. Therefore, the stereochemistry of these two compounds became the basis for the determination of the stereochemistry of other compounds reported here including the remainder of the novel cylindrols. The stereochemistry of ascochlorin has been thoroughly elucidated by X-ray analysis of a *p*-bromosulfonate derivative.[23] The absolute stereochemistry of LL Z1272ϵ, a member of the ascochlorin family, was carefully correlated to that of ascochlorin by CD methods by Takamatsu and co-workers.[19] The absolute stereochemistry of **1f** is identical to that of LL-Z1272ϵ as evidenced by their identical specific rotation and CD spectrum.[17,19] Like LL-Z1272ϵ, the CD spectrum of **1f** showed a negative Cotton effect at 287 nm ($\Delta\epsilon = -1.48$). Likewise, the CD spectrum of **1a** showed a negative Cotton band at 286 nm ($\Delta\epsilon = -1.47$) due to the cyclohexanone ring and another band at 242 nm ($\Delta\epsilon = +2.03$) presumably due to the additional chiral center at C-12, thus establishing identical stereochemistry to the cyclohexanone part of the molecule in **1a**. Since the relative stereochemistry of all the stereocenters were established by X-ray analysis,[22] the absolute stereochemistry of cylindrol A was assigned as 12*R*,14*S*,15*R*,19*R*. The stereochemistry of the novel cylindrol A_1 to A_4 (**1b**−**e**) were accordingly assigned as 12*R*,14*S*,15*R*,19*R* by comparison of their NMR spectral data and specific rotation values. The stereochemistries of cylindrols B and B_1 were similarly assigned as 14*R*,15*R*,19*R*, and 14*S*15*S*16*S*,19*R*. These cylindrols represent novel members of the ascochlorin family.[24]

Chemical Modifications of 1f. The availability of a number of natural products gave us an entry into the FPTase structure−activity relationship (SAR) in the series (*vide infra*). To further this and address certain key issues, a number of semisynthetic modifications were carried out on the most abundant compound, **1f**.

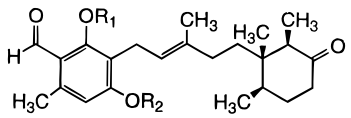
Methylation of a mixture of compounds **1f** and **2a** with diazomethane in methylene chloride at 0 °C followed by purification gave monomethyl ethers **2d** and **6b** selectively. The same reaction in methanol resulted in a complex mixture. On the other hand **1f** reacted smoothly with MOMCl to afford bis-MOM ether **6d**. Similarly, the

(21) (a) Kosuge, Y.; Suzuki, A.; Tamura, S. *Agric. Biol. Chem.* **1974**, *38*, 1265. (b) Saimoto, H.; Ueda, J.; Sashiwa, H.; Shigemasa, Y.; Hiyama, T. *Bull. Chem. Soc. Jpn.* **1994**, *67*, 1178.

(22) (a) Compound **1f**, $C_{23}H_{32}O_4$, $M_r = 372.509$, monoclinic, $P2_1$, $a = 7.7524(8)$, $b = 12.252(2)$, $c = 22.020(3)$ Å, $\beta = 94.69(1)°$ $V = 2084.5(9)$ Å^3, $Z = 4$, $D_x = 1.187$ g cm^{-3}, monochromatized radiation λ(Cu K_α) = 1.541838 Å, μ =0.60 mm^{-1}, $F(000) = 808$, $T = 294$ K. Data were collected on a Rigaku AFC5 diffractometer to a θ limit of 72.5° which yielded 4378 unique reflections. The observed reflections, those having $I \geq 3\sigma(I)$, number 3035. The structure was solved by direct methods (SHELXS-86, Sheldrick, G. M. *Acta Crystallogr.* **1990**, *A46*, 467−473) and refined using full-matrix least-squares on *F* (SDP-PLUS, Structure Determination Package Version 3, Enraf-Nonius, Delft, 1985). There are two independent molecules in a unit cell. The final model was refined using 486 parameters and the observed data. All non-hydrogen atoms were refined with anisotropic thermal displacements. Hydrogen atoms are included at their calculated positions and constrained to ride with their attached atom. The final agreement statistics are: $R = 0.049$, $wR = 0.047$, $S = 2.23$ with $(\Delta/\sigma)_{max} = 0.07$. The least-squares weights were defined using $1/\sigma^2(F)$. The maximum peak height in a final difference Fourier map is 0.30(4) eÅ^{-3}, and this peak is without chemical significance. (b) The atomic coordinates for **1a** and **1f** structure have been deposited with the Cambridge Crystallographic Data Centre. The coordinates can be obtained on request from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK..

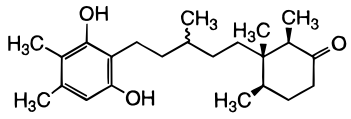
(23) (a) Nawata, Y.; Ando, K.; Tamura, G.; Arima, K.; Iitaka, Y. *J. Antibiot.* **1969**, *22*, 511. (b) Natawa, Y.; Iitaka, Y. *Bull. Chem. Soc. Jpn.* **1971**, *44*, 2652.

(24) (a) Tamura, G. A.; Suzuki, S.; Takasuki, A.; Ando, K.; Arima, K. *J. Antibiot.* **1968**, *21*, 539. (b) Sasaki, H.; Hosokawa, T.; Nawata, Y.; Ando, K. *Agric. Biol. Chem.* **1974**, *38*, 1463 and references cited therein.



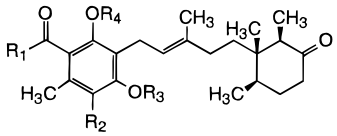
6a: $R_1 = R_2 = OCOCH_3$
6b: $R_1 = H$, $R_2 = CH_3$
6c: $R_1 = H$, $R_2 = TBS$
6d: $R_1 = R_2 = MOM$

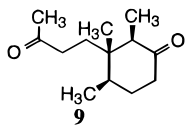
reaction with TBS chloride initially formed a bis-TBS ether (observed by TLC) but during aqueous work-up it furnished a mono TBS ether **6c**. Acetylation with acetic anhydride and pyridine gave the somewhat labile diacetate **6a**. The C-2 acetate was hydrolytically labile and yielded the C-2 free hydroxy group. This hydrolytic cleavage is not surprising in view of the chelation of C-2 hydroxy group with the C-1 aldehyde. Catalytic hydrogenation of **1f** using 10% Pd/C in ethyl acetate gave compound **7** exclusively.



7

Oxidative Studies. We wished to determine the effect of an acidic group on the SAR and thus attempted direct oxidations of the aldehyde group. Numerous methods of direct oxidations were attempted but failed to yield the desired compound.

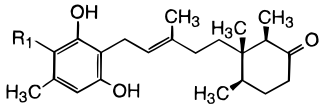




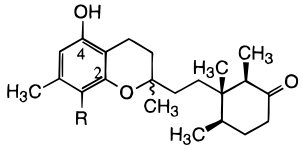
9

8a: $R_1 = OH$, $R_2 = R_3 = R_4 = H$
8b: $R_1 = OCH_3$, $R_2 = R_3 = R_4 = H$
8c: $R_1 = OCH_3$, $R_2 =$ H, $R_3 = R_4 = MOM$
8d: $R_1 = OCH_3$, $R_2 = H$, $R_3 = MOM$, $R_4 = H$
8e: $R_1 = H$, $R_2 =$ Cl, $R_3 = R_4 = H$

Oxidation of **1f** with chromium trioxide-based reagents initially did not produce any product, but when an excess of reagent was used compound **9** was obtained as the only identifiable product. A similar product was prepared from ascochlorin by ozonolysis.[17] Due to the difficulty in direct oxidation of the aldehyde group to acid **8a**, an alternative method was chosen to prepare the methyl ester **8b**. The aldehyde group of **1f** was indirectly converted to the ester *via* manganese dioxide oxidation of cyanohydrin intermediate followed by *in-situ* reaction with methanol.[25] A similar reaction with bis-MOM ether **6d** gave methyl ester **8c** and hydrolyzed product **8d**. Hydrolysis of **8b** and **8c** turned out to be extremely difficult due to either hydrolytic inactivity of the ester or decarboxylation of the resulting carboxyl group. A number of basic hydrolytic methods including lithium hydroxide and potassium carbonate failed to give the carboxyl derivative. Heating of **8b** with dilute aqueous NaOH gave the decarboxy compound **10a** in a small amount, as the only isolable product. The hydrolysis of **8b** *via* demethylation[26] using TMSCl−NaI gave a mixture of cyclic ethers **11a** and iodo derivative **11b**. This type of cyclization process is well documented in the case of reactions with sulfuric acid.[17,18] The lack of a chelated hydroxy group signal in the ^{1}H NMR spectrum of **11a** led us to suggest cyclization at C2 rather than at C4.

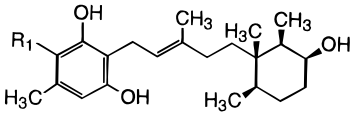


10a: $R_1 = H$
10b: $R_1 = CH_3$
10c: $R_1 = CH_2OH$



11a: $R = CO_2CH_3$
11b: R = I

Oxidation of **1f** using Wuts[27] procedure through a bisulfite adduct yielded only the diacetate **6a** despite the quantitative formation of the desired bisulfite adduct. Sodium hypochlorite−H_2O_2 oxidation[28] likewise did not result in the oxidation of the benzaldehyde group and as expected gave **8e** (illicicolin, LL-Z 1272δ, a compound that was previously reported as a natural product).[17,18]



12a: $R_1 = CH_2OH$
12b: $R_1 = CH_3$

Borohydride Reductions. Reduction of **1f** with sodium borohydride resulted in instantaneous reduction of both the aldehyde and ketone groups to afford diol **12a**. Reaction of **1f** with 4 equivalents of sodium cyanoborohydride in methanol gave a mixture of products **10b**, **10c**, **12a** and **12b**. The ratio of these products were dependent on the quantity of the reducing agent used and the reaction time. Selectivity was achieved when the reaction was performed in a mixture of THF−methanol (1:1) with approximately 3 equiv of sodium cyanoborohydride. This reaction favored the reduction of the aldehyde and furnished compounds **10b** and **10c** in a 1:2.5 ratio. The hydroxy group at C-18 in compounds **12a** and **12b** was assigned as β (axial) in view of the lack of any axial−axial coupling and the presence of equatorial−equatorial and equitorial−axial coupling ($J = \sim 3$ Hz) in their ^{1}H spectrum. These compounds were important for the structure−activity studies as described below.

FPTase Structure−Activity Relationship. The compounds presented in the current study were isolated by bioassay-guided separation using bovine FPTase.[15] The IC_{50} of the natural products and the synthetic derivatives are listed in Table 3. The most active compound **1f** inhibited the bovine FPTase with an IC_{50} of 0.7 μM. The other compounds were all significantly less active than **1f**. Acyl substitution at C12 reduced the activity by 3 to 20 fold. Cylindrol A_1 (**1b**), having a C12 acetate, was the least active among the series A cylindrols. The activity improved with the increase in the length of the ester side chain. The propionate side chain (**1a**) showed the maximal activity. The chloro compound

(25) Corey, E. J.; Gilman, N. W.; Ganem, B. E. *J. Am. Chem. Soc.* **1968**, *90*, 5616.

(26) (a) Ayer, W. A., McCaskill, R. H. *Can. J. Chem.* **1981**, *59*, 2159. (b) Jung, M. E.; Lyster, M. A. *J. Am. Chem. Soc.* **1977**, *99*, 968. (c) Olah, G. A.; Narang, S. C.; Gupta, B. G. B.; Malhotra, R. *J. Org. Chem.* **1979**, *49*, 1247.

(27) Wuts, P. G. M.; Bergh, C. L. *Tetrahedron Lett.* **1986**, *27*, 3995.

(28) Dalcanale, E.; Montanari, F. *J. Org. Chem.* **1986**, *51*, 567.

Table 3. Bovine FPTase Activity of Cylindrols and Analogs 1−12

compound no.	IC_{50} (μM)	rel activity
	Natural Products	
1f	0.7	1.000
1a	2.2	0.320
2c	2.8	0.250
1d	4.6	0.150
2b	5.6	0.130
1e	6.5	0.110
2a	6.5	0.110
1b	13.0	0.050
3	15.0	0.047
5	77.0	0.009
4b	155.0	0.005
4a	170.0	0.004
	Derivatives	
6b	2.0	0.350
6a	6.5	0.110
7	18.6	0.040
10a	20.0	0.035
10b	30.7	0.020
2d	31.0	0.020
10c	75.0	0.010
12a	101.0	0.007
12b	>140.0	0.005
6c	>140.0	0.005
8b	>140.0	0.005
9	>140.0	0.003

2c having a $\Delta^{12,13}$ olefin was two fold more potent when compared to cylindrols B (**2a**) and B_1 (**2b**), the latter two being almost equipotent. Compounds missing the cyclohexanone ring (**4**, **5**) were basically inactive. This result clearly illustrates that the FPTase inhibitory activity is not only due to the benzaldehyde unit but is also modulated by other parts of the bicyclic molecule. Monomethylation (**6b**) or acetylation (**6a**) of **1f** reduced the activity by 3 fold and 8−9 fold, respectively. The oxidation of the aldehyde to a methyl ester (**8b**) abolished activity.

The most interesting observation was made when comparing the activities of the reduced compounds (**10** and **12**). The FPTase inhibitory activity of **1f** was diminished >40 fold by complete reduction of the aldehyde to the methyl group (**10b**), >28 fold by substitution of aldehyde with a hydrogen (**10a**), and was virtually nil in the case of the hydroxymethyl compound (**10c**). The reduction of both aldehyde and ketone (**12a** and **12b**) had the greatest effect on the activity. It is interesting to note that the methyl analogs (**10b** and **12b**) are somewhat more active than their hydroxymethyl (**10c** and **12a**) counterparts. It is quite clear from this limited study that both the aldehyde and ketone play a role in the biological activity of this series of compounds.

Compound **1f** inhibited the reaction of recombinant human FPTase[29] with Ras-CVLS with an IC_{50} of 0.43 μM. To determine the mechanism of inhibition of **1f**, a more detailed kinetic analysis was performed. Compound **1f** was a noncompetitive inhibitor of bovine FPTase with respect to both FPP and the ras peptide (Ras-CVLS) exhibiting K_i values[6d] of 1.2 and 1.5 μM, respectively.

Conclusion

We have described a number of novel compounds in the ascochlorin family isolated from *C. lucidum*. These compounds have been evaluated for their potential as inhibitors of FPTase. The structure−activity relationship indicated that the bicyclic system, aldehyde, and ketone are all important for biological activity. Small changes in the molecule had a profound effect on FPTase activity. The absolute stereochemistry of the compounds is identical to that of the ascochlorins. The literature ascochlorins are mostly chlorinated at C5. This has been attributed to the presence of the chloride ions present in the production medium of the fermentation.[18] Most of our compounds are not chlorinated since the production media used for the fermentation of *C. lucidum* did not contain any significant amounts of halogen ions. The absence of halogenated compounds supports this hypothesis.[18] In addition to the FPTase inhibitory activity reported here, a number of different compounds of the ascochlorin family have previously been shown to inhibit 5α-reductase,[19] to be cytotoxic to HeLa cells,[30] and to have hypolipidemic activities.[24b,31]

Experimental Section

General Procedure. For general experimental procedure see reference 7. [^{3}H]-farnesyl diphosphate (FPP) was obtained from New England Nuclear.

Fermentation of *C. lucidum*. *C. lucidum* (MF 5710) was isolated from dried cow dung near Weed, Lincoln National Forest, Otero County, New Mexico. Cultures were maintained as mixtures of spores and hyphae in sterile soil and stored at 4 °C until ready for use. Seed cultures were inoculated by using a small portion of the preserved soil aseptically transferred into a 250 mL Erlenmeyer flask containing 50 mL of seed medium of the following composition (in g/L); corn steep liquor, 5.0; tomato paste, 40.0; oat flour, 10.0; glucose, 10.0; and trace elements solution, 10 mL/L (consisting of, in g/L $FeSO_4 \cdot 7H_2O$, 1.0; $MnSO_4 \cdot 4H_2O$, 1.0; $CuCl_2 \cdot 2H_2O$, 0.025; $CaCl_2 \cdot 2H_2O$, 0.1; H_3BO_3, 0.056; $(NH_4)_6MoO_{24} \cdot 4H_2O$, 0.019; $ZnSO_4 \cdot 7H_2O$, 0.2; dissolved in 0.6 N HCl). The pH of the medium was adjusted to 6.8 by addition of NaOH before sterilization. Seed medium was prepared using distilled water and was dispensed into Erlenmeyer flasks that were capped with cotton plugs before being autoclaved at 121 °C for 20 min. Seed cultures were incubated at 25 °C, on a gyrotory shaker (220 rpm, 5.1 cm throw) for 74 h prior to inoculation of fermentation flasks.

Fermentations were performed on either solid substrate or in liquid production media. The solid substrate production medium was formulated as follows: millet, 15.0 g/250 mL Erlenmeyer flask to which was added 15 mL containing 0.5 g yeast extract, 0.1 g of sodium tartrate, 0.5 g of sucrose, 0.5 g of alfalfa, 0.1 mL of corn oil, and 0.01 g of $FeSO_4 \cdot 7H_2O$. Solid substrate production flasks were capped with cotton plugs and sterilized at 121 °C for 15 min. Immediately prior to inoculation, distilled water (15.0 mL) was added to each flask, the flasks were resterilized at 121 °C for 20 min and then cooled. Each production flask was inoculated with 2.0 mL of vegetative seed growth mixed throughout the solid substrate. The production flasks were incubated without agitation at 25 °C for 21 days. Individual fermentation flasks were extracted with 50 mL of methyl ethyl ketone.

The liquid production medium was formulated as follows (in g/L): sucrose, 80.0; yellow corn meal, 50.0; and yeast extract, 1.0. This medium was prepared using distilled water; 50 mL of medium was dispensed into 250 mL Erlenmeyer flasks that were capped with cotton plugs before being autoclaved at 121 °C for 20 min. Production flasks were inoculated with 2.0 mL of vegetative seed growth and were incubated at 25 °C, on a gyrotory shaker (220 rpm, 5.1 cm throw) for 21

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(30) Hayakawa, S.; Minato, H.; Katagiri, K. *J. Antibiot.* **1971**, *24*, 654.

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days. At time of harvest, liquid fermentation flasks were homogenized and pooled.

Isolation of Cylindrols 1a−e, 2a,b) and Compounds 1f, 2−5). Fermentation broth (1.9 L) was extracted with methyl ethyl ketone (1.0 L) by shaking on a shaker at room temperature for 2 h. Celite was added to the agitated thick mixture and was filtered using sintered glass funnel. The filtrate was transferred into a separatory funnel, and methyl ethyl ketone layer was separated. The aqueous layer was washed with 400 mL of methyl ethyl ketone. The combined methyl ethyl ketone extract was concentrated to a small volume on a rotary evaporator under reduced pressure and lyophilized to remove residual water. The residue thus obtained was suspended in 100 mL of methanol and filtered. The filtrate contained all of the Ras farnesyl-protein transferase activity. Methanol was removed from filtrate under reduced pressure to give 6.5 g of a dark viscous gum.

The crude gum was dissolved in 50 mL of methanol and was chromatographed over a Sephadex LH-20 (2.0 L) column in methanol. Elution with methanol at 10 mL/min afforded the active components after 1350 mL. The active region was split into six successive fractions (100 mL each) to give: fraction A (0.53 g), B (0.7 g), C (1.07 g), D (1.62 g), E (0.73 g), and F (0.14 g).

Fractions A and B (1.23 g) were combined and flash chromatographed on a silica gel column (2 × 20 cm). Elution with 10 to 30% ethyl acetate−hexane gave the following compounds as amorphous solids listed in order of elution: **1e** (83 mg), **1d** (139 mg), fraction G (112 mg), and **5** (115 mg).

Fraction C (250 mg) was chromatographed by a reverse phase HPLC using a Whatman Partisil-10 column (50% acetonitrile−water for 20 min followed by a gradient to 70% acetonitrile over 80 min at 10 mL/min). The fractions were freeze-dried to give amorphous powders of **5** (20.7 mg t_R 25.1 min), **2b** (6.6 mg, t_R 37.1 min), **2a** (9.6 mg, t_R 41 min), **1f** (30.2 mg, t_R 43 min), and **2c** (11.5 mg, t_R 52 min).

Fraction D was crystallized from acetone to give colorless rosettes of 90% pure **1f** (0.34 g) that upon recrystallization from hot methanol gave colorless needles of pure **1f.**

Crystallization of fraction E from methanol gave **1f** (240 mg) and chromatography of the mother liquor on a flash silica gel column (2 x 20 cm) and elution with 10 to 30% ethyl acetate in hexane gave amorphous solids of **4b** (17.5 mg), and **4a** (92.2 mg).

Fraction F (130 mg) was chromatographed on a 50 mL silica gel column and eluted with 5 to 15% acetone in hexane to give chromatographically homogeneous **4b** (12.4 mg), **4a** (27.7 mg), and fraction H (3.3 mg). The latter fraction was chromatographed on a Whatman Partisil-10 22 × 250 mm HPLC column and eluted with a 60 min gradient of 50 to 70% acetonitrile−water at a flow rate of 10 mL/min. The fractions eluted at 30 min yielded amorphous solid of **3** (2.0 mg) after lyophilization.

Fraction G (112 mg) was rechromatographed on a reverse phase preparative Whatman Partisil 10 (C-18) HPLC column (22 × 250 mm) eluted initially for 10 min with 50% acetonitrile in water followed by a gradient to 60% acetonitrile over 60 min at a flow rate of 10 mL/min. Lyophilization afforded **1b** (11.6 mg, t_R 30 min), **1a** (22.5 mg, t_R35 min), **1c** (4.4 mg, t_R 42 min), and **1d** (5.4 mg, t_R 48.7 min) all as powders.

Physical Properties. 1a: For other physical data see reference 14. HREIMS (*m/z*): 444.2614 (<1%, M^+, calcd for $C_{26}H_{36}O_6$: 444.2510), 370.2115 (93%, M-$C_2H_5CO_2H$, calcd for $C_{23}H_{30}O_4$: 370.2144), 257.1173 (15%, calcd for $C_{16}H_{17}O_3$: 257.1178), 231.1042 (99%, calcd for $C_{14}H_{15}O_3$: 231.1021), 217.0838 (93%, calcd for $C_{13}H_{13}O_3$: 217.0864), 214.0967 (72%, calcd for $C_{14}H_{14}O_2$: 214.0993), 203.0744 (98%, calcd for $C_{12}H_{11}O_3$: 203.0708), 177.0561 (35%, calcd for $C_{10}H_9O_3$: 177.0552), 139.1135 (72%, calcd for $C_9H_{15}O$: 139.1123), 97.0646 (50%, calcd for C_6H_9O: 97.0653), CD (MeOH) λ_{max} 242 ($\Delta\epsilon$ = +2.03), 286 ($\Delta\epsilon$ = −1.47); **1b:** Rods from acetone−hexane, mp. 157−58 °C, $[\alpha]^{23}_D$ +23.65° (*c* 0.52, MeOH); UV (MeOH) λ_{max} 204 (log ϵ = 4.19), 223 (4.15), 296 (4.16), 340 (sh) nm, IR: ν_{max} (ZnSe): 3274, 2971, 1731, 1708, 1626, 1493, 1430, 1372, 1342, 1307, 1284, 1250, 1174, 1104, 1059, 1033, 1017, 913, cm^{-1}; NMR spectral data is presented in Tables 1 and 2; HREIMS (*m/z*): 430.2470 (M^+, calcd for $C_{25}H_{34}O_6$: 430.2354), due to identical values and assignments to **1a**, only low resolution data is listed 430 (1%, M^+), 370 (25%), 257 (5%), 231 (100%), 217 (65%), 203 (45%), 177 (17%), 165 (55%), 139 (70%), 97 (90%); **1c:** Colorless gum, $[\alpha]^{23}_D$ +20.6° (*c* 0.3, MeOH); IR: ν_{max} (ZnSe): 3265, 2930, 1731, 1708, 1626, 1493, 1430, 1373, 1307, 1252, 1185, 1104, 1016, 968 cm^{-1}; See Table 2 for ^1H NMR spectral data; HREIMS (*m/z*): 458.2768 (M^+, calcd for $C_{27}H_{38}O_6$: 458.2667), 458 (<1%, M^+), 370 (20%), 257 (3%), 231 (100%), 217 (60%), 203 (45%), 177 (15%), 165 (55%), 139 (70%), 97 (85%); **1d:** colorless gum, $[\alpha]^{23}_D$ +26.2° (*c* 0.71, MeOH); UV (MeOH) λ_{max} 204 (log ϵ = 4.26), 222 (4.19), 296 (4.19), 340 (sh) nm, IR: ν_{max} (ZnSe): 3275, 2962, 1731, 1708, 1626, 1493, 1430, 1372, 1343, 1306, 1252, 1189, 1171, 1104, 1060, 1016, 978 cm^{-1}; NMR spectral data is presented in Tables 1 and 2; HREIMS (*m/z*): 472.2824 (M^+, calcd for $C_{28}H_{40}O_6$: 472.2824), 472 (<1%, M^+), 370 (25%), 257 (5%), 231 (100%), 217 (55%), 203 (35%), 177 (15%), 165 (45%), 139 (65%), 97 (80%); **1e:** Colorless gum, $[\alpha]^{23}_D$ +20.9° (*c* 0.69, MeOH); UV (MeOH) λ_{max} 204 (log ϵ = 4.37), 230 (sh), 259 (3.92), 291 (3.81), 348 (4.22) nm, IR: ν_{max} (ZnSe): 3373, 2960, 1731, 1708, 1626, 1461, 1421, 1375, 1327, 1285, 1248, 1190, 1168, 1111, 1061, 1015, 974, 911, 796, 732, 713 cm^{-1}; For NMR spectral data see Tables 1 and 2; HREIMS (*m/z*): 506.2463 (M^+, calcd for $C_{28}H_{39}ClO_6$: 506.2434), 506 (<1%, M^+), 404 (20%), 266 (65%), 251 (40%), 237 (15%), 211 (10%), 199 (30%), 139 (100%), 97 (85%); **1f:** mp. 175−77 °C (acetone-hexane), Lit[17] mp. 171.5−172.5), $[\alpha]^{23}_D$ +6.5° (*c* 0.83, MeOH); lit.[17] +6° (c, 0.93, MeOH); IR: ν_{max} (ZnSe): 3241, 2968, 1694, 1625, 1490, 1428, 1376, 1341, 1306, 1252, 1170, 1103, 1016, 964 cm^{-1}; See Tables 1 and 2 for NMR spectral data; HREIMS (*m/z*): 372.2414 (M^+, calcd for $C_{23}H_{32}O_4$: 372.2300); CD (MeOH) λ_{max} 286 ($\Delta\epsilon$ = −1.48); **2a:** amorphous powder, $[\alpha]^{23}_D$ −11.9° (*c* 0.81, MeOH); UV (MeOH) λ_{max} 223 (log ϵ = 4.23), 232 (4.22), 296 (4.09), 340(sh) nm, IR: ν_{max} (ZnSe): 3258, 2972, 2935, 2876, 1708, 1625, 1490, 1429, 1373, 1306, 1252, 1105, 971, 904, 838, 736 cm^{-1}; For ^1H and ^{13}C NMR assignments see Tables 1 and 2; HREIMS (*m/z*): 370.2161 (M^+, calcd for $C_{23}H_{30}O_4$: 370.2144). **2b:** amorphous powder, $[\alpha]^{23}_D$ −11° (*c* 0.35, MeOH); UV (MeOH) λ_{max} 223 (log ϵ = 4.31), 233 (4.32), 295 (4.12), 340(sh) nm, IR: ν_{max} (ZnSe): 3314, 2977, 2939, 2876, 1740, 1715, 1626, 1490, 1429, 1371, 1251, 1224, 1104, 1064, 1026, 975, 942, 904, 838, 803, 737 cm^{-1}; For ^1H and ^{13}C NMR assignments see Tables 1 and 2; HREIMS (*m/z*): 428.2287 (M^+, calcd for $C_{25}H_{32}O_6$: 428.2198). **2c:** ^1H NMR ($CDCl_3$) δ: 0.74 (3H, s), 0.87 (3H, d, *J* = 6.6 Hz), 0.84 (3H, d, *J* = 6.6 Hz), 1.80 (1H, m), 1.92 (3H, s), 2.00 (1H, m), 2.06 (3H, s), 2.40 (1H, dd, *J* = 12.6, 6.3 Hz), 2.49 (1H, m), 2.61 (3H, s), 2.87 (1H, dd, *J* = 13.5, 5.7 Hz), 3.54 (2H, d, *J* = 7.5 Hz), 4.90 (1H, dd, *J* = 5.7, 11.1 Hz), 5.32 (1H, *J* = 16.2 Hz), 5.5 (1H, t, *J* = 6.9 Hz), 5.92 (1H, d, *J* = 15.9 Hz), 6.39 (1H, brs), 10.15 (1H, s), 12.71 (1H, s), ^{13}C NMR ($CDCl_3$) δ: 8.83, 11.51, 12.43, 12.61, 14.46, 21.02, 22.23, 45.36, 45.54, 47.20, 53.85, 73.64, 111.50, 113.15, 113.67, 128.26, 133.76, 134.20 (2C), 137.82, 156.11, 162.20, 170.05, 193.20, 207.81; HREIMS (*m/z*): 462.1723 (M^+, calcd for $C_{25}H_{31}ClO_6$: 462.1803). **3:** ^1H NMR ($CDCl_3$) δ: 0.80 (3H, s), 0.95 (3H, d, *J* = 6.8 Hz), 0.97 (3H, d, *J* = 7.6 Hz), 1.93 (3H, brs), 2.45 (1H, q, *J* = 6.8 Hz), 2.60 (3H, s), 2.63 (1H, m), 3.54 (2H, d, *J* = 7.2 Hz), 5.42 (1H, d, *J* = 16 Hz), 5.54 (1H, brt, *J* = 7.2 Hz), 5.98 (1H, d, *J* = 16 Hz), 5.99 (1H, d, *J* = 10 Hz), 6.36 (1H, brs), 6.55 (1H, dd, *J* = 10, 2 Hz), 10.15 (1H, s), 11.71 (1H, brs); HREIMS (*m/z*): 402.1701 (M^+, calcd for $C_{23}H_{27}ClO_4$: 402.1598). **4a:** ^1H NMR ($CDCl_3$) δ: 1.59 (3H, s), 1.67 (3H, s), 1.81 (3H, s), 2.08 (4H, m), 2.49 (3H, s), 3.40 (2H, d, *J* = 7.2 Hz), 5.05 (1H, m), 5.26 (1H, t, *J* = 7.2 Hz), 6.21 (1H, s), 6.26 (1H, brs), 10.07 (1H, s), 12.75 (1H, s), C-13 NMR ($CDCl_3$) δ: 16.22, 17.69, 17.96, 25.65, 26.33, 39.70, 110.87, 111.56, 113.22, 120.95, 123.68, 132.10, 139.76, 139.76, 141.97, 162.65, 163.61, 192.96; EIMS (*m/z*): 288 (M^+). **4b:** ^1H NMR ($CDCl_3$) δ: 1.57 (3H, s), 1.65 (3H, s), 1.79 (3H, s), 2.03 (4H, m), 2.60 (3H, s), 3.40 (2H, d, *J* = 7.2 Hz), 5.06 (1H, m), 5.23 (1H, qt, *J* = 7.2, 1.2 Hz), 6.41 (1H, brs), 10.14 (1H, s), 12.69 (1H, s), ^{13}C NMR ($CDCl_3$) δ: 14.42, 16.17, 17.65, 22.02, 25.63, 26.60, 39.75, 113.61, 114.40, 120.70, 124.17, 131.45, 137.00, 137.58, 156.43, 162.18, 193.19; HREIMS (*m/z*): 322.1345 (M^+, calcd for $C_{18}H_{23}ClO_3$: 322.1336). **Cylindrocarpol (5):** Colorless gum, $[\alpha]^{23}_D$ −11.7° (*c* 0.35, MeOH); UV (MeOH) λ_{max}

205 (log ϵ = 4.34), 295 (4.21), 340(sh) nm, IR: ν_{max} (ZnSe): 3349, 2925, 1625, 1490, 1430, 1371, 1305, 1284, 1254, 1166, 1105, 1058, 1034, 1011 cm^{-1}; for NMR spectral data see Tables 1 and 2; HREIMS (*m*/*z*): 390.2416 (M^+, calcd for $C_{23}H_{34}O_5$: 390.2660).

Acetylation of 1f. To a solution of **1f** (20 mg) in pyridine (0.2 mL) was added acetic anhydride (0.1 mL), and the solution was stirred at room temperature for 48 h. After completion of the reaction, pyridine and excess acetic anhydride were removed under a stream of nitrogen and finally dried under vacuum. The crude product was purified on a pipette filled with silica gel and eluted with 20% ethyl acetate in hexane to give 20 mg of chromatographically homogeneous **6a** as an amorphous powder. ^{1}H NMR ($CDCl_3$) δ: 0.57 (3H, s), 0.87 (3H, d, J = 6.6 Hz), 0.90 (3H, d, J = 6.9 Hz), 1.35 (3H, m), 1.61 (1H, m), 1.75 (3H, s), 1.82 (2H, m), 1.97 (1H, m), 2.30 (3H, s), 2.33 (2H, m), 2.35 (3H, s), 2.43 (1H, q, J = 6.6 Hz), 2.61 (3H, s), 3.18 (2H, d, J = 6.6 Hz), 5.01 (1H, t, J = 6.6 Hz), 6.91 (1H, s), 10.27(1H, s); HREIMS (*m*/*z*): 413.2354 (M^+ − Ac; calcd for $C_{25}H_{33}O_5$: 413.2328), 371.2247 (M^+ − 2 − Ac; calcd for $C_{23}H_{31}O_4$: 371.2222).

Methylation of 1f and 2a. To a cooled (0 °C) solution of a 2:3 mixture of **1f** and **2a** in methylene chloride (1 mL) was added an excess (5 mL) of an ethereal solution of diazomethane, and the solution was stirred overnight at 0 °C. Reaction was complete, and a polar (SiO_2: TLC, hexane−ethyl acetate, 7:3) product was formed. Solvents were carefully evaporated under a stream of nitrogen, and the products were purified on a silica gel column (1 × 10 cm) eluted with 10% ethyl acetate−hexane. Both compounds were finally separated by reverse phase HPLC using Whatman ODS-3 (22 × 250 mm) column. Elution with a 50% to 70% gradient of acetonitrile−water at 10 mL/min gave **2d** (t_R 16 min) and **6b** (t_R 18 min). The fractions were lyophilized to give an amorphous colorless powder. **2d:** ^{1}H NMR ($CDCl_3$) δ: 0.69 (3H, s), 0.81 (3H, d, J = 6.6 Hz), 0.84 (3H, d, J = 6.6 Hz), 1.63 (2H, m), 1.90 (3H, s), 1.95 (1H, m), 2.38 (3H, m), 2.56 (3H, s), 3.45 (2H, d, J = 7.2 Hz), 3.90 (3H, s), 5.34 (1H, d, J = 15.9 Hz), 5.49 (1H, t, J = 7.2 Hz), 5.89 (1H, d, J = 15.9 Hz), 6.29 (1H, s), 10.13 (1H, s), 12.41 (1H, s); HREIMS (*m*/*z*): 384.2329 (M^+, calcd for $C_{24}H_{32}O_4$: 384.2301); **6b:** ^{1}H NMR ($CDCl_3$) δ: 0.55 (3H, s), 0.87 (3H, d, J = 6.9 Hz), 0.90 (3H, d, J = 6.6 Hz), 1.38 (2H, m), 1.63 (1H, m), 1.79 (3H, brs), 1.83 (2H, m), 1.98 (2H, m), 2.31 (2H, m), 2.45 (1H, q, J = 6.6 Hz), 2.56 (3H, s), 3.30 (2H, d, J = 6.6 Hz), 3.89 (3H, s), 5.20(1H, t, J = 6.6 Hz), 6.28 (1H, s), 10.12 (1H, s), 12.39 (1H, brs); HREIMS (*m*/*z*): 386.2492 (M^+, calcd for $C_{24}H_{34}O_4$: 386.2457).

Preparation of TBS ether of 1f. To a solution of **1f** (100 mg, 0.27 mmol) in dimethylformamide (1 mL) was added diisopropylethylamine (0.21 mL, 1.2 mmol) followed by *tert*-butyldimethylsilyl chloride (121 mg, 0.8 mmol). The solution was stirred under nitrogen overnight. Ice−water (50 mL) was added, and the mixture was stirred for 20 min. The product was extracted with ethyl acetate (100 mL) and washed sequentially with water, 10% aqueous citric acid, water, 10% aqueous sodium bicarbonate, and water. The extract was dried over sodium sulfate, ethyl acetate was removed under reduced pressure, and the product was crystallized from methanol as colorless crystals of **6c** (115 mg, 88.5%), **6c:** ^{1}H NMR ($CDCl_3$) δ: 0.29 (6H, s), 0.56 (3H, s), 0.87 (3H, d, J = 6.9 Hz), 0.90 (3H, d, J = 6.9 Hz), 1.01 (9H, s), 1.30−1.65 (4H, m), 1.77 (3H, s), 1.82 (1H, m), 1.98 (2H, m), 2.31 (2H, m), 2.44 (1H, q, J = 6.9 Hz), 2.49 (3H, s), 3.30 (2H, d, J = 6.9 Hz), 5.19 (1H, t, J = 6.6 Hz), 6.19 (1H, s), 10.11 (1H, s), 12.47 (1H, brs, OH), HREIMS (*m*/*z*): 486.3151 (M^+, calcd for $C_{29}H_{46}O_4Si$: 486.3165).

Preparation of *Bis*-MOM Ether of 1f. To a solution of **1f** (117 mg, 0.32 mmol) in CH_2Cl_2 (2 mL) was added diisopropylethylamine (0.35 mL, 1.92 mmol) followed by MOM chloride (0.15 mL, 1.92 mmol), and the solution was stirred at room temperature under nitrogen for 5 h. After completion of the reaction, ice was added followed by ethyl acetate (100 mL). The ethyl acetate layer was washed with water, 10% aqueous citric acid, and water, dried (Na_2SO_4), and evaporated under reduced pressure to give crude product which was chromatographed over a small silica gel column eluted with 5% ethyl acetate−hexane to give **6d** as a colorless gum (145 mg).^{1}H NMR ($CDCl_3$) δ: 0.54 (3H, s), 0.85 (3H, d, J = 6.6 Hz), 0.88 (3H, d, J = 6.6 Hz), 1.35 (2H, m), 1.60 (2H, m), 1.78 (3H, s), 1.80 (1H, m), 1.95 (2H, m), 2.31 (2H, m), 2.45 (1H, q, J = 6.6 Hz), 2.56 (3H, s), 3.46 (2H, d, J = 6.9 Hz), 3.45 (3H, s), 3.57 (3H, s), 5.00 (2H, s), 5.15(1H, t, J = 6.6 Hz), 5.24 (2H, s), 6.74 (1H, s), 10.36 (1H, s); HREIMS (*m*/*z*): 460.2816 (M^+, calcd for $C_{27}H_{40}O_6$: 460.2825).

Hydrogenation of 1f. Palladium/carbon (10%, 10 mg) was added to a solution of **1f** (40 mg) in ethyl acetate (1.5 mL). The mixture was evacuated under vacuum and flushed with hydrogen from a balloon. This process was repeated three times and finally the stirred reaction mixture was connected to a hydrogen-filled balloon and was allowed to continue overnight. After completion of the reaction, catalyst was removed by filtration through Celite, and the product was purified on a 1 × 10 cm silica gel column. Elution with 20% ethyl acetate−hexane yielded diastereomeric mixture of **7** (20 mg) as an amorphous powder. ^{1}H NMR ($CDCl_3$) δ: 0.57 (3H, s), 0.87, 0.91 (3H, d, J = 6.6 Hz), 0.88, 0.90 (3H, d, J = 6.9 Hz), 1.00 (3H, d, J = 6.0 Hz), 1.21−1.36 (5H, m), 1.41 (1H, m), 1.61 (2H, m), 1.84 (1H, m), 2.00 (1H, m), 2.09 (3H, s), 2.19 (3H, s), 2.34 (2H, m), 2.47 (1H, brq, J = ∼5 Hz), 2.54−2.70 (2H, m), 4.70 (2H, brs, 2 x OH), 6.25 (1H, s); HREIMS (*m*/*z*): 360.2624 (M^+, calcd for $C_{23}H_{36}O_3$: 360.2664).

Chromium Trioxide Oxidation of 1f. To a cooled (0 °C) solution of **1f** (10 mg) in acetone (0.5 mL) was added Jones reagent (20 μL, ∼2 equiv). The solution was stirred at 0 °C for 30 min followed by room temperature for 4 h. No reaction was observed (TLC and HPLC); therefore, additional amounts of the Jones reagent was added. The starting compound was now consumed and a product was formed. 2-Propanol was added to quench the reagent, and the product was taken up in ethyl acetate, washed with water, and dried over sodium sulfate. (Another reaction with 10 mg of **1f** was performed in identical conditions using CrO_3 in acetic acid as a reagent, and this gave the same product. Both reactions were combined after workup and chromatographed together.) Solvent was removed under reduced pressure, and product was purified on a small silica gel column. Elution of the column with 20% ethyl acetate−hexane gave chromatographically homogeneous gum of **9** (5.8 mg). ^{1}H NMR ($CDCl_3$) δ: 0.63 (3H, s), 0.89 (3H, d, J = 6.6 Hz), 0.92 (3H, d, J = 6.6 Hz), 1.54−1.70 (3H, m), 1.83−1.91 (2H, s), 2.17 (3H, s), 2.26−2.50 (5H, m).

MnO_2 Oxidation of Cyanohydrin of 1f. To a solution of **1f** (200 mg, 0.54 mmol) in methanol (8 mL) were added manganese dioxide (936 mg, 20 equiv) and potassium cyanide (338 mg, 10 equiv) followed by acetic acid (0.31 mL, 10 equiv), and the mixture was stirred at room temperature under nitrogen overnight. Progress of the reaction was slow, therefore, an additional 2 equiv of potassium cyanide and acetic acid was added, and the mixture was heated at 70 °C for 6 h whereupon most of the starting compound was consumed and a major and few minor products were formed (silica gel, TLC, hexane−ethyl acetate, 7:3). After completion of the reaction, the mixture was filtered through Celite, and the filtrate was diluted with 50 mL of ethyl acetate and washed with water, dried over sodium sulfate, and evaporated under reduced pressure. The product mixture was chromatographed over a silica gel column eluted with 20% ethyl acetate−hexane to give methyl ester **8b** (60 mg) as an amorphous powder. ^{1}H NMR ($CDCl_3$) δ: 0.57 (3H, s), 0.88 (3H, d, J = 6.6 Hz), 0.91 (3H, d, J = 6.6 Hz), 1.30−1.65 (4H, m), 1.84 (3H, s), 1.75−2.1 (3H, m), 2.32 (2H, m), 2.45 (1H, q, J = 6.9 Hz), 2.46 (3H, s), 3.42 (2H, d, J = 7.2 Hz), 3.92 (3H, s), 5.30 (1H, t, J = 6.6 Hz), 5.66 (1H, brs, OH), 6.23 (1H, s), 12.00 (1H, brs, OH); ^{13}C NMR ($CDCl_3$) δ: 7.6, 15.12, 15.37, 16.52, 22.20, 24.02, 31.09, 32.83, 35.83, 36.28, 41.63, 43.59, 50.58, 51.73, 105.5, 111.34, 111.70, 121.84, 138.29, 140.88, 159.17, 162.79, 173.0, 213.0; HREIMS (*m*/*z*): 402.2423 (M^+, calcd for $C_{24}H_{34}O_5$: 402.2406).

MnO_2 Oxidation of Cyanohydrin of 6d. Reaction was repeated as described for the preparation of **8b**. **6d** (130 mg, 0.28 mmol) in 3 mL of methanol was reacted with MnO_2 (486 mg, 20 equiv), KCN (182 mg, 10 equiv), and AcOH (0.13 mL, 8 equiv) for 4 days. Two products were formed and were purified on preparative TLC using hexane-ethyl acetate (4:1).

Bands were eluted (listed in order of their elution) with acetone to give **8d** (5 mg) and **8c** (20 mg) both as a colorless gum. **8c:** ^{1}H NMR ($CDCl_3$) δ: 0.55 (3H, s), 0.85 (3H, d, J = 6.6 Hz), 0.88 (3H, d, J = 6.9 Hz), 1.35 (2H, m), 1.63 (2H, m), 1.77 (3H, s), 1.82 (1H, m), 1.96 (2H, m), 2.32 (2H, m), 2.44 (1H, q, J = 6.6 Hz), 2.47 and 2.58 (3H, s), 3.35 (2H, brd, J = 6.9 Hz), 3.44 (3H, s), 3.52 (3H, s), 3.88 (3H, s), 4.95 (2H, s), 5.18 (1H, t, J = 6.6 Hz), 5.18 (2H, s), 6.73 (1H, s); HREIMS (m/z): 490.2925 (M^+, calcd for $C_{28}H_{42}O_7$: 490.2930); **8d:** ^{1}H NMR ($CDCl_3$) δ: 0.55 (3H, s), 0.86 (3H, d, J = 6.6 Hz), 0.89 (3H, d, J = 6.9 Hz), 1.35 (2H, m), 1.63 (2H, m), 1.81 (3H, s), 1.80 (1H, m), 1.97 (2H, m), 2.31 (2H, m), 2.45 (1H, q, J = 6.6 Hz), 2.50 (3H, s), 3.36 (2H, d, J = 6.9 Hz), 3.46 (3H, s), 3.92 (3H, s), 5.23 (1H, t, J = 6.6 Hz), 5.23 (2H, s), 6.47 (1H, s), 10.50 (1H, brs, OH); HREIMS (m/z): 446.2715 (M^+, calcd for $C_{26}H_{38}O_6$: 446.2668).

Hydrolysis−Decarboxylation of 8b. To a solution of **8b** (30 mg) in THF (3 mL), methanol (3 mL), and water (4 mL) was added LiOH (10 mg), and the solution was stirred at room temperature for 2 h. TLC examination indicated no reaction; addition of 20 mg of K_2CO_3 and a further 2 h of stirring did not promote reaction either. NaOH (4 N, 0.1 mL) was then added, and the solution was heated at 70 °C for overnight. The starting material was now consumed, the reaction mixture was acidified at −78 °C with dilute HCl. The products were extracted with ethyl acetate (2 × 50 mL), washed with water, dried (Na_2SO_4), and evaporated to give a complex mixture of products. These products were chromatographed on a reverse phase Whatman C-18 (9.4 × 250 mm) column and eluted with a gradient of 40 to 60% acetonitrile−water containing 0.2% TFA. Elution at 4 mL/ min gave **10a** (0.7 mg) and a number of unidentified orange compounds of higher MW (than **8b**) compounds. **10a:** ^{1}H NMR ($CDCl_3$) δ: 0.57 (3H, s), 0.88 (3H, d, J = 6.9 Hz), 0.91 (3H, d, J = 6.6 Hz), 1.41 (2H, m), 1.80 (2H, m), 1.84 (3H, s), 1.90 (1H, m), 1.96 (2H, m), 2.22 (3H, s), 2.34 (2H, m), 2.45 (1H, q, J = 6.6 Hz), 3.39 (2H, d, J = 6.6 Hz), 4.92 (1H, s), 5.27 (1H, t, J = 7.2 Hz), 6.24 (2H, s); HREIMS (m/z): 344.2412 (M^+, calcd for $C_{22}H_{32}O_3$: 344.2351).

Reaction of 8b with TMS Iodide. To a solution of **8b** (4.8 mg, 0.013 mmol) in anhydrous acetonitrile (1 mL) were added trimethylsilyl chloride (0.026 mL) and sodium iodide (28 mg), and the solution was heated at 60 °C for 8 h followed by stirring at room temperature for 4 days. After completion of the reaction, ethyl acetate (50 mL) was added and the solution was washed with 5% aqueous sodium thiosulfate (20 mL) and water (20 mL), dried (Na_2SO_4), and evaporated under reduced pressure. Products were purified on silica gel plates (hexane−ethyl acetate, 4:1) and bands were eluted with acetone to give **11a** (0.8 mg) and **11b** (2.0 mg) both as amorphous solids. **11a:** ^{1}H NMR ($CDCl_3$) δ: 0.61 (3H, s), 0.84, 0.86, 0.88, 0.92 (6H, d, J = 6.9 Hz), 1.29 (3H, s), 1.4−1.64 (6H, m), 1.80 (2H, m), 2.00 (1H, m), 2.34 (2H, m), 2.42 (1H, q, J = 7.2 Hz), 2.45 (3H, s), 2.65 (2H, m), 3.91 (3H, s), 6.19 (1H, s), HREIMS (m/z): 402.2403 (M^+, calcd for $C_{24}H_{34}O_5$: 402.2406); **11b:** ^{1}H NMR ($CDCl_3$) δ: 0.63 (3H, s), 0.84, 0.86, 0.93, 0.97 (6H, d, J = 6.9 Hz), 1.30 (3H, s), 1.4−1.64 (6H, m), 1.82 (2H, m), 2.00 (1H, m), 2.33 (2H, m), 2.35 (3H, s), 2.45 (1H, q, J = 7.2 Hz), 2.64 (2H, m), 4.82 (1H, brs, OH), 6.36 (1H, s); HREIMS (m/z): 470.1330 (M^+, calcd for $C_{22}H_{31}IO_3$: 470.1277).

Oxidation of 1f through Sulfite Adduct. To a solution of **1f** (32 mg, 0.086 mmol) in ethanol (1 mL) was added an aqueous solution of sodium hydrogen sulfite (100 mg in 0.5 mL of water, 10 equivalent), and the solution was stirred at room temperature for 30 min. The adduct formed was filtered. The filtrate was heated at 60 °C overnight, and further amounts of precipitate were collected. Combined precipitate was dried in a vacuum desiccator to give adduct as a colorless powder (40 mg, 97.7%).

The adduct was dissolved in anhydrous DMSO (0.3 mL), and acetic anhydride (0.1 mL) was added. The mixture was stirred overnight which finally gave a homogeneous solution. The reaction was quenched with aqueous K_2CO_3. Ethyl acetate (50 mL) was added and acidified with 4 N aqueous HCl to pH 2, and the organic solution was washed with water, dried (Na_2SO_4), and evaporated to give diacetate **6a.**

Reaction of 1f with $NaClO_3$−H_2O_2. A solution of **1f** (10 mg, 0.027 mmol) in acetonitrile (0.5 mL), NaH_2PO_4 (10 mg) in water (0.5 mL), and H_2O_2 (0.01 mL) was added to a solution of $NaClO_3$ (6 mg, 0.054 mmol) in water (0.5 mL), and the solution was stirred at room temperature for 4 h. There was no product formed, and the starting material was intact (TLC, hexane−ethyl acetate, 7:3); therefore, an additional 3 equiv of $NaClO_3$ was added and the solution was stirred overnight. A faster moving product was formed. The reaction mixture was acidified and extracted with ethyl acetate (100 mL). Ethyl acetate extract was washed with water, dried (Na_2SO_4), evaporated under reduced pressure, and chromatographed over a small silica gel column eluted with 10% ethyl acetate−hexane to give a powder of chloro compound **8e** which was identified as illicicolin C by comparison of NMR spectrum[19] and mass spectral analysis.

Sodium Borohydride Reduction of 1f. To a solution of **1f** (40 mg, 0.11 mmol) in a 3:2 mixture of tetrahydrofuran−methanol (1 mL) was added sodium borohydride (5.2 mg, 0.11 mmol), and the solution was stirred at ambient temperature for 10 min. The product diol was formed almost instantaneously. The reaction mixture was concentrated to dryness, and ethyl acetate (100 mL) was added. The ethyl acetate solution was washed with water (2 × 50 mL), dried (Na_2SO_4), and evaporated under reduced pressure to give a gummy residue which was chromatographed on a 1 × 10 cm silica gel column. Elution of the column with 25% ethyl acetate−hexane afforded **12a** (20 mg) as an amorphous powder. ^{1}H NMR ($CDCl_3$) δ: 0.81 (3H, d, J = 6.3 Hz),0.84 (3H, s), 0.95 (3H, d, J = 7.5 Hz), 1.30 (3H, m), 1.41−1.63 (5H, m), 1.74−1.91 (2H, m), 1.83 (3H, s), 2.19 (3H, s), 3.40 (2H, d, J = 6.9 Hz), 3.84 (1H, dd, J =3.0, 3.0 Hz), 4.84 (2H, brs), 5.27 (1H, t, J = 6.9 Hz), 5.29 (1H, brs, OH), 6.22 (1H, s), 7.66 (1H, brs, OH), ^{13}C NMR ($CDCl_3$) δ: 12.40, 15.71, 16.49, 17.28, 19.12, 22.25, 25.51, 32.91, 33.89, 36.18, 36.53, 38.51, 39.48, 60.52, 73.29, 109.40, 112.13, 115.58, 121.51, 134.19, 139.28, 154.70, 155.39; HREIMS (m/z): 376.2580 (M^+, calcd for $C_{23}H_{36}O_4$: 376.2613).

Sodium Cyanoborohydride Reduction of 1f. Sodium cyanoborohydride (10 mg, 0.16 mmol) was added to a solution of **1f** (16 mg, 0.043 mmol) in methanol (2 mL) and acetic acid (0.02 mL), and the solution was stirred overnight at room temperature. After all of the starting material was consumed, ethyl acetate (100 mL) was added to the reaction mixture, washed with water (2 × 50 mL), dried (Na_2SO_4), and evaporated under reduced pressure to give a mixture of four products. These products were purified on preparative silica gel plates (250 μm) developed in ethyl acetate−hexane (7:3). Four bands were eluted with acetone to give **10b** (2.1 mg), **12b** (3.3 mg), **10c** (1.7 mg), and **12a** (2.6 mg) mostly as a gum.

When this reaction was carried out in controlled conditions, the reduction of the ketone was minimized. Typical experiment: To a solution of **1f** (70 mg, 0.19 mmol) in a mixture of tetrahydrofuran-methanol (1:1, 4 mL) and acetic acid (0.1 mL) was added sodium cyanoborohydride (11.9 mg, 0.19 mmol), and the solution was stirred overnight under nitrogen. The progress of the reaction was extremely slow and most of the starting material was left intact. Additional amounts of sodium cyanoborohydride (17.9 mg, 0.28 mmol) were added, and reaction mixture was stirred for additional 24 h. Two major products **10b** and **10c** were formed, and starting material was consumed. The reaction mixture was worked up as described in the previous experiment, and products were purified on a small silica gel column eluted with 10 to 20% ethyl acetate−hexane to give clean gums of **10b** (16 mg) and **10c** (40 mg). **10b:** ^{1}H NMR ($CDCl_3$) δ: 0.58 (3H, s), 0.88 (3H, d, J = 6.9 Hz), 0.92 (3H, d, J = 6.6 Hz), 1.34−1.46 (2H, m), 1.50−1.69 (2H, m), 1.79−2.10 (3H, m), 1.86 (3H, brs), 2.07 (3H, s), 2.19 (3H, s), 2.34 (2H, m), 2.45 (1H, q, J = 6.6 Hz), 2.56 (3H, s), 3.41 (2H, d, J = 7.5 Hz), 4.68 (1H, brs, OH), 5.08 (1H, brs, OH), 5.28 (1H, brt, J = 6.9 Hz), 6.27 (1H, s), HREIMS (m/z): 358.2473 (M^+, calcd for $C_{23}H_{34}O_3$: 358.2508); **10c:** ^{1}H NMR ($CDCl_3$) δ: 0.57 (3H, s), 0.88 (3H, d, J = 6.6 Hz), 0.91 (3H, d, J = 6.6 Hz), 1.30−1.65 (4H, m), 1.85 (3H, s), 1.87−2.1 (3H, m), 2.20 (3H, s), 2.32 (2H, m), 2.46 (1H, q, J = 7.2 Hz), 3.41 (2H, d, J = 6.9 Hz), 4.88 (2H, brs), 5.05 (1H, brs, OH), 5.30 (1H, t, J = 6.9 Hz), 6.23 (1H, s), 7.68 (1H, brs, OH); HREIMS (m/z): 374.2455 (M^+, calcd for $C_{23}H_{34}O_4$: 374.2457); **12b:** ^{1}H NMR ($CDCl_3$) δ: 0.82 (3H, d, J = 6.6 Hz), 0.84 (3H,

s), 0.96 (3H, d, J = 7.2 Hz), 1.27−1.40 (3H, m), 1.41−1.62 (5H, m), 1.74−1.91 (2H, m), 1.84 (3H, s), 2.08 (3H, s), 2.19 (3H, s), 3.40 (2H, d, J = 6.9 Hz), 3.84 (1H, dd, J =2.7, 2.7 Hz), 4.69 (1H, brs, OH), 5.14 (1H, brs, OH), 5.25 (1H, t, J = 6.9 Hz), 6.23 (1H, s); HREIMS (m/z): 360.2702 (M^+, calcd for $C_{23}H_{36}O_3$: 360.2664).

Assay of Farnesyl-Protein Transferase. Recombinant human FPTase and the Ras peptide were prepared as described by Omer et al.[29] Bovine FPTase assay was performed as described elsewhere.[6d] The human transferase assay was carried out in a volume of 100 μL containing 100 mM *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid) (HEPES), pH 7.4, 5 mM $MgCl_2$, 10 μM $ZnCl_2$, 5 mM dithiothreitol (DTT), 0.1% (w/v) polyethylene glycol 20,000, 50 nM [^{3}H]-FPP, 400 nM Ras-CVLS, and 2 nM FPTase at 31 °C for 20 min. Reactions were initiated with FPTase and quenched with 0.1 mL of 30% (v/v) trichloroacetic acid in ethanol. Precipitates were collected onto glass fiber filtermats using a Tomtec Mach II cell harvestor, washed thoroughly with 100% ethanol, dried, and counted in an LKB Betaplate counter. The assay was linear with respect to both substrates, enzyme level, and time. Less than 10% of the [^{3}H]-FPP was utilized during the reaction period. Compounds were dissolved in 100% dimethyl sulfoxide and were diluted twenty fold into the assay.

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Supporting Information Available: ^{1}H (400 or 300 MHz) and ^{13}C (100 or 75 MHz) NMR spectra of **1a**, **1b**, **1d**, **1f**, **2a**, **2b**, **5**; ^{1}H (400 or 300 MHz) NMR spectra of **1c**, **1e**, **2d**, **6b**, **6c**, **6d**, **7**, **8b**, **8d**, **10a**, **10b**, and **12a**; a 400 MHz HMBC correlation of **1b**; and ORTEP diagrams of **1a** and **1f** from single-crystal X-ray structure determination (30 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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