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Synthetic Modifications of Ascomycin - I. A Chemoselective Removal of the Cyclohexyl Residue of Ascomycin

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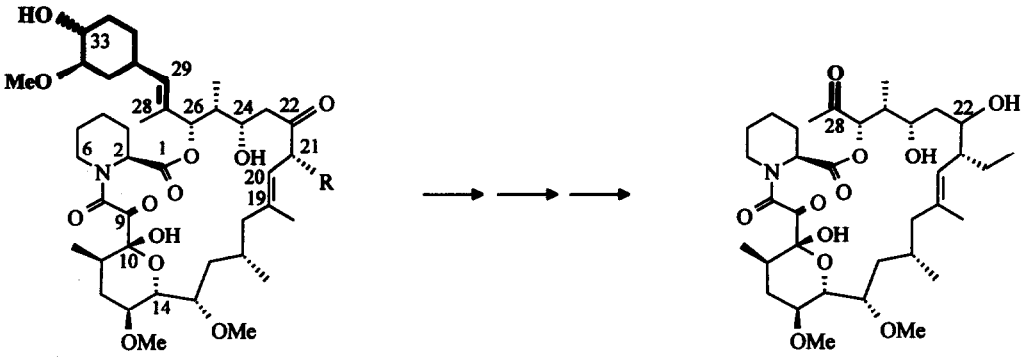
Abstract: An efficient semisynthetic preparation of des-28-(cyclohexyl)methylene-28-oxo-ascomycin derivatives starting from 24,33-*O*-bis(*tert*-butyldimethylsilyl)-ascomycin (**1**) is described. The strategy for preparing 28-oxo-ascomycin derivatives involves the reduction of C-22 carbonyl group, followed by 5-endo-cyclization of the resulting C-22 alcohol with the C-19/C-20 double bond using an oxymercuration reaction; ozonolysis of the C-28/C-29 double bond and regeneration of the C-19/C-20 double bond. Further, the 20-mercury-substituted ascomycin derivatives could be reduced to the corresponding metal free cyclic ethers using n-Bu_3SnH.

Introduction. The 23-membered macrolide FK 506 (tacrolimus, Prograf®), a potent immunosuppressant, isolated from *Streptomyces tsukubaensis* 9993,[1] has been the subject of intensive investigations in synthetic modifications,[2] biological and pharmacological examinations[3] as well as structural assignments.[4] Several groups have investigated partial[5] and total[6] syntheses of FK 506 and its analogues in the last few years. It has been suggested and verified by structural studies, that this immunosuppressant agent possesses two functional molecular domains: a binding domain containing a tricarbonyl region (the left half of the molecule) and an effector domain (the right half).[7] A number of reports on the chemical modifications and biological evaluations of both domains (e.g. binding domain,[8-15] effector domain[16-20]) have appeared. The role of the cyclohexyl moiety is, however, not completely clear,[21] although there have been numerous reports describing modification of this part of the structure in FK 506 and its 21-ethyl analogue ascomycin.[22] As a part of our research program we aimed at the synthesis and biological evaluation of FK 506 and ascomycin derivatives in which the cyclohexyl residue is not simply modified but replaced by other substituents.

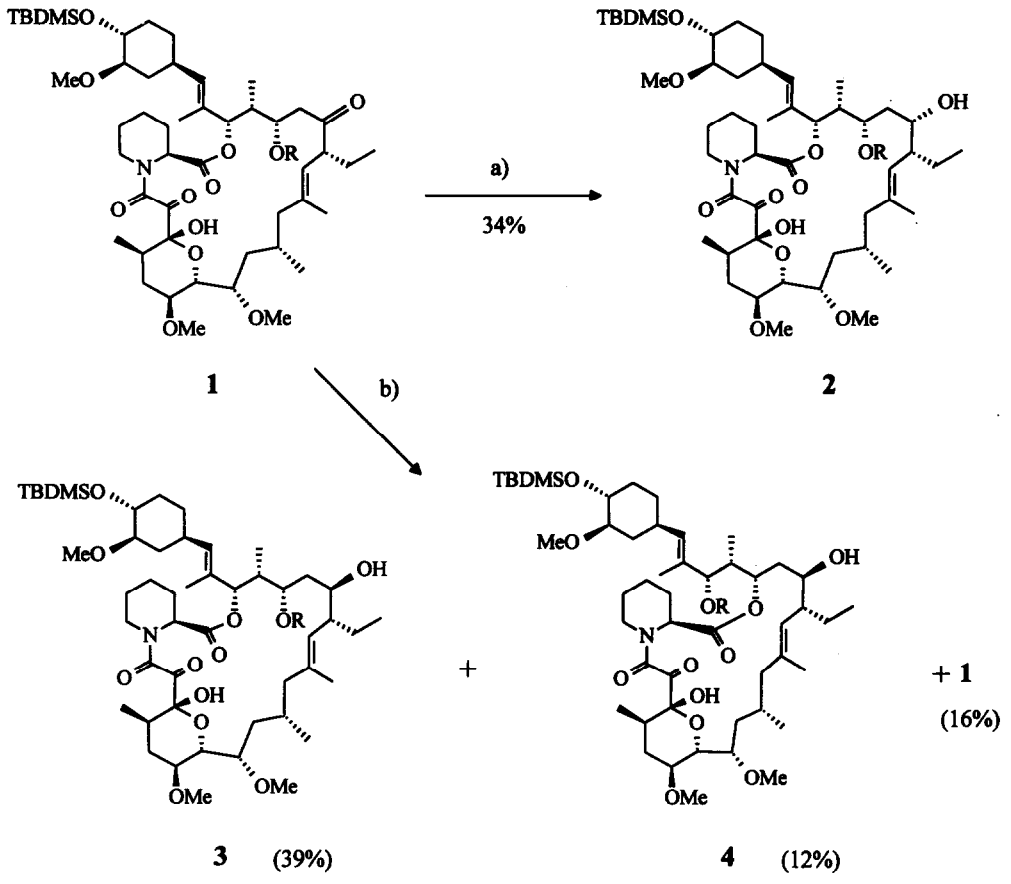
We wish to report herein our strategy for preparing des-28-(cyclohexyl)methylene-28-oxo-ascomycin derivatives, which has been accomplished in four steps, starting from the 24,33-*O*-bis-silylated ascomycin **1**. The reaction sequence for these derivatives involves 1) the protection of the C-19/C-20 double bond through reduction of the C-22 carbonyl group followed by 2) 5-endo-cyclization of the resulting C-22 alcohol with the C-19/C-20 double bond using an oxymercuration reaction 3) ozonolysis of the exocyclic C-28/C-29 double bond, and 4) regeneration of the C-19/C-20 double bond (Scheme 1). Restoration of the 22-ketofunction is envisaged after introduction of new substituents in position 28 in order to avoid competing reactions at position 22.



FK 506 (R = allyl)
Ascomycin (R = ethyl)

Scheme 1

Regio- and stereoselective reduction of the C-22 carbonyl group: It is known that the C-22 carbonyl group in 24,33-*O*-bis(*tert*-butyldimethylsilyl)-ascomycin (**1**) can be reduced regio- und stereoselectively to the 22(*S*)-alcohol **2** with L-Selectride® (Scheme 2).[23] Surprisingly, when K-Selectride® was used instead, the 22(*R*)-isomer **3** was formed predominantly (39% yield) together with 26,33-*O*-bis(*tert*-butyldimethylsilyl)-22(*R*)-dihydro-*iso*-ascomycin (**4**, 12%) and trace amounts of the 22(*S*)-dihydro-ascomycin **2**. The exact isomeric ratio was found to be 22-*R* : 22-*S* ≥ 97 : 3 by NMR analysis of the isomeric mixture obtained in the next step. Compound **4** is formed by an acyl-shift from O-26 to O-24 with concomitant migration of the O-24-silyl group to O-26. Similar reactions are known to occur with ascomycins under basic reaction conditions.[23] The structure of the 22(*R*)-compound **4** was established by ^{1}H NMR spectroscopy (26-H: δ = 4.15; 24-H: δ = 4.93) as well as by comparing with the ^{1}H/^{13}C NMR data of the already known 22(*S*)-epimer.[23b]

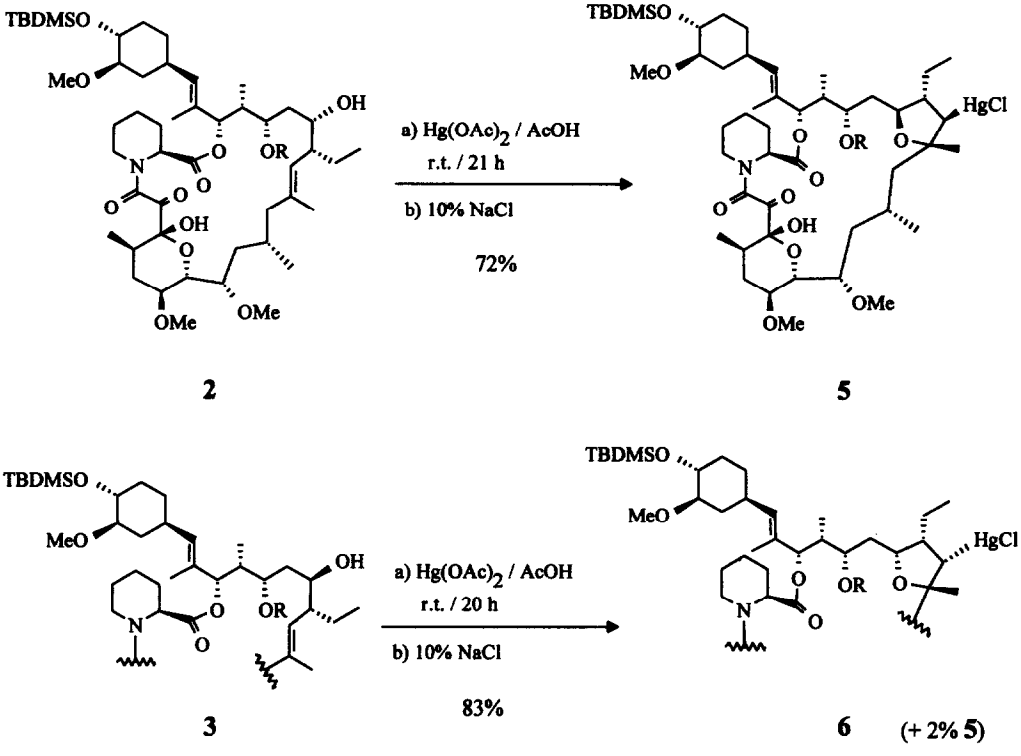


Reaction conditions: a) L-Selectride; -45 °C to -25 °C / 4 h. b) K-Selectride; -45 °C / 0.5 h, -25 °C / 4.5 h.

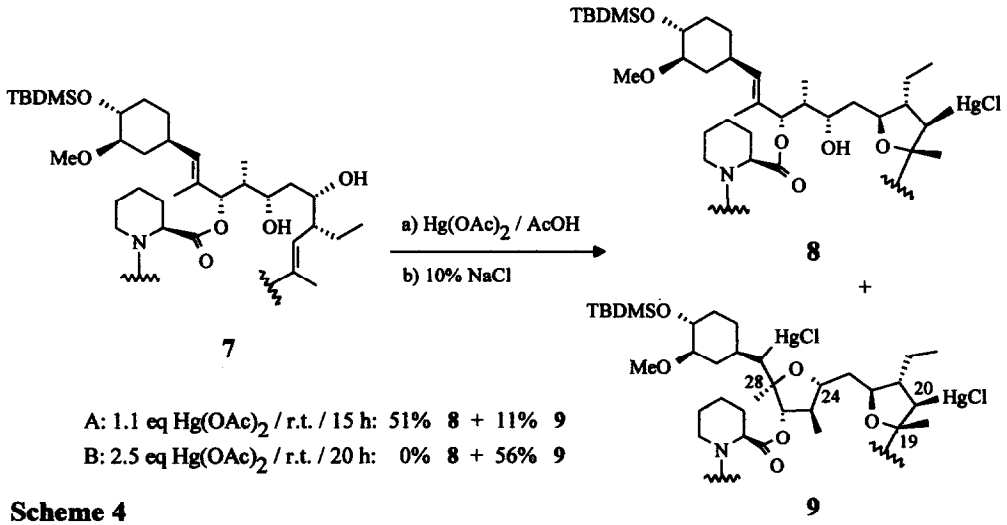
Scheme 2 R = TBDMS

Protection of the C-19/C-20 double bond: The two epimeric homoallylic alcohols **2** and **3** were converted stereospecifically to the corresponding tetrahydrofuran structures **5** (72%) and **6** (83%), respectively, using mercury(II) acetate in acetic acid (Scheme 3). The configurations of the tetrahydrofuran partial structures in **5** and **6**, as depicted in Scheme 3, were determined by $^{1}H/^{13}C$ NMR investigations (ROESY).

In the case of the 22(*S*)-dihydro-ascomycin derivative **7** (Scheme 4) with a free 24-hydroxyl group oxymercuration using 1.1 equivalents of mercury(II) acetate led to the mono- and the bis-tetrahydrofuran structures **8** (51%) and **9** (11%), respectively, indicating that the C-19/C-20 double bond is attacked preferentially. With 2.5 equivalents of mercury(II) acetate the bis-tetrahydrofuran derivative **9** was obtained in 56% yield. Compound **9** is configurationally homogeneous with respect to the newly generated stereocenters (see Scheme 4).



Scheme 3 R = TBDMS

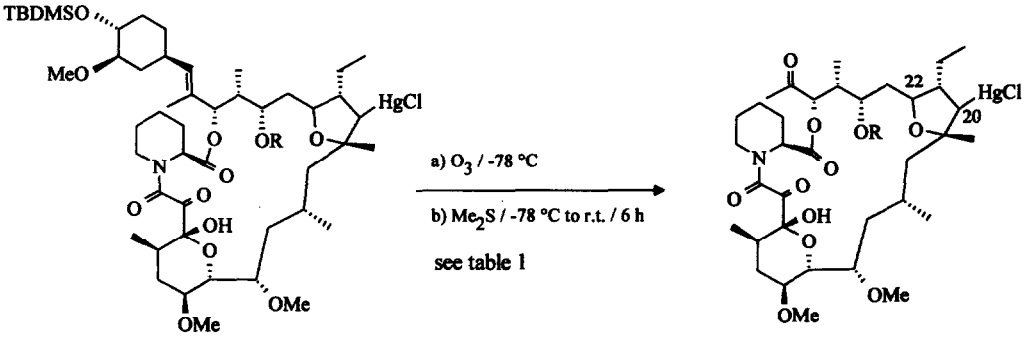


7

A: 1.1 eq $Hg(OAc)_2$ / r.t. / 15 h: 51% **8** + 11% **9**
B: 2.5 eq $Hg(OAc)_2$ / r.t. / 20 h: 0% **8** + 56% **9**

Scheme 4

Removal of the cyclohexyl fragment: Having protected the C-19/C-20 double bond removal of the cyclohexyl moiety was envisaged by oxidative cleavage of the C-28/C-29 double bond. Thus, the mercury-containing protected compounds **5**, **6** and **8** were subjected to ozonolysis (-78 °C) and the ozonides were decomposed with dimethyl sulfide leading to the products **10-12** in good to excellent yields (Scheme 5, Table 1). It is known that secondary organomercury compounds can react with ozone to give the corresponding ketones.[24] We found, however, no indication for the formation of 20-ketoderivatives.



5, 6, 8 **10, 11, 12**

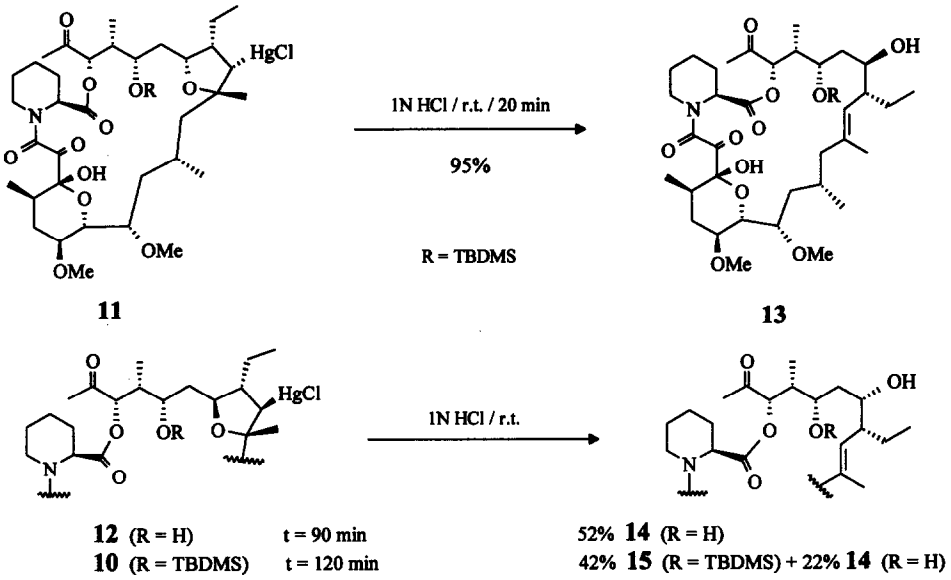
Scheme 5

Table 1. Preparation of **10** - **12**

Educt	R	Configuration at C-20	Configuration at C-22	Product	Yield [%]
5	TBDMS	*R*	*S*	**10**	80
6	TBDMS	*S*	*R*	**11**	98
8	H	*R*	*S*	**12**	54

Regeneration of the C-19/C-20 double bond: The restoration of the C-19/C-20 double bond could be easily achieved by treatment of the mercury-substituted compounds with 1N aqueous hydrochloric acid in acetonitrile at room temperature for a few minutes (Scheme 6). Thus, the compounds **10**, **11** and **12**, respectively, led to the expected 28-oxo-ascomycin derivatives **13-15** in good yields. With **10** additional

desilylation to product **14** was observed after longer reaction times (120 min). The configuration at the C-19/C-20 double bond in **13** was determined by means of ^{1}H NMR-NOE measurement. A positive NOE was observed at the signals of 18-H, 21-H and 22-H with irradiation at 4.88 ppm (20-H), whereas no NOE was found between 20-H and 19-Me protons. This confirms that the restored C-19/C-20 double bond has the same configuration (*E*) as in the parent compound.

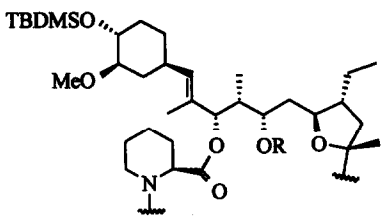


11 **13**

12 (R = H) t = 90 min 52% **14** (R = H)
10 (R = TBDMS) t = 120 min 42% **15** (R = TBDMS) + 22% **14** (R = H)

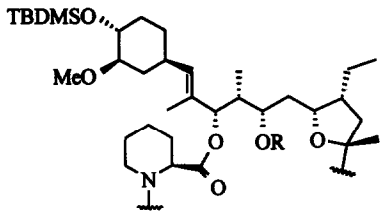
Scheme 6

Preparation of C-19/C-22 cyclic ethers: We have also explored the demetallation of 20-mercury-substituted ascomycin derivatives for the preparation of the corresponding metal free cyclic ethers (Scheme 7). Thus, the derivatives **5**, **6** and **9** were converted into **16**, **17** and **18**, respectively, using *n*-tributylstannyl hydride under mild reaction conditions (room temperature / short reaction time) in reasonable yields (54-75%). These transformations demonstrate a suitable preparation of useful ascomycin derivatives with a modified effector domain. Interestingly, Edmunds et al.[19] have recently described a C-19/C-22 cyclic ether derivative which binds to macrophilin-12, the cytosolic receptor of FK 506, with an affinity similar to that of FK 506.

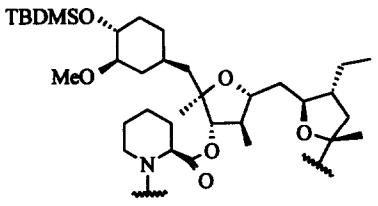


16 (64%)

R = TBDMS



17 (54%)

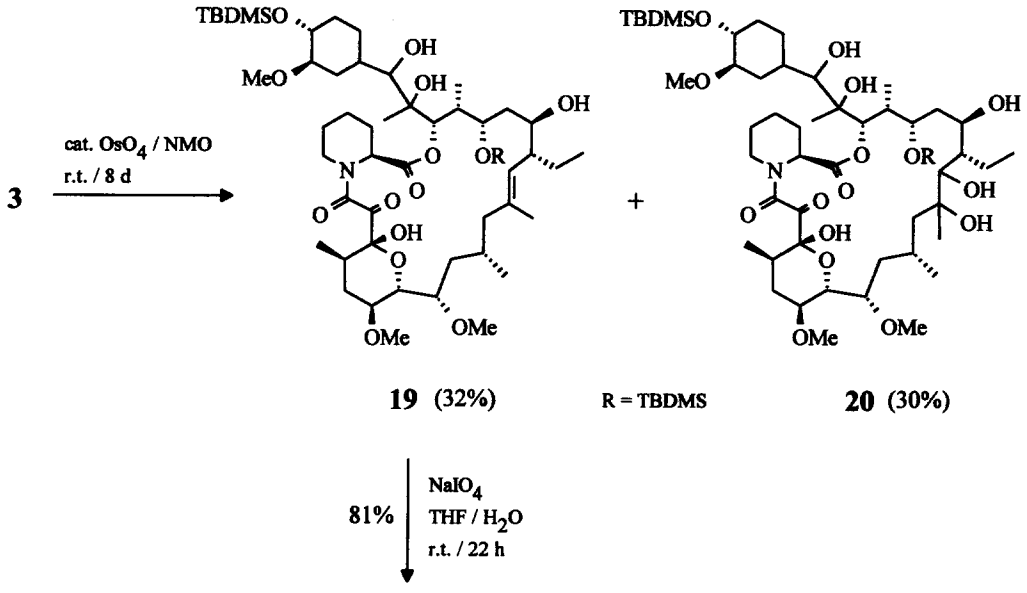


18 (75%)

Scheme 7

Alternative Synthesis of 28-oxo-ascomycin derivative 13: In connection with our efforts to the preparation of 28-oxo-ascomycin **13** starting from the bis-silylated compound **3**, we also studied an alternative route. Thus **3** was subjected to *cis*-hydroxylation using osmium tetroxide and *N*-methylmorpholine *N*-oxide (Scheme 8). The reaction, however, was unselective affording about equal amounts of the two products: **19** (32%) and **20** (30%). Ozonolytic cleavage of unprotected ascomycin was observed to lack regioselectivity as well.[25] Compound **19** reacted smoothly with sodium periodate to give the required product **13** (81%).

Summary. In conclusion, a convenient semisynthetic strategy for preparing des-28-(cyclohexyl)-methylene-28-oxo-ascomycin derivatives **13-15** has been developed. Starting from **1** 24-*O*-(*tert*-butyldimethylsilyl)-22(*R*)-dihydro-28-oxo-ascomycin (**13**) was prepared in an overall yield of 30%. We have also demonstrated, that **13** could alternatively be accessed from **3** via **19**, albeit in a lower overall yield (10%). Further reactions employing 28-oxo-ascomycin derivatives as key intermediates will be presented in a forthcoming paper.[26]



Scheme 8

EXPERIMENTAL SECTION

General: ^1H and ^{13}C NMR spectra were recorded on a Bruker WM 250 and Bruker AMX 500. The solvent ($CDCl_3$) was used as internal standard ($\delta_H = 7.27$, $\delta_C = 77.0$). Since ^1H NMR spectra always show complicated overlapping multiplets, only relevant absorptions are reported. All mass spectra are fast atom bombardment (FAB) spectra and were recorded on a VG 70-SE instrument (VG analytical) operating at 8 kV accelerating voltage. Melting points were determined on a Reichert Thermovar microscope and are uncorrected. Column chromatography was carried out on silica gel (0.040 - 0.063 mm, E. MERCK). All reactions were monitored by TLC on glass backed silica gel plates with fluorescent stain for UV absorption indication ($\lambda_{max} = 254$ nm). Visualization of the reaction components was obtained by spraying with a solution of molybdatophosphoric acid (20% in EtOH/H_2O, 3:1). All reactions were performed in flame dried reaction vessels under a slight pressure of dry argon. Solvents were dried by standard methods. All other commercially available reagents were applied without further purification. The ozonolyses were carried out with an apparatus from Gebr. Herrmann, Cologne, Germany.

24,33-O-Bis(tert-butyldimethylsilyl)-22(S)-dihydro-ascomycin (2): By analogy with ref.[23], a solution of 1.00 g (0.980 mmol) of **1** and 2.21 ml (2.21 mmol) of L-Selectride® (1M in THF) in 20 ml of dry THF was stirred for 4 h at -45 °C → -25 °C. After work up as described in ref.[23] the crude product was purified by column chromatography (hexane/ethyl acetate, 5:1). Yield: 0.34 g (34%) of **2** as colourless foam. Mixture of two conformers (4:1), major conformer: ^1H NMR ($CDCl_3$): δ 5.22 (s, 26-H), 5.10 (d, J=9Hz, 29-H), 3.40,

3.38, 3.28 (3 s, 3 OMe), 0.91, 0.88 (2 s, SitBu), 0.10, 0.07, 0.06, 0.04 (4 s, $SiMe_2$). ^{13}C NMR ($CDCl_3$): δ 196.0 (C-9), 169.0 (C-1), 165.1 (C-8), 135.9 (C-19), 132.6 (C-28), 130.2 (C-29), 125.8 (C-20), 97.1 (C-10), 84.1 (C-32), 75.3, 75.2 (C-15, -33), 73.8, 73.5, 72.9, 69.8 (C-13, -14, -22, -24), 58.0 (C-2), 57.0, 56.8, 56.2 (3 OMe), 49.0 (C-18), 45.8 (C-21), 40.4, 39.4 (C-6, -25), 36.6 (C-23), 35.0, 34.9, 33.8, 32.8, 30.7 (C-12, -16, -30, -34, -35), 27.0 (C-3), 25.9 (SitBu), 26.2, 25.4 (C-5, -17), 24.4 (C-36), 21.6 (C-4), 20.3 (17-Me), 18.1, 18.0 (Si-*C*), 16.1 (11-Me), 15.3 (19-Me), 14.3 (28-Me), 12.3 (C-37), 10.8 (25-Me), -4.3, -4.5, -4.6, -4.7 ($SiMe_2$). FAB-MS: m/z = 1028 ($[M+Li]^+$), 894, 870, 670, 560, 522, 383, 266, 225.

24,33-O-Bis(tert-butyldimethylsilyl)-22(R)-dihydro-ascomycin (3) and its iso-ascomycin derivative (4): To a solution of 25.0 g (24.5 mmol) of 24,33-*O*-bis(*tert*-butyldimethylsilyl)-ascomycin (**1**) in 250 ml of THF 110 ml (55.0 mmol) of K-Selectride® (0.5M in THF) was slowly added within 30 min at -45 °C. Then the reaction mixture was warmed up to -25°C and was stirred for 4.5 h. 80 ml of 1N HCl were added, the organic layer was separated, followed by the extraction of the aqueous phase with diethyl ether (3 x 50 ml). Drying of the combined extracts ($MgSO_4$) and evaporation of the solvents provided 31.8 g of crude product, which was purified by column chromatography (hexane/ethyl acetate, 5:1 → 4:1 → 3:1): fraction I: 9.83 g (39%) of **3** (22-*R*:22-*S* ≥ 97:3); fraction II: 0.98 g of a mixture of **3** and starting material **1**; fraction III: 4.07 g (16%) of **1**; fraction IV: 2.94 g (12%) of **4**.

22(*R*)-Dihydro-ascomycin **3**: ^1H NMR ($CDCl_3$): δ 5.49 (d, J=1Hz, 2-H), 5.38 (d, J=2Hz, 26-H), 4.98 (d, J=9Hz, 29-H), 4.89 (d, J=10Hz, 20-H), 4.41 (br d, J=11Hz, 6-H_e), 3.42, 3.35, 3.29 (3 s, OMe), 0.91, 0.90 (2 s, SitBu), 0.17, 0.15, 0.08, 0.07 (4 s, $SiMe_2$). ^{13}C NMR ($CDCl_3$): δ 198.5 (C-9), 168.5 (C-1), 166.1 (C-8), 136.3 (C-19), 131.8 (C-28), 128.5 (C-29), 125.5 (C-20), 99.1 (C-10), 84.2 (C-32), 76.5 (C-26), 75.8, 75.3 (C-24, -33), 74.5, 74.4, 73.8, 73.6 (C-22, -13, -14, -15), 58.2 (C-2), 57.0, 56.4, 56.2 (3 OMe), 49.3 (C-18), 44.7 (C-21), 38.7 (C-25), 38.1 (C-6), 36.8 (C-30), 35.5, 34.9, 34.8 (C-11, -12, -31), 33.9 (C-23), 33.0, 32.5 (C-16, -34), 31.1 (C-35), 27.9 (C-3), 26.9, 26.5 (C-5, -17), 26.0, 25.9 (SitBu), 24.2 (C-36), 22.0 (17-Me), 21.8 (C-4), 18.2, 18.1 (Si-*C*), 16.1 (11-Me), 15.4 (19-Me), 14.7 (28-Me), 12.1 (C-37), 10.6 (25-Me), -3.6, -3.9, -4.5, -4.7 ($SiMe_2$). FAB-MS: m/z = 1028 ($[M+Li]^+$), 872, 560, 522, 493.

22(*R*)-Dihydro-*iso*-ascomycin **4**: ^1H NMR ($CDCl_3$): δ 5.31 (d, J=9Hz, 29-H), 5.09 (d, J=10Hz, 20-H), 4.93 (dt, J=4, 10Hz, 24-H), 4.59 (br d, J=4Hz, 2-H), 4.48 (br d, J=12Hz, 6-H_e), 4.23 (s, 10-OH), 4.15 (br s, 26-H), 3.78 (dd, J=1.5, 10Hz, 14-H), 2.96 (ddd, J=4.5, 8.5, 11Hz, 32-H), 2.65 (dt, J=2, 12Hz, 6-H_a), 0.89 (s, SitBu), 0.09, 0.08, 0.07, 0.06 (4 s, $SiMe_2$). ^{13}C NMR ($CDCl_3$): δ 195.8 (C-9), 169.9 (C-1), 165.3 (C-8), 134.8 (C-19), 133.6 (C-28), 130.5 (C-29), 129.2 (C-20), 96.9 (C-10), 84.2 (C-32), 76.9, 75.3, 75.2, 73.4, 73.1, 72.3 (C-13, -14, -15, -22, -24, -26, -33), 57.9, 56.7, 56.4 (3 OMe), 57.1 (C-2), 49.6, 39.7, 38.8, 36.8, 36.5, 35.1, 34.8 (C-6, -16, -18, -21, -23, -25, -30, -31), 33.9 (C-34), 32.8, 32.3 (C-11, -12), 30.9 (C-35), 26.5 (C-3), 26.0 (SitBu), 25.7 (C-17), 24.6 (C-15, -36), 21.6 (C-4), 20.2 (17-Me), 20.1 (Si-*C*), 18.2 (11-Me), 16.3 (19-Me), 15.0 (28-Me), 13.4 (C-37), 8.2 (25-Me), -3.7, -4.2, -4.5, -4.7 ($SiMe_2$). FAB-MS: m/z = 1028 ($[M+Li]^+$), 1004, 986, 854, 822, 716, 690, 636, 493.

Reaction with mercury(II) acetate; General Procedure: The corresponding 22-dihydro-ascomycin derivative (1 equivalent) was dissolved in acetic acid (20 ml/1 mmol) and mercury(II) acetate (1.1 - 2.5 equivalents) was added under argon atmosphere. The solution was stirred mechanically at room temp. for the time indicated. After consumption of starting material, the resulting orange solution was concentrated in vacuo. The residue was dissolved in toluene (20 ml/1 mmol) and the resulting solution was subsequently washed with 10% NaCl solution (5 ml/1 mmol) and H_2O (5 ml/1 mmol). The organic phase was dried ($MgSO_4$) and the solvent was evaporated in vacuo. The resulting crude product was purified by column chromatography (hexane/ethyl acetate, 5:1). For individual experimental data see Table 2.

Table 2. Synthesis of mercury-containing ascomycin derivatives **5**, **6**, **8**, and **9**

Precursor	g (mmol)	$Hg(OAc)_2$ g (mmol)	Reaction time [h]	Product	Yield g (%)
2	0.523 (0.511)	0.203 (0.639)	21	**5**	0.453 (72)
3[a]	6.64 (6.50)	2.59 (8.13)	20	**6** **5**	6.72 (83) 0.160 (2)
7	0.300 (0.33)	0.116 (0.363)	15	**8** **9**	0.190 (51) 0.048 (11)
7	0.113 (0.125)	0.100 (0.313)	20	**9**	0.096 (56)

[a] **3** contains 22(*S*)-dihydro-ascomycin **2** (< 3%).

Characterizations of 5, 6, 8 and 9: Compound **5**: ^{1}H NMR ($CDCl_3$): δ 5.04 (s, 26-H), 5.02 (d, J=9Hz, 29-H), 4.66 (s, 2-H), 4.48 (d, J=13Hz, 6-H_e), 4.09 (s, 10-OH), 3.77 (d, J=9Hz, 14-H), 3.41, 3.38, 3.28 (3 s, OMe), 2.96 (m_c, 32-H), 2.47 (br t, J=13Hz, 6-H_a), 2.32 (d, J=12.5Hz, 20-H), 1.63 (s, 28-Me), 1.16 (s, 19-Me), 1.00 (t, J=6.5Hz, 37-H), 0.91, 0.88 (2 s, SitBu), 0.13, 0.07, 0.06, 0.05 (4 s, $SiMe_2$). ^{13}C NMR ($CDCl_3$): δ 195.7 (C-9), 168.8 (C-1), 165.2 (C-8), 131.1 (C-28), 129.1 (C-29), 96.7 (C-10), 84.1, 83.9 (C-19, -32), 79.4, 79.1 (C-22, -26), 75.2, 74.9 (C-15, -33), 73.5 (C-13), 72.2 (C-14), 71.6, 71.0 (C-20, -24), 58.1, 56.6, 56.4 (3 OMe), 57.0 (C-2), 51.0 (C-18), 48.8 (C-21), 42.3, 42.1 (C-23, -25), 39.7 (C-6), 36.7 (C-31), 34.9, 34.85 (C-11, -30), 33.8 (C-34), 33.0, 32.9 (C-12, -16), 31.0 (C-35), 30.4 (C-3, 19-Me), 26.7 (C-17), 26.2, 25.8 (SitBu), 24.7 (C-5), 23.8 (C-36), 21.7 (C-4), 20.6 (17-Me), 18.3, 18.1 (Si-*C*), 16.3 (11-Me), 14.5 (28-Me), 13.2 (C-37), 11.1 (25-Me), -3.5, -3.6, -4.5, -4.7 ($SiMe_2$). FAB-MS: m/z = 1356 ($[M+Li]^+$), 1222, 1028, 994, 678, 516.
Compound **6**: ^{1}H NMR ($CDCl_3$): δ 5.17 (s, 26-H), 5.00 (d, J=9Hz, 29-H), 4.45 (d, J=13Hz, 6-H_e), 4.41 (s, 2-H), 4.23 (s, 10-OH), 3.72 (d, J=10Hz, 14-H), 3.41, 3.38, 3.29 (3 s, OMe), 2.96 (m_c, 32-H), 1.61 (s, 28-Me), 1.24 (s, 19-Me), 1.07 (t, J=7Hz, 37-H), 0.93, 0.90 (2 s, SitBu), 0.09, 0.08, 0.07, 0.06 (4 s, $SiMe_2$). ^{13}C NMR ($CDCl_3$): δ 195.5 (C-9), 168.5 (C-1), 165.1 (C-8), 131.1 (C-28), 129.1 (C-29), 97.6 (C-10), 84.15, 84.1 (C-19, -32), 77.5, 76.5, 75.4, 75.2, 73.7, 72.4 (C-13, -14, -15, -22, -26, -33), 70.5, 69.8 (C-20, -24), 58.1, 56.7, 56.3 (3 OMe), 57.4 (C-2), 50.9 (C-18), 46.1 (C-21), 41.8, 39.5, 38.0, 36.8, 34.9, 34.8, 33.8, 33.5, 32.7 (C-6, -11, -12, -16, -23, -25, -30, -31, -34), 31.0, 28.4 (C-3, -35, 19-Me), 26.8 (C-17), 26.0, 25.9 (SitBu), 24.6 (C-5), 22.6, 21.6 (C-4, -36), 18.1 (Si-*C*), 16.0 (11-Me), 14.8, 14.5 (C-37, 28-Me), 11.0 (25-Me), -3.9, -4.0, -4.5, -4.7 ($SiMe_2$). FAB-MS: m/z = 1356 ($[M+Li]^+$), 1222, 1028, 994, 678, 614, 400, 266, 227.
Compound **8**: mixture of two conformers (4:1), major conformer: ^{1}H NMR ($CDCl_3$): δ 5.12 (d, J=9Hz, 26-H), 5.08 (s, 29-H), 4.66 (d, J=5Hz, 2-H), 4.46 (d, J=13Hz, 6-H_e), 4.20 (s, 10-OH), 3.74 (dd, J=1, 8Hz, 24-H), 3.41, 3.37, 3.29 (3 s, OMe), 2.97 (m_c, 32-H), 2.61 (br t, J=12.5Hz, 6-H_a), 2.35 (d, J=12Hz, 20-H), 1.64 (s, 28-Me), 1.21 (s, 19-Me), 1.06 (t, J=7Hz, 37-H), 0.88 (s, SitBu), 0.08, 0.07 (2 s, $SiMe_2$). ^{13}C NMR ($CDCl_3$): δ 196.8 (C-9), 168.9 (C-1), 165.2 (C-8), 131.1 (C-28), 129.5 (C-29), 99.8 (C-10), 84.0 (C-32), 83.9 (C-19), 80.1 (C-22), 78.8 (C-26), 75.0 (C-15), 74.9 (C-33), 73.8 (C-13), 72.2 (C-14), 71.3 (C-20), 70.0 (C-24), 58.1, 57.0, 56.3 (3 OMe, C-2), 50.6 (C-18), 49.0 (C-21), 41.7, 41.5 (C-6, -23), 39.5 (C-25), 36.5 (C-31), 34.8 (C-30), 34.7 (C-16), 33.6, 33.0, 32.9 (C-11, -12, -34), 30.8 (C-35), 30.3 (C-3), 27.0 (C-17), 25.8 (SitBu), 24.7 (C-5), 23.9 (C-36), 21.6 (C-4), 20.8 (17-Me), 18.1 (Si-*C*), 16.3 (11-Me), 14.8 (28-Me), 13.4 (C-37), 9.8 (25-Me), -4.5, -4.7 ($SiMe_2$). FAB-MS: m/z = 1242 ($[M+Li]^+$), 914, 678, 516.
Compound **9**: m.p. 173-175 °C; ^{1}H NMR ($CDCl_3$): δ 4.99 (d, J=5.5Hz, 2-H), 4.65 (s, 26-H), 4.41 (d, J=13.5Hz, 6-H_e), 4.15 (dd, J=4, 10Hz, 24-H), 3.83 (s, 10-OH), 3.61 (d, J=9.5Hz, 14-H), 3.60 (dd, J=2, 11.5Hz, 15-H), 3.51 (dt, J=1.5, 10Hz, 22-H), 2.95 (ddd, J=4.5, 8.5, 11Hz, 32-H), 2.91 (d, J=5Hz, 29-H), 1.49, 1.28 (2 s, 19-, 28-Me), 1.08 (t, J=7Hz, 37-H), 1.06 (d, J=6Hz, 11-Me), 1.04 (d, J=7Hz, 25-Me), 0.94 (d, J=6Hz, 17-Me), 0.89 (s, SitBu), 0.08, 0.06 (2 s, $SiMe_2$). ^{13}C NMR ($CDCl_3$): δ 197.7 (C-9), 170.4 (C-1), 165.6 (C-8), 96.0 (C-10), 88.2 (C-26), 85.3 (C-28), 84.3 (C-32), 83.2 (C-19), 80.9 (C-24), 75.0, 74.8 (C-15, -33), 73.9,

73.0 (C-13, -14) 71.4 (C-20), 67.1 (C-29), 58.0, 57.5, 56.4 (3 OMe), 56.2 (C-2), 50.0 (C-18), 49.3 (C-21), 45.5 (C-25), 36.7, 36.0 (C-23, -30), 34.4, 33.8, 33.2, 33.0 (C-11, -12, -16, -34), 30.5 (19-Me), 29.6 (28-Me), 28.5 (C-3), 26.5 (C-17), 25.8 (SitBu), 24.3, 24.1 (C-5, -36), 21.2, 21.1 (C-4, 17-Me), 18.2 (Si-*C*), 16.5 (11-Me), 13.6 (C-37), 11.5 (25-Me), -4.5, -4.8 ($SiMe_2$). FAB-MS: m/z = 1392, 1341, 1305, 1200, 930, 564, 522, 365, 322, 266.

Ozonolysis of ascomycin derivatives; General Procedure: A solution of the corresponding ascomycin derivative in a 9:1 mixture of methanol/dichloromethane (50 ml/1 mmol of ascomycin derivative) was treated with ozone (reaction time see Table 3) at -78 °C. After addition of dimethyl sulfide (10 equivalents), the reaction mixture was allowed to warm up to room temp. within 6 h. Evaporation of the solvents and the volatile components, and purification of the crude product by column chromatography (hexane/ethyl acetate, 3:1) provided the 28-oxo derivative as a colourless foam. For experimental details see Table 3.

Table 3. Preparation of 28-oxo ascomycin derivatives **10** - **12** by ozonolysis

Precursor	g (mmol)	Reaction time [min]	Me_2S µl (mmol)	Product	Yield g (%)
5	0.391 (0.315)	5	235 (3.15)	**10**	0.253 (80)
6	6.70 (5.40)	57	4000 (54.0)	**11**	5.34 (98)
8	0.171 (0.152)	3	113 (1.52)	**12**	0.072 (54)

*Characterization of 28-oxo-ascomycin derivatives **10** - **12**:* Compound **10**: ^{1}H NMR ($CDCl_3$): δ 5.15 (d, J=2.5Hz, 26-H), 4.82 (s, 2-H), 4.44 (d, J=11Hz, 6-H_e), 4.07 (d, J=1.5Hz, 10-OH), 3.77 (dd, J=1.5, 9.5Hz, 14-H), 3.68 (dt, J=1.5, 9.5Hz, 24-H), 3.64 (ddd, J=1.5, 5, 11.5Hz, 15-H), 3.56 (dt, J=3, 4Hz, 22-H), 3.39, 3.30 (2 s, OMe), 2.71 (dt, J=3, 13Hz, 6-H_a), 2.34 (d, J=12.5Hz, 20-H), 2.16 (td, J=4.5, 12.5Hz, 12-H), 2.15 (s, 28-Me), 1.68 (dd, J=3, 14.5Hz, 18-H), 1.16 (s, 19-Me), 1.07 (t, J=7.5Hz, 37-H), 1.03 (d, J=6.5Hz, 11-Me), 0.90 (s, SitBu), 0.88 (d, J=7Hz, 25-Me), 0.86 (d, J=6.5Hz, 17-Me), 0.15, 0.06 (2 s, $SiMe_2$). ^{13}C NMR ($CDCl_3$): δ 201.9 (C-28), 196.3 (C-9), 169.2 (C-1), 165.3 (C-8), 96.5 (C-10), 83.9 (C-19), 80.2 (C-26), 79.2 (C-22), 74.7 (C-15), 73.7 (C-13), 72.2 (C-14), 71.4 (C-20), 70.6 (C-24), 56.9 (C-2), 56.6, 56.3 (2 OMe), 50.9 (C-18), 48.8 (C-21), 42.2 (C-23), 40.4 (C-25), 39.5 (C-6), 34.8 (C-11), 33.1, 33.0 (C-12, -16), 30.3 (19-Me), 28.0 (C-3), 26.1 (SitBu), 26.0 (C-17), 24.8 (C-5), 23.9 (C-36), 21.0 (C-4), 20.6 (17-Me), 16.3 (11-Me), 13.3 (C-37), 11.9 (25-Me), -3.5, -3.6 ($SiMe_2$). FAB-MS: m/z = 1116 ($[M+Li]^+$), 788, 762, 638.

Compound **11**: ^{1}H NMR ($CDCl_3$): δ 5.19 (d, J=2.5Hz, 26-H), 4.66 (br s, 2-H), 4.40 (d, J=13Hz, 6-H_e), 4.19 (s, 10-OH), 3.37, 3.28 (2 s, OMe), 2.89 (dt, J=2.5, 13Hz, 6-H_a), 2.28 (d, J=11Hz, 20-H), 2.15 (s, 28-Me), 1.19 (s, 19-Me), 1.07 (t, J=7Hz, 37-H), 0.97, 0.94 (2 d, J=6.5Hz each, 17-, 25-Me), 0.90 (s, SitBu), 0.09, 0.06 (2 s, $SiMe_2$). ^{13}C NMR ($CDCl_3$): δ 201.8 (C-28), 196.3 (C-9), 169.3 (C-1), 165.1 (C-8), 97.2 (C-10), 84.2 (C-19), 79.5 (C-26), 77.1 (C-22), 75.4 (C-15), 73.6 (C-13), 72.6 (C-14), 69.8, 69.3 (C-20, -24), 56.8 (C-2), 56.6, 56.3 (2 OMe), 50.6 (C-18), 45.8 (C-21), 40.9, 39.4, 38.9 (C-6, -23, -25), 34.8 (C-11), 33.2, 32.6 (C-12, -16), 28.0, 27.6, 26.8 (C-3, 19-, 28-Me), 26.1 (SitBu), 25.9 (C-17), 24.7 (C-5), 22.6, 21.3, 20.8 (C-4, -36, 17-Me), 18.1 (Si-*C*), 16.1 (11-Me), 14.6 (C-37), 11.5 (25-Me), -3.7, -4.3 ($SiMe_2$). FAB-MS: m/z = 1018 ($M^+ + H_2O$), 1000 (M^+), 764, 632, 614, 400, 266, 227.

Compound **12**: mixture of two conformers (3:2), major conformer: ^{1}H NMR ($CDCl_3$): δ 5.15 (d, J=2.5Hz, 26-H), 4.79 (br s, 2-H), 4.42 (d, J=13Hz, 6-H_e), 3.35, 3.32 (2 s, OMe), 2.77 (dt, J=3, 13Hz, 6-H_a), 2.18 (s, 28-Me). FAB-MS: m/z = 1002 ($[M+Li]^+$), 614, 400, 266.

24-O-tert-Butyldimethylsilyl-22(R)-dihydro-28-oxo-ascomycin (13): 5.18 g (5.18 mmol) of mercury-substituted ascomycin derivative **11** was stirred with 5 ml of 1N HCl in 75 ml of acetonitrile at room temp. for 20 min. 100 ml of dichloromethane and 25 ml of sat. $NaHCO_3$ solution were added, the organic layer separated, and the aqueous phase extracted with dichloromethane (3 x 30 ml). The combined extracts were dried ($MgSO_4$) and concentrated to provide 4.63 g crude product. Purification by recrystallization from hexane/acetone afforded 3.16 g (78%) **13** (single conformer) as colourless crystals; m.p. 150-152 °C. The concentration of the mother liquor provided further 0.70 g (17%) **13**; m.p. 148-152 °C. ^{1}H NMR ($CDCl_3$): δ 5.56 (d, J=3Hz, 26-H), 5.28 (d, J=2Hz, 10-OH), 4.88 (d, J=10Hz, 20-H), 4.41 (d, J=14Hz, 6-H_e), 4.32 (m_c, 2-H), 3.98 (ddd, J=4.5, 6.5, 11Hz, 24-H), 3.78 (td, J=2.5, 7.5Hz, 22-H), 3.37, 3.30 (2 s, OMe), 3.05 (dt, J=3, 13Hz, 6-H_a), 2.83 (d, J=2Hz, 22-OH), 2.48 (dquint, J=3, 7Hz, 25-H), 2.15 (28-Me), 2.10 (br d, J=15Hz, 18-H), 1.61 (s, 19-Me), 1.05 (d, J=6.5Hz, 17-Me), 1.02 (s, SitBu), 0.90 (d, J=7Hz, 11-Me), 0.88 (d, J=6.5Hz, 25-Me), 0.21, 0.20 (2 s, $SiMe_2$). ^{13}C NMR ($CDCl_3$): δ 204.2 (C-28), 198.4 (C-9), 169.8 (C-1), 166.1 (C-8), 136.5 (C-19), 125.3 (C-20), 98.9 (C-10), 78.6 (C-26), 76.4 (C-15), 74.4 (C-13), 73.9 (C-14), 73.7 (C-24), 73.3 (C-22), 56.6 (C-2), 56.4, 56.3 (2 OMe), 49.2 (C-18), 44.7 (C-21), 38.8 (C-6), 38.1 (C-25), 35.2 (C-23), 34.6 (C-11), 33.1 (C-16), 32.5 (C-12), 27.9 (C-17), 26.7, 26.6 (C-3, -36), 26.1 (28-Me), 25.9 (SitBu), 24.1 (C-5), 21.9 (17-Me), 21.2 (C-4), 18.1 (Si-*C*), 16.1 (19-Me), 15.4 (11-Me), 12.0 (C-37), 10.6 (25-Me), -4.1, -4.2 ($SiMe_2$). FAB-MS: m/z = 788 ([M+Li]$^+$), 764, 632, 614.

22(S)-Dihydro-28-oxo-ascomycin (14): According to the procedure as described above, a mixture of 0.791 g (0.893 mmol) **12** and 3 ml of 1N HCl in 10 ml of acetonitrile was stirred for 90 min at room temp. and then worked up. Purification of the crude product by chromatography (toluene/ethyl acetate, 1:1) yielded 0.307 g (52%) **14**. ^{1}H NMR ($CDCl_3$): δ 5.21 (d, J=3Hz, 26-H), 4.48 (d, J=13Hz, 6-H_e), 3.38, 3.34 (2 s, OMe), 2.18 (s, 28-Me), 1.61 (s, 19-Me). FAB-MS: m/z = 675 ([M+Li]$^+$), 657, 225.

By analogy with the preparation of **13**, the treatment of 0.201 g (0.201 mmol) **10** with 1 ml of 1N HCl in 2 ml of acetonitrile (reaction time: 120 min, r.t.) provided after chromatography (hexane/ethyl acetate, 3:1 → 1:1) 0.066 g (42%) of 24-*O*-silylated product **15** and 0.030 g (22%) of desilylated compound **14**.
Compound **15**: mixture of two conformers (9:1), major conformer: ^{1}H NMR ($CDCl_3$): δ 5.32 (s, 26-H), 5.13 (d, J=9Hz, 20-H), 4.37 (d, J=13Hz, 6-H_e), 4.34 (s, 2-H), 4.08 (br s, 10-OH), 3.35, 3.24 (2 s, OMe), 2.80 (dt, J=2.5, 13Hz, 6-H_a), 2.16 (s, 28-Me), 1.48 (s, 19-Me), 0.91 (s, SitBu), 0.12, 0.06 (2 s, $SiMe_2$). ^{13}C NMR ($CDCl_3$): δ 203.3 (C-28), 196.5 (C-9), 169.8 (C-1), 164.7 (C-8), 135.7 (C-19), 124.9 (C-20), 97.5 (C-10), 77.1, 75.1, 73.7, 72.9, 71.6 (C-13, -14, -15, -22, -26), 68.3 (C-24), 57.0, 56.5, 56.3 (C-2, 2 OMe), 48.5 (C-18), 45.6 (C-21), 39.3, 37.8 (C-23, -25), 34.7, 32.6, 31.9 (C-6, -11, -12), 27.4, 26.3 (C-3, -17), 25.9 (SitBu), 24.7 (C-5), 20.7 (C-4), 18.0 (Si-*C*), 16.1 (19-Me), 14.9 (11-Me), 11.9, 10.3 (25-, 28-Me), -4.1, -4.9 ($SiMe_2$). FAB-MS: m/z = 788 ([M+Li]$^+$), 764, 632, 227.

Treatment of mercury substituted compounds with n-tributylstannyl hydride; General Procedure: A solution of the corresponding mercury substituted compound (1 equivalent) in dichloromethane (3 ml/0.1 mmol) was treated with *n*-tributylstannyl hydride (1 or 2 equivalents) at room temp. for 15 min. Then the reaction mixture was filtered, the solvent evaporated and the residue was purified by chromatography (hexane/ethyl acetate, 5:1). For individual data see Table 4.

Table 4. Synthesis of **16** - **18**

Precursor	mg (mmol)	*n*-Bu_3SnH [mg (mmol)]	Product	Yield [mg (%)]
5	171 (0.138)	40.2 (0.138)	**16**	90 (64)
6	114 (0.092)	26.8 (0.092)	**17**	51 (54)
9	251 (0.187)	109 (0.374)	**18**	127 (75)

Characterization of compounds 16 - 18: Compound **16**: ^{1}H NMR ($CDCl_3$): δ 5.05 (s, 26-H), 5.01 (d, J=9Hz, 29-H), 4.58 (d, J=4.5Hz, 2-H), 4.48 (d, J=13.Hz, 6-H_e), 4.11 (s, 10-OH), 3.78 (dd, J=1.5, 9.5Hz, 14-H), 3.43, 3.38, 3.29 (3 s, OMe), 2.97 (ddd, J=4.5, 8.5, 11Hz, 32-H), 2.46 (dt, J=2.5, 13Hz, 6-H_a), 1.62 (s, 28-Me), 0.90, 0.88 (2 s, SitBu), 0.15, 0.08, 0.07, 0.06 (4 s, $SiMe_2$). ^{13}C NMR ($CDCl_3$): δ 195.4 (C-9), 168.8 (C-1), 165.1 (C-8), 131.1 (C-28), 128.7 (C-29), 96.9 (C-10), 84.0 (C-32), 81.3 (C-19), 79.7, 79.1 (C-22, -26), 75.2 (C-13, -15), 73.6 (C-33), 72.4, 72.2 (C-14, -24), 58.1, 56.6, 56.3 (3 OMe), 57.1 (C-2), 51.3 (C-20), 48.4 (C-18), 45.4 (C-21), 41.8, 41.6 (C-23, -25), 39.6 (C-6), 36.8 (C-31), 34.9 (C-11, -30), 33.9 (C-34), 33.0, 32.7 (C-12, -16), 31.1 (C-35), 26.4 (C-3), 26.2, 26.1 (SitBu), 25.9, 25.8 (C-17, 19-Me), 24.8, 24.5 (C-5, -36), 21.6 (C-4), 20.9 (17-Me), 18.1, 18.0 (Si-*C*), 16.3 (11-Me), 14.5 (28-Me), 12.9 (C-37), 11.0 (25-Me), -3.4, -3.5, -4.6, -4.7 ($SiMe_2$). FAB-MS of the desilylated compound of **16** (HF/ MeCN; r.t./4 h): m/z = 800 ([M+Li]$^+$), 776, 615, 590, 536, 399, 355.

Compound **17**: ^{1}H NMR ($CDCl_3$): δ 5.33 (s, 26-H), 4.97 (d, J=9Hz, 29-H), 4.54 (s, 10-OH), 4.42 (d, J=13Hz, 6-H_e), 4.30 (br s, 2-H), 3.72 (d, J=9Hz, 14-H), 3.42, 3.38, 3.30 (3 s, OMe), 1.62 (s, 28-Me), 1.14 (s, 19-Me), 0.93 (s, SitBu), 0.08, 0.07 (2 s, $SiMe_2$). ^{13}C NMR ($CDCl_3$): δ 195.5 (C-9), 168.6 (C-1), 165.6 (C-8), 131.9 (C-28), 128.6 (C-29), 98.2 (C-10), 84.2 (C-32), 81.5 (C-19), 77.9, 76.5, 75.7, 75.3, 74.0, 72.2, 71.1 (C-13, -14, -15, -22, -23, -24, -26), 58.2, 57.4, 56.6, 56.3 (C-2, 3 OMe), 51.9 (C-20), 45.2 (C-18), 42.6 (C-21), 40.6, 39.2, 36.8, 34.9, 34.7, 34.3, 33.8, 33.0, 31.0 (C-6, -11, -12, -16, -25, -30, -31, -34, -35), 26.5, 26.0, 25.9 (C-3, -17, 19-Me), 25.85 (SitBu), 25.3, 24.5 (C-5, -36), 22.2, 21.9 (C-4, 17-Me), 18.2, 18.1 (Si-*C*), 15.9 (11-Me), 14.8 (28-Me), 13.0 (C-37), 10.8 (25-Me), -3.8, -4.4, -4.5, -4.7 ($SiMe_2$). FAB-MS of the desilylated compound of **17** (HF/ MeCN; r.t./3 h): m/z = 800 ([M+Li]$^+$), 590, 566, 536, 522.

Compound **18**: mixture of two conformers (4:1), major conformer: ^{1}H NMR ($CDCl_3$): δ 4.96 (d, J=5.5Hz, 2-H), 4.57 (s, 26-H), 4.41 (d, J=13.5Hz, 6-H_e), 4.12 (s, 10-OH), 3.31, 3.28 (2 s, OMe), 1.28 (s, 28-Me), 0.91 (s, SitBu), 0.09, 0.07 (2 s, $SiMe_2$). ^{13}C NMR ($CDCl_3$): δ 197.5 (C-9), 170.5 (C-1), 165.9 (C-8), 96.2 (C-10), 87.7 (C-26), 84.2 (C-32), 80.6 (C-22), 76.1 (C-24), 75.4, 75.3 (C-15, -33), 74.1 (C-13), 73.0 (C-14), 57.2, 56.8, 56.3 (3 OMe), 55.9 (C-2), 49.5 (C-29), 48.6 (C-18), 46.0 (C-30), 45.1 (C-25), 41.0 (C-16), 39.1 (C-6), 37.8 (C-31), 35.2, 35.0, 34.2 (C-11, -23, -34), 33.3 (C-35), 33.1, 32.8 (C-12, -21), 31.9 (C-20), 27.7 (C-3), 26.8 (C-17), 26.5, 26.4 (19-, 28-Me), 26.0 (SitBu), 24.5, 24.4 (C-5, -36), 21.4 (17-Me), 20.9 (C-4), 18.1 (Si-*C*), 16.4 (11-Me), 12.8 (C-37), 11.6 (25-Me), -4.5, -4.7 ($SiMe_2$). FAB-MS: m/z = 914 ([M+Li]$^+$), 732, 616, 537, 400.

Hydroxylation of 24,33-O-bis(tert-butyldimethylsilyl)-22(R)-dihydro-ascomycin (3) with osmium tetroxide: To a solution of 0.230 g (0.225 mmol) of 22(*R*)-dihydro-ascomycin **3** in 6 ml of acetone/THF (5:1) was added successively 0.030 g (0.259 mmol) of *N*-methylmorpholine *N*-oxide, 0.5 ml of H_2O and 0.06 µl of osmium tetroxide solution (100 mg/1 ml THF). The reaction mixture was stirred at room temp. for 8 d. The reaction mixture was quenched with 1 ml of sat. $NaHSO_3$ solution. After addition of 0.5 g of florisil and filtration the solution was concentrated. The residue was dissolved in 5 ml of toluene and the resulting solution was subsequently washed with brine and H_2O. The organic phase was dried ($MgSO_4$) and purified by column chromatography (hexane/ethyl acetate, 6:1 → 4:1). Fraction I: 0.075 g (32%) of 28,29-dihydroxy-ascomycin **19**; fraction II: 0.073 g (30%) of slight impure **20** (mixture of four isomers).

28,29-Dihydroxy-ascomycin derivative **19**: mixture of two conformers (3:1), major conformer: ^{1}H NMR ($CDCl_3$): δ 5.28 (s, 26-H), 4.78 (d, J=10Hz, 20-H), 4.62 (d, J=1.5Hz, 10-OH), 3.52 (d, J=9.5Hz, 14-H), 3.40, 3.38, 3.23 (3 s, OMe), 3.12 (dd, J=2, 6Hz, 29-H), 2.66 (s, OH), 2.60 (dt, J=2, 13Hz, 6-H_a), 2.55 (s, OH), 2.20 (d, J=13Hz, 18-H), 1.59 (s, 19-Me), 1.12 (s, 28-Me), 1.00 (d, J=7Hz, 25-Me), 0.99 (d, J=7Hz, 17-Me), 0.95 (d, J=6.5Hz, 11-Me), 0.93, 0.89 (2 s, SitBu), 0.81 (t, J=7Hz, 37-H), 0.13, 0.08, 0.06, 0.01 (4 s, $SiMe_2$). ^{13}C NMR ($CDCl_3$): δ 197.7 (C-9), 171.6 (C-1), 165.7 (C-8), 136.5 (C-19), 126.4 (C-20), 97.9 (C-10), 84.5 (C-32), 75.9 (C-28), 75.7 (C-15), 75.6 (C-22), 75.3 (C-33), 74.0 (C-24), 73.8 (C-13), 73.6, 73.5 (C-26, -29), 73.2 (C-14), 58.0, 56.5, 56.4 (3 OMe), 57.7 (C-2), 49.2 (C-18), 45.4 (C-21), 39.4 (C-6), 37.0 (C-25), 36.6, 36.5 (C-23, -30), 36.0 (C-31), 34.8 (C-11), 33.7 (C-34), 32.6 (C-12), 32.2 (C-16), 27.1 (C-17), 26.7, 26.6 (C-3, -36), 26.0, 25.9 (SitBu), 24.4 (C-5), 23.2 (C-35), 22.4 (C-4), 21.4 (17-Me), 18.2, 17.9 (Si-*C*), 17.8 (28-Me), 15.8 (11-Me), 15.7 (19-Me), 13.1 (25-Me), 11.7 (C-37), -3.8, -4.4, -4.5, -4.7 ($SiMe_2$). FAB-MS: m/z = 1062 ([M+Li]$^+$), 906, 888, 522, 337.

Cleavage of diol 19 with $NaIO_4$: To a stirred solution of 0.049 g (0.046 mmol) **19** in 2 ml of THF was added at room temp. dropwise a solution of 0.098 g (0.46 mmol) of $NaIO_4$ in 0.2 ml of H_2O. After 22 h the reaction mixture was evaporated, diluted with 5 ml of H_2O and extracted several times with ethyl acetate. The combined organic phases were washed with brine, dried ($MgSO_4$) and evaporated to gave the crude product, which was purified by column chromatography (hexane/ethyl acetate, 3:1). Yield of 28-oxo-ascomycin **13**: 0.029 g (81%) as colourless foam.

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