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On the reactivity of ascomycin at the binding domain. Part 1: Liberation of the tricarbonyl portion of ascomycin

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Abstract—Within the binding domain, ascomycin features the unusual pattern of a masked tricarbonyl moiety, which potentially allows for high structural diversity via simple isomerisation events. Herein, methodologies, allowing the liberation of the tricarbonyl unit by blocking the 14-hydroxy group are reported.

1. Introduction

Ascomycin (**1**, Fig. 1) and the related compound FK 506 (**2**), represent highly functionalized 23-membered macrocycles, containing a pipecolate residue linked by an amide (C8) and an allylic ester moiety (C26–29) to a polyketide backbone.[1] Elidel®, a topical formulation of ASM 981 (**3**), the 33-*epi*-chloro derivative of ascomycin, heralds major advances in the treatment of inflammatory skin diseases as compared to traditional treatment schedules.[2] As confirmed by X-ray crystal structure analysis and NMR-studies, the left hand part of the macrolactams mediate binding to their common immunophilin (macrophilin, FK 506-binding protein, FKBP) and has therefore been termed the 'binding domain'. The right hand part of the macrocycles, together with elements of the immunophilin, interact with the protein phosphatase calcineurin, which plays a key role in the Ca^{2+} dependent activation of lymphocytes, and thus represents the 'effector domain'.[3]

Most notably, the macrolactams feature the unusual pattern of three adjacent carbonyl groups within the binding domain (C8–C10, tricarbonyl portion, α,β-diketoamide moiety), whereby one carbonyl group (C10) is involved in hemiketal formation with the secondary hydroxyl group at C14 to form the tetrahydropyrane unit (C10–14). Although the structure at the binding domain of ascomycin shown by formula **A** (Fig. 2) is the main isomeric form adopted in organic solution,[4] the close proximity of the tricarbonyl portion to the hydroxyl group at C-14 could potentially lead to the formation of numerous alternative isomers. Thus, via liberation and enolisation of the tricarbonyl portion (**T**, **E**), hemiketal formation at C9 or C10 could potentially lead to the formation of eight diastereomeric six- or seven-membered hemiketal forms (**A**, **B**).[5] Furthermore, anticipating an 1,4-addition of the 14-hydroxy group to the enolised tricarbonyl form (**E**) would allow the generation of a set of isomeric 'furano-ascomycins' (**F**).[6] As observed for ascomycin itself, each potential equilibria product may exhibit a mixture of rotamers with respect to the geometry of the amide bond. The structural flexibility of ascomycin in the binding domain translates into a tremendous reactivity within this portion. In this context, oxidative, reductive and nucleophilic induced cleavage reactions,[5c,7] unexpected base induced rearrangement reactions,[8] unusual deoxygenation and amination reactions,[9] a surprisingly high reactivity towards diazomethane[10] as well as a pronounced photosensitivity[11] have been reported. For some of these reactions, it is not yet clear whether they proceed via the six- or seven-membered hemiketal form or whether the liberated tricarbonyl portion is essential. In order to study the reactivity of ascomycin in the binding domain in more detail, we planned the synthesis of derivatives bearing the unmasked tricarbonyl portion. Herein, several protocols leading to such derivatives are reported.

2. Results and discussion

2.1. Liberation of the tricarbonyl portion via silylation

As published earlier, reaction of ascomycin (**1**) with an excess of *t*-butyldimethylsilyl chloride (TBDMSCl) and imidazole in DMF furnishes 24,33-bis-OTBDMS-ascomycin **4** in high yield.[12] No further silylation was observed,

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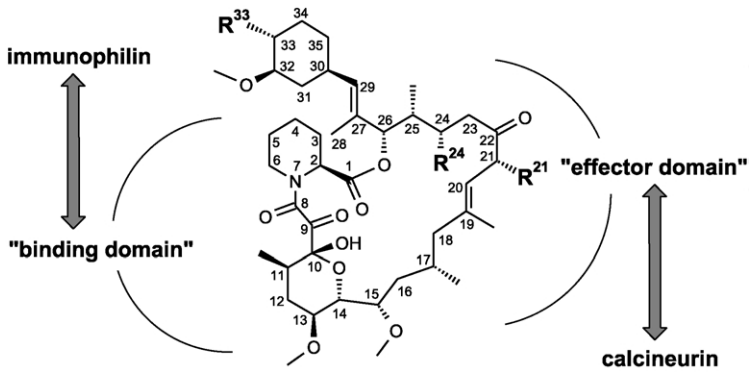

1 Ascomycin (R^{21} = ethyl; R^{33} = R^{24} = OH)
2 FK 506 (R^{21} = allyl; R^{33} = R^{24} = OH)
3 ASM 981 (R^{21} = ethyl; R^{33} = *epi*-chloro; R^{24} = OH)
4 24,33-bis-OTBDMS-ascomycin (R^{21} = ethyl; R^{33} = R^{24} = OTBDMS)

Figure 1.

even when a vast excess of reagents was used. However, screening different silylation reagents, bases and solvent systems led to the development of a suitable method for the liberation of the tricarbonyl portion via selective protection of the 14-hydroxy group (Fig. 3). Thus, addition of *t*-butyldimethylsilyl triflate (TBDMSOTf, 10 equiv.) to a solution of ascomycin and 2,6-lutidine (33 equiv.) in dichloromethane furnished the yellow colored 14,24,33-tris-OTBDMS-ascomycin **5** as the main product in 72% yield. The major byproduct **9** (12.5%) also contains the free tricarbonyl portion, but in addition a silyl enol ether formation at the C22-carbonyl has taken place. The formation of the minor tris-silylated byproducts (ketal **6**, the 10,11-silyl enol ether **7** and the seven-membered ketal **8**) can be explained by trapping some of the previously discussed potential equilibria products of ascomycin. The same holds true for the tetra-silylated 22,23-silyl enol ether derivatives **10** and **11**. Quenching the reaction after a short time (15 min) allowed the isolation of 24,33-bis-OTBDMS-ascomycin **4** in excellent yield (93%), thus indicating that silylation occurs primarily at the 33-and 24-hydroxyl followed by the slow liberation and silylation of the 14-hydroxy group.

2.2. Liberation of the tricarbonyl portion with trichloromethyl chloroformate (diphosgene)

Next, we attempted to block the 14-hydroxy group via carbamate formation (Fig. 4). For this purpose, a solution of 24,33-bis-OTBDMS-ascomycin **4** in acetonitrile was reacted with diphosgene (1.5 equiv.) in the presence of an excess 4-dimethylaminopyridine (DMAP, Steglich-base). Surprisingly, quenching the crude reaction mixture with an aqueous solution of ammonia, methylamine or imidazole did not result in the formation of the corresponding *O*-14-carbamates. Instead, the 24-OTBDMS-14,33-bis-carbamates **14**, **15** and **16** were generated in substantial yields. Obviously, action of diphosgene blocked not only the 14-hydroxy group, but also led to a desilylation and subsequent acylation of OH-33. Probably due to steric hindrance, the 24-OTBDMS-group remained unchanged, even after prolonged reaction times. Attempts to avoid concomitant desilylation of the 33-OTBDMS group failed. For example, applying a larger excess of DMAP only led to a significantly decreased reaction rate and did not result in suppression of the desilylation. This problem could be avoided by replacing the comparatively labile 33-OTBDMS-group

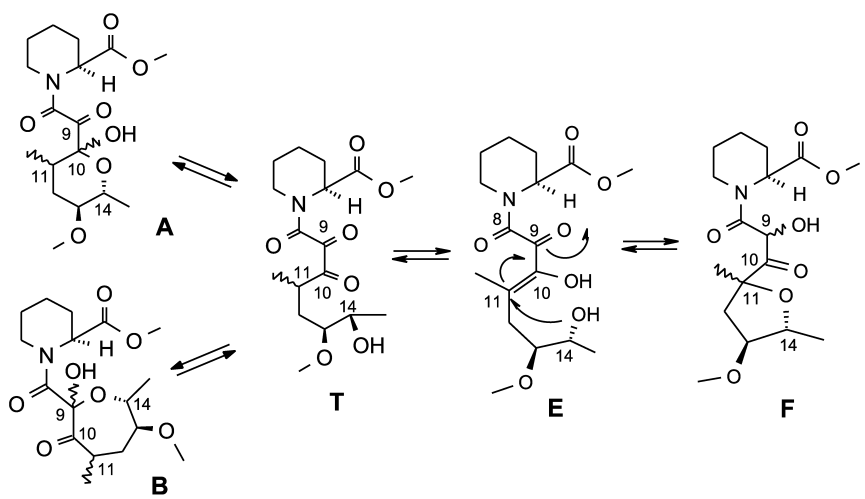

Figure 2.

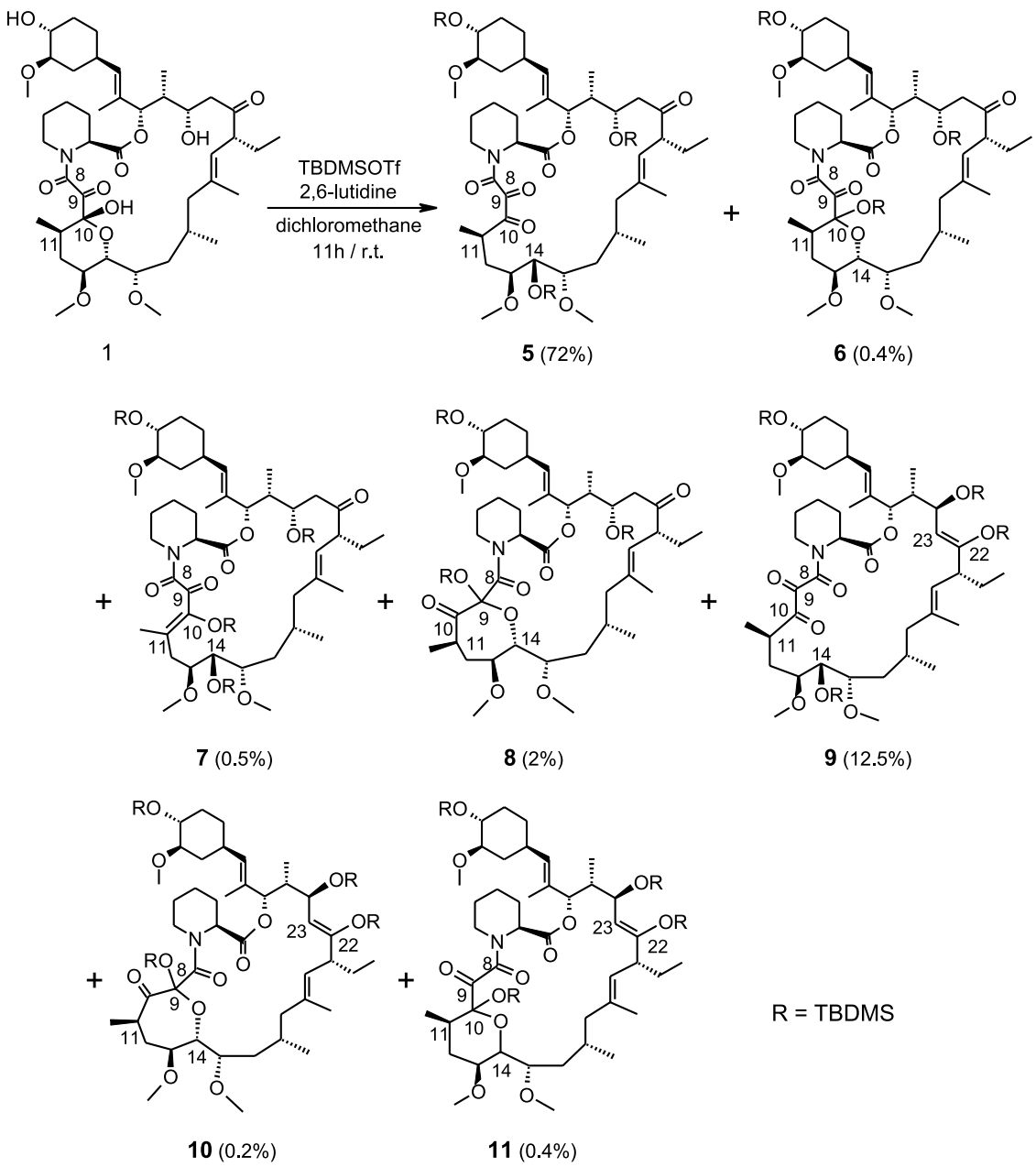

Figure 3.

with a 33-*O*-formyl group via regioselective desilylation (**4→12**), followed by formylation with the mixed anhydride of acetic acid and formic acid[13] to give 33-*O*-formyl-24-OTBDMS derivative **13**. Thus, treatment of **13** with diphosgene/DMAP provided compound **17** in excellent yield after an aqueous work-up in presence of ammonia. An alternative work-up using an excess α-amino ethyl acetate resulted in the formation of the 14-($OCONHCH_2COOEt$)-derivative **18**.

2.3. Liberation of the tricarbonyl portion with iminium chlorides

(Chloromethylene)dimethyliminium chloride (generated in situ from DMF and diphosgene)[14] gave similar reactions on protected ascomycin derivatives as described above for diphosgene. Thus, independent of the protecting group at R^{33} (**4**, **12** or **13**, R^{33}=OTBDMS, OH or OCHO; R^{24}=OTBDMS), (chloromethylene)dimethyliminium chloride in acetonitrile at ambient temperature furnished the 24-OTBDMS-14,33-bis-*O*-formyl ascomycin derivative **20** in good yield after a basic, aqueous work up. Once again, starting from the 33-OTBDMS-protected ascomycin derivative **4**, capture of OH-14 is accompanied by a simultaneous silyl/acyl exchange at OH-33. Monitoring the course of the reaction by TLC revealed that silyl/acyl exchange occurs prior to the acylation of OH-14. Applying the same reaction conditions to the readily available 24,33-bis-OCHO-ascomycin **19** (prepared from ascomycin by acylation with the mixed anhydride of formic acid and acetic acid) allowed the preparation of 14,24,33-tris-OCHO-ascomycin **21** in excellent yield. The same product is also obtained starting from unprotected ascomycin **1**, albeit in low yield (Fig. 5).

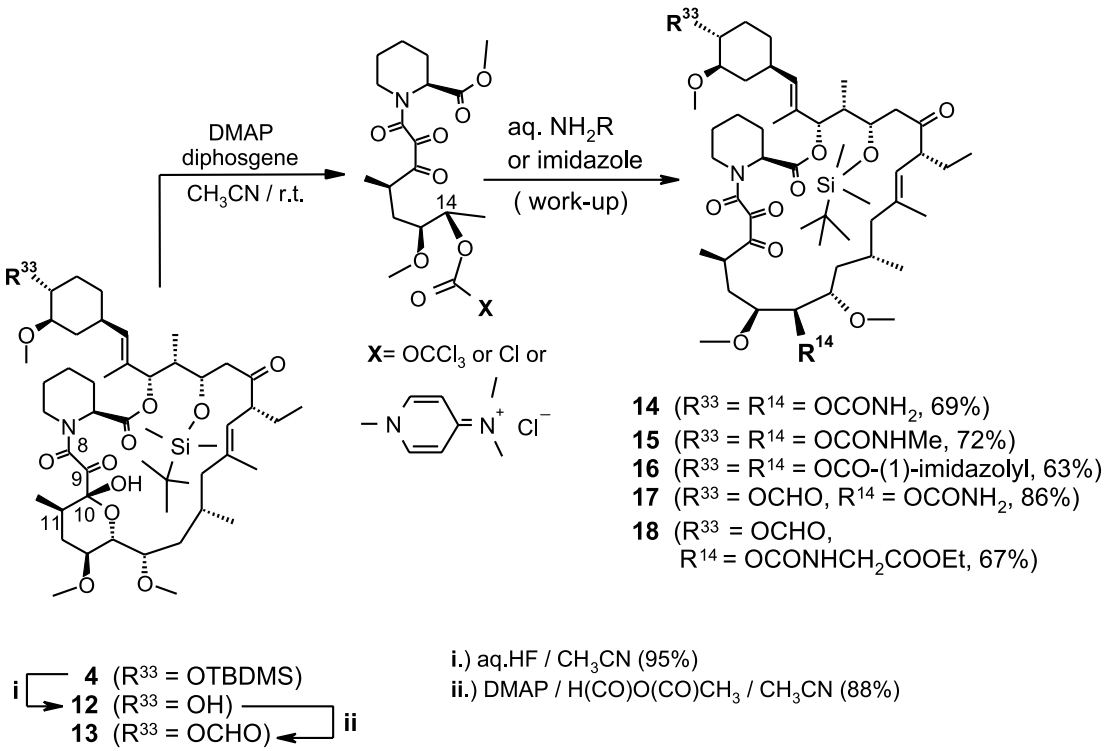

Figure 4.

Attempts to introduce an acetyl group in an analogous manner gave an unexpected result. Thus, attempting the preparation of (1-chloroethylidene)dimethylammonium chloride[15] from *N*,*N*-dimethyl acetamide, we added a solution of phosgene in toluene to a solution of excess *N*,*N*-dimethyl acetamide in diethyl ether and reacted the resulting solution with 24,33-bis-*O*-formyl-ascomycin **19**. Surprisingly, after several hours at room temperature the unexpected ascomycin derivatives **22** and **23** instead of the desired 14-*O*-acetyl compound could be isolated (Fig. 6). Inspection of these structures clearly suggests that the initially formed 1-(chloroethylidene)dimethylammonium chloride **P** reacted further with phosgene to give the phosgeneimmonium complex **Q**,[16] which in turn reacts with the 10-hydroxyl of the six-membered hemiketal form or the liberated 14-hydroxyl of the tricarbonyl form of **19** to finally give the isolated structures **22** and **23**, respectively.

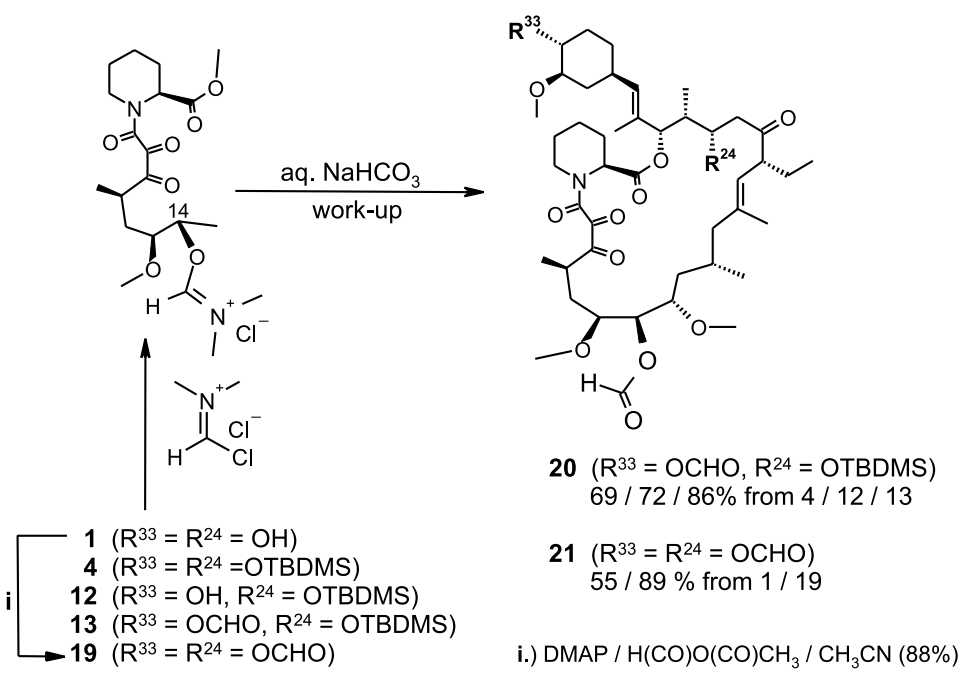

Figure 5.

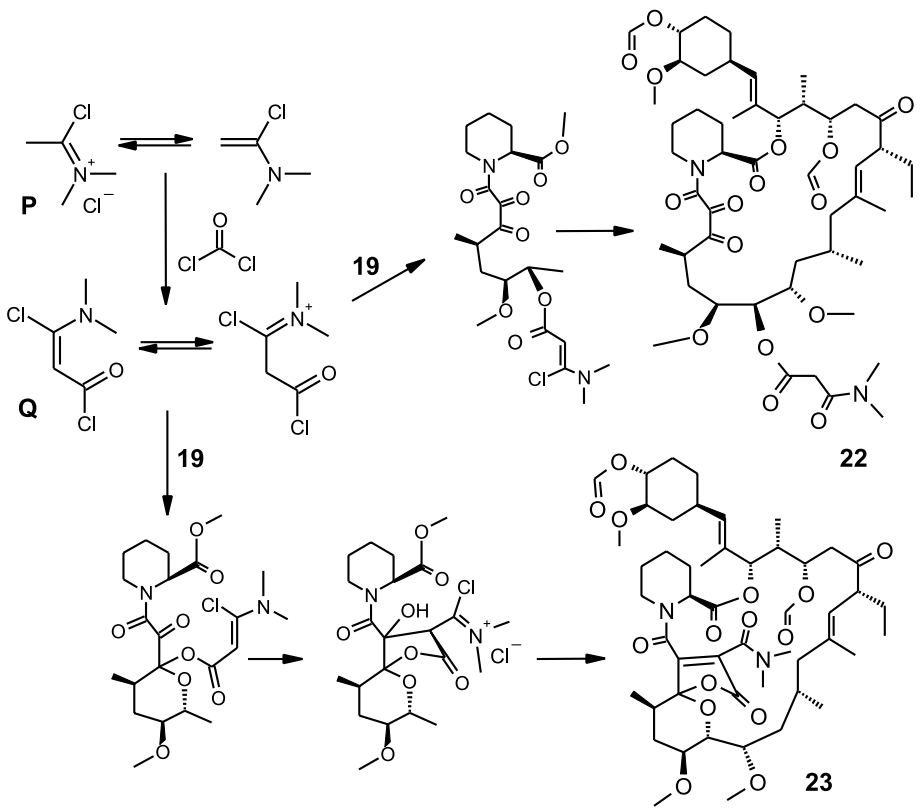

Figure 6.

3. Analytical evidence and structural features

All compounds described herein represent single isomers. The presence of an unmasked tricarbonyl unit can easily be recognized due to its characteristic yellow color, caused by the conjugation of three adjacent carbonyls. Further evidence could be obtained from one- and two-dimensional NMR-techniques: (a) the 10-hemiketal resonance is replaced by an additional carbonyl resonance (C10; 199–202 ppm), (b) the C9 resonance (184–187 ppm) is shifted upfield by approximately 10 ppm, with respect to ascomycin, due to the additional conjugation with the C10 carbonyl, (c) the H11 resonance is shifted downfield due to the adjacent C10 carbonyl and (d) $^{3}J_{CH}$ and $^{2}J_{CH}$ long range cross peaks from H11 to the C9 and C10 carbonyl signals and from 11-methyl to C11 and C10 are observed. Analogously, the presence of a fixed seven-membered ketal form (compounds **8**, **10** in Figure 3) could also be supported by NMR. Thus, as H11 is α- to a carbonyl (C-10), the signal is significantly shifted downfield and the C10 carbonyl, now lacking conjugation to an adjacent carbonyl appears at 206–207 ppm as expected for isolated carbonyls. Furthermore, all relevant $^{3}J_{CH}$ long range cross peaks, especially from H14 to C9 (which appears at 99–102 ppm, in accordance with the proposed ketal structure) substantiate the assigned seven-membered cyclic structure in the binding domain. With the exception of obvious differences caused by different substitution patterns at the 24- and 33-hydroxyl, the ^{1}H and ^{13}C NMR-spectra of the trapped six-membered ketal forms (**6**, **11** in Figure 3) are comparable to the spectra of the parent compound ascomycin. The absolute configuration at C9 or C10 of the silylated, isomerically pure six- and seven-membered compounds **6**, **8**, **10** and **11** could not be determined. The 10,11-silyl-enol ether motif in compound **7** (Fig. 3) could be confirmed by the absence of H11, the simplification of the 11-methyl signal to a singlet at 1.93 ppm and the presence of two additional quaternary olefinic carbon resonances (143.22 and 137.79 ppm) which show $^{3}J_{CH}$ and $^{2}J_{CH}$ cross peaks to H12a, H12b and the 11-methyl group. The geometry at the 10,11-double bound (*E* or *Z*) could not be deduced due to the absence of diagnostically relevant NOEs. In contrast, cross-peaks between H21/H23 in the T-Roesy spectra of the tetra-silyl derivatives **9**, **10** and **11** confirmed the *Z*-geometry of the 22,23-silyl enol ether motif in these compounds. For the rigid compound **23**, the absence of the C9 carbonyl resonance, a downfield shift of C10 and the appearance of a double bond with two quaternary substituted carbon atoms together with two additional carbonyl resonances ($CONMe_2$, and –C(O)O– of the furanone system) are characteristic. In contrast to the parent compound ascomycin, which adopts preferentially the *cis*-geometry at the amide bond (*cis*–*trans*=2:1 in $CDCl_3$ solution),[4] liberation of the tricarbonyl unit forces the macrocycles to adopt preferentially the *trans*-conformation (*cis*–*trans*<2:10). Interestingly, the 22-silyl enol ether derivatives **9**–**11** as well as the 10,11-silylenolether **7** adopt exclusively the trans geometry, whereas the rigid derivative **23** exists completely in the *cis*-configuration in $CDCl_3$ solution. Assignment of the amide configuration could easily be done by inspection of the C2/C6 and the H-2/H-6_{ax}/H-6_{eq} resonances. Thus, as we have reported earlier,[17] the ^{13}C-resonances of C2 and C6 do not spread over several ppm units but clearly form two distinct groups: transition from *cis* to *trans* causes the C6 resonances to shift to lower field (from 39–39.5 to 43–45 ppm), whereas the C2 resonances are shifted to higher

field (from 56–56.5 to 50.5–52 ppm). An analogous trend is found for the protons: the transition from *cis* to *trans* shifts H2 and $H6_{ax}$ to lower field and H-6_{eq} to higher field.

4. Conclusion

In summary, suitable methods allowing the chemoselective liberation of the tricarbonyl unit in the binding domain of ascomycin have been discovered. In addition, an unexpected rigid ascomycin derivative (**23**) in which the flexible binding domain is blocked via furanone formation, has been identified. Having accomplished the synthesis of ascomycin derivatives bearing the unmasked tricarbonyl moiety, more detailed investigations addressing the reactivity of this peculiar unit, can now be performed. The results of these future studies will be reported in due course.

5. Experimental

5.1. General

All NMR spectra were recorded on a BRUKER AVANCE 500 MHz spectrometer (resonance frequencies 500.13 MHz for ^{1}H, 125.76 MHz for ^{13}C), equipped with a broadband inverse probe head with *z*-gradients, in 0.6 ml $CDCl_3$ (Merck Uvasol®, 99.8% D) at 301 K. Chemical shifts are given in values of ppm, referenced to residual $CHCl_3$ signals (7.26 for ^{1}H, 77.0 for ^{13}C). Proton and carbon-13 signal assignments were deduced from ^{1}H, ^{13}C, gradient-selected $^{1}H,^{1}H$-COSY (correlated spectroscopy), gradient-selected inverse $^{1}H,^{13}C$-HSQC (heteronuclear single-quantum correlation), and gradient-selected inverse $^{1}H,^{13}C$-HMBC (heteronuclear multiple-bond correlation) experiments.[18] Stereochemical information was extracted from two-dimensional T-ROESY (transverse rotating-frame Overhauser effect spectroscopy)[19] or selective one-dimensional ROESY[20] experiments. Mass spectroscopy (ESI, electrospray ionization) was performed on a Finnigan Navigator AQA mass spectrometer with HP 1100 LC system, using methanol (Merck LiChrosolv®, gradient grade) as solvent. Solutions of approx. 50–100 μg/ml of the test compound in acetonitrile (Merck LiChrosolv®) were used for injection. Two scans in each experiments were applied, with 25 and 50 V cone voltages, respectively. The probe temperature was 523 K. All reactions were monitored by HPTLC (Merck HPTLC-plates, silica gel 60, F_{254}). Visualisation of the reaction components was obtained by spraying with a solution of molybdatophosphoric acid (20% in EtOH/H_2O, 3:1). Flash column chromatography was performed on silica gel (Merck, silica gel 60, 0.04–0.063 mm, 230–400 mesh ASTM) at approximately 3–5 bar. The solvents used for chromatography (reagent grade) were used as purchased. Isolated minor by-products were subjected to molecular size exclusion chromatography (Sephadex® LH20, ethyl acetate) in order to remove low molecular weight impurities (originating from the solvent used for chromatography) which might have been enriched during chromatographic isolation. *Caution*. Phosgene and diphosgene are health and environmental hazards. All reactions with phosgene or diphosgene were carried out in a well-ventilated hood under a slight stream of inert gas (argon). The gas outlet was bubbled through a solution of 20 w/w% aq. potassium hydroxide solution. Commercially available reagents were applied without further purification. Formic acetic anhydride was prepared following a procedure described in the literature.[13]

5.1.1. Preparation and isolation of the compounds 5–11. To a cooled (ice-bath), magnetically stirred solution of 30 g (0.38 mol) ascomycin (1) and 145 ml (33 equiv., 1.252 mmol) 2,6-lutidine in 600 ml dichloromethane 88 ml (10 equiv., 0.38 mol) *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBDMSOTf) were added in such a manner that the temperature was kept below 10°C. After complete addition (15 min), the cooling bath was removed and the reaction mixture was stirred for 11 h at room temperature. The mixture was concentrated under reduced pressure to approximately one half of its volume and partitioned between ethyl acetate and saturated aq. $NaHCO_3$. The organic layer was separated and sequentially washed with 3×300 ml 1N HCl and 2×200 ml brine, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure at max. 40°C. The residual yellow oil was subjected to flash column chromatography (silica gel, *n*-heptane–ethyl acetate=7:1) to give 10 g of a mixture of the compounds **6**, **7**, **9**, **10** and **11** and the separated compounds **8** and **5**. The first fraction was further separated (silica gel, *n*-heptane–ethyl acetate=10:1) to give a mixture of **7**, **10** and **11** and the separated compounds **6** and **9**. The mixture of **7**, **10** and **11** was separated by a third flash chromatography (silica gel, toluene–acetonitrile=70:1). In order to get rid of low molecular weight impurities, all fractions <1 g were subjected to size exclusion chromatography (Sephadex® LH20, ethyl acetate), evaporated to dryness and lyophilized from dioxane. The following products were obtained:

Compound **5**. 30.95 g, 72%. Yellow powder; MS: 1156 (MNa^+), 1197 ($MNa^+ \cdot CH_3CN$); CHN ($C_{61}H_{111}NO_{12}Si_3$) calcd: 64.56/9.86/1.23, found: 64.60/10.12/1.05; ^{13}C NMR ($CDCl_3$, *E*–*Z*=1:4), δ (*E*/*Z*-isomer, ppm): 168.66/168.83 (C1); 56.15/51.87 (C2); 25.9 (br)/25.9 (br) (C3); 20.72/21.15 (C4); 25.36/25.36 (C5); 39.44/43.89 (C6); 165.01/165.54 (C8); 187.19/185.37 (C9); 203.6 (br)/199.4 (C10); 38.55/36.17 (C11); 31.7 (br)/34.66 (C12); 81.92/81.11 (C13); 75.53/75.45 (C14); 79.11/78.0 (br) (C15); 41.27/41.22 (C16); 27.33/27.46 (C17); 46.85/46.59 (C18); 139.62/139.14 (C19); 123.78/123.39 (C20); 55.62/55.39 (C21); 209.43/209.24 (C22); 48.00/47.6 (br) (C23); 67.97/68.16 (C24); 38.74/38.86 (C25); 83.69/82.8 (br) (C26); 130.98/131.34 (C27); 11.24/11.33 (C28); 136.73/135.75 (C29); 35.13/35.02 (C30); 36.17/36.17 (C31); 84.11/84.11 (C32); 75.13/75.13 (C33); 33.96/33.96 (C34); 30.64/30.73 (C35); 23.69/23.69 (C36); 11.63/11.63 (C37); 57.03/57.48 (13-OMe); 60.17/59.07 (15-OMe); 57.85/57.76 (32-OMe); 15.58/15.69 (11-CH_3); 19.52/19.52 (17-CH_3); 16.52/16.52 (19-CH_3); 8.83/9.65 (25-CH_3); 26.06, 26.02, 25.87, 25.63, 18.41, 18.37, 18.17, 18.04 (Si–*t*-Bu); −3.98, −4.17, −4.43, −4.49, −4.76 ($SiMe_2$). ^{1}H NMR ($CDCl_3$, *E*–*Z*=1:4, selected data), δ (*E*-isomer, ppm): 4.26 (d, *J*=5.1 Hz, H-2); 4.41 (br d, *J*=13 Hz, H-6a); 3.06 (H-6b); 3.20 (H-11); 3.02 (H-13); 3.69 (dd, *J*=7.3+1.7 Hz, H-14); 3.11 (H-15); 4.70 (d, *J*=10.4 Hz, H-20); 3.22 (H-21); 2.85 (H-23a); 2.23

(H-23b); 4.09 (dd, J=10.0+4.5 Hz, H-24); 5.08 (d, J=10.2 Hz, H-26); 5.34 (d, J=8.8 Hz, H-29); 2.92 (ddd, J=11.3+8.4+4.4 Hz, H-32); 3.39 (H-33); 1.24 (d, 3H, J=7.1 Hz, 11-Me); 1.75 (s, 3H, 19-Me); 1.54 (d, 3H, J=1.1 Hz, 27-Me); 0.02, 0.04, 0.06, 0.07, 0.09 (s, each 3H, Si–Me); δ (Z-isomer, ppm): 5.18 (d, J=5.0 Hz, H-2); 3.44 (H-6a); 3.20 (H-6b); 3.42 (H-11); 3.22 (H-13); 3.73 (dd, J=5.7+3.7 Hz, H-14); 3.19 (H-15); 4.83 (d, J=10.3 Hz, H-20); 3.22 (H-21); 2.82 (dd, J=16.8+10.0 Hz, H-23a); 2.28 (H-23b); 4.16 (dd, J=9.2+4.5 Hz, H-24); 5.15 (d, J=9.2 Hz, H-26); 5.30 (d, J=9.0 Hz, H-29); 2.92 (ddd, J=11.3+8.4+4.4 Hz, H-32); 3.39 (H-33); 1.16 (d, 3H, J=7.0 Hz, 11-Me); 1.74 (s, 3H, 19-Me); 1.51 (d, 3H, J=1.0 Hz, 27-Me); 0.02, 0.05, 0.06, 0.07, 0.08, 0.10 (s, each 3H, Si–Me); 0.09 (s, 6H, Si–Me); 0.88 (s, 18H, $(CH_3)_3$); 0.91, 0.90 (s, each 9H, $(CH_3)_3$).

Compound **6**. 175 mg, 0.4%. White powder; MS: 1156 (MNa^+), 1197 ($MNa^+ \cdot CH_3CN$); CHN ($C_{61}H_{111}NO_{12}Si_3$) calcd: 64.56/9.86/1.23, found: 64.46/9.95/1.58; ^{13}C NMR ($CDCl_3$, E–Z<1:10), δ (Z-isomer, ppm): 169.25 (C1); 50.54 (C2); 26.39 (C3); 20.88 (C4); 24.90 (C5); 43.21 (C6); 165.19 (C8); 198.10 (C9); 99.21 (C10); 39.94 (C11); 33.64 (C12); 73.94 (C13); 76.10 (C14); 76.8 (br) (C15); 35.13 (C16); 26.16 (C17); 47.89 (C18); 141.25 (C19); 121.54 (C20); 56.53 (C21); 211.25 (C22); 43.21 (C23); 73.0 (br) (C24); 41.8 (br) (C25); 76.5 (br) (C26); 133.47 (C27); 13.32 (C28); 125.02 (C29); 34.93 (C30); 36.47 (C31); 84.15 (C32); 75.15 (C33); 33.93 (C34); 30.74 (C35); 22.74 (C36); 11.25 (C37); 56.43 (13-OMe); 57.10 (15-OMe); 57.87 (32-OMe); 15.58 (11-CH_3); 18.90 (17-CH_3); 15.51 (19-CH_3); 10.49 (25-CH_3); 25.91, 25.87, 18.16, 17.86 (Si–t-Bu); −2.61, −2.91, −3.96, −4.49, −4.75, −5.07 ($SiMe_2$). ^1H NMR ($CDCl_3$, E–Z<1:10, selected data), δ (Z-isomer, ppm): 5.16 (d, J=5.3 Hz, H-2); 3.40 (m, 2H, H-6a,b); 1.81 (H-11); 3.47 (H-13); 3.50 (H-14); 3.41 (H-15); 4.62 (d, J=10.1 Hz, H-20); 3.21 (H-21); 2.40 (dd, J=14+9.3 Hz, H-23a); 2.18 (H-23b); 4.05 (ddd, J=9.3+3.3+3.3 Hz, H-24); 5.38 (br s, H-26); 5.20 (d, J=9.0 Hz, H-29); 2.93 (H-32); 3.38 (H-33); 1.05 (d, 3H, J=6.8 Hz, 11-Me); 1.56 (s, 3H, 19-Me); 1.60 (s, 3H, 27-Me); −0.06, −0.01, 0.06, 0.07, 0.18, 0.20 (s, each 3H, Si–Me); 0.86 (s, 9H, $(CH_3)_3$), 0.88 (s, 18H, $(CH_3)_3$).

Compound **7**. 240 mg, 0.5%. White powder; MS: 1270 (MNa^+), 1311 ($MNa^+ \cdot CH_3CN$); CHN ($C_{67}H_{125}NO_{12}Si_4$) calcd: 64.43/10.09/1.12, found: 64.44/9.83/0.87; ^{13}C NMR ($CDCl_3$, E–Z=0:1), δ (ppm): 169.12 (C1); 51.11 (C2); 25.07 (C3); 20.95 (C4); 24.88 (C5); 44.59 (C6); 165.84 (C8); 189.94 (C9); 137.79 (C10); 143.22 (C11); 32.93 (C12); 85.20 (C13); 76.78 (C14); 79.12 (C15); 41.06 (C16); 26.17 (C17); 45.64 (C18); 140.40 (C19); 123.60 (C20); 55.76 (C21); 208.37 (C22); 46.94 (C23); 67.34 (C24); 37.83 (C25); 83.77 (C26); 130.77 (C27); 10.81 (C28); 137.11 (C29); 35.09 (C30); 36.21 (C31); 84.32 (C32); 75.18 (C33); 34.05 (C34); 30.75 (C35); 22.42 (C36); 11.65 (C37); 57.35 (13-OMe); 61.27 (15-OMe); 57.72 (32-OMe); 22.93 (11-CH3); 20.43 (17-CH_3); 16.23 (19-CH_3); 8.42 (25-CH_3); 25.98, 25.89, 25.88, 18.49, 18.19, 18.17, 18.08 (Si–t-Bu); −3.57, −3.75, −4.15, −4.31, −4.56 ($SiMe_2$). ^1H NMR ($CDCl_3$, E–Z=0:1, selected data), δ (ppm): 5.20 (d, J=4.2 Hz, H-2); 3.28 (H-6a); 2.83 (H-6b); 2.61 (dd, J=12.6+3.1 Hz, H-12a); 2.48 (H-12b); 3.34 (H-13); 3.72 (d, J=9.2 Hz, H-14); 3.22 (H-15); 4.66 (d, J=10.6 Hz, H-20); 3.17 (H-21); 2.90 (dd, J=15.7+11.2 Hz, H-23a); 2.12 (dd, J=15.7+3.8 Hz, H-23b); 4.16 (dd, J=11.2+3.8 Hz, H-24); 4.86 (d, J=11.2 Hz, H-26); 5.37 (d, J=8.8 Hz, H-29); 2.92 (H-32); 3.40 (H-33); 1.93 (s, 3H, 11-Me); 1.81 (s, 3H, 19-Me); 1.48 (s, 3H, 27-Me); 0.02, 0.07, 0.12, 0.20 (s, each 3H, Si–Me); 0.06, 0.13 (s, each 6H, Si–Me); 0.87, 0.89, 0.96, 0.96 (s, each 9H, $(CH_3)_3$).

Compound **8**. 860 mg, 2%. White powder; MS: 1156 (MNa^+), 1197 ($MNa^+ \cdot CH_3CN$); CHN ($C_{61}H_{111}NO_{12}Si_3$) calcd: 64.56/9.86/1.23, found: 64.14/9.65/1.12; ^{13}C NMR ($CDCl_3$, E–Z=2:1), δ (E/Z-isomer, ppm): 168.55/170.24 (C1); 55.84/52.74 (C2); 31.39/26.51 (C3); 21.30/20.97 (C4); 25.58/24.78 (C5); 39.72/43.60 (C6); 166.38/167.75 (C8); 102.73/102.10 (C9); 207.10/207.86 (C10); 38.43/38.01 (C11); 39.77/39.03 (C12); 78.44/77.42 (C13); 75.94/nd (C14); 82.02/nd (C15); 36.70/36.36 (C16); 25.27/28.75 (C17); 47.37/47.90 (C18); 139.70/137.77 (C19); 124.33/123.70 (C20); 57.03/54.48 (C21); 208.90/210.50 (C22); 49.92/47.44 (C23); 68.71/68.56 (C24); 40.94/39.75 (C25); 85.33/nd (C26); 129.98/nd (C27); 10.90/12.90 (C28); 138.09/nd (C29); 35.17/35.05 (C30); 35.91/35.91 (C31); 84.08/84.20 (C32); 75.03/75.15 (C33); 33.91/33.91 (C34); 30.57/30.72 (C35); 23.09/24.69 (C36); 11.46/11.91 (C37); 56.68/56.25 (13-OMe); 59.26/58.22 (15-OMe); 57.64/57.91 (32-OMe); 17.20/17.20 (11-CH_3); 20.72/20.95 (17-CH_3); 15.83/16.80 (19-CH_3); 9.97/10.90 (25-CH_3); 26.50, 25.96, 19.22, 18.99, 18.16, 18.06, 18.00 (Si–t-Bu); −2.38, −2.47, −3.59, −3.94, −4.05, −4.13, −4.32, −4.50, −4.75 ($SiMe_2$). ^1H NMR ($CDCl_3$, E–Z=2:1, selected data), δ (E-isomer, ppm): 5.51 (br d, J=4.0 Hz, H-2); 4.28 (br d, J=13.5 Hz, H-6a); 3.28 (H-6b); 2.80 (H-11); 3.38 (H-13); 3.59 (dd, J=9.6+2.5 Hz, H-14); 3.45 (H-15); 4.59 (d, J=10.8 Hz, H-20); 3.39 (H-21); 2.86 (H-23a); 2.19 (H-23b); 4.03 (dd, J=11.0+3.4 Hz, H-24); 4.88 (d, J=10.3 Hz, H-26); 5.40 (d, J=8.9 Hz, H-29); 2.92 (H-32); 3.39 (H-33); 1.18 (d, 3H, J=6.4 Hz, 11-Me); 1.79 (s, 3H, 19-Me); 1.42 (s, 3H, 27-Me); 0.02, 0.03, 0.06, 0.07, 0.12, 0.25 (s, each 3H, Si–Me); 0.83, 0.87, 0.91 (s, each 9H, $(CH_3)_3$); δ (Z-isomer, ppm): 5.08 (br d, J=4.0 Hz, H-2); 4.70 (br d, J=13.7 Hz, H-6a); 3.17 (H-6b); 2.97 (H-11); 3.36 (H-13); 4.91 (d, J=9.9 Hz, H-20); 3.19 (H-21); 2.88 (H-23a); 2.29 (H-23b); 4.22 (H-24); 5.35 (br s, H-26); 5.24 (br d, J=8.5 Hz, H-29); 2.92 (H-32); 3.39 (H-33); 1.18 (d, 3H, J=6.4 Hz, 11-Me); 1.65 (s, 3H, 19-Me); 1.58 (s, 3H, 27-Me); 0.05, 0.06, 0.07, 0.08, 0.16, 0.22 (s, each 3H, Si–Me); 0.85, 0.88, 0.90 (s, each 9H, $(CH_3)_3$).

Compound **9**. 5.95 g, 12.5%. Yellow powder; MS: 1270 (MNa^+), 1311 ($MNa^+ \cdot CH_3CN$); CHN ($C_{67}H_{125}NO_{12}Si_4$) calcd: 64.43/10.09/1.12, found: 64.35/10.10/0.78; ^{13}C NMR ($CDCl_3$, E–Z=0:1), δ (ppm): 167.86 (C1); 52.14 (C2); 26.73 (C3); 21.56 (C4); 25.19 (C5); 43.66 (C6); 165.87 (C8); 185.66 (C9); 204.0 (br) (C10); 36.90 (C11); 32.92 (C12); 80.89 (C13); 75.89 (C14); 79.79 (C15); 40.92 (C16); 26.17 (C17); 45.54 (C18); 136.11 (C19); 127.24 (C20); 45.40 (C21); 153.36 (C22); 108.14 (C23); 69.53 (C24); 42.09 (C25); 75.59 (C26); 133.37 (C27); 14.81 (C28); 128.04 (C29); 34.96 (C30); 36.78 (C31); 84.21 (C32); 75.21 (C33); 33.91 (C34); 31.08 (C35); 28.55 (C36); 11.84 (C37); 57.01 (13-OMe); 60.33 (15-OMe); 57.95 (32-OMe); 17.01 (11-CH3); 20.01 (17-CH3); 15.98 (19-CH_3); 9.04

(25-CH_3); 26.07, 25.92, 25.90, 25.86, 25.19, 18.38, 18.15, 18.04 (Si–*t*-Bu); −2.72, −3.55, −3.82, −4.42, −4.51, −4.65, −4.73 ($SiMe_2$). ^{1}H NMR ($CDCl_3$, *E*–*Z*=0:1, selected data), δ (ppm): 5.18 (d, *J*=5 Hz, H-2); 3.52 (br d, *J*=13 Hz, H-6a); 3.43 (H-6b); 3.38 (H-11); 3.03 (dd, *J*=6.6+6.6 Hz, H-13); 3.68 (d, *J*=8.2 Hz, H-14); 3.08 (ddd, *J*=9.5+8.2+2.7 Hz, H-15); 5.13 (d, *J*=8.8 Hz, H-20); 2.65 (ddd, *J*=9.2+9.2+2.7 Hz, H-21); 4.32 (d, *J*=9.2 Hz, H-23); 4.69 (dd, *J*=9.2+5.3 Hz, H-24); 5.53 (br s, H-26); 4.98 (d, *J*=9.0 Hz, H-29); 2.94 (ddd, *J*=11.3+8.6+4.6 Hz, H-32); 3.37 (H-33); 1.22 (d, 3H, *J*=7.0 Hz, 11-Me); 1.52 (s, 3H, 19-Me); 1.62 (s, 3H, 27-Me); −0.04, −0.01, 0.06, 0.07, 0.07, 0.08, 0.18, 0.23 (s, each 3H, Si–Me); 0.85, 0.88, 0.91, 0.96 (s, each 9H, $(CH_3)_3$).

Compound **10**. 95 mg, 0.2%. White powder; MS: 1270 (MNa^+), 1311 ($MNa^+ \cdot CH_3CN$); CHN ($C_{67}H_{125}NO_{12}Si_4$) calcd: 64.43/10.09/1.12, found: 64.17/9.99/0.95; ^{13}C NMR ($CDCl_3$, *E*–*Z*=0:1), δ (ppm): 168.52 (C1); 53.30 (C2); 27.32 (C3); 21.28 (C4); 25.63 (C5); 43.98 (C6); 170.50 (C8); 99.33 (C9); 206.16 (C10); 35.03 (C11); 37.50 (C12); 78.47 (C13 or C15); 79.36 (C14); 78.41 (C15 or C13); 36.74 (C16); 30.38 (C17); 49.12 (C18); 135.27 (C19); 126.17 (C20); 45.38 (C21); 152.78 (C22); 109.00 (C23); 69.50 (C24); 41.41 (C25); 74.50 (C26); 133.17 (C27); 14.88 (C28); 126.94 (C29); 34.95 (C30); 36.91 (C31); 84.31 (C32); 75.34 (C33); 34.01 (C34); 31.07 (C35); 29.09 (C36); 11.25 (C37); 56.98 (13-OMe); 56.77 (15-OMe); 57.91 (32-OMe); 16.82 (11-CH_3); 19.27 (17-CH_3); 19.72 (19-CH_3); 9.00 (25-CH_3); 26.19, 26.04, 25.87, 25.63, 18.33, 18.16, 18.04, 18.01 (Si–*t*-Bu); −2.13, −2.86, −3.72, −3.89, −4.50, −4.58 ($SiMe_2$). ^{1}H NMR ($CDCl_3$, *E*–*Z*=0:1, selected data), δ (ppm): 5.15 (d, *J*=5.0 Hz, H-2); 4.02 (br d, *J*=13 Hz, H-6a); 3.32 (H-6b); 3.14 (H-11); 3.58 (H-13); 3.97 (dd, *J*=9.0+1.8 Hz, H-14); 3.60 (H-15); 5.48 (d, *J*=9.8 Hz, H-20); 2.70 (ddd, *J*=9.8+9.0+3.3 Hz, H-21); 4.50 (d, *J*=8.8 Hz, H-23); 4.74 (dd, *J*=8.8+5.9 Hz, H-24); 5.58 (br s, H-26); 4.88 (d, *J*=9.0 Hz, H-29); 2.94 (ddd, *J*=11.2+8.4+4.4 Hz, H-32); 3.37 (H-33); 1.15 (d, 3H, *J*=6.8 Hz, 11-Me); 1.67 (s, 3H, 19-Me); 1.57 (s, 3H, 27-Me); −0.11, −0.04, 0.06, 0.07, 0.15, 0.17, 0.22, 0.31 (s, each 3H, Si–Me); 0.79, 0.88, 0.89, 0.96 (s, each 9H, $(CH_3)_3$).

Compound **11**. 191 mg, 0.4%. White powder; MS: 1270 (MNa^+), 1311 ($MNa^+ \cdot CH_3CN$); ^{13}C NMR ($CDCl_3$, *E*–*Z*=0:1), δ (ppm): 169.29 (C1); 50.98 (C2); 26.35 (C3); 20.62 (C4); 24.48 (C5); 43.06 (C6); 165.70 (C8); 197.43 (C9); 99.33 (C10); 39.68 (C11); 33.11 (C12); 74.23 (C13); 77.21 (C14); 77.84 (C15); 34.43 (C16); 29.40 (C17); 47.79 (C18); 133.02 (C19); 128.52 (C20); 46.37 (C21); 155.14 (C22); 106.96 (C23); 69.81 (C24); 42.58 (C25); 74.14 (C26); 134.21 (C27); 14.68 (C28); 127.11 (C29); 34.86 (C30); 36.81 (C31); 84.25 (C32); 75.24 (C33); 33.91 (C34); 31.05 (C35); 28.80 (C36); 12.74 (C37); 56.14 (13-OMe); 57.43 (15-OMe); 57.92 (32-OMe); 15.50 (11-CH_3); 20.20 (17-CH_3); 19.59 (19-CH_3); 9.32 (25-CH_3); 26.01, 25.92, 25.88, 18.42, 18.17, 18.05, 17.93 (Si–*t*-Bu); −2.81, −2.99, −3.34, −3.86, −4.62, −4.74 ($SiMe_2$). ^{1}H NMR ($CDCl_3$, *E*–*Z*=0:1, selected data), δ (ppm): 5.03 (d, *J*=5.7 Hz, H-2); 3.53 (ddd, *J*=12.8+12.8+3.3 Hz, H-6a); 3.34 (H-6b); 1.77 (H-11); 3.43 (H-13); 3.31 (H-14); 3.38 (H-15); 5.58 (d, *J*=9.5 Hz, H-20); 2.61 (ddd, *J*=9.5+9.5+3.5 Hz, H-21); 4.28 (d, *J*=9.0 Hz, H-23); 4.63 (dd, *J*=9.0+5.0 Hz, H-24); 5.48 (s, H-26); 4.95 (d, *J*=9.0 Hz, H-29); 2.94 (ddd, *J*=11.2+8.2+4.4 Hz, H-32); 3.37 (H-33); 1.07 (d, 3H, *J*=7.0 Hz, 11-Me); 1.58 (s, 3H, 19-Me); 1.61 (s, 3H, 27-Me); −0.07, −0.04, 0.06, 0.07, 0.13, 0.18, 0.20, 0.22 (s, each 3H, Si–Me); 0.83, 0.95 (s, each 9H, $(CH_3)_3$), 0.88 (s, 18H, $(CH_3)_3$).

5.1.2. 24-*O*-TBDMS-ascomycin 12. To a magnetically stirred solution of 5 g (4.9 mmol) 24,33-bis-*O*-TBDMS-ascomycin (**4**) in 130 ml acetonitrile were added 5 ml of an aq. hydrogen fluoride solution (40 w/w%) in one portion. After 15 min, the mixture was partitioned between saturated aqueous sodium hydrogen carbonate solution (200 ml) and ethyl acetate. The organic layer was washed three times with brine, dried over anhydrous sodium sulfate, filtered end concentrated. The residual crude product was purified by flash chromatography (silica gel, ethyl acetate–*n*-heptane=2:1). The product fraction was concentrated under reduced pressure and dried for 15 h under high vacuum.

4.45 g (95%). Colorless foam; MS: 906 (MH^+), 928 (MNa^+); CHN ($C_{49}H_{83}NO_{12}Si$) calcd: 64.94/9.23/1.55, found: 64.52/9.27/1.43; ^{13}C NMR ($CDCl_3$, *E*–*Z*=2:1), δ (*E*/*Z*-isomer, ppm): 169.00/168.25 (C1); 56.36/52.70 (C2); 27.62/26.49 (C3); 20.57/20.83 (C4); 24.22/24.64 (C5); 39.00/43.64 (C6); 164.62/165.74 (C8); 196.48/191.30 (C9); 97.53/98.69 (C10); 34.72/33.33 (C11); 32.57/32.75 (C12); 73.54/73.66 (C13); 72.78/71.70 (C14); 75.35/76.36 (C15); 34.85/35.98 (C16); 25.44/26.18 (C17); 49.33/48.31 (C18); 138.07/139.32 (C19); 123.78/122.73 (C20); 55.53/55.62 (C21); 210.24/210.80 (C22); 48.31/44.63 (C23); 69.73/70.80 (C24); 40.51/41.18 (C25); 81.3 (br)/77.1 (br) (C26); 131.74/132.79 (C27); 12.14/14.14 (C28); 134.4 (br)/129.69 (C29); 35.00/34.89 (C30); 34.55/34.55 (C31); 84.08/84.16 (C32); 73.44/73.44 (C33); 31.24/31.19 (C34); 30.37/30.62 (C35); 24.38/23.15 (C36); 11.58/11.60 (C37); 57.12/57.43 (15-OMe); 16.02/16.09 (11-CH_3); 19.49/18.85 (17-CH_3); 15.33/16.37 (19-CH_3); 10.32/10.36 (25-CH3); 56.51/56.29/56.24/56.23 (both conformers, 32-OMe and 15-OMe); 25.83, 25.79, 17.97, 17.89 (Si–*t*-Bu); −4.20, −4.35, −4.49, −4.90 ($SiMe_2$). ^{1}H NMR ($CDCl_3$, *E*–*Z*=2:1, selected data), δ (*E*-isomer, ppm): 4.83 (H-2); 4.40 (br d, *J*=13.5 Hz, H-6a); 3.10 (H-6b); 2.26 (H-11); 3.46 (H-13); 3.79 (dd, *J*=9.6+1.6 Hz, H-14); 3.60 (ddd, *J*=11.2+4.1+1.8 Hz, H-15); 4.80 (d, *J*=10.3 Hz, H-20); 3.29 (H-21); 2.78 (dd, *J*=14.2+7.6 Hz, H-23a); 2.22 (dd, *J*=14.2+5.3 Hz, H-23b); 4.04 (ddd, *J*=7.3+5.7+1.8 Hz, H-24); 5.19 (d, *J*=7.3 Hz, H-26); 5.26 (d, *J*=9.2 Hz, H-29); 3.00 (H-32); 3.38 (H-33); 0.95 (d, 3H, *J*=6.6 Hz, 11-Me); 1.63 (s, 3H, 19-Me); 1.48 (s, 3H, 27-Me); 0.02, 0.03 (s, each 3H, Si–Me); 0.86 (s, 9H, $(CH_3)_3$); 4.20 (s, 10-OH); δ (*Z*-isomer, ppm): 4.99 (br dd, *J*=5.5+2.0 Hz, H-2); 3.88 (br d, *J*=13.5 Hz, H-6a); 3.28 (H-6b); 2.19 (H-11); 3.46 (H-13); 3.93 (dd, *J*=9.4+2.5 Hz, H-14); 3.57 (ddd, *J*=11.0+4.6+2.8 Hz, H-15); 5.07 (d, *J*=10.1 Hz, H-20); 3.08 (H-21); 2.46 (dd, *J*=16.5+6.9 Hz, H-23a); 2.41 (dd, *J*=16.5+4.1 Hz, H-23b); 4.23 (H-24); 5.25 (d, *J*=2.8 Hz, H-26); 5.04 (d, *J*=8.9 Hz, H-29); 3.00 (H-32); 3.38 (H-33); 1.00 (d, 3H, *J*=6.6 Hz, 11-Me); 1.69 (s, 3H, 19-Me); 1.63 (s, 3H, 27-Me); −0.04, 0.03 (s, each 3H, Si–Me); 0.85 (s, 9H, $(CH_3)_3$); 5.42 (br s, 10-OH).

5.1.3. 33-*O*-formyl-24-*O*-TBDMS-ascomycin 13. To a stirred solution of 4 g (4.41 mmol) 24-*O*-TBDMS-ascomycin (**11**) and 5.4 g DMAP (10 equiv., 44.1 mmol) in 130 ml acetonitrile were added at room temperature 1.94 g (5 equiv., 22 mmol) of the mixed anhydride of acetic acid and formic acid ($H(CO)O(CO)CH_3$).[21] After TLC analysis confirmed completion of the reaction, the mixture was partitioned between ethyl acetate and saturated aqueous sodium hydrogen carbonate. The organic layer was washed twice with 1N hydrochloric acid (to remove DMAP) and brine, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residual crude product was purified by flash chromatography (silica gel, ethyl acetate–*n*-heptane=3:2). The product fraction was concentrated under reduced pressure and dried for 15 h under high vacuum.

3.63 g (88%). Colorless foam; MS: 934 (MH^+), 956 (MNa^+), 997 ($MNa^+\cdot CH_3CN$); CHN ($C_{50}H_{83}NO_{13}Si$) calcd: 64.28/8.95/1.50 found: 63.93/8.82/1.50; ^{13}C NMR ($CDCl_3$, *E*–*Z*=2:1), δ (*E*/*Z*-isomer, ppm): 168.98/168.23 (C1); 56.23/52.71 (C2); 27.57/26.21 (C3); 20.84/20.64 (C4); 24.62/24.24 (C5); 39.03/43.65 (C6); 164.62/165.80 (C8); 196.43/191.22 (C9); 97.55/98.72 (C10); 34.62/33.36 (C11); 32.78/32.59 (C12); 73.57/73.67 (C13); 72.83/71.71 (C14); 75.37/76.35 (C15); 34.6 (br)/36.02 (C16); 25.52/25.83 (C17); 49.29/48.34 (C18); 138.11/139.29 (C19); 123.73/122.76 (C20); 55.60/55.50 (C21); 210.72/210.25 (C22); 48.34/44.71 (C23); 69.74/70.67 (C24); 40.54/41.05 (C25); 80.8 (br)/76.9 (br) (C26); 132.3 (br)/133.21 (C27); 12.34/14.22 (C28); 133.5 (br)/128.95 (C29); 34.72/34.50 (C30); 35.82/36.13 (C31); 80.43/80.48 (C32); 75.81/75.88 (C33); 29.72/29.66 (C34); 30.22/30.43 (C35); 24.40/23.22 (C36); 11.58/11.61 (C37); 56.33/56.23 (13-OMe); 57.10/57.41 (15-OMe); 56.97/57.15 (32-OMe); 16.01/16.09 (11-CH_3); 19.57/18.85 (17-CH_3); 15.37/16.38 (19-CH_3); 10.26/10.26 (25-CH_3); 160.96/160.96 (33-OCHO); 25.80/25.83 and 17.97/17.89 (Si–*t*-Bu); −4.20, −4.33, −4.50, −4.89 ($SiMe_2$). ^1H NMR ($CDCl_3$, *E*–*Z*=2:1, selected data), δ (*E*-isomer, ppm): 4.42 (br s, H-2); 4.41 (br d, *J*=13 Hz, H-6a); 3.09 (H-6b); 2.28 (H-11); 3.46 (H-13); 3.79 (dd, *J*=9.5+1.5 Hz, H-14); 3.60 (ddd, *J*=11.4+4.0+1.5 Hz, H-15); 4.80 (d, *J*=10.1 Hz, H-20); 3.29 (H-21); 2.77 (dd, *J*=14.5+7.6 Hz, H-23a); 2.22 (dd, *J*=14.5+5.5 Hz, H-23b); 4.05 (br dd, *J*=6+6 Hz, H-24); 5.19 (d, *J*=7.1 Hz, H-26); 5.25 (d, *J*=10.0 Hz, H-29); 3.24 (H-32); 4.71 (H-33); 0.96 (d, 3H, *J*=6.6 Hz, 11-Me); 1.63 (s, 3H, 19-Me); 1.49 (s, 3H, 27-Me); 0.02, 0.03 (s, each 3H, Si–Me); 0.86 (s, 9H, $(CH_3)_3$); 4.20 (s, 10-OH); 8.12 (s, 33-CHO). δ (*Z*-isomer, ppm): 4.99 (br d, *J*=5.5 Hz, H-2); 4.90 (br d, *J*=13.5 Hz, H-6a); 3.28 (H-6b); 2.20 (H-11); 3.46 (H-13); 3.93 (dd, *J*=9.6+2.6 Hz, H-14); 3.58 (ddd, *J*=11.3+4.5+2.6 Hz, H-15); 5.09 (d, *J*=10.1 Hz, H-20); 3.07 (H-21); 2.46 (dd, *J*=16.5+7.1 Hz, H-23a); 2.41 (dd, *J*=16.5+3.6 Hz, H-23b); 4.24 (H-24); 5.23 (s, H-26); 5.02 (d, *J*=8.8 Hz, H-29); 3.24 (H-32); 4.71 (H-33); 1.01 (d, 3H, *J*=6.6 Hz, 11-Me); 1.69 (s, 3H, 19-Me); 1.63 (s, 3H, 27-Me); −0.04, 0.03 (s, each 3H, Si–Me); 0.85 (s, 9H, $(CH_3)_3$); 5.49 (s, 10-OH); 8.11 (s, 33-CHO).

5.1.4. 14,33-Bis-*O*-(aminocarbonyl)-24-*O*-TBDMS-ascomycin 14 (representative run). 0.355 ml (0.58 g, 3 equiv., 2.94 mmol) trichloromethyl-chloroformate (diphosgene) was dropped via syringe into a solution of 1.0 g (0.98 mmol) 24,33-bis-OTBDMS-ascomycin (**4**) and 0.96 g (8 equiv., 7.84 mmol) 4-(*N*,*N*-dimethylamino)-pyridine (DMAP) in 70 ml acetonitrile. The reaction was allowed to stir at room temperature until TLC indicated the complete consumption of starting material (6 h). The reaction mixture was poured into a rapidly stirred mixture of 100 ml aq. ammonia (15 w/w%) and 200 ml ethyl acetate. After 10 min the organic phase was separated, washed twice with 1N hydrochloric acid and brine, dried over anhydrous sodium sulfate, filtered and evaporated at reduced pressure. The residual crude product was purified by flash chromatography (silica gel, ethyl acetate–*n*-heptane=7:1), followed by filtration through Sephadex® LH20 (ethyl acetate). The product containing fraction was evaporated at reduced pressure and dried via lyophilization from dioxane at high vacuum.

670 mg (69%). Yellow powder; MS: 1014 (MNa^+), 1055 ($MNa^+\cdot CH_3CN$); CHN ($C_{51}H_{85}N3O_{14}Si$) calcd: 61.73/8.63/4.23, found: 61.51/8.41/4.04; ^{13}C NMR ($CDCl_3$, *E*–*Z*<1:10), δ (*Z*-isomer, ppm): 169.08 (C1); 52.01 (C2); 25.66 (C3); 21.26 (C4); 25.42 (C5); 43.83 (C6); 165.82 (C8); 184.46 (C9); 200.17 (C10); 32.06 (C11); 35.07 (C12); 78.07 (C13); 73.02 (C14); 76.49 (C15); 38.31 (C16); 27.68 (C17); 46.87 (C18); 138.72 (C19); 123.11 (C20); 54.80 (C21); 208.63 (C22); 48.67 (C23); 66.93 (C24); 38.29 (C25); 84.31 (C26); 131.61 (C27); 10.97 (C28); 135.95 (C29); 34.78 (C30); 35.82 (C31); 80.78 (C32); 76.64 (C33); 30.21 (C34); 30.32 (C35); 23.63 (C36); 11.81 (C37); 56.93 (13-OMe); 58.03 (15-OMe); 56.63 (32-OMe); 16.98 (11-CH_3); 18.91 (17-CH_3); 17.71 (19-CH_3); 9.91 (25-CH_3); 156.65 (33-$OCONH_2$); 156.02 (14-$OCONH_2$); 25.87, 18.07 (Si–*t*-Bu); −3.77, −4.15 ($SiMe_2$). ^1H NMR ($CDCl_3$, *E*–*Z*<1:10, selected data), δ (*Z*-isomer, ppm): 5.19 (br d, *J*=5.4 Hz, H-2); 3.47 (H-6a); 3.17 (H-6b); 3.84 (H-11); 3.45 (H-13); 5.31 (d, *J*=9.0 Hz, H-14); 3.48 (H-15); 5.00 (d, *J*=10.2 Hz, H-20); 3.17 (H-21); 2.98 (dd, *J*=17.2+10.2 Hz, H-23a); 2.29 (dd, *J*=17.2+3.4 Hz, H-23b); 4.20 (dd, *J*=10.2+3.4 Hz, H-24); 5.11 (d, *J*=10.7 Hz, H-26); 5.32 (d, *J*=8.9 Hz, H-29); 3.15 (H-32); 4.56 (ddd, *J*=11.3+9.4+4.9 Hz, H-33); 1.09 (d, 3H, *J*=6.7 Hz, 11-Me); 1.74 (s, 3H, 19-Me); 1.52 (s, 3H, 27-Me); 0.01, 0.04 (s, each 3H, Si–Me); 0.88 (s, 9H, $(CH_3)_3$); 4.69, 4.85 (s, each 1H, $CONH_2$).

5.1.5. 14,33-Bis-*O*-(*N*-methylaminocarbonyl)-24-*O*-TBDMS-ascomycin 15. Starting from 0.5 g (0.49 mmol) 24,33-bis-OTBDMS-ascomycin (**4**), compound **15** was prepared as described above for compound **14**, with the exception that a mixture of an aq. solution of methylamine (15 w/w%) and ethyl acetate was used for quenching the reaction. Purification of the crude reaction mixture was performed by flash chromatography (silica gel, ethyl acetate–*n*-heptane=5:1).

720 mg (72%). Yellow powder; MS: 1020 (MH^+), 1042 (MNa^+), 1083 ($MNa^+\cdot CH_3CN$); CHN ($C_{53}H_{89}N_3O_{14}Si$) calcd: 62.39/8.79/4.12, found: 62.37/8.85/3.95; ^{13}C NMR ($CDCl_3$, *E*–*Z*<1:10), δ (*Z*-isomer, ppm): 169.08 (C1); 51.96 (C2); 25.69 (C3); 21.26 (C4); 25.44 (C5); 43.79 (C6); 165.83 (C8); 184.47 (C9); 200.13 (C10); 32.1 (br) (C11); 35.09 (C12); 78.28 (C13); 72.92 (C14); 76.74 (C15); 38.55

(C16); 27.47 (C17); 46.92 (C18); 138.74 (C19); 123.24 (C20); 54.86 (C21); 208.51 (C22); 48.65 (C23); 67.00 (C24); 38.41 (C25); 84.19 (C26); 131.55 (C27); 10.90 (C28); 136.00 (C29); 34.82 (C30); 35.83 (C31); 80.80 (C32); 76.22 (C33); 30.42 (C34); 30.34 (C35); 23.63 (C36); 11.81 (C37); 56.9 (br) (13-OMe); 58.07 (15-OMe); 56.64 (32-OMe); 16.95 (11-CH_3); 18.93 (17-CH_3); 17.53 (19-CH_3); 9.90 (25-CH_3); 156.89, 27.72 (33-O*C*ONH*C*H_3); 156.45, 27.84 (14-O*C*ONH*C*H_3); 25.87, 18.07 (Si–*t*-Bu); −3.82, −4.15 ($SiMe_2$). ^{1}H NMR ($CDCl_3$, *E*–*Z*<1:10, selected data), δ (*Z*-isomer, ppm): 5.19 (br d, *J*=5.5 Hz, H-2); 3.47 (H-6a); 3.18 (H-6b); 3.86 (H-11); 3.42 (H-13); 5.32 (d, *J*=9.0 Hz, H-14); 3.47 (H-15); 5.01 (d, *J*=10.4 Hz, H-20); 3.17 (H-21); 2.97 (dd, *J*=17.0+10.0 Hz, H-23a); 2.27 (dd, *J*=17.0+3.3 Hz, H-23b); 4.20 (dd, *J*=10.0+3.3 Hz, H-24); 5.12 (d, *J*=10.5 Hz, H-26); 5.32 (d, *J*=8.8 Hz, H-29); 3.15 (H-32); 4.57 (H-33); 1.08 (d, 3H, *J*=6.8 Hz, 11-Me); 1.74 (s, 3H, 19-Me); 1.50 (d, 3H, *J*=1.0 Hz, 27-Me); 0.01, 0.05 (s, each 3H, Si–Me); 0.88 (s, 9H, $(CH_3)_3$); 4.76 (br q, *J*=5.0 Hz, 14-CON*H*CH_3); 2.82 (d, *J*=5.0 Hz, 14-CONH*C*H_3); 4.59 (br s, 33-CON*H*CH_3); 2.79 (d, *J*=5.0 Hz, 33-CONH*C*H_3).

5.1.6. 14,33-Bis-*O*-(1-imidazolyl-carbonyl)-24-*O*-TBDMS-ascomycin 16. Starting from 1.0 g (0.98 mmol) 24,33-bis-OTBDMS-ascomycin (**4**), compound **16** was prepared as described above for compound **14**, with the exception that a mixture of an aq. solution of imidazole (25 w/w%) and ethyl acetate was used for quenching the reaction. Purification of the crude reaction mixture was performed by flash chromatography (silica gel, ethyl acetate–acetonitrile=20:1).

1.07 g (72%). Yellow powder; MS: 1094 (MH^+), 1042 (MNa^+); CHN ($C_{57}H_{87}N_5O_{14}Si$) calcd: 62.56/8.01/6.40 found: 62.32/7.85/6.26; ^{13}C NMR ($CDCl_3$, *E*–*Z*=1:10), δ (*Z*-isomer, ppm): 168.87 (C1); 52.00 (C2); 25.70 (C3); 21.18 (C4); 25.39 (C5); 43.97 (C6); 165.53 (C8); 184.83 (C9); 199.9 (br) (C10); 33.9 (br) (C11); 34.62 (C12); 77.68 (C13); 77.79 (C14); 76.20 (C15); 39.34 (C16); 27.51 (C17); 46.65 (C18); 138.64 (C19); 123.54 (C20); 55.07 (C21); 208.78 (C22); 47.0 (br) (C23); 67.40 (C24); 38.61 (C25); 83.59 (br) (C26); 132.17 (C27); 11.28 (C28); 135.00 (C29); 34.57 (C30); 35.73 (C31); 80.53 (C32); 80.27 (C33); 29.69 (C34); 30.18 (C35); 23.60 (C36); 11.72 (C37); 57.35 (13-OMe); 58.50 (15-OMe); 57.18 (32-OMe); 16.62 (11-CH_3); 19.14 (17-CH_3); 17.22 (19-CH_3); 9.78 (25-CH_3); 148.47, 148.36 (14,33-bis-O*C*=O), 137.0 (br), 130.87, 130.47, 117.25, 117.19 (2×1-imidazolyl); 25.86, 18.06 (Si–*t*-Bu); −3.82, −4.13 ($SiMe_2$). ^{1}H NMR ($CDCl_3$, *E*–*Z*=1:10), δ (*Z*-isomer, ppm): 5.20 (d, *J*=5.6 Hz, H-2); 3.45 (H-6a); 3.20 (H-6b); 3.70 (H-11); 3.59 (H-13); 5.49 (dd, *J*=7.0+3.5 Hz, H-14); 3.56 (H-15); 4.94 (d, *J*=10.0 Hz, H-20); 3.21 (H-21); 2.92 (H-23a); 2.31 (H-23b); 4.20 (dd, *J*=9.8+4.0 Hz, H-24); 5.13 (d, *J*=10.0 Hz, H-26); 5.34 (d, *J*=8.9 Hz, H-29); 3.33 (H-32); 4.88 (H-33); 1.14 (d, 3H, *J*=7.0 Hz, 11-Me); 1.75 (s, 3H, 19-Me); 1.55 (s, 3H, 27-Me); 0.03, 0.06 (s, each 3H, Si–Me); 0.90 (s, 9H, $(CH_3)_3$); 8.16, 8.15, 7.45, 7.44, 7.07, 7.10 (s, each 1H, imidazole-H).

5.1.7. 14-*O*-Formyl-33-*O*-(aminocarbonyl)-24-*O*-TBDMS-ascomycin 17. Starting from 0.8 g (0.86 mmol) 33-*O*-formyl-24-*O*-TBDMS-ascomycin (**13**), compound **17** was prepared as described above for compound **14** (8 equiv. DMAP, 3 equiv. diphosgene, reaction time 5 h). Purification of the crude product was performed by flash chromatography (silica gel, ethyl acetate–*n*-heptane=1:1).

720 mg (86%). Yellow powder; MS: 977 (MH^+), 999 (MNa^+), 1040 (MNa^+·CH_3CN); CHN ($C_{51}H_{84}N_2O_{14}Si$) calcd: 62.68/8.66/2.87, found: 62.35/8.51/2.78; ^{13}C NMR ($CDCl_3$, *E*–*Z*=1:10), δ (*Z*-isomer, ppm): 169.14 (C1); 52.01 (C2); 25.67 (C3); 21.27 (C4); 25.42 (C5); 43.82 (C6); 165.83 (C8); 184.47 (C9); 200.16 (C10); 32.06 (C11); 35.09 (C12); 78.07 (C13); 73.08 (C14); 76.48 (C15); 38.32 (C16); 27.67 (C17); 46.89 (C18); 138.74 (C19); 123.12 (C20); 54.82 (C21); 208.57 (C22); 48.69 (C23); 66.93 (C24); 38.32 (C25); 84.25 (C26); 131.89 (C27); 11.01 (C28); 135.66 (C29); 34.62 (C30); 35.61 (C31); 80.48 (C32); 76.08 (C33); 29.83 (C34); 30.27 (C35); 23.62 (C36); 11.82 (C37); 56.64 (13-OMe); 58.06 (15-OMe); 56.94 (32-OMe); 16.99 (11-CH_3); 18.93 (17-CH_3); 17.69 (19-CH_3); 9.91 (25-CH_3); 161.15 (33-OCHO); 155.89 (14-O*C*ONH_2); 25.87, 18.08 (Si–*t*-Bu); −3.74, −4.14 ($SiMe_2$). ^{1}H NMR ($CDCl_3$, *E*–*Z*=1:10, selected data), δ (*Z*-isomer, ppm): 5.20 (d, *J*=5.5 Hz, H-2); 3.48 (H-6a); 3.18 (H-6b); 3.85 (H-11); 3.45 (H-13); 5.32 (d, *J*=9.0 Hz, H-14); 3.49 (H-15); 5.00 (d, *J*=10.0 Hz, H-20); 3.18 (H-21); 2.99 (dd, *J*=17.2+10.4 Hz, H-23a); 2.28 (H-23b); 4.21 (dd, *J*=10.2+3.4 Hz, H-24); 5.12 (d, *J*=10.5 Hz, H-26); 5.33 (d, *J*=9.1 Hz, H-29); 3.22 (H-32); 4.70 (H-33); 1.10 (d, 3H, *J*=6.7 Hz, 11-Me); 1.75 (s, 3H, 19-Me); 1.53 (d, 3H, *J*=1.1 Hz, 27-Me); 0.02, 0.05 (s, each 3H, Si–Me); 0.89 (s, 9H, $(CH_3)_3$); 8.13 (s, 33-CHO); 4.6–4.9 (br s, 2H, 14-C*O*NH_2).

5.1.8. 14-*O*-(Ethoxycarbonylmethyl)-33-*O*-formyl-24-*O*-TBDMS-ascomycin 18. 0.39 ml (0.64 g, 3 equiv., 3.21 mmol) trichloromethyl-chloroformate (diphosgene) was dropped via syringe into a solution of 1.0 g (1.07 mmol) 33-*O*-formyl-24-*O*-TBDMS-ascomycin (**13**) and 1.05 g (8 equiv., 8.56 mmol) 4-(*N*,*N*-dimethylamino)-pyridine (DMAP) in 60 ml acetonitrile. The reaction was stirred at room temperature until TLC indicated a complete consumption of the starting material (5 h). The reaction mixture was poured into a rapidly stirred mixture of 100 ml water/10 g glycine ethyl ester hydrochloride/10 ml saturated aq. potassium carbonate and 250 ml ethyl acetate. After 20 min, the organic phase was separated, washed twice with 1N hydrochloric acid and brine, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residual crude product was purified by flash chromatography (silica gel, ethyl acetate–*n*-heptane=1:1) followed by filtration through Sephadex® LH20 (ethyl acetate). The purified product was lyophilized from benzene under high vacuum.

730 mg (67%) yellow powder; MS: 1085 (MNa^+); CHN ($C_{55}H_{90}N_2O_{16}Si$) calcd: 62.12/8.53/2.63 found: 61.95/8.65/2.59; ^{13}C NMR ($CDCl_3$, *E*–*Z*=1:10), δ (*Z*-isomer, ppm): 169.15 (C1); 51.98 (C2); 25.65 (C3); 21.27 (C4); 25.42 (C5); 43.80 (C6); 165.88 (C8); 184.54 (C9); 200.12 (C10); 32.0 (br) (C11); 34.95 (C12); 78.08 (C13); 73.30 (C14); 76.44 (C15); 38.28 (C16); 27.72 (C17); 46.90 (C18); 138.81 (C19); 123.10 (C20); 54.78 (C21); 208.45 (C22); 48.71 (C23); 66.89 (C24); 38.39 (C25); 84.24 (C26); 131.92 (C27); 10.96 (C28); 135.65 (C29); 34.61 (C30);

35.61 (C31); 80.48 (C32); 76.10 (C33); 29.83 (C34); 30.27 (C35); 23.59 (C36); 11.81 (C37); 56.63 (13-OMe); 58.02 (15-OMe); 56.93 (32-OMe); 16.85 (11-CH_3); 18.83 (17-CH_3); 17.68 (19-CH_3); 9.92 (25-CH_3); 161.12 (33-OCHO); 155.98, 42.98, 169.78, 61.45, 14.13 (14-OCONHCH$_2$COOCH$_2$CH$_3$); 25.86, 18.07 (Si–*t*-Bu); −3.77, −4.15 ($SiMe_2$). ^{1}H NMR ($CDCl_3$, *E*–*Z*=1:10, selected data), δ (*Z*-isomer, ppm): 5.20 (d, *J*=5.5 Hz, H-2); 3.48 (H-6a); 3.18 (H-6b); 3.83 (H-11); 3.47 (H-13); 5.36 (d, *J*=8.9 Hz, H-14); 3.48 (H-15); 5.01 (d, *J*=10.0 Hz, H-20); 3.17 (H-21); 2.99 (dd, *J*=17.2+10.3 Hz, H-23a); 2.28 (dd, *J*=17.2+3.4 Hz, H-23b); 4.22 (H-24); 5.12 (d, *J*=10.6 Hz, H-26); 5.32 (H-29); 3.21 (H-32); 4.70 (H-33); 1.08 (d, 3H, *J*=6.9 Hz, 11-Me); 1.74 (s, 3H, 19-Me); 1.52 (d, 3H, *J*=1.1 Hz, 27-Me); 0.02, 0.05 (s, each 3H, Si–Me); 0.89 (s, 9H, $(CH_3)_3$); 5.30 (br s, 14-CON*H*); 4.01 (dd, *J*=18.1+6.2 Hz, 14-CONH*CH*$_2$-*a*); 3.90 (dd, *J*=18.1+5.4 Hz, 14-CONH*CH*$_2$-*b*); 4.20 (q, *J*=7.1 Hz, COO*CH*$_2$CH$_3$); 1.28 (t, *J*=7.1 Hz, COOCH$_2$*CH*$_3$); 8.12 (s, 33-CHO).

5.1.9. 24,33-Bis-*O*-formyl-ascomycin 19. To a stirred solution of 5 g (6.31 mmol) ascomycin (**1**) and 15.46 g DMAP (20 equiv., 0.126 mol) in 150 ml acetonitrile, 5.55 g (10 equiv., 63.1 mmol) of the mixed anhydride of acetic acid and formic acid (H(CO)O(CO)CH_3 were added at room temperature.[21] After TLC showed completion of the reaction (70 min) the mixture was worked up as described above for the preparation of compound **12**. Purification of the crude product was accomplished using flash column chromatography (silica gel; ethyl acetate–toluene=1:2).

4.71 g (88%). Colorless foam; 848 (MH^+), 870 (MNa^+), 1081 (MNa^+·CH_3CN); ^{13}C NMR ($CDCl_3$, *E*–*Z*=2:1), δ (*E*/*Z*-isomer, ppm): 169.08/168.79 (C1); 56.35/52.74 (C2); 27.53/26.25 (C3); 20.84/20.66 (C4); 24.49/24.29 (C5); 39.16/43.92 (C6); 164.75/166.08 (C8); 196.42/192.42 (C9); 97.35/98.53 (C10); 34.63/33.61 (C11); 32.62/32.62 (C12); 73.66/73.86 (C13); 73.02/72.49 (C14); 75.32/76.98 (C15); 33.99/35.54 (C16); 26.01/26.59 (C17); 48.98/48.17 (C18); 138.40/139.28 (C19); 123.57/123.20 (C20); 55.23/54.60 (C21); 208.34/208.05 (C22); 43.05/41.87 (C23); 71.44/71.56 (C24); 37.86/37.04 (C25); 79.31/78.51 (C26); 131.39/131.58 (C27); 12.77/13.52 (C28); 132.36/130.72 (C29); 34.54/34.51 (C30); 35.84/35.93 (C31); 80.45/80.45 (C32); 75.82/75.82 (C33); 29.66/29.66 (C34); 30.11/30.24 (C35); 24.38/23.87 (C36); 11.66/11.60 (C37); 57.10/57.15 (13-OMe); 57.15/57.42 (15-OMe); 56.23/56.067 (32-OMe); 16.08/15.99 (11-CH_3); 19.90/19.68 (17-CH_3); 15.67/16.27 (19-CH_3); 10.28/10.10 (25-CH_3); 160.9 (33-OCHO); 159.68/169.88 (24-OCHO). ^{1}H NMR ($CDCl_3$, *E*–*Z*=2:1, selected data), δ (*E*-isomer, ppm): 4.54 (br dd, *J*=4+3 Hz, H-2); 4.43 (br d, *J*=13.5 Hz, H-6a); 3.06 (ddd, *J*=13.5+13.3+2.8 Hz, H-6b); 2.27 (H-11); 3.45 (H-13); 3.74 (dd, *J*=9.7+1.5 Hz, H-14); 3.60 (ddd, *J*=10.9+3.6+1.5 Hz, H-15); 4.89 (dq, *J*=10.1+1.2 Hz, H-20); 3.27 (H-21); 2.85 (dd, *J*=15.2+6.3 Hz, H-23a); 2.42 (dd, *J*=15.2+6.6 Hz, H-23b); 5.22 (ddd, *J*=6.6+6.3+2.8 Hz, H-24); 5.10 (d, *J*=5.5 Hz, H-26); 5.13 (d, *J*=9.1 Hz, H-29); 3.25 (H-32); 4.71 (ddd, *J*=11.3+9.5+4.8 Hz, H-33); 0.98 (d, 3H, *J*=6.5 Hz, 11-Me); 1.64 (d, 3H, *J*=1.2 Hz, 19-Me); 1.54 (d, 3H, *J*=1.2 Hz, 27-Me); 8.00 (s, 14-CHO); 8.12 (s, 33-CHO); 4.21 (s, 10-OH); δ (*Z*-isomer, ppm): 4.98 (dd, *J*=6.2+2.2 Hz, H-2); 3.77 (br d, *J*=13.2 Hz, H-6a); 3.32 (H-6b); 2.27 (H-11); 3.45 (H-13); 3.87 (dd, *J*=9.7+2.8 Hz, H-14); 3.55 (H-15); 5.05 (d, *J*=9.0 Hz, H-20); 3.15 (H-21); 2.76 (dd, *J*=17.6+5.0 Hz, H-23a); 2.63 (dd, *J*=17.6+6.9 Hz, H-23b); 5.35 (H-24); 5.10 (d, *J*=5.5 Hz, H-26); 5.05 (d, *J*=9.1 Hz, H-29); 3.25 (H-32); 4.71 (ddd, *J*=11.3+9.5+4.8 Hz, H-33); 0.99 (d, 3H, *J*=6.5 Hz, 11-Me); 1.68 (d, 3H, *J*=1.2 Hz, 19-Me); 1.63 (d, 3H, *J*=1.2 Hz, 27-Me); 8.00 (s, 14-CHO); 8.12 (s, 33-CHO); 5.04 (s, 10-OH).

5.1.10. 14,33-Bis-*O*-formyl-24-*O*-TBDMS-ascomycin 20. *Starting from* **4**. To a cooled (ice-bath), magnetically stirred solution of 4 ml (30 equiv., 44.1 mmol) *N*,*N*-dimethyl formamide in 100 ml acetonitrile were added drop wise via a syringe 1.77 ml (10 equiv., 14.7 mmol, 2.91 g) trichloromethyl-chloroformate (diphosgene) in such a rate, that the temperature was kept below 5°C. After an additional 30 min at 0–5°C, 1.5 g (1.47 mmol) 24,33-bis-*O*-TBDMS-ascomycin (**4**) were added in one portion and the cooling bath was removed. The course of the reaction was monitored by TLC. After 20 min, the complete consumption of the starting material and the formation of 33-*O*-formyl-24-OTBDMS-ascomycin (**13**) together with minor amounts of the target compound **20** could be detected. After 6 h, the complete conversion of the intermediate **13** into **20** was observed. The reaction mixture was partitioned between a saturated aqueous sodium hydrogen carbonate solution (200 ml) and ethyl acetate (400 ml). The organic layer was washed twice with brine, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified using flash column chromatography (silica gel, toluene–acetonitrile=5:1) to give 0.98 g (69%) of the title compound **20**.

Starting from **12**. Using 0.5 g (0.552 mmol) 24-*O*-TBDMS-ascomycin (**12**), 1.53 ml (30 equiv., 16.55 mmol) DMF and 0.67 ml (10 equiv., 5.52 mmol) diphosgene, the reaction was performed as described above to give 385 mg (72%) **20**.

Starting from **13**. Using 0.5 g (0.53 mmol) 33-*O*-formyl-24-*O*-TBDMS-ascomycin (**13**), 1.47 ml (30 equiv., 15.9 mmol) DMF and O.64 ml (10 equiv., 5.3 mmol) diphosgene, the reaction was performed as described above to give 440 mg (86%) **20**.

980/385/440 mg (69/72/86% from **4/12/13**) yellow foam; 962 (MH^+), 984 (MNa^+), 1025 (MNa^+·CH_3CN); CHN ($C_{51}H_{83}NO_{14}Si$) calcd: 63.66/8.69/1.46, found: 63.75/8.48/1.43; ^{13}C NMR ($CDCl_3$, *E*–*Z*=1:10), δ (*Z*-isomer, ppm): 168.97 (C1); 52.00/56.24 (C2); 25.62 (C3); 21.23 (C4); 25.38 (C5); 43.90 (C6); 165.69 (C8); 184.61 (C9); 199.95 (C10); 32.6 (br) (C11); 34.61 (C12); 77.67 (C13); 72.41 (C14); 75.92 (C15); 38.57 (C16); 27.50 (C17); 46.86 (C18); 138.63 (C19); 123.26 (C20); 54.83 (C21); 208.59 (C22); 48.41 (C23); 67.06 (C24); 38.23 (C25); 84.05 (C26); 131.89 (C27); 11.09 (C28); 135.57 (C29); 34.64 (C30); 35.65 (C31); 80.48 (C32); 76.06 (C33); 29.82 (C34); 30.17 (C35); 23.60 (C36); 11.76 (C37); 56.81 (13-OMe); 58.09 (15-OMe); 56.95 (32-OMe); 16.80 (11-CH_3); 18.91 (17-CH_3); 17.60 (19-CH_3); 9.88 (25-CH_3); 161.09 (33-OCHO); 160.11 (14-OCHO); 25.86, 18.07 (Si–*t*-Bu);

−3.79, −4.15 ($SiMe_2$). ^{1}H NMR ($CDCl_3$, E–Z=1:10), δ (Z-isomer, ppm): 5.19 (d, J=5.0 Hz, H-2); 3.44 (br d, J=13 Hz, H-6a); 3.17 (H-6b); 3.73 (H-11); 3.49 (H-13); 5.56 (dd, J=8.2+1.7 Hz, H-14); 3.50 (H-15); 4.98 (d, J=10.3 Hz, H-20); 3.18 (H-21); 2.97 (dd, J=17.1+10.2 Hz, H-23a); 2.29 (dd, J=17.1+3.8 Hz, H-23b); 4.21 (dd, J=10.2+3.8 Hz, H-24); 5.11 (d, J=10.4 Hz, H-26); 5.33 (dq, J=8.9+1.2 Hz, H-29); 3.21 (H-32); 4.70 (ddd, J=11.5+9.5+5.0 Hz, H-33); 1.10 (d, 3H, J=6.7 Hz, 11-Me); 1.75 (s, 3H, 19-Me); 1.54 (d, 3H, J=1.2 Hz, 27-Me); 0.02, 0.05 (s, each 3H, Si–Me); 0.89 (s, 9H, $(CH_3)_3$); 8.19 (s, 14-CHO); 8.12 (s, 33-CHO).

5.1.11. 14,24,33-Tris-*O*-formyl-ascomycin 21. *Starting from* **19**. Using 1.2 g (1.42 mmol) 24,33-bis-*O*-formyl-ascomycin (**19**), 4 ml (30 equiv., 15.9 mmol) DMF and 1.72 ml (10 equiv., 5.3 mmol) diphosgene, the reaction was performed as described above for the preparation of compound **20**. Flash chromatography (silica gel, ethyl acetate–*n*-heptane=2:1) provided 1.11 g (89%) **21**.

Starting from **1**. Using 1.0 g (1.26 mmol) ascomycin (**1**), 4.7 ml (40 equiv., 50.4 mmol) DMF and 2.28 ml (15 equiv., 18.9 mmol) diphosgene, the reaction was performed as described above to give 0.61 g (55%) **21**.

1.11/0.61 g (89/55% from **19**/**1**) yellow foam; 876 (MH^+), 898 (MNa^+), 939 ($MNa^+\cdot CH_3CN$); CHN ($C_{46}H_{69}NO_{15}$) calcd: 63.07/7.94/1.60 found: 62.98/7.79/1.65; ^{13}C NMR ($CDCl_3$, E–Z=1:10), δ (Z-isomer, ppm): 168.88 (C1); 51.82 (C2); 25.24 (C3); 21.18 (C4); 25.03 (C5); 44.09 (C6); 165.85 (C8); 184.34 (C9); 199.84 (C10); 31.9 (br) (C11); 34.52 (C12); 77.31 (C13); 71.03 (C14); 75.66 (C15); 37.22 (C16); 27.65 (C17); 46.60 (C18); 138.81 (C19); 122.93 (C20); 54.23 (C21); 206.97 (C22); 43.80 (C23); 69.46 (C24); 35.42 (C25); 84.27 (C26); 130.68 (C27); 10.81 (C28); 135.87 (C29); 34.45 (C30); 35.44 (C31); 80.53 (C32); 75.96 (C33); 29.69 (C34); 29.91 (C35); 23.58 (C36); 11.77 (C37); 56.72 (13-OMe); 57.65 (15-OMe); 56.92 (32-OMe); 16.93 (11-CH_3); 18.86 (17-CH_3); 18.53 (19-CH_3); 10.55 (25-CH_3); 161.11 (33-OCHO); 159.96 (14 and 24-OCHO). ^{1}H NMR ($CDCl_3$, E–Z=1:10), δ (Z-isomer, ppm): 5.22 (d, J=5.5 Hz, H-2); 3.43 (br d, J=13 Hz, H-6a); 3.06 (H-6b); 3.74 (H-11); 3.54 (H-13); 5.57 (d, J=9.2 Hz, H-14); 3.49 (H-15); 5.01 (d, J=10.1 Hz, H-20); 3.22 (H-21); 3.00 (dd, J=17.9+10.8 Hz, H-23a); 2.64 (dd, J=17.9+3.2 Hz, H-23b); 5.27 (dd, J=10.8+3.2 Hz, H-24); 4.93 (d, J=10.3 Hz, H-26); 5.18 (dd, J=9.0 Hz, H-29); 3.20 (H-32); 4.67 (H-33); 1.10 (d, 3H, J=6.9 Hz, 11-Me); 1.77 (s, 3H, 19-Me); 1.62 (s, 3H, 27-Me); 8.19 (s, 14-CHO); 8.10 (s, 33-CHO); 7.95 (s, 24-CHO).

5.1.12. 24,33-Bis-*O*-formyl-14-*O*-(*N*,*N*-dimethylcarbamoylacetyl)-ascomycin 22 and compound 23. To a cooled and magnetically stirred solution of 1.6 ml (30 equiv., 17.3 mmol) *N*,*N*-dimethyl acetamide in 50 ml diethyl ether were added 10 ml (30 equiv., 17.7 mmol) of a solution of phosgene (20 w/w% in toluene) in such a rate (10 min), that the temperature was kept below 5°C. After additional 2 h at 0–5°C, 0.5 g (5.89 mmol) 24,33-bis-*O*-formyl-ascomycin (**19**) was added in one portion, the cooling bath was removed and the mixture was stirred for 20 h. The reaction mixture was partitioned between a saturated solution of aqueous sodium hydrogen carbonate (100 ml) and ethyl acetate (200 ml). The organic layer was washed twice with brine, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The crude mixture was separated by using flash column chromatography (silica gel, ethyl acetate–*n*-heptane=2:1→5:1). The two fractions isolated were further purified by size exclusion chromatography (Sephadex® LH20, ethyl acetate) and dried via lyophilization from dioxane under high vacuum.

Compound **22**. 42 mg, 7.4%. Yellow powder; 983 (MNa^+), 1024 ($MNa^+\cdot CH_3CN$); ^{13}C NMR ($CDCl_3$, E–Z<1:10), δ (Z-isomer, ppm): 168.95 (C1); 51.78 (C2); 25.09 (C3); 21.19 (C4); 25.28 (C5); 44.06 (C6); 166.01 (C8); 184.57 (C9); 200.21 (C10); 32.3 (br) (C11); 35.1 (br) (C12); 77.78 (C13); 72.44 (C14); 76.03 (C15); 37.56 (C16); (C17); 46.66 (C18); 139.04 (C19); 123.02 (C20); 54.33 (C21); 206.73 (C22); 43.86 (C23); 69.51 (C24); 37.70 (C25); 84.18 (C26); 130.75 (C27); 10.80 (C28); 135.85 (C29); 34.48 (C30); 35.47 (C31); 80.57 (C32); 76.03 (C33); 29.72 (C34); 29.92 (C35); 23.51 (C36); 11.77 (C37); 56.64 (13-OMe); 57.73 (15-OMe); 56.90 (32-OMe); 16.76 (11-CH_3); 18.64 (17-CH_3); 18.64 (19-CH_3); 10.50 (25-CH_3); 161.07 (33-OCHO); 159.94 (24-OCHO); 166.81, 41.17, 165.64, 35.55, 35.52 (14-$OCOCH_2CON(CH_3)_2$). ^{1}H NMR ($CDCl_3$, E–Z<1:10, selected data), δ (Z-isomer, ppm): 5.22 (d, J=5.4 Hz, H-2); 3.44 (H-6a); 3.06 (H-6b); 3.75 (H-11); 3.51 (H-13); 5.51 (dd, J=9.0+1.8 Hz, H-14); 3.47 (H-15); 5.01 (d, J=10.2 Hz, H-20); 3.20 (H-21); 2.99 (H-23a); 2.62 (dd, J=17.9+3.2 Hz, H-23b); 5.27 (br d, J=10.8 Hz, H-24); 4.93 (d, J=10.3 Hz, H-26); 5.17 (d, J=9.1 Hz, H-29); 3.20 (H-32); 4.66 (H-33); 1.11 (d, 3H, J=6.6 Hz, 11-Me); 1.76 (s, 3H, 19-Me); 1.60 (s, 3H, 27-Me); 8.10 (s, 33-CHO); 7.95 (s, 24-CHO); 3.49, 3.52 (each d, 1H, J=15.2 Hz, 14-$OCOCH_2$); 2.99, 2.94 (s, each 3H, NCH_3).

Compound **23**. 362 mg, 65%. Colorless powder; 943 (MH^+), 965 (MNa^+), 1006 ($MNa^+\cdot CH_3CN$); CHN ($C_{50}H_{74}N_2O_{15}$) calcd: 63.68/7.91/2.97 found: 63.51/7.82/2.86; ^{13}C NMR ($CDCl_3$, E–Z>10:1), δ (E-isomer, ppm): 168.82 (C1); 58.27 (C2); 26.81 (C3); 21.75 (C4); 24.65 (C5); 39.56 (C6); 161.25 (C8); 150.82 (C9); 108.53 (C10); 34.17 (C11); 32.85 (C12); 73.01 (C13); 76.61 (C14); 75.18 (C15); 32.56 (C16); 26.20 (C17); 48.00 (C18); 138.26 (C19); 123.49 (C20); 53.53 (C21); 210.09 (C22); 41.76 (C23); 72.08 (C24); 38.26 (C25); 77.34 (C26); 132.51 (C27); 14.77 (C28); 129.01 (C29); 34.45 (C30); 36.07 (C31); 80.40 (C32); 75.79 (C33); 29.62 (C34); 30.40 (C35); 24.17 (C36); 11.50 (C37); 56.44 (13-OMe); 56.70 (15-OMe); 57.19 (32-OMe); 15.30 (11 or 19-CH_3); 20.40 (17-CH_3); 15.27 (19 or 11-CH_3); 10.41 (25-CH_3); 166.50 (C9a); 126.09 (C9); 160.09 (C9c); 160.93 (33-OCHO); 160.31 (24-OCHO): 37.81, 34.51 ($CON(CH_3)_2$). ^{13}C NMR ($CDCl_3$, E–Z>10:1), δ (E-isomer, ppm): 4.42 (H-2); 4.44 (H-6a); 2.77 (ddd, (J=13+13+3 Hz, H-6b); 2.30 (H-11); 3.48 (H-13); 3.69 (d, J=9.5 Hz, H-14); 3.55 (d, J=11.5 Hz, H-15); 4.94 (d, J=9.3 Hz, H-20); 3.33 (H-21); 3.02 (dd, J=17.4+9.1 Hz, H-23a); 2.73 (dd, J=17.4+3.0 Hz, H-23b); 5.41 (H-24); 5.05 (H-26); 5.06 (H-29); 3.23 (H-32); 4.71 (H-33); 1.03 (d, 3H, J=6.6 Hz, 11-Me); 1.61 (s, 3H, 19-Me); 1.63 (d, 3H, J=1.0 Hz, 27-Me); 8.11 (s,

33-CHO); 8.02 (s, 24-CHO); 2.93, 2.94 (s, each 3H, $N-CH_3$).

References

1. For reviews see: (a) Grassberger, M. A.; Baumann, K. *Curr. Opin. Ther. Patents* **1993**, 931–937. (b) Stuetz, A.; Grassberger, M. A.; Baumann, K.; Edmunds, A. J. F.; Hiestand, P.; Meingassner, J. G.; Nussbaumer, P.; Schuler, W.; Zenke, G. Immunophilins as Drug Targets. In *Perspectives in Medicinal chemistry*; Testa, B., Kyburz, E., Fuhrer, W., Giger, R., Eds.; YHCA: Basle, 1993; pp 427–444 Chapter 27.
2. (a) Harper, J.; Green, A.; Scott, G.; Gruendl, E.; Dorobek, B.; Cardno, M.; Burtin, P. *Br. J. Dermatol.* **2001**, *144*, 781–787. (b) Luger, T.; Van Leent, E. J. M.; Graeber, M.; Hedgecock, S.; Thurston, M.; Kandra, A.; Berth-Jones, J.; Bjerke, J.; Christophers, E.; Knop, J.; Knulst, A. C.; Morren, M.; Morris, A.; Reitamo, S.; Roed-Petersen, J.; Schoepf, E.; Thestrup-Pedersen, K.; Van der Valk, P. G. M.; Bos, J. D. *Br. J. Dermatol.* **2001**, *144*, 788–794. (c) Rappersberger, K.; Komar, M.; Eberlin, M. E.; Scott, G.; Büche, M.; Burtin, P.; Wolff, K. *J. Invest. Dermatol.* **2000**, *110*, 114.
3. (a) Van Duyne, G. D.; Standaert, R. F.; Karplus, P. A.; Schreiber, S. L.; Clardy, J. *Science* **1991**, *252*, 839–842. (b) Lepre, C. A.; Thomson, J. A.; Moore, J. M. *FEBS Lett.* **1992**, *302*(1), 89–96. (c) Van Duyne, G. D.; Standaert, R. F.; Karplus, P. A.; Schreiber, S. L.; Clardy, J. *J. Mol. Biol.* **1993**, *229*(1), 105–124. (d) Griffith, J. P.; Kim, J. L.; Kim, E. E.; Sintchak, M. D.; Thomson, J. A.; Fitzgibbon, M. J.; Fleming, M. A.; Caron, P. R.; Hsiao, K.; Navia, M. A. *Cell* **1995**, *82*(3), 507–522. (e) Kissinger, C. R.; Parge, H. E.; Knighton, D. R.; Lewis, C. T.; Pelletier, L. A.; Tempczyk, A.; Kalish, V. J.; Tucker, K. D.; Showalter, R. E.; Moomaw, E. W. *Nature* **1995**, *378*(6557), 641–644. (f) Liu, J.; Farmer, J. D., Jr.; Lane, W. S.; Friedman, J.; Weissman, I.; Schreiber, S. L. *Cell* **1991**, *66*(4), 807–815.
4. Karuso, P.; Kessler, H.; Mierke, D. F. *J. Am. Chem. Soc.* **1990**, *112*(25), 9434–9436.
5. (a) Namiki, Y.; Kihara, N.; Koda, S.; Hane, K.; Yasuda, T. *J. Antibiot.* **1993**, *46*(7), 1149–1155. (b) Gailliot, P.; Natishan, T. K.; Ballard, J. M.; Reamer, R. A.; Kuczynski, D.; McManemin, G. J.; Egan, R. S.; Buckland, B. C. *J. Antibiot.* **1994**, *47*(7), 806–811. (c) Baumann, K.; Oberhauser, B.; Grassberger, M. A.; Haidl, G.; Schulz, G. *Tetrahedron Lett.* **1995**, *36*(13), 2231–2234.
6. For the synthesis of a set of furano-ascomycins see: Baumann, K.; Oberhauser, B.; Strnadt, G.; Knapp, H.; Schulz, G.; Grassberger, M. A. *Synlett* **1999**(Spec.), 877–880.
7. (a) Fisher, M. J.; Chow, K.; Villalobos, A.; Danishefsky, S. J. *J. Org. Chem.* **1991**, *56*(8), 2900–2907. (b) Coleman, R. S.; Danishefsky, S. J. *Heterocycles* **1989**, *28*(1), 157–161. (c) Goulet, M. T.; Mills, S. G.; Parsons, W. H.; Rupprecht, K. M.; Wyvratt, M. J. *Rec. Prog. Chem. Synth. Antibiot. Relat. Microb. Prod.* **1993**, 141–212. (d) Luengo, J. I.; Rozamus, L. W.; Holt, D. A. *Tetrahedron Lett.* **1993**, *34*(29), 4599–4602.
8. (a) Koch, G.; Jeck, R.; Hartmann, O.; Kuesters, E. *Org. Process Res. Dev.* **2001**, *5*(3), 211–215. (b) Baumann, K. Eur. Pat. Appl. EP 569337, 52 pp, 1993. (c) Askin, D.; Reamer, R. A.; Joe, D.; Volante, R. P.; Shinkai, I. *Tetrahedron Lett.* **1989**, *30*(45), 6121–6124. (d) Askin, D.; Jones, T. K.; Reamer, R. A.; Volante, R. P.; Shinkai, I. Eur. Pat. Appl. EP 364031, 16pp, 1990.
9. (a) Emmer, G.; Weber-Roth, S. *Tetrahedron* **1992**, *48*(28), 5861–5874. (b) Nussbaumer, P.; Grassberger, M. A.; Schulz, G. *Tetrahedron Lett.* **1992**, *33*(27), 3845–3846. (c) Baumann, K.; Knapp, H.; Strnadt, G.; Schulz, G.; Grassberger, M. A. *Tetrahedron Lett.* **1999**, *40*(44), 7761–7764.
10. (a) Högenauer, K. Diploma work, Department of Organic Chemistry, Karl Franzens University, Graz, Ausria, 1997. (b) Edmunds, A. J. F.; Baumann, K.; Grassberger, M.; Schulz, G. *Tetrahedron Lett.* **1991**, *32*(48), 7039–7042.
11. Bulusu, M. A. R. C.; Haidl, E.; Schulz, G.; Waldstatten, P.; Grassberger, M. *Indian J. Chem., Sect. B: Org. Chem. Incl. Med. Chem.* **1999**, *38B*(10), 1159–1164.
12. Zimmer, R.; Grassberger, M. A.; Baumann, K.; Schulz, G.; Haidl, E. *Tetrahedron* **1994**, *50*(48), 13655–13670.
13. Krimen, L. I. *Org. Synth.* **1970**, *50*, 1–3.
14. To our knowledge, the preparation of (chloromethylene)dimethylammonium chloride using diphosgene is not yet described in the literature. For the preparation of this compound using phosgene, see: Zemlicka, J.; Owens, J. *Nucl. Acid Chem.* **1978**, *2*, 989–992.
15. Belzecki, C.; Rogalska, E. *J. Chem. Soc., Chem. Commun.* **1981**, *2*, 57–58.
16. For the chemistry of phosgeneiminium salts see: (a) Viehe, H. G.; Le Clef, B.; Elgavi, A. *Angew. Chem.* **1977**, *89*(3), 189. (b) Gorissen, J.; Viehe, H. G. *Bull. Soc. Chim. Belg.* **1978**, *87*(5), 391–401. (c) Viehe, H. G.; Buijle, R.; Fuks, R.; Merenyi, R.; Oth, J. F. M. *Angew. Chem. Int. Ed.* **1967**, *6*(1), 77–78, and references cited therein.
17. Baumann, K.; Högenauer, K.; Schulz, G.; Steck, A. *Magn. Reson. Chem.* **2002**, *40*, 443–448.
18. Croasmun, W. R.; Carlson, R. M. K. *Two-Dimensional NMR Spectroscopy*. VCH: New York, 1994.
19. Hwang, T.-L.; Shaka, A. J. *J. Am. Chem. Soc.* **1992**, *114*, 3157–3159.
20. Neuhaus, D.; Williamson, M. P. *The Nuclear Overhauser Effect in Structural and Conformational Analysis*; Wiley-VCH: New York, 2000.
21. Damle, S. B. *Chem. Engng News* **1993**, *71*(6), 4.