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On the reactivity of ascomycin at the binding domain. Part 2: Hydroxide mediated rearrangement reactions

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Abstract—The natural product ascomycin represents a highly functionalised 23-membered macrocycle with a polyketide backbone. 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, highly stereoselective, hydroxide mediated rearrangement reactions at the binding domain are reported.

1. Introduction

FK 506 **1** is a 23-membered macrolactam isolated from the fermentation broth of *Streptomyces tsukubaensis 9993*.[7–9] Interestingly, ascomycin **2**, a compound which had been isolated earlier as a consequence of its antifungal activities, and whose structure had originally not been elucidated, was later shown to be a close structural analogue of FK 506 (ethyl in position 21 instead of allyl).[10–13] Pimecrolimus (Elidel®, SDZ ASM 981, **3**), derived from ascomycin and featuring a more lipophilic cyclohexyl-part, has been shown to possess a high therapeutic potential for the treatment of

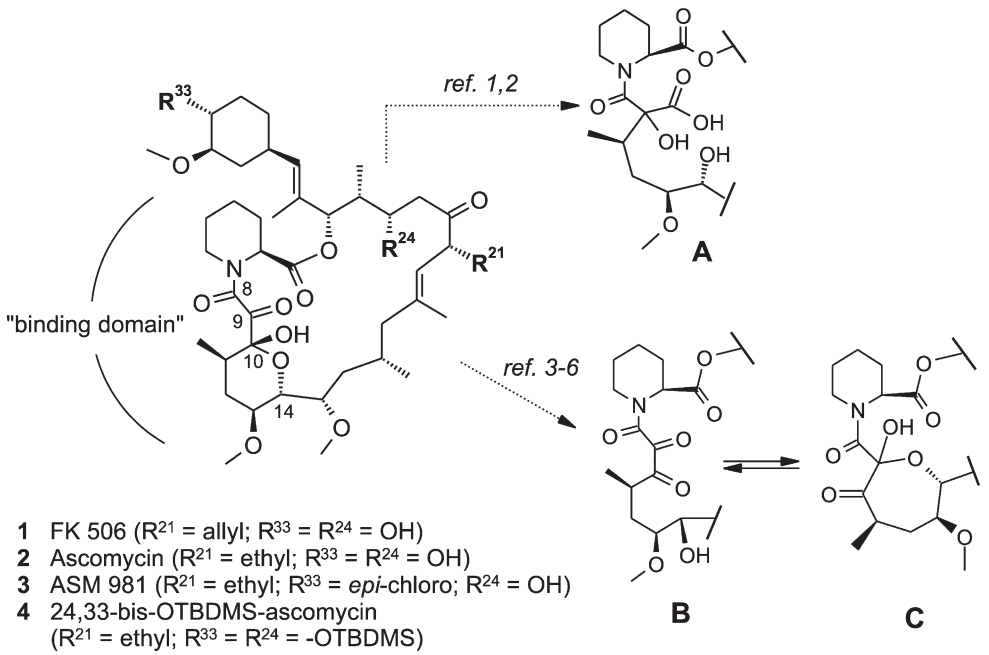

Figure 1.

Keywords: Ascomycin; Ring contraction; Rearrangement; Binding domain; Tricarbonyl.
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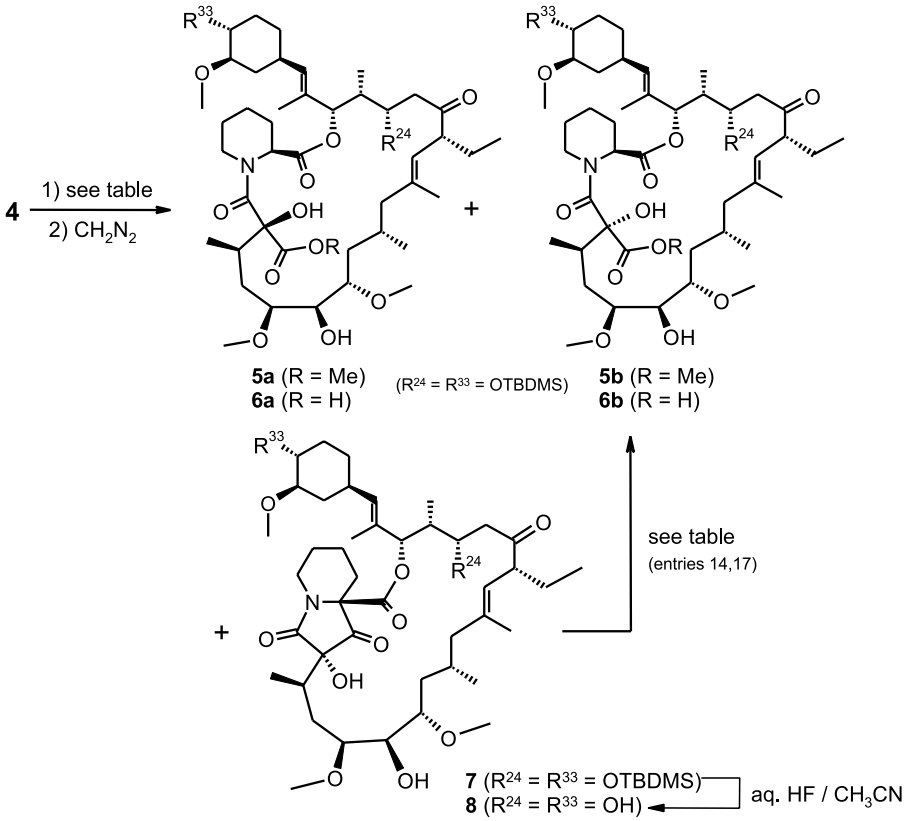
inflammatory skin diseases. Pimecrolimus cream 1%, which combines a skin selective, anti-inflammatory activity with a low risk for systemic side effects, has successfully been introduced into the market for the treatment of atopic dermatitis and contact dermatitis.[14–16] In the left hand part ('binding domain', Fig. 1),[17–21] ascomycin features the unusual pattern of three adjacent carbonyl groups (C8–C10), whereby one carbonyl group (C10) is involved in hemiketal formation. The inherently labile hemiketal structure at C10 potentially allows the formation of numerous alternative isomers via liberation of the tricarbonyl portion, tautomerisation, enolisation and non-specific



Scheme 1.

Table 1.

Entry[a]	Educt	Base	Solvent	Time	**5a/5b**[b]	Products (isol. %)[c]
1	**4**	5 equiv. LiOH	THF/H_2O=3:1	12 min	82:18	**5a** (72), **5b** (11)
2	**4**	5 equiv. KOH	THF/H_2O=3:1	10 min	83:17	**5a** (70), **5b** (12)
3	**4**	5 equiv. NaOH	THF/H_2O=3:1	10 min	86:14	**5a** (69), **5b** (9)
4	**4**	10 equiv. $Ca(OH)_2$	THF/H_2O=3:1	30 min	>98:2	**5a** (91) or **6a** (95%)[d]
5	**4**	10 equiv. $Ca(OH)_2$	THF/H_2O=7:1	60 min	>98:2	**5a** (91)
6	**4**	10 equiv. $Ca(OH)_2$	THF/H_2O=20:1	3 h	>98:2	**5a** (86)
7	**4**	10 equiv. $Ca(OH)_2$	THF/H_2O=80:1	30 h	>98:2	**5a** (74)
8	**4**	1.2 equiv. LiOH	DMSO	20 min	<2:98	**5b** (75) or **6b** (78)[d]
9	**4**	1.2 equiv. KOH	DMSO	30 min	<2:98	**5b** (78)
10	**4**	1.2 equiv. KOH	THF/18-crown-6	40 min	<2:98	**5b** (82), **7**(trace)
11	**4**	1.2 equiv. KOH	THF/18-crown-6	30 min	n.d.	**5b** (75), **7** (6)
12	**4**	1.2 equiv. KOH	THF/18-crown-6	10 min	n.d.	**5b** (52), **7** (24)
13	**4**	1.2 equiv. KOH	THF/18-crown-6	1.5 min	n.d.	**7** (74),[d] **4** (11), **6b** (trace)
14	**7**	1.2 equiv. KOH	THF/18-crown-6	40 min	0:100	**5b** (85) or **6b** (78)[d]
15	**4**	5 equiv. NaOD	THF/D_2O=3:1	12 min	81:19	**5a** (71),[e] **5b** (9)[f]
16	**4**	10 equiv. $Ca(OH)_2$	THF/D_2O=3:1	30 min	>98:2	**5a** (93)[e]
17	**7**	5 equiv. NaOD	THF/D_2O=3:1	40 min	0:100	**5b** (71%)[f]
18	**6a**	5 equiv. NaOD	THF/D_2O=3:1	15 min	100:0	**5a** (91)[e]
19	**6b**	5 equiv. NaOD	THF/D_2O=3:1	15 min	0:100	**5b** (89)[e]

[a] All reactions were carried out at room temperature.
[b] Determined by ^1H NMR of the crude reaction mixture after esterification with diazomethane.
[c] Isolated yields (not optimised) after esterification with diazomethane.
[d] Acidic work up without esterification.
[e] No deuterium incorporations detected.
[f] >95% deuterium incorporation at C2.

re-hemiketalsation events (**B**, **C** in Fig. 1).[3–6] The resulting structural diversity at the binding domain translates into a tremendous reactivity towards manifold reaction conditions.[22–27] As a consequence, selective reactions directed to other parts of the macrocycle are not easily achieved without provoking concomitant transformations within this peculiar unit. Thus, several attempts to cleave the endocyclic ester linkage led to a facile ring contraction via rearrangement of the C9/C10 (**A** in Fig. 1) region instead of yielding the desired 1,26-*seco*-derivative. For this reaction, a benzilic acid type rearrangement process has been proposed.[1,2] As part of our research program on the ascomycins, aiming at the generation of more stable and structurally less flexible binding domain mimics, we decided to investigate this reaction in more detail, in order to assess its scope and limitations and to get more insight into the reaction pathway(s) involved.

2. Results and discussion

2.1. Hydroxide mediated rearrangement reactions

In order to prevent cross-reactivity at the potentially base sensitive β-hydroxyketone portion of ascomycin (C22–C24),[28] 24,33-bis-OTBDMS-protected ascomycin **4** was chosen as the starting material for our investigations (Scheme 1, Table 1). First, we treated **4** with lithium hydroxide in THF–water solution (Table 1, entry 1). After an acidic work up, followed by esterification of the crude reaction mixture with diazomethane, two diastereoisomers, the ring contracted 10(*S*)-α-hydroxy acid methyl ester **5a** together with minor amounts of the not yet known 10(*R*)-epimer **5b** could be isolated (for stereo chemical determinations see Section 2.3). Replacement of lithium hydroxide by potassium or sodium hydroxide led to similar results (entries 2 and 3). Notably, replacement of lithium hydroxide by calcium hydroxide resulted in an almost complete diastereoselectivity of the rearrangement reaction. Thus, the reaction of **4** with 10 equiv. calcium hydroxide in THF/water (3:1) solution, afforded after esterification almost exclusively the 10(*S*)-isomer **5a** in high yield (entry 4). Changing the amount of water in the calcium hydroxide mediated rearrangement reaction had a strong influence on the reaction times required but no effect on stereo selectivity (entries 5–7). Remarkably, a complete change in stereo selectivity is observed under aprotic conditions. Thus, the treatment of **4** with powdered lithium or potassium hydroxide in dimethylsulfoxide (DMSO) solution or with potassium hydroxide in anhydrous tetrahydrofuran in the presence of 18-crown-6, yielded, dependent on the work up procedure applied, the diastereoisomerically pure 10(*R*)-hydroxy acid **6b** or the corresponding methyl ester **5b** in reasonable yields (entries 8–10). Careful monitoring of the latter reaction revealed that the 10(*R*)-hydroxy acid **6b** is formed via the unexpected novel intermediate **7**. Thus, short treatment of **4** with KOH/18-crown-6 led preferentially to **7** together with minor amounts of **5b**, whereas prolonged reaction times resulted in an increase in **6b** at the expense of **7** (entries 10–13). As could be anticipated from these results, treatment of pure **7** under identical reaction conditions, gave diastereoisomerically pure **6b** or (after esterification) **5b** (entry 14). In order to answer the question whether or not a similar intermediate might also be involved in the formation of the 10(*S*)-hydroxy-acid **6a**, 24,33-bis-OTBDMS-ascomycin **4** was treated with calcium deuteroxide or sodium deuteroxide in THF–D_2O solution. Analysis of the NMR spectra of the products **5a**,**b** showed no deuterium–hydrogen exchange in **5a** but a quantitative deuterium incorporation at C2 of **5b** (entries 15 and 16). Complete deuteration at C2 of **5b** could be confirmed by the absence of the H2-signal, a highfield shift and signal broadening of $H6_{eq}$ and $H3_{eq}$ in the ^1H NMR and a downfield shift of the C2-resonance by 0.2 ppm. As expected, analogous treatment of **7** gave **5b** deuterated almost quantitatively at C2 (entry 17). Treatment of isolated **6a** and **6b** with NaOD in THF–D_2O resulted in no deuteration at any position, thus giving evidence that the deuterium incorporation at C2 of **6b** when starting from **4**, occurs not after the formation of **6b**. Furthermore, as no

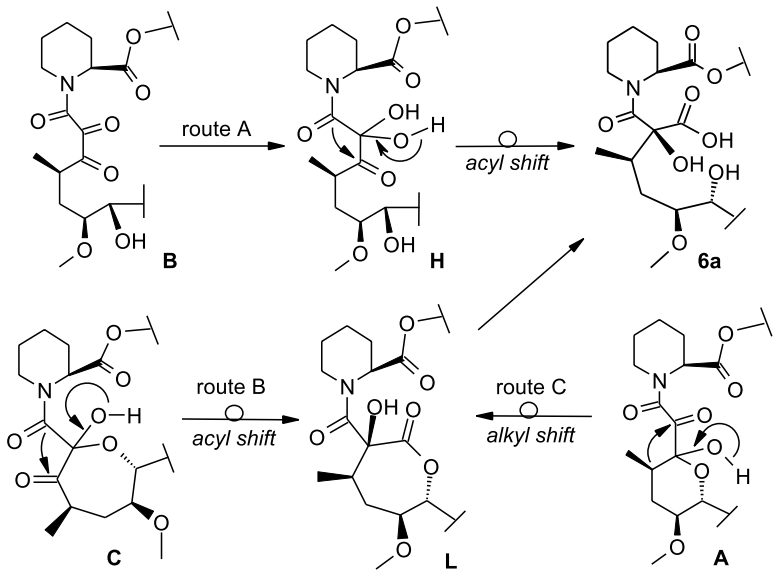
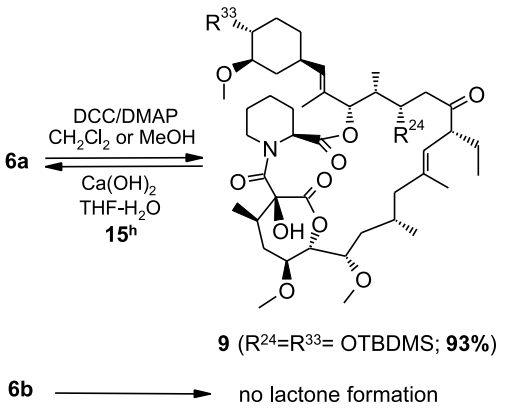


Figure 2.

hydrogen–deuterium exchange is observed when **4** is converted to **6a**, using calcium hydroxide in THF–D_2O solution (entry 16), this confirms that the configurations at all other potentially base labile positions (i.e., C2, C21 and C11) of **5a** and **6a** are not affected and thus are identical with the corresponding configurations in the starting material **4**. In summary, these experiments clearly demonstrate that involvement and deprotonation at C2 occurs in the formation of the 10(*R*)-hydroxy-acid **6b**, but can be excluded for the 10(*S*)-hydroxy-acid **6a**. The diastereoisomers **6a** and **6b** are thus formed via distinct, highly diastereoselective reaction pathways.

2.2. Discussion of reaction mechanisms

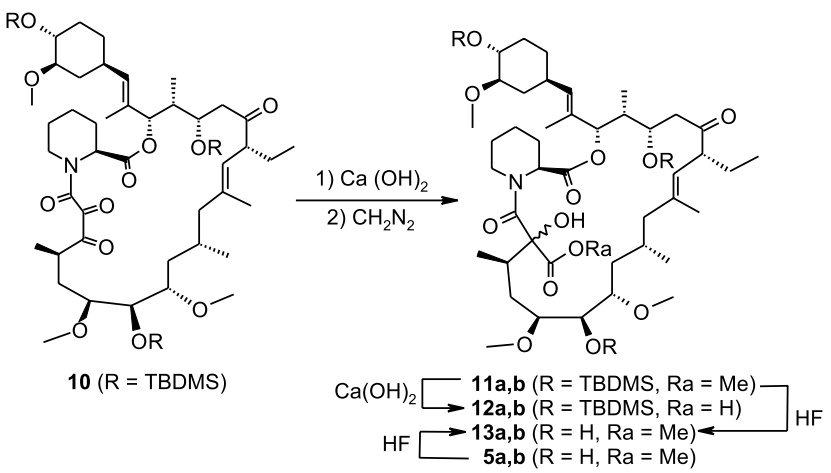
2.2.1. Formation of 6a. Taking into consideration the ketone/hemiketal equilibrium at the binding domain (compare Fig. 1), at least three different pathways for the formation **6a** (Fig. 2) can be formulated. Route A suggests an acyl migration as the key step of the rearrangement process, initiated by a nucleophilic attack of a hydroxide ion at the most reactive central carbonyl of the tricarbonyl form (**B→H→6a**, intermolecular nucleophile induced acyl shift). This benzilic acid type rearrangement reaction has already been proposed from the results of a study with 9-^{13}C-labelled material: the ^{13}C-label was mostly found in the carboxyl group which is in agreement with an acyl(C8)-migration.[1,2] However, the labelling study fits also to route B, which supposes essentially the same mechanism, but with participation of the 14-hydroxy group as internal nucleophile (**C→L→6a**, intramolecular nucleophile induced acyl shift), thus leading to a seven-membered lactone intermediate which might yield the final product on lactone hydrolysis. Such a lactone intermediate could also be formed if the hydroxyl ion acts rather as a base than as a nucleophile (route C; **A→L→6a**, base induced alkyl shift). Although a seven-membered lactone derivative (**L**) was not found in the rearrangement reactions, it cannot be excluded as intermediate because its formation may be much slower than its hydrolysis to the final product. In order to shed some light on this, we attempted to prepare such lactone derivatives, starting from the corresponding acids **6a** and **6b** (Scheme 2).



Scheme 2.

Lactonisation of **6a** was performed with *N*,*N*′-dicyclohexylcarbodiimide (DCC) in the presence of catalytic amounts of 4-dimethylaminopyridine (DMAP) in dichloromethane to provide **9** in excellent yield. Apparently, the carboxyl function and the 14-hydroxy group in **6a** are in a perfect orientation for lactonisation, since even in methanol only **9** instead of the expected methyl ester **5a** was obtained. In contrast, all attempts to convert the 10(*R*)-hydroxy acid **6b** to the corresponding lactone failed. Hydrolysis of **9** with calcium hydroxide (10 equiv.) in THF/water (3:1) resulted in the acid **6a** as expected, but the reaction time was markedly longer (15 h) than the conversion of **4** to **6a** under comparable reaction conditions (30 min; compare Table 1, entry 4). This clearly rules out **9** as an intermediate during the rearrangement process and supports the proposed intermolecular benzilic acid type rearrangement event (compare Fig. 2). Additional evidence for this suggestion was gained starting from the easily available 14,24,33-tris-OTBDMS-protected ascomycin derivative **10**,[29] in which the binding domain is fixed in the tricarbonyl form by blocking the 14-hydroxy group (Scheme 3).

Thus, treatment of the characteristically yellow coloured **10** with calcium hydroxide (10 equiv.) in THF/water (3:1) led almost instantaneously to the disappearance of the yellow colour and afforded in a fast reaction (<5 min) and with

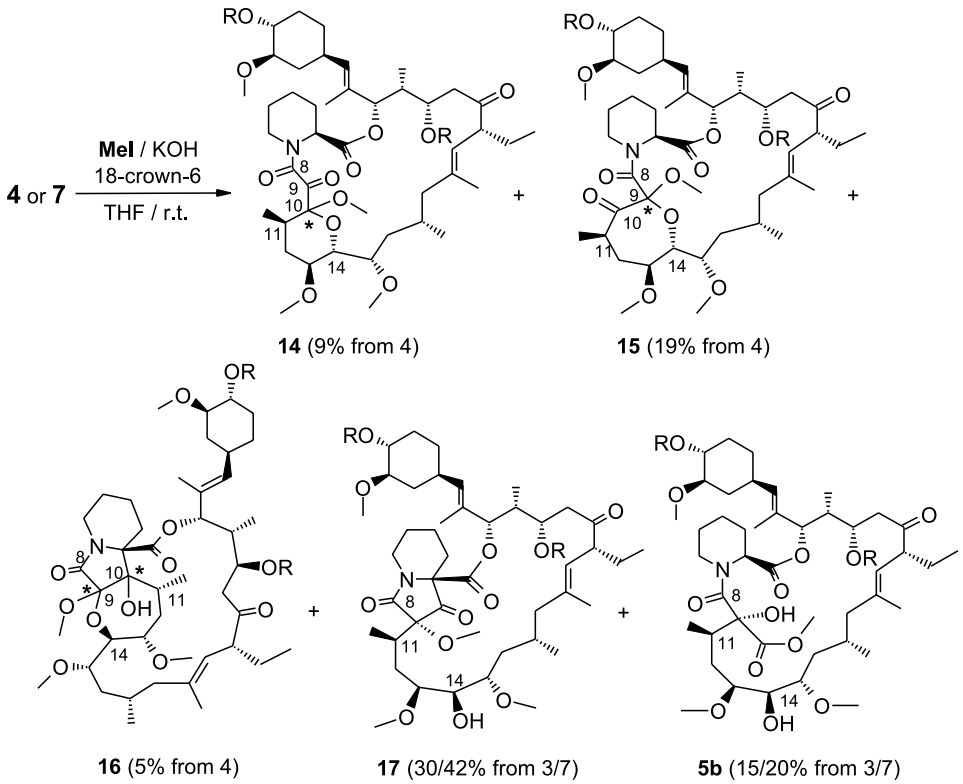


Scheme 3.

excellent diastereoselectivity after esterification with diazomethane (10(*S*)/10(*R*)=96:4) the 14,24,33-tris-OTBDMS-protected hydroxy acid methyl esters **11a** and **11b**. Saponification of the separated esters with excess calcium hydroxide in tetrahydrofuran–water solution allowed the isolation of the corresponding acids **12a** and **12b** as well. Desilylation of **11a** and **5a**, using aqueous hydrogen fluoride in acetonitrile solution furnished the same hydroxy acid methyl ester **13a**, thus corroborating that **5a**, **11a**, **12a** and **13a** differ only with respect to their protection pattern but exhibit the same stereochemistry at C10. The same relationship could be shown for the 10(*S*)-epimers **5b**, **11b**, **12b** and **13b**, respectively.

2.2.2. Formation of 6b and 7. Inspection of the structure of **7** clearly reveals that a rearrangement process and a cyclisation event are required for its formation. Therefore, two general pathways, a cyclisation prior to a rearrangement or vice versa should be considered. In order to gain some insight into the reaction pathway involved, we repeated the reaction leading to **7** and **6b** in the presence of excess methyl iodide as trapping reagent (Scheme 4).

In the event, 24,33-bis-OTBDMS-ascomycin **4** was added in one portion at room temperature to a well stirred suspension of powdered potassium hydroxide (1.5 equiv.), 18-crown-6 (0.5 equiv.) and methyl iodide (10 equiv.) in tetrahydrofuran. After an acidic work up a complex mixture was obtained which could be separated by column chromatography on silica gel to afford the O-methylated derivatives **14**-**17** and the 10(*R*)-hydroxy ester **5b**. Using **7** as a starting material and applying the same reaction conditions led in an overall yield of 62% to the compounds **17** and **5b** as well, thus emphasising once again the key role of **7** as intermediate and further confirming that **17** has the same configurations as **7** at all chiral positions. The compounds **14** and **15** represent trapped versions of the potential equilibrium products of **4** and thus their formation is explicable. Inspective is the formation of the O-methyl-derivative **16** since in this compound a cyclisation between C2 and C10 together with an intact (not rearranged) carbon chain (C8–C11) can easily be recognised. Interestingly, compound **15** is completely stable under the conditions of its formation. Thus, no further cyclisation to **16** is observed. Taking this into consideration, a reaction mechanism involving a cyclisation prior to a rearrangement can be proposed (Fig. 3). Thus, cyclisation between C2 and C10 may start from the O-deprotonated seven-membered hemiketal form **C** via a, most probably, intramolecular assisted deprotonation at C2 (no intramolecular assistance is possible in the O-methylated derivative **15**) followed by ring closure to give the intermediate **D**, which has the possibility to be in a hemiketal/ketone equilibrium with the unmasked α-ketoamide form **F**. Taking into consideration intermediate **F**, an α-ketol-type rearrangement easily explains the formation of **7** which in turn, upon a hydroxide mediated *retro*-ester condensation event, provides the hydroxy acid **6b**. The rearrangement process **F** to **7** is not unlikely, since thereby the destabilising electronic repulsion of two adjacent carbonyls (C8, C9) is removed. However, although the suggested reaction pathway is supported by the quenching experiment, other reaction



Scheme 4.

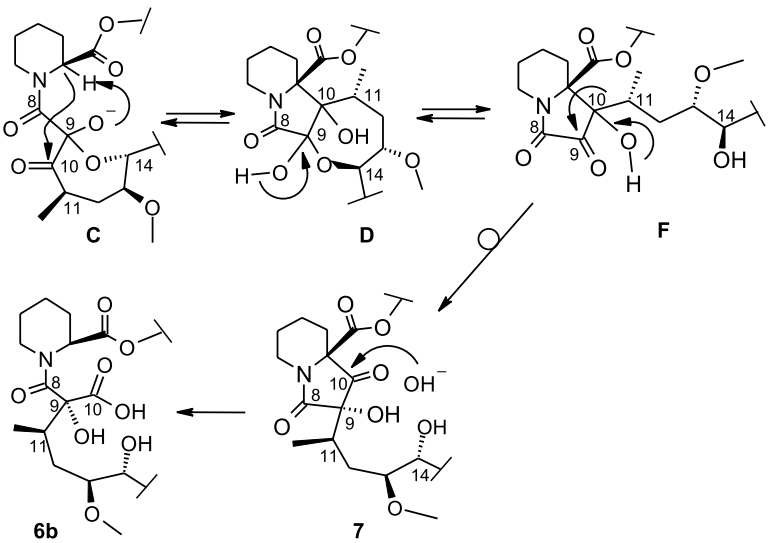
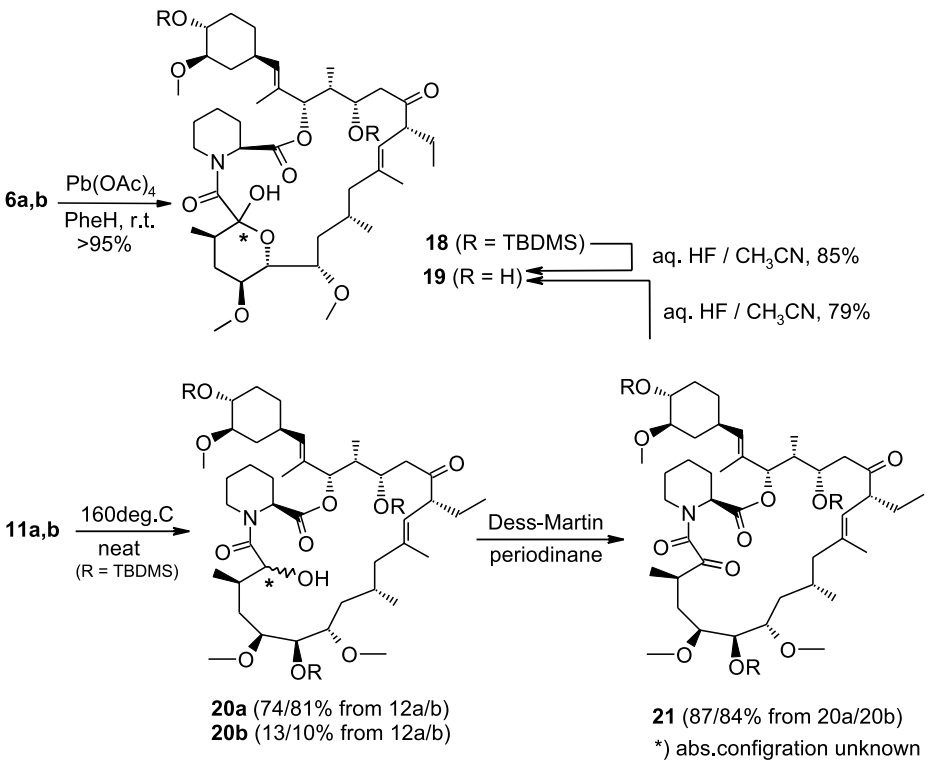


Figure 3.

pathways can be proposed as well. Further attempts to identify decisive experiments which allow the mechanism to be unambiguously defined are ongoing.

2.3. Stereochemical assignments

2.3.1. Stereochemical correlations via regioselective degradation. As already shown above, the ring contracted hydroxy acid derivatives **5a**, **6a**, **11a**, **12a** and **13a** differ only with respect to their substitution pattern and not in their stereochemical arrangement. The same applies to the series **5b**, **6b**, **11b**, **12b** and **13b**. From the mechanistic investigations and the trapping experiments it is also clear that the rearrangement product **7** and its O-methylated congener **17** relate to the 10(*R*) hydroxy acid **6b**. However, since the formation of the **6b** evidently involves position C2, **6a** and **6b** may not only differ at the newly created chiral center (C10), but also in the configuration at C2. In order to compare those isomers with respect to their relative configurations, it was necessary to remove or modify the chirality at C10 without affecting the remaining chiral positions. This could be achieved by an oxidative decarboxylation of the diastereoisomeric acids **6a** and **6b** or by a thermal decarboxylation of the 14,24,33-tri-s-OTBDMS-protected hydroxy acids **12a** and **12b** (Scheme 5).



Scheme 5.

Thus, reacting **6a** or **6b** with excess lead tetra acetate in benzene solution afforded, independent of the starting material applied, the common degradation product **18**, which upon desilylation provided the nor-9-carbonyl-ascomycin derivative **19** in high overall yield. It is interesting to note, that **18** exists in deuterochloroform solution exclusively in the 9(*R*)-hemiketal form (NOE from 9-OH to H14) and as a single conformer with *E*-configuration at the amide bond. In contrast, the deprotected congener **19** is in equilibrium with the free 9-keto form (hemiketal form/keto-form=5:1) whereby the hemiketal form adopts most probably exclusively the *E*-amide and the keto form the *Z*-amide orientation.[30] Alternatively, heating up **12a** or **12b** in the absence of solvent to 160 °C for 10 min accomplished a clean decarboxylation to give mixtures of the α-hydroxy amides **20a** and **20b**, which differ only in their configuration at C10. As expected, oxidative conversion of both, applying the Dess–Martin protocol,[31] gave the 14,24,33-tris-OTBDMS-protected α-keto amide **21**, which could be deprotected to **18** as well, thus once again corroborating that **6a** and **6b** (or **12a** and **12b**) differ only in their configuration at C10. Together with the deuteration experiments, listed in Table 1, this also substantiate, that all other chiral positions of **6a**,**b** and its differently protected congeners are identical as compared to those in natural ascomycin.

2.3.2. X-ray analysis of the compounds 8 and 13a. After having carefully established the relative stereochemical relationships, crystalline material for X-ray analysis was required in order to determine absolute configurations. Gratifyingly, suitable crystals could be obtained from the compounds **8** (**8**·3H_2O from methanol–water) and **13a** (**13a**·THF from tetrahydrofuran). ORTEP-plots of the structures (atomic displacement ellipsoids drawn at the 50% probability level, hydrogen atoms drawn as spheres of arbitrary radius) are depicted in Figures 4 and 5.[32]

The rearrangement product **13a** (and consequently all of its

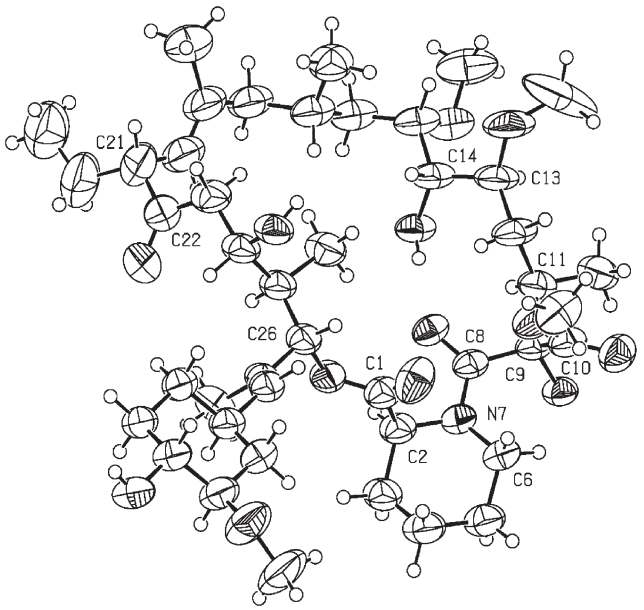


Figure 4.

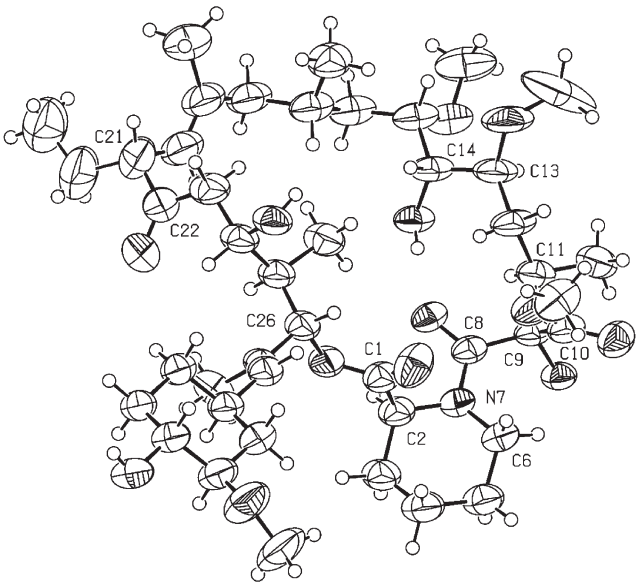


Figure 5.

only differently protected congeners: i.e., **5a**, **6a**, **11a** and **12a**) exhibits the *S*-configuration at the quaternary carbon C10 and the natural *S*-stereochemistry at C2. Consequently, and as deduced from the selective degradations, the series **5b**, **6b**, **11b** and **12b** should exhibit the same stereochemistry at C2 (*S*) but the opposite configuration (*R*) at C10. The latter is in agreement with the X-ray structure of **8** (Fig. 5), which exhibits the 2(*R*), 10(*S*)-configuration. With the aid of the X-ray-structure of **8**, the absolute configurations of congeners **7** and **17** at C2 and C10, together with the 2-(*R*)-configuration at C2 of the cyclised but not yet rearranged compound **16** could be assigned unambiguously as well.

3. Summary

Starting from 24,33-bis-OTBDMS-ascomycin **4** carefully chosen reaction conditions allow either the diastereoselective preparation of the 10(*S*)-α-hydroxy acid **6a** or its 10(*R*)-congener **6b** in reasonable yields. Mechanistic investigations confirmed, that **6a** is formed via an benzilic acid type rearrangement process as proposed earlier,[1,2] whereas, trapping experiments and the isolation of the novel rearrangement product **7** clearly suggest, that the 10(*R*)-isomer **6b**, which has been isolated for the first time, is formed through a cascade of reaction steps including tautomerisation, cyclisation and a acyloin-rearrangement followed by a *retro*-ester condensation. The relative and absolute stereochemistry at newly formed chiral centers of most of the compounds disclosed herein could be determined unambiguously via regioselective degradation reactions and X-ray analysis. The structures of all compounds are fully supported by one- and two-dimensional NMR data. Since the tricarbonyl portion of ascomycin is a source of lability, the findings described herein may be of use for researchers in the field, who are attempting to replace the tricarbonyl by more stable binding domain mimics.

4. Experimental

4.1. 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. Stereochemical information was extracted from two-dimensional T-ROESY (transverse rotating-frame Overhauser effect spectroscopy) or selective one-dimensional ROESY[20] experiments. Routine mass spectroscopy (ESI, electrospray ionisation) 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 experiment were applied, with 25 and 50 V cone voltages, respectively. The probe temperature was 523 K. High-resolution mass spectra (HRMS) were measured on a Finnigan MAT900 S mass spectrometer or on a 9.4T Bruker APEX III Fourier Transform mass spectrometer in positive-ESI mode. Unit cell determination and intensity data collection for compound **8** were performed on an Enraf Nonius CAD4 with graphite monochromatised Cu(K_α) radiation. Non-hydrogen atoms were refined with anisotropic displacement parameters, hydrogen atoms were calculated in idealised positions and refined using a riding model. Unit cell determination and intensity data collection for **13b** were performed on a Bruker AXS SMART 6000 CCD, with Cu(K_α) radiation from rotating anode generator with Osmic multilayer mirrors. A semi-empirical absorption correction was applied, based on the intensities of symmetry-related reflections measured at different angular settings. The structures were solved by direct methods and refined by full-matrix least-squares on F^2. 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 approx. 3–5 bar. Solvents and reagents (reagent grade) were used as purchased. Samples for micro-elementary analysis were subjected to size exclusion chromatography (Sephadex® LH20, in order to get rid of minor low molecular weight impurities which might originate from the solvents used for chromatography) and lyophilised from dioxane or benzene at high vacuum.

4.2. Preparation of 5a, 5b, 6a, 6b and 7 and 2-deuterio-5b according to Table 1

Compounds **5a**,**b** (*entry* 1). To a solution of 5.1 g 24,33-bis-OTBDMS-ascomycin **4** (5 mmol) in 300 ml tetrahydrofuran and 35 ml water were added 25 ml (25 mmol, 5 equiv.) of an aq. 1 N-lithium hydroxide solution and the resultant slightly turbid mixture was magnetically stirred for 12 min at room temperature. For work up the mixture was partitioned between ethyl acetate (600 ml) and 1 N hydrochloric acid (150 ml). The aqueous layer was separated and washed twice with ethyl acetate (100 ml). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate and concentrated at reduced pressure to a volume of about 100 ml. The resultant solution was titrated with an approx. 1 M ethereal diazomethane solution until the yellow colour did not disappear. Excess diazomethane was removed in a slight stream of nitrogen and the remaining solution was evaporated to dryness at the rotary evaporator. Flash column chromatography (silica gel, toluene/acetonitrile=5:1) provided **5b** as the first and **5a** as the second fraction. The product fractions were evaporated under reduced pressure and dried for 15 h under high vacuum to give 3.71 g (72%) **5a** and 0.57 g (11%) **5b** as amorphous powders.

Compounds **5a**,**b** (*entries* 2 and 3). Starting from 5.1 g 24,33-bis-OTBDMS-ascomycin **4**, but using 1 N aqueous potassium hydroxide (or sodium hydroxide) solution, the reaction, work up, esterification and purification was performed as described above to give the title compounds as colourless foams in yields as indicated in Table 1.

Compound **5a** *or* **6a** (*entry* 4). To a solution of 10.2 g 24,33-bis-OTBDMS-ascomycin **4** (10 mmol) in 300 ml tetrahydrofuran and 50 ml water were added 7.41 g (100 mmol, 10 equiv.) calcium hydroxide in one portion and the resultant suspension was stirred at room temperature until TLC indicated the complete consumption of the starting material (30 min). The mixture was partitioned between ethyl acetate (1200 ml) and 1 N hydrochloric acid (400 ml). The aqueous layer was separated and extracted twice with ethyl acetate (200 ml). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate and evaporated to dryness at reduced pressure to give the crude acid **6a**.

Isolation as hydroxy acid **6a**. Half of the above crude product was subjected to a short flash column chromatography (silica gel, dichloromethane/methanol=10:1). The product containing fraction was evaporated at the rotary evaporator, re-dissolved in 200 ml ethyl acetate, washed with 20 ml 1 N-hydrochloric acid (to remove salt forming impurities originating from the silica gel) and brine, dried over anhydrous sodium sulfate and filtered through a Whatman® (A) glass microfibre filter. The filtrate was evaporated at reduced pressure and dried on high vacuum to give 4.93 g (95%) **6a** as an amorphous powder.

Isolation as hydroxy acid, *methyl ester* **5a**. The remaining crude acid **6a** was dissolved in 100 ml ethyl acetate, titrated with an approx. 1 M ethereal diazomethane solution until the yellow colour remained. Excess diazomethane was removed in a slight stream of nitrogen and the remaining solution was evaporated to dryness at the rotary evaporator. Flash column chromatography (silica gel, toluene/ethyl

acetate=2:1) provided 4.79 g (91%) **5b** as amorphous powder.

Compound **5a** (*entries* 5–7). To solutions of in each case 2.0 g (1.96 mmol) **4** in mixtures of 70 ml tetrahydrofuran/water=7:1, 20:1 and 80:1 were added 1.45 g (10 equiv., 19.6 mmol) calcium hydroxide and the reactions were stirred at room temperature until TLC indicated complete consumption of the starting material (1, 3 and 30 h). Work up, esterification and purification as described above provided 1.88 (91%), 1.77 (86%) and 1.53 g (74%) **5a** as colorless foams.

Compound **5b** *and* **6b** (*entry* 8). To a solution of 3.0 g (2.94 mmol) 24,33-bis-OTBDMS-ascomycin **4** in 30 ml dimethyl sulfoxide (DMSO) were added 85 mg (1.2 equiv.; 3.53 mmol) powdered lithium hydroxide, monohydrate in one portion and the resultant suspension was vigorously stirred at room temperature (30 min). The reaction mixture was partitioned between ethyl acetate (200 ml) and 1 N-hydrochloric acid (50 ml). The organic layer was five times washed with water, dried over anhydrous sodium sulfate and evaporated at reduced pressure. The residual oil was subjected to size exclusion chromatography (Sephadex® LH20, ethyl acetate) in order to remove DMSO completely and the relevant fraction was evaporated to dryness. The crude product was divided into two equal parts and further manipulated as described above (entry 4) to give 1.19 g (78%) **6b** and 1.16 g (75%) **5b** as amorphous powders.

Compound **5b** (*entry* 9). Starting from 1.0 g (0.98 mmol) 24,33-bis-OTBDMS-ascomycin **4** in 10 ml DMSO and 66 mg (1.2 equiv.; 1.18 mmol) powdered potassium hydroxide, the reaction was performed and worked up as described above (30 min reaction time) to give after esterification and purification 0.81 g (78%) **5b** as amorphous powder.

Compounds **5b** *and* **7** (*entries* 11–14). To solutions of in each case 2.0 g (1.96 mmol) 24,33-bis-OTBDMS-ascomycin **4** and 100 mg (0.38 mmol, 20 mol%) 18-crown-6 in 100 ml tetrahydrofuran were added 132 mg (1.2 equiv.; 2.36 mmol) powdered potassium hydroxide and the suspensions were allowed to stir at room temperature for 1.5/10/30 and 40 min, respectively. For work up, the mixtures were partitioned between ethyl acetate (300 ml) and 1 N-hydrochloric acid (60 ml). The organic layers were washed with brine, dried over anhydrous sodium sulfate and evaporated at reduced pressure to give the crude products as slightly brownish foams.

The crude products of the 40/30 and 10 min runs were redissolved in 40 ml dichloromethane and titrated with an approx. 1 M ethereal solution of diazomethane until the characteristic yellow colour remained. The resultant solutions were evaporated and subjected to flash column chromatography (silica gel, toluene/ethyl acetate=2:1 to 1:1) to give the title compounds **5b** and **7** as amorphous powders. 40 min run (entry 10): 1.69 g (82%) **5b**; 30 min run (entry 11): 1.55 g (75%) **5b** and 0.12 g (6%) **7**; 10 min run (entry 12): 1.07 g (52%) **5b** and 0.48 g (24%) **7**. The crude product of the 1.5 min run (entry 13) was directly subjected to flash column chromatography (silica gel, toluene/ethyl acetate=1:1) to give 0.22 g (11%) of recovered starting material **4** and 1.48 g (74%) **7** as amorphous powders.

Compound **5b** *from* **7** (*entry* 14). To a solution of 0.5 g (0.49 mmol) **7** and 25 mg (0.098 mmol, 20 mol%) 18-crown-6 in 25 ml tetrahydrofuran were added 33 mg (1.2 equiv.; 0.59 mmol) powdered potassium hydroxide and the suspensions were allowed to stir at room temperature for 40 min. Work up, esterification and purification as described above provided 0.44 g (85%) **5b** as amorphous powder.

Compound **5a** and 2-deuterio-**5b** (*entry* 15). To a solution of 0.51 g 24,33-bis-OTBDMS-ascomycin **4** (0.5 mmol) in 30 ml tetrahydrofuran and 3.5 ml deuterium oxide were added 2.5 ml (2.5 mmol, 5 equiv.) of an aq. 1 N-sodium deuterium oxide solution in deuterium oxide and the resultant mixture was stirred for 12 min at room temperature. Work up, esterification and purification as described for entry 1 provided 0.38 g (71%) **5a** and 0.047 g (9%) 2-deuterio-**5b**.

Compound **5a** (*entry* 16). 1.02 g 24,33-bis-OTBDMS-ascomycin **4** (1 mmol) were added in one portion to a pre-stirred (10 min) suspension of 0.74 g (10 mmol, 10 equiv.) calcium hydroxide in 30 ml tetrahydrofuran and 5 ml deuterium oxide. After 30 min, an acidic work up, followed by esterification and purification as described for entry 4 provided 0.98 g (93%) **5a**. No deuteration could be seen by MS and ^{1}H NMR.

Compound 2-deuterio-**5b** *from* **7** (*entry* 17). To a solution of 0.51 g **7** (0.5 mmol) in 30 ml tetrahydrofuran and 3.5 ml deuterium oxide were added 2.5 ml (2.5 mmol, 5 equiv.) of a 1 N-sodium deuteride solution in deuterium oxide and the resultant mixture was magnetically stirred for 40 min at room temperature. Work up, esterification and purification as described for entry 1 provided 0.37 g (71%) 2-deuterio-**5b** as amorphous powder.

Compound **5a** *and* **5b** *from* **6a** *and* **6b** (*entries* 18 *and* 19). To a solution of 0.52 g (0.5 mmol) **6a** or **6b** in 30 ml tetrahydrofuran and 3.5 ml deuterium oxide were added 2.5 ml (2.5 mmol, 5 equiv.) of a 1 N-sodium deuteride solution in deuterium oxide and the resultant mixtures was stirred for 15 min at room temperature. Work up, esterification and purification as described for above provided 0.48 g (91%) **5a** and 0.47 g (89%) **5b** as amorphous powders.

4.2.1. Compound 5a. CHN ($C_{56}H_{101}NO_{13}Si_2$) calcd: 63.90/9.67/1.33, found: 63.64/9.51/1.24. HRMS (M+Na; calcd/found): 1074.6709/1074.6713. ^{13}C NMR ($CDCl_3$, *Z*/*E*=3:2), δ (*Z*/*E*-isomer, ppm): 168.38/168.93 (C1); 53.99/56.63 (C2); 26.32/28.20 (C3); 20.95/20.60 (C4); 24.93/25.01 (C5); 43.58/42.00 (C6); 169.47/168.93 (C8); 81.61/81.43 (C9); 172.16/172.16 (C10); 35.08/36.02 (C11); 36.10/36.08 (C12); 79.23/82.19 (C13); 74.39/73.48 (C14); 77.69/77.51 (C15); 41.0br/n.d. (C16); 28.80/26.55 (C17); 47.22/47.43 (C18); 139.17/139.72 (C19); 122.92/123.89 (C20); 55.03/56.25 (C21); 209.29/209.24 (C22); 45.40/48.40 (C23); 67.70/68.28 (C24); 38.98/39.47 (C25); 83.95/

81.25 (C26); 131.19/131.57 (C27); 12.25/11.30 (C28); 136.46/135.72 (C29); 35.08/35.11 (C30); 36.37/36.16 (C31); 84.19/84.10 (C32); 75.18/75.11 (C33); 33.93/33.87 (C34); 30.81/30.67 (C35); 23.25/23.53 (C36); 11.63/11.57 (C37); 52.86/52.75 (10-OMe); 56.76/56.63 (13-OMe); 57.42/59.07 (15-OMe); 57.89/57.83 (32-OMe); 14.33/13.43 (11-Me); 20.05/20.61 (17-Me); 18.50/16.60 (19-Me); 9.00/9.35 (25-Me); 25.91, 25.86, 25.78, 18.13, 18.04, −4.03, −4.36, −4.52, −4.75 (2×TBDMS). ^{1}H NMR ($CDCl_3$, selected data), δ (*Z*-isomer, ppm): 5.22 (d, *J*=5.5 Hz, H-2); 4.03 (d, *J*=12.5 Hz, H-6a); 3.09 (H-6b); 2.66 (H-11); 3.29 (H-13); 3.52 (H-14); 3.51 (H-15); 4.92 (d, *J*=10.2 Hz, H-20); 3.13 (H-21); 2.75 (dd, *J*=17.5+7.9 Hz, H-23a); 2.36 (dd, *J*=17.5+5.4 Hz, H-23b); 4.17 (dd, *J*=7.9+5.4 Hz, H-24); 5.13 (d, *J*=9.9 Hz, H-26); 5.25 (d, *J*=9.7 Hz, H-29); 2.94 (H-32); 3.40 (H-33); 1.01 (d, 3H, *J*=6.7 Hz, 11-Me); 1.74 (s, 3H, 19-Me); 1.62 (s, 3H, 27-Me); 0.07, −0.03, (s, each 3H, Si−Me), 0.06 (s, 6H, Si−Me); 0.89 (s, 18H, $(CH_3)_3$); 3.82 (s, 3H, 10-OMe); 5.48 (s, 9-OH); δ (*E*-isomer, ppm): 5.00 (br s, H-2); 4.56 (d, *J*=13.5 Hz, H-6a); 2.98 (H-6b); 2.35 (H-11); 3.20 (H-13); 3.48 (H-14); 3.44 (H-15); 4.69 (d, *J*=10.0 Hz, H-20); 3.31 (H-21); 2.85 (dd, *J*=14.7+10.0 Hz, H-23a); 2.28 (dd, *J*=14.7+4.4 Hz, H-23b); 4.08 (dd, *J*=10.0+4.4 Hz, H-24); 5.19 (d, *J*=8.0 Hz, H-26); 5.31 (d, *J*=8.6 Hz, H-29); 2.94 (H-32); 3.40 (H-33); 0.93 (d, 3H, *J*=6.6 Hz, 11-Me); 1.83 (s, 3H, 19-Me); 1.55 (s, 3H, 27-Me); 0.07, 0.02, (s, each 3H, Si−Me), 0.01 (s, 6H, Si−Me); 0.87, 0.85 (s, each 9H, $(CH_3)_3$); 3.62 (s, 3H, 10-OMe); 4.20 (br s, 9-OH).

4.2.2. Compound 5b. CHN ($C_{56}H_{101}NO_{13}Si_2$) calcd: 63.90/9.67/1.33, found: 63.72/9.61/1.28. HRMS (M+Na; calcd/found): 1074.6709/1074.6716. ^{13}C NMR ($CDCl_3$, single rotamer), δ (ppm): 169.68 (C1); 53.32 (C2); 25.97 (C3); 20.59 (C4); 25.13 (C5); 44.28 (C6); 168.25 (C8); 82.17 (C9); 172.92 (C10); 36.66 (C11); 34.53 (C12); 79.64 (C13); 75.31 (C14); 77.59 (C15); 35.35 (C16); 28.06 (C17); 48.07 (C18); 139.09 (C19); 123.60 (C20); 55.15 (C21); 209.60 (C22); 45.83 (C23); 67.85 (C24); 39.93 (C25); 80.56 (C26); 131.74 (C27); 12.49 (C28); 134.92 (C29); 35.07 (C30); 36.41 (C31); 84.19 (C32); 75.17 (C33); 33.93 (C34); 30.82 (C35); 23.54 (C36); 11.62 (C37); 52.99 (10-OMe); 56.37 (13-OMe); 57.50 (15-OMe); 57.88 (32-OMe); 15.43 (11-Me); 20.32 (17-Me); 17.82 (19-Me); 9.37 (25-Me); 25.93, 25.87, 18.15, 18.06, −4.33, −4.52, −4.74 (2×TBDMS). ^{1}H NMR ($CDCl_3$, selected data), δ (ppm): 5.27 (d, *J*=4.8 Hz, H-2); 3.97 (d, *J*=13.5 Hz, H-6a); 3.24 (H-6b); 2.69 (H-11); 3.26 (H-13); 3.40 (H-14); 3.46 (ddd, *J*=9.4+4.6+1.7 Hz, H-15); 4.88 (d, *J*=9.9 Hz, H-20); 3.15 (H-21); 2.60 (dd, *J*=17.5+5.5 Hz, H-23a); 2.46 (dd, *J*=17.5+6.5 Hz, H-23b); 4.17 (ddd, *J*=6.5+5.5+2.1 Hz, H-24); 5.13 (d, *J*=7.5 Hz, H-26); 5.24 (d, *J*=8.9 Hz, H-29); 2.93 (ddd, *J*=11.4+8.5+4.6 Hz, H-32); 3.40 (H-33); 0.93 (d, 3H, *J*=6.7 Hz, 11-Me); 1.71 (d, 3H, *J*=1.2 Hz, 19-Me); 1.60 (d, 3H, *J*=1.2 Hz, 27-Me); 0.08, 0.06, 0.03, −0.06 (s, each 3H, Si−Me); 0.89, 0.86 (s, each 9H, $(CH_3)_3$); 4.06 (br s, 9-OH); 3.80 (s, 3H, 10-OMe).

4.2.3. Compound 2-deuterio-5b. ^{13}C NMR ($CDCl_3$, single rotamer), δ (ppm): 169.67 (C1); 53.10 (C2); 25.9 (br) (C3); 20.53 (C4); 25.13 (C5); 44.24 (C6); 168.23 (C8); 82.15 (C9); 172.92 (C10); 36.64 (C11); 34.56 (C12); 79.59 (C13); 75.32 (C14); 77.59 (C15); 35.33 (C16); 28.11 (C17); 48.07 (C18); 139.10 (C19); 123.59 (C20); 55.13 (C21); 209.58 (C22); 45.91 (br) (C23); 67.81 (C24); 39.88 (C25); 80.63 (br) (C26); 131.73 (C27); 12.46 (C28); 133.5 br (C29); 35.06 (C30); 36.40 (C31); 84.18 (C32); 75.16 (C33); 33.92 (C34); 30.81 (C35); 23.52 (C36); 11.61 (C37); 53.00 (10-OMe); 56.35 (13-OMe); 57.49 (15-OMe); 57.88 (32-OMe); 15.41 (11-Me); 20.31 (17-Me); 17.83 (19-Me); 9.34 (25-Me); 25.93, 25.86, 18.14, 18.06, −4.24, −4.34, −4.52, −4.75 (2×TBDMS). ^{1}H NMR ($CDCl_3$, selected data), δ (ppm): H2 absent; 3.94 (d, *J*=13.9 Hz, H-6_{eq}); 3.23 (m, overlapped, H-6_{ax}); 2.68 (m, H-11); 3.24 (m, H-13); 3.37 (m, H-14); 3.46 (ddd, *J*=9.4+4.6+1.3 Hz, H-15); 4.87 (d, *J*=9.7 Hz, H-20); 3.14 (m, H-21); 2.60 (dd, *J*=17.8+5.3 Hz, H-23a); 2.44 (dd, *J*=17.8+6.6 Hz, H-23b); 4.16 (ψtd, *J*=6.1+1.5 Hz, H-24); 5.13 (d, *J*=7.6 Hz, H-26); 5.24 (d, *J*=8.7 Hz, H-29); 2.25 (m, H-30); 2.92 (m, H-32); 3.38 (m, H-33); 0.82 (t, *J*=7.3 Hz, CH_3-37); 0.07, 0.06, 0.02, −0.07 (4s, 24H, 24-Si(t.Bu)*Me*$_2$+32-Si(t.Bu)*Me*$_2$); 0.88, 0.85 (2s, 18H, 24-Si(*t*Bu)Me$_2$+32-Si(*t*Bu)Me$_2$); ~4.0 (s, very broad, 9-O*H*); 3.80 (s, 10-OMe); 0.92 (d, 3H, *J*=6.9 Hz, 11-Me); 3.31 (s, 3H, 13-OMe); 3.36 (s, 3H, 15-OMe); 1.70 (s, 3H, 19-Me); 1.59 (s, 3H, 27-Me); 3.38 (s, 3H, 32-OMe).

4.2.4. Compound 6a. CHN ($C_{55}H_{99}NO_{13}Si_2$) calcd: 63.61/9.61/1.35, found: 63.39/9.58/1.21. HRMS (M+Na; calcd/found): 1060.6553/1060.6549. ^{13}C NMR (CD_3OD, single rotamer), δ (ppm): 169.55 (C1); 53.39 (C2); 26.18 (C3); 20.50 (C4); 24.53 (C5); 43.41 (C6); 170.14 (C8); 81.54 (C9); 172.05 (C10); 35.75 (C11); 32.99 (C12); 79.71 (C13); 74.26 (C14); 77.90 (C15); 37.36 (C16); 27.63 (C17); 46.74 (C18); 139.82 (C19); 122.36 (C20); 55.44 (C21); 210.37 (C22); 44.3 br (C23); 69.03 (C24); 39.96 (C25); 78.8 (C26); 132.07 (C27); 11.95 (C28); 132.7br (C29); 34.84 (C30); 36.03 (C31); 84.13 (C32); 75.06 (C33); 33.57 (C34); 30.62 (C35); 23.29 (C36); 10.63 (C37); 55.44 (13-OMe); 57.14 (15-OMe); 56.76 (32-OMe); 13.55 (11-Me); 19.25 (17-Me); 16.92 (19-Me); 8.57 (25-Me); 25.09, 24.99, 17.57, 17.48, −5.46, −5.56, −5.65, −5.89 (2×TBDMS). ^{1}H NMR (CD_3OD, selected data), δ (ppm): 5.15 (br d, *J*=4.5 Hz, H-2); 4.31 (d, *J*=12.5 Hz, H-6a); 3.10 (ddd, *J*=12.5+12.5+2.9 Hz, H-6b); 2.53 (H-11); 3.30 (H-13); 3.56 (dd, *J*=5.7+3.9 Hz, H-14); 3.51 (H-15); 4.94 (d, *J*=9.9 Hz, H-20); 3.26 (H-21); 2.71 (br dd, *J*=17.0+5.2 Hz, H-23a); 2.48 (dd, *J*=17.0+6.0 Hz, H-23b); 4.21 (ddd, *J*=6.0+5.2+2.6 Hz, H-24); 5.23 (H-26); 5.23 (H-29); 3.00 (ddd, *J*=11.2+8.5+4.5 Hz, H-32); 3.43 (H-33); 1.07 (d, 3H, *J*=6.6 Hz, 11-Me); 1.75 (s, 3H, 19-Me); 1.65 (d, 3H, *J*=1.2 Hz, 27-Me); 0.09, 0.08, 0.07, −0.01 (s, each 3H, Si−Me); 0.90, 0.89 (s, each 9H, $(CH_3)_3$).

4.2.5. Compound 6b. CHN ($C_{55}H_{99}NO_{13}Si_2$) calcd: 63.61/9.61/1.35, found: 63.40/9.54/1.39. HRMS (M+Na; calcd/found): 1060.6553/1060.6551. ^{13}C NMR ($CDCl_3$, mixture of rotamers >9:1), δ (major rotamer, ppm): 170.19 (C1); 55.38 (C2); 26.23 (C3); 21.57 (C4); 25.30 (C5); 45.87 (C6); 173.83 (C8); 83.48 (C9); 173.35 (C10); 40.57 (C11); 33.92 (C12); 78.03 (C13); 74.10 (C14); 77.27 (C15); 34.40 (C16); 25.86 (C17); 47.20 (C18); 137.33 (C19); 122.14 (C20); 54.53 (C21); 208.88 (C22); 49.41 (C23); 68.22 (C24); 42.65 (C25); 79.22 (C26); 130.54 (C27); 13.77 (C28); 130.54 (C29); 34.94 (C30); 36.49 (C31); 84.13 (C32); 75.10 (C33);

33.73 (C34); 30.95 (C35); 22.74 (C36); 11.76 (C37); 56.22 (13-OMe); 57.09 (15-OMe); 57.99 (32-OMe); 13.77 (11-Me); 20.62 (17-Me); 18.92 (19-Me); 10.61 (25-Me); 2×25.86, 18.13, 17.95, −4.52, −4.57, −4.68, −4.73 (2×TBDMS). ^{1}H NMR ($CDCl_3$, selected data), δ (major rotamer, ppm): 5.65 (m, H-2); 5.20 (d, J=12.4 Hz, H-6$_{eq}$); 2.75 (ψt, J=12.6 Hz, H-6$_{ax}$); 2.60 (m, H-11); 3.28 (m, H-13); 3.54 (m, H-14); 3.51 (m, H-15); 4.95 (d, J=10.9 Hz, H-20); 3.22 (m, H-21); 2.89 (dd, J=18.8+7.9 Hz, H-23a); 2.24 (dd, J=18.8+2.2 Hz, H-23b); 4.20 (ψtd, J=8.4+2.5 Hz, H-24); 4.54 (s, broad, H-26); 5.02 (d, J=8.8 Hz, H-29); 2.22 (m, H-30); 2.95 (m, H-32); 3.40 (m, H-33); 0.79 (t, J=7.4 Hz, CH_3-37); 0.08, 0.07, 0.06, 0.01, 0.89, 0.87 (6s, 42H, 2×TBDMS); 4.53 (s, 9-O*H*); 13.5 (broad, 10-COO*H*); 0.96 (d, 3H, J=6.9 Hz, 11-Me); 3.30 (s, 3H, 13-OMe); 3.02 (s, very broad, 14-O*H*); 3.32 (s, 3H, 15-OMe); 1.78 (s, 3H, 19-Me); 1.49 (s, 3H, 27-Me); 3.40 (s, 3H, 32-OMe).

4.2.6. Compound 7. HRMS (M+Na; calcd/found): 1042.6447/1042.644. ^{13}C NMR ($CDCl_3$), δ (ppm): 163.89 (C1); 72.86 (C2); 33.19 (C3); 20.67 (C4); 23.72 (C5); 37.28 (C6); 170.54 (C8); 203.27 (C9); 76.70 (C10); 33.92 (C11); 30.00 (C12); 77.94 (C13); 71.81 (C14); 79.48 (C15); 39.42 (C16); 26.35 (C17); 47.79 (C18); 140.21 (C19); 123.88 (C20); 56.42 (C21); 208.50 (C22); 47.35 (C23); 68.10 (C24); 39.31 (C25); 86.06 (C26); 130.93 (C27); 11.03 (C28); 137.59 (C29); 35.17 (C30); 36.13 (C31); 84.08 (C32); 75.12 (C33); 33.85 (C34); 30.64 (C35); 22.21 (C36); 11.48 (C37); 55.12 (13-OMe); 59.74 (15-OMe); 57.91 (32-OMe); 16.94 (11-Me); 20.50 (17-Me); 16.16 (19-Me); 9.59 (25-Me); 25.87, 25.83, 18.16, 18.04, −3.78, −4.27, −4.51, −4.73 (2×TBDMS). ^{1}H NMR ($CDCl_3$, selected data), δ (ppm): 2.47 (br d, J=13.6 Hz, H-3a); 1.48 (H-3b); 4.36 (dd, J=13.4+4.8 Hz, H-6a); 3.20 (H-6b); 2.52 (H-11); 3.87 (H-13); 3.88 (H-14); 3.22 (H-15); 4.72 (d, J=10.8 Hz, H-20); 3.24 (H-21); 2.82 (dd, J=15.0+11.1 Hz, H-23a); 2.17 (dd, J=15.0+4.1 Hz, H-23b); 4.11 (dd, J=11.1+4.1 Hz, H-24); 5.17 (d, J=10.6 Hz, H-26); 5.36 (d, J=8.9 Hz, H-29); 2.95 (ddd, J=11.2+8.5+4.5 Hz, H-32); 3.40 (H-33); 0.76 (d, 3H, J=7.3 Hz, 11-Me); 1.82 (s, 3H, 19-Me); 1.62 (s, 3H, 27-Me); 0.08, 0.07 (s, each 3H, Si–Me); 0.01 (s, 6H, Si–Me); 0.89, 0.85 (s, each 9H, $(CH_3)_3$); 4.25 (s, 10-OH); 2.42 (s, 14-OH).

4.2.7. Compound 8. To a solution of 3.0 g (2.94 mmol) **7** in 120 ml acetonitrile were added 10 ml aqueous hydrogen fluoride (40 w/w%). The mixture was stirred for 6 h at room temperature and then partitioned between ethyl acetate and a saturated solution of aqueous sodium hydrogen carbonate. The organic layer was separated, washed with brine, dried over anhydrous sodium sulfate and evaporated at reduced pressure. Flash column chromatography (silica gel, ethyl acetate) provided 2.07 g (89%) **8** as amorphous powder. 1.0 g of the amorphous material was dissolved in 5 ml methanol and water (~10 ml) was added until the solution got slightly turbid. The solution was filtered through a Whatman® glass filter (type GF) and the clear solution was allowed to stand at room temperature for 2 weeks. The crystals thereby formed were used for X-ray analysis. CHN ($C_{43}H_{69}NO_{12}$, from the amorphous powder) calcd: 65.21/8.78/1.77, found: 64.92/8.43/1.59. HRMS (M+Na; calcd/found): 814.4714/814.4719. ^{13}C NMR ($CDCl_3$/d_6-DMSO=6:1), δ (ppm): 164.50 (C1); 72.05 (C2); 30.27 (C3); 20.72 (C4); 23.55 (C5); 37.59 (C6); 170.46 (C8); 76.25 (C9); 204.24 (C10); 34.18 (C11); 31.79 (C12); 78.64 (C13); 73.66 (C14); 78.67 (C15); 37.07 (C16); 27.13 (C17); 48.18 (C18); 139.42 (C19); 124.08 (C20); 55.46 (C21); 209.97 (C22); 46.25 (C23); 66.82 (C24); 38.75 (C25); 84.90 (C26); 130.76 (C27); 11.9 (C28); 134.45 (C29); 34.84 (C30); 34.68 (C31); 83.89 (C32); 73.19 (C33); 31.71 (C34); 30.24 (C35); 22.56 (C36); 11.38 (C37); 56.11 (13-OMe); 58.55 (15-OMe); 56.43 (32-OMe); 14.89 (11-Me); 20.69 (17-Me); 16.47 (19-Me); 9.03 (25-Me). ^{1}H NMR ($CDCl_3$/d_6-DMSO=6:1, selected data), δ (ppm): 2.50 (br d, J=13.5 Hz, H-3a); 2.00 (H-3b); 4.35 (br dd, J=13.7+3.0 Hz, H-6a); 3.07 (H-6b); 2.44 (H-11); 3.59 (H-13); 3.54 (H-14); 3.29 (H-15); 4.78 (d, J=10.4 Hz, H-20); 3.21 (ddd, J=10.4+8.4+5.5 Hz, H-21); 2.79 (dd, J=16.1+8.6 Hz, H-23a); 2.45 (H-23b); 3.99 (ddd, J=7.9+5.5+1.7 Hz, H-24); 5.17 (d, J=9.0 Hz, H-26); 5.36 (d, J=9.2 Hz, H-29); 3.02 (ddd, J=11.5+8.8+4.3 Hz, H-32); 3.39 (H-33); 0.87 (d, 3H, J=7.1 Hz, 11-Me); 1.77 (s, 3H, 19-Me); 1.62 (s, 3H, 27-Me).

4.2.8. Compound 9. A solution of 1.5 g (1.44 mmol) **6a** and 0.70 g (3 equiv., 4.33 mmol) 1,1′-carbonyldiimidazole (CDI) and 5 mg (2.8 mol%) 4-dimethylaminopyridine (DMAP) in 25 ml dichloromethane was stirred for 2 h at room temperature. For work up the mixture was partitioned between ethyl acetate and 1 N-hydrochloric acid. The organic layer was separated, washed with brine, dried over anhydrous sodium sulfate and evaporated to dryness at reduced pressure. The residual foam was subjected to flash column chromatography (silica gel, toluene/ethyl acetate=5:1) to give 1.37 g (93%) **9** as amorphous powder. CHN ($C_{55}H_{97}NO_{12}Si_2$) calcd: 64.73/9.58/1.37, found: 64.59/9.40/1.21. HRMS (M+Na; calcd/found): 1042.6447/1042.6441. ^{13}C NMR ($CDCl_3$, mixture of rotamers <1:9), δ (major rotamer, ppm): 168.50 (C1); 56.94 (C2); 27.78 (C3); 20.15 (C4); 24.97 (C5); 41.54 (C6); 169.89 (C8); 80.79 (C9); 168.22 (C10); 33.36 (C11); 36.42 (C12); 76.55 (C13); 80.51 (C14); 76.5 br (C15); 31.66 (C16); 25.98 (C17); 49.20 (C18); 136.34 (C19); 125.89 (C20); 53.23 (C21); 212.98 (C22); 46.46 (C23); 70.45 (C24); 42.04 (C25); 76.5 br (C26); 133.88 (C27); 12.47 (C28); 132.62 (C29); 34.90 (C30); 36.41 (C31); 84.18 (C32); 75.13 (C33); 33.91 (C34); 30.60 (C35); 25.44 (C36); 11.45 (C37); 57.31 (13-OMe); 56.32 (15-OMe); 57.83 (32-OMe); 17.93 (11-Me); 21.30 (17-Me); 15.96 (19-Me); 11.81 (25-Me); 26.00, 25.86, 18.14, 17.96, −4.39, −4.52, −4.75 (2×TBDMS). ^{1}H NMR ($CDCl_3$, selected data), δ (major rotamer, ppm): 4.14 (br s); 4.48 (d, J=13.3 Hz, H-6a); 3.85 (br dd, J=13.3+13.3 Hz, H-6b); 2.06 (H-11); 3.49 (H-13); 4.63 (d, J=8.7 Hz, H-14); 3.59 (d, J=11.4 Hz, H-15); 4.94 (d, J=9.7 Hz, H-20); 3.33 (H-21); 3.02 (d, J=19.1 Hz, H-23a); 2.63 (dd, J=19.1+8.7 Hz, H-23b); 4.13 (H-24); 5.57 (s, H-26); 5.24 (d, J=9.2 Hz, H-29); 2.93 (ddd, J=11.4+8.6+4.6 Hz, H-32); 3.38 (H-33); 0.92 (d, 3H, J=6.3 Hz, 11-Me); 1.36 (s, 3H, 19-Me); 1.63 (s, 3H, 27-Me); 0.07, 0.06 (s, each 3H, Si–Me); 0.03 (s, 6H, Si–Me); 0.89, 0.82 (s, each 9H, $(CH_3)_3$); 5.49 (br s, 9-OH).

4.2.9. Compounds 11a and 11b. To a solution of 20 g (17.62 mmol) 14,24,33-tris-OTBDMS-ascomycin **10** in 250 ml tetrahydrofuran were added 15 ml water and 20 g

(15.3 equiv., 270 mmol) calcium hydroxide and the resultant suspension was stirred for 30 min at room temperature (TLC-monitoring revealed completion of the reaction in <5 min). For work up the mixture was partitioned between ethyl acetate (800 ml) and 1 N-hydrochloric acid (200 ml). The organic layer was separated, washed twice with brine, dried over anhydrous sodium sulfate and evaporated to dryness at reduced pressure. The residue was dissolved in 200 ml dichloromethane and titrated with an approx. 1 M ethereal solution of diazomethane until the characteristic yellow colour remained. After evaporation at reduced pressure the mixture of **11a**,**b** was subjected to flash column chromatography (silica gel, cyclohexane/ethyl acetate=10:1) to give 17.69 g (86%) **11a** and 514 mg (2.5%) **11b** as amorphous powders.

4.2.10. Compound 11a. CHN ($C_{62}H_{115}NO_{13}Si_3$) calcd: 63.82/9.93/1.20, found: 63.73/9.82/1.00. HRMS (M+Na; calcd/found): 1188.7574/1188.7578. ^{13}C NMR ($CDCl_3$, *Z*/*E*~3:7), δ (*Z*(only selected data due to extreme signal broadening)/*E*-isomer, ppm): n.d./168.32 (C1); 53.53/56.35 (C2); n.d./28.50 (C3); n.d./20.95 (C4); n.d./25.07 (C5); 43.14/41.81 (C6); n.d./168,45 (C8); 82.44/81.88 (C9); n.d./171.84 (C10); n.d./39.59 (C11); n.d./31.77 (C12); 81.33/85.89 (C13); 76.98/76.14 (C14); 79.46/78.16 (C15); n.d./42.29 (C16); n.d./26.54 (C17); 46.50/46.70 (C18); n.d./140.39 (C19); n.d./123.38 (C20); n.d./56.59 (C21); n.d./209.07 (C22); n.d./48.29 (C23); n.d./68.24 (C24); n.d./39.59 (C25); n.d./84.16 (C26); n.d./131.21 (C27); 12.64/11.10 (C28); n.d./136.61 (C29); 35.03/35.13 (C30); 36.48/36.15 (C31); n.d./84.10 (C32); n.d./75.12 (C33); n.d./33.87 (C34); 30.84/30.65 (C35); 23.37/23.10 (C36); 11.54/11.54 (C37); 53.11/52.70 (10-OMe); n.d./55.85 (13-OMe); 60.20/60.38 (15-OMe); 57.84/57.84 (32-OMe); 14.74/13.82 (11-Me); 20.42/20.14 (17-Me); 16.61/16.14 (19-Me); n.d./9.19 (25-Me); 26.03, 25.86, 25.78, 18.42, 18.14, 18.03, −3.67, −4.35, −4.52, −4.75 (3×TBDMS). ^{1}H NMR ($CDCl_3$, selected data), δ (*Z*-isomer, ppm): 5.16 (H-2); 4.08 (H-6a); 3.35 (H-6b); 2.50 (H-11); 3.72 (d, *J*=8.4 Hz, H-14); 4.87 (d, *J*=10.2 Hz, H-20); 3.19 (H21); 2.47 (H-23a); 2.42 (H-23b); 4.08 (H-24); 2.95 (ddd, *J*=11.3+8.5+4.4 Hz, H-32); 3.40 (H-33); 1.06 (d, 3H, *J*=6.6 Hz, 11-Me); 1.72 (s, 3H, 19-Me); 1.62 (s, 3H, 27-Me); 0.90, 0.89,0.85 (s, each 9H, $(CH_3)_3$); 0.07, 0.06, 0.06 (s, each 6H, Si–Me); 3.84 (s, 3H, 10-OCH_3); δ (*E*-isomer, ppm): 4.99 (br s, H-2); 4.58 (H-6a); 3.04 (H-6b); 2.20 (H-11); 2.85 (H-13); 3.67 (d, *J*=8.4 Hz, H-14); 3.05 (H-15); 4.60 (d, *J*=9.8 Hz, H-20); 3.30 (H-21); 2.84 (dd, *J*=14.8+10.8 Hz, H-23a); 2.24 (dd, *J*=14.8+4.1 Hz, H-23b); 4.09 (dd, *J*=10.8+4.1 Hz, H-24); 5.14 (d, *J*=10.3 Hz, H-26); 5.31 (d, *J*=8.9 Hz, H-29); 2.95 (ddd, *J*=11.3+8.5+4.4 Hz, H-32); 3.40 (H-33); 0.95 (d, 3H, *J*=6.6 Hz, 11-Me); 1.83 (s, 3H, 19-Me); 1.55 (s, 3H, 27-Me); 0.90, 0.89,0.85 (s, each 9H, $(CH_3)_3$); 0.08, 0.07, 0.02 (s, each 6H, Si–Me); 3.62 (s, 3H, 10-OCH_3); 5.43 (s, 9-OH).

4.2.11. Compound 11b. CHN ($C_{62}H_{115}NO_{13}Si_3$) calcd: 63.82/9.93/1.20, found: 63.63/9.89/1.01. HRMS (M+Na; calcd/found): 1188.7574/1188.7580. ^{13}C NMR ($CDCl_3$, *Z*/*E*=4:1), δ (*Z*/*E*-isomer, ppm): 169.02/169.78 (C1); 53.84/55.89 (C2); 26.23/n.d. (C3); 20.97/n.d. (C4); 25.33/25.05 (C5); 43.72/39.96 (C6); 167.39/166.49 (C8); 83.13/81.74 (C9); 172.99/173.35 (C10); 38.60/n.d. (C11); 31.57/30.68 (C12); 84.75/85.5 br (C13); 76.34/n.d. (C14); 79.24/78.72 (C15); 41.49/n.d. (C16); 27.00/26.41 (C17); 46.56/46.90 (C18); 140.49/139.96 (C19); 123.75/123.75 (C20); 56.24/57.77 (C21); 211.78/208.73 (C22); 42.74/47.92 (C23); 72.29/67.87 (C24); 41.90/39.25 (C25); 77.08/82.6 br (C26); 132.70/131.20 (C27); 14.38/11.05 (C28); 130.37/136.58 (C29); 34.94/35.17 (C30); 36.65/36.13 (C31); 84.12/84.12 (C32); 75.10/75.08 (C33); 33.77/33.92 (C34); 30.93/n.d. (C35); 22.83/22.68 (C36); 11.47/11.47 (C37); 52.95/52.94 (10-OMe); 56.95/55.89 (13-OMe); 60.88/60.88 (15-OMe); 57.98/n.d. (32-OMe); 15.977n.d. (11-Me); 20.56/20.2 br (17-Me); 16.60/16.10 (19-Me); 10.06/n.d. (25-Me); 26.09, 26.00, 25.92, 25.86, 18.41, 18.13, 17.88, −4.47, −4.52, −4.72, −4.93 (3×TBDMS). ^{1}H NMR ($CDCl_3$, selected data), δ (*Z*-isomer, ppm): 5.31 (br s, H-2); 4.22 (d, *J*=14.5 Hz, H-6a); 2.90 (H-6b); 2.51 (H-11); 3.30 (H-13); 3.67 (d, *J*=8.4 Hz, H-14); 3.05 (H-15); 4.83 (d, *J*=10.2 Hz, H-20); 3.14 (ddd, *J*=10.2+9.7+4.2 Hz, H-21); 2.57 (dd, *J*=14.1+9.5 Hz, H-23a); 2.16 (H-23b); 4.03 (br d, *J*=9.5 Hz, H-24); 5.15 (H-26); 5.14 (d, *J*=9.8 Hz, H-29); 2.94 (H-32); 3.40 (H-33); 0.94 (d, 3H, *J*=6.9 Hz, 11-Me); 1.71 (s, 3H, 19-Me); 1.65 (s, 3H, 27-Me); 0.08, 0.07 (s, each 6H, Si–Me), 0.03, −0.05 (s, each 3H, Si–Me); 0.90, 0.89, 0.88 (s, each 9H, $(CH_3)_3$); 3.81 (s, 3H, 10-OCH_3); δ (*E*-isomer, ppm): 5.14 (H-2); 4.43 (d, *J*=13.2 Hz, H-6a); 3.12 (H-6b); 3.65 (H-14); 2.92 (H-15); 4.57 (d, *J*=10.2 Hz, H-20); 3.25 (H-21); 2.84 (H-23a); 2.19 (H-23b); 4.10 (H-24); 5.20 (d, *J*=10.0 Hz, H-26); 5.35 (d, *J*=9.0 Hz, H-29); 2.94 (H-32); 3.40 (H-33); 1.80 (s, 3H, 19-Me); 1.55 (s, 3H, 27-Me); 0.08 (s, 9H, Si–Me), 0.07 (s, 6H, Si–Me), 0.02 (s, 3H, Si–Me); 0.90, 0.89, 0.88 (s, each 9H, $(CH_3)_3$); 3.84 (s, 3H, 10-OCH_3).

4.2.12. Compound 12a. A suspension of 2.0 g (1.71 mmol) **11a** and 1.9 g (15 equiv., 25.7 mmol) calcium hydroxide in 50 ml tetrahydrofuran and 10 ml water was stirred for 40 h at room temperature. For work up the mixture was partitioned between ethyl acetate and 1 N-hydrochloric acid. The organic layer was separated, washed with brine, dried over anhydrous sodium sulfate and evaporated to dryness at reduced pressure to give 1.9 g (96%) **12a** as an amorphous powder which required no further purification. HRMS (M+Na; calcd/found): 1174.7418/1174.7413. ^{13}C NMR ($CDCl_3$, mixture of rotamers <1:9), δ (major rotamer, selected data, ppm): n.d. (C1); 57.18 (C2); 28.63 (C3); 20.79 (C4); 25.24 (C5); 42.62 (C6); n.d. (C8); 83.53 (C9); n.d. (C10); 40.37 (C11); 31.72 (C12); n.d. (C13); 75.16 (C14); 79.58 (C15); 41.19 (C16); 27.18 (C17); 47.25 (C18); 140.19 (C19); 123.42 (C20); 56.42 (C21); 209.20 (C22); 48.58 (C23); 68.45 (C24); 39.89 (C25); 84.14 (C26); 131.37 (C27); n.d. (C28); n.d. (C29); 35.14 (C30); 36.20 (C31); 84.14 (C32); 75.16 (C33); 33.91 (C34); 30.67 (C35); 23.09 (C36); 11.53 (C37); 55.79 (13-OMe); 60.13 (15-OMe); 57.84 (32-OMe); 14.05 (11-Me); 20.04 (17-Me); 16.11 (19-Me); 8.8 br (25-Me); 26.05, 25.94, 25.87, 25.82, 18.30, 18.15, 18.03, −3.77, −4.30, −4.52, −4.74, −4.79 (3×TBDMS). ^{1}H NMR ($CDCl_3$, selected data), δ (major rotamer, ppm): 5.73 (br s, H-2); 4.53 (H-6a); 3.26 (H-6b); 2.26 (H-11); 3.00 (H-13); 3.71 (d, *J*=7.9 Hz, H-14); 2.90 (H-15); 4.57 (H-20); 3.19 (H-21); 2.87 (dd, *J*=14.7+10.8 Hz, H-23a); 2.20 (H-23b); 4.09 (dd, *J*=11.0+3.3 Hz, H-24); 5.15 (H-26); 5.32 (H-29); 2.95 (ddd, *J*=11.4+8.7+4.6 Hz, H-32); 3.39 (H-33); 0.94 (d, 3H,

J=6.8 Hz, 11-Me); 1.80 (s, 3H, 19-Me); 1.54 (s, 3H, 27-Me); 0.08, 0.01 (s, each 6H, Si−Me), 0.07, 0.06 (s, each 3H, Si−Me); 0.90, 0.86 (s, each 9H, $(CH_3)_3$), 0.89 (s, 18H, $(CH_3)_3$); 6.66 (br s, COOH).

4.2.13. Compound 12b. Starting from 0.35 g (0.3 mmol) **11b**, the reaction was performed as described above to give 0.33 g (96%) **12b**. CHN ($C_{61}H_{113}NO_{13}Si_3$) calcd: 63.55/9.88/1.21, found: 63.55/9.70/1.21. HRMS (M+Na; calcd/found): 1174.7418/1174.7420. ^{13}C NMR ($CDCl_3$, *Z*/*E*=3:1), δ (*Z*/*E*-isomer, ppm): 168.16/169.18 (C1); 55.31/57.05 (C2); 27.09/28.63 (C3); 21.17/20.38 (C4); 25.01/24.94 (C5); 45.25/40.96 (C6); 171.86/172.84 (C8); 83.97/81.26 (C9); n.d./n.d. (C10); 41.50/42.97 (C11); 30.44/30.00 (C12); 80.55/79.54 (C13); 75.68/74.85 (C14); 82.05/84.53 (C15); 40.67/41.11 (C16); 29.21/27.28 (C17); 47.26/47.26 (C18); 139.41/139.88 (C19); 124.00/123.87 (C20); 55.86/56.21 (C21); 209.75/208.95 (C22); 43.15/48.20 (C23); 70.36/67.90 (C24); 34.98/39.30 (C25); 78.10/83.33 (C26); 132.33/130.98 (C27); 13.68/11.04 (C28); 131.10/136.95 (C29); 34.93/35.18 (C30); 36.45/36.19 (C31); 84.09/84.15 (C32); 75.09/75.17 (C33); 33.83/33.92 (C34); 30.84/30.65 (C35); 24.44/22.88 (C36); 11.65/11.50 (C37); 60.48/60.18 (13-OMe); 56.10/55.86 (15-OMe); 57.87/57.87 (32-OMe); 14.06/15.98 (11-Me); 21.35/20.01 (17-Me); 17.05/16.41 (19-Me); 10.89/8.64 (25-Me); 25.95, 25.86, 25.81, 18.30, 18.15, 18.03, −3.68, −4.36, −4.47, −4.52, −4.62, −4.76 (3×TBDMS). ^1H NMR ($CDCl_3$, selected data), δ (*Z*-isomer, ppm): 5.30 (br s, H-2); 4.56 (H-6a); 3.25 (H-6b); 1.81 (H-11); 3.00 (H-13); 3.74 (d, *J*=7.9 Hz, H-14); 2.95 (H-15); 4.60 (H-20); 3.27 (H-21); 2.85 (dd, *J*=15.0+11.1 Hz, H-23a); 2.20 (H-23b); 4.09 (dd, *J*=11.1+3.8 Hz, H-24); 5.19 (d, *J*=10.9 Hz, H-26); 5.35 (d, *J*=9.0 Hz, H-29); 2.93 (H-32); 3.39 (H-33); 1.12 (d, 3H, *J*=7.0 Hz, 11-Me); 1.81 (s, 3H, 19-Me); 1.55 (s, 3H, 27-Me); 0.90 (s, 18H, Si−Me); 0.0–0.1 (overlapped, $(CH_3)_3$); δ (*E*-isomer, ppm): 5.11 (H-2); 4.96 (br d, *J*=13.0 Hz, H-6a); 3.13 (H-6b); 1.85 (H-11); 3.18 (H-13); 3.72 (d, *J*=7.9 Hz, H-14); 3.02 (H-15); 5.05 (d, *J*=10.3 Hz, H-20); 3.13 (H-21); 2.51 (dd, *J*=17.0+7.4 Hz, H-23a); 2.44 (dd, *J*=17.0+4.0 Hz, H-23b); 4.16 (H-24); 5.14 (H-26); 5.11 (H-29); 2.93 (H-32); 3.39 (H-33); 0.97 (d, 3H, *J*=7.0 Hz, 11-Me); 1.71 (s, 3H, 19-Me); 1.59 (s, 3H, 27-Me); 0.90 (s, 18H, Si−Me); 0.0–0.1 (overlapped, $(CH_3)_3$).

4.2.14. Compound 13a. (1) from **5a**. To a solution of 2.1 g (2 mmol) **5a** in 100 ml acetonitrile were added 9 ml aqueous hydrogen fluoride (40 w/w%). The mixture was stirred for 5 h at room temperature and then partitioned between ethyl acetate and a saturated solution of aqueous sodium hydrogen carbonate. The organic layer was separated, washed with brine, dried over anhydrous sodium sulfate and evaporated at reduced pressure. Flash column chromatography (silica gel, ethyl acetate) provided 1.50 g (93%) of analytically pure **13a** as amorphous powder. 1.0 g of the amorphous powder was dissolved in 30 ml tetrahydrofuran and concentrated to a volume of about 15 ml and the resulting clear solution was stored for 1 week at 4 °C. The crystals formed thereof (0.4 g) were used for X-ray analysis. (2) from **11a**. Starting from 0.4 g (0.34 mmol) **11a**, the reaction, work up and purification was performed as described above (reaction time 7 h) to provide 0.22 g (79%) **12a** as amorphous powder. HRMS (M+Na; calcd/found): 846.4980/846.4975. ^{13}C NMR ($CDCl_3$, *Z*/*E*=1:1), δ (*Z*/*E*-isomer, ppm): 169.51/n.d. (C1); 54.33/56.8 br (C2); 26.16/n.d. (C3); 20.74/n.d. (C4); 25.02/24.9 br (C5); 44.12/41.61 (C6); 168.90/n.d. (C8); 81.85/n.d. (C9); 171.96/n.d. (C10); 33.56/n.d. (C11); 30.42/n.d. (C12); 79.42/77.67 (C13); 72.86/n.d. (C14); 77.67/76.83 (C15); 36.53/35.17 (C16); 29.92/27.86 (C17); 47.65/48.78 (C18); 139.48/139.3 br (C19); 123.92/124.47 (C20); 54.48/n.d. (C21); 211.71/n.d. (C22); 45.41/45.65 (C23); 66.67n.d. (C24); 38.73/39.42 (C25); 82.54/n.d. (C26); 130.94/131.41 (C27); 12.34/13.3 br (C28); 133.86/130.49 (C29); 34.96*/34.90* (C30); 34.51/34.74 (C31); 84.17/84.17 (C32); 73.49/73.49 (C33); 31.22/31.22 (C34); 30.62/30.53 (C35); 23.70/n.d. (C36); 11.69*/11.57* (C37); 52.84/53.4 br (10-OMe); 57.65/56.14 (13-OMe); 57.97/n.d. (15-OMe); 56.48/56.48 (32-OMe); 15.26/19.76 (11-Me); 19.74/21.3 br (17-Me); 18.28/16.3 br (19-Me); 9.20/8.7 br (25-Me). ^1H NMR ($CDCl_3$, selected data), δ (*Z*-isomer, ppm): 5.31 (d, *J*=5 Hz, H-2); 4.08 (d, *J*=13.3 Hz, H-6a); 3.00 (H-6b); 2.66 (H-11); 3.30 (H-13); 3.38 (H-14); 3.49 (H-15); 4.99 (d, *J*=9.8 Hz, H-20); 3.09 (H-21); 2.64 (dd, *J*=18.3+4.7 Hz, H-23a); 2.48 (dd, *J*=18.3+8.4 Hz, H-23b); 3.98 (H-24); 5.10 (d, *J*=8.5 Hz, H-26); 5.29 (d, *J*=10.0 Hz, H-29); 2.98 (H-32); 3.40 (H-33); 1.01 (d, 3H, *J*=6.5 Hz, 11-Me); 1.72 (s, 3H, 19-Me); 1.58 (s, 3H, 27-Me); 3.81 (s, 3H, 10-OMe); 5.53 (br s, OH); 4.62 (br s, OH); δ (*E*-isomer, ppm): 4.88 (br s, H-2); 2.66 (H-11); 3.35 (H-13)*; 3.38 (H-14); 3.30 (H-15)*; 4.94 (br s, H-20); 2.75 (dd, *J*=16.8+5.8 Hz, H-23a); 2.60 (H-23b); 5.07 (d, *J*=9.5 Hz, H-29); 2.98 (H-32); 3.40 (H-33); 0.97 (d, *J*=6.5 Hz, 11-Me); 1.64 (s, 3H, 19-Me); 1.58 (s, 3H, 27-Me); 3.71 (br s, 3H, 10-OMe); 5.72 (br s, OH); 4.58 (br s, OH); (*) opposite assignment possible.

4.2.15. Compound 13b. Starting from 0.9 g (0.86 mmol) **5b** or 0.1 g (0.086 mmol) **11b** the deprotections were carried as described above to give 0.64 g (93%) or 81 mg (89%) **13b** as amorphous powders. HRMS (M+Na; calcd/found): 846.4980/846.4972. ^{13}C NMR ($CDCl_3$, single rotamer), δ (ppm): 169.92 (C1); 53.26 (C2); 25.25 (C3); 20.89 (C4); 25.15 (C5); 45.06 (C6); 168.28 (C8); 81.55 (C9); 172.46 (C10); 37.48 (C11); 33.99 or 33.93 (C12); 81.07 (C13); 75.68 (C14); 76.91 (C15); 33.99 or 33.93 (C16); 27.34 (C17); 49.19 (C18); 138.31 (C19); 125.09 (C20); 54.53 (C21); 211.92 (C22); 46.43 (C23); 66.04 (C24); 38.73 (C25); 83.78 (C26); 130.56 (C27); 12.03 (C28); 134.44 (C29); 34.99 (C30); 34.44 (C31); 84.25 (C32); 73.49 (C33); 31.27 (C34); 30.35 (C35); 23.18 (C36); 11.55 (C37); 52.68 (10-OMe); 57.26 (13-OMe); 56.93 (15-OMe); 56.38 (32-OMe); 17.17 (11-Me); 20.95 (17-Me); 16.86 (19-Me); 9.65 (25-Me). ^1H NMR ($CDCl_3$, selected data), δ (ppm): 5.32 (d, *J*=4.9 Hz, H-2); 3.88 (br d, *J*=14.2 Hz, H-6a); 3.02 (br dd, *J*=14.2+13.5 Hz, H-6b); 2.65 (H-11); 3.17 (H-13); 3.17 (H-14); 3.37 (H-15); 4.80 (d, *J*=9.9 Hz, H-20); 3.17 (H-21); 2.62 (H-23a); 2.55 (dd, *J*=18.6+9.1 Hz, H-23b); 3.96 (br d, *J*∼9 Hz, H-24); 4.93 (d, *J*=9.4 Hz, H-26); 5.36 (d, *J*=9.0 Hz, H-29); 2.98 (H-32); 3.41 (H-33); 1.02 (d, 3H, *J*=6.9 Hz, 11-Me); 1.70 (s, 3H, 19-Me); 1.49 (s, 3H, 27-Me); 4.75 (br s, 9-OH); 3.83 (s, 3H, 10-OCH_3).

4.2.16. Compounds 14-17 and 5b. (a) from **4**. To a magnetically stirred solution of 5.0 g (4.9 mmol) 24,33-bis-OTBDMS-ascomycin **4**, 500 mg (0.39 equiv., 1.9 mmol)

18-crown-6 and 3.48 g (5 equiv., 24.5 mmol, 1.53 ml) iodomethane in 150 ml tetrahydrofuran were added 0.41 g (1.5 equiv.; 7.35 mmol) powdered potassium hydroxide. After stirring for 40 min at room temperature, the mixture was partitioned between ethyl acetate (500 ml) and 1 N-hydrochloric acid (100 ml). The organic layer was washed with brine, dried over anhydrous sodium sulfate and evaporated at reduced pressure. Separation by flash column chromatography (silica gel, gradient/toluene/ethyl acetate=7:1 to 2:1) afforded 0.96 g (19%) **15**, 0.26 g (5%) **16**, 0.46 g (9%) **14**, 1.52 g (30%) **17** and 0.77 g (15%) **5b** (in the given order) as amorphous powders. (b) from **7**. Starting from 0.5 g **7** (0.49 mmol) the reaction and work up was performed as described above to give a mixture of **17** and **5b**. Flash column chromatography (toluene/ethyl acetate=3:1) afforded 213 mg (42%) **17** and 103 mg (20%) **5b**, respectively (Scheme 4).

4.2.17. Compound 14. CHN ($C_{56}H_{99}NO_{12}Si_2$) calcd: 65.01/9.65/1.35, found: 65.33/9.45/1.21. HRMS (M+Na; calcd/found): 1056.6604/1056.6605. ^{13}C NMR ($CDCl_3$, *Z*/*E*=3:1), δ (*Z*/*E*-isomer, ppm): 168.93/168.93 (C1); 50.81/56.17 (C2); 26.70/27.74 (C3); 21.21/20.72 (C4); 25.034/25.55 (C5); 43.1 br/38.35 (C6); 164.99/165.91 (C8); 196.54/196.54 (C9); 100.31/100.95 (C10); 32.35/31.29 (C11); 32.81/30.65 (C12); 74.02/74.21 (C13); 76.16/76.72 (C14); 76.98/79.48 (C15); 35.31/36.78 (C16); 26.55/28.55 (C17); 47.46/42.5 br (C18); 141.13/134.86 (C19); 121.45/126.61 (C20); 56.39/55.61 (C21); 211.15/210.66 (C22); 43.49/50.2 (C23); 72.54/67.87 (C24); 41.55/39.30 (C25); 76.16/82.8 br (C26); 133.28/131.08 (C27); 13.72/11.6 br (C28); 130.8 br/136.4 br (C29); 34.97/35.16 (C30); 36.54/36.14 (C31); 84.17/84.17 (C32); 75.15/75.15 (C33); 33.92/33.92 (C34); 35.31/35.31 (C35); 22.61/24.50 (C36); 11.31/11.65 (C37); 49.17/48.95 (10-OMe); 56.49/55.16 (13-OMe); 57.05/60.05 (15-OMe); 57.86/57.77 (32-OMe); 15.01/15.88 (11-Me); 18.55/21.46 (17-Me); 15.96/19.4 br (19-Me); 10.12/9.78 (25-Me); 25.86, 25.83, 18.13, 18.01, 17.87, −4.09, −4.51, −4.75, −4.99 (2×TBDMS). ^{1}H NMR ($CDCl_3$, selected data), δ (*Z*-isomer, ppm): 5.14 (d, *J*=6.0 Hz, H-2); 3.40 (H-6a); 3.32 (H-6b); 2.10 (H-11); 3.45 (H-13); 3.62 (dd, *J*=9.5+1.6 Hz, H-14); 3.43 (H-15); 4.74 (d, *J*=10.2 Hz, H-20); 3.19 (ddd, *J*=10.0+10.0+4.2 Hz, H-21); 2.39 (H-23a); 2.24 (H-23b); 4.08 (ddd, *J*=9.0+3.4+3.4 Hz, H-24); 5.27 (br s, H-26); 5.12 (d, *J*=9.4 Hz, H-29); 2.93 (ddd, *J*=11.2+8.5+4.4 Hz, H-32); 3.37 (H-33); 1.05 (d, 3H, *J*=6.9 Hz, 11-Me); 1.62 (s, 3H, 19-Me); 1.61 (s, 3H, 27-Me); 0.07, 0.06, 0.01, −0.06 (s, each 3H, Si−Me); 0.88, 0.87 (s, each 9H, $(CH_3)_3$); 3.38 (s, 3H, 10-OMe); δ (*E*-isomer, ppm): 4.26 (d, *J*=5.4 Hz, H-2); 4.38 (br dd, *J*=13.0+3.4 Hz, H-6a); 3.20 (H-6b); 2.10 (H-11); 3.37 (H-13); 3.73 (H-14); 3.76 (H-15); 4.94 (d, *J*=10.1 Hz, H-20); 3.34 (H-21); 2.93 (H-23a); 2.35 (H-23b); 4.12 (dd, *J*=10.2+4.3 Hz, H-24); 5.22 (d, *J*=10.2 Hz, H-26); 5.33 (d, *J*=9.0 Hz, H-29); 2.93 (ddd, *J*=11.2+8.5+4.4 Hz, H-32); 3.37 (H-33); 1.09 (d, 3H, *J*=6.8 Hz, 11-Me); 1.74 (d, 3H, *J*=1.2 Hz, 19-Me); 1.53 (d, 3H, *J*=1.2 Hz, 27-Me); 0.07, 0.06, 0.02, 0.01 (s, each 3H, Si−Me); 0.89, 0.87 (s, each 9H, $(CH_3)_3$); 3.34 (s, 3H, 10-OMe).

4.2.18. Compound 15. CHN ($C_{56}H_{99}NO_{12}Si_2$) calcd: 65.01/9.65/1.35, found: 64.78/9.59/1.30. HRMS (M+Na; calcd/found): 1056.6604/1056.6603. ^{13}C NMR ($CDCl_3$, mixture of rotamers=1:4), δ (major rotamer, ppm): 169.92 (C1); 56.03 (C2); 28.02 (C3); 21.00 (C4); 25.17 (C5); 39.77 (C6); 165.39 (C8); 103.13 (C9); 209.31 (C10); 39.17 (C11); 39.59 (C12); 77.68 (C13); 78.67 (C14); 77.2 br (C15); 35.65 (C16); 25.7 br (C17); 40.2 br (C18); 139.17 (C19); 123.70 (C20); 56.03 (C21); 212.5 br (C22); n.d. (C23); 70.8 br (C24); 41.43 (C25); 78.67 (C26); 132.18 (C27); 12.6 br (C28); 134.5 br (C29); 35.04 (C30); 36.29 (C31); 84.09 (C32); 75.09 (C33); 33.85 (C34); 30.67 (C35); 23.66 (C36); 11.26 (C37); 55.25 (9-OMe); 57.13 (13-OMe); 56.15 (15-OMe); 57.84 (32-OMe); 16.92 (11-Me); 19.87 (17-Me); 15.88 (19-Me); 10.65 (25-Me); 25.86, 18.14, 17.94, −4.10, −4.46, −4.52, −4.74 (2×TBDMS). ^{1}H NMR ($CDCl_3$, selected data), δ (major rotamer, ppm): 5.59 (br d, *J*=4.5 Hz, H-2); 4.37 (d, *J*=13.5 Hz, H-6a); 2.84 (br s, H-6b); 2.94 (H-11); 3.42 (H-13); 3.49 (H-14); 3.62 (H-15); 4.68 (d, *J*=10.3 Hz, H-20); 3.30 (H-21); 2.57 (br s, H-23a); 2.27 (H-23b); 4.05 (ddd, *J*=6.6+6.6+2.3 Hz, H-24); 5.22 (H-26); 5.32 (d, *J*=9.1 Hz, H-29); 2.94 (H-32); 3.38 (H-33); 1.19 (d, 3H, *J*=6.6 Hz, 11-Me); 1.74 (s, 3H, 19-Me); 1.59 (s, 3H, 27-Me); 0.07, 0.06, 0.02, 0.01 (s, each 3H, Si−Me); 0.89, 0.87 (s, each 9H, $(CH_3)_3$); 3.62 (s, 3H, 9-OMe); δ (minor rotamer, ppm): 5.10 (H-2); 2.94 (H-11); 4.91 (d, *J*=10.0 Hz, H-20); 3.20 (H-21); 2.75 (dd, *J*=17.5+5.5 Hz, H-23a); 2.36 (dd, *J*=17.5+5.9 Hz, H-23b); 4.28 (H-24); 5.22 (H-29); 2.94 (H-32); 3.38 (H-33); 1.15 (d, 3H, *J*=6.6 Hz, 11-Me); 0.06, 0.05, 0.05, 0.01 (s, each 3H, Si−Me); 0.88, 0.85 (s, each 9H, $(CH_3)_3$); 3.32 (s, 3H, 9-OMe).

4.2.19. Compound 16. CHN ($C_{56}H_{99}NO_{12}Si_2$) calcd: 65.01/9.65/1.35, found: 65.06/9.57/1.17. HRMS (M+Na; calcd/found): 1056.6604/1056.6603. ^{13}C NMR ($CDCl_3$), δ (ppm): 170.58 (C1); 73.17 (C2); 28.11 (C3); 21.58 (C4); 23.42 (C5); 39.71 (C6); 166.4 (C8); 99.04 (C9); 80.28 (C10); 33.36 (C11); 34.15 (C12); n.d. (C13); 75.06 (C14); 78.46 (C15); n.d. (C16); 27.97 (C17); 38.7 br (C18); 136.3 br (C19); 124.71 (C20); 54.04 (C21); ~214 br (C22); n.d. (C23); n.d. (C24); n.d. (C25); n.d. (C26); 131.2 br (C27); 13.8 br (C28); 127.9 br (C29); 35.35 (C30); 36.38 (C31); 84.22 (C32); 75.23 (C33); 33.99 (C34); 30.85 (C35); 25.14 (C36); 11.88 (C37); 54.41 (9-OMe); ~58 br (13-OMe); 56.04 (15-OMe); 57.63 (32-OMe); 17.9 br (11-Me); 21.46 (17-Me); 17.58 (19-Me); 10.04 (25-Me); 25.85, 25.84, 18.17, 18.00, −4.16, −4.53, −4.82 (2×TBDMS). ^{1}H NMR ($CDCl_3$, selected data), δ (ppm): 2.19 (d, *J*=13.7 Hz, H-3a); 2.02 (H-3b); 4.01 (d, *J*=12.5 Hz, H-6a); 2.57 (br s, H-6b); 1.71 (H-11); 3.02 (br s, H-13); 3.68 (br d, *J*~9.0 Hz, H-14); 3.50 (br s, H-15); 4.97 (d, *J*=8.5 Hz, H-20); 3.40 (H-21); 4.22 (br s, H-24); 4.83 (br s, H-26); 5.41 (br d, *J*=6.5 Hz, H-29); 2.96 (ddd, *J*=11.4+8.5+4.6 Hz, H-32); 3.38 (H-33); 0.97 (br s, 3H, 11-Me); 1.74 (s, 3H, 19-Me); 1.61 (s, 3H, 27-Me); 0.06, 0.06, 0.04, 0.01 (s, each 3H, Si−Me); 0.88, 0.86 (s, each 9H, $(CH_3)_3$); 3.77 (s, 3H, 9-OMe); 3.69 (s, 10-OH).

4.2.20. Compound 17. CHN ($C_{56}H_{99}NO_{12}Si_2$) calcd: 65.01/9.65/1.35, found: 64.70/9.54/1.23. HRMS (M+Na; calcd/found): 1056.6604/1056.6599. ^{13}C NMR ($CDCl_3$), δ (ppm): 163.87 (C1); 72.44 (C2); 31.82 (C3); 20.40 (C4); 24.33 (C5); 37.24 (C6); 168.06 (C8); 204.20 (C9); 84.34 (C10); 34.21 (C11); 30.33 (C12); 79.46 (C13); 74.07 (C14); 78.83 (C15); 39.06 (C16); 26.69 (C17); 47.73 (C18); 140.31 (C19); 123.72 (C20); 56.20 (C21); 208.67 (C22); 47.32

(C23); 67.98 (C24); 39.30 (C25); 86.26 (C26); 130.76 (C27); 11.11 (C28); 137.65 (C29); 35.17 (C30); 36.09 (C31); 84.07 (C32); 75.09 (C33); 33.84 (C34); 30.60 (C35); 22.31 (C36); 11.47 (C37); 54.26 (10-OMe); 57.10 (13-OMe); 59.60 (15-OMe); 57.88 (32-OMe); 16.45* (11-Me); 20.50 (17-Me); 16.41* (19-Me); 9.89 (25-Me); 25.86, 25.83, 18.15, 18.04, −3.83, −4.28, −4.52, −4.74 (2×TBDMS). ^{1}H NMR ($CDCl_3$), δ (ppm): 2.44 (br d, J=12.5 Hz, H-3a); 1.42 (H-3b); 4.44 (br dd, J=13.0+3.0 Hz, H-6a); 3.23 (H-6b); 2.57 (H-11); 3.57 (H-13); 3.68 (dd, J=7.0+3.6 Hz, H-14); 3.26 (H-15); 4.73 (d, J=10.7 Hz, H-20); 3.23 (H-21); 2.80 (dd, J=15.0+10.5 Hz, H-23a); 2.21 (dd, J=15.0+4.5 Hz, H-23b); 4.10 (dd, J=10.5+4.5 Hz, H-24); 5.10 (d, J=10.4 Hz, H-26); 5.36 (d, J=9.0 Hz, H-29); 2.95 (ddd, J=11.3+8.6+4.5 Hz, H-32); 3.40 (H-33); 0.80 (d, 3H, J=7.5 Hz, 11-Me); 1.82 (s, 3H, 19-Me); 1.60 (s, 3H, 27-Me); 0.07, 0.06, 0.01, 0.00 (s, each 3H, Si−Me); 0.89, 0.85 (s, each 9H, $(CH_3)_3$); 3.16 (s, 3H, 10-OMe).

4.2.21. Compound 18. (a) from **6a**. To a solution of 0.5 g (0.48 mmol) hydroxy acid **6a** in 30 ml benzene were added 1.0 g (4.7 equiv., 2.26 mmol) lead tetra acetate in one portion. The resultant suspension was stirred for 30 min at room temperature and then partitioned between ethyl acetate and a saturated aqueous sodium hydrogen carbonate solution. The aqueous layer was removed and the organic layer was washed twice with brine, dried over sodium sulfate and evaporated to dryness at reduced pressure. The residue was subjected to a short flash column chromatography to give 0.46 g (96%) **18** as an amorphous powder. (b) from **6b**. Starting from 0.5 g **6b** the reaction, work up and purification was performed as described above to give 0.47 g (98%) **18**, CHN ($C_{54}H_{97}NO_{11}Si_2$) calcd: 65.35/9.85/1.41, found: 65.30/9.83/1.22. HRMS (M+Na; calcd/found): 1014.6498/1014.6508. ^{13}C NMR ($CDCl_3$, single rotamer), δ (ppm): 169.74 (C1); 57.00 (C2); 27.53 (C3); 20.65 (C4); 25.85 (C5); 42.8 br (C6); 169.39 (C8); 97.43 (C10); 36.69 (C11); 32.71 (C12); 73.72 (C13); 72.87 (C14); 75.59 (C15); 31.84 (C16); 25.56 (C17); 49.65 (C18); 137.2 br (C19); 123.37 (C20); 54.40 (C21); 210.86 (C22); 46.4 br (C23); 70.40 (C24); 39.4 br (C25); n.d. (C26); n.d. (C27); 10.9 br (C28); 135.35 (C29); 35.06 (C30); 36.25 (C31); 84.27 (C32); 75.11 (C33); 34.02 (C34); 30.65 (C35); 25.79 (C36); 11.44 (C37); 56.21 (13-OMe); 57.23 (15-OMe); 57.75 (32-OMe); 15.77 (11-Me); 20.08 (17-Me); 14.43 (19-Me); 10.9 br (25-Me); 25.93, 25.79, 18.13, 18.04, −4.11, −4.52, −4.75 (2×TBDMS). ^{1}H NMR ($CDCl_3$, selected data), δ (ppm): 5.37 (H-2); 4.47 (br d, J=13.0 Hz, H-6a); 2.76 (H-6b); 1.82 (H-11); 2.07 (ddd, J=12.3+4.6+4.6 Hz, H-12a); 3.39 (H-13); 3.81 (dd, J=9.7+1.8 Hz, H-14); 3.54 (ddd, J=11.5+4.7+1.4 Hz, H-15); 5.05 (br s, H-20); 3.31 (H-21); 2.72 (br s, H-23a); 2.54 (br s, H-23b); 4.00 (br s, H-24); 5.05 (br s, H-26); 5.35 (d, J=8.7 Hz, H-29); 2.92 (ddd, J=11.2+8.5+4.5 Hz, H-32); 3.40 (H-33); 0.81 (d, 3H, J=6.6 Hz, 11-Me); 1.52 (s, 3H, 19-Me); 1.46 (s, 3H, 27-Me); 0.07, 0.06, 0.05, 0.03 (s, each 3H, Si−Me); 0.88, 0.85 (s, each 9H, $(CH_3)_3$); 5.64 (10-OH).

4.2.22. Compound 19. Starting from 0.3 g (0.27 mmol) **21** or 0.4 g (0.4 mmol) **18**, the deprotection and work up was performed as described above for **13a** to give after a flash column chromatography (silica gel, ethyl acetate) 0.17 g (82%) or 0.24 g (78%) **19** as amorphous powders. HRMS (M+Na; calcd/found): 786.4768/786.4757. ^{13}C NMR ($CDCl_3$, hemiketal form/ketone form=5:1), δ (hemiketal/ketone, ppm): 169.81 or 169.73/169.43 (C1); 56.92/52.63 (C2); 28.39/26.11 (C3); 21.06/21.29 (C4); 25.57/25.20 (C5); 42.05/44.38 (C6); 169.81 or 169.73/166.55 (C8); 97.64/202.68 (C10); 37.10/40.08 (C11); 32.31/32.21 (C12); 73.48/80.79 (C13); 73.11/73.19 (C14); 75.94/78.09 (C15); 32.86/35.58 (C16); 25.11/27.55 (C17); 49.34/48.51 (C18); 139.01/138.56 (C19); 123.84/124.76 (C20); 53.76/55.11 (C21); 213.94/211.87 (C22); 47.51/45.35 (C23); 67.01/69.94 (C24); 41.90/38.50 (C25); 78.31/82.79 (C26); 132.51/130.92 (C27); 13.53/12.46 (C28); 130.61/133.47 (C29); 34.92/34.99 (C30); 34.73/34.49 (C31); 84.17/84.21 (C32); 73.52/73.52 (C33); 31.22/31.21 (C34); 30.54/30.41 (C35); 24.43/23.29 (C36); 11.55/11.61 (C37); 55.89/57.35 (13-OMe); 57.84/57.05 (15-OMe); 56.50/56.43 (32-OMe); 15.75/14.70 (11-Me); 20.09/21.04 (17-Me); 14.70/14.78 (19-Me); 9.77/9.84 (25-Me). ^{1}H NMR ($CDCl_3$, selected data), δ (hemiketal form, ppm): 5.53 (s, H-2); 4.40 (d, J=13.0 Hz, H-6a); 3.05 (br dd, J=13.0+13.0 Hz, H-6b); 1.79 (H-11); 3.49 (H-13); 3.83 (H-14); 3.54 (ddd, J=11.2+5.0+2.1 Hz, H-15); 4.88 (d, J=10.1 Hz); 3.41 (H-21); 2.98 (dd, J=14.8+4.0 Hz, H-23a); 2.53 (dd, J=14.8+9.0 Hz, H-23b); 3.80 (H-24); 5.25 (s, H-26); 5.27 (d, J=9.2 Hz, H-29); 2.99 (H-32); 3.40 (H-33); 0.80 (d, 3H, J=6.6 Hz, 11-Me); 1.54 (s, 3H, 19-Me); 1.62 (s, 3H, 27-Me); 5.62 (s, 10-OH); δ (ketone form, ppm): 5.21 (d, J=5.5 Hz, H-2); 3.80 (H-6a); 3.00 (H-6b); 3.40 (H-11); 3.80 (H-14); 3.26 (H-15); 4.85 (d, J=10.2 Hz, H-20); 3.39 (H-21); 2.71 (dd, J=18.0+3.3 Hz, H-23a); 2.53 (H-23b); 3.98 (H-24); 5.08 (d, J=8.0 Hz, H-26); 5.32 (d, J=9.3 Hz, H-29); 2.99 (H-32); 3.40 (H-33); 1.21 (d, 3H, J=7.0 Hz, 11-Me); 1.59 (s, 3H, 19-Me); 1.57 (s, 3H, 27-Me).

4.2.23. Compounds 20a and 20b. (a) from **12a**. A small glass tube was charged with 0.5 g (0.43 mmol) **12a** and a magnetic stirring bar and immersed in a preheated (160 °C) oil bath. After approx. 2 min, carbon dioxide formation occurred in the clear liquid which ceased after two additional minutes. After 8 min, the mixture was cooled down to room temperature, diluted in 3 ml dichloromethane and subjected to flash column chromatography (silica gel, dichloromethane/acetone=20:1) to give after evaporation and drying of the relevant fractions at high vacuum 0.36 g (74%) **20a** and 63 mg (13%) **20b** as amorphous powders. (b) from **12b**. Starting from 100 mg (0.087 mmol) **12b**, the decarboxylation was performed as described above to give 78 mg (81%) **20a** and 10 mg (10%) **20b**.

4.2.24. Compound 20a. CHN ($C_{60}H_{113}NO_{11}Si_3$) calcd: 64.99/10.27/1.26, found: 65.20/10.05/1.22. HRMS (M+Na; calcd/found): 1130.7519/1130.7525. ^{13}C NMR ($CDCl_3$, Z/E=1:2), δ (Z/E-isomer, ppm): 169.73/169.34 (C1); 52.88/54.66 (C2); 26.60/27.50 (C3); 21.01/20.32 (C4); 26.38/24.68 (C5); 43.60/40.01 (C6); 172.99/174.20 (C8); 71.50/69.05 (C10); 34.40/33.04 (C11); 33.41/32.23 (C12); 79.64/79.72 (C13); 78.11/72.67 (C14); 80.36/81.53 (C15); 40.69/37.28 (C16); 31.20/32.33 (C17); 47.08/45.88 (C18); 139.16/140.91 (C19); 124.10/123.43 (C20); 54.88/55.20 (C21); 208.80/209.65 (C22); 45.88/47.92 (C23); 68.37/67.52 (C24); 39.21/38.58 (C25); 81.74/83.13 (C26); 131.65/130.81 (C27); 12.04/11.58 (C28); 135.30/136.76 (C29); 35.01/35.15 (C30); 36.23/36.15 (C31); 84.09/84.09 (C32);

75.11/75.11 (C33); 33.88/33.88 (C34); 30.73/30.66 (C35); 23.34/23.36 (C36); 11.49/11.49 (C37); 59.92/55.83 (13-OMe); 57.11/58.26 (15-OMe); 57.84/57.90 (32-OMe); 12.98/13.82 (11-Me); 20.23/21.33 (17-Me); 16.54/18.54 (19-Me); 11.49/9.70 (25-Me); 26.14, 26.00, 25.91, 25.86, 25.85, 18.15, 18.06, 18.02, −3.62, −4.16, −4.34, −4.52, −4.75, −4.90 (3×TBDMS). ^{1}H NMR ($CDCl_3$, selected data), δ (*Z*-isomer, ppm): 5.39 (d, *J*=4.8 Hz, H-2); 3.73 (br d, *J*=13.3 Hz, H-6a); 3.20 (H-6b); 4.40 (d, *J*=2.1 Hz, H-10); 1.85 (H-11); 3.27 (H-13); 3.71 (dd, *J*=8.0+1.4 Hz, H-14); 3.29 (H-15); 4.78 (d, *J*=10.5 Hz, H-20); 3.19 (H-21); 2.78 (dd, *J*=17.4+8.0 Hz, H-23a); 2.36 (dd, *J*=17.4+4.6 Hz, H-23b); 4.14 (ddd, *J*=8.0+4.6+2.3 Hz, H-24); 5.19 (d, *J*=8.7 Hz, H-26); 5.27 (d, *J*=8.9 Hz, H-29); 2.95 (H-32); 3.40 (H-33); 0.79 (d, 3H, *J*=6.6 Hz, 11-Me); 1.78 (s, 3H, 19-Me); 1.57 (s, 3H, 27-Me); 0.10, 0.09, 0.08, 0.06, 0.05, 0.01 (s, each 3H, Si−Me); 0.92, 0.89, 0.88 (s, each 9H, $(CH_3)_3$); δ (*E*-isomer, ppm): 4.46 (d, *J*=5.1 Hz, H-2); 4.39 (d, *J*=12.0 Hz, H-6a); 3.16 (H-6b); 4.22 (s, H-10); 1.85 (H-11); 3.27 (H-13); 3.95 (dd, *J*=8.0+1.6 Hz, H-14); 3.08 (H-15); 4.66 (d, *J*=10.3 Hz, H-20); 3.23 (H-21); 2.85 (dd, *J*=16.7+10.3 Hz, H-23a); 2.31 (dd, *J*=16.7+4.0 Hz, H-23b); 4.09 (ddd, *J*=10.3+4.0+1.4 Hz, H-24); 5.16 (d, *J*=9.6 Hz, H-26); 5.34 (d, *J*=8.7 Hz, H-29); 2.95 (H-32); 3.40 (H-33); 0.80 (d, 3H, *J*=6.6 Hz, 11-Me); 1.80 (d, 3H, *J*=1.0 Hz, 19-Me); 1.64 (d, 3H, *J*=1.1 Hz, 27-Me); 0.09, 0.08, 0.08, 0.07, 0.02, 0.01 (s, each 3H, Si−Me); 0.91, 0.89, 0.87 (s, each 9H, $(CH_3)_3$).

4.2.25. Compound 20b. CHN ($C_{60}H_{113}NO_{11}Si_3$) calcd: 64.99/10.27/1.26, found: 65.22/10.11/1.25. HRMS (M+Na; calcd/found): 1130.7519/1130.7520. ^{13}C NMR ($CDCl_3$, mixture of rotamers >9:1), δ (major rotamer, ppm): 168.69 (C1); 53.13 (C2); 26.40 (C3); 21.24 (C4); 25.06 (C5); 43.16 (C6); 174.94 (C8); 72.56 (C10); 34.96 (C11); 29.96 (C12); 83.35 (C13); 75.01 (C14); 79.53 (C15); 42.61 (C16); 27.48 (C17); 48.01 (C18); 139.66 (C19); 123.57 (C20); 55.16 (C21); 210.21 (C22); 45.83 (C23); 68.99 (C24); 40.53 (C25); 78.70 (C26); 132.34 (C27); 13.70 (C28); 131.17 (C29); 34.96 (C30); 36.61 (C31); 84.14 (C32); 75.17 (C33); 33.87 (C34); 30.95 (C35); 23.55 (C36); 11.50 (C37); 56.68 (13-OMe); 60.64 (15-OMe); 57.99 (32-OMe); 21.31 (11-Me); 20.27 (17-Me); 15.97 (19-Me); 9.71 (25-Me); 25.99, 25.86, 25.80, 18.39, 18.16, 17.97 (3×TBDMS). ^{1}H NMR ($CDCl_3$, selected data, δ (major rotamer, ppm): 5.17 (d, *J*=5.3 Hz, H-2); 3.66 (br d, *J*=13.0 Hz, H-6a); 3.11 (H-6b); 4.22 (s, H-10); 1.85 (H-11); 2.87 (d, *J*=11.0 Hz, H-13); 3.66 (d, *J*=8.5 Hz, H-14); 2.97 (H-15); 4.87 (d, *J*=10.3 Hz, H-20); 3.11 (H-21); 2.67 (dd, *J*=17.2+5.0 Hz, H-23a); 2.35 (dd, *J*=17.2+6.6 Hz, H-23b); 4.27 (H-14); 5.11 (d, *J*=4.4 Hz, H-26); 5.10 (d, *J*=9.4 Hz, H-29); 2.95 (H-32); 3.39 (H-33); 1.16 (d, 3H, *J*=7.1 Hz, 11-Me); 1.76 (d, 3H, *J*=0.7 Hz, 19-Me); 1.59 (d, 3H, *J*=1.0 Hz, 27-Me); 0.08, 0.08, 0.07, 0.06, 0.05, −0.01 (s, each 3H, Si−Me); 0.91, 0.89, 0.85 (s, each 9H, $(CH_3)_3$); 3.48 (br s, 10-OH).

4.2.26. Compound 21. (a) from **20a**. To a solution of 0.3 g (0.27 mmol) **20a** in 10 ml dichloromethane were added 0.34 g (8.12 mmol, 3 equiv.). Dess−Martin periodinane and the suspension was stirred for 5 h at room temperature. The resultant mixture was directly subjected to a short flash column chromatography (silica gel, dichloromethane/acetone=50:1) to afford 0.26 g (87%) **21**. (b) from **20b**. Starting from 45 mg (0.041 mmol) **20b** and applying the same reaction conditions and work up as described above provided 38 mg (84%) **21** as amorphous powder: CHN ($C_{60}H_{111}NO_{11}Si_3$) calcd: 65.11/10.11/1.27, found: 65.00/9.92/1.15. HRMS (M+Na; calcd/found): 1128.7363/1128.7363. ^{13}C NMR ($CDCl_3$, mixture of rotamers >9:1), δ (major rotamer, ppm): 168.84 (C1); 51.36 (C2); 25.53 (C3); 20.65 (C4); 25.73 (C5); 44.58 (C6); 167.08 (C8); 203.70 (C10); 40.46 (C11); 31.78 (C12); 82.37 (C13); 75.75 (C14); 78.24 (C15); 42.93 (C16); 26.56 (C17); 46.40 (C18); 139.92 (C19); 123.48 (C20); 55.79 (C21); 208.97 (C22); 47.73 (C23); 67.70 (C24); 38.75 (C25); 82.95 (C26); 130.87 (C27); 11.25 (C28); 136.17 (C29); 35.08 (C30); 36.27 (C31); 84.19 (C32); 75.18 (C33); 34.03 (C34); 30.72 (C35); 23.61 (C36); 11.69 (C37); 56.85 (13-OMe); 61.09 (15-OMe); 57.78 (32-OMe); 16.48 (11-Me); 20.54 (17-Me); 16.02 (19-Me); 8.87 (25-Me); 26.05, 25.87, 25.73, 18.41, 18.18, 18.05, −3.95, −4.17, −4.40, −4.49, −4.74, −4.85 (3×TBDMS). ^{1}H NMR ($CDCl_3$, selected data), δ (major rotamer, ppm): 5.24 (br d, *J*=4.0 Hz, H-2); 3.43 (H-6a); 3.05 (ddd, *J*=13.8+13.8+2.8 Hz, H-6b); 3.28 (H-11); 2.13 (dd, *J*=14.7+10.5 Hz, H-12a); 2.79 (d, *J*=9.6 Hz, H-13); 3.63 (d, *J*=9.2 Hz, H-14); 3.20 (H-15); 4.61 (d, *J*=10.3 Hz, H-20); 3.22 (H-21); 2.87 (dd, *J*=16.0+9.9 Hz, H-23a); 2.22 (dd, *J*=16.0+4.0 Hz, H-23b); 4.17 (dd, *J*=9.9+4.0 Hz, H-24); 4.92 (d, *J*=9.9 Hz, H-26); 5.35 (d, *J*=8.7 Hz, H-29); 2.93 (ddd, *J*=11.2+8.5+4.4 Hz, H-32); 3.40 (H-33); 1.19 (d, 3H, *J*=7.6 Hz, 11-Me); 1.78 (s, 3H, 19-Me); 1.44 (s, 3H, 27-Me); 0.10, 0.08, 0.06, 0.03 (s, each 3H, Si−Me), 0.07 (s, 6H, Si−Me); 0.93, 0.89, 0.88 (s, each 9H, $(CH_3)_3$).

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32. Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 219392 (compound **13a**) and CCDC 219393 (compound **8**). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1 EZ, UK [fax:+44-1223-336033 or email: deposit@ccdc.cam.ac.uk].