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Synthesis and Biological Properties of the Cytotoxic 14-Membered Macrolides Aspergillide A and B

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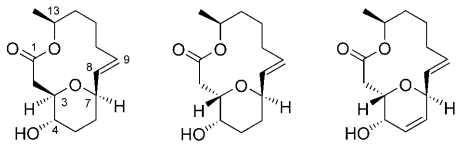
Dedicated to Professor José Barluenga on the occasion of his 70th birthday

Abstract: Total, stereoselective syntheses of the naturally occurring, cytotoxic macrolides aspergillide A and B are described. Olefin metatheses and asymmetric allylations were key steps in the synthetic sequences. Cytotoxicity assays against several tumor cell lines have been performed for the two aspergillides and some of the intermediates or side products of the synthetic sequence. One of these intermediates has been found markedly active against the human leukemia cancer cell line HL-60, with an IC_{50} value comparable with that of the clinical drug fludarabine.

Keywords: antitumor agents · asymmetric allylation · cytotoxicity · macrolides · olefin metathesis

Introduction

The aspergillides A, B and C (Scheme 1, **1**–**3**) are three 14-membered macrolides isolated from a strain of the marine-derived fungus *Aspergillus ostianus* cultivated in a bromine-modified medium.[1,2] The compounds showed cytotoxic activity in the micromolar range against mouse lymphocytic leukemia cells (L1210). Their stereostructures show some unusual features. For instance, only two recent examples have been reported of naturally occurring, 14-membered macrolides that possess a tetrahydropyran ring not forming part of a hemiacetal or acetal moiety.[3,4] This and the aforementioned bioactivities prompted us and other groups to perform total syntheses of these compounds. As commented below, these synthetic efforts have led to a correction of the originally published structures of aspergillides A and B, as well as to a confirmation of the structure of aspergillide C.



Scheme 1. Correct stereostructures of aspergillides A (**1**), B (**2**) and C (**3**).

In the beginning of 2009, Uenishi et al. published their synthesis of a compound with structure **2**, which at that time was believed to correspond to aspergillide A.[5] As a matter of fact, these authors found that their synthetic compound had spectral properties identical with those reported for aspergillide B. The latter compound was thus assigned structure **2**, which led to the need of a revised structure for aspergillide A. Shortly afterwards, we published our own synthesis of compound **2**, which was found to be identical with aspergillide B,[6] therefore confirming the findings of Uenishi and his group.[5] Eventually, the group who isolated these natural compounds succeeded in carrying out X-ray diffraction analyses[7] of suitable derivatives of aspergillides A and B. This led to the assignment of **1** and **2**, respectively, as the correct stereostructures of these natural compounds.[8] Very recently, we have communicated our own synthesis of asper-

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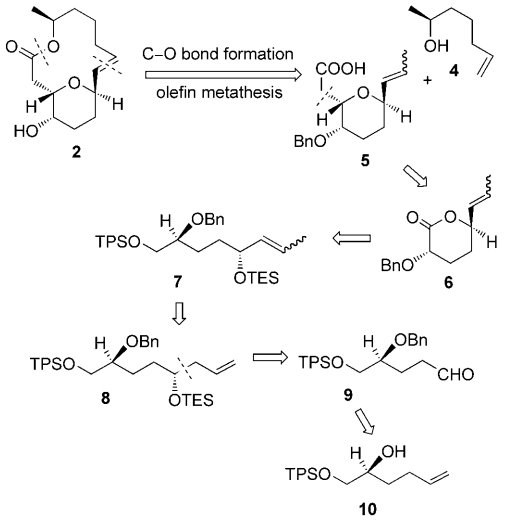
gillide A, which confirms the structure deduced from the diffraction analysis.[9] Finally, the structure of aspergillide C has recently been confirmed by two total syntheses.[10]

In the present manuscript, we wish to publish the full details of our total syntheses of aspergillides A (**1**) and B (**2**). We will also report on the cytotoxic activity of these two compounds and of some of the intermediates of the synthetic sequence against several cancer cell lines.

Results and Discussion

At the onset of our research, aspergillide A was still assumed to have structure **2**. Its retrosynthetic analysis is depicted in Scheme 2. Hydrolytic opening of the lactone ring and inverse metathesis yields the known alcohol **4**[11] and tetrahydropyran **5**. The reconstitution of the lactone ring from precursors **4** and **5** was feasible in principle either by means of esterification (intermolecular C–O bond formation) followed by ring-closing metathesis (RCM, intramolecular C=C-bond formation) or, conversely, through initial cross metathesis (CM) followed by intramolecular C–O-bond formation (macrolactonization). We opted for the latter alternative because of the stereochemical uncertainties of the *E*/*Z* stereoselectivity in the RCM step, CM being expected to give a higher proportion of the required *E* configuration of the olefinic bond.[12,13]



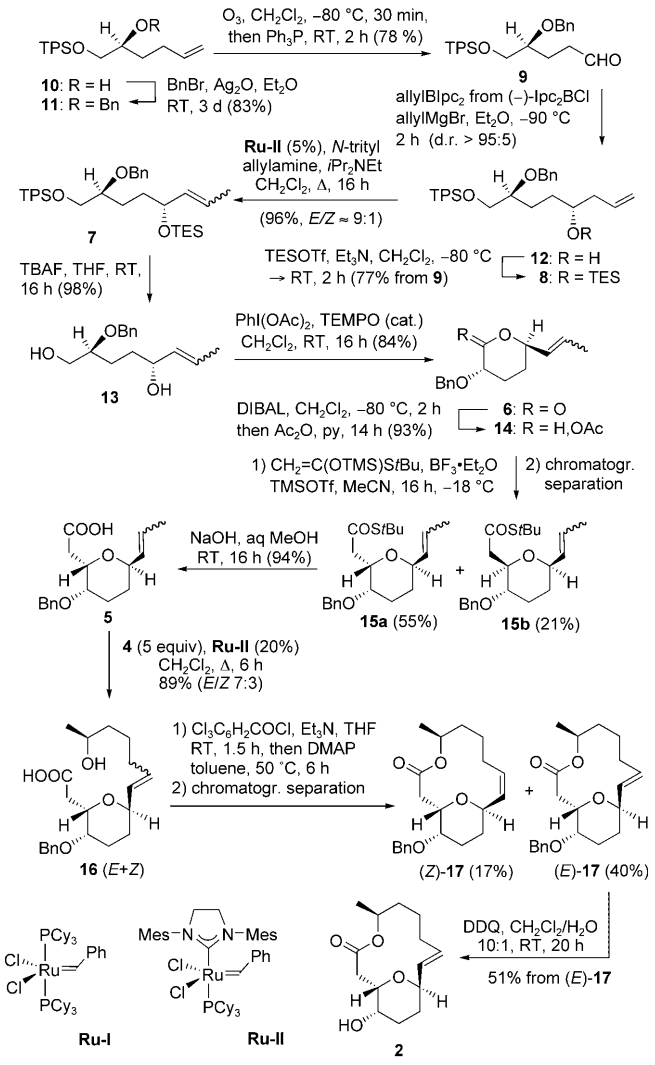
Scheme 2. Retrosynthetic analysis of **2**, later shown to be aspergillide B. Bn = benzyl; TPS = *tert*-butyldiphenylsilyl; TES = triethylsilyl.

We planned the stereoselective preparation of the 2,6-*trans*-disubstituted tetrahydropyran[14–16] **5** by means of a Mukaiyama-type C-glycosidation[17] of a suitable lactol derivative prepared through reduction of lactone **6**. The latter was to be obtained by functional modification of **7**, in turn derived from **8** through a metal-catalyzed double bond migration.[18] Following this, retrosynthetic asymmetric allylation to aldehyde **9** and retro-oxidative cleavage of the olefinic bond finally leads to the known compound **10**.[19]

Scheme 3 shows the details of the synthesis. Alcohol **10** was benzylated to **11** under mild, nonbasic conditions.[20] Ozonolytic cleavage of the olefinic bond yielded aldehyde **9**, which was first purified and then subjected to Brown's asymmetric allylboration.[21] This gave homoallyl alcohol **12** in a very high diastereomeric purity (d.r. >95:5 by NMR), which was subsequently protected as its triethylsilyl derivative **8**. Isomerization of the terminal olefinic bond to the internal position was achieved by means of Wipf's adaptation of the catalytic method reported by Roy et al.[22] With the aid of this procedure, compound **8** was converted into its isomer **7** in high yield.[23] Compound **7** was obtained as a 9:1 *E*/*Z* mixture[24] which proved difficult to separate and was thus carried as such until the CM step. Cleavage of the two silyl groups and selective oxidation of the primary alcohol with $PhI(OAc)_2$/TEMPO[16g,25] afforded δ-lactone **6**. Reduction of **6** with DIBAL followed by acetylative quenching yielded the acetylated lactol **14** as a mixture of stereoisomers,[26] which were not separated. The mixture was subsequently treated with the trimethylsilyl enolate of *tert*-butyl thioacetate[27] in the presence of the Lewis acids BF_3-etherate and TMSOTf.[15a,28] This furnished the *trans*-2,6-disubstituted[16,29] tetrahydropyran **15a** in 55% yield, accompanied by its epimer at C-3, **15b** (21%). After chromatographic separation, alkaline hydrolysis of **15a** provided acid **5** in high yield.

Treatment of **5** with five equivalents of olefinic alcohol **4** in the presence of 20% of the second-generation ruthenium catalyst **Ru-II** caused cross-metathesis[13] and afforded hydroxy acid **16** in 89% yield as a 7:3 *E*/*Z* mixture. Macrolactonization was performed on the mixture by means of the Yamaguchi procedure[30] and gave a separable mixture of (*E*)-**17** and (*Z*)-**17**. Cleavage of the benzyl group in (*E*)-**17** was performed with DDQ[31] in wet CH_2Cl_2 and yielded lactone **2** in 51% yield.[32,33] The synthetic compound showed physical and spectral properties identical to those published for aspergillide B,[1] as also found by Hande and Uenishi.[5]

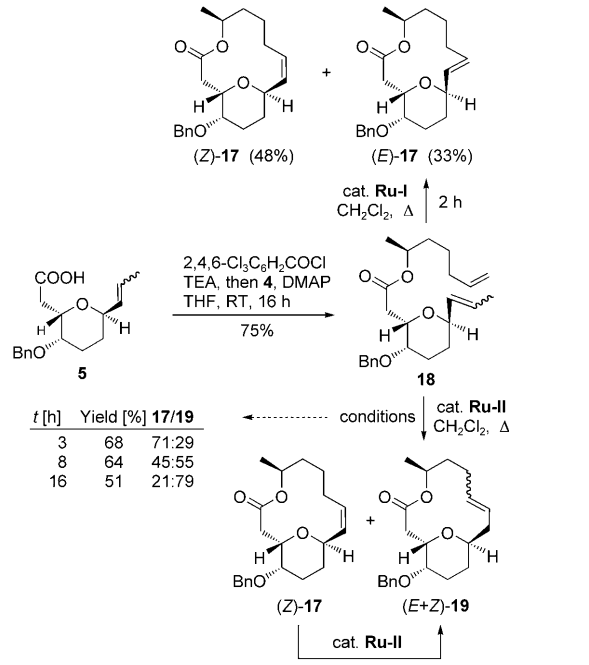
Even though the yield of the CM step **5** → **16** was good (89%), both the stereoselectivity of this step and the chemical yield of the macrolactonization step **16** → **17** were not as satisfactory as desired. For this reason, we decided to investigate whether the change of order of these two steps might, despite our previous concern, lead to higher yields of lactone (*E*)-**17**. The results of these efforts are shown in Scheme 4. Yamaguchi esterification[30] of acid **5** with alcohol **4** gave ester **18** in 75% yield. RCM of this ester catalyzed by the first-generation Grubbs catalyst **Ru-I** led to a mixture of lactones (*E*+*Z*)-**17** with predominance of the undesired *Z* isomer. When catalyst **Ru-II** was used, two lactones were formed, too, together with decomposition products. Surprisingly, while one of the lactones was (*Z*)-**17**, the other was found to have structure **19** (~85:15 *E*/*Z* mixture), a product of double bond migration (from $\Delta^{8,9}$ to $\Delta^{9,10}$, aspergillide numbering). The longer the reaction time, the higher the percentage of **19** and also of decomposition products. Lactone (*E*)-**17** was formed, if at all, in a very small amount.



Scheme 3. Synthesis of aspergillide B (**2**). Ipc = isopinocampheyl; TBAF = tetra-*n*-butylammonium fluoride; DMAP = 4-(*N*,*N*-dimethylamino)-pyridine; Tf = trifluoromethanesulfonyl; TMS = trimethylsilyl; TEMPO = 2,2,6,6-tetramethylpiperidine-1-oxyl; DIBAL = diisobutylaluminum hydride; DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone.

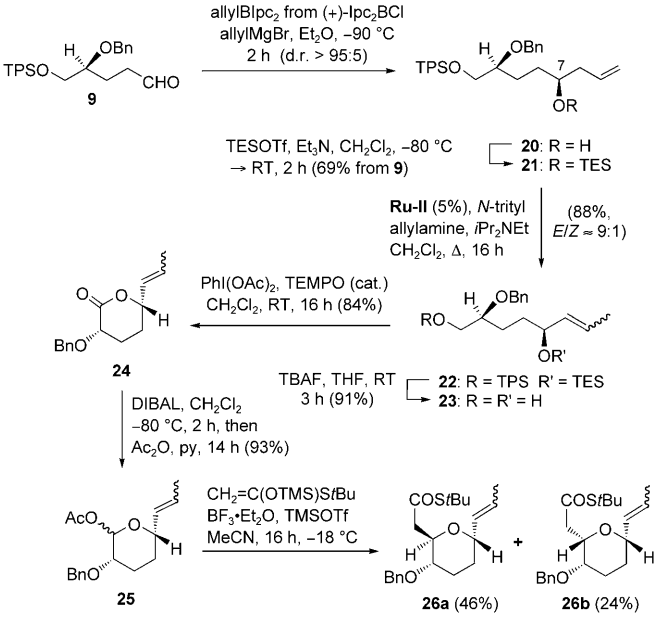
Furthermore, when subjected to RCM reaction conditions with **Ru-II**, lactone (*Z*)-**17** was progressively converted into (*E*+*Z*)-**19**. These facts suggest that lactone **19** is thermodynamically more stable than either (*E*)-**17** or (*Z*)-**17**, a conclusion supported by theoretical calculations.[34] Indeed, such double-bond migrations and other various "non-metathesis" side reactions in Ru-catalyzed processes have been attributed to the in situ formation of ruthenium hydrides, this being more frequent with second-generation catalysts like **Ru-II**.[35] In any case, this alternative route does not lead to an improvement in the yield of the required (*E*)-**17**.

When we initiated the synthesis of aspergillide A, we were still unaware of the actual structure of this natural macrolide. A comparative study of the NMR data of several of our synthetic compounds with those of the natural product suggested that aspergillide A might contain, in contrast



Scheme 4. Attempts at an improved preparation of intermediate (*E*)-**17**.

to **2**, a *cis*-2,6-disubstituted tetrahydropyran moiety. Under the assumption that aspergillide A and B could possibly be epimeric at C-7 (Scheme 1), we tried to adapt to the preparation of aspergillide A the same route that was successful for aspergillide B (Scheme 3). Thus, aldehyde **9** was subjected as previously to Brown's asymmetric allylation but now with the chiral allylborane prepared from allylmagnesium bromide and (+)-Ipc$_2$BCl (Scheme 5). This gave secondary alcohol **20**, with the opposite configuration at C-7 (aspergil-

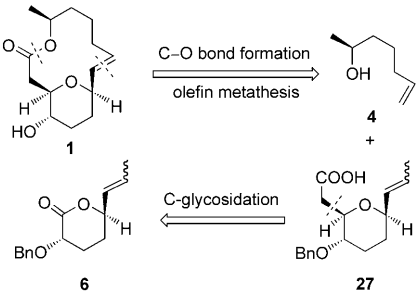


Scheme 5. Initial route towards possible structures of aspergillide A.

lide numbering) as compared with **12** (Scheme 3). Alcohol **20** was then subjected to the same reaction sequence depicted in Scheme 3 for **12**. However, this led to a disappointment, as the C-glycosidation step provided in 70% overall yield a mixture **26a**/**26b** in which the targeted *cis*-2,6-disubstituted tetrahydropyran **26b** was the minor component. Variation of the reaction conditions did not bring much change in the diastereomeric ratio.

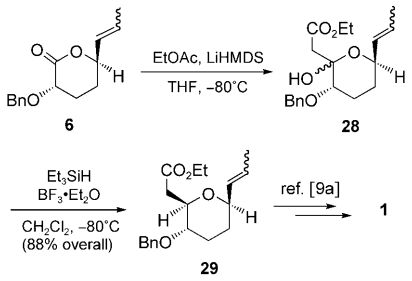
Just at this moment, we learned the results of X-ray diffraction analyses[7] of aspergillide A, which showed that the compound had actually structure **1** (Scheme 1). Thus, aspergillides A and B are epimeric at C-3, not at C-7. This apparently minor stereochemical difference between **1** and **2** affects the retrosynthetic concept in a crucial way, as it involves the methodology needed to create the relative configuration of the stereocenters C-3/C-7.[14–16]

The retrosynthetic analysis as depicted in Scheme 6 relies on that proposed for aspergillide B (**2**).[6] Retrosynthetic opening of the lactone ring by means of hydrolytic cleavage of the ester moiety and olefin metathesis[12,13] gives rise to the known alcohol **5**[11] as well as to acid **27**. Although the results depicted in Scheme 5 suggested that the formation of the tetrahydropyran ring[14] of **27** through a stereocontrolled C-glycosidation[17] of a lactol derivative prepared via reduction of δ-lactone **7**[6] was not a promising way, we made some attempts in this direction. However, all attempts to obtain **27** or a similar derivative using a Mukaiyama-type C-glycosidation on a lactol derivative prepared from **6** were not satisfactory. Mixtures of *cis*-/*trans*-2,6-disubstituted tetrahydropyrans were always obtained, with the desired *cis* isomer never being the major compound.[36]



Scheme 6. Retrosynthetic analysis of aspergillide A (**1**).

In the strategy we have followed until now for the conversion of the δ-lactone moiety into a tetrahydropyran ring, we first introduced the hydrogen atom (DIBAL reduction to the lactol) and then the acetic acid side chain (Mukaiyama C-glycosidation). We then investigated the result of a change in the order of steps, that is, by introducing first the acetic acid chain and then the hydrogen atom. In accordance with this idea, we finally reached success in the way shown in Scheme 7. Addition of the lithium enolate of ethyl acetate to lactone **6** at low temperature gave lactol **28** as a mixture of stereoisomers at the olefinic bond and at the hemiketal carbon. Treatment of this mixture with $Et_3SiH/BF_3{\cdot}Et_2O$[15a,b]

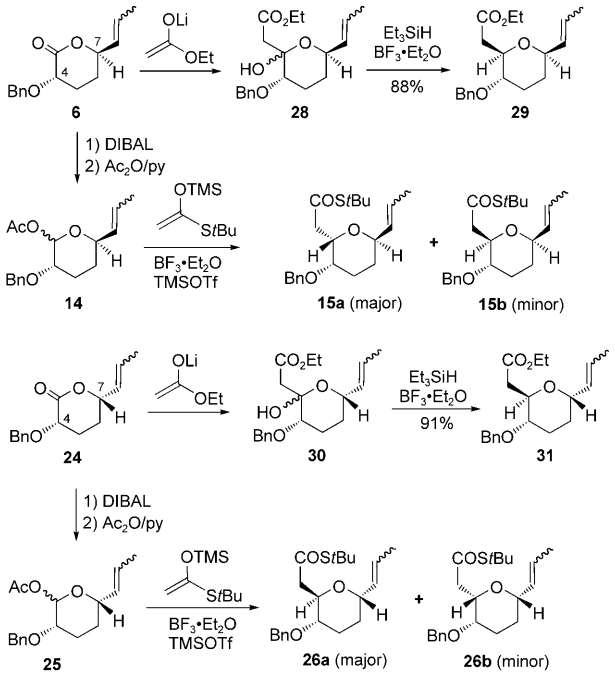


Scheme 7. Synthesis of aspergillide A (**1**). LiHMDS = lithium hexamethyldisilazide.

provided **29**, which displayed an exclusively *cis* relationship at the stereocenter pair C-3/C-7 (aspergillide numbering, Scheme 1), as demonstrated by the observation of a NOE between H-3 and H-7.[9a] As the precursor lactone **6**, **29** was also a ~9:1 *E*/*Z* mixture. Ester **29** was then converted into aspergillide A (**1**) along a reaction sequence described in our previous paper.[9a]

Some particular aspects of the syntheses disclosed above deserve comment. One of these aspects is the stereochemical features observed in the formation of tetrahydropyran rings (Scheme 8). C-Glycosidation of the lactol derivative **14** gives rise mainly to tetrahydropyran **15a** (Scheme 3), where the acetic acid chain and the propenyl group are *trans* with respect to the ring. As regards to lactol derivative **25**, epimeric of **14** at C-7 (aspergillide numbering), the same reaction conditions give rise mainly to tetrahydropyran **26a**, where the acetic acid moiety and the propenyl group are also *trans* with respect to the ring. Furthermore, treatment of lactol **28** with $Et_3SiH/BF_3{\cdot}Et_2O$ gives only tetrahydropyran **29** (Scheme 8), where the acetic acid moiety and the propenyl group are *cis* with respect to the ring. In contrast, lactol **30**, obtained from lactone **24** (Scheme 5), was found to yield under the same reaction conditions tetrahydropyran **31** (Scheme 8), with the acetic acid moiety and the propenyl group *trans* with respect to the ring.

These results should be examined in the light of previous findings of Kishi and co-workers,[15a] as well as of other groups. According to the common view, Mukaiyama-type C-glycosidations and reductions of lactols with Et_3SiH, both Lewis-acid mediated reactions, take place via a kinetically controlled, nucleophilic attack of the enol silane and the silicon hydride, respectively, on an intermediate cyclic oxocarbenium cation.[37,38] The reactions are markedly exergonic and are thus believed to occur through reactant-like transition states.[39] Thus, lactol **28** and lactol acetate **14**, both derived from δ-lactone **6** (Scheme 9), are transformed into the corresponding oxocarbenium cations where the main half-chair conformations, **A** and **C**, respectively, display the propenyl and benzyloxy groups in the energetically more favorable pseudoequatorial positions. Furthermore, these conformations are favored by the pseudoaxial hydrogen at C-2, as a σ_{C-H} bond is a better hyperconjugative donor to the $C{=}O^+$ bond than a σ_{C-O} bond.[39a] It may be expected that reactions occur preferentially via axial attack of the nucleophile on



Scheme 8. Comparison of stereochemical outcomes in the formation of C-glycosides carried out with different methods on different substrates.

either of these two conformations, rather than on the alternative half-chair conformations **B** and **D**, where the same groups are in a pseudoaxial orientation. Axial attack at C-1 in conformations **A** and **C** may appear counterintuitive at first sight, as it takes place *syn* to the benzyloxy group and gives rise to a developing *gauche* interaction between the latter and the nucleophile. However, such an interaction is not considered to be quantitatively very important in the case of a benzyloxy group.[39]

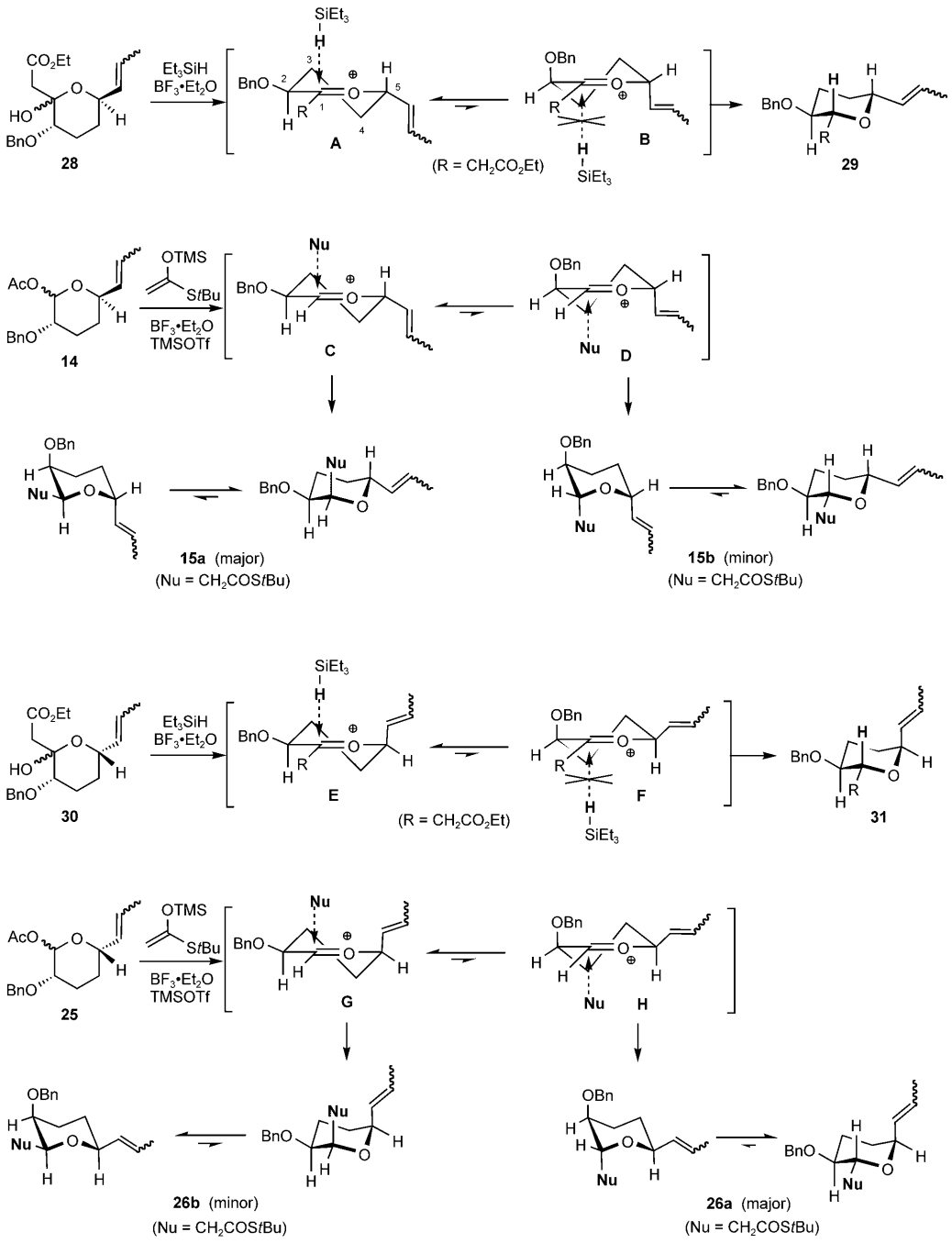
All these considerations fit well with the result of the reduction of lactol **28** with $Et_3SiH/BF_3{\cdot}Et_2O$, where the *cis*-2,6-substituted tetrahydropyran **29** was the sole product isolated. Since reactions mediated by $BF_3{\cdot}Et_2O$ are believed to occur via an S_N1-like mechanism, oxocarbenium ion with conformation **A** is assumed to be the key intermediate. In fact, no product formed through axial attack on conformation **B** is detected. Not only ground state but also transition-state effects (Curtin–Hammett kinetics) disfavor the last possibility, as axial attack on **B** would develop a 1,3-diaxial interaction.[39] Likewise, enol silane attack on the corresponding oxocarbenium cation **C** leads to the *trans*-2,6-substituted tetrahydropyran **15a** as the major product. Accordingly, the minor *cis*-2,6-substituted tetrahydropyran **15b** may be formed through a pathway via conformation **D**, which is less favorable for the same reasons discussed above with **B**.

This mechanistic rationale notwithstanding, it is not yet clear why the C-glycosidation is much less stereoselective than the lactol reduction with triethylsilane. Various explanations have been advanced to explain such situations in nucleophilic substitutions of cyclic acetals. It has been proposed that this type of reactions takes place through a continuum of mechanisms which range from S_N2-like to S_N1-like processes.[39e,g] Key-influencing factors are the type of nucleophile and Lewis acid used. Low diastereoselectivities may be due to competition between these two mechanisms, this being in turn related to the formation of ion pairs[39g,i] with counteranions formed from the leaving group or else to nucleophilic attacks taking place at the diffusion limit. Even the possibility of nonaxial attacks to the oxocarbenium ion cannot be excluded, either.[39e,g] In the present case, the appreciable electronic and steric differences between the enol silane, a strong π-nucleophile,[39g,40] and the weak nucleophile Et_3SiH may play a relevant role in the stereochemical outcome of the reactions discussed here. Another factor worth considering is the fact that the C-glycosidation is carried out at −18 °C whereas the lactol reduction is performed at −80 °C.[41] Furthermore, differences in solvent polarity (MeCN in the C-glycosidation vs CH_2Cl_2 in the lactol reduction) may also play a role, as observed in previous instances.[42]

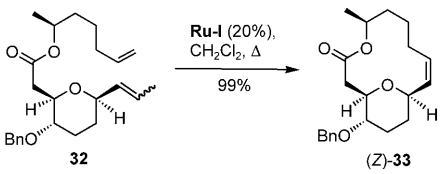
The same reactions were carried out on the corresponding lactol derivatives prepared from lactone **24**, epimeric of **6** at C-7. Lactol **30**, the epimer of **28**, gave the *trans*-2,6-substituted tetrahydropyran **31** as the sole reaction product. As regards to lactol acetate **25**, the epimer of **14**, it gave mainly the *trans*-2,6-substituted tetrahydropyran **26a** under the same C-glycosidation conditions as above. In order to explain the formation of **26a** and its minor isomer **26b**, we may assume that axial attack of the enol silane takes place on an oxocarbenium ion having the two half-chair conformations **G** and **H**, both showing a pseudoaxial and a pseudoequatorial substituent. According to the previous reasoning,[39] the former should be slightly predominant (pseudoaxial hydrogen at C-2) but, at the same time, less reactive due to the developing 1,3-diaxial interaction between the incoming nucleophile and the 1-propenyl group in the transition state. Apparently, these two factors compensate each other, as no high stereoselectivity is observed (d.r. ~1.9:1).

More surprising, and as yet unexplained, is the stereochemical outcome of the silane reduction of lactol **30**, where only the *trans*-2,6-substituted tetrahydropyran **31** is formed.[43] On the basis of axial hydride attack on an intermediate oxocarbenium cation, only conformation **E** can explain the observed result. As commented above, **E** should be slightly predominant but less reactive than **F**. No convincing reasons therefore can be presented for the observed stereochemical outcome but, once again, differences in temperature and solvent[41,42] between both reaction types may have an influence.

Another aspect worth discussing is the result of some of the metathesis reactions, those depicted in Schemes 4 and 10, the latter showing the key RCM step of the synthetic sequence leading to **1**.[9a] The very high stereoselectivity of the conversion of **32** into (*Z*)-**33** (Scheme 10) deserves some mention since the stereochemical outcome of RCM reactions that give rise to medium-sized or large rings (≥10 atoms)[44] is not always predictable with full certainty. In the present case, molecular mechanics calculations showed that



Scheme 9. Mechanistic discussion of the reactions depicted in Scheme 8 (S_N1-like processes with formation of intermediate oxocarbenium ions are assumed).

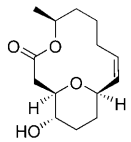


Scheme 10. Key RCM step in the synthesis of **1** (ref. [9a]).

(*Z*)-**33** is much more stable than (*E*)-**33**.[34] This suggests that the formation of (*Z*)-**33** in the RCM process is thermodynamically controlled. However, even though the macrolide ring of **1** is formally 14-membered from the point of view of the atom periphery and thus relatively flexible, the presence of the tetrahydropyran ring may impose certain conformational restraints which make the formation of (*E*)-**33** kinetically disfavored. Thus, the formation of (*Z*)-**33** in the RCM may also be kinetically controlled as well. Indeed, compound (*Z*)-**33** is obtained in very high yield when using catalyst **Ru-I**, which is assumed to work under kinetic conditions.[12] The more active, second-generation catalyst **Ru-II**

gave also (*Z*)-**33** but in a lower yield (67%). Catalyst **Ru-II** is known to cause in some cases *E*/*Z* isomerizations,[35] thus approaching thermodynamic (equilibrium) conditions. Furthermore, compound (*E*)-**33** may show some instability under these conditions, what could explain the eroded yield observed when using **Ru-II**. Finally, it also worth noting that, in contrast to that observed with ester **18** (Scheme 4), no C=C bond migration took place in the RCM of **32** with catalyst **Ru-II**. This may be related to the fact that, according to our theoretical calculations, C=C bond migration leads in the present case to a less stable compound.[34]

Biological assays: Aspergillides A (**1**) and B (**2**) have been described as moderately cytotoxic against murine lymphoma L1210 cells.[1] A more detailed characterization of their activity and selectivity profile, however, is still missing. In order to further characterize the biological profile of these two natural macrolides, their cytotoxicity has now been examined against five different human cancer cell lines: promyelocytic leukemia, breast carcinoma, fibrosarcoma, colon adenocarcinoma and osteosarcoma, as well as in a primary culture of non-transformed bovine aorta endothelial cells. In addition, some of the synthetic intermediates prepared in the courses of the syntheses have also been tested. Lactone **34** is the *Z* stereoisomer of aspergillide A,[9a] while lactone **35** is the dihydro derivative of aspergillide B (its preparation



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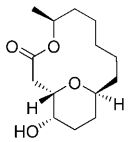

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is described in the Experimental Section). The results of the cytotoxicity tests are shown in Table 1.

The data of Table 1 indicate that aspergillide A (**1**) is the compound that exhibits the lowest cytotoxicity for all the cell types, with aspergillide B (**2**) being at least 1.5 times more active throughout the entire panel. Consistent in all series is also the fact that the HL-60 (human leukemia) cell line showed the highest sensitivity to treatment with the different aspergillide analogues, whereas the non-transformed endothelial cells (BAE) showed the lowest sensitivity to the tested compounds.

Most of the tested non-natural aspergillide derivatives were found to be more active than the natural metabolites **1** and **2**. The best results were obtained with compound **34**, which showed the highest cytotoxicity for all cell types assayed, most particularly for human leukemia (18 times more cytotoxic than aspergillide B and 45 times more cytotoxic than aspergillide A) and breast cancer cells (24 times more cytotoxic than aspergillide B and 34 times more cytotoxic than aspergillide A). In the case of aspergillide B (**2**), the saturation of the olefinic bond (compound **35**) leads to a small decrease of the cytotoxicity in a degree which depended of the cell line tested.

Another aspect to note is the influence of the *O*-benzyl group. For aspergillide B, its cytotoxicity is very similar to those of its benzylated derivative (*E*)-**17** and the stereoisomer (*Z*)-**17** (except in the case of the HL-60 human leukemia cell line, where the two benzylated derivatives are 2.5–3 times more active than **2**). In contrast, the benzylated derivative of aspergillide A, (*E*)-**33**, and its stereoisomer (*Z*)-**33**, are at least three times more active than the parent lactone **1** against all cell lines tested, and in some cases much more.

The observation that all tested compounds showed their highest activity against leukemia cells suggests a potential usefulness as antileukemia drugs, most particularly in the case of lactone **34**, which shows a cytotoxic activity comparable with that of the clinical drug fludarabine.[45] In view of the potential interest of this and other aspergillide analogues for cancer treatment, we are now preparing a series of such compounds in order to establish more extended structure–activity relationships.

Table 1. Cytotoxic activity IC_{50} [$\mu g\,mL^{-1}$] of macrolides **1**, **2** and some synthetic intermediates against several cell types.[a]

	Cell lines[b]					
Compound	HL-60	MDA-MB-231	HT-1080	HT-29	U2OS	BAE
1	81.2±17.5	99.0±7.9	84.3±13.7	107.7±9.3	>100	>100
2	32.8±7.6	68.2±6.5	45.0±4.5	64.3±1.5	54.5±6.6	60.6±6.5
(*E*)-**17**	12.3±1.5	46.7±5.7	48.1±2.7	54.6±5.1	53.8±4.1	33.4±3.0
(*Z*)-**17**	11.4±0.2	77.4±19.0	60.3±2.1	51.3±2.8	72.8±5.2	41.7±4.7
(*E*)-**33**	12.3±0.3	18.5±2.6	27.9±2.9	31.1±2.9	22.8±3.7	34.3±2.2
(*Z*)-**33**	6.7±0.9	16.2±2.2	19.7±3.3	20.4±0.8	18.3±3.5	21.5±2.4
34	1.8±0.2	2.9±0.2	6.5±0.4	6.7±0.8	4.2±0.9	7.7±1.3
35	62.8±10.9	71.2±10.2	92.3±13.3	95.0±8.7	>100	>100

[a] Cells were incubated for 72 h in the presence of each compound, and the ratios of viable cells were determined by MTT assay. The drug concentration required to inhibit cell growth by 50% was determined from semilogarithmic dose-response plots, and results represent the means + SDs of three independent experiments with triplicate samples each. [b] The cell lines used were: HL-60 (human promyelocytic leukemia), MDA-MB-231 (human breast carcinoma), HT1080 (human fibrosarcoma), HT29 (human colon adenocarcinoma), U2OS (human osteosarcoma), and the non-transformed bovine aorta endothelial (BAE) cells.

Experimental Section

General methods: $^1H/^{13}C$ NMR spectra were recorded at 500/125 MHz in the indicated solvent at 30 °C. Signals of the deuterated solvent ($CDCl_3$ in all but one case) were taken as the reference (in $CDCl_3$, the singlet at δ 7.27 and the triplet centered at 77.0 ppm for ^1H and ^{13}C NMR, respectively; in C_6D_6, the singlet at δ 7.16 and the triplet centered at 128.0 ppm for ^1H and ^{13}C NMR, respectively). Carbon atom types (C, CH, CH_2, CH_3) were determined with the DEPT pulse sequence. ^1H and ^{13}C signals were assigned with the aid of 2D methods. Mass spectra were run by the electron impact mode (EIMS, 70 eV), by the fast atom bombardment mode (FABMS, *m*-nitrobenzyl alcohol matrix) or by the electro-

spray mode (ESMS). IR data are given only for compounds with significant functions (OH, C=O) and were recorded as oily films on NaCl plates (oils) or as KBr pellets (solids). Optical rotations were measured at 25 °C. Reactions which required an inert atmosphere were carried out under N_2 with flame-dried glassware. Et_2O and THF were freshly distilled from sodium/benzophenone and transferred via syringe. Dichloromethane was freshly distilled from CaH_2. Tertiary amines were freshly distilled from KOH. Toluene was freshly distilled from sodium wire. Commercially available reagents were used as received. Unless detailed otherwise, "work-up" means pouring the reaction mixture into brine, followed by extraction with the solvent indicated in parenthesis. If the reaction medium was acidic, an additional washing with 5% aq $NaHCO_3$ was performed. If the reaction medium was basic, an additional washing with aq NH_4Cl was performed. New washing with brine, drying over anhydrous Na_2SO_4 and elimination of the solvent under reduced pressure were followed by chromatography on a silica gel column (60–200 μm) and elution with the indicated solvent mixture. Where solutions were filtered through a Celite pad, the pad was additionally washed with the same solvent used, and the washings incorporated to the main organic layer.

(*S*)-5-Benzyloxy-6-(*tert*-butyldiphenylsilyloxy)-hex-1-ene (11): A solution of alcohol **10** (23.04 g, 65 mmol) in dry Et_2O (250 mL) was treated under N_2 with benzyl bromide (35.7 mL, 300 mmol) and Ag_2O (45 g, ca. 195 mmol). The reaction mixture was then protected from light and stirred for 3 d at room temperature. After this time, the mixture was filtered through Celite, and the volatiles were removed under reduced pressure. Column chromatography of the residue on silica gel (hexanes/toluene 7:3) furnished benzyl ether **11** (24 g, 83%) as a colourless oil. $[\alpha]_D = -20.6$ ($c = 0.86$, $CHCl_3$); ^{1}H NMR: $\delta = 7.70$–7.65 (m, 4H), 7.45–7.30 (br m, 11H), 5.83 (ddt, $J = 17.2$, 10.3, 6.5 Hz, 1H), 5.05–4.95 (m, 2H), 4.70 (d, $J = 11.7$ Hz, 1H), 4.54 (d, $J = 11.7$ Hz, 1H), 3.81 (dd, $J = 10.5$, 5.5 Hz, 1H), 3.71 (dd, $J = 10.5$, 5.5 Hz, 1H), 3.58 (m, 1H), 2.30–2.10 (br m, 2H), 1.75–1.65 (br m, 2H), 1.11 ppm (s, 9H); ^{13}C NMR: $\delta = 139.0$, 133.6 (×2), 19.2 (C), 138.7, 135.6 (×4), 129.7 (×2), 128.3 (×2), 127.8 (×2), 127.7 (×4), 127.5, 79.2 (CH), 114.6, 72.2, 66.2, 31.0, 29.7 (CH_2), 26.9 ppm (×3) (CH_3); HR FABMS: m/z: calcd for $C_{29}H_{36}O_2Si$: 444.2484; found: 444.2465 [M^+].

(*S*)-4-(Benzyloxy)-5-(*tert*-butyldiphenylsilyloxy)pentanal (9): A solution of olefin **11** (22.23 g, 50 mmol) in CH_2Cl_2 (500 mL) was cooled to −80 °C. A stream of ozone-containing air was then bubbled through the solution until complete consumption of the starting material (ca. 30 min, TLC monitoring). Ozone residues were then eliminated by bubbling a stream of N_2 for 5 min and the mixture was allowed to reach room temperature. After addition of PPh_3 (26.23 g, 100 mmol), the mixture was then stirred for 2 h. After solvent removal under reduced pressure, the crude residue was stirred for 15 min under pentane (3×300 mL) and filtered. The solution was then concentrated under reduced pressure and the crude residue was purified by means of column chromatography on silica gel (pentane/Et_2O 9:1). This provided aldehyde **9** (17.42 g, 78%) as a colourless oil. $[\alpha]_D = -26.3$ ($c = 0.86$, $CHCl_3$); ^{1}H NMR: $\delta = 9.70$ (br s, 1H), 7.70–7.65 (m, 4H), 7.45–7.30 (br m, 11H), 4.65 (d, $J = 11.6$ Hz, 1H), 4.48 (d, $J = 11.6$ Hz, 1H), 3.82 (dd, $J = 10.7$, 5.2 Hz, 1H), 3.70 (dd, $J = 10.7$, 5.2 Hz, 1H), 3.55 (m, 1H), 2.49 (br t, $J = 7.3$ Hz, 2H), 2.05–1.95 (m, 1H), 1.90–1.80 (m, 1H), 1.11 ppm (s, 9H); ^{13}C NMR: $\delta = 138.4$, 133.3, 133.2, 19.2 (C), 202.2, 135.6 (×4), 129.7 (×2), 128.3 (×2), 127.8 (×2), 127.7 (×4), 127.5, 78.5 (CH), 72.0, 65.7, 39.8, 24.3 (CH_2), 26.8 ppm (×3) (CH_3); IR: $\nu_{max} = 1710\ cm^{-1}$ (C=O); HR FABMS: m/z: calcd for $C_{28}H_{34}O_3Si$: 446.2277; found: 446.2299 [M^+].

(4*R*,7*S*)-7-Benzyloxy-8-(*tert*-butyldiphenylsilyloxy)-oct-1-en-4-ol (12): Allylmagnesium bromide (commercial 1 M solution in Et_2O, 37.5 mL, 37.5 mmol) was added dropwise under N_2 at 0 °C via syringe to a solution of (−)-Ipc_2BCl (14.4 g, 45 mmol) in dry Et_2O (250 mL). The mixture was further stirred for 1 h at 0 °C. The solution was then allowed to stand, which caused precipitation of magnesium chloride. The supernatant solution was then carefully transferred to another flask via cannula. After cooling this flask at −90 °C, a solution of aldehyde **9** (13.4 g, 30 mmol) in dry Et_2O (60 mL) was added dropwise via syringe. The resulting solution was further stirred at the same temperature for 2 h. The reaction mixture was then quenched through addition of phosphate pH 7 buffer solution (200 mL), MeOH (200 mL) and 30% H_2O_2 (100 mL). After stirring for 30 min at room temperature, the mixture was poured onto satd. aq $NaHCO_3$ and worked up (extraction with EtOAc). Column chromatography on silica gel (hexane/EtOAc 9:1) afforded **12**, still contaminated with boron-containing side products, which was used as such in the next step. An aliquot was carefully purified for analytical purposes. Colourless oil. $[\alpha]_D = -16.7$ ($c = 1.6$, $CHCl_3$); ^{1}H NMR: $\delta = 7.75$–7.70 (m, 4H), 7.45–7.30 (br m, 11H), 5.83 (ddt, $J = 17.2$, 10.3, 7 Hz, 1H), 5.15–5.10 (m, 2H), 4.69 (d, $J = 11.8$ Hz, 1H), 4.54 (d, $J = 11.8$ Hz, 1H), 3.82 (dd, $J = 10.5$, 5.6 Hz, 1H), 3.70 (dd, $J = 10.5$, 5 Hz, 1H), 3.65–3.60 (m, 1H), 3.60–3.55 (m, 1H), 2.30–2.25 (m, 1H), 2.20–2.15 (m, 1H), 2.00 (br s, 1H, OH), 1.80–1.75 (m, 1H), 1.70–1.60 (m, 2H), 1.50–1.45 (m, 1H), 1.10 ppm (s, 9H); ^{13}C NMR: $\delta = 138.7$, 133.5 (x 2), 19.2 (C), 135.6 (×4), 135.0, 129.6 (×2), 128.3 (×2), 127.8 (×2), 127.7 (×4), 127.5, 79.8, 70.8 (CH), 117.8, 72.1, 66.1, 41.9, 32.6, 27.8 (CH_2), 26.9 ppm (×3) (CH_3); IR: $\nu_{max} = 3420\ cm^{-1}$ (br, OH); HR FABMS: m/z: calcd for $C_{31}H_{40}O_3Si$: 488.2746; found: 488.2730 [M^+].

(4*R*,7*S*)-7-Benzyloxy-8-(*tert*-butyldiphenylsilyloxy)-4-(triethylsilyloxy)oct-1-ene (8): The alcohol **12** obtained above was dissolved in dry CH_2Cl_2 (400 mL), cooled to −80 °C and treated dropwise with Et_3N (12.5 mL, 90 mmol) and TESOTf (17 mL, 75 mmol). The mixture was stirred for 15 min at the same temperature and then for 2 h at room temperature. Work-up (extraction with CH_2Cl_2) was followed by column chromatography of the residue on silica gel (hexanes/Et_2O 49:1) to yield **8** (13.93 g, 77% overall from **9**) as a colourless oil. $[\alpha]_D = -9$ ($c = 1.65$, $CHCl_3$); ^{1}H NMR: $\delta = 7.75$–7.70 (m, 4H), 7.45–7.30 (br m, 11H), 5.82 (ddt, $J = 17.2$, 10.3, 7 Hz, 1H), 5.10–5.00 (m, 2H), 4.67 (d, $J = 11.7$ Hz, 1H), 4.52 (d, $J = 11.7$ Hz, 1H), 3.78 (dd, $J = 10.7$, 5.4 Hz, 1H), 3.72 (br quint, $J \sim 6$ Hz, 1H), 3.70 (dd, $J = 10.7$, 4.7 Hz, 1H), 3.50 (m, 1H), 2.24 (dd, $J = 7$, 6 Hz, 2H), 1.80–1.70 (m, 1H), 1.70–1.60 (m, 1H), 1.60–1.50 (m, 1H), 1.45–1.35 (m, 1H), 1.10 (s, 9H), 0.97 (t, $J = 7.7$ Hz, 9H), 0.62 ppm (q, $J = 7.7$ Hz, 6H); ^{13}C NMR: $\delta = 139.0$, 133.6 (×2), 19.2 (C), 135.6 (×4), 135.2, 129.6 (×2), 128.2 (×2), 127.7 (×2), 127.6 (×4), 127.3, 80.1, 72.2 (CH), 116.8, 72.0, 66.2, 42.2, 32.7, 27.7, 5.1 (×3) (CH_2), 26.9 (×3), 7.0 ppm (×3) (CH_3); HR FABMS: m/z: calcd for $C_{37}H_{53}O_3Si_2$: 601.3533; found: 601.3520 [$M-H^+$].

(4*R*,7*S*)-7-Benzyloxy-8-(*tert*-butyldiphenylsilyloxy)-4-(triethylsilyloxy)oct-2*E*,*Z*-ene (7): *N*-Tritylallylamine (12 g, 40 mmol) and catalyst **Ru-II** (850 mg, 1 mmol) were dissolved under N_2 in dry, deoxygenated CH_2Cl_2 (50 mL). Following this, a solution of compound **8** (12.06 g, 20 mmol) and *i*Pr_2NEt (3.5 mL, 20 mmol) in dry, deoxygenated CH_2Cl_2 (350 mL) were added dropwise under N_2, and the mixture was heated at reflux for 16 h. After removal of all volatiles under reduced pressure, the residue was purified by means of column chromatography on silica gel (hexanes/Et_2O 49:1) to give **7** (11.58 g, 96%) as a ca. 9:1 *E*/*Z* mixture as a colourless oil. ^{1}H NMR (signals from the major *E* isomer): $\delta = 7.75$–7.70 (m, 4H), 7.45–7.30 (br m, 11H), 5.60–5.50 (m, 1H), 5.50–5.40 (m, 1H), 4.68 (d, $J = 11.7$ Hz, 1H), 4.56 (d, $J = 11.7$ Hz, 1H), 4.04 (m, 1H), 3.79 (br dd, $J = 10.8$, 5.5 Hz, 1H), 3.70 (br dd, $J = 10.8$, 4.8 Hz, 1H), 3.54 (br m, 1H), 1.70 (br d, $J \sim 6.3$ Hz, 3H), 1.70–1.55 (br m, 4H), 1.11 (s, 9H), 0.97 (t, $J = 7.7$ Hz, 9H), 0.62 ppm (q, $J = 7.7$ Hz, 6H); ^{13}C NMR (signals from the major *E* isomer): $\delta = 139.0$, 133.6 (×2), 19.2 (C), 135.6 (×4), 134.8, 129.6 (×2), 128.2 (×2), 127.7 (×2), 127.6 (×4), 127.3, 125.2, 80.1, 73.9 (CH), 72.0, 66.2, 34.2, 27.7, 5.1 (×3) (CH_2), 26.8 (×3), 17.5, 6.9 ppm (×3) (CH_3); HR FABMS: m/z: calcd for $C_{37}H_{53}O_3Si_2$: 601.3533; found: 601.3544 [$M-H^+$].

(2*S*,5*R*)-2-(Benzyloxy)-oct-6*E*,*Z*-ene-1,5-diol (13): A solution of compound **7** (10.8 g, ca. 18 mmol) in dry THF (180 mL) was treated at room temperature under N_2 with solid TBAF trihydrate (12.6 g, 40 mmol). The mixture was stirred for 16 h at room temperature. After removal of all volatiles under reduced pressure, the residue was purified by means of column chromatography on silica gel (hexanes/EtOAc 1:1) to yield **13** (4.41 g, 98%) as a ~9:1 *E*/*Z* mixture: colourless oil. ^{1}H NMR (signals from the major *E* isomer): $\delta = 7.30$–7.20 (br m, 5H), 5.60–5.50 (m, 1H), 5.40–5.35 (m, 1H), 4.50 (AB system, $J = 11.7$ Hz, 2H), 3.92 (m, 1H), 3.59 (br dd, $J = 11.3$, 2 Hz, 1H), 3.50–3.40 (br m, 2H), 2.70 (br s, 1H, OH), 2.50 (br s, 1H, OH), 1.62 (br d, $J \sim 6.3$ Hz, 3H), 1.60–1.45 ppm (br m, 4H); ^{13}C NMR (signals from the major *E* isomer): $\delta = 138.3$ (C), 133.9, 128.3

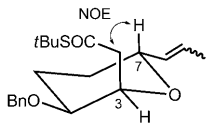
(×2), 127.7 (×2), 127.6, 126.6, 79.6, 72.7 (CH), 71.5, 63.8, 32.8, 26.7 (CH_2), 17.6 ppm (CH_3); IR: ν_{max} = 3400 cm^{-1} (br, OH); HR ESMS: *m/z*: calcd for $C_{15}H_{22}O_3Na$: 273.1467; found: 273.1466 [M+Na^+].

(3*S*,6*R*)-3-(Benzyloxy)-6-(prop-1*E*,*Z*-enyl)tetrahydro-2*H*-pyran-2-one (6): A solution of diol **13** (3.75 g, 15 mmol) in dry CH_2Cl_2 (150 mL) was cooled under N_2 to 0 °C and treated sequentially with TEMPO (470 mg, 3 mmol) and then, portionwise, with $PhI(OAc)_2$ (10.6 g, 33 mmol). The mixture was stirred for 16 h at room temperature. The reaction was then quenched by addition of saturated aqueous $Na_2S_2O_3$ (20 mL) and worked up (extraction with CH_2Cl_2). **CAUTION**: evaporation of volatiles under reduced pressure has to be performed at room temperature, due to the appreciable volatility of lactone **6**. Column chromatography on silica gel (pentane/Et_2O 9:1) furnished **6** (3.1 g, 84 %) as a ~9:1 *E*/*Z* mixture as a colourless oil. ^{1}H NMR (signals from the major *E* isomer): δ = 7.40–7.25 (br m, 5H), 5.77 (dqd, *J* = 15.6, 6.5, 1 Hz, 1H), 5.49 (ddq, *J* = 15.6, 6.7, 1.5 Hz, 1H), 4.94 (m, 1H), 4.92 (d, *J* = 11.8 Hz, 1H), 4.66 (d, *J* = 11.8 Hz, 1H), 3.96 (dd, *J* = 7.5, 6 Hz, 1H), 2.20–1.95 (br m, 3H), 1.72 (ddd, *J* = 6.5, 1.5, 1 Hz, 3H), 1.70 ppm (m, 1H); ^{13}C NMR (signals from the major *E* isomer): δ = 170.7, 137.4 (C), 129.7, 128.9, 128.4 (×2), 128.0 (×2), 127.8, 80.3, 73.6 (CH), 72.6, 27.4, 26.3 (CH_2), 17.6 ppm (CH_3); IR: ν_{max} = 1744 cm^{-1} (C=O); HR EIMS: *m/z* (%): calcd for $C_{15}H_{19}O_3$: 247.1334; found: 247.1324 [M+H^+] (4), 91 (100).

(2*R*/*S*,3*S*,6*R*)-3-(Benzyloxy)-6-(prop-1*E*,*Z*-enyl)tetrahydro-2*H*-pyran-2-yl acetate (14): A solution of lactone **6** (2.46 g, 10 mmol) in dry CH_2Cl_2 (100 mL) was cooled under N_2 to −80 °C and treated dropwise with DIBAL (1 M solution in CH_2Cl_2, 13 mL, 13 mmol). The reaction mixture was then stirred at −80 °C for 2 h. After this time, a solution of DMAP (7.33 g, 60 mmol) in dry CH_2Cl_2 (60 mL) was slowly added dropwise, followed by addition of Ac_2O (8.5 mL, 90 mmol). After stirring overnight at −80 °C, the temperature was allowed to reach 0 °C and the reaction was quenched by addition of saturated aqueous NH_4Cl (100 mL) and saturated sodium potassium tartrate (65 mL), followed by stirring at room temperature for 30 min. Work-up (extraction with CH_2Cl_2) and column chromatography on silica gel (hexane/EtOAc 19:1) afforded the acetylated lactol **14** (2.70 g, 93 %) as a mixture of stereoisomers at both the anomeric carbon (~2:1) and the olefinic bond (~9:1) as a colourless oil. ^{1}H NMR: δ = 7.35–7.25 (br m, 5H), 6.38 (d, 1H, *J* = 3.1 Hz, minor anomer, *E* isomer)/6.33 (d, 1H, *J* = 3.1 Hz, minor anomer, *Z* isomer), 5.64 (d, 1H, *J* = 7.9 Hz, major anomer, *E* isomer)/5.59 (d, 1H, *J* = 7.9 Hz, major anomer, *Z* isomer), 5.75–5.65 (br m, 1H), 5.50–5.40 (br m, 1H), 4.64 (s, 2H, major anomer)/4.63, 4.53 (AB system, 2H, *J* = 11.7 Hz, minor anomer), 4.24 (m, 1H, minor anomer)/4.04 (m, 1H, major anomer), 3.57 (m, 1H, minor anomer)/3.38 (m, 1H, major anomer), 2.20 (m, 1H, major anomer), 2.15 (s, 3H, minor anomer), 2.10 (s, 3H, major anomer), 1.92 (m, 1H, minor anomer), 1.68 (br d, *J* ~6.4 Hz, 3H), 1.80–1.45 ppm (br m, 3H); IR: ν_{max} = 1752 cm^{-1} (C=O).

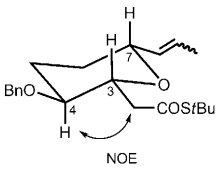
***S*-*tert*-Butyl 2-[(2*S*,3*S*,6*R*)-3-(benzyloxy)-6-(prop-1*E*,*Z*-enyl)tetrahydro-2*H*-pyran-2-yl]ethanethioate (15a) and *S*-*tert*-butyl 2-[(2*R*,3*S*,6*R*)-3-(benzyloxy)-6-(prop-1*E*,*Z*-enyl)tetrahydro-2*H*-pyran-2-yl]ethanethioate (15b)**: The trimethylsilyl enolate of *tert*-butyl thioacetate, CH_2=C(OS*t*Bu)OSiMe$_3$, was prepared according to a literature procedure.[27] This enol silane (2.04 g, 10 mmol) and compound **14** (1.16 g, 4 mmol) were dissolved under N_2 in dry MeCN (20 mL), mixed with powdered, activated 4 Å molecular sieves (2 g) and then cooled to −30 °C. Following this, a solution of freshly distilled BF_3-etherate (1 mL, ca. 8 mmol) and TMSOTf (145 μL, 0.8 mmol) in dry MeCN (3 mL) was added dropwise to the reaction mixture, which was subsequently stirred at −18 °C for 16 h. Work-up (extraction with CH_2Cl_2) and column chromatography on silica gel (hexanes/Et_2O 49:1 to 19:1) furnished first **15b** (305 mg, 21 %) and then **15a** (798 mg, 55 %), both as ~9:1 *E*/*Z* mixtures.

15a: colourless oil; ^{1}H NMR (signals from the major *E* isomer): δ = 7.35–7.25 (br m, 5H), 5.75–5.60 (m, 1H), 5.50–5.40 (m, 1H), 4.56 (d, *J* = 11.7 Hz, 1H), 4.50–4.40 (m, 2H), 4.20 (m, 1H), 3.53 (m, 1H), 2.90 (dd, *J* = 15.2, 8.4 Hz, 1H), 2.75 (dd, *J* = 15.2, 5.3 Hz, 1H), 1.95–1.70 (br m, 3H), 1.69 (br d, *J* ~6.4 Hz, 3H), 1.45 (s, 9H), 1.45–1.40 ppm (m, 1H); ^{13}C NMR (signals from the major *E* isomer): δ = 198.1, 138.3, 48.0 (C), 130.6, 128.3 (×2), 127.7, 127.6 (×2), 127.5, 73.5, 70.8, 70.7 (CH), 70.6, 43.4, 27.3, 23.5 (CH_2), 29.8 (×3), 17.8 ppm (CH_3); IR: ν_{max} = 1682 cm^{-1} (C=O); HR ESMS: *m/z*: calcd for $C_{21}H_{30}O_3SNa$: 385.1813; found: 385.1808 [M+Na^+]. The NOE indicated above between H-7 (aspergillide numbering) and the acetate side-chain methylene is diagnostic of the relative configuration at the pair of stereocenters C-3/C-7 in this molecule.



15a (*E*+*Z*)

15b: colourless oil; ^{1}H NMR (signals from the major *E* isomer): δ = 7.35–7.25 (br m, 5H), 5.70 (dqd, *J* = 15.7, 6.4, 1.5 Hz, 1H), 5.47 (ddq, *J* = 15.7, 5.8, 1.5 Hz, 1H), 4.64 (d, *J* = 11.7 Hz, 1H), 4.46 (d, *J* = 11.7 Hz, 1H), 3.82 (m, 2H), 3.16 (td, *J* = 9.7, 4.2 Hz, 1H), 3.00 (dd, *J* = 15.2, 4 Hz, 1H), 2.62 (dd, *J* = 15.2, 8.3 Hz, 1H), 2.28 (m, 1H), 1.78 (m, 1H), 1.69 (br d, *J* ~6.4 Hz, 3H), 1.47 (s, 9H), 1.55–1.35 ppm (br m, 2H); ^{13}C NMR (signals from the major *E* isomer): δ = 197.6, 138.2, 47.7 (C), 131.2, 128.3 (×2), 127.6 (×2), 127.5, 126.5, 77.4, 77.2, 76.6 (CH), 70.5, 47.7, 30.8, 29.0 (CH_2), 29.7 (×3), 17.7 ppm (CH_3); IR: ν_{max} = 1684 cm^{-1} (C=O); HR ESMS: *m/z*: calcd for $C_{21}H_{30}O_3SNa$: 385.1813; found: 385.1811 [M+Na^+]. Due to the overlapping of the signals of H-3 and H-7 at δ 3.80, this highly diagnostic NOE was not amenable to measurement. However, when a chair conformation is assumed for **15b**, what seems very likely in view of the three equatorial substituents, the observed NOE between H-4 and the acetate side-chain methylene strongly suggests that the molecule shows the configuration depicted below.



15b (*E*+*Z*)

2-[(2*S*,3*S*,6*R*)-3-(Benzyloxy)-6-(prop-1*E*,*Z*-enyl)-tetrahydro-2*H*-pyran-2-yl]acetic acid (5): A solution of thioester **15a** (362 mg, 1 mmol) in MeOH (200 mL) was treated at room temperature with a solution of NaOH (8 g, 200 mmol) in water (50 mL). The reaction mixture was then stirred at room temperature for 16 h, then concentrated under reduced pressure to eliminate most MeOH and washed with CH_2Cl_2 to eliminate neutral impurities. The aqueous layer was then acidified with HCl and extracted with EtOAc. The organic layer was dried over dry $MgSO_4$ and evaporated under reduced pressure. The oily residue was acid **5** (273 mg, 94 %, ~9:1 *E*/*Z* mixture), pure enough for the next reaction. ^{1}H NMR (signals from the major *E* isomer): δ = 11.00 (br s, 1H, OH), 7.45–7.35 (br m, 5H), 5.80 (m, 1H), 5.60 (m, 1H), 4.70 (d, *J* = 11.8 Hz, 1H), 4.58 (d, *J* = 11.8 Hz, 1H), 4.60–4.50 (m, 1H), 4.32 (br s, 1H), 3.67 (dt, *J* = 7.8, 3.8 Hz, 1H), 2.90 (dd, *J* = 15.7, 8.8 Hz, 1H), 2.77 (dd, *J* = 15.7, 5.4 Hz, 1H), 2.10–1.80 (br m, 3H), 1.80 (d, *J* ~6.5 Hz, 3H), 1.55 ppm (m, 1H); ^{13}C NMR (signals from the major *E* isomer): δ = 177.2, 138.0 (C), 130.3, 128.2 (×2), 127.8, 127.5 (×2), 127.4, 73.1, 71.0, 70.2 (CH), 70.6, 33.7, 26.9, 23.1 (CH_2), 17.7 ppm (CH_3); IR: ν_{max} = 3500–2500 (br, COOH), 1714 cm^{-1} (C=O); HR ESMS: *m/z*: calcd for $C_{17}H_{22}O_4Na$: 313.1416; found: 313.1419 [M+Na^+].

2-[(2*S*,3*S*,6*R*)-3-(Benzyloxy)-6-[(*S*)-6-hydroxyhept-1*E*,*Z*-enyl]tetrahydro-2*H*-pyran-2-yl]acetic acid (16): Compound **4**[11] (342 mg, 3 mmol) and catalyst **Ru-II** (100 mg, 0.12 mmol) were dissolved under N_2 in dry, deoxygenated CH_2Cl_2 (20 mL). The mixture was then heated at reflux. A solution of acid **5** (175 mg, 0.6 mmol) in dry, deoxygenated CH_2Cl_2 (5 mL) was added dropwise, and the mixture was maintained at reflux for 6 h. After cooling to room temperature, the reaction was quenched through addition of DMSO[46] (0.4 mL) followed by stirring overnight. Removal of all volatiles under reduced pressure and filtration of the residue through silica gel (CH_2Cl_2/MeOH 19:1) yielded acid **16** (193 mg, 89 %) as a ~70:30 *E*/*Z* mixture, which was used as such in the next step. An ali-

quot was rechromatographed for analytical purposes, which led to an enrichment in the *E* isomer: colourless oil; ^{1}H NMR: δ = 7.35–7.25 (m, 5H), 5.75–5.65 (m, 1H), 5.55–5.45 (m, 1H), 4.80–4.65 (brs, 2H, OH), 4.62 (d, J=12 Hz, 1H), 4.48 (d, J=12 Hz, 1H), 4.35 (dt, J=8.5, 4 Hz, 1H), 4.30 (brq, J ~5 Hz, 1H), 3.80 (m, 1H), 3.52 (brq, J ~4.5 Hz, 1H), 2.80 (dd, J=15.7, 9.2 Hz, 1H), 2.56 (dd, J=15.7, 4.5 Hz, 1H), 2.15–2.00 (brm, 3H), 1.82 (m, 2H), 1.55–1.40 (m, 5H), 1.18 ppm (d, J=6.5 Hz, 3H); ^{13}C NMR: δ = 175.3, 138.2 (C), 133.5, 129.1, 128.4 (×2), 127.8 (×2), 127.7, 73.2, 71.6, 70.2, 68.1 (CH), 70.8, 38.4, 34.7, 32.2, 26.1, 25.0, 23.0 (CH_2), 23.3 ppm (CH_3); IR: ν_{max} = 3400–2500 (br, COOH), 1722 cm^{-1} (C=O); HR ESMS: m/z: calcd for $C_{21}H_{30}O_5Na$: 385.1991; found: 385.1989 [M+Na$^+$].

(1*S*,5*S*,11*R*,14*S*)-14-(Benzyloxy)-5-methyl-4,15-dioxabicyclo[9.3.1]pentadec-9*E*-en-3-one [(*E*)-17] and (1*S*,5*S*,11*R*,14*S*)-14-(benzyloxy)-5-methyl-4,15-dioxabicyclo [9.3.1]pentadec-9*Z*-en-3-one [(*Z*)-17]: A solution of acid **16** (*E*/*Z* mixture, 181 mg, 0.5 mmol) in dry THF (4 mL) was cooled to 0 °C under N_2. Then, triethylamine (700 μL, 5 mmol) and 2,4,6-trichlorobenzoyl chloride (470 μL, 3 mmol) were added dropwise, followed by stirring at room temperature for 1.5 h. The reaction mixture was then diluted with dry toluene (100 mL) and added dropwise along 6 h over a solution of DMAP (733 mg, 6 mmol) in dry toluene (250 mL) at 50 °C. Work-up (extraction with EtOAc) and column chromatography on silica gel (hexanes/EtOAc 19:1 to 9:1) furnished first (*Z*)-**17** (29 mg, 17%) and then (*E*)-**17** (69 mg, 40%). Physical and spectral properties of both compounds described in ref. [6].

(1*S*,5*S*,11,14*S*)-14-Hydroxy-5-methyl-4,15-dioxabicyclo[9.3.1]pentadec-9*E*-en-3-one (aspergillide B, 2): A solution of lactone (*E*)-**17** (62 mg, 0.18 mmol) was dissolved in CH_2Cl_2/H_2O 10:1 (15 mL) and treated with DDQ (1.22 g, 5.4 mmol). The reaction mixture was stirred for 20 h at room temperature. Work-up (extraction with CH_2Cl_2) and column chromatography on silica gel (hexane/EtOAc 7:3) furnished **2** (24 mg, 51%). Physical and spectral properties described in ref. [6] (see also correction).

(*S*)-Hept-6-en-2-yl 2-[(2*S*,3*S*,6*R*)-3-(benzyloxy)-6-(prop-1*E*,*Z*-enyl)tetrahydro-2*H*-pyran-2-yl]acetate (18): A solution of acid **5** (174 mg, 0.6 mmol) in dry THF (15 mL) was cooled to 0 °C under N_2. Then, triethylamine (210 μL, 1.5 mmol) and 2,4,6-trichlorobenzoyl chloride (188 μL, 1.2 mmol) were added dropwise, followed by stirring at room temperature for 2 h. Alcohol **4** (82 mg, 0.72 mmol) and DMAP (183 mg, 1.5 mmol) were dissolved in dry THF (9 mL) and added slowly via syringe to the reaction mixture, with further stirring for 16 h at room temperature. Work-up (extraction with Et_2O) and column chromatography on silica gel (hexanes/EtOAc 19:1) furnished **18** (174 mg, 75%) as a ca. 9:1 *E*/*Z* mixture: colourless oil; ^{1}H NMR (signals from the major *E* isomer): δ = 7.35–7.25 (m, 5H), 5.85–5.60 (m, 2H), 5.50–5.40 (m, 1H), 5.05–4.90 (m, 3H), 4.60 (d, J=12 Hz, 1H), 4.55–4.40 (m, 2H), 4.20 (m, 1H), 3.58 (m, 1H), 2.76 (dd, J=15.2, 9 Hz, 1H), 2.60 (dd, J=15.2, 5 Hz, 1H), 2.05 (m, 2H), 1.95–1.60 (brm, 3H), 1.69 (brd, J ~6.2 Hz, 3H), 1.60–1.30 (brm, 5H), 1.29 ppm (d, J=6.3 Hz, 3H); ^{13}C NMR (signals from the major *E* isomer): δ = 171.5, 138.4 (C), 138.5, 130.8, 128.3 (×2), 127.6 (×2), 127.5, 127.4, 73.7, 70.9 (×2), 70.6 (CH), 114.7, 70.7, 35.4, 34.1, 33.5, 27.7, 24.6, 23.6 (CH_2), 20.0, 17.8 ppm (CH_3); IR: ν_{max} = 1730 cm^{-1} (C=O); HR ESMS: m/z: calcd for $C_{24}H_{34}O_4Na$: 409.2355; found: 409.2357 [M+Na$^+$].

Ring-closing metathesis of 18: *a) With catalyst **Ru-I***: Grubbs ruthenium catalyst **Ru-I** (16.5 mg, ca. 0.02 mmol) was dissolved under N_2 in dry, deoxygenated CH_2Cl_2 (90 mL). After heating the solution to reflux, diene **18** (39 mg, ca. 0.1 mmol) dissolved in dry, deoxygenated CH_2Cl_2 (10 mL) was added slowly via syringe (within 1 h) to the reagent solution. The reaction mixture was then additionally stirred at reflux for 2 h. After cooling to room temperature, the reaction was quenched through addition of DMSO[46] (75 μL) followed by stirring overnight. Removal of all volatiles under reduced pressure and column chromatography of the residue on silica gel (hexanes/EtOAc 19:1 to 9:1) yielded first (*Z*)-**17** (16.5 mg, 48%) and then (*E*)-**17** (11 mg, 33%).

*b) With catalyst **Ru-II***: the reaction was carried out under the same conditions as above. Column chromatography on silica gel (hexanes/EtOAc 19:1 to 9:1) gave first (*Z*)-**17** and then **19** as a ca. 85:15 *E*/*Z* mixture. Yields and relative proportions of the two lactones depended on the reaction time as indicated in Scheme 4.

When compound (*Z*)-**17** was subjected to the same reaction conditions with catalyst **Ru-II** (reaction time 16 h), **19** was formed in 76% yield (based on recovered starting material) together with unreacted (*Z*)-**17**.

19: colourless oil; ^{1}H NMR (signals from the major *E* isomer): δ = 7.35–7.25 (m, 5H), 5.45–5.30 (m*, 2H), 4.87 (m, 1H), 4.68 (d, J=12 Hz, 1H), 4.38 (d, J=12 Hz, 1H), 4.03 (brdt, J ~13, 4.5 Hz, 1H), 3.97 (brd, J ~11.5 Hz, 1H), 3.28 (brs, 1H), 2.77 (dd, J=14, 12 Hz, 1H), 2.68 (brq, J ~12 Hz, 1H), 2.30 (m, 1H), 2.20–1.90 (brm, 5H), 1.80–1.60 (brm, 4H), 1.17 ppm (d, J=6.5 Hz, 3H) (* the signals of each of the two olefinic protons may be interpreted as double doublets, each peak being further subdivided by many small coupling constants of less than 2 Hz; this makes the extraction of the individual J values difficult, see Supporting Information); ^{13}C NMR (signals from the major *E* isomer): δ = 170.6, 138.5 (C), 133.3, 128.3 (×2), 127.9 (×2), 127.6, 125.0, 73.3, 72.3, 70.0, 67.3 (CH), 70.5, 39.3, 35.0, 34.0, 31.6, 22.1, 21.5 (CH_2), 21.2 ppm (CH_3); IR: ν_{max} = 1728 cm^{-1} (C=O); HR ESMS: m/z: calcd for $C_{21}H_{28}O_4Na$: 367.1885; found: 367.1886 [M+Na$^+$].

(4*S*,7*S*)-7-Benzyloxy-8-(*tert*-butyldiphenylsilyloxy)-oct-1-en-4-ol (20): Asymmetric allylation of aldehyde **9** was carried out under the same conditions followed for the preparation of **12**, except that (+)-Ipc$_2$BCl was now the chiral reagent. Column chromatography on silica gel (hexanes/EtOAc 9:1) afforded alcohol **20**, still contaminated with boron-containing side products, which was used as such in the next step. An aliquot was carefully purified for analytical purposes: colourless oil. $[\alpha]_D$ = −20.9 (c = 2, $CHCl_3$); ^{1}H NMR: δ = 7.75–7.70 (m, 4H), 7.50–7.30 (brm, 11H), 5.85 (ddt, J=17.2, 10.3, 7 Hz, 1H), 5.20–5.10 (m, 2H), 4.72 (d, J=11.5 Hz, 1H), 4.56 (d, J=11.5 Hz, 1H), 3.85 (dd, J=10.6, 5.5 Hz, 1H), 3.74 (dd, J=10.6, 5 Hz, 1H), 3.65–3.55 (m, 2H), 2.30–2.25 (m, 1H), 2.20–2.15 (m, 1H), 2.00 (brs, 1H, OH), 1.80–1.70 (m, 2H), 1.60–1.50 (m, 2H), 1.14 ppm (s, 9H); ^{13}C NMR: δ = 138.7, 133.5 (×2), 19.2 (C), 135.6 (×4), 134.9, 129.6 (×2), 128.3 (×2), 127.8 (×2), 127.7 (×4), 127.5, 79.5, 70.6 (CH), 117.8, 72.0, 66.1, 41.9, 32.4, 27.7 (CH_2), 26.9 ppm (×3) (CH_3); IR: ν_{max} = 3415 cm^{-1} (br, OH); HR ESMS: m/z: calcd for $C_{31}H_{40}O_3SiNa$: 511.2644; found: 511.2645 [M+Na$^+$].

(4*S*,7*S*)-7-Benzyloxy-8-(*tert*-butyldiphenylsilyloxy)-4-(triethylsilyloxy)oct-1-ene (21): The silylation of alcohol **20** was carried out under the same conditions followed for alcohol **12**. Column chromatography on silica gel (hexanes/Et_2O 49:1) furnished **21** (69% overall yield from **9**). Colourless oil. $[\alpha]_D$ = −10.5 (c = 2.4, $CHCl_3$); ^{1}H NMR: δ = 7.75–7.70 (m, 4H), 7.45–7.30 (brm, 11H), 5.82 (ddt, J=17.2, 10.3, 7 Hz, 1H), 5.10–5.00 (m, 2H), 4.68 (d, J=11.7 Hz, 1H), 4.54 (d, J=11.7 Hz, 1H), 3.78 (dd, J=10.6, 5.5 Hz, 1H), 3.75–3.65 (m, 2H), 3.53 (m, 1H), 2.25–2.20 (m, 2H), 1.65–1.45 (m, 4H), 1.10 (s, 9H), 0.98 (t, J=8 Hz, 9H), 0.62 ppm (q, J=8 Hz, 6H); ^{13}C NMR: δ = 139.1, 133.6 (×2), 19.2 (C), 135.6 (×4), 135.2, 129.6 (×2), 128.2 (×2), 127.7 (×2), 127.6 (×4), 127.4, 80.0, 72.0 (CH), 116.7, 71.9, 66.3, 41.9, 32.5, 27.3, 5.1 (×3) (CH_2), 26.9 (×3), 7.0 ppm (×3) (CH_3); HR ESMS: m/z: calcd for $C_{37}H_{54}O_3Si_2Na$: 625.3509; found: 625.3510 [M+Na$^+$].

(4*S*,7*S*)-7-Benzyloxy-8-(*tert*-butyldiphenylsilyloxy)-4-(triethylsilyloxy)oct-2*E*,*Z*-ene (22): The double-bond isomerization in olefin **21** to yield **22** was carried out under the same conditions followed for the conversion of **8** into **7**. Column chromatography on silica gel (hexanes/Et_2O 49:1) gave **22** (88%) as a ca. 9:1 *E*/*Z* mixture: colourless oil. ^{1}H NMR: δ = 7.75–7.70 (m, 4H), 7.50–7.30 (brm, 11H), 5.55–5.45 (m, 1H), 5.40–5.30 (m, 1H), 4.65 (d, J=11.8 Hz, 1H), 4.50 (d, J=11.8 Hz, 1H), 4.00 (m, 1H), 3.73 (dd, J=10.6, 3.5 Hz, 1H), 3.64 (dd, J=10.6, 4.4 Hz, 1H), 3.50 (m, 1H), 1.65 (brd, J ~6.3 Hz, 3H), 1.65–1.40 (brm, 4H), 1.05 (s, 9H), 0.92 (t, J=7.7 Hz, 9H), 0.57 ppm (q, J=7.7 Hz, 6H); ^{13}C NMR (signals from the major *E* isomer): δ = 139.1, 133.6 (×2), 19.2 (C), 135.6 (×4), 134.7, 129.6 (×2), 128.2 (×2), 127.7 (×2), 127.6 (×4), 127.4, 125.3, 80.0, 73.6 (CH), 72.0, 66.3, 34.1, 27.3, 5.0 (×3) (CH_2), 26.8 (×3), 17.5, 6.9 ppm (×3) (CH_3); HR ESMS: m/z: calcd for $C_{37}H_{54}O_3Si_2Na$: 625.3509; found: 625.3502 [M+Na$^+$].

(2*S*,5*S*)-2-(Benzyloxy)-oct-6*E*,*Z*-ene-1,5-diol (23): Desilylation of compound **22** was carried out under the same conditions followed for the preparation of **13**. Column chromatography on silica gel (hexanes/EtOAc

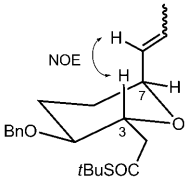
1:1) afforded diol **23** as a ca. 9:1 *E*/*Z* mixture (91%). Colourless oil; ^{1}H NMR (signals from the major *E* isomer): δ = 7.35–7.25 (br m, 5H), 5.60–5.55 (m, 1H), 5.45–5.40 (m, 1H), 4.54 (s, 2H), 3.95 (m, 1H), 3.62 (dd, J = 11.5, 3.8 Hz, 1H), 3.52 (dd, J = 11.5, 5.5 Hz, 1H), 3.47 (m, 1H), 3.10 (br s, 1H, OH), 2.90 (br s, 1H, OH), 1.67 (br d, J ~6.5 Hz, 3H), 1.65–1.45 ppm (br m, 4H); ^{13}C NMR (signals from the major *E* isomer): δ = 138.2 (C), 133.9, 128.2 (×2), 127.6 (×2), 127.4, 126.2, 79.3, 72.3 (CH), 71.2, 63.5, 32.4, 26.4 (CH_2), 17.4 ppm (CH_3); IR: ν_{max} = 3380 cm^{-1} (br, OH); HR ESMS: *m*/*z*: calcd for $C_{15}H_{22}O_3Na$: 273.1467; found: 273.1468 [M+Na$^+$].

(3*S*,6*S*)-3-(Benzyloxy)-6-(prop-1*E*,*Z*-enyl)tetrahydro-2*H*-pyran-2-one (24): The oxidation of diol **23** to lactone **24** was carried out under the same conditions followed for the preparation of **6. CAUTION**: evaporation of volatiles under reduced pressure has to be performed at room temperature, due to the appreciable volatility of lactone **24**. Column chromatography on silica gel (pentane/Et_2O 9:1) provided **24** as a ca. ~9:1 *E*/*Z* mixture (84%). Colourless oil; ^{1}H NMR (signals from the major *E* isomer): δ = 7.45–7.30 (br m, 5H), 5.79 (br dq, J = 15.5, 6.8 Hz, 1H), 5.49 (ddq, J = 15.5, 6.5, 1.5 Hz, 1H), 4.98 (d, J = 11.8 Hz, 1H), 4.72 (m, 1H), 4.65 (d, J = 11.8 Hz, 1H), 4.05 (t, J = 8.2 Hz, 1H), 2.25–2.20 (m, 1H), 2.00–1.85 (br m, 3H), 1.73 ppm (br d, J = 7 Hz, 3H); ^{13}C NMR (signals from the major *E* isomer): δ = 171.5, 137.5 (C), 130.0, 128.6, 128.4 (×2), 128.0 (×2), 127.8, 78.7, 71.5 (CH), 72.4, 26.6, 25.1 (CH_2), 17.6 ppm (CH_3); IR: ν_{max} = 1745 cm^{-1} (C=O); HR ESMS: *m*/*z*: calcd for $C_{15}H_{18}O_3Na$: 269.1154; found: 269.1154 [M+Na$^+$].

(2*R*/*S*,3*S*,6*S*)-3-(Benzyloxy)-6-(prop-1*E*,*Z*-enyl)tetrahydro-2*H*-pyran-2-yl acetate (25): Reduction of lactone **24** to lactol acetate **25** was carried out under the same conditions followed for the preparation of **14**. Column chromatography on silica gel (hexane/EtOAc 19:1) afforded **25** (93%) as a mixture of stereoisomers at both the anomeric carbon and the olefinic bond: colourless oil; ^{1}H NMR: δ = 7.35–7.20 (br m, 5H), 5.75–5.50 (br m, 3H, olefinic and anomeric protons), 4.64 (br s, 2H), 4.05 (ddd, J = 10, 6.5, 2.5 Hz, 1H), 3.52 (br s, 1H), 2.10–2.00 (m, 1H), 2.06 (s, 3H), 1.85–1.75 (m, 1H), 1.65 (br d, J ~6 Hz, 3H), 1.65–1.55 (m, 1H), 1.50–1.40 ppm (m, 1H); IR: ν_{max} = 1750 cm^{-1} (C=O).

***S*-*tert*-Butyl 2-[(2*R*,3*S*,6*S*)-3-(benzyloxy)-6-(prop-1*E*,*Z*-enyl)tetrahydro-2*H*-pyran-2-yl]ethanethioate (26a) and *S*-*tert*-butyl 2-[(2*S*,3*S*,6*S*)-3-(benzyloxy)-6-(prop-1*E*,*Z*-enyl)tetrahydro-2*H*-pyran-2-yl]ethanethioate (26b)**: The C-glycosidation of **25** was carried out under the same conditions followed for the preparation of thiol esters **15a** and **15b**. Work-up (extraction with CH_2Cl_2) and column chromatography on silica gel (hexanes/Et_2O 49:1 to 19:1) furnished first **26b** (24%) and then **26a** (46%), both as ~9:1 *E*/*Z* mixtures.

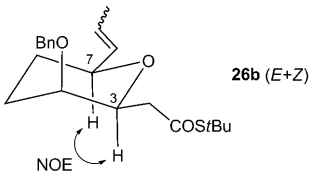
26a: oil; ^{1}H NMR (signals from the major *E* isomer): δ = 7.35–7.20 (m, 5H), 5.75–5.65 (br m, 1H), 5.55–5.50 (m, 1H), 4.60 (d, J = 12 Hz, 1H),



26a (*E*+*Z*)

4.47 (d, J = 12 Hz, 1H), 4.30 (br s, 1H), 4.15 (td, J = 7.7, 4.5 Hz, 1H), 3.20 (m, 1H), 2.90 (dd, J = 14.7, 4.4 Hz, 1H), 2.60 (dd, J = 14.7, 8.3 Hz, 1H), 1.95 (m, 1H), 1.80 (m, 1H), 1.75–1.60 (br m, 2H), 1.71 (br d, J ~6.5 Hz, 3H), 1.44 ppm (s, 9H); ^{13}C NMR (signals from the major *E* isomer): δ = 197.5, 138.2, 47.8 (C), 130.1, 128.2 (×2), 127.8, 127.5 (×2), 127.4, 75.8, 71.6, 71.0 (CH), 70.3, 47.1, 26.9, 24.2 (CH_2), 29.6 (×3), 17.8 ppm (CH_3); IR: ν_{max} = 1683 cm^{-1} (C=O); HR ESMS: *m*/*z*: calcd for $C_{21}H_{31}O_3S$: 363.1994; found: 363.1999 [M+H$^+$]. The NOE indicated above is diagnostic of the relative configuration at C-3 and C-7.

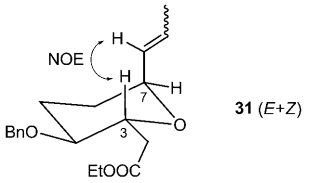
26b: oil; ^{1}H NMR (signals from the major *E* isomer): δ = 7.30–7.15 (m, 5H), 5.60 (br dq, J = 15.2, 6.5 Hz, 1H), 5.42 (ddq, J = 15.2, 6.5, 1 Hz, 1H), 4.54 (d, J = 12 Hz, 1H), 4.35 (d, J = 12 Hz, 1H), 3.81 (br t, J ~6.5 Hz, 1H), 3.76 (br dd, J = 10.8, 6 Hz, 1H), 3.30 (br s, 1H), 2.80–2.70 (m, 2H), 2.05



26b (*E*+*Z*)

(br d, J ~15 Hz, 1H), 1.65 (br q, J ~13.5 Hz, 1H), 1.58 (br d, J ~6.5 Hz, 3H), 1.50–1.30 (m, 2H), 1.35 ppm (s, 9H); ^{13}C NMR (signals from the major *E* isomer): δ = 198.2, 138.4, 47.8 (C), 131.9, 128.2 (×2), 128.0 (×2), 127.5, 126.8, 78.5, 76.0, 71.2 (CH), 70.9, 46.5, 26.0, 25.8 (CH_2), 29.7 (×3), 17.7 ppm (CH_3); IR: ν_{max} = 1679 cm^{-1} (C=O); HR ESMS: *m*/*z*: calcd for $C_{21}H_{30}O_3SNa$: 385.1813; found: 385.1800 [M+Na$^+$]. The NOE indicated above is diagnostic of the relative configuration at C-3 and C-7.

Ethyl 2-[(2*R*,3*S*,6*S*)-3-(benzyloxy)-6-(prop-1*E*,*Z*-enyl)tetrahydro-2*H*-pyran-2-yl]acetate (31): A solution of ethyl acetate (0.1 mL, ~1 mmol) in dry THF (3 mL) was cooled under N_2 to −80 °C and treated dropwise



31 (*E*+*Z*)

with lithium hexamethyldisilazide (1 M in hexane, 0.7 mL, 0.7 mmol). After stirring at this temperature for 30 min, a solution of lactone **24** (25 mg, ca. 0.1 mmol) in dry THF (1 mL) was added dropwise. The reaction mixture was then stirred at −80 °C for 15 min. Work-up (extraction with EtOAc) gave crude lactol **30**, which was dried and used directly in the next step.

The oily lactol from above was dissolved under N_2 in dry CH_2Cl_2 (2 mL) and treated with triethylsilane (160 μL, 1 mmol). After cooling to −80 °C, freshly distilled BF_3·Et_2O (62 μL, 0.5 mmol) was added dropwise. The reaction mixture was then stirred at the same temperature for 16 h. Work-up (extraction with CH_2Cl_2) and column chromatography on silica gel (hexanes/EtOAc 9:1) afforded **31** (29 mg, 91%) as a ca. 9:1 *E*/*Z* mixture as a colourless oil. ^{1}H NMR (signals from the major *E* isomer): δ = 7.35–7.25 (br m, 5H), 5.75–5.65 (m, 1H), 5.57 (ddq, J = 15.5, 3, 1.7 Hz, 1H), 4.62 (d, J = 11.6 Hz, 1H), 4.47 (d, J = 11.6 Hz, 1H), 4.30 (m, 1H), 4.15–4.05 (m, 3H), 3.22 (td, J = 8, 4 Hz, 1H), 2.80 (dd, J = 15.3, 4 Hz, 1H), 2.44 (dd, J = 15.3, 9 Hz, 1H), 2.02 (m, 1H), 1.85–1.65 (br m, 3H), 1.72 (br d, J ~6.5 Hz, 3H), 1.24 ppm (t, J = 7.3 Hz, 3H); ^{13}C NMR (signals from the major *E* isomer): δ = 171.5, 138.3 (C), 130.0, 128.3 (×2), 128.1, 127.7 (×2), 127.6, 76.5, 72.0, 70.7 (CH), 70.5, 60.3, 38.0, 27.2, 24.4 (CH_2), 18.0, 14.2 ppm (CH_3); IR: ν_{max} = 1735 cm^{-1} (C=O); HR ESMS: *m*/*z*: calcd for $C_{19}H_{26}O_4Na$: 341.1729; found: 341.1728 [M+Na$^+$]. The NOE indicated above (see Supporting Information) is diagnostic of the relative configuration at C-3 and C-7.

The experimental procedures for the preparation of compounds **28**, **29**, **32**, (*Z*)-**33** and **34**, as well as the corresponding spectral data, can be taken from ref. [9a].

(1*S*,5*S*,11*S*,14*S*)-14-Hydroxy-5-methyl-4,15-dioxabicyclo-[9.3.1]pentadecan-3-one (35): Palladium catalyst (10% Pd/C, 10 mg) was suspended in EtOAc (1 mL) and stirred under H_2 at room temperature for 10 min. Subsequently, a solution of compound **17**, either as a single stereoisomer or as the *E*/*Z* mixture (17 mg, 0.05 mmol) in EtOAc (2 mL) was added via syringe, followed by stirring under H_2 at room temperature for 2 h. The reaction mixture was then filtered through Celite, and the solvent was removed under reduced pressure. Column chromatography of the residue on silica gel (hexanes/EtOAc 7:3) yielded **35** (12 mg, 95%) as a colourless oil. $[\alpha]_D$ = −28.1 (c = 0.1, $CHCl_3$); ^{1}H NMR: δ = 5.02 (m, 1H), 4.20 (br d, J ~11.3 Hz, 1H), 3.94 (br dd, J ~11, 6 Hz, 1H), 3.60

(m, 1H), 2.66 (brt, J ~13 Hz, 1H), 2.42 (brd, J ~13 Hz, 1H), 2.15–2.05 (brm, 3H), 1.90–1.75 (brm, 2H), 1.75–1.50 (brm, 6H), 1.40–1.20 (brm, overlapping a methyl doublet, 4H), 1.20 ppm (m, 1H); ^{13}C NMR: δ = 170.7 (C), 75.1, 69.8, 69.4, 66.8 (CH), 39.3, 32.9, 26.6, 26.0, 25.6, 25.1, 23.9, 21.6 (CH_2), 20.0 ppm (CH_3); IR: ν_{max} = 3450 (br, OH), 1735 cm^{-1} (C=O); HR ESMS: m/z: calcd for $C_{14}H_{22}O_4Na$: 279.1572; found: 279.1575 [M+Na$^+$].

Materials and methods for the biological work

Cell culture: Cell culture media were purchased from Gibco (Grand Island, NY, USA) and Cambrex (Walkersville, MD, USA). Fetal bovine serum (FBS) was a product of Harlan-Seralab (Belton, UK). Supplements and other chemicals not listed in this section were obtained from Sigma Chemicals Co. (St. Louis, Mo., USA). Plastics for cell culture were supplied by NUNC (Roskilde, Denmark). Aspergillides and their analogues (for structures, see Schemes 1, 3 and 10) were dissolved in DMSO and stored at −20 °C until use.

Bovine aortic endothelial (BAE) cells were obtained by collagenase digestion and maintained in Dulbecco's modified Eagle's medium (DMEM) containing glucose (1 g L^{-1}), glutamine (2 mM), penicillin (50 IU mL^{-1}), streptomycin (50 μg mL^{-1}), and amphoterycin (1.25 μg mL^{-1}), supplemented with 10% FBS. All the cancer cell lines used in this study were obtained from the American Type Culture Collection (ATCC). Human fibrosarcoma HT1080 cells were maintained in DMEM containing glucose (4.5 g L^{-1}), glutamine (2 mM), penicillin (50 IU mL^{-1}), streptomycin (50 μg mL^{-1}), and amphoterycin (1.25 μg mL^{-1}), supplemented with 10% FBS. Human colon adenocarcinoma HT29 and human osteosarcoma U2-OS cells were maintained in McCoy's 5A medium containing glutamine (2 mM), penicillin (50 IU mL^{-1}), streptomycin (50 μg mL^{-1}), and amphoterycin (1.25 μg mL^{-1}), supplemented with 10% FBS. Human breast cancer carcinoma MDA-MB-231 and human promyelocytic leukemia HL60 cells were maintained in RPMI1640 medium containing glutamine (2 mM), penicillin (50 IU mL^{-1}), streptomycin (50 μg mL^{-1}), and amphoterycin (1.25 μg mL^{-1}), supplemented with 10 and 20% FBS, respectively.

Cytotoxicity assays: The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma Chemical Co., St. Louis, MO) dye reduction assay in 96-well microplates was performed by Drug Discovery Biotech S.L. (Málaga, Spain).[47] 3×10^3 BAE or 2×10^3 tumor cells in a total volume of 100 μL of their respective growth media were incubated with serial dilutions of the tested compounds. After 3 d of incubation (37 °C, 5% CO_2 in a humid atmosphere) 10 μL of MTT (5 mg mL^{-1} in PBS) were added to each well and the plate was incubated for further 4 h (37 °C). The resulting formazan was dissolved in 0.04 N HCl in isopropanol (150 μL) and read at 550 nm. All determinations were carried out in triplicate. IC_{50} values were calculated from semilogarithmic dose-response plots as the concentration of compound yielding a 50% of cell survival.

Acknowledgements

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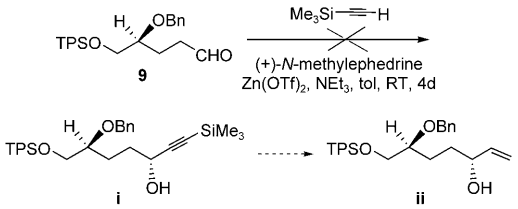

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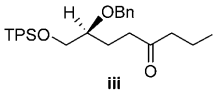

iii

[24] Both *E*/*Z* isomers were synthetically productive. However, in order to avoid working with mixtures, we tried to convert compound **7** into its nor-methyl derivative (vinyl instead of propenyl) by means of cross metathesis under an ethylene atmosphere. This goal was achieved but yields were not satisfactory (50–55% at <1 mmol scale) and difficult to reproduce at a larger scale.

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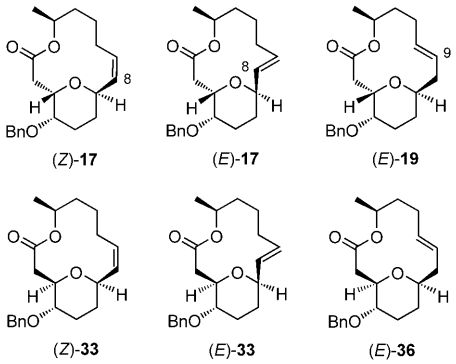
[34] The energy contents and relative stabilities of synthetic lactones (*E*)-**17**, (*Z*)-**17**, (*E*)-**19**, (*E*)-**33** and (*Z*)-**33**, as well as of the nonprepared lactone (*E*)-**36**, have been evaluated with the aid of theoretical calculations. For the results of these, see the Supporting Information.



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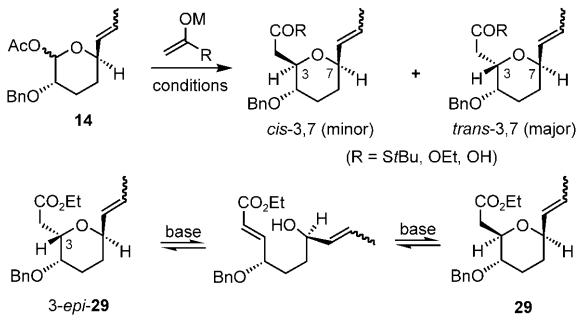
[36] With respect to the reaction conditions used in our previous synthesis of aspergillide B from lactol **14**,[6] where the *trans* (major) to *cis* (minor) ratio was about 2.6:1, changes in temperature, solvent and Lewis acid did not lead to mixtures in which the desired *cis*-3,7-disubstituted tetrahydropyran (aspergillide numbering) was clearly the major component. The enolsilanes of acetic acid and ethyl acetate were tried in addition to *tert*-butyl thioacetate. In all cases, mixtures of the *cis* and *trans* isomers were obtained but the *cis*/*trans* ratio was not ameliorated in this way. Finally, we tried to equilibrate the undesired C-3 epimer of **29** with **29** via a base-catalyzed retro-Michael/Michael sequence (equilibration). Again, mixtures of variable composition were obtained but no synthetically satisfactory percentages of **29**.



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