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Syntheses and Biological Properties of Brefeldin Analogues

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Total and partial syntheses of brefeldin analogues are described. (6*R*)-Hydroxy-BFA (**5**) was obtained through a total synthesis from (1*S*,2*R*)-2-[(trityloxy)methyl]cyclopent-3-ene-1-carbonitrile (*cis*-**8**) in 13 steps. The BFA lactam analogue **6** was prepared via the key building block **25**, which was accessed in four steps from BFA (**1**). (7*S*)-Amino-BFC (**7**) was obtained from 7-dehydro-BFA (**3**) by reductive amination. The structures of the brefeldin analogues were determined by X-ray analyses. The activities of the brefeldin analogues in blocking protein traffic between the endoplasmatic reticulum and the Golgi apparatus were determined both for mammalian cells and for plant cells. Molecular mechanics calculations and docking into the Arf1-GEF protein complex, an established receptor of BFA, were carried out.

Introduction

Brefeldin A (BFA, Figure 1) is a secondary metabolite of several *Ascomycetes* and was first isolated in 1958 from *Penicillium decumbens*.[1] It exhibits antifungal,[2] antiviral,[3] nematodic,[4] and cytostatic activity against numerous human cancer cell lines.[5] The cytostatic effect is caused by the induction of apoptosis in a *p*-53-independent manner.[6] After the discovery of this quite unusual effect in the 1990s, many research groups became interested in BFA. The full mechanism of apoptosis induction still remains to be elucidated, however, and so the molecular target responsible for that mode of action is not known. Although BFA exhibits strong cytostatic activity accompanied by low toxicity,[2,7] which is important for the development of drugs, BFA could not be established as an anticancer agent because of its low bioavailability and poor pharmacokinetics.[8]

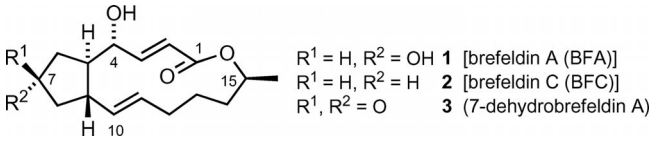

Figure 1. Various brefeldins.

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Another interesting and well-investigated effect of BFA is its ability to block protein traffic from the endoplasmatic reticulum (ER) to the Golgi apparatus. Because of this effect, treatment with BFA leads to dramatic morphological changes of the Golgi apparatus in eukaryotic cells.[9] In particular, the cis-Golgi redistributes into the endoplasmatic reticulum and becomes part of it. Investigation of this mode of action revealed that the molecular target of BFA is a protein complex of a GDP-bound adenosine ribosylation factor (Arf1) and a guanidine exchange factor (GEF).[10] Arf1 belongs to the family of small G-proteins and is responsible for vesicle budding at the donor membrane at the ER.[11] Generally, G-proteins are switched between active and inactive states by exchange of the nucleotide GDP for GTP. This exchange is catalyzed by GEFs. In 2003, Cherfils et al.[12] and Goldberg et al.[13] independently published X-ray crystal structures of a GDP-bound Arf1/Sec7 protein complex. The Sec7 domain is the catalytically active part of the GEF, and BFA binds at the interface of the interacting proteins. It thus appears that BFA is a noncompetitive inhibitor of the Arf1 activation.

The two modes of action displayed by BFA are indicative of several molecular targets. A first hint of this was found by Nojiri et al.,[6c] who showed that the induction of apoptosis is caused by a modulatory effect of BFA on the biosynthesis of gangliosides. They demonstrated that forskolin did not affect the terminal differentiation of cancer cells treated with BFA, whereas it antagonizes the effects of BFA on protein traffic and the Golgi apparatus. Furthermore, Cushman et al.[14] developed BFA analogues that evoked the same morphologic changes to the Golgi apparatus as BFA but did not induce apoptosis. On the other hand, it was found by Ellerby, Bredesen et al. that continuous BFA-induced stress on the endoplasmatic reticulum

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leads to cell death through caspase activation.[15] Accordingly, the induction of apoptosis might be related to the perturbation of protein traffic.

Our aim was to search for brefeldin analogues[16] with cytostatic activity similar to that of BFA but improved in vivo stability and solubility in aqueous systems. The new brefeldin analogues were submitted to the National Cancer Institute (Maryland, USA) for tests of their cytostatic activities in the Developmental Therapeutics Program (dtp).[17] Furthermore, the effects of the brefeldin analogues on the Golgi apparatus were investigated. Both plant and mammalian cells were used. To gain a deeper understanding of the interactions of the brefeldin analogues with the Arf1-GEF protein complex, the structures of the brefeldin analogues were modeled and docked into the BFA binding site.

The following brefeldin analogues were prepared. The first target was (6*R*)-hydroxy-BFA (**5**, cf. Figure 2). This compound was expected to have a higher solubility in water than BFA. Furthermore, previous work by Nojiri et al.[16a] and Cushman et al.[14] had indicated that structural alteration in the vicinity of the 7-OH group without loss of activity might be possible.[18] Remarkably, 8-hydroxy-BFA has been isolated from the broth of an unknown fungus,[19] although no information on its biologic properties is available. Another target was the BFA lactam analogue **6**.[20] Obviously, lactone hydrolysis would be expected to be a major path of deactivation for BFA. Accordingly, the corresponding lactam **6** was a logical target (Figure 2).[21]

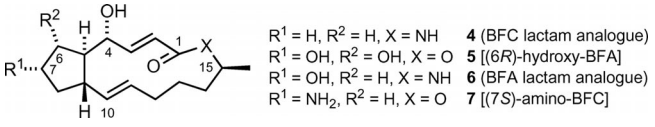


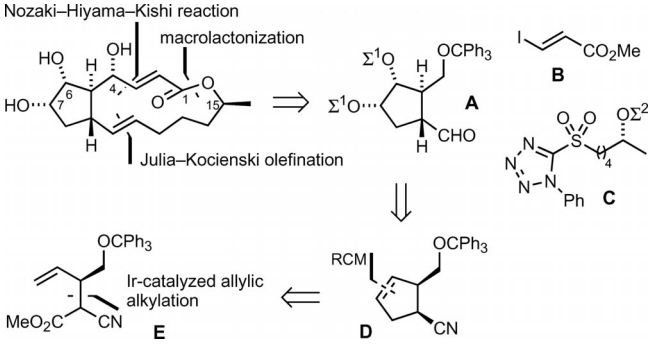
Figure 2. Brefeldin analogues described in this article.

Another possibility was the replacement of a hydroxy group by an amino group, and so the synthesis of (7*S*)-amino-BFC (**7**) was carried out. Apparently, the compound has also been prepared elsewhere, because test results are recorded in the NCI database.[17] Nevertheless, we want to present the synthesis and the X-ray crystal structure of this compound, because no report on it has yet been published.

Results and Discussion

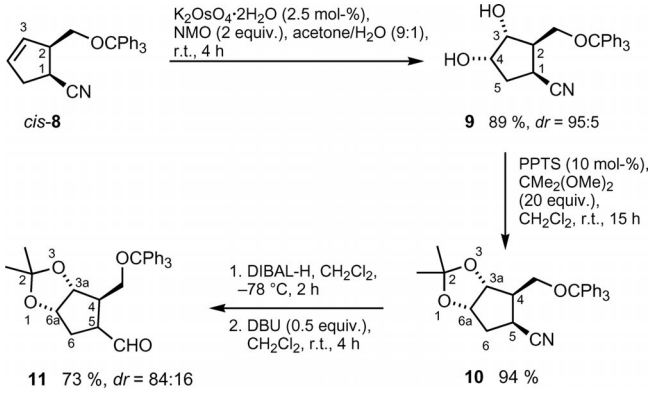
Total Synthesis of (6*R*)-Hydroxybrefeldin A

(6*R*)-Hydroxy-BFA (**5**) was synthesized according to a strategy previously applied by us in a synthesis of the structurally simpler BFC lactam analogue **4** (Scheme 1).[16g] The building blocks **A**–**C** were combined in a convergent manner. Of these, the tetrazolyl sulfones **C** are established compounds.[16f,16g,22] As the cyclopentanoid building block, the nitrile **D** with a *cis* configuration was chosen in order to allow a diastereoselective dihydroxylation. Epimerization to the required *trans* configuration was carried out at the stage of the aldehyde **A**. The nitrile was prepared from compound **E**, obtained by Ir-catalyzed allylic substitution as already described.[16g] The protecting group scheme was based on the use of a trityl group because of convenience in the allylic substitution. The trityl group can be removed by a Birch-type reduction; as further orthogonal protecting groups, an *O*-silyl group (Σ^2) and an acetonide (Σ^1) were chosen.



Scheme 1. Retrosynthetic analysis for (6*R*)-hydroxy-BFA (**5**). Σ: Protecting group.

The starting point of the synthesis was the dihydroxylation of the nitrile *cis*-**8** with *N*-methylmorpholine *N*-oxide (NMO)/$(K_2OsO_4)_{cat}$ (Scheme 2). The reaction proceeded with high diastereoselectivity and in high yield. The resulting diol **9** was protected as an acetonide, and the cyano group of **10** was reduced with DIBAL-H at low temperature to give the aldehyde **11**, which was epimerized as the crude product under basic conditions in order to establish the desired *trans* configuration. The diastereomeric ratio (84:16) was determined by ^{1}H NMR spectroscopy. The isomers could not be separated, and the further synthesis was carried out with the mixture; the products resulting from the minor epimer were removed at a later stage.



Scheme 2. Synthesis of the five-membered ring compound **11**.

The lower side chain was introduced through a Julia–Kocieński olefination, with use of the aldehyde **11** and the tetrazolyl sulfone **12** as starting materials (Scheme 3). The product **13** was a mixture of C-5 epimers (*dr* = 84:16), which we could not separate; the double bond was exclusively formed in the (*E*) configuration according to ^{13}C NMR spectroscopy. The trityl protecting group was re-

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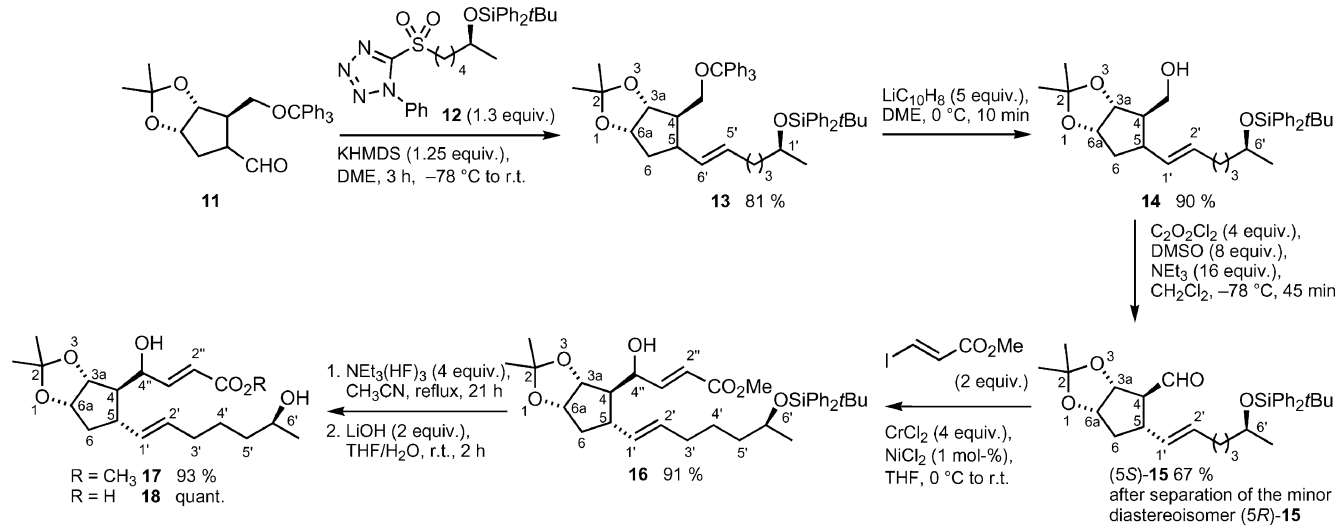
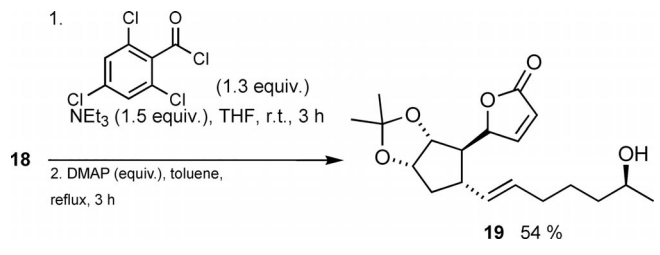


Scheme 3. Assembly of the carbon skeleton of (6*R*)-hydroxy-BFA through a Julia–Kocieński olefination and a Nozaki–Hiyama–Kishi reaction.

ductively removed ($LiC_{10}H_8$ in DME) to give the alcohol **14** in high yield. Swern oxidation gave the aldehyde **15**. At this stage the minor epimer (5*R*)-**15** was removed by column chromatography. The enoate moiety was introduced through a Nozaki–Hiyama–Kishi reaction[23] between the isomerically pure aldehyde (5*S*)-**15** and methyl iodoacrylate. No diastereoselectivity was observed in this step, but the mixture **16** was used for further synthesis, and the stereochemical problem was rectified at a later stage.

Attempts to protect the 4′′-OH group of **16** were unsuccessful, presumably because of steric shielding around the 4′′-OH group. It was decided to make positive use of this effect by carrying out the macrolactonization without an *O*-protecting group in this position. Accordingly, the silyl protecting group was removed by treatment with NEt_3-$(HF)_3$ in CH_3CN at reflux, and the methyl ester **17** was saponified to give the macrocyclization precursor **18** in quantitative yield. Initially, we tested the Yamaguchi method[24] for macrolactonization, but the desired cyclization product was not formed. Instead, the γ-lactone **19** was obtained (Scheme 4). Obviously, a double-bond isomerization of the enoate moiety, induced by conjugate addition of a nucleophile, and a ring closure had occurred. Analogous reactions have been observed by Trost et al.[25] and Fürstner et al.[26]



Scheme 4. Formation of the γ-lactone **19** under Yamaguchi conditions.

In order to find a method that would avoid this reaction, we used (2*E*)-4-hydroxyundec-2-enoic acid as a model substrate and screened several macrolactonization methods;[27] the corresponding γ-lactone – 5-heptylfuran-2(5*H*)-one – was formed in high yield on use of the methods of Yamaguchi, Corey/Nicolaou,[28] and Mukaiyama[29] and upon activation with HBTU. As pointed out previously,[25,26] nucleophilic reaction conditions must be avoided in order to prevent lactone formation. There are only a few corresponding methods, developed by Trost et al.[30] and by Gais et al.[31] We investigated the latter method, for which a push-pull-substituted alkyne – 4-(dimethylamino)but-3-yn-2-one – is used as reagent (Scheme 5), reacting with a carboxylic acid to give an enol ester of *N*,*N*-dimethyl-3-oxobutanamide. This rather stable intermediate can react with an alcohol under acid catalysis.

Application of the Gais method to our problem led to the clean formation of the macrocycle in 78% yield. Furthermore, the C-4-epimeric macrolactones **21a** and **21b** could readily be separated by column chromatography. The assignment of the configuration is based on an X-ray crystal structure of **21b**. In addition, complete epimerization of **21a** was possible by oxidation with MnO_2 followed by reduction of the crude ketone with $NaBH_4$ at –78 °C. Finally, the acetonide protecting group was removed under acidic conditions to give (6*R*)-hydroxy-BFA (**5**) in 78% yield.

Partial Synthesis of the BFA Lactam Analogue 6

The BFA lactam analogue **6** was prepared from BFA (**1**, Scheme 6). The strategy was aimed at an intermediate, the aldehyde **25**, that would be expected to allow introduction of modified southern side chains through Julia–Kocieński olefinations. Compounds **22**–**25** are known intermediates of various total syntheses of BFA, but had not so far been derived from BFA itself.[32] Firstly, BFA was transformed

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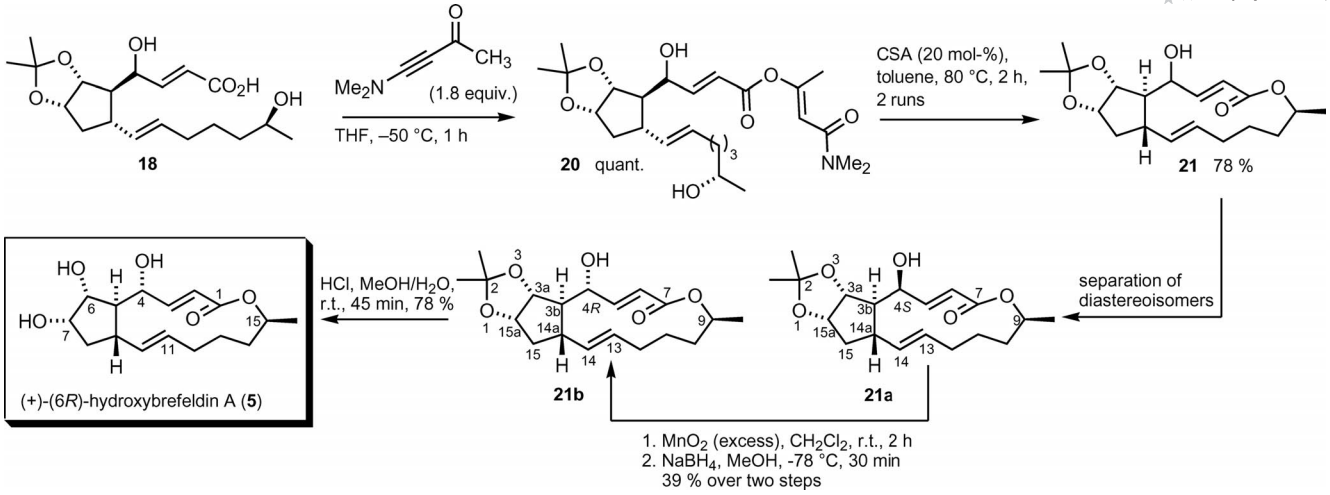
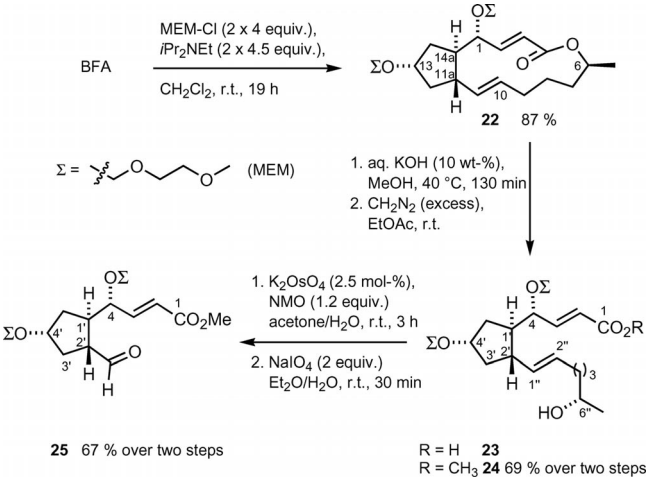


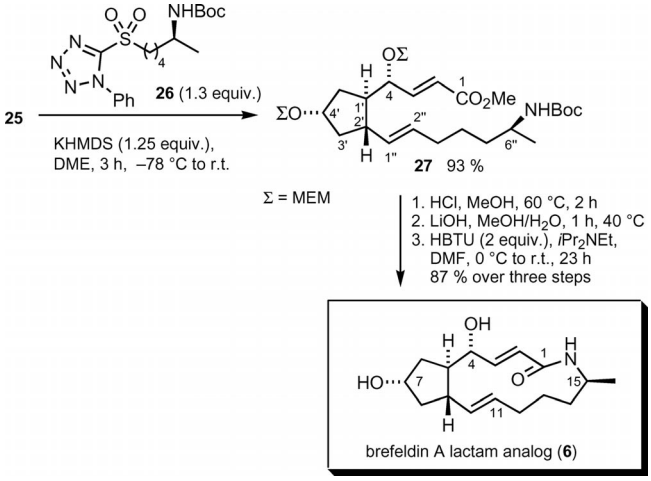
Scheme 5. Final steps of the synthesis of (6*R*)-hydroxy-BFA (**5**).

into the MEM derivative **22**[33] in 87% yield. Saponification and treatment of the resulting carboxylic acid with diazomethane then gave the methyl ester **24**[33a] in 69% yield. The southern side chain was cleaved by dihydroxylation/oxidation. As anticipated, treatment with NMO/$(K_2OsO_4)_{cat}$ preferentially led to dihydroxylation at the electron-rich double bond. The oxidation product was treated with $NaIO_4$ in a stirred mixture of water and diethyl ether. Epimerization (ca. 10%) occurred at C-2′ of the aldehyde **25**, despite careful control of the pH during aqueous workup.



Scheme 6. Synthesis of the key building block **25**.

The aldehyde **25** was subjected to a Julia–Kocieński olefination (Scheme 7) with the tetrazolyl sulfone **26**[16g] to give the (*E*) olefin **27** exclusively according to ^{13}C NMR spectroscopy. The MEM and Boc protecting groups were then removed under acidic conditions, and the methyl ester was saponified to an amino acid, which was subjected to macrolactamization. A method that had been successful in the synthesis of the BFC lactam analogue, was used first.[16g] However, the macrolactamization product **6** was formed in very low yield. Next, amide formation with HBTU/NE*t*iPr_2 was examined. This reaction was very clean and gave the BFA lactam analogue **6** in excellent yield.



Scheme 7. Synthesis of the BFA lactam analogue **6**.

Partial Synthesis of (7*S*)-Amino-BFC

7-Dehydro-BFA (**3**) was used as the starting material for the synthesis of (7*S*)-amino-BFC (**7**, Scheme 8). A reductive amination protocol developed by Ellman et al.[34] was employed: a solution of **3**, *tert*-butyl sulfinamide, and Ti(OEt)$_4$ in THF was heated at reflux. The resulting sulfinimine was reduced with $NaBH_4$ without isolation at –15 °C. As anticipated, the reaction proceeded with very low selectivity to give a mixture of the diastereoisomers **28a** and **28b**, from which the latter was separated by column chromatography (46% yield). Finally, treatment with methanolic HCl gave the amine **7**. The configuration at C-7 was assigned through an X-ray crystal structure analysis of **7**.

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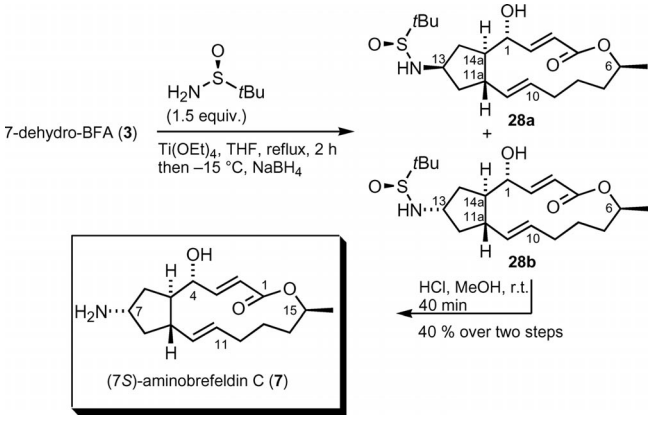

Scheme 8. Reductive amination of 7-dehydro-BFA (**3**).

Biologic Properties of the Brefeldin Analogues

Golgi Disruption

HeLa cells were treated with solutions of the brefeldin analogues (compounds **4**,[16g] **5**, **6**, **7**, and **21b**) in DMSO (1 $\mu g\,mL^{-1}$), and Golgi disrupting effects were determined after 30 min and 60 min. Furthermore, the reversibility of the morphologic changes was confirmed by washout of the brefeldin analogues after 2 h. Immunofluorescence (IF) with the Golgi marker GM130 was performed in order to monitor the Golgi disruption. Representative images were taken with a spinning disk confocal microscope (Figure 3). Activity was observed for (6*R*)-hydroxy-BFA (**5**) and the BFA lactam analogue **6**. In the case of **5**, Golgi reassembly is already starting at an incubation time of 60 min (without washout). This inactivation is likely caused by secretion from cells or metabolization. (7*S*)-Amino-BFC (**7**) showed a very weak effect, whereas the BFC lactam analogue and **21b** did not show activity.

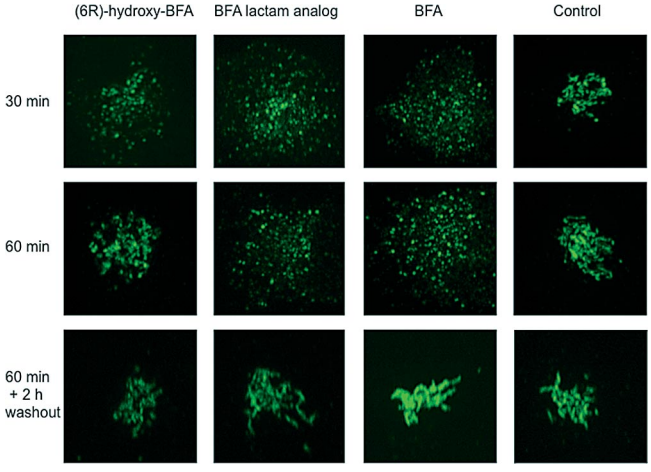

Figure 3. Treatment of HeLa cells with BFA analogues. Cells were incubated with (6*R*)-hydroxy-BFA (**5**), the BFA lactam analogue **6**, or BFA or were mock-treated (control) for the times indicated. Washout experiments were performed after incubation (60 min) in the presence of BFA or BFA analogues, followed by incubation (2 h) in fresh growth medium. Golgi structures were analyzed with the aid of an anti-GM130 antibody.

Anticancer Activity

The brefeldin analogues were submitted to the National Cancer Institute (NCI, Maryland, USA) of the U.S. National Institutes of Health and tested for their cytostatic activities in the Developmental Therapeutics Program (dtp). Modest cytostatic activities were found. The detailed screening results are given in the Supporting Information; the data will also soon be published in the database of the NCI.[35]

X-ray Crystal Structures of the Brefeldin Analogues

X-ray crystal structures of the brefeldin analogues **3**, **5**, **6**, **7**, and **21b** were determined (Table 1). Structural deviations from BFA are negligible for (6*R*)-hydroxy-BFA (**5**) in the 13-membered ring moiety (Figure 4A). Conformational differences for the five-membered ring, however, are substantial. Whereas the 7-OH group is found in an axial disposition in the crystal of BFA, in **5** it occupies an equatorial position. Similarly to **5**, (7*S*)-amino-BFC (**7**) only shows a deviation from BFA with respect to the five-membered ring. The amino group at C-7 is found in an equatorial disposition (Figure 4B).

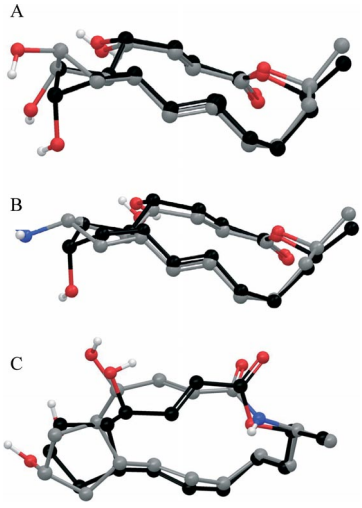

Figure 4. Superposition of the X-ray crystal structures of the brefeldin analogues (gray) and BFA (black). A: (6*R*)-hydroxy-BFA (**5**); B: (7*S*)-amino-BFC (**7**); C: BFA lactam analogue **6**.

A closer look at the crystal lattice of BFA indicates that the axial orientation of the 7-OH group in the solid state is caused by hydrogen bonds. This assumption is supported by two observations. (a) The BFA conformation of lowest energy obtained by force-field calculations (see below) shows an equatorial position of the 7-OH. (b) The X-ray crystal structure of the aminoacetyl derivative of BFA at 7-OH (Breflat) also exhibits an equatorial position of the O-acyl group at C-7. In this case a hydrogen bond is not possible because of the esterification.[36] It thus appears that the axial orientation in the solid state is a crystal-packing effect and that the five-membered ring is flexible, with a small energy difference for the axial and equatorial disposition of the 7-OH group.

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The conformation of the BFA lactam analogue **6** differs considerably from that of BFA (Figure 4C). The main difference is found for the enoate moiety, which possesses an *s-trans* conformation [τ(O=C–C=C) = 177°] in the case of BFA and an *s-cis* conformation [τ(O=C–C=C) = 26°] in the case of **6**.

Molecular Modeling of the Brefeldin Analogues

The structures of the brefeldin analogues were also investigated by molecular modeling, with use of the Macromodel software. Conformational searches based on the MMFFS force field and a Monte Carlo Multiple Minimum (MMCM) search were performed. This procedure was developed by Still et al., who used 7-dehydro-BFA for its validation.[37,38] The geometric parameters of the low-energy structures were used as starting geometries for ligand docking (see below).

The (6*R*)-hydroxy-BFA (**5**) conformer of lowest energy fits the X-ray structure well with regard to the macrocyclic ring. In the case of the five-membered ring, an envelope conformation with an axial disposition of the 7-OH group was identified by a conformational search for the conformer of lowest energy, although a conformer with an equatorial orientation of the 7-OH group was only less stable by 3 kJ mol^{-1}. It is the latter that is present in the X-ray crystal structure of **5**. As pointed out above for BFA, the conformational situation of the five-membered ring is strongly influenced by crystal packing effects, and in solution an equilibrium of both conformers is likely. The coupling constant ^3J(5-H/6-H) = 8.8 Hz was determined by ^{1}H NMR spectroscopy. This value is in good agreement with the value calculated [^3J(5-H/6-H) = 9.2 Hz] for the conformer of lowest energy.

The X-ray crystal structure of (7*S*)-amino-BFC (**7**) was almost perfectly reproduced by the force-field calculations. Reproduction of the structure of the BFA lactam analogue **6** was also satisfactory. All the conformers with energies of up to 10 kJ mol^{-1} were calculated. Two sets of conformers were found, differing mainly in the enoate moiety (Figure 5), one exhibiting the *s-cis* and the other the *s-trans* conformation. These conformers are structurally very similar to the low-energy structure of BFA. Overall, the molecular mechanics calculations reproduced the structures of brefeldin A and its analogues very well.

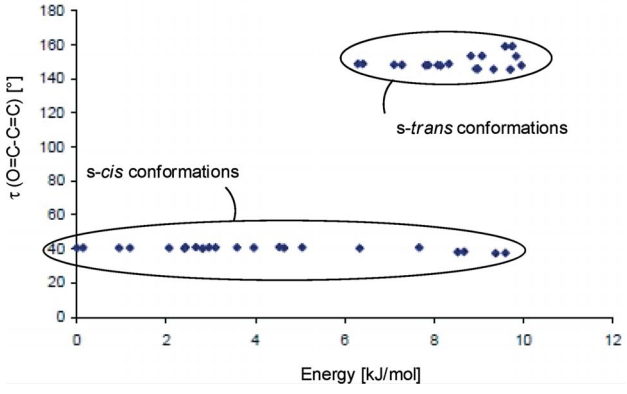

Figure 5. Conformational plot for the BFA lactam analogue **6** calculated by molecular modeling.

Ligand Docking of the Brefeldin Analogues into the Binding Site of BFA

Methods

The starting geometries of the ligands were built by the method described above. Docking was performed with GOLD v. 4.1,[39] and the results were visualized with Chimera.[40] The X-ray structure of the protein complex was downloaded from the PDB (code 1RE0) and prepared for docking with the auxiliary programs of the GOLD package. The BFA ligand was removed from the structure, and the resulting cavity was defined as binding pocket in the docking runs. Water molecules near the binding pocket were included in the definition of the binding site and were defined as flexible. Different rotamers were allowed for three amino acids (Ser198, Tyr256, and Met260; numbering from 1RE0), which appeared to have an increased degree of flexibility and were thought to be relevant for the binding of the brefeldin analogues. Otherwise, the default settings of the GOLD software were used for the docking runs.

Results

All ligands were docked in a position and orientation (pose) that resembles the pose of the brefeldin molecule. This is demonstrated in Figure 6, in which the docked pose of (6*R*)-hydroxy-BFA is overlaid with the brefeldin X-ray structure 1RE0. High docking scores, which indicate a good geometric fit and favorable interactions in the binding pocket, were obtained for all analogues except for the acetonide **21b** of hydroxy-BFA. This compound fits into the pocket, but has a significantly lower hydrogen-bonding score than the other compounds.

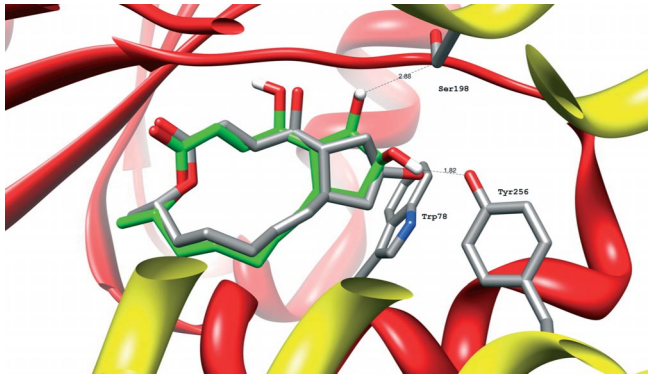

Figure 6. Close-up view of BFA in the binding pocket between Arf1 (red ribbon) and Sec7 (yellow ribbon), based on the X-ray structure 1RE0. The key hydrophilic residues Tyr256, Ser198, and Trp78 are represented as sticks. Overlaid is the docked pose of (6*R*)-hydroxy-BFA (**5**). This compound can, depending on the conformation of the cyclopentane ring, enter into hydrogen-bonding interactions with Tyr256 and Trp78. A rotation of the Ser198 side chain results in additional interactions with the 6-hydroxy group of **5**.

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Discussion of Structure–Activity Relationships and Docking

Significant activity is observed for BFA and its hydroxylated analogues [i.e., (6*R*)-hydroxy-BFA (**5**) and the BFA lactam analogue **6**]. The binding pocket of BFA is characterized by a large lipophilic area, occupied by the macrolactone ring, and a smaller hydrophilic region. The latter is formed by the residues Tyr256 and Ser198 from Sec7 and Trp78 from ARF1 (numbering from 1RE0). Although Ser198 is not directly involved in the binding of BFA, a rotation of the side chain of this residue results in an additional hydrogen-bonding interaction to ligands in the pocket. In Figure 6, the X-ray structure of BFA bound to Sec7 and Arf1 is compared with the docked pose of (6*R*)-hydroxy-BFA (**5**). The conformations of the two ligands are clearly very similar. It is also obvious that the additional hydroxy group of **5** has the potential to enter into additional interactions with Ser198 if the side chain is rotated towards the ligand, which can easily explain the high activity of this analogue.

There are three inactive compounds in the data set: (7*S*)-amino-BFC (**7**), the BFC lactam analogue, and the acetonide-protected (6*R*)-hydroxy-BFA **21b**. The hydrogen-bonding capacity of the cyclopentane moiety of the last two compounds is either nonexistent or severely restricted. The inactivity of these compounds can be explained unproblematically in accordance with the previous reasoning.

(7*S*)-Amino-BFC (**7**) is inactive in the cellular assays, although it has a hydrogen-bonding potential similar to that of BFA and consequently obtains high scores in the docking runs. It appears likely that the basic functionality, which under physiological conditions is completely protonated and positively charged, confers a different extra- and intracellular "pharmacokinetic" profile to this compound. This can result, through various mechanisms, in a diminished presence of this derivative at the locus of action.

Conclusions

We have synthesized (6*R*)-hydroxy-BFA through a total synthesis by a route previously developed for the BFC lactam analogue. The BFA lactam analogue and (7*S*)-amino-BFC were prepared by partial syntheses starting from natural BFA and 7-dehydro-BFA. In biological tests, (6*R*)-hydroxy-BFA induced morphological changes to the Golgi apparatus in mammalian cells and in *Arabidopsis* cells similar to those observed with BFA. The BFA lactam analogue **6** was only active in mammalian cells. Furthermore, conformation analyses by molecular mechanics calculations and docking of the brefeldin analogues into the BFA binding site of the Arf1-GEF protein complex were carried out.

Experimental Section

General Information: Melting points are uncorrected. Optical rotations were measured with a Perkin–Elmer 341 Polarimeter and a mercury lamp. ^{1}H NMR spectra were recorded at room temperature with Bruker DRX 200 (199.92 MHz), Bruker Avance DRX 300 (300.13 MHz), Bruker Avance DRX 500 (500.13 MHz), or Bruker Avance III 600 (600.13 MHz) spectrometers. Chemical shifts are reported in δ units relative to the solvent residual peak ($CHCl_3$ in $CDCl_3$ at δ_H = 7.26 ppm, CD_2HOD in CD_3OD at δ_H = 3.31 ppm).[41] The following abbreviations are used for description of the signal multiplicity: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), dt (doublet of triplets), ddd (doublet of doublets of doublets), etc., br. s (broad signal), m (multiplet). Diastereotopic hydrogen atoms are described with superscripts 1 and 2 (CH^1H^2). Chemical shifts are not reported for acidic protons (NH, OH) if CD_3OD was used as solvent. ^{13}C NMR spectra were recorded at room temperature with Bruker DRX 200 (50.27 MHz), Bruker Avance DRX 300 (75.47 MHz), Bruker Avance DRX 500 (125.76 MHz), or Bruker Avance III 600 (150.90 MHz) spectrometers. Chemical shifts are reported in δ units relative to the solvent signal: $CDCl_3$ [δ_C = 77.16 ppm (central line of the triplet)], CD_3OD [δ_C = 49.00 ppm (central line of the septet)].[41] The following abbreviations were used: s (singlet, quaternary C-atom), d (doublet, CH group), t (triplet, CH_2 group), q (quartet, CH_3 group). The multiplicity stated refers to {^{1}H}-decoupled spectra. In every case, the assignments of signals were confirmed through ^{1}H,^{1}H-COSY, ^{1}H,^{13}C-COSY, and DEPT spectra. High-resolution mass spectra were recorded with a JEOL JMS-700 (EI^+, FAB^+) or a Bruker ApexQe FT-ICR (ESI^+) mass spectrometer. Elemental analyses were carried out at the Organisch-Chemisches Institut, Universität Heidelberg. Analytical thin-layer chromatography was performed with precoated TLC plates (Polygram® SIL G/UV_{254}, Macherey & Nagel). Spots were visualized with UV light (λ = 254 nm) or by dipping plates into an aqueous solution of $KMnO_4$ [$KMnO_4$ (1.5 g), K_2CO_3 (2.5 g), in H_2O (250 mL)] and subsequent heating. Flash column chromatography was carried out with silica gel (0.032–0.062) (Macherey, Nagel and Co.). Tetrahydrofuran was dried with benzophenone ketyl, and the water content was determined by Karl Fischer titration. The following compounds were prepared according to published procedures: 5-{[(5*S*)-5-{[*tert*-butyl(diphenyl)silyl]oxy}hexyl]sulfonyl}-1-phenyl-1*H*-tetrazole (**12**),[16f] 4-(dimethylamino)but-3-yn-2-one,[42] (1*S*,2*R*)-2-[(trityloxy)methyl]cyclopent-3-ene-1-carbonitrile (*cis*-**8**),[16g] methyl (2*E*)-3-iodoacrylate,[43] and *tert*-butyl {(1*S*)-1-methyl-5-[(1-phenyl-1*H*-tetrazol-5-yl)sulfonyl]pentyl}carbamate (**27**).[16g] IUPAC names were generated with the aid of the program ACD/Labs 6.0 from Advanced Chemistry Development Inc.

Golgi Disruption: HeLa cells were cultivated in DMEM medium [supplemented with fetal calf serum (10%), penicillin (100 units mL^{-1}), streptomycin (100 µg mL^{-1}), and L-glutamine (2 mM)]. BFA or BFA analogues (final concentration 5 µg mL^{-1}) were added at 37 °C for 30 or 60 min. For reassembly studies cells were incubated in the presence of BFA or BFA analogues for 60 min. After washout, the cells were incubated at 37 °C in DMEM medium for an additional 2 h. Cells were processed for immunofluorescence and microscopy with the aid of an anti-GM130 antibody (BD Transduction) as a Golgi marker as described.[44] Images were taken with a Perkin–Elmer Ultra-view spinning disk confocal microscope at the Nikon Imaging Center, Heidelberg.

Analytical Data for (1*S*,2*R*)-2-[(Trityloxy)methyl]cyclopent-3-ene-1-carbonitrile (*cis*-8): $[\alpha]_D^{20}$ = –61.0 (*c* = 0.435, $CHCl_3$, 97% *ee*). ^{1}H NMR (300.13 MHz, $CDCl_3$): δ = 2.69–2.81 (m, 2 H, 5-H), 3.02–3.15 (m, 1 H, 2-H), 3.17–3.40 (m, 3 H, 1-H, CH_2O), 5.66–5.75 (m, 1 H, 3-H or 4-H), 5.75–5.84 (m, 1 H, 3-H or 4-H), 7.15–7.36 (m, 9 H, Ar-H), 7.48–7.53 (m, 6 H, Ar-H) ppm. ^{13}C NMR (75.48 MHz, $CDCl_3$): δ = 29.79 (d, C-1), 37.55 (t, C-5), 47.73 (d, C-2), 64.12 (t, CH_2O), 87.22 (s, CPh_3), 120.83 (s, CN), 127.16, 127.90, 128.91 (3 × d, C-Ar), 129.84, 131.34 (2 × d, C-3, C-4), 144.02 (s, C-Ar) ppm. HR-MS (FAB^+): *m*/*z* calcd. for $C_{26}H_{23}NO^+$ 365.1774 $[M]^+$;

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found 365.1779. $C_{26}H_{23}NO$ (365.47): calcd. C 85.45, H 6.34, N 3.83; found C 85.37, H 6.37, N 3.77. HPLC [Chiralpak AD-H, *n*-hexane/*i*PrOH (99:1), flow 0.5 mL min^{-1}, room temp., λ = 210 nm]: t_R[(+)-(1*R*,2*S*)-**8**] = 24.8 min, t_R[(–)-(1*S*,2*R*)-**8**] = 32.8 min.

(1*S*,2*R*,3*R*,4*S*)-3,4-Dihydroxy-2-[(trityloxy)methyl]cyclopentanecarbonitrile (9): $K_2OsO_4{\cdot}2H_2O$ (55 mg, 0.15 mmol) and NMO [2.53 mL, 12.00 mmol (50 wt.-% solution in water)] were added consecutively to a solution of *cis*-**8** (2.193 g, 6.00 mmol) in acetone/water (9:1, 30 mL). The mixture was stirred at room temperature for 3 h [TLC monitoring: petroleum ether/ethyl acetate (3:2); R_f(**9**) = 0.14]. $Na_2S_2O_4$ (100 mg) was then added, and the mixture was stirred for a further 30 min. A black precipitate was removed by filtration through a pad of Celite, which was rinsed with diethyl ether (100 mL). The solvent was evaporated in vacuo, and the crude product was purified by flash chromatography on silica gel [100 g; petroleum ether/ethyl acetate (1:1); R_f(**9**) = 0.22] to give **9** (2.134 mg, 89%) as a white powder. $[\alpha]_D^{20}$ = 21.0 (*c* = 1.42, $CHCl_3$). ^{1}H NMR (300.13 MHz, $CDCl_3$): δ = 2.08 (ddd, *J* = 14.4, *J* = 6.9, *J* = 4.9 Hz, 1 H, 5-H^1), 2.18 (ddd, *J* = 14.4, *J* = 8.9, *J* = 2.3 Hz, 1 H, 5-H^1), 2.45 (ddd, *J* = 17.6, *J* = 8.5, *J* = 6.4 Hz, 1 H, 2-H), 2.55 (br. s, 1 H, OH), 2.85 (d, *J* = 2.4 Hz, 1 H, OH), 3.26 (ddd, *J* = 9.2, *J* = 9.2, *J* = 7.1 Hz, 1 H, 1-H), 3.46 (m_c, 1 H, CH_2O), 3.55 (dd, *J* = 9.5, *J* = 6.3 Hz, 1 H, CH_2O), 3.80–3.88 (m, 1 H, 3-H), 4.11–4.17 (m, 1 H, 4-H), 7.21–7.36 (m, 9 H, Ar-H), 7.39–7.50 (m, 6 H, Ar-H) ppm. ^{13}C NMR (75.48 MHz, $CDCl_3$): δ = 26.70 (d, C-1), 35.76 (t, C-5), 44.08 (d, C-2), 64.24 (t, CH_2O), 71.55 (d, C-4), 77.19 (d, C-3), 87.92 (s, CPh_3), 120.40 (s, CN), 127.50, 128.18, 128.73 (3×d, C-Ar), 143.51 (s, C-Ar) ppm. HR-MS (FAB$^+$): *m*/*z* calcd. for $C_{26}H_{25}O_3NNa^+$ 422.1727 [M + Na]$^+$; found 422.1748. $C_{26}H_{25}NO_3$ (399.48): calcd. C 78.17, H 6.31, N 3.51; found C 78.34, H 6.29, N 3.55.

(3a*R*,4*R*,5*S*,6a*S*)-2,2-Dimethyl-4-[(trityloxy)methyl]tetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxole-5-carbonitrile (10): 2,2-Dimethoxypropane (13.2 mL, 107.4 mmol) and pyridinium toluene-4-sulfonate (134 mg, 0.533 mmol) were added at room temperature to a solution of **9** (2.134 g, 5.342 mmol) in CH_2Cl_2 (21 mL). After 2.5 h, TLC monitoring showed complete conversion [petroleum ether/ethyl acetate (1:1); R_f(**9**) = 0.22, R_f(**10**) = 0.76]. The solution was added to a mixture of saturated aqueous $NaHCO_3$ (50 mL) and CH_2Cl_2 (50 mL). The aqueous layer was separated and extracted with CH_2Cl_2 (3×20 mL). The combined organic layers were washed with brine, dried with Na_2SO_4, filtered, and concentrated in vacuo. The crude product was subjected to flash chromatography on silica gel [petroleum ether/ethyl acetate (5:1); UV detection; R_f(**10**) = 0.35] to give **10** (2.219 g, 94%) as a glass. $[\alpha]_D^{20}$ = –25.5 (*c* = 0.970, $CHCl_3$). ^{1}H NMR (300.13 MHz, $CDCl_3$): δ = 1.23, 1.40 [2×s, 6 H, $C(CH_3)_2$], 2.12–2.35 (m, 2 H, 6-H), 2.45 (dd, *J* = 11.6, *J* = 5.4 Hz, 1 H, 4-H), 3.20 (ddd, *J* = 12.1, *J* = 7.2, *J* = 7.2 Hz, 1 H, 5-H), 3.37 (dd, *J* = 10.2, *J* = 5.7 Hz, 1 H, CH_2O), 3.42 (dd, *J* = 10.2, *J* = 4.6 Hz, 1 H, CH_2O), 4.32 (d, *J* = 5.7 Hz, 1 H, 3a-H), 4.60 (dd, *J* = 5.5, *J* = 5.5 Hz, 1 H, 6a-H), 7.21–7.36 (m, 6 H, Ar-H), 7.41–7.50 (m, 9 H, Ar-H) ppm. ^{13}C NMR (75.48 MHz, $CDCl_3$): δ = 23.81, 26.41 [2×q, $C(CH_3)_2$], 28.60 (d, C-5), 37.36 (t, C-6), 47.85 (d, C-4), 61.88 (t, CH_2O), 79.61 (d, C-6a), 82.98 (d, C-3a), 88.00 (s, CPh_3), 109.88 [s, $C(CH_3)_2$], 119.92 (s, CN), 127.38, 128.10, 128.83 (3×d, C-Ar), 143.58 (s, C-Ar) ppm. HR-MS (ESI$^+$): *m*/*z* calcd. for $C_{29}H_{29}NO_3Na^+$ 462.2040 [M + Na]$^+$; found 462.2043. $C_{29}H_{29}NO_3$ (439.55): calcd. C 79.24, H 6.65, N 3.19; found C 79.61, H 6.66, N 2.86.

(3a*R*,4*R*,5*S*,6a*S*)-2,2-Dimethyl-4-[(trityloxy)methyl]tetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxole-5-carbaldehyde (11): A solution of DIBAL-H in hexane (4.4 mL, 1 M) was added dropwise to a cooled (–78 °C) solution of **10** (647 mg, 1.47 mmol) in dry CH_2Cl_2 (6 mL). After the mixture had been stirred for 2 h, MeOH (4.4 mL) was added, and stirring was continued at –78 °C for further 10 min. The mixture was then added to a saturated aqueous solution of sodium potassium tartrate. The aqueous layer was extracted with CH_2Cl_2 (3×20 mL). The combined organic layers were dried with Na_2SO_4, filtered, and concentrated in vacuo. The crude product was dissolved in CH_2Cl_2 (15 mL), and DBU was added (0.11 mL, 0.74 mmol). After 2 h of stirring, the solvent was evaporated in vacuo. The ^{1}H NMR spectrum of the crude product showed incomplete isomerization. More DBU (0.08 mL, 0.05 mmol) was added to a solution of the crude product in CH_2Cl_2 (4.5 mL). After 2 h of stirring, the solvent was evaporated in vacuo, and the residue was subjected to flash column chromatography on silica gel [petroleum ether/ethyl acetate (5:1); UV detection; R_f(**11**) = 0.38] to yield **11** (473 mg, 1.069 mmol, 73%) as a colorless oil. The ratio of diastereoisomers was determined by ^{1}H NMR to be 84:16. ^{1}H NMR (300.13 MHz, $CDCl_3$): δ = 1.22, 1.28 [2×s, 6 H, $C(CH_3)_2$], 1.74 (ddd, *J* = 13.9, *J* = 12.2, *J* = 5.2 Hz, 1 H, 6-H^1), 1.96 (dd, *J* = 14.0, *J* = 6.1 Hz, 1 H, 6-H^1), 2.07–2.20 (m, 1 H, 4-H), 2.64–2.78 (m, 1 H, 5-H), 3.22 (dd, *J* = 9.0, *J* = 9.0 Hz, 1 H, CH_2O), 3.55 (dd, *J* = 9.5, *J* = 5.7 Hz, 1 H, CH_2O), 4.56–4.68 (m, 2 H, 3a-H, 6a-H), 7.18–7.34 (m, 9 H, Ar-H), 7.36–7.44 (m, 6 H, Ar-H), 9.67 (d, *J* = 3.1 Hz, 1 H, CHO) ppm. ^{13}C NMR (75.48 MHz, $CDCl_3$): δ = 24.04, 26.00 [2×q, $C(CH_3)_2$], 33.97 (t, C-6), 47.97 (d, C-4), 52.55 (d, C-5), 62.28 (t, CH_2O), 79.74, 80.81 (2×d, C-3a, C-6a), 87.43 (s, CPh_3), 109.80 (s, C-2), 127.09, 127.89, 128.86 (3×d, C-Ar), 144.06 (s, C-Ar), 203.13 (d, CHO) ppm. HR-MS (FAB$^+$): *m*/*z* calcd. for $C_{29}H_{30}O_4Na^+$ 465.2036 [M + Na]$^+$; found 465.2056 . $C_{29}H_{30}O_4$ (442.55): calcd. C 78.71, H 6.83; found C 78.92, H 7.01.

***tert*-Butyl{[(1*S*,5*E*)-6-{(3a*R*,4*R*,5*S*,6a*S*)-2,2-dimethyl-4-[(trityloxy)methyl]tetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxol-5-yl}-1-methylhex-5-en-1-yl]oxy}diphenylsilane (13):** A solution of KHMDS in toluene (0.5 M, 6.66 mL, 3.33 mmol) was added dropwise to a cooled (–78 °C) solution of **12** (1.895 g, 3.453 mmol) in dry DME (13.3 mL, freshly dried with sodium), and the mixture was stirred at this temperature for 30 min. A solution of **11** (1.18 g, 2.67 mmol) in dry DME (13.3 mL) was then added dropwise. After stirring at –78 °C for 2 h, the solution was allowed to warm to room temperature and was stirred for a further 1 h. Afterwards, water (30 mL), brine (30 mL), and diethyl ether (60 mL) were added. The aqueous layer was separated and extracted with diethyl ether (3×50 mL). The combined organic layers were dried with Na_2SO_4, filtered, and concentrated in vacuo. The residue was subjected to flash column chromatography on silica gel [petroleum ether/ethyl acetate (20:1); UV detection; R_f(**13**) = 0.36] to yield **13** as a colorless, viscous oil (1.658 g, 2.167 mmol, 81%). The product was a mixture of diastereoisomers at C-5 (84:16) that could not be separated. ^{1}H NMR (300.13 MHz, $CDCl_3$): δ = 1.03–1.10 (m, 3 H, C-1′-CH_3), 1.08 [s, 9 H, $C(CH_3)_3$], 1.20–1.54 (m, 4 H, 2′-H, 3′-H), 1.34, 1.53 [2×s, 6 H, $C(CH_3)_2$], 1.65 (ddd, *J* = 13.8, *J* = 10.5, *J* = 5.1 Hz, 1 H, 6-H^1), 1.72–1.88 (m, 2 H, 4′-H), 1.95 (ddd, *J* = 8.8, *J* = 8.7, *J* = 4.3 Hz, 1 H, 4-H), 2.17 (ddd, *J* = 13.2, *J* = 6.6, *J* = 6.6 Hz, 1 H, 6-H^1), 2.57 (dddd, *J* = 9.8, *J* = 9.8, *J* = 7.3, *J* = 7.3 Hz, 1 H, 5-H), 3.10 (dd, *J* = 9.2, *J* = 4.5 Hz, 1 H, CH_2O), 3.27 (dd, *J* = 9.2, *J* = 4.4 Hz, 1 H, CH_2O), 3.76–3.92 (m, 1 H, 1′-H), 4.52–4.70 (m, 2 H, 3a-H, 6a-H), 5.12–5.38 (m, 2 H, 5′-H, 6′-H), 7.18–7.50 (m, 21 H, Ar-H), 7.67–7.74 (m, 4 H, Ar-H) ppm. ^{13}C NMR (75.48 MHz, $CDCl_3$): δ = 19.40 [s, $C(CH_3)_3$], 23.37 (q, C-1′-CH_3), 25.10 (t, C-3′), 25.21, 27.74 [2×q, $C(CH_3)_2$], 27.19 [q, $C(CH_3)_3$], 32.49 (t, C-4′), 39.10 (t, C-2′), 39.15 (t, C-6), 44.41 (d, C-5), 51.86 (d, C-4), 62.11 (t, CH_2O), 69.60 (d, C-1′), 80.03, 83.24 (2×d, C-3a or C-6a), 86.47 (s, CPh_3), 111.92 (s, C-2), 127.02, 127.51, 127.59, 127.83, 128.90, 129.51,

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129.58 (7 × d, C-Ar), 130.57 (d, C-5′), 132.50 (d, C-6′), 134.71, 135.05 [2 × s, OSi*Ph*$_2$C(CH$_3$)$_3$], 135.98, 136.00 (2 × d, C-Ar), 144.29 (s, C*Ph*$_3$) ppm. HR-MS (ESI$^+$): *m*/*z* calcd. for $C_{51}H_{60}O_4SiNa^+$ 787.4153 [M + Na]$^+$; found 787.4152. $C_{51}H_{60}O_4Si$ (765.11): calcd. C 80.06, H 7.90; found C 80.22, H 8.03.

{(3a*R*,4*R*,5*S*,6a*S*)-5-[(1*E*,6*S*)-6-{[*tert*-Butyl(diphenyl)silyl]oxy}hept-1-en-1-yl]-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxol-4-yl}methanol (14): Granular lithium (152 mg, 21.9 mmol) was added to a solution of naphthalene (1.402 g, 10.94 mmol) in dry DME (55 mL), and the mixture was sonicated at room temperature for 1 h to give a dark green stock solution of $LiC_{10}H_8$ in DME (0.2 M). Part of this solution (38.3 mL, 7.62 mmol of $LiC_{10}H_8$) was then added dropwise by syringe to a cooled (0 °C) solution of **13** (1.340 g, 1.751 mmol) in dry DME (7.7 mL). After 10 min of stirring, TLC monitoring showed complete conversion of **13** [petroleum ether/ethyl acetate (10:1); UV detection; R_f(**13**) = 0.47, R_f(**14**) = 0.10]. Brine (50 mL), water (50 mL), and diethyl ether (50 mL) were then added. The aqueous layer was separated and extracted with diethyl ether (3 × 80 mL). The combined organic layers were dried with Na_2SO_4, filtered, and concentrated in vacuo. The residue was subjected to flash column chromatography on silica gel (petroleum ether/ethyl acetate (10:1 to 1:1)] to yield **14** (826.5 mg, 1.581 mmol, 90%) as a colorless oil. The product was a mixture of diastereoisomers (84:16), which were not separated by column chromatography. However, pure fractions could be obtained for analytical evaluation. $[\alpha]_D^{20}$ = –20.2 (*c* = 1.35, $CHCl_3$). ^{1}H NMR (300.13 MHz, $CDCl_3$): δ = 1.05 [s, 9 H, C(CH$_3$)$_3$], 1.01–1.10 (m, 3 H, C-6′-C*H*$_3$), 1.20–1.52 (m, 4 H, 4′-H, 5′-H), 1.32, 1.52 [2 × s, 6 H, C(CH$_3$)$_2$], 1.62 (ddd, *J* = 13.0, *J* = 11.2, *J* = 5.6 Hz, 1 H, 6-H^1), 1.80–1.98 (m, 3 H, 3′-H^1, 4-H), 2.17 (ddd, *J* = 13.4, *J* = 6.8, *J* = 6.8 Hz, 1 H, 6-H^1), 2.30 (dddd, *J* = 10.7, *J* = 10.7, *J* = 7.0, *J* = 7.0 Hz, 1 H, 5-H), 3.55 (dd, *J* = 10.7, *J* = 6.7 Hz, 1 H, CH$_2$O), 3.75 (dd, *J* = 10.7, *J* = 4.6 Hz, 1 H, CH$_2$O), 3.79–3.89 (m, 1 H, 6′-H), 4.42 (dd, *J* = 6.5, *J* = 6.5 Hz, 1 H, 3a-H), 4.58 (dd, *J* = 12.8, *J* = 6.5 Hz, 1 H, 6a-H), 5.25–5.45 (m, 2 H, 1′-H, 2′-H), 7.32–7.47 (m, 6 H, Ar-H), 7.64–7.74 (m, 4 H, Ar-H) ppm. ^{13}C NMR (75.48 MHz, $CDCl_3$): δ = 19.40 [s, *C*(CH$_3$)$_3$], 23.37 (q, C-6′-*C*H$_3$), 25.11 (t, C-4′), 25.13 [q, C(*C*H$_3$)$_2$], 27.17 [q, C(*C*H$_3$)$_3$], 27.67 [q, C(*C*H$_3$)$_2$], 32.42 (t, C-3′), 38.92 (t, C-6), 39.02 (t, C-5′), 44.64 (d, C-5), 53.35 (d, C-4), 63.17 (t, CH$_2$O), 69.54 (d, C-6′), 79.53 (d, C-6a), 83.57 (d, C-3a), 112.73 [s, *C*(CH$_3$)$_2$], 127.51, 127.59, 129.52, 129.58 (4 × d, C-Ar), 131.47, 132.06 (2 × d, C-1′, C-2′), 134.71, 135.00 (2 × s, C-Ar), 135.99 (d, C-Ar) ppm. HR-MS (ESI$^+$) calcd. for $C_{32}H_{46}O_4SiNa^+$ 545.3058 [M + Na]$^+$; found 545.3066. $C_{32}H_{46}O_4Si$ (522.79): calcd. C 73.52, H 8.87; found C 73.30, H 8.89.

(3a*R*,4*S*,5*S*,6a*S*)-5-[(1*E*,6*S*)-6-{[*tert*-Butyl(diphenyl)silyl]oxy}hept-1-en-1-yl]-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxole-4-carbaldehyde (15): DMSO (0.51 mL, 7.12 mmol) was added to a cooled (–78 °C) solution of oxalyl chloride (0.31 mL, 3.56 mmol) in dry CH_2Cl_2 (4.5 mL). After 10 min of stirring, a solution of **14** (465 mg, 0.89 mmol) in dry CH_2Cl_2 (4.5 mL) was added to the mixture. After further 20 min of stirring, NEt_3 (1.98 mL, 14.2 mmol) was added dropwise. After 45 min, TLC monitoring [petroleum ether/ethyl acetate (3:1)] showed complete conversion. The solution was treated with phosphate buffer solution (10 mL, 0.5 M solution of NaH_2PO_4/Na_2HPO_4), and the mixture was extracted with CH_2Cl_2 (3 × 25 mL). The combined organic layers were dried with Na_2SO_4, filtered, and concentrated in vacuo. The residue was subjected to flash column chromatography on silica gel [petroleum ether/ethyl acetate (10:1); UV detection] to yield (5*R*)-**15** {minor diastereoisomer: 77.5 mg, 17%, R_f[(5*R*)-**15**] = 0.35 [petroleum ether/ethyl acetate (10:1)]} and (5*S*)-**15** {major diastereoisomer: 311 mg, 67%, R_f[(5*S*)-**15**] = 0.25 [petroleum ether/ethyl acetate (10:1)], 85% overall yield}. $[\alpha]_D^{20}$ = –39.8 (*c* = 1.11, $CHCl_3$). ^{1}H NMR (300.13 MHz, $CDCl_3$): δ = 1.04 [s, 9 H, C(CH$_3$)$_3$], 1.04 (d, *J* = 6.0 Hz, 3 H, 7′-H), 1.32, 1.52 [2 × s, 6 H, C(CH$_3$)$_2$], 1.32–1.51 (m, 4 H, 4′-H, 5′-H), 1.68–1.80 (m, 1 H, 6-H^1), 1.83–1.93 (m, 2 H, 3′-H), 2.14 (ddd, *J* = 11.4, *J* = 6.5, *J* = 6.5 Hz, 1 H, 6-H^1), 2.70–2.83 (m, 2 H, 5-H, 4-H), 3.76–3.88 (m, 1 H, 6′-H), 4.63 (ddd, *J* = 6.6, *J* = 6.6, *J* = 4.5 Hz, 1 H, 6a-H), 4.82 (dd, *J* = 6.7, *J* = 4.7 Hz, 1 H, 3a-H), 5.31–5.59 (m, 2 H, 1′-H, 2′-H), 7.32–7.45 (m, 6 H, Ar-H), 7.63–7.71 (m, 4 H, Ar-H), 9.72 (d, *J* = 1.1 Hz, 1 H, CHO) ppm. ^{13}C NMR (75.48 MHz, $CDCl_3$): δ = 19.41 [s, *C*(CH$_3$)$_3$], 23.37 (q, C-6′-*C*H$_3$), 24.79 [q, C(*C*H$_3$)$_2$], 24.95 (t, C-4′), 27.17 [3 × q, C(*C*H$_3$)$_3$], 27.29 [q, C(*C*H$_3$)$_2$] 32.38 (t, C-3′), 38.60 (t, C-6), 39.01 (t, C-5′), 43.92 (d, C-5), 63.46 (d, C-4), 69.53 (d, C-6′), 79.95 (d, C-6a), 80.49 (d, C-3a), 112.63 (s, C-2), 127.52, 127.60, 129.53, 129.59 (4 × d, C-Ar), 130.85, 131.87 (d, C-1′, C-2′), 134.70, 135.01 (2 × s, C-Ar), 136.00, 136.01 (2 × d, C-Ar), 201.52 (d, CHO) ppm. HR-MS (ESI$^+$): *m*/*z* calcd. for $C_{32}H_{45}O_4Si^+$ 520.3082; found 521.3085 [M + H]$^+$. $C_{32}H_{44}O_4Si$ (520.77): calcd. C 73.80, H 8.52; found C 74.05, H 8.53.

Methyl (2*E*)-4-{(3a*R*,4*R*,5*S*,6a*S*)-5-[(1*E*,6*S*)-6-{[*tert*-Butyl(diphenyl)silyl]oxy}hept-1-en-1-yl]-2,2-dimethyl-tetrahydro-3a*H*-cyclopenta-[*d*][1,3]dioxol-4-yl}-4-hydroxybut-2-enoate (16a/b): $CrCl_2$ (350 mg, 2.85 mmol) was added to a cooled (0 °C) mixture of methyl (2*E*)-3-iodoacrylate (253 mg, 1.19 mmol), a catalytic amount of $NiCl_2$, and **15** (311 mg, 0.597 mmol) in dry THF (6.0 mL). After 10 min at 0 °C, the ice bath was removed, and stirring was continued at room temperature for 1 h. TLC monitoring [petroleum ether/ethyl acetate (5:1)] showed complete conversion. The solution was treated with diethyl ether (10 mL) and water (2 mL), and, after 5 min of stirring, the mixture was filtered through a pad of Celite, which was washed with Et_2O. The solution was concentrated in vacuo, and the residue was subjected to flash column chromatography on silica gel {petroleum ether/ethyl acetate (5:1); detection with $KMnO_4$; R_f(**16**) = 0.07, R_f[dimethyl (2*E*,4*E*)-hexa-2,4-dienedioate] = 0.30} to yield **16** (328 mg, 91%) as a colorless oil. The product was a 1:1 mixture of diastereoisomers, which could not be separated. ^{1}H NMR (300.13 MHz, $CDCl_3$): δ = 1.05 [s, 9 H, C(CH$_3$)$_3$], 1.03–1.08 (m, 3 H, C-6′-CH$_3$), 1.28, 1.29, 1.49, 1.51 [4 × s, 6 H, C(CH$_3$)$_2$], 1.22–1.51 (m, 4 H, 5′-H, 4′-H), 1.54–1.66 (m, 1 H, 6-H^1), 1.80–1.93 (m, 2 H, 3′-H), 1.93–2.00 (m, 1 H, 4-H), 2.04–2.23 (m, 1 H, 6-H^1), 2.29–2.58 (m, 1 H, 5-H), 3.73, 3.74 (2 × s, 3 H, OCH$_3$), 3.77–3.88 (m, 1 H, 6′-H), 4.28–4.58 (m, 3 H, 4′′-H, 3a-H, 6a-H), 5.20–5.46 (m, 2 H, 1′-H, 2′-H), 6.08, 6.15 (2 × dd, *J* = 15.6, *J* = 1.7, *J* = 15.6, *J* = 1.8 Hz, 1 H, 2′′-H), 6.97, 7.02 (2 × dd, *J* = 15.3, *J* = 4.6, *J* = 15.5, *J* = 4.7 Hz, 1 H, 3′′-H), 7.32–7.45 (m, 6 H, Ar-H), 7.66–7.70 (m, 4 H, Ar-H) ppm. ^{13}C NMR (75.48 MHz, $CDCl_3$): δ = 19.40 [s, *C*(CH$_3$)$_3$], 23.37 (q, C-6′-CH$_3$), 25.01, 25.04 (2 × t, C-4′), 25.21 [q, C(*C*H$_3$)$_2$], 27.16 [q, C(*C*H$_3$)$_3$], 27.72, 27.75 [2 × q, C(*C*H$_3$)$_2$], 32.38, 32.42 (2 × t, C-3′), 38.83, 39.07, 39.18 (3 × t, C-6, C-5′), 44.44, 44.61 (2 × d, C-5), 51.72, 51.77 (2 × q, OCH$_3$), 56.07, 56.20 (2 × d, C-4), 69.51, 69.53 (2 × d, C-6′), 70.50, 71.29 (d, C-4′′), 79.25, 79.44, 81.00, 81.74 (4 × d, C-3a, C-6a), 112.60, 113.05 (2 × s, C-2), 120.49, 121.57 (2 × d, C-2′′), 127.52, 127.60, 129.52, 129.59 (4 × d, C-Ar), 131.84, 132.08, 132.11, 132.20 (4 × d, C-1′, C-2′), 134.70, 134.72, 134.99 (3 × s, C-Ar), 135.99 (d, C-Ar), 147.59, 149.43 (2 × d, C-3′′), 166.88 (s, CO$_2$) ppm. HR-MS (ESI$^+$): *m*/*z* calcd. for $C_{36}H_{51}O_6Si^+$ 607.3449 [M + H]$^+$; found 607.3459. $C_{36}H_{50}O_6Si$ (606.86): calcd. C 71.25, H 8.30; found C 71.03, H 8.44.

Methyl (2*E*)-4-Hydroxy-4-{(3a*R*,4*R*,5*S*,6a*S*)-5-[(1*E*,6*S*)-6-hydroxyhept-1-en-1-yl]-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]-dioxol-4-yl}but-2-enoate (17): A solution of **16** (194 mg,

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0.319 mmol) and $NEt_3(HF)_3$ (208 μL, 1.275 mmol) in dry CH_3CN (3.2 mL) was heated at reflux under argon for 21 h. TLC monitoring showed complete conversion [petroleum ether/ethyl acetate (1:1); R_f(**16**) = 0.64/0.56; R_f(**17**) = 0.14]. The reaction mixture was allowed to cool to room temperature and subjected directly to flash column chromatography on silica gel [petroleum ether/ethyl acetate (3:1 to 0:1)] to yield **17** (109 mg, 93%) as a colorless oil and as a mixture of diastereoisomers that could not be separated. ^{1}H NMR (300.13 MHz, $CDCl_3$): δ = 1.16 (d, J = 6.2 Hz, 3 H, 7′-H), 1.26, 1.27 [2 × s, 3 H, $C(CH_3)_2$], 1.32–1.52 (m, 4 H, 5′-H, 4′-H), 1.47, 1.49 [2 × s, 3 H, $C(CH_3)_2$], 1.54–1.68 (m, 1 H, 6-H^1), 1.82–2.23 (m, 5 H, 4-H, 3′-H^2, 6-H^1, OH), 2.34–2.78 (m, 2 H, 5-H, OH), 3.72, 3.73 (2 × s, 3 H, OCH_3), 3.70–3.83 (m, 1 H, 6′-H), 4.24–4.34 (br. s, 0.5 H, 4′′-H), 4.38 (dd, J = 6.5, J = 6.5 Hz, 0.5 H, 3a-H), 4.42–4.58 (m, 2 H, 4′′-H, 3a-H, 6a-H), 5.27–5.57 (m, 2 H, 1′-H, 2′-H), 6.07 (dd, J = 15.6, J = 1.6 Hz, 0.5 H, 2′′-H), 6.10 (dd, J = 15.6, J = 1.6 Hz, 0.5 H, 2′′-H), 6.96 (dd, J = 15.5, J = 4.4 Hz, 0.5 H, 3′′-H), 7.02 (dd, J = 15.7, J = 4.6 Hz, 0.5 H, 3′′-H) ppm. ^{13}C NMR (75.48 MHz, $CDCl_3$): δ = 23.54, 23.55 (2 × q, C-7′), 25.18, 25.18 [2 × q, C(CH$_3$)$_2$], 25.50, 25.56 (2 × t, C-4′), 27.69, 27.70 [2 × q, C(CH$_3$)$_2$], 32.33, 32.35 (2 × t, C-3′), 38.80, 38.83 (2 × t, C-5′), 38.93, 39.20 (2 × t, C-6), 43.97, 44.71 (2 × d, C-5), 51.76, 51.80 (2 × q, OCH_3), 56.11, 56.21 (2 × d, C-4), 67.97, 67.97 (2 × d, C-6′), 70.72, 71.07 (2 × d, C-4′′), 79.40, 79.34 (2 × d, C-6a), 81.98, 81.46 (2 × d, C-3a), 112.58, 112.88 [2 × s, C(CH$_3$)$_2$], 121.27, 120.28 (2 × d, C-2′′), 131.74, 131.74, 132.43, 132.50 (4 × d, C-1′, C-2′), 148.18, 149.80 (2 × d, C-3′′), 166.99, 167.08 (2 × s, CO_2) ppm. HR-MS (ESI$^+$): m/z calcd. for $C_{20}H_{33}O_6^+$ 369.2272 [M + H]$^+$; found 369.2269.

(3a*R*,3b*R*,9*S*,14a*S*,15a*S*)-4-Hydroxy-2,2,9-trimethyl-3a,3b,4,9,10,11,12,14a,15,15a-decahydro-7*H*-[1,3]dioxolo[4,5]cyclopenta[1,2-*f*]-oxacyclotridecin-7-one (21): A solution of **17** (53 mg, 0.144 mmol) in a mixture of THF (1.5 mL) and water (1.2 mL) was treated with an aqueous solution of LiOH (290 μL, 1 M), and the mixture was stirred at room temperature for 2 h. The mixture was then neutralized with the strongly acidic ion exchange resin Amberlite IR-120 and filtered through a pad of Celite, and the filtrate was concentrated in vacuo. The crude acid **18** was placed in a Schlenk tube and dissolved in dry THF (2.9 mL). The solution was cooled to –50 °C and treated with 4-(dimethylamino)but-3-yn-2-one (29 μL, 0.261 mmol). After 1 h at –50 °C, the solution was allowed to warm to room temperature, and the solvent was evaporated in vacuo. The crude product was dissolved in ethyl acetate and filtered through a short column of silica gel, which was eluted with ethyl acetate/methanol (20:1) [R_f(**20**) = 0.23]. After evaporation of the solvent in vacuo, **20** was dissolved in dry toluene (20 mL), and the solution was added dropwise by syringe at 80 °C to a solution of camphorsulfonic acid (6.7 mg, 0.029 mmol) in dry toluene (20 mL) over a period of 40 min. After the addition, the syringe was flushed with dry toluene (4 mL). After 2 h, the solution was allowed to cool to room temperature, and a saturated aqueous solution of $NaHCO_3$ (20 mL) and ethyl acetate (20 mL) were added. The aqueous layer was separated and extracted with ethyl acetate (3 × 20 mL). The combined organic layers were dried with Na_2SO_4 and filtered, and the solvent was removed in vacuo. A mixture of the product **21** and unreacted **20** was obtained and separated by flash column chromatography {petroleum ether/ethyl acetate (3:1) to ethyl acetate/MeOH (10:1); R_f(**21**) = 0.16; R_f(**20**) = 0.10 [petroleum ether/ethyl acetate (3:1)]}. Recovered **20** (24.9 mg, 0.053 mmol) was treated in the same way as described above (18 mL of toluene, 6.7 mg of CSA). The total yield of **21a** (22.3 mg), as a colorless oil, was 46%, and that of **21b** (15.7 mg) was 32%. Crystallization of **21b** from ethyl acetate afforded colorless needles (m.p. 164–166 °C) appropriate for X-ray analysis. Analytical data for **21a**: $[\alpha]_D^{20}$ = 59.2 (c = 1.105, $CHCl_3$). ^{1}H NMR (500.13 MHz, $CDCl_3$): δ = 0.96–1.06 (m, 1 H, 11-H^1), 1.23 (d, J = 6.4 Hz, 3 H, CH_3), 1.30, 1.50 [2 × s, 6 H, C(CH_3)$_2$], 1.46–1.61 (m, 2 H, 15-H^1, 10-H^1), 1.65–1.88 (m, 3 H, 12-H^1, 11-H^1, 10-H^1), 1.91–2.00 (m, 2 H, 3b-H, 12-H^1), 2.16 (m_c, 1 H, 15-H^1), 2.24 (br. s, 1 H, OH), 2.87 (m_c, 1 H, 14a-H), 4.50 (ddd, J = 6.7, J = 6.7, J = 6.7 Hz, 1 H, 15a-H), 4.64–4.72 (m, 2 H, 4-H, 3a-H), 4.93 (dqd, J = 12.5, J = 6.3, J = 1.5 Hz, 1 H, 9-H), 5.22 (m_c, 1 H, 14-H), 5.64 (ddd, J = 14.9, J = 9.7, J = 4.9 Hz, 1 H, 13-H), 5.79 (dd, J = 15.8, J = 0.6 Hz, 1 H, 6-H), 7.03 (dd, J = 15.9, J = 8.3 Hz, 1 H, 5-H) ppm. ^{13}C NMR (125.48 MHz, $CDCl_3$): δ = 20.69 (q, CH_3), 25.66 [q, C(CH$_3$)$_2$], 26.17 (t, C-11), 28.21 [q, C(CH$_3$)$_2$], 31.76 (t, C-12), 34.69 (t, C-10), 40.41 (t, C-15), 41.47 (d, C-14a), 56.29 (d, C-3b), 70.73 (d, C-4), 71.98 (d, C-9), 80.02 (d, C-15a), 83.11 (d, C-3a), 112.13 [s, C(CH$_3$)$_2$], 119.97 (d, C-6), 130.84 (d, C-13), 136.83 (d, C-14), 150.58 (d, C-5), 166.53 (s, CO_2) ppm. HR-MS (ESI$^+$): m/z calcd. for $C_{19}H_{29}O_5^+$ 337.2010 [M + H]$^+$; found 337.2019.

Inversion of the Stereocenter at C-4. (3a*R*,3b*R*,4*R*,9*S*,14a*S*,15a*S*)-4-Hydroxy-2,2,9-trimethyl-3a,3b,4,9,10,11,12,14a,15,15a-decahydro-7*H*-[1,3]dioxolo[4,5]cyclopenta[1,2-*f*]oxacyclotridecin-7-one (21b): MnO_2 (491 mg) was added to a solution of **21a** (22.3 mg, 0.066 mmol) in CH_2Cl_2 (1.3 mL), and the resulting suspension was stirred at room temperature for 2 h [TLC monitoring; petroleum ether/ethyl acetate (3:1); R_f(**21a**) = 0.16]. The mixture was then filtered through a pad of Celite, which was washed with ethyl acetate, and the solvent was evaporated in vacuo. A solution of the crude product (9.5 mg) in dry methanol (1.3 mL) was cooled to –78 °C and treated with $NaBH_4$ (5 mg, 0.132 mmol). After the mixture had been stirred at –78 °C for 30 min, TLC monitoring showed complete conversion [petroleum ether/ethyl acetate (3:1); R_f(**21b**) = 0.10]. Water (5 mL) was then added, and the mixture was allowed to warm to room temperature. After a further 10 min of stirring, ethyl acetate (10 mL) was added, and the aqueous layer was separated and extracted with ethyl acetate (3 × 10 mL). The combined organic layers were dried with Na_2SO_4 and filtered, and the solvent was removed in vacuo. The crude product was purified by flash column chromatography on silica gel [petroleum ether/ethyl acetate (3:1 to 1:1)] to yield **21b** (8.7 mg, 39%) as small white needles. $[\alpha]_D^{20}$ = 71.9 (c = 0.755, $CHCl_3$). ^{1}H NMR (300.13 MHz, $CDCl_3$): δ = 0.93–1.07 (m, 1 H, 11-H^1), 1.24 (d, J = 6.2 Hz, 3 H, CH_3), 1.32, 1.52 [2 × s, 6 H, C(CH_3)$_2$], 1.46–1.90 (m, 7 H, 3b-H, 15-H^1, 12-H^1, 10-H^2, 11-H^1, OH), 1.92–2.08 (m, 1 H, 12-H^1), 2.21 (ddd, J = 13.9, J = 6.9, J = 6.9 Hz, 1 H, 15-H^1), 2.32–2.50 (m, 1 H, 14a-H), 4.23 (d, J = 10.1 Hz, 1 H, 4-H), 4.47–4.55 (m, 1 H, 3a-H), 4.55–4.62 (m, 1 H, 15a-H), 4.87 (m_c, 1 H, 9-H), 5.23 (m_c, 1 H, 14-H), 5.69 (ddd, J = 14.9, J = 9.5, J = 5.1 Hz, 1 H, 13-H), 5.95 (dd, J = 15.7, J = 1.8 Hz, 1 H, 6-H), 7.23 (dd, J = 15.8, J = 3.4 Hz, 1 H, 5-H) ppm. ^{13}C NMR (75.48 MHz, $CDCl_3$): δ = 20.85 (q, CH_3), 25.52 [q, C(CH$_3$)$_2$], 26.72 (t, C-11), 27.97 [q, C(CH$_3$)$_2$], 31.53 (t, C-12), 34.45 (t, C-10), 39.73 (t, C-15), 45.45 (d, C-14a), 58.77 (d, C-3b), 71.64 (d, C-9), 74.72 (d, C-4), 79.50 (d, C-15a), 87.27 (d, C-3a), 113.19 [s, C(CH$_3$)$_2$], 118.21 (d, C-6), 131.33 (d, C-13), 135.78 (d, C-14), 150.79 (d, C-5), 166.29 (s, CO_2) ppm. HR-MS (ESI$^+$): m/z calcd. for $C_{19}H_{29}O_5^+$ 337.2010 [M + H]$^+$; found 337.2025.

(1*R*,6*S*,11a*S*,13*S*,14*R*,14a*R*)-1,13,14-Trihydroxy-6-methyl-1,6,7,8,9,11a,12,13,14,14a-decahydro-4*H*-cyclopenta[*f*]oxacyclotridecin-4-one (5): A solution of **21b** (15.7 mg, 0.047 mmol) in methanol (930 μL) was treated with an aqueous solution of HCl (94 μL, 1 M) and stirred at room temperature for 15 min. More HCl solution (24 μL) was added, and the mixture was stirred for another 30 min. TLC showed complete conversion [petroleum ether/ethyl acetate (1:2); R_f(**21b**) = 0.56, R_f(**5**) = 0.14]. The mixture was filtered

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through a small pad of the strongly basic anion exchange resin DOWEX MONOSPHERE 550A (OH), and the filtrate was concentrated in vacuo. The crude product was purified by flash column chromatography on silica gel [petroleum ether/ethyl acetate (1:2 to 0:1)] to give **5** (10.8 mg, 78%) as a white solid. Recrystallization from ethyl acetate afforded colorless needles suitable for X-ray analysis (m.p. 128–131 °C). $[\alpha]_D^{20}$ = 80.9 (c = 0.485, $CHCl_3$). ^1H NMR (500.13 MHz, $CDCl_3$): δ = 0.82–0.97 (m, 1 H, 13-H^1), 1.26 (d, J = 6.2 Hz, 3 H, CH_3), 1.46–1.56 (m, 1 H, 14-H^1), 1.59 (ddd, J = 14.8, J = 5.2, J = 1.6 Hz, 1 H, 8-H^1), 1.66–1.90 (m, 4 H, 12-H^1, 5-H, 13-H^1, 14-H^1), 1.97–2.05 (m, 1 H, 12-H^1), 2.14 (ddd, J = 14.7, J = 9.9, J = 4.9 Hz, 1 H, 8-H^1), 2.32 (dddd, J = 9.6, J = 9.6, J = 9.6, J = 5.0 Hz, 1 H, 9-H), 3.51, 3.30, 2.67 (3 × br. s, 3 H, OH), 3.97 (dd, J = 8.8, J = 4.2 Hz, 1 H, 6-H), 4.08 (br. s, 1 H, 7-H), 4.39 (d, J = 9.8 Hz, 1 H, 4-H), 4.81–4.90 (m, 1 H, 15-H), 5.29–5.37 (m, 1 H, 10-H), 5.65 (ddd, J = 14.9, J = 10.2, J = 4.6 Hz, 1 H, 11-H), 5.94 (dd, J = 15.7, J = 1.7 Hz, 1 H, 2-H), 7.25 (dd, J = 15.6, J = 3.1 Hz, 1 H, 3-H) ppm. ^{13}C NMR (125.77 MHz, $CDCl_3$): δ = 20.97 (q, CH_3), 26.85 (t, C-13), 31.85 (t, C-12), 34.32 (t, C-14), 37.55 (t, C-8), 41.05 (d, C-9), 55.96 (d, C-5), 72.04 (d, C-15), 72.77 (d, C-7), 75.88 (d, C-4), 80.64 (d, C-6), 117.98 (C-2), 130.42 (d, C-11), 136.93 (d, C-10), 151.13 (d, C-3), 166.44 (s, CO_2) ppm. HR-MS (EI$^+$): m/z calcd. for $C_{16}H_{24}O_5^+$ 296.1618 [M]$^+$; found 296.1591 and m/z calcd. for $C_{16}H_{22}O_4^+$ 278.1513 [M – H_2O]$^+$; found 278.1516.

Cultivation of *Eupenicillium brefeldianum*. Isolation of Brefeldin A (1) and 7-Dehydrobrefeldin A (3): A sample of *Eupenicillium brefeldianum* was purchased from the ATCC® (American Type Culture Collection, ATCC-58665) and cultivated on a potato dextrose agar medium. The fungus grew rapidly, and BFA was produced efficiently.[45] The agar slants were extracted with ethyl acetate, and the crude product was subjected to column chromatography on silica gel. BFA (662 mg m^{-2}) was isolated, and the analytical data were in accordance with reported data.[46] After 2 months, the fungus started to produce 7-dehydrobrefeldin A (**3**), which is probably a catabolite of BFA and occurs in the biosynthesis.[47] Again the analytical data were in accordance with reported data.[48] In addition, the structure was unambiguously assigned by X-ray crystal structure analysis (Table 1). CCDC-797341 (**3**), -797342 (**5**), -797343 (**6**), -797344 (**7**), and -797345 (**21b**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

(1*R*,6*S*,11a*S*,13*S*,14a*R*)-1,13-Bis[(2-methoxyethoxy)methoxy]-6-methyl-1,6,7,8,9,11a,12,13,14,14a-decahydro-4*H*-cyclopenta[*f*]oxacyclotridecin-4-one (22): Analogously to a small-scale procedure by Taber et al.,[33] iPr$_2$EtN (1.66 mL, 9.70 mmol) was added dropwise to a cooled (0 °C) suspension of BFA (672 mg, 2.397 mmol) in dry CH_2Cl_2 (25 mL). After the mixture had been stirred at 0 °C for 10 min, MEM-Cl (1.10 mL, 9.56 mmol) was added dropwise. The mixture was allowed to warm to room temperature and was stirred for a further 3 h. The solution was then cooled (0 °C), and iPr$_2$EtN (1.7 mL, 9.90 mmol) and MEM-Cl (1.10 mL, 9.56 mmol) were added again. The resulting solution was allowed to warm to room temperature and stirred overnight. TLC monitoring showed complete conversion of BFA [petroleum ether/ethyl acetate (1:3); R_f(**22**) = 0.30, R_f(BFA) = 0.10]. Water (20 mL) and brine (20 mL) were added, and the aqueous layer was separated and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried with Na_2SO_4, filtered, and concentrated in vacuo. The crude product was subjected to flash chromatography on silica gel [100 g; petroleum ether/ethyl acetate (1:1)] to yield fully protected **22** (1.18 g) and a mixture of **22** and the mono-MEM-protected BFA (100 mg) as colorless oils. Note: The major fraction of **22** was accompanied by two inseparable side products derived from the excess of MEM-Cl. These compounds were removed in the next step. The analytical data were consistent with reported data.[32,33] $[\alpha]_D^{20}$ = –21.8 (c = 1.23, in $CHCl_3$). NMR data for the side products derived from the excess of MEM-Cl: ^1H NMR (300.13 MHz, $CDCl_3$): δ = 3.38 (s, OCH_3), 3.52–3.74 (m, CH_2O), 4.77, 4.83 (2 × s, CH_2O_2) ppm. ^{13}C NMR (75.48 MHz, $CDCl_3$): δ = 59.15 (q, OCH_3), 71.87, 71.89 (2 × t, CH_2O), 92.47, 95.79 (2 × t, CH_2O_2) ppm.

Methyl (2*E*,4*R*)-4-{(1*R*,2*S*,4*S*)-2-[(1*E*,6*S*)-6-Hydroxyhept-1-en-1-yl]-4-[(2-methoxyethoxy)methoxy]cyclopentyl}-4-[(2-methoxyethoxy)-methoxy]but-2-enoate (24): An aqueous solution of KOH (10 wt.-%, 7.4 mL) was added dropwise to a solution of **22** (598 mg, 1.31 mmol) in methanol (13.1 mL). After the mixture had been stirred at 40 °C for 1 h, TLC monitoring showed complete conversion [ethyl acetate; R_f(**22**) = 0.55]. The solution was allowed to cool to room temperature and was treated with the strongly acidic ion exchange resin Amberlite IR-120 until the mixture reached pH = 7. The solution was then filtered through a pad of Celite, which was washed with ethyl acetate/methanol (1:1). The filtrate was concentrated in vacuo, and residual water was removed by co-evaporation with toluene (3 × 4 mL). The crude acid **23** was dissolved in ethyl acetate (13 mL), and a solution of CH_2N_2 in diethyl ether (ca. 0.4 M, 8 mL) was added dropwise until the mixture turned yellow. Subsequently, silica gel (ca. 1 g) was added in small portions until bubbling of nitrogen ceased. The colorless mixture was filtered, and the filtrate was concentrated in vacuo. The crude product was subjected to flash chromatography on silica gel {60 g; petroleum ether/ethyl acetate (1:2); R_f(**24**) = 0.21 [petroleum ether/ethyl acetate (1:2)]} to yield **24** (441 mg, 69%) as a colorless oil. Note that the starting material **22** was accompanied by two side products from the MEM protection, which could not be removed in the previous step. Analytical data were consistent with reported data.[33] $[\alpha]_D^{20}$ = –18.0 (c = 0.61, $CHCl_3$).

Methyl (2*E*,4*R*)-4-{(1*R*,2*R*,4*R*)-2-Formyl-4-[(2-methoxyethoxy)-methoxy]cyclopentyl}-4-[(2-methoxyethoxy)methoxy]but-2-enoate (25): A mixture of $K_2OsO_4{\cdot}2H_2O$ (9.2 mg, 25.0 μmol) and 4-methylmorpholine N-oxide (aqueous solution 50 wt.-%, 0.212 mL, 1.02 mmol) in water (1 mL) was added dropwise to a solution of **24** (490 mg, 1.00 mmol) in acetone (9 mL). After the mixture had been stirred at room temperature for 1 h, TLC monitoring showed incomplete conversion [ethyl acetate/MeOH (10:1); R_f(**24**) = 0.69]. Again, 4-methylmorpholine N-oxide (0.09 mL, 0.43 mmol) was added in two portions after 0.5 h and 1 h. After an overall reaction time of 3 h, conversion was complete. $Na_2S_2O_4$ (200 mg) was then added, and, after stirring for further 20 min, the solution was filtered through a pad of Celite and then a pad of silica gel, which was rinsed with ethyl acetate/MeOH (10:1). The filtrate was concentrated in vacuo to give the dihydroxylation product (436 mg, 83%) as a colorless oil and as a 1:1 mixture of diastereomers (^{13}C NMR), which was dissolved in a mixture of diethyl ether/water (2:1; 8.4 mL). $NaIO_4$ (357 mg, 1.67 mmol) was then added, and the mixture was stirred at room temperature for 30 min. TLC monitoring showed complete conversion [ethyl acetate/MeOH (10:1); R_f(**25**) = 0.65]. Phosphate buffer (10 mL, 0.5 M NaH_2PO_4/Na_2HPO_4) and diethyl ether (10 mL) were added, and the aqueous layer was separated and extracted with diethyl ether (3 × 10 mL). The combined organic layers were dried with Na_2SO_4 and concentrated in vacuo. The crude product was subjected to flash chromatography on silica gel [40 g; petroleum ether/ethyl acetate (1:2 to 1:1)] to yield **25** (271 mg, 67% over two steps) as a colorless oil. The compound contained 10% of the C-2′ epimer (^{13}C NMR). Analytical data were not fully reported.[32] $[\alpha]_D^{20}$ = –66.2 (c = 0.54, $CHCl_3$). ^1H NMR (300.13 MHz, $CDCl_3$): δ = 1.69 (ddd, J = 14.2, J = 9.6, J =

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Table 1. Data for the crystal structure analyses of **3**, **5**, **6**, **7**, and **21b**.

	3	**5**	**6**	**7**	**21b**
Empirical formula	$C_{16}H_{22}O_4$	$C_{16}H_{24}O_5$	$C_{16}H_{25}NO_3$	$C_{16}H_{26}ClNO_3$	$C_{19}H_{28}O_5$
Formula mass	278.34	296.35	279.37	315.83	336.41
Crystal size [mm]	0.23 × 0.13 × 0.12	0.36 × 0.18 × 0.08	0.35 × 0.08 × 0.05	0.21 × 0.15 × 0.05	0.28 × 0.06 × 0.02
Crystal system	monoclinic	monoclinic	orthorhombic	orthorhombic	monoclinic
Space group	$P2_1$	$P2_1$	$P2_12_12_1$	$P2_12_12_1$	$C2$
a [Å]	6.8697(9)	6.9802(7)	4.8652(6)	5.3107(6)	31.078(14)
b [Å]	5.2886(7)	5.4898(5)	7.3722(9)	6.9620(8)	5.811(3) Å
c [Å]	20.663(3)	20.474(2)	42.240(5)	47.239(5)	10.122(4)
α [°]	90	90	90	90	90
β [°]	99.090(3)	99.552(2)	90	90	96.306(18)
γ [°]	90	90	90	90	90
V [Å^3]	741.29(17)	773.68(13)	1515.0(3)	1746.6(3)	1817.0(14)
Z	2	2	4	4	4
$\rho_{calcd.}$ [Mg m^{-3}]	1.25	1.27	1.23	1.20	1.23
μ [mm^{-1}]	0.09	0.09	0.08	0.23	0.09
Max/min transmission	0.99/0.98	0.99/0.97	1.00/0.97	0.99/0.95	1.00/0.98
Index ranges					
h	–9 to 9	–9 to 9	–5 to 5	–6 to 6	–31 to 31
k	–7 to 7	–7 to 7	–8 to 8	–8 to 8	–5 to 5
l	–27 to 27	–26 to 27	–50 to 49	–55 to 56	–10 to 10
θ [°]	2.0 to 28.4	2.0 to 28.4	2.8 to 25.1	2.6 to 25.1	2.3 to 21.3
T [K]	200(2)	200(2)	200(2)	200(2)	200(2)
Reflections, collected	7739	8202	12508	14144	4560
Reflections, independent (R_{int})	3632 (0.0229)	2120 (0.0302)	1634 (0.0571)	3099 (0.0522)	1138 (0.0797)
Reflections, observed [$I > 2\sigma(I)$]	3318	2034	1516	2776	981
Data/restraints/parameters	3632/1/269	2120/1/203	1634/0/193	3099/0/196	1138/1/218
GOF on F^2	1.09	1.13	1.22	1.17	1.11
Final R indices [$I > 2\sigma(I)$]					
R_1	0.050	0.041	0.056	0.061	0.065
wR_2	0.107	0.101	0.116	0.118	0.134
Absolute structural parameter	0.0(12)	0.5(11)	2(3)	0.11(12)	0(4)
Max/min residual electron density [e Å^{-3}]	0.21/–0.17	0.33/–0.16	0.20/–0.22	0.26/–0.32	0.21/–0.27

4.9 Hz, 1 H, 5′-H^1), 1.84–2.20 (m, 3 H, 3′-H^2, 5′-H^1), 2.63–2.85 (m, 2 H, 2′-H, 1′-H), 3.36, 3.37 (2 × s, 6 H, OCH_3), 3.48–3.83 (m, 8 H, CH_2O), 3.73 (s, 3 H, CO_2CH_3), 4.23 (m_c, 1 H, 4′-H), 4.33 (m_c, 1 H, 4-H), 4.60–4.72 (m, 4 H, CH_2O_2), 6.00 (dd, J = 15.8, J = 1.2 Hz, 1 H, 2-H), 6.79 (dd, J = 15.8, J = 6.4 Hz, 1 H, 3-H), 9.63 (d, J = 1.5 Hz, 1 H, CHO) ppm. ^{13}C NMR (75.48 MHz, $CDCl_3$): δ = 33.85, 33.95 (2 × t, C-3′, C-5′), 42.12 (d, C-1′), 51.82, 51.84 (d,q, C-2′, CO_2CH_3), 59.13, 59.18 (2 × q, OCH_3), 67.10, 67.72, 71.80, 71.87 (4 × t, CH_2O), 76.69 (d, C-4), 77.35 (d, C-4′), 93.85, 93.89 (2 × t, CH_2O_2), 123.14 (d, C-2), 145.92 (d, C-3), 166.38 (s, CO_2), 203.14 (d, CHO) ppm. HR-MS (ESI$^+$): m/z calcd. for $C_{19}H_{32}O_9Na^+$ 427.1939; found 427.1936 [M + Na]$^+$. $C_{19}H_{32}O_9$ (404.45): calcd. C 56.42, H 7.97; found C 56.30, H 7.80.

Methyl (2*E*,4*R*)-4-{(1*R*,2*S*,4*S*)-2-{(1*E*,6*S*)-6-[(*tert*-butoxycarbonyl)-amino]hept-1-en-1-yl}-4-[(2-methoxyethoxy)methoxy]cyclopentyl}-4-[(2-methoxyethoxy)methoxy]but-2-enoate (27): A solution of KHMDS in toluene (1.5 mL, 0.5 M) was added dropwise to a cooled solution (–78 °C) of **26** (328 mg, 0.801 mmol) in DME (3.1 mL, freshly dried with sodium). After 30 min, a solution of **25** (244 mg, 0.603 mmol) in dry DME (3.1 mL) was added dropwise over a period of 15 min. After 2 h at –78 °C, the solution was allowed to warm to room temperature and was stirred for a further 60 min. Water (10 mL) and brine (10 mL) were then added, and the mixture was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were dried with Na_2SO_4, filtered, and concentrated in vacuo. The residue was subjected to flash column chromatography on silica gel {40 g; petroleum ether/ethyl acetate (3:1 to 2:1 to 1:1); R_f(**27**) = 0.25 [petroleum ether/ethyl acetate (1:1), $KMnO_4$]} to provide **27** (329 mg, 93%) as a colorless oil. $[\alpha]_D^{20}$ = –46.7 (c = 0.77, $CHCl_3$). ^{1}H NMR (300.13 MHz, $CDCl_3$): δ = 1.10 (d, J = 6.6 Hz, 3 H, 7′′-H), 1.30–1.54 (m, 5 H, 3′-H^1, 4′′-H, 5′′-H), 1.64–2.06 (m, 5 H, 1′-H, 5′-H, 3′′-H), 1.43 [s, 9 H, $C(CH_3)_3$], 2.17 (ddd, J = 14.0, J = 8.0, J = 6.2 Hz, 1 H, 3′-H^1), 2.35 (m_c, 1 H, 2′-H), 3.37, 3.38 (2 × s, 6 H, OCH_3), 3.50–3.82 (m, 9 H, CH_2O, 6′′-H), 3.73 (s, 3 H, CO_2CH_3), 4.10–4.17 (m, 1 H, 4′-H), 4.17–4.23 (m, 1 H, 4-H), 4.37 (br. s, 1 H, NH), 4.64–4.71 (m, 4 H, CH_2O_2), 5.20–5.44 (m, 2 H, 1′′-H, 2′′-H), 5.95 (dd, J = 15.8, J = 1.2 Hz, 1 H, 2-H), 6.81 (dd, J = 15.8, J = 6.1 Hz, 1 H, 3-H) ppm. ^{13}C NMR (75.48 MHz, $CDCl_3$): δ = 21.45 (q, C-7′′), 26.05 (t, C-4′′), 28.57 [q, $C(CH_3)_3$], 32.44 (t, C-3′′), 33.08 (t, C-5′), 36.95 (t, C-5′′), 40.35 (t, C-3′), 43.18 (d, C-2′), 46.52 (d, C-6′′), 48.33 (d, C-1′), 51.71 (q, CO_2CH_3), 59.13, 59.17 (2 × q, OCH_3), 66.94, 67.65, 71.81, 71.92 (4 × t, CH_2O), 75.80 (d, C-4), 76.81 (d, C-4′), 78.98 [s, $C(CH_3)_3$], 94.26, 94.39 (2 × t, CH_2O_2), 121.37 (d, C-2), 130.93 (d, C-2′′), 133.70 (d, C-1′′), 148.36 (d, C-3), 155.51 (s, CO_2tBu), 166.74 (s, C-1) ppm. HR-MS (ESI$^+$): m/z calcd. for $C_{30}H_{54}NO_{10}^+$ 588.3742 [M + H]$^+$; found 588.3744. $C_{30}H_{53}NO_{10}$ (587.74): calcd. C 61.31, H 9.09, N 2.38; found C 61.31, H 9.10, N 2.46.

(1*R*,6*S*,11a*S*,13*S*,14a*R*)-1,13-Dihydroxy-6-methyl-5,6,7,8,9,11a,12,13,14,14a-decahydrocyclopenta[*f*]azacyclotridecin-4(1*H*)-one (6): A solution of HCl in MeOH (1 M, 5.1 mL) was added to **27** (301 mg, 512 μmol), and the resulting solution was heated at 60 °C. After 2 h, the volatile components were removed under reduced pressure. Traces of water and HCl were removed by co-evaporation of toluene (2 × 2 mL). A solution of the residue in MeOH (2.6 mL) was treated with an aqueous solution of LiOH (5.1 mL, 1 M), and the mixture was heated at 40 °C. After 1 h and cooling to room temperature, water was added (5 mL). The mixture was poured onto a

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short column loaded with the strongly acidic ion exchange resin Dowex 50Wx8 and eluted with a solution of NH_3 in acetonitrile/water (3:2; 1 M to 4 M). The volatile components were removed in vacuo, and a suspension of the crude product in dry DMF (26 mL) was cooled to 0 °C. iPr$_2$NEt (260 μL, 1.52 mmol) and HBTU (388 mg, 1.02 mmol) were then added. After 15 min at 0 °C, a clear solution resulted. This was allowed to warm to room temperature and stirred for 23 h. Water (3 mL) was then added, and, after 10 min of stirring, DMF and water were removed in vacuo at 60 °C. Subsequently, a solution of the residue in MeOH was filtered through a column of the strongly basic ion-exchange resin DOWEX MONOSPHERE® 550 (OH). The filtrate containing the crude product was taken up on Celite and subjected to chromatography on silica gel {ethyl acetate/methanol (15:1 to 10:1); R_f(**6**) = 0.23 [ethyl acetate/MeOH (10:1)]} to yield **6** as a white powder (125 mg, 87%). Recrystallization from MeOH/1,2-dichloroethane gave small colorless needles (decomposition at 248 °C) suitable for X-ray crystal structure analysis. $[\alpha]_D^{20}$ = –40.6 (c = 0.51, MeOH). ^{1}H NMR (500.13 MHz, CD_3OD): δ = 0.99–1.06 (m, 1 H, 13-H^1), 1.15 (d, J = 6.8 Hz, 3 H, CH_3), 1.22–1.32 (m, 1 H, 14-H^1), 1.42 (m$_c$, 1 H, 8-H^1), 1.75–1.84 (m, 4 H, 6-H^1, 12-H^1, 13-H^1, 14-H^1), 1.86–2.02 (m, 3 H, 6-H^1, 12-H^1, 5-H), 2.11 (m$_c$, 1 H, 8-H^1), 2.28 (m$_c$, 1 H, 9-H), 3.87–3.95 (m, 1 H, 15-H), 3.90 (ddd, J = 9.8, J = 6.1, J = 1.2 Hz, 1 H, 4-H), 4.19 (ddd, J = 10.9, J = 5.5, J = 5.5 Hz, 1 H, 7-H), 5.23 (dd, J = 15.0, J = 9.5 Hz, 1 H, 10-H), 5.61 (ddd, J = 15.0, J = 10.7, J = 4.1 Hz, 1 H, 11-H), 5.92 (dd, J = 16.0, J = 1.2 Hz, 1 H, 2-H), 6.61 (dd, J = 16.0, J = 6.0 Hz, 1 H, 3-H) ppm. ^{13}C NMR (125.75 MHz, CD_3OD): δ = 21.26 (q, CH_3), 27.63 (t, C-13), 33.78 (t, C-12), 37.59 (t, C-14), 41.32 (t, C-6), 44.60 (t, C-8), 45.93 (d, C-9), 47.55 (d, C-15), 51.70 (d, C-5), 72.84 (d, C-7), 77.83 (d, C-4), 123.88 (d, C-2), 131.33 (d, C-11), 138.05 (d, C-10), 147.11 (d, C-3), 170.46 (s, CONH) ppm. HR-MS (ESI$^+$): m/z calcd. for $C_{16}H_{26}NO_3^+$ 280.1907 [M + H]$^+$; found 280.1913.

(1*R*,6*S*,11a*S*,13*S*,14a*R*)-13-Amino-1-hydroxy-6-methyl-1,6,7,8,9,11a,12,13,14,14a-decahydro-4*H*-cyclopenta[*f*]oxacyclotridecin-4-one (7): Ti(OEt)$_4$ (140 mg, 0.614 mmol) was added to a solution of 7-dehydro-BFA (**3**, 57 mg, 0.205 mmol) and (*S*)-2-methylpropane-2-sulfinamide (37 mg, 0.305 mmol) in dry THF (2.1 mL), and the mixture was heated at reflux for 2 h [TLC monitoring; ethyl acetate; R_f(**3**) = 0.69]. The solution was then cooled to –30 °C, and $NaBH_4$ (15.5 mg, 0.410 mmol) was added. The mixture was warmed to –15 °C and stirred at this temperature for 2 h [TLC monitoring; ethyl acetate; R_f(**28a**) = 0.33, R_f(**28b**) = 0.22]. Subsequently, water (5 mL) and THF (2 mL) were added, and the mixture was allowed to warm to room temperature. After the mixture had been stirred for 5 min, ethyl acetate (10 mL) was added, and the aqueous layer was separated and extracted with ethyl acetate (3 × 10 mL). The combined organic layers were dried with Na_2SO_4 and filtered, and the solvent was removed in vacuo. The crude product was subjected to column chromatography on silica gel [ethyl acetate/methanol (20:1); R_f(**28a**) = 0.44, R_f(**28b**) = 0.36]. The nonpolar fractions contained two components, which could not be separated. The polar fractions contained **28b** accompanied by a trace of (*S*)-2-methylpropane-2-sulfinamide (36 mg). A solution of this mixture in methanolic HCl (1 M, 1.88 mL) was stirred at room temperature for 45 min and then filtered through a short column of the strongly basic ion exchange resin DOWEX 550A MONOSPHERE (OH), which was rinsed with methanol (20 mL). The solvent was removed under reduced pressure to yield **7** (23 mg, 40% from **3**) as small colorless plates (decomposition at 157 °C). $[\alpha]_D^{20}$ = 52.1 (c = 0.81, CH_3OH). ^{1}H NMR (300.13 MHz, $CDCl_3$): δ = 0.82–0.97 (m, 1 H, 13-H^1), 1.20–1.34 (m, 1 H, 8-H^1), 1.24 (d, J = 6.3 Hz, 3 H, CH_3), 1.50–1.95 (m, 6 H, 5-H, 6-H^1, 13-H^1, 14-H^2, 12-H^1), 1.95–2.13 (m, 3 H, 12-H^1, 8-H^1, 6-H^1), 2.30–2.46 (m, 1 H, 9-H), 3.19–3.34 (m, 1 H, 7-H), 4.03 (m$_c$, 1 H, 4-H), 4.73–4.87 (m, 1 H, 15-H), 5.23 (dd, J = 15.1, J = 9.5 Hz, 1 H, 10-H), 5.73–5.87 (m, 1 H, 11-H), 5.82 (dd, J = 15.6, J = 1.9 Hz, 1 H, 2-H), 7.44 (dd, J = 15.6, J = 3.0 Hz, 1 H, 3-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 21.07 (q, CH_3), 27.97 (t, C-13), 32.99 (t, C-12), 34.94 (t, C-14), 41.11 (t, C-6), 45.42 (t, C-8), 46.91 (d, C-9), 52.60 (d, C-7), 53.11 (d, C-5), 73.18 (d, C-15), 76.38 (d, C-4), 117.96 (d, C-2), 131.93 (d, C-11), 137.16 (d, C-10), 154.86 (d, C-3), 168.32 (s, CO_2) ppm. HR-MS (ESI$^+$): m/z calcd. for $C_{16}H_{26}NO_3^+$ 280.1907 [M + H]$^+$; found 280.1908.

Supporting Information (see footnote on the first page of this article): Copies of ^{13}C NMR spectra of compounds **5**, **6**, **7**, **17**, **21a**, and **21b**, X-ray crystal data for compounds **3**, **5**, **6**, **7**, and **21b**, and test results from the National Cancer Institute, Bethesda, USA, for compounds **4**, **5**, and **6**.

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