Synthesis and Conformational Analysis of Fusidic Acid Side Chain Derivatives in Relation to Antibacterial Activity

Tore Duvold,* Morten Dahl Sørensen, Fredrik Björkling, Anne S. Henriksen, and Niels Rastrup-Andersen

Leo Pharmaceutical Products, Industriparken 55, DK-2750 Ballerup, Denmark

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Novel fusidic acid type antibiotics having flexible side chains are described. Saturation of the double bond between C-17 and C-20 of fusidic acid produces four stereoisomers differing in the configuration at C-17 and C-20. The structure−activity relationship of the stereoisomers was studied using computer-assisted analyses of low-energy conformations and crystallographic data. Only one of the four stereoisomers showed potent antibiotic activity comparable with that of fusidic acid, whereas the other three stereoisomers retained little or no activity. The orientation of the side chain is crucial, and there is only a limited space for bioactive side chain conformations. This investigation demonstrates the essential role of the side chain conformations in relation to antibacterial activity and contradicts earlier assumptions that the Δ17(20) bond is an essential feature in the molecule.

Introduction

Fusidic acid belongs to a family of naturally occurring antibiotics, the fusidanes, having in common a tetracyclic ring system with the unique chair−boat−chair conformation separating them from steroids (Figure 1). These compounds also have in common the same carboxylic acid bearing side chain linked to the ring system at C-17 via a double bond and an acetate group at C-16. They show a high degree of antibacterial activity and have a similar spectrum. Fusidic acid, the most potent of the fusidanes, was first isolated from *Fusidium coccineum* in 1960[1−3] and has since 1962 been used clinically in the treatment of both topical and systemic infections caused by staphylococci. Although fusidic acid is commonly used against staphylococci, it is also efficient against several other Gram-positive species.[4] The clinical value of fusidic acid is also due to its excellent distribution in various tissues, low degree of toxicity and allergic reactions, and the absence of cross-resistance with other clinically used antibiotics.[5,6] The structure−activity relationship (SAR) of fusidic acid has been extensively studied (Figure 1),[7,8] and a large number of analogues have been prepared. However, only a few of these analogues showed activities comparable with that of fusidic acid. Most of these have a similar antibacterial spectrum and are cross-resistant. Despite the extensive SAR studies, the potential of side chain modifications has not earlier been explored.[9] As part of our renewed interest in improving the antibacterial and pharmacokinetic properties of fusidic acid type antibiotics, we decided to further investigate the relatively unexplored side chain. In particular, we wanted to investigate the importance of the Δ17(20) double bond which has been assumed to be essential for antibacterial activity.[10,11] In our earlier work, only two of the four possible epimers of analogues with a single bond between C-17 and C-20 have been prepared. These analogues have in common the 17R configuration but are epimers at C-20. They retain little or no antibacterial activity. We suggested that the Δ17,20 double bond is not an essential feature for the biological activity of the molecule and that the loss of activity of the 17*R* analogues previously synthesized is due primarily to an unfavorable configuration of the side chain. In the following, we present the synthesis of new 17,20-dihydrofusidic acid analogues, the elucidation of their absolute configurations, conformational analyses of the side chains by means of molecular modeling, and their in vitro antibacterial activity.

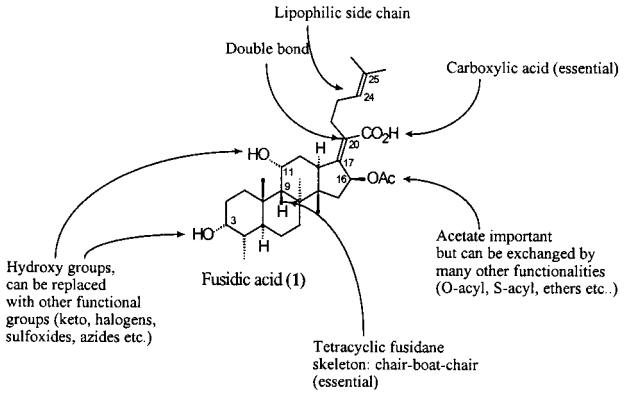

Figure 1. An overview of structure−activity relationships of fusidic acids showing important and essential structural and functional features.

Chemistry

The tetrahydrofusidic acid analogues **2** and **3** with the 17*R* configuration had been prepared previously by means of catalytic hydrogenation of fusidic acid and its Δ17(20) isomer, lumifusidic acid, successively.[12] For the preparation of the 17*S* analogues, we took advantage of the $NaBH_4$ reduction of the corresponding fusidic acid lactone proceeding solely with the attack from the α-face[12] of the molecule yielding the desired 17*S*, 20*R*

* To whom correspondence should be addressed. Tore Duvold, Department of Medicinal Chemistry, Leo Pharmaceutical Products, Industriparken 55, DK-2750 Ballerup, Denmark. Tel: +45-44 92 38 00. Fax: +45-44 94 55 10. E-mail: tore.duvold@leo-pharma.com.

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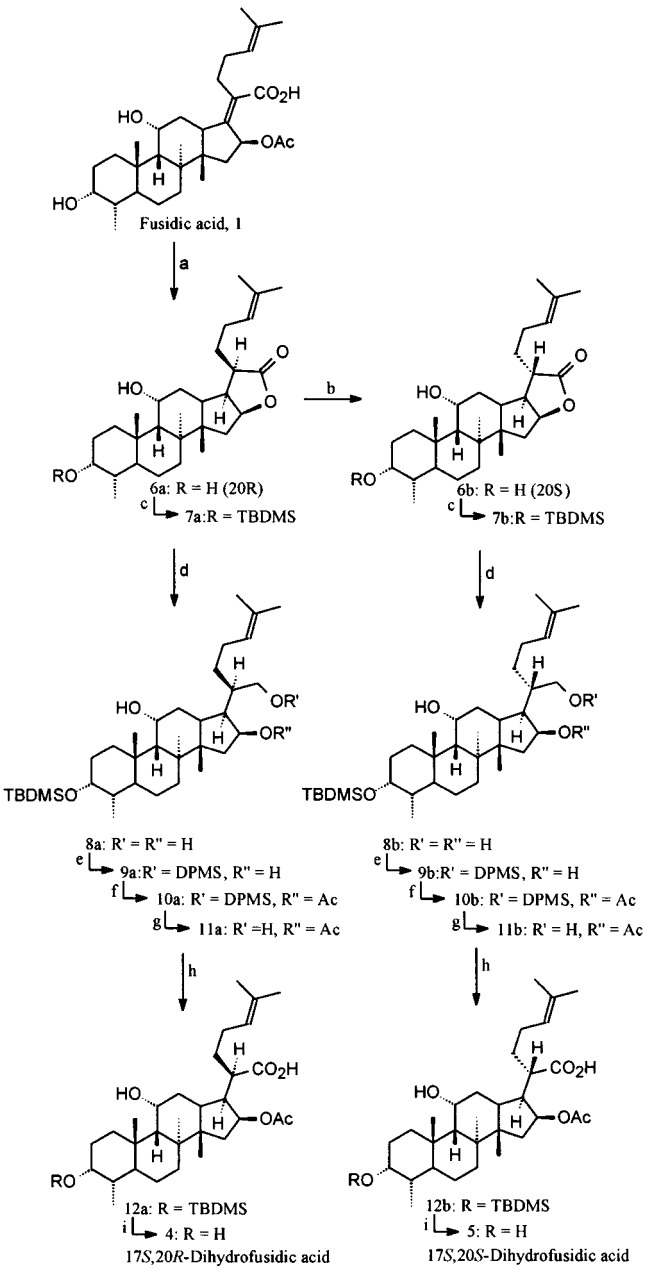
Scheme 1[a]



[a] (a) (i) aq. NaOH in EtOH, reflux, (96%), (ii) $NaBH_4$ in MeOH/water, rt, 3 h, (94%); (b) aq. 28% NaOH, EtOH, reflux, 1 h, (93%); (c) TBDMSCl, imidazole in DMF, rt, overnight, (96%); (d) $LiAlH_4$, THF, reflux, (quant); (e) DPMSCl, Et_3N, CH_2Cl_2, −20 °C, (quant); (f) Ac_2O/pyridine, (90%); (g) TBA^+F^-, AcOH, THF, (90%); (h) (i) Dess−Martin periodinane, CH_2Cl_2, 0 °C, 3 h, (quant); (ii) $NaClO_2$, *t*-BuOH, (81%); (i) aq. HF in THF, rt, 24 h (87%).

stereoisomer (Scheme 1). Inversion of the C-20 configuration was obtained by refluxing the saturated lactone **6a** in the presence of concentrated aqueous NaOH. This resulted in quantitative C-20 epimerization to lactone **6b** with the 17*S*,20*S* configuration. Synthesis of 17*S*,20*R*- and 17*S*,20*S*-dihydrofusidic acid were then carried out starting from lactones **6a** and **6b**, respectively. We were unable to isolate the open form of the saturated lactones, either as the corresponding free acids or trapped in situ as corresponding carboxylic esters. Instead, we employed a stepwise synthetic strategy to restore the free C-21 carboxyl group and the C-16 acetoxy group (Scheme 1). We first protected the 3-hydroxy group of lactones **6a** and **6b** with a *tert*-butyldimethylsilyl (TBDMS) group and reduced the resulting protected lactones **7a** and **7b** to the corresponding diols **8a** and **8b** with $LiAlH_4$. The C-21 primary hydroxy group of **8a** and **8b** were selectively protected with the bulky and moderately stable diphenylmethyl silyl (DPMS) group, and the 16-hydroxy group was thereafter acetylated with acetic anhydride and excess of pyridine. The diphenylmethylsilyl group in **10a** and **10b** was cleaved with TBA^+F^- buffered with acetic acid, and the resulting primary hydroxy group of **11a** and **11b** was oxidized in a two-step manner to the corresponding carboxylic acid, first to the aldehyde with Dess−Martin reagent and further to the acid with sodium chlorite. The TBDMS group of **12a** and **12b** was finally cleaved with aqueous HF yielding 17,20-dihydrofusidic acid **4** and **5**, respectively.

Structure Elucidation. The chemical structure of compound **4** and **5** could be derived unambiguous from the synthetic route and from NMR and MS data. Total assignment of the ^1H and ^{13}C data were performed for key compounds, and in addition, the stereochemistry in positions 17 and 20 could be derived from NOESY experiments (Figure 5). In **6a** (20R) and **6b** (20S), H-17 clearly showed NOEs to H-13 as well as H-16 proving the configuration of position 17 to be 17*S*. A strong NOE between H-20 and H-17 was observed in **6a**. In contrast to this observation, no NOE was observed between these two protons in **6b**. Instead, a strong NOE between H-20 and the H-18 protons was observed. These observations proved the stereochemistry in position 20 to be 20*R* for **6a** and 20*S* for **6b**, respectively.

Molecular Modeling. Conformational analysis of the four side chain analogues **2**−**5** were carried out using computer-assisted molecular modeling and compared with fusidic acid. The conformation of the fusidane ring system was in all calculations kept in the conformation found in the crystal structure of fusidic acid methyl ester 3-*p*-bromobenzoate[13] and was assumed to be relatively unaffected by side chain modifications. This assumption was later confirmed by the obtained crystal structure of 17*S*,20*S*-dihydrofusidic acid, **5**.[14] Thus, in the calculations of low-energy conformations, only the side chain and the C-16 acetoxy group were allowed to change. The schematic overview of fusidic acid SAR previously established (Figure 1) explains that the carboxyl group in the side chain is essential for activity, the C-16 acetoxy group is important but can be exchanged with other functional groups, and the Δ24(25) bond is of little or no importance. Superposition of the lowest energy conformations (global minimum) of fusidic acid, 17*R*,20*S*-tetrahydrofusidic acid (**2**), 17*R*,20*R*-tetrahydrofusidic acid (**3**), 17*S*,20*R*-dihydrofusidic acid (**4**), and 17*S*,20*S*-dihydrofusidic acid (**5**) is shown in Figure 4. As suspected, the C-16 acetoxy group is located in approximately the same position in all five structures. It should be noted, however, that the position of the acetate group is slightly different in the 17*R* compounds **2** and **3** as compared to fusidic acid and the 17*S* compounds (Figure 1 and Table 1). In contrast, the positions of the carboxyl group in the global minimum conformations of **3** and **4** are very different from the position found for fusidic acid, whereas the position of the carboxyl group of **2** is more similar to that of fusidic

Table 1. Comparison of the Positions of the C16 Acetoxy and COOH Groups in the Four Side Chain Analogues **2**−**5** and Fusidic Acid

compound	acetoxy RMS (Å)[a]	COOH RMS (Å)[a]	COOH plane angle[b]
fusidic acid (**1**)	0.35 ± 0.06	0.37 ± 0.16	11.1 ± 9.5
2	0.89 ± 0.09	2.39 ± 1.30	18.9 ± 7.0
3	0.87 ± 0.26	3.61 ± 0.81	19.1 ± 11.0
4	0.25 ± 0.10	2.85 ± 1.00	61.6 ± 1.7
5	0.18 ± 0.05	1.10 ± 0.65	20.1 ± 7.4

[a] The C16 acetoxy and COOH RMS values are measured with respect to the crystal structure of fusidic acid methyl ester 3-*p*-bromobenzoate. All conformations within 3 kcal/mol of the global minimum were included. [b] The plane angle of the COOH group was only calculated for those conformations in the 0−3 kcal/mol energy window which have a COOH RMS less than 2 Å. The plane angle is measured with respect to the global minimum conformation of fusidic acid (see text). The carbon atoms of the cyclohexyl rings of the tetracyclic ring system was used for superposition of the modeled conformations to the crystral structure of fusidic acid methyl ester 3-*p*-bromobenzoate prior to all calculations.

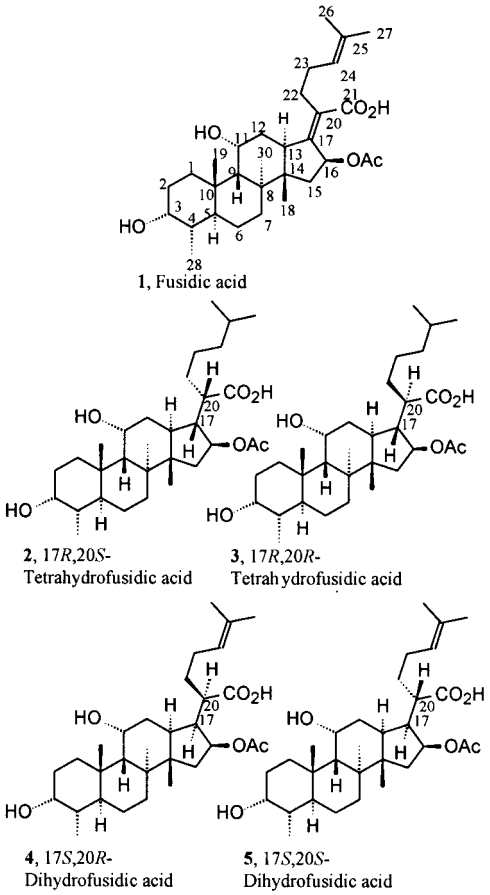


Figure 2. Fusidic acid and derivatives.

acid, and a high resemblance to fusidic acid is observed for **5** (Figure 3). A comparison of the positions of the carboxyl group and the corresponding group in the crystal structure of fusidic acid methyl ester 3-*p*-bromobenzoate was carried out for all conformations within 3 kcal/mol of the global minimum of compounds **1**−**5**. The RMS values obtained from this analysis are listed in Table 1. It is obvious when looking at all conformations in this energy window that the position of the carboxyl group in fusidic acid is generally best emulated in the conformations found for compound **5**. However, all four analogues have several conformations within 3 kcal/mol of the global minimum where the RMS

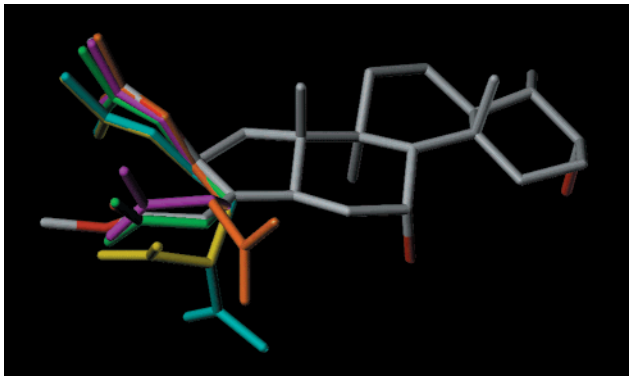


Figure 3. Superposition of the global minimum conformations of fusidic acid and the four side chain analogues on the crystal structure of fusidic acid methyl ester 3-*p*-bromobenzoate. The carbon atoms of the cyclohexyl rings of the tetracyclic ring system were superimposed. The crystal structure is shown in atom colors, **1** is shown in green, **2** is shown in yellow, **3** is shown in cyan, **4** shown in orange, and **5** is shown in magenta. The lipophilic part of the side chain of all conformations has been omitted for clarity. The 3-*p*-bromobenzoate moiety of the crystal structure has also been omitted.

value of the carboxyl group is less than 2 Å. To further analyze these conformations, we determined the angle between the plane defined by the carboxyl group of the conformation in question and the plane defined by the carboxyl group of the global minimum conformation of fusidic acid. The crystal structure of fusidic acid methyl ester 3-*p*-bromobenzoate was not used for this analysis because it contains a methyl ester instead of a carboxyl group. The extra methyl probably affects the plane angle slightly. The results show that the carboxyl groups of fusidic acid (**1**) and compounds **2**, **3**, and **5** are presented in a manner similar to the interacting elongation factor G (EF-G) receptor,[11,15] whereas the carboxyl group of **4** is presented significantly different (Table 1). Finally, we wanted to examine the position of the lipophilic moiety of the side chain. The position of this moiety of the side chain in all low energy conformations where the RMS value of the carboxyl group is less than 2 Å is shown in Figure 4. Clearly, only for compound **5** does the lipophilic moiety occupy the same conformational space as fusidic acid. For compound **3**, the side chain can also attain conformations similar to that of fusidic acid.

Discussion

Extensive SAR work has been made on fusidic acid, including a limited number of side chain modifications. In this early work, only two of four possible isomers of tetrahydrofusidic acid were obtained by means of catalytic hydrogenation of fusidic acid and its Δ17(20) isomer, lumifusidic acid. However, these analogues have no or little antibiotic activity. It was assumed that the altered conformations, resulting from the more flexible saturated side chain and the absence of a conjugated carboxylic acid, were responsible for the loss of activity. In our present conformational analysis of tetrahydrofusidic acid and dihydrofusidic acid stereoisomers, we found a common orientation of the carboxyl group in compounds **2** (17*R*,20*S*), **3** (17*R*,20*R*), and **5** (17*S*,20*S*) in comparison with fusidic acid. However, only in compound **5** do both the carboxyl group and the lipophilic moiety of the side chain cover the conformational

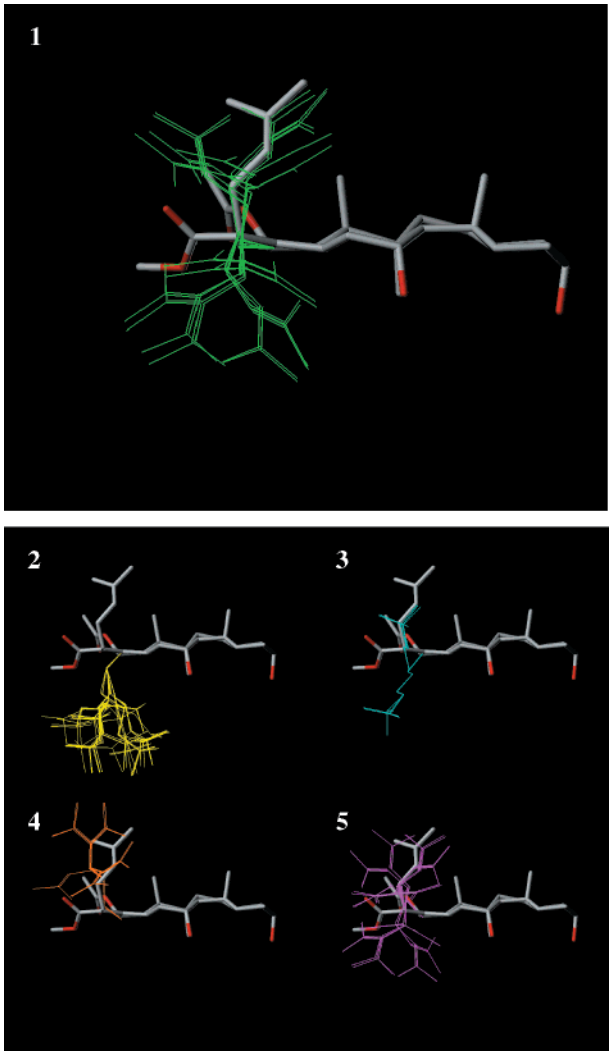


Figure 4. Superposition of all conformations within 3 kcal/mol of the global minimum where the RMS value of the carboxyl group is less than 2 Å (see text). Only the lipophilic part of the side chain is shown. The color scheme and atoms superimposed are the same as in Figure 3.

space found for fusidic acid (Figure 4). The carboxyl group in low-energy conformations of compound **4** (17S,-20R) could not be oriented similarly to fusidic acid. Therefore, the importance of a Δ17(20) double bond for antibacterial activity was further investigated. The two isomers, 17*R*,20*R* and 17*R*,20*S*, previously prepared, have in common the 17*R* configuration and are epimers at C-20. We were now able to prepare the remaining isomers, compounds **4** and **5**, by means of $NaBH_4$ reduction of fusidic acid lactone yielding the saturated 17*S*,20*R* lactone (**6a**). The corresponding 20S epimer (**6b**) was obtained by base catalyzed epimerization of lactone **6a**. The C-21 carboxyl group and the C-16 acetoxy group could then be reintroduced in a stepwise manner.

In good correlation with our conformational modeling studies, the antibacterial assays showed potent activity of compound **5** and no activity of compound **4** (Table 2). We could confirm previous reports showing low activity for compound **2**. The antibacterial spectrum and potency of compound **5** was found to be similar to fusidic acid. Our conformational modeling studies provide a rational explanation for the observed differences in the antibac-

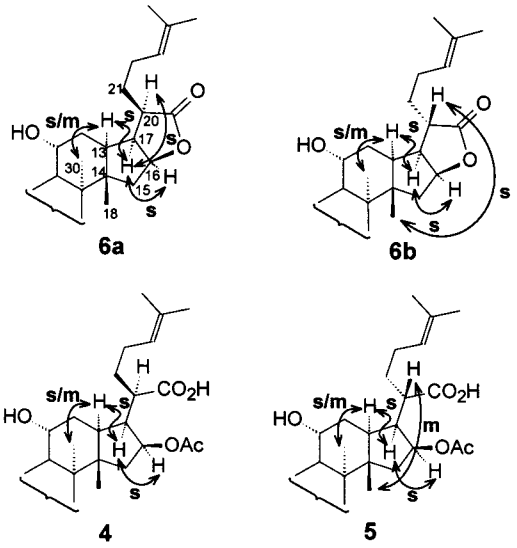


Figure 5. Assignment of stereochemistry by nuclear overhauser effect obtained in 2D NOESY and 2D TROESY experiments. s, NOESY cross-peak with high intensity; m, NOESY cross-peak with medium intensity.

terial activities of the four fusidic acid analogues. The inactivity of compound **4** is most likely due to different orientation of the carboxyl group. Furthermore, it is interesting to note that the lipophilic moiety of the side chain is positioned significantly different in compound **2** as compared to fusidic acid and compound **5** (Figure 4). Thus, the low activity of compound **2** as compared to the high activity of both fusidic acid and compound **5** suggests that the lipophilic moiety of the side chain is positioned above the ring plane in a bioactive conformation. In conclusion, the correlation between low energy conformations and antibacterial activity demonstrates that the double bond between C-17 and C-20 is not essential for the antibacterial activity of the molecule but rather that the carboxyl group and the lipophilic moiety of the side chain must be oriented in a constrained conformational space similar to that of fusidic acid. Only one of the side chain analogues, with the best conformational fit to fusidic acid, 17*S*,20*S*-dihydrofusidic acid (**5**), was found equipotent to fusidic acid. However, it is not known whether the side chains of **5** and fusidic acid have the optimal bioactive conformations. These studies demonstrate the importance of both the carboxyl group and the lipophilic moiety for the antibacterial activity of fusidic acid. Further investigations for the search of more active side chains are in progress.

Experimental Section

Molecular Modeling. All calculations were performed on a Silicon Graphics O2 R10000 workstation. The conformational analyses of the C-16 acetoxy and the C-17 side chains of the fusidic acid analogues were carried out using the Monte Carlo (Mcrlo) routine of MacroModel 7.0 (Schrödinger Inc.). The conformation of the tetracyclic ring system was kept fixed in the conformation observed in the crystal structure of fusidic acid methyl ester 3-*p*-brombenzoate.[13] It should be noted that the torsion angles C5−C6−C7−C8 and C5−C10−C9−C8 describing the conformation of the boat-shaped B-ring are −30° and −4°, respectively. These two torsion angles were constrained using the FXTA command, the force constant being the default 1000 kJ/mol. The structures in the conformational analyses were energy-minimized with the truncated Newton

Table 2. Antimicrobial Activity of Fusidic Acid and Derivatives[a]

organism/strain	MIC (μg/mL)			
	fusidic acid (**1**)	**2**	**4**	**5**
Staphylococcus aureus ATCC 2977	0.006 (0.0007−0.05)	4	>125	0.03 (0.008−0.128)
Staphylococcus aureus CJ 232 (MRSA)	0.015 (0.005−0.05)	4 (2.5−5.5)	>125	0.03 (0.01−0.06)
Staphylococcus aureus CJ 234 (R) (MRSA)	0.006 (0.002−0.017)	0.9 (0.4−2)	>125	0.03 (0.02−0.04)
Staphylococcus aureus CJ 234 (F) (MRSA)	>125	>125	>125	>125
Staphylococcus aureus ATCC 6538P	0.007 (0.001−0.036)	4	>125	0.012 (0.005−0.03)
Staphylococcus aureus ATCC 29213	0.007 (0.003−0.016)	2 (0.6−7)	>125	0.01 (0.007−0.02)
Staphylococcus epidermidis ATCC 12228	0.0007 (0.00004−0.01)	3.8 (2.8−5)	>125	0.01 (0.003−0.03)
Propionibacterium acnes NCTC 737	0.003 (0.0009−0.007)	4	4	0.03 (0.015−0.05)
Corynebacterium xerosis NCTC 9755	0.01 (0.001−0.1)	4	>125	0.03 (0.007−0.1)
Streptococcus faecium EI 119 (P)	0.01 (0.001−0.1)	16	>125	0.025 (0.008−0.08)
Streptococcus sp. EF 6	4	>125	>125	4
Streptococcus pyogenes EC	4	>125	>125	16
Streptococcus thermophilus EG 5	4	>125	>125	16
Streptococcus zooepidermidis ED (gr.C)	16	>125	>125	16
Streptococcus salivarius EG 7 (gr.A)	4	>125	>125	4
Clostridium perfringens KT 13	16	>125	>125	16
Micrococcus luteus ATCC 9341	1 (0.3−3)	>125	>125	1 (0.4−2.5)
Bacillus cereus ATCC 10876	4	>125	>125	1
Escherichia coli HA 44	>125	>125	>125	>125
Saccharomyces cerevisiae ZZ 7	4	>125	>125	4
Candida albicans ATCC 10231	16	>125	>125	64
Aspergillus niger ATCC 16404	16	16	64	64

[a] Numbers in brackets represent the concentration interval containing the real MIC value with 95% confidence. MRSA = meticilline resistant *S. aureus*, R = rifampicin resistant, P = penicillin resistant, F = fus. resistant.

conjugate gradient (TNGG) method using MMFF94s force field, until the default derivative convergence criterion of 0.05 kJ/mol/Å were met. All structures converged within 300 cycles of minimization. The solvent was set to water using the SLVNT command. The resulting structures were visualized with Sybyl 6.6 (Tripos Inc.).

Antimicrobial Activity. Minimum inhibitory concentrations (MICs) were estimated using an agar cup assay.[16] Bacterial strains were obtained from the American Type Culture Collection or from our own collection of clinical isolates. Colonies from fresh overnight culture were resuspended in saline water to 0.5 MacFarland corresponding to 10^8 CFU/mL. A total of 200 mL Mueller Hinton agar (Oxoid) at 48 °C was inoculated at a concentration of 10^6 CFU/mL and poured into square Petri dishes (245 × 245 mm). Holes were made in the inoculated plates and 200 μL of each sample was disposed into each hole. Dilution series for fusidic acid and fusidic acid analogues contained six dilutions between 0.25 and 125 μg/mL. For *Streptococci*, Mueller Hinton agar was supplemented with 5% sheep blood. Plates were appropriately incubated and zone diameters of growth inhibition were measured using an electronic caliper. MICs were estimated using a linear regression curve between the zone diameter of growth inhibition and the $\log_2$ of the sample concentration. A 95% confidence interval was estimated when possible using Statgraphics Softwear −Plus for Windows 4.1, (Statistical Graphics Corp.).

General Experimental Procedures. Melting points were measured on a Büchi 535 apparatus and are uncorrected.

NMR spectra were recorded at 300° K on either a Bruker ARX300 or a Bruker DRX500 spectrometer equipped with a 5 mm qnp and a 5 mm broadband inverse probe, respectively.

In most cases, $CDCl_3$ was used as solvent. All chemical shifts are given in ppm δ scale using either tetramethylsilane (unsilylated compounds, TMS δ = 0.00 ppm), or chloroform (silylated compounds ^{1}H δ = 7.25 ppm, ^{13}C δ = 76.81 ppm, respectively) as internal reference.

Conventional ^{1}H, ^{13}C, and DEPT135 spectra were obtained on all compounds. In addition HMQC, HMBC, COSY, HHTOCSY, and CHTOCSY experiments were performed on key compounds to make total assignments.

The stereochemistry was elucidated by comparing NOESY and TROESY experiments. Mass spectra were recorded on either a Micromass LC-QuattroII or a high-resolution Micromass AutoSpec sector instrument. Elementary analyses were obtained on a home built combustion equipment.

All solvents and reagents were of highest available quality and used as such. Reactions were monitored by TLC analyses using 0.25-mm glass-coated silica plates (E. Merck 60 F254). Chromatography was performed on silica gel 60, 230−400 mesh (E. Merck) using mixtures of ethyl acetate and low boiling petroleum ether as eluant. For chromatography of compounds having a carboxylic acid group, the eluants contained 0.5−1% of formic acid. Anhydrous solvents were prepared by storing analytical grade solvents over 4 Å molecular sieves a few days prior to use. The water content was measured before use on a Carl Fisher apparatus (typical water content: 5−12 ppm for THF, dichloromethan and 20−40 ppm for DMF).

17*S*,20*R*-Dihydrofusidic Acid Lactone (6a). Fusidic acid (6 g, 11.3 mmol) was dissolved in EtOH (90 mL) and 2 N NaOH (30 mL) was added. The resulting reaction mixture was refluxed for 1 h, cooled to room temperature, and acidified with diluted HCl causing crystallization. The crystals were collected by filtration and recrystallized from MeOH to yield 5.1 g (96%) of colorless crystals, mp 154−155 °C (Lit. 158.5−159.5 °C).[17]

MS Calc. for $C_{29}H_{44}O_4$ *m*/*z* 456.3240, observed *m*/*z* 456.3238. Anal. ($C_{29}H_{44}O_4$) C, H.

(ii) 16-Deacetylfusidic acid lactone from (i) (5.0 g, 10.9 mmol) was dissolved in MeOH (100 mL), and a solution of $NaBH_4$ (0.83 g, 22.0 mmol) in water (20 mL) was added dropwise. The resulting reaction mixture was vigorously stirred for 2 h at room temperature. The reaction was quenched by diluted HCl and concentrated under reduced pressure. The mixture was suspended in water and extracted with EtOAc (2 × 100 mL). The combined organic extracts were washed with brine (2 × 50 mL), dried (Na_2SO_4), and concentrated to a white powder which was recrystallized from MeOH to yield 4.7 g (94%) of colorless crystals, mp 160−161 °C. (Lit. 168−171 °C)[12]

^{1}H NMR ($CDCl_3$): 0.90 (s, 3H), 0.92 (d, *J* = 6.9 Hz, 3H), 0.96 (s, 3H), 1.34 (s, 3H), 1.63 (bs, 3H), 1.70 (bs, 3H), 2.66 (m, 1H), 2.79 (m, 1H), 3.15 (m, 1H), 3.76 (m, 1H), 4.30 (m, 1H), 4.99 (dd, 1H), 5.09 (bt, 1H). MS (EI+): *m*/*z* 458, 376, 389.

17*S*,20*S*-Dihydrofusidic Acid Lactone (6b). Lactone **3** (2 g, 4.4 mmol) was dissolved in ethanol (10 mL) and 28% aqueous NaOH (5 mL). The resulting yellow solution was heated at 60 °C for 1 h. The reaction mixture was allowed to cool, and the mixture was acidified to pH 4 with concentrated acetic acid resulting an almost colorless solution. Water was added under continuous stirring until precipitation of colorless crystals. Crystals were collected by filtration yielding 1.95 g of 16-deacetyl-17*S*,20*S*-dihydrofusidic acid lactone (**6b**). Re-

crystallization from methanol−water afforded 1.85 g (93%), mp 167−169 °C.

^{1}H NMR ($CDCl_3$): 0.88 (s, 3H), 0.92 (d J = 6.9 Hz, 3H), 0.96 (s, 3H), 1.36 (s, 3H), 1.61 (bs, 3H), 1.69 (bs, 3H), 2.52 (m, 1H), 2.65 (m, 1H), 2.70 (m, 1H), 3.75 (m, 1H), 4.36 (m, 1H), 5.00 (t, 1H), 5.08 (bt, 1H). MS: Calc. for $C_{29}H_{46}O_4$ m/z 458.3396, observed: m/z 458.3396. Anal. Calcd for $C_{29}H_{46}O_4$: C, 75.94; H, 10.11. Found: C, 75.38; H, 10.11.

3-*O*-TBDMS-17*S*,20*R*-Dihydrofusidic Acid Lactone (7a). 16-Deacetyl-17*S*,20*R*-dihydrofusidic acid lactone (**6a**) (2.0 g, 4.4 mmol) was dissolved in anhydrous DMF (10 mL). To the solution was added imidazole (0.6 mg, 8.8 mmol) and TBDMSCl (1.3 g, 8.8 mmol), and the reaction mixture was stirred overnight under an atmosphere of argon. Water (50 mL) was added to the reaction mixture followed by extraction with EtOAc (2 × 50 mL), and the combined organic layers were washed successively with water and brine. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure resulting in a colorless solid. Recrystallization from methanol yielded 2.4 g (96%) of **7a** as a colorless powder, melting point 138.5−140 °C.

NMR ($CDCl_3$): 0.01 (s,3H), 0.02 (s, 3H), 0.79 (d J = 6.9 Hz, 3H), 0.88 (s, 3H), 0.89 (s, 9H), 0.93 (s, 3H), 1.31 (s, 3H), 1.62 (bs, 3H), 1.69 (bs, 3H), 2.64 (m, 1H), 2.78 (m, 1H), 3.13 (m, 1H), 3.69 (m, 1H), 4.27 (m, 1H), 4.97 (dd, 1H), 5.08 (m, 1H). MS: (EI+) m/z 572, 497, 423.

3-*O*-TBDMS-17*S*,20*S*-Dihydrofusidic Acid Lactone (7b). The reaction was carried out as described for the preparation of **7a**. Mp 163−163.5 °C. ^{1}H NMR ($CDCl_3$): 0.01 (s, 3H), 0.02 (s, 3H), 0.80 (d J = 6.5 Hz, 3H), 0.87 (s, 3H), 0.89 (s, 9H), 0.93 (s, 3H), 1.33 (s, 3H), 1.59 (bs, 3H), 1.67 (bs, 3H), 2.52 (m, 1H), 2.61 (m, 1H), 2.67 (m, 1H), 3.69 (m, 1H), 4.34 (m, 1H), 4.99 (t, 1H), 5.07 (m, 1H).

3-*O*-TBDMS-17*S*,20*R*-Dihydrofusidin-3,11,16,21-tetrol (8a). Lithium aluminum hydride (0.4 g, 100 mmol) was suspended in anhydrous THF (30 mL) under argon in an oven-dried two-necked round-bottom flask fitted with a condenser. To the stirred suspension was added a solution of lactone **7a** (1.75 g, 3.0 mmol) in anhydrous THF (10 mL) in such a rate causing gentle reflux. The reaction mixture was refluxed under vigorous stirring for 3 h and then allowed to attain room temperature. Excess lithium aluminum hydride was destroyed with EtOAc, and water was then added slowly. The resulting suspension was acidified with diluted hydrochloric acid to pH 5. The mixture was extracted with EtOAc (3 × 50 mL), and the combined organic layers were washed with brine (2 × 50 mL). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure yielding 1.76 g (quantitative) of essentially pure title compound diol (5) as a colorless white powder. An analytically pure sample was obtained by recrystallization from methanol, mp 147−150 °C.

NMR ($CDCl_3$): 0.018 (s, 3H), 0.002 (s,3H), 0.80 (d J = 6.8 Hz, 3H), 0.89 (s, 9H), 0.94 (s, 3H), 1.11 (s, 3H), 1.31 (s, 3H), 1.60 (bs, 3H), 1.67 (bs, 3H), 2.42 (m, 1H), 2.64 (m, 1H), 3.67 (m, 1H), 3.68 (m, 1H), 3.92 (dd, 1H), 4.29 (m, 1H), 4.53 (bt, 1H), 5.12 (bt, 1H). MS: (EI+) m/z 540, 522, 409.

3-*O*-TBDMS-17*S*,20*S*-Dihydrofusidin-3,11,16,21-tetrol (8b). The reaction was carried out as described for the preparation of **8a**. Mp 122−124 °C. NMR ($CDCl_3$): 0.004 (s, 3H), 0.02 (s, 3H), 0.80 (d J = 6.8 Hz, 3H), 0.89 (s, 9H), 0.93 (s, 3H), 1.60 (bs, 3H), 1.67 (bs, 3H), 2.61 (m, 1H), 3.59 (dd, 1H), 3.68 (m, 1H), 3.74 (dd, 1H) 4.29 (m, 1H), 4.48 (dt, 1H), 5.09 (bt, 1H).

3-*O*-TBDMS-21-*O*-Diphenylmethylsilyl-17*S*,20*R*-dihydrofusidin-3,11,16,21-tetrol (9a). Diol (**8a**) (1.5 g, 2.5 mmol) was dissolved in anhydrous dichloromethane (20 mL) and triethylamine (0.7 mL, 5 mmol) under argon and cooled at −25 °C. To the cooled solution was added over a period of 15 min a solution of diphenylmethylchlorosilane (0.57 mL, 2.75 mmol) in anhydrous dichloromethane (5 mL) so that the temperature did not exceed −20 °C and stirring was continued for 15 min. Water (50 mL) was added to the reaction mixture followed by extraction with EtOAc (2 × 100 mL). The combined extracts were washed successively with a solution of saturated $NaHCO_3$ (50 mL), water (50 mL), and brine (50 mL). The organic solution was dried (Na_2SO_4) and solvents were evaporated under reduced pressure yielding 1.96 g (quantitative) of a colorless foam of 3-*O*-TBDMS-21-*O*-diphenylmethylsilyl-17*S*,-20*R*-methanofusidin-3,11,16,21-tetrol (**9a**).

NMR ($CDCl_3$): 0.01 (s, 3H), 0.02 (s, 3H), 0.67 (s, 3H), 0.80 (d J = 6.9 Hz, 3H), 0.89 (s, 9H), 0.93 (s, 3H), 1.12 (s, 3H), 1.31 (s, 3H), 1.49 (bs, 3H), 1.65 (bs, 3H), 2.38 (m, 1H), 2.64 (m, 1H), 3.41 (bd, 1H), 3.67 (m, 1H), 3.69 (m, 1H), 3.93 (dd, 1H), 4.26 (m, 1H), 4.53 (dt, 1H), 5.04 (bt, 1H), 7.32−7.46 (m, 6H), 7.54−7.62 (m, 4H).

3-*O*-TBDMS-21-*O*-Diphenylmethylsilyl-17S,20S-dihydrofusidin-3,11,16,21-tetrol (9b). The reaction was carried out as described for the preparation of **9a**. NMR ($CDCl_3$): 0.01 (s, 3H), 0.02 (s, 3H), 0.66 (s, 3H), 0.81 (d J = 6.9 Hz, 3H), 0.90 (s, 9H), 0.93 (s, 3H), 1.05 (s, 3H), 1.33 (s, 3H), 1.50 (bs, 3H), 1.64 (bs, 3H), 2.57 (m, 1H) 3.61 (dd, 1H), 3.68 (bs, 1H), 3.86 (dd, 1H), 3.98 (d, 1H), 4.25 (bs, 1H), 4.40 (m, 1H), 5.00 (bt, 1H), 7.39 (m, 6H), 7.57 (m, 4H).

3-*O*-TBDMS-21-*O*-Diphenylmethylsilyl-16β-acetoxy-17*S*,-20*R*-dihydrofusidin-3,11,16,21-tetrol (10a). Compound **9a** acetylated by dissolving in pyridine (10 mL) and acetic anhydride (5 mL). The resulting mixture was stirred overnight at room temperature in a stoppered bottle. After this time, the reaction mixture was concentrated under reduced pressure yielding a pale yellow oil. Essentially pure title compound (**10a**), 1.85 g (90%), was obtained as a white foam after column chromatography using a mixture of EtOAc and low boiling petroleum ether as eluant.

NMR ($CDCl_3$): 0.065 (s, 3H), 0.023 (s, 3H), 0.63 (s, 3H), 0.79 (d J = 6.9 Hz), 0.89 (s, 9H), 0.92 (s, 3H), 0.94 (s, 3H), 1.29 (s, 3H), 1.50 (bs, 3H), 1.64 (bs, 3H), 1.96 (s, 3H), 2.19 (m,2H), 2.57 (m, 2H), 3.68 (bs, 1H), 3.73 (m, 2H), 4.15 (bs, 1H), 4.97 (bt, 1H), 5.34 (bt, 1H), 7.37 (m, 6H), 7.56 (m, 4H). MS (EI+) m/z 814, 736, 522.

3-*O*-TBDMS-21-*O*-Diphenylmethylsilyl-16β-acetoxy-17*S*,-20*S*-dihydrofusidin-3,11,16,21-tetrol (10b). The reaction was carried out as described for the preparation of **10a**. NMR ($CDCl_3$): 0.005 (s,3H), 0.022 (s, 3H), 0.61 (s, 3H), 0.79 (d J = 6.5 Hz, 3H), 0.90 (s, 9H), 0.93 (s, 3H), 1.29 (s, 3H), 1.56 (bs, 3H), 1.68 (bs, 3H), 1,80 (s, 3H), 2.17 (m, 2H), 2.57 (m, 2H), 3.66 (dd, 1H), 3.69 (bs, 1H), 3.70 (dd, 1H), 4.20 (bs, 1H), 5.08 (bt, 1H), 5,25 (bt, 1H), 7.56 (m, 4H), 7.36 (m, 6H).

3-*O*-TBDMS-16β-Acetoxy-17*S*,20*R*-dihydrofusidin-3,-11,16,21-tetrol (11a). Compound **10a** (1.9 g, 2.3 mmol) was dissolved in tetrahydrofuran (30 mL) and glacial acetic acid (0.25 mL). To this solution was added TBA^+F^- (1.17 g, 4.6 mmol), and the reaction mixture was stirred at room temperature for 15 min. The reaction mixture was diluted with EtOAc (100 mL) and the organic solution was washed with water (2 × 25 mL) and brine (2 × 25 mL). The organic solution was dried (Na_2SO_4) and concentrated under reduced pressure yielding a colorless syrup. Pure title compound (**11a**), 1.3 g (90%), was obtained as a colorless foam after column chromatography using a mixture of EtOAc and low boiling petroleum ether as eluant.

NMR (C_6D_6): 0.08 (s, 3H), 0.09 (s, 3H), 0.91 (d J = 6 Hz, 3H), 0.91 (s, 3H), 1.03 (s, 3H), 1.06 (s, 9H), 1.39 (s, 3H), 1.62 (bs, 3H), 1.68 (bs, 3H), 1.79 (s, 3H), 3.55 (m, 1H), 3.57 (dd, 1H), 3.66 (dd, 1H), 4.08 (m, 1H), 5.25 (bt, 1H), 5.52 (t, 1H).

3-*O*-TBDMS-16β-Acetoxy-17*S*,20*S*-dihydrofusidin-3,11,-16,21-tetrol (11b). The reaction was carried out as described for the preparation of **11a**. NMR ($CDCl_3$): 0.001 (s, 3H), 0.017 (s, 3H), 0.79 (d J = 6.5 Hz, 3H), 0.89 (s, 9H), 0.93 (s, 3H), 1.00-(s, 3H), 1.32 (s,3H), 1.60 (bs, 3H), 1.67 (bs, 3H), 2.02 (s, 3H), 2.54 (m, 1H), 2.66 (m, 1H), 3.59 (dd, 1H), 3.66 (bs, 1H), 3.67 (dd, 1H), 4.30 (m, 1H), 5.09 (bt, 1H), 5.33 (dt, 1H).

3-*O*-TBDMS-17*S*,20*R*-Dihydrofusidic Acid (12a). (i) Dess−Martin periodinane (0.89 g, 2.1 mmol) was added portionwise to a solution of compound **11a** (1.26 g, 2.0 mmol) in anhydrous THF (20 mL) under argon at 0 °C. The resulting reaction mixture was stirred for 3 h at 0 °C. The reaction was stopped by adding a solution of saturated $NaHCO_3$ (50 mL) and 1 N sodium thiosulfate (50 mL), and the resulting two

layers were vigorously stirred for 30 min. The mixture was extracted with EtOAc (2 × 100 mL), and the combined organic extracts were washed with saturated $NaHCO_3$ (50 mL) and brine (50 mL). The organic solution was dried (Na_2SO_4) and concentrated under reduced pressure yielding 1.25 g (quantitative) of a colorless syrup.

NMR ($CDCl_3$): 0.0002 (s, 3H), 0.012 (s, 3H), 0.79 (d $J = 6.5$ Hz, 3H), 0.88 (s, 9H), 0.90 (s, 3H), 1.28 (s, 3H), 1.55 (bs, 3H), 1.65 (bs, 3H), 2.03 (s, 3H), 2.53 (m, 1H), 2.67 (m, 1H), 2.69 (m, 1H),3.67 (m, 1H), 4.25 (bs, 1H), 4.99 (bt, 1H), 5.43 (t, 1H), 9.50 (d, $J = 5.0$, 1H).

(ii) Aldehyde (1.25 g, 2.0 mmol) from (i) was used without purification dissolved in *tert*-butanol (15 mL). To this solution was added 2-methyl-2-butene (0.48 mL, 5.4 mmol), 1 N sodium dihydrogenphosphate (5.5), and sodium chlorite (0.46 g, 5.0 mmol) in water (20 mL), and the resulting reaction mixture was stirred vigorously overnight at room temperature. The reaction mixture was acidified to pH 4 with acetic acid and transferred to a separatory funnel with EtOAc. The two layers were shaken and separated. The aqueous layer was re-extracted twice with EtOAc. The combined organic extracts were washed twice with brine, dried (Na_2SO_4), and concentrated under reduced pressure yielding 1.2 g of a pale yellow foam. Purification by column chromatography using a mixture of EtOAc, low boiling petroleum ether, and a trace of formic acid as eluant yielded 1.05 g (81% from 11a) of pure acid **12a**, the title compound, as a semicrystalline compound.

NMR ($CDCl_3$): 0.002 (s, 3H), 0.007 (s, 3H), 0.79 (d $J = 6.9$ Hz), 0.88 (s, 9H), 0.91 (s, 3H), 1.003 (s, 3H), 1.28 (s, 3H), 1.56 (bs, 3H), 1.65 (bs, 3H), 1.01 (s, 3H), 2.47 (m, 1H), 2,66 (m, 1H), 2.71 (m, 1H), 3.67 (bs, 1H), 4.28 (bs, 1H), 5,05 (bt, 1H), 5.39 (bt, 1H). MS (ES-): 631. Anal. ($C_{43}H_{74}O_8Si$) C, H.

3-*O*-TBDMS-17*S*,20*S*-Dihydrofusidic Acid (12b). The reaction was carried out as described for the preparation of **12a**. Mp 210−212 °C. NMR ($CDCl_3$): 0.007 (s, 3H), 0.009 (s, 3H), 079 (d $J = 6.8$ Hz, 3H), 0.88(s, 9H), 0.93 (s, 3H), 0.98 (s, 3H), 1.29 (s, 3H), 1.57 (bs, 3H), 1.66 (s, 3H), 1.92 (s, 3H), 3.67 (bs, 1H), 4.31 (bs, 1H), 5.05 (bt, 1H), 5.30 (bt, 1H).

17*S*,20*R*-Dihydrofusidic Acid (4). 3-*O*-TBS-17*S*,20*R*-dihydrofusidic acid (**12a**) (1.0 g, 1.55 mmol) was dissolved in THF (12 mL) and 40% aqueous HF (3 mL) in a round-bottom Teflon flask, and the resulting mixture was stirred vigorously at room temperature for 24 h. After this time, water (25 mL) was added and the pH of the mixture was adjusted to 5 with 2 N NaOH. The mixture was extracted with EtOAc (3 × 50 mL). The combined organic extracts were washed with brine (3 × 25 mL), dried (Na_2SO_4), and concentrated under reduced pressure yielding 1.1 g of compound **4** as a colorless solid. Recrystallization from methanol−water yielded 0.72 g (87%) of a dihydrate as colorless crystals, mp 243−245 °C.

NMR ($CDCl_3$): 0.91 (d $J = 6.8$ Hz 3H), 0.97 (s, 3H), 1.01 (s, 3H), 1.31 (s, 3H), 1.57 (bs, 3H), 1.66 (bs, 3H), 2.01 (s, 3H), 2.51 (m, 1H), 2,68 (m, 2H), 3.75 (bs, 1H), 4.24 (bs, 1H), 5.05 (bt, 1H), 5.39 (bt, 1H). MS: Calc. for $C_{31}H_{46}O_4$ (M-2xH_2O) *m*/*z* 482.3396, Observed *m*/*z* 482.3400. Anal. ($C_{31}H_{50}O_6$, $2H_2O$) C, H.

17*S*,20*S*-Dihydrofusidic Acid (5). The reaction was carried out as described for the preparation of **4**. Mp 195−195.5 °C. NMR ($CDCl_3$): 0.91 (d, $J = 6.8$ Hz, 1H), 0.97 (s, 3H), 1.01 (s, 3H), 1.32 (s, 3H), 1.58 (bs, 3H), 1.68 (bs, 3H), 1.95 (s, 3H), 2.60 (m, 1H), 2.71 (m, 2H), 3.75 (bs, 1H), 4.34 (bs, 1H), 5.06 (bt, 1H), 5.32 (bs, 1H). MS: Calc. for $C_{31}H_{46}O_4$ (M-$2H_2O$) *m*/*z* 482.3396, Observed *m*/*z* 482.3391 Anal. ($C_{31}H_{50}O_6$, $2H_2O$) C, H.

17*S*,20*R*-Dihydrofusidic Acid Lactone (6a). Anal. ($C_{29}H_{44}O_4$) C,H; C: Calcd: 76.27; found: 76.20; H: calcd. 9.71; found, 9.77.

17*S*,20*S*-Dihydrofusidic Acid Lactone (6b). Anal. ($C_{29}H_{46}O_4$) C,H: C: calcd. 75.94; found 75.38; H: calcd. 10.11; found 10.11.

3-*O*-TBDMS-17*S*,20*R*-Dihydrofusidic Acid (12a). Anal. ($C_{43}H_{74}O_8Si$) C,H; C: Calcd. 70.21; found, 70.05; H: calcd. 10.19; found, 10.19.

17*S*,20*R*-Dihydrofusidic Acid (4). Anal. ($C_{31}H_{50}O_6$, $2H_2O$) C,H; C: Calcd. 67.12; found, 67.35; H: calcd. 9.81; found, 9.52.

17*S*,20*S*-Dihydrofusidic Acid (5). Anal. ($C_{31}H_{50}O_6$, $2H_2O$) C,H; C: Calcd. 71.78; found 70.90; H: calcd. 9.72; found, 9.66.

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