Synthesis and Structure−Activity Relationships of Bafilomycin A₁ Derivatives as Inhibitors of Vacuolar H⁺-ATPase

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The macrolide antibiotic bafilomycin A₁ is a highly potent and selective inhibitor of all the vacuolar ATPases (V-ATPases). With the aim of obtaining novel analogues specific for the osteoclast subclass of vacuolar ATPase, 31 derivatives of bafilomycin A₁ were synthesized and tested for their ability to inhibit differentially the V-ATPase-driven proton transport in membrane vesicles derived from chicken osteoclasts (cOc) and bovine chromaffin granules (bCG). Although none of the new analogues were more potent than the parent compound, the obtained data provided a significant amount of information about the structural requirements for the inhibitory activity of bafilomycin A₁. The different effects of a few analogues on the two enzymes could also suggest the possibility of a selective modulation of the V-ATPases in different tissues.

Introduction

Vacuolar H⁺-ATPases (V-ATPases)[1] are a class of proton-translocating pumps which provide discrete compartments of the cell with an acidic lumen. In eukaryotic cells, V-ATPases are mainly located in most intracellular organelles of both constitutive and specialized secretory pathways and play an important role in membrane trafficking and protein sorting as well as in protein degradation.[2−4] In addition, these enzymes participate in urinary acidification[5] and osteoclast-mediated bone resorption.[6] Considerable interest has thus developed in studying the pathophysiological role of vacuolar proton pumps as well as in identifying novel inhibitors that might serve as effective therapeutic agents.

Our interest in this field was aimed at identifying selective inhibitors of the vacuolar H⁺-ATPase located on the ruffled border of the osteoclasts in order to obtain novel agents potentially useful for treating diseases associated with excessive bone resorption, e.g., osteoporosis. It is known, in fact, that this V-ATPase is essential for the bone resorption process since it provides the acidic environment necessary for the degradation of both the inorganic and organic components of bone.[7]

Bafilomycin A₁ (**1**) (Chart 1), a macrolide antibiotic produced by *Streptomyces griseus*,[8] is a potent and highly specific inhibitor of the V-ATPases.[9,10] This compound, however, is not selective for any particular subclass and, when administered to animals, is toxic due to a generalized inhibition of all essential V-ATPases.

Starting from the natural macrolide lead, we undertook a program of chemical modifications in order to find the minimal structural requirements for V-ATPase inhibitory activity. The ability of the new derivatives to inhibit the ATP-driven proton transport was assessed in two different enzyme systems, namely, chicken osteoclasts (cOc) and bovine chromaffin granule membrane vesicles (bCG). The obtained structure−activity relationships (SARs) afforded useful indications on the possibility of producing new inhibitors selective for the osteoclast proton pump.

Chart 1. Bafilomycin A₁ (**1**)

Bafilomycin A₁ (**1**)
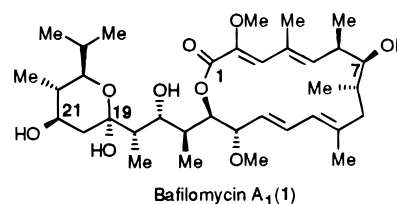
Biochemistry

The exact structure of V-ATPases is not yet completely known. These are multisubunit enzyme complexes with two functional domains:[11,12] a 500-kDa cytoplasmatic catalytic sector that contains at least eight different proteins,[13] including three copies each of A and B catalytic subunits, and an integral domain containing the proton channel formed by a hexamer of 16-kDa proteolipids and at least three accessory subunits. Notwithstanding their high complexity, V-ATPases are evolutionarily conserved. Thus, the A and B catalytic subunits have greater than 75% conservation of primary structure extending from *archaebacteria* to humans.[14]

It is still controversial whether the osteoclast plasma membrane possesses a unique proton pump: sequence differences between the chicken osteoclast enzyme and V-ATPases in other cell types have been reported,[15,16] as well as a peculiar sensitivity of osteoclast V-ATPase to vanadate and nitrate.[15] The latter findings, however, have not been confirmed by other investigators in either chicken[17,18] or mammalian[19,20] osteoclasts. Therefore, the only possible strategy for finding selective inhibitors consisted in comparing the potency of the new derivatives against vacuolar enzymes from different origins. Sufficient amounts of osteoclast membranes can be obtained only from the medullary bone of calcium-starved egg-laying hens, while other V-ATPases can be prepared from several sources, e.g., brain,[21] kidney,[22] or adrenal glands[23] from bovines. Since literature reports indicate a close similarity of the characterized subunits of the osteoclast V-ATPase with the homolo-
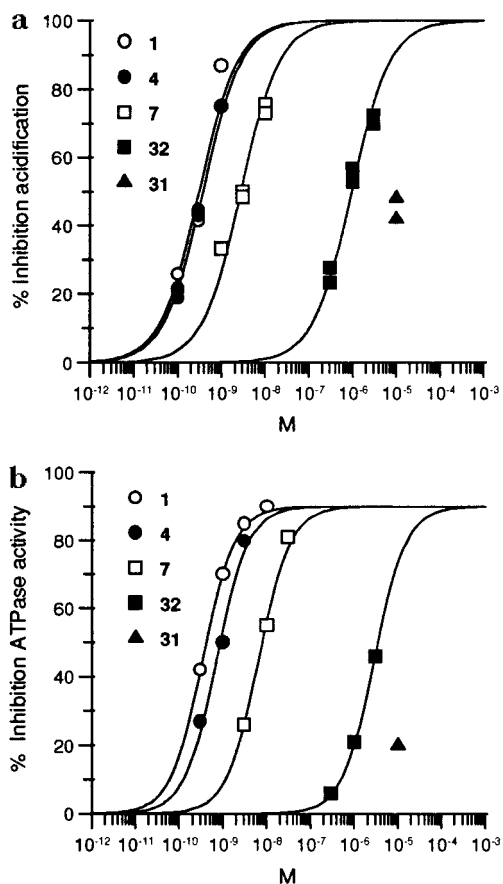
Figure 1. Dose−response curves of proton transport (a) and ATPase assay (b) in bovine chromaffin granules. Simultaneous fitting of curves of **1**, **4**, **7**, **32**, and **31**.

gous subunits from bovine brain or bovine chromaffin granules, the latter was chosen as a comparator for selectivity, owing to its abundance and easy preparation from the medulla of bovine adrenals.

The inhibitory activity of the compounds was determined by using the proton transport assay. In this functional test the proton-pumping activity of the enzyme is assessed by measuring the entry of protons into the inside-out vesicles reconstituted from cOc plasma membranes or from bCG membranes. For this purpose, acridine orange (AO), a fluorescent weak base, was used as a probe. The rate of fluorescence quench, produced by entry of AO into the vesicles, is proportional to the net rate of inward proton transport. Inhibition of proton transport activity was expressed as the percentage versus DMSO-matched controls. IC_{50} values were derived by analyzing at least three duplicated dose levels. Since the bafilomycin IC_{50} is strictly dependent from the protein concentration, actual IC_{50} values may vary with the concentration of the enzyme.[10] To compare data from different experiments, performed with different batches of membranes, IC_{50} values of all our compounds were normalized against the IC_{50} of bafilomycin A_1 obtained in the same experiment.

The validity of this approach to predict V-ATPase inhibition was verified for some selected compounds (**1**, **4**, **7**, **31**, and **32**) showing a wide range of potency in the proton transport assay. The effect of these derivatives on bafilomycin-sensitive ATPase activity in bCG has been measured, and the good correlation between the two sets of results (Figure 1) clearly indicated that

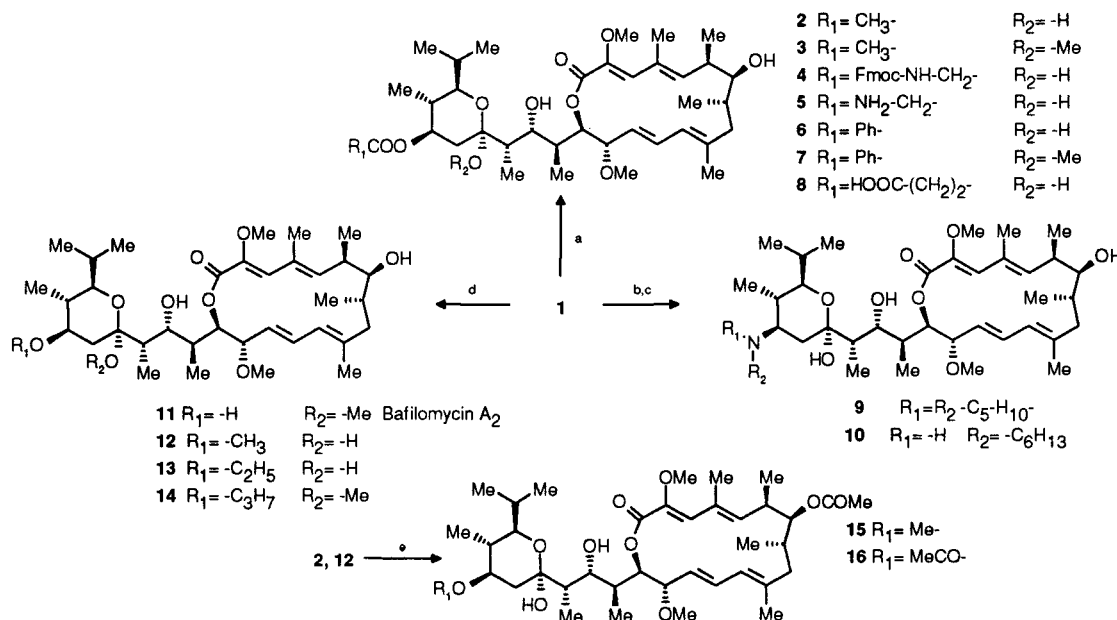the inhibition of acidification was the consequence of a direct action on the enzyme activity.

Moreover, the same bafilomycin analogues (**1**, **4**, **31**, and **32**) were tested for their ability to inhibit in vitro bone resorption by isolated rat osteoclasts. Bafilomycin A_1 (**1**) was able to inhibit completely bone resorption at 1 nM; compound **4**, which was 60-fold less potent than **1** in the osteoclast proton transport assay, was inactive at 10 nM but fully inhibited resorption at 100 nM. The two analogues **31** and **32**, which were inactive in the proton transport assay, did not display any activity in the resorption assay at 100 nM. Although very preliminary, these data clearly suggest that inhibition of proton transport in chicken osteoclast is correlated to, and predictive of, the antiresorptive activity of bafilomycin derivatives in the mammalian osteoclast system.

Chemistry

Little information is available in the literature about chemical modifications of the functional groups of bafilomycin A_1.[24,25] The chemical work was mainly focused on modifying the most reactive hydroxy groups at positions 7 and 21, producing esters, ethers, and amines or oxidized derivatives. The 21-hydroxy is the most reactive moiety, since the 7-OH is sterically hindered and the 17-OH is involved in a strong hydrogen-bonding system with the 19-OH and the lactone carbonyl.[26] In addition, fragmentation, ring opening, and hydrogenation were performed to allow assessment of the role of macrolactone and tetrahydropyran rings, and of the dienic systems as well.

Bafilomycin derivatives **2**,[27] **4**, and **6**,[27] acylated at position 21 (Scheme 1), were prepared by reaction of **1** with the appropriate acyl chloride (2.5 equiv) in pyridine with a large amount of DMAP (2 equiv). A lower amount of acyl chloride or DMAP gave an incomplete reaction. The 21-*O*-hemisuccinate **8** was obtained by reaction with succinic anhydride in dichloromethane. The 21-alkylamino derivatives **9** and **10** were prepared from bafilomycin A_1 by derivatization with 4-nitrobenzenesulfonyl chloride and subsequent treatment of the activated, not isolated, intermediate with the corresponding amine. Surprisingly, compounds **9** and **10** retained the same configuration at C-21 of the natural macrolide **1**, as demonstrated by ^{1}H NMR. A possible mechanism could entail a double inversion of this center, resulting initially by the intramolecular displacement of the 21-nitrobenzenesulfonyloxy group by the hemiketalic α-oriented hydroxy at C-19. The intermediate oxetane could be subsequently opened by the appropriate amine from the β face of the molecule restoring the initial configuration. A similar mechanism was postulated for the stereoselective generation of 21β-alkoxides **12**−**14** produced by treating bafilomycin A_1 with the appropriate alcohol in the presence of aqueous oxalic acid.[28] 7-Acetoxy derivatives **15** and **16** were obtained by refluxing **12** or **2**, respectively, in acetic anhydride. 19-*O*-Methyl derivatives **3**[27] and **7** were obtained from the corresponding 21-*O*-acylbafilomycin A_1 derivatives in the presence of MeOH and acetic acid. Bafilomycin A_2 (**11**) was synthesized following the procedure described in the literature.[24]

Ketones **17**−**21** and **32** (Scheme 2) were obtained by oxidation of **1**, **2**, or **6**, respectively, as reported previously.[27]

Scheme 1[a]

11 R_1= -H R_2= -Me Bafilomycin A_2
12 R_1= -CH₃ R_2= -H
13 R_1= -C₂H₅ R_2= -H
14 R_1= -C₃H₇ R_2= -Me

2 R_1= CH₃- R_2= -H
3 R_1= CH₃- R_2= -Me
4 R_1= Fmoc-NH-CH₂- R_2= -H
5 R_1= NH₂-CH₂- R_2= -H
6 R_1= Ph- R_2= -H
7 R_1= Ph- R_2= -Me
8 R_1=HOOC-(CH₂)₂- R_2= -H

9   R_1=R_2 -C₅-H₁₀-
10   R_1= -H R_2= -C₆H₁₃

15 R_1= Me-
16 R_1= MeCO-

[a] Reagents: (a) RCOCl, DMAP, pyridine; (2 → 3 and 6 → 7) MeOH, acetic acid; (4 → 5) piperidine, EtOAc; (8) succinic anhydride, DMAP, CH₂Cl₂; (b) 4-nitrobenzenesulfonyl chloride, CH₂Cl₂; (c) R₁R₂NH, CH₂Cl₂ or EtOAc; (d) ROH, 10% oxalic acid; (e) acetic anhydride, reflux.

Scheme 2[a]

17 R_1= -OH
18 R_1= =O

32

19 R_1= H-
20 R_1= MeCO-
21 R_1= PhCO-
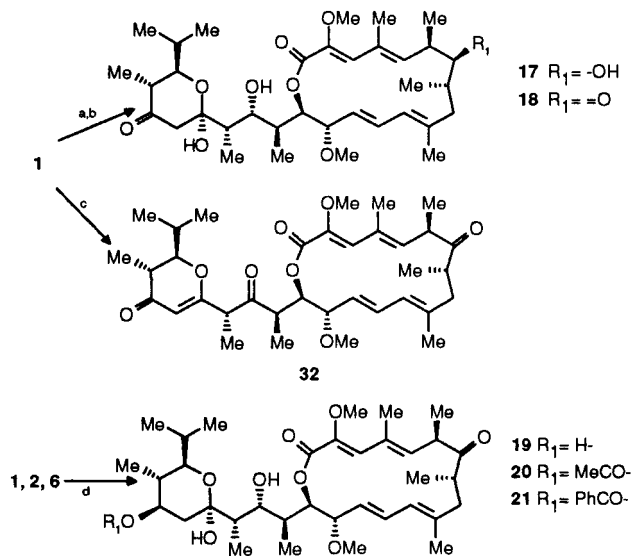
[a] Reagents: (a) DMSO, oxalyl chloride, CH₂Cl₂, −70 °C; (b) Dess−Martin periodinane (1,1,1-triacetoxy-1,1-dihydro-1,2-benzodioxol-3(1*H*)-one) (3 mol), CH₂Cl₂, rt; (c) Dess−Martin periodinane (7.5 mol), CH₂Cl₂, rt; (d) PCC, AcONa, CH₂Cl₂.

Reaction of **1** with diethylaminosulfur trifluoride (DAST) in CH₂Cl₂ at −78 °C (Scheme 3) afforded, in 60% yield, the β,γ-unsaturated ketone **22** formally resulting by the elimination of isobutanal from the tetrahydropyran ring.[29] Reduction of **22** with NaBH₄ in MeOH at room temperature gave the corresponding alcohol **23** as an 80:20 epimeric mixture. The configuration of the predominant epimer was not determined.

A different fragmentation of the side chain of bafilomycin A₁ (Scheme 4) was obtained by reaction of 21-oxo derivatives **17** and **18** with an excess of sodium cyanoborohydride and ammonium acetate in methanol.[29] After 4 days at room temperature, the acids **24** and **25** were isolated in 25−30% yield. Esterification
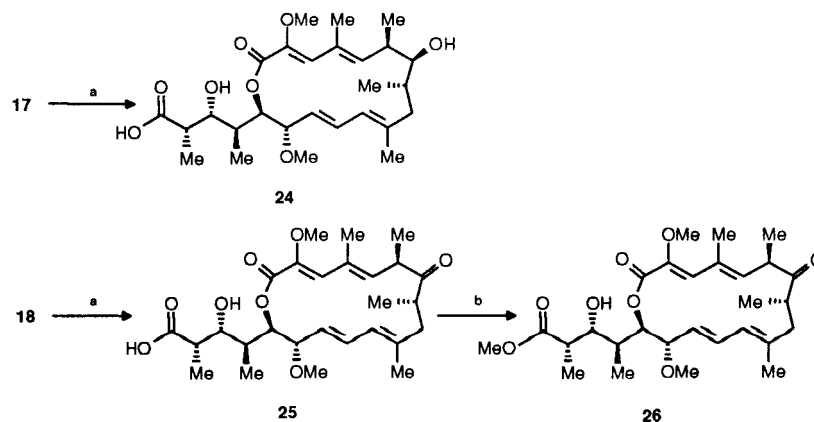
Scheme 3[a]


22

23
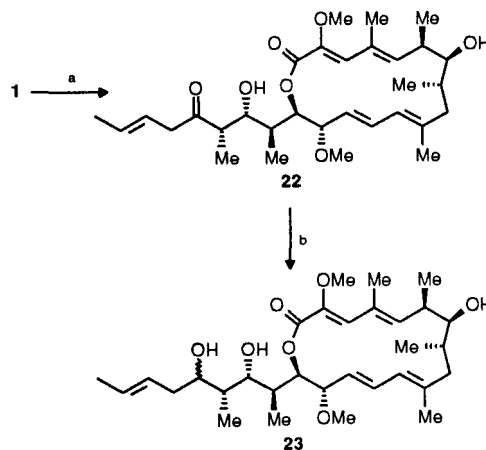
[a] Reagents: (a) DAST, CH₂Cl₂; (b) NaBH₄, MeOH.

with diazomethane of the carboxylic acid **25** gave the corresponding methyl ester **26**.

Bafilomycin D (**28**)[30] and bafilomycin V₁ (**29**)[24] were prepared according to the methods known in the literature. Compound **27**, in which the tetrahydropyran ring was opened and the 19-ketone reduced to the corresponding alcohol, was obtained as an epimeric mixture by reduction of bafilomycin A₁ with sodium borohydride in ethanol (Scheme 5).

To assess the importance of the dienic systems in the macrolactone ring, partial or total hydrogenation was performed (Scheme 6). Treatment of **1** with Mg in methanol resulted in the 1,4-addition of hydrogen to the two dienic systems, affording compound **30** in which two isolated double bonds, shifted to positions 3 and 11, are present. Hydrogenation with 10% Pd on activated carbon of the 21-benzoyl derivative **7**, used to allow UV/HPLC detection of reaction products, provided the totally hydrogenated derivative of bafilomycin A₁, **31**. Both compounds **30** and **31** were obtained as diastere-

Scheme 4[a]

24

25 → **26**

[a] Reagents: (a) NaBH$_3$CN, MeCOONH$_4$, MeOH; (b) diazomethane.

Scheme 5[a]

27

28, Bafilomycin D

29, Bafilomycin V$_1$
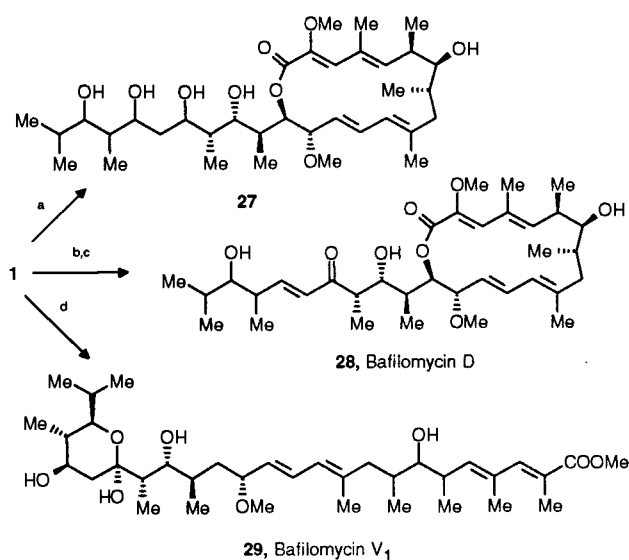
[a] Reagents: (a) NaBH$_4$, EtOH; (b) MeCOCl, DMAP, pyridine; (c) NaOH; (d) MeOH, NaOH.

Scheme 6[a]

30

31
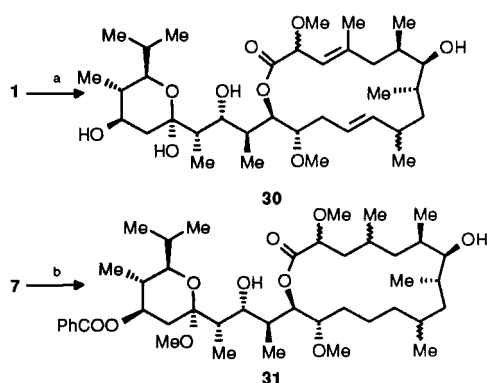
[a] Reagents: (a) Mg, MeOH; (b) 10% Pd/C, MeOH.

oisomeric mixtures. The identification of the compounds was done on the basis of MS data and NMR spectra even if the assignment of each chemical shift was impossible because of the complexity of the NMR spectra.

Results and Discussion

Although none of the 31 derivatives of bafilomycin A$_1$ were more potent than the natural macrolide, several

Table 1. Bafilomycin Derivatives Modified at Position 21 or/and 7: Inhibition of Proton Transport in Chicken Osteoclast and Bovine Chromaffin Granule[a]

compd	R$_1$	R$_2$	R$_3$	potency ratio (IC$_{50}$ compd/IC$_{50}$ baf A$_1$) cOc	bCG
1	OH	OH	OH	1	1
2	MeCO$_2$	OH	OH	2	1.4
3	MeCO$_2$	OMe	OH	1.3	1.8
4	Fmoc-NH-CH$_2$CO$_2$	OH	OH	60	1.2
5	NH$_2$CH$_2$CO$_2$	OH	OH	3.5	3.2
6	PhCO$_2$	OH	OH	4	1.6
7	PhCO$_2$	OMe	OH	42.1	9.1
8	HO$_2$C(CH$_2$)$_2$CO$_2$	OH	OH	1.6	2.3
9	C$_5$H$_{10}$N	OH	OH	5.5	6.7
10	C$_6$H$_{11}$NH	OH	OH	9.3	9.2
11	OH	OMe	OH	1.4	1.4
12	MeO	OH	OH	1.3	1.2
13	EtO	OH	OH	1.4	1.2
14	PrO	OMe	OH	4	5
15	MeO	OH	MeCO$_2$	430	738
16	MeCO$_2$	OH	MeCO$_2$	49	280
17	=O	OH	OH	2	2.2
18	=O	OH	=O	103	112
19	OH	OH	=O	34	32
20	MeCO$_2$	OH	=O	531	552
21	PhCO$_2$	OH	=O	na	na

[a] IC$_{50}$ of bafilomycin A$_1$ was 1−3 nM in chicken osteoclasts (cOc) and 0.6−1.5 nM in bovine chromaffin granules (bCG); na, IC$_{50}$ greater than 10 μM.

chemical modifications could be performed without any significant prejudice for V-ATPase inhibitory activity. Furthermore, in the majority of the new derivatives, no significant differences of potency, relative to bafilomycin A$_1$, were observed in the two biological systems used, i.e., cOc and bCG membranes.

In Table 1, the relative potencies of compounds obtained by derivatization or modification of the hydroxy groups at position 21, 7, or 19 are reported. Acylation at position 21 with acetyl (**2**) or benzoyl (**6**) groups was well-tolerated with only a slight decrease in potency of **6** against the osteoclast enzyme. Bulkier acyl groups, such as Fmoc-aminoacetyl derivative **4**, caused a marked (60-fold) reduction of potency in cOc, whereas in bCG,

the compound was as potent as the parent. Compound **4**, therefore, displayed a 50-fold preference for the V-ATPase in bCG; this result seems to indicate that a differential pharmacological modulation of the two V-ATPases in cOc and bCG is possible.

Introduction of basic or acidic moieties in the acylating group (e.g., the 21-*O*-aminoacetyl derivative **5** or the 21-*O*-hemisuccinate **8**) did not greatly affect the potency against both enzymes. The direct replacement of 21-OH by piperidine (**9**) or hexylamine (**10**) groups, however, slightly decreased the potency (10- and 5-fold, respectively) in both systems, while 21-O-alkylation as in **12** and **13** afforded compounds with potency and selectivity comparable to that of the parent **1**.

The effect of methylation of the hemiketalic 19-OH was more unpredictable. While in 21-OH (**11**), 21-OAc (**3**), and 21-OPr (**14**) derivatives such a methylation had little or no effect, in the 21-benzoylated derivative (**7**) the potency was significantly reduced by 40-fold in osteoclasts and 10-fold in chromaffin granules in comparison with bafilomycin A₁ (**1**) or 10-fold in cOc and about 6-fold in bCG when compared with the corresponding 21-*O*-benzoyl analogue (**6**).
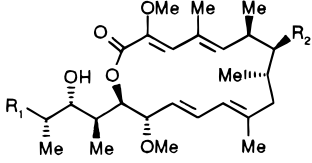
Selective oxidation of 21-OH, to produce the 21-oxo analogue **17**, caused only a slight reduction of potency, indicating that this hydroxy group does not play a fundamental role in the interaction of bafilomycin with the enzyme. Conversely, the presence of the hydroxy group at position 7 looks very important for activity, as it appears from data in Table 1. Either acetylation or oxidation of 7-OH caused a significant drop in potency. The 7-oxo derivative **19** was 30-fold less potent than **1**; moreover, acylation or oxidation of **19** at 21-OH caused a further reduction of potency. The 7,21-dioxo derivative **18** and the 21-acetylated analogue **20** displayed a potency reduced by 100- and 500-fold, respectively, while the 21-*O*-benzoyl derivative **21** was almost inactive, with a proton transport inhibition less than 50% at 10 μM. Extensive oxidation (positions 21, 17, and 7) with dehydration of the hydroxy at position 19 (**32**) gave a poorly active compound (about 1000-fold less potent than bafilomycin A₁) in cOc enzyme (Table 3).
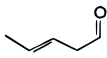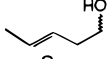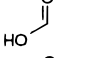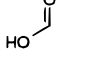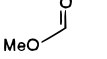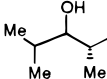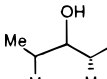
Furthermore, the 7,21-disubstituted bafilomycins **15** and **16** showed only weak activity; however, the diacetyl derivative **16** was the only compound showing some preference for the chicken osteoclast V-ATPase, being about 6-fold more potent than in the bCG system.

Opening and/or partial degradation of the tetrahydropyran ring had only a moderate effect on potency (Table 2), indicating that this moiety is not essential for V-ATPase interaction. Compound **22** in which the side-chain ring is replaced by a *β,γ*-pentenone moiety was about 20-fold less potent than **1**; interestingly, **23**, in which the ketone was reduced and the 19-OH restored, was almost equipotent to bafilomycin A₁. Analogously, opening the tetrahydropyran ring with concomitant NaBH₄ reduction, as in **27**, or dehydration, as in **28**, caused about 10-fold reduction of potency.

Greater or total loss of activity was observed when the side-chain ring was removed and replaced by a carboxylic group as in **24**−**26**. Drastic modifications of the macrolactone ring (Table 3), such as partial (**30**) or total (**31**) hydrogenation, practically abolished the activity, while opening of this ring did not remove completely

Table 2. Bafilomycin Derivatives with a Modified Tetrahydropyran Ring: Inhibition of Proton Transport in Chicken Osteoclast and Bovine Chromaffin Granule[a]

compd	R₁	R₂	potency ratio (IC₅₀ compd/IC₅₀ baf A₁) cOc	potency ratio (IC₅₀ compd/IC₅₀ baf A₁) bCG
22		OH	21	21
23		OH	3.2	2.6
24		OH	na	na
25		=O	na	na
26		=O	na	na
27		OH	13.1	8.2
28		OH	9	15

[a] IC₅₀ of bafilomycin A₁ was 1−3 nM in chicken osteoclasts (cOc) and 0.6−1.5 nM in bovine chromaffin granules (bCG); na, IC₅₀ greater than 10 μM.

the activity. Compound **29**, indeed, maintained a submicromolar potency which, although 200−300-fold lower than that of **1**, is still promising and suggests that a rigid macrocyclic ring might not be strictly required.

Conclusions

Extensive modifications of the structure of bafilomycin A₁ did not allow to identify any new analogue more potent or significantly more selective for the cOc V-ATPase. However, this work has provided a significant amount of information about the structural requirements for inhibitory activity of bafilomycins. The most important features can be summarized in the following points:

(1) The hydroxy group at position 21 can be acylated, alkylated, and oxidized, and therefore, it does not seem essential for the interaction with the enzyme. On the contrary the same modifications at position 7 cause a significant reduction of potency. This indicates that hydroxy at position 7 is crucial for the bafilomycin activity.

(2) Partial and total hydrogenations strongly reduce the activity, indicating that the dienic systems of the macrolactone ring are essential.

(3) Extensive chemical modifications can be performed on the tetrahydropyran ring without affecting biological activity. However, the replacement of the whole ring by carboxylic or carbomethoxy groups afforded inactive compounds indicating that this part of the molecule does indeed contribute to the interaction with the V-ATPase complex, although it was not possible to identify any particular groups involved in this interaction.

Table 3. Bafilomycin Derivatives with a Modified Macrolide Ring: Inhibition of Proton Transport in Chicken Osteoclast and Bovine Chromaffin Granule[a]

compd		potency ratio (IC$_{50}$ compd/IC$_{50}$ baf A$_1$)	
		cOc	bCG
29		200	330
30		2160	2110
31		na	na
32		1040	3050

[a] IC$_{50}$ of bafilomycin A$_1$ was 1–3 nM in chicken osteoclasts (cOc) and 0.6–1.5 nM in bovine chromaffin granules (bCG); na, IC$_{50}$ greater than 10 μM.

The differential effects of a few analogues on the two enzymes, albeit small, might suggest that selective modulation of V-ATPases in different tissues might be hopefully achieved. This piece of information could constitute the basis for a novel approach of medicinal chemistry aimed at the de novo design of simplified molecules, which could afford a novel class of antiresorptive agents useful for the treatment of osteoporosis and similar osteopenic diseases.

Experimental Section

Melting points were determined on a Büchi 535 apparatus and are uncorrected. Optical rotations were determined at 20 °C on a Perkin-Elmer at *c*% solution in the solvent indicated, using a 100-mm optical cell. NMR were recorded on a Bruker ARX-300 spectrometer in CDCl$_3$ (7.26 and 77.7 ppm). ^{1}H NMR spectra are reported as follows: chemical shifts (multiplicity, coupling constants in Hz, integration, interpretation). ^{13}C NMR are reported as chemical shifts. Two-dimensional COSY-90 experiments were carried out using standard software. FAB mass spectra were recorded with a Finnigan MAT TSQ 70 spectrometer; experimental conditions were Xe gas, 8 kV, source temperature 50 °C, using diethanolamine in negative ion mode or thioglycerol in positive ion mode. Positive electrospray was recorded with a FinniganTSQ 700 instrument. Bafilomycin A$_1$ was isolated from the mycelium of *S. griseus*. Reagents and solvents were purchased from common commercial suppliers and were used as received or distilled from the appropriate drying agent. Column chromatography (CC) was performed with silica gel 0.063–0.200 mm. TLC was performed on Merck silica gel 60 F$_{254}$ precoated plates eluting with solvent A (*n*-hexane/EtOAc, 3:2), B (*n*-hexane/EtOAc, 4:1), or C (EtOAc) and detected by 5% vanillin/H$_2$SO$_4$. Analytical HPLC method: C-18 column (Vydac RP18), 250- × 4.6-mm i.d., flow rate 1 mL/min, λ = 254 nm; mobile phase A, 0.05 M NH$_4$OAc, pH 6.5; B, MeOH; linear gradient indicated. Preparative HPLC was performed using the same conditions on a C-18 column (Vydac RP18), 250- × 22-mm i.d., and a flow rate of 24 mL/min.

21-*O*-[2-[(9-Fluorenylmethoxycarbonyl)amino]acetyl]-bafilomycin A$_1$ (4). To a solution of bafilomycin A$_1$ (157.6 mg, 0.25 mmol) in pyridine (2.5 mL) were added 4-DMAP (61.8 mg, 0.51 mmol) and *N*-(9-fluorenylmethoxycarbonyl)ami-noacetyl chloride (199.8 mg, 0.51 mmol) at room temperature. After 2 h the reaction mixture was diluted with EtOAc and washed with a solution of 3% citric acid, a saturated solution of NaHCO$_3$, and brine. After evaporating to dryness the crude product was chromatographed over silica gel eluting with *n*-hexane/EtOAc (9:1) affording 106.1 mg (47%) of the title compound, mp = 107–110 °C. TLC (A): R_f = 0.46. [α]$_D$ = −15.4 (*c* = 1.12%, MeOH). HPLC: gradient 75–90% of B in 40 min, t_R 38 min. ^{1}H NMR (CDCl$_3$): 7.69 (d, *J* = 7 Hz, 1H, Fmoc); 7.54 (d, *J* = 7 Hz, 1H, Fmoc); 7.31 (dd, *J* = 7, 7 Hz, 1H, Fmoc); 7.21 (dd, *J* = 7, 7 Hz, 1H, Fmoc); 6.60 (s, 1H, 3-H); 6.43 (dd, *J* = 14, 10 Hz, 1H, 12-H); 5.73 (dd, *J* = 10, 1 Hz, 1H, 11-H); 5.69 (dd, *J* = 9, 1 Hz, 1H, 5-H); 5.44 (d, *J* = 2 Hz, 1H, 19-OH); 5.26 (t br, *J* = 5 Hz, 1H, 21-*NH*CH$_2$); 5.10 (d, *J* = 14, 9 Hz, 1H, 13-H); 5.01 (ddd, *J* = 10, 10, 5 Hz, H, 21-H); 4.89 (dd, *J* = 9, 1 Hz, 1H, 15-H); 4.57 (s br, 1H, 17-OH); 4.32 (m, 2H, −CH$_2$OCO); 4.12 (m, 1H, -*CH*CH$_2$OCO); 4.04 (d br, *J* = 9 Hz, 1H, 17-H); 3.92 (m, 2H, 21-NH*CH$_2$*); 3.80 (dd, *J* = 9, 9 Hz, 1H, 14-H); 3.57 (s, 3H, 2-OMe); 3.53 (dd, *J* = 11, 2 Hz, 1H, 23-H); 3.21 (m, 1H, 7-H); 3.16 (s, 3H, 14-OMe); 2.51−2.41 (m, 1H, 6-H); 2.30 (dd, *J* = 12, 5 Hz, 1H, 20-Heq); 2.10−2.02 (m, 1H, 9-H$_{eq}$); 2.10−2.02 (m, 1H, 16-H); 1.95−1.85 (m, 1H, 9-H$_{ax}$); 1.90−1.80 (m, 1H, 8-H); 1.90−1.80 (m, 1H, 24-H); 1.90 (d, *J* = 1 Hz, 3H, 4-Me); 1.84 (d, *J* = 1 Hz, 3H, 10-Me); 1.75−1.67 (m, 1H, 18-H); 1.57−1.47 (m, 1H, 22-H); 1.12 (dd, *J* = 12, 10 Hz, 1H, 20-H$_{ax}$); 1.00 (d, *J* = 7 Hz, 3H, 6-Me); 0.96 (d, *J* = 7 Hz, 3H, 18-Me); 0.89 (d, *J* = 7 Hz, 3H, 8-Me); 0.88 (d, *J* = 7 Hz, 3H, 24-Me); 0.76 (d, *J* = 7 Hz, 3H, 16-Me); 0.76 (d, *J* = 7 Hz, 3H, 22-Me); 0.70 (d, *J* = 7 Hz, 3H, 24-Me). FAB-MS (negative): *m/z* 900 (M − H).

21-*O*-(2-Aminoacetyl)bafilomycin A$_1$ (5). Piperidine (1 mL, 10.11 mmol) was added to a solution of 21-*O*-[2-[(9-fluorenylmethoxycarbonyl)amino]acetyl]bafilomycin A$_1$ (4) (106.1 mg, 0.12 mmol) in ethyl acetate (5 mL) at 0 °C. The reaction mixture was stirred for 1 h at room temperature, diluted with EtOAc, and washed with a solution of 3% citric acid, a

saturated solution of NaHCO₃, and brine. After evaporating to dryness the crude product was chromatographed over silica gel eluting with EtOAc obtaining 43 mg (53%) of the title compound as a white powder, mp = 112−118 °C. TLC (C): $R_f = 0.05$. $[\alpha]_D = -8$ ($c = 0.24\%$, THF). HPLC: gradient 75−90% of B in 40 min, t_R 20 min. ^{1}H NMR (CDCl₃): 6.66 (s, 1H, 3-H); 6.50 (dd, $J = 15, 10$ Hz, 1H, 12-H); 5.81 (dd, $J = 10, 1$ Hz, 1H, 11-H); 5.78 (dd, $J = 9, 1$ Hz, 1H, 5-H); 5.50 (s br, 1H, 19-OH); 5.17 (dd, $J = 15, 9$ Hz, 1H, 13-H); 5.03 (ddd, $J = 11, 11, 5$ Hz, 1H, 21-H); 4.96 (dd, $J = 9, 1$ Hz, 1H, 15-H); 4.12 (m, 1H, 17-H); 3.89 (dd, $J = 9, 9$ Hz, 1H, 14-H); 3.64 (s, 3H, 2-OMe); 3.61 (dd, $J = 11, 1$ Hz, 1H, 23-H); 3.41 (s br, 2H, 21-COCH₂N); 3.31−3.26 (m, 1H, 7-H); 3.22 (s, 3H, 14-OMe); 2.59−2.49 (m, 1H, 6-H); 2.35 (dd, $J = 11, 5$ Hz, 1H, 20-H$_{eq}$); 2.20−2.10 (m, 1H, 16-H); 2.18−2.10 (m, 1H, 9-H$_{eq}$); 2.00−1.95 (m, 1H, 9-H$_{ax}$); 1.99 (d, $J = 1$ Hz, 3H, 4-Me); 1.95−1.85 (m, 1H, 8-H); 1.94−1.85 (m, 1H, 24-H); 1.92 (d, $J = 1$ Hz, 3H, 10-Me); 1.79−1.70 (m, 1H, 18-H); 1.65−1.50 (m, 1H, 22-H); 1.20 (dd, $J = 11, 11$ Hz, 1H, 20-H$_{ax}$); 1.07 (d, $J = 7$ Hz, 3H, 6-Me); 1.02 (d, $J = 7$ Hz, 3H, 18-Me); 0.93 (d, $J = 7$ Hz, 3H, 8-Me); 0.91 (d, $J = 7$ Hz, 3H, 24-Me); 0.81 (d, $J = 7$ Hz, 3H, 16-Me); 0.80 (d, $J = 7$ Hz, 3H, 22-Me); 0.77 (d, $J = 7$ Hz, 3H, 24-Me). FAB-MS (positive): m/z 718 (MK⁺).

21-*O*-Benzoyl-19-*O*-methylbafilomycin A₁ (7). A solution of 21-*O*-benzoylbafilomycin A₁[27] (940 mg, 1.3 mmol) in methanol (150 mL) was stirred at 60 °C for 7 h in the presence of acetic acid (0.2 mL). After cooling, the solution was made neutral with NaHCO₃, and the solvent was removed under vacuum. The residue was diluted with EtOAc, washed with brine, dried, and evaporated. The crude product was chromatographed eluting with *n*-hexane/EtOAc (4:1) to afford 442 mg (46%) of the title compound 7 as a white foam. TLC (A): $R_f = 0.75$. ^{1}H NMR (CDCl₃): 8.05 (d, $J = 6$ Hz, 2H, 21-*o*Ph-H); 7.55 (dd, $J = 6, 6$ Hz, 1H, 21-*p*Ph-H); 7.42 (dd, $J = 6, 6$ Hz, 2H, 21-*m*Ph-H); 6.61 (s, 1H, 3-H); 6.49 (dd, $J = 15, 10$ Hz, 1H, 12-H); 5.81 (dd, $J = 10, 1$ Hz, 1H, 11-H); 5.75 (dd, $J = 9, 1$ Hz, 1H, 5-H); 5.20 (dd, $J = 15, 9$ Hz, 1H, 13-H); 5.10 (dd, $J = 9, 1$ Hz, 1H, 15-H); 3.88 (dd, $J = 9, 9$ Hz, 1H, 14-H); 3.78 (d, $J = 5$ Hz, 1H, 17-OH); 3.70 (s, 3H, 2-OMe); 3.51 (dd, $J = 11, 5$ Hz, 1H, 17-H); 3.32−3.28 (m, 1H, 7-H); 3.25 (dd, $J = 9, 1$ Hz, 1H, 23-H); 3.25 (s, 3H, 14-OMe); 3.10 (s, 3H, 19-OMe); 2.59−2.49 (m, 1H, 6-H); 2.35 (dd, $J = 12, 5$ Hz, 1H, 20-H$_{eq}$); 2.12 (d br, $J = 12$ Hz, 1H, 9-H$_{eq}$); 2.08−1.98 (m, 1H, 16-H); 2.05−1.95 (m, 1H, 18-H); 2.00−1.98 (m, 1H, 9-H$_{ax}$); 2.00−1.89 (m, 1H, 24-H); 1.99 (d, $J = 1$ Hz, 3H, 4-Me); 1.98−1.89 (m, 1H, 8-H); 1.91 (d, $J = 1$ Hz, 3H, 10-Me); 1.79−1.69 (m, 1H, 22-H); 1.75 (dd, $J = 12, 11$ Hz, 1H, 20-H$_{ax}$); 1.50 (d, $J = 6$ Hz, 1H, 7-OH); 1.08 (d, $J = 7$ Hz, 3H, 6-Me); 1.07 (d, $J = 7$ Hz, 3H, 24-Me); 1.02 (d, $J = 7$ Hz, 3H, 16-Me); 0.94 (d, $J = 7$ Hz, 3H, 8-Me); 0.93 (d, $J = 7$ Hz, 3H, 24-Me); 0.91 (d, $J = 7$ Hz, 3H, 18-Me); 0.88 (d, $J = 7$ Hz, 3H, 22-Me). FAB-MS (negative): m/z 739 (M − H).

21-*O*-Succinylbafilomycin A₁ (8). To a solution of bafilomycin A₁ (130 mg, 0.21 mmol) in CH₂Cl₂ (5 mL) were added succinic anhydride (50 mg) and DBU (0.1 mL) at 0 °C. After 30 min the reaction mixture was diluted with CH₂Cl₂ and washed with a solution of 3% citric acid, a saturated solution of NaHCO₃, and brine. After evaporating to dryness 150 mg of a solid was obtained. Trituration with diisopropyl ether and filtration gave 140 mg (81%) of the desired compound, mp = 118−120 °C. $[\alpha]_D = -29.9$ ($c = 1.27\%$, MeOH). HPLC: gradient 75−90% of B in 40 min, t_R 10 min. ^{1}H NMR (CDCl₃): 6.67 (s, 1H, 3-H); 6.51 (dd, $J = 15, 10$ Hz, 1H, 12-H); 5.82 (dd, $J = 10, 1$ Hz, 1H, 11-H); 5.78 (dd, $J = 9, 1$ Hz, 1H, 5-H); 5.49 (d, $J = 1$ Hz, 1H, 19-OH); 5.19 (dd, $J = 15, 9$ Hz, 1H, 13-H); 5.01 (ddd, $J = 12, 12, 4$ Hz, 1H, 21-H); 4.98 (d, $J = 9$ Hz, 1H, 15-H); 4.61 (d, $J = 2$ Hz, 1H, 17-OH); 4.14 (dd br, $J = 10, 2$ Hz, 1H, 17-H); 3.90 (dd, $J = 9, 9$ Hz, 1H, 14-H); 3.64 (s, 3H, 2-OMe); 3.61 (dd, $J = 10, 2$ Hz, 1H, 23-H); 3.30 (d br, $J = 6$ Hz, 1H, 7-H); 3.25 (s, 3H, 14-OMe); 2.72−2.60 (m, 4H, 21-OCH₂CH₂); 2.61−2.50 (m, 1H, 6-H); 2.34 (dd, $J = 12, 4$ Hz, 1H, 20-H$_{eq}$); 2.20−2.16 (m, 1H, 16-H); 2.15 (d br, $J = 12$ Hz, 1H, 9-H$_{eq}$); 2.00−1.92 (m, 1H, 9-H$_{ax}$); 2.00 (d, $J = 1$ Hz, 3H, 4-Me); 1.95−1.90 (m, 1H, 8-H); 1.95−1.85 (m, 1H, 24-H); 1.94

(d, $J = 1$ Hz, 3H, 10-Me); 1.76 (dt, $J = 6, 7$ Hz, 1H, 18-H); 1.62−1.50 (m, 1H, 22-H); 1.21 (dd, $J = 12, 12$ Hz, 1H, 20-H$_{ax}$); 1.08 (d, $J = 7$ Hz, 3H, 6-Me); 1.03 (d, $J = 7$ Hz, 3H, 18-Me); 0.95 (d, $J = 7$ Hz, 3H, 8-Me); 0.92 (d, $J = 7$ Hz, 3H, 24-Me); 0.83 (d, $J = 7$ Hz, 3H, 16-Me); 0.82 (d, $J = 7$ Hz, 3H, 22-Me); 0.78 (d, $J = 7$ Hz, 3H, 24-Me). FAB-MS (negative): m/z 721 (M − H), 491, 419.

21-Deoxy-21-piperidinobafilomycin A₁ (9). To a solution of bafilomycin A₁ (273 mg, 0.44 mmol) in pyridine (2 mL) was added 4-nitrobenzenesulfonyl chloride (243.5 mg, 1.1 mmol). The reaction mixture was stirred overnight, then diluted with EtOAc, and washed with citric acid 3%, a saturated solution of NaHCO₃, and brine. The organic layer was dried with Na₂SO₄ and evaporated under vacuum but not to complete dryness. The residue was dissolved in EtOAc (6 mL), and piperidine (1.5 mL) was added dropwise at 0 °C. The reaction mixture was left at room temperature overnight and then diluted with EtOAc, and the organic layer was washed with citric acid 3%, a saturated solution of NaHCO₃, and brine. After drying and evaporating to dryness the residue was chromatographed on silica gel eluting with *n*-hexane/EtOAc (3:2) obtaining 83 mg (80%) of the desired compound, mp = 114−118 °C. $[\alpha]_D = -23.5$ ($c = 0.25\%$, THF). HPLC: gradient 40−80% of B in 30 min, t_R 23 min. ^{1}H NMR (CDCl₃): 6.65 (s, 1H, 3-H); 6.50 (dd, $J = 15, 10$ Hz, 1H, 12-H); 5.81 (dd, $J = 10, 1$ Hz, 1H, 11-H); 5.76 (dd, $J = 9, 1$ Hz, 1H, 5-H); 5.36 (d, $J = 2$ Hz, 1H, 19-OH); 5.17 (dd, $J = 15, 9$ Hz, 1H, 13-H); 4.97 (dd, $J = 9, 1$ Hz, 1H, 15-H); 4.54 (d, $J = 4$ Hz, 1H, 17-OH); 4.14 (ddd, $J = 13, 4, 2$ Hz, 1H, 17-H); 3.88 (dd, $J = 9, 9$ Hz, 1H, 14-H); 3.63 (s, 3H, 2-OMe); 3.49 (dd, $J = 10, 2$ Hz, 1H, 23-H); 3.29 (dd, $J = 11, 1$ Hz, 1H, 7-H); 3.24 (s, 3H, 14-OMe); 2.60−2.55 (m, 1H, 21-H); 2.57−2.50 (m, 1H, 6-H); 2.55−2.50 (m, 1H, 21-NC*H*H); 2.35−2.25 (m, 1H, 21-NCH*H*); 2.18−2.10 (m, 1H, 9-H$_{eq}$); 2.18−2.10 (m, 1H, 16-H); 2.08−2.02 (m, 1H, 20-H$_{eq}$); 1.99 (d, $J = 1$ Hz, 3H, 4-Me); 1.98−1.92 (m, 1H, 9-H$_{ax}$); 1.95−1.90 (m, 1H, 8-H); 1.95−1.82 (m, 1H, 24-H); 1.93 (d, $J = 1$ Hz, 3H, 10-Me); 1.77−1.70 (m, 1H, 18-H); 1.55−1.45 (m, 6H, (CH₂)₃); 1.50 (d, $J = 11$ Hz, 1H, 7-OH); 1.45−1.35 (m, 1H, 22-H); 1.10−1.03 (m, 1H, 20-H$_{ax}$); 1.06 (d, $J = 7$ Hz, 3H, 6-Me); 1.03 (d, $J = 7$ Hz, 3H, 18-Me); 0.92 (d, $J = 7$ Hz, 3H, 8-Me); 0.88 (d, $J = 7$ Hz, 3H, 24-Me); 0.86 (d, $J = 7$ Hz, 3H, 22-Me); 0.82 (d, $J = 7$ Hz, 3H, 16-Me); 0.74 (d, $J = 7$ Hz, 3H, 24-Me). FAB-MS (negative): m/z 688 (M − H).

21-Deoxy-21-(hexylamino)bafilomycin A₁ (10). To a solution of bafilomycin A₁ (102 mg, 0.16 mmol) in pyridine (1 mL) was added 4-nitrobenzenesulfonyl chloride (88 mg, 0.397 mmol). Following the procedure described above, the crude 4-nitrobenzenesulfonyl derivative was treated with hexylamine (1 mL), and after purification on silica gel, 10 mg (9%) of the desired compound was obtained, as a white foam. $[\alpha]_D = -25.6$ ($c = 0.35\%$, MeOH). HPLC: gradient 75−90% of B in 40 min, t_R 19.6 min. ^{1}H NMR (CDCl₃): 6.68 (s, 1H, 3-H); 6.51 (dd, $J = 15, 11$ Hz, 1H, 12-H); 5.82 (dd, $J = 11, 1$ Hz, 1H, 11-H); 5.77 (dd, $J = 9, 1$ Hz, 1H, 5-H); 5.40 (d, $J = 2$ Hz, 1H, 19-OH); 5.18 (dd, $J = 15, 9$ Hz, 1H, 13-H); 4.98 (dd, $J = 9, 1$ Hz, 1H, 15-H); 4.59 (d, $J = 4$ Hz, 1H, 17-OH); 4.16 (ddd, $J = 10, 4, 2$ Hz, 1H, 17-H); 3.90 (dd, $J = 9, 9$ Hz, 1H, 14-H); 3.65 (s, 3H, 2-Me); 3.53 (dd, $J = 10, 2$ Hz, 1H, 23-H); 3.33−3.28 (m, 1H, 7-H); 3.25 (s, 3H, 14-Me); 2.75−2.65 (m, 1H, 21-H); 2.60−2.48 (m, 1H, 6-H); 2.60−2.48 (m, 2H, 21-NH*CH₂*CH₂); 2.29 (dd, $J = 12, 4$ Hz, 1H, 20-H$_{eq}$); 2.18−2.10 (m, 1H, 9-H$_{eq}$); 2.18−2.10 (m, 1H, 16-H); 1.99 (d, $J = 1$ Hz, 3H, 4-Me); 1.95−1.88 (m, 1H, 8-H); 1.95−1.88 (m, 1H, 9-H$_{ax}$); 1.95−1.83 (m, 1H, 24-H); 1.93 (d, $J = 1$ Hz, 3H, 10-Me); 1.78−1.70 (m, 1H, 18-H); 1.53−1.45 (m, 2H, 21-NHCH₂*CH₂*); 1.52−1.45 (m, 1H, 22-H); 1.40−1.25 (m, 6H, -(CH₂)₃CH₃); 1.08 (d, $J = 7$ Hz, 3H, 6-Me); 1.05 (d, $J = 7$ Hz, 3H, 18-Me); 1.00−0.95 (m, 1H, 20-H$_{ax}$); 0.94 (d, $J = 7$ Hz, 3H, 8-Me); 0.91 (d, $J = 7$ Hz, 3H, 24-Me); 0.90 (d, $J = 7$ Hz, 3H, 22-Me); 0.89 (t, $J = 6$ Hz, 3H, CH₃); 0.83 (d, $J = 7$ Hz, 3H, 16-Me); 0.74 (d, $J = 7$ Hz, 3H, 24-Me). FAB-MS (negative): m/z 704 (M − H), 419 [(M − C₁₁H₂₄O₃) − H].

21-*O*-Methylbafilomycin A₁ (12). To a solution of bafilomycin A₁ (600 mg, 0.96 mmol) in methanol (95 mL) at room temperature was added 10% aqueous oxalic acid (24 mL). The

solution was stirred at room temperature for 3 h, concentrated under vacuum, and diluted with EtOAc. The organic layer was washed with a saturated solution of NaHCO$_3$ and brine and dried over MgSO$_4$. Evaporation of the solvent afforded 576 mg (93%) of the title compound **12**, mp = 108–110 °C. TLC (A): R_f = 0.7. [α]$_D$ = −2.5 (c = 0.23%, THF). HPLC: gradient 75–90% of B in 40 min, t_R 22 min. ^{1}H NMR (CDCl$_3$): 6.67 (s, 1H, 3-H); 6.51 (dd, J = 15, 10 Hz, 1H, 12-H); 5.81 (dd, J = 10, 1 Hz, 1H, 11-H); 5.78 (dd, J = 9, 1 Hz, 1H, 5-H); 5.50 (d, J = 2 Hz, 1H, 19-OH); 5.17 (dd, J = 15, 9 Hz, 1H, 13-H); 4.96 (d, J = 9 Hz, 1H, 15-H); 4.63 (d, J = 4 Hz, 1H, 17-OH); 4.15 (ddd, J = 10, 4, 2 Hz, 1H, 17-H); 3.89 (dd, J = 9, 9 Hz, 1H, 14-H); 3.64 (s, 3H, 2-OMe); 3.49 (dd, J = 10, 2 Hz, 1H, 23-H); 3.38 (s, 3H, 21-OMe); 3.29 (m, 1H, 7-H); 3.25 (s, 3H, 14-OMe); 3.22 (ddd, J = 12, 12, 4 Hz, 1H, 21-H); 2.59–2.49 (m, 1H, 6-H); 2.44 (dd, J = 12, 4 Hz, 1H, 20-H$_{eq}$); 2.20–2.10 (m, 1H, 9-H$_{eq}$); 2.20–2.10 (m, 1H, 16-H); 1.99 (d, J = 1 Hz, 3H, 4-Me); 1.93 (d, J = 1 Hz, 3H, 10-Me); 1.92–1.82 (m, 1H, 24-H); 1.91 (m, 1H, 9-H$_{ax}$); 1.90 (m, 1H, 8-H); 1.81–1.72 (m, 1H, 18-H); 1.65 (d, J = 6 Hz, 1H, 7-OH); 1.45–1.32 (m, 1H, 22-H); 1.09 (d, J = 7 Hz, 3H, 6-Me); 1.08 (d, J = 7 Hz, 3H, 18-Me); 1.00 (ddd, J = 12, 12, 2 Hz, 1H, 20-H$_{ax}$); 0.94 (d, J = 7 Hz, 3H, 22-Me); 0.91 (d, J = 7 Hz, 3H, 8-Me); 0.89 (d, J = 7 Hz, 3H, 24-Me); 0.82 (d, J = 7 Hz, 3H, 16-Me); 0.75 (d, J = 7 Hz, 3H, 24-Me). FAB-MS (negative): m/z 635 (M − H), 621, 419.

21-*O*-Ethylbafilomycin A$_1$ (13). To a solution of bafilomycin A$_1$ (200 mg, 0.32 mmol) in ethanol (32 mL) at room temperature was added 10% aqueous oxalic acid (8 mL). The solution was stirred at room temperature for 8 h, concentrated, and diluted with ethyl acetate. The organic layer was washed with a saturated solution of NaHCO$_3$ and brine and dried over MgSO$_4$. After evaporation of the solvent, the crude product was chromatographed over silica gel (*n*-hexane/ethyl acetate, 4:1) obtaining the title compound **13** (116 mg, 56%), mp = 97–102 °C. TLC (A): R_f = 0.7. [α]$_D$ = +8.72 (c = 0.22%, MeOH). HPLC gradient 75–90% of B in 40 min, t_R 27 min. ^{1}H NMR (CDCl$_3$): 6.67 (s, 1H, 3-H); 6.51 (dd, J = 14, 10 Hz, 1H, 12-H); 5.81 (dd, J = 10, 1 Hz, 1H, 11-H); 5.78 (dd, J = 10, 1 Hz, 1H, 5-H); 5.46 (d, J = 2 Hz, 1H, 19-OH); 5.16 (dd, J = 14, 9 Hz, 1H, 13-H); 4.96 (dd, J = 9, 1 Hz, 1H, 15-H); 4.61 (d, J = 4 Hz, 1H, 17-OH); 4.14 (ddd, J = 10, 4, 2 Hz, 1H, 17-H); 3.89 (dd, J = 9, 9 Hz, 1H, 14-H); 3.68 (dq, J = 10, 7 Hz, 1H, 21-OCHH); 3.64 (s, 3H, 2-OMe); 3.49 (dd, J = 10, 2 Hz, 1H, 23-H); 3.41 (dq, J = 10, 7 Hz, 1H, 21-OCHH); 3.36–3.25 (m, 1H, 21-H); 3.32–3.25 (m, 1H, 7-H); 3.23 (s, 3H, 14-OMe); 2.54–2.49 (m, 1H, 6-H); 2.40 (dd, J = 10, 4 Hz, 1H, 20-H$_{eq}$); 2.20–2.09 (m, 1H, 16-H); 2.152.08 (m, 1H, 9-H$_{eq}$); 1.99 (d, J = 1 Hz, 3H, 4-Me); 1.95–1.85 (m, 1H, 8-H); 1.95–1.85 (m, 1H, 9-H$_{ax}$); 1.95–1.82 (m, 1H, 24-H); 1.93 (d, J = 1 Hz, 3H, 10-Me); 1.80–1.72 (m, 1H, 18-H); 1.50 (d, J = 7 Hz, 1H, 7-OH); 1.47–1.33 (m, 1H, 22-H); 1.19 (t, J = 7 Hz, 3H, 21-OCH$_2$CH_3); 1.07 (d, J = 7 Hz, 3H, 6-Me); 1.05 (m, 1H, 20-H$_{ax}$); 1.05 (d, J = 7 Hz, 3H, 18-Me); 0.93 (d, J = 7 Hz, 3H, 8-Me); 0.91 (d, J = 7 Hz, 3H, 22-Me); 0.89 (d, J = 7 Hz, 3H, 24-Me); 0.83 (d, J = 7 Hz, 3H, 16-Me); 0.75 (d, J = 7 Hz, 3H, 24-Me). FAB-MS (negative): m/z 649 (M − H).

19-*O*-Methyl-21-*O*-propylbafilomycin A$_1$ (14). To a solution of 19-*O*-methylbafilomycin A$_1$ (bafilomycin A$_2$) (11) (177 mg, 0.28 mmol) in propanol (32 mL) at room temperature was added 10% aqueous oxalic acid (8 mL). The solution was stirred at room temperature for 8 h, concentrated under vacuum, and diluted with ethyl acetate. The organic layer was washed with a saturated solution of NaHCO$_3$ and brine and dried over MgSO$_4$. After evaporation of the solvent, the crude product was purified by preparative HPLC affording 66 mg (35%) of the title compound **14**, mp = 96–98 °C. TLC (B): R_f = 0.47. [α]$_D$ = +7.92 (c = 0.5%, MeOH). HPLC: gradient 75–90% of B in 40 min, t_R 32 min. ^{1}H NMR (CDCl$_3$): 6.65 (s, 1H, 3-H); 6.49 (dd, J = 15, 10 Hz, 1H, 12-H); 5.81 (dd, J = 10, 1 Hz, 1H, 11-H); 5.76 (dd, J = 9, 1 Hz, 1H, 5-H); 5.20 (dd, J = 15, 9 Hz, 1H, 13-H); 5.11 (dd, J = 9, 1 Hz, 1H, 15-H); 3.86 (dd, J = 9, 9 Hz, 1H, 14-H); 3.70 (d, J = 4 Hz, 1H, 17-OH); 3.70 (s, 3H, 2-OMe); 3.59 (dt, J = 9, 5 Hz, 1H, 21-OCHH); 3.49 (ddd,

J = 10, 4, 1 Hz, 1H, 17-H); 3.32–3.26 (m, 1H, 7-H); 3.28–3.11 (m, 1H, 21-OCHH); 3.23 (s, 3H, 14-OMe); 3.19 (ddd, J = 10, 10, 3 Hz, 1H, 21-H); 3.06 (dd, J = 9, 2 Hz, 1H, 23-H); 3.02 (s, 3H, 19-OMe); 2.60–2.49 (m, 1H, 6-H); 2.35 (dd, J = 13, 3 Hz, 1H, 20-H$_{eq}$); 2.14 (d br, J = 12 Hz, 1H, 9-H$_{eq}$); 2.07–1.99 (m, 1H, 16-H); 2.07–1.99 (m, 1H, 18-H); 2.05–1.98 (m, 1H, 9-H$_{ax}$); 1.99 (d, J = 1 Hz, 3H, 4-Me); 1.98–1.87 (m, 1H, 8-H); 1.95–1.83 (m, 1H, 24-H); 1.91(d, J = 1 Hz, 3H, 10-Me); 1.61–1.52 (m, 2H, 21-OCH$_2$CH_2); 1.52–1.39 (m, 1H, 22-H); 1.44 (dd, J = 13, 10 Hz, 1H, 20-H$_{ax}$); 1.07 (d, J = 7 Hz, 3H, 6-Me); 1.02 (d, J = 7 Hz, 3H, 24-Me); 0.98 (d, J = 7 Hz, 3H, 8-Me); 0.98 (d, J = 7 Hz, 3H, 16-Me); 0.93 (t, J = 7 Hz, 3H, 21-OCH$_2$CH$_2$CH_3); 0.92 (d, J = 7 Hz, 3H, 18-Me); 0.91 (d, J = 7 Hz, 3H, 22-Me); 0.86 (d, J = 7 Hz, 3H, 24-Me). FAB-MS (negative): m/z 677 (M − H), 634 [(M − C$_3$H$_7$) − H], 419 [(M − C$_{11}$H$_{24}$O$_3$) − H].

7-Acetyl-21-*O*-methylbafilomycin A$_1$ (15). To a solution of 21-*O*-methylbafilomycin A$_1$ (11) (100 mg, 016 mmol) in dichloromethane (25 mL) were added triethylamine (0.5 mL, 3.6 mmol), acetic anhydride (0.5 mL, 3.6 mmol), and (dimethylamino)pyridine (catalytic amount) under nitrogen, at room temperature. The mixture was stirred at 40 °C for 16 h. After this time, an additional amount of acetic anhydride (1 mL, 10.6 mmol) was added and the solution was heated for an additional 8 h. After cooling, the solvent was evaporated, and the residue was diluted with EtOAc and washed with 10% citric acid, a saturated solution of NaHCO$_3$, and brine. The organic layer was dried over MgSO$_4$ and evaporated. The residue was chromatographed over silica gel (*n*-hexane/acetate, 4:1) to afford 49 mg (45%) of the compound as a white foam. HPLC: gradient 75–90% of B in 40 min, t_R 30 min. ^{1}H NMR (CDCl$_3$): 6.64 (s, 1H, 3-H); 6.50 (dd, J = 15, 11 Hz, 1H, 12-H); 5.84 (dd, J = 11, 1 Hz, 1H, 11-H); 5.77 (dd, J = 7, 1 Hz, 1H, 5-H); 5.46 (d, J = 3 Hz, 1H, 19-OH); 5.20 (dd, J = 15, 9 Hz, 1H, 13-H); 4.98 (dd, J = 9, 1 Hz, 1H, 15-H); 4.75 (dd, J = 4, 3 Hz, 1H, 7-H); 4.63 (d, J = 4 Hz, 1H, 17-OH); 4.16 (m, 1H, 17-H); 3.90 (dd, J = 9, 9 Hz, 1H, 14-H); 3.63 (s, 3H, 2-OMe); 3.50 (dd, J = 8, 2 Hz, 1H, 23-H); 3.37 (s, 3H, 21-OMe); 3.27–3.17 (m, 1H, 21-H); 3.25 (s, 3H, 14-OMe); 2.68–2.58 (m, 1H, 6-H); 2.45 (dd, J = 11, 4 Hz, 1H, 20-H$_{eq}$); 2.18 (m, 1H, 9-H$_{eq}$); 2.15 (m, 1H, 16-H); 2.15 (s, 3H, 7-OCOMe); 2.00 (m, 1H, 8-H); 1.99 (d, J = 1 Hz, 3H, 4-Me); 1.90 (m, 1H, 24-H); 1.87 (d, J = 1 Hz, 3H, 10-Me); 1.76 (m, 1H, 18-H); 1.70 (dd, J = 15, 11 Hz, 1H, 9-H$_{ax}$); 1.45–1.31 (m, 1H, 22-H); 1.10 (d, J = 7 Hz, 3H, 18-Me); 1.07 (d, J = 7 Hz, 3H, 8-Me); 1.00 (m, 1H, 20-H$_{ax}$); 0.95 (d, J = 7 Hz, 3H, 6-Me); 0.95 (d, J = 7 Hz, 3H, 22-Me); 0.94 (d, J = 7 Hz, 3H, 24-Me); 0.89 (d, J = 7 Hz, 3H, 16-Me); 0.78 (d, J = 7 Hz, 3H, 24-Me). FAB-MS (negative): m/z 677 (M − H), 461 (C$_{26}$H$_{37}$O$_7$).

7,21-Di-*O*-acetylbafilomycin A$_1$ (16). Acetic anhydride (1.5 mL, 15.9 mmol) was added to a solution of 21-*O*-acetylbafilomycin A$_1$^{27} (325 mg, 0.49 mmol) and triethylamine (1.5 mL, 10.7 mmol) in dichloromethane (50 mL) at room temperature. The solution was refluxed for 12 h. After cooling, the mixture was washed with 10% citric acid, a saturated solution of NaHCO$_3$, and brine and dried over MgSO$_4$. After evaporation of the solvent, the crude product was chromatographed over silica gel (*n*-hexane/acetate, 4:1) to afford 131 mg (38%) of a crude compound that was purified by preparative HPLC obtaining 78 mg (22%) of the title compound, mp = 82–94 °C. HPLC: gradient 80–90% of B in 30 min, t_R 11 min. ^{1}H NMR (CDCl$_3$): 6.65 (s, 1H, 3-H); 6.50 (dd, J = 15, 10 Hz, 1H, 12-H); 5.84 (dd, J = 10, 1 Hz, 1H, 11-H); 5.77 (dd, J = 9, 1 Hz, 1H, 5-H); 5.45 (d, J = 3 Hz, 1H, 19-OH); 5.20 (dd, J = 15, 9 Hz, 1H, 13-H); 4.97 (ddd, J = 11, 11, 4 Hz, 1H, 21-H); 4.96 (d, J = 9 Hz, 1H, 15-H); 4.75 (dd, J = 7, 3 Hz, 1H, 7-H); 4.61 (dd, J = 4, 1 Hz, 1H, 17-OH); 4.14 (ddd, J = 10, 4, 1 Hz, 1H, 17-H); 3.90 (dd, J = 9, 9 Hz, 1H, 14-H); 3.63 (s, 3H, 2-OMe); 3.60 (dd, J = 10, 1 Hz, 1H, 23-H); 3.25 (s, 3H, 14-OMe); 2.69–2.59 (m, 1H, 6-H); 2.34 (dd, J = 11, 4 Hz, 1H, 20-H$_{eq}$); 2.16 (s, 3H, 7-OCOMe); 2.15 (m, 1H, 16-H); 2.11 (m, 1H, 9-H$_{eq}$); 2.04 (s, 3H, 21-OCOMe); 1.98 (m, 1H, 8-H); 1.98 (d, J = 1 Hz, 3H, 4-Me); 1.88 (m, 1H, 24-H); 1.88 (d, J = 1 Hz, 3H, 10-Me); 1.78 (m, 1H, 18-H); 1.70 (dd, J = 15, 11 Hz, 1H, 9-H$_{ax}$); 1.55 (m, 1H, 22-H); 1.18 (ddd, J = 11,

11, 2 Hz, 1H, 20-H$_{ax}$); 1.04 (d, J = 7 Hz, 3H, 8-Me); 1.03 (d, J = 7 Hz, 3H, 18-Me); 0.92 (d, J = 7 Hz, 3H, 6-Me); 0.91 (d, J = 7 Hz, 3H, 24-Me); 0.85 (d, J = 7 Hz, 3H, 16-Me); 0.82 (d, J = 7 Hz, 3H, 22-Me); 0.77 (d, J = 7 Hz, 3H, 24-Me). FAB-MS (negative): DAU 705 (M − H), DAU ∼ 705, 645, 461.

[6R-(2Z,4E,6R*,7S*,8S*,10E,12E,14S*,15R*,17R*,18S*,-21E)]-7,17-Dihydroxy-2,14-dimethoxy-4,6,8,10,16,18-hexamethyl-19-oxo-2,4,10,12,21-tricosapentenoic Acid 1,15-Lactone (22). To a solution of bafilomycin A$_1$ (1 g, 1.6 mmol) in dichloromethane (30 mL) at −70 °C, under nitrogen was added diethylaminosulfur trifluoride (0.43 mL, 3.2 mmol) dropwise. The reaction mixture was stirred at −70 °C for 2 h, warmed to −50 °C, and poured in a saturated solution of Na$_2$CO$_3$ (60 mL). The layers were separated, and the aqueous phase was extracted with CH$_2$Cl$_2$ (3 × 15 mL). The combined organic layers were washed with brine, dried over MgSO$_4$, filtered, concentrated, and chromatographed (*n*-hexane/acetate, 8:2) to yield 620 mg (73%) of the desired compound **22**. [α]$_D$ = −19.95 (c = 0.36%, THF). HPLC: gradient 75−90% of B in 40 min, t_R 11 min. ¹H NMR (CDCl$_3$): 6.65 (s, 1H, H-3); 6.5 (dd, J = 14, 11 Hz, 1H, H-12); 5.82 (dd, J = 11, 1 Hz, 1H, H-11); 5.77 (dd, J = 8, 1 Hz, 1H, H-5); 5.63−5.45 (m, 2H, H-21, H-22); 5.18 (dd, J = 14, 8 Hz, 1H, H-13); 5.04 (dd, J = 8, 2 Hz, 1H, H-15); 3.84 (dd, J = 8, 8 Hz, 1H, H-14); 3.74 (ddd, J = 9, 3, 1 Hz, 1H, H-17); 3.72 (d, J = 1 Hz, 1H, 17-OH); 3.69 (s, 3H, 2-OMe); 3.32 (m, 1H, H-7); 3.30−3.19 (m, 2H, H-20); 3.24 (s, 3H, 14-OMe); 2.73 (dq, J = 3, 7 Hz, 1H, H-18); 2.55 (ddq, J = 8, 2, 7 Hz, 1H, H-6); 2.14 (dd, J = 14, 1 Hz, 1H, H-9$_{ax}$); 2.05 (dd, J = 14, 8, 1H, H-9$_{eq}$); 2.00−1.86 (m, 2H, H-8, H-16); 1.99 (d, J = 1 Hz, 3H, 10-Me); 1.92 (d, J = 1 Hz, 3H, 4-Me); 1.70 (d, J = 4 Hz, 3H, 22-Me); 1.18 (d, J = 7 Hz, 3H, 18-Me); 1.08 (d, J = 7 Hz, 3H, 6-Me); 0.95 (d, J = 7 Hz, 3H, 8-Me); 0.91 (d, J = 7 Hz, 3H, 16-Me). ESI-MS (solvent methanol/spray 4.5 kV/skimmer 60 V/capillary 220 °C): m/z 555 (MNa$^+$).

[6R-[2Z,4E,6R*,7S*,8S*,10E,12E,14S*,15R*,17R*,18S*,-19(R,S), 21E]]-2,14-Dimethoxy-4,6,8,10,16,18-hexamethyl-7,17,19-trihydroxy-2,4,10,12,21-tricosapentenoic Acid 1,-15-Lactone (23). To a solution of 7,17-dihydroxy-2,14-dimethoxy-4,6,8,10,16,18-hexamethyl-19-oxo-2,4,10,12,21-tricosapentenoic acid 1,15-lactone (**22**) (473 mg, 0.89 mmol) in MeOH (35 mL) at room temperature under nitrogen was added NaBH$_4$ (40 mg, 1.05 mmol). The mixture was stirred at room temperature for 3 h. After this time the solvent was evaporated under vacuum, and the residue was diluted with ethyl acetate, washed with a saturated solution of NaHCO$_3$ and brine, and dried with MgSO$_4$. After evaporation, the crude product was chromatographed over silica gel (*n*-hexane/acetate, 4:1) to yield 225 mg (47%) of compound **23** as a diastereomeric mixture. TLC (A): R_f = 0.6. HPLC: gradient 75−90% of B in 40 min, t_R 9.2 min (minor component) and 10.2 min (major component). ¹H NMR (CDCl$_3$): 6.66 (d, J = 1 Hz, 1H, 3-H); 6.50 (dd, J = 15, 10 Hz, 1H, 12-H); 5.81 (dd, J = 10, 1 Hz, 1H, 11-H); 5.78 (dd, J = 9, 1 Hz, 1H, 5-H); 5.56 (dq, J = 15, 5 Hz, 1H, 22-H); 5.18 (dd, J = 15, 9 Hz, 1H, 13-H); 4.55 (m, 1H, 21-H); 4.49 (d, J = 9 Hz, 1H, 15-H); 3.89 (dd, J = 9, 9 Hz, 1H, 14-H); 3.86−3.79 (m, 1H, 19-H); 3.79 (s, 3H, 2-OMe); 3.46−3.38 (m, 1H, 17-H); 3.32−3.25 (m, 1H, 7-H); 3.24 (s, 3H, 14-OMe); 2.59−2.48 (m, 1H, 6-H); 2.32−2.22 (m, 1H, 20-H$_{eq}$); 2.15−2.05 (m, 1H, 9-H$_{eq}$); 2.15−2.05 (m, 1H, 16-H); 2.15−2.00 (m, 1H, 20-H$_{ax}$); 2.00−1.95 (m, 1H, 9-H$_{ax}$); 1.99 (d, J = 1 Hz, 3H, 4-Me); 1.95−1.85 (m, 1H, 8-H); 1.92 (d, J = 1 Hz, 3H, 10-Me); 1.75−1.65 (m, 1H, 18-H); 1.67 (dd, J = 5, 1 Hz, 3H, 22-Me); 1.51 (m, 1H, 7-OH); 1.08 (d, J = 7 Hz, 3H, 6-Me); 0.95 (d, J = 7 Hz, 3H, 18-Me); 0.94 (d, J = 7 Hz, 3H, 8-Me); 0.85 (d, J = 7 Hz, 3H, 16-Me). FAB-MS (negative): m/z 533 (M − H), 638 [(M·DEA) − H].

[6R-(2Z,4E,6R*,7S*,8S*,10E,12E,14S*,15R*,17R*,18S*)]-7,15,17-Trihydroxy-2,14-dimethoxy-4,6,8,10,16,18-hexamethyl-2,4,10,12-nonadecatetrene-1,19-dioic Acid 1,15-Lactone (24). To a solution of 21-deoxy-21-oxobafilomycin A$_1$[27] (100 mg, 0.16 mmol) in methanol (30 mL) were added ammonium acetate (950 mg, 12.3 mmol) and sodium cyanoborohydride (950 mg, 15.2 mmol) at room temperature. The mixture was stirred for 96 h at room temperature. After this

time the mixture was concentrated under vacuum, diluted with EtOAc, and washed with a saturated solution of NaHCO$_3$ and brine. The organic layer was dried over MgSO$_4$ and evaporated to give the crude product. After purification by preparative HPLC (gradient 70−75% of B in 40 min, t_R 4.5 min) the compound was obtained as a white lyophile (23 mg, 29%). ¹H NMR (CDCl$_3$): 6.66 (s, 1H, H-3); 6.51 (dd, J = 15, 11 Hz, 1H, H-12); 5.81 (dd, J = 11, 1 Hz, 1H, H-11); 5.78 (dd, J = 9, 1 Hz, 1H, H-5); 5.15 (dd, J = 15, 9 Hz, 1H, H-13); 5.02 (d, J = 9 Hz, 1H, H-15); 3.87 (dd, J = 9, 9 Hz, 1H, H-14); 3.80−3.65 (m, 2H, H-17, 17-OH); 3.67 (s, 3H, 2-OMe); 3.30 (m, 1H, H-7); 3.25 (s, 3H, 14-OMe); 2.73−2.62 (m, 1H, H-18); 2.60−2.50 (m, 1H, H-6); 2.11 (dd, J = 13, 13 Hz, 1H, H-9$_{ax}$); 2.15−2.08 (m, 1H, H-16); 2.04 (d br, J = 13, 1H, H-9$_{eq}$); 1.98 (d, J = 1 Hz, 3H, 4-Me); 1.94−1.90 (m, 1H, H-8); 1.93 (d, J = 1 Hz, 3H, 10-Me); 1.21 (d, J = 7 Hz, 3H, 18-Me); 1.07 (d, J = 7 Hz, 3H, 6-Me); 0.93 (d, J = 7 Hz, 3H, 8-Me); 0.88 (d, J = 7 Hz, 3H, 16-Me). FAB-MS (negative): m/z 493 (M − H).

[6R-(2Z,4E,6R*,8S*,10E,12E,14S*,15R*,17R*,18S*)]-15,-17-Dihydroxy-2,14-dimethoxy-4,6,8,10,16,18-hexamethyl-7-oxo-2,4,10,12-nonadecatetrene-1,19-dioic Acid 1,15-Lactone (25). To a solution of 7,21-dideoxy-7,21-dioxobafilomycin A$_1$[27] (200 mg, 0.32 mmol) in methanol (30 mL) at room temperature were added ammonium acetate (493 mg, 6.4 mmol) and sodium cyanoborohydride (403 mg, 6.4 mmol). The mixture was stirred for 8 h at room temperature; after this time additional amounts of ammonium acetate (1 g, 12.9 mmol) and sodium cyanoborohydride (1 g, 15.9 mmol) were added. The mixture was stirred for an additional 24 h. The mixture was concentrated under vacuum, diluted with ethyl acetate, and washed with a saturated solution of NaHCO$_3$ and brine. The organic layer was dried over MgSO$_4$ and evaporated to give the crude product. After purification by preparative HPLC, the title compound was obtained as a white lyophile (14 mg, 9%). [α]$_D$ = −374 (c = 0.2%, MeOH). HPLC: 65% of B, t_R 5 min. ¹H NMR (CDCl$_3$): 6.47 (dd, J = 15, 11 Hz, 1H, H-12); 6.44 (s, 1H, H-3); 5.83 (dd, J = 11, 1 Hz, 1H, H-11); 5.23 (dd, J = 15, 9 Hz, 1H, H-13); 5.22 (dd, J = 10, 1 Hz, 1H, H-5); 5.04 (d, J = 9 Hz, 1H, H-15); 3.84 (dd, J = 9, 9 Hz, 1H, H-14); 3.80−3.55 (m, 2H, H-17, 17-OH); 3.69 (s, 3H, 2-OMe); 3.41 (dq, J = 10, 7 Hz, 1H, H-6); 3.24 (s, 3H, 14-OMe); 2.85−2.73 (m, 1H, H-8); 2.73−2.62 (m, 1H, H-18); 2.30 (dd, J = 12, 12 Hz, 1H, H-9$_{ax}$); 2.20−2.08 (m, 1H, H-16); 2.13 (dd, J = 12, 3 Hz, 1H, H-9$_{eq}$); 2.08 (d, J = 1 Hz, 3H, 4-Me); 1.73 (d, J = 1 Hz, 3H, 10-Me); 1.21 (d, J = 7 Hz, 3H, 18-Me); 1.09 (d, J = 7 Hz, 3H, 6-Me); 1.02 (d, J = 7 Hz, 3H, 8-Me); 0.88 (d, J = 7 Hz, 3H, 16-Me). FAB-MS (negative): m/z 491 (M − H).

[6R-(2Z,4E,6R*,8S*,10E,12E,14S*,15R*,17R*,18S*)]-15,-17-Dihydroxy-2,14-dimethoxy-4,6,8,10,16,18-hexamethyl-7-oxo-2,4,10,12-nonadecatetrene-1,19-dioic Acid 1,15-Lactone 19-Methyl Ester (26). To a solution of 15,17-dihydroxy-2,14-dimethoxy-4,6,8,10,16,18-hexamethyl-7-oxo-2,4,10,12-nonadecatetrene-1,19-dioic acid 1,15-lactone (**25**) (102 mg, 0.24 mmol) in methanol (15 mL) at 0 °C was added an etheral solution of diazomethane (∼3 mmol). The solution was stirred for 1 h at 0 °C. The evaporation of the solvent afforded 104 mg of the crude compound that was purified by preparative HPLC: gradient 75−90% of B in 40 min, t_R 7.5 min, obtaining 15 mg of the title compound as a white lyophile (12%). ¹H NMR (CDCl$_3$): 6.46 (dd, J = 1, 4, 11 Hz, 1H, 12-H); 6.44 (s, 1H, 3-H); 5.83 (dd, J = 1, 1, 1 Hz, 1H, 11-H); 5.23 (dd, J = 14, 9 Hz, 1H, 13-H); 5.21 (dd, J = 11, 1 Hz, 1H, 5-H); 5.17 (dd, J = 9, 1 Hz, 1H, 15-H); 3.81 (dd, J = 9, 9 Hz, 1H, 14-H); 3.80−3.70 (m, 1H, 17-H); 3.70 (s, 3H, 2-OMe); 3.70 (s, 3H, 18-COOMe); 3.41 (dq, J = 11, 7 Hz, 1H, 6-H); 3.24 (s, 3H, 14-OMe); 2.85−2.75 (m, 1H, 8-H); 2.68 (dq, J = 3, 7 Hz, 1H, 18-H); 2.30 (dd, J = 12, 12 Hz, 1H, 9-H$_{eq}$); 2.20−2.12 (m, 1H, 9-H$_{ax}$); 2.20−2.12 (m, 1H, 16-H); 2.09 (d, J = 1 Hz, 3H, 4-Me); 1.72 (d, J = 1 Hz, 3H, 10-Me); 1.21 (d, J = 7 Hz, 3H, 18-Me); 1.09 (d, J = 7 Hz, 3H, 6-Me); 1.02 (d, J = 7 Hz, 3H, 8-Me); 0.91 (d, J = 7 Hz, 3-H 16-Me). FAB-MS (negative): m/z 505 (M − H), 475 [(M − CH$_2$OH) − H]. FAB-MS (positive): m/z 507 (M + H)$^+$, 513 (M + Li)$^+$, 529 (M + Na)$^+$, 545 (M + K)$^+$.

21-Deoxy-20,21-*O*-dihydro-19,21-dihydroxybafilomycin D (27). To a solution of bafilomycin A₁ (300 mg, 0.40 mmol) in ethanol (30 mL), under nitrogen was added NaBH₄ (150 mg, 3.9 mmol) at room temperature. The mixture was stirred at room temperature for 24 h. After this time the mixture was concentrated under vacuum, diluted with ethyl acetate, and washed with a solution of 3% citric acid, a saturated solution of NaHCO₃, and brine. The organic layer was dried over MgSO₄, concentrated, and chromatographed over silica gel eluting with *n*-hexane/EtOAc (4:1) to afford 106 mg (42%) of the desired compound as a diastereomeric mixture, mp = 98−102 °C. HPLC: gradient 75−90% of B in 40 min, t_R 10.3 min (major component) and 12 min (minor component). ¹H NMR (CDCl₃): 6.66 (s, 1H, 3-H); 6.51 (dd, $J = 15, 11$ Hz, 1H, 12-H); 5.82 (dd, $J = 11, 1$ Hz, 1H, 11-H); 5.79 (dd, $J = 10$, 1 Hz, 1H, 5-H); 5.17 (dd, $J = 15, 9$ Hz, 1H, 13-H); 4.96 (dd, $J = 9, 2$ Hz, 1H, 15-H); 4.73 (d, $J = 3$ Hz, 1H, 17-OH); 4.60 (d, $J = 1$ Hz, 1H, 19-OH); 4.57 (s, 1H, 23-OH); 4.17 (d br, $J = 10$ Hz, 1H, 23-H); 4.11 (d br, $J = 10$ Hz, 1H, 19-H); 3.93 (d, $J = 4$ Hz, 1H, 21-OH); 3.89 (dd, $J = 9, 9$ Hz, 1H, 14-H); 3.70 (s, 3H, 2-OMe); 3.48 (ddd, $J = 10, 3, 2$ Hz, 1H, 17-H); 3.38−3.31 (m, 1H, 21-H); 3.35−3.27 (m, 1H, 7-H); 3.25 (s, 3H, 14-OMe); 2.59−2.49 (m, 1H, 6-H); 2.28−2.22 (m, 1H, 9-H$_{eq}$); 2.28−2.19 (m, 1H, 16-H); 2.05−1.95 (m, 1H, 9-H$_{ax}$); 2.00−1.90 (m, 1H, 8-H); 2.00−1.90 (m, 1H, 20-H$_{eq}$); 2.00 (d, $J = 1$ Hz, 3H, 4-Me); 1.92 (d, $J = 1$ Hz, 3H, 10-Me); 1.82−1.72 (m, 1H, 22-H); 1.82−1.72 (m, 1H, 24-H); 1.68−1.61 (m, 1H, 18-H); 1.52 (d, $J = 7$ Hz, 1H, 7-OH); 1.33−1.26 (m, 1H, 20-H$_{ax}$); 1.07 (d, $J = 7$ Hz, 3H, 6-Me); 1.00 (d, $J = 7$ Hz, 3H, 18-Me); 0.94 (d, $J = 7$ Hz, 3H, 8-Me); 0.94 (d, $J = 7$ Hz, 3H, 22-Me); 0.93 (d, $J = 7$ Hz, 3H, 24-Me); 0.89 (d, $J = 7$ Hz, 3H, 24-Me); 0.85 (d, $J = 7$ Hz, 3H, 16-Me). FAB-MS (negative): *m/z* 623 (M − H).

Tetrahydrobafilomycin A₁ (30). To a solution of bafilomycin A₁ (200 mg, 0.32 mmol) in methanol (25 mL) at room temperature and under nitrogen were added 10% Pd on activated carbon (20 mg) and Mg turnings (40.9 mg, 1.68 mmol). The mixture was stirred at reflux temperature for 24 h, cooled, filtered on Celite, and evaporated. The residue was diluted with ethyl acetate (30 mL) and washed with 10% citric acid, a saturated solution of HCO₃, and brine. The crude product was obtained by evaporation of the solvent. Purification by CC eluting with *n*-hexane/EtOAc (3:2) afforded 38 mg (19%) of tetrahydrobafilomycin A₁. TLC (A): $R_f = 0.2$. FAB-MS (negative): *m/z* 625 (M − H).

21-*O*-Benzoyl-19-*O*-methyl-2,3,4,5,10,11,12,13-octahydrobafilomycin A₁ (31). To a solution of 21-*O*-benzoyl-19-*O*-methylbafilomycin A₁ (7) (320 mg, 0.43 mmol) in methanol (100 mL) at room temperature and under nitrogen was added 10% Pd on activated carbon (30 mg). The mixture was hydrogenated in a Parr apparatus at 40 psi at room temperature for 24 h. The catalyst was filtered off and the solvent removed under vacuum to afford 161 mg (50%), mp 63−68 °C. FAB-MS (negative): *m/z* 747 (M − H).

Preparation of Membrane Vesicles. Bone microsomes were prepared from long bones of calcium-starved egg-laying hens, as reported in the literature.[31] Briefly, medullary bone from tibiae and femurs was dissected and homogenized in 10 mM Hepes buffer, pH 7.4, 0.2 M sucrose, 50 mM KCl, 1 mM EGTA, and 2 mM DTT, with a glass Teflon homogenizer. An initial centrifugation at $6500g_{max}$ for 20 min was performed to remove mitochondria and lysosomes. The supernatant was then centrifuged at $100000g_{max}$ for 1 h to pellet the microsomal fraction.

Bovine chromaffin granule membranes were obtained from bovine adrenal medulla as described in the literature.[23] Medulla was separated from cortex, homogenized in 10 mM Hepes buffer, pH 7.5, 0.3 M sucrose, 5 mM EGTA, 1 mM DTT, 1 mM ATP, 2 μg/mL pepstatin A, and 4 μg/mL leupeptin. After a centrifugation at $1000g_{max}$ for 15 min, the supernatant was centrifuged at $10000g_{max}$ for 20 min. The pellet, applied on a two-step sucrose gradient (1.2 and 1.8 M), was centrifuged overnight at $200000g_{max}$. The resultant pellet was resuspended in sucrose-free medium a centrifuged at $3000g_{max}$ for 10 min, and the resultant supernatant was again centrifuged

at $200000g_{max}$ for 60 min to pellet the purified microsomal fraction. All the procedures were performed at 4 °C.

Mg²⁺-ATP-Dependent Proton Transport by Isolated Inside-Out Vesicles. Proton transport was determined using the fluorescent weak base acridine orange. The vesicles (40−100 μg of protein) were suspended in a final volume of 1 mL containing 10 mM Hepes buffer, pH 7.4, 0.2 M sucrose, 50 mM KCl, 1 mM Na₂ATP, 1 mM CDTA, and 5 μM acridine orange. The reaction was started with 5 mM MgSO₄. Bafilomycin derivatives were dissolved in DMSO and added as 100× solutions to give 1% final DMSO. The initial rate of quenching (ex, 490 nm; em, 530 nm) was estimated as the minimum of a continuous first-derivative plot of the quenching trace performed on sets of 25 points (15 s of quenching trace) and is expressed as the rate of quenching/min. Inhibition of proton transport activity is expressed as the percentage versus DMSO-matched controls. IC₅₀ values were derived by analyzing at least three duplicated dose levels.

Data Analysis. IC₅₀'s were obtained from nonlinear regression analyses using the best fit to the four-parameter logistic equation with fixed responses at zero and infinite doses, performed using the GRAFIT software (Erithacus Software Ltd.).

Bafilomycin-Sensitive ATPase Assay. Crude microsomal vesicles were further purified in three-step sucrose gradient (15%, 30%, 45% w/w) and centrifuged at $280000g_{max}$ for 2 h. Enrichment in V-ATPase activity was found at 30−45 interface. This activity was determined by measuring the release of inorganic phosphate from ATP at 37 °C during 30 min of incubation using the colorimetric method described in the literature.[32] The reaction medium contained 0.2 M sucrose, 50 mM KCl, 1 mM ATPNa₂ (buffered at pH 7), and 10 mM Hepes-Tris, pH 8. The reaction was initiated by the addition of MgSO₄ (final concentration of 5 mM). The assay media also contained oligomycin (5 μg/mL) and vanadate (1 mM), to dump F- and P-ATPase activities. The presence of these inhibitors did not interfere with bafilomycin activity.

Bone Resorption Assay by Isolated Rat Osteoclast. This assay uses a very low number of cells freshly prepared from neonatal rat bone. The cells are settled on slices of bone and incubated for 48 h in a standard culture medium. Cellular activity is quantified by measuring the number of pits formed. Neonatal Wistar rats (2−4 days old) were decapitated, and the long bones were removed, freed of adherent soft tissues, and curetted into medium 199 containing 2 mM glutamine and 10% FCS. The resulting cell suspension was settled into microwells containing devitalized bovine cortical bone slices (4 × 4 × 0.2 mm) for 30 min at 37 °C. The bone slices were removed, rinsed briefly in medium 199, and then incubated in D-MEM containing 10% FCS, 50 IU/mL penicillin, and 50 μg/mL streptomycin at 37 °C in an atmosphere of 10% CO₂. The compounds were added after 1 h to give the final concentration of 1, 10, and 100 nM. For each experiment eight bone slices were used per group. After 47 h, the bone slices were fixed in 2% glutaraldehyde and stained for the enzyme tartrate-resistant acid phosphatase (TRAP) using the method described in the literature.[33] The slices were mounted on glass slides, and the number of osteoclasts (Oc) was determined by defining Oc's as large, TRAP-stained (red) multinucleate cells. Cells were removed by 5-min incubation in 5% bleach; the slices were washed, dried, and sputter-coated with gold, and the number of excavations (pits) was counted by light microscopy. The results are expressed as pits number/bone slice.

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