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Convenient Synthesis of a [1-^{14}C]Diazirinylbenzoic Acid as a Photoaffinity Label for Binding Studies of V-ATPase Inhibitors

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Dedicated to Prof. Axel Zeeck

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Diazirine-tagged systems are considered reliable compounds for photoaffinity labeling (PAL) in biochemical studies as they enable investigation and understanding of biological mechanisms through covalent bonding to the target and subsequent detection. ^{14}C-labeled 4-(3-trifluoromethyl-3*H*-diazirin-3-yl)benzoic acid (**11**) was prepared by a lithium-bromide exchange on the bis-silylated 4-bromophenyldiaziridine **19** with subsequent transformations with electrophiles as key steps of the synthesis. Using $^{14}CO_2$, which was generated from rather inexpensive $Ba^{14}CO_3$, the desired diazirinylbenzoic acid **21** was obtained in 78 % yield based on the employed radioactive material. Oxidation under mild conditions then yielded diazirine **11** in a 100 mg scale. This versatile photoaffinity label was selectively attached to the tetrahydropyrane ring of bafilomycin A_1 (**2**) and concanamycin A-derived **3**, which both specifically inhibit the V-ATPases. Inhibition assays were performed and revealed that the inhibitory capacities of the labeled derivatives are suitable for PAL studies on this important group of enzymes to elucidate the as yet unknown binding sites.

Introduction

Photoaffinity labeling (PAL)[1] is a versatile and reliable tool in molecular biology and finds a growing interest as reflected by the ever-increasing number of papers and reviews on this topic.[2] PAL is needed for the investigation of molecular machines; for example, for the determination of the precise binding site of biologically active compounds and the mode of action of certain peptide domains. The experimental procedure comprises the attachment of a photosensitive group to either natural ligands or inhibitors and the subsequent exposure to the biological target to form a ligand-receptor complex. This is followed by photoactivation leading to a highly reactive intermediate, which then forms a covalent, thus irreversible bond between ligand and receptor. The photosensitive molecule has to meet certain requirements including a high stability of the precursor prior to activation together with a very high reactivity of the photolytically released intermediate to allow for fast reactions even with C–H bonds. Additionally, byproducts formed during irradiation must be chemically inert. The label should be small to prevent steric perturbations in the target-binding process, and the activation should occur at wavelengths which do not cause damage to the examined biological system ($\lambda \geq 300$ nm). Different types of photoreactive moieties generally suitable for PAL are azides, diazo compounds, benzophenones, diazonium salts, and diazirines. The 3-trifluoromethyl-3-aryldiazirines, which generate carbenes upon 300 nm irradiation are considered to "meet most of the criteria of an ideal photoreactive group".[2d] Yet, azides have found widespread use since they are readily obtainable and show a high stability in the dark.[2b] For tracing and identification of the tagged target, the photophore must bear an easily detectable group like a radioactive label, a biotin tag, magnetic micro spheres, or a fluorescent tag.[2h] Although the biotinylation technique was especially successful in several studies, all but the radioactive labels suffer from the same potential drawback; the use of sterically demanding tracer groups causes perturbations between the biological target and the labeled reagent, and thus, can inhibit formation of the ligand-receptor complex.[3]

Such radioactive labels have been used for PAL studies on the aglycon **3** (Figure 1) derived from the natural product concanamycin A (**1**), which together with bafilomycin A_1 (**2**) is one of the most potent inhibitors of vacuolar

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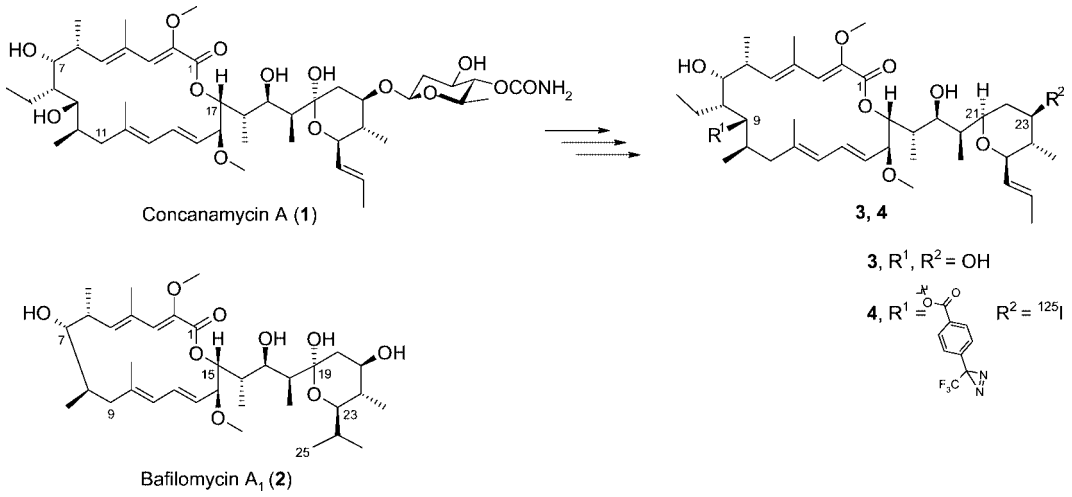


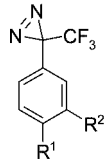
Figure 1. Structures of concanamycin A (**1**), bafilomycin A_1 (**2**), and concanolides **3** and **4**.

ATPase (V-ATPase).[4] This proton-translocating enzyme is a component of almost all eukaryotic cells and is essential in many processes including bone degradation, acid secretion, receptor-mediated endocytosis, and cell proliferation.[5] The malfunction of V-ATPases is considered to cause several severe diseases such as osteopetrosis, deafness, and male infertility. Furthermore, due to its function in bone metabolism and in invading tumors, the enzyme is a promising pharmacological target for osteoporosis and cancer therapy.[6,7] Therefore, the determination of the binding sites of the inhibitors is an important aim. In 2001 Zeeck et al. reported on the preparation of the concanolide **4**, bearing both a diazirine group and ^{125}I as the tracer,[8] and PAL studies using this compound[9] as well as genetic approaches by Bowman and Bowman employing **1** and **2**[10] pointed to the c-subunit of heteromultimeric V-ATPases as the binding site of both plecomacrolides. Yet, the specific inhibitory potential of derivative **4** on V-ATPase of *Manduca sexta* is three orders of magnitude lower than that of the natural product **1**, thus complicating the elucidation of the precise site of the covalent modification. This decreased potency might stem from the fact that in **4**, hydrophobic substituents are placed at both C-9 of the macrocyclic ring and C-23 of the tetrahydropyrane moiety. In contrast, the 21-deoxyconcanolide **3** itself as well as derivatives modified at either C-9 or C-23 retain the high inhibition potential of concanamycin A.[11a] Studies on the structure-activity relationships of bafilomycin A_1 revealed that modifications at C-21 of the tetrahydropyrane ring but not at the hydroxy moiety at C-7 of this molecule have no decreasing influence on the inhibitory activity.[11b] Therefore, we became interested in the synthesis of a radioactively labeled 3-trifluoromethyl-3-aryldiazirine and its attachment to the tetrahydropyrane ring of inhibitors **2** and **3** for subsequent detailed PAL studies on the V-ATPase.

Results and Discussion

Preparation of the Photoaffinity Label

A few syntheses of 3-trifluoromethyl-3-aryldiazirines containing radioactive isotopes have already been described. They incorporate ^{125}I as the tracer in compounds like **5**[12] or tritium in compounds like **6**–**8**[13] and **9**[14] (Figure 2). Both isotopes—particularly ^{125}I—possess a high specific activity, an attribute desirable for photoaffinity studies, but ^{125}I has a half-life of only 60 d, which limits its practical use. Furthermore, the use of tritium as a marker can lead to problems due to its low traceability as a consequence of the low energy of its β radiation.[15] The synthesis of the ^{14}C-labeled compound **10** has already been described,[14] but as for compounds **5**–**9**, their preparation required the use of highly toxic thallium reagents and were altogether quite tedious. In addition, the purification of **10** was performed by preparative HPLC, and the employed $^{14}CH_3I$ is rather expensive. Since the photoaffinity label for compounds **2** and **3** has to possess a carboxy moiety, we envisaged the synthesis of ^{14}C-labeled 4-(3-trifluoromethyl-3*H*-diazirin-3-yl)benzoic acid (**11**) by carboxylation starting



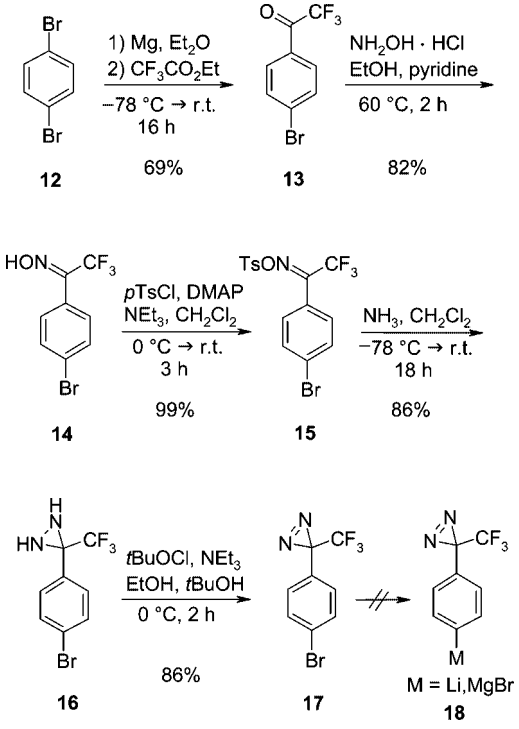
	R^1	R^2
5	OR	^{125}I
6	CH_2NH_2	^{3}H
7	CH_2OH	^{3}H
8	COOH	^{3}H
9	CO_2CH_3	$OC^{3}H_3$
10	CO_2CH_3	$O^{14}CH_3$
11	$^{14}COOH$	H

Figure 2. Selected diazirinyl photoaffinity labels bearing a radioactive tracer.

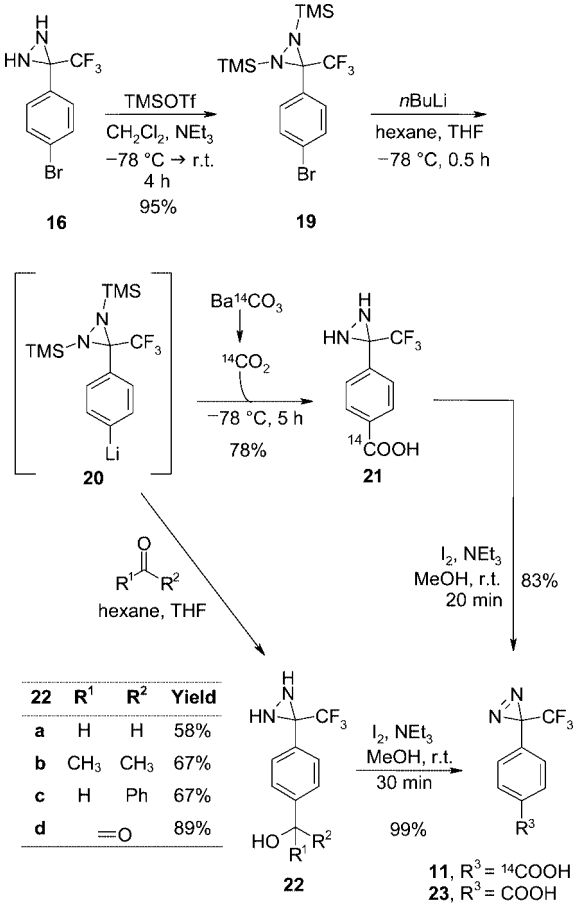
from $Ba^{14}CO_3$ as the cheapest source of ^{14}C.[16] The non-radioactive analogue of **11** has already been used as a reliable tool in several PAL studies.

For this purpose inexpensive 1,4-dibromobenzene (**12**) was chosen as the starting material, which was transformed into the trifluoromethyl ketone **13** by the reaction of the corresponding Grignard reagent with ethyl trifluoroacetate according to a reported procedure (Scheme 1).[17] The obtained ketone **13** was efficiently transformed into diaziridine **16** following protocols described in the literature (through the formation of the oxime **14**, its tosylation to furnish **15**, and finally treatment with liquid ammonia in an autoclave).[18] This led to the isolation of diaziridine **16** in multigram scale with an overall yield of 48%. Diaziridine **16** was successfully dehydrogenated to the corresponding diazirine **17** using *tert*-butyl hypochlorite (*t*BuOCl) as the oxidant,[18c] yet later on, iodine/triethylamine proved to be a more convenient oxidation system and resulted in higher yields.[18d] Although the trifluoroethyldiazirine moiety is stable under a broad range of reaction conditions including strong bases, the attempted metal-halogen exchange of **17** with *n*-, *sec*-, or *tert*-butyllithium as well as with isopropylmagnesium chloride[19] failed. While *n*BuLi preferentially underwent nucleophilic attack on the diazirine ring, complex mixtures were obtained from the other reactions. In addition, no conversion took place when treating aryl bromide **17** with standard magnesium turnings or highly reactive Rieke magnesium prepared by the reduction of $MgCl_2$ with potassium.[20a] This failure of the oxidative addition and thus formation of the respective Grignard reagent might be caused by coordination of the nitrogens of the diazirine moiety to the activated metal surface, as previously observed in the case of a cyano group.[20b]

Since diazirine **17** turned out to be unsuitable for metallation and subsequent carboxylation, this transformation was attempted at the stage of diaziridine **16**. For the intermediate protection of the diaziridine moiety, the trimethylsilyl group was chosen, which promised to be readily cleavable upon aqueous work up. While incomplete conversions occurred when using $TMSCl/NEt_3$, the more reactive trimethylsilyl triflate (TMSOTf) led to the formation of the bis-silylated **19** in excellent yield (Scheme 2). Though rather sensitive to moisture, compound **19** could be stored for up to 10 d at –20 °C. Treatment of **19** with *n*BuLi (1 equiv.) resulted in a smooth lithiation, and the versatility of intermediate **20** was examined by its reaction with several electrophiles furnishing derivatives **22a–c** in reasonable yields.[21] For the carboxylation yielding non-radioactive **22d**, excess carbon dioxide was generated from dry ice, passed through molecular sieves (3 Å) and then introduced into the solution of lithium organyl **20**. After aqueous workup, essentially pure **22d** was isolated in high yield (89%) and directly oxidized to diazirine **23** using iodine and NEt_3. Purification of the product just required a basic extraction of the reaction mixture and led to the isolation of **23** in quantitative yield. Thus, in addition to a methyl substituent,[18b] protected hydroxymethyl groups[18b,22] and



Scheme 1. Synthesis of *p*-bromophenyldiazirine **17**.



22	R^1	R^2	Yield
a	H	H	58%
b	CH_3	CH_3	67%
c	H	Ph	67%
d	=O		89%

Scheme 2. Completion of the synthesis.

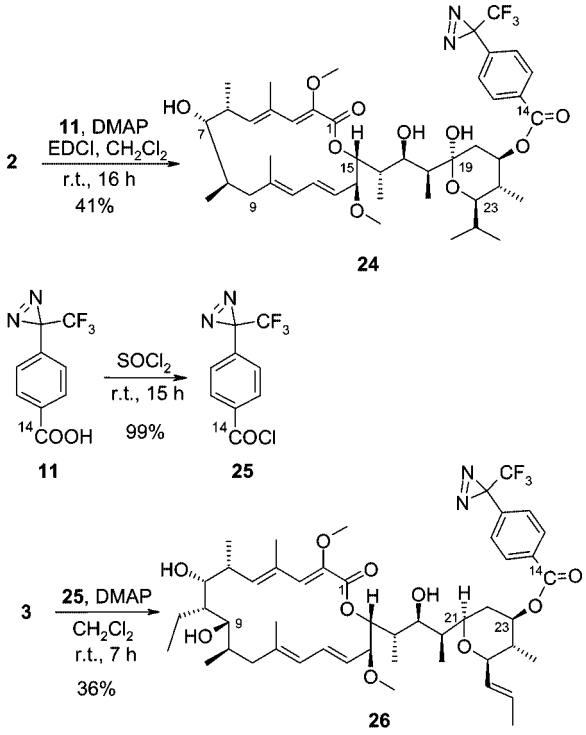
an oxazoline ring[18c] a bromo substituent is a suitable "latent" carboxy moiety for the preparation of diazirinylbenzoic acids.

Having succeeded with the preparation of unlabeled diazirine **23** in high yields, the synthesis of the radioactive analogue **11** was started with an extensive testing of different experimental procedures in order to achieve a maximum yield based on liberated carbon dioxide. This led to the development of a rather simple setup, as the $^{14}CO_2$ was generated by adding concentrated H_2SO_4 directly to the glass vial in which the $Ba^{14}CO_3$ was purchased. The gas stream was passed through a cooling bath to freeze out traces of acid and then introduced into a solution containing only 3 equiv. of the lithium organyl **20**.[23] Starting from 50 mCi (0.91 mmol) of $Ba^{14}CO_3$, 165 mg of the ^{14}C-diaziridine **21** was thus obtained, which corresponds to a yield of 78%. The oxidation of **21** was performed as described above affording the pure diazirine **11** in 83% yield after a basic extraction as the sole purification step. This radioactive photoaffinity label possessed a specific activity of 44.11 mCi/mmol and showed no signs of decomposition when stored for several months at –18 °C in CH_2Cl_2 at a concentration of approximately 4 mCi/mL.

Labeling of the Plecomacrolides and Inhibition Assays

Based on the structure-activity relationships of the plecomacrolide derivatives discussed above, the secondary hydroxy moieties on the tetrahydropyrane ring in both bafilomycin A_1 (**2**) and 21-deoxyconcanolide A (**3**) appeared to be the most promising sites for the attachment of the photoaffinity label **11**. Fortunately, these groups are significantly more reactive towards esterification than the hydroxy moieties at C-9 in compound **3** and C-7 in metabolite **2**, while all the other hydroxy groups are rather unreactive, presumably due to intramolecular conformational constraints and hydrogen bonding.[8,11] Thus, regioselective esterification of **2** and **3** seemed to be possible without intermediate protection. The secondary metabolites **1** and **2** were obtained from fermentations of the *Streptomyces* sp. strains Gö 22/15 and Gö3822 14F, respectively. Efficient isolation of concanamycin A (**1**) from a 50 L cultivation was achieved by silica gel chromatography of the acetone cell extract,[24] and subsequent methylation, reduction, and deglycosylation were performed as described to yield **3** in multi-mg scale.[8] Bafilomycin A_1 was obtained from shaking-flask cultivations in yields of 4 mg/L.[24]

The transformation of macrolide **2** was performed by a standard EDCI-based protocol employing only 2.1 equiv. of **11** (Scheme 3). After aqueous workup and column chromatography on silica gel, the desired labeled bafilomycin **24** was isolated in 41% yield (59% yield based on a 69% conversion) with a specific activity of 33.7 mCi/mmol. In contrast, the attempted esterification of 21-deoxyconcanolide **3** using DCC or EDCI and various bases only led to decomposition. Therefore, benzoic acid **11** was converted into benzoyl chloride **25** by a reaction with thionyl chloride for 15 h and the distillative removal of the latter at 40 mbar. This sophisticated step had to be performed very carefully, since on one hand, benzoyl chloride **25** is quite volatile and on the other hand, traces of remaining thionyl chloride in crude **25** led to the decomposition of macrolide **3**. The desired labeled 21-deoxyconcanolide **26** was obtained in 36% yield (52% yield based on a 69% conversion) with a specific activity of 40.0 mCi/mmol by reacting **3** with freshly prepared **25** in the presence of an excess of 4-*N*,*N*-dimethylaminopyridine. Thus, both plecomacrolides were selectively tagged with the necessary functionalities for PAL studies in a single operational step and with only one photoaffinity tracer group. This is an apparent experimental advantage of radioactively labeled photophores such as **11**, especially when working with ligands of limited availability.



Scheme 3. Coupling reaction of diazirine **11** with ATPase inhibitors **2** and **3**.

The inhibitory potential of the labeled derivatives **24** and **26** on the purified V-ATPase was examined, and the results are shown in Table 1. Remarkably, the inhibition by the

Table 1. Inhibitory activity of **1**, **2**, **3**, **4**, **24**, and **26** against purified V-ATPase form *M. sexta*. The V-ATPase holoenzyme for the inhibition assays was purified and its activity was determined according to the literature.[9]

Entry	Compound	K_i [nM]	I_{50} [nmol/mg]
1	**1**	13	0.7
2	**2**	1.5	0.08
3	**3**	4	0.2
4	**4**	20000[a]	600[a]
5	**24**	100	5.3
6	**26**	20	1

[a] Data taken from the literature.[9]

benzoylated **26** is almost equal to the natural concanamycin A (**1**) and close to that of concanolide **3**, thus representing a substantial improvement compared to the previously used, ^{125}I-labeled compound **4**.[8,9] In contrast, the labeled bafilomycin **24** is approximately seventy times less active than the natural product **2**, yet it is still effective enough to be used for the first PAL studies of this important inhibitor. Recent reports of **2** increasing the therapeutic effectiveness of anticancer drugs fuel the interest in elucidating the detailed mode of action of the plecomacrolides.[25]

Conclusions

An effective and convenient synthesis for both the frequently used photoaffinity label **23** and its ^{14}C-labeled analogue **11** has been elaborated, which features high chemical yields in combination with low prices of all the starting materials, particularly the ^{14}C source. All transformations can be performed on a large scale and thus furnish multi-gram amounts of products. Furthermore, the experimental simplicity of all the procedures including the purifications is noteworthy. Crucial for this achievement was the observation that while the diazirine moiety seems to be incompatible with Grignard reagents and lithium organyls, the protected diaziridine **19** can easily be lithiated and then reacted with electrophiles in the usual manner. This new reaction mode of aziridines together with their smooth oxidation to azirines should provide access to a variety of known and new photoaffinity labels. The ^{14}C-labeled benzoic acid **11** is generally suitable for attachment to alcohols and amines, yet it can also lead to a photoaffinity label for carboxylic acids, since the carboxy moiety in **11** can easily be reduced to a hydroxymethyl group.[26] We expect that the convenient synthesis of **11** together with the long half-life of ^{14}C will make it the photoaffinity label of choice in several future binding studies.

For a first application, the photoaffinity label **11** was selectively attached to bafilomycin A_1 (**2**) and the stable concanamycin analogue 21-deoxyconcanolide A (**3**). The preliminary inhibition studies suggest the products **24** and **26** are ideal candidates to further enlighten the precise binding site of these potent V-ATPase inhibitors. The PAL studies are still in progress, but first results have already disclosed that the specific activity of the ^{14}C tracer is sufficient for easy detection of the photolabeled components. The full results will be reported in due course.

Experimental Section

General: ^{1}H NMR spectra were recorded at 300 MHz or 600 MHz using a Varian Unity 300 or a Varian Inova 600 spectrometer. Chemical shifts in $CDCl_3$, [D$_6$]benzene, CD_2Cl_2, or [D$_6$]acetone are reported as δ values relative to $CHCl_3$ (δ = 7.26 ppm), [D$_5$]benzene (δ = 7.15 ppm), $CHDCl_2$ (δ = 5.32 ppm), or [D$_5$]acetone (δ = 2.05 ppm) as an internal reference unless stated otherwise. ^{13}C NMR spectra were recorded at 75.5 MHz (Unity 300), 125.7 MHz (Inova 500), or 150.8 MHz (Inova 600). Chemical shifts in $CDCl_3$, [D$_6$]benzene, CD_2Cl_2, or [D$_6$]acetone are reported as δ values relative to $CDCl_3$ (δ = 77.00 ppm), [D$_6$]benzene (δ = 128.0 ppm), CD_2Cl_2 (δ = 53.37 ppm), or [D$_6$]acetone (δ = 29.84 ppm). Chemical shift assignments were made using COSY and HSQC. If not stated otherwise, spectra were recorded at ambient temperature and with normal detection (300 MHz) or inverse detection (600 MHz). 600 MHz COSY spectra were collected using a gCOSY pulse sequence. HSQC spectra were collected using a gHSQC pulse sequence. IR spectra were recorded with a Bruker IFS 66 (FT-IR) spectrometer. HRMS (ESI) was performed with a Bruker APEX-Q 7T IV spectrometer with preselected ion peak matching at R >> 10000 to be within ±2 ppm of the exact masses. Elemental analyses were performed by Mikroanalytisches Labor des Instituts für Organische und Biomolekulare Chemie der University of Göttingen, Germany. Specific radioactivity was measured using a Beckmann bF Betaszint 5000/300 scintillation counter. Medium-pressure liquid-chromatography was performed with a Knauer MaxiStar pump using a RP18-Lobar LiChroprep column size B. Melting points are uncorrected. Solvents for extraction and chromatography were of technical grade and distilled before use. All moisture-sensitive reactions were carried out under dry nitrogen or argon in oven- and/or flame-dried glassware. Column chromatography was performed with silica gel 60 (0.040–0.063 mm, Machery–Nagel). Flash column chromatography was performed with silica gel 60 (0.025–0.040 mm, Machery–Nagel). TLC was performed with silica gel 60 F_{254} precoated plates (Merck, 0.25 mm). THF was distilled from sodium benzophenone ketyl, and dichloromethane, NEt_3, and pyridine were distilled from CaH_2. 1-(4-Bromophenyl)-2,2,2-trifluoroethanone (**13**)[15] and the THF solution of formaldehyde[19] were prepared as described in the literature, and $Ba^{14}CO_3$ was used as delivered by Moravek Biochemicals and Radiochemicals, 577 Mercury Lane, Brea, CA 92821, USA. Plecomacrolides **1** and **2** were obtained from fermentations of the *Streptomyces* sp. strains Gö 22/15 and Gö3822 14F and purified by chromatography as described previously.[8,24]

1-(4-Bromophenyl)-2,2,2-trifluoroethanone Oxime (14): A solution of 1-(4-bromophenyl)-2,2,2-trifluoroethanone (**13**, 1.00 g, 3.95 mmol) and hydroxylamine hydrochloride (0.270 g, 3.89 mmol) in absolute ethanol (1 mL) and pyridine (2 mL) was heated to 60 °C for 2 h. After evaporation of the solvents, the residue was partitioned between water (20 mL) and diethyl ether (200 mL). The organic layer was washed with water (10 mL) and brine (10 mL) and then dried with Na_2SO_4. After evaporation of the solvent, the crude oxime was purified by column chromatography (silica gel, hexane/EtOAc, 10:1) to furnish 0.870 g (82% yield) of the title compound as a colorless solid (1:1 mixture of isomers). R_f = 0.57 (hexane/EtOAc, 3:1); m.p. 75–80 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.32–7.42 (m, 2 H, Ar-H), 7.52–7.64 (m, 2 H, Ar-H), 8.89 (s, OH, isomer 1), 8.92 (s, OH, isomer 2) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 117.2 (q, $^1J_{C,F}$ = 175.3 Hz, CF_3, isomer 1), 121.0 (q, $^1J_{C,F}$ = 166.1 Hz, CF_3, isomer 2), 116.2, 118.5, 120.0, 122.2, 124.6, 125.3, 129.9, 130.3, 131.9, 145.9–148.2 (m, CNOH, both isomers) ppm. IR (KBr): $\tilde{\nu}$ = 3287 (OH), 1885, 1620 (C=N), 1587, 1493, 1466, 1000, 806 cm^{-1}. MS (DCI, 70 eV): *m*/*z* (%) = 266/268 (50) [M – H]$^-$, 533/535/537 (100) [2M – H]$^-$. $C_8H_5BrF_3NO$ (268.03): calcd. C 35.85, H 1.88, N 5.23; found C 35.87, H 2.00, N 5.12.

1-(4-Bromophenyl)-2,2,2-trifluoroethanone *O*-(*p*-Tolylsulfonyl)oxime (15): To a solution of oxime **14** (58.1 g, 217 mmol), NEt_3 (26.3 g, 260 mmol), and DMAP (2.65 g, 21.7 mmol) in CH_2Cl_2 (250 mL) was added in portions *p*-toluenesulfonyl chloride (47.6 g, 250 mmol) with stirring at 0 °C. When the addition was complete, the reaction mixture was stirred at room temp. for 3 h. After evaporation of the solvent, diethyl ether (500 mL) was added, and the organic layer was washed with water (3 × 50 mL) and brine

(10 mL). The organic phase was dried with Na_2SO_4 and concentrated to yield 91.0 g (99% yield) of a yellowish solid. The crude product was of sufficient purity for further transformations; an analytical sample was purified by recrystallization from $CHCl_3$. R_f = 0.43 (hexane/EtOAc, 2:1); m.p. 133–135 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.46 (s, 3 H, C*H*$_3$), 7.26 (d, $^3J_{H,H}$ = 8.6 Hz, 2 H, Ar-H), 7.37 (d, $^3J_{H,H}$ = 8.2 Hz, 2 H, Ar-H), 7.61 (d, $^3J_{H,H}$ = 8.6 Hz, 2 H, Ar-H), 7.86 (d, $^3J_{H,H}$ = 8.2 Hz, 2 H, Ar-H) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 21.8 (CH_3), 119.4 (q, $^1J_{C,F}$ = 277.4 Hz, CF_3), 123.3 (C-4), 126.6 (C-1), 129.3 (C-Ar), 129.9 (4 C, C-Ar), 131.0 (C-1′), 132.2 (C-Ar), 146.3 (C-4′), 153.0 (q, $^2J_{C,F}$ = 33.8 Hz, CNO) ppm. IR (KBr): $\tilde{\nu}$ = 1927, 1917, 1637 (C=N), 1589, 1489, 1447, 1390, 1179, 901, 820 cm^{-1}. MS (DCI, 70 eV): *m*/*z* (%) = 439/441 (100) $[M + NH_4]^+$, 456/458 (20) $[M + NH_3 + NH_4]^+$, 860/862/864 (40) $[2M + NH_4]^+$. $C_{15}H_{11}BrF_3NO_3S$ (422.22): calcd. C 42.67, H 2.63, N 3.32; found C 42.63, H 2.73, N 3.25.

3-(4-Bromophenyl)-3-(trifluoromethyl)diaziridine (16): Tosylate **15** (90.9 g, 215 mmol) was placed in an autoclave and dissolved in CH_2Cl_2 (200 mL). After cooling to –78 °C, gaseous ammonia was slowly introduced over a period of 4 h. The resulting mixture was stirred for 18 h and warmed to room temp. during this time. Excess ammonia was carefully evaporated, and the residue was partitioned between water (50 mL) and CH_2Cl_2 (500 mL). The organic layer was separated, washed with brine (10 mL), and dried with Na_2SO_4. After evaporation of the solvent, the resulting solid was recrystallized repeatedly from a mixture of pentane and CH_2Cl_2 (1:5) to give 49.4 g (86% yield) of the title compound as a colorless solid. R_f = 0.33 (hexane/EtOAc, 2:1); m.p. 47–48 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.18 (d, $^3J_{H,H}$ = 8.4 Hz, 1 H, NH), 2.79 (d, $^3J_{H,H}$ = 8.4 Hz, 1 H, NH), 7.48 (d, $^3J_{H,H}$ = 8.6 Hz, 2 H, Ar-H), 7.55 (d, $^3J_{H,H}$ = 8.6 Hz, 2 H, Ar-H) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 57.6 [q, $^2J_{C,F}$ = 36.5 Hz, $C(NH)_2$], 123.3 (q, $^1J_{C,F}$ = 278.2 Hz, CF_3), 124.6 (C-4), 129.8 (C-Ar), 130.7 (C-1), 132.0 (C-Ar) ppm. IR (KBr): $\tilde{\nu}$ = 3197 (NH), 1921, 1596 (NH), 1495, 1407, 1398, 1191, 1012, 829 cm^{-1}. MS (ESI): *m*/*z* (%) = 267/269 (100) $[M + H]^+$; analysis calcd. for $C_8H_7BrF_3N_2$: 266.97392 $[M + H]^+$ (correct mass according to ESI-HRMS). $C_8H_6BrF_3N_2$ (267.05): calcd. C 35.98, H 2.26, N 10.49; found C 35.98, H 2.19, N 10.42.

3-(4-Bromophenyl)-3-(trifluoromethyl)-3*H*-diazirine (17): A solution of diaziridine **16** (6.01 g, 22.5 mmol), NEt_3 (3.14 mL, 22.5 mmol), and ethanol (22 mL) was cooled to 0 °C. Under vigorous stirring, a solution of freshly prepared *tert*-butyl hypochlorite (7.34 g, 67.6 mmol) in *tert*-butanol (9.5 mL) was slowly added. The mixture was stirred at 0 °C for 2 h and then quenched with 10% aqueous $Na_2S_2O_5$ (150 mL). The aqueous phase was extracted with diethyl ether (3 × 50 mL), and the combined organic phases were dried with Na_2SO_4. The solvent was evaporated leaving 5.14 g (86% yield) of a colorless oil, whose purity was >95%. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.05 (d, $^3J_{H,H}$ = 8.4 Hz, 2 H, Ar-H), 7.51 (d, $^3J_{H,H}$ = 8.4 Hz, 2 H, Ar-H) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 28.2 (q, $^2J_{C,F}$ = 43.5 Hz, CN_2), 121.9 (q, $^1J_{C,F}$ = 274.7 Hz, CF_3), 124.2 (C-4), 128.0 (C-Ar), 132.0 (C-Ar) ppm. The signals of C-1 were not observed. MS (EI, 70 eV): *m*/*z* (%) = 71 (82) $[HCF_3]^+$, 107 (20) $[C_7H_9N]^+$, 137 (40), 155/157 (100) $[C_6H_4Br]^+$, 171 (20), 236/238 (45) $[M – N_2]^+$, 500/502/504 (20) $[C_{16}H_8Br_2F_6N_2]^+$. $C_8H_4BrF_3N_2$ (265.03).

3-(4-Bromophenyl)-3-(trifluoromethyl)-1,2-bis(trimethylsilyl)diaziridine (19): Diaziridine **16** (1.35 g, 5.06 mmol) was added to a solution of NEt_3 (4.2 mL, 30 mmol) in CH_2Cl_2 (25 mL). After stirring at room temp. for 1 h, the solution was cooled to –78 °C, and TMSOTf (2.0 mL, 2.5 g, 11 mmol) was added slowly. After 1 h at –78 °C, the cooling bath was removed, and the solution was stirred at room temp. The reaction progress was monitored by ^{1}H NMR analysis; for this purpose, 0.1 mL samples were taken from the reaction mixture and concentrated in vacuo. After 3 h, the conversion was complete, and all volatiles were removed in vacuo. The remaining yellow oil was extracted with benzene (4 × 10 mL), and the extracts were concentrated in vacuo. For purification of the product this extraction procedure was repeated twice with hexane (3 × 10 mL each) to furnish 1.97 g (95% yield) of the title compound as colorless crystals. ^{1}H NMR (300 MHz, C_6D_6): δ = 0.01 [s, 18 H, $Si(CH_3)_3$], 7.15 [d, $^3J_{H,H}$ = 8.1 Hz, 2 H, 3(5)-H], 7.28 [d, $^3J_{H,H}$ = 8.1 Hz, 2 H, 2(6)-H] ppm. ^{13}C NMR (75.5 MHz, C_6D_6): δ = –0.75 [$Si(CH_3)_3$], –0.76 [$Si(CH_3)_3$], 66.4 [q, $^2J_{C,F}$ = 35.2 Hz, $C(NTMS)_2$], 124.0 (C-4), 125.3 (q, $^1J_{C,F}$ = 282.4 Hz, CF_3), 131.2 (C-Ar), 131.4 (C-Ar), 133.1 (C-1).

3-[(4-Hydroxymethyl)phenyl]-3-(trifluoromethyl)diaziridine (22a): The protected diaziridine **19** (211 mg, 513 μmol) was dissolved in THF (15 mL) and cooled to –78 °C. After the addition of *n*BuLi (257 μL, 514 μmol, 2.00 M in hexane), the orange solution was stirred at this temperature for 1 h. Freshly prepared formaldehyde solution (2.93 mL, 2.05 mmol, 0.70 M in THF) was then added slowly, and the resulting mixture was stirred for 6 h and warmed to –50 °C during this time. After the addition of saturated aqueous NH_4Cl (500 μL), the THF was removed in vacuo. The residue was diluted with diethyl ether (250 mL), washed with water (15 mL), saturated aqueous $NaHCO_3$ (10 mL) and brine (10 mL), and dried with Na_2SO_4. The crude product (302 mg) was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 20:1) and medium-pressure liquid-chromatography (CH_3CN/H_2O, 1:4) to yield 65.0 mg (58%) of the title compound as a colorless oil. R_f = 0.42 (CH_2Cl_2/MeOH, 20:1). ^{1}H NMR (300 MHz, [D$_6$]acetone): δ = 3.26 (d, $^3J_{H,H}$ = 8.2 Hz, 1 H, NH), 3.60 (d, $^3J_{H,H}$ = 8.2 Hz, 1 H, NH), 4.42 (br. s, 1 H, OH), 4.66 (s, 2 H, C*H*$_2$OH), 7.43 [d, $^3J_{H,H}$ = 8.2 Hz, 2 H, 3(5)-H], 7.58 [d, $^3J_{H,H}$ = 8.2 Hz, 2 H, 2(6)-H] ppm. ^{13}C NMR (75.5 MHz, [D$_6$]acetone): δ = 58.4 [q, $^2J_{C,F}$ = 35.3 Hz, $C(NH)_2$], 64.1 (CH_2OH), 125.2 (q, $^1J_{C,F}$ = 277.4 Hz, CF_3), 127.2 (C-Ar), 129.1 (C-Ar), 131.7 (C-1), 145.2 (C-4) ppm. IR (KBr): $\tilde{\nu}$ = 3391, 3217, 1601, 1495, 1450, 1416, 1391, 1260, 1234, 1199, 1181, 1147, 1026, 945, 859, 736, 710, 621 cm^{-1}. MS (DCI, 70 eV): *m*/*z* (%) = 219 (100) $[M + H]^+$, 236 (80) $[M + NH_4]^+$. $C_9H_9F_3N_2O$ (218.18).

3-[4-(2-Hydroxyprop-2-yl)phenyl]-3-(trifluoromethyl)diaziridine (22b): The protected diaziridine **19** (286 mg, 695 μmol) was dissolved in THF (15 mL) and cooled to –78 °C. *n*BuLi (297 μL, 689 μmol, 2.32 M in hexane) was then added, and the resulting mixture was stirred for 40 min at –78 °C. Dry acetone (62 μL, 49 mg, 0.84 mmol) was added, and the mixture was warmed to –45 °C within 4.5 h and quenched by the addition of H_2O (0.5 mL). The solvents were removed in vacuo, and the residue was diluted with diethyl ether (150 mL), washed with H_2O (10 mL) and brine (2 × 10 mL), and dried with Na_2SO_4. The crude product (165 mg) was purified by column chromatography (silica gel, hexane/EtOAc, 2:1) to yield 114 mg (67% yield) of the title compound as a colorless solid. R_f = 0.26 (hexane/EtOAc, 2:1); m.p. 78 °C. ^{1}H NMR (300 MHz, [D$_6$]acetone): δ = 1.58 (s, 6 H, CH_3), 1.66 (br. s, OH), 2.22 (d, $^3J_{H,H}$ = 8.2 Hz, 1 H, NH), 2.80 (d, $^3J_{H,H}$ = 8.2 Hz, 1 H, NH), 7.44–7.68 (m, 4 H, Ar-H) ppm. ^{13}C NMR (75.5 MHz, [D$_6$]-acetone): δ = 31.7 (CH_3), 57.8 [q, $^2J_{C,F}$ = 35.8 Hz, $C(NH)_2$], 72.4 (COH), 125.2 (q, $^1J_{C,F}$ = 277.4 Hz, CF_3), 127.2 (C-Ar), 129.1 (C-Ar), 131.7 (C-1), 145.2 (C-4) ppm. IR (KBr): $\tilde{\nu}$ = 3371, 3237, 3214, 2983, 2932, 1615, 1515, 1463, 1403, 1236, 1155, 1118, 1020, 952, 832, 693 cm^{-1}. MS (DCI, 70 eV): *m*/*z* (%) = 229 (38) $[M – OH]^+$, 246 (100) $[M – H_2O + NH_4]^+$, 263 (40) $[M – H_2O + NH_3 +$

$NH_4]^+$. $C_{11}H_{13}F_3N_2O$ (246.23): calcd. C 53.66, H 5.32, N 11.38; found C 53.76, H 5.07, N 11.12.

3-[4-(Hydroxyphenylmethyl)phenyl]-3-(trifluoromethyl)diaziridine (22c): The protected diaziridine **19** (271 mg, 659 μmol) was dissolved in THF (15 mL) and cooled to –78 °C. *n*BuLi (281 μL, 652 μmol, 2.32 M in hexane) was added, and the resulting mixture was stirred for 40 min at –78 °C. A solution of freshly distilled benzaldehyde (77 mg, 730 μmol) in THF (2 mL) was then added, and the mixture was warmed to –40 °C within 4.5 h and quenched by the addition of H_2O (0.5 mL). The solvents were removed in vacuo, and the residue was diluted with diethyl ether (150 mL), washed with H_2O (10 mL) and brine (2 × 10 mL), and dried with Na_2SO_4. The crude product (205 mg) was purified by column chromatography (silica gel, hexane/EtOAc, 5:1) to yield 130 mg (67%) of the title compound as a colorless solid. R_f = 0.16 (hexane/EtOAc, 5:1); m.p. 82–84 °C. ^{1}H NMR (300 MHz, [D$_6$]acetone): δ = 3.29 (d, $^3J_{H,H}$ = 8.1 Hz, 1 H, NH), 3.59 (d, $^3J_{H,H}$ = 8.1 Hz, 1 H, NH), 5.03 (d, $^3J_{H,H}$ = 2.7 Hz, 1 H, OH), 5.88 (d, $^3J_{H,H}$ = 2.7 Hz, 1 H, C*H*OH), 7.18–7.36 (m, 3 H, Ar-H), 7.40–7.60 (m, 6 H, Ar-H) ppm. ^{13}C NMR (125.7 Hz, [D$_6$]acetone): δ = 58.4 [q, $^2J_{C,F}$ = 35.2 Hz, C(NH)$_2$], 75.7 (CHOH), 125.1 (q, $^1J_{C,F}$ = 277.7 Hz, CF_3), 127.1 (C-Ar), 127.2 (C-Ar), 127.8 (C-Ar), 129.0 (C-Ar), 129.2 (C-Ar), 131.8 (C-Ar), 146.0 (C-Ar), 148.2 (C-Ar) ppm. IR (KBr): $\tilde{\nu}$ = 3434, 3213, 2984, 1615, 1515, 1403, 1236, 1155, 1118, 951, 831, 693 cm^{-1}. MS (DCI, 70 eV): *m*/*z* (%) = 229 (100), 246 (90), 264 (10). $C_{15}H_{13}F_3N_2O$ (294.28): calcd. C 61.22, H 4.45, N 9.52; found C 60.99, H 4.26, N 9.40.

4-[(3-Trifluoromethyl)diaziridin-3-yl]benzoic Acid (22d): The protected diaziridine **19** (320 mg, 778 μmol) was dissolved in THF (30 mL) and cooled to –78 °C. After the addition of *n*BuLi (409 μL, 818 μmol, 2.00 M in hexane), the orange-red solution was stirred at –78 °C for 30 min. CO_2 was generated from approximately 3 g of dry ice, passed through a drying tube containing molecular sieves (3 Å), and introduced into the solution. After 30 min the addition was complete, and the solution was stirred for 16 h at –78 °C. The reaction was quenched with H_2O (0.5 mL), and the solvents were removed in vacuo. Diethyl ether (150 mL) was added, and the organic phase was extracted with saturated aqueous $NaHCO_3$ (3 × 15 mL). The combined aqueous phases were acidified with 50% hydrochloric acid to approximately pH 1. A colorless solid precipitated, which was extracted with diethyl ether (4 × 50 mL). The combined organic phases were washed with water (3 × 20 mL) and brine (2 × 10 mL). Evaporation of the solvent yielded 161 mg (89%) of the title compound as a colorless solid. The product was essentially pure and was directly used in further transformations. An analytical sample was purified by recrystallization from acetone; m.p. 181 °C (decomposition). ^{1}H NMR (300 MHz, [D$_6$]acetone): δ = 3.50 (d, $^3J_{H,H}$ = 7.6 Hz, 1 H, NH), 3.78 (d, $^3J_{H,H}$ = 7.6 Hz, 1 H, NH), 7.77 [d, $^3J_{H,H}$ = 8.3 Hz, 2 H, 2(6)-H], 8.09 [d, $^3J_{H,H}$ = 8.3 Hz, 2 H, 3(5)-H] ppm. ^{13}C NMR (75.5 MHz, [D$_6$]acetone): δ = 58.4 [q, $^2J_{C,F}$ = 35.7 Hz, C(NH)$_2$], 125.0 (q, $^1J_{C,F}$ = 277.7 Hz, CF_3), 129.6 (C-Ar), 130.5 (C-Ar), 132.8 (C-1), 137.7 (C-4), 167.0 (COOH) ppm. IR (KBr): $\tilde{\nu}$ = 3407, 3192, 3015, 2639, 2518, 1950, 1711, 1617, 1581, 1424, 1386, 1263, 1166, 1110, 1021, 959, 900, 859, 714 cm^{-1}. MS (ESI): *m*/*z* (%) = 231 (100) [M – H]$^-$, 485 (45) [2M – 2H + Na]$^-$. $C_9H_7F_3N_2O_2$ (232.16): calcd. C 46.56, H 3.04, N 12.07; found C 46.59, H 2.89, N 12.00.

4-[(3-Trifluoromethyl)diaziridin-3-yl]-[1-^{14}C]benzoic Acid (21):[23] A 25 mL Schlenk flask with an attached 100 mL glass syringe was charged with the protected diaziridine **19** (1.23 g, 2.99 mmol) and THF (20 mL) and cooled to –78 °C. After the addition of *n*BuLi (1.23 mL, 2.85 mmol, 2.32 M in hexane), the orange-red solution was stirred for 30 min at –78 °C. The plastic cap of the glass vial in which $Ba^{14}CO_3$ was delivered was pierced, and an additional rubber septum was introduced between the cap and vial. The carboxylation was then initiated by the addition of two drops of concentrated H_2SO_4 to the solid $Ba^{14}CO_3$ (50 mCi, 0.91 mmol), and the gas was introduced into the THF solution of the aryllithium compound using Teflon™ tubing. To remove traces of acid in the $^{14}CO_2$ stream, a 10 cm piece of the Teflon™ tubing was cooled in a –78 °C acetone/dry ice bath. When the gas evolution had ceased, additional concentrated H_2SO_4 was added dropwise (2 mL). After all the $Ba^{14}CO_3$ had been consumed, the Teflon™ tubing was disconnected with a clamp, and the solution was stirred for 5 h at –78 °C. The dark-blue solution was quenched with water (0.5 mL) and warmed to room temp. The whole apparatus was flushed with nitrogen, and the gas stream was passed through a wash bottle containing water and a tube containing soda-lime to remove unreacted $^{14}CO_2$. The reaction mixture was diluted with diethyl ether (250 mL), and the organic layer was extracted with NaOH solution (1 M, 3 × 20 mL). The combined aqueous phases were acidified with hydrochloric acid (6 N) to approximately pH 1. A colorless solid precipitated, which was extracted with diethyl ether (4 × 50 mL). The combined organic phases were washed with water (3 × 20 mL) and brine (1 × 10 mL). Evaporation of the solvent yielded 164 mg (78% calculated on $Ba^{14}CO_3$) of the title compound as a colorless solid, which was essentially pure and directly used in the subsequent oxidation.

4-[3-(Trifluoromethyl)-3*H*-diazirin-3-yl]benzoic Acid (23) and 4-[3-(Trifluoromethyl)-3*H*-diazirin-3-yl]-[1-^{14}C]benzoic Acid (11). General Procedure: The diaziridine (**21** or **22d**, respectively, 0.59 mmol) was dissolved in MeOH (15 mL), and NEt_3 (6 equiv.) was added. A solution of iodine (30 mg/mL in MeOH) was then added dropwise at room temp. to the stirred solution until the orange-red color of iodine persisted for more than 1 min (ca. 1.2 equiv. of I_2). After the addition of iodine was complete, the solution was stirred for 20–30 min in the dark at room temp. The methanol was removed under reduced pressure, and the residue was diluted with diethyl ether. The product was extracted from the organic phase with aqueous NaOH (1 M). The combined aqueous phases were acidified with hydrochloric acid (6 N) to approximately pH 1, and the product was extracted with diethyl ether. The combined organic phases were washed with water and brine. Evaporation of the solvent yielded the desired products **11** and **23** as colorless solids in yields ranging from 90% to 99%. The yield in the oxidation of the ^{14}C-labeled diaziridine **21** was 83%, and the specific activity of the diazirine **11** was determined to be 44.11 mCi/mmol using a liquid scintillation counter. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 28.4 (q, $^2J_{C,F}$ = 39.6 Hz, CN_2), 121.8 (q, $^1J_{C,F}$ = 274.7 Hz, CF_3), 126.4 (C-Ar), 130.2 (C-1), 130.5 (C-Ar), 134.8 (C-4), 171.3 (COOH) ppm. $C_9H_5F_3N_2O_2$ (230.15): calcd. C 46.97, H 2.19, N 12.17; found C 46.73, H 2.17, N 12.28. All other analytical data and the melting point were consistent with published data.[18b,18c]

21-*O*-[4-(3-Trifluoromethyl-3*H*-diazirin-3-yl)benzoyl]bafilomycin A_1 (24): Bafilomycin A_1 (**2**, 16.4 mg, 26.3 μmol), DMAP (7.8 mg, 64 μmol), diazirine **23** (12.6 mg, 54.7 μmol), and ethyl-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDCI, 11.1 mg, 57.9 μmol) were dissolved in dry CH_2Cl_2 (1.5 mL). The reaction mixture was stirred for 16 h in the dark at room temp. and monitored by TLC (hexane/EtOAc, 2:1). When the reaction had ceased, the solution was diluted with diethyl ether (150 mL) and washed with saturated aqueous $NaHCO_3$ (5 mL), H_2O (5 mL), and brine (5 mL). After drying the organic layer with $NaSO_4$, the solvent was removed in vacuo. The crude product was purified by column chromatography (silica gel, hexane/acetone, 6:1) to yield 9.1 mg

(41%) of the desired product as colorless oil and 3.6 mg (22%) of the starting material **2**. R_f = 0.25 (hexane/acetone, 6:1). ^{1}H NMR (600 MHz, CD_2Cl_2): δ = 0.80 (d, $^3J_{H,H}$ = 7.0 Hz, 3 H, 24-CH_3), 0.83 (d, $^3J_{H,H}$ = 7.9 Hz, 3 H, 16-CH_3), 0.83 (d, $^3J_{H,H}$ = 6.7 Hz, 3 H, 8-CH_3), 0.91 (d, $^3J_{H,H}$ = 6.1 Hz, 3 H, 24-CH_3), 0.92 (d, $^3J_{H,H}$ = 6.1 Hz, 3 H, 22-CH_3), 0.99 (d, $^3J_{H,H}$ = 7.2 Hz, 3 H, 18-CH_3), 1.03 (d, $^3J_{H,H}$ = 7.0 Hz, 3 H, 6-CH_3), 1.11 (dd, $J_{H,H}$ = 13.2, 7.4 Hz, 1 H, 20a-H), 1.76–1.81 (m, 1 H, 18-H), 1.82–1.94 [m, 4 H, 8(9a,22,24)-H], 1.92 (s, 3 H, 10-CH_3), 1.98 (d, $^3J_{H,H}$ = 1.1 Hz, 3 H, 4-CH_3), 2.06–2.16 [m, 2 H, 9b(16)-H], 2.23 (dd, $^3J_{H,H}$ = 11.8, 4.8 Hz, 1 H, 20b-H), 2.53 (ddq, $J_{H,H}$ = 9.1, 7.1, 2.0 Hz, 1 H, 6-H), 3.22 (s, 3 H, 14-OCH_3), 3.27 (m_c, 1 H, 7-H), 3.61 (s, 3 H, 2-OCH_3), 3.64 (dd, $J_{H,H}$ = 10.3, 2.2 Hz, 1 H, 23-H), 3.88 (t, $^3J_{H,H}$ = 9.0 Hz, 1 H, 14-H), 4.12 (ddd, $J_{H,H}$ = 10.7, 4.0, 1.9 Hz, 1 H, 17-H), 4.65 (dd, $J_{H,H}$ = 4.0, 0.7 Hz, 1 H, 17-OH), 4.88 (dd, $^3J_{H,H}$ = 8.6, 1.2 Hz, 1 H, 15-H), 5.07–5.17 [m, 2 H, 13(21)-H], 5.46 (d, $^3J_{H,H}$ = 2.1 Hz, 1 H, 19-OH), 5.77 (d, $^3J_{H,H}$ = 9.1 Hz, 1 H, 5-H), 5.81 (d, $^3J_{H,H}$ = 10.8 Hz, 1 H, 11-H), 6.53 (dd, $J_{H,H}$ = 15.0, 10.8 Hz, 1 H, 12-H), 6.68 (d, $^4J_{H,H}$ = 0.6 Hz, 1 H, 3-H), 7.26 (d, $^3J_{H,H}$ = 8.2 Hz, 2 H, Ar-H), 8.05 (d, $^3J_{H,H}$ = 8.2 Hz, 2 H, Ar-H) ppm. ^{13}C NMR (150.8 MHz, CD_2Cl_2): δ = 7.1 (18-CH_3), 9.8 (16-CH_3), 12.4 (22-CH_3), 14.0 (4-CH_3), 14.3 (24-CH_3), 17.3 (6-CH_3), 20.2 (10-CH_3), 21.2 (24-CH_3), 21.7 (8-CH_3), 28.3 (C-24), 37.0 (C-6), 37.5 (C-16), 40.5 (C-22), 41.5 (C-8), 41.5 (C-9), 42.2 (C-18), 53.4 (C-20), 55.7 (14-OCH_3), 60.2 (2-OCH_3), 71.0 (C-17), 75.6 (C-21), 76.0 (C-23), 77.0 (C-15), 81.2 (C-7), 82.6 (C-14), 99.2 (C-19), 125.4 (C-11), 126.6 (C-Ar), 127.1 (C-13), 130.1 (C-Ar), 132.3 (C-Ar), 133.2 (C-4), 133.5 (C-12), 133.6 (C-Ar), 134.0 (C-3), 141.5 (C-2), 143.5 (C-5), 143.6 (C-10), 165.2 (CO_2), 167.6 (C-1) ppm, signals of CF_3 and CN_2 were not observed due to low intensity. IR (KBr): $\tilde{\nu}$ = 3422, 2930, 1718, 1275, 1194, 1159, 1101 cm^{-1}. MS (ESI): *m*/*z* (%) = 853 (34) [M + NH_4]$^+$, 858 (100) [M + Na]$^+$; analysis calcd. for $C_{44}H_{61}F_3N_2NaO_{10}$: 857.41705 [M + Na]$^+$ (correct mass according to ESI-HRMS).

21-*O*-[4-(3-Trifluoromethyl-3*H*-diazirin-3-yl)-[1-^{14}C]benzoyl]bafilomycin A_1 (24): The reaction was performed as described above employing bafilomycin A_1 (**2**, 15.7 mg, 25.2 μmol) and label **11** (11.6 mg, 50.4 μmol). The product yield was 8.6 mg (41%), and 4.9 mg (31%) of bafilomycin A_1 (**2**) were re-isolated. The ^{1}H NMR spectroscopic data were consistent with the data of the unlabeled compound **24**. The specific activity was determined to be 33.7 mCi/mmol using a liquid scintillation counter.

23-*O*-[4-(3-Trifluoromethyl-3*H*-diazirin-3-yl)benzoyl]-21-deoxyconcanolide A (26): Diazirine **23** (36.0 mg, 156 μmol) was dissolved in thionyl chloride (0.50 mL), and the resulting solution was stirred for 15 h at room temp. in the dark. Then, the thionyl chloride was carefully removed under reduced pressure (40 mbar) to yield 38.5 mg (99%) of 4-(3-trifluoromethyl-3*H*-diazirin-3-yl)benzoyl chloride (**25**) which was dissolved in CH_2Cl_2 (3 mL). 21-Deoxyconcanolide A (**3**, 12.0 mg, 17.7 μmol) and DMAP (11.5 mg, 94.1 μmol) were dissolved in CH_2Cl_2 (1.5 mL), and NEt_3 (50 μL) was added. Then, a solution of benzoyl chloride **25** (5.3 mg, 21 μmol) in CH_2Cl_2 (0.41 mL) was added dropwise and the resulting mixture was stirred at room temp. in the dark. Due to incomplete conversion after 1.5 h as detected by TLC, more benzoyl chloride **25** (2.7 mg, 11 μmol) dissolved in CH_2Cl_2 (0.21 mL) was added. After 4.5 h, another portion of DMAP (11.0 mg, 90.0 μmol) was added, and the reaction was stopped after 7 h by the addition of water (1 mL). The mixture was diluted with diethyl ether (150 mL), and the organic layer was washed with aqueous $NaHCO_3$ (1 M, 5 mL), H_2O (5 mL), and brine (5 mL). Drying with Na_2SO_4 and evaporation of the solvent yielded a crude product (51 mg) which was purified by column chromatography (silica gel, hexane/acetone, 10:1) to yield 5.0 mg (32%) of the title compound as a colorless oil and 4.6 mg (38%) of the starting material **3**. R_f = 0.25 (hexane/acetone, 6:1). ^{1}H NMR (600 MHz, CD_2Cl_2): δ = 0.80 (d, $^3J_{H,H}$ = 7.6 Hz, 3 H, 18-CH_3), 0.81 (d, $^3J_{H,H}$ = 6.7 Hz, 3 H, 20-CH_3), 0.85 (d, $^3J_{H,H}$ = 7.6 Hz, 3 H, 24-CH_3), 0.85 (m_c, 3 H, 8-CH_2CH_3), 1.00–1.10 (m, 3 H, 6-CH_3), 1.05 (d, $^3J_{H,H}$ = 7.0 Hz, 3 H, 10-CH_3), 1.13–1.22 (m, 2 H, 8-CH_2CH_3), 1.22–1.30 (m, 1 H, 24-H), 1.48 (m_c, 1 H, 8-H), 1.60 (m_c, 1 H, 20-H), 1.64 (dd, $J_{H,H}$ = 6.5, 1.5 Hz, 3 H, 28-H_3), 1.64–1.68 (m, 2 H, 22-H_2), 1.84 (br. s, 3 H, 12-CH_3), 1.92–1.99 (m, 2 H, 11-H_2), 1.96 (s, 3 H, 4-CH_3), 2.02 (m_c, 1 H, 18-H), 2.23 (m_c, 1 H, 10-H), 2.72 (m_c, 1 H, 6-H), 3.13–3.22 (m, 1 H, 9-H), 3.22 (s, 3 H, 16-OCH_3), 3.47 (dd, $^3J_{H,H}$ = 9.7, 7.8 Hz, 1 H, 25-H), 3.55 (s, 3 H, 2-OCH_3), 3.56–3.61 [m, 2 H, 7(19)-H], 3.76 (m_c, 1 H, 21-H), 3.82 (t, $^3J_{H,H}$ = 8.9 Hz, 1 H, 16-H), 4.88 (dt, $^3J_{H,H}$ = 10.8, 4.8 Hz, 1 H, 23-H), 5.14 (m_c, 1 H, 17-H), 5.21 (m_c, 1 H, 15-H), 5.36 (ddq, $J_{H,H}$ = 15.0, 7.6, 1.6 Hz, 1 H, 26-H), 5.63 (dq, $^3J_{H,H}$ = 15.0, 6.4 Hz, 1 H, 27-H), 5.67 (m_c, 1 H, 5-H), 5.79 (d, $^3J_{H,H}$ = 10.5 Hz, 1 H, 13-H), 6.37 (br. s, 1 H, 3-H), 6.54 (dd, $^3J_{H,H}$ = 15.0, 10.5 Hz, 1 H, 14-H), 7.26 (d, $^3J_{H,H}$ = 8.3 Hz, 2 H, Ar-H), 8.05 (d, $^3J_{H,H}$ = 8.3 Hz, 2 H, Ar-H) ppm. ^{13}C NMR (150.8 MHz, CD_2Cl_2): δ = 8.0 (20-CH_3), 9.4 (18-CH_3), 11.5 (8-CH_2CH_3), 13.3 (24-CH_3), 14.1 (4-CH_3), 16.2 (12-CH_3), 16.7 (6-CH_3), 17.7 (C-28), 21.4 (10-CH_3), 22.8 (8-CH_2CH_3), 28.4 (q, $^2J_{C,F}$ = 41.6 Hz, CN_2), 34.6 (C-6), 35.3 (C-10), 36.4 (C-22), 37.3 (C-18), 39.1 (C-20), 40.7 (C-11), 43.4 (C-24), 44.5 (C-8), 55.7 (16-OCH_3), 59.1 (2-OCH_3), 69.1 (C-19), 74.2 (C-7), 75.7 (C-17), 76.4 (C-21), 77.1 (C-23), 79.4 (C-9), 82.1 (C-16), 82.6 (C-25), 121.8 (q, $^1J_{C,F}$ = 274.8 Hz, CF_3), 122.9 (C-13), 126.1 (C-Ar), 127.1 (C-15), 129.1 (C-27), 129.8 (C-Ar), 130.1 (C-Ar), 130.2 (C-26), 131.4 (C-3), 132.0 (C-4), 133.1 (C-Ar), 133.4 (C-14), 139.6 (C-12), 141.97 (C-2), 142.02 (C-5), 164.9 (CO_2), 165.7 (C-1) ppm. IR (KBr): $\tilde{\nu}$ = 3446, 2967, 2930, 1718, 1700, 1458, 1382, 1343, 1276, 1196, 1159, 1106 cm^{-1}. MS (ESI): *m*/*z* (%) = 825 (20) [M–2 MeOH]$^+$, 858 (100) [M – MeOH + H]$^+$, 912 (30) [M + Na]$^+$; analysis calcd. for $C_{48}H_{67}F_3N_2NaO_{10}$: 911.46455 [*M* + Na]$^+$ (correct mass according to ESI-HRMS).

23-*O*-[4-(3-Trifluoromethyl-3*H*-diazirin-3-yl)-[1-^{14}C]benzoyl]-21-deoxyconcanolide A (26): The reaction was performed as described above employing 21-deoxyconcanolide A (**3**, 12.5 mg, 18.5 μmol) and label **25** (7.17 mg, 28.9 μmol). The product yield was 4.6 mg (36%), and 3.9 mg (31%) of 21-deoxyconcanolide A (**3**) were re-isolated. The ^{1}H NMR spectroscopic data were consistent with the data of the unlabeled compound **26**. The specific activity was determined to be 40.0 mCi/mmol using a liquid scintillation counter.

Supporting Information (see also the footnote on the first page of this article): A figure showing the experimental setup for the carboxylation using $Ba^{14}CO_3$ and ^{1}H NMR spectra of all new compounds.

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