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New Fluorous Photoaffinity Labels (F-PAL) and Their Application in V-ATPase Inhibition Studies

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Dedicated to Professor Christian Reichardt on the occasion of his 75th birthday

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(Trifluoromethyl)diazirines are well established photoaffinity labels (PAL) used in biochemical investigations to create covalent ligand-receptor bonds. Two new diazirinylbenzoic acids **8b**,**c** with perfluorobutyl and perfluorooctyl chains (F-PAL) were efficiently prepared from *p*-bromobenzaldehyde and attached to the highly potent and specific V-ATPase inhibitors 21-deoxyconcanolide A (**2**) and bafilomycin A_1 (**5**), deriving from the natural product pool from *Streptomyces* producer strains. The labelled derivatives **17** and **18** were efficiently purified by fluorous solid-phase extraction. Functional biochemical assays with the V-ATPase holoenzymes proofed strong inhibition activities. So far, radioactive isotopes or biotin-tags have mainly been used for tracing compounds in photoaffinity studies. The C_4F_9- and C_8F_{17}-fluorous tags aim to enable advantageous separation and identification of labelled peptide fragments by fluorous chromatography followed by MS analysis. Therefore, F-PAL represents an innovative new concept for binding site determination and should significantly accelerate and simplify such biochemical investigations.

Introduction

Fundamental research and development of new drugs demand a detailed molecular understanding of ligand-target recognition and interaction between pharmacophore and target molecule. The V-type ATPase – an important molecular proton transporting machine and pH regulator in almost all eukaryotic cells – shows a great potential as target in osteoporosis and cancer therapy.[1] This was shown in a number of studies with the microbial metabolites concanamycin A (**1**) from *Streptomyces* strain Gö 22/15, its derivative 21-deoxyconcanolide A (**2**) or bafilomycin A_1 (**5**) from strain Gö 3822-F4 (Figure 1) which all belong to the most selective and potent V-ATPase inhibitors with IC_{50} values at nanomolar range.[2] In order to determine the mechanism of inhibition, Bowman et al. investigated the binding site of these plecomacrolides using site-directed mutagenesis.[3] In addition, Zeeck and co-workers prepared concanolide **3** for photoaffinity labelling studies.[4] The 3-aryl-3-(trifluoromethyl)diazirine moiety at R^2 served as photoactivatable group in these experiments and led to a covalent bond between inhibitor and binding site. Tracing and detection was realized with the radioactive ^{125}I at R^3. These studies strongly suggested subunit c of the V-ATPase as the binding site of concanamycins, however, were hampered by a seriously reduced inhibitory potential of compound **3** (IC_{50} = 15–20 μM) possibly due to modification of two hydroxy groups in concanolide analogue **3**. Other tracing methods like voluminous fluorescent-tagged PAL were not suitable for these V-ATPase studies.[4c] Recently, this problem was solved by our development of the new photoaffinity label (PAL) **7** which contains a radioactive ^{14}C atom in the photosensitive substituent itself and offers the additional advantage of the very long half-life of ^{14}C (τ = 5730 *y*). Indeed, inhibitors **4** and **6**, modified only at the tetrahydropyrane rings, retain most of the high inhibitory activities of the natural products **1** and **5**.[5] Photoactivation of these compounds in the presence of the V-ATPase holoenzyme from the tobacco hornworm *Manduca sexta*, separation of the subunits by SDS PAGE and detection by autoradiography furnished further insights into involved subunits (details will be reported in due course). Yet, attempts to analyze tagged, very non-polar peptide fragments by HPLC-MS analysis proofed to be difficult – this is a general

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problem in PAL studies since the efficiency of crosslinking is usually low and only traces of labelled fragments need to be identified among a vast excess of unlabelled ones.[6]

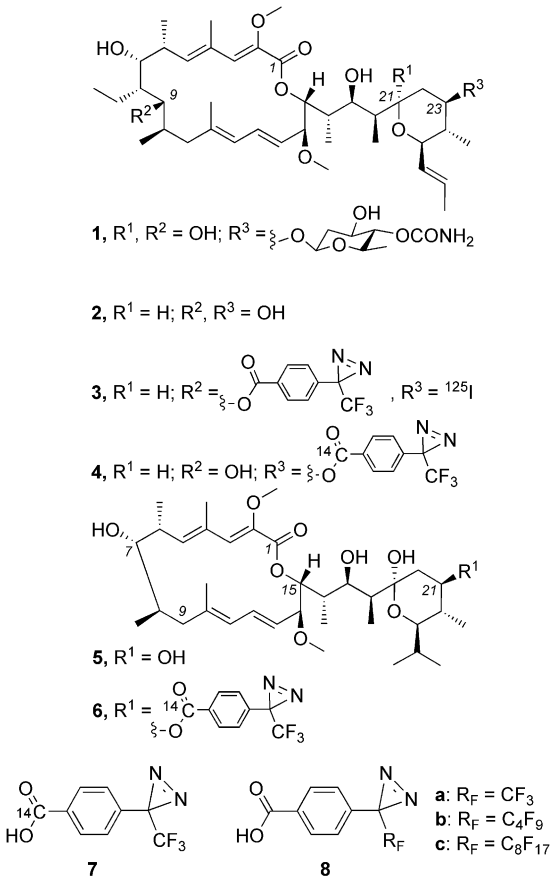


Figure 1. Structures of V-ATPase inhibitors **1**–**6** and photoaffinity labels **7**, **8**.

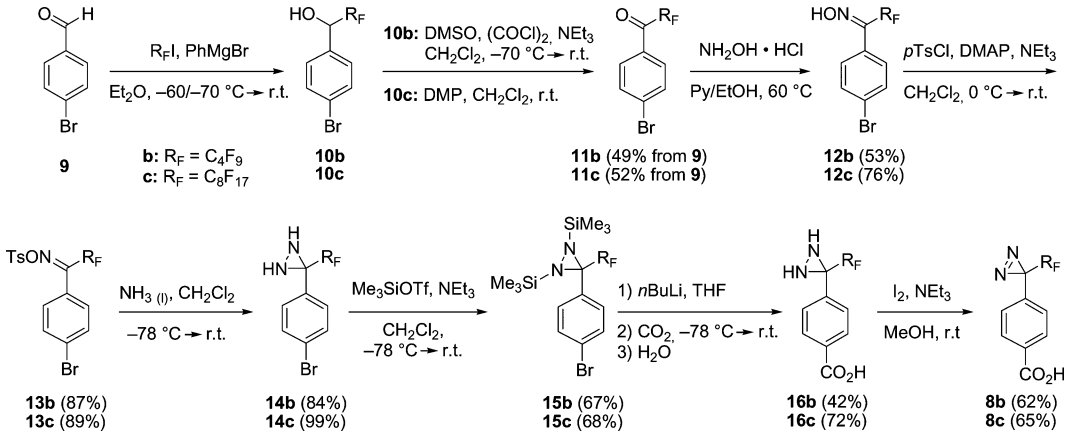
An elegant possibility to overcome this difficulty is the "fishing approach" for ligand-protein target studies.[7] Frequently, a biotin molecule is attached to the bioactive ligand or the photosensitive group itself, e.g., to diazirine-based PAL. After photoactivation and digestion, labelled fragments are easily "fished" from unlabelled peptide fragments using avidin- or streptavidin-based matrices and analysed by mass spectrometry. Yet, in some cases this sterically demanding tracer group unfavourably interferes with the ligand-target complex.[8]

Therefore, we became interested in the development of a new non-voluminous tracer group and herewith bring the young method of fluorous chromatography and fluorous solid phase extraction (FSPE) into play.[9] The outstanding property of this separation technique is that only fluorinated compounds are retarded on the fluorinated silica gel while all other organic material – (mostly) regardless of its physicochemical properties – is washed off a column with the appropriate organic mobile phase. Thus, we speculated that substitution of the trifluoromethyl group in the non-radioactive PAL **8a** by a longer perfluoroalkyl residue would lead to a new fluorous photoaffinity label (F-PAL) containing both, the photosensitive moiety and the tracer unit in a single compound. Again, only one operational step is then necessary for their attachment to the bioactive ligand. Identification of the labelled fragments would then be achieved by fluorous chromatography and HPLC-MS analysis. The chemical synthesis of such photoaffinity labels has been devised independently by Zhang et al. and us,[10] and we here present the first successful practical use of the new fluorous photoaffinity labels (F-PAL) in a ligand-target binding study exemplified with the functional V-ATPase inhibition.

Results and Discussion

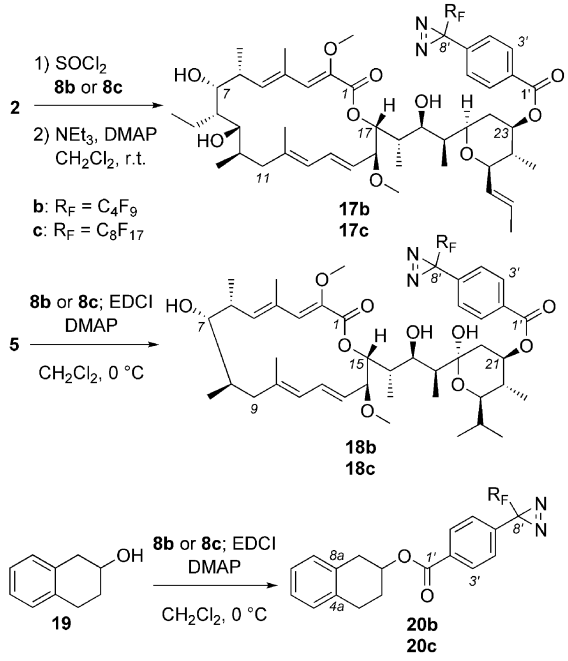
The interaction of fluorinated compounds with fluorous stationary phases is critically dependent on the balance between their organic and fluorous character.[9] Therefore, we decided to prepare F-PAL **8b** and **8c** with different lengths of the perfluoroalkyl chains, namely perfluorobutyl and perfluorooctyl while perfluoropropyl and perfluorohexyl residues were chosen independently by Zhang et al.[10a] Our synthesis commenced with metallation of the respective perfluoroalkyl iodides with phenylmagnesium bromide and addition to *p*-bromobenzaldehyde (**9**) furnishing alcohols **10b**,**c** which were oxidized using the Swern protocol or Dess–Martin periodinane (DMP, Scheme 1). Ketones **11b**,**c** were then transformed into diazirines **14b**,**c** along the common approach[11] consisting of formation of oximes **12b**,**c**, their tosylation and treatment with liquid ammonia in an autoclave at room temp. Conversion to benzoic acids **16b**,**c** was then performed following our protocol[5] of temporary silylation of the diaziridine moiety with trimethylsilyl triflate, bromide-lithium exchange and carboxylation with carbon dioxide. Finally, oxidation to the diazirines **8b**,**c** with iodine/NEt_3 completed an eight-step synthesis with overall yields of 3% (**8b**) and 11% (**8c**), respectively.

The potent microbial natural products concanamycin A (**1**) and bafilomycin A_1 (**5**) were isolated and purified starting from cultures of *Streptomyces* sp. Gö 22/15 and Gö 3822-F4, respectively.[12] Preparation of the chemically more stable aglycon 21-deoxyconcanolide A (**2**) was performed via methylation, reduction and deglycosylation as described.[4a] In former coupling reactions of PAL **7** and **8a** with these inhibitors, the hydroxy groups on the tetrahydropyran rings of **2** (at C-23) and **5** (at C-21) had shown to be most reactive and were thus selectively esterified.[5,13] Accordingly, the desired derivatives **17b**,**c** were obtained from esterification with the respective acid chlorides obtained by treatment of **8b**,**c** with thionyl chloride (Scheme 2). Derivatives **18b**,**c** of bafilomycin A_1 (**5**) carrying the fluorous photoaffinity labels (F-PAL) were obtained from mild EDCI-mediated reactions. Direct light was obstructed from all reactions and products. Purification of the crude F-PAL inhibitor fractions from standard silica gel chromatography already took advantage of fluorous solid phase extraction. Thus, concanamycin derivatives **17b**,**c** were applied to C_8F_{17}-modified silica gel and eluted with MeOH/H_2O (80:20) as fluorophobic, and MeOH as fluoro-



Scheme 1. Synthesis of the new fluorous photoaffinity labels (F-PAL) **8b**,**c**.

philic solvents to afford highly pure compounds based on TLC and NMR analysis in yields of 0.6 mg (**17b**) and 1.0 mg (**17c**).[13]



Scheme 2. Attachment of F-PAL **8b** or **8c** to V-ATPase inhibitors **2**, **5** and model compound **19**.

Since the macrolide bafilomycin A_1 (**5**) is known to be highly unstable in MeOH solution an alternative FSPE procedure had to be established for purification of compounds **18**. Among mixtures containing THF, acetone or DMF, best results were observed with MeCN/H_2O (70:30) as fluorophobic and pure MeCN as fluorophilic solvents,[13] and photoaffinity agents **18b** and **18c** were obtained in yields of 2.2 mg and 4.6 mg, respectively (Table 1). Moreover, the tetrahydronaphth-2-ol derived agents **20b**,**c** were prepared: Since one of the binding sites at the V-ATPase is extremely apolar we aimed to unambiguously exclude a misleading inhibitory activity of the apolar fluorous tails themselves, that is, the possibility of unspecific inhibitory interactions with the hydrophobic enzyme surface. The thus obtained six F-PAL-substituted probes **17**, **18** and **20** as well as the native natural products concanamycin A (**1**) and bafilomycin A_1 (**5**) as further controls were subjected to the functional V-ATPase holoenzyme inhibition assay performed as described by Huss et al.[4b]

Table 1. Conditions of fluorous solid phase extraction (FSPE).

Compound	Solvent [fluorophobic/fluorophilic]
17b	MeOH/H_2O 80:20/MeOH
17c	MeOH/H_2O 80:20/MeOH
18b	MeCN/H_2O 70:30/MeCN
18c	MeCN/H_2O 70:30/MeCN
20b	MeOH/H_2O 80:20/MeOH
20c	MeOH/H_2O 80:20/MeOH

The results of the biochemical investigation are shown in Figure 2 and Figure 3 and point out that concanamycin analogues **17b**,**c** have a very high inhibition potential with IC_{50} values of approximately 0.06 μM. Thus, they are about one order of magnitude inferior to the values of the natural product concanamycin A (**1**) (IC_{50} = 0.005 μM) and of concanolide derivative **17a**[5,14] (IC_{50} = 0.01 μM) modified with the trifluoromethyl-based PAL **8a**. The longer perfluoroalkyl chains in agents **17b**,**c** might therefore slightly interfere with the binding sites of the V-ATPase. However, there is no significant inhibition of the V-ATPase by model compounds **20b**,**c** which excludes the possibility of the F-PAL interacting unspecifically with the relevant binding site of the holoenzyme to give a false positive result. Contrary, the inhibition potential of bafilomycin analogues **18b**,**c** is lower than that of the natural product as drawn from the IC_{50} values, both >> 100 μM. Taking into account that introduction of trifluoromethyl-based PAL **8a** leads to a decrease of the activity by the factor 20 (IC_{50} = 0.08 μM for **18a**),[5,14] bafilomycin A_1 (**5**) seems to be more sensitive than concanolide **2** for disturbing substituents like the fluorous tags. Hitherto, the exact reasons for these differences in activity are unknown; there might be an interaction between the photoaffinity label, the fluorous tail and the macrolide ring of bafilomycin A_1 (**5**) in a different way compared to the similar structures of the concanamycins.

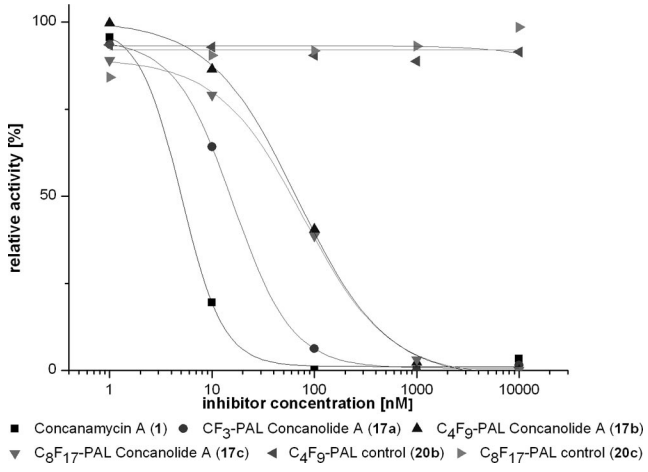


Figure 2. Inhibition of the V-ATPase with concanamycin A (**1**), derived F-PAL agents **17a**,**b**,**c** and F-PAL control compounds **20b**,**c**.

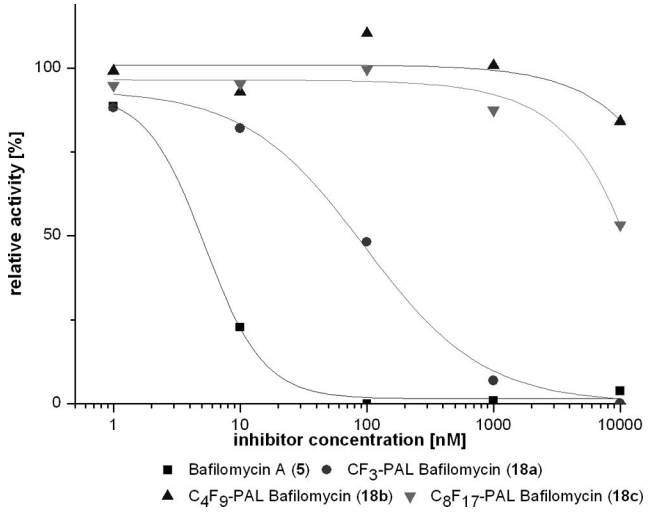


Figure 3. Inhibition of the V-ATPase with bafilomycin A_1 (**5**) and derived F-PAL-agents **18a**,**b**,**c** (control compounds **20b**,**c**; see Figure 2).

One can only speculate if the fluorous tail might change the folding of the macrocyclic ring in structure **5**, but anyway, the binding of inhibitor analogues **18b**,**c** is too low to allow for successful photoaffinity studies. Concanamycin derivatives **17b**,**c**, however, are perfectly suited for this application and first experiments to separate and identify labelled protein fragments using fluorous chromatography and MS-MS analyses are currently under way.

Conclusions

A new type of fluorous photoaffinity label (F-PAL) has been developed equipped with both, a photoactivatable group and a new tracer unit. While diazirines are generally meant to be the most suitable photoreactive moiety[6] the perfluoroalkyl chains are promising anchors for the "fishing technique" using fluorous chromatography. Combination of both entities in a single molecule offers the advantage of requiring only one operational step for functionalization of the bioactive ligand thus causing less modification of its structure.

Moreover, we have shown the applicability of this new concept in two aspects: First, the F-PAL could easily be attached to potent V-ATPase inhibitors, and as an example the concanamycin derivatives **17b**,**c** retained the high and specific binding affinity. This was not obvious from the beginning due to the highly apolar character of the perfluoroalkyl chains which could either inhibit formation of the ligand-receptor complex or even lead to an unspecific binding to the enzyme surface – the later possibility was excluded by the thoroughly applied control substances **20b**,**c**. Second, the fluorous tails on the photoaffinity label add sufficient fluorous character to the complex photoaffinity probes **17** and **18** with molecular weights of more than 1 kDa to enable their efficient and reliable separation and purification by fluorous solid phase extraction of the crude mixtures. This is a strong indication for a successful future separation of the labelled peptide fragments after photoactivation and digestion of the ligand-receptor complex, especially since van Boom et al. already described purification of a synthetic oligopeptide of 22 amino acids using a perfluorooctyl anchor.[15]

Thus, we expect that the concept of F-PAL will afford significant improvements in elucidating the exact peptide sequences of the sites of the V-ATPase to which inhibitors bind and, by doing so, will contribute to the overall long-term aim to unravel the function of the V-ATPase proton pumping machinery. Additionally, linkage of the concepts of photoaffinity labelling and fluorous chromatography is thought to replace less convenient labels and generate a method to make binding studies much faster and easier.

Experimental Section

General: See Supporting Information.

General Procedure for Esterification with 21-Deoxyconcanolide A (2): Diazirinyl compounds were all handled under low light conditions. The respective F-PAL **8b** (19.0 mg, 49.9 μmol) or **8c** (30.8 mg, 53.1 μmol) and thionyl chloride (0.50 mL, 6.9 mmol) were stirred for 15 min at room temp., excess thionyl chloride was removed under reduced pressure (20 mbar) and the residue was dissolved in dichloromethane (CH_2Cl_2, 2 mL). Concanolide A (**2**) and DMAP were dissolved in CH_2Cl_2 (1.5 mL) and treated with triethylamine (35.0 mg, 346 μmol) and 4-[(3-perfluorobutyl)diazirin-3-yl]benzoyl chloride or 4-[(3-perfluorooctyl)diazirin-3-yl]benzoyl chloride dissolved in CH_2Cl_2, respectively. The mixture was stirred for 3 h and treated with a second portion of the respective benzoyl chloride dissolved in CH_2Cl_2. The reaction was stirred for 24 h and then diluted with water (1 mL) and ethyl ether (150 mL). The mixture was washed with saturated aqueous $NaHCO_3$ (1 × 5 mL), brine (1 × 5 mL) and water (1 × 5 mL). The organic layer was dried with Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was initially purified by column chromatography (silica gel, $CHCl_3$/EtOAc/MeOH, 10:5:0.5).

General Procedure for Esterification with Bafilomycin A (5): Diazirinyl compounds were all handled under low light conditions. 4-[(3-

Perfluorobutyl)diazirin-3-yl]benzoic acid (**8b**) or 4-[(3-perfluorooctyl)diazirin-3-yl]benzoic acid (**8c**), respectively, were dissolved in dry CH_2Cl_2 (0.8 mL) and cooled to 0 °C. EDCI was added and the solution was stirred for 5 min. After addition of DMAP, the reaction mixture was stirred for 3 min and a solution of bafilomycin A_1 (**5**) in CH_2Cl_2 (0.4 mL) was added dropwise. The reaction mixture was stirred for 18 h in the dark and then diluted with ethyl ether (150 mL). The mixture was washed with saturated aqueous $NaHCO_3$ (1 × 5 mL), brine (1 × 5 mL) and water (1 × 5 mL). The organic layer was dried with Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was initially purified by column chromatography (silica gel, cyclohexane/acetone, 6:1).

Fluorous Solid Phase Extraction: The extractions of the concanamycin derivatives **17b** and **17c** were performed with a solvent mixture of MeOH/H_2O (80:20) for the non-fluorous and pure MeOH for the fluorous wash. The extractions of the bafilomycin derivatives **18b** and **18c** were performed with a solvent mixture of MeCN/H_2O (70:30) for the non-fluorous and pure MeCN for the fluorous wash.[13]

23-*O*-[4-(3-Perfluorobutyl-3*H*-diazirin-3-yl)benzoyl]-21-deoxyconcanolide (17b): The reaction was performed as described above with reduced reaction time of 5 h employing 21-deoxyconcanolide (**2**) (9.7 mg, 14 μmol), the benzoyl chloride of F-PAL **8b** (21.0 mg, 52.7 μmol) and DMAP (11.7 mg, 95.7 μmol) to furnish 0.65 mg (0.63 μmol, 4.5%) of **17b** and 5.8 mg (8.5 μmol, 60%) of the re-isolated starting material **2**. R_f = 0.48 (cyclohexane/acetone, 6:1). ^{1}H NMR (600 MHz, CD_2Cl_2/CD_3OD, 2:1): δ = 0.86 (m, 3 H, 6-CH_3), 0.86 (m, 3 H, 8-CH_2CH_3), 0.86 (m, 3 H, 18-CH_3), 0.86 (m, 3 H, 20-CH_3), 0.86 (m, 3 H, 24-CH_3), 1.05 (m, 3 H, 10-CH_3), 1.15 (m, 1 H, 22-H_a), 1.19 (m, 1 H, 24-H), 1.24 (m, 2 H, 8-CH_2CH_3), 1.46 (m, 1 H, 8-H), 1.62 (dd, ^3J = 7.2, 1.2 Hz, 3 H, 28-H), 1.66 (m, 1 H, 20-H), 1.84 (s, 3 H, 12-CH), 1.94 (s, 3 H, 4-CH_3), 1.99 (m, 2 H, 11-H_2), 2.06 (m, 1 H, 18-H), 2.26 (m, 1 H, 22-H_b), 2.30 (m, 1 H, 10-H), 2.68 (m, 1 H, 6-H), 3.14 (m, 1 H, 9-H), 3.22 (s, 3 H, 16-OCH_3), 3.25 (m, 1 H, 25-H), 3.48 (dt, ^3J = 8.0, 0.3 Hz, 1 H, 21-H), 3.54 (s, 3 H, 2-OCH_3), 3.62 (dd, ^3J = 12.0, 1.0 Hz, 1 H, 19-H), 3.68 (m, 1 H, 7-H), 3.86 (dd, ^3J = 6.0, 6.0 Hz, 1 H, 16-H), 4.88 (m, 1-H, 23-H), 5.15 (m, 1 H, 17-H), 5.19 (m, 1 H, 15-H), 5.32 (m, 1 H, 26-H), 5.63 (m, 1 H, 27-H), 5.74 (m, 1 H, 5-H), 5.76 (m, 1 H, 13-H), 6.42 (s, 1 H, 3-H), 6.58 (dd, ^3J = 14.4, 10.4 Hz, 14-H), 7.45 (d, ^3J = 10.8 Hz, 2 H, Ph*H*, 4′, 6′-H), 8.04 (d, ^3J = 10.8 Hz, 2 H, Ph*H*, 3′, 7′-H) ppm. ^{13}C NMR (125.7 MHz, CD_2Cl_2/CD_3OD, 2:1): δ = 8.2 (20-CH_3), 9.8 (18-CH_3), 13.5 (24-CH_3), 14.1 (4-CH_3), 17.7 (C-28), 23.1 (8-CH_2CH_3), 29.7 (C-8′, m_C), 36.0 (C-10), 40.0 (C-20), 41.5 (C-11), 55.9 (16-OCH_3), 60.0 (2-OCH_3), 70.0 (C-19), 76.9 (C-21), 77.9 (C-23), 83.0 (C-25), 128.6 (C-15), 129.5 (C-27), 130.4 (C-Ar), 132.5 (C-Ar), 133.54 (C-Ar), 134.0 (C-Ar), 165.6 (C-1′) ppm. ESI-MS (positive ions): *m*/*z* (%) = 1061.5 $[M + Na]^+$. ESI-MS (negative ions): *m*/*z* (%) = 1083.5 $[M + HCOO]^-$. MW calcd. = 1039.1, $C_{51}H_{67}F_9N_2O_{10}$. Not all ^{13}C-NMR signals could be detected at room temp. as described previously.[16]

23-*O*-[4-(3-Perfluorooctyl-3*H*-diazirin-3-yl)benzoyl]-21-deoxyconcanolide (17c): The reaction was performed as described above employing 21-deoxyconcanolide (**2**) (8.6 mg, 13 μmol), the benzoyl chloride of F-PAL **8c** (20 mg, 33 μmol) and DMAP (9.9 mg, 81 μmol) to furnish 0.95 mg (0.77 μmol, 5.9%) of **17c** and 4.8 mg (7.1 μmol, 56%) of the re-isolated starting material **2**. R_f = 0.52 (cyclohexane/acetone, 6:1). ^{1}H NMR (600 MHz, CD_2Cl_2/CD_3OD, 2:1): δ = 0.86 (m, 3 H, 6-CH_3), 0.86 (m, 3 H, 8-CH_2CH_3), 0.86 (m, 3 H, 18-CH_3), 0.86 (m, 3 H, 20-CH_3), 0.86 (m, 3 H, 24-CH_3), 1.05 (m, 3 H, 10-CH_3), 1.15 (m, 1 H, 22-H_a), 1.20 (m, 1 H, 24-H), 1.28 (m, 2 H, 8-CH_2CH_3), 1.38 (m, 1 H, 8-H), 1.60 (dd, ^3J = 7.0, 1.0 Hz, 28-H_3), 1.66 (m, 1 H, 20-H), 1.84 (s, 3 H, 12-CH_3), 1.95 (s, 3 H, 4-CH_3), 2.01 (m, 2 H, 11-H_2), 2.01 (m, 1 H, 18-H), 2.22 (m, 1 H, 22-H_b), 2.30 (m, 1 H, 10-H), 2.68 (m, 1 H, 6-H), 3.14 (m, 1 H, 9-H), 3.22 (s, 3 H, 16-OCH_3), 3.30 (m, 1 H, 25-H), 3.48 (dt, ^3J = 8.0, 0.3 Hz, 1 H, 21-H), 3.54 (s, 3 H, 2-OCH_3), 3.62 (dd, ^3J = 12.0, 1.0 Hz, 1 H, 19-H), 3.68 (m, 1 H, 7-H), 3.86 (dd, ^3J = 6.0, 6.0 Hz, 1 H, 16-H), 4.88 (m, 1 H, 23-H), 5.18 (m, 1 H, 17-H), 5.22 (m, 1 H, 15-H), 5.32 (m, 1 H, 26-H), 5.38 (m, 1 H, 27-H), 5.63 (m, 1 H, 13-H), 5.74 (m, 1 H, 5-H), 6.42 (s, 1 H, 3-H), 6.56 (dd, ^3J = 14.4, 10.4 Hz, 14-H), 7.45 (d, ^3J = 10.8 Hz, 2 H, Ph*H*, 4′, 6′-H), 8.04 (d, ^3J = 10.8 Hz, 2 H, Ph*H*, 3′, 7′-H) ppm. ^{13}C NMR (125.7 MHz, CD_2Cl_2/CD_3OD, 2:1): δ = 8.2 (20-CH_3), 9.7 (18-CH_3), 13.5 (24-CH_3), 17.7 (C-28), 29.8 (C-8′, m_C), 35.9 (C-10), 39.8 (C-11), 41.4 (C-20), 55.8 (16-OCH_3), 59.6 (2-OCH_3), 70.0 (C-19), 77.8 (C-23), 83.0 (C-16), 128.5 (C-15), 129.5 (C-27), 130.4 (C-Ar), 132.4 (C-Ar), 133.5 (C-Ar), 134.0 (C-Ar), 165.6 (C-1′) ppm. ESI-MS (positive ions): *m*/*z* (%) = 1261.4 $[M + Na]^+$. ESI-MS (negative ions): *m*/*z* (%) = 1237.5 $[M - H]^-$. MW calcd. = 1239.1, $C_{55}H_{67}F_{17}N_2O_{10}$. Not all ^{13}C-NMR signals could be detected at room temp. as described previously.[16]

21-*O*-[4-(3-Perfluorobutyl-3*H*-diazirin-3-yl)benzoyl]bafilomycin A_1 (18b): The reaction was performed as described above employing bafilomycin A_1 (**5**) (17.3 mg, 27.8 μmol), DMAP (8.0 mg, 65.5 μmol), EDCI (11.4 mg, 59.5 μmol) and label **8b** (8.5 mg, 22 μmol). The reaction time was elongated to 22 h to give 2.2 mg (2.2 μmol, 8.0%) of **18b**. R_f = 0.32 (cyclohexane/acetone, 6:1). ^{1}H NMR (600 MHz, CD_2Cl_2): δ = 0.76–0.82 (m, 3 H, 22-CH_3), 0.76–0.82 (m, 3 H, 16-CH_3), 0.76–0.82 (m, 3 H, 25-H_3), 0.96 (m, 3 H, 8-CH_3), 0.96 (m, 3 H, 26-H_3), 1.01 (d, ^3J = 10.0 Hz, 3 H, 18-CH_3), 1.04 (d, ^3J = 10.0 Hz, 3 H, 6-CH_3), 1.24 (m, 1 H, 20-H_a), 1.56 (m, 1 H, 18-H), 1.68 (m, 1 H, 8-H), 1.76 (m, 1 H, 22-H), 1.90 (m, 1 H, 24-H), 1.92 (m, 1 H, 9-H_a), 1.93 (s, 3 H, 10-CH_3), 1.96 (d, ^3J = 1.0 Hz, 3 H, 4-CH_3), 2.12 (m, 1 H, 9-H_b), 2.12 (m, 1 H, 16-H), 2.41 (dd, ^3J = 13.2, 7.0 Hz, 1 H, 20-H_b), 2.54 (m, 1 H, 6-H), 3.10 (s, 3 H, 14-OCH_3), 3.24 (m, 1 H, 7-H), 3.62 (s, 3 H, 2-OCH_3), 3.64 (m, 1 H, 23-H), 3.86 (dd, ^3J = 9.0, 9.0 Hz, 1 H, 14-H), 4.12 (m, 1 H, 17-H), 4.94 (d, ^3J = 9.0 Hz, 1 H, 15-H), 5.18 (m, 1 H, 13-H), 5.18 (m, 1 H, 21-H), 5.53 (m, 1 H, 19-O*H*), 5.80 (d, ^3J = 9.0 Hz, 1 H, 5-H), 5.81 (d, ^3J = 10.0 Hz, 1 H, 11-H), 6.48 (dd, ^3J = 13.0, 10.0 Hz, 1 H, 12-H), 6.66 (s, 1 H, 3-H), 7.40 (d, ^3J = 10.8 Hz, 2 H, Ph*H*, 4′, 6′-H), 8.11 (d, ^3J = 10.8 Hz, 2 H, Ph*H*, 3′, 7′-H) ppm. ^{13}C NMR (125.7 MHz, CD_2Cl_2): δ = 7.1 (18-CH_3), 9.9 (16-CH_3), 12.5 (22-CH_3), 14.1 (4-CH_3), 14.4 (24-CH_3), 17.3 (6-CH_3), 20.2 (10-CH_3), 21.1 (24-CH_3), 21.7 (8-CH_3), 29.7 (C-24), 36.7 (C-6), 37.2 (C-16), 55.6 (14-OCH_3), 60.1 (2-OCH_3), 70.6 (C-17), 75.3 (C-21), 75.6 (C-23), 77.6 (C-15), 81.2 (C-7), 82.2 (C-14), 98.8 (C-19), 124.7 (C-11), 128.0 (C-13), 130.0 (C-Ar), 133.2 (C-4), 142.9 (C-2), 167.8 (C-1) ppm. ^{19}F NMR (282 MHz, CD_2Cl_2): δ = –80.88 (m, 3 F, CF_3), –109.41 (m, 2 F, CF_2), –121.14 (m, 2 F, CF_2), –126.16 (m, 2 F, CF_2) ppm. MW calcd. = 985.0, $C_{47}H_{61}F_9N_2O_{10}$. Not all ^{13}C-NMR signals could be detected.[12]

21-*O*-[4-(3-Perfluorooctyl-3*H*-diazirin-3-yl)benzoyl]bafilomycin A_1 (18c): The reaction was performed as described above employing bafilomycin A_1 (**5**) (10.4 mg, 16.7 μmol), DMAP (4.8 mg, 39 μmol), EDCI (6.9 mg, 36 μmol) and label **8c** (7.8 mg, 13 μmol) to furnish 4.6 mg (3.9 μmol, 23%) of **18c**. R_f = 0.34 (cyclohexane/acetone, 6:1). ^{1}H NMR (600 MHz, CD_2Cl_2): δ = 0.76–0.89 (m, 3 H, 22-CH_3), 0.76–0.89 (m, 3 H, 16-CH_3), 0.76–0.89 (m, 3 H, 25-H_3), 0.90 (m, 3 H, 8-CH_3), 0.90 (m, 3 H, 26-CH_3), 0.98 (d, ^3J = 10.0 Hz, 3 H, 18-CH_3), 1.02 (d, ^3J = 10.0 Hz, 3 H, 6-CH_3), 1.26 (m, 1 H, 20-H_a), 1.58 (m, 1 H, 18-H), 1.72 (m, 1 H, 8-H), 1.80 (m, 1 H, 22-H), 1.90 (m, 1 H, 24-H), 1.90 (m, 1 H, 9-H_a), 1.93 (s, 3 H, 10-CH_3), 1.98 (d, ^3J = 1.0 Hz, 3 H, 4-CH_3), 2.13 (m, 1 H, 9-H_b), 2.13 (m, 1

H, 16-H), 2.39 (dd, ^3J = 13.2, 7.0 Hz, 1 H, 20-H_b), 2.54 (m, 1 H, 6-H), 3.24 (s, 3 H, 14-OCH_3), 3.24 (m, 1 H, 7-H), 3.62 (s, 3 H, 2-OCH_3), 3.64 (dd, ^3J = 12.0, 2.4 Hz, 1 H, 23-H), 3.90 (dd, ^3J = 9.0, 9.0 Hz, 1 H, 14-H), 4.15 (m, 1 H, 17-H), 4.91 (d, ^3J = 9.0 Hz, 1 H, 15-H), 5.12 (m, 1 H, 13-H), 5.14 (m, 1 H, 21-H), 5.44 (m, 1 H, 19-O*H*), 5.78 (d, ^3J = 9.0 Hz, 1 H, 5-H), 5.82 (d, ^3J = 10.0 Hz, 1 H, 11-H), 6.54 (dd, ^3J = 13.0, 10.0 Hz, 1 H, 12-H), 6.68 (s, 1 H, 3-H), 7.44 (d, ^3J = 10.8 Hz, 2 H, Ph*H*, 4′, 6′-H), 8.02 (d, ^3J = 10.8 Hz, 2 H, Ph*H*, 3′,7′-H) ppm. ^{13}C NMR (125.7 MHz, CD_2Cl_2): δ = 7.2 (18-CH_3), 9.9 (16-CH_3), 12.5 (22-CH_3), 14.1 (4-CH_3), 14.3 (24-CH_3), 17.4 (6-CH_3), 20.2 (10-CH_3), 21.3 (24-CH_3), 21.8 (8-CH_3), 28.3 (C-24), 37.1 (C-6), 37.6 (C-16), 40.5 (C-22), 41.6 (C-8), 41.6 (C-9), 42.3 (C-18), 55.7 (14-OCH_3), 60.2 (2-OCH_3), 71.0 (C-17), 75.6 (C-21), 76.0 (C-23), 77.9 (C-15), 81.2 (C-7), 82.7 (C-14), 99.1 (C-19), 125.3 (C-11), 127.1 (C-13), 128.3 (C-Ar), 130.2 (C-Ar), 132.6 (C-Ar), 133.1 (C-4), 133.4 (C-12), 133.8 (C-3), 141.4 (C-2), 143.4 (C-5), 143.5 (C-10), 167.5 (C-1) ppm. ^{19}F NMR (282 MHz, CD_2Cl_2): δ = –81.12 (m, 3 F, CF_3), –109.48 (m, 2 F, CF_2), –120.35 (m, 2 F, CF_2), –121.90 to –123.10 (m, 8 F, CF_2), –126.37 (m, 2 F, CF_2) ppm. ESI-MS (positive ions): *m*/*z* (%) = 1207.4 $[M + Na]^+$. ESI-MS (negative ions): *m*/*z* (%) = 1183.4 $[M - H]^-$. MW calcd. = 1185.0, $C_{51}H_{61}F_{17}N_2O_{10}$. Not all ^{13}C-NMR signals could be detected.

Supporting Information (see also the footnote on the first page of this article): Experimental procedures and analytical data for the synthesis of F-PAL **8b**,**c** and compounds **20b**,**c**, details of the purification by fluorous solid phase extraction and ^{1}H NMR spectra of compounds **17b**,**c**.

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[13] Details are described in the Supporting Information.

[14] Compounds **17a** and **18a** are the non-radioactive analogues of compounds **4** and **6**, respectively.

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