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Bafilomycin analogue site-specifically fluorinated at the pharmacophore macrolactone ring has potent vacuolar-type ATPase inhibitory activity

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ABSTRACT

Based on previously reported structure–activity relationships, an analogue of bafilomycin A_1 with a site-specific fluorine label at the C2 position was designed and efficiently synthesized. The fluorinated compound exhibited potent vacuolar-type ATPase (V-ATPase) inhibitory activity, comparable to that of the natural product, representing the first example of highly bioactive analogues with a modified macrolactone core from the plecomacrolide family compounds.

A vacuolar-type ATPase (V-ATPase) is an ATP-driven proton pump occurring in eukaryotic cells. V-ATPase acts upon acidification of endomembrane organelles such as lysosomes, endosomes, and Golgi apparatus to regulate protein degradation and transport.[1] Subtypes of V-ATPase are also distributed in the plasma membranes of various cells, including osteoclasts, renal intercalated cells, and cancer cells, and are correspondingly deeply involved in bone resorption, pH homeostasis and cell growth.[1–4] Consequently, specific inhibitors of V-ATPase have received significant attention as useful tools for functional analysis, and also as drug candidates. For example, it has been suggested that suppression of V-ATPase activity induces the apoptotic death of cancer cells.[5,6]

One of the most potent and specific V-ATPase inhibitors, bafilomycin A_1 (Baf, **1**, Scheme 1), was first isolated from *Streptomyces griseus* sp. *sulphurus*.[7] Although it has been widely utilized as a diagnostic agent for V-ATPase-implicated biological events, the molecular mechanism of action is not fully elucidated; the binding site has been deduced to be the subunit c ring of the transmembranous V_o domain.[8,9]

To elucidate the exact binding site for attachment of Baf to V-ATPase, we utilize structural analysis using $^{13}C\{^{19}F\}$REDOR,[10] which is a distance measurement solid-state NMR technique often used for complicated non-crystalline systems such as membrane proteins.[11] Preparation of a bioactive fluorine-labeled derivative is essential as the first step of this strategy; we recently successfully synthesized 24,24-didesmethyl-24-F_3-Baf (24-F-Baf) possessing a CF_3 group at the C23 position instead of the *i*-propyl group. This structure was carefully designed based on consideration of previously reported structure–activity relationship (SAR) data.[12] As anticipated we confirmed the potent V-ATPase inhibitory activity of 24-F-Baf.[13] Another objective for the synthesis of fluorine-labeled Baf is to elucidate the exact conformation of Baf in the V-ATPase bound form since knowledge of the active structure of a specific inhibitor upon binding to a potential drug target protein generally provides important information for drug development. The significance of the macrolide conformation of Baf to the overall binding structure could be determined using a site-specifically fluorinated Baf analogue at this moiety. However the potential perturbation induced by fluorine substitution[14] was of particular concern since the macrolactone ring was thought to be the pharmacophore for V-ATPase binding. Previous SAR experiments demonstrated that any modifications on the macrolactone resulted in loss of activity. For example, opening of the lactone ring,

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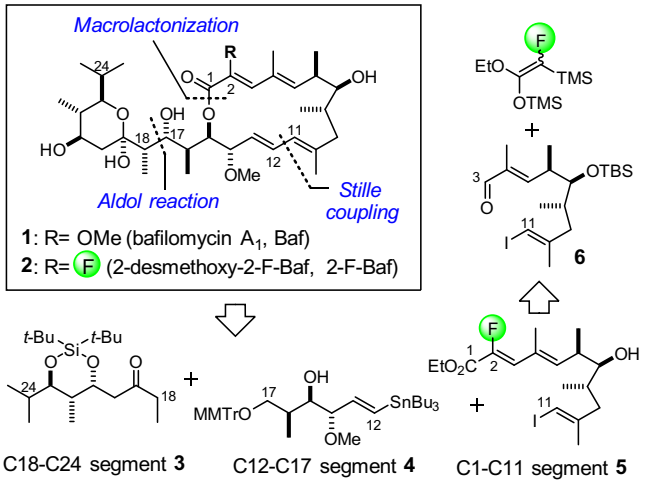
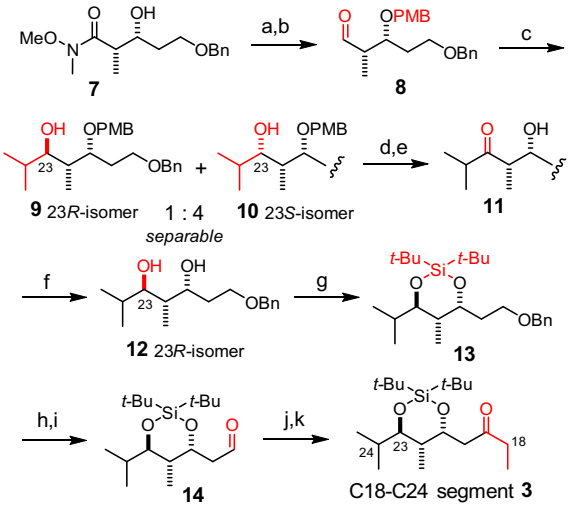


Scheme 1. Retrosynthesis of 2-F-Baf.



Scheme 2. Synthesis of the C18–C24 segment **3**. Reagents and conditions: (a) $PMBOCNHCCl_3$, $Sc(OTf)_3$, toluene, rt, 85%; (b) DIBAL, CH_2Cl_2, −78 °C, 92%; (c) *i*-PrMgBr, THF, 0 °C, 81%; (d) AZADOL, TBAB, $NaClO{\cdot}5H_2O$, KBr, CH_2Cl_2, $NaHCO_3$ aq, 0 °C, 99%; (e) DDQ, CH_2Cl_2, PBS buffer (pH 7.0), rt, 74%; (f) $Me_4NBH(OAc)_3$, AcOH, rt, 79% and 10% 23*S*-isomer; (g) $(t\text{-Bu})_2Si(OTf)_2$, 2,6-lutidine, DMF, rt, 85%; (h) H_2, Pd black, EtOH, rt, 68%; (i) Dess-Martin periodinane, $NaHCO_3$, CH_2Cl_2, rt, 86%; (j) EtMgBr, THF, rt, 99%; (k) Dess-Martin periodinane, $NaHCO_3$, CH_2Cl_2, rt, 92%.

oxidation of the 7-hydroxy group to the ketone, and even demethylation of the two methyl groups at the C6 and C8 positions remarkably diminished the activity.[12c,13b]

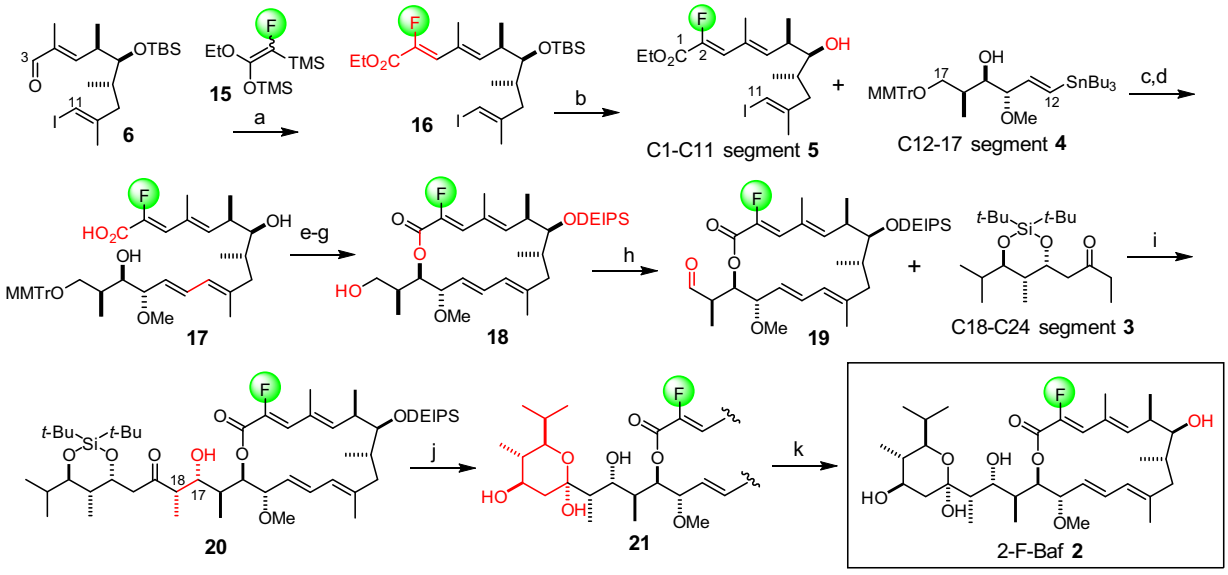
Considering the previous results, we designed and synthesized a novel fluorine-labeled Baf analogue modified at the macrolactone backbone; this analogue exhibited V-ATPase inhibitory activity comparable to that of the native structure. Compound **2** (2-F-Baf, **2**) is the first analogue with the modified macrolactone that possesses potent bioactivity.

In selecting the position for fluorine substitution, we first chose the hydroxy or methoxy group to minimize perturbation of the polarity. Among the three possible positions, the 7-hydroxy group and 14-methoxy group were ruled out because of the known biological importance and the expected instability, respectively. Hence, the 2-F-Baf derivative (2-F-Baf, **2**) was selected as a synthesis target; this moiety was expected to be easier to synthesize despite concerns about the effect of fluorine in destabilizing the surrounding dienoate moiety (Scheme 1). The strategy involved the convergent synthetic method established for synthesis of 24-F-Baf from three key segments via the Stille coupling, macrolactonization, and diastereoselective aldol reactions.[13] For synthesis of the C18–C24 segment, **3**, which was already reported by two groups,[15] we examined a new route by modifying our scheme for synthesis of the CF_3-C18–C24 segment to improve the efficiency.[13] The 2-F-labeled C1–C11 segment, **5**, could be synthesized from the known aldehyde **6** via Z-selective fluoroolefination.

Initially, the C18–C24 segment, **3**, was prepared by starting from the known Weinreb amide **7**[16] as shown in Scheme 2. After several attempts at direct alkylation to **7**,[17] it was found that transformation to the aldehyde **8** and the subsequent addition of isopropyl magnesium bromide afforded the isopropylated alcohols **9** and **10** as 1:4 diastereomer mixtures. This undesired stereoselectivity could be explained by Felkin-Ahn model applied to α-chiral aldehydes. The undesired 23*S*-epimer **10** was separately oxidized to the ketone,[18] and removal of the *p*-methoxybenzyl (PMB) group yielded the β-hydroxyketone **11**, which was followed by 1,3-*anti*-selective reduction reported by Evans to furnish the desired 23*R*-isomer **12** with good selectivity.[19] After cyclic silyl protection of the diols and silica gel purification, the desired silyl ether **13** was obtained in 85% yield, followed by a conventional sequence in four steps to afford the C18–C24 segment, **3**. The overall steps were shortened and modification of the C23 substituent was more facile than in previous reports.[15]

Subsequently, synthesis of the 2-fluorinated C1–C11 segment, **5**, and the ensuing coupling reactions leading to 2-F-Baf, **2**, were performed as shown in Scheme 3. At first, the fluoroolefination of aldehyde **6**, which was synthesized as outlined in our previous report,[13] was examined using fluoro(trimethylsilyl)ketene ethyl trimethylsilyl acetal **15** (Z:E = 1:1) in the presence of a catalytic amount of *n*-Bu_4NOAc as a Lewis base according to Michida's procedure.[20] The reaction proceeded smoothly to afford the desired (*Z*)-α-fluoroacrylate product selectively and the subsequent deprotection of *t*-butyldimethylsilyl (TBS) ether gave the C1–C11 segment, **5** in 48% yield in two steps. The Stille coupling reaction with the separately prepared C12–C17 segment, **4**[13b] and hydrolysis of the ethyl ester furnished the carboxylic acid **17**; this process was followed by macrolactonization under Yamaguchi conditions[21] to successfully furnish the fluorine-containing macrolactone in a similar yield to that obtained with the non-fluorinated seco acid.[13] Protection of the secondary alcohol with a diethylisopropylsilyl (DEIPS) group via a previously optimized process[15a] and selective removal of the monomethoxytrityl (MMTr) group with pyridinium *p*-toluenesulfonate (PPTS) furnished the primary alcohol **18** in 83% yield despite the expected lability of the fluorinated dienoate moiety under acidic conditions. After oxidation to aldehyde **19**, the (*E*)-boron enolate generated from ketone **3** was added and stirred at −78 °C for 3 h to give the desired hydroxyketone **20** with excellent stereoselectivity in 60% yield,[22] which was comparable to the data for natural Baf synthesis.[15a,23] Finally, deprotection of the two silyl groups was performed by using carefully tuned conditions, including the use of 18% HF·pyridine and TBAF with AcOH, leading to completion of the synthesis of 2-F-Baf.[24]

To evaluate the biological activity of 2-F-Baf, **2**, the inhibitory effect of V-ATPase on the acidification of acidic organelles was examined using HeLa cells (Fig. 1).[13] The control cells exhibited the red fluorescence of acidic vesicles, which were acidified by properly functioning V-ATPase. In contrast, treatment with 2-F-Baf, **2** markedly reduced the red fluorescence to a similar extent to that achieved with Baf, **1**, which implied that 2-F-Baf, **2** possessed potent V-ATPase inhibitory activity, comparable to that of the natural product. To evaluate the inhibition potency of **2**, the inhibitory effect on V-ATPase activity was evaluated based on quantification of the inorganic phosphate produced by ATP hydrolysis (Table 1).[13,25] The results demonstrated that 2-F-Baf, **2**, inhib-



Scheme 3. Synthesis of 2-F-Baf **2**. Reagents and conditions: (a) **15**, *n*-Bu_4NOAc, CH_2Cl_2, rt; (b) TBAF, THF, rt, 48% (2 steps); (c) $[Pd_2(dba)_3]\cdot CHCl_3$, Ph_3As, LiCl, NMP, rt, 63%; (d) KOH, dioxane, 80 °C; (e) 2,4,6-trichlorobenzoyl chloride, *i*-Pr_2NEt, toluene, rt, then diluted with toluene, DMAP, 34% (2 steps); (f) DEIPSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C, 90%; (g) PPTS, THF, MeOH, rt, 83%; (h) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C to 0 °C; (i) **3**, $PhBCl_2$, *i*-Pr_2NEt, CH_2Cl_2, −78 °C, 60% (2 steps); (j) 18% HF·Py, THF, rt, 67%; (k) TBAF, AcOH, THF, rt, 48%.

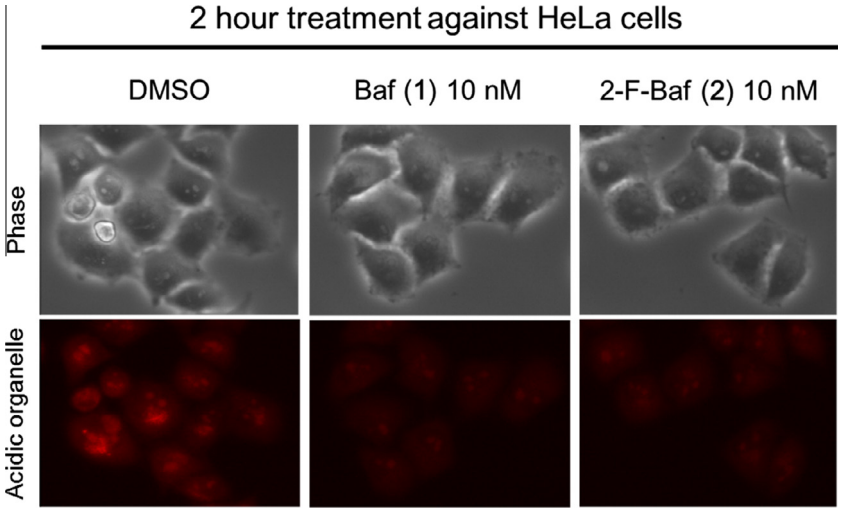


Figure 1. Effect of 2-F-Baf on acridine orange stain of HeLa cells.

ited V-ATPase function as potently as natural Baf, **1**, (IC_{50} = 5.3 and 4.3 nM, respectively), indicating that fluorine substitution at the C2 position of Baf induced no perturbation effect.

After confirming the potent activity of 2-F-Baf, we next examined whether the analogue maintained enough of the structural features of Baf to be used for accurate conformational analysis in solid-state NMR by comparing their structures in solution. The ^1H NMR data of **2** were compared with those of **1** as shown in Figure 2. Most of the ^1H chemical shifts of these two species were similar ($\Delta\delta < 0.05$ ppm), except for those of the protons close to the fluorine atom and susceptible hydroxy protons (H3, H5,17-OH, 19-OH) (see Supporting information for detailed data). Moreover, all coupling constants of the protons on the ring shown in Figure 2A were consistent with those of Baf, and NOE correlations attributed to H3/H5 and H8/H11, characteristic to Baf, were observed (Fig. 2B). These observations suggested that the entire conformation of the macrolactone ring is virtually the same as that of Baf. To confirm the conformation, a detailed structural analysis was performed using Macromodel with some restrictions derived from NMR experiments (Fig. 2B) (see Supporting information for detailed data). The superimposed structures clearly showed that 2-F-Baf and Baf have a similar conformation in the macrolactone moiety, especially in the direction of the 7-hydroxy group; this conformation was largely altered in non-active analogues such as the 6,8-desmethylated derivative.[13b] These results suggest that 2-F-Baf retains not only the V-ATPase activity but also the conformation of natural Baf in $CDCl_3$ solution.

Table 1
V-ATPase inhibition of budding yeast.

	IC_{50} (nM)
Baf **1**	4.3
2-F-Baf **2**	5.3

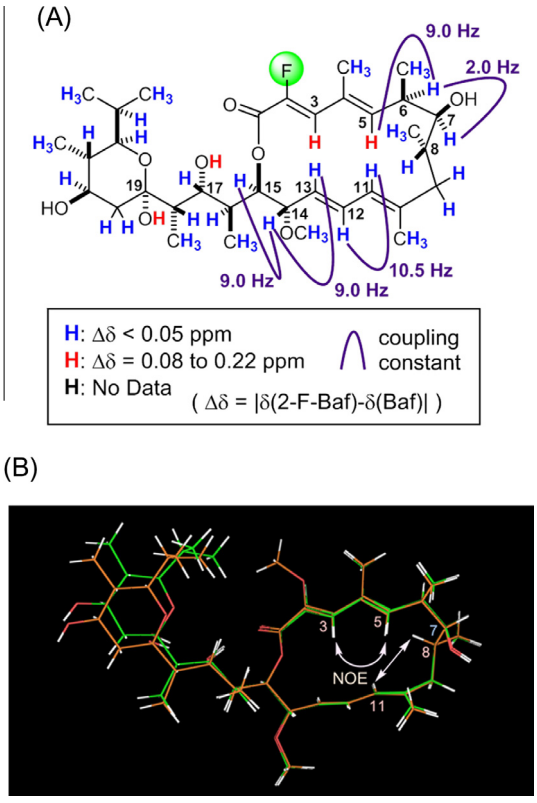


Figure 2. (A) Differences of ^{1}H NMR chemical shifts between 2-F-Baf **2** and Baf **1** and observed coupling constants in $CDCl_3$. (B) Lowest-energy conformation of Baf (orange) and 2-F-Baf (green) and experimentally observed NOE correlations of them.

It is recognized that there must be some differences between the conformation in solution and the active 3D structure upon binding to V-ATPase in the membrane, because the former is largely influenced by intramolecular hydrogen bonding, whereas, the latter is potentially affected by interaction with protein. Nevertheless, we deduce that there may be some correlations since Baf is thought to bind to the hydrophobic interface in the α-helix domains in subunit c of V-ATPase, where the formation of multiple intermolecular hydrogen bonds is less likely.[8]

In summary, we successfully synthesized site-specifically fluorinated bafiromycin A_1 at the C2 position of the macrolactone ring. This analogue exhibits potent V-ATPase inhibitory activity, and is the first bioactive agent of the plecomacrolide family compounds in which the macrolactone core is modified as far as we know. In addition, fluorine-labeling of Baf backbone with no structural perturbation would pave the way to a better understanding of the mechanism underlying V-ATPase activity and to the development of a specific inhibitor of the enzyme. Solid-state NMR experiments are now underway in our laboratory.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2016.04.075.

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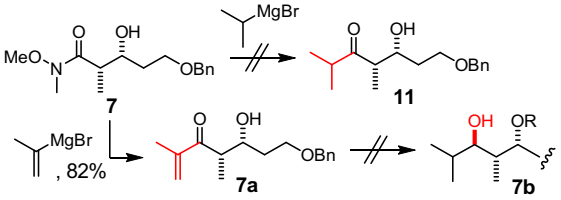

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