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Design and Synthesis of 24-Fluorinated Bafilomycin Analogue as an NMR Probe with Potent Inhibitory Activity to Vacuolar-type ATPase

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A fluorine-labeled bafilomycin analogue was designed and convergently synthesized from three segments via the Stille coupling, macrolactonization, and diastereoselective aldol reaction. The V-ATPase inhibitory activity of the analogue was comparable to that of the natural product, indicating its utility as a potential molecular probe for investigating the inhibition mechanism of bafilomycin by NMR.

Vacuolar-type ATPase (V-ATPase) is a ubiquitous proton pump that occurs in the endomembrane systems of all eukaryotic cells and in the plasma membranes of diverse animal cells.[1] This protein family has various functions including the regulation of intracellular or intraorganellar pH and the facilitation of transport processes across the membrane. In recent years, it has become more evident that the malfunction of V-ATPase is correlated with an increasing number of age-related diseases such as osteoporosis, renal tubular acidosis, and cancer. Therefore, the inhibitors of V-ATPase play a pivotal role in understanding the molecular pathology of these diseases and in developing corresponding molecular-targeting drugs. Since bafilomycin A_1 (Baf, **1**) (Figure 1) was first isolated in 1983 from *Streptomyces griseus* ssp. *sulphurus*,[2] the antibiotic has long been regarded as a representative V-ATPase inhibitor.[3] The potent and specific activity of Baf has accelerated mechanism-of-action studies, resulting in the elucidation of its putative binding site to be the transmembrane V_o domain of V-ATPase.[4] However, the precise location of the binding site remains unclear. The largest problem lies in the structural analysis of such a complicated membrane-bound system. We aimed to solve this problem using solid-state NMR techniques, especially REDOR[5] known as a powerful tool for interatomic distance measurements. For solid-state NMR measurements, labeled compounds with NMR-sensitive nucleus are essential; among several elements and isotopes, ^{19}F is often regarded as the most appropriate nucleus, despite its higher perturbations in biology, because of its characteristic properties such as a nuclear spin of 1/2, high gyromagnetic ratio, and low background signals in biological samples.[6] We have demonstrated that REDOR provides important evidence for elucidating the structure of a channel complex of ^{19}F-labeled amphotericin B in lipid bilayers.[7] In this study we designed and synthesized a novel ^{19}F-labeled Baf analogue that has potent inhibitory activity toward V-ATPase and could be used for investigating the molecular interactions between Baf and the protein.

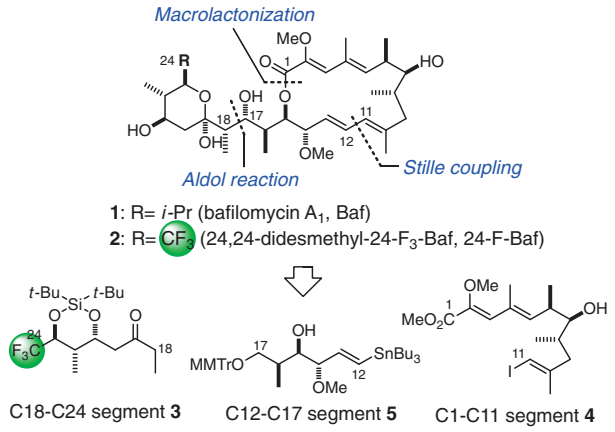


Figure 1. Structure of bafilomycin and its fluorinated analogue.

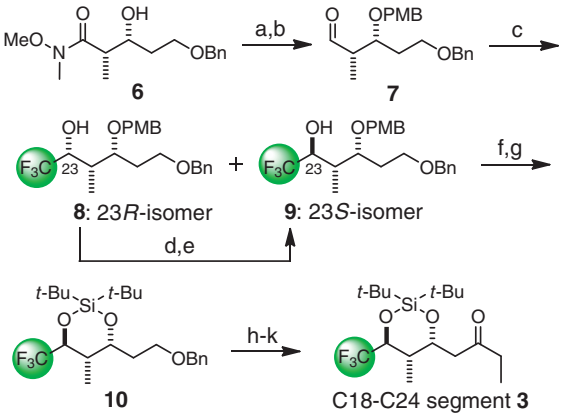
As a target molecule, 24,24-didesmethyl-24-F_3-Baf (24-F-Baf, **2**), where the isopropyl unit of **1** was replaced by a trifluoromethyl group, was designed in consideration of the following points. (i) To minimize perturbations on bioactivity brought by fluorine substitution, the tetrahydropyran (THP) portion was selected based upon previous structure–activity relationship studies.[8] (ii) Fluorine substitution for hydroxy groups in the THP ring was avoided because the destabilization of products was expected. (iii) A CF_3 group could be easily introduced and equivalently replace the *i*-Pr group in terms of the bulkiness.[9]

The efficient synthesis of **2** was planned by using the diastereoselective aldol reaction of a novel CF_3-labeled C18–C24 segment **3** and the known C1–C17 macrocyclic core,[10b] which could be constructed via the Stille coupling and macrolactonization from C1–C11 segment **4** and C12–C17 segment **5** by following previous synthetic studies of **1**.[10]

Synthesis of **3** commenced with PMB protection of the known Wienreb amide **6** prepared from 1,3-propanediol in 4 steps,[11] followed by DIBAL reduction to afford **7** (Scheme 1). Next, diastereoselective trifluoromethylation was attempted by the exposure of the aldehyde **7** with $TMSCF_3$ in the presence of TBAF, which resulted in formation of undesired 23*R*-epimer **8** as a major product. Since the chelation-controlled conditions using the β-hydroxy group of **7** was not successful, we attempted to invert the C23 configuration of **8**. After several trials,[12] the Dess–Martin oxidation of **8** followed by treatment with L-Selectride was found to give the desired epimer **9** preferentially in 4.2:1 ratio.[13] After removal of the PMB group, the resulting diol was protected as a cyclic silyl ether to furnish **10**. Hydrogenolysis of the benzyl group followed by the Dess–Martin oxidation afforded an aldehyde, which was then treated

with the Grignard reagent and the produced alcohol was oxidized to give **3** in 80% yield for 4 steps.

Next, preparation of the C1–C11 segment **4** was carried out via carbon elongations from the known hydroxyketone **11**[14] as shown in Scheme 2. Enantiopure alcohol **11** was silylated with TBSOTf and 2,6-lutidine, and the reduction of 9-ketone

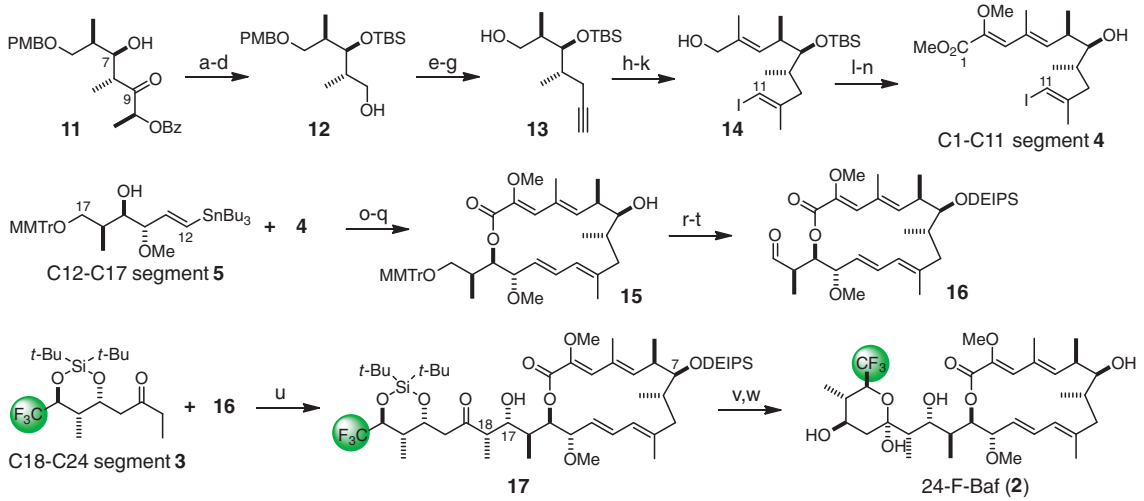


Scheme 1. Synthesis of the C18–C24 segment **3**. Reagents and conditions: a) $PMBOCNHCCl_3$, $Sc(OTf)_3$, toluene, rt, 70%; b) DIBAL, CH_2Cl_2, −78 °C, 80%; c) $TMSCF_3$, TBAF, THF, rt, 49% for **8**, 27% for **9**; d) Dess–Martin periodinane, $NaHCO_3$, CH_2Cl_2, rt, 92%; e) L-Selectride, THF, −78 °C, 68% for **9**, 16% for **8**; f) DDQ, CH_2Cl_2, H_2O, rt, then AcOH, THF, 40 °C, 79%; g) $(t\text{-Bu})_2Si(OTf)_2$, 2,6-lutidine, DMF, rt, 89%; h) Pd/C, H_2, EtOAc, rt, 98%; i) Dess–Martin periodinane, $NaHCO_3$, CH_2Cl_2, rt, 94%; j) EtMgBr, THF, rt, 93%; k) Dess–Martin periodinane, $NaHCO_3$, CH_2Cl_2, rt, 90%.

followed by one-pot treatment with K_2CO_3 furnished the diol. Then oxidative cleavage and $NaBH_4$ reduction resulted in the formation of **12**. Then, the primary alcohol **12** was converted to alkyne **13**[15] via tosylation, addition of lithium acetylide and removal of the PMB group. Then the resultant alcohol **13** was converted to **4**, chiefly following a report by Marshall;[10f,16] after three carbon elongation of the allylic alcohol moiety in 3 steps, the formation of the vinyl iodide was performed under Negishi's conditions[17] to produce **14**. The TEMPO oxidation of the allylic alcohol to aldehyde and the subsequent Horner–Wadsworth–Emmons reaction proceeded in a *Z*-selective manner, which was followed by removal of the TBS group to afford the C1–C11 segment **4** successfully.

Next, the coupling reaction between **4** and separately prepared C12–C17 segment **5**[18] and construction of the macrocyclic structure were performed following the previous protocol.[10] The Stille coupling of segments **4** and **5** under the conditions reported by Marshall et al.[10f] efficiently gave the *E*,*E*-diene product in 80% yield. Then, saponification of methyl ester[10b] afforded the corresponding seco acid and the following macrolactonization was examined under Yamaguchi conditions[10e,19] to furnish the desired macrolactone **15** in moderate yield.

Since macrolactone **15** was successfully obtained, connection with **3** by diastereoselective aldol reaction established by Evans et al.[10a] was next attempted. First, the secondary alcohol of **15** was protected as a diethylisopropylsilyl ether, which was necessary for the following reactions.[10b] The deprotection of the primary alcohol with PPTS followed by the Swern oxidation provided **16**, which was treated with **3** under the reported conditions.[10a,10b] As a result, the diastereoselective aldol reaction proceeded smoothly to afford the desired β-hydroxyketone **17** in



Scheme 2. Synthesis of fluorinated bafilomycin derivative **2**. Reagents and conditions: a) TBSOTf, 2,6-lutidine, −78 °C; b) $NaBH_4$, THF, MeOH, rt, then K_2CO_3, rt, 79% (2 steps); c) $NaIO_4$, CH_2Cl_2, pH 7.0 buffer, rt; d) $NaBH_4$, THF, MeOH, rt, 91% (2 steps); e) TsCl, Py, rt, 99%; f) $LiCCH\cdot H_2NCH_2CH_2NH_2$, DMSO, rt, 64%; g) DDQ, CH_2Cl_2, pH 7.0 buffer, 94%; h) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C; i) $Ph_3P{=}C(Me)CO_2Et$, toluene, 100 °C; j) DIBAL, CH_2Cl_2, −78 °C, 83% (3 steps); k) Cp_2ZrCl_2, $AlMe_3$, $ClCH_2CH_2Cl$, 60 °C then I_2, THF, −30 °C, 66%; l) TEMPO, TBACl, NCS, CH_2Cl_2, pH 8.6 buffer, rt, 88%; m) $(i\text{-PrO})_2P(O)CH(OMe)CO_2Me$, KHMDS, 18-crown-6 ether, THF, rt, 49%; n) TBAF, THF, rt, 84%; o) $[Pd_2(dba)_3]\cdot CHCl_3$, Ph_3As, LiCl, NMP, rt, 85%; p) KOH, dioxane, 80 °C; q) 2,4,6-trichlorobenzoyl chloride, $i\text{-Pr}_2NEt$, toluene, rt, then diluted with toluene, DMAP, rt, 51% (2 steps); r) DEIPSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C, 85%; s) PPTS, THF, MeOH, rt, 78%; t) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C; u) $PhBCl_2$, $i\text{-Pr}_2NEt$, CH_2Cl_2, −78 °C, 32% (2 steps); v) 18% HF·Py, THF, rt; w) $TsOH\cdot H_2O$, CH_2Cl_2, MeCN, H_2O, rt, then HPLC purification, 36% (2 steps).

32% yield over 2 steps with 20:1 diastereomer ratio.[20] This result clearly revealed that the CF_3 moiety had no adverse effect on the high stereoselectivity reported in the syntheses of **1**.[10a,10b] Finally, stepwise removal of silyl groups was achieved using HF·Py, followed by TsOH·H_2O, to afford 24-F-Baf (**2**) in 36% yield after HPLC purification.

The V-ATPase inhibitory activity of 24-F-Baf (**2**) was evaluated by two methods (See Supporting Information for details).[21,22] First, the effect on the acidification of intracellular acidic organelles by V-ATPase in rat 3Y1 fibroblasts was examined. Treatment with 100 nM **2** caused the complete disappearance of red fluorescence in acridine orange staining, indicating that **2** strongly inhibited V-ATPase in the cells. Next, to evaluate the inhibition of V-ATPase directly and quantitatively, we tested the effect of **2** on V-ATPases obtained from the purified vacuole membrane of budding yeast by measuring the liberated inorganic phosphate from ATP. The V-ATPase activity was inhibited by **2** in a dose-dependent manner and its IC_{50} value (2.5 nM) was comparable with that of natural bafilomycin **1** (2.3 nM). These results demonstrated that our rational design led to the preparation of the first fluorine-labeled analogue of bafilomycin with potent ATPase inhibitory activity.

In summary, the fluorine-labeled Baf analogue (24-F-Baf, **2**) was properly designed and convergently synthesized from three key segments. 24-F-Baf (**2**) was shown to possess potent V-ATPase inhibition activity comparable with that of **1** and expected to be a potential molecular probe for elucidating the inhibition mechanism of bafilomycin using solid-state NMR and other spectroscopic techniques.

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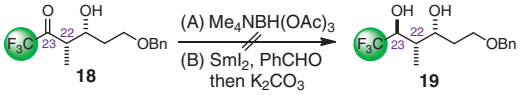

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