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Synthesis of 28-^{19}F-amphotericin B methyl ester

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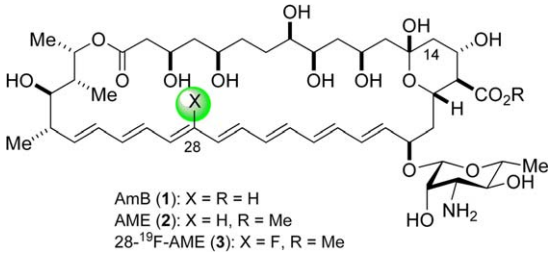
Abstract—A fluorinated amphotericin B (AmB) derivative, 28-^{19}F-AmB methyl ester (**3**), labeled at the polyene moiety, was synthesized by combining chemical synthesis with degradation of a natural product via cross-coupling reactions and macrolactonization. The fluorinated derivative **3** showed antifungal activity similar to that of AmB, and is expected to be a powerful tool for NMR-based investigation of the mechanism of ion-channel formation.

Amphotericin B (AmB, **1**) is a polyene macrolide antibiotic which, despite its severe side effects, has long been used as a standard drug for treatment of deep-seated systemic fungal infections.[1] A variety of modified AmB products, such as amphotericin B methyl ester (AME, **2**) and various amide, *N*-alkyl and *N*-acyl derivatives, have been prepared with the aim of developing more effective and less toxic drugs.[2] Although it is widely accepted that AmB associates with sterols in the phospholipid bilayer membrane of the target cell to form barrel-stave type pores,[3] details of the molecular architecture of the ion-channel assembly remain unclear.[4,5] We have been investigating the mode of action of the drug in lipid bilayer membranes, particularly the mechanism of ion-channel formation by AmB and sterol molecules, and molecular recognition between AmB/AmB,[6] AmB/phospholipid,[7] and AmB/sterol.[8] In these experiments, ^{19}F-labeled AmB derivatives are expected to be a versatile tool for examining intermolecular interactions via NMR, due to the particular properties of ^{19}F: its nuclear spin of 1/2, high gyromagnetic ratio, 100% natural abundance, and low background signal in biological systems.[9] Recently, we succeeded in preparing a bioactive fluorinated AmB derivative, (14*S*)-14-^{19}F-AmB, labeled at the polyol side.[10] We then turned our attention to fluorinated AME, which is expected to possess biological activities comparable to those of AmB, and should serve as a versatile intermediate for the preparation of covalent conjugates of AmB. Herein, we report a practical synthesis of 28-^{19}F-amphotericin B methyl ester (28-^{19}F-AME, **3**), labeled at the polyene moiety, which is expected to be useful in NMR-based investigations of the mechanism of ion-channel formation.



Although total synthesis of AmB was achieved by Nicolaou in 1987,[11,12] and remarkable progress has recently been made in the total synthesis of polyene macrolides,[13] full assembly of the complex molecule can only be achieved over a large number of steps. For a practical and versatile synthesis of labeled AmB derivatives, we envisaged a hybrid synthetic strategy combining chemical synthesis and degradation of the natural product, as shown in Scheme 1. Compound **3**, 28-^{19}F-AME which seems to be most expeditiously prepared, was to be synthesized via a Stille coupling[14]–macrolactonization[15] sequence, although until very recently there has been no precedent for this in the synthesis of polyene–polyol macrolides.[16] The C1–C21 segment **4** was to be prepared

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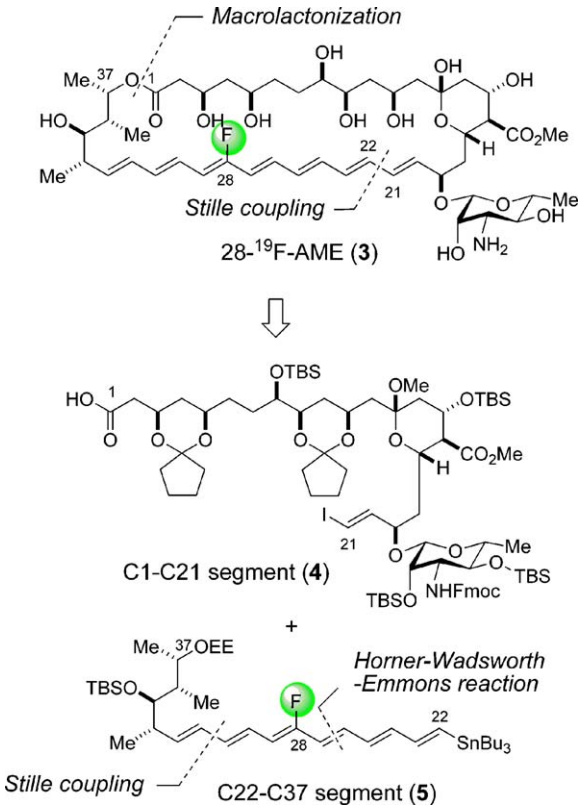
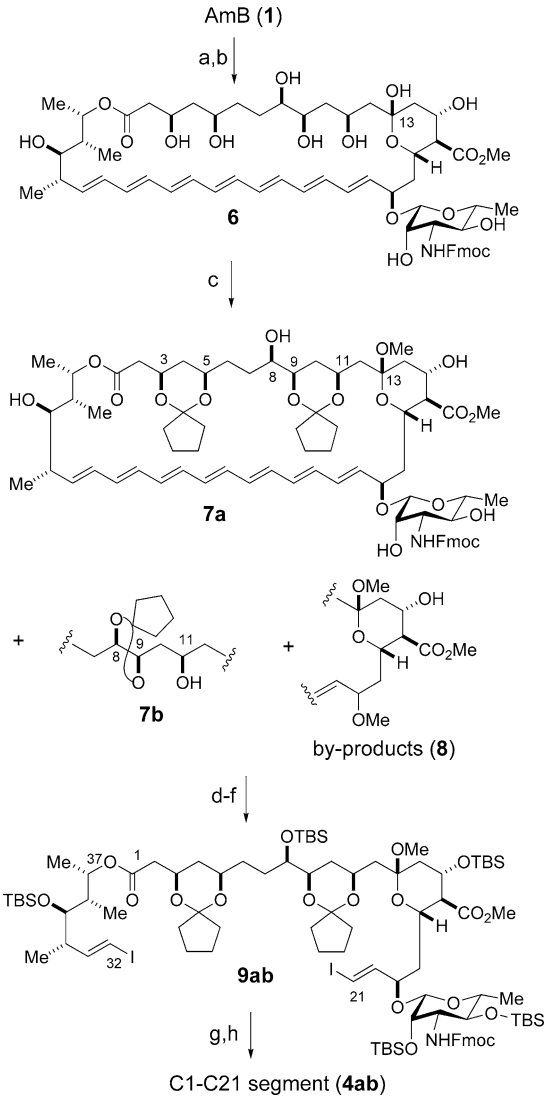


Scheme 1. Plan for synthesis of 28-^{19}F-AME.

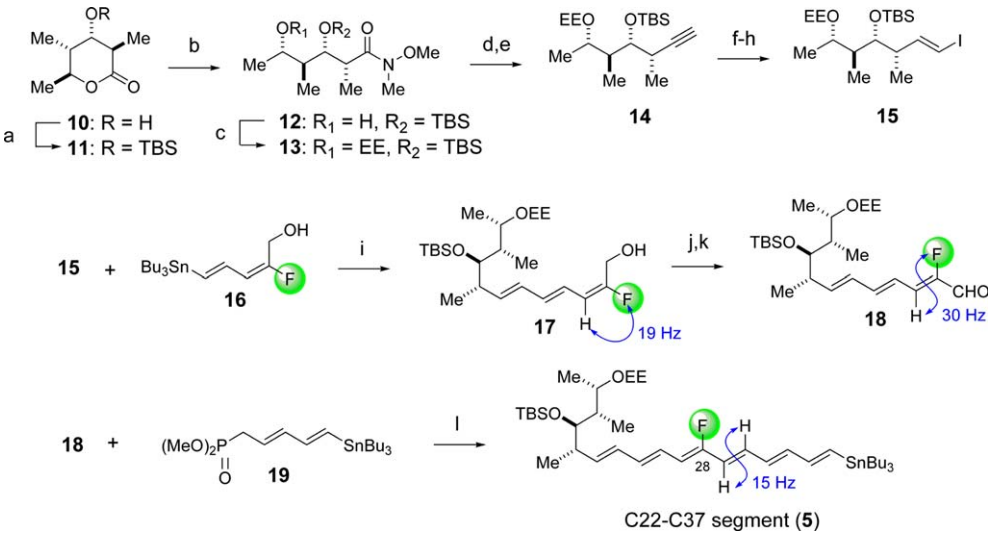
via degradation from natural AmB,[17] which is commercially available in large quantities.[18] In the synthesis of the ^{19}F-labeled C22–C37 segment **5**, Stille coupling and Horner–Wadsworth–Emmons reactions[19] were envisioned for construction of the heptaene moiety.

Preparation of the C1–C21 segment **4** is depicted in Scheme 2. Treatment of AmB with 9-fluorenylmethylsuccinimidylcarbonate (Fmoc-OSu) followed by methyl iodide afforded **6** in 79% yield over two steps. In the synthesis of **3**, the final deprotection steps, especially hydrolysis of the ketals under acidic conditions, were expected to be problematic due to the acid-labile property of AmB.[11f] After considerable experimentation under both reported and modified conditions, cyclopentylidene ketals were chosen as protecting groups for the 1,3- or 1,2-diols (vide infra).[20] Thus, treatment of the polyol **6** with CSA and dimethoxycyclopentane in MeOH resulted in the formation of bis-ketals **7a** and **7b** as an inseparable mixture (**7a**:**7b** = 1:1), with conversion of the hemiketal at C13 to methyl ketal, and concomitant formation of deglycosidated by-products **8** (and/or other isomers, **7ab**:**8** = 1:0.2–1:0.4). For synthesis of the C1–C21 segment, the remaining hydroxy groups of **7ab** were protected as TBS ethers. Exhaustive ozonolysis of the heptaene moiety[11c] followed by Takai olefination[21] of the resulting dialdehyde gave bis(*trans*-iodoolefin) **9ab**.[17] Selective saponification in the presence of methyl ester with LiOH afforded the C1–C21 segment **4ab** as an inseparable mixture of ketal regioisomers (removal of the Fmoc group occurred under saponification conditions, but reprotection was then carried out using Fmoc-OSu).



Scheme 2. Reagents and conditions: (a) Fmoc-OSu, pyridine, DMF, rt, 14.5 h; (b) CH_3I, Na_2CO_3, DMF, rt, 3.5 h, 79% (2 steps); (c) cyclopentanone dimethyl acetal, CSA, MeOH, 30 min; (d) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C, 50 min, 57% (2 steps); (e) O_3, −78 °C, then PPh_3, rt, 14 h, 57%; (f) $CrCl_2$, CHI_3, THF, rt, 18 h, 65%; (g) LiOH, THF, H_2O, MeOH, rt, 24 h; (h) Fmoc-OSu, pyridine, DMF, rt, 11 h, 50% (2 steps).

Synthesis of the C22–C37 segment **5** commenced with hydroxy δ-lactone **10**, reported by Carreira,[22] as shown in Scheme 3. Protection of the secondary alcohol of **10** as a TBS ether, followed by ring-opening of the lactone **11** by the action of Me_3Al, gave Weinreb amide **12**.[23] The resulting secondary alcohol was protected as ethoxyethyl (EE) ether **13**, which was converted to terminal alkyne **14** in two steps, that is, reduction with DIBAL and treatment of the resulting aldehyde with lithium trimethylsilyldiazomethane.[24] Hydrostannation[25] of **14**, followed by treatment of the resulting alkenylstannane with iodine, afforded *E*-iodoolefin **15** (partial removal of the EE group occurred under these reaction conditions, but further treatment with ethyl vinyl ether resulted in recovery of **15**). Stille coupling of **15** with fluorinated alkenylstannane **16**[26] yielded 28*E*-fluoroolefin **17**, which was further converted to unsaturated

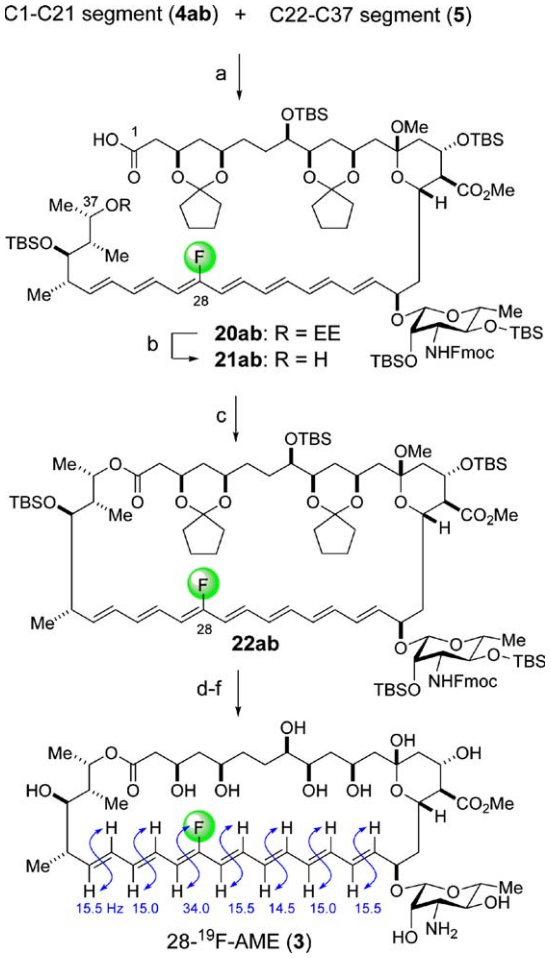


Scheme 3. Reagents and conditions: (a) TBSOTf, 2,6-lutidine, CH_2Cl_2, −45 °C, 40 min, 80%; (b) HNMeOMe HCl, Me_3Al, CH_2Cl_2, rt, 6 h; (c) ethyl vinyl ether, PPTS, CH_2Cl_2, rt, 2.5 h, 90% (2 steps); (d) DIBAL, THF, −78 °C, 50 min, 92%; (e) $TMSCHN_2$, *n*-BuLi, THF, −78 °C then 0 °C, 80%; (f) *n*-Bu_3SnH, $PdCl_2(PPh_3)_2$, THF, 0 °C, 20 min, 89%; (g) I_2, THF, 0 °C, 10 min; (h) ethyl vinyl ether, PPTS, CH_2Cl_2, rt, 15 min, 86% (2 steps); (i) $PdCl_2(MeCN)_2$, DMF, rt, 1 h, 97%; (j) Dess–Martin periodinane, CH_2Cl_2, rt, 30 min; (k) PhSeSePh, hν, CH_2Cl_2, rt, 35 min, 68% (2 steps); (l) LiHMDS, THF, −78 °C then 0 °C, 84%.

aldehyde 28*Z*-**18**, as a single isomer, by Dess–Martin oxidation[27] and subsequent isomerization with PhSeSePh under irradiation by a tungsten lamp.[28] Horner–Wadsworth–Emmons reaction of stannylphosphonate **19**[29] with aldehyde **18** produced the fluorinated polyene segment **5** as a single isomer.

The critical sections of the present synthesis—Stille coupling of large segments **4ab** (C1–C21) and **5** (C22–C37), and subsequent macrolactonization—were conducted as shown in Scheme 4. A mixture of iodide **4ab** and stannane **5** in DMF was treated with $Pd_2(dba)_3{\cdot}CHCl_3$ in the presence of Ph_3As and *i*-Pr_2NEt.[30] The coupling reaction, even in the complex polyene–polyol system, proceeded smoothly to afford heptaene **20ab** in 70% yield.[17] Selective removal of the EE group with PPTS in MeOH in the presence of cyclopentanone dimethylacetal (to avoid removing the cyclopentylidene ketals under the reaction conditions) gave **21ab** in 79% yield. The next critical step was the unprecedented macrolactonization of the nonconjugated carboxylic acid, which was achieved using the method of Shiina et al.[31] Lactonization of the seco acid **21ab** by treatment with MNBA (2-methyl-6-nitrobenzoic anhydride) in the presence of DMAP in CH_2Cl_2 proceeded smoothly to afford the fluorinated macrolactone **22ab** in 69% yield.

The final global deprotection steps were carried out under carefully controlled conditions. The TBS groups of **22ab** were removed by treatment with 18% HF–pyridine in MeOH at 50 °C to yield a pentaol (82%), after which the Fmoc group was removed with piperidine (79%) prior to ketal hydrolysis to prevent the formation of deglycosidated by-products. Removal of the ketals with PPTS under mildly acidic conditions[20] proved to be too sluggish, and treatment with CSA in MeOH[11f] followed by addition of water resulted in the formation of considerable amounts of mono-ketals with



Scheme 4. Reagents and conditions: (a) $Pd_2(dba)_3{\cdot}CHCl_3$, $AsPh_3$, *i*-Pr_2NEt, THF, rt, 3 h, 70%; (b) PPTS, cyclopentanone dimethyl acetal, MeOH, rt, 1.5 h, 79%; (c) 2-methyl-6-nitrobenzoic anhydride, DMAP, CH_2Cl_2, rt, 1.5 h, 69%; (d) HF–pyridine, pyridine, MeOH, 50 °C, 27 h, 82%; (e) piperidine, MeOH, rt, 3 h, 79%; (f) HCl, MeOH, 0 °C, 34 h, then HCl, H_2O, *t*-BuOH, rt, 1 h, 53%.

decomposed by-products; however, stepwise removal of the ketals with HCl[16] gave satisfactory results. The pentaol was treated with HCl in MeOH (86 mM) at 0 °C for methanolysis of the cyclopentylidene groups, and the reaction was then quenched by addition of solid $NaHCO_3$. After removal of the salts and solvents, the residue was treated with aqueous HCl (86 mM) at room temperature for hydrolysis of the methyl ketal, affording 28-^{19}F-AME **3** in 53% yield.

The configuration of the heptaene moiety was unambiguously determined based on $^3J_{H,H}$ and $^3J_{H,F}$ values (Scheme 4) by 920 MHz ^{1}H NMR analysis.[32] The antifungal activity of **3** against *Aspergillus niger* is comparable to that of AmB (MIC = 10 μg/disc)[10,33] which suggests that the use of **3** may be a powerful tool for NMR-based investigations of the mechanism of ion-channel formation.

In summary, we have developed a practical and versatile method for synthesizing a fluorinated AmB derivative by combining chemical synthesis with degradation of a natural product. The present method should be applicable to the preparation of ^{13}C-labeled AmB derivatives for solid-state NMR measurements; investigations in this area are currently in progress in our laboratory.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2006.06.159.

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32. Compound **3**: Chemical shifts are reported in δ (ppm) using residual DMSO as an internal standard of δ 2.50 for ^{1}H NMR, and DMSO-d_6 as that of δ 39.50 for ^{13}C NMR, respectively. ^{1}H NMR (920 MHz, DMSO-d_6) δ 6.56 (1H, dd, $J = 14.5$, 12.0 Hz, H24), 6.56 (1H, dd, $J = 15.5$, 11.0 Hz, H26), 6.43 (1H, dd, $J = 15.0$, 10.5 Hz, H22), 6.34 (1H, dd, $J = 14.5$, 11.0 Hz, H25), 6.33 (1H, dd, $J = 15.0$, 11.0 Hz, H30), 6.29 (1H, dd, $J = 15.0$, 12.0 Hz, H23), 6.23 (1H, dd, $J = 15.0$, 10.5 Hz, H31), 6.17 (1H, dd, $J = 28.0$, 15.5 Hz, H27), 6.12 (1H, dd, $J = 15.5$, 10.5 Hz, H32), 6.12 (1H, dd, $J = 15.5$, 10.5 Hz, H21), 5.96 (1H, dd, $J = 15.5$, 9.0 Hz, H20), 5.75 (1H, dd, $J = 34.0$, 11.0 Hz, H29), 5.51 (1H, dd, $J = 15.5$, 9.5 Hz, H33), 5.18 (1H, m, H37), 4.37 (1H, m, H19), 4.25 (1H, s, H1′), 4.23 (2H, m, H11, H17), 4.05 (1H, m, H3), 4.03 (1H, m, H15), 3.62 (3H, s, $-CO_2CH_3$), 3.59 (1H, m, H5), 3.51 (1H, br s, H2′), 3.46 (1H, m, H9), 3.12 (1H, m, H35), 3.11 (1H, m, H8), 3.05 (1H, m, H5′), 2.86 (1H, m, H4′), 2.29 (2H, m, H3′, H34), 2.20 (1H, dd, $J = 16.5$, 9.0 Hz, H2a), 2.14 (1H, dd, $J = 16.5$, 3.0 Hz, H2b), 2.08 (1H, m, H16), 1.90 (1H, dd, $J = 12.0$, 5.0 Hz, H14$_{eq}$), 1.87 (1H, dd, $J = 16.0$, 5.5 Hz, H18a), 1.71 (1H, m, H36), 1.55 (1H, m, H18b), 1.48–1.57 (2H, m, H10), 1.45–1.53 (2H, m, H7), 1.26–1.40 (6H, m, H4, H6, H12), 1.15 (3H, d, $J = 6.0$ Hz, H6′), 1.13 (1H, m, H14$_{ax}$), 1.12 (3H, d, $J = 6.5$ Hz, H38), 1.04 (3H, d, $J = 6.5$ Hz, H40), 0.91 (3H, d, $J = 7.0$ Hz, H39); ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.82, 170.15, 156.20 (d, $^{1}J_{CF} = 252$ Hz), 137.22, 137.13, 136.72, 136.11, 135.97, 134.51, 132.45, 131.36, 131.04, 130.68, 129.75 (d, $^{2}J_{CF} = 14.5$ Hz), 128.73, 123.08, 121.65, 97.38, 97.24, 96.66, 73.80, 73.36, 73.14, 72.96, 70.02, 69.84, 69.28, 69.13, 67.15, 66.56, 65.87, 64.58, 56.82, 56.46, 45.90, 44.52, 44.14, 42.44, 42.26, 41.07, 36.80, 34.90, 28.98, 18.46, 17.97, 17.06, 11.96.; ESI-MS, m/z 978 ($M+Na^{+}$); UV: λ_{max}(DMSO) 416 nm ($\varepsilon_{max} = 1.02 \times 10^{5}$); HPLC conditions: column, COSMOSIL® 5C$_{18}$-MS-II ($\Phi 10 \times 250$ mm, Nacalai Tesque Inc.); flow rate, 1.5 mL/min; solvent system, 70% MeOH in ammonium acetate buffer to 100% MeOH in 30 min,-$t_R = 24.7$ min; detection, $\lambda = 408$ nm.
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