Synthesis of 25-^{13}C-Amphotericin B Methyl Ester: A Molecular Probe for Solid-state NMR Measurements

Naohiro Matsushita, Yukiko Matsuo, Hiroshi Tsuchikawa, Nobuaki Matsumori, Michio Murata, and Tohru Oishi*
Department of Chemistry, Graduate School of Science, Osaka University, 1-1 Machikaneyama, Toyonaka, Osaka 560-0043

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A ^{13}C-labeled amphotericin B (AmB) derivative was synthesized based on a hybrid strategy combining chemical synthesis with degradation of a natural product through successive cross-coupling reactions and macrolactonization. The specimen regiospecifically ^{13}C-labeled (99% enrichment) at C25 position corresponding to the polyene moiety would be a powerful tool for structural analysis of the molecular assembly formed by AmB based on solid-state NMR measurements.

Amphotericin B (AmB, **1**) is a polyene macrolide antibiotic produced by *Streptomyces nodosus*, which has been used as a standard drug for treatment of deep-seated systemic fungal infections for over 40 years.[1] 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,[2] details of the molecular architecture of the ion-channel assembly remain unclear.[3] During the course of our studies on the structure of the assembly based on solid-state NMR measurements,[4] we have investigated the interaction between AmB and sterols using an AmB–sterol conjugate,[5] in which the AmB moiety was uniformly enriched with ^{13}C (≈50% average labeling index) prepared by fermentation in the presence of U–^{13}C glucose.[6] However, specimens labeled at specific positions are required to estimate the accurate inter-atomic distances between ^{13}C and ^{19}F nuclei by solid-state NMR. Although it is known that ^{13}C-enriched AmB selectively labeled at C39, C40, and C41 corresponding to the terminal of the molecule is produced by feeding with 3-^{13}C-propionate (7–10% labeling index),[6] selective incorporation of ^{13}C at the middle of the molecule is unachievable by fermentation. Therefore, chemical synthesis[7] would be a practical way to provide regioselectively labeled specimens with high labeling index (≈99%), since we have already developed a versatile method for synthesizing the AmB derivative in which H28 was substituted with ^{19}F.[8] Herein we report a synthesis of 25-^{13}C-AmB methyl ester **2** labeled at the central part of the polyene moiety based on the hybrid synthetic strategy combining chemical synthesis and degradation of the natural product.

The ^{13}C-labeled derivative **2** was to be constructed by the Stille coupling of the C1–C21 and C22–C37 segments followed by macrolactonization (Figure 1). Synthesis of the polyene part corresponding to the C22–C37 segment **9** commenced with the Stille coupling of the iodoolefin **3**[8,9] and stannane **4**[10] to afford phosphonate **5** in 86% yield (Scheme 1). On the other hand, the Wittig reaction of aldehyde **7**[11] with ^{13}C-labeled ylide **6**[12] prepared from commercially available 2-^{13}C-bromoacetic acid (99% labeling index), followed by reduction of the resulting α,β-unsaturated ester with DIBAL-H (85%, 2 steps) and the Dess–Martin oxidation afforded aldehyde **8** (87%). The Horner–Emmons reaction of **8** with phosphonate **5** resulted in the formation of heptaene **9** as a single isomer (68%).

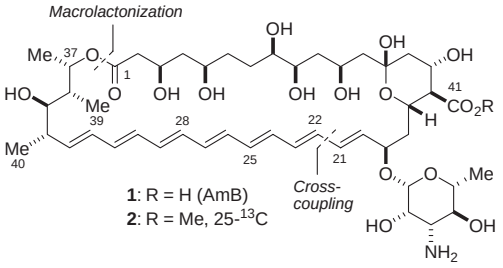
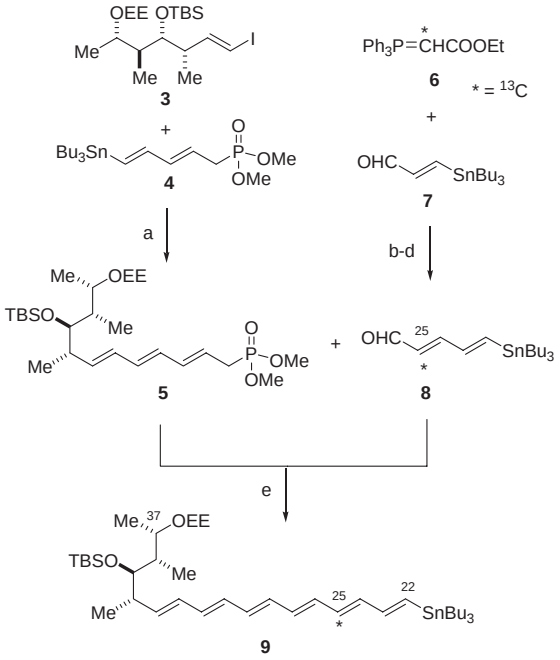
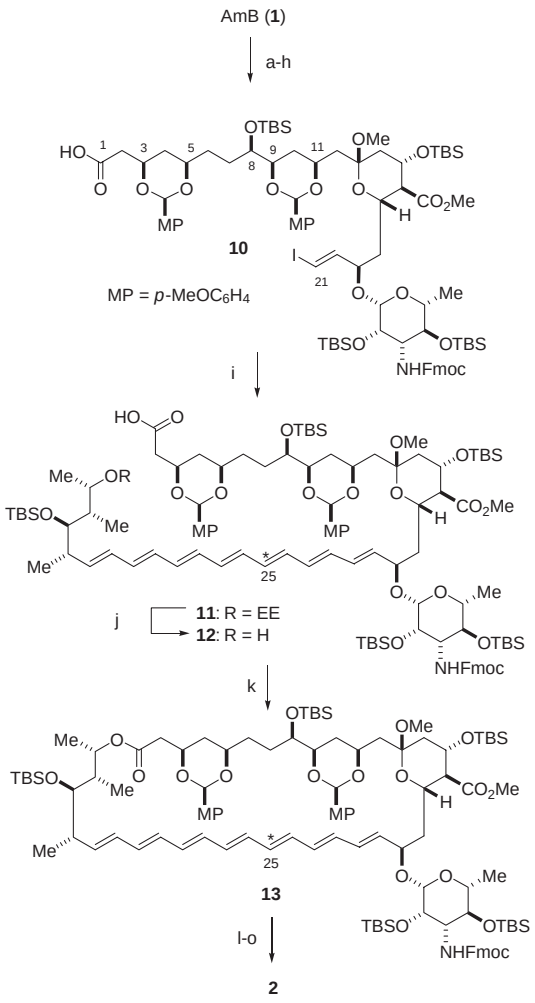


Figure 1. Structures of AmB and 25-^{13}C-AmB methyl ester.



Scheme 1. Synthesis of the C22–C37 segment **9**. Reagents and conditions: a) $[Pd_2(dba)_3]\cdot CHCl_3$, $(i\text{-Pr})_2NEt$, DMF, rt, 86%; b) toluene, 65 °C; c) DIBAL-H, CH_2Cl_2, −78 °C, 85% (2 steps); d) Dess–Martin periodinane, pyridine, CH_2Cl_2, 0 °C to rt, 87%; e) LHMDS, THF, 0 °C, 68%.

Synthesis of the C1–C21 segment **10** and coupling with the C22–C37 segment **9** was carried out as shown in Scheme 2. Degradation of natural AmB[8,13] via (i) protection of the amino group with an Fmoc group and the carboxylic acid as methyl ester (86%, 2 steps), (ii) selective protection of the 1,3-diols (3,5- and 9,11-positions) as *p*-methoxybenzylidene (MP) acetals and remaining hydroxy groups as TBS ethers (68%, 2 steps), (iii) exhaustive ozonolysis of the heptaene moiety (65%) and the subsequent Takai olefination[14,15] of the resulting aldehyde (63%), and (iv) selective saponification in the presence of methyl ester and



Scheme 2. Synthesis of 25-^{13}C-AmB methyl ester **2**. Reagents and conditions: a) Fmoc-OSu, pyridine, DMF, rt; b) CH_3I, Na_2CO_3, DMF, rt, 86% (2 steps); c) CSA, *p*-$MeOC_6H_4CH(OMe)_2$, MeOH, rt; d) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C, 68% (2 steps); e) O_3, CH_2Cl_2, MeOH, −78 °C, 2 h, then PPh_3, rt, 65%; f) $CrCl_2$, CHI_3, THF, rt, 63%; g) LiOH, THF, H_2O, MeOH, rt; h) Fmoc-OSu, pyridine, DMF, rt, 63% (2 steps); i) **9**, $[Pd_2(dba)_3]\cdot CHCl_3$, Ph_3As, $(i\text{-}Pr)_2NEt$, THF, rt; j) PPTS, *p*-$MeOC_6H_4CH(OMe)_2$, MeOH, rt; k) 2-methyl-6-nitrobenzoic anhydride, DMAP, CH_2Cl_2, rt, 26% (3 steps); l) HF·pyridine, MeOH, 50 °C; m) piperidine, MeOH, rt, 64% (2 steps); n) HCl, MeOH, 0 °C, 1 h, quenched with $NaHCO_3$ (solid); o) solvent exchange to *t*-BuOH, H_2O, then HCl, 0 °C, 5 h, 53% (2 steps); then HPLC purification (16%).

reprotection of the amino group afforded the C1–C21 segment **10** (63%, 2 steps). The Stille coupling of the iodide **10** with stannane **9** by treating with $[Pd_2(dba)_3]\cdot CHCl_3$ in the presence of Ph_3As and $(i\text{-}Pr)_2NEt$[16] resulted in the formation of **11**. Selective removal of the EE group with PPTS was followed by macrolactonization of the seco acid **12** by the Shiina method[17] to afford **13** (26%, 3 steps). The final global deprotection steps were carried out under carefully controlled conditions[8] via (i) removal of the TBS groups of **13** with 18% HF–pyridine in MeOH at 50 °C to yield a pentaol, (ii) removal of the Fmoc group with piperidine (64%, 2 steps), (iii) methanolysis of the *p*-methoxybenzylidene acetals by treating with HCl in MeOH (86 mM) at 0 °C for 1 h, and (iv) hydrolysis of the methyl ketal with aqueous HCl (86 mM) at 0 °C for 5 h, to afford 25-^{13}C-amphotericin B methyl ester **2** (53%, 2 steps), which was further purified by HPLC. The ^{13}C NMR spectra of **2** showed a high intensity signal at 134.23 ppm corresponding to C25.[18]

In summary, we have developed a practical method for synthesizing regioselectively ^{13}C-labeled AmB derivatives in high labeling index. Preparation of AmB derivatives labeled at other positions and solid-state NMR measurements using **2** are currently in progress in our laboratory.

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References and Notes

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18 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

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