Natural Products (1)

DOI: 10.1002/ange.200800589

Synthesis of 35-Deoxy Amphotericin B Methyl Ester: A Strategy for Molecular Editing**

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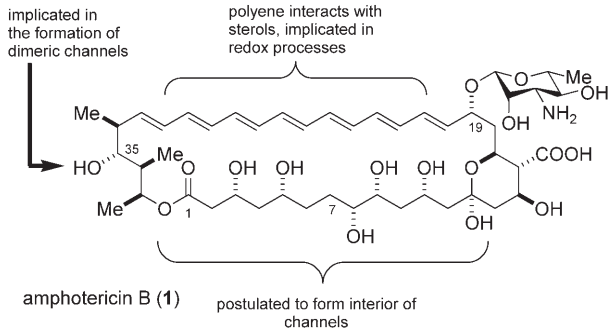
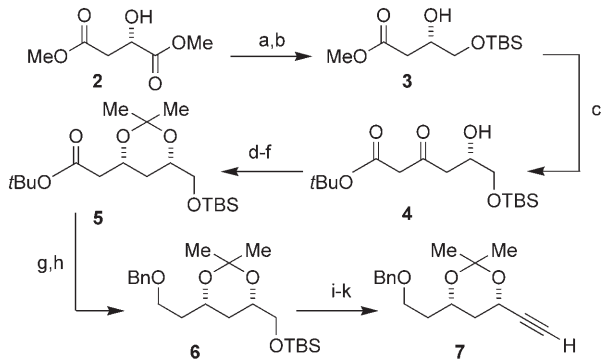
Amphotericin B (**1**) is the most prominent of the mycosamine polyene macrolides and is of great importance in medicine.[1] Various formulations of **1** have allowed its successful use as a fungicide against aspergillosis, candidemia mucormycosis, and multidrug resistant forms of leishmania.[2] Despite four decades of research, there remain numerous questions concerning its mechanism of action; the two most commonly cited include its ability to induce oxidative damage and its role in loss of electrochemical membrane potential.[3,4] The latter is widely accepted and involves the formation of barrel-stave ion channels that result in efflux of electrolytes through the fungal cell membrane. Numerous experimental and computational studies have led to various proposals for the structure of these channels.[3,4] We have selected 35-deoxy amphotericin B as a synthesis target because of the pivotal role the hydroxy group at C35 has been suggested to play in stabilizing channels. Herein we disclose the synthesis of 35-deoxy amphotericin B aglycone, a necessary condition for the subsequent biological studies described in the accompanying paper.[5] More broadly, the approach we document should enable access to designed analogues as powerful probes for additional studies of the mechanism of action.



Modifications of the amphotericin B polyketide synthase machinery through genetic engineering by Caffrey and co-workers as well as semisynthetic alterations by other researchers have provided access to derivatives that highlight the importance of the polyene backbone, the carboxylic acid, and the free amino group.[6–8] In an effort to access variants not addressed by the reported approaches we chose to pursue a modular strategy that would render the synthetic scheme amenable to modification of various functional groups at will.[9] It is important to note that numerous approaches to the subunits of **1** have been developed,[4] in addition to the synthesis of the related aglycones of candidin and rimocidin.[10,11] However, only one total synthesis of amphotericin B (**1**) has been reported to date.[12]

The synthesis of the C1–C7 fragment **7** commenced with dimethyl (*S*)-malate (**2**; Scheme 1).[13] Selective ester reduction[14] was followed by protection of the primary alcohol



Scheme 1. a) $BH_3{\cdot}SMe_2$, cat. $NaBH_4$, THF, 0 °C; b) TBSCl, imidazole, CH_2Cl_2, 0–23 °C; c) LDA, *tert*-butyl acetate, THF, −78 to −10 °C; 54.5% over three steps; d) Bu_3B, $NaBH_4$, MeOH, THF, −78 °C; e) H_2O_2, water/THF; f) PPTS, 2-methoxypropene, −35 °C to RT; 69% over three steps, d.r. = 15:1; g) $LiAlH_4$, THF, −10 °C; h) NaH, BnBr, Bu_4NI, THF/DMF (10:1); 88% over two steps; i) HF/pyridine, THF, 0 °C; j) cat. TEMPO, cat. KBr, NaOCl, pH 8.6 buffer, CH_2Cl_2, 0–7 °C; k) $EtO_2CC(N_2)P(O)(OEt)_2$, K_2CO_3, MeOH; 51% over three steps. LDA = lithium diisopropylamide; TBS = *tert*-butyldimethylsilyl; PPTS = pyridinium *para*-toluenesulfonate, Bn = benzyl.

group. Chain elongation by Claisen condensation of **3** and *tert*-butyl acetate gave β-ketoester **4** (54.5%, three steps). Prasad reduction afforded the desired *syn*-3,5-diol with 15:1 selectivity.[15] Protection of the diol was effected by PPTS and 2-methoxypropene at low temperature (69%, three steps). The ester **5** was converted into the benzyl ether **6**. Subsequent

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[**] This research was supported by ETH and agrant from the Swiss National Science Foundation. Postdoctoral scholarships were provided by the Carlsberg foundation (A.M.S.), Fonds NATEQ (J.M.M.), and NSF (N.R.W.).

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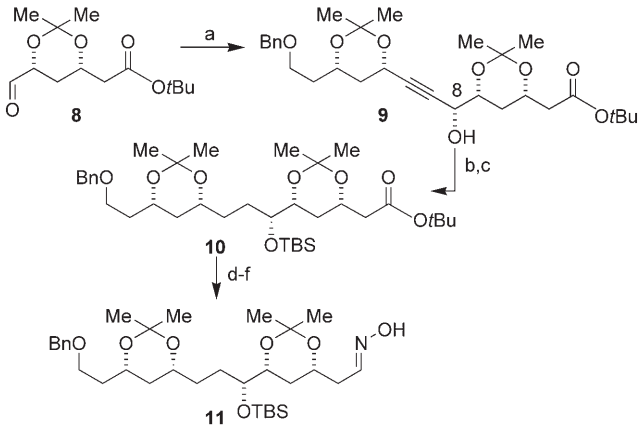
removal of the TBS group gave the free alcohol which was oxidized to the corresponding aldehyde. Finally, treatment with Ohira's reagent[16] in the presence of potassium carbonate afforded the desired alkyne **7** in 51% overall yield. Only four chromatographic purification steps were necessary for the entire sequence, a fact that expedited the synthesis of more than one hundred grams of this material.

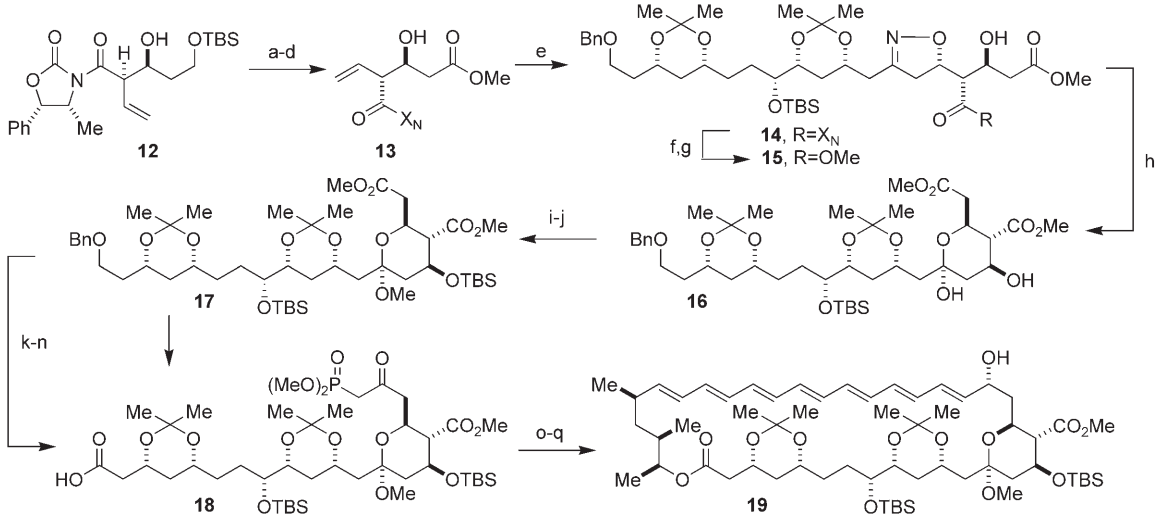
Aldehyde **8** (Scheme 2), corresponding to the C8–C13 part of amphotericin B, was synthesized in an analogous manner in eight steps starting from readily available diethyl (*R*)-malate.[17]



Scheme 2. a) **7**, $Zn(OTf)_2$, (−)-NME, Et_3N, toluene, then **8**; 98%, d.r. = 16:1; b) H_2, cat. Pd/C, $NaHCO_3$, MeOH; c) TBSCl, imidazole, DMF, 40 °C; d) $LiAlH_4$, THF, 0 °C; e) cat. TEMPO, cat. KBr, NaOCl, pH 8.6 buffer, CH_2Cl_2, 0 °C; f) $HONH_2$·HCl, pyridine; 84% over five steps. NME = *N*-methyl ephedrine; TEMPO = 2,2,6,6-tetramethylpiperidine-1-oxyl.

Studies in our laboratory had shown that coupling of lithiated **7** to **8** would produce the (*S*)-C8 epimer of **9** as the major and undesired product.[18] This step thus presented an opportunity to examine the asymmetric zinc acetylide addition to aldehydes in a highly complex setting.[19] Accordingly, alkyne **7** was treated with $Zn(OTf)_2$, (−)-*N*-methyl-ephedrine, Et_3N, and aldehyde **8** to afford propargylic alcohol **9** in 98% yield and d.r. = 16:1 (Scheme 2). Hydrogenation of the alkyne was followed by protection of the secondary alcohol function. Ester **10** was converted to oxime **11** in 84% overall yield in three steps without purification of intermediates.

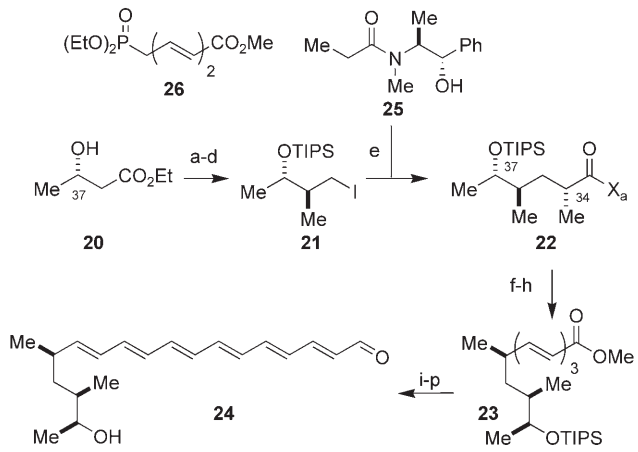
Alcohol **12** was accessed through an Evans aldol addition reaction and converted to **13** following deprotection, oxidation, and esterification.[20] Coupling of **11** and **13** was then performed by a nitrile oxide cycloaddition (Scheme 3), in analogy to that reported by McGarvey et al. for a different homoallylic alcohol.[21] Under these conditions, the desired isoxazoline **14** was formed as a single regioisomer in 95% yield and d.r. = 88:12. Treatment of **14** with LiOOH afforded the carboxylic acid, which was converted to bis-methyl ester **15**. Reductive opening of the isoxazoline ring with $[Mo(CO)_6]$ led to unmasking of a hydroxy ketone,[22] which underwent cyclization during purification to hemiketal **16**. Protection of **16** as the methyl ketal proved difficult because of low reactivity combined with the acid sensitivity of the acetonides. This problem was solved by employing 2-chloropyridinium camphorsulfonate ($pK_a = 0.8$) in a mixture of 2,2-dimethoxypropane and MeOH. Protection of the secondary alcohol using TBSOTf afforded the fully protected C1–C19 polyol **17**. The treatment of **17** with 1.8 equiv of $(MeO)_2P(O)CH_2Li$ at 0 °C gave the desired keto-phosphonate in 71% yield. Removal of the benzyl group and two-step oxidation of the resulting C1 alcohol afforded carboxylic acid **18** (99%), which



Scheme 3. a) HF/pyridine, THF, 0 °C; b) cat. TEMPO, cat. KBr, NaOCl, pH 8.6 buffer/CH_2Cl_2, 0 °C: c) $NaClO_2$, *t*BuOH/2-methyl-2-butene/2 M NaH_2PO_4, 0 °C; d) $TMSCHN_2$, MeOH/EtOAc; 63% over three steps; e) $(Bu_3Sn)_2O$, **11**, then **13**, *t*BuOCl, CH_2Cl_2, −35 to 23 °C; 95%, d.r. = 88:12; f) LiO_2H, water/dioxane; g) $TMSCHN_2$, MeOH/EtOAc, 67% over two steps; h) $[Mo(CO)_6]$, MeCN/water, 80 °C, 86%; i) 2-chloropyridine·CSA, $(MeO)_2CMe_2$, MeOH; j) TBSOTf, 2,6-lutidine, CH_2Cl_2, 75% over two steps; k) $(MeO)_2PCH_2Li$, THF, −35–0 °C; 71% at 73% conversion; l) cat. $Pd(OH)_2$, H_2, EtOAc; m) DMP, pyridine, CH_2Cl_2; n) $NaClO_2$, *t*BuOH, 2-methyl-2-butene, 2 M NaH_2PO_4; 99% over three steps; o) **24**, 2,4,6-trichlorobenzoylchloride, pyridine, CH_2Cl_2, 0 °C; 48%; p) K_2CO_3, [18]crown-6, toluene, 60 °C; 52%; q) $NaBH_4$, MeOH, 0 °C; 73%. TMS = trimethylsilyl; CSA = camphor sulfonic acid; DMP = Dess–Martin periodonane; X_N = Evans' nor-ephedrine derived auxiliary.

intercepts the Nicolaou synthesis of Amphotericin B (**1**).[12] Using this route, more than five grams of **18** were produced. The work outlined in Scheme 1–3 constitutes the shortest (27 steps from dimethyl malate) and most efficient (4.1% yield) synthesis of **18** to date. Importantly, we had fulfilled the first objective of our strategy, namely to have access to all three major fragments of amphotericin B by efficient synthetic routes on a gram-scale.[9]

The assembly of the 35-deoxy analogue of the C21–C38 fragment **24** commenced with a Fráter–Seebach alkylation of (*S*)-3-hydroxy-butyric acid ethyl ester (**20**, Scheme 4). After



Scheme 4. a) LDA, MeI, HMPA/THF (1:10), −78 °C; 92%, d.r. = 95:5; b) TIPSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C; c) DIBAL, Et_2O, −78 to 0 °C; 47% over two steps; d) Ph_3P, I_2, THF; 86%; e) **25**, LDA, LiCl, THF, −78 to 0 °C; 80%, d.r. = 95:5; f) LDA, $BH_3{\cdot}NH_3$, THF; 72%; g) cat. TEMPO, cat. KBr, NaOCl, pH 8.6 buffer/CH_2Cl_2, 0 °C; h) **26**, LDA, THF, −78 to 0 °C; 94%; i) HF, aq. MeCN; 67%; j) TESOTf, 2,6-lutidine, CH_2Cl_2, 0 °C; 81%; k) DIBAL, CH_2Cl_2, −78 °C; l) MnO_2, CH_2Cl_2; m) **26**, LDA, THF, −78 to 0 °C; 55% over three steps; n) HF/pyridine, THF, 0 °C; o) DIBAL, CH_2Cl_2, −78 °C; p) MnO_2, CH_2Cl_2; 62% over three steps. TIPS = triisopropylsilyl; Tf = trifluoromethanesulfonyl; TES = triethylsilyl; DIBAL = diisobutylaluminum hydride; X_a = Myers' pseudo-ephedrine derived auxiliary.

alcohol protection, ester reduction, and conversion of the resulting primary alcohol to iodide **21**, Myers diastereoselective alkylation[23] was employed to set the remaining stereogenic center. Reductive removal of the auxiliary provided an alcohol that was converted into hexaene-aldehyde **24**.

Coupling of carboxylic acid **18** with alcohol **24** using dicyclohexylcarbodiimide (DCC) and 4-(*N*,*N*-dimethylamino)pyridine (DMAP)[12d] as well as under a host of other conditions afforded none of the desired ester product. An apparent problem was the low reactivity of **24** in combination with the marked tendency of activated anhydrides and esters of **18** to undergo β-elimination with concomitant opening of the 3,5-acetonide. In addition, the low solubility of **24** in any other solvent than dichloromethane severely hampered optimization efforts. After extensive experimentation, it was found that esterification could be achieved via the mixed Yamaguchi anhydride[24] by performing the anhydride formation and esterification in one pot and avoiding the use of DMAP or other strong bases. Subsequent HWE-macrocyclization afforded protected 19-keto 35-deoxy amphotericin B aglycone.

In a final step, substrate-controlled reduction[10,12a] of the resulting macrocyclic ketone afforded protected 35-deoxy amphoteronolide as a single diastereomer.[12a]

In summary, we have reported a modular strategy that is adaptable to the efficient assembly of amphotericin B analogues bearing modifications in the macrolactone ring. This strategy relies on the gram-scale efficient synthesis of all the subunits.[9] For example, we have prepared more than 100 grams of each of the C1–7 and C8–C13 units and more than five grams of the complex C1–C20 polyol **18**. Additionally, the reagent-controlled coupling of alkyne and aldehyde provides full configurational control over the relevant fragment assembly. The ready availability of these subunits should expedite the preparation of a large variety of amphotericin B analogues that may not be accessed by semi-synthesis or genetic engineering.[6–8] The implementation of the approach is showcased with the preparation of the aglycone en route to the 35-deoxy analogue of amphotericin B.[25]

Received: February 5, 2008
Revised: March 18, 2008
Published online: May 2, 2008

Keywords: amphotericin B · antifungal agents · asymmetric synthesis · natural products · total synthesis

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