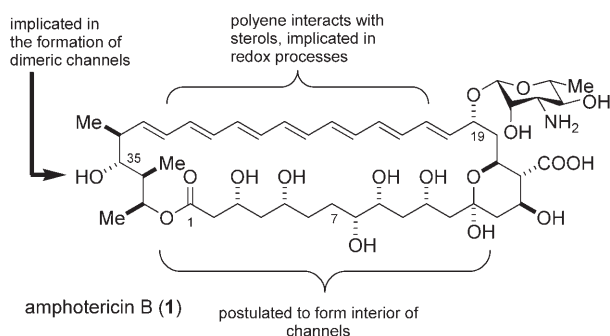


Synthesis of 35-Deoxy Amphotericin B Methyl Ester: A Strategy for Molecular Editing**

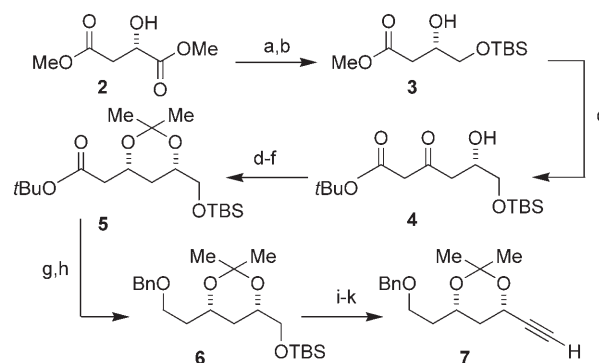
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Amphotericin B (**1**) is the most prominent of the mycosamine polyene macrolides and is of great importance in medicine.^[1] Various formulations of **1** have allowed its successful use as a fungicide against aspergillosis, candidemia mucormycosis, 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) $\text{BH}_3\text{-SMe}_2$, cat. NaBH_4 , THF, 0°C ; b) TBSCl, imidazole, CH_2Cl_2 , $0\text{--}23^\circ\text{C}$; 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 , $0\text{--}7^\circ\text{C}$; k) $\text{EtO}_2\text{CC}(\text{N}_2)\text{P}(\text{O})(\text{OEt})_2$, 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

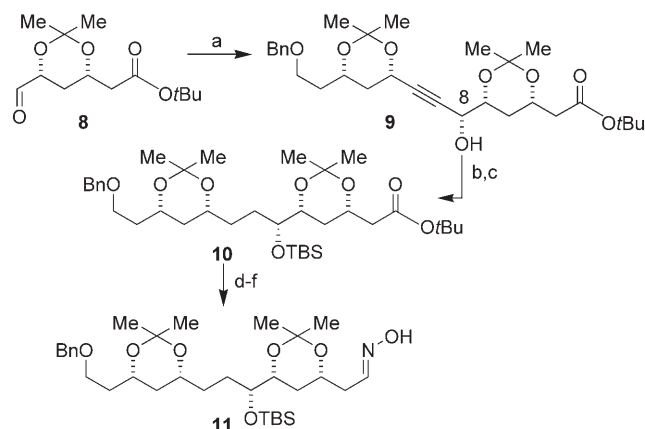
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[**] This research was supported by ETH and a grant from the Swiss National Science Foundation. Postdoctoral scholarships were provided by the Carlsberg foundation (A.M.S.), Fonds NATEQ (J.M.M.), and NSF (N.R.W.).

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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.

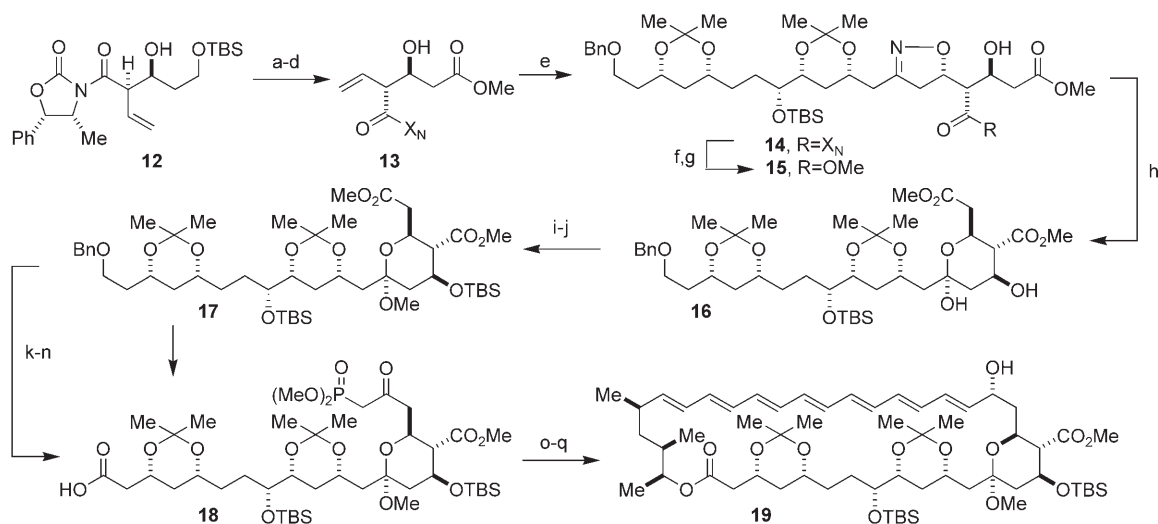
Aldehyde **8** (Scheme 2), corresponding to the C8–C13 part of amphotericin B, was synthesized in an analogous manner in eight steps starting from readily available diethyl (*R*)-malate.^[17]



Scheme 2. a) **7**, Zn(OTf)₂, (–)-NME, Et₃N, toluene, then **8**; 98%, d.r. = 16:1; b) H₂, cat. Pd/C, NaHCO₃, MeOH; c) TBSCl, imidazole, DMF, 40 °C; d) LiAlH₄, THF, 0 °C; e) cat. TEMPO, cat. KBr, NaOCl, pH 8.6 buffer, CH₂Cl₂, 0 °C; f) HONH₂·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, Et₃N, 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.

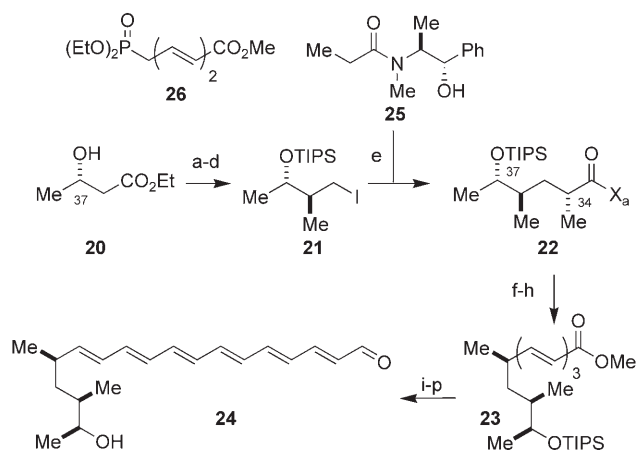
Alcohol **12** was accessed through an Evans aldol addition reaction and converted to **13** following deprotection, oxidation, and esterification.^[20] Coupling of **11** and **13** was then performed by a nitrile oxide cycloaddition (Scheme 3), in analogy to that reported by McGarvey et al. for a different 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 acetanides. This problem was solved by employing 2-chloropyridinium camphorsulfonate (p*K*_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)CH₂Li 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/CH₂Cl₂, 0 °C; c) NaClO₂, *t*BuOH/2-methyl-2-butene/2 M NaH₂PO₄, 0 °C; d) TMSCHN₂, MeOH/EtOAc; 63% over three steps; e) (Bu₃Sn)₂O, **11**, then **13**, *t*BuOCl, CH₂Cl₂, –35 to 23 °C; 95%, d.r. = 88:12; f) LiO₂H, water/dioxane; g) TMSCHN₂, MeOH/EtOAc, 67% over two steps; h) [Mo(CO)₆], MeCN/water, 80 °C, 86%; i) 2-chloropyridine-CSA, (MeO)₂CMe₂, MeOH; j) TBSOTf, 2,6-lutidine, CH₂Cl₂, 75% over two steps; k) (MeO)₂PCH₂Li, THF, –35–0 °C; 71% at 73% conversion; l) cat. Pd(OH)₂, H₂, EtOAc; m) DMP, pyridine, CH₂Cl₂; n) NaClO₂, *t*BuOH, 2-methyl-2-butene, 2 M NaH₂PO₄; 99% over three steps; o) **24**, 2,4,6-trichlorobenzoylchloride, pyridine, CH₂Cl₂, 0 °C; 48%; p) K₂CO₃, [18]crown-6, toluene, 60 °C; 52%; q) NaBH₄, 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-butiric 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, $\text{BH}_3\cdot\text{NH}_3$, 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-macrocycli-

zation 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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