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SYNTHESIS AND BIOLOGICAL EVALUATION OF NON-POLYENE ANALOGS OF AMPHOTERICIN B[1]

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Abstract: Synthesis of the first analog of a polyene macrolide antibiotic containing a rigid, non-polyene backbone has been accomplished. The sterol recognition surface of amphotericin B has been modified in an effort to better understand the role of the polyene backbone. Its antifungal activity is reduced significantly compared with amphotericin B. © 1997 Elsevier Science Ltd.

Polyene macrolide antibiotics such as amphotericin B (**1**) are an important class of biologically active natural products.[2] Amphotericin B (AmB) was first isolated from *Streptomyces nodosus*,[3] and due to its proven clinical efficacy and lack of fungal resistance, AmB remains the drug of choice for serious systemic mycoses.[4,5] This heptaene macrolide, whose stereochemistry was proven by X-ray crystallography,[6] contains a 38-membered lactone ring with a polar polyhydroxylated region, a non-polar rigid polyene region, and zwitterionic amino acid functional groups at one end. The polyol region is capable of forming a hydrogen bonded network, and the polyene acts as a rigid backbone to enforce a rod shape to the macrolide. The structural elements found in AmB are common to many of the polyene macrolides, and are presumed to play an important role in biological activity.

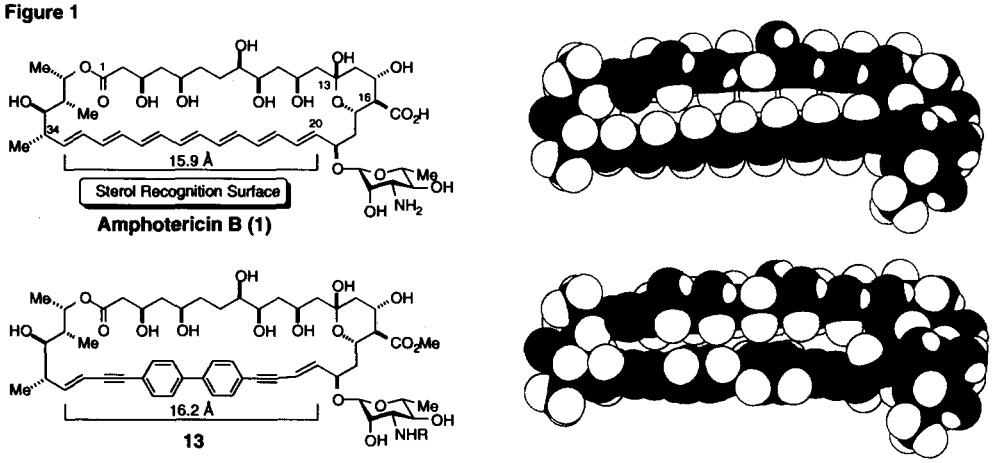
The antifungal activity and thus the mode of action of the polyene macrolide antibiotics has been of interest since their discovery over 40 years ago.[7] Amphotericin B acts in the membrane and is proposed to aggregate and form ion channels in fungal cell walls; these ion channels lead to the loss of electrolytes and fungal cell death.[8] The aggregation of AmB to form ion channels requires the presence of sterols in the membrane, and evidence from our group has shown that sterols are incorporated into the ion-channel structure.[9] The rigid polyene backbone is required for antifungal activity and is believed to confer the rod shape necessary for ion-channel activity. The polyene is also thought to play a key role in sterol recognition, but this interaction is poorly understood.[10] In an effort to better understand the role of the rigid polyene backbone of polyene macrolide antibiotics, we chose to modify this region (C20 to C33) of AmB (**1**). We report here the first synthetic analogs of amphotericin B in which the proposed sterol recognition surface has been modified.

Tremendous efforts have gone into chemical studies of the polyene macrolide antibiotics,[11] in particular amphotericin B. Most of the chemical studies of AmB have been directed toward alteration of the parent structure to attain a less toxic antifungal agent. Early modifications were performed only at the C16 carboxylate and the C3' mycosamine positions due to the ease of manipulation at these centers. The carboxylate at C16 has been successfully converted to esters,[12] amides,[13] and acyl hydrazides.[14] Modifications at the C3' amine have included the preparation of *N*-alkyl,[15] *N*-acyl,[16,17] and *N*-ornithyl derivatives.[18] Mixed C16 esters and C3' acyl derivatives

have also been reported.[19] A group at SmithKline Beecham Pharmaceuticals has recently carried out extensive protection and derivatization studies on AmB,[20] which led to the synthesis and biological evaluation of a series of C13,[21] C14,[22] and C16[23] analogs of amphotericin B. Some of the derivatization studies have produced analogs with reduced nephrotoxcity, but each was eventually dropped due to increased neurotoxicity.[24]

To find a suitable non-polyene backbone for AmB, attention was first turned to molecular mechanics calculations. Starting from the X-ray structure of AmB, minimization using the MM2 force field illustrated that the length of the extended polyene (C20 to C33) was ca. 15.9 Å. Our goal was to retain the rigidity of the backbone while changing the sterol recognition surface; thus our modeling focused on aromatic backbones. The two analogs that looked most promising were the *p*-terphenyl chain (contained in compound **7**), and the biphenyl-bis(acetylene) backbone (in **13**) both having an overall length of ca. 16.2 Å. As shown by the CPK models in Figure 1, these modifications provide AmB analogs with the same overall shape while providing a considerably different sterol recognition surface.

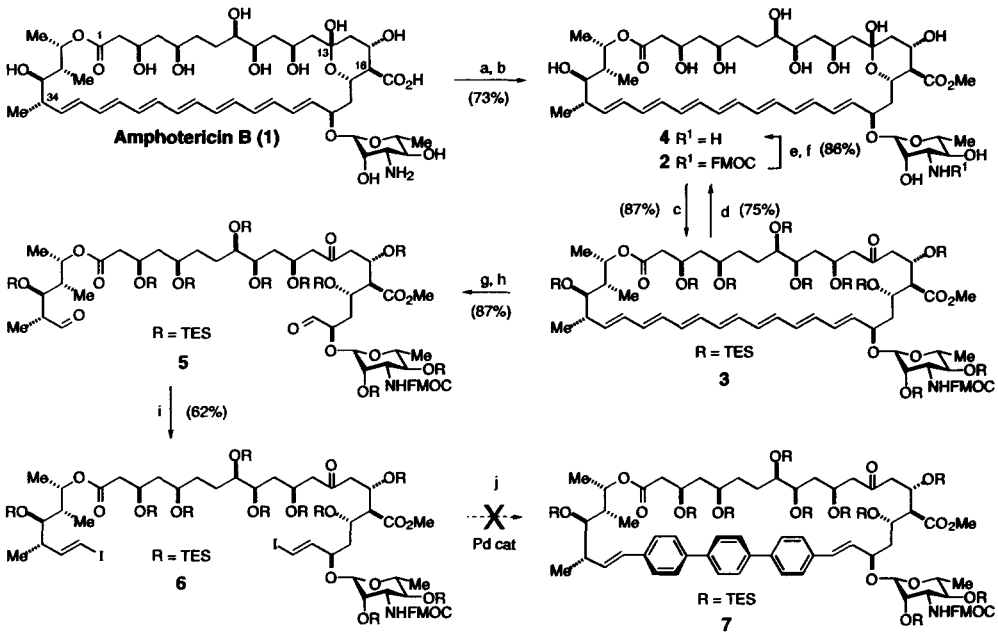
Figure 1



The protection and degradation of AmB is illustrated in Scheme 1. We chose to utilize the FMOC group, *N*-(9-fluorenylmethoxycarbonyl-oxy), to protect the C3' amine of the mycosamine sugar and the methyl ester to protect the C16 carboxylic acid.[23a] Methyl ester derivatives of AmB were selected for evaluation since it has been demonstrated that the AmB methyl ester is as active an antifungal agent as natural AmB.[25] Amphotericin B (**1**) was first treated with FMOC-Succinimide, followed immediately by treatment with excess diazomethane to give the *N*-FMOC amphotericin B methyl ester **2**. The hydroxyls of compound **2** were then protected by treatment with triethylsilyl chloride to give ketone **3**. In the original evaluation of **3** it was assumed that the six-membered acetal ring was closed to give the fully silylated product as Nicolaou had reported in a similar TMS protection of amphotericin B.[26] The ^{13}C spectrum of **3** clearly showed the presence of a ketone (205 ppm) and the absence of the expected acetal carbon (ca. 100 ppm). Under these conditions, the acetal ring of AmB opens to the corresponding hydroxy ketone, which is then trapped by TES-Cl. The reverse sequence, desilylation with

HF/pyridine and removal of the FMOC with piperidine, all proceeded cleanly to provide AmB methyl ester **4**. Thus the protection *and deprotection* sequence was an effective method to block the reactive functional groups of AmB during backbone manipulation. The polyene backbone was then removed by treatment with ozone followed by reduction with triphenylphosphine to afford bis(aldehyde) **5** in 87% yield. This compound was converted to bis(vinyliodide) **6** by treatment under modified Takai conditions.[27] The successful protection and degradation sequence outlined in Scheme 1 provided a complex precursor derived from the natural product that could be converted into an amphotericin B backbone analog.

Scheme 1



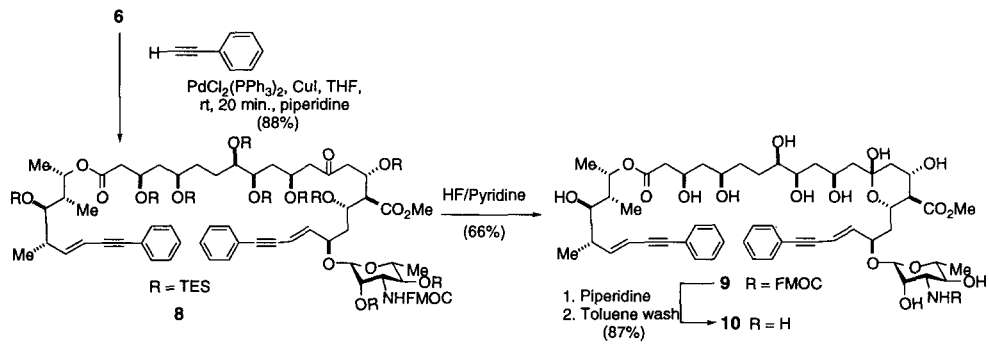
Scheme 1. (a) FMOC-Succinimide, pyridine, rt, 4 h, DMF/MeOH; (b) CH_2N_2, rt, 30 min; (c) TES-Cl, imidazole, DMF, rt, 4 h; (d) HF/pyridine, MeOH/THF/pyridine; (e) Piperidine; (f) Wash with toluene; (g) O_3, –78 °C, CH_2Cl_2/MeOH (h) PPh_3, –78 °C to rt, over night; (i) $CrCl_2$, CHI_3, THF/ dioxane, rt, 5 h; (j) 4,4''-bis(trimethylstannyl)-*p*-terphenyl.

Initial efforts focused on the preparation of compound **7** by a Stille cyclization using palladium catalysis as shown in Scheme 1.[28] Synthesis of the bis(trialkylstannyl)-*p*-terphenyl spacer was performed by standard methods.[29] Model studies performed on non-AmB compounds demonstrated that the best conditions for the Stille coupling for simple substrates are $Pd(PPh_3)_4$, THF at reflux. When these conditions and others were applied to effect the desired transformation, none of *p*-terphenyl analog **7** was obtained. The decomposition of **6** was facile at elevated temperatures and it was necessary to find a milder, lower temperature reaction to obtain any

hydrophobic analogs of this nature. A palladium-zinc catalyst system did indeed provide coupling to iodide **6** with excess phenyltrimethyltin,[30] but coupling and cyclization of a bis-(trimethylstannyl)-*p*-terphenyl backbone under these conditions failed to provide desired analog **7**. Other catalysts, including Liebeskind's copper(I) 2-thiophenecarboxylate,[31] did effect coupling, but were unsuccessful in producing desired cyclic analog **7**. Though we were able to effect coupling of simple aryl stannanes with **6**, the direct cyclization with the *p*-terphenyl distannane did not occur.

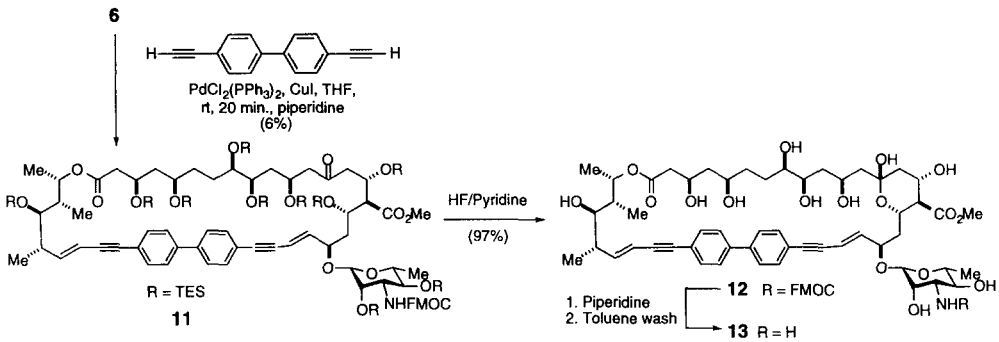
When the Stille cyclization to produce **7** was unsuccessful, alternative analog **13** was investigated. Vinyl iodides couple with acetylenes in the presence of a palladium-copper catalyst at room temperature in a Sonogashira reaction to afford enynes.[32] The only potential problem we anticipated in this transformation was that the base required for the reaction could potentially remove the FMOC protecting group found on the amino sugar. An acyclic case was first investigated to determine if the chemistry was viable (Scheme 2). Upon treatment of **6** with phenylacetylene in the presence of a palladium catalyst at room temperature, acyclic analog **8** was isolated in excellent yield. The triethyl silyl protecting groups were removed using the conditions described earlier, followed by removal of FMOC to afford the non-cyclic amphotericin B analog **10**.

Scheme 2



With this successful coupling and deprotection scheme worked out, attempts were made to form the desired cyclic analog **13** (Scheme 3). Treatment of compound **6** with the biphenylacetylene backbone[33] shown in Scheme 3 in the presence of a palladium catalyst afforded the desired cyclic analog **11**. Though the coupling process of an acetylene with a complex vinyl iodide was quite facile as demonstrated by the model study (Scheme 2), the cyclization event proved to be inefficient. All attempts to improve the yield of **11** were unsuccessful. Alternative protecting group strategies, such as methyl acetal formation or acetonide protection that would reduce the number of degrees of freedom in the macrocyclization precursor should lead to a more effective cyclization, but these alternatives were not explored. Deprotection of **11** as described previously produced cyclic analog **13**. Compound **13** is the first analog of a polyene macrolide antibiotic containing a rigid non-polyene backbone.

Scheme 3



The amphotericin B analogs were evaluated in a well diffusion assay against the pathogenic fungi *Candida albicans* and *Cryptococcus neoformans* (Table 1).[34] The cyclic analog **13** showed measurable but poor activity as an antifungal agent, while the acyclic analog **10** showed no measurable activity. Since biphenyl analog **13** has the same overall shape as AmB as judged by molecular modeling, its poor antifungal properties are likely a reflection of poor sterol binding and ion-channel activity. Thus a simple aromatic backbone enforces the rod shape of macrolide **13** and confers limited antifungal activity. The absence of a good sterol recognition surface is presumed to be responsible for the much reduced activity with respect to AmB. A more detailed explanation of the biological activity of **13** must await its evaluation in membrane permeability or sterol binding assays.

Table 1: Antifungal Activity of Amphotericin B and its Analogs[a]

Compound	Minimum Inhibitory Concentration (mg/mL)	
	Candida albicans (16820)	*Cryptococcus neoformans* (271A)
Amphotericin B	4	4
Amphotericin B Me Ester	8	8
10	>1280	>1280
13	1280	1280

[a]Diffusion assay performed by introducing sample into lawn agar composed of 1% agarose containing 0.03% (w/v) Sabouraud dextrose medium which had been seeded with ca. 1 million CFU of the appropriate organism. After 2-3 hours of incubation at 37 °C, lawn agar was overlaid with 1% agarose containing 6% (w/v) Sabouraud dextrose medium and allowed to incubate for 18 h (Candida) or 32 h (Crypto) at 37 °C. Zones of inhibition were measured at that point.

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