Natural Product Synthesis

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Spirofungin A: Stereoselective Synthesis and Inhibition of Isoleucyl-tRNA Synthetase**

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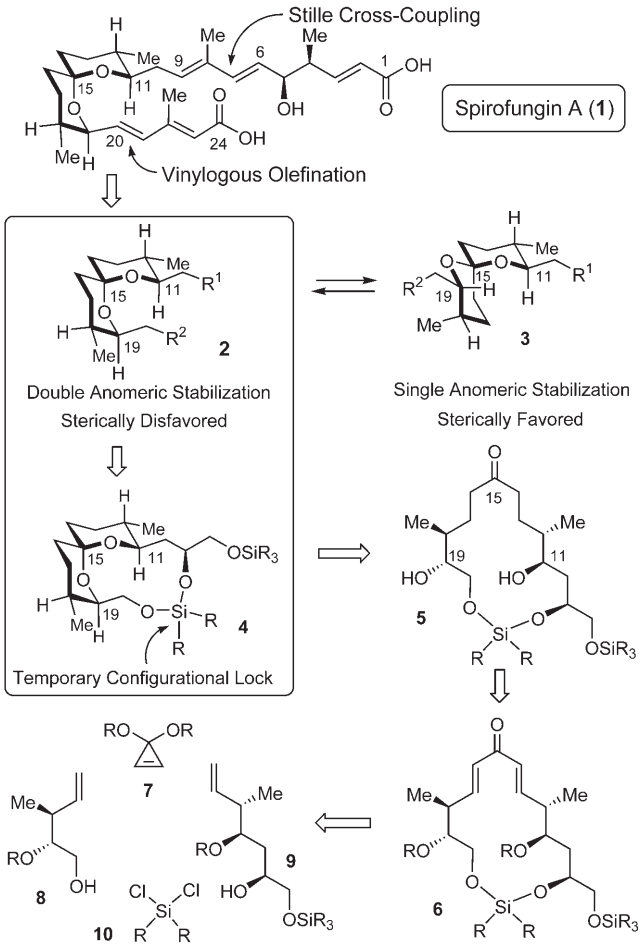
Spirofungins A and B constitute a family of secondary metabolites from *Streptomyces violaceusniger* Tü 4113.[1] The two natural products arise from epimerization of the spiroketal subunit[2] and were initially isolated as a mixture, which was reported to inhibit growth of *Candida albicans*.[1] While the antifungal activity of each congener has not been assessed, the structure–activity relationship of the closely related reveromycins[3,4] strongly suggested that spirofungin A (**1**) should display antiproliferative activity not only in yeast, but also in mammalian cells, possibly by specific inhibition of isoleucyl-tRNA synthetase.[5,6] The first syntheses of spirofungins A and B were recently reported by Shimizu et al.[7] The assembly process, however, required chromatographic separation of the two diastereomeric spiroketals en route to the final targets. Our synthetic strategy was uniquely designed to solve a challenging spiroketalization problem and to provide a fully stereoselective access to spirofungin A (**1**). Herein we report the development of a highly stereocontrolled and efficient synthesis of this natural product. We further demonstrate that spirofungin A (**1**) displays notable antiproliferative activity in a panel of cancer cell lines, and selectively inhibits the activity of isoleucyl-tRNA synthetase in mammalian cells.

The retrosynthetic analysis of spirofungin A (**1**) involves the initial detachment of the two unsaturated side arms from the spiroketal subunit at the C(20)–C(21) alkene and the diene fragments at C(7) and C(8) (Scheme 1). Control of the spiroketalization event entailed the most challenging aspect of the synthesis.[8] While the desired spiroketal **2** is favored stereoelectronically, the axial disposition of the C(19) substituent leads to significant steric congestion. As a result, a mixture of two spiroketals **2** and **3** is expected to form upon spontaneous spiroketalization.[9] To enable the exclusive formation of spiroketal **2**, we exploited different spatial orientation of the side arms (R^1 and R^2) in the two spiroketal units. Indeed, if the two arms were held by a temporary connection (that is, using a cyclic silane **4**),[10] this would force



Scheme 1. Retrosynthetic analysis of spirofungin A (**1**).

the spiroketalization of the 15-membered silacyclic ketone **5** to produce spiroketal **4** exclusively.[11] Cyclic ketone **5**, in turn, would derive from dienone **6**, which would be assembled from four simple building blocks (**7**–**10**) by employing our cyclopropenone acetal metathesis-based approach for polyketide assembly, which was initially developed during the synthesis of bistramide A.[12]

The synthesis began by subjecting alkene **11**[13] to cyclopropenone acetal **12** in the presence of the Grubbs catalyst **13**,[14] which promoted the ring-opening metathesis to give diene **14** upon subsequent desilylation (Scheme 2). Sequential exposure of a mixture of alcohols **14** and **15**[13] to dichlorodiisopropylsilane and imidazole introduced the requisite dialkoxysilane connector. Chemoselective removal of the 1,3-dioxane was efficiently achieved using oxalic acid to give ketone **16**. Exposure of **16** to the Grubbs catalyst **13** resulted

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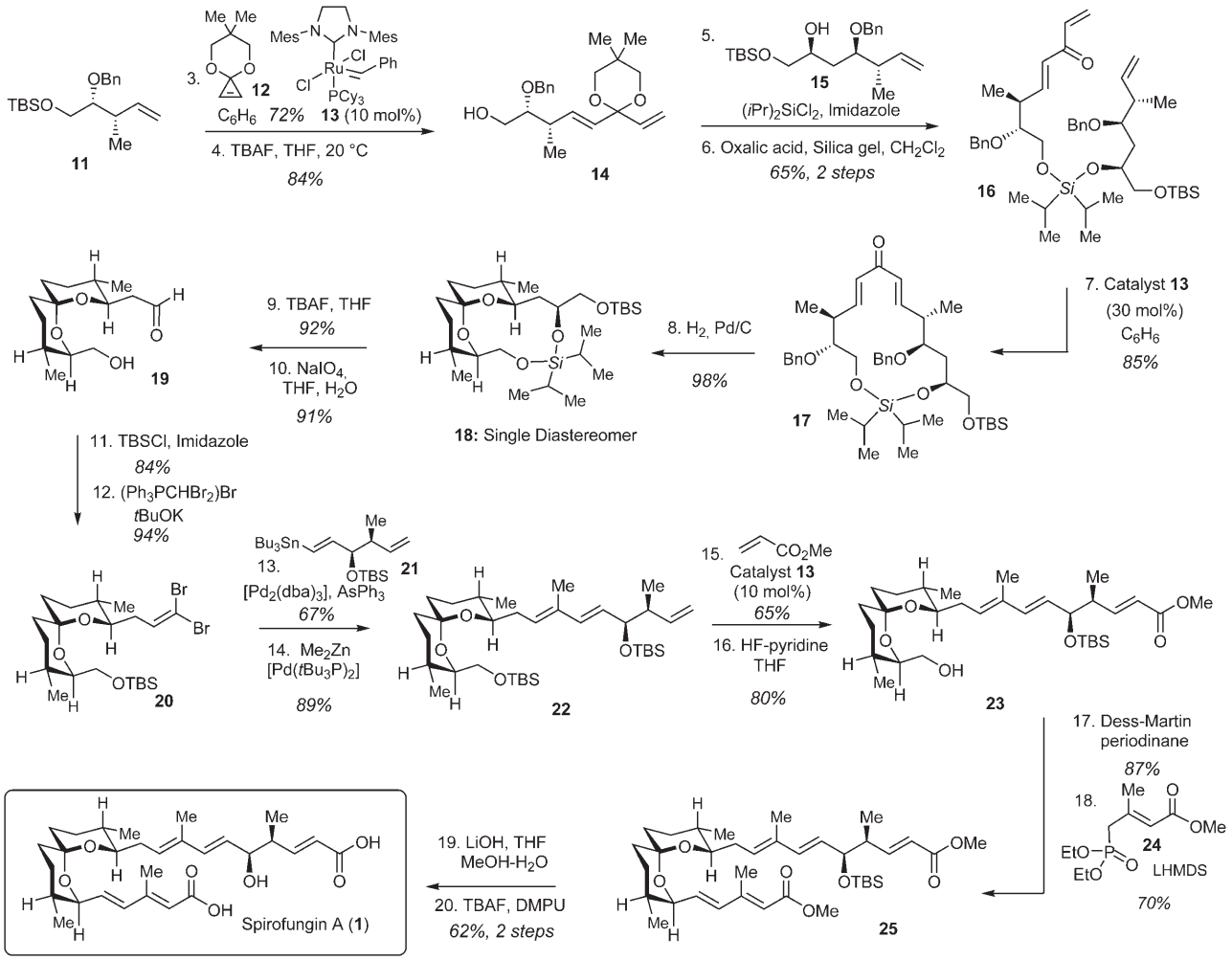

Scheme 2. Stereocontrolled synthesis of spirofungin A (**1**). Bn = benzyl, Mes = 2,4,6-trimethyl phenyl, Cy = cyclohexyl, TBAF = tetrabutylammonium fluoride, TBSCl = *tert*-butyldimethylsilyl chloride, dba = dibenzylideneacetone, LHMDS = lithium hexamethyldisilazanide, DMPU = *N*,*N*′-dimethyl-*N*,*N*′-propylene urea.

in the cyclization to afford the 15-membered dienone **17** in 85% yield.[15] The ring-closing metathesis proceeded with complete chemoselectivity only at the two terminal alkenes of trienone **16**. Hydrogenation of **17** with concomitant hydrogenolysis of the two benzyl ethers resulted in a spontaneous formation of tricyclic spiroketal **18** as a single detectable diastereomer in 98% yield. The structure of **18** was established by a combination of COSY and NOESY NMR spectroscopy, as well as X-Ray crystallographic analysis of the structurally analogous spiroketal **26** obtained during our exploratory study.[16] Fluoride-mediated cleavage of three O–Si bonds afforded the corresponding triol, which was treated with $NaIO_4$ to give hydroxy aldehyde **19**. Installation of the TBS protecting group, and conversion of the aldehyde into dibromoalkene **20** allowed the introduction of the diene subunit. The first stage of this process entailed the *E*-selective Stille cross-coupling reaction[17] of dibromide **20** with stannane **21**.[13] The resulting bromodiene was subjected to Negishi cross-coupling[18] using Me_2Zn and $[Pd(tBu_3P)_2]$ to give triene **22**, which was further elaborated via cross-metathesis with methyl acrylate[19] and chemoselective removal of the primary TBS ether. The final diene subunit was introduced by Dess–Martin oxidation of alcohol **23** and treatment of the resulting aldehyde with phosphonate **24**.[7] Completion of the synthesis of spirofungin A entailed the saponification of the two methyl esters in **25**, followed by removal of the TBS group according to the protocol developed by Shimizu et al.[7] The NMR and mass spectra, as well as the optical rotations of (−)-spirofungin A (**1**) and diester **25** were in agreement with those reported.[7]

While the inhibition of the yeast growth by spirofungin A (**1**) has been previously reported,[1] we were interested in probing the antiproliferative activity of this natural product in mammalian cells. By using the standard ATP-monitoring luciferase-based protocol,[13] we established that spirofungin A inhibited the growth of several human cancer cell lines, including HL-60 (leukemia), HCT116 (colon), PC3 (prostate), and A549 (lung), with IC_{50} values of 1.0, 0.64, 1.9, and 6.4 μM, respectively (Figure 1). It is noteworthy that this activity profile was consistent with the previously observed cell-based behavior of reveromycin A.[3b]

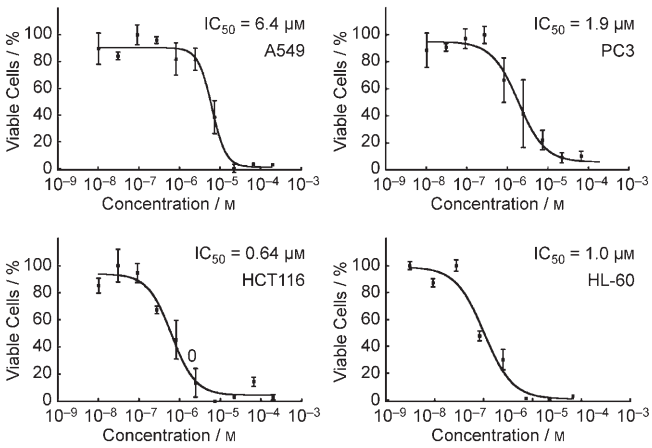

Figure 1. Dose-dependent suppression of cancer cell proliferation by spirofungin A (**1**). The cell viability assays were performed with 1000 cells per well by using an ATP-monitoring luciferase-based protocol at a variable concentration of spirofungin A (**1**).

Reveromycin A has been previously demonstrated to specifically inhibit the activity of isoleucyl-tRNA synthetase, which was identified by a combination of yeast genetics and biochemical methods to be the cellular target of this natural product.[5,6] The significant degree of structural homology between reveromycin A and spirofungin A (**1**), as well as the similar cell-based antiproliferative activities of the two natural products, strongly suggested that spirofungin A may act as a specific inhibitor of isoleucyl-tRNA synthetase. To test this hypothesis we examined the ability of spirofungin A to inhibit the synthesis of isoleucyl-tRNA and leucyl-tRNA in vitro by monitoring the incorporation of the corresponding [^{3}H]-labeled amino acids into the aminoacyl-tRNAs in HL-60 cell lysates. We found that spirofungin A (**1**) inhibited isoleucyl-tRNA synthetase in a dose-dependent manner (Figure 2a). Importantly, no effect of spirofungin A (**1**) on the activity of a homologous leucyl-tRNA synthetase was observed (Figure 2b), which strongly indicated a highly selective mode of inhibition of isoleucyl-tRNA synthetase by spirofungin A (**1**).

In closing, we have developed a fully stereocontrolled synthesis of (−)-spirofungin A (**1**) with a longest linear sequence of 20 steps. The strategy exploits our approach based on cyclopropenone acetal metathesis for rapid polyketide assembly. We further demonstrated that the natural product suppressed proliferation of several human cancer cell lines and selectively inhibited the activity of isoleucyl-tRNA synthetase in vitro. This study sets the stage for determining the nature of the remarkably specific inhibition of isoleucyl-tRNA synthesis by spirofungin A (**1**) at the molecular level and the investigation of potential therapeutic applications of this natural product.[6]

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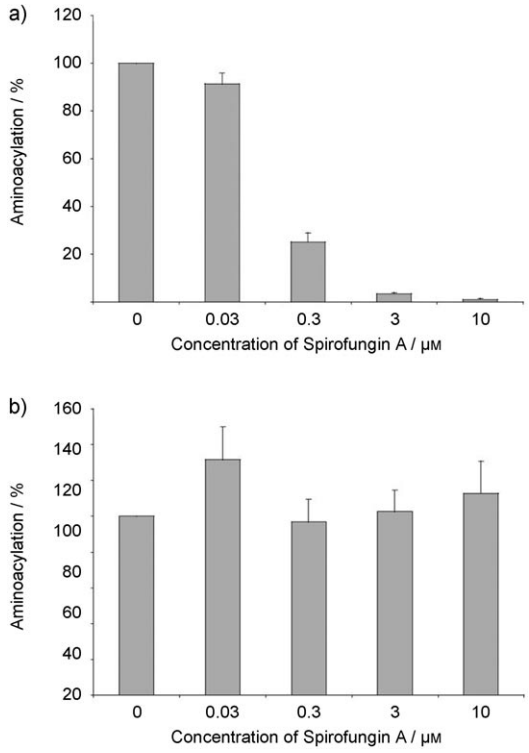

Figure 2. Dose-dependent effects of spirofungin A (**1**) on the activity of isoleucyl-tRNA synthetase (a) and leucyl-tRNA synthetase (b) in HL-60 cell lysates. The data is expressed as mean values from two cultures.

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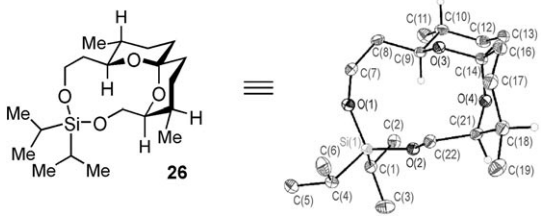

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