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Synthesis of the *trans*-fusarinine scaffold

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ABSTRACT

The *trans*-fusarinine backbone is a common feature encountered in many fungal siderophores. This monomer is notably the structural base of N^{α}-methyl coprogen B and dimerumic acid. Both siderophores are known to be secreted by *Scedosporium apiospermum*, an emerging pathogenic fungus studied for its high involvement in invasive infections of immunocompromised patients. The strategy developed here for the synthesis of the *trans*-fusarinine scaffold relies on the preparation of both *N*-hydroxyornithine and 3-anhydroxymevalonic acid subunits starting from L-ornithine and 3-butyn-1-ol, respectively. The coupling of these two building blocks led to the expected protected backbone.

Iron is an essential nutriment for all eukaryotes and almost all prokaryotes as it is required in a large variety of fundamental metabolic and informational cellular pathways.[1] Despite being one of the most abundant elements in the Earth's crust, the bioavailability of iron either in many environments (soil or sea) or in the human hosts is limited by the very low solubility of ferric ion. To circumvent this problem, many microorganisms synthesize, secrete and utilize very specific high-affinity iron uptake systems called siderophores.[2] These species, known to be amongst the strongest binders to Fe^{3+} ion, are able to withdraw iron from human protein–iron complexes, solubilize and transport it back to the cells. Because of this high binding affinity, siderophores have attracted much interest as they can be potentially used for a wide variety of therapeutic applications such as iron detoxification agent, removal of excess iron resulting from the supportive therapy for β-thalassaemia, antioxidant, antimicrobial and anticancer drugs.[3]

Siderophores can be divided into three main classes depending on the chemical nature of the moieties donating the oxygen ligands for Fe^{3+} coordination, which are either phenolates/catecholates, hydroxamates, or (α-hydroxy)-carboxylates.

From a chemical point of view, a wide range of natural mycobactins and related analogues with an hydroxamate function has already been prepared.[4] Nevertheless, to the best of our knowledge, examples of syntheses of hydroxamate-based siderophores also bearing a (3*E*)-anhydroxymevalonic acyl chain are quite rare.[5] This specific chain, coupled with N^5-hydroxyornithine appendage, gives *trans*-fusarinine **1**.[6] This particular siderophore can be recognized as the main building block of various siderophores, mainly secreted by fungi, such as coprogens, ferrichromes and obviously the different members of the fusarinine family (Fig. 1).[7,8]

As part of a previous study from our group on *Scedosporium apiospermum*, an emerging opportunistic fungus, we have shown that two siderophores are secreted under iron stress: N^{α}-methyl coprogen B (**2**) and dimerumic acid.[9] In order to study their physicochemical properties and their interactions with the human host, we decided to prepare quantities of these iron-binders. Thus, we wanted first to focus on the development of a synthesis of their common structural monomer, the *trans*-fusarinine scaffold.

trans-Fusarinine **1** is a small complex molecule bearing four different functions: a carboxylic acid, an amine, a primary alcohol and a hydroxamate. Moreover, prior to embarking on this synthesis, two critical points have to be highlighted: the configuration of both the double bond and the asymmetric carbon 2 of the fusarinine chain.

The key step of our retrosynthetic approach, depicted in Figure 2, consisted in coupling two subunits: (3*E*)-anhydroxymevalonic acid (**5**) and *N*-hydroxyornithine (**6**), respectively, obtained from 3-butyn-1-ol (**7**) and L-ornithine (**8**). Regarding our strategy, appropriate protecting groups have to be chosen to allow a specific cleavage of each one. Carboxylic acid and N^{α}-amine functions of *N*-hydroxyornithine (**6**) were converted to *t*-butyl ester and *t*-butylcarbamate groups whereas the primary alcohol function of (3*E*)-anhydroxymevalonic acid (**5**) was protected with *t*-butyldiphenylsilyl chloride. In this case other protecting groups such as tetrahydropyranyl ether and tosylate were investigated. None proved to be successful with our approach.

The primary alcohol **9** was protected according to Nicolaou et al. procedure (Scheme 1).[10] Then 4-(*t*-butyldiphenylsilyloxy)-but-1-

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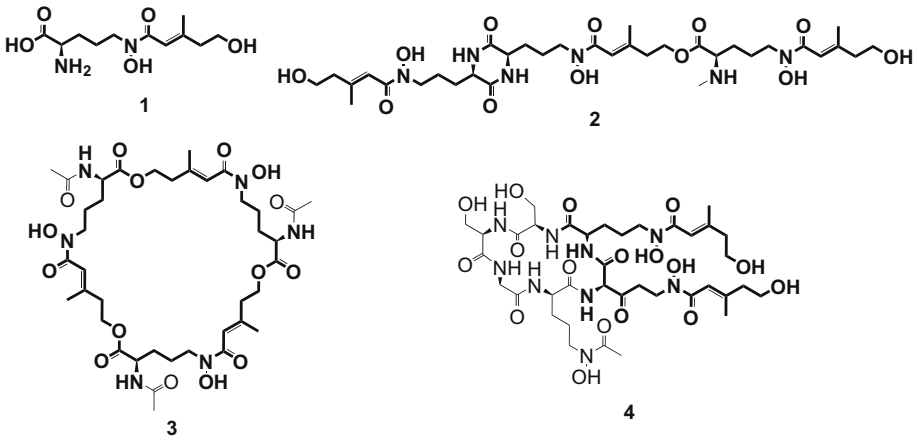


Figure 1. Structures of siderophores based on the *trans*-fusarinine scaffold (1): N^{α}-methyl coprogen B (2), N,N',N''-triacetylfusarinine C (3) and asperchrome B1 (4).

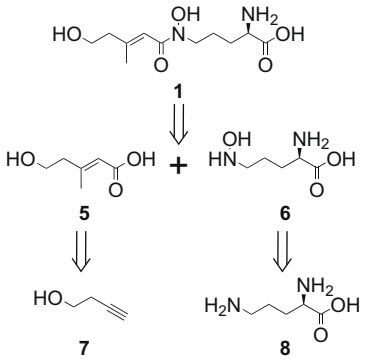
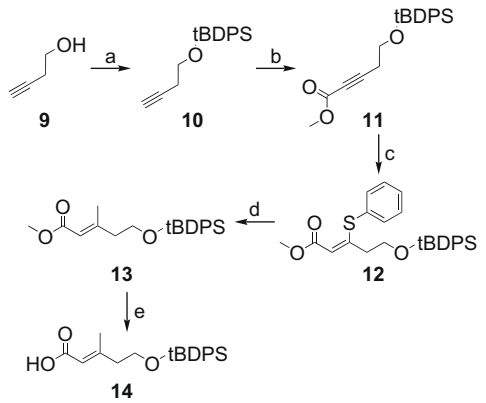


Figure 2. Retrosynthetic approach for *trans*-fusarinine **1**.

yne (**10**) was converted to ester **13**, bearing a *E*-double bond, in three steps with a 47% overall yield using Hollowood et al. strategy.[11] Following a basic deprotonation with LDA, **10** underwent a carbanionic attack of methyl chloroformate to yield the unsaturated ester **11**. A [1–4] Michael-type addition of thiophenolate on the alkyne bond afforded the unsaturated ester **12**. Both *Z* and *E* configurations were obtained for the resulting double bond. Formation of the *Z*-isomer as the major product (*Z*/*E* ratio of 4.8) might



Scheme 1. Synthesis of (3*E*)-anhydroxymevalonic acid appendage **14**. Reagents and conditions: (a) *t*BDPS-Cl, imidazole, *p*-dimethylaminopyridine, CH_2Cl_2, 0 °C, 30 min, 99%; (b) (1) LDA, THF, (2) methyl chloroformate, −78 °C to rt, overnight, 83%; (c) thiophenol, sodium methanolate (cat.), MeOH, rt, overnight, 58%; (d) MeCuBr, THF, −78 °C to rt, 99%; (e) LiOH 2 M, *t*-BuOH, 100%.

be explained by the steric interactions of the introduced thiophenyl appendage with both the silyl-protected hydroxyethyl chain and the ester function. To confirm this mechanistic hypothesis, we showed that the use of a bulkier ester group such as fluorenylmethyl induced the formation of the *Z*-isomer as the only product. Unfortunately, we were unable to achieve properly the next reaction step with this protected ester. Therefore, we kept working with the methyl ester **11**. Separation of the two isomers of **12** was easily achieved by column chromatography. The thiophenyl group of **12** was converted to a methyl group using freshly prepared methyl copper bromide. The basic hydrolysis of ester **13** was achieved quantitatively using lithium hydroxide affording the expected (3*E*)-anhydroxymevalonic acid **14**.[12,13] The double bond configuration of compounds **12**, **13** and **14** was systematically assessed by NOESY NMR spectrometry (Fig. 3).

The synthesis of the *N*-hydroxy-L-ornithine (**18**), based on biomimetic hypothesis, was carried out by oxidizing the terminal amine of L-ornithine (Scheme 2).[14,15] In order to cleave separately the protecting groups of the two amine functions, commercial N^2-Boc-N^5-carbobenzyloxy-L-ornithine was chosen as the starting material (**15**).[16] The use of this protected naturally occurring amino acid was essential as it gave us the opportunity to introduce the chiral centre with the expected configuration found in the final *trans*-fusarinine structure. All along our multi-step synthesis, our attention has been mainly focused on the retention of this stereochemical parameter. Then amino ester **16** was synthesized using dimethylformamide diterbutylacetal under microwave radiation.[17] During this reaction the methyl ester is unexpectedly formed with a 55% yield. Then the pallado-catalyzed cleavage of the benzylcarbamate group (*Z*) under hydrogen atmosphere was almost quantitative. The oxidation of the free terminal amine function with benzoyl peroxide eventually led to appropriate N^2-benzylcarbonyloxy-L-ornithine (**18**).[18] This oxidation procedure, previously reported by Berman and Johnson,[19] allowed us to obtain the oxidized product only under anhydrous conditions. Usual biphasic conditions gave rise to the acetylated amine as a side product.[20] Using this strategy, **18** was obtained with a 21% overall yield over three steps.

To synthesize the desired *trans*-fusarinine **19**, a coupling reaction had to be achieved with **18** and **14**. Thionyl chloride in the presence of an highly substituted base such as diisopropylethylamine was the first procedure used to activate the carboxylic acid derivative **14** (Scheme 3).[21] Albeit with a low yield (4%), this procedure allowed us to isolate and characterize **19** for the first time. Coupling agents such as DCC and EDCI were investigated unsuccessfully.

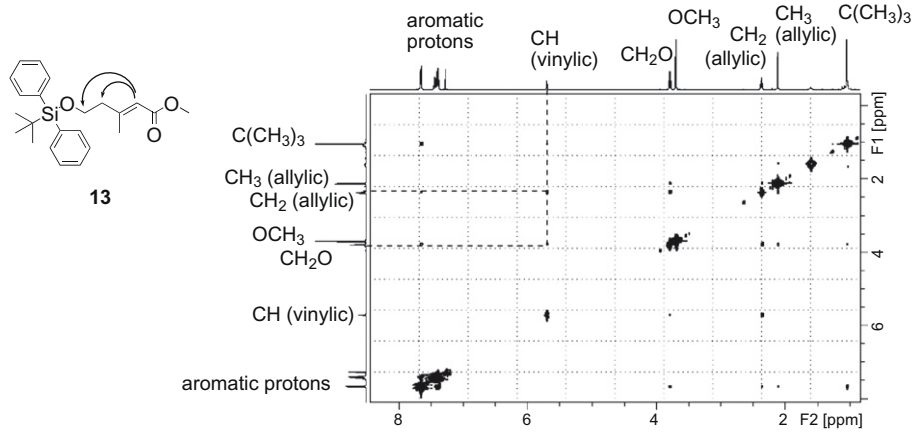
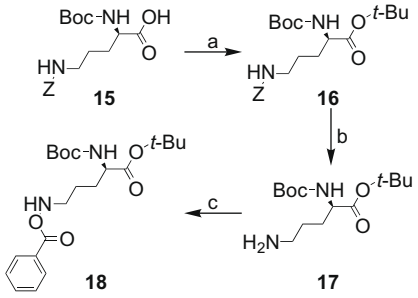
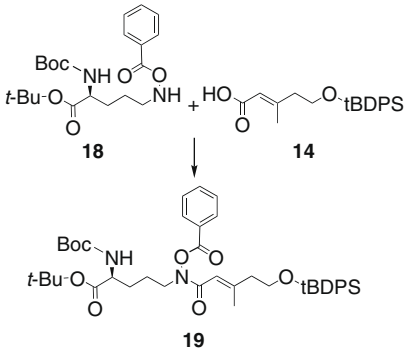


Figure 3. NOESY NMR spectrum of the unsaturated ester **13**.



Scheme 2. Synthesis of *N*-hydroxyornithine subunit **18**. Reagents and conditions: (a) DMF–DBA, toluene, MW, 160 °C, 10 min, 45%; (b) H_2, Pd/C 10%, 99%; (c) benzoyl peroxide, DMF, rt, 1.5 h, 47%.



Scheme 3. Synthesis of protected *trans*-fusarinine **19**. Reagents and conditions: $SOCl_2$, *i*Pr_2EtN, CH_2Cl_2, 4%.

We reported here an original strategy for the total synthesis of *trans*-fusarinine. So far, we described an advanced protected precursor of this scaffold. As the final coupling stage has to be improved in terms of yield, we are currently investigating other coupling reaction alternatives.[22] Moreover, this strategy should also be adapted for the synthesis of the *cis*-fusarinine scaffold. Thus addition of dimethyl lithium cuprate to the unsaturated ester **11** should afford the (*Z*) isomer of **13** as a key subunit of *cis*-fusarinine.[23]

The yield of the final coupling reaction has to be improved to turn this route into a general synthetic pathway to many major fungal siderophores so far not prepared using the usual peptide-chemistry strategies. These derivatives and their synthetic analogues will be the essential tools to study the iron acquisition mechanism known to be mediated by secreted iron chelators. Dimers built from a siderophore and a fluorescent marker should also be accessible using the synthetic approach described herein.[24] Such probe-adducts would also be of a great interest to further understand the specific mechanisms developed by fungi such as *Aspergillus fumigatus*,[25] *Histoplasma capsulatum*[26] and *S. apiospermum*.[9]

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