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Trojan Horse Strategy : synthesis of piperazine-based siderophores

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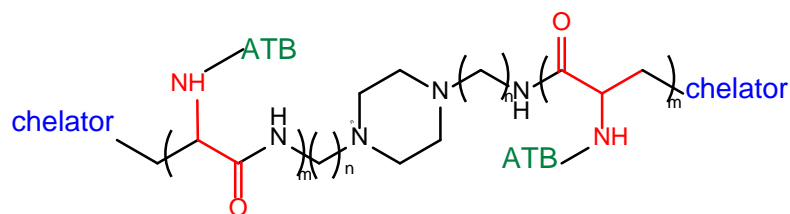
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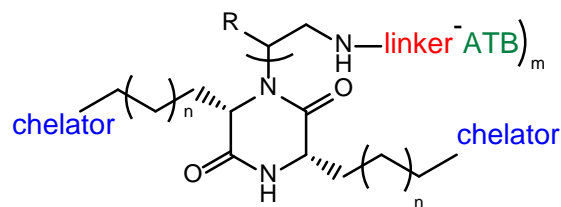
Trojan Horse Strategy : Synthesis of piperazine-based siderophores

Graphical Abstract



A

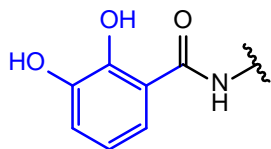
1,4-disubstituted piperazines



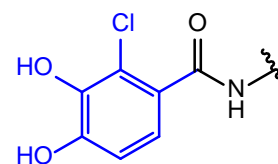
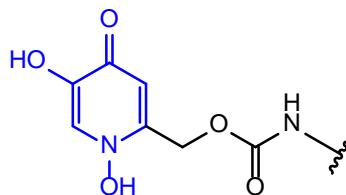
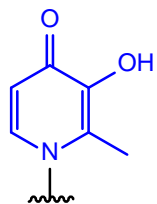
B

3,6-disubstituted dioxopiperazines

Iron chelator :



MPPS0225
(A; n = 3; m=0)



Abstract

Gram-negative bacteria's resistance such as *Pseudomonas aeruginosa* and the *Burkholderia* group to conventional antibiotics leads to therapeutic failure. Use of iron transport systems is a promising strategy to overcome resistance phenomenon. These TonB-dependent receptors, essential for the survival of microorganisms, allow specific recognition of ferric siderophore complexes to transport iron within bacteria. Bacteria express different receptors allowing them to recognize endogenous siderophores and xenosiderophores. These specific systems may allow the introduction of antibacterial agents by forming antibiotic-siderophore conjugates or toxic complexes. Previous work has shown that piperazine 1,4-dicatechol structures could be recognized by *P. aeruginosa* strains. To further investigate this platform, we synthesized iron chelators bearing 3-hydroxypyridin-4-ones and 1,3-dihydroxypyridin-4-one ligands. At the same time, we were interested in the synthesis of a more complex 2,5-dioxopiperazine platform, part of the rhodotorulic acid (RA), siderophore of *Rhodotorula* (pFe = 21,8). A RA synthesis will be developed as well as the corresponding 3,6-disubstituted analogs. We would like to study the influence, on the iron complexation, of the nitrogenous platform, the presence of stereogenic centers and the nature of the iron ligands. The best siderophores analogs will be highlighted through the evaluation of the siderophore-like potential as well as physicochemical studies of synthesized compounds.

Keywords: Trojan Horse; siderophores; iron.



Introduction

The resistance of bacteria to antibiotics is an emerging phenomenon and a real health problem. ESKAPE multi-drug resistant bacteria are a major problem in hospitals and especially for immunocompromised patients. We are particularly interested in Gram-negative bacteria such as *Pseudomonas aeruginosa* and *Burkholderia* group, previously classified in the genus *Pseudomonas*, which are resistant to antibiotics *via* a lack of membrane permeability or efflux. The use of bacterial iron transport systems is a promising strategy to overcome this resistance phenomenon by restoring the activity of conventional antibiotics. Iron is a micronutrient necessary for the survival of bacteria. It is essential to many biological processes such as respiration and DNA synthesis. However, the ferric iron is not very bioavailable due to its low solubility in water and sequestration in host-protein. An iron complex could be linked to a conventional antibiotic, as a Trojan Horse strategy, to restore its activity.

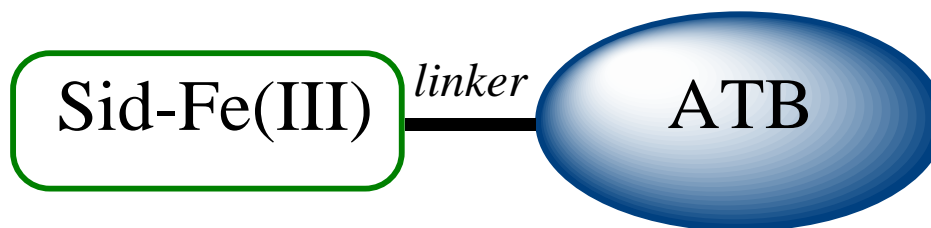


Figure 1 : Trojan Horse Strategy



Introduction

Under iron limited conditions, many bacteria then synthesize molecules of low molecular weight called siderophores able to chelate the surrounding iron. These siderophore-Fe (III) complexes are then recognized by specific receptors responsible for bringing the essential iron element to the bacteria. Interestingly, the bacteria is able to recognize its endogenous siderophores but also siderophores synthesized by other bacteria or synthetic siderophores¹.

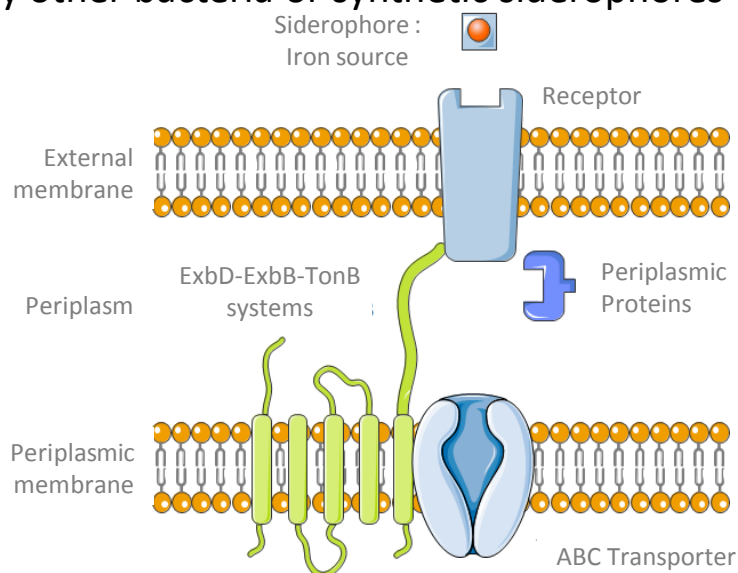


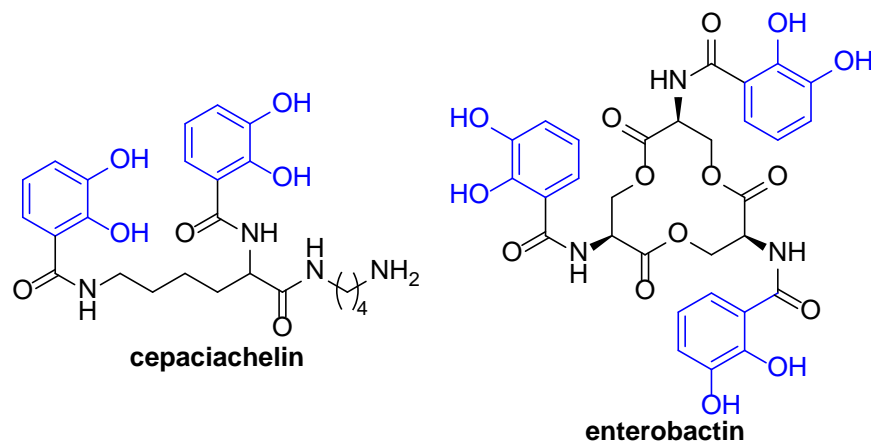
Figure 2 : Siderophore pathway

1. Page MGP, Dantier C, Desarbre E.. *Antimicrob. Agents Chemother.* **2010**, *54*, 2291–2302.



Introduction

In particular, *P. aeruginosa* and *B. pseudomallei* both possess FptA receptors for the recognition of pyocheline, the endogenous siderophore of *P. aeruginosa*. These two types of bacteria are also capable of recognizing catechol siderophores such as cepaciachelin for *B. pseudomallei*², and enterobactin for *P. aeruginosa*³ (Fig. 3). These specific systems may allow the introduction of antibacterial agents by forming antibiotic-siderophore conjugates or toxic complexes such as gallium complexes, in the bacteria.



[Figure 3 : Siderophores recognized by *B. pseudomallei* and *P. aeruginosa*](#)

2. Butt AT.; Thomas MS. *Frontiers in Cellular and Infection Microbiology* , 2017.

3. J.B. Neilands, T.J. Erickso, W.H. Rastetter. Stereospecificity of the ferric enterobactin receptor of *Escherichia coli* K-12. *The Journal of Biological Chemistry*. 1981, 256(8), 3831-3832.



Results and discussion

Previous work in the laboratory has shown that piperazine 1,4-dicatechol structures (**MPPS0225**) can be recognized by strains of *Pseudomonas aeruginosa*. Bacterial growth has been observed as a function of the ratio **MPPS0225**/Fe(III) in Medium Minimum Succinate. With a ratio **MPPS0225**/Fe(III) equal to 1,5, bacterial growth has been observed for DSM1117 strains, producing pyoverdine and pyocheline (Fig. 4).

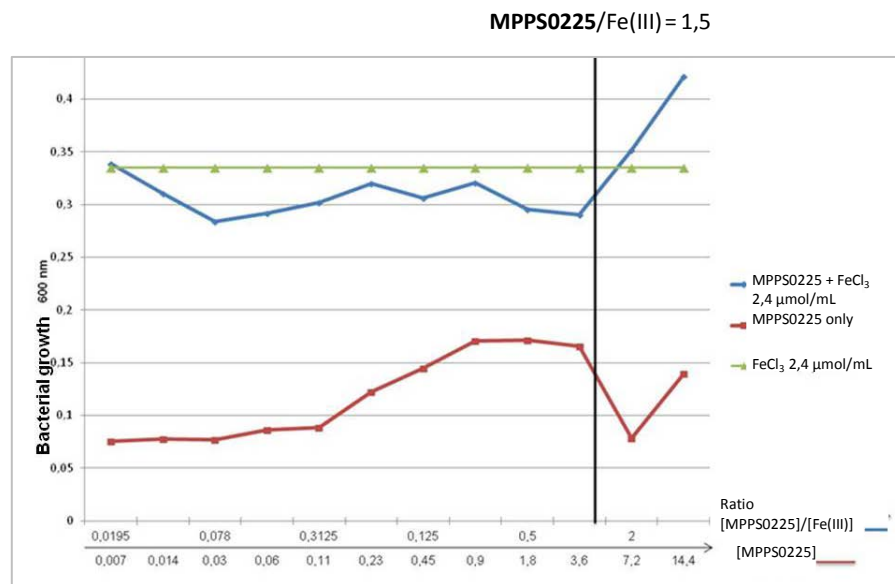
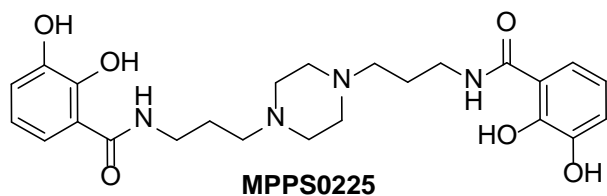


Figure 4: Representation of bacterial growth (in Medium Minimum Succinate) as a function of the ratio MPPS0225/Fe(III) for DSM1117 strains



Results and discussion

The same observation was found for PAD07 strains, which don't produce pyoverdine and pyocheline. We can assume that our compound is recognized by the bacteria (Fig. 5) and there is a competition between **MPPS0225** and the endogenous siderophores of *P. aeruginosa* (Fig. 4).

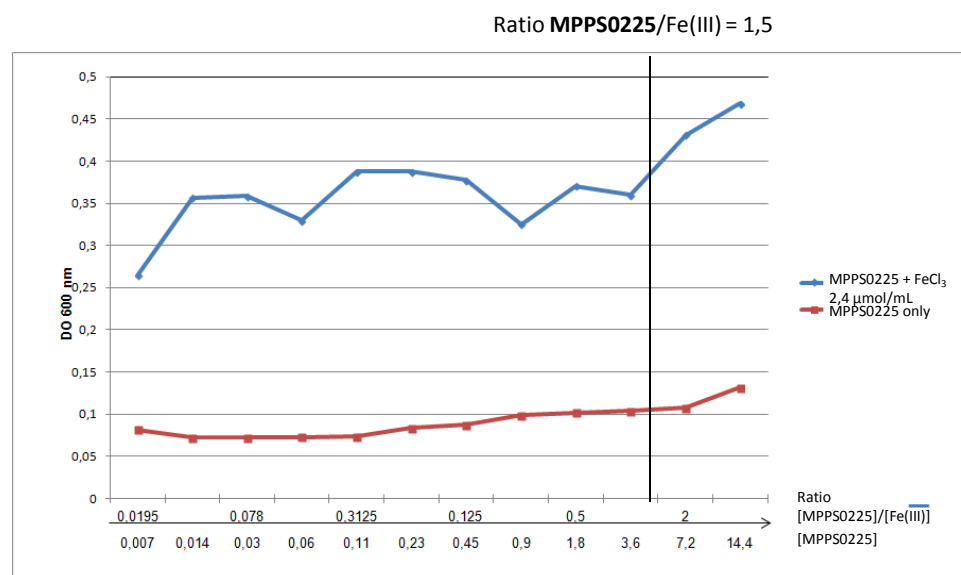
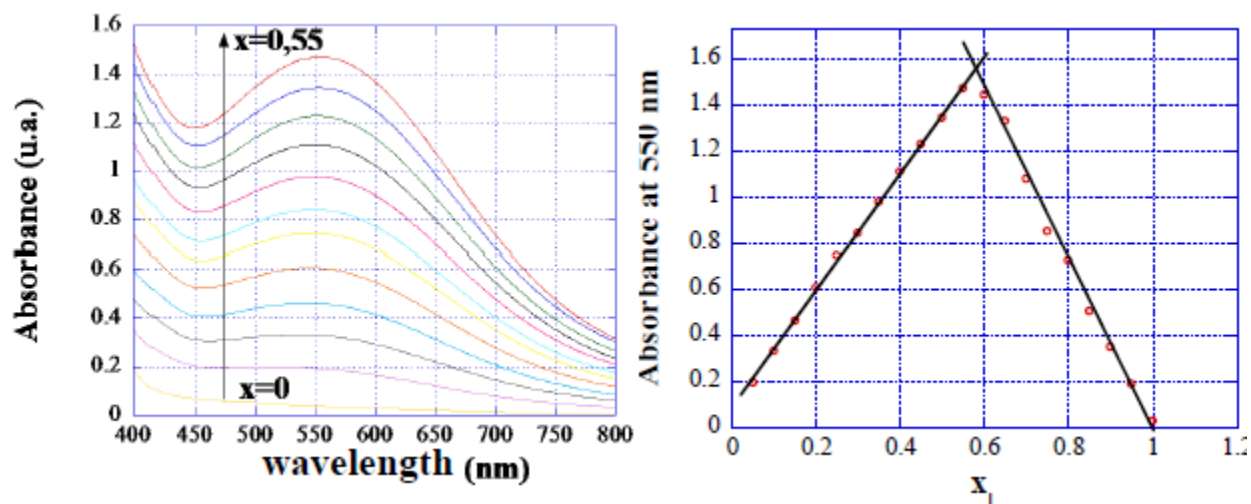


Figure 5: Representation of bacterial growth (in Medium Minimum Succinate) as a function of the ratio MPPS0225/Fe(III) for PAD07 strains



Results and discussion

Figure 6 illustrates the determination of the stoichiometry of the complex Fe(III)-**MPPS0225** at pH = 5.70 using the JOB plot method. We can see the evolution of the ligand-to-metal charge-transfer (LMCT) absorption at 550 nm up to a 0.58 molar fraction of ligand, corresponding to an iron(III)/**MPPS0225** 2:3 stoichiometry. At physiological pH this stoichiometry is kept.



*Figure 6 : Determination of the stoichiometry of the complex **MPPS0225-iron(III)***



Results and discussion

To complete these results, **MPPS0225** has been synthesized for physicochemicals studies like the pFe measurement. In order to further investigate this piperazine platform, we have synthesized iron chelators bearing 3-hydroxypyridin-4-ones ligands, bioisosteres of catechol groups. The bidentate ligands precursors **3** and **4** were synthesized with *para*-methoxybenzyl (PMB) as a protective group in order to be coupled with the 1,4-bis(3-aminopropyl)piperazine **1**.

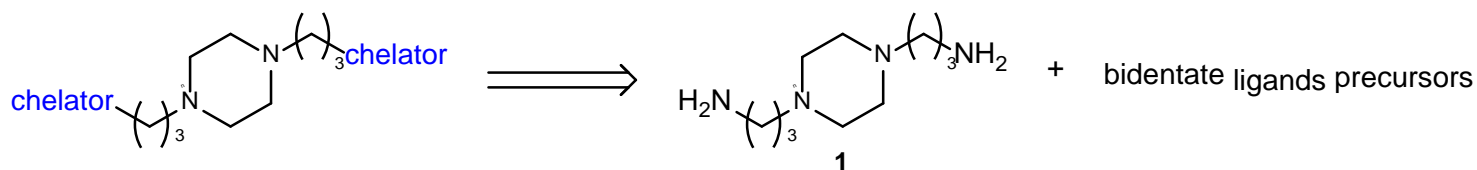


Figure 7 : Retrosynthesis of 1,4 disubstituted piperazines



Results and discussion

The bidentate ligands precursors **3** and **4** were synthesized with *para*-methoxybenzyl (PMB) as a protective group with a 70% and 65% yield.

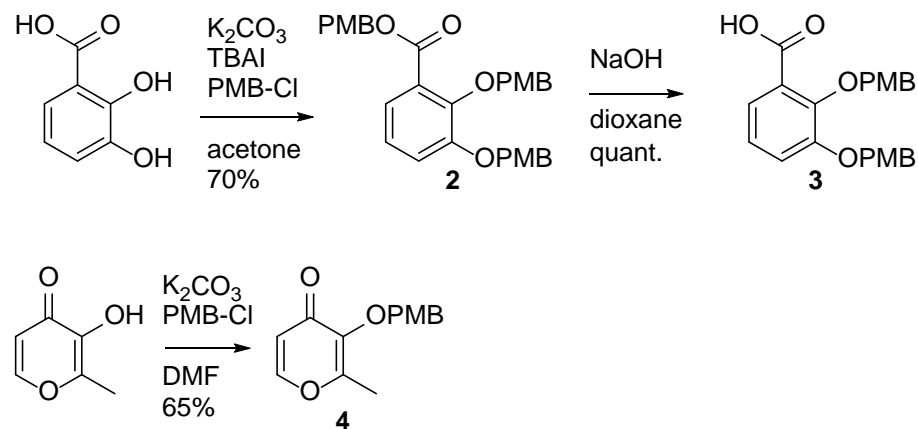


Figure 8: Synthesis of the bidentate ligands precursors



Results and discussion

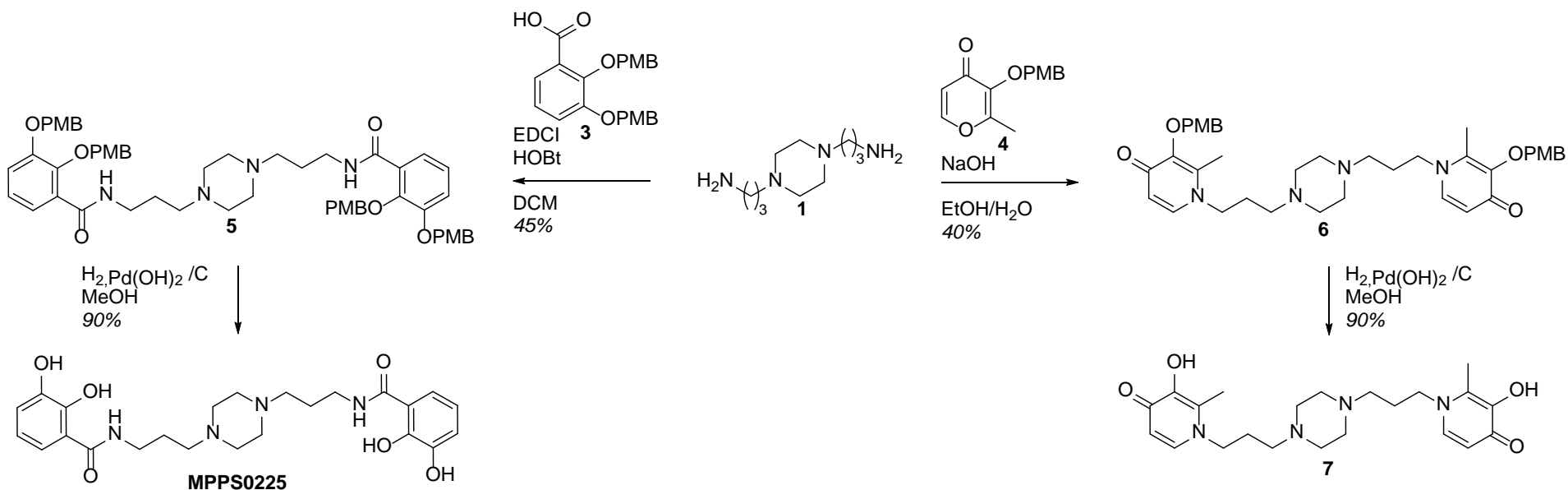


Figure 9: Synthesis of MPPS0225 and the hydroxypyridinone analog

As mentioned before, **3** and **4** were synthesized to be coupled with the 1,4-bis(3-aminopropyl)piperazine **1**. The common hydrogenation step was optimized and carried out using a H-cube system generating hydrogen by electrolysis of water. **MPPS0225** and **7** were, respectively, obtained with a 35% and 30% yield.



Results and discussion

At the same time, we were interested in the synthesis of a more complex 2,5-dioxopiperazine platform, with analogs of the rhodotorulic acid (**RA**), a siderophore recognized by Gram-negative bacteria, showing an interesting iron affinity ($pFe = 21,8$). Different ways to obtain **RA** have been described⁴ but the one we propose should be more efficient. Indeed, it is a convergent strategy which could lead to the synthesis of analogs thanks to asymmetric alkylations of a key intermediate carrying two cleavable chiral inductors (**13**). **RA** should be obtained in ten steps from the commercially available (*S*)-phenylglycinol (Fig. 10).

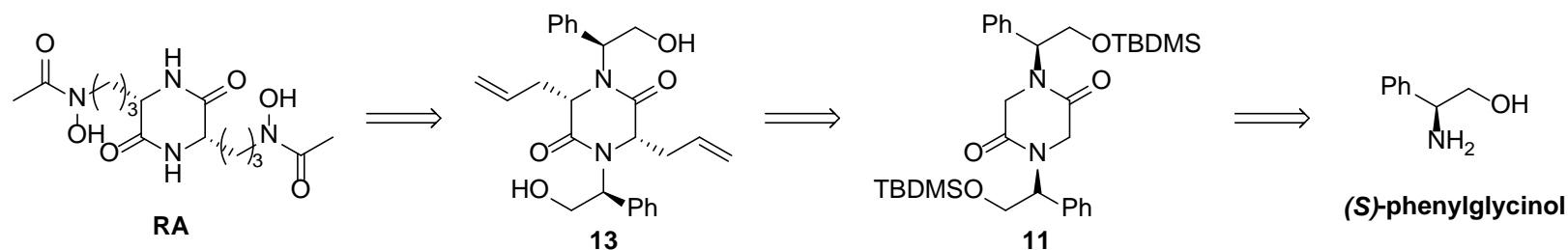


Figure 10: Retrosynthesis of rhodotorulic acid (RA)

4. a) Y. Isowa, T. Takashima, M. Ohmori, H. Kurita, M. Sato, K. Mori. *Bulletin of the chemical society of Japan*, **1972**, 45, 1467-1471. b) T. Fujii, Y. Hatanaka. *Tetrahedron*. **1973**, 29, 3825-3831. c) J. Wiedmer, W. Keller-Schierlein. *Helvetica Chimica Acta*. **1969**, 52(2), 388-396. d) M. Nakao, S. Fukayama, S. Kitaike, S. Sano. *Heterocycles*, **2015**, 90(2), 1309-1316.



Results and discussion

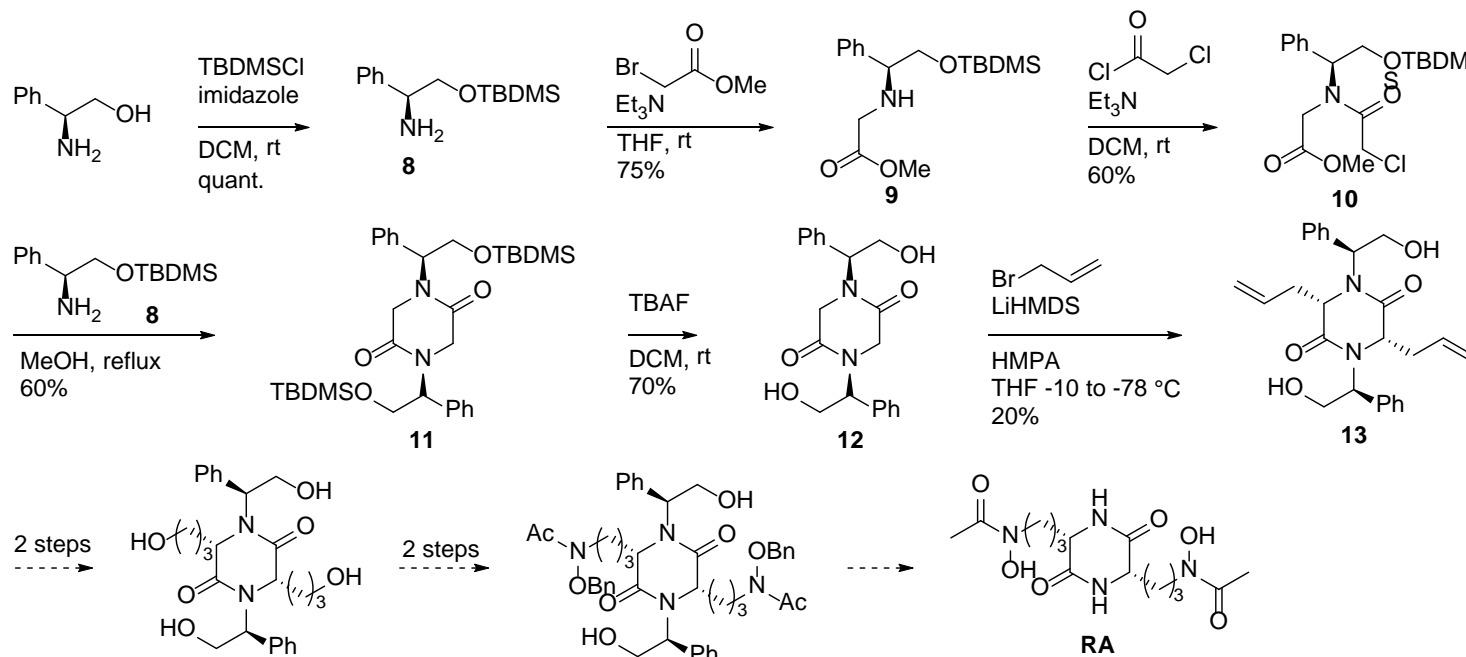


Figure 11: Synthesis of rhodotorulic acid

At this time, we synthesized the dioxopiperazine **13** in 6 steps from the (S)-phenylglycinol. This one was protected with TBDMS to afford **8** which was alkylated in presence of methyl 2-bromoacetate to afford the secondary amine **9**. This product was engaged in an amidification reaction to give **10** which was cyclized in presence of **8** to dioxopiperazine **11**. Then, the TBDMS protective group was cleaved with TBAF to afford **12**. Thanks to the (S)-phenylglycinol chiral inductors, the *cis*-dialkylation product **13** was obtained in a 4% global yield.



Conclusions

We managed to synthesize two 1,4-disubstituted piperazines, **MPPS0225** and **7** in, respectively 3 and 4 steps with 35% and 30% yields. The **RA** synthesis has been carried out until the formation of a key intermediate **13** in 6 steps with a 4% yield.

As for the dicatechol siderophore analogs, **MPPS0225**, we will first study the bacterial recognition of synthesized chelators **7** and **RA** by measuring the siderophore-like effect and evaluate their ability to complex iron by physicochemical methods. The siderophores having shown the best capacity for recognition and complexation of iron will then be linked to an antibiotic, *via* a cleavable spacer or not depending on whether the target of the antibiotic is periplasmic or cytoplasmic. The antibacterial activity of these conjugates (or complexes) will then be evaluated and compared to the antibiotic alone to estimate the vectorization capacity of the siderophore. In parallel, cytotoxicity studies will also be conducted on these compounds to determinate their therapeutic index.



Acknowledgments

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