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Efficient synthesis of a bacterial translocase MraY inhibitor

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Abstract—The bacterial translocase MraY has recently been demonstrated as a prime target for the development of new antibiotics. We describe a straightforward synthesis of a new inhibitor **1** of this enzyme. The two key steps involve a tandem nucleophilic epoxide ring opening of C2-symmetrical bis-epoxide and subsequent O-heterocyclisation, followed by O-glycosylation. The in vitro biological evaluation of **1** at 2 mM showed an 81% of inhibition of the MraY activity. Therefore, congeners of **1** should permit detailed SAR investigations for the discovery of novel antibacterials.

1. Introduction

In the context of the world-wide emergence of antibiotic resistance,[1] the bacterial cell wall peptidoglycan is a key target for antibiotic development. In particular, the inhibition of new targets having been little exploited is a challenging objective. Thus, the translocase MraY, which catalyses (Fig. 1) the first membrane step of peptidoglycan biosynthesis,[2] represents such a target, since it has been demonstrated to be essential and specific to bacteria,[3] and is currently not the target of therapeutic drugs, notably due to its transmembrane localisation[4] making it difficult to purify and to study. However, this enzyme has been recently purified to homogeneity[5] and tests allowing high-throughput screening of inhibitors have been achieved.[6]

This enzyme catalyses the transfer of uridine monophosphate-*N*-acetyl-muramoyl pentapeptide from its precursor UDP-Mur-*N*-Ac-pentapeptide onto undecaprenyl phosphate, a lipid carrier, resulting in the formation of lipid **I**. In an ongoing programme directed towards MraY inhibition,[7] we have already developed the synthesis of compounds[8] displaying analogous structures to those of naturally known inhibitors of that enzyme, such as liposidomycins[9] and caprazamycins[10] (Fig. 2).

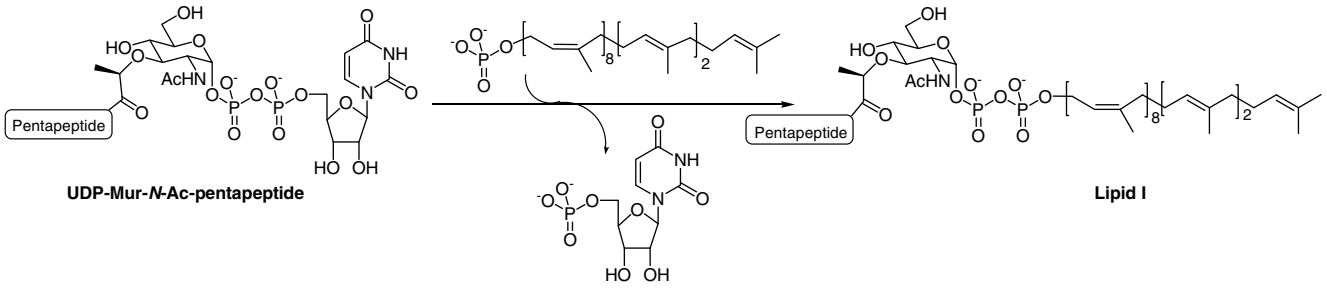

Figure 1. Enzymatic reaction catalysed by the bacterial translocase MraY.

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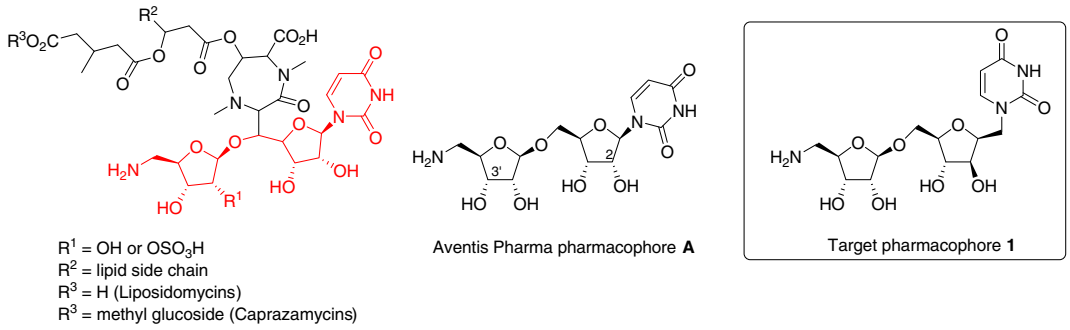


Figure 2. Natural inhibitors of MraY and pharmacophores.

Indeed, we have recently described a powerful synthesis of polyfunctionalised diazepanone rings[11] as promising scaffolds. The work described herein is related to a complementary approach, aimed at developing a straightforward access to a pharmacophore from which further modifications will permit SAR investigations for the discovery of new antibacterials.

This pharmacophore has been designed on the basis of model studies performed by Aventis Pharma[12] and taking into consideration the superimposing of UDP-Mur-*N*Ac-pentapeptide, one of the MraY substrates, with two known inhibitors, liposidomycins and tunicamycins.[13] Following these studies, Aventis developed a synthesis for pharmacophore **A**, including the common residues to the three compounds, that might represent the minimal part responsible for activity. With regards to these results, our goal was to perform an efficient and convergent synthesis of pharmacophore **1**.

As compared to the known pharmacophore **A**, which retains residues, such as aminoribose and uridine, having been demonstrated to be crucial for biological activity,[14] target **1** presents two differences. Firstly, the introduction of an extra methylene group between uracil and ribose-like moieties should improve both the flexibility and stability towards the hydrolysis of the resulting inhibitor. Indeed, an enhanced flexibility of such a compound is expected to help in its positioning within the active site of the enzyme.[15] Secondly, related to our strategy, an inversion of the absolute configuration at C2 has been introduced while keeping in mind that the absolute configuration at this stereogenic centre in pharmacophore **A** has been proven to be of weak importance for biological activity.[16] Thus, the targeted pharmacophore **1** should present the advantage of being both efficiently synthesised in only a few steps and more stable with regard to hydrolysis as compared to the Aventis pharmacophore. Furthermore, it represents an interesting scaffold for the future development of a library of related putative inhibitors.

2. Results and discussion

Retrosynthetic analysis towards pharmacophore **1** relies on two key steps (Fig. 3), which are the O-glycosylation of a uridine-like compound **3** with an aminoribose derivative

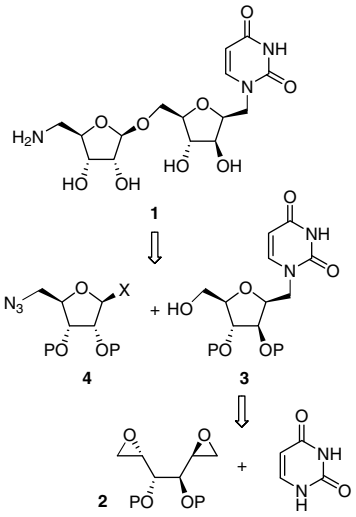
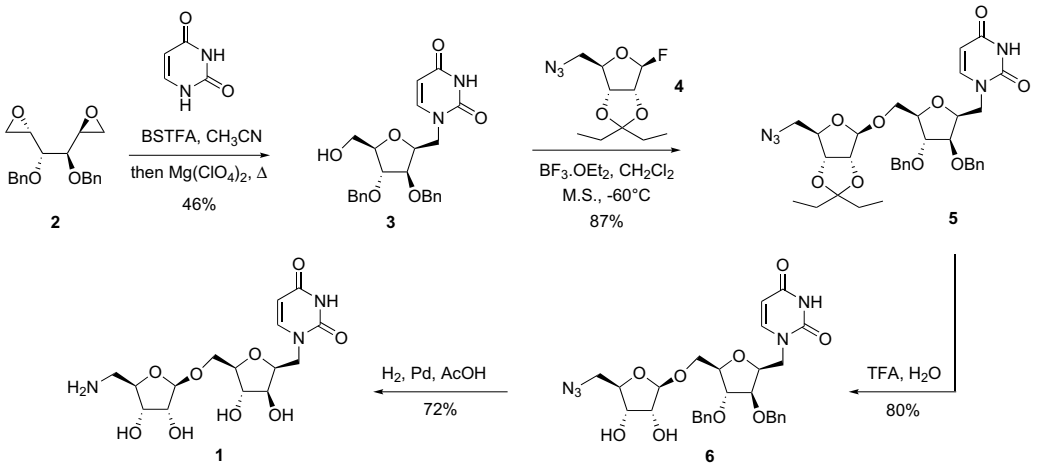


Figure 3. Retrosynthetic analysis of the target pharmacophore **1**.

4 activated at its anomeric position and a tandem nucleophilic epoxide ring opening of C2-symmetrical bis-epoxide **2** followed by concomitant O-heterocyclisation leading to **3**.

Ring opening of 1,2:5,6-dianhydro-3,4-di-*O*-benzyl-L-iditol **2** (Scheme 1), readily available from D-mannitol,[17] by bis(trimethylsilylated) uracil, in situ prepared from uracil and *N*,*O*-bis[trimethylsilyl] trifluoroacetamide (BSTFA), was performed in the presence of magnesium(II) perchlorate in refluxing acetonitrile to afford the uridine-like **3** in 46% yield. Its formation results from the regiospecific ring opening of the first epoxide functionality at the least substituted side, followed by a subsequent O-heterocyclisation of the resulting alcoholate on the other epoxide functionality[18,19] according to a *5-exo-tet* process. Owing to the presence of the C2-axis of symmetry within the bis-epoxide **2**, the opening of one or the other epoxide function leads to the same compound.

The second key step involved an O-glycosylation between the primary alcohol function of **3** and 5-azido-1,5-dideoxy-2,3-di-*O*-ethylpropylidene-1-fluoro-D-ribofuranose **4**.[20,21] The presence of an isopentylidene group on the α face of the sugar was intended to promote major glycosylation on its β face due to the steric hindrance of the α face.



Scheme 1. Synthesis of the pharmacophore **1**.

The reaction was performed in the presence of boron trifluoride etherate and molecular sieves in excess in CH_2Cl_2 at −60 °C and led to a 13:1 β:α mixture of the expected protected pharmacophore **5**. The pure β-anomer could be easily obtained in 87% yield after flash chromatographic separation from its α-anomer. Then, acidic hydrolysis of the di-*O*-ethylpropylidene protective group of **5** was performed with aqueous trifluoroacetic acid to afford the corresponding diol **6** in 80% yield. Finally, hydrogenolysis of the benzyl protecting groups and simultaneous azide reduction in the presence of Pd black in acetic acid gave the targeted pharmacophore **1**. Careful analysis of ^{1}H and ^{13}C NMR spectra revealed a partial reduction of the uracil double bond (15%).[22]

The in vitro biological evaluation of pharmacophore **1** on purified MraY from *Bacillus subtilis* was carried out in the conditions previously described.[23] An 81% inhibition of the MraY activity was observed when **1** was tested at a concentration of 2 mM.

3. Conclusion

In conclusion, we have described a short and efficient access to an aminoribose uridine like scaffold which is obtained in two main key steps from uracil and L-ido bis-epoxide in 23% overall yield. It should be pointed out that by comparison with the Aventis Pharmacophore **A**, the introduction of an exocyclic methylene group was not prejudicial for biological activity. Furthermore, inversion of the absolute configuration at C2 confirms that the configuration at this stereogenic centre does not seem to be crucial for biological activity. This promising result is now the starting point for a library synthesis of related compounds for further SAR investigations. In particular, the introduction of hydrophobic moieties on the primary amine function should improve the cell penetration of the resulting inhibitors, thereby endowing them with antibacterial properties. Further work is currently in progress towards this goal.

Acknowledgement

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References

1. For example, see: (a) Walsh, C.; Wright, G. *Chem. Rev.* **2005**, *105*, 391, and all references of this volume dedicated to antibiotic resistance; (b) Talbot, G. H.; Bradley, J.; Edwards, J., Jr.; Gilbert, D.; Scheld, M.; Bartlett, J. G. *Clin. Infect. Dis.* **2006**, *42*, 657.
2. van Heijenoort, J. *Nat. Prod. Rep.* **2001**, *8*, 503.
3. Boyle, D. S.; Donachie, W. D. *J. Bacteriol.* **1998**, *180*, 6429.
4. Bouhss, A.; Mengin-Lecreulx, D.; Le Beller, D.; van Heijenoort, J. *Mol. Microbiol.* **1999**, *34*, 576.
5. Bouhss, A.; Crouvoisier, M.; Blanot, D.; Mengin-Lecreulx, D. *J. Biol. Chem.* **2004**, *279*, 29974.
6. Stachyra, T.; Dini, C.; Ferrari, P.; Bouhss, A.; van Heijenoort, J.; Mengin-Lecreulx, D.; Blanot, D.; Bitton, J.; Le Beller, D. *Antimicrob. Agents Chemother.* **2004**, *48*, 897.
7. For MraY inhibitors, see for example: (a) Kimura, K.-I.; Bugg, T. D. H. *Nat. Prod. Rep.* **2003**, *20*, 252; (b) Dini, C. *Curr. Topics Med. Chem.* **2005**, *5*, 1221; (c) Bugg, T. D. H.; Lloyd, A. J.; Roper, D. I. *Infect. Disord. Drug Targets* **2006**, *6*, 85.
8. Le Corre, L.; Gravier-Pelletier, C.; Le Merrer, Y. *Eur. J. Org. Chem.* **2007**, 5386.
9. Isono, K.; Uramoto, M.; Kusakabe, H.; Kimura, K.; Izaki, K.; Nelson, C. C.; McCloskey, J. A. *J. Antibiot.* **1985**, *38*, 1617.
10. (a) Takeuchi, T.; Igarashi, M.; Naganawa, H.; Hamada, M. PCT Int. Appl. **2001**, WO 2001012643, 73 pp; (b) Igarashi, M.; Nakagawa, N.; Doi, N.; Hattori, S.; Naganawa, H.; Hamada, M. *J. Antibiot.* **2003**, *56*, 580.
11. Monasson, O.; Ginisty, M.; Bertho, G.; Gravier-Pelletier, C.; Le Merrer, Y. *Tetrahedron Lett.* **2007**, *48*, 8149.
12. Dini, C.; Colette, P.; Drochon, N.; Guillot, J.-C.; Lemoine, G.; Mauvais, P.; Aszodi, J. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1839.

13. Brandish, P. E.; Kimura, K.; Inukai, M.; Southagate, R.; Lonsdale, J. T.; Bugg, T. D. H. *Antimicrob. Agents. Chemother.* **1996**, *40*, 1640.
14. Dini, C.; Didier-Laurent, S.; Drochon, N.; Feteanu, S.; Guillot, J. C.; Monti, F.; Uridat, E.; Zhang, J.; Aszodi, J. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1209.
15. For correlation of molecular flexibility and inhibitory activities, see for example: (a) Mai, A.; Massa, S.; Ragno, R.; Cerbara, I.; Jesacher, F.; loidl, P.; Brosch, G. *J. Med. Chem.* **2003**, *46*, 512; (b) Narsinghani, T.; Chaturvedi, S. C. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 461.
16. Dini, C.; Drochon, N.; Guillot, J.-C.; Mauvais, P.; Walter, P.; Aszodi, J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 533.
17. Poitout, L.; Le Merrer, Y.; Depezay, J.-C. *Tetrahedron Lett.* **1994**, *35*, 3293.
18. For analogous tandem reaction on bis-epoxides, see: (a) Poitout, L.; Le Merrer, Y.; Depezay, J.-C. *Tetrahedron Lett.* **1995**, *36*, 6887; (b) Le Merrer, Y.; Sanière, M.; McCort, I.; Dupuy, C.; Depezay, J.-C. *Tetrahedron Lett.* **2001**, *42*, 2661; (c) Saladino, R.; Ciambecchini, U.; Hanessian, S. *Eur. J. Org. Chem.* **2003**, 4401; (d) Busca, P.; McCort, I.; Prangé, T.; Le Merrer, Y. *Eur. J. Org. Chem.* **2006**, 2403.
19. There are some typographical errors in Ref. 18c. For example, in Scheme 1, bis-epoxide **3** and related compounds **4**–**9** are described with a L-*manno* and a D-*gulo* configuration, respectively, instead of a D-*manno* or a L-*gulo* configuration and in Scheme 2, compounds **13**–**18** should have an L-*gluco* configuration.
20. Hirano, S.; Ichikawa, S.; Matsuda, A. *Angew. Chem., Int. Ed.* **2005**, *44*, 1854.
21. For similar glycosylation reaction involving the 5-azido-1,5-dideoxy-2,3-di-*O*-isopropylidene-1-fluoro-D-ribofuranose, see: Ginisty, M.; Gravier-Pelletier, C.; Le Merrer, Y. *Tetrahedron: Asymmetry* **2006**, *17*, 142.
22. Nevertheless, a sample of pure compound **1** could be obtained after HPLC and flash chromatographic purification.
23. Bouhss, A.; Crouvoisier, M.; Blanot, D.; Mengin-Lecreulx, D. *J. Biol. Chem.* **2004**, *279*, 29974.