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Synthesis of a β-Ketophosphonate Bioisostere of UDP-*N*-acetylglucosamine

Nicolas Auberger,[a] Christine Gravier-Pelletier,*[a] and Yves Le Merrer*[a]

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A concise route to a new β-ketophosphonate analog of glycosyl nucleotides is described. Such a diphosphate bioisostere is a stable mimic of enzyme substrates involved in peptidoglycan biosynthesis and will be the starting point for the development of new potential antibiotics. The synthesis is carried out by condensing a lithiomethylenephosphonate derivative on a methyl *N*-acetylglucosaminylacetate followed by esterification with a uridine derivative.

Introduction

The cell wall is a unique and essential feature for bacteria and it is a key target for antibiotics development. Its biosynthesis[1] (Figure 1) takes place first at the cytoplasm from fructose-6-phosphate, which is converted under the successive action of three enzymes (GlmS, GlmM, and GlmU) into UDP-*N*-acetylglucosamine (UDP-Glc*N*Ac). Its further transformation into UDP-*N*-acetylmuramic acid pentapeptide (UDP-Mur*N*Ac-pentapeptide) is catalyzed by the Mur enzymes (MurA, MurB, MurC, MurD, MurE, and MurF). Then, at the plasma membrane two distinctive proteins (MraY and MurG) subsequently synthesize polyprenyl-linked precursors (Lipids I and II) that carry one complete cell wall subunit. Thereafter, a transport protein flips lipid II across the membrane to deliver the cell wall subunit to the polymerization enzymes, that is, the penicillin-binding proteins (PBPs). The latter catalyze both the polymerization of the lipid bearing monomer units of peptidoglycan to make long polysaccharide chains and their cross linking at the peptide unit. Owing to their high specificity and their sole occurrence in bacteria, the enzymes implicated in peptidoglycan biosynthesis are potential targets of particular interest for the search of novel antibacterial agents. Furthermore, no counterparts have been identified in eukaryotes, and this renders these enzymes ideal targets for new antibiotics. Several natural products with various chemical structures have been identified as inhibitors[2] of the enzymes involved in peptidoglycan biosynthesis, such as fosfomycin for MurA, tunicamycins, muraymycins, mureidomycins and liposidomycins for MraY, ramoplanin for MurG, vancomycin for transglycosylases, and, for instance, moenomycin or penicillins for PBPs.

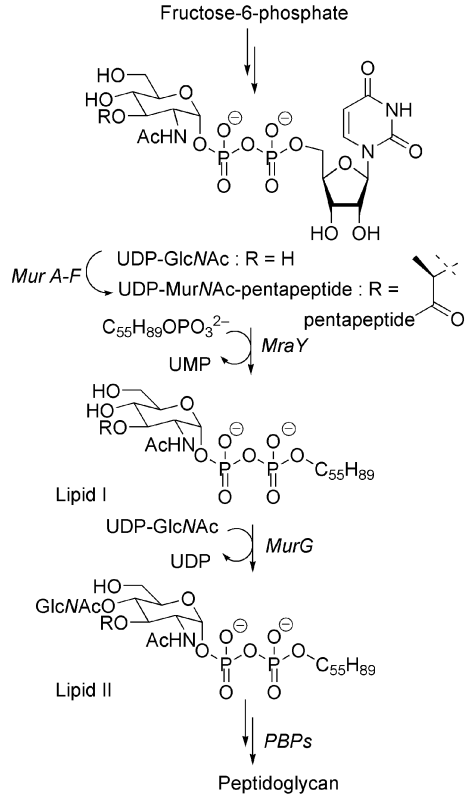


Figure 1. Biosynthesis of the bacterial peptidoglycan.

Results and Discussion

In an ongoing program[3] aiming at the inhibition of new targets for fighting antibiotics resistance,[4] the synthesis of stable substrate analogs (Figure 2) for the enzymes involved in this biosynthesis (MurA-G and MraY) is currently under study.

[a] Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, Université Paris Descartes, CNRS UMR 8601, 45 rue des Saints-Pères, 75006 Paris, France
Fax: +33-142868387
E-mail: christine.gravier-pelletier@parisdescartes.fr
yves.le-merrer@parisdescartes.fr

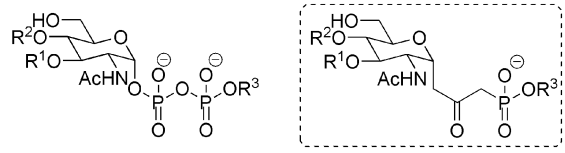


Figure 2. Enzyme substrates and structure of the targeted β-ketophosphonate bioisosteres.

Indeed, the central core of these substrates is based on a pyrophosphate moiety linked on the one hand to an *N*-acetylglucosamine derivative, and on the other hand to either uridine or undecaprenol. Within the targeted analogs, the oxygen atom at the anomeric position of the sugar is replaced by a methylene group in order to enhance the stability of the resulting inhibitors towards hydrolysis. Furthermore, a carbonyl group is introduced to replace the phosphate at the sugar anomeric position, the latter being the site[1d,5] of the enzymatic reaction catalyzed by MraY. The resulting ketone is proposed as an alternative electrophilic center for the corresponding phosphate. Finally, the central oxygen atom of the pyrophosphate moiety is exchanged for a methylene group expected to prevent the release of UMP in the particular case of the reaction catalyzed by MraY. One can assume that the resulting glycosyl β-ketophosphonate bioisostere, stable to hydrolysis, should have the required electronic properties to coordinate the metallic cofactor of this reaction (Mg^{2+} or Mn^{2+}). Therefore, it should be a pertinent substrate mimic. Moreover, such a pyrophosphate bioisostere can also serve as a relevant stable surrogate of most substrates involved in various steps of peptidoglycan biosynthesis.

A few different glycosyl nucleotide analogs have already been described[6] as mimics of the pyrophosphate moiety (Figure 3). However none of them displays a glycosyl β-ketophosphonate skeleton.

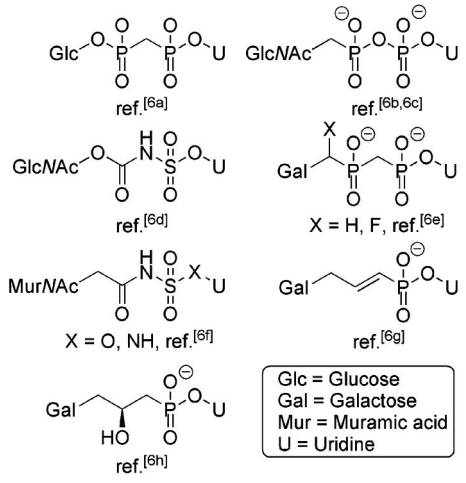


Figure 3. Pyrophosphate analogs of glycosyl nucleotides previously described.

To reach these new analogs of UDP-Glc*N*Ac and their derivatives, the retrosynthetic analysis (Figure 4) relies on two complementary strategies. The first one involves nucleophilic opening of conveniently protected epoxide **3** by trialkylphosphite followed by the oxidation of the resulting secondary alcohol function. The second one relies on the condensation of a dialkyl lithiomethylenephosphonate on the ester function of conveniently protected methyl *N*-acetylglucosaminylacetate **4**. Then, esterification with uridine should afford in both cases the targeted UDP-Glc*N*Ac mimic. Both epoxide and ester building blocks result from the single orthogonally protected α-1*C*-allyl-*N*-acetylglucosamine **1**, readily obtained in four steps (50% overall yield as an α/β mixture of anomers) from commercially available *N*-acetylglucosamine.[7]

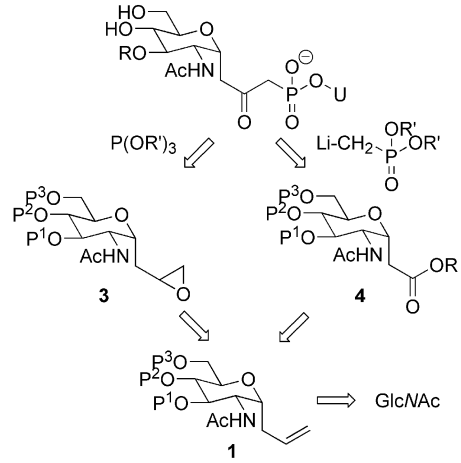
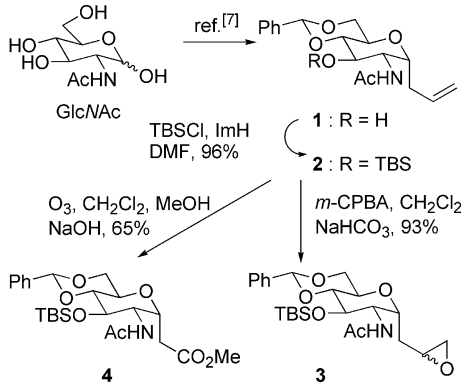


Figure 4. Retrosynthetic analysis.

The preparation of these key building blocks (Scheme 1) required the protection of the secondary alcohol function of **1** as its *tert*-butyldimethylsilyl ether **2**, which was isolated by flash chromatography as a pure α anomer from the 24:1 α/β mixture.

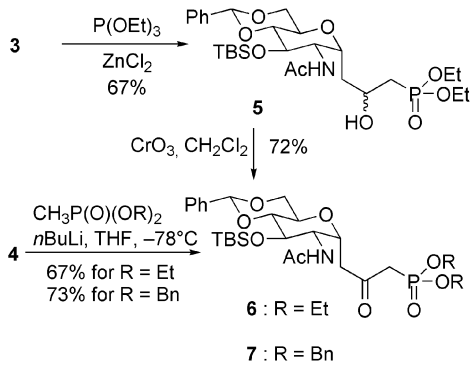


Scheme 1. Preparation of the building blocks.

The subsequent *m*-CPBA oxidation of the double bond of **2** led to **3** as a 3:2 mixture of epimers. Alternatively, the ozonolysis of the alkene under basic conditions in the presence of methanol led to methyl ester **4**.[8]

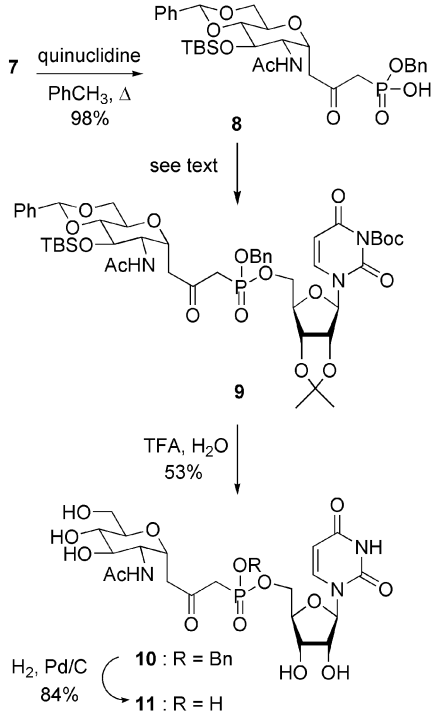
The synthesis of *N*-acetylglucosamine derivative **6** (Scheme 2) involved the nucleophilic opening of epoxide **3** by triethylphosphite in the presence of activated zinc chlo-

ride under microwave irradiation at 60 °C[9] (CEM discover®) followed by Collins oxidation of resulting alcohol **5**.



Scheme 2. Formation of the β-ketomethylenephosphonate key moieties.

In a complementary and more efficient way, compound **6** could also result from ester **4** through condensation of diethyl lithiomethylenephosphonate at –78 °C. This lithio derivative was readily generated at –78 °C by addition of butyllithium to the commercially available diethyl methylphosphonate. Furthermore, analogous dibenzyl β-ketophosphonate **7** could be obtained from ester **4** under similar conditions to those used for dibenzyl methylphosphonate, which could be prepared from commercial dibenzyl *H*-phosphonate by NaH treatment in the presence of methyl iodide.[10] The monodeprotection of diethyl phosphonate **6** in the presence of sodium azide in DMF could not be controlled, whereas that of dibenzyl phosphonate **7** could be efficiently achieved in the presence of quinuclidine[11] in refluxing toluene to give monobenzyl β-ketophosphonate **8** (Scheme 3).



Scheme 3. Synthesis of a β-ketophosphonate bioisostere of UDP-Glc*N*Ac.

We next turned to the introduction of the isopropylidene-protected uridine, which was tentatively carried out under Mitsunobu conditions[12] in the presence of triphenylphosphane and diisopropyl azodicarboxylate. However, the latter conditions were unsuccessful. Interestingly, esterification of **8** with isopropylidene *N*-Boc uridine[13] under the above-mentioned Mitsunobu conditions afforded the targeted protected glycosyl nucleotide analog **9** in 20% yield. Alternative conditions for uridine introduction were then studied and we showed that coupling of isopropylidene *N*-Boc uridine with β-ketophosphonate **8** could also be achieved in the presence of a coupling agent (PyBOP, BOP, or HATU)[14] and a base (diisopropylethylamine or triethylamine) in moderate yield (30%).

Simultaneous acidic hydrolysis of the benzylidene and isopropylidene ketals, the *tert*-butyldimethylsilyl ether, and the *tert*-butylcarbamate groups of **9** afforded **10** (53% yield). Finally, subsequent hydrogenolysis of benzyl phosphonate gave targeted compound **11** (84% yield).

Conclusions

In conclusion, we describe a concise and straightforward route to a new β-ketophosphonate mimic of nucleotide sugars. It is a key intermediate towards substrate analogs of enzymes involved in peptidoglycan biosynthesis and identified as a target for the development of new antibiotics. The methodology reported herein should serve as a major tool for the further elaboration of a related series of inhibitors. Indeed, the introduction of a peptidic chain at C3 of the glycosyl part should afford substrate analogs of both cytoplasmic enzymes and MraY, whereas that of polyisoprenol residues in place of uridine should afford substrate analogs of MurG. Current work is in progress towards that goal.

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