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INHIBITORS OF THE BACTERIAL CELL WALL BIOSYNTHESIS ENZYME MUR D

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Abstract: A series of transition-state analog inhibitors of the D-glutamic acid-adding enzyme (MurD) of bacterial peptidoglycan biosynthesis has been synthesized and evaluated for inhibition of the *E. coli* enzyme. © 1998 Elsevier Science Ltd. All rights reserved.

The bacterial cell wall peptidoglycan layer consists of alternating *N*-acetyl muramic acid (MurNAc) and *N*-acetyl glucosamine units that are crosslinked through pentapeptide chains. The disruption of this structure leads to cell lysis; peptidoglycan biosynthesis is therefore an essential pathway and an important target for antibiotics research.¹ The final cytoplasmic precursor for peptidoglycan biosynthesis is the uridine diphosphate-MurNAc-pentapeptide **2** (Figure 1). This compound is synthesized from UDP-MurNAc **1** by four amino acid ligases, Mur C, D, E, and F, that add L-Ala, D-Glu, *meso*-DAP (or L-Lys in some Gram-positive bacteria), and D-Ala-D-Ala, respectively. MurC through F are believed to function in a similar manner to the ATP-dependent amide-forming enzymes glutamine synthetase,² glutathione synthetase,³ and D-Ala-D-Ala ligase.⁴ These latter enzymes each catalyze the formation of an acyl phosphate that is attacked by the incoming amino acid to form a tetrahedral species that collapses to the amide product. Recent mechanistic studies on MurC⁵ and MurD⁶ support the hypothesis that the Mur ligases also proceed by the acyl phosphate mechanism. This information is especially useful in designing potential inhibitors of the Mur enzymes, as it is known that phosphinates act as slow-binding inhibitors of glutamine synthetase⁷, glutathione synthetase⁸ and D-Ala-D-Ala ligase.^{9,10}





0960-894X/98/\$19.00 © 1998 Elsevier Science Ltd. All rights reserved. *Pll:* S0960-894X(98)00285-6 MurD, the second enzyme in the series of peptidoglycan biosynthesis ligases, catalyzes the addition of Dglutamic acid to UDP-MurNAc-L-alanine 3 to afford UDP-MurNAc-L-Ala-D-Glu 4 (Figure 2). Recently, Tanner and coworkers reported the first effective inhibitors of MurD (Compounds 5 and 6, Figure 3).¹¹ Tanner's transition-state analog inhibitors 5 and 6 feature a phosphinate transition-state analog at the center of amide bond formation and a methylene chain as a replacement for the *N*-acetyl muramic acid moiety. Compound 5 was found to be a good inhibitor of MurD with an IC₅₀ value of 680 nM, while phosphate 6 was a weaker inhibitor with IC₅₀ of 29 μ M.¹¹

Figure 2



We were interested in preparing MurD inhibitors that would be more potent than those that had already been reported. We hypothesized that the *N*-acetyl muramic acid structure could be an important contributor to potency and we therefore chose 7 as our initial MurD inhibitor target molecule. In our first-generation inhibitor 7, we have retained the proven phosphinate transition-state mimic design. However, we have incorporated the carbohydrate moiety and controlled the stereochemical configuration of the α -amino phosphinate. Here we report the synthesis and biological evaluation of 7 and its synthetic precursors.





Our starting material, the known methyl phosphinate **8**, was synthesized in six steps according to the procedure of Baylis.¹² This included a resolution of the free phosphinous acid by recrystallization of its salt with R- α -methylbenzylamine. Following Tanner's precedent, compound **8** was treated with sodium methoxide and dimethyl 2-methylene pentanedioate¹³ to afford the fully protected dipeptide isostere as a mixture of four diastereomers (Scheme 1). The CBz group was removed via hydrogenolysis in preparation for formation of the amide bond to link the phosphinate and sugar fragments. The amine **9** was added to benzyl-N-acetyl-4,6-O-benzylidene muramic acid¹⁴ activated by *N*-hydroxysuccinimide/DCC. The amide product **10** was isolated in 59% yield and was also found to be a mixture of four diastereomers by ³¹P NMR. Removal of the benzyl and benzylidene protecting groups from amide **10** proceeded quantitatively and was followed by saponification of the triol **11** to afford the phosphinate **12** as its trisodium salt. This compound containing a free anomeric hydroxyl group was of particular interest to us in establishing our preliminary SAR for the inhibition of MurD.



We then turned our attention to the phosphorylation of the anomeric position. All of our attempts to phosphorylate triol **11** were unsuccessful, despite literature precedent for the phosphorylation of unprotected glucoses.¹⁵ It was therefore necessary to return to the fully protected derivative **10** and deprotect the muramic acid in a stepwise manner (Scheme 2). The benzyl group was selectively removed from amide **10** by catalytic transfer hydrogenation using ammonium formate as the hydrogen donor¹⁶ to afford the anomeric alcohol **13**. The alcohol **13** was phosphorylated¹⁷ by treatment with *n*-BuLi and dibenzylchlorophosphate to afford the unstable muramic acid dibenzyl phosphate. Therefore, immediately upon consumption of **13**, the reaction mixture was allowed to warm to room temperature and stir under an atmosphere of H₂ in the presence of Pd/C catalyst. This phosphorylation procedure selectively produced the 1- α -phosphate **14** in 97% yield. Once again, this compound was a mixture of four diastereomers due to mixtures at the phosphinate chiral center and at the carbon chiral center located at the β -position to the phosphinate.



In order to reach our desired target, the benzylidene group was removed from 14 via hydrogenolysis in the presence of acetic acid (Scheme 3). The resulting diol was saponified to afford phosphate 16 as its pentasodium salt. At this stage, only formation of the pyrophosphate bond was required to complete the synthesis of the fully elaborated inhibitor 7. However, in order to probe the effect of functionality at the 4,6-position on potency, the substituted phosphoric acid 14 was saponified to yield the benzylidene-protected pentasodium salt 15.



In the final step of the synthesis, the pyrophosphate bond was formed via Khorana's standard morpholidate-activated coupling conditions.¹⁸ The free acid form of phosphate **16** was allowed to react with uridine 5'-monophosphomorpholidate to afford the desired diphosphate **7** (Scheme 4) as a mixture of two diastereomers. In addition, the 5-iodo analog **17** was synthesized in a similar manner by allowing **16** to react with 5-iodo-5'-monophosphomorpholidate. Our isolated yields were poor (7%, 3%) and we were not able to improve them by use of 1*H*-tetrazole as a catalyst.¹⁹



We tested compounds 12, 15, 16, 17, and 7 for inhibition against the MurD enzyme isolated from *E. coli.* The reaction catalyzed by MurD was followed by the formation of radiolabelled UDP-MurNAc-L-Ala-D-Glu from radiolabelled D-[2,3,4-³H]-glutamic acid (25 μ M) and UDP-MurNAc-L-Ala (25 μ M). The IC₅₀s were determined in the presence of 850 nM enzyme for compound 12 and 1 nM enzyme for all other compounds. Our results are summarized in Table 1; Tanner's data¹¹ have been included for comparison.



We conclude that the incorporation of muramic acid and the resolution of one stereocenter significantly increase potency, perhaps by more than three orders of magnitude. This increase in potency is evident when Tanner's terminal phosphate inhibitor **6** (29 μ M) is compared to our terminal phosphate **16** (20 nM). The IC₅₀ of our uridine-containing inhibitor **7** (<1 nM) lies below the sensitivity of the enzyme assay; we see that it is at least approaching a value three orders of magnitude below the IC₅₀ of Tanner's analogous compound **5** (680 nM). Within our series, the phosphate group is critical (**16**, 20 nM) as its presence adds more than four orders of magnitude in potency to the terminal hydroxy compound **12** (782 μ M). It is interesting to note that the 4,6-*O*-benzylidene group is reasonably well tolerated, with only a 20-fold difference in potency observed between compounds **15** and **16**.

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