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A new approach to the synthesis of peptidoglycan fragments

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Introduction

- Peptidoglycans are central structural components of the cell wall of bacteria. Several animal and plant receptors are known to recognize peptidoglycan fragments. It is believed that these receptors form part of the defense mechanism against bacterial infections in these species.

- Peptidoglycans (PGN) consist of long chains of alternating β(1-4)-linked GlcNAc and MurNAc moieties that are crosslinked by short, non-ribosomal peptides. These peptides consist of several D-amino acids, and for gram-negative bacteria the symmetrical (*R*,*S*)-diaminopimelic acid (*meso*-DAP).

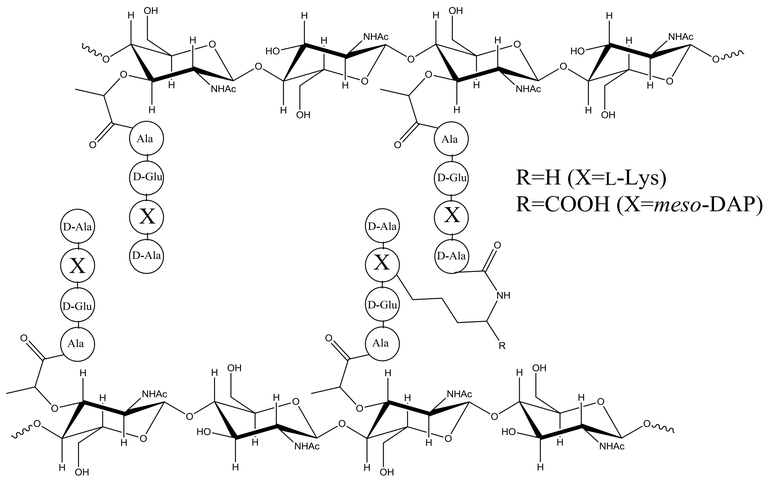


Figure 1: Generalized structure of peptidoglycan

Our approach to peptidoglycan synthesis is based on the use of mostly commercially available building blocks and the use of manual and automated peptide synthesis.

The fragments were synthesized using SPPS (on semi- and fully automated peptide synthesizers) and solution phase strategies. Fragment 1 was assembled on the Biotage® Initiator+ SP *Wave*, fragment 2 was assembled partially on the Biotage® Syro *Wave*™ and Initiator+ SP *Wave*. Fragment 3 and 4 were both synthesized using the Biotage® Initiator+ Alstra™.

Peptidoglycan from *T. thermophilus*

- Peptidoglycan fragments from *T. thermophilus* containing Orn(Gly-Gly) cross-links of the peptide stems were synthesized as putative substrates of Tth-P60, a D,L-endo-peptidase involved in PGN re-modelling during bacterial growth.

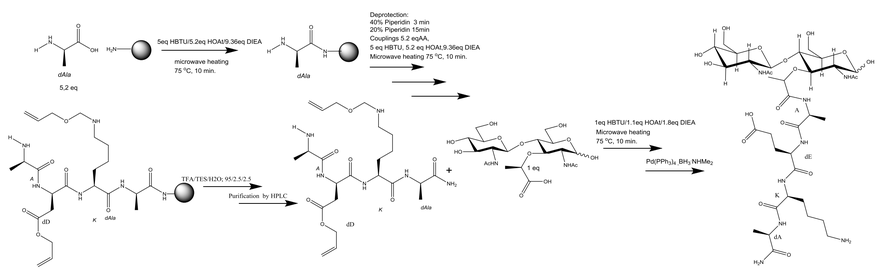


Figure 2: Reaction scheme showing the synthesis of **Fragment 1**

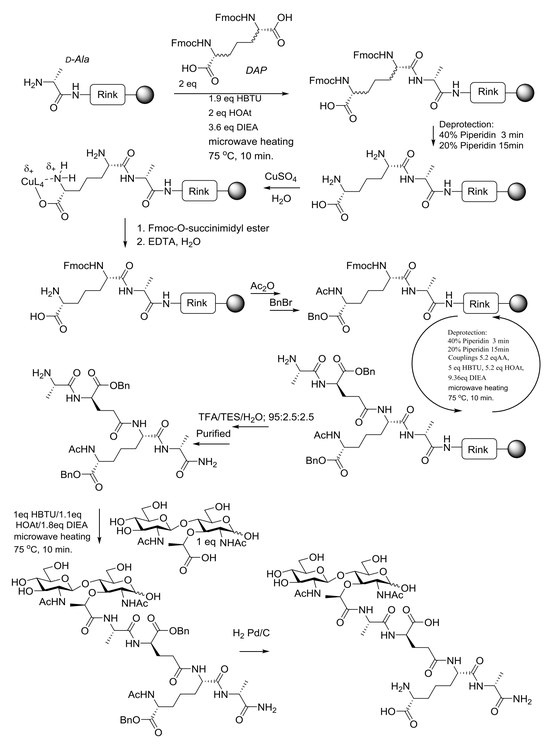


Figure 3: Reaction scheme showing the synthesis of **Fragment 2**

T. thermophilus PGN peptides were synthesized on a Trityl resin by coupling with Dde-L-Orn(Fmoc)-OH at the branching points (Figure 5). The Nα-Dde groups were removed by treatment with 1% hydrazine hydrate in DMF for 2 x 250 minutes. Subsequently was coupled Fmoc-D-Glu-OBn through the glutamic acid side-chains, followed by Fmoc-L-Ala-OH, and Fmoc-L-Tyr-OH. After deprotection of the two N-terminal amino groups, the peptide was cleaved from the resin, and purified by HPLC.

- We labelled the PGN peptides with Tyr in the C- or N-terminus in order to facilitate analysis of enzyme digestion by LC-MS.

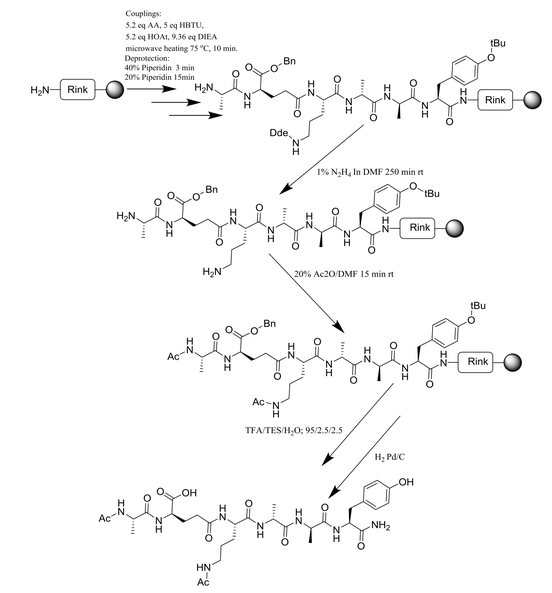


Figure 4: Reaction scheme showing the synthesis of **Fragment 3**

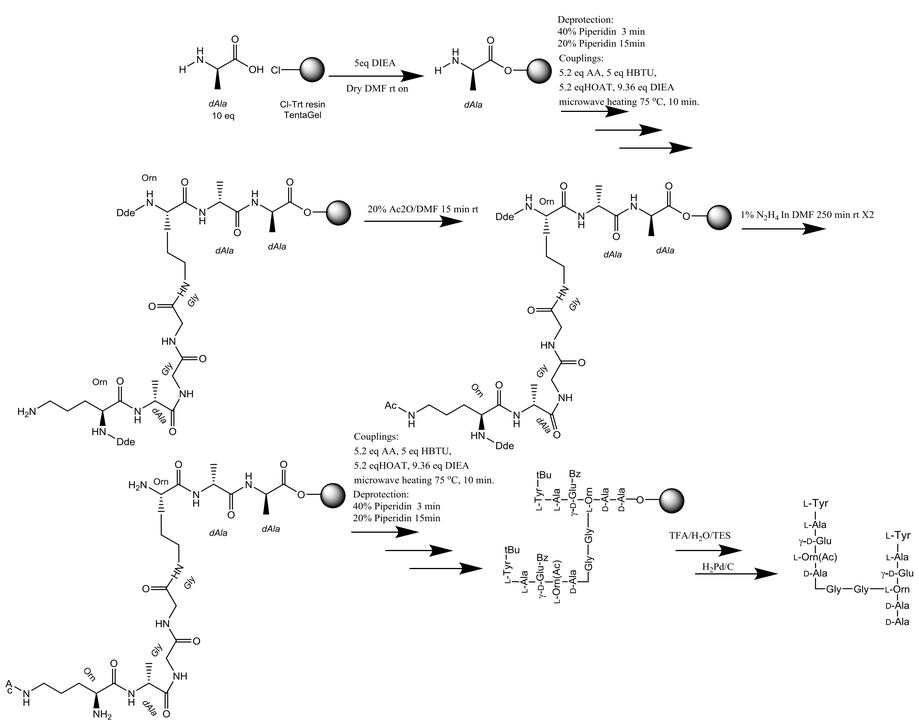
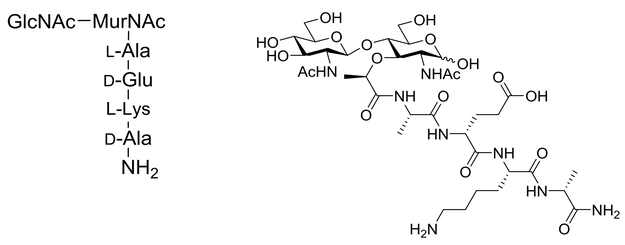
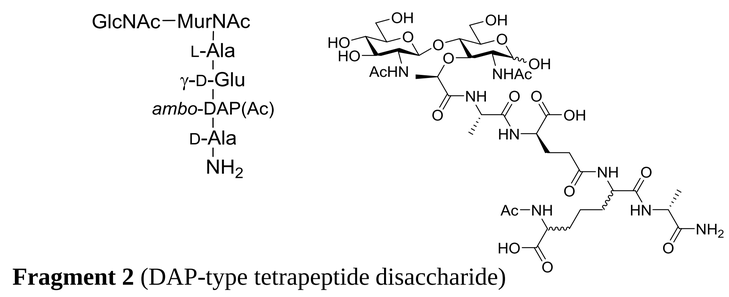


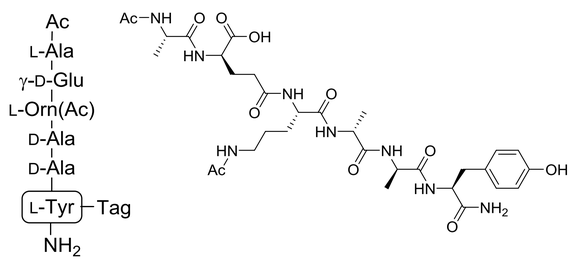
Figure 5: Reaction scheme showing the synthesis of **Fragment 4**



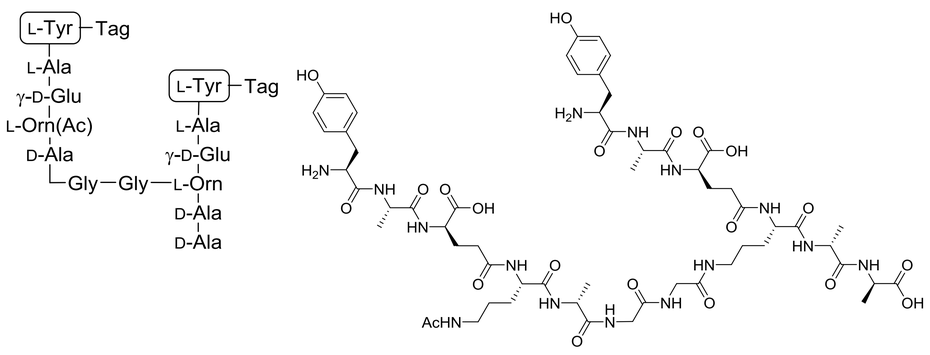
Fragment 1 (Lys-type tetrapeptide disaccharide)



Fragment 2 (DAP-type tetrapeptide disaccharide)



Fragment 3 (tagged *T. thermophilus* pentapeptide)



Fragment 4 (tagged *T. thermophilus* cross-linked peptide)

Conclusion

- DAP-type tetrapeptide disccharide PGN fragments were synthesized on solid-phase followed by solution-phase coupling of the GlcNAc-MurNAc disaccharide.

- Two PGN fragments from *T. thermophilus* were synthesized on solid-phase by using Dde-Orn(Fmoc)-OH at the branching points.

- These PGN fragments are evaluated as putative P60 D,L-endopeptidase substrates.

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