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Multi Gram Synthesis of UDP-*N*-Acetylmuramic Acid

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Abstract—As part of an effort to discover novel antibacterial agents, a new and efficient synthesis was established in order to provide a large amount of UDP-*N*-acetylmuramic acid (UDP-MurNAc).

Resistance to antibiotics has become a major problem for the treatment of nocosominal infections.[1] One way to overcome this phenomenon would be to discover new chemical entitities acting on novel targets. Among the enzymes of the peptidoglycan biosynthesis pathway,[2] which is specific to bacteria[3] and essential for bacteria survival,[4] MurC[5–7] was identified as a relevant target by M.T. Bocquel et al.[8] This prompted us to initiate a programme for the discovery of specific inhibitors of MurC, able to induce an antibacterial effect on whole cells. This enzyme catalyses the amide bond formation berween the carboxylic group of UDP-MurNAc, and the amino function of L-alanine (Scheme 1).

Large quantities of UDP-MurNac, which is not commercially available, were needed for the programme envisaged. Three approaches could be used to produce this nucleotide substrate: (A) isolation from cell wall precursors leads to limited amounts,[9] (B) enzymatic production using upstream enzymes of the peptidoglycan biosynthesis, namely MurA and MurB (Scheme 2) and the commercially available substrate of MurA, UDP-GlcNAc.[10,11] However, this necessitates large quantities of both proteins, (C) chemical synthesis, which is often delicate in these series, but gives the prospect of the preparation of gram quantities. Of these, the last option was retained.

Blanot et al. described in 1995 the first full chemical synthesis of UDP-MurNAc.[12,13] Unfortunately, we found the scale-up of the phosphorylation step (according to MacDonald's procedure[14]), gave neither a reproductive yield nor a satisfactory α/β ratio (Scheme 3). These results prompted us to establish an alternative and efficient access to a MurNac 1-P derivative.

Lactone **4** was identified as a relevant key intermediate, because of its ease of synthesis. It was prepared from **3**,[15] by hydrogeneolysis of the benzyl protecting group.

Phosphorylation in the anomeric positon was accomplished by using commercially available diphenyl chlorophosphate in the presence of 4-pyrrolidinopyridine (which was found to be the best acylating catlayst). After work up and purification on a short silica gel column (15 parts), only the expected α anomer was recovered.[16] It is worth noting that a good and reproducible yield (43%) of **5α**[17] was only obtained by limiting the amount of crude material applied to silica gel, corresponding to the use of 10 g of **4**. PtO_2 catalysed hydrogenolysis of **5α** in THF for 48 h, gave the intermediate **6** which was immediately treated with 4 equivalents of aqueous lithium hydroxide. The crude material was purified through a Dowex 50WX2 column (triethylammonium form) providing **7** in 72% yield for the two steps. The final coupling step was accomplished with an improved Roseman et al.[18] procedure: activated 4 Å molecular sieves and dry commercially available uridine 5′-monophosphomorpholidate **8** were successively added to a solution of **7** in dry DMF. The mixture was heated to 70 °C for 10 h. The rather high reaction yield (52%) with respect to values from literature is a result of thorough drying of **8** at 100 °C under vacuum for 18 h, prior to use. Compound **8** is sold as a hydrate and this coupling reaction is known to be water-sensitive.[19] Under these conditions, heating was possible, reducing the reaction time from 1 week (rt) to 10 h (70 °C). Purification was simplified by limiting the amount of **10** (product of auto-condensation of **8** formed under these conditions). Furthermore, a reduced amount of this expensive

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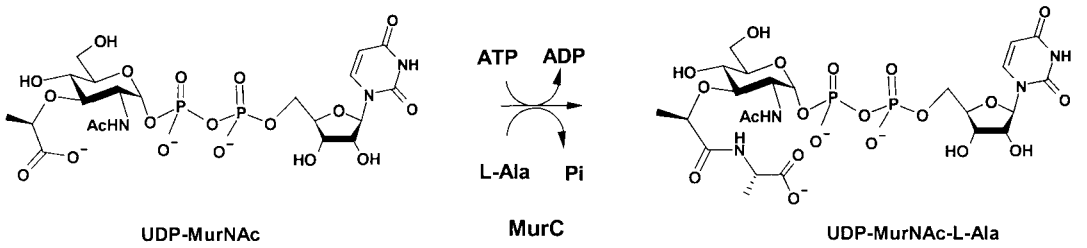
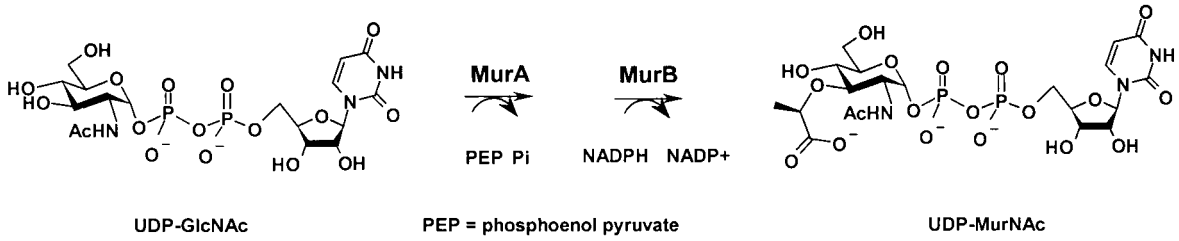
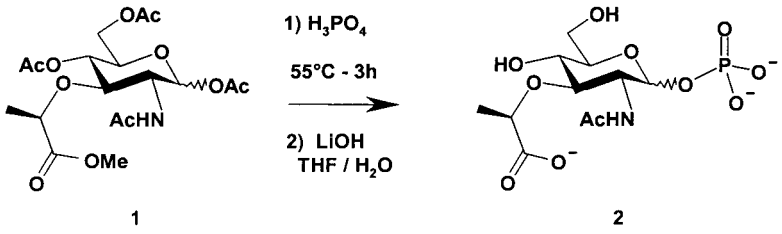


Scheme 1.

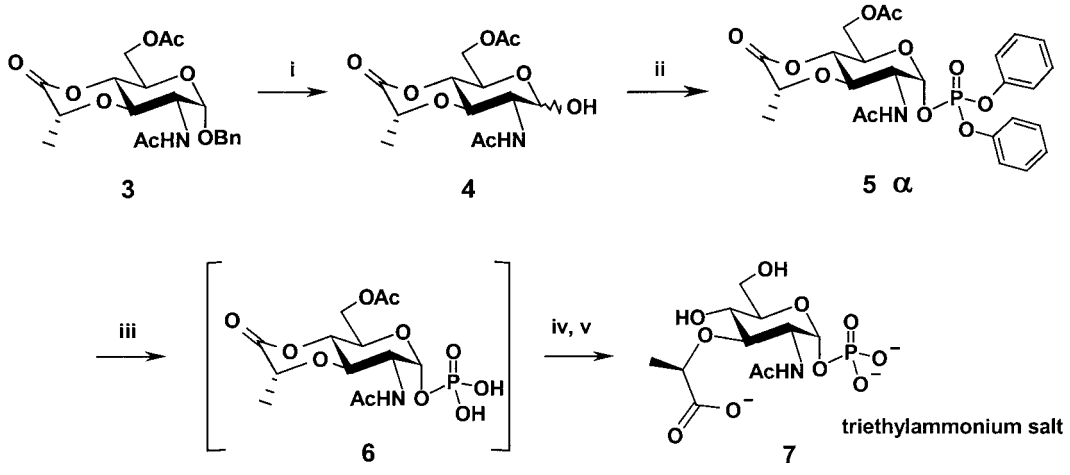


Scheme 2.

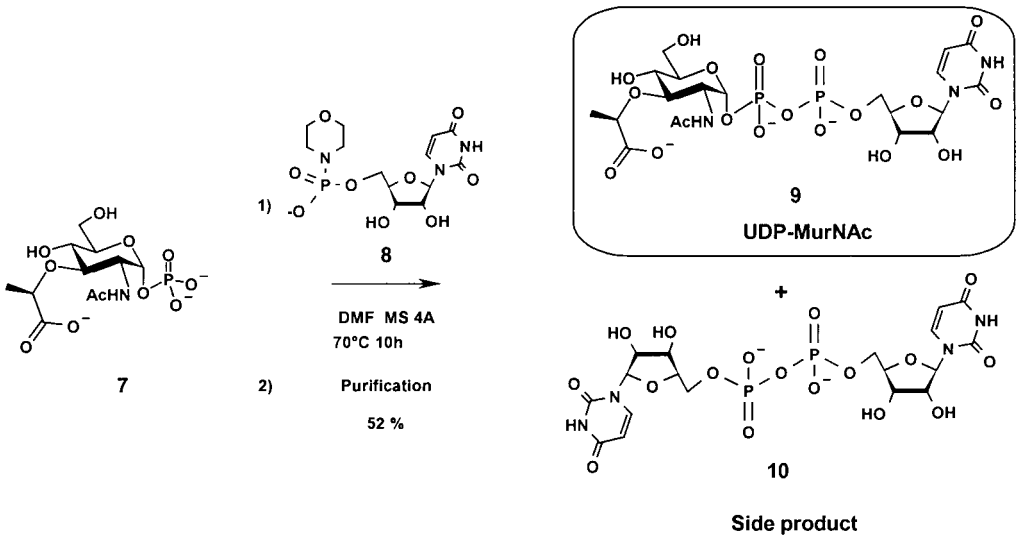


Scheme 3.

reagent **8** was required (1.5 eq. instead of a large excess used previously). After usual work-up the UDP-MurNAc was purified by reverse phase and desalting chromatography. Thus, the crude product containing UDP-MurNAc was dissolved in 2 M ammonium formate and then applied on a C18 Hyperprep (SHANDON) column equilibrated with 50 mM ammonium formate at pH 4.0. Pure fractions were pooled, concentrated by distillation and then applied on a G25 Sephadex gel (Pharmacia) column equilibrated with water. The main fraction was lyophilised and the pure compound **9** was isolated in 52% yield.



i) Pd/C (10%) , H_2 , MeOH, 98% ; ii) $ClP(O)(OPh)_2$, 4-pyrrolidinopyridine, CH_2Cl_2, -30 °C, overnight, 43% ; iii) PtO_2, H_2, THF, 48h , R.T. iv) LiOH 4eq. , H_2O, overnight. v) Dowex 50WX2, TEA form, 72% from **5α**.



Physical analyses are in accordance with data from the literature.[9–11] This new and efficient synthesis of UDP-MurNAc was carried out on a multi gram scale and more than 25 g of pure MurC substrate were prepared this way.

References and Notes

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17. Data for **5α**;: ^{1}H NMR ($CDCl_3$): 1.52 (d, 3H, CH_3 lactoyl), 1.86 (s, 3H, Ac), 2.02 (s, 3H, Ac), 4.72 (q, 1H, C$\underline{H}CH_3$ lactoyl), 3.86 (dd, 1H, J=9 Hz, 10.5 Hz, H3 or H4), 4.03 (dm, 1H, H5), 4.13 (dd ,1H, J=3.5, 12.5 Hz, H6), 4.26 (dd, 1H, J=3.5, 12.5 Hz, H6′), 4.44 (m, 2H, H2 + H3 or H4), 5.59 (d, 1H, J=9 Hz, N$\underline{H}$Ac), 5.59 (dd, 1H, J=3 Hz, 6Hz, H1, α anomer), 7.15–7.44 (m, 10H, OPh). MS (ES): 572 $(M+Na)^+$, 550 $(M+H)^+$.
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