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An improved total synthesis of UDP-*N*-acetyl-muramic acid

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Abstract—Biochemical testing for novel inhibitors of Mur ligases requires several commercially unavailable and structurally complex substrates. We describe a modified synthetic strategy for the total chemical synthesis of the MurC ligase substrate UDP-*N*-acetyl-muramic acid which includes several improvements over published methods, especially with regard to purification procedures. The synthetic strategy is applicable for the synthesis of further Mur ligase substrates.

Antibiotic resistance is becoming an overwhelming public health problem and there is a need for novel classes of antimicrobial agents.[1] Targeting the enzymes involved in the biosynthesis of the bacterial cell wall appears to be an attractive strategy since it should ensure selective toxicity in humans. Mur ligases are enzymes which catalyse early essential steps of cell wall biosynthesis. They have been well characterised and constitute a promising group of targets for antimicrobial research.[2–5] However, biochemical testing of potential Mur ligases inhibitors requires several commercially unavailable and structurally complex substrates.[6,7] UDP-*N*-acetyl-muramic acid (UDP-Mur-*N*-Ac) is the substrate for MurC ligase and is thus needed for testing inhibition of MurC. It is also commonly used for further enzymatic synthesis of MurD, E and F substrates where L-Ala, D-Glu and *m*-dap are attached consecutively to the carboxy end of the peptidoglycan precursor. Here we describe the total synthesis of UDP-Mur-*N*-Ac, using a modified synthetic pathway which could be applied to the total synthesis of further Mur ligase substrates, as well as exhibiting several improvements over the published synthetic and purification procedures.[8–11]

We started the synthesis from the inexpensive α-D-glucosamine hydrochloride **1** which was *N*-acetylated to give α-D-*N*-acetyl-glucosamine **2**, which is also available commercially.[12] In the two following steps, 1-*O*-benzyl and 4′-, 6′-benzylidene protecting groups were introduced according to known procedures, leaving the 3′-hydroxy group of **4** unprotected.[13] The muramic acid moiety was introduced through Williamson synthesis using racemic 2-chloropropionic acid. Under appropriate conditions the reaction gave predominantly the desired *R*-muramic acid derivative (diastereoisomeric ratio 4:1). Treatment of the sodium salt of which with methyl iodide in DMF at room temperature gave methyl ester **5** in quantitative yield as shown in Scheme 1.

To our knowledge, the only reports on purifying a diastereoisomeric mixture of muramic and isomuramic acid derivatives make use of complex gradient column chromatography with toxic carbon tetrachloride and dioxane as eluents.[13] Column chromatographic purification was substantially simplified by using a tri-component mobile phase with isocratic elution, which eliminated the need for a stereo-controlled Williamson reaction[14] and gave higher overall yield.

For further synthesis of UDP-Mur-*N*-Ac, compound **6** was synthesised from **5** in three simple and straightforward steps, according to reported procedures.[9,15] Removal of the benzyl aglycon from lactone **6** under hydrogenation conditions in methanol, as depicted in Scheme 2, presented an overwhelming problem. The reaction gave a dead-end methyl ester derivative **7** with a free 4′-hydroxy group, instead of **8**, as the main reaction product. It has been well established that removal of the benzyl aglycon from muramic acid can sometimes be problematic[15] and our attempts to obtain **8** in good and reproducible yields failed.

Keywords: UDP-Mur-*N*-Ac; Total synthesis; MurC; Diphosphates.
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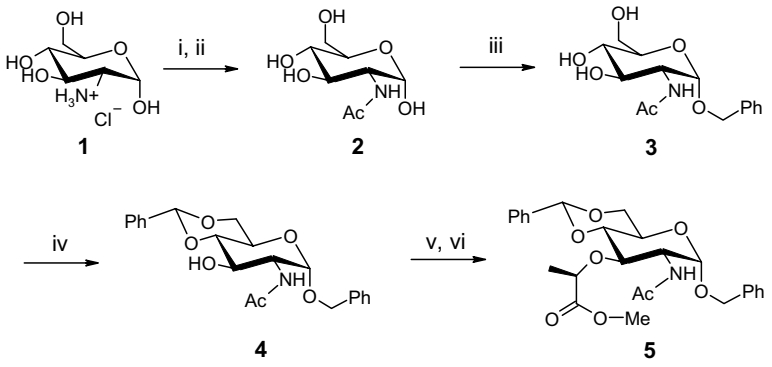
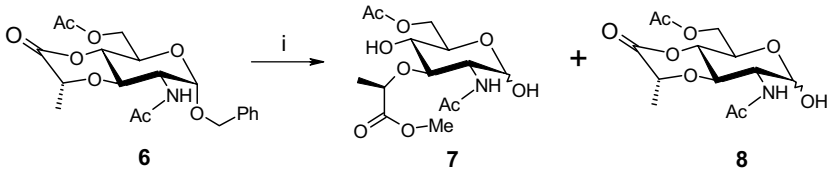


Scheme 1. Reagents and conditions: (i) Na/MeOH (ii) $(Ac)_2O$, rt, 98%; (iii) BnOH, PTSA, benzene, reflux, 12 h, 63%; (iv) Ph-CHO, $(EtO)_3CH$, PTSA, DMF, dioxane, rt, 12 h, 76%; (v) NaH, (*R*,*S*)-$CH_3CHClCOOH$, 50 °C, dioxane, 4 h; (vi) MeI, DMF, rt, 6 h, 63% (for steps v and vi).



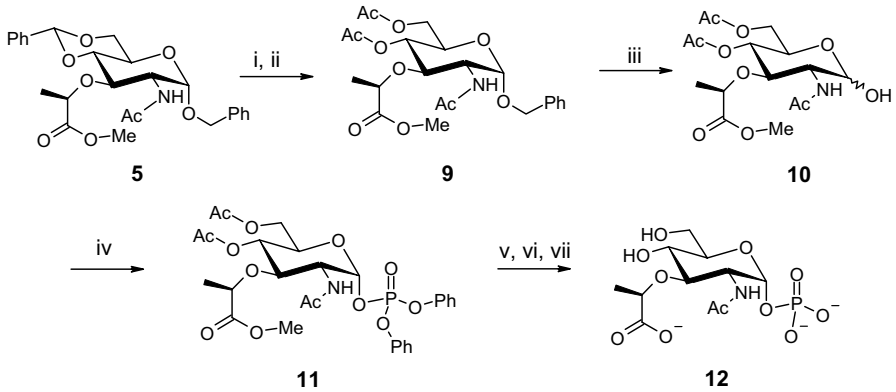
Scheme 2. Reagents and conditions: (i) H_2, 10% Pd/C, MeOH, rt.

At this stage, we hoped that a very simple modification of the muramic acid protecting groups would make the further synthesis straightforward, bypassing the relatively labile internal lactone **6**.

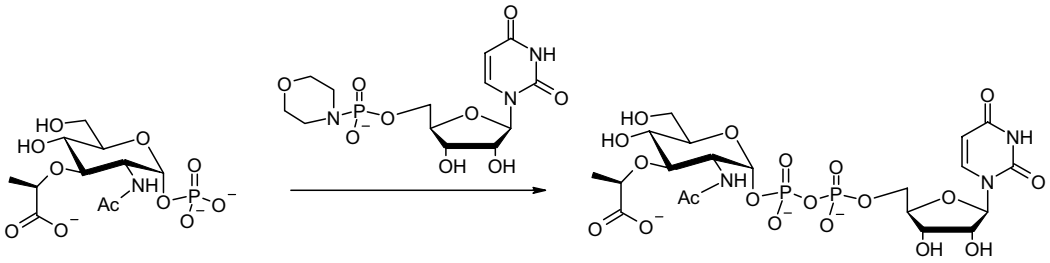
The methyl ester moiety was hence left intact and served as a carboxy protective group in further synthetic steps. Instead, the benzylidene group was removed in aqueous acetic acid and the free 4′- and 6′-hydroxyl groups acetylated with acetic acid anhydride in dry pyridine, giving **9** as depicted in Scheme 3. Compound **9** represents a synthetic substitute for the internal lactone **6**. Indeed, methyl ester **9** was stable under hydrogenation conditions in THF and **10** was obtained in a very short reaction time and in an excellent yield (96%) compared to reported procedures for removing the anomeric benzyl protective group.[15,16] Phosphorylation of the aglycon hydroxyl group catalysed by 4-pyrrolidinopyridine gave only the α-anomer of **11**[17] in good yield.[18]

The phenyl groups of **11** were deprotected under hydrogenation conditions using platinum dioxide as the catalyst[19] immediately followed by hydrolysis with lithium hydroxide which removed the remaining hydroxyl and carboxy protecting groups. The triethylamine salt of **12** was obtained via elution through a Dowex 50W-X2 ion-exchange column.

In a fashion similar to the procedure reported by Dini et al.,[9] coupling of **12** with uridine-5′-monophosphomorpholidate proceeded in sufficiently good yield only when considerable attention was given to drying the reaction glassware and both reagents over phosphorus pentoxide under high vacuum. The reaction was performed under argon using anhydrous DMF. Interestingly, a good reaction yield was achieved despite the fact that no tetrazole was used as the catalyst.[20,21] HPLC analysis of the crude product revealed UDP-Mur-*N*-Ac **13** as the major product with small amounts



Scheme 3. Reagents and conditions: (i) 60% AcOH, H_2O, 100 °C; (ii) $(Ac)_2O$, pyridine, 92%; (iii) H_2, Pd/C, THF, 96%; (iv) $ClPO(OPh)_2$, 4-pyrrolidinopyridine, CH_2Cl_2, −30 °C, 55%; (v) H_2, PtO_2, THF; (vi) 1 M LiOH, THF, rt; (vii) DOWEX 50W-X2-TEA form.



12 13

Scheme 4. Reagents and conditions: DMF, 4 Å molecular sieves, 70 °C, 20 h, 51%.

of UMP and UMP-UMP dimer present. A prolonged reaction time of 20 h compared to reported procedures and the use of a smaller excess of uridine-5'-monophosphomorpholidate (1.3 equiv) improved the outcome of the reaction. After work-up, crude product **13** was purified by gel filtration. It was applied consecutively to G-10 and G-15 Sephadex columns equilibrated with bidistilled water. Very good separation was achieved by careful control of the flow rate and the amount of crude product applied to the columns. Fractions containing the product were pooled and lyophilized, thereby omitting the usual preparative HPLC purification (Scheme 4).

In conclusion, we have presented an improved total synthesis of UDP-Mur-*N*-Ac. Several key synthetic intermediates were modified and novel purification methods for muramic acid derivative **5** and UDP-Mur-*N*-Ac were introduced. Perhaps most importantly, the synthetic strategy is applicable to the synthesis of other Mur ligase substrates. This is currently under investigation in our laboratory and will be published in due course.

Acknowledgements

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17. Spectroscopic data for **11**: Yield 55%; colourless oil; IR (KBr) υ_{max}/cm^{-1}: 2956, 1745, 1594, 1490, 1376, 1213, 1092, 1035, 916, 777; ^1H NMR 300 MHz [$CDCl_3$], δ_H 1.36 (d, 3H, CH_3, $J = 7.0$ Hz), 2.02 (s, 3H, CH_3CONH), 2.08 (s, 3H, CH_3CO), 2.09 (s, 3H, CH_3CO), 3.82 (s, 3H, CH_3–OCO), 3.71–4.03 (m, 4H, H-2, H-3, H-5, H-6), 4.10 (dd, 1H, H-6′, $J = 4.5$, $J = 12.5$ Hz), 4.27 (q, 1H, $CHCH_3$, $J = 7.0$ Hz), 5.18 (t, 1H, H-4, $J = 9.5$ Hz), 6.38 (dd, 1H, H-1, $J = 2.8$, 6.0 Hz), 7.17–7.38 (m, 10H, H-Ar), 7.88 (d, 1H, NH, $J = 3.9$ Hz); ^{13}C NMR 75 MHz [$CDCl_3$], δ_C 18.72, 20.63, 20.79, 22.77, 53.88, 61.28, 70.37, 71.06, 74.56, 75.35, 96.96, 119.94, 120.00, 120.12, 120.19, 120.33, 125.49, 129.48, 129.80, 129.85, 169.02, 170.71, 171.41, 175.12. ^{31}P NMR 121 MHz [$CDCl_3$], δ_P − 13.29. MS (ESI) 646 $(M+Na)^+$; HRMS Calcd for $C_{28}H_{34}NO_{13}NaP$ *m/z*: 646.1665 $(M+Na)^+$, found 646.1677. $C_{28}H_{34}NO_{13}P_1 \times 1.4\ H_2O$ calcd: C 51.79, H 5.67, N 2.16. Found: C 52.18, H 6.08, N 2.14.
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