Drug Discovery Department[1], Lek Pharmaceuticals d.d., Ljubljana; Jenko d.o.o.[2], Ig, Slovenia

Optimization of UDP-*N*-acetylmuramic acid synthesis

J. Humljan,[1] Š. Starčević,[1] V. Car,[1] P. Štefanič Anderluh,[1] D. Kocjan,[1] B. Jenko,[2] U. Urleb[1]

Received June 26, 2007, accepted July 18, 2007

Dr. Jan Humljan, Drug Discovery Department, Lek Pharmaceuticals, Verovškova 57, 1526 Ljubljana, Slovenia
jan.humljan@sandoz.com

Pharmazie 63: 102–106 (2008) *doi: 10.1691/ph.2008.7675*

UDP-*N*-acetylmuramic acid (UDP-Mur*N*Ac) is a substrate of MurC, an important enzyme in the intracellular pathway of bacterial peptidoglycan biosynthesis. Various approaches towards preparation of UDP-Mur*N*Ac have been published but these synthetic preparations were shown to include many problematic steps. An optimization study with the focus on muramyl phosphate and UMP-morpholidate coupling was performed, resulting in a synthetic procedure enabling robust and easily reproducible production on a multi-gram scale.

1. Introduction

The biosynthetic pathway of bacterial peptidoglycan, an essential building block of bacterial cell walls, is a rich source of attractive enzyme targets for development of new antibacterials. The intracellular pathway of peptidoglycan biosynthesis encompasses transformations of UDP-*N*-acetylglucosamine (UDP-Glc*N*Ac) via a cascade of consecutive biosynthetic steps catalyzed by the enzymes MurA-MurG and MraY (van Heijenoort 2001; Katz and Caufield 2003; Zoeiby et al. 2003; Kotnik et al. 2007). The main focus of many research groups involved in peptidoglycan biosynthesis is MurC ligase and its substrate UDP-*N*-acetylmuramic acid (UDP-Mur*N*Ac) (**1**) (Reck et al. 2001; Benson et al. 1993). UDP-Mur*N*Ac is a useful intermediate in the preparation of building blocks of later steps of peptidoglycan biosynthesis which are substrates for the consecutive MurD-MurG enzymes, and which are indispensable components of Mur ligase inhibitor assays (Katz et al. 2003).

UDP-Mur*N*Ac, which is not commercially available, may be isolated from bacteria (Flouret et al. 1981) or obtained in a bioenzymatic process from commercially available UDP-Glc*N*Ac transformed by MurA and MurB (Benson et al. 1993; Reddy et al. 1999). However, the described procedures proved to yield only milligram quantities of UDP-Mur*N*Ac, emphasizing the need for large scale synthetic preparation. Thus, Blanot et al. (1994) developed a synthetic procedure for UDP-Mur*N*Ac by introduction of a phosphate group with molten phosphoric acid, but which turned out to give the product in low and poorly reproducible yield and afforded mixtures of α and β anomers of UDP-Mur*N*Ac, which in addition demanded a time-consuming separation procedure by preparative HPLC. In contrast, Dini's group (Dini et al. 2000) managed to develop a synthetic route by employing lactone as a key intermediate and performing a phosphorylation step to give solely the α anomer of UDP-Mur*N*Ac. However, the published procedure gives neither specific reaction conditions nor spectroscopic data for all intermediates. In addition, it includes the hydrogenation of benzyl lactone which in practice turned out to be problematic, and coupling with 5′-uridinemonophosphomorpholidate leading to many side products.[3]

In this paper we report optimization of the synthesis of UDP-Mur*N*Ac and provide a detailed synthetic procedure for UDP-Mur*N*Ac on a multi-gram scale.

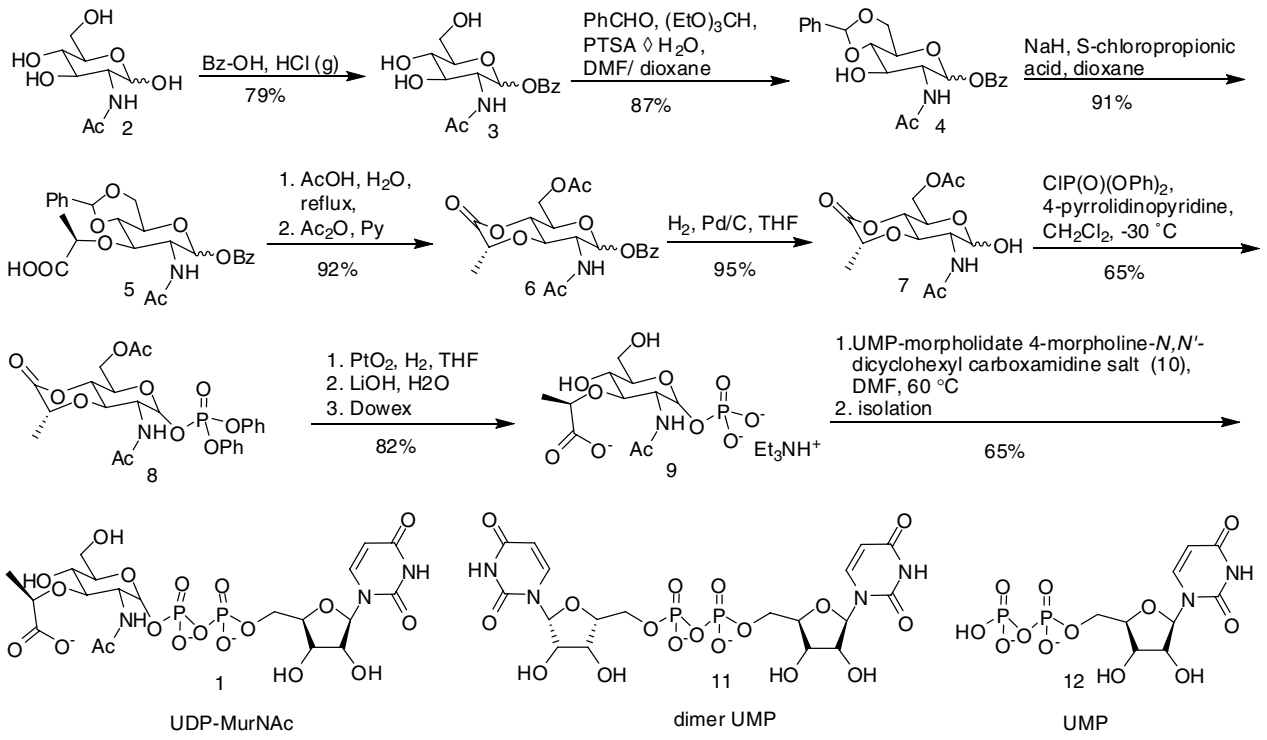
2. Investigations, results and discussion

2.1. Chemistry

The synthesis of UDP-Mur*N*Ac is outlined in the Scheme. The starting material was commercially available *N*-acetyl-D-glucosamine (Glu*N*Ac) (**2**), which was protected as the benzyl ether **3** using benzyl alcohol and gaseous HCl (Gross and Rimpler 1986). Treatment of the corresponding ether **3** with triethyl orthoformate, benzaldehyde and *p*-toluenesulphonic acid (PTSA) in DMF : dioxane = 1 : 1 afforded protected Glu*N*Ac **4** (Gross and Rimpler 1986). The intermediate **5** was prepared by alkylation of **4** by *S*-chloropropionic acid (Imoto et al. 1986). The benzylidene group of **5** was removed in boiling 60% acetic acid, followed by acetylation with acetic acid anhydride in pyridine to give lactone **6** (Osawa et al. 1969). The original work-up (Osawa et al. 1969) was slightly modified. The addition of methanol for neutralization of the excess of acetic acid anhydride was omitted due to observed transesterification of the lactone ring of **6** with methanol.

In the next step, lactone **7** was prepared by removal of the benzyl group by catalytic hydrogenolysis. Initially, the hydrogenolysis was conducted in anhydrous MeOH (Dini et al. 2000). The observed yield (60%) was much lower than that reported (Dini et al. 2000: 98%) and this appeared to be mostly due to transesterification of the target

Scheme



compound **7** with methanol. The observed methanolysis of lactone at room temperature was also reported previously (Keglević and Pongračić 1984). When the reaction was carried out in anhydrous acetic acid only 50% of target compound **7** was isolated and compound **7** with the hydrolyzed internal ester ring was identified as the major by-product. An excellent and reproducible yield (95%) of **7** was finally achieved by performing the hydrogenation in anhydrous THF.

Phosphorylation of **7** in the α-anomeric position was achieved by using a 10-fold excess of diphenyl chlorophosphate and by conducting the reaction at −30 °C (Sabesan and Neira 1992). 4-Pyrrolidinopyridine, which was reported to be the best acylating catalyst (Dini et al. 2000), was used in the reaction. After work-up and purification by flash column chromatography on silica elution with EtOAc : hexane = 2 : 1 and EtOAc : acetone = 10 : 3, the α-anomer of **8** was obtained in 65% yield. Product **8** was unstable at room temperature, especially in solvents, and was therefore stored at −15 °C. The phenyl protecting groups of the phosphate **8** were removed by hydrogenation over PtO_2 in THF (Sabesan and Neira 1992). When the phosphate **8** could no longer be detected by TLC and HPLC, the catalyst was filtered off and the reaction mixture immediately treated with 6 equivalents of aqueous 0.6 M LiOH. The crude material was purified on a Dowex 50WX2 100–200 mesh column (Et_3NH^+ form) affording muramyl phosphate **9** in 82% yield for two steps.

Muramyl phosphate **9** and uridine 5′-monophospho-morpholidate (UMP-morpholidate) **10** were coupled at 70 °C, after thorough drying of UMP-morpholidate **10** at 100 °C under vacuum (Dini et al. 2000). During the coupling reaction formation of the product UDP-Mur*N*Ac (**1**) and side products, e.g. dimer UMP (**11**) (product of auto-condensation of UMP-morpholidate), uridine 5′-monophosphate (UMP) (**12**) and other phosphorus containing side products were detected (Scheme). Since we also wanted to improve the yield, the impurity profile and shorten the reaction times, we considered changing the reaction conditions. The coupling reactions of nucleoside monophosphomorpholidates with various glycosylphosphates have been reported in different solvents (DMF, pyridine), at various temperatures ranging from room temperature to 70 °C and using 1*H*-tetrazole, which has been recognized as an efficient catalyst in phosphoramidate coupling reactions (Blanot et al. 1994; Dini et al. 2000; Roseman et al. 1961; Wittmann and Wong 1997; Heidlas et al. 1992; Liu et al. 2001). In order to optimize the reaction conditions and to get more information about the influence of the temperature and catalyst applied, we performed a muramyl phosphate **9** and UMP-morpholidate **10** coupling study.

2.2. Muramyl phosphate and UMP-morpholidate coupling study

The coupling reaction was carried out in a 9 : 1 mixture of DMF and DMSO-d_6 as solvent, with 1*H*-tetrazole or no catalyst, and performing the transformation at temperatures of 25, 50 and 75 °C. The course of the reaction was monitored by ^{31}P and ^{1}H NMR at appropriate intervals for 3 days. The consumption of the starting materials muramyl phosphate **9** and UMP-morpholidate **10** and the formation of the product UDP-Mur*N*Ac (**1**) and side products dimer UMP (**11**), UMP (**12**) and other phosphorus containing side products were followed (Fig. 1).

It turned out that 1*H*-tetrazole significantly accelerated the coupling reaction at all temperatures. It also increased the formation of the side products dimer UMP (**11**) and UMP (**12**) from UMP-morpholidate **10**, leading to a poorer impurity profile and indicating that a larger excess of UMP-morpholidate **10** would be needed for the completion of the coupling reactions (Fig. 1A-C). The observed reaction rate at room temperature was relatively slow and led to reaction times longer that 1 week (Fig. 1A, 1D). With in-

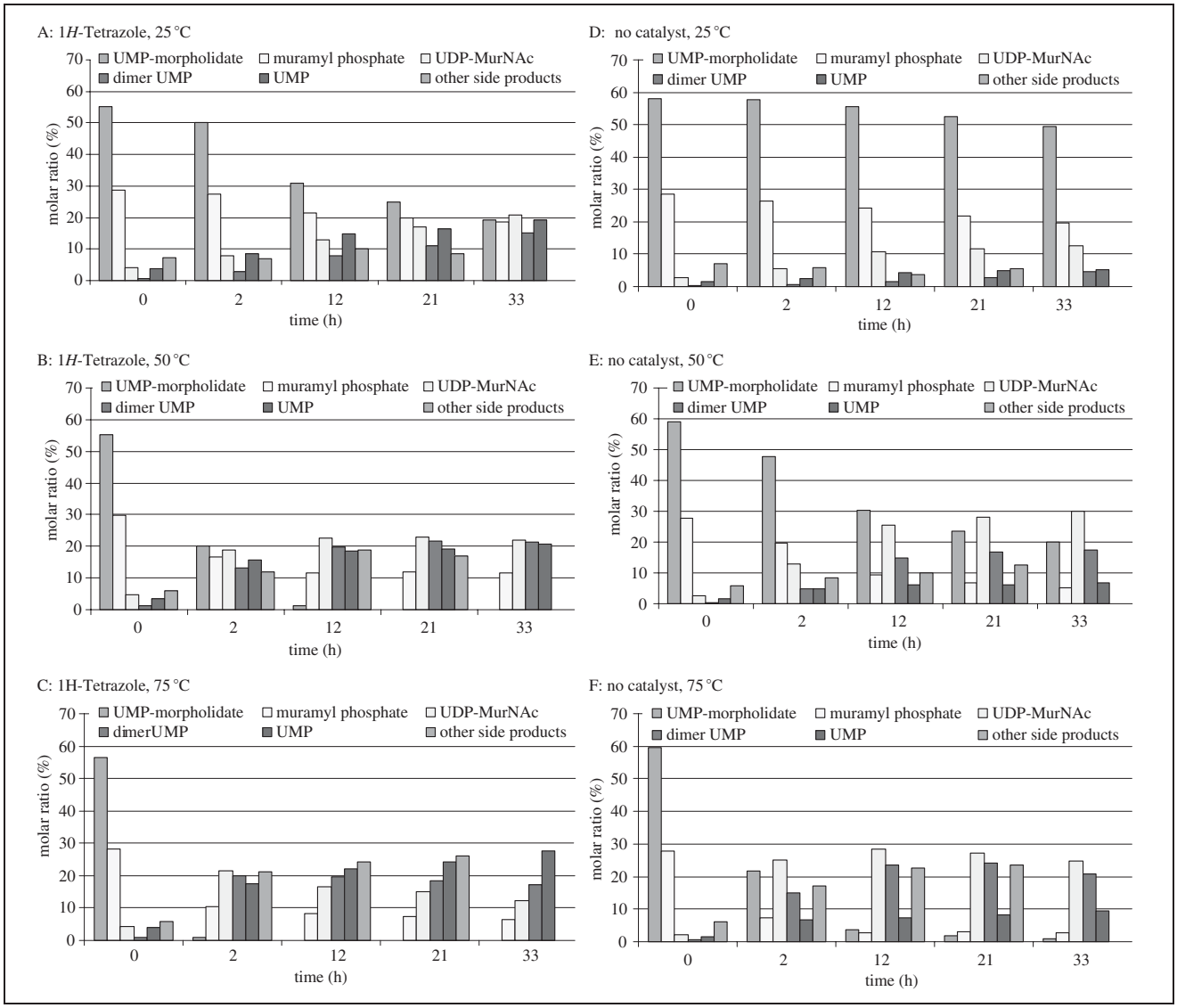


Fig. 1: Representation of the course of the coupling reactions between muramyl phosphate **9** and UMP-morpholidate **10** carried out under the following conditions: A) 1*H*-tetrazole, 25 °C; B) 1*H*-tetrazole, 50 °C; C) 1*H*-tetrazole, 75 °C; D) no catalyst, 25 °C; E) no catalyst, 50 °C; F) no catalyst, 75 °C as monitored by ^{1}H and ^{31}P-NMR

creasing temperature the reaction rate significantly increased, but at 75 °C the formation of side products **11** and **12** considerably increased (Fig. 1C, 1F). Furthermore, a significant impurity increase was observed due to irreversible transformation of the product UDP-Mur*N*Ac (**1**) at 75 °C (up to 10%), (Fig. 1F), a process which occurred to an even higher extent (up to 50%) when 1*H*-tetrazole was used (Fig. 1C)[4]. Regarding the observed turnover rate and the impurity profile, the best result of the coupling study was achieved by performing the coupling reaction without catalyst at 50 °C (Fig. 1E).

From the above findings we were able to define the optimal coupling conditions and perform the coupling on a larger scale. The triethylammonium salt of muramylphosphate (**9**) was coupled under anhydrous conditions in DMF at 60 °C, with 1.5 equivalents of commercially available UDP-morpholidate **10**. Furthermore, we decided not to use molecular sieves due to their strong inhibition of

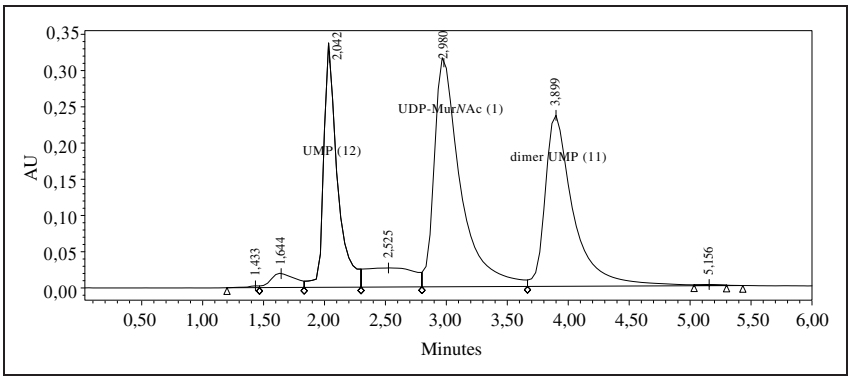


Fig. 2:
Analytical HPLC chromatogram of crude product UDP-Mur*N*Ac (**1**)

the coupling reaction (Wittmann and Wong 1997). The solvent was removed and the resulting residue dissolved in 100 mM ammonium formate, lyophilized and directly applied to a preparative reverse phase column to afford UDP-Mur*N*Ac (**1**) in good yield (65%).

2.3. Preparative HPLC purification of UDP-MurNAc

UDP-Mur*N*Ac (**1**) is fairly hydrophilic and thus poorly retained on a conventional reversed-phase column. Atlantis dC18, a fully endcapped stationary phase, appeared to be the most appropriate column for an efficient purification with a relatively short run time (Fig. 2). The best separation was scaled up to a 30×150 mm, 5 μm, Atlantis dC 18 OBD preparative column. Ammonium formate mobile phase at pH = 4 was selected to achieve good separation, as well as good fraction volatility during lyophilization. To improve sample load and throughput, we increased the sample size and actually overloaded the column until peaks started to overlap and purity was compromised.

2.4. Conclusions

In this work the synthesis of UDP-Mur*N*Ac was optimized, enabling a robust procedure to be performed on a multi-gram scale. In addition, a coupling study of muramyl phosphate and UMP-morpholidate and the optimal conditions obtained for the reaction provide a good starting point towards more efficient coupling of nucleoside monophosphates with various glycosylphosphates.

3. Experimental

3.1. Materials

Anhydrous solvents were purchased from Fluka and used without further purification. Chemicals from Sigma-Aldrich, Acros Organics or Merck were of analytical or HPLC grade and were used without further purification. Deionised water for preparative HPLC was purified by a MilliQ water purification system (Millipore Corporation, Massachusetts, USA). Analytical TLC was performed on Merck silica ($60F_{254}$) plates (0.25 mm); compounds were visualized with ultraviolet light or by heating the plate at 250 °C. Column chromatography was carried out on silica 60 (particle size 240–400 mesh). Cation exchange resin Dowex 50WX2 100–200 mesh (H^+ form) was purchased from Acros and converted to Et_3N salt form prior to use. Melting points were determined on a Reichert hot stage microscope and are uncorrected. IR spectra were obtained with a Nicolet FT-IR Nexus spectrometer and optical rotation was measured on a Perkin Elmer 1241 MC polarimeter. ^{1}H NMR and ^{31}P NMR spectra were recorded on a Varian Inova300 spectrometer in $CDCl_3$, DMSO-d_6, CD_3OD or D_2O solution, with TMS as the internal standard and phosphoric acid as the external standard. Mass spectra were obtained using a Autospec Q Micromass mass spectrometer. HPLC analyses were performed using a Waters 2695 Separation Module with a Waters 2996 PDA detector. For intermediates **3–6** and **8** analytical column XTerra RP C18 (150×4.6 mm I.D., 3.5 μm) and a gradient elution method combining mobile phase A with 25 mM ammonium acetate (pH = 6)/acetonitrile (95/5 v/v) and mobile phase B with 25 mM ammonium acetate (pH = 6)/acetonitrile (10/90 v/v) was used. For compounds **1**, **11**, **12** and fractions from preparative HPLC analytical column Atlantis dC18 OBD (100×4.6 mm I.D., 5 μm) and isocratic elution method with mobile phase 50 mM ammonium formate (pH = 4) was used. The preparative chromatography was performed using a Varian PrepStar SD-1 (Walnut Creek, USA) HPLC solvent delivery system, Atlantis dC18 OBD (150×30 mm I.D., 5 μm) preparative column, UV detector (Model ProStar 320) and a Rheodyne preparative injection valve (Model 3725i-038) with a 5 mL sample loop. The elution was carried out using 50 mM ammonium formate (pH = 4) with the flow rate 40 mL/min. The main fractions were neutralized with a 25% ammonia solution, as soon as they were eluted, and then lyophilized. All reported yields are yields of purified products.

3.2. Synthetic procedures

3.2.1. Benzyl 2-acetamido-6-O-acetyl-3-O-[(R)-1-carboxyethyl]-2-deoxy-α-D-glucopyranoside 1′,4-lactone (**6**)

Compound **5** (13.21 g, 28.04 mmol) was suspended in 60% AcOH (130 mL), and heated under reflux for 2 h, during which time it became a clear light yellow solution. The solvent was evaporated under reduced pressure, and the oily residue was taken up in water (60 mL). Evaporation of water removed the last traces of acetic acid and benzaldehyde, and gave a white solid, which was dried by addition of dry toluene and distillation. The white solid was dissolved in pyridine (75 mL) and Ac_2O (60 mL), and the mixture was stirred at room temperature overnight. The solvents were evaporated, and the last traces of the solvents were removed by addition of toluene, followed by evaporation. The residue was recrystallized from ethanol to afford **6** as a white solid (10.51 g, 92%). Rf = 0.70 (EtOAc : acetone = 2 : 1); m.p. 168–170 °C (lit. 170–172 °C (Osawa et al. 1969)); $[\alpha]_D^{23}$ + 189 (c 0.135, MeOH); IR (KBr, cm^{-1}) 3314, 2939, 1751, 1653, 1549, 1237, 1038 and 697; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 1.50 (d, 3 H, J = 7.0 Hz, $\underline{CH_3}CH$), 1.99 (s, 3 H, CH_3CON), 2.12 (s, 3 H, CH_3COO), 3.83 (dd, 1 H, $\overline{J = 10.5}$ and 9.1 Hz, CH), 3.92–4.02 (m, 1 H, CH), 4.22 (dd, 1 H, J = 12.3 and 4.3 Hz, CH), 4.30–4.44 (m, 3 H, CH_2 and CH), 4.48–4.76 (2 × d, 2 H, J = 11.7 Hz, $\underline{CH_2}Ph$), 4.71 (q, 1 H, J = 7.0 Hz, $\underline{CH}CH_3$), 4.97 (d, 1 H, J = 3.7 Hz, CH), $\overline{5.60}$ (d, 1 H, J = 9.4 Hz, NH) and $\overline{7.28}$–7.45 (m, 5 H, Ph); m/z (ES+) 408.1659 ($[M + H]^+$ $C_{20}H_{26}N0_8$ requires 408.1658); HPLC purity 98% (t = 16.6 min).

3.2.2. 2-Acetamido-6-O-acetyl-3-O-[(R)-1-carboxyethyl]-2-deoxy-α-D-glucopyranoside 1′,4-lactone (**7**)

A solution of benzyl lactone **6** (5.10 g, 12.52 mmol) in anhydrous THF (150 mL) was hydrogenated for 24 h in the presence of 10% Pd/C (1.50 g). The catalyst was filtered off, the solvent was evaporated under reduced pressure, and the residue was purified by dry-column flash chromatography through 15 parts of silica (ethyl acetate/acetone = 2/1) to afford lactone **7** as a white solid (3.77 g, 95%). Rf = 0.45 (EtOAc : acetone = 2 : 1); m.p. 188–190 °C (lit. 197–199 °C (Osawa et al. 1969)); $[\alpha]_D^{23}$ + 118 (c 0.165, MeOH); IR (KBr, cm^{-1}) 3427, 3292, 2945, 1759, 1660, 1552, 1375, 1233, 1127 and 1048; ^{1}H NMR (300 MHz, $CDCl_3 : CD_3OD = 1 : 1$) δ = 1.48 (d, 3 H, J = 7.2 Hz, $\underline{CH_3}CH$), 1.99 (s, 3 H, CH_3CON), 2.06 (s, 3 H, CH_3COO), 3.85 (dd, 1 H, $\overline{J = 10.4}$ and 9.2 Hz, CH), 4.10–4.53 (m, 5 H, 3 × CH and CH_2), 4.68 (q, 1 H, J = 7.2 Hz, $\underline{CH}CH_3$) and 5.15 (d, 1 H, J = 3.6 Hz, CH); m/z (ES+) 318.1196 ($[M + H]^+$ $C_{13}H_{20}NO_8$ requires 318.1189).

3.2.3. 2-Acetamido-6-O-acetyl-3-O-[(R)-1-carboxyethyl]-2-deoxy-α-D-glucopyranoside-1-α-(diphenyl phosphate) 1′,4-lactone (**8**)

A solution of 4-pyrrolidinopyridine (16.30 g, 109.98 mmol) in anhydrous CH_2Cl_2 (20 mL) was added to a solution of **7** (3.10 g, 9.77 mmol) in anhydrous CH_2Cl_2 (250 mL) at −30 °C under argon. The mixture was stirred for 20 min, followed by dropwise addition of diphenyl chlorophosphate (15.0 mL, 72.03 mmol) and stirring was continued for 5 h between −25 to −30 °C. The mixture was then diluted with CH_2Cl_2 (100 mL) and washed with ice cold H_2O (2 × 150 mL), saturated $NaHCO_3$ (2 × 150 mL), and brine (100 mL). The organic phase was dried over $MgSO_4$, filtered and evaporated under reduced pressure at room temperature. The resulting residue was purified by flash column chromatography on silica, eluting with EtOAc : hexane = 2 : 1 and then EtOAc : acetone = 10 : 3 to afford the α-anomer of **8** as a colourless solid. The product **8** was dried with anhydrous ether and stored at −15 °C (3.49 g, 65%). ^{1}H NMR was in agreement with reference (Dini et al. 2000). Rf = 0.70 (EtOAc : acetone = 2 : 1); m.p. 50–57 °C; $[\alpha]_D^{23}$ + 105 (c 0.500, MeOH), IR (KBr, cm^{-1}) 3427, 3071, 1747, 1591, 1490, 1228, 962, 776 and 690; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 1.53 (d, 3 H, J = 7.0 Hz, $\underline{CH_3}CH$), 1.88 (s, 3 H, CH_3CON), 2.03 (s, 3 H, CH_3COO), 3.87 (dd, 1 H, $\overline{J = 10.6}$ and 9.3 Hz, CH), 3.98–4.07 (m, 1 H, CH), 4.13 (dd, 1 H, J = 12.5 and 3.5 Hz, CH_2), 4.26 (dd, 1 H, J = 12.5 and 3.5 Hz, CH_2), 4.39–4.53 (m, 2 H, 2 × CH), 4.72 (q, 1 H, J = 7.0 Hz, $\underline{CH}CH_3$), 5.57 (d, 1 H, J = 9.0 Hz, NH), 6.00 (dd, 1 H, J = 6.1 and 3.3 Hz, $\overline{CH}OPO_3$, α anomer) and 7.10–7.45 (m, 10 H, 2 × Ph); m/z (ES+) 572.1303 ($[M + Na]^+$ $C_{25}H_{28}NO_{11}NaP$ requires 572.1298); HPLC purity 92% (t = 19.3 min).

3.2.4. Triethylammonium muramyl phosphate (**9**)

PtO_2 hydrate (Pt content 79–84%, 200 mg) was added to a solution of diphenyl phosphate **8** (2.50 g, 4.55 mmol) in THF (40 mL) under argon. Hydrogen was bubbled into the reaction mixture for 15 min, and then a positive hydrogen pressure was maintained in the flask overnight. After the catalyst was filtered, 0.6 M aq. LiOH (40 mL) was added immediately. The resulting mixture was stirred for 24 h at room temperature and then evaporated to dryness under reduced pressure. The residue was redissolved in water and lyophilized to give lithium muramyl phosphate. The solution of lithium muramyl phosphate in water (5 mL) was slowly adsorbed onto a resin bed of Dowex 50WX2, 100–200 mesh (Et_3NH^+ form, 3.0 × 25 cm) and slowly washed with water. Fractions containing the desired compound were pooled and lyophilized. The procedure of lyophilization was repeated to afford **9** as a highly hygroscopic colourless solid (2.34 g, 82%). The content of Et_3N was determined to be 2.5 equiv (^{1}H NMR). Rf = 0.35 (i-PrOH : H_2O : 25% NH_4OH = 6 : 1 : 3); IR (KBr, cm^{-1}) 3375, 2977, 2937, 2676, 2491, 1652, 1568, 1398, 1037 and 950; ^{1}H NMR (300 MHz, D_2O) δ = 1.23 (t, 9 H, J = 7.3 Hz, Et_3N), 1.31 (d, 3 H, J = 7.0 Hz, $\underline{CH_3}CH$), 2.00 (s, 3 H, CH_3CON), 3.15 (q, 6H, J = 7.3 Hz, Et_3N), 3.50–$\overline{3.88}$ (m,

6 H, CH_2 and 4 × CH), 4.33 (q, 1 H, J = 7.0 Hz, $\underline{CH}CH_3$) and 5.58 (dd, 1 H, J = 7.5 and 2.0 Hz, $CHOPO_3$, α anomer); m/z (ES−) 372.0703 ($[M - H]^-$ $C_{11}H_{19}NO_{11}P$ requires 372,0696).

3.2.5. UDP-MurNAc (**1**)

Uridine 5′-monophosphomorpholidate 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidine salt (3.90 g, 5.68 mmol) (**10**) was dried for 24 h at 100 °C and P ≤ 1 mbar and then dried by concentrating in vacuo from anhydrous pyridine (3 × 20 mL) and releasing the vacuum each time to argon. UMP-morpholidate **10** was dissolved in anhydrous DMF (15 mL) and one equivalent of UMP-morpholidate **10** solution (10 mL) was added to triethylammonium muramyl phosphate (**9**) (2.30 g, 3.67 mmol, 2.5 equiv. of Et_3N) in anhydrous DMF (7 mL). Muramyl phosphate **9** had been previously dried from anhydrous pyridine in an identical fashion. The reaction was allowed to proceed for 3 h at 60 °C and then the rest of the UMP-morpholidate **10** in anhydrous DMF (5 mL) was added, and stirred for an additional 12 h at 60 °C under argon. The solvent was removed and the resulting residue dissolved in 100 mM ammonium formate (10 equiv, pH = 7), lyophilized and directly applied to a preparative reverse phase column to afford UDP-Mur*N*Ac (**1**) as a highly hygroscopic white solid (1.65 g, 65%). Rf = 0.30 (i-PrOH : H_2O : 25% NH_4OH) = 6 : 1 : 3; HPLC purity 98% (t = 3.7 min). Spectral analyses were in agreement with references (Benson et al. 1993; Flouret et al. 1981; Reddy et al. 1999; Blanot et al. 1994).

3.2.6. ^{31}P NMR monitoring of the coupling reaction

Uridine 5′-monophosphomorpholidate 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidine salt (560 mg, 0.82 mmol) (**10**) and triethylammonium muramyl phosphate (**9**) (250 mg, 0.40 mmol) were dried as described previously. A solution of UMP-morpholidate **10** in anhydrous DMF : DMSO-d_6 = 9 : 1 (10 mL) was added to triethylammonium muramyl phosphate (**9**) under argon. The stock solution obtained was transferred to six NMR tubes (1 mL) under argon, with 1*H*-tetrazole (1.23 mmol) or no catalyst, and the reaction performed at different temperatures (25, 50 and 75 °C). The course of the reaction was monitored by ^{31}P NMR at appropriate intervals for 33 h. Phosphoric acid in DMF : DMSO-d_6 = 9 : 1 was used as an external standard. Signals at ^{31}P NMR (300 MHz; DMF : DMSO-d_6 = 9 : 1) δ = +5.58 (s, 1P, UMP-morpholidate **10**), +0.76 (s, 1P, UMP (**12**)), −0.02 (s, 1P, muramyl phosphate **9**), −10.46 (d, 1P, J = 20.5 Hz, UDP-Mur*N*Ac (**1**)), −11.70 (d, 1P, J = 20.5 Hz, UDP-Mur*N*Ac (**1**)), −11.91 (s, 2P, dimer UMP (**11**)) and signals for other side products were integrated. The molar ratio of monitored components was determined by dividing the integral of a specific component by the sum of all integrals from δ = 10.00 to −22.00. The molar ratio for other phosphorus containing impurities corresponds to the phosphate impurity.

Acknowledgements: This work was supported by the European Union FP6 Integrated Project EUR-INTAFAR (Project n° LSHM-CT-2004-512138) under the thematic priority Life Sciences, Genomics and Biotechnology for Health, the Ministry of Education, Science and Sport of the Republic of Slovenia. The authors thank Dr. Anthony R. Byrne for critical reading of the manuscript.

[3] We would like to inform the reader that during preparation of the manuscript an alternative synthesis of UDP-Mur*N*Ac, starting from an oxazoline intermediate obtained from Glc*N*Ac, was published (Kurosu et al. 2007).

[4] New signals in the ^{31}P NMR (300 MHz; DMF : DMSO-d_6 = 9 : 1) δ = −10.53 (d, 1P, J = 22.5 Hz) and −12.78 (d, 1P, J = 22.5 Hz) were observed due to irreversible transformation of UDP-Mur*N*Ac [−10.46 (d, 1P, J = 20.5 Hz) and −11.70 (d, 1P, J = 20.5 Hz)].

References

Benson TE, Marquardt J L, Etzkorn FA, Walsh CT (1993) Overexpression, purification, and mechanistic study of UDP-*N*-acetylenolpyruvylglucosamine reductase. Biochemistry 32: 2024–2030.

Blanot D, Auger G, Liger D, van Heijenoort J (1994) Synthesis of α and β anomers of UDP-*N*-acetylmuramic acid. Carbohydr Res 252: 107–115.

Dini C, Drochon N, Ferrari P, Aszodi J (2000) Multi gram synthesis of UDP-*N*-acetylmuramic acid. Bioorg Med Chem Lett 10: 143–145.

Flouret B, Mengin-Lecreulx D, van Heijenoort J (1981) Reverse-phase high-pressure liquid chromatography of uridine diphosphate *N*-acetylmuramyl peptide precursors of bacterial cell wall peptidoglycan. Anal Biochem 114: 59–63.

Gross PH, Rimpler M (1986) Stereochemically pure derivatives of muramic and isomuramic acids. Liebigs Ann Chem 1: 37–45.

Heidlas JE, Lees WJ, Pale P, Whitesides GM (1992) Gram-scale synthesis of uridine 5′-diphospho-*N*-acetylglucosamine: comparison of enzymic and chemical routes. J Org Chem 57: 146–151.

van Heijenoort J (2001) Recent advances in the formation of the bacterial peptidoglycan monomer unit. Nat Prod Rep 18: 503–519.

Imoto M, Kageyama S, Kusumoto S, Kohno M, Matsumoto K, Hashimoto S, Tohgo A, Shiba T (1986) Synthesis and antitumor activity of *N*-acetylmuramyl-L-alanyl-D-isoglutamine 6-phosphate and its lipophilic derivatives. Bull Chem Soc Jpn 59: 3207–3212.

Katz AH, Caufield CE (2003) Structure-based design approaches to cell wall biosynthesis inhibitors. Curr Pharm Des 9: 857–866.

Keglević D, Pongračić M (1984) Methanolysis and aminolysis of *N*-acetylmuramic acid lactones: evidence for the retention of the D-gluco configuration. Carbohydr Res 135: 85–99.

Kotnik M, Štefanič Anderluh P, Preželj A (2007) Development of novel inhibitors targeting intracellular steps of peptidoglycan biosynthesis. Curr Pharm Des – in press.

Kurosu M, Mahapatra S, Narayanasamy P, Crick DC (2007) Chemoenzymatic synthesis of Park's nucleotide: toward the development of high-throughput screening for MraY inhibitors. Tetrahedron Lett 48: 799–803.

Liu H, Sadamoto R, Sears PS, Wong CH (2001) An efficient chemoenzymatic strategy for the synthesis of wild-type and vancomycin-resistant bacterial cell-wall precursors: UDP-*N*-acetylmuramyl-peptides. J Am Chem Soc 123: 9916–9917.

Osawa T, Sinay P, Halford M, Jeanloz RW (1969) *O*-Acetyl derivatives of *N*-acetylmuramic acid. Biochemistry 8: 3369–3375.

Reck F, Marmor S, Fisher S, Wuonola MA (2001) Inhibitors of the bacterial cell wall biosynthesis enzyme MurC. Bioorg Med Chem Lett 11: 1451–1454.

Reddy SG, Waddell ST, Kuo DW, Wong KK, Pompliano DL (1999) Preparative enzymic synthesis and characterization of the cytoplasmic intermediates of murein biosynthesis. J Am Chem Soc 121: 1175–1178.

Roseman S, Distler JJ, Moffatt JG, Khorana HG (1961) Nucleoside polyphosphates. XI. An improved general method for the synthesis of nucleotide coenzymes. Synthesis of uridine-5′, cytidine-5′, and guanosine-5′ diphosphate derivatives. J Am Chem Soc 83: 659–663.

Sabesan S, Neira S (1992) Synthesis of glycosyl phosphates and azides. Carbohydr Res 223: 169–185.

Wittmann V, Wong CH (1997) 1*H*-Tetrazole as catalyst in phosphoromorpholidate coupling reactions: efficient synthesis of GDP-fucose, GDP-mannose, and UDP-galactose. J Org Chem 62: 2144–2147.

Zoeiby A, Sanschagrin F, Levesque RC (2003) Structure and function of the Mur enzymes: development of novel inhibitors. Mol Microbiol 47: 1–12.