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Research paper

Synthetic tripeptides as alternate substrates of murein peptide ligase (Mpl)

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ABSTRACT

Murein peptide ligase (Mpl) is an enzyme found in Gram-negative bacteria. It catalyses the addition of tripeptide L-Ala-γ-D-Glu-*meso*-diaminopimelate to nucleotide precursor UDP-*N*-acetylmuramic acid during the recycling of peptidoglycan. Although not essential, this enzyme represents an interesting target for antibacterial compounds through the synthesis of alternate substrates whose incorporation into peptidoglycan might be deleterious for the bacterial cell. Therefore, we have synthesised 10 tripeptides L-Ala-γ-D-Glu-Xaa in which Xaa represents amino acids different from diaminopimelic acid. Tripeptide with Xaa = ε-D-Lys proved to be an excellent substrate of *Escherichia coli* Mpl *in vitro*. Tripeptides with Xaa = *p*-amino- or *p*-nitro-L-phenylalanine were poor substrates, while tripeptides with Xaa = D- or L-2-aminopimelate, DL-2-aminoheptanoic acid, L-Glu, L-norleucine, L-norvaline, L-2-aminobutyric acid or L-Ala were not substrates at all. Although a good Mpl substrate, the D-Lys-containing tripeptide was devoid of antibacterial activity against *E. coli*, presumably owing to poor uptake.

1. Introduction

Peptidoglycan (murein) is a major component of the cell wall of most eubacteria. It is composed of long glycan chains cross-linked by short peptides [1]. Its main function is to preserve cell integrity by withstanding the internal osmotic pressure. Its biosynthesis is a complex process occurring in different cell compartments: in the cytoplasm, in the membrane and on the outer side of the membrane (periplasm in Gram-negative bacteria) (Fig. 1) [2–4]. Due to the essential role of the macromolecule, the enzymes involved in this biosynthetic process represent important targets for new antibacterial compounds.

The cytoplasmic steps include *inter alia* a series of four enzymes, the Mur ligases, responsible for the assembly of the peptide stem of peptidoglycan [2]. These enzymes catalyse the successive addition of L-Ala (MurC), D-Glu (MurD), *meso*-diaminopimelic acid (A_2pm) or L-lysine (MurE), and D-Ala-D-Ala (MurF) to UDP-MurNAc, leading to the synthesis of Park's nucleotide, UDP-MurNAc-pentapeptide (Fig. 1). Nevertheless, besides this *de novo* synthesis, there exists in Gram-negative bacteria another process called recycling [5,6]: tripeptide L-Ala-γ-D-Glu-*meso*-A_2pm, which results from the action of hydrolase and permease activities, is directly added to UDP-MurNAc, generating UDP-MurNAc-tripeptide which can re-enter the main synthetic process through MurF (Fig. 1). The demonstration that a defect in peptidoglycan recycling in *Escherichia coli* brings about bacteriolysis suggests that this process is a promising target for novel antibacterial agents [7].

Murein peptide ligase (Mpl) is the enzyme which catalyses the addition of the tripeptide to UDP-MurNAc. Mpl has been identified in and purified from *E. coli* [8,9], and the tridimensional structure of

Abbreviations: Abu, 2-aminobutyric acid; AcOH, acetic acid; Apm, 2-aminopimelic acid; A_2pm, 2,6-diaminopimelic acid; Bn, benzyl; Boc, *t*-butyloxycarbonyl; DCC, *N*,*N*′-dicyclohexylcarbodiimide; DCM, dichloromethane; DMF, dimethylformamide; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; Hep, heptyline (2-aminoheptanoic acid); HOBt, hydroxybenzotriazole; HOSu, *N*-hydroxysuccinimide; HPLC, high-performance liquid chromatography; IPTG, isopropyl β-D-thiogalactopyranoside; MALDI-TOF, matrix-assisted laser desorption/ionisation time-of-flight; Me, methyl; Mpl, murein peptide ligase; MppA, murein peptide permease A; MurNAc, *N*-acetylmuramoyl; Nle, norleucine; NMM, *N*-methylmorpholine; Nva, norvaline; TFA, trifluoroacetic acid; TLC, thin-layer chromatography; Z, benzyloxycarbonyl.

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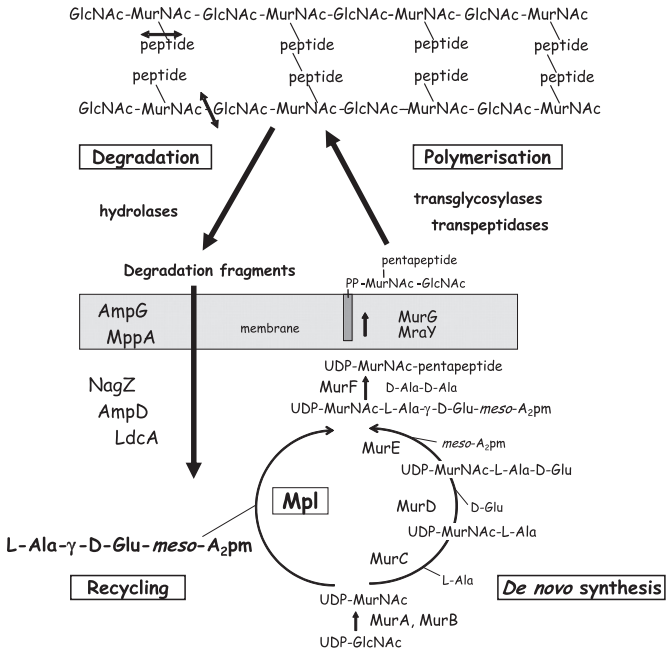


Fig. 1. Metabolism of peptidoglycan. Mature peptidoglycan is produced by *de novo* synthesis (right hand side). These reactions occur in the cytoplasm (synthesis of UDP-MurNAc-pentapeptide from UDP-GlcNAc), at the level of the cytoplasmic membrane (enzymes MraY and MurG), and in the periplasm (polymerisation reactions). During bacterial growth, peptidoglycan is partly degraded by numerous hydrolases. The degradation fragments are re-imported by permeases (AmpG, MppA) and further degraded by cytoplasmic enzymes (NagZ, AmpD, LdcA). The final degradation product, tripeptide L-Ala-γ-D-Glu-*meso*-A_2pm, is re-introduced into the synthetic pathway by enzyme Mpl (left hand side), a process named recycling.

the orthologue from *Psychrobacter arcticus* has been solved [10]. Although the *mpl* gene is not essential for bacterial growth [8], Mpl is interesting from a medicinal chemistry standpoint for two reasons. First, alternate Mpl peptide substrates might be incorporated into peptidoglycan and display an antibacterial effect through inhibition of subsequent steps, in particular transpeptidases. The fact that tetrapeptide L-Ala-γ-D-Glu-*meso*-A_2pm-D-Ala, or tri- and tetrapeptide containing L-Lys in lieu of *meso*-A_2pm, were good or moderate substrates of *E. coli* Mpl [9] indicates that the enzyme is permissive enough to accept other compounds than the regular L-Ala-γ-D-Glu-*meso*-A_2pm tripeptide. Second, such alternate substrates might increase the susceptibility to existing antibiotics: indeed, it has been shown that the deletion of the *mpl* gene in *Acinetobacter baylyi* brings about a hypersensitivity to β-lactam antibiotics [11]. In this paper, we wish to report on the synthesis and biological evaluation of tripeptides as alternate substrates of Mpl from *E. coli*.

2. Materials and methods

2.1. Chemicals

UDP-MurNAc and UDP-[^{14}C]MurNAc were obtained according to published procedures [12,13]. The Mpl enzyme from *E. coli* was produced as the C-terminal His-tagged form according to Hervé et al. [9].

2.2. Peptide synthesis

The peptides were synthesised as described in the Supplementary material.

2.3. Assay for Mpl activity

The assay mixture (50 μL) contained 100 mM Tris–HCl buffer (pH 8.4), 5 mM ATP, 15 mM $MgCl_2$, 0.4 mM UDP-[^{14}C]MurNAc (500 Bq), purified Mpl enzyme (20 ng of protein), and peptide **1**, **2** or **12** (variable concentrations). Mixtures were incubated at 37 °C for 30 min and reactions were stopped by the addition of 10 μL of acetic acid, followed by lyophilisation. The residue was dissolved in 50 mM ammonium formate, pH 3.8 (buffer A) and injected onto a Nucleosil 100C_{18} 5 μm column (4.6 × 150 mm; Grace Davison Discovery Sciences) using buffer A at 0.6 mL min^{-1} as the mobile phase. Detection was performed with a radioactive flow detector (model LB506-C1, Berthold) using the Quicksafe Flow 2 scintillator (Zinsser Analytic) at 0.6 mL min^{-1}. Quantification was carried out with the Winflow software (Berthold).

For the determination of the kinetic constants, the same assay was used with various concentrations of one substrate and fixed concentrations of the others. In all cases, the substrate consumption was <20%, the linearity being ensured within this interval even at the lowest substrate concentration. The data were fitted to the equation $v = V_{max}S/(K_m + S)$ or $v = V_{max}S/(K_m + S + S^2/K_i)$ using the MDFitt software developed by M. Desmadril (IBBMC, Orsay, France).

Identical assay conditions were employed when other peptides were tested as substrates. However, in order to make sure that the putative product is eluted from the column, isocratic elution with buffer A (10 min) was followed by a gradient of methanol in buffer A (from 0 to 40% over 35 min).

2.4. Microbiology

The *E. coli* BW25113 strain [14] was used to test the antibacterial activity of tripeptide **12**. Cells were grown in 2YT rich medium or M63 minimal medium [15]. When required, media were supplemented with ampicillin (25 mg mL^{-1}) and IPTG (0.001–1 mM).

2.5. Docking simulation

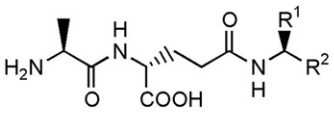
The docking procedure is described in the Supplementary material.

3. Results

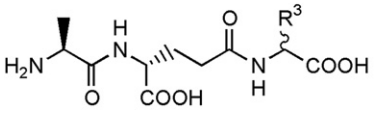
3.1. Tripeptide design and synthesis

Starting from the structures of known tripeptide substrates of Mpl, L-Ala-γ-D-Glu-*meso*-A_2pm (**1**) and L-Ala-γ-D-Glu-L-Lys (**2**) (Fig. 2), we decided to synthesise peptides of core structure L-Ala-γ-D-Glu-Xaa. Amino acids at position 3 (Xaa) were selected so that they lacked a proximal (*i.e.*, on the main chain) or distal (*i.e.*, on the lateral chain) function, thereby possibly hampering subsequent biosynthetic reactions (MurF or transpeptidases). These amino acids were 2-aminopimelic acid (Apm) (tripeptide **3**) and its lower homologue glutamic acid (tripeptide **4**); heptyline (Hep) and its lower homologues norleucine (Nle), norvaline (Nva), 2-aminobutyric acid (Abu) and alanine (tripeptides **5**, **6**, **7**, **8** and **9**, respectively); *p*-aminophenylalanine (tripeptide **10**) and its synthetic precursor, *p*-nitrophenylalanine (tripeptide **11**); and D-lysine acylated on its ε-amino function (tripeptide **12**) (Fig. 2).

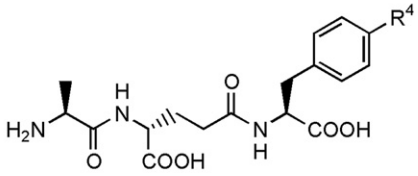
Apm in compound **3** represents *meso*-A_2pm lacking the distal NH_2 group. Hep in compound **5** is an isostere of lysine with the distal NH_2 group replaced by methyl. *p*-Aminophenylalanine in compound **10** is also an isostere of lysine, but the distal NH_2 group is present while the aliphatic side-chain is replaced by a phenyl ring. Finally, in tripeptide **12**, the distal functions (amino and



1 (R^1 = (*R*)$CH_2CH_2CH_2CH(NH_2)COOH$, R^2 = COOH)
2 (R^1 = $CH_2CH_2CH_2CH_2NH_2$, R^2 = COOH)
4 (R^1 = CH_2CH_2COOH, R^2 = COOH)
6 (R^1 = $CH_2CH_2CH_2CH_3$, R^2 = COOH)
7 (R^1 = $CH_2CH_2CH_3$, R^2 = COOH)
8 (R^1 = CH_2CH_3, R^2 = COOH)
9 (R^1 = CH_3, R^2 = COOH)
12 (R^1 = (*R*)$CH_2CH_2CH_2CH(NH_2)COOH$, R^2 = H)



3 (R^3 = $CH_2CH_2CH_2CH_2COOH$)
5 (R^3 = $CH_2CH_2CH_2CH_2CH_3$)



10 (R^4 = NH_2)
11 (R^4 = NO_2)

Fig. 2. Formulae of the tripeptides synthesised.

carboxyl) of *meso*-A_2pm are maintained while the proximal carboxyl group is absent.

The peptides were synthesised by the classical methods of peptide synthesis. Amino acids Xaa were of the L configuration, except Apm and Hep, for which only the racemic compound is commercially available, and of course D-lysine. A first series of compounds (**4**, **9**) were synthesised according to the scheme of Fig. 3. Boc-D-Glu-OBn **15** was coupled with benzyl-protected amino acids **13** and **14** by the EDC/HOBt method, yielding protected dipeptide **16** or **17**, respectively. The Boc group was eliminated by acidolysis, and resulting derivatives **18** and **19** were coupled with Boc-L-Ala **20**. Finally, the protecting groups of tripeptide derivatives **21** and **22** were removed by trifluoroacetic acid treatment followed by catalytic hydrogenolysis.

A second series of tripeptides (**3**, **5**–**8**, **12**) was synthesised according to the scheme of Fig. 4. Z-L-Ala **23** was activated with DCC/HOSu and coupled with D-Glu-OBn **24**, yielding partially protected dipeptide derivative **25**. The γ-carboxyl group of **25** was activated and coupled with benzyl-protected amino acids **26**–**30**, or Z-D-Lys-OBn **31**. Finally, the protecting groups of

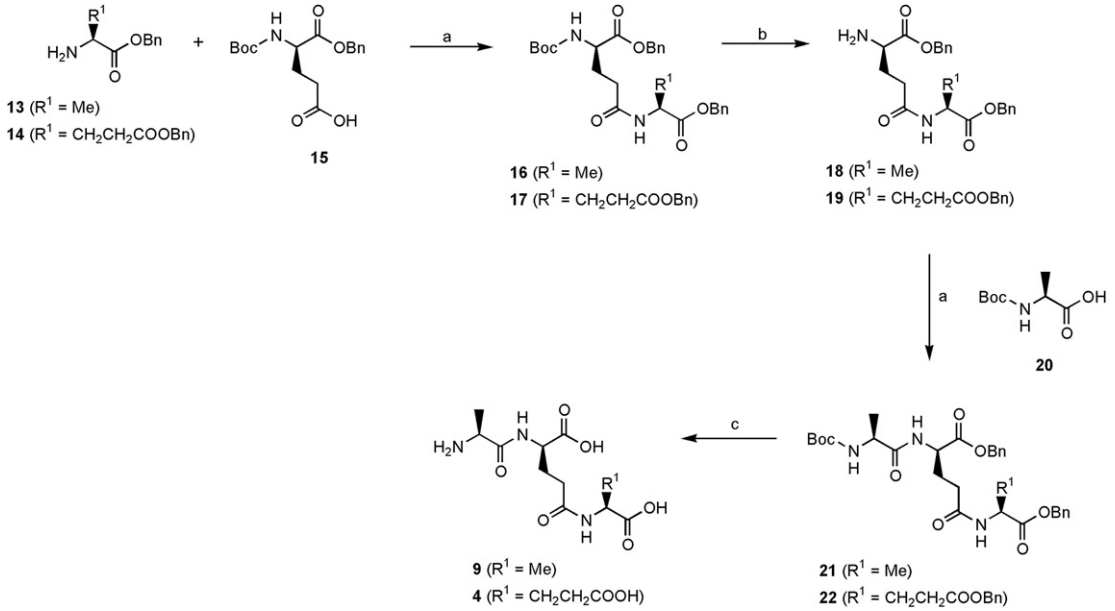


Fig. 3. Synthesis of tripeptides **4** and **9**. Reagents and conditions: (a) EDC, HOBt, NMM, DMF; (b) TFA, DCM; (c) 1. TFA, DCM; 2. H_2, Pd/C, MeOH.

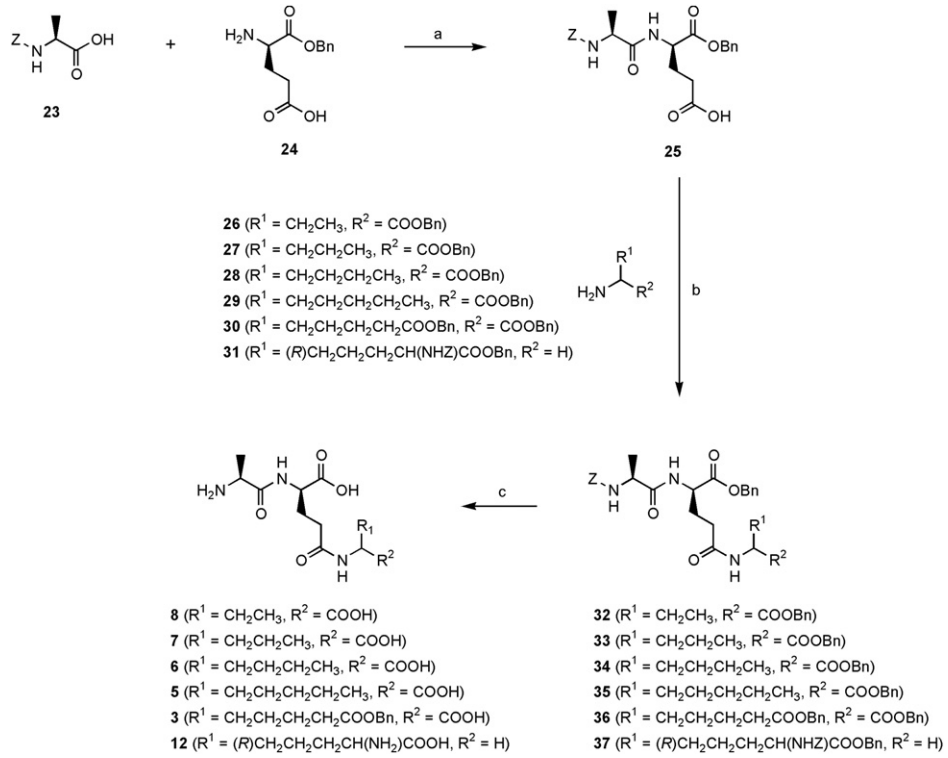


Fig. 4. Synthesis of tripeptides **3**, **5**–**8** and **12**. Reagents and conditions: (a) 1. **23**, DCC, HOSu, DCM; 2. **24**, NMM; (b) EDC, HOBt, NMM, DMF; (c) H_2, Pd/C, glacial AcOH.

tripeptide derivatives **32**–**37** were removed by catalytic hydrogenolysis.

A similar scheme (Fig. 5) was used for tripeptides **11** and **10**, but owing to the presence of the *p*-nitrophenyl group, carboxyl groups were protected as methyl esters. Dipeptide derivative Boc-L-Ala-D-Glu-OMe **39** was synthesised from Boc-L-Ala **20** and D-Glu-OMe **38** as described above. Coupling to *p*-nitro-L-phenylalanine methyl ester **40** yielded tripeptide derivative **41**. The protecting groups were removed by alkaline hydrolysis followed by acidolysis, giving *p*-nitro-L-phenylalanine-containing tripeptide **11**. A small amount of **11** was submitted to catalytic hydrogenation to obtain *p*-amino-L-phenylalanine-containing tripeptide **10**.

The tripeptides were purified by HPLC. They were characterised by TLC, analytical HPLC, and MALDI-TOF mass spectrometry using carbon nanotubes [16]. This method has recently been shown to give good spectra for low molecular weight compounds without interference of intrinsic matrix ions. Tripeptides appeared mainly as cationised species in the positive mode, and as the molecular ion in the negative mode.

Tripeptides **3** and **5** are mixtures of LDL and LDD diastereoisomers. While diastereoisomers of **5** were eluted on the semi-preparative column as a largely unresolved peak, those of **3** could be separated. The stereochemistry of resulting compounds **3a** and **3b** was determined by chiral chromatography of acid hydrolysates to be LDD and LDL, respectively.

3.2. Tripeptides as Mpl substrates

Tripeptides were tested for their ability to be added to radioactive UDP-MurNAc by purified Mpl. When classical conditions of incubation were used (30 min incubation time, 20 ng enzyme), tripeptide **12** gave rise to the appearance of a radiolabelled product which had the same retention time as authentic UDP-MurNAc-L-Ala-γ-D-Glu-ε-D-Lys [17]. The kinetic parameters of **12** were determined and found to be similar to those of peptide **1** (Table 1).

With tripeptide **10** or **11** at 1 mM, a radiolabelled product appeared only upon prolonged incubation (16 h) with a large amount of enzyme (2 μg): in these conditions, *ca.* 50% (**11**) or 10% (**10**) conversion with respect to UDP-MurNAc occurred. In order to verify the structure of these new products, the reactions were performed with non-labelled UDP-MurNAc and the products were purified by HPLC. MALDI-TOF mass spectrometry was in agreement with the structures UDP-MurNAc-L-Ala-γ-D-Glu-*p*-amino-L-phenylalanine (*m*/*z* ratio of the $[M - H]^-$ ion, 1040.31; calculated molecular mass of the nucleotide product, 1041.26) and UDP-MurNAc-L-Ala-γ-D-Glu-*p*-nitro-L-phenylalanine (*m*/*z* ratio of the $[M - H]^-$ ion, 1070.32; calculated molecular mass of the nucleotide product, 1071.24).

No radiolabelled product could be detected with tripeptides **3a**, **3b**, **4**–**9** at 1 mM after 16 h with 2 μg Mpl.

3.3. Attempts to incorporate tripeptide *12* into the peptidoglycan via Mpl

Since tripeptide **12** is an excellent substrate of Mpl, attempts were made to test a possible antibacterial effect on *E. coli* cells. Indeed, its *in vivo* incorporation would produce a nucleotide which could not be a substrate for the subsequent biosynthetic enzyme, MurF, owing to the absence of proximal carboxyl group. In this purpose, the BW25113 strain was plated on rich 2YT medium or glucose minimal medium, and different concentrations of tripeptide **12** were spotted. However, even the addition of large amounts of peptide (up to 5 mg mL^{-1}) had no effect on cell growth. The BW25113 strain was transformed either with the pMLD131 [9] or pMLD1285 [18] plasmid, allowing overexpression of the *mpl* or *mppA* gene, respectively, upon IPTG induction. The aim of these experiments was to stimulate the recycling process (Mpl overproduction) or to increase tripeptide **12** uptake (MppA overproduction, see Section 4). But even in these conditions, no deleterious effect of the tripeptide was observed.

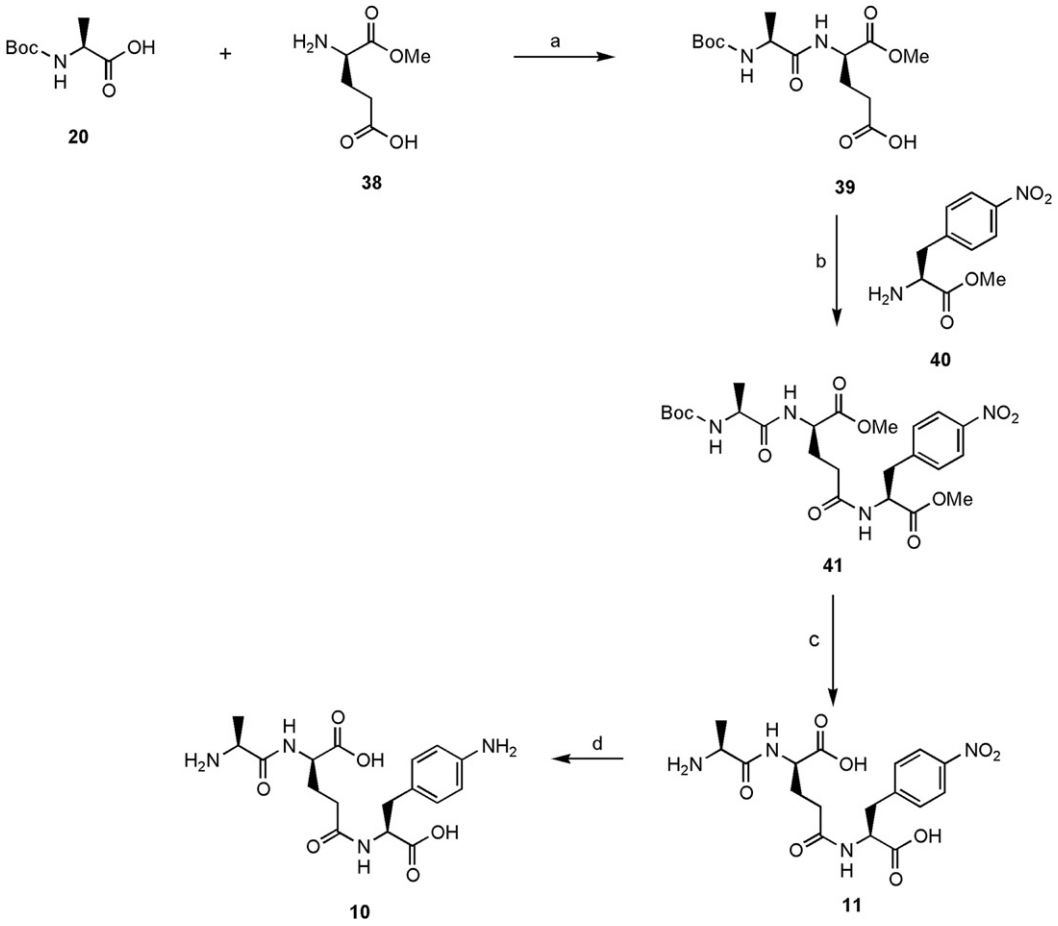


Fig. 5. Synthesis of tripeptides **10** and **11**. Reagents and conditions: (a) **20**, DCC, HOSu, DCM; 2. **38**, NMM; (b) EDC, HOBt, NMM, DMF; (c) 1. NaOH, dioxane; 2. TFA, DCM; (d) H_2, Pd/C, 10% AcOH.

4. Discussion

The Mpl enzyme intervenes in peptidoglycan recycling by catalysing the addition of *meso*-A_2pm-containing tripeptide **1** to UDP-MurNAc. We had previously shown that it can also utilise L-Lys-containing tripeptide **2** *in vitro* [9]. The most remarkable result of the present study is that D-Lys-containing tripeptide **12** constitutes another substrate for Mpl. The examination of the kinetic parameters (Table 1) shows that tripeptide **12** is as good a substrate as **1**, the specificity constant k_{cat}/K_m being of the same order of magnitude.

A more disappointing result is that no other peptide (apart from **10** and **11**, see below) is a substrate of the enzyme. Nevertheless, we can draw a few conclusions concerning the interactions of Mpl and its tripeptide substrate. As mentioned in Section 3.1, the residue at position 3 possesses two recognition sites: the proximal site (the one which is in the main peptide chain) and the distal site (the one which is in the lateral chain) (Fig. 6) [19]. Mpl recognises the amino and carboxyl groups in the distal site of the natural substrate **1**. The carbon atom bearing these groups should be of the D configuration. The absence of carboxyl group in **2** brings about a decrease of specificity constant (700-fold); nevertheless, **2** remains a fairly good substrate. By contrast, the absence of amino group in **3b** or **5** prevents the recognition between enzyme and peptide (Fig. 6). This is *a fortiori* the case when an additional modification, namely shortening of the lateral chain, occurs (**4**, **6**–**9**).

Table 1
Kinetic parameters of Mpl for tripeptide substrates.

Compound	K_m (mM)	k_{cat} (min^{-1})	k_{cat}/K_m (min^{-1} mM^{-1})
1[a]	0.10 ± 0.02	290 ± 70	2900
2[a]	4.1 ± 0.4	16 ± 1	3.9
12[b]	0.15 ± 0.04	360 ± 40	2400

[a] Ref. [9].
[b] The concentration range of tripeptide **12** was 0.1–1.2 mM. Owing to inhibition by excess tripeptide, data were fitted to the equation $v = V_{max}S/(K_m + S + S^2/K_i)$; the K_i value determined was 0.70 ± 0.20 mM.

The case of peptides **10** and **11** is particular. Peptide **10** is a poor substrate of the enzyme. Interestingly, it contains an amino group (borne by a phenyl ring) at the C-terminal position. This would suggest that an amino group on the distal site is essential for a peptide to be a substrate. However, peptide **11** with a nitro group at the same place is also a substrate, albeit even less efficient than **10**. Moreover, Olrichs et al. have shown that peptide **2** labelled at the lysine residue with *N*-7-nitro-2,1,3-benzoxadiazol-4-yl (NBD) is substrate of Mpl *in vitro* [20]. As for **10** and **11**, a large amount of enzyme had to be added. Together, these results indicate that a primary amine at the distal site of position 3 is not absolutely necessary.

Tripeptide **12** is very interesting. Its D-lysine residue is linked "upside down" to the D-glutamyl residue with a γ → ε bond. Such a structure has been found in the peptidoglycan of *Thermotoga maritima* [17,21]. The distal site of **12** is constituted by amino and carboxyl functions borne by a D carbon, *i.e.* identical to that of peptide **1** (Fig. 6). This is the reason why the enzyme recognises peptide **12** quite well. It should be noted that the modification of

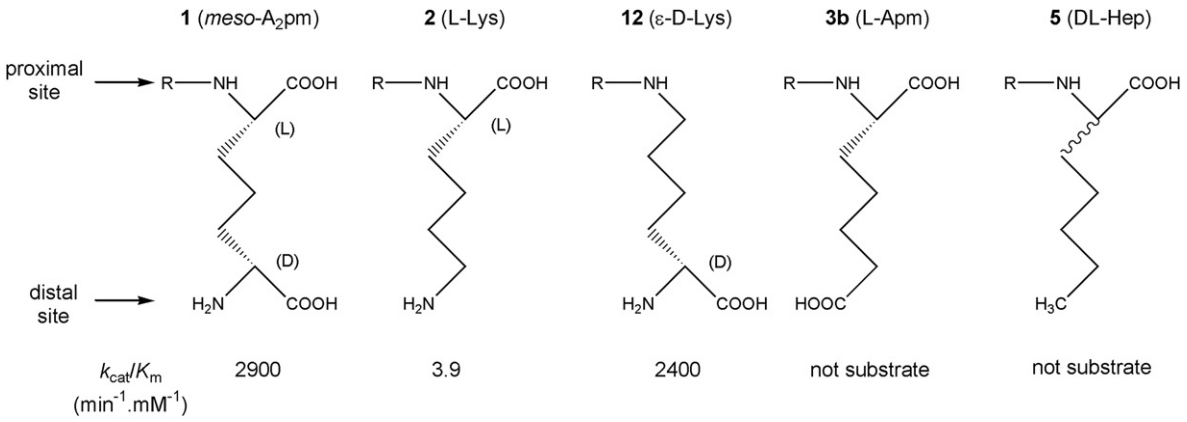


Fig. 6. Proximal and distal sites of amino acids found at position 3 of tripeptides. The specificity constant of those tripeptides which are substrates for Mpl is shown in the lower part of the figure. R, L-Ala-D-Glu.

the proximal site (suppression of the carboxyl group and transformation of an L carbon to an achiral one) has no influence on the interaction between the peptide and the enzyme. This is in keeping with the fact that tetra- and pentapeptides, in which the carboxyl function of the proximal site is substituted, are as good substrates as the tripeptide [9].

In spite of being an excellent substrate for Mpl, tripeptide **12** was devoid of antibacterial activity against *E. coli*. Such a disappointing result had already been encountered with tripeptide **2**, a less efficient Mpl substrate [9]. An explanation could be the poor uptake of **12** by *E. coli* cells. Elements in favour of this hypothesis can be found upon examination of the murein peptide permease A (MppA). This protein is responsible for the internalisation of tripeptide **1** during the recycling process of peptidoglycan [18]. Maqbool et al. [22] have solved the crystal structure of MppA in complex with **1** (PDB entry: 3O9P). They have found that the tripeptide forms multiple interactions with the active site of the protein, in particular through all of its ionised groups (N-terminal amino group, α-carboxylate of D-Glu, distal amino group and both carboxylates of *meso*-A_2pm). Interestingly, peptide **2**, which differs from **1** by the absence of distal carboxylate at position 3, has 500-fold less affinity than **1** for MppA [22]. It is likely that the lack of proximal carboxylate at the C-terminus brings about a reduced affinity of **12** for MppA. The absence of effect of **12** on the MppA-overproducing strain is consistent with this notion. This is also substantiated by the docking of **12** into the 3O9P structure followed by scoring, which confirms that the peptide has lost an important interaction with MppA with respect to **1** (Supplementary Fig. 1). Therefore, since all the ionised groups of tripeptide **1** are necessary for a good affinity with MppA, a solution for obtaining antibacterial tripeptides might be the synthesis of analogues of **1** possessing at position 3 an anionic proximal group other than carboxylate (phosphonate, sulfonate, tetrazole).

Acknowledgements

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Appendix A. Supplementary material

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.biochi.2012.12.011.

References

[1] W. Vollmer, D. Blanot, M.A. de Pedro, Peptidoglycan structure and architecture, FEMS Microbiol. Rev. 32 (2008) 149–167.
[2] H. Barreteau, A. Kovač, A. Boniface, M. Sova, S. Gobec, D. Blanot, Cytoplasmic steps of peptidoglycan biosynthesis, FEMS Microbiol. Rev. 32 (2008) 168–207.
[3] A. Bouhss, A.E. Trunkfield, T.D.H. Bugg, D. Mengin-Lecreulx, The biosynthesis of peptidoglycan lipid-linked intermediates, FEMS Microbiol. Rev. 32 (2008) 208–233.
[4] E. Sauvage, F. Kerff, M. Terrak, J.A. Ayala, P. Charlier, The penicillin-binding proteins: structure and role in peptidoglycan biosynthesis, FEMS Microbiol. Rev. 32 (2008) 234–258.
[5] E.W. Goodell, Recycling of murein by *Escherichia coli*, J. Bacteriol. 163 (1985) 305–310.
[6] J.T. Park, Why does *Escherichia coli* recycle its cell wall peptides? Mol. Microbiol. 17 (1995) 421–426.
[7] M.F. Templin, A. Ursinus, J.V. Höltje, A defect in cell wall recycling triggers autolysis during the stationary growth phase of *Escherichia coli*, EMBO J. 18 (1999) 4108–4117.
[8] D. Mengin-Lecreulx, J. van Heijenoort, J.T. Park, Identification of the *mpl* gene encoding UDP-*N*-acetylmuramate:L-alanyl-γ-D-glutamyl-*meso*-diaminopimelate ligase in *Escherichia coli* and its role in recycling of cell wall peptidoglycan, J. Bacteriol. 178 (1996) 5347–5352.
[9] M. Hervé, A. Boniface, S. Gobec, D. Blanot, D. Mengin-Lecreulx, Biochemical characterization and physiological properties of *Escherichia coli* UDP-*N*-acetylmuramate:L-alanyl-γ-D-glutamyl-*meso*-diaminopimelate ligase, J. Bacteriol. 189 (2007) 3987–3995.
[10] D. Das, M. Hervé, J. Feuerhelm, C.L. Farr, H.J. Chiu, M.A. Elsliger, M.W. Knuth, H.E. Klock, M.D. Miller, A. Godzik, S.A. Lesley, A.M. Deacon, D. Mengin-Lecreulx, I.A. Wilson, Structure and function of the first full-length murein peptide ligase (Mpl) cell wall recycling protein, PLoS One 6 (2011) e17624.
[11] M.J. Gomez, A.A. Neyfakh, Genes involved in intrinsic antibiotic resistance of *Acinetobacter baylyi*, Antimicrob. Agents Chemother. 50 (2006) 3562–3567.
[12] A. Babič, S. Pečar, An improved total synthesis of UDP-*N*-acetylmuramic acid, Tetrahedron Lett. 48 (2007) 4403–4405.
[13] A. Bouhss, S. Dementin, J. van Heijenoort, C. Parquet, D. Blanot, MurC and MurD synthetases of peptidoglycan biosynthesis: borohydride trapping of acyl-phosphate intermediates, Methods Enzymol. 354 (2002) 189–196.
[14] K.A. Datsenko, B.L. Wanner, One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 6640–6645.
[15] J.H. Miller, Experiments in Molecular Genetics, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1972.
[16] S. Xu, Y. Li, H. Zou, J. Qiu, Z. Guo, B. Guo, Carbon nanotubes as assisted matrix for laser desorption/ionization time-of-flight mass spectrometry, Anal. Chem. 75 (2003) 6191–6195.
[17] A. Boniface, A. Bouhss, D. Mengin-Lecreulx, D. Blanot, The MurE synthetase from *Thermotoga maritima* is endowed with an unusual D-lysine adding activity, J. Biol. Chem. 281 (2006) 15680–15686.
[18] J.T. Park, D. Raychaudhuri, H. Li, S. Normark, D. Mengin-Lecreulx, MppA, a periplasmic binding protein essential for import of the bacterial cell wall peptide L-alanyl-γ-D-glutamyl-*meso*-diaminopimelate, J. Bacteriol. 180 (1998) 1215–1223.
[19] G. Auger, J. van Heijenoort, J.C. Vederas, D. Blanot, Effect of analogues of diaminopimelic acid on the *meso*-diaminopimelate-adding enzyme from *Escherichia coli*, FEBS Lett. 391 (1996) 171–174.
[20] N.K. Olrichs, M.E. Aarsman, J. Verheul, C.J. Arnusch, N.I. Martin, M. Hervé, W. Vollmer, B. de Kruijff, E. Breukink, T. den Blaauwen, A novel in vivo cell-wall labeling approach sheds new light on peptidoglycan synthesis in *Escherichia coli*, ChemBioChem 12 (2011) 1124–1133.

[21] A. Boniface, C. Parquet, M. Arthur, D. Mengin-Lecreulx, D. Blanot, The elucidation of the structure of *Thermotoga maritima* peptidoglycan reveals two novel types of cross-link, J. Biol. Chem. 284 (2009) 21856–21862.

[22] A. Maqbool, V.M. Levdikov, E.V. Blagova, M. Hervé, R.S. Horler, A.J. Wilkinson, G.H. Thomas, Compensating stereochemical changes allow murein tripeptide to be accommodated in a conventional peptide-binding protein, J. Biol. Chem. 286 (2011) 31512–31521.