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The synthesis of diaminopimelic acid containing peptidoglycan fragments using metathesis cross coupling

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Abstract—Properly protected diaminopimelic acid (DAP), a component of peptidoglycan of Gram-negative bacteria, was prepared by a metathesis cross coupling between properly protected allyl and vinyl glycine derivatives using Grubb's second-generation catalyst followed by reduction of the double bond of the resulting compound. The DAP derivatives were used in the solution- and polymer-supported synthesis of biological active peptides.

Peptidoglycan (PGN) is an essential component of the cell wall of virtually all bacteria that preserves cell integrity by withstanding the internal osmotic pressure. It is also responsible for the maintenance of cell shape and is intimately involved in cell division. PGN is especially abundant in Gram-positive bacteria, in which it accounts for approximately half of the cell wall mass. On the other hand, Gram-negative bacteria contain only a relatively thin PGN layer found in the periplasmic space.[1,2]

PGN are large polymers composed of alternating β-(1–4)-linked *N*-acetylglucosamine (Glc*N*Ac) and *N*-acetylmuramic acid (Mur*N*Ac) residues, which are cross linked by short peptide bridges (Fig. 1). These peptides consist of four or five alternating L- and D-amino acids, which are attached to the carboxylic acid of Mur*N*Ac. The third amino acid of the peptides moiety of PGN of Gram-positive bacteria is commonly lysine while Gram-negative bacteria have a diaminopimelic acid (DAP) residue at this position.

The biosynthesis of PGN is a well-recognized target for antibiotic development.[3–6] For example, penicillins, cephalosporins, and vancomycin all act by inhibiting key steps in the assembly of the PGN layers. Recently, PGN has also attracted considerable attention as a ligand for receptors of the innate immune system of eukaryotes.[7,8]

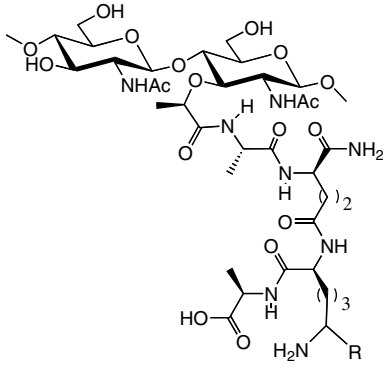

Figure 1. R = H (Lys) Gram-positive PGN, R = COOH (DAP) Gram-negative PGN.

For example, CD14 and Toll-like receptor 2 (TLR2), which are expressed on the surface of host monocytes and macrophages, can bind to PGN resulting in the secretion of several pro-inflammatory cytokines through the activation of transcription factors such as nuclear factor κB (NF-κB). These cytokines provide a first line of defense against invading pathogens. Over production of these mediators is known, however, to result in the clinical symptoms of septic shock.[9,10] Recently, it has been shown that nucleotide-binding oligomerization domain proteins 1 and 2 (NOD1 and 2), which are intracellular pattern recognition receptors, can detect small

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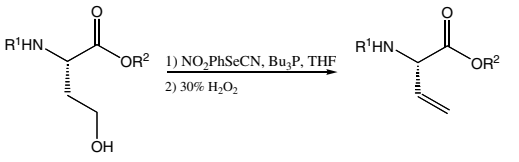
fragments of PGN initiating a pro-inflammatory response.[11–14]

In order to determine the molecular details of PGN-mediated cytokine production, well-defined synthetic fragments of PGN are required.[15] Furthermore, synthetic analogs of PGN part structures may provide a lead compound for the development of novel antibiotics.

Although lysine-containing compounds can be conveniently synthesized by standard solution- or polymer-supported approaches,[16,17] the chemical synthesis of DAP containing fragments is hampered by difficulties in obtaining this unusual amino acid. For example, Vederas and co-workers attempted the synthesis of DAP by a ring closing metathesis (RCM) of *N*-(benzyloxycarbonyl)-L-vinyl glycine and *N*-(*tert*-butylcarbonyl)-D-allylglycine that were esterified to ethylene glycol. This approach failed, however, due to the formation of an, α,β-unsaturated ester during vinyl glycine preparation. DAP was successfully synthesized by multi-step approach in which the key C–C bond-forming step was an ene reaction of methyl glyoxylate with methyl *N*-(benzyloxycarbonyl)-L-allylglycine catalyzed by a chiral Cu-catalyst to give a 96/4 mixture of diastereoisomers.[18] Several other lengthy synthetic approaches have been reported, which required separation of enantiomers or epimers.[19–24]

We envisaged that DAP could be conveniently prepared by a cross metathesis reaction between readily available allyl and vinyl glycine followed by a reduction of the double bond of the resulting compound. High selectivity in olefin cross metathesis reactions has been achieved when two olefins have significantly different reactivities.[25] In this respect, it has been shown that a cross metathesis of vinyl glycine with terminal alkenes using Grubb's first-generation catalyst gives mainly the recovery of the vinyl glycine, homo-dimerization of the alkene and a very low yield of cross coupling product.[26] On the other hand, the use of allyl glycines gave reasonable yields of cross metathesis products when applied under similar reaction conditions. The lower reactivity of vinyl glycines is probably due to a combination of steric and electronic factors. The introduction of the Grubb's second-generation catalyst has made it possible to perform cross metathesis reactions with sterically more demanding substrates. Therefore, it was expected that using this catalyst, a reaction of properly protected vinyl with allyl glycine would give a cross coupling product in good yield.

Several protected vinyl glycine (**5**–**8**) derivatives were prepared by treatment of corresponding homoserines (**1**–**4**) with 2-nitrophenyl selenocyanate and tributylphosphine in THF followed by the addition of hydrogen peroxide (Scheme 1).[27,28] A reaction of **5** with a stoichiometric quantity of **9** in the presence of Grubb's second-generation catalyst gave, after a reaction time of 24 h, the expected cross coupling product **13** in an acceptable yield of 46% (Table 1). Apart from this compound, unreacted vinyl glycine and homo-dimerized allyl glycine were formed which could be easily separated by silica gel column chromatography. The amount of homo-dimerized allyl glycine varied from 17% to 26%. Surprisingly, a prolonged reaction time failed to increase the yield of the cross coupling. The latter finding indicates that the homo-dimerized allyl glycine is not activated by the Grubb's catalyst to give compound **13**. Indeed, when a mixture of the homo-dimer of allyl and vinyl glycine **5** were subjected to metathesis conditions for two days, no cross coupling product **13** was formed. Fortunately, the use of an excess of vinyl glycine resulted in a significant improvement of the yield.



1 R^1 = Z, R^2 = Bn
2 R^1 = Z, R^2 = *t*-Bu
3 R^1 = Fmoc, R^2 = Bn
4 R^1 = Boc, R^2 = *t*-Bu

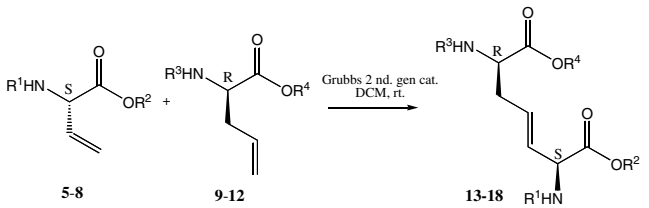
5 R^1 = Z, R^2 = Bn (60%)
6 R^1 = Z, R^2 = *t*-Bu (55%)
7 R^1 = Fmoc, R^2 = Bn (52%)
8 R^1 = Boc, R^2 = *t*-Bu (55%)

Scheme 1. Synthesis of vinyl glycine derivatives.

Previously, it has been shown that protecting groups may influence the outcome of cross metathesis reactions.[25] Therefore, several different allyl and vinyl glycines were used containing benzyl carbamate (*Z*), 9-fluorenylmethylcarbamate (Fmoc) and *tert*-butyl carbamate (Boc) as amino protecting groups and benzyl (Bn), *tert*-butyl (*t*-Bu) and methyl esters for carboxylic acid protection. The reaction combinations of vinyl (**5**–**8**) and allyl glycine (**9**–**12**) were chosen in such a manner that the resulting DAP derivatives (**13**–**18**) had an appropriate protecting group pattern for the synthesis of biologically relevant peptides. As can be seen in Table 1, the protecting groups had a small effect on the yields of the reactions ranging from 52% to 60%. In each case, the use of an excess of vinyl glycine led to an improvement in the yield of the cross metathesis product.

Having established a convenient approach for the preparation of properly protected DAP derivatives, attention was focused on the synthesis of biologically active PGN fragments. Recently, it was shown that the dipeptide *i*-Glu-DAP can induce activation of the transcription factor NF-κB in HEK-293 cells transfected with the gene encoding for NOD1.[11] We envisaged that hydrogenation of **14** over Pd–C would lead to a reduction of the double bond with concomitant removal of the benzyl carbamate group. Coupling of the resulting compound with an appropriately protected isoglutamic acid **20** would, after removal of the protecting groups, give the targeted dipeptide (Scheme 2). Thus, the double bond of **14** was reduced with concomitant removal of benzyl carbamate by catalytic hydrogenation over Pd–C to afford compounds **19** in an almost quantitative yield. The resulting compound was coupled with isoglutamic acid **20** using *N*,*N*,*N*′,*N*′-tetramethyl-*O*-(7-azabenzotriazol-1-yl)uronium hexafluorophosphate (HATU) as the

Table 1. Cross metathesis reactions of vinyl and allyl glycine derivatives

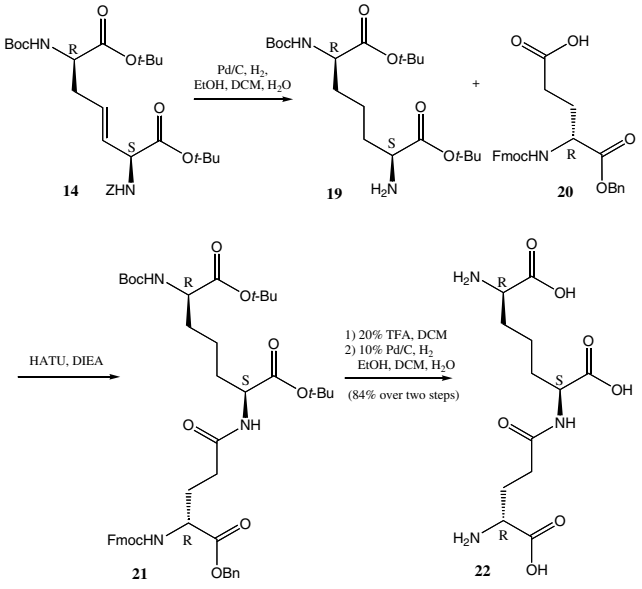


5-8 9-12 13-18

Substrates	R^1	R^2	R^3	R^4	Comp./yield, %
5:9 (1:1)	*Z*	Bn	Boc	Me	**13**/46
5:9 (1.8:1)	*Z*	Bn	Boc	Me	**13**/60
6:10 (1:1)	*Z*	*t*-Bu	Boc	*t*-Bu	**14**/42
6:10 (1.8:1)	*Z*	*t*-Bu	Boc	*t*-Bu	**14**/55
7:11 (1:1)	Fmoc	Bn	Boc	Bn	**15**/34
7:11 (1.8:1)	Fmoc	Bn	Boc	Bn	**15**/49
8:12 (1:1)	Boc	*t*-Bu	Fmoc	Bn	**16**/31
8:12 (1.8:1)	Boc	*t*-Bu	Fmoc	Bn	**16**/59
5:11 (1.8:1)	*Z*	Bn	Boc	Bn	**17**/61
7:10 (1.8:1)	Fmoc	Bn	Boc	*t*-Bu	**18**/64

activation reagent to give the dipeptide **21** in a yield of 86%. The Fmoc protecting group of **21** can be selectively removed for further extension. However, target compound **22**[29] was obtained by a two-step deprotection procedure involving cleavage of the *tert*-butyl carbamate and *tert*-butyl ester by treatment with 20% TFA in DCM followed by the simultaneous removal of the Fmoc and benzyl ester by catalytic hydrogenation over Pd–C to give **22** in an overall yield of 84%.

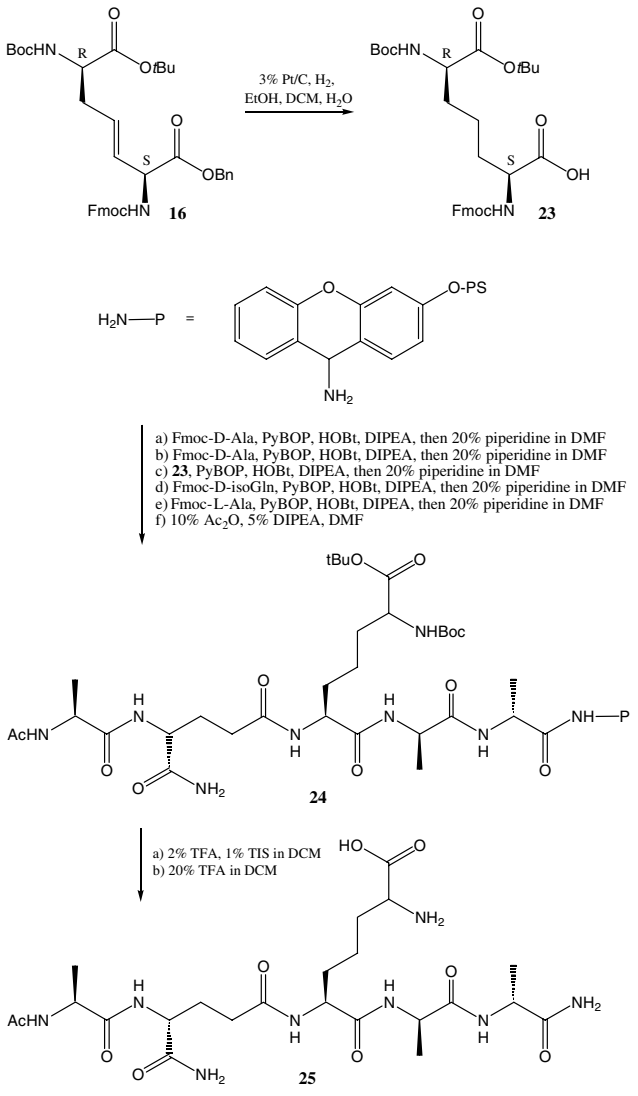
Derivative **16** is suitably protected for use in solid supported synthesis of DAP containing peptides. To illustrate the use of this compound, pentapeptide **25**,[30] which is a fragment of PGN of Gram-negative bacteria, was prepared by a polymer-supported approach (Scheme 3).



Scheme 2. Synthesis of biological active Glu-(7*S*,11*R*)-7-11 diaminopimelic acid.

Thus, catalytic hydrogenation of compound **16** over Pt–C resulted in a clean reduction of the double bond and removal of the benzyl ester to give DAP derivative **23** in a yield of 98%. Next, the dipeptide D-Ala-D-Ala attached to a hyper acid sensitive Sieber amide linker was prepared using classical Fmoc chemistry and benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP)/*N*-hydroxybenzotriazole(HOBT)/*N*-ethyldiisopropyl amine (DIPEA) as the coupling reagent. In the next step of peptide assembly, DAP derivative **23** was incorporated using the same activation reagent to give a polymer bound tripeptide. Treatment of this derivative with 20% piperidine in DMF led to the removal of the Fmoc group as judged by a positive Kaiser test. Subsequently, an isoglutamine moiety was incorporated using Fmoc-D-isoglutamine and PyBOP/HOBT/DIPEA as the coupling reagent. Finally, removal of the Fmoc protecting group of the tetrapeptide and coupling of Fmoc-L-Ala gave the fully assembled peptide. The Fmoc protecting group was removed using standard conditions and the terminal amine acetylated with acetic anhydride to give peptide **24**. The peptide was cleaved from the solid support using 2% TFA and 1% triisopropyl silane (TIS). Under these conditions the side chain protecting groups were not removed which facilitated the purification of the compound. Finally, global deprotection with 20% TFA gave the target pentapeptide **25**.

In conclusion, the unusual amino acid, diaminopimelic acid, could conveniently be prepared by a metathesis reaction between appropriately protected vinyl and allyl glycine derivatives followed by reduction of the double bond of the resulting compounds. By careful selection of protecting groups, the latter reaction selectively removed a benzyl carbamate or benzyl ester to give DAP derivatives that were used in the solution- or polymer-supported synthesis of biologically active PGN part structures.



Scheme 3. Polymer-supported synthesis of DAP containing peptide derived from Gram-negative PGN.

Acknowledgements

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29. *Analytical data of compound* **22**: $[\alpha]_D^{26}$ +5.7, ^{1}H NMR (500 MHz, CD_3OD): δ 4.32 (1H, q, α-CH, DAP), 3.94 (1H, t, α-C*H*, Glu), 3.86 (1H, q, α-C*H*, DAP), 2.47 (2H, γ-CH_2, Glu), 2.10–2.19 (1H, m, β-CH_2, Glu), 2.04–2.10 (1H, m, β-CH_2, Glu), 1.78–1.96, 1.65–1.69, 1.45–1.57 (6H, m, β,γ,δ-CH_2CH_2CH_2, DAP). ^{13}C NMR (125 MHz, CD_3OD): 175.12, 174.45, 53.92, 53.71, 53.34, 32.14, 32.01, 31.22, 31.11, 27.24, 22.65. HRMS-MALDI-TOF calcd for $C_{12}H_{21}N_3O_7$ (M+Na): 342.1380, found 342.0795.
30. *Analytical data of compound* **25**: $[\alpha]_D^{26}$ +26.25, ^{1}H NMR (500 MHz, CD_3OD): δ 4.21–4.29 (4H, m, α-C*H*, Glu, α-C*H*, DAP, 2 × α-C*H*, Ala), 3.71–3.74 (1H, m, α-C*H*, Ala), 3.20 (1H, q, α-C*H*, DAP), 2.38–2.40 (2H, m, γ-CH_2, Glu), 2.12–2.15 (1H, m, β-CH_2, Glu), 2.03 (3H, s, NHCOCH_3), 1.79–1.98 (5H, m, β-CH_2, Glu, β-CH_2, DAP, δ-CH_2, DAP), 1.45–1.50 (2H, m, γ-CH_2, DAP), 1.34–1.42 (9H, m, 3 × CH_3, Ala). HRMS-MALDI-TOF calcd for $C_{23}H_{40}N_8O_9$ (M+Na): 595.6122, found 595.4370.