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Solid Phase Synthesis of Peptidoglycan Monomers for the Generation of a Combinatorial Library

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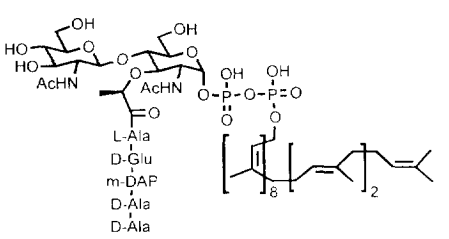
Abstract: *Solid phase synthesis of peptidoglycan monomers was conducted by coupling the corresponding lipid-bearing glycocarboxylic acids to resin-bound peptides. The efficiency of coupling reagents was found to be in descending order of HATU>HBTU>PyBOP>EEDQ. There was no obvious difference in coupling yield between conducting the solid phase synthesis on polystyrene resins or on polyethylene glycol grafted polystyrene resins.* Copyright © 1996 Elsevier Science Ltd

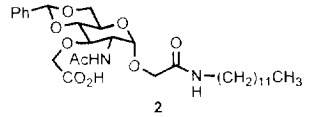
Inhibition of bacterial cell wall biosynthesis is a well established strategy for the identification of clinically viable antibiotics.[1] Bacterial cell wall peptidoglycan consists of a network of peptide containing polysaccharide which is cross linked by the peptide chain to provide cell wall rigidity.[1] A typical peptidoglycan monomer for bacterial cell wall biosynthesis is a triunit made up of a lipid, a carbohydrate and a peptide unit, such as the lipid-bearing 1,4-linked N-acetylglucosamine N-acetylmuramoyl pentapeptide **1**.[2] A rational approach to inhibit bacterial cell wall biosynthesis would be to prepare analogs of the peptidoglycan monomer triunit **1**. It is expected that these analogs would be potential inhibitors of the transglycosylases or transpeptidases involved in bacterial cell wall biosynthesis.

In an effort to identify potential inhibitors of peptidoglycan biosynthesis, we wanted to construct a peptidoglycan monomer combinatorial library in which we could vary the nature of the peptide, carbohydrate and lipid units. In addition, we wanted to construct the library on the solid phase followed by the release of the peptidoglycan products into solution for biological assay. Since there are only a few reports describing the solution synthesis of the lipid-carbohydrate[3] and the carbohydrate-peptide[4] fragments of the peptidoglycan monomer, to accomplish a library construction required that we develop efficient solid phase chemistry for the synthesis of the peptidoglycan systems.

The basic solid phase strategy which we chose required that protected lipid-bearing glycocarboxylic acid building blocks be coupled to resin-bound peptides by solid phase amide bond formation. Subsequently, any base labile carbohydrate protecting groups would be removed before a one-step acid cleavage of the lipid-carbohydrate-peptide product from the solid support and removal of acid sensitive carbohydrate or peptide protecting groups. In this report, we describe the development of efficient solid phase chemistry for the construction of peptidoglycan monomer systems employing both monosaccharide **2** and disaccharide **3** building blocks.



1



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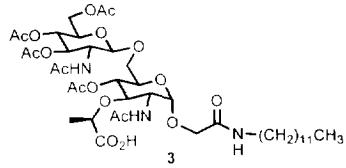
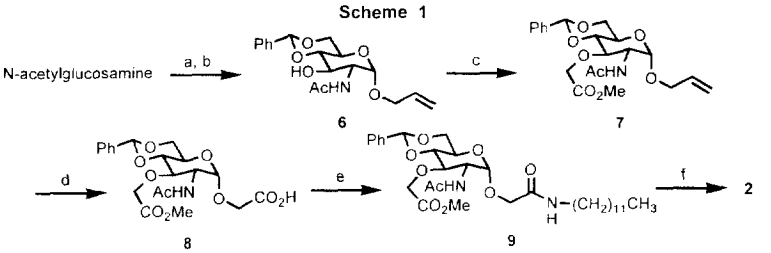


3

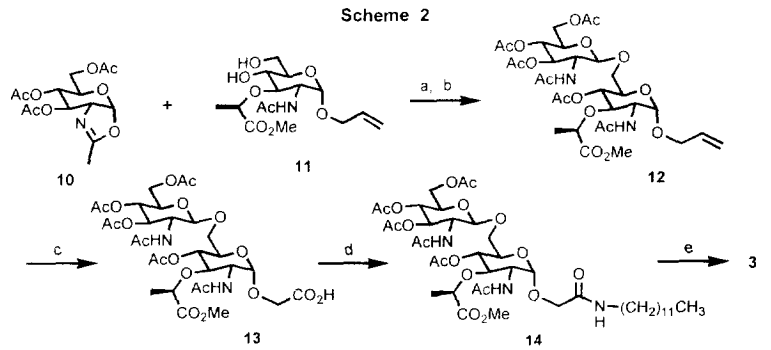
The synthesis of the monosaccharide **2** and disaccharide **3** building blocks are shown in Scheme 1 and 2 respectively. In both cases, the allyl glycosides **7** and **12** were oxidized to the corresponding acids **8** and **13** by a $RuCl_3$ mediated oxidation[5] which were subsequently coupled to 1-dodecylamine in the presence of HOBt and a water-soluble CDI[6] (WSCDI) to yield **9** and **14** respectively. The desired building blocks **2** and **3** were obtained by selective saponification[7] of the corresponding esters **9** and **14**.

Scheme 1



a) allyl alcohol, BF_3-OEt_2 (qt. yd., α:β = 4:1). b) $PhCH(OMe)_2$, TsOH, DMF, column separation (47 %).
c) NaH, THF, methyl bromoacetate (qt. yd.). d) $RuCl_3$, $NaIO_4$, CCl_4, MeCN, H_2O (73 %).
e) 1-dodecylamine, HOBt, WSCDI, CH_2Cl_2 (50 %). f) 0.1 M KOH, dioxane (qt. yd.)

Scheme 2



a) *p*-TsOH, toluene, reflux (70 %). b) Ac_2O, DMAP, py (qt. yd.). c) $RuCl_3$, $NaIO_4$, CCl_4, MeCN, H_2O (86 %).
d) 1-dodecylamine, HOBt, WSCDI, CH_2Cl_2 (73 %). e) 0.1 M KOH, dioxane, 0°C (91 %)

Although solid phase peptide synthesis is well established, the coupling reaction involving structurally different lipid-bearing glycocarboxylic acid substrates deserved extensive investigation. We found that the pentafluorophenyl (Pfp) esters of glycocarboxylic acids **2** and **3** were very labile and the Pfp-ester/Dhbt-OH[8] solid phase amide bond formation did not afford reproducible coupling yield. These results rendered the direct coupling of glycocarboxylic acids **2** and **3** to resin-bound peptides a simpler and better alternative. The solid phase synthesis of peptidoglycan monomer analogs **4** and **5** is illustrated in Scheme 3. The Fmoc protected resin-bound tripeptide[9] was treated with 20% piperidine in DMF to generate the free amino terminal. The disaccharide building block **3** was coupled[10] to a tripeptide bound to polystyrene resin *via* a chlorotrityl linker, **AKA-(Clt)-(PS)**. The monosaccharide building block **2** was coupled to **AKA-(Clt)-(PS)** as well as to a tripeptide bound to polyethylene glycol grafted polystyrene resin, **AKA-(PEG)-(PS)**. The resin-bound monosaccharide coupling products, **2-AKA-(Clt)-(PS)** and **2-AKA-(PEG)-(PS)**, were treated with 90% aqueous trifluoroacetic acid to complete the cleavage and global deprotection processes. The monosaccharide peptidoglycan monomer analog **4** was obtained by precipitation with diethyl ether. RP-HPLC analysis[11] showed that the deprotection was complete (Figure 1). On the other hand, the resin-bound disaccharide coupling product, **3-AKA-(Clt)-(PS)**, was first treated with sodium methoxide to remove the acetate protecting groups. It was followed by the 90% TFA cleavage and peptide deprotection. The disaccharide peptidoglycan monomer analog **5** was finally isolated by precipitation with diethyl ether. The coupling yield of each combination of solid support and coupling reagent was determined by RP-HPLC analysis[11] of the isolated products, and the results are summarized in Table 1. To find

the most efficient coupling conditions, four coupling reagents, EEDQ,[12] HATU,[13] HBTU,[14] and PyBOP[15] (Figure 2), were evaluated. We found that HATU consistently afforded the highest coupling yield between glycocarboxylic acids, **2** or **3**, and resin bound peptides. Despite the larger size of the dissaccharide building block **3**, coupling yields match those observed with the monosaccharide building block **2**.

Scheme 3

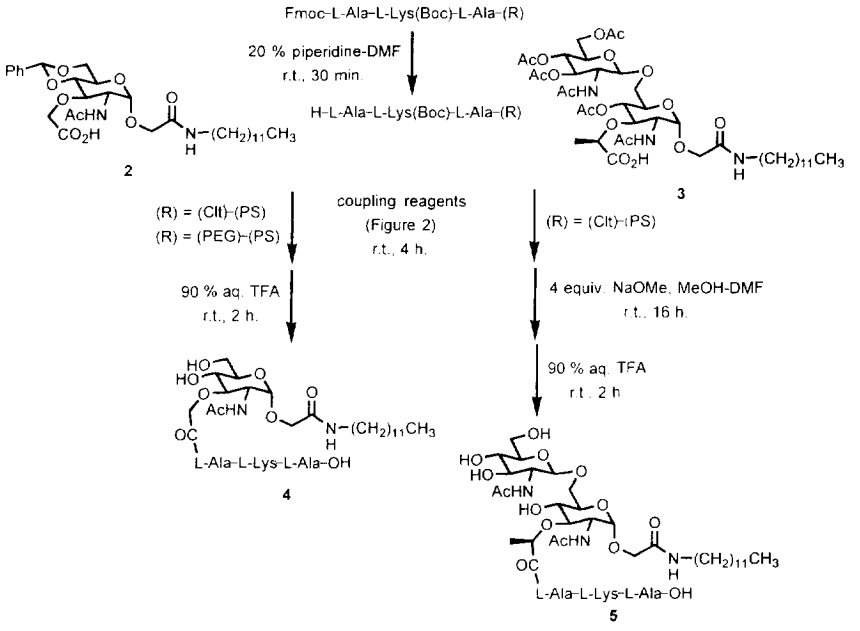


Figure 1. Chromatogram of isolated products

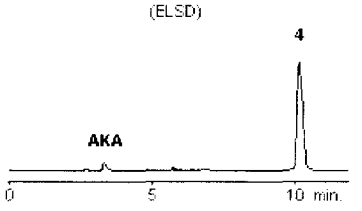


Figure 2. Coupling reagents

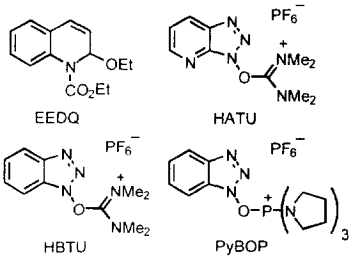


Table 1. Coupling yield (%) of solid phase reactions.[10]

	2 + AKA-(Clt)-(PS)	2 + AKA-(PEG)-(PS)	3 + AKA-(Clt)-(PS)
EEDQ/DCM	74	80	67
HATU/DIPEA/DMF	84	85	89
HBTU/DIPEA/DMF	79	77	89
PyBOP/DIPEA/DMF	75	76	82

In summary, we have demonstrated the efficient solid phase construction of peptidoglycan monmer analogs by coupling lipid-bearing glycocarboxylic acids to resin-bound peptides. The most effective coupling reagent in the study was found to be HATU followed by HBTU, PyBOP and EEDQ respectively. These findings agree with the role of HATU in coupling hindered amino acids. We also found that there was no obvious difference in coupling yield when conducting the solid phase synthesis on polyethylene glycol grafted polystyrene resins or chlorotrityl polystyrene resins. This result shows that there is no advantage in substituting polystyrene resins with more expensive PEG-grafted resins when solid phase reactions could be carried out in solvents with good swelling capability, such as dichloromethane or dimethylformamide. Utilization of both monosaccharide and disaccharide building blocks was compatible with the solid phase chemistry. The difference in size and protecting groups on the monosaccharide and disaccharide building blocks did not significantly alter yields on the solid phase coupling reaction. It implies that resins with adequate pore size provide similar access to both monosaccharide and disaccharide substrates. Benzylidene as well as acetate protecting groups are compatible with the solid phase synthesis conditions on chlorotrityl resins. Since the synthetic scheme allows the use of acid or base labile protecting groups, the scope of glycocarboxylic acid building blocks is not limited by the protecting group strategy. Consequently, a larger number of carbohydrate variants could be incorporated in the construction of a peptidoglycan monomer library.

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9. Resin-bound peptides are custom synthesized by AnaSpec Inc., 2149 O'Toole Ave., San Jose, CA 95131.
10. Solid phase coupling reaction was carried out with 2 equiv. of glycocarboxylic acid and 2 equiv. of coupling reagents, and shaken for 4 h. at room temperature.
11. RP-HPLC analysis was performed on a C18 column (5 micron, 4.6x250 mm) with gradient elution (10% to 50% MeCN-H_2O/0.1%TFA) and monitored by UV (205 nm) and ELSD (evaporative light scattering detector).
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