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Preparation of Potential Inhibitors of the Mur-Pathway Enzymes on Solid Support Using an Acetal Linker

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Abstract—Here, we report a novel strategy for the combinatorial or parallel solid-phase synthesis of potential inhibitors of the mur-pathway enzymes. The strategy involves the efficient use of *p*-alkoxybenzylidene acetal linker for reversible immobilization of sugar scaffolds to solid phase. This methodology was used to synthesize several glycopeptides on solid phase in good yields.

The bacterial cell wall was first identified as a target for antibacterial agents, namely β-lactams, almost half a century ago.[1] However, some of the enzymes involved in the early, cytoplasmic steps (Fig. 1) of the cell wall assembly have not been exploited for antibacterial research until very recently,[2] primarily due to the difficulties in obtaining their substrates. Bacterial cell wall is a polymeric peptidoglycan consisting of alternating *N*-acetylmuramic acid (MurNAc) and *N*-acetylglucosamine (GlcNAc) units that are cross-linked through the pentapeptide chains. The monomeric precursor for peptidoglycan biosynthesis (**3**) is assembled in the cytoplasm starting with the condensation of UDP-*N*-acetylglucosamine (**1**) and phosphoenolpyruvate (PEP) catalyzed by MurA. Carboxylate (**2**), generated by reduction with NADPH and MurB, serves as the point of attachment for the pentapeptide chain, which is in turn synthesized by a series of ATP-dependent amino acid ligases (MurC-F).[3] Recently, a high throughput coupled enzyme assay has been developed to simultaneously screen for an inhibitor of any one of the enzymes in the pathway.[4] With such a potent tool for identifying inhibitors in hand, the challenge remains to develop an efficient synthesis of inhibitors for these enzymes, since they are all valid antibacterial targets.

In the past decade solid-phase combinatorial chemistry has emerged as a very powerful tool for discovery of biologically active compounds and for rapid optimization of lead structures.[5] The split–pool[6] technique allows for rapid synthesis of large libraries of compounds, but assaying mixtures may give false results and requires labor-intensive deconvolution. Parallel synthesis of single compounds avoids these screening problems, but is limited to the synthesis of smaller libraries.[7] Since each bead in the split-pool combinatorial synthesis undergoes a set of unique chemical reactions, at the end of the sequence it carries only a single

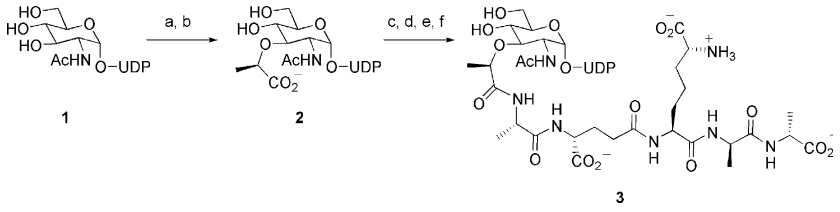

Figure 1. Cytoplasmic steps of bacterial cell wall biosynthesis: (a) MurA, PEP; (b) MurB, NADPH; (c) MurC, ATP, L-Ala; (d) MurD, ATP, D-Glu; (e) MurE, ATP, mDAP; (f) MurF, ATP, D-Ala-D-Ala.

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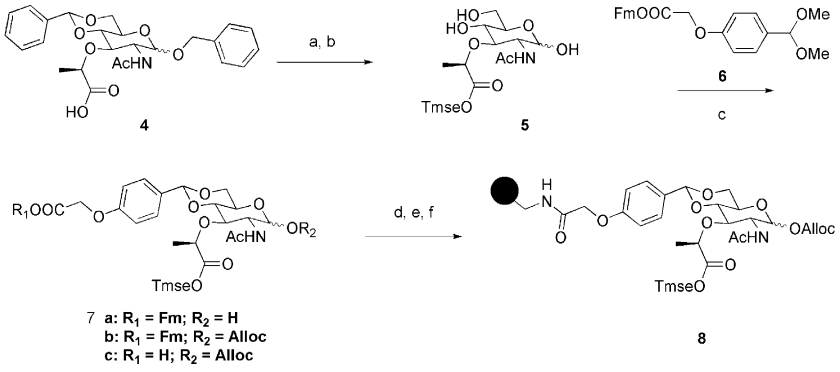
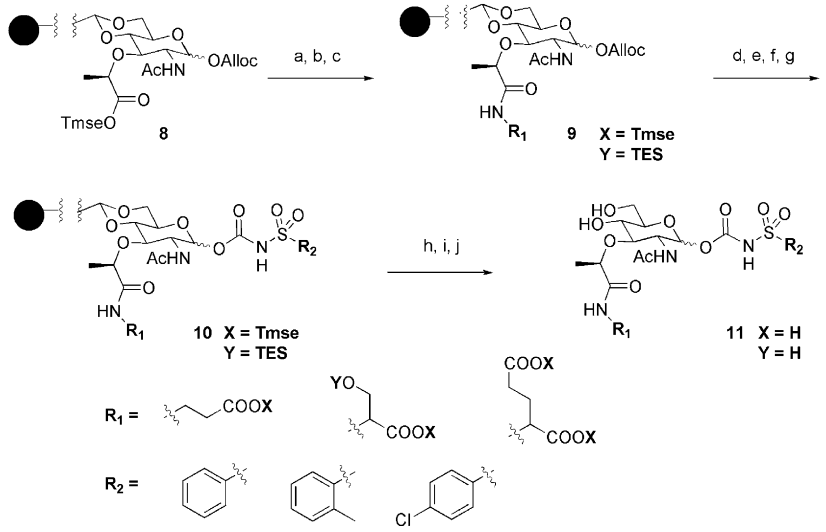
chemical entity.[5e] Creating the solid phase library using the split–pool technique, but separating the beads prior to cleavage and assay, retains the synthetic advantages of split–pool technique while also having the analytical advantages of parallel synthesis.[8] In this paper, we report on our efforts to develop new strategies for combinatorial synthesis of glycoside-based potential inhibitors of MurA-F enzymes on solid phase. In this process, we developed an efficient linker for the reversible immobilization of monosaccharides to solid phase and, as a proof of principle, synthesized two small prototypical glycoside-based libraries on solid phase.

It has been shown that the MurNAc moiety is an important contributor to the potency of some MurD inhibitors,[2a] since it displays the UDP, *N*-acyl, and pentapeptide moieties around a rigid scaffold in a predictable orientation. Therefore, in designing our prototype libraries we used the unaltered sugar scaffold, but allowed for modifications at three other sites: the peptide position, the *N*-acyl position, and the polar anomeric UDP position. That left the 4,6-diol unit unfunctionalized, and an ideal point for anchoring to the resin. Although, an initial report on suspending sugars to solid phase as 4,6-benzylidene acetals appeared in the literature over 25 years ago,[9] the utility of this linker in solid phase synthesis has not been widely explored.[10]

The goal of our first prototype split–pool library was to evaluate the stability and cleavage conditions of the benzylidene acetal anchor group and to establish that good loading and recovery of compound from individual beads could be achieved.[11] Initially we synthesized resin bound MurNAc (**8**) as depicted in Scheme 1, then functionalized it using the split–pool method (Scheme 2) to make a nine-compound library. A commercially available MurNAc derivative (**4**) was protected as a 2-(trimethylsilyl)ethyl (TMSE) ester using standard conditions. Treatment of the diol (**5**) with dimethyl acetal (**6**) and a catalytic amount of *p*-toluenesulfonic acid (TsOH) gave the 9-fluorenylmethyl (Fm)-protected benzylidene acetal (**7a**). Following several protecting-group manipulation steps, acid (**7c**) was coupled to the resin using EDC/HOBt conditions. The loading was assessed qualitatively using the Kaiser test for free amine residues[12] and gravimetrically by releasing the product off the resin with 90% aqueous acetic acid



Scheme 1. Synthesis of orthogonally protected MurNAc and attachement to the aminomethyl polystyrene resin through the *p*-alkoxybenzylidene acetal anchor group **3**: (a) TMSethanol, EDC, DMAP, cat., 80%; (b) $Pd(OH)_2$, H_2, EtOAc, MeOH, 90%; (c) $TsOH{\cdot}H_2O$, DMF, 67%; (d) allyl chloroformate, Et_3N, CH_2Cl_2, 80%; (e) 7% diethylamine, CH_2Cl_2, CH_3CN; (f) PS-CH_2NH_2, EDC, HOBt, CH_2Cl_2, DMF.



Scheme 2. Synthesis of a nine compound library: (a) TBAF, THF; (b) split into three vessels; (c) R_1-NH_2, EDC, HOBt, DMF; (d) mix; (e) $Pd(PPh_3)_4$, $PhSiH_3$, CH_2Cl_2; (f) split into three vessels; (g) R_2-SO_2NCO; (h) mix; (i) TBAF, THF; (j) 15% TFA, CH_2Cl_2, 1% H_2O (or glacial acetic acid, 40 °C).

(AcOH) containing 1% trifluoroacetic acid (TFA). Released product showed no detectible cleavage of the acid labile anomeric allyloxycarbonyl (Alloc) group.

Using a split–pool combinatorial technique we prepared a 1 × 3 × 3 library of compounds following Scheme 2. The carboxylate of the resin bound MurNAc derivative (**8**) was unmasked with tetrabutylammonium fluoride (TBAF) in THF. The resin was split into three vessels and coupled with different protected amino acids (β-alanine, L-serine and D-glutamate) (**9**), then mixed and the anomeric position deprotected. Next, the resin was split into three vessels and treated with three aryl-sulfonyl isocyanates (phenyl, *o*-toluyl, and *p*-chlorophenyl) (**10**). Finally, the resin was pooled, treated with TBAF and the compounds (**11**) released from solid phase using either TFA in methylene chloride at room temperature, or glacial acetic acid at 40 °C. These cleavage conditions were sufficiently mild to leave the acid-labile anomeric acylsulfonamides intact.[13] Average yield was determined gravimetrically to be 53%.[14] By successful preparation of this 9-component library, we demonstrated the usefulness and versatility of the alkoxybenzylidene acetal linker, its stability during synthesis, and desirable susceptibility to weak acid that allows for reversible immobilization of sugars to solid phase.

Having to functionalize the monosaccharide with the linker (**7c**) prior to resin attachment makes this methodology less useful when preparing larger libraries, due to the laborious synthesis of the appropriately protected sugars. A more efficient approach would involve condensing the resin-bound linker directly with the 4,6-diol of the monosaccharide. In order to employ that approach, we developed methodology outlined in Scheme 3. The resin-bound 2-(4-formylphenoxy) acetate (**12**) was activated as a dimethyl acetal using standard methods, trimethylorthoformate (TMOF) and catalytic TsOH.[15] MurNAc derivative (**5**) was directly attached onto the activated resin (**13**) by reacting only two equivalents of sugar with respect to the reactive sites on the resin to accomplish high loading.[16]

To showcase the usefulness of this approach in immobilizing not very readily available mono-saccharides on solid phase and to synthesize useful building blocks for a larger library of mur-pathway inhibitors, we prepared four analogues of **5** (**17a–d**) by diversifying the acyl moiety (Scheme 4). Commercial 2-acetamido-2-deoxy-glucopyranoside (**15**), was deacetylated using potassium hydroxide (KOH) in ethanol at elevated temperature. Using standard conditions, the amino group was selectively acylated, in 75–85% yields, to give four amides (**16a–d**) which were then elaborated to triols (**17a–d**). Each of the glycosides was attached to the solid phase using procedures outlined in Scheme 3. These resins were then subjected to the series of steps analogous to the ones described in Scheme 2 to elaborate fully to the potential inhibitors (**18 a–e**) shown in Figure 2.

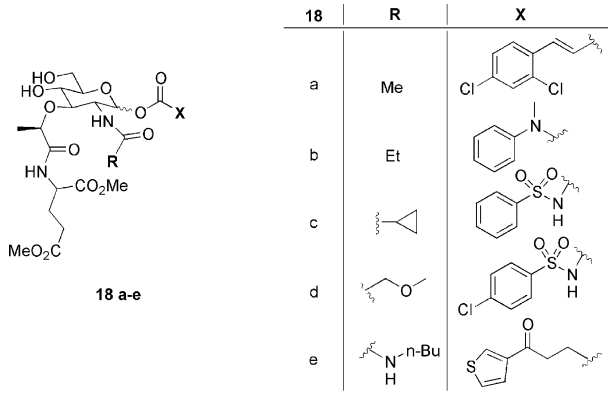
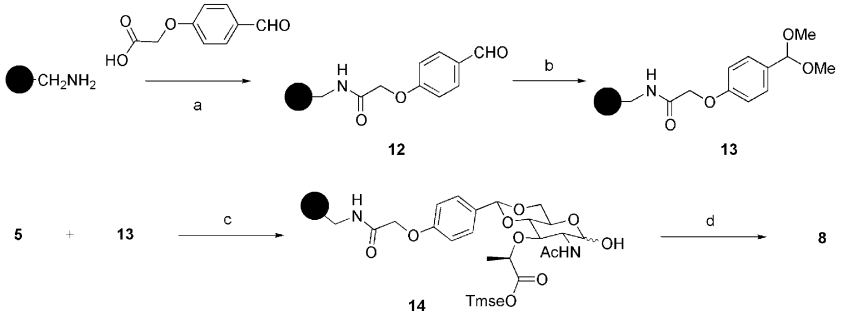
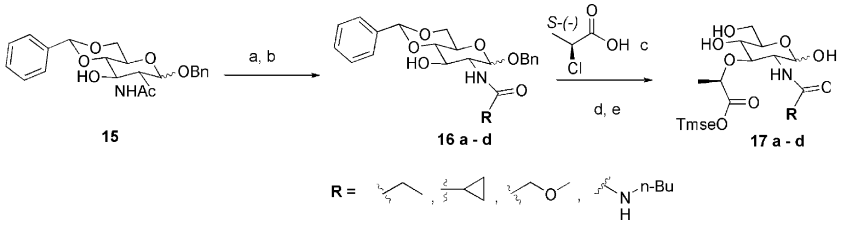


Figure 2. Potential inhibitors of the mur-pathway enzymes prepared on solid phase using the procedures outlined in Schemes 2 and 3.



Scheme 3. An alternative method for immobilization of MurNAc to the resin using a preattached linker: (a) EDC, HOBt, DMF; (b) TMOF, TsOH, CH_2Cl_2; (c) TsOH, DMF; (d) allylchloroformate, CH_2Cl_2.



Scheme 4. Synthesis of acyl derivatives of **5**: (a) KOH, EtOH, 130 °C (87%); (b) RCOCl, CH_2Cl_2, pyridine (75–85%); (c) NaH, dioxane (30–50%); (d) EDC, DMAP, TMSethanol (80–95%); (e) $Pd(OH)_2/C$, EtOAc, MeOH, 40 psi.

In conclusion, we developed a new synthetic strategy for the combinatorial or parallel synthesis of muramic acid glycopeptides as potential inhibitors of the mur- pathway enzymes. The strategy involves reversible attachment of the sugars through the 4,6-diol to the resin using an acid-sensitive linker. This efficient methodology for immobilizing sugars to a resin, which reduces the number of synthesis steps prior to attachment, makes this strategy very appealing for immobilizing not very readily available sugar scaffolds. Work is in progress to miniaturize the mur-pathway enzyme assay and to develop usable tagging methodology that will facilitate identification of compounds on bead. Coupled with these powerful technological tools, this synthetic strategy could allow for identification of novel inhibitors for some of the mur-pathway enzymes.

Acknowledgements

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11. Our solid support was: LCC Engineering aminomethyl polystyrene, uniform beads, with 1.1 mmol/g (4.4 nmol/bead) loading.
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13. The library was characterized by single bead cleavage of compounds followed by LC/MS analysis. Beads were picked, suspended in 50 μL of cleavage cocktail for 15 min, then dried under a nitrogen stream, resuspended in 15 μL of acetonitrile and analyzed by LC/MS. Individual cleavage of 20 beads resulted in finding all of the nine desired compounds. The HPLC trace of each compound confirmed the high purity (>90%) or each analogue.
14. Forty milligrams of resin were treated with 15%TFA in methylene chloride for 10 min. The resin was filtered off and washed with three washes of methylene chloride and acetonitrile. The filtrates were combined and solvent was removed under reduced pressure to yield 9 mg of oily residue (53% yield).
15. While this conversion could be followed by IR (observing the disappearance of the carbonyl stretch) on the Wang aldehyde resin, it could not be followed by the same method on the methyl amino resin due to the presence of the amide bond. Instead, the optimized conditions for the Wang aldehyde resin were applied to the aminomethyl polystyrene resin.
16. Loading was assessed gravimetrically, by weighing the cleaved product from the known amount of resin (assuming 1.1 mmol/g loading) and also by comparison of cleavage product HPLC trace with the cleavage product of resin **8**.