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Synthesis of labeled meropenem for the analysis of *M. tuberculosis* transpeptidases

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ABSTRACT

A concise synthesis of ^{14}C labeled meropenem prepared from ^{14}C dimethylamine hydrochloride is described. Using a similar reaction sequence, the meropenem nucleus was also attached to biotin providing a probe for protein interaction studies.

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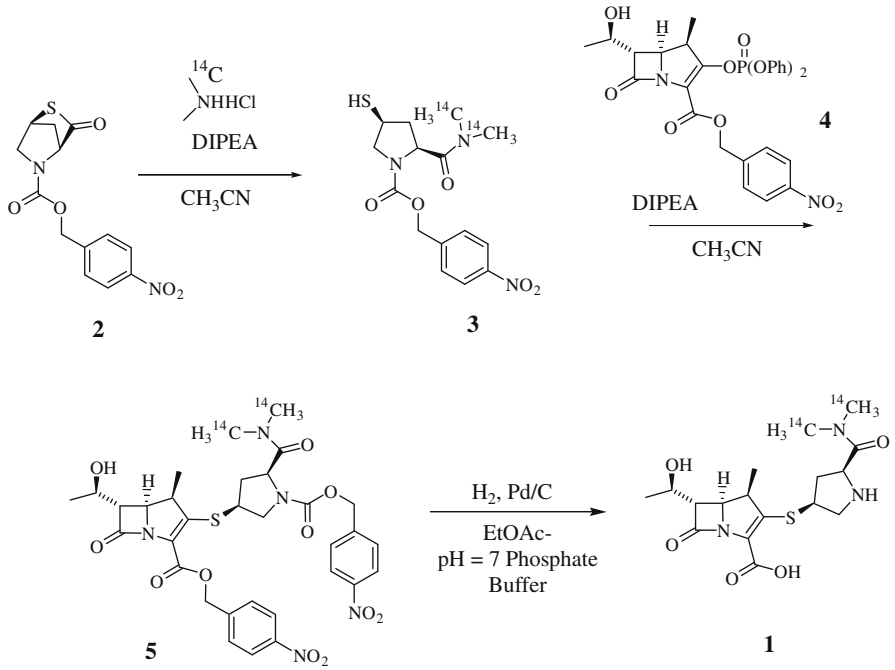
The mycobacterial cell wall is a complex, multi-layered heteropolymer composed of a peptidoglycan core that is covalently bound to arabinogalactan and mycolic acids.[1–3] Although the size and hydrophobic nature of this complex contribute to the impermeability of mycobacteria to many antibiotics,[4,5] other features are likely to contribute to the resistance to specific classes of antibiotics. The β-lactams (penicillins, cephalosporins, and carbapenems) are the most widely prescribed anti-infectives and derive their biologic activity through the acylation and deactivation of the transpeptidases involved in peptidoglycan crosslinking.[6–8] *Mycobacterium tuberculosis*



Scheme 1. ^{14}C labeled meropenem synthesis.

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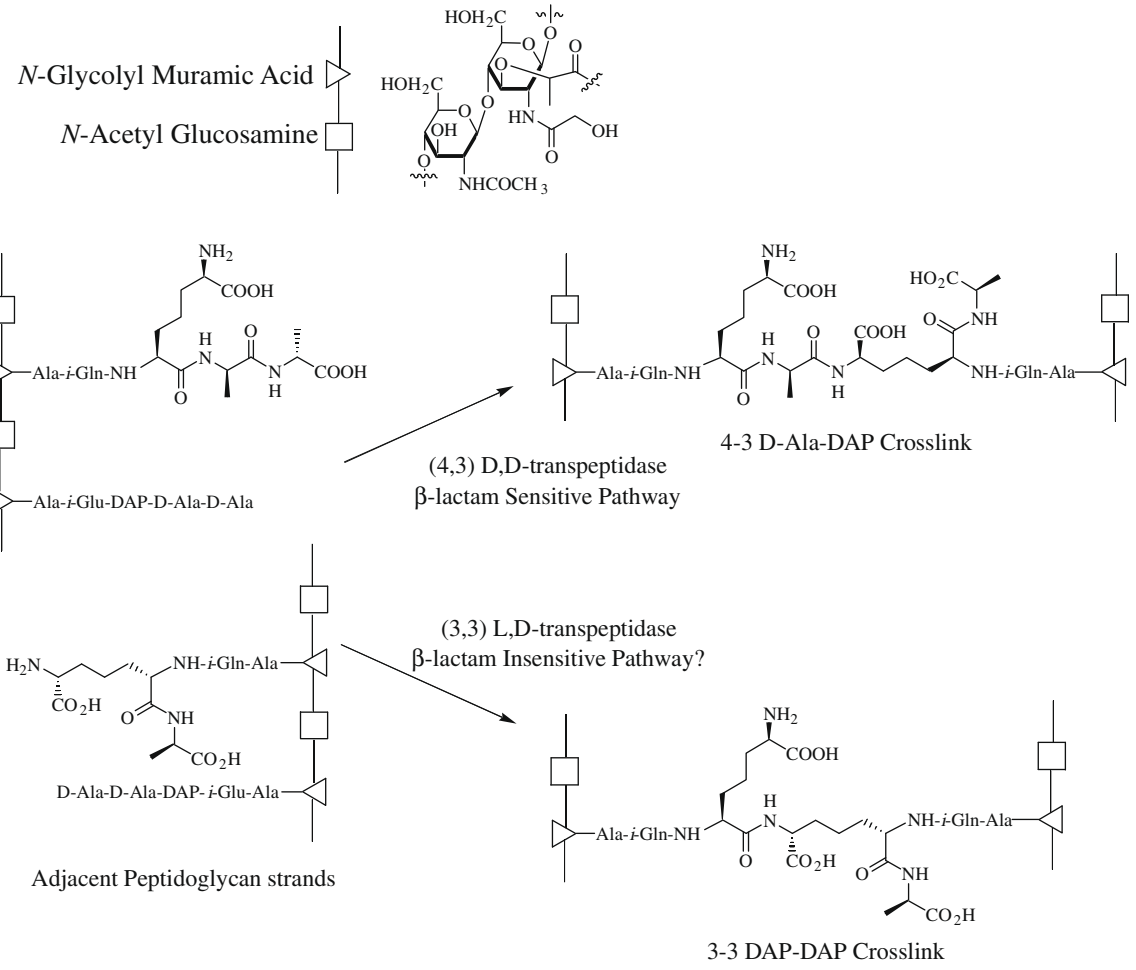


Figure 1. Two types of peptidoglycan crosslink in *M. tuberculosis*.

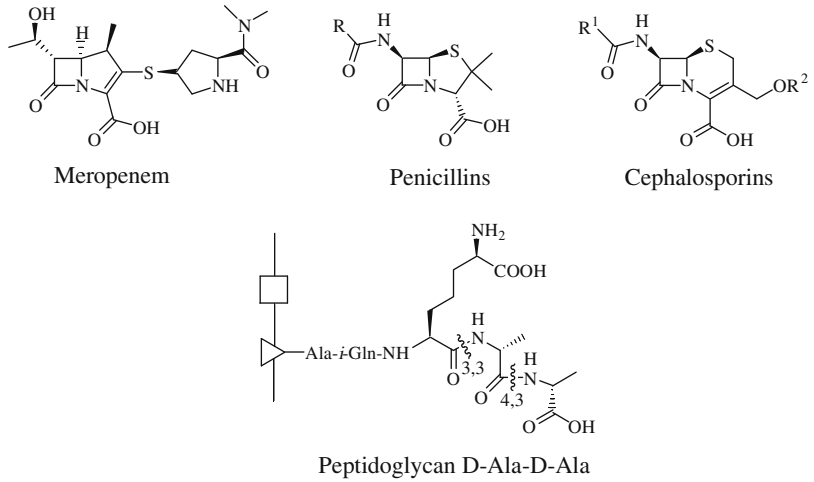


Figure 2. Common β-lactam structures.

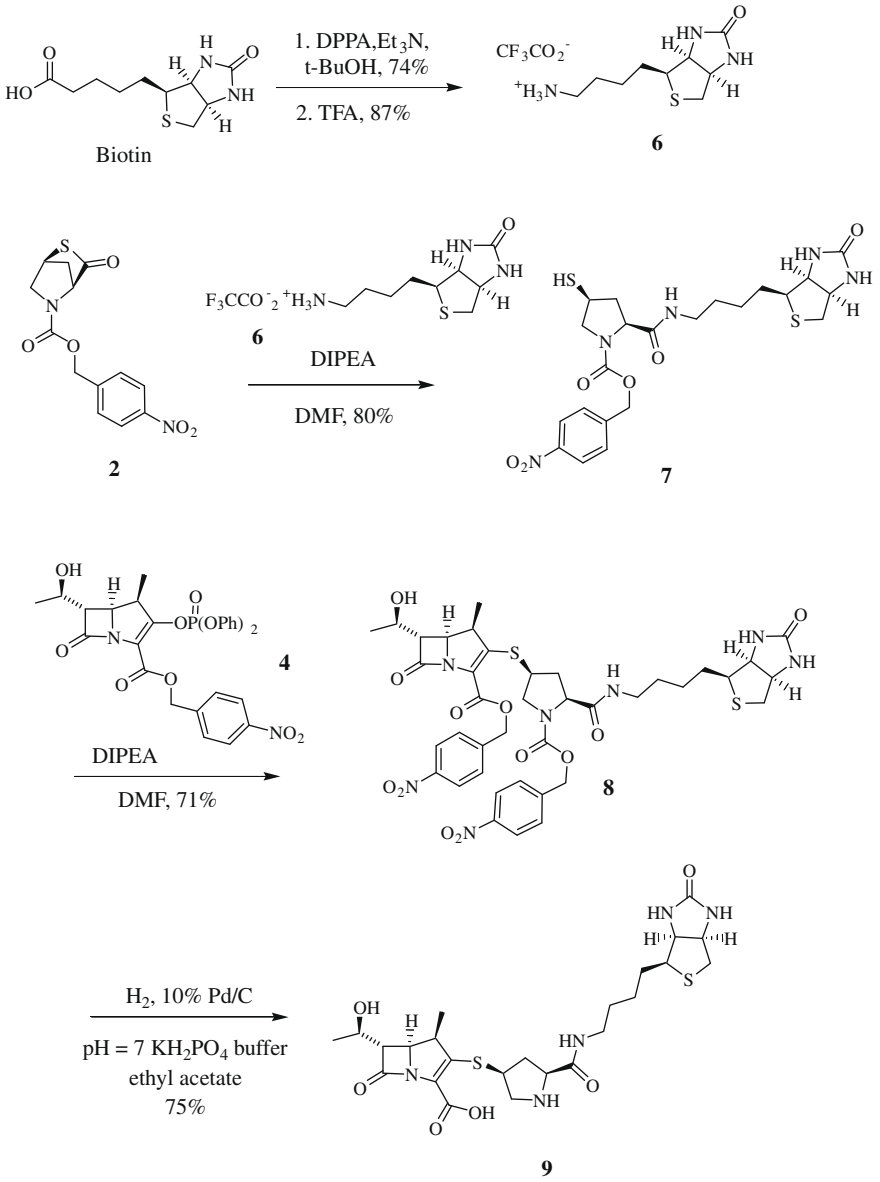
(Mtb) contains at least one chromosomal β-lactamase, Rv2068c, a Class A, extended spectrum β-lactamase.[9] Rv2068c can be inhibited by clavulanic acid and thus combinations of antibiotic and β-lactamase inhibitor or newer classes of β-lactamase resistant antibiotics should prove effective for treating tuberculosis.[10]

Historically the β-lactams have not been used in treating tuberculosis despite the fact that the peptidoglycan of mycobacteria is extensively crosslinked and that β-lactams penetrate the cell wall and inhibit transpeptidase targets in *Mtb*.[11,12] A recent explanation for this paradox suggested that the mycobacterial cell wall actually contains two distinct types of crosslinks.[13] The more intensively studied 4–3 crosslinks (Fig. 1) are synthesized by a class of D,D transpeptidases called penicillin-binding proteins that are inhibited by the β-lactams. In several species of bacteria including *M.*

tuberculosis, a different type of 3–3 crosslink (Fig. 1) has been observed. These crosslinks are formed by L,D transpeptidases, a new class of cysteine transpeptidase previously thought to be insensitive to β-lactams but now known to be inhibited by the carbapenem class of β-lactam antibiotics. Conceivably, the 3–3 crosslinks provide an alternative architectural modification and some evidence suggests that these linkages play a role in stationary phase rigidification providing benefits for the long term survival of nonreplicating bacilli.[14–20]

The carbapenems, a class of four FDA-approved antibiotics (imipenem, meropenem, ertapenem, and doripenem) are β-lactams with a structure derived from the natural product thienamycin.[21] This class received much attention due to its broad spectrum potency notably toward gram negative and anaerobic bacteria, its stability to clinically significant β-lactamases, and its rapid, bactericidal activity.[22] The penicillins and cephalosporins contain fused bicyclic structures, and are suicide substrates reminiscent of D-Ala-D-Ala, the substrate for the 4–3 transpeptidation reaction, as originally suggested by Tipper and Strominger (Fig. 2).[6] The saturated five-membered thiazolidine ring of the penicillins and the saturated six-membered dihydrothiazoline ring of the cephalosporins reveal common precursors in valine and cysteine. While the differences in core structure and *N*-acyl substitution account for differences in reactivity, β-lactamase stability, and spectrum of activity, the putative targets of these compounds are still D,D transpeptidases. The structural similarity of the carbapenem core, the hydroxyethyl and sulfide-linked proline sidechains excepted, suggested that the carbapenems also inhibited the D,D transpeptidases. This fact was corroborated in several studies,[23–25] however recent evidence suggests the L,D transpeptidases are another possible target.

The recent discovery that the peptidoglycan of stationary phase cultures of *M. tuberculosis* contains up to 80% 3–3 crosslinks contrasted strikingly with the traditional view of mycobacterial peptidoglycan and strongly supported the notion that the L,D transpeptidases contribute to the resistance to the β-lactam antibiotics.[26] More intriguingly, a putative L,D transpeptidase, *Mtb* Rv0116c, (Ldt_{Mtb}), was inhibited by meropenem, and from studies conducted in our laboratory, meropenem demonstrated efficacy against extensively drug resistant (XDR)-TB when co-administered with the β-lactamase inhibitor clavulanic acid.[27] This combination



Scheme 2. Synthesis of a biotinylated carbapenem.

was also active against nonreplicating bacilli, suggesting an essential remodeling or recycling function of these enzymes in metabolically static bacteria. With the immediate goal of identifying the protein targets of the carbapenems in whole cells of *M. tuberculosis*, we undertook the synthesis of two labeled forms of meropenem for use as probes.

The synthesis of ^{14}C labeled meropenem (**1**, Scheme 1) utilizes bicyclic intermediate **2** (prepared in 2 steps from *trans* 4-hydroxy-L-proline)[28,29] which is synthesized in kilogram quantities for the production of Merrem®. From **2**, the synthesis was optimized at milligram scale for introduction of the radiolabel. Unlabeled intermediates were prepared as TLC standards and spectral data are contained in the supporting information. A 250 μCi sample of ^{14}C-labeled Me_2NH–HCl (4.0 mg) was obtained from American Radiolabeled Chemicals specially prepared in acetonitrile. This reagent was treated with DIPEA (18 μL, 107 μmol) and **2** (16 mg, 52 μmol) for 2 h at 0 °C. The solution was subsequently treated with unlabeled Me_2NH–HCl (4.4 mg, 54 μmol), DIPEA (18 μL, 107 μmol) and stirred for an additional 2 h at 0 °C. While there are reports of in situ opening of **2** and coupling to **4**, we purified the thiol intermediate **3** by flash chromatography (SiO_2, 5% MeOH–CH_2Cl_2). Labeled **3** was coupled to the carbapenem enol phosphate **4** (35 mg, 59 μmol, Bosche Scientific) with DIPEA (20 μL, 112 μmol) in acetonitrile (0.5 mL, 3 h, 25 °C) to provide **5** (19 mg, 56% two steps). Hydrogenolysis of **5** (10 mg, H_2, 10% Pd/C, 1 atm, 2 h), in a biphasic (1:1, 2 mL) solvent system of ethyl acetate/aqueous potassium phosphate buffer (0.050 M, pH 7) followed by the removal of the catalyst by filtration through Celite, and washing of the aqueous layer with ethyl acetate (2 × 1 mL) provided an aqueous solution of labeled meropenem **1** which was pure by nonradiographic TLC (R_f = 0.25) 7:3 MeOH–CH_2Cl_2), radiographic TLC is contained in the supporting info. The aqueous phase containing the pure, labeled antibiotic was adjusted with additional phosphate buffer to a volume of 1.0 mL (29.3 μCi/mL, 22% radioactive yield) and was stored in 100 μL aliquots and frozen at −30 °C for future use.

We also considered the use of an alternative, nonradioactive label for utilization in a protein pull-down strategy (Scheme 2). Since the radiolabeled meropenem synthesis relied on the introduction of a labeled amine, it would be advantageous to introduce an alternative label in a similar fashion with the only constraints that the label be compatible with the carbapenem nucleus and not impart any significant steric demand. Biotin does not contain an amine handle, but previous reports documented its introduction by Curtius rearrangement of the carboxylic acid.[30] Treatment of biotin (1.0 g, 4.2 mmol) in *t*-BuOH (15 mL, distilled from CaH_2) with DPPA (1.0 mL, 4.6 mmol) and Et_3N (0.64 mL, 4.6 mmol) at 95 °C for 14 h provides *N*-Boc norbiotinamine (1.0 g, 74%). Deprotection of this compound (1.6 g, 5.1 mmol) in neat TFA (3.0 mL) at 25 °C provides the salt **7** isolated as a white solid (1.2 g, 87%). Insoluble in acetonitrile, the reaction of **7** (0.56 g, 1.7 mmol) with **2** (0.50 g, 1.6 mmol) in DMF (8.0 mL) with DIPEA (310 μL, 1.8 mmol) at 25 °C for 2 h provides the thiol **8** (0.450 g, 80%). Coupling of **8** (0.181 g, 0.347 mmol) to **4** (0.206 g, 0.347 mmol) with DIPEA (63 μL, 0.364 mmol) provided **9** (0.215 g, 71%). Hydrogenolysis (H_2, 10% Pd/C, 45 psi, 2 h) of **9** (0.233 g, 0.268 mmol) in the biphasic solvent system detailed above (5 mL ethyl acetate: 5 mL phosphate buffer) removed the *p*-nitrobenzyl ester and carbamate. In this case, the aqueous layer was collected, washed with ethyl acetate (2 × 5 mL), and concentrated in vacuo. The crude residue was dissolved in methanol, filtered to remove the precipitated salts, and concentrated again to provide the biotin-labeled meropenem **9** (111 mg, 75%) as a yellow solid, pure by ^1H NMR (supporting info). Studies to indentify the targets of meropenem in *M. tuberculosis* are underway. It is worth noting that ^{14}C and biotin-labeled meropenems offer tools to identify and characterize the targets of the carbapenems in other organisms.

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Supplementary data

Supplementary data (^1H NMR spectra and experimental information) associated with this Letter can be found, in the online version, at doi:10.1016/j.tetlet.2009.10.124.

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