Antibacterial activity of synthetic analogues based on the disaccharide structure of moenomycin, an inhibitor of bacterial transglycosylase

Eugene R. Baizman, Arthur A. Branstrom, Clifford B. Longley, Nigel Allanson,† Michael J. Sofia,‡ David Gange and Robert C. Goldman§

Moenomycin is a natural product glycolipid that inhibits the growth of a broad spectrum of Gram-positive bacteria. In Escherichia coli, moenomycin inhibits peptidoglycan synthesis at the transglycosylation stage, causes accumulation of cell-wall intermediates, and leads to lysis and cell death. However, unlike Esc. coli, where 5–6 log units of killing are observed, 0–2 log units of killing occurred when Gram-positive bacteria were treated with similar multiples of the MIC. In addition, bulk peptidoglycan synthesis in intact Gram-positive cells was resistant to the effects of moenomycin. In contrast, synthetic disaccharides based on the moenomycin disaccharide core structure were identified that were bactericidal to Gram-positive bacteria, inhibited cell-wall synthesis in intact cells, and were active on both sensitive and vancomycin-resistant enterococci. These disaccharide analogues do not inhibit the formation of N-acetylglucosamine-β-1,4-MurNAc-pentapeptide-pyrophosphoryl-undecaprenol (lipid II), but do inhibit the polymerization of lipid II into peptidoglycan in Esc. coli. In addition, cell growth was required for bactericidal activity. The data indicate that synthetic disaccharide analogues of moenomycin inhibit cell-wall synthesis at the transglycosylation stage, and that their activity on Gram-positive bacteria differs from moenomycin due to differential targeting of the transglycosylation process. Inhibition of the transglycosylation process represents a promising approach to the design of new antibacterial agents active on drug-resistant bacteria.

Keywords: moenomycin, peptidoglycan synthesis, transglycosylation

INTRODUCTION

Bacterial peptidoglycan synthesis initiates in the cytoplasm with the synthesis of UDP-N-acetyl-muramyl-pentapeptide (UDP-MurNAc-pentapeptide). The MurNAc-pentapeptide is transferred via the mraY gene product to a C55 undecaprenol phosphate carrier lipid generating MurNAc-pentapeptide-pyrophosphoryl-undecaprenol (lipid I). N-Acetylglucosamine is then transferred from UDP-N-acetylglucosamine (UDP-GlcNAc) to lipid I via MurG, generating N-acetylglucosamine-β-1,4-MurNAc-pentapeptide-pyrophosphoryl-undecaprenol (lipid II). Lipid II is then polymerized into peptidoglycan by specific transglycosylases. These enzymes can be bifunctional, containing associated transpeptidase activity (e.g. PBP1A, PBP1B and PBP1C) or monofunctional (product of the mgt gene). Note that the monofunctional glycosyl-transferase was referred to as mgt (Di Berardino et al., 1996), but that this designation is used in the genome databases for the Mg" transport system, also referred to as corA. It now appears that databases are listing the
Moenomycin is a natural product inhibitor of bacterial transglycosylation and transpeptidation. It inhibits the transglycosylation reaction of Escherichia coli (van Heijenoort et al., 1987), but not the monofunctional transglycosylase from Staphylococcus aureus (El-Abadla et al., 1996). Although moenomycin is not as potent against Gram-positive bacteria (due to the outer-membrane barrier), its mode of action on whole cells is consistent with inhibition of cell-wall synthesis. Moenomycin causes rapid lysis of growing *E. coli* cells (van Heijenoort et al., 1987), but not stationary-phase cells. Lysis, but not killing, was inhibited by the addition of Mg²⁺, consistent with the functioning of the autolytic system in *E. coli*. UDP-MurNAc-pentapeptide and the lipid-linked precursors lipid I and II accumulated when *E. coli* was treated with moenomycin (Kohlrausch & Holte, 1991a). All of these data with *E. coli* are consistent with the hypothesis that inhibition of transglycosylation leads to growth inhibition and killing even in the absence of lysis.

Much less is known about the effects of moenomycin on Gram-positive organisms. Given the poor activity of moenomycin on *E. coli* compared to Gram-positive bacteria, it seems likely that moenomycin evolved as an inhibitor of the much more sensitive Gram-positive organisms. Moenomycin does induce the vancomycin-resistance pathway in *Enterococcus faecalis* (Mani et al., 1998) and *Enterococcus faecium* (Lai & Kirsch, 1996), consistent with inhibition of cell-wall synthesis; however, there are few additional data on the biochemical and physiological consequence of exposure of Gram-positive organisms to moenomycin.

There is a growing need for new antibacterial agents that are active on drug-resistant Gram-positive pathogens. Our effort to discover novel analogues of moenomycin prepared by combinatorial synthesis (Kakarla et al., 1999; Sofia et al., 1999) led to identification of several related disaccharide analogues with unique antibacterial properties. Synthetic disaccharide analogues were identified that: (1) were bactericidal for Gram-positive organisms; (2) were active against antibiotic-resistant bacteria (including vancomycin-resistant enterococci); (3) killed growing, but not stationary-phase cells; and (4) inhibited peptidoglycan synthesis in intact *Ent. faecalis* cells.

### METHODS

**Strains and growth conditions.** *E. coli* ATCC 23226 was grown aerobically at 37 °C in a 10 l fermenter Brain Heart Infusion medium (BHI) supplemented with 0.1% (w/v) Casamino acids (BHI/CAA) plus 1% (w/v) glucose. The culture was grown exponentially to an OD₆₀₀ value of 6 (1 cm cell, Perkin Elmer Lambda 25 spectrophotometer) using aeration (1 l min⁻¹) with air/oxygen to maintain a dissolved O₂ concentration of 5%. The pH was maintained at 6.5. The culture was then cooled on ice, harvested by centrifugation at 6000 g for 20 min, and the cell pellet was stored at −80 °C. Cells were used for synthesis of peptidoglycan following permeabilization with ether as described below.

*E. coli* OV58 (pTA9) overexpressing MurG (Ikeda et al., 1992) was grown to an OD₆₀₀ of 1.0 at 37 °C in a 10 l batch fermenter in BHI/CAA using 1 l min⁻¹ aeration with air and 150 r.p.m. agitation. The pH was not controlled. The cells

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**Fig. 1.** Structure of moenomycin A and its disaccharide degradation product TS0514. The various structural units of moenomycin referred to in the text are labelled A–I.

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monofunctional glycosyltransferase as mtgA. This pathway in *Escherichia coli* was recently reviewed (Holte, 1998).

Moenomycin is a natural product inhibitor of bacterial peptidoglycan synthesis, acting at the stage of transglycosylation. Structurally it consists of a pentasaccharide chain linked to a C₂₅ hydrophobic tail, moenocinol, via a phosphoric acid diester and a glycerol acid unit (Fig. 1). Degradation studies reveal that some of the structural elements present in the parent molecule can be removed with retention of varying degrees of biological activity (for review see El-Abadla et al., 1999). Although trisaccharide analogues of moenomycin are potent inhibitors of the *in vitro* transglycosylation reaction in *Escherichia coli*, they are 50-fold less active as antibacterial agents against *Staphylococcus aureus* (El-Abadla et al., 1999). In contrast, specific disaccharide analogues maintain the ability to inhibit *E. coli* transglycosylase activity *in vitro*, while losing almost all (> 500-fold against *Sta. aureus*, and at least 700-fold against *Streptococcus pyogenes*) antibacterial activity (El-Abadla et al., 1999).

*E. coli* is the only organism used thus far in the study of the precise mechanism of antibacterial activity of moenomycin and its analogues. Moenomycin inhibits the *in vitro* transglycosylation reaction in *E. coli* (van Heijenoort et al., 1978; van Heijenoort & van Heijenoort, 1980) where the main cell-wall polymerization and cross-linking activity is due to penicillin-binding protein (PBP) 1B. The activity of PBP1A (and arguably PBP3; Goffin & Ghuysen, 1998; Adam et al., 1997) may also be sensitive to moenomycin-like inhibitors (Ishino & Matsuhashi, 1981; Matsuhashi et al., 1981), as may PBP1C (Schiffer & Holte, 1999), but these data are not as definitive as the data for inhibition of PBP1B. In any event, moenomycin does not appear to inhibit the monofunctional transglycosylase from *E. coli* (Di Berardino et al., 1996). Although moenomycin is not as potent against *E. coli* compared to Gram-positive organisms (due to the outer-membrane barrier), its mode of action on whole cells is consistent with inhibition of cell-wall synthesis. Moenomycin causes rapid lysis of growing *E. coli* cells (van Heijenoort et al., 1987), but not stationary-phase cells. Lysis, but not killing, was inhibited by the addition of Mg²⁺, consistent with the functioning of the autolytic system in *E. coli*. UDP-MurNAc-pentapeptide and the lipid-linked precursors lipid I and II accumulated when *E. coli* was treated with moenomycin (Kohlrausch & Holte, 1991a). All of these data with *E. coli* are consistent with the hypothesis that inhibition of transglycosylation leads to growth inhibition and killing even in the absence of lysis.

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**Fig. 1.** Structure of moenomycin A and its disaccharide degradation product TS0514. The various structural units of moenomycin referred to in the text are labelled A–I.
were chilled on ice and harvested by centrifugation at 6000 g for 20 min. Cells were washed once with 5 mM Tris/HCl, pH 8.0 (50 ml; g wet pellet wt-1), then resuspended in the same buffer to a concentration of 0.06 g wet wt ml-1. Membranes were prepared following lysis of cells at 20000 p.s.i. (138 MPa) with a French press. The lysate was centrifuged at 6000 g for 10 min, and the resulting supernatant was ultracentrifuged at 200000 g for 1 h. The pellet was resuspended in assay buffer and used as a source of enzymes for evaluation of lipid II formation (see below).

**Determination of MIC values.** The MIC of test compounds was determined in 96-well microtitre plates using twofold dilutions in BHI/CAA medium. Exponentially growing cells were diluted to approximately 5 x 10^5 c.f.u. ml^-1 and subjected to test compounds solubilized and serially diluted in DMSO. At a final concentration of 5% (v/v), DMSO had no effect on cell growth or viability. Following an 18 h incubation at 37 °C, the OD_{690} values were read on a microplate reader (Dynatec, model MR5000) immediately after plate mixing. The MIC was determined using the following criteria: (OD_{690} untreated cells – OD_{690} test concentration)/(OD_{690} untreated cells – OD_{690} media blanks) x 100 ≥ 90%.

**Determination of bactericidal activity.** Cells were grown in BHI/CAA at 37 °C and test compounds were added to exponentially growing cells (OD_{690} 0.08–0.1). Samples were incubated with shaking at 37 °C for 4 h, and viable counts determined over time by plating a dilution series in triplicate onto BHI/CAA agar plates that were incubated at 37 °C for 18–36 h prior to counting. Bactericidal activity was also determined using stationary cells prepared by overnight growth in BHI/CAA. Cell growth was shut off with either chloramphenicol (50 µg ml^-1), tetracycline (20 µg ml^-1) or moenomycin (10 µg ml^-1) added to the culture. Viable counts were determined at the time of growth shut-off, then challenge compounds were added. Viable bacteria remaining after a 4 h incubation at 37 °C with shaking were determined by plating for c.f.u.

**Preparation of ether permeabilized Esc. coli cells.** Esc. coli ATCC 23226 cells were exposed to diethyl ether as described (Mirelman et al., 1976; Vosberg & Hoffmann-Berling, 1971) with minor modifications. Frozen bacterial cell pellets (grown and harvested as described above) were thawed on ice, washed twice by centrifugation (8000 g for 10 min) in Basic Medium (Vosberg & Hoffmann-Berling, 1971), then permeabilized by a 1 min ether treatment. Cell suspensions were stored at -80 °C in the presence of DMSO (1%, v/v) in 1 ml aliquots of 5–10 mg protein ml^-1. A new aliquot was thawed immediately prior to use for each assay, washed with and resuspended in peptidoglycan polymerization assay buffer (see below).

**Purification of UDP-MurNac-pentapeptide from bacteria.** Ent. faecium strain MT10 Rev (Billot-Klein et al., 1997), or Bacillus cereus ATCC 11778 was grown aerobically in a 10 l fermenter in BHI/CAA medium. The substrate UDP-MurNAc-pentapeptide, containing either L-lysine or meso-diaminopimelic acid, was isolated from these cells as described (Kohlrausch & Holtje, 1991b), with minor modifications. The concentration of purified nucleotide sugars was determined using the molar extinction coefficient of uridine, \(\varepsilon_{262} = 1 \times 10^4\). The UDP-MurNAc-pentapeptides were authenticated by mass spectrometry, and lyophilized for storage at -80 °C.

**Biotin labelling of UDP-MurNAc-pentapeptide.** UDP-MurNAc-pentapeptide (containing L-lysine) was labelled (Men et al., 1998) using solid suIo-NHS-LC Biotin (Pierce). The UDP-MurNAc-pentapeptide-biotin conjugate was purified by HPLC using a Supelcosil C-18 (46 mm x 25 cm) column eluted at 10 ml min^-1 with an elution profile of potassium phosphate (10 min), water (5 min), followed by a 30 min linear gradient to 50% methanol/water (v/v) (Bransstrom et al., 2000b).

**Peptidoglycan polymerization assay.** Peptidoglycan synthesis was determined in ether-treated bacteria (ETB) as described (Allen et al., 1992, 1996), with modifications for adaptation to automated liquid handling equipment. GFC filter-bottom 96-well microplates (Millipore) were used throughout. Assay buffer was prepared fresh daily from 10 x stock solutions, and contained: 50 mM Tris/HCl (pH 8.3); 50 mM NH_4Cl; 20 mM MgSO_4; 0.15 mM d-aspartic acid, 100 µg tetacycline ml^-1 and 0.5 mM β-mercaptoethanol (reagents from Sigma Chemical). Reaction mixtures (100 µl) also contained: 5 mM ATP (Tris salt), 1.0 mM UDP-N-acetyl-L[3-¹⁴C]glucosamine [UDP-GlcNAc, DuPont/NEN or Amersham; 200–300 mCi (74–111 GBq) mmol^-1], and 15–25 µM UDP-MurNAc-pentapeptide (containing meso-diaminopimelic acid). Assay buffer (10 µl), ATP (20 µl), UDP-MurNAc-pentapeptide (10 µl) and UDP-[¹⁴C]GlcNAc (20 µl) were mixed and added as a single 60 µl aliquot to all wells with a Tecan Genesis 150 liquid handler, followed by either 20 µl test compound, reference standard or buffer/vessel. Reactions were initiated by adding 20 µl ETB suspension (25–30 µg bacterial protein well^-1). Plates were covered, mixed and incubated for 120 min at 37 °C. A 100 µl aliquot of ice-cold 20% (w/v) TCA was added to terminate the reaction, and plates were held at 4 °C for 30 min to ensure complete precipitation of peptidoglycan. Each plate was then filtered under vacuum and washed rapidly 5–6 times with 200 µl 10% (w/v) ice-cold TCA. A 30 µl aliquot of Optiphase Supermix (EG & G Wallac) was added to each well for overnight equilibration and plates were counted in a Microbeta TriLux LSC (model 1450; EG & G Wallac). Reaction blanks were defined as corrected c.p.m. retained on the filter either in the absence of UDP-MurNAc-pentapeptide or in the presence of a concentration of reference compound (moenomycin) completely suppressing incorporation of radiolabel. Concentration-response curves were analysed by non-linear regression using a four-parameter logistic model fitted and plotted with GraphPad Prism v. 2.01, GraphPad Software. IC_{50} values given in the text represent means from 2–10 separate experiments, each curve using at least 5–7 concentrations of test compound in duplicate wells.

**Effect of drugs on peptidoglycan degradation.** The stability of peptidoglycan made during various time periods of the reaction using ether permeabilized Esc. coli cells was examined in the presence of the disaccharide analogues. Reactions were set as given above, and initiated by addition of warmed ether permeabilized cells (20 µl, 30 µg protein per well) to all reaction wells. Vehicle or test compound (20 µl) was then added sequentially to appropriate wells at \(t_0\) (120 min incubation with drug), \(t_{30}\) min (90 min incubation with drug), \(t_{45}\) min (60 min incubation with drug), \(t_{60}\) min (30 min incubation with drug) and \(t_{120}\) min (30 min incubation with drug). At \(t_{120}\) min, reactions were terminated by addition of 100 µl 20% (w/v) TCA to all wells, followed by shaking for 30 s on an orbital plate shaker. Samples were then processed (see above) and radioactivity incorporated into peptidoglycan determined as described above.

**Peptidoglycan synthesis in intact cells.** Ent. faecalis ATCC 29212 was grown in BHI/CAA medium at 37 °C and used to measure incorporation of radiolabelled lysine into peptidoglycan as described by Allen et al. (1996) with minor modifications for adaptation to microtitre filtration. Briefly,
this involved treatment of cells with 50 μg tetracycline ml⁻¹ plus 100 μg chloramphenicol ml⁻¹ for 30 min to inhibit protein synthesis. Varying concentrations of drugs were added to cells, and reactions were initiated by addition of [³⁵S]lysine (0-25 μCi (9.25 kBq) per reaction, 329 mCi (12.2 GBq) mmol⁻¹; Amersham). Reactions were stopped, samples processed and data analysed as described above for the peptidoglycan polymerization assay. Incorporation was linear for 60 min, and 15000–20000 d.p.m. were incorporated into control samples.

Inhibition of transglycosylation and accumulation of lipid intermediates. Inhibition of mature (cross-linked) and immature (nascent) peptidoglycan, and accumulation of lipid intermediates was determined as described (Ge et al., 1999). Briefly, this method uses 100 μg penicillin G ml⁻¹ to inhibit cross-linking of peptidoglycan into mature strands, and follows accumulation of lipid intermediates by extraction into butanol- pyridinium acetate.

Lipid II formation assay. The assay was performed as described by Branstrom et al. (2000b). Reaction components consisted of bacterial membranes (25 μg protein), 0.5 μM UDP-[¹⁴C]GlcNAc (≈ 20000 d.p.m. (333 Bq)), 10 μM biotinylated UDP-MurNAc-pentapeptide and 0.1% (v/v) Triton X-100, all in 50 μl assay buffer (50 mM Tris/Cl, pH 8.0, 42 mM Mg(Ac)₂ and 208 mM KCl). Reactions were preincubated without radiolabelled UDP-GlcNAc for 10 min at room temperature to allow formation of lipid I and then lipid II formation was initiated by addition of UDP-GlcNAc. Lipid II synthesis was allowed to proceed for 15 min at room temperature before being terminated with 25 μl 1% (v/v) SDS. Incorporation of labelled GlcNAc continued for 40–50 min in control reactions. Lipid II was identified by mobility using paper chromatography (van Heijenoort et al., 1992) and specific capture with Softlink avidin resin (Promega) (see below).

Streptavidin-bead capture. Capture of biotinylated lipid II was as previously described by Branstrom et al. (2000b). Binding buffer (10 mM Tris/HCl, pH 8.0, 150 mM NaCl, 0.2% v/v Triton X-100) either 500 μl or 100 μl was added to Eppendorf tubes or 96-well filter plates, respectively, containing lipid II reaction mixtures. Tetralink (Promega) tetramer avidin resin (35 μl as supplied) was added to each reaction to allow for capture of product. For the tube assay, samples were gently mixed for 1 h at room temperature, centrifuged for 3 min at 1500 g, and then washed four times with 500 μl binding buffer. Reactions run in filter plates were vacuum filtered and washed five times with 200 μl binding buffer. The washed beads from Eppendorf tubes were resuspended in buffer, mixed with scintillation cocktail, and counted in a scintillation counter to determine the amount of lipid II product made. Softlink monomeric avidin resin (Promega) was substituted for Tetralink resin when the captured product needed to be released for further analysis by paper chromatography (Anderson et al., 1966).

Polymerization of lipid II into peptidoglycan. Polymerization of lipid II into peptidoglycan was monitored in situ (Branstrom et al., 2000a). The first step of this assay allowed synthesis and accumulation of lipid II in a reaction mixture containing Esc. coli membranes (0.5 mg ml⁻¹), UDP-MurNAc-pentapeptide (20 μM) and radiolabelled UDP-GlcNAc (0.5 μM) in reaction buffer (50 mM Tris; pH 8.0; 42 mM Mg(Ac)₂; 208 mM KCl; 0.1% v/v Triton X-100; and 10% v/v DMSO). Reactions were incubated for 2 h at room temperature to accumulate endogenous lipid II. Inhibitors were added prior to removal of Triton X-100 by the addition of Detergent-Out resin (Geno-Technology). Reactions proceeded for an additional 2 h at room temperature to allow conversion of lipid II into peptidoglycan, which was monitored by ascending paper chromatography (Anderson et al., 1966).

Effect on Candida albicans growth and glucan synthesis. Candida albicans strain CCH442 was grown in BHI/CAA to OD₅₀₀ 0.1, and drugs added. Growth and viable counts were monitored. Microsomes were prepared as previously described for assay of 1,3-β-glucan synthesis (Frost et al., 1994), monitoring incorporation of [³⁵S]glucose from UDP-glucose into glucan.

In vitro cytotoxicity. LD₅₀ values for cytotoxicity of moenomycin and the synthetic disaccharide analogues were determined in three mammalian cell lines– NIH3T3, HL-60 and HBL-100. Test compounds diluted in OptiMEM (Gibco-BRL), or medium alone were incubated for 6 h at 37 °C. Medium was removed and cells were incubated with fresh medium for 18 h. A Cytolux (EG & G Wallac) luminescent assay kit and Wallac Trilux plate reader were used to screen for cell viability as described by the manufacturer. At least eight concentrations of test compound were used to determine the LD₅₀ computed by a four-parameter logistic fit of percentage cell viability versus drug concentration.

Antibiotics. Moenomycin A was isolated and purified by standard preparative HPLC from commercial sources (Flavomyces; Hoechst). Reference standard antibiotics were purchased from Sigma. All synthetic disaccharide analogues were prepared at Incara Research Laboratories as described by Soha et al. (1999).

RESULTS

Antibacterial activity of moenomycin analogues

Moenomycin possesses potent antibacterial activity against Gram-positive bacteria, but is much less active against Gram-negative bacteria (van Heijenoort et al., 1987). However, moenomycin is quite active against Esc. coli B5A849, containing a defect in outer-membrane structure (Table 1). This strain of Esc. coli was isolated as a mutant hypersusceptible to antibiotics (Sampson et al., 1989). The disaccharide product of moenomycin degradation, TS0514 (Fig. 1), was devoid of antibacterial activity (MIC > 100 μg ml⁻¹) as reported by El-Abadla et al. (1999; see p. 706 therein) in their later assays.

Starting with the basic lipid-linked disaccharide moenomycin core, we initiated a programme to produce synthetic disaccharide analogues that regained antibacterial activity. Analogues TS30153, TS30663 and TS30888 (Fig. 2) are related in structure to the moenomycin E-F disaccharide TS0514 (Fig. 1). All contain a lipid anchor (C₁₄ lipid), and possess a β-linked disaccharide similar to the moenomycin E-F disaccharide. They differ from the moenomycin disaccharide by the substitution of a nitrogen functionality in place of the C₃-sugar of moenomycin and the absence of the C₄-methyl group of the F-sugar. In addition, TS30153 replaces the moenomycin glucosamine E-unit with a galactosamine sugar. All contain aromatic moieties attached to the saccharide units (units E and F, see Figs 1 and 2). The disaccharide analogues were active on Gram-positive bacteria (Table 1) and two showed
Table 1. MIC values (µg ml⁻¹) for moenomycin analogues and reference compounds

MICS were determined by microbroth dilution in BHI/CAA media on two or more occasions, and were identical or within twofold. Note: TS30153, TS30663 and TS30888 are given as compounds 23, 21 and 18, respectively, by Sofia et al. (1999). MIC values for TS0514 (moenomycin disaccharide degradation product) on these strains was >100 µg ml⁻¹ (from El-Abadla et al., 1999).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Esc. coli BAS 849*</th>
<th>Ent. faecalis ATCC 29212</th>
<th>Ent. faecalis ATCC 49624</th>
<th>Sta. aureus ATCC 29213</th>
<th>Sta. epidermidis ATCC 12228</th>
<th>Ent. faecalis CL 4877 (VanB)</th>
<th>Ent. faecalis CL 5244 (VanB)</th>
<th>Ent. faecium RLA†</th>
<th>Ent. faecium CL 4931 (VanA)</th>
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<tbody>
<tr>
<td>TS30663</td>
<td>12.5</td>
<td>6.25</td>
<td>6.25&lt;12.5</td>
<td>6.25&lt;12.5</td>
<td>3.13</td>
<td>3.13</td>
<td>6.25</td>
<td>6.25</td>
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<tr>
<td>TS30888</td>
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<td>6.25</td>
<td>12.5</td>
<td>6.25</td>
<td>25</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0.78–1.56</td>
<td>3.12</td>
<td>0.39</td>
<td>1.56</td>
<td>1.56</td>
<td>1250</td>
<td>15–25</td>
<td>0.78</td>
<td>&gt;1250</td>
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<tr>
<td>Moenomycin</td>
<td>0.025</td>
<td>0.078</td>
<td>&gt;200</td>
<td>0.05</td>
<td>0.025–0.05</td>
<td>0.13</td>
<td>0.06</td>
<td>0.78</td>
<td>0.25</td>
</tr>
</tbody>
</table>

*Strain BAS 849 is supersensitive to antibiotics.
†Strain RLA1 is ampicillin resistant.

Bactericidal activity of moenomycin and disaccharide analogues

Our studies revealed that moenomycin had differential bactericidal activity on Gram-negative and Gram-positive bacteria. Moenomycin is bactericidal to Esc. coli and will cause rapid cell lysis (van Heijenoort et al., 1987). Killing was also observed in the absence of lysis when Mg²⁺ was added to cells to prevent autolysis. We obtained similar results with our hypersusceptible strain of Esc. coli (data not shown). The survival frequency was 3·3×10⁻⁴ and 2·1×10⁻⁵ in the presence and absence of Mg²⁺, respectively, 4 h after treatment with 0·25 µg moenomycin ml⁻¹ (10 times the MIC level of drug).

In contrast, moenomycin showed only slight bactericidal
activity on Gram-positive bacteria. Moenomycin gradually inhibited growth of *Ent. faecalis* at concentrations ranging from 4 to 600 times MIC, and the optical density gradually increased threefold by 4 h after drug addition (data not shown). *Sta. aureus* and *Sta. epidermidis* showed slightly faster growth shut-off occurring 1 h after treatment with moenomycin at 10 or 100 times the MIC, and the optical density declined slightly between 1 and 4 h post-drug addition (Fig. 3). The timing of growth shut-off was similar at 100 times MIC, but the decline in optical density was slightly faster (data not shown).

In addition to moenomycin being weakly bactericidal to Gram-positive bacteria, the degree of bactericidal activity was also dependent on the species tested. Less than 1 log unit of killing occurred after 4 h when *Ent. faecalis* strain RLA1 (Fig. 4b) or *Ent. faecalis* (not shown) were treated with up to 500 times the MIC of moenomycin. Similar data were obtained with *Sta. aureus* when treated with 10–100 times MIC of moenomycin (Fig. 4a and data not shown). We tested high levels of moenomycin to validate that the low level of killing was inherent to moenomycin, and not just dependent on the amount used. Three log units of killing occurred when *Sta. epidermidis* was treated with 100 times the MIC of moenomycin (Fig. 4a) and this was reduced to 2 log units of killing at 10 times MIC (data not shown). In contrast, TS30663 (Fig. 4a) killed 4–6 log units when Gram-positive bacteria were treated with four to eight times MIC for 4 h. Similar results were obtained with TS30153 and TS30888 (data not shown). The multi-drug-resistant *Ent. faecium* strain RLA1 (Fig. 4b) and the vancomycin-resistant *Ent. faecalis* strain CL5244 (not shown) were also killed 3–5 log units when treated with four to eight times MIC of the disaccharide analogue TS30663. *Ent. faecium* strain ATCC 49624 was resistant to moenomycin (Table 1) and thus not killed by moenomycin, but was killed by TS30663 (Fig. 4b).

Non-growing bacterial cells are characteristically re-
**Table 2. Effect of protein synthesis inhibition and moenomycin treatment on killing by disaccharide analogues**

c.f.u. ml\(^{-1}\) were determined at time 0 and the first drug was added (50 µg chloramphenicol ml\(^{-1}\), 20 µg tetracycline ml\(^{-1}\) or 10 µg moenomycin ml\(^{-1}\)). After growth shut-off due to the first drug, cultures were diluted in medium (to OD\(_{600}\) = 0-1) containing the same concentration of first drug and c.f.u. ml\(^{-1}\) were determined in triplicate. Challenge drug was then added (TS30153, TS30663) at 4–8 times MIC. c.f.u. ml\(^{-1}\) were determined again at 4 h after addition of challenge drug. Growing cells were also challenged with test drug (TS30153 and TS30663), 50 µg ml\(^{-1}\) at OD\(_{600}\) = 0-1 for 4 h, and survival frequency determined. Values in parentheses are from a repeat experiment.

<table>
<thead>
<tr>
<th>Drug treatment</th>
<th>Survival frequency*</th>
<th>Survival non-growing/growing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloramphenicol</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Chloramphenicol plus TS30153</td>
<td>0-81 (0-91)</td>
<td>2-7 \times 10^4 (1-1 \times 10^4)</td>
</tr>
<tr>
<td>Chloramphenicol plus TS30663</td>
<td>0-52</td>
<td>&gt; 5-2 \times 10^3</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Tetracycline plus TS30153</td>
<td>0-26 (0-38)</td>
<td>8-7 \times 10^3 (4-7 \times 10^3)</td>
</tr>
<tr>
<td>Tetracycline plus TS30663</td>
<td>0-32</td>
<td>&gt; 3-2 \times 10^3</td>
</tr>
<tr>
<td>Moenomycin</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Moenomycin plus TS30153</td>
<td>0-57 (1-1)</td>
<td>1-9 \times 10^4 (1-4 \times 10^4)</td>
</tr>
<tr>
<td>Moenomycin plus TS30663</td>
<td>0-12</td>
<td>&gt; 1-2 \times 10^5</td>
</tr>
<tr>
<td>TS30153 alone</td>
<td>3-0 \times 10^{-5}</td>
<td></td>
</tr>
<tr>
<td>TS30663 alone</td>
<td>&lt; 1-0 \times 10^{-6}</td>
<td></td>
</tr>
</tbody>
</table>

*The viable cell count after treatment with the first drug (growth shut-off) was normalized to 1 and survival frequency determined for treatment with the challenge drug.

**Table 3. Inhibition of peptidoglycan synthesis**

Values in parentheses are 95% confidence intervals from data for 2–10 separate experiments, using duplicate wells for each compound concentration in all experiments.

<table>
<thead>
<tr>
<th>Compound</th>
<th><em>In vitro</em> peptidoglycan synthesis*</th>
<th><em>In vitro</em> peptidoglycan synthesis†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC(_{50}) (µg ml(^{-1}))</td>
<td>IC(_{50}) (µM)</td>
</tr>
<tr>
<td>TS30153</td>
<td>15-2 (9-1–17-4)</td>
<td>14-3</td>
</tr>
<tr>
<td>TS30888</td>
<td>6-9 (6-3–7-5)</td>
<td>6-7</td>
</tr>
<tr>
<td>TS30663</td>
<td>10-6 (8-7–12-8)</td>
<td>9-8</td>
</tr>
<tr>
<td>Moenomycin</td>
<td>0-025 (0-017–0-036)</td>
<td>0-0156</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>5-31 (4-44–6-36)</td>
<td>3-66</td>
</tr>
</tbody>
</table>

*Measured as incorporation of UDP-[\(^{14}\)C]GlcNAc into peptidoglycan using ether-permeabilized *Esc. coli*. Incorporation of label reached 5–10% and represented 3000–6000 d.p.m. (50–100 Bq) in the control sample.
†Measured as incorporation of [\(^{14}\)C]lysine into peptidoglycan in *Ent. faecalis* over the linear range of 60 min. Control values were 15000–20000 d.p.m. (250–333 Bq).

Sistant to killing by cell-wall synthesis inhibitors. Accordingly, stationary-phase cells were resistant to killing by the disaccharide analogues, yielding survival frequencies of 0-18–0-41 that were 10^2–10^4 greater than survival frequencies for growing cells (data not shown). In addition, the bactericidal activity of disaccharide analogues was abolished when bacteria were pretreated with tetracycline, chloramphenicol or moenomycin (Table 2). Tetracycline and chloramphenicol inhibited growth, though both caused minimal killing by themselves (< 1 log unit). Bactericidal activity of the disaccharide analogues was abolished in chloramphenicol- and moenomycin-pretreated cells, and nearly abolished in tetracycline-pretreated cells by factors of 10^2–10^6 compared to growing cells.

**Inhibition of peptidoglycan synthesis in permeabilized *Esc. coli* cells**

The disaccharide analogues TS30663, TS30153 and TS30888 all inhibited peptidoglycan synthesis in ether-permeabilized *Esc. coli* cells (Table 3) with IC\(_{50}\) values of
Fig. 5. Effect of moenomycin and disaccharide analogues on lipid II formation and peptidoglycan synthesis in *E. coli*. Bacterial membranes were incubated with UDP-MurNAc-pentapeptide (biotinylated on lysine) and UDP-[14C]GlcNAc to form lipid II and subsequent peptidoglycan (a). Lipid II was synthesized and accumulated in the presence of 10 µg moenomycin ml⁻¹, but inhibition of transglycosylation blocked peptidoglycan formation (b). Similarly, lipid II formation occurred in the presence of 100 µg TS30153 ml⁻¹, while peptidoglycan synthesis was inhibited (c). Reaction products were separated by ascending paper chromatography.

Fig. 6. Effect of disaccharide analogues on stability of peptidoglycan. Ether-permeabilized *E. coli* were incubated with UDP-MurNAc-pentapeptide and UDP-[14C]GlcNAc, in the presence of test compound added at different times following initiation of the reaction. Samples were processed in triplicate and the amount of [14C]GlcNAc incorporated into peptidoglycan determined. The reaction was linear for 120 min. Disaccharide analogues (100 µg ml⁻¹) were added at 0, 30, 60, 90 and 120 min following initiation of the reaction. All samples were terminated at 120 min. Buffer controls showed a constant level of incorporation. Peptidoglycan made during the first 30, 60 or 90 min was stable during the remainder of the incubation, as the expected level of incorporation was observed. Values in the figure represent means (±SE) of triplicate wells at each data point. Control (■); TS30153 (▲); TS30888 (▼).

10⁻⁶, 15⁻² and 6⁻⁹ µg ml⁻¹, respectively. This assay is sensitive to a range of known inhibitors (including moenomycin and vancomycin) of peptidoglycan synthesis (Table 3). Moenomycin is the most potent of these inhibitors on both a mass and molar basis with an IC₅₀ of 0.025 µg ml⁻¹ (0.016 µM). The disaccharide analogues were 400–600-fold (based on mass and molarity, respectively) less potent compared to moenomycin in their ability to inhibit peptidoglycan synthesis in ether-treated *E. coli*. However, the disaccharide analogues were of the same relative potency as vancomycin in this *in vitro* system. In all cases, complete inhibition of peptidoglycan synthesis at high doses of drug was documented by spotting a sample of the reaction mix for paper chromatography (Anderson et al., 1966) and determining the amount of radioactivity in peptidoglycan present at the origin.

**Inhibition of cell-wall synthesis in Ent. faecalis**

The disaccharide analogues also inhibited cell-wall synthesis in intact *Ent. faecalis* cells (Table 3) at concentrations near their MIC values. Vancomycin was used as a control compound that also inhibited incorporation of [14C]lysine into cell walls. The disaccharide analogues were within 3–10-fold of the activity...
of vancomycin on both a mass and molar basis in this assay (Table 3). Surprisingly, moenomycin itself did not show inhibition even when tested at 200 µg ml⁻¹.

**Lipid II and peptidoglycan synthesis using Esc. coli membranes**

Neither moenomycin, TS30153, TS30663, nor TS30888 inhibited formation of lipid II from UDP-MurNAc-pentapeptide and UDP-GlcNAc in an assay designed to measure only lipid II synthesis (see Branstrom et al., 2000b; data not shown). By contrast, in the same assay, tunicamycin (an inhibitor of MraY) inhibited lipid II formation by 50% at 1 µg ml⁻¹, as expected. We confirmed that peptidoglycan was in fact formed by Esc. coli membranes from biotinylated UDP-MurNAc-pentapeptide during a 2 h incubation (Fig. 5a). As expected, moenomycin, at 400 times its IC₅₀, inhibited peptidoglycan formation and caused accumulation of lipid II (Fig. 5b). TS30153 also inhibited peptidoglycan formation at six times its IC₅₀ (highest concentration testable), but not formation of lipid II (Fig. 5c), and no accumulation of lipid II relative to controls was observed. Similar results were obtained for TS30888 and TS30663 (data not shown). Lipid II was identified by mobility, incorporation of labelled GlcNAc and the presence of biotin (from UDP-MurNAc-pentapeptide) as assessed by capture with Softlink avidin resin (not shown).

**Direct analysis of inhibition of lipid II polymerization into peptidoglycan**

Inhibition of transglycosylation was monitored by following conversion of endogenous, radiolabelled lipid II into peptidoglycan. Moenomycin was a potent inhibitor with an IC₅₀ of 9·6 ng ml⁻¹ (95% confidence interval = 8·2–11·2 ng ml⁻¹). TS30663 also gave a typical sigmoidal concentration-response curve in the assay and an IC₅₀ value of 27·8 µg ml⁻¹ (95% confidence interval = 20·33–38·04 µg ml⁻¹). IC₅₀ values for TS30153 and TS30888 were estimated at 40 and 15 µg ml⁻¹, respectively, by single point analysis (data not shown).

**Analysis of potential peptidoglycan degradation**

The disaccharide analogues inhibited incorporation into peptidoglycan of radiolabelled [¹⁴C]GlcNAc from UDP-GlcNAc (Table 3). This could be due to either inhibition of peptidoglycan synthesis, or stimulation of peptidoglycan degradation. We thus examined the stability of newly synthesized peptidoglycan made during various time periods over the course of the 2 h incubation. Peptidoglycan synthesis was allowed to occur for various times, and then disaccharide inhibitors were added. Peptidoglycan made prior to disaccharide addition, even during the first 30 min, was stable during the remainder of the 2 h incubation (Fig. 6). These data show that the disaccharide analogues inhibit formation of peptidoglycan, but do not stimulate its degradation.

**Inhibition of mature and immature peptidoglycan in Esc. coli**

Like moenomycin, TS30663 inhibited synthesis of both mature and immature peptidoglycan (Fig. 7), consistent with inhibition at the transglycosylation stage. Whereas
moenomycin caused a 50% increase in butanol-extractable lipid II, only slight accumulation of lipid intermediates occurred with TS30663 treatment when penicillin was included to inhibit cross-linking of peptidoglycan.

C. albicans growth and glucan synthesis

Growth of C. albicans strain CCH442 was not altered by 50 µg disaccharide analogues ml\(^{-1}\) (data not shown). In addition, disaccharide analogues failed to inhibit L,3-β-glucan synthesis by microsomes from the same strain, when tested at 50 µg ml\(^{-1}\). Thus, the antimicrobial effects of these novel disaccharide analogues appeared specific for bacterial cell-wall synthesis.

In vitro cell viability

The LD\(_{50}\) for cytotoxicity in three mammalian cell lines (NIH3T3, HL-60 and HBL-100) averaged 15 µg ml\(^{-1}\) for TS30663 and >50 µg ml\(^{-1}\) for both TS30888 and TS30153. A concentration of 100 µg moenomycin ml\(^{-1}\) failed to exhibit significant effects on viability in any of these cell lines (data not shown). The differential antimicrobial effects that were observed at MIC levels for moenomycin versus novel disaccharide analogues reported in the present studies are, therefore, unlikely to be due to non-specific cytotoxicity.

DISCUSSION

Moenomycin is one of the few known inhibitors of the essential transglycosylation reaction catalysing polymerization of lipid II into bacterial peptidoglycan. Moenomycin has a broad spectrum of activity against Gram-positive bacteria, and is active in vivo in mouse models of bacterial infection (von Wasielewski et al., 1965). However, moenomycin itself has no clinical utility because it is poorly absorbed. It shows low systemic toxicity in animals and is used as an animal feed ‘growth promoter’ under the Hoechst trademark Flavomycin (Huber, 1979). The minimal structural requirements for antibacterial activity consist of a trisaccharide linked to a C\(_{25}\) hydrophobic tail, moenocinol, via a phosphoric acid diester and a glycerol acid unit, with the disaccharide (Fig. 2) being nearly devoid of antibacterial activity (El-Abadla et al., 1999). Essential components of the basic moenomycin structure likely interact with the donor or acceptor sites on the transglycosylase enzyme, thus inhibiting enzyme interaction with the normal lipid II substrate and/or nascent lipid-linked peptidoglycan. However, most of the studies investigating the mode of action of moenomycin on bacteria were conducted with the Gram-negative bacterium Esc. coli.

A programme was initiated based on synthesis of analogues of the moenomycin disaccharide structure (Kakarla et al., 1999; Sofia et al., 1999). Our rationale was that creating new sites of interaction with amino acid residues adjacent to the active site on a target enzyme could enhance even the weak activity reported for disaccharide analogues related to the moenomycin core structure (El-Abadla et al., 1999). Simplified lipids were also included in the synthetic scheme in place of the C\(_{25}\) moenocinol. Combinatorial chemistry, using synthetic strategies developed in our laboratory (Sofia et al., 1999; Kakarla et al., 1999) was used to explore chemical modification of the disaccharide core. Several active compounds were identified and three (TS30153, TS30663 and TS30888) were investigated in detail.

The three disaccharide analogues studied all possess antibacterial activity against Gram-positive bacteria, including strains resistant to vancomycin, with MIC values ranging from 3 to 12 µg ml\(^{-1}\). Since the moenomycin disaccharide degradation product (TS5014) is inactive as an antibacterial agent, our data demonstrate that appropriate derivatization of the E and F units can lead to antibacterial agents active on both sensitive and resistant bacteria. These disaccharide analogues are active on vancomycin-resistant strains and are nearly as potent as vancomycin on vancomycin-sensitive strains.

Almost all the data in the literature pertaining to inhibition of transglycosylase activity by moenomycin and its physiological consequences on bacteria, comes from the study of Esc. coli (Hara & Suzuki, 1984; Kohrausch & Holte, 1991a; Tamura et al., 1980; van Heijenoort et al., 1978, 1987; van Heijenoort & van Heijenoort, 1980). Moenomycin is rapidly bactericidal to growing Esc. coli, resulting in bacterial lysis and cell death, although lysis is not required for loss of viability. In contrast, our data reveal that the bactericidal activity of moenomycin is limited for Gram-positive bacteria. Only 1–2 log units of killing occurred when Gram-positive bacteria were treated with 5–10 times the MIC value of moenomycin. Killing was increased to 3 log units when the most sensitive species, Sta. epidermidis, was treated with 100 times the MIC of moenomycin. In contrast, the disaccharide analogues killed 3–6 log units when Gram-positive bacteria were treated with four to eight times the MIC. Thus, modifications to the disaccharide core resulted in the discovery of novel bactericidal analogues contrasting with the parent compound that had only bacteriostatic effects in Gram-positive bacteria.

Our data revealed that disaccharide analogues blocked peptidoglycan synthesis in ether-permeabilized Esc. coli at some point between the synthesis of lipid II and its polymerization into peptidoglycan by transglycosylation. This in vitro system requires the concerted action of MraY, MurG, lipid II translocation and recycling, and polymerization of lipid II into peptidoglycan. Evidence supporting inhibition of the transglycosylation stage by the synthetic disaccharide analogues is as follows. Neither the disaccharide analogues nor moenomycin inhibited lipid II synthesis in a system that depended on the functioning of MraY and MurG. However, both moenomycin and the disaccharide analogues did inhibit conversion of lipid II into peptidoglycan. Disaccharide analogues and moenomycin in-
hibited synthesis of both mature and immature peptido-
 glycan, data consistent with inhibition at the trans-
glycosylase stage. However, the disaccharide analogues
caused less accumulation of lipid II than moenomycin.
In addition, stimulation of peptidoglycan degradation
was ruled out, since peptidoglycan made during various
time periods in ether permeabilized Esc. coli was stable
following addition of the disaccharide inhibitors. Thus,
one may conclude that the site of inhibition is between
lipid II formation and its polymerization into peptido-
glycan via the transglycosylolation process.

The synthetic disaccharide analogues target cell-wall
synthesis in vivo, in intact Gram-positive bacteria, as
assessed by both direct and indirect experimental results.
They all inhibited incorporation of lysine into cell-wall
material in intact Ent. faecalis with IC_{50} values within
twofold of their respective MIC values on the same
strain. In addition, the disaccharide analogues were
bactericidal only to actively growing cells. Stationary-
phase cells and cells pretreated with protein synthesis
inhibitors or moenomycin were resistant to the bac-
tericidal effects of the disaccharide analogues. Such
differential killing is indicative of cell-wall synthesis
inhibitors. Although little is known about the stringent
response in Gram-positive bacteria, our data indicate
that protein synthesis may be required for killing caused
by the disaccharide analogues. Disaccharide analogues
did not inhibit growth of C. albicans CCH442, or synthesis of 1,3-β-glucan from microsomes prepared
from this strain (data not shown). The glucan-synthesis
complex is known to be sensitive to agents which non-
specifically perturb membrane structure (Goldman et al., 1995; Ko et al., 1994). Testing for overt cytotoxicity
at concentrations well above the MIC in several mam-
malian cell lines suggests that these novel compounds
(and moenomycin itself) are unlikely to induce non-
specific bacterial cell killing.

Our data are consistent with the following hypotheses
and current base of knowledge regarding bacterial
transglycosylases and their inhibition by moenomycin.
Moenomycin is not a universal transglycosylase in-
hibitor, since it does not inhibit the monofunctional
glycosyltransferase from Esc. coli (Di Berardino et al.,
1996; Hara & Suzuki, 1984), or the transglycosylase
activity from Micrococcus luteus (Park & Matsuhashi,
1984). Thus one would expect differential effects on
bacteria depending on (a) the repertoire of trans-
glycosylases inhibited, (b) the degree of inhibition and
(c) the physiological consequences of inhibition. The
pronounced bactericidal activity and subsequent rapid
lysis of Esc. coli following treatment with moenomycin
might result from inhibition of the transglycosylase
activity of PBP1A and PBP1B, but it may also inhibit
other transglycosylase enzymes such as PBP1C (Schiﬄer
& Holtje, 1999). Our data are consistent with the
hypothesis that these synthetic disaccharide analogues
of moenomycin target specific components of the
transglycosylation process in Gram-positive bacteria in
a unique manner not shared by the parent compound,
moenomycin. Perhaps moenomycin inhibits an essential
transglycosylase activity in Gram-positive bacteria, but
one that accounts for only a minor fraction of the total
peptidoglycan. Given the complexity of the interactions
between the biosynthetic components involved in
peptidoglycan synthesis (Holtje, 1996a, b, 1998; Koch,
1998; Vollmer et al., 1999; von Rechenberg et al., 1996),
further work will be required to understand the physio-
logical consequences of inhibition of the trans-
glycosylation process by different inhibitors. We are
currently using these and other synthetic disaccharide
analogues to clarify some of these processes.

ACKNOWLEDGEMENTS

The authors wish to thank Drs Nicole Hatzenbuhler, Dusan
Liu and Ramesh Kakarla for synthesis and puriﬁcation of the
novel moenomycin-based disaccharides and degradation
products reported in the text. We also thank Dr Helena
Axelrod for her guidance and contributions during the early
part of this project.

REFERENCES

Adam, M., Fraipont, C., Rhazi, N. & B others (1997). The
bimolecular G57-V577 polypeptide chain of the class B penicillin-
binding protein 3 of Escherichia coli catalyzes peptide bond
formation from thiolesters and does not catalyze glycan chain
polymerization from the lipid II intermediate. J Bacteriol 179,
6005–6009.

Allen, N. E., Hobbs, J. N., Jr, Richardson, J. M. & Riggin, R. M.
(1992). Biosynthesis of modiﬁed peptidoglycan precursors by
vancocycin-resistant Enterococcus faecium. FEMS Microbiol

peptidoglycan biosynthesis in vancocycin-susceptible and
resistant bacteria by a semisynthetic glycopeptide antibiotic.

Anderson, J. S., Meadow, P. M., Haskin, M. A. & Strominger, J. L.
(1966). Biosynthesis of the peptidoglycan of bacterial cell wall.
I. Utilization of uridine diphosphate acetylmuramyl pentapeptide
and uridine diphosphate acetylgulcosamine for peptidoglycan
synthesis by particulate enzymes from Staphylococcus aureus
and Micrococcus lysodeikticus. Arch Biochem Biophys 116,
487–515.

Billot-Klein, D., Shlaes, D., Bryant, D., Bell, D., Legrand, R.,
acetylmuramyl-hexapeptides and -heptapeptides in enterococci
and staphylococci after treatment with ramoplanin, tunicamycin,

assay for identifying inhibitors of bacterial transglycosylase.

Branstrom, A. A., Midha, S., Longley, C. B., Han, K., Baizman, E. R.
specific for bacterial MraY translocase and MurG transferase.
Anal Biochem 280, 315–319.

Di Berardino, M., Dijkstra, A., Stuber, D., Keck, W. & Gubler, M.
(1996). The monofunctional glycosyltransferase of Escherichia
coli is a member of a new class of peptidoglycan-synthesising
enzymes. FEMS Lett 392, 184–188.

El-Abadla, N., Lampilis, M., Hennig, L., Findeisen, M., Welzel, P.,
Muller, D., Markus, A. & van Heijenoort, J. (1999). Moenomycin
A: the role of the methyl group in the moenuronamide unit and a
general discussion of structure-activity relationships. Tetra-
Biol Rev Biochim Biophys Acta 62

murein hydrolases in

Inhibition of yeast (1,3)-β-glucan synthase by phos- 


