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# Differential Antibacterial Activity of Moenomycin Analogues on Gram-Positive Bacteria

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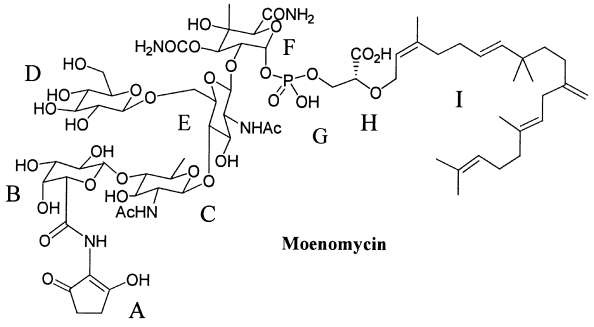
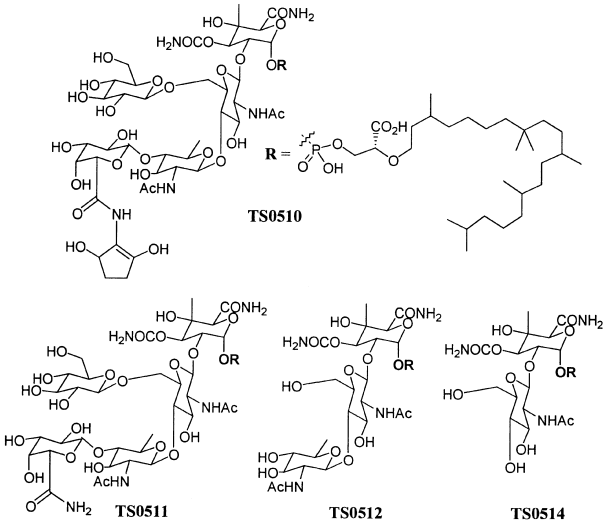
**Abstract**—The moenomycin trisaccharide degradation product and synthetic disaccharide analogues based on the disaccharide core were bactericidal to Gram-positive bacteria, inhibited lipid II polymerization, and inhibited cell wall synthesis in *Entercoccus faecalis*. Truncating moenomycin to the trisaccharide, and building upon the core disaccharide have both led to molecules possessing properties not shared with their respective parent structures. © 2000 Elsevier Science Ltd. All rights reserved.

The natural product moenomycin is the only well studied inhibitor of the essential transglycosylase reaction that polymerizes *N*-acetylglucosamine-β-1,4-MurNAc-pentapeptide-pyrophosphoryl-undecaprenol (lipid II) into bacterial peptidoglycan.[1]




However, most studies evaluating the biochemical mechanism of antibacterial activity were conducted with the Gram-negative bacterium *Escherichia coli*, which is rather insensitive to moenomycin due to the presence of the outer membrane. Moenomycin does lyse *E. coli* BAS849 (a supersensitive strain)[2] and is bactericidal, killing 4–5 logs. In contrast, it does not lyse Gram-positive bacteria, and only kills 0–2 logs depending on bacterial species.[3] In addition, degradation of moenomycin (following hydrogenation) revealed that the trisaccharide (lacking unit A, and sugar units B and D) was the smallest fragment that possessed antibacterial activity, and that the disaccharide (lacking units A, B, C, and D) inhibited the transglycosylase in vitro, but did not possess antibacterial activity even though an earlier report indicated that it did.[1]




We now demonstrate that although moenomycin, TS0510 (its hydrogenation product), and TS0511 (hydrogenation product lacking unit A) possess antibacterial activity, they only cause 0–2 logs killing of Gram-positive bacteria. In contrast, TS0512 (the trisaccharide containing units C and E–I) is more bactericidal to Gram-positive bacteria, and the inactive disaccharide can be chemically modified to yield novel compounds with antibacterial efficacy and bactericidal activity on Gram-positive bacteria.

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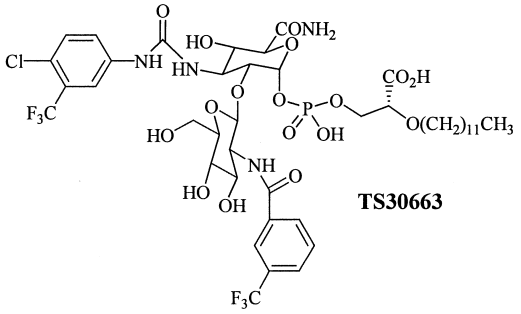
**Table 1.** Antibacterial activity[a]

| Compound | *E. coli* BAS849[b] | *E. faecalis* ATCC 29212 | *E. faecium* ATCC 49624 | *S. aureus* ATCC 29213 | *S. epidermidis* ATCC 12228 | *E. faecalis* CL4877 (Van B) | *E. faecalis* CL5244 (Van B) | *E. faecium* RLA1[c] | *E. faecium* CL4931 (Van A) |
|---|---|---|---|---|---|---|---|---|---|
| **Moenomycin** | 0.025 | 0.078 | >200 | 0.05 | 0.025 | 0.13 | 0.06 | 0.78 | 0.25 |
| **TS0510** | 0.31 | 0.08 | >25 | 0.16 | 0.31 | 0.25 | 0.25 | 0.25 | 0.78 |
| **TS0511** | 0.08 | 0.16 | >25 | 0.08 | 0.16 | 0.25 | 0.25 | 0.25 | 0.39 |
| **TS0512** | 0.31 | 0.78 | 25 | 0.63 | 0.53 | 1.56 | 1.56 | 1.56 | 1.56 |
| **TS0514** | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **TS30663** | 12.5 | 6.25 | 6.25 | 6.25 | 6.25 | 3.13 | 3.13 | 6.25 | 6.25 |
| **Vancomycin** | 0.78 | 3.12 | 0.39 | 1.56 | 1.56 | 1250 | 25 | 0.78 | >1250 |

[a]MICs were determined by microbroth dilution in BHI-CAA media and are given in μg/mL.
[b]*E. coli* BAS849 is supersensitive to antibiotics, due to outer membrane wall defects (see refs 2 and 3).
[c]Strain RLA1 is ampicillin resistant.

Thus, molecular trimming of moenomycin to the trisaccharide, and specific additions to the disaccharide both yield molecules with a unique spectrum of activity not shared with their respective parent structures.

We chemically degraded moenomycin following hydrogenation (yielding TS0510), giving TS0511 (lacking unit A), TS0512 (trisaccharide lacking unit A and sugars B and C), and TS0514 (disaccharide lacking unit A and sugars B–D). Purity of compounds was assessed by LC/MS.[4] We confirmed that all except the disaccharide possessed antibacterial activity (Table 1), as reported previously.[1] Our solid-phase synthetic combinatorial library using various disaccharide cores,[3,5,6] yielded compounds with a unique activity profile distinct from the moenomycin disaccharide degradation product.




**TS30663**

Unlike the inactive moenomycin disaccharide, the library derived disaccharide analogue TS30663 possessed antibacterial activity (Table 1), as did other related analogues.[3,6] Synthetic disaccharide analogues such as TS30663 also killed Gram-positive bacteria, while moenomycin killing was less effective and species dependent.[3,7] In addition, among the moenomycin degradation products that possessed antibacterial activity, TS0510 and TS0511 were also less effective at killing Gram-positive bacteria, and killing was again species dependent as it was for moenomycin (Table 2).

**Table 2.** Bactericidal activity of compounds

| Treatment | % Survival[a] | | |
|---|---|---|---|
| | *E. faecalis* | *S. aureus* | *S. epidermidis* |
| **Control** | 100 | 100 | 100 |
| **Moenomycin** | 143[b] | 125 | 0.48 |
| **TS0510** | 141 | 62 | 0.33 |
| **TS0511** | 176 | 61 | 0.40 |
| **TS0512** | 147 | 0.035 | 0.022 |
| **TS30663** | 0.00024 | < 0.00005 | < 0.00005 |

[a]Bacteria were treated with 10× the MIC for 4 h, and viable counts determined in triplicate by plating for colony-forming units.
[b]Growth was suppressed without killing.

In contrast, the moenomycin trisaccharide TS0512 was more effective at killing *S. aureus* and *S. epidermidis* (Table 2). Thus, degrading moenomycin to the trisaccharide, and building up the structure of the basic disaccharide (TS30663), both led to compounds with antibacterial properties not shared with their parent structures. Our synthetic disaccharide analogue also inhibited peptidoglycan synthesis in intact *Entercoccus faecalis* as did vancomycin and bacitracin, while moenomycin, TS0510 and TS0511 were inactive even when tested at 700 μM (Table 3). In contrast, the trisaccharide degradation product TS0512 not only killed Gram-positive bacteria more extensively than TS0510 and TS0511 (Table 2) but also inhibited peptidoglycan synthesis in intact *E. faecalis* cells (Table 3).

As expected,[1] moenomycin and all of its degradation products inhibited cell wall synthesis and transglycosylation in vitro using a system derived from *E. coli*. All of the moenomycin degradation products were within 10-fold of the molar potency of moenomycin for inhibition of peptidoglycan synthesis and the transglycosylation reaction (Table 3). To our knowledge this is the first time actual $IC_{50}$ values have been reported for inhibition of the transglycosylation reaction by moenomycin degradation products. Unlike the moenomycin disaccharide, synthetic disaccharide analogue TS30663 showed antibacterial activity (Table 1), inhibited peptidoglycan synthesis and had detectable inhibition of transglycosylation in vitro (Table 3). The enzymatic conversion of Lipid II to peptidoglycan by transglycosylase was performed as described[11] with modifications.[13]

We observed, as did others,[1] that the moenomycin disaccharide degradation product TS0514 was a potent inhibitor of peptidoglycan synthesis and polymerization of lipid II, but was inactive on the supersensitive *E. coli* strain BAS849. One possibility that has not yet been

**Table 3.** Inhibition of peptidoglycan synthesis in ether permeabilized cells, intact cells, and membranes

| | Inhibition of peptidoglycan synthesis[a] | | |
|---|---|---|---|
| | *E. coli* | *E. coli* | *E. faecalis* |
| Compound | $IC_{50}$ (μM)[b] | $IC_{50}$ (μM)[c] | $IC_{50}$ (μM)[d] |
| **Moenomycin** | 0.0156 | 0.031 | >700 |
| **TS0510** | 0.0081 | 0.098 | >700 |
| **TS0511** | 0.0086 | 0.087 | >700 |
| **TS0512** | 0.016 | 0.131 | 50.4 |
| **TS0514** | 0.033 | 0.241 | NA |
| **TS30663** | 9.8 | 100 | 2.5 |
| **Vancomycin** | 3.7 | 4.5[e] | 2.6 |
| **Bacitracin** | 0.65 | >77 | 28.3 |

[a]Values are the mean of two to 10 separate experiments.
[b]Measured as incorporation of UDP-[$^{14}$C]-GlcNAc into peptidoglycan using ether permeabilized *E. coli* cells.[3,8]
[c]Measured as incorporation of [$^{14}$C]-lipid II into peptidoglycan using *E. coli* membranes (see text), followed by filtration to separate substrate from product.[9]
[d]Measured as incorporation of [$^{14}$C]-lysine into peptidoglycan in intact cells after shut-off of protein synthesis with chloramphenicol and tetracycline.[10]
[e]Measured as incorporation of [$^{14}$C]-lipid II into peptidoglycan using *E. coli* membranes and paper chromatography to separate substrate from product.[1,11,12]

raised in the literature is that the stripped down disaccharide may be metabolized by bacteria via presentation of susceptible bonds not exposed in the larger degradation fragments TS0510, 0511, and 0512. All of these data combined most likely reflect the fact that multiple transglycosylases exist in bacteria, and thus differential targeting by moenomycin and its analogues may have different physiological effects.[14] Moenomycin does not inhibit the monofunctional transglycosylase from *E. coli*[9,15] or transglycosylase from *Micrococcus luteus*,[16] and thus, does not represent a universal transglycosylase inhibitor.

Moenomycin does inhibit the transglycosylase activity of penicillin binding protein (PBP) 1B[11,12] and 1C[17] from *E. coli*. It may also inhibit the activity of PBP2 and 3 from *E. coli*, but the data are not as complete as for PBP1B.[18,19] If moenomycin inhibits a transglycosylase in Gram-positive bacteria that synthesizes a small, but essential fraction of the peptidoglycan (e.g., a specific site at the septum), moenomycin would give little inhibition in the intact *E. faecalis* system, but would still function by inhibition of peptidoglycan synthesis. This same scenario could also explain minimal killing of Gram-positive bacteria by moenomycin and TS0510 and TS0511. Thus, both the bactericidal activity and ability to inhibit peptidoglycan synthesis in intact *E. faecalis*, could both be due to targeting key transglycosylases in Gram-positive bacteria.[14] These hypotheses would be best addressed by a combination of molecular genetic and biochemical experiments, work that is in progress in our laboratory.

In summary, derivatization of the basic moenomycin disaccharide core led to molecules with activity that differed from the moenomycin disaccharide and moenomycin itself, as did molecular dissection of moenomycin down to the trisaccharide TS0512. The synthetic disaccharide TS30663 was active on Gram-positive bacteria, and on *E. coli* that contains a mutation that renders it sensitive to antibiotics, including moenomycin, its degradation products, and vancomycin.

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4. TS0512 and TS0514 were assessed for purity by HPLC using a $C_{18}$ column (250×4.6 mm) coupled with light-scattering detection, followed by mass spectrometry (PE Sciex model API 100). Compounds were judged 96% and 90% pure, respectively, using a linear gradient of either methanol or acetonitrile in 10 mM $NH_4OAc$ buffer. TS0510 and TS0511 were analyzed with MS using direct infusion in MeOH/10 mM $NH_4OAc$. Moenomycin-A starting material (MW 1582) was not observed in any MS trace for these compounds. TS30663, resynthesized and purified similarly, was assessed >95%.
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13. Typical procedure: Reaction mixtures (40 μL) contained: 15 μL 50 mM Tris buffer pH 8.3 with 10 mM $MgCl_2$; 10 μL of *E. coli* OV58 (pUG 18) French press membranes (1 mg/mL);

10 μL test compound in DMSO; and 5 μL (23,000 dpm, 100 pmol) $^{14}$C-labeled Lipid II precursor in methanol. Reactions were incubated for 60 min at 37 °C, in 96-well Millipore hydrophilic PTFE filter-bottom microplates (Cat. No. MAR1), then all wells washed six times by vacuum filtration with 200 μL 0.4 M ammonium acetate in methanol. Scintillation cocktail was added and plates counted. Percent inhibition was calculated as: (Control$_{[untreated]}$ CPM−Test CPM)/(Control$_{[untreated]}$ CPM−Blank$_{[moenomycin\ treated]}$ CPM)×100. Concentration–response curves were analyzed and fit by a four-parameter logistic model and the $IC_{50}$ values determined. Each plate contained at least eight concentrations of test compound in duplicate wells.

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