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Communications to the Editor

Discovery of Novel Disaccharide Antibacterial Agents Using a Combinatorial Library Approach

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The increase in bacterial resistance to conventional chemotherapy has resulted in a resurgent interest in the discovery and development of antibacterial agents.[1−4] The search for novel antibiotics active against resistant phenotypes is increasingly focused on identification of novel chemotypes or antibiotics with novel mechanisms of action.[5,6]

The bacterial cell wall is an attractive target for developing novel antibacterial agents. The cell wall of both Gram-positive and Gram-negative bacteria is essential for cell viability. Of the many enzymes involved in bacterial cell wall biosynthesis, only transpeptidases responsible for cross-linking the growing glycan chain are targeted by existing clinically useful chemotherapeutic agents. As a strategy for developing novel antibacterial agents that would be effective against resistant phenotypes, we focused on the transglycosylase enzyme activity associated with the penicillin-binding proteins (PBPs). This activity functions to lengthen the peptidoglycan polymer and may also be required to initiate synthesis of a new chain. Our approach for developing novel inhibitors of transglycosylase focused on exploring moenomycin A (**1**) (Chart 1) as a lead.

The moenomycins are a family of natural product antibiotics which are known to inhibit the synthesis of bacterial cell wall peptidoglycan through inhibition of transglycosylase.[7−11] Moenomycin A is a pentasaccharide containing a long lipid attached to the reducing sugar (F) through a phosphoglycerate unit. By degradation studies and limited directed analogue synthesis, Welzel and co-workers showed that cell wall inhibitory activity was retained in a disaccharide core structure **2**.[12−22] In addition, they showed that certain structural elements were important for retaining transglycosylase inhibitory activity. The activity of moenomycin-derived disaccharides encouraged us to investigate the construction and screening of a library of disaccharides related to moenomycin A with the goal of identifying novel bacterial transglycosylase inhibitors.

Access to a library of moenomycin disaccharides required that we develop a general solid-phase synthetic strategy that would allow us to explore carbohydrate diversity and chemical diversity at sites known to be linked to biological activity. Welzel had shown that the carbamate at C-3, the amide at C-2′, and the phosphoglycerate moiety at C-1 were important in overall biological activity.[12−22] However, structural variations at these sites were never explored. The complexity of the moenomycin disaccharide system in combination with our desire to investigate multiple structural variations combinatorially posed a formidable synthetic challenge. Although solution syntheses of several moenomycin-type disaccharides had been reported by Welzel and others, these syntheses lacked the generality and efficiency necessary for constructing complex libraries.[12−22] In this paper, we describe the solid-phase synthesis of a library of moenomycin disaccharide analogues and the identification of novel antibacterial agents from this library.

Our basic synthetic approach envisioned constructing appropriately functionalized and protected disaccharide lactols that would allow us to explore modifications at C-1, C-3, and C-2′ and explore limited variation in the

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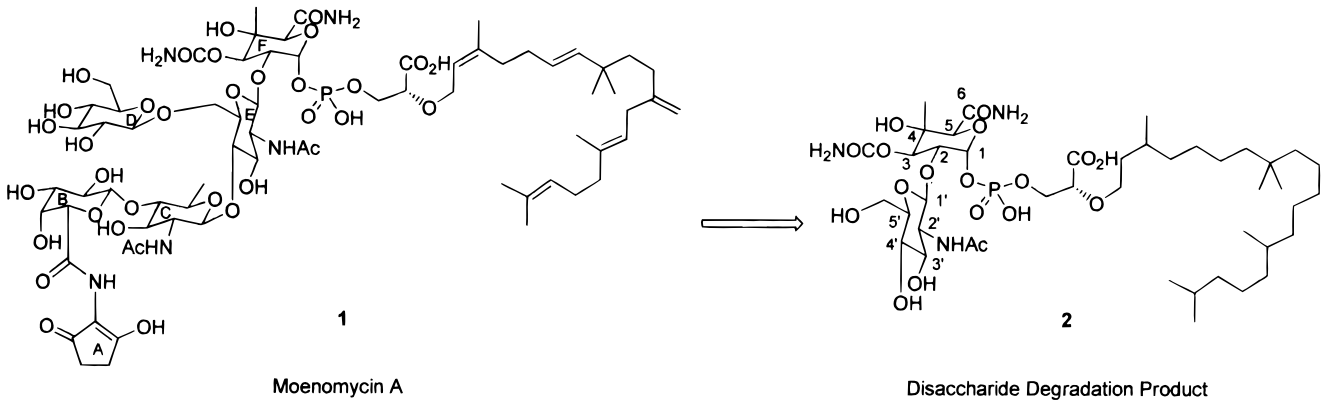
Chart 1



Scheme 1

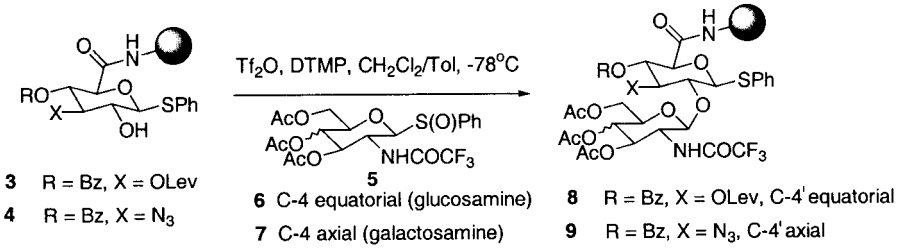
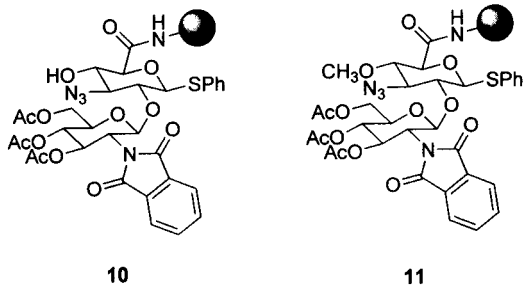


Chart 2



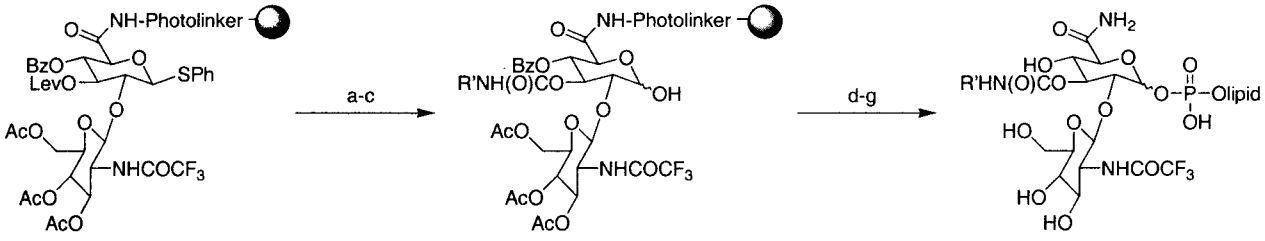
basic structure of the disaccharide core. This approach would also allow us to attach the potentially sensitive phospholipid side chain in the last functionalization step prior to deprotection and cleavage of the product from the resin. To construct functionalized lactols on the solid phase, we pursued both direct solid-phase glycosylation of resin-bound acceptor sugars followed by disaccharide derivatization and solid-phase derivatization of disaccharides preconstructed in solution and attached to the desired solid support. Although solid-phase glycosylation of acceptors **3** and **4** using glycosyl sulfoxide donors **6** and **7**[23] provided the disaccharides **8** and **9** (Scheme 1) in high yield (90−95%), with other acceptor−donor pairs, we were not able to completely eliminate the presence of monosaccharide byproducts. Attempts to achieve high yields of other target disaccharides by repeated exposure of resin-bound acceptors to glycosyl sulfoxide glycosylation were not always successful. Since we wanted to maximize product purity for screening without having to execute multiple parallel purifications, we ultimately chose to bypass the solid-phase glycosylation step and preconstructed the other target disaccharides **10** and **11** (Chart 2) using glycosyl bromide solution chemistry. In all cases, attachment to aminoethyl-photolinker AM resin[24] of either a monosaccharide acceptor or a disaccharide core occurred by amide bond formation through their respective C-6 carboxylate groups.

The solid-phase chemistry used to build our disaccharide library is outlined in Schemes 2 and 3. Each of the β-linked disaccharides utilized a base-labile protecting group (trifluoroacetamido[25] or phthalimido) on the C-2′ amino group not only for controlling β-stereochemistry at the glycosidic linkage but also as an easily removable group that would allow for further amine derivatization. To explore chemical diversity at the C-3 position of the reducing sugar, we employed either a 3-*O*-levulinate-protected sugar (Scheme 2) or a sugar containing an azido group at C-3 (Scheme 3). In the case of resin-bound disaccharide **12**, we were able to remove the levulinate protecting group under conditions that would not result in the loss of the other ester protecting groups (Scheme 2), thus allowing for regioselective derivatization with isocyanates to give carbamate derivatives.

For disaccharides containing a C-3 azido group (Scheme 3), we accomplished regioselective derivatization by first removing the base labile protecting groups on the phenyl thioglycoside intermediates **15** and then reacting the resulting free C-2′ amino group with carboxylic acids to provide the corresponding amides. Reacetylation followed by azide reduction provided the C-3 amine ready for reaction with isocyanates or acids to give the corresponding ureas or amides.

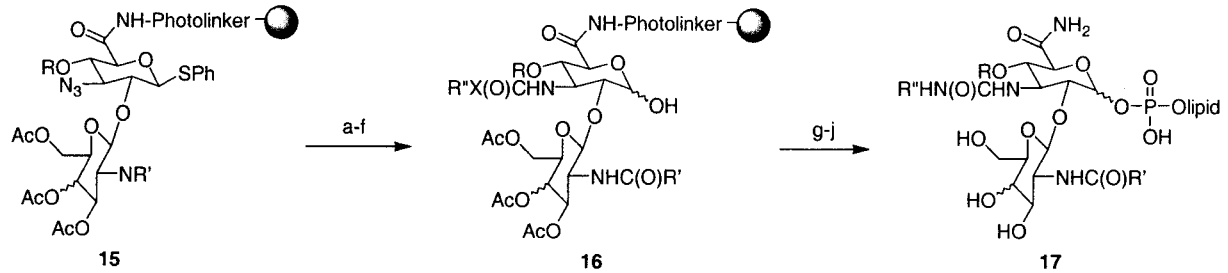
Each disaccharide was designed to contain an anomeric thiophenyl group at the reducing terminus. This thiophenyl group acted as a masked anomeric hydroxyl group. Therefore, after derivatization of the C-3 and C-2′ sites was accomplished, cleavage of the anomeric thiophenyl group produced the desired lactol intermediate **13** or **16** ready for attachment of the phospholipid unit. To attach the phospholipid, we relied on phosphoramidite chemistry using modified conditions to accomplish efficient solid-phase oxidation which gave the desired

Scheme 2[a]



[a] Reagents and conditions: (a) NH_2NH_2·AcOH; (b) R′NCO, DMF, rt, O.N.; (c) $Hg(OCOCF_3)_2$, CH_2Cl_2, rt, 1.5 h; (d) lipid amidite, tetrazole, CH_2Cl_2−THF, rt, 3 h; (e) O_2, THF, rt; (f) 0.1 M LiOH·H_2O/THF−MeOH (4:1), rt, 1 h; (g) hν_{365nm}, THF, O.N.

Scheme 3[a]



[a] Reagents and conditions: (a) 1 M hydrazine/THF or 0.5 M LiOH/THF−MeOH (1:1), rt, O.N.; (b) R′CO_2H, HATU, DIPEA, DMF, rt, O.N.; (c) Ac_2O, DMAP, CH_2Cl_2, rt, O.N.; (d) Me_3P, H_2O, THF−EtOH (1:1), rt, 2 h; (e) R″NCO, DMF, rt, 4 h, or R″CO_2H, HATU, DMF, rt; (f) $Hg(OCOCF_3)_2$, CH_2Cl_2, rt, 1.5 h; (g) lipid amidite, tetrazole, CH_2Cl_2−THF (1:1), rt, 3 h; (h) *m*CPBA, CH_2Cl_2, rt, 30 min; (i) 0.1 M LiOH/THF−H_2O−MeOH (7:2:1), rt, 1 h; (j) $h\nu_{365nm}$, THF, O.N.

Table 1. Inhibition of Bacterial Cell Wall Biosynthesis and Bacterial Growth on Antibiotic Sensitive and Resistant Strains

		MIC (μg/mL)[31]										
		sensitive strains					resistant strains					
compd	PGP[30] IC_{50} (μg/mL) X ± SE	*E. faecalis* ATCC 29212	*E. faecium* ATCC 49624	*S. aureus* ATCC 29213	*S. epi.* ATCC 12228	*E. coli* BAS849[a]	*S. aureus* ATCC 43300[b]	*E. faecium* CL4931 (VanA)[c]	*E. faecalis* CL5244 (VanB)[d]	*E. faecalis* CL4877 (Van B)[d]	*E. faecalis* ATCC 51575[d]	*E. faecium* ATCC 51559[c]
18	6.8 ± 0.3	12.5	12.5	12.5	12.5	25	25	25	12.5	6.25	25	12.5
19	9.2 (n = 2)	3.12	3.12	6.25	6.25	>25	3.12	6.25	6.25	3.12	6.25	3.12
20	8.2 (n = 2)	6.25	6.25	6.25	6.25	12.5	6.25	6.25	6.25	3.12	6.25	6.25
21	10.6 ± 1.1	3.12	6.25	12.5	12.5	12.5	12.5	6.25	3.13	3.13	12.5	6.25
22	15.4 ± 3.2	3.12	12.5	6.25	12.5	12.5	6.25	3.13	3.13	3.13	12.5	12.5
23	9.2 ± 3.9	6.25	6.25	6.25	6.25	>25	6.25	6.25	6.25	6.25	25	12.5
moenomycin	0.025 ± 0.014	0.078	>200	0.05	0.025	0.025	0.062	0.39	0.062	0.062	0.062	1.56
vancomycin	5.2 ± 0.08	6.25	0.78	3.13	3.13	0.78	3.13	>125	15.6	>125	>125	>125

[a] Super-sensitive permeability mutant of *E. coli*. [b] Methicillin-resistant *S. aureus*. [c] Vancomycin-resistant *E. faecium*. [d] Vancomycin-resistant *E. faecalis*.

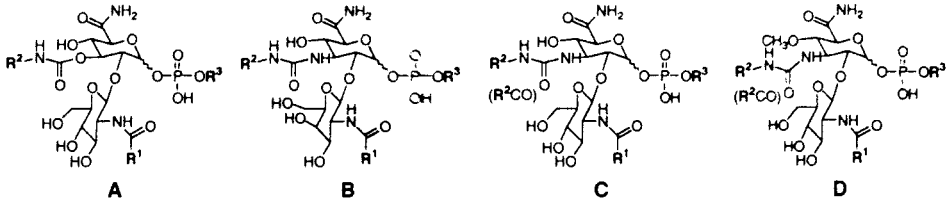
phosphate intermediate. Following treatment under basic conditions to remove all base labile protecting groups, photolytic cleavage provided the desired C-6 carboxamide disaccharides **14** and **17**.

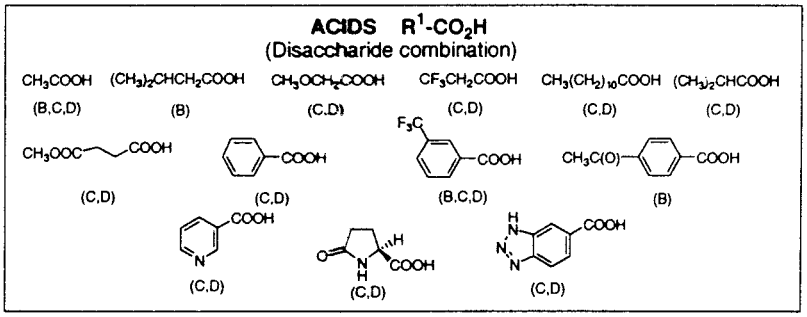
A library of 1300 disaccharides was prepared using the acid, isocyanate, and lipid building blocks shown in Chart 3.[26] The library was prepared using the IRORI technology for directed-sorting mix-and-split synthesis.[27] Each member of the library was obtained as a discrete product. The library was screened for both inhibition of bacterial cell wall biosynthesis and inhibition of bacterial growth. The bacterial cell wall biosynthesis inhibition assay was performed in a 96-well microplate format and used *E. coli* permeabilized cell membranes. This assay measured incorporation of exogenously added [^{14}C]-*N*-acetylglucosamine into bacterial peptidoglycan.[28,29] Inhibition of bacterial growth for a panel of both Gram-positive and Gram-negative microbes was initially determined using an agar lawn assay where approximately 2.5 μL of each compound solubilized in DMSO was applied to the agar in a microplate cover, using a 384 pin applicator, and the zones of inhibition of bacterial growth were determined. Initial screening identified compounds that inhibited both cell wall biosynthesis and bacterial growth at a screening concentration averaging 10 and 25 μg/mL, respectively. The maximum concentration of each compound testable in the lawn assay was based on library compound supply and DMSO vehicle interference.

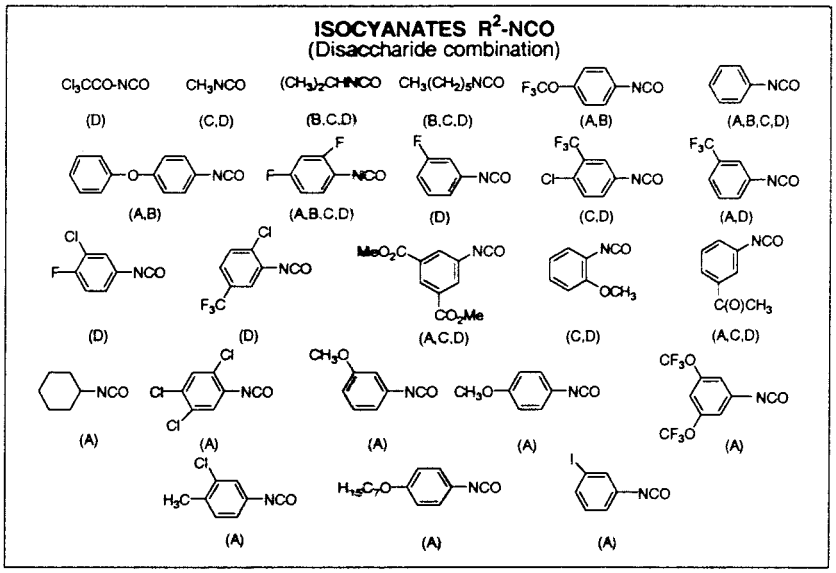
General analysis of the SAR for the library showed that the three disaccharide cores **9**−**11** provided compounds with both cell wall inhibition and whole cell antibacterial activity. When combined with substitutions at C-3 and C-2′, we were able for the first time to identify active moenomycin-like disaccharide analogues where the moenomycin glycerate-lipid unit was replaced

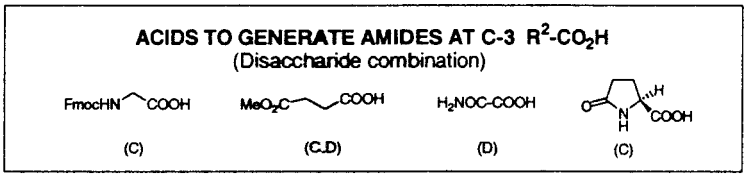
Chart 3

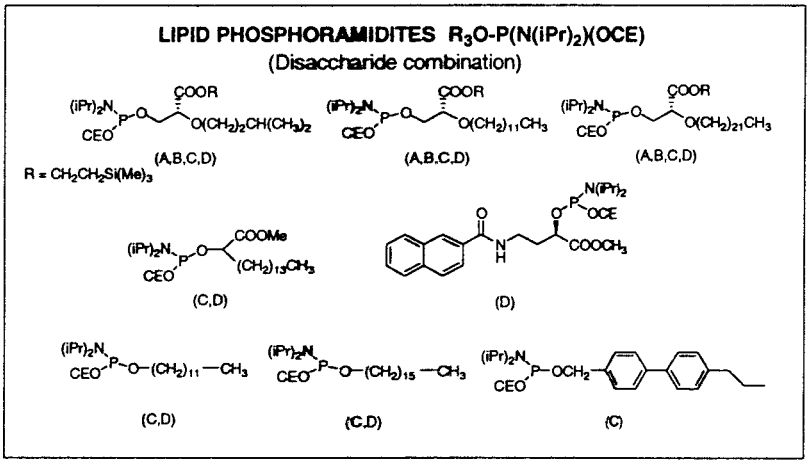
Library Building Blocks





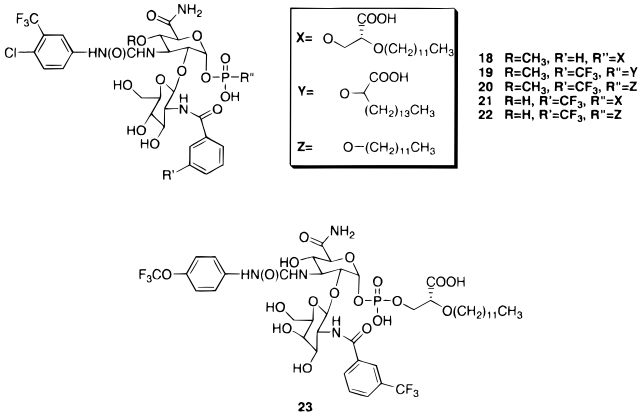






with either a 2-hydroxypropionic acid unit or a simple straight chain C-12 lipid. In addition, none of the compounds containing the moenomycin-like phosphoglycerate with a C-5 lipid moiety demonstrated any significant potency either as cell wall synthesis inhibitors or as antibacterial agents. All active disaccharides contained a substituted urea at the C-3 position, with a substituted aromatic urea as the preferred substituent. Unsubstituted ureas related to the natural moenomycin disaccharide C-3 substituent were shown to be inactive. None of the active compounds contained carbamates at the C-3 position.

Confirmation of the activity of the screening hits was accomplished by determining IC_{50}'s for inhibiting bacterial cell wall biosynthesis and by MIC determination using a broth dilution assay. Compounds **18**−**23** had IC_{50}'s for inhibiting bacterial cell wall biosynthesis that were below 15 μg/mL and had MICs below 25 μg/mL. These compounds were resynthesized and purified by HPLC chromatography. Each compound was resynthesized using the solid-phase protocol described in Scheme



3. In each case, crude product was obtained in approximately 30% overall yield starting with 1−4 g of disaccharide functionalized resin. Purification gave 20−150 mg of pure product as the α-anomeric phosphate derivative as determined by NMR spectroscopy. The resynthesized and purified compounds were shown to have IC_{50} values for inhibition of cell wall biosynthesis of 8−10 μg/mL and MIC values of 3.12−12.5 μg/mL that were similar to those obtained from the initial screening set (see Table 1). Compounds **18**−**23** were also screened against a panel of clinically relevant antibiotic sensitive and resistant Gram-positive bacteria and demonstrated antibacterial activity with MICs in the 3−25 μg/mL range.

Although Table 1 shows that moenomycin A is still the more potent inhibitor of bacterial cell wall biosynthesis, we have been able to identify structurally more simple and novel disaccharides that inhibit both cell wall biosynthesis and bacterial growth. The identification of these compounds supports the idea that the entire moenomycin pentasaccharide is not necessary for target recognition and efficacy. All six compounds were also effective against the *E. faecium* strain naturally resistant to moenomycin. In addition, when compared to the clinically used antibiotic vancomycin, compounds **18**−**23** were shown to be equipotent to vancomycin as inhibitors of cell wall biosynthesis (Table 1). The formation of lipid II was shown to be unaffected by the presence of the disaccharide inhibitors, suggesting that nonspecific perturbations of the cell wall by these compounds are unlikely (data not shown). These compounds were also shown to have antibacterial activity comparable to that of vancomycin (see Table 1) when evaluated against the panel of antibiotic sensitive Gram-positive bacteria. Against clinically relevant antibiotic resistant *Enterococcus* organisms, these compounds were shown to be more effective than vancomycin.

In conclusion, we have shown that using a combinatorial library strategy and the moenomycin A disaccharide as a template, we were able to identify a novel class of potent inhibitors of bacterial cell wall biosynthesis that for the first time also exhibit potent antibacterial activity. Additional studies on this unique class of antibacterial agents will be reported elsewhere.

Acknowledgment. We thank Dan Kahne for helpful discussions and Lynn Silver and Joyce Kohler for supplying *Enterococcus* strains CL4931, CL5244, and CL4877 for comparative testing.

Supporting Information Available: Solid-phase synthesis procedure for library synthesis and spectral data for compounds **18**−**23**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(26) Based on the analysis of 10% of the library products, the average yield of the desired products was determined to be 22% and the average product purity was determined to be 78%. Product yield was calculated from standard curves developed with several HPLC-purified disaccharide products. The product purity was determined based on the relative HPLC peak areas using evaporative light scattering detection.

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(30) Peptidoglycan polymerization assay: A minimum of six concentrations of test compound were incubated each in duplicate wells of a Millipore GFC 96-well filter plate with [^{14}C]UDPGlcNAc (1 μM), UDPMurNAc pentapeptide (15 μM), and ether-permeabilized *E. coli*. (30 μg protein/well) in a buffer containing Tris HCl (pH 8.3), NH_4Cl (50 mM), $MgSO_4$ (20 mM), ATP (5 mM), D-Asp (0.15 mM), tetracycline (100 μg/mL), and β-mercaptoethanol (0.5 mM). Final incubation volume was 100 μL. Incubations were terminated by addition of 100 μL of 20% TCA to precipitate both nascent and mature peptidoglycan. Radioactivity incorporated into the precipitated peptidoglycan was determined using a Wallac Trilux plate counter, and IC_{50} values were computed from nonlinear regression analysis of the concentration−response curves. Vancomycin and moenomycin A were used as internal reference compounds for each assay. Only compounds showing ≥50% inhibition of incorporation of radiolabel at the initial screening concentration (10 μg/mL) were chosen for IC_{50} determination.

(31) The minimum inhibitory concentrations (MIC) of test compounds were determined using brain heart infusion media (BHI) supplemented with 0.1% casamino acids. Logarithmically growing cells were diluted to approximately 5×10^5 CFU/mL and subjected to test compounds solubilized and serially diluted in DMSO. A 5% final DMSO concentration had no effect on cell viability or killing; 96-well microtiter plates were read on a microplate reader, and the OD_{600} was determined. For a given concentration, an MIC determination was made if [OD_{600} control − OD_{600} test concentration]/[OD_{600} control − OD_{600} media] × 100 > 90%.

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