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Degradation and Reconstruction of Moenomycin A and Derivatives: Dissecting the Function of the Isoprenoid Chain

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Abstract

Moenomycin A is the only known natural product that inhibits peptidoglycan biosynthesis by binding the bacterial transglycosylases. We describe a degradation/reconstruction route to manipulate the reducing end of moenomycin A. A comparison of the biological and enzyme inhibitory activity of moenomycin and an analog containing a nerol lipid in place of the natural C₂₅ lipid chain provides insight into the role of the moenocinol unit. Our results show that a lipid chain having ten carbons in moenocinol is sufficient for enzyme inhibition, but a longer chain is required for biological activity, apparently because the molecule must partition into biological membranes to reach its target in bacterial cells.

Moenomycin A (**1**, Scheme 1) is a natural product that inhibits peptidoglycan biosynthesis by binding to the bacterial transglycosylases (TGases).¹ On a molar basis, moenomycin is a thousand times more potent than vancomycin, but poor pharmacokinetic properties related to the C₂₅ isoprenoid chain have prevented its use in humans.² Removing this unit completely abolishes biological activity.³ Whether this portion of the molecule can be replaced by a shorter lipid is unclear for two reasons: first, until now there have been no methods to remove the natural lipid chain and replace it with other lipids without also altering other structural features of the molecule;⁴ second, assays to evaluate the TGase activity of moenomycin and derivatives in the absence of biological membranes⁵ have only recently become available⁶, making it difficult to dissect the contribution of the isoprenoid chain to enzyme binding versus membrane anchoring⁷. Here we describe a degradation/reconstruction route to manipulate the reducing end of moenomycin. We evaluate the enzyme inhibitory activity of moenomycin and an analog containing a nerol chain against

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Supporting Information Available: Experimental procedures and spectral data for all compounds. Over-expression and purification conditions for *E. faecalis* PBP2a are also described. This material is available free of charge via the Internet at <http://pubs.acs.org>.

membrane-free TGases from the clinically relevant pathogens *Staphylococcus aureus* and vancomycin resistant *Enterococcus faecalis*. This work provides insight into the different structural requirements for TGase inhibition and biological activity.

We faced three challenges in developing a degradation/reconstruction route to moenomycin A. First, although there has been considerable work on the degradation of moenomycin A,⁸ a synthetic route to degrade **1** to the intact pentasaccharide **3** has not been reported. Second, we needed to develop a synthesis of the 2-*O*-moenocinyl glycerate (**11**, Scheme 2). Model systems for the lipid glycerate have been synthesized previously, but the chemistry could not be extended to the natural lipid.⁹ Third, we needed an efficient method to form the phosphoglycerate linkage to **3**.

Previous studies on the degradation of moenomycin A have shown that under protic acid conditions the glycosidic bonds of the pentasaccharide core begin to decompose before the anomeric phosphate bond is cleaved.^{8a} We envisioned a solution to this problem that takes advantage of the known lability of allyl ethers such as that found in the glycidyl ether linkage of moenomycin A. Moenomycin A was fully protected by sequential acetylation of hydroxyls followed protected by esterification of the acids on the phosphoglycerate moiety. The glycidyl ether linkage was then cleaved by treatment with TMSOTf to produce **2**. Workup in the presence of saturated sodium bicarbonate produced the desired anomeric lactol **3** in 75% yield, presumably via ejection of the cyclic glycerol phosphate (Scheme 1).

We next required a route to moenocinyl glycerate **11**. Our synthetic approach was patterned after the routes to moenocinol developed by Coates¹⁰ and Schmidt¹¹. To form the glycidyl ether linkage, moenocinol was converted to the corresponding allylic bromide and then alkylated to a 1,3-protected glycerol **7**, which was obtained using the elegant chemistry developed by Jacobsen¹². Efficient etherification required a specific 7:1 ratio of THF : DMF and the use of the triisopropyl silyl (TIPS) group to prevent silyl protecting group migration. Under these conditions, alkylation proceeded in 66% yield. Following formation of the allyl ether linkage, enantiopure **8** was treated with TBAF to remove TIPS group. Oxidation of the deprotected glycerol **9**, followed by esterification to generate **10**, and finally oxidative deprotection gave the desired compound **11**.

The final challenge involved developing chemistry to attach **11** via a phosphodiester linkage to the core pentasaccharide. Using H-phosphate chemistry¹³, derivative **12**, generated from **3**, was coupled with **11** using 1-adamantanecarbonyl chloride. Mild oxidation proceeded regioselectively in the presence of NMM/CCl₄¹⁴ to produce the corresponding phosphate in 53% yield from **3** without affecting the lipid chain olefins or the 2-amino-3-hydroxycyclopent-3-enone unit (Scheme 2). Global deprotection using LiOH afforded moenomycin A (**1**).

Using the same route, we were also able to prepare neryl derivative **15** in good yield (Scheme 3). We evaluated the ability of **15** and moenomycin A to inhibit TGases from *S. aureus*⁶ and *E. faecalis*, which was over-expressed and purified for the first-time in this paper (Table 1). The inhibitory activity of **15** is comparable to that of moenomycin A. However, the MICs for **15** against *S. aureus*⁶ and *E. faecalis* are several orders of magnitude higher than for moenomycin A. Thus, a lipid chain having only ten carbons (**15**) is sufficient for enzyme inhibition in a membrane- and detergent-free assay, but a longer chain is required for biological activity. Access to the transglycosylases, which are anchored to the bacterial membrane and operate on membrane-bound substrates, may require that moenomycin partition into membranes^{3,7,15}. Whether the entire C25 chain is necessary for activity remains to be established.

The degradation/reconstruction route described here, combined with approaches to derivatives of the moenomycin pentasaccharide and the ability to evaluate compounds against purified transglycosylases, may enable the preparation of active moenomycin analogs with shorter lipid chains.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Scheme 1.

Degradation of moenomycin Aa

^a Conditions: (a) Ac_2O , Py, rt; (b) TMSCHN_2 , $\text{CH}_2\text{Cl}_2/\text{MeOH}$, $-78\text{ }^\circ\text{C}$, 61% two steps; (c) TMSOTf , MS $4\text{\AA}$, CH_2Cl_2 , $-78\text{ }^\circ\text{C}$ to $0\text{ }^\circ\text{C}$; (d) sat. NaHCO_3 , rt, 1 h, 75% two steps.

Scheme 2.**Reconstruction of moenomycin Aa**

^a Conditions: (a) LDA, PhSeCl, THF, -78°C to -5°C , 77%; (b) LDA, geranyl bromide, -78°C to -5°C ; (c) DIBAL, toluene, 0°C , 54% two steps; (d) $(\text{CF}_3\text{CO})_2\text{O}$, TEA, then NaOMe, MeOH, 63%; (e) TBAF, THF, rt, 99%; (f) ref. 10; (g) PBr_3 , Et_2O , 0°C , 80%; (h) **7**, NaH, THF/DMF (7:1), 0°C to rt, 66%; (i) TBAF, THF, 0°C , 97%; (j) Dess-Martin periodinane, NaHCO_3 , CH_2Cl_2 , rt; (k) NaClO_2 , NaH_2PO_4 , 2-methyl-2-butene, *t*-BuOH, H_2O ; (l) TMSCHN_2 , $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (1:1), -60°C , 57% three steps; (m) DDQ, $\text{CH}_2\text{Cl}_2/\text{pH}$ 7 buffer (10:1), 61% (recovered starting material 34%); (n) 2-Chloro-1,3,2-benzodioxaphosphorin-4-one, 85%; (o) Py, MS $4\text{\AA}$, rt, 1-adamantanecarbonyl chloride, then NMM/ $\text{CCl}_4/\text{Py}/\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (1:2.5:6:1:1), 2 h, 62%; (p) 0.1 N LiOH, THF/ H_2O (1:1), rt, then AcOH, 47%.

Scheme 3.

Synthesis of moenomycin A analog (**15**) a

^a Conditions: (a) PBr₃, Et₂O, 0 °C, 100%; (b) **7**, NaH, THF/DMF (7:1), 0 °C to rt, 96%; (c) TBAF, THF, 0 °C, 92%; (d) Dess-Martin, NaHCO₃, CH₂Cl₂, rt; (e) NaClO₂, NaH₂PO₄, 2-methyl-2-butene, *t*-BuOH; (f) TMSCHN₂, CH₂Cl₂/MeOH (1:1), –60 °C, 49% three steps; (g) DDQ, CH₂Cl₂/pH 7 buffer (10:1), 70%; (h) **12**, adamantanecarbonyl chloride, Py, MS 4Å, rt, then NMM/CCl₄/Py/ CH₃CN/H₂O (1:2.5:6:1:1), 51%; (i) 1.3 M KOH, THF/H₂O (2:1), rt, then AcOH, 92%.

Table 1Biological activity and transglycosylase inhibition for moenomycin A **1** and derivative **15**^a

	IC ₅₀ (μg/ml)		MIC (μg/ml)	
	<i>S. aureus</i> PBP2	<i>E. faecalis</i> PBP2a	<i>S. aureus</i> ^b	<i>E. faecalis</i> ^c
1	0.035	0.015	0.016	0.063
15	0.057	0.022	> 50	25

^aExperimental procedures see supporting information.^bBaterial strain 29213.^cBacterial strain 29212.