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

Masaatsu Adachi,[†] Yi Zhang,[†] Catherine Leimkuhler,[†] Binyuan Sun,[‡] John V. LaTour,[‡] and Daniel E. Kahne*[,†,§]

Department of Chemistry and Chemical Biology, Harvard University, Cambridge, Massachusetts 02138, Department of Chemistry, Princeton University, Princeton, New Jersey 08544, and Department of Biological Chemistry and Molecular Biology, Harvard Medical School, Boston, Massachusetts 02115

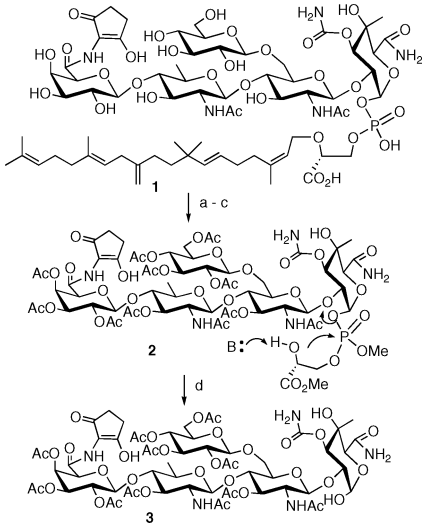
Received August 14, 2006; E-mail: kahne@chemistry.harvard.edu

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 A 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 A and derivatives in the absence of biological membranes[5] have only recently become available,[6] making it difficult to dissect the contribution of the isoprenoid chain to enzyme binding versus membrane anchoring.[7] Here we describe a degradation/reconstruction route to manipulate the reducing end of moenomycin A. We evaluate the enzyme inhibitory activity of moenomycin A and an analogue containing a nerol chain against 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[10] was fully protected by sequential acetylation of hydroxyls followed 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).

Scheme 1. Degradation of Moenomycin A[a]



[a] Conditions: (a) Ac_2O, Py, room temperature; (b) $TMSCHN_2$, CH_2Cl_2/MeOH, −78°C, 61%, two steps; (c) TMSOTf, MS 4 Å, CH_2Cl_2, −78 to 0 °C; (d) saturated $NaHCO_3$, room temperature, 1 h, 75%, two steps.

We next required a route to moenocinyl glycerate **11**. Our synthetic approach was patterned after the routes to moenocinol developed by Coates[11] and Schmidt.[12] 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 Jacobsen.[13] 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 the 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 chemistry,[14] derivative **12**, generated from **3**, was coupled with **11** using 1-adamantanecarbonyl chloride. Mild oxidation proceeded regioselectively in the presence of NMM/CCl_4[15] to produce the corresponding phosphate in 53% yield from **3** without affecting the lipid chain olefins or the 2-amino-3-hydroxycyclopent-2-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

[†] Harvard University.
[‡] Princeton University.
[§] Harvard Medical School.

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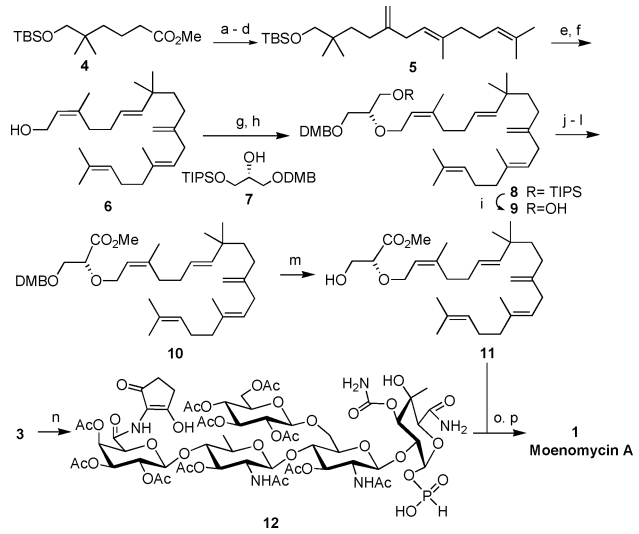
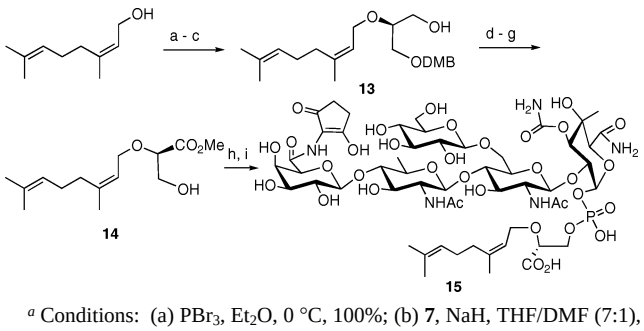
Scheme 2. Reconstruction of Moenomycin A[a]



[a] Conditions: (a) LDA, PhSeCl, THF, −78 to −5 °C, 77%; (b) LDA, geranyl bromide, −78 to −5 °C; (c) DIBAL, toluene, 0 °C, 54%, two steps; (d) $(CF_3CO)_2O$, TEA, then NaOMe, MeOH, 63%; (e) TBAF, THF, room temperature, 99%; (f) ref 10; (g) PBr_3, Et_2O, 0°C, 80%; (h) **7**, NaH, THF/DMF (7:1), 0°C to room temperature, 66%; (i) TBAF, THF, 0°C, 97%; (j) Dess−Martin periodinane, $NaHCO_3$, CH_2Cl_2, room temperature; (k) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*-BuOH, H_2O; (l) $TMSCHN_2$, CH_2Cl_2/MeOH (1:1),-60 °C, 57%, three steps; (m) DDQ, CH_2Cl_2/pH 7 buffer (10:1), 61% (recovered starting material 34%); (n) 2-chloro-1,3,2-benzodioxaphosphorin-4-one, 85%; (o) Py, MS 4 Å, room temperature, 1-adamantanecarbonyl chloride, then NMM/CCl_4/Py/CH_3CN/H_2O (1:2.5:6:1:1), 2 h, 62%; (p) 0.1 N LiOH, THF/H_2O (1:1), room temperature, then AcOH, 47%.

Scheme 3. Synthesis of Moenomycin A Analog (**15**)[a]



[a] Conditions: (a) PBr_3, Et_2O, 0 °C, 100%; (b) **7**, NaH, THF/DMF (7:1), 0 °C to room temperature, 96%; (c) TBAF, THF, 0 °C, 92%; (d) Dess−Martin, $NaHCO_3$, CH_2Cl_2, room temperature; (e) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*-BuOH; (f) $TMSCHN_2$, CH_2Cl_2/MeOH (1:1), −60 °C, 49%, three steps; (g) DDQ, CH_2Cl_2/pH 7 buffer (10:1), 70%; (h) **12**, adamantanecarbonyl chloride, Py, MS 4 Å, room temperature, then NMM/CCl_4/Py/ CH_3CN/H_2O (1:2.5:6:1:1), 51%; (i) 1.3 M KOH, THF/H_2O (2:1), room temperature, then AcOH, 92%.

of **15** and moenomycin A to inhibit TGases from *S. aureus*[6] and *E. faecalis*, which was overexpressed 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 A partition into membranes.[3,7,16]

Table 1. Biological Activity and Transglycosylase Inhibition for Moenomycin A **1** and Derivative **15**[a]

	IC_{50} (μg/mL)		MIC (μg/mL)	
	S. aureus PBP2	*E. faecalis* PBP2a	*S. aureus*[b]	*E. faecalis*[c]
1	0.035	0.042	0.016	0.063
15	0.057	0.022	>250	>250

[a] For experimental procedures see Supporting Information. [b] Baterial strain 29213. [c] Bacterial strain 29212.

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 analogues with shorter lipid chains.

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Supporting Information Available: Experimental procedures and spectral data for all compounds. Overexpression 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.

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