Antibiotics

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A Long Research Story Culminates in the First Total Synthesis of Moenomycin A**

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Bacteria are surrounded by the netlike polymer peptidoglycan, which is responsible for a defined cell shape and preserves cell integrity by withstanding internal osmotic pressure. Peptidoglycan consists of repeating β-1,4-linked *N*-acetylglucosaminyl-*N*-acetylmuramyl units cross-linked by short peptide chains.[1] In the biosynthesis the last monomeric intermediate is lipid II (Scheme 1), from which peptidoglycan is formed by two (polymerization) reactions that occur at the outside surface of the cytoplasmic membrane:[1,2] First, the sugar chains are assembled by a so-called transglycosylation reaction—a nucleophilic substitution reaction involving the displacement of the diphosphoundecaprenyl group by a GlcNAc 4-OH group.[3,4] The subsequent transpeptidation consists (formally) of the nucleophilic attack of the free amino group of one peptide chain (see *meso*-diaminopimelic acid (m-DAP) and L-lysine in Scheme 1) with the terminal peptide bond of another chain, resulting in loss of the terminal D-Ala. This leads to the formation of a cross-linking peptide bond between the two strands.[5]

The transglycoslyation reaction is catalyzed by a number of membrane-associated multimodular bifunctional polymerases, class A high-molecular-mass penicillin-binding proteins (PBPs), which also catalyze the transpeptidation reaction. In addition, both Gram-positive and Gram-negative bacteria have monofunctional transglycosylases that show a high degree of similarity to the transglycosylase domains of class A PBPs.[2] A number of antibiotics inhibit the transglycosylation reaction by targeting lipid II.[6] The only compounds known to date that exert their activity by interacting with the enzyme (e.g. the transglycosylation domain of *E. coli* PBP 1b) are the moenomycin antibiotics. It was found that the binding of moenomycin A (**1**) to *E. coli* PBP 1b is reversible,[3] and photoaffinity labeling has recently provided evidence that binding occurs indeed at the transglycosylation domain.[7] Moenomycin A (**1**) and related antibiotics[8] were discovered following extensive industrial screening in the 1960s and 1970s.[9] Although the moenomycins are among the most active antibiotics known, define a new target, and are not prone to resistance development, industrial research has largely ignored both the target and the inhibitors.[2]

The moenomycin structures were originally elucidated by a combination of chemical degradation reactions and spectroscopic studies[10] and can nowadays be deduced more easily without chemical degradation by (nontrivial[11]) high-field ^{1}H NMR spectroscopy[12] and even more readily by modern mass spectrometry (which, of course, does not provide stereochemical details).[13] Besides ordinary building blocks (B: D-galacturonic acid, C: 2-acetamido-2,6-dideoxy-D-glucose, D: D-glucose, E: 2-acetamido-2-deoxy-D-glucose, G-H: D-phosphoglycerate), moenomycin A contains 2-aminocyclopentane-1,5-dione (unit A, a compound biogenetically derived from aminolevulinic acid that has been found also in other natural products[14]) and building blocks F and I, which are unique to the moenomycin antibiotics (Scheme 2). The 4-methyl group of unit F is derived from *S*-adenosylmethionine[15] (and is probably transferred by a radical mechanism[16]), and the C_{25} unit I is assembled from a C_{10} and a C_{15} terpenoid precursor, which are formed by the nonmevalonate pathway.[17] The moenomycin biosynthetic genes in *Streptomyces ghanaensis* (ATCC14672) have been identified recently, and a biosynthetic scheme has been proposed.[16] By stepwise and selective degradation of moenomycin A,[18] the structural features (shown in blue in Scheme 2) essential for antibiotic activity (the lipid part may be hydrogenated) were determined. All structure–activity relationships correlate well with the strength of the binding to the enzyme, as deduced from surface plasmon resonance (competition) experiments.[19] The synthetic trisaccharide **2a** (Scheme 3) was shown to inhibit the transglycosylase and to have antibiotic activity, whereas the corresponding $1^F\beta$ isomer **3** and compound **2b** turned out to be inactive in both test systems.[8] This underscores the importance of the configuration at C1 of unit F and the acetylamino function in position 2 of unit C (verified by STD NMR[20]) and confirms[8] that both the presence of the methyl group and the configuration at C4 of unit F are of lesser importance. Synthetic **2c** was inactive, too, most probably because the lipid chain is too short to provide sufficient binding energy on interaction with the cytoplasmic membrane.[21] Based on the structure–

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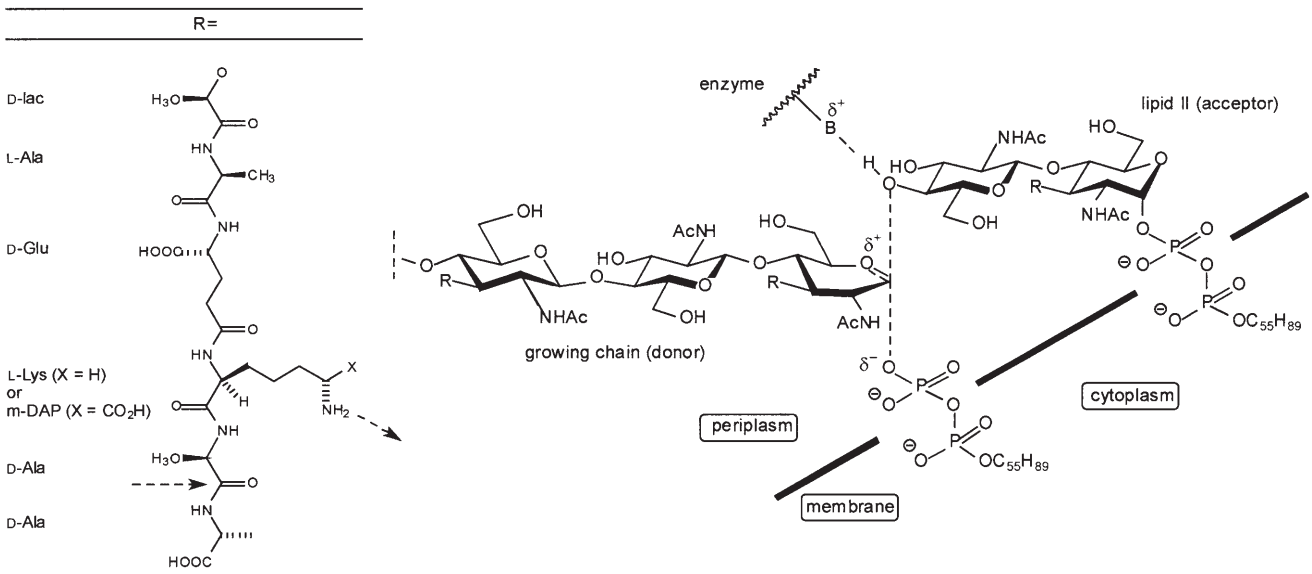
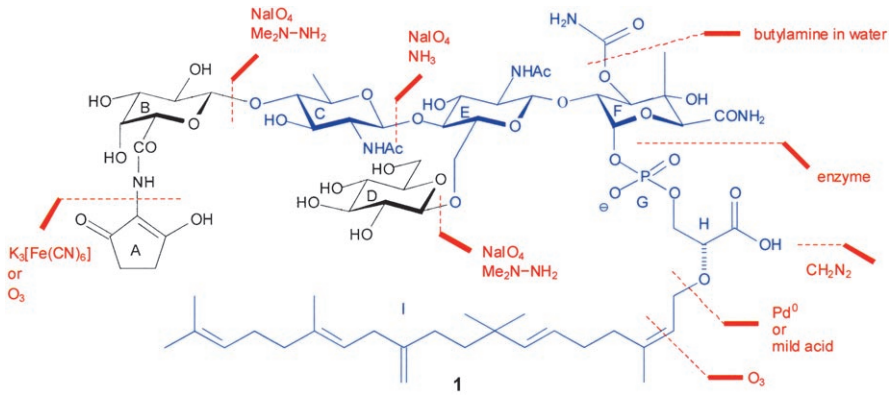
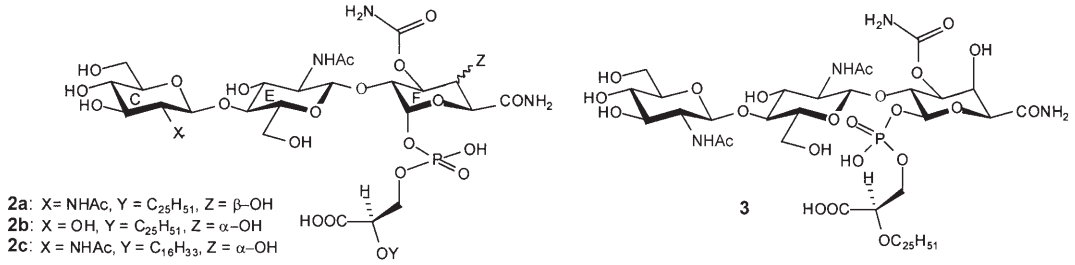


Scheme 1. Transglycosylation and groups involved in the transpeptidation.



Scheme 2. Structure of moenomycin A (**1**), selective degradation reactions (in red), and minimum structural requirements for antibiotic activity (in blue).

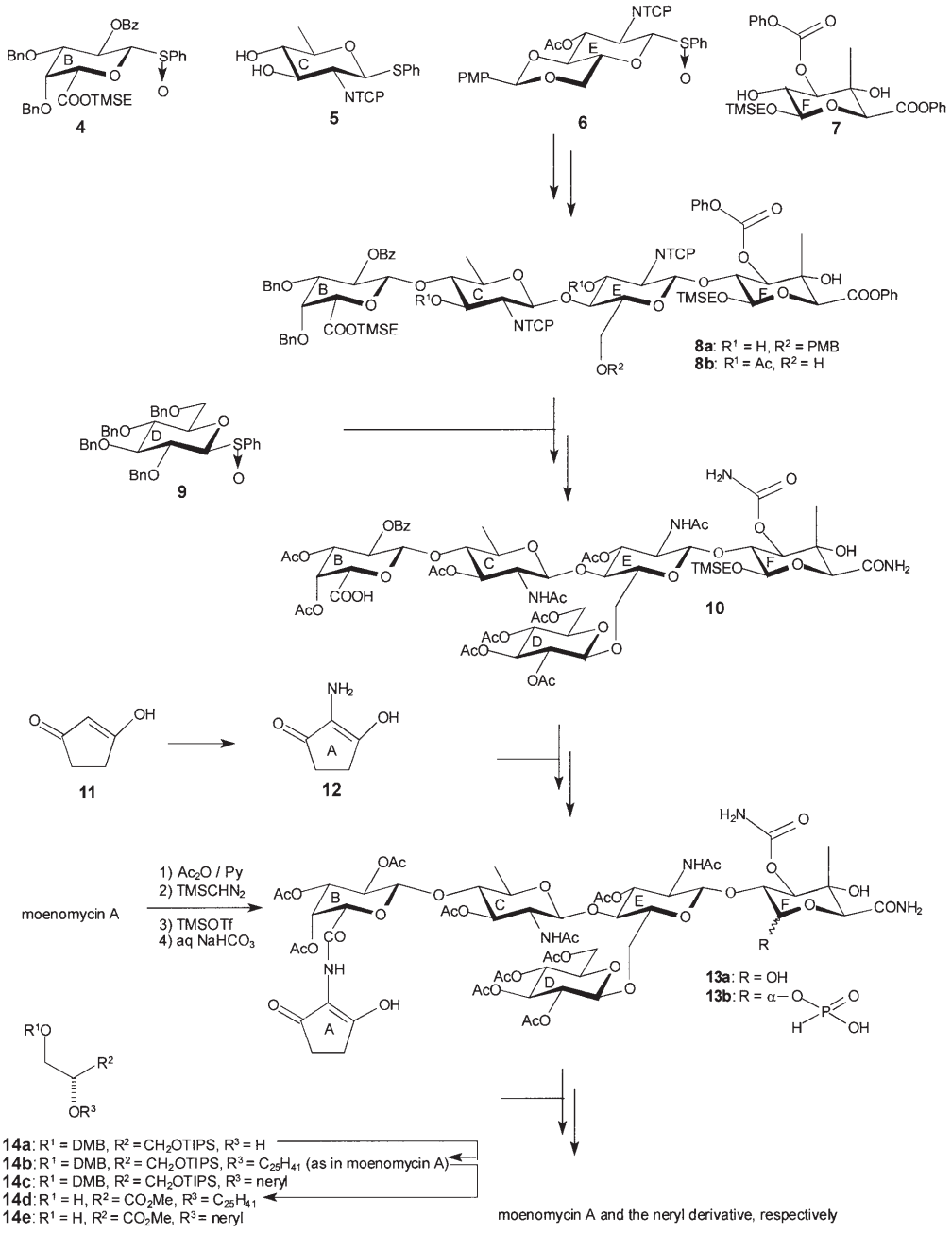


Scheme 3. Synthetic trisaccharide analogues of moenomycin A.

activity studies it was concluded that moenomycin A and the related antibiotics exert their action by first binding to the cyctoplasmic membrane through their lipid moiety followed by a highly selective binding of the sugar (trisaccharide) part to the donor binding site of the enzyme. A detailed model for the mode of action has been suggested[22] and is supported by recent X-ray crystallographic studies.[23]

Synthetic efforts in the moenomycin area[8] recently culminated in the first total synthesis of moenomycin A, reported by Kahne and co-workers (Scheme 4).[24] Building blocks **4**–**7**, which were assembled to give the tetrasaccharide intermediate **8a**, reveal some of the special features of the synthesis. Glycosidic bonds were formed making use of Kahne's powerful sulfoxide meth-

od[25,26] (see **4**, **6**, and also **5**). However, with one exception, the standard protocol was not successful, and special, optimized conditions were required. The amino groups were tetrachlorophthimido-protected,[27] which ensured formation of 1,2-*trans* (β-) glycosidic bonds by neighboring-group participation and, because of the bulkiness of the protecting group, permitted a selective 4-*O*-glycosidation of 3,4-diol **5**. The moenuronic acid building block **7** bears a phenyl carbonate and a phenyl ester group as latent equivalents of a carbamoyl and an amide group, respectively. These protecting groups solved many polarity (solubility) problems found in previous synthetic attempts.[28] The 4-C-methyl group in unit F was introduced by nucleophilic attack of methyllithium to a 4-ulose unit.[29] (Methyllithium and methylmagnesium iodide are known add with complementary stereoselectivity to hexopyranosid-4-uloses.[30]) Trifluoromethanesulfonic anhydride (Tf_2O) mediated B–C and E–F coupling was followed by BC–EF coupling to provide tetrasaccharide **8a**, from which **8b** was obtained by protecting-group manipulations. For the Tf_2O-mediated glycosidation forming the 1→6 bond between units E and D, tetra-*O*-benzyl-D-glucose derived donor **9** was chosen.



Scheme 4. Total synthesis of moenomycin A reported by Kahne et al. and degradation of moenomycin A into **13a**. Bn = benzyl, Bz = benzoyl, DMB = 2,4-dimethoxybenzyl, NTCP = tetrachlorophthalimido, PMP = 4-methoxyphenyl, Py = pyridine, Tf = trifluoromethanesulfonyl, TIPS = triisopropylsilyl, TMS = trimethylsilyl, TMSE = 2-(trimethylsilyl)ethyl.

The reaction was performed in propionitrile solution. Under these conditions the reaction proceeds via an intermediate α-nitrilium–nitrile conjugate[31] securing β-glycoside formation. Again, special glycosidation conditions had to be used to avoid extensive side-product formation. Conversion of the phenyl ester and the phenyl carbonate in unit F into the uronamide and the urethane, respectively, with NH_3 under proper conditions and subsequent protecting-group adjustments provided **10**, which on HATU-mediated coupling with **12** (available from cyclopentane-1,3-dione (**11**) by attack of a suitable nitrogen electrophile[32,33]), and some further protecting-group manipulations furnished **13a** with a free anomeric hydroxy group in unit F (HATU = *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate). Building block **14d** (see also Ref. [8]) was available by alkylation of 1,3-di-O-protected optically active glycerol derivative **14a** with moenocinyl bromide.[34]The former was prepared making use of Jacobsen's epoxide resolution,[35] and the synthesis of the latter was patterned after a synthetic scheme developed by Coates and by Schmidt.[8] Building blocks **14d** and **13a** were tied together by means of a phosphoric acid diester linkage making use of H-phosphonate chemistry[36] (see **13b**). Finally, ester hydrolysis under basic conditions furnished moenomycin A (**1**). This synthesis is a beautiful achievement, combining in a most imaginative way new synthetic methods with moenomycin chemistry uncovered previously. Probably the most interesting compound in this approach is lactol **13a**, which is available either by synthesis as summarized above or from moenomycin A by a four-step degredation sequence (see Scheme 4).[34] Pentasaccharide derivative **13a** should be a very useful intermediate for the replacement of the phosphoglycerate unit by isosteric connecting modules and for probing the structure–activity relationships within the lipid part. As a first step towards these goals Kahne and co-workers converted **13a** into the moenomycin A neryl analogue (using **14e**), which turned out to be antibiotically inactive,[34] in agreement with previous observations on analogues with short lipid parts (vide supra).

To cope with the problem of antibiotic resistance,[37,38] antiinfectives must be developed with novel modes of action. The moenomycins are promising lead compounds for several reasons: 1) They exert their activity at the extracellular surface of the cytoplasmic membrane; 2) their chemistry is now well-developed, allowing the preparation of active analogues with suitable pharmacokinetic properties; 3) new and efficient test systems have recently become available which rely on the fact that the target transglycosylase(s) can now be overexpressed and purified readily[39] and that the monomeric peptidoglycan precursor lipid II (and useful analogues) can be made in sufficient amounts by total synthesis and by chemoenzymatic and purely enzymatic methods;[8,40] and 4) the X-ray structures of two transglycosylases (even one with moenomycin) were published very recently.[23,41]

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