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Moenomycin-Type Transglycosylase Inhibitors: Inhibiting Activity vs. Topology around the Phosphoric Acid Diester Group

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Abstract: The correct configuration at C-1 of the uronamide carrying the phosphate group is essential for antibiotic activity.

A number of bifunctional enzymes (penicillin binding proteins, PBP's) have been identified that catalyse both transglycosylation and transpeptidation, the two final reactions of the biosynthesis of crosslinked peptidoglycan from the membrane intermediate GlcNAc-MurNAc-(pentapeptide)-PP-undecaprenol.[1] With cell free systems from *E.coli* it was demonstrated that the antibiotic moenomycin A selectively inhibits the transglycosylation step by its inhibitory effect on PBP 1b.[2] Already many years ago it has been speculated that moenomycin A interacts with the enzyme due to its structural similarity with the membrane intermediate mentioned above.[3] This view has found support by studying the structure-activity relations of many moenomycin degradation products.[4] In addition, van Heijenoort has demonstrated that an antibiotically active derivative of moenomycin A forms reversibly a complex with the enzyme.[5]

Recently, we have been able to synthesize compound **7a**, a structural analogue of moenomycin A which is *in vitro* a highly active inhibitor of the transglycosylase.[6] **7a** is closely related to **7b**, the smallest moenomycin C_1 degradation product with full transglycosylase inhibiting potency.[7] With transglycosylase inhibitors being synthetically accessible, structure-activity relations can now be investigated that were hitherto out of reach. In the Scheme the individual steps are summarized which have been used to prepare **7a** from trisaccharide intermediate **1**.[9] Key step was the formation of phosphoric acid triester **2** employing a version of the phosphite methodology that was adapted to the synthesis of moenomycin A analogues.[4] This procedure leads to the formation of an α-oriented phosphate group at the anomeric center of the uronamide moiety.[4,6] In the present Communication we describe the synthesis of **8** (the β-diastereoisomer of **7a**) and its inhibiting activity in the transglycosylase test system.

The synthesis of **8** commenced from **1**[6] which was converted into the α-trichloroacetimidate **3** (the configuration at C-1 was apparent from $J_{1,2}$ = 3.5 Hz[8]). Phosphoric acid monoester **4** was obtained[9] by hydrogenation of compound MB derived from moenomycin A by enzymatic degradation[10] followed by methyl ester formation. Reaction of **3** and **4** in CH_2Cl_2 solution (25°C, 1h) provided **6a** in 54% yield. The β configuration was confirmed[11] by the following coupling constants: $J_{P,1} = J_{1,2}$ = 7.5 Hz. Treatment of **6a**

with p-toluenesulfonic acid in THF (24 h, 20°C) led to the formation of a 4:3 mixture of **6a** and its α-diastereoisomer (39% total yield after column separation). For the removal of the TrOC protecting group from **6a** we used the reaction that we have recently discovered.[12] Thus, irradiation of **6a** in the presence of triethylamine (CH_3CN solution, mercury high pressure lamp HPK 125, quartz vessel) provided **6b** in 45% yield. Final deprotection (**6b**→**8**) was achieved by ester hydrolysis (LiOH, THF-H_2O 2.5:1). It may be noted at this point, that **8** is the minor compound that has been mentioned in the paper on the synthesis of **7a**.[6]

Inhibition of the UDP-N-acetylmuramyl pentapeptide-dependent incorporation of [^{14}C]UDP-N-acetylglucosamine into cross-linked high-molecular weight peptidoglycan by **8** was studied with a slightly modified[13] version of the assay described by Izaki, Matsuhashi, and Strominger.[14] Furthermore, the minimum inhibitory concentrations (MIC's) of compound **8** against various microorganisms have been determined by a serial two-fold agar dilution method (Müller Hinton Agar). The results are compared with those obtained for **7a** (see Tables 1 and 2). In contrast to the α-phosphate **7a**, the β-diastereoisomer is almost inactive.

This observation again supports the idea that the transglycosylase inhibiting activity of the moenomycin-type compounds is based on their structural similarity with the last membrane intermediate of the peptidoglycan biosynthesis as discussed above. In this intermediate the diphosphate moiety is **α-connected** to the muramic acid unit.[15]

Table 1:

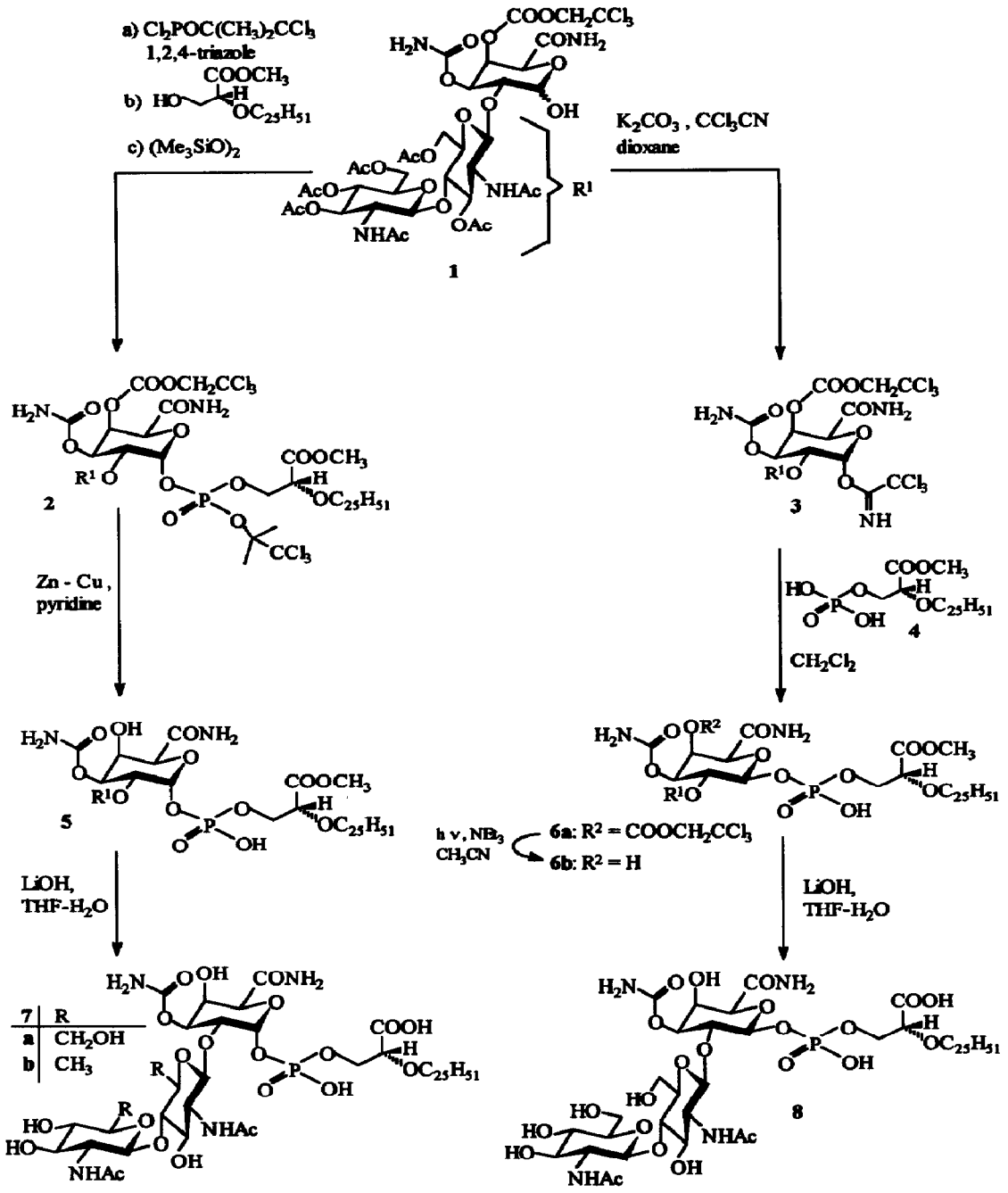
Effect of compounds **7a** and **8** on the *in-vitro* UDP-N-acetylmuramyl pentapeptide-dependent incorporation of [^{14}C]UDP-N-acetylglucosamine into cross-linked high-molecular weight peptidoglycan.

	%inhibition	
concentration (mg/L)	**7a**	**8**
10	93	64
1	86	0
0.1	18	0

Table 2:

Minimum inhibitory concentrations (in mg/L) of compound **7a** and **8** against various test organisms.

test organism	**7a**	**8**
Staph. aureus SG 511	12.5	>50
Staph.aureus 503	12.5	>50
Strept. pyogenes A77	0.781	1.56
Pseud. aerug. 1771m	50	>50
E. coli DC 2	>100	>50


a) Cl2POC(CH3)2CCl3
1,2,4-triazole
b) HO COOCH3 H OC25H51
c) (Me3SiO)2
K2CO3 , CCl3CN
dioxane
1
2
3
4
Zn - Cu ,
pyridine
CH2Cl2
5
hν, NBu3
CH3CN
6a: R2 = COOCH2CCl3
6b: R2 = H
LiOH,
THF-H2O
7 | R
a | CH2OH
b | CH3
8

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