Pergamon

Tetrahedron Letters, Vol. 37, No. 29, pp. 5087-5090, 1996

0040-4039/96 $15.00 + 0.00

PII: S0040-4039(96)01035-0

A Selective Reaction that can be used to attach Moenomycin to Solid Supports and Proteins

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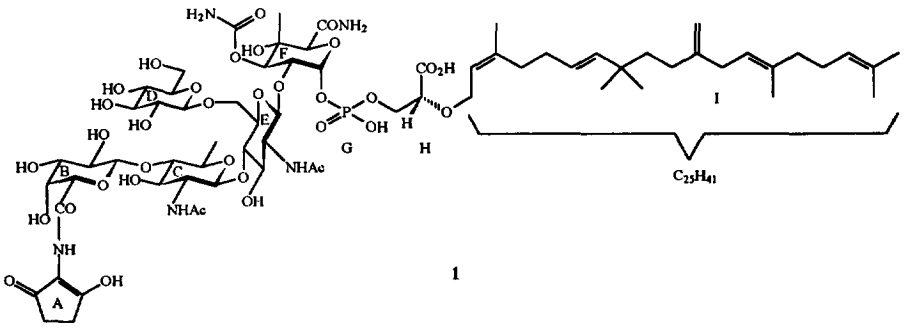
Abstract - A selective reaction at unit A of the antibiotic moenomycin A (**1**) has been developed: Reaction of **1** with *o*-nitrobenzenediazonium chloride cleanly yielded **12**. This compound is as active as moenomycin against *Staph. aureus* SG 511 and as an *in-vitro* transglycosylase inhibitor. Copyright © 1996 Elsevier Science Ltd

In the final stage of *E. coli* peptidoglycan biosynthesis, polymerization with the use of lipid II (GlcNAc-MurNAc(pentapeptide)-pyrophosphoryl-undecaprenol) involves transglycosylation reactions, which lead to linear glycan strands and subsequent transpeptidation reactions which cross-link the peptide units of different strands. These reactions are catalyzed by high molecular mass penicillin-binding proteins (PBP's), membrane-bound proteins that are located on the outer face of the cytoplasmic membrane. PBP's such as PBP's 1a and 1b are bifunctional enzymes with two separate active sites, one for transglycosylation and the other one for transpeptidation. The transpeptidase binding site is located in the C-terminal module, and the enzyme activity is inhibited by covalent binding of penicillin. The few antibiotics that interfere with the transglycosylase[1] (located in the N-terminal module) are reversible-binding inhibitors. The best-studied ones, the moenomycins, are assumed to compete with lipid II for the binding site as a results of structural similarities.[2]

Investigations on the moenomycins until now have concentrated on structural determinations and structure-activity relations.[3] The potential of the moenomycins as tools to identify and characterize the transglycosylase active site has not yet been exploited. A first step into this direction is the subject of the present publication. We are interested to find reactions that would allow to couple moenomycin A (**1**) to solid supports (for affinity chromatography), to reactive or activatable groups (for affinity labeling), to proteins (for raising antibodies), and so forth.

Work on structure-activity relations has shown that units E, F, G, H, I of moenomycin A (**1**) are indispensable for transglycosylase inhibiting properties. Very few structural changes in this part of the molecule are



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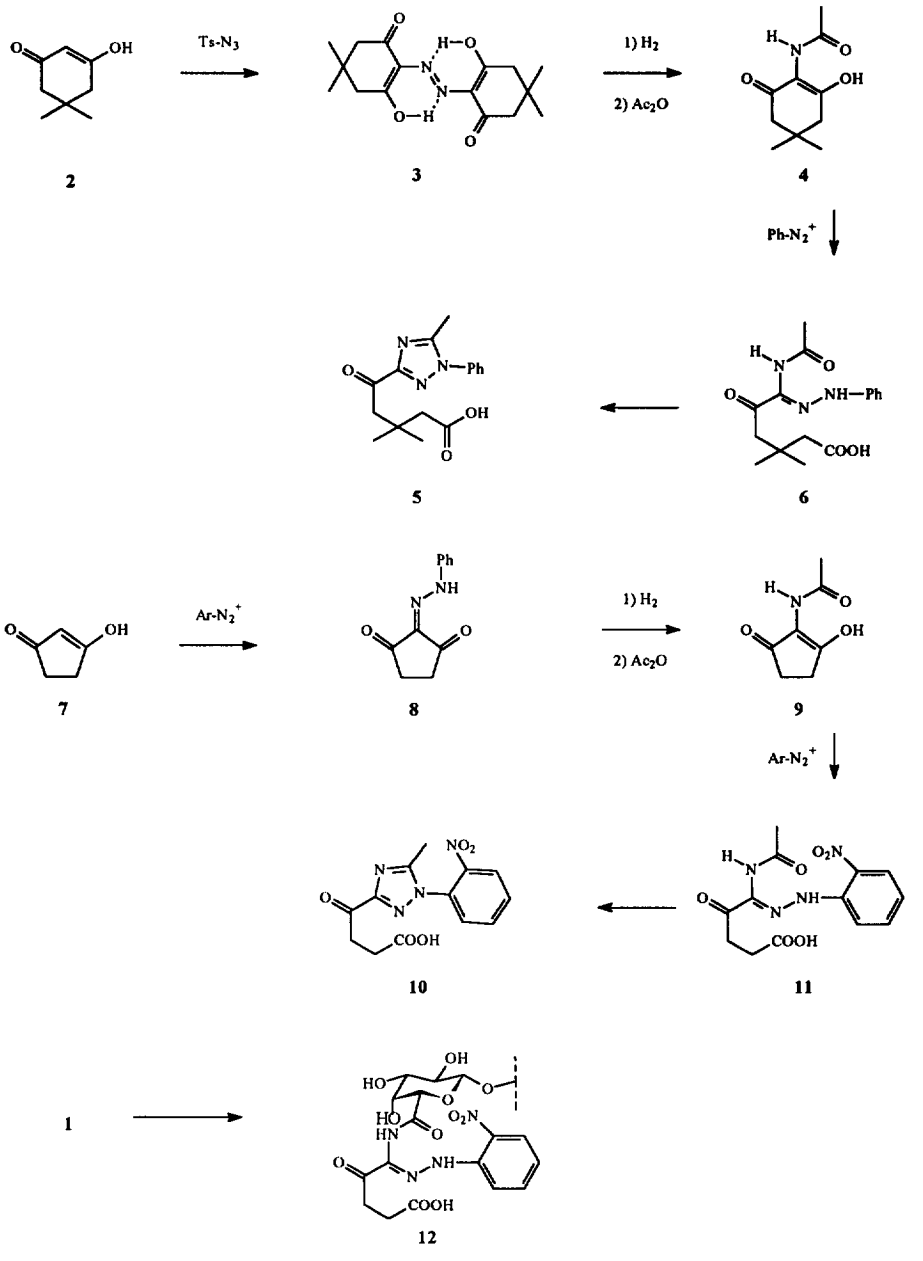
tolerated without loss of activity.[3] This means that selective reactions for the above-mentioned purposes should occur in units A to D. Of these, the enolised diketone unit A seems, of course, to be the first choice for reactions with soft electrophiles which would not attack all the other nucleophilic groups in **1**. Unit A, the so-called moenomycin chromophore[4,5,6] has been found in a number of antibiotics.[7] Biogenetically it is derived from glycine and succinic acid.[8]

We based our studies on selective reactions of moenomycin A (**1**) on work described some 30 years ago by Stetter,[9] Eistert and Regitz.[10] Thus, model compound **4** (prepared from dimedone **2** by (i) reaction with tosyl azide (**2**→**3**)[11] and (ii) hydrogenation in the presence of acetic anhydride[12]) on treatment with benzenediazonium chloride in aqueous solution in the presence of an excess of sodium acetate (0°C, 15 min) yielded triazole derivative **5**,[13,14] which according to the results of Regitz and Eistert is formed by Japp-Klingemann reaction[15] of the first reaction intermediate to give **6**[16] and subsequent cyclization. For the synthesis of **9** the tosyl azide route failed. Therefore, **7** was treated with benzenediazonium chloride whereupon **8** was formed which on hydrogenation/acetylation yielded **9**.[17] This compound did not react with benzenediazonium chloride, but with the more electrophilic *o*-nitro derivative nicely **11**[18] was obtained which on prolonged standing under the reaction conditions cyclized to provide **10**.[19]

When moenomycin A (**1**) under the same conditions was treated with *o*-nitrobenzenediazonium chloride a clean reaction occurred, and the desired derivative **12**[20] was isolated by reversed phase chromatography (HP 20, water-methanol gradient) in about 56% yield.

A prerequisite for the further application of this method in the sense indicated in the introductory part is that compounds of type **12** inhibit the transglycosylase. Therefore, both the the antibiotic activity and the *in-vitro* transglycosylase inhibiting properties were determined as described previously.[21] The following results were obtained: inhibition of the transglycosylase at 1 μg/ml: 87% (for comparison, **1**: 94%), MHK against *Staph.auresus* SG 511: 0.015 μg/ml (for comparison, **1**: 0.062 μg/ml).



Use of appropriately functionalized diazonium salts will allow to couple moenomycin derivatives of type **12** to suitable carriers, reactive or activatable groups, and proteins, respectively. This will be reported in due course.

Acknowledgements - Financial support by the Deutsche Forschungsgemeinschaft (Innovationskolleg „Chemisches Signal und biologische Antwort"), the Fonds der Chemischen Industrie, Roussel Uclaf and the Hoechst AG is gratefully acknowledged.

References and Notes

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13 All compounds were characterized by ^{1}H NMR, ^{13}C NMR, and MS.

14 Characteristic ^{13}C chemical shifts (in $CDCl_3$): δ = 193.6 (ketone), 177.3 (COOH), 160.1 (triazole C-3), 154.7 (triazole C-5), 13.6 (triazole CH_3).

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16 Only the methyl ester of **6** could be isolated in pure form (after addition of methanol). Characteristic ^{13}C chemical shifts (in $CDCl_3$): δ = 194.5 (ketone), 172.9 ($COOCH_3$), 170.3 (amide), 144.0, 130.0 (amidrazone-C, C-1^{Ar}).

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18 Characteristic ^{13}C chemical shifts (in DMSO-d_6): δ = 194.3 (ketone), 174.2 (COOH), 169.8 (amide), 139.6, 135.2, 133.4 (amidrazone-C, C-1^{Ar}, C-2^{Ar}). UV (methanol)): λ_{max} (ε) = 220 (16557), 276 (7709), 321 (12081), 402 (8010). see for comparison ref. [10]

19 Characteristic ^{13}C chemical shifts (in DMSO-d_6): δ = 192.7 (ketone), 175.5 (COOH), 159.4 (triazole C-3), 155.9 (triazole C-5), 12.2 (triazole CH_3).

20 $C_{75}H_{113}O_{37}N_8P$ (1748.69), FAB MS: m/z = 1749.4 ($[M+H]^+$), 1771.4 ($[M+Na]^+$), 1787.4 ($[M+K]^+$).-

21 UV (methanol)): λ_{max} (ε) = 272 (9213), 322 (12861), 406 (8908). see for comparison ref. [10]
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(*Received in Germany* 2 *May* 1996; *accepted* 22 *May* 1996)