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A FORMAL SYNTHESIS OF ALTHIOMYCIN

Peter L. Toogood,* Jessica J. Hollenbeck, Huong M. Lam, and Li Li.

Willard H. Dow Laboratory, Department of Chemistry, University of Michigan, Ann Arbor, MI 48109-1055.

Abstract: A formal synthesis of the antibiotic althiomycin has been completed preliminary to studies of the interaction of this compound with prokaryotic ribosomes.

Althiomycin (**1**) is a naturally occurring antibiotic, active against both gram-positive and gram-negative bacteria.[1,2] Its mechanism of action involves inhibition of protein synthesis at the peptidyltransferase stage.[2-4] Althiomycin is selective for prokaryotic organisms and does not inhibit protein synthesis in rabbit reticulocyte lysates. The structure of althiomycin was determined through chemical analysis, degradation studies, and X-ray diffraction.[1,5] Biosynthetically, althiomycin appears to be derived from the pentapeptide H_2N-gly-cys-ser-cys-gly-OH by post-translational modification, although this pathway has not been proven.[5b] The organization of these residues into the tricyclic structure of althiomycin gives the molecule an arc-like structure in three-dimensions that suggests it could bind to the major groove of double helical RNA.[6] The fact that althiomycin is a peptidyltranferase inhibitor further suggests that it might bind specifically to a region of helical rRNA located close to the peptidyltransferase active site in 23S rRNA. Thus, we became interested in studying the mode of recognition of ribosomal RNA by althiomycin.

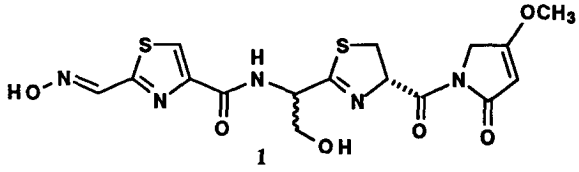

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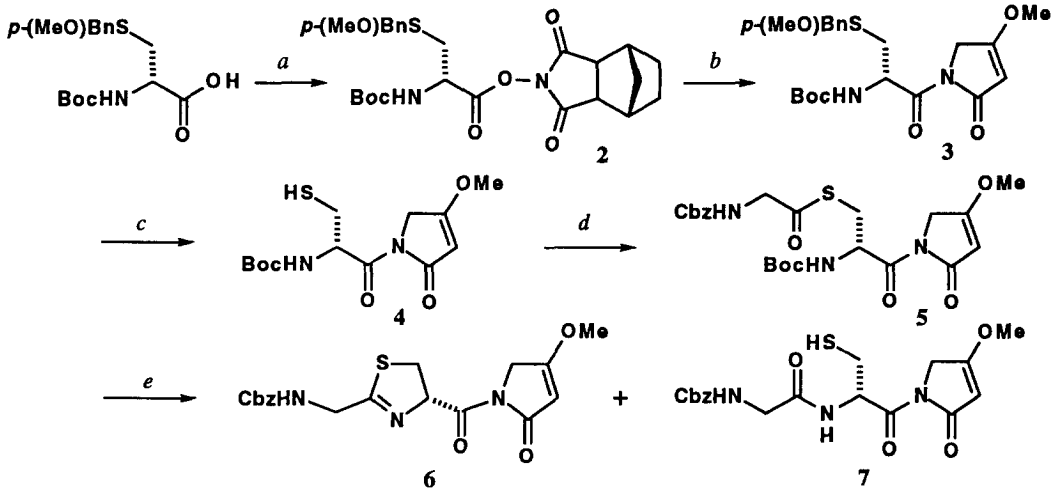
Figure 1. Althiomycin

Using althiomycin (~90% pure) obtained from the Upjohn Pharmaceutical Co. we have attempted to footprint *E. coli* rRNA using dimethyl sulfate (DMS) and the primer extension reaction as described for other antibiotics by Noller and coworkers.[7] While we were able to reproduce the literature results for chloramphenicol, experiments with althiomycin were never sufficiently clean to give interpretable results. This problem appeared to stem from reaction between DMS and the antibiotic. However, another potential problem was the relatively weak affinity of althiomycin for ribosomes, although this compound is a more potent inhibitor of protein synthesis than chloramphenicol.[2] Consequently, we chose to employ an alternative

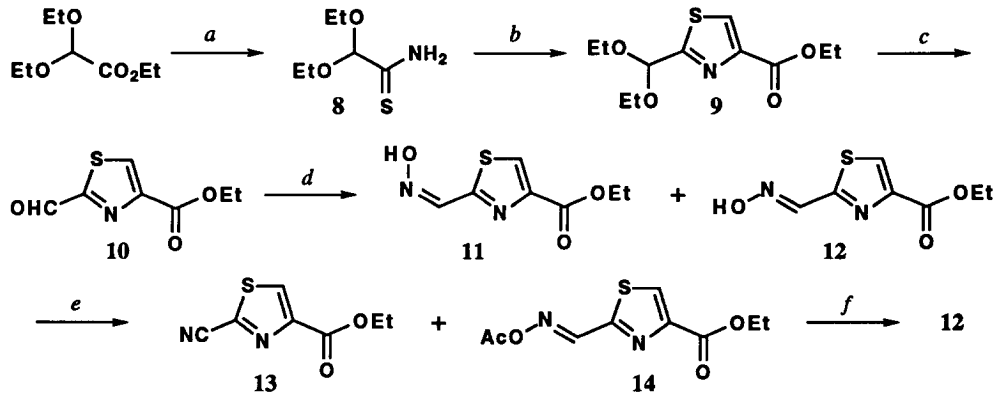
approach wherein an althiomycin affinity label would be used to covalently modify the rRNA so that its location could be determined by sequencing. We describe here our initial studies in this project, culminating in a formal synthesis of althiomycin.

Our original plan was to employ the synthetic scheme used by Shiba in the only reported total synthesis of althiomycin.[8] However, upon encountering problems in attempting to reproduce this chemistry we sought an alternative method for construction of the required thiazoline ring. In their synthesis of tantazole, Fukuyama and Xu prepared two thiazolines by dehydration of *S*-acyl-cysteines.[9] We decided to adapt this approach to our synthesis of althiomycin. To this end, *N*-Boc-*S*-*p*-methoxybenzyl-D-cysteine (Bachem) was converted to the activated ester **2** by condensation with *N*-hydroxy-5-norbornene-*endo*-2,3-dicarboxamide (Scheme 1).



Scheme 1. *a*. DCC, *endo*-*N*-hydroxy-5-norbornene-2,3-dicarboxamide, THF. *b*. NaH, 4-methoxy-3-pyrrolin-2-one, THF, 47% (two steps). *c*. i. $Hg(OCOCF_3)_2$, AcOH. ii. $H_2S_{(g)}$, 74%. *d*. *N*-Cbz-glycine *N*-hydroxy succinimide ester, Et_3N, THF, 73%. *e*. i TFA. ii. PhH, 80 °C.

To form the C-terminal imide, the anion of 4-methoxy-3-pyrrolin-2-one was prepared using sodium hydride in THF under reflux for 30 min. Deprotonation proceeds to give a yellow solution, which was cooled to 0 °C, then added *via* canula to a solution of succinimide ester **2**. Compound **3** was obtained in 47% yield. Removal of the thiol protecting group was achieved using $Hg(OCOCF_3)_2$ in acetic acid. Once all of the starting material had reacted (as indicated by tlc), H_2S gas was bubbled into the reaction mixture to regenerate the free thiol. The mixture turns black immediately upon addition of the gas, however, treatment was continued for 10 min to ensure complete reaction. The thiol (**4**), obtained in 74% yield, gave a positive Ellman's test and was used without further purification. Acylation of the thiol with the *N*-hydroxy succinimide ester of *N*-Cbz-glycine (formed from *N*-Cbz-glycine using DCC and *N*-hydroxy succinimide) gave thioester **5**, which was stable to column chromatography on silica gel. Removal of the Boc group using TFA, and heating in benzene under reflux for 2 h, provided thiazoline **6** in yields ranging from 20-30%.[10] The major product from this reaction (43%) was identified as compound **7**, which presumably results from an acid-catalyzed intramolecular *S*- to *N*-acyl migration reaction.



Scheme 2. *a*. i. $NH_4OH_{(aq)}$, NH_4Cl. ii. P_2S_5, benzene, rt 64%. *b*. EtOH, 4 Å sieves, 89%. *c*. 10% 1 M $HCl_{(aq)}$, acetone, reflux, 76%. *d*. $HCl.H_2NOH$, pyridine, EtOH, reflux, 79%. *e*. Ac_2O, pyrdine, 40% compound **14**. *f*. LiOH, MeOH, 90%.

The thiazole-containing fragment was prepared following the route described by Shiba and coworkers with the following modifications.[8] Ethyl diethoxyacetate was converted to thioamide **8** in two steps. First, treatment with aqueous ammonium hydroxide provided the amide in 55% yield. Then, reaction with P_2S_5 converted the amide to the thioamide in 64% yield. Condensation of this thioamide with ethyl bromopyruvate provided thiazole **9** as reported previously. Acid-catalyzed hydrolysis of the diethyl acetal yielded aldehyde **10**, which was converted to a 1:1 mixture of aldoximes **11** and **12**. Although these diastereomeric products display slightly different chromatographic properties on silica gel they are difficult to separate by column chromatography; it was found that separation could be achieved most readily by reaction of the aldoximes with acetic anhydride in pyridine. Under these conditions, the *Z*-aldoxime undergoes dehydration to form the corresponding nitrile (**13**), while the *E*-aldoxime is converted to the acetyl-derivative **14**. These two products are easily separated by silica gel column chromatography (30% ethyl acetate-70% hexane) and compound **14** can be converted back to diastereomerically pure aldoxime **12** by treatment with lithium methoxide (90%).[11,12]

Compounds **6** and **12** are identical to the intermediates prepared by Shiba and coworkers.[8] Thus, we have completed a formal synthesis of althiomycin *via* a route that is similar to the one described previously with the exception of (a) a modified synthesis of the thiazoline ring and (b) the addition of a practical method for separation of the diastereomeric aldoximes. Future efforts will be directed to employing this new synthetic approach to althiomycins in the construction of potential peptidyltransferase affinity labels.

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6. Molecular modeling of althiomycin was performed using the INSGHT-II/DISCOVER software package (Biosym). A starting structure was generated from the coordinates reported for dehydroalthiomycin.[1b]
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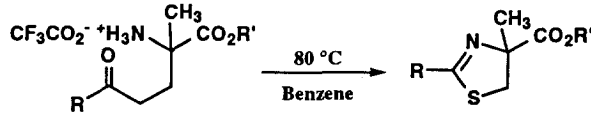
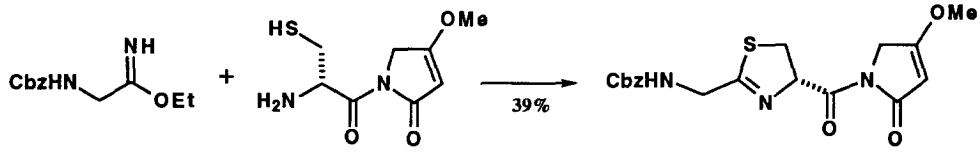


10. Shiba and coworkers prepared the same thiazoline in 39% yield using the following condensation reaction.[8]



11. The two aldoximes may be differentiated by ^{1}H NMR in d_6-DMSO, based on the chemical shift of the oxime proton: *Z*-, δ_{CH} = 13.12 ppm ; *E*-, δ_{CH} = 12.24 ppm.
12. This reaction was found to proceed more cleanly in methanol than in ethanol, even though transesterification occurs with longer reaction times.

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