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A DEHYDROALANINE ROUTE TO AN ACTIVATED PHENOLIC SPARSOMYCIN ANALOG

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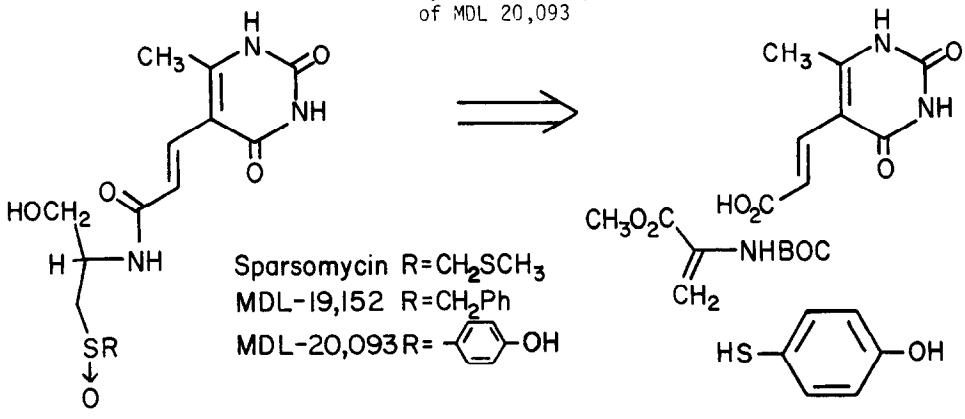
ABSTRACT: The addition of p-mercaptophenol to an N-protected dehydroalanine methyl ester is utilized in the synthesis of novel activated sparsomycin analog MDL 20,093.

Sparsomycin* is a sulfoxide-containing antibiotic of natural origin[1] which is a novel "A-site" inhibitor of ribosomal protein biosynthesis.[2] We have recently proposed a rationale[3] for the observed "preincubation effect" which sparsomycin and its sulfoxide containing analogs demonstrate.[4] Our hypothesis suggests that these unusual kinetics result from a Pummerer rearrangement initiated by a histidine mediated acylation of the sulfoxide moiety at the active site of peptidyl transferase. In our preliminary report[3], the necessity of the sulfoxide moiety for the "preincubation effect" was demonstrated and the incorporation of radiolabel into ribosomes of E. coli incubated in the presence of tritium labeled inhibitor MDL 19,152 was discussed.

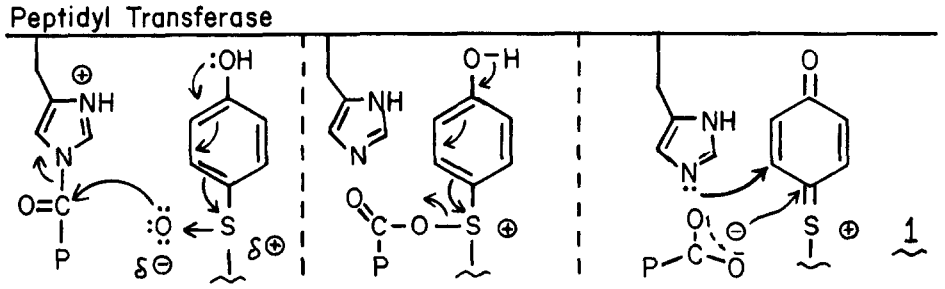
Since the rate-limiting step in the Pummerer rearrangement of sulfoxides is O-acylation, the electron rich phenolic sulfoxide MDL 20,093 was designed to favor this process through direct involvement of the phenol ring which could lead to the reactive sulfenium ion 1 possessing multiple electrophilic sites, see Scheme 1.

Retrosynthetic Analysis of MDL 20,093



*The absolute configuration of sparsomycin is (R_S, S_C).

Scheme 1



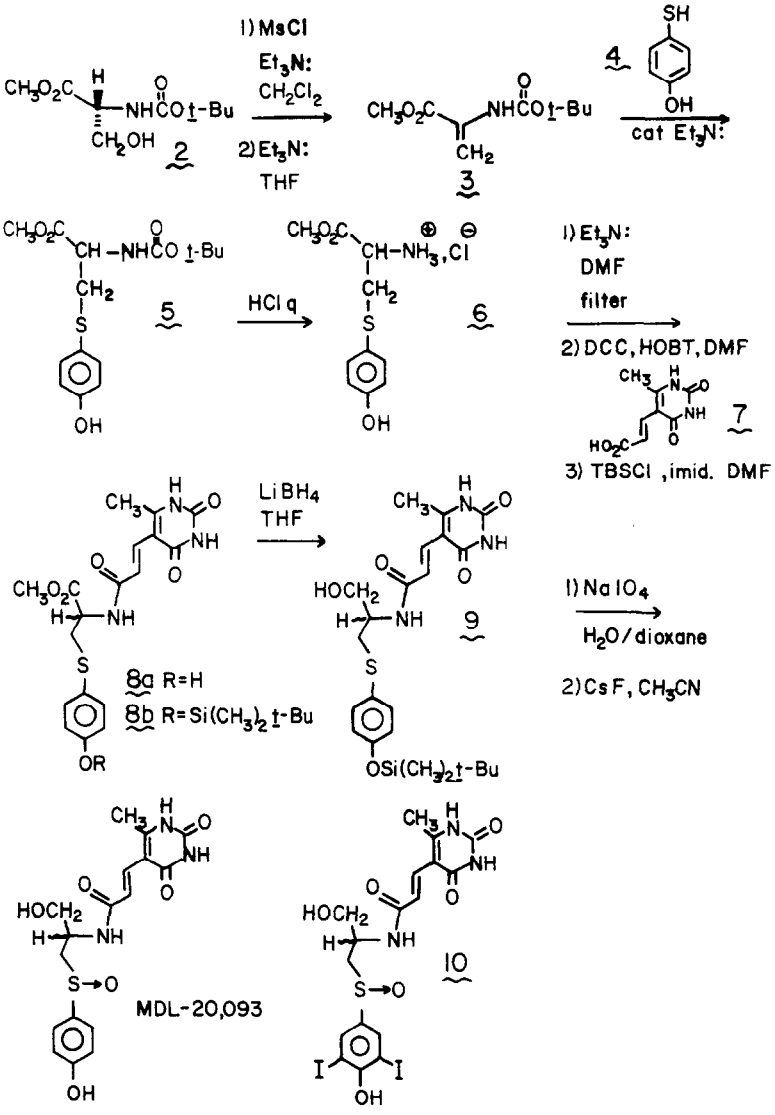
Our choice of the dehydroalanine route, Scheme 2, to phenolic sulfoxide MDL 20,093 was necessitated by the lack of compatible existing methodology[4,6,7] and facilitated by the commercial availability of p-mercaptophenol. N-t-Butoxycarbonyldehydroalanine methyl ester 3 was easily prepared from N-t-butoxycarbonyl-L-serine methyl ester via elimination (Et_3N, THF) of its mesylate (MsCl, Et_3N, CH_2Cl_2,0°) in 87% overall yield. Dehydroalanine 3 could be stored for extended periods at -15°C as a solution in dichloromethane stabilized with hydroquinone.

Conjugate addition of selected thiols to dehydroalanine 3, see Table, indicated that the ease of addition was dependent on the electron density of the nucleophile. Treatment of stoichiometric amounts of p-mercaptophenol 4 and the dehydroalanine 3 in methyl alcohol containing a catalytic amount of triethylamine consistently gave the adduct 5 in 75% chromatographed yield. Treatment of an ethereal solution of 5 with a stream of gaseous HCl quantitatively precipitated the hydrochloride salt 6. Coupling of 6 with 6-methyluracilacrylic acid 7 was affected in 49% yield using standard conditions: A) Et_3N, DMF and B) DCC, HOBT, 7, DMF. The resulting amide 8a was converted to silyl ether 8b (TBSCl, imid., DMF, 73% yield) to facilitate handling. Ester reduction of 8b ($LiBH_4$, THF, 93% yield) afforded alcohol 9 which underwent periodate oxidation ($NaIO_4$, aq. CH_3OH) and silyl ether cleavage (CsF, CH_3CN, ▽) to give a 30-45% yield of diastereomeric MDL 20,093 as an insoluble hygroscopic white powder.[8]

Table 1

Thiol	Conditions	Yield of Adduct
HS-C$_6$H$_4$-OH	18 h	75%
HS-C$_6$H$_5$	24 h	57%
HS-C$_6$H$_4$-NO_2	48 h	58%
$HSCH_2$-C$_6$H$_5$	48 h	81%

Scheme 2


1) MsCl
Et3N:
CH2Cl2
2) Et3N:
THF
4
cat Et3N:
1) Et3N:
DMF
filter
2) DCC, HOBT, DMF
3) TBSCl, imid. DMF
LiBH4
THF
1) NaIO4
H2O/dioxane
2) CsF, CH3CN
8a R=H
8b R=Si(CH3)2t-Bu
MDL-20,093

Example Procedure: Conjugate addition of p-mercaptophenol. To a stirred solution of 25.7 mL (73.3 mM) of a 2.85 M stock solution of N-t-butoxycarbonyl dehydroalanine methyl ester 3 in 50 mL of degassed abs. methanol under argon at 25°C was added 9.25 g (73.3 mM) of p-mercaptophenol 4 and 1 mL of triethylamine. The reaction was monitored by TLC which indicated complete conversion after 18 h. The mixture was concentrated in vacuo and the residue was chromatographed with 5% acetone/CH_2Cl_2 to give 18.0 g (55.0 mM), 75% yield, of adduct 5. A small sample was recrystallized from CH_2Cl_2, m.p. 113-114°C, IR (KBr) 3380, 3310, 1720, 1675, 1530, 1215 cm^{-1}; NMR ($CDCl_3$) δ 1.45 (s, 9H), 3.18 (d, 2H, J=7 Hz), 3.52 (s, 3H), 4.40 (q, 1H, J=7 Hz), 5.37 (d, 1H, J=7 Hz), 6.70 (d, 2H, J=11 Hz), 6.89 (s, 1H), 7.27 (d, 2H, J=11 Hz).

The dehydroalanine route described here complements existing syntheses of sparsomycin analogs and is particularly suited for the synthesis of MDL 20,093. Further investigation into the kinetics of inhibition are required before conclusions can be drawn. The potential for providing an enzyme activated labeling of peptidyl transferase through the radioactive bis-iodo derivative 10 is under active study.

Preliminary kinetic data for MDL 20,093 is summarized in Table 2. Although MDL 20,093 is a poor competitive inhibitor of peptidyl transferase compared to either sparsomycin or MDL 19,152, this compound exhibits a significantly enhanced "preincubation effect." This observation is consistent with our Pummerer mechanism rationale.

Table 2

		% Inhibition[9]		
Compound	Concentration	(-) preincubation	(+) preincubation	(+)/(-)
Sparsomycin	0.25 µM	28	39	1.39
MDL 19,152*	1.0 µM	29	59	2.03
MDL 20,093*	5.0 µM	18	80	4.44

*Compounds are mixtures of all four possible stereoisomers.

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8. As previously reported[6], these coupled products are poorly soluble in organic solvents and purification of end products in this system was exceedingly cumbersome.
9. Inhibitory values were determined in the peptidyl-puromycin reaction described in reference 3.

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