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Studies Toward the Synthesis of a- and B- Mercapto Alanine Derivatives, and of a,B- and B, B- Dimercapto Alanine Derivatives

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STUDIES TOWARD THE SYNTHESIS OF α- AND β-MERCAPTO ALANINE DERIVATIVES, AND OF α,β- AND β,β- DIMERCAPTO ALANINE DERIVATIVES

by

Aldean James Kolar

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I wish to dedicate this thesis to my two sisters, Mary Ann and Irene Marie, and my brother, Dr. Frank who gave both financial and moral support towards the completion of this thesis.

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Aldean J. Kolar

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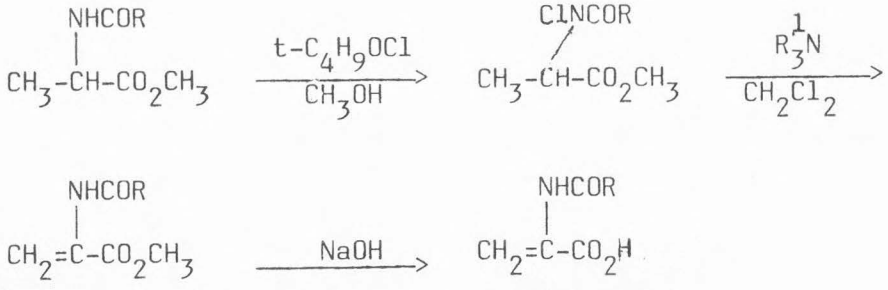
by

Aldean James Kolar, Doctor of Philosophy

Utah State University, 1978

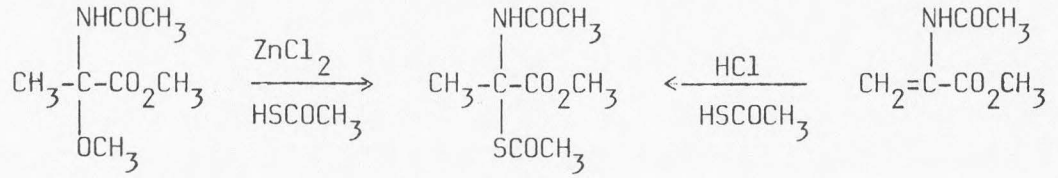
Major Professor: Dr. Richard K. Olsen
Department: Chemistry and Biochemistry

A convenient, economical, large scale synthesis of N-acetyldehydroalanine (α-acetamidoacrylic acid) and its methyl ester was developed via a sequence of N-chlorination-dehydrochlorination. The method was extended to the synthesis of the corresponding N-benzoyl, N-phenylacetyl and N-benzyloxycarbonyl derivatives.

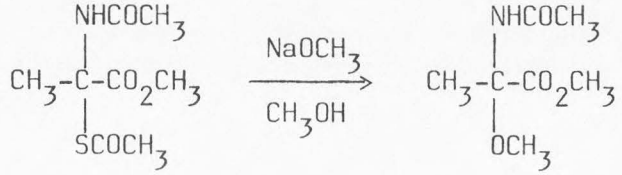


The conversion of α-methoxy-N-acetyl alanine derivatives to the corresponding α-mercapto alanine derivatives, using zinc chloride and an appropriate mercaptan, was investigated. Methyl α-methoxy-N-acetyl-D,L-alaninate was successfully converted to the α-acetylthio derivative,

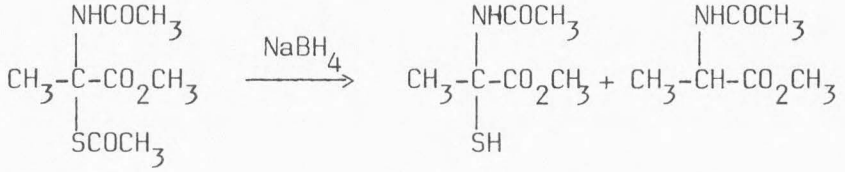
in 24% yield; however, a 90% yield could be obtained by treatment of the dehydroalanine derivative with hydrogen chloride gas in neat thiolacetic acid.



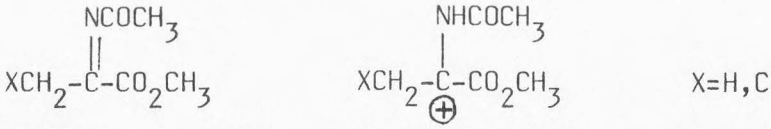
A facile conversion of the α-acetylthio derivative to the α-methoxy derivative, using sodium methoxide in methanol, was observed to occur.



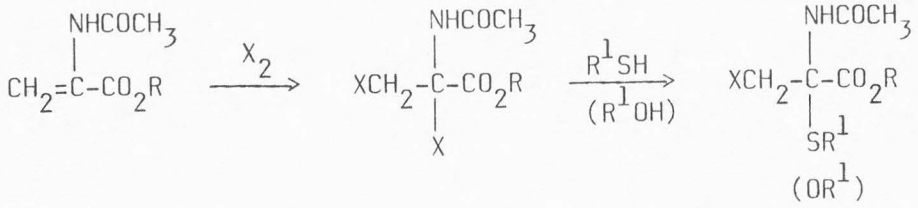
The normally facile conversion of an acetylthio group to a mercapto group, using sodium borohydride, gave mixtures of the α-mercapto derivative and alanine derivative.



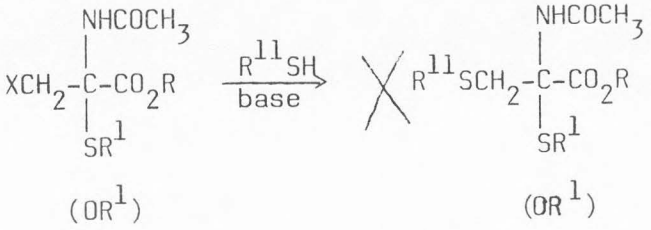
The reactions at the α position of α-hetero-N-acetyl-D,L-alanines and α,β-disubstituted N-acetyl-D,L-alanine derivatives seemed consistent with the formation of an acylimine intermediate under basic conditions and a carbonium ion intermediate under acidic conditions.



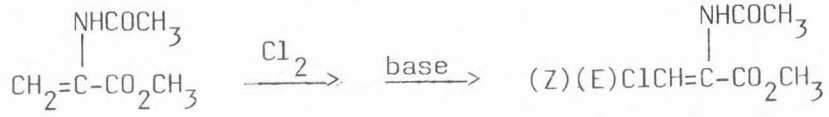
From these studies, a facile, clean synthesis of β-halo-α-mercapto- and α-alkoxy-N-acetyl-D,L-alanine derivatives was accomplished.



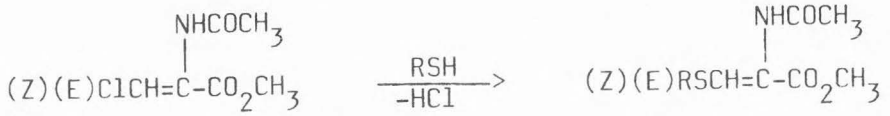
All attempts to synthesize α,β-dimercapto derivatives failed because the β-halogen could not be replaced with a mercapto group when the α position was a mercapto or methoxy derivative. Attempts to generate a β-mercapto-α-halo derivative also failed.



A facile synthesis of the E and Z isomers of methyl β-chloro-N-acetyldehydroalaninate was developed. The ratio of Z to E isomers was found to vary with the base used for the elimination.

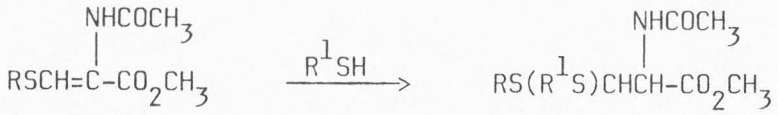


The E and Z isomers of methyl β-chloro-N-acetyldehydroalaninate were converted to the β-mercapto derivatives by reaction with mercaptan. The reaction proceeded with retention of stereochemistry.

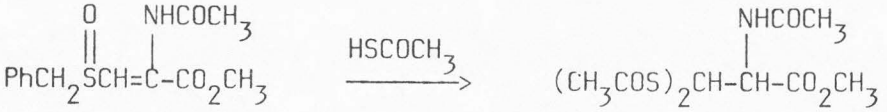


The formation of β-substituted N-acetyldehydroalanine derivatives seemed consistent with an acylimine intermediate followed by a sequence of Michael-type addition and dehydrochlorination.

A study of the conversion of the β-mercapto-N-acetyldehydroalanine derivatives to mixed dithioacetals, which would be useful in the synthesis

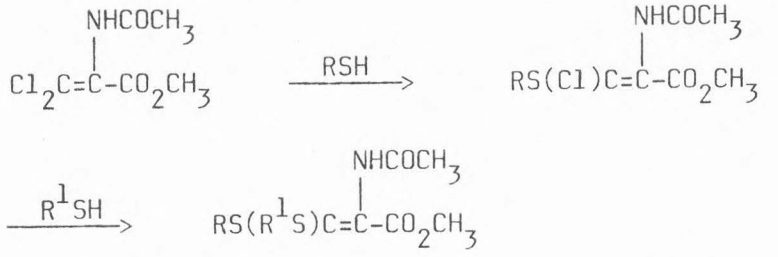


of natural antibiotics, was undertaken. This approach to the synthesis of mixed dithioacetals was unsuccessful because an exchange of mercapto groups was observed, the addition of a second, different mercaptan



failed or the yield was too low to be synthetically useful.

A synthesis of β,β-dimercapto-N-acetyldehydroalanine (mixed, unsaturated dithioacetals) derivatives was accomplished. However, because

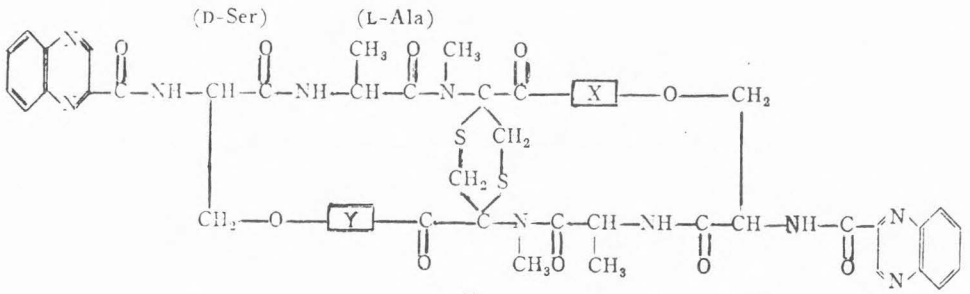


of low yields, a reduction to β,β-dimercapto-N-acetyl-D,L-alanine derivatives (mixed dithioacetals) was not investigated.

(166 pages)

INTRODUCTION

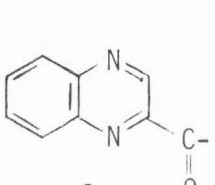
The generic name "quinoxaline antibiotics" was originated by Kuroya and coworkers (1a), in 1961, to designate those "actinomycin-type" antibiotics from streptomyces all of which possessed a quinoxaline moiety. The origination of this generic name coincided with the isolation of the quinoxaline antibiotics quinomycins A, B and C (1). However, it wasn't recognized until 1965 (2), that quinomycin A, echinomycin (3a,b), antibiotic X-948 (3b), levomycin (3c) and actinoleukin (3d,e) were all the same antibiotic. By 1968, the quinomycin family of quinoxaline antibiotics had been isolated: quinomycins A, B_o, C, D, B and E (4) (see Figure 1).



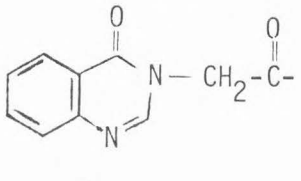
	X	Y
Echinomycin (Quinomycin A)	MeVal	MeVal
Quinomycin B_o	MeVal	DimeAlloileu
Quinomycin C	DimeAlloileu	DimeAlloileu
Quinomycin D	MeVal	MeAlloileu
Quinomycin B	MeAlloileu	MeAlloileu
Quinomycin E	MeAlloileu	DimeAlloileu

Figure 1. Old structure of quinomycins.

Because of their biological properties, the quinomycin family of quinoxaline antibiotics have been of continuing interest since their isolation. The quinomycin family has been reported to be active against gram-positive bacteria (1-5) and gram-negative bacteria (1-4,6) to a lesser extent. Echinomycin has been reported to inhibit the growth of certain protozoa (3a,5a). Echinomycin (3a) and the quinomycin-complex (a 5:1:44 mixture of A:B_o:C) (7) were thought to function as a in vitro virucide with polio and influenza viruses. The quinomycin family of quinoxaline antibiotics were reported to be powerful antitumor agents, in vitro and/or in vivo (1,3-6,8). Although these biological properties were not observed with 2-quinoxalinecarboxylic acid (7), echinomycin with one or both of the 2-quinoxalinecarboxyl residues (1) replaced by a quinazol-4-one-3-acetyl residue (2) or by a 2-quinolinecarboxyl residue (3) possessed similar biological properties (8c,9).



1



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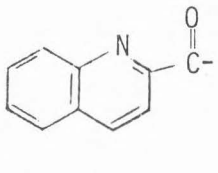

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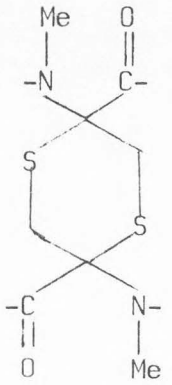
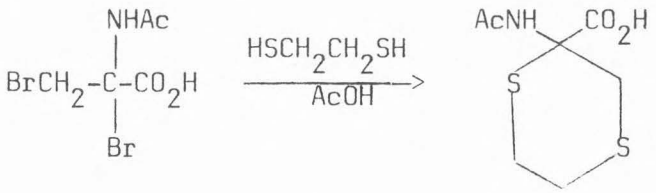
Investigations, since 1965, have shown that echinomycin inhibits DNA directed enzymic RNA synthesis (4c) by preferential inhibition of chain elongation at low concentrations; high concentrations also inhibited chain initiation (6b,10). This inhibition of RNA synthesis by echinomycin was reported, in 1974 (5b,8f), to involve intercalation, i.e., insertion, during the binding to DNA. Furthermore, this intercalation was of a bifunctional nature. Thus, echinomycin is the first bifunctional intercalating agent ever found to occur in nature.

In 1959, Keller-Schierlein and coworkers (11) reported the in depth structural determination of echinomycin by chemical means. The structure of echinomycin was reported as in Figure 1. In 1966, based upon Keller-Schierlein and coworkers' works, Otsuka and Shoji (4b,12) assigned the complete structures for all the quinomycins (see Figure 1). The main point of interest in the structure of the quinomycin family of quinoxaline antibiotics, as far as this research project was concerned, was the presence of the 1,4-dithiane (4) moiety interconnecting the amino acids.



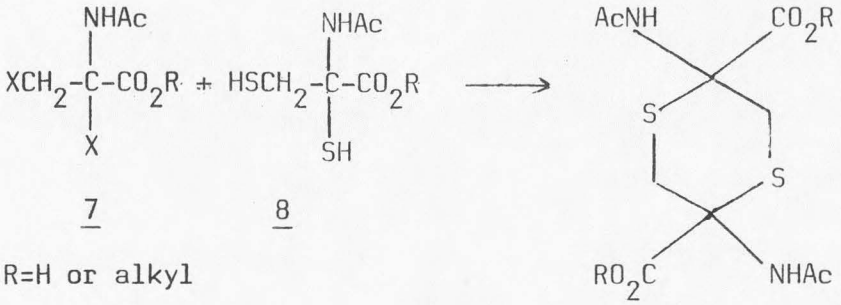
4

Olsen (13) prepared 2-acetamido-1,4-dithiane-2-carboxylic acid (6) by the reaction between α,β-dibromo-N-acetylalanine (5) and 1,2-ethanedithiol in acetic acid. Similarly, it may be proposed that



5 6

reaction between the α,β-dihalo-N-acetylalanine derivative (7) and the α,β-dimercapto-N-acetylalanine derivative (8) might give the 2,5-diacetamido-1,4-dithiane-2,5-dicarboxylic acid or ester derivative (9).



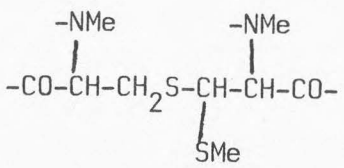
7 8

R=H or alkyl

9

Therefore, it was of interest to undertake studies toward the synthesis of α,β-dimercapto-N-acetylalanine derivatives. Furthermore, extending the work of Patel (14), the synthesis of α-substituted N-acetylalanine derivatives were studied as a basis for a stepwise preparation the α,β-derivatives.

However, this interest in α,β- and α-alanine derivatives immediately ended in mid 1975 when Martin and coworkers (8h) followed by Dell and coworkers (15) published undisputed results correcting the structure of the quinomycin family of quinoxaline antibiotics. Specifically, echinomycin and quinomycin C were observed to contain a thioacetal moiety (10) instead of the 1,4-dithiane (4) moiety interconnecting the amino acids. This "new structure" (see Figure 2) was supported by PMR, ^{13}C N.M.R.



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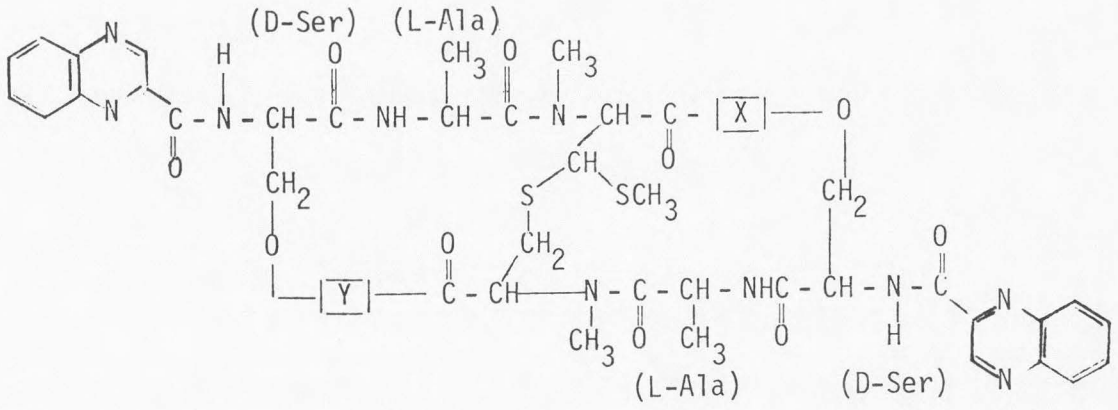


Echinomycin (Quinomycin A)	X	Y
Q-B	MeVal	MeVal
Q-C	MeVal	Dime Alloileu
Q-D	Dime Alloileu	Dime Alloileu
Q-B	MeVal	MeAlloileu
Q-E	MeAlloileu	MeAlloileu
	MeAlloileu	Dime Alloileu

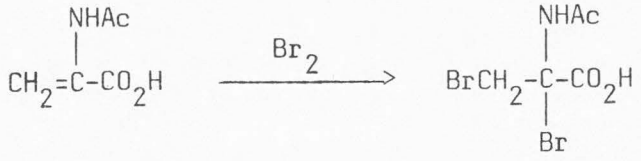
Figure 2. New structure of quinomycins.

and field desorption mass spectrometry (M^+ at 1100 for new echinomycin versus 1085 old structure). Furthermore, this structural revision allowed acceptable explanations of a few abnormalities noted by Keller-Schierlein and coworkers in their previously proposed structure (15).

Since this structural revision may be visualized as a β,β-disubstituted alanine derivative, it was of interest to undertake studies toward the synthesis of β and β,β-dimercapto-N-acetylalanine derivatives.

Our approach to the synthesis of both the α,β-derivatives and the β,β-derivatives involved the reactions of the unsaturated double bond of N-acetyldehydroalanine (11) and its esters. The syntheses were either direct additions to this double bond, or substitutions in the halo

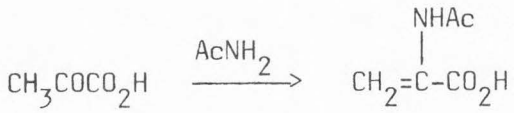
derivatives like 5, which was prepared by halogenation of the double bond of N-acetyldehydroalanine (11). Therefore, the preparation of



11 5

N-acetyldehydroalanine (11) and its esters was a basic starting point in our syntheses.

Since N-acetyldehydroalanine (11) was prepared from pyruvic acid, the loss of a commerical source of high purity pyruvic acid made



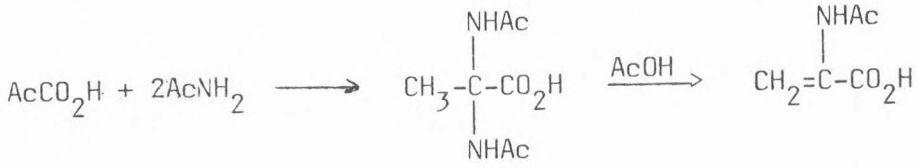
11

it necessary to seek an alternate synthesis of 11 and its esters. The method developed accomplishes this goal. Furthermore, it is relatively convenient and is presently the most economical of the available alternatives.

LITERATURE REVIEW

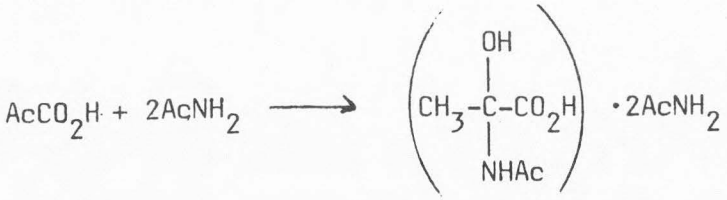
Syntheses of N-acetyldehydroalanine (α-acetamidoacrylic) derivatives

Bergmann and Grafe (16) prepared N-acetyldehydroalanine (11) by the condensation reaction between pyruvic acid and acetamide. Two equivalents of acetamide were heated, neat, under vacuum with one equivalent of pyruvic acid. The intermediate bis compound (12) was separated from some 11, which also formed, and was refluxed with glacial acetic acid to produce 11. This approach has been reported by several



12 11

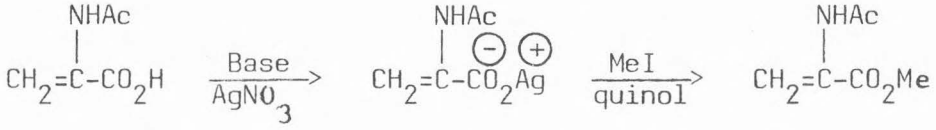
researchers using various conditions of temperature and pressure (17). The scale of the reaction was generally 0.2 moles with a production of 11, in about 50% (Rothstein (17a) reported a 4.4 mole scale, but only a 17% yield). Herbst (17b) reported complex (13) as the intermediate in the reaction, which could be selectively converted to the bis compound (12) or to the product (11).



13

Kildisheva and coworkers (18a) reported the most comprehensive study of the only other common method of performing the condensation to N-acetyldehydroalanine (11). Excess pyruvic acid was refluxed with acetamide in benzene in a Dean-Stark apparatus to effect azeotropic removal of the water formed during the reaction to 11 without isolation of 12. Wieland and coworkers (18b) reported similar results using 1,1,2-trichloroethane as solvent. However, when toluene (19) was used as solvent, the bis compound (12) was isolated and had to be converted to 11 by refluxing with glacial acetic acid.

Rothstein (17a) prepared methyl N-acetyldehydroalaninate (methyl α-acetamidoacrylate) (14) by formation of the silver salt of the acid (11) and refluxing the salt with methyl iodide in the presence of quinol as a polymerization inhibitor. Wieland and coworkers (18b) also prepared 14 by this method but no polymerization inhibitor was added.



11 14

Not only did Adams and coworkers (20) report the preparation of 14 using the silver nitrate, methyl iodide sequence, they noted that attempts to form 14 via other reactions failed or formed an undesired product (see Figure 3). Furthermore, they reported that methyl N-acetyldehydroalaninate (14) polymerized easily upon standing at room temperature.

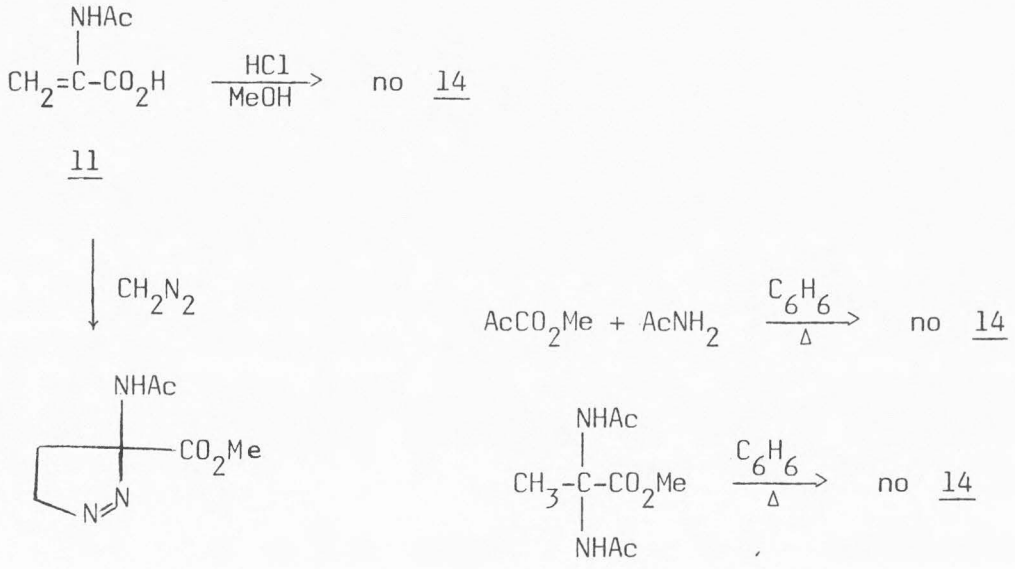


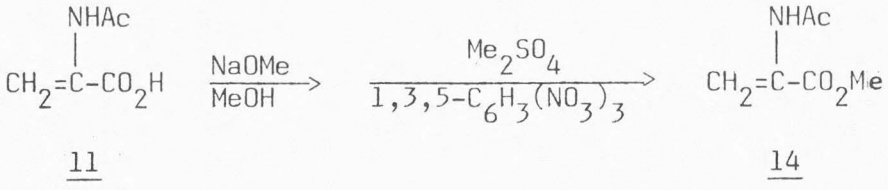
Figure 3. Unproductive attempts to prepare 14.

Rothstein (17a) also prepared methyl N-acetyldehydroalaninate (14) by the reaction of the acid (11) with sodium carbonate, sodium methoxide and dimethyl sulfate by refluxing in methanol with quinol as inhibitor. Increasing the reflux time from two to twenty hours reduced the observed

11 14

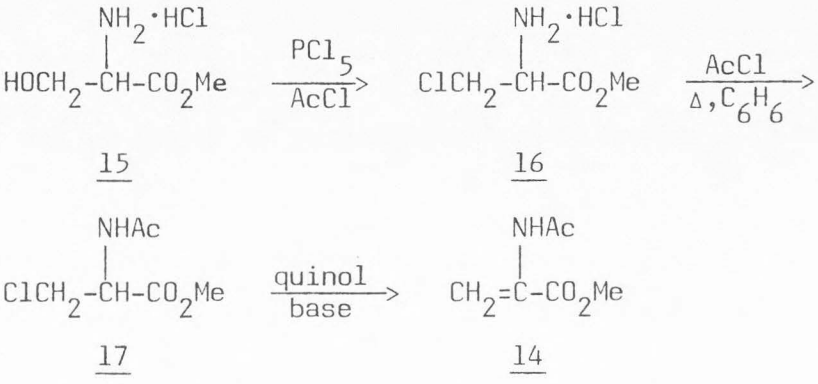
yield. Rothstein noted that 14 polymerized without the presence of quinol.

Coover and Dickey (21) prepared the ester (14) by the reaction of the acid (11) with sodium methoxide and dimethyl sulfate in refluxing methanol with 1,3,5-trinitrobenzene as a polymerization inhibitor. However, their reported melting point was twenty-five degrees higher than that normally observed for the ester (14).



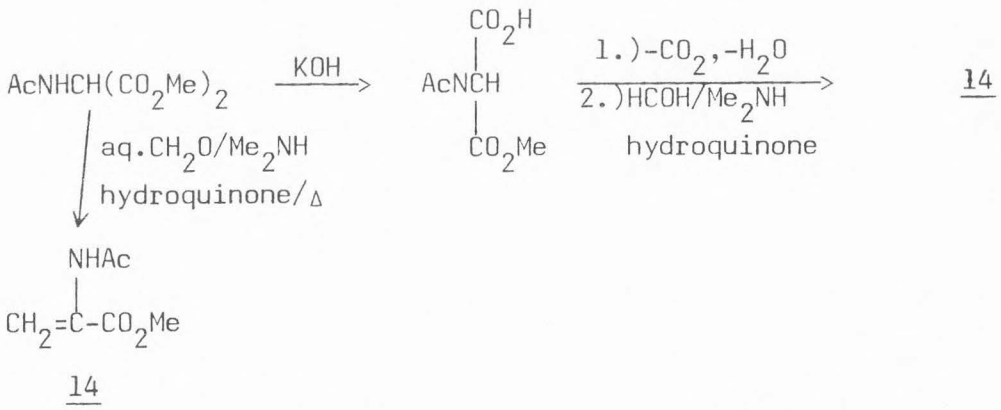
11 14

Additionally, Rothstein (17a) prepared methyl N-acetyldehydroalaninate (14) from D,L-serine methyl ester hydrochloride (15) by the following procedure.



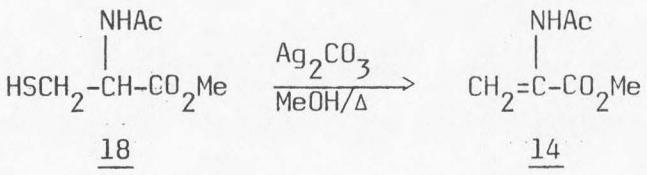
15 16 17 14

Hellmann and coworkers (22) prepared the ester (14) from the acetamide of dimethyl malonate according to the following procedures.



14

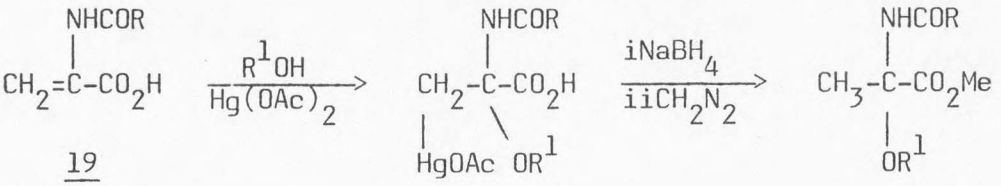
Finally, Gravel and coworkers (23) reported the preparation of the ester (14) by the elimination of hydrogen sulfide from the corresponding cysteine derivative (18) with silver carbonate.



18 14

Syntheses of α-substituted N-acylalanine derivatives

Gallina and coworkers (24) prepared the methyl α-alkoxy-N-acylalaninates (20) from the corresponding N-acyldehydroalanines (19) by a sequence of alkoxymercuration-demercuration followed by esterification with diazomethane. The alkoxymercuration-demercuration sequence was

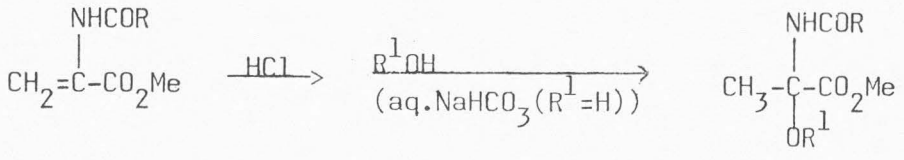


19 20

R	R^1	Yield(%)
C_6H_{11}	Me	12
Ph	Me	20
CH_2OPh	Me	29
CH_2Ph	Me	82
CH_2Ph	Et	16
CH_2Ph	CH_2Ph	0
CH_2Ph	$CHMe_2$	0

very sensitive to structural changes in both the N-acyl- and the α-alkoxy groups.

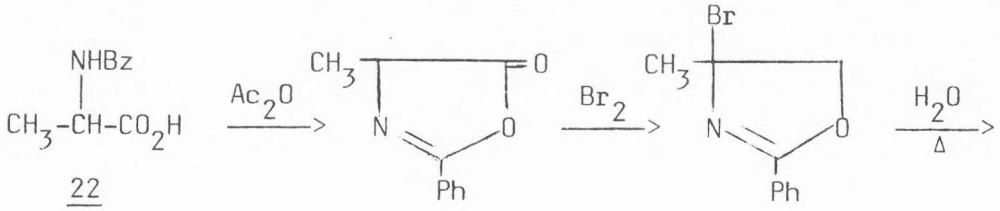
Lucente and Rossi (25) synthesized the methyl α-alkoxy- and α-hydroxy-N-acylalaninates (20) from the corresponding methyl N-acyldehydroalaninates (21) by a sequence of hydrochlorination-substitution. This synthetic approach was sensitive to the nature of the N-acyl moiety.



21 20

R	R^1	Yield(%)
CH_2Ph	H	60
CH_2Ph	Me	70
CH_2Ph	Et	45
Me	Me	15

Chaman and Shemyakin (26) prepared α-hydroxy-N-benzoylalanine (23) from N-benzoylalanine (22) via the azlactone. It was reported that 23



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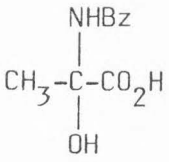
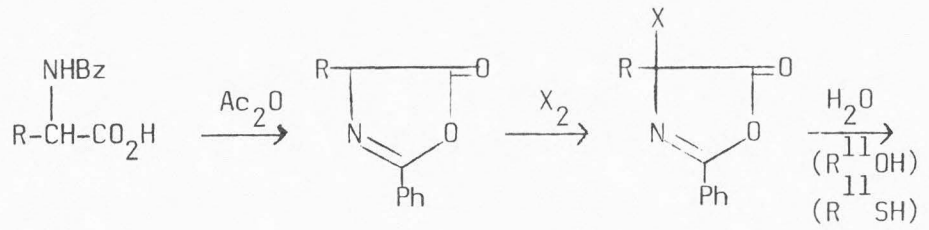
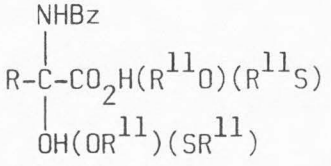


23

was readily hydrolyzed to pyruvic acid and benzamide in the presence of aqueous sodium bicarbonate or aqueous alcoholic hydrogen chloride.

Chemiakine and coworkers (27) reported the synthesis of various α-hydroxy, α-alkoxy and α-arylthio-N-benzoylamino acid derivatives from the azlactones by a sequence of halogenation followed by treatment with water, alcohols or mercaptans. Some of the free acids of the α-hydroxy



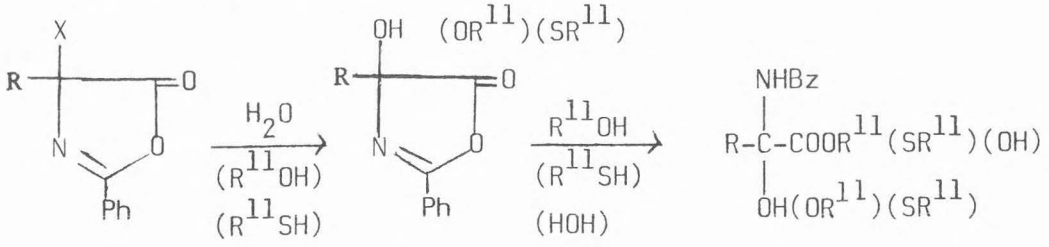


$R^{11}O$=OMe; OEt; $O(CH_2)_2Me$; $O(CH_2)_{11}Me$; $OCHMe_2$; $OCMe_3$; OCH_2Ph; OPh

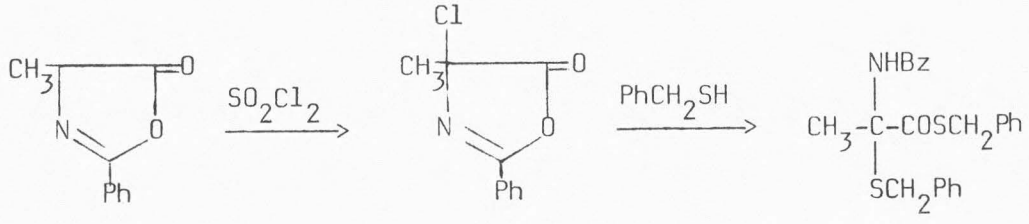
$R^{11}S$=SCH_2Ph; SPh

R=H; Me; Et; $CHMe_2$; $(CH_2)_3Me$

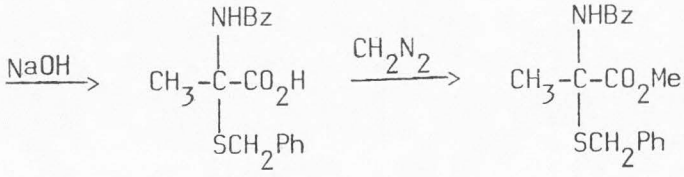
compounds were converted to the corresponding methyl esters with diazomethane. Some of the α-alkoxy and α-arylthio-compounds were converted to the corresponding free acids with dilute sodium hydroxide solution. Alternately, a sequence of substitution of the 4-halo group for a hydroxy, alkoxy or arylthio group followed by hydrolysis of the azlactone in the presence of water, an alcohol or a mercaptan gave respectively an acid, an ester or a thiolester.



Pojer and Rae (28) attempted to prepare methyl α-mercapto-N-benzoyl-alaninate (27) from 4-methyl-2-phenyl-2-oxazolin-5-one (24). They reported that attempts to prepare the free acid (25) directly instead of

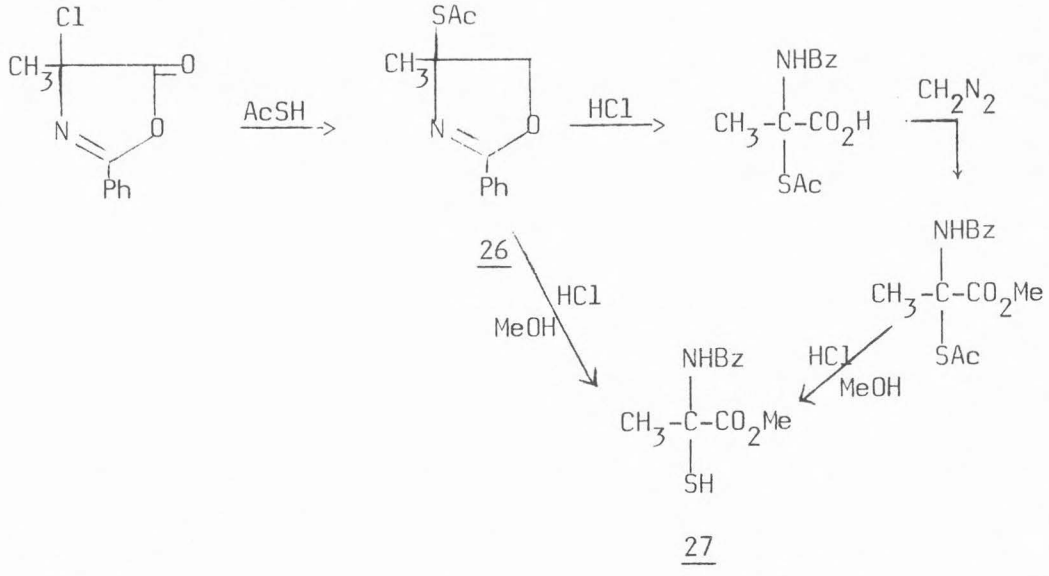


24



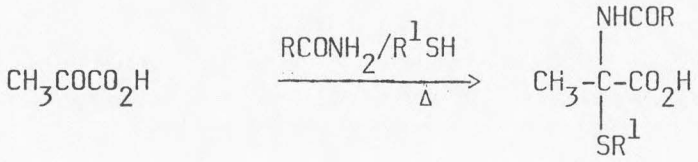
25

via the benzylthiol ester failed, 25 decomposed under other esterification reagents and all attempts to cleave the benzyl group failed. However, the corresponding acetylthio derivative could easily be converted



to product 27. Alternatively, 4-acetylthio-4-methyl-2-phenyl-2-oxazolin-5-one (26) could be converted directly to 27 with saturated methanolic hydrogen chloride solution.

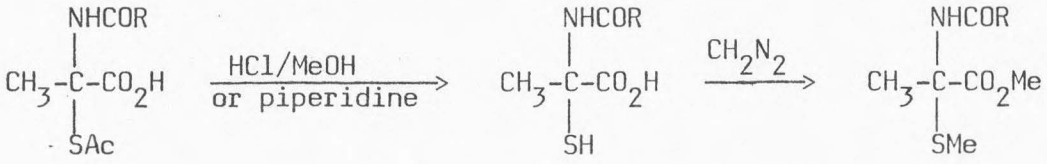
Several workers (29) have prepared α-alkylthio-N-acylalanines by the reaction of pyruvic acid, an appropriate amide and a mercaptan. Although the yields were low, only the trifluoro derivative did not



R=Me, H, OEt, OCH_2Ph, Ph, CF_3

R^1=Ac, CH_2Ph

form. The corresponding free thiol derivative was prepared by cleavage of the acetyl group with methanolic hydrogen chloride or piperidine (29b) (sodium methoxide and sodium tetrahydridoborate gave tars). Direct esterification of the α-mercapto derivative with diazomethane gave the O,S dimethylated product.



Patel and coworkers (30) synthesized α-alkylthio-N-acetyl- and N-benzoylalanines from the corresponding dehydroalanine derivative by a sequence of hydrogen chloride addition to the double bond followed by substitution with the appropriate mercaptan. The α-acetylthio derivative

19

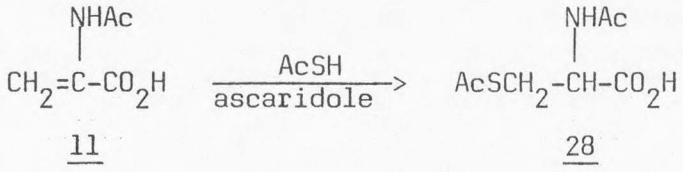
R=Me, Ph, OCH_2Ph

R^1=H, Ac, $CHPh_2$

could be converted to the α-mercapto derivative (R^1=H) by cleavage with methanolic hydrochloric acid. Similarily, the α-benzhydrylthio derivative (R^1=$CHPh_2$) could be cleaved to the α-mercapto derivative (R^1=H) with trifluoroacetic acid (TFA). The α-mercapto-N-benzyloxycarbonyl derivative (R=OCH_2Ph, R^1=H) was unstable when isolated but could be generated in situ (see 29b also).

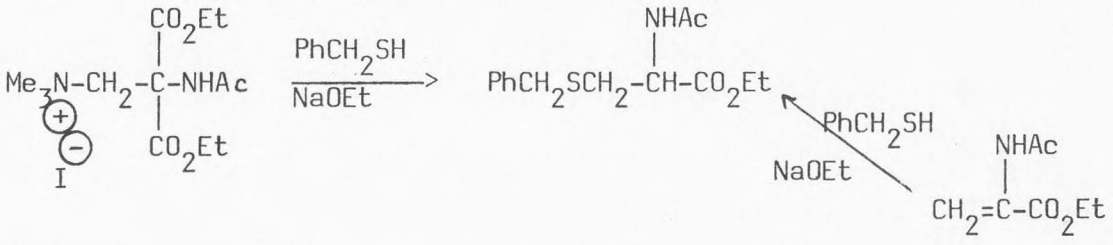
Syntheses of
β-substituted alanine derivatives

Farlow (31) prepared β-acetylthio-N-acetyl-D,L-alanine (28) by the free radical addition of thiolacetic acid to N-acetyldehydroalanine (11).

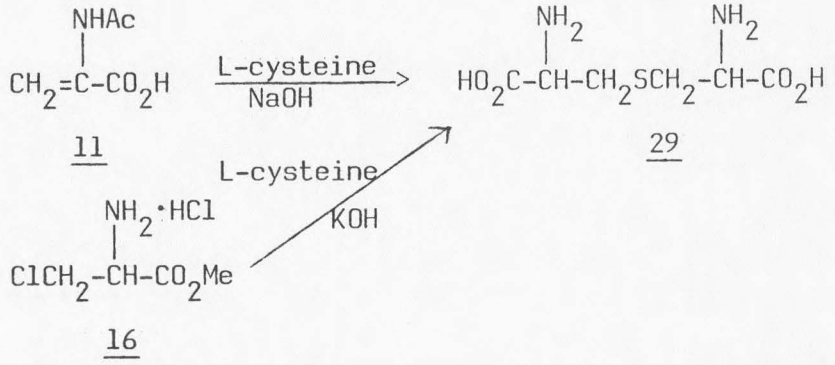


11 28

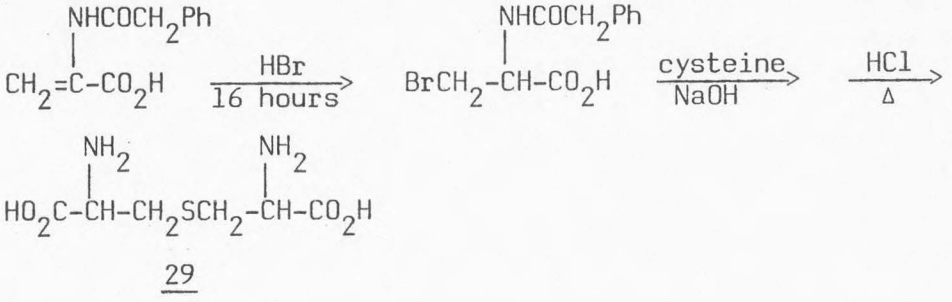
Similarily, Hellmann and Folz (32) made ethyl β-benzylthio-N-benzoyl-alaninate by the addition of benzyl mercaptan to both the corresponding dehydroalanine derivative and the Mannich base of dimethyl aminomethyl-acetaminomalonic acid diethyl ester under basic conditions.



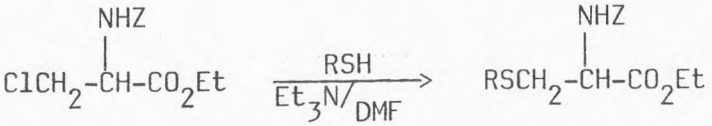
Stereochemical derivatives of the amino acid lanthionine (29) have been prepared both by the addition of L-cysteine to N-acetyldehydroalanine (11) (33) and by the substitution reaction between L-cysteine and β-chloro-D,L-alanine methyl ester hydrochloride (16) (34).



Knunyants and Shokina (35) prepared lanthionine (29) by a combination of addition of hydrogen bromide to N-phenylacetyldehydroalanine followed by substitution with cysteine.

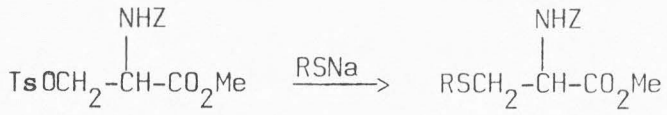


Wilchek and coworkers (36a) reported preparing various β-sulfides of ethyl N-benzyloxycarbonylalaninate using the general approach of nucleophilic substitution in the β-chloro derivative. This nucleophilic substitution approach was also applied by these workers (36b) to the



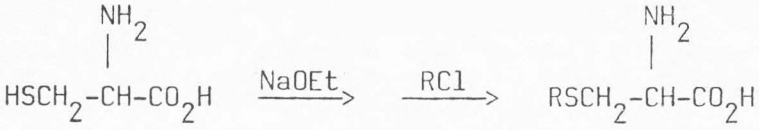
R=Ac, PhCO, $PhCH_2$

β-tosyl derivatives of serine. The mercaptans were first converted to their sodium salts with sodium methoxide in methanol before reaction.



R=Ac, $PhCH_2$, CH_2CO_2H

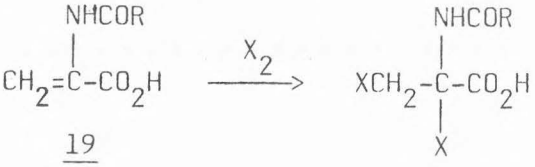
Theodoropoulos (37) reported the preparation of β-sulfides of alanine by the substitution reaction between the anion of cysteine and an added alkyl chloride.



R=CH_2Ph, Bu, Et, Me, CPh_3

Syntheses of selected α,β-disubstituted alanine derivatives

Kildisheva and coworkers (18a) prepared the α,β-dihalo derivatives of N-acylalanine by the addition of chlorine or bromine to the double bond of the corresponding N-acyldehydroalanine derivatives (19). The dichloro derivatives were more stable than the dibromo derivatives to

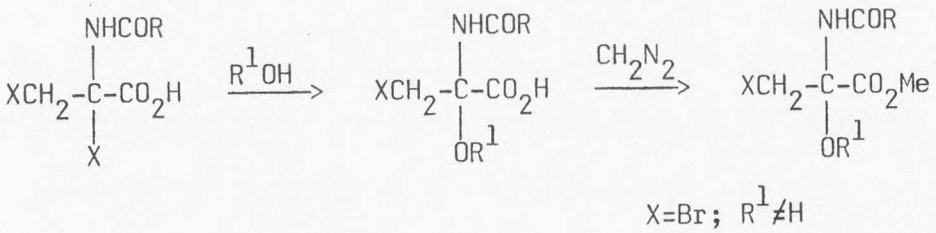


R=Ph, CH_2Ph, OCH_2Ph

X=Cl, Br

hydrolysis with hot dilute base (both bromine atoms could be removed, but only one chlorine atom was removed under these conditions).

These dihalo derivatives were easily converted to the corresponding β-halo-α-alkoxy or α-hydroxy derivatives with water (aqueous sodium bicarbonate) or alcohols (37). The β-chloro-α-hydroxy derivatives



X=Br; $R^1 \neq H$

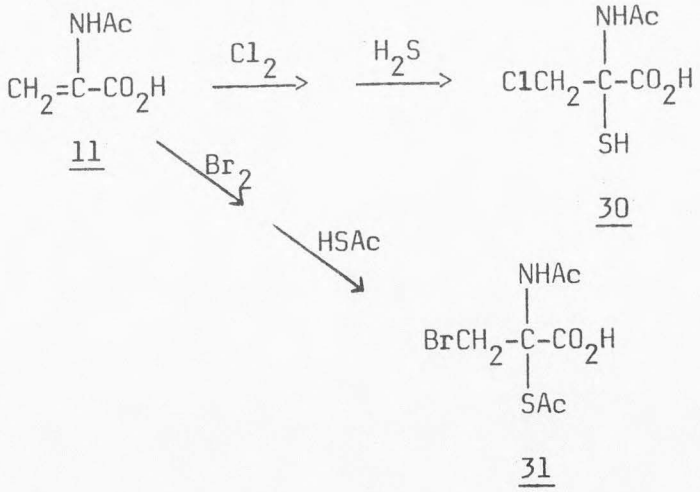
R^1=H, Me, Et

R=Ph, CH_2Ph

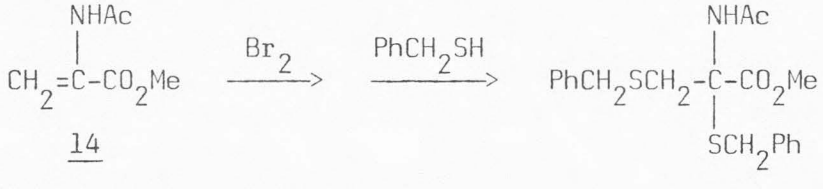
X=Cl, Br

were more stable than the β-bromo-α-hydroxy derivatives to alkaline and acid hydrolysis. The β-halo-α-alkoxy derivatives were all more stable towards purification than the corresponding α-hydroxy derivative and could be converted to their methyl esters with diazomethane (in contrast, only the β-chloro-α-hydroxy derivatives underwent esterification with diazomethane).

Patel and coworkers (30) synthesized β-chloro-α-mercapto-N-acetylalanine (30) and β-bromo-α-acetylthio-N-acetylalanine (31) by the addition of halogen to N-acetyldehydroalanine (11) followed by treatment with hydrogen sulfide or thiolacetic acid. The β-bromo-α-mercapto-N-acetylalanine derivative was unstable and could not be isolated.

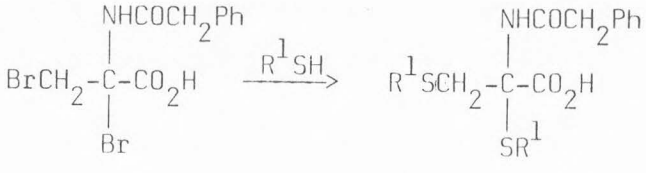


Patel (14) prepared methyl α,β-dibenzylthio-N-acetylalanine (32) by the addition-substitution sequence from methyl N-acetyldehydroalaninate (14). Only the mono-α-acetylthio derivative (31) was obtained with excess thiolacetic acid.



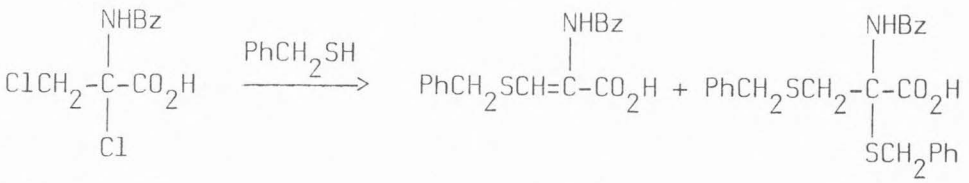
14 32

Similarly, Kildisheva and coworkers (38a) prepared the α,β-dibenzylthio (34) and the α,β-diethylthio (35)-N-phenylacetylalanine derivatives from the dibromo compound (33). An attempt to prepare the



33 34(R^1=$PhCH_2$) 35(R^1=Et)

α,β-dibenzylthio derivative from α,β-dichloro-N-benzoylalanine (36) gave a mixture of the unsaturated derivative (37) and product (38). Excess ethylmercaptan and the dichloro compound (36) gave only the



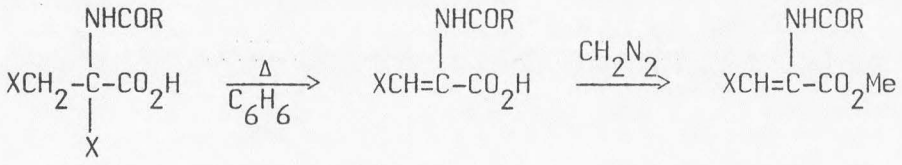
36 37 38

α-ethylthio derivative (39) (38b).

36 39

Syntheses of
β-substituted N-acyldehydroalanines

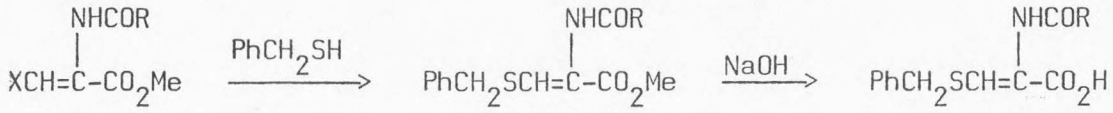
Kildisheva and coworkers (39a) reported the synthesis of β-halo-N-acyldehydroalanines from the corresponding α,β-dihalo derivatives by refluxing in benzene. The free acids were converted to their methyl esters with diazomethane.



R=Ph, CH_2Ph, OCH_2Ph

X=Cl, Br

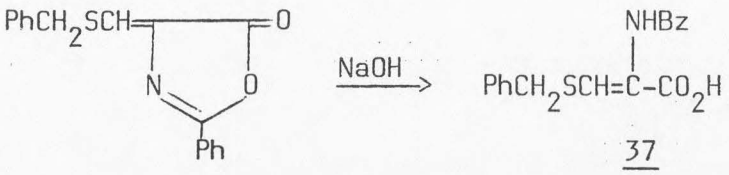
Treatment of the methyl β-halo-N-acyldehydroalaninates with benzyl mercaptan in liquid ammonia gave the corresponding β-benzylthio derivatives (39b). Alkaline hydrolysis gave the corresponding free acids. The stereochemistry of the double bond was not determined.



R=Ph, CH_2Ph

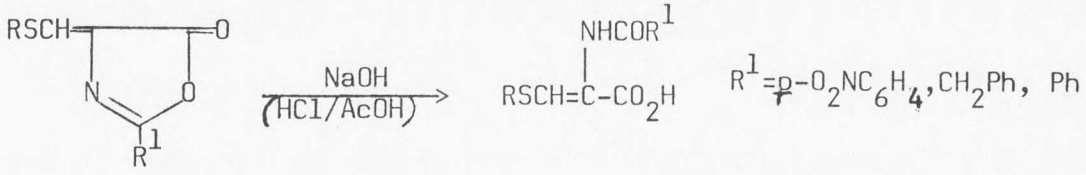
X=Cl, Br

Baltazzi and Davis (40) prepared β-benzylthio-N-benzoyldehydroalanine (37) from 2-phenyl-4-benzylthiomethylene-5-oxazolone by alkaline hydrolysis. This general approach for the preparation of β-sulfides of



37

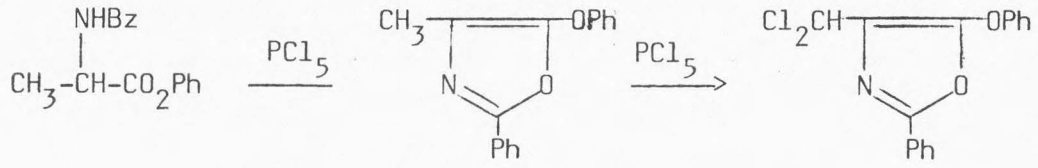
N-acyldehydroalanine derivatives had been used by Cornforth (41). Both alkaline and acid hydrolysis of the oxazolone gave the same product for



R=Et, $PhCH_2$

which the stereochemistry was not determined.

Wieland and coworkers (42) prepared phenyl β-chloro-N-benzoyldehydroalaninate from phenyl N-benzoylalaninate via oxazole (40). The phenyl ester was converted to the methyl ester by acid catalyzed transesterification, however, the stereochemistry of the double bond was not established.



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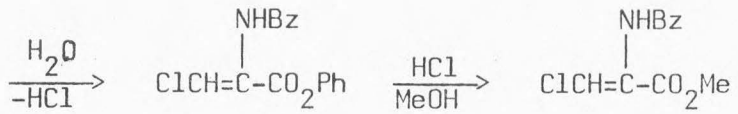
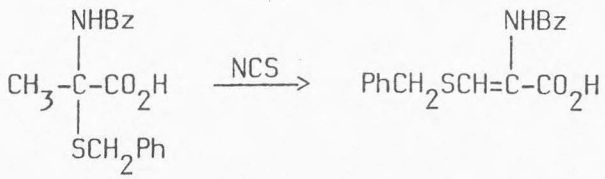
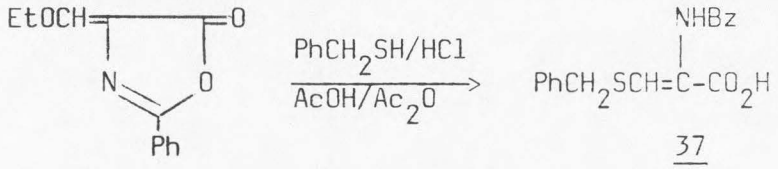


Pojer and Rae (43) reported that an attempt to cleave the benzyl protecting group from the sulfur of the α-benzylthio derivative (25) with N-chlorosuccinimide (NCS) gave β-benzylthio-N-benzoyldehydroalanine (37) (an E,Z mixture of isomers). This compound could also be prepared



25 37(E,Z)

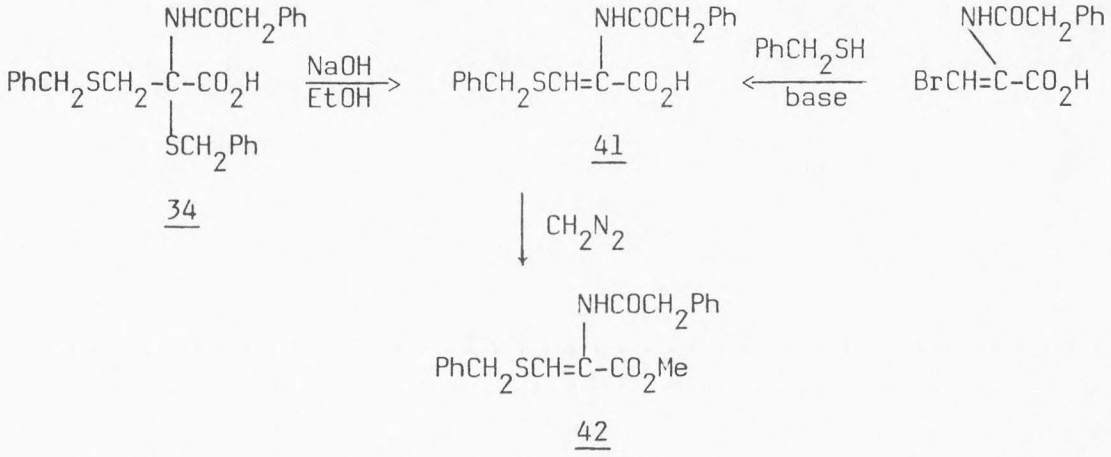
from the appropriate azlactone, however, only a single isomer was obtained. The methyl ester of 25 did not undergo this reaction with NCS



37

and 37 could not be prepared by the addition of toluene sulfenyl chloride to N-benzoyldehydroalanine (19, R=Ph).

Kildisheva and coworkers (38a) reported that the α,β-dibenzylthio compound (34) was converted to the β-benzylthio unsaturated compound (41) when treated with ethanolic sodium hydroxide. A sample of this compound was also prepared by the reaction between benzyl mercaptan and

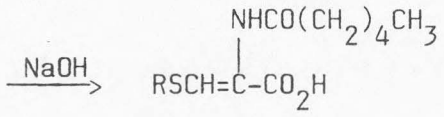


34 41 42

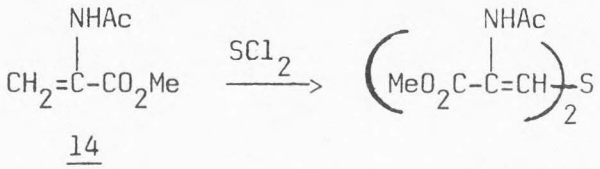
β-bromo-N-phenylacetyldehydroalanine. The methyl ester (42) was made by esterification with diazomethane, however, the stereochemistry of the acid and its ester was not determined.

Cornforth and Huang (44) synthesized the β-sulfides of N-acyldehydroalanines from the potassium salt of ethyl α-(1-ethoxyhexylideneamino)-β-hydroxyacrylate with hydrogen chloride gas and a mercaptan. Alkaline hydrolysis gave the corresponding free acids.

R=$PhCH_2$, Et

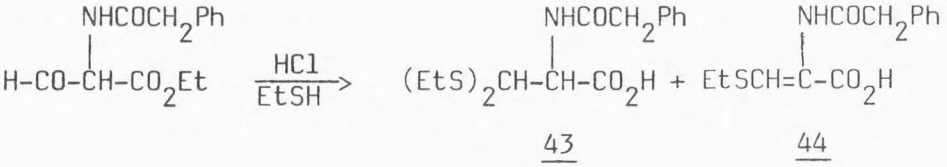


Love and Olsen (45) reported the preparation of the β-sulfide of methyl N-acetyldehydroalanine (14) by treatment of 14 with sulfur dichloride



14

Brown (46) reported that an attempt to prepare the dithioacetal (43) of ethyl benzylpenaldate with hydrogen chloride in ethyl mercaptan gave a mixture of products. However, the dithioacetal (43) was separable from β-ethylthio-N-phenylacetyldehydroalanine (44).

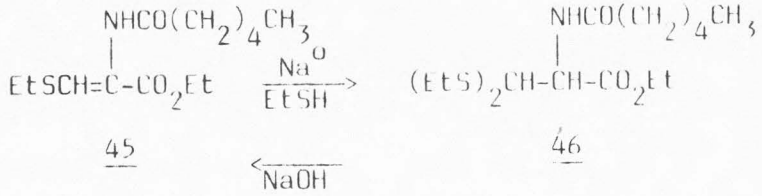


43 44

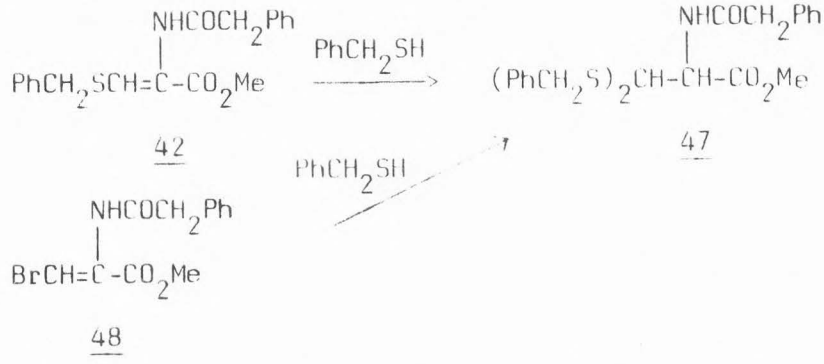
Syntheses of
β,β-disubstituted N-acylalanines

Cornforth and Huang (44) reported that ethyl β-ethylthio-N-caproyldehydroalaninate (45) when treated neat with sodium metal and ethyl mercaptan gave the β,β-diethylthio derivative (46). However, hot one normal sodium hydroxide solution converted the diethylthio-

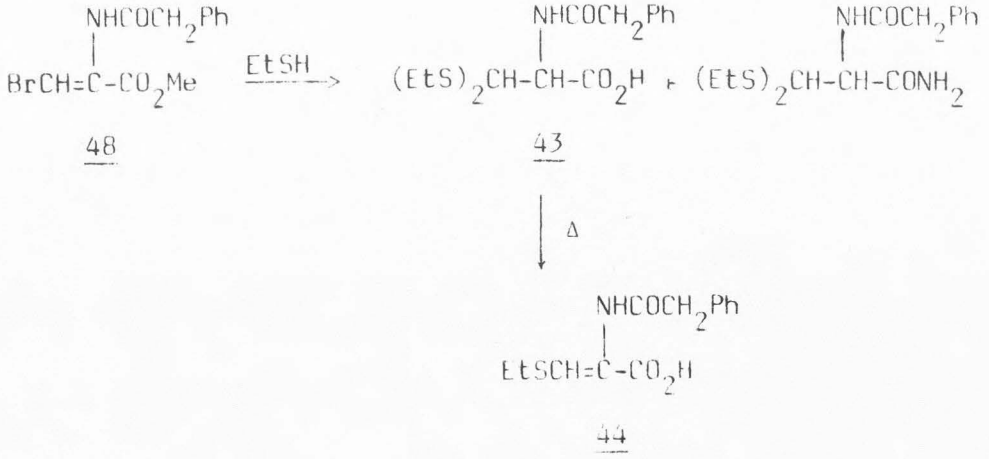
acetal (46) back to 45. Only ethyl n-amylpenaldate was obtained when the diethylthioacetal (46) was cleaved with mercuric chloride.



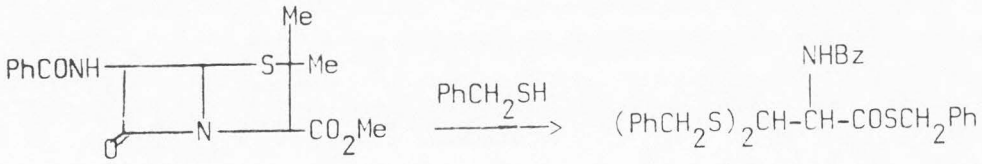
Similarly, Kildisheva and coworkers (39b) reported that methyl β,β-dibenzylthio-N-phenylacetylalaninate (47) could be prepared by treatment of methyl β-benzylthio-N-phenylacetyldehydroalaninate (42) with benzyl mercaptan or methyl β-bromo-N-phenylacetyldehydroalaninate (48) with benzyl mercaptan in liquid ammonia. However, treatment of 48



with ethyl mercaptan in liquid ammonia gave the free acid and amide of the desired product. Furthermore, warming the acid (43) to 80 degrees gave the acid (44).

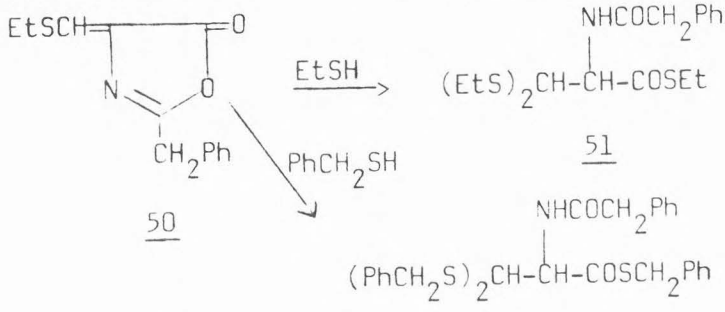


Cornforth (41) prepared the benzylthiol ester of β,β-dibenzylthio-N-benzoylalanine (49) by treating methyl D,L-phenylpenicillinate with benzyl mercaptan and sodium metal. Treatment of the azlactone (50) with sodium metal and ethyl mercaptan in hot benzene gave the ethylthiol ester of β,β-diethylthio-N-phenylacetylalanine (51). However,



49

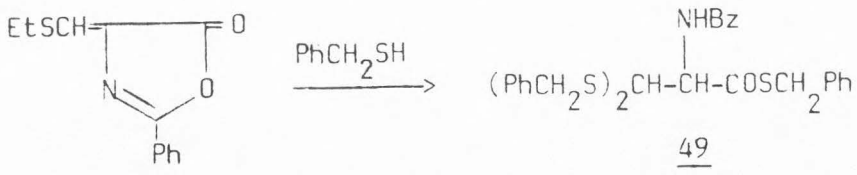
similar treatment of the azlactone (50) with benzyl mercaptan gave the



50 51 52

unexpected benzylthiol ester of β,β-dibenzylthio-N-phenylacetylalanine (52), thus, not only addition had occurred but also exchange of sulfides.

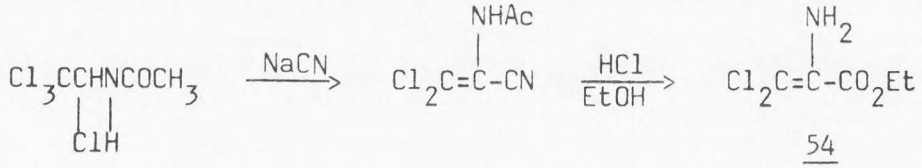
Similarly, Brown (46) reported that the azlactone (53) when treated with benzyl mercaptan in hot benzene gave the unexpected benzylthiol ester of β,β-dibenzylthio-N-benzoylalanine (49).



53 49

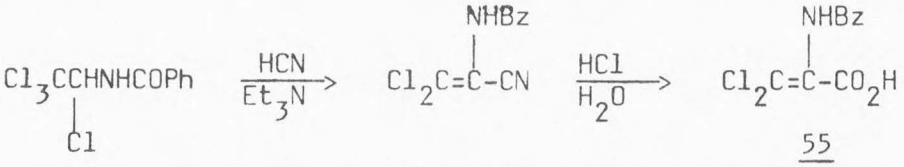
Syntheses of
β,β-disubstituted dehydroalanines

Dovlatyan and Ambartsumyan (47) synthesized ethyl β,β-dichloro-dehydroalaninate (54) by the reaction between sodium cyanide and N-1,2,2,2-tetrachloroethylacetamide. Acid hydrolysis of the nitrile in ethanol gave the ethyl ester and deprotected the amino group of alanine.



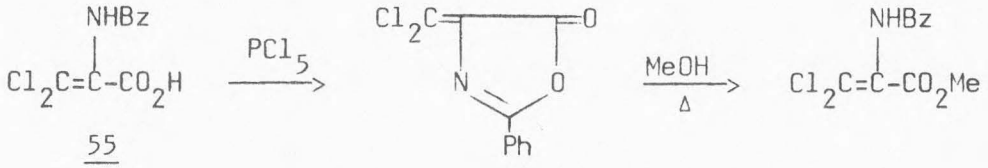
54

Drach and Miskevich (48) prepared β,β-dichloro-N-benzoyldehydro-alanine (55) from the reaction between N-1,2,2,2-tetrachloroethyl-benzamide and hydrogen cyanide in the presence of triethylamine. Acid hydrolysis of the nitrile with concentrated hydrochloric acid gave the



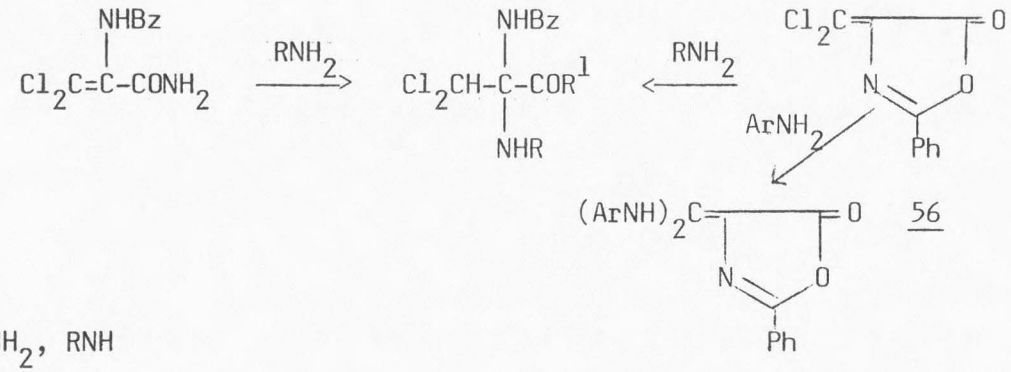
55

acid. The corresponding methyl ester was obtained by conversion of the acid to the azlactone (56) and treatment of the azlactone with refluxing



56

methanol. Reaction of the azlactone (56) or the amide of the acid (55) with an aliphatic primary amine gave the α-substituted product.



$R^1=NH_2$, RNH

$R=CH_3$, Et, isopropyl

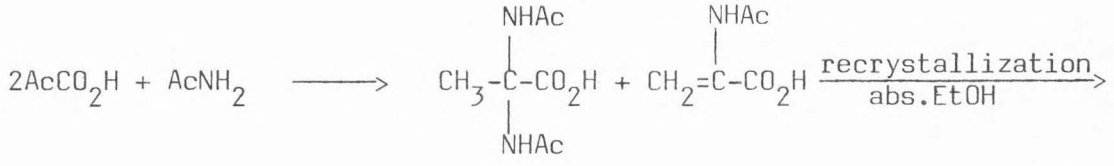
Ar=Ph, $CH_3C_6H_4$

Reaction of the azlactone (56) with an aromatic primary amine gave the β,β-diarylamine derivative, but the azlactone did not undergo ring opening.

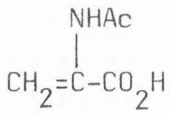
RESULTS AND DISCUSSION

Previous synthesis of N-acetyldehydroalanine (11)

N-Acetyldehydroalanine (α-acetamidoacrylic acid) (11) was prepared following the procedure of Kildisheva and coworkers (18a) using a 2:1 mole ratio of pyruvic acid to acetamide. Analysis by PMR spectroscopy indicated a mixture of 11 and the bis compound (12). Pure N-acetyldehydroalanine (11) was obtained by crystallization from absolute ethanol.



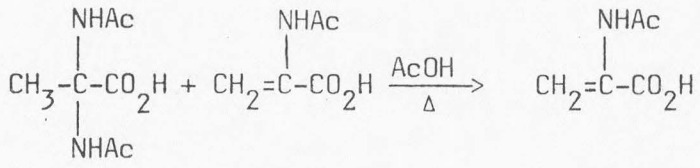
12 11



11

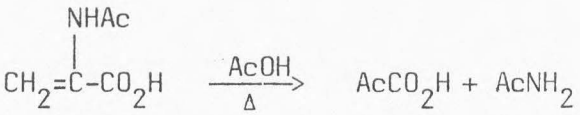
After three experiments under these conditions, because the yield was only 17 to 34% (lit. 53%); the procedure of Kildisheva and coworkers was altered to involve a 1:1 ratio of pyruvic acid and acetamide. The reaction was repeated fifteen times using the 1:1 ratio on scales of 0.3 to 2.36 moles with yields from 18 to 45%. The work up always involved recrystallization of the crude reaction product from alcohol yielding pure 11. Additional N-acetyldehydroalanine (11) was obtained by

heating the remaining mixture of 12 and 11 (after removal of pure 11) with glacial acetic acid (16) and recrystallization from alcohol. Coincidental with the elimination of acetamide from 12 to 11 was always



12 11 11

hydrolysis of 11 to pyruvic acid; therefore, the treatment with glacial acetic acid was done with care. It was discovered that methanol was a better solvent for purification of crude bis (12) and 11 because of higher solubilities.



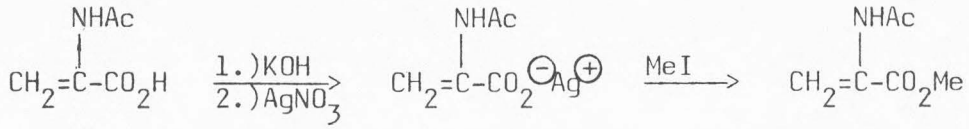
11

This reaction was also tried once with toluene as solvent instead of benzene (19); however, the yield fell to 5%. Since pyruvic acid is unstable and heat aids decomposition, it was felt that refluxing toluene (b.p.110°) versus benzene (b.p.80°) should favor decomposition and the decreased production of 11.

The yield of N-acetyldehydroalanine (11) was from 5 to 10% higher, generally, if the pyruvic acid was pretreated with Norit just before use, especially if the bottle had sat more than a month in the refrigerator. Freshly distilling the pyruvic acid was generally not helpful and always wasteful since pyruvic acid is hard to distill even under high vacuum (normally, only about 50% recovery).

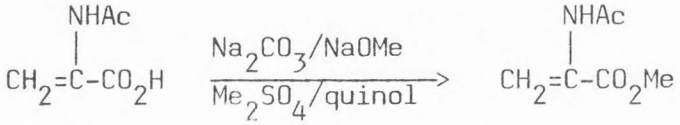
Synthesis of
methyl N-acetyldehydroalaninate (14)

The methyl ester of N-acetyldehydroalanine (14) was prepared by the method of Wieland and coworkers (18b) without a polymerization inhibitor and as a consequence the product polymerized occasionally during purification (17a). However, when polymerization did not occur, pure product was prepared in 10 to 47% yield.



11 14

Because of the high cost of silver nitrate, the method of Rothstein (17a) seemed a more attractive alternate for synthesis of methyl N-acetyldehydroalaninate (14). N-Acetyldehydroalanine (11) was treated with a mixture of sodium carbonate, sodium methoxide and dimethyl sulfate in methanol with quinol as a polymerization inhibitor. It was observed

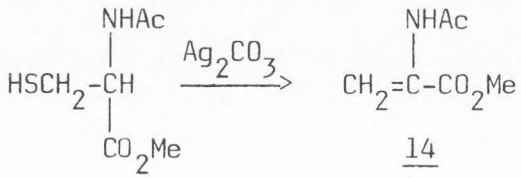


11 14

that this reaction worked as well when refluxed two hours or stirred at room temperature for fifteen to twenty hours. Since dimethyl sulfate is an insidious poison with few symptoms before death, the work up procedure reported by Rothstein was considered too cavalier and not followed. Since dimethyl sulfate undergoes rapid hydrolysis in the presence of water above 20°, water was added to the crude reaction mixture and it was stirred one hour at room temperature. Several PMR spectra of the reaction residues after removal of 14 showed no evidence of residual dimethyl sulfate (singlets between 3 and 4δ).

This reaction was done twenty times on scales of 0.15 to 0.7 moles in 36 to 68% yields. Use of the quinol (hydroquinone) polymerization inhibitor imparted a brown tint to the product (14), however, no evidence indicated reduced reactivity, except of course polymer formation, for the product with quinol present.

The method of Gravel and coworkers (23) was also tried. Methyl N-acetylcysteinate (18) and silver carbonate were refluxed in methanol without a polymerization inhibitor. In our hands, this reaction was



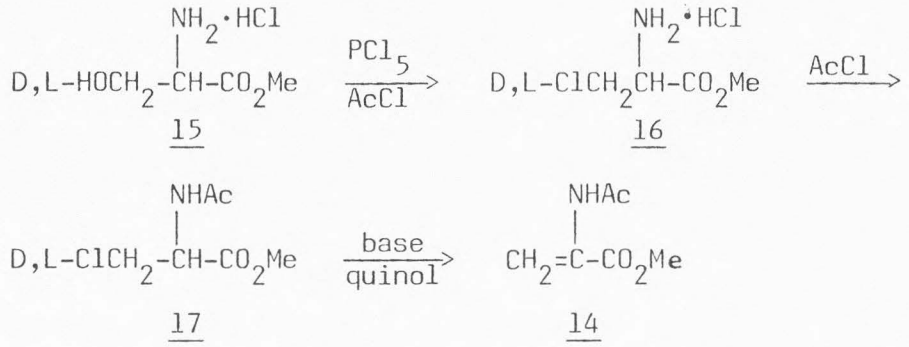
18 14

never fruitful. One out of six reactions gave methyl N-acetyldehydroalaninate (14), but in this case hydroquinone had been added to the reaction and the yield was only enough for a PMR spectrum (the authors report 62% yield).

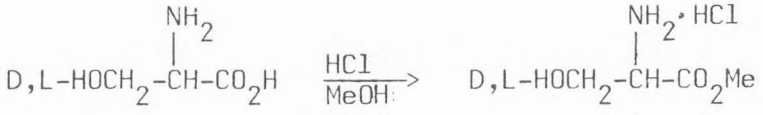
Alternate syntheses of 11 and 14

In 1975, commerical pyruvic acid of high purity and reasonable price was no longer available. These reasons coupled with the constant presence of 2,2-bis (acetamido)propanoic acid (12) in the product encouraged us to seek an alternate synthesis of N-acetyldehydroalanine (11) and its methyl ester (14).

The first method considered was that reported by Rothstein (17a) utilizing the following series of reactions.

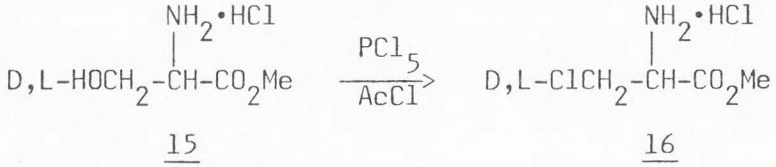


Serine methyl ester hydrochloride (15) was prepared following the method of Fischer and Suzuki (49). Racemic serine was suspended in methanol; saturation with hydrogen chloride gas gave D,L- serine methyl ester hydrochloride (15). Initially, reaction time was overnight at room temperature, however, fifteen minutes of rapid hydrogen chloride flow (serine dissolved) followed by forty-five minutes of slow flow and one hour stirring at room temperature gave a higher yield than the overnight method (86% versus 70%).



Since Srinivasan (50a) and also Wood and Middlesworth (50b) had reported the preparation of β-chloroalanine acid and ester hydrochloride using phosphorus pentachloride in chloroform, this procedure was used to prepare β-chloro-D,L-alanine methyl ester hydrochloride (16). However, much to my surprise, not only was the melting point ten degrees low but PMR indicated a mixture of product (16) and starting material (15). Since Srinivasan had worked with the L- serine derivative, and Wood and Middlesworth had reported obtaining the free acid, this method was abandoned for the moment.

The procedure of Rothstein (17a) using phosphorus pentachloride in acetyl chloride was used to prepare β-chloro-D,L-alanine methyl ester hydrochloride (16). However, the melting point was low and had a range of ten degrees.



15 16

Because of the impurity of the product and the amount of acetyl chloride consumed, the method of Srinivasan (50a) was again considered. Referring to Table 1, runs 1-3 indicated that the phosphorus pentachloride reaction was relatively slow (sufficient product to be visible in the PMR spectrum required over four hours). Entries 4 and 5 seemed to indicate that too much solvent disfavored complete reaction, however, entry 6 indicated that too little solvent led to decomposition. This probably indicated that phosphorus oxychloride, the assumed reaction product when an alcohol reacts with phosphorus pentachloride, was too corrosive to 15 and/or 16 in high concentration. Entries 7 and 8 support the above assumption. Entry 9 indicated the nonlinearity between solvent volume and the scale of the reaction. However, it must be pointed out that the product (16), when the reaction went to completion, was of greater purity than when acetyl chloride was used as the solvent (by melting point and PMR).

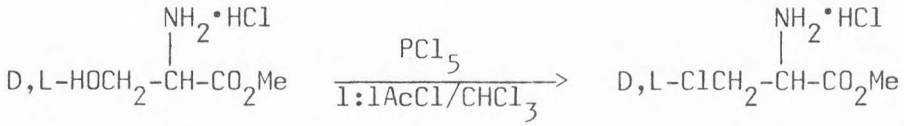
TABLE 1

EFFECT OF VARYING THE CONDITIONS FOR β-CHLORINATION OF SERINE METHYL ESTER WITH PCl_5 IN CHLOROFORM

Run	PCl_5 equivalents	$CHCl_3$ volume in ml	Time in hours	Isolated Products
1	1.2	20	4	15
2	1.2	60	4	15
3	1.2	50	5.5	15+16
4	1.2	20	12	16
5	1.1	50	12	15+16
6	2	30	20	brown tar
7	2	40	19	16
8	2	50	12	16
9	1.2	200[a]	12	15+16

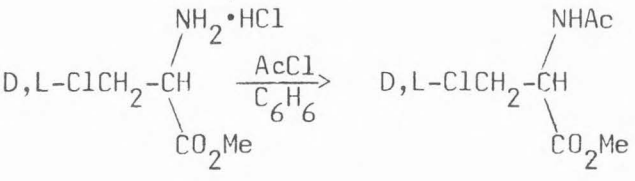
[a]Reaction on ten times the scale of others.

Because of the inability to increase the scale of the reaction to prepare (16) with a reasonable certainty of completion using chloroform as solvent, another procedure was sought. When acetyl chloride was used as solvent, a fast reaction occurred (complete in two hours), but the product (16) was not of high purity. When chloroform was used as solvent, the product was of higher purity, but this solvent did not readily lend itself to reactions on various scales. Therefore, it was decided to try a 1:1 mixture of acetyl chloride and chloroform as solvent. Not only was the reaction fast (two to three hours), but the product (16) was of high purity. The reaction was done on scales of 0.1 moles to 0.4 moles with 72-75% yield.



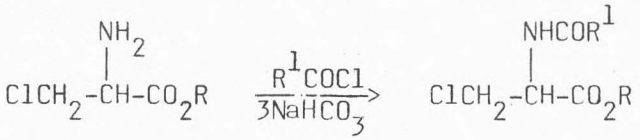
15 16

Initially, methyl β-chloro-N-acetyl-D,L-alaninate (17) was prepared by the method of Rothstein (17a). β-Chloro-D,L-alanine methyl ester hydrochloride (16) was refluxed in a mixture of acetyl chloride and benzene. However, the yield was low (33%, Rothstein reported 46%).

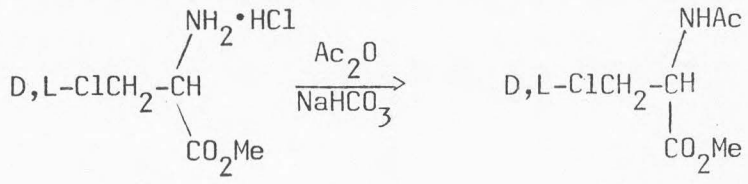


16 17

Benoiton (51) reported the conversion of β-chloroalanine derivatives to the corresponding N-acyl derivative with sodium bicarbonate and acyl chloride. Because of excess sodium bicarbonate, the reaction gave a

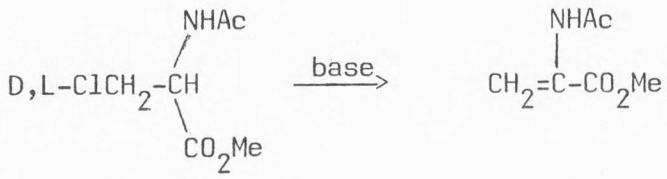


mixture of products (sodium bicarbonate was a strong enough base to eliminate hydrogen chloride). Therefore, acetic anhydride was substituted for acetyl chloride, which allowed the use of water as the solvent, and only one equivalent of sodium bicarbonate was used (only enough base to free the amino group from its hydrochloride salt).



16 17

Rothstein (17a) converted methyl β-chloro-N-acetyl-D,L-alaninate (17) to methyl N-acetyldehydroalaninate (14) in the presence of quinol as a polymerization inhibitor with potassium hydroxide in methanol, ammonia in methanol, piperidine in ether and potassium methoxide in methanol. Methyl N-acetyldehydroalaninate (14) was prepared in the presence of hydroquinone (quinol) with sodium methoxide in methanol, sodium bicarbonate in methanol plus water, sodium carbonate plus triethylamine in acetonitrile plus water and triethylamine in ether or ethyl acetate.



17 14

The reaction of 17 to 14 in the presence of sodium bicarbonate gave a mixture of product (14) and starting material (17) after five hours at room temperature. From this mixture, 30% of methyl β-chloro-N-acetyl-D,L-alaninate (17) was isolated; the remainder was a 2:1 ratio of 14 to 17 by PMR. Therefore, although sodium bicarbonate (pka 6.4)

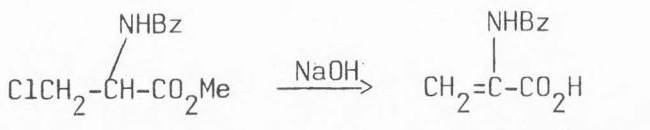
was a strong enough base to promote the elimination of hydrogen chloride, the reaction was too slow to be of synthetic utility.

Using triethylamine in ethyl acetate as Srinivasan (50a) reported led to complete reaction in three hours, but the isolated yield was only 21%. The solvent was switched to ether and complete reaction occurred in thirty minutes at reflux temperature. The crude yield was about 50%. The crude product was used in subsequent reactions without further purification.

The reaction using sodium carbonate (pKa 10.3) and triethylamine (pKa 11) as base gave a 84% crude yield of 14. The product (14) was used in subsequent reactions without further isolation. This appeared to be the best method both for purity and yield.

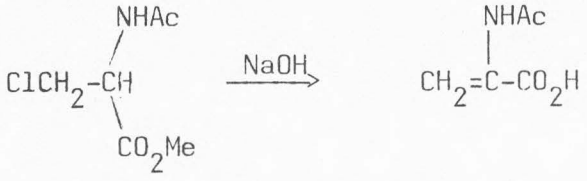
The reaction using sodium methoxide as the base in methanol was complete in thirty minutes with a yield of 52%. Potassium methoxide as Rothstein (17a) reported was not used because he reported a mixture of methyl N-acetyldehydroalaninate (14) and what he thought was methyl β-methoxy-N-acetylalaninate (57) as product. Furthermore, the mixture could not be completely separated, although some of the β-methoxyalanine ester (57) was isolated.

Fry (52) had reported the direct conversion of methyl β-chloro-N-benzoylalaninate to N-benzoyldehydroalanine (19) using sodium hydroxide in water. Therefore, methyl β-chloro-N-acetyl-D,L-alaninate (17) was



19 (R=Ph)

directly converted to N-acetyldehydroalanine (11). The reaction proceeded smoothly in a 70% yield.



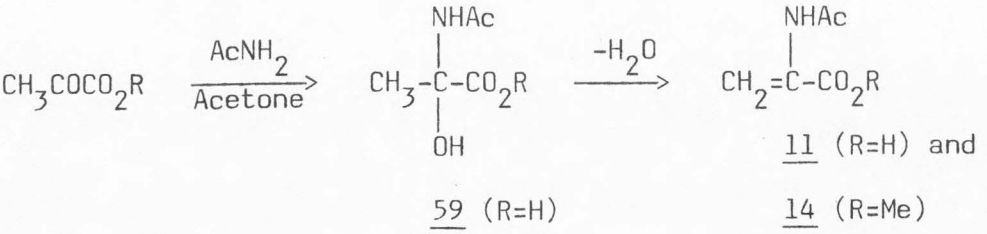
17 11

Although the synthesis from D,L- serine was indeed successful, the best overall yield from serine was 44% for methyl N-acetyldehydroalaninate (14) and 37% for N-acetyldehydroalanine (11), which compared well with the 45% for 11 and 36% for 14 from pyruvic acid; however, the serine method lacked the economy desired in any synthesis of so basic a starting material. Therefore, as time progressed, the chemical literature was reviewed for a still better method.

Zoller and Ben-Ishai (53) reported the preparation of α-hydroxy-N-benzoylglycine (58) from glyoxylic acid and benzamide in refluxing acetone. It was hoped that pyruvic acid and its ester would undergo

58

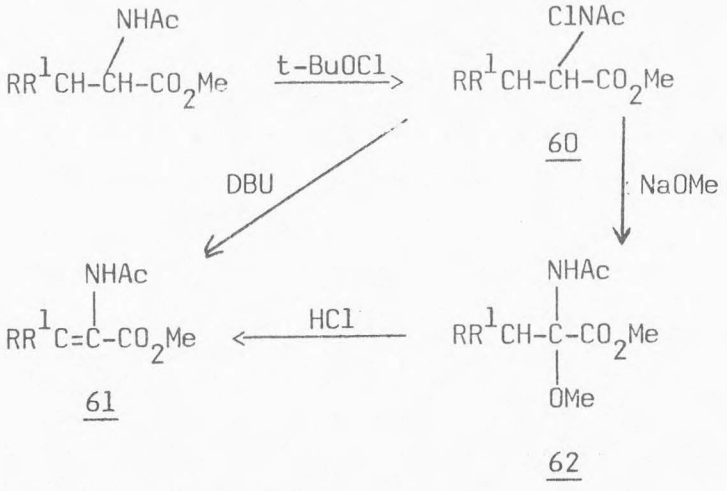
a similar reaction to produce highly pure α-hydroxy-N-acetylalanine (59) and its esters, which upon dehydration would produce N-acetyldehydroalanine (11) and its esters.



59 (R=H) 11 (R=H) and 14 (R=Me)

Although the methyl ester and the ethyl ester of pyruvic acid reacted readily and very cleanly under these conditions in quantitative crude yields, the α-hydroxy methyl and ethyl esters were so unstable at room temperature that reversion to the corresponding pyruvate was observed to even occur as acetone was removed in vacuo. The α-hydroxy-N-acetyl alanine (59) derivative was stable at room temperature, however, dehydration with refluxing benzene and refluxing benzene plus glacial acetic acid as a catalyst in a Dean-Stark apparatus gave less than 30% of a N-acetyldehydroalanine (11) and 2,2-bis (acetamido)-propionic acid (12) mixture. All other attempts to dehydrate 59 with various reagents and condition produced tars or reverted 59 back to pyruvic acid.

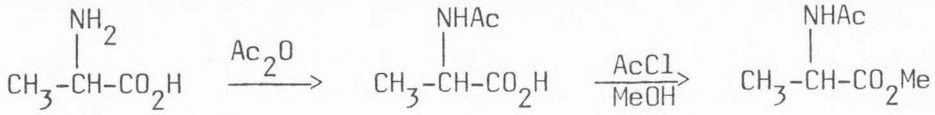
Finally, Poisel and Schmidt (54) reported the synthesis of dehydroamino esters (61) and amides via N-chloro-N-acylamino esters (60). The substituents in the beta position were always at least one alkyl group.



If sodium methoxide in methanol was used instead of 1,5-diazabicyclo [5.4.0] undec-5-ene (DBU) as the base, the corresponding α-methoxy compound (62) was isolated. The α-methoxy compound could be converted by hydrogen chloride gas in ether to the corresponding dehydroamino ester (61). This report and the low cost of D,L-alanine, coupled with

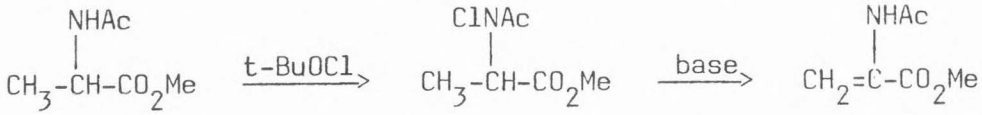
the shortcomings of the serine and pyruvic acid methods, prompted us to investigate this alternative.

Since this approach required the availability of methyl N-acetyl-D,L-alaninate (64), a high yield synthesis was developed. The acetylation of D,L-alanine by the method of Greenstein and Winitz (55) proceeded smoothly and in high yield. Initially, 63 was isolated in 78% yield, however, more commonly it was used as a crude paste in the esterification method of Riegel and coworkers (56) using acetyl chloride in methanol. The esterification gave 64 in 82% isolated yield or 96% crude yield

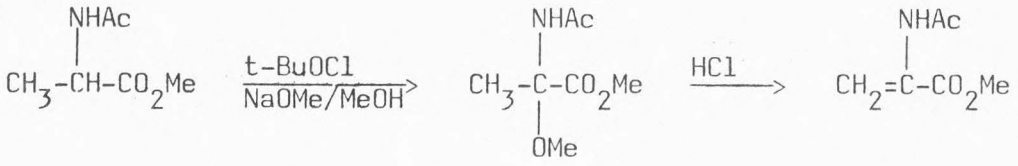


after removal of material boiling lower than 100^{o}/12mm. The yield of 64 was always 10-20% higher if Aldrich brand acetic anhydride was used for the acetylation.

The initial attempts to prepare methyl N-acetyldehydroalaninate (14) failed; (a mixture of 14 and 64 was obtained in at best a 1:1 ratio). This failure was determined to be because of the inability to force the N-chlorination to go to completion using t-butyl hypochlorite in



benzene or ether with potassium t-butoxide as catalyst (54). Therefore, the alternate synthesis indicated by Poisel and Schmidt (54) was investigated.



64 66 14

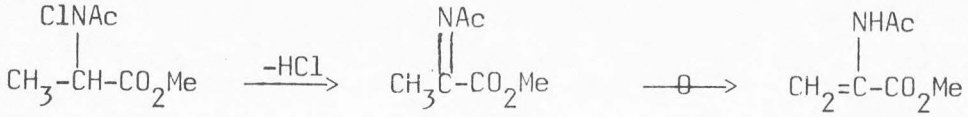
The preparation of methyl α-methoxy-N-acetyl-D,L-alaninate (66) was accomplished in 38% isolated and as high as 51% crude yield following the method of Poisel and Schmidt (54) or substituting methanol for Et_2O as the initial solvent (see below). It was assumed that the low yield resulted from a failure of complete N-chlorination.

The elimination of methanol from 66, using hydrogen chloride gas in ether, to form methyl N-acetyldehydroalaninate (14) always resulted in the formation of a mixture of 66 and 14. Although, the product (14) was normally the major component (in excess of 50%); the low yield obtained for the preparation of methyl α-methoxy-N-acetyl-D,L-alaninate (66) disfavored this approach as a viable synthetic alternative.

Since the difficulties with this approach were thought to be linked to the inability to get complete N-chlorination under the conditions of Poisel and Schmidt, several other methods of N-chlorination were tried. Of these, only the method of Johnson and Greene (57) (methanol as solvent) resulted in complete N-chlorination. Because the reaction underwent an initiation period, t-butyl hypochlorite had to be added in portions to prevent decomposition. An attempt to avoid the initiation period by catalysis with potassium t-butoxide resulted in a very loud and colorful explosion. By the time the conditions for the synthesis of the N-acyldehydroalanines were established, a report by Poisel and Schmidt (58) using 1% sodium methoxide in methanol as a catalyst for their reaction induced me to try this catalyst for the reaction of

Johnson and Greene (57). With this catalyst the N-chlorination reaction proceeded smoothly when the t-butyl hypochlorite was added in only one portion. Completeness of reaction was determined by T.L.C. analysis.

Once methyl N-chloro-N-acetylalaninate (65) was formed, the elimination-isomerization (54) to methyl N-acetyldehydroalaninate (14) was considered in detail. Non-amine type bases were ineffective for the



65 67 14

elimination step probably because of poor solubilities in the appropriate solvent. It was discovered that 1,4-diazabicyclo [2.2.2] octane (DABCO), triethylamine (2 equivalents) and 1,5-diazabicyclo [5.4.0] undec-5-ene (DBU) all effected the elimination step. Of these, DBU was disfavored because of a greatly enhanced tendency for 14 to polymerize. Less than two equivalents of triethylamine gave methyl N-acetyldehydroalaninate (14) in decreased yields (see Table 2).

TABLE 2

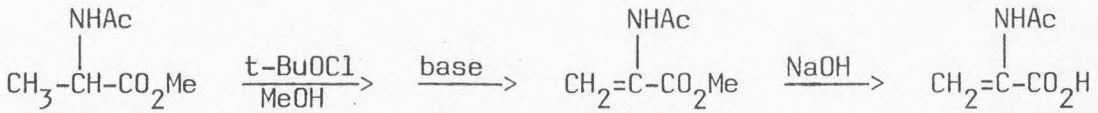
EFFECT OF EXCESS TRIETHYLAMINE IN THE PREPARATION OF METHYL N-ACETYLDEHYDROALANINATE (14)

Et_3N equivalents	crude 14
1.2	34%
1.5	54%
2	72%

Methylene chloride was the solvent of choice for this elimination reaction because of its ease of purification (washing with 10% sodium carbonate solution and drying over molecule sieves (Linde 3A)) and lack of alcoholic impurities, which as illustrated later, reacted faster with the intermediate acylimine (67) than isomerization occurred (t-butyl alcohol did not react with acylimine (67)).

By this method methyl N-acetyldehydroalaninate (14) on a scale of 0.1 to 0.9 moles was isolated as an oil in 72-79% yield simply by effecting an aqueous wash of the product mixture in methylene chloride. Analysis by PMR indicated the oil to be approximately 95% pure with some methyl N-acetyl-D,L-alaninate (64) present. This material was satisfactory for all subsequent syntheses. Crystalline 14 was obtained in 40-55% yield by a procedure involving Kugelrohr distillation and/or recrystallization.

The preparation of N-acetyldehydroalanine (11) was accomplished on a scale of 0.1 to 2 moles in 50-62% yield from methyl N-acetyl-D,L-alaninate (64) by treating the crude reaction after elimination with sodium hydroxide solution. Because of the greater water solubility



64 14 11

of N-acetyl-D,L-alanine (63), N-acetyldehydroalanine (11) could be easily purified.

t-Butyl hypochlorite was made simply and easily by the method of Mintz and Walling (59) from t-butyl alcohol and bleach in 75% yield. t-Butyl hypochlorite and methyl N-chloro-N-acetyl-D,L-alaninate (65) were unstable in light and at room temperature for long periods, however,

$$t\text{-BuOH} \xrightarrow[AcOH]{\text{bleach}} t\text{-BuOCl}$$

both are stable at temperatures below 20° for short periods and below 0° indefinitely.

Preparation of
N-acyldehydroalanines and their esters

In order to determine the scope of our synthesis of methyl N-acetyldehydroalaninate (14) and N-acetyldehydroalanine (11), it was decided to attempt to prepare N-benzoyldehydroalanine (19a), N-phenylacetyldehydroalanine (19b), N-benzyloxycarbonyldehydroalanine (19c) and their respective methyl esters by this method.

$$\underset{\underline{68(a\text{-}c)}}{CH_3\text{-}\overset{\overset{NHCOR}{|}}{CH}\text{-}CO_2Me} \xrightarrow{t\text{-BuOCl}} \underset{\underline{69(a\text{-}c)}}{CH_3\text{-}\overset{\overset{ClNCOR}{|}}{CH}\text{-}CO_2Me} \xrightarrow{base} \underset{\underline{21(a\text{-}c)}}{CH_2=\overset{\overset{NHCOR}{|}}{C}\text{-}CO_2Me}$$

$$\xrightarrow{NaOH} \underset{\underline{19(a\text{-}c)}}{CH_2=\overset{\overset{NHCOR}{|}}{C}\text{-}CO_2H}$$

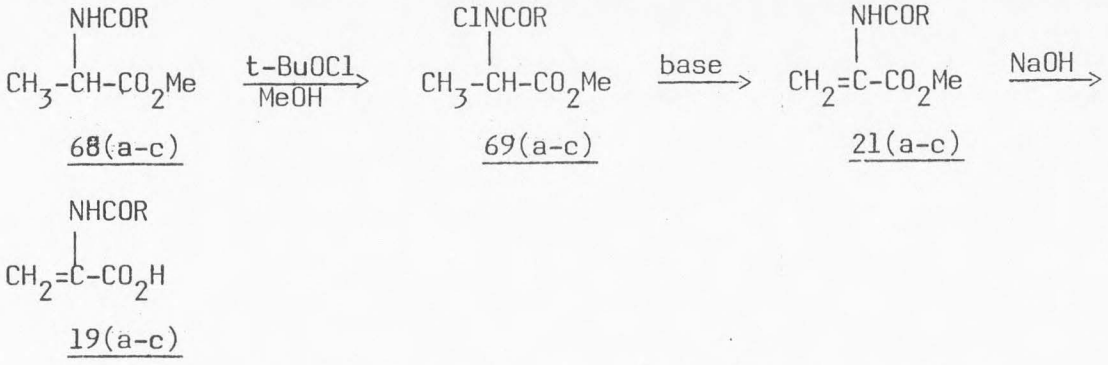
	a	b	c
R	Ph	$PhCH_2$	$PhCH_2O$

A general synthesis of methyl N-acylalaninates (68(a-c)) was developed from the available literature methods. The method of Dunn and coworkers (60) for the preparation of N-benzoylalanine (70a) was used to acylate D,L-alanine except sodium bicarbonate was used as the base instead of sodium hydroxide. The N-acylalanines (70(a-c)) were obtained in yields of 75 to 92%, first isolated but later used as a crude

$$\underset{}{CH_3\text{-}\overset{\overset{NH_2}{|}}{CH}\text{-}CO_2H} \xrightarrow[NaHCO_3]{RCOCl} \underset{\underline{70(a\text{-}c)}}{CH_3\text{-}\overset{\overset{NHCOR}{|}}{CH}\text{-}CO_2H} \xrightarrow[MeOH]{AcCl} \underset{\underline{68(a\text{-}c)}}{CH_3\text{-}\overset{\overset{NHCOR}{|}}{CH}\text{-}CO_2Me}$$

paste in the esterification method of Riegel and coworkers (56). This esterification, using acetyl chloride and methanol, proceeded smoothly

in 80-90% yield from 70(a-c) or 60 to 81% from D,L-alanine when the N-acyl-D,L-alanines (70(a-c)) were used crude. Once again, the methyl N-acyl-D,L-alaninates (68(a-c)) were at first isolated but later, more conveniently, used crude after removal of volatiles boiling lower than 100^{o}/1mm. These volatiles included methyl phenylacetate or methyl benzoate which were hard to remove in the previous or subsequent steps in the synthesis. Unlike the methyl N-acetyl derivative (64), the methyl N-acyl-D,L-alaninates (68(a-c)) were insoluble in water, thus, easier to isolate.



68(a-c) 69(a-c) 21(a-c) 19(a-c)

It was discovered that the N-chlorination of the methyl N-acyl-D,L-alaninates (68(a-c)) was most rapid with methyl N-benzyloxycarbonyl-D,L-alaninate (68c) and most difficult in the case of methyl N-benzoyl-D,L-alaninate (68a). The conditions developed (see Table 3) led to complete N-chlorination of the methyl N-acylalaninates (69(a-c)) as measured by T.L.C. and PMR analysis. The N-chloro derivatives (69(a-c)) were also water insoluble and easily isolated from methanol and t-butyl alcohol.

TABLE 3

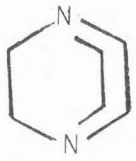
CONDITIONS FOR THE PREPARATION AND T.L.C. DATA FOR THE N-CHLORO COMPOUNDS 69(a-c) and 65

Compound	Reaction Time		T.L.C. R_f Values		T.L.C. Solvent
	at 10-20°	at ∿ 8°	NH	N-Cl	
65	2-4h	8-12h	0.40	0.72	ethyl acetate
69a	——[a]	40-48h	0.36	0.64	chloroform
69b	5-6h	20-24h	0.47	0.56	ethyl acetate
69c	1-2h	8-12h	0.50	0.65	chloroform

[a] 6 hours at 10-20° and 20 hours at ∿ 8°.

The dehydrochlorination step also was found to require some variations. Once again, only the amine-type bases were appropriate. Where as triethylamine, 1,4-diazabicyclo [2.2.2] octane (DABCO) and 1,5-diazabicyclo [5.4.0] undec-5-ene (DBU) could be used for dehydrochlorination of methyl N-chloro-N-acyl-D,L-alaninates (69a,b), DBU was not

DBU



DABCO

normally used because of the greater tendency of the resulting methyl N-acyldehydroalaninates (21a,b) towards polymerization (indeed, DBU is a very effective polymerization catalyst according to the recent patent literature).

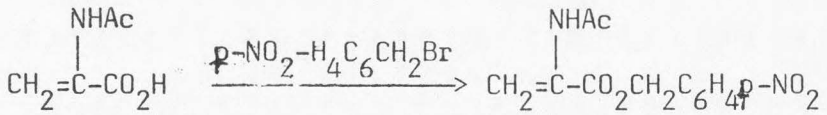
In the case of methyl N-chloro-N-benzyloxycarbonylalaninate (69c), DABCO was not a strong enough base for dehydrochlorination. Only triethylamine and DBU could be used for this derivative. Furthermore, both the resulting methyl N-benzyloxycarbonyldehydroalaninate (21c) and its acid (19c) were found to polymerize if appropriate care was not exercised when either DBU or triethylamine were used as the base.

DABCO was a more effective base for dehydrochlorination (1 equivalent) than triethylamine (2 equivalents), although its pKa is relatively low (DABCO, 8.7; triethylamine, 11.01), because its alkyl substituents are "tied back" allowing the unshared pair of electrons on nitrogen to more easily abstract protons. DBU also has this feature but the pKa is much higher than DABCO.

The methyl N-acyldehydroalaninates (21(a-c)) were isolated as crude oils in 50 to 75% yield from the methyl N-acyl-D,L-alaninates (68(a-c)). For this study, these crude esters were treated directly with sodium hydroxide solution, converted to the N-acyldehydroalanines (19(a-c)) and isolated as the acid in 30-40% yield from 68(a-c). The unsaturated esters (21(a-c)) were insoluble in water allowing a simplified isolation procedure as compared to methyl N-acetyldehydroalaninate (14).

Preparation of
p-nitrobenzyl N-acetyldehydroalaninate (71)

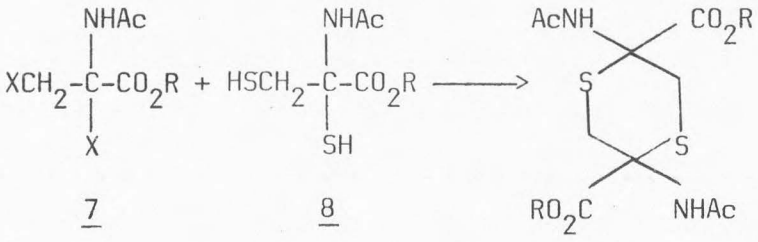
The p-nitrobenzyl ester (71) was prepared from N-acetyldehydroalanine (11) and p-nitrobenzyl bromide in 35 to 82% yield by the method of Olsen (13).



11 71

Studies toward the synthesis of
α-mercapto-N-acetyl-D,L-alanine derivatives

As stated previously, a possible synthesis of the 2,5-diacetamido-1,4-dithiane-2,5-dicarboxylic acid or ester derivative (9) of the 1,4-dithiane (4) moiety in the structure of the quinomycin family of quinoxaline antibiotics could involve the reaction between 7 and 8.

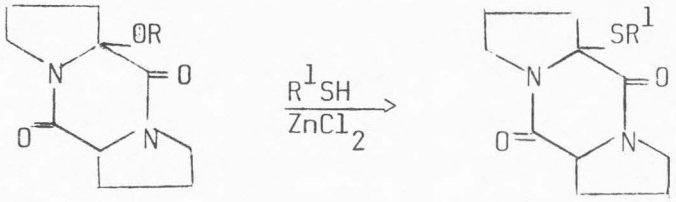


7 8 9

R=H or alkyl

Since both 7 and 8 involved substituents at the α and β positions of alanine, the reactivity of the α and β positions was of interest. Patel and coworkers (30) had done preliminary studies of the reactivity of the α position of alanine derivatives. Using these preliminary studies as a basis, it was of interest to extend our knowledge of the reactivity of the α position, especially to those types of reactions which could, perhaps, be applied to the β position as well as the α position.

Ohler and coworkers (61) reported the facile conversion of hydroxy and methoxy derivatives to the corresponding sulfides using zinc chloride as a catalyst. Because this synthesis involved the α position and mild

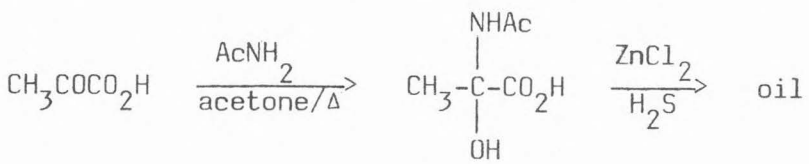


R=H, Me

R^1=H, Ac, Et

conditions, this method was investigated as a possible alternative route to α-mercapto-N-acetyl-D,L-alanine derivatives. For this and other reasons, which will become obvious later, the preparation of α-methoxy and α-hydroxy-N-acetyl alanine and its esters was attempted.

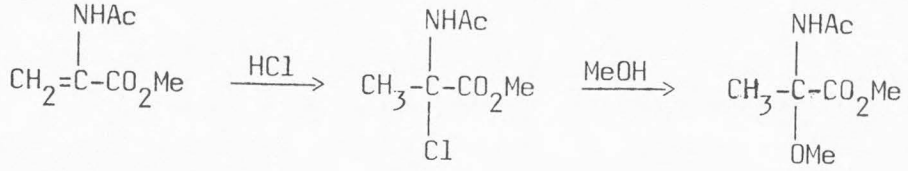
All attempts to prepare the methyl and p-nitrobenzyl esters of α-hydroxy-N-acetyl-D,L-alanine (59) failed. This failure was assumed to be a result of the instability of these compounds, which was subsequently, experimentally observed (see p. 39) for the methyl and ethyl esters using the pyruvic acid method of Zoller and Ben-Ishai (53). α-Hydroxy-N-acetyl-D,L-alanine (59) was prepared as mentioned previously, but the



59

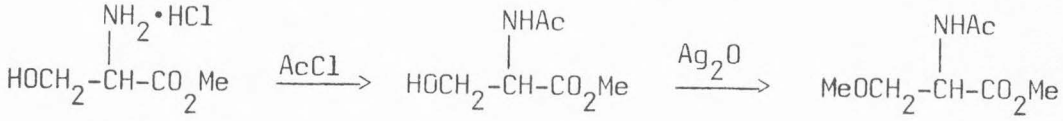
reaction of 59 with hydrogen sulfide and zinc chloride gave a foul smelling yellow oil, which did not contain the desired product in isolable amounts (a positive sodium nitroprusside test did indicate some free SH).

The initial attempts to prepare methyl α-methoxy-N-acetyl-D,L-alaninate (66) were based on the sequence of addition of hydrogen chloride to the double bond of methyl N-acetyldehydroalaninate (14) followed by displacement with methanol (see Table 4).



14 72 66

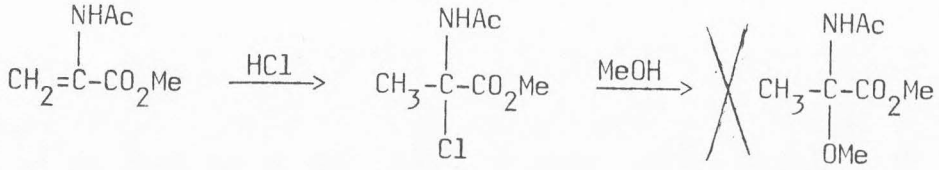
The structure of 57 (methyl β-methoxy-N-acetyl-D,L-alaninate) formed in Run 11 was verified by comparison to a known sample prepared by the method of Synge (65) from methyl N-acetyl-D,L-serinate (73), silver oxide and methyl iodide (the method of Rothstein (17a) gave only inseparable mixtures of 57 and 14). Methyl N-acetyl-D,L-serinate (73)



15 73 57

was prepared from D,L- serine methyl ester hydrochloride (15) by an adaptation of the method of Benoiton (51).

Because the general sequence of reactions for the preparation of 66 had involved the initial addition of hydrogen chloride to the double bond of 14 (except for Runs 10 and 13), the addition of hydrogen chloride to 14, was investigated in detail.



14 72 66

TABLE 4

ATTEMPTED PREPARATION OF 66

Run	Solvent for hydrochlorination	Base (equivalents) with methanol	Product	Reference
1	AcOH/dioxane	none	$AcNH_2$	a
2	TFA/dioxane	none	green tar	a
3	TFA	none	polymer[b]	a
4	CCl_4	none	polymer[b]	--
5	CCl_4	1 NaOMe	c	--
6	CH_2Cl_2	none	14+polymer[b]	25
7	TFA	none	14	a
8	AcOH	none	polymer	a
9	DMF/dioxane	2 NaOMe	DMF+14	--
10	d	none	--	62
11	CH_2Cl_2	2 NaOMe	57	--
12	THF/dioxane	LiOMe	14	63
13	e	e	--	64

[a]Patel and coworkers' (30) approach to α-sulfide synthesis. [b]Generally, 14 was not recovered because it polymerized during work up. [c]Small amount of material of unknown structure. [d]$BF_3 \cdot Et_2O$, no hydrochlorination. [e]Hydroxymercuration-demercuration procedure.

Love and Olsen (45) had reported the very rapid addition of hydrogen bromide to 14. Furthermore, after several hours isomerization to the β-bromo derivative began (this isomerization was complete in 3 days).

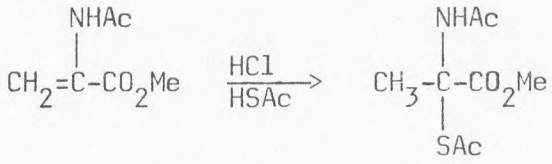
Following the method of Love and Olsen, the addition of hydrogen chloride to 14 was investigated. Dry hydrogen chloride was bubbled into a sample of 14 dissolved in deuteriochloroform and the PMR spectrum was taken. PMR analysis after ten minutes indicated a mixture of 70% 14 and 30% 72 (methyl α-chloro-N-acetylalaninate). After fourteen hours, the mixture by PMR analysis was 60 to 65% 72 and 35 to 40% 14 (no evidence of the isomerization to the β-chloro derivative was found after fourteen hours). As Love and Olsen (45) reported, the complete disappearance of 14 was observed within five minutes after the addition of hydrogen bromide to 14.

The failure of hydrogen chloride to readily add to the double bond of methyl N-acetyldehydroalaninate (14) perhaps can explain, in part, the poor yield of 66 reported by Lucente and Rossi (25) (see literature section) in which the nature of the N-acyl moiety greatly altered the yield of the corresponding methyl α-alkoxy-N-acylalaninate (20). Similarly, Gallina and coworkers (24) (literature review section) also noted this dependence of yield and the nature of the N-acyl group in their alkoxymercuration-demercuration sequence for the synthesis of 20.

However, the failure of hydrogen chloride to readily add to 14 was not the only reason for the poor yields (the 15% yield obtained for 66 by Lucente and Rossi (25) did not correlate with the 60 to 65% of methyl α-chloro-N-acetyl-D,L-alaninate (72) possible under their reaction conditions). This second reason must be related to the nature of methanol

since the synthesis of methyl α-acetylthio-N-acetyl-D,L-alaninate (74) (see below) using this approach proceeded in excess of these amounts. Furthermore, this general approach of addition of hydrogen chloride followed by substitution with mercaptans proceeded smoothly with N-acetyldehydroalanine (11) (14,30).

The best procedure for preparation of 74 was to use thiolacetic acid as a solvent and bubble hydrogen chloride continuously through the reaction mixture (89% verses 60% by the usual approach). However, a similar approach using methanol as solvent immediately hydrolyzed 14 to acetamide and methyl pyruvate with both hydrogen chloride and hydrogen bromide.

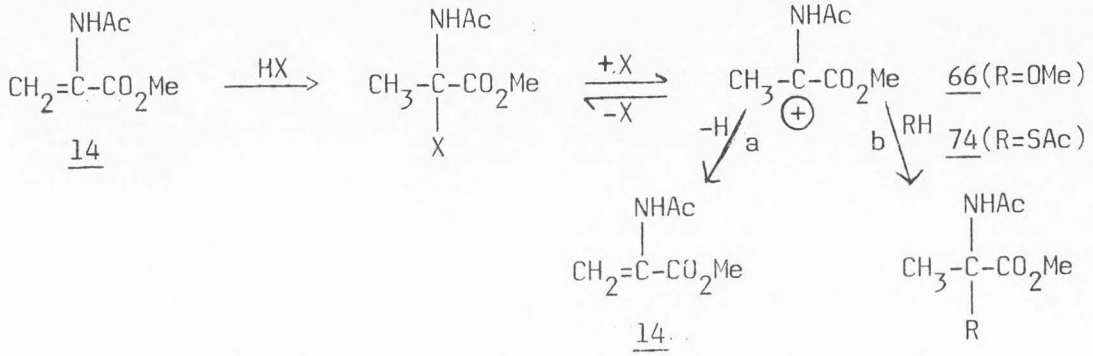


14 74

The preparation of methyl α-methoxy-N-acetyl-D,L-alaninate (66) proceeded smoothly with hydrogen bromide or even hydrogen chloride with sodium bicarbonate as added base (37). The yield was 20 to 30% regardless of the hydrogen halide used (hydrogen bromide gave characteristics to the product (66) which made purification more difficult with greater loss). Alternately, esterification of N-acetyldehydroalanine (11) with acetyl chloride in methanol (56) gave 66 in 24% yield. However, the subsequent appearance of the method of Poisel and Schmidt (54) via the N-chloride (65) was the best method for the preparation of 66 (see previous discussion).

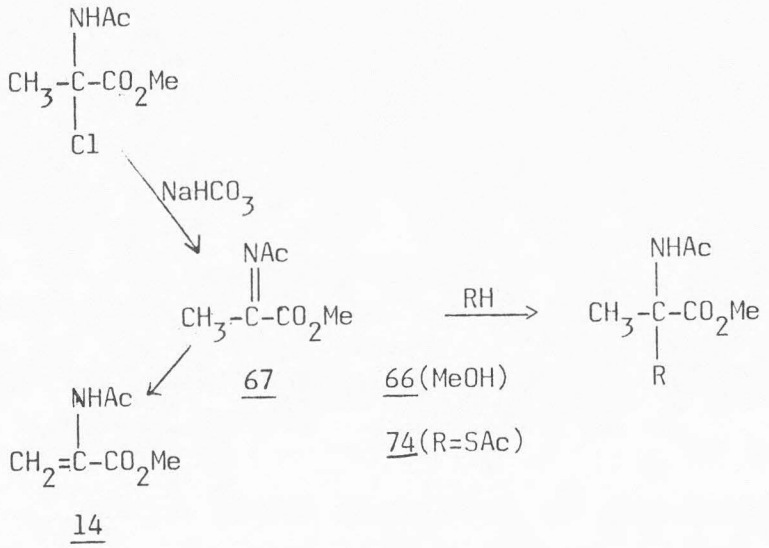
One approach to the explanation of the above results would be a dual mechanism, one sequence for reaction with added base (sodium

bicarbonate) and one sequence for reaction without base. When no base was added, the reaction conditions involved the addition of hydrogen halide to the double bond of 14 in methylene chloride and evaporation of solvent followed by the addition of methanol or thiolacetic acid. The carbonium ion, which was presumably formed, could react by two pathways; (a) loss of a proton to reform 14 or (b) reaction



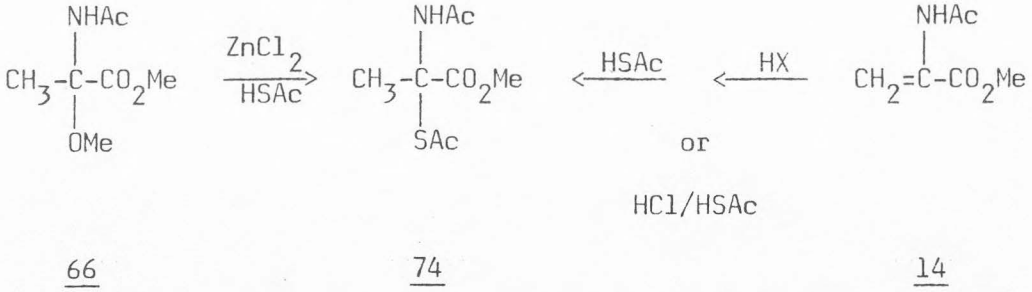
with a nucleophile to form product. Because of the difficulties observed when methanol was the nucleophile the rate of formation of the carbonium ion was, apparently, slower than the rate of carbonium ion formation when thiolacetic was the nucleophile. This description was supported by the formation of product (66) with acetyl chloride in methanol (overnight reaction). Furthermore, Patel (14) noted that hydrogen sulfide formed product one sixth as fast as either benzyl mercaptan and benzhydryl mercaptan using the approach of hydrochlorination-displacement on N-acetyldehydroalanine (11).

When base was added (sodium bicarbonate) the products obtained (66 or 74) were consistent with an acylimine intermediate (67) formed from the α-halo compound. This acylimine, besides reacting with a nucleophile to form product, could isomerize to starting marterial (14). It must



be noted that this acylimine (67) was the same intermediate that made the synthetic sequence of Poisel and Schmidt (54) possible. Furthermore, their synthesis of the α-methoxy derivatives was a sufficient example to illustrate that the reaction with nucleophiles was faster than isomerization of the acylimine to 14 (see also the discussion of our synthesis of 11 and 14, p. 43). When the base was sodium methoxide (see Table 4) the α-methoxy derivative (66) could not be isolated.

The reaction of methyl α-methoxy-N-acetyl-D,L-alaninate (66) and thiolacetic acid with zinc chloride gave methyl α-acetylthio-N-acetyl-D,L-alaninate (74) in 18% yield (61). This approach compared poorly

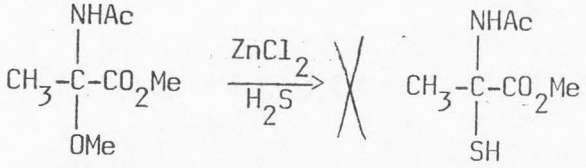


66 74 14

with the maximum 63% yield of 74 using the general approach of Patel and coworkers (30) via 14 and the maximum 89% yield of 74 via 14 by the

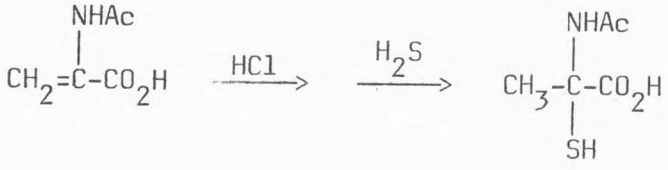
method developed here, i.e., use of thiolacetic acid as solvent and continuous addition of hydrogen chloride to the reaction mixture.

However, similar treatment of 66 with hydrogen sulfide and zinc chloride did not give the corresponding α-mercapto derivative (75).



66 75

This failure to prepare the methyl ester of α-mercapto-N-acetyl-D,L-alanine (76) was not surprising since all attempts to isolate 75 have failed (see Table 5) and 75 remains an unknown compound except as will be noted later. The free acid (76) is a known, stable compound prepared by Patel and coworkers (30).



11 76

All attempts to prepare the free acid of 66, i.e., α-methoxy-N-acetyl-alanine, also failed (unchanged N-acetyldehydroalanine (11) was recovered in every case).

Although these studies resulted in the observation of important mechanistic information, the conversion of α-methoxy or α-hydroxyl derivatives to the α-thio derivative using zinc chloride was only moderately successful.

TABLE 5

RESULTS OF ATTEMPTS TO SYNTHESIZE METHYL α-MERCAPTO-N-ACETYLALANINATE (75)

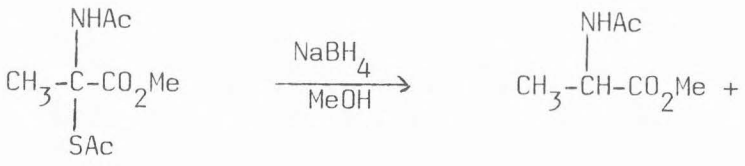
Starting material	Reagents	Product	Reference
14	HBr/NaSH	$AcNH_2 \cdot HBr$	--
14	$HBr/NaSH/NaHCO_3$	a	--
14	$HBr/H_2S/NaHCO_3$	tar	--
14	$HBr/NaSH/NaHCO_3$	b	--
14	$HBr/H_2S/HOAc$	$AcNH_2 \cdot HBr$	30

[a]T.L.C. evidence indicated product present. [b]A small amount of product by PMR analysis, however, it decomposed during further purification.

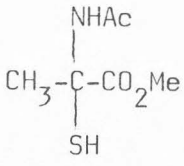
$$RSAc \xrightarrow[MeOH]{NaBH_4} RSH$$

Ohler and coworkers (66) reported the facile reductive hydrolysis of the acetylthio moiety to the corresponding mercapto derivative with sodium borohydride in methanol. Because of the successful preparation of 74 by the above zinc chloride and other methods mentioned above, this reductive hydrolysis seemed a convenient method to convert an α-acetylthio group to a α-mercapto group.

However, when this procedure was applied to methyl α-acetylthio-N-acetyl-D,L-alaninate (74), a mixture of methyl N-acetyl-D,L-alaninate (64) and what was thought to be methyl α-mercapto-N-acetyl-D,L-alaninate (75) was obtained. The saturated ester (64) was identified by T.L.C. and PMR comparisons with an authentic sample of 64.

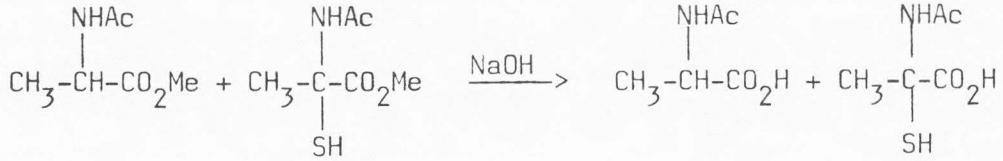


74 64



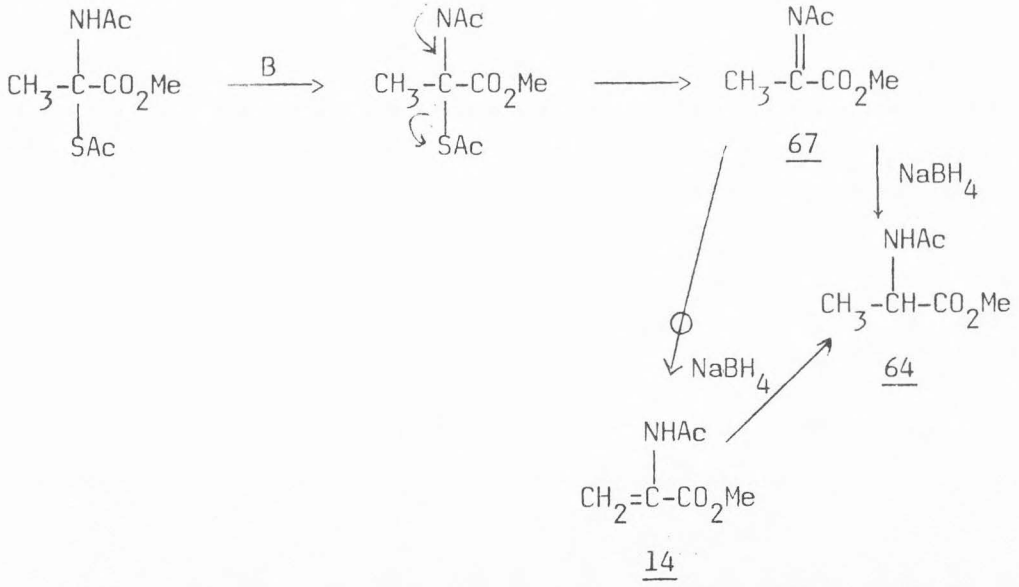
75

The identity of 75 was less clear, although PMR and T.L.C. comparisons with a crude sample (see Table 5) of 75 indicated this structure was probably valid. Further evidence for the presence of 75 was obtained by basic hydrolysis of the 64 and 75 mixture. PMR and T.L.C. comparisons confirmed the presence of 63 and 76.



64 75 63 76

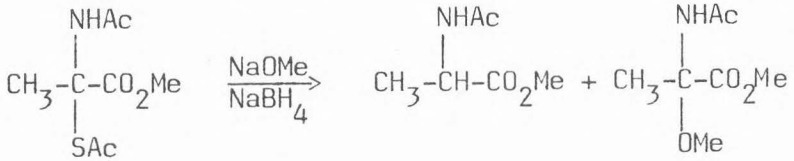
One way to explain the formation of 64 was to invoke a basic elimination of sodium thiolacetate with the formation of the acylimine intermediate (67) which was subsequently reduced by sodium borohydride (67 was, of course, the reactive intermediate upon which the synthesis of the N-acyldehydroalanine derivatives of Poisel and Schmidt (54,58) was based). In order to exclude the possibility that the acylimine (67)



isomerized to methyl N-acetyldehydroalaninate (14), which then underwent reduction to 64, methyl N-acetyldehydroalaninate (14) was treated with

sodium borohydride in methanol. Unfortunately, 14 was smoothly reduced to 64 under these conditions. Methyl N-acetyldehydroalaninate (14) was not reduced to 64 with sodium cyanoborohydride, but the reductive hydrolysis of 74 was also not effected with this reducing agent and was recovered unchanged.

Since it was felt that the formation of methyl α-mercapto-N-acetyl-D,L-alaninate (75) occurred before enough base accumulated to effect elimination to the acylimine (67), the reduction was performed with one equivalent of added sodium methoxide. A mixture of methyl N-acetyl-D,L-alaninate (64) and methyl α-methoxy-N-acetyl-D,L-alaninate (66) was obtained. However, this could be a result of the physical impossibility of getting sodium methoxide and sodium borohydride to react



74 64 66

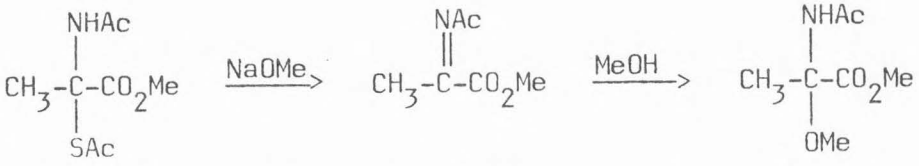
simultaneously. This was therefore indirect evidence that the acylimine (67) was reduced by sodium borohydride since evidence discussed earlier indicated the formation of the α-methoxy derivative (66) was faster than isomerization to methyl N-acetyldehydroalaninate (14) (i.e., if 14 was reduced by sodium borohydride, only the α-methoxy compound (66) would have been found in the product mixture).

The reductive hydrolysis of 74 using sodium borodeuteride was less clear. The reduction of 14 was apparently slower with $NaBD_4$ than with $NaBH_4$. The reaction of 74 with $NaBD_4$ gave a singlet for the methyl group

in the PMR spectrum of 64; however, the reaction was less clean and integration of the protons on the methyl group was indefinite.

Although the reductive hydrolysis of methyl α-acetylthio-N-acetyl-D,L-alaninate (74) failed, additional evidence for the acylimine intermediate was observed.

The ability of sodium methoxide to convert the α-acetylthio group of methyl α-acetylthio-N-acetyl-D,L-alaninate (74) to an α-methoxy group had been previously observed by us. This conversion seems best explained via the acylimine intermediate (67). Not only was the preparation of 66 complete (i.e., all evidence for 74 was absent), no evidence for the presence of methyl N-acetyldehydroalaninate (14) was observed (reaction of the acylimine (67) with methanol was faster than isomerization to 14). The identity of 66 was verified by PMR, T.L.C. and melting point comparisons to an authentic sample of 66.



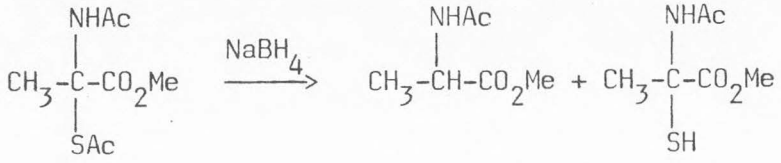
74 67 66

Summary

The conversion of α-hydroxy and α-methoxy-alanine derivatives to the corresponding α-mercapto-alanine derivatives was successful, using the zinc chloride method of Ohler and coworkers (61), only with thiolacetic acid and methyl α-methoxy-N-acetyl-D,L-alaninate (66). The failure of this method with the α-hydroxy deriviatives of 66 could be due to the instability of the α-hydroxy derivatives. Similarly, the

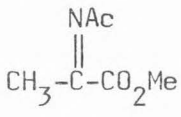
failure of this method to prepare methyl α-mercapto-N-acetyl-D,L-alaninate (75) was apparently due to the instability of this compound in the pure state.

The conversion of 74 to its free mercapto derivative (75), using the reductive hydrolysis method of Ohler and coworkers (66), was not synthetically useful because reduction of α-acetylthio-N-acetyl-D,L-alanine in the presence of basic sodium borohydride gave a mixture of 64 and 75. Reactions at the α position of α-hetero-N-acetyl-D,L-alanine



74 64 75

were apparently governed by the acylimine intermediate (67) under basic conditions.



67

Similarly, the reactivity of the α position in α-hetero-N-acetyl-D,L-alanine deriviatives under acidic conditions was apparently governed by a carbonium ion mechanism. A difference in the rate of formation of this carbonium ion with methanol and mercaptans was seemingly responsible for the difference in the ease of product formation, i.e., the formation of α-mercapto derivatives was more facile and complete than the formation of α-methoxy derivatives.

Using the mechanistic proposals of the α position of α-hetero-N-acetyl-D,L-alanine deriviatives, facile and synthetically useful,

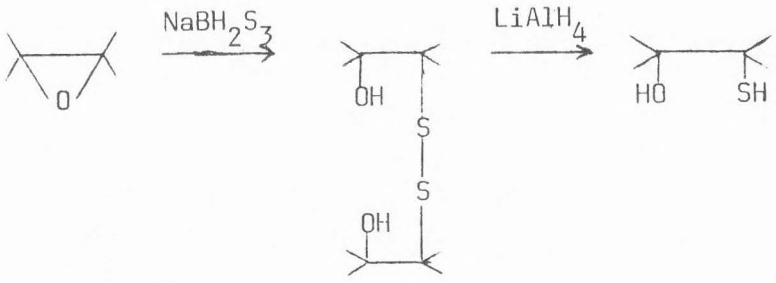
successful syntheses of α-methoxy and α-mercapto derivatives were accomplished. However, the synthetic method for the preparation of the α -methoxy-N-acetyl-D,L-alanine derivatives was not as synthetically useful as the literature method of Poisel and Schmidt (54,58). However, a synthesis of 74 in a 89% yield was developed and appeared to be a

14 74

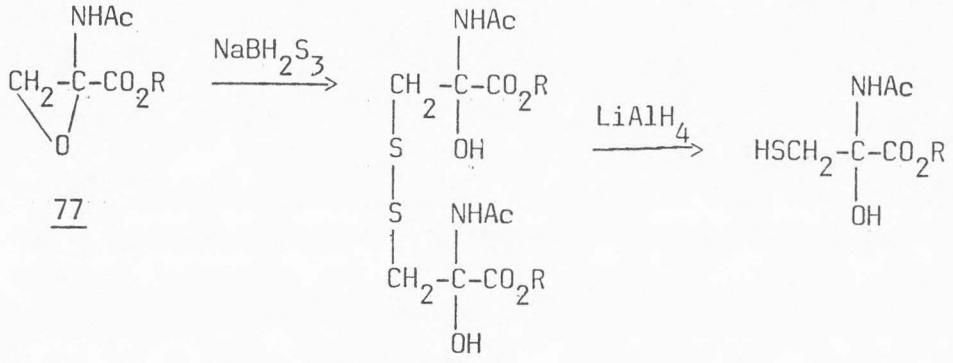
useful synthetic approach to the α-thioethers of N-acetyl-D,L-alanine derivatives.

Studies toward the synthesis of α,β-dimercapto-N-acetyl-D,L-alanine derivatives

Lalancette and Freche (67) reported the preparation of vicinal mercapto hydroxides from the corresponding epoxides. Since the mercapto group was found at the less hindered side of the molecule, it was hoped



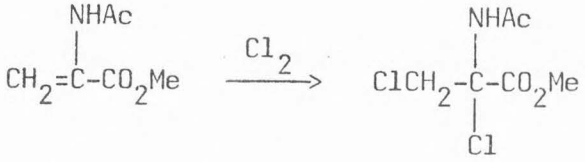
that a similar procedure would result in the synthesis of β-mercapto-α-hydroxy-N-acetyl-D,L-alanine derivatives. For this reason, a



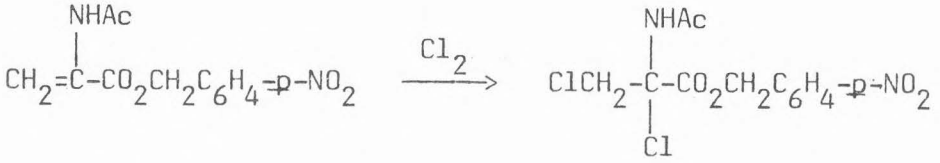
77

stepwise synthesis of the epoxide of N-acetyl-D,L-alanine derivatives (77) was attempted.

The α,β-dichloro-N-acetyl-D,L-alanine methyl ester (7a) and p-nitrobenzyl ester (7b) could be easily prepared from 14 and 71 by the addition of chlorine, both quantitatively and cleanly. However, contrary



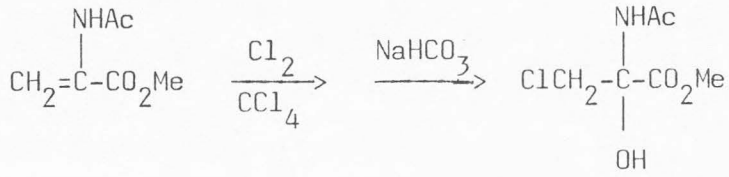
14 7a



71 7b

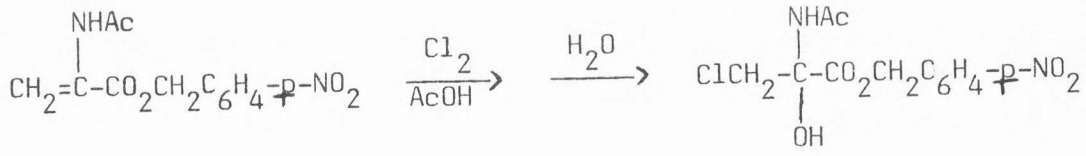
to the α,β-dichloro and α,β-dibromo-N-acyl-D,L-alanine derivatives reported by Kildisheva and coworkers (18a), 7a and 7b were unstable at room temperature and could not be isolated. For this reason, 7a 7b and the corresponding α,β-dibromo derivatives were generated in situ immediately before further reaction.

The method of Kildisheva and coworkers (37) was used to prepare methyl β-chloro-α-hydroxy-N-acetyl-D,L-alaninate (78). However, this approach failed with the corresponding p-nitrobenzyl ester because



14 78

of the insolubility of 71 in carbon tetrachloride. Therefore, the approach of Patel and coworkers (30) was used to prepare p-nitrobenzyl β-chloro-α-hydroxy-N-acetyl-D,L-alaninate (79). Although, distinctly

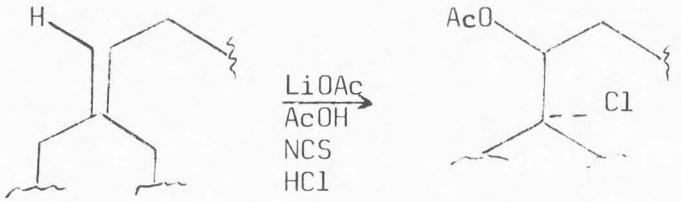


71 79

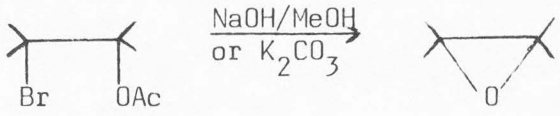
more stable than the corresponding dichloro derivatives, 78 and 79 decomposed during recrystallization and were thereafter used crude (79 was the more stable of the two).

However, all attempts to prepare the corresponding epoxide by generation of the alkoxide anion followed by substitution with ring closure failed. Since it was not known if the relative instability of 78 and 79 was responsible for this failure, an alternative system was sought involving a more stable intermediate.

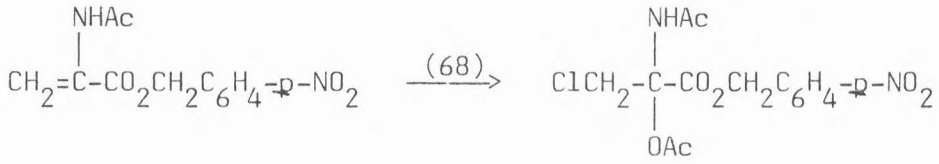
Robinson and coworkers (68) prepared vicinal chloro acetates from the corresponding alkene.



Levine and Wall (69) reported the facile preparation of epoxides from the corresponding vicinal bromo acetates.

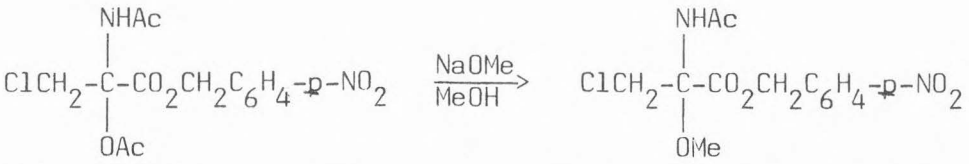


The preparation of the β-chloro-α-acetate (80) proceeded smoothly in 78% yield from p-nitrobenzyl N-acetyldehydroalaninate (71) using the procedure of Robinson and coworkers.



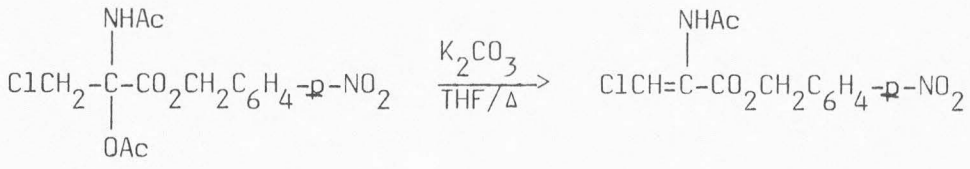
71 80

Attempts to prepare the corresponding epoxide from 80 using sodium methoxide in methanol gave p-nitrobenzyl β-chloro-α-methoxy-N-acetyl-D,L-alaninate (81). The identity of 81 was confirmed by comparison of melting point and PMR spectra with an authentic sample of 81, prepared as will be discussed later (see p. 71).



80 81

Attempts to prepare the corresponding epoxide from 80 using potassium carbonate as base gave p-nitrobenzyl (Z)-β-chloro-N-acetyldehydroalaninate (82). The identity of 82 was confirmed by melting

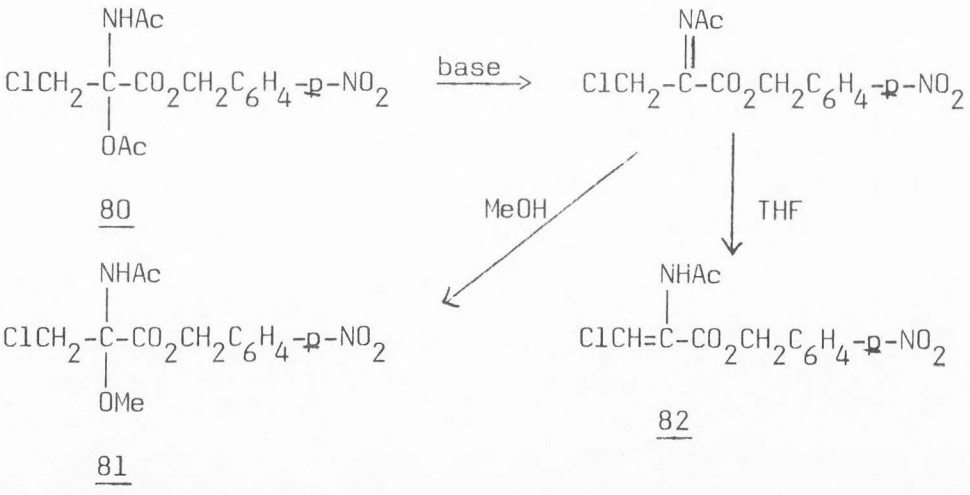


80 82

point and PMR comparisons with an authentic sample, which was prepared by a known method that will be discussed in detail later in this thesis as well as the stereochemistry (see p. 81).

7b 82

The rationale for the preparation of both 81 and 82 was the occurrence of the acylimine intermediate previously mentioned. The base (sodium methoxide or potassium carbonate) generated the acylimine, which then reacted with a nucleophilic solvent (methanol) to form 81 or isomerized to 82 in the absence of a nucleophile in sufficient concentration.



80 82 81

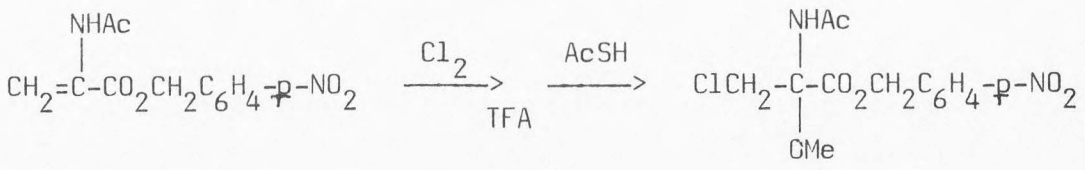
Because of the operation of the acylimine intermediate, this stepwise approach to the synthesis of epoxide (77) was discontinued. The

direct preparation of the epoxide (77) from the corresponding N-acetyldehydroalanine derivatives using m-chloroperoxybenzoic acid (MCPBA) (70), t-butyl hydroperoxide (71), or mercuric oxide-iodide (72) failed (i.e., inseparable mixtures, except for N-acetyldehydroalanine (11) in which unchanged 11 was recovered, were obtained).

Although all attempts to synthesize an epoxide of N-acetyldehydroalanine derivatives failed (the synthetic sequence of Lalancette and Freche (67) was not applicable to the N-acetyldehydroalanine system), important mechanistic information was derived from this approach. The acylimine intermediate was apparently operative in the α,β-disubstituted N-acetylalanine system.

The α,β-dibenzylthio derivative of methyl N-acetyl-D,L-alaninate (32) (Patel(14)) and of N-acetylphenylalanine (34) (Kildisheva and coworkers(38a)) (see literature review) had been prepared. Since Pojer and Rae (28,43) and Olsen (13) had demonstrated the inability to successfully deprotect the sulfur of the benzylthio moiety, the stepwise addition of non-benzylthio groups was investigated.

Several attempts to prepare p-nitrobenzyl β-chloro-α-acetylthio-N-acetyl-D,L-alaninate (83), using the general approach of Patel and coworkers (30), gave, unexpectedly, p-nitrobenzyl β-chloro-α-methoxy-N-acetyl-D,L-alaninate (81). Since methanol had been added during work

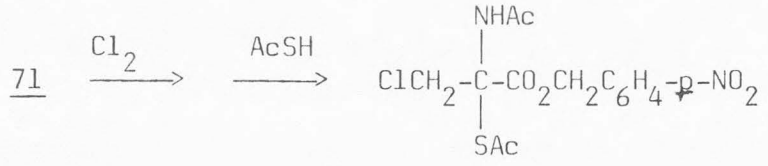


71 81

up to help eliminate traces of trifluoroacetic acid (TFA), the origin of the methoxy group could be explained. Furthermore, one experiment

using added ethanol gave an isolated solid whose PMR spectrum was consistent with the corresponding α-ethoxy derivative.

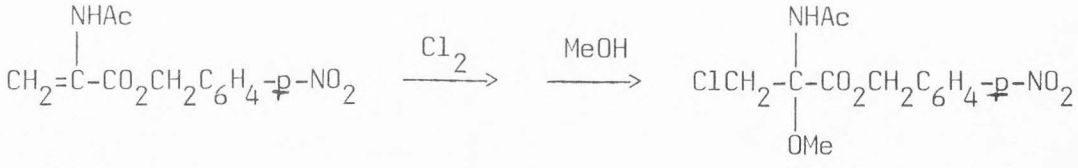
The failure to obtain 83 was thought to be due, in part, to the instability of this compound since 83 prepared by exclusion of TFA and



83

use of thiolacetic acid as solvent was a clear film which was extremely hygroscopic in air and had only limited stability in an evacuated desiccator. However, PMR spectra of crude 83 which had been stirred at room temperature with methanol, methanol plus TFA and sodium bicarbonate plus methanol all appeared to be unchanged 83; thus, 81 was not formed from 83 during the work up. The formation of 81 can be rationalized, but the failure of 83 to form was not understood, i.e., why the presumed intermediate carbonium ion does not react with thiolacetic acid in the presence of TFA.

The identity of 81 was established by PMR and melting point comparisons with an authentic sample of 81 prepared by the addition of chlorine to 71 followed by treatment with methanol.



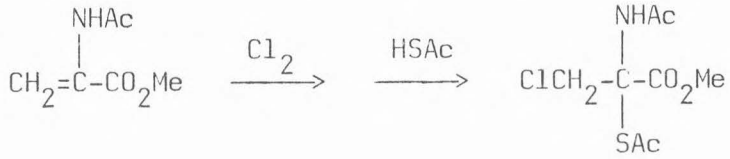
71 81

Attempts to find evidence for the acylimine intermediate by treatment of 83 with sodium methoxide in methanol were complicated by the formation of 71 and, therefore, although some PMR evidence existed for

the formation of 81 via the acylimine no claim is made in support of the evidence previously noted above.

In an attempt to observe additional evidence for the acylimine intermediate in the reactions of the α-position of the methyl ester of α,β-disubstituted N-acetyl-D,L-alanines, the conversion of methyl β-chloro-α-acetylthio-N-acetyl-D,L-alaninate (84) to methyl β-chloro-α-methoxy-N-acetyl-D,L-alaninate (85), using sodium methoxide in methanol, was investigated.

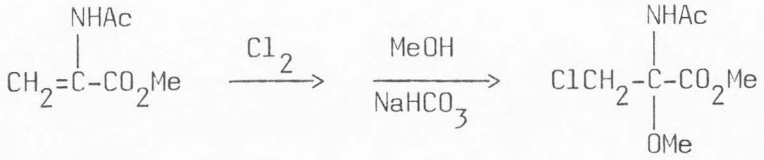
Methyl β-chloro-α-acetylthio-N-acetyl-D,L-alaninate (84) was prepared from methyl N-acetyldehydroalaninate (14) via a sequence of chlorine addition followed by treatment with neat thiolacetic acid (probably, via the carbonium ion). The preparation of 84 with one equivalent



14 84

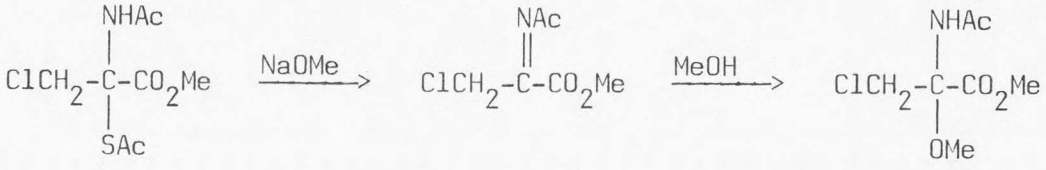
of sodium methoxide or sodium bicarbonate was also successful (yields were about 30% with or without base). The product (84) was unstable in the presence of Norit (charcoal).

Methyl β-chloro-α-methoxy-N-acetyl-D,L-alaninate (85) was smoothly prepared in 45% yield by the general approach of Kildisheva and coworkers (37).



14 85

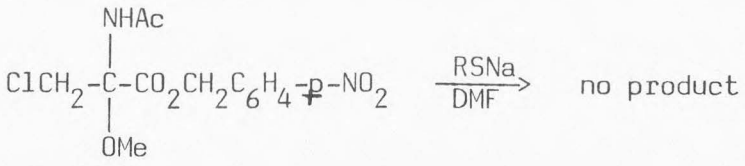
As expected, the α-acetylthio derivative (84) was smoothly converted to the α-methoxy derivative (85) by treatment with sodium methoxide in methanol. Thus, strong evidence exists for the acylimine as the



84 85

intermediate in the base catalyzed reactions of the α-position of α,β-disubstituted N-acetyl-D,L-alanine derivatives. This latter evidence correlated with that obtained for the α substituted system.

Since the methoxide anion is a strong base, and thus, a poor leaving group, one would not expect the acylimine to form from 81 (or 85) in the presence of the anions of mercaptans (indeed, 84 or 74 was not formed from 85 or 66 when treated with one equivalent of potassium t-butoxide in thiolacetic acid as solvent; unchanged 85 or 66 was recovered). Therefore, several attempts to substitute the acetylthio or the benzylthio group for the β-chloro group of p-nitrobenzyl β-chloro-α-methoxy-N-acetyl-D,L-alaninate (81) were done following the nucleophilic substitution procedure of Wilchek and coworkers (36) (see literature review). Only starting material or other non-productive



81

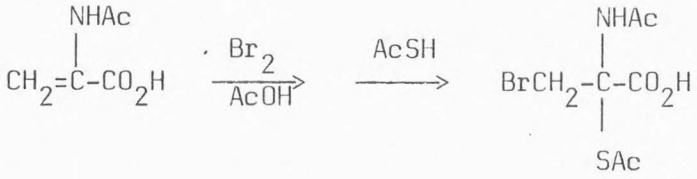
R=$PhCH_2$, Ac

materials were isolated; no evidence of the desired products was ever obtained. Thus, the protection of the α position (the α-methoxy group

eliminates interference to nucleophilic reaction at the β position by reactions at the α position via the acylimine) did not lead to successful nucleophilic displacements at the β position of 81.

Although nucleophilic substitution in the β-chloro position of p-nitrobenzyl β-chloro-α-methoxy-N-acetyl-D,L-alaninate (81) had failed, the observation of Kildisheva and coworkers (38) of the difference in reactivity of α,β-dichloro-N-benzoylalanine (36) and α,β-dibromo-N-acetylphenylalanine (33) (see literature review) was still an encouragement. Since Patel and coworkers (30) had prepared β-bromo-α-acetylthio-N-acetyl-D,L-alanine (31), this compound was chosen for further attempts to synthesize α,β-dimercapto derivatives.

β-Bromo-α-acetylthio-N-acetyl-D,L-alanine (31) was prepared by the method of Patel and coworkers (30) in 79% yield. Interestingly, one attempt to prepare 31 using TFA as solvent as in the attempted

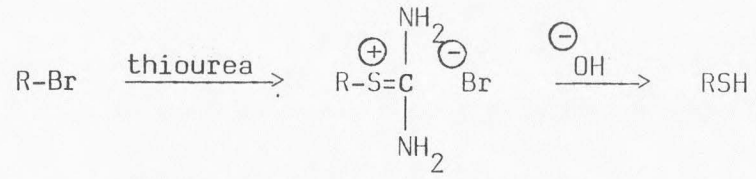


11 31

synthesis of 83 did not give isolable product (31). Similarly, attempts to convert 31 to its methyl ester with diazomethane or via the silver salt failed.

All attempts to synthesize the α,β-diacetylthio derivative by nucleophilic displacement of the β-bromo group of 31 failed (only starting material (31) was recovered).

Attempts to prepare the β-mercapto-α-acetylthio derivative of 31 via the isothiuronium salt (73) or via the Bunte salt (74) gave



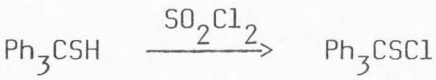
only unchanged starting material (31). Thus, reactions of both a

nucleophilic nature and other various conversions of alkyl halides to mercaptans were unsuccessful at the β position of N-acetyl-D,L-alanine derivatives.

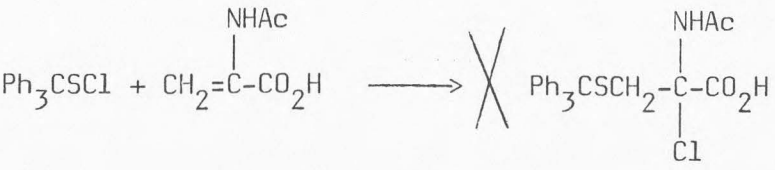
Since replacement of the β-halo moiety failed and replacement of the α-halo moiety was comparatively easy, a report by Gundermann and Huchting (75) of the facile addition of methyl sulfenyl chloride to methyl acrylate seemed an appropriate method of synthesis of α,β-

dimercapto derivatives.

Trityl sulfenyl chloride was prepared from trityl mercaptan with sulfurly chloride (76).

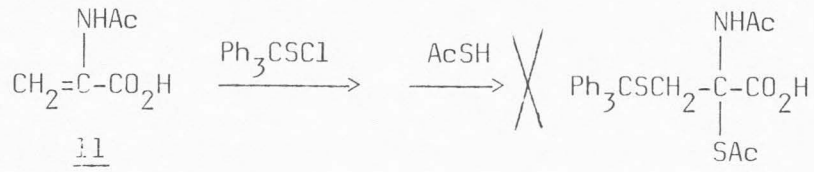


The attempted addition of trityl sulfenyl chloride to 11 gave only recovered 11. To avoid the possibility that 86 was unstable to



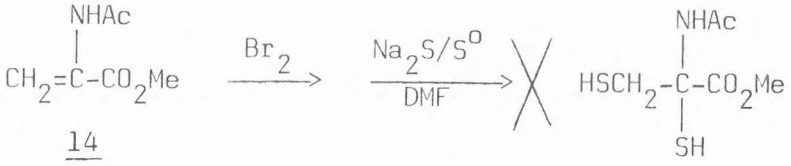
11 86

isolation (no examples of these compounds were found in the literature), 86 was generated in situ and treated with one equivalent of thiolacetic acid. However, only 11 was recovered in as high as 85% yield. Thus,



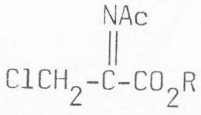
an attempt to prepare an α,β-disubstituted N-acetyl-D,L-alanine derivative containing a chloride group at the highly reactivity α position and a mercapto derivative at the unreactive β position failed.

Finally, because of the report by Eliel and coworkers (77) that sodium sulfide/sulfur in DMF was a very superior reagent for the preparation of mercaptans, this reagent was applied to the dibromo derivative of 14. However, this reaction also failed (only polymerized 14 was recovered).



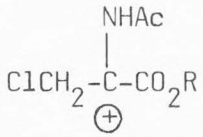
Summary

Compelling evidence was observed for the occurrence of the acylimine intermediate in the reactions of the α position of α,β-disubstituted N-acetyl-D,L-alanine derivatives under basic conditions. Similarly,

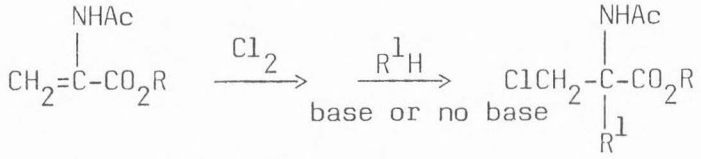


R=Me, p-$O_2NC_6H_4CH_2$

evidence for the carbonium ion intermediate was also observed. Both of these intermediates were apparently more reactive than the corresponding derivative in the previously investigated α system, i.e., the ease and completeness of product formation was markedly improved over the



α system. However, the ability of trifluoroacetic acid (TFA) to prevent formation of β-halo-α-acetylthio derivatives (presumably when the carbonium ion intermediate was operative) was noted but not understood. The preparation of β-halo-α-alkoxy and α-mercapto derivatives were, in general, facile and led to relatively pure products.

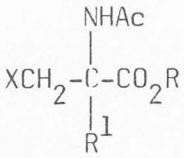


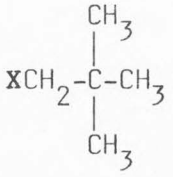
R^1=alkyl S, aryl S, alkyl O

R=Me, H, p-nitrobenzyl

All attempts to synthesis α,β-dimercapto-N-acetyl-D,L-alanine derivatives failed. This failure was the result of an inability to convert the β-halo moiety to a β-mercapto derivative.

One explanation for the failure to obtain nucleophilic substitution of the S_N2 type in the β position of α,β-disubstituted N-acetyl-D,L-alanine derivatives would be that the β-halo-α-substituted alanine system is a pseudo-neopentyl system. It is known that the relative

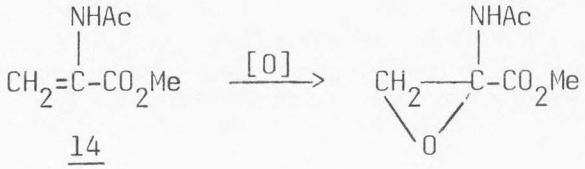




neopentyl halide

rate of S_N2 substitution at a primary carbon decreases with increasing β branching. The rate is slowest in the neopentyl system and is due to steric hindrance; i.e., a physical blockage to backside attack of the nucleophile.

Similarly, all attempts to form the epoxide of methyl N-acetyl-dehydroalanine (14) failed. This may not be surprising since the corresponding episulfides were known to be unstable and easily decomposed.

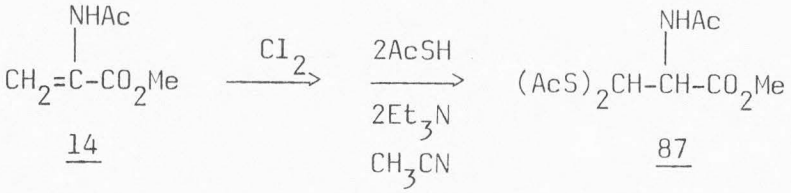


14

However, further study of the synthesis of α,β-dimercapto-N-acetyl-D,L-alanine derivatives was interrupted by the correction of the structure of the quinomycin family of quinoxaline antibiotics in 1975. Since at that time, this research was federally supported, further study of the synthesis of the α,β-dimercapto derivatives could not be justified.

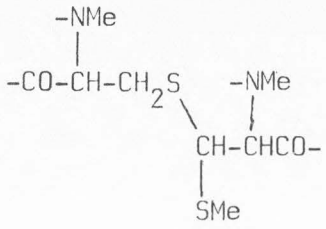
Studies toward the synthesis of β,β-dimercapto-N-acetyl-D,L-alanine derivatives

Six months before the structure of the quinomycin family of quinoxaline antibiotics was revised (8h,15), an attempt to prepare methyl α,β-diacetylthio-N-acetyl-D,L-alaninate using the method of Endo and coworkers (78) gave methyl β,β-diacetylthio-N-acetyl-D,L-alaninate (87) in 19% yield. Since the corrected structure involved the dithioacetal moiety (10), which can be visualized as a β,β-disubstituted



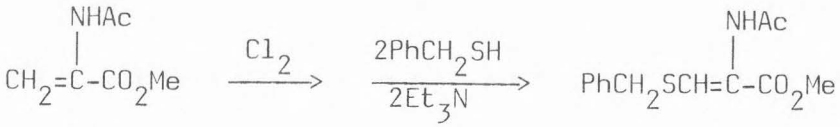
14 87

alanine, the preparation of 87 appeared to allow a smooth transition from the study towards the synthesis of the α,β-structure to the synthesis of the β,β-structure.



10

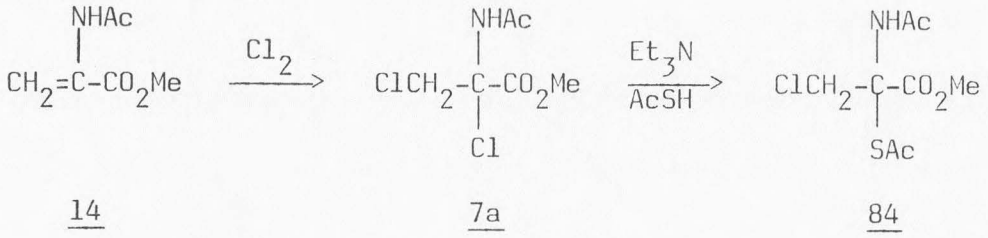
Using the procedure that gave 87, the preparation of methyl β,β-dibenzylthio-N-acetyl-D,L-alaninate was attempted. However, only methyl β-benzylthio-N-acetyldehydroalaninate (88) was obtained in 32% yield.



14 88

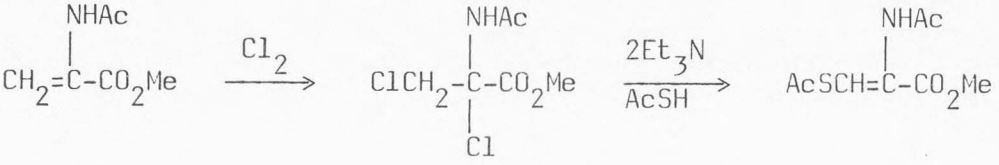
This failure to form the β,β-dibenzylthio derivative, the relative low yields, and a desire to understand the mechanism of the reaction caused us to investigate the formation of 87 in detail.

The α,β-dichloro compound (7a) gave methyl β-chloro-α-acetylthio-N-acetyl-D,L-alaninate (84) when treated with one equivalent of triethylamine and one equivalent of thiolacetic acid.



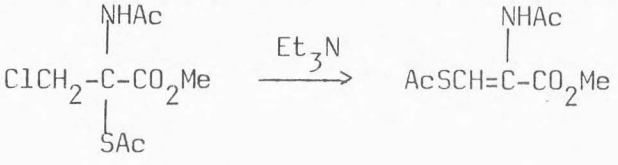
14 7a 84

Similarly, 7a treated with two equivalents of triethylamine and one equivalent of thiolacetic acid gave a 24% yield of methyl β-acetylthio-N-acetyldehydroalaninate (89).



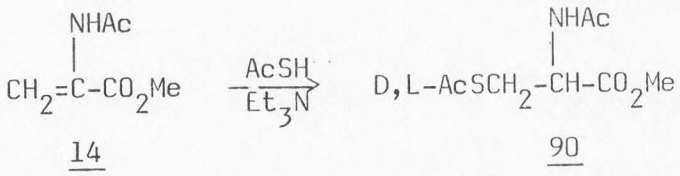
14 7a 89

Treatment of 84 with one equivalent of triethylamine under the same reaction conditions gave 89. The identity of 89 was established by PMR and T.L.C. comparisons. However, 89 could not be isolated since it was unstable on a silica gel column and was not formed in sufficient quantity to allow isolation by crystallization.



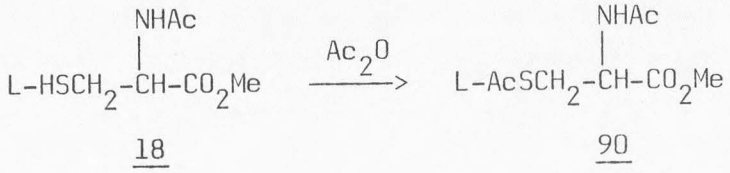
84 89

To rule out the possibility that 89 was formed from 14, 14 was treated with one equivalent of thiolacetic acid. Both one equivalent or two equivalents of triethylamine gave methyl β-acetylthio-N-acetyl-D,L-alaninate (90).



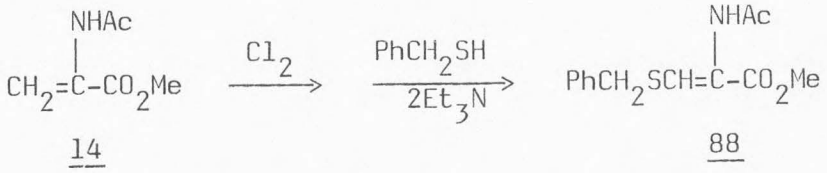
14 90

The identity of 90 was established by PMR and IR comparisons with the known L-isomer of 90. The L-isomer of 90 was prepared by the method of Wirth (79) except sodium bicarbonate was used as the base.



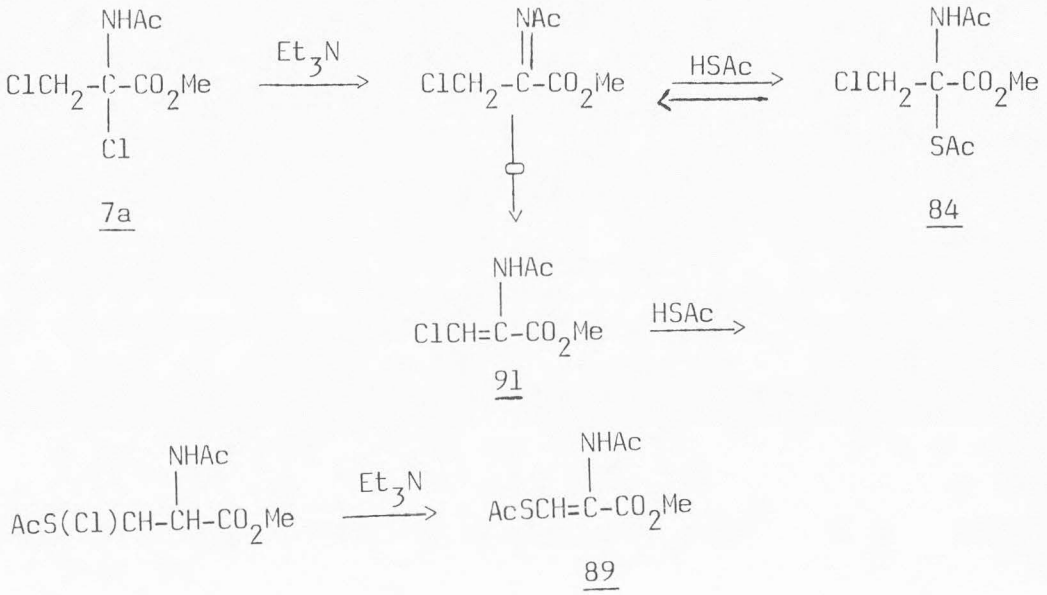
18 90

Similarly, one equivalent of benzyl mercaptan and two equivalents of triethylamine gave a 12% yield of 88 from 14 via the dichloro intermediate.



14 88

It appears the mechanism of formation of 89 and probably 88 involved the initial formation of the α-derivative (84) via the acylimine. The second equivalent of base then eliminated thiolacetic acid reforming the acylimine which isomerized to 91. Methyl β-chloro-N-acetyldehydroalaninate (91) then underwent a Michael type addition followed by elimination of hydrogen chloride to form 89.

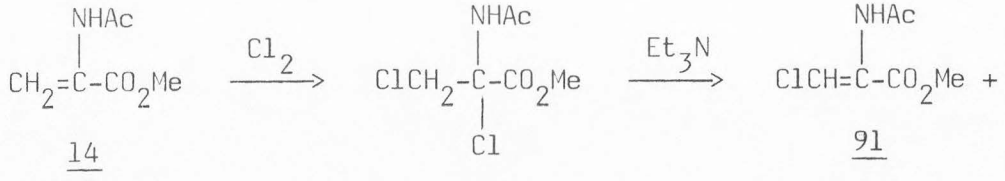


7a 84 91

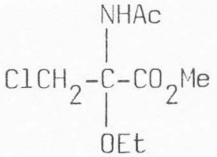
89

Because of the involvement of methyl β-chloro-N-acetyldehydroalaninate (91) in the apparent mechanism and the knowledge that compounds of this type were stable, the preparation of 91 was attempted in the hope of increasing the yield and obtaining further mechanistic information.

Treatment of the α,β-dichloro compound (7a) with one equivalent of triethylamine or by refluxing in benzene (Kildisheva and coworkers (39a)) gave methyl β-chloro-N-acetyldehydroalaninate (91) in low yield (18% maximum). Large scale reactions (0.1 mol) using triethylamine gave, in addition to 91, isolable amounts of methyl β-chloro-α-ethoxy-N-acetyl-D,L-alaninate (92).

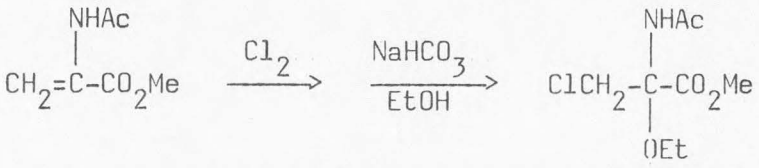


14 7a 91



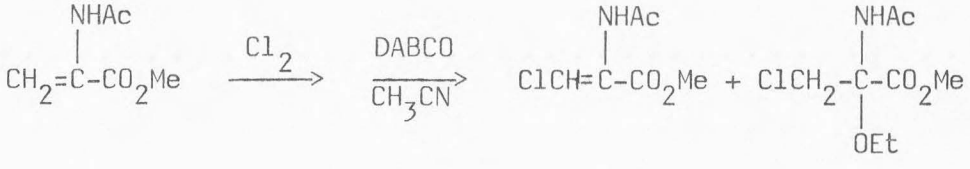
92

The identity of 92 was confirmed by melting point and PMR comparisons with an authentic sample prepared from 7a with sodium bicarbonate in ethanol.



14 92

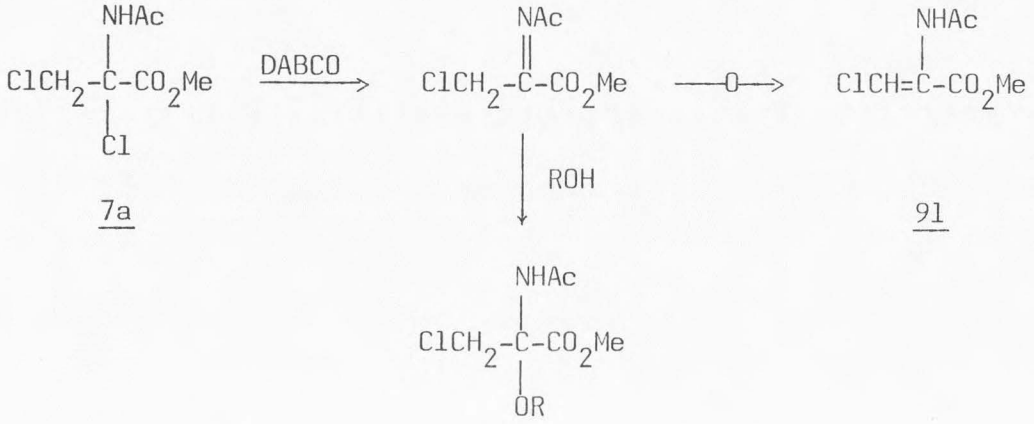
Because of the low yields and the slowness of the reaction (overnight at room temperature or refluxing benzene), an alternate approach under mild conditions was sought. Because of the superiority of DBU-like amine bases in dehydrohalogenation (bases like sodium methoxide were disregarded because of the acylimine intermediate) over other amine type bases (see previous discussions of this point), DABCO was chosen for the synthesis of (91). Although the yield of 91 increased to 40% (isolated), the formation of 92 continued to plague the synthesis.



14 91 92

Since it was unlikely that both DABCO (a solid) and triethylamine contained ethanol as an impurity, the solvent (acetonitrile) was suspected. Substitution of purified methylene chloride (see previous discussion) for acetonitrile solved this problem (in one experiment using methylene chloride as solvent, the synthesis of 91 was hindered by formation of methyl β-chloro-α-methoxy-N-acetyl-D,L-alaninate (85) but the origin of the methanol was never determined).

The formation of both 92 and 85 can be cited as compelling evidence that the formation of 91 involved the acylimine intermediate. The acylimine could isomerize to the normal product (91) or react with

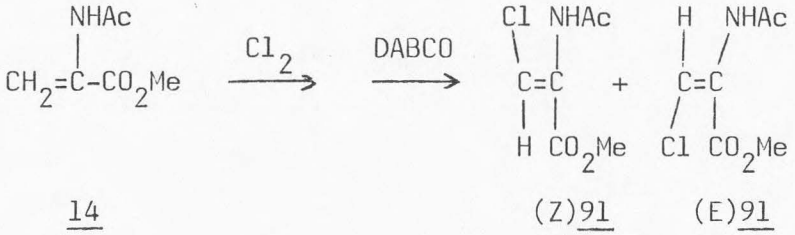


R=Et, Me

alcohols to form the undesired α-alkoxy compounds. The anti-Michael type addition of ethanol to 91 was ruled out because 91, when treated overnight at room temperature with triethylamine and ethanol in acetonitrile, gave only unchanged 91. Similarly, 7a treated with triethylamine and ethanol in acetonitrile gave only 92.

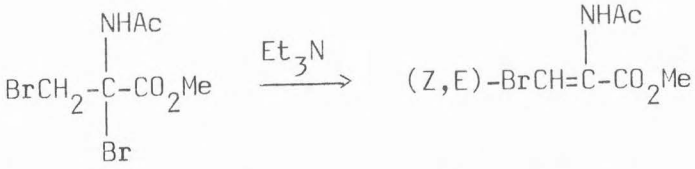
Thus, the above investigation not only established the mechanism of reaction (the acylimine intermediate), but led to a higher yield preparation of 91.

Once the problem with low yields was solved, the formation of the stereoisomers of methyl β-chloro-N-acetyldehydroalaninate (91) was investigated. Use of DABCO as the base gave a 4:1 mixture of (Z) and (E) methyl β-chloro-N-acetyldehydroalaninate (91). The



stereochemistry of the double bond was assigned by use of PMR spectroscopy. It has been shown (80) that for α-acylaminoacrylate (N-acetyldehydroalaninates) or crotonate derivatives, a vinylic proton cis to the acylamino group was downfield compared to a proton trans to that function. Furthermore, a proton trans to the acylamino group (Z isomer) was shifted downfield 0.34 to 0.80 ppm in going from $CDCl_3$ to TFA, while a proton cis to this function (E isomer) underwent an upfield shift of 0.0 to 0.50 ppm (80a) (sometimes this shift in TFA can result in an exchange of positions of protons, i.e., the Z isomer has the low-field vinyl absorption). This second criterion allowed assignment of stereochemistry when only one isomer was present. Additionally, it was observed that the chemical shift positions of the vinylic protons for mixtures of E and Z isomers were not reproducible among various samples; however, the relative positions of the vinylic protons for the two isomers did not change (a single, isolated isomer did have reproducible chemical shift positions for the vinylic proton).

Nakatsuka and coworkers (81) had reported the preparation of the bromo derivative of 91 in a 2:1 Z/E ratio using triethylamine as base. Following this procedure, a 1:1 Z/E ratio using triethylamine was obtained. When repeated, but using DABCO as base, a 3:1 Z/E ratio



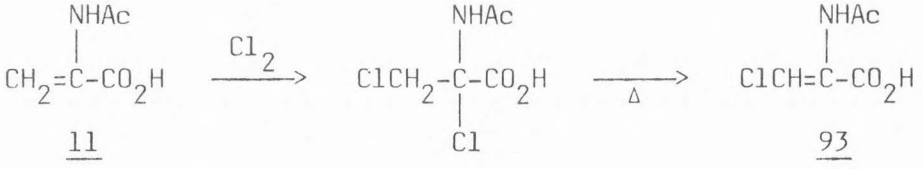
was observed. The Z isomer was separated and purified to give a solid, while purification of the E isomer gave an oil.

Because of this difference in Z/E ratios, 91 was prepared using DBU as the base. With DBU as base the ratio of Z/E was as high as

9:1 (the reaction time was the same as with DABCO, i.e., 20 minutes at room temperature). Similarly, potassium t-butoxide when used as the base gave a 9:1 ratio of Z/E isomers. Thus, some selectivity can be obtained in the preparation of the Z and E isomers of 91 by an appropriate selection of the base used to effect elimination of HCl.

The Z isomer of 91 could be easily and selectively crystallized from the Z/E mixture until the ratio of isomers approached 1:1. Since the E isomer of 91 could only be obtained with great difficulty, as a semi-pure oil, the normal procedure was to remove as much of the Z isomer as conveniently possible and use the 1:1 ratio of Z/E mixture for further reaction. Separation of the Z isomer and the E isomer was then effected on the new product produced.

Unlike 91, the corresponding free acid (93) was best formed by warming a chloroform-acetonitrile solution of the dichloro derivative. Not only was this compound insoluble in $CDCl_3$, it was unstable in TFA



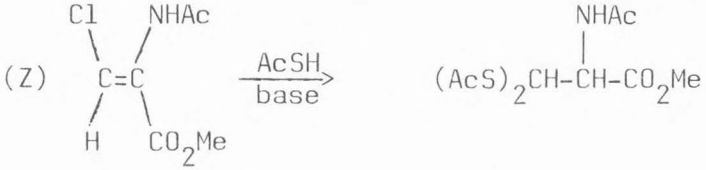
except for short periods. The isomer obtained was assumed to be the Z isomer. Furthermore, the product was not sufficiently stable to allow preparation of an analytical sample.

Once the synthesis of 91 and its E and Z isomers was accomplished, the conversion of 91 to the corresponding β- and β,β-dimercapto derivatives was investigated. Pure methyl (Z)-β-chloro-N-acetyldehydroalaninate (91) gave the corresponding methyl (Z)-β-acetylthio-N-acetyldehydroalaninate (89) in 30 to 40% yield (the presence of the E isomer was never detected). Similarly, the approximate 1:1 ratio

of Z/E 91 gave a Z/E mixture of 89. Methyl (E)-β-acetylthio-N-acetyldehydroalaninate (89) was easily separated from the Z isomer because the Z isomer decomposed on a silica gel column. Only the E isomer could be purified for elemental analysis (several attempts to prepare an analytical sample of the Z isomer resulted in decomposition).

91 89

Methyl (Z)-β-chloro-N-acetyldehydroalaninate (91), when stirred at room temperature with excess thiolacetic acid and slightly more than one equivalent of sodium bicarbonate plus DABCO, gave 87 in a 53% yield.



91 87

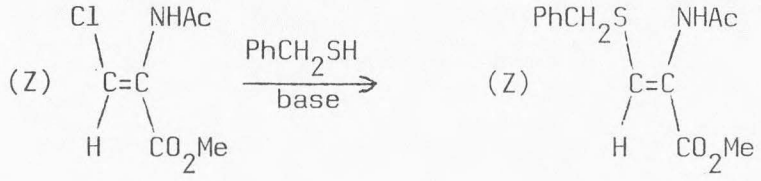
The Z isomer of 89 gave a 70% isolated yield of 87 in 4 hours at room temperature with only a catalytic amount of DABCO and excess thiolacetic acid. Indeed, PMR analysis showed 87 formed from (Z)-89

89 87

even without catalytic amounts of DABCO. Surprisingly, the E isomer of 89, when treated with excess thiolacetic acid and a catalytic amount of DABCO, did not readily form 87 (after 2.5 hours at room temperature,

(E)-89 was isolated in 50% recovery. After an additional 7.5 hours, a PMR spectrum of the crude reaction mixture still contained a prominent vinyl proton peak). These results indicated a markedly different reactivity for the Z and E isomers of 89.

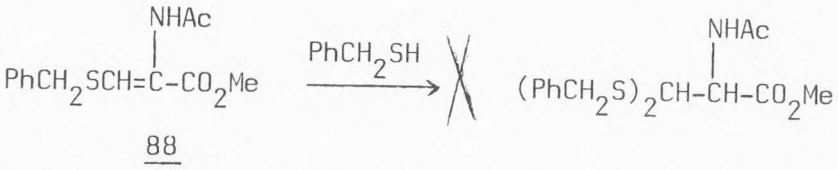
Once the reactions of 89 and 91 towards thiolacetic acid were determined, the reactivity of 91 toward other mercaptans was investigated.



91 88

Methyl (Z)-β-chloro-N-acetyldehydroalaninate (91) and benzyl mercaptan gave methyl (Z)-β-benzylthio-N-acetyldehydroalaninate (88). The yield was 50 to 70% regardless of the base used (KOH, Et_3N or DABCO). Similarly, a Z/E mixture of 91 gave Z/E-88. The E isomer of 88 was separated from the Z isomer by selective crystallization and Norit treatment. Both isomers were stable and analytical samples were easily prepared.

All attempts to add a second molecule of benzyl mercaptan to 88,

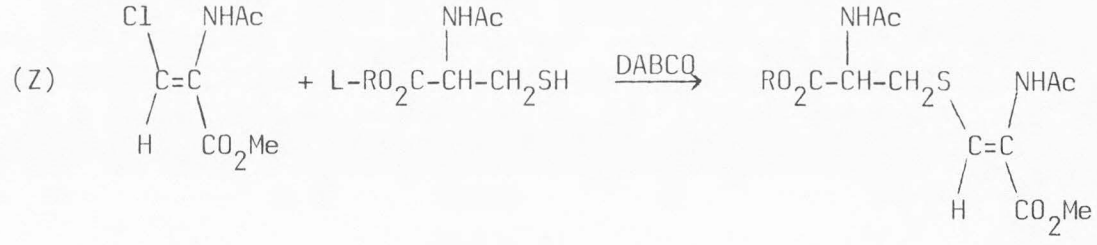


88

i.e., to form the β,β-dibenzylthio derivative, failed. Only starting material (88) was recovered under basic or free radical conditions.

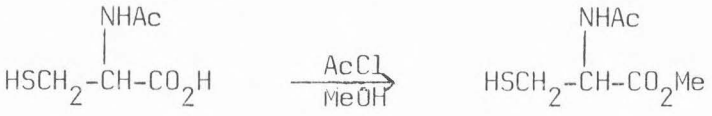
Treatment of methyl (Z)-β-chloro-N-acetyldehydroalaninate (91) with methyl N-acetyl-L-cysteinate or N-acetyl-L-cysteine gave methyl (Z)-β-(methyl N-acetyl-L-alaninate-β-thio)-N-acetyldehydroalaninate (94) and methyl (Z)-β-(β-N-acetyl-L-alanylthio)-N-acetyldehydroalaninate

(95), respectively. Only DABCO gave product (neither KOH nor Et_3N gave isolable product). The dimethyl ester (94) was a gel-like solid which could not be purified by recrystallization or column chromatography. Therefore, 94 was used crude in subsequent reactions.



91 18, R=Me 94, R=Me 95, R=H

Methyl N-acetyl-L-cysteinate (18) was prepared by esterification of N-acetyl-L-cysteine using acetyl chloride in methanol (56).



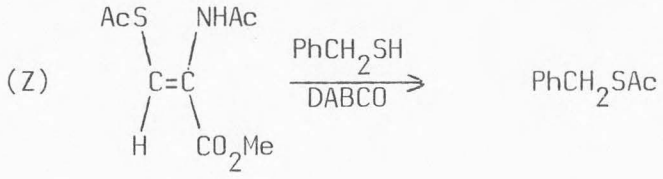
18

Thus, the reactivity of 91 to various mercaptans was similar in the preparation of the β-mercapto-N-acetyldehydroalaninate derivatives. However, this similarity was not observed in the reaction of the β-mercapto-N-acetyldehydroalaninates and a second molecule of the given mercaptan (i.e., only methyl β-acetylthio-N-acetyldehydroalaninate (89) would add a second molecule of thiolacetic acid).

Because of the difference in reactivity of thiolacetic acid to other mercaptans and because of the need to synthesize mixed dithioacetals (see structure 10), the addition of thiolacetic acid to 88 and 95 was attempted. The addition of thiolacetic acid to 88 and 95 failed, i.e., only unreacted 88 or 95 was identified by T.L.C. analysis.

No evidence for the desired product was ever found (no characteristic doublet or quartet between 5 and 6 δ (the methine protons) (see experimental for β,β-diacetylthio derivative (87)).

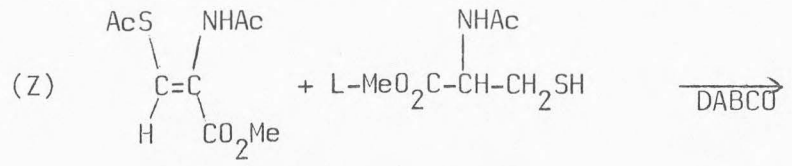
Since the addition of thiolacetic acid to 88 and 95 failed, additions to 89 were attempted (it was hoped that the facile addition of a second HSAc to 89 indicated a greater reactivity for the double bond in 89). An attempt to add benzyl mercaptan to (Z)89 gave an oil containing benzyl mercaptan (detected by odor). The main component of the oil appeared to be benzylthiol acetate. A second material



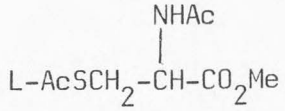
89

of only limited stability at room temperature was isolated, but its structure was not determined (the N-acetyl singlet was absent from the PMR spectrum).

Similarly, (Z)89 and methyl N-acetyl-L-cysteinate under basic conditions gave methyl S,N-diacetyl-L-cysteinate (90) and a second material which appeared to be the same as that obtained above with benzyl mercaptan by PMR comparisons. Methyl S,N-diacetyl-L-cysteinate



89 18



90

(90) was identified by melting point and PMR comparisons with an authentic sample prepared as previously described.

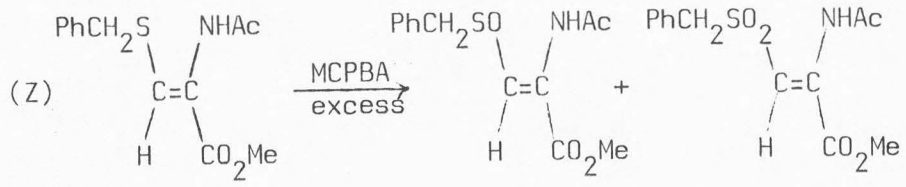
Under free radical and acidic conditions, methyl N-acetyl-L-cysteinate (18) and methyl (Z)-β-acetylthio-N-acetyldehydroalaninate (89) gave mixtures. Only 89 and 18 were identified by T.L.C. and PMR comparisons. No evidence for the desired product was ever found, i.e., no characteristic doublet or quartet between 5 and 6 δ due to the methine protons (see experimental for 87).

Since a mixed dithioacetal was not obtained under the above conditions, several attempts were made to alter the structure of methyl β,β-diacetylthio-N-acetyl-D,L-alaninate (87) by treatment with one equivalent of base and methyl iodide. In all cases, 87 was identified in the reaction mixture by T.L.C. comparisons with an authentic sample,

87

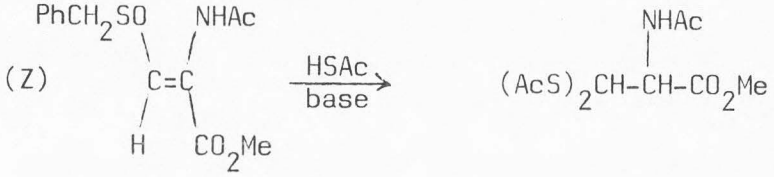
while the reaction mixture was shown to contain several components by T.L.C. analysis. None of the components isolated had PMR spectra consistent with the assumed appearance of the desired product. However, this was one area of study that was not investigated, because of time considerations, as thoroughly as one would have liked.

Because of the inability to obtain a clean, selective preparation of a mixed dithioacetal from 87, the structure of thiolacetic acid suggested the reactivity of 88 may be enhanced by conversion to its sulfoxide. The sulfoxide of 88 was prepared using MCPBA as oxidant. A slight excess of MCPBA gave a mixture of the sulfoxide and sulfone of (Z)88. The stereochemistry of 96 was (Z) as expected.



88 96

The addition of excess thiolacetic acid to 96 with DABCO as base gave 87 in 64% yield. Only unchanged 96 was recovered under free

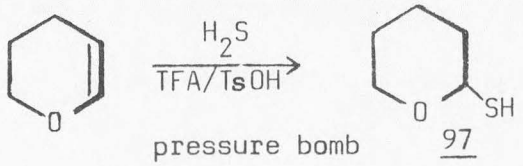


96 87

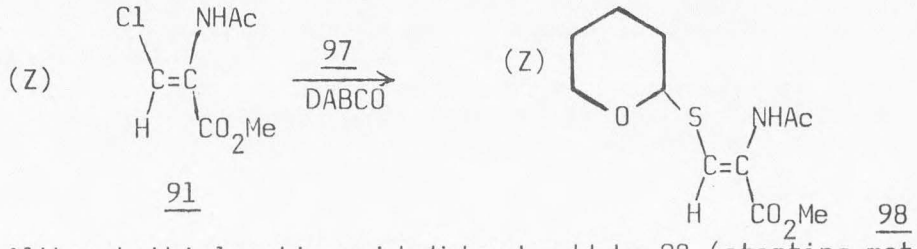
radical or non-catalyzed conditions. Thus, not only did thiolacetic acid add to (Z)96 under basic conditions, but the interchange of sulfides was observed (this phenomenon has previously been noted in the literature review section (41,46)). Thus, although the reactivity of 88 was enhanced by conversion to its sulfoxide (96), the previously observed interchange of sulfides was also enhanced by the presence of the sulfoxide moiety.

Because of the poor selectivity in the cleavage of only one of the acetyl groups of the β,β-diacetylthio compound (87) and the apparent need for a carbon-oxygen function adjacent to sulfur for successful reaction, α-tetrahydropyranthiol (97) was chosen as a possible answer to both of these problems. α-Tetrahydropyranthiol (97) contains both an oxygen bearing carbon adjacent to sulfur and is stable to basic or mild acidic cleavage (the conditions needed to deprotect the sulfur of the acetylthio group).

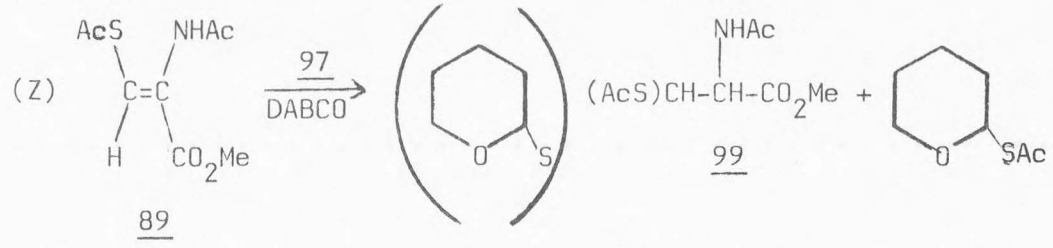
α-Tetrahydropyranthiol (97) was prepared by the method of Missakian and coworkers (83). The addition of α-tetrahydropyranthiol (97) to



methyl (Z)-β-chloro-N-acetyldehydroalaninate (91) gave a 53% yield of methyl (Z)-β-(α-tetrahydropyranylthio)-N-acetyldehydroalaninate (98).



Although thiolacetic acid did not add to 98 (starting material was only recovered) under basic conditions, α-tetrahydropyranthiol (97) did add to methyl (Z)-β-acetylthio-N-acetyldehydroalaninate (89). In addition to a 4% yield of 99, a 39% yield of α-tetrahydropyranylthiol

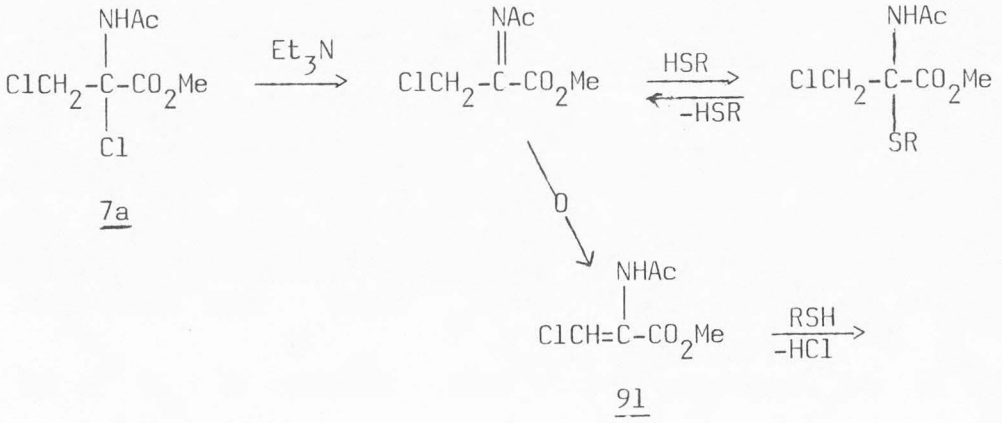


acetate was identified by boiling point and PMR comparisons to the data of this known compound (84). However, because of the low yield, selective cleavage of the acetyl group from sulfur with base was never tried.

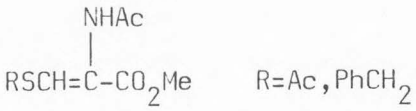
Summary

The synthesis of β-mercapto-N-acetyldehydroalanine derivatives from the α,β-dichloro derivative appeared to involve the acylimine intermediate, which isomerized to the unsaturated analog. A

Michael-type addition of mercaptan followed by an elimination of HCl gave product (100).



7a

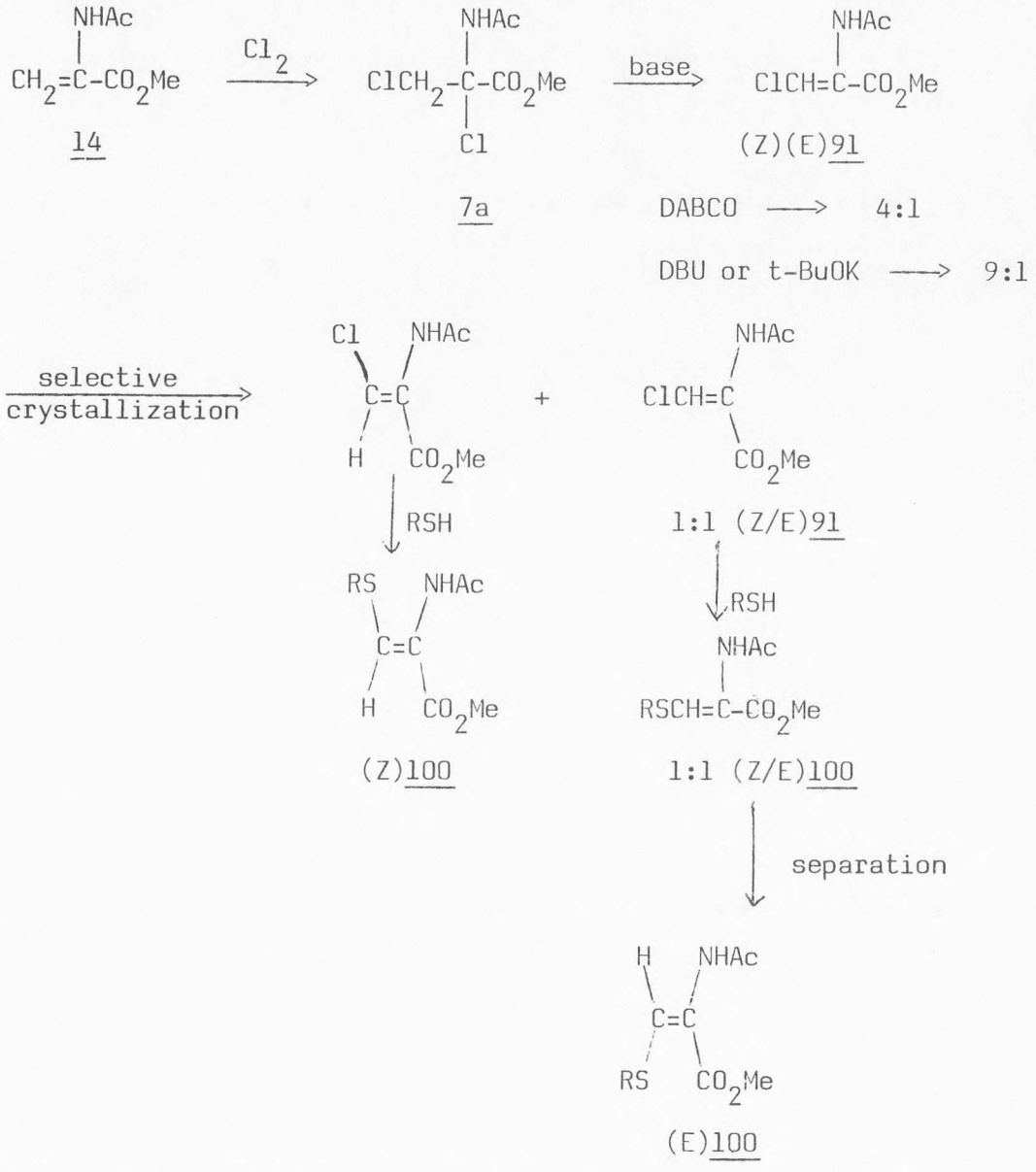


100

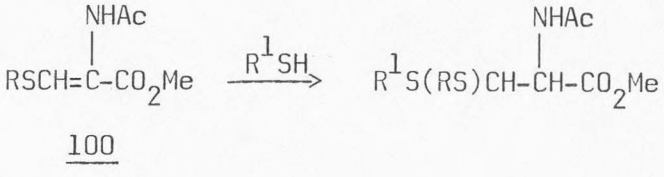
Taking advantage of the formation of 91 in this reaction sequence, a high yield direct synthesis of 91 was developed. The occurrence of the acylimine intermediate in the direct preparation of 91 was assumed. The presence of the E and Z isomers of 91 was noted in this synthesis.

Methyl (Z)-β-chloro-N-acetyldehydroalaninate (91) was observed to undergo the expected Michael-type addition-elimination sequence with various mercaptans (the corresponding reaction was also possible with the E isomer of 91). The reaction proceeded with retention of stereochemistry, i.e., (Z)91 gave the (Z)100 and (E)91 gave (E)100. Thus, a facile synthesis of β-substituted N-acetyldehydroalanine derivatives was developed. Stereochemical control of the synthesis was possible by appropriate selection of starting material, which,

itself, could be prepared with sufficient selectivity by choice of the base used in its synthesis.

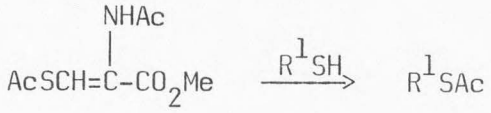


However, to be useful in the synthesis of dithioacetal (10), mixed dithioacetals must be obtained from 100. Attempts to add thiolacetic acid (R^1=Ac) to 100 failed except when R=Ac (i.e., 100



was methyl β-acetylthio-N-acetyldehydroalaninate (89)). This β,β-diacetylthio derivative (i.e., 87) could not be selectively cleaved to produce a mixed dithioacetal.

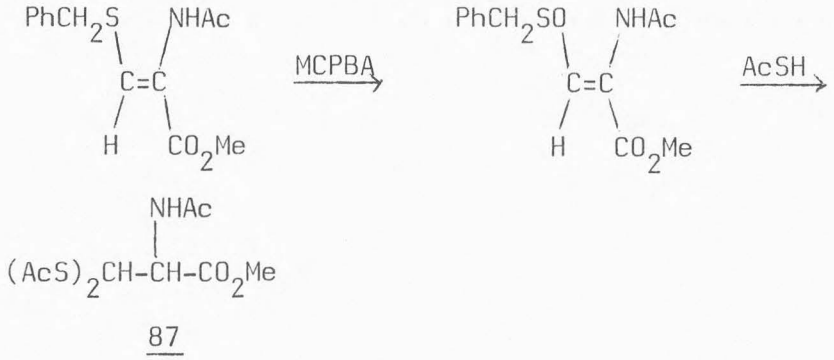
The addition of mercaptans to 89 (i.e., $R^1 \neq Ac$) resulted in the formation of the corresponding thiol acetate (presumably by cleavage of the acetyl group from the sulfur of 89). In one case (R^1=α-tetrahydropyran), in addition to the thiol acetate, a low yield of the



89

mixed dithioacetal was obtained. However, the low yield prevented attempts to cleave it and form the desired derivative of 10.

The reactivity of the double bond in 100 could be enhanced by conversion to the corresponding sulfoxide. However, this unsaturated sulfoxide was more susceptible to an interchange phenomenon and thus of little use in the preparation of mixed dithioacetals.

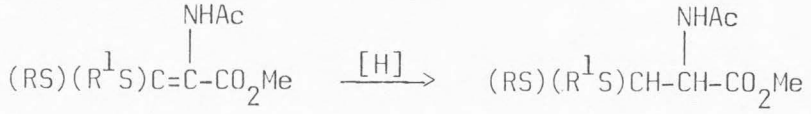


87

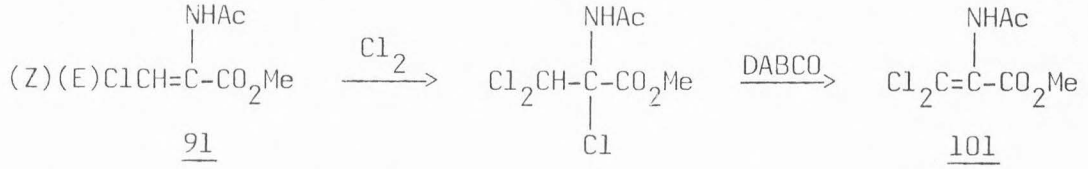
The preparation of
β,β-dimercapto-N-acetyldehydroalanine derivatives

Because of the poor results in our attempts to synthesize mixed dithioacetals in the N-acetylalanine system (i.e., low yields, no or

unuseful reactions), the preparation of mixed unsaturated β,β-dimercapto derivatives was investigated. Presumably, once formed, conditions could be developed to reduce the β,β-dimercapto-N-acetyldehydroalanine derivatives to the corresponding mixed dithioacetals.

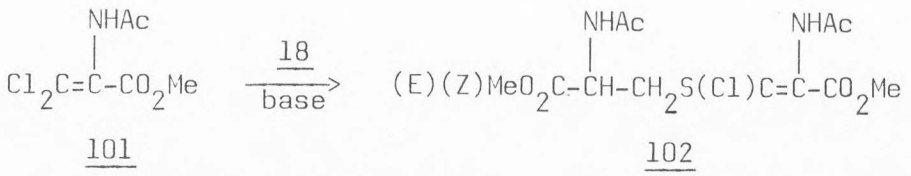


It had been noticed that insufficient care in the removal of excess chlorine during the synthesis of methyl β-chloro-N-acetyldehydroalaninate (91) gave isolable amounts of methyl β,β-dichloro-N-acetyldehydroalaninate (101). Thus, 101 was prepared from a crude mixture of Z/E-91 by the addition of chlorine followed by elimination with DABCO.



The mechanism presumably involved the formation of the acylimine intermediate followed by isomerization to 101. The yield of 101 was 16% to 54%.

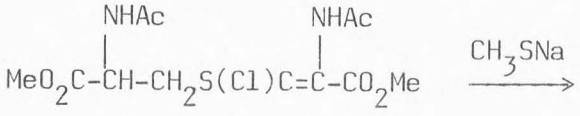
The addition of methyl N-acetyl-L-cysteinate (18) to 101 gave a mixture of presumably the Z and E isomers of methyl β-chloro-β-(methyl N-acetyl-L-alaninate-β-thio)-N-acetyldehydroalaninate (102). DBU was the base of choice for preparation of 102 in a crude yield of 32%



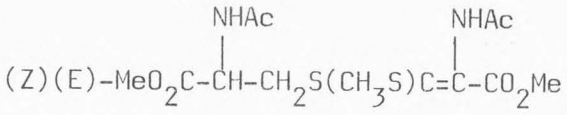
(the major isomer was isolated in 9% yield from the crude E/Z mixture).

Unfortunately, there are no known literature methods to determine the stereochemistry about the double bond of this type of compound (i.e., 102) (except, of course, by a determination of the crystal structure), when one of the substituents is not an alkyl group. Thus, although the major isomer was isolated as a stable, pure solid and the minor isomer was isolated as a semi-pure oil by column chromatography, the stereochemistry could not be assigned with certainty.

The major isomer of 102 was reacted with the sodium salt of methyl mercaptan to give an 8% isolated yield of product corresponding to the major isomer plus a second isomer. The major isomer of 102 was assumed to correspond to the major isomer of 103 because of a solvent



102



103

effect in $CDCl_3$, i.e., the major isomers of both 102 and 103 displayed a multiplet for the methylene protons in the PMR spectra in $CDCl_3$. The minor isomer of 102 and 103 displayed the normal doublet for these methylene protons in the PMR spectra in $CDCl_3$. In solvents other than $CDCl_3$, the multiplet collapsed to the normal doublet or a broad singlet.

The stereochemistry could not again be assigned to these isomers for the same reasons as already mentioned for 102. Thus, the

stereochemistry of the different isomers of 102 and 103 is an important objective left for future study.

Although as predicted, mixed β,β-dimercapto-N-acetyldehydroalanine derivatives could be formed via the Michael-type addition of mercaptans followed by the elimination of HCl, the yields were low. Because of the low yields, the reduction to the mixed dithioacetal was not investigated.

EXPERIMENTAL

Methods

A Thomas-Hoover Capillary Melting Point Apparatus was used to determine melting points. All melting points and boiling points are uncorrected. Evaporation in vacuo was carried out with a Buchler Rotary Flash Evaporator with the vacuum provided by a water aspirator. A Beckman Model 20A Infrared Spectrophotometer was used to record infrared spectra in potassium bromide pellets, chloroform deposited films or neat liquids. Proton magnetic resonance spectra were recorded on a Varian XL-100-12, Varian A-60 or Varian EM-360 Spectrometers. The format of the data is reported: chemical shifts (multiplicity, integral intensity, source). High pressure liquid chromatography was done on a Waters Associates ALC/GPC201 R401 instrument. Elemental analyses were performed by M-H-W Laboratories, Phoenix, Arizona. A Hitachi Perkin-Elmer RMU-6E single focusing instrument was used to obtain mass spectral data. Thin layer chromatography (T.L.C.) was performed on Quantum Industries Silica Gel MQ6F 1"x3" plates or Brinkmann Precoated Silica Gel plates. Spots were detected in iodine vapor and/or UV light. T.L.C. solvents were: A, chloroform: methanol: acetic acid (85:10:5); B, chloroform: acetic acid (95:5); C, chloroform: methanol: acetic acid (10:5:1) or as noted. Solvents were sometimes dried before use over Linde 3A Molecular Sieves (MS-3A). Methylene chloride was purified by washing with 10% sodium carbonate and drying over MS-3A.

Preparation of
N-acetyldehydroalanine (α-acetamidoacrylic acid) (11)

a. The method of Kildisheva and coworkers (18a) was followed as far as possible. A mixture of 35g (0.4 mol) of pyruvic acid (Aldrich gold label), 12g (0.2 mol) of acetamide and 300 ml of dry benzene was boiled for 4 hours in a Dean-Stark apparatus with vigorous stirring. The reaction mixture was allowed to cool to room temperature and the precipitate was filtered, washed with ether and then with acetone. Recrystallization from ethanol (25 ml/g) gave 6.5g (25%) of 11, mp 191-192° (lit. 198-199° (18a)) and ∿ 4g of a bis (12)-11 mixture, mp 184-185° (lit. 189-190° for pure 12 (16)). This mixture of 12 and 11 was refluxed 10 minutes in 25 ml glacial AcOH and rapidly cooled in an ice bath. The precipitate was filtered and washed with ether. Recrystallization from ethanol gave 2.2g (total: 33%) of 11, mp 190-192°.

11 PMR(TFA)δ 2.4(s,3,Ac); 6.47 and 6.73(s,2,vinyl); 8.5(b,1,NH).

11 and 12 PMR(TFA)δ 2.1(s,CH_3); 2.3(s,bisAc); 2.4 (s,Ac); 6.47 and 6.73(s,vinyl); 8.57(b,NH).

b. A mixture of 123.3g (1.40 mol) of pyruvic acid (Aldrich gold label), 82.7g (1.4 mol) of acetamide and 2.5 l of dry benzene was boiled for 12 hours in a Dean-Stark apparatus with overhead stirring. The precipitate was filtered hot, washed with cold benzene and cold ether. Recrystallization from methanol (15 ml/g) gave 60.5g (33%) of 11, mp 194-195° and 26g of 12-11 mixture. The bis (12) and 11 mixture was refluxed 10 minutes in 100 ml of glacial AcOH and rapidly cooled in an ice bath. The precipitate was filtered and washed with ether. Recrystallization from methanol gave 10.0g (total: 39%) of 11, mp 191-192°.

Preparation of methyl N-acetyldehydroalaninate (methyl α-acetamidoacrylate) (14)

a. Following the method of Wieland and coworkers (18b), 9.0g (0.16 mol) of potassium hydroxide was dissolved in 400 ml of water and 10.0g (0.077 mol) of N-acetyldehydroalanine (11) was added. To this solution was added 25.5g (0.15 mol) of silver nitrate. After stirring for 1 hour at room temperature in the dark, the brown precipitate was filtered, washed with water and air dried for 60 hours in the dark. The dry salt was pulverized and refluxed for 5 hours with excess methyl iodide in an oil bath at 45°. The precipitate was filtered and thoroughly washed with chloroform. After stirring for 2 hours with Norit at room temperature, the Norit was removed by filtration and the filtrate evaporated in vacuo. The residue was extracted with hot pet ether (bp 60-70°); cooling the solvent gave 5.2g (47%) of 14, mp 50-51.7° (lit. 52-54° (17a)).

PMR($CDCl_3$)δ 2.17(s,3,Ac); 3.87(s,3,Me); 5.93 and 6.60 (s,2, vinyl); 8.23(b,1,NH).

b. To a mixture 25.1g (0.465 mol) of sodium methoxide, 24.6g (0.232 mol) of sodium carbonate and 50.0g (0.387 mol) of 11 in 800 ml of methanol (MS-3A) was added 73.2g (0.581 mol) of dimethyl sulfate. After stirring 20 hours at room temperature (or refluxing 2 hours in an oil bath at 70°), 400 ml of water was added and the reaction stirred at room temperature 1 hour. Then the reaction was filtered and the methanol evaporated in vacuo. The aqueous residue was extracted with ether, saturated with sodium chloride and extracted with ether. The combined extracts were dried over $MgSO_4$, filtered and evaporated in

vacuo to give a yellow oil. The oil was extracted several times with hot pet ether (bp 30-75°) (1800 ml). Cooling the solvent gave 31.2g (56%) of 14, mp 51-53°.

Preparation of
D,L-serine methyl ester hydrochloride (15)

In 150 ml of dry methanol (MS-3A) was suspended 10.5g (0.1 mol) of D,L-serine. Hydrogen chloride gas was bubbled into the stirred reaction at a very fast rate for 15 minutes; the reaction became a complete solution and was exothermic during this time. The HCl flow was reduced to a slow, but continuous, flow rate for another 45 minutes; then the flow of HCl was stopped and the reaction stirred at room temperature for 1 hour. The methanol was concentrated in vacuo to a slush and excess abs. ether was added. Cooling gave 13.5g (86%) of 15, mp 132-133° (lit. 133-134° (49)).

PMR(TFA)δ 4.03(s,3,Me); 4.5 and 4.6(s,m,3,CH_2,CH); 7.77(b,3,NH_3).

Preparation of
β-chloro-D,L-alanine methyl ester hydrochloride (16)

a. In 50 ml of acetyl chloride was suspended 4.8g (0.03 mol) of D,L-serine methyl ester hydrochloride (15), the suspension was stirred and cooled in an ice bath while 7.5g (0.036 mol) of phosphorus pentachloride was added. After the vigorous reaction subsided, the mixture was stirred at room temperature for 2 hours. The precipitate was filtered, washed with acetyl chloride and pet ether (bp 60-90°) to give 3.8g (70%) of 16, mp 124-134° (lit. 134° (85)).

b. In 20 ml of chloroform, 1.6g (0.01 mol) of 15 and 2.5g (0.01 mol) of PCl_5 were stirred at room temperature for 12 hours. The

precipitate was filtered, washed with $CHCl_3$ and pet ether (bp 60-90°) to give 1.2g (67%) of 16, mp 129-132°.

PMR(TFA)δ 4.07(s,3,Me); 4.33(d,2,CH_2); 4.97(m,1,CH); 7.90(b,3,NH_3).

c. In 200 ml of chloroform, 15.7g (0.1 mol) of 15 and 25.0g (0.12 mol) of PCl_5 were stirred at room temperature for 12 hours. The precipitate was filtered, washed with $CHCl_3$ and pet ether (bp 60-90°) to give 11,3g of 15 + 16, mp approximately 120°.

PMR(TFA)δ 4.08(s,Me); 4.30(d,CH_2); 4.56 and 4.9 (s,m,CH_2,2CH); 7.8(b,NH_3); from the ratio of peaks from δ 4.3 to 4.9, the mixture was very roughly 40% 15 and 60% 16.

d. To a suspension of 15.6g (0.1 mol) of 15 in 50 ml of AcCl and 50 ml of $CHCl_3$, 25.0g (0.12 mol) of PCl_5 was added with stirring in an ice bath. After the addition, the reaction was stirred 2 hours at room temperature. The precipitate was filtered, washed with $CHCl_3$ and pet ether (bp 60-90°) to give 12.7g (73%) of 16, mp 130-132°.

Preparation of
methyl β-chloro-N-acetyl-D,L-alaninate (17)

a. To a suspension of 3.6g (0.02 mol) of 16 in 40 ml of benzene, 6 ml of AcCl was added. The mixture was refluxed until all of 16 dissolved (4 hours) and excess pet ether (bp 30-60°) was added. The precipitate was recrystallizated from ether-pet ether to give 1.25g (33%) of 17, mp 75-77° (lit. 79-80° (17a)).

PMR($CDCl_3$)δ 2.10(s,3,Ac); 3.90 and 3.97(s,d,5,Me,CH_2); 5.07 (m,1,CH); 6.46(b,1,NH).

b. To a cold solution of 83.5g (0.48 mol) of 16 in 300 ml of water was added 40.3g (0.48 mol) of $NaHCO_3$, slowly, allowing complete

reaction after each addition. The solution was stirred a few minutes at room temperature and 75g (0.7 mol) of acetic anhydride was added. After stirring overnight at room temperature, the reaction was carefully neutralized with solid $NaHCO_3$ and extracted with methylene chloride. After drying over $MgSO_4$, the mixture was filtered and the solvent evaporated in vacuo. Crystallization from ether-pet ether (bp 30-60°) gave 68.0g (79%) of 17, mp 76-78°.

Preparation of
methyl N-acetyldehydroalaninate (14) from 17

a. To a solution of 3.0g (.017 mol) of methyl β-chloro-N-acetyl-D,L-alaninate (17) in 25 ml of methanol (MS-3A) was added 0.9g (0.017 mol) of commerical sodium methoxide and a speck of hydroquinone. After stirring at room temperature for 30 minutes, the solvent was evaporated in vacuo. The residue was dissolved in 25 ml of water and extracted with ether. The ether extracts were dried over $MgSO_4$, filtered and evaporated in vacuo. Crystallization from pet ether (bp 30-60°) gave 1.27g (52%) of 14, mp 49-50°.

b. To a solution of 3.0g (17 mmol) of 17 in 25 ml of ethyl acetate was added 1.7g (17 mmol) of triethylamine and a speck of hydroquinone. After stirring 3 hours at room temperature, the solvent was removed in vacuo. The residue was extracted with hot pet ether (bp 30-60°) and cooled to give 0.5g (21%) of 14, mp 49-53°.

c. To a solution of 7.6g (42 mmol) of 17 in 100 ml of ether was added 8.6g (85 mmol) of Et_3N and a speck of hydroquinone. The reaction was refluxed 30 minutes, filtered and evaporated in vacuo to give 3.6g (59%) of crude 14. PMR indicated some impurity but no trace of starting material (17).

d. To a solution of 68.0g (0.38 mol) of 17 in 500 ml of water plus 100 ml of acetonitrile was added 40.2g (0.38 mol) of Na_2CO_3 and a spatula of hydroquinone. The reaction was stirred for 30 minutes at room temperature, 20 ml (∿ 0.14 mol) of Et_3N was added and the reaction warmed at 40° for 20 minutes. The reaction was saturated with NaCl and extracted with ether. The ether extracts were dried over $MgSO_4$, filtered and evaporated in vacuo to give 45.5g (84%) of crude 14. PMR indicated an even purer crude product than with Et_3N/ether (method c).

Preparation of
N-acetyldehydroalanine (11) from 17

To a solution of 2.0g (11 mmol) of methyl N-acetyl-β-chloro-D,L-alaninate (17) in 3 ml of ethanol was added a speck of hydroquinone and 23 ml of 1N sodium hydroxide solution. The solution was stirred 30 minutes at room temperature, acidified to pH2 with 3N HCl solution and cooled. A second crop of precipitate was obtained by concentration in vacuo and cooling. Recrystallization from methanol gave 1.0g (70%) of 11, mp 189-190°.

Preparation of
α-hydroxy-N-acetyl-D,L-alanine (59)

To a solution of 8.8g (0.1 mol) of pyruvic acid in 50 ml of acetone was added 5.8g (0.098 mol) of acetamide. After refluxing for 5 hours on a steam bath, the solvent was evaporated in vacuo to give 14.4g (100%) of 59 as an impure solid.

PMR($CDCl_3$)δ 2.19(s,3,Me); 2.55(s,3,Ac); 7.42(b,1,NH); impurity at δ 1.33(s) equivalent to 0.3 protons.

Preparation of
methyl N-acetyl-D,L-alaninate (64)

N-acetyl-D,L-alanine (63) was prepared by the method of Greenstein and Winitz (55); 89.1g (1.0 mol) of D,L-alanine was dissolved in 900 ml of glacial acetic acid and the solution heated to boiling. After cooling 2 minutes at room temperature, 160 ml (1.7 mol) of Aldrich acetic anhydride was added dropwise with rapid stirring. After the addition was complete, the solution was boiled 10 minutes and cooled to room temperature. The solvent was evaporated in vacuo; water was added 3 to 5 times and evaporated in vacuo (odor of acetic acid and anhydride was gone). In some experiments, N-acetyl-D,L-alanine (63) was isolated by recrystallization from ethyl acetate to give 102.6g (78%) of 63, mp 136-137^{o} (lit. 136^{o} (55)). More commonly, the crude paste (63) was twice dissolved in dry acetone (MS-3A) and evaporated in vacuo to remove any residual water. This residue was dissolved in 900 ml of dry methanol (MS-3A) and cooled in an ice bath. To the cold solution was slowly added 80 ml (1.1 mol) of acetyl chloride with vigorous stirring. After the addition was complete, the reaction was stoppered and stirred overnight (12-20 hours) at room temperature. The solvent was evaporated in vacuo and the residual hydrogen chloride was neutralized with 150 ml of saturated sodium bicarbonate followed by solid $NaHCO_3$. The aqueous solution was extracted with chloroform, saturated with NaCl and again extracted with $CHCl_3$. The combined $CHCl_3$ extracts were dried over $MgSO_4$, filtered and evaporated in vacuo. A small amount of lower boiling material was removed under vacuum by heating to 100^{o}/12mm. The 139g (96% from D,L-alanine) of 64 that remained in the distillation flask was of sufficient purity for use in

subsequent reactions. Methyl N-acetyl-D,L-alaninate (64) could be distilled to give 119g (82% from D,L-alanine) of 64, bp 78-85°/0.2mm, mp 45-47° from carbon tetrachloride (lit. bp 126-127°/10mm (86)).

PMR($CDCl_3$)$_\delta$ 1.42(d,3,CH_3); 2.05(s,3,Ac); 3.78(s,3,Me ester); 4.5(m,1,CH); 7.55(b,1,NH).

Preparation of
methyl α-methoxy-N-acetyl-D,L-alaninate (66) via (64)

To a solution of 43.7g (0.3 mol) of methyl N-acetyl-D,L-alaninate (64) in 100 ml of dry methanol (MS-3A) was added 40 ml (about 0.33 mol) of t-butyl hypochlorite. After stirring 5 minutes at room temperature, 16.5g (0.31 mol) of sodium methoxide in 200 ml dry methanol was added with stirring in an ice bath. After the addition, the reaction was stirred at room temperature for 1 hour and the solvent evaporated in vacuo. The residue was suspended in ethyl acetate and filtered. The filtrate was evaporated in vacuo to give an oil. Crystallization from ether gave 20.0g (38%) of 66, mp 121-122° (lit 117-122° (25)).

PMR($CDCl_3$)$_\delta$ 1.20(s,3,Me); 2.10(s,3,Ac); 3.37(s,3,OMe); 3.87(s, 3,Me ester); 7.30(b,1,NH).

Preparation of
methyl N-acetyldehydroalaninate (14) from 64

A solution of 97.8g (0.67 mol) of crude methyl N-acetyl-D,L-alaninate (64) in 125 ml of dry methanol (MS-3A) containing a trace of hydroquinone was stirred in the dark in a 10-20° cold water bath. To this solution was added in one portion 100 ml (0.83 mol) of t-butyl hypochlorite and 2 ml of a freshly prepared 1% solution of sodium in methanol. The reaction mixture was stirred at 10-20° for 2 to 4 hours,

the reaction being followed by T.L.C. (developed with ethyl acetate). Alternatively, the reaction flask was wrapped in aluminum foil and stored overnight in a refrigerator at approximately 8^{o}. The excess t-butyl hypochlorite and methanol were removed in vacuo at a water bath temperature below 40^{o}. The resulting oil was dissolved in 400 ml of purified methylene chloride, washed once with 100 ml of saturated sodium chloride solution (removed traces of methanol and most of the t-butyl alcohol) and stored in ice while drying over $MgSO_4$. The cold, dried solution was filtered and stored in an ice bath until ready for the elimination reaction. A sample of the N-chloro compound (65) was isolated by evaporation of the methylene chloride in vacuo.

PMR($CDCl_3$)δ 1.50(d,3,Me); 2.30(**s**,3,Ac); 3.77(**s**,3,Me ester); 5.33(m,1,CH).

a. The solution was diluted to 1500 ml with dry, alcohol-free methylene chloride and 75.2g (0.67 mol) of 1,4-diazabicyclo [2.2.2] octane (DABCO) was added slowly with stirring at room temperature at a rate that a gentle reflux was maintained. After the addition was complete, the reaction was stirred at room temperature until the vigorous reaction had subsided (10-20 minutes) and then heated at $40\text{-}50^{o}$ in a hot water bath for 10-20 minutes with vigorous stirring.

b. To 135.6g (1.34 mol) of neat triethylamine was added in portions the methylene chloride-N-chloro compound (65) with stirring at room temperature. After the addition was complete, the reaction was stirred at room temperature until the vigorous reaction had subsided (10-20 minutes) and then heated at $40\text{-}50^{o}$ in a hot water bath for 10-20 minutes with vigorous stirring. The excess triethylamine and solvent were evaporated in vacuo and the residue diluted with 300 ml of ether.

The cold organic mixtures (ether from b. and methylene chloride from a.) were filtered and washed once with 50 ml of water, once or twice with 1N hydrochloric acid so that the aqueous phase remained acidic, once with 50 ml of saturated sodium bicarbonate, once with 50 ml of water. After drying the respective organic phases over $MgSO_4$, they were filtered, a speck of hydroquinone added and the solvent evaporated in vacuo. Methyl N-acetyldehydroalaninate (14) was obtained in yields of 72-79% as an oil 95% pure by PMR analysis. If less than 95% pure, the oil was dissolved in 400 ml of ether, washed once with 100 ml of water and dried over $MgSO_4$. Evaporation of the ether gave 14 in 95% purity.

Crystalline 14 was obtained by Kugelrohr distillation under high vacuum (hydroquinone was added to both the distillation and receiving flasks to prevent polymerization of 14). The distillate was recrystallized from pet ether (bp 30-60°) to give 14 in yields of 20-40%, mp 50-52°. Crystalline 14 was also obtained by initial crystallization of the crude oil from ether, washing the crystalline material with pet ether (bp 30-60°) and recrystallization from pet ether (bp 30-60°) to give 14 in yields of 30-55%.

Preparation of
N-acetyldehydroalanine (11) from 64

After dehydrochlorination of methyl N-acetyl-N-chloro-D,L-alaninate (65) as described above for (14), the mixture was filtered and the solvent removed in vacuo. The residue was dissolved in 200 ml of dioxane and 680 ml (0.68 mol) of 1N sodium hydroxide was slowly added with stirring at room temperature. After the addition, the reaction was

stirred at room temperature for 30-45 minutes, acidified to pH2 with concentrated hydrochloric acid and cooled. Filtration gave 51.0g (58% from 64) of 11, mp 195-196°. In small scale reactions, this product was of lesser purity and had to be recrystallized from methanol following treatment with Norit. A second crop of 11 could be obtained by evaporation of the dioxane in vacuo, cooling and recrystallization from methanol after treatment with Norit of this second crop. Another 3.1g of 11 was obtained.

Preparation of
t-butyl hypochlorite

In a 1-l flask, 450 ml (0.39 mol) of Purex laundry bleach was cooled to about 0° in the dark. A freshly prepared solution of 37 ml (0.39 mol) of t-butyl alcohol and 24.5 ml (0.43 mol) of glacial acetic acid was added in one portion. The solution was stirred 3 minutes at less than 10° in the dark. The layers were separated. The upper organic phase was washed once with 50 ml of 10% sodium carbonate solution and once with 50 ml of water. The yellow oil was dried over $CaCl_2$, decanted and stored in a freezer over fresh $CaCl_2$ in a tightly sealed brown bottle. The oil represented 29g (75%) of t-butyl hypochlorite which was normally in excess of 90% purity (59).

Preparation of
methyl N-acyl-D,L-alaninates (68(a-c))

To a solution of 8.9g (0.1 mol) of D,L-alanine in 100 ml of water and 50 ml of dioxane was added 21.0g (0.25 mol) of sodium bicarbonate. With vigorous stirring at room temperature, a solution of the appropriate acyl chloride (0.11 mol) in 25 ml of dioxane was added dropwise. After the addition was complete (1 hour), the mixture was stirred 1

hour at room temperature and neutralized with 1N hydrochloric acid. The dioxane was removed in vacuo and the remaining aqueous solution was extracted twice with ether. The aqueous solution was acidified to pH2 with 6N hydrochloric acid, extracted with ethyl acetate, saturated with sodium chloride and extracted with ethyl acetate. The combined ethyl acetate extracts were dried over $MgSO_4$, filtered and evaporated in vacuo. By recrystallization, 70(a-c) could be isolated.

N-benzoyl-D,Lalanine (70a): 79%; mp 159-160° (from acetone) (lit. mp 160-161° (60)); PMR($CDCl_3$ + 5 drops DMSO-d_6)δ 1.30(d,3,Me,); 3.57(S,2,CH_2Ph); 4.47(m, 1,CH); 7.37(S,b,6,Ph,NH).

N-phenylacetyl-D,L-alanine (70b): 77%; mp 151-153° (from ethyl acetate) (lit. mp 150-152° (87)); PMR($CDCl_3$ + 5 drops DMSOd_6)δ 1.30 (d,3,Me); 3.57(s,2,CH_2Ph); 4.47(m,1,CH); 7.37(s,b,6,Ph, NH)

N-benzyloxycarbonyl-D,L-alanine (70c): 78%; mp 111-112° (from benzene) (lit. mp 113.5° (88)); PMR($CDCl_3$)δ 1.45(d,3,Me); 4.45(m,1,CH); 5.15 and 5.4(s,b,3,CH_2Ph,NH); 7.42(s,5,Ph)

The crude, dry precipitate (70(a-c)) was dissolved in dry methanol (MS-3A) and 9 ml (0.13 mol) of acetyl chloride was slowly added with vigorous stirring in an ice bath. After the addition was complete, the solution was stoppered and stirred at room temperature 16-24 hours. The solvent was removed in vacuo and the residual hydrogen chloride neutralized with 50 ml of saturated sodium bicarbonate followed by solid sodium bicarbonate. The resulting mixture was diluted to 250 ml with

ethyl acetate, separated and washed twice with water. After drying over $MgSO_4$, the reaction was filtered and evaporated in vacuo. The crude ester (68(a-c)) were vacuum distilled until 100°/1.0mm to remove lower boiling impurities. By vacuum distillation followed by crystallization, 68(a-c) could be isolated.

Methyl benzoyl-D,L-alaninate (68a): 60-76% from D,L-alanine; bp 131-140°/0.15 mm, mp 81-82.5° (from methylene chloride-pet ether (bp 30-60°)) (lit. mp 82° (89)); PMR($CDCl_3$)δ 1.52(d,3,Me); 3.83(s,3, Me ester); 4.87(m,1,CH); 7.03(b,1,NH); 7.63(m,5,Ph).

Methyl phenylacetyl-D,L-alaninate (68b): 59-82% from D,L-alanine; bp 135-140°/0.2mm, mp 66-68° (from methylene chloride-pet ether (bp 30-60°)) (no lit.); PMR($CDCl_3$)δ 1.36(d,3,Me); 3.63(s,2,CH_2Ph); 3.77 (s,3,Me ester); 4.60(m,1,CH); 6.16(b,1,NH); 7.38(s,5,Ph).

Methyl benzyloxycarbonyl - D,L - alaninate(68c): 60-86% from D,L-alanine; bp 135-140°/0.3mm, mp 50-51° (from methylene chloride-pet ether (bp 30-60°)) (lit. 46° (89)); PMR($CDCl_3$)δ 1.39(d,3,Me); 3.77(s, 3,Me ester); 4.42(m,1,CH); 5.15(s,2,CH_2Ph); 5.57(b,1,NH); 7.40(s,5,Ph)

Preparation of
N-acyldehydroalanines (19(a-c))

To a solution of crude methyl N-acyl-D,L-alaninates (68(a-c)) (0.05 mol) in 25 ml of dry methanol (MS-3A) was added a speck of hydroquinone and the solution was stirred in a cold water bath at 10-20°. To the cold mixture, 10 ml (0.08 mol) of t-butyl hypochlorite was added followed by 1 ml of a 1% solution of sodium in methanol. The solution was stirred in the dark in a cold water bath at 10-20° until the N-chlorination was completed (followed by T.L.C.), or the reaction

was wrapped in aluminum foil and stored in a refrigerator at approximately 8^{o} (see Table 3 for time and T.L.C. data). The excess t-butyl hypochlorite and methanol were removed in vacuo maintaining water bath temperature less than 40^{o}. The crude yellow oil was dissolved in 200 ml of methylene chloride, washed twice with water, stored in an ice bath while drying over $MgSO_4$, filtered and the filtrate stored in ice until ready to use. A sample of the N-chloro compounds (69(a-c) was isolated by evaporation of the methylene chloride in vacuo.

N-benzoyl derivative (69a): PMR($CDCl_3$)δ 1.61(d,3,Me); 3.83(s,3, Me ester); 5.15(m,1,CH); 7.60 (m,5,Ph).

N-phenylacetyl derivative (69b): PMR($CDCl_3$)δ 1.51(d,3,Me); 3.75 (s,2,CH_2Ph); 4.0(s,3,Me ester); 5.45(m,1,CH); 7.40(s,5,Ph).

N-benzyloxycarbonyl derivative (69c): PMR($CDCl_3$)δ 1.49(d,3, Me); 3.68(s,3,Me ester); 5.04(m,1,CH); 5.22(s,2,CH_2Ph); 7.37(s,5,Ph).

a. To the methylene chloride solution 5.6g (0.05 mol) of DABCO was slowly added with stirring at room temperature at a rate that a gentle reflux was maintained.

b. To 10.1g (0.1 mol) of neat triethylamine was slowly added the methylene chloride solution with stirring at room temperature.

c. To the methylene chloride solution 7.6g (0.05 mol) of DBU was added with vigorous stirring in an ice bath at a rate that a gentle reflux was maintained.

After the addition was complete, the reaction was stirred at room temperature for 10-20 minutes until the vigorous reaction had subsided and then heated at $40-50^{o}$ in a hot water bath for 10-20 minutes with vigorous stirring. The reaction was cooled by the addition of ice,

washed once with water, once or twice with 1N hydrochloric acid so that the aqueous phase remained acidic, once with saturated sodium bicarbonate solution and once with water. After drying over $MgSO_4$, a speck of hydroquinone was added and the solvent removed in vacuo to give crude oil. Crude yields were measured by PMR analysis and are reported from 68(a-c).

Methyl N-benzoyldehydroalaninate (21a): method a, 70%; method b, 54%; method c, 67%

Methyl N-phenylacetyldehydroalaninate (21b): method a, 77%; method b, 56%.

Methyl N-benzyloxycarbonyldehydroalaninate (21c): method b, 53%; method c, 73%.

This crude oil or more usually the dehydrochlorinated organic mixture after filtration and solvent removal in vacuo was dissolved in enough dioxane or methanol (methanol must be used with 21c) to produce a complete solution. To the solution was added 1.1 equivalents of 1N sodium hydroxide and the resulting solution was stirred at room temperature for 30-60 minutes. The organic solvent was removed in vacuo (for 21c, the water bath was maintained below 40^{o} to prevent polymerization). The remaining aqueous solution was acidified to pH2 with concentrated hydrochloric acid and extracted with ethyl acetate. After drying over $MgSO_4$, the solvent was removed in vacuo and the resulting brown oily paste was initially crystallized from minimum mls of cold ethanol by the repeated addition of water, cooling and filtration (this procedure easily separated acids 19(a-c) from their saturated analogs 70(a-c) since it was shown by removal of ethanol and concentration of the remaining aqueous solution in vacuo produced precipitates

that PMR analysis indicated to be the corresponding 70(a-c)). The crude brown precipitate was dissolved in the appropriate solvent, treated with Norit and crystallized to yield pure product.

N-benzoyldehydroalanine (19a): 39% from 68a; mp 161-163° (from aqueous ethanol) (lit. 158-160° (18a)); PMR(TFA)δ 6.60 and 6.97 (2s,2,vinyl); 7.70(m,5,Ph); 8.80(b,1,NH).

N-phenylacetyldehydroalanine (19b): 33% from 68b; mp 179-181° (from aqueous ethanol) and mp 187-189° (from ethyl acetate) (lit. 189-190° (18a)); PMR(TFA)δ 3.97(s,2,CH_2Ph); 6.45 and 6.80(2s,2,vinyl); 7.45(s,5,Ph); 8.38(b,1,NH).

N-benzyloxycarbonyldehydroalanine (19c): 40% from 68c; mp 107-109° (from methylene chloride-pet ether (bp 30-60°)) (lit. mp 100-101° (18a)); PMR($CDCl_3$)δ 5.20(s,2,CH_2Ph); 6.0 and 6.37(2s,2,vinyl); 7.33 (b,1,NH); 7.40(s,5,Ph).

Preparation of
p-nitrobenzyl N-acetyldehydroalaninate (71)

To a solution of 3.0g (23 mmol) of N-acetyldehydroalanine (11) and 7.5g (34.8 mmol) of freshly recrystallized p-nitrobenzyl bromide in 100 ml of acetonitrile, 4.9 ml (34.8 mmol) of triethylamine was added. After refluxing in an oil bath at 90° for 7 hours, the reaction was cooled to room temperature and the solvent removed in vacuo. The residue was dissolved in chloroform, washed with water, 1N hydrochloric acid, water, 10% sodium bicarbonate and water. The chloroform solution was dried over $MgSO_4$, filtered and evaporated in vacuo. Recrystallization from ethyl acetate gave 5g (82%) of 71, mp 142.5-144°.

PMR(TFA)$_\delta$ 2.42(s,3,Ac); 5.55(s,2,CH_2Ph); 6.49 and 6.70(2s,2,vinyl); 7.68 and 8.36(A_2B_2 pattern,4,Ph); 8.79(b,1,NH).

Anal. Calcd. for $C_{12}H_{12}N_2O_5$: C,54.54; H,4.58; N,10.60

Found: C,54.28; H,4.38; N,10.36

Other preparations of
methyl α-methoxy-N-acetyl-D,L-alaninate (66)

a. A solution of 2.0g (14 mmol) of methyl N-acetyldehydroalaninate (14) in 40 ml of methylene chloride was saturated with hydrogen chloride gas over 20 minutes with stirring at room temperature. The solution was evaporated for 1 minute in vacuo to remove excess HCl and a mixture of 3.5g (42 mmol) of sodium bicarbonate in dry methanol (MS-3A) was added. After stirring overnight at room temperature, the solvent was removed in vacuo and the residue dissolved in saturated sodium chloride solution. The solution was extracted with chloroform. The $CHCl_3$ extract was dried over $MgSO_4$, filtered and the $CHCl_3$ removed in vacuo. Crystallization from ether gave 0.7g (28%) of 66, mp 121-122.5°.

b. To an ice cold mixture of 1.3g (0.01 mol) of N-acetyldehydroalanine (11) in 20 ml dry MeOH (MS-3A) was added 2 ml of acetyl chloride with stirring in an ice bath. After the addition was completed, the reaction was stirred overnight at room temperature, neutralized with solid $NaHCO_3$ and the solvent removed in vacuo. Crystallization from ether gave 0.34g (23%) of 66, mp 121-123°.

c. To a solution of 0.65g (3mmol) of methyl α-acetylthio-N-acetyl-D,L-alaninate (74) in 25 ml dry MeOH (MS-3A) was added 0.16g (3mmol) of sodium methoxide. After stirring overnight at room temperature, the solvent was removed in vacuo to give a yellow oil. The oil

was diluted with ethyl acetate and one gram of Amberlite CG-50 was added. After stirring 5 minutes, the mixture was filtered and evaporated in vacuo. Crystallization from $CHCl_3$-pet ether (bp 60-90°) after Norit treatment gave 0.08g (15%) of 66, mp 119-121°.

Preparation of
methyl N-acetyl-D,L-serinate (73)

To an ice cold mixture of 1.9g (12.2mmol) of D,L-serine methyl ester hydrochloride (15) and 3.0g (36mmol) of solid sodium bicarbonate in 30 ml of acetonitrile was slowly added 1.4g (18mmol) of acetyl chloride with stirring in an ice bath. After the addition was completed, the reaction was stirred 3 hours at room temperature and evaporated in vacuo. Crystallization from dry acetone (MS-3A)-Et_2O, after pre-filtration of NaCl, gave 1.1g (55%) of 73, mp 99-100.5° (lit. 100-101.6° (90)).

PMR($CDCl_3$)δ 2.14(s,3,Ac); 3.82(s,3,Me); 4.68(d,2,CH_2); 5.00(m, 1,CH); 7.50(b,1,NH).

Preparations of
methyl β-methoxy-N-acetyl-D,L-alaninate (57)

a. A mixture of 2.5g (15.5mmol) of methyl N-acetyl-D,L-serinate (73), 10.0g (43mmol) of silver oxide and 20 ml of methyl iodide in 40 ml of acetone was stirred in an oil bath at 37° for 10 hours. The precipitate was filtered with Celite and the solvent removed in vacuo. The residue was distilled in vacuum and the fraction collected at 100-105°/0.05mm was treated with Norit. Crystallization from ether gave 0.3g (11%) of 57, mp 69.5-70° (lit. 70-71°, bp 100-140°/ 0.05mm (65)).

PMR($CDCl_3$)δ 2.05(s,3,Ac); 3.35(s,3,OMe); 3.64(d,2,CH_2); 3.78 (s,3,Me ester); 4.72(m,1,CH); 6.34(b,1,NH).

b. Hydrogen chloride was bubbled into a solution of 1g (7mmol) of methyl N-acetyldehydroalaninate (14) in 40 ml methylene chloride for 30 minutes with stirring at room temperature. The solvent was removed in vacuo and a solution of 0.8g (14mmol) of sodium methoxide in methanol was added. After stirring 21 hours at room temperature, 2 ml of glacial acetic acid was added and the solvent was removed in vacuo. The residue was extracted with ether and the ether was evaporated in vacuo. The crude residue was separated on a Porasil A prep hplc column with chloroform (flow rate: 8 ml/minute). Crystallization from ether gave 0.1g (8%) of 57, mp 67-68.5°. The PMR spectrum was superimposable on the PMR spectrum of 57 by method a. T.L.C. R_f(B)= 0.26 for a and b preparations of 57.

Addition of hydrogen chloride to methyl N-acetyldehydroalaninate (14)

Hydrogen chloride was bubbled into a solution of 0.5g of 14 in 10 ml of deuteriochloroform for 10 minutes. A sample was placed in a PMR tube and the spectrum taken: δ 2.08(s,Me); 2.18(s,Ac); 2.33 (s,Ac); 3.89(s,Me ester); 6.08 and 6.50(2s,vinyl); 8.67(b,NH); comparison of intergration indicated 30% of the α-chloro and 70% of 14. After standing 14 hours at room temperature, the PMR spectrum was retaken: δ 2.17(s,Me); 2.33(s,Ac); 2.50(s,Ac); 3.97(s,Me ester); 6.30 and 6.50(2s,vinyl); 9.73(b,NH); comparison of integration indicated 60% to 65% of the α-chloro and 35% to 40% of 14.

Preparation of
methyl α-acetylthio-N-acetyl-D,L-alaninate (74)

a. Hydrogen chloride was bubbled into a solution of 1g (7mmol) of methyl N-acetyldehydroalaninate (14) in a mixture of 10 ml of chloroform and 10 ml of carbon tetrachloride for 15 minutes with stirring at room temperature. The solvent was evaporated in vacuo and the residue was dissolved in 10 ml of thiolacetic acid. After stirring for 4 hours at room temperature, 10 ml of ethanol was added and the reaction was evaporated to dryness in vacuo. The residue was triturated with ether. Crystallization from ethyl acetate gave 0.96g (63%) of 74, mp 166.5-167.5°.

PMR($CDCl_3$)δ 1.92(s,3,Me); 2.02(s,3,Ac); 2.29(s,3,SAc); 3.85(s, 3,Me ester); 7.38(b,1,NH); ($CDCl_3$ + 2 drops TFA) 2.00(s,3,Me); 2.18 (s,3,Ac); 2.36(s,3,SAc); 3.89(s,3,Me ester); 8.11(b,1,NH).

Anal. Calcd for $C_8H_{13}NO_4S$: C,43.82; H,5.98; N,6.39; S,14.62

Found: C,43.69; H,5.96; N,6.09; S,14.49

b. Hydrogen chloride was bubbled continuously into a solution of 4.3g (30mmol) of 14 in 50 ml of HSAc while stirring at room temperature for 1 hour. The flow of HCl was stopped and the reaction stirred an additional 3 hours at room temperature. The solvent was removed in vacuo, chloroform was added twice and removed in vacuo. Recrystallization from EtOAc gave 5.9g (89%) of 74, mp 165-167°.

c. To a solution of 0.5g (2.9mmol) of methyl α-methoxy-N-acetyl-D,L-alaninate (66) in 25 ml of $CHCl_3$ was added 3 ml of HSAc and a spatula of zinc chloride. After stirring 2.5 hours at room temperature, the reaction was diluted with $CHCl_3$ and washed with 1N potassium hydroxide and water. The solvent was dried over Na_2SO_4, filtered and evaporated

in vacuo. Recrystallization from EtOAc gave 0.11g (18%) of 74, mp 165-167°.

Preparation of
α-mercapto-N-acetyl-D,L-alanine (76)

To a solution of 1g (7.7mmol) of N-acetyldehydroalanine(11) in 150 ml of glacial acetic acid and 25 ml of trifluoroacetic acid was added 5 ml of 4N HCl in dioxane. After stirring for 15 minutes at room temperature, hydrogen sulfide was bubbled into the reaction for 20 minutes. After stirring overnight at room temperature, the solvent was removed in vacuo. Crystallization from ethyl acetate gave 0.88g (69%) of 76, mp 155.5-156.5° (lit. mp 154-156° (30)).

T.L.C. R_f(A)=0.17 (characteristic violet color)

PMR(TFA)δ 1.98(s,3,Me); 2.29(s,3,Ac); 7.92(b,1,NH)

Attempted preparation of
methyl α-mercapto-N-acetyl-D,L-alaninate (75)

Hydrogen bromide was bubbled into a solution of 0.5g (3.5mmol) of methyl N-acetyldehydroalaninate (14) in 50 ml of chloroform for 7 minutes with stirring at room temperature. The flow of gas was stopped and the reaction stirred an additional 20 minutes. The solvent was removed in vacuo and a mixture of 1.9g of sodium bisulfide hydrate, 2g of Na_2SO_4 and 2 spatulas of sodium bicarbonate in 50 ml of tetrahydrofuran was added. After stirring 18 hours at room temperature, the reaction was filtered and the filtrate was evaporated in vacuo. The residue was dissolved in chloroform, washed with water, dried over $MgSO_4$ and evaporated in vacuo to give a yellow oil.

PMR($CDCl_3$)δ 1.43(s,3,Me); 2.08(s,3,Ac); 3.82(s,3,Me ester); 7.05(b,1,NH); impurities occurred at 5.0, 3.25 and 2.28

Reactions with
sodium borohydride and sodium borodeuteride

a. To a solution of 3.0g(13.7mmol) of methyl α-acetylthio-N-acetyl-D,L-alaninate (74) in 40 ml of dry methanol (MS-3A) was added 1.6g (41mmol) of sodium borohydride in portions over 10 minutes. After stirring 1 hour at room temperature, the solvent was removed in vacuo and the residue was dissolved in chloroform. After washing with water, the $CHCl_3$ was dried over $MgSO_4$ filtered and evaporated in vacuo to give 0.15g of crude methyl N-acetyl-D,L-alaninate (64). The PMR spectrum was superimposable on that of a known sample of 64; furthermore, T.L.C. data matched that of known 64.

T.L.C. R_f(B)=0.38; (A)=0.67; (C)=0.43; (1:1 EtOH:$CHCl_3$)=0.67; ($CHCl_3$)=0.13

The combined water washings were acidified, saturated with sodium chloride and extracted with $CHCl_3$. The $CHCl_3$ was dried over $MgSO_4$, filtered and evaporated to give 1.3g of a crude oil. PMR analysis indicated a 2:1 mixture of 64 and probably methyl α-mercapto-N-acetyl-D,L-alaninate (75) (the SAc singlet was absent); furthermore, T.L.C. comparisons to known 64 and crude 75 (as prepared elsewhere) matched.

75 crude and reaction T.L.C. R_f(B)=0.61; (C)=0.60; (A)=0.67; ($CHCl_3$)=0.11; (1:1 EtOH:$CHCl_3$)=0.67

To a solution of 0.85g of this crude oil in 100 ml of 75% aqueous dioxane was added 15 ml of 1N sodium hydroxide solution. After stirring 30 minutes at room temperature, the reaction was acidified with 1N HCl

and the solvent was evaporated in vacuo. The residue was extracted with a spectra grade of acetone and the acetone was evaporated in vacuo to give 0.67g of a crude oil. PMR analysis was consistent with a 1:1 mixture of α-mercapto-N-acetyl-D,L-alanine (76) and N-acetyl-D,L-alanine (63); furthermore, T.L.C. comparisons to known 63 and 76 matched.

T.L.C. of 63 R_f(C)=0.14; (B)=0.13; (A)=0.36

T.L.C. of 76 R_f(C)=0.17; (B)=0.10; (A)=0.16; (1:1 EtOH:$CHCl_3$)= 0.15 (characteristic violet color)

b. To a solution of 0.5g (3.5mmol) of methyl N-acetyldehydro-alaninate (14) in 25 ml of dry MeOH was added 0.4g (10.6mmol) of $NaBH_4$. After stirring 30 minutes at room temperature, the solvent was removed in vacuo and the residue was extracted with $CHCl_3$. The $CHCl_3$ was evaporated in vacuo to give 0.18g (35%) of crude 64. The PMR spectrum was superimposable on that of a known sample of 64 and T.L.C. data matched that of known 64.

c. To a solution of 0.25g (1.8mmol) of 14 in 25 ml of dry MeOH was added 0.2g (4.8mmol.) of sodium borodeuteride. After stirring at room temperature for 1 hour, the reaction was acidified with 1N HCl and the solvent removed in vacuo. The residue was dissolved in water and extracted with $CHCl_3$. The $CHCl_3$ was dried over $MgSO_4$, filtered and evaporated in vacuo to give 0.15g of an oil. PMR analysis indicated a 2:3 mixture of 14 and 64.

d. To a solution of 0.2g (0.9mmol) of 74 in 10 ml of dry MeOH was added 0.13g (3.1mmol) of $NaBD_4$. After stirring overnight at room temperature, the reaction was worked up as in procedure c to give

0.15g of an oil. Although the methyl doublet of 64 had collapsed to a singlet in the PMR spectrum, integration comparisons did not differentiate between 2 and 3 protons.

e. To a solution of 2.2g (0.01 mol) of 74 in 100 ml of dry MeOH was added 1.0g (26mmol) of $NaBH_4$ and 1 equivalent of freshly prepared sodium methoxide solution. After stirring at room temperature for 1 hour, the solvent was removed in vacuo and the residue was diluted with $CHCl_3$. The $CHCl_3$ was dried over $MgSO_4$ and evaporated in vacuo to give 0.7g of an oil. PMR analysis indicated a mixture of 64 and 66 (the α-methoxy derivative), the methyl peak at 1.92δ and the SAc peak at 2.29δ of 74 were absent.

Preparation of
p-nitrobenzyl β-chloro-α-acetoxy-N-acetyl-D,L-alaninate (80)

To a mixture of 5.0g (18.9mmol) of p-nitrobenzyl N-acetyldehydroalaninate (71), 19.3g (0.19 mol) of lithium acetate and 2.8g (20.8mmol) of N-chlorosuccinimide in 200 ml of glacial acetic acid was added 5 ml of 4N HCl in dioxane. After stirring 20 hours at room temperature, the solvent was removed in vacuo and the residue was diluted with chloroform. The chloroform was washed with water, saturated sodium bicarbonate solution and water, dried over $MgSO_4$, filtered and evaporated in vacuo. Crystallization from acetone–pet ether (bp 60-90°) gave 5.3g (78%) of 80, mp 125-126.5°.

PMR(DMSO-d6)δ 1.90(s,3,Ac); 2.06(s,3,O-Ac); 4.26 and 4.50(AB doublets,2,CH_2); 5.34(s,2,$PhCH_2$); 7.66 and 8.25(A_2B_2,4,Ph); 9.16 (s,1,NH).

Anal. Calcd. for $C_{14}H_{15}N_2O_7$: C,46.87; H,4.21; N,7.81

Found: C,46.76; H,4.37; N,7.66

Preparation of
p-nitrobenzyl β-chloro-α-methoxy-N-acetyl-D,L-alaninate (81)

a. To a solution of 1.0g (3.8mmol) of p-nitrobenzyl N-acetyl-dehydroalaninate (71) in 20 ml of trifluoroacetic acid was added chlorine in carbon tetrachloride until a permanent yellow color was obtained with stirring at room temperature. After stirring for 10 minutes, excess methanol was added. After stirring 1.5 hours at room temperature, precipitation occurred. Filtration and recrystallization from ethyl acetate gave 0.53g (42%) of 81, mp 150-151° (lit. 151.5-152° (13)).

PMR($CDCl_3$ + 3 drops TFA)δ 2.30(s,3,Ac); 3.34(s,3,OMe); 3.95 and 4.48(AB doublet,2,CH_2); 5.46(s,2,$PhCH_2$); 7.42(b,1,NH); 7.63,8.33(A_2B_2,4,Ph).

b. A solution of 3.0g (11.4mmol) of 71 in 20 ml of TFA was treated as above with Cl_2/CCl_4. After stirring for 10 minutes at room temperature, excess thiolacetic acid was added and the reaction was stirred for 3.5 hours. The solvent was removed in vacuo and MeOH was added once and removed in vacuo. Recrystallization from EtOAc gave 2.5g (66%) of 81, mp 153-154°.

c. To a solution of 1.0g (2.8mmol) of p-nitrobenzyl β-chloro-α-acetoxy-N-acetyl-D,L-alaninate (80) in 50 ml of dry MeOH was added 0.15g (2.8mmol) of sodium methoxide. After stirring 20 hours at room temperature, the solvent was evaporated in vacuo and the residue was diluted with chloroform. The $CHCl_3$ was washed with water, dried over Na_2SO_4, filtered, and evaporated in vacuo. Recrystallization from acetone gave 0.22g (24%) of 81, mp 144-145.5°; with known 81, mmp 150.8-152°. The PMR spectrum was superimposable on that of known 81; T.L.C. data corresponded to known 81.

T.L.C. R_f(A)=0.59; (B)=0.18; (C)=0.49; (1:1 EtOH:$CHCl_3$)=0.66

Preparation of
p-nitrobenzyl β-chloro-α-ethoxy-N-acetyl-D,L-alaninate

The procedure for 81 method b was followed except ethanol was added once and removed in vacuo. Recrystallization from EtOAc gave 0.4g (24%) of product, mp 167.5-168°.

PMR(TFA)δ 1.16(t,3,Me); 2.22(s,3,Ac); 3.4(m,2,OCH_2); 3.90 and 4.46(AB doublet,2,CH_2); 5.38(s,2,$PhCH_2$); 7.38(b,1,NH); 7.56 and 8.24 (A_2B_2,4,Ph).

Preparation of
p-nitrobenzyl(Z)-β-chloro-N-acetyldehydroalaninate (82)

a. To a solution of 0.5g (1.4mmol) of p-nitrobenzyl β-chloro-α-acetoxy-N-acetyl-D,L-alaninate (80) in 20 ml of tetrahydrofuran was added 0.11g (0.8mmol) of potassium carbonate. After refluxing for 7 hours, the solvent was evaporated in vacuo and the residue was diluted with chloroform. The $CHCl_3$ was washed with water, dried over Na_2SO_4, filtered and evaporated in vacuo. Crystallization from ethyl acetate-pet ether (bp 60-90°) gave 0.1g (23%) of (Z)82, mp 148-149°.

PMR($CDCl_3$)δ 2.18(s,3,Ac); 5.40(s,2,$PhCH_2$); 7.08(s,1,vinyl); 7.63 and 8.33(A_2B_2,4,Ph); 9.75(b,1,NH)

PMR(TFA)δ 2.42(s,3,Ac); 5.54(s,2,$PhCH_2$); 7.60(s,1,vinyl); 7.68 and 8.38(A_2B_2,4,Ph).

b. To a solution of 2.6g (0.01 mol) of p-nitrobenzyl N-acetyldehydroalaninate (71) in 50 ml of purified methylene chloride was added chlorine gas until a permanent yellow solution was obtained. After stirring 20 minutes at room temperature, the solvent was evaporated

in vacuo and the residue was redissolved in purified CH_2Cl_2. With stirring at room temperature, 1.1g (0.01 mol) of DABCO was added. After stirring for 20 minutes, the reaction was acidified with 1N HCl and the solvent was evaporated in vacuo. The residue was diluted with water and extracted with ethyl acetate. The EtOAc was dried over $MgSO_4$, filtered and removed in vacuo. Crystallization from EtOAc-pet ether (bp 60-90°) gave 1.3g (42%) of 82, mp 145-148°; mmp with 82 by method a was 146-148°. The PMR spectrum was superimposable with that of 82 by method a.

Preparation of
p-nitrobenzyl β-chloro-α-acetylthio-N-acetyl-D,L-alaninate (83)

Chlorine gas was added to 71 as described above and the crude, dichloro derivative (7b) was dissolved in 25 ml of thiolacetic acid. After stirring overnight at room temperature, the solvent was evaporated in vacuo and the residue was dissolved in ethyl acetate. The EtOAc was washed with water and saturated sodium bicarbonate solution, dried over $MgSO_4$, filtered and evaporated in vacuo. Crystallization from ether-pet ether (bp 30-60°) gave 0.7g (49%) of 83, mp 85-95°.

PMR(1:1 $CDCl_3$/TFA)δ 2.32(s,3,Ac); 2.42(s,3,SAc); 4.07 and 4.90 (AB doublets,2,CH_2); 5.55(s,2,$PhCH_2$); 7.72 and 8.40(A_2B_2,4,Ph); 8.25 (b,1,NH)

To demonstrate that 81 was not formed from 83, the PMR spectrum was taken after stirring at room temperature for 20 minutes with methanol, 1:1 MeOH/TFA, or MeOH plus 1 equivalent of $NaHCO_3$ (work up involved evaporation of solvent in vacuo and extraction of residue with EtOAc. EtOAc was removed in vacuo and the PMR was taken. 0.11g of

83 was used and 0.09g was typically recovered). Since the PMR spectra was unchanged after each treatment (i.e., unchanged 83 was recovered), the formation of 81 in the presence of TFA (see above) did not involve the prior formation of 83.

General preparation of
methyl β-chloro-α-acetylthio-N-acetyl-D,L-alaninate (84)

To a solution of 0.5g (3.5mmol) of methyl N-acetyldehydroalaninate (14) in 20 ml of carbon tetrachloride was added chlorine gas in CCl_4 until a permanent yellow solution was obtained with stirring at room temperature. After stirring 10 minutes, the solvent was removed in vacuo. The crude dichloro derivative (7a) was dissolved in 25 ml of thiolacetic acid and 0.19g (3.5mmol) of sodium methoxide or 0.29g (3.5mmol) of sodium bicarbonate or no base was added. After stirring 3 hours at room temperature, the solvent was evaporated in vacuo and the residue was diluted with ethyl acetate. The EtOAc was washed with water and saturated $NaHCO_3$ solution, dried over $MgSO_4$ and evaporated in vacuo. Crystallization from ether-pet ether (bp 60-90°) gave 0.28g (30%) of 84, mp 113-114.5° (lit. 118.5-120° (13)).

PMR($CDCl_3$)δ 2.10(s,3,Ac); 2.30(s,3,SAc); 3.86(s,3,Me ester); 3.90 and 4.85(AB doublets,2,CH_2); 7.27(b,1,NH).

General preparation of
methyl α,β-dichloro-N-acetyl-D,L-alaninate (7a)

To a solution of methyl N-acetyldehydroalaninate (14) in carbon tetrachloride or purified methylene chloride was added chlorine gas or chlorine in CCl_4 until a permanent yellow solution was obtained. After stirring for 10-30 minutes at room temperature, the solvent was

evaporated in vacuo to give a quantitative yield of 7a as a yellow oil.

PMR($CDCl_3$)δ 2.17(s,3,Ac); 4.02(s,3,Me ester); 4.21 and 5.19(AB doublets,2,CH_2); 7.65(b,1,NH)

General preparation of methyl β-chloro-α-alkoxy-N-acetyl-D,L-alaninates

To a solution of methyl α,β-dichloro-N-acetyl-D,L-alaninate (7a) in a dry alcohol (MS-3A) was added 1.1 equivalents of sodium bicarbonate. After stirring 2 hours at room temperature, the solvent was evaporated in vacuo and the residue was extracted with ethyl acetate. The EtOAc was filtered and evaporated in vacuo. Crystallization from solvent gave product.

Ethanol gave 0.37g (47%) of methyl β-chloro-α-ethoxy-N-acetyl-D,L-alaninate (92), mp 117-117.5° (ether).

T.L.C. R_f($CHCl_3$)=0.20; (C)=0.61; (B)=0.61; (A)=0.93

PMR($CDCl_3$)δ 1.20(t,3,Me); 2.08(s,3,Ac); 3.43(m,2,OCH_2); 3.87(s,3,Me ester); 3.86 and 4.60(AB doublets,2,CH_2); 6.80(b,1,NH)

Anal. Calcd. for $C_8H_{14}ClNO_4$: C,42.96; H,6.31; N,6.26

Found: C,42.94; H,6.38; N,6.35

Methanol gave 3.4g (46%) of methyl β-chloro-α-methoxy-N-acetyl-D,L-alaninate (85), mp 112-114° (ethyl acetate-pet ether (bp 60-90°)) (lit. 112-112.5° (13)).

PMR($CDCl_3$)δ 2.12(s,3,Ac); 3.27(s,3,OMe); 3.88(s,3,Me ester); 3.84 and 4.60(AB doublets,2,CH_2); 6.90(b,1,NH)

Preparation of the
β-chloro-α-ethoxy derivative (92) during the
synthesis of methyl β-chloro-N-acetyldehydroalaninate (91)

The dichloro compound (7a) was prepared from 10.0g (69.9mmol) of 14 . The crude residue was dissolved in 50 ml of dry acetonitrile (MS-3A) and 4.5g (40.1mmol) of DABCO or 7.1g (69.9mmol) of triethylamine was added. After stirring 30 minutes at room temperature (12 hours for Et_3N), the solvent was evaporated in vacuo and the residue was diluted with ethyl acetate, filtered and evaporated in vacuo. Crystallization from ether gave 2.9g (23%) of methyl β-chloro-N-acetyldehydroalaninate (91), mp 95-96.5° and 0.5g of 92, mp 115-117°. The PMR spectrum was superimposable on that of known 92 and T.L.C. data were consistent with known 92.

Preparation of the
β-chloro-α-methoxy derivative (85) from the
β-chloro-α-acetylthio derivative (84)

A solution of 0.05g (0.2mmol) of methyl β-chloro-α-acetylthio-N-acetyl-D,L-alaninate (84) in 5 ml of dry methanol was flushed with nitrogen and 0.012g (0.22mmol) of sodium methoxide was added. After stirring for 7 minutes at room temperature, 2 ml of glacial acetic acid was added and the solvent was removed in vacuo. Ethanol was added and evaporated in vacuo to give a white solid. A PMR spectral comparison to a known sample of 85 was superimposable. A PMR spectrum of a mixture of known 85 and this solid also was unchanged.

Preparation of
methyl N-acetyl-L-cysteinate (18)

To an ice cold solution of 16.3g (0.1 mol) of N-acetyl-L-cysteine (Sigma grade) in 100 ml of dry methanol (MS-3A) was added 8 ml of

acetyl chloride with stirring in an ice bath. After stirring for 24 hours at room temperature, the solvent was evaporated in vacuo and the residue was neutralized with saturated sodium bicarbonate solution and solid $NaHCO_3$. The mixture was saturated with sodium chloride and extracted with methylene chloride. The extracts were dried over $MgSO_4$, filtered and evaporated in vacuo. Crystallization from isopropyl ether gave 9.6g (54%) of 18, mp 78-79.2° (lit. 79-80° (91)).

PMR($CDCl_3$)δ 1.37(t,1,SH); 2.10(s,3,Ac); 3.03(q,2,CH_2); 3.87(s, 3,Me ester); 4.90(m,1,CH); 6.47(b,1,NH)

Preparation of
methyl S,N-diacetyl-L-cysteinate (90)

To a solution of 0.5g (2.8mmol) of 18 in 25 ml of water was added 0.47g (5.6mmol) of sodium bicarbonate followed by 0.3g (2.8mmol) of acetic anhydride. After stirring 1 hour at room temperature, the reaction was extracted with chloroform. The $CHCl_3$ was dried over $MgSO_4$, filtered and evaporated in vacuo. Crystallization from ethyl acetate gave 0.22g (36%) of 90, mp 94.5-96.5° (lit. 100° (79)).

PMR($CDCl_3$)δ 1.95(s,3,Ac); 2.28(s,3,SAc); 3.30(d,2,CH_2); 3.70 (s,3,Me ester); 4.74(m,1,CH); 6.30(b,1,NH)

IR (film) 3200, 1690, 1610, 1530, 1420, 1350, 1270, 1210, 1100, 1010, 960, 930 and 820 cm^{-1}

Preparation of
methyl β-chloro-α-acetylthio-N-acetyl-D,L-alaninate (84)
with triethylamine

The dichloro compound (7a) was prepared from 0.5g (3.5mmol) of 14. The crude residue was dissolved in 30 ml of acetonitrile (MS-3A),

and 0.35g (3.5mmol) of Et_3N and 0.27g (3.6mmol) of thiolacetic acid were added. After stirring overnight at room temperature, the solvent was evaporated in vacuo and the residue diluted with chloroform. The $CHCl_3$ was washed with water, dried over $MgSO_4$, filtered and evaporated in vacuo. Crystallization from ether-pet ether (bp 30-60°) gave 0.13g (15%) of 84, mp 115.5-116.5°.

Preparations of
methyl (Z)-β-acetylthio-N-acetyldehydroalaninate (89)

a. The dichloro compound (7a) was prepared from 0.5g (3.5mmol) of 14. The crude residue was dissolved in 25 ml of acetonitrile (MS-3A), and 0.75g (7.4mmol) of triethylamine and 0.27g (3.6mmol) of thiolacetic acid were added. After stirring at room temperature for 12 hours, the solvent was evaporated in vacuo and the residue was diluted with chloroform. The $CHCl_3$ was washed with water, dried over $MgSO_4$, filtered and evaporated in vacuo. Crystallization from ethyl acetate gave 0.72g (24%) of (Z)89, mp 135-137°.

b. To a solution of 0.5g (2mmol) of methyl β-chloro-α-acetylthio-N-acetyl-D,L-alaninate (84) in 25 ml of acetonitrile (MS-3A) was added 0.2g (2mmol) of Et_3N. After stirring for 12 hours at room temperature, the solvent was evaporated in vacuo and the residue diluted with $CHCl_3$. The $CHCl_3$ was washed with water, dried over $MgSO_4$, filtered and evaporated in vacuo to give an oil. A PMR spectrum was superimposable with a PMR spectrum of known (Z)89. T.L.C. data was consistent with known (Z)89.

T.L.C. R_f (EtOAc)=0.48; (1:1 EtOH:$CHCl_3$)=0.80; (C)=0.75; (B)=0.30; (A)=0.61

Preparations of
methyl (Z)-β-benzylthio-N-acetyldehydroalaninate (88)

a. The dichloro compound (7a) was prepared from 1.0g (7mmol) of 14. The crude residue was dissolved in 80 ml of CH_3CN (MS-3A), and 1.7g (14mmol) of benzyl mercaptan and 1.4g (14mmol) of Et_3N were added. After stirring for 12 hours at room temperature, the solvent was evaporated in vacuo and the residue diluted with $CHCl_3$. The $CHCl_3$ was washed with water, dried over $MgSO_4$, filtered and evaporated in vacuo. Crystallization from ethyl acetate-pet ether (bp 30-60°) gave 0.59g (32%) of (Z)88, mp 100.5-102.5°.

b. The dichloro compound (7a) was prepared from 5.0g (34.9mmol) of 14. The crude residue was dissolved in 100 ml of CH_3CN, and 7.2g (70.7mmol) of Et_3N and 4.3g (34.9mmol) of benzyl mercaptan were added. After stirring for 12 hours at room temperature, the reaction was worked up as above. Crystallization from EtOAc-pet ether (bp 30-60°) gave 1.7g (18%) of (Z)88, mp 103.5-104.5°.

Preparations of
methyl S,N-diacetyl-D,L-cysteinate (90)

To a solution of 0.5g (3.5mmol) of methyl N-acetyldehydroalaninate (14) in 25 ml of CH_3CN (MS-3A) were added 0.27g (3.6mmol) of thiolacetic acid and 0.75g (7.4mmol) or 0.35g (3.5mmol) of Et_3N. After stirring for 12 hours at room temperature, the solvent was removed in vacuo and the residue was diluted with $CHCl_3$. The $CHCl_3$ was washed with water, dried over $MgSO_4$, filtered and evaporated in vacuo. Crystallization from ethyl acetate-pet ether (bp 30-60°) gave 0.29g (37%) or 0.33g (43%) of 90, mp ∿ 70°. Recrystallization of combined precipitates

gave 0.44g (29%) of 90, mp 72-73°. Comparison of PMR spectra and IR spectra with L-(90) indicated the structure of D,L-(90) was correct.

Preparations of
methyl β,β-diacetylthio-N-acetyl-D,L-alaninate (87)

a. The dichloro compound (7a) was prepared from 0.5g (3.5mmol) of 14. The crude residue was dissolved in 20 ml of CH_3CN (MS-3A), and 0.7g (6.9mmol) of Et_3N and 0.53g (7mmol) of HSAc were added. After stirring for 3 hours at room temperature, the solvent was evaporated in vacuo and the residue was diluted with $CHCl_3$. The $CHCl_3$ was washed with water, dried over $MgSO_4$, filtered and evaporated in vacuo. Crystallization from methylene chloride-pet ether (bp 30-60°) after Norit treatment, gave 0.14g (14%) of 87, mp 113-114° (alternate crystalline modification mp 94-95°).

PMR($CDCl_3$)δ 2.07(s,3,Ac); 2.37(s,6,SAc); 3.80(s,3,Me ester); 5.17(m,1,αCH); 5.60(d,1,βCH); 6.57(b,1,NH)

T.L.C. R_f (A)=0.75; (B)=0.36; (C)=0.60; (1:1 EtOH:$CHCl_3$)=0.63

b. To a solution of 5.33g (0.03 mol) of methyl (Z)-β-chloro-N-acetyldehydroalaninate in 100 ml of CH_3CN (MS-3A) were added 5 ml of HSAc, 2.5g (0.03 mol) of sodium bicarbonate and several specks of DABCO. After stirring overnight at room temperature, the reaction was worked up as above. Crystallization from CH_2Cl_2-pet ether (bp 30-60°), after Norit treatment, gave 6.2g (71%) of 87, mp 94-96°.

c. To a solution of 0.5g (2.3mmol) of methyl (Z)-β-acetylthio-N-acetyldehydroalaninate (89) in 30 ml of CH_3CN (MS-3A) were added 1/3 spatula of DABCO and 1 ml of HSAc. After stirring for 4 hours at room temperature, 1 ml of 4N HCl in dioxane was added and the solvent

removed in vacuo. After the above work up, crystallization from CH_2Cl_2-pet ether (bp 30-60°), after Norit treatment, gave 0.47g (70%) of 87, mp 94-96°.

d. To a solution of 0.2g (0.9mmol) of methyl (E)-β-acetylthio-N-acetyldehydroalaninate (89) in 10 ml of CH_3CN (MS-3A) were added a speck of DABCO and 0.2 ml of HSAc. After stirring for 2.5 hours at room temperature, the solvent was removed in vacuo and the residue was dissolved in CH_2Cl_2. The CH_2Cl_2 was washed with water, dried over $MgSO_4$, filtered and evaporated in vacuo. Crystallization from CH_2Cl_2-pet ether (bp 30-60°) gave 0.1g (50% recovery) of (E)89, mp 106.8-108° (lit. 106.8-107.4°)(see below) and 0.1g of an oil which PMR analysis indicated was a mixture of (E)89 and 87.

The precipitate and crude oil (0.2g total) was redissolved in 10 ml of CH_3CN; a speck of DABCO and 0.5 ml of HSAc were added. After stirring for 7.5 hours at room temperature, the same reaction work up gave 0.15g of an oil. PMR analysis indicated (E)89 was still present, i.e., an intense singlet at 8.87δ.

e. To a solution of 0.5g (1.8mmol) of methyl (Z)-β-benzyl-sulfinyl-N-acetyldehydroalaninate (96) in 30 ml of CH_3CN (MS-3A) were added 0.2g (1.8mmol) of DABCO and 1 ml of HSAc. After stirring for 2 hours at room temperature, the solvent was evaporated in vacuo and the residue dissolved in $CHCl_3$. The $CHCl_3$ was washed with water, dried over $MgSO_4$, filtered and evaporated in vacuo. Crystallization from ether-pet ether (bp 30-60°) gave 0.34g (64%) of 87, mp 94-96°. The PMR spectrum was superimpossable on that of an authentic sample of 87.

Preparation of
α-tetrahydropyranthiol (97)

To 45g (1.3 mol) of liquefied hydrogen sulfide in a 100 ml stainless high pressure bomb in a liquid nitrogen bath was added 15.0g (0.18 mol) of dihydropyran, 25 mgm of p-toluenesulfonic acid and 5 drops of trifluoroacetic acid. The bomb was sealed and stored at room temperature for 20 hours. The H_2S was released and the remaining oil distilled under vacuum to give 9.3g (44%) of 97, bp 50-51°/aspirator (lit. bp 58°/15mm (83)).

PMR($CDCl_3$)δ 1.67(bd.s,6,CH_2); 2.20(d,1,SH); 3.90(m,2,CH_2O); 5.13(m, 1,CHS)

Oxidation of
methyl (Z)-β-benzylthio-N-acetyldehydroalaninate (88)

To a solution of 2.3g (8.7mmol) of methyl (Z)-β-benzylthio-N-acetyldehydroalaninate (88) in 100 ml of purified methylene chloride was added 2.3g (11.3mmol) of 85% m-chloroperoxybenzoic acid. After stirring for 3 hours at room temperature, the reaction was diluted with chloroform. The $CHCl_3$ was washed with water and saturated sodium bicarbonate solution, dried over $MgSO_4$, filtered and evaporated in vacuo. Crystallization from ethyl acetate gave 1.5g (63%) of the (Z) sulfoxide (96), mp 127.5-129°, and 0.22g of the (Z) sulfone, mp 126.5-127.5°. A similar preparation with 1 equivalent of 85% MCPBA gave 62% yield of the (Z) sulfoxide (96) (see Table 6 for physical data, Table 7 for PMR data and Table 8 for T.L.C. data).

TABLE 6

PHYSICAL DATA FOR METHYL β-SUBSTITUTED -N-ACETYLDEHYDROALANINATES

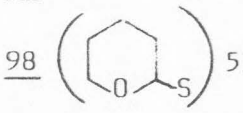
Method (p. 141)	Compound Yield (β-substituent) %	Crystallization Solvent	Melting Point °C	Molecular Formula	Analysis Calculated / Found C%	H%	N%	S%
	(Z) ($PhCH_2SO_2$)	EtOAc	126.5-127.5	$C_{13}H_{15}NO_5S$	52.52 52.69	5.09 5.12	4.71 4.66	10.78 10.66
	(Z) 91 (Cl) 28	Et_2O	96-96.5	$C_6H_8ClNO_3$	40.58 40.64	4.54 4.60	7.89 7.88	
A	(Z) 89 (AcS) 45	$CHCl_3$	140-142					
A	(E) 89 (AcS)[b] 12	$CHCl_3$-PE[a]	106.8-107.4	$C_8H_{11}NO_4S$	44.23 44.24	5.10 5.12	6.45 6.41	
A	(Z) 88 ($PhCH_2S$) 64	EtOAc-PE[a]	102.8-104	$C_{13}H_{15}NO_3S$	58.85 58.57	5.70 5.78	5.28 5.18	12.09 11.92
A	(E) 88 ($PhCH_2S$)[c] 3	Et_2O	99.5-101	$C_{13}H_{15}NO_3S$	58.85 58.75	5.70 5.81	5.28 5.07	
B	95 (N-Ac·Cys) 51	acetone-Et_2O	179.5-180.5	$C_{11}H_{16}N_2O_6S$	43.42 43.28	5.30 5.38	9.21 9.13	10.54 10.25
C	(Z) 94 (N-Ac·Cys·OMe) 80[d]	EtOAc-[d] Et_2O						
A	(Z) 98  53	EtOAc-PE[a]	97-98.5	$C_{11}H_{17}NO_4S$	50.95 51.01	6.61 6.71	5.40 5.21	12.36 12.23

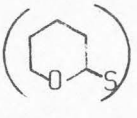
TABLE 6 continued

DATA ON METHYL β-SUBSTITUTED-N-ACETYLDEHYDROALANINATES

[a]Petroleum ether (bp 30-60°). [b]Separated from Z isomer by column chromatography on Silica Gel 60 (E. Merck) 230-400 mesh eluted with solvent B. [c]Separated from E isomer by selective crystallization. [d]94 is a crude gelatin material.

TABLE 7

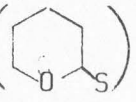
PMR DATA OF METHYL β-SUBSTITUTED-N-ACETYLDEHYDROALANINATES

Compound (β-substituent)	δ values in $CDCl_3$ (in TFA) for N-acetyl[a]	Me ester[a]	vinyl[a]	NH[b]	others		
(Z) 96 ($PhCH_2SO$)	2.07 (2.40)	3.83 (4.03)	6.33 (7.07)	8.60 (8.97)	4.10, 4.47[c] (4.53, 4.80)[c]	7.40[d] (7.47)[d]	
(Z) ($PhCH_2SO_2$)	1.87 (2.08)	3.87 (4.02)	5.73 (6.30)	8.97 (9.07)	4.37[e] (4.60)[e]	7.47[d] (7.52)[d]	
(Z) (Br)	2.17 (2.43)	3.83 (4.03)	7.17 (7.87)	7.30 (8.33)			
(E) (Br)	2.15 (2.35)	3.97 (4.07)	8.13 (7.63)	7.70 (8.60)			
(Z) 91 (Cl)	2.13 (2.40)	3.83 (4.03)	7.00 (7.53)	7.13 (8.33)			
(E) 91 (Cl)	3.33	3.90	7.43	8.77			
(Z) 89 (AcS)	2.15 (2.40)	3.87 (4.03)	7.93 (8.40)	7.53 (8.57)	2.45[f] (2.60)[f]		
(E) 89 (AcS)	2.18 (2.45)	3.95 (4.12)	8.80 (8.80)	8.86 (8.72)	2.48[f] 2.65[f]		
(Z) 88 ($PhCH_2S$)	2.03 (2.38)	3.70 (3.90)	7.43 (8.10)	7.30-7.43 (8.30)	3.98[e] (4.13)[e]	7.30[d] (7.37)[d]	
(E) 88 ($PhCH_2S$)	2.05 (2.33)	4.82 (3.96)	8.33 (7.97)	7.58 (8.52)	3.93[e] (4.03)[3]	7.33[d] (7.33)[d]	
95 (N-Ac·Cys)	(2.16)	(3.73)	(7.83)	(8.22)[i]	(3.40)[g]	(4.93)[h]	(7.73)[i]
(Z) 94 (N-Ac·Cys·OMe)	2.10 (2.37)[j]	3.88 (4.0)	7.47 (8.07)	8.12[i] (8.50)[i]	3.40[g] (3.60)[g]	4.93[h] (5.17)[h]	7.56[i] (8.18)[i]
(Z) 98 	2.15 (2.43)	3.82 (4.00)	7.70 (8.26)	7.10 (8.47)	1.73[k] (1.90)[k]	3.90[l] (4.17)[l]	5.26[m] (5.60)[m]

[a]Singlet. [b]Broad peak. [c]AB doublets, CH_2Ph. [d]Singlet, Ph. [e]Singlet, CH_2Ph. [f]Singlet, SAc. [g]Doublet, CH_2. [h]Multiplet, CH. [i]Broad, NH of cysteine or alanine. [j]N-Ac of cysteine and alanine, doublet. [k]Broad singlet, CH_2. [l]Multiplet, CH_2O. [m]Multiplet, CHS.

TABLE 8

T.L.C. DATA FOR METHYL β-SUBSTITUTED-N-ACETYLDEHYDROALANINATES

Compound (β-substituent)	R_f values in Solvent A	Solvent B	Solvent C	Others
(Z) 96 ($PhCH_2SO$)	0.64			
(E) (Br)	0.63	0.39	0.85	0.74[a]
(Z) (Br)	0.61	0.32	0.85	0.72[a]
(Z) 91 (Cl)	0.87	0.49	0.44	0.14[b]
(E) 91 (Cl)		0.40		
(Z) 89 (AcS)	0,68	0.36	0.84	0.79[a]
(E) 89 (AcS)	0.70	0.44	0.84	0.78[a]
(Z) 88 ($PhCH_2S$)	0.72			
(E) 88 ($PhCH_2S$)	0.79			0.76[a]
95 (N-Ac·Cys)	0.42		0.32	0.23[a]
(Z) 94 (N-Ac·Cys·OMe)		0.25		
(Z) 98 	0.63	0.40		0.37[c] 0.12[b] 0.63[d]

[a]Ethanol:chloroform (1:1). [b]Chloroform. [c]Ethyl acetate. [d]Acetone.

General preparation of
methyl (Z)(E)-β-bromo-N-acetyldehydroalaninate

To a solution of 1.0g (7mmol) of methyl N-acetyldehydroalaninate (14) in 20 ml of purified methylene chloride was added bromine in CH_2Cl_2 with stirring at room temperature until a permanent brown solution was obtained. After stirring for 10 minutes at room temperature, 0.7g (7mmol) of triethylamine or 0.78g (7mmol) of DABCO was added. The reaction was stirred at room temperature for 22 hours (Et_3N) or 40 minutes (DABCO) and the precipitate was filtered through Celite. The solvent was evaporated in vacuo and the residue was diluted with anhydrous ether. The ether was filtered and evaporated in vacuo to give 1.6g of an oil. PMR analysis of the (E) and (Z) vinyl protons of this oil indicated a 1:1 ratio of (Z/E isomers (Et_3N) or a 3:1 ratio of Z/E isomers(DABCO). Preparative T.L.C. using $CHCl_3$-AcOH (95:5) as elutant separated the Z and E isomers (upper band was E isomer). The Z isomer was isolated as a pure solid, mp 77-78.5° (Et_2O-pet ether (bp 30-60°)). The E isomer was isolated as a pure oil (see Table 7 for PMR data and Table 8 for T.L.C. data).

Preparation of
β-chloro-N-acetyldehydroalanine (93)

Chlorine gas was bubbled into a solution of 0.5g (3.9mmol) of N-acetyldehydroalanine (11) in 50 ml of chloroform and 10 ml of acetonitrile until a permanent yellow solution was obtained. After stirring for 10 minutes at room temperature, the reaction was refluxed on a steam bath for 1 hour. The solvent was removed in vacuo. Crystallization from ethyl acetate-pet ether (bp 30-60°) gave 0.21g (33%) of 93, mp 132.5-133.5°.

PMR(TFA)δ 2.40(s,3,Ac); 7.70(s,1,vinyl); 8.30(b,1,NH)

PMR(acetone-d_6)δ 2.07(s,3,Ac); 7.07(s,1,vinyl); 8.63(b,1,NH)

General preparation of
methyl (Z,E)-β-chloro-N-acetyldehydroalaninate (91)

The dichloro compound (7a) was prepared from 42.7g (0.3 mol) of methyl N-acetyldehydroalaninate (7a). The crude residue was dissolved in 300 ml of purified methylene chloride and 33.7g (0.3 mol) of DABCO or 45.0g (0.3 mol) of DBU was added (or the residue was dissolved in t-butyl alcohol and added to 33.7g (0.3 mol) of potassium t-butoxide in 200 ml of t-BuOH). After stirring for 20 minutes at room temperature, the solvent was removed in vacuo and the residue was dissolved in water. The aqueous solution was acidified with 1N HCl, extracted with ether, saturated with sodium chloride and extracted with ether. The ether extracts were dried over $MgSO_4$, filtered and evaporated in vacuo. PMR analysis of the crude reaction indicated a 4:1 Z/E mixture (DABCO) or a 9:1 Z/E mixture (DBU or t-BuOK) of 91. Crystallization from ether gave 15.1g (28%) of (Z) 91, mp 96-96.5°, and 13.6g (25%) of a 1:1 mixture of (Z/E) 91. A partial separation of the E isomer could be effected by column chromatography on silica gel (Sigma Type I) eluted with solvent B, however, this was generally not done (the 1:1 mixture of (Z/E) 91 was used for further reaction) (see Table 6 for physical data, Table 7 for PMR data and Table 8 for T.L.C. data).

General preparations of
methyl β-mercapto-N-acetyldehydroalaninate derivatives

a. To a solution of methyl (Z)- or 1:1 (Z/E)-β-chloro-N-acetyldehydroalaninate (91) in acetonitrile (MS-3A) were added 1 equivalent of DABCO and 1 equivalent of the appropriate mercaptan. After stirring

for 2 to 4 hours at room temperature, the solvent was evaporated in vacuo and the residue was diluted with ethyl acetate. After filtering the precipitate, the EtOAc was washed with water, 1N HCl and saturated $NaHCO_3$ solution, dried over $MgSO_4$, filtered and evaporated in vacuo. Crystallization gave product.

b. Same procedure as A except after stirring at room temperature, the reaction was acidified with 4N HCl in dioxane and filtered through celite. The solvent was evaporated in vacuo and selective crystallization gave product.

c. Same procedure as A except after stirring at room temperature, the solvent was removed in vacuo and the residue diluted with water. The aqueous solution was extracted with EtOAc, saturated with sodium chloride and extracted with EtOAc. The EtOAc was dried over $MgSO_4$, filtered and evaporated in vacuo. Crystallization gave product (see Table 6 for physical data, Table 7 for PMR data and Table 8 for T.L.C data).

Reaction of α-tetrahydropyranthiol (97) with methyl (Z)-β-acetylthio-N-acetyldehydroalaninate (89)

To a solution of 4.3g (0.02 mol) of 89 in 100 ml of dry acetonitrile (MS-3A) were added 2.4g (0.02 mol) of 97 and a speck of DABCO. After stirring at room temperature for 6 days in a screw capped bottle, the reaction was acidified with 2.1N HCl in THF and the solvent evaporated in vacuo. The residue was diluted with ethyl acetate, dried over $MgSO_4$, filtered and the solvent removed in vacuo. The crude residue was separated by column chromatography on silica gel (Sigma IV) eluted with EtOAc to give 1.5g (47%) of an oil. The oil was distilled in vacuum to give 0.97g (30%) of α-tetrahydropyranylthiol acetate; bp

54-57°/0.38mm, n_D^{23}1.5011 (lit. bp 61°/0.4mm, n_D^{23}1.5012 (84)).
T.L.C. R_f (EtOAc)=0.69

PMR($CDCl_3$)δ 1.70(bd.s,6,CH_2); 2.37(s,3,SAc); 3.83(m,2,CH_2O); 5.73(m,1,SCH)

Besides the oil, a second material was obtained (T.L.C. (EtOAc)= 0.64 + 2 other spots near origin). Crystallization from ether-pet ether (bp 30-60°) gave 0.26g (3.9%) of product 99, mp 106-117°. An analytical sample of methyl β-(α-tetrahydropyranylthio)-β-acetylthio-N-acetyl-D,L-alaninate (99) was prepared by column chromatography on Silica Gel 60 (E. Merck) 230-400 mesh eluted with EtOAc followed by recrystallization from ether, mp 122-132°.

PMR($CDCl_3$)δ 1.70(bd.s,6,CH_2); 2.07(s,3,Ac); 2.37(s,3,SAc); 3.87 (s,3,Me ester); 3.77(m,2,CH_2O): 5.27(m,3,SCH,2CH); 6.43(b,1,NH)

Anal. Calcd. for $C_{13}H_{21}NO_5S_2$: C,46.55; H,6.31; N,4.18; S,19.12
Found: C,46.29; H,6.19; N,4.13; S,19.19

Reaction of
methyl N-acetyl-L-cysteinate (18) with 89

To a solution of 0.5g (2.3mmol) of (Z)89 in 30 ml of dry acetonitrile (MS-3A) were added 1/3 of a spatula or 0.25g (2.2mmol) of DABCO and 0.41g (2.3mmol) of 18. After stirring for 20 hours at room temperature, the solvent was evaporated in vacuo. The residue was diluted with chloroform. The $CHCl_3$ was washed with water, dried over $MgSO_4$, filtered and evaporated in vacuo. Crystallization from ethyl acetate-pet ether (bp 30-60°) gave 0.38g (76%) of methyl S,N-diacetyl-L-cysteinate (90), mp 91-93.5° (lit. 100° (79)).

PMR and IR spectral data corresponded to previously prepared 90; T.L.C. R_f (C)=0.80

Reaction of
benzyl mercaptan with 89

To a solution of 1.09g (5mmol) of (Z)89 in 50 ml of dry acetonitrile (MS-3A) were added 0.62g (5mmol) of benzyl mercaptan and 0.76g (5mmol) of DBU. After refluxing for 3 hours, the reaction was acidified with 1N HCl and the solvent evaporated in vacuo. Column chromatography on Silica Gel 60 (E. Merck) 230-400 mesh eluted with ethyl acetate gave 0.5g of benzylthiol acetate contaminated with benzyl mercaptan (odor-oil of Leek).

T.L.C. R_f (EtOAc)=0.46 for benzyl mercaptan; 0.74 for benzylthiol acetate (PMR analysis indicated 30% benzyl mercaptan and 70% benzylthiol acetate in the mixture.

Benzylthiol acetate PMR($CDCl_3$)δ 2.27(s,3,SAc); 4.10(s,2,CH_2Ph); 7.33(s,5,Ph)

Benzyl mercaptan PMR($CDCl_3$)δ 3.62(s,2,CH_2PH); 7.33(s,5,Ph)

Preparation of
methyl β,β-dichloro-N-acetyldehydroalaninate (101)

To a solution of 44.5g (0.25 mol) of methyl (Z)(E)-β-chloro-N-acetyldehydroalaninate (91) in 350 ml of purified methylene chloride was added chlorine gas until a permanent yellow solution was obtained. After stirring for 20 minutes at room temperature, the solvent was evaporated in vacuo. The yellow residue was dissolved in 300 ml of purified CH_2Cl_2 and 28.0g (0.25 mol) of DABCO was slowly added with stirring at room temperature. After the addition was completed, the reaction was stirred for 10 minutes at room temperature and refluxed for 10 minutes. The reaction was cooled, washed with water, dried over $MgSO_4$ and filtered. The solvent was evaporated in vacuo and the

residue crystallized from water (a hot water insoluble gum was removed by filtration) to give 29.0g (54%) of 101, mp 102.5-103.5°.

T.L.C. R_f (EtOAc)=0.50; (B)=0.34; (C)=0.79; ($CHCl_3$)=0.18 (I_2 vapors do not color the spot on a T.L.C. plate)

PMR($CDCl_3$)δ 2.15(s,3,Ac); 3.90(s,3,Me ester); 7.83(b,1,NH)

Anal. Calcd for $C_6H_7Cl_2NO_3$: C,33.99; H,3.33; N,6.61

Found: C,33.89; H,3.31; N,6.47

Preparation of methyl β-chloro-β-(methyl N-acetyl-L-alaninate-β-thio)-N-acetyldehydroalaninate (102)

To a solution of 2.12g (0.01 mol) of 101 in 50 ml of dry acetonitrile (MS-3A) were added 1.77g (0.01 mol) of methyl N-acetyl-L-cysteinate (18) and 1.6g (0.01 mol) of 96% DBU. After refluxing for 3 hours, 2 ml of 1N HCl was added and the solvent was removed in vacuo. The residue was dissolved in water and extracted with chloroform. The $CHCl_3$ was dried over $MgSO_4$, filtered and evaporated in vacuo. Column chromatography on Silica Gel 60 (E. Merck) 230-400 mesh eluted with ethyl acetate gave 0.5g of recovered 101, 0.63g of the major isomer of 102, 0.81g of a mixture of major/minor isomers of 102 and several other unidentified fractions. Recrystallization of the major isomer from EtOAc-pet ether (bp 30-60°) gave 0.3g of 102 (major isomer), mp 134.5-135.5°.

T.L.C. R_f (40% acetone-EtOAc)=0.68; (acetone)=0.84; (EtOAc)=0.41

PMR($CDCl_3$)δ 2.13 and 2.20(2s,6,Ac); 3.13(m,2,CH_2); 3.87 and 3.93 (2s,6,Me esters); 4.93(m,1,CHS); 6.90(bd.d,1,NH cys); 8.83(b,1,NH)

PMR(DMSO-d_6)δ 2.03 and 2.13(2s,6,Ac); 3.50(bd.s,2,CH_2); 3.83 and 3.87(2s,6,Me esters); 4.67(m,1,CHS); 8.73(bd.d,1,NH cys); 9.87(b,1,NH)

Anal. Calcd. for $C_{12}H_{17}ClN_2O_6S$: C,40.85; H,4.86; N,7.94; S,9.09

Found: C,40.75; H,4.84; N,7.77; S,9.03

In another experiment, 0.01g of the minor isomer was also isolated, mp 58-70° (EtOAc-pet ether (bp 30-60°))

T.L.C. R_f (40% acetone-EtOAc)=0.64; R_f's same as major isomer for rest of solvents tried.

PMR($CDCl_3$)δ 2.03 and 2.17(2s,6,Ac); 3.40(bd.d,2,CH_2); 3.83 and 3.93(2s,6,Me esters); 4.97(m,1,CHS); 7.40(bd.d,1,NH cys); 8.17(b,1,NH)

Preparation of methyl β-methylthio-β-(methyl N-acetyl-L-alaninate-β-thio)-N-acetyldehydroalaninate (103)

To a solution of 0.07g (3.0mmol) of sodium metal in 5 ml of methanol (MS-3A) were added 50 ml of acetonitrile (MS-3A) and 0.2g (4.2mmol) of methyl mercaptan. After stirring a few minutes at room temperature, 1.0g (2.8mmol) of the major isomer of 102 was added. After stirring for 70 hours at room temperature, 1N HCl was added and the solvent was evaporated in vacuo. The residue was diluted with ethyl acetate, dried over $MgSO_4$, filtered and evaporated in vacuo. Column chromatography on Silica Gel 60 (E. Merck) 230-400 mesh eluted with EtOAc gave 0.08g (7.8%) of the major isomer of 103, mp 126-127°.

T.L.C. R_f (EtOAc)=0.48; (40% acetone-EtOAc)=0.63

PMR($CDCl_3$)δ 2.10(s,3,CH_3S); 2.20 and 2.27(2s,6,Ac); 3.13(m,2,CH_2); 3.83 and 3.93(2s,6,Me esters); 4.87(m,1,CHS); 6.67(bd.d,1,NH cys); 8.87(b,1,NH)

Anal. Calcd. for $C_{13}H_{20}N_2O_6S_2$: C,42.84; H,5.53; N,7.69; S,17.60

Found: C,42.96; H,5.47; N,7.60; S,17.09

Another fraction, isolated as an oil, appeared to be the minor isomer of 103 (0.02g).

T.L.C. R_f (40% acetone-EtOAc)=0.57

PMR($CDCl_3$)δ 2.03(s,3,CH_3S); 2.17 and 2.33(2s,6,Ac); 3.40(bd.s, 2,CH_2); 3.83 and 3.97(2s,6,Me ester); 4.97(m,1,CHS); 7.63(bd.d,1,NH cys); 7.97(b,1,NH)

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