THE TOTAL SYNTHESIS OF
A CEPHAM DERIVATIVE

A THESIS
BY
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The Total Synthesis of a Cepham Derivative

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Abstract

The total synthesis of a cepham derivative was accomplished, starting from the readily available glyceraldehyde acetonide. The Asinger cyclization and the Ugi condensation reactions, formed the basis of the approaches. During the course of the synthesis, a new aldehyde protecting group was developed which was stable to acylating agents, anhydrous bases and thiolate anion displacements and could be easily hydrolyzed with dilute aqueous acetic acid. Many erroneously assigned structures, published by Worrall, were corrected. In addition, several new compounds were prepared and characterized.

La Synthèse Totale d'un Dérivé
du Cépham
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Résumé

Basée sur une cyclisation du type Asinger et sur la réaction d'Ugi, une synthèse totale d'un dérivé du cépham est réalisée à partir de l'acétonide du glycéraldéhyde. Un nouveau groupement protecteur pour aldéhydes, résistant à certains agents d'acylation, à des bases anhydres et à des déplacements par les anions thiolates, et qui est hydrolysé par l'acide acétique aqueux, a été mis au point. Des structures erronées, publiées par Worrall, sont corrigées. Par ailleurs plusieurs nouveaux composés sont préparés et caractérisés.

To my parents and Marilou

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I cannot fully express my profound gratitude to my research director, Dr. George E. Just, for his continuous encouragement, stimulating discussions and constructive criticism. His interest, guidance and friendship throughout my research made my stay in his laboratory a most interesting phase in my career.

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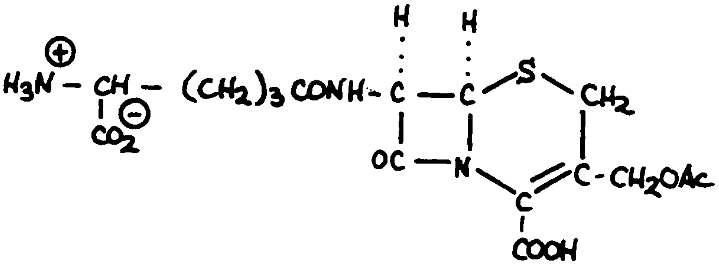
TABLE OF CONTENTS

RESULTS AND DISCUSSION

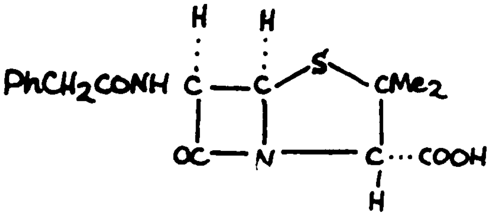
INTRODUCTION

In the course of a search for antibiotic-producing micro-organisms, Professor Guiseppe Brotzu, Rector in the University of Cagliari, Sardinia, sampled sea water where municipal sewage was discharged. This location was chosen because, Brotzu reasoned, "self-purification" occurred in sewage and "antagonistic organisms" should therefore have been abundant. He identified, in 1945, one isolated fungus as a species of cephalosporium. Because of the inadequate facilities available locally he was able to carry out only a limited investigation on which he reported in 1948. A culture of the fungus was then sent to Sir Howard Florey at Oxford University who had played an active role in penicillin research.

A detailed examination of all the antibiotics produced by this cephalosporium sp. was started by E.P. Abraham and G. G. F. Newton[2], also of Oxford University. In 1955 they isolated from cultures of a mold called cephalosporium acremonium a new antibiotic substance cephalosporin C [1] whose structure[3], determined by the same authors in 1961 and confirmed by X-ray studies of Prof. Dorothy Hodgkin[4], turned out to be closely related to that of the penicillins [2].



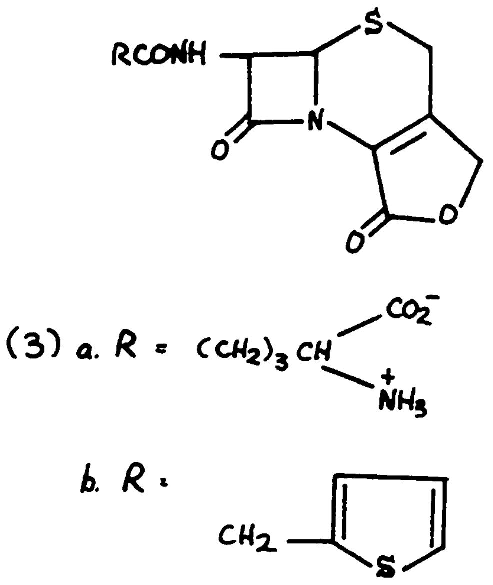
(1) Cephalosporin 'C'



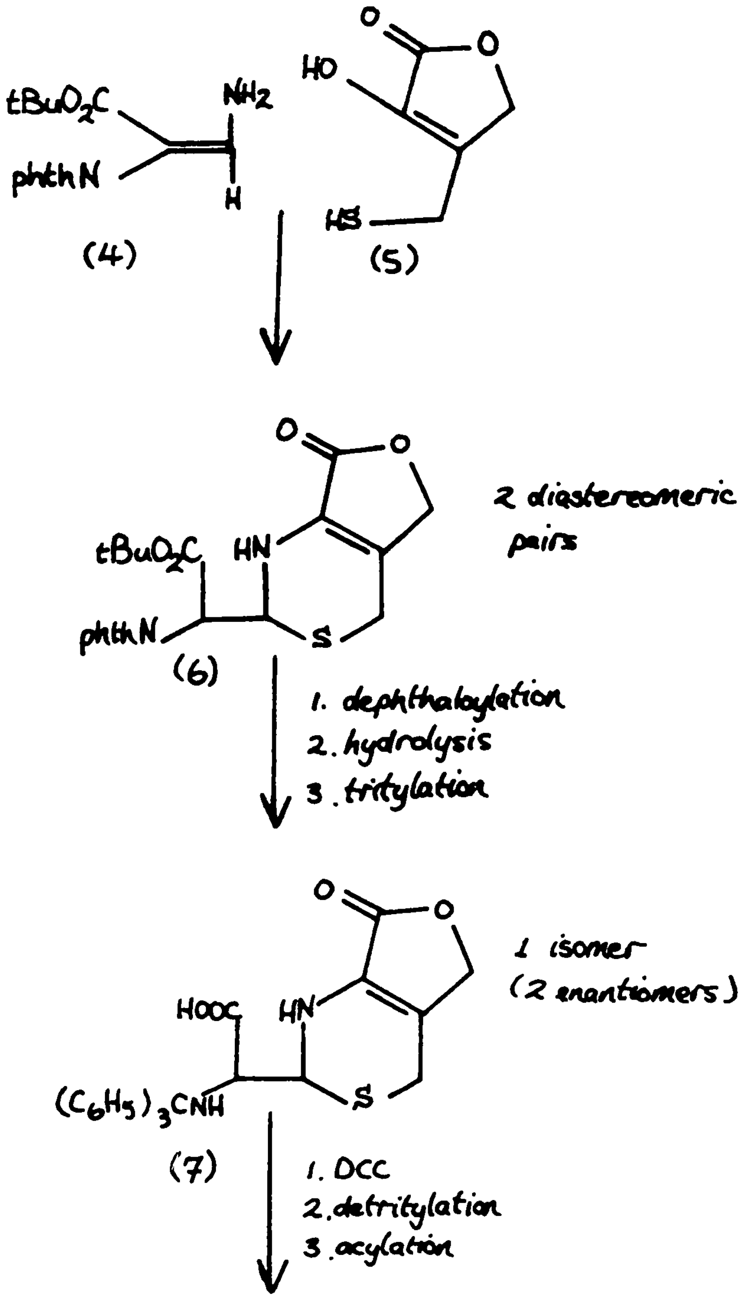
(2) Penicillin 'G'

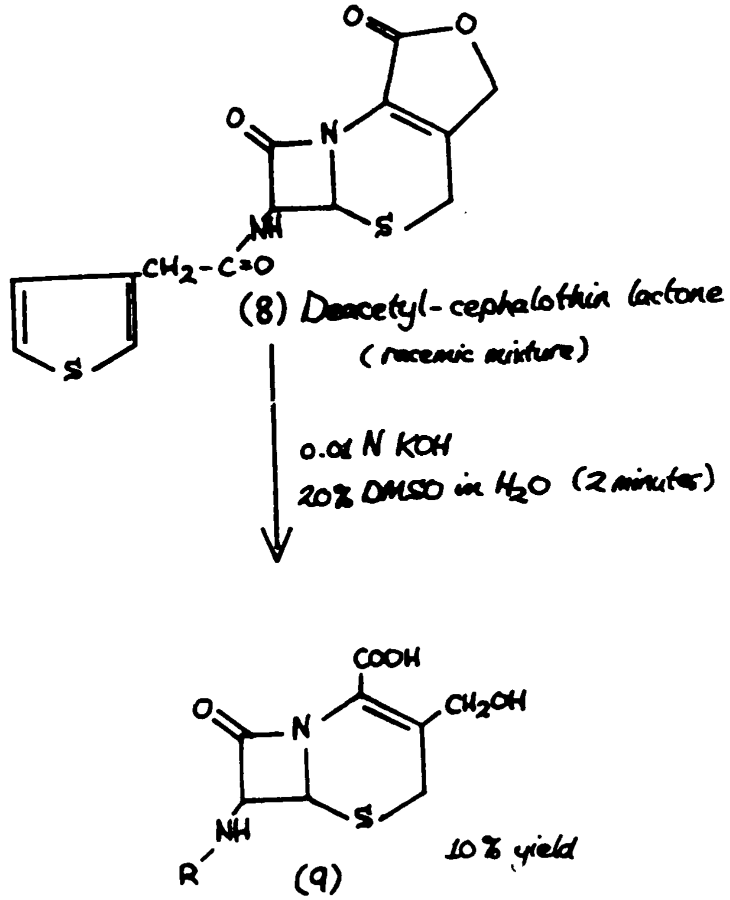
The total synthesis of penicillins proved to be a lost cause for the synthetic chemists mainly because microbiologists had been able to train micro-organisms to produce penicillins so efficiently in a single fermentation step that a multi-step chemical synthesis could not compete. The situation with respect to the related cephalosporins seemed more in favor of the synthetic chemists. Micro-organisms were less cooperative in producing high yields of cephalosporin C. An efficient and simple total synthesis, aiming directly at pharmacologically interesting derivatives of cephalosporin C, seemed to be a worthwhile enterprise.

A successful total synthesis of a derivative of cephalosporin C_c [3a], which has no biological activity, has been reported in 1966 by a French team of scientists[5] (Heymès, Amiard and Nominé). Their work was the culmination of attempts by several groups to apply Sheehan[6] and Henery-Logan's approach to penicillin to the synthesis of cephalosporin C_c [3b].



A major advantage in choosing this unsaturated γ-lactone as the synthetic goal is that the carboxy and the hydroxy groups in the dihydrothiazine ring are protected and the double bond is immobilized. The general scheme is depicted on the following page. In the condensation of [4] and [5], two isomers of structure [6] are formed. It is claimed [5] however that after the removal of the N-protecting group the two isomeric tertiary butyl esters give on acid treatment and tritylation one single amino acid [7]. Closure of the β-lactam ring, removal of the trityl group and acylation with thiophene acetyl chloride finally produced deacetylcephalothin lactone [8]. This synthesis suffers from a grave disadvantage. Up to the present there has been only one method to open the lactone ring without destruction of the sensitive β-lactam ring. In 1969, Neidleman and Dolfini [7] managed to obtain a 10% yield of the deacetylcephalothin [9] from the corresponding lactone. This hydroxy acid could be converted only with great difficulty to cephalothin [8].


tBuO$_2$C
NH$_2$
phthN
H
(4)
HO
O
O
HS
(5)
O
O
tBuO$_2$C
HN
phthN
S
(6)
2 diastereomeric pairs
1. dephthaloylation
2. hydrolysis
3. tritylation
O
O
HOOC
HN
(C$_6$H$_5$)$_3$CNH
S
(7)
1 isomer (2 enantiomers)
1. DCC
2. detritylation
3. acylation



(8) Deacetyl-cephalothin lactone

(racemic mixture)

0.01 N KOH

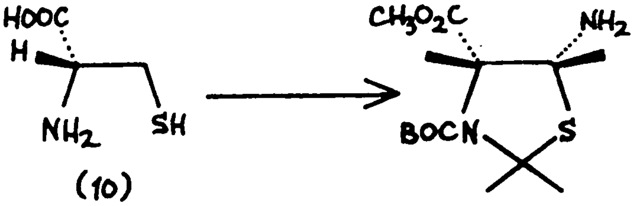
20% DMSO in H_2O (2 minutes)

(9)

10% yield

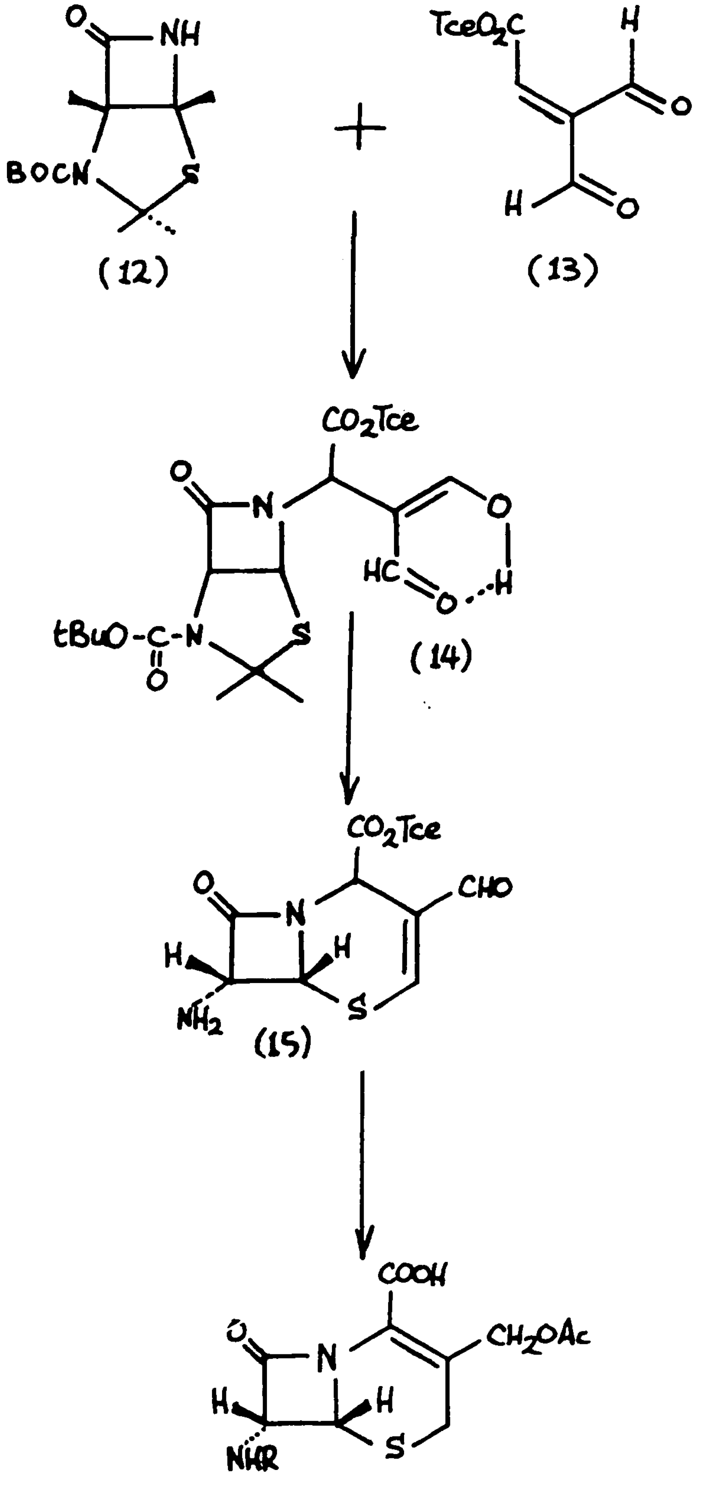
S.L. Neidleman & J.E. Dolfini (1969)

The only successful total synthesis of cephalosporin C and cephalothin was achieved by R. B. Woodward[9] at the Woodward Research Institute in Basel in 1966. The synthesis is depicted on the next scheme. The starting point of the synthesis was L-cysteine [10], ensuring thereby the correct absolute configuration at C-7. The amino group in the β-position to the carbonyl, required for completing the β-lactam ring was introduced stereospecifically in a novel fashion. The cis aminoester [11] was transformed to the β-lactam [12]. Condensation of [12] with the malonaldehyde derivative [13] produced an intermediate [14] which afforded the dihydrothiazine-β-lactam [15] on deblocking the thio function. Modification of the appropriate substituents led to the natural antibiotic [16] without the need of optical resolution.



(10)

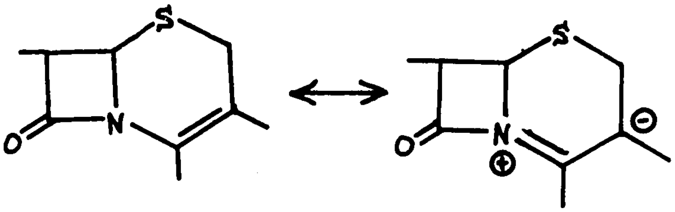
(11)



(16) *Cephalosporin*

Several other attempts have been made to synthesize cepham derivatives, without much success. Several reviews [10-20] have dealt in detail with these approaches and extensively with the chemistry and biological activity of the cephalosporin group. Thousands of semisynthetic cephalosporins have been synthesized in an effort to find antibiotics with superior antimicrobial and pharmacological properties [21].

Some interesting work published by Sweet and Dahl [19] in 1970 can be briefly discussed at this point, to answer the question of why the β-lactam of the cephalosporins is so susceptible to ring opening. Enamine resonance (see diagram below) plus the lack of planarity of the nitrogen atom combine to decrease the amide resonance in the lactam amide bond and therefore the carbonyl group is more susceptible to hydrolysis.



Extensive studies have shown that the biological activity of the cephalosporins and the penicillins increase as the β-lactam carbonyl stretching frequency in the ir increases (ie., less electron delocalization). It increases as the nitrogen planarity decreases and as the ease of basic hydrolysis of the lactam amide bond increases. The carbonyl stretching frequency for the β-lactam carbonyl in penicillin G, and cephalosporin C is about 1785-1790 cm^{-1} compared with 1775-1780 cm^{-1} in 2-deacetoxy-cephalosporin and approximately 1765-1770 cm^{-1} in the Δ^2-isomers of the latter [19a].

In the case of the biologically inactive Δ^2-cephalosporins where there is less ring strain to force the lactam nitrogen to a nonplanar configuration and no possibility for electron delocalization outside the lactam ring, the β-lactam amide bond is more like a free amide and thus more stable to hydrolytic cleavage.

The impression should not be left that the cephalosporins are affective against all infectious diseases. None of the virus diseases, like influenza, measles, chicken pox, herpes or mumps, is affected by this drug. None of the protozoal and parasitic diseases, such as malaria, African sleeping sickness, dysentery or schistosomiasis, can be cured with cephalosporins. Fungal infections, like ringworm, athlete's foot,or histoplasmosis, do not respond to its administration. They are not useful for the treatment of a number of important bacterial diseases, including tuberculosis, cholera, brucellosis and bubonic plague, or the rickettsial diseases, such as typhus fever. The list of diseases that are successfully treated with cephalosporins is quite impressive however. These are the drugs of first choice for treating the respiratory diseases, like pneumonia, scarlet fever, sore throat, and ear infections; for rheumatic fever and many infections of the urinary tract; for wound infections, boils, abscesses and gas gangrene; for meningitis and tetanus; and for syphilis and gonorrhea. There are three clinically available derivatives of cephalosporin C. The Glaxo-Allenburys Pharmaceutical Company markets cephaloridine (Ceporan),7-(2-thienyl) acetamido - 3(1-pyridylmethyl) - cephem-4-carboxylic acid betaine. Lilly produces sodium cephalothin (Keflin) and and orally active derivative cephalexin (Keflex). The latter is 7-(D-α-amino- α-phenylacetamido)-3-methyl-3-cephem-4-carboxylic acid.

The intriguing characteristics of cephalosporin are that [1] like penicillin, it is non-toxic; [2] it is more acid stable than penicillin; [3] more chemical variations are possible; [4] it has good gram-positive and some gram-negative antibacterial activity; [5] it is active against penicillin-resistant staphylococci; and [6] it is less liable to cause allergic reactions (probably the greatest advantage over the penicillins). Penicillins and cephalosporins seem to have similar modes of action, interfering with cell wall synthesis[19,22].

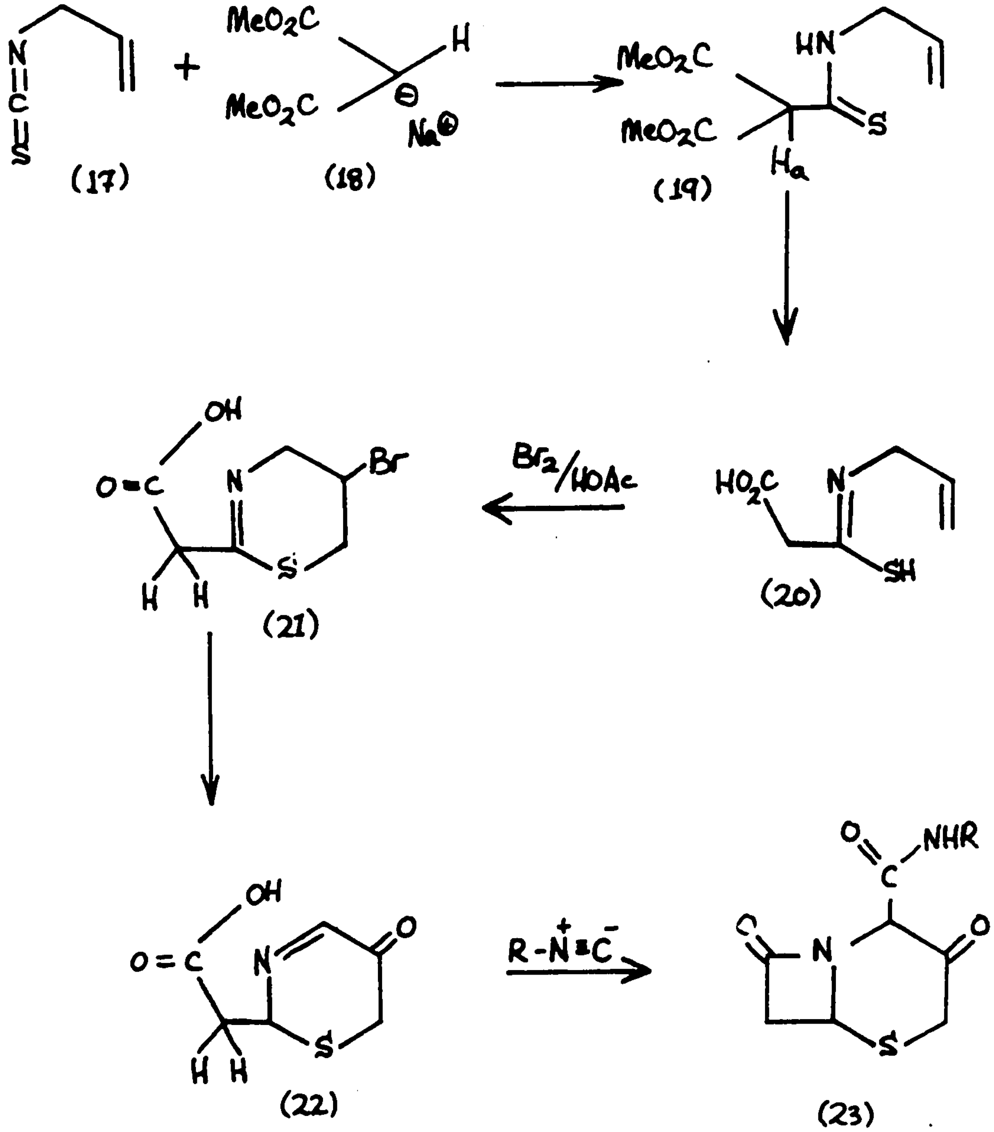
The objective of this work was to devise a novel and adaptable scheme for the synthesis of cephalosporin derivatives, using inexpensive and readily available starting materials.

RESULTS AND DISCUSSION

CHAPTER I

The Action of Hydrazine and its Derivatives on the Addition Products of Allyl Isothiocyanate and Dimethyl Malonate [27].

The early attempt at the synthesis of the cephalosporin skeleton was based on some work done by David Worrall [28] in 1932 (see following page). Carbomethoxy methyl malonate monothioallylamide [19] was prepared by condensing the sodium salt of dimethyl malonate [18] and allyl isothiocyanate [17]. Worrall treated the diester [19] with hydrazine and claimed he obtained the acid [20] in 20% yield. He treated this substituted thioamide [20] with bromine in acetic acid and got the dihydrothiazine [21]. This compound would have been the starting material for the synthetic scheme. Oxidation of [21] and isomerization of the double bond would have given the α, β-unsaturated ketone [22]. At this point, treatment of [22] with an isonitrile (Ugi reaction) would have afforded a compound [23] having a cephalosporin skeleton.

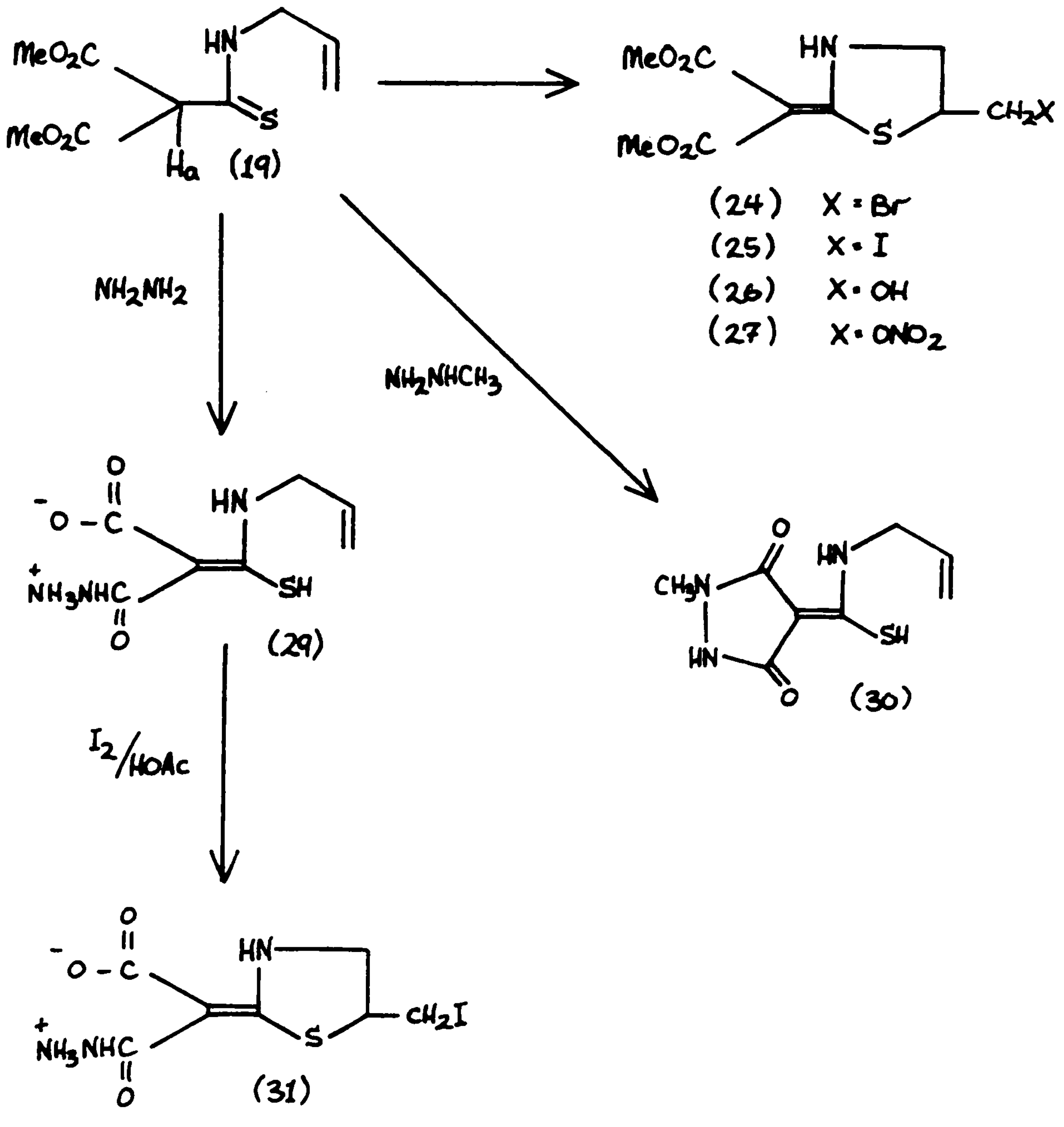

N
C
S
(17)
+
MeO2C
MeO2C
H
Na
(18)
MeO2C
MeO2C
HN
S
Ha
(19)
HO2C
N
SH
(20)
Br2/HOAc
OH
O=C
N
Br
S
H H
(21)
OH
O=C
N
O
S
H H
(22)
R-N≡C
O
NHR
C
O
O
N
S
(23)

Attempts to repeat the work of Worrall led to problems. Condensation of dimethyl malonate and allyl isothiocyanate gave [19] (see following page). Hydrazinolysis of [19], followed by reaction with hydrochloric acid, did not, however yield [20], mp 120-121°, as described, but the hydrazide [29], mp 120-121°. No attempt was made to differentiate between the two possible geometric isomers.

The structure of [20] had been based on its sulfur analysis (Calcd for $C_6H_9O_2NS \cdot 2H_2O$: S, 16.4. Found: S, 16.4), which is close to that of [29] (S, 15.95). Microanalysis indicated formula $C_7H_{11}O_2N_3S$ rather than $C_6H_{13}O_4NS$. The nmr spectrum of [29] showed bands characteristic of the allyl group, which were in the same position as in the starting material [19], one low-field exchangeable proton at 10.1 ppm, and a broad band (5H), the position of which was concentration dependent ($NH\text{-}N^+H_3$, NH, and SH). The major feature of the infrared spectrum was a very broad band at 3500-2300 cm^{-1}, typical of an amine salt.

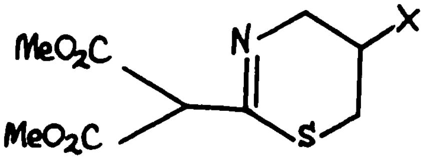
Reaction of [29] with iodine gave, as described, a compound, mp 213-214°, which does not have the structure [21] (Br = I), but [31]. The proof of structure for the five-rather than six-membered ring will be discussed later.

In order to confirm the structure of the hydrazinolysis product [29], the diester [19] was treated in an analogous manner with methylhydrazine. The unstable product obtained appeared to have the cyclic structure [30], as evidenced by its elemental


MeO2C
MeO2C
HN
S
Ha
(19)
NH2NH2
NH2NHCH3
HN
CH2X
(24) X = Br
(25) X = I
(26) X = OH
(27) X = ONO2
O
-O-C
HN
+NH3NHC
SH
(29)
CH3N
HN
SH
(30)
I2/HOAc
HN
CH2I
S
(31)

analysis and nmr spectrum, which indicated the presence of an N-methyl group (δ 3.2 ppm, singlet), an allyl group, and only three exchangeable protons.

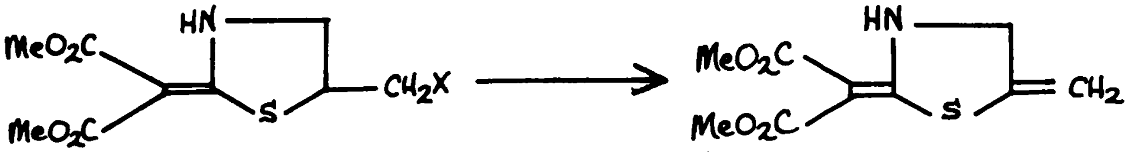
Bromination or iodination of [19] led to the thiazolidine [24,25] and not the dihydrothiazine [32] as had been described by Worrall.



(32) X = Br or I

Worrall suggested an intermediate dibromo compound. It has been shown [29-31] in analogous cases that the intermediate is a bromonium ion and the mechanism involves an ionic intermediate. The product of this type of cyclization is usally the five-membered and not the six-membered ring [32-35], even if the carbonium ion leading to the six-membered ring is more favored [29-30].

Careful integration of the signal at 4.7 ppm in the nmr spectrum of the nitrate [27] indicated the presence of two protons, rather than one, thus favouring the thiazolidine structure. Reduction of the iodide [25] with palladium on charcoal gave a compound which showed the presence of a methyl group (nmr, doublet at 1.4 ppm). Finally, treatment of [24] with diethylamine led to a compound [28] having a terminal methylene group (3030, 1645, 870 cm^{-1} in the ir spectrum). Similar results were obtained for the hydrazido-acid [29], thus ruling out the possibility of the six-membered ring.



(24) X = Br

(28)

Treatment of the bromide [24] or iodide [25] with alcoholic silver nitrate did not give the corresponding alcohol [26], as had been described, but the nitrate [27] (diphenylamine and sulfuric acid)[36]. The nmr and mass spectral data and elemental analysis were consistent with the structure.

It appears that the position of the double bond in [24] and [25] was exocyclic and not endocyclic [32], as had been described. The assignment for the position of the double bond was based mainly on the nmr spectra of [24] and [25], which did not show the low field proton H_a of compound [19] (δ 5.6 ppm singlet), but indicated the presence of a typical N-H proton at 3.0-6.0 ppm (broad band). In addition, [24] and [25] absorbed at longer wavelength [λ_{max} (EtOH) 285 nm (ε 12,500)] than [19] [λ_{max} (EtOH) 274 nm (ε 13,400)].

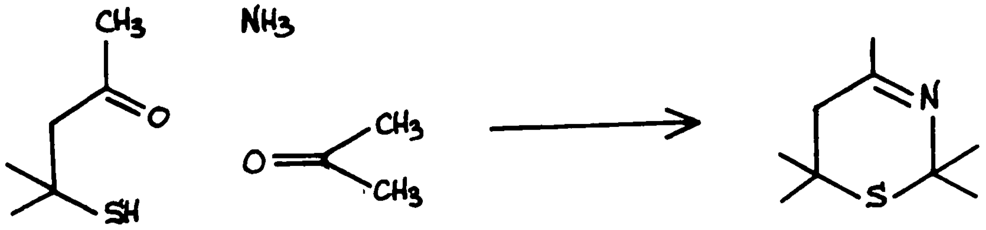
The position of the double bond in [29] and [30] was based on the fact that both compounds form a cuprous salt (thiol) [37], and both showed a secondary amine absorption in their ir spectra. No experimental work was done to find out why reaction of [19] with hydrazine and methylhydrazine gave the hydrazido acid [29] and the pyrazolidinedione [30], respectively.

Although the chemistry of these heterocyclic compounds was interesting, we abandoned this approach, since it did not lead to the desired six-membered heterocycle.

CHAPTER 2

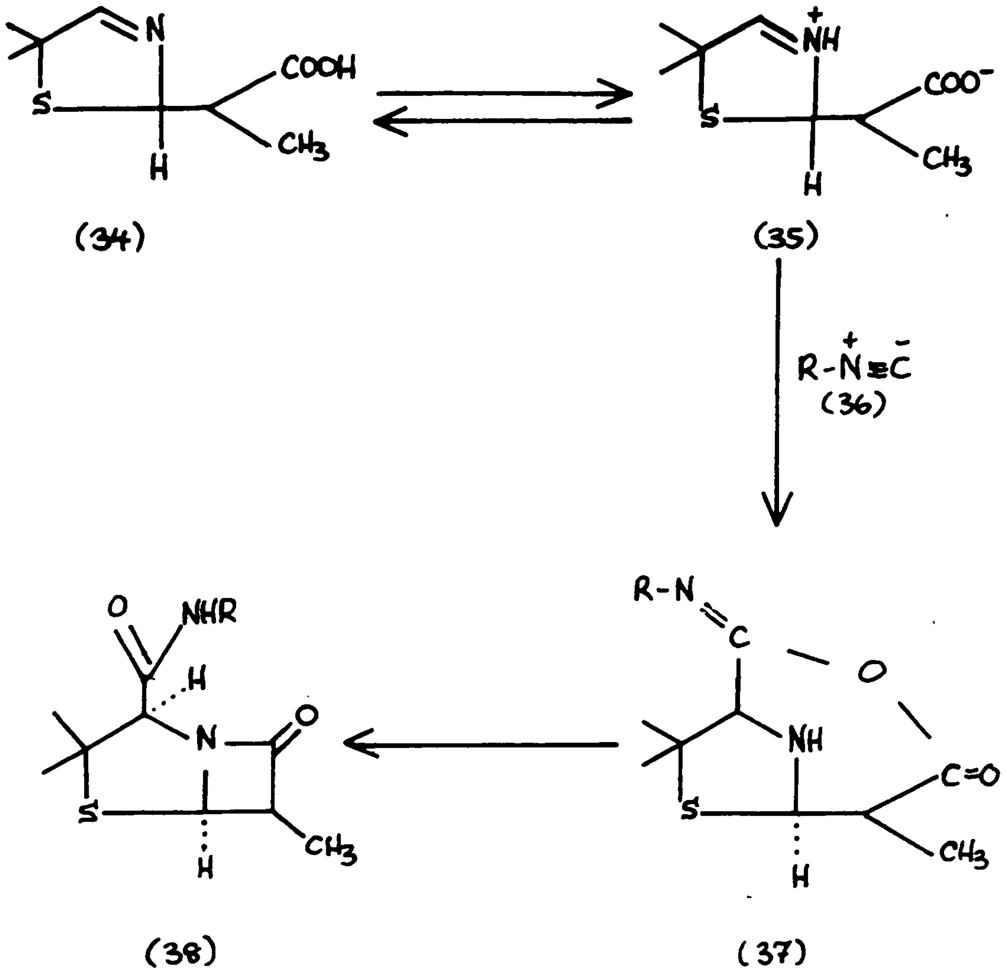
The Asinger Cyclization and Ugi Reaction

In the 1950's, F. Asinger [38] and his co-workers prepared Δ^3-dihydrometathiazine derivatives by condensing β-mercapto ketones, ammonia and an aldehyde or ketone component. For example Δ^3-2, 2, 4, 6, 6-pentamethyldihydrometathiazine [33] was prepared from acetone, diacetone mercaptan and ammonia.



(33)

This type of condensation product can be converted to a penicillin analogue of wrong stereochemistry by means of an Ugi reaction [23-26]. Compound [34] is reacted with an isonitrile [36] in a two-phase mixture of water and petroleum ether. If the reaction is carried out in a single organic phase, the formation of resins predominates. In aqueous solution, [34] is in equilibrium with the zwitterion [35], which adds to the isonitrile to form the bicyclic adduct [37]. The latter is converted into [38] by transannular acyl migration. It follows from this mechanism that the reaction is stereospecific and that the carbonamide group and the β-lactam are in cis relation. The yields range from 17% [39] to 57% [40]. A possible explanation for the relatively low yields and formation of resins is that since a three-fold excess of isonitrile is used, one can envisage the attack of the isonitrile on the imino anhydride [37] affording undesired reactive side-products, which could lead to polymer formation.



(39) $R = c\text{-}C_6H_{11}$
(40) $R = i\text{-}C_3H_7$

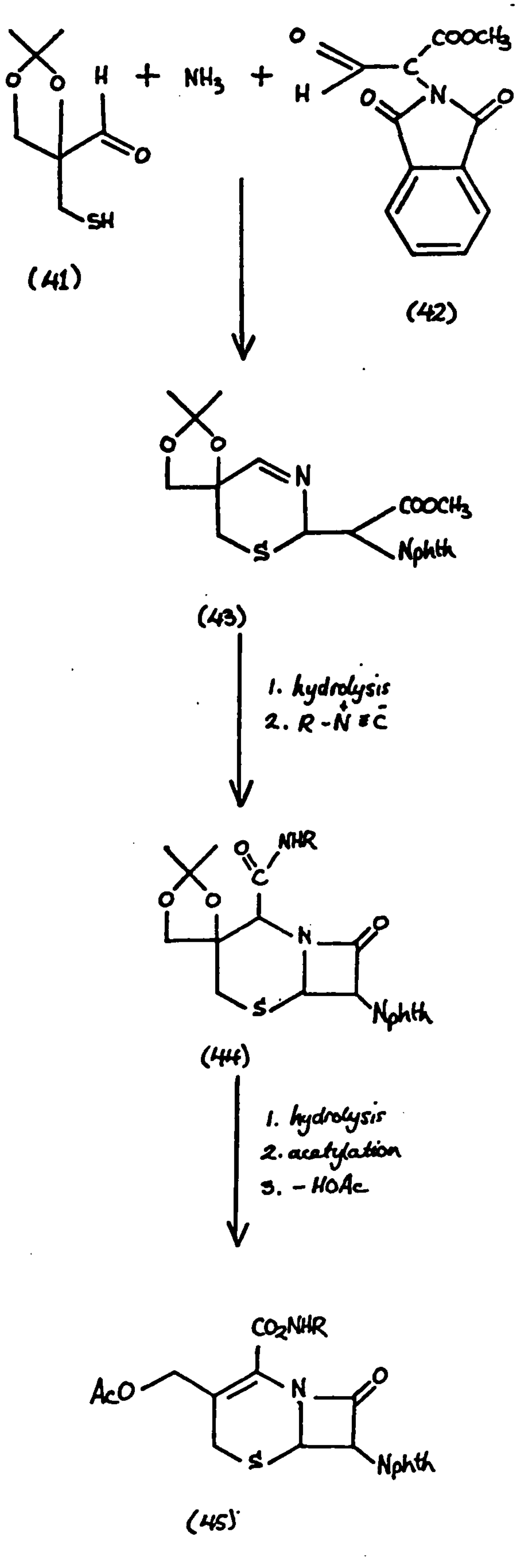
These reactions suggested a new approach to the synthesis of β-lactam antibiotics of the cephalosporin class. The general proposal is depicted on the following pages.

Compound [41] and compound [42] are the proposed sub-units to be reacted together in an Asinger type condensation with ammonia. The product [43] would be an imino ester which after mild hydrolysis and reaction with an isonitrile would yield to cepham derivative [44]. Hydrolysis of the acetonide, acetylation and elimination would afford a cephalosporin amide derivative [45].

One can envisage modification of the typical Asinger condensation (to be referred to as type A) to include the ammonia function in either of the two sub-units, as depicted below. In the type A' scheme, the ammonia function is part of the right-hand sub-unit and in the type A" scheme, the ammonia is incorporated in the left-hand sub-unit. A third type of modification (type A"') would allow for the thiol-aldehyde to be condensed with the right-hand side, followed by reaction with ammonia. In the last approach, the aldehyde function could be protected first and then the free aldehyde liberated at a later stage.

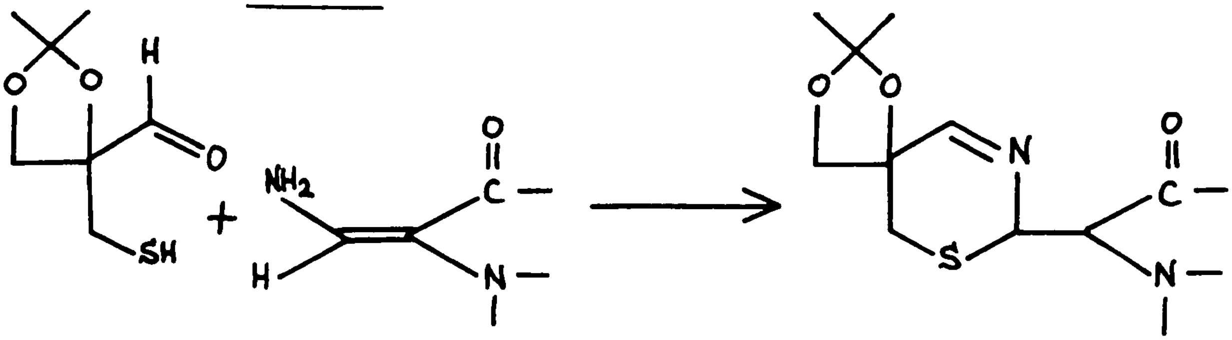
General Scheme using Asinger Ring Closure

(Type A)

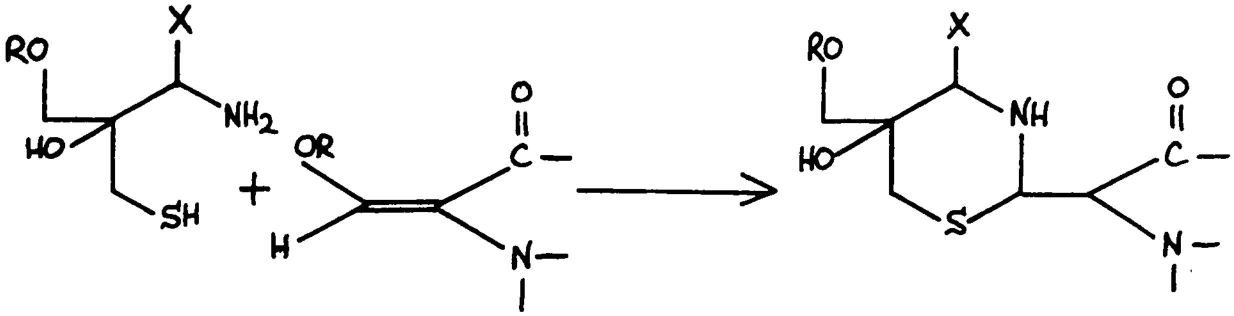


General Types of Asinger Modifications to be Attempted

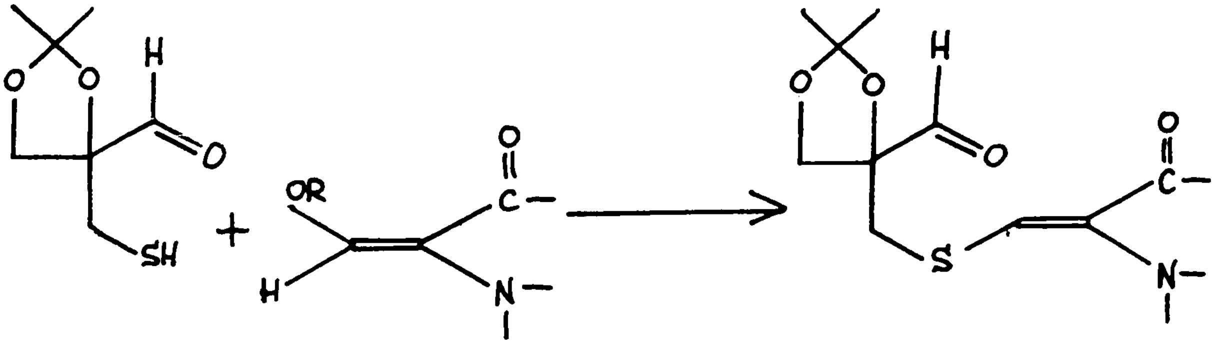
TYPE A'



TYPE A"

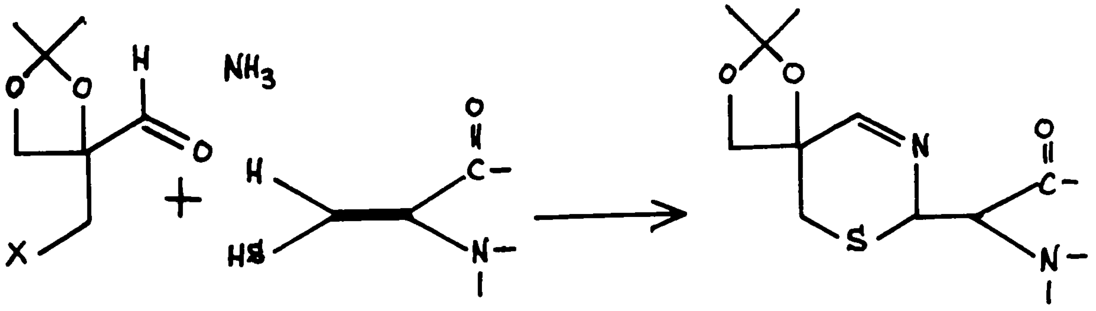


TYPE A"'



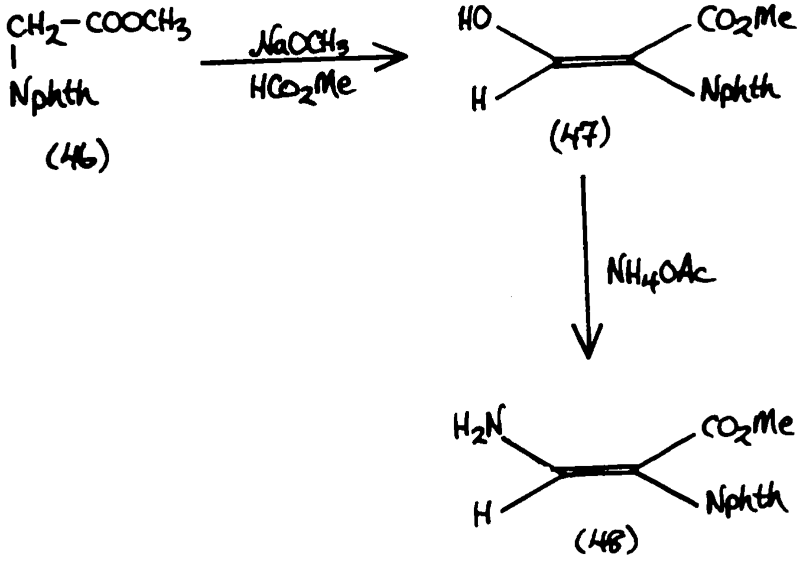
The instability of the thiol-aldehyde (A' and A'''), as will be seen later, induced us to examine other possibilities. Another modification in the general scheme was made, so as to have the thio-functionality incorporated in the right-hand moiety (see below).

TYPE B



The synthesis of a modified version of compound [42], in which ammonia is already incorporated in the form of the aminomethylene ester [48], will be discussed first.

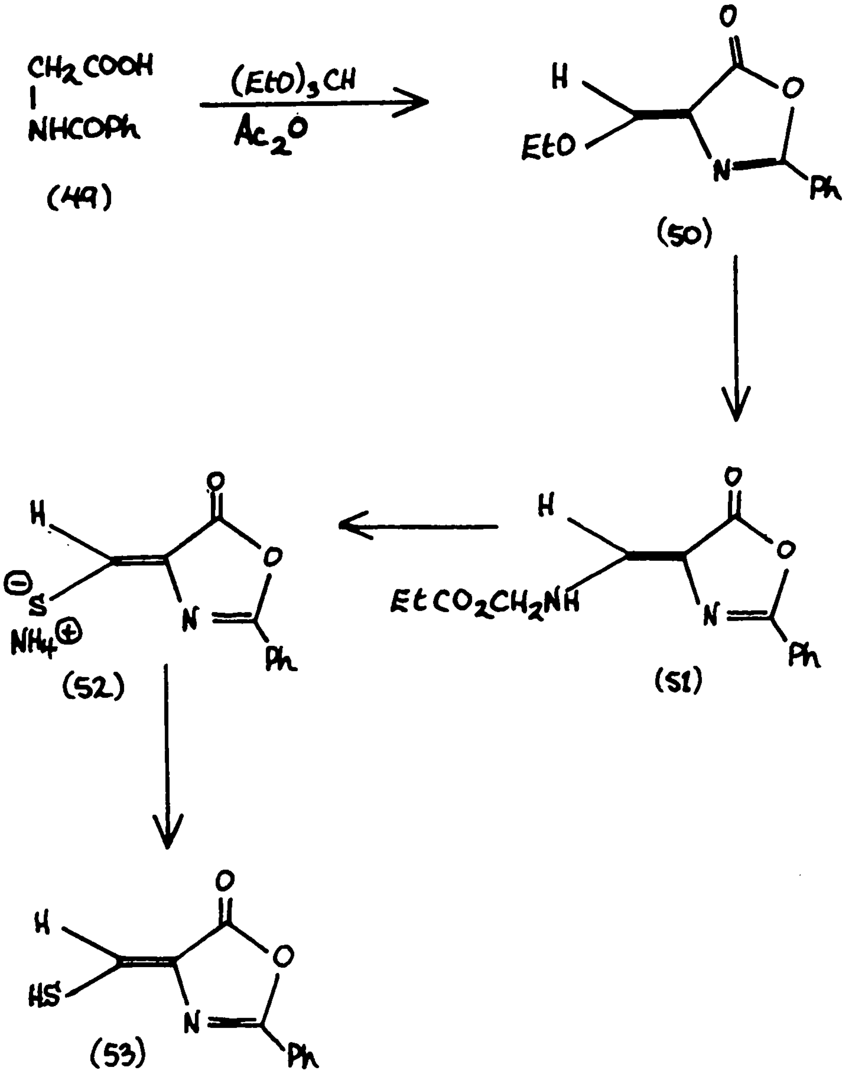
Sheehan and Johnson[39] condensed methyl phthalimidoacetate [46] and methyl formate in the presence of sodium methoxide and obtained the enolic aldehyde [47]. Heymes et al[5] reacted the aldehyde with ammonium acetate and obtained a poor yield of [48]. After much experimentation, it was found that by fusing the aldehyde with ammonium acetate at 150° for 15 minutes in a nitrogen atmosphere, crystalline [48] could be obtained in acceptable yield (50-60%).



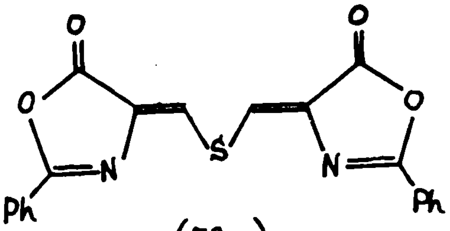
The only available right-hand sub-unit containing a thio-function (see type B reaction) was 2-phenyl-4-thiomethylene-5-oxazolone [53]. Its preparation is described below.

Hippuric acid [49] was transformed to the ethoxy-methylene oxazolone [50] using literature methods [46].

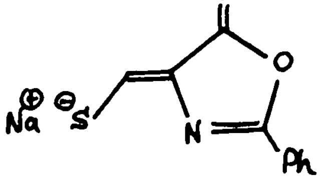
Displacement of the glycine ethyl ester derivative [51] with ammonia and hydrogen sulfide in a pressure reactor afforded the ammonium salt [52] [46] (see diagrams below). Neutralization, using hydrochloric acid gave the thiomethylene derivative [53].



The sodium salt of the thiomethylene-oxazolone was prepared by known methods [46]. Analysis of the salt showed the existence of bis-sulfide [53a] contamination, as evidenced by the ultraviolet spectrum, which showed absorption bands at 4200 and 3100 nm. It was reported by Berlin and co-workers [51] that these bands were characteristic of [53a]. By treating pure ethoxymethylene-oxazolone [50] with anhydrous sodium hydrogen sulfide in anhydrous methanol (literature procedure for the potassium salt was used [51]), pure 2-phenyl-4-mercaptomethylene-(5)-oxazolone sodium salt [53b] was prepared (85-90% yield). Hydrolysis using acetic acid in water afforded pure 2-phenyl-4-mercaptomethylene-5-oxazolone, mp 172-175°, with decomposition (lit [51] 172-176°, with decomposition). This second method gave a much purer product than the first one.

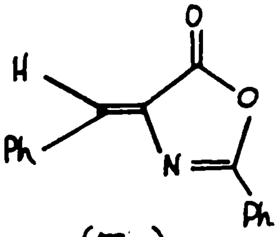


(53a.)



(53b.)

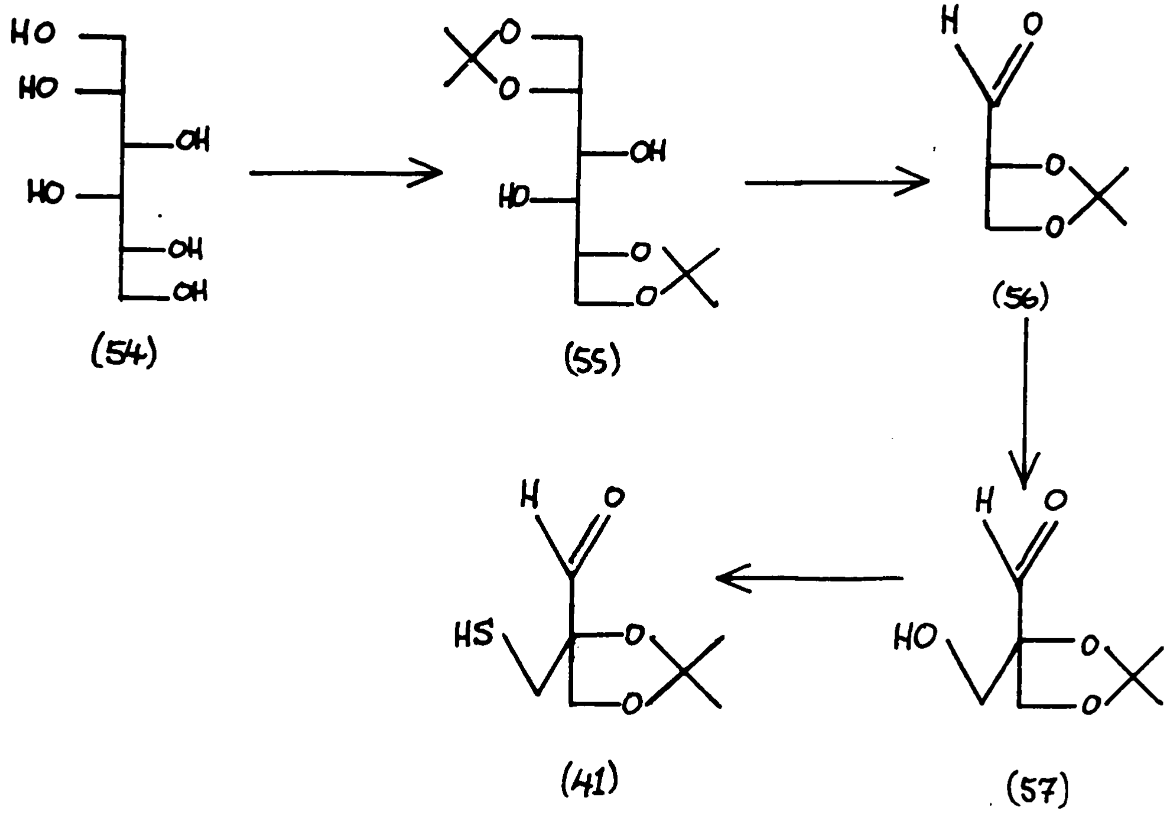
A brief discussion of the geometric configuration of the ethoxymethylene and thiomethylene oxazolones is appropriate at this stage. Brocklehurst [59] has shown, by a crystallographic analysis, that the more stable isomer of 4-benzylidene-2-phenyl-Δ^2-oxazolin-5-one [53c] has the phenyl substituent trans with respect to the ring carbonyl function. It was shown by examination of the nmr spectrum of [50] and [53] that only one isomer is formed. Since it was proven that the more stable isomer is the trans one, we can conclude, by analogy, that we have the trans isomer, as depicted in the structural formulas of these compounds.



(53c)

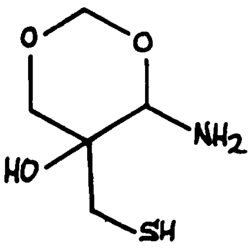
Having suitable methods for the preparation of the right-hand sub-units, attempts to synthesize the thiol-aldehyde [41] (left-hand sub-unit) was undertaken. The plan required the synthesis of glyceraldehyde acetonide, which had been already described by Fischer and Baer [40].

Mannitol [54] was transformed to its diacetonide [55] which was cleaved to glyceraldehyde acetonide using lead tetraacetate [40]. We proposed to treat compound [56] with potassium carbonate and formaldehyde in the manner of a Tollens' condensation [41,41a], to give us the aldol [57]. Treatment of this aldol with mesyl chloride and triethylamine, followed by displacement with sodium hydrogen sulfide should afford compound [41], which would have been the desired compound required for the Asinger ring closure (type A).



Difficulties arose in the conversion of glyceraldehyde acetonide [56] to the hydroxyaldehyde [57] (see scheme, page 32). A crystalline compound was obtained when [56] was treated with formaldehyde and potassium carbonate. The ir spectrum showed no evidence of carbonyl absorption and only a characteristic band at 3400 cm^{-1} for the hydroxyl group. Nmr indicated no aldehyde functionality. Structure [58] was proposed for this unknown substance. Microanalytical and spectral data were congruent with the structure. Hydroxy-dioxanes are known to arise by the action of aldehydes on aldols [42].

Since this reaction provided for the protection of the aldehyde function, it was decided to block the anomeric center at C-6, in order to cleave the acetonide and carry out transformations at C-4'. The general idea was to try to synthesize a molecule of the type I depicted below. A modified Asinger condensation of type A" would then be attempted (see page 23).

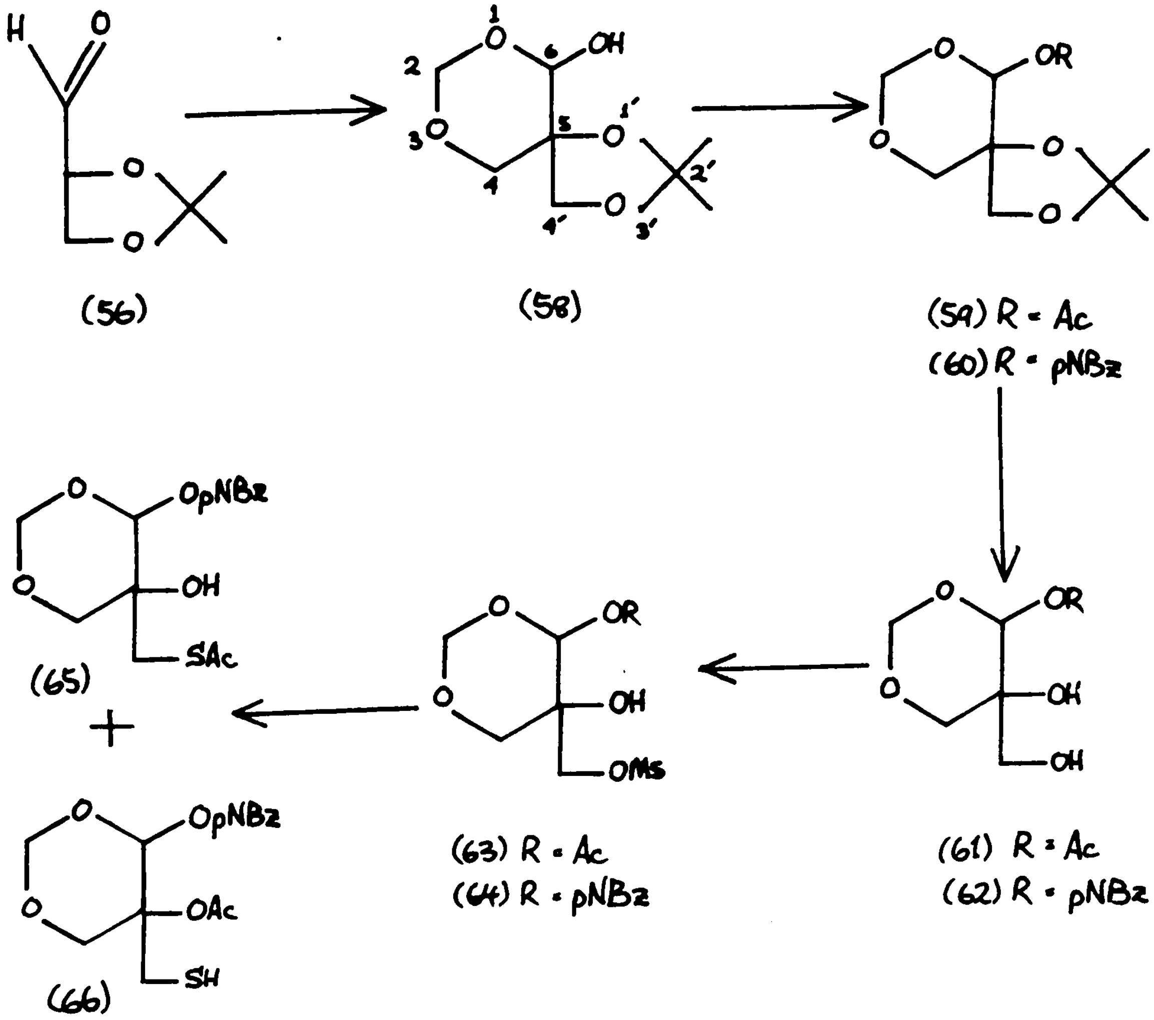


I

The acetate [59] was prepared, but was difficult to obtain in crystalline form. This was due to the fact that two isomers were present in the reaction mixture, as evidenced by the nmr spectrum which showed double singlets for the anomeric proton at 5.65 and 5.85 ppm in a ratio of 3:1. After much experimentation a method was devised to achieve selective hydrolysis of the acetonide function of the acetate [59] and the diol [61] was obtained in very good yield. The method consisted of treatment of the acetonide with 90% aqueous trifluroacetic acid for 2 minutes. The diol-acetate [61] was converted to its mesylate [63] but no further work was done, since these compounds could not be obtained crystalline.

The p-nitrobenzoate [60] was easily prepared in crystalline form, even though two isomers were obtained (double singlet at 6.3, 6.5 ppm; ratio 3:1). It is evident from the nmr that the isomer in which the p-nitrobenzoate is equatorial (axial hydrogen) is favored. Compound [60] could be converted to a crystalline diol[62] and a crystalline mesylate [64].

It has been shown [43] that mesylates and tosylates can be converted to thiols by the action of potassium thioacetate and subsequent hydrolysis of the resulting thioacetate using ammonia or a catalytic amount of sodium methoxide.



(56)

(58)

(59) R = Ac
(60) R = pNBz

(61) R = Ac
(62) R = pNBz

(63) R = Ac
(64) R = pNBz

(65)

\+

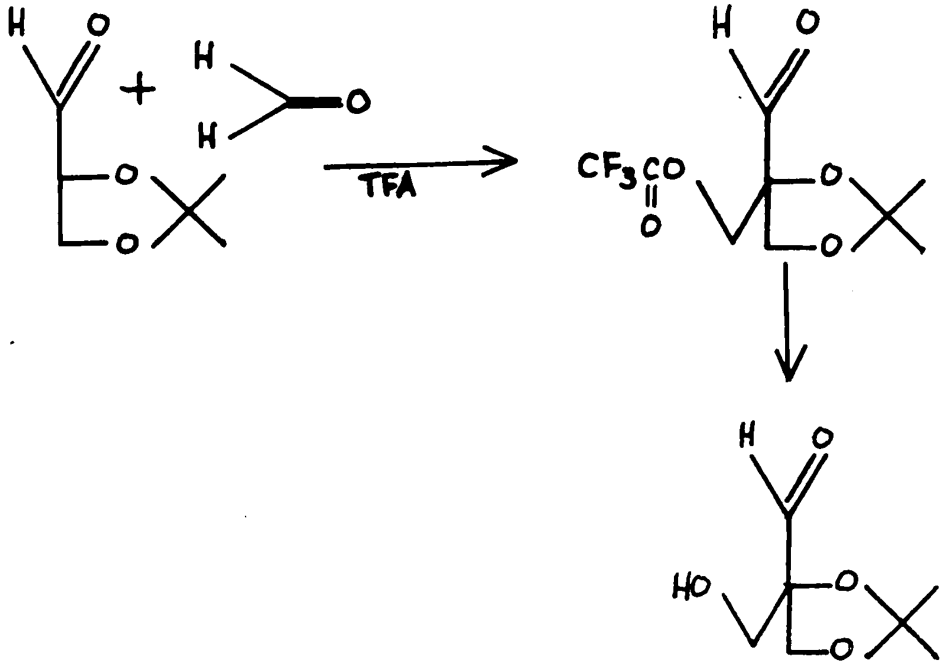
(66)

Displacement of the mesylate [64] with potassium thioacetate in acetone at 60^{o} gave a mixture which contained thioacetate [65] and thiol [66]. Because of the proximity of the thioacetate and alcohol function in compound [65], a transacetylation is not unlikely, and explains its conversion to the thiol [66]. Attempts to displace the mesylate function of [64] with sodium hydrogen sulfide gave a mixture from which pure desired thiol could not be obtained in good yield.

In a parallel series of reactions, an attempt was made to replace the p-nitrobenzoate group in [60], used as a model compound, by an amine or potential amino group. As described previously such compounds could perhaps be used in an Asinger type A" condensation (see page 23) after the appropriate modification of the acetonide group. Reaction of p-nitrobenzoate [60] with ammonia or with sodium azide led, however to the hydrolysis of the benzoate function, and subsequent further degradation of the product.

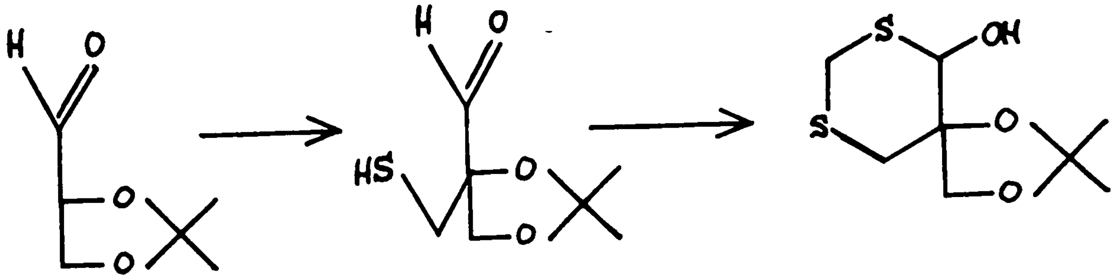
The fact that the above displacement reaction did not succeed and the fact that the thiol [66] could not be obtained in a pure state, led us to abandon the Asinger type A" approach.

It has been reported that formaldehyde reacts with aldehydes and ketones in an acid medium (trifluroacetic acid) to form β-hydroxyaldehydes or ketones [44]. This led us to attempt the following scheme:



The above reaction was followed by nmr spectroscopy using trifluroacetic acid-d_1. After a few minutes, it was noticed that the acetonide singlet at 1.4 ppm collapsed, indicating its hydrolysis. No identifiable products were recovered, probably due to dimerization or polymerization of the intermediates. The approach was therefore abandoned.

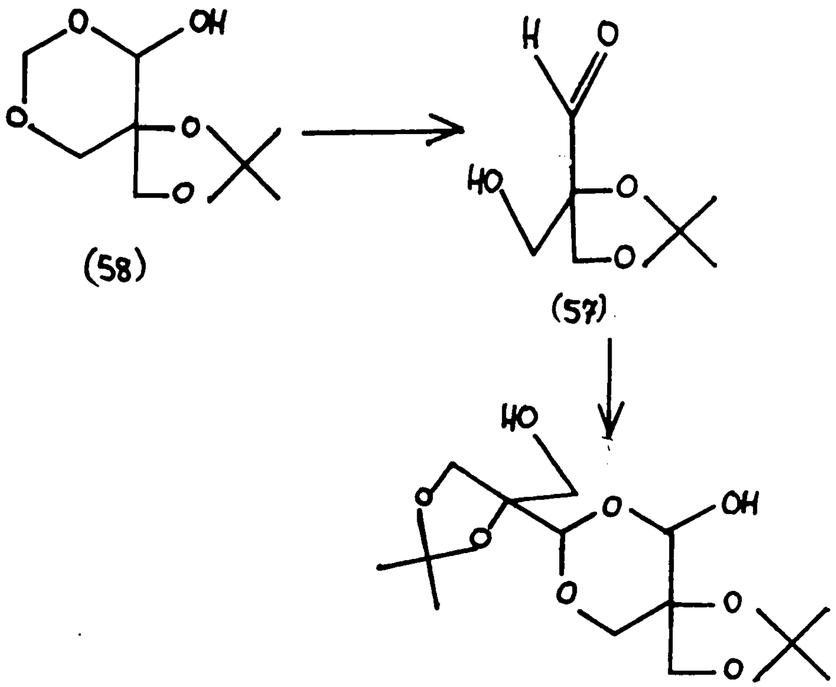
Since indirect methods to introduce a methylene thiol group failed, attempts were made to introduce that functionality in a more direct manner. Glyceraldehyde acetonide was treated with potassium carbonate, formaldehyde and hydrogen sulfide, conditions under which the formation of methanedithiol has been postulated[58]. However no useful products could be isolated from this reaction.



CHAPTER 3

Aldehyde Protecting Groups - An Interesting Development.

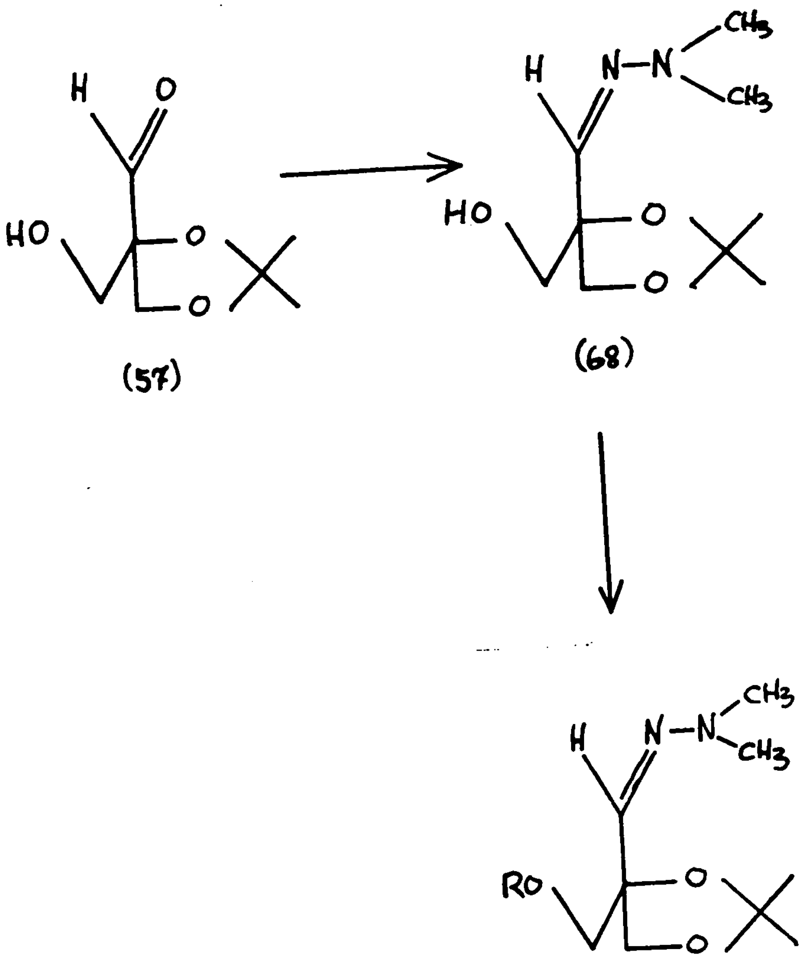
Whilst carrying out the preceding and related reactions, it was noticed that high temperature distillation of compound [58] gave the aldehyde [57] which we had wanted in the first place (see scheme p. 29). The ir spectrum indicated the presence of an aldehyde group (1730 cm^{-1}) and an absorption at 3400 cm^{-1}, typical of the hydroxyl group.



(58) (57) (67)

The nmr spectrum was consistent with the proposed structure [57]. Upon standing at room temperature for a few hours, the hydroxyaldehyde became very viscous and the carbonyl absorption decreased considerably in the ir spectrum. Aldols are known [42] to polymerize or dimerize on standing and give inter alia compounds of the type [67].

It was thus necessary to protect the aldehyde function and then try to convert the β-hydroxy group to a thio-functionality. Removal of the protecting group would afford a thiol aldehyde which could be used in the Asinger type A' or A''' condensations (see page 23). The first derivative to be considered was a N, N-dimethylhydrazone. They are known to be hydrolysed [45] rapidly by treatment with 95% ethanol after quarternization with methyl iodide. The aldol [57] was treated with dimethylhydrazine in ethanol and a 75% yield of [68] was obtained. It was easily converted to its acetate [69c], tosylate [69b] and mesylate [69a]. However, attempted displacement of the mesylate group with potassium thioacetate or sodium hydrogen sulfide gave intractable materials, presumably because of intramolecular cyclization. It was shown by an nmr study that the tosylate decomposed on standing in methanol for a few hours. No attempts were made to isolate the several products of the deterioration of this compound.



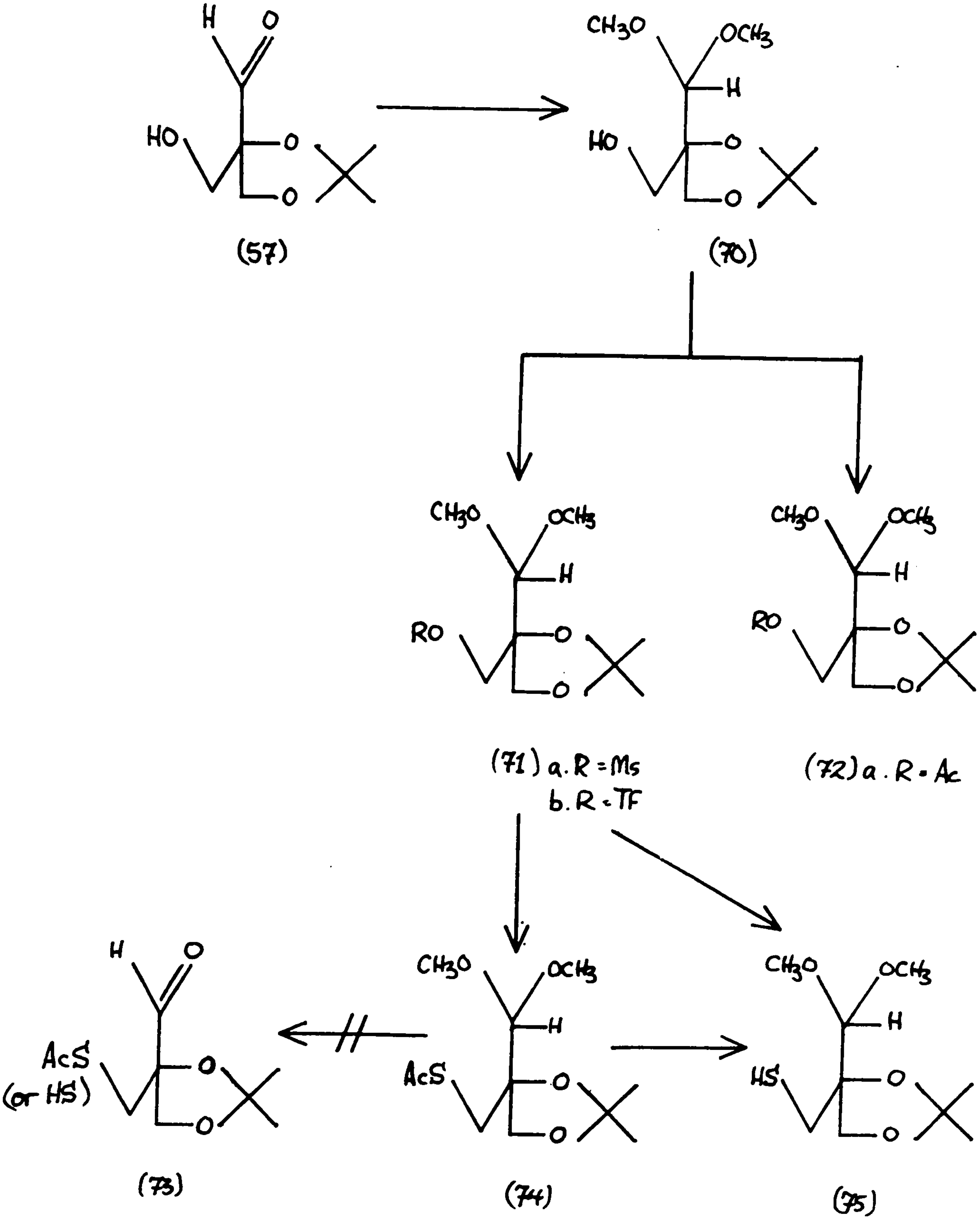
(57)

(68)

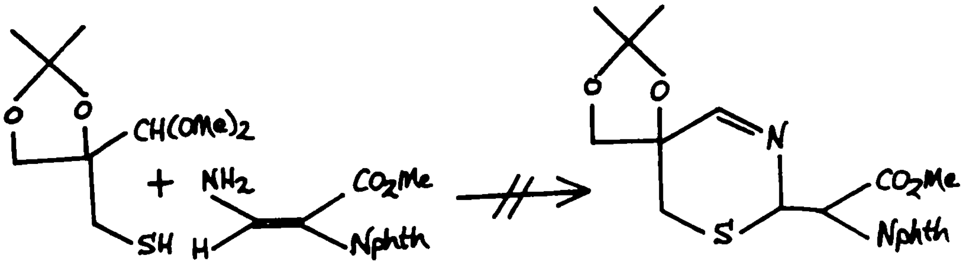
(69) a. R = Ms
b. R = Ts
c. R = Ac

Next, the aldehyde function was protected as its dimethylacetal [70]. Conventional methods for the preparation of acetals (ie., alcohol and trace of acid using a water separator) gave tarry materials. The only method which gave a clean product consisted of a room temperature reaction of [57] in a large excess of anhydrous methanol and a trace of p-toluenesulfonic acid. The water formed in the reaction was eliminated by the use of molecular sieves. The best yield obtained was 60-65% of distilled product.

Compound [70] was converted easily to its acetate [72a], mesylate [71a] and trifluoromethanesulfonate[49] (triflate) [71b]. Displacement of the mesylate was accomplished by refluxing for several days with potassium thioacetate or sodium hydrogen sulfide in an acetone or methanol solution. Displacement of the triflate group in [71b] could be carried out in a few hours, giving a good thioacetate [74]. The thiol [75] could be obtained directly from the triflate [71b] by reaction with sodium hydrogen sulfide or by hydrolysis, using sodium methoxide, of the thioacetate [74].

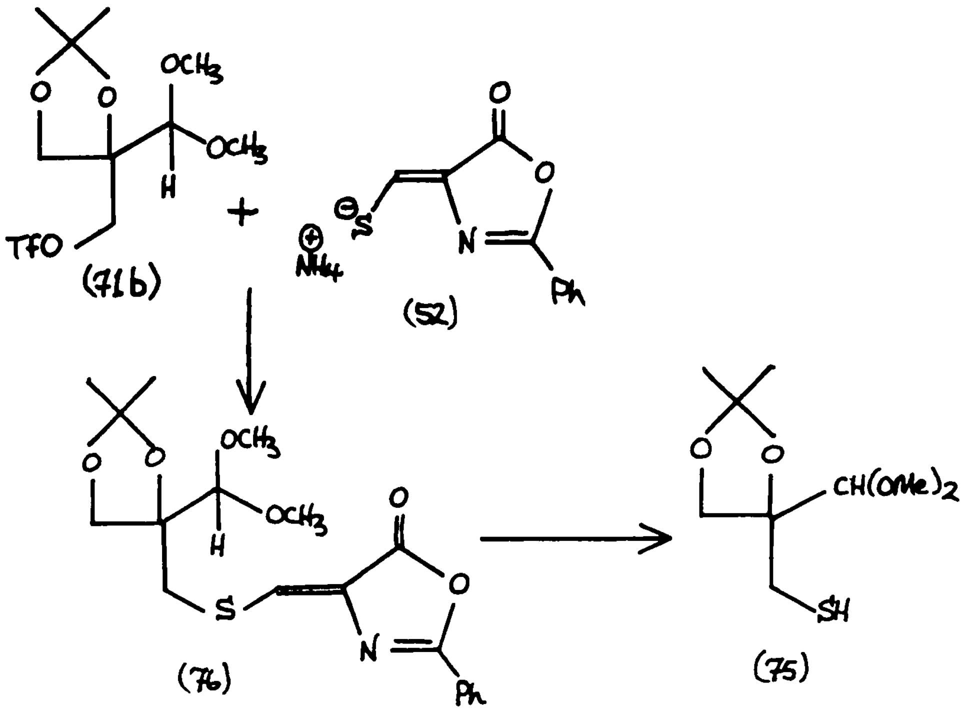

H
O
HO
O
O
(57)
CH3O
OCH3
H
HO
O
O
(70)
CH3O
OCH3
H
RO
O
O
(71) a. R = Ms
b. R = TF
CH3O
OCH3
H
RO
O
O
(72) a. R = Ac
H
O
AcS
(or HS)
O
O
(73)
CH3O
OCH3
H
AcS
O
O
(74)
CH3O
OCH3
H
HS
O
O
(75)

Attempts to condense the thiol [75] with compound [48] in an effort to obtain the required heterocycle resulted only in recovery of the starting materials (see Asinger type A' condensation, page 23).



(75) (48)

We therefore tried an Asinger type B condensation (see page 24) using the triflate [71b] and the azlactone [52]. This reaction was followed in acetone-d_6 by nmr spectrocopy. The signal due to the methylene protons adjacent to the triflate (4.7 ppm) shifted upfield by 1.5 ppm to 3.2 ppm, indicating that the triflate had been quantitatively displaced by the thiol, to give the sulfide [76].

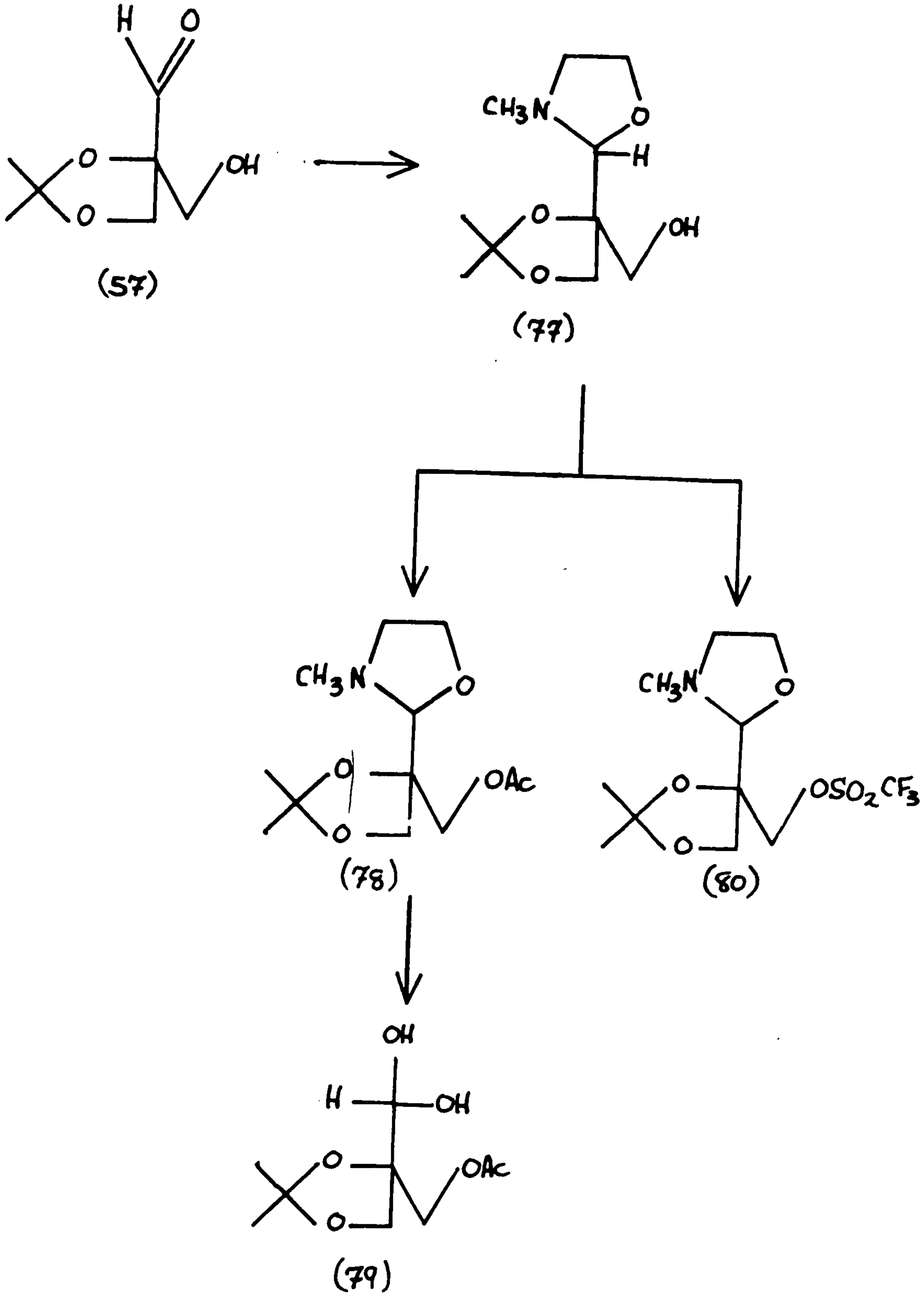


The crude sulfide [76] was treated with hydrochloric acid in order to hydrolyse the methoxy groups of the acetal function. The reaction was monitored by tlc and when no starting material was left, the new product formed was isolated. Spectral data and comparison with an authentic sample indicated that the product had structure [75].

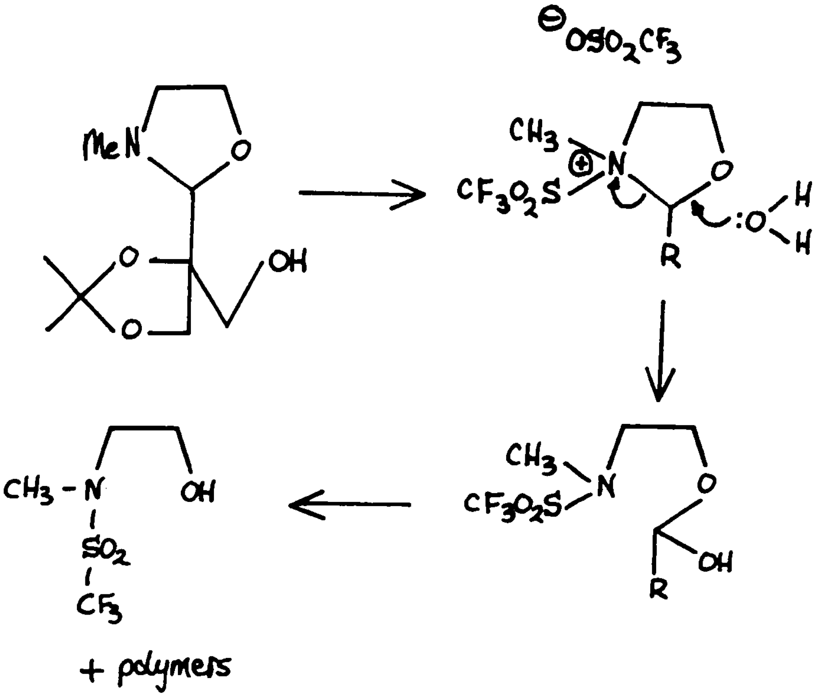
This approach was dropped when it was realized that attempts to selectively hydrolyse the acetal function of [74] or [75] (used as models) to [73], using the usual hydrolytic methods (80% acetic acid, 10% hydrochloric acid, 2% sulfuric acid and 10% aqueous oxalic acid at room temperature) led to hydrolysis of both the acetonide and acetal groups. No selectivity was evident and all the above mentioned conditions led to polymerization products.

Transketalization is a very effective method in removal of ketals. Only the starting material was recovered when [74] was treated at room temperature with acetone and a trace of p-toluenesulfonic acid for prolonged periods of time. The reaction was followed by nmr using acetone-d_6 as solvent. Exchange of the acetonide function by a deutero-acetonide group was observed, but there was no change in the methoxy absorption.

Kjell Sjoberg[47] brought to our attention the possibility of using oxazolidines[52] as protecting groups for aldehydes. It was decided to investigate this protecting group. Treatment of the hydroxyaldehyde [57] with N-methylethanolamine in diethyl ether afforded the oxazolidine [77]. Examination of the product using vpc analysis showed the existence of one major component and an impurity of approximately 10%. Distillation did not remove the impurity. Purification on a silica column led to destruction of the protecting group. Filtration on alumina, using benzene as eluent, afforded pure [77] in an overall yield of 55% which could be converted to acetate [78] in quantitative yield. A study was made to find out the ease of hydrolysis of the oxazolidine. The hydrolysis of acetate [78] was followed by nmr spectroscopy in 50% deuterium oxide and acetic acid-d_4. The reaction was complete in 15 minutes as evidehced by the shift of the N-methyl absorption from 2.5 ppm to 2.9 ppm. Another method of hydrolysis involved a two phase system. The oxazolidine was dissolved in chloroform and to this solution was added an excess of a 50% solution of acetic acid in water. Following the diappearance of the N-methyl absorption in the nmr (due to the solubility of N-methylethanolamine in water), it was shown that the hydrolysis was complete in 1 hour at room temperature.


(57)
(77)
(78)
(80)
(79)
CH3N
OH
OAc
OSO2CF3
H

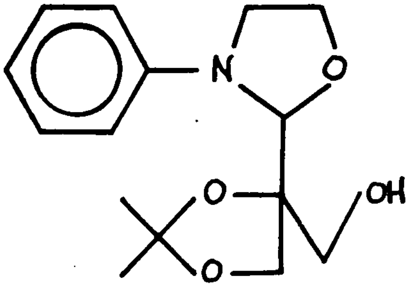
Having developed a mild method for the cleavage of the oxazolidine protecting group, it was decided to continue the scheme as previously attempted using the hydrazone and the acetal protecting groups. Attempts to prepare the triflate [80] using trifluromethanesulfonic anhydride and pyridine, led to an impure triflate. The nmr spectrum indicated that the protecting group had been cleaved. A probable explanation is that since the nitrogen in the oxazolidine ring is more basic ($pk_a \approx 9.6$) than that of pyridine ($pk_a \approx 5.2$) triflation of the nitrogen on the heterocycle occurs with formation of a sulfonamide (see mechanism below).



An attempt was made to prepare a pure triflate by using an excess of an amine of basicity comparable to the basicity of the ring nitrogen. Oxazolidine [77] was treated with trifluromethanesulfonic anhydride and an excess of triethylamine. The nmr spectrum of the resulting product indicated that, although the triflate had been formed in relatively high yield, there had occurred hydrolysis of the protecting group to the extent of approximately 20%, making purification very difficult.

Since the two preceding experiments indicated that the high basicity of the oxazolidine nitrogen was responsible for undesired side-reactions, we attempted to prepare the N-phenyloxazolidine [81] in which the pk_a of the ring nitrogen is considerably lower (≃ 5.8).

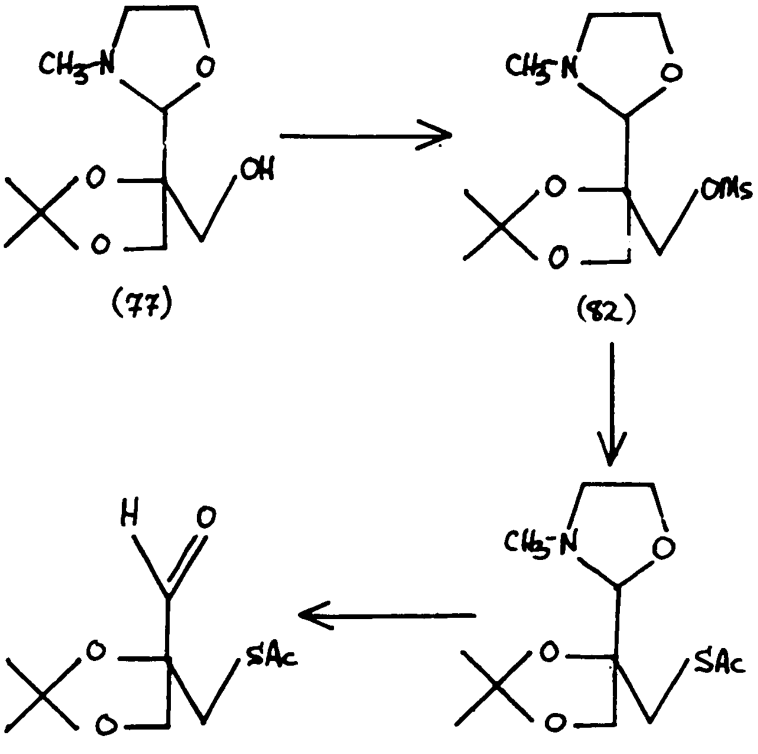
N-phenylethanolamine [48] was allowed to react with the hydroxyaldehyde [57]. Vpc analysis of the crude reaction product showed the presence of at least fifteen compounds and no major product. This approach was quickly abandoned.



(81)

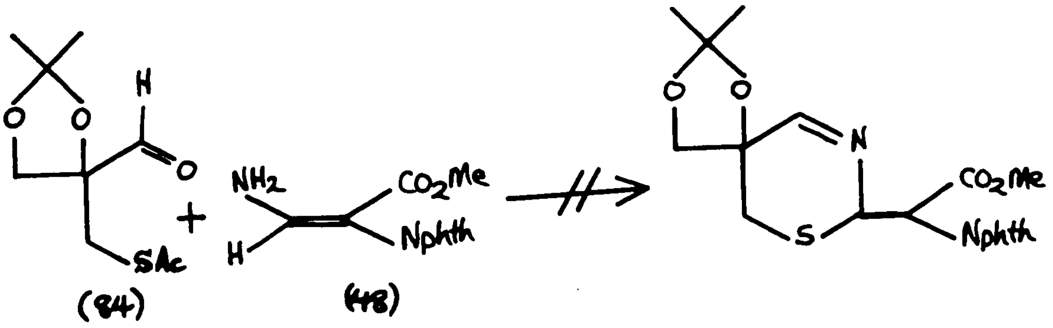
Since all attempts to form a trifluromethanesulfonate in the presence of an oxazolidine ring failed, we prepared the less reactive methanesulfonate [82], which was obtained in 80% yield, in a crystalline form.

It was converted to the thioacetate [83] by displacement using potassium thioacetate in refluxing acetone for 2-3 days. The aldehyde acetate [84] was easily prepared by acetic acid hydrolysis.

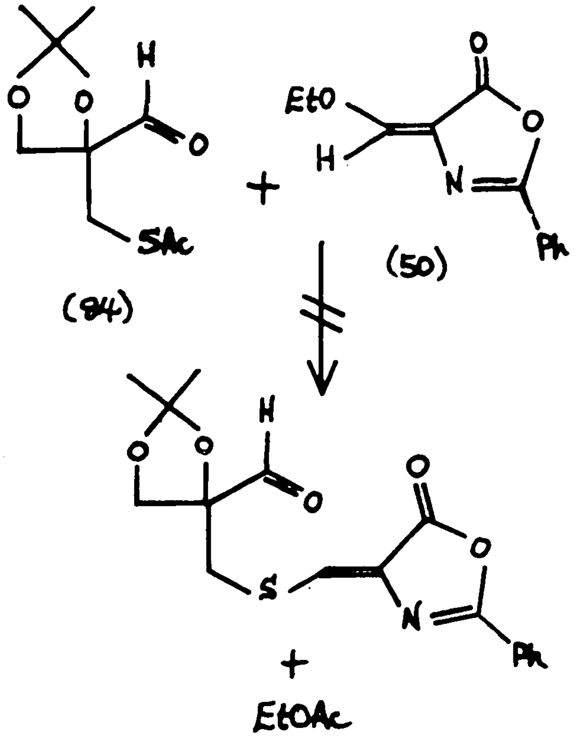


(77) (82) (83) (84)

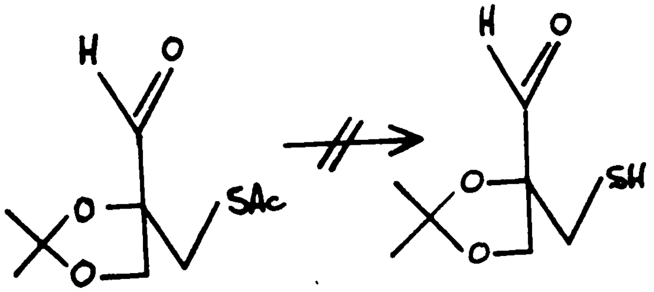
Probably due to the non-nucleophilic nature of the thioacetate group, endeavors to condense compound [84] and the aminomethylene ester [48] (Asinger type A' condensation, see page 23), resulted in the recovery of starting materials only.



The same results were obtained when [84] was allowed to react with the ethoxymethylene oxazolone [50] (Asinger type A''' condensation, page 23).

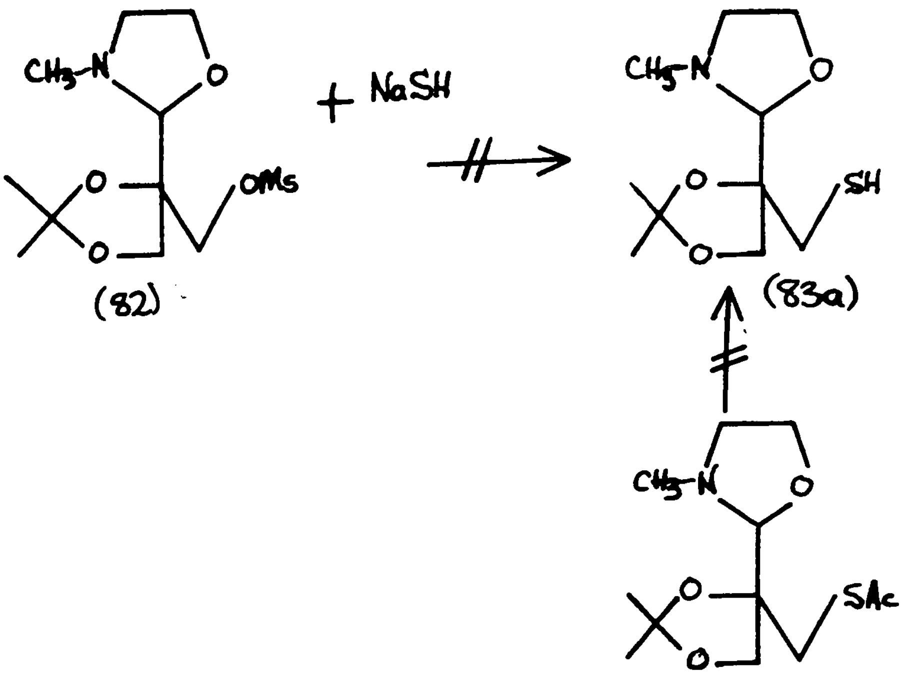


An effort to hydrolyse [84] to the thiol aldehyde [41] using ammonia in methanol or a trace of sodium methoxide in methanol gave intractable mixtures from which no products were isolated.



(84) (41)

The mesylate [82] was treated with sodium hydrogen sulfide to try to obtain the free thiol [83a]. Upon work up of the crude reaction mixture, the protecting group was no longer apparent (nmr) and the resulting thiol aldehyde must have polymerized. No pure product could be isolated.



(82) (83a)

(83)

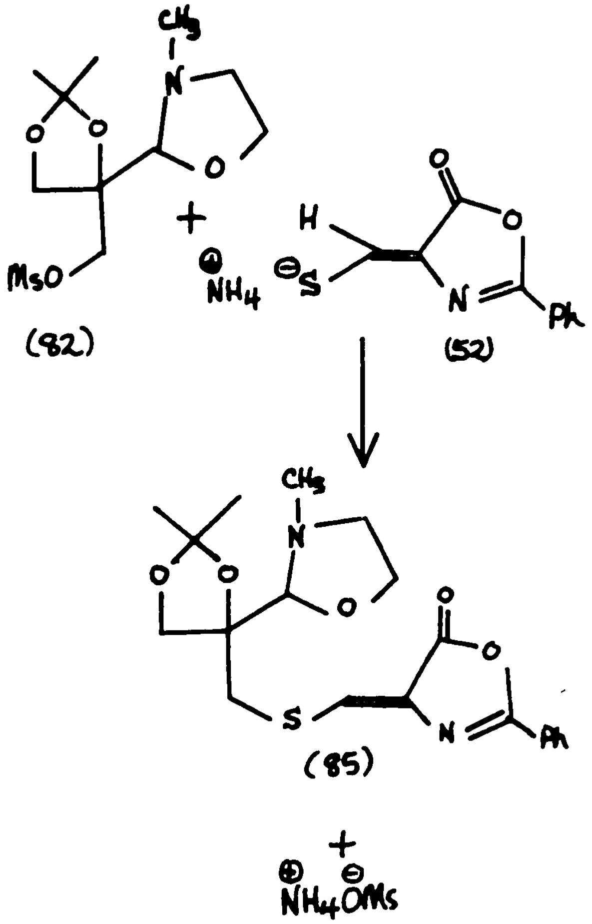
An unsuccessful effort was made to hydrolyze the thioacetate [83], using sodium in methanol (catalytic amount), to the free thiol-oxazolidine [83a]. The usual work up, involving addition of water, led to hydrolysis of the protecting group (nmr), and no pure compounds could be isolated from the mixture.

Since pure thiol aldehyde [41] or the protected thiol aldehyde [83a] could not be obtained, the Asinger type A' and A''' approaches were abandoned (see page 23). In the following chapter attempts at Asinger type B condensations will be discussed (see page 24).

CHAPTER 4

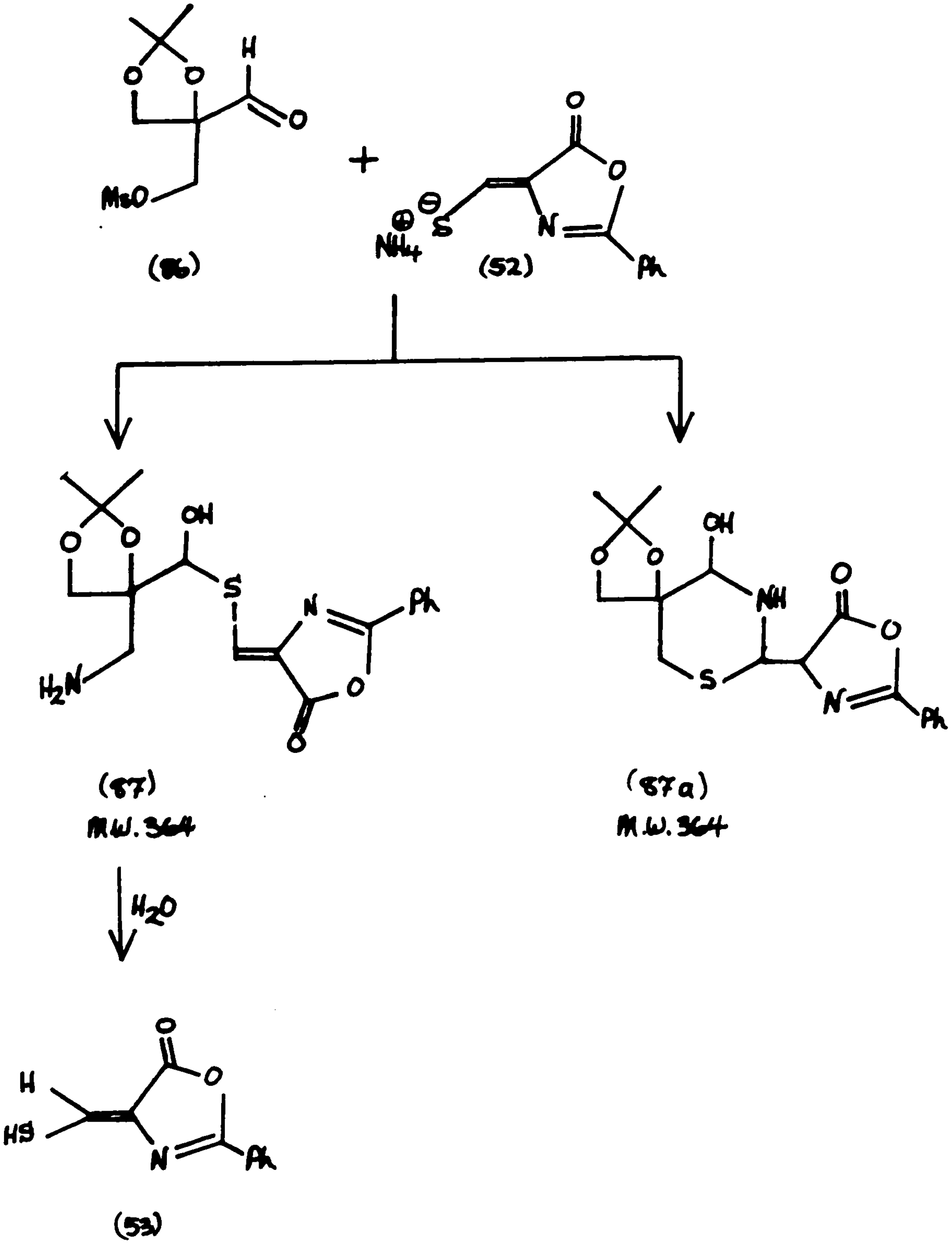
Asinger Type B Condensations

The first approach involved the condensation of the ammonium salt of the thiomethyleneoxazolone [52] with the mesylate [82].



The crystalline mesylate [82] and the ammonium salt [52] (1 equivalent) were dissolved in dimethylsulfoxide-d_6 and placed in an nmr tube. The clear solution was heated to 60-70^o and the reaction was followed periodically by nmr spectroscopy. After about one hour, the signal for the two protons α to the mesylate (approx. 4 ppm) had disappeared and a new signal at 2.6 ppm (methylene group adjacent to sulfur) had appeared. It was also noticed that the signal due to the N-methyl of the protecting group (149 cps) had diminished in size and a new absorption, at higher field was appearing (144 cps). It was thus concluded that the displacement of the mesylate had occurred and the protecting group was being cleaved at the same time. The ammonium mesylate which was formed must have been acidic enough to protonate the nitrogen in the oxazolidine ring and the moisture in the air (anhydrous conditions were difficult to maintain) hydrolysed the group to the aldehyde.

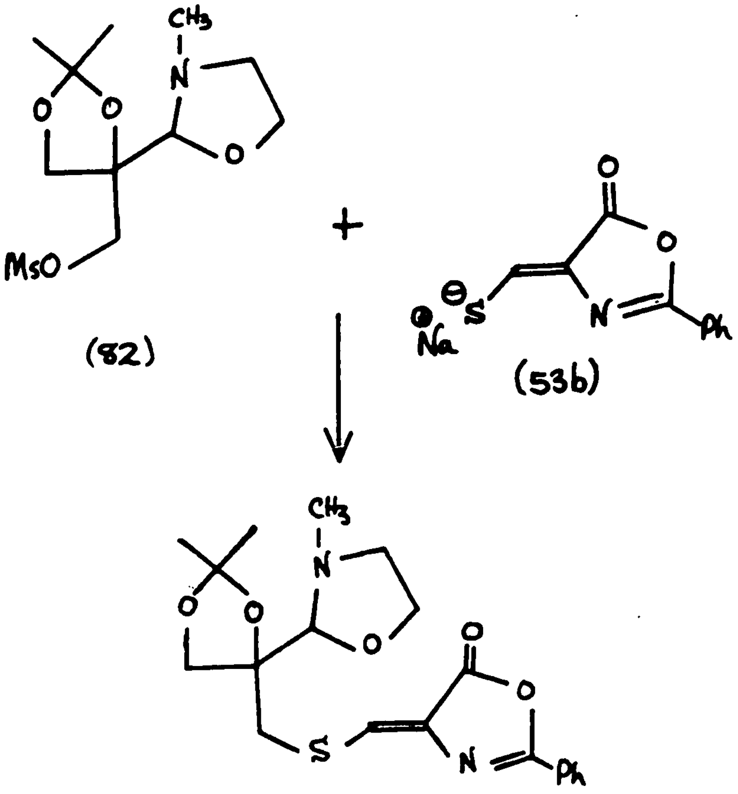
In a second approach, the oxazolidine mesylate [82] was hydrolysed to the aldehyde mesylate [86]. Compound [86] was dissolved in anhydrous dimethylsulfoxide and the solution was saturated with anhydrous ammonia. An equimolar amount of oxazolone [52] was added and the clear solution was heated to 70^o for 1 hour, in an attempt to synthesize compound [87a]. Evaporation and methylene chloride extraction


H
O
MsO
(86)
+
$NH_4^{\oplus}$ $S^{\ominus}$
N
Ph
(52)
OH
S
H_2N
(87)
M.W. 364
NH
(87a)
M.W. 364
H_2O
H
HS
(53)

gave an oily product [87] from which the crystalline thiomethyleneoxazolone [53] was isolated upon crystallization from water. This result is best accounted for by assuming that the thiol function of [52] had first reacted with the aldehyde group of [86] and that ammonia had displaced the mesylate group. The mass spectrum [m/e 364 (M^+), 347 (M^+-OH), 302 (M^+ - OH - $CH_2\dot{O}$ - CH_3)] was consistent with either structures [87] or [87a]. However, formation of [53] rule out structure [87a].

It can be concluded from the above reactions that the protected aldehyde must be used. Also, a salt of the thiomethyleneoxazolone must be prepared so that the corresponding salt of the mesylate does not hydrolyze the protecting group.

The mesylate oxazolidine [82] was therefore allowed to react with 1.5 equivalents of the pure sodium salt of the thiomethyleneoxazolone [53b] in dimethylsulfoxide (anhydrous conditions, nitrogen atmosphere) at 70-80^o for 2 hours. After the dark red solution was worked-up, the resulting orange-red oil was filtered through an aluminum oxide column (neutral, activity I) using methylene chloride-methanol (9:1). A slightly yellow oil was obtained in 76% yield. Analytical and spectral data of the product showed that the desired compound [85] had been obtained.



(82)

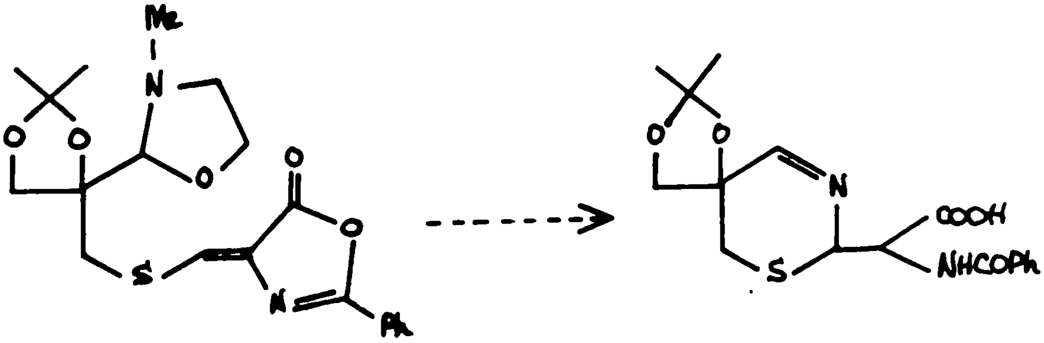
(53b)

(85)

CHAPTER 5

Synthesis of a Cepham Derivative

The first immediate objective was to obtain the heterocycle [95] depicted below.

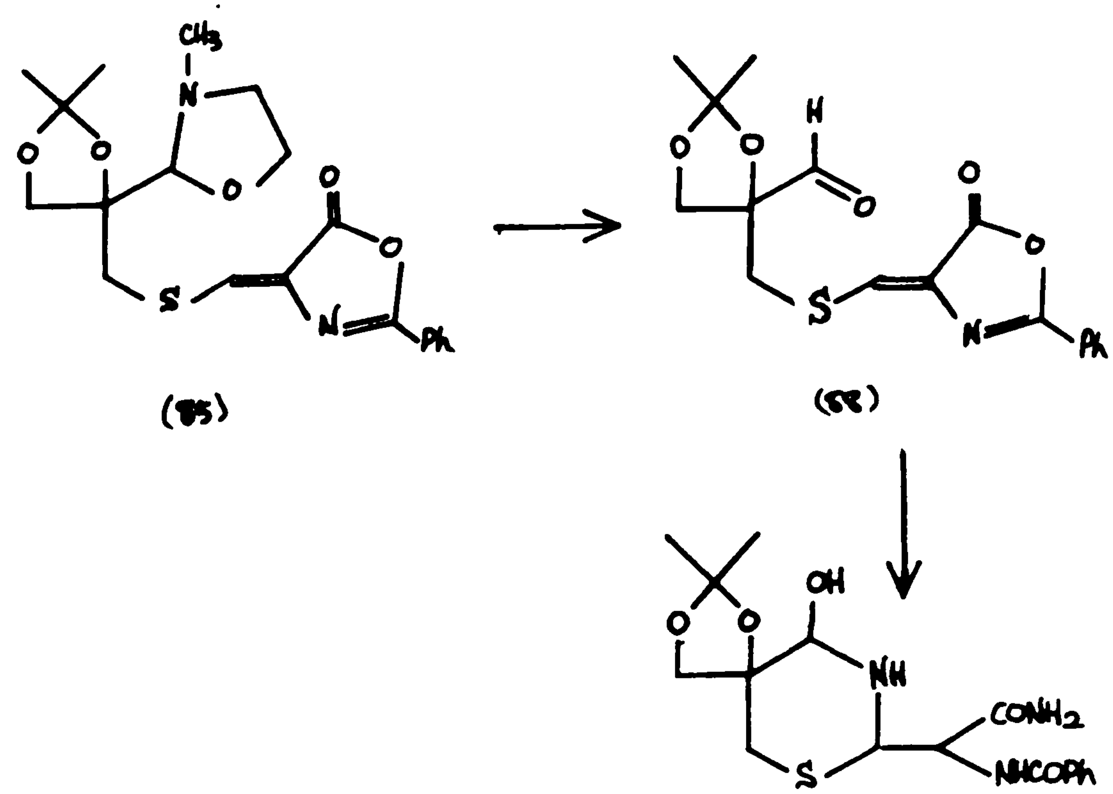


(85) (95)

In the first attempt, the protecting group of the oxazolidine azlactone [85] was hydrolyzed by the usual procedure using acetic acid. This afforded a mixture of the aldehyde [88] and its hydrate. Careful chromatography on silica gel (activity III) using carbon tetrachloride - ether (8:2) gave a crystalline aldehyde. The ir spectrum indicated the presence of the azlactone (1800 cm^{-1}) and the aldehyde functions (1740 cm^{-1}). A singlet at 10.3 ppm, characteristic of the aldehyde, was observed in the nmr spectrum. The compound had a melting range of 79-81^{o} and was analytically pure. A positive test specific for aldehydes [55] was obtained when compound [88] was treated with 4-amino-3-hydrazino-5-mercapto-1, 2, 4-triazole.

For synthetic purposes, the crude aldehyde and aldehyde hydrate could be obtained in acceptable purity by filtration on an aluminum oxide column.

In order to transform the aldehyde to imine [95], anhydrous ammonia was introduced into a refluxing benzene solution of the aldehyde for 4-6 hours. Evaporation of the solvent gave a yellow oil. Spectral data, in particular the mass spectrum confirmed the assigned structure [91]: m/e 333 ($M^+ - H_2O - H_2CO$), 318 ($M^+ - H_2O - H_2CO - CH_3$), 303 ($M^+ - H_2O - H_2CO - CH_3 - CH_3$), 259 ($303 - NH_3 - HCN$). The ir spectrum had no absorptions at 1800 cm^{-1} (azlactone carbonyl) or at 1735-1725 cm^{-1} (aldehyde).



(85)

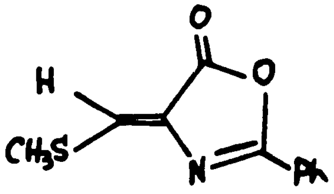
(88)

(91)
M.W. 381

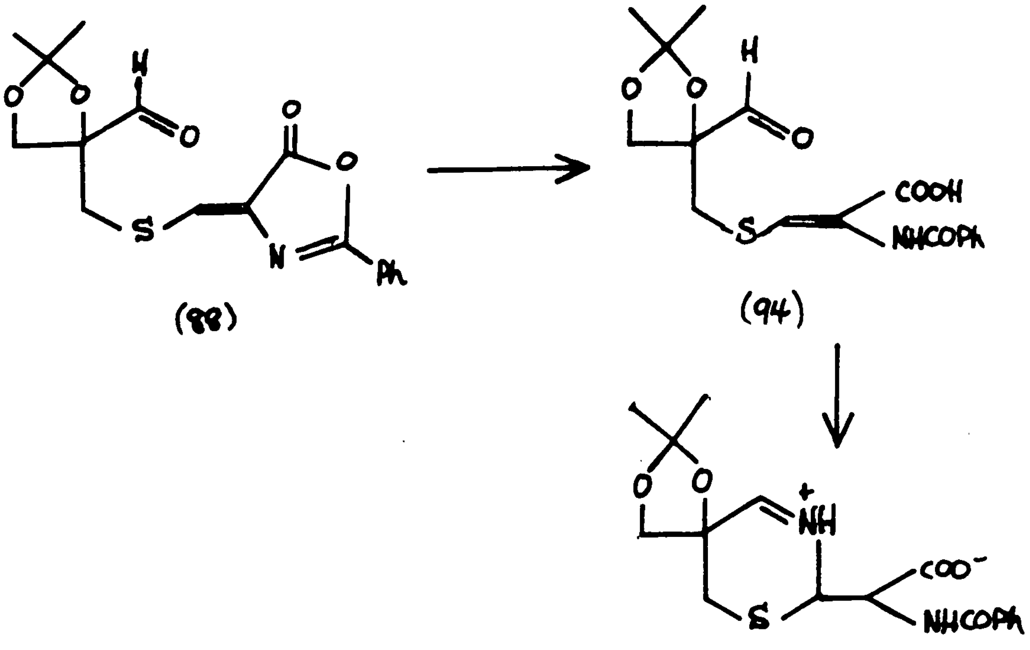
Since the reaction with ammonia cleaved the azlactone ring in an undesired manner, it was decided to first hydrolyze the azlactone to the benzamido-acid [94] and then to carry out the ammonia reaction. The aldehyde-azlactone [88] was treated with 2N sodium hydroxide (20 equivalents) for 2-3 hours, according to literature procedure [46] for 2-phenyl-4-methylthiomethylene-5-oxazolone [93].

The reaction was followed by monitoring the disappearance of the band at 355 nm characteristic [56] of the phenyl azlactone. The reaction was worked up when the absorption dropped to 5% of its initial intensity. The normal work-up procedure, involving acidification and extraction, gave a low yield of desired product. A modified work-up of the sodium salt of the acid using ion exchange resin (Rexyn 102 H^+) gave the free aldehyde-acid [94] in acceptable yield. The crude product [60-65% pure by tlc, methanol-methylene chloride (1:1)], was treated with ammonia in a refluxing benzene solution for 24 hours. Evaporation of the benzene yielded an amorphous pale brown solid, for which structure [95] or its hydrated form was proposed. Addition of anhydrous hydrogen chloride to a solution of [95] in anhydrous tetrahydrofuran, afforded a crystalline hydrochloride salt.

The hydrolysis sequence of the oxazolidine azlactone [85] could be reversed. The yield of the product [aldehyde-acid (94)] was however slightly lower.



(93)

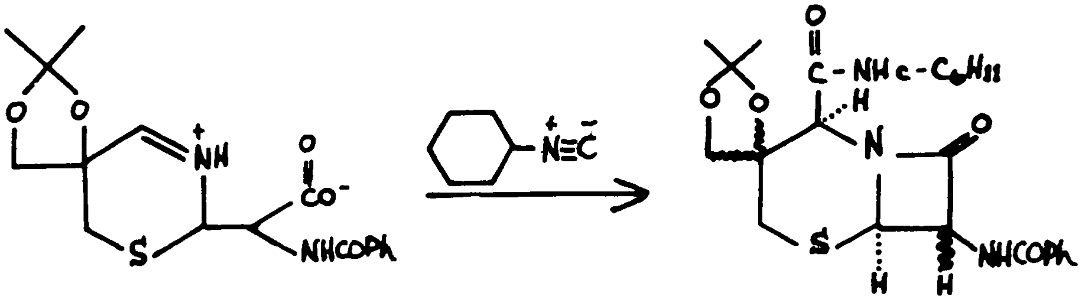


(88)

(94)

(95)

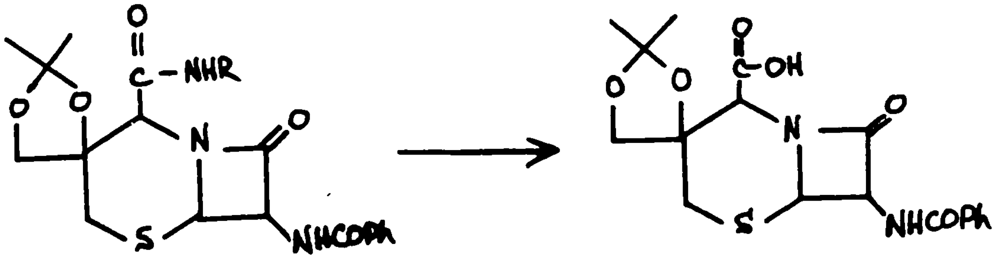
Having synthesized the imino-acid [95], the remaining task was to condense this compound with an isonitrile, to give the title compound. Crude [95] was treated in a two phase system with cyclohexyl isonitrile (prepared by the procedure of Ugi [54]). The crude product contained a β-lactam carbonyl group as evidenced by the infrared spectrum (1745-1750 cm^{-1}). The complete characterization of the compound [96] will be discussed later.



(95)

(96)

A necessary condition for the total synthesis of a cephalosporin derivative was the hydrolysis of the amide generated in the Ugi reactions. This is depicted below.



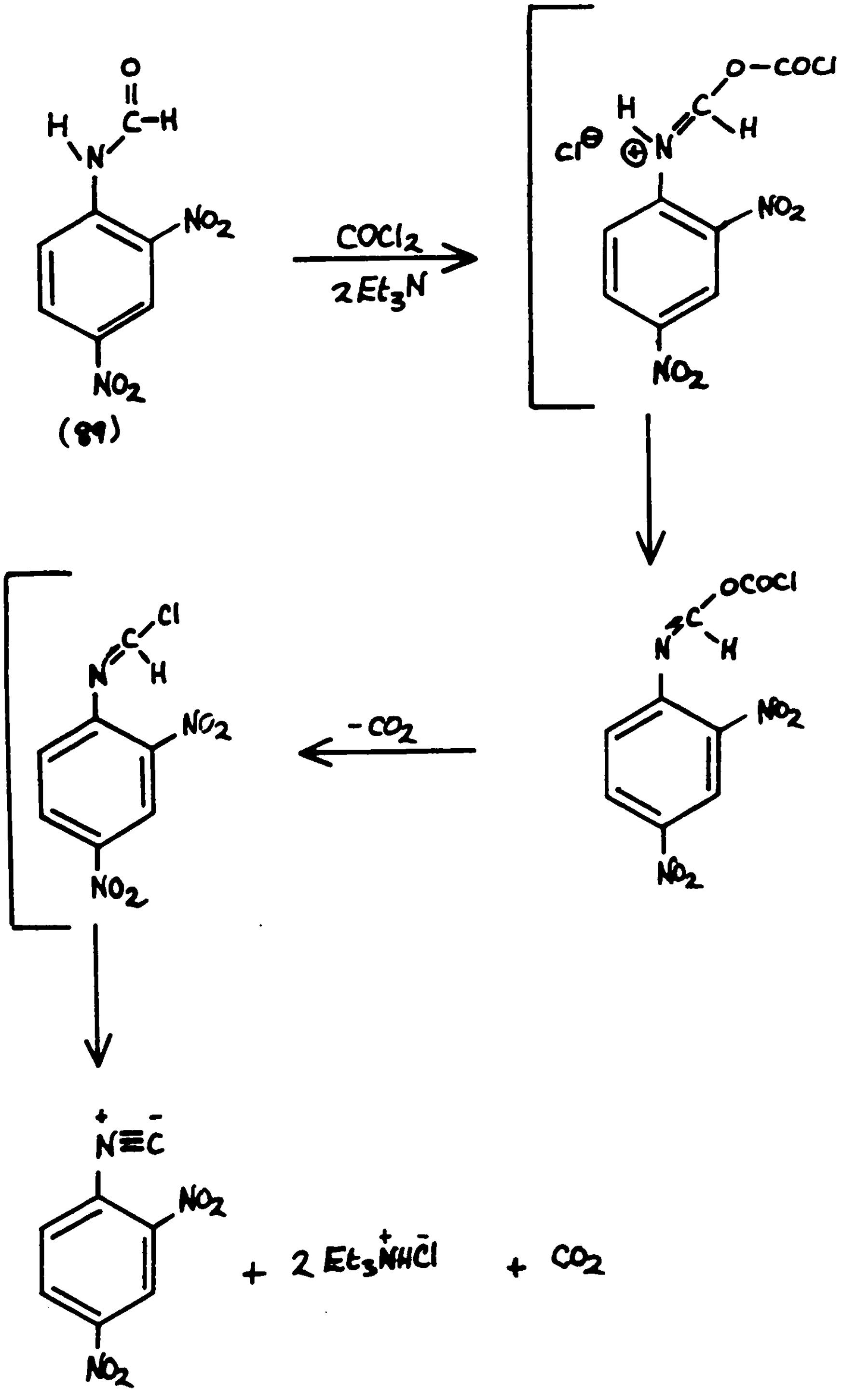
(97) a. R = o,p-dinitrophenyl
b. R = o-nitrophenyl
c. R = isopropyl

(97) d.

From theoretical considerations one can predict that o,p-dinitroanilides can be hydrolyzed under mild conditions. If the anilide [97a] could be prepared there would be a good chance that the hydrolysis to [97d] would be possible without destruction of the β-lactam ring. A model study was made to find out the ease of hydrolysis of o,p-dinitroacetanilide, as compared to that of the β-lactam ring of the cephalosporin C. Stirring a methanol-water solution of the anilide overnight, at room temperature with 2 equivalent of potassium bicarbonate - conditions to which cephalosporin is known to be stable - afforded complete hydrolysis to o,p-dinitroaniline. Attempts to prepare o,p-dinitrophenyl isonitrile [90] using Ugi's phosgene procedure [24], failed. The crude reaction mixture showed the existence of an isonitrile absorption in the ir (2030 cm^{-1}) but upon work-up only the starting formamide was isolated. This is probably due to the enhanced electrophilicity of the isonitrile to α addition of water because of the strong electron withdrawing groups in the phenyl ring.

The proposed mechanism for the preparation of an isonitrile using phosgene is depicted in the scheme on the following page. The starting material is the formamide [89] obtained from the corresponding aniline and formic acid.

Since o,p-dinitrophenyl isonitrile could not be formed, the possibility of preparing the o-nitrophenyl derivative was studied. The hydrolysis of o-nitroacetanilide, required more severe conditions (3 hours, potassium bicarbonate, 50-60°). We were able to synthesize o-nitrophenyl isonitrile, for the first time, using Ugi's phosgene procedure [24]. The crude β-lactam [97b] was prepared. Attempts to purify the product (30-40% pure by ir analysis) by hydrolysis of the anilide to the potassium salt of the carboxylic acid failed. The conditions worked out previously had been too severe and led to complete destruction of the β-lactam ring, as evidenced by the ir spectrum.



COCl2
2Et3N
O-COCl
Cl⊖
H
⊕N
H
NO2
NO2
OCOCl
-CO2
Cl
H
N≡C
+ 2 Et3NHCl
+ CO2

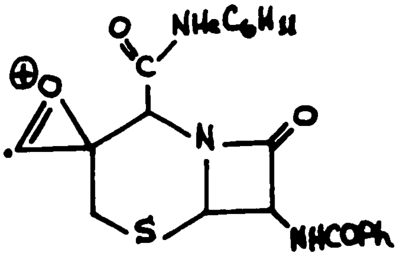
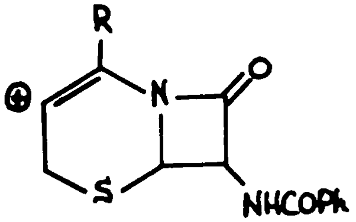
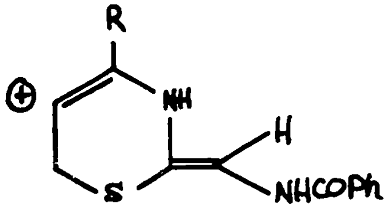
(89)

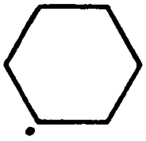
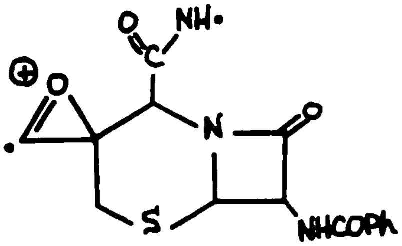
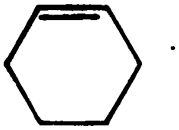
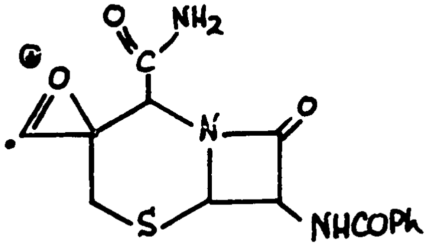
(90)

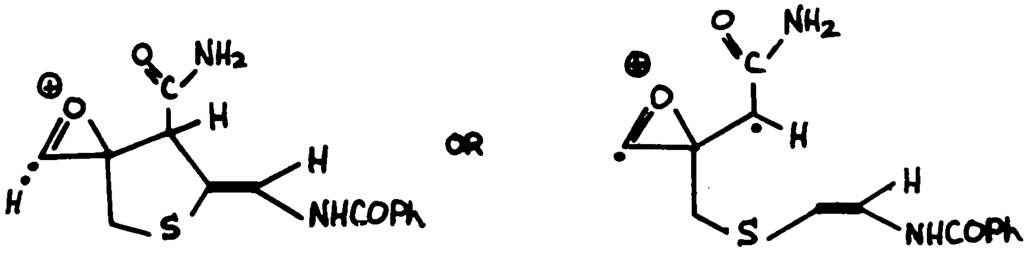
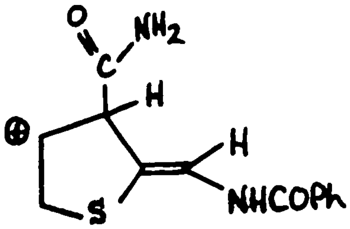
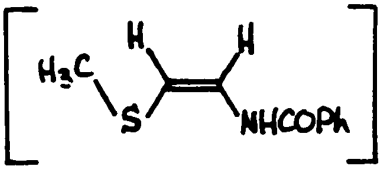
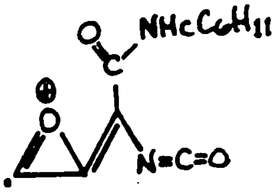
It has recently been shown by Fechtig [60] that amides in cephalosporin type compounds can be hydrolyzed quite readily, by using phosphorus pentachloride, to the corresponding acid and amine. The ease and mild conditions involved in the above reaction led us to abandon the study to find an easily hydrolyzable amide.

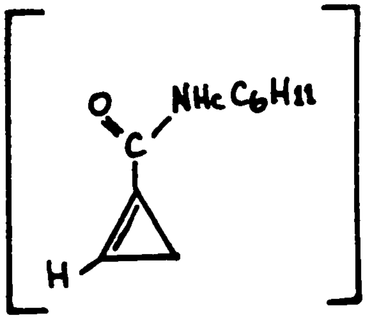
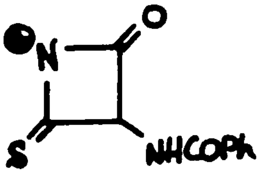
The isopropyl amide [97c] was prepared using isopropyl isonitrile and the imino-acid [95]. This derivative was chosen because of the ease of removal of any excess isonitrile (bp 82-83°/750 mm) in the work-up of the reaction mixture. The crude yield of the product [97c] was low (< 20%) as estimated from the ir spectrum and it was decided to continue with the cyclohexyl amide [96] because it was obtained in the best overall yield. The crude product was chromatographed on a silica gel column (activity III) and eluted with benzene-acetone (7:3). A light yellow semi-crystalline oil was obtained in 20-25% yield. The compound was characterized by careful analysis of the mass spectrum and by infrared spectroscopy (1745-1750 cm^{-1}, β-lactam carbonyl). A negative thiol [37] test (cuprous salt), a negative aldehyde test [55] and the fact that the compound did not dissolve in 10% sodium bicarbonate or 10% hydrochloric acid, led to the exclusion of other possible structures. (eg starting materials or unfused β-lactam derivatives). Not enough material was available for thorough purification and microanalysis.

Interpretation of the Major Fragments of the Mass Spectrum of Compound [96]

m/e	Suggested Fragment
413	$M^+ - CH_2O - CH_3 - CH_3$ 
384	413 - CHO 
356	384 - CO 

m/e	Suggested Fragment
332	413 -  
331	413 -  

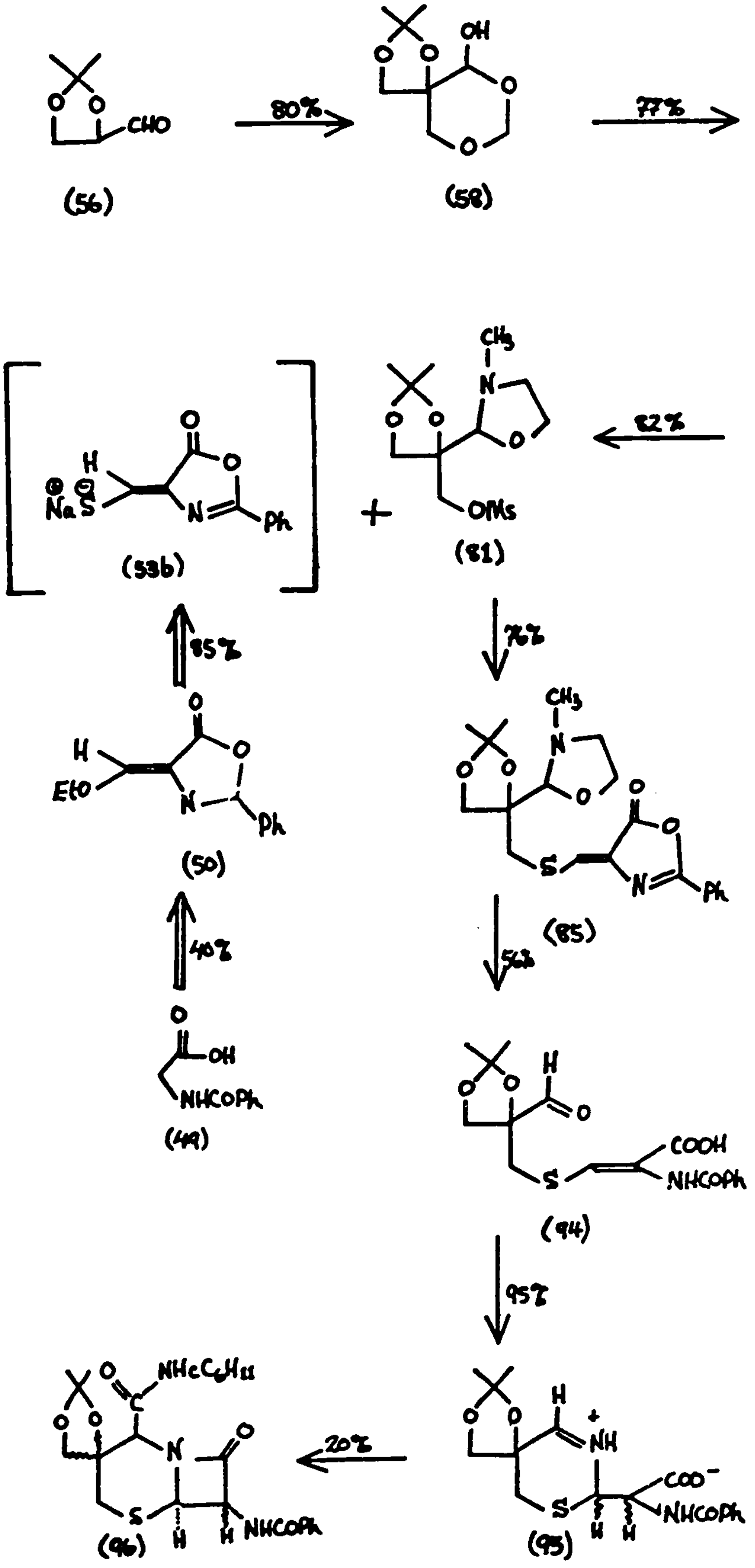
m/e	Suggested Fragment
289	331 - NCO (characteristic of β-lactams [57]) 
260	289 - HCO 
220	413 -  

m/e	Suggested Fragment
219	384 - 
	

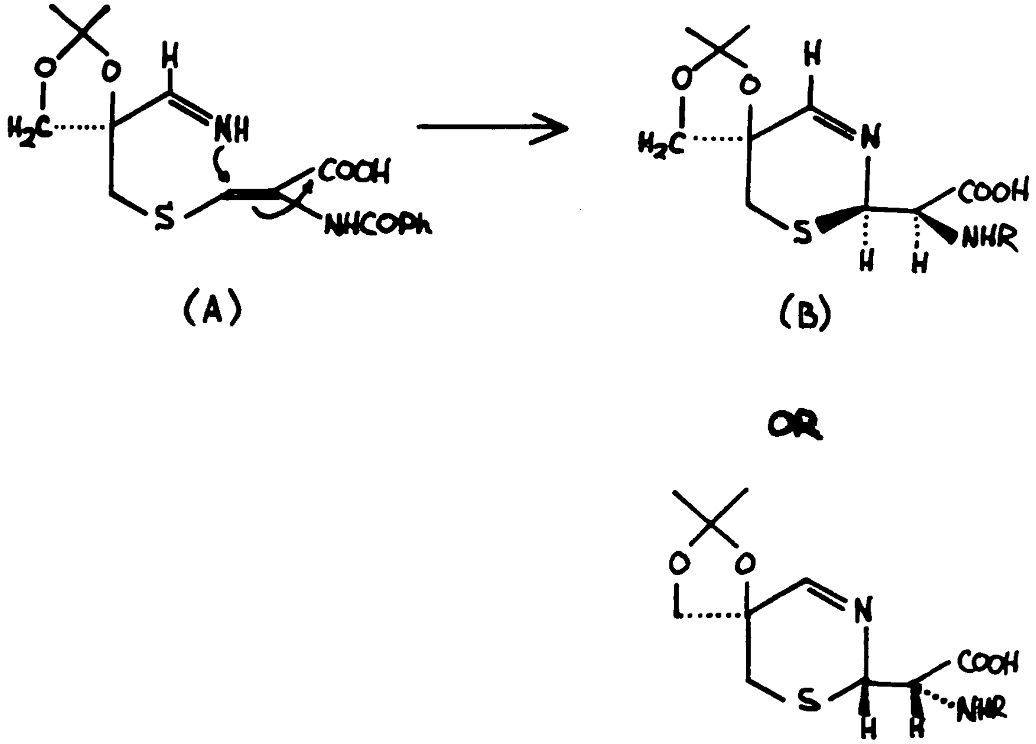
Because of the multiplicity of unsuccessful approaches reported, it is appropriate at this time to outline, on a flowsheet (see following page), the shortest path from glyceraldehyde acetonide to the cepham [96]. The overall yield for the sequence of reactions, based on glyceraldehyde acetonide, is 2.3%. It has recently been found in our laboratories that treatment of the formaldehyde adduct [58] with N-methylethanolamine gives directly the protected aldehyde [77] in 85% yield. The overall yield, replacing this reaction for the ones depicted, is 4.5%.

A brief discussion of the stereochemistry of the product [96] might be pertinent at this time. Cephalosporin C has two asymmetric centers only, which bear a cis-relationship. All other asymmetric centers in compounds [95] and [96] are removed in the course of the introduction of the double bond in the Δ^3 position.

Assuming that the sulfide function and the carbonyl group of [A] bear a trans relationship (for discussion see page 28), a concerted trans addition of the imine (or carbinolamine) onto the α, β-unsaturated acid would give the desired cis relation at C-6 and C-7. If the addition takes place in a non-concerted manner, both the cis and trans compounds would be obtained.


(56)
CHO
80%
OH
(58)
77%
H
OH
(57)
55%
CH3
N
OH
(77)
82%
(81)
OMs
+
(53b)
H
Na S
N
Ph
85%
H
EtO
(50)
Ph
40%
OH
NHCOPh
(49)
76%
(85)
S
56%
COOH
NHCOPh
(94)
95%
H
NH
COO-
NHCOPh
(95)
20%
NHcC6H11
C
N
S
H
H NHCOPh
(96)

The influence of the dioxolane ring on the mode of addition of the imine of compound [A] is unknown, so that either diastereomer [B] or [C] or most likely both, would be obtained in a concerted reaction. In the non-concerted case, two more diastereomers would be formed.



(A)

(B)

OR

(C)

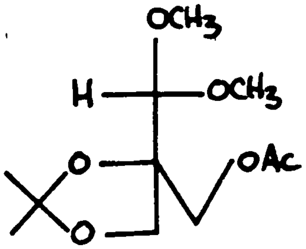
The same arguments pertain to the stereochemistry of the isonitrile adduct [96]. However, no additional isomers would be formed, since the carboxyamido-function introduced is cis with respect to the C_6 - C_7 bond.

The β-lactam [96] exists therefore as a mixture of two, and more likely four diastereomers. Since the asymmetric centers at C-3 and C-4 are removed in the course of the introduction of the double bond, the concerted addition would give one correct isomer and the non-concerted addition would afford two isomers of the cephalosporin amide derivative.

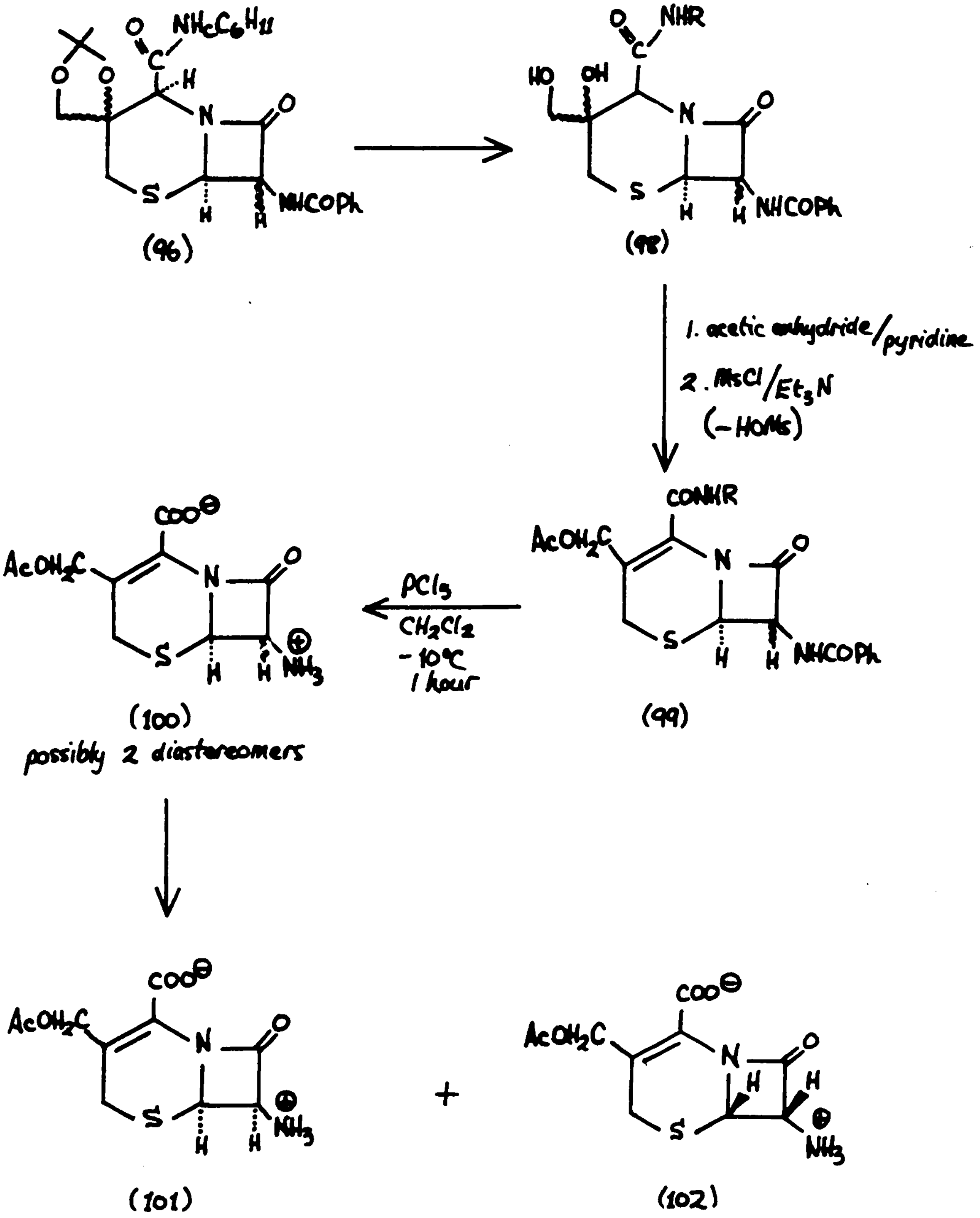
Although the nmr spectrum of the cepham [96] was consistent with the proposed structure, no evidence as to the configuration as C_6 and C_7 could be derived, because of the complexity of the spectrum in the region under examination.

PROPOSAL FOR FUTURE STUDY

The hydrolysis of the acetonide function of compound [96] is a critical step for the synthesis of a cephalosporin derivative. By using the acetate [72a], as a model compound, the minimum conditions for the hydrolysis of acetonide group was found. This consisted of treatment of the compound with 10% trifluroacetic acid in aqueous methanol (1:1), at room temperature. By monitoring the reaction by nmr spectroscopy, it was found that the acetonide was completely cleaved to the diol, in seventy-five minutes. No evidence of hydrolysis of the β-lactam function of cephalothin was observed when it was treated under the above mentioned conditions. It is known [10,12,13] that the β-lactam ring of cephalosporin derivatives, not having a Δ^3 double bond, are more acid stable. It can thus be assumed that compound [96] could be converted to the diol [98] (see scheme on following page).



(72a)


(96)
(98)
1. acetic anhydride/pyridine
2. MsCl/Et3N
(–HOMs)
(99)
PCl5
CH2Cl2
-10°C
1 hour
(100)
possibly 2 diastereomers
(101)
+
(102)

Acetylation, elimination, possibly by treatment with methanesulfonyl chloride in triethylamine and hydrolysis of the two amide functions using phosphorus pentachloride would probably lead to compound [100]. Separation of the two possible diastereomers of [100] using physical methods and optical resolution of the racemic 7-aminocephalosporanic acid (101, 102) would afford optically pure 7-aminocephalosporanic acid [101].

CONTRIBUTIONS TO KNOWLEDGE

Many erroneously assigned structures published by Worrall were corrected.

A new aldehyde protecting group was developed which can be hydrolyzed under mild acid conditions and is stable to anhydrous bases, acylating agents and thiolate anion displacements.

A total synthesis of a cepham derivative starting from mannitol was accomplished.

Several new compounds were prepared and characterized.

EXPERIMENTAL

Melting points were determined on a Gallenkamp block and are uncorrected. Mass spectra were obtained on an AE1-MS-902 mass spectrometer at 70eV using a direct-insertion probe. Nmr spectra were recorded on a Varian Associates T-60 spectrometer and tetramethylsilane was used as the internal standard. The multiplets and quartets, in the nmr spectral data, were recorded as the center of the peaks. Ir spectra were obtained on a Unicam SP1000 and a Perkin-Elmer 257 infrared spectrophotometer. Ultraviolet spectra were determined with a Unicam SP-800 spectrophotometer. Optical rotations were measured with a Perkin-Elmer 141 polarimeter.

Thin layer chromatography was performed on silica gel coated plates (Eastman). Woelm aluminum oxide (neutral) and silica gel were used for column chromatography. Microanalysis were carried out by A. Bernhardt, Mikroanalytisches Laboratorium, Elbach uber Engelskirchen, C. Daessle, Montreal, and F. Pascher, Bonn, West Germany.

CHAPTER I

Carbomethoxy Methyl Malonate Monothioallylamide [19]. ——

This compound was prepared according to the procedure of Worrall [28] with the following modification: the mixture of the sodium salt of dimethyl malonate and allyl isothiocyanate was refluxed for 24 hours with vigorous stirring. This ensured that most of the sodium metal had reacted and minimized the possibility of any large excess of sodium igniting when the mixture was poured into ice water. After recrystallization from ethyl alcohol-water, a 56% yield of IV was obtained: mp 42-43° (lit [28] yield 66%; mp 42-43°); ir (KBr) 3385, 3335 (NH), 1750, 1715 cm^{-1} (C=O); nmr ($CDCl_3$) δ 3.9 (s, 6), 4.4 (t, 2), 5.6 (s, 1) 5.2-6.4 (m, 3), 4.0-6.0 (s,broad, NH); uv max (95% C_2H_5OH) 274 nm (ε 13,400); mass spectrum (70eV) m/e 231 (M^+).

Anal. Calcd for $C_9H_{13}O_4NS$: C, 46.75; H, 5.62; N, 6.06; S, 13.85. Found: C, 47.03; H, 5.48; N, 5.87; S, 14.03.

Reaction of [19] with Bromine [24]. ——

The procedure of Worrall was followed exactly: yield 68%; mp 152-154° (lit [28] yield 68% mp 153-154°);

ir (KBr) 3200, 1615, 1650 cm^{-1}; nmr (DMSO-d_6) δ 3.9 (s, 6), 4.1 (m, 2), 3.6 (m, 3), 3.0-6.0 (broad, 1, NH); uv max (95% ethanol) 285 nm (ε 12,500); mass spectrum (70eV) m/e 309 (M^+), 311 (M^+ + 2).

Anal. Calcd for $C_9H_{12}O_4NSBr$: C, 34.95; H, 3.88; N, 4.53; S, 10.36; Br, 25.85. Found: C, 34.92; H, 3.83; N, 4.53; S, 10.61; Br, 25.96.

The iodo derivative [25] was prepared in a similar manner, mp 156-157° (lit [28] mp 156-157°).

Reaction of [24] with Silver Nitrate [27]. ——

This compound was prepared according to Worrall: yield 65%; mp 104-105° (lit [28] mp 104-105°) (after drying under vacuum at 40°, mp 81-82°); ir (KBr) 3220, 1650, 1660, 1630 (ONO_2), 1285, 870-855 (O-N str) cm^{-1}; nmr (DMSO-d_6) δ 3.8 (s, 6), 3.95 (m, 3), 4.7 (m, 2), 9.8 (broad, 1, NH); mass spectrum (70eV) m/e 292 (M^+), 229 (M^+ - HNO_3). It was difficult to obtain good analytical values because of the instability of [27].

Anal. Calcd for $C_9H_{12}O_7N_2S$: C, 36.99; H, 4.11; N, 9.59; S, 10.95. Found: C, 37.48; H, 4.29; N, 9.63; S, 10.87.

Reaction of [19] with Hydrazine [29]. ——

The procedure of Worrall was followed except for the following modification in the work-up: the concentrated deep red solution was added slowly, with vigorous stirring, to an iced solution of 5 N hydrochloric acid. If this was not strictly followed an intractable gum was obtained which could not be crystallized. During the reaction a strong odor of hydrogen sulfide was detected, probably due to gross decomposition of the thioamide. A 20% yield of crystalline product was obtained. It turned yellow upon standing for several weeks. Water of hydration was removed by drying the compound under vacuum at 60° for 4-6 days: mp 120-121°; ir (KBr) 3300, 3000-2300 (NH_3^+ str), 1630-1520 (C-O str), 1480, 1430, 1230, 1040, 1000, 940, 775 cm^{-1}; nmr (DMSO-d_6) δ 4.3 (t, 2), 5.35 (m, 1), 5.1 (m 1), 6.0 (m, 1), 3.0-6.0 (broad, 5), 10.1 (broad, 1); uv max (95% C_2H_5OH) 293 nm (ε 16,500), 255 (14,300).

Anal. Calcd for $C_7H_{11}O_3N_3S$: C, 41.79; H, 5.47; N, 20.89; S, 15.95. Found: C, 42.02; H. 5.27; N, 21.16; S, 15.72.

Reaction of [19] with Methylhydrazine [30]. ——

[30] was prepared according to the procedure described for [29] except that the deep red solution was worked up after 2-3 hours. The resulting crystalline solid (25%) was recrystallized from ether-hexane: mp 186-188^o (dried under vacuum at 40^o overnight); ir (KBr) 3320, 2800-2700 (NCH_3); nmr ($DMSO-d_6$) δ 3.2 (s, 3), 4.5 (t, 2), 5.3 (m, 1), 5.6 (m, 1), 5.9 (m, 1), 4.0-9.0 (broad, 3, exchangeable with D_2O); uv max (95% EtOH) 295 nm (ε 18,800), 258 (16,000).

Anal. Calcd for $C_8H_{11}O_2N_3S$: C, 45.07; H, 5.16; N, 19.72; S, 15.02. Found: C, 45.53; 44.51; H, 5.13, 5.31; N, 19,96; S, 14.83

Reaction of [29] with Iodine [31]. ——

The compound was prepared according to the published procedure [28]. The product was dried overnight under vacuum at 100^o to yield 75% of a colorless crystalline product: mp 213-214^o (lit [28] 213-214^o); ir (KBr) 3300, 3000-2500 (NH_3^+), 1640 cm^{-1}; nmr ($DMSO-d_6$) δ 3.0 (m, 2), 3.3 (m, 2) 3.6 (m, 1), 5.0-10.0 (broad, 5, exchangeable with D_2O); uv max (95% EtOH) 295 nm (ε 22,400).

Anal. Calcd for $C_7H_{10}O_3N_3SI$: C, 24.50; H, 2.94; N, 12.25; S, 9.33; I, 37.01. Found: C, 24.67; H, 2.97; N, 12.43; S, 9.60; I, 37.11.

CHAPTER 2

Methyl α-phthalimidomalonaldehydate [47]. ——

The procedure of Sheehan [39] was followed exactly. A yield of 35% was obtained. The compound was recrystallized from benzene, mp 139-141° (lit [39] mp 140.5-142.0°); ir (KBr) 3500-3200 (enol form of aldehyde), 1770, 1720 (phthalimide system), 1735 (ester), 1680 cm^{-1} (formyl group); nmr ($DMSO-d_6$) δ 3.6 (s, 3, ester), 8.0 (s, 4), 8.05 (s, 1), 9.0-10.0 ppm (s, 1, OH); mass spectrum (70eV) m/e 247 (M^+).

Methyl α-phthalimido-β-aminoacrylate [48]. ——

To a 50 ml round bottom flask was added 2.5 g of methyl α-phthalimidomalonaldehydate [47] and 3.8 g (5 equivalents) of ammonium acetate. The mixture was heated to 150° under a nitrogen atmosphere for 15 minutes. The reddish-brown mass was extracted with methylene chloride and washed several times with water, dried over sodium sulfate and evaporated to give a red oil. Upon warming the oil in benzene, an amorphous product precipitated. Thin layer chromatography on silica gel G ($CHCl_3$ - Et_2O, 6:4) showed the product to be homogeneous (Rf 0.35). Yield 13.5 g (54%); mp 191-193° (crystallized from acetone); ir (KBr) 3300-3400 (NH_2 str), 1780, 1720 (phthalimide system), 1735 (ester), 1680-1670 (formyl group); nmr (acetone-d_6) δ 3.7 (s, 3, OCH_3), 6.4-7.0 (d, broad, 2, NH_2), 7.95 (s, 1), 8.0 ppm (s, 4, phthalimide system); uv max (95% C_2H_5OH) 266 nm (ε 25,000), 270 (ε 21,500); mass spectrum (70eV) m/e 246 (M^+).

Anal. Calcd for $C_{12}H_{10}N_2O_4$: C, 58.53; H, 4.07; N, 11.37. Found: C, 58.59; H, 4.02; N, 11.29.

Ammonium salt of 2-phenyl-4-ethoxythiomethylene-5-oxazolone [52].

The procedure of Barber and Slack [46] was followed. An overall yield of 20% was obtained (lit [46] 25-30%). The fact that the ethoxymethylene derivative [50] causes severe allergic reactions should be considered when attempting to synthesize these compounds. Mp 219-222° (lit, not recorded); ir (nujol mull) 3300-3330 (NH_4^+), 1700 (C=O str), 1580, 1470, 1370, 1000, 770, 690 cm^{-1}; nmr (D_2O) δ 7.4, 7.8 (m, 5), 7.9 ppm (s, 1).

The ammonium salt could be converted to the free thiol by dissolving it in water and neutralizing with hydrochloric acid. The precipitated product (quantitative) was washed with water and dried. Mp 165-173° (lit [51] 172-176° dec.); mass spectrum (70eV) m/e 205 (M^+).

Reaction of 2-Phenyl-4-ethoxymethylene-5-oxazolone with Sodium Hydrogen Sulfide. ——

To an ice cold slurry of 2.17 g of the ethoxymethylene derivative [50] in 5 ml of absolute methanol was added at once 565 mg (1.01 equiv) of anhydrous sodium hydrogen sulfide. Anhydrous reaction conditions were maintained. After stirring at 5° for 30 minutes, the red-orange solution was added dropwise to vigorously stirred, ice cooled anhydrous ether (300 ml). The ether was decanted and the orange precipitate was washed several times with anhydrous ether. Last traces of ether were removed on a vacuum line. The amorphous orange solid (193 mg , 85%) was stored in a desiccator. The sodium salt was dissolved in water and neutralized with acetic acid, to give the free thiol in quantitative yield. Nmr (acetone-d_6) δ 7.65, 8.2 (m, 6), 8.6 ppm (s, 1, H-C=C); mass spectrum (70eV) m/e 205 (M^+); uv max (95% ethanol) 345 nm (ε 31,600).

Preparation of Mannitol Diacetonide [55]. ——

The procedure of Baer and Fischer [40] was followed except for the following modification: after destroying the zinc chloride using potassium carbonate, the decanted ether-acetone solution was dried rigorously with anhydrous sodium sulfate. The concentrate was heated for 2 hours under high vacuum at 70-80°C, to ensue a completely dried product. It was essential that the reaction was carried out at 20°,otherwise triacetone mannitol resulted. Yield 48% (lit [40] 55%); mp 117-118° (lit [40] 119°).

Preparation of Glyceraldehyde Acetonide [56]. ——

The procedure of Baer and Fischer [40], using lead tetraacetate, was followed. The product was a colorless oil and distilled at 52-56° at 17-18 mm (lit [40] 35-42° / 8-11 mm). The consistency of the oil changed after a few hours. The ir spectrum of the latter showed a weak absorption at 1725 cm^{-1}. Yield 70% (lit [40] 79.3%); ir (NaCl film) 3450 (OH str), 1725 (C=O str), 1260, 1220 1080 (C-O); nmr (neat) δ 1.25 (d, 6), 3.9 (m, 2), 4.2 (m, 1), 9.7 ppm (d, 1, aldehyde).

Formaldehyde Adduct [58]. ——

Freshly distilled glyceraldehyde acetonide (18 g) was added to a stirred solution of 18 g (2 equiv) of anhydrous potassium carbonate, 50 ml of a 40% formaldehyde solution and 100 ml of distilled water and 200 ml of methanol. The clear solution was stirred at room temperature overnight. The next day the solution was concentrated on a rotatory evaporator connected to a high vacuum pump (bath temperature < 40°). The crystalline mass (product and potassium carbonate) was extracted several times with methylene chloride. The solvent was dried and evaporated to a clear oil which crystallized spontaneously when the last traces of solvent were removed under high vacuum. The colorless crystalline compound was recrystallized from ether-methylene chloride mixtures. An analytical sample was prepared by sublimation (high vacuum, 90° bath temperature). Yield 20.8 g (80%); mp 89.5-91°; ir (KBr) 3400 (OH str), 1380 1220, 1160, 1090, 1080, 1050, 1000 cm^{-1}. Nmr ($CDCl_3$) δ 1.5 (s, 6), 3.9 [ABq, 2, J=10 Hz, CH_2-O-C $(CH_3)_2$], 4.2 (ABq, 2, J=10 Hz), 5.0 (ABq, 2, J=7 Hz, C-O-CH_2-O), 5.1 ppm (s, 1), 4.0-6.0 ppm (broad, 1, OH); mass spectrum (70eV) m/e 190 (M^+).

Anal. Calcd for $C_8H_{14}O_5$: C, 50.52; H, 7.42. Found: C, 50.57; H, 7.52.

Acetate [59]. ——

The hydroxydioxane [58] was acetylated using pyridine and acetic anhydride. Yield 89%; mp 30-31.5° (ether-carbon tetrachloride); ir (NaCl film) 1750 (C=O str), 1380, 1170-1280 (C-O str), 1120, 1080, 1010, 930 cm^{-1}; nmr (CCl_4) δ 1.3 (s, 6), 2.1 (s, 3, CH_3CO-), 3.7 (ABq, 2, J=10 Hz), 3.9 (ABq, 2, J=10 Hz), 4.8 (ABq, 2, J=7 Hz), 5.7, 5.85 (double singlets, 1, CHOAc, 2 isomers); mass spectrum (70eV) m/e 232 (M^+), 133 (M^+-$OCOCH_3$).

Anal. Calcd for $C_{10}H_{16}O_6$: C, 51.72; H, 6.94. Found: C, 51.51; H, 6.98.

Hydrolysis of Acetonide Acetate [59] to Diol [61]. ——

The acetate [59] (2.3 g, 10 mM) was dissolved in 250 cc of 80% acetic acid and heated at 60° for 15 hours. The acetic acid was evaporated under high vacuum to a half of the original volume, diluted with water and extracted with three portions of methylene chloride. The solvent was dried (sodium sulfate) and evaporated to yield 1.5 g of a light yellow oil. The oil crystallized only with difficulty (methanol-ether). Yield 79%; mp 109-110.5°; ir (NaCl film) 3300-3500 (OH str), 1740 (C=O str), 1390, 1260, 1050 cm^{-1}; nmr (pyridine-d_5) δ 2.0 (s, 3), 4.2 (ABq, 2, J=10 Hz), 4.3 (s, 2, $\underline{CH_2}OH$), 4.8 (ABq, 2, J=7 Hz), 5.6 ppm (double s, CHOAc, 2 isomers).

Anal. Calcd for $C_7H_{12}O_6$: C, 43.75; H, 6.29. Found: C, 43.97; H. 6.09.

Another method was used to hydrolyse the acetonide. This consisted of dissolving the compound (100 mg) in 1 ml of 90% trifluoroacetic acid and stirring at room temperature for 2 minutes. Flash evaporation under high vacuum and evaporation several times with methanol and toluene removed

removed last traces of acid. The resulting oil crystallized from methanol and ether mixtures. Yield 66 mg (80%).

Preparation of Mesylate [63]. ——

The diol (890 mg) was added to a solution of 2 ml of dry pyridine and 580 mg (1.1 equiv.) of mesyl chloride. Precipitation of pyridine hydrochloride was evident immediately. The usual work up, after the mixture was stirred for half an hour, yielded 1.05 g (83.5%) of a light yellow oil, which did not crystallize. Nmr ($CDCl_3$) δ 2.2 (d, 3, $COCH_3$), 3.1 (d, 3, OMs), 4.0 (m, 2), 4.2 (m, 3), 4.9 (ABq, 2, J=7 Hz), 5.9 ppm (s, 1).

Preparation of p-Nitrobenzoate [60]. ——

The alcohol [58] (4.75 g) was dissolved in 25 ml of dry pyridine and 5.2 g of recrystallized p-nitrobenzoyl chloride (from CCl_4) (1 equiv) was added at once. The reaction mixture was stirred at 0° for 2 hours and 12 hours at 25°. The precipitated mass was stored at -20° for 24 hours. Saturated sodium bicarbonate solution (30 ml) was added whilst being ice cooled. Stirring was continued for 15 minutes and then the mixture was poured into 300 mls of ice cold water. Rapid stirring was continued for 1 hour and then the precipitate was filtered and air dried and recrystallized from chloroform. Yield 7.0 g (82.5%); mp 228-229.5^{o}; ir (KBr) 1740, 1610, 1540, 1360, 1270, 1100, 1060 cm^{-1}; nmr (CDCl$_3$) δ 1.6 (d, 6), 3.85 (ABq, 2, J=10 Hz), 4.1 (s, 2), 5.1 (ABq, 2, J=7 Hz,OCH$_2$O-) 6.4, 6.55 (double s, 1), 8.5 ppm (s, 4); mass spectrum (70eV) m/e 339 (M^{+}). It was apparent from the nmr spectrum that the 2 epimers were in a ratio of 3:1.

Anal. Calcd for $C_{15}H_{17}NO_8$: C, 53.10; H, 5.05; N, 4.13. Found C, 52.92; H, 4.01; N, 4.25.

Hydrolysis of Acetonide [60] to diol [62]. ——

The procedure for the hydrolysis of acetonide [59] using trifluroacetic acid, was followed. From 300 mg of [60] and 2 ml of trifluroacetic acid, 227 mg of [62]was obtained (84%). Crystallized from methanol, mp 122-124^{o} (softens 115^{o}); ir (NaCl film) 3400-3300, 1740, 1610, 1530, 1350, 1270, 1170, 1100, 1090, 1050, 880, 720 cm^{-1}; nmr (DMSO-d$_6$) δ 3.8 (s, 2, CH$_2$OH), 3.8-4.0 (broad singlet, 2, OH), 4.0 (ABq,2, J=10 Hz), 5.0 (ABq 2, J=7 Hz, OCH$_2$O), 6.1, 6.4 ppm (double singlet, 1, epimeric hydrogen α to pNBz), 8.7 ppm (s, 4).

Anal. Calcd for $C_{12}H_{13}NO_8$: C, 43.75; H, 6.29; N, 4.68. Found: C, 43.97; H, 6.09; N, 4.79.

Preparation of the Mesylate [64]. ——

The normal procedure for mesylations was used. Yield from 2.99 g , 3.1 g or 80% yield, mp 146-148° (methanol); ir (KBr) 3500, 1740, 1610, 1540, 1340 (SO_2 str), 1260, 1250, 1170, 1180, 830, 720 cm^{-1}; nmr (acetone d_6) δ 3.13 (s, 3, OMs), 4.0 (ABq, 2, J=10 Hz, $O-CH_2-C$), 4.5 (s, 2, $\underline{CH_2}OMs$), 5.0 (ABq, 2, J=7 Hz), 4.8-5.2 (broad, 1, OH), 6.2 ppm (s, 1, $\underline{CH}OBzNp$), 8.4 ppm (s, 4); mass spectrum (70eV) m/e 281 ($M^+-HOSO_2CH_3$).

Reaction of Mesylate [64] with Potassium Thioacetic Acid. ——

The mesylate (.38 g, 1 mm) was dissolved in 10 ml of dry acetone and to this solution was added 125 mg (1.1 equiv) of potassium thioacetic acid (Eastman, recrystallized from water). The suspension was stirred, under nitrogen, at 60-80° for 24-48 hours. The precipitated potassium mesylate was filtered and the yellow-red solution was evaporated to dryness, dissolved in methylene chloride, washed with water, dried and evaporated to afford a red semi-crystalline oil (300 mg , 84% of theory). Tlc showed the presence of 2 major compounds (SiO_2, $CHCl_3$). The nmr spectrum indicated the existence of an acetate (2.1 ppm) and a thioacetate (2.4 ppm) in an approximate ratio of 1:1. The mass spectrum (70eV) was highlighted by the following major peaks: m/e 323 (M^+-H_2S) and 281 ($M^+-H_2S-CH_2CO$).

CHAPTER 3

Preparation of Hydroxy-Aldehyde [57]. ——

The formaldehyde adduct [58] was placed in a 35 ml round botton flask, and immersed in an oil bath at 140°. The crystalline material melted and a strong odor of formaldehyde was detected. Partial vacuum was applied, while the temperature of the bath was raised to 160°. An odorless, colorless oil distilled at 101-104°/0.7 mm. Yield 10.4 g (77%). Runs of 20-25 g are recommended, as the product is unstable to heat and a rapid distillation is necessary, otherwise the yield of polymerized materials increases. The nmr showed the following peaks: δ 1.7 (s, 6, acetonide), 4.0 (ABq, 2, J=8 Hz CH_2OH), 3.9 (s, 1, OH), 4.25 (ABq, J=10 Hz) and 10.4 ppm (s, 1, aldehyde). The ir (NaCl film) had a strong absorption at 3300-3500 cm^{-1} (hydroxyl) and a strong aldehyde absorption at 1735 cm^{-1}.

Upon standing for a few hours, the hydroxyaldehyde polymerized, thus reactions involving this intermediate must be carried out immediately.

N, N-Dimethylhydrazone [68].

Into a 50 ml 2-necked flask equipped with a reflux condenser, drying tube and rubber septum was added 2-3 g of barium oxide and a solution of 2.1 ml (1.5 equiv) of 1, 1-dimethylhydrazine (distilled twice over barium oxide) in 20 ml of absolute ethanol. To the cooled solution, was added dropwise, 2.9 g of aldehyde dissolved in 10 ml of absolute ethanol. The addition was made over a period of 30 minutes. After the initial exothermic reaction had subsided the reaction mixture was refluxed for 1 hour. The cooled solution was filtered and evaporated to yield a light yellow oil. Yield after distillation was 2.7 g (75%); bp 98-99^{o} at 0.7 mm; ir (NaCl film) 3600-3300 (OH str), 2830, 2820, 2800 [$N(CH_3)_2$] 1600, 1480, 1460, 1370, 1260, 1220, 1070, 1050, 1030 cm^{-1}; nmr ($CDCl_3$) δ 1.5 (s, 6), 2.9 [s, 6, $N(CH_3)_2$], 3.5 (s, 1, OH), 3.7 (s, 2, $\underline{CH_2}OH$), 4.1 ppm [s,2, $\underline{CH_2}OC(CH_3)_2$] 6.6 ppm (s, 1, H-C=N); mass spectrum (70eV) m/e 202 (M^+).

Anal. Calcd for $C_9H_{18}N_2O_3$: C, 53.44; H, 8.97; N, 13.85. Found: C, 53.63; H, 8.79; N, 13.34.

Preparation of the Acetate [69c]. ——

The acetate was prepared in the usual manner using acetic anhydride and pyridine. The yield of crude product was quantitative. Ir (NaCl film) 2830, 2816, 2790 [$N(CH_3)_2$], 1740 (C=O str), 1600, 1460, 1450, 1380, 1370, 1250, 1050 cm^{-1}; nmr ($CDCl_3$) δ 1.45 (s, 6), 2.1 (s, 3), 2.8 (s, 6), 4.15 (ABq, 2, J=9 Hz, CH_2OAc), 4.3 (s, 2), 6.6 ppm (s, 1).

Anal. Calcd for $C_{11}H_{20}N_2O_4$: C, 54.09; H, 8.14; N, 11.47. Found: C, 53.98; H, 8.01; N, 11.34.

Mesylate Dimethylhydrazone [69a]. ——

The usual method for the preparation of mesylates was utilized. Yield from 400 mg of the alcohol was 343 mg (62%). An analytical sample could not be prepared. Ir (NaCl film) 2890, 2800, 1600, 1460 (SO_2 str), 1180, 1060, 1000 cm^{-1}; nmr ($CDCl_3$) δ 1.6 (d, 6), 3.0 (s, 6), 3.2 (s, 3), 4.3 (ABq, 2, J=8 Hz, CH_2-O-C), 4.5 (s, 2 CH_2OMs), 6.6 (s, 1).

Tosylate [69b]. ——

Treatment of [68] with tosyl chloride and pyridine gave [69b] as a pale yellow oil (yield 55%). Ir (NaCl film) 2880, 2790, 1600, 1450, 1370 (SO_2 str), 1180, 1100, 1060, 1040, 990, 810 cm^{-1}; nmr ($CDCl_3$) δ 1.4 (s, 6), 2.5 (s, 3, C_6H_4-$\underline{CH_3}$), 2.8

(s, 6), 4.15 (ABq, 2, J=8 Hz, CH_2-O-C), 4.25 (s, 2, CH_2OTs), 6.5 (s, 1), 7.7 ppm (m, 4, C_6H_4-); mass spectrum (70eV) m/e 356 (M^+), 184 (M^+-HOTs).

Preparation of Dimethylacetal [70]. ——

To 100 ml of absolute methanol (distilled over magnesium turnings) and 10 mg of p-toluenesulfonic acid was added 10 g of molecular sieves 3A and 1.6 g (10 mm) of the aldehyde. The mixture was stirred (with exclusion of moisture) for 12-15 hours at room temperature. After neutralization of the acid with ion exchange resin [Rexyn 203 (OH)], filtration, evaporation and distillation, pure acetal was obtained in 60% yield (1.24 g) Bp 100-102°/0.5 mm; ir (NaCl film) 3600-3300 (OH str), 1470, 1410, 1390, 1270, 1230, 1120, 1100, 1080 cm^{-1}; nmr ($CDCl_3$) δ 1.55 (s, 6), 3.4 (s, 1, OH), 3.65 [s, 6,$(OCH_3)_2$], 3.8 (s, 2, CH_2-OH), 4.1 (ABq, 2, J=6 Hz, CH_2O-C), 4.5 [s, 1, <u>H</u>-C $(OCH_3)_2$].

Anal. Calcd for $C_9H_{18}O_5$: C, 52.41; H, 8.80. Found: C, 52.31; H, 8.68.

Acetate [72a]. ____

The compound was prepared in a 95% yield from the alcohol, by means of pyridine and acetic anhydride. Evaporation of the reagents afforded analytically pure acetate. Ir (NaCl film) 1745, 1460, 1390, 1380, 1250 (C-O str), 1120, 1090, 1060 cm^{-1}; nmr ($CDCl_3$) δ 1.7 (s, 6), 2.4 (s, 3), 3.85 (d, 6) 4.2 (ABq, 2, J=8 Hz, CH_2-O-C), 4.5 (ABq, 2, J=8 Hz, CH_2OAc), 4.55 ppm [s, 1, CH $(OCH_3)_2$].

Anal. Calcd for $C_{11}H_{20}O_6$: C, 53.21; H, 8.12. Found: C, 53.30; H, 7.94.

Mesylate [71a]. ____

This compound was prepared by the usual method with methanesulfonyl chloride and pyridine at 0°. An 85% yield of light yellow foam was obtained after the usual work-up procedure . Nmr ($CDCl_3$) δ 1.45 (s, 6), 3.1 (d, 3 OMs), 3.6 (s, 6), 4.0 (ABq, 2, J=10 Hz, CH_2-O-C) 4.3 [s, 1, $\underline{H}$-C $(OCH_3)_2$], 4.35 ppm (ABq, 2, J=9 Hz, $\underline{CH_2}OMs$).

Anal. Calcd for $C_{10}H_{20}O_7S$: C, 42.25; H, 7.04; S, 11.26. Found: C, 42.01; H, 7.15; S, 11.12.

Triflate [71b]. ——

The alcohol [70] (200 mg , 1 mM) was dissolved in 3 ml of absolutely dry pyridine and the solution was cooled to -5° and protected from moisture by the use of a drying tube (calcium chloride). Freshly prepared trifluoromethanesulfonic anhydride (850 mg , 5 equiv.) (prepared [50] by flame distillation of the acid over phosphorus pentoxide, bp 85°) was added slowly to the cooled mixture. A color change from colorless to green to red was observed. The red solution was stirred for 5 minutes and immediately evaporated to dryness on rotatory evaporator connected to a high vacuum pump (bath temperature must not exceed 35°). The deep red oil was dissolved in methylene chloride and washed with 3 portions of ice cold water. The solvent was dried and evaporated to afford an orange-red oil (yield 85.5%, 290 mg). Because of the instability of the product no attempts were made at its purification. The ir spectrum showed no hydroxyl absorption and a large absorption band at 1420 and 1390 cm^{-1} (SO_2 str). Nmr ($CDCl_3$) δ 1.5 (s, 6), 3.6 (s, 6), 4.1 [ABq, 2, J=10 Hz, CH_2-O-C $(CH_3)_2$] 4.45 [s, 1, CH-C $(OCH_3)_2$], 4.65 ppm (ABq, 2, J=1 Hz, CH_2OTf).

The triflate was used in further reactions without purification.

An alternate procedure using Crossland's method [53] for mesylates (methylene chloride, triethylamine) gave an identical product.

Preparation of Thioacetate Dimethylacetal [74].

Into a 50 ml round bottom flask was placed 340 mg (1 mm) of the triflate [71b], 30 ml of dry acetone and 230 mg of potassium thioacetic acid (2 equiv). The suspension was stirred under a nitrogen blanket at 60-65° for 5 hours. The reaction was followed by tlc (SiO_2, $CHCl_3$). The potassium triflate was filtered off and the red solution was evaporated. The oil was dissolved in methylene chloride and washed with water. The usual work-up afforded 200 mg (75.5%) of a reddish-orange oil.

A similar procedure was used to convert the mesylate [71a] to the thioacetate, except that the suspension was refluxed in acetone for 72 hours. The resulting oil was pale yellow (78% of theory) and was identical in spectral properties to the product from the triflate. Ir (NaCl film) 1695 (SAc), 1380, 1270, 1220, 1110, 1090 cm^{-1}; nmr ($CDCl_3$) δ 1.45 (s, 6), 2.4 (s, 3, SAc), 3.4 (s, 2, CH_2SAc), 3.6 (s, 6), 4.0 (ABq, 2, J=10 Hz, CH_2-O-C), 4.25 ppm [s, 1, H-C $(OCH_3)_2$]; mass spectrum (70eV) m/e 264 (M^+), 188 (M^+-SAc).

Preparation of Thiol [75] from Triflate [71b]. ——

To a solution of 112 mg (2 equiv) of anhydrous sodium hydrogen sulfide dissolved in 25 ml of absolute methanol was added 340 mg (1 mm) of the triflate. The solution was heated to 60° under a nitrogen atmosphere, for 12 hours. The resulting orange solution was evaporated to dryness and dissolved in methylene chloride. The usual work-up procedure afforded a light orange oil (164 mg , 74%). Ir (NaCl film) 2700-2500 (-SH str), 1470, 1400, 1390, 1280, 1230, 1190, 1100, 1010 cm^{-1}; nmr (acetone-d_6) δ 1.45 (d, 6), 2.8-3.1 (s, broad, 1, SH), 3.25 (s, 2, CH_2SH), 3.5 (s, 6), 4.0 (ABq, 2, J=10 Hz), 4.5 ppm (s, 1); mass spectrum (70eV) m/e 222 (M^+), 188 (M^+ - SH).

Hydrolysis of Thioacetate [74]. ——

To a solution of 0.2 mg of sodium (.01 equiv) in 20 ml of absolute methanol was added 264 mg (1 mm) of the thioacetate. The clear solution was heated to 60° for 15-20 minutes. The cooled solution was evaporated, extracted with methylene chloride, washed with water, the organic layer dried and evaporated to yield 17.5 mg of a pale yellow oil (79% of theory). The spectral data were identical to that of the thiol obtained by displacement of the triflate with sodium hydrogen sulfide.

The thiol was not purified for the use in subsequent reactions.

Protection of Aldol [57] using N-Methylethanolamine. ——

Anhydrous sodium carbonate (5 g) was suspended in an ice cold solution of 1 g (1.1 equiv.) of N-methylethanolamine in 30 cc of dry ether. 1.95 g of freshly prepared aldol, dissolved in 10 cc of anhydrous ether, was added dropwise over a period of 30 minutes. The mixture was stirred for an additional 30 minutes at 0° and then 1 hour at room temperature. The filtered solution was evaporated and distilled (105-108°/ 0.75 mm). Crude yield was 2.1 g (80%). Vpc analysis (0.125 in. x 6 ft., 3% OV-25 column of 80-100 mesh Chromosorb W at 150° using a Hewlet Packard Model 5750 B gas chromatograph) showed one major component(90%)and one minor component (10%). Filtration on an alumina (activity 1) column using benzene as eluent afforded pure material (3 g of alumina for 1 g crude product). After purification a yield of 1.8 g (overall 55%) was obtained. Ir (NaCl film) 3600-3300 (OH str), 2810 (N-CH_3), 1470, 1380, 1260, 1225, 1150, 1080, 1070, 1050 cm^{-1}; nmr ($CDCl_3$) δ 1.45 (s, 6), 2.55, 2.60 (d, 3, N-CH_3), 2.7 (m, 1, part of AA' BB'system of N-CH_2-CH_2-O), 3.3 (m, 1, N-CH_2-CH_2-O), 4.0 ppm (m, 8, part of AA' BB' system, N-$\underline{CH}$-O, two AB systems for CH_2OH and CH_2OC-, and OH); mass spectrum (70eV) m/e 217 (M^+).

Anal. Calcd for $C_{10}H_{19}NO_4$: C, 55.28; H, 8.82; N, 6.45. Found: C, 55.16; H, 8.72; N, 6.25.

Acetate [78]. ——

The usual procedure was used to prepare the acetate in quantitative yield. The sample was analytically pure after evaporation of the reagents. Ir (NaCl film) 2810 (N-CH_3), 1745 (C=O str), 1460, 1380, 1270-1210 (C-O str), 1110, 1080, 1060 cm^{-1}; nmr ($CDCl_3$) δ 1.5 (s, 6), 2.25 (s, 3, OAc), 2.55 (s, 3, N-CH_3), 2.8 (m, 1), 3.3 (m, 1), 4.2 ppm (m, 7).

Anal. Calcd for $C_{12}H_{19}NO_5$: C, 56.02; H, 7.44; N, 5.44 Found: C, 56.28; H, 7.39; N, 5.24.

Mesylate oxazolidine [82]. ——

The method of Crossland and Servis [53] was used to prepare [82]. To a solution of 217 mg (1 mm) of the alcohol in 10 cc of methylene chloride containing a 50% molar excess of triethylamine (220 μl) at 0° to -10° was added a 10% excess of methanesulfonyl chloride (84 μl) over a period of 30 minutes. Anhydrous reaction conditions were maintained. Stirring for an additional 15-30 minutes completed the reaction. The mixture was transferred to a separatory funnel with the aid of more methylene chloride. The mixture was washed with ice water. Drying of the organic phase followed by solvent removal gave an oil. The oil was crystallized from methylene chloride-ether. Yield 242 mg (82%); mp 79.5-81°; tlc, Rf 0.6 (ether, SiO_2); ir (CCl_4 solution) 2810 ($N-CH_3$ str), 1470, 1380 (OMs), 1220, 1180, 1080, 1010. Nmr ($CDCl_3$) δ 1.5 (s, 6), 2.6 (s, 3, $N-CH_3$), 2.7 (m, 1), 3.2 (s, 3, OMs), 3.3 (m, 1), 3.8-4.6 ppm (m, 7).

Anal. Calcd for $C_{11}H_{21}NO_6S$: C, 44.75; H, 7.12; N, 4.75; S, 10.85. Found: C, 44.63; H, 7.18; N, 4.66 S, 10.63.

Displacement of Mesylate [82] using Potassium Thioacetate. ——

To a solution of 295 mg (1 mm) of the mesylate in 20 cc of dry acetone was suspended 171 mg (1.5 equiv) of recrystallized thioacetic acid. The mixture was refluxed, under a nitrogen atmosphere, for 3 days. Filtration of the cooled solution and evaporation of the acetone, gave a pale yellow oil. The oil was dissolved in methylene chloride and washed with ice water. Drying of the organic layer and evaporation afforded 264 mg (96% of theory), of a pale yellow semi-crystalline oil [83]. The sample was analytically pure. Nmr ($CDCl_3$) δ 1.5 (double singlet, 6), 2.4 (s, 3, SAc), 2.55 (s, 3, N-CH_3), 2.7 (m, 1), 3.2 (m, 1), 3.35 (s, 2, CH_2SAc), 4.0 [ABq, 2, J=10 Hz, $CH_2OC(CH_3)_2$], 4.0 ppm (s, 1).

Anal. Calcd for $C_{12}H_{21}NO_4S$: C, 52.36; H, 7.64; N, 5.09; S, 11.64. Found: C, 52.11; H, 7.53; N, 4.95; S, 11.22.

Hydrolysis of Thioacetate Oxazolidine [83] to Thioacetate [84]. ——

The compound (57 mg) was treated with 10 ml of a 1:1 mixture of acetic acid and water for 15-30 minutes at room temperature. The aqueous solution was extracted with three portions of methylene chloride and the dried extracts were evaporated first on a rotatory evaporator (water aspirator) and then on a high vacuum line, to remove last traces of acetic acid. In this way, 41 mg (91% yield) of analytically pure material was obtained. Nmr ($CDCl_3$) δ 1.45, 1.5 (double singlet, 6), 2.45 (s, 3, SAc), 3.3 (s, 2, CH_2SAc), 4:1 (ABq, 2, J=10 Hz, CH_2OC), 10 ppm (s, 1, CHO).

Anal. Calcd for $C_9H_{14}O_4S$: C, 49.54; H, 6.42; S, 14.68. Found: C, 49.41; H, 6.34; S, 14.48.

CHAPTER 4

Preparation of Mesylate Aldehyde [86]. ———

The oxazolidine [82] (295 mg, 1mM) was dissolved in 10 ml of a 50% acetic acid-water solution. The mixture was stirred rapidly for 1½ hours at room temperature. The aqueous solution was extracted three times with methylene chloride. The combined organic layers were dried and evaporated. Last traces of acetic acid were removed on a high vacuum pump. The colorless oil was not purified for the subsequent reactions. Yield 232 mg (97.5%). Nmr ($CDCl_3$) δ 1.55 (s, 6), 3.1 (s, 3, OMs), 4.15 (ABq, 2, J=8 Hz, CH_2OC) 4.5 (s, 2, CH_2OMs), 10.1 ppm (s, 1, CHO).

Preparation of the Oxazolidine Azlactone [85]. ———

The oxazolidine mesylate [82] (295 mg, 1 mm) was added to a solution of 340 mg of the sodium salt of the thiomethyleneazlactone [53b] (1.5 equiv) in 2-3 ml of absolutely dry dimethyl sulfoxide. The orange solution was heated to 70-80^{o} for two hours, with precautions taken to exclude moisture and under a nitrogen blanket. The solution turned dark red during the course of the heating. The cooled reaction mixture was poured into ice water and extracted with methylene chloride. The extracts were washed several times with ice cold brine solution until the water layer was only slightly colored. Emulsions could be broken by passing the mixture through glass wool. The organic layer was dried over sodium sulfate and evaporated. The red-orange oil was filtered through an aluminum oxide column (Woelm, activity I) using methylene chloride - methanol in a ratio of 9:1. An analytical sample was prepared by careful chromatography on silica gel (act. III) using carbon tetrachloride-ether, 8:2. A poor yield of product was obtained from the latter purification, probably due to hydrolysis of the protecting group. The overall yield from the aluminum oxide filtration was 310 mg (76%) of a yellow oil. Ir (CCl_4 solution) 2810 ($N-CH_3$), 1800, 1700 (azlactone), 1650 (N=C str), 1490, 1460, 1390, 1380, 1270, 1230, 1170, 1080 cm^{-1}; nmr ($CDCl_3$) δ 1.45 (s, 6), 2.5 (s, 3, NCH_3), 2.7 (m, 1), 3.4 (m, 4), 3.9 (m, 4), 7.5, 8.0 ppm (m, 6, aromatic, H-C=C); mass spectrum (70eV) m/e 346 (M^+-acetone), 330 (346-16), 303 [M^+ - 58 - CH_3 - (CH_2=CH_2)], 276 (306-NO); uv max (95% ethanol) 354 (ε 1300), 355 nm (ε 1300).

Anal. Calcd for $C_{20}H_{24}N_2O_5S$: C, 59.40; H, 5.98; N, 6.93; S, 7.91. Found: C, 59.59; H, 6.03; N, 6.71; S, 8.20.

CHAPTER 5

Preparation of Aldehyde Azlactone [88]. ——

To 100 ml of a 50% mixture of acetic acid-water was added 1.26 g of the oxazolidine azlactone [85]. The clear solution was stirred for 1 hour at room temperature. The solution was then transferred to a separatory funnel and extracted three times with methylene chloride. The dried extracts were evaporated on a rotatory evaporator and the resulting oil was rid of traces of acetic acid by co-evaporation with methanol. The orange oil was dried on a high vacuum pump for several hours. The crude aldehyde was chromatographed on an aluminum oxide column (act. I) using methylene chloride and methanol (95:5). Yield of a light orange oil, 1.02 g (85%). An analytical sample was prepared by careful chromatography on silica gel (act. III) using benzene-chloroform (1:1). A crystalline aldehyde was obtained in this way, mp 79-81°. A positive aldehyde test (4-amino-3-hydrazino-5-mercapto-1, 2, 4-triazole) [55] was obtained for the above compound. Ir (CCl_4 solution), 3000, 2940, 2900, 1800 (azlactone C=O), 1740 (aldehyde) 1650(azlactone C=N), 1460, 1390, 1300, 1280, 1230, 1170, 1070 cm^{-1};

nmr ($CDCl_3$) δ 1.5, 1.55 (double singlet, 6), 3.3-3.7 (broad 2, OH), 3.95 (s, 2, CH_2S), 4.4 [m, 2½, CH_2OC and <u>CH</u> $(OH)_2$], 7.8, 8.2 (m, 6), 10.3 (s,½, CHO). It was evident from the nmr data that the aldehyde and the hydrated aldehyde were in a ratio of 1:1. Mass spectrum (70eV) for hydrate m/e 350 (M^+-CH_3), 335 (M^+-CH_2O), 321 (M^+-CO_2), 303 (M^+-H_2O-CO_2), 263 (M^+-CO_2-acetone).

Anal. Calcd for $C_{17}H_{17}NO_5S$: C, 58.79; H, 4.93; N, 4.03; S. 9.21. Found: C, 58.70; H, 5.01; N, 4.13; S, 9.08.

Preparation of Aldehyde Acid [94]. ——

The azlactone [88] (365 mg) was stirred in 10 ml of 2N sodium hydroxide (20 equiv) for 2 hours. The clear solution was extracted with three 5 ml portions of methylene chloride to remove any starting materials and the resulting basic aqueous solution was passed through a column containing 12 ml of Rexyn 102 H^+ (2.2 equiv) using 50% methanol-water as eluent. The slightly acidic eluent was evaporated on a rotatory evaporator connected to a high vacuum pump (bath temperature < 40°). Yield of a yellow amorphous solid, 252 mg (66%). Tlc analysis (MeOH/CH_2Cl_2-1:1) showed a major spot at R_f 0.7 (60-65%). An analytically pure sample was not obtained. Ir (NaCl film) 3500-2500 (OH str of hydrate, acid) 1725 (aldehyde), 1710 (C=O str of unsaturated acid), 1670 (amide C=O), 1590, 1490, 1395, 1385, 1300-1220 (C-O str), 1070 cm^{-1}. A positive aldehyde test was obtained using the reagent described previously [55]. Uv analysis showed no absorption for the azlactone at 355 nm.

Preparation of Imino-Acid [95]. ——

The aldehyde [94] (380 mg , 1 mM) was placed in a 250 ml 3-necked round bottom flask, equipped with a water separator, and two serum stoppers. Anhydrous benzene (200 ml) was introduced and the sample was rigorously dried for 4-6 hours, by azeotropic removal of water. Anhydrous ammonia was then introduced into the refluxing benzene solution by a gas inlet tube through one of the serum stoppers. The introduction was continued for 24 hours. The cooled mixtures was evaporated to dryness to yield 345 mg (95% based on the non-hydrated form of the imino-acid) of a pale brown amorphous solid. This crude product was dissolved in a minimum amount of dry tetrahydrofuran and dry hydrogen chloride gas was introduced to the cooled solution. A recovery of 62.5% (336 mg) of the precipitated hydrochloride salt was obtained. The ir spectrum (CCl_4 solution) of the imino-acid showed characteristic absorptions for the carboxyl group (3500-2500 cm^{-1}) and the ammonium band (2500-2300 cm^{-1}). The aldehyde absorption at 1725 cm^{-1} was no longer apparent. The small discrepancy in the analysis of the hydrochloride salt can be attributed to the fact that the compound is extremely hygroscopic. The calculated values for the imine hydrochloride and the imine hydrochloride hydrate are compared with the found values. The analysis is consistent if ¼ of a mole of water of hydration is taken into account.

Anal. Calcd for $C_{17}H_{21}N_2O_5SCl$: C, 50.94; H, 5.24; N, 6.99; S, 7.99; Cl, 8.86.

Calcd for $C_{17}H_{21}N_2O_5SCl \cdot H_2O$: C, 48.75; H, 5.50; N, 6.69; S, 7.65; Cl, 8.48.

Calcd for $C_{17}H_{21}N_2O_5SCl \cdot \frac{1}{4}H_2O$: C, 50.37; H, 5.31; N, 6.91; S, 7.90; Cl, 8.77.

Found: C, 50.29; H, 5.29; N, 6.81; S, 7.78; Cl, 8.63.

Procedure for Preparation of Cyclohexyl Isonitrile. ——

The procedure used by Ugi [54] was exactly followed. A yield of 65% of a colorless foul-smelling liquid was obtained. Bp 56-58°/11 mm (67-72%). Ir (NaCl film) 2940, 2880, 2140 (C=N str), 1450, 1370, 1330 cm^{-1}.

o-Nitrophenyl Isonitrile. ——

To a cooled suspension of 1.66 g (1 equiv) of o-nitrophenyl formamide (prepared from the aniline derivative and formic acid, mp 122°) in 50 cc of dry methylene chloride was added 1.8 cc of triethylamine (2.2 equiv). Under a nitrogen atmosphere, 0.5 g of phosgene, dissolved in 5 ml of methylene chloride, was added dropwise. After the addition was complete, 50 cc of water was added and the organic layer was separated, dried and evaporated. The crude material was filtered through a silica gel (activity I) column as a benzene solution. A 40% yield (590 mg) of an analytically pure light yellow amorphous solid was obtained. Ir (KBr) 2135 (CN str), 1610, 1580, 1540, 1350, 860, 780, 740 cm^{-1}.

Anal. Calcd for $C_7H_4N_2O_2$: C, 56.76; H, 2.72; N, 18.91. Found: C, 57.03; H, 2.85; N, 19.04.

A similar procedure was used to prepare isopropyl isonitrile. A yield of 30% of a colorless foul smelling liquid was obtained [bp 86-87^{o} (lit ^{61} 86-87^{o})].

Cepham Cyclohexylamide [96]. ——

The crude imino-acid [95] (364 mg , 1 mM) was dissolved in a mixture of 10 ml of methylene chloride and 10 ml of carbon tetrachloride. An equivalent amount of water was added and the 2 phase system was vigorously stirred. 350 mg of cyclohexyl isonitrile (3 equiv) was added and the mixture was stirred under a nitrogen atmosphere, at room temperature for 12-15 hours. The two phases were separated and the water layer was extracted once with methylene chloride (caution - fumehood). The collected organic layers were washed with ice cold hydrochloric acid (5%), water, ice cold bicarbonate (5%) and finally water, to remove any starting materials. The dried extracts ($MgSO_4$) were evaporated. The resulting yellow oil was rid of any excess isonitrile on a high vacuum pump (traps filled with liquid nitrogen). Chromatography on silica gel (activity III) using a 7:3 mixture of benzene and acetone afforded 110 mg of the product (23% of theory). Ir (CCl_4 solution) 3440 (NH str, secondary amide), 3100, 3040, 3000, 2940, 2880, 1750 (β-lactam carbonyl) 1690 (amide), 1680 (amide), 1550-1520, 1490, 1460, 1390, 1380 (isopropyl doublet), 1270, 1220, 1070 cm^{-1}. Mass spectrum was previously discussed. The nmr spectrum (CCl_4) showed the presence of three groups of complex bands at 8.4-7.4, 5.0-2.8 and 2.0-1.0 ppm. The integration of the two former bands were consistent (5:9) but an accurate integration of the peaks at 2.0-1.0 ppm was not obtained due to contamination of the product with cyclohexyl isonitrile and cyclohexyl formamide, which could not be completely removed.

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