A NOVEL ANTIBACTERIAL AGENT BY DESIGN
2-CARBOXYMETHYL-5-HYDROXY-1,2-OXAZIN-3-ONE

by

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Abstract

The title compound is predicted to be the prototype of a new class of antibacterial agents targeted to the penicillin receptor. It and a number of its congeners have been synthesized for the first time by a double condensation of N-hydroxyglycine esters with a variety of 4-carbon synthons, including vinylacetic acid, maleic anhydride, succinic anhydride, hydroxysuccinic anhydride, butyrolactone, diketene and malic acid, followed by removal of protecting groups. Most of the synthetic routes examined lead, not to the desired six-membered 1,2-oxazin-3-ones, but to the five-membered 1,2-isoxazolidin-3-ones. Two of these isoxazolidinones, the 3-carboxymethyl-5-hydroxymethyl and the 3-carboxymethyl-5-hydroxy-5-methyl compounds, are structural isomers of each other and of the target compound, and exhibit no antibacterial activity versus *Micrococcus luteus*. The six-membered ring is chemically stable and antibacterially active, as predicted. Although the level of activity is not high, it is expected that this will be improved significantly by a number of obvious modifications of the initial structure, including the syntheses of optically active compounds. Some potential routes have been examined during this work. Others are proposed.

The successful route to the title compound proceeds in seven steps from diketene and the t-butyl ester of N-hydroxyglycine.

Dedication

To my kids

Acknowledgements

Goals and objectives are often achieved through sharing, dependence and assistance of human beings. It is therefore with a deep sense of appreciation that I acknowledge the following persons.

First of all my Senior Supervisor, Dr. Saul Wolfe, for his patience, financial support, close guidance and supervision. I also wish to thank Marcy Tracey, Greg Owens and M. K. Yang for providing the nmr, mass spectra and microanalyses, respectively, and Dr. G. Eigendorf of the University of British Columbia for providing the high resolution mass spectra.

Special thanks to my husband Dr. I. N. Iwuagwu, for his love, understanding and support and my laboratory colleague Stephen Ro for his assistance and friendship. I also wish to thank Rasmus Storjohann and Fred Chin for all their help and encouragement. Finally, I wish to express my gratitude to all my colleagues, friends and relatives, who encouraged me in one way or another throughout the duration of this work.

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List of Abbreviations

AD	asymmetric dihydroxylation
Bn	benzyl group
BOC	tert-butoxycarbonyl group
tBDMS	tert-butyldimethylsilyl group
mCPBA	meta-chloroperbenzoic acid
DBU	1,8-diazabicyclo [5.4.0] undec-7-ene
DCC	dicyclohexylcarbodiimide
DEAD	diethylazodicarboxylate
DMF	dimethylformamide
EI-MS	electron impact mass spectrometry
EtOAc	ethyl acetate
NBA	N-bromoacetamide
NBS	N-bromosucciniimide
Ph	phenyl
THF	tetrahydrofuran
TMSI	trimethylsilyl iodide

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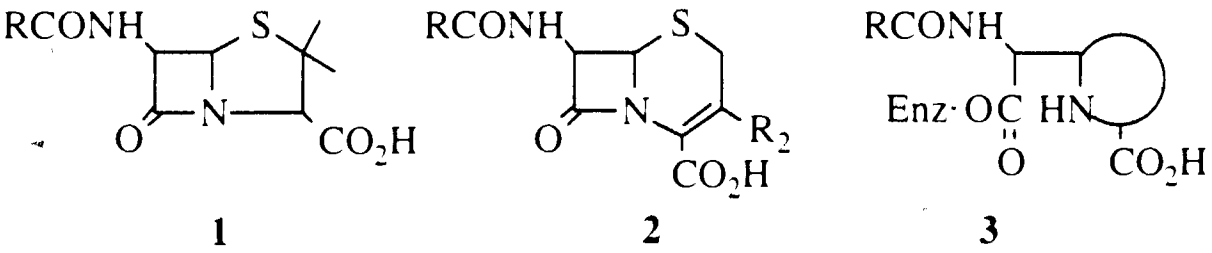
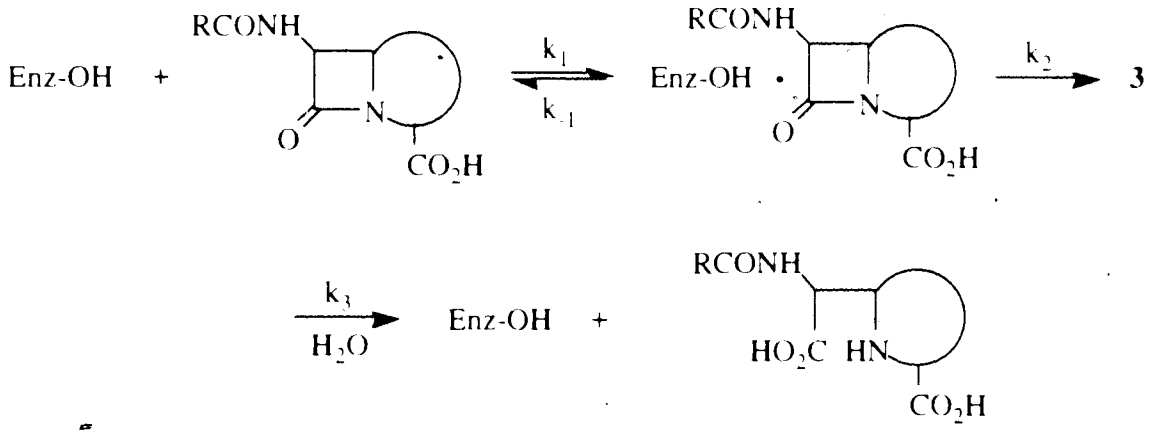
Introduction

β-Lactam antibiotics, whose members include the penicillins **1** (1) and cephalosporins **2** (2), function by interfering with the biosyntheses of bacterial cell walls (1). The target enzymes, known as penicillin-binding-proteins (PBP's) (2), are serine proteases, and the chemical reaction that inhibits these enzymes is the acylation of the hydroxyl group of the active site serine by the β-lactam ring (Scheme 1). The resulting acylenzyme (**3**) is unable to carry out the final step in the biosynthesis of the bacterial cell wall. The wall is weakened, becomes permeable to water, and the cell swells, bursts, and dies.



1 **2** **3**



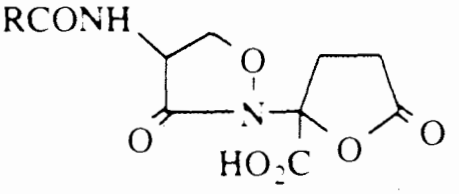
Scheme 1

In addition to the PBP's, many bacteria also produce a second type of penicillin-recognizing enzyme, known as a β-lactamase (3). The two enzymes exhibit the same kinetics (Scheme 1), but with different rate constants (4), and this difference in rate constants has important consequences. In the case of the PBP's, $k_2 >> k_3$. The result is that the enzyme is inhibited, and antibacterial activity may be observed. In the case of a β-lactamase, $k_2 \cong k_3$. These kinetics lead to regeneration of the enzyme, and inactivation of the antibiotic as a result of the net hydrolysis of the β-lactam bond. The latter sequence of reactions comprises the principal mechanism of bacterial resistance to β-lactam antibiotics.

Resistance to antibiotics is a problem of much current concern (5). Because of the relatively low cost and relative safety of the β-lactam group of antibiotics, and because many details of their mechanism of action and the mechanism of bacterial resistance are understood, one approach to the problem of resistance is to discover or invent new classes of compounds targeted to the penicillin receptor. Lactivicin (**4**) (6) is the first example of a non-β-lactam containing natural product that complexes to and reacts with penicillin-recognizing enzymes, and exhibits antibacterial activity. The pyrazolidinones (**5**), developed by the Lilly group (7) are the first examples of synthetic compounds to behave similarly.

In our laboratory, the design of new compounds targeted to the penicillin receptor has been based on three considerations: (i) the compound must be able to complex to the active site of a penicillin-recognizing enzyme. A model of the active site and a strategy for the docking of candidate structures to this model have been developed

(8-13); (ii) the compound must then be properly positioned within this site so as to be able to react with the hydroxyl group of the active site serine by a mechanism and with a rate constant comparable to those exhibited by a penicillin or cephalosporin. This mechanism has been elucidated (12-15); (iii) the resulting acyl-enzyme or its equivalent must then be unable to participate in the k_3 step of Scheme 1 (16). A first



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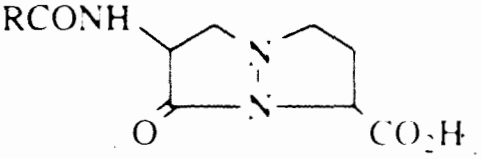

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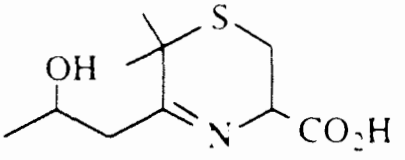

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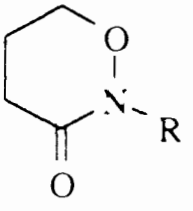

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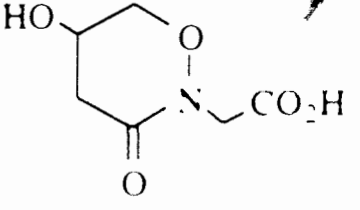


8

class of compounds (**6**) meeting these conditions has been synthesized and found to exhibit antibacterial activity. These compounds, and also the design strategy, have been patented (17).

Continued research in this laboratory by Dr. C. K. Kim has now led to the set of activation energies for reaction with a hydroxyl group summarized in Figure 1, relative to that of the bicyclic ring system of penicillin. The antibacterial activities relative to

penicillin of all known classes of β-lactam compounds and also of the ring systems of lactivicin and the pyrazolidinones parallel these reactivities exactly. The Figure includes the previously unknown 1,2-oxazin-3-one (**7**). Subsequent work by Dr. K. Yang suggested that **8** is a specific example of a compound containing this ring system which exhibits an acceptable fit to the model of the penicillin receptor. However, this work was unable to distinguish between the two enantiomers of **8**.

The work described here was initiated to explore potential synthetic approaches to the novel ring system **7**, to extend this work to the synthesis of **8**, and hopefully to provide both enantiomers of **8** of known absolute configuration.

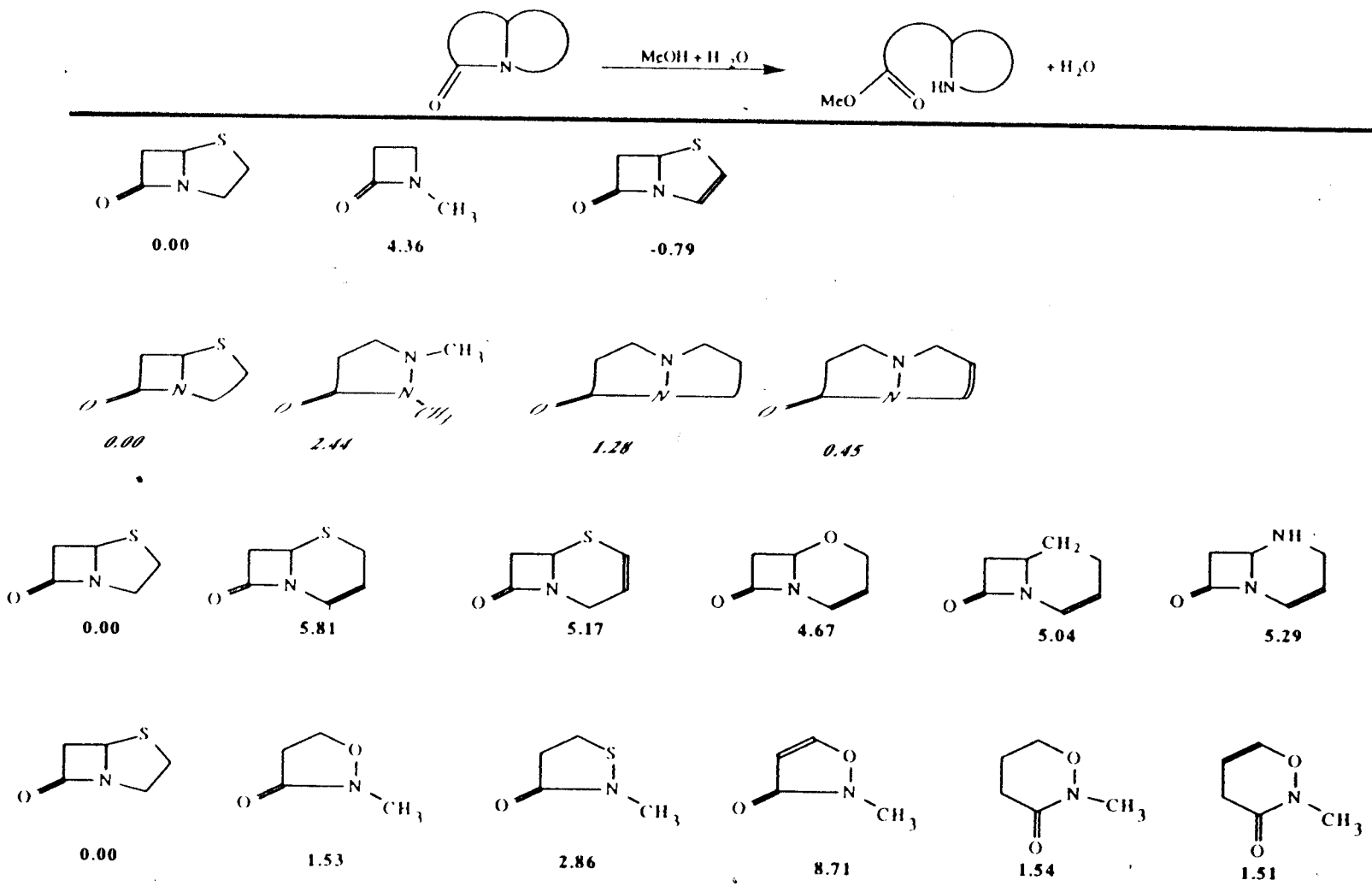
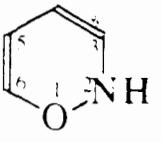


Figure 1: Calculated relative activation energies (kcal / mol) for the methanolysis of various lactams with catalysis by one molecule of water.

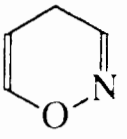
6-Membered Heterocyclic Rings Containing Oxygen and Nitrogen

Structural Types and Nomenclature

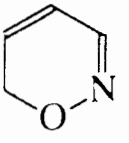
Six-membered heterocyclic rings containing oxygen and nitrogen are termed oxazines. There are numerous structural possibilities, depending on the relative positions of the two heteroatoms and the degree of oxidation of the ring system.



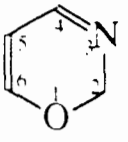
2H-1,2-



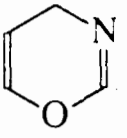
4H-1,2-



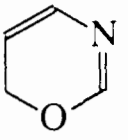
6H-1,2-



2H-1,3

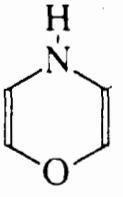


4H-1,3-



6H-1,3-

2H-1,4-



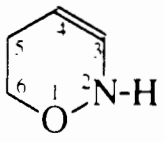
4H-1,4-

Further structural variation occurs when a single benzene ring is fused to the heterocycle, leading to benzoxazines. The dibenzo series are known as phenoxazines.

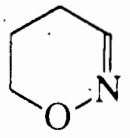
Although some dibenzo-1,2-oxazines are known, those based on the 1,4-system are more common and have a long history (18-21).

Hydrogenated derivatives of the monocyclic oxazines have also been widely studied. Some of them are so familiar that they are known by their trivial names; for

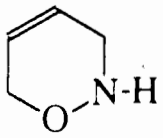
example, tetrahydro-1,4-oxazine is better known as morpholine. Some of the structural variations are shown below.



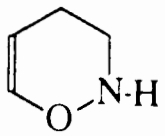
2H-5,6-



4H-5,6-

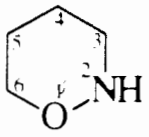


2H-3,6

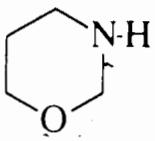


2H-3,4-

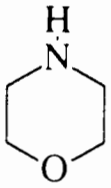
Dihydro-1,2-oxazines



1,2-

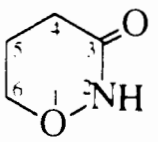


1,3-

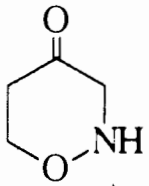


1,4-

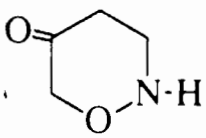
Tetrahydroxazines



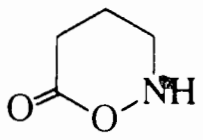
1,2-Oxazin-3-one



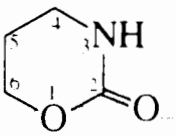
1,2-Oxazin-4-one



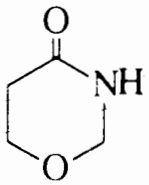
1,2-Oxazin-5-one



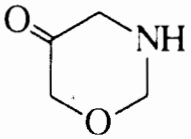
1,2-Oxazin-6-one



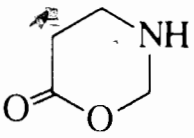
1,3-Oxazin-2-one



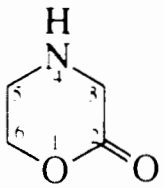
1,3-Oxazin-4-one



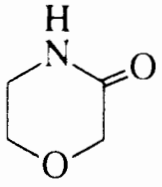
1,3-Oxazin-5-one



1,2-Oxazin-6-one



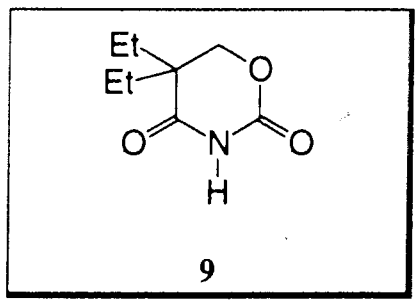
1,4-Oxazin-2-one



1,4-Oxazin-3-one

Oxazinones

Although there are many patents describing the antibacterial, antifungal and antiprotozoal activites of 1,3-oxazines (22-27), only one 1,3-oxazine derivative, 5,5-diethyltetrahydro-1,3-oxazine-2,4-dione (**9**) has been introduced into medicine. The compound has the trade names Dioxone, Dietadion and Diethadion. Some of its uses are as an analeptic and anticonvulsant (28-32). The compound also has central nervous system and antiinflamatory activity (33-35).



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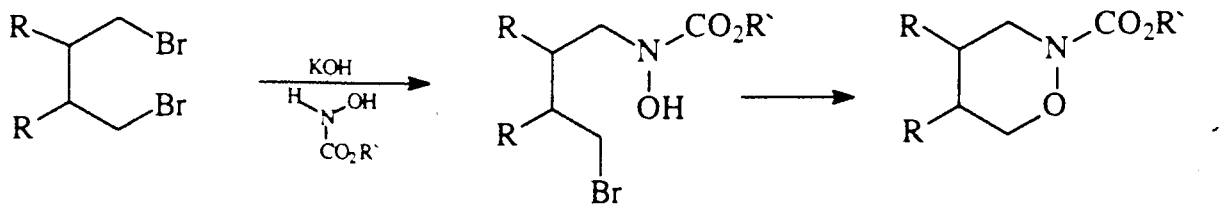
1.1.1 Syntheses of Tetrahydro-1,2-Oxazine

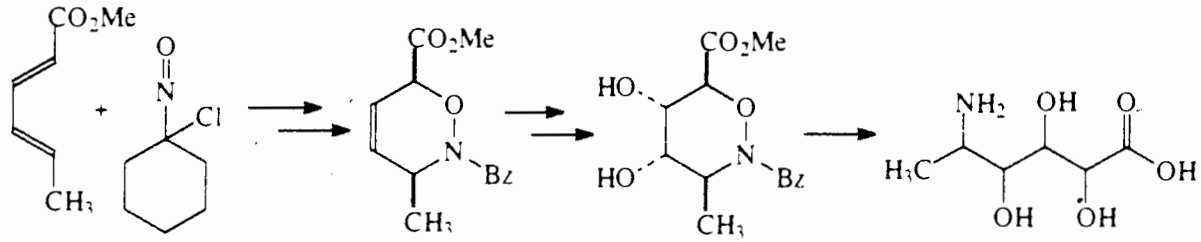
The first synthesis of the tetrahydro-1,2-oxazine ring system was accomplished by King in 1942 (36) by reaction of 1,4- and 2,5-dibromoalkanes with N-hydroxyurethanes. Riddell (37) later synthesized a series of tetrahydro-1,2-oxazines using this method.



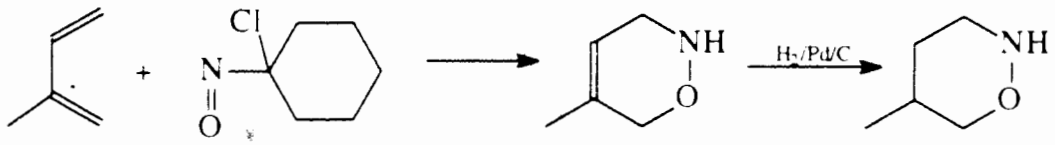
The tetrahydro-1,2-oxazines have also been synthesized by hydrogenation of dihydro-1,2-oxazines, accessible by Diels-Alder reactions of nitroso compounds, as summarized below.

Reaction of Butadiene with Nitroso Compounds (38, 39)

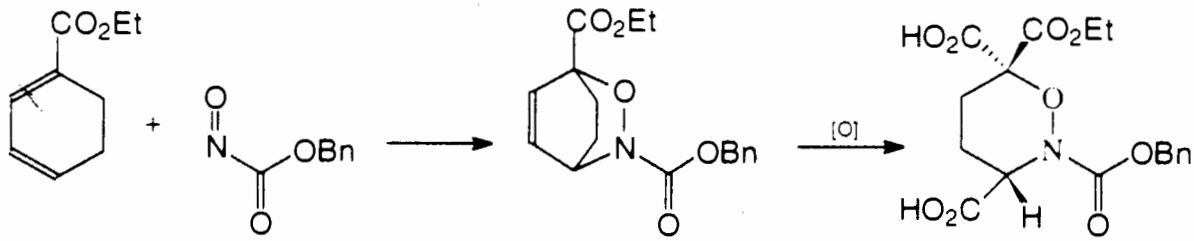
(*i*)



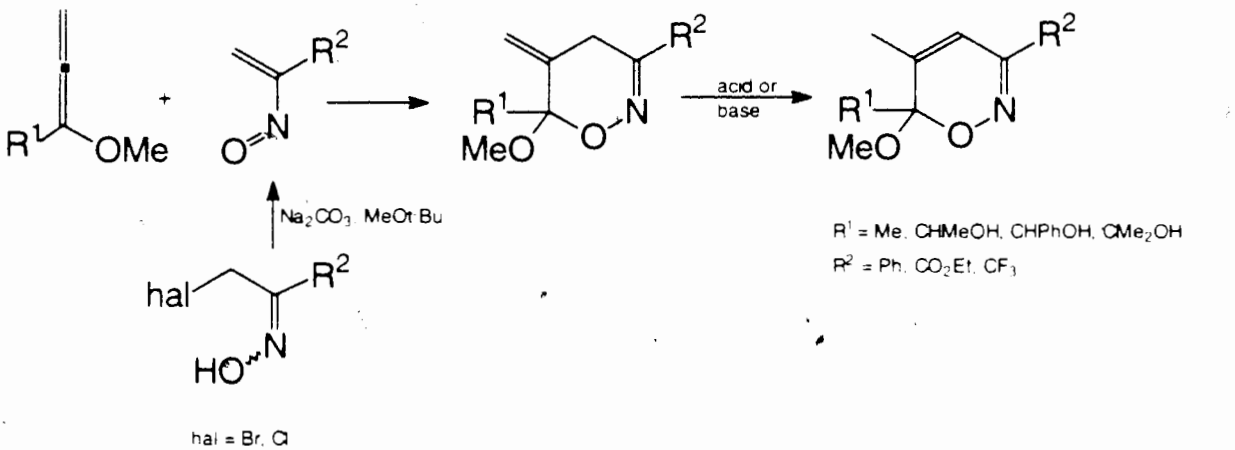
(*ii*) (40, 41)



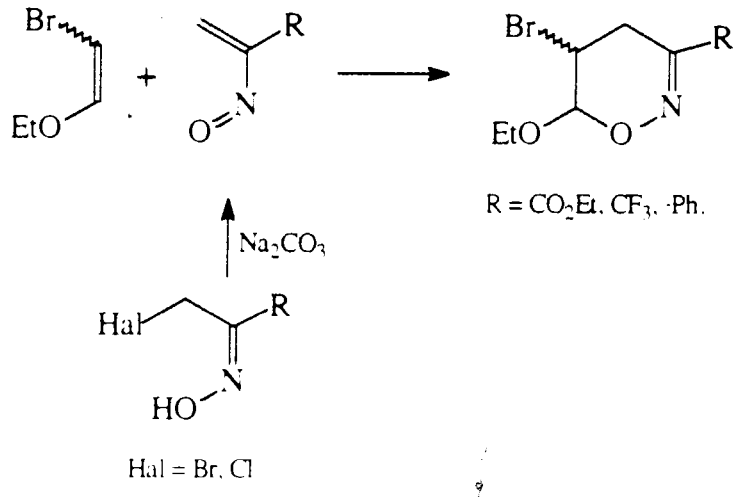
(*iii*) (42)



Reaction of Methoxyallenes with Nitrosoalkenes (43)

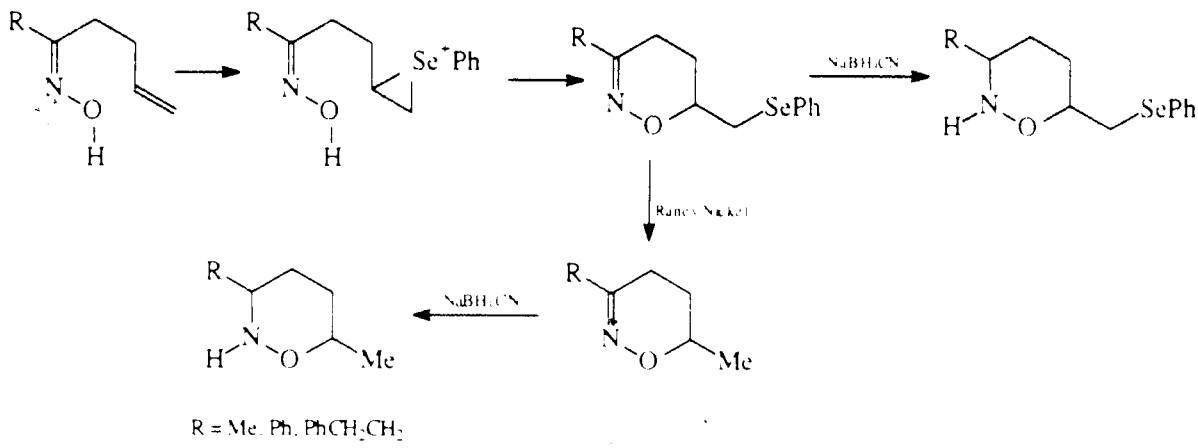


Reaction of β-Bromoenol Ether with Nitrosoalkenes (44)

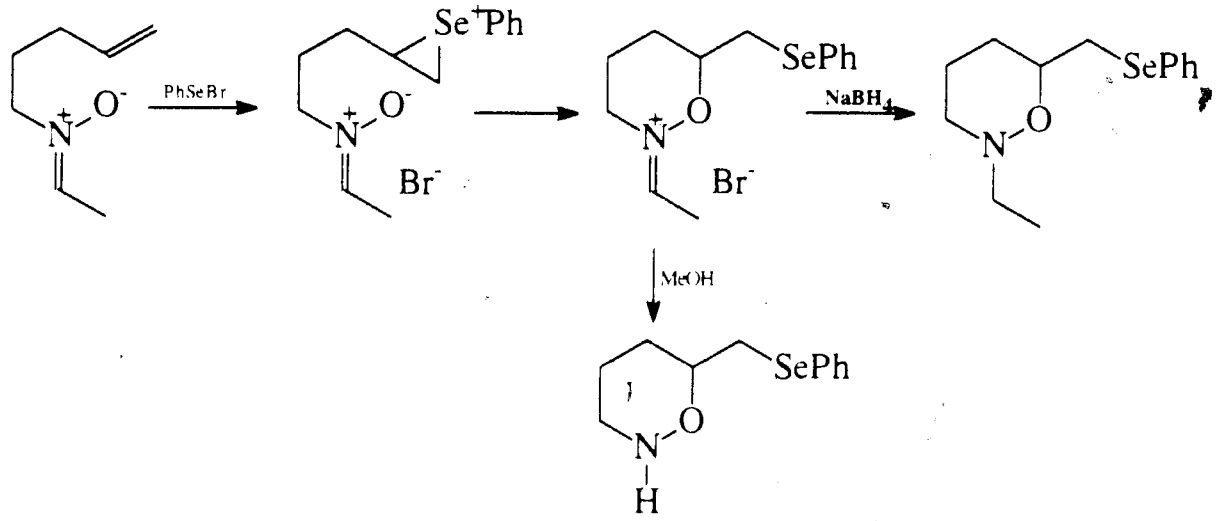


Other approaches to the tetrahydro-1,2-oxazine ring system include the reaction of phenylselenylating agents with alkenyl oximes (45) and nitrones (46) and by 9,10-dicyanoanthracene (DCA)-sensitized irradiation of γ,δ-unsaturated oximes (47) as exemplified in *i*, *ii* and *iii* below.

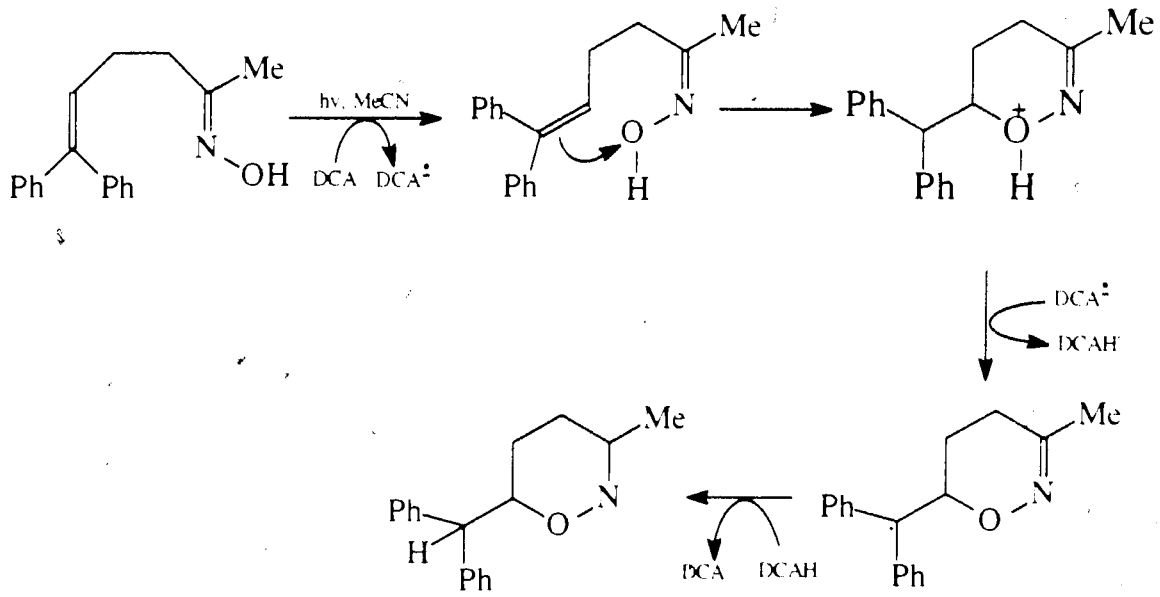
(*i*)



(ii)



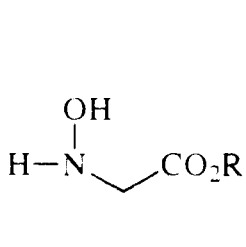
(iii)



None of the above methods was applicable to the synthesis of the 1,2-oxazin-3-one **8**, and a different approach to this ring system had to be devised.

2. Results and Discussion

Retrosynthetic analysis of **8** suggested N-hydroxyglycine **10**, R = H, or one of its esters, and a four-carbon synthon as the major building blocks. The four-carbon synthons examined in this work were γ-butyrolactone (**11**), vinylacetic acid (**12**), 3,4-dihydroxybutanoic acid (**13**), succinic anhydride (**14**), hydroxysuccinic anhydride (**15**), maleic anhydride (**16**), diketene (**17**) and 2,2,6-trimethyl-4H-1,3-dioxin-4-one (**18**).



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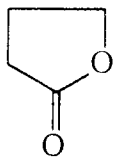

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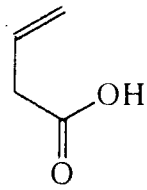

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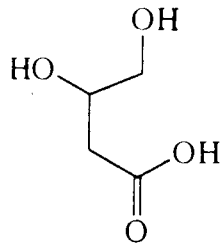

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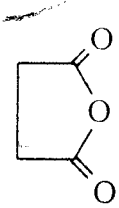

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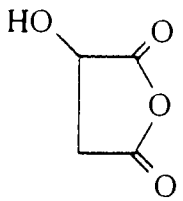

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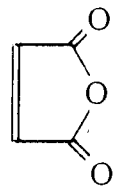
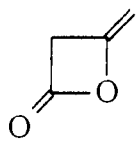
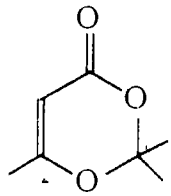


16



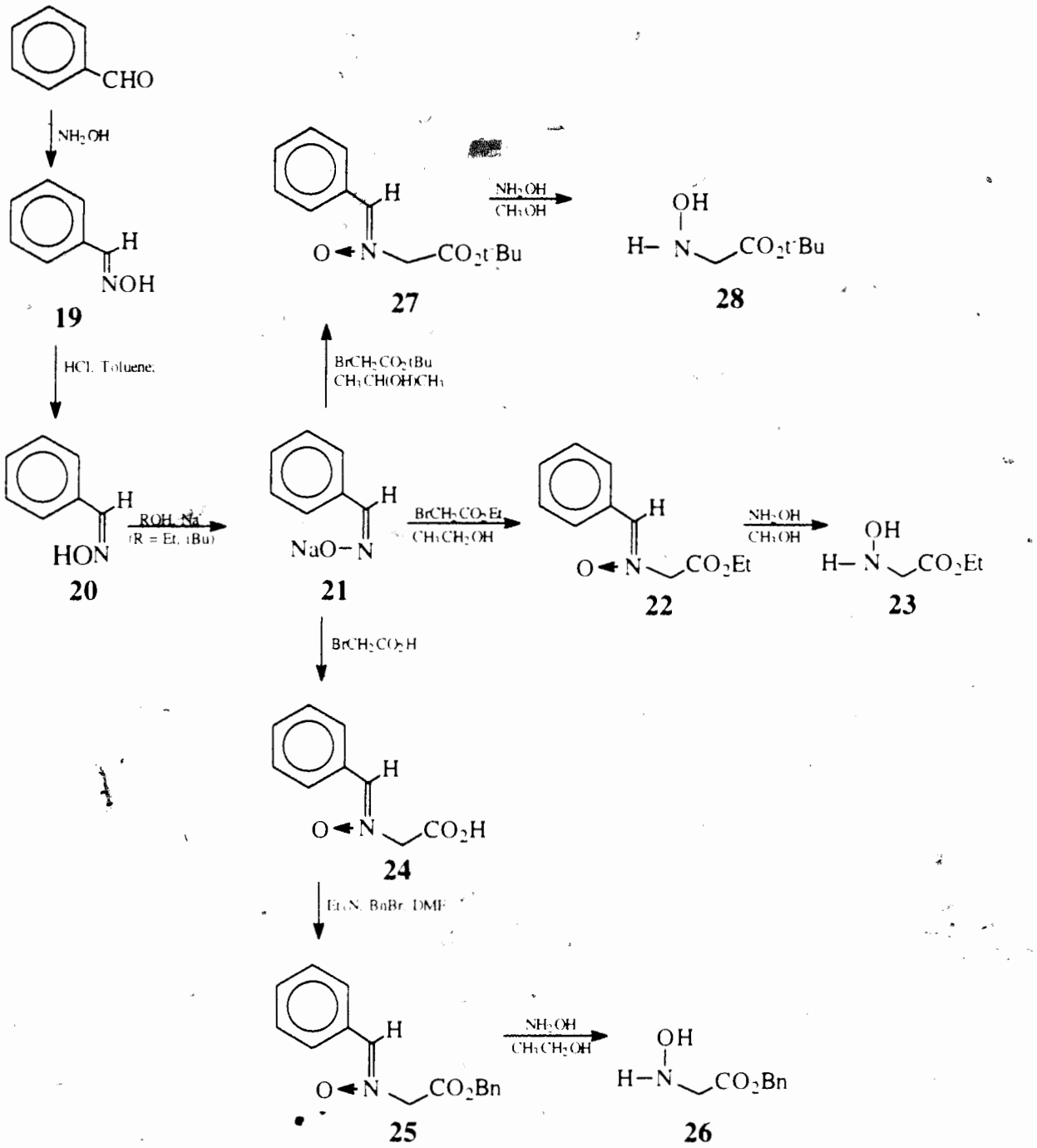
17



18

Scheme 2 summarizes the syntheses of the ethyl, benzyl and t-butyl esters of N-hydroxyglycine. Treatment of benzaldehyde with hydroxylamine gave E-benzaldoxime **19**, which upon treatment with hydrogen chloride in refluxing toluene afforded Z- benzaldoxime hydrochloride. Neutralization afforded Z-benzaldoxime **20** (48) in 70% overall yield from benzaldehyde. Reaction of the sodium salt of Z-benzaldoxime (**21**) with ethyl bromoacetate in ethanol produced N-benzylideneglycine N-oxide ethyl ester **22** (49), and N-hydroxyglycine ethyl ester **23** was obtained in 80% yield

when **22** was treated with hydroxylamine (50). The benzyl ester **26** was synthesized by

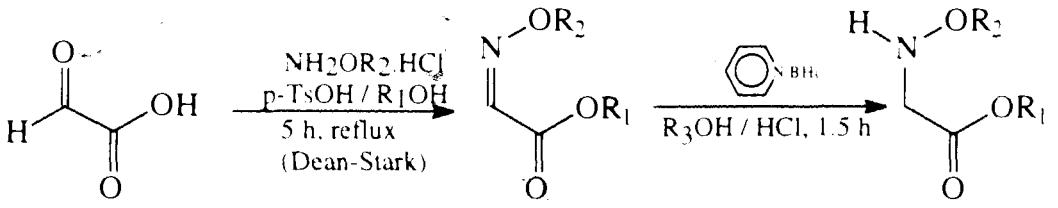


Scheme 2

the reaction of **21** with bromoacetic acid in ethanol, esterification of the acid **24** and treatment of the nitrone **25** with hydroxylamine. The t-butyl ester **28** was obtained by

treatment of **21** with t-butyl bromoacetate in 2-propanol, followed by reaction with hydroxylamine (50).

The methyl ester **30** was obtained by reaction of glyoxylic acid with hydroxylamine hydrochloride in refluxing methanol (51) and reduction of the resulting oxime **29** (R_1 = Me, R_2 = H) with pyridine-borane (52). The use of ethanol as the solvent for the initial step led to the ethyl ester **23**. The O-benzyl ethyl ester **31** (R_1 = Et, R_2 = OBn) was obtained using O-benzylhydroxylamine hydrochloride in the first step.

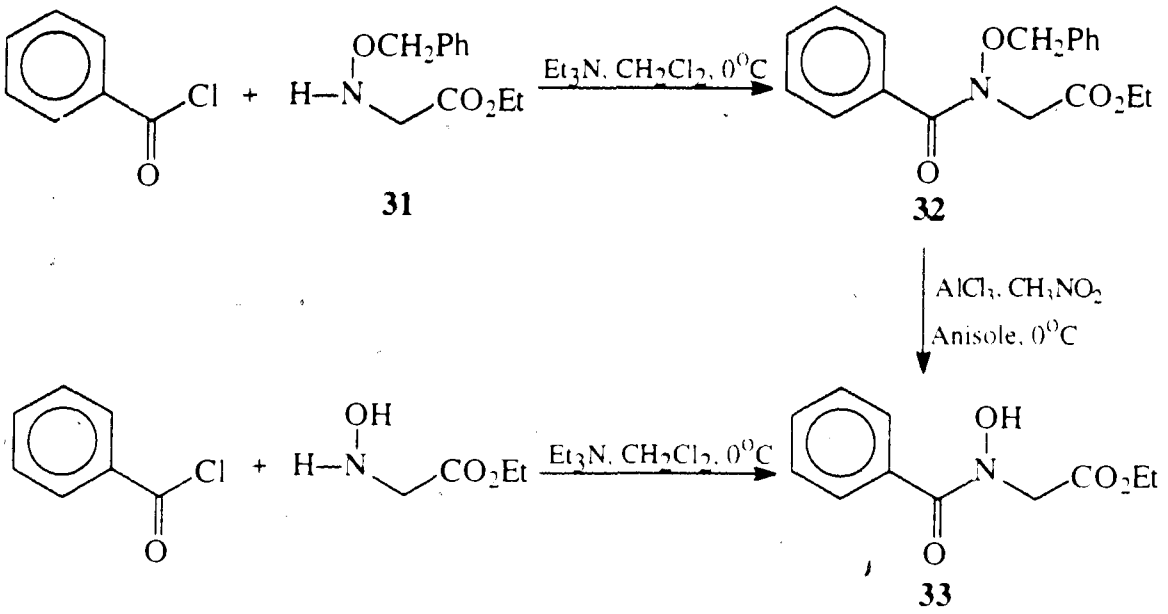


29

23 $R_1 = R_3$ = Et, R_2 = H
30 $R_1 = R_3$ = Me, R_2 = H
31 $R_1 = R_3$ = Et, R_2 = CH_2Ph

2.1. Vinylacetic Acid and Cyclic Anhydrides as Synthons

The reaction of **31** with benzoyl chloride gave **32**, which was debenzylated by treatment with aluminium trichloride and anisole in nitromethane to give **33** (53). The same compound was obtained upon reaction of N-hydroxyglycine ethyl ester with benzoyl chloride, demonstrating that the N-hydroxyglycine function undergoes N- and not O-acylation.

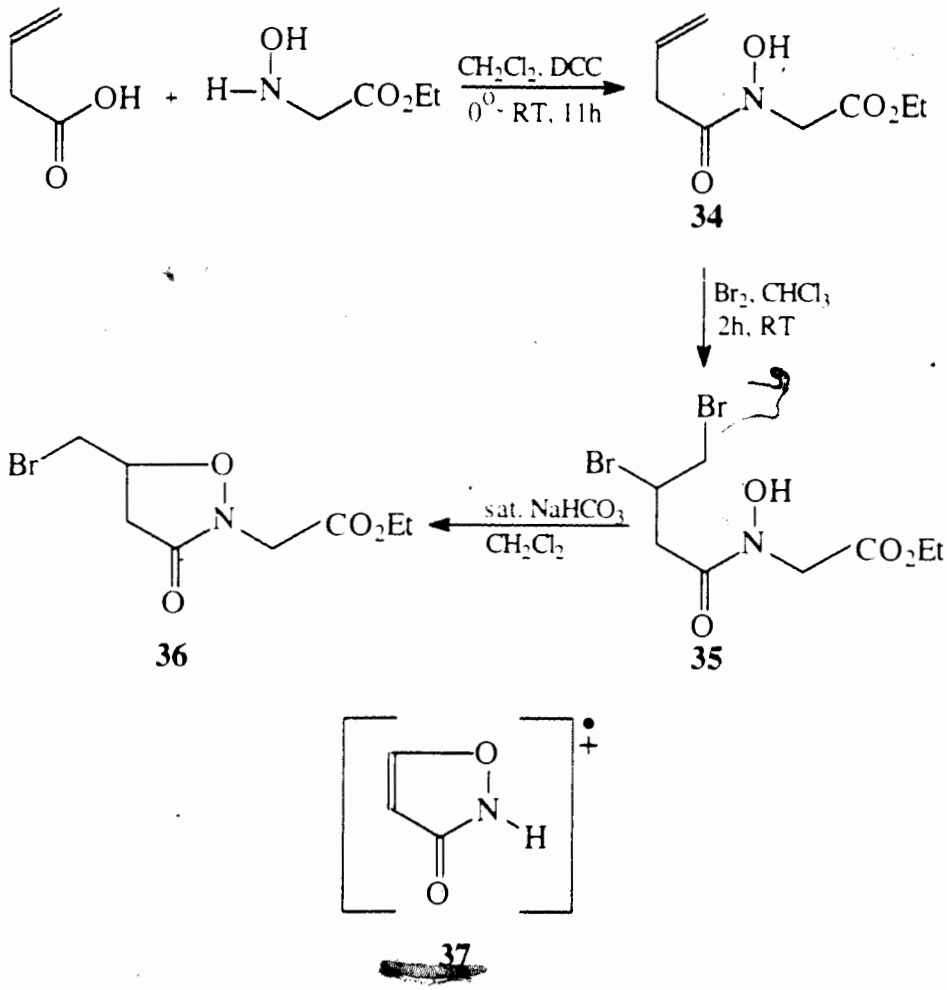


Following these preliminary experiments, vinylacetic acid and **23** were coupled in dichloromethane, using dicyclohexylcarbodiimide (DCC), to give vinylacetamido N-hydroxyglycine ethyl ester **34**. The infrared absorption at 1626 cm^{-1} typical of an amide functional group was the same as that seen in **33**.

Bromination of **34** in chloroform at room temperature gave a dibromide **35** which upon treatment with sodium bicarbonate yielded a colourless oil. The elemental analysis and the mass, infrared and proton nmr spectra of this compound were consistent with the isoxazolidinone structure **36**. The infrared absorptions at 1710 and 1755 cm^{-1} were assigned to the carbonyl groups of the ester and isoxazolidinone ring. The proton nmr spectrum showed a downfield shift of the tertiary hydrogen from 4.55 ppm in the dibromide **35** to 4.80 ppm in **36**.

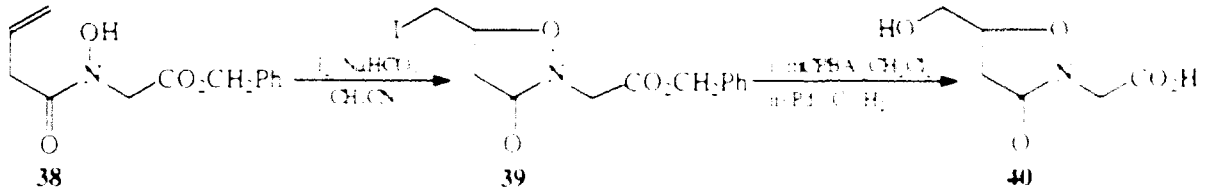
The EI mass spectrum showed peaks at m/z 85, 93 and 95. These were assigned to the five-membered ring **37** and the $BrCH_2$ side chain of **36**. The presence of a

peak at m/z 85 was used subsequently to confirm the presence of a 5-substituted isoxazolidinone ring.



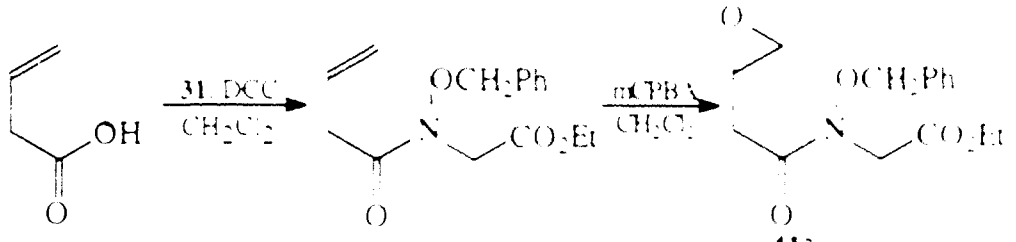
The bromide **36** was stable to the action of silver acetate in refluxing acetic acid. Attempts to displace the bromine atom of **36** using sodium formate in dimethylformamide (54), potassium phthalimide in refluxing toluene (55), sodium azide and tetrabutylammonium bromide in refluxing benzene (56) and triethylamine and formic acid in acetonitrile (57) were not successful (58). Therefore, the benzyl ester **39** was synthesized by coupling of N-hydroxyglycine benzyl ester with vinylacetic acid, followed

by iodolactonisation (59, 60) with iodine and sodium bicarbonate in acetonitrile. Peracid oxidation of **39** (61), followed by hydrogenolysis, yielded the hydroxyacid **40**. This acid, which is an isomer of the target compound **8**, exhibited no antibacterial activity versus *E. coli* or *M. luteus*.



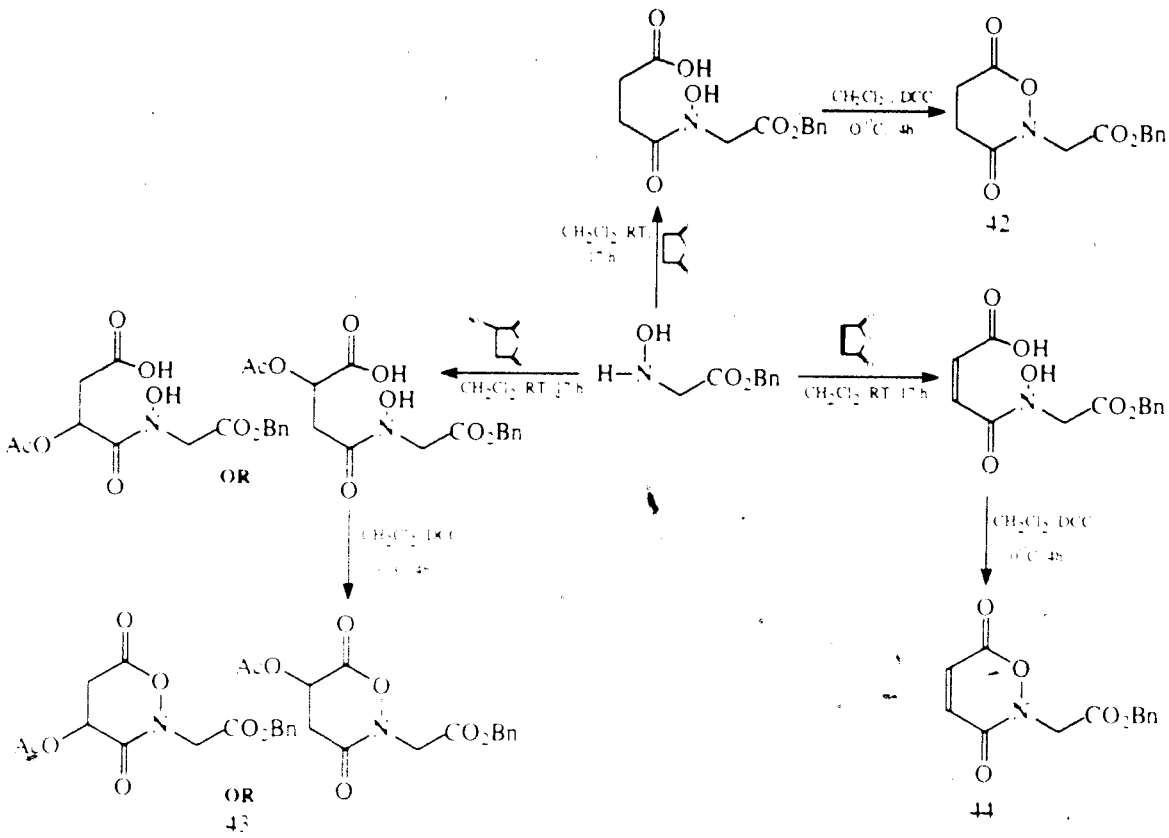
38 **39** **40**

It was then reasoned that benzylation of the hydroxyl group of an *N*-hydroxyglycine ester followed by condensation with vinylacetic acid, conversion of the olefin to a $CH(OR)$-CH_2X functionality and removal of the protecting group would allow cyclization to a six-membered ring. Coupling of **31** with vinylacetic acid followed by epoxidation with m-chloroperbenzoic acid in dichloromethane gave **41**



41

Attempts to open the epoxide and remove the benzyl group of **41** simultaneously by treatment with aluminium trichloride and anisole in nitromethane (53) or oxalyl chloride and triethylamine (62) failed. Other non-hydrogenolytic debenzylation methods employed, e.g., reaction with *N*-bromosuccinimide and calcium carbonate in water (63), and treatment with iodotrimethylsilane in acetonitrile also failed.

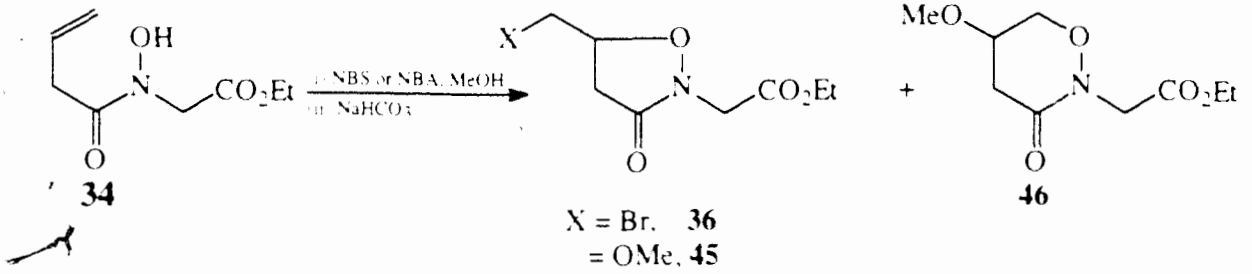


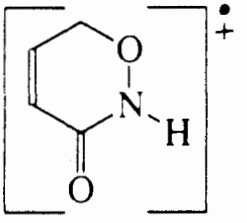
Scheme 3

The first successful syntheses of oxazinones were achieved in the present work by condensation of N-hydroxyglycine esters with cyclic anhydrides in dichloromethane, followed by carbodiimide cyclization in the same solvent (64). The reactions leading to **42**, **43** and **44**, are summarized in Scheme 3.

Reaction of the alkene **34** with N-bromosuccinimide or N-bromoacetamide in methanol, followed by a bicarbonate wash, afforded a mixture of three products. Separation by preparative layer chromatography (plc) yielded **36** (7.4%) and a mixture of two isomers (22%). The ^{1}Hmr spectrum of this mixture is shown in Figure 2. The isomers were identified as **45** and **46**, initially on the basis of their gc-ms fragmentation patterns. Using a DB-1 column (30 meters), the gc exhibited peaks at 8.07 and 8.43 min. The peak

at 8.07 min exhibited an EI-MS fragmentation at m/z 99, assigned to **47**, and the peak at 8.43 min exhibited the EI-MS fragmentation at m/z 85 previously assigned to **37**. These observations suggested that the two isomers were **45** and **46**, and detailed analysis of the ^{1}Hmr spectrum of **45** and **46** as shown in Figure 2 and Tables 1 and 2, was in agreement with this assignment.





47

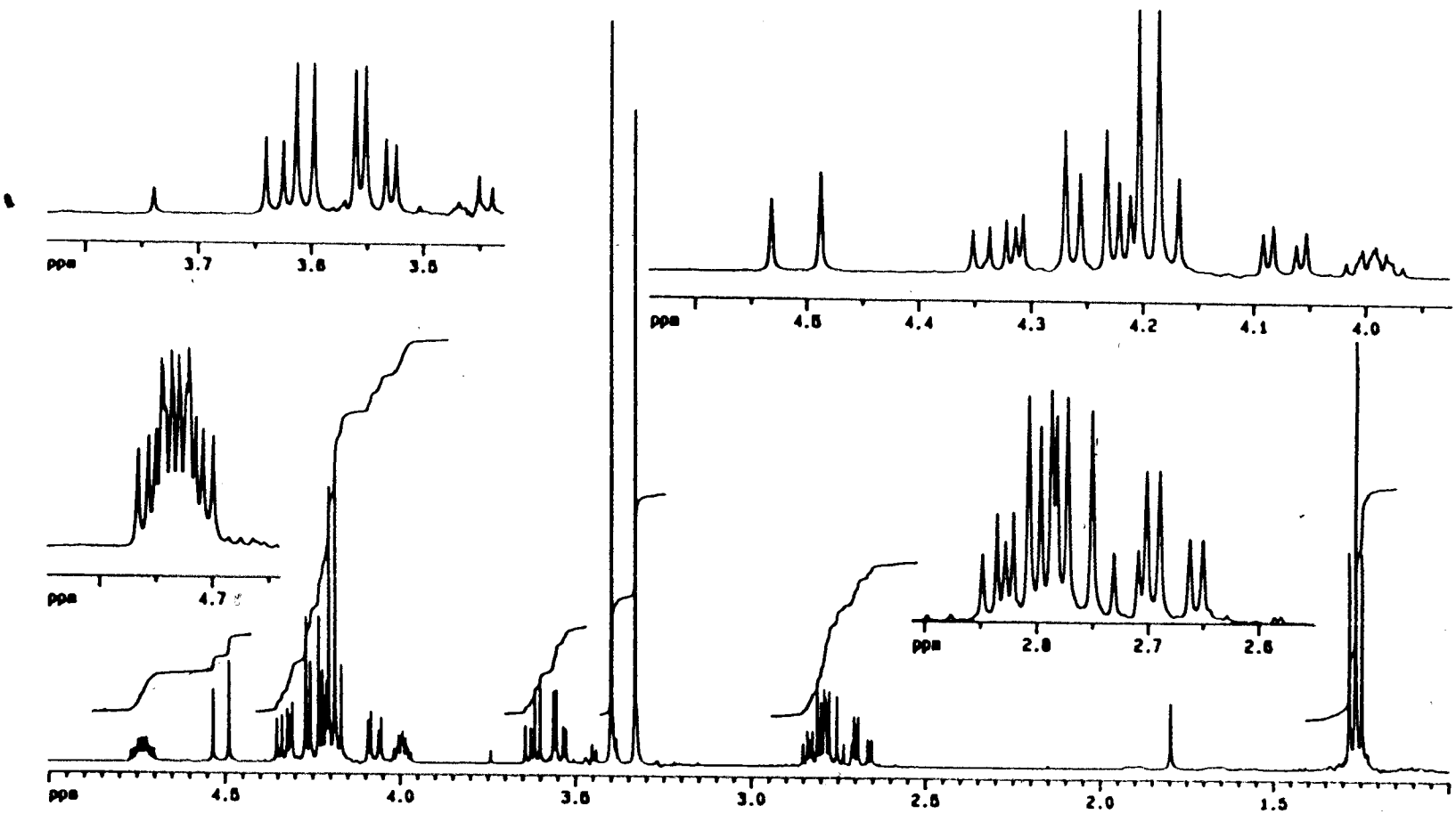
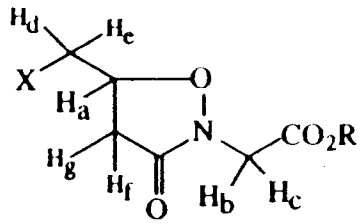


Figure 2: ^{1}Hmr spectrum of an approximately 1:1 mixture of 5- and 6-membered rings (ethyl ester).

Table 1: Summary of the ^{1}Hmr spectra of 1,2-Isoxazolidin-3-ones having a CH_2X substituent at C5

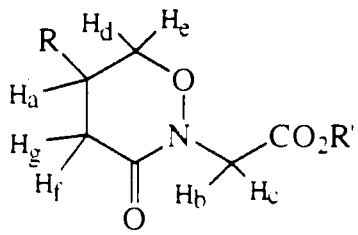


	R = Me[a] X = Br		R = Et[a] X = Br		R = Et[a] X = I		R = Bn[a] X = I		R = Et[a] X = OH		R = Bn[a] X = OH		R = H[b] X = OH		R = Me[a] X = OMe		R = Et[a] X = OMe		R = t-Bu[a] X = OMe	
	δ_{ppm}	J_{Hz}	δ_{ppm}	J_{Hz}	δ_{ppm}	J_{Hz}	δ_{ppm}	J_{Hz}	δ_{ppm}	J_{Hz}	δ_{ppm}	J_{Hz}	δ_{ppm}	J_{Hz}	δ_{ppm}	J_{Hz}	δ_{ppm}	J_{Hz}	δ_{ppm}	J_{Hz}
H_a	4.78		4.78		4.67		4.60		4.66		4.63		4.72		4.74		4.74		4.73	
H_b	4.29	17.0	4.29	17.0	4.26	17.9	4.33	17.8	4.57	18.0	4.57	18.0	4.36	18.2	4.28	17.8	4.28	17.8	4.20	17.7
H_c	4.28	17.0	4.28	17.0	4.24	17.9	4.30	17.8	4.06	18.0	4.13	18.0	4.34	18.2	4.22	17.8	4.22	17.8	4.12	17.7
H_d	3.58	10.8 5.5	3.58	10.8 5.5	3.39	10.4 5.5	3.33	10.4 5.5	3.91	12.8 2.4	3.87	12.8 2.5	3.78	12.9 3.2	3.62	10.9 6.2	3.62	10.9 6.2	3.63	10.9 6.2
H_e	3.53	10.8 6.7	3.53	10.8 6.8	3.35	10.4 7.4	3.28	10.4 7.6	3.67	12.8 4.0	3.66	12.8 4.2	3.74	12.9 5.6	3.54	10.9 3.6	3.54	10.9 3.6	3.55	10.9 3.6
H_f	3.01	16.8 8.2	3.01	16.8 8.2	3.00	16.8 8.0	2.98	16.8 8.0	2.96	16.6 8.7	2.92	16.7 8.6	3.02	17.0 8.8	2.81	16.6 8.2	2.81	16.6 8.2	2.81	16.6 8.2
H_g	2.83	16.8 7.0	2.83	16.8 7.0	2.76	16.8 7.2	2.73	16.8 7.2	2.90	16.6 6.2	2.87	16.7 6.7	2.79	17.0 7.6	2.75	16.6 8.8	2.75	16.6 8.8	2.74	16.6 8.7

[a] The solvent was $CDCl_3$

[b] The solvent was D_2O

Table 2: Summary of the ^{1}Hmr spectra of 1,2-Oxazin-3-ones

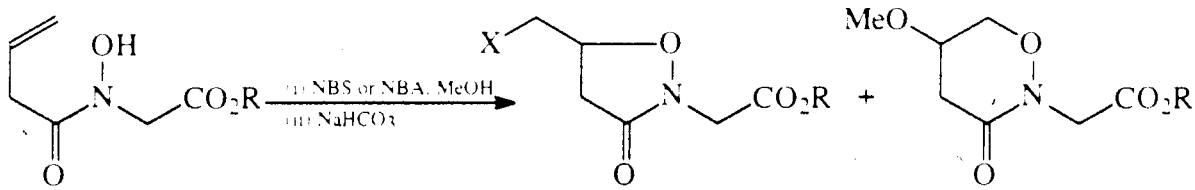


	R = OMe[a] R' = Me		R = OMe[a] R' = Et		R = OMe[a] R' = t-Bu		R = OH[b] R' = Na	
	δ_{ppm}	J_{Hz}	δ_{ppm}	J_{Hz}	δ_{ppm}	J_{Hz}	δ_{ppm}	J_{Hz}
H_a	3.99		3.99		4.01		4.49	
H_b	4.51	17.8	4.51	17.8	4.43	17.7	4.28	17.4
H_c	4.22	17.8	4.23	17.8	4.13	17.7	4.09	17.4
H_d	4.32	11.9 6.0	4.33	11.9 6.0	4.34	11.9 6.0	4.29	12.0 4.6
H_e	4.07	11.9 3.2	4.07	11.9 3.2	4.07	11.9 3.6	4.02	12.0 3.2
H_f	2.79	16.0 5.8	2.80	16.0 5.8	2.90	15.6 5.9	2.92	16.8 5.9
H_g	2.67	16.0 4.7	2.68	16.0 4.7	2.75	15.6 4.8	2.52	16.8 3.2

[a] The solvent was $CDCl_3$
[b] The solvent was D_2O

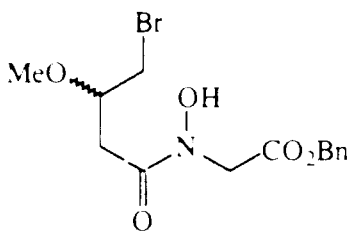
Attempts to separate **45** and **46** by chromatographic methods (plc and hplc) failed. Treatment of the mixture with trimethylsilyl chloride and sodium iodide in acetonitrile (65) at room temperature or at reflux temperature led to partial separation of **46**. The same result was obtained using lithium iodide in refluxing dimethylformamide (66).

The reaction of **48** with N-bromosuccinimide or N-bromoacetamide in methanol, followed by a sodium bicarbonate wash, gave the methyl esters of the 5- and 6-membered rings (Figure 3) while the same sequence of reactions with **38** and **49** gave a mixture of the acyclic bromomethoxy isomers as the major products and only a trace of cyclized products. The high resolution chemical ionization mass spectrum of the product from **38** had a peak for $C_{14}H_{19}BrNO_5$, assigned to **38a** or **38b.** The high resolution chemical ionization mass spectrum of the product from **49** contained peaks for $C_7H_{11}NO_5$, assigned to **49a** or **49b** and $C_{11}H_{19}BrNO_5$ assigned to **49c** or **49d**. The synthetic route to these mixtures from the various alkenes is summarized below.

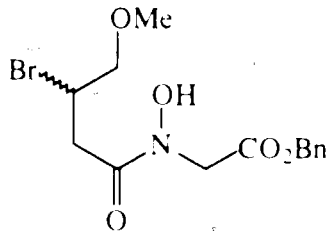


R = Bn, **38**
Me, **48**
t-Bu, **49**

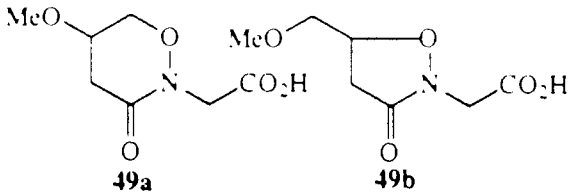
X = Br, OMe



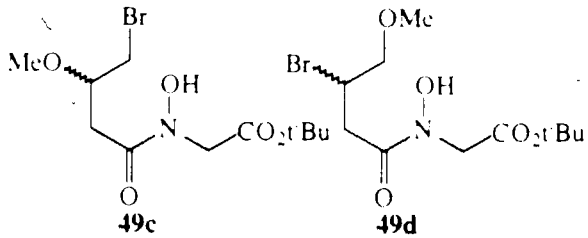
38a



38b



49a **49b**



49c **49d**

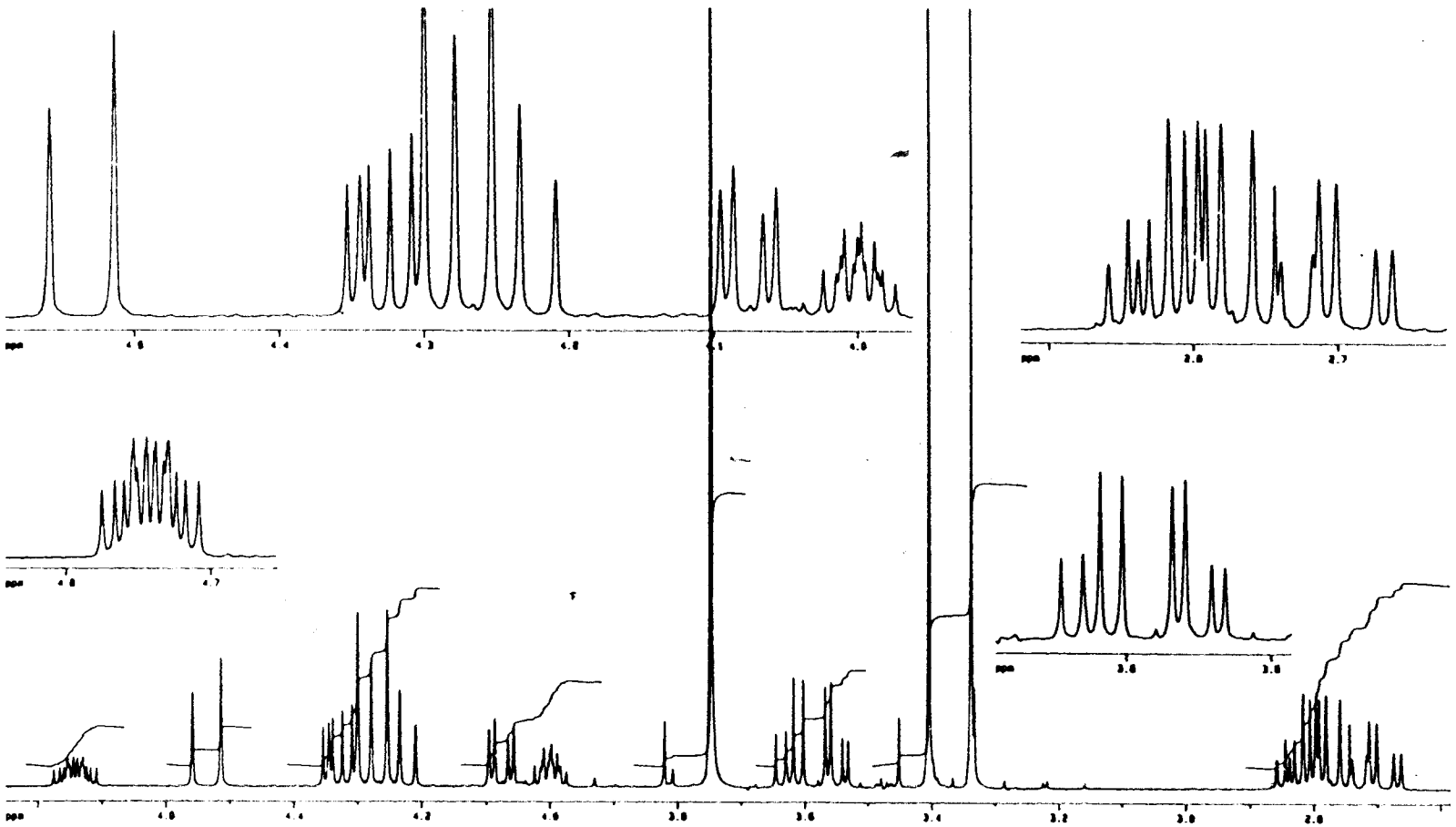
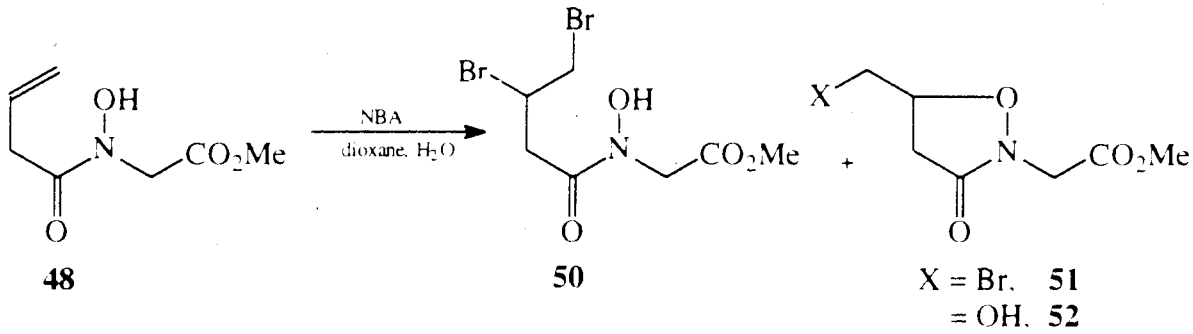


Figure 3: ^{1}Hmr spectrum of an approximately 1:1 mixture of 5- and 6-membered rings (methyl ester).

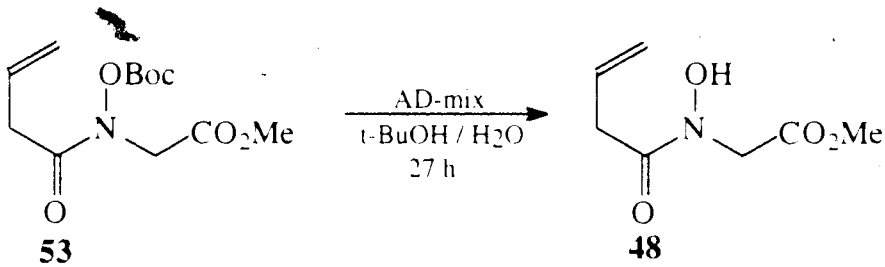
A mixture of **50**, m/z 331, 333, 335, **51**, m/z 251, 253 and **52**, m/z 189 was obtained when the alkene **48** was treated with N-bromoacetamide in 50% aqueous dioxane. The m/z 85 peak was seen in **52**, but the absence of a peak at m/z 99 indicated that a six-membered ring was not formed.



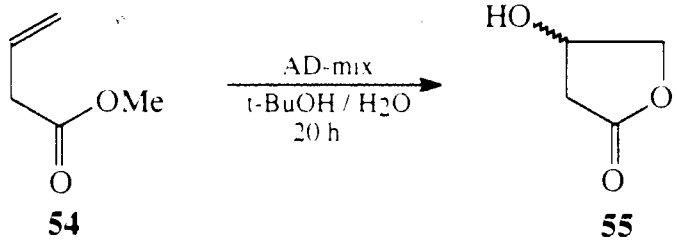
48 **50** X = Br, **51**
= OH, **52**

2.2. 3,4-Dihydroxybutanoic acid as a Synthon

Sharpless dihydroxylation of the alkene **48** (67) was not successful, and **48** and the hydroxylactone **55** were obtained when the hydroxylation was performed on the alkenes **53** and **54**, respectively.

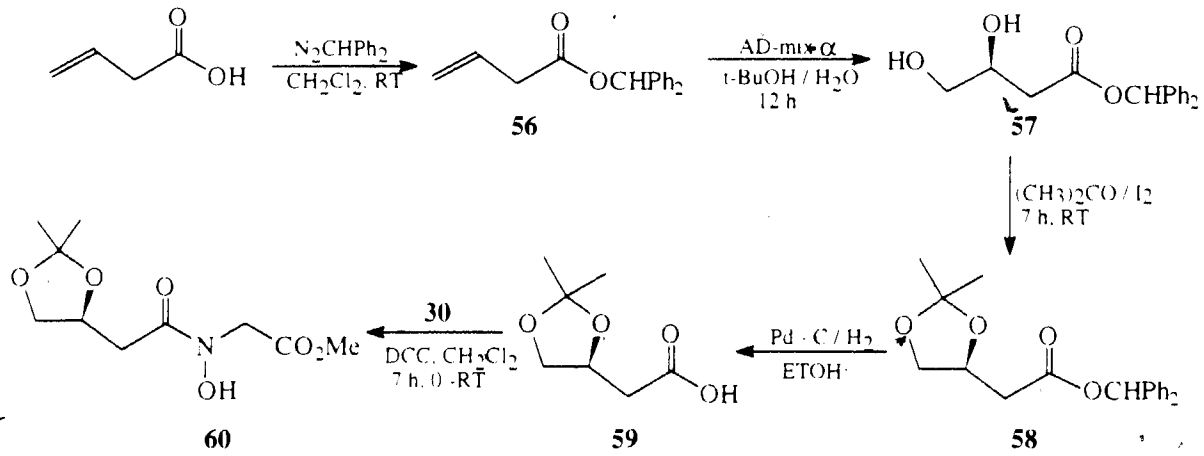


53 **48**



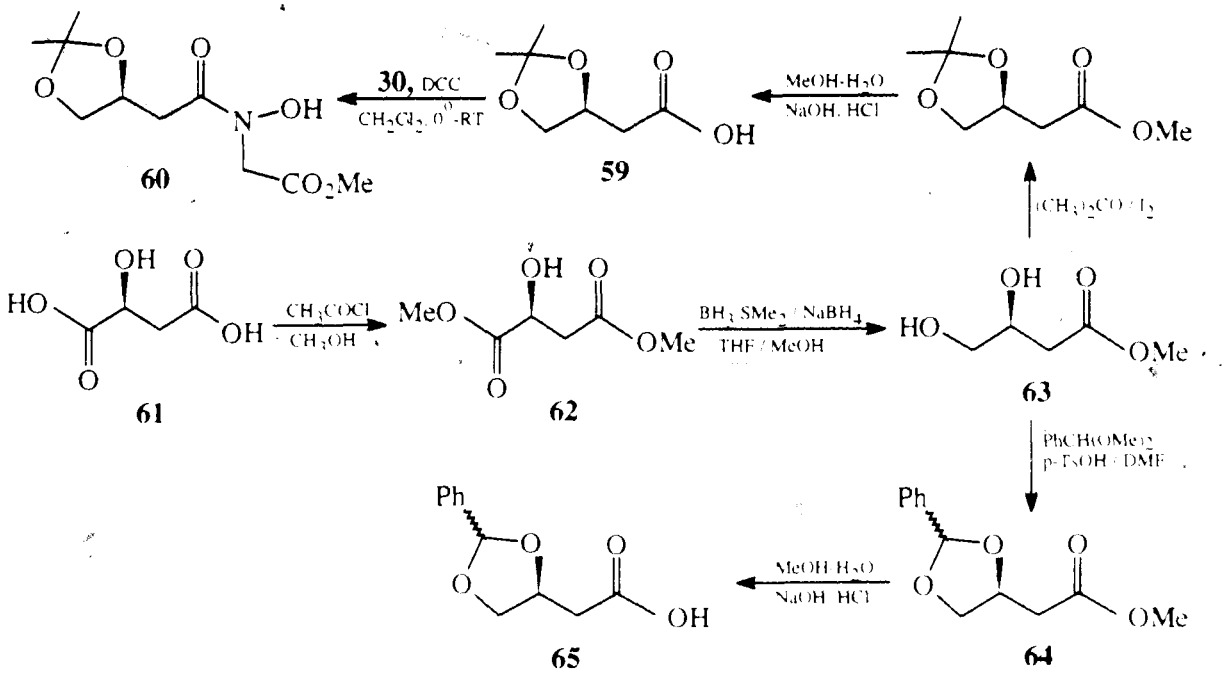
54 **55**

Treatment of vinylacetic acid with diphenyldiazomethane in dichloromethane afforded **56**, which upon dihydroxylation with AD-mix α in 50% aqueous t-butanol gave the diol **57** $[\alpha]_D^{21}$ - 6.33° (c 1.26°, EtOH). Hydrogenolysis of the benzyhydryl ester gave an acid which, upon attempted coupling with N-hydroxyglycine esters in 25% aqueous acetonitrile (68), cyclized to the lactone **55**. Therefore, **57** was treated with iodine in acetone (69) to give the acetonide **58** $[\alpha]_D^{22}$ + 3.25° (c 1.23, CH_2Cl_2). Hydrogenolysis of **58** afforded the acid **59** $[\alpha]_D^{21}$ - 3.95° (c 0.253, CH_2Cl_2).

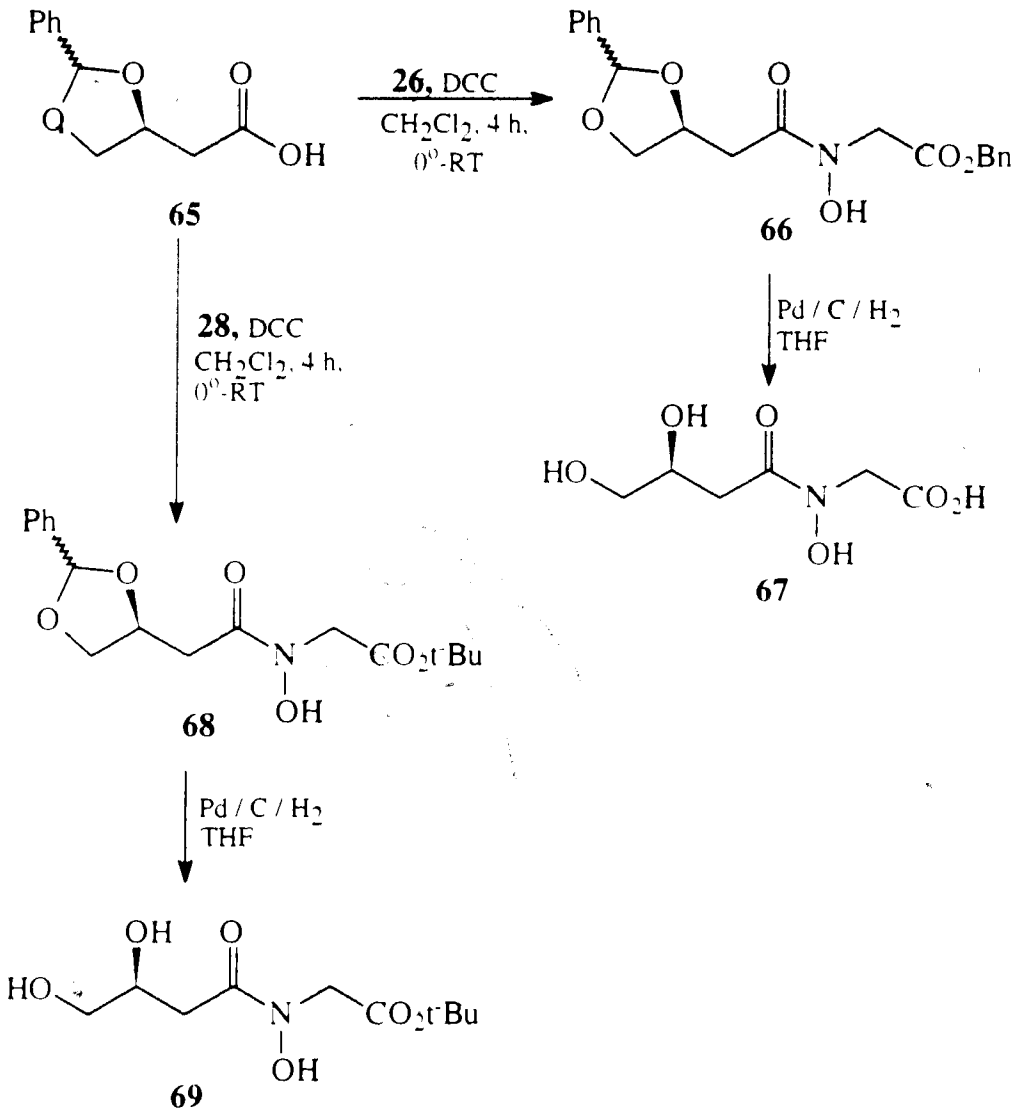


An alternative route to optically active **59** began with (*S*)-(-)-malic acid **61** which was converted to (S)-(-)-dimethyl malate **62** $[\alpha]_D^{21}$ -11° (c 2.2, EtOH) by treatment with acetyl chloride in methanol (70). Selective reduction of the ester group alpha to the hydroxyl group of **62** with borane-dimethyl sulfide and a catalytic amount of sodium borohydride in tetrahydrofuran afforded methyl (3S)-3,4-dihydroxybutanoate **63** $[\alpha]_D^{20}$ - 24.9° (c 2.44, EtOH) (71). Reaction of **63** with iodine in acetone, followed by hydrolysis gave **59**, and coupling of **59** with N-hydroxyglycine methyl ester gave **60** which yielded

the hydroxylactone **55** on treatment with trifluoroacetic acid either neat or dissolved in dichloromethane.

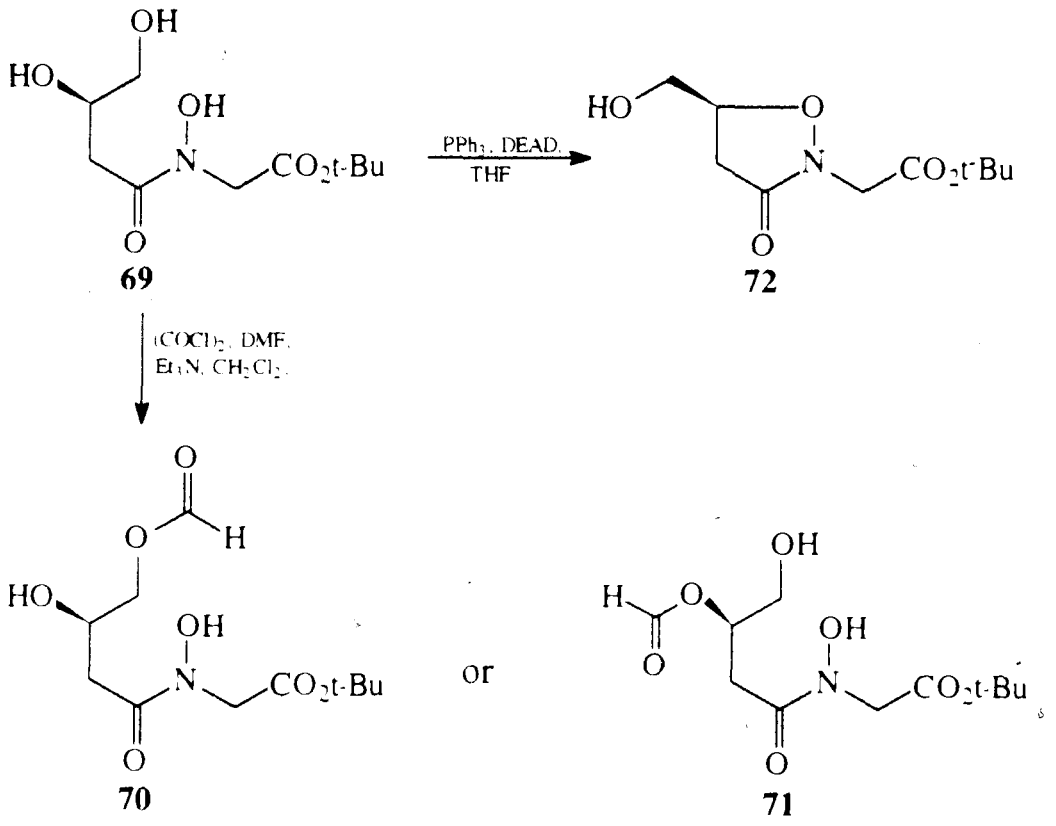


To circumvent this problem, the benzylidene derivative **64** $[\alpha]_D^{23}$ + 6.8° (c 0.76, EtOH) was prepared by reaction of the diol **63** with benzaldehyde dimethyl acetal and a catalytic amount of p-toluenesulfonic acid monohydrate in dimethylformamide (72). Alkaline hydrolysis of **64** gave the acid **65** $[\alpha]_D^{22}$ - 3.70° (c 0.11, CH_2Cl_2). This was coupled to N-hydroxyglycine benzyl ester to give **66** $[\alpha]_D^{20}$ - 11° (c 0.3, $CHCl_3$). Debenzylation of **66** by hydrogenolysis afforded **67**. Attempts to cyclize **67** by reacting it with methanesulfonyl chloride and triethylamine in acetone-d_6, methanesulfonyl chloride and sodium carbonate in deuterium oxide-acetone-d_6 or p-toluenesulfonyl chloride in pyridine-d_5, led to the hydroxylactone **55**.



Carbodiimide coupling of **65** with N-hydroxyglycine t-butyl ester in dichloromethane, gave **68** $[\alpha]_D^{22}$ + 6.3° (c 0.32, CH_2Cl_2), which upon hydrogenolysis yielded **69** $[\alpha]_D^{20}$ -10.7° (c 2.21, CH_2Cl_2). Reaction of **69** with oxalyl chloride, triethylamine and dimethyformamide in dichloromethane (73) afforded only the formate ester **70** or **71**, while treatment with triphenylphosphine and diethyl azodicarboxylate in refluxing tetrahydrofuran (73) gave the isoxazolidinone **72**. Trifluoroacetic acid treatment of **72** yielded an acid whose ^{1}Hmr and mass spectra were identical to those of the

hydroxyacid **40**. There was no reaction when **69** was reacted with triphenylphosphine, triethylamine and carbon tetrachloride in acetonitrile (73).



All of the results so far indicate that competition between cyclization to a 6-membered ring and cyclization to a 5-membered ring favours the latter (74). In the reactions of the alkenes **34**, **48** and **49** with N-bromosuccinimde or N-bromoacetamide in methanol, cyclization to a 6-membered ring was possible because of the formation of regioisomeric methoxybromides. This reaction was found to be temperature dependent. In the methyl ester series a 1:1 ratio of isomers was obtained following bicarbonate treatment when the reaction was perfomed at 0 °C, the 5-membered ring predominated when methoxybromination was performed at temperatures greater than 25 °C, and an

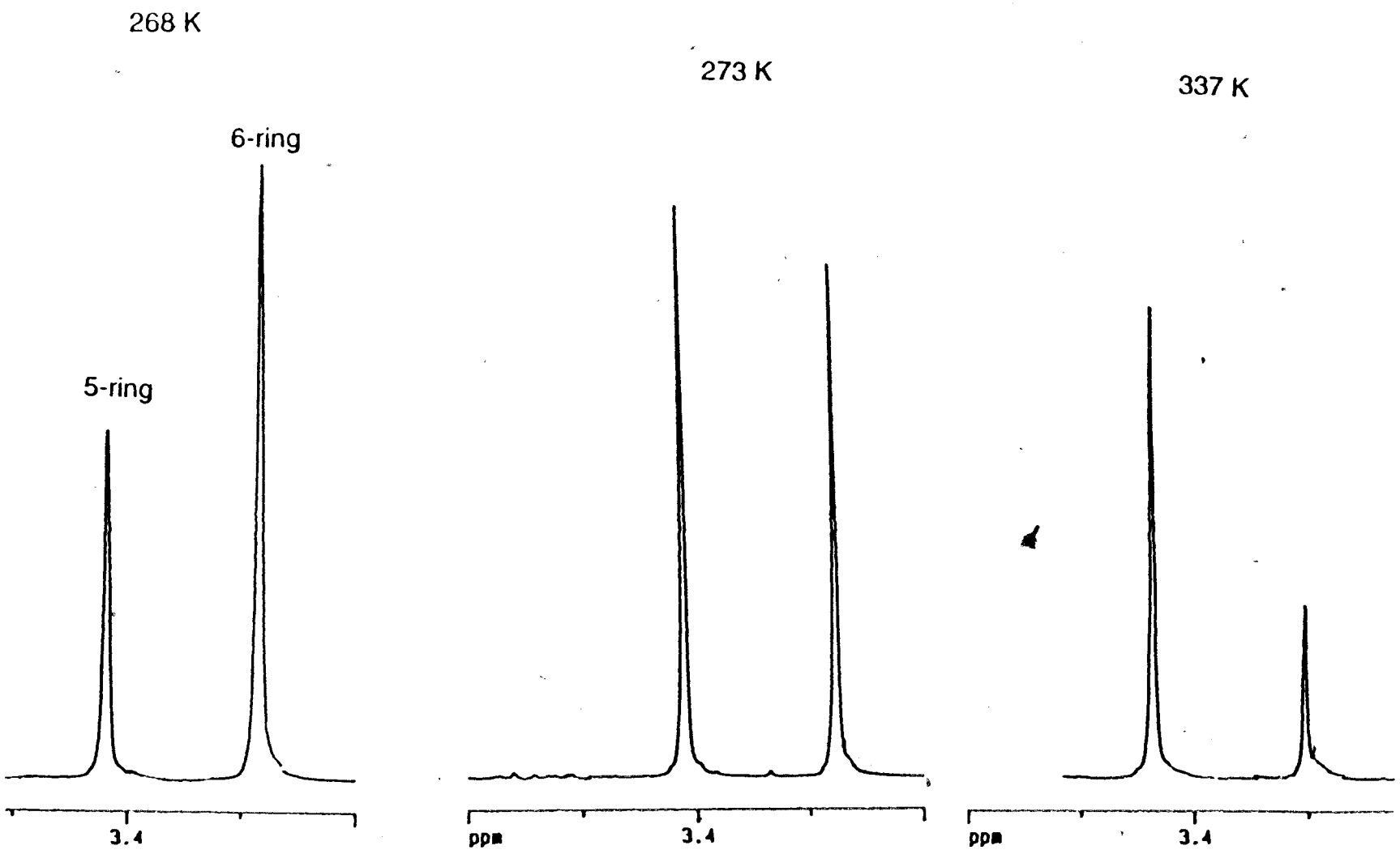


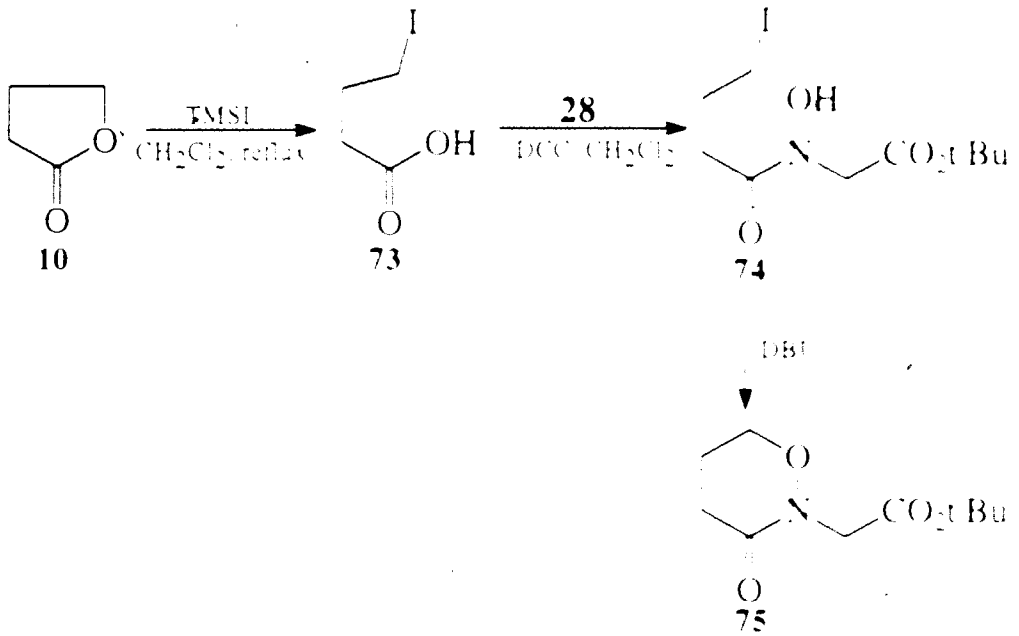
Figure 4: Temperature dependence of the methoxybromination of **48** (methoxy groups shown).

excess of the 6-membered ring was obtained when methoxybromination was carried out below 0 °C (Figure 4).

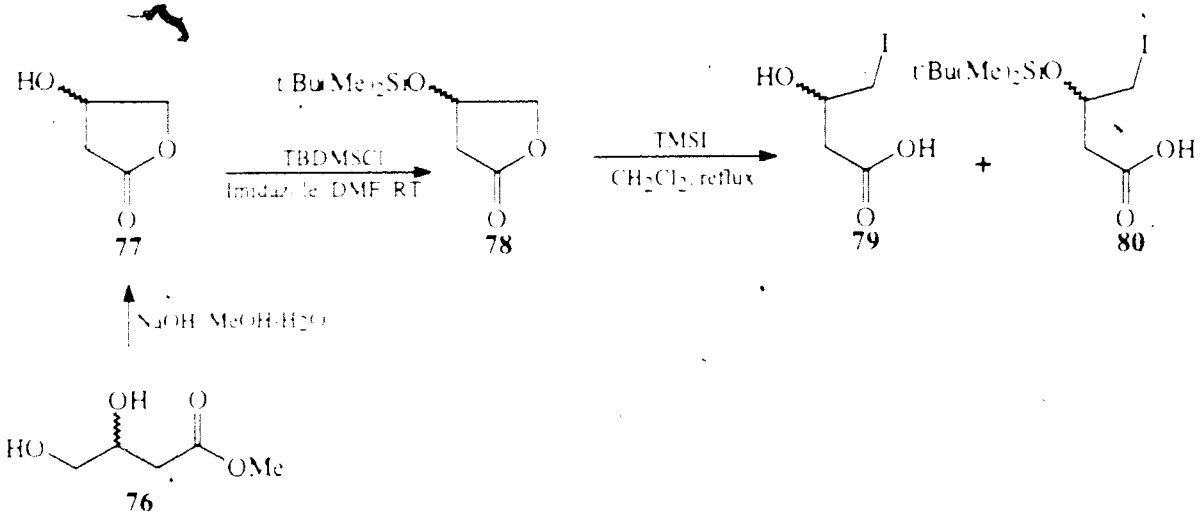
2.3. Butyrolactones as Synthons

It was now clear that the competition between the formation of the 5- and 6-membered rings had to be avoided. To accomplish this, an initial series of reactions was carried out with N-hydroxyglycine benzyl ester and butyrolactone, using trimethylaluminium (75) and aluminium trichloride (76) as catalysts. In both cases, either the starting materials were recovered or a complex mixture of products was obtained.

Success was finally achieved using the method of Larcheveque and Henrot (77). Treatment of the lactone **10** with trimethylsilyl iodide in refluxing dichloromethane gave the acid **73**, which was coupled to N-hydroxyglycine t-butyl ester to give **74**. Cyclisation to **75** was accomplished using DBU.

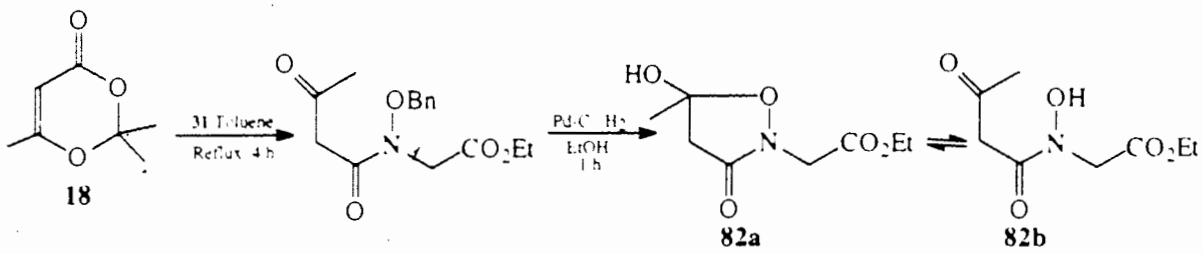


Following this successful result, the hydroxylactone **77** was synthesized by hydrolysis of methyl (R,S)-3,4-dihydroxybutanoate **76**, and protected as the t-butyldimethylsilyl ether **78** (78). Treatment of **78** with trimethylsilyl iodide in refluxing dichloromethane gave a mixture of products believed to include **79** and **80**.

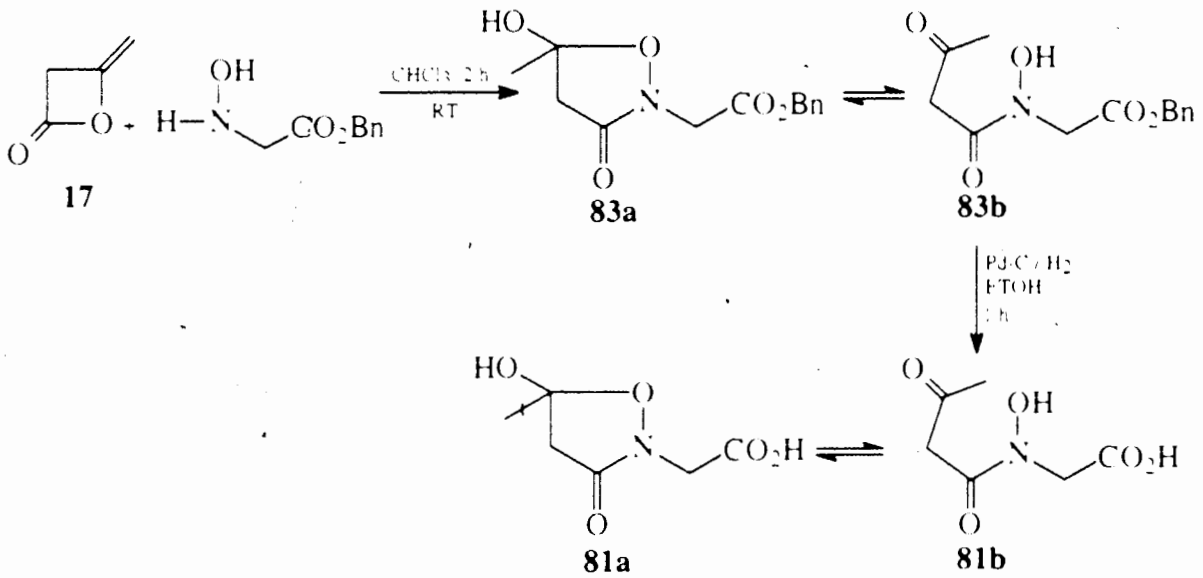


2.4. 2,2,6-Trimethyl-4H-1,3-Dioxin-4-one and Diketene as Synthons

As noted earlier, **40**, a structural isomer of **8**, showed no antibacterial activity against *E. coli or M. luteus*. A second structural isomer **81** also showed no antibacterial activity. The synthesis of the ethyl ester **82** was accomplished by treating O-benzyloxyglycine ethyl ester with 2,2,6-trimethyl-4H-1,3-dioxin-4-one (**18**) in refluxing toluene (79) followed by hydrogenolysis. The compound exists in chloroform-d as an 83:17 mixture of the tautomers **82a** and **82b** (see Experimental).



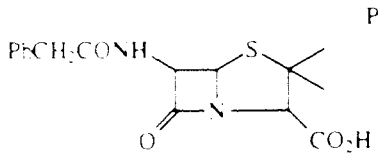
Since N-hydroxyglycine benzyl ester decomposed at elevated temperatures, the synthesis of **81** was carried out using diketene (**17**) in place of **18**. The reaction of diketene with N-hydroxyglycine benzyl ester in chloroform (80) afforded an 83:17 mixture of **83a** and **83b** in chloroform-d which, upon hydrogenolysis, gave the acid **81**, which exists in water as a 2:1 (**81a** : **81b**) mixture of tautomers (see Experimental). Table 3 summarizes the ^{1}Hmr spectra of **81a**, **82a** and **83a**.



The reaction of diketene with bromine and benzyl alcohol in dichloromethane gave **84**, which was reduced to **85** by treatment with sodium borohydride in a tetrahydrofuran-methanol mixture. Protection of **85** as its tetrahydropyranyl ether **86** was achieved by treatment with dihydropyran and p-toluenesulfonic acid in dichloromethane (81). Hydrogenolysis of **86** gave the acid **87**, and carbodiimide coupling of **87** with N-

hydroxyglycine t-butyl ester produced **88**, which cyclized to **89** when treated with DBU (scheme 4).

The sodium salt **90** (≡ **8**) was isolated by successive treatment of **89** with trifluoroacetic acid and sodium bicarbonate. This salt is chemically stable and shows antibacterial activity versus *M. luteus*. The ^{1}Hmr spectrum is shown in Figure 5 and summarized in Table 2. Figures 6 and 7 show the results of antibacterial assays versus *M. luteus* in which **40**, **81** and **90** were compared to penicillin G (**91**) and desacetoxycephalosporin G (**92**).



91

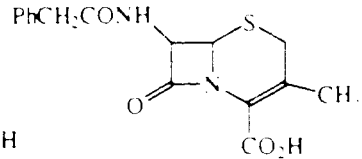


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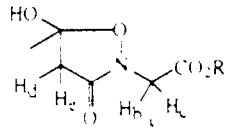
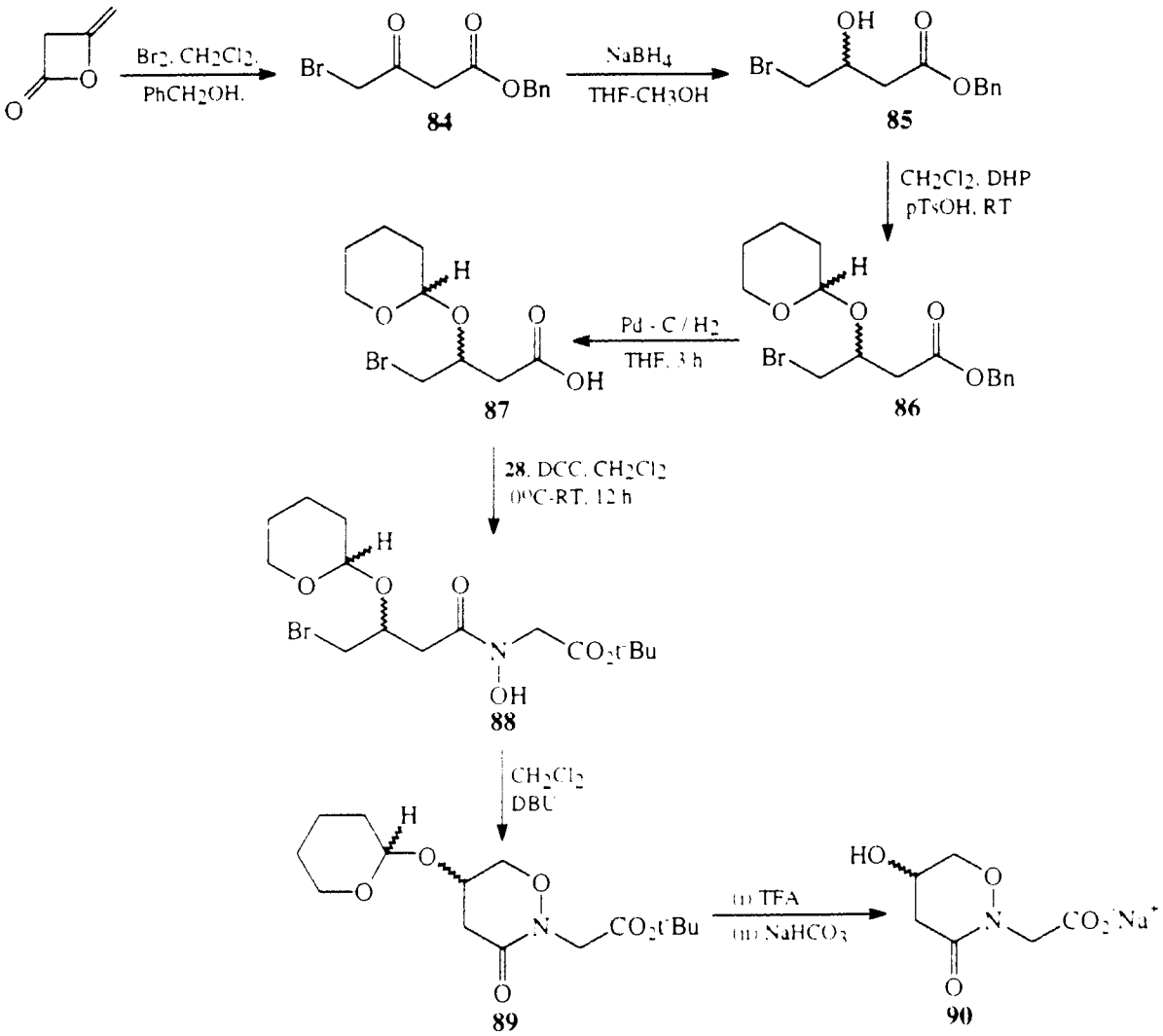
Table 3: Summary of the ^{1}Hmr spectra of 5-hydroxy-5-methyl isoxazolidinones



	R = H[b] **81a**		R = Et[a] **82a**		R = Bn[a] **83a**	
	δ_{ppm}	J_{Hz}	δ_{ppm}	J_{Hz}	δ_{ppm}	J_{Hz}
H_b	4.39	17.7	4.51	18.2	4.53	18.1
H_c	4.20	17.7	4.12	18.2	4.19	18.1
H_d	3.17	17.2	3.02	16.8	3.00	16.8
H_e	2.77	17.2	2.76	16.8	2.76	16.8

[a] The solvent was $CDCl_3$. [b] The solvent was D_2O


Br2, CH2Cl2,
PhCH2OH.
NaBH4
THF-CH3OH
84
85
CH2Cl2, DHP
pTsOH, RT
Pd - C / H2
THF, 3 h
86
87
28, DCC, CH2Cl2
0°C-RT, 12 h
88
CH2Cl2
DBU
89
(i) TFA
(ii) NaHCO3
90

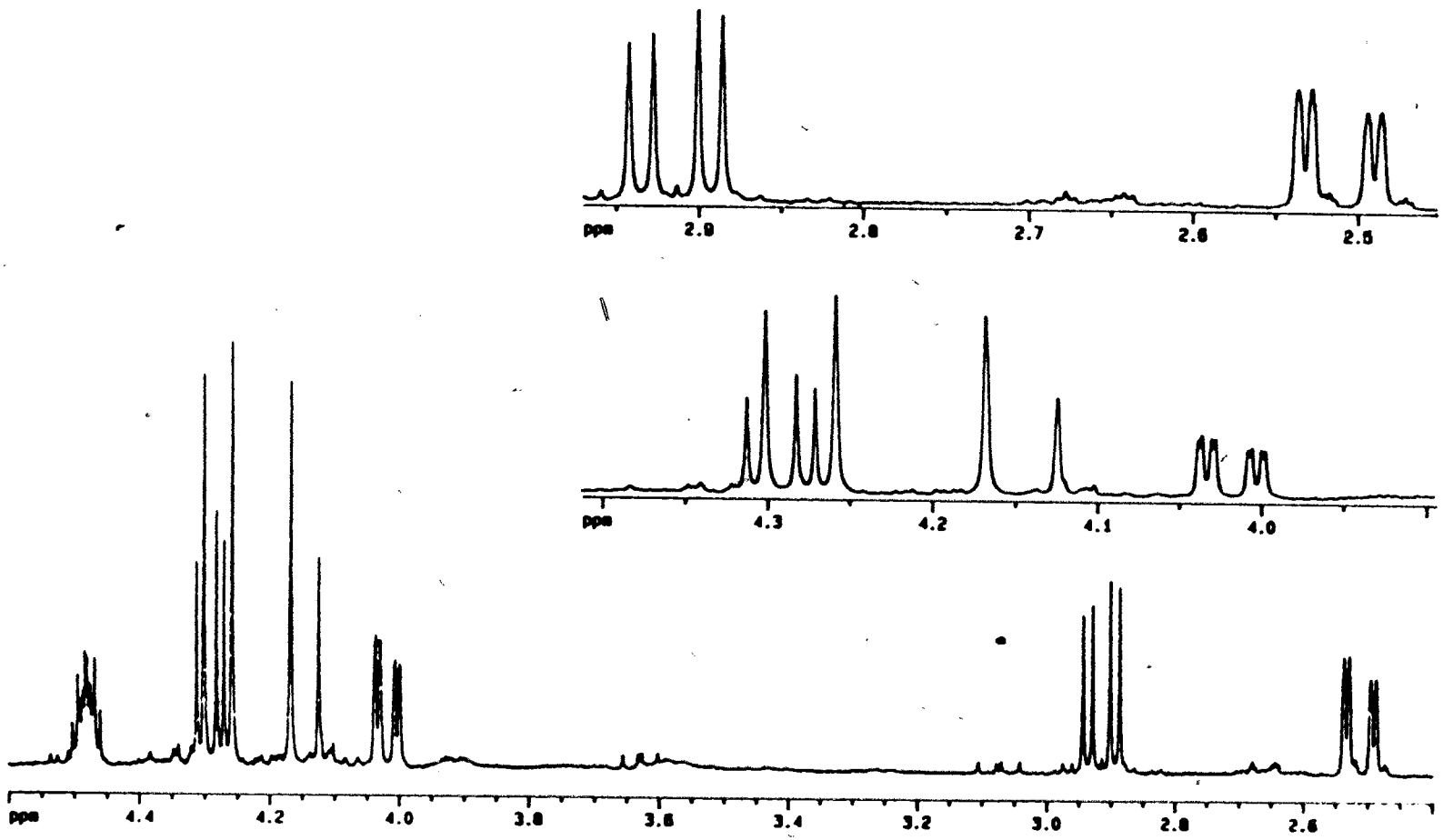


Figure 5: ^{1}Hmr spectrum of 2-carboxymethyl-5-hydroxy-1,2-oxazin-3-one (**90**)

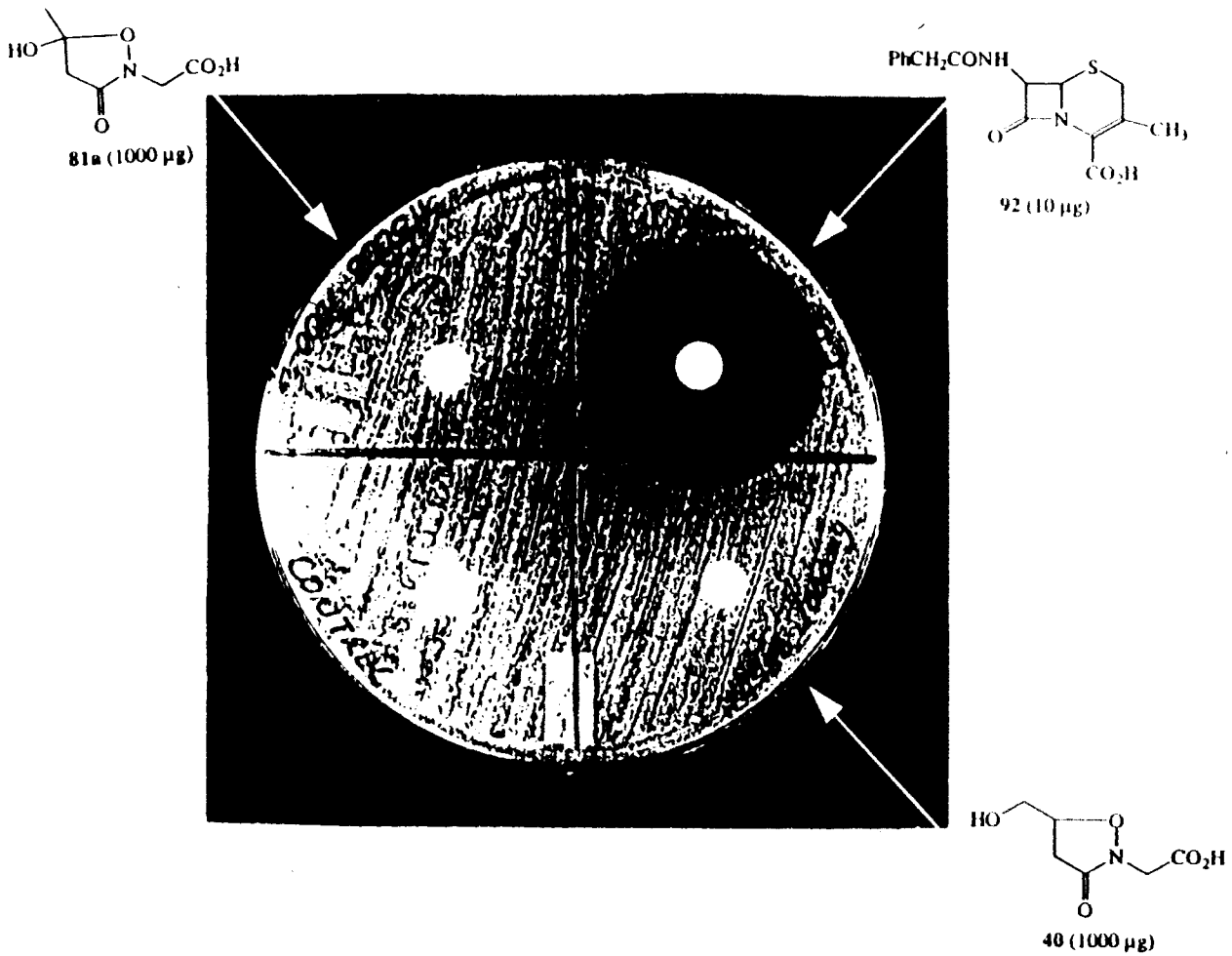


Figure 6: Zone of inhibition versus *M. luteus* using **40**, **81a** and **92**

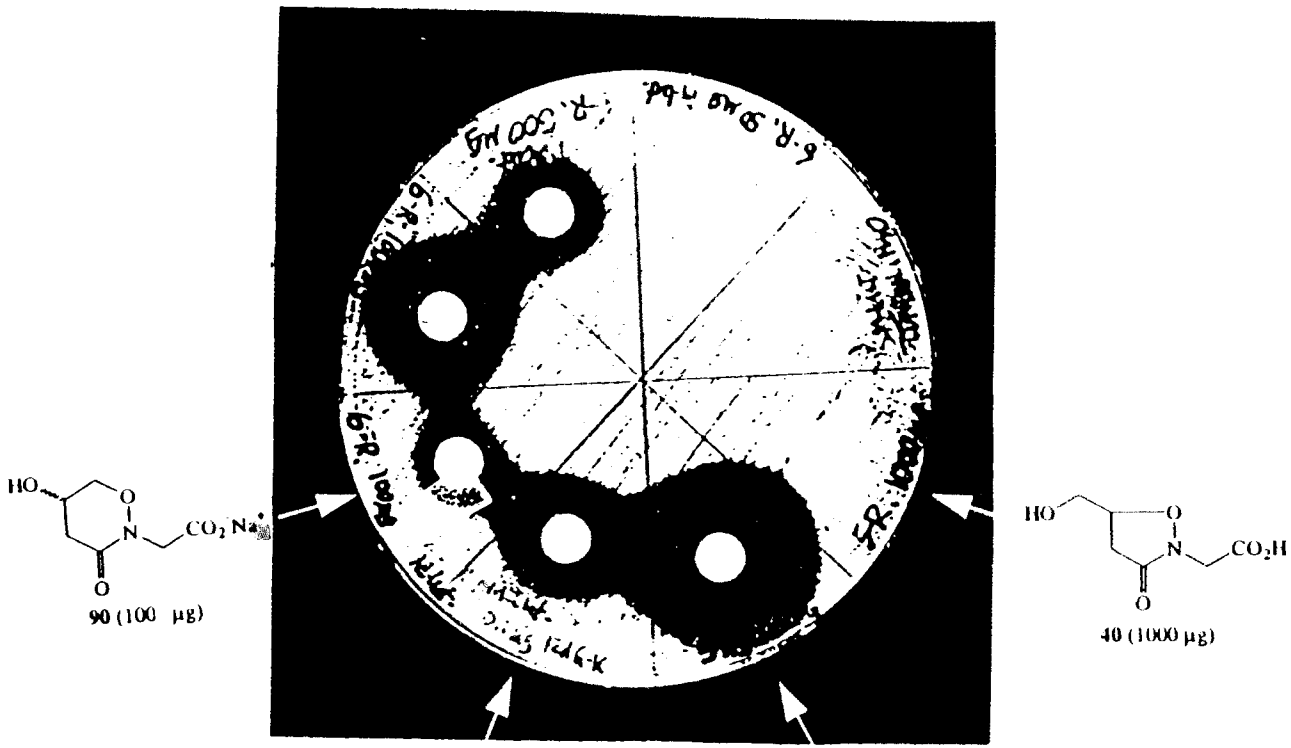


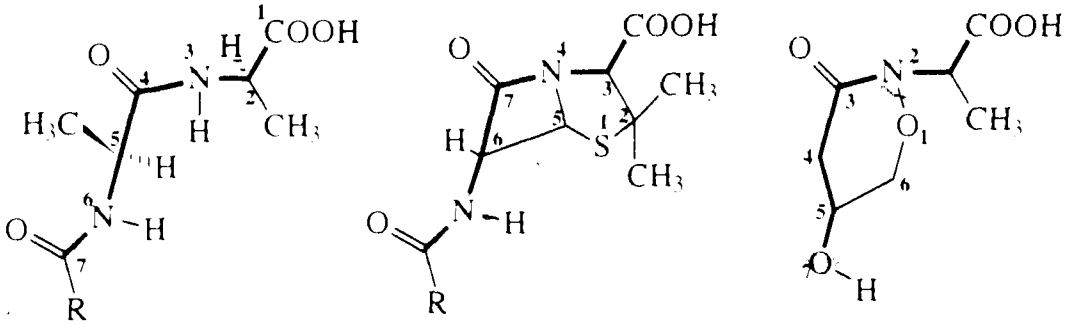
Figure 7: Zone of inhibition versus *M. luteus* using **40**, **90**, **91** and **92**

3. Conclusions and Suggestions for Further Work

5-Hydroxy-1,2-oxazin-3-one **90** (≡ **8**) has been synthesized. The compound is chemically stable, shows antibacterial activity versus *M. luteus*, and can be prepared easily from readily available starting materials. Having achieved one of the objectives of this research, I therefore put forward the following suggestions for future work.

The two enantiomers of **90** with known absolute configuration have to be synthesized. It is expected that this can be accomplished by the reduction of **84** with one of Corey's oxazaborolidines (82).

β-Lactam compounds manifest their antibacterial activity through an ability to interfere with cross-linking of peptidoglycan chains, a step mediated by the PBPs (1). The peptide segment of these chains terminates in the sequence R-X-D-Ala^1-D-Ala^2, which is a structural analogue of penicillin (11).

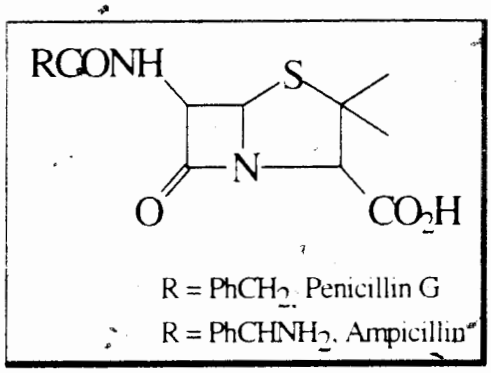


A **B** **C**

Application of Strominger's proposal (83) regarding the structural relationship between the peptide segment of the peptidoglycan (**A**) and penicillin (**B**) to **90**, leads to

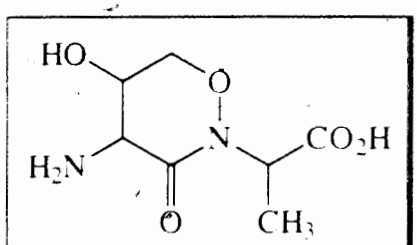
(**C**), which should be accessible by the use of N-hydroxy-D-alanine in place of N-hydroxyglycine. Obviously, N-hydroxyglycine can be replaced by other amino acids.

The substituents on the ring can be varied. Like penicillin G, **90** is not active against *E. coli*. However, ampicillin, a derivative of penicillin G (11), is a broad spectrum antibiotic. If the same structural variation is applied to **90,** then the analogue of ampicillin would be **D**.



R = $PhCH_2$, Penicillin G

R = $PhCHNH_2$, Ampicillin



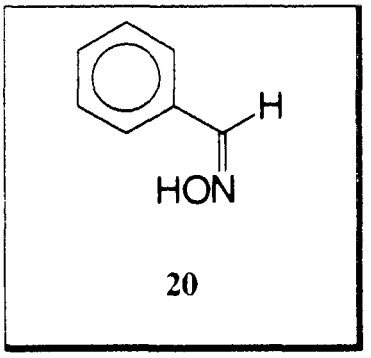
D

4. Experimental

All solvents were dried and distilled before use according to standard literature procedures (84). Except where indicated, chemicals were of the highest available purity from Aldrich. Melting points were determined on a Fisher-Johns apparatus, and are uncorrected. Infrared (ir) spectra were obtained on a Perkin-Elmer 599B spectrometer (neat film, 1-2% KBr pellet or 1% solution). ^{1}H nuclear magnetic resonance spectra (nmr) were obtained from a Bruker AMX 400 NMR spectrometer. Chemical shifts are recorded in parts per million (ppm) downfield from tetramethylsilane. Mass spectra were recorded on a Hewlett-Packard 5985 GC/MS/IS system. Elemental analyses were performed by Mr. M. Yang on a Carlo Erba model 1106 elemental analyzer. Optical rotations were determined with a Rudolph automatic polarimeter model Autopol II, with a cell length of 10 cm. Concentrations are reported in g/100 mL of solvent. Analytical and preparative thin layer chromatography (tlc, plc) were carried out using precoated Merck silica gel 60 F-254 plates with aluminium backing. Spots were observed under ultraviolet light and visualized with a solution of 1% ceric sulfate and 2% molybdic acid in 10% sulfuric acid. Flash chromatography was carried out with 230-400 mesh Merck silica gel 60.

Z-Benzaldoxime (48)

Benzaldehyde (5.3 g, 0.05 mole) [Fisher] and hydroxylamine hydrochloride (3.6 g, 0.052



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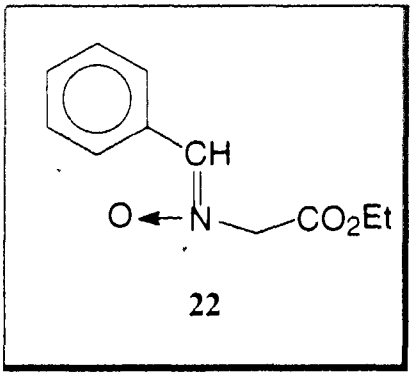
mole) [Mallinckrodt] were added successively at room temperature to a stirred solution of sodium hydroxide (5 g, 0.125 mole). Stirring was continued until a clear solution was obtained and the temperature had increased to 70 °C. The reaction mixture was cooled to 35 °C, and carbon dioxide was passed in until the pH had decreased from 14 to 10. The two layers were then separated. The aqueous layer was extracted with toluene (4 x 25 mL) and the combined organic phase was dried over anhydrous magnesium sulfate, filtered and poured into a 250 mL three necked flask equipped with a large-bore gas inlet tube, stirrer, thermometer and condenser. The flask was heated on an oil bath and 20 mL of toluene were removed by distillation. The remaining solution was reheated to boiling, the oil bath was removed and a strong flow of anhydrous hydrogen chloride was sparged through the vigorously stirred solution. Addition of the gas was continued until the oil which first separated had solidified and the temperature had fallen to 50 °C. The resulting white slurry was cooled to 10 °C in an ice-water bath, and filtered. The white crystalline solid was washed with toluene (2 x 25 mL) and hexane, and stored in a desiccator over potassium hydroxide pellets. Diethyl ether (50 mL) was added to a stirred solution of sodium hydroxide (4 g, 0.1 mole) in water (40 mL). The mixture was cooled to 10 °C and the crude Z-benzaldoxime hydrochloride was added rapidly with stirring until all of the solid had dissolved. A solution of ammonium chloride (10 g, 0.187 mole) [Fisher] in water (40 mL) was added, and the mixture was stirred until a clear solution was obtained (1 min). The two layers were separated and the aqueous layer was extracted with ether (2 x 25 mL). The combined ethereal extracts were dried over anhydrous magnesium sulfate, and concentrated. Hexane (40 mL) was added to the white slurry and

the mixture concentrated again. Another 40 mL of hexane were added, the mixture was filtered, and the solid was air dried until a constant weight was obtained. The Z-benzaldoxime was obtained as white needles (4.26 g, 70%) m.p. 131 °C, (lit.(48) m.p. 130 °C). **^{1}Hmr** ($CDCl_3$, δ): 8.19 (1H, s, CH=N), 7.60 (2H, m, ArH), 7.45 (3H, m, ArH), 4.71 (2H, s), 4.26 (2H, q) 1.29 (3H, t). **Mass spectrum** (EI, m/z): 121 (M^+). **Anal. Calcd. for C_7H_7NO**: C, 69.42; H, 5.79; N, 11.57. **Found**: C, 69.44; H, 5.96; N, 10.99.

N-Benzylideneglycine N-Oxide Ethyl Ester (49)

Ethyl bromoacetate (0.244 mL, 0.367 g, 2.2 mmoles) was added to a stirred solution of



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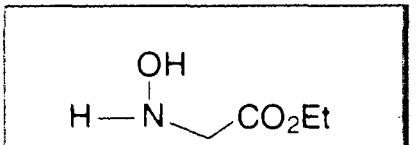
sodium (0.46 g, 0.02 g-atom) and Z-benzaldoxime (0.242 g, 2.0 mmoles) in ethanol (15 mL). Stirring was continued until a wet pH 7 was reached. The reaction mixture was poured into water (20 mL), extracted with ether (2 x 20 mL), dried, and concentrated under reduced pressure. The golden yellow oil was purified by flash chromatography on silica gel (4.5 g) (40% ethyl acetate-hexane), and subsequently crystallized from ethyl acetate-hexane. The pale yellow crystals weighed 0.298 g (73%), m.p. 53°C. **^{1}Hmr** ($CDCl_3$, δ): 8.19 (1H, s, (CH=N), 7.60 (2H, m, ArH), 7.45 (3H, m, ArH), 4.71 (2H, s), 4.26 (2H, q), 1.29 (3H, t). **IR** (Film, cm^{-1}): 1743 (C=O), 1584 (C=N). **Mass spectrum**

(EI, m/z): 207 (M^+). **Anal. Calcd. for $C_{11}H_{13}NO_3$**: C, 63.76; H, 6.32; N, 6.76. **Found**: C, 64.04; H, 6.41; N, 6.60.

N-Hydroxyglycine Ethyl Ester (50)

Method A. N-Benzylideneglycine N-oxide ethyl ester (180 mg, 0.87 mmole) was added to a stirred suspension of sodium methoxide (64.8 mg, 1.2 mmoles) [Anachemia] and



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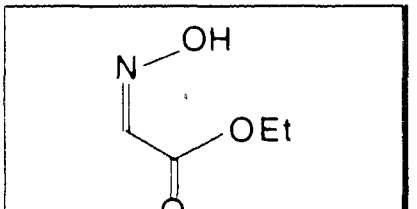
hydroxylamine hydrochloride (83.4 mg, 1.2 mmoles) [Mallinckrodt] in methanol (3 mL). The reaction mixture was heated on a water bath (40 °C) with stirring until the wet pH was slightly acidic (2 min), and then concentrated under reduced pressure. The residue was triturated with toluene (2 x 10 mL), concentrated, and purified by flash chromatography (5 g silica gel and 40% ethyl acetate-hexane) to give a pale yellow oil (82.7 mg, 80%). **^{1}Hmr** ($CDCl_3$, δ): 5.89 (2H, br, NH-OH), 4.23 (2H, q, OCH_2CH_3), 3.67 (2H, s), 1.29 (3H, t, OCH_2CH_3). **IR** (film, cm^{-1}): 3319 (O-H, br), 3267 (N-H, m), 1735 (C=O).

Method B (51, 52). A mixture of glyoxylic acid (1.094 g, 0.014 mole) [Eastman],



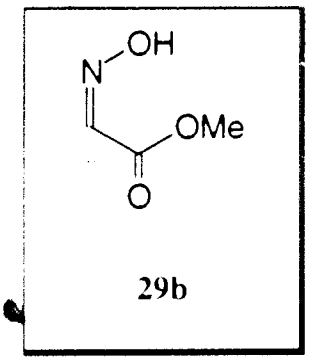
29a

hydroxylamine hydrochloride (1.00 g, 14 mmoles) [Mallinckrodt] and p-toluenesulfonic acid (0.273 g, 1 mmole) [Fisher] in dry ethanol (8 mL) was heated under reflux for 5 h with removal of water using a Dean-Stark trap. The reaction mixture was concentrated, ether (100 mL) and saturated sodium bicarbonate (100 mL) were added, and the mixture was stirred until the phases became clear. The organic layer was separated, washed with saturated ammonium chloride, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure to yield ethyl glyoxylate oxime (1.11 g, 66%). **^{1}Hmr** ($CDCl_3$, δ): 9.55 (1H , b, =NOH), 7.56 (1H, s, *HC*=NOH), 4.31 (2H, q, $CO_2CH_2CH_3$), 1.33 (3H, t, $CO_2CH_2CH_3$). **IR** (film, cm^{-1}): 3347 (OH, br), 1725 (C=O, s), 1623 (C=N, m). **Mass spectrum** (CI, m/z): 118 (M+1). **Anal. Calcd. for $C_4H_7NO_3$**: C, 41.03; H, 5.98; N, 11.96. **Found**: C, 41.41; H, 6.12; N, 11.43.

Ethanolic hydrogen chloride (6.76 mL of a 7 N solution prepared by diluting 30 mL of 36% HCl to 50 mL with ethanol) was added to a stirred solution of the oxime ethyl ester (1.0 g, 8.5 mmoles), and pyridine-borane complex (2.16 mL of 8 M, 17.3 mmoles) in ethanol (10 mL) was added at such a rate that the reaction temperature remained below 40 °C. Stirring was continued for 8 h, while the reaction was monitored by tlc. The reaction mixture was then concentrated and the pale yellow syrupy residue was dissolved in dichloromethane (50 mL). Sodium carbonate (4 g) was added and the mixture was stirred at room temperature for 30 h, filtered, and the filtrate concentrated under reduced pressure. The crude oil was purified by flash chromatography (60% ethyl acetate-hexane) to give a pale yellow oil (0.673 g, 67%). **Mass spectrum** (CI, m/z): 120 (M+1). **Anal. Calcd. for $C_4H_9NO_3$**: C, 40.34; H, 7.56; N, 11.76. **Found**: C, 40.21; H, 7.55; N, 11.54.

Methylglyoxylate Oxime (51)

A mixture of glyoxylic acid (1.094 g, 0.014 mole) [Eastman], hydroxylamine hydrochloride (1.00 g, 0.014 mole) [Mallinckrodt] and p-toluenesulfonic acid (0.273 g,

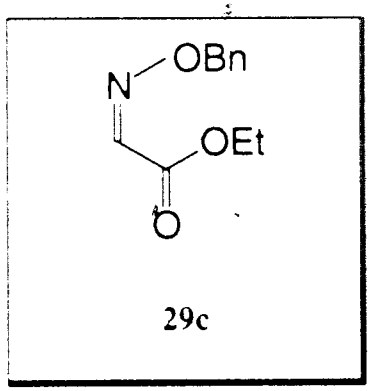


29b

0.001 mole) [Fisher] in methanol (8 mL) was heated under reflux for 5 h with removal of water using a Dean-Stark trap. The reaction mixture was then concentrated, ether (20 mL) was added, and the suspension was cooled (0 °C) and filtered. The filtrate was concentrated under reduced pressure to yield a pale yellow oil (1.12 g , 76%). **^{1}Hmr** ($CDCl_3$, δ): 9.76 (1H, b, =N-OH), 7.58 (1H, d, *HC*=NOH), 3.88 (3H, s). **IR** (film, cm^{-1}): 3346 (OH, b), 1726 (C=O, s), 1625 (C=N, m). **Mass spectrum** (CI, m/z): 104 (M+1).

Ethyl Glyoxylate N-Benzyloxyoxime (51)

A mixture of glyoxylic acid (0.476 g, 6.26 mmoles) [Eastman], O-benzylhydroxylamine



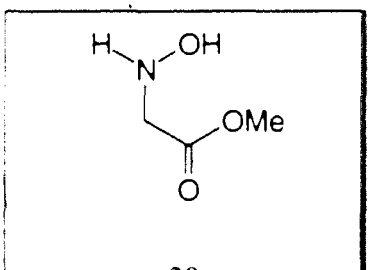
29c

hydrochloride (1.00 g, 6.26 mmoles) and p-toluenesulfonic acid (0.12 g, 0.626 mmole)

[Fisher] in dry ethanol (8 mL) was refluxed for 6 h, with removal of water using a Dean-Stark trap. The reaction mixture was concentrated and dichloromethane (50 mL) was added to the yellow oily residue. The solution was washed successively with water (30 mL), saturated sodium bicarbonate (30 mL) and saturated sodium chloride (30 mL), dried over anhydrous magnesium sulfate, and concentrated under reduced pressure to give a pale yellow oil (1.08 g, 83%). **^{1}Hmr** ($CDCl_3$, δ): 7.57 (1H, s, N=CH), 7.39 (5H, s, OCH_2Ph), 5.32 (2H, s, OCH_2Ph), 4.33 (2H, q, OCH_2CH_3), 1.36 (3H, t, OCH_2CH_3). **IR** ($CDCl_3$, cm^{-1}): 1727 (C=O), 1600 (C=N). **Mass spectrum** (CI, m/z): 208 (M+1). **Anal. Calcd. for $C_{11}H_{13}NO_3$**: C, 63.77; H, 6.28; N, 6.76; **Found**: C, 63.06; H, 6.33; N, 7.34.

N-Hydroxyglycine Methyl Ester (52)

A methanolic hydrogen chloride solution (18 mL of 7 N, prepared by diluting 15 mL of 36% HCl to 25 mL with methanol) was added dropwise to a stirred solution of the oxime



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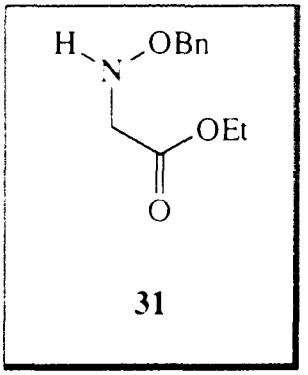
(2.25 g, 21.84 mmoles), and pyridine-borane (5.52 mL of 8M, 44.2 mmoles) in methanol (50 mL) was added at such a rate that the reaction temperature did not exceed 40 °C. Stirring was continued for 1.5 h, and the solvent was then removed. The pale yellow syrup was dissolved in dichloromethane (100 mL) and sodium carbonate (11.6 g, 0.11 mole) was added. The mixture was stirred for 2 h at room temperature, stored overnight at 0 °C, filtered, and the filtrate concentrated. The product was purified by flash

chromatography (60% ethyl acetate-hexane) to give a pale yellow oil (1.55 g , 67%). **^{1}Hmr** ($CDCl_3$, δ): 5.95 (2H, br, NH-OH), 3.78 (3H, s), 3.70 (2H, s). **IR** (film, cm^{-1}): 3319 (O-H, br), 3267 (N-H, m), 1735 (C=O). **Mass spectrum** (EI, m/z): 105 (M^+). **Anal. Calcd. for $C_3H_7NO_3$**: C, 34.29; H, 6.67; N, 13.33. **Found**: C, 35.16; H, 6.62; N, 12.70.

N-Benzyloxyglycine Ethyl Ester (52)

Ethanolic hydrogen chloride solution (6.76 mL of a 7 N solution prepared by diluting 30



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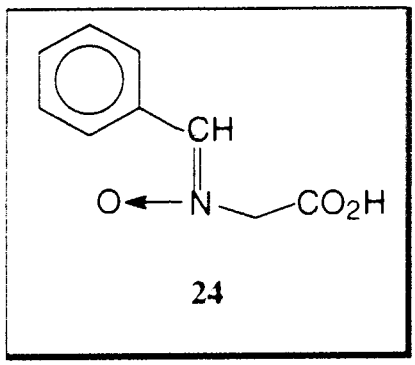
mL of 36% HCl to 50 mL with ethanol) was added to a stirred solution of the oxime (3.5 g, 16.91 mmoles), and pyridine-borane (8.54 mL of 8 M, 68.3 mmoles) in ethanol (10 mL) was added at such a rate that the reaction temperature did not exceed 40 °C. Stirring was continued for 13.5 h, with monitoring by tlc, and the solvent was then removed. The pale yellow syrup was dissolved in dichloromethane (50 mL) and the solution was washed with N sodium hydroxide, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The crude oil was purified by flash chromatography using 2% ethanol in dichloromethane as the eluent. The pale yellow oil thereby obtained weighed 3.0 g, 85%. **^{1}Hmr** ($CDCl_3$, δ): 7.36 (5H, s, OCH_2Ph), 6.13 (1H, t, N-H), 4.75 (2H, s, OCH_2Ph), 4.24 (2H, q, OCH_2CH_3), 3.62 (2H, d, $NCH_2C(O)$), 1.30 (3H, t, OCH_2CH_3). **IR** ($CDCl_3$, cm^{-1}): 3273 (N-H , w), 1739 (C=O, s). **Mass spectrum** (CI, m/z): 210

(M+1). **Anal. Calcd. for $C_{11}H_{15}NO_3N$**: C, 63.16; H, 7.18; N, 6.70; **Found**: C, 63.37; H, 7.01; N, 7.50.

N-Benzylidene Glycine N-Oxide (50)

Sodium (0.46 g, 0.02 g-atom) and Z-benzaldoxime **20** (1.21 g, 10 mmoles) in ethanol (40 mL) were stirred until the sodium dissolved. Then α-bromoacetic acid (1.53 g, 11

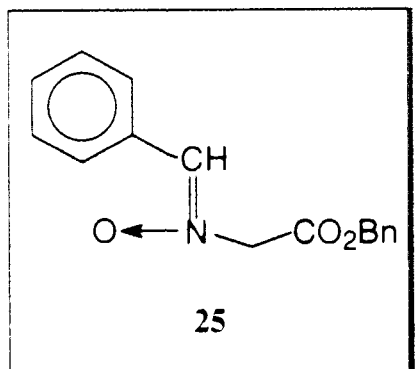


24

mmoles) [Lancaster] was added in one portion, with stirring. Stirring was continued for 1 h at 70 °C and the solvent was removed. A suspension of the residue in water (10 mL), was cooled, acidified with 0.1 N hydrochloric acid (9.5 mL) and filtered. The white crystals were collected and dried at 1 torr to give 1.21 g, (66%) of product, m.p 175-177 °C (lit (50): 178-179 °C). **IR** (KBr, cm^{-1}): 3069, 1724, 1617. **Mass spectrum** (EI, m/z): 179 (M^+).

N-Benzylidene-Glycine N-Oxide Benzyl Ester (50)

To a cloudy solution of N-benzylideneglycine N-oxide (0.181 g, 1.01 mmoles) in



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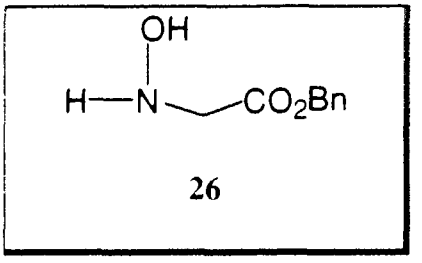
dimethylformamide (2 mL) was added triethylamine (0.141 mL, 103 mg, 1.01 mmoles), followed by dropwise addition of benzyl bromide (0.120 mL, 0.173 g, 1.01 mmoles) [Sigma]. The reaction mixture was stirred for 13 h, poured into water (10 mL) and extracted with chloroform (2 × 10 mL). The chloroform extract was dried over anhydrous sodium sulfate, concentrated, and the residue was purified by column chromatography (60% ethyl acetate-hexanes) to give 0.191 g (70%) of product. **^{1}Hmr** ($CDCl_3$, δ): 8.25 (2H, m, Ar + =CH), 7.45-7.34 (9H, m, Ar), 5.27 (2H, s, CH_2Ph), 4.76 (2H, s, CH_2). **IR** (CH_2Cl_2, cm^{-1}): 3054, 1753, 1587. **Mass spectrum** (EI, m/z): 269 (M^+). **Anal. Calcd. for $C_{16}H_{15}NO_3$:** C, 71.37; H, 5.58; N, 5.20. **Found**: C, 71.13; H, 5.80; N, 5.40.

N-Hydroxyglycine Benzyl Ester (50)

A suspension of N-benzylideneglycine N-oxide benzyl ester (1.35 g, 5.02 mmoles) and hydroxylamine hydrochloride (352 mg, 5.02 mmoles) [Mallinckrodt] in ethanol (10 mL)



26

was brought into solution by heating. The solution was concentrated and the residue taken up in ether (50 mL) and washed with saturated sodium bicarbonate (20 mL). The ether layer was separated, dried over anhydrous magnesium sulfate and the solvent was removed under reduced pressure. The product was purified by flash chromatography (60% ethyl acetate-hexanes) to give 772 mg (85%) of white solid. **^{1}Hmr** ($CDCl_3$, δ): 7.36 (5H, s, Ar), 5.22 (2H, s, PhCH_2), 3.73 (2H, s, NCH_2). **IR** (CH_2Cl_2, cm^{-1}): 3290,

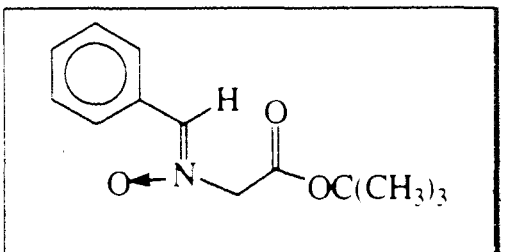
3055, 1741. **Mass spectrum** (EI, m/z): 181 (M^+). **Anal. Calcd. for $C_8H_{11}NO_3$:** C, 59.67; H, 6.08; N, 7.73. **Found**: C, 59.74; H, 6.07; N, 7.11.

t-Butyl Bromoacetate (50)

A solution of oxalyl chloride (6.18 mL, 8.99 g, 0.071 mole) in dichloromethane (10 mL) was added to a cooled (0 °C) solution of bromoacetic acid (8.21 g, 0.059 mole) [Lancaster] and dimethylformamide (3 drops) in dichloromethane (40 mL). The pale yellow solution was stirred for 1 h at 0 °C , then at room temperature for 3 h, concentrated to about 10 mL and slowly added to a cooled solution of t-butanol (5.255 g, 0.071 mole) and triethylamine (10.71 mL, 7.78 g, 0.077 mole) in dichloromethane (45 mL). The dark brown suspension was stirred for 45 min at 0 °C, then for 3 h at room temperature, and poured into water (60 mL). The organic layer was separated and the aqueous layer extracted with dichloromethane (2 x 15 mL). The organic extract was washed with saturated sodium bicarbonate (50 mL), dried over anhydrous magnesium sulfate and concentrated to give 4.48g (39%) of crude product. **^{1}Hmr** ($CDCl_3$, δ): 3.74 (2H, s), 1.47 (9H, s). **Mass spectrum** (CI, m/z): 197:195 (1:1).

N-Benzylidene Glycine N-Oxide t-Butyl Ester (50)

Z-Benzaldoxime (5.64 g, 46.61 mmoles) and t-butyl bromoacetate (7.58 mL, 10 g, 51.27 mmoles) were added successively to a solution prepared by dissolving 1.07 g (0.047 g-

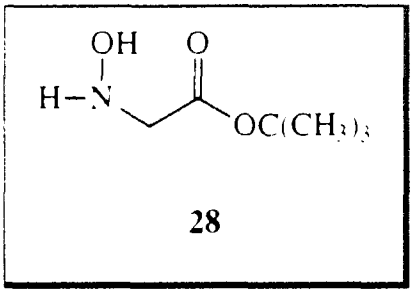


27

atom) of sodium metal in 2-propanol (120 mL). The dark brown suspension was stirred for 2 h at room temperature, poured into water (100 mL) and extracted with dichloromethane (5 x 20 mL). The combined organic extract was dried over anhydrous magnesium sulfate and concentrated. The crude product was purified by flash chromatography (60% ethyl acetate-hexanes) to give 8.74 g (80%) of an oil. **^{1}Hmr** ($CDCl_3$, δ): 8.24 (2H, m, Ar + =CH), 7.42 (4H, m, Ar), 4.62 (2H, s, CH_2), 1.51 (9H, s, t-Bu). **IR** (CH_2Cl_2, cm^{-1}): 3054, 1746, 1580, 1260. **Mass spectrum** (CI, m/z): 236 (M+1). **Anal. Calcd. for $C_{13}H_{17}NO_3$:** C,66.38; H, 7.23; N, 5.96. **Found**: C, 66.78; H, 7.10; N 6.01.

N-Hydroxyglycine t-Butyl Ester (50)

The nitrone **27** (1.3 g, 5.53 mmoles) was added to a stirred suspension of sodium methoxide (0.418 g, 7.74 mmoles) [Anachemia] and hydroxylamine hydrochloride (0.538



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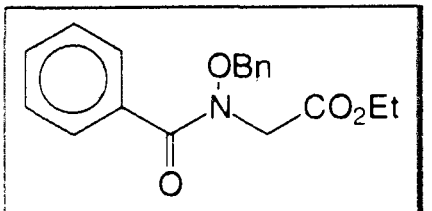
g, 7.74 mmoles) [Mallinckrodt] in methanol (6 mL). The reaction mixture was placed in a water bath (50 °C) and stirred until all of the nitrone had dissolved, then concentrated and the residue was dissolved in dichloromethane (50 mL), filtered and concentrated. The resultant oil was purified by flash chromatography (60% ethyl acetate-hexanes) to give 0.650 g (80%) of the product. **^{1}Hmr** ($CDCl_3$, δ): 5.46 (2H, br, HNOH), 3.62 (2H, s, CH_2), 1.49 (9H, s, t-Bu). **IR** (film, cm^{-1}): 3279, 1735. **Mass spectrum** (CI, m/z): 148 (M+1).

Anal. Calcd. for $C_6H_{13}NO_2$: C.48.98; H. 8.84; N. 9.52. **Found**: C. 48.13; H. 8.65; N 9.44.

N-Benzoyl, N-Benzyloxyglycine Ethyl Ester

Benzoyl chloride (0.028 mL, 0.034 g, 0.23 mmole) [Fluka] was added dropwise to a cooled (0 °C) solution of N-benzyloxyglycine ethyl ester (48 mg , 0.23 mmole) and



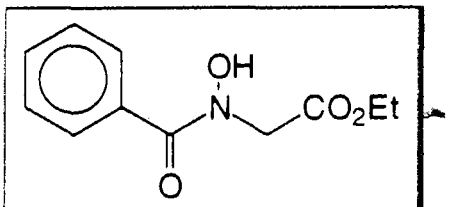
32

triethylamine (0.032 mL, 0.023 g, 0.023 mmole) in dichloromethane (0.5 mL). The cloudy solution was stirred for 20 min, diluted with dichloromethane (25 mL) and washed successively with saturated sodium bicarbonate (10 mL) and saturated sodium chloride (10 mL), dried over anhydrous magnesium sulfate and concentrated to a pale yellow oil (70.7 mg, 98%). **^{1}Hmr** ($CDCl_3$, δ): 7.71-7.07 (10H, m, Ar), 4.71 (2H, s, OCH_2Ar), 4.42 (2H, s, CH_2), 4.24 (2H, q, $CO_2CH_2CH_3$), 1.29 (3H, t, $CO_2CH_2CH_3$). **IR** (film, cm^{-1}): 1746 (C=O, s), 1626. **Mass spectrum** (CI, m/z): 314 (M+1).

Synthesis of 33

Method A. A solution of **32** (38.2 mg, 0.12 mmole) and anisole (0.053 mL, 0.49 mmole) [BDH] in dichloromethane (1 mL) was added dropwise at 0 °C to a solution of aluminium trichloride (48.8 mg, 0.37 mmole) [Anachemia] in nitromethane (1 mL). The resultant pink solution was stirred at 0 °C for 90 min, and then at room temperature for 10

min. The reaction mixture was diluted with ethyl acetate (30 mL) and washed successively with N hydrochloric acid (10 mL) and 4 % sodium bicarbonate (40 mL). The



33

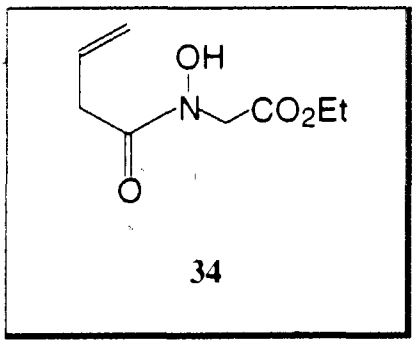
organic layer was dried over anhydrous magnesium sulfate and concentrated to give a bright orange oil which was purified by plc (40% EtOAc-hexane). The product weighed 12.5 mg (45%) and was identical to the product obtained from method B.

Method B. Benzoyl chloride (0.016 mL, 0.019 g, 0.14 mmole) [Fluka] was added dropwise to a cooled (0 °C) solution of N-hydroxyglycine ethyl ester (16.4 mg, 0.14 mmole) and triethylamine (0.019 mL, 0.014 g, 0.014 mmole) in dichloromethane (0.5 mL). The suspension was stirred for 1 h and the solvent was then removed. The residue was redissolved in ether (10 mL), filtered and the filtrate concentrated. The oil was subjected to plc (60% ethyl acetate-hexanes) to give 14.8 mg (41%) of the product. **^{1}Hmr** ($CDCl_3$, δ): 7.72 (5H, m, Ar), 4.47 (2H, s), 4.25 (2H, q), 1.30 (3H, t). **IR** (film, cm^{-1}): 1743 (C=O, s), 1626. **Mass spectrum** (CI, m/z): 224 (M+1).

Vinylacetamido N-Hydroxyglycine Ethyl Ester

To a stirred solution of N-hydroxyglycine ethyl ester (117 mg, 0.98 mmole) in dichloromethane (2 mL) was added vinylacetic acid (0.088 ml, 0.089 g, 1.03 mmoles) in one portion. The solution was cooled to 0 °C and a solution of dicyclohexylcarbodiimide (213 mg, 1.03 mmoles) [BDH] in dichloromethane (2 mL) was added dropwise during 10

min. Stirring was continued for 11 h while the mixture warmed to room temperature. The mixture was then concentrated under reduced pressure and the residue was extracted with



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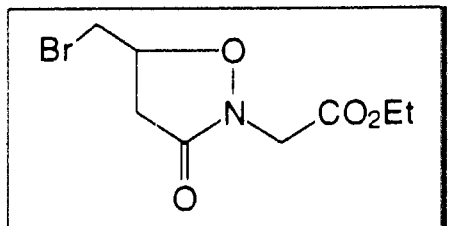
ether (2 x 10 mL). The combined organic extracts were dried, concentrated, and the crude product was purified by flash chromatography (30 g silica gel and 40% ethyl acetate-hexane) to give a pale yellow oil (94 mg, 51%). **^{1}Hmr** ($CDCl_3$, δ): 5.98-5.91 (1H, m, *CH*=CH_2), 5.21-5.18 (2H, d, CH=*CH_2*), 4.48 (2H, s), 4.22 (2H, q), 3.36 (2H, d), 1.29 (3H, t). **IR** (film, cm^{-1}): 3318 (O-H, br), 3083 (C=C, w), 1742 (C=O, s), 1626 (N-C(O), s). **Mass spectrum** (EI, m/z): 187 (M^+).

Bromination of 34

A solution of bromine (0.041 mL, 0.128 g, 0.80 mmole) [Fisher] in chloroform (1 mL) was added dropwise, under nitrogen, at room temperature to a stirred solution of vinylacetamido N-hydroxyglycine ethyl ester (0.125 g, 0.66 mmole) in chloroform (0.6 mL). Stirring was continued for 2 h and the solvent was then removed to give a deep orange oil (0.148 g, 100%). **^{1}Hmr** ($CDCl_3$, δ): 7.87 (1H, O-H, br), 4.55 (1H, m), 4.50 (2H, s), 4.33 (2H, q), 3.86 (2H, dd), 3.38 (2H, dd), 1.30 (3H, t). **IR** (film, cm^{-1}): 3211 (O-H, br), 1745 (C=O), 1650 (N-C(O)). **Mass spectrum** (EI, m/z): 345 : 347 : 349 (1:2:1). The oil was dissolved in dichloromethane (2 mL), and saturated sodium bicarbonate (3 mL) was added. The mixture was stirred for 2 h, washed with saturated

sodium chloride (5 mL), dried, and concentrated. The crude product was purified by plc

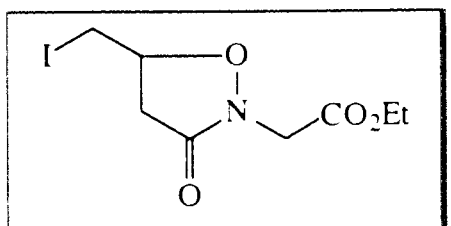


36

(60% ethyl acetate-hexane) to give a colourless oil **36** (0.077g, 50%). **^{1}Hmr** ($CDCl_3$, δ): 4.80-4.75 (1H, m), 4.29 (1H, d, C*H*H, 17 Hz), 4.28 (1H, d, CH*H*, 17Hz), 3.58 (1H, dd, 10.78, 5.52 Hz), 3.53 (1H, dd, 10.78, 6.76 Hz), 3.02 (1H, dd, 16.8, 8.24 Hz), 2.83 (1H, dd, 16.8, 7.04 Hz), 4.23 (2H, q, $CO_2CH_2CH_3$), 1.29 (3H, t, $CO_2CH_2CH_3$). **IR** (film, cm^{-1}): 1750, 1718 (C=O). **Mass spectrum** (EI, m/z):(relative intensity): 267 (12.1), 265 (12.6), 194 (78.4), 192 (68.3), 149 (58.2), 147 (57.4), 95 (7.4), 93 (8.7), 85 (17.8). **Anal. Calcd. for $C_8H_{12}NO_4Br$**: C, 36.11; H, 4.55; N, 5.26. **Found**: C, 35.75; H, 4.58; N, 5.25.

Iodocyclization of 34

Iodine (0.41 g, 1.60 mmoles) and sodium bicarbonate (0.27 g, 3.21 mmoles) were added to a solution of **34** (0.20 g, 1.07 mmoles) in acetonitrile (5 mL). The solution was stirred



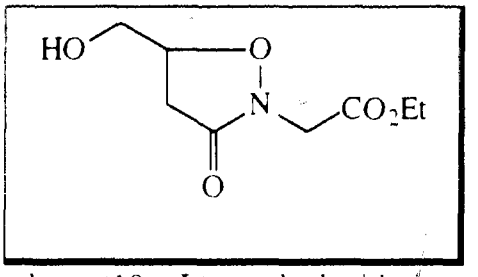
39b

for 7 h at 0 °C and then diluted with ether (50 mL) and washed successively with

saturated sodium thiosulfate (30 mL), water (30 mL) and saturated sodium chloride (30 mL), dried over anhydrous magnesium sulfate and evaporated under reduced pressure. The product was purified by flash chromatography (60% ethyl acetate-hexanes) to give an oil 93 mg (28%). **^{1}Hmr** ($CDCl_3$, δ): 4.70-4.63 (1H, m), 4.26 (1H, d, C*H*H, 17.9 Hz), 4.24 (1H, d, CH*H*, 17.9 Hz), 4.22 (2H, q), 3.39 (1H, dd, 10.40, 5.5 Hz), 3.35 (1H, dd, 10.40, 7.44 Hz), 3.0 (1H, dd, 16.8, 8.0 Hz), 2.76 (1H, dd, 16.8, 7.24 Hz), 1.28 (3H, t). **IR** (film, cm^{-1}): 1750, 1718 (C=O).

Oxidation of 39b

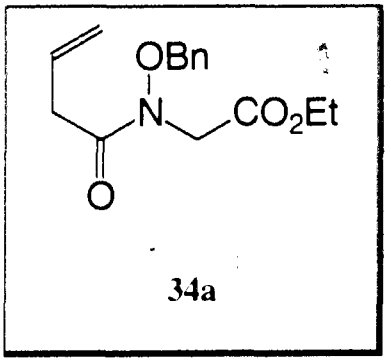
m-Chloroperbenzoic acid (45 mg of 85%, 0.22 mmoles) was added to a solution of **39b** (46 mg, 0.15 mmole) in dichloromethane (2 mL). The purple solution was stirred for 5 h,



then diluted with dichloromethane (10 mL), washed with saturated sodium thiosulfate (10 mL) and dried over anhydrous magnesium sulfate. The residue was purified by plc (80% ethyl acetate-hexanes) to give 8 mg of product (25%). **^{1}Hmr** ($CDCl_3$, δ): 4.69-4.63 (1H, m), 4.57 (1H, d, C*H*H, 18 Hz), 4.24 (2H, q), 4.06 (1H, d, CH*H*, 18 Hz), 3.91 (1H, dd, 12.80, 2.40 Hz), 3.67 (1H, dd, 12.80, 4.0 Hz), 2.96 (1H, dd, 16.60, 8.70 Hz), 2.90 (1H, dd, 16.60, 6.20 Hz), 1.30 (3H, t). **IR** ($CHCl_3$, cm^{-1}): 1747, 1703 (C=O). **Mass spectrum** (CI, m/z): 204 (M+1), (EI, m/z): 203 (M^+)

Vinylacetamido N-Benzyloxyglycine Ethyl Ester

Method A. Dicyclohexylcarbodiimide (0.197 g, 0.96 mmole) [BDH] was added in one



34a

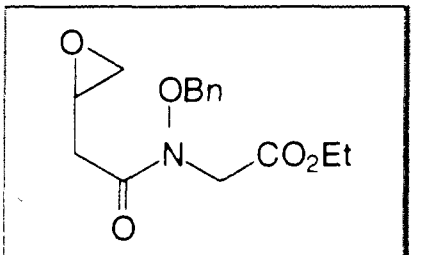
portion to a cooled (0 °C) solution of vinylacetic acid (0.08 mL, 0.081 g, 0.96 mmole) and N-benzyloxyglycine ethyl ester (0.200 g, 0.96 mmole) in dichloromethane (5 mL). The mixture was stirred for 30 min at 0 °C, and then for 3.5 h at room temperature, and filtered. The filtrate was concentrated to give a deep orange oil which was purified by flash chromatography (60% ethyl acetate-hexane) to give an orange oil (0.255 g, 96%).

Method B. A mixture of vinylacetamido N-hydroxyglycine ethyl ester. (27.6 mg, 0.15 mmole) and 1,8-diazabicyclo[5.4.0]undec-7-ene (0.022 mL, 0.022 g, 0.15 mmole) in dichloromethane (1 mL) was stirred at 0 °C for 5 min and benzyl bromide (0.018 mL, 0.026 g, 0.15 mmole) [Sigma] was added in one portion. The cooling bath was removed and stirring of the golden yellow solution was continued for 2.5 h. The reaction mixture was then diluted with dichloromethane (30 mL), washed successively with water (20 mL), 0.5 M hydrochloric acid, saturated sodium bicarbonate (10 mL) and saturated sodium chloride (10 mL), dried over anhydrous magnesium sulfate, and concentrated. The product was purified by plc (40% ethyl acetate-hexane) to give an orange oil, 11.1 mg (28%). **^{1}Hmr** ($CDCl_3$, δ): 7.40 (5H, s, OCH_2Ph), 5.98-5.91 (1H, m, CH=CH_2), 5.21-5.10 (2H, dd, CH=CH_2), 4.90 (2H, s, OCH_2Ph), 4.29 (2H, s, NCH_2CO_2Et), 4.20 (2H, q,

OCH_2CH_3), 3.24 (2H, dd, $CH_2CH{=}CH_2$), 1.27 (3H, t, OCH2CH_3). **IR** ($CDCl_3$, cm^{-1}): 3086 (C=C, w), 1748 (C=O, s), 1670 (NC(O), s). **Mass spectrum** (CI, m/z): 278 (M+1). **Anal. Calcd. for $C_{15}H_{19}NO_4$**: C, 64.98; H, 6.86. **Found**: C, 64.99; H, 7.00.

Epoxidation of Vinylacetamido N-Benzyloxyglycine Ethyl Ester

m-Chloroperbenzoic acid (315 mg of 85%, 1.56 mmoles) [Sigma] was added in one

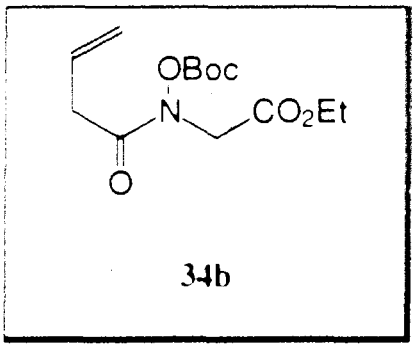


41a

portion to a cooled (0 °C) dichloromethane (5 mL) solution of vinylacetamido N-benzyloxyglycine ethyl ester (174.4 mg, 0.63 mole). The cloudy reaction mixture was stirred for 23 h and allowed to warm to room temperature. Then dichloromethane (20 mL) was added and the mixture was washed with saturated sodium bicarbonate (20 mL). The organic layer was separated, dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude product was purified by flash chromatography (40% ethyl acetate-hexane) to give 137 mg (74%) of a yellow oil. **^{1}Hmr** ($CDCl_3$, δ): 7.38 (5H, m, OCH_2Ph), 4.86 (2H, s, OCH_2Ph), 4.45 (2H, q, NCH_2CO) 4.28 (2H, q, $COCH_2CH_3$), 3.31 (1H, m), 2.86-2.83 (2H, m), 2.57-2.49 (2H, m) 1.28 (3H, t, $COCH_2CH_3$). **IR** (film, cm^{-1}): 1743 (C=O, s), 1672 (C=O, amide, s). **Mass spectrum** (CI, m/z): 294 (M+1). **Anal. Calcd. for $C_{15}H_{19}NO_5$**: C, 61.43; H, 6.48. **Found**: C, 61.62; H, 6.73.

Vinylacetamido N-t-Butoxycarbonyloxyglycine Ethyl Ester

A solution of the alkene **34** (371.4 mg, 1.98 mmoles) and t-butylcarbonate (433 mg, 1.98 mmoles) [Fluka] in tetrahydrofuran (3 mL) was stirred vigorously at room temperature

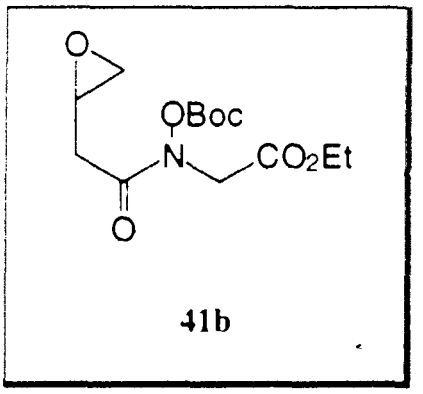


34b

for 5 min. Then 1M sodium hydroxide (1.98 mL, 1.98 mmoles) was added dropwise with stirring during 40 min. Stirring was continued for 2 h and the reaction mixture was then diluted with ether (40 mL), washed with saturated sodium chloride (20 mL), dried over anhydrous magnesium sulfate, concentrated under reduced pressure and the product purified by flash chromatography (40% ethyl acetate-hexane) to give a colourless oil (247 mg, 43%). **^{1}Hmr** ($CDCl_3$, δ: 5.98-5.91 (1H, m, *CH*=CH_2), 5.21-5.16 (2H, m, CH=*CH_2*), 4.43 (2H, s, *CH_2*$CO_2CH_2CH_3$), 4.20 (2H, q, CO_2*CH_2*CH_3), 3.20 (2H, d, *CH_2*-CH=CH_2), 1.53 (9H, s, $(CH_3)_3C$-), 1.27 (3H, t, CO_2CH_2*CH_3*). **IR** (film, cm^{-1}): 3083 (C=C, w), 1788 (CO_2, s), 1702, 1755 (C=O, s), 1642 (C=O, amide, s). **Mass spectrum** (CI, m/z): 288 (M+1)

Epoxidation of Vinylacetamido N-t-Butoxycarbonyloxyglycine Ethyl Ester

m-Chloroperbenzoic acid (133 mg, 0.66 mmole) [Sigma] was added in one portion to a cooled (0 °C) dichloromethane (1 mL) solution of vinylacetamido N-t-



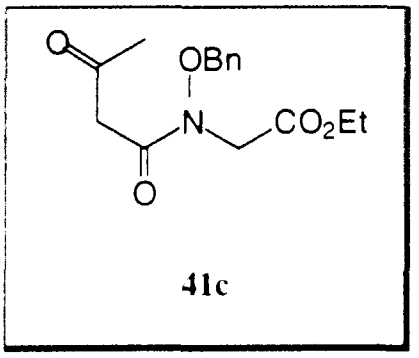
41b

butoxycarbonyloxyglycine ethyl ester (94.2 mg, 0.33 mole). The cloudy reaction mixture was stirred for 24 h and allowed to warm to room temperature. Then dichloromethane (20 mL) was added and the mixture was washed with saturated sodium bicarbonate (20 mL). The organic layer was separated, dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude product was purified by flash chromatography (40% ethyl acetate-hexane) to give 63 mg (63%) of a yellow oil. **^{1}Hmr** ($CDCl_3$, δ): 7.38(5H, m, OCH_2Ph), 4.86 (2H, s, OCH_2Ph), 4.45 (2H, q, NCH_2CO), 4.28 (2H, q, $COCH_2CH_3$), 3.31 (1H, m), 2.86-2.83 (2H, m), 2.57-2.49 (2H, m), 1.53 (9H, s, $(CH_3)_3C$-), 1.28 (3H, t, $COCH_2CH_3$). **IR** (film, cm^{-1}): 1743 (C=O, s), 1672 (C=O, amide, s). **Anal. Calcd. for $C_{13}H_{21}NO_7$**: C, 51.49; H, 6.93; N, 4.62. **Found**: C, 51.42; H, 7.13; N, 4.83.

Acetoacetamido N-Benzyloxyglycine Ethyl Ester (79)

A solution of N-benzyloxyglycine ethyl ester (0.159 g, 0.76 mmole) and 2,2,6-trimethyl-4H-1,3-dioxin-4-one (0.1 mL, 0.76 mmole) in toluene (0.76 mL) was immersed in a

preheated (110 °C) oil bath and the solution was stirred vigorously for 4 h and monitored by tlc. The reaction mixture was then concentrated under reduced pressure and further

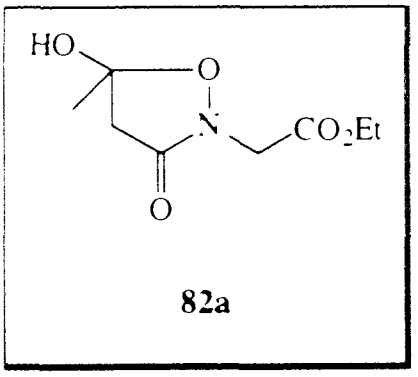


41c

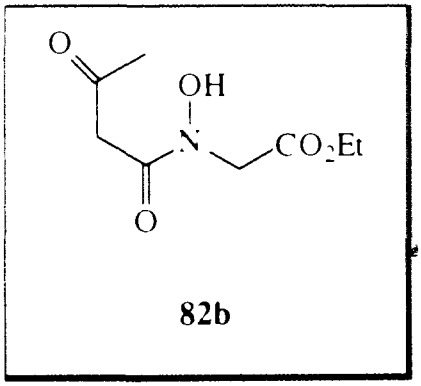
dried at 1 torr for 2h to give a viscous orange oil (0.222 g, 100%). **^{1}Hmr** ($CDCl_3$, δ): 7.40 (5H, s, OCH_2Ph), 4.88 (2H, s, OCH_2Ph), 4.32 (2H, s, (O)CCH_2C(O)), 4.22 (2H, q, OCH_2CH_3), 3.55 (2H, s, NCH_2CO_2Et), 2.25 (3H, s, CH_3C(O)), 1.29 (3H, t, OCH_2CH_3). **IR** ($CDCl_3$, cm^{-1}): 1749 (C=O , s), 1670 (NC(O)). **Mass spectrum** (CI, m/z): 294 (M+1). **Anal. Calcd. for $C_{15}H_{19}NO_5$**: C, 61.43; H, 6.48; N, 4.78; **Found**: C, 62.21; H, 6.74; N, 5.14.

Hydrogenolysis of Acetoacetamido N-Benzyloxyglycine Ethyl Ester

Palladium on carbon (10%) (97 mg) was added to a solution of acetoacetamido N-



82a



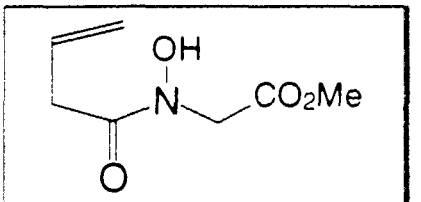
82b

benzyloxyglycine ethyl ester (104 mg, 0.35 mmole) in ethanol (2 mL). The resulting suspension was fitted with a balloon, flushed thrice with hydrogen and then stirred under hydrogen for 1 h at room temperature. After filtration through Celite and and evaporation

of the filtrate, the oil was purified by chromatography through a short column of silica gel using 60% ethyl acetate- hexanes. The colourless oil isolated weighed 65.2 mg (91%) and was identified as an 83:17 mixture of **82a** and **82b**. **^{1}Hmr** ($CDCl_3$, δ): **82a**: 4.51 (1H, d, 18.2 Hz), 4.24 (2H, q), 4.12 (1H, d, 18.2 Hz), 3.03 (1H, d, 16.8 Hz), 2.75 (1H, d, 16.8 Hz), 1.60 (3H, s), 1.29 (3H, s), **82b**: 4.54 (2H, s), 4.24 (2H, q), 3.75 (2H, s), 2.29 (3H, s), 1.29 (3H, s). **IR** (film, cm^{-1}): 3390, 1746, 1687. **Mass spectrum** (CI, m/z): 204 (M+1). **Anal. Calcd. for $C_8H_{13}NO_5$**: C, 47.29; H, 6.70; N, 6.20; **Found**: C, 47.57; H, 6.40; N, 6.90.

Vinylacetamido N-Hydroxyglycine Methyl Ester

Vinylacetic acid (0.834 mL, 0.845 g, 9.52 mmoles) was added in one portion to a stirred solution of N-hydroxyglycine methyl ester (1.0 g, 9.52 mmoles) in dichloromethane



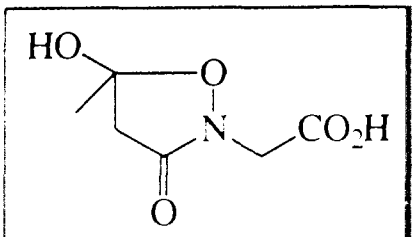
48

(60 mL). The resulting solution was cooled to -5 °C and a solution of DCC (1.97 g, 9.52 mmoles) [BDH] in dichloromethane (20 mL) was added dropwise, with stirring, during 10 min. Stirring was continued for 3 h while the reaction was allowed to warm to room temperature. The solvent was then removed under reduced pressure and the product was purified by flash chromatography (70% ethyl acetate-hexane) to give a pale yellow oil (0.864 g, 52%). **^{1}Hmr** ($CDCl_3$, δ): 5.97-5.88 (1H, m), 5.20-5.16 (2H, m, $=CH_2$), 4.43 (2H, s), 3.78 (3H, s, CH_3), 3.33 (2H, d, $CH_2C(O)$). **IR** (film, cm^{-1}): 3196 (OH, s), 1752

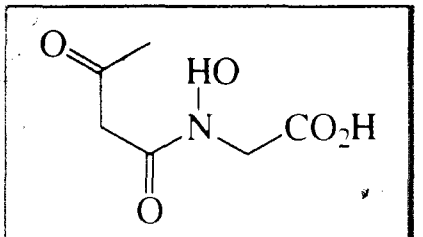
(C=O, s), 1630 (C=O, amide, s). **Mass spectrum** (CI, m/z): 174 (M+1). **Anal. Calcd. for $C_7H_{11}NO_4$**: C, 48.55; H, 6.36; N, 8.09. **Found**: C, 48.94; H, 6.55; N, 7.80.

Synthesis of 81 (80)

Diketene (0.13 mL, 0.12 g, 1.67 mmoles) was added to a solution of N-hydroxyglycine



81a

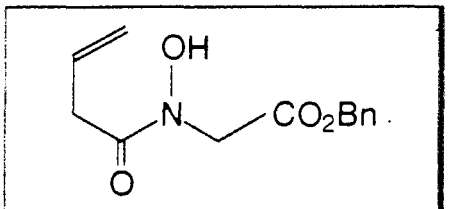


81b

benzyl ester (0.303 g, 1.67 mmoles) in chloroform (3 mL). The solution was stirred for 3 h at room temperature, concentrated under reduced preesure and purified by plc (60% EtOAc-hexanes). The yellow solid weighed 0.16 g (36%). **^{1}Hmr** ($CDCl_3$, δ): 7.37-7.32 (5H, m, Ar), 5.18 (2H, s, CH_2Ph), 4.53 (1H; d, 18.1 Hz), 4.19 (1H, d, 18.1 Hz), 3 (1H, d, 16.8 Hz), 2.76 (1H, d, 16.8 Hz), 1.60 (3H, s, CH_3). **Mass spectrum** (CI, m/z): 266 (M+1). The solid (150 mg, 0.57 mmole) was dissolved in ethanol and palladium on carbon (150 mg, 10%) was added. The suspension was fitted with a balloon, flushed thrice with hydrogen and then stirred under hydrogen for 1 h at room temperature, filtered through Celite and evaporated. The oil was redissolved in water (10 mL), filtered and the filtrate was lyophilized to give a brown solid 80 mg (81%), identified as an 83:17 mixture of **81a** and **81b**. **^{1}Hmr** (D_2O, δ): **81a**: 4.39 (1H, d, 17.7 Hz), 4.20 (1H, d, 17.7 Hz), 3.17 (1H, d, 17.2 Hz), 2.77 (1H, d, 17.2 Hz), 1.60 (3H, s), **81b**: 4.38 (2H, s), 3.38 (2H, s), 2.29 (3H, s). **Mass spectrum** (CI, m/z): 176 (M+1).

Coupling of N-Hydroxyglycine Benzyl Ester and Vinylacetic acid

Dicyclohexylcarbodiimide (1.38 g, 6.68 mmoles) [BDH] was added to a cooled solution of N-hydroxyglycine benzyl ester (1.15 g, 6.36 mmoles) and vinylacetic acid (0.57 mL,

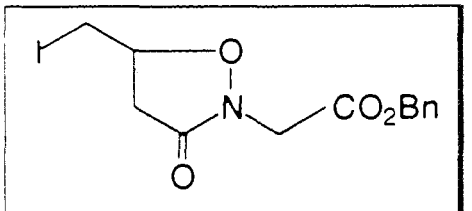


38

575 mg, 6.68 mmoles) in dichloromethane (30 mL). The cloudy mixture was stirred for 0.5 h at 0 °C and then for 3.5 h at room temperature, filtered and concentrated. The resulting oil was purified by flash chromatography (60% ethyl acetate-hexane) to give 912 mg (58%) of the product. **^{1}Hmr** ($CDCl_3$, δ): 7.38-7.33 (5H, m, Ar), 6.02-5.89 (1H, m), 5.21-5.17 (2H, m, $=CH_2$), 5.19 (2H, s, CH_2Ph), 4.52 (2H, s), 3.34 (2H, d, $CH_2C(O)$. **IR** (film, cm^{-1}): 3200 (OH, s), 1749 (C=O, s), 1633 (C=O, amide, s). **Mass spectrum** (CI, m/z): 250 (M+1). **Anal. Calcd. for $C_{13}H_{15}NO_4$**: C, 62.65; H, 6.02; N, 5.62. **Found**: C, 62.88; H, 6.22; N,6.00.

Iodocyclization of 38 (59, 60)

Iodine (1.23 g, 4.83 mmoles) and sodium bicarbonate (0.41 g, 8.83 mmoles) were added to a solution of **38** (0.40 g, 1.61 mmoles) in acetonitrile (10 mL). The solution was stirred

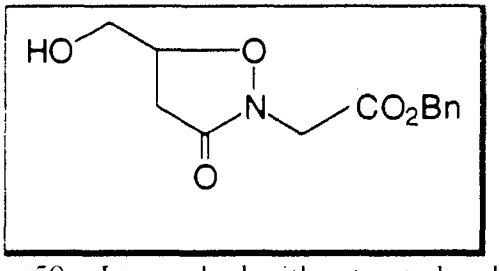


39

for 3 h at 0 °C and then diluted with ether (20 mL) and washed successively with saturated sodium thiosulfate (20 mL) and saturated sodium chloride (20 mL), dried over anhydrous magnesium sulfate and evaporated under reduced pressure. The product was purified by flash chromatography (60% ethyl acetate-hexanes) to give an oil 0.345 g (57%). **^{1}Hmr** ($CDCl_3$, δ): 7.38-7.33 (5H, m, Ar), 5.21 (1H, d, CH*H*Ph, 12.08 Hz), 5.19 (1H, d, C*H*HPh, 12.08 Hz), 4.61-4.58 (1H, m), 4.33 (1H, d, C*H*H, 17.8 Hz), 4.30 (1H, d, CH*H*, 17.8 Hz), 3.33 (1H, dd, 10.36, 5.52 Hz), 3.28 (1H, dd, 10.36, 7.56 Hz), 2.98 (1H, dd, 16.80, 8.04 Hz), 2.73 (1H, dd, 16.80, 7.24 Hz). **IR** (film, cm^{-1}): 1750, 1718 (C=O). **Mass spectrum** (EI, m/z): 375 (M^+).

Oxidation of 39 (61)

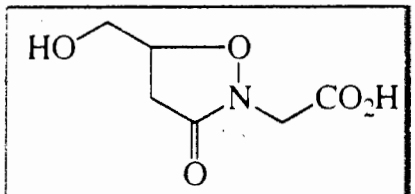
m-Chloroperbenzoic acid (68%), (351 mg, 1.38 mmoles) was added to a solution of **39** (345 mg, 0.92 mmole) in dichloromethane (6 mL). The purple solution was stirred for 24



h, then diluted with ether (50 mL), washed with saturated sodium thiosulfate (10 mL) and dried over anhydrous magnesium sulfate. The residue was purified by plc (80% ethyl acetate-hexanes) to give 66 mg of product (27%). **^{1}Hmr** ($CDCl_3$, δ): 7.38-7.33 (5H, m, Ar), 5.21 (1H, d, CH*H*Ph, 12.08 Hz), 5.19 (1H, d, C*H*HPh, 12.08 Hz), 4.66-4.60 (1 H, m), 4.57 (1H, d, C*H*H, 18 Hz), 4.13 (1H, d, CH*H*, 18 Hz), 3.87 (1H, dd, 12.80, 2.48 Hz), 3.66 (1H, dd, 12.80, 4.24 Hz), 2.92 (1H, dd, 16.7, 8.56 Hz), 2.87 (1H, dd, 16.7, 6.68 Hz). **IR** ($CHCl_3$, cm^{-1}): 1750, 1718 (C=O). **Mass spectrum** (CI, m/z): 266 (M+1).

Synthesis of 40

Palladium (10% on carbon) (250 mg) was added to a solution of the benzyl ester (190.5 mg, 0.72 mmole) in ethanol (10 mL). The resulting suspension was fitted with a balloon,



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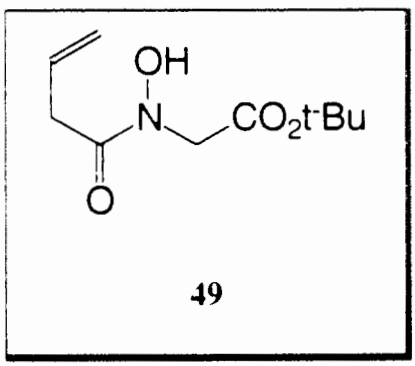
flushed thrice with hydrogen and then stirred under hydrogen for 1 h at room temperature. After filtration through Celite and and evaporation of the filtrate, the oil was redissolved in water (10 mL), filtered and the filtrate was lyophilized to give a pale green viscous oil 120 mg (95%). **^{1}Hmr** (D_2O, δ): 4.74 (1H,m), 4.36 (1H, d, 18.2 Hz), 4.34 (1H, d, 18.2 Hz), 3.78 (1H, dd, 12.9, 3.2 Hz), 3.74 (1H, dd, 12.9, 5.64 Hz), 3.02, (1H, dd, 17.04, 8.76 Hz), 2.79 (1H, dd, 17.04, 7.68 Hz). **Mass spectrum** (CI, m/z): 176 (M+1).

Vinylacetamido N-Hydroxyglycine t-Butyl Ester

Dicyclohexylcarbodiimide (0.79 g, 3.83 mmoles) [BDH] was added to a cooled solution of **28** (0.563 g, 3.83 mmoles) and vinylacetic (0.33 mL, 0.33 g, 3.83 mmoles) in



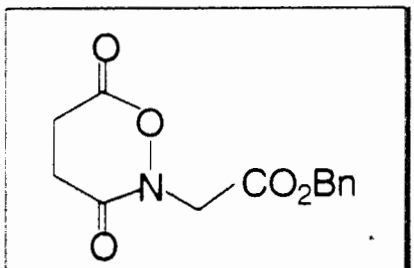
49

dichloromethane (20 mL). The cloudy mixture was stirred for 0.5 h at 0 °C and then for

3.5 h at room temperature, filtered and concentrated. The resulting oil was purified by flash chromatography (60% ethyl acetate-hexane) to give 425 mg (52%) of the product. **^{1}Hmr** ($CDCl_3$, δ): 6.00-5.92 (1H, m), 5.22-5.17 (2H, m), 4.40 (2H, s), 3.34 (2H, d), 1.49 (9H, s). **IR** (film, cm^{-1}): 3200 (OH), 1749 (C=O), 1633 (C=O, amide). **Mass spectrum** (CI, m/z): 216 (M+1).

Synthesis of 42 (64)

Succinic anhydride (100 mg, 1 mmole) was suspended in dichloromethane (0.5 mL) and a solution of N-hydroxyglycine benzyl ester (181 mg, 1 mmole) in dichloromethane



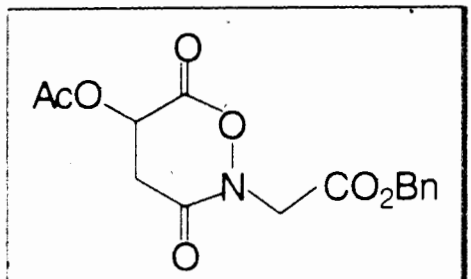
42

(0.5 mL) was added with stirring. Stirring was continued for 16 h. The suspension was then filtered and the white solid was dried at 1 torr to give 183 mg (65%) of the product. **^{1}Hmr** ($(CD_3)_2CO$, δ): 7.41-7.33 (5H, m, Ar), 5.17 (2H, s, CH_2Ph), 4.41 (2H, s), 2.82-2.55 (4H, m, $(CH_2)_2$). The hydroxysuccinamic acid (175.6 mg, 0.62 mmole) was suspended in dichloromethane (0.65 mL) under nitrogen at 0 °C and dicyclohexylcarbodiimide (128 mg, 0.62 mmole) was added in one portion. Stirring was continued for 4 h at 0 °C. The mixture was then filtered through Celite and the filtrate concentrated under reduced pressure to afford an oily residue. Purification by plc (40% ethyl acetate-hexanes) gave 105 mg (64%) of the product as an oil. **^{1}Hmr** ($CDCl_3$, δ): 7.41-7.33 (5H, m, Ar), 5.21

(2H, s, CH_2Ph), 4.49 (2H, s), 2.91-2.87 (2H, m), 2.77-2.74 (2H, m). **Mass spectrum** (CI, m/z): 264 (M+1).

5-Acetoxy-Tetrahydro-1,2-Oxazin-3,6-dione (64)

N-Hydroxyglycine benzyl ester (362 mg, 2 mmoles) in dichloromethane (1 mL) was added with stirring to a solution of acetoxysuccinic anhydride (4.11 mg, 2.6 mmoles) in



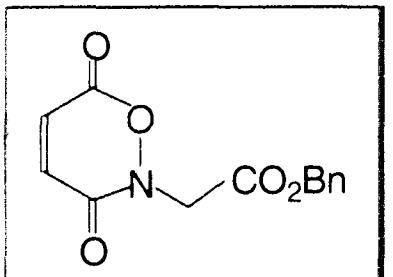
43

dichloromethane (1 mL). Stirring was continued for 16 h. The suspension was then filtered and the white solid was collected and dried at 1 torr to give 518.2 mg (80%) of the product. **^{1}Hmr** ($(CD_3)_2CO$, δ): 7.41-7.33 (5H, m, Ar), 5.87 (1H, dd, 2.68, 2.76 Hz), 5.20 (2H, s, CH_2Ph), 4.66 (1H, d, 17.6 Hz), 4.18 (1H, d, 17.6 Hz), 3.10 (1H, dd, 17.1, 2.72 Hz), 2.59 (1H, dd, 17.1 Hz). **Mass spectrum** (CI, m/z): 340 (M+1). The above product (500 mg, 1.55 mmoles) was suspended under nitrogen in dichloromethane (1.5 mL), the suspension was cooled 0 °C and dicyclohexylcarbodiimide (319 mg, 1.55 mmoles) was added in one portion. Stirring was continued at 0 °C for 4 h, and the slurry was then filtered through Celite and the filtrate concentrated under reduced pressure. The oily residue was purified by plc (40% ethyl acetate-hexanes) to give 417.3 mg (88%) of the product as an oil. **^{1}Hmr** ($CDCl_3$, δ): 7.41-7.32 (5H, m, Ar), 5.61 (1H, dd, 5.28, 11.2

Hz), 5.20 (2H, s, CH_2Ph), 4.58 (1H, d, 18.1 Hz), 4.49 (1H, d, 18.1 Hz), 3.22 (1H, dd, 16.7, 11.2 Hz), 3.34 (1H, dd, 16.7, 5.28 Hz). **Mass spectrum** (CI, m/z): 322 (M+1).

Synthesis of 44

Maleic anhydride (98 mg, 1 mmole) was suspended in dichloromethane (0.5 mL) and a solution of N-hydroxyglycine benzyl ester (181 mg, 1 mmole) in dichloromethane (0.5

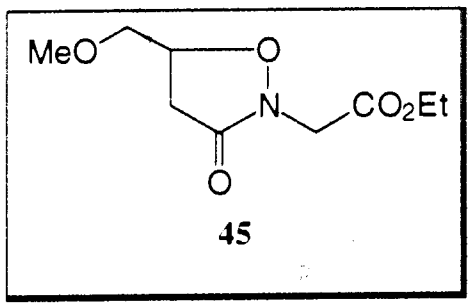


44

mL) was added with stirring. Stirring was continued for 16 h. The suspension was then filtered and the white solid was dried at 1 torr to give 178 mg (64%). **^{1}Hmr** ($CDCl_3$, δ): 7.41-7.32 (5H, m, Ar), 7.11 (1H, d, 12.9 Hz), 6.35 (1H, d, 12.9 Hz), 5.22 (2H, s, CH_2Ph), 4.59 (2H, s). The solid (171 mg, 0.61 mmole) was suspended under nitrogen in dichloromethane (1 mL), the suspension was cooled 0 °C, and dicyclohexylcarbodiimide (126.5 mg, 0.61 mmole) was added in one portion. Stirring was continued at 0 °C for 4 h. The slurry was then filtered through Celite and the filtrate concentrated under reduced pressure to afford an oily residue. Purification by plc (40% ethyl acetate-hexanes) gave 154 mg (96%) of the product as a yellow solid. **^{1}Hmr** ($CDCl_3$, δ): 7.39-7.32 (5H, m, Ar), 7.01 (1H, d, 10.2 Hz), 6.78 (1H, d, 10.2 Hz), 5.20 (2H, s, CH_2Ph), 4.66 (2H, s). **Mass spectrum** (CI, m/z): 262 (M+1).

Reaction of 34 with NBS in Methanol

A solution of the alkene **34** (100 mg, 0.53 mmole) in dry methanol (5 mL) was cooled in



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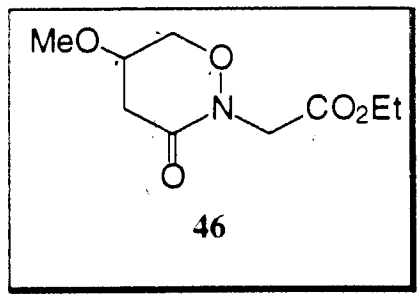


46

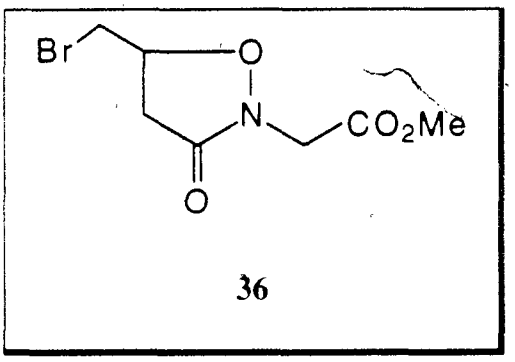
an ice-water bath, and N-bromosuccinimide (95 mg, 0.53 mmole) [BDH] was added in one portion. Stirring was continued at 0 °C for 15 min and then at room temperature for 45 min. The reaction mixture was concentrated, and the residue was subjected to flash chromatography (60% ethyl acetate -hexanes). Two products were separated. These were dissolved in dichloromethane (3 mL), washed with saturated sodium bicarbonate (2 mL), dried over anhydrous sodium sulfate and concentrated to give **36** (9.6 mg) and a mixture of **45** and **46** (25 mg). **^{1}Hmr** ($CDCl_3$, δ): **46**: 4.51 (1H, d, 17.8 Hz, C*H*H), 4.23 (1H, d, 17.8 Hz, CH*H*), 4.33 (1H, dd, 11.9, 6.0 Hz, C*H*H), 4.23 (2H, q, OCH_2CH_3), 4.07 (1H, dd, 11.9, 3.6 Hz, CH*H*), 4.02-3.98 (1H, m, CH), 2.80 (1H, dd, 16.0 Hz, 5.8 Hz, C*H*H), 2.68 (1H, dd, 16.0 Hz, 4.7 Hz, CH*H*), 1.30 (3H, t, OCH_2CH_3). **45**: 4.75-4.73 (1H, m, CH), 4.28 (1H, d, 17.8 Hz, C*H*H), 4.23 (2H, q, OCH_2CH_3), 4.22 (1H, d, 17.8 Hz, CH*H*), 3.62 (1H, dd, 10.9, 6.2 Hz, C*H*H), 3.54 (H, dd, 10.9, 3.6 Hz, CH*H*), 2.81 (1H, dd, 16.6, 8.2 Hz, C*H*H), 2.75 (1H, dd, 16.6, 8.8 Hz, CH*H*), 1.30 (3H, t, OCH_2CH_3). **IR** (film, cm^{-1}): 1793, 1751, 1700. **Mass spectrum** (EI, m/z): 217 (M^+), 144, 112, 99, 85, (CI, m/z): 218 (M+1), GC-MS (CI, m/z): 218 (M+1), 99, 218 (M+1), 85.

Reaction of 38 with NBS in Methanol

A solution of **38** (120.3 mg, 0.44 mmole) in methanol (3 mL) was cooled in an ice-water bath and N-bromosuccinimide (164 mg, 0.92 mmole) [BDH] was added in one portion. Stirring was continued at 0 °C for 15 min then at room temperature for 3 h. The solvent was removed and the residue was redissolved in dichloromethane (20 mL), and washed successively with water (10 mL) and saturated sodium bicarbonate (30 mL), dried over anhydrous sodium sulfate and concentrated. Purification by plc (60% ethyl acetate-hexane) afforded the product. **Mass Spectrum**: **Calcd**. for $C_{14}H_{19}{}^{79}BrNO_5$ (M+1): 360.0403. **Found** (HR-CIMS): 360.0435. **Calcd**. for $C_{14}H_{19}{}^{81}BrNO_5$ (M+1): 362.0378. **Found** (HR-CIMS): 362.0414.

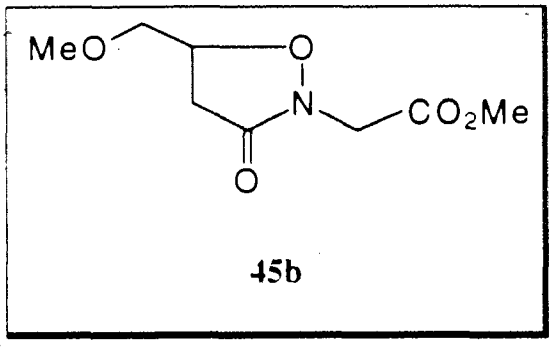
Reaction of 48 with NBS in Methanol

A solution of **48** (601 mg, 3.47 mmoles) in methanol (10 mL) was cooled in an ice-water

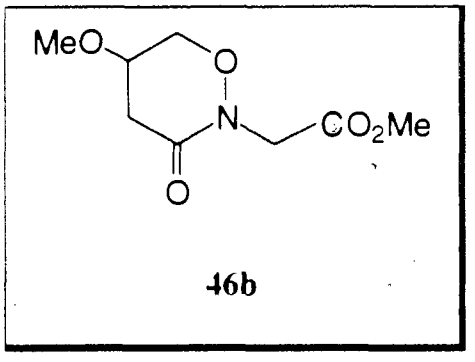


36

bath and N-bromosuccinimide (618 mg, 3.47 mmoles) [BDH] was added in one portion.



45b



46b

Stirring was continued at 0 °C for 15 min then at room temperature for 2.25 h. The solvent was removed and the residue was redissolved in dichloromethane (50 mL), and washed successively with water (20 mL) and saturated sodium bicarbonate (30 mL), dried over anhydrous sodium sulfate and concentrated. Column chromatography (60% ethyl acetate-hexane) afforded the products **36** (155 mg), and a mixture of **45b** and **46b** (282 mg). **^{1}Hmr** ($CDCl_3$, δ): **36**: 4.78 (1H, m), 4.29 (1H, d, 17.0 Hz), 4.28 (1H, d, 17.0 Hz), 3.58 (1H, dd, 10.8, 5.5 Hz), 3.53 (1H, dd, 10.8, 6.7 Hz), 3.01 (1H, dd, 16.8, 8.2 Hz), 2.83 (1H, dd, 16.8, 7.0 Hz); **46b**: 4.51 (1H, d, 17.8 Hz, C*H*H), 4.23 (1H, d, 17.8 Hz, CH*H*), 4.33 (1H, dd, 11.9 Hz, 6 Hz, C*H*H), 4.07 (1H, dd, 11.9 Hz, 3.6 Hz, CH*H*), 4.02-3.98 (1H, m, CH), 2.80 (1H, dd, 16 Hz, 5.8 Hz, C*H*H), 2.68 (1H, dd, 16 Hz, 4.7 Hz, CH*H*), 3.39 (3H, s, CO_2CH_3); **45b**: 4.78-4.72 (1H, m, CH), 4.28 (1H, d, 17.8Hz, C*H*H), 4.22 (1H, d, 17.8 Hz, CH*H*), 3.62 (1H, dd, 10.9 Hz, 6.2 Hz, C*H*H), 3.54 (H, dd, 10.9 Hz, 3.6 Hz, CH*H*), 2.81 (1H, dd, 16.6 Hz, 8.2 Hz, C*H*H), 2.75 (1H, dd, 16.6 Hz, 8.8 Hz, CH*H*), 3.32 (3H, s, CO_2CH_3). **IR** ($CDCl_3$, cm^{-1}): 1755 (C=O), 1694 (C=O, amide). **Mass spectrum** (GC-MS, m/z): 203, 144, 99, 71, 45, 203, 171, 144, 112, 102, 85, 75, 58, 45. **Anal. Calcd. for $C_8H_{13}NO_5$:** C, 47.29; H, 6.40; N, 6.90. **Found**: C, 47.04; H, 6.55; N, 7.33.

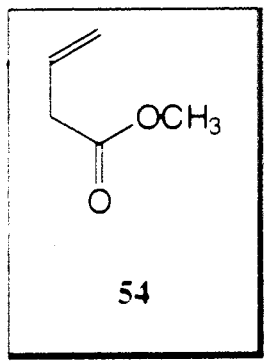
Reaction of 49 with NBS in Methanol

A solution of **49** (197.7 mg, 0.92 mmole) in methanol (3 mL) was cooled in an ice-water bath and N-bromosuccinimide (164 mg, 0.92 mmole) [BDH] was added in one portion. Stirring was continued at 0 °C for 15 min then at room temperature for 1 h. The solvent was removed and the residue was redissolved in dichloromethane (50 mL), and washed successively with water (20 mL) and saturated sodium bicarbonate (30 mL), dried over

anhydrous sodium sulfate and concentrated. Purification by plc (60% ethyl acetate-hexane) afforded the products. **A**: **Mass Spectrum**: **Calcd**. for $C_7H_{12}NO_5$ (M-C_4H_8) 190.0682. **Found** (HR-CIMS): 190.0710. **B**: **Calcd**. for $C_{11}H_{21}{}^{79}BrNO_5$ (M+1): 326.0558. **Found** (HR-CIMS): 326.0592. **Calcd**. for $C_{11}H_{21}{}^{81}BrNO_5$ (M+1): 328.0523. **Found** (HR-CIMS): 328.0570.

Methyl Vinylacetate

A solution of vinylacetic acid (1 mL, 1.013 g, 1 mmole) in dichloromethane (80 mL) was cooled in an ice-water bath and diazomethane [prepared by passing nitrogen gas over



54

a suspension of p-toluenesulphonylmethyl nitroso amide (2 g) in ethanol (12.5 mL)] was introduced until the solution remained bright yellow. A drop of glacial acetic acid was added and the solvent was then removed. The oil was distilled and the product was collected at 108 °C (1.176 g, 100%). **^{1}Hmr** ($CDCl_3$, δ): 5.97-5.82 (1H, m), 5.19-5.13 (2H, m), 3.68 (3H, s), 3.10 (1H, t, 1.44, 1.36 Hz, C*H*H), 3.09 (1H, t, 1.44, 1.36 Hz, CH*H*). **IR** (film, cm^{-1}): 3083, 1742, 1644. **Mass spectrum** (EI, m/z): 100 (M^+).

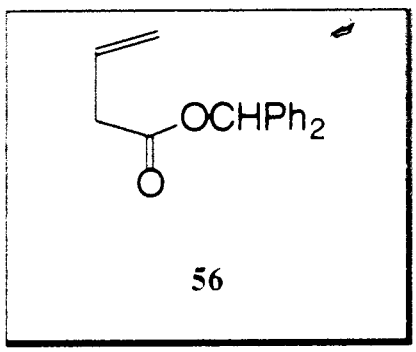
Diphenyldiazomethane

Hydrazine monohydrate (4.68 mL, 4.81 g, 0.096 mole) [Anachemia] was added to a suspension of benzophenone (12.5 g, 0.069 mole) in ethanol (10 mL). The mixture was refluxed overnight, cooled to room temperature and filtered. Recrystallisation from hot

ethanol gave diphenylhydrazone as white needles m.p. 98-100°C Activated manganese (IV) oxide (3 g of 85% purity, 0.03 mole) was added in portions to a cooled (0 °C) suspension of diphenylhydrazone (1.61 g, 0.008 mole) and anhydrous magnesium sulfate (0.886 g, 0.006 mole) in dry dichloromethane (17 mL). The purple reaction mixture was stirred at 0 °C for 2.5 h, at room temperature for 2 h, filtered and concentrated. The product was recrystallized from hexane to give 1.59 g (100%) of diphenyldiazomethane.

Benzhydryl Ester of Vinylacetic Acid

A solution of diphenyldiazomethane (495 mg, 2.55 mmoles in dichloromethane (5 mL) was added dropwise to a solution of vinylacetic acid (0.206 mL, 0.209 g, 2.42 mmoles) in

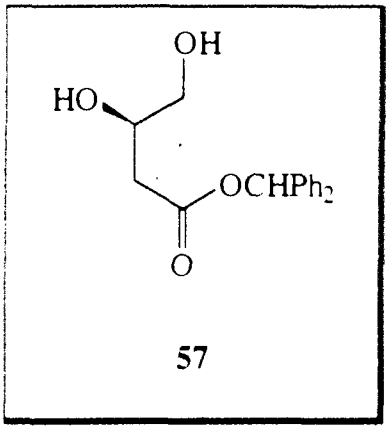


56

dichloromethane (5 mL). The reaction mixture was stirred at room temperature. The colour became yellow after 10 min. Stirring was continued for 70 min and the solvent was then removed (0.667 g, 100%). **^{1}Hmr** ($CDCl_3$, δ): 7.39 (10H, s, CH*Ph*$_2$), 6.90 (1H, s, C*H*Ph$_2$), 6.01-5.87 (1H, m), 5.23-5.16 (2H, m), 3.21 (1H, dt, 6.96, 1.4 Hz, C*H*H), 3.15 (1H, dt, 6.96, 1.4 Hz, CH*H*). **IR** (film, cm^{-1}): 3031, 1739. **Mass spectrum** (CI, m/z): 253 (M+1).

Sharpless Hydroxylation of 56 (α-mix) (67)

A mixture of t-butyl alcohol (20 mL) and water (20 mL) were added to AD-mix α (5.42



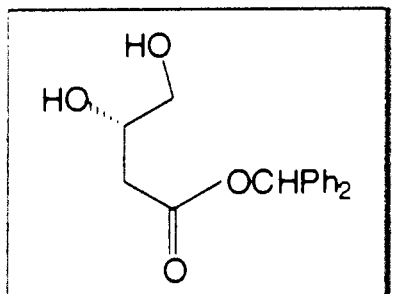
57

g). The suspension was stirred at room temperature until most of the solid had dissolved, cooled in an ice-water bath, and **56** (1.008 g, 4 mmoles) was added in one portion. The mixture was stirred at 0 °C for 12 h, sodium sulfite (1.5 g, 11.90 mmoles) was added, and the ice-bath was removed. Stirring was continued for 1 h. The resulting grey slurry was extracted with ethyl acetate (3 x 15 mL), and the extract was dried over anhydrous sodium sulfate and evaporated. The crude product was purified by flash chromatography (60% ethyl acetate-hexane) to give 708 mg (62%) of the product. **^{1}Hmr** ($CDCl_3$, δ): 7.39 (10 H, s, CH*Ph*$_2$), 6.90 (1H, s, C*H*Ph$_2$), 4.15 (1H, m), 3.67 (1H, dd, 11.2, 3.28 Hz, C*H*H), 3.52 (1H, dd, 11.2, 6.12 Hz, CH*H*), 2.69 (1H, dd, 16.6, 3.92 Hz, C*H*H), 2.63 (1H, dd, 16.6, 8.64 Hz, CH*H*). **IR** (film, cm^{-1}): 3394 (OH, b), 3088 (C-H, aromatic), 1732 (C=O). **Mass spectrum** (EI, m/z): 209 (M-Ph), 184 (Ph_2CHOH), 167 ($CHPh_2$), 103 (M-$OCHPh_2$). **Rotation:** $[\alpha]_D^{25}$ - 6.33° (c 1.26, EtOH).

Sharpless Hydroxylation of 56 (β-mix) (67)

A mixture of t-butyl alcohol (20 mL) and water (20 mL) were added to AD-mix β

(5.42 g). The suspension was stirred at room temperature until most of the solid had dissolved, cooled in an ice-water bath and **56** (1.008 g, 4 mmoles) was added in one



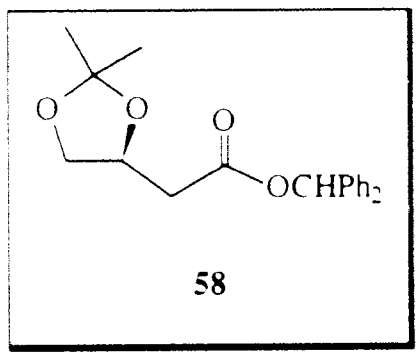
57b

portion. The mixture was stirred at 0 °C for 12 h, sodium sulfite (1.5 g, 11.90 mmoles) was added, and the ice-bath was removed. Stirring was continued for 1 h. The resulting grey slurry was extracted with ethyl acetate (3 x 15 mL), and the extract was dried over anhydrous sodium sulfate and evaporated. The crude product was purified by flash chromatography (60% ethyl acetate-hexane) to give 708 mg (62%) of the product.

Rotation: $[\alpha]_D^{21}$ + 6.33° (c 1.26, EtOH).

Conversion of 57 to the Acetonide (69)

Iodine (67 mg, 0.78 mmole) was added to a solution of **57** (223 mg, 0.78 mmole) in



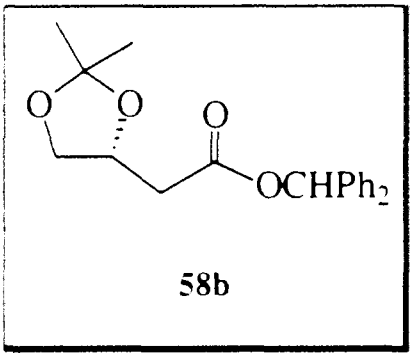
58

acetone (9 mL). The dark brown mixture was stirred at room temperature for 7 h, and 10% sodium thiosulfate (5 mL) was then added. The resulting colourless mixture was

extracted with chloroform (3 x 20 mL). The extract was washed with saturated sodium chloride (10 mL), dried over anhydrous sodium sulfate and concentrated to give 203 mg (80%) of product after purification by flash chromatography (75% ethyl acetate-hexane). **^{1}Hmr** ($CDCl_3$, δ): 7.39 (10 H, s, CH*Ph*$_2$), 6.90 (1H, s, C*H*Ph$_2$), 4.50 (1H, m), 4.12 (1H, dd, 8.36, 5.96 Hz, C*H*H), 3.64 (1H, dd, 8.36, 6.4 Hz, CH*H*), 2.84 (1H, dd, 15.8, 6.24 Hz, C*H*H), 2.63 (1H, dd, 15.8, 7.36 Hz, CH*H*). **IR** (film, cm^{-1}): 3088, 3031, 1738 (C=O). **Mass spectrum** (CI, m/z): 167 ($CHPh_2$), 184 ($HOCHPh_2$). **Rotation:** $[\alpha]_D^{22}$ +3.25° (c 1.23, CH_2Cl_2).

Conversion of 57b to the Acetonide (69)

Iodine (157 mg, 0.62 mmole) was added to a solution of the diol **57b** (522 mg, 1.83 mmoles) in acetone (12 mL). The dark brown mixture was stirred at room temperature for



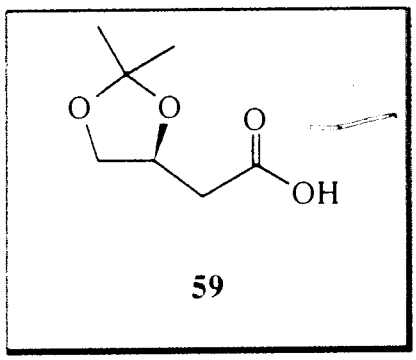
58b

h, and 10% sodium thiosulfate (10 mL)was then added. The resulting colourless mixture was extracted with chloroform (3 x 20 mL). The extract was washed with saturated sodium chloride (20 mL), dried over anhydrous sodium sulfate and concentrated to give 521 mg (88%) of product after isolation by flash chromatography (75% ethyl acetate-hexane). **^{1}Hmr** ($CDCl_3$, δ): 7.39 (10 H, s, CH*Ph*$_2$), 6.90 (1H, s, C*H*Ph$_2$), 4.50 (1H, m), 4.12 (1H, dd, 8.36, 5.96 Hz, C*H*H), 3.64 (1H, dd, 8.36, 6.4 Hz, CH*H*), 2.84 (1H,

dd, 15.84, 6.24 Hz, C*H*H), 2.63 (1H, dd, 15.84, 7.36 Hz, CH*H*). **IR** (film, cm^{-1}): 3088, 3031, 1738 (C=O). **Mass spectrum** (CI, m/z): 167 ($CHPh_2$), 184 ($HOCHPh_2$).

Hydrogenolysis of 58

Palladium (550 mg, 10%) was added to a solution of the benzhydryl ester **58** (564 mg,

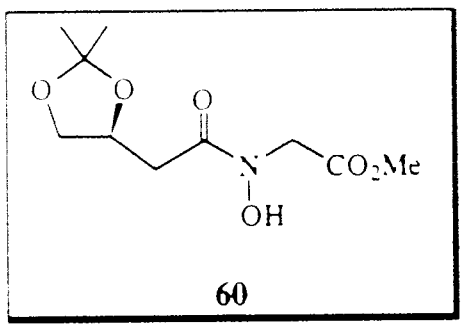


59

1.731 mmoles) in ethanol (15 mL). The resulting suspension was fitted with a balloon, flushed thrice with hydrogen and then stirred under hydrogen for 1 h at room temperature. After filtration through Celite and and evaporation of the filtrate, the oil was washed with ether and dried at 1 torr to give 277 mg (95%) of product. **^{1}Hmr** ($CDCl_3$, δ): 4.43 (1H, m), 4.12 (1H, dd, 8.44, 6 Hz), 3.62 (1H, dd, 8.44, 6.16 Hz), 2.69 (1H, dd, 16.2, 6.88 Hz), 2.54 (1H, dd, 16.2, 5.52 Hz), 1.41 (3H, s), 1.36 (3H, s). **IR** (film, cm^{-1}): 3460 (OH), 1715 (C=O). **Mass spectrum** (CI, m/z): 161 (M+1). **Rotation:** $[\alpha]_D^{21}$ -3.95° (c 0.253, CH_2Cl_2).

Coupling of 59 with N-Hydroxyglycine Methyl Ester

Dicyclohexylcarbodiimide (32.25 mg, 0.16 mmole) [BDH] was added to a cooled (0 °C)

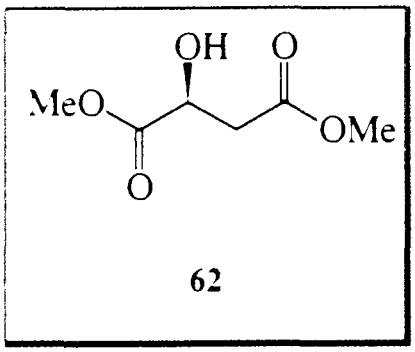


60

solution of **59** (25 mg, 0.16 mmole) and N-hydroxyglycine methyl ester (16.4 mg, 0.16 mmole) in dichloromethane (2 mL). The resulting suspension was stirred for 7 h, allowed to warm to room temperature, and then filtered and concentrated under reduced pressure. The crude product was purified by column chromatography (60% ethyl acetate-hexane). **^{1}Hmr** ($CDCl_3$, δ): 4.47 (2H, d), 4.18 (1H, dd, 8.4, 6.04 Hz, C*H*H), 3.69 (1H, dd, 8.4, 7.04 Hz, CH*H*), 3.77 (3H, s, CO_2CH_3), 3.00 (1H, dd, 15.2, 6.36 Hz, C*H*H), 2.79 (1H, dd, 15.2, 5.96 Hz, CH*H*), 1.42 (3H,s), 1.35 (3H, s). **IR** ($CDCl_3$, cm^{-1}): 3246, 1750 (C=O), 1654 (NC=O). **Mass spectrum** (CI, m/z): 248 (M+1).

Dimethyl (S)-(-)-Malate (70)

L (-) Malic acid (10 g, 0.075 mole), was dissolved in a solution prepared by addition of acetyl chloride (3.33 mL) [Fisher] to methanol (67 mL). The solution was stirred at room

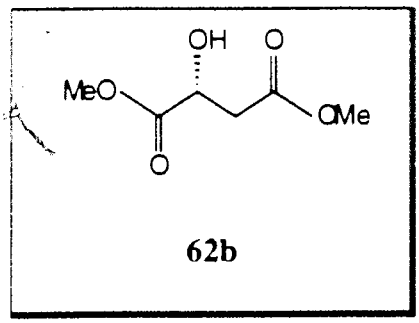


62

temperature for 12 h, and then concentrated. The product was purified by flash chromatography (60% ethyl acetate-hexanes) to give a colourless oil (10.5 g, 87%). **^{1}Hmr** ($CDCl_3$, δ): 4.44 (1H, b), 3.75 (3H, s), 3.65 (3H, s), 3.18 (1H, b), 2.81 (1H, dd, 16.48, 4.36 Hz), 2.74 (1H, dd, 16.48, 6.12 Hz). **IR** (film, cm^{-1}): 3470, 1732. **Mass spectrum** (CI, m/z): 163 (M+1). **Anal. Calcd. for $C_6H_{10}O_5$:** C, 44.44; H, 6.17. **Found**: C, 44.20; H 6.13. **Rotation**: $[\alpha]_D^{25}$ -11.01° (c 2.21, EtOH).

Dimethyl (R)-(+)-Malate

R-(+)-Malic acid (5 g, 0.037mole), was dissolved in a solution prepared by addition of



62b

acetyl chloride (2 mL) [Fisher] to methanol (34 mL). The solution was stirred at room temperature for 13 h, and then concentrated. The product was purified by flash chromatography (60% ethyl acetate-hexanes) to give a colourless oil 5.89 g (98%).

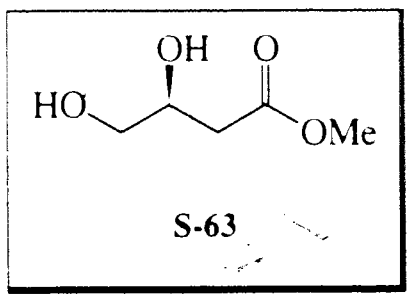
Rotation: $[\alpha]_D^{20}$ + 8.76° (c 2.22, EtOH), *Lit.* (72), $[\alpha]_D^{20}$ + 9.1° (c 2.22, EtOH).

Dimethyl (R,S)-Malate

D,L-Malic acid (20 g, 0.15 mole), was dissolved in a solution prepared by addition of acetyl chloride (6.7 mL) [Fisher] to methanol (134 mL). The solution was stirred at room temperature for 13 h, and then concentrated. The product was purified by flash chromatography (60% ethyl acetate-hexanes) to give a colourless oil (18.5 g, 77%).

(S)-(-)-3,4 Dihydroxybutanoate Methyl Ester (71)

Borane-dimethylsulfide (6.25 mL of 10 M, 0.063 mole), was added dropwise to a



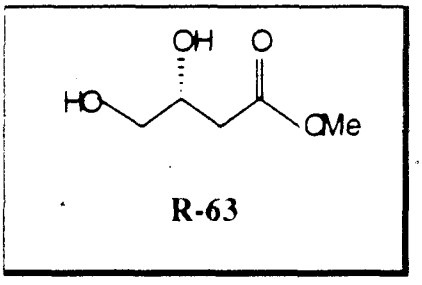
S-63

solution of S-(-)-dimethylmalate (10 g, 0.062 mole) in tetrahydrofuran (125 mL). The

solution was stirred at 20 °C for 30 min and sodium borohydride (0..117 g, 3.1 mmole) [BDH] was added in one portion. Stirring was continued for 30 min, methanol (40 mL) was added dropwise, and after an additional 30 min the solvent was removed under reduced pressure. The product was purified by flash chromatography (60% ethyl acetate-hexanes) to give a colourless viscous oil (5.88 g, 71%). **^{1}Hmr** ($CDCl_3$, δ): 4.07 (1H, m), 3.66 (3H, s), 3.61 (1H, dd, 11.4, 3.52 Hz), 3.46 (1H, dd, 11.4, 6.2 Hz), 2.80 (2H, br), 2.50 (1H, dd, 16.6, 8.52 Hz), 2.44 (1H, dd, 16.6, 4.16 Hz). **IR** (film, cm^{-1}): 3414, 1738. **Mass spectrum** (CI, m/z): 135 (M+1). **Anal. Calcd. for $C_5H_{10}O_4$:** C, 44.78; H, 7.46. **Found**: C, 44.12; H, 7.55. **Rotation**: $[\alpha]_D^{20}$ - 24.9° (c 2.44, EtOH).

(R)-(+)-3,4 Dihydroxybutanoate Methyl Ester

Borane-dimethylsulfide (3.4 mL of 10 M, 0.034 mole), was added dropwise to a solution

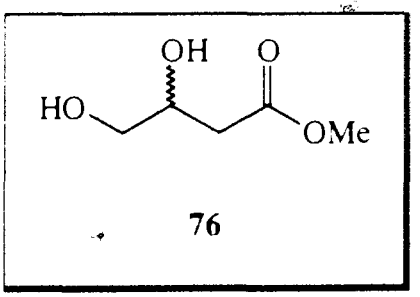


R-63

of R-(+)-dimethylmalate (5.482 g, 0.034 mole) in tetrahydrofuran (68 mL). The solution was stirred at 20 °C for 30 min and sodium borohydride (0.063 g, 1.7 mmole) [BDH] was added in one portion. Stirring was continued for 30 min, methanol (22 mL) was added dropwise, and after an additional 30 min the solvent was removed under reduced pressure. The product was purified by flash chromatography (60% ethyl acetate-hexanes) to give a colourless viscous oil (2.8 g, 63%). **Rotation**: $[\alpha]_D^{20}$ + 22.4° (c 2.44, EtOH).

3,4-Dihydroxybutanoate Methyl Ester

Borane-dimethylsulfide (11 mL of 10 M, 0.11 mole), was added dropwise to asolution of R,S-dimethylmalate (17.80 g, 0..11 mole) in tetrahydrofuran (220 mL). The solution was

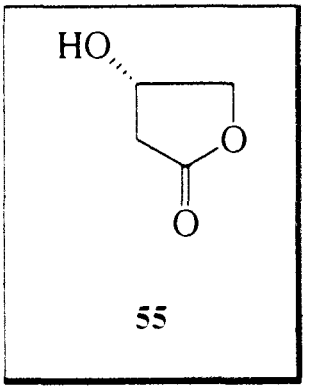


76

stirred at 20 °C for 30 min and sodium borohydride (0.208 g, 5.5 mmole) [BDH] was added in one portion. Stirring was continued for 30 min, methanol (70 mL) was added dropwise and after an additional 30 min the solvent was removed under reduced pressure. The product was purified by flash chromatography (60% ethyl acetate-hexanes) to give a colourless viscous oil (12.27 g, 83%).

Lactonization of (R)-(+)-3,4-Dihydroxybutanoate Methyl Ester

Sodium hydroxide (202.5 mg, 5.06 mmoles) was added to a solution of methyl (R)-(+)-



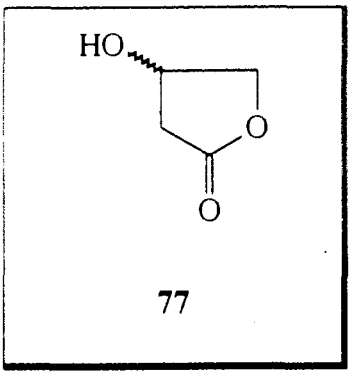
55

3,4-dihydroxybutanoate (R)-**63** (678.3 mg, 5.06 mmoles) in methanol:water (10:3 mL). The solution was stirred for 30 min and the solvent was then removed. The residue was washed with ethyl acetate (20 mL) and redissolved in water (20 mL). The solution was reacidified with N hydrochloric acid and extracted with dichloromethane (3 × 15 mL).

The organic extract was dried over anhydrous sodium sulfate and concentrated to give 380 mg of a golden yellow oil (74%). **^{1}Hmr** ($CDCl_3$, δ): 4.73-4.70 (1H, m), 4.54 (1H, dd, 10.48, 4.16 Hz), 4.36 (1H, dt, 10.48, 1.04 Hz), 2.97 (1H, dd, 18.3, 5.96 Hz), 2.52 (1H, dt, 18.3, 1.2 Hz). **IR** (Film, cm^{-1}): 3429, 1770. **Mass Spectrum** (EI, m/z): 102 (M^+). **Rotation**: $[\alpha]_D^{21}$ + 76.5° (c 2.3, EtOH), (Lit. (72) **Rotation**: $[\alpha]_D^{23}$ + 77.3° (c 2.0, EtOH)).

Lactonization of 3,4-dihydroxybutanoate Methyl Ester

Sodium hydroxide (238 mg, 5.94 mmoles) was added to a solution of (R,S)-3,4-dihydroxybutanoate methyl ester (795.8 mg, 5.94 mmoles) in methanol:water (10:3 mL).



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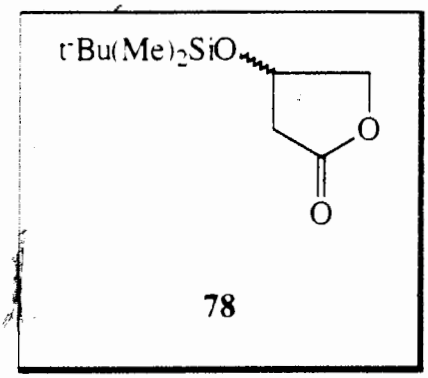
The solution was stirred for 30 min and the solvent was then removed. The residue was washed with ethyl acetate (20 mL) and redissolved in water (20 mL). The solution was reacidified with N hydrochloric acid and extracted with dichloromethane (3 × 15 mL). The organic extract was dried over anhydrous sodium sulfate and concentrated to give 507 mg of a golden yellow oil (84%).

t-Butyldimethylsilyl Ether (78)

(R,S)-3-Hydroxybutyrolactone (378.5 mg, 3.71 mmoles was dissolved under nitrogen in DMF (1 mL). Imidazole (632 mg, 9.28 mmoles) and t-butyldimethylsilyl chloride (671

mg, 4.45 mmoles) were added. The solution was stirred for 34 h and diluted with dichloromethane (50 mL), then washed successively with saturated ammonium chloride (2 x 20 mL) and saturated sodium chloride (2 x 20 mL), dried over anhydrous magnesium

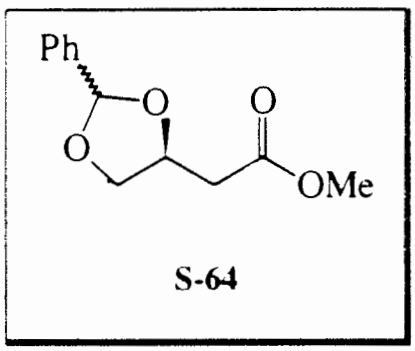


78

sulphate and concentrated. Purification by flash chromatography (60% EtOAc-hexanes) gave 593 mg (85%) of product. ^{1}**Hmr** ($CDCl_3$, δ): 4.61-4.57 (1H, m), 2.68 (1H, dd, 17.5, 6.56 Hz), 2.44 (1H, ddd, 17.5, 2.88, 0.8 Hz), 0.88 (9H, s), 0.08 (6H, s).

Benzylidene Derivative of S-63 (72)

A solution of (S)-**63** (5.88 g, 43.85 mmoles), benzaldehyde dimethyl acetal (6.3 mL, 6.39



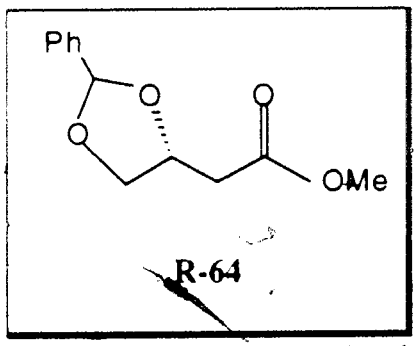
S-64

g, 41.97 mmoles) and p-toluenesulfonic acid monohydrate (413.5 mg, 2.17 mmole) [Fisher] in dimethylformamide (20 mL) was stirred at room temperature for 72 h and then poured into ice-water (25 mL) and extracted with ethyl acetate (3 × 40 mL). The organic extract was washed with saturated sodium chloride (20 mL), dried over anhydrous sodium sulfate and concentrated. Purification by column chromatography (60% ethyl acetate-hexanes) gave 6.35 g (65%) of a colourless oil. ^{1}**Hmr** ($CDCl_3$, δ):

7.49-7.35 (5H, m, Ar), 5.96 (1H, s), 5.86 (1H, s), 4.68-4.60 (1H, m), 4.21 (1H, dd, 8.28, 6.72 Hz), 4.12 (1H, dd, 8.44, 6.16 Hz), 3.98 (1H, dd, 8.44, 6.60 Hz), 3.86 (1H, dd, 8.28, 5.68 Hz), 2.85 (1H, dd, 16.06, 6.36 Hz), 2.82 (1H, dd, 16.06, 6.36 Hz), 2.66 (1H, dd, 16.06, 7.20 Hz), 2.65 (1H, dd, 16.06, 7.32 Hz). **IR** (film, cm^{-1}): 3034, 1737 (C=O). **Mass spectrum** (CI, m/z): 223 (M+1). **Rotation**: $[\alpha]_D^{23}$ + 6.6° (c 0.76, CH_2Cl_2).

Benzylidene Derivative of R-63

A solution of the methyl ester (R)-**63** (423 mg, 3.16 mmoles), benzaldehyde dimethyl acetal (0.474 mL, 3.16 mmoles) and p-toluenesulfonic acid monohydrate (30 mg, 0.16



R-64

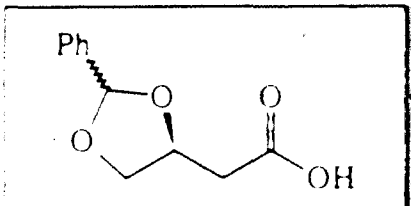
mmole) [Fisher] in dimethylformamide (2 mL) was stirred at room temperature for 24 h and then poured into ice-water (3 mL) and extracted with ethyl acetate (3 × 10 mL). The organic extract was washed with saturated sodium chloride (10 mL), dried over anhydrous sodium sulfate and concentrated. Purification by column chromatography (60% ethyl acetate-hexanes) gave 710 mg (65%) of a colourless oil. **^{1}Hmr** ($CDCl_3$, δ): 7.49-7.35 (5H, m, Ar), 5.96 (1H, s), 5.86 (1H, s), 4.68-4.60 (1H, m), 4.21 (1H, dd, 8.28, 6.72 Hz), 4.12 (1H, dd, 8.44, 6.16 Hz), 3.98 (1H, dd, 8.44, 6.60 Hz), 3.86 (1H, dd, 8.28, 5.68 Hz), 2.85 (1H, dd, 16.06, 6.36 Hz), 2.82 (1H, dd, 16.06, 6.36 Hz), 2.66 (1H, dd, 16.06, 7.20 Hz), 2.65 (1H, dd, 16.06, 7.32 Hz). **IR** (film, cm^{-1}): 3034, 1737 (C=O). **Mass spectrum** (CI, m/z): 223 (M+1).

Benzylidene derivative of (RS)-63

A solution of methyl 3,4-dihydroxybutanoate (11.44 g, 85.36 mmoles), benzaldehyde dimethyl acetal (11 mL, 11.15 g, 3.16 mmoles) and p-toluenesulfonic acid monohydrate (812 mg, 4.27 mmole) [Fisher] in dimethylformamide (20 mL) was stirred at room temperature for 24 h and then poured into ice-water (50 mL) and extracted with ethyl acetate (5 × 40 mL). The organic extract was washed with saturated sodium chloride (30 mL), dried over anhydrous sodium sulfate and concentrated. Purification by column chromatography (60% ethyl acetate-hexanes) gave 18.5 g (77%) of a colourless oil.

Alkaline Hydrolysis of S-64

Sodium hydroxide (24.77 mL of 1M, 24.77 mmoles) was added to a solution of the methyl ester (S)-**64** (5.5g, 24.77 mmoles) in methanol (30 mL). The reaction mixture was



S-65

stirred for 1h and the solvent was removed. The residue was taken up in ethyl acetate (50 mL) and water (30 mL). The aqueous phase was acidified with N hydrochloric acid and extracted with dichloromethane (4 × 30 mL). The organic extract was dried over anhydrous sodium sulfate and concentrated to give a golden yellow oil, 2.11 g (40%). ^{1}**Hmr** ($CDCl_3$, δ): ^{1}**Hmr** ($CDCl_3$, δ): 7.49-7.36 (5H, m, Ar), 5.96 (1H, s), 5.82 (1H, s), 4.68-4.60 (1H, m), 4.37 (1H, dd, 8.50, 6.20 Hz), 4.11 (1H, dd, 8.32, 6.72 Hz), 3.99 (1H, dd, 8.32, 5.48 Hz), 3.75 (1H, dd, 8.50, 6.60 Hz), 2.88 (1H, dd, 16.4, 6.60 Hz), 2.71 (1H,

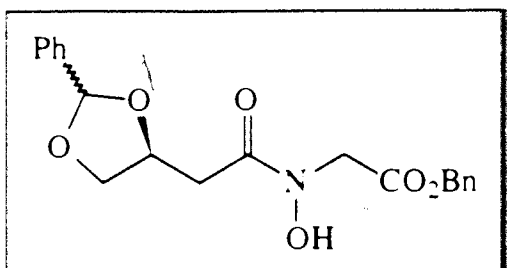
dd, 16.4, 6.96 Hz), 2.68 (1H, dd, 16.4, 6.80 Hz). **IR** (film, cm^{-1}): 3037, 1713 (C=O). **Mass spectrum** (CI, m/z): 209 (M+1). **Rotation**: $[\alpha]_D^{22}$ -3.70° (c 0.11, CH_2Cl_2).

Alkaline Hydrolysis of Racemic 64

Sodium hydroxide (25 mL of 1 M, 25 mmoles) was added to a solution of the methyl ester (5.75 g, 25.9 mmoles) in methanol (40 mL). The reaction mixture was stirred for 30 min and the solvent was then removed. The residue was taken up in ethyl acetate (50 mL) and water (30 mL). The solution layer was acidified with N hydrochloric acid and extracted with dichloromethane (4 × 30 mL). The organic extract was dried over anhydrous sodium sulfate and concentrated to give 3.16 g, (59%) of product. **^{1}Hmr** ($CDCl_3$, δ): 7.49-7.36 (5H, m, Ar), 5.96 (1H, s), 5.82 (1H, s), 4.68-4.60 (1H, m), 4.37 (1H, dd, 8.50, 6.20 Hz), 4.11 (1H, dd, 8.32, 6.72 Hz), 3.99 (1H, dd, 8.32, 5.48 Hz), 3.75 (1H, dd, 8.50, 6.60 Hz), 2.88 (1H, dd, 16.4, 6.60 Hz), 2.71 (1H, dd, 16.4, 6.96 Hz), 2.68 (1H, dd, 16.4, 6.80 Hz). **IR** (film, cm^{-1}): 3037, 1713 (C=O). **Mass spectrum** (CI, m/z): 209 (M+1).

Coupling of S-65 and N-Hydroxyglycine Benzyl Ester

Dicyclohexylcarbodiimide (0.992 g, 4.81 mmoles) [BDH] was added to a cooled solution of **S-65** (1 g, 4.81 mmoles) and N-hydroxyglycine benzyl ester (0.870 g, 4.81 mmoles) in

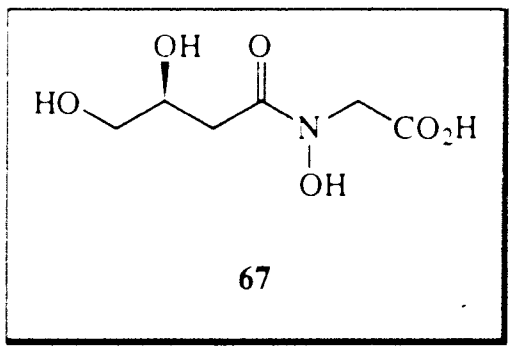


66

dichloromethane (40 mL). The cloudy mixture was stirred under nitrogen at 0 °C for 0.5 h and then at room temperature for 3.5 h, filtered and the filtrate concentrated. Purification by flash chromatography (60% ethyl acetate-hexane) gave 0.364 g (20%) of the product. **IR** (film, cm^{-1}): 3250, 1749, 1650 **Mass spectrum** (CI, m/z): 372 (M+1). **Anal. Calcd. for $C_{20}H_{21}NO_6$:** C, 64.70; H, 5.70; N, 3.80. Found: C, 64.58; H, 6.25; N, 4.11. **Rotation**: $[\alpha]_D^{20}$ + 10° (c 0.3, $CHCl_3$).

Hydrogenolysis of 66

Palladium on carbon (10%) (0.364 g) was added to a solution of **66** (0.364 g, 0.98



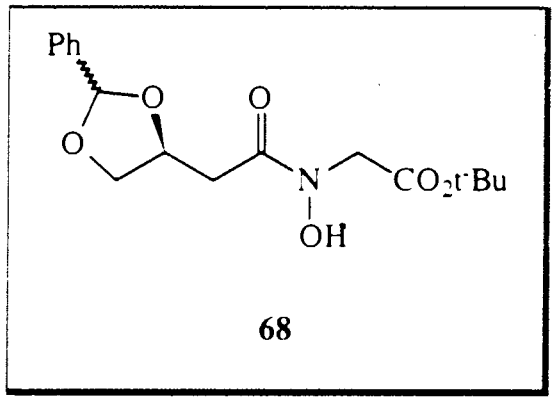
67

mmole) in tetrahydrofuran (12 mL). The system was flushed thrice with hydrogen and then stirred under hydrogen for 48 h, filtered through Celite and the filtrate was concentrated. The oil was dissolved in water, filtered and the filtrate was lyophilized to give 0.144 g (76%) of a pale yellow viscous oil. **^{1}Hmr** (D_2O, δ): 4.36 (1H, d, 18 Hz, C*H*H), 4.43 (1H, d, 18 Hz, CH*H*), 4.10 (1H, m), 3.58 (1H, dd, 11.8, 4.04 Hz, C*H*H), 3.50 (1H, dd, 11.8, 6.48 Hz, CH*H*), 2.75 (1H, dd, 15.5, 8.4 Hz, C*H*H), 2.68 (1H, dd, 15.5, 4.8 Hz, CH*H*). **Mass spectrum** (CI, m/z): 194 (M+1).

Coupling of 28 and S-65

Dicyclohexylcarbodiimide (0.859 g, 4.16 mmoles) [BDH] was added to a cooled solution of **S-65** (0.87 g, 4.16 mmoles) and N-hydroxyglycine t-butyl ester **28** (0.60 g, 4.09

mmoles) in dichloromethane (20 mL). The cloudy mixture was stirred at 0 °C for 0.5 h and at room temperature for 4 h and then filtered and the filtrate concentrated.

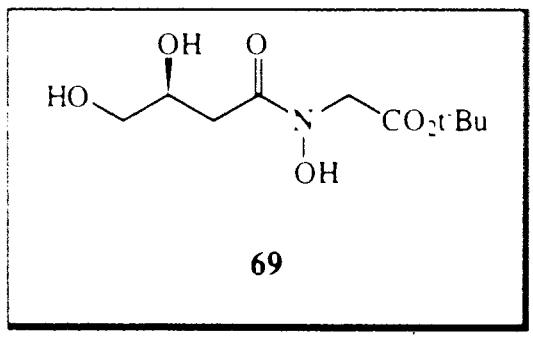


68

Purification of the oil by flash chromatography (60% ethyl acetate-hexane) gave 0.650 g (47%) of the product. **^{1}Hmr** ($CDCl_3$, δ): 7.49-7.35 (5H, m, Ar), 5.96 (1H, s), 5.86 (1H, s), 4.59-4.62 (1H, m), 4.45 (1H, d, 17.7 Hz), 4.39 (1H, dd, 8.56, 6.08 Hz), 4.38 (1H, d, 17.8 Hz), 4.31 (1H, d, 17.8 Hz), 4.29 (1H, d, 17.7 Hz), 4.25 (1H, dd, 8.20, 6.80 Hz.), 3.89 (1H, dd, 8.20, 5.84 Hz), 3.79 (1H, dd, 8.56, 7.12 Hz), 3.20 (1H, dd, 15.9, 6.12 Hz), 3.02 (1H, dd, 15.5,5.60 Hz), 2.92 (1H, dd, 15.5, 7 Hz), 2.81 (1H, dd, 15.9, 7.16 Hz), 1.48 (9H, s, t-Butyl). **IR** (film, cm^{-1}): 3249, 1739, 1641. **Mass spectrum** (CI, m/z): 338 (M+1). **Anal. Calcd. for $C_{17}H_{23}NO_6$:** C,60.53; H, 6.82; N, 4.15. **Found**: C, 60.84; H, 7.07; N 4.36. **Rotation:** $[\alpha]_D^{22}$ + 6.31° (c 0.32, CH_2Cl_2).

Hydrogenolysis of 68

Palladium on carbon (10%) (0.400 g) was added to a solution of **68** (0.400 g, 1.19



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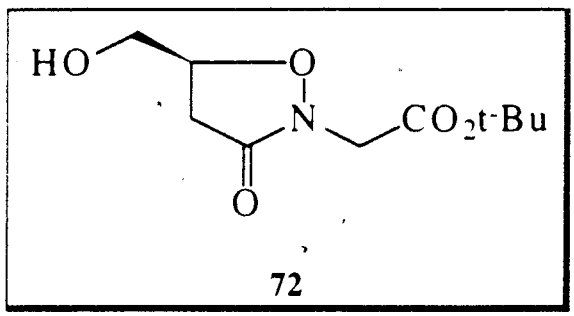
mmoles) in tetrahydrofuran (15 mL). The suspension was flushed thrice with hydrogen, then stirred under hydrogen for 3 h, filtered through Celite, and the filtrate concentrated. The residue was purified by plc (80% ethyl acetate-hexanes) to give a viscous golden oil 0.150 g (51%). **^{1}Hmr** (D_2O, δ): 4.37 (1H, d, 17.7 Hz), 4.30 (1H, d, 17.7 Hz), 4.13 (1H, m), 3.62 (1H, dd, 11.8, 4 Hz), 3.53 (1H, dd, 11.8, 6.88 Hz), 2.78 (1H, dd, 15.2, 8.24 Hz), 2.71 (1H, dd, 15.2, 4.96 Hz), 1.49 (9H, s). **IR** (film, cm^{-1}): 3362, 1737, 1635. **Mass spectrum** (CI, m/z): 250 (M+1). **Anal. Calcd. for $C_{10}H_{19}NO_6.1.5H_2O$:** C,43.47; H, 7.37; N, 5.07. **Found**: C, 43.45; H,7.29; N 5.26. **Rotation:** $[\alpha]_D^{20}$ -10.7° (c 0.28, CH_2Cl_2).

Synthesis of 72 (75)

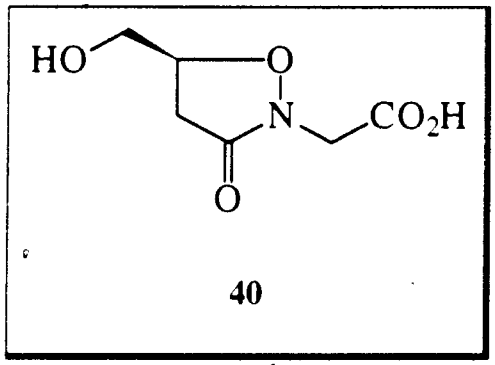
A mixture of the diol **69** (68.8 mg, 0.28 mmole), triphenylphosphine (78.3 mg, 0.30



72

mmole) and diethylazodicarboxylate (65 µL, 72 mg, 0.41 mmole) in tetrahydrofuran (9 mL) was stirred for 24 h at room temperature and then concentrated under reduced pressure. The residue was purified by plc (60% ethyl acetate-hexanes) to give **72** (68 mg). **^{1}Hmr** ($CDCl_3$, δ): 4.64-4.59 (1H, m), 4.37 (1H, d, 17.8 Hz), 3.98 (1H, d, 17.8 Hz), 3.86 (1H, dd, 12.6, 2.68 Hz), 3.65 (1H, dd, 12.6, 4.16 Hz), 2.89 (1H, dd, 16.6, 8.52 Hz), 2.85 (1H, dd, 16.6, 6.64 Hz), 1.47 (9H, s). **IR** (film, cm^{-1}): 3279, 1735. **Mass spectrum** (EI, m/z): 231 (M^+), (CI, m/z): 232. The above compound was dissolved in trifluoroacetic

acid (0.5 mL) and stirred under a nitrogen atmosphere for 1.5 h. The solution was then



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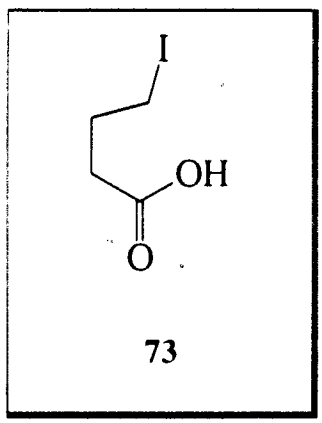
freeze-dried to give 28 mg (57%) of product. **^{1}Hmr** (D_2O, δ): 4.74 (1H, m), 4.36 (1H, d, 18.2 Hz), 4.34 (1H, d, 18.2 Hz), 3.78 (1H, dd, 12.9, 3.2 Hz), 3.74 (1H, dd, 12.9, 5.64 Hz), 3.02, (1H, dd, 17.0, 8.76 Hz), 2.79 (1H, dd, 17.0, 7.68 Hz). **Mass spectrum** (CI, m/z): 176 (M+1).

4-Iodobutanoic acid (77)

Trimethylsilyl chloride (5.0 mL, 4.28 g, 33.4 mmoles) was added dropwise under nitrogen to a suspension of sodium iodide (5.84 g, 34.0 mmoles) in dichloromethane (60



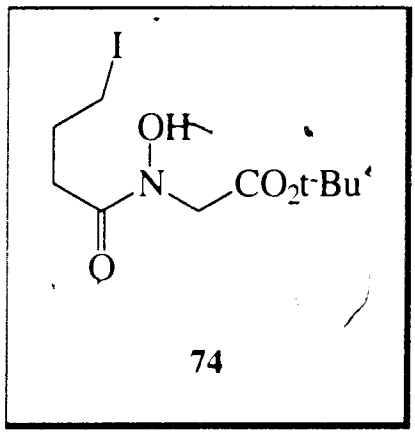
73

mL). The mixture was stirred for 15 min and γ-butyrolactone (2.0 mL, 2.24 g, 26.0 mmoles) was then added dropwise (15 min). Stirring was continued at room temperature for 12 h. Water (50 mL) and ether (100 mL) were added and the organic layer was separated and washed successively with 10% sodium thiosulfate (50 mL), saturated sodium chloride (50 mL), dried over anhydrous sodium sulfate and evaporated under

reduced pressure to give 2.06 g (74%) of a yellow oil. **^{1}Hmr** ($CDCl_3$, δ): 3.25 (2H, t, 6.72 Hz), 2.52 (2H, t, 7.28 Hz), 2.13 (2H, q). **Mass spectrum** (CI, m/z): 215 (M+1).

Reaction of 73 with N-Hydroxyglycine t-Butyl Ester

Dicyclohexylcarbodiimide (51 mg, 0.25 mmole) [BDH] was added under nitrogen to a



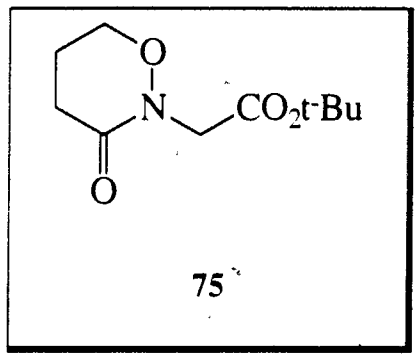
74

cooled (0 °C) solution of the acid **73** (65 mg, 0.30 mmole) in dichloromethane (2 mL), and a solution of N-hydroxyglycine t-butyl ester (34 mg, 0.23 mmole) in dichloromethane (2 mL) was added dropwise, with stirring. The cloudy mixture was stirred for 12 h at 0 °C - room temperature and was then filtered. The filtrate was evaporated and the residue was purified by flash chromatography (40% ethyl acetate-hexane) to give 16.8 mg (21%) of a yellow oil which gave a positive Beilstein test. **^{1}Hmr** ($CDCl_3$, δ): 4.38 (2H, s, CH_2), 3.28 (2H, t, 6.72 Hz, OCH_2), 2.69 (2H, t, 7.12 Hz, CH_2CO), 2.16 (2H, m), 1.47 (9H, s). **IR** (film, cm^{-1}): 3224, 1739, 1626. **Mass spectrum** (CI, m/z): 344 (M+1).

Cyclization of 74

This oil **74** (4 mg, 0.012 mmole) was dissolved under nitrogen, in dichloromethane (5 mL), triethylamine (13 µL, 9.44 mg,, 0.093 mmole) was added, and the solution stirred

for 3 h. Then DBU (4 µL, 4 1 mg, 0.027 mmole) was added and stirring was continued for 3.5 h.. The solvent was then removed. The residue was dissolved in ethyl acetate (5



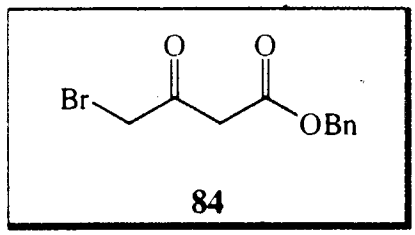
75

mL), washed with saturated sodium chloride, dried over anhydrous sodium sulfate, and concentrated to give 3 mg of **75**. **^{1}Hmr** ($CDCl_3$, δ): 4.28 (2H, s, CH_2), 4.16 (2H, t, 6.84 Hz, OCH_2), 2.55 (2H, t, 7.2 Hz, CH_2CO), 2.14 (2H, m), 1.47 (9H, s). **IR** (film, cm^{-1}): 1743, 1668. **Mass spectrum** (EI, m/z): 215 (M^+). **Anal. Calcd. for $C_{10}H_{17}NO_4$:** C, 55.81; H, 7.91; N, 6.51. **Found**: C, 55.65; H, 7.95; N, 6.88.

The oil (72 mg, 0.34 mmole) was treated with trifluoroacetic acid (1 mL) for 1h and concentrated. The residue was dissolved in water (2 mL), filtered and lyophilized to give green sticky solid. **^{1}Hmr** ($CDCl_3$, δ): 4.18 (2H, s, CH_2), 4.14 (2H, t, 6.52 Hz, OCH_2), 2.52 (2H, t, 7.16 Hz, CH_2CO), 2.10 (2H, m). **Mass spectrum** (EI, m/z): 159 (M^+).

Benzyl-γ-bromoacetoacetate (80)

Diketene (500 µL, 545 mg, 6.5 mmoles) was dissolved, under nitrogen, in methylene chloride (30 mL), cooled to -25°C, and treated dropwise with a solution of bromine (333 µL, 1.02 g, 6.5 mmoles) in methylene chloride (2 mL). After the addition was complete



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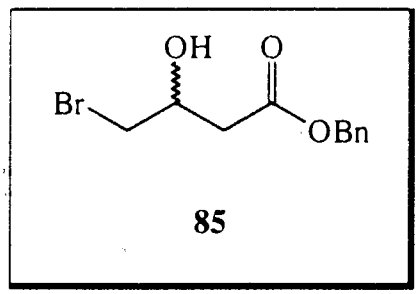
(10 min) the solution was stirred at -25°C for 15 min, and benzyl alcohol (700 μL, 732 mg, 6.8 mmoles) was added dropwise. Stirring was continued for 15 min, and the pale yellow solution was warmed to room temperature and evaporated. The residue was dissolved in ether (20 mL), washed successively with saturated sodium bicarbonate (2 x 20 mL), water (20 mL) and saturated sodium chloride (20 mL), dried over anhydrous magnesium sulfate and concentrated to give benzyl γ-bromoacetoacetate. **^{1}Hmr** ($CDCl_3$, δ): 7.36 (5H, m), 5.19 (2H, s), 4.02 (2H, s), 3.75 (2H, s). **IR** (film, cm^{-1}): 3033, 1734, 1654. **Mass spectrum** (CI, m/z): 273:271 (1:1). **Anal. Calcd. for $C_{11}H_{11}BrO_3$:** C, 48.69; H, 4.05. **Found**: C, 48.55; H, 3.94.

Benzyl-3-Hydroxy-4-Bromobutanoate

The benzyl γ-bromoacetoacetate (500 mg, 1.84 mmoles) was dissolved in a mixture of tetrahydrofuran (9 mL) and methanol (1 mL), the solution was cooled to 0°C, and sodium



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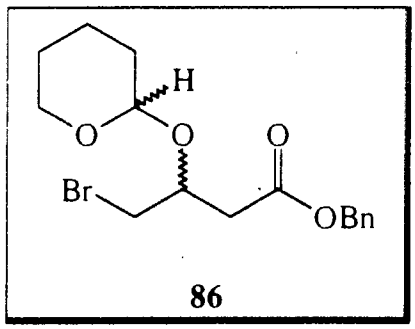
borohydride (72 mg, 1.90 mmoles) was added in one portion. Stirring was continued for 15 min at 0°C, and ethyl acetate (50 mL) and M HCl (1.5 mL) were added. The aqueous layer was separated, extracted with ethyl acetate (20 mL), and the combined organic layers were washed with saturated bicarbonate (40 mL), dried over anhydrous magnesium sulfate and evaporated to give benzyl 3-hydroxy-4-bromobutanoate (433 mg, 86%). **^{1}Hmr** ($CDCl_3$, δ): 7.36 (5H, s), 5.17 (2H, s), 4.26 (1H, m), 3.50 (1H, dd, 10.5, 5.0 Hz),

3.47 (1H, dd, 10.5, 5.6 Hz), 3.06 (1H, d, 5.1 Hz), 2.72 (1H, dd, 16.6, 5.0 Hz), 2.69 (1H, dd, 16.6, 7.3 Hz). **IR** (film, cm^{-1}): 3443, 3064, 1731, 1624. **Mass spectrum** (CI, m/z): 275:273 (1:1). **Anal. Calcd. for $C_{11}H_{13}BrO_3.0.5H_2O$:** C, 46.82; H, 5.0. **Found**: C, 46.51; H, 4.88.

Tetrahydropyranyl Derivative of Benzyl 3-Hydroxy-4-Bromobutanoate (81)

The bromohydrin (160 mg , 0.59 mmol) was dissolved in methylene chloride (2 mL), and dihydropyran (56 µL, 52 mg, 0.61 mmol) and p-toluenesulfonic acid monohydrate (3 crystals) were added. The solution was stirred at room temperature for 35 min, an



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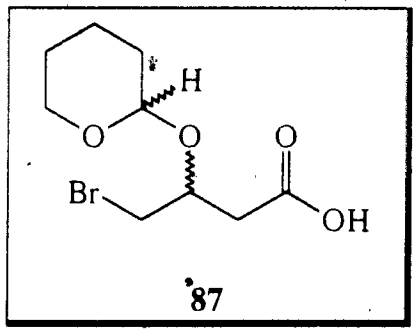
additional 20 µL of dihydropyran were added, and stirring was continued for 1.5 h. Ether (20 mL) was then added, and the solution was washed with saturated bicarbonate (2 x 25 mL), dried over anhydrous sodium sulfate and evaporated. The product was purified by chromatography on silica gel, and eluted with 30:70 ethyl acetate:hexane to give 182 mg (87%) of **86** as a 1:1 mixture of diasteromers. **IR** (film, cm^{-1}): 3033, 1737. **Mass spectrum** (CI, m/z): 359:357 (1:1). **Anal. Calcd. for $C_{16}H_{21}BrO_4$:** C, 53.75; H, 5.88. **Found**: C, 53.65; H, 5.60.

Tetrahydropyranyl Derivative of 3-Hydroxy-4-Bromobutyric Acid

The benzyl 3-O-tetrahydropyranyl-4-bromobutanoate (95 mg, 0.27 mmol) was dissolved

in tetrahydrofuran (2.6 mL) and 10% Pd/C (54 mg) was added. The mixture was flushed

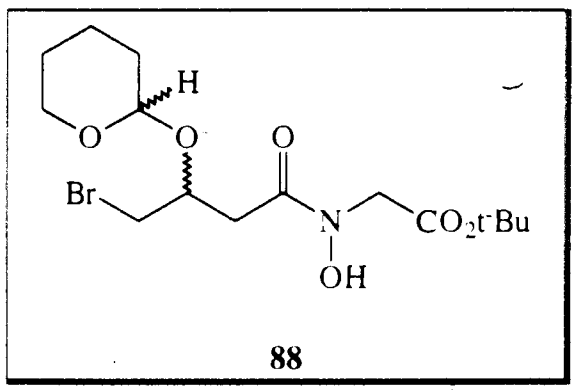


87

several times with hydrogen and then stirred under hydrogen for 2 h. An additional 30 mg of catalyst was added and stirring was continued, under hydrogen, for 1 h. The mixture was filtered through Celite, the Celite was rinsed with ethyl acetate (20 mL), and the filtrate was evaporated to give **87**, which was taken directly to the next step. **IR** (film, cm^{-1}): 1715. **Mass spectrum** (CI, m/z): 269:267 (1:1).

Coupling of 87 with t-Butyl-N-Hydroxyglycine

The acid **87** (85 mg, 0.32 mmol) was dissolved, under nitrogen, in methylene chloride (3 mL), the solution was cooled in an ice-bath, stirred, and dicyclohexylcarbodiimide (70



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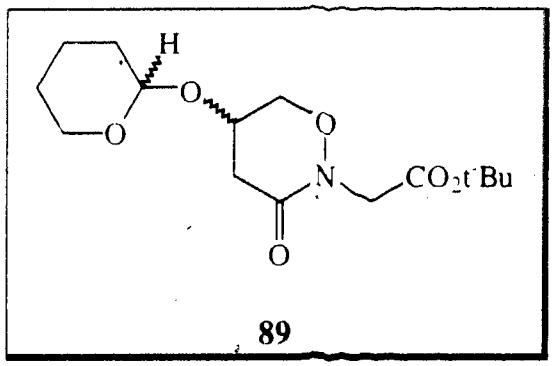
mg, 0.34 mmol) was added, followed by a solution of **28** (46.8 mg, 0.32 mmol) in methylene chloride (3 mL). The cloudy mixture was stirred under nitrogen, initially at 0 °C, and allowed to warm to room temperature. After 12 h, ether (20mL) was added, the mixture was filtered, and the filtrate was evaporated. The product was purified by chromatography on silica gel. Elution with 30:70 ethyl acetate:hexane gave 81 mg (64%)

of a viscous oil **88**. **IR** (film, cm^{-1}): 3240, 1740, 1636. **Mass spectrum** (CI, m/z): 398:396 (1:1). **Anal. Calcd. for $C_{15}H_{26}BrNO_6$:** C, 45.42; H, 6.56; N, 3.53. **Found**: C, 45.42; H, 6.96; N, 3.45.

Protected 2-Carboxymethyl-5-Hydroxy-1,2-Oxazine-3-one

A portion of this oil **88** (37 mg, 0.093 mmol) was dissolved in methylene chloride (2 mL), and triethylamine (14 µL, 10.2 mg, 0.10 mmol) was added. The solution was left, under nitrogen, for 1 h and diazabicycloundecene (DBU) (7.0 µL, 0.047 mmol) was added followed, after 2.5 h, by an additional 5.0 µL (0.033 mmol) of DBU. Stirring was continued under nitrogen for 3.2 h, and the reaction mixture was then diluted with



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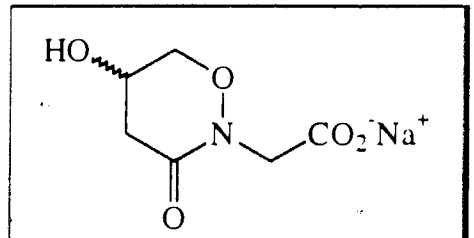
methylene chloride (10 mL), washed with water (10 mL), dried over anhydrous sodium sulfate and evaporated. Chromatography on silica gel and elution with 45:55 ethyl acetate:hexane gave the protected oxazinone as a semisolid as a 1:1 mixture of diastereomers. **^{1}Hmr** ($CDCl_3$, δ): 4.66 (1H, m, THP proton, one isomer), 4.41 (1H, m, THP proton, second isomer), 4.32-4.45 (2H, m, both isomers), 3.83 (1H, m, 5-H of one isomer), 2.94-2.62 (2H, both isomers, two ddd), 1.8 (2H, m), 1.7 (2H, m), 1.55 (2H, m), 1.47 (9H, s). **IR** (film, cm^{-1}): 1737, 1681. **Mass spectrum** (CI, m/z): 316 (M+1). **Anal. Calcd. for $C_{15}H_{25}NO_6$:** C, 57.08; H, 7.99; N, 4.44. **Found**: C, 56.92; H, 8.24; N, 4.51.

2-Carboxymethyl-5-Hydroxy-1,2-Oxazine-3-one

The protected oxazinone (11 mg, 0.035 mmol) was dissolved, under nitrogen, in



90

methylene chloride (1 mL), cooled in an ice-bath, and trifluoroacetic acid (3 µL) was added. Additional trifluoroacetic acid (300 µL) was added after 15 min, after an additional 20 min (500 µL), and after an additional 30 min (300 µL). After an additional 55 min, the solvent was removed. The residue was redissolved in trifluoroacetic acid (500 µL), evaporated after 30 min to give semi-solid residue. **^{1}Hmr** (D_2O, δ): 4.51 (1H, m), 4.49 (1H, d, 18.04 Hz), 4.38 (1H, d, 18.04 Hz), 4.31 (1H, dd, 12.1, 4.72 Hz), 4.04 (1H, dd, 12.1, 3.2 Hz), 2.93 (1H, dd, 16.8, 5.8 Hz), 2.53 (1H, dd, 16.8, 3.3 Hz). **Mass spectrum** (CI, m/z): 176 (M+1). **Anal. Calcd. for $C_6H_9NO_5.0.25H_2O$:** C, 40.11; H, 5.29; N, 7.80. **Found**: C, 39.85; H, 5.28; N; 7.90.

The residue shaken with ethyl acetate (2 mL), water (1 mL) and sodium bicarbonate (3 mg). The aqueous phase was separated, washed with ethyl acetate, and lyophilized to give 2-carboxymethyl-5-hydroxy-1,2-oxazin-3-one as the sodium salt. **^{1}Hmr** (D_2O, δ): 4.49 (1H, m), 4.29 (1H, dd, 12.1, 4.6 Hz), 4.28 (1H, d, 18.2 Hz), 4.15 (1H, d, 18.2 Hz), 4.02 (1H, ddd, 12.1, 3.24, 0.8 Hz), 2.92, (1H, dd, 16.8, 5.9 Hz), 2.52 (1H, ddd, 16.8, 3.16 Hz).

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