To my parents Halimah and Hamzah

STUDIES TOWARDS THE TOTAL SYNTHESIS OF LACTACYSTIN AND ITS DERIVATIVES

by

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Abstract

This thesis has been divided into three main sections. The first chapter contains a review of the total synthesis of lactacystin and also its derivatives. Chapter two consists of our own synthetic work, and experimental details are provided in chapter three.

Our synthetic approach towards the total synthesis of lactacystin requires the Dieckmann cyclisation reaction of an appropriate carbamate, formed between *N*-glycine ethyl ester with alkyl or aryl malonyl chlorides and also alkyl malonates. Dieckmann cyclisation of the carbamates with the exception of the *N*-Boc protected compounds gave us the required pyrrolidinone ring.

In the next step of the work, we attempted the alkylation of β-β-ketoester lactam at C-3, but this task turned out to be problematic. The problem was solved by carrying out the reaction with tetrabutylammonum fluoride, which acts as a base and as a phase transfer catalyst. Treatment of this methylated compound with lithium bis(trimethylsilyl)amide followed by benzyl cyanoformate furnished the corresponding ester with 100% regioselectivity. Reaction of this keto ester with isobutyrylchloride in the presence of pyridine furnishes intermediate **98** as only one diastereoisomer.

Attempted decarboxylation of the methyl ester C-3 using different conditions failed to give our target compound. We then prepared a different substrate using similar conditions to introduce a benzyl ester at C-3 rather than methyl ester **104**. Hydrogenolysis followed by decarboxylation of this compound with palladium hydroxide in tetrahydrofuran gave us the advanced intermediate **105** in quantitive yield. In summary, we have successfully synthesised the important advanced intermediate **105** in six steps.

Full experimental details for the synthetic studies are given in Chapter Three.

Abbreviations used in the text

Ac	acetyl
AcOH	acetic acid
AIBN	azo-bis-isobutyronitrile
aq.	aqueous
Ar	aromatic
atm.	atmosphere
BAIB	bis(acetoxy)iodobenzene
Bn	benzyl
Boc	tert-butyloxycarbonyl
BOPCl	bis(2-oxo-3-oxazolidinyl)phosphinic chloride
b.p.	boiling point
^{n}Bu	n-butyl
^{t}Bu	tert-butyl
°C	degrees Celsius
CAN	ceric ammonium nitrate
cat.	catalytic
CI	chemical ionization
cm^{-1}	wave number
conc.	concentration
DABCO	1,4-Diazabicyclo[2.2.2]octane
DBU	1,5-Diazabicyclo[5.4.0]undec-7-ene
DCC	N,N-dicyclohexylcarbodiimide
DCM	dichloromethane
δ	chemical shift
DIBAL	diisobutylaluminium hydride
DMAP	4-dimethylaminopyridine
DMF	N, N-dimethylformamide
DMPU	N, N-dimethylpropyleneurea
DMSO	dimethylsulfoxide
eq.	equivalents
E	ester group
EI	electron impact
Et	ethyl
EtOH	ethanol

g	grams
h	hour, hours
HMPA	hexamethylphosphoramide
HMPT	hexamethyl phosphortriamide
iPr	isopropyl
IR	infra red
LDA	lithium diisopropylamide
LHMDS	lithium bis(trimethylsilyl)amide
M	molar
Me	methyl
min.	minute(s)
mg	milligram(s)
ml	millilitre(s)
mmol	millimole(s)
m.p.	melting point
m/z	mass to charge ratio
NMO	4-methylmorpholine-N-oxide
NMR	nuclear magnetic resonance
n.O.e	nuclear Overhauser enhancement
NGF	nerve growth factor
PCC	pyridinium chlorochromate
PDC	pyridinium dichromate
Ph	phenyl
PivCl	pivaloyl chloride
PLE	porcine liver esterase
PMB	p-methoxy benzyl
quant.	quantitative
r.t.	room temperature
TBAB	tetrabutylammonium bromide
TBAF	tetrabutylammonium fluoride
TBDMS	tert-butyldimethylsilyl
TBSOTf	tert-butyldimethylsilyl trifluoromethanesulfonate
TCDI	thiocarbonyldiimidazole
TEMPO	2,2,6,6-tetramethyl-1-piperidinyloxyl
TESCl	triethylsilyl chloride
TFA	trifluoroacetic acid
THF	tetrahydrofuran

TMS	trimethylsilyl
TPAP	tetrapropylammonium perruthenate VII
Ts	tosyl
uv	ultra violet

CONTENTS

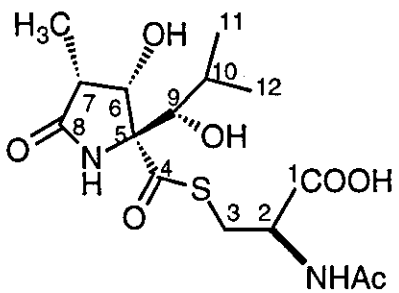
CHAPTER ONE

STUDIES TOWARDS THE TOTAL SYNTHESIS OF LACTACYSTIN AND ITS DERIVATIVES

1.0 INTRODUCTION

Lactacystin (**1**) is a novel microbial natural product isolated by Omura *et al,* from *Streptomyces* species after screening several thousand microbial cultures.[1] Lactacystin inhibits cell growth or proliferation and also induces neurite outgrowth and differentiation in a mouse neuroblastoma cell line, Neuro 2A. In other words, lactacystin is considered to be an important mimic of the nerve growth factor (NGF).

Nerve growth factors (NGF) are valuable compounds that are responsible and essential for the survival and function of nerve cells or neurons.[2] Deficiency of these compounds is thought to cause various nerve related diseases which include Alzheimer's and Parkinson's. The findings of these investigations suggested that NGF-like compounds might be therapeutically useful for the treatment of these diseases.[3] Nerve growth factors are also important as therapeutic agents and as neuroscience research tools which are in great demand in many biological laboratories today.

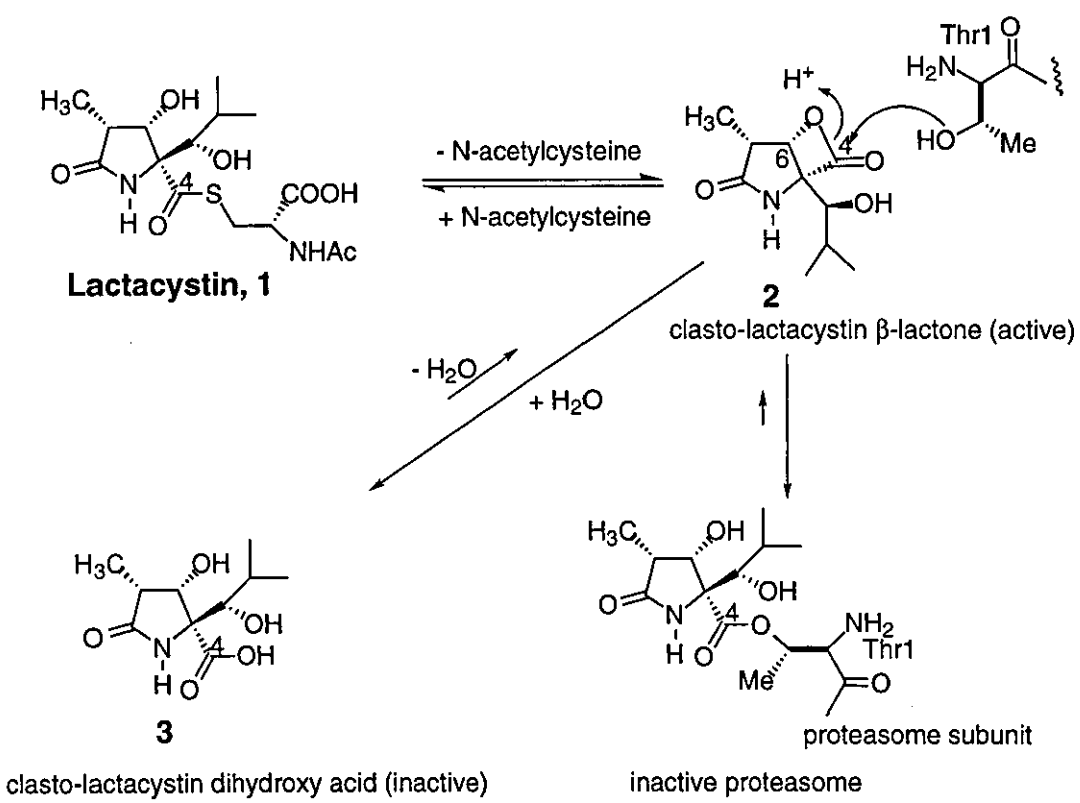


1

Although the early work of Omura suggested that lactacystin is a mimic of the nerve growth factor, recent work suggests that this is not exactly the case. Work carried out by the Schreiber group on the effect of this compound on mammalian cells showed that the biological effects of lactacystin were in fact due to its ability to inhibit the 20 S proteasome.[4]

The 20 S proteasome is a very large (700 kg mol^{-1}) cylindrical complex of 28 protein-subunits which is responsible for the hydrolytic fragmentation of ubiquitinated proteins. This proteasome is essential for the normal turnover of cellular proteins, for removal of damaged or mutated proteins, and also plays an important role in the processing and degradation of regulatory proteins that control cell growth and metabolism.[5]

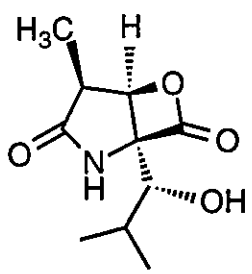
The thioester function of lactacystin is reactive enough to undergo spontaneous conversion to the active β-lactone (**2**), *via* the intramolecular attack on the C-4 carbonyl of the thioester by the C-6-OH, suggesting that these groups have to be in *syn* relationship to one another. Lactacystin inactivation of the proteasome is thought to occur through the acylation of the threonine subunit, which is the key participant in the proteolytic catalysis (**Scheme 1**).[6]



Scheme 1

Confirmation of the proteasome inhibition by lactacystin was obtained by the X-ray analysis of the inactive proteasome at 2.4 Å resolution.[7]

In another related study, lactacystin was found to undergo spontaneous hydrolysis at pH 8 to give *N*-acetyl-*L*-cysteine and the inactive lactacystin acid, clasto-lactacystin dihydroxy acid (**3**).[8] The mechanism of hydrolysis of lactacystin has been studied and was shown to proceed through the intermediacy of the active lactacystin analogue, clasto-lactacystin β-lactone (**2**). Therefore, conditions that stabilize lactacystin and thus prevent the transient accumulation of the intermediate β-lactone, negate the ability of lactacystin to inactivate the proteasome. These findings suggest that lactacystin acts as a precursor for clasto-lactacystin β-lactone (**2**) and that the latter is the active species that reacts with the proteasome.



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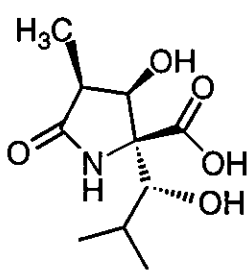


3

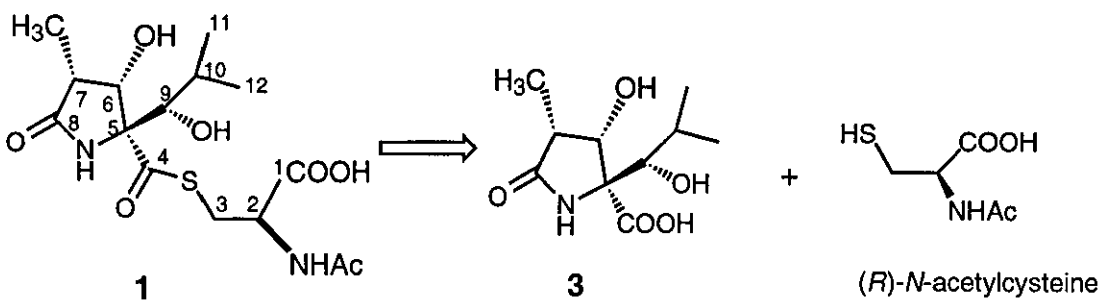
Thus, lactacystin or its intermediate β-lactone has been recognised as a small but potent molecule for the inhibition of the 20 S proteasome. It is highly likely that the substituent at the β-lactone bridgehead mediates a key interaction at the S1 subsite of the protease and that variation of this group would yield inhibitors of serine proteases other than the proteasome itself.

Stimulated by the unique structure of lactacystin, this remarkably specific inhibitor of the 20 S proteasome, the scarcity of the natural material, and also the rapid demand for this compound, we have undertaken the challenge to develop a more efficient and economical approach towards the total synthesis of lactacystin. Furthermore, recent studies have shown that lactacystin has now become one of the most important tools for the study of protein biochemistry and cell biology.[9]

The aim of this project is to develop a flexible and stereocontrolled synthesis of lactacystin and its analogues.

1.1 LITERATURE REVIEW ON LACTACYSTIN

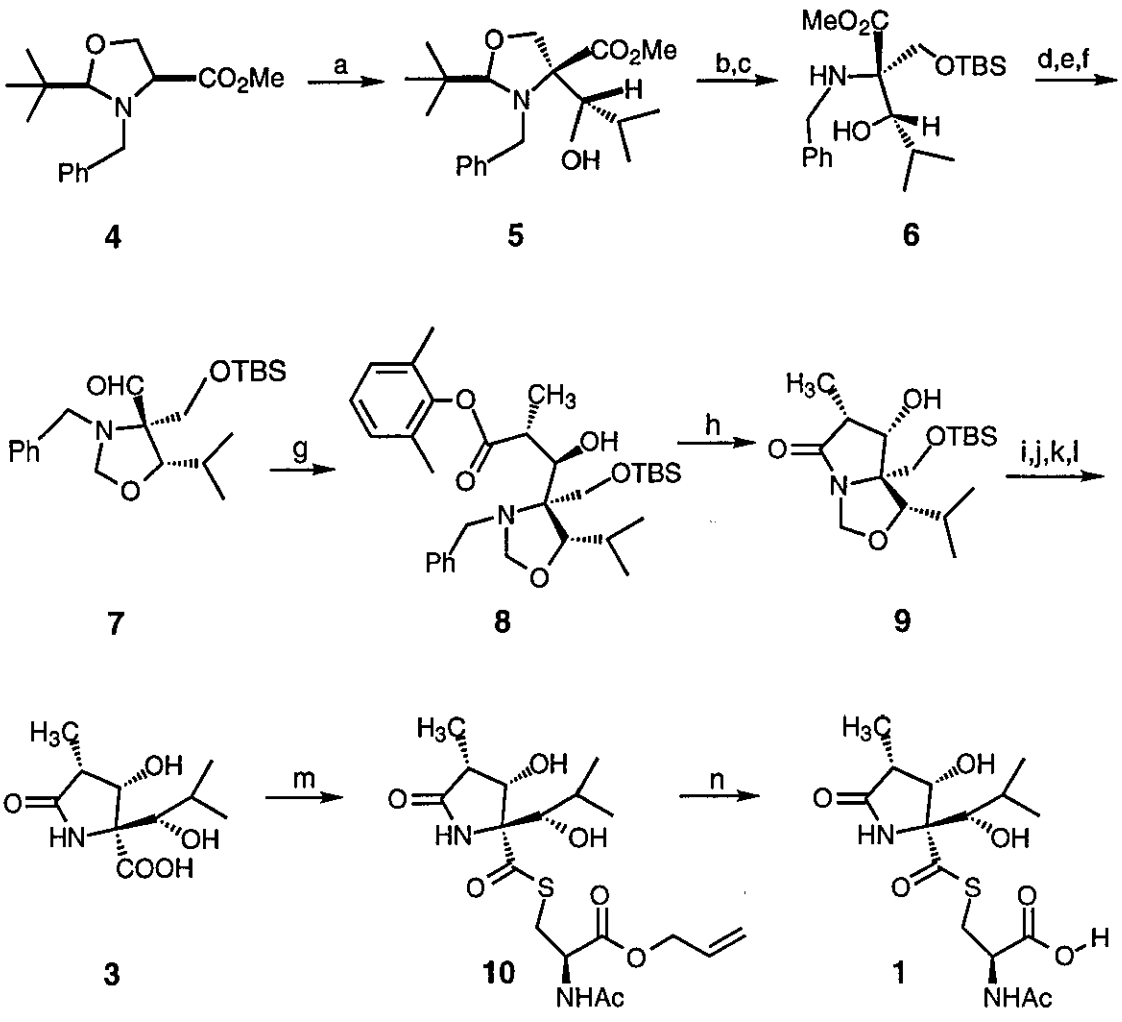
Despite its small molecular weight, consisting of two α-amino acids, namely (*R*)-N-acetylcysteine and a novel α-substituted pyroglutamic acid, joined through a thioester, lactacystin possesses four stereogenic centres, one of which is a quite demanding quaternary carbon atom (C5). As a synthetic target, the γ-substituted pyroglutamic acid portion presents several potential problems, the most challenging being the construction of the C5 quaternary centre with the correct relative stereochemistry. Within a couple of years after the discovery of lactacystin, four research groups developed multistep syntheses to assemble lactacystin.



In their first reported synthesis of lactacystin, Corey and Reichard utilized (2*S*)-*N*-benzyl-L-serine methyl ester **4** as the source of chirality (**Scheme 2**).[10] The protected amino acid was transformed into the corresponding *cis* ^{t}Butyl-oxazolidine using Seebach's protocol.[11] Aldol reaction between the oxazolidine and isobutyraldehyde in the presence of lithium bromide furnished the aldol product **5** with high d.e. but in moderate yield (51% after recrystallisation)). In the absence of lithium bromide, the aldol condensation gave the product in very poor yield and selectivity, suggesting that the lithium cation played an important role, perhaps by coordinating with isobutyraldehyde to make it more susceptible towards the attack by the lithium enolate.

Following the aldol reaction, aminal cleavage of **5** using methanol/triflic acid gave **6** in which the liberated primary alcohol was selectively protected using tert-butyl dimethyl silyl chloride (TBSCl). Treatment of product with paraformaldehyde in the presence of $TsOH/C_6H_6$ under reflux conditions transformed the compound into

the secondary oxazolidine. Reduction of the methyl ester using lithium borohydride furnished the corresponding primary alcohol which was then oxidised under Swern conditions to give the aldehyde **7**.



Scheme 2

Conditions: (a) LDA, LiBr (5 eq.), Isobutyraldehyde, THF, -78 °C, 8 h; 51% (b) MeOH, TfOH, 4 eq, 80 °C, 6.5 h; 91% (c) TBSCl, Imidazole, DMF, 23 °C, 3.5 h; 97% (d) TsOH, $(CH_2O)_n$, benzene, heat, 30 min; 96%. (e) $LiBH_4$/THF, MeOH, 23 °C, 24 h (f) DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2, -78 °C, 1.5 h; 85% (g) LDA, THF, -78 °C, 2,6-dimethylphenylpropionate; 48% (h) H_2, Pd/C, EtOH, 23 °C, 1 d; 87% (i)

5% HF/CH_3CN, 23 °C, 9h; 90% (j) DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2, -78 °C, 2 h; 73% (k) $NaClO_2$, NaH_2PO_4, ^tBuOH, 2-methyl-2-butene, 23 °C, 15 min; >95% (l) 1-3-propanedithiol, 2%HCl (g), CF_3CH_2OH, 50 °C, 6 h; >95% (m) (*R*)-*N*-Acetylcysteine allyl ester. BOPCl, Et_3N, CH_2Cl_2, 23 °C, 1 h; 79% (n) $Pd(PPh_3)_4$, HCO_2H, Et_3N, THF, 23 °C, 1 h; 84%

Aldol reaction of **7** using Pirrung-Heathcock's *anti*-aldol conditions[12] gave the desired aldol stereoisomer **8** with moderate selectivity (3:2). The other *syn* product of this aldol reaction, which was derived from the attack of the enolate from the opposite face of the aldehyde, was also formed and can be separated from the major compound by column chromatography.

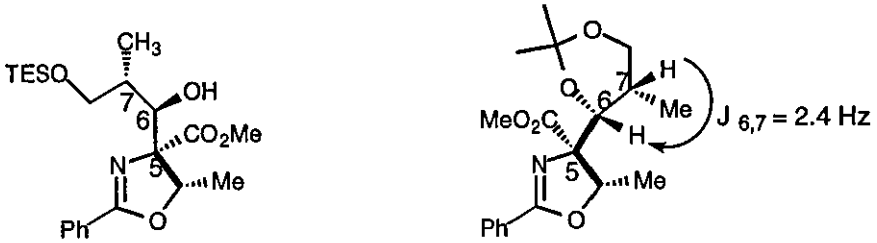
Catalytic hydrogenolysis of **8** gave the free amine which then attacked the ester and cyclised to give the bicyclic lactam **9**. Removal of the silyl group with hydrogen fluoride gave the primary alcohol which was oxidised to the carboxylic acid in two steps, using Swern conditions followed by $NaClO_2$. Removal of the N/O methylene bridge using acid catalysed transfer of the methylene group to 1,3-propanedithiol gave the acid precursor **3**.

The carboxylic acid function was then esterified with *N*-acetylcysteine allyl ester using BOPCl[13] and triethylamine to give **10** without the protection of the hydroxyl groups. The final step to the conversion of lactacystin was achieved by deallylation of **10** using triethylammonium formate-Pd(0). The overall procedure encompassed 16 steps (from *L*-serine-based-oxazolidine **4**) and produced lactacystin in *ca* 6% yield.

Corey and Choi later employed a totally different approach to assemble the unnatural (6*R*)-lactacystin (**Scheme 3**).[14] The key operation utilized chiral 2-phenyl-1,3-oxazoline **16** which was synthesized from (2*R*, 3*S*)-3-hydroxyleucine in two steps. Aldol coupling of **16** using KHMDS as the base, in the presence of $ZnCl_2$ as Lewis acid with silyl protected (*S*)-2-methyl-3-hydroxypropanal **13,** (prepared from (*S*)-(+)-methyl-3-hydroxy-2-methylpropionate in two steps) was effected in good yield to form **17.**

The confirmation of the stereochemistry of **17** was achieved by carrying out a parallel aldol reaction of the threonine analog of **16** (isopropyl group replaced by methyl) with the same aldehyde **13** to give **17(a).** The stereochemistry was

confirmed by an nOe study after removal of the silyl group and conversion into the acetonide **17(b)** (**Figure 1**).



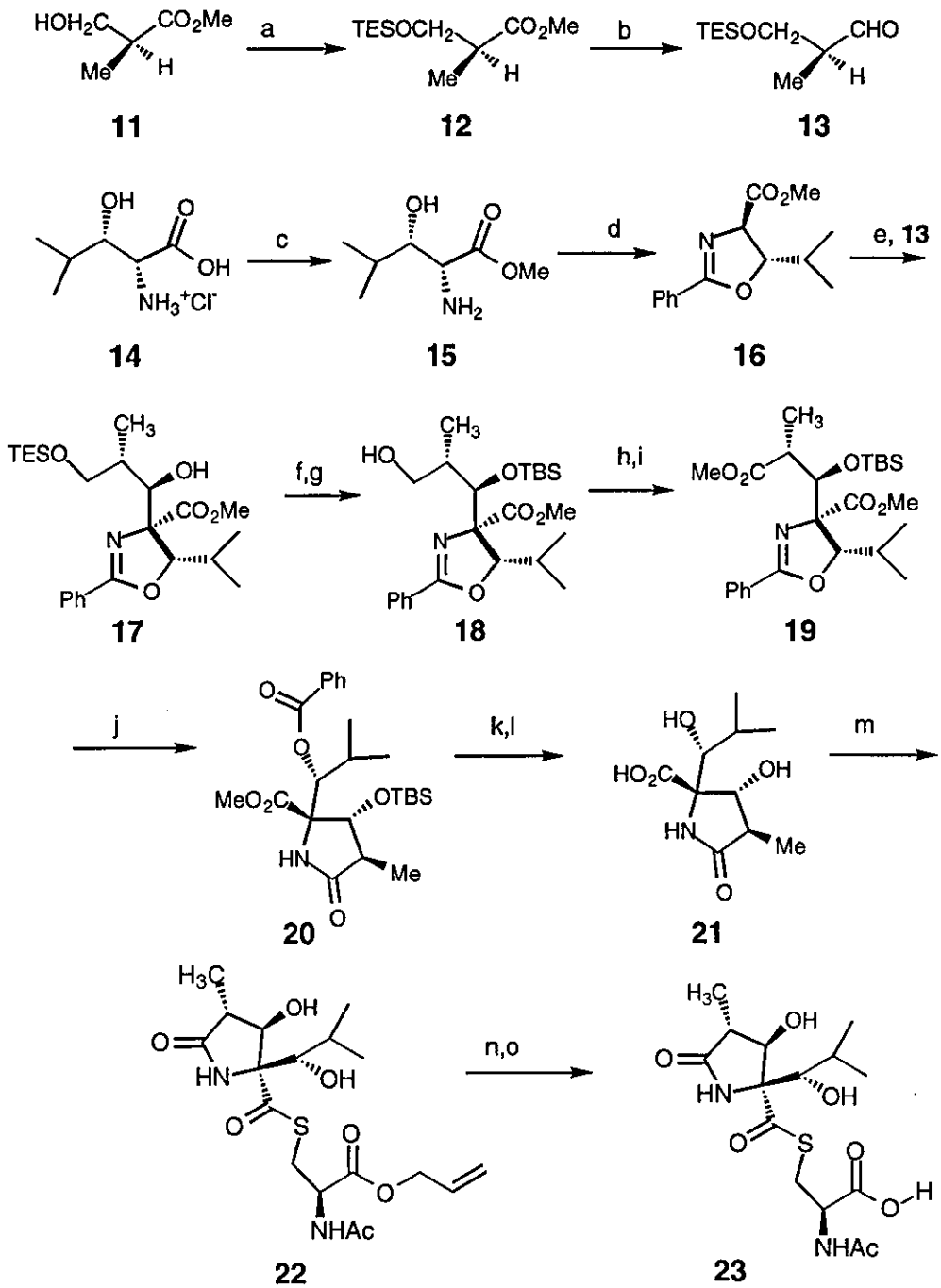
17(a) **17(b)**

Figure 1

In the aldol product of the threonine derivative, the face selectivity at C-5 is determined by the steric influence of the methyl group at the vicinal stereocentre on the oxazolidine ring. The stereochemistry at C-6 is controlled by a combination of the preferred ester enolate and the transition state geometry. The latter is thought to go through the boat six-membered ring transition state with the enolate with *cis* configuration (*cis* O^- and N substituents). Based on these assumptions, the geometry of the aldol product **17** should follow that of the threonine analog, giving the correct stereochemistry.

With the aldol product in hand, the secondary alcohol was then protected with TBSOTf and the silyl protected primary alcohol was removed using HCl-THF. Oxidation of the primary alcohol under Swern conditions followed by $NaClO_2$ gave the carboxylic acid which was then esterified as the methyl ester using diazomethane to give ester **19**. Treatment of **19** with HCl in methanol cleaved the oxazoline and cyclisation afforded the lactam **20**.

Saponification of the methyl ester using $LiOH\text{-}H_2O_2$ followed by removal of both silyl protecting groups and the cleavage of the benzoate ester using CsF in hydrazine furnished **21**. Finally, coupling of **21** with *N*-acetylcysteine allyl ester to generate **22** was carried out as previously described for natural (+)-lactacystin **1** using BOPCl and triethylamine. Deallylation of **22** using triethylammonium formate with $Pd(PPh_3)_4$ as catalyst gave the unnatural lactacystin **23**. The complete synthesis comprises 16 steps [from (2*R*-3*S*)-3-hydroxyleucine] and gives (6*R*)-lactacystin in *ca.* 13% yield.



Scheme 3

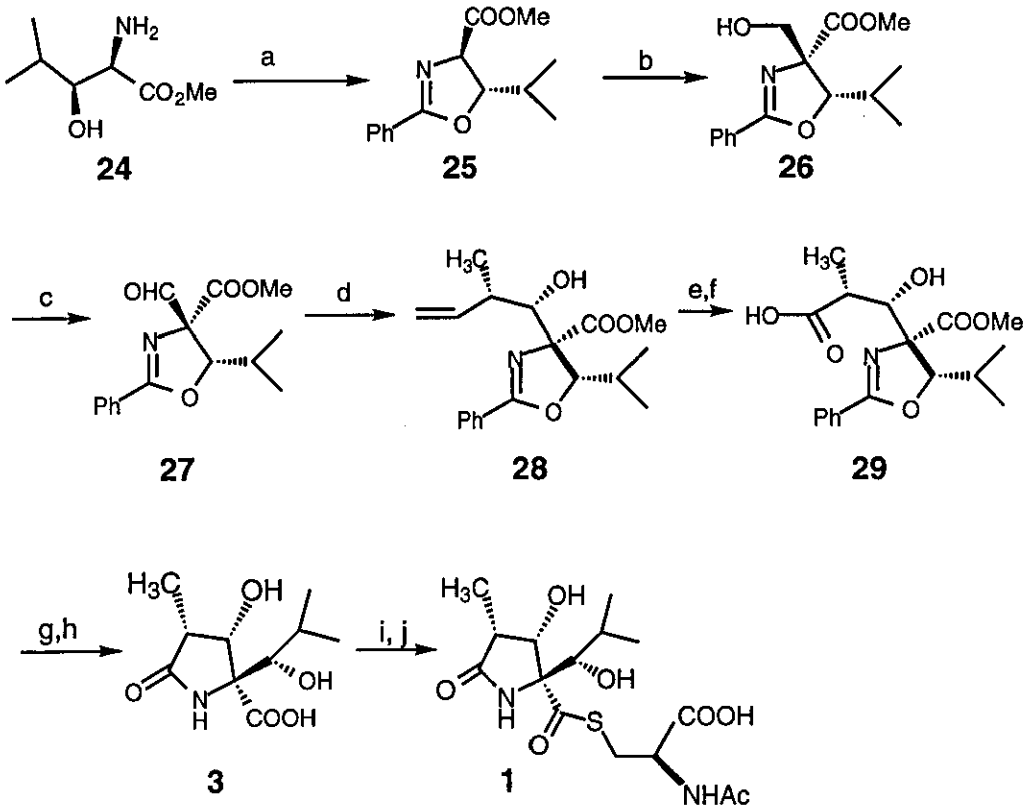
Conditions: (a) TESOTf, 2,6-lutidine, CH_2Cl_2, 0 °C, 1 h, >95% (b) DIBAL, CH_2Cl_2,-78 °C, 66% (c) HCl, MeOH, 72% (d) $PhC(OMe)_3$, TsOH, DME, reflux, 1h, 87% (e) KHMDS, $ZnCl_2$, **13**, 84% THF; (f) TBSOTf, 2,6-

lutidine, CH_2Cl_2, r.t., 97% (g) HCl-THF (1:1), 0 °C, quant. (h) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, -78 °C to 0 °C, 92%; (i) $NaClO_2$, then CH_2N_2, MeOH, 97% (j) 0.48M HCl, MeOH, rt, 96% (k) LiOOH, THF-H_2O, 96% (l) CsF, H_2NNH_2 (m) (*R*)-*N*-acetylcysteine allyl ester, Et_3N, BOPCl, CH_2Cl_2,70-80% (n) Et_3N-HCOOH, $Pd(PPh_3)_4$,THF; (o) 4:2:1 THF-EtOAc-AcOH (64%).

The synthetic approach of Omura and Smith[15] towards lactacystin made use of the enantiopure oxazoline **25**, the same enantiomer exploited by Corey during the synthesis of (6*R*)-lactacystin (**Scheme 4**). Oxazoline **25** was prepared by the reaction of 2(*R*), 3(*S*)-β-hydroxyleucine methyl ester **24** (prepared in five steps from *E*-4-methyl-2-pentene-1-ol[16]) with methyl benzimidate. Aldol condensation of this compound with formaldehyde and lithum bis(trimethylsilyl)amide *via* the Seebach's protocol[17] gave exclusively **26**, a compound possessing the correct absolute configuration at the C-5 quaternary carbon, the stereochemistry of which was confirmed using nOe studies.

After various oxidation conditions (e.g. Swern[18], Dess Martin[19], Parikh-Doering[20], PCC[21]) proved unsuccessful, Moffatt oxidation[22] of **26** afforded aldehyde **27**. The aldehyde was then subjected without purification to Brown asymmetric allylboration using (*E*)-crotyldiisopinocamphenylborane to give a 4:1 mixtures, favouring the desired β-methyl homoallylic alcohol **28**.[23] Alternative crotonylation procedures gave lower selectivities. For example, both Hiyama's (*E*)-crotylchromium (II) reagent[24] and Roush's (*E*)-crotylpinacol borane,[25] gave 2:1 mixtures of the homoallylic alcohols. Ozonolysis of **28** to the aldehyde followed by chlorite oxidation gave acid **29**.

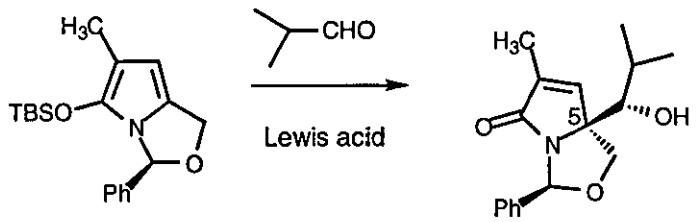
Hydrogenolysis of acid **29** using the standard Pd/C, $Pd(OH)_2$, and Pd black followed by acid hydrolysis failed to cleave the oxazoline moiety. However, when catalystic transfer hydrogenation[26] (Pd, HCO_2NH_4) was employed, compound **29** was successfully cyclised to the key lactam **2** by oxazoline opening and base-promoted intramolecular lactam formation. After saponification of the methyl ester using sodium hydroxide, final transformation of **3** into (+)-lactacystin was carried out using Corey's method. The overall synthesis involved 15 steps and gave a 13% overall yield of lactacystin (**1**).



Scheme 4

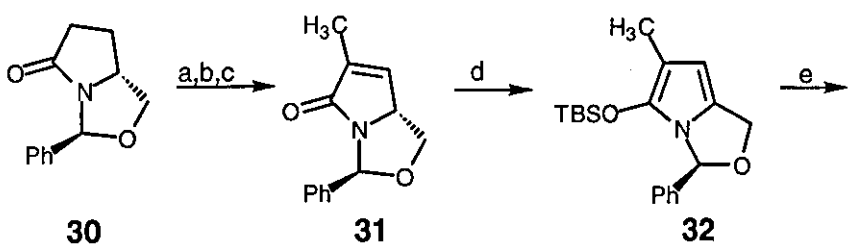
Conditions: (a) Ph(MeO)C=NH, 72% (b) LiHMDS, HCHO; 85% (c) DMSO-Benzene, TFA, DCC, pyridine r.t. (d) (*E*)-crotyldiisopinocamphenylborane, 2 steps ,70% (e) O_3, DMS; (f) $NaClO_2$. NaH_2PO_4; 56% (g) Pd, HCO_2NH_4 (h) 0.1N NaOH; 82% (i) BOPCl, Et_3N, (*R*)-*N* -acetylcysteine allyl ester; 79% (j) $Pd(PPh_3)_4$; 88%

The key step in Baldwin's synthesis of lactacystin involved the stereoselective aldol reaction of a siloxypyrrole, (readily available from pyroglutamate), with isobutyraldehyde, in which the both quaternary carbon C-5 and the exocyclic secondary alcohol were formed with the correct stereochemistry (**Scheme 5**).[27]

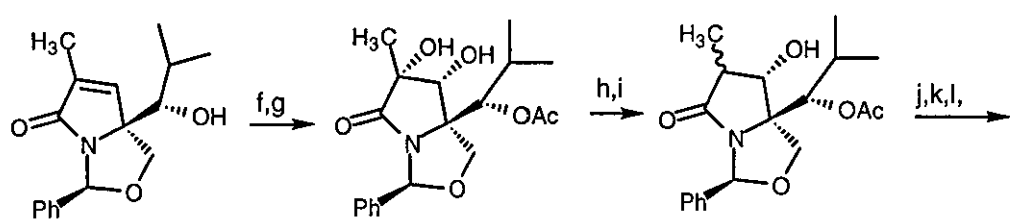


Scheme 5

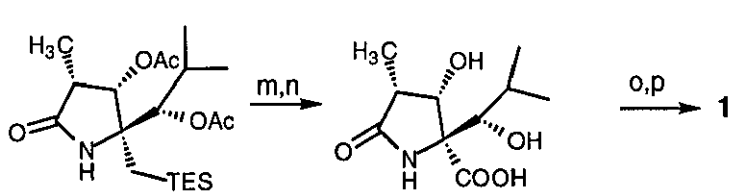
Baldwin made use of oxazolidine **30** as precursor (prepared in three steps)[28] in his strategy (**Scheme 6**). Methylation of the oxazolidine followed by selenylation and ozonolysis gave the unsaturated derivative **31** which was further elaborated to the silyloxypyrrole **32.**



30 **31** **32**



33 **34** **35**

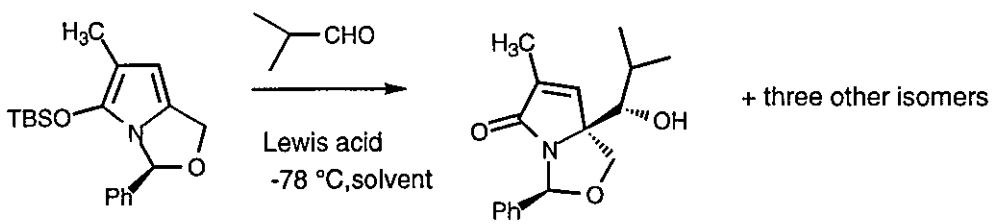


36 **3**

Scheme 6

Conditions: (a) LDA, MeI, THF, -78 °C; 95% (b) LDA, PhSeBr, THF, -78 °C; 79% (c) O_3, CH_2Cl_2, -78 °C; pyridine, to room temp; 87% (d) TBSOTf, 2,6-lutidine, CH_2Cl_2, room temp; 89% (e) *i*-PrCHO, $SnCl_4$, ether, -78 °C; 55%. (f) Ac_2O, pyridine, room temp; 99% (g) OsO_4, *N*-methylmorpholine*N*-oxide, aqueous acetone, room temp; 87% (two cycles) (h) *N*-*N'*-Thiocarbonyldiimidazole, THF, room temp; 91% (i) Bu_3SnH, AIBN, toluene, reflux; 94%. (j) 2N NaOH/MeOH (1:3), 0-3°C; 94%. (k) H_2, Pd/C.,HCl, MeOH, room temp; 87% (l) Et_3SiCl, pyridine; Ac_2O,room temp; 89%.(m) 40% HF, CH_3CN, room temp; 91%. (n) Jones' reagent, acetone, 0 °C to room temp; 91% (o) 0.2 N NaOH, room temp; quantitative (p) (*R*)-*N* -acetylcysteine allyl ester. BOPCl, Et_3N, CH_2Cl_2, room temp; 60% (q) $Pd(PPh_3)_4$, HCO_2H, Et_3N, THF, room temp; 88%

The key aldol reaction between the silyloxypyrrole **32** and isobutyraldehyde had to be explored rather extensively in order to give the correct stereochemistry at both the quaternary carbon and the secondary alcohol. Various Lewis acids and solvents were tried, and eventually the use of $SnCl_4$ (2 equiv.) with ether as a solvent gave the required compound **33.** Other Lewis acids used, such as BF_3.etherate, $ZnCl_2$ or $TiCl_4$ in various solvents gave poor yields of the desired aldol product.[29] (**Scheme 7**, **Table 1**).



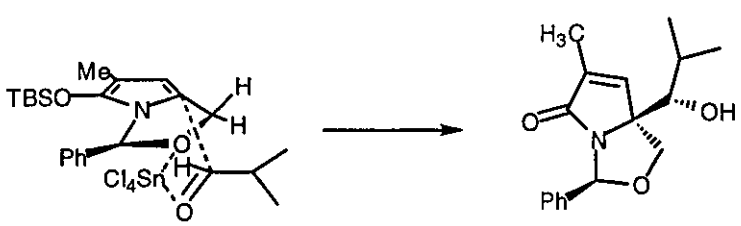
33

Scheme 7

Table 1 Aldol reaction of **32** with isobutyraldehyde using different Lewis acids

Lewis acid (equiv.)	solvent	yield of **33**
BF_3 etherate (1)	diethyl ether	17
BF_3 etherate(1)	dichloromethane	32
$SnCl_4$ (2)	diethyl ether	63
$SnCl_4$ (2)	dichloromethane	no product
$TiCl_4$ (1.5)	^{t}butyl methyl ether/diethyl ether	28
$ZnCl_2$ (2.6)	^{t}butyl methyl ether/	8

The stereochemistry of the product obtained when $SnCl_4$ is used can be rationalised in terms of the reaction taking place at the more hindered *si*-face, thereby forming 5(*R*)-compound. It is assumed that $SnCl_4$ binds to both aldehyde and the aminal oxygen in the transition state (**Figure 2**).



(transition state of aldol reaction)

33

Figure 2

The transition state shown in **Figure 2** is expected to be more favoured due to the pseudoequatorial orientation of the phenyl group, which in turn would align the α lone pair of electrons on oxygen orthogonal to the plane of the pyrrole. This change from the usual *erythro* to the *threo* relative stereochemistry at the newly created chiral centres can be understood in terms of the bulkiness of the phenyl group.

The secondary alcohol formed was first protected by acetylation and then dihydroxylation with osmium tetroxide and NMO to give **34** as a single diastereoisomer. Removal of the tertiary hydroxyl group at C-6 using Barton's procedure[30] i.e. *via* the cyclic thiocarbonate followed by reflux with Bu_3SnH in toluene, gave **35**. This transformation resulted in the formation of 1:1 epimers at

C-6. Treatment of **35** with sodium hydroxide in aqueous methanol causes epimerisation at C-6 (lactacystin skeleton). This process increases the ratio of the desired *syn* isomer (referring to Me/OH substituents) with respect to the *anti* isomer, which is also obtained in small amounts, together with some of the dehydrated product **33.**

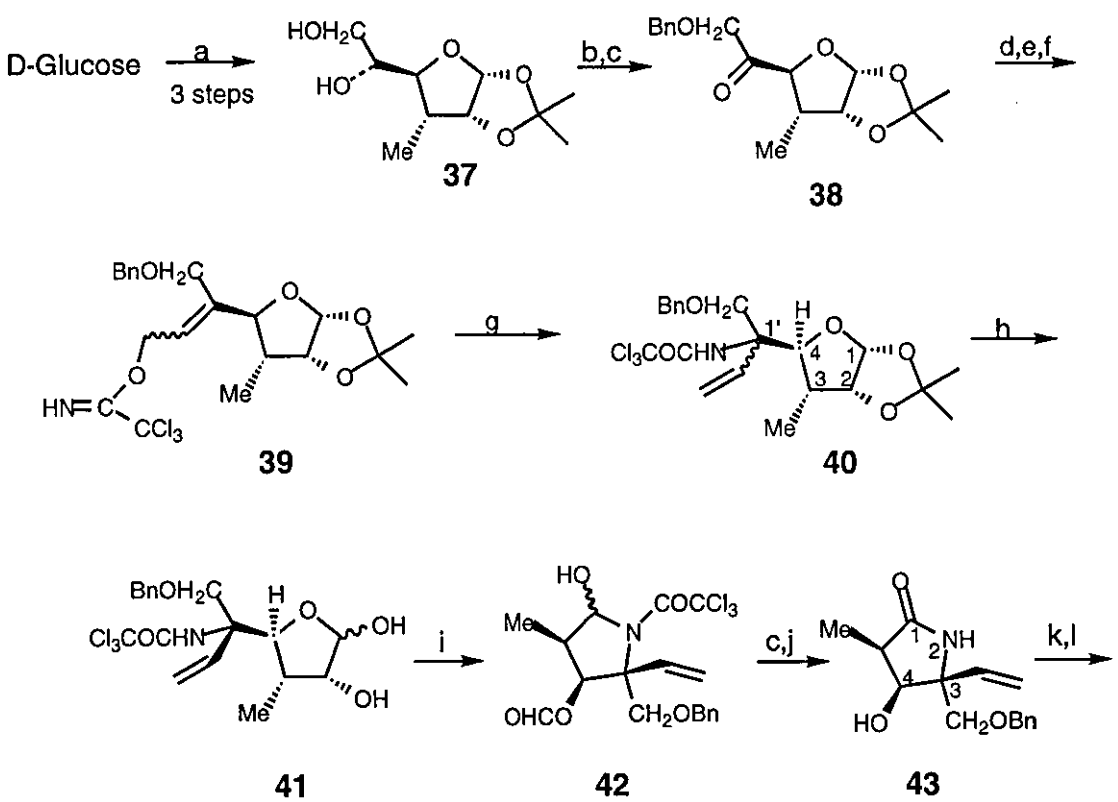
Hydrogenolytic cleavage of the benzylidene protecting group under acidic conditions (Pd/C, HCl, MeOH) followed by protection of the primary alcohol with triethylsilyl chloride and the secondary alcohol with excess acetic anhydride (performed in one operation) gave compound **36.** Removal of the silyl protecting group with HF followed by oxidation of the primary alcohol with Jones' reagent gave the carboxylic acid. Saponification of the diacetate of the acid with sodium hydroxide gave the important acid precursor **2.** The transformation of **2** into **1** was achieved using Corey's protocol. This synthetic route comprises 17 steps (starting with a bicyclic oxazolidine derived from (*R*)-glutamic acid in three steps), and gives 7.5% overall yield of lactacystin.

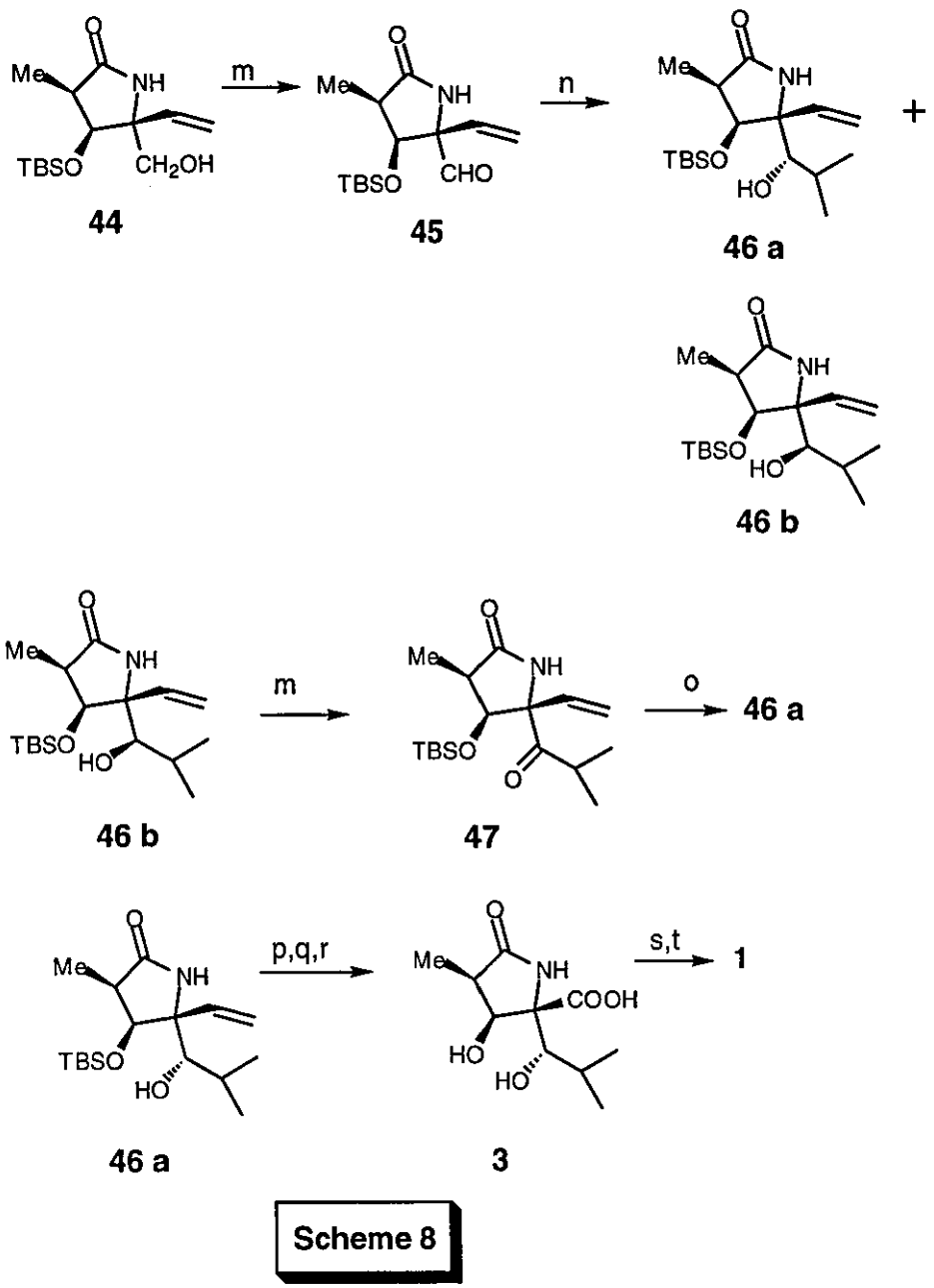
In another study, the known 3-deoxy-1,2-*O*-isopropylidene-3-*C*-methyl-a-D-allofuranose **37** (prepared from diacetone -D-glucose in three steps),[31] was chosen as the starting material by Chida and co-workers in their approach towards lactacystin (**Scheme 8**).[32] Reaction of **37** with dibutyltin oxide[33] followed by alkylation with benzyl bromide gave **38** in moderate yield. Oxidation of the secondary alcohol using Jones' reagent furnished the ketone which was was then subjected to a Wittig reaction to give the allylic ester as an inseparable mixture of (*E*) and (*Z*) isomers (1:1).

Reduction of the ester with DIBAL gave the allylic alcohols also as a mixtures of *E* and *Z* isomers. Reaction with trichloroacetonitrile provided the allylic imidates **39** which were then subjected to a variety of conditions in order to access the allylic amines *via* the Overman rearrangement.[34] The Overman rearrangement however, proceeded with limited success, and addition of Lewis acids such as mercury salts, BF_3.etherate or palladium reagents failed to improve the efficiency of this step. In the end, separation of the two isomers of the allylic alcohol and conversion into the corresponding imidates, followed by the rearrangement process, gives better yields of **40.** Compound **40** is obtained as a 2.7:1 mixture of isomers from the *E*-imidate and as a 5:1 mixture from the *Z*-imidate. It is perhaps strange that in both cases the same isomer is formed as the predominant product.

Acid hydrolysis of the mixture afforded **41** which exists as an equilibrium mixture of C-2 epimers (6:1). Periodate oxidation of **41** gave the hemiaminal derivative **42** which underwent Jones' oxidation. Removal of both *N*-trichloroacetyl and *O*-formyl groups by $NaBH_4$ furnished γ-lactam **43**. Protection of the OH group as the TBDMS ether followed by the removal of the *O* - benzyl group with sodium in liquid ammonia gave **44**, which was then oxidised under Moffatt conditions to give aldehyde **45**. Treatment of **45** with isopropylmagnesium bromide gave adducts **46 a** and **46 b** which were separated by column chromatography.

The undesired isomer **46 b** was converted to **46 a** by an oxidation-reduction procedure using Moffat oxidation and triisobutylaluminiumhydride. Use of other reducing agents such as Red-Al, $Zn(BH_4)_2$ or DIBAL gave low selectivity although the yields were very high (>95% for all these three reagents). Treatment of **46 a** with aqueous trifluoroacetic acid removed the silyl protecting group and ozonolysis of the vinyl group at C-5 followed by oxidation with $NaClO_2$ gave the key carboxylic acid **3**. Final transformation of **3** into (+)lactacystin was carried out by the method developed by Corey. The total synthesis of lactacystin by Chida involves 23 steps with an overall yield of *ca.* 2%.





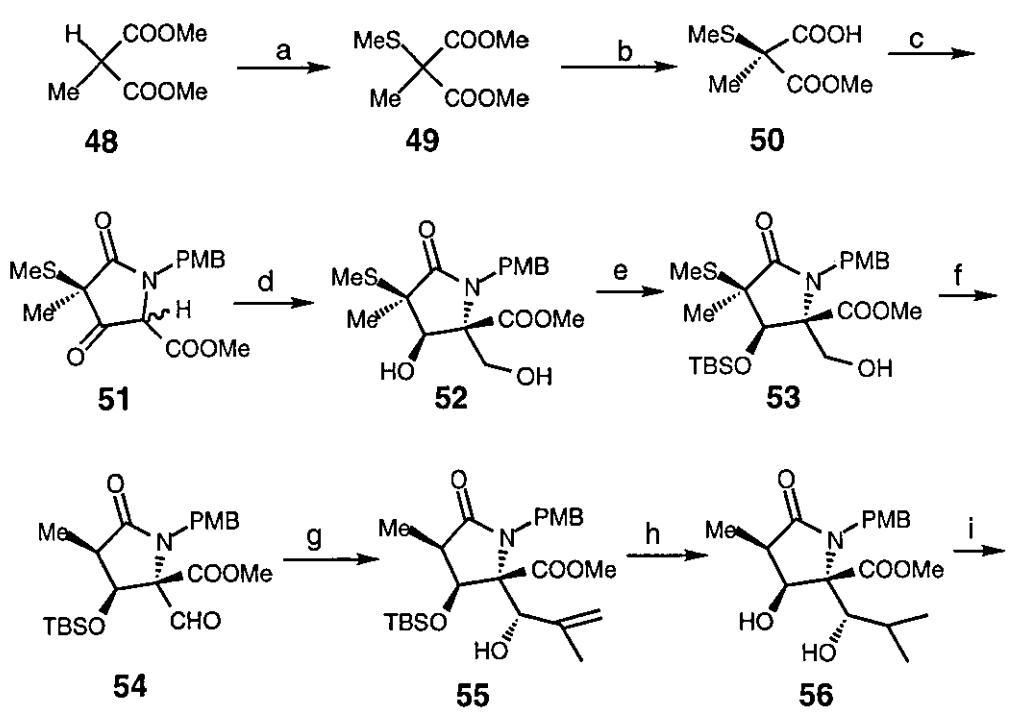
Scheme 8

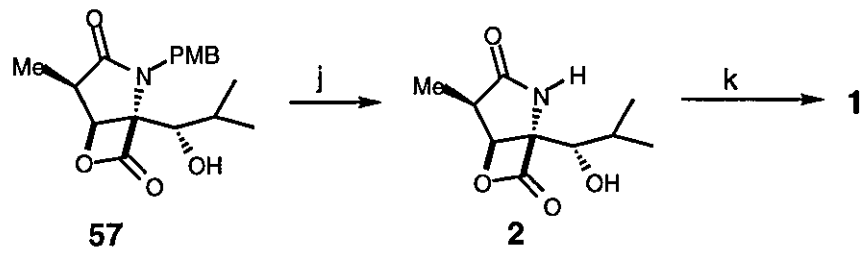
Conditions; (a) see Ref 31 (b) Bu_2SnO, toluene, reflux, then BnBr, CsF, DMF, room temp. (c) Jones' reagent , acetone, 0 °C (d) $Ph_3P{=}CHCO_2Et$, toluene, 60 °C (e) DIBAL-H, CH_2Cl_2, -15 °C (f) trichloroacetonitrile, NaH (60 mol%), Et_2O, room temp. (g) heat at 150 °C, toluene (in a sealed tube), 89 h (h) TFA-H_2O (3:2), 0 °C (i) $NaIO_4$, MeOH-H_2O (1:1), room temp. (j) $NaBH_4$, MeOH, 0°C; (k) tert-butyldimethylsilyl trifluoromethane-

sulfonate, 2,6-lutidine, CH_2Cl_2, room temp. (l) Na, liq. NH_3-THF, -78 °C; (m) Me_2SO, DCC, TFA, pyridine, benzene, room temp. (n) isopropylmagnesium bromide, THF, 20 °C to room temp. (o) triisobutyl-aluminiumhydride, CH_2Cl_2-hexane, 0 °C; (p) TFA-H_2O (4:1), 50 °C; (q) O_3, CH_2Cl_2, -78 °C, then Me_2S (r) $NaClO_2$, NaH_2PO_4, $HOSO_2NH_2$, tert-butanol-H_2), room temp. (s) bis(2-oxo-3-oxazolidinyl)phosphonic chloride, *N*-acetyl-L-cysteine allyl ester, Et_3N, CH_2Cl_2, 0 °C to room temp. (t) $Pd(PPh_3)_4$, HCO_2H, Et_3N, THF, room temp.

Corey's next approach towards the total synthesis of lactacystin was accomplished using a totally different strategy (**Scheme 9**).[35] Dimethyl methyl malonate **48** was first treated with sodium hydride followed by methyl sulfenyl chloride[36] to give the achiral α-methyl thio derivative **49.**

The α-methyl thio derivative **49** was then desymmetrised into the chiral monoester **50** using porcine liver esterase (PLE). Coupling of the acid chloride of **50** with *N*-p-methoxybenzylglycinate methyl ester (PMB-$NHCH_2COOMe$) furnished the amide ester which was then subjected to Dieckmann cyclisation using lithium diisopropylamide to give the keto lactam **51** as a 1:1 mixture of diastereoisomers.





57 2

Scheme 9

Conditions: (a) NaH, MeSCl, THF, 23 °C, 12 h (b) 1. PLE, H_2O, pH 7.3, 23°C, 24 h, 97%; 2. recrystallisation of the quinine salt, 62%, 95% ee (c) 1. $(COCl)_2$. DMF(cat.), 23 °C, 1 h; 2. PMB-$NHCH_2COOMe$, Et_3N, CH_2Cl_2, 23 °C, 1 h, 99%; 3. LDA, THF, -78 °C, 2 h, 93%; (d) 1. DBU, THF, -78 °C, then aq. CH_2O, -78 °C, 0.5 h, 90%; 2. $NaBH(OAc)_3$, HOAc, 23 °C, 1 h, recrystallisation, 95%, 99% ee (e) PivCl, pyridine, 23 °C, 10 h, 85%; 2. TBSOTf, 2-6-lutidine, 23 °C, 12 h, 98%; 3. NaOMe, MeOH, 23 °C, 5 h, 92% (f)1. Raney Ni, EtOH, 0 °C, 1 h, 82%; 2. Dess-Martin reagent, CH_2Cl_2, 23 °C, 1 h, 95% (g) H_2C=C(Me)MgBr, TMSCl, THF, -40 °C, 0.5 h, 97% (h) 1. H_2/Pd-C, EtOH, 23 °C, 12 h, 99%; 2. CF_3COOH/H_2O, 4/1, 50 °C, 4 h, 87% (i) 1. LiOH, THF/H_2O, 1:1, 23 °C, 0.5 h; 2. BOPCl, Et_3N, CH_2Cl_2, 23 °C, 0.5 h, 90% (j) Ce^{iv}, CH_3CN/H_2O, 3/1, 23 °C, 1 h, 62%;(k) *N*-acetyl *L*-cysteine, Et_3N, CH_2Cl_2, 23 °C, 4 h, 99%

Stereoselective α-hydroxymethylation of **51** using a catalytic amount of DBU in the presence of formalin gave the hydroxymethylated product in 9:1 mixtures, which subsequently underwent stereospecific reduction of the keto group with sodium triacetoxyborohydride[37] to give the crystalline dihydroxy lactam **52**. Lactam **52** was then converted to the mono tert-butyldimethylsilyl ether **53** by selective protection of the primary alcohol using pivaloyl chloride, protection of the secondary OH group with tert-butyldimethylsilyl triflate, and finally cleavage of the pivalate ester, using sodium methoxide /methanol.

Removal of the sulfur group using Raney nickel followed by Dess-Martin periodinane oxidation gave aldehyde **54**. The final construction of the isopropyl alcohol moiety was attempted by addition of Grignard or organolithium reagents to the aldehyde, but it turned out to be problematic, presumably due to the tendency for a retro aldol reaction at the quaternary centre.

Corey managed to overcome the problem, however, by the addition of trimethylchlorosilane to a mixture of **54** and 2-propenyl Grignard reagent. This gave the desired addition product **55** stereospecifically in high yield. Apparently, chlorotrimethyl silane acts as a trapping agent by reacting with the alkoxide anion resulting from the addition of the Grignard agent to the substrate, and the rate of this process is much higher than that of the retro aldol reaction. The trimethylsilyl ether of **55** is also stable during the reaction but is rapidly cleaved during aqueous work up.

The stereochemistry of the reaction is thought to be controlled by the steric effects generated by a bidentate complex involving the Mg ions and the carbonyl groups of the ester and formyl moieties. The enantiopure **55** was then subjected to hydrogenation and desilylation to give **56**, which after saponification and subsequent treatment with BOPCl gave β-lactone **57**. Removal of the para-methoxybenzyl (PMB) protecting group using ceric ammonium nitrate produced the known lactone **2**, which in turn was converted into lactacystin using *N*-acetylcysteine and triethylamine. The total synthesis by this route, which consist of 16 steps from **49**, gave lactacystin in 13% overall yield.

Corey recently reported an improved route towards the total synthesis of lactacystin (**Scheme 10**).[38] In this modified version of their original work, the synthesis begins with **58** (available in large scale from (*S*)-serine methyl ester hydrochloride in three steps).[10] The next step in the synthesis involves an aldol reaction between an aldehyde and the ester enolate to give the desired *anti* aldol product **60**.

In his first reported synthesis of lactacystin,[10] Corey had encountered several problems in their approach, notably the aldol coupling coupling of a chiral tertiary α-amino aldehyde **59** and the 2,6-dimethylphenyl propanoate to give the *anti* aldol product. The aldol reaction, carried out under Pirrung-Heathcock procedure,[12] was less than satisfactory since the ratio of *anti* to *syn* aldol products was only 2:1.

Furthermore, the separation of *anti* and *syn* aldol compounds was very difficult and the optimum combined yield was only 50%. The difficulties associated with these reactions necessitated further investigation of the conditions for the formation of the *anti* aldol product, which was required in large quantity in order to proceed with the synthesis.

In the aldol reaction using Braun's chiral controller in conjuntion with a zirconium enolate,[39] the formyl face selectivity was greatly increased, but the drawback was that a four-fold excess of the chiral enolate was required for complete conversion. Furthermore, the bulkiness of the Braun ester would render the subsequent reactions problematic due to steric hindrance (**Figure 3**).

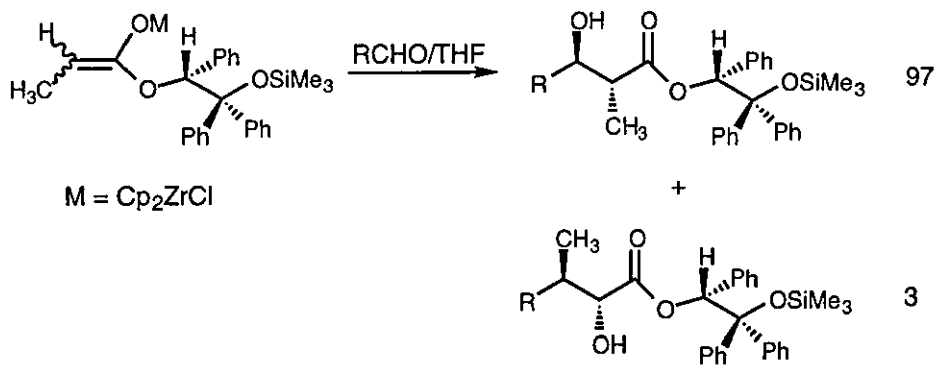
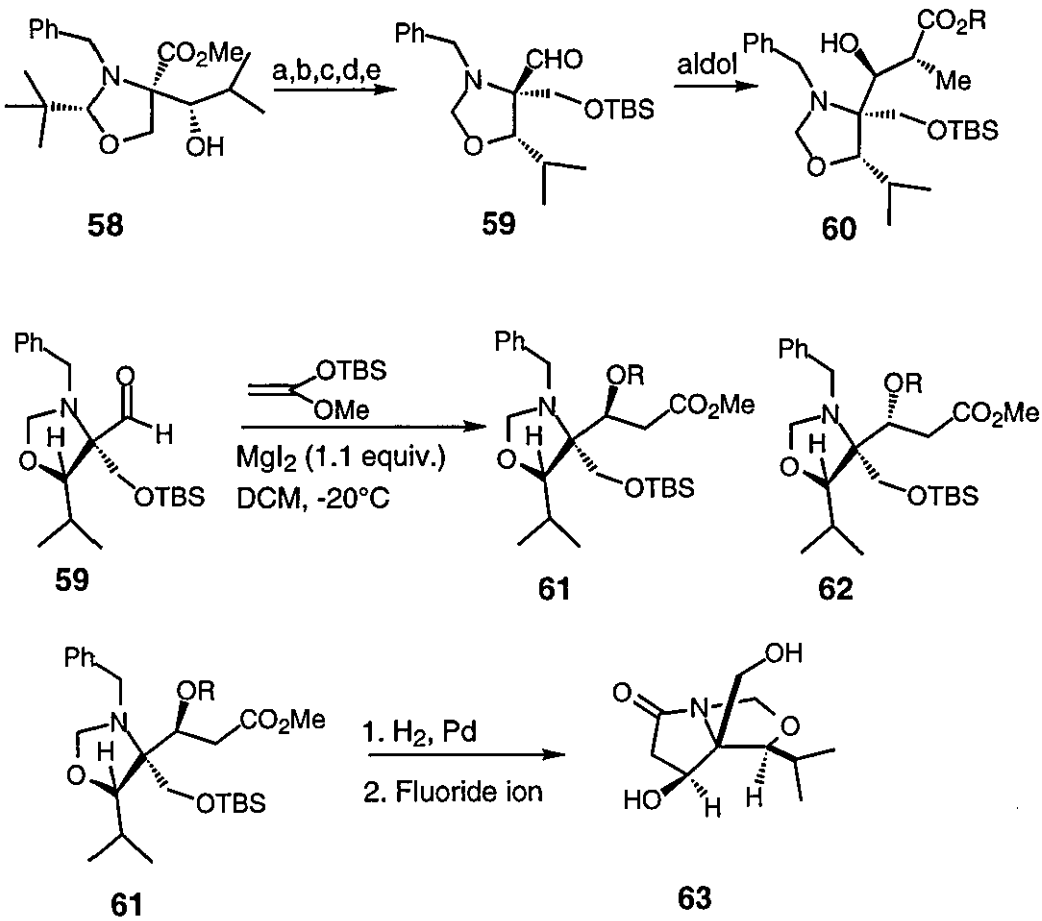


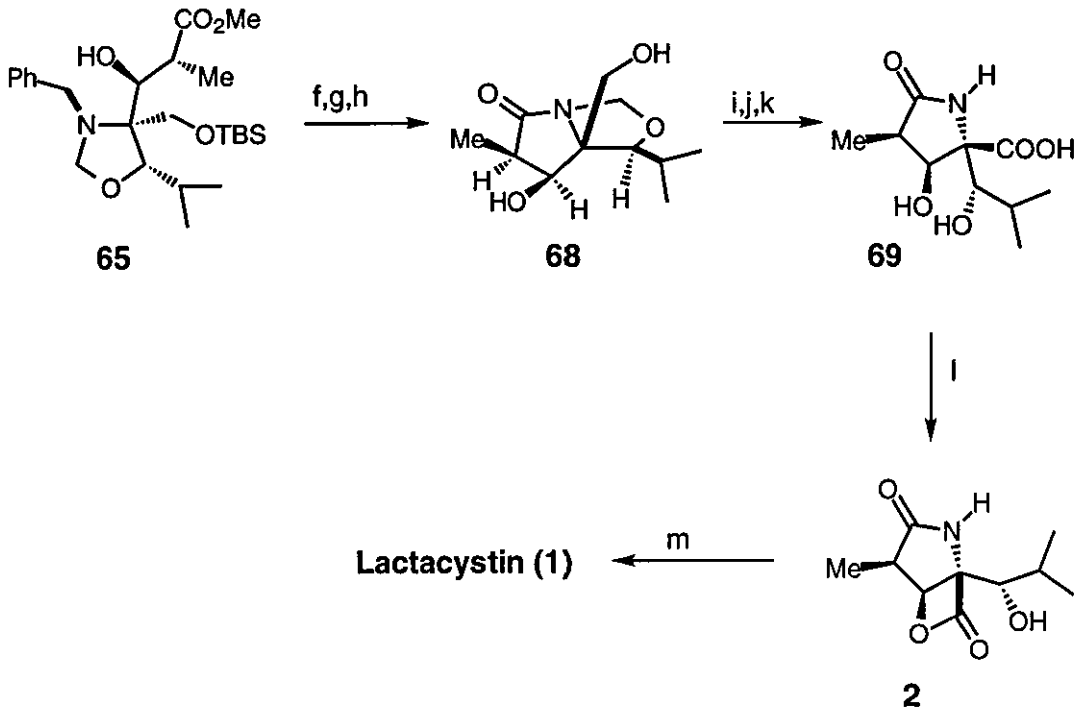
Figure 3 Anti-aldol reaction using Braun chiral controller

Because of the difficulties encountered in using both of the methods described above, control in the aldol reaction of **59** was attempted by the use of metal chelation involving the nitrogen atom and the carbonyl of the formyl group. Reetz has reported using a doubly facially selective aldol reaction between chiral *N,N* -dibenzylalanal and the trimethylsilyl ketene acetal of phenyl acetate using $TiCl_4$, but the yield was low (25%).[40]

Corey has also investigated the aldol reaction of **59** using $TiCl_4$ and various other Lewis acids such as $SnCl_4$, $ZnCl_2$ and $Cu(OTf)_2$. With $TiCl_4$ as a catalyst, the aldol reaction was carried out at -78 °C, but the aldehyde **59** decomposed without giving any aldol product. Other Lewis acids, however, gave neither substrate decomposition nor aldol products. Corey therefore suggested that the benzyl protected nitrogen cannot bind to the metal, and perhaps steric effects may also be partially responsible for the observations.

Interestingly, with MgI_2 as a catalyst, the aldol reaction was found to proceed with complete stereoselectivity to give the *anti* aldol product **61** in high yield (90%). None of the isomeric product **62** was isolated from the reaction mixture, suggesting that the *anti* aldol transition state is much more favourable over the *syn* (**Scheme 10**). The stereochemistry of the aldol product was proved by converting it to the dihydroxy γ-lactam **63** by hydrogenolysis followed by removal of the silyl protecting group. Replacing iodine with bromine in the Lewis acid also gave the same product but the rate of reaction was slower and the yield was still low.





Scheme 10

Conditions: (a) TfOH, 80 °C (b) TBSCl, imidazole (c) $(CH_2O)_n$, H^+, 80 °C (d) $LiBH_4$, THF-MeOH (e) Swern oxidation (overall yield from steps a to e, 83%), (f) H_2, Pd-C, EtOH, $(^iPr)_2NEt$, 23 °C, 30 h (g) MeOH, 55 °C, 20 h (h) 5% HF-CH_3CN, 23 °C, 24 h, (overall yield from steps f to g, 76%) (i) Et_3N, DMSO-$(COCl)_2$, CH_2Cl_2, -78 °C (j) $NaClO_2$, NaH_2PO_4, ^tBuOH, 23 °C (k) $HS(CH_2)_3SH$, HCl, CF_3CH_2OH, 55 °C, 8 h (overall yield from steps i to k, 77%) (l) BOPCl, Et_3N, CH_2Cl_2, 23 °C, 1 h (93%) (m) *N*-Acetyl-*L*-cysteine, Et_3N, CH_2Cl_2, 23 °C, 4 h (99%).

The superiority of MgI_2 over $MgBr_2$ is attributed to the facile dissociation of I^- from species such as L_2MgI_2, and this phenomenon was observed by Corey in his work on the catalytic enantioselective Diels-Alder reactions using a chiral *bis* -oxazoline as ligand.[41] The preferred transformation from **59** to **61** is expected to occur first by chelation of the formyl oxygen and the α-tert-amino group with MgI_2, followed by the attack of the nucleophilic silyl ketene acetal on the *si* face of the formyl carbon. Attack on the *re* face of the formyl group is not favourable due to blocking by the *N*-benzyl and OTBS groups (**Figure 4**)

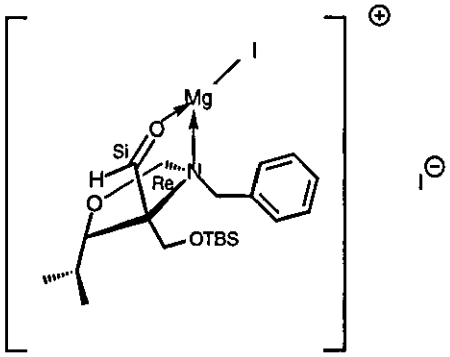
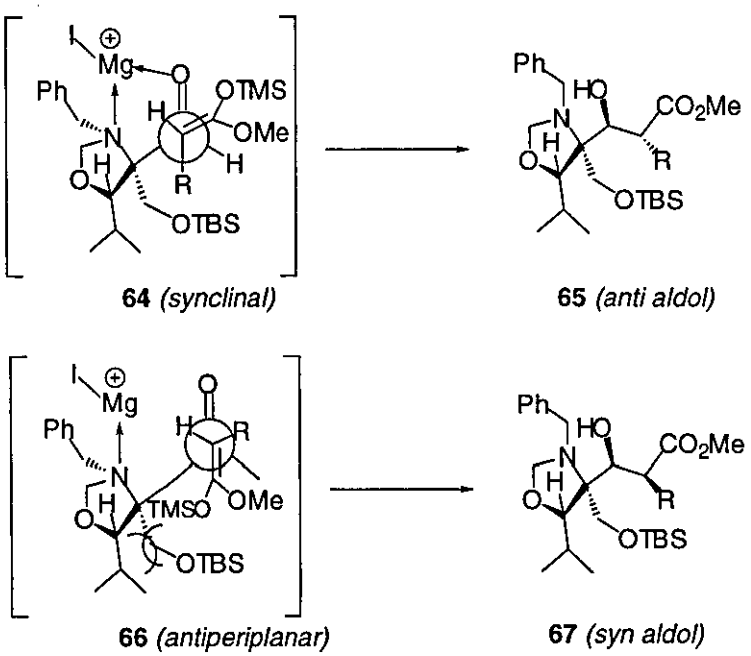


Figure 4

The corresponding aldol reaction between aldehyde **59** and the trimethylsilyl ketene acetal of methyl propionate in the presence of MgI_2 proceeded with high diastereoselectivity at both newly created chiral centres. The effectiveness of MgI_2 in the reaction can be explained on the basis of the IMg^+ chelate structure which allows a low energy pathway *via* the transition state assembly **64** as shown in **Figure 5**.

This low energy arrangement involves a *synclinal* relationship between the formyl group and the C=C double bond of the ketene acetal to give **65**. Transition state **64** shows that the intermediate structure suffers less steric repulsions compared to the alternative transition state **66**, which involves an *anti*periplanar arrangement.



64 *(synclinal)* **65** *(anti aldol)*

66 *(antiperiplanar)* **67** *(syn aldol)*

Figure 5

Although Mukaiyama-type aldol reactions usually proceed *via* an open transition state and are generally considered to occur preferentially through *anti*periplanar geometry,[42] the *anti*periplanar pathway **66**, which would lead to *syn* aldol product, is apparently disfavoured due to the steric repulsions between the bulky groups shown. Corey also reported that the effectiveness of the doubly diastereoselective aldol reaction was maintained on a larger scale, which is important for the total synthesis of lactacystin. The conversion of the aldol product **65** into lactacystin follows the original procedure as depicted in **Scheme 10**. The overall yield with this modification of the original work increased from 6% to 11 %.

Recently, Kang and Jun [43] also reported an enantiocontrolled synthesis of an intermediate of lactacystin **83** (**Scheme 11**). The key steps in this approach involved amination of the olefinic double bond by the Overman-type rearrangement of an allylic trichloroacetimidate. The differentiation of the hydroxymethyl group in ring formation was also crucial in this approach.

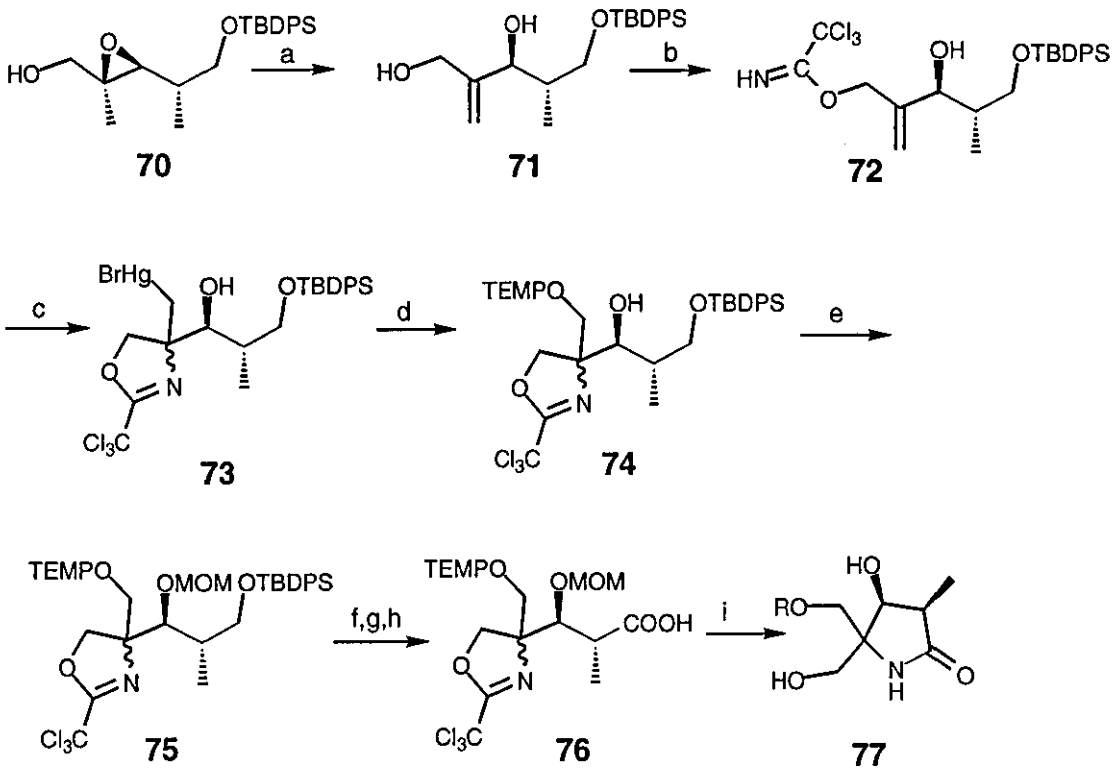
This synthesis begins with the known epoxide **70**, prepared by the method of Sharpless,[44] which was first treated with lithium diisopropylamide to give the allylic diol **71**. The primary alcohol was converted into the trichloroacetimidate using trichloroacetonitrile and DBU. The crude product monoimidate **72** was then subjected to intramolecular mercurioamidation ($Hg(O_2CCF_3)_2/K_2CO_3$ to give a 1:1 mixture of diastereoisomeric oxazolidines **73**.

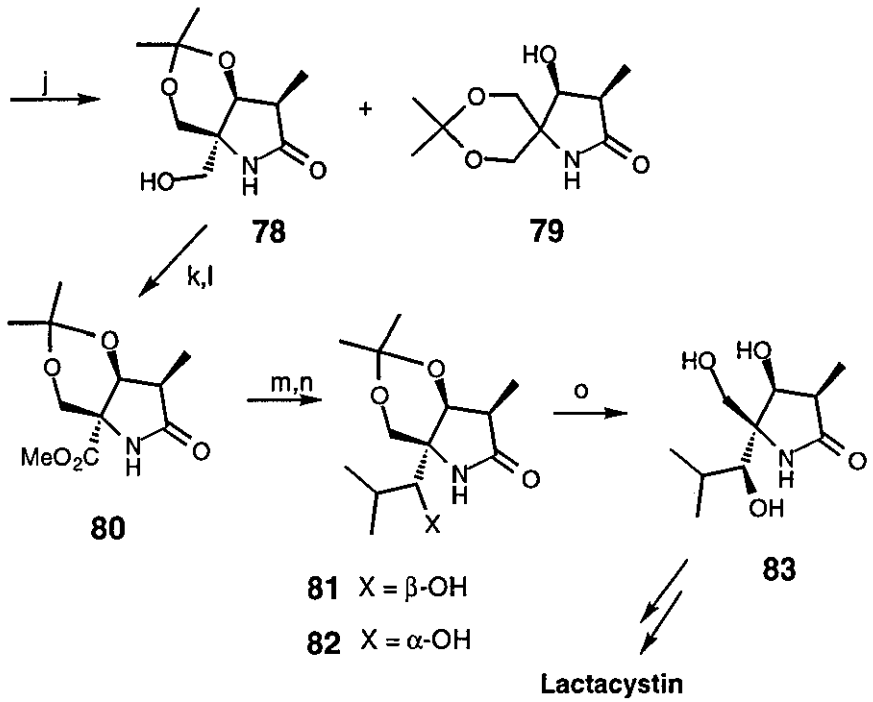
The next step, which involved the oxidative demercuration of the oxazolidine using O_2, was problematic. Although various conditions were tried, the demercuration failed. However, treatment of the mercuro-oxazolidine with TEMPO in the presence of $LiBH_4$ gave the desired oxidised product **74**. The secondary alcohol was then protected with MOMCl to give **75** and the silylated primary alcohol was deprotected. Oxidation of this alcohol to the carboxylic acid with PDC was partially successful, but Swern oxidation followed by $KMnO_4$ gave carboxylic acid **76** in good yield. The N-O bond of the TEMPO moiety was cleaved by zinc/acetic acid reduction to give the trihydroxy pyrrolidinone **77**.

Reaction of **77** with acetone under acidic conditions gave a mixture of acetonides **78** and **79** in 7:1 ratio. The primary alcohol in the desired product **78** was then oxidised using Swern conditions to give the aldehyde, which upon treatment with isopropylmagnesium bromide gave a 1:1 mixture of alcohols **81** and **82**. Because

of the low selectivity in the Grignard reaction, **78** was first converted into the methyl ester **80** and then subsequently treated with isopropyl magnesium bromide to give the ketone.

Stereoselective reduction of the ketone to the desired alcohol **81** was problematic in terms of accessing the alcohol with the correct stereochemistry. Reducing agents such as oxazaborolidine,[45] Ipc_2Cl[46] and sodium triacetoxyboro-hydride[37] failed to give the product in good yield and with high stereoselectivity. The best diastereoselectivity was achieved using sodium borohydride in methanol at 0 °C, which favoured the desired alcohol **81** in a 5:1 ratio. Finally, hydrolysis of the acetonide using p-toluenesulphonic acid gave intermediate **83**. The overall yield of this 15 step synthesis, from **70** to the intermediate **83**, is 19%.





Scheme 11

Conditions: (a) LDA, THF, 0-24 °C (b) Cl_3CCN, DBU, CH_3CH_2CN, -78 °C (c) $Hg(O_2CCF_3)_2$, K_2CO_3, THF, 0 °C, then aq. KBr (d) TEMPO, $LiBH_4$, THF, 24 °C (e) MOMCl, $^iPr_2NEt$, CH_2Cl_2, 0-24 °C; (f) TBAF, H_2O, THF, 45 °C (g) $(COCl)_2$, DMSO, Et_3N (h) 1M $KMnO_4$, NaH_2PO_4, ^tBuOH, 24 °C (i) conc. HCl, EtOH, AcOH, reflux then Zn, reflux (j) TsOH, acetone, 24 °C (k) Jones' reagent, acetone, 0 °C; (l) CH_2N_2, THF, 0 °C (m) Pr^iMgBr, THF, -20 to 0 °C (n) $NaBH_4$, MeOH, 0 °C (n) TsOH, MeOH, 60 °C

Kang *et al* [47] also reported the synthesis of the same intermediate, trihydroxy pyrrolidinone **98**, but using a different approach (**Scheme 12**). The key steps involved stereoselective crotylboration of an aldehyde and the intramolecular mercurioamidation of the allylic trichloroacetimidate (Overman-type rearrangement), a strategy used by them in their earlier work.

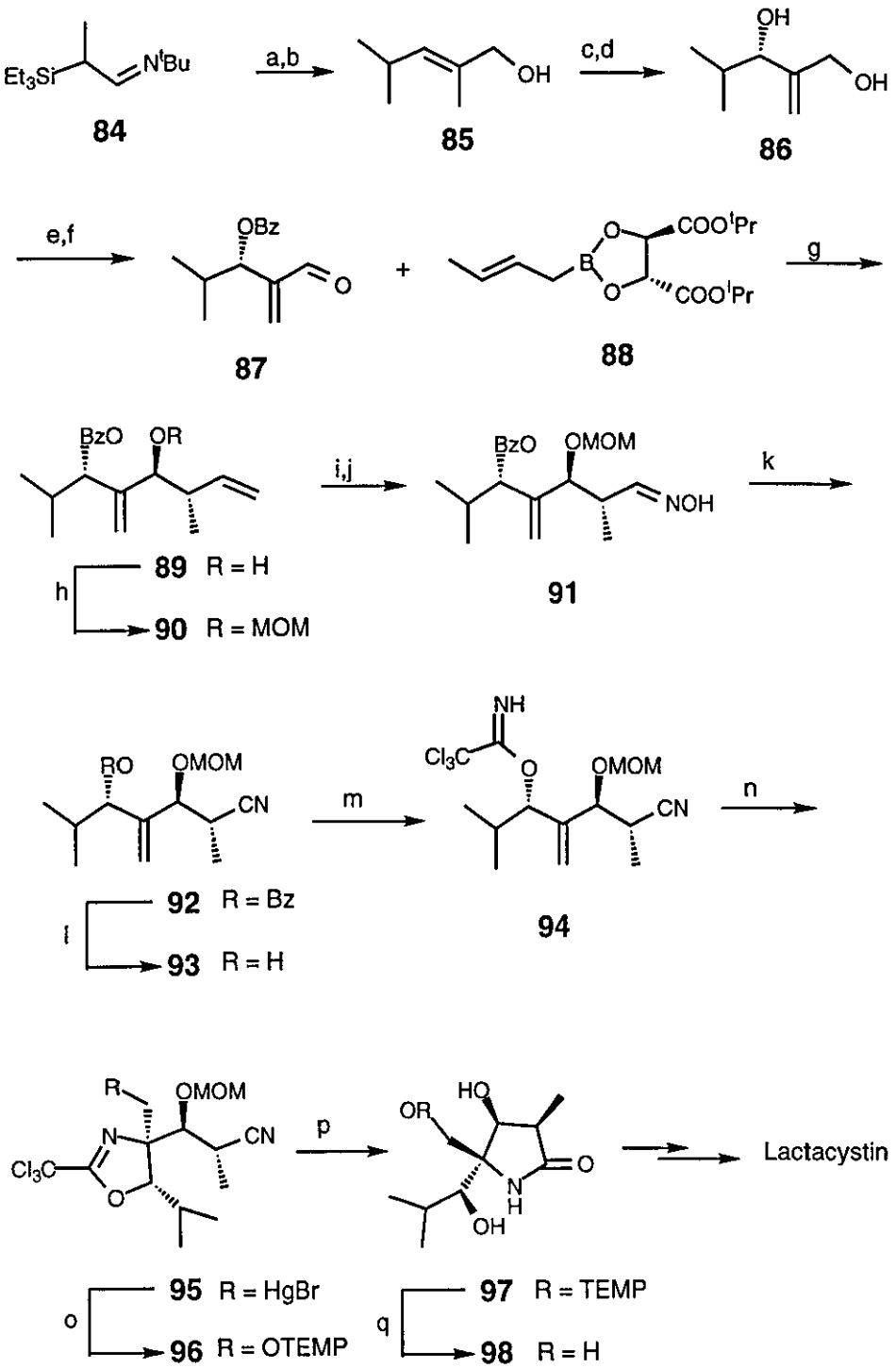
The allylic alcohol **85** was prepared by Peterson olefination of tert-butylimine **84** with isobutyraldehyde using a known procedure,[48] followed by reduction of the carbonyl group using sodium borohydride. Sharpless epoxidation of the allylic alcohol using diethyl L-tartrate, followed by ring opening of the epoxide using

lithium diisopropylamide, furnished diol **86**. Chemoselective oxidation of the primary allylic alcohol using manganese dioxide furnished the aldehyde.

Protection of the secondary alcohol as the benzoate ester gave conjugated aldehyde **87**, which was coupled with the chiral crotylboronate **88** (derived from diisopropyl L-tartrate).[49] This reaction gave diastereoisomerically pure alcohol **89** (>50:1 diastereoselectivity). The observed selectivity, favouring the *anti*-homoallylic alcohol over the *syn* homoallylic alcohol, is thought to be controlled by the cyclic transition state **88**. The use of (*E*)-crotylboronate will give the *anti* alcohol whereas the (*Z*)-crotylboronate will preferentially give the *syn* homoallylic alcohol.

The secondary hydroxyl group of the homoallylic alcohol was then protected using MOMCl, before the terminal olefin was oxidatively cleaved with osmium tertroxide and sodium periodate to give the aldehyde. Nitrile **92** was prepared by dehydration of the corresponding oxime by methanesulfonic anhydride and DBU. Hydrolysis of the benzoyl group using potassium carbonate in methanol furnished alcohol **93**.

The free hydroxyl group was then treated with trichloroacetonitrile and base, and the resulting allylic trichloroacetimidate **94** was ring-closed using mercuric trifluoroacetate to furnish the oxazoline **95**. Oxidative demercuration of **95** with TEMPO in the presence of $LiBH_4$ gave the oxidised compound **96**. Treatment of **96** with hydrochloric acid effected deprotective cyclisation of the the molecule to give the pyrrolidinone **97**. This was followed by the addition of zinc to the hot mixture in order to cleave the TEMPO group and to give trihydroxypyrrolidinone **98**. The overall yield of this 17 step synthesis of **98** is 7.6 %.

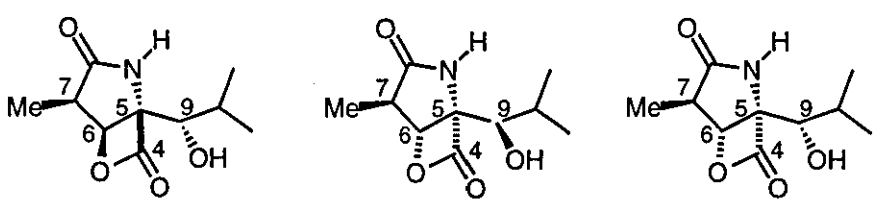


Scheme 12

Conditions: (a) LDA, THF, -78 °C; Me_2CHCHO, -78 °C to 20 °C (b) $NaBH_4$, MeOH, 0 °C, yield for steps a to b, 76% (c) Diethyl L-tartrate,

$Ti(O^iPr)_4$, ^tBuOOH, CH_2Cl_2, -15 °C (d) LDA, THF, 0 °C to r.t., 66% for steps c to d (e) MnO_2, CH_2Cl_2, reflux (f) $(PhCO)_2O$, DMAP, Et_3N, CH_2Cl_2, 0 °C (g) 4Å mol. sieve, toluene, -78 °C, 71% for steps e to g (h) $CH_2(OMe)_2$, P_2O_5, $CHCl_3$, r.t. 93% (i) OsO_4, $NaIO_4$, H_2O, THF, 0 °C (j) $HONH_2$:HCl, pyridine, r.t. 80% from steps i to j (k) $(MeSO_2)_2O$, DBU, CH_2Cl_2, -20 °C, 85% (l) K_2CO_3, MeOH, r.t. 100% (m) Cl_3CCN, DBU, EtCN, -20 °C (n) $Hg(OOCCF_3)_2$, THF, PhH, r.t. then aq. KBr, 64% for steps l to n (o) TEMPO, $LiBH_4$, THF, -20 °C, 70% (p) 6N HCl reflux (q) Zn, AcOH, reflux, 77% for steps p to q.

Analogues of lactacystin β-lactone **110** and **111** have also been synthesised by Corey[50] using the strategy described earlier (**Scheme 13**).[35] The main objectives of their study were to investigate the reactivity of these lactones by modifying the stereochemistry at C-5, C-6 and C-9 in the active β-lactone **2**, hence establishing the structural requirements for optimum biological activity (**Figure 6**).



2 (β-lactone) **110** **111**

Figure 6

The synthesis of **110** started with racemic dimethyl isopropenylmethylmalonate **99** prepared by using the reported procedure.[51] Enzymatic hydrolysis of the malonate using porcine liver esterase (PLE) gave the enantiopure mono acid **100**. Corey reported that this transformation was quite efficient, as the minor isomer can be removed by recrystallisation. Conversion of **100** into the keto lactam **101** was achieved by first converting the mono acid into the corresponding acid chloride, coupling with *N* -(4-methoxybenzyl)glycine methyl ester, followed by Dieckmann cyclisation reaction.

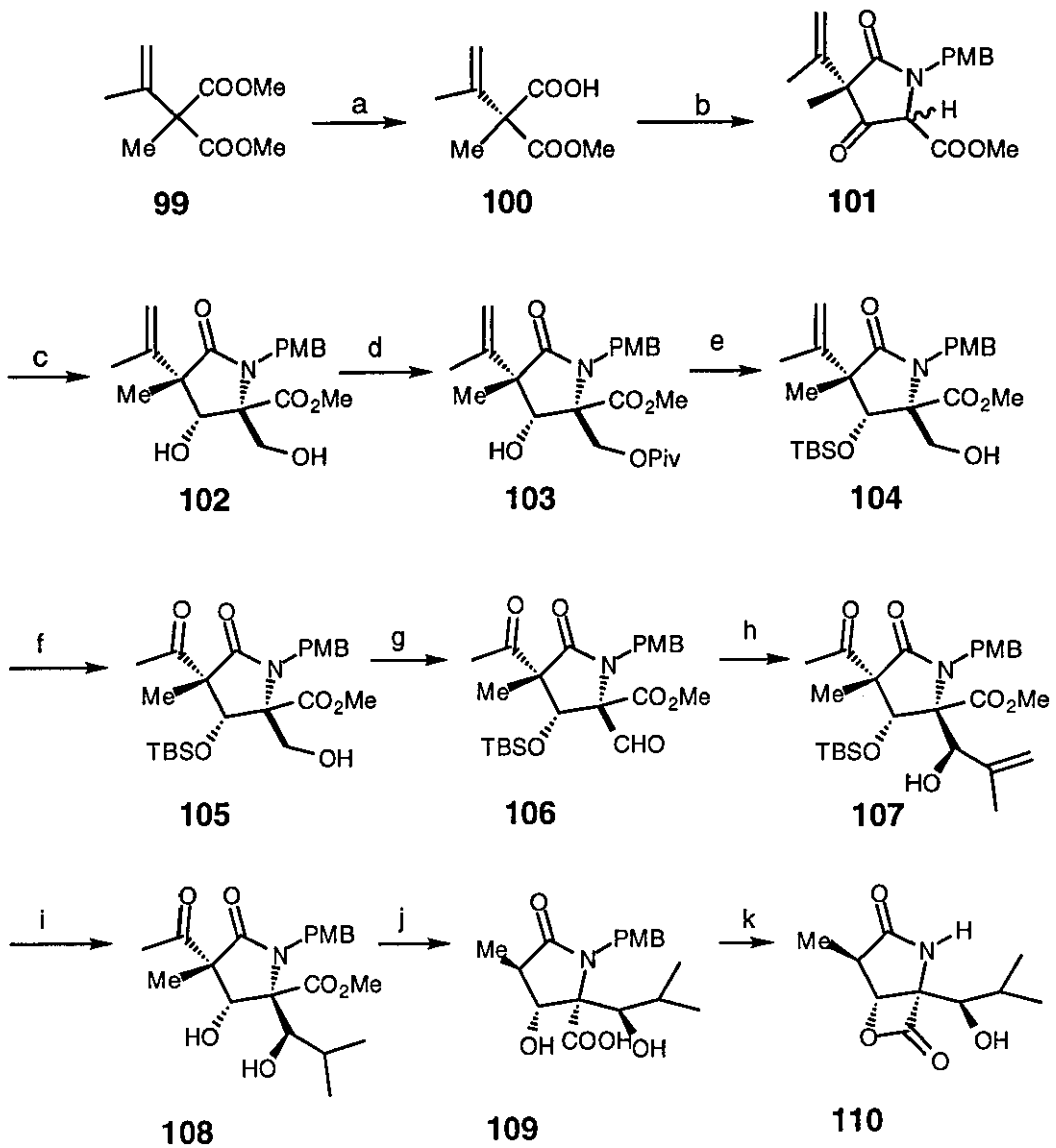
Base-catalysed reaction of the keto lactam with formalin, followed by stereoselective reduction of the keto group using sodium triacetoxyborohydride, gave the diol **102** with 80% ee. Selective protection of the primary alcohol with pivaloyl chloride followed by recrystallization, provided enantiomerically pure **103**

(>99% ee). The high level of stereocontrol at the quaternary carbon during the aldol step is thought to be due to the steric effects associated with the larger isopropenyl group over those of the methyl group. The diastereoselective reduction of the C-6 keto function is then controlled by the 5-hydroxymethyl moiety through internal delivery of the hydride.[52]

Protection of the secondary alcohol of **103** with tert-butyldimethylsilyl triflate followed by the cleavage of the pivalate ester with sodium methoxide gave **104**. Oxidation of **104** with sodium periodate and a catalytic amount of osmium tetroxide furnished the keto alcohol **105**, which was then further oxidised using Dess-Martin periodinane to give keto aldehyde **106**. Treatment of the aldehyde with 2-propenyl magnesium bromide in the presence of trimethylsilyl chloride[37] (to prevent the retro aldol reaction) afforded the unsaturated keto alcohol **107**.

Hydrogenation followed by desilylation of **107** gave the saturated keto alcohol **108** together with the minor contaminant of **108** (the 9*S* -diastereoisomer, 10% total). Fortunately the diastereoisomers can be separated by column chromatography. Hydrolysis of the methyl ester was achieved by treating **108** with LiOH in 1:1 mixture of THF:H_2O to give the lithium carboxylate, which was then heated in aqueous LiOH to induce deacetylation, followed by acidification to give the dihydroxyacid **109**. Conversion into the β-lactone **110** was achieved by treatment of **109** with bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOPCl) followed by deprotection of the PMB group using ceric ammmonium nitrate (CAN). In a similar manner, the other diastereoisomer of **108** (i.e. the 9*S* diastereoisomer) was converted into the lactone **111**.

Measurement of the biological activities of lactones **110** and **111** on the 20 S proteasome showed that these compounds were less active than the natural β-lactone **2**. Although these lactone derivatives have the same topology and functionality, the specific geometry of the functional groups are clearly important for the activation of the proteasome. The only modifications that preserved the activity of the lactone were the replacement of the C-7 methyl group by other alkyl substituents such as isopropyl, ethyl, n-propyl or isopropyl, or the replacement of the 7-hydrogen by a methyl group.

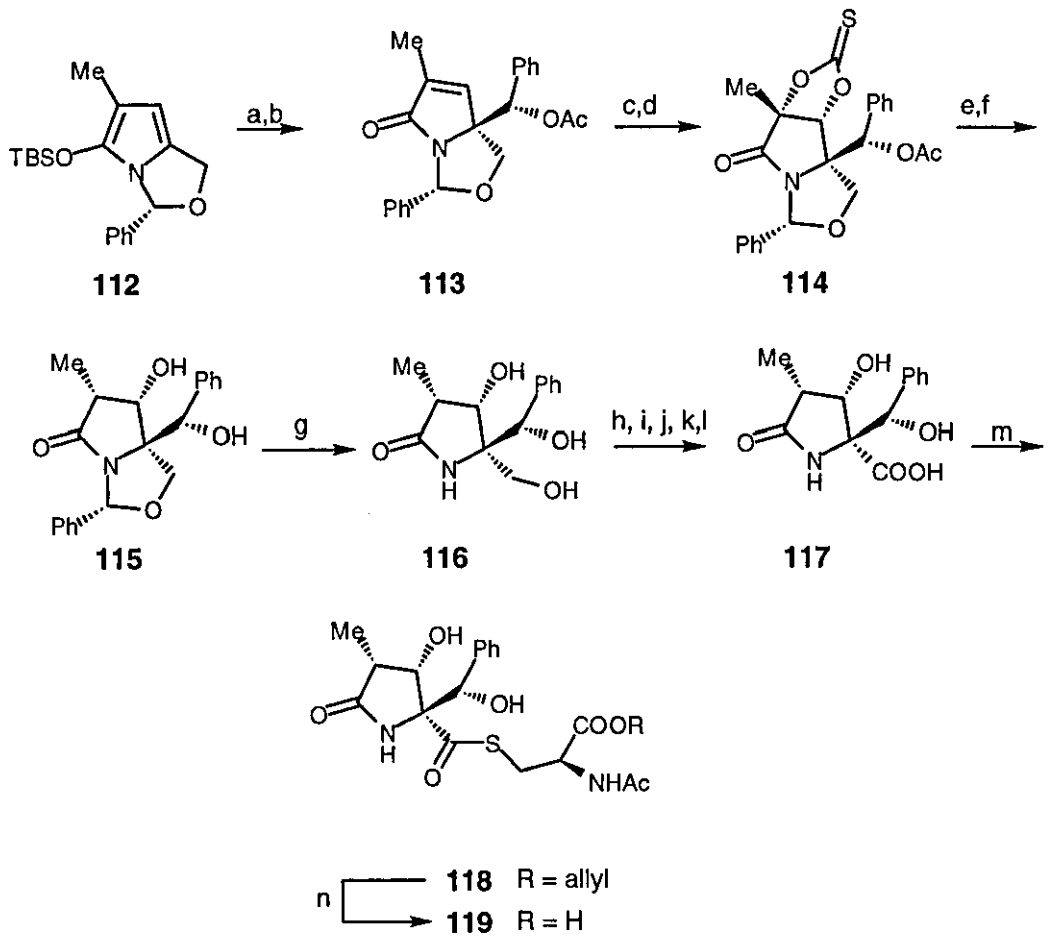


Scheme 13

Conditions: (a) PLE, NaOH, pH 7.3, 23 °C, 30 h (b) 1. $(ClCO)_2$, cat. DMF, PhH, 23 °C, 0.5 h; 2. $PMBNHCH_2COOMe$, Et_3N, CH_2Cl_2, 0 °C, 1 h; 3. LDA, THF, -78 °C, 0 °C, 2.5 h (c) 1. HCHO, cat. DBU, THF, -78 °C, 1h; 2. $NaBH(OAc)_3$, AcOH, 23 °C, 1h (d) PivCl, Pyridine, 23 °C, 12 h; recrystallisation from hexane/ethyl acetate (1:1) (e) 1. TBSOTf, 2,6-lutidine, CH_2Cl_2, 23 °C, 5 h; 2. NaOMe, MeOH, 23 °C, 4 h (f) OsO_4 (5 mol %), $NaIO_4$, Dioxane-H_2O (1:1), 23 °C, 48 h (g) Dess-Martin periodinane, CH_2Cl_2, 23 °C, 1h (h) CH_2=C(Me)MgBr, TMSCl, THF, -50

°C, 0.5 h (i) 1. H_2, Pd-C (10%), EtOH, 23 °C, 1 h; 2. TFA-H_2O (4:1), 60 °C, 2 h (j) 1. LiOH, THF-H_2O (1:1), 23 °C, 2 h; 2. LiOH, H_2O, 80 °C, 1 h; 3. BOPCl, Et_3N, CH_2Cl_2, 23 °C, 1 h; (k) CAN, CH_3CN-H_2O (3:1), 23 °C, 2 h.

The total synthesis of lactacystin analogue **119** has also been reported by Uno,[53] based on their original work on the total synthesis of lactacystin.[27] The key step in this approach is the Lewis acid-mediated reaction of 2-silyloxypyrrole, derived from (*L*)-glutamic acid, with benzaldehyde (**Scheme 14**).



Scheme 14

Conditions: (a) benzaldehyde, $BF_3.OEt_2$, ether, -78 °C (b) Ac_2O, pyridine, 73% (from a to b) (c) OsO_4, NMO, ^tBuOH (d) TCDI, THF, 80% for steps c to d (e) n-Bu_3SnH, AIBN, toluene, reflux, 93% (f) 0.5N MeOH-H_2O, 3 days, 71% (g) conc.HCl, MeOH, 88% (h) Et_3SiCl, pyridine (i) Ac_2O, pyridine, (j) 50% HF, MeCN 69% from steps h to j (k) Jones reagent (l) 0.2N NaOH, 46% from steps k to l (m) *N*-acetylcysteine allyl ester, BOPCl (n) $Pd(Ph_3P)_4$, HCOOH/NEt_3

The silyloxypyrrole **112** was treated with benzaldehyde in the presence of $BF_3.OEt_2$, to give the aldol product, which is derived by attack on the *si*-face of benzaldehyde. Changing the Lewis acid to $SnCl_4$, however, gave a very poor yield and also low diastereoselectivity, despite $SnCl_4$ being effective in the original work with isobutyraldehyde. This dramatic change in the Lewis acid-mediated aldol reaction may perhaps be attributed to the nature of the aldehyde (aromatic or aliphatic).

The formation of the observed *syn* adduct **113** formed in the reaction of siloxypyrrole **112** and benzaldehyde in the presence of of $BF_3.OEt_2$ may be explained by considering the transition state of the reaction. In the aldol reaction of an aromatic aldehyde, the π-π interaction between the pyrrole moiety and the aromatic ring may become the major controlling factor and would lead to the observed *threo* isomer. On the other hand, the reaction of silyloxypyrrole with an aliphatic aldehyde would proceed *via* a the Diels-Alder-like transition state that would give rise to the *erythro* product (**Figure 7**).

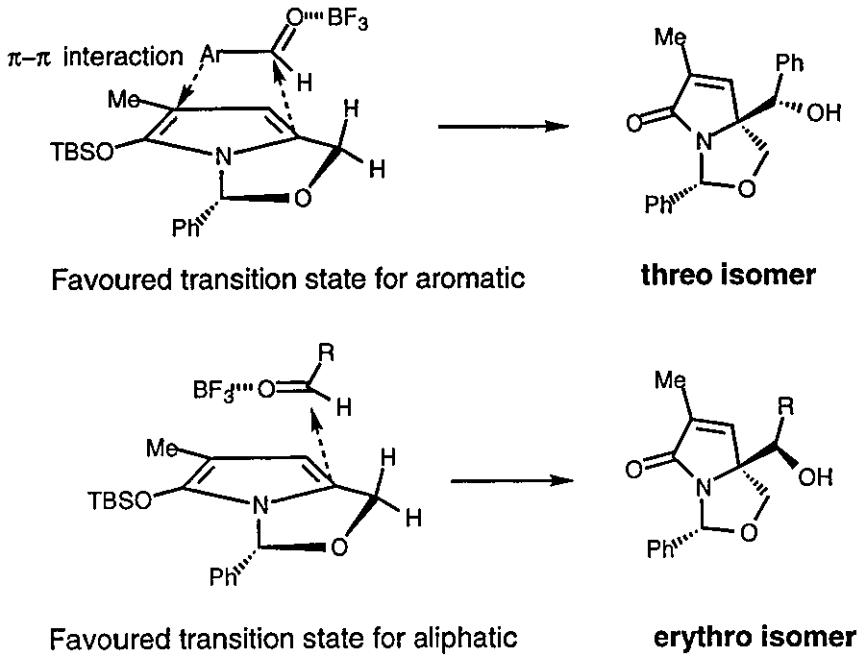


Figure 7

The aldol product obtained was acylated and then converted into the cyclic thiocarbonate **114**, by dihydroxylation with osmium tetroxide, followed by treatment with thiocarbonyldiimidazole (TCDI). Reduction of the cyclic thiocarbonate with tributyltin hydride resulted in the formation of the diastereoisomeric β-hydroxy lactams in a *syn* to *anti* ratio of 1:2.8. Treatment of this mixture with aqueous base in a methanolic solution caused epimerization of the methyl group as well as saponification of the ester, to give the desired *syn* **115** in 7.7:1 ratio. Following purification of the *syn* isomer by recrystallisation, treatment with acid in methanol cleaved the *N*-acyloxazolidine to give the triol **116**.

The primary alcohol was then protected using triethyl silyl chloride (TESCl), and the other two secondary alcohols were protected as the acetate esters with acetic anhydride. Selective deprotection of the primary alcohol using hydrogen fluoride in acetonitrile, followed by Jones' oxidation and hydrolysis of the acetate esters furnished the pyrroglutamic acid derivative **117**. Final conversion of the acid into lactacystin derivative **119** was accomplished using Corey's protocol.[10]

Recently, Corey investigated the structural requirements of lactacystin **1** to inhibit the 20 S proteasome.[9] Various analogues of lactacystin and also the active β-lactone **2** were synthesised and tested for their activities. From the findings of this

investigation, both synthetic and biological information for these analogues was gained. Some of these analogues were found to be more active than lactacystin itself in the ability to inhibit the 20 S proteasome. The structural changes that affect the activity of lactone **2** are given in **Figure 8**.

Most of the functionalities present in the natural coumpound are essential in order for the lactone to have activity against the proteasome, with the exception of that at C-7. Replacement of the methyl group by ethyl, n-propyl or iso-propyl led to increased activity towards the proteasome by a factor of 2 or 3.[9,35] On the other hand, replacement of the hydrogen by a methyl group gives rise to lactone **120** , which exhibits the same activity as lactone **2**.

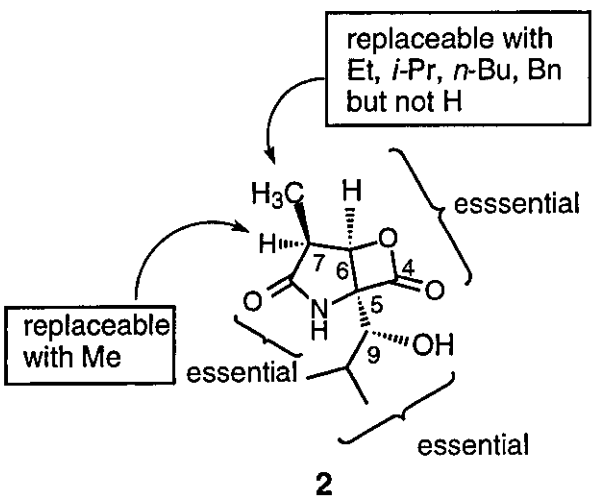
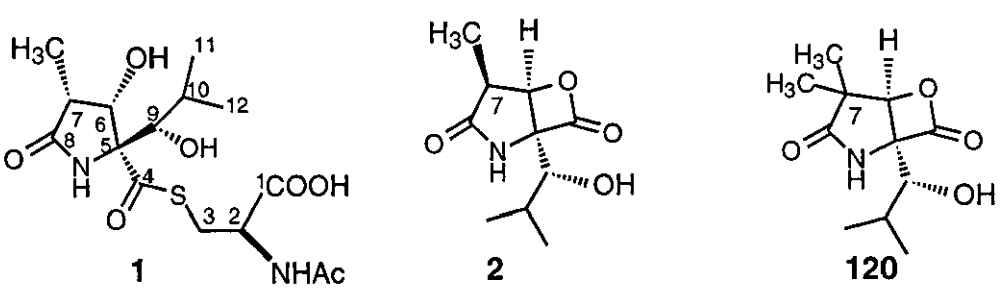


Figure 8

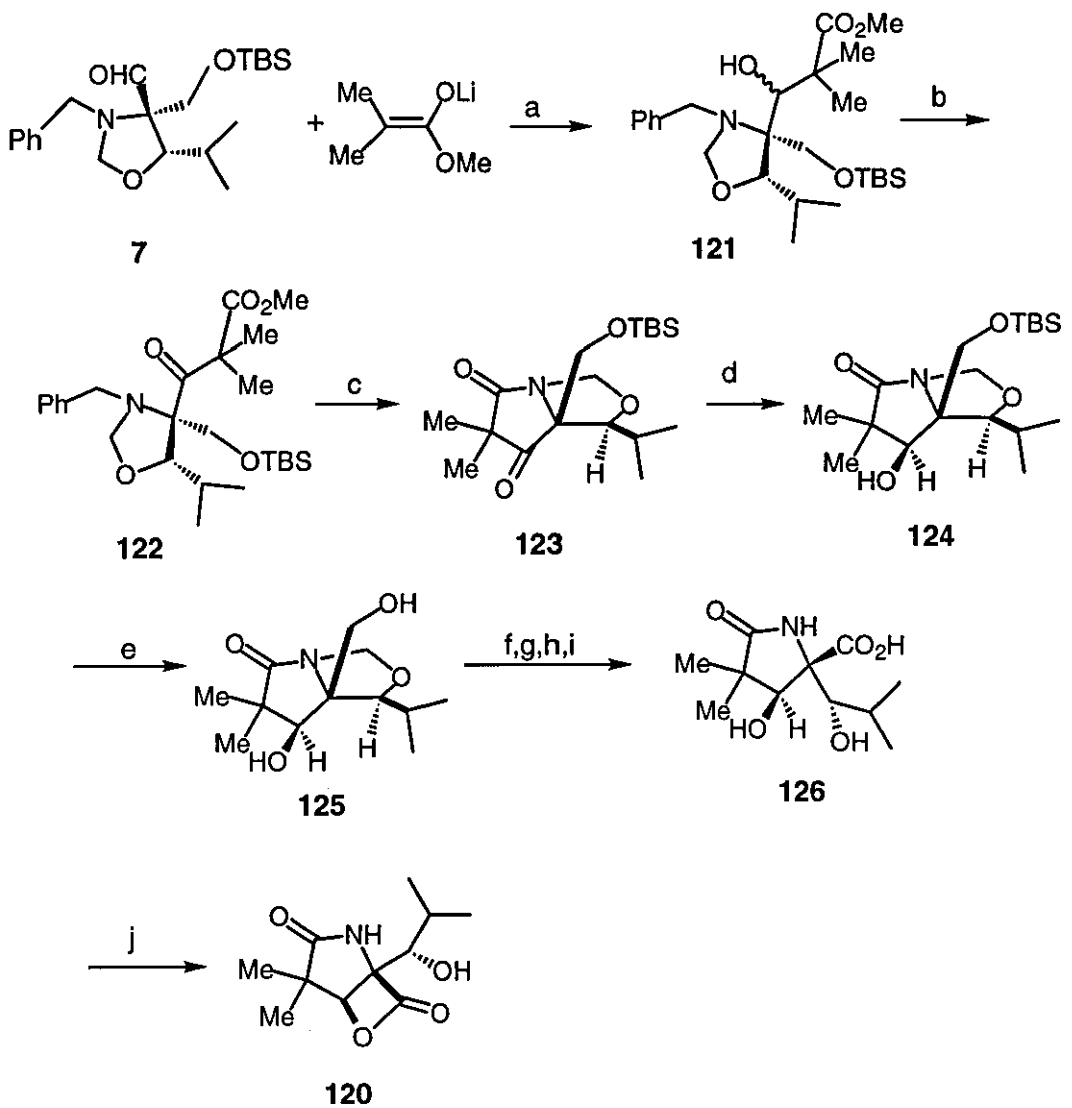


The total synthesis of the lactone **120** was recently reported by Corey in a study of the biological activity of analogues of the lactone **2**[54] (**Scheme 15**). The synthesis of **120** started from the readily available aldehyde **7**.[10,38] Treatment of aldehyde **7** with the lithium enolate of methyl isobutyrate at -78 °C gave a mixture

of two diastereoisomeric β-hydroxy esters, **121** (ratio 1.2 :1) which was oxidised using Dess-Martin periodinane to give the β-keto ester **122** .

Hydrogenation of the keto ester using Pd-C in ethanol, effected debenzylation and concurrent γ-lactam formation to afford the keto lactam **123**. Reduction of the lactam with sodium borohydride at -10 °C in MeOH/THF afforded the desired β-hydroxy γ-lactam **124** in 12:1 ratio. The stereochemistry of the major isomer was confirmed by nOe studies. The crude γ-lactam **124** was then desilylated to afford diol **125**, and then the primary alcohol was selectively oxidised to the acid, *via* the corresponding aldehyde. Exchange of the methylene group using 1,3-propanedithiol gave the acid precursor **126**.

Finally, treatment of the γ-lactam dihydroxy acid **126** with BOPCl and triethylamine resulted in rapid and selective formation of the β-lactone **120**. The results of biological testing on **120** towards the inhibition of 20 S proteasome showed that it is as active as the β-lactone **2**. These findings are useful, since the lactone **120** can be made easily and with a better yield than lactone **2**. Furthermore, it is more stable towards β–elimination, and from a synthetic point of view, there is one less stereogenic centre that has to be considered.



Scheme 15

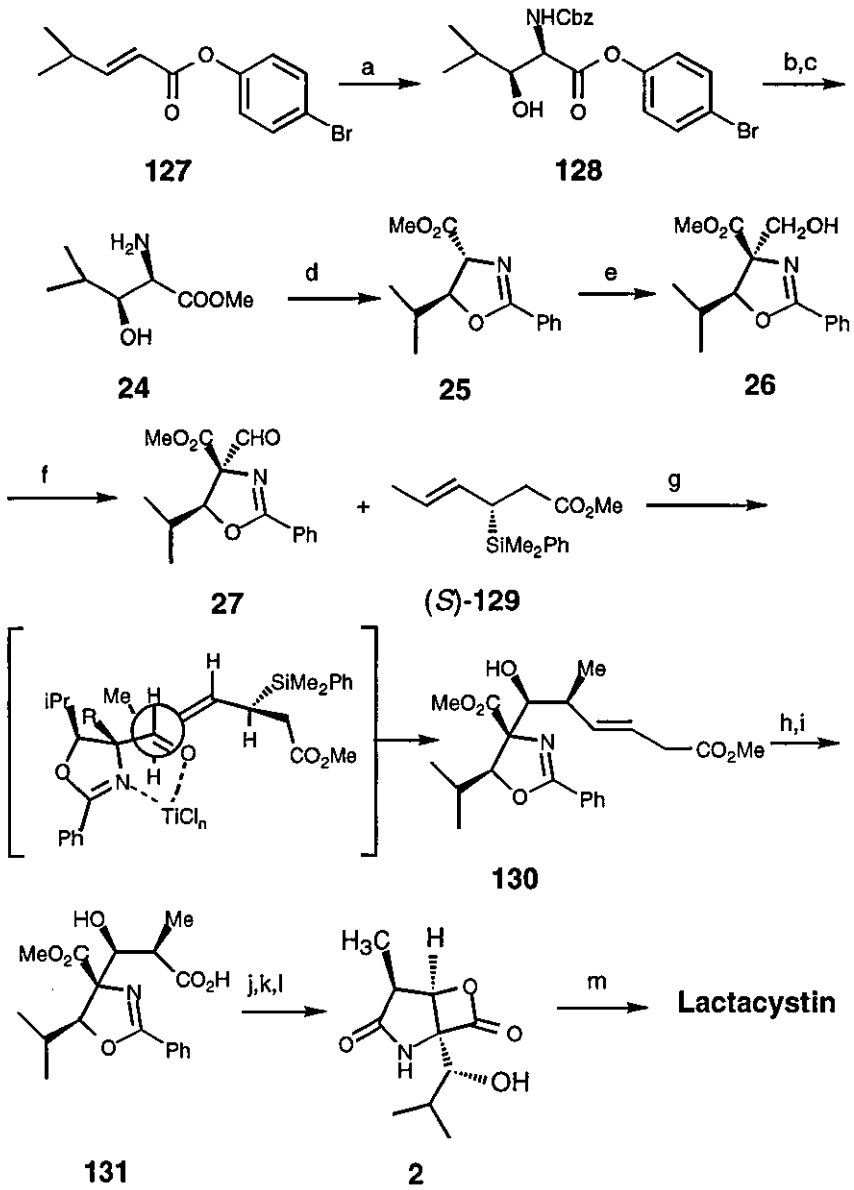
Conditions: (a) THF, -78 °C, 0.5 h, 96% (b) Dess-Martin Periodinane, CH_2Cl_2, 23 °C, 0.5 h, 100% (c) H_2, Pd-C (10%), EtOH, 23 °C, 24 h, 87% (d) $NaBH_4$, MeOH-THF, -10 °C to 0 °C, 2.5 h, 91% (e) 5% HF-CH_3CN, 23 °C, 12 h, 95% (f) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, -78 °C, 2.5 h (g) $NaClO_2$, NaH_2PO_4, ^tBuOH/2-methyl-2-butene, 23 °C, 0.5 h (h) $HS(CH_2)_3SH$, HCl (i), CF_3CH_2OH, 60 °C, 15 h, 64% for steps f to h (j) BOPCl, Et_3N, CH_2Cl_2, 23 °C, 0.5 h, 90%

Recently, Panek[55] also reported a total synthesis of lactacystin, based on the approach of Omura and Smith.[15] The crucial step is the *anti* crotylation reaction of aldehyde **25** using a chiral silane (**Scheme 18**). In the original work by Omura and Smith, the *anti* crotylation reaction was carried out by treating **27** with a chiral *E*-crotolboron reagent, but this method gave a low selectivity of the desired *anti* product (4:1). Because of the importance of this step, in which the methyl and the hydroxy groups at C-6 and C-7 have to be *anti* to one another, Panek's found that by using a chiral silane reagent, better selectivity can be achieved (30:1).

Panek started the synthesis of lactacystin with asymmetric aminohydroxylation of (p-bromophenyl)-4-methyl-2-pentenoate **127** using the benzylcarbamate-based Sharpless reaction.[56] This gave **128** with good selectivity (7:1) in favour of the α-amino ester ester with 87% ee. The enantiopurity could be increased to 99% when the crude product was recrystallised twice from ethanol/water (1:1).

Subsequent transesterification of **128** to give the methyl ester using methanol in the presence of $Ti(O^iPr)_4$,[57] followed by removal of the benzyloxycarbonyl group by hydrogenolysis, afforded **24**. Treament of **24** with trimethylorthobenzoate in the presence of p-toluenesulfonic acid gave the *trans*-oxazoline **25**.[58] Aldehyde **27** was then obtained according to the method of Omura and Smith,[15] namely by the aldol condensation of **25** using Seebach's method to give **26** as a single diastereoisomer, followed by Moffat oxidation.

The aldehyde was then subjected to the crucial *anti* crotylation using the chiral silane reagent[59] **129** in the presence of $TiCl_4$, to give the allylic alcohol **130**. This doubly stereodifferentiating reaction, which gave **130** with high level of diastereoselectivity (*anti*:*syn* >30:1), involves simultaneous coordination of the aldehyde oxygen atom and the nitrogen atom of the oxazoline ring with the Lewis acid. The Lewis acid-promoted reactions of allylsilanes are thought to proceed through an open transition state, to generate a five-membered chelate with $TiCl_4$ through a *syn*clinical transition state approach (**Scheme 16**).[60]



Scheme 16

Conditions: (a) $K_2[OsO_2(OH)_4]$ (5 mol%), $(DHQ)_2AQN$ (5 mol%), CBzNNaCl, nPrOH, H_2O, r.t. 4 h, 60% (b) $Ti(O^iPr)_4$, MeOH, r.t. (c) H_2, 10% Pd/C, MeOH, 100% (2 steps) (d) $PhC(OMe)_3$, p-TsOH, DME, reflux 4 h, 85% (e) LHMDS, HCHO, THF, -78 °C, 85% (f) Moffat [O], (g) $TiCl_4$, -78 °C to -35 °C, 60%, (h) O_3, Me_3S (i) $NaClO_2$, NaH_2PO_4, 90% (2 steps) (j) Pd, HCO_2NH_4 (k) 0.1N NaOH, (l) BOPCl, TEA, 80% (3 steps) (m) *N*-acetylcysteine, TEA, CH_2Cl_2, r.t. 70%.

Following the crotylation reaction, **130** was subjected to ozonolysis, and subsequent oxidation with sodium chlorite[61] afforded the carboxylic acid **131**. Conversion of **131** into lactacystin was carried out using the method of Omura and Smith[15] and also that of Corey[10]. Thus, catalytic transfer hydrogenolysis of the oxazoline moiety using Pd-black gave the γ-lactam methyl ester after *in situ* cyclisation. Saponification of the methyl ester using dilute sodium hydroxide, afforded the dihydroxy acid which was then converted directly to the β-lactone **2** using bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOPCl). Final conversion to lactacystin **1** was achieved by treating the lactone with *N*-acetylcysteine in the presence of triethylamine. This synthetic route comprises 13 steps and gives an overall yield of 13.1 %.

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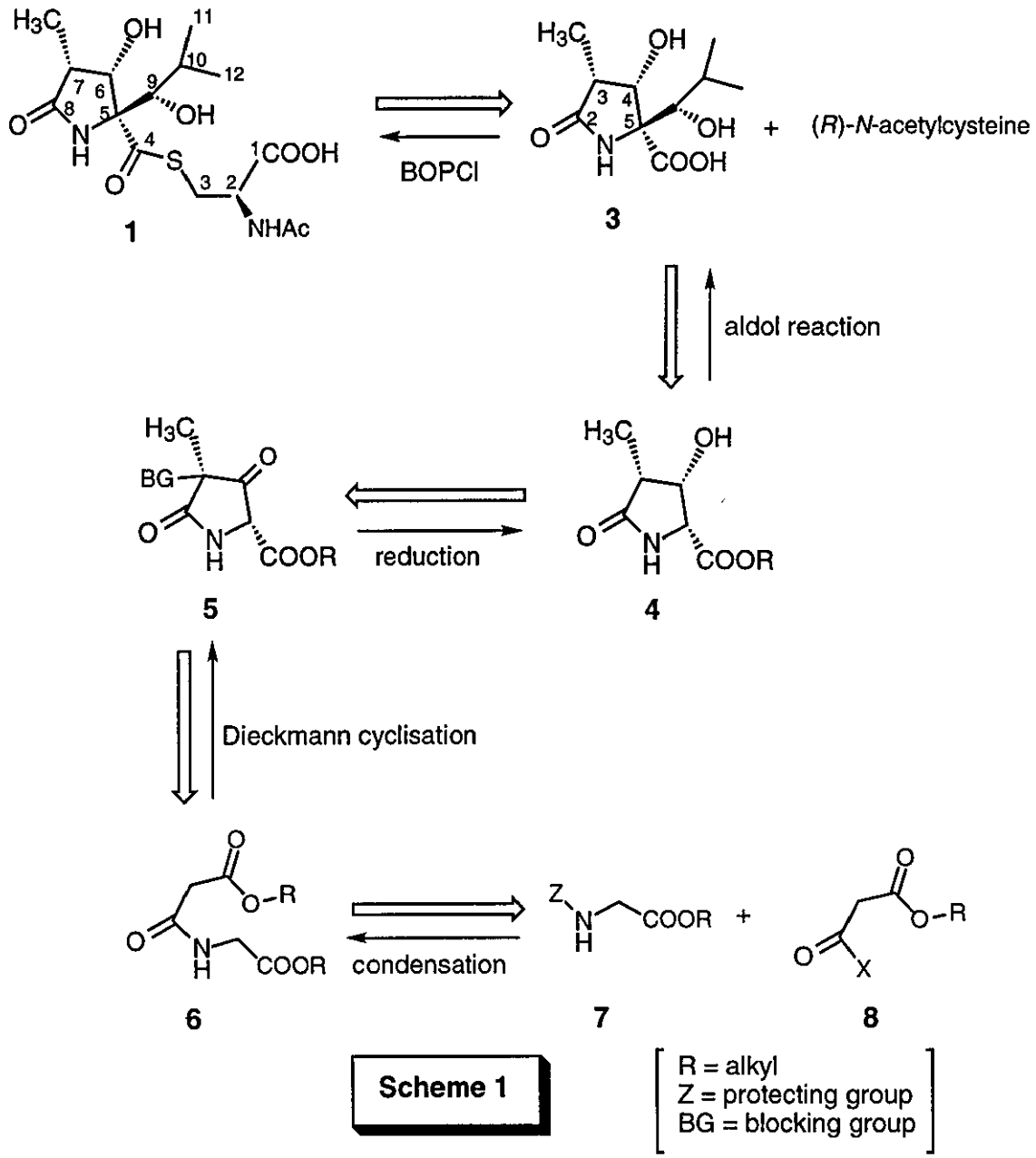
CHAPTER TWO

2.0 RETROSYNTHETIC OUTLINES TOWARDS THE TOTAL SYNTHESIS OF LACTACYSTIN AND ITS DERIVATIVES

2.1 TOWARDS THE TOTAL SYNTHESIS OF LACTACYSTIN

Retrosynthetic analysis of lactacystin

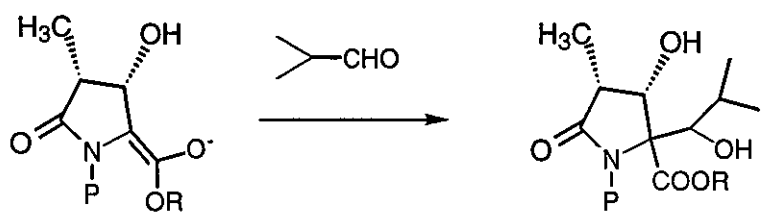


Scheme 1

The numbering system used for the precursor of lactacystin **3** has been adopted for all the subunits used in the following discussion, and all compounds containing asymmetric centres are racemic unless otherwise stated. Our retrosynthetic analysis of lactacystin **1** is outlined in **Scheme 1.**

Disconnection of the thiol ester linkage leads to the two main components of the molecule, the important α-substituted pyroglutamic acid **3** and (*R*)-*N*-acetylcysteine. Since Corey has developed a method of coupling between this pyroglutamic acid and (*R*)-*N*-acetylcysteine using BOPCl to give lactacystin,[1] and this methodology has been adopted by other groups, our main objective is to synthesise the highly functionalised α-substituted pyroglutamic acid **3.**

The acid **3** can in principle be derived by disconnection of the isobutyl group to give the 2-oxo-4-hydroxypyrrolidinone **4.** This aldol reaction would be the key step of our synthetic approach towards the total synthesis of the pyroglutamic acid **3.** The reaction would be based around the hydroxyalkylation of a suitable 4-hydroxy pyroglutamic acid derivative, protected as necessary (**Scheme 2**). It is perhaps remarkably that none of the synthetic approaches previously described have addressed this concept. This strategy should permit the development of a highly flexible, but short, synthesis which would allow the rapid preparation of a range of derivatives and stereoisomers of lactacystin.



P = protecting group

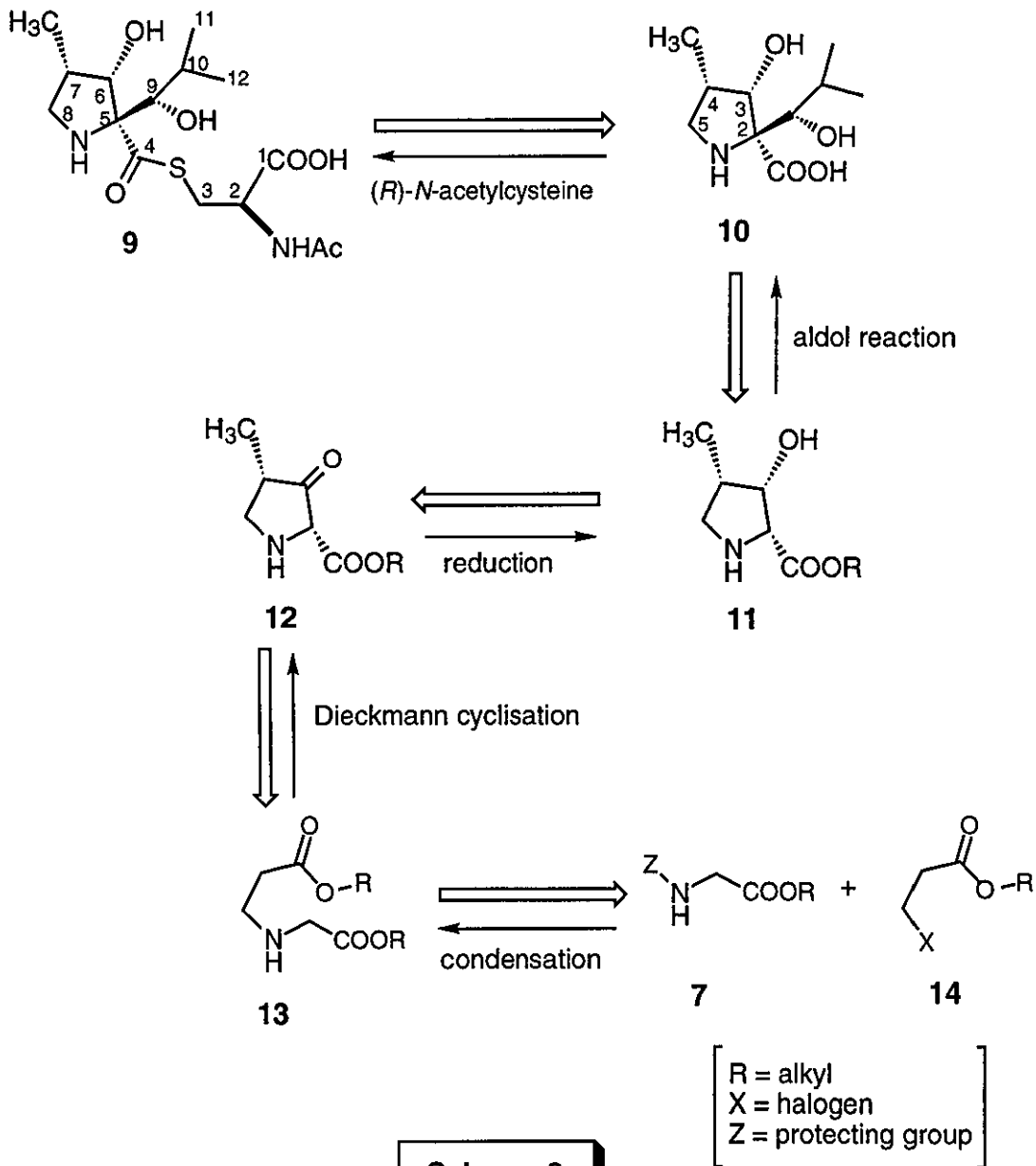
Scheme 2

The hydroxypyroglutamic acid **4** can be derived from the reduction of the 2,4-dioxo pyrrolidinone **5.** This strategy however, required some important considerations, particularly due to the acidity of the proton at C-3. The carbon at this position has to be blocked by a suitable group that can be removed at a later stage of the reaction. While serving as a blocking group, it might also function as a control to dictate the stereochemistry of later reactions in the synthesis of lactacystin.

Disconnection of **5** can be envisaged through a Dieckmann cyclisation reaction of a suitable diester **6**. The diester **6** can be further disconnected to glycine ethyl ester **7** and a suitable malonate ester such as methyl malonyl chloride, diethyl malonate or methyl diethylmalonate **8**.

2.2 TOWARDS THE TOTAL SYNTHESIS OF A DERIVATIVE OF LACTACYSTIN

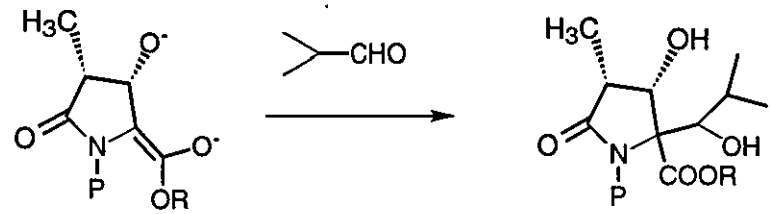
Retrosynthetic analysis of 8-deoxolactacystin



9 **10** **11** **12** **13** **7** **14**

Scheme 3

The numbering system used for the 8-deoxolactacystin derivative **10** has been adopted for all the subunits used in the discussion. The retrosynthetic analysis of **9** is outlined in **Scheme 3**. Disconnection of the thiol ester linkage again gives the (*R*)-N-acetylcysteine and the 5-deoxopyroglutamic acid derivative **10**. The acid derivative **10** could be derived from the aldol reaction of **11**, for example using dianion chemistry (**Scheme 4**).



P = protecting group

Scheme 4

Disconnection of **11** can be envisaged through the reduction of a β-keto ester **12** to give the right *cis* configuration. One well known reagent that can be used to perform this stereoselective reduction also in an enantioselective fashion is Baker's yeast.[2] The β-keto ester **12** can be derived from the Dieckmann cyclisation of an appropriate diester **13**. Disconnection of the diester gives our two main precursors, glycine ethyl ester **7** and a suitable ester **14**, e.g. ethyl 3-bromopropionate.

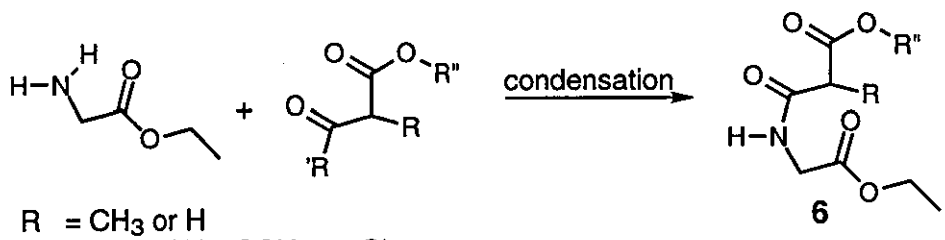
In summary, our synthetic strategy of both lactacystin and its derivatives involves three important tactical points:

i. **Synthesis of 4-oxoprolines and pyroglutamates**
ii. **Control of cyclisation regiochemistry in pyroglutamate formation by adjustment of relative acidities through the correct choice of the *N* - protecting group**
iii. **Investigation of stereocontrol in the introduction of the substituent groups using enolate alkylation and aldol reactions**

2.2.1 Synthesis of 4-oxoprolines and pyroglutamates

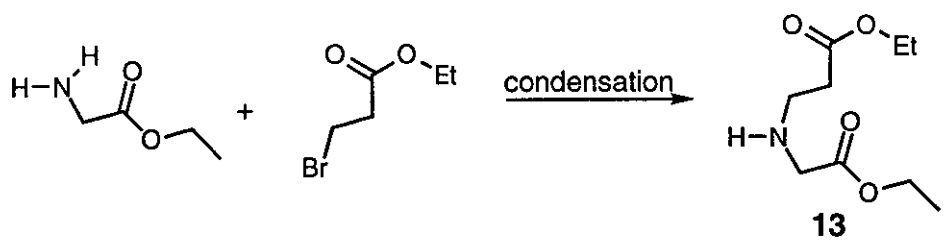
As the total synthesis of both lactacystin and its derivatives requires the diesters **6** and **13**, our first step was to synthesise both compounds in large quantity. The starting material used for both compounds is the readily available and cheap

glycine ethyl ester hydrochloride. The diester would be synthesised through a condensation reaction between glycine ethyl ester with an appropriate malonate ester such as ethyl hydrogen malonate, methyl diethyl malonate, or methyl or ethyl malonyl chloride to give **6** (for lactacystin, **Scheme 5**) or coupling with ethyl 3-bromopropionate to give **13** (for 8-deoxolactacystin, **Scheme 6**).



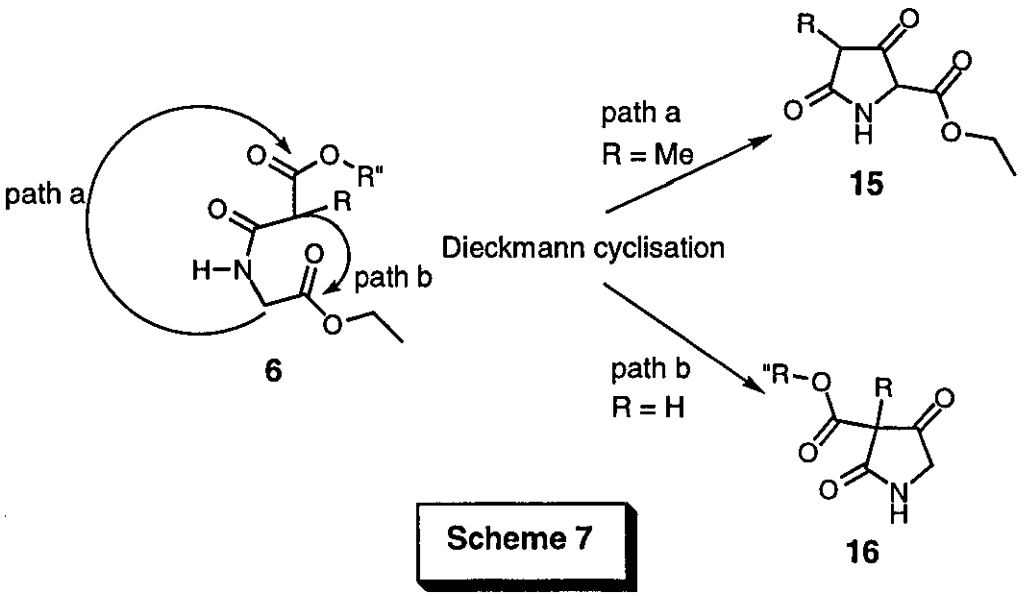
R = CH_3 or H
R' = OCH_2CH_3, OCH_3 or Cl
R" = CH_2CH_3, CH_3

Scheme 5



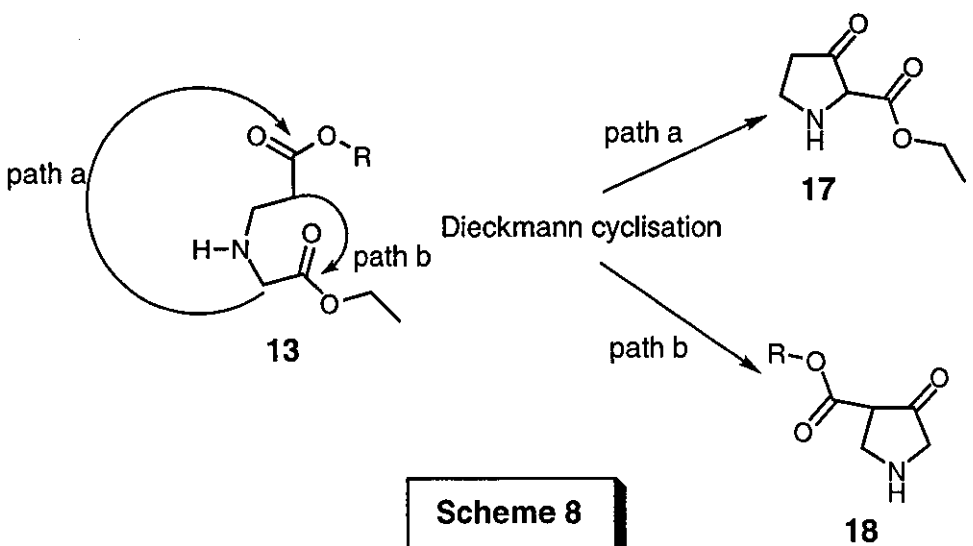
Scheme 6

Once the diesters are made, the next step would be the Dieckmann cyclisation reaction to give the required 4-oxopyroglutamates. For the preparation of lactacystin, the cyclisation of the diester would be expected to occur with the major pathway (path a, R = Me, **Scheme 7**) producing the desired regiochemistry on the basis of a closely related literature precedent.[3] On the other hand, if the Dieckmann cyclisation did not give the compound with the correct regiochemistry **15**, but instead gave the alternative product **16** (path b), the compound could still be used for further reactions to take us to the target compound. It is expected that when R = H, then the Dieckmann cyclisation of **6** should follow pathway b and not pathway a since the product from pathway b is more stable due to formation of the enolate as the final step driving the equilibrium process.



Scheme 7

As for the preparation of 8-deoxolactacystin, Dieckmann cyclisation of **13** would be expected to give two products **17** and **18** by two different pathways. Only one of these products, **17**, would be useful for the total synthesis of the lactacystin derivative (**Scheme 8**)

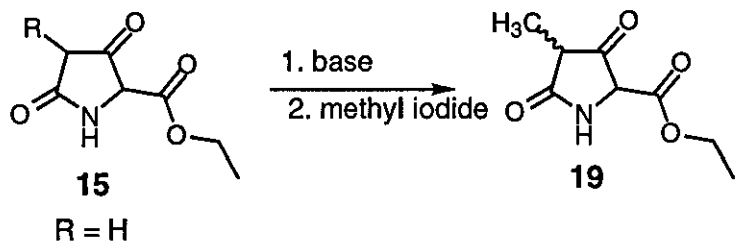


Scheme 8

2.2.2 Techniques for alkylation and aldol reaction

Having made the pyroglutamates **15** and **16**, the next step in the synthesis of lactacystin would be the methylation (if R is not methyl) (**Scheme 9**). Methylation of **15** should be straightforward due to the acidity of the C-3 proton, which makes it readily removable by a base, by treating the enolate formed with a suitable

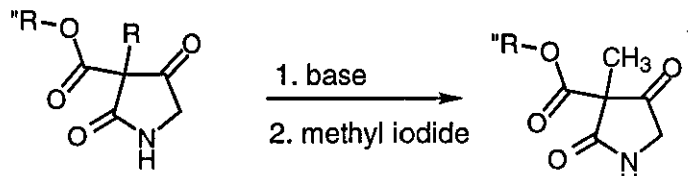
methylating agent. One major problem would be to minimise the formation of a dimethylated product. Hopefully, use of only one equivalent of the electrophile would give mainly the desired methylated product **19**. Similarly, methylation of **16** should also be straightforward to give the expected product **20**.



15

R = H

19



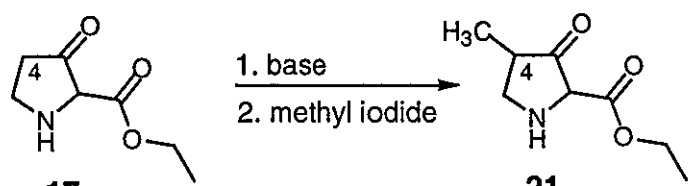
16

R = H

20

Scheme 9

As for the synthesis of 8-deoxolactacystin, the 3-oxoproline **17** would be methylated regioselectively at position C-4 using dianion chemistry. This method for example would involve the treatment of the *N*-protected compound with sodium hydride followed by butyllithium or by using two equivalents of lithium diisopropylamide followed by methyl iodide to give the desired compound **21** (**Scheme 10**).



17

21

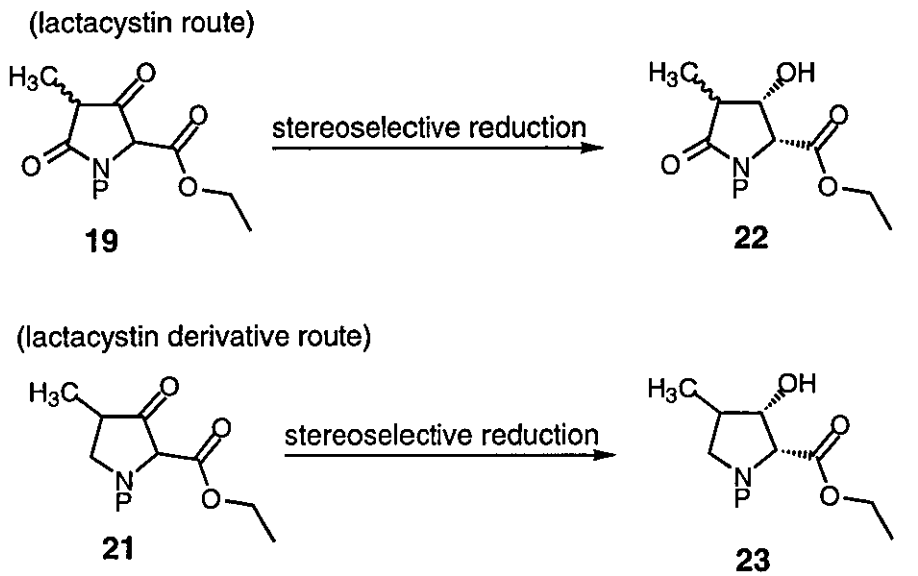
Scheme 10

With the methylated product in hand, the next step would be the important stereoselective reduction of **19** (for lactacystin) and **21** (for lactacystin derivative). As for the other methylated pyroglutamate **20**, a different approach would have to be employed, such as the introduction of an appropriate 5-carboxyl group using

anion chemistry, in order to construct the the important quaternary C-5 at a later stage.

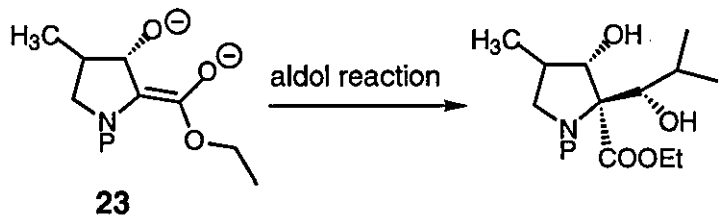
2.2.3 Stereoselective reduction and aldol reactions of 19 and 21

The desired absolute stereocontrol in the synthesis of compounds **22** and **23** could potentially be obtained by yeast reduction of both β-keto esters **19** and **21** to give the desired (*S*)-stereochemistry at the newly created chiral centres (**Scheme 11**).



Scheme 11

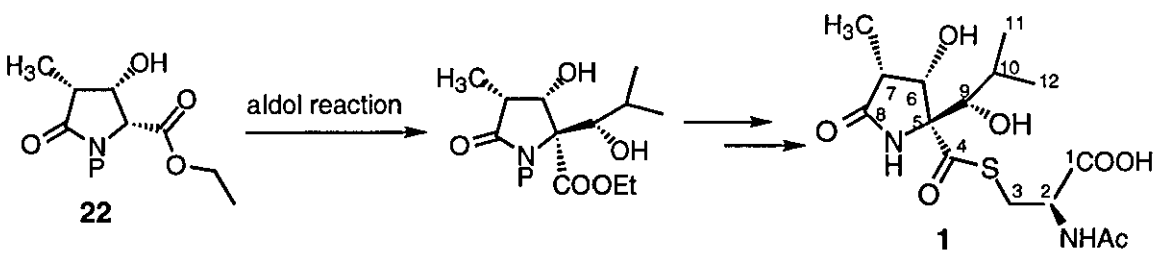
Aldol reaction of the two systems **22** and **23** is also an interesting problem since this is an aldol reaction of an unusually substitued enolate, producing two new asymmetric centres. It is important that dianion chemistry should probably be employed in order to avoid elimination of water from the molecule (**Scheme 12**). Techniques for controlling the stereochemistry of the aldol reaction are of course well established in related heterocycles[4] and indeed Corey has investigated aldol reactions in related systems using lithium counter ions.[1] We believe that it will be important to employ not only the usual group I metals (Li, Mg) but also boron enolates, known to provide good stereoselectivity *via* tighter Zimmerman-Traxler transition states, and zinc and titanium enolates, which are known to provide *anti* and *syn* selectivity regardless of the enolate geometry.



23

Scheme 12

The use of a silyl enol ether in place of a metal enolate, catalysed by Lewis acid would also be examined in the aldol reaction. Introduction of the lactacystin side chain will be the final phase of the work to complete the formal synthesis (**Scheme 13**). Conversion of the acid residue into the natural product thiol ester has already been reported.[1]



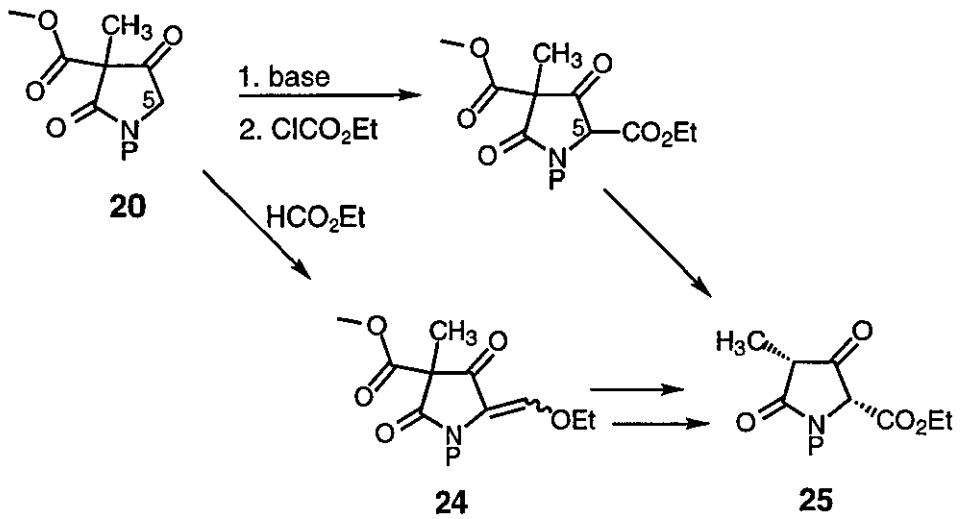
22 **1**

lactacystin

Scheme 13

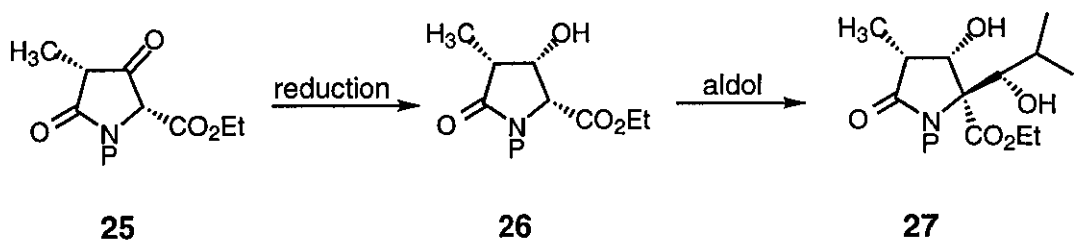
2.2.3.1 Introduction of carboxyl function, reduction and aldol reactions of 20

The methylated product **20**, derived from **16** (See **Scheme 9**) can also be used in our approach towards the total synthesis of lactacystin. The introduction of a suitable carboxyl group at the important C-5 centre would be carried out by direct *C*-acylation, for example using ethyl chloroformate, or acid anhydride, or through initial condensation with ethyl formate (**Scheme 14**). Subsequent decarboxylation of the methyl ester and functional group interconversion of compound **24** would produce the desired 4-oxo-3-methylpyroglutamate **25**.



Scheme 14

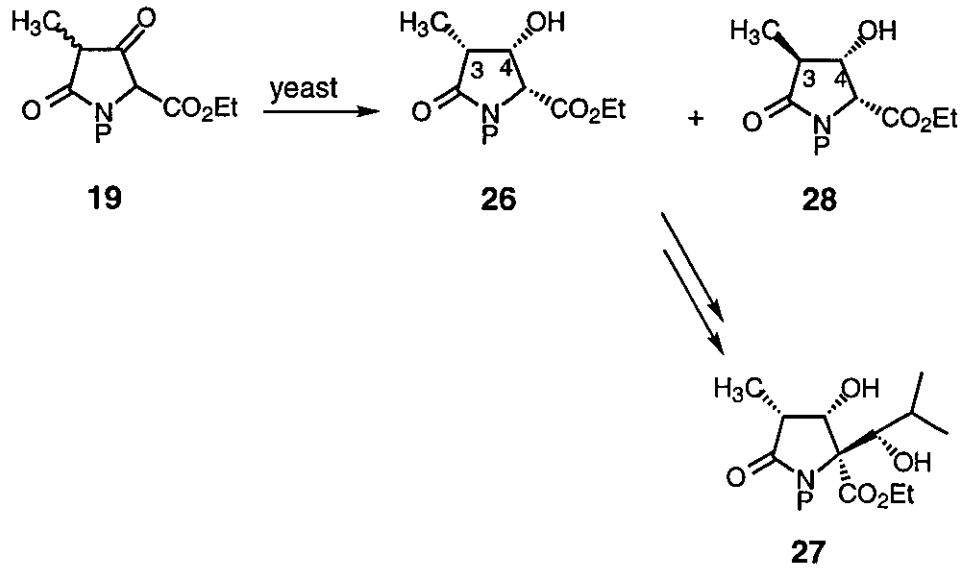
Compound **25** is expected to be the more stable *cis* isomer, but any *trans* compound formed could be equilibrated to give the desired stereochemistry. Chemoselective reduction of the ketone **25** with standard reagents would then give rise to the 4,5-*cis* compound **26** in racemic form, together with the 4,5-*trans* stereoisomer. Aldol reaction of **26** would provide the advanced intermediate **27** and thus provide a very short synthesis of racemic lactacystin (**Scheme 15**).



Scheme 15

The absolute stereochemistry of the ketone reduction of **19** following cyclisation might be controlled using yeast or modified hydride reagents to give the desired (*S*)- stereochemistry at the resulting C-4 hydroxy esters in **26** and **28.** If feasible, this (*S*)-stereocentre will dictate the methyl group stereochemistry using our knowledge of preferred conformations, or perhaps by equilibrating **27** to give the *cis* material **25** only. Again, the aldol reaction of the planar ester enolate will follow to provide advanced intermediate **27** in enantiomerically pure form in a very short

route (**Scheme 16**). Alternatively, if the methyl group stereochemistry cannot be sufficiently well controlled, the *cis* material **26** will be taken on to the natural product as above, while the *trans* material **28** will be used to provide 3-*epi* materials for evaluation or may be recycled by reoxidation to the ketone and equilibrated under the acidic conditions.



Scheme 16

In summary, our synthetic strategy towards the total synthesis of lactacystin and its derivatives involves the Dieckmann cyclisation of an appropriate diester followed by methylation, stereoselective reduction, and finally the important aldol reaction. Once the important intermediate pyroglutamic acid is made, final transformation to lactacystin and its derivatives can be achieved by coupling with (*R*)-*N*-acetylcysteine using BOPCl, the method developed by Corey.[1]

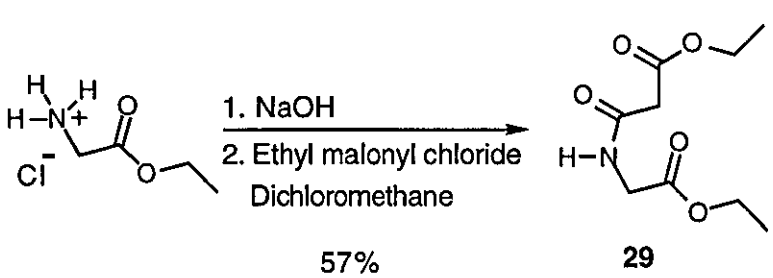
2.3 RESULTS AND DISCUSSION

Initially we aimed to prepare the carbon skeleton of the pyroglutamic acid derivative and then use synthetic methodologies to introduce further functionalization for the total synthesis of lactacystin and its derivatives (See **Schemes 1** and **2**). This chapter is divided into two main parts, the first concerning our chemical studies towards the total synthesis of lactacystin and the second our studies towards the synthesis of 8-deoxolactacystin.

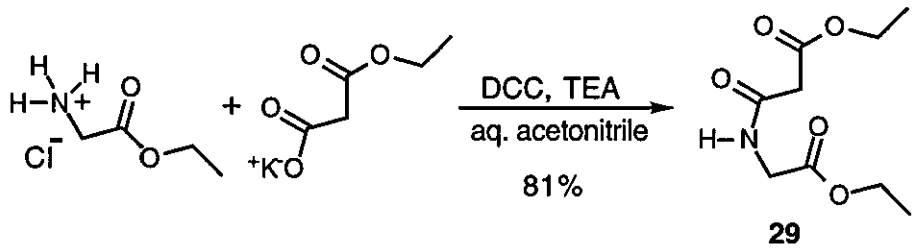
Towards the total synthesis of lactacystin.

2.3.1 FORMATION OF DIESTER, A PRECURSOR FOR DIECKMANN CYCLISATION REACTION.

In the first step of the synthesis, we initially required the diester, diethyl 3-aza 4-oxo 1,6-dicarboxylate **29**, in large quantity before we could proceed with the Dieckmann cyclisation reaction. Our first attempt was to carry out the procedure previously reported by Rapoport,[5] which involves treatment of the glycine ethyl ester salt with sodium hydroxide followed by the addition of ethyl malonyl chloride. This gave the desired diester but in a moderate yield (**Scheme 17**). We managed to improve the yield of the diester **29** (up to 81 %) by treating glycine ethyl ester with potassium ethyl malonate in the presence of dicyclohexylcarbodiimide (DCC) in aqueous acetonitrile using the method of Heniecke[6] (**Scheme 18**). Furthermore, using this procedure the reaction can easily be controlled since there is no violent release of hydrogen chloride as in the first method.



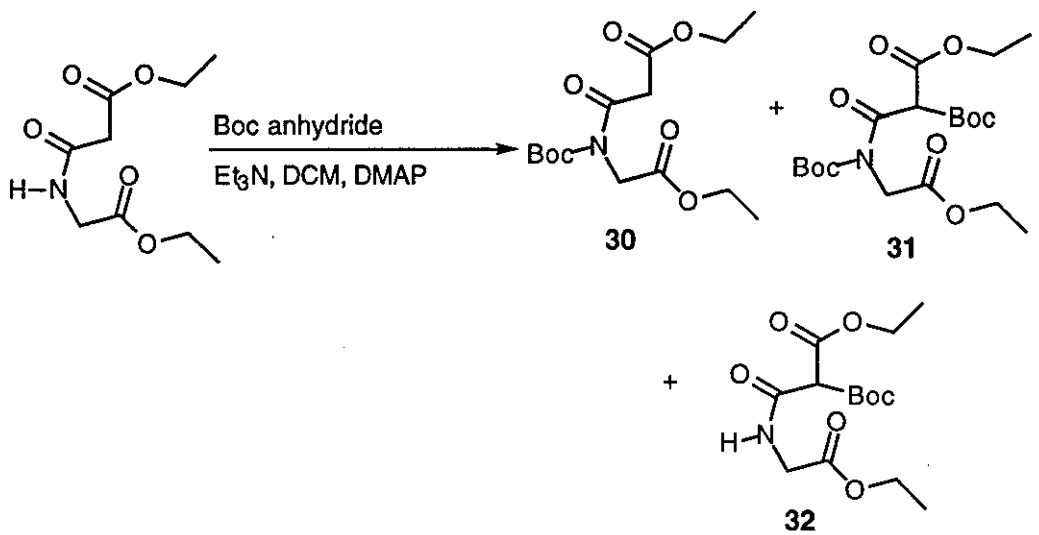
Scheme 17



29

Scheme 18

Having made **29** in large quantities, we decided to protect the nitrogen before proceeding with the Dieckmann cyclisation in order to prevent any possible side reactions. Although they are many protecting groups available for the protection of amides[7], we decided to make use of the Boc group which can easily be introduced and later removed using trifluoroacetic acid. Hence, treatment of **29** with Boc anhydride/triethylamine in dichloromethane as the solvent gave us the desired *N*-protected diester **30** together with two side products, **31** and **32** (**Scheme 19**).



30 31 32

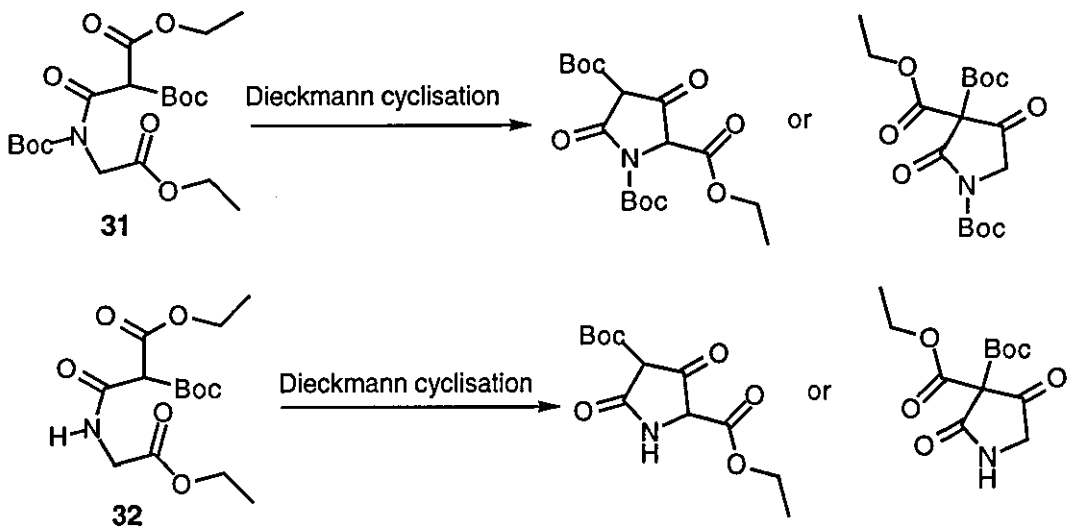
Scheme 19

Evidently, the Boc anhydride had reacted with the methylene group to give the side product **32.** Apparently, the protection of the nitrogen atom does not proceed smoothly due to the acidity of the methylene protons.

The other side product **31**, in which the Boc group was incorporated at both nitrogen and carbon ,was also formed. These results suggest that in order to

optimise the yield of **30**, we need to use a different approach. Furthermore, the three products were formed in roughly equal amounts.

In addition, since one of these acidic protons will have to be replaced by a methyl group at a later stage, this might lead to difficulty as the group might interfere in the methylation step by steric hindrance. Nevertheless, both side products **31** and **32** can be used in the Dieckmann cyclisation, as the Boc group alternatively can serve both as a protecting group and as a group for controlling the stereochemistry of the reactions (**Scheme 20**).



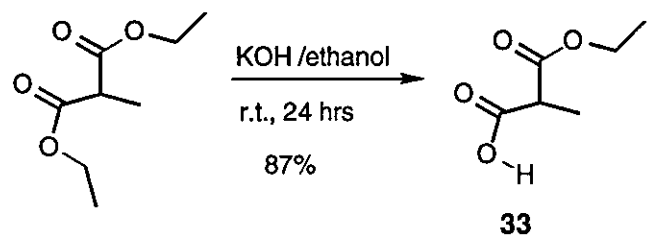
Scheme 20

Because of the problems associated with the regiospecific deprotonation of the Dieckmann cyclisation we decided to investigate different conditions. The use of triethylamine might not be suitable for the reaction above as it might easily remove the acidic methylene protons of **29** and this in turn may attack Boc anhydride. Ragnarsson[8] reported that amides can efficiently be protected with Boc anhydride when the reaction is carried out in acetonitrile in the presence of catalytic DMAP. The same authors also found that the Boc/DMAP adduct is five times more readily available in acetonitrile than in dichloromethane.

When we tried the above conditions with our substrate **29**, again both side products were formed, suggesting that the compound is very acidic, and that the formation of *C*-Boc products is unavoidable. Because of these problems, the original plan to cyclise the *N*-Boc protected compound **29** was abandoned. In

order to prevent the formation of the side products during the protection of the diester, we decided that introduction of a methyl group at the methylene carbon may supress the reaction with Boc anhydride.

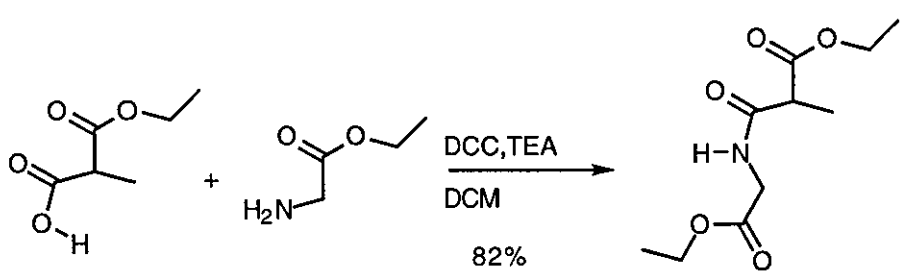
We also thought that introducing the methyl group atom at an earlier stage would prevent the formation of the *C*-Boc product due to steric hindrance. In this strategy, ethyl methylmalonate **33** was first prepared by mono hydrolysis of the methylmalonate diethylester with potassium hydroxide in aqueous ethanol (**Scheme 21**).



33

Scheme 21

The mono acid ethyl methylmalonate **33** was treated with glycine ethyl ester in the presence of dicyclohexylcarbodiimide to give *N*-methylethoxycarbonyl-acetyl glycine ethyl ester **34** in 82% yield (**Scheme 22**).

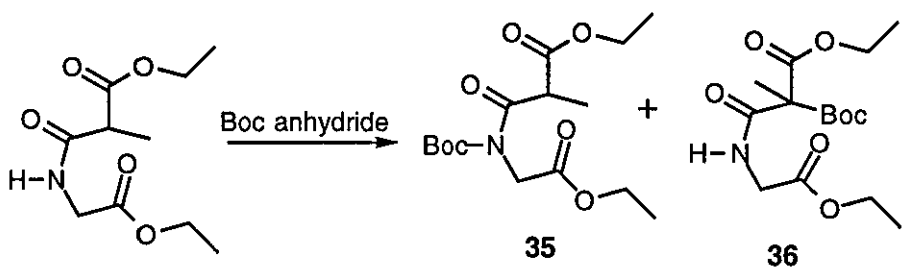


33 **34**

Scheme 22

With **34** in hand, Dieckmann cyclisation could be carried out with or without the nitrogen protection. Because of the strong tendency for **34** to undergo self-condensation, we decided to protect it first with Boc anhydride. Treatment of **34** with Boc anhydride/triethylamine and catalytic amount of DMAP in dichloromethane gave us the desired product **35** together with the side product **36** in a 3:1 ratio.

These findings suggested that although the methyl group is affecting reactivity at the methylene carbon, the remaining proton being next to two carbonyl groups is still very acidic and thus **35** is easily deprotonated. The anion in tum may attack the incoming electrophile, Boc anhydride. Changing the solvent system to acetonitrile in the presence of catalytic amount of DMAP, dramatically increased the yield of **35** from 49% to 71% (**Scheme 23, Table 1**). This observations might due to the Boc/DMAP adduct being readily formed in the more polar solvent i.e. acetonitrile.

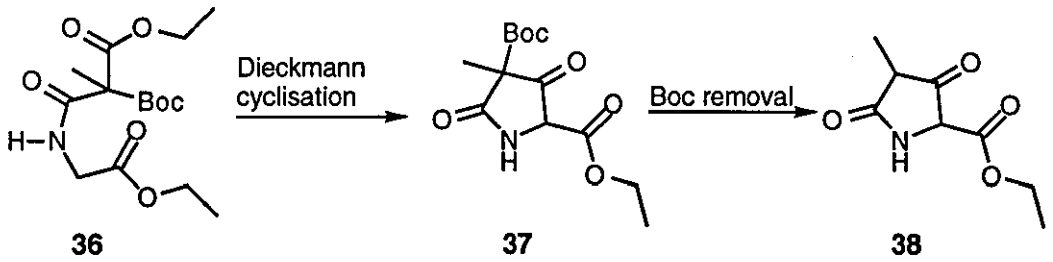


Scheme 23

Table 1

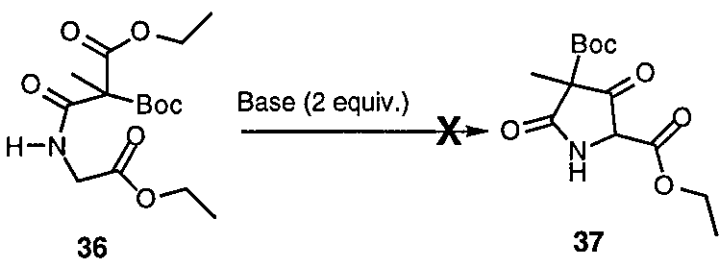
conditions	yield of **35**	yield of **36**
TEA, DMAP, DCM, r.t.	30	18
DMAP, acetonitrile, r.t.	60	11

With multigram quantities of compounds **35** and **36** available, we then proceeded with the Diekmann cyclisation of both these materials. It is worth noting that with compound **36**, only one mode of cyclisation can occur, and this will give correct skeleton for our main target. Removal of the Boc protecting group of **37** will furnish the important pyroglutamate precursor **38** in just three steps (**Scheme 24**).



Scheme 24

The Dieckmann cyclisation of **36** using different bases, however, did not give us the desired product **37**. In each reaction, two equivalents of base were used, because of the free N-H but no clean product was detected, only the formation of complex mixtures (**Scheme 25, Table 2**). It is perhaps strange that even the non-nucleophilic base sodium hydride failed to give any cyclised product. We believe that the cyclisation may be unsuccessful due to a self condensation reaction between the molecules. In order to prevent the side reaction, we decided to protect both the nitrogen and the carbon bearing the acidic proton. With both functionalities protected, the cyclisation of the diester could, we hoped, proceed without substantial difficulty.

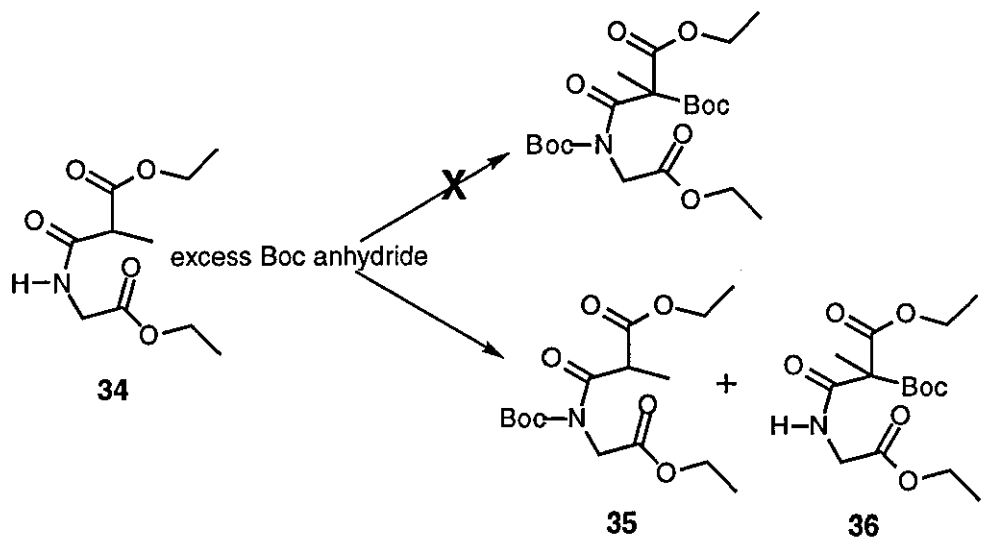


Scheme 25

Table 2

conditions	product **37**
NaH, toluene, 0 °C	complex mixture
NaH, THF, 0 °C	complex mixture
^{t}BuOK, toluene, 0 °C	complex mixture
LDA, -78 °C to 0 °C, THF	complex mixture

Thus, compound **34** was treated with excess Boc anhydride in order to protect both the nitrogen and carbon bearing the methyl group. To our surprise, we did not observed the formation of the desired product but instead, only products **35** and **36** were formed (**Scheme 26, Table 3**). Although various conditions were tried, we still failed to protect both the nitrogen and carbon. One possible explanation is that when one Boc group is incorporated on the N or C atoms, the second Boc group may not be incorporated at the other position, presumably due to the steric hindrance.



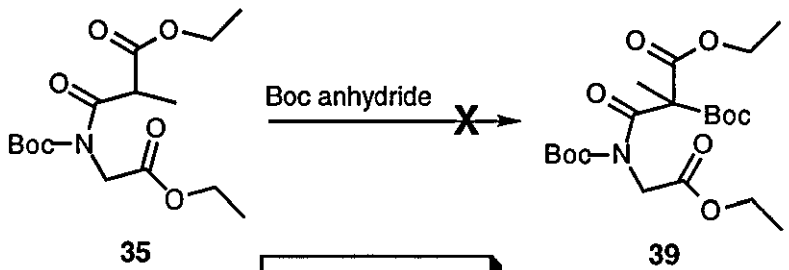
Scheme 26

Table 3

conditions	products
DMAP, acetonitrile, 0 °C	**35** and **36**
DMAP, acetonitrile, r.t.	**35** and **36**
DMAP, TEA, DCM, 0 °C	**35** and **36**
DMAP, TEA, DCM, r.t.	**35** and **36**
K_2CO_3, DMAP, acetonitrile, r.t.	no reaction
K_2CO_3, DMAP, acetonitrile, reflux	no reaction

Since we had failed to carry out the protection, we then decided to take both products **35** and **36** and treat each of them separately with Boc anhydride in an attempt to access both N and C protected compounds. Hence, compound **35** was treated with excess of Boc anhydride, but again the active carbon did not

react. Even though the reaction was left stirring for forty-eight hours at room temperature, none of the desired compound **39** was formed (**Scheme 27, Table 4**).



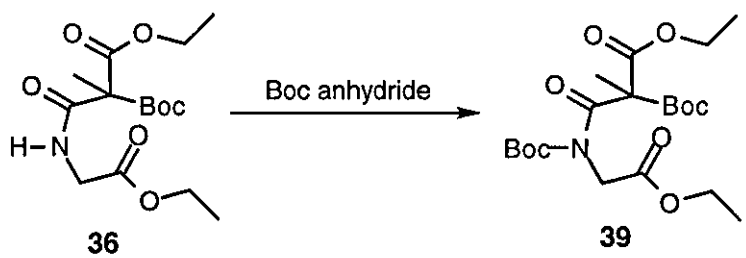
35 **39**

Scheme 27

Table 4

conditions	product **39**
DMAP, acetonitrile, r.t. , 24 h	no reaction
DMAP, acetonitrile, r.t. and then reflux for 12 h	no reaction
DMAP, TEA, DCM, r.t., 48 h	no reaction

Having failed to *C*-protect the *N*-Boc diester **35**, we then attempted to *N*-protect compound **36**. Suprisingly, when the reaction was carried out in dichloromethane with triethylamine as a base and DMAP as a catalyst, we managed to isolate the desired product, **39** (**Scheme 28, Table 5**). The only setback was the yield which was very low, and there was insufficient material to take through to the next step of the synthesis. Because of these problems, we decided to abandon the idea of protecting both nitrogen and carbon atoms before the Dieckmann cyclisation.



36 **39**

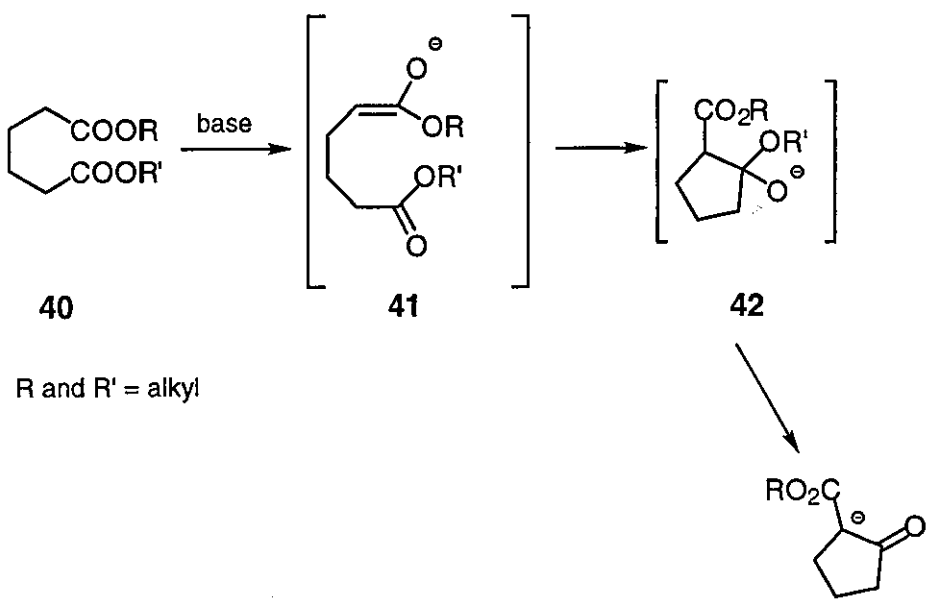
Scheme 28

Table 5

conditions	yield of **39**
DMAP, acetonitrile, r.t., 24 h	no reaction
DMAP, TEA, DCM, r.t., 10 days	4%

2.3.2 The Dieckmann Condensation Reaction

The Dieckmann condensation is an intramolecular version of an acylation reaction which involves cyclisation of an α,ω diester such as **40**. The reaction is usually carried out under thermodynamic conditions, although kinetic control conditions can also be used. The enolate initially formed, **41**, attacks the ester group at the other end of the molecule to give the ring intermediate **42** (an alkoxide). The expulsion of alkoxide leads to the formation of β-keto ester **43** (**Scheme 29**).

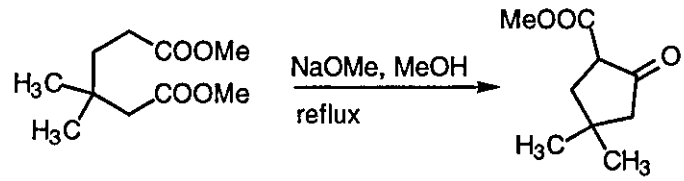


40 **41** **42** **43**

R and R' = alkyl

Scheme 29

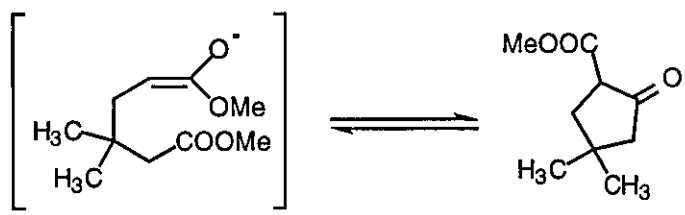
If two different enolates can be formed, steric effects usually control the enolisation and the cyclisation processs. For example the Dieckmann cyclisation of **44** gave only one single product **45** (**Scheme 30**).



44 45

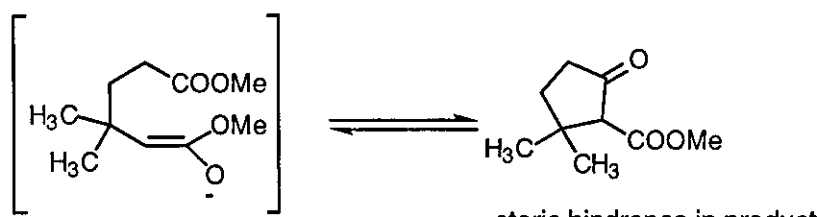
Scheme 30

This selectivity can be explained by looking at the two possible formation of the enolates **46** and **47** (**Scheme 31**). In enolate **47**, the geminal dimethyl groups are close to the enolate carbon, hence inhibiting the approach of the ester carbonyl through steric hindrance. In compound **46**, however, both dimethyl groups are removed from the enolate carbon and approach to the ester group is facile, leading to the thermodynamically more stable product, **45**.



46 **45**

more stable product

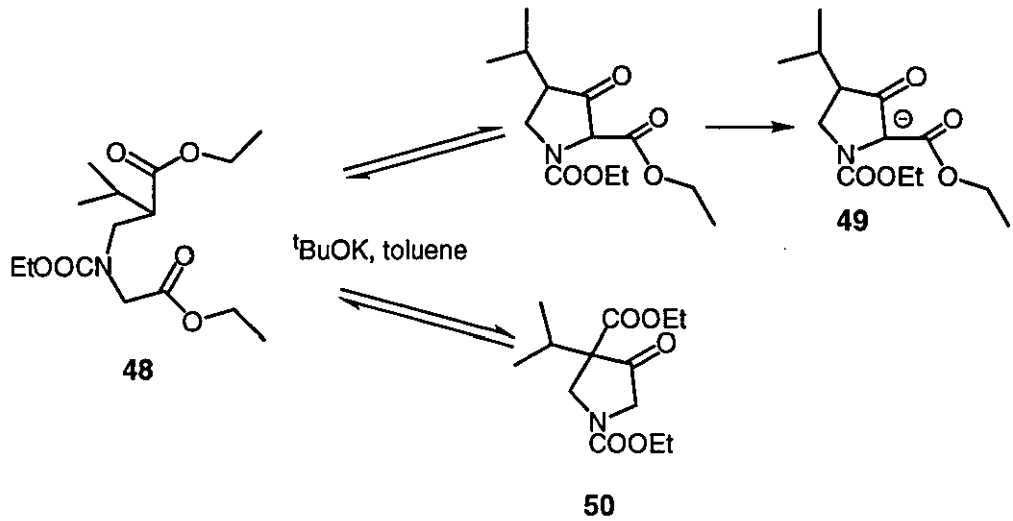


47

steric hindrance in product

Scheme 31

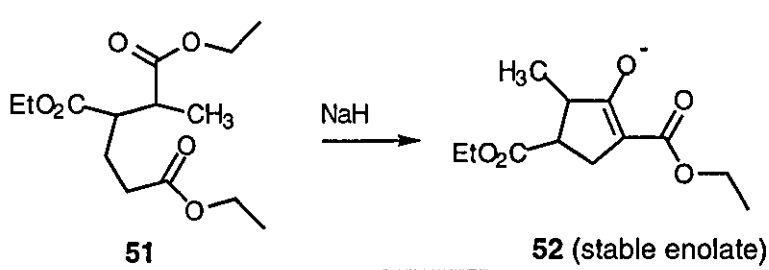
Rapoport[5] reported that if the α-methylene of the diester **48** is monosubstituted, then the Dieckmann condensation reaction would be likely to occur at the unsubstituted methylene giving the product **49** and not **50**. The main reason for the lack of condensation at the substituted methylene is attributed to the inability of the resulting β-keto ester to enolize and also partly to a rate retardation due to steric and inductive effect (**Scheme 32**).



Scheme 32

Any resulting β-keto ester from Dieckmann cyclisation that cannot form a stable enolate anion under equilibrating conditions is susceptible to ring opening and reclosure to β-keto ester which can form a stable enolate ion. Rapoport also reported that if both of the methylenes are monosubstituted, then neither β-keto ester product will be capable of forming the enolate. Then the enolate and the Dieckmann cyclisation occurs in such a way so as to produce the more stable β-keto ester enolate. If one of the methylene is disubstituted, condensation at that methylene is of course impossible at that position.

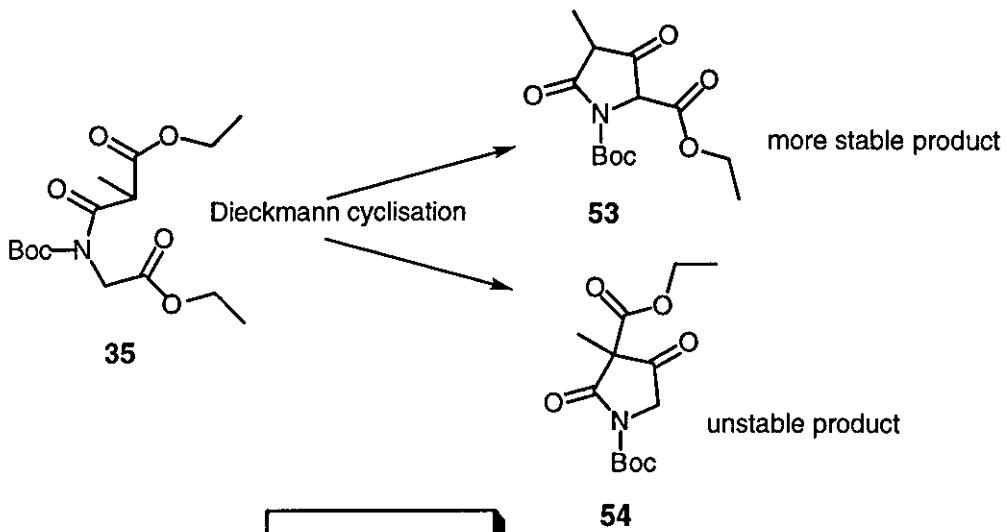
A similar system was also reported by Newman[9] in which a monosubstituted methylene diester **51** which underwent the Dieckmann cyclisation only gave the most stable five membered ring product β-keto ester enolate **52**, based on the arguments discussed above (**Scheme 33**).



Scheme 33

2.3.2.1 Dieckmann condensation of 35

Having failed to cyclised the *C*- Boc protected form of **36**, we then turned our attention to the other Dieckmann cyclisation of **35**, the major product that was formed during the protection of the diester **34** with Boc anhydride. Based on the earlier analysis, in the Dieckmann cyclisation of **35**, one would expect to observe one major pathway based on the stability of the enolate ion of the β-keto ester formed, giving the product **53**, and not **54** (**Scheme 34**).

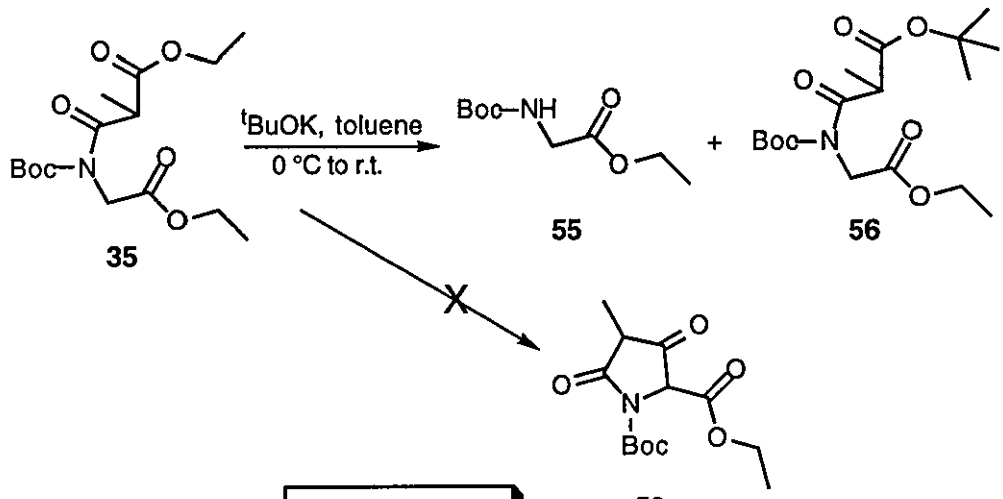


Scheme 34

In our system, the carbon bearing the methyl group has an acidic proton since it is located between the two carbonyl groups. We expected that this acidic proton will be removed first and then the resulting enolate will attack the carbonyl of the ester *via* Dieckmann condensation to form a ring. However, due to the inability of the β-keto ester product to form a stable enolate through this pathway, the product would be susceptible to ring opening and reclosure to a more stable β-keto ester product **53**, which is what we required in this work.

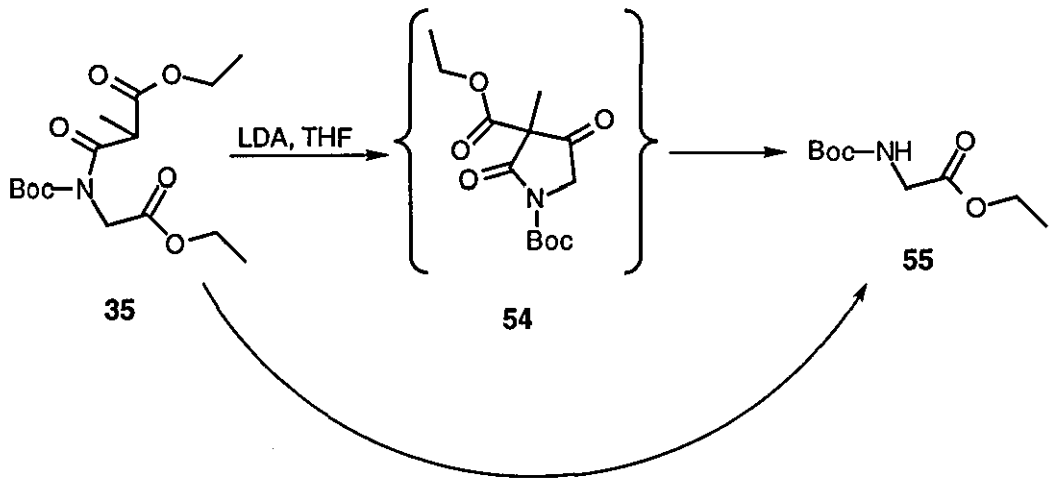
In our first attempt, **35** was treated with potassium tertiary butoxide in toluene and the reaction was carried out at -20 °C, but no reaction occured. When the temperature of the reaction condition was raised to 0 °C, we observed another product formed in the reaction mixture (tlc). After five hours at that temperature, the tlc of the reaction mixture still showed the same profile suggesting that whatever the reaction, it was very slow at 0 °C. The reaction was brought to room temperature and stirred until all the starting material disappeared (tlc). After the usual work up, we managed to isolate two major products, the Boc protected

glycine ethyl ester **55** and a transesterified product **56**, but not the desired Dieckmann cyclisation product **53** (**Scheme 35**).



Scheme 35

In an attempt to avoid transesterification products, we decided to use LDA as the base. When LDA was used, again we could not detect any formation of the cyclised product. Only the formation of the *N* -protected glycine ethyl ester **55** was observed, possibly suggesting cleavage of the intermediate **54** had occured to give **55** (**Scheme 36**). The reaction was very sluggish at both -78 °C and 0 °C, but when the reaction was warmed to room temperature the *N* -Boc glycine ethyl ester started to form, although the starting material was never completely consumed (tlc). The transesterified product, however, was not formed in this Dieckmann reaction using LDA.



Scheme 36

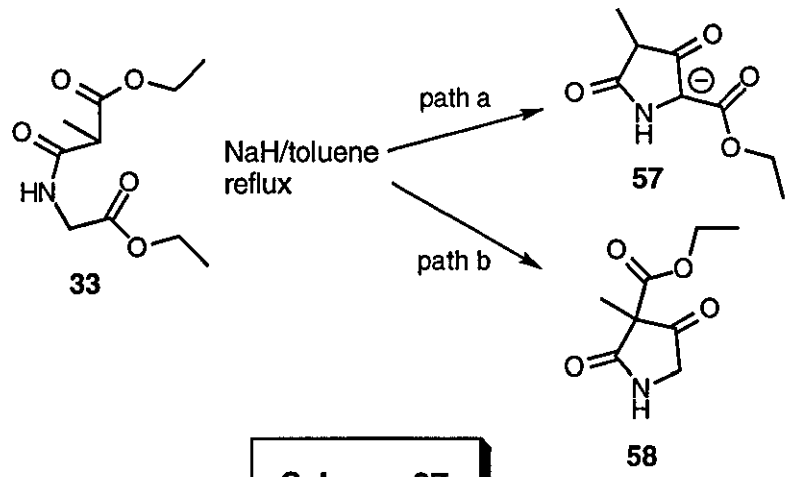
In order to confirm that the cleavage of the diester **35** had taken place, we then tried using a nucleophilic base such as sodium ethoxide in ethanol (equilibrating conditions). Alkoxide is well-known to cleave the ring of any non enolizable Dieckmann cyclisation product.[10] Hence, treament of **35** with sodium ethoxide in ethanol gave the *N*-Boc glycine ethyl ester **55** exclusively in thirty minutes (**Table 6**).

Table 6

conditions	product	reaction time
^{t}BuOK, toluene, 0 °C	**55** and **56**	18 h
LDA, THF, -78 °C	**55**	48 h
LDA, THF, 0 °C	**55**	36 h
NaOEt, EtOH, r.t.	**55**	0.5 h

2.3.2.2 Dieckmann condensation of 33

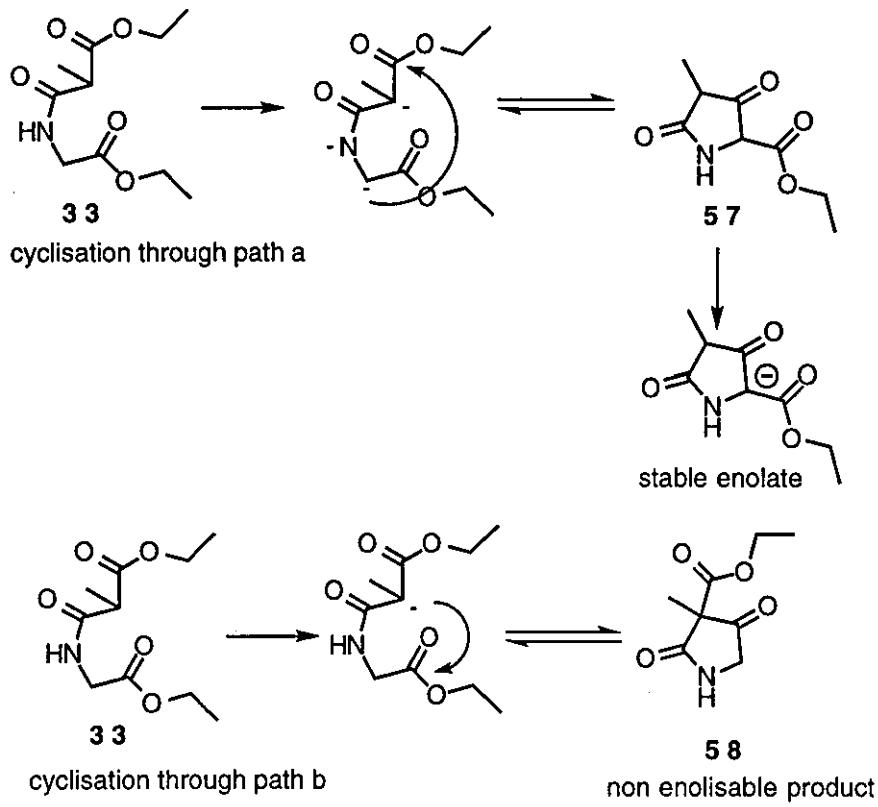
Since we had failed to cyclise the *N*-protected diester **35**, we decided to carry out a Dieckmann cyclisation of the unprotected diester **33**. Although two modes of cyclisation (pathways a and b) can occur to give two different products **57** and **58**, **57** is expected to dominate due to the stability of the resulting enolate (**Scheme 37**).



Scheme 37

Since the nitrogen in **33** is not protected, there may be a tendency for intramolecular reaction. In order to prevent this, the reaction was carried out in dilute solution. Unfortunately, the Dieckmann cyclisation of **33** did not give any of the cyclised products but only a complex mixture.

In pathway a, obviously the reaction is more favourable because the product formed is thermodynamically favoured due to its ability to enolise. But, for the cyclisation to take place, several equivalents of base might be required as several acidic protons are present. The other mode of cyclisation through pathway b, however, should be less favourable because of the inability of the product to form a stable enolate. (**Scheme 38**).



Scheme 38

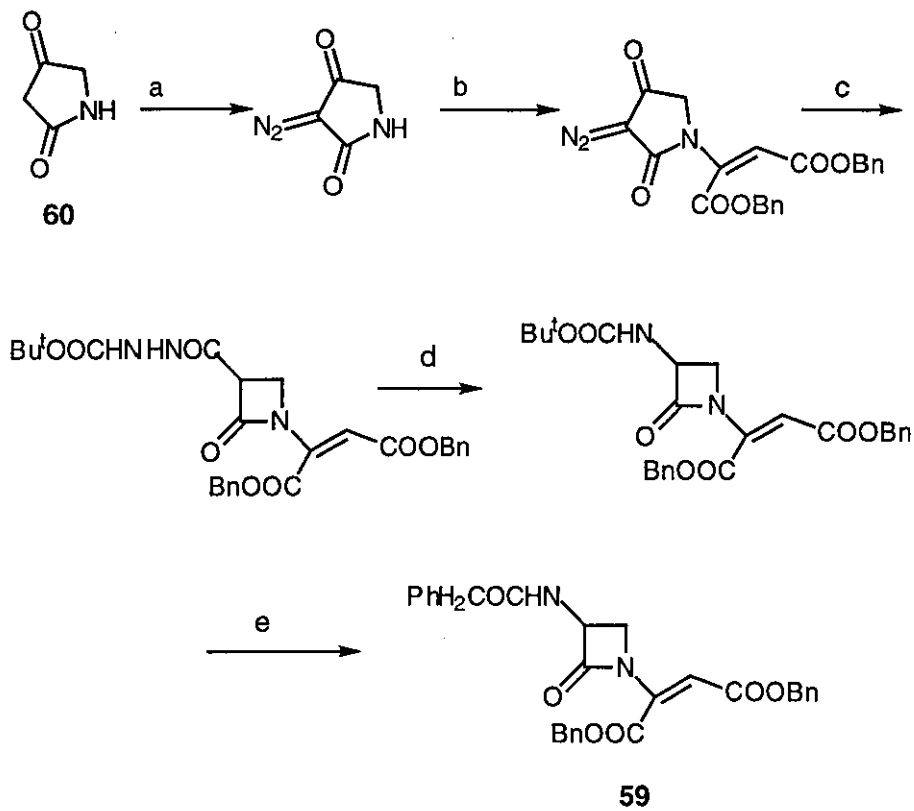
In the attempted cyclisation reaction above, various bases were tried and the reactions were carried out under several different sets of conditions. In each reaction, the amount of base and the concentration of the reaction mixture used were optimized in order to prevent any side reactions from taking place. However, under all the conditions examined, only complex mixtures were formed (**Table 7**).

Table 7

base	no.of equiv.	solvent	temp.	product **57** or **58**
^{t}BuOK	3	Toluene	0 °C	complex mixture
^{t}BuOK	3	DMSO	r.t.	complex mixture
^{t}BuOK	2	Toluene	0 °C	complex mixture
NaH	2	Toluene	reflux	complex mixture
NaH	3	THF	reflux	complex mixture
LDA	2	THF	-78 °C	complex mixture
NaOEt	2	EtOH	reflux	complex mixture
NaOEt	3	EtOH	r.t.	complex mixture

2.3.2.3 Dieckmann condensation of diester 28

In 1973, Lowe reported the cyclisation of the synthesis of a β-lactam which is related to the cephalosporins **59**.[11] The important precursor pyrrolidine-2,4-dione **60** was prepared from the demethoxycarbonylation of the diester formed by the Dieckmann cyclisation of *N*-(ethoxycarbonylacetyl)glycine ethyl ester, which is the precursor also required in our work (**Scheme 39**).



60 **59**

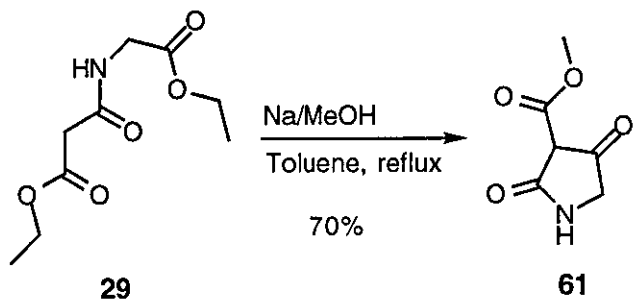
Scheme 39

Conditions: (a) $MeSO_2N_3$-TEA (b) NaH-$PhCH_2O_2CCCO_2CH_2Ph$ (c) *hν*-$^tBuO_2CNHNH_2$ (d) 1. TFA, 2. $NaNO_2$-HCl, 3. heat in benzene, 4. heat with ^tBuOH (e) 1. TFA, 2. $PhCH_2COCl$-TEA, 3. Pd-H_2

Since we had failed to cyclise the Boc protected diester **35**, **36** and also the unprotected diester **33**, we then decided to attempt the cyclisation of the diester **29**, with an unsubstituted methylene unit. This compound can be made in large quantity by treating glycine ethyl ester with potassium ethyl malonate in the

presence of DCC using aqueous acetonitrile as the solvent. The urea which is formed as the by-product in the reaction is easily removed by filtration (see **Scheme 18**).

We then carried out the Dieckmann cyclisation of **29** using the method of Lowe, but changing the solvent from benzene to toluene with sodium methoxide as the base. The reaction proceed smoothly to give us the cyclised product, methyl-2,4-dioxopyrrolidinone-3-carboxylate **61** in good yield (**Scheme 40**). The white amorphous product was not soluble in any of the common organic solvents and was therefore used without any purification. The proton nuclear magnetic resonance in deuteriated dimethylsulfoxide and the high resolution mass spectra, however, confirmed the identity of the compound.

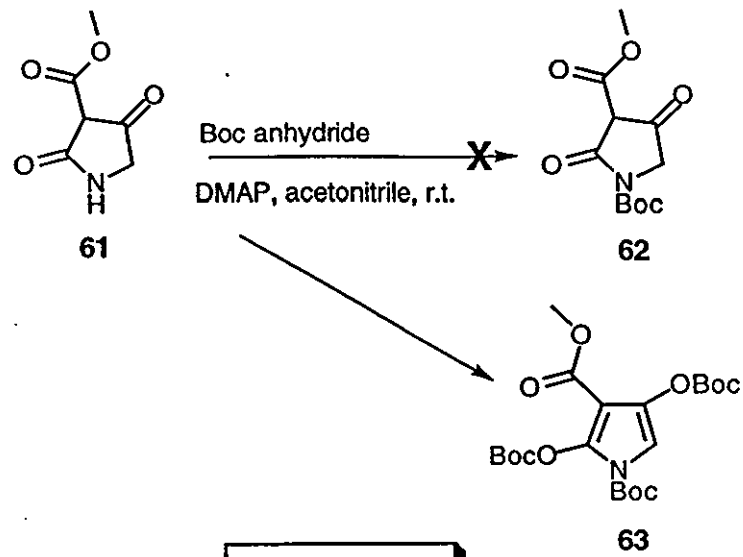


29 **61**

Scheme 40

With the 2,4-dioxopyrrolidinone **61** in hand, we decided first to protect the nitrogen atom. It was also hoped that by protecting the nitrogen, the compound would become more soluble in organic solvent so that it would be easier to carry out further reactions towards our target.

In our first attempted nitrogen protection of **61**, the compound was treated with Boc anhydride in the presence of DMAP and acetonitrile as the solvent. The reaction however, did not produce the desired *N*-Boc protected dioxopyrrolidinone **62** but instead the *N*-Boc-trisubstituted pyrrole **63**. Changing the reaction conditions by using triethylamine in dichloromethane in the presence of DMAP also gave the same crystalline compound (**Scheme 41**). The crystal structure of **63** determined by single crystal X-ray analysis is given in **Figure 2.1**



Scheme 41

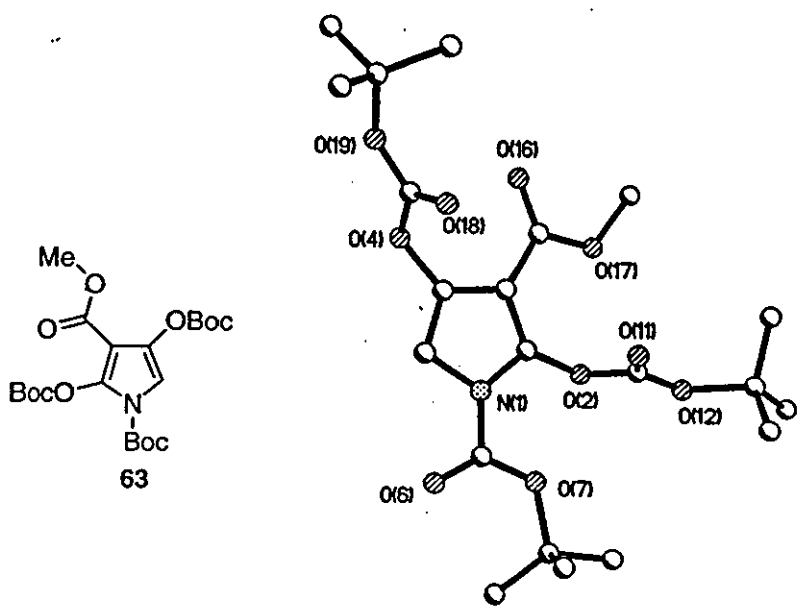
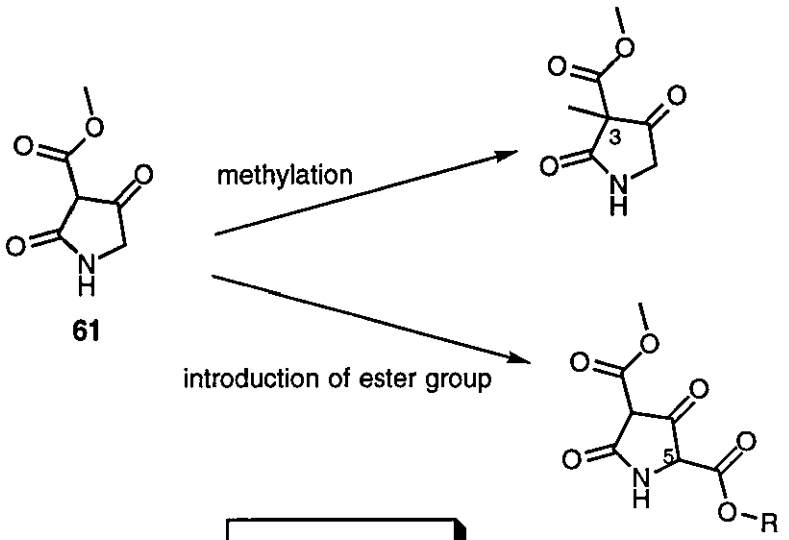


Figure 2.1 X-ray structure of **63**

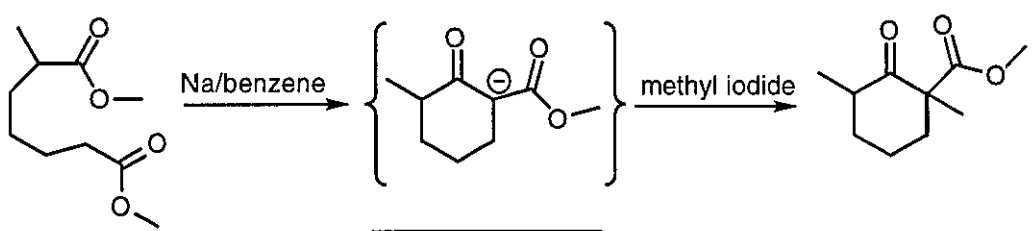
We needed to proceed with the next step which involves either the methylation at the C-3 carbon or introduction of an ester function at C-5 position (**Scheme 42**).



Scheme 42

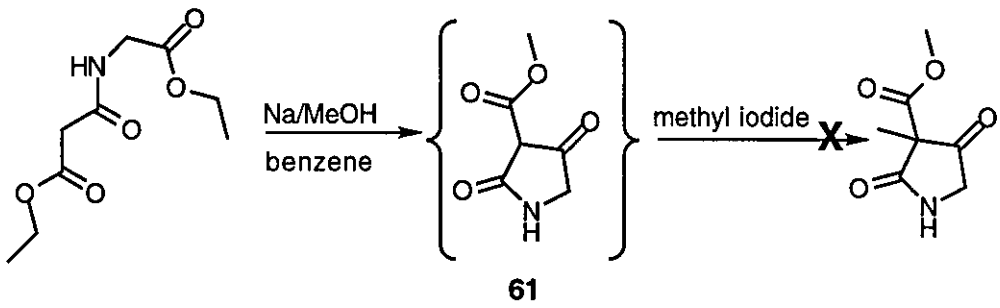
The methylation of the dioxo compound **61** at C-3 should be easier to perform in comparison to the introduction of the ester group at C-5 since trianion chemistry might have to be employed for the latter. Obviously, to carry out this reaction using trianion chemistry would be difficult.

In 1960, Banerjee[12] reported the methylation of a similar cyclised product without the isolation of the Dieckmann cyclised intermediate (**Scheme 43**).



Scheme 43

We attempted the methylation reaction and using the conditions described by Banerjee, but we did not obtain the expected methylated product, but only the cyclised intermediate **61** (**Scheme 44**). We suspect that the methylation reaction was not successful because of the insolubility of the compound and/or the stability of the enolate.

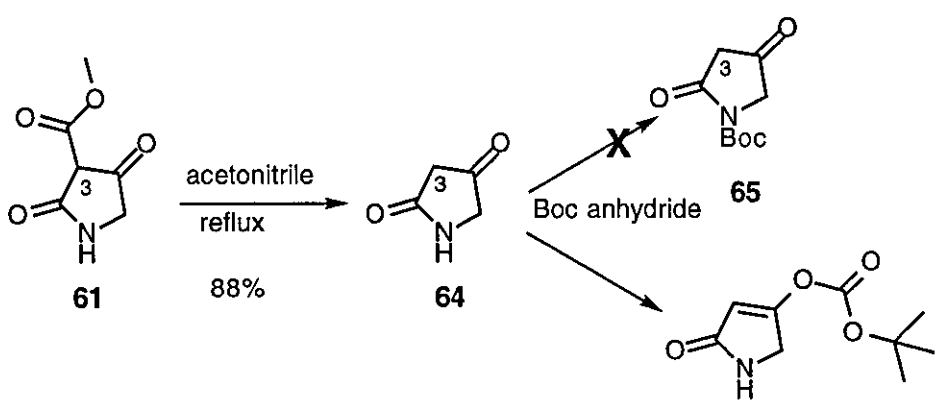


61

Scheme 44

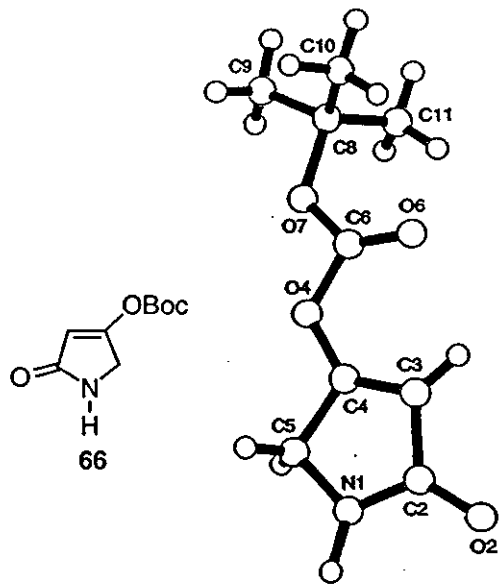
We had been fascinated to discover that the methyl ester at C-3 of **61** can be removed by refluxing the compound in acetonitrile to give **64** in a decarboxylation reaction. This yellowish compound is soluble in most conventional organic solvents and thus would help us to carry out further reactions towards the target molecule. Initially, we decided to protect the nitrogen atom of **64** in order to prevent any side reactions from occuring.

Treatment of **64** with Boc anhydride however, did not give the desired product **65** but instead the *O*-Boc pyrrrolidone **66** (**Scheme 45**). The structure of **66** was confirmed by X-ray analysis and is given in **Figure 2.2**.



61 64 65 66

Scheme 45

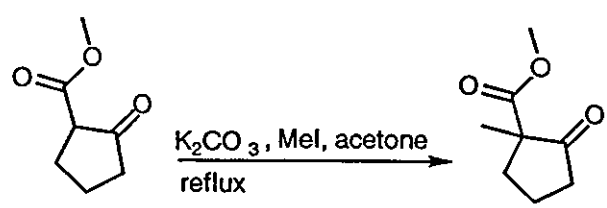


66

Figure 2.2 X-ray structure of **66**

2.3.3 Methylation of 2,4-dioxopyrrolidinone ester 61

Because of the difficulty in protecting the nitrogen of ester **61**, we decided to carry out the methylation at C-3 without any protection. Alkylation of this β, β–ketoester amide system has not been reported before, although numerous examples have been reported of the alkylation of β–ketoesters. For example, Baro reported the methylation of a five membered ring β–ketoester using potassium carbonate as the base in refluxing acetone, giving the methylated product in 100% yield (**Scheme 46**).[13]

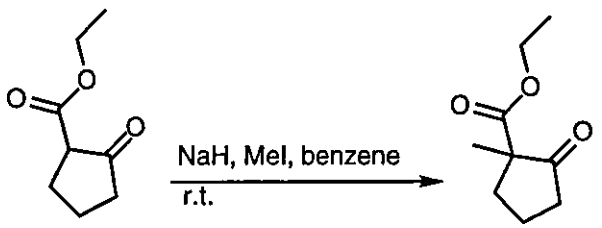


Scheme 46

Other alkylating agents such as isopropyl iodide, n-bromopentane, allyl bromide and ethyl-1-bromoethanoate gave a low yield of the alkylated products. With the exception of the reaction with isopropyl iodide, exclusive *C*-alkylation was

observed. With isopropyl iodide, however, almost 20% of the *O*-alkylated product was formed presumably due to steric hindrance.

In another example, Rhoads made used of sodium hydride as the base in an attempt to methylate a cyclic β–ketoester[14] (**Scheme 47**).

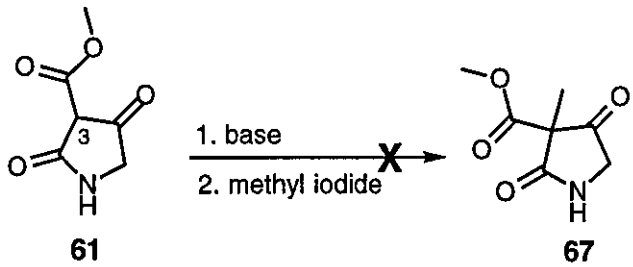


Scheme 47

Other reported work of the alkylation of diketoesters includes that of Fedorynski, in which the ester was treated with alkylating reagent in the presence of K_2CO_3, tetrabutylammonium bromide (TBAB) or 18-crown-6 in refluxing acetonitrile to give the alkylated product.[15]

Since we could not find any relevant work on the alkylation of the β,β–ketoester amide **61**, we had to develop the best conditions for this transformation ourselves. In our first attempt, the substrate was first treated with sodium hydride followed by the addition of methyl iodide. After the usual work up, we did not detect any formation of the methylated product, but only starting material was recovered. Initially, the reaction was performed at 0 °C but even at room temperature, no product was observed.

We then tried the method of Baro by treating **61** with potassium carbonate/methyl iodide in refluxing acetone. Again, no methylated product was detected but instead only the starting material was recovered: in this case the problem with compound **61** could be its insolubility in acetone. We then tried different conditions for the alkylation reaction, but still no product was detected (**Scheme 48, Table 8**).



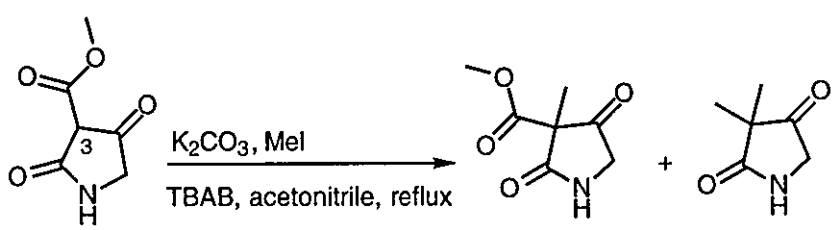
61 67

Scheme 48

Table 8

conditions	product **67**
NaH, THF, r.t.	none
K_2CO_3, acetone, reflux	none
NaOMe, MeOH, reflux	none
^tBuOK, BuOH, reflux	none
NaOH, MeOH, r.t.	none

Since solubility was a serious problem in the alkylation reaction, we decided to use a phase transfer catalyst as used by Fedorynski in the alkylation of diethyl malonate. Hence, treatment of **61** with potassium carbonate, tetrabutylammonium bromide and methyl iodide, indeed gave us the methylated product **67**, but in a very low yield (<5%). The major product under these conditions was the 3,3-dimethylated product **68** (**Scheme 49**).

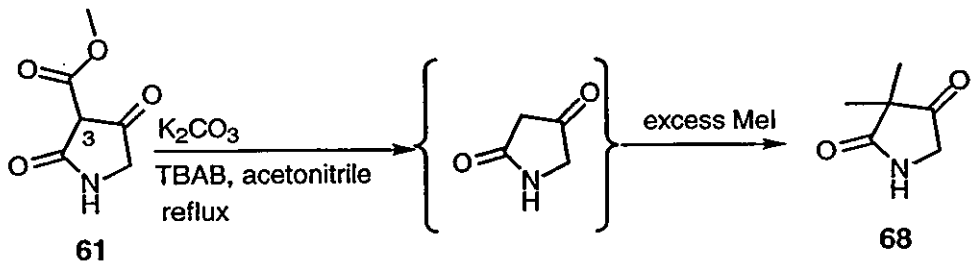


61 67 68

Scheme 49

This observation suggested that using a phase transfer catalyst helped the diester **61** react with the methyl iodide to give **67** as the minor product. The major product **68** is presumably formed through decarboxymethylation when it is treated in refluxing acetonitrile and subsequent reaction with excess of methyl iodide to give

68 (**Scheme 50**). X-ray analyses on the products of this reaction confirmed their structures as **67** and **68** (**Figure 2.3**).



Scheme 50

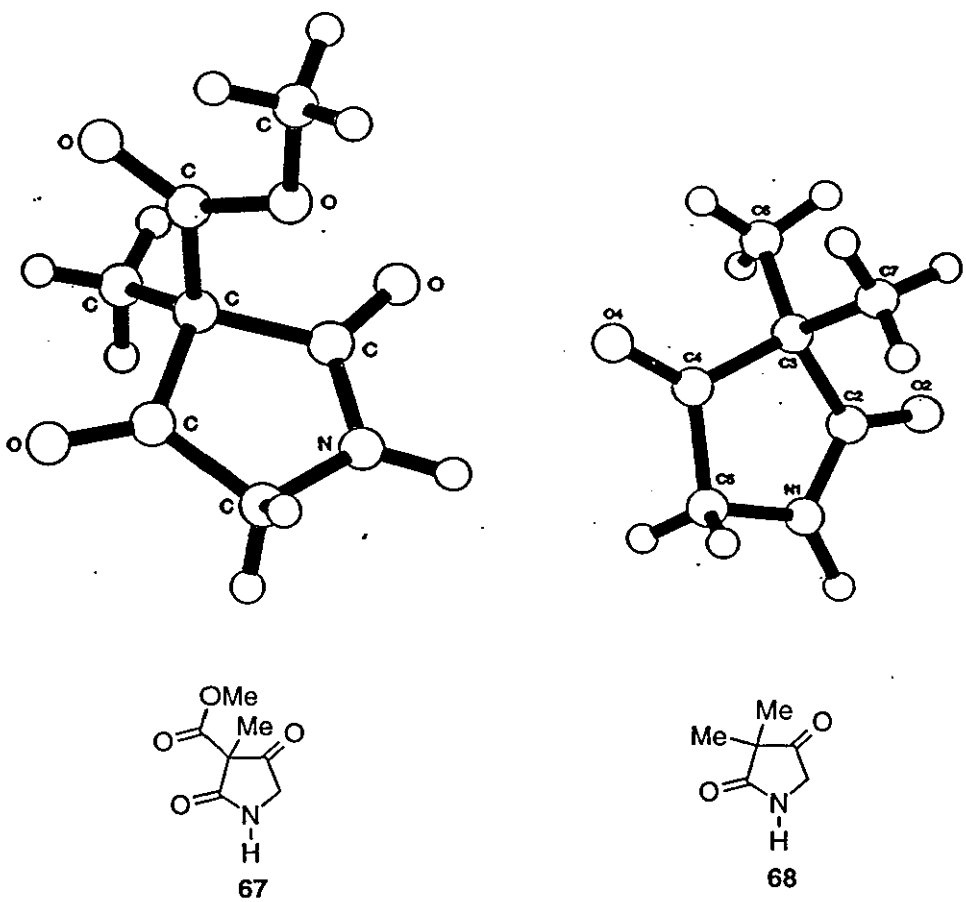
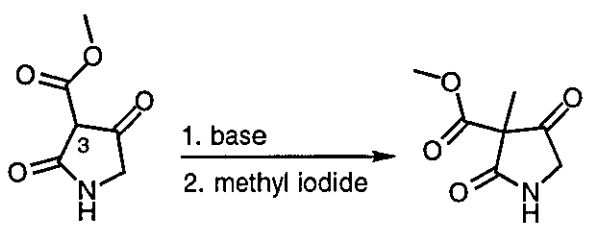


Figure 2.3 X-ray structures of **67** and **68**

Since the desired methylated product was formed when TBAB was used as catalyst, we decided to use water as a cosolvent in order to increase the solubility of the starting material. Hence, treatment of **61** with sodium hydroxide followed by methyl iodide in aqueous methanol under reflux conditions also gave us the product, but the yield was again low. Since a high yield is crucial in order to take us further in our synthesis of lactacystin, we presevered with seeking more successful reaction conditions. We investigated the use of TBAF, which can act as both phase transfer catalyst and base. Under these conditions and using tetrahydrofuran as the solvent we managed to obtain the desired product in good yield. It is perhaps surprising that when methyl toluene sulfonate was used instead of methyl iodide, no product was formed from the reaction (**Scheme 51**, **Table 9**).



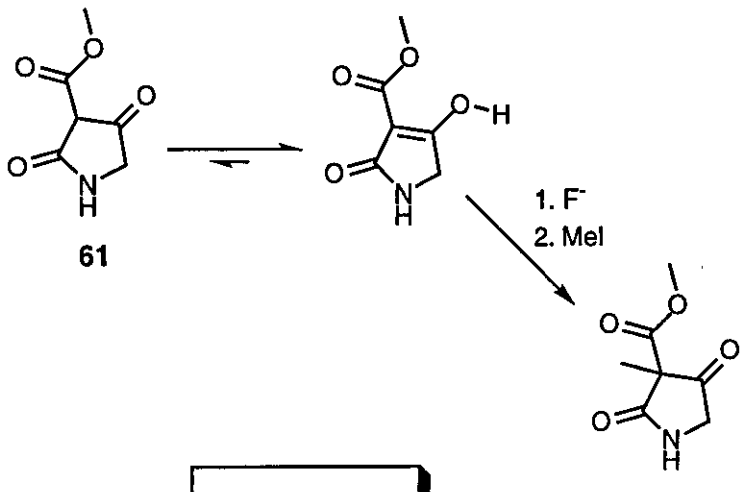
61 **67**

Scheme 51

Table 9

conditions	yield of product **67** (%)
NaOH, aq. MeOH, reflux	10
TBAF, THF, r.t.	82
TBAF, methyl p-toluenesulfonate	no reaction

The reason why methyl iodide was more successful than methyl p-toluene sulfonate as electrophile may be attributed to the 'softer' nature of methyl iodide. This is crucial, since the carbon at C-3, being attached to three carbonyl groups, is a soft nucleophiles thus favouring the interaction with the softer electrophile methyl iodide. Besides acting as a phase transfer catalyst, TBAF also acts as a base. (**Scheme 52**).



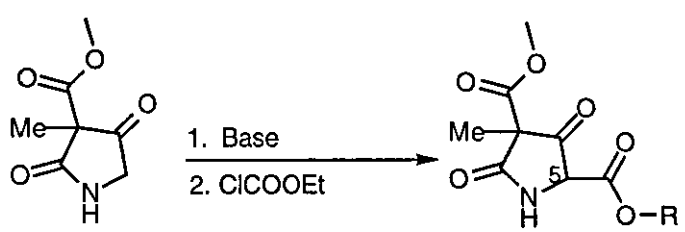
61 67

Scheme 52

This alkylation step is very versatile since with this method we can introduce a different alkyl group at the C-3 position and thus access various analogues of lactacystin using the same approach. In a study by Corey regarding the structure-activity requirements in lactacystin, he showed that replacement of the methyl group by ethyl, isopropyl or benzyl increases the activity of the compound against the proteasome.[16] In a similar study by Omura on the biological activity of analogues of lactacystin, the ethyl analogue was shown to be more potent than lactacystin towards the malarial parasite *Plasmodium berghei*.[17]

2.3.4 Introduction of the ester function at C-5

Having successfully introduced the methyl group at position C-3 of the 2,4-dioxopyrrolidinone, our next step was the introduction of the ester group at the C-5 position of lactacystin to give **69** (**Scheme 53**).

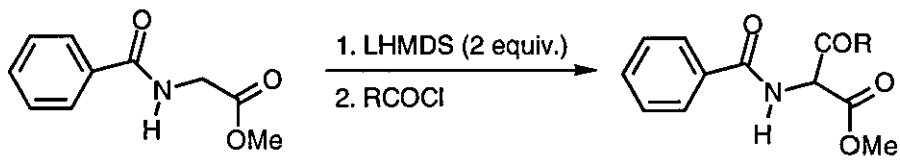


67 69

Scheme 53

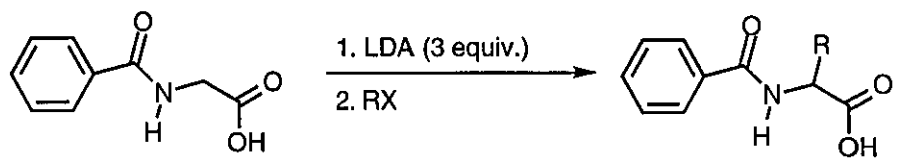
Since the nitrogen of the methylated compound is not protected, obviously we would need to carry out the ester insertion reaction without *N*-protection on the free pyrrolidone. It is in principle possible to do this by the use of dianion chemistry. The proton attached to nitrogen is expected to be removed first, followed by a proton at C-5, which then reacts with an appropriate ester donor.

In 1981, Harding reported a similar acylation of methyl hippurate using lithium hexamethyldisilazide (LHMDS) as a base for the double deprotonation (**Scheme 54**).[18]



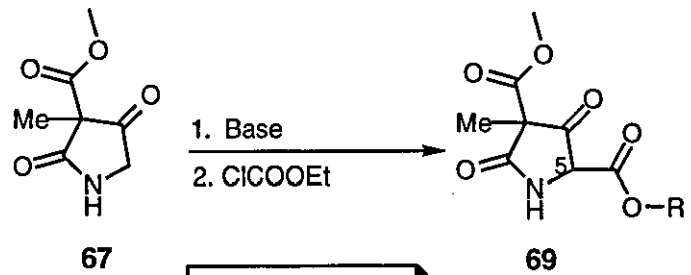
Scheme 54

Related work has also been reported by Krapcho in which the trianion of hippuric acid was alkylated at the carbon next to the acid function to give the *C*-alkylated (**Scheme 55**).[19] Evans has reported the acetylation of a ethyl hippurate using acid anhydrides, but generally the yields were low.[20]



Scheme 55

We attempted the introduction of the ester function using ethyl chloroformate and employing similar dianion chemistry, but the reaction failed to give desired product (**Scheme 56, Table 10**). Although different bases were tried, in each case complex mixtures were obtained.



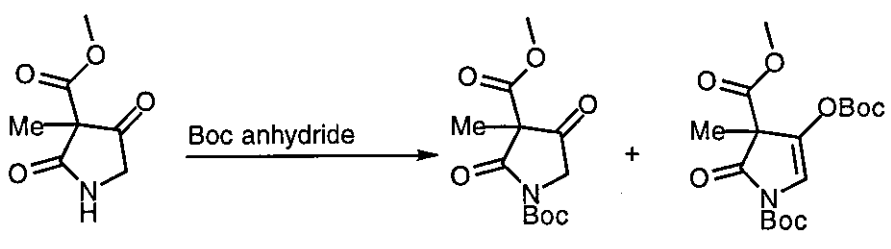
67 69

Scheme 56

Table 10

conditions	product **69**
LHMDS (2 equiv.) -78 °C, THF	complex mixture
LDA (2 equiv.), -78 °C, THF	complex mixture
NaH (2 equiv.), -78 °C, THF	complex mixture

Tlc of the crude mixture showed the presence of at least six different products, which suggested that several side reactions had also taken place. In order to prevent any side reaction, we decided to protect the nitrogen before the introduction of the ester group. Treatment of **67** with Boc anhydride and triethylamine gave the *N*-Boc protected compound **70** in low yield (22%). The major product proved to be the *N,O*-diBoc protected compound **71** (**Scheme 57**).



67 70 71

Scheme 57

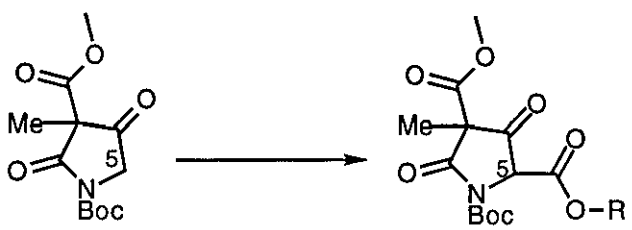
This findings suggested that the protons at C-5 are acidic and therefore easily deprotonated by triethylamine to form an enolate. Fortunately, upon changing the reaction base and solvent to dimethylaminopyridine and acetonitrile respectively,

the desired product **70** was obtained in 76% yield, and only a trace of the side product **71** was observed (**Table 11**).

Table 11

conditions	% yield of *N*-Boc **70**	% yield of *N,O*-diBoc **71**
TEA, DCM, r.t.	22	39
DMAP, acetonitrile, r.t.	76	trace

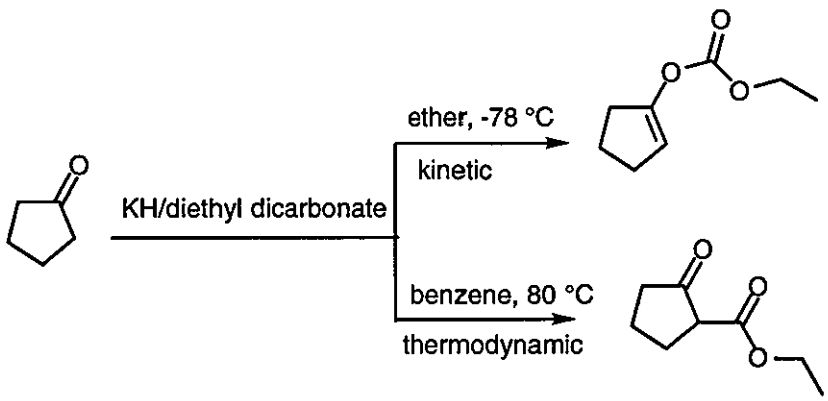
With the *N*-protected compound **70** available in good yields, the next step in our synthesis was the introduction of the ester group at C-5 (**Scheme 58**). With the nitrogen protected, we were hoping that the acetylation would not give any side reactions. Furthermore, since there can only be one site of deprotonation, the introduction of an ester group should be straightforward. The formation of a β-ketoester in this way is very well documented and many examples have been reported in the literature.



70 **72**

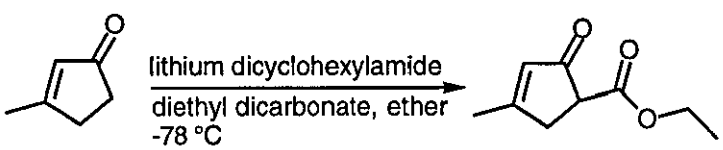
Scheme 58

In 1984, Hellou reported the synthesis of a β-ketoester of a cyclic ketone using potassium hydride as a base and diethylcarbonate as the electrophile.[21] In these studies he showed that the reaction conditions are very important in determining whether *C*- or *O*-acylation occurs. For example, treatment of cyclopentanone with potassium hydride and diethylcarbonate under thermodynamic conditions gave the *C*-acylated product, whereas when the same reaction was carried out at -78 °C with diethyl ether as the solvent, the *O*-acylated product was exclusively formed (**Scheme 59**).



Scheme 59

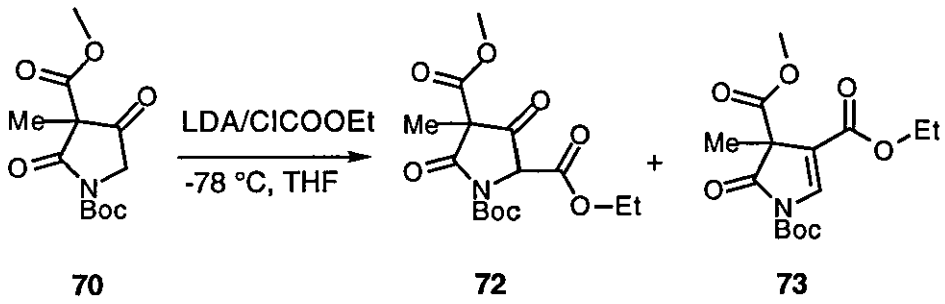
Hellou also reported that when an unsaturated cyclic ketone was treated under the same thermodynamic conditions (KH/benzene) the reaction failed to give any *C*-acylated product. With this unsaturated system however, lithium dicyclohexylamide seems to be the most suitable base in diethyl ether as the solvent in order to obtain the *C*-acylated product (**Scheme 60**).



Scheme 60

Other bases such as potassium tertiary butoxide failed to give any *C*-acylated product, but instead only complex mixtures were formed, presumably due to the tendency for the compound to self-condense. Similar work on the introduction of an ester group adjacent to a ketone was also reported by Nubbemeyer,[22] Marino[23] and Skarzewski.[24]

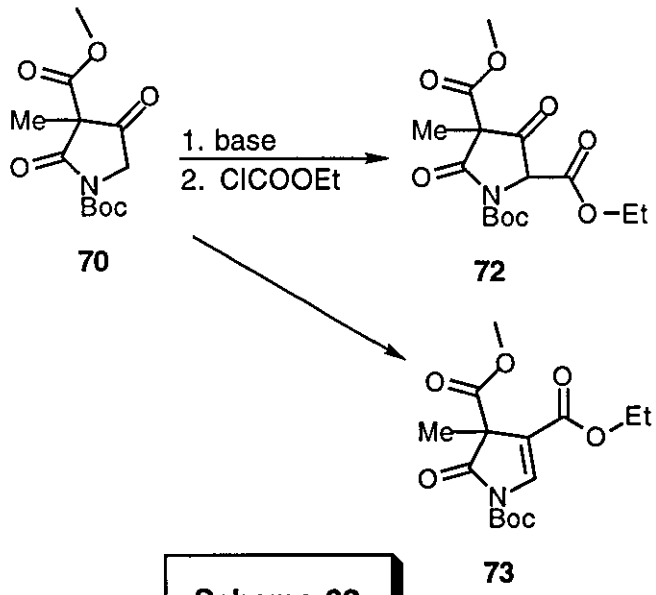
With the information available to us for introducing an ester group at an acidic position, we then attempted reactions with our substrate **70**. In our first attempt, **70** was treated with lithium diisopropylamide and ethyl chloroformate, and gave the desired product **72**, but in very low yield (10%), together with the *O*-acylated product **73** (12%) (**Scheme 61**). Changing the base to lithium bis(trimethylsilyl)amide did not give any of these products, only recovered starting material.



70 **72** **73**

Scheme 61

In view of the large amount of **72** required, we decided to explore different reagents in an attempt to increase the yield of the compound. With potassium tertiary butoxide and sodium hydride as the base and ethyl chloroformate as the electrophile only the *O*-acylated product **73** was formed. These observations are expected since both cations form a rather strong covalent bond with the oxygen thus favouring the *O*-acylated product. It is perhaps strange that with lithium tertiary butoxide as the base, the reaction also gave the *O*-acylated product, although one would expect the *C*-acylated compound to predominate from the reaction of the lithium enolate with the electrophile (**Scheme 62, Table 12**).



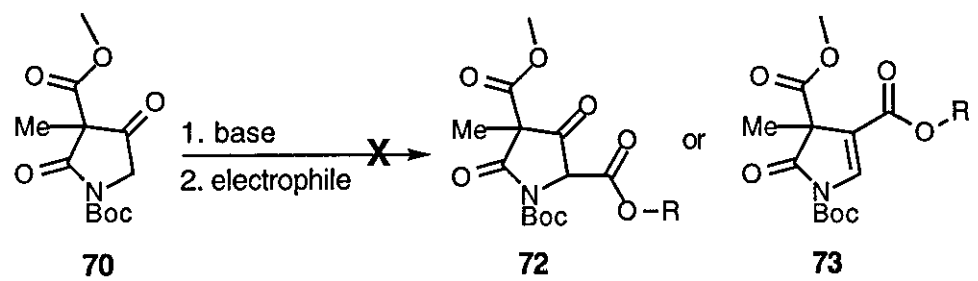
70 **72** **73**

Scheme 62

Table 12

conditions	72	73
^{t}BuOK, THF, -78 °C	No	Yes
NaH, THF, -78 °C	No	Yes
^{t}BuOLi, THF, -78 °C	No	Yes

Since the electrophile ethyl chloroformate gave the required product only in low yield, we turned our attention to different electrophiles such as diethyl carbonate and diethyl dicarbonate. **70** was treated with these electrophiles in the presence of different bases and under a variety of conditions, but again neither the required *C*-acylated nor *O*-acylated compound were formed, in any of the reactions (**Scheme 63, Table 13**).



70 **72** **73**

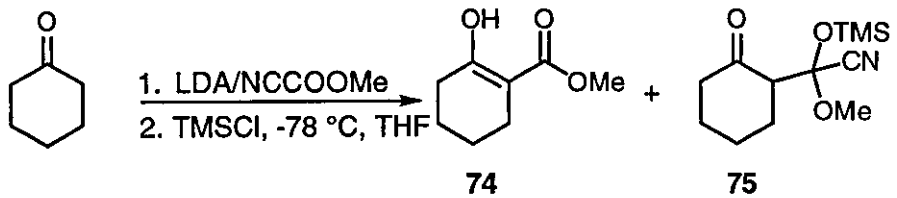
Scheme 63

Table 13

conditions	72	73
LDA, diethyl carbonate,THF, -78 °C	no	no
^{t}BuOK, diethyl carbonate, THF, -78 °C	no	no
NaH, diethyl carbonate, THF, reflux	no	no
KH, diethyl dicarbonate, benzene, reflux	no	no
Pyridine, $MgCl_2$, diethyl dicarbonate, r.t.	no	no

In a more desperate attempt to find the best conditions for the acylation reaction, we came across the work of Mander who reported that the formation of β-keto esters can be achieved with 100% regioselectivity using either alkyl or aryl cyanoformates as the ester donor.[25] The mechanism of this reaction was proposed by Ziegler to proceed through an aldol type intermediate which it is possible to trap.[26] For example, when cyclohexanone was treated with LDA,

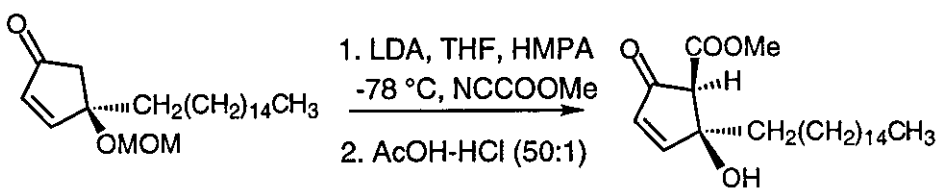
followed by the successive addition of methyl cyanoformate and trichloromethysilane the reaction mixture afforded three products. The first was the carbomethoxylated product **74** and the other two were diastereoisomers of **75**. Treament of **75** with tetrabutylammonium fluoride (TBAF) gave β-keto ester **74**, suggesting that the intermediate was indeed formed through the attack of the lithum enolate on methyl cyanoformate (**Scheme 64**).



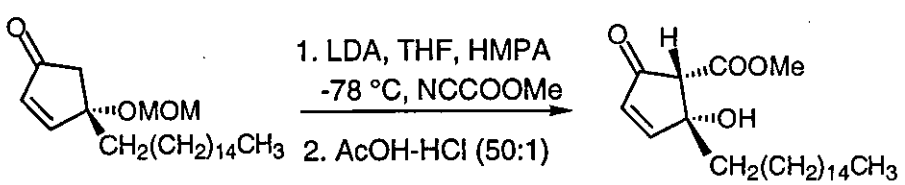
74 **75**

Scheme 64

In 1995, Miyaoka also reported the use of methyl cyanoformate as acylating agent in the total synthesis of (-) and (+) untenone A, an anti-cancer compound isolated from a marine sponge *Plakortis* sp.[27] The final step in this work required the formation of a β-keto ester which was successfully prepared using Mander's procedure to give the target compounds **76** and **77**. Miyaoka also reported that the *O*-acylated compound was not formed in this reaction (**Scheme 65**).



(-) untenone A, **76**

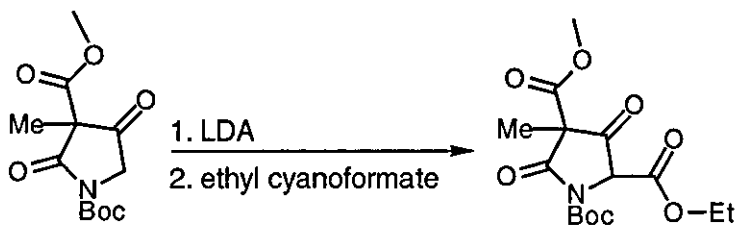


(+) untenone A, **77**

Scheme 65

Other examples that used the same alkyl or aryl cyanoformate methodology with lithium enolates to give exclusively the β-keto ester were reported by Kraus[28], Williams[29], Tremblay[30], Ziegler[31], Hirai[32] and Ikegami.[33]

Our initial attempt on the acylation of **70** using lithium diisopropylamide and ethyl cyanoformate gave us the desired product but only in 10% yield (**Scheme 66**). Addition of HMPA or DMPU did not have any significant effect on the yield of the product. No reaction was observed when the base was changed from lithium diisopropyl amide to lithium bis(trimethylsilyl) amide (**Table 14**).



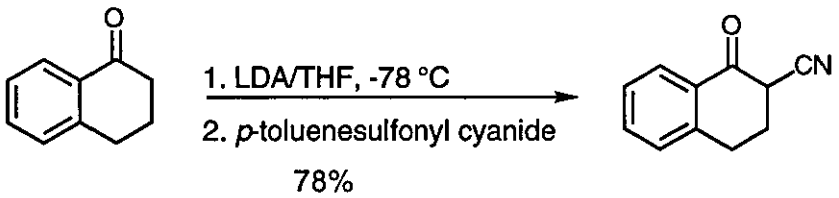
70 **72**

Scheme 66

Table 14

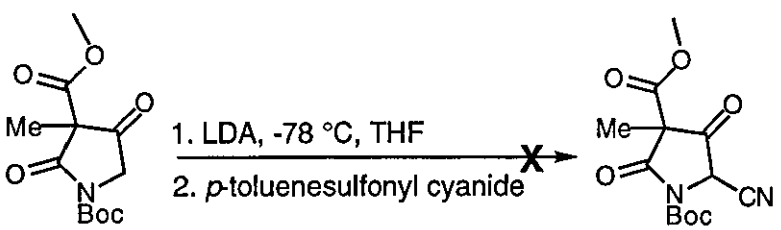
conditions	product **72**
LDA (1.2 equiv.), THF, -78 °C	low yield
LDA (2.0 equiv.), THF, -78 °C	low yield
LDA (1.2 equiv.), HMPA,THF, -78 °C	low yield
LDA (1.2 equiv.), DMPU, THF, -78 °C	low yield
LDA (1.2 equiv.), THF, 0 °C	low yield
LHMDS (1.2 equiv.), THF, -78 °C	no reaction
LHMDS (1.2 equiv.), DMPU, THF, -78 °C	no reaction
LHMDS(1.2 equiv.), HMPA, THF, -78 °C	no reaction

In another approach, instead of introducing the ester group directly we decided to introduce a cyano group at C-5, since the latter can also be hydrolysed to the carboxylic acid. Kahne has reported that the synthesis of α-cyano ketones is possible with the use of *p*-toluene sulfonyl cyanide at -78 °C in good yield[34] (**Scheme 67**).



Scheme 67

When we attempted Kahne's protocol on substrate **70** under the same conditions, however, we failed to observe the corresponding 5-cyano pyrrolidinone **78** (**Scheme 68**).



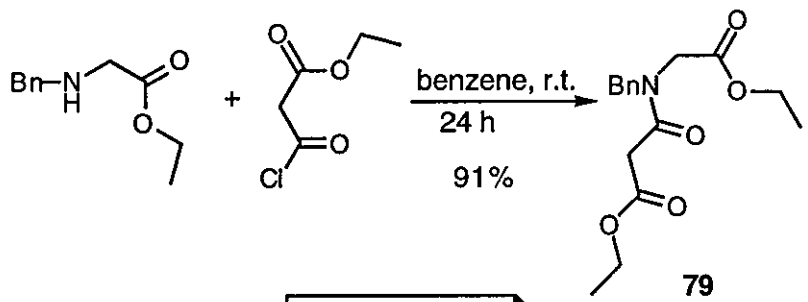
70 **78**

Scheme 68

2.3.5 Synthetic approaches using *N*-protected glycine ethyl ester

Since the yield of the β-keto ester **72** was low, we decided to change our synthetic strategy by using an *N*-protected glycine ethyl ester as the starting material. By using the *N*-benzyl protected glycine ethyl ester we avoid Boc anhydride, which was found to give rise to the *O*-Boc material (See **Scheme 57**). We also felt that the steric hindrance of the Boc group made the introduction of the final ester group more difficult.

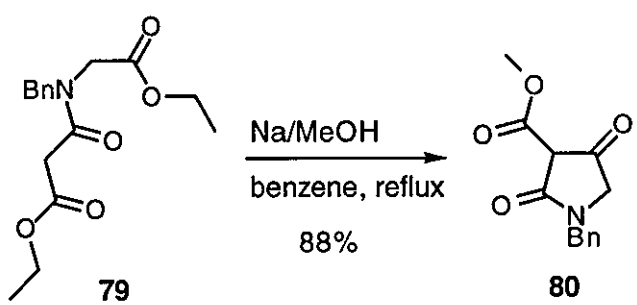
The diester **79** was prepared by treating *N*-benzylated glycine ethyl ester with ethyl malonyl chloride to give the desired carbamate in 91% yield (**Scheme 69**).



79

Scheme 69

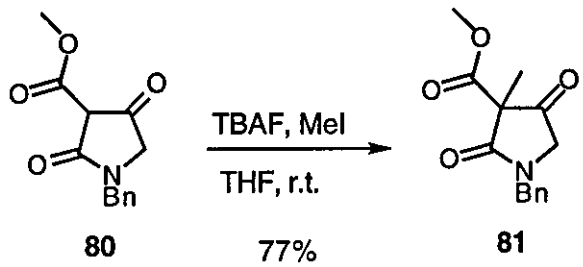
Dieckmann cyclisation of **79** using sodium/methanol afforded the *N*-benzylated pyrrolidinone **80** in 88% yield (**Scheme 70**). Sodium methoxide was used as the base in the reaction in order to give the methyl ester at the C-3 position through transesterification. This is important since the methyl ester can be selectively removed at a later stage.



79 **80**

Scheme 70

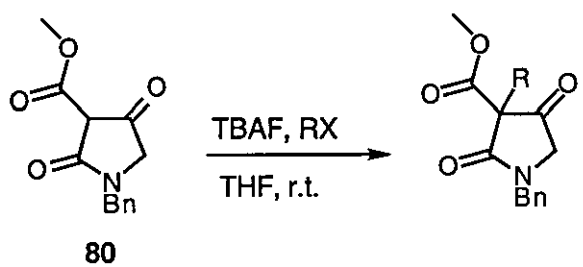
Using the same conditions as described above, the *N*-protected pyrrolidinone **80** was methylated using tetrabutylammonium flouride and methyl iodide to furnish the methylated product **81** in 77% yield (**Scheme 71**).



80 77% **81**

Scheme 71

As indicated above, this method enables us to synthesise a variety of alkylated analogues of lactacystin (**Scheme 72**). In **Table 15** we present some of the compounds that we have accessed successfully with this method.



80

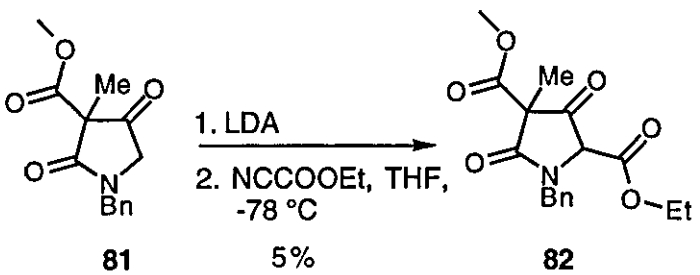
Scheme 72

Table 15

R	yield (%)
CH_3	77
CH_2CH_3	63
CH_2Ph	89
$CH_2CH{=}CH_2$	60
$CH_2CH{=}CHPh$	64
CH_2COOEt	46

With the methylated compound in hand, the next step in our synthesis was the introduction of the ester group at C-5. We had shown that alkyl cyanoformates are

the reagents of choice for the transformation of **81** to the β-keto ester **82**, and we therefore carried out the reaction using the same conditions. Treatment of compound **81** with lithium diisopropylamide followed by ethyl cyanoformate affords the desired product **82** in low yield. Addition of lithium chelating agents such as HMPA or DMPU did not, however, have a significant effect on the yield of this reaction (**Scheme 73**).

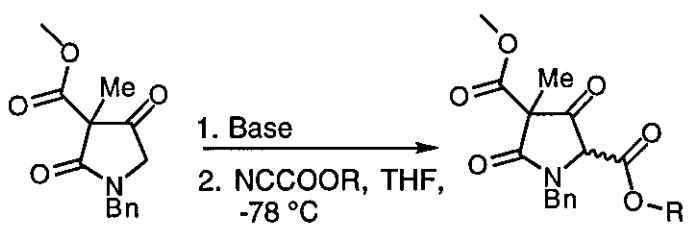


81 5% **82**

Scheme 73

Since the yield of the keto ester **82** was low when lithium diisopropyl amide was used, we decided to use to lithium bis(trimethysilyl) amide and to our delight the yield of product increased slightly. Because of the importance of this step to take us further in our synthesis, we had to optimize the reaction conditions. Incorporation of HMPA in the reaction mixture, again did not increase the yield of the keto ester.

Next we used methyl cyanoformate instead of the ethyl analogue, and, after several attempts, we found that by using two equivalents of LHMDS, methyl cyanoformate and HMPA, the reaction proceeded smoothly and gave the desired β-keto ester as a 2:1 mixture of diastereoisomers with 76% yield (**Scheme 74**, **Table 16**).



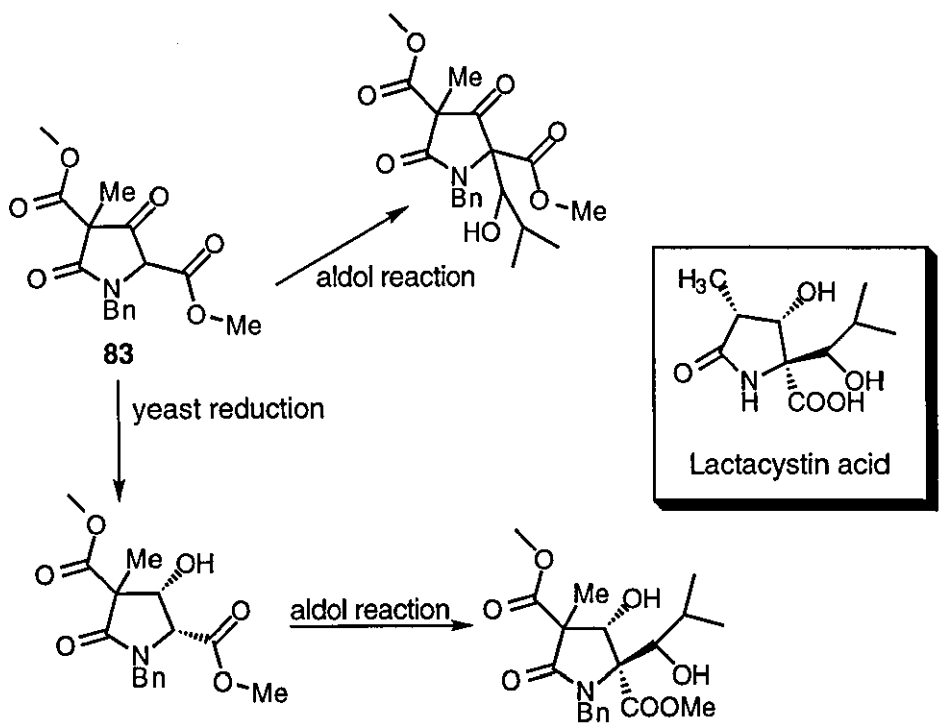
R = Me, **83**

Scheme 74

Table 16

conditions	R = Et or Me	product (yield)
LDA (1.1 equiv.)	Et	5%
LDA (1.1 equiv.), HMPA	Et	5%
LDA (1.1 equiv.), DMPU	Et	5%
LHMDS (1.1 equiv.)	Et	5%
LHMDS (1.1 equiv.), HMPA	Et	10%
LDA (1.1 equiv.)	Me	5%
LHMDS (1.1 equiv.)	Me	10%
LHMDS (2 equiv.), HMPA (2 equiv.)	Me	76%
LHMDS (2 equiv.), HMPA (2 equiv.)	Et	56%
LDA (2 equiv.), HMPA (2 equiv.)	Me	No

Having made β-keto ester **83**, the next step of the reaction was either the aldol reaction or keto reduction of **83.** If the isopropyl group was introduced successfully into **83**, we would have constructed the full basic skeleton of lactacystin acid in just five steps. At this stage we do not know which of the two approaches outlined in **Scheme 75** would allow us the greatest stereocontrol. Thus, we attempted to investigate both strategies, first with the reduction of the keto group at C-4 using yeast reduction.



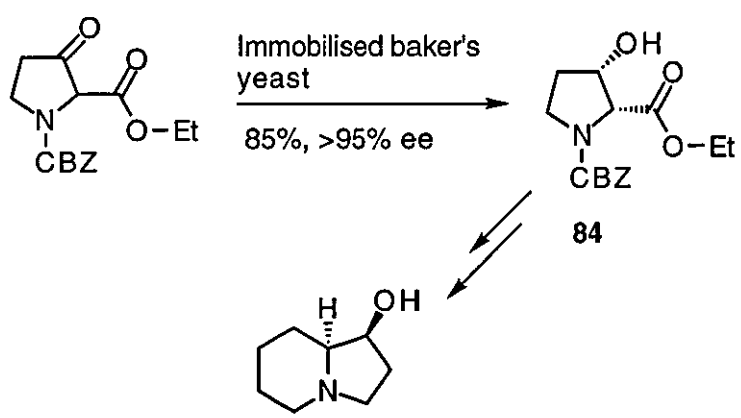
Scheme 75

2.3.6 Yeast reduction of keto ester 83

The use of enzymatic catalysis in preparative organic chemistry has been recognised for decades, as enzymes are capable in carrying out diastereoselective and enantioselective transformations.[35]

The use of bakers' yeast as a reagent in organic synthesis has been applied since the beginning of the century. Bakers' yeast is inexpensive, versatile and most important of all it is easy to use in organic reactions. The reduction of β-keto ester using bakers' yeast is one of the most extensively studied small molecule microbial transformations leading to chiral intermediates in asymmetric synthesis. Although the reaction has been known since the 30's, only when the reduction of 3-oxobutanoates were re-examined by Deol did it become again an important research tool in organic chemistry.[36] In his work, Deol successfully carried out the reductions of various α- and β-keto esters and amides to produce the corresponding optically active α- and β-hydroxy esters and amides.

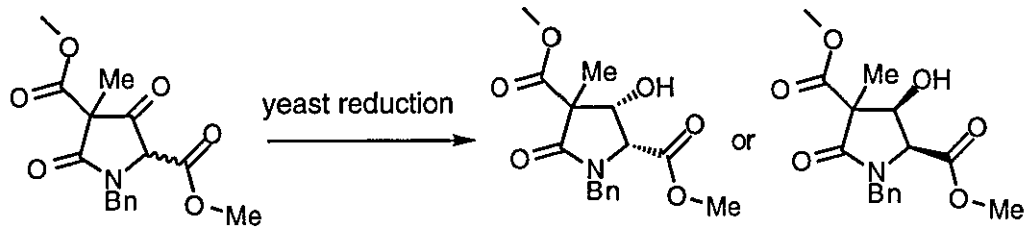
In 1990, Sibi reported the synthesis of 1-hydroxyindolizidine, an important precursor used in the total synthesis of alkaloid slaframine.[37] The important precursor 3*S*-hydroxy proline **84** was synthesised using bakers' yeast reduction, a procedure developed by Knight.[38] Improvement of the chemical yield and also the optical yield (ee) was achieved using immobilised bakers' yeast (**Scheme 76**).



Scheme 76

Other examples of the reduction of β-ketoesters using bakers' yeast were reported by Sakai[39] and Williams.[40]

In our first attempt at the yeast reduction of our substrate **83**, the reaction proceeded to give exclusively the desired *cis* hydroxy ester, but in low yield (10 %). Increasing the reaction time followed by the addition of more sugar seems to have no effect on the yield of the hydroxy product. In another attempt to increase the yield of the product, we used modified yeast based on the procedure of Rauk[41], but again the yield remained low (**Scheme 77**, **Table 17**).



83 **85**

Scheme 77

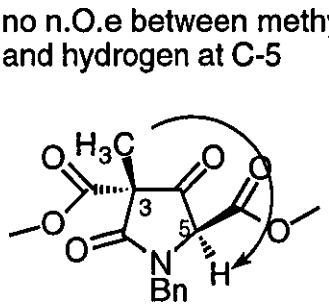
Table 17

yeast type	time	yield
free bakers' yeast	2 days	5%
modified yeast	4 days	10%

One of the main problems asociated with the reductions using both free and modified bakers' yeast, is that only one of the distereoisomers **83** was reduced by the enzyme to give the hydroxy ester. The other isomer, which unfortunately is the major diastereoisomer, did not react with yeast. Furthermore, since the proportion of the major to minor isomer is 2:1 obviously the yield of the product was very low. We tried to separate both isomers, but proved unsuccessful.

In one instance, the major isomer of **83** crystallised out when a small amount of the mixture of diastereoisomers was left in the fridge for a long time, but an attempt to repeat the procedure with a large quantity of material failed to separate the mixture. Although we were able to isolate the major product, unfortunately we could not obtain the X-ray structure to confirm the structure of the compound. n.O.e studies on the compound, however, suggested that the major product has the methyl group *trans* to the C-5 proton. We could not confirm this however because there

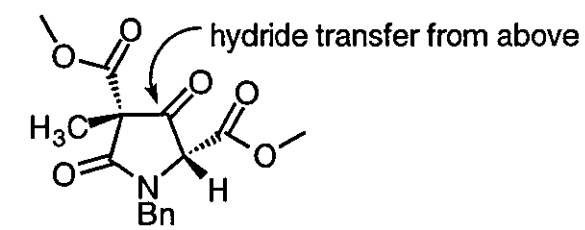
are no other useful interactions between hydrogen atoms in the molecule (**Figure 2.4**).



major isomer + enantiomer

Figure 2.4

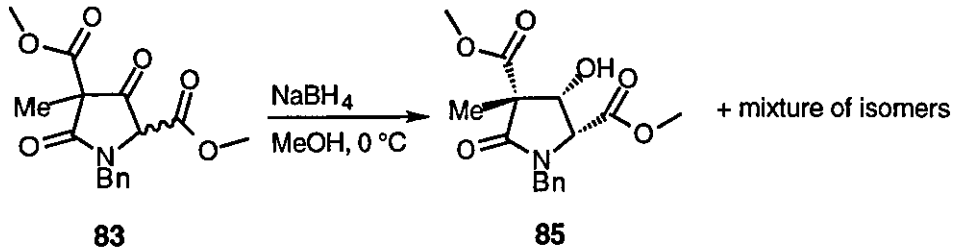
A reason why this major isomer might not be reduced by yeast is the steric hindrance associated with the *anti* ester groups. These may block by yeast both sides of the molecule and therefore hinder the reduction of the keto group. One enantiomer of the minor isomer, however, with the methyl esters *syn* to one another would allow the enzyme to deliver the hydride from the less hindered side of the molecule (**Figure 2.5**). The other enantiomer of the *syn* isomer would also be expected not to be reduced by the yeast.



minor isomer + enantiomer

Figure 2.5

Since the yeast reduction of **83** gave a low yield of the hydroxy ester **85** (10%) even though the correct stereochemistry was attained, we decided to use sodium borohydride. The reduction of the keto ester **83** with sodium borohydride at 0 °C occured smoothly, and all the starting material was used up. But instead of getting the desired hydroxy ester **85** exclusively, a mixture of products was obtained and separation of these was extremely difficult (**Scheme 78**).



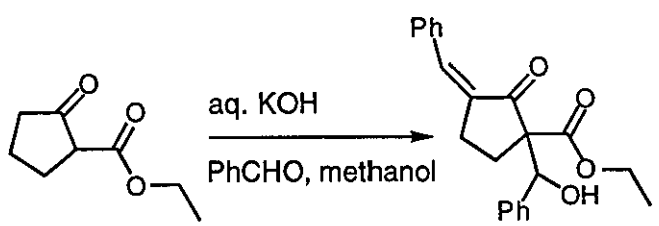
83 **85**

Scheme 78

2.3.7 Aldol reaction of keto ester 83

Since the reduction of **83** using both the yeast and sodium borohydride reagents failed to give the desired product **85** in good yield, we decided to carry out the aldol reaction of **83**. It is interesting to note that should the aldol reaction work on compound **83**, we will then have constructed the important carbon skeleton of the pyroglutamic acid in only five steps. Although the aldol reaction of β-keto esters is not well documented, presumably due to the tendency of the system to undergo retro aldol, nevertheless we still decided to attempt the transformation. Also, following the aldol reaction, the reduction of the keto group may become more selective.

The aldol reaction of a β-keto ester had been reported by Mayer, in which 2-oxo-cyclopentanecarboxylic acid ethyl ester was treated with benzaldehyde using aqueous potassium hydroxide as a base.[42] Although the aldol reaction works well at the carbon bearing the acidic proton, another molecule of benzaldehyde was also attacked by the carbon adjacent to the keto group to give the double aldol product which was then dehydrated to give compound **86** (**Scheme 79**).

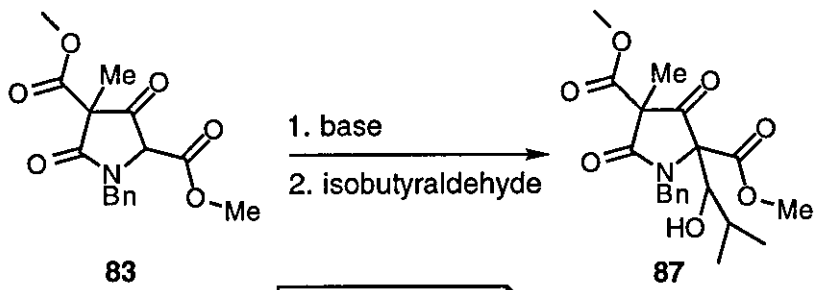


86

Scheme 79

In our first attempted aldol reaction, **83** was treated with lithium diisopropylamide at -78 °C followed by isobutyraldehyde, but unfortunately we could not detect any

formation of the aldol product **87**, only the formation of complex mixtures. The presence of HMPA or DMPU in the reaction mixture did not have any significant effect on the products formed. Changing the base from lithium diisopropylamide to lithium bis(trimethysilyl) amide, lithium tertiary butoxide or sodium hydride also failed to give the aldol product. Instead, complex mixtures were observed from all reactions that were carried out (**Scheme 80, Table 18**).



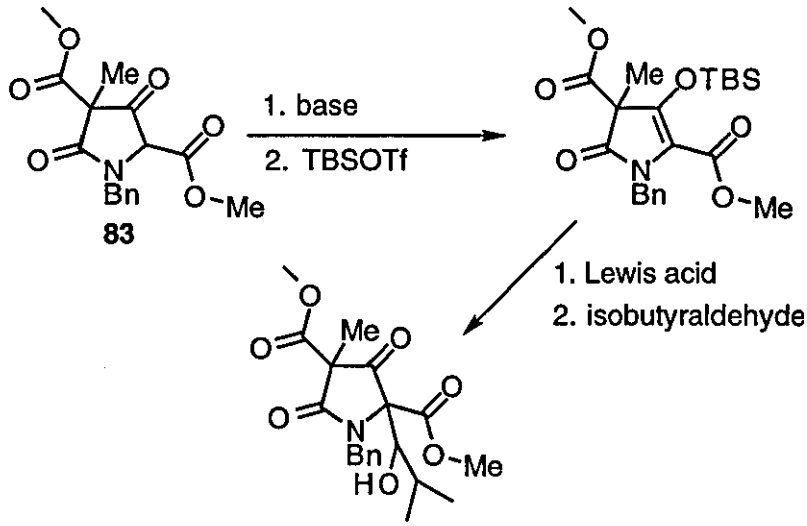
83 **87**

Scheme 80

Table 18

conditions	product
1. LDA (1 equiv.), -78 °C, THF	complex mixture
2. LDA, DMPU, -78 °C, THF	complex mixture
3. LHMDS, -78 °C, THF	complex mixture
4. LHMDS, DMPU, -78 °C, THF	complex mixture
5. LHMDS, HMPA, -78 °C, THF	complex mixture
6. NaH, -78 °C, THF	complex mixture
7. ^{t}BuOLi, -78 °C, THF	complex mixture

Since the aldol reaction failed, we decided to convert the β-keto ester into the silyl enol ether and allow that to react with isobutyraldehyde in the presence of a Lewis acid. We hope that by using this strategy, the retro aldol reaction could be surpressed, thus providing us with the desired product (**Scheme 81**).

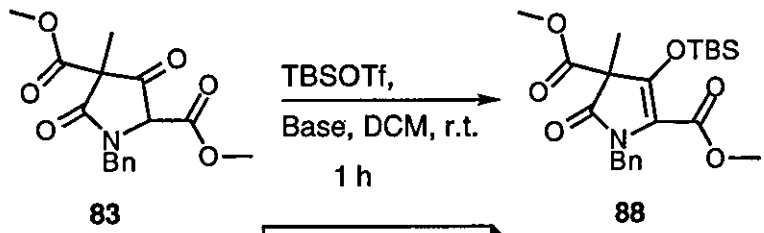


Scheme 81

2.3.7.1 Aldol reactions of 83 using silyl enol ethers

The aldol condensation is one of the most versatile synthetic tools for the creation of new carbon-carbon bonds, but its utility is often limited due to the difficulty in controlling the course of the reaction. Some of the common problems in these reactions include the formation of self or poly-condensation products, and also the stereoselectivity of the overall process. Furthermore, forcing reaction conditions may lead to dehydration products, which may then suffer from further side reactions. Some of the reported work on the aldol reactions of silyl enol ethers with Lewis acids or fluoride induction were carried out by Mukaiyama[43], Oppolzer[44] and Noyori.[45]

The silyl enol ether **88** was first prepared by treating compound **83** with tert-butyldimethylsilyl triflate, and with 2,6-lutidine as a base. The yield of the silyl enol ether was increased from 76 to 83% by replacing lutidine with triethylamine (**Scheme 82, Table 19**).



83 **88**

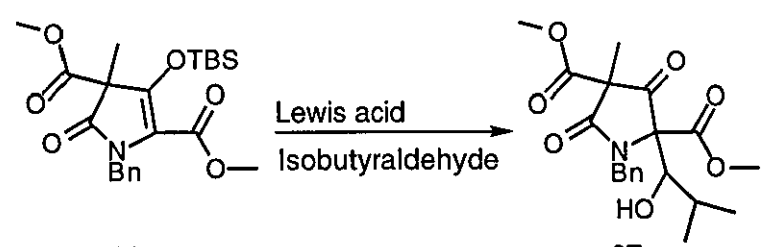
Scheme 82

Table 19

base	yield of **88** (%)
2,6-lutidine	76
triethylamine	83

Having prepared the silyl enol ether **88** in good yield, we then investigated the aldol reaction of **88** with isobutyraldehyde using titanium tetrachloride as the Lewis acid. Although other Lewis acids such as boron etherate and tin(IV) chloride can also be used, titanium chloride is thought to be the most favourable Lewis acid to prevent the formation of retro aldol products.[46]

In our first attempted reaction, silyl enol ether **88** was treated with one equivalent of Lewis acid and isobutyraldehyde (one equiv.) at -78 °C, but no reaction was observed. Warming up the reaction to room temperature also failed to give any reaction. Since no reaction was observed when one equivalent of Lewis acid was used, we then tried using two equivalents of the Lewis acids. This time some of the starting materials were consumed (tlc), but no aldol product **87** was detected in the reaction by NMR spectroscopy (the product failed to show the presence of the isopropyl group) (**Scheme 83**, **Table 20**).



88 **87**

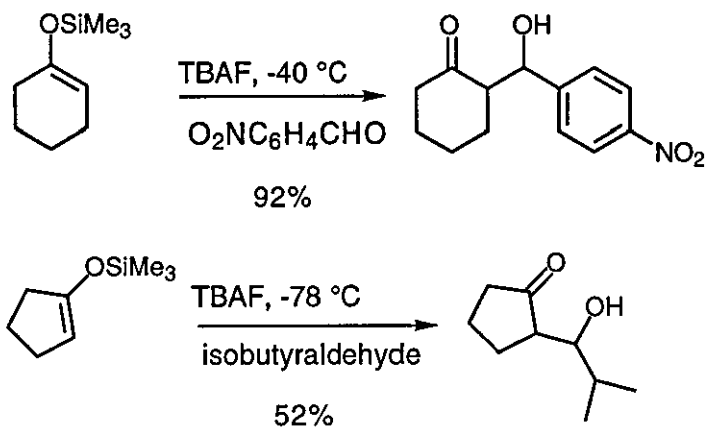
Scheme 83

Table 20

conditions	product **87**
$TiCl_4$, (1 equiv.), -78 °C, 2 hr	no reaction
$TiCl_4$, (1 equiv.), -78 °C to r.t.	no reaction
$SnCl_4$, (1 equiv.), -78 °C to r.t.	no reaction
$TiCl_4$, (2 equiv.), -78 °C to r.t.	complex mixture

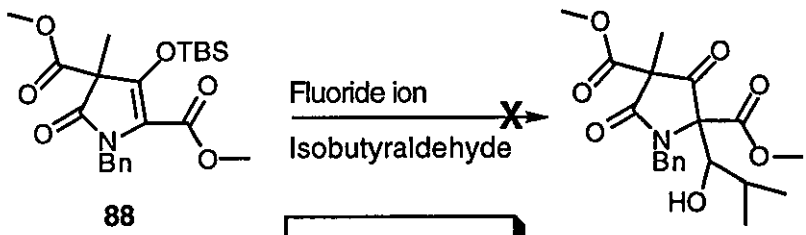
2.3.7.2 Fluoride induced aldol reaction of 88

Having failed to perform the aldol reaction of **88** using a Lewis acid, we then decided to use fluoride ion as the inducer. Noyori[45] reported that the aldol reaction of 1-(trimethylsiloxy)cyclohexene with *p*-nitrobenzaldehyde affords the aldol product with high yield. A similar reaction has also been reported for trimethyl siloxy cyclopentene and isobutyraldehyde, but the yield was slightly lower (**Scheme 84**).



Scheme 84

We used the same conditions as Noyori, but still we could not detect any formation of the aldol product. Tlc of the reaction mixture showed the presence of complex mixtures together with some of the unreacted starting material. Changing the fluoride source from tert-butylammonium fluoride to benzyltrimethylammonium fluoride (BTAF) and using dichloromethane as the reaction solvent also failed to give the aldol product (**Scheme 85, Table 21**).



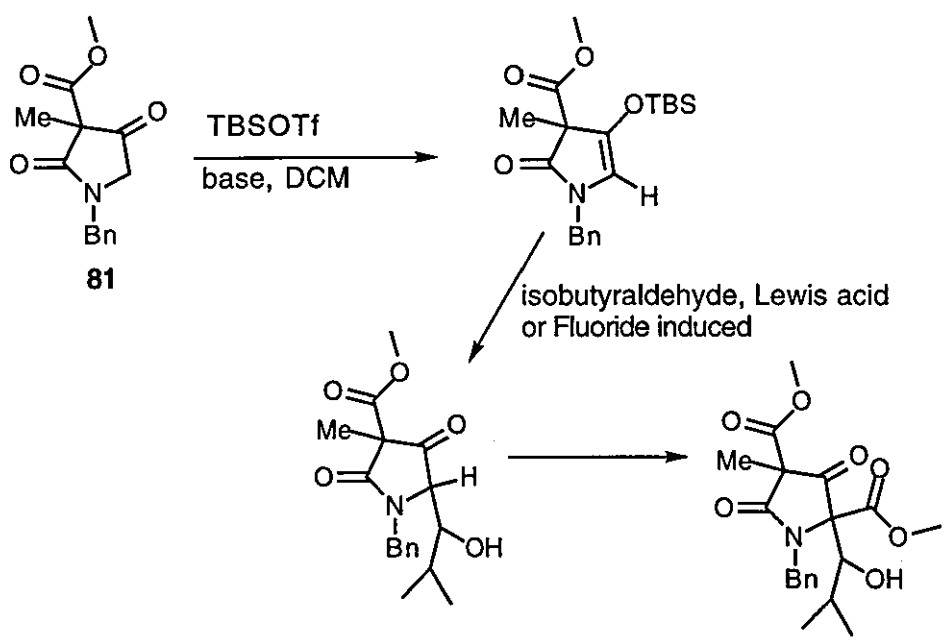
88

Scheme 85

Table 21

conditions	results
TBAF, THF, -78 °C	complex mixture
TBAF, THF, 0 °C to r.t.	complex mixture
BTAF, DCM, -78 °C, mol. sieves	complex mixture

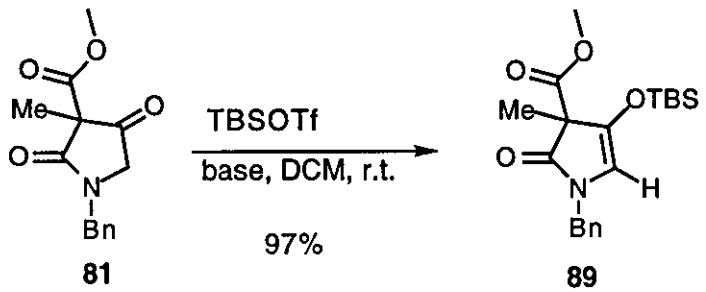
Since we had failed to carry out the aldol reaction of **88** using both Lewis acid and fluoride ion induced reaction, presumably due to the steric hindrance or retro aldol reaction, we decided to introduce the bulky isobutyryl group into **81** using a silyl enol ether aldol, before the incorporation of the smaller methyl ester group (**Scheme 86**).



81

Scheme 86

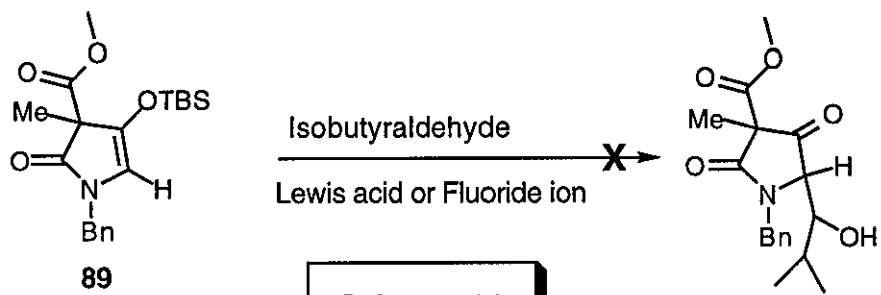
The silyl enol ether of **89** was prepared in high yield by treating ketone **81** with tert-butyldimethylsilyl triflate in the presence of triethylamine (**Scheme 87**).



81 **89**

Scheme 87

With **89** in hand, we carried out the aldol reaction with titanium (IV) chloride as the Lewis acid, but again no aldol product was detected in the reaction. Upon changing to the fluoride induced aldol reaction, we obtained mixtures of products one of which is compound **81**, possibly arising from the retro aldol reaction (**Scheme 88, Table 22**).



89

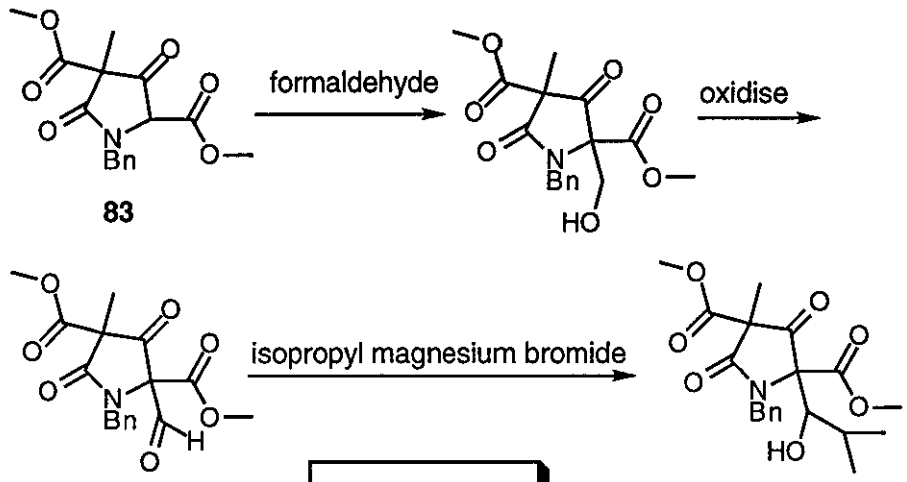
Scheme 88

Table 22

conditions	product
$TiCl_4$ (1 equiv.), DCM, -78 °C to r.t.	complex mixture
$TiCl_4$ (2 equiv.), DCM, -78 °C to r.t.	complex mixture
TBAF, THF, -78 °C	complex mixture + **81**
BTAF, DCM, mol. sieves, -78 °C	complex mixture + **81**

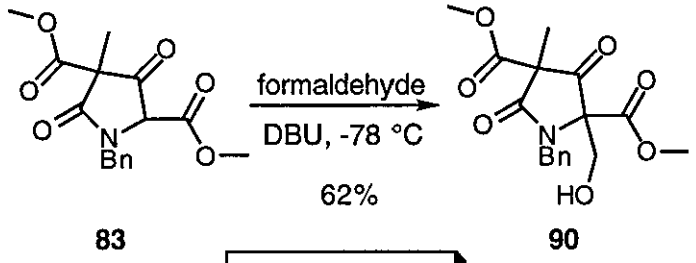
Since we had failed to introduce the isopropyl group into the silyl enol ether **89**, we then decided to test the aldol reaction of **83** with a less bulky aldehyde such as formaldehyde. If the reaction with formaldehyde proved successful we could then

oxidise the primary alcohol to the corresponding aldehyde. Then, addition of isopropyl magnesium bromide or chloride to the aldehyde would give us the required aldol product. This stepwise approach, however, adds two extra steps to our synthetic route (**Scheme 89**).



Scheme 89

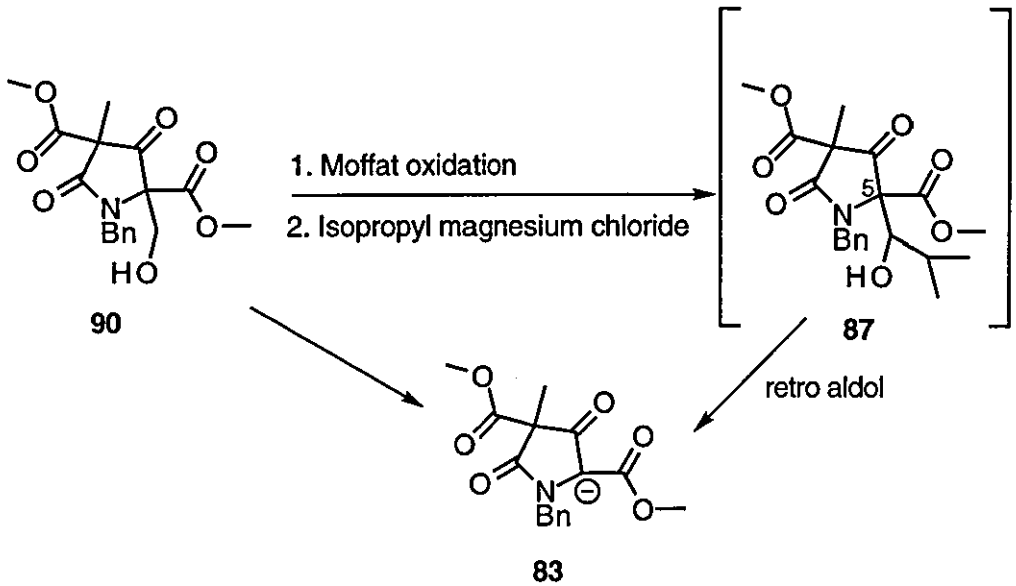
Hence treatment of **83** with excess formaldehyde (formalin) in tetrahydrofuran and DBU as the base at -78 °C gave us the desired hydroxymethyl derivative compound **90** as a mixture of diastereoisomers (2:1) with 62% yield (**Scheme 90**).



Scheme 90

With compound **90** in hand, we then oxidised the alcohol to aldehyde using Moffat conditions and, without isolating the aldehyde, we proceeded with the Grignard reaction using isopropyl magnesium chloride.[47] Although the starting material was consumed completely, purification of the crude products did not give us the expected product **87**, but instead compound **83** was obtained, suggesting perhaps that the retro aldol was occuring. This is not unusual since elimination of

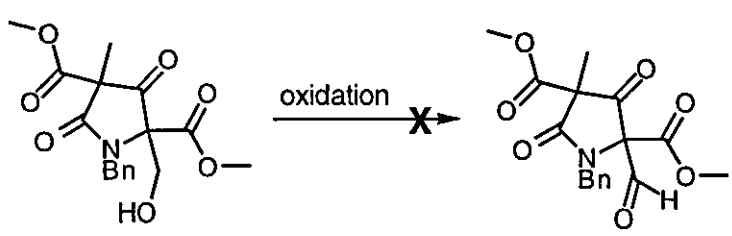
isobutyryl aldehyde from **87**, relieves C-5 from steric congestion, a stabilised carbanion can be formed (**Scheme 91**).



90 **87** **83**

Scheme 91

Since we could not carry out the aldol reaction in one pot (without isolating the aldehyde) we then decided to isolate it first before we proceeded with the Grignard reaction. We carried out the oxidation of the primary alcohol **90** to aldehyde using various reaction conditions such as Dess-Martin, PDC, PCC, TPAP and Moffat, but in each case no aldehyde was detected (**Scheme 92**, **Table 23**).



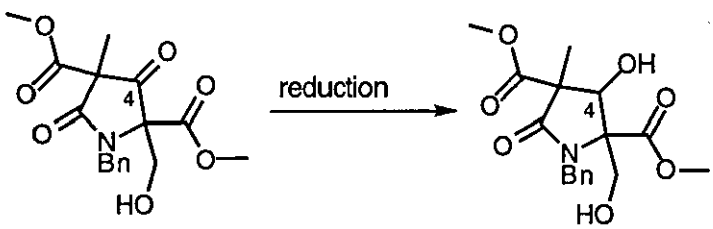
90 **91**

Scheme 92

Table 23

conditions	**91**
Dess-Martin periodinane, DCM, r.t.	no product
PDC, DCM, r.t.	no product
PCC, DCM, r.t.	no product
DMSO, DCC, TFAA, pyridine, r.t.	no product
TPAP, NMO, DCM, mol. sieves, r.t.	no product

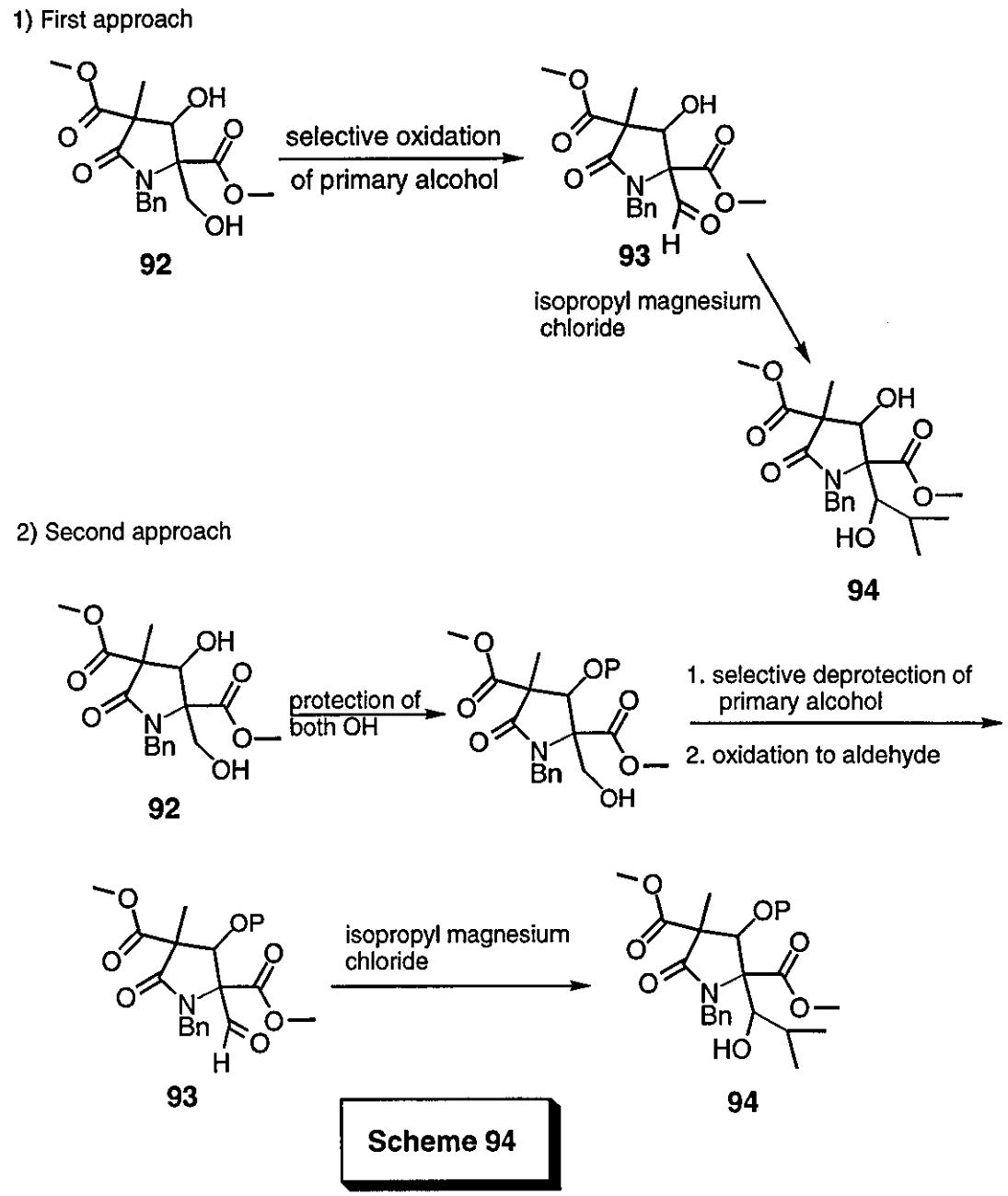
This failure to oxidise **90** could be due to its tendency to undergo retro aldol reaction for the reasons we mentioned above, and we therefore decided to reduce the keto group at C-4 before we attempt the oxidation of the carbinol moiety (**Scheme 93**). It was hoped that with the reduction of carbonyl group at C-4, the tendency for the molecule to undergo retro aldol would be less since the acidity of the C-5 proton of β-keto ester **83** would be reduced (See **Scheme 91**).



90 **92**

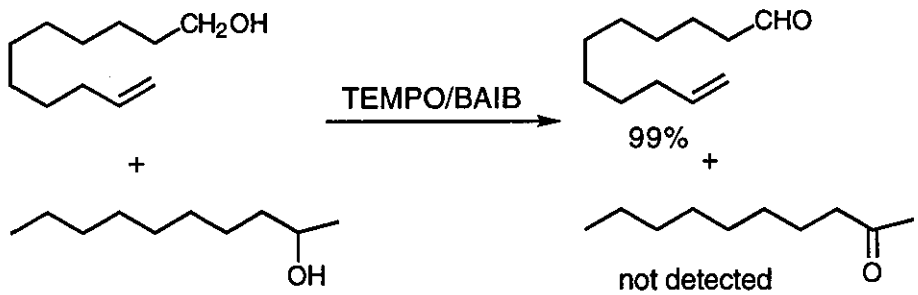
Scheme 93

With the dihydroxy compound **92** in hand, it would be possible to investigate two different approaches towards the synthesis of the lactacystin acid. The first is the selective oxidation of the primary alcohol over the secondary to give aldehyde **93** followed by the addition of isopropyl magnesium chloride to give the desired product **94**. The second approach would be protection of both primary and secondary alcohols followed by selective deprotection of the primary alcohol and then oxidation to the aldehyde. Addition of the isopropyl magnesium chloride to the aldehyde would furnish the aldol **94**. Because the latter approach makes three more synthetic steps we decided to concentrate on the former (**Scheme 94**).



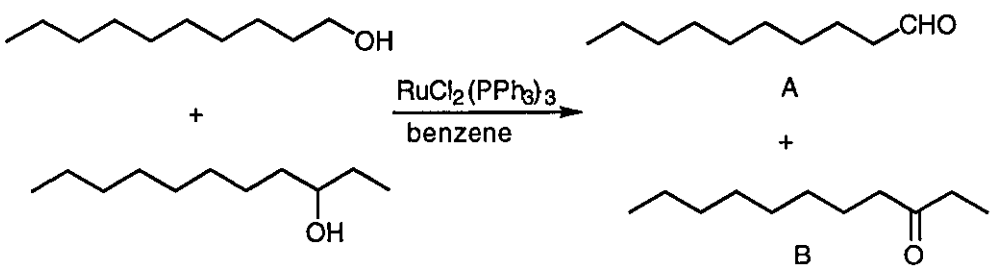
Scheme 94

The selective oxidation of a primary alcohol to the aldehyde in the presence of a secondary alcohol has been well established. In 1997, Mico reported that the oxidation of a primary aliphatic alcohol in the presence of secondary alcohol gives the aldehyde in 99% yield using TEMPO/BAIB [(bis(acetoxy)iodo)]benzene.[48] The reaction was so efficient that the reaction was complete in just six minutes. The corresponding ketone from the secondary alcohol was not detected in this reaction (**Scheme 95**).



Scheme 95

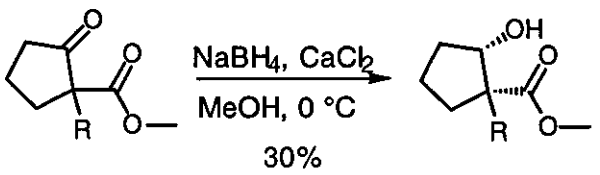
Another example has been reported by Tomioka, in which a primary alcohol was selectively oxidised to the aldehyde in the presence of a secondary alcohol using $RuCl_2(PPh_3)_3$ as the oxidant[49] (**Scheme 96**). Again, in this reaction only a negligible amount of the ketone was formed through the oxidation of the secondary alcohol.



A:B = 50:1

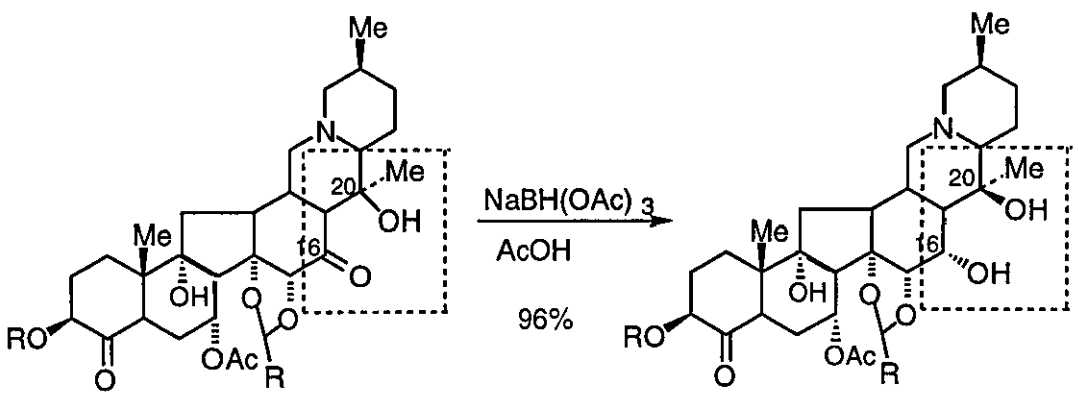
Scheme 96

Before we can carry out the selective oxidation of the primary alcohol to the aldehyde in the presence of a secondary alcohol, we first have to reduce the keto group at C-4 of compound **90** (See **Scheme 93**). The reduction of **90** can be carried out stereoselectively since in lactacystin the OH group at C-4 is *syn* to the ester at C-5. Reduction of a similar type of system to give the desired *syn* β-hydroxy ester has been reported by several authors. For example, Fraga[50] reported that the reduction of α-substituted β-keto ester using sodium borohydride in the presence of calcium chloride gives the *syn* product (**Scheme 97**).



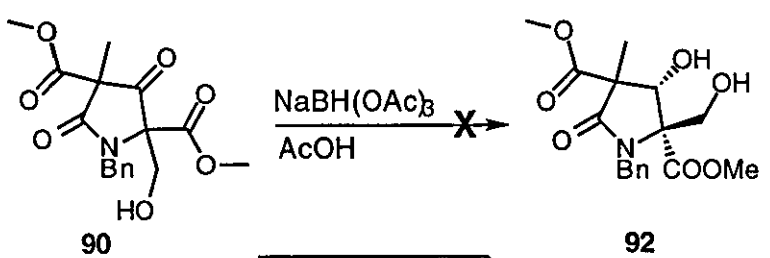
Scheme 97

Another option is to take advantage of the hydroxymethyl group for the stereoselective reduction of the keto group using sodium tricetoxyborohydride. This method was used by Saksena in his work on the total synthesis of veratrum alkaloids.[51] This stereoselective reduction is thought to proceed by intramolecular hydroxyl-directed hydride, delivery which results in the hydroxyl group at C-16 to be *trans* to the hydroxy group at C-20 (**Scheme 98**).



Scheme 98

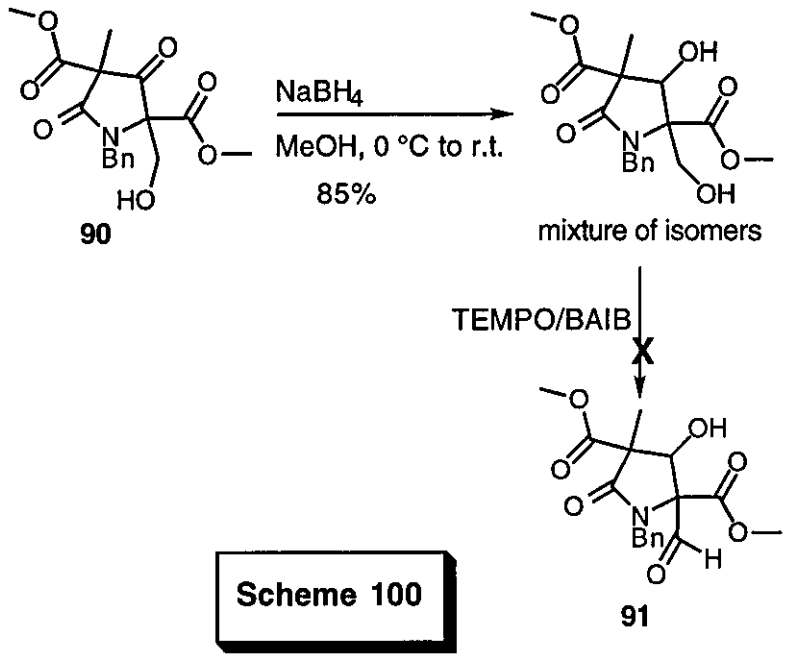
In our first attempted reduction of compound **90**, we used the method of Saksena, but unfortunately we did not detect the formation of the required *syn* β-hydroxy ester **92**. Instead a complex mixture of products was obtained (**Scheme 99**).



90 **92**

Scheme 99

However, when the reduction of **90** was carried out using sodium borohydride in methanol, it did give us the desired compound, but as a mixture of isomers which was difficult to separate. An attempt to selectively oxidised the primary alcohol over the secondary alcohol using TEMPO/BAIB, however, failed to give the desired aldehyde **91** (**Scheme 100**).

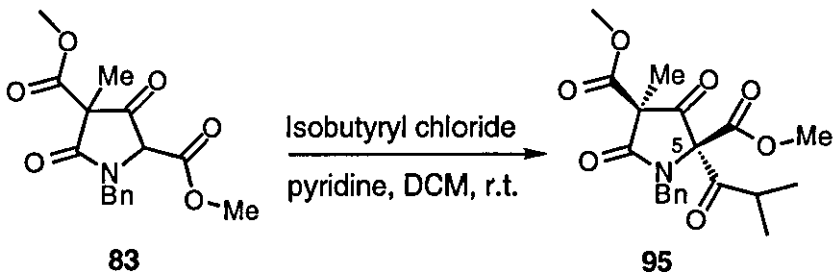


90 mixture of isomers **91**

Scheme 100

2.3.8 Acylation reaction of 83

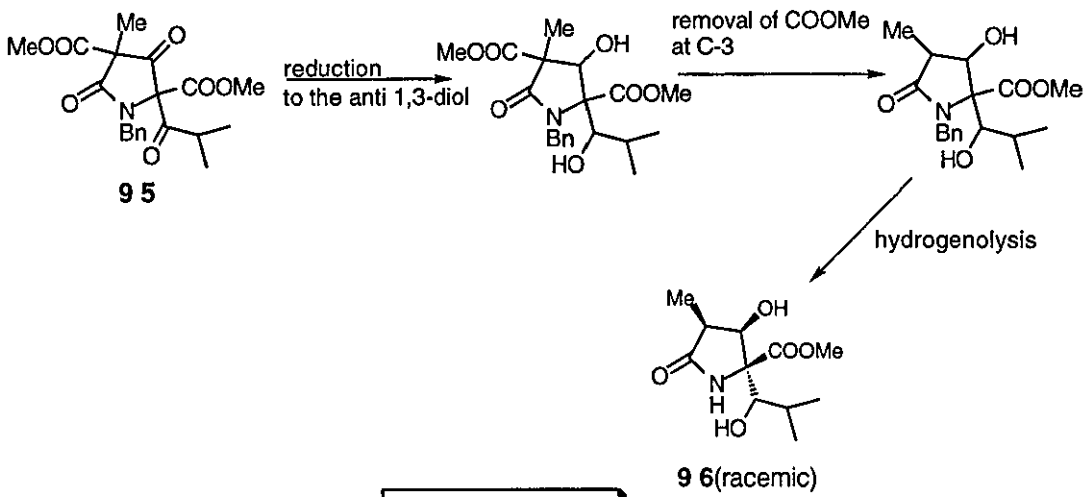
Since we had failed in carrying out the aldol reactions, we envisaged that the isobutyryl group might be introduced by an acylation reaction. Treatment of **83** with the isobutyryl chloride and pyridine gave the pyrroglutamate ester **95** in 90% yield and as one diastereoisomer (**Scheme 101**). With the introduction of the isobutyryl group on **83** we have successfully assembled the full carbon skeleton of the carboxylic acid precursor of lactacystin in just five steps.



83 **95**

Scheme 101

In order to obtain the important acid precursor **96**, we only needed to carry out three transformations: the first is the reduction of both keto groups, the second and third involve the removal of both methyl ester at C-3 and the benzyl protecting group on nitrogen. It is also worth pointing out that the ketone reductions might be carried out stereoselectively (**Scheme 102**).

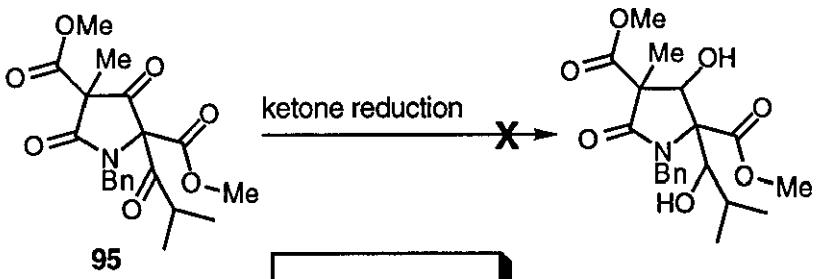


9 5

9 6(racemic)

Scheme 102

With compound **95** in hand, we attempted the reduction of the both ketone groups. To our surprise, none of the methods we tried with various reducing agents gave us the expected diol. Instead either no reaction (entries 1 and 6) , or complex mixtures, were obtained (**Scheme 103**, **Table 24**).

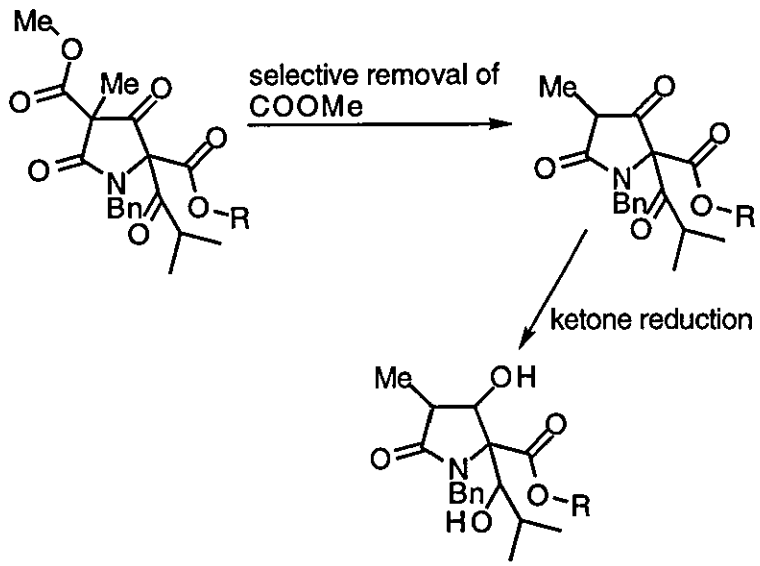


Scheme 103

Table 24

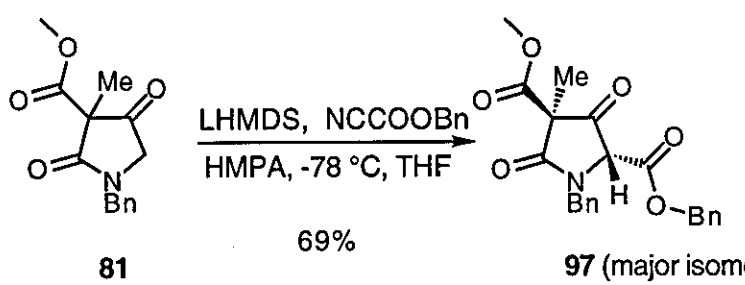
conditions	results
1. $NaBH_4$, MeOH, -78 °C	no reaction
2. $NaBH_4$, MeOH, r.t.	complex mixture
3. $NaBH(OAc)_3$, AcOH, r.t.	complex mixture
4. $NaBH(OAc)_3$, CH_3CN, AcOH, r.t. then reflux	complex mixture
5. $NaBH_4$, $MnCl_2$, r.t.	complex mixture
6. Ph_2SiH_2, $[Rh(COD)_2]BF_4$, THF, r.t.	no reaction

One possible explanation for the reduction being unsuccessful might be due to the steric effect associated with both methyl esters at C-3 and C-5, and the isopropyl group at C-5. These may prevent the reducing agent from delivering the hydride ion to the carbonyl functions. If this is the case, obviously we have to remove some of the steric congestions before we carry out the reduction. One way of accomplishing this may be through the removal of the ester group at C-3 by decarboxylation. In order to discriminate between the two ester groups however, we decided that they should be derived from different alcohols (**Scheme 104**).



Scheme 104

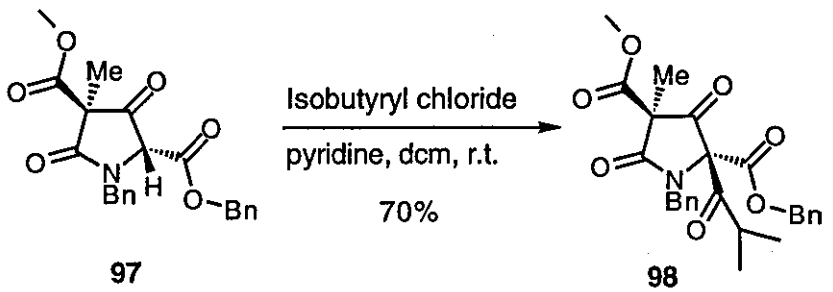
We decided to keep the methyl ester at C-3 because we knew that such esters can easily be decarboxylated. Since we have to introduce a different type of ester at C-5 which survives the removal of the methyl ester at C-3, we decided to use a benzyl ester. The introduction of this ester can easily be accomplished using the commercially available benzyl cyanoformate. Furthermore, the ester can be easily removed or hydrolysed under acidic conditions. Hence, treatment of **81** with lithium bis(trimethylsilyl) amide, benzyl cyanoformate and HMPA gave the keto ester **97** which was obtained as mixture of diastereoisomers (2:1) (**Scheme 105**).



81 **97** (major isomer)

Scheme 105

Using the same conditions described earlier, **97** was treated with isobutyrylchloride and pyridine to give us the important intermediate **98** in the synthesis of the acid portion of lactacystin (**Scheme 106**). Although the starting material initially consists of mixture of diastereoisomers, the product however, turned out to be a single diastereoisomer as in the case of the methyl ester derivative **95** (See **Figure 2.6** for the NMR of **97** and **98**)



97 **98**

Scheme 106

Having successfully introduced the isobutyryl group at C-5, the next step of the synthesis involved the selective removal of the methyl ester at C-3 before reducing both the keto groups. Selective removal of the methyl ester or demethoxycarbonylation in the presence of different esters in β-keto ester systems is well documented.[52] Some of the most commonly used reagents for this transformation include lithium iodide/dimethylformamide, lithium iodide/pyridine, sodium cyanide/hexamethyl phosphoramide, sodium chloride/wet dimethyl sulfoxide.

Using the synthetic route we have described, it is possible to obtain **98** in large amounts. We attempted several reactions for the removal of the methyl ester, but unfortunately all of them failed to give the desired product **99**. Most of the products obtained were either complex mixtures, and in one case no reaction had taken place (**Scheme 107, Table 25**).

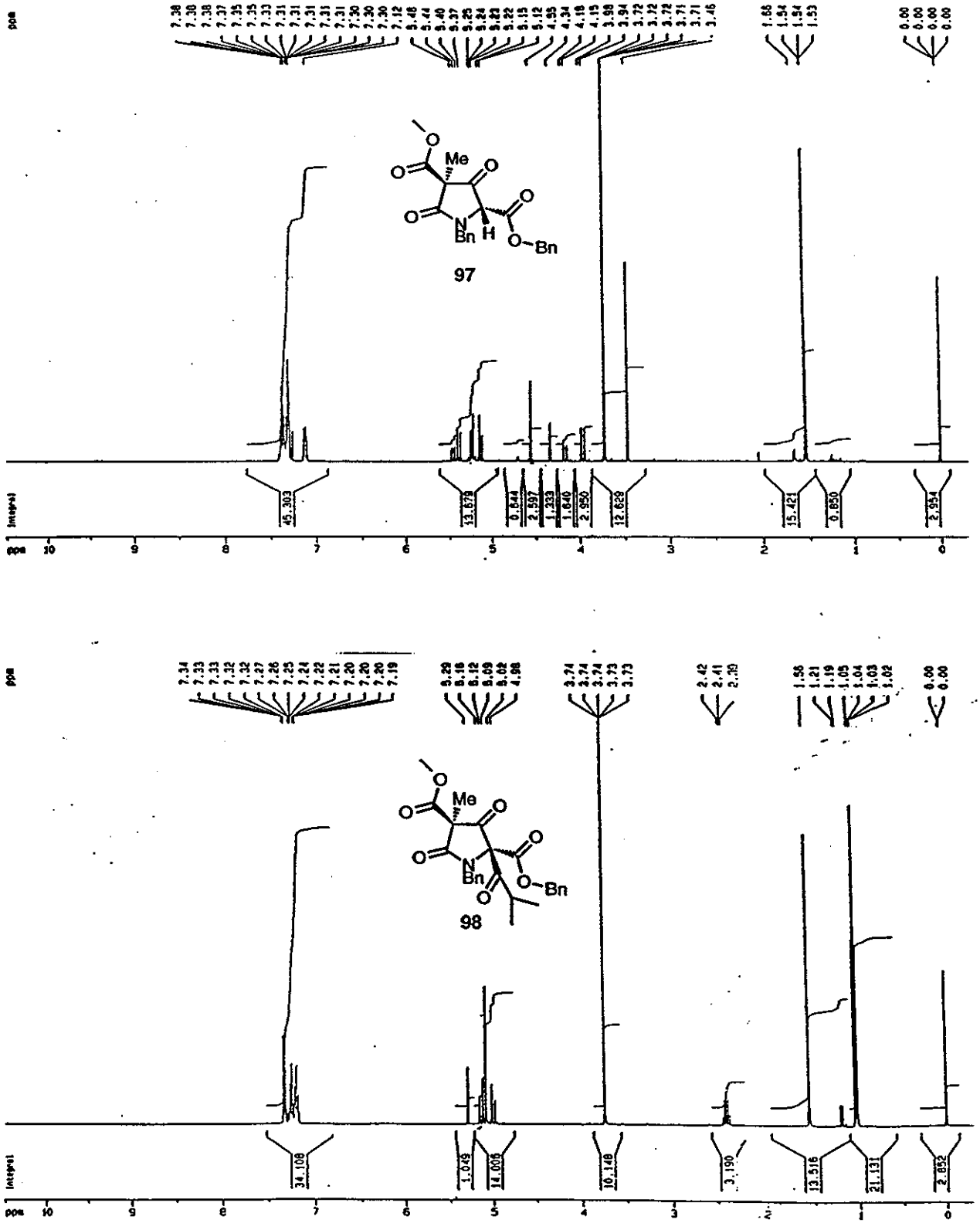
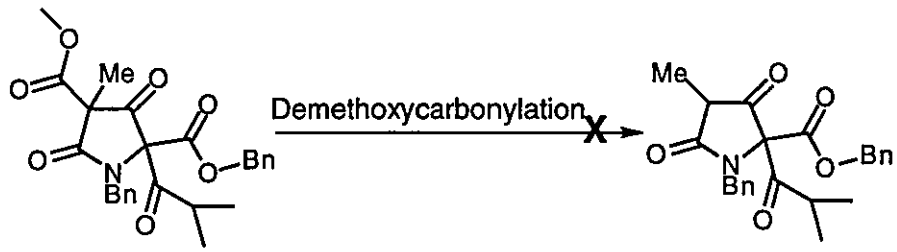


Figure 2.6 NMR spectra of **97** and **98**



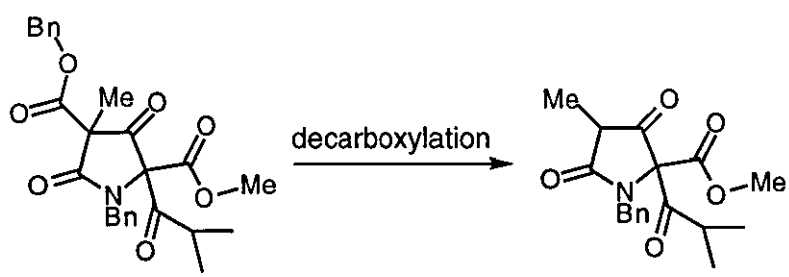
98 99

Scheme 107

Table 25

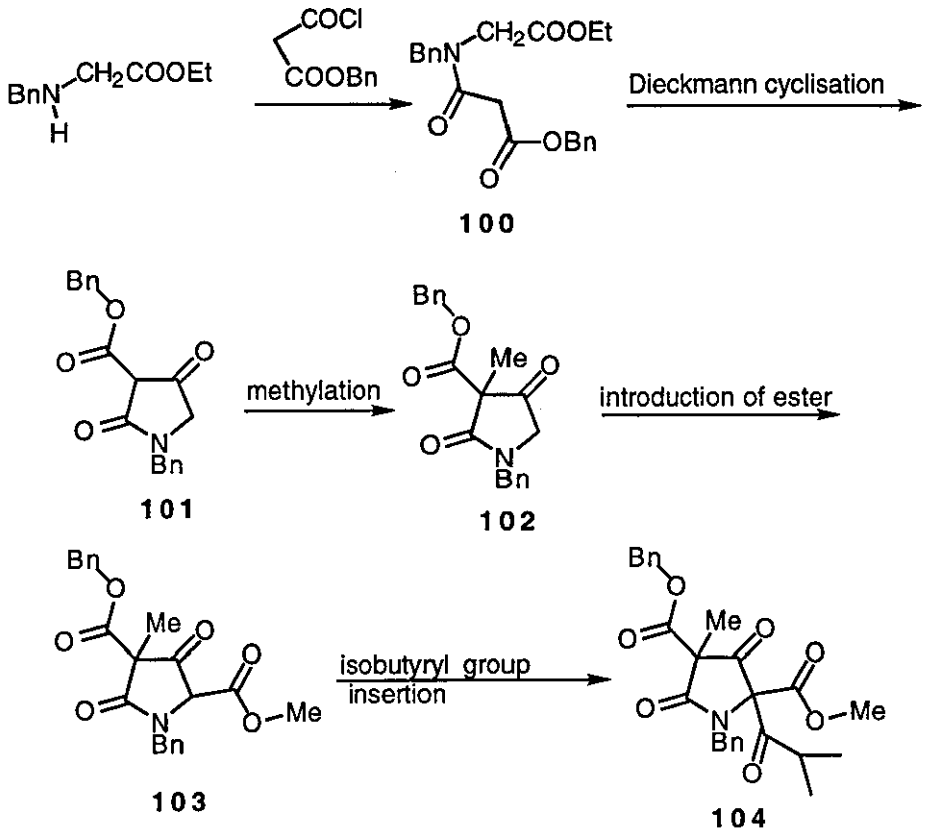
conditions	results
CH_3CN, reflux	no reaction
LiI, CH_3CN, reflux	complex mixture
LiI, DMF, reflux	complex mixture
NaCl, wet DMSO, reflux	complex mixture
KOH, MeOH, r.t.	complex mixture
LiCl, HMPA, reflux	complex mixture
NaCN, HMPT, reflux	complex mixture

Since we have failed to remove the methyl ester at C-3 we decided to continue our synthetic route with a different derivative. Instead of having a methyl ester group at the C-3 position, we decided to replace it with a benzyl ester and have the methyl ester at C-5. We hoped that the benzyl ester would be easily removed under mild conditions such as hydrogenolysis. Furthermore, since the benzyl ester is located between two carbonyl groups, the decarboxylation should be fast (**Scheme 108**).



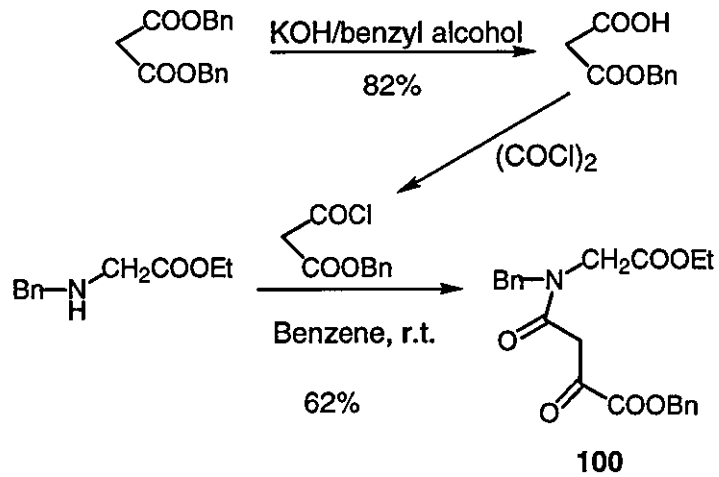
Scheme 108

Since we have optimised the conditions for the synthesis of **98**, it should be straightforward to prepare the intermediate **104**, in which the esters at C-3 and C-5 are switched around. The only main difference is that the starting precursor has to be changed from methyl malonyl chloride to benzyl malonyl chloride (**Scheme 109**).



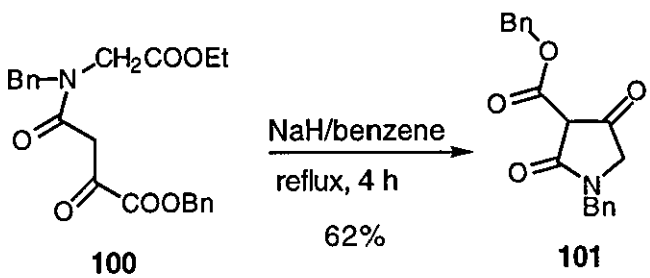
Scheme 109

Our synthesis started by treating *N*-benzylglycine ethyl ester with benzyl malonyl chloride (prepared from dibenzyl malonate in two steps[53]) to give the condensation product **100** in 62% yield (**Scheme 110**).



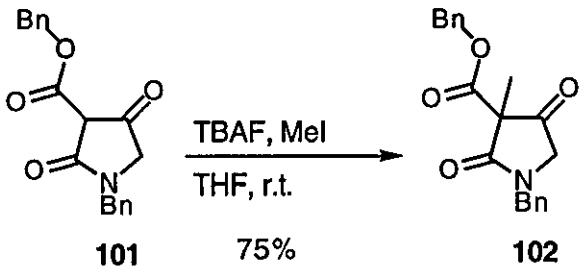
Scheme 110

Compound **100** was then subjected to Dieckmann cyclisation conditions using sodium in benzyl alcohol, to give the cyclised product **101**, but in poor yield (14%). Fortunately, changing the conditions from sodium/benzyl alcohol to sodium hydride/benzene, increased the yield of the cyclised product to 62% (**Scheme 111**).



Scheme 111

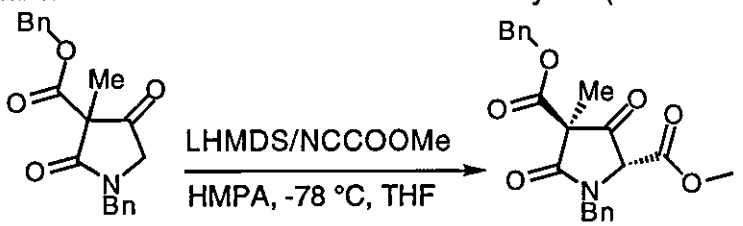
Treatment of **101** with methyl iodide and tetrabutylammonium fluoride furnished the methylated compound **102** in 75% yield (**Scheme 112**).



101 **102**

Scheme 112

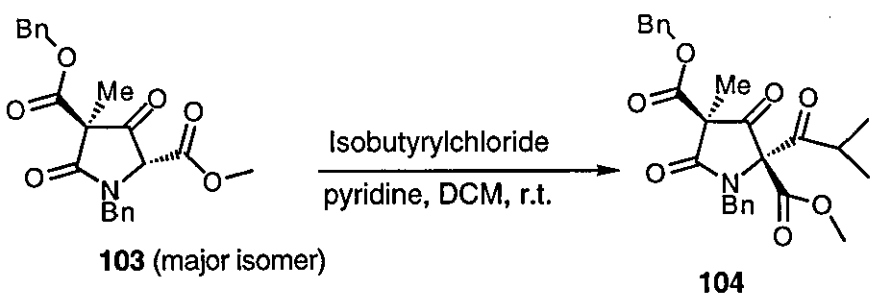
With compound **102** in hand, the methyl ester group was introduced at C-5 using lithium bis(trimethylsilyl)amide and methyl cyanoformate to give the β-keto ester **103** as a mixture of diastereoisomers 2:1 in 70% yield (**Scheme 113**).



102 **103** (major isomer)

Scheme 113

Under the conditions described above, the isobutyryl group was introduced at C-5 using isobutyrylchloride and pyridine. This provided us with the important intermediate **104** as one diastereoisomer in 66% yield (**Scheme 114**).

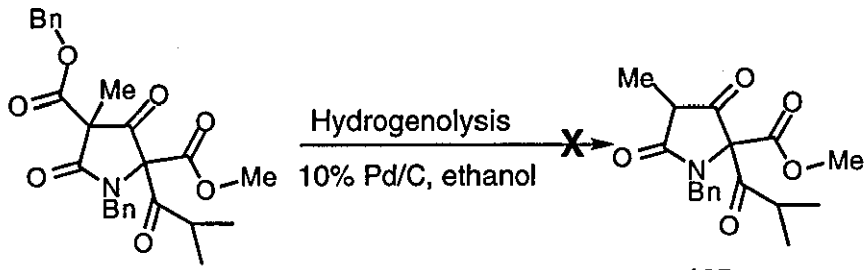


103 (major isomer) **104**

Scheme 114

The next steps of our synthesis involved the removal of the benzyl ester at C-3 followed by the reduction of both keto groups. In our first attempt at

hydrogenolysis of **104**, the reaction was carried out using standard conditions i.e. 10% Pd/C in ethanol. Although the starting material was totally consumed within thirty minutes, the product that was isolated turned out to be the C-5 deacylated material judging from the absence of the isopropyl group in the ^{1}H NMR spectrum of the crude product, perhaps through a retro Claisen process (**Scheme 115**).

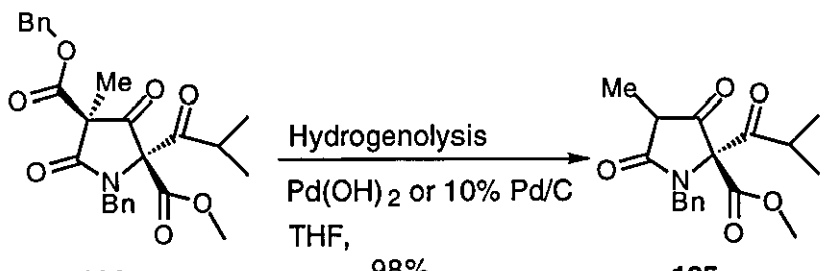


104 **105**

Scheme 115

This observations suggested that a nucleophilic solvent such as ethanol is not suitable for this type of transformation. Although we are not certain what happens to the substrate, presumably ethanol acts as a nucleophile and attacks the isopropyl ketone, leading to a retro Claisen reaction. It is possible that the extensive decomposition we observed during the decarboxylation of the methyl ester at C-3, may arise from similar reactions at the isopropyl keto group, under the strongly nucleophilic conditions used.

Since ethanol was not suitable for the hydrogenolysis we decided to use tetrahydrofuran as the reaction solvent using $Pd(OH)_2$ or 10% Pd on carbon. This time the hydrogenolysis went smoothly to furnish the desired product **105** in quantitative yield. The reaction took just thirty minutes to complete using $Pd(OH)_2$, and three hours with 10% Pd/C (**Scheme 116**).



104 **105**

Scheme 116

Interestingly, the NMR spectrum of compound **105** indicates that the compound exists entirely in the keto form, although one might expect that the preferred structure would be the enol form due to the acidity of the hydrogen at C-3 (See **Figure 2.7** for proton and carbon NMR data of **105**). At this stage, we have successfully synthesised the full carbon skeleton of the advanced intermediate **105** of lactacystin in just six steps, and no column chromatography was required.

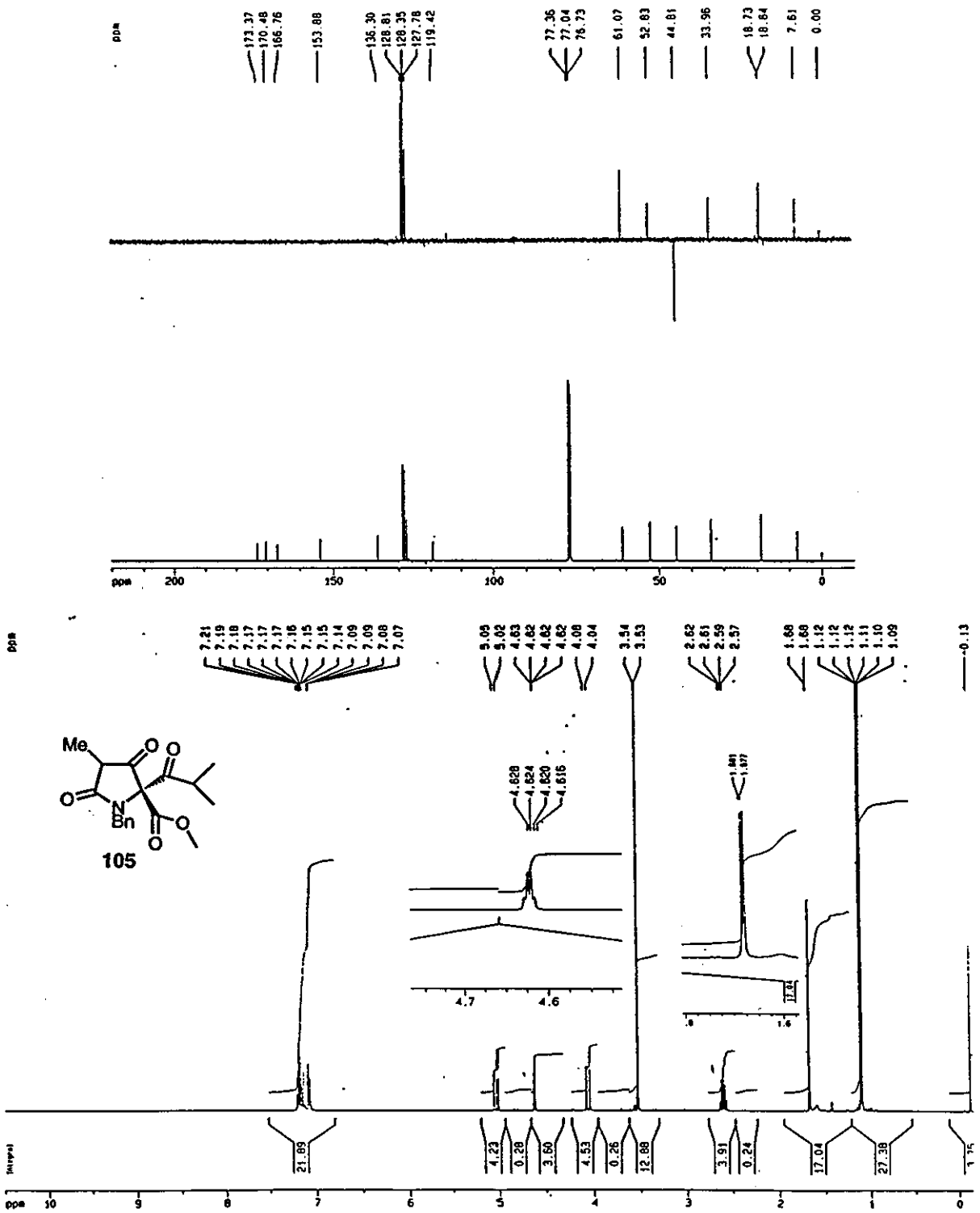


Figure 2.7 NMR spectra of **105**

2.4 CONCLUSION

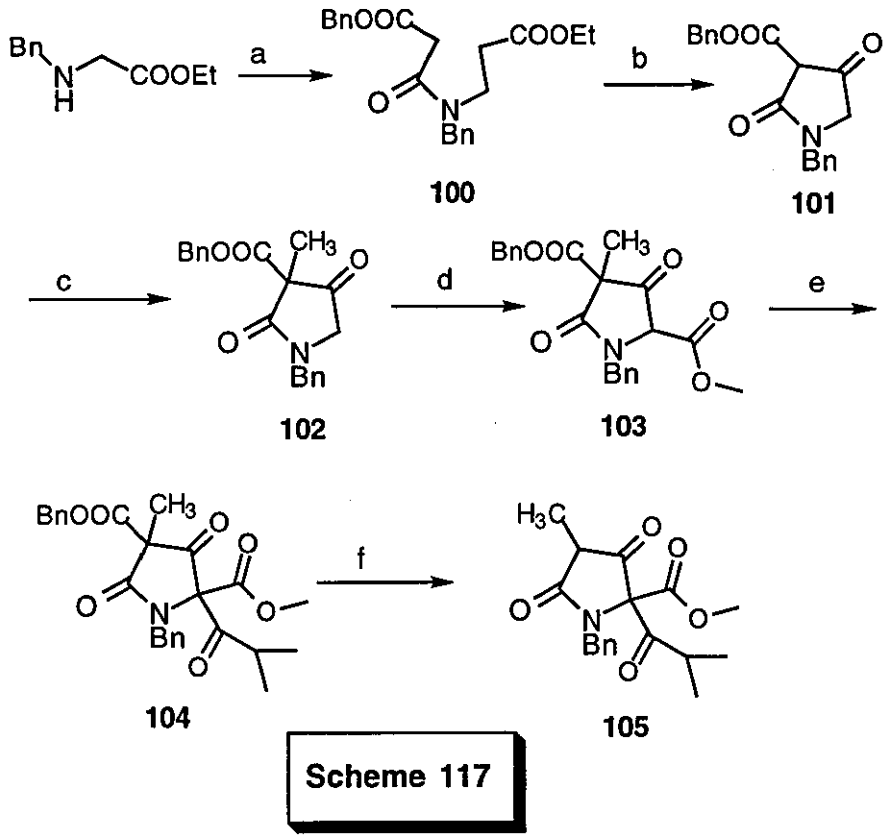
Our synthetic appproached towards the synthesis of lactacystin started with the condensation between glycine ethyl ester and methyl or benzyl malonyl chloride and also alkyl malonates. Attempts to protect the nitrogen atom of the carbamate with Boc anhydride failed to give *N*-protected carbamate due to competing side reactions. We solved this problem by condensing *N*-benzylated glycine ethyl ester with methyl or benzyl malonyl chloride to give the carbamates **79** and **100** with 88% and 62% yield, respectively.

Dieckmann cyclisation of the *N*-benzylated carbamates using sodium hydride or sodium methoxide in benzene furnished the pyrrolidinone ring. Alkylation of this β-β-ketoester amide pyrrolidinones **80** and **101** was rather troublesome since efforts to alkylate with methyl iodide in the presence of bases such as sodium hydride, potassium tertiary butoxide and potassium carbonate, failed to give the methylated compounds **81** and **102**. We believed that the lack of reactivity of this system is due to the insolubility of the compound in most conventional solvents and also to the stability of the resulting anion. Fortunately, the methylation reaction was successfully when TBAF was used since it acts as both the base and phase transfer agent.

Following methylation of the pyrrolidinone, the compound was then treated with benzyl or methyl cyanoformate to give us the corresponding β-keto esters **83**, **97** and **103** in 2:1 diastereoisomeric ratio. Attempts to obtain the aldol products directly from the β-keto esters and isobutyraldehyde were not successful due to retro-aldol reaction. However, reaction of the esters with isobutyryl chloride occurs readily in the presence of pyridine to give us the advanced intermediates **95**, **98** and **104**, each as one diastereoisomer in good yield.

Attempts to decarboxylate the methyl ester at C-3 of **98** using different conditions failed to give the desired product. In some cases examined, loss of the isobutyryl group was observed under the nucleophilic reaction conditions. Using the schemes described above, we also prepared compound **104** in 70% yield, and in one diastereoisomeric form. We hoped that the mild conditions required for the benzyloxy group at C-3 would not cause cleavage of the isobutyryl group. Intially, when we carried out the hydrogenolysis using Pd/C in methanol we still observed the cleavage of the isobutyryl group. However, switching to tetrahydrofuran as the reaction solvent with palladium hydroxide as the catalyst gave us the desired product **105** in quantitative yield.

In summary, the advanced intermediate **105** in the synthesis of lactacystin has been prepared in diastereomerically pure form in six steps with 14% overall yield on a gram scale (**Scheme 117**).



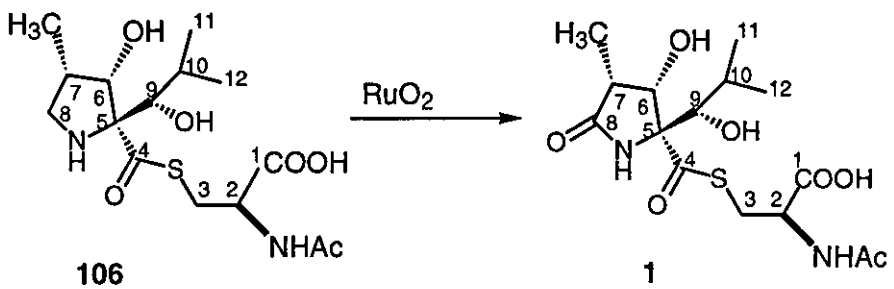
Scheme 117

Conditions: (a) $ClCOCH_2COOBn$, benzene, 0 °C to r.t. 24 h, 62% (b) NaH/benzene, reflux, 4 h, 62% (c) TBAF, MeI, r.t. 12 h, 75% (d) LHMDS, HMPA, CNCOOMe, -78 °C, 2 h, 70% (e) Isobutyryl chloride, pyridine, r.t. 1 h, 73% (f) $Pd(OH)_2$, H_2, 15 mins, 96%.

2.5 RESULTS AND DISCUSSION

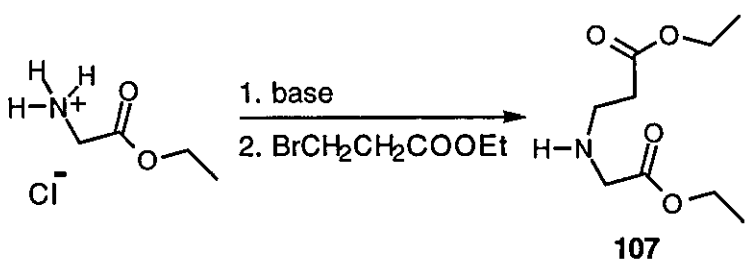
2.5.1 Towards the total synthesis of 8-deoxoactacystin (106)

Although the main objective of this research work is to synthesise the natural product lactacystin, we are also interested synthesise a derivative of lactacystin 8-deoxolactacystin **106.** It is interesting to note that this compound can also be transformed into natural lactacystin by oxidation of the C-8 carbon to ketone using ruthenium oxide.[54] (**Scheme 118**).



Scheme 118

The synthesis of 8-deoxolactacystin started with the condensation between glycine ethyl ester salt and 3-bromoethyl propionate to give diethyl 3-azahexane-1,6-dicarboxylate, **107 (Scheme 119).** Initial attempted reaction using the procedure of Takada[55] failed to give the carbamate in good yield. We managed to overcome this problem by treating the glycine ethyl ester salt with sodium hydroxide to liberate the free amine, and reacted this directly with the bromo compound (**Table 26**).

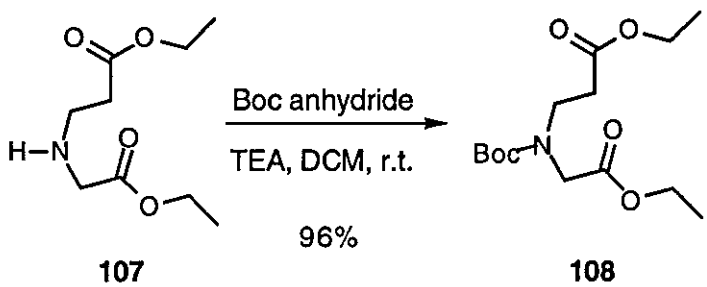


Scheme 119

Table 26

conditions	yield of **107**
K_2CO_3, EtOH, reflux	10%
NaOH, DCM, reflux	60%

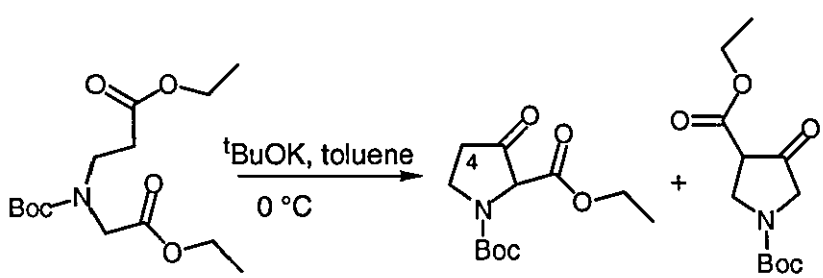
With the carbamate **107** in hand, the nitrogen atom was first protected with BOC anhydride in the presence of triethylamine to give the *N*-Boc protected carbamate in 96% yield (**Scheme 120**).



107 **108**

Scheme 120

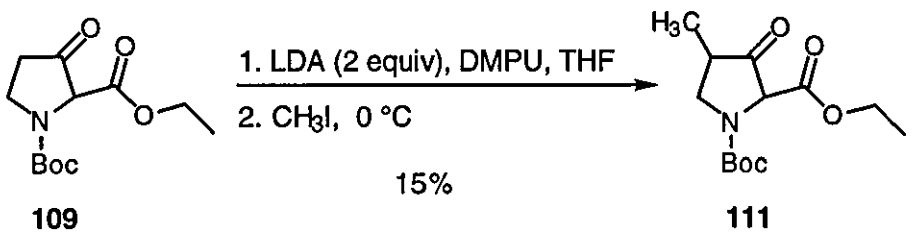
Dieckmann cyclisation of the *N*-protected diester **108** using Rapoport's procedure[19] gave a 1:1 mixture of the desired regioisomer **109** and the alternative product **110** (**Scheme 121**). Fortunately, both isomers can be separated by extraction from aqueous pH 9.5 carbonate buffer in which the side product **110** was more soluble.



108 **109** **110**

Scheme 121

The *N*-protected pyrrolidine carboxylate **109** was then methylated specifically at C-4 using dianion chemistry.[56] This was achieved by treatment of **109** with two equivalents of LDA, methyl iodide and DMPU to give the methylated compound **111** in 15% yield (**Scheme 122**).

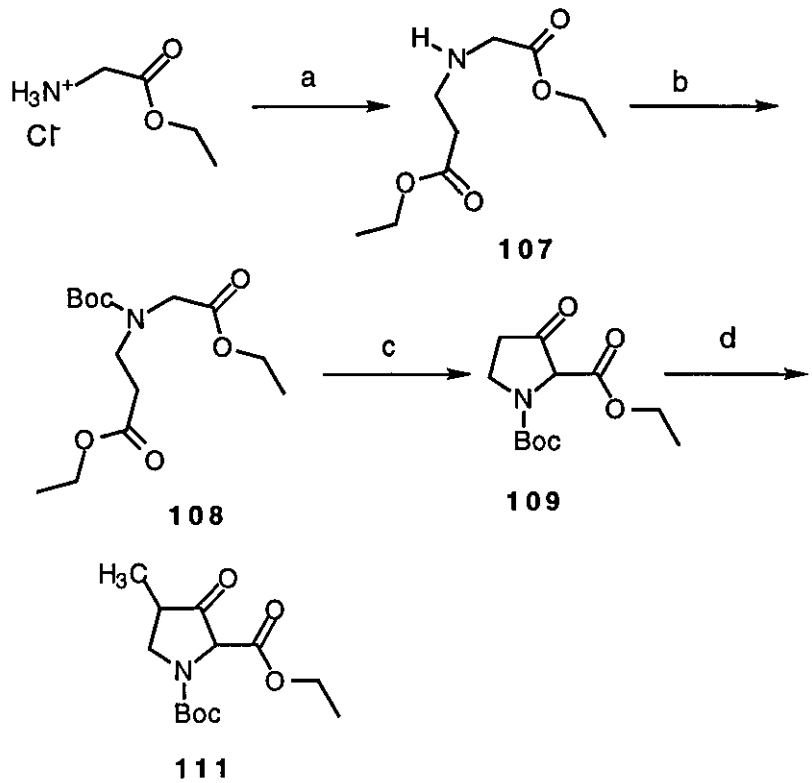


109 **111**

Scheme 122

2.5.2 CONCLUSION

Our synthetic approach towards the synthesis of 8-deoxolactacystin started with coupling between glycine ethyl ester and 3-bromoethyl propionate gave the carbamate **108** with moderate yield. Protection of the nitrogen atom using Boc anhydride furnished the *N*-protected carbamate in quantitative yield. Dieckmann cyclisation of this carbamate gave a 1:1 mixture of the 3-oxo and 4-oxo pyrrolidinones **109** and **110** which fortunately can be separated using buffer solution. Methylation of **109** using dianion chemistry furnished the 4-methylated compound **111**. In conclusion, the important intermediate **111** for the synthesis of 8-deoxolactacystin was synthesised in four steps (**Scheme 123**).

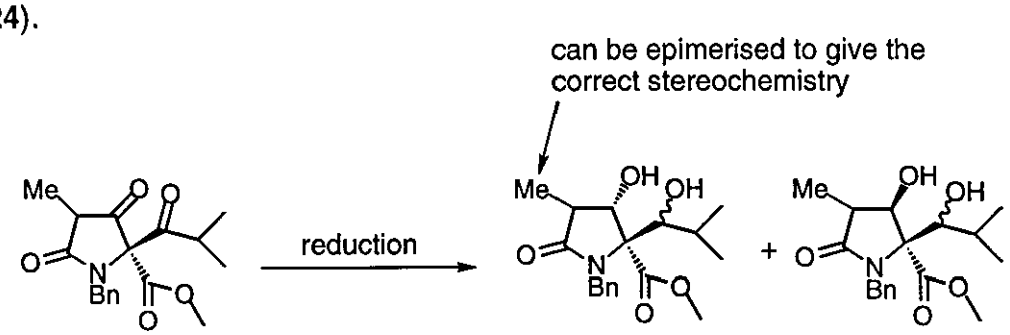


Scheme 123

Conditions: (a) $BrCH_2CH_2COOEt$, NaOH, DCM, reflux, 60% (b) Boc anhydride, TEA, DCM, r.t. 96% (c) ^tBuOK, toluene, 0 °C, 40% (d) LDA, DMPU, 0 °C, 15%

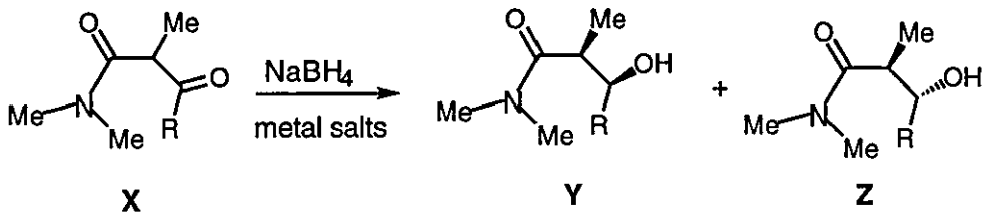
2.6 RECOMMENDED FUTURE WORK

With the important advanced intermediate **105** already synthesised, we are just three steps away from the target molecule lactacystin. The first of these steps would be the stereoselective reduction of **105** at C-4 to give us the required dihydroxy acid precursors **112a** and possibly the other isomers **112b** (**Scheme 124**).



Scheme 124

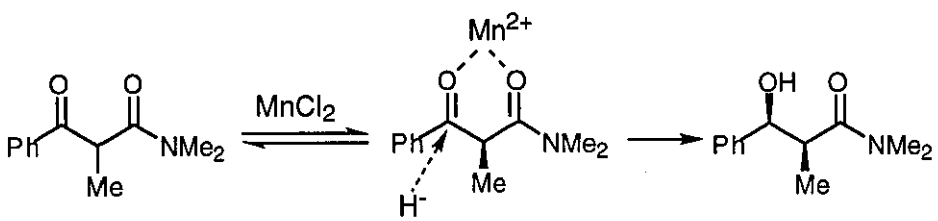
Taniguchi has reported the stereoselective reduction of 2-methyl-3-oxo amides with $NaBH_4$ in the presence of various metal salts.[57] The nature of the reduction products, either the *erythro* Y or *threo* Z, can be selectively controlled by using different metal salts **(Scheme 125).**



X Y Z

Scheme 125

The results showed that when $MnCl_2$ was used as the catalyst, most of the reduction products gave the **Y** isomer whereas with *n*-Bu_4NBH_4, the **Z** isomer predominates. The selective reduction to *erythro* when $MnCl_2$ was used as the catalyst can be rationalised by assuming that the hydride attacks the carbonyl from the opposite side of the methyl group (**Scheme 126**). When *n*-Bu_4NBH_4 was used as the catalyst, the *threo* product dominates because there is no counter ion present that coordinates with the two carbonyls and obviously the hydride anion will attack the carbonyl from the opposite side of the bulky carboalkoxy group.

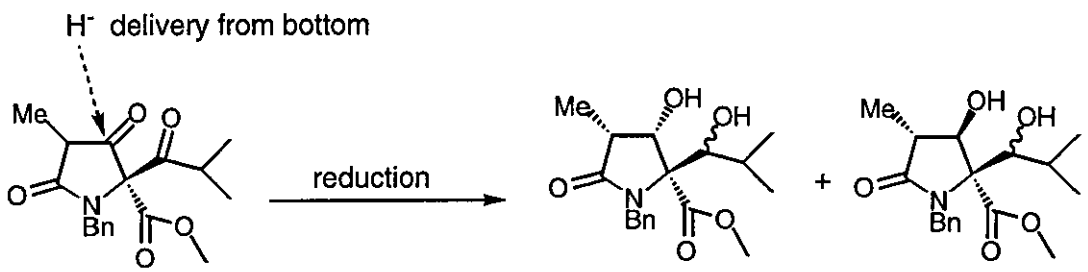


Y

Scheme 126

As for the reduction of **105**, we will have to try different reaction conditions in order to achieve the stereoselective transformation to the required product. Although we may be able to control the reduction of the C-4 carbonyl adjacent to the methyl group to give us the configuration with the alcohol at C-4 *cis* to the ester (**112a**), we are not certain whether the reduction of the exocyclic keto group will give the desired stereochemistry as shown in **Scheme 127.**

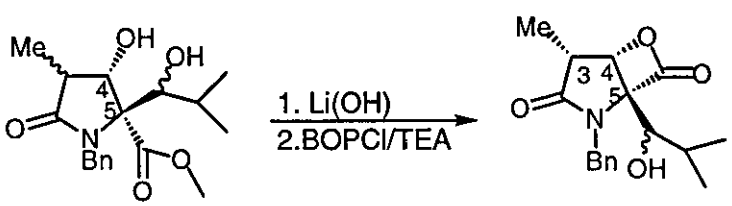
It is expected that the bulky isobutyl group will dictate the outcome of the reduction of the ketone groups. If this is the case, then the delivery of the hydride would have to be from the same side of the isobutyl group to the keto at C-4 in order to give **112a**. But it would be expected that the hydride would be delivered from the less hindered side of the molecule i.e. from the bottom to give **112b** as the major product and **112a** as the minor. Nevertheless, even if we obtained both isomers **112a** and **112b**, we might be able to separate them using chromatographic techniques. Alternatively, the other side product **112b** can be used to prepare derivatives of lactacystin (**Scheme 127**).



105 **112a** minor **112b** major

Scheme 127

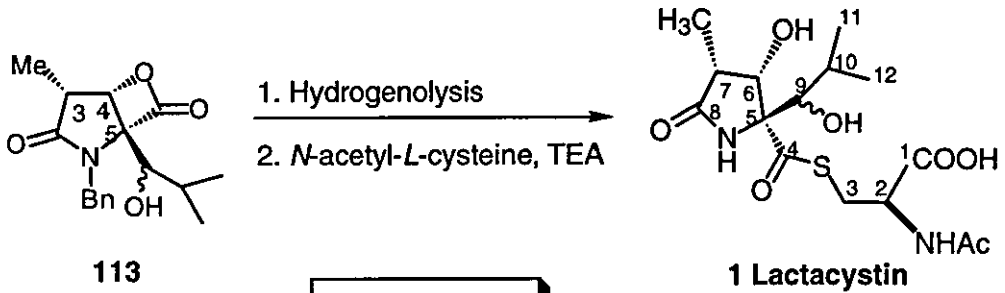
With compound **112a** in hand, the next step of the synthesis would be the epimerization at C-3 followed by the formation of the important lactone **2** using Corey's protocol.[1] In this lactonization process, only one of the isomers will be able to form the lactone since both the hydroxyl at C-4 and the methyl ester at C-5 have to be *cis* to one another. This transformation will be carried out by saponification of **112a** with $Li(OH)_2$ followed by treatment with BOPCl to give the lactone **113** (**Scheme 128**).



112a **113**

Scheme 128

Removal of the benzylic group by hydrogenolysis followed by coupling with *N*-acetyl-*L*-cysteine will give us the target molecule lactacystin **1**. In summary, only nine steps will be required to synthesise the natural product lactacystin by this route if the stereochemistry is controllable including the stereochemistry at the isopropyl alcohol (**Scheme 129**).

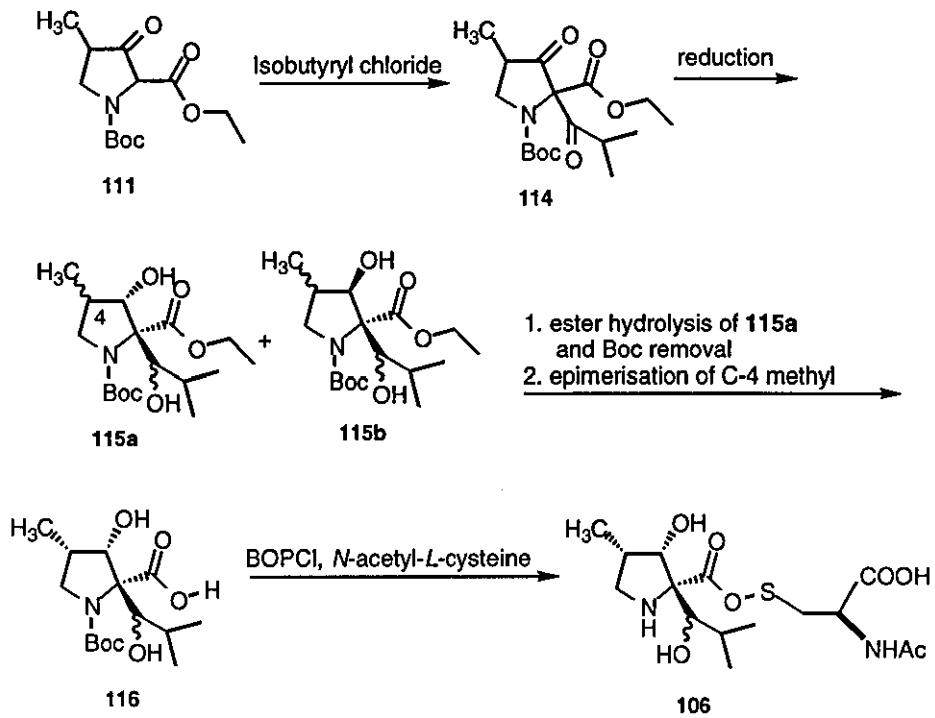


113 **1 Lactacystin**

Scheme 129

As for the synthesis of 8-deoxolactacystin **106**, the next step of the reaction would be the introduction of the isobutyryl group to give **114** using the conditions described earlier. The introduction of the isobutyryl group hopefully will give us one diastereoisomer as we have discovered in our earlier work. Following this, the compound will be reduced stereoselectively either using yeast or modified hydride reagents to give the reduced products **115a** and **115b**. It is expected that reduction of the excylic keto group would give mixture of isomers as in the case of lactacystin.

Separation of these isomers followed by hydrolysis of the ester and removal of the Boc group will furnish the acid skeleton **116**. Following epimerisation of the methyl group at C-4, the final transformation to 8-deoxolactacystin would be carried out using Corey's protocol i.e. BOPCl with *N*-acetyl-*L*-cysteine (**Scheme 130**).


Isobutyryl chloride
reduction
Boc
111
114
115a
115b
1. ester hydrolysis of 115a
and Boc removal
2. epimerisation of C-4 methyl
116
BOPCl, N-acetyl-L-cysteine
106
COOH
NHAc

Scheme 130

CHAPTER THREE

3.0 EXPERIMENTAL

3.1 GENERAL EXPERIMENTAL PROCEDURES

3.1.1 Purification of reagents, compounds and solvents

Commercially available reagents were used as supplied, without further purification, unless otherwise stated. Air and sensitive compounds were stored in a dessicator over self-indicating silica pellets, under nitrogen atmosphere.

Flash chromatography was carried out using Merck 9385 Kieselgel 60-45 (230-400 mesh) and hand bellows to apply pressure to the column. Analytical thin layer chromatography was carried out using aluminium backed plates coated with Merck Kieselgel 60 GF_{254}. Plates were visualised under UV light (at 254 nm), staining with potassium permanganate solution followed by heating or by exposure to an ethanolic solution of phosphomolybdic acid, (acidified with concentrated sulfuric acid), followed by charring where appropriate.

Light petroleum refers to the fraction of petroleum ether which boils between 40 °C and 60 °C and was distilled from anhydrous $CaCl_2$ before used. Ethyl acetate and dichloromethane were distilled from anhydrous $CaCl_2$ and phosphorous pentoxide, respectively. Tetrahydrofuran was distilled from the sodium/benzophenone ketyl radical or lithium aluminium hydride before use. Triethylamine and diisopropyl-ethylamine were stored over potassium hydroxide pellets.

3.1.2 Preparation of glassware

Highly air- and moisture- sensitive reactions were carried out using glassware that had been dried overnight in an oven at 150 °C. These were allowed to cool in a desiccator over self-indicating silica pellets, under a nitrogen atmosphere. All organometallic and air-sensitive reactions were carried out under slight static positive pressure of nitrogen, and reagents and solvents were introduced using syringe or cannula techniques, through a septum cap.

3.1.3 Elemental analyses and melting points

Microanalyses were performed on a Perkin Elmer Elemental Analyser 2400 CHN, and melting points were measured on a Leica Gallin hot plate melting point apparatus or using an Electrothermal-IA 9100 apparatus and were uncorrected.

3.1.4 Infrared and Mass spectra (IR, MS)

Fourier transformed infrared absortion spectra were recorded on a Perkin Elmer Paragon 2001 instrument in the range 600-4000 cm^{-1}. Solid samples were run as nujol mulls on sodium chloride discs or as thin films of their solution usually in dichloromethane. Liquid samples were run neat on sodium chloride discs.

High and low-resolution mass spectra were recorded on a Kratos MS80 or Jeol (JMX)SX102 instrument using electron impact (EI), ionisation technique.

3.1.5 Nuclear Magnetic Resonance (NMR)

Proton nuclear magnetic resonance spectra was recorded using Bruker AC-250 and Bruker DPX-400 instruments operating at 250.13 and 400.13 MHz, respectively. The experiments were conducted in deuteriated solvents with tetramethylsilane as the internal standard. Multiplicities were recorded as broad signals (br.s), singlets (s), doublets (d), triplets (t), doublets of doublets (dd) and multiplets (m).

Carbon-13 nuclear magnetic resonance spectra were recorded on a Bruker AC250 and Bruker DPX-400, operating at 62.86 and 100.62 MHz, respectively. Normally, the ^{13}C NMR spectrum for each compound was recorded in the same deuteriated solvent as that used for the ^{1}H NMR spectrum, unless otherwise stated. Tetramethylsilane was used as the internal standard. DEPT, nOe and COSY analyses were also recorded on the same instruments.

3.1.6 X-ray reports

The crystallographic data for the structures presented in the text are given in this section. The crystallographic analyses were carried out at Loughborough University by Dr. A. M. Z. Slawin.

Crystal data for 1-(tert-butyl)3-methyl, 2,4-di[(tert-butoxycarbonyl)oxy]-1*H*-pyrrole-1,3-dicarboxylate (**Figure 2.1**, page 75), $C_{21}H_{31}NO_{10}$, monoclinic, a = 14.932 (2) Å, b = 10.593 (10) Å, c = 15.684 (2) Å, V = 2478.32 (5) A^3, space group $P_{2/n}$, z = 4, d = 1.226 g/cm^3, u (CuKα) = 9.82 cm^{-1}, F_{000} = 976. Reflections were measured on a Rigaku AFC7S diffractometer. The structure was solved by direct methods. Crystal and electronic stability was observed and therefore no decay correction was applied. R = 0.018.

Crystal data for 1,1-dimethylethyl (5-oxo-2,5-dihydro-1*H*-pyrrol-3-yl) carbonate (**Figure 2.2**, page 78), $C_9H_{13}NO_4$, triclinic, a = 9.089 (4) Å, b = 9.340 (3) Å, c = 6.161 (4) Å, V = 571.7 (4) A^3, space group P_1, z = 2, d = 1.278 g/cm^3, u (CuKα) = 8.13 cm^{-1}, F_{000} = 212. Reflections were measured on a Rigaku AFC7S diffractometer. The structure was solved by direct methods and expanded using Fourier techniques. Linear corrections were applied to account for a 1.3 % decrease in the standard. R = 0.072, R_w = 0.047.

Crystal data for Methyl 3-methyl-2,4-dioxotetrahydro-1*H*-pyrrole-3-carboxylate (**Figure 2.3**, page 81), $C_7H_9NO_4$, monoclinic, a = 12.059 (4) Å, b = 7.562 (4) Å, c = 19.320 (4) Å, V = 1642.6 (9) A^3, space group C2/c, z = 8, d = 1.384 g/cm^3, u (CuKα) = 9.41cm^{-1}, F_{000} = 720. Reflections were measured on a Rigaku AFC7S diffractometer. The structure was solved by direct methods and expanded using Fourier techniques. Linear corrections were applied to account for a 1.3 % decrease in the standard. R = 0.051, R_w = 0.047.

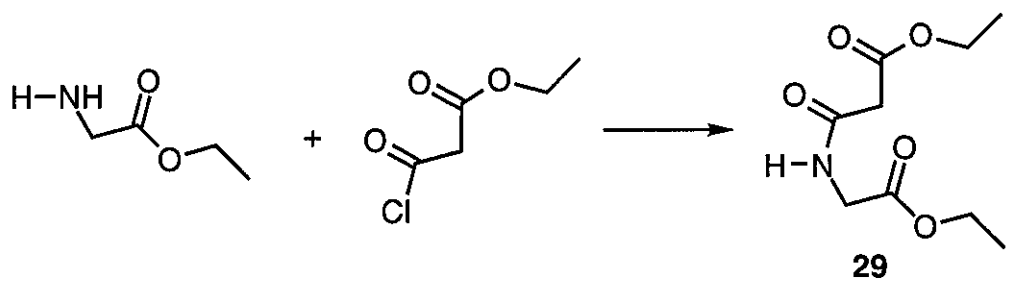
Crystal data for 3,3-dimethyltetrahydro-1*H*-pyrrole-2,4-dione (**Figure 2.3**, page 81), $C_6H_9NO_2$, monoclinic, a = 28.744 (5) Å, b = 12.650 (6) Å, c = 11.583 (5) Å, V = 4120 (3) A^3, space group C2/c, z = 24, d = 1.230 g/cm^3, u (CuKα) = 7.74cm^{-1}, F_{000} = 1632. Reflections were measured on a Rigaku AFC7S diffractometer.

The structure was solved by direct methods and expanded using Fourier techniques. Linear corrections were applied to account for a 8.4 % decrease in the standard. R = 0.027, R_w = 0.026.

3.2 LACTACYSTIN

3.2.1 INDIVIDUAL EXPERIMENTAL PROCEDURES

Ethyl 3-[(2-ethoxy-2-oxoethyl)amino]-3-oxopropanoate 29

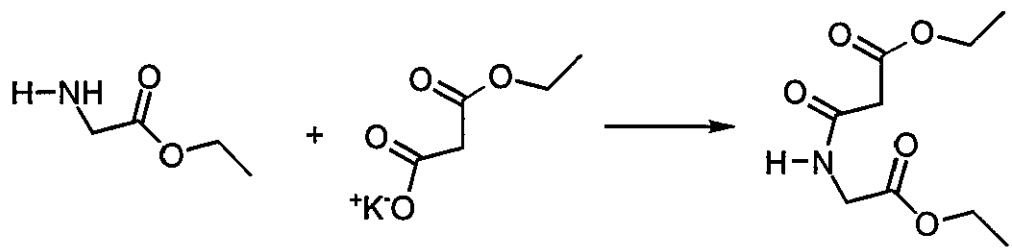


29

Method A

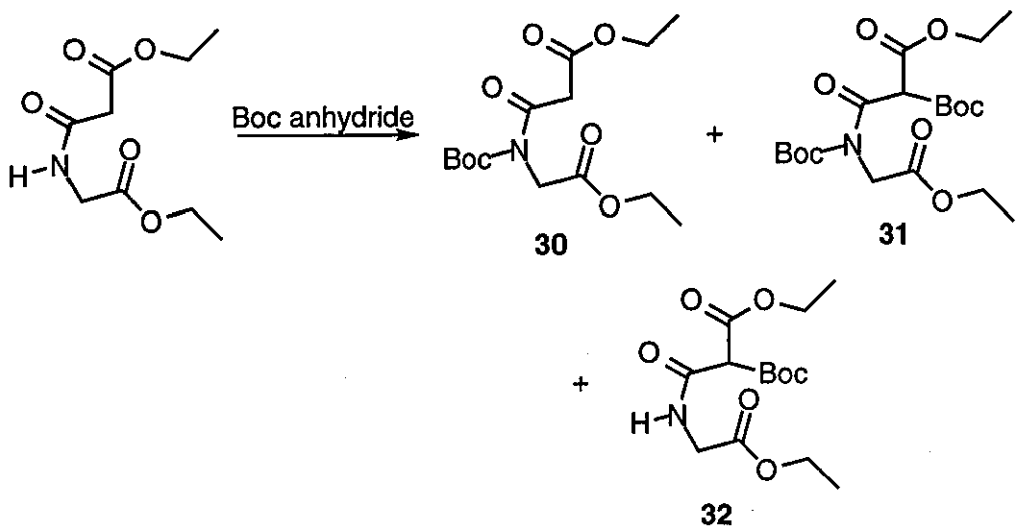
Glycine ester chloride (41.3 g, 0.29 mol) was dissolved in water (125 ml) and a solution of 10 M sodium hydroxide (40 ml) was added until the pH reached 9.5. The aqueous solution was extracted with (200 ml x 2) and (100 ml x 4) portions of dichloromethane, readjusting the pH to 9.5 after every extraction. The fractions collected were then combined, dried and the solvent was removed under vacuum. To the glycine ethyl ester was added carefully ethyl malonyl chloride (9.51g, 0.060 mol) over forty-five minutes with strong evolution of HCl gas. After two hours, the reaction was stopped and the white precipitate formed was filtered. The filtrate was concentrated *in vacuo*, washed with water, dried over anhydrous magnesium sulfate and the residue subjected to short column chromatography on silica gel using ethyl acetate as the solvent. The products were combined and recrystallised from ethyl acetate/petroleum-ether to afford colourless crystals (7.53 g, 57% based on ethyl malonyl chloride) (See **Method B** for spectroscopic data)

Method B



Dicyclohexylcarbodiimide (6.20 g, 30 mmol) was added to a cooled, stirred solution of glycine ethyl ester hydrochloride (4.20 g, 30 mmol) and potassium ethyl malonate (5.10 g, 30 mmol) in acetonitrile/water (70/20 ml) at 0 °C, and the mixture was stirred for a further two hours. The precipitate formed was filtered and washed throughly with dichloromethane. The combined filtrates were evaporated and the residue was recrystallised from acetone/petroleum-ether to give the ester as colourless needles (5.28 g, 81%), m.p. 70-72 °C (lit. 71.5-72 °C).[6] IR ν cm^{-1} : 3265 (N-H), 1745 (C=O), 1726 (C=O), 1645 (C=O); δ_H ($CDCl_3$, 400 MHz): 1.28 (6H, t, J = 7 Hz, 2 x CH_2CH_3), 3.36 (2H, s, $COCH_2CO$), 4.06 (2H, s, NCH_2CO), 4.23 (4H, q, J = 7 Hz, 2 x CH_2CH_3), 7.58 (1H, br s, N-H); ^{13}C ($CDCl_3$, 100 MHz): 14.1 (CH_3), 14.2 (CH_3), 40.8 ($COCH_2CO$), 41.3 (NCH_2CO), 61.5 (OCH_2CH_3), 61.7 (OCH_2CH_3), 165.2 (C=O), 169.1 (C=O), 169.5 (C=O); M/S: (Found M^+ 217.0946, $C_9H_{15}NO_5$ requires M^+ 217.0950) (Found C, 49.64%; H, 6.86%; N, 6.53% $C_9H_{15}NO_5$ requires C, 49.75%; H, 6.96%; N, 6.45%); (m/z): 217 (M^+, 15%), 172 (40), 144 (71) and 30 (93).

Ethyl-3-[(tert-butoxycarbonyl)(2-ethoxy-2-oxoethyl)amino]-3-oxopropanoate 30



30 31 32

Ethyl 3-[(2-ethoxy-2-oxoethyl)amino]-3-oxopropanoate (1.29 g, 5.94 mmol) in acetonitrile (10 ml) was treated with DMAP (7.2 mg, 1 mmol), followed by a solution of Boc anhydride (1.43 g, 6.53 mmol) in dry acetonitrile (5 ml). Evolution of carbon dioxide started and the light brown reaction mixture was then left overnight with stirring at room temperature and exclusion of atmospheric moisture. The next day a second equivalent of BOC anhydride was added and the reaction mixture was left for three days after which the starting material had been consumed. The reaction mixture was diluted with ethyl acetate, washed with 10% aqueous citric acid (10 ml x 1), water (10 ml x 1), saturated brine (10 ml x 1) and dried over anhydrous Na_2SO_4. The solvent was removed *in vacuo* and the residue was purified by chromatography on silica gel (dichloromethane/ethyl acetate, 90/10) giving the title compound **30** as colourless oil (0.29 g, 15%) together with two side products **31** (0.26 g, 11%) and **32** (0.25 g, 13%). **For 30**: IR ν cm^{-1} :1757 (C=O), 1731 (C=O), 1653 (C=O); δ_H ($CDCl_3$, 400 MHz): 1.26 (6H, t, J =7 Hz, 2 x CH_3CH_2), 1.47 (9H, s, $C(CH_3)_3$), 3.95 (s, 2H, $COCH_2CO$), 4.21 (4H, q, J = 7 Hz, 2 x CH_2CH_3), 4.48 (2H, s, NCH_2COO);^{13}C ($CDCl_3$, 100 MHz):14.0 (CH_3), 14.1 (CH_3), 27.6 ($C(CH_3)_3$), 45.0 ($COCH_2CO$), 45.4 (CH_2COO), 61.1 (OCH_2CH_3), 61.2 (OCH_2CH_3), 84.5 (quat.C, $C(CH_3)_3$), 152.3 (C=O), 168.4 (C=O), 170.6 (C=O), 172.3 (C=O); M/S: (Found MH^+, 318.1512, $C_{14}H_{24}NO_7$ requires MH^+ 318.1507); (m/z): 318 (M^+, 9%), 218 (100), 172 (39) and 57 (100).

1-(tert-butyl) 3-ethyl 2- {[(tert-butoxycarbonyl)(2-ethoxy-2-oxo-ethyl)amino] carbonyl} malonate 31

For 31:

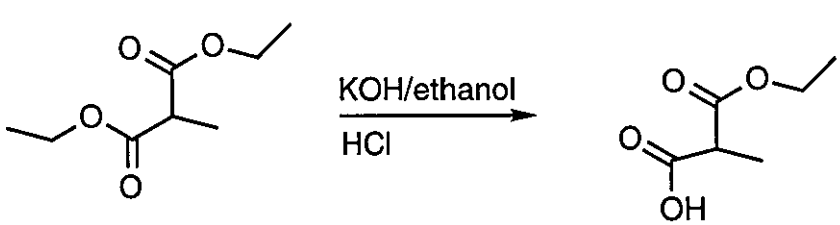
IR ν cm^{-1} : 1740 (C=O), 1703 (C=O); δ_H ($CDCl_3$, 400 MHz): 1.27-1.29 (6H, m, 2 x C*H* $_3CH_2$), 1.47 (9H, s, $C(CH_3)_3$), 1.48 (9H, s, $C(CH_3)_3$), 4.19-4.28 (4H, m, 2 x C*H* $_2CH_3$), 4.48 (2H, s, NCH_2COO), 5.20 (1H, s, COCH (Boc)); ^{13}C ($CDCl_3$, 100 MHz): 13.9 (CH_3), 14.2 (CH_3), 27.7 ($C(CH_3)_3$), 45.1 (CH_2COO), 61.3 (OCH_2CH_3), 61.9 (OCH_2CH_3), 62.9 (CH(Boc)), 82.7 (quat.C, $C(CH_3)_3$), 84.5 (quat.C, $C(CH_3)_3$), 152.5 (C=O), 164.5 (C=O), 165.0 (C=O), 167.1 (C=O), 169.2 (C=O); M/S: (Found MH^+, 418.2071, $C_{19}H_{32}NO_9$ requires MH^+ 418.2076); (m/z): 418 (M^+, 16), 362 (85), 262 (100), 218 (16) and 57 (80).

1-(tert-butyl)3-ethyl 2-{[2-ethoxy-2-oxoethyl)amino]carbonyl}malonate 32

For 32:

IR ν cm^{-1} : 3406 (N-H), 1745 (C=O), 1704 (C=O); δ_H ($CDCl_3$, 400 MHz): 1.27-1.32 (6H, m, 2 x C*H* $_3CH_2$), 1.48 (9H, s, $C(CH_3)_3$), 3.37 (1H, s, CH (Boc)CO), 4.08 (2H, s, NCH_2COO), 4.20-4.28 (4H, m, 2 x C*H* $_2CH_3$), 7.87 (N-H); ^{13}C ($CDCl_3$, 100 MHz): 14.3 (CH_3), 14.4 (CH_3), 28.1 ($C(CH_3)_3$), 41.8 (NCH_2CO), 59.7 (COCH (Boc)), 61.4 (OCH_2CH_3), 62.4 (OCH_2CH_3), 84.5 (quat.C, $C(CH_3)_3$), 164.9 (C=O), 169.6 (C=O), 170.3 (C=O), 171.5 (C=O); M/S: (Found M^+, 317.1484, $C_{14}H_{23}NO_7$ requires M^+ 317.1490); (m/z): 317 (M^+, 2%), 261 (100), 218 (82) and 57 (96).

Ethyl methyl malonate 33

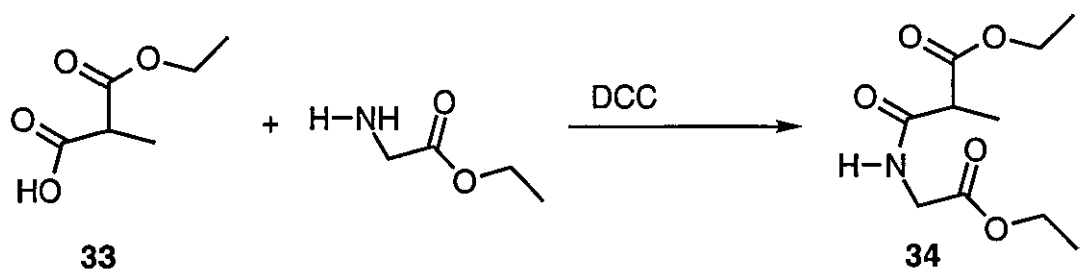


33

Potassium hydroxide (6.60g, 0.10 mol) in absolute ethanol (150 ml) was added to diethyl methyl malonate (20 g, 0.1 mol) in ethanol (50 ml) and the mixture was stirred for twenty-four hours. The ethanol was removed under vacuum and the mono potassium salt precipitated out. The salt was washed with petroleum-ether

(30 ml x 5) and dried *in vacuo* overnight to give a creamy white solid. The salt was dissolved in water (100 ml) and then acidified to pH 3 using concentrated HCl and extracted with dichloromethane (50 ml x 5). The combined organic extracts were dried over anhydrous $MgSO_4$ and evaporated to give the title compound as colourless oil (14.72 g, 87%). IR ν cm^{-1} : 2500-3500 (COOH), 1716 (C=O); δ_H ($CDCl_3$, 400 MHz): 1.27 (3H, t, J = 7 Hz, OCH_2CH_3), 1.44 (3H, d, J = 7.3 Hz, CH_3CH), 3.49 (1H, q, J = 7 Hz, $CH\,CH_3$), 4.21 (2H, q, J = 7 Hz, OCH_2CH_3). 10.48 (1H, br s, COOH); ^{13}C ($CDCl_3$, 100MHz): 13.6 (CH_3), 14.0 (CH_3), 46.2 (CH), 61.4 (CH_2CH_3), 170.0 (C=O), 174.8 (C=O); M/S: (Found M^+, 146.0583, $C_6H_{10}O_4$ requires M^+ 146.0579); (m/z): 146 (M^+, 3%), 129, (30), 101 (98), 74 (100), 49 (84)

Ethyl 3-[(2-ethoxy-2-oxoethyl)amino]-2-methyl-3-oxopropanoate 34

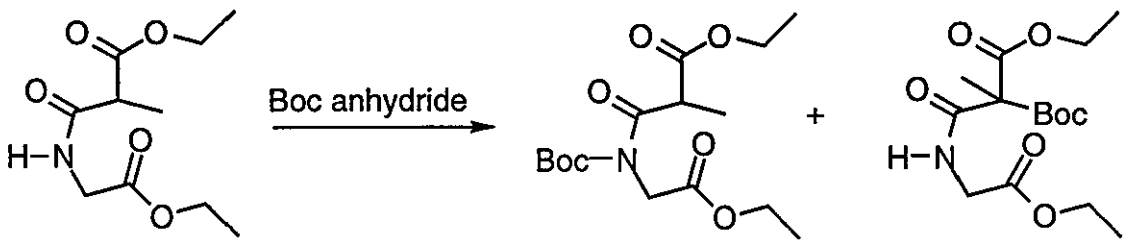


33 **34**

To a stirred solution of glycine ethyl ester hydrochloride (20.60 g, 0.14 mol) in dichloromethane (300 ml) at 0 °C was added triethylamine (14.93 g, 0.14 mol). To this solution were added methyl ethyl malonate (21.58 g, 0.14 mol) in dichloromethane (200 ml) followed by dicyclohexylcarbodiimide (30.45 g, 0.1476 mol) in dichloromethane (100 ml). The mixture was stirred at 0 °C for fifteen minutes and then for two hours at room temperature after which the suspension was filtered and the precipitate washed thoroughly with dichloromethane. The combined filtrates were washed with water, dried and evaporated to give a solid mass which was dissolved in acetone. The solution was filtered, concentrated under vacuum to about 100 ml and petroleum-ether was then added to the solution giving the title compound as colourless needles (28.2 g, 82%), m.p. 65-67 °C. IR ν cm^{-1} :3276 (N-H), 1746 (C=O), 1736 (C=O), 1652 (C=O); δ_H ($CDCl_3$, 400 MHz): 1.29 (6H, t, J = 7 Hz, 2 x CH_3CH_2), 1.47 (3H, d, J = 7.3 Hz, CH_3CH), 3.35 (1H, q, J =7 Hz, $CH\,CH_3$), 4.04 (2H, s, NCH_2CO_2), 4.22 (4H, q, J = 7 Hz, 2 x CH_2CH_3), 7.07 (1H, br s, N-H); ^{13}C ($CDCl_3$, 100MHz): 14.0 (CH_3), 14.1 (CH_3), 14.9 (CH_3), 41.5 ((NCH_2CO), 46.5 (CH), 61.5 (OCH_2CH_3), 61.6 (OCH_2CH_3), 169.2 (C=O), 169.6 (C=O), 172.0 (C=O); M/S: (Found M^+, 231.1108, $C_{10}H_{17}NO_5$

requires M^+ 231.1106), (Found C, 52.17%; H, 7.38%; N, 6.06% $C_{10}H_{17}NO_5$ requires C, 51.92%; H, 7.41%; N, 6.06%); (m/z); 231 (M^+, 9%), 186 (48), 158 (72), 140 (13) and 129 (63).

Ethyl 3-[(tert-butoxycarbonyl)(2-ethoxy-2-oxoethyl)amino-2-methyl-3-oxopropanoate 35



34 **35** **36**

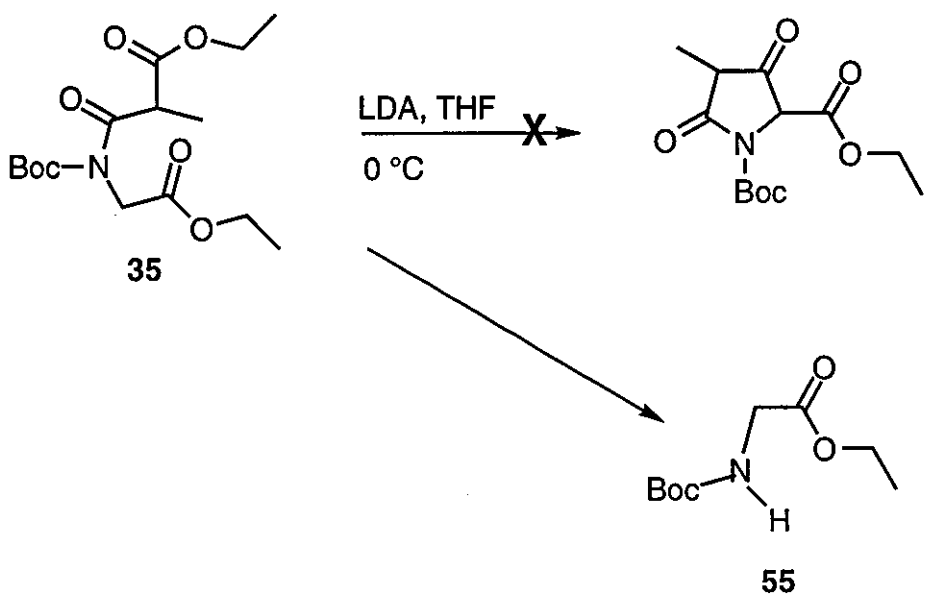
To a stirred solution of Boc anhydride (3.18 g, 14.6 mmol) and DMAP (0.15 g, 0.13 mmol) in dry dichloromethane (35 ml) was added a solution of *N*-(Methylethoxycarbonylacetyl) glycine ethyl ester (3.07 g, 13 mmol), and dry triethylamine (1.3 ml, 13 mmol) in dry dichloromethane (20 ml) over ten minutes. The resulting solution was stirred at ambient temperature for two hours and left strring overnight. The next day the reaction mixture was washed with 2M HCl (25 ml x 3) and water (25 ml), dried and evaporated to give the crude product which was chromatographed on silica gel using petroleum-ether/ethyl acetate (6/4) to afford the two compounds, **35** (1.3 g, 30%) and **36** (0.8 g, 18%) both as colourless oil. **For 35:** IR ν cm^{-1} : 1755 (C=O), 1742 (C=O), 1732 (C=O), 1704 (C=O); δ_H ($CDCl_3$, 400 MHz): 1.26 (6H, t, J =7 Hz, 2 x C*H*$_3$CH$_2$), 1.45 (3H, d, J = 7.3 Hz, C*H*$_3$CH), 1.48 (9H, s, C(CH$_3$)$_3$), 4.15 (4H, q, J =7 Hz, 2 x C*H*$_2$CH$_3$), 4.42 - 4.55 (3H, m, C*H*CH$_3$ and NC*H*$_2$CO$_2$); ^{13}C ($CDCl_3$, 100MHz): 14.0 (CH_3), 14.1 (CH_3), 14.2 (CH_3), 27.7 ((CH$_3$)$_3$), 45.4 (NCH$_2$CO), 48.1 (CH), 61.1 (OCH$_2$CH$_3$), 61.2 (OCH$_2$CH$_3$), 84.2 (quat.C, C(CH$_3$)$_3$), 152.0 (C=O), 168.6 (C=O), 170.8 (C=O), 172.0 (C=O); M/S: (Found MH^+, 332.1706 $C_{15}H_{26}NO_7$ requires MH^+ 332.1709); (m/z): 332 (MH^+ , 9%), 276 (30), 232 (100), and 57 (100).

1-(tert-butyl)3-ethyl,2-{[2-ethoxy-2-oxoethyl)amino]carbonyl}-2-methylmalonate 36

For 36:

IR ν cm^{-1} : 3388 (N-H),1738 (C=O), 1732 (C=O); δ_H ($CDCl_3$, 400 MHz): 1.27 (6H, t, J = 7 Hz, 2 x CH_3CH_2), 1.47 and 1.49 (9H, 2 x s, $C(CH_3)_3$), 1.71 (3H, s, CH_3), 4.07 (2H, s, CH_2N), 4.28 (4H, q, J = 7 Hz, 2 x CH_2CH_3), 8.27 (1H, br s, N-H); ^{13}C ($CDCl_3$, 100MHz): 13.9 (CH_3), 14.1 (CH_3), 20.6 (CH_3), 27.6 ($(CH_3)_3$), 41.7 (NCH_2CO), 60.8 (quat. C), 61.5 (OCH_2CH_3), 62.1 (OCH_2CH_3), 83.4 (quat. C, $C(CH_3)_3$), 167.5 (C=O), 168.6 (C=O), 169.3 (C=O), 169.4 (C=O); M/S: (Found MH^+ ,332.1715 $C_{15}H_{26}NO_7$ requires MH^+ 332.1709); (m/z): 332 (MH^+ ,17%), 276 (83), 202 (40), 186 (13), and 57 (100).

Attempted cyclisation of ethyl 3-[(tert-butoxycarbonyl)(2-ethoxy-2-oxoethyl)amino-2-methyl-3-oxopropanoate 35



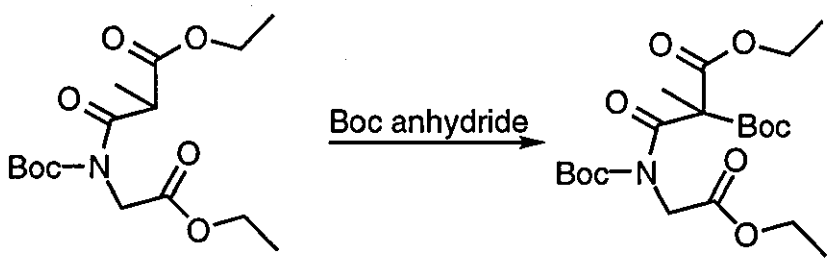
35

55

N-tert-butyl (Methylethoxycarbonylacetyl) glycine ethyl ester (0.224 g, 0.067 mmol) in THF (5 ml) was added slowly to a cooled stirred solution of lithium diisopropylamide (0.067 mmol) and DMPU (0.067 mmol) over a period of five minutes. After stirring for thirty minutes at 0 °C, the reaction was warmed slowly to room temperature and then left overnight. After that period, the mixture was quenched with concentrated HCl (2 ml) and the reaction was worked up by the addition of water (5 ml) and diethyl ether (15 ml). The aqueous layer was

separated and further extracted with ether (15 ml x 2). The organic extracts were combined, washed with saturated sodium chloride solution, dried over anhydrous sodium sulfate and filtered. The solvent was removed under reduced pressure to give a crude extract which was chromatographed on silica gel using petroleum ether/ethyl acetate (8/2) to give compound **55** as a colourless oil (98 mg, 52 %). IR ν cm^{-1} : 1748 (C=O), 1731 (C=O), 1715 (C=O), 1699 (C=O); δ_H ($CDCl_3$, 400 MHz): 1.25 (3H, t, J = 7 Hz, CH_3CH_2), 1.45 (9H, s,$C(CH_3)_3$) , 3.89 (2H, s, CH_2N), 4.20 (2H, q, J = 7 Hz, CH_2CH_3); ^{13}C ($CDCl_3$, 100MHz): 14.1 (CH_3), 27.9 (($CH_3)_3$), 42.4 (CH_2N), 61.3 (OCH_2CH_3), 62.4 (quat. C), 79.9 (quat. C, $C(CH_3)_3$), 155.7 (C=O), 170.4 (C=O); M/S: (Found M^+, 203.1151 $C_9H_{17}NO_4$ requires M^+ 203.1157); (m/z): 203 (M^+ ,19%), 158 (32), 144 (22), 129 (60), 104 (100), 74 (100),

1-(tert-butyl) 3-ethyl 2-{[(tert-butoxycarbonyl)(2-ethoxy-2-oxoethyl)amino] carbonyl}-2-methylmalonate 39

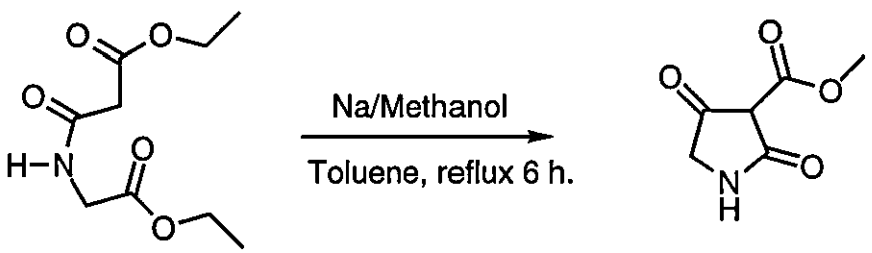


35 39

To a stirred solution of Boc anhydride (1.68 g, 0.73 mmol) and DMAP (0.08 g, 0.067 mmol) in dry dichloromethane (20 ml) was added a solution of *N*--tert-butyl (Methylethoxycarbonylacetyl) glycine ethyl ester (2.22 g, 0.67 mmol) and dry triethylamine (0.92 ml, 0.67 mmol) in dry dichloromethane (20 ml) over a period of five minutes. The resulting solution was stirred at room temperature for seven days after which it was stopped since the starting material was not completely consumed. The reaction mixture was washed with 2M HCl (15 ml x 3) and water (20 ml), dried over anhydrous magnesium sulfate and evaporated to give the crude product which was chromatographed on silica gel using petroleum-ether/ethyl acetate (8/2) to give the title compound as colourless oil (0.12 g, 4%). IR ν cm^{-1} : 1783 (C=O), 1731 (C=O), 1704 (C=O); δ_H ($CDCl_3$, 400 MHz): 1.26 (6H, t, J =7 Hz, 2 x CH_3CH_2),1.48 (9H, s, $C(CH_3)_3$), 1.76 (3H, s, CH_3Boc), 4.18 (4H, q, J = 7 Hz, CH_2CH_3), 4.49 (2H, s,NCH_2CO); ^{13}C ($CDCl_3$, 100MHz): 14.1 (CH_3), 14.2 (CH_3), 21.0 (CH_3), (27.7 ($CH_3)_3$), 45.5 (CH_2CO), 60.3 (quat. C, C(Boc)(CH_3), 61.1 (OCH_2CH_3), 61.5 (OCH_2CH_3), 82.3 (quat. C, $C(CH_3)_3$), 84.0 (quat. C,

$C(CH_3)_3$), 151.8 (C=O), 165.9 (C=O), 167.6 (C=O), 168.4 (C=O), 170.1 (C=O); M/S: (Found MH^+, 432.2232, $C_{20}H_{34}NO_9$ requires MH^+ 432.2233); (m/z): 376 (MH^+-C_4H_8, 10%), 320 (5), 276 (7), 231 (20), 202 (20) and 57 (100).

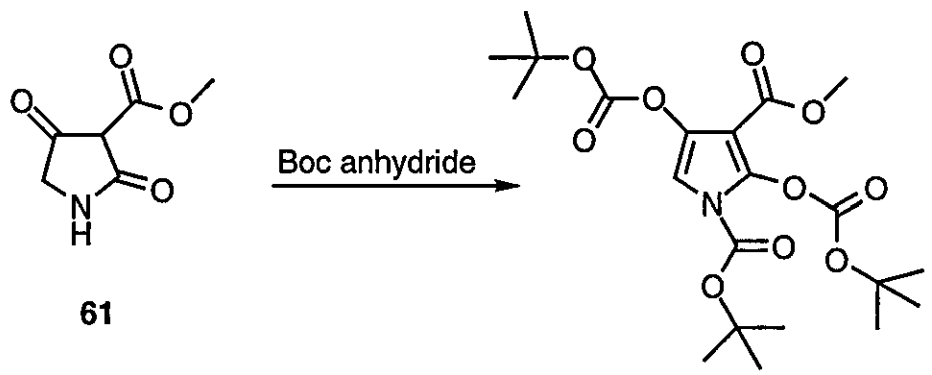
Methyl 2,4-dioxopyrrolidone-3-carboxylate 61



29 **61**

To a solution of sodium methoxide [from sodium (0.897 g, 0.013 mol) in dry methanol (15 ml)] was added a solution of the diester (3.01 g, 0.013 mol) in dry toluene (100 ml). The mixture was refluxed for six hours under nitrogen, cooled, and diluted with water, and the two phases were separated. The organic layer was washed twice with water and the combined aqueous layers were carefully acidified with concentrated HCl and the dioxopyrrolidone ester slowly precipitated as a white powder (2.17 g, 70% yield) m.p. >360 °C (decomposed). Because of the insolubility of the compound in most conventional organic solvents, it was used without prior purification. IR ν cm^{-1} :3307 (NH amide), 1693 (β-keto ester), 1614-1650 (β-diketone and amide); δ_H $(CD_3)_2SO$, 400 MHz): 3.1 (1H, s, $CHCO_2CH_3$), 3.60 (3H, s, OCH_3), 3.76 (2H, s, NCH_2C); M/S: Found M^+, 157.0374 $C_6H_7NO_4$ requires M^+ 157.0375; (m/z): 157 (M, 10%), 125 (70) , 97 (40), 44 (45), 31 (100).

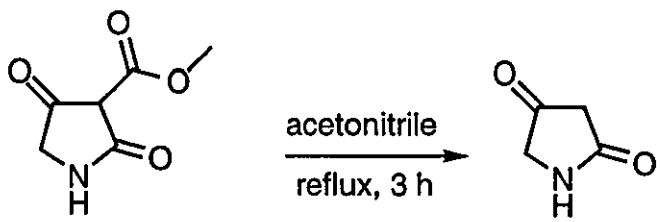
1-(tert-butyl)3-methyl2,4-di[(*tert*-butoxycarbonyl)oxy]-1*H*-pyrrole-1,3-dicarboxylate 63



61 **63**

To a stirred solution of the methyl 2,4-dioxopyrrolidone-3-carboxylate (0.679 g, 4.3 mmol) and DMAP (0.052 g, 0.43 mmol) in acetonitrile (20 ml) was added a solution of Boc anhydride (1.038 g, 4.75 mmol) in acetonitrile (10 ml). After stirring at room temperature for one hour the reaction mixture turned deep purple and it was left stirring overnight. After that period the reaction mixture was diluted with ethyl acetate, washed with 10% aqueous citric acid (15 ml x 1), water (15 ml x 1), saturated brine (20 ml x 1) and dried over anhydrous sodium sulfate. The solvent was removed *in vacuo* and the residue was purified by column chromatography on silica gel (petroleum-ether/ethyl acetate, 8/2) giving the title compound as colourless crystals (0.272 g, 14% yield), m.p. 139-141 °C. IR ν cm^{-1} :1784 (C=O), 1763 (C=O), 1758, (C=O), 1725 (C=O); δ_H ($CDCl_3$, 400 MHz): 1.54 (27H, s, 3 x $C(CH_3)_3$), 3.78 (3H, s, OCH_3), 6.60 (1H, s, CH aromatic); ^{13}C ($CDCl_3$, 100MHz): 27.5 ($CH_3)_3$), 51.4 (OCH_3), 83.8 (quat.C, $C(CH_3)_3$), 85.1 (quat. C, $C(CH_3)_3$), 85.8 (quat. C, ($C(CH_3)_3$), 101.0 (quat. C), 106.6 (CH aromatic), 134.8 (quat. C), 137. 9 (quat. C), 146.5 (C=O), 149.3 (C=O), 151.3 (C=O), 161.4 (C=O); M/S: Found M^+, 457.1869 $C_{21}H_{31}NO_{10}$ requires M^+ 457.1869; (Found C, 55.23%; H, 6.69%; N, 2.73% $C_{21}H_{31}NO_{10}$ requires C, 55.12%; H, 6.83%; N, 3.06%); (m/z): 457 (M^+, 11%), 356 (42), 300 (80), 244 (100), 201 (72), 156 (80), 57 (83).

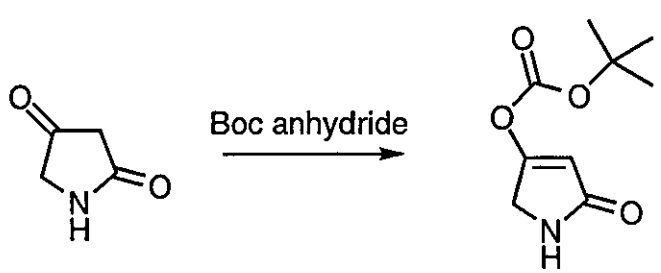
Tetrahydro-1*H*-pyrrole-2,4-dione 64



61 **64**

Methyl-2,4-dioxopyrrolidone-3-carboxylate (1.281 g, 8.15 mmol) was dispersed in acetonitrile (200 ml) and the mixture was heated at reflux for three hours. The ester dissolved gradually to give a clear solution. The solvent was removed under vacuum and the title compound was obtained as yellow solid which exists in keto and enol forms (0.709 g, 88%). m.p. >360 °C. IR ν cm^{-1} : 3217 (NH), 1760 (C=O), 1690 (C=O); δ_H ($CDCl_3$, 400 MHz): 2.92 (2H, s, $COCH_2CO$), 3.74 (2H, s, $CH_2C{=}C$), 3.78 (2H, s, CH_2CO), 4.76 (1H, s, C=CH), 7.05 (1H, br s, NH), 8.20 (1H, br s, OH), enol form; ^{13}C ($CDCl_3$, 100MHz): 50.5 (CH_2), 53.8 (CH_2), 104.7 (quat.C), 121 (CH olefinic), 154.5 (C=O), 177.4 (C=O), 178.5 (C=O); M/S: Found M^+, 99.0320, $C_4H_5NO_2$ requires M^+ 99.0320; (Found C, 49.89%; H, 4.89%; N, 15.08% $C_4H_5NO_2$ requires C, 48.47%; H, 5.09%; N, 14.14%); (m/z): 99 (M^+, 78%), 71 (100), 42 (60), 30 (34).

1,1-dimethylethyl (5-oxo-2,5-dihydro-1*H*-pyrrol-3-yl) carbonate 66

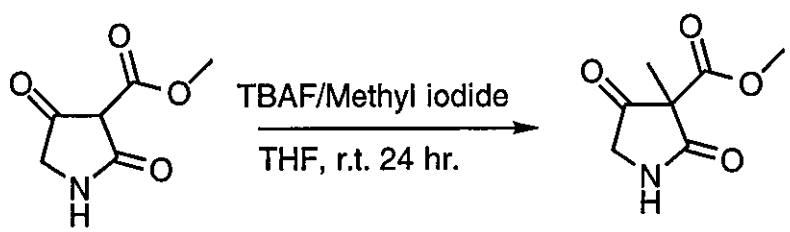


64 **66**

To a stirred solution of the tetrahydro-1*H*-pyrrole-2,4-dione (0.24 g, 24 mmol) and DMAP (0.1 g, 0.24 mmol) in acetonitrile (20 ml) was added a solution of Boc anhydride (0.58 g, 26 mmol) in acetonitrile (10 ml). After stirring at room temperature for one hour the reaction mixture turned brownish and it was left stirring overnight. The reaction mixture was diluted with ethyl acetate, washed with 10% aqueous citric acid (10 ml x1), water (10 ml x 1), saturated brine (15 ml x

1) and dried over anhydrous sodium sulfate. The solvent was removed *in vacuo* and the residue was purified by column chromatography on silica gel (petroleum-ether/ethyl acetate, 8/2) giving the title compound as yellowish crystals, 0.31 g (65 %), m.p. 185-187 °C. IR ν cm^{-1} : 3204 (NH), 1772 (C=O), 1672 (C=O), 1622 (C=O); δ_H ($CDCl_3$, 400 MHz): 1.55 (9H, s, $C(CH_3)_3$), 4.11 (2H, s, NCH_2), 5.96 (1H, s, C=CH), 6.53 (1H, br s, NH); ^{13}C ($CDCl_3$, 100MHz): 27.4 ($C(CH_3)_3$), 46.8 (CH_2), 85.1 (quat. C), 106.2 (C=CH), 147.2 (quat. C), 164.7 (C=O), 174.9 (C=O); M/S: (Found MH^+, 200.0923, $C_9H_{14}NO_4$ requires MH^+ 200.0922); (m/z): 200 (MH^+ 100%), 156 (13), 100 (27), 100 (100), 57 (98), 41 (69).

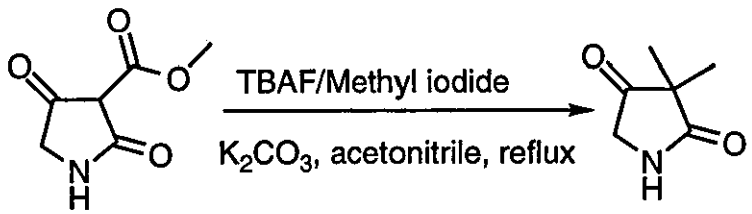
Methyl 3-methyl-2,4-dioxotetrahydro-1*H*-pyrrole-3-carboxylate 67



61 **67**

To a stirred solution of methyl 2,4-dioxopyrrolidone-3-carboxylate (0.836 g, 5.32 mmol) in THF (15 ml), was added TBAF (6.38 ml, 6.38 mmol) and the reaction was stirred until the solid dissolved. Methyl iodide (3.77 ml, 26.6 mmol) was then added and the mixture was left stirring overnight. The reaction mixture was evaporated under reduced pressure and the residue partitioned between water and dichloromethane. The dichloromethane fraction was washed with water, dried over anhydrous magnesium sulfate and evaporated to give a brownish residue which was then chromatographed on silica gel using ethyl acetate to give the title compound as yellowish crystals, 0.52 g (67%), m.p. 132-134 °C. IR ν cm^{-1} :3252 (N-H), 1786 (C=O), 1746 (C=O), 1707 (C=O); δ_H ($CDCl_3$, 400 MHz): 1.51 (3H, s, CH_3), 3.76 (3H, s, OCH_3), 4.09 (1H, d, J = 17 Hz, NC*H* H), 3.98 (1H, d, J = 17 Hz, NCH*H*), 6.78 (1H, br s, NH); ^{13}C ($CDCl_3$, 100MHz): 15.1 (CH_3), 51.5 (CH_2), 53.4 (OCH_3), 57.5 (quat.C), 165.8 (C=O), 172.8 (C=O), 203.6 (C=O); M/S: (Found M^+ , 171.0532 $C_7H_9NO_4$ requires M^+ 171.0531); m/z: 171 (M^+ , 21%), 144 (15), 139 (32), 87 (100),

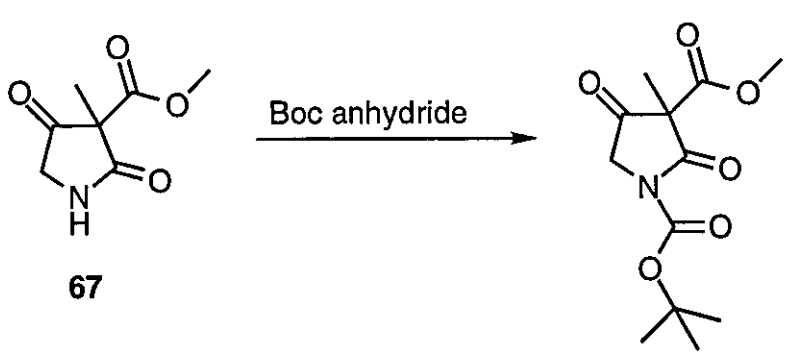
3,3-dimethyltetrahydro-1*H*-pyrrole-2,4-dione 68



61 **68**

Methyl 2,4-dioxopyrrolidone-3-carboxylate (0.438 g, 0.0027 mol), potassium carbonate (0.385 g, 0.0027 mol), TBAB (0.05 g, 0.15 mmol) in acetonitrile (30 ml) was heated at reflux for thirty minutes after which methyl iodide (1.73 ml, 0.027 mol) was added and the mixture was heated at reflux for another twelve hours. After that period, the mixture was diluted with ethyl acetate (50 ml) and partitioned with water. The organic layer was washed with brine, filtered and evaporated under vacuum to a give a yellowish residue which was chromatographed on silica gel using petroleum-ether/ethyl acetate (80/20) to afford yellowish crystals, 0.14 g (40%), m.p. 100-102 °C. IR ν cm^{-1} : 3428 (NH), 1761(C=O), 1686 (C=O); δ_H ($CDCl_3$, 250 MHz): 1.26 (6H, s, 2 x CH_3), 3.94 (2H, s, CH_2), 7.79 (1H, s, NH); ^{13}C ($CDCl_3$, 100MHz): 20.3 (CH_3), 46.2 (quat.C), 50.6 (CH_2), 179.3 (C=O), 211.6 (C=O); M/S: Found M^+, 127.0634, $C_6H_9NO_2$ requires M^+ 127.0633); (Found C, 56.86%; H, 7.06%; N, 10.89% $C_6H_9NO_2$,requires C, 56.66%; H, 7.14%; N, 11.02%); (m/z): 127 (M^+, 86 %), 99 (21), 70 (100), 57 (65), 42 (68).

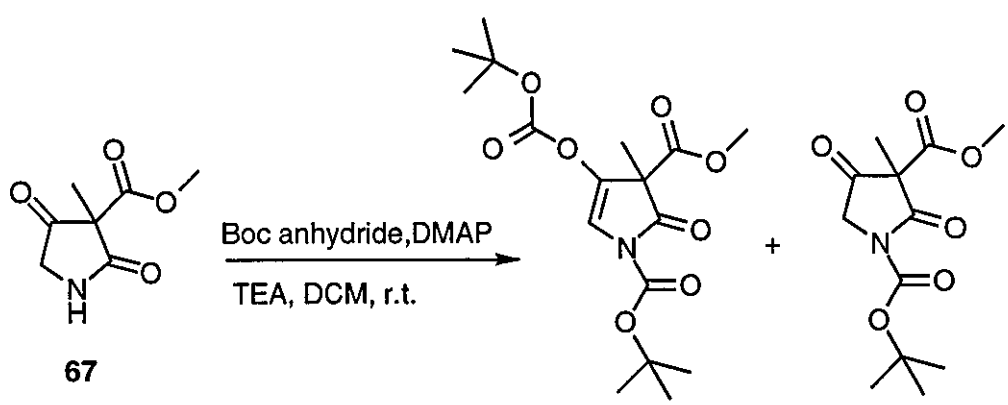
1-(1,1-dimethylethyl) 3-methyl 3-methyl-2,4-dioxotetrahydro-1*H*-pyrrole-1,3-dicarboxylate 70



67 **70**

To a stirred solution of methyl 3-methyl-2,4-dioxotetrahydro-1H-pyrrole-3-carboxylate (0.160 g, 0.93 mmol) and DMAP (0.011 g, 0.093 mmol) in acetonitrile (10 ml) was added a solution of Boc anhydride (0.22 g, 1.008 mmol) in acetonitrile (5 ml) and the reaction mixture was left stirring overnight. The reaction mixture was diluted with ethyl acetate, washed with 10% aqueous citric acid (10 ml x 1), water (10 ml x 1), saturated brine (15 ml x 1) and dried over anhydrous sodium sulfate. The solvent was removed *in vacuo* and the residue was purified by column chromatography on silica gel (petroleum-ether/ethyl acetate, 8/2) giving the title compound as a yellowish white powder (0.193 g, 76 % yield), m.p. 85-87 °C. IR ν cm^{-1} : 1803 (C=O), 1768 (C=O), 1749 (C=O), 1722 (C=O); δ_H ($CDCl_3$, 400 MHz): 1.52 (3H, s, CH_3), 1.56 (9H, s, $C(CH_3)_3$), 3.75 (3H, s, OCH_3), 4.29 (1H, d, J = 18 Hz, C*H*HCO), 4.43 (1H, d, J = 18 Hz, CH*H* CO); ^{13}C ($CDCl_3$, 100MHz): 15.4 (CH_3), 27.8 ($C(CH_3)_3$), 53.7 (OCH_3), 55.2 (CH_2), 61.0 (quat. C), 84.8 (quat. C), 148.2 (C=O), 165.1 (C=O), 168.3 (C=O), 200.0 (C=O); M/S: (Found M^+ 271.1053 $C_{12}H_{17}NO_6$ requires 271.1055) (Found C, 53.26%; H, 6.20%; N, 5.00% $C_{12}H_{17}NO_6$ requires C, 53.12%; H, 6.32%; N, 5.17%); (m/z): M^+ (271, 80%), 216 (100), 192 (80), 172 (83) 57 (89).

1-(1,1-dimethylethyl) 3-methyl 4-({[(1,1-dimethylethyl)oxy]carbonyl}oxy)-3-methyl-2-oxo-2,3-dihydro-1*H*-pyrrole-1,3-dicarboxylate 71

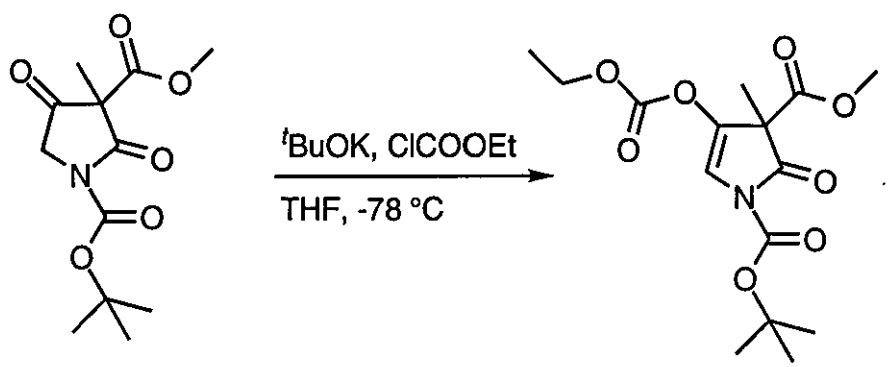


67 71 70

Boc anhydride (3.709 g, 0.016 mol) was added to a stirred solution of ester (2.642 g, 0.015 mol), triethylamine (2.15 ml, 0.015 mol) and DMAP (0.188 g, 1.69 mmol) in dichloromethane (30 ml). The yellowish solution first turned brown and then black upon the addition of Boc anhydride. After stirring for twelve hours, the reaction was washed with 2M HCl (15 ml x 3) and water (25 ml x 1), dried over

anhydrous magnesium sulfate and evaporated to give the crude extract which was chromatographed on silica gel using petroleum-ether/ethyl acetate (80/20) to give compound **71** as colourless solid (1.626 g, 39 %) together with compound **70** (0.933 g, 22%), m.p. 127-129 °C. IR ν cm^{-1} : 1803 (C=O), 1768 (C=O), 1745 (C=O), 1732 (C=O) ; δ_H ($CDCl_3$, 400 MHz): 1.50 (9H, s, $C(CH_3)_3$), 1.54 (9H, s, $C(CH_3)_3$), 1.57 (3H, s, CH_3), 3.73 (3H, s, OCH_3), 7.09 (1H, s, CH olefinic); ^{13}C ($CDCl_3$, 100MHz): 17.4 (CH_3), 27.4 ($C(CH_3)_3$),27.8 ($C(CH_3)_3$), 53.2 (OCH_3), 57.9 (quat. C), 84.4 (quat. C), 84.7 (quat. C), 115.9 (=CH), 135.5 (C=C), 147.4 (C=O), 149.8 (C=O), 167.0 (C=O), 168.7 (C=O); M/S: (Found M^+ + 2H, 373.1736, $C_{17}H_{27}NO_8$ requires M^+ + 2H, 373.1736), (Found C, 55.03%; H, 6.65%; N, 3.58% $C_{17}H_{25}NO_8$ requires C, 54.96%; H, 6.79%; N, 3.58%); (m/z): 371 (M^+ , 49%), 315 (98), 259 (100), 215 (83), 170 (82), 57 (93).

1-(1,1-dimethylethyl) 3-methyl 4-{[(ethyloxy]carbonyl}oxy}-3-methyl-2-oxo-2, 3 dihydro-1*H*-pyrrole-1,3-dicarboxylate 73

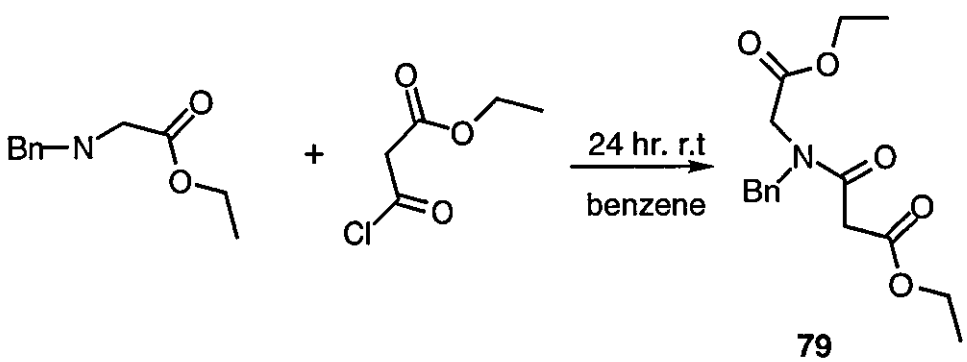


70 **73**

To a stirred solution of ^{t}BuOK (0.130 g, 11.59 mmol) in dry THF (10 ml) at -78 °C, was added a solution of the ester **70** (0.3 g, 11.07 mmol) in THF (3 ml) and the mixture was stirred for thirty minutes at -78 °C. Ethyl chloroformate (0.125 g, 11.59 mmol) in THF (2 ml) was then added and stirring was continued for another hour at -78 °C. After that period the reaction mixture was allowed to warm to room temperature. Removal of the solvent under reduced pressure led to a yellowish oil which was partitioned between water (10 ml) and diethyl ether (15 ml). The organic layer was decanted and the aqueous phase was extracted with ether (10 ml x 3). The combined organic layers were washed with brine until neutral pH and dried over anhydrous magnesium sulfate. Evaporation of the solvent under reduced pressure gave a yellowish brown oil which was purified by flash

chromatography on silica gel (petroleum-ether/ethyl acetate (80/20)) to give the title compound as a colourless oil, 0.11 g (35%). IR ν cm^{-1} :1786 (C=O), 1749 (C=O), 1732 (C=O); δ_H ($CDCl_3$, 400 MHz): 1.36 (3H, t, J = 7.1 Hz, CH_3CH_2), 1.50 (9H, s, $C(CH_3)_3$), 1.54 (3H, s, CH_3), 3.75 (3H, s, OCH_3), 4.27 (2H, q, 2H, J =7.1 Hz, CH_2CH_3), 7.15 (1H, s, C=CH); ^{13}C ($CDCl_3$, 100MHz):13.9 (CH_3), 17.4 (CH_3), 27.8 ($C(CH_3)_3$), 53.3 (OCH_3), 65.5 (CH_2), 57.6 (quat. C), 84.5 (quat. C),115.9 (C=CH), 135.7 (C=C), 147.2 (C=O), 150.2 (C=O), 167.4 (C=O), 168.8 (C=O); M/S: Found (M^+, 343.1268, $C_{15}H_{21}NO_8$ requires M^+, 343.1267; m/z: 343 (M^+, 4%), 270 (3), 199 (9), 171 (74), 139 (48), 57 (100).

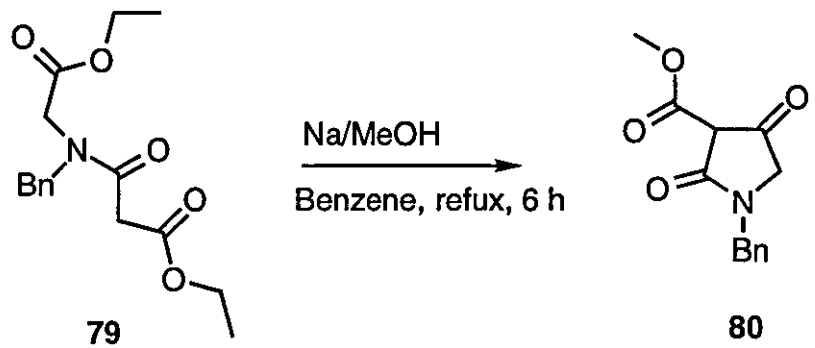
Ethyl 3-[[2-(ethyloxy)-2-oxoethyl](phenylmethyl)amino]-3-oxopropanoate 79



79

Ethyl malonyl chloride (1.55 g, 0.010 mol) in dry benzene (40 ml) was added over a period of ten minutes to a stirred solution of benzyl glycine ethyl ester (4.0 g, 0.020 mol) in dry benzene (60 ml), cooled to 0 °C. The resulting solution was stirred at room temperature for twenty-four hours after which the mixture was filtered, and the filtrate washed with 10% sodium bicarbonate solution (15 ml x 4), and with dilute hydrochloric acid (10 ml x 3). The organic phase was dried over anhydrous magnesium sulfate and the solvent was removed under vacuum to give the diester **79** as an orange oil, (2.89 g, 91%). IR ν cm^{-1} :1747 (C=O), 1660 (C=O); δ_H ($CDCl_3$, 250 MHz): 1.27 (6H, t, J = 7 Hz, CH_3CH_2), 3.55 (2H, s, NCH_2CO_2), 3.95 - 4.20 (6H, m, CH_2CO and 2 x CH_2CH_3), 4.64 (2H, s, CH_2Ph), 7.29-7.38 (5H, m, ArH); ^{13}C ($CDCl_3$, 100MHz): 13.9 (2 x CH_3), 41. 0 (CH_2), 46.9 (CH_2), 52.6 (CH_2), 61.1 (OCH_2), 61.5 (OCH_2), 127.6-128.5 (aromatic CH), 135 (quat. olefinic C), 166.5 (C=O), 167.0 (C=O), 168.8 (C=O); M/S: Found (M^+, 307.1420, $C_{16}H_{21}NO_5$ requires M^+, 307.1418), 307 (M^+, 6%); m/z: 192 (100), 120 (30), 118 (14), 106 (13), 91 (70).

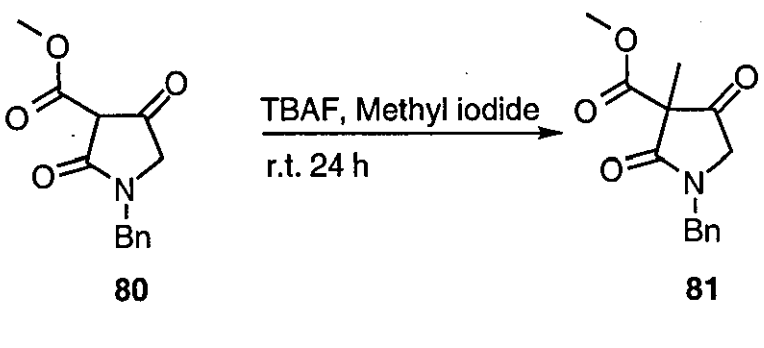
Methyl 2,4-dioxo-1-(phenylmethyl) tetrahydro-1*H*-pyrrole-3-carboxylate 80



79 **80**

To a stirred solution of sodium methoxide [prepared from sodium (0.18 g, 0.0079 mol) in 10 ml methanol] was added the diester (2.44 g, 0.0079 mole) in benzene (50 ml). The mixture was refluxed under nitrogen for six hours and the resulting white solid was collected, dissolved in 10% sodium bicarbonate solution (30 ml), washed with benzene (20 ml x 1) and then acidified with dilute sulfuric acid to give a white solid. The white solid was dried over phosphorus pentoxide to give the product **80** as white powder (1.012 g, 55%), m.p. 121-122 °C. IR ν cm^{-1} : 1705 (C=O), 1663 (C=O); δ_H ($CDCl_3$, 250 MHz): 3.47 (1H, s, $CHCO_2$), 3.74 (3H, s, OCH_3), 3.92 (2H, s, NCH_2CO), 4.59 (2H, s, NCH_2Ph), 7.29-7.36 (5H, m, ArH); ^{13}C ($CDCl_3$, 100MHz): 45.3 (CH_2), 52.0 (OCH_3), 56.6 (CH_2), 127.6-128.9 (aromatic CH), 136 (quat. C), 166.5 (C=O), 167.0 (C=O), 182.0 (C=O); M/S: (Found M^+, 247.0850, $C_{13}H_{13}NO_4$ requires M^+, 247.0845); m/z: 247 (M^+, 3%), 245 (46), 244 (28), 221 (33), 130 (14), 106 (30), 91 (100).

Methyl-3-methyl-2,4-dioxo-1-(phenylmethyl) tetrahydro-1*H*-pyrrole-3-carboxylate 81

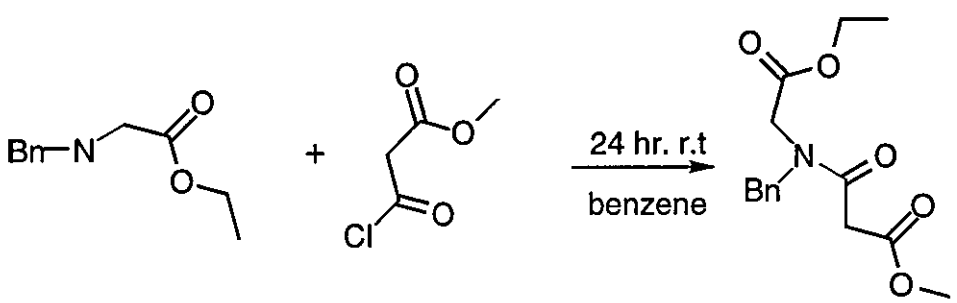


80 **81**

To a stirred solution of the keto ester (0.220 g, 0.89 mmol) in THF (10 ml), was added TBAF (1.06 ml, 1.06 mmol) and the reaction was stirred until the solid

dissolved. Methyl iodide (1.26 ml, 8.90 mmol) was then added and the reaction mixture was left stirring overnight. After that period the reaction mixture was evaporated under reduced pressure to give a yellowish residue. Ethyl acetate was added to the residue and the solid formed was filtered and the filtrate was then concentrated to give a yellowish residue which was then chromatographed on silica gel using petroleum-ether/ethyl acetate (6/4) to give the title compound as colourless oil , 0.176 g (77%). IR ν cm^{-1} : 1782 (C=O), 1747 (C=O), 1693 (C=O); δ_H ($CDCl_3$, 400 MHz): 1.52 (3H, s, CH_3), 3.73 (3H, s, OCH_3), 3.68 (1H, d, J = 9 Hz, NC*H* HCO), 3.99 (1H, d, J = 9 Hz, CH*H*CO), 4.47 (1H, d, J = 14 Hz, NC*H* HPh), 4.84 (1H, d, J = 14 Hz, NCH*H* Ph), 7.27-7.39 (5H, m, ArH); ^{13}C ($CDCl_3$, 100MHz): 15.1 (CH_3), 46.1 (CH_2), 53.3 (OCH_3), 54.8 (CH_2Ph), 58.8 (quat. C), 128.0-128.9 (Ar CH), 134.6 (quat. olefinic C), 166.8 (C=O), 169.8 (C=O), 202.1 (C=O); M/S: Found M^+ 261.1004 $C_{14}H_{15}NO_4$ requires 261.1001; m/z; 261 (M^+, 32%), 233 (5), 202 (62), 91 (100), 83 (13).

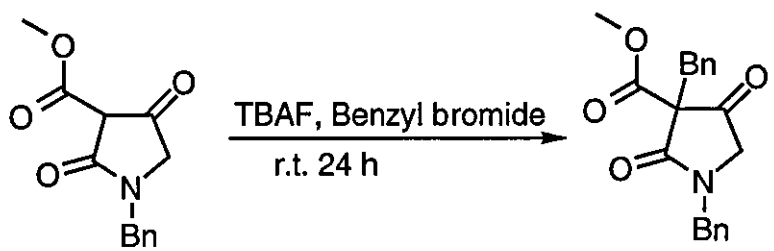
Methyl 3-[[2-(ethyloxy)-2-oxoethy](phenylmethyl)amino]-3-oxopropanoate



Methyl malonyl chloride (5.47 g, 0.04 mol) in dry benzene (60 ml) was added over a period of ten minutes to a stirred solution of benzyl glycine ethyl ester (15.49 g, 0.08 mol) in dry benzene (90 ml), cooled to 0 °C. The resulting solution was then stirred at room temperature for twenty-four hours. After that period the mixture was filtered, and the filtrate washed with 10 % sodium bicarbonate solution (15 ml x 3), and then with dilute hydrochloride acid (10 ml x 3). The organic phase was dried and the solvent was removed under vacuum to give the diester as an orange oil, (10.27 g, 88 %). IR ν cm^{-1} :1744 (C=O), 1660 (C=O); δ_H ($CDCl_3$, 250 MHz): 1.24 (3H, t, J = 7 Hz, C*H* $_3CH_2$), 3.57 (2H, s, NCH_2CO), 3.76 (3H, s, OCH_3), 4.06 (2H, s, $COCH_2CO$), 4.14 (2H, q, J = 7 Hz, C*H* $_2CH_3$), 4.64 (2H, s, CH_2Ph), 7.29-7.39 (5H, m, ArH); ^{13}C ($CDCl_3$, 100MHz):14.0 (CH_3), 41.0 (CH_2), 47.0 (CH_2), 49.8 (CH_2), 52.6 (OCH_3), 61.6 (OCH_2), 126-128 (Ar-H), 135.1 (quat.C), 166.4 (C=O),

167.2 (C=O), 168.7 (C=O); ((M/S: Found M^+, 293.1268 $C_{15}H_{19}NO_5$ requires 293.1263 ; m/z: 293 (M^+, 9%), 192 (90), 120 (45), 118 (12), 106 (23), 91 (100).

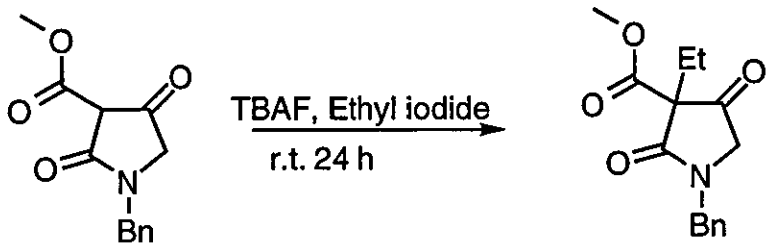
Methyl-3-benzyl-2,4-Dioxo-1-(phenylmethyl)tetrahydro-1*H*-pyrrole-3-carboxylate



80

To a stirred solution of methyl 2,4-dioxo-1-(phenylmethyl) tetrahydro-1*H*-pyrrole-3-carboxylate (0.146 g, 0.59 mmol) in THF (10 ml), was added TBAF (0.7 ml, 0.7 mmol) and the reaction was stirred until the solid dissolved. Benzyl bromide (1.01 g, 5.9 mmol) was added and the reaction mixture was left stirring overnight. The mixture was evaporated under reduced pressure to give a yellowish residue which was then chromatographed on silica gel using petroleum-ether/ethyl acetate (6/4) to give the title compound as a colourless oil, 0.17 g (89 %). IR ν cm^{-1} : 1780 (C=O), 1747 (C=O), 1697 (C=O); δ_H ($CDCl_3$, 250 MHz): 2.90 (1H, d, J = 17 Hz, NC*H* HCO), 3.40 (1H, d, J = 12 Hz, C*H* HPh), 3.64 (1H, d, J = 12 Hz, CH*H* Ph), 3.71 (1H, d, J = 17 Hz, NCH*H* CO), 3.77 (3H, s, OCH_3), 4.31 (1H, d, J= 14 Hz, NC*H* HPh), 4.70 (1H, d, J = 14 Hz, NCH*H* Ph), 7.12-7.26 (10H, m, ArH); ^{13}C ($CDCl_3$, 100MHz): 36.5 (CH_2), 45.8 (CH_2), 53.4 (OCH_3), 55.3 (CH_2), 65.0 (quat.C), 127.3-128.9 (Ar CH), 133.8 (quat. C), 134.0 (quat. C), 166.6 (C=O), 169.8 (C=O), 202.6 (C=O); M/S: Found M^+ 337.1310, $C_{20}H_{19}NO_4$ requires 337.1310; m/z: 337 (M^+, 6%), 292 (9), 278 (13), 246 (20), 214 (39), 130 (14), 91 (100).

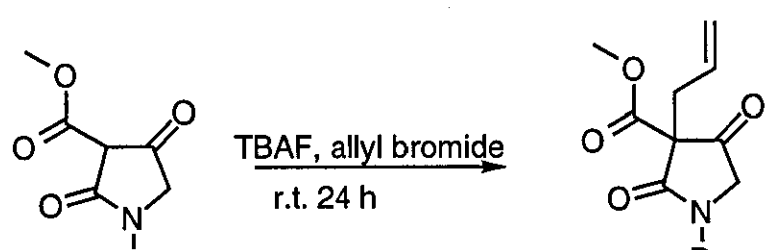
Methyl-3-ethyl-2,4-dioxo-1-(phenylmethyl)tetrahydro-1H-pyrrole-3-carboxylate



80

To a stirred solution of methyl 2,4-dioxo-1-(phenylmethyl) tetrahydro-1*H*-pyrrole-3-carboxylate (0.201 g, 0.81 mmol) in THF (10 ml), was added TBAF (0.97 ml, 0.97 mmol) and the reaction was stirred until the solid dissolved. Ethyl iodide (1.26 g, 8.13 mmol) was then added and the reaction mixture was left stirring overnight. The reaction mixture was evaporated under reduced pressure to give a yellowish residue which was then chromatographed on silica gel using petroleum-ether/ethyl acetate (6/4) to give a colourless oil , 0.14 g (63 %). IR ν cm^{-1} : 1780 (C=O), 1748 (C=O), 1693 (C=O); δ_H ($CDCl_3$, 250 MHz): 0.83 (3H, t, J = 7 Hz, C*H* $_3CH_2$), 2.22 (2H, q, J = 7 Hz, C*H* $_2CH_3$), 3.61 (1H, d, J = 17 Hz, C*H*HCO), 3.72 (3H, s, OCH_3), 3.91 (1H, d, J = 17 Hz, CH*H* CO), 4.73 (2H, s, CH_2Ph), 7.33-7.39 (m, 5H, ArH); ^{13}C ($CDCl_3$, 100MHz): 8.35 (CH_3), 23.9 (CH_2), 46.1 (CH_2), 53.2 (OCH_3), 55.4 (CH_2), 64.2 (quat. C), 128.1-128.9 (Ar CH), 134.2 (quat. C), 166.2 (C=O), 168.7 (C=O), 202.4 (C=O); M/S: Found M^+ 275.1164, $C_{15}H_{17}NO_4$ requires 275.1158; m/z :275 (M^+, 36%), 247 (2), 230 (4), 216 (50), 214 (20), 118 (14), 91 (100).

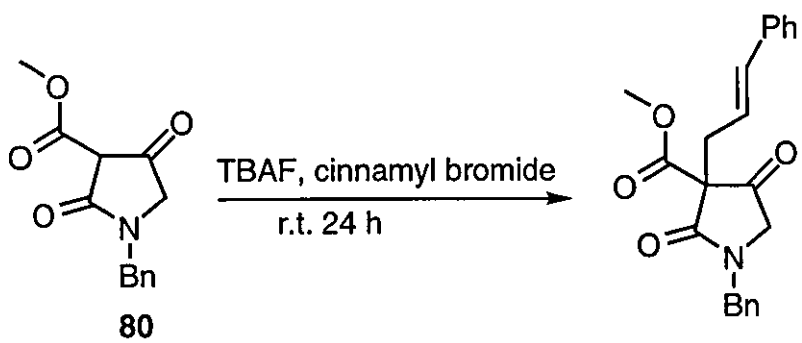
Methyl 2,4-dioxo-1-(phenylmethyl)-3-prop-2-enyltetrahydro-1H-pyrrole-3-carboxylate



80

To a stirred solution of methyl 2,4-dioxo-1-(phenylmethyl) tetrahydro-1*H*-pyrrole-3-carboxylate (2.1 g, 8.5 mmol) in THF (20 ml), was added TBAF (10.2 ml, 10.2 mmol) and the reaction was stirred until the solid dissolved. Allyl bromide (2.05 g, 17 mmol) was then added and the reaction mixture was left stiring overnight. The mixture was evaporated under reduced pressure to give a yellowish residue which was then chromatographed on silica gel using petroleum-ether/ethyl acetate (7/3) to give the title compound as a colourless oil, 1.4 g (60 %). IR ν cm^{-1} : 1781 (C=O), 1738 (C=O), 1698 (C=O); δ_H ($CDCl_3$, 400 MHz): 2.86 (1H, dd, J = 7.6 Hz and 8 Hz, C*H*HCH=C), 2.96 (1H, dd, J = 7.2 Hz, and 8 Hz, CH*H*CH=C), 3.53 (1H, d, J =17.6 Hz, CC*H*HCO), 3.74 (3H, s, OCH_3), 3.88 (1H, d, J = 17.6 Hz, CH*H*CO), 4.66 (1H, d, J = 14 Hz, C*H*HPh), 4.72 (1H, d, J = 14 Hz, CH*H*Ph), 5.06 (1H, d, J = 8 Hz, CH*H*=CH), 5.19 (1H, d, J = 16 Hz, C*H* H=CH), 5.56-5.58 (1H, m, CH=C), 7.25-7.36 (5H, m, ArH); ^{13}C ($CDCl_3$, 100MHz): 34.8 (CH_2), 46.2 (CH_2), 53.4 (OCH_3), 55.5 (CH_2), 63.4 (quat. C), 121.1 (CH_2), 121.4 (CH olefinic), 128.2-130.1 (Ar CH), 134.7 (quat. C), 165.3 (C=O), 168.2 (C=O), 208.6 (C=O); M/S: Found M^+ 287.1156, $C_{15}H_{17}NO_4$ requires 287.1157; m/z :287 (M^+, 8%), 246 (19), 214 (71), 91 (100), 65 (10).

Methyl 2,4-dioxo-1-(phenylmethyl)-3-(3phenylprop-2-enyl)tetrahydro-1*H*-pyrrole

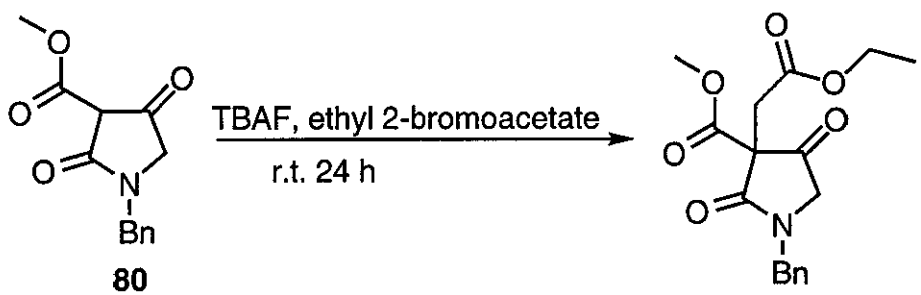


80

To a stirred solution of the ketoester (1.5 g, 6.07 mmol) in THF (20 ml) was added TBAF (7.3 ml, 7.3 mmol) followed by cinnamyl bromide (2.39 g, 12.1 mmol). The reaction mixture was stirred overnight after which the solvent was removed under reduced pressure to give a yellowish crude product. The crude product was purified over a short column of silica gel using petroleum-ether/ethyl acetate (70/30) to give the title product as yellowish oil, (1.4 g, 64 %). IR ν cm^{-1} : 1780 (C=O), 1747 (C=O), 1694 (C=O); δ_H ($CDCl_3$, 400 MHz): 3.00 (1H, dd, J = 7Hz and 9 Hz, C*H*HCH=C), 3.13 (1H, dd, J = 7Hz and 9 Hz, CH*H*CH=C), 3.50 (1H, d, J =

20 Hz, C*H* HCO), 3.76 (3H, s, OCH_3), 3.86 (1H, d, J = 20 Hz, CH*H*CO), 4.38 (1H, d, J = 16 Hz, CH*H* Ph), 4.97 (1H, d, J = 16 Hz, C*H* HPh), 5.94-5.96 (1H, m, C*H* (CH_2)=CH), 6.52 (1H, d, J = 16 Hz, PhC*H* =CH), 7.28 -7.39 (m, 10H, ArH); ^{13}C ($CDCl_3$, 100MHz): 34.1 (CH_2), 46.2 (CH_2), 53.4 (OCH_3), 55.4 (CH_2), 63.6 (quat. C), 127.6 -128.9 (aromatic C), 134.4 (quat.C), 136.1 (CH olefinic), 136.4 (quat. C), 165.3 (C=O), 168.2 (C=O), 202.1 (C=O); M/S: Found M^+ 363.1473, $C_{22}H_{21}NO_4$ requires 363. 1470; m/z :363 (M^+, 12%), 304 (36), 128 (19), 117 (24), 91 (100), 65 (11).

Methyl3-[2-(ethyloxy)-2,4-dioxo-1-(phenylmethyl)tetrahydro-1*H*-pyrrole-3-carboxylate

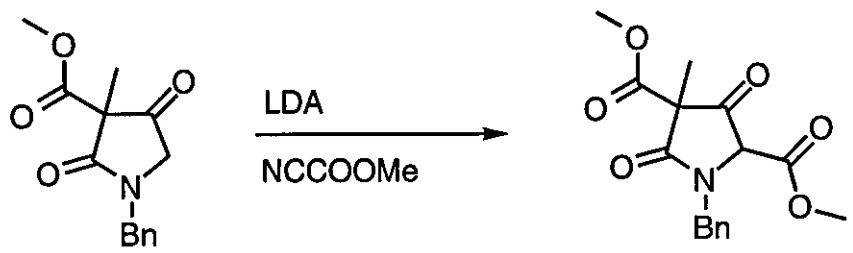


80

To a stirred solution of the ketoester (1.04 g, 4.2 mmol) in THF (15 ml), was added TBAF (5.05 ml, 5.05 mmol) followed by ethyl 2-bromoacetate (1.4 g, 8.4 mmol). The reaction mixture was stirred overnight after which the solvent was removed under reduced pressure to give an orange crude product. The crude product was purified over a short column of silica gel using petroleum-ether/ethyl acetate (70/30) to give the title product as colourless oil, (0.58 g, 43 %). IR ν cm^{-1}: 1785 (C=O), 1747 (C=O), 1724 (C=O), 1701 (C=O); δ_H ($CDCl_3$, 250 MHz): 1.22 (3H, t, J = 7 Hz, C*H* $_3CH_2$), 3.34 (1H, d, J = 18 Hz, C*H* HCO), 3.43 (1H, d, J = 18 Hz, CH*H* CO), 3.73 (3H, s, OCH_3), 3.87 (1H, d, J = 17 Hz, NC*H* HCO), 3.96 (1H, d, J = 17 Hz, NCH*H*CO), 4.09 (2H, q, J = 7 Hz, C*H* $_2CH_3$), 4.54 (1H, d, J = 14.9 Hz, NC*H* HPh), 4.91 (1H, d, J = 14.9 Hz, NCH*H* Ph), 7.27-7.37 (m, 5H, Ar H); ^{13}C ($CDCl_3$, 100MHz): 14.0 (CH_3), 35.9 (CH_2), 46.5 (CH_2), 53.6 (OCH_3), 55.6 (CH_2), 60.2 (quat.C), 61.6 (CH_2), 128.0-128.8 (aromatic C), 134.8 (quat. olefinic C), 164.5 (C=O), 168.3 (C=O), 170.4 (C=O), 200.8 (C=O); M/S: (Found M^+, 333.1218, $C_{17}H_{19}NO_6$ requires 333.1212); m/z: 333 (M^+, 17%), 274 (11), 200 (31), 118 (10), 91 (100)

Dimethyl-4-methyl-3,5-dioxo-1-(phenylmethyl) tetrahydro-1*H*-pyrrole-2,4-dicarboxylate 83

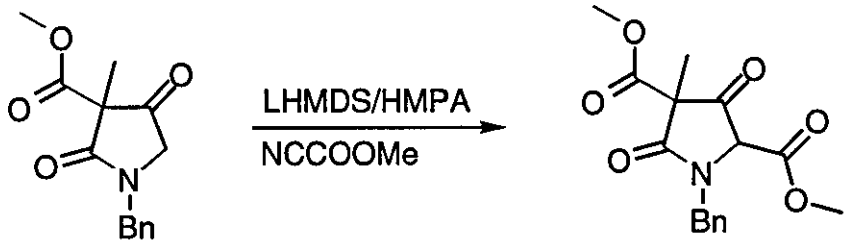
Method A:



81 **83**

To diisopropylamine (0.88 ml, 6.27 mmol) in tetrahydrofuran (15 ml) at 0 °C was added n-butyllithium (4.0 ml, 6.27 mmol), of a 1.6 M solution in hexane), and the reaction stirred at the same temperature for fifteen minutes. The reaction mixture was cooled to -78 °C and the ester **81** (1.44 g, 5.55 mmol) was added in THF (10 ml). The reaction mixture was stirred at 0 °C for one hour and then recooled to -78 °C, after which methyl cyanoformate (0.47 g, 5.55 mmol) was added. The reaction mixture was allowed to warm to room temperature over one hour and then poured into saturated aqueous ammonium chloride solution (80 ml), extracted with ethyl acetate (50 ml x 2), and dried over anhydrous sodum sulfate. Column chromatography on silica gel of the crude extract using ethyl acetate/petroleum-ether (6:4) gave the title compound as colourless oil, (0.1g, 5%) (2:1 mixture of diastereomers) . IR ν cm^{-1} :1789 (C=O), 1748 (C=O), 1731 (C=O), 1713 (C=O); δ_H ($CDCl_3$, 250 MHz): 1.54 (major isomer) (3H, s, CH_3), 1.57 (minor isomer) (3H, s, CH_3), 1.60 (3H, s, CH_3), 3.76 (minor isomer) (3H, s, OCH_3), 3.77 (major isomer) (3H, s, OCH_3), 3.78 (major isomer) (3H, s, OCH_3), 3.81 (minor isomer) (3H, s, OCH_3), 4.06 (major isomer) (1H, d, J = 17 Hz, C*H* HPh), 4.19 (minor isomer) (1H, d, J = 17 Hz, C*H* HPh), 4.35 (minor isomer) (s, 1H, CHCO), 4.57 (major isomer) (1H, s, CHCO), 5.38 (major isomer) (1H, d, J = 17 Hz, CH*H* Ph), 5.46 (minor isomer) (1H, d, J = 17 Hz, CH*H* Ph), 7.25-7.39 (10H, m, ArH); ^{13}C ($CDCl_3$, 100MHz): 14.0 (major isomer) (CH_3), 15.7 (minor isomer) (CH_3), 45.1(major isomer) (CH_2), 46.6 (minor isomer) (CH_2), 53.3 (OCH_3), 53.4 (OCH_3), 58.1 (quat. C), 60.1 (quat. C), 66.4 (major isomer) (CH), 67.4 (minor isomer) (CH), 128.1-128.9 (Ar CH), 133.1 (major isomer) (quat. olefinic C), 34.0 (minor isomer) (quat. olefinic C), 163.8 (C=O), 164.8 (C=O), 165.2 (C=O), 169.3 (C=O), 195.3 (C=O), 196.7 (C=O); M/S: (Found M^+, 319.1060, $C_{16}H_{17}NO_6$ requires M^+ 319.1056); (m/z); 319 (M^+ , 29%), 260(49), 177 (15), 109 (19), 91 (100).

Method B:



To a stirred solution of LHMDS (2.2 ml, 2.2 mmol) in 10% HMPA/THF (10 ml) at -78 °C, was added the ester (0.296 g, 1.1 mmol) in THF (2 ml). The solution was stirred for forty-five minutes at that temperature when methyl cyanoformate (0.19 g, 2.2 mmol) was added in one portion. The reaction was stirred at -78 °C for two hours and then quenched with saturated aqueous ammonium chloride (4 ml), diluted with dichloromethane (40 ml) and then washed with water and brine. The organic layer was dried over anhydrous magnesium sulfate, filtered, evaporated to give a residue which was chromatographed on silica gel (petroleum-ether/ethyl acetate, 6/4) to give the title compound as colourless oil (0.23 g, 65 %). The spectroscopic data is the same as **83** above.

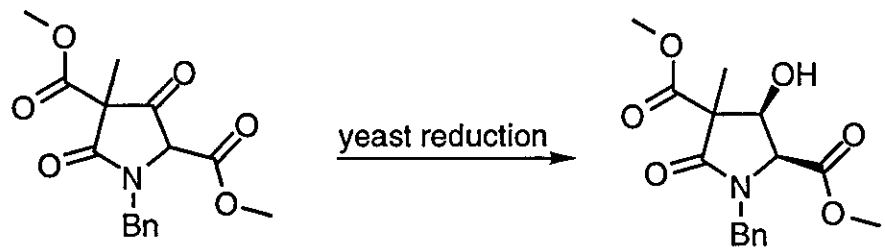
2-ethyl-4-methyl-4-methyl-3,5-dioxo-1-(phenylmethyl) tetrahydro-1*H*-pyrrole-2,4-dicarboxylate

81

To a stirred solution of LHMDS (3.3 ml, 3.3 mmol) in 10% HMPA/THF (15 ml) at -78 °C, was added the ester (0.432 g, 1.65 mmol) in THF (4 ml). The solution was stirred for forty-five minutes at that temperature when ethyl cyanoformate (0.32 g, 3.3 mmol) was added in one portion. The reaction was stirred at -78 °C for two hours and quenched with saturated aqueous ammonium chloride (6 ml), diluted with dichloromethane (60 ml) and then washed with water and brine. The organic layer was dried over anhydrous magnesium sulfate, filtered, evaporated to give a residue which was chromatographed on silica gel (petroleum-ether/ethyl acetate,

6/4) to give the title compound as colourless oil (0.32 g, 56 %). IR ν cm^{-1} :1790 (C=O), 1747 (C=O), 1712 (C=O), 1698 (C=O); δ_H ($CDCl_3$, 250 MHz): 1.27 (major and minor isomers) (6H, t, J = 7.2 Hz, 2 x C*H*$_3$CH_2), 1.53 (minor isomer) (3H, s, CH_3), 1.56 (major isomer) (3H, s, CH_3), 3.71 (major isomer) (3H, s, OCH_3), 3.75 (minor isomer) (3H, s, OCH_3), 4.06 (minor isomer) (1H, d, J = 17 Hz, C*H* HPh), 4.20 (m, 4H, 2 x C*H*$_2$CH_3), 4.24 (major isomer) (1H, d, J = 17 Hz, C*H* HPh), 4.51 (major isomer) (1H, s, CHCOOCH_3), 4.92 (minor isomer) (1H, s, CHCOOCH_3), 5.36 (minor isomer) (1H, d, J = 17 Hz, C*H* HPh), 4.71 (minor isomer) (1H, d, J = 17 Hz, CH*H* Ph), (6.46 (major isomer) (1H, d, J = 17 Hz, CH*H* Ph), 7.20-7.37 (m, 10H, ArH); ^{13}C ($CDCl_3$, 100MHz): 13.1 (minor) (CH_3), 13.9 (major) (CH_3), 15.7 (CH_3), 45.1 (CH_2), 53.4 (OCH_3), 58.2 (quat. C), 66.4 (minor isomer) (CH), 67.4 (major isomer) (CH), 128.1-128.9 (Ar CH), 133.9 (major isomer)(quat. olefinic C), 134.1 (minor isomer) (quat. olefinic C), 163.4 (C=O), 164.4 (C=O), 165.3 (C=O), 169.4 (C=O), 195.3 (C=O), 196.9 (C=O); M/S:Found M^+ 333.1208, $C_{17}H_{19}NO_6$ requires 333.1212; m/z: 333 (M^+ , 5%), 276 (16), 105 (28), 91 (100), 86 (22).

Dimethyl (2*S*, 3*R*)-3-hydroxy-4-methyl-5-oxo-1-(phenylmethyl)tetrahydro-1*H*-pyrrole-2,4-dicarboxylate 85

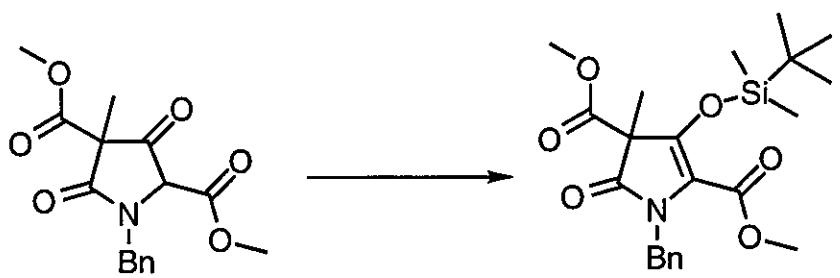


83 **85**

To the keto ester **83** (0.9 g) was added tap water (80 ml), sucrose (14 g), and dried bakers' yeast (*Saccharomyces cerevisiae*) (10 g) and the resulting mixture was stirred at 30 °C for twenty-four hours. After that period, Celite was added to the reaction mixture and the mixture was filtered and the solid washed thoroughly with water. The filtrate was saturated with sodium chloride and extracted with ether (20 ml x 5). The combined extracts were washed with water, dried over anhydrous magnesium sulfate and evaporated to give the hydroxy ester (50 mg, 5%). IR ν cm^{-1} :3414 (OH), 1747 (C=O), 1714 (C=O), 1693 (C=O); δ_H ($CDCl_3$, 400 MHz): 1.51 (3H, s, CH_3), 3.73 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 4.00 (1H, d, J

=7.2 Hz, CHC*H* COO), 4.23 (1H, d, J =7.2 Hz, C*H* CHCOO), 4.18 (1H, d, J = 17 Hz, C*H* HPh), 5.37 (1H, d, J = 17 Hz, CH*H* Ph), 7.20-7.35 (m, 5H, ArH); $^{13}C(CDCl_3$, 100MHz): 19.5 (CH_3), 45.6 (CH_2), 53.3 (OCH_3), 53.5 (OCH_3), 61.6 (CH), 62.6 (quat.C), 74.4 (CH), 127.8-129.1 (Ar CH), 134.9 (quat. olefinic C), 166.2 (C=O), 169.0 (C=O), 171.1 (C=O); M/S:(Found M^+ , 321.1215 $C_{16}H_{19}NO_6$ requires M^+ 321.1210); (m/z): 321 (M^+, 25%), 262 (32), 219 (17), 218 (39), 178 (42), 177 (68), 116 (28), 106 (19), 91 (100), 65 (23).

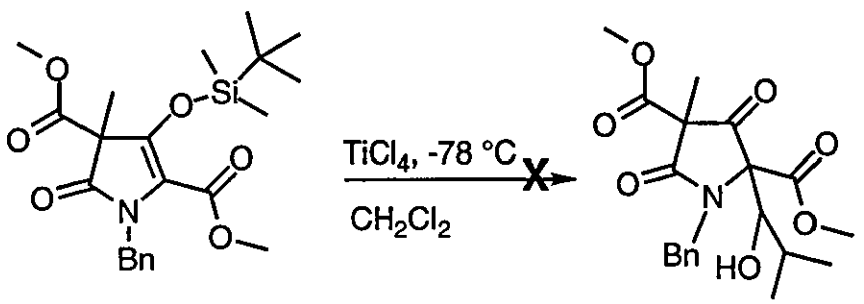
Dimethyl 3-{[1-(1,1-dimethylethyl)-1,1-dimethylsilyl]oxy}-4-methyl-5-oxo-1-(phenylmethyl)-4,5-dihydro-1*H*-pyrrole-2,4-dicarboxylate 88



83 **88**

To a stirred solution of diester (0.147 g, 0.46 mmol) in dry dichloromethane (10 ml) were added 2,6-lutidine (0.147 g, 1.38 mmol) and TBSOTf (0.133 g, 0.506 mmol) under nitrogen at room temperature. After the reaction mixture was stirred for four hours, the solvent was evaporated and the residue was chromatographed on silica gel using petroleum-ether/ethyl acetate (70/30) to give the silylated compound **88** as colourless oil (0.13 g, 70%). IR ν cm^{-1} :1759 (C=O), 1715 (C=O), 1614 (C=C); δ_H ($CDCl_3$, 400MHz): 0.09 (3H, s, CH_3), 0.18 (3H, s, CH_3), 0.84 (9H, s, $(CH_3)_3$), 1.58 (3H, s, CH_3), 3.64 (3H, s, OCH_3), 3.73 (3H, s, OCH_3), 4.93 (1H, d, J = 15 Hz, CH*H* Ph), 5.07 (1H, d, J = 15 Hz, C*H* HPh), 7.17-7.30 (m, 5H, ArH); ^{13}C ($CDCl_3$, 100MHz): -4.56 (CH_3), -4.23 (CH_3), 17.0 (CH_3), 18.5 (quat. C), 25.5 ($(CH_3)_3$), 44.7 (CH_2), 51.3 (OCH_3), 53.0 (OCH_3), 58.6 (quat. C), 115.3 (quat. C=C), 127.2- 128.3 (aromatic C), 137.4 (quat. C), 150.0 (quat. C), 159.9 (C=O), 167.5 (C=O), 170.8 (C=O); M/S: (Found M^+, 433.1922 $C_{22}H_{31}NO_6Si$ requires M^+ 433.1921; (m/z): 433 (M^+, 6%), 377 (25), 376 (100), 189 915), 147 (46), 91 (72), 73 (24).

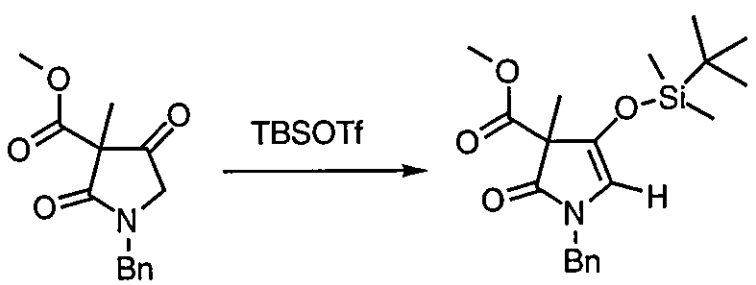
Attempted Mukaiyama type aldol reaction of dimethyl 3-{[1-(1,1-dimethylethyl)-1,1-dimethylsilyl]oxy}-4-methyl-5-oxo-1-(phenylmethyl)-4,5-dihydro-1*H*-pyrrole-2,4-dicarboxylate 88



88

A solution of the silyl enol ether (0.237 g, 0.54 mmol) in dichloromethane (2 ml) was added dropwise to a solution of isobutyraldehyde (0.11 ml, 0.82 mmol) and $TiCl_4$ (0.10 ml, 0.82 mmol) in dichloromethane (10 ml), cooled to -78 °C, and the resulting mixture was stirred for two hours. After that period, the reaction mixture was quenched with aqueous potassium carbonate solution, and allowed to warm at room temperature. The mixture was extracted with ether (10 ml x 3), and the combined organic extracts were washed with water and dried with anhydrous magnesium sulfate. Purification of the crude extract with column chromatography on silica gel gave the starting material 0.19 g (80%). All analytical data agreed with that obtained for the product **88** of the above reaction .

Methyl 4-{[1-(1,1-dimethylethyl)-1,1-dimethylsilyl]oxy}-3-methyl-2-oxo-1-(phenylmethyl)-2,3-dihydro-1*H*-pyrrole-3-carboxylate 89

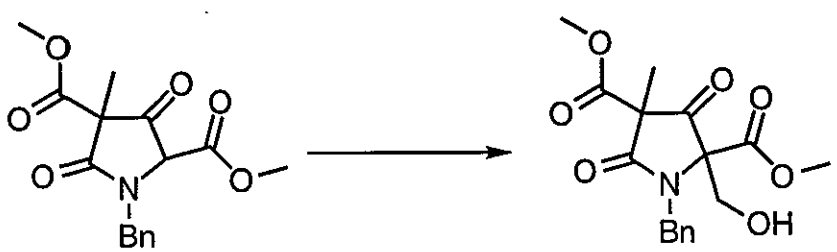


81 **89**

TBSOTf (0.36 ml, 1.3 mmol), then triethylamine (0.26 ml, 1.8 mmol) were added at 0 °C to a solution of the ester (0.241 g, 0.9 mmol) in dry dichloromethane (20 ml)

under nitrogen atmosphere. The solution was warmed to room temperature and left stirring for another twelve hours. Evaporation of the solution and column chromatography on silica gel of the crude extract using petroleum-ether/ethyl acetate (8/2) afforded the title compound as white solid, 0.33 g (97%), m.p. 90-91 °C. IR ν cm^{-1} :1748 (C=O), 1714(C=O), 1659 (C=C); δ_H ($CDCl_3$, 250 MHz): 0.08 (3H, s, CH_3), 0.11 (3H, s, CH_3), 0.87 (9H, s, $C(CH_3)_3$), 1.53 (3H, s, CH_3), 3.73 (3H, s, OCH_3), 4.58 (1H, d, J = 15 Hz, NC*H*HPh), 4.68 (1H, d, J = 15 Hz, NCH*H*Ph), 5.45 (1H, s, CH=C), 7.22-7.35 (5H, m, ArH); ^{13}C ($CDCl_3$, 100MHz): -5.2 (CH_3), -4.7 (CH_3), 16.8 (CH_3), 17.9 (quat.C), 25.3 ($C(CH_3)$, 45.3 (CH_2), 52.7 (OCH_3), 57.6 (quat. C), 108.5 (CH olefinic), 127.3-129.0 (aromatic C), 136.3 (quat. C), 141.6 (quat. C), 168.7 (C=O), 171.6 (C=O); M/S: (Found M^+, 375.1867 $C_{20}H_{29}NO_4Si$ requires 375.1866; Found C, 63.97%; H, 7.75%; N, 3.46% $C_{17}H_{25}NO_8$ requires C, 63.97%; H, 7.75%; N, 3.73%); (m/z): 375 (M^+, 70%), 318 (37), 316 (47), 227 (40), 171 (19), 91 (100).

Dimethyl 2-(hydroxymethyl)-4-methyl-3,5-dioxo-1-(phenylmethyl)tetrahydro-1*H*-pyrrole-2,4-dicarboxylate 90

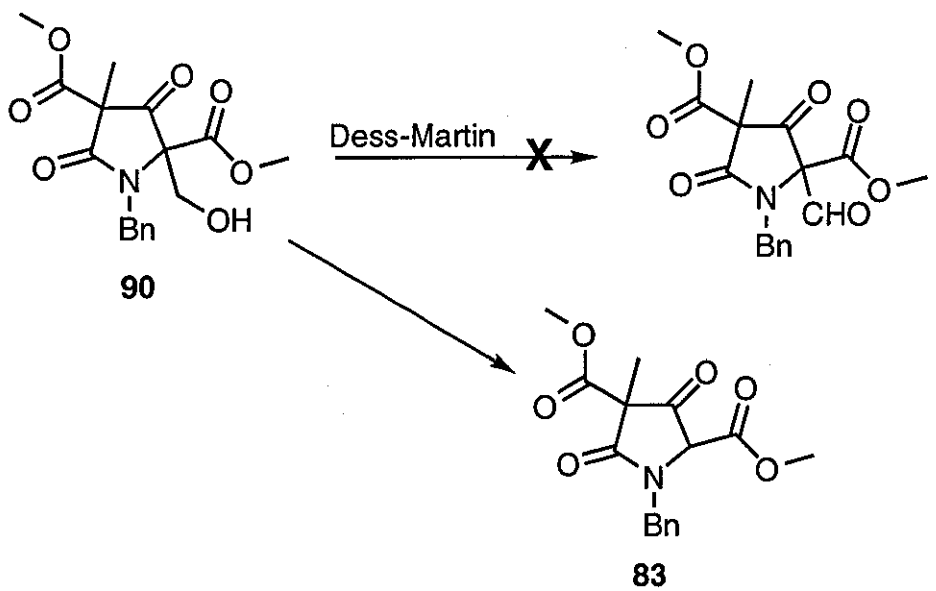


83 **90**

A stirred solution of β-keto ester, 0.352 g (1.1 mmol) in THF (8 ml) was treated with DBU (0.033 ml, 0.22 mmol) at 0 °C for fifteen minutes. The solution was then cooled to -78 °C and treated with a solution of formalin (37 wt %, 12 M aqueous solution, 0.91 ml, 11 mmol) in THF (5 ml). The reaction was stirred for thirty minutes at that temperature and then quenched with 10% aqueous $CuSO_4$ solution. The reaction mixture was extracted with diethyl ether (10 ml x 3) and the organic extracts were washed with brine and dried. Evaporation of the solvent *in vacuo* gave the crude product, which was purified by flash chromatography on silica gel using petroleum-ether/ethyl acetate (6/4) to give the title compound as colourless oil , (0.235 g, 62 %). IR ν cm^{-1}: 3421 (OH), 1789 (C=O), 1748 (C=O), 1698 (C=O); δ_H ($CDCl_3$, 250 MHz): 1.59 (3H, s, CH_3), 1.59 (1H, s, OH), 3.57 (3H,

s, OCH_3), 3.74 (3H, s, OCH_3), 3.77-3.89 (2H, m, C*H*$_2$OH), 4.57 (1H, d, J = 15 Hz, NC*H* HPh), 5.03 (1H, d, J = 15 Hz, NCH*H* Ph), 7.20-7.39 (5H, m, ArH); ^{13}C ($CDCl_3$, 100MHz): 16.2 (CH_3), 44.7 (CH_2), 53.0 (OCH_3), 53.4 (OCH_3), 57.3 (quat.C), 61.0 (CH_2), 78.5 (quat.C), 127.8-129.2 (aromatic C), 136.3 (quat. C), 165.2 (C=O), 166.4 (C=O), 171.5 (C=O), 198.6 (C=O); M/S: (Found M^+ 349.1164, $C_{17}H_{19}NO_7$ requires 349.1161; (m/z): 349 (M^+, 14%), 319 (94), 287 (38), 260 (56), 221 (21), 177 917), 132 (17), 106 (92), 91 (100), 83 (25).

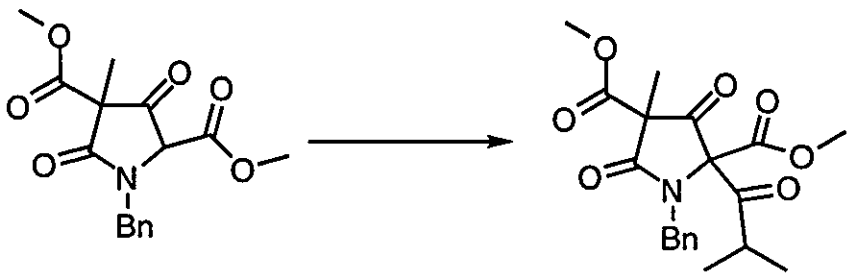
Attempted Dess-Martin oxidation of dimethyl 2-(hydroxymethyl)-4-methyl-3,5-dioxo-1-(phenylmethyl)tetrahydro-1*H*-pyrrole-2,4-dicarboxylate 90



90 **83**

To a stirred solution of the alcohol (0.156 g, 0.44 mmol) in dichloromethane (12 ml) under the atmosphere of nitrogen was added a suspension of Dess-Martin periodinane (0.272 g, 0.64 mmol) in dichloromethane (4 ml). The solution was stirred for one hour at room temperature and then washed with 1:1 sat. $NaHCO_3$:10% $Na_2S_2O_4$, water and brine. The crude product was purified by column chromatography on silica gel (petroleum-ether/ethyl acetate, 70/30) to give the β-keto ester **83** as colourless oil, 90 mg (64 %). All analytical data agreed with that obtained previously for compound **83** .

Dimethyl-4-methyl-2-(2-methylpropanoyl)-3,5-dioxo1(phenylmethyl) tetrahydro-1*H*-pyrrole-2,4-dicarboxylate 95



83 **95**

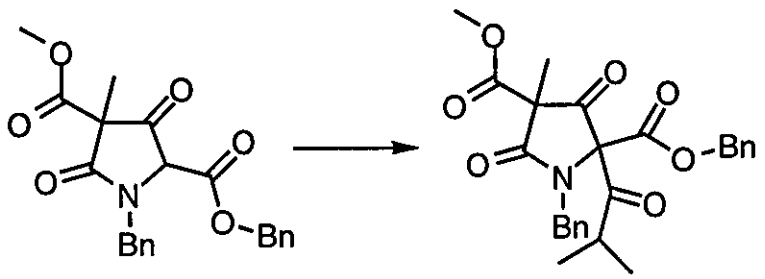
To a stirred solution of the keto-ester (0.81 g, 2.5 mmol) in dry dichloromethane (30 ml) was added pyridine (0.4 ml, 5.0 mmol) and the solution was stirred at room temperature for five minutes. Isobutyrylchloride (0.32 g, 3.0 mmol) in dichloromethane (5 ml) was added and the reaction mixture was allowed to stir for another twenty minutes. After that period, the mixture was quenched with water and the organic layer was washed with aqueous $CuSO_4$ (15 ml x 4). The combined organic fractions was then dried over anhydrous sodium sulfate and the solvent removed under vacuum to give a yellowish crude product. Column chromatography on silica gel of the crude product using petroleum-ether/ethyl acetate (70/30) afforded the title compound as colourless oil, 0.83 g (90%). IR ν cm^{-1} : 1775 (C=O), 1754 (C=O), 1724 (C=O); δ_H ($CDCl_3$, 250 MHz): 1.24 (6H, d, J = 7.0 Hz, (C*H* $_3)_2$CH), 1.57 (3H, s, CH_3), 2.72 (1H, sextet, J = 7.0 Hz, C*H* $(CH_3)_2$) 3.68 (3H, s, OCH_3), 3.75 (3H, s, OCH_3), 4.98 (1H, d, J = 15 Hz, C*H* HPh), 5.15 (1H, d, J = 15 Hz, CH*H* Ph), 7.22- 7.33 (5H, m, ArH); ^{13}C ($CDCl_3$, 100MHz): 16.6 (CH_3), 18.7 ($2CH_3$), 33.9 (CH), 44.4 (CH_2Ph), 52.1 (OCH_3), 53.3 (OCH_3), 57.4 (quat. C), 127.2-128.5 (aromatic CH), 137.1 (quat.C), 141.0 (quat.C), 159.0 (C=O), 167.1 (C=O), 171.9 (C=O), 173.6 (C=O ; M/S: (Found M^+, 389.1468, $C_{20}H_{23}NO_7$ requires 389.1474; (m/z): 389 (M^+, 5%), 320 (14), 319 (78), 228 (10), 91 (100), 71 (65).

4-methyl-2-phenylmethyl-4-methyl-3,5-dioxo-1-(phenylmethyl) tetrahydro-1*H*-pyrrole-2,4-dicarboxylate 97

81 **97**

To a stirred solution of LHMDS (3.7 ml, 3.73 mmol) in 10% HMPA/THF (15 ml) at -78 °C, was added the ester (0.487 g, 1.86 mmol) in THF (3 ml). The solution was stirred for forty-five minutes at that temperature and benzyl cyanoformate (0.60 g, 3.73 mmol) was added in one portion. The reaction was stirred at -78 °C for two hours and quenched with saturated aqueous ammonium chloride (5 ml), diluted with dichloromethane (30 ml) and then washed with water and brine. The organic layer was dried over anhydrous magnesium sulfate, filtered and evaporated to give a residue which was chromatographed on silica gel (petroleum-ether/ethyl acetate, 8/2) to give the title compound as colourless oil (0.47 g, 60 %). IR ν cm^{-1} :1791 (C=O), 1751 (C=O), 1708 (C=O) ; δ_H ($CDCl_3$, 250 MHz): 1.54 (3H, s, CH_3), 3.71 (3H, s, OCH_3), 3.95 (major isomer) (1H, d, J = 14.4 Hz, NC*H* HPh), 4.16 (minor isomer) (1H, d, J = 14.6 Hz, NC*H* HPh), 4.33 (minor isomer) (1H, s, CHCOO), 4.55 (major isomer) (1H, s, CHCOO), 5.13 (minor isomer) (1H, d, J = 12.3 Hz, OC*H* H_2Ph), 5.22 (major isomer)(1H, d, J = 12. 4 Hz, OC*H* HPh), 5.39 (major) (1H, d, 14.4 Hz, NCH*H* Ph), 5.46 (minor) (1H, d, J = 14.6 Hz, NCH*H* Ph), 7.30-7.38 (10H, m, ArH); ^{13}C ($CDCl_3$, 100MHz): 15.7 (CH_3), 45.2 (CH_2), 53.3 (minor isomer) (CH), 53.5 (major isomer) (CH), 58.3 (quat. C), 65.3 (minor isomer) (OCH_3), 66.6 (major isomer), (OCH_3), 68.1 (minor isomer) (OCH_2), 68.4 (major isomer) (OCH_2), 128.1-129.1 (Ar CH), 133.8 (major isomer), quat. C), 134.0 (minor isomer), quat. C), 134.1 (major isomer) (quat. C), 163.3 (C=O), 164.4 (C=O), 165.3 (C=O), 168.4 (C=O) and 169.4 (C=O), 195.8 (C=O) and 196.8 (C=O); M/S:Found M^+ 395.1369, $C_{22}H_{21}NO_6$ requires 395.1368 m/z: 395 (M^+ , 30%), 319 (15), 304 (87), 260 (100), 107 (13), 91 (100), 65 (22).

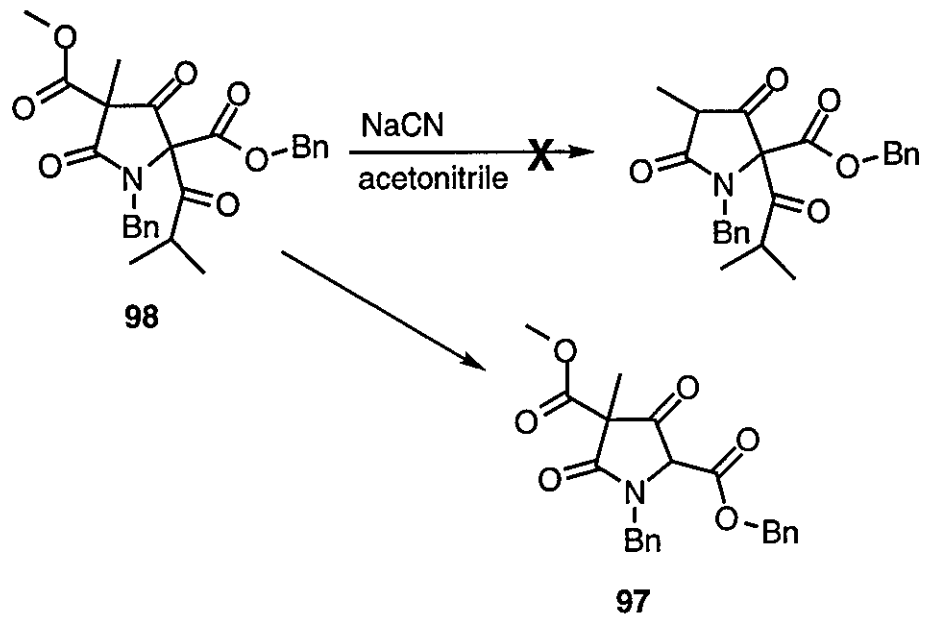
4-methyl-2-phenylmethyl-4-methyl-2-(2-methylpropanoyl)-3,5-dioxo-1-(phenylmethyl) tetrahydro-1*H*-pyrrole-2,4-dicarboxylate 98



97 **98**

To a stirred solution of the keto-ester **97** (0.211g, 0.53 mmol) in dry dichloromethane (10 ml) was added pyridine (10.1 ml, 1.32 mmol) and the solution was stirred at room temperature for five minutes. Isobutyrylchloride (0.129 g, 0.63 mmol) in dichloromethane (5 ml) was added and the reaction mixture was allowed to stir for another thirty minutes. After that period, the mixture was quenched with water and then the organic layer was washed with aqueous $CuSO_4$ (10 ml x 3) The organic fractions were combined and dried over anhydrous sodium sulfate and the solvent was removed under vacuum to give the crude product. Column chromatography on silica gel of the crude extract using petroleum-ether/ethyl acetate (80/20) afforded the title compound as colourless oil, 0.17 g (69 %). IR ν cm^{-1} : 1774 (C=O), 1752 (C=O), 1720 (C=O); δ_H ($CDCl_3$, 250 MHz): 1.01 (3H, d, J = 6.8 Hz, C*H*$_3$CH), 1.04 (3H, d, J = 6.9 Hz, C*H*$_3$CH), 1.55 (3H, s, CH_3), 2.40 (sextet, 1H, J = 6.9 Hz, C*H*(CH_3)$_2$), 3.73 (s, 3H, OCH_3), 5.02 (1H, d, J = 15 Hz, C*H*HPh), 5.15 (1H, d, J = 15 Hz, CH*H*Ph), 5.08 (2H, s, OCH_2Ph), 7.20-7.34 (10H, m, ArH); ^{13}C ($CDCl_3$, 100MHz): 16.7 (CH_3), 18.4 (2 x CH_3), 33.7 (CH), 44.4 (CH_2Ph), 53.3 (OCH_3), 57.4 (OCH_2), 134.5 (quat.C), 137.1 (quat.C), 141.2 (quat.C), 158.5 (C=O), 167.1 (C=O), 172.0 (C=O), 173.4 (C=O); M/S: Found M^+, 465.1787 $C_{26}H_{27}NO_7$ requires 465.1787; (m/z): 465 (M^+, 16%), 395 (100), 377 (14), 351 (22), 287 (72), 91 (100).

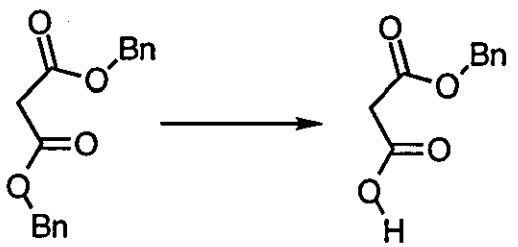
Attempted decarboxymethylation of 4-methyl-2-phenylmethyl-4-methyl-2-(2-methylpropanoyl)-3,5-dioxo-1-(phenylmethyl) tetrahydro-1*H*-pyrrole-2,4-dicarboxylate



98 **97**

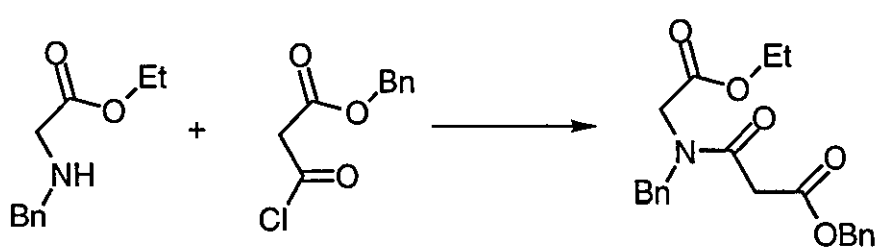
To a stirred solution of sodium cyanide (0.137 g, 2.80 mmol) in acetonitrile (20 ml) at 78 °C was added the ester (1.089 g, 2.34 mmol) in one portion. The solution turned yellow after fifteen minutes and the reaction mixture was further reflux at that temperature. After all the starting material was consumed, the reaction was stopped, cooled and the solvent was removed under vacuum to give a yellowish solid. The solid was dissolved in water and extracted with dichloromethane (15 ml x 3). The organic phase was washed with brine and dried over anhydrous magnesium sulfate and the solvent was removed to give a yellowish crude extract. Purification of the crude using column chromatography on silica gel (petroleum-ether/ethyl acetate, 70/30) gave the β-keto ester **97** (0.483 g, 52%). All analytical data agreed with that obtained previously for compound **97** .

3-oxo-3-[phenylmethyl)oxy]propanoic acid



To a stirred solution of dibenzylmalonate (28.6 g, 0.1 mol) in benzyl alcohol (250 ml) was added gradually a solution of KOH (6.5 g, 0.1 mol) in benzyl alcohol (100 ml). The reaction mixture was stirred under nitrogen for six hours at room temperature after which it was diluted with diethyl ether (1.5 L) and the monoester benzyl malonate formed was then filtered, washed with diethyl ether and then air dried to give the title compound as a white solid (18.3 g, 82 %).[53] m.p. 209-210 °C. IR ν cm^{-1} :1725 (C=O), 1644 (C=O); δ_H (D_2O, 400 MHz): 3.26 (2H, s, CH_2), 5.12 (2H, s, CH_2Ph), 7.33-7.38 (5H, m, aromatic protons); ^{13}C (D_2O, 100MHz): 44.7 (CH_2), 67.6 (CH_2), 128-129 (aromatic CH), 135.9 (quat. C), 171.5 (C=O), 174.3 (C=O); (M/S: Found M^+ - 44, 150.0682 $C_9H_{10}O_2$ requires 150.0681; M/S 150 (M^+ - 44, 10%) , 108 (100), 91 (40), 79 (90), 51 (22)

Phenylmethyl 3-[[2-(ethyloxy)-2-oxoethtyl](phenylmethyl)amino]-3-oxopropanoate 100

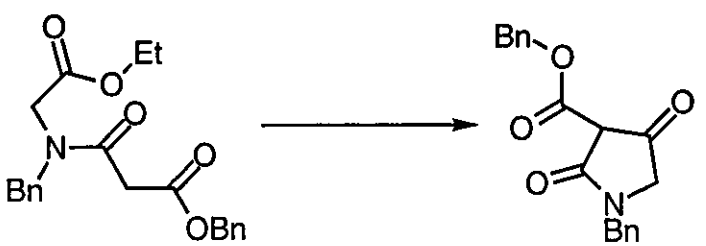


100

Monobenzyl ester malonate (11.0 g, 0.056 mol) was dissolved in toluene (100 ml) and excess oxalyl chloride (15 ml) was then added. The solution was stirred at 34 °C for twenty-four hours after which the solvent and excess oxalyl chloride was removed to give the acid chloride of malonic acid monobenzylester (11.4 g). The crude acid chloride was dissolved in benzene (50 ml) and then added dropwise to

a stirred solution of *N*-benzylglycine ethyl ester (35.0 g, 0.18 mol) in benzene (70 ml) at 0 °C. After the addition was completed, the resulting solution was stirred at room temperature for twenty-four hours. The mixture was filtered, and the filtrate was washed with 10 % sodium bicarbonate solution (15 ml x 3), and then with dilute hydrochloride acid (10 ml x 3). The organic phase was dried over anhydrous magnesium sulfate and the solvent was removed under vacuum to give the diester (21.0 g, 62%) as an orange oil. IR ν cm^{-1} :1736 (C=O), 1654 (C=O); δ_H ($CDCl_3$, 250 MHz): 1.23 (3H, t, J = 7 Hz, C*H* $_3CH_2$), 3.92 (2H, s, NCH_2CO), 4.10 (2H, s, $COCH_2CO$), 4.14 (2H, q, J = 7 Hz, C*H* $_2CH_3$), 4.61 (2H, s, NCH_2Ph), 5.16 (2H, s, OCH_2Ph), 7.19-7.35 (10H, m, aromatic H); ^{13}C ($CDCl_3$, 100MHz): 14.1 (CH_3), 41.3 (CH_2), 47.1 (CH_2), 52.7 (CH_2), 61.2 (CH_2), 67.3 (CH_2), 126-129 (Aromatic C), 135.3 (quat.C), 136.6 (quat.C), 166.9 (C=O), 167.1 (C=O), 168.7 (C=O); (M/S: Found M^+, 369.1576 $C_{21}H_{23}NO_5$ requires 369.1582 ; m/z: 369 (M^+, 12%), 278 (17), 192 (100), 118 (33), 92 (24), 91 (100), 65 (23)

Phenylmethyl 2,4-dioxo-1-(phenylmethyl)tetrahydro-1H-pyrrole-3-carboxylate 101



100 **101**

Method A

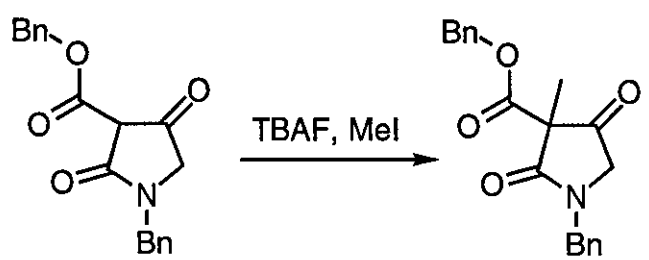
To a stirred solution of benzyl alcohol (5 ml) under nitrogen was added sodium metal (0.077 g, 3.3 mmol) and the solution was stirred until the sodium dissolved. A solution of the diester (1.124 g, 3.04 mmol) in benzene (10 ml) was slowly added and the solution was refluxed for six hours. The solvent was removed under vacuum and the yellowish soild was dissolved in aqueous sodium bicarbonate solution (20 ml) and then washed with benzene (15 ml). The aqueous layer was acidified with dilute sulfuric acid to give a white suspension which became a white precipitate slowly. The precipitate was filtered and dried under phosphorus pentoxide to give the title compound as white amorphous powder, 0.132 g (14%), m.p. 201 °C (decomposed). Since the compound is not soluble in

most common organic solvent it was used without any purification. IR ν cm^{-1}: 1703 (C=O), 1664 (C=O); M/S: Found M^+, 323.1157 $C_{19}H_{17}NO_4$ requires M^+ 323.1159 (m/z): 323 (M^+, 3%), 278 (13), 189 (10), 108 (50), 91 (100).

Method B

To a suspension of sodium hydride (0.14 g, 5.8 mmol) in benzene (15 ml) was added slowly a solution to the diester (1.474 g, 4.0 mmol) in benzene (10 ml). The reaction mixture was refluxed for five hours, cooled and water (30 ml) was slowly added to the solution. The two phases were separated and the organic phase was further extracted with water (20 ml x 2). The aqueous layers were combined and then slowly acidified using dilute sulfuric acid to give a white suspension which slowly became a white powder. The product was filtered and washed with water and then dried over phosphorus pentoxide to give the title product as a white powder (0.75 g, 62 %). The spectroscopic data is the same as above.

Phenylmethyl 3-methyl-2,4-dioxo-1-(phenylmethyl)tetrahydro-1*H*-pyrrole-3-carboxylate 102

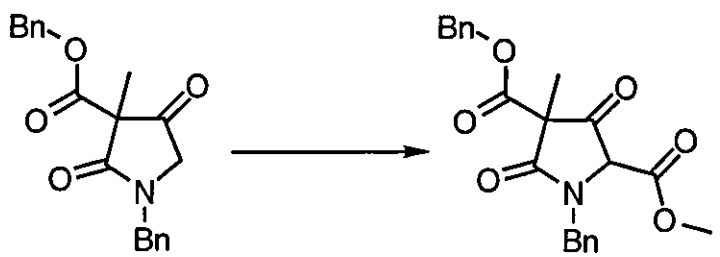


101 **102**

To a stirred solution of the ester (0.13 g, 0.4 mmol) in THF (10 ml), was added TBAF (0.5 ml, 0.5 mmol) followed by methyl iodide (0.11 g, 0.8 mmol). The solution was stirred overnight after which the solvent was removed under vacuum to give a yellowish crude extract. The crude extract was purified by column chromatography on silcia gel using petroleum-ether/ethyl acetate (7/3) to give the title compound as a yellowish oil (71 mg , 54%). IR ν cm^{-1} : 1781 (C=O), 1747 (C=O), 1697 (C=O); δ_H ($CDCl_3$, 250 MHz): 1.56 (3H, s, CH_3), 3.38 (1H, d, J = 17.5 Hz, NC*H* HCO), 3.87 (1H, d, J = 17.5 Hz, NCH*H* CO), 4.36 (1H, d, J = 14.8 Hz, NC*H* HPh),), 4.93 (1H, d, J = 14.7 Hz, NCH*H* Ph), 5.12 (1H, d, J = 12 Hz, OC*H* HPh), 5.18 (1H, d, J = 12 Hz, OCH*H* Ph), 7.13-7.36 (10H, m, ArH); ^{13}C ($CDCl_3$,

100MHz): 25.6 (CH_3), 56.5 (CH_2), 65.3 (CH_2), 69.4 (quat. C), 78.5 (CH_2), 137-139 (aromatic C), 144.8 (quat. C), 145.0 (quat. C), 175.6 (C=O), 179.7 (C=O), 212.4 (C=O); M/S: Found M^+, 337.1314 $C_{20}H_{19}NO_4$ requires 337.1313; m/z; 337 (M^+, 1%), 246 (12), 202 (36), 91 (100), 83 (9), 65 (15)

2-methyl 4-phenylmethyl 4-methyl-3,5-dioxo-1-(phenylmethyl)tetrahydro-1*H*-pyrrole-2,4-dicarboxylate 103

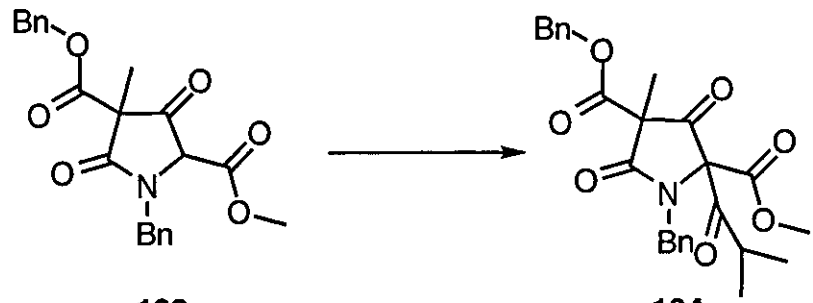


102 **103**

To a stirred solution of LHMDS (8.6 ml, 86 mmol) in THF (30 ml) at -78 °C, was added HMPA (1.54 ml, 86 mmol) and then the ester (1.44 g, 43 mmol) dissolved in THF (3 ml). The solution was stirred for forty-five minutes at that temperature when methyl cyanoformate (0.73 g, 86 mmol) was added in one portion. The reaction was stirred at -78 °C for two hours and then quenched with saturated aqueous ammonium chloride (10 ml), diluted with dichloromethane (60 ml) and then washed with water and brine. The organic layer was dried over anhydrous magnesium sulfate, filtered, evaporated and the residue obtained was chromatographed on silica gel (petroleum-ether/ethyl acetate, 70/30) to give the keto ester **103** as colourless oil (1.20 g, 70 %). IR ν cm^{-1} :1789 (C=O), 1748 (C=O), 1706 (C=O) ; δ_H ($CDCl_3$, 250 MHz): 1.57 (minor isomer) (3H, s, CH_3), 1.61 (major isomer) (3H, s, CH_3), 3.44 (minor isomer) (3H, s, OCH_3), 3.78 (major isomer) (3H, s, OCH_3), 4.09 (major isomer) (1H, d, J = 16Hz, NC*H* HPh), 4.11 (minor isomer) (d, 1H, J = 16Hz, NCH*H* Ph), 4.30 (minor isomer) (1H, s, CHCOO), 4.48 (major isomer) (1H, s, CHCOO), 5.09 (minor isomer) (1H, d, J = 12 Hz, OC*H* HPh), 5.18 (major isomer) (1H, d, J = 12 Hz, OC*H* HPh), 5.19 (major isomer) (1H, d, J = 12 Hz, OCH*H* Ph), 5.20 (minor isomer) (1H, d, J = 12 Hz, OCH*H* Ph), 5.37 (major isomer) (d, 1H, J = 16 Hz, NCH*H* Ph), 5.41 (minor isomer) (d, 1H, J = 16 Hz, NC*H* HPh), 7.07 -7.36 (10H, m, aromatic H); ^{13}C ($CDCl_3$, 100MHz):16.0 (major isomer) (CH_3), and 18.1 (minor isomer) (CH_3), 45.1 (major isomer) (CH_2Ph), 45.3 (minor isomer) (CH_2Ph), 53.4 (minor isomer) (OCH_3), 53.5 (major isomer) (OCH_3),

60.4 (quat. C), 66.6 (minor isomer) (CH), 67.4 (major isomer) (CH), 68.3 (major isomer) (OCH_2Ph), 68.4 (minor isomer) (OCH_2Ph), 127-129 (aromatic C), 133.7 (quat. C), 134.7 (quat. C), 163 (C=O), 164.7 (C=O), 164.9 (C=O), 167.8 (C=O), 169. 4(C=O), 195.9 (C=O), 196.9 (C=O) ; M/S:Found M^+ 395.1367, $C_{22}H_{21}NO_6$ requires 395.1368 ; m/z: 395 (M^+ , 10%), 320 (18), 304 (71), 260 (100), 180 (17), 132 (20), 106 (22), 91 (100).

2-methyl 4-phenylmethyl 4-methyl-2-(2-methylpropanoyl)-3,5-dioxo-1-(phenylmethyl)tetrahydro-1*H*-pyrrole-2,4-dicarboxylate 104

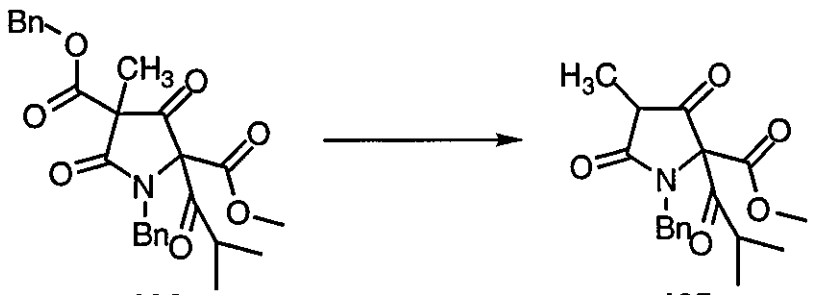


103 **104**

To a stirred solution of the keto-ester (0.301 g, 0.76 mmol) in dry dichloromethane (10 ml) was added pyridine (7.2 ml, 3.04 mmol) and the solution was stirred at room temperature for five minutes. Isobutyrylchloride (0.097 g, 0.91 mmol) in dichloromethane (5 ml) was added and the reaction mixture was allowed to stir for another thirty minutes. After that period, the mixture was quenched with water and then the organic layer was washed with aqueous $CuSO_4$ (10 ml x 3). The organic fraction was dried over anhydrous magnesium sulfate and the solvent was removed under vacuum to give the crude product which was purified by column chromatography on silica gel using peroleum-ether/ethyl acetate (80/20) to give the title compound as colourless oil which slowly solidified to give a white solid, 0.23 g (66 %), m.p. 93-95 °C. IR υ cm^{-1}: 1773 (C=O), 1751 (C=O), 1735 (C=O), 1720 (C=O); δ_H ($CDCl_3$, 250 MHz): 1.19 (3H, d, J = 6.9 Hz, C*H*$_3$CH), 1.20 (3H, d, J = 6.9 Hz, C*H*$_3$CH), 1.59 (3H, s, CH_3), 2.68 (1H, sextet, J = 6.9 Hz, C*H* $(CH_3)_2$), 3.64 (3H, s, OCH_3), 5.11 (1H, d, J = 12Hz, OC*H* HPh), 5.13 (1H, d, J = 12 Hz, OCH*H*Ph), 5.18 (1H, d, J = 15 Hz, NC*H* HPh), 5.23 (1H, d, J = 15 Hz, NCH*H* Ph), 7.05 -7.37 (10H, m, aromatic H); ^{13}C ($CDCl_3$, 100MHz): 16.9 (CH_3), 18.6 (CH_3), 18.8 (CH_3), 33.9 (CH), 44.4 (CH_2), 52.0 (OCH_3), 57.6 (quat. C), 68.1 (OCH_2), 127.3 -128.7 (aromatic CH), 134.6 (quat. C), 135.5 (quat. C), 141.0 (quat. C), 158.9 (C=O), 166.6 (C=O), 171.8 (C=O), 173.4 (C=O) ; M/S: Found M^+, 465.1780

$C_{26}H_{27}NO_7$ requires 465.1787; (Found C, 67.09%; H, 5.83%; N, 3.01% $C_{26}H_{27}NO_7$ requires C, 66.92%; H, 5.85%; N, 3.01%) ;(m/z): 465 (M^+, 1 %), 395 (27), 304 (20), 260 (12), 175 (5), 91 (100), 71 (36), 43 (26)

Methyl 4-methyl -2-(2-methylpropanoyl)-3,5-dioxo-1-(phenylmethyl)tetra-hydro-1*H*-pyrrole-2,-carboxylate 105



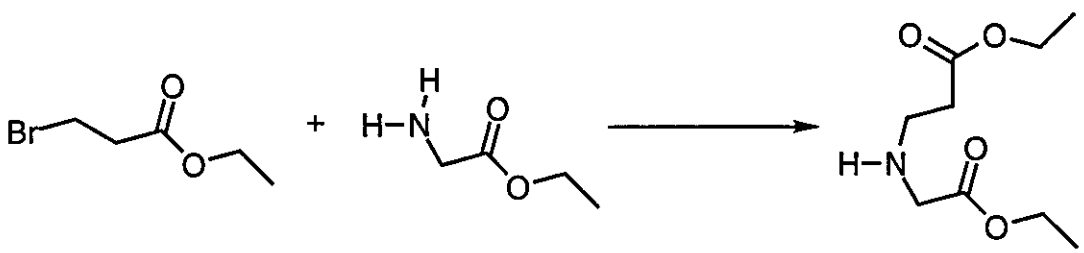
104 **105**

To a solution of the ester **104** (0.253 g, 0.54 mmol) in THF (15 ml) was added 20% palladium hydroxide (0.1 g). The resulting mixture was degassed and hydrogenated under balloon pressure for thirty minutes at room temperature. The reaction mixture was filtered through a pad of Celite, and the resulting filtrate was concentrated *in vacuo* to give the title product as colourless oil, 0.17 g (96 %). IR ν cm^{-1}: 1776 (C=O), 1751 (C=O), 1700 (C=O) ; δ_H ($CDCl_3$, 400 MHz): 1.11 (6H, d, J = 7.2 Hz, (C*H*$_3$)$_2$CH), 1.67 (3H, d, J = 1.6 Hz, C*H*$_3$CH), 2.60 (1H, sextet, J = 7.2 Hz, C*H* (CH_3)$_2$), 3.53 (3H, s, OCH_3), 4.04 (d, 1H, J = 15 Hz, NC*H* HPh), 4.62 (q, 1H, J = 1.6 Hz, C*H* (CH_3), 5.03 (d, 1H, J = 15 Hz, NCH*H* Ph), 7.06 -7.20 (m, 5H, aromatic H); ^{13}C ($CDCl_3$, 100MHz): 7.61 (CH_3), 18.6 (CH_3), 18.7 (CH_3), 33.9 (CH), 44.4 (CH_2), 52.8 (OCH_3), 61.0 (CH), 119.4 (quat. C), 127.7-128.8 (aromatic CH), 136.3 (quat. C), 141.0 (quat. C), 153.8 (C=O), 166.7 (C=O), 170.4 (C=O), 173.3 (C=O) ; M/S: Found M^+, 331.1419 $C_{18}H_{21}NO_5$ requires 331.1419 (m/z): 331 (M^+, 12 %), 261 (87), 202 (20), 106 (18), 91 (100), 71 (47).

3.3 SYNTHESIS OF 8-DEOXOLACTACYSTIN

3.3.1 INDIVIDUAL EXPERIMENTS

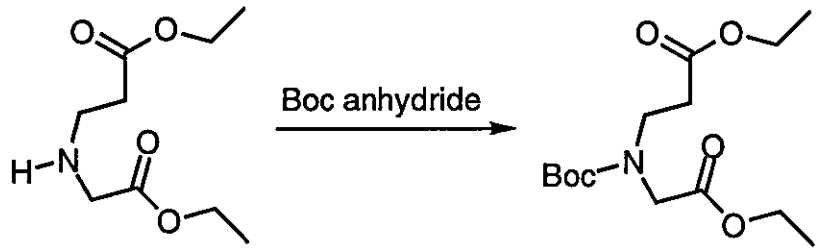
Ethyl 3-[(2-ethoxy-2-oxoethyl)amino]propanoate 107



107

Glycine ester chloride (60 g, 0.429 mol) was dissolved in water (160 ml), and a solution of 10 M sodium hydroxide (60 ml) was added until the pH reached 9.5. The aqueous solution was extracted with (200 ml x 2) and (100 ml x 4) portions of dichloromethane, readjusting the pH to 9.5 after every extraction. The fractions collected were combined, dried over magnesium sulfate and the solvent was removed under vacuum of which 3-bromo-ethyl propionate (27.7 g , 0.15 mol) in dichloromethane (35 ml) was added. The solution was refluxed for one hour under nitrogen after which a white precipitate appears. The mixture was filtered and the solid was thoroughly washed with dichloromethane. The combined filtrates were washed with water, dried over anhydrous magnesium sulfate and evaporated under vacuum to give a yellowish oil (24.5 g). The crude extract was purified by column chromatography on silica gel using petroleum-ether:ethyl acetate (8:2) to give the title compound as colourless oil (18.2 g, 60% based on 3-bromoethyl propionate).[38] IR ν cm^{-1} : 3450 (N-H), 1798 (C=O), 1761 (C=O); δ_H ($CDCl_3$, 400 MHz): 1.23- 1.30 (6H, m, 2 x CH_2CH_3), 2.43 (2H, t, J = 7 Hz, CH_2CH_2N) , 2.98 (2H, t, J = 7 Hz, NCH_2CH_2), 3.4 (2H, s, NCH_2CO_2), 4.09-4.18 (4H, m, 2 x CH_2CH_3); ^{13}C ($CDCl_3$, 100 MHz): 14.2 (CH_3), 14.2 (CH_3), 33.6 (CH_2CO), 49.6 (NCH_2C), 54.7 (CH_2CO), 60.3 (OCH_2CH_3), 171.2 (C=O), 172.3 (C=O); M/S: (Found M^+, 203.1196, $C_9H_{17}NO_4$ requires M^+ 203.1197); (m/z): 203 (M^+, 1%), 202 (100), 144 (30), 130 (31), 59 (31) and 29 (65).

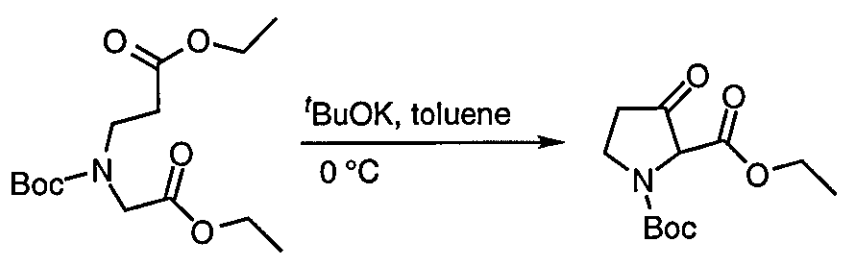
Ethyl 3-[(tert-butoxycarbonyl)(2-ethoxy-2-oxoethyl)amino]propanoate 108



107 **108**

To a stirred solution of Boc anhydride (9.6 g, 44 mmol) in dry dichloromethane (35 ml) was added a solution of diethyl 3-azahexane-1,6-dicarboxylate (8.13 g, 40 mmol) and dry triethylamine (5.75 ml, 41 mmol) in dry dichloromethane (20 ml) over ten minutes. The resulting solution was stirred at ambient temperature for two hours after which the evolution of carbon dioxide had ceased. The solution was washed with 2M HCl (25 ml x 3) and water (25 ml x 1), dried over anhydrous magnesium sulfate and the solvent was evaporated to give the carbamate as colourless oil (11.7 g, 96%).[38] IR ν cm^{-1} : 1753 (C=O), 1737 (C=O), 1703 (C=O); δ_H ($CDCl_3$, 400 MHz): 1.23-1.29 (6H, m, 2 x CH_2CH_3), 1.41 and 1.48 (9H, 2 x s, $C(CH_3)_3$), 2.62 (2H, t, J = 7 Hz, NCH_2CH_2), 3.55 (2H, t, J = 7 Hz, NCH_2CH_2), 3.95 (1H, s, NCH_2CO_2), 4.02 (1H, s, NCH_2CO_2), 4.12-4.20 (4H, m, 2 x CH_2CH_3); ^{13}C ($CDCl_3$, 100 MHz): 14.15 (CH_3), 14.18 (CH_3), 28.1 ($C(CH_3)_3$), 33.4 (CH_2CO), 44.8 (NCH_2), 50.6 (CH_2CO), 60.6 (OCH_2CH_3), 80.5 (quat. C, $C(CH_3)_3$), 155.0 (C=O), 170.3 (C=O), 172.1 (C=O); M/S: (Found M^+, 303.1680 $C_{14}H_{26}NO_6$ requires M^+, 303.1681); (m/z): 304 (MH^+, 25%), 248 (58), 204 (96), 130 (92), 116 (41), 84 (64) and 57 (100).

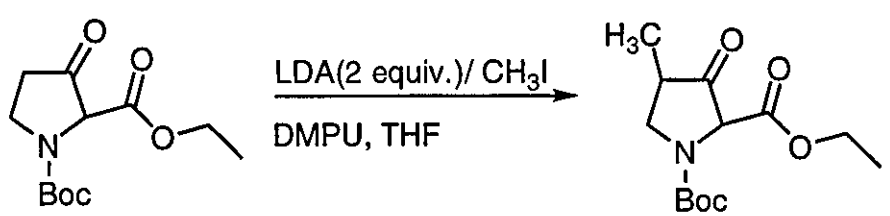
1-tert-butyl-2-ethyl 3-oxopyrrolidine-1,2-dicarboxylate 109



108 **109**

To a stirred solution of potassium tert-butoxide (2.44 g, 0.021 mol) in dry toluene (60 ml), maintained at 0 °C under dry nitrogen, was added a solution of diester (4.46 g, 0.014 mol) in dry toluene (20 ml) during ten minutes. The solution was stirred for thirty minutes at 0 °C and quenched with glacial acetic acid (4 ml) and a solution of sodium dihydrogen phosphate (11.7 g) in ice cold water (100 ml). The resulting mixture was then extracted with dichloromethane (150 ml x 2) and the combined extracts was washed with pH 7 phosphate buffer (20 ml x 2), dried over magnesium sulfate and evaporated to give the two isomers. The mixture was dissolved in toluene (100 ml) and the resulting solution was extracted with pH 9.5 carbonate buffer (125 ml x 5). The aqueous extracts were brought to pH 3 with concentrated phosphoric acid and extracted with dichloromethane (100 ml x 2). The combined extracts were dried and evaporated to give the undesired 1,3-diester (0.72 g, 19%). The toluene fraction was washed with water (20 ml), dried and evaporated to give a yellowish oil which was chromatographed on silica gel (petroleum-ether/ethyl acetate 80/20) to give the desired 1,2-diester **109**, as colourless oil, (1.413 g, 37%).[38] IR ν cm^{-1} : 1775 (C=O), 1743 (C=O), 1713 (C=O); δ_H ($CDCl_3$, 400 MHz): 1.30 (3H, t, J = 7.1 Hz, CH_3CH_2), 1.43 (9H, s, $C(CH_3)_3$), 2.68 (2H, t, J = 7 Hz, NCH_2CH_2CO), 3.76-3.90 (2H, m, NCH_2CH_2CO), 4.24 (2H, q, J = 7 Hz, CH_2CH_3), 4.53 (1H, s, CHCOO); ^{13}C ($CDCl_3$, 100 MHz): 14.2 (CH_3), 28.2 ($C(CH_3)_3$), 36.4 (CH_2CO), 41.6 (NCH_2), 61.2 (OCH_2CH_3), 81.1 (quat. C, $C(CH_3)_3$), 153.8 (C=O), 166.6 (C=O), 204.7 (C=O); M/S: (Found M^+, 257.1258 $C_{12}H_{19}NO_5$ requires 257.1263); (m/z): 257 (M^+, 2%), 201 (33), 156 (7), 84 (31) and 57 (100).

1-tert-butyl-2-ethyl 4-methyl -3-oxopyrrolidine-1,2-dicarboxylate 111



109 **111**

A solution of the keto-ester (0.824 g, 3.2 mmol) and DMPU (1.16 ml) in dry THF (0.5 ml) is added dropwise over five minutes to a stirred solution of lithium diisopropyl amide (3.2 ml, 6.4 mmol) in dry THF (20 ml) at 0 °C under nitrogen. After stirring the solution for twenty minutes at 0 °C, methyl iodide (0.21 ml, 3.52

mmol) was added to the reaction mixture. After thirty minutes, the reaction was quenched with concentrated HCl (3 ml) and with water (10 ml) and the reaction mixture was diluted with diethyl ether (20 ml). The aqueous extract was further extracted with diethyl ether (15 ml x 2). The organic extracts were combined, washed with water until neutral, dried over anhydrous magnesium sulphate and filtered and the solvent was removed under reduced pressure to give a crude product which was then chromatographed on silica gel (petroleum-ether/ethyl acetate, 70/30) to give the title compound as colourless oil (80 mg, 18%). IR ν cm^{-1} : 1773 (C=O), 1745 (C=O), 1712 (C=O); δ_H ($CDCl_3$, 400 MHz): 1.18-1.22 (3H, m, C*H*$_3$CH), 1.30 (3H, t, J = 7 Hz, C*H*$_3$CH$_2$), 1.47 (9H, s, C(CH$_3$)$_3$), 2.66-2.80 (1H, m, C*H*(CH$_3$), 3.26-3.36 (2H, m, NC*H*$_2$CH(CH$_3$), 4.13-4.27 (2H, m, C*H*$_2$CH$_3$), 4.55 (1H, s, CHCOOEt); ^{13}C ($CDCl_3$, 100 MHz): 12.7 (CH_3), 14.4 (CH_3), 28.5 (C(CH_3)$_3$), 42.9 (CH), 49.4 (CH_2), 62.3 (OCH_2), 66.1 (CH), 81.4 (quat. C, C(CH_3)$_3$), 154.8 (C=O), 166.6 (C=O), 206.7 (C=O); M/S: (Found M^+, 271.1411, $C_{13}H_{21}NO_5$ requires 271.1419) ; (m/z): 271 (M^+, 6%), 215 (35), 170 (19), 98 (44) and 57 (100).

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