NEW SYNTHETIC APPROACHES TOWARDS CARBOCYCLIC ANALOGUES OF NATURAL NUCLEOSIDES & NUCLEOTIDES

Thesis submitted for the degree of

Doctor of Philosophy

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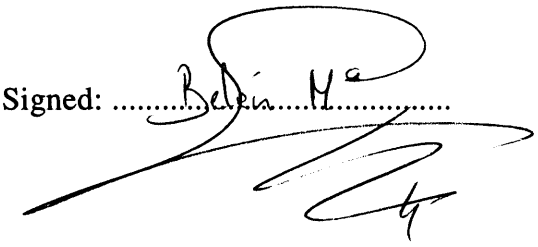
STATEMENT

This thesis, submitted for the degree of Doctor of Philosophy, entitled "**New Synthetic Approaches Towards Carbocyclic Analogues of Natural Nucleosides and Nucleotides**", is based on the work carried out by the author, Belén Mª Domínguez Fernández, in the Department of Chemistry, at The University of Leicester, between September 1995 and September 1998. All the work presented herein, is original unless otherwise stated and referenced. None of this thesis has been submitted for any other degree at this, or any other university or establishment.

Signed: .....Belén Mª.....



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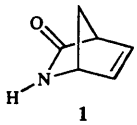
"NEW SYNTHETIC APPROACHES TOWARDS CARBOCYCLIC ANALOGUES OF NATURAL NUCLEOSIDES & NUCLEOTIDES"

by *Belén Mª Domínguez Fernández*

ABSTRACT

This thesis describes approaches towards the synthesis of some carbocyclic analogues of nucleosides and nucleotides starting from the bicyclic lactam **1**.



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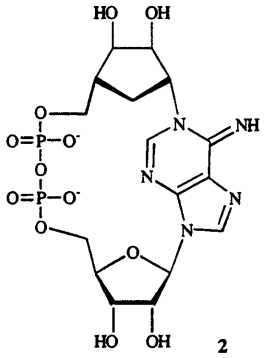

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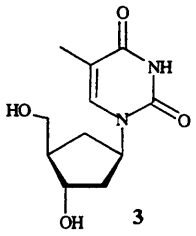

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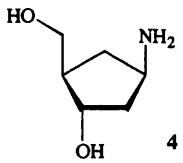
The first compound considered was the analogue of cyclic adenosine diphosphate ribose **2**. A crucial step in one of the retrosynthetic analyses involved conversion of a primary amine into a different functionality *via* a nucleophilic displacement of an amine derivative. Using a range of simple model compounds, attempts were made to achieve such a displacement *via* formation of a diazonium salt, a ditosyl imide or a pyridinium salt. Many side reactions such as elimination or rearrangement had taken place rather than nucleophilic substitution at the carbon bearing the amine derivative. These results led to the conclusion that this approach is not a suitable strategy to consider for further investigations into the synthesis of carbocyclic nucleotide analogues.

The development of a new synthetic route towards carbocyclic thymidine **3** is described in chapter 3. Its synthesis was successfully performed in 8 steps *via* a linear approach which consisted of the construction of the thymine moiety on the carbocyclic cyclopentylamine **4**. The amine **4**, was made *via* a flexible pathway which also could be adapted to give a wide range of carbocyclic sugar analogues. In addition, enantiomerically pure (+)-thymidine analogue **3** was prepared from (-) **1** according to the strategy developed for the racemic series, and then converted into the phosphoramidite derivative **5**. This monomer will be used to prepare hybrid DNA strands and then to undertake some biological studies on the stability compared to that of the natural DNA.



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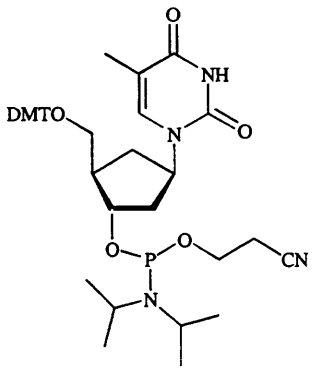

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ACKNOWLEDGEMENTS

I would initially like to thank my supervisor *Prof. Paul M. Cullis*, firstly having given me the opportunity to undertake here my PhD studies and secondly, for all the guidance and advice during the last three years.

My thanks and appreciation to *Dr. Gerry Griffiths* for all his help running all the High Field and 2-Dimensional NMR spectra, to *Dr. John Fawcett* for the X-Ray structures, to *Dr. Graham Eaton* for running all the Mass spectra and to *Mick Lee* for his technical assistance and to all the other members of *the staff of the Chemistry Department* who, in one way or other, very kindly have helped me during my PhD.

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This thesis is especially dedicated to my *Mum,* my two *sisters* and the closest *family*, for their love, emotional support and loads of encouragement in all my studies and anything I have decided to pursue.

ABBREVIATIONS

δ	Chemical shift
μ	10^{-6} units
ΔG	Gibbs free energy
18-Crown-6	1,4,7,10,13,16-Hexa-oxaoctadecane
4-DMAP	4-Dimethylaminopyridine
9-BBN	9-Boranebicyclo[3.3.1]nonane
A	Adenosine
Ac	Acetyl
ADP	Adenosine 5'-diphosphate
ADP	Adenosine diphosphate
ADPR	Adenosine diphosphate ribose
AICAR	5-Amidoimidazole-4-carboxamide-1-β-*D*-ribofuranoside
AIDS	Acquired immune deficiency syndrome
AMP	Adenosine 5'-monophosphate
Ar.	Aromatic ring
Ara-A	Arabinosyladenine
Ara-C	Arabinosylcytosine
ATP	Adenosine 5'-triphosphate
AZT	3'-Azido-2'-deoxythymidine
Boc	*tert*-Butoxycarbonyl
Boc-on	2-(*tert*-Butoxycarbonyl-oxymino)-2-phenyl acetonitrile
br	Broad
br s	Broad singlet
br t	Broad triplet
C	Cytosine
$c(dT)_n$	Carbocyclic oligothymidylates; n: units number
cADPR	Cyclic adenosine diphosphate ribose
cADPR	Cyclic adenosine diphosphate ribose

CI	Chemical impact
Cl-Mes	2-Mesitylenesulfonyl chloride
Cl-Tpp	2,4,6-Triisopropylbenzenesulfonyl chloride
cm^{-1}	Wavenumber
Co-A	Coenzyme A
COSY	Correlation spectroscopy
CTP	Cytidine triphosphate
d	Doublet
DCC	*N,N'*-Dicyclohexylcarbodiimide
dd	Double doublet
Dib-Al	Diisobutylaluminium hydride
DMF	Dimethyl formamide
DMSO	Dimethyl sulfoxide
DMSO-d_6	Hexadeutared dimethylsulfoxide
DMT	Dimethoxytrityl
DMT-Cl	Dimethoxytrityl chloride
DNA	Deoxyribonucleic acid
dquint	Double quintuplet
dt	Double triplet
dTMP	2'-Deoxythymidine monophosphate
dUMP	2'-Deoxyuridine monophosphate
EI	Electronic impact
ENZA 1	Rhodococcuss equi NCIB 40213
ENZA 2	Pseudomonas solanacearum NCIB 40249
Et	Ethyl
FAB	Fast atom bombardment
FAD	Flavin adenine dinucleotide
G	Guanosine
GC	Gas chromatography
GMP	Guanosine monophosphate
h	Hour
Hα	Hydrogen down
Hβ	Hydrogen up

HIV	Human inmunodeficiency virus
HPLC	High performance liquid chromatography
Hz	Hertz
IMP	Inosine monophosphate
iPr	Isopropyl
IR	Infrared
J	Coupling constant
Kcal	Kilocalories
m	Multiplet
m	Medium
M	Molar
m.p.	Melting point
MCPBA	*m*-Chloroperbenzoic acid
Me	Methyl
Mes	2-Mesitylenesulfonyl
MS	Mass spectroscopy
NAD^+	Nicotinamide adenin dinucleotide (Reduced)
NADases	Nicotinamidases
NADH	Nicotinamide adenin dinucleotide
NADPH	Nicotinamide adenin dinucleotide diphosphate
NMO	*N*-Methylmorpholine oxide
NMR	Nuclear magnetic spectroscopy
NOE	Nuclear Overhauser effect
NOESY	Nuclear Overhauser spectroscopy
Pht	Pthalyl
Pi	Inorganic monophosphate
PMA	Phosphomolybdic acid
PPi	Inorganic diphosphate
ppm	Parts per million
PRPP	5-Phosphoribosyl-1-pyrophosphate
psi	Pounds per square inch
Py	Pyridine
q	Quadruplet

quint.	Quintuplet
Red-Al	Sodium *bis*-(2-methoxyethoxy)aluminium hydride
R_f	Front retention
Ribose-P	Ribose 5'-phosphate
RNA	Ribonucleic acid
ROESY	Rotating frame overhauser effect spectroscopy
Rt	Retention time
rt	Room temperature
s	Singlet
s	Strong
SM	Starting material
sol.	Solution
t	Triplet
T	Thymidine
t-Bu	*tert*-Butyl
TBDMSCl	4,4-*tert*-Butyldimethylsilyl chloride
TfOTBDMS	4,4-*tert*-Butyldimethylsilyl trifluoromethanesulfonate
THF	Tetrahydrofuran
Thy	Thymidine moiety
TIPS-Cl_2	1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane
TLC	Thin layer chromatography
Tpp	2,4,6-Triisopropylbenzenesulfonyl
Tr-Cl	Triphenylmethyl chloride
Ts	*p*-Toluenesulphonyl
Ts-Cl	*p*-Toluenesulphonyl chloride
tt	Triple triplet
U	Uridine
UMP	Uridine monophosphate
UTP	Uridine triphosphate
UV	Ultraviolet
w	Weak

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CHAPTER 1

CHAPTER 2

CHAPTER 3

CHAPTER 4

CHAPTER 1

NUCLEOSIDES & NUCLEOTIDES

1. NUCLEOSIDES AND NUCLEOTIDES

1.1 INTRODUCTION

The general terms nucleotide and nucleoside are given to the monomeric building blocks which form the nucleic acids RNA and DNA. The catalytic cleavage or degradation of a nucleic acid by specific enzymes, yields nucleotide monomeric units. Further catalytic hydrolysis of the nucleotide removes a phosphate unit and converts it into the corresponding nucleoside. Therefore, a nucleoside is composed of two parts, a pentose sugar and a heterocyclic base (Fig. 1).

There are two basic structural differences between DNA and RNA. One is the sugar, which is ribose in RNA and 2'-deoxyribose in DNA, and the other involves the heterocyclic base thymine which is replaced by uracil in RNA. The purine or pyrimidine base is bonded to the C-1' of the sugar by an *N*-glycosyl linkage. Nucleoside monomers are linked to form oligomers *via* phosphate diesters involving the 5' and 3' hydroxyl groups (Fig. 1).

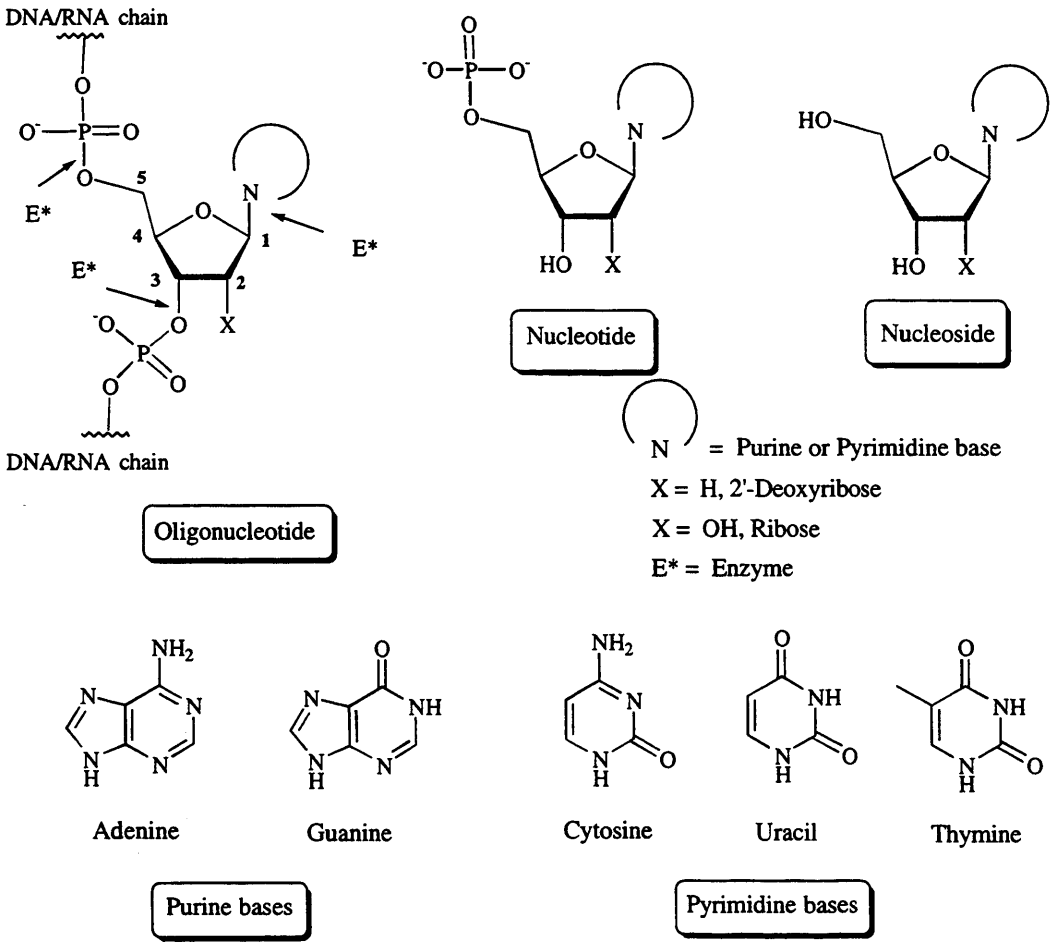


Figure 1

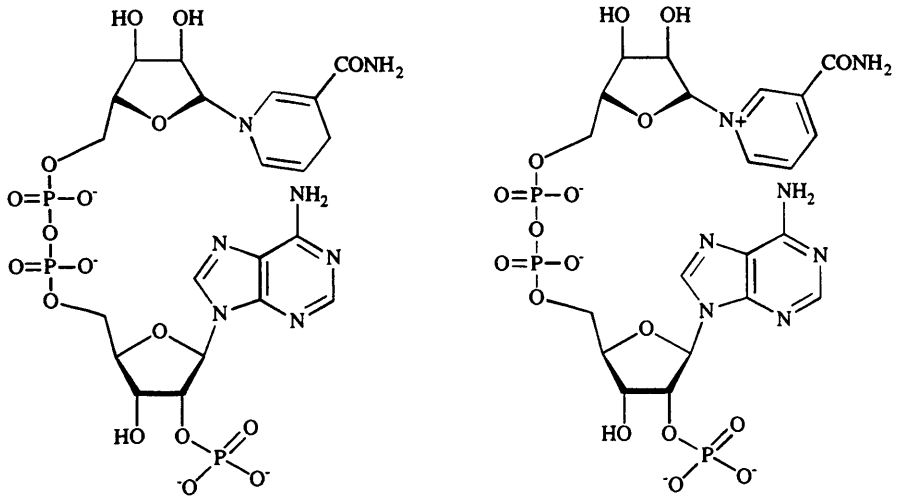
The enormous importance of these structures is due to the crucial biological roles of DNA/RNA. Hence, the sequence in which the individual nucleotides are arranged in the DNA, holds all the hereditary information of an individual, and the RNA carries out the expression of that information in terms of the synthesis of the enzymes and receptors necessary for all function.

Nucleotides may play other roles in addition to being components of DNA or RNA. For instance, adenosine units are part of the structure of two important coenzymes: nicotinamide adenin dinucleotide (NADH) and acetyl coenzyme A (acetyl Co-A). The first is the major electron donor in reductive biosynthesis and the second operates as an acylating agent in many biochemical reactions and is an essential source of C_2 units in secondary metabolism (fatty acids, polyketides, etc.). Other important nucleotides are adenosine 5'-triphosphate (ATP), as the universal currency of free energy in biological systems, adenosine 5'-monophosphate (AMP), an important regulator of hormone activity or cyclic adenosine diphosphate ribose (cADPR) a novel nucleotide with intracellular Ca^{2+} mobilising activity.

1.2 BIOSYNTHESIS OF NUCLEOTIDES

1.2.1 NADPH

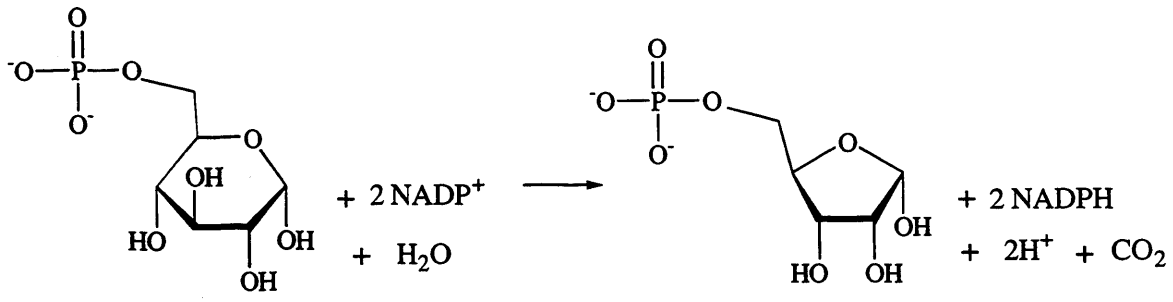
NADPH is the source of reducing power in cells, being an electron donor in reductive biosyntheses to give $NADP^+$. A phosphate group at the C-2 of one of the ribose units (Fig. 2) makes the difference with NADH.



NADPH $NADP^+$

Figure 2

NADPH is generated when glucose 6-phosphate is oxidised to ribose 5-phosphate, primarily formed by the pentose phosphate pathway (Scheme 1). This sugar and its derivatives lead to the biosynthesis of molecules as important as ATP, Co-A, NAD^+, FAD (Flavin adenine dinucleotide), RNA and DNA.



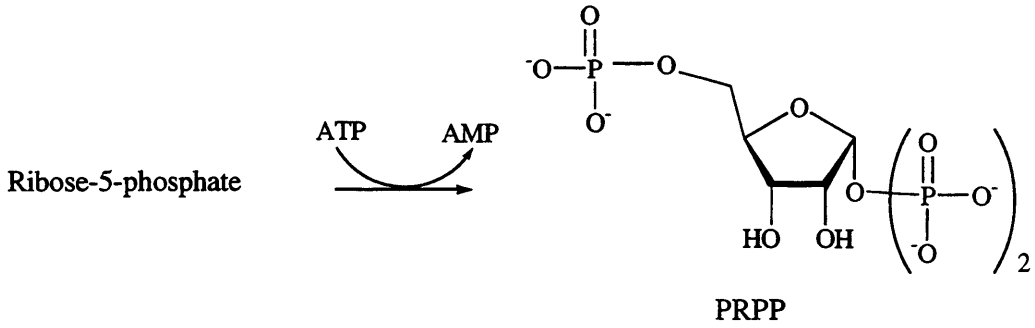
Glucose-6-phosphate Ribose-5-phosphate

Scheme 1

1.2.2 PURINE AND PYRIMIDINE NUCLEOTIDES

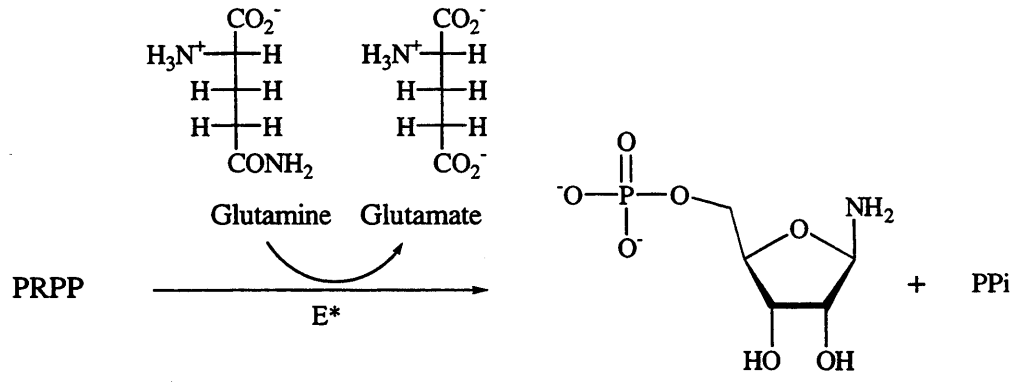
1.2.2.1 PURINE NUCLEOTIDES

The formation of the ribose phosphate portion of purine and pyrimidine nucleotides comes from 5-phosphoribosyl-1-pyrophosphate (PRPP), synthesised from ATP and ribose-5-phosphate (Scheme 2).



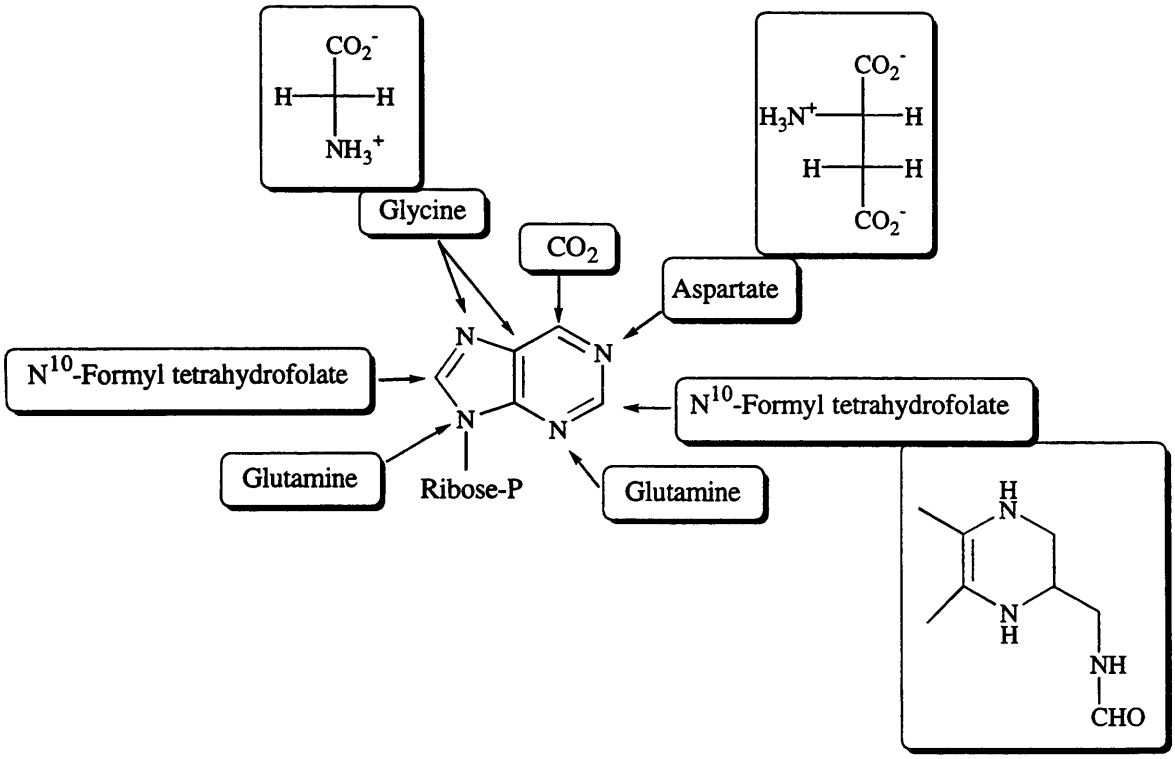
Scheme 2

Displacement of the diphosphate unit gives 5-phosphoribosyl-1-amine (Scheme 3). The amino group, which presents the β-configuration characteristic of naturally occurring nucleotides, comes from glutamine which is converted into glutamate.



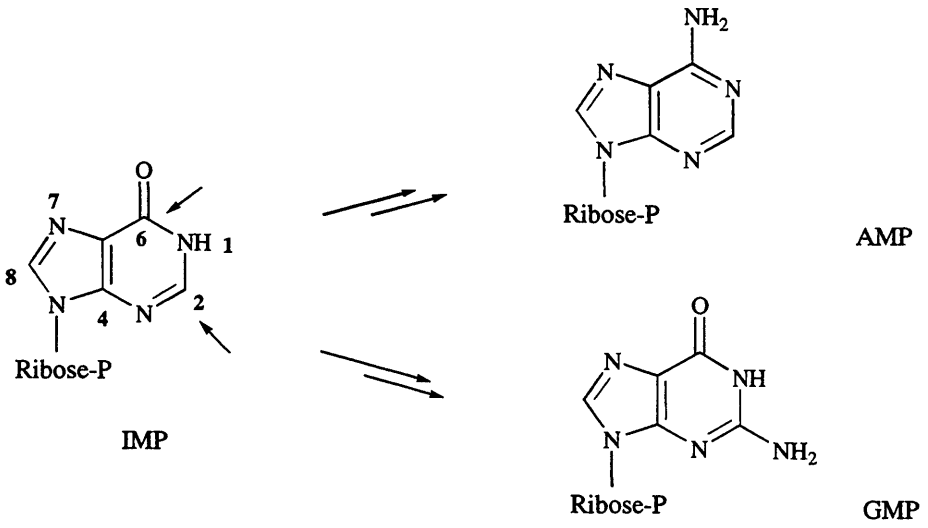
Scheme 3

The five membered ring of purine is the first part of the skeleton to be synthesised. Apart from glutamine, it is made from glycine and N^{10}-formyl tetrahydrofolate, glycine being the first assembled amino acid on 5-phosphoribosyl-1-amine (Scheme 4).



Scheme 4

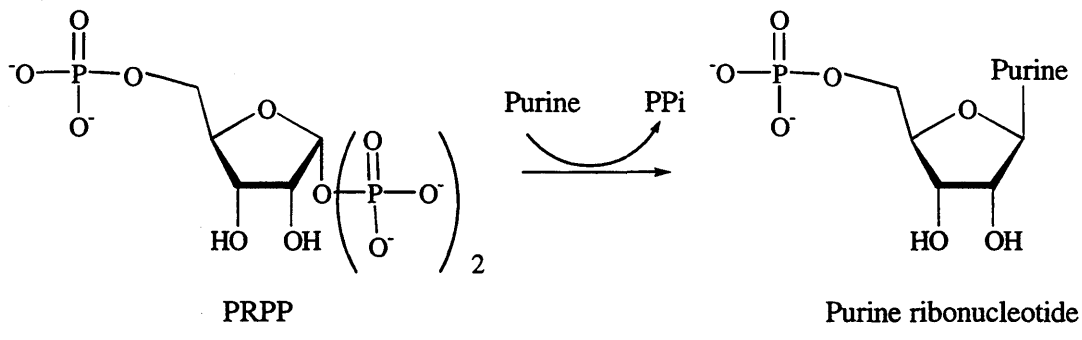
The formation of the six membered ring of the purine skeleton is completed with CO_2, aspartate and formyl tetrahydrofolate. The purine base formed is inosine monophosphate (IMP) (Scheme 5). On its formation two molecules of ATP are consumed.



Scheme 5

Inosine monophosphate can then be converted into AMP and guanosine monophosphate (GMP). Thus, AMP is synthesised by substitution of the carbonyl for an amino group and GMP by insertion of an amino group at C-2. NAD^+ is the H^- acceptor in the oxidation of the C-2 of IMP and ATP is converted into AMP producing a diphosphate unit (PPi) which is consequently hydrolysed.

An alternative pathway to the "de novo synthesis" of purines previously described is the "salvage reaction" where the purine base can be obtained by hydrolytic cleavage of nucleic acids or nucleotides. This process is more "energy cost effective" than the "de novo synthesis". It takes place by transferring the ribose phosphate moiety of PRPP to a specific heterocyclic base to yield the corresponding purine ribonucleotide (Scheme 6).



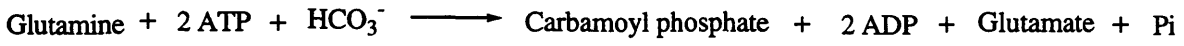
PRPP

Purine ribonucleotide

Scheme 6

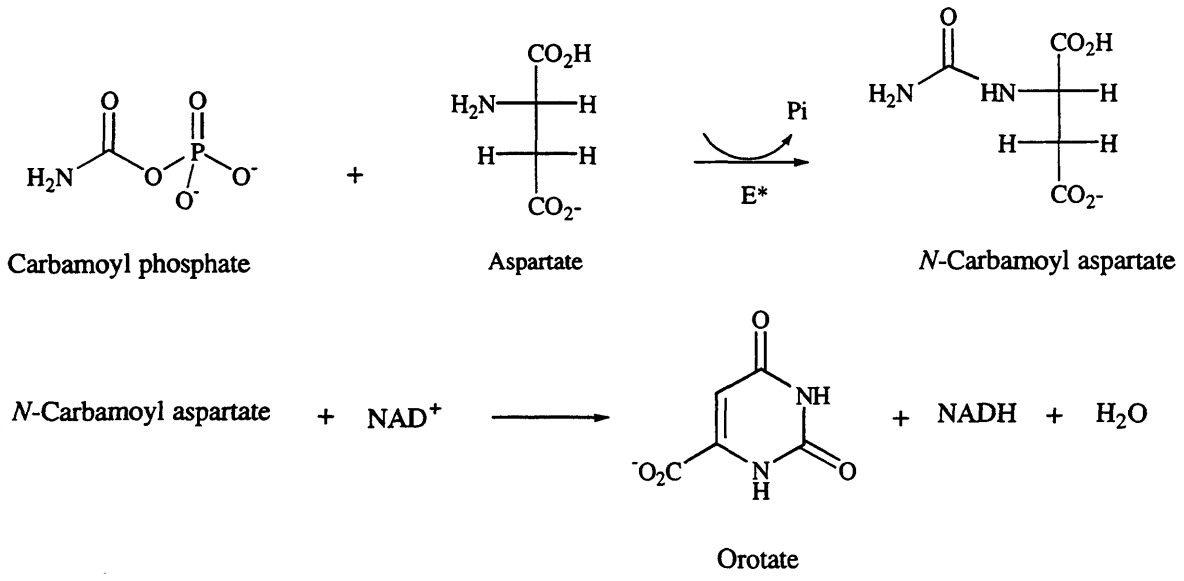
1.2.2.2 PYRIMIDINE NUCLEOTIDES

In contrast with purines, pyrimidine bases are first biosynthesised and then linked to PRPP. The pyrimidine biosynthesis starts with formation of carbamoyl phosphate (Scheme 7).



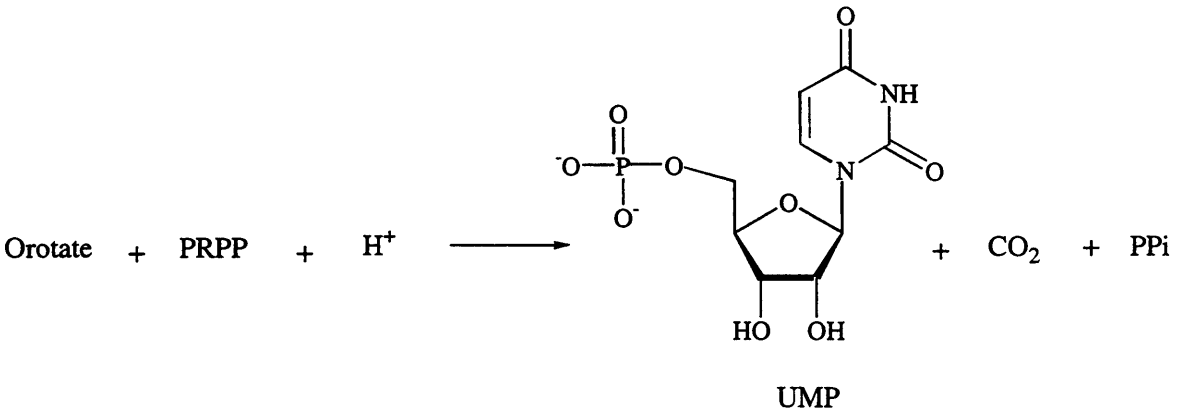
Scheme 7

Reaction of carbamoyl phosphate with aspartate gives *N*-carbamoyl aspartate which then cyclises with loss of water to yield orotate after further dehydrogenation (Scheme 8).



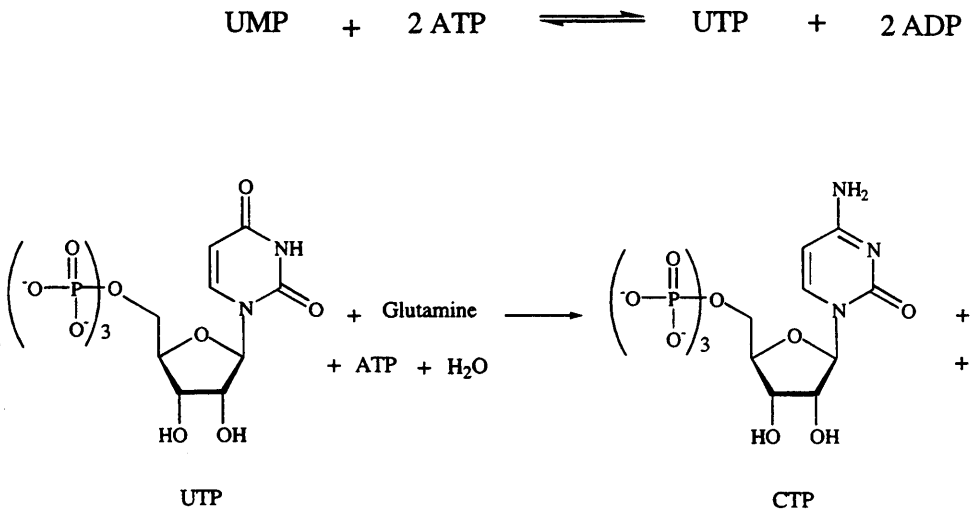
Scheme 8

The formation of the C-N glycosyl linkage between the pyrimidine ring and PRPP takes place at this stage with loss of diphosphate and CO_2 to give uridylate (UMP) (Scheme 9).



Scheme 9

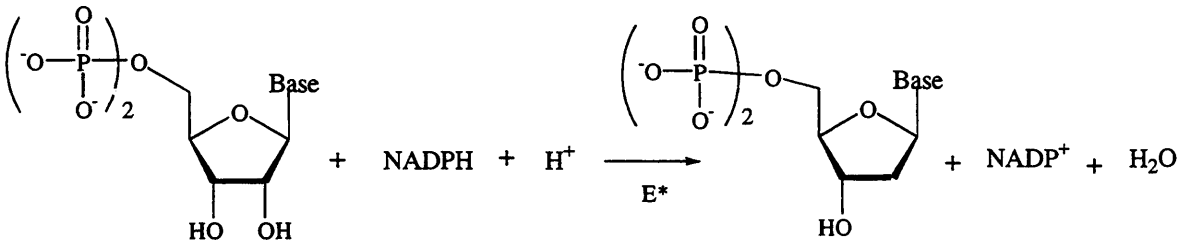
The catalytic conversion of UMP into UTP involves two molecules of ATP (Scheme 10). Biosynthesis of cytidine triphosphate (CTP) then takes place by amination of UTP using glutamine as the amine donor.



Scheme 10

1.2.2.3 FROM RIBOSE NUCLEOTIDES TO DEOXYRIBONUCLEOTIDES

The formation of deoxyribonucleotides is carried out *via* reduction of the corresponding ribonucleoside diphosphate catalysed by ribonucleotide reductase. NADPH is the source of electrons with an overall stoichiometry shown in Scheme 11. Electrons are transfered to the substrate through a series of free radical intermediates.[1]



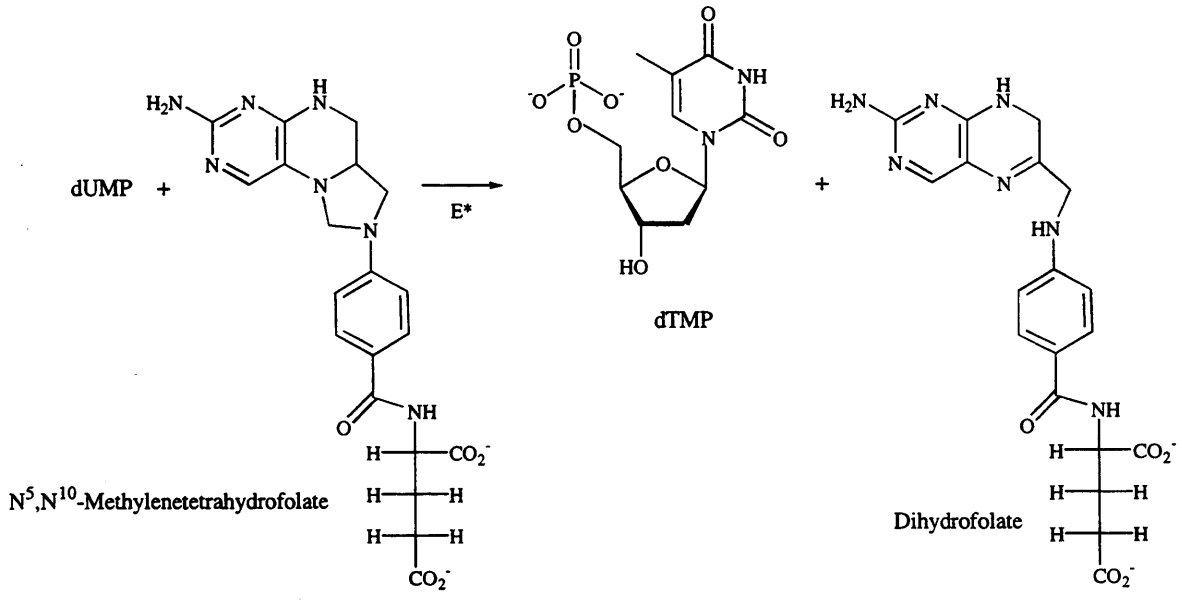
Ribonucleoside diphosphate

Deoxyribonucleoside diphosphate

Scheme 11

1.2.2.4 BIOSYNTHESIS OF THYMIDINE

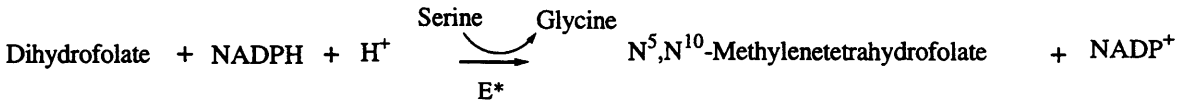
Bearing in mind that thymine is a specific base of DNA, the biosynthesis of thymidine takes place at a different level than the rest of the nucleotides described because the sugar ribose is now replaced by deoxyribose. Thus, thymidine is formed *via* the deoxyribonucleoside monophosphate dUMP (Scheme 12).



N^5,N^{10}-Methylenetetrahydrofolate

Scheme 12

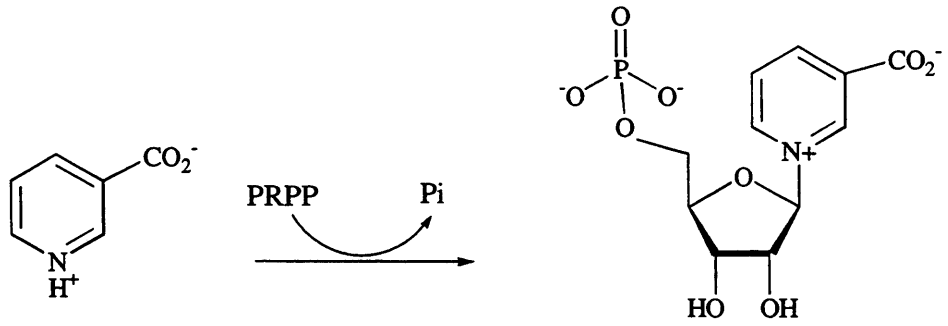
Catalytic methylation by a synthase converts dUMP into dTMP. The source of electrons and the carbon donor is in this case the same: N^5,N^{10}-methylenetetrahydrofolate which is oxidised to dihydrofolate. Reduction of dihydrofolate is accomplished by a reductase, NADPH and serine, regenerating tetrahydrofolate (Scheme 13).



Scheme 13

1.2.3 NAD^+

The formation of NAD^+ starts from nicotinate and PRPP (Scheme 14). Nicotinate is naturally synthesised if the dietary intake of the amino acid tryptophan is adequate.

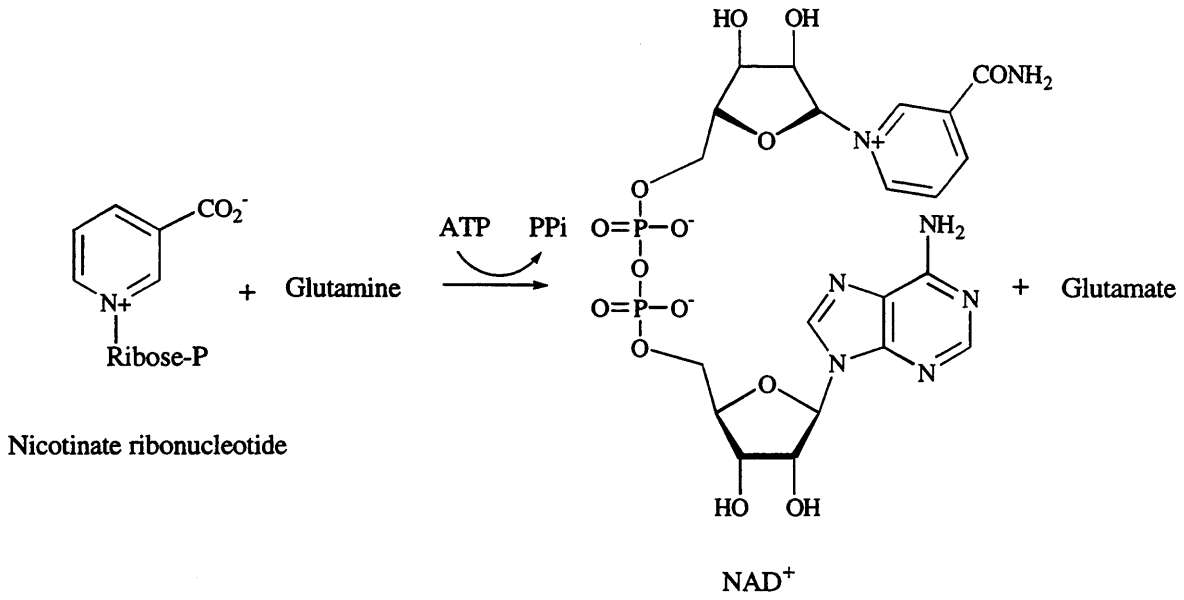


Nicotinate

Nicotinate ribonucleotide

Scheme 14

ATP is the donor of AMP which is transfered to nicotinate ribonucleotide forming the new phosphodiester linkage. In the last step, glutamine transfers an amino group yielding NAD^+ (Scheme 15).



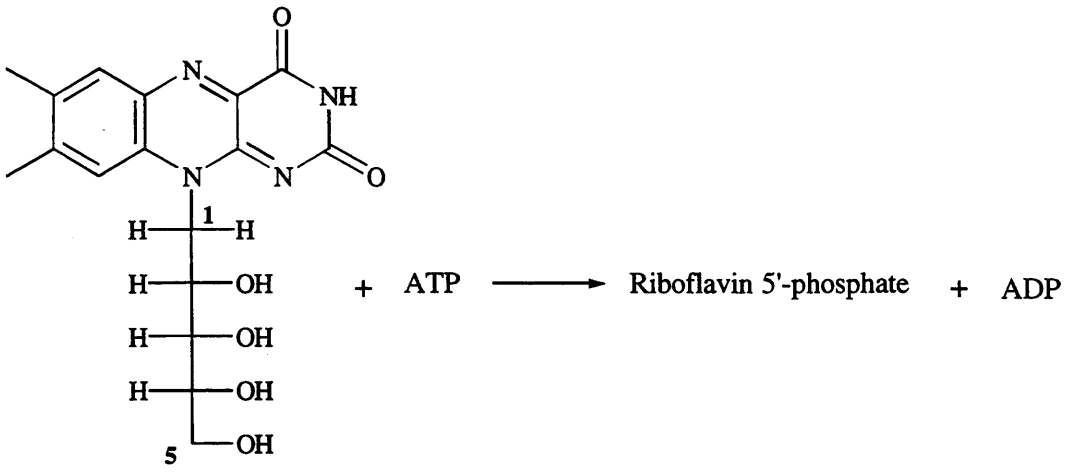
Nicotinate ribonucleotide

NAD^+

Scheme 15

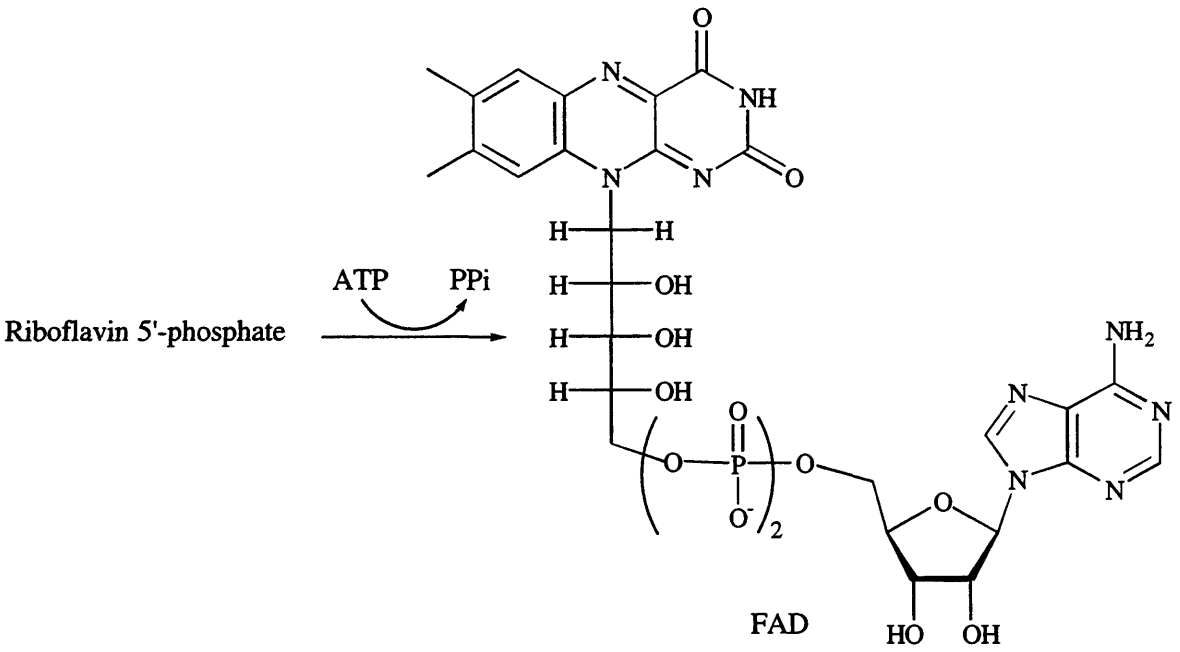
1.2.4 FAD

Flavin adenine dinucleotide (FAD) is synthesised from vitamin B_2 or riboflavin (Scheme 16). Phosphorylation of the sugar moiety by ATP at the 5' position gives riboflavin 5'-phosphate. Further donation of AMP by ATP yields the corresponding FAD (Scheme 17).



Riboflavin

Scheme 16



Scheme 17

The reduced form of FAD is $FADH_2$ (Fig. 3), one of the major electron carriers in the oxidation of molecules together with NADH.

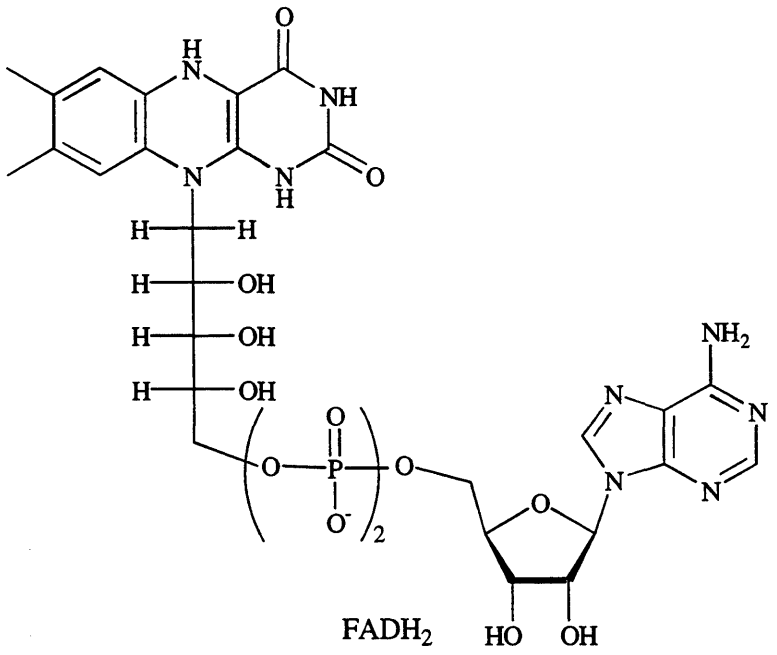
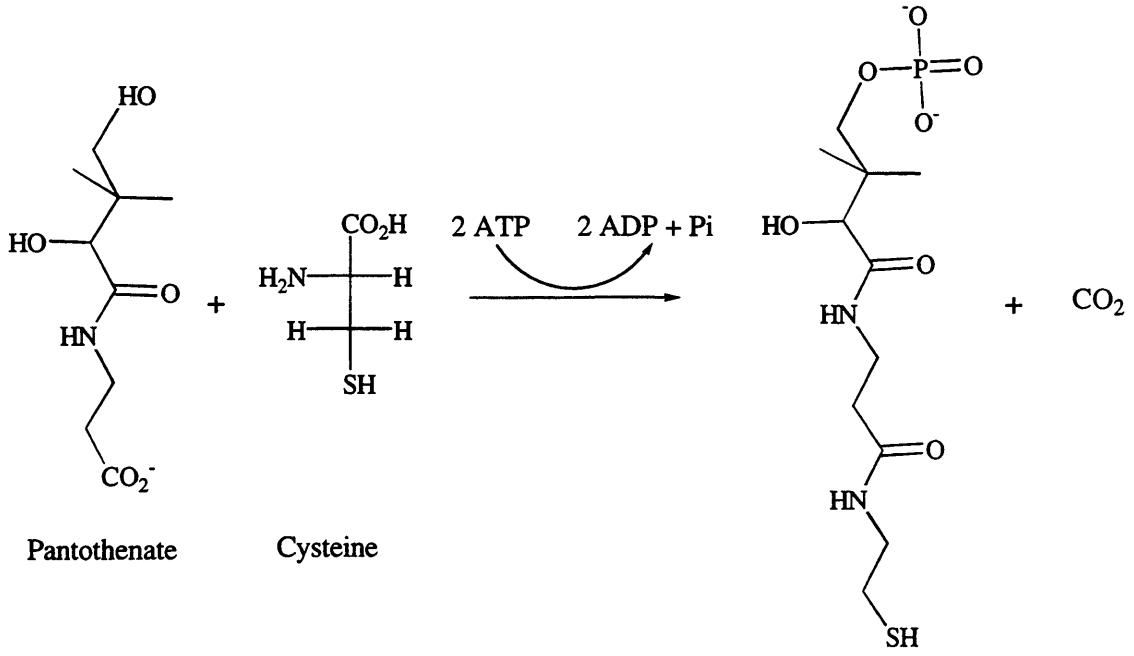


Figure 3

1.2.5 COENZYME A

The biosynthesis of Co-A starts from pantothenate (Scheme 18). After phosphorylation of the primary hydroxyl group by ATP, cysteine is linked to the carboxyl group by a peptidic bond. Loss of the carboxylic group of the cysteine moiety, leads to the intermediate 4-phosphopantetheine.

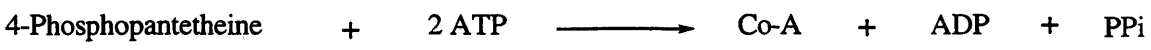


Pantothenate Cysteine

4-Phosphopantetheine

Scheme 18

AMP is transfered from ATP to 4-phosphopantetheine to form the pyrophosphate moiety. This gives a metabolite which is later phosphorylated by ATP at the C-3' yielding Co-A (Fig. 4). The stoichiometry of this proccess is shown (Scheme 19).



Scheme 19

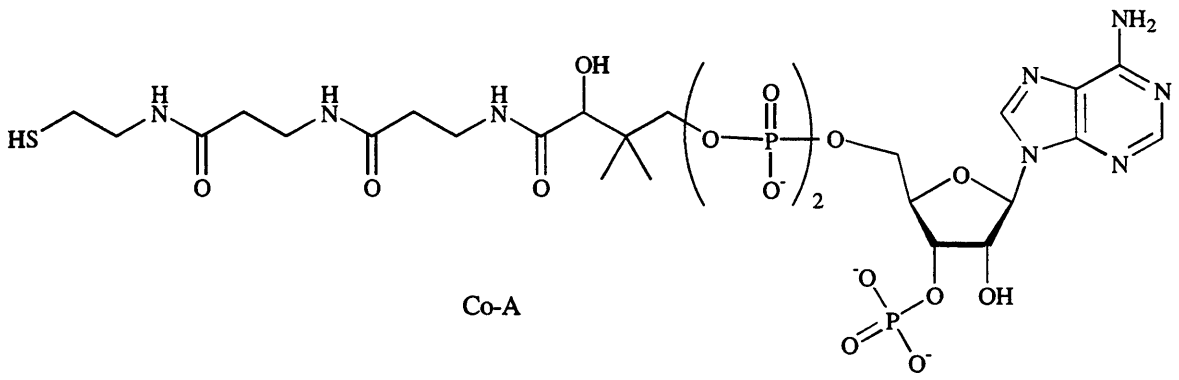


Figure 4

The terminal sulfhydryl group in Co-A is the reactive site of this coenzyme. Acyl units can be linked to Co-A forming a thioester bond yielding acyl Co-A (or acetyl Co-A if the acyl group is an acetyl). The transfer of an acetyl group is thermodynamically very favourable. The hydrolysis of acetyl Co-A represents a ΔG value of - 7.5 kcal/mol and serves as auniversal carrier of the acyl group, just as ATP is considered to be the universal carrier of the phosphoryl group.

1.2.6 ATP

ATP (Fig. 5) is synthesised in a transmembrane assembly in the mitochondrial matrix, leading to the generation of a membrane potential. An electron flow through the molecular assembly is generated by oxidation of NADH (Scheme 20). The energy released in this process is used to synthesise ATP.

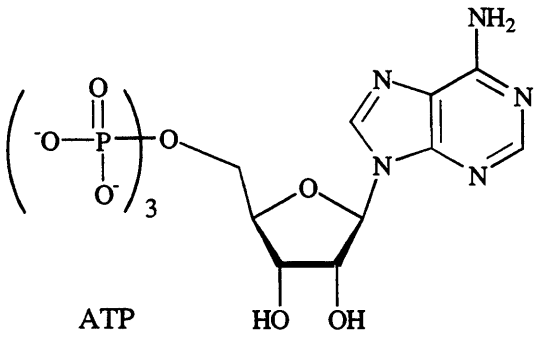
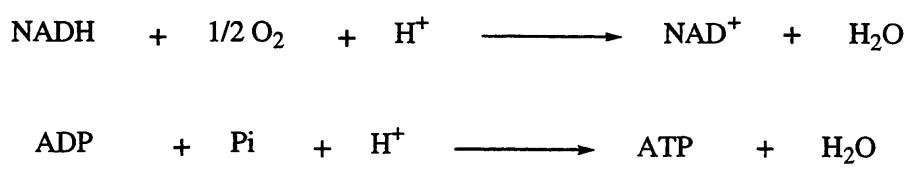


Figure 5



Scheme 20

In the early 1960s it was postulated that the electron transfer through the respiratory chain leads to the pumping of protons from the inner mitochondrial membrane to the other side of the inner membrane. The pH gradient between both sides generates an electric potential that is the driving force for the ATP synthesis. Thus, this proton gradient couples oxidation and phosphorylation together.

The flow of electrons does not occur unless ATP needs to be synthesised. This phosphorylation implies a supply of NADH, O_2, ADP and Pi, the level of ADP being the most important factor determining ATP synthesis.

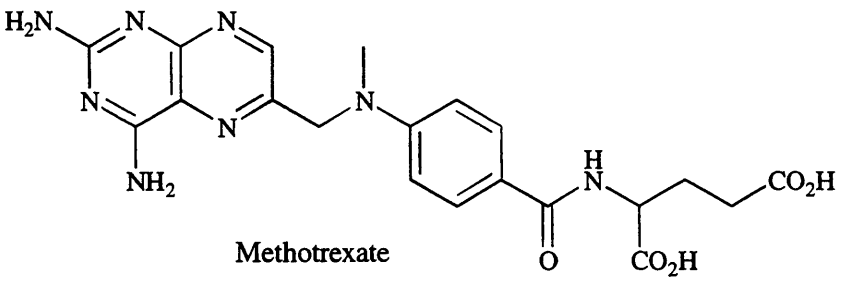
1.3 INHIBITORS OF NUCLEOTIDE BIOSYNTHESIS

One of the most current important challenges in medicinal chemistry involves the design of drugs which are capable of interfering with the formation and function of normal cellular metabolites. This is usually done by synthesis of stable analogues of the natural metabolites (antimetabolites) and investigating their effects in cellular systems or by selective inhibition of specific enzymes involved in the biosynthetic pathway.

Some of the compounds that are currently being used as anticancer drugs are inhibitors of nucleotide biosynthesis. Among others, some examples are methotrexate, azidothymidine (AZT), arabinosylcytosine (Ara-C), arabinosyladenine (Ara-A), fluorouracil and acyclovir. Their activity is described in this Section.

1.3.1 METHOTREXATE

Methotrexate (Fig. 6), inhibits the formation of deoxythymidylate, a precursor of one of the four building blocks of DNA. This drug is used for the treatment of many rapidly growing tumours such as acute leukaemia and choriocarcinoma. Nevertheless, it is quite toxic because it does not show any significant selectivity between the normal and malignant cells.



Methotrexate

Figure 6

1.3.2 AZT

AZT (Fig. 7), is also an inhibitor of DNA biosynthesis. The presence of an azido group replacing the 3'-OH, stops the DNA chain elongation because of the lack of the hydroxyl group on which to add the next deoxynucleotide.

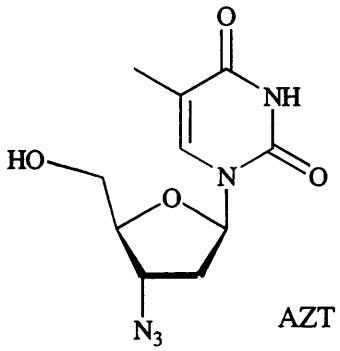


Figure 7

Cellular enzymes convert AZT to the 5'-triphosphate derivative and the viral RNA-dependent DNA-polymerase (reverse transcriptase) recognises it as a normal deoxythymidine 5'-triphosphate. Because the AZT 5'-triphosphate is a good substrate for the viral enzyme but not the human DNA-polymerase, selective inhibition of viral replication is achieved. In this way AZT is active against HIV-1 (human immune deficiency virus-1), the retrovirus that causes AIDS (acquired immune deficiency syndrome).

1.3.3 ARA-C AND ARA-A

Another important antimetabolite is Ara-C (Fig. 8) in which the sugar moiety is an epimer of D-ribose at C-2. Once Ara-C is activated *in vivo* to the corresponding 5'-triphosphate derivative, it is an inhibitor of DNA-polymerase. Its principal use is in the treatment of certain types of acute leukaemia and non-Hodgkin's lymphoma. The corresponding adenosine analogue Ara-A in its activated phosphorylated form, also inhibits DNA-polymerase.

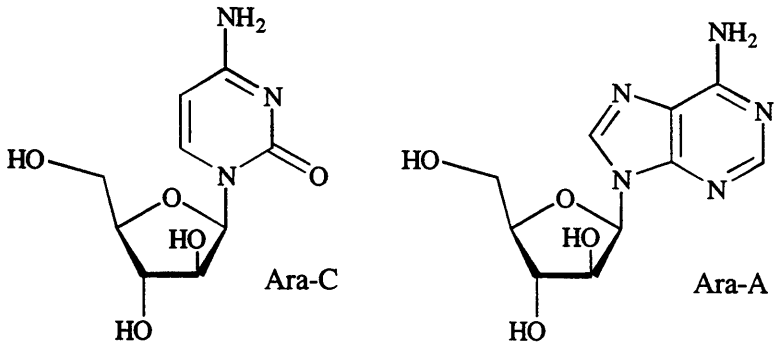
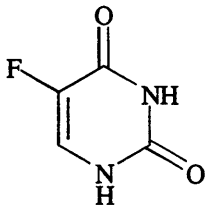


Figure 8

1.3.4 FLUOROURACIL

An analogue of uracil, fluorouracil (Fig. 9), is converted *in vivo* to its active form 5-fluorodeoxyuridylate (5-F-dUMP). This compound is able to block the enzyme thymidylate synthase. This synthase converts 5-F-dUMP into a reactive inhibitor that inactivates the enzyme, there by preventing the biosynthesis of thymidine.



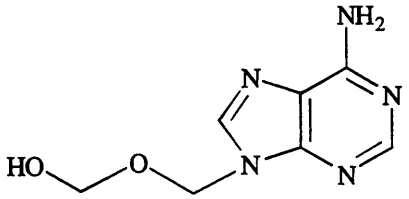
Fluorouracil

Figure 9

1.3.5 ACYCLOVIR

Acyclovir (Figure 10) constitutes a new family of antimetabolites whose structure lacks carbon atoms in the ribose ring of the nucleoside. The activated triphosphate derivative, inhibits the synthesis of DNA by binding to DNA-polymerase and forming an enzyme-

complex where the absence of 3'-OH does not allow the replication to continue. Acyclovir is highly effective against herpes simplex infections: type 1 which give rise to mouth and eye sores and type 2, which produce genital infections.

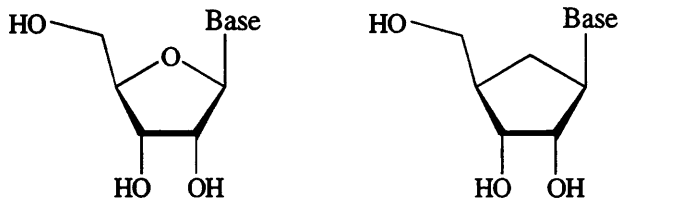


Acyclovir

Figure 10

1.4 NATURALLY OCCURRING CARBOCYCLIC NUCLEOSIDES

The term of "carbocyclic nucleoside" is generally given to compounds with structures similar to the nucleoside components of the nucleic acids, but in which the oxygen of the furanose has been replaced by a methylene unit (Fig. 11). Further modifications on the sugar moiety and/or in the heterocyclic base may also be incorporated and these are all generally referred to as carbocyclic nucleosides.

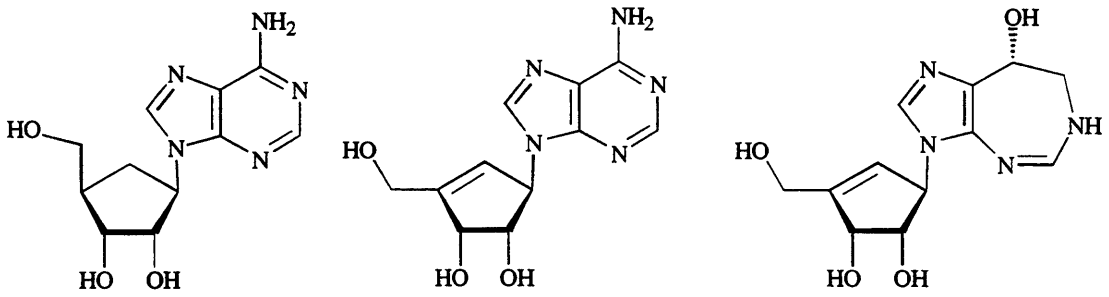


Natural nucleoside Carbocyclic nucleoside

Figure 11

The lack of the oxygen in the five membered ring converts the N-glycosyl linkage in the natural nucleoside into a normal C-N bond. The importance of this modification is the increase in the resistance towards hydrolases and phosphorylases, enzymes that break the glycosyl linkage in the natural nucleosides.

The search for potentially selective antiviral drugs led to the synthesis of aristeromycin (Fig. 12) for first time in 1966.[2] This carbocyclic analogue of adenosine, was isolated from *Streptomyces citricolor* IFO 13005 B-16575 as the first naturally occurring pseudo-nucleoside[3] and it became of interest in regard to its biological activity. This nucleoside showed a number of activities, among them, the inhibition of AMP synthesis in mammalian cells, inhibition of cell division and elongation in rice plants and, inhibition of the enzyme *S*-adenosyl homocysteine hydrolase.[4]



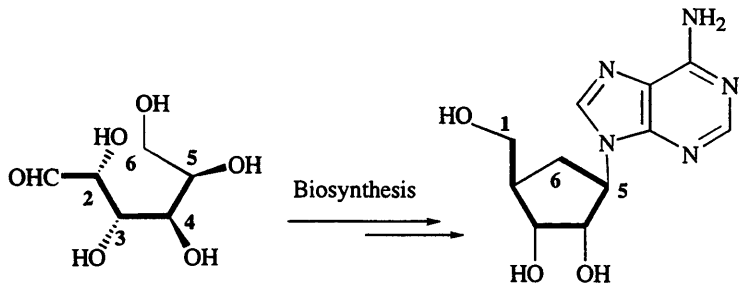
Aristeromycin Neplanocin A Adecypenol

Figure 12

Neplanocin A (Fig. 12), was first isolated in 1981 from *Ampullariella regularis*.[5,6] More recently it has been shown to be co-produced alongside aristeromycin by *Streptomyces citricolor*.[7] Neplanocin A exhibits potent antitumor[5] and antiviral[8] activity and it is a powerful inhibitor of *S*-adenosyl homocysteine hydrolase.[8] Moreover, an analogue of neplanocin A, adecypenol (Fig. 12) was also isolated from *Streptomyces spp.*[9] and it has shown to be a potent inhibitor of adenosine deaminase.

1.4.1 BIOSYNTHESIS OF CARBOCYCLIC NUCLEOSIDES

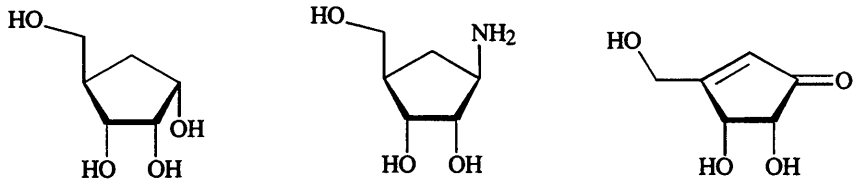
The biosynthetic origin of these carbocyclic nucleosides received attention for first time in 1980 through the work of Parry[7,10,11] but further studies[12,13] in collaboration with the Natural Products Group at Glaxo Research and Development were undertaken and have led to a better understanding. The first biological studies,[7,10] used isotopically labelled precursors to prove that the origin of the carbocyclic skeleton of aristeromycin and neplanocin A was D-glucose (Scheme 21).



D-Glucose Aristeromycin

Scheme 21

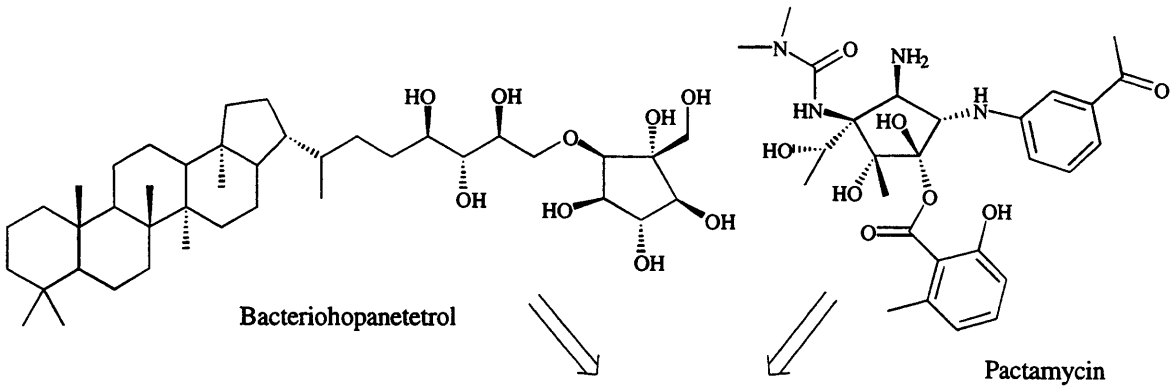
Further experiments suggested that carbocycles **6** and **7** (Fig. 13), are present as intermediates on the biosynthetic pathway. This hypothesis was later disproved by the Glaxo Group by experiments using mutants of *S-citricolor*. Their results proved that the enone **8** is the first carbocyclic intermediate towards the biosynthesis of aristeromycin and neplanocin A.

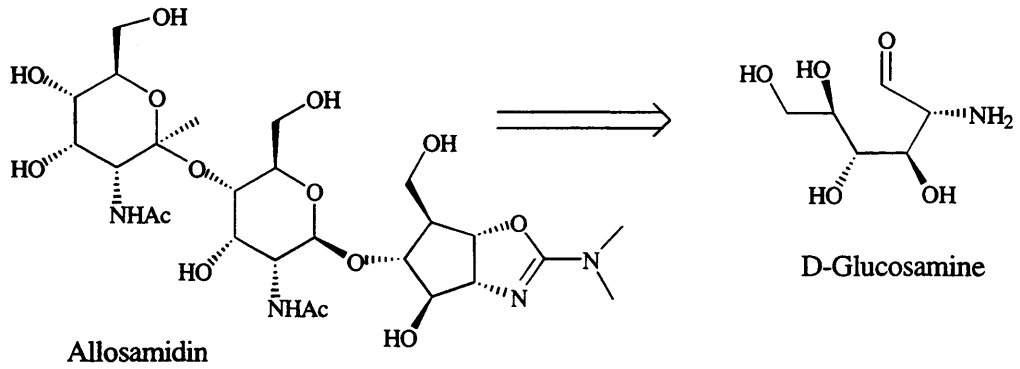


6 **7** **8**

Figure 13

Few biosynthetic studies with natural products containing cyclopentane rings have been reported in the literature, apart from those with aristeromycin and neplanocin A. Thus, metabolites pactamycin[14,15,16] and bacteriohopanetetrol,[17,18,19] contain a carbocyclic ring derived from D-glucose (Scheme 22). Moreover, both the disaccharide part and the carbocyclic ring of allosamidin,[20,21] are derived from D-glucosamine.





Scheme 22

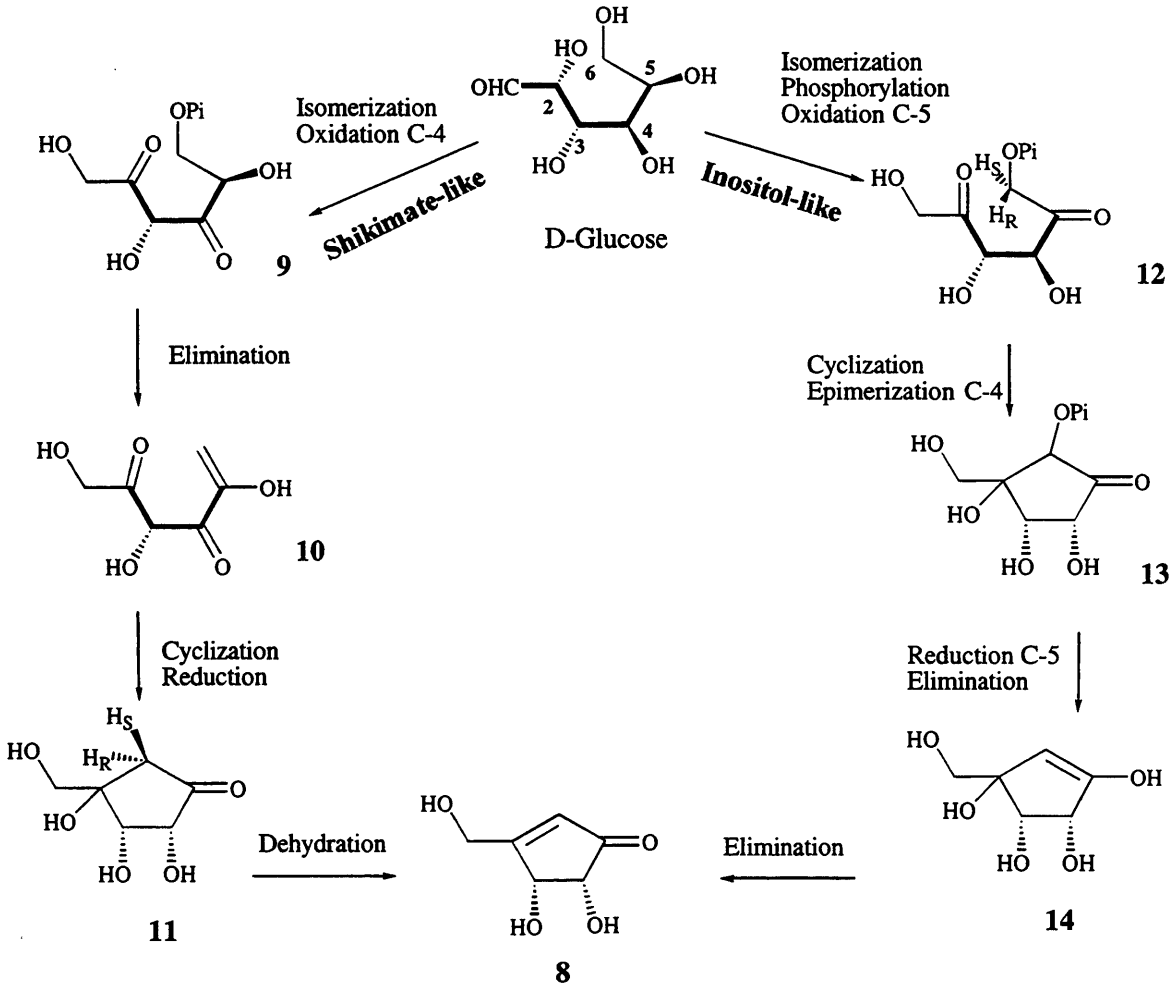
1.4.1.1 ARISTEROMYCIN AND NEPLANOCIN A

1.4.1.1.1 CONVERSION OF D-GLUCOSE TO THE CYCLOPENTANE RING

The formation of the five membered carbocycle in nature was proposed by analogy with the biosynthesis of six membered rings from carbohydrates. Two synthetic routes are possible, the "shikimate-like" and the "inositol-like" both involving an aldol-like condensation to effect cyclization (Scheme 23). They differ on the method by which the enol is generated.

The shikimate-like pathway would proceed *via* isomerization of glucose to fructose. Phosphorylation followed by oxidation at C-4 would lead to the intermediate **9**. Elimination of the phosphate group then yields the enolate **10** which undergoes cyclization and

reduction at C-4. The metabolite **11** is considered as the first carbocyclic compound generated. Further reduction by removal of the H-6 *pro*-S gives the enone **8**.

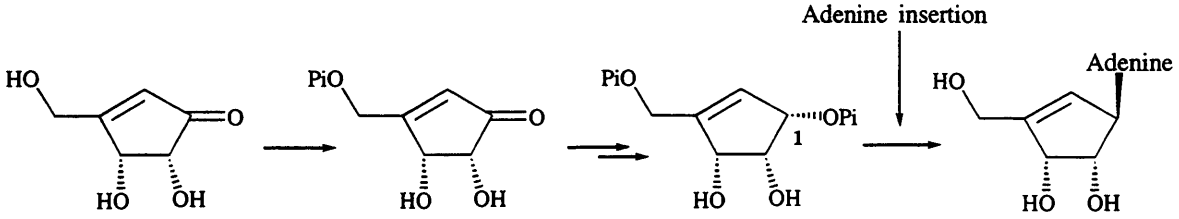


Scheme 23

The second pathway, analogous to the inositol process would involve isomerization of glucose to fructose *via* enolate formation as in the shikimate-like pathway followed by phosphorylation and oxidation at C-5 (Scheme 23). Loss of the H-6 *pro*-S in the substrate **12** previously formed, would lead to an aldol cyclization. Epimerization at C-4 would give **13** which would be reduced at C-5 to the alcohol and eliminating phosphate affording **14**. A further elimination reaction yields the conjugated ketone **8**.

1.4.1.1.2 ATTACHMENT OF THE ADENINE RING

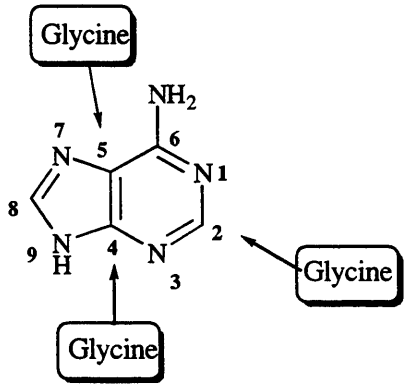
In contrast to the biosynthesis of normal adenosine where the adenine ring is built on PRPP, labelling experiments with precursors of aristeromycin, have shown that the heterocycle is added intact to a carbocyclic intermediate at the C-1 activated by pyrophosphate (Scheme 24) *via* a "salvage pathway". The possibility of a minor route *via* a parallel "de novo" biosynthesis of the adenine base, can also be considered upon an amine intermediate at C-5.



8 Neplanocin A

Scheme 24

Isotopic labelling experiments have also shown that carbon 2, 4, 5 and 8 of the adenine moiety (Fig. 14), were derived from glycine and C-6 from bicarbonate.[7]

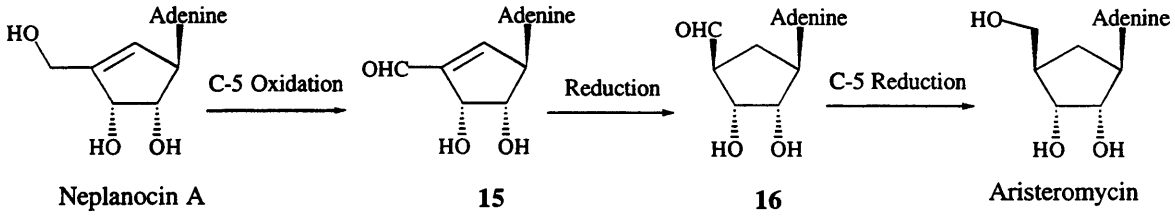


Adenine

Figure 14

1.4.1.1.3 FROM NEPLANOCIN A TO ARISTEROMYCIN

The close similarity between aristeromycin and neplanocin A and the fact that both metabolites can be isolated from the same micro-organism *S. citricolor*, suggested that neplanocin A is the derived precursor of aristeromycin. This proposal was confirmed by several studies carried out by Parry[22,23,24] and co-workers at Exeter.[13] These studies suggested that the mechanism for the conversion of neplanocin A into aristeromycin might involve oxidation of the primary hydroxyl group to an aldehyde **15** and then reduction of the conjugated double bond would give **16**. In the last step, the aldehyde would be reduced to yield aristeromycin (Scheme 25).



Scheme 25

Further studies have still to be undertaken in order to gain more detail of the precursor of the enone **8** obtained from D-glucose and of the intermediates that lead to the adenine addition. No detail concerning the enzymes involved in the conversion of glucose into the five-membered ring have been reported.

CHAPTER 2

SYNTHESIS OF A CARBOCYCLIC ANALOGUE OF CYCLIC ADENOSINE DIPHOSPHATE RIBOSE

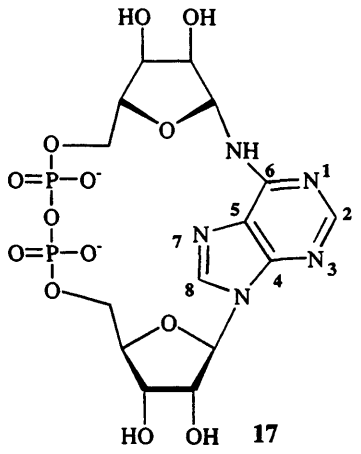
2. SYNTHESIS OF A CARBOCYCLIC ANALOGUE OF CYCLIC ADENOSINE DIPHOSPHATE RIBOSE

2.1 CYCLIC ADENOSINE DIPHOSPHATE RIBOSE, A DIFFERENT METABOLITE OF NICOTINAMIDE-ADENOSINE DIPHOSPHATE

The nicotinamidases (NADases) are a diverse group of enzymes that have been grouped together because of their common activity. They cleave the nicotinamide-ribose bond of nicotinamine adenosine diphosphate (NAD^+) to produce nicotinamide and adenosine diphosphate ribose (ADPR). However, apparently all NADases do not generate ADPR. In 1987, Clapper and co-workers[25] discovered a metabolite of NAD^+ that releases Ca^{2+} from internal stores, identified as cyclic adenosine diphosphate ribose (cADPR).

Lee and co-workers[26] used different approaches to determine the real structure of this Ca^{+2} releasing metabolite of NAD^+. By radiollabeling NAD^+ at different positions, they demonstrated that this is the metabolic precursor of cADPR ribose.

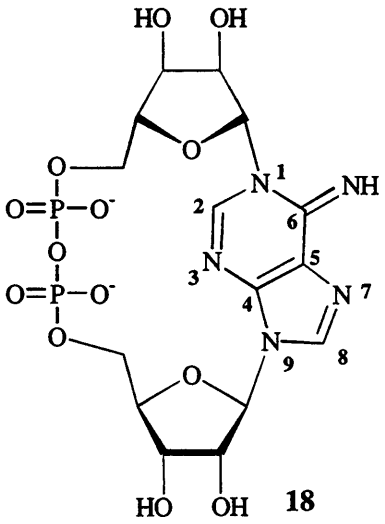
The novel metabolite was characterised by NMR spectroscopy including 2-dimensional COSY, and showed 12 protons for the ribosyl units and 2 for the adenine ring whereas those for the nicotinamide group had been lost. The chemical shift for the anomeric carbon was still consistent with linkage to nitrogen. Mass spectroscopy gave the most characteristic peaks $(M-H)^+$ and $(M+Na)^+$ and phosphate determination uniquely specified a molecular composition of $C_{15}H_{21}N_5O_{13}P_2$. But the most important evidence that they presented was the analysis of the major breakdown product of cADPR, identified by HPLC as ADPR. According to all these results the only structure for cADPR they found to be consistent corresponded to compound **17** (Fig. 15).



17

Figure 15

Further structural characterisation[27] showed that cADPR contained a free amino group at position 6 (Fig. 16) and this suggested that the substitution was at N-1 position of adenine ring rather than to N-6 as had been previously proposed (Fig. 15). Thus, the crystallographic analysis and the UV spectral properties of cADPR were studied as a function of pH and compared to other compounds containing an adenine ring, with substitutions at known positions. All the results reached the conclusion that the real structure of this novel nucleotide corresponded to compound **18** (Fig. 16).



18

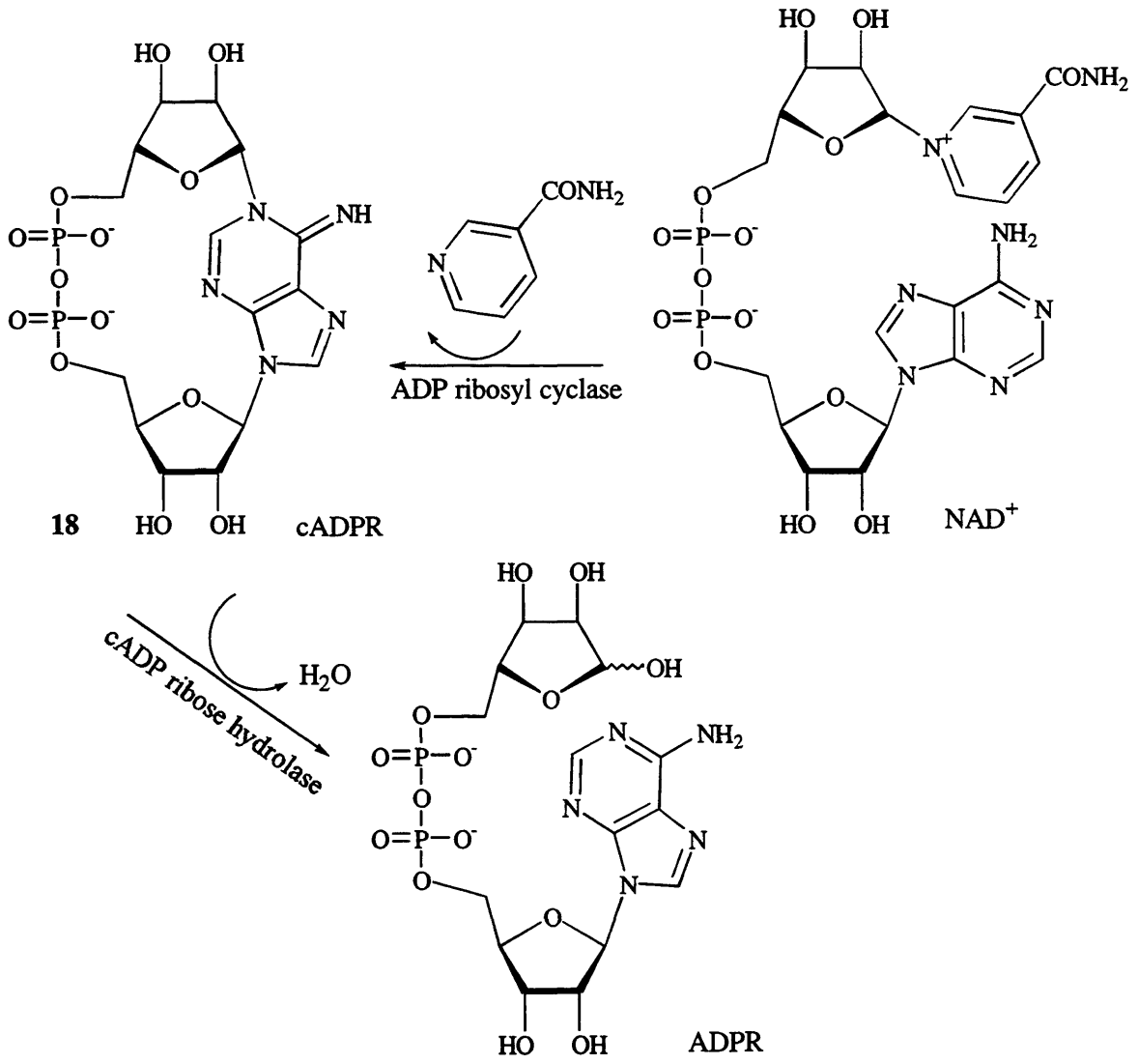
Figure 16

The resolution of the structure of this metabolite was relevant in order to carry out further studies of the function of this nucleotide, including the synthesis of analogues as inhibitors of cADPR metabolism.

2.2 BIOSYNTHESIS AND BIODEGRADATION OF cADP RIBOSE

Hellmich and Strumwasser[28] purified and characterised for the first time the enzyme that generates cADPR **18**. This NADase, cleaves the nicotinamide ribose bond of NAD^+ to produce nicotinamide and cADPR, rather than ADPR as observal with other NADases. Further enzymatic studies[29] identified the enzyme involved in the hydrolysis of cADPR to

ADPR. Thus, the overall reaction would be the conversion of NAD^+ to ADPR and nicotinamide (Scheme 26).



Scheme 26: Enzymatic process of synthesis and degradation of cADPR.

2.3 BIOLOGICAL ACTIVITY

A similar activity of the enzyme responsible for the synthesis of cADPR was found in various mammalian tissue extracts and this suggested that this metabolite might be a general messenger for calcium mobilisation in cells, with minimal or no change in endogenous inositol triphosphate (IP_3) level. Thus, both IP_3 and cADPR release calcium from

intracellular stores but by different mechanisms. In order to distinguish the other NADases from this enzyme which metabolises NAD^+ to give cADPR, it was named ADP ribosyl cyclase.

The role of cADPR in insulin secretion from pancreatic cells was also reported,[30] showing that glucose is a primary stimulus of insulin secretion and synthesis from the pancreatic islets of Langerhans. Increase in intracellular Ca^{2+} mediate the biochemical events that link glucose stimulation to insulin secretion by the islets and the mobilisation of Ca^{2+} from intracellular stores in the endoplasmic reticulum. Cyclic ADPR is a mediator of this release, being generated in the islets from glucose stimulation and serving as second messenger for the calcium mobilisation in the endoplasmic reticulum.

2.4 CYCLIC ADP RIBOSE ANALOGUES

2.4.1 BIOLOGICAL IMPORTANCE

The knowledge of the events involved in cellular communications and response is always difficult because these chemical messengers are continually being synthesised and broken down. One of the ways to probe these pathways is to synthesise stable analogues of cADPR, to investigate their effects in cellular systems or to selectively inhibit the enzymes responsible for their synthesis or degradation.

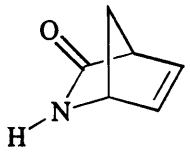
The first non-enzymatic stereoselective synthesis of cADPR from NAD^+ was reported in 1994[31] with the highest yield of 28%. In addition, a chemoenzymatic route to the cyclization of analogues of NAD was developed, using the enzyme ADP ribosyl cyclase.[32]

The urgent need for new substrates to investigate the Ca signalling pathways, has increased the interest in the synthesis of stable analogues of cADPR, and several studies recently reported, have been focused on this objective.

2.5 CARBOCYCLIC SUGARS: IMPORTANT TOOLS TOWARDS cADPR ANALOGUES

Carbocyclic sugars have been employed in many syntheses of nucleoside analogues, since the replacement of the oxygen in the sugar ribose by a methylene group greatly improves the metabolic and chemical stability of the C-N bond. This linkage becomes more resistant than a glycosylic bond towards enzymatic hydrolysis and this change is expected to selectively inhibit the enzyme responsible for the synthesis and degradation of cADPR **18**.

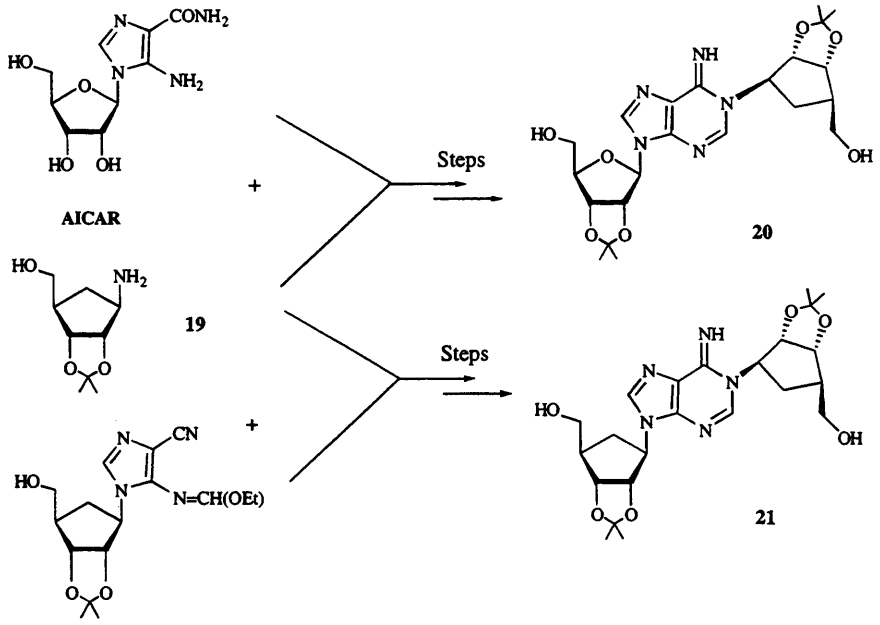
The synthesis of three stable precursors of cADPR analogues have been reported recently.[33,34] They have in common that the carbocyclic sugar moiety incorporated into their structure was prepared from a common source of chirality, the lactam **1** (Fig. 17).



1

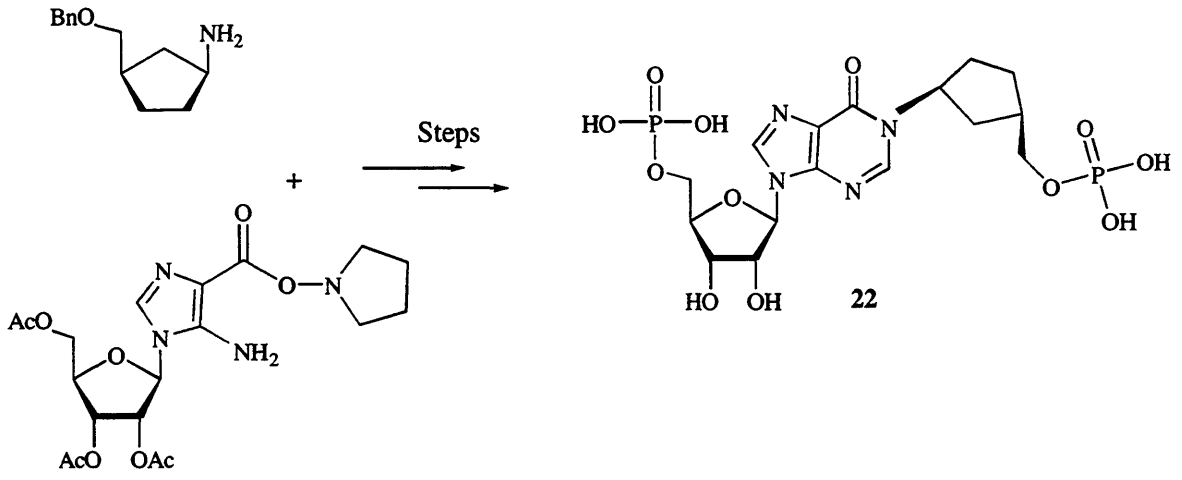
Figure 17

Hutchinson and co-workers[33] made use of the same substrate **19** to prepare the cADPR analogues **20** and **21** (Scheme 27).



Scheme 27

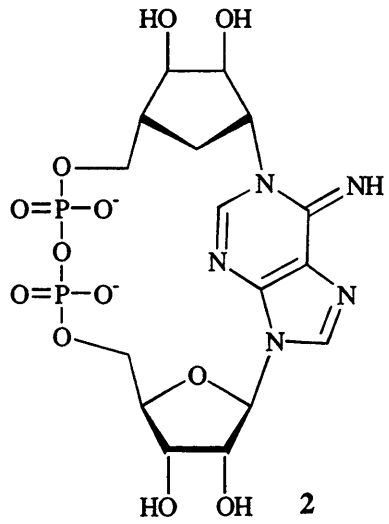
Fortt and Potter[34] reported the synthesis of compound **22** *via* a coupling reaction between a different carbocyclic moiety and a derivative of the commercially available AICAR[35] (Scheme 28).



Scheme 28

2.6 PROPOSAL

Although some studies on the complete synthesis of stable analogues of cADPR have been reported, it is still a big challenge to pursue a cyclic analogue *via* a synthetic pathway. Hence, the proposal of this investigation was focused on the synthesis of compound **2** (Fig. 18).



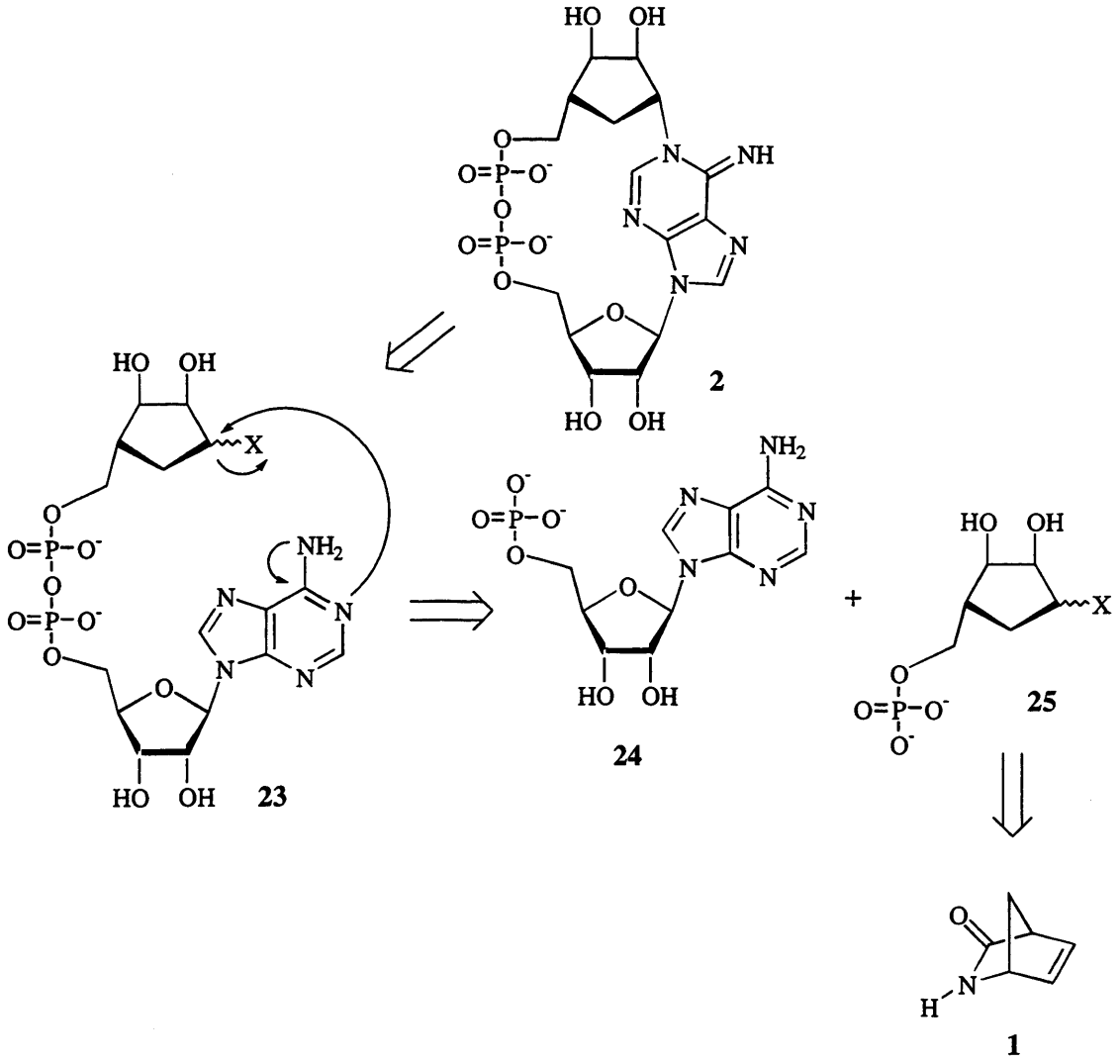
2

Figure 18

The main feature of this molecule is the modification in one of the furanose rings. Thus, the oxygen of the ribose attached to the N-1 of adenine in the natural metabolite **18**, has been replaced by a methylene unit in **2**. This change is expected to increase the resistance towards the enzymes responsible for the degradation of cADPR.

2.6.1 FIRST APPROACH

The retrosynthesis of our target molecule **2** is shown (Scheme 29).



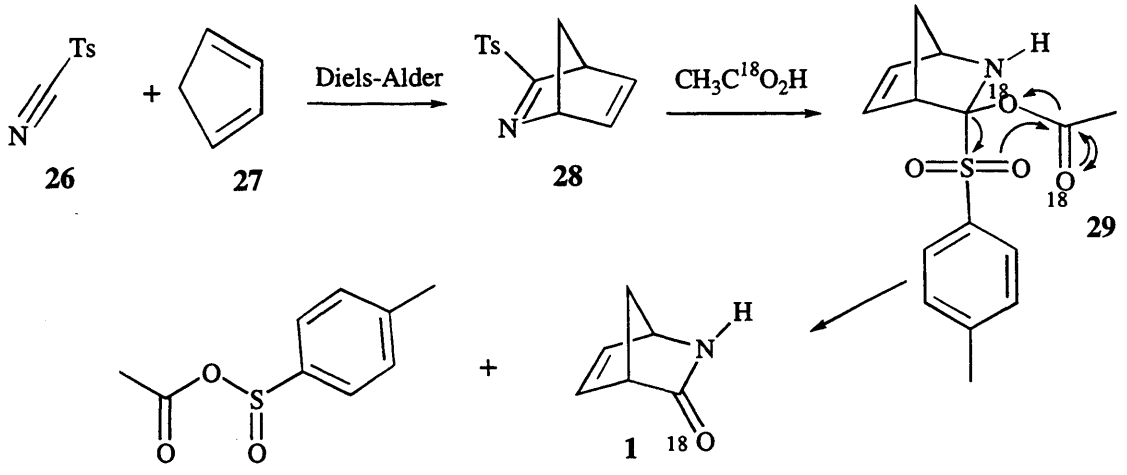
Scheme 29

The principal challenge of the proposed route towards our target molecule **2** was the cyclization step. That "key reaction" could involve the substitution of a good leaving group, represented as X in compounds **23** and **25**, *via* an intramolecular reaction promoted by N-6 of the heterocyclic base leading to the expected cADPR analogue **2**. Moreover, **23** could be prepared *via* formation of a phosphodiester link between AMP **24** and a cyclopentyl-monophosphate derivative **25** with DCC as coupling reagent. Compound **25** may be

prepared from the bicyclic lactam **1** which is commercially available. The defined configuration of this substrate, offered an unique starting point of this synthetic work. The lactam **1** contains two chiral centres with the appropriate relative configuration for the preparation of the carbocyclic moiety of **2**. Our experiments were carried out with the racemic series with the intention to undertake the synthesis with either of the pure enantiomers once the conditions for the preparation of our target molecule **2** had been optimised. The pure enantiomers of the lactam **1** are also commercially available.

2.6.2 SOURCES OF (±) AND (-) LACTAM 1

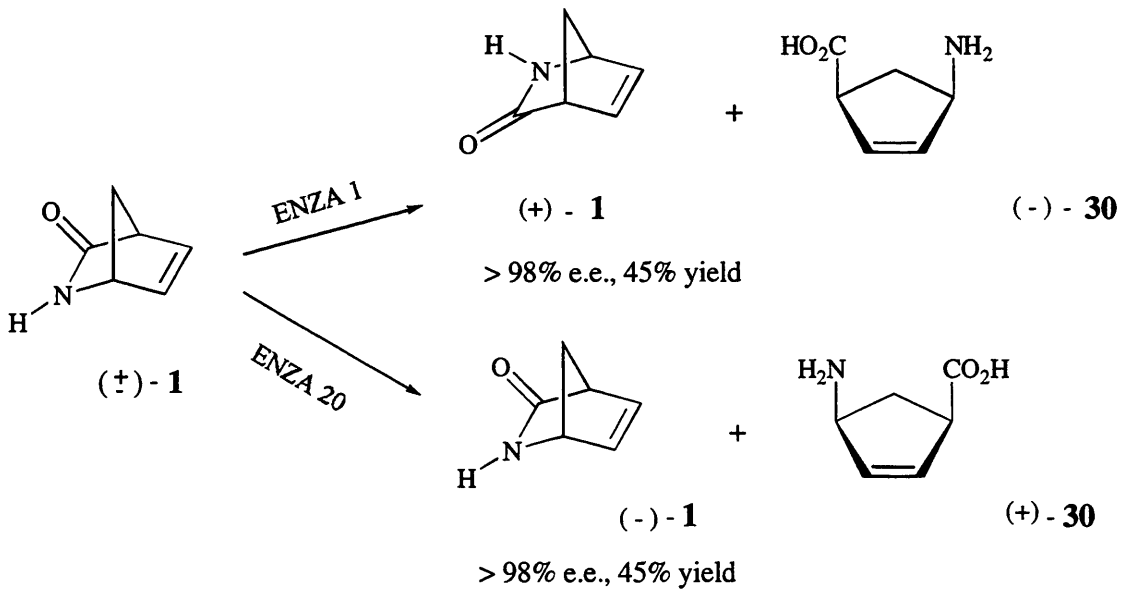
The lactam **1** can be prepared by addition of tosyl cyanide **26** to cyclopentadiene **27** *via* a Diels-Alder reaction, followed by acidic work-up.[36] Originally it was proposed that the conversion of the tosyl cyanide adduct **28** into **1** occurr by addition of water in the presence of acetic acid as catalyst. Recent studies by Morgan and co-workers[37] using ^{18}O labelling experiments, showed that the oxygen of the carbonyl group in the lactam **1** comes from the acetic acid, with the formation of the intermediate **29** (Scheme 30), and not from water as was initially assumed.[36]



Scheme 30

The resolution of racemic lactam **1**, can be achieved through enantioselective hydrolysis using fermentation with two micro-organisms:[38] ENZA 1 (*Rhodococcuss equi* NCIB 40213) gives the (+) enantiomer and (-) **30** whereas ENZA 20 (*Pseudomonas solanacearum* NCIB 40249) produces the (-) enantiomer and (+) **30** (Scheme 31).

Both substrates **1** and the amino acids **30**, have considerable potential for the synthesis of carbocyclic nucleosides.

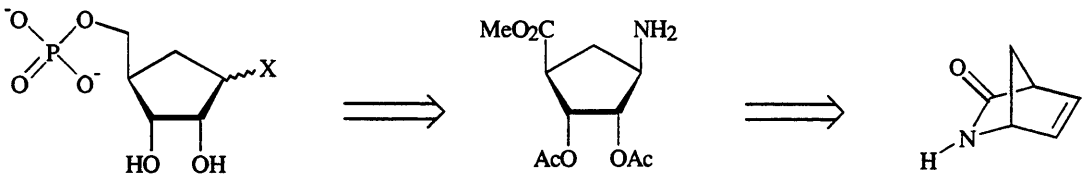


Scheme 31

2.6.3 PREPARATION OF THE CARBOCYCLIC MOIETY OF THE cADPR ANALOGUE 2

Considering the synthetic plan to prepare compound **25** from lactam **1** (Scheme29), X must ultimately be derived from a primary amine or amide. This consideration will lead to investigate different methods to undertake that conversion.

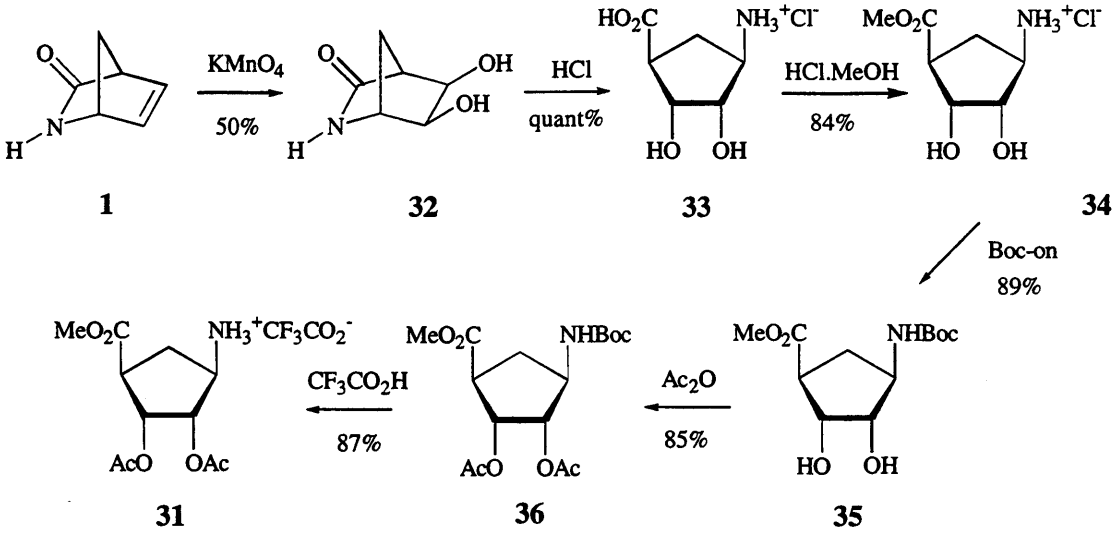
The first cyclopentane derivative precursor of compound **25** planned, was **31** (Scheme 32). Our particular interest in this compound, specially the presence of an acetate unit in α-position to the primary amine, will be explained in the synthetic strategies that follow (Section 2.6.3.1).



25 31 1

Scheme 32

Preparation of **31** as the trifluoroacetate salt derivative was performed from **1** in six steps (Scheme 33). The acetylation of the diol in **34** without any effect on the primary amine is problematic, since the basic conditions needed for this reaction would afford acetylation of the amine also. Therefore, it was necessary to proceed through a strategy involving selective amine protection, diol acetylation and finally selective amine deprotection.

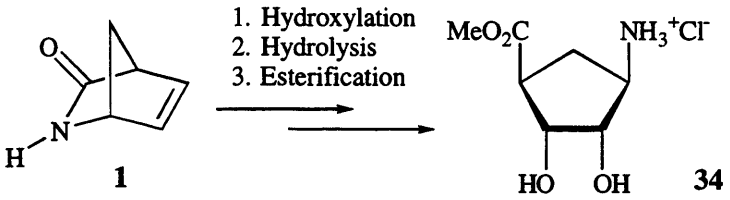


1 32 33 34 35 36 31

Scheme 33

2.6.3.1 SYNTHESIS OF COMPOUND 31

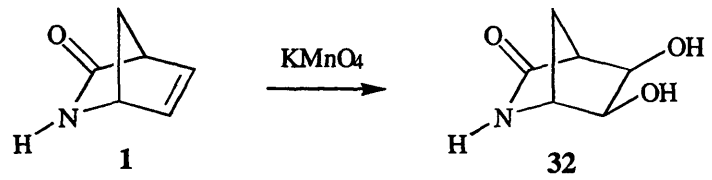
The first three steps to obtain **33** were reported by Oppenheimer[39] and they were undertaken with the modification described in this section (Scheme 34).



1 **34**

Scheme 34

Dihydroxylation of the lactam **1** can be undertaken using osmium tetroxide[40] or potassium permanganate.[39] The use of $KMnO_4$ was chosen for this oxidation for the following reasons: a) the alternative reagent, OsO_4, is highly toxic; b) the reaction could be followed colorimetrically and c) it is a stereoselective reaction and according to literature, yielded 91% of the desired 2,3- *cis*-dihydroxy lactam **32**. In our experience, the procedure described in the literature, gave yields that never exceeded 30%. Different modifications to this procedure were made, varying the pH, the rate of addition of the oxidant and the base, the volume of solvent and the temperature. The results are shown in Table 1 and the conclusions are described as follows: a) At room temperature, the experimental conditions reported led to an important ratio of products of over oxidation; b) the effect of a base such as NaOH, increased the yield but still an important amount of over reacting product was observed; c) an excess of oxidising agent increased the ratio of over oxidation product although did not have an important effect in the percentage of expected **32**; d) the effect of the temperature seemed to be important, though. Thus, at 40 °C or 50 °C although yields of **32** were not very high because of the high percentage of unreacted starting material, products of over oxidations were never detected. Nevertheless, decreases in temperature seemed to be more efficient, since no further oxidations had taken place and the conversion is total. Yields obtained for the expected **32** were 33% at 0 °C and the highest 50% at - 78 °C.



1 32

Table 1: Results obtained using $KMnO_4$ as dihydroxylating agent for the preparation of compound **32**.*

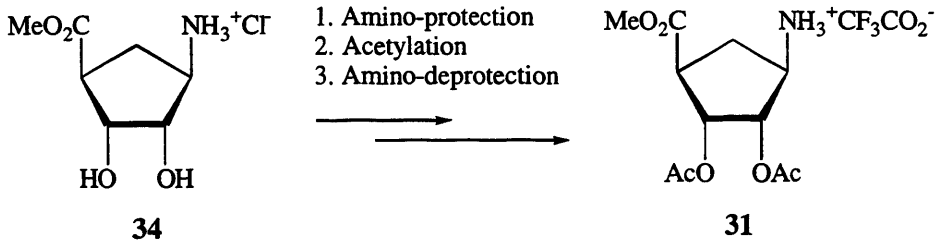
T (°C)	NaOH (M)	t (min.)	$KMnO_4$ (M)	Acetone (cm^3)	Mass ratio 1/OO/32	Crude mass (mg) (% 32)
25		60	1	2	1:4.1:5	200 (38)
25	0.01[a]	60	1	2	1:1:1	210 (25)
25	0.01[b]	60	1	2	1:1.7:6.6	180 (48)
25		60	1.1	2.5	0:1.9:1	100 (13)
25		60	1.2	2.5	0:1:2.2	46 (12)
25		120	1	7	0:1:5.5	150 (48)
40		75	1.1	16	1:0:1.9	197 (49)
50		75	1.1	16	1:0:3	133 (38)
0		75	1.1	16	0:0:1	86 (33)
-78		60	1.1	16	0:0:1	131 (50)

*All the experiments were performed on a 250 mg scale of starting material. The percentage of yield indicates the products obtained. **a**, simultaneous addition of base and oxidant. **b**, the addition of the base followed slowly by addition of the oxidant. OO = over oxidations; **t** indicates the time taken for adding the oxidant.

As final conclusion to the data described in Table 1 it is important to remark that, although a general pattern is not observed in the yield of the expected compound **32**, the conditions of - 78 °C were chosen as the most convenient for our purposes since the formation of by-products were not detected in the crude mass. The lack of a greater yield might be attributed to the loss of organic product retained with the inorganic material through the Celite pad during the work-up.

Preparation of compounds **33** and **34** were undertaken under the acidic conditions described in the literature[39] with similar yields to those reported.

Three further steps were neccesary for the preparation of compound **31** (Scheme 35).



34 **31**

Scheme 35

Selective acetylation of an hydroxyl group in the presence of an amino group is rather difficult. Nevertheless, reaction of **34** with Boc-on (2-*tert*-butoxycarbonyloxymino-2-phenyl acetonitrile), one of the most important amino-protecting groups in peptide synthesis,[41] proceeded chemoselectively yielding **35** (Scheme 33). Acetylation of this compound in pyridine with acetic anhydride, readily gave **36**.

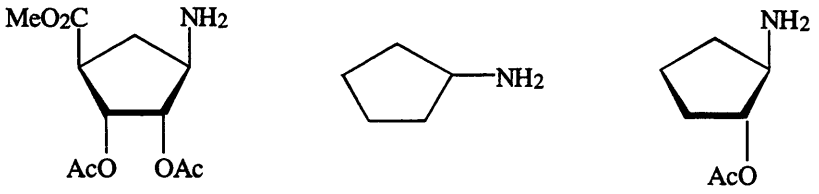
The Boc group has the advantage of being easily removed by treatment with trifluoroacetic acid in the presence of triethylsilane.[42] This method was applied to prepare **31** from **36**, mainly because a simple work-up procedure was required and all the secondary products were volatile. Hence, the synthesis of **31** was achieved in six steps with 28% overall yield.

2.6.4 ATTEMPTS TO CONVERT A PRIMARY AMINE INTO A DIFFERENT FUNCTIONALITY BY NUCLEOPHILIC SUBSTITUTION

2.6.4.1 USE OF SIMPLE MODEL COMPOUNDS

Different strategies for the conversion of the primary amine into a different functional group have been undertaken. For this purpose, it was considered more convenient to work with substrates with fewer functional groups than compound **31**, but keeping its structural

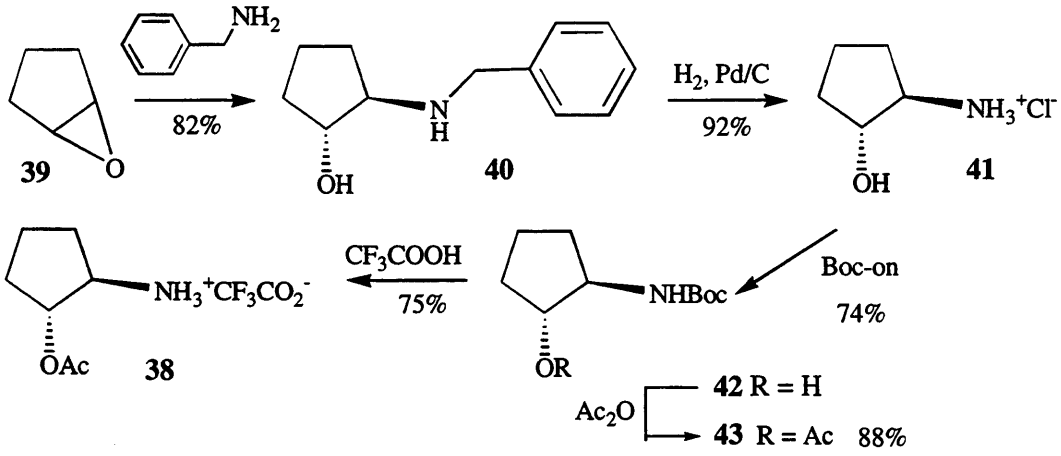
similarity. Thus, cyclopentylamine **37** and compound **38** were used as the simplest readily available models (Fig. 19).



31 **37** **38**

Figure 19

The synthesis of **38** as the trifluoroacetate salt, was performed from cyclopentane epoxide **39**, which is commercially available (Scheme 36).



Scheme 36

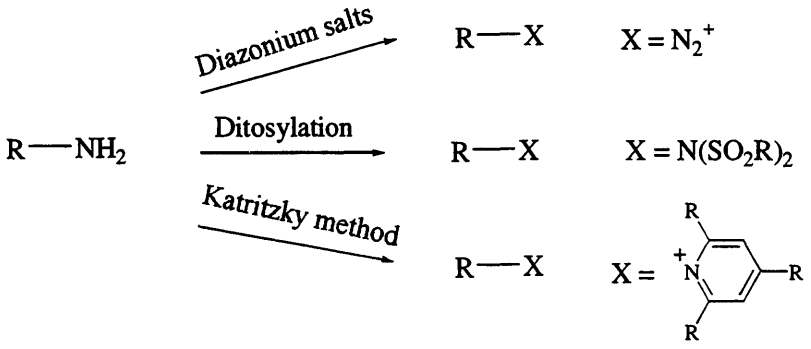
Compounds **40** and **41**, were prepared according to literature methods[43] with similar yields. Once that the aminoalcohol **41** was obtained, protection of the primary amine with Boc-on to obtain **42** was slow, and in order to increase the rate of reaction it was necessary to raise the temperature to 80 °C. Acetylation of the hydroxyl group gave **43** and deprotection of the amine afforded the expected **38** in 38% overall yield.

Three different methods for the displacement of the amino group have been investigated involving conversion of the amine into different functional groups. The results and discussion is described in the sections that follow:

2.6.4.2. Diazonium salts

2.6.4.3. Ditosylation

2.6.4.4. Pyridinium salts

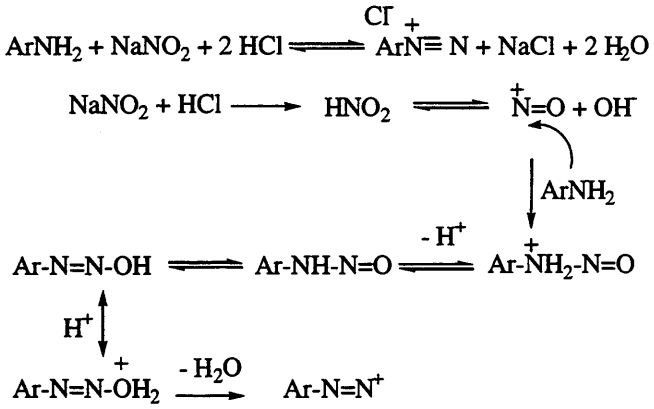


2.6.4.2 DIAZONIUM SALTS

In the chemistry of aromatic compounds, nucleophilic substitution is important *via* formation of a diazonium salt from a primary amine. Hence, this is a very useful and versatile method for preparing different substituted benzenes, such as halogen-arenes, aryl-cyanides, arenes or phenols. All those examples have found many applications in synthetic organic chemistry. The reaction involves treatment of the primary aryl-amine with sodium nitrite under acidic conditions and low temperature to yield a diazonium salt as a discrete intermediate (Scheme 37). This undergoes substitution with any nucleophile including water.

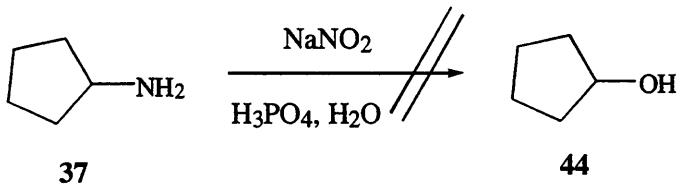
Aliphatic amines, treated under those conditions lead to the formation of a diazonium salt, releasing nitrogen and a carbonium ion which readily decomposes. This intermediate, is a "high energy" or unsolvated carbonium ion which does not have a counterion or group that might contribute to its stabilisation. The great feature of nitrogen as a leaving group, leads to a lower activation energy required for the decomposition of the aliphatic diazonium ion

and consequently a wide variety of side products resulting from solvolysis, elimination and rearrangements are produced.



Scheme 37: Mechanism of formation of diazonium salts.

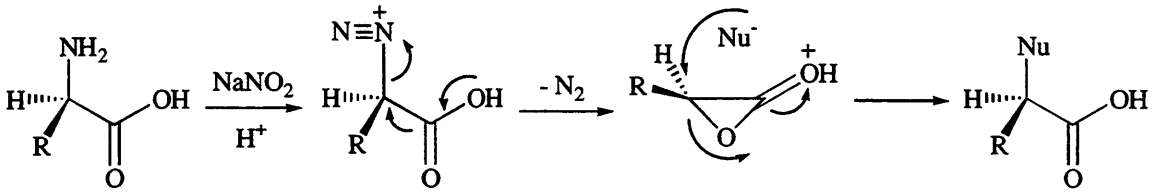
Huckel and Kupka,[44] claimed to obtain maximum yields of 65% in the conversion of cyclopentylamine **37** into cyclopentanol **44** under the conditions for the formation of the diazonium salt (although they also reported a significant amount of cyclopentene). Unfortunately, all attempts to reproduce their procedure were unsuccessful and TLC analysis of the crude reaction showed severe streaking which indicated possible decomposition or rearrangements, as expected for a primary amine of these characteristics (Scheme 38).



Scheme 38

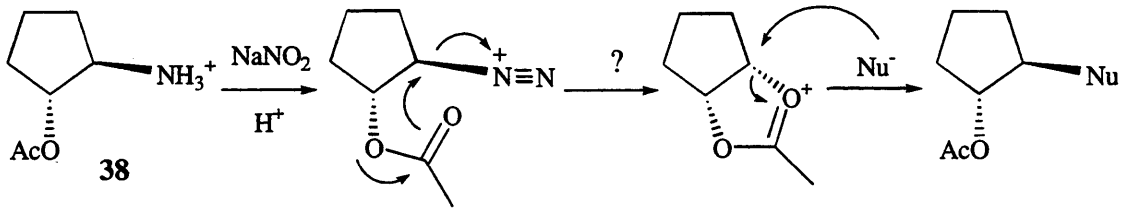
Although the diazonium salts derived from aliphatic primary amines decompose readily even at low temperatures, an important exception occurs in amino acids. In these compounds, the oxygen of the carboxylic group may act as an internal nucleophile *via* a process known as

"*neighbouring group-participation*".[45] With the formation of the diazonium salt, release of nitrogen follows formation of a three membered ring lactone with inversion of the configuration at the chiral centre. The presence of water or a more effective nucleophile, opens the lactone with overall retention of configuration (Scheme 39).



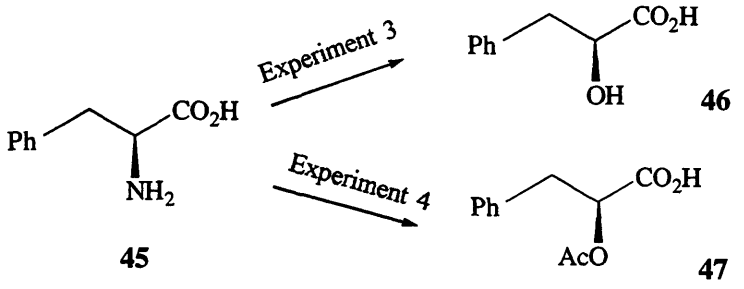
Scheme 39: Conversion of the amino group into a different functionality in amino acids by formation of the diazonium salt.

One of the important features present in the carbocyclic analogue **31** and in our model substrate **38**, is the presence of an acetate group in an α-*trans*-position to the primary amine. Thus, it was reasoned that this group might undergo *neighbouring group participation* as seen with amino acids, and this may control the reactivity of the carbonium ion generated (Scheme 40). The acetoxonium ion intermediate would lead, after the attack by a nucleophile, to a compound with retention of configuration.



Scheme 40: Expected behaviour of compound **38** under the conditions for the formation of the diazonium salts.

L-Phenylalanine **45** (Scheme 41) was used as a simple substrate to establish optimal conditions towards the diazonium salt formation and further attack by a nucleophile. Different reported procedures for amino acids were attempted but only the experimental conditions 3 and 4 were successful (Table 2) leading to compounds **46** and **47**, respectively.

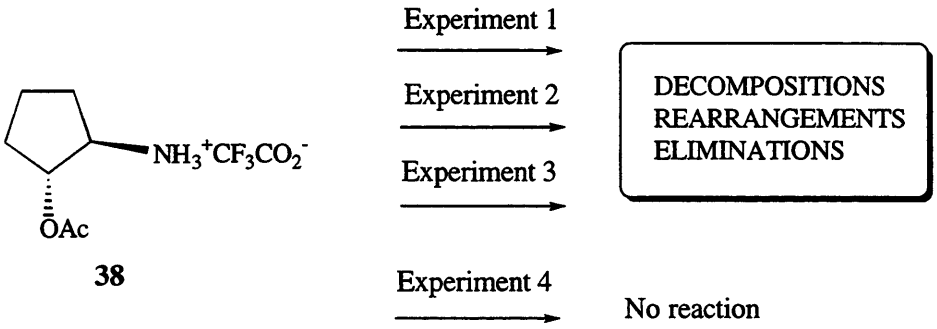


Scheme 41

Table 2: Results of diazotization using L-phenylalanine **45** as substrate. All the experiments were run on a < 100 mg scale.

Experiment	1[46]	2[47]	3[45]	4[48]
Acid	$H_2SO_{4(aq)}$	$HCl_{(c)}$, $H_2SO_{4(aq)}$	$HCl_{(aq)}$	CH_3CO_2H glacial
Oxidant	$NaNO_2$	$NaNO_2$	$AgNO_2$	$NaNO_2$
Product	–	–	**46**	**47**
Yield (%)	–	–	45	40

In order to test if the participation of the acetate group into the displacement of nitrogen was possible, the same procedures 1-4 were the followed with the substrate **38** (Scheme 42). Reaction of this compound under conditions 1-3, were unsuccessful with no isolation of any organic material into the organic layer. The aqueous phase was concentrated to dryness and analysed by ^1H NMR. The spectrum from that residue showed no evidence of the methyl group. This indicated that the hydrolysis of the ester had taken place. Furthermore, the acidic conditions used in experiment 4, were not efficient for the formation of the diazonium salt even when addition of the oxidant was done at room temperature, and most of the starting material did not react.



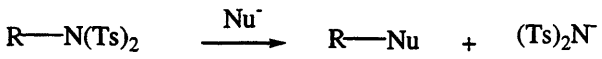
Scheme 42

None of the attempts to form the diazonium salt effected nucleophilic substitution. There was no evidence of *neighbouring group-participation*, *via* the acetoxyl group in α-position to the primary amine, and further side reactions such as decomposition or rearrangements due to the low stability of the secondary carbocation generated, might be predominant.
It is important to mention that the displacement of nitrogen might have also ocurred *via* an elimination reaction, with the consequent formation of volatile products such as cyclopentanone. Those compounds were not detected and may have been missed with the evaporation of the solvent.

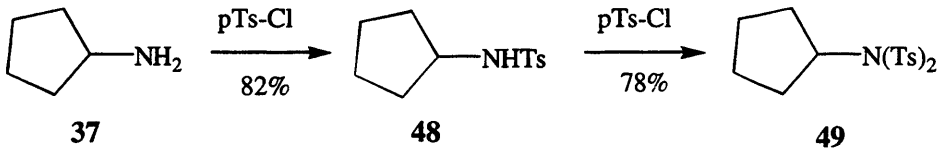
2.6.4.3 DITOSYLATION

One of the most useful reactions that promote C-O bond cleavage consist of the displacement of a tosyl group by an appropiate nucleophile. This is based on the fact that tosylate and related anions are much better leaving groups than the hydroxyl group. An analogous prediction may be made for disulfonimide anions. Thus, the pKa of *N,N*-di-(*p*-nitrobenzenesulfonamide) is 0.3[49] whereas the pKa of parent amine is in the range of 35. Hence, synthetic methods appeared in the literature for displacement of ditosylamine by iodide, bromide, chloride or acetoxide.[50] Nevertheless, it should be considered that the reaction was described for primary amines and in some cases not only nucleophilic substitution but also elimination products were identified. This leads to the conclusion that steric problems might decrease the yield of substitution in hindered primary amines, in favour of an elimination process.

The lack of success in our previous attempts, led to investigate the S_N2 nucleophilic substitution of ditosylamine.



With this aim, cyclopentylamine **37** was converted into the corresponding ditosylate **49** in two steps (Scheme 43). Monotosylation of the primary amine to give **48**, readily took place. However, the formation of **49** was slower possibly due to the steric hindrance which might decrease the rate of reaction towards a second substitution.



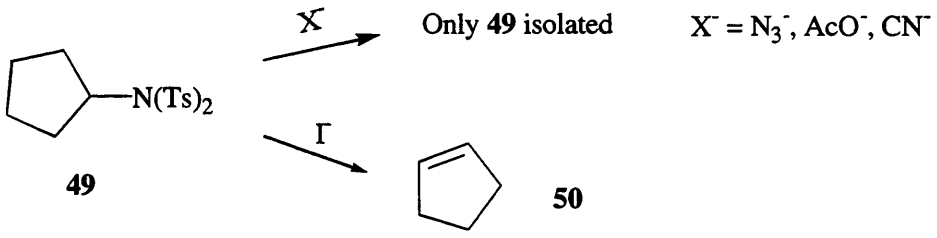
Scheme 43

The results obtained by treatment of compound **49** with different nucleophiles are shown in Table 3. DMF, CH_3CN and DMSO were the solvents used in the experiments here described. It was expected that they would favour nucleophilic substitution due to their polar aprotic nature by solvation of the cation, leaving the anion free to react.

Table 3: Results obtained using different nucleophiles to displace disulfonamide group in **49**.
• represents the use of dibenzo-18-Crown-6.

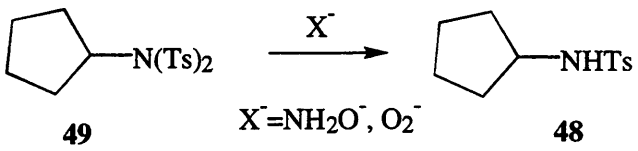
Experiment	1	2	3	4	5	6
Reagent	NaN_3	KOAc	KCN	KI	NH_2OH-HCl, KO^tBu	KO_2
Catalyst	•	•	•	–	–	–
T[a]	rt→100	rt→100	rt→100	120	rt	rt
Solvent	DMF	DMF CH_3CN	CH_3CN	DMF DMSO	^{t}BuOH	DMF
Product	**49**	**49**	**49**	**50**	**48** **49**	**48** **49**

Experimental conditions, 1, 2 and 3 which involved reaction with azide, acetate or cyanide, did not affect the starting material (Scheme 44). Reaction of compound **49** with KI was carried out in an open flask. Analysis by TLC of the crude product did not show the presence of either starting material or any other product. This could only mean that the product was volatile. Thus, a reacti-vial was used and the reaction was monitored directly by ^{1}H NMR (DMSO-d_6). Only fifteen minutes at 120 °C were enough to achieve about 50% elimination, detected by a decrease in the integration at the methine proton signal between 4.41-4.27 ppm and the appearance of an apparent singlet at 5.77 ppm, which should correspond to the formation of cyclopentene **50** (in agreement with the Aldrich NMR catalogue). Thus, the difficulty in the approach to a secondary carbon and the bulky size of iodide, made the elimination faster rather than the substitution.



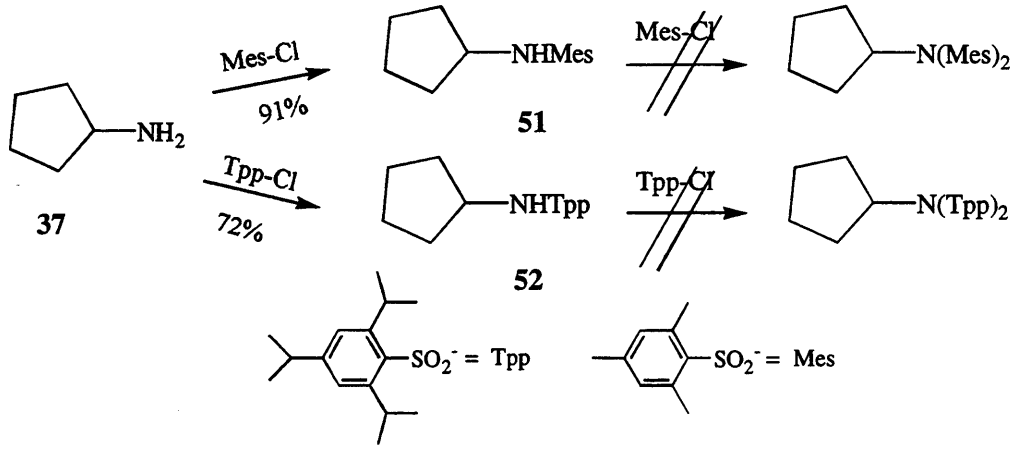
Scheme 44

In addition, when the reaction was carried out with good oxygen nucleophiles such as O_2^- and NH_2O^-, the predominant reaction was the attack to the sulphone leading to the formation of **48** as the main product but also an important amount of the starting material **49** (Scheme 45). This behaviour was mentioned by De Christopher and co-workers[50b] but specific examples were not described.



Scheme 45

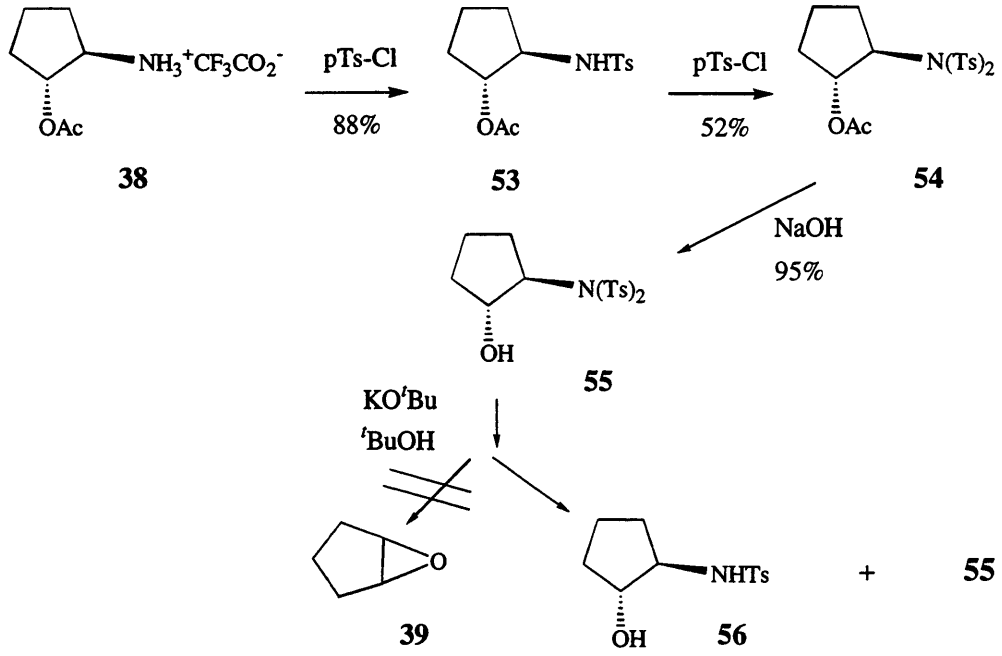
According to these results it was decided to study the behaviour of these oxygen nucleophiles with a different substrate which could make the attack at sulphur more difficult. Hence, disulfonylation of **37** was attempted using 2,4,6-triisopropylbenzenesulfonyl chloride (Tpp-Cl) and 2-mesitylenesulfonyl chloride (Mes-Cl) as sulfonating agents, respectively. Monosulfonylation readily took place leading to **51** and **52** but disulfonylation was too slow, possibly because of the steric hindrance and in both cases, the starting material was isolated (Scheme 46).



Scheme 46

The last attempt to effect nucleophilic displacement of a disulfonamide was made *via* an intramolecular nucleophilic substitution *via* an adjacent hydroxyl group. With this aim, **54** was prepared in an acceptable yield from compound **38** in two steps, using *p*-toluenesulfonyl chloride as tosylating agent (Scheme 47). Hydrolysis of the acetyl group was carried out successfully in aqueous NaOH to give **55**.

Reaction of **55** with potassium *tert*-butoxide in ^{t}BuOH at room temperature did not give the expected cyclopentane-epoxide **39**. The compounds isolated were **56** and starting material **55**.

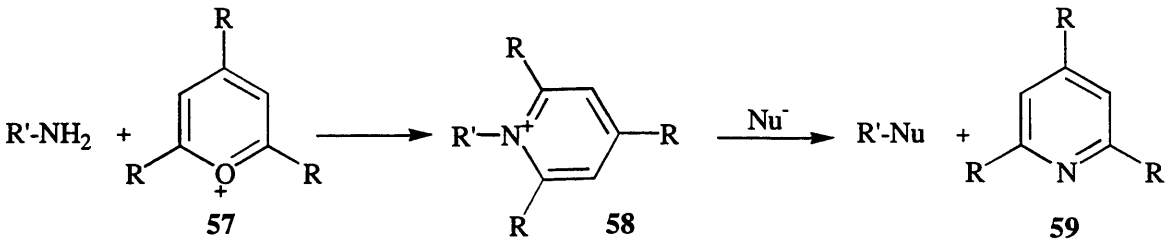


Scheme 47

Although displacements of ditosylamide attached to a primary carbon are well documented, the results obtained proved that nucleophilic substitution at a secondary carbon is too slow. When starting material was not recovered, products from elimination reactions were predominant.

2.6.4.4 PYRIDINIUM SALTS

The lack of success encouraged the investigation of a new method to effect the nucleophilic displacement of a primary amine derivative. In the literature is described a method to effect C-N bond cleavage developed by Katritzky,[51] which consisted of the conversion of a primary amine into a pyridinium salt, *via* a two step sequence (Scheme 48).

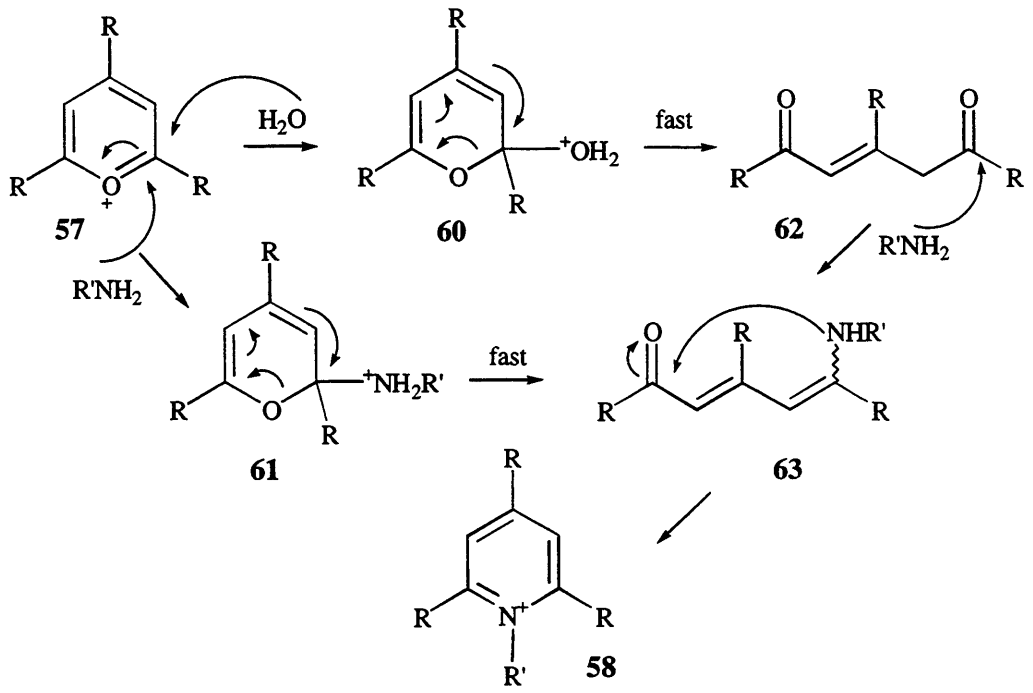


Scheme 48

The first step involves reaction of a primary amine with a 2,4,6-substituted pyrylium cation **57** to yield a pyridinium salt **58**, and this is followed by nucleophilic attack at the alkyl carbon moiety. This nucleophilic displacement affords the alkyl-derivative and the substituted 2,4,6 pyridine **59** as by-product.

Initial reaction of the amine with the pyrylium salt is rapid as is deprotonation and ring-opening (Scheme 49).[51] The amine-pyrylium ratio should be at least 2:1 for obtaining full conversion of the pyrylium salt into **63**. Lower amine:pyrylium ratios, afford the diketone **62**, presumably from presence of traces of water. Intermediate **63** is converted at a measurable rate into the pyridinium salt **58**, and **62** also reacts with the amine giving **58** but at a slow rate. The function of the second molecule of amine is to deprotonate the intermediate **61** but its substitution by triethylamine gives identical results. The last step is subject to acid catalysis where the rate could be increased one thousand times by addition of acetic acid. Protonations and deprotonations could be assisted by reaction with the solvent which could be water, an alcohol or dichloromethane with a specific amount of acetic acid.

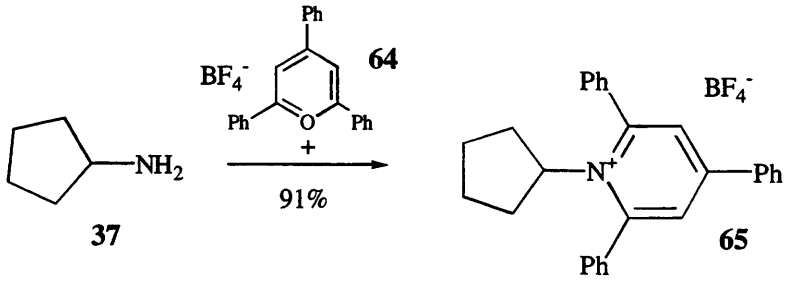
This method was chosen assuming that nucleophilic displacement of the amino derivative could be easier than in previous reactions. It was reasoned that the fact that the leaving group is a neutral species together with its high stability, might favour nucleophilic displacement.



Scheme 49: Mechanism of formation of pyridinium salts from primary amines.

The conversion of alkyl and benzyl primary amines into the corresponding acetates,[52] chlorides,[53] iodines[54] and azides[55] *via* displacement on pyridinium cations have been reported. Reaction of cyclohexylpyridinium with iodide was described to afford high percentage of elimination.[54] Considering the size-relation between a five and six membered ring, the use of iodine was discarded in our experiments. Steric hindrance on the approach of the nucleophile may possibly lead to elimination as it was experienced on previous attempts to displace the disulfonamide.

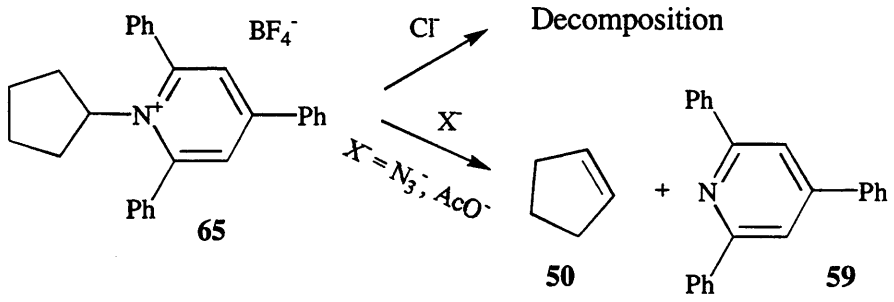
The readily available 2,4,6-triphenylpyrylium tetrafluoroborate **64** was used as the pyrylium salt in our experiments. Cyclopentylamine **37** and compound **38**, were the substrates for the preparation of the pyridinium salts and these were subsequently used for exploring the nucleophilic substitution.



Scheme 50

Reaction of an excess of **37** with 2,4,6-triphenylpyrylium tetrafluoroborate **64** in ethanol (Scheme 50), showed severe streaking on analysis by TLC. This could be a consequence of the stabilisation of intermediates of reaction formed in such a polar solvent, reducing the rate of formation of the expected salt. This result encouraged us to use one equivalent of triethylamine for each of amine, with acetic acid as catalyst and dichloromethane as solvent. Those conditions afforded **65**, in good yield.

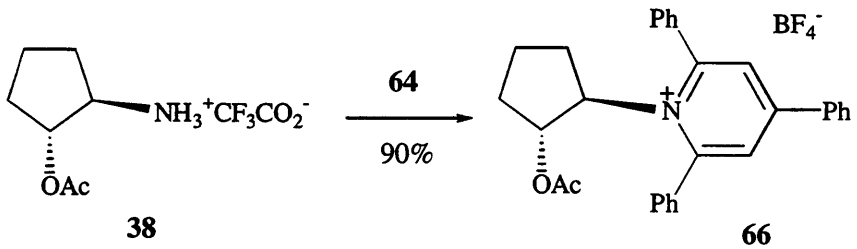
N-Cyclopentyl[2,4,6-triphenylpyridinium]tetrafluoroborate **65** was the substrate used in our experiments with azide, acetate and chloride as nucleophiles. The procedures described in literature with chloride[53] or acetate[52] involved pyrolysis of the *N*-substituted pyridinium salt. Thus, chloride should be added to the reaction as an eutectic mixture of $ZnCl_2$:NaCl:KCl at 250 °C whereas reaction with acetate requires 2,4,6-triphenylpyridine as co-reagent, possibly in order to reduce the pyrolysis temperature. Therefore, reaction with chloride at 250 °C was carried out in a Kugel-oven linked to a condenser refrigerated at -78 °C. Analysis by TLC of the crude residue and ^{1}H NMR, indicated unsuccessful reaction with decomposition, possibly due to the high temperature conditions.



Scheme 51

Reaction of compound **65** with sodium azide at room temperature did not succeed and the starting material was recovered. Nevertheless when the same reaction was carried out between 100 and 150 °C at atmospheric pressure, analysis by TLC showed a product with higher R_f than the starting material. After extraction only a solid, identified as 2,4,6-triphenylpyridine **59**, was isolated (Scheme 51). This result suggested that the other product must have been cyclopentene **50** (boiling point of 44 °C). In order to support this conclusion, the reaction was run in a reacti-vial with C_6D_6 as solvent and monitored by ^1H NMR spectroscopy. The ^1H NMR spectrum showed a peak at 5.77 ppm for the alkene protons of **50**, which confirmed our prediction. In addition, the crude product was analysed by GC and the chromatogram revealed a major peak with retention time of 2.32 minutes which corresponded to cyclopentene **50** (an analytical sample of the pure compound injected as reference under the same conditions, showed a Rt of 2.30 minutes). Furthermore, when the pyridinium salt **65** was reacted with acetate in the presence of **59** at 100 °C (as reported in the literature),[52] an increase in the weight of this compound **59**, was observed. This showed once more, that under these experimental conditions, elimination rather than substitution was predominant.

The next attempt was made using **66** as starting material (Scheme 52). This compound was prepared from **38** following a similar procedure to the preparation of **65** with the exception that, one more equivalent of base was added to provide complete deprotonation of the amine salt.

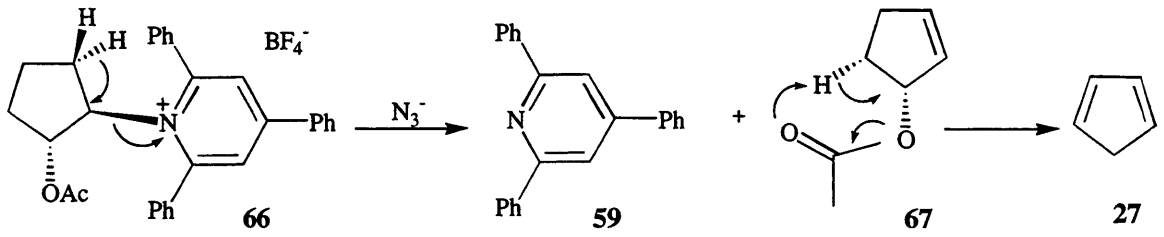


38 **66**

Scheme 52

Compound **66** was dissolved in DMF and azide was used as the nucleophile. Running the reaction in a reacti-vial between 100-140 °C, TLC analysis showed the presence of different

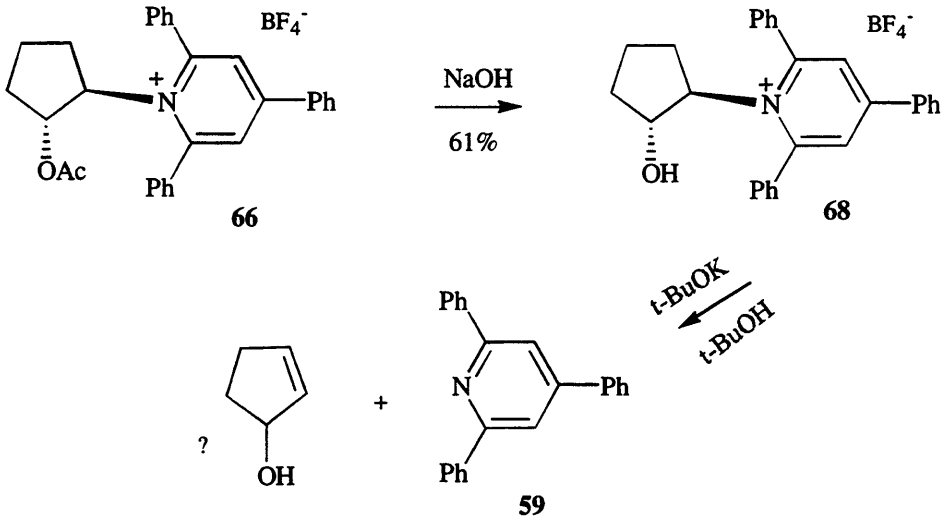
products. ^{1}H NMR of the crude product did not show a peak corresponding to the methyl group of the acetate but, amongst others, new resonances between 4 and 5 ppm. However, attempts to isolate any compound by flash chromatography on silica gel, afforded only **59**. A possible mechanism that might explain the loss of the acetylated moiety is shown below (Scheme 53). The first elimination process, might be straightforward considering the result obtained with other nucleophiles, essentially because of the high stability of the leaving group **59**. The second one could be favoured by the acetoxy group in compound **67** leading to formation of cyclopentadiene **27** (or dicyclopentadiene as dimer) which would account for the alkene protons observed in the ^{1}H NMR spectrum.



Scheme 53

The conclusion reached was that nucleophilic substitution did not successfully compete under these experimental conditions. The steric hindrance that impedes the nucleophilic approach made elimination faster and more appropriate. The two phenyl groups on *ortho* positions may further impede approach of a nucleophile and in consequence elimination reactions may be favoured.

The last approach considered was regarding an intramolecular nucleophilic displacement which would undergo formation of cyclopentane epoxide. Therefore, treatment of **66** with aqueous NaOH in methanol, yielded **68** in 61% (Scheme 54).



Scheme 54

Reaction of **68** with potassium *tert*-butoxide in ^{t}BuOH was performed at room temperature. Analysis of the extracted crude product by TLC, showed severe streaking and the ^{1}H NMR spectrum gave peaks corresponding to 2,4,6-triphenylpyridine **59** as major product but among other signals, the presence of alkene protons was evident. Unfortunately, purification by chromatography only isolated the by-product **59**.

According to these last experimental results, it seemed that substitution of a pyridinium salt attached to a secondary carbon in a cyclopentane ring, is rather difficult and elimination appeared to dominate.

2.7 GENERAL CONCLUSIONS

1.-Three different reactions were carried out with the aim of converting an amino group into a good leaving group which would undergo nucleophilic substitution. These were diazotization, ditosylation and pyridinium salt formation.

2.-Diazotization did not allow the isolation or characterisation any discrete products. This was attributed to the low stability of the carbocation generated under these conditions needed.

3.-Attempts to displace a ditosylamide group lead to elimination (when iodide was the nucleophile) or the monotosylated starting material rather than nucleophilic substitution products. Potential reasons could be either steric hindrance on the approach to a secondary carbon through an S_N2 reaction or low stability of a secondary carbocation on a S_N1 process.

4.-Substitution of the pyridinium moiety by different nucleophiles was unsuccessful. The high temperature conditions required to effect the displacement of 2,4,6-triphenylpyridine **59** did not allow the isolation or identification of the product formed from the cyclopentane moiety, during reaction. Steric hindrance might prevent the approach of the nucleophile and favour mainly elimination rather than substitution.

5.-All the previous results have shown that deamination *via* nucleophilic substitution of an amino-derivative such as a diazonium salt, ditosylamide or a pyridinium salt attached to a secondary carbon, is an unsuitable methodology to follow towards the formation of the analogue of cADPR **2**, and a new synthetic strategy should be developed with that purpose.

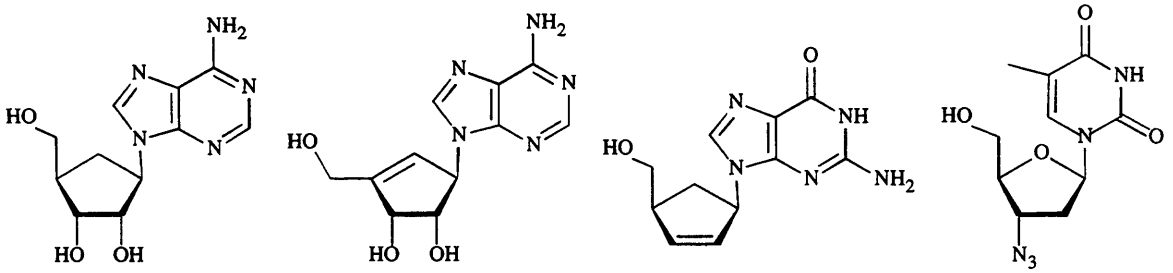
CHAPTER 3

SYNTHESIS OF CARBOCYCLIC ANALOGUES OF DNA & RNA NUCLEOSIDES

3. SYNTHESIS OF CARBOCYCLIC ANALOGUES OF DNA & RNA NUCLEOSIDES

3.1 BIOLOGICAL IMPORTANCE

Carbocyclic analogues of nucleosides are of considerable importance because of their significant antitumor and antiviral activity which is evident from the large amount of work published in this field during the last 10 years.[56] The biological activity must be related to the greatly increased stability of the C-N bond joining the purine or pyrimidine base to the cyclopentane ring making such analogues less susceptible to enzymatic cleavage than the glycosidic bond in the natural nucleosides. In addition to being synthetic targets, carbocyclic nucleosides have also been identified as natural products. Hence, aristeromycin[57] and neplanocin[58] (Scheme 55), two naturally occurring metabolites produced by certain prokaryotic organisms are two of the compounds that have been widely studied. Thus, (-) aristeromycin has been successfully used for the synthesis of Carbovir[59] which together with another chemotherapeutic agent, AZT,[60] showed a potent and selective anti-HIV activity, preventing DNA chain elongation.



Aristeromycin Neplanocin Carbovir AZT

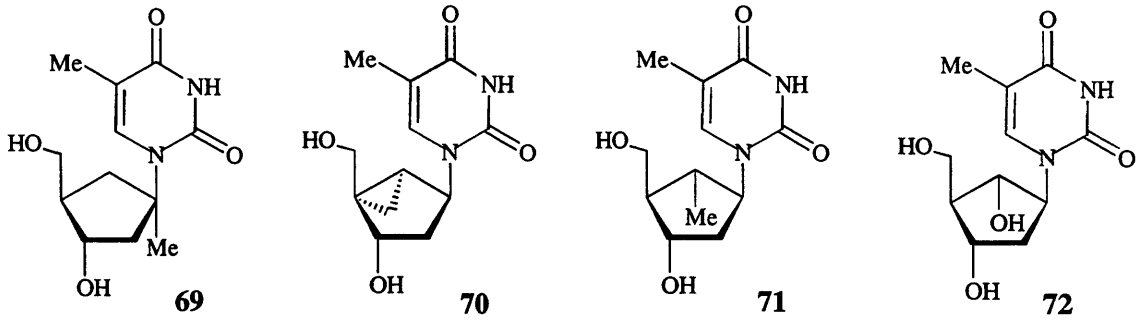
Scheme 55: Some carbocyclic analogues of nucleosides with important properties as antiviral and antineoplastic agents.

AZT has been used as an anti-AIDS agent. Nevertheless, its short life in the body together with some important side effects,[61] led to the development of new potential HIV drugs. Therefore, alterations to the structure to retain biological activity but to increase resistance

to biodegradation have been introduced either in the sugar moiety by varying the size of the ring, the substituents, or even the heterocyclic base.

The nucleotide derivatives themselves are too polar, and in consequence are not capable of penetrating into cells at a rate sufficient to produce an efficient therapeutic effect. Thus, they have to enter the cell as nucleosides and then are converted into the corresponding nucleotides by viral or host enzymes. Strategies focused on this approach include the preparation of 3',5'-cyclic phosphates,[62] phosphodiester derivatives,[63] cyclic phosphodiesters[64] and phosphoramidite derivatives.[65]

But one of the most recent applications of the carbocyclic analogues of nucleosides is their incorporation into short segments of DNA or RNA and the study of the effect on the nucleic acid stability. The synthesis and resulting stability of oligonucleotides bearing carbocyclic analogues, their recognition by the complementary strands and also the susceptibility of those hybrid strands to be cleaved by restriction enzymes, has been reported. For instance, Altmann and Kesselring[66] synthesised compound **69** (Scheme 56). Studies on its effect on DNA/RNA duplex showed that the presence of a methyl group produces a decrease in the duplex stability.



Scheme 56: Some carbocyclic analogues of thymidine incorporated into DNA/RNA strands.

The same authors and co-workers[67] described the increase in the thermodynamic stability of the DNA/RNA heteroduplex by hybridising oligonucleotides with compound **70**. Sági and co-workers,[68] reported on hybridisation and stability properties of oligo (+)-carbocyclic-thymidylates. Among them, $c(dT)_n$ inhibits DNA replication, possibly because this

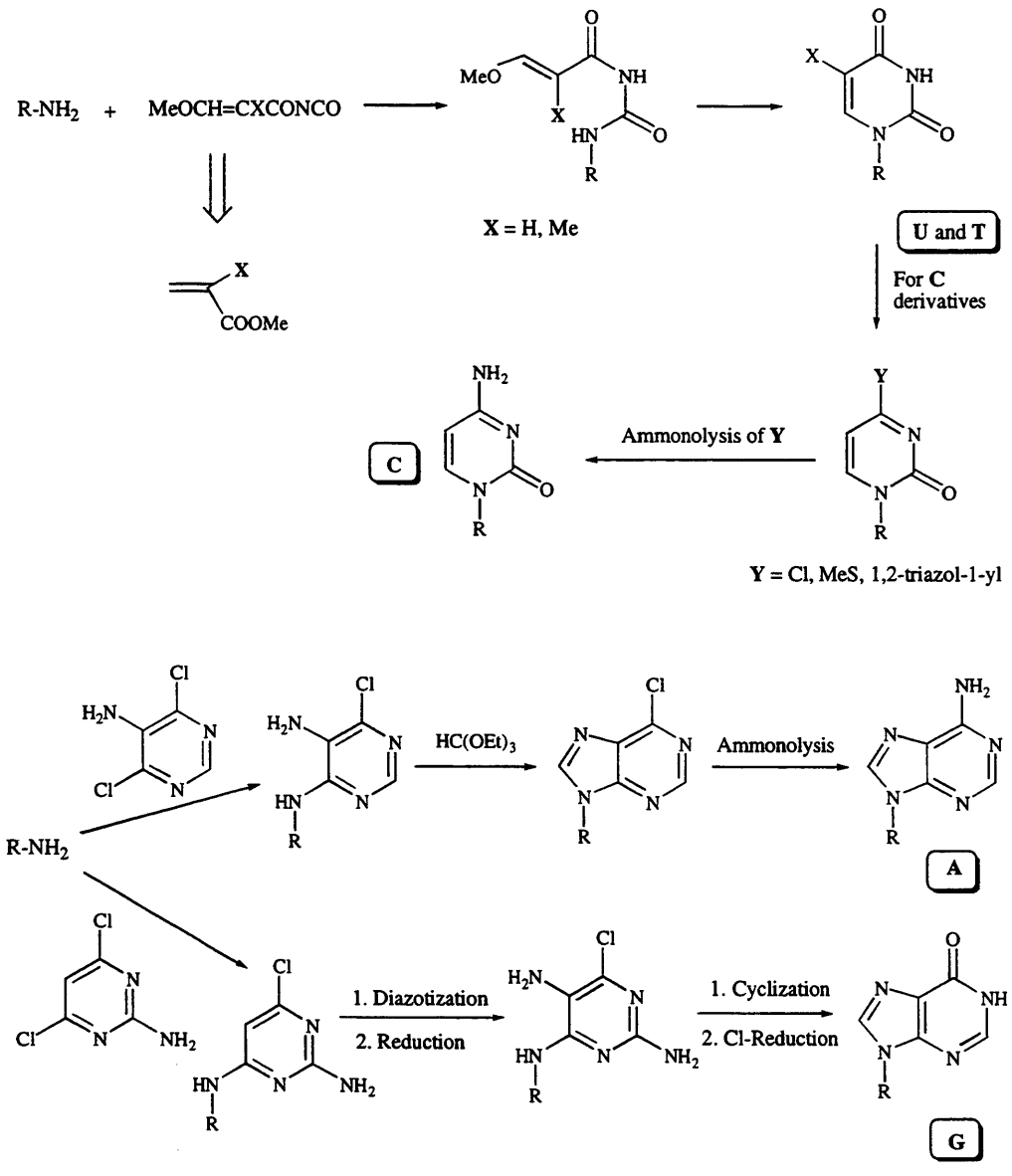
oligonucleotide binds strongly to the polymerase enzyme, therefore, replication fails. Furthermore, $c(dT)_n$ possesses a helical structure in solution of appropriate ionic strength.

Egli[69] described some structural aspects of the Dickerson-Drew self-complementary dodecamer d(CGCGAATTCGCG), containing the carbocyclic nucleotide **70** and more recently the crystal structure of an oligodeoxyribonucleotide containing **71** and **72** respectively, has been reported.[70]

The great interest in modified nucleosides or nucleotides increases the demand for the development of synthetic routes not only towards new analogues, but to improve the reported methodologies leading to existing nucleoside analogues. Biological testing cannot be carried out unless considerable amounts of synthetic nucleosides are available. This has become an important challenge pursued by many chemists.

3.2 STANDARD APPROACHES TOWARDS PURINE AND PYRIMIDINE NUCLEOSIDES

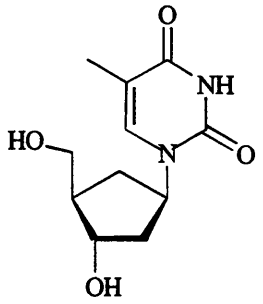
The importance of these analogues led to the development of syntheses with the emphasis on convergent approaches, where the intact heterocyclic base is coupled directly to a suitable funtionalised carbocyclic moiety. Linear approaches have the inconvenience that laborious stepwise construction of each purine or pyrimidine moiety has to be made. Syntheses of pyrimidine (Uridine and Thymidine) derivatives employ methodology developed originally by Shaw and Warrener.[71] The construction of purines (Adenines and Guanines) is based on the Traube synthesis (Scheme 57).



Scheme 57

3.3 PROPOSAL

Based on the huge importance of these substrates and in the urgent need to discover new and efficient synthetic routes towards them, the first aim of this work was focused on the synthesis of the carbocyclic analogue of thymidine **3** (Fig. 20). Once the synthesis was completed, studies of the stability of hybrid DNA containing this carbocyclic nucleotide, were planned.



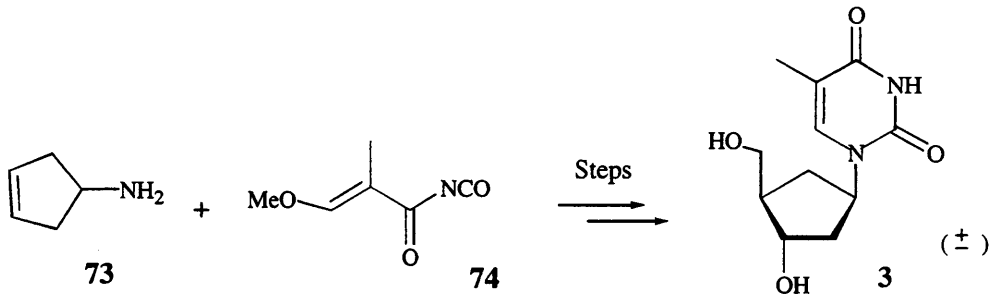
3

Figure 20

3.4 SYNTHESES OF CARBOCYCLIC THYMIDINE 3

3.4.1 BACKGROUND

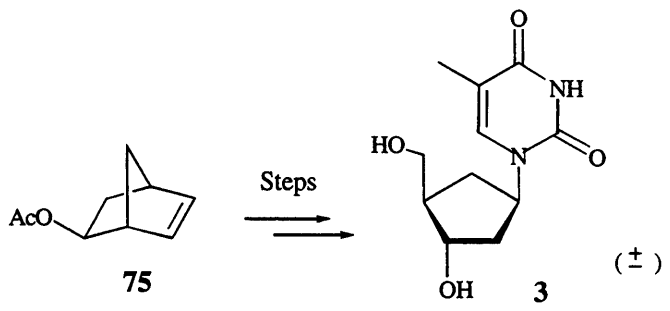
In 1962, Murdock and Angier,[72] claimed to have prepared for first time the carbocyclic analogue of thymidine **3** starting the synthesis from a condensation reaction between 3-cyclopentene amine **73** and the isocyanate **74** (Scheme 58). No antiviral properties were found for the compound claimed by the authors to be **3**.



73 74 3

Scheme 58

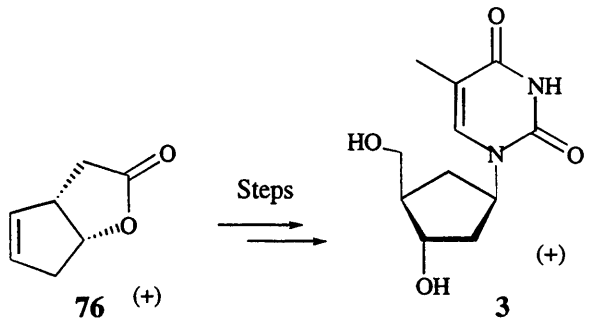
Shealy[73] and co-workers, challenged the claims made by Murdock and Angier. Different comparative studies with their carbocyclic thymidine (prepared from compound **75**) and the product obtained by Murdock and Angier, showed that both compounds were different. Therefore, the synthesis of the real compound was reported for the first time in 1981, according to the scheme below (Scheme 59). A modest but reproducible activity of this compound against leukaemia *in vivo* was announced.



75 3

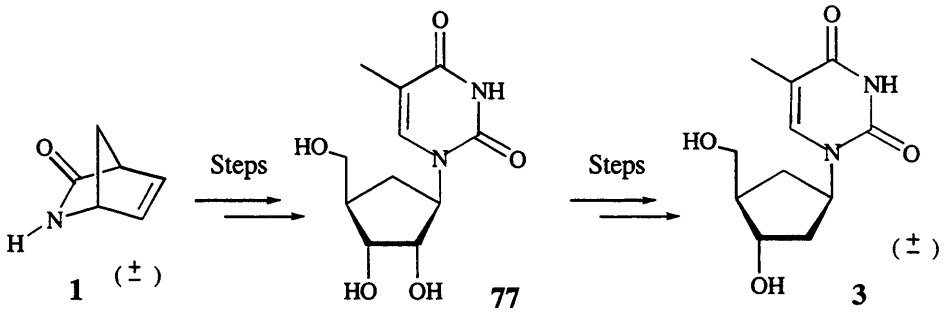
Scheme 59

The enantioselective synthesis of carbocyclic thymidine appeared in 1987 for the first time.[74] It was prepared from the lactone **76** (Scheme 60) and the antiviral properties of this carbocyclic nucleoside were reported, too.



Scheme 60

In 1991, a new synthetic strategy was decribed for racemic **3** (Scheme 61).[75] The intermediate **77** was prepared from **1** followed by further steps to effect deoxygenation.

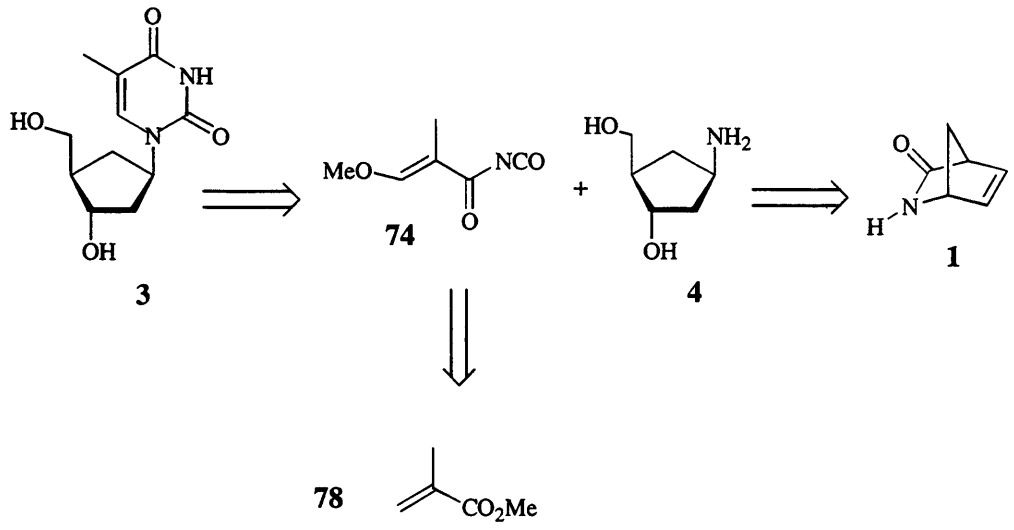


Scheme 61

In conclusion, the biological importance of this substrate encouraged further investigations with the aim of discovering flexible and shorter synthetic pathways than those previously developed.

3.4.2 GENERAL APPROACH

The general strategy planned towards the preparation of our final target molecule **3** is shown below (Scheme 62). Thus, the carbocyclic deoxyribose analogue **4** could be made from lactam **1**, and **74** from methyl methacrylate **78**, both compounds being commercially available. A coupling reaction between **4** and the isocyanate **74** would be performed for building the heterocyclic base moiety, according to the methodology developed by Shaw and Warrener.[76]



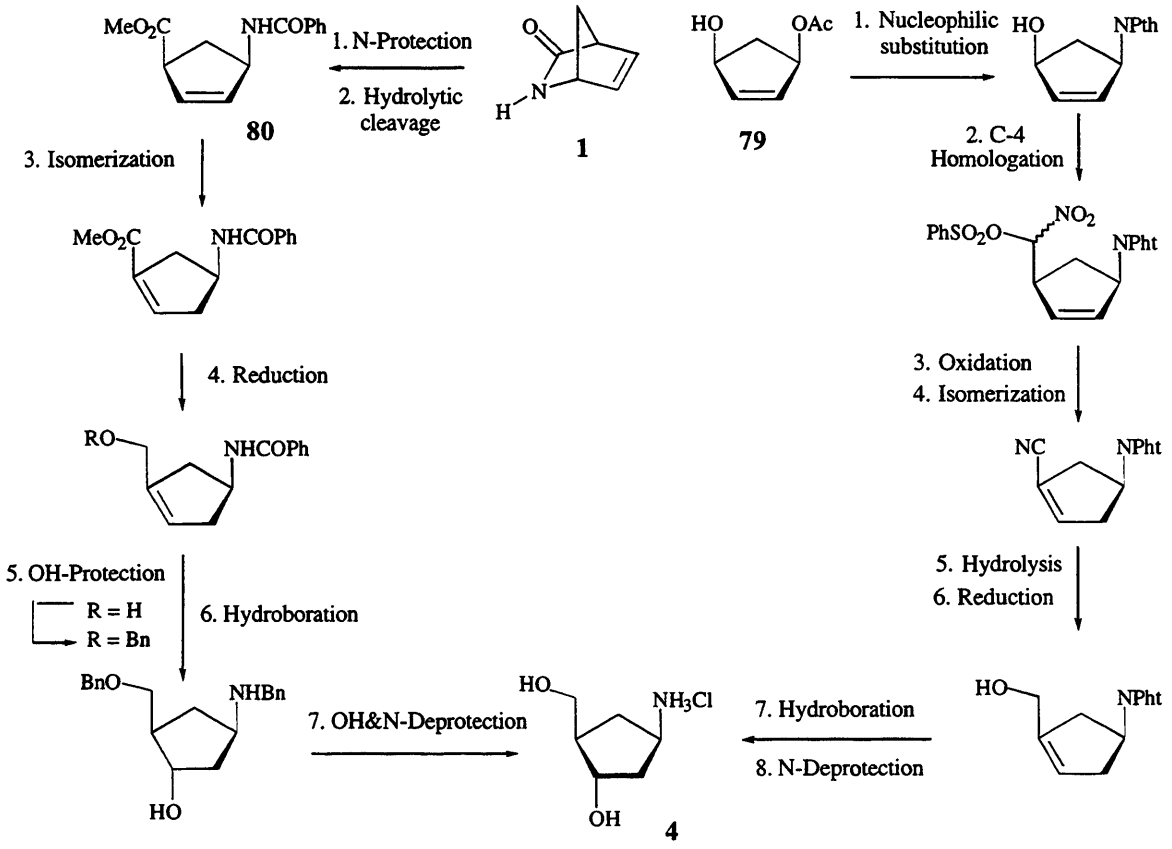
Scheme 62

3.4.3 SYNTHESES OF CARBOCYCLIC DEOXYRIBOSE 4

3.4.3.1 BACKGROUND

The preparation of carbocyclic deoxyribose **4** was found in the literature *via* 8 steps[77] or 13 steps,[51a] as a key intermediate towards the synthesis of carbocyclic thymidine. Two different approaches have been recently reported from the homochiral lactam **1** and the cyclopentene **79**[78] (Scheme 63). Some considerable improvements to the former method have been

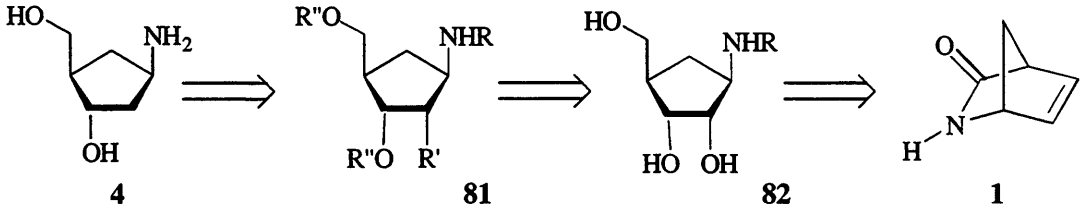
achieved since the synthetic route reported in this Chapter is considerably shorter with greater regio and stereochemical control.



Scheme 63

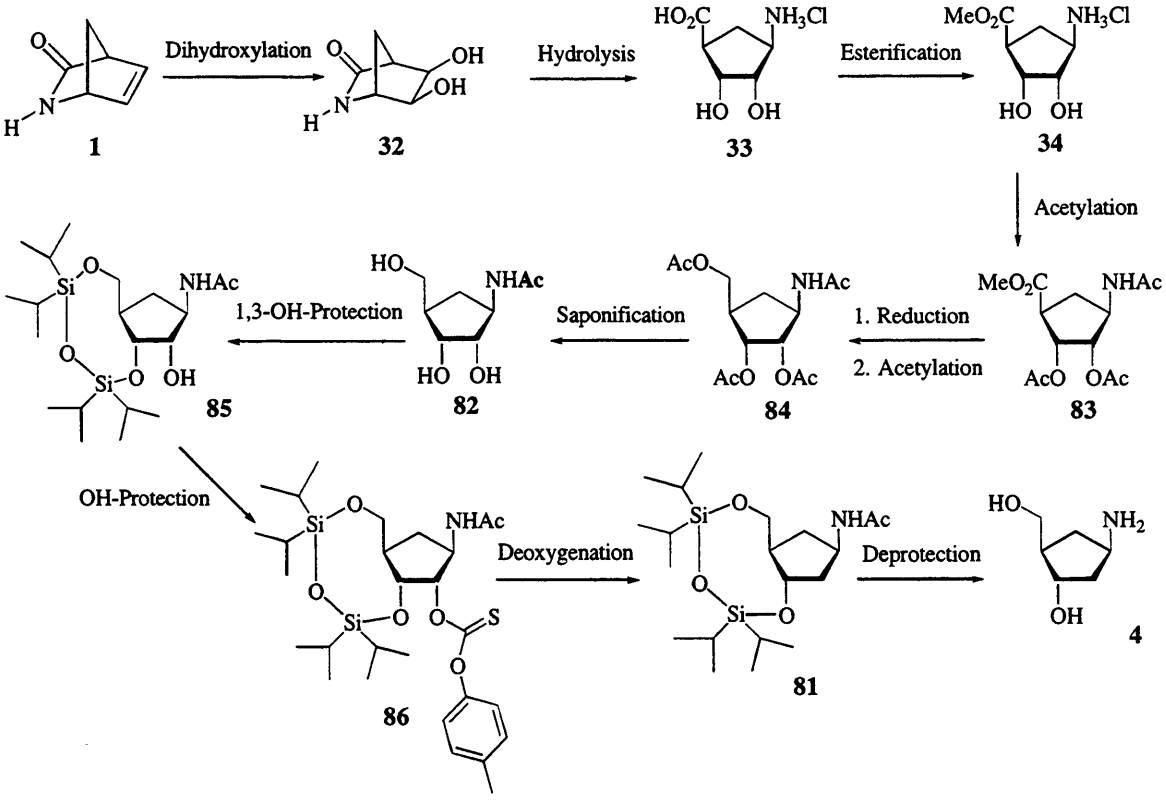
3.4.3.2 INITIAL APPROACH

The synthesis of **4** could be made *via* formation of the carbocyclic ribose derivative **82** (Scheme 64). Selective protection of the primary hydroxyl group and the adjacent secondary hydroxyl group would give **81**; further deoxygenation, would give the desired carbocyclic deoxyribose **4**.



Scheme 64

The bicyclic lactam **1** was proposed as the starting point of this route due to its defined and convenient stereochemistry. The synthetic pathway planned and undertaken is shown (Scheme 65). Attempts to complete the synthesis, after the preparation of compound **82**, were unsuccessful and a short and new methodology was later designed and will be discussed (Section 3.4.3.3).

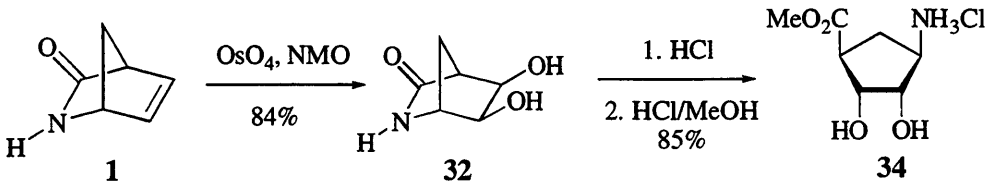


Scheme 65

3.4.3.2.1 RESULTS

The conversion of the racemic lactam **1** into the ribose derivative **82** has been described by Oppenheimer[39] but modifications to that methodology were made. One of them was the formation of **32** from lactam **1** already discussed (Section 2.6.3.1). Some others will be mentioned in the present section.

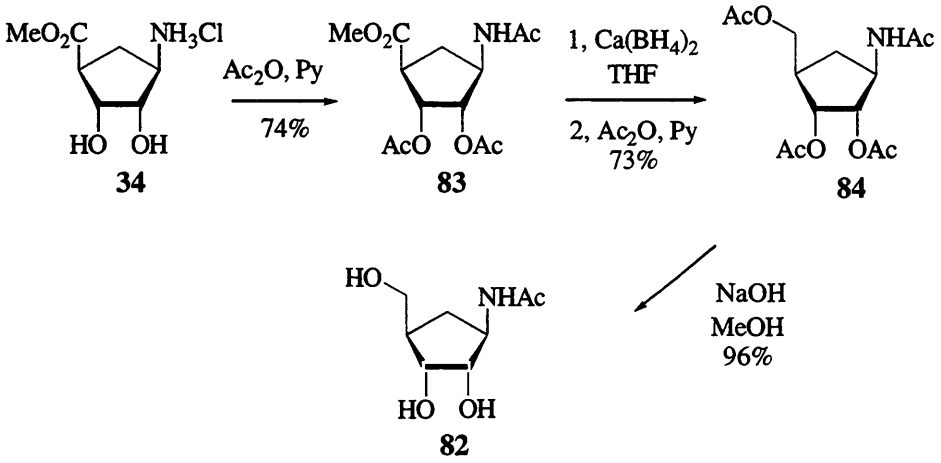
Preparation of **34** (Scheme 66) was undertaken in three steps as described in Section 2.6.3.1, with the difference that the preparation of the intermediate *exo*-diol lactam **32** was carried out using OsO_4 as the dihydroxylating agent according to the method of Cermark and Vince.[40]



Scheme 66

$KMnO_4$, previously used for this conversion, was substituted by OsO_4 mainly for two reasons: a) it affords a higher yield and b) a very straightforward experimental procedure is needed. *N*-Methyl morpholine oxide (NMO) acts as co-oxidant, regenerating the reduced osmium back to Os(VIII), in order to proceed with the oxidation. When the reaction was completed, sodium metabisulphite ($Na_2S_2O_5$) was added to reduce the osmium species in solution which could then be removed *via* filtration. This procedure afforded the *exo*-diol **32** in 84% yield. The preparation of **34** was carried out from **32** following the same conditions described in Section 2.6.3.1, without purification of the intermediate **28** generated.

The synthesis of **82** was described by Oppenheimer *via* the intermediate **84**.[39] Some attempts performed in our laboratories for the preparation of **84**, by reduction of the ester **34** with lithium superhydride and further acetylation, gave very poor yields of the expected product. Hence, the procedure followed was the one developed by Cermark[40] in a three steps sequence (Scheme 67).



Scheme 67

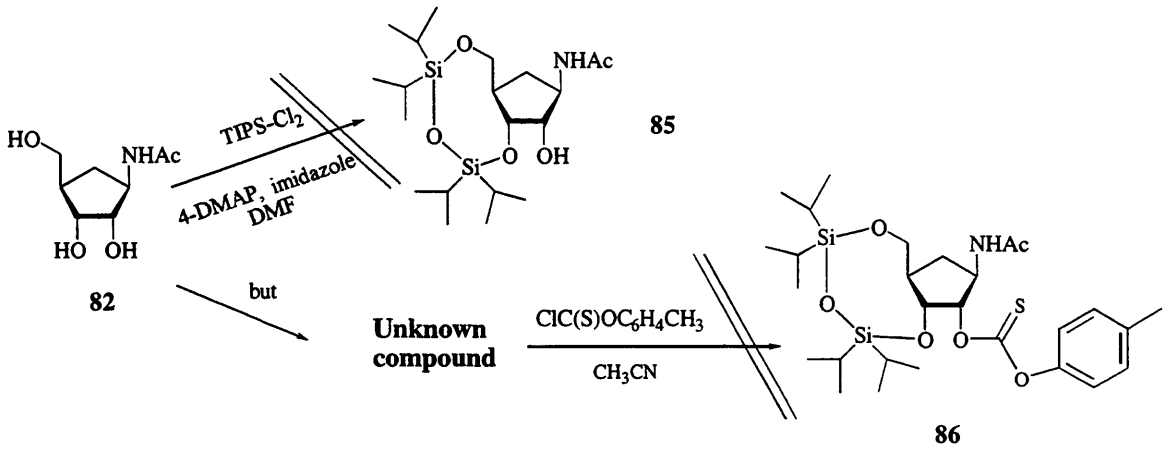
Thus, treatment of **34** with acetic anhydride yielded compound **83** in 74%. Reaction of that substrate with calcium borohydride (previously prepared by reaction of calcium chloride and sodium borohydride) followed treatment of the crude product with acetic anhydride. This afforded compound **84** in 73% yield from **83**.[40]

Sodium hydroxide 1.5 M in methanol, was used to deprotect the acetylated alcohols and gave compound **82** in 96% yield. The saponification was complete in two hours at room temperature but all the attempts to isolate the product into an organic solvent were unsuccessful, due to its high polarity. Thus, evaporation of the volatile components, followed by purification by column chromatography was found to be a more convenient method for isolation of **82**.

Selective protection of the 1,3 diol in **82** was attempted with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (TIPS-Cl_2). This protecting group was reported in syntheses of furanose nucleosides, for the protection of the 3',5'-dihydroxyl group.[79] Hence, compound **82** was dissolved in dry DMF and treated with imidazole and 4-DMAP followed by addition of the silyl protecting agent. After reaction for six hours, the solvent was evaporated and the crude product was purified by column chromatography. That afforded a pure compound in 65% yield. The ^{1}H NMR spectrum seemed to correspond to the expected product **85** (Scheme 68) but the integration for the isopropyl groups (0.9-1.2 ppm), was much higher than expected. Moreover, this was also supported by the ^{13}C NMR spectrum, which presented six different *C*H isopropyl (15.9-16.5 ppm) and six different (*C*H_3)$_2$-CH carbons (12.6-11.7 ppm). Furthermore, the accurate mass of this compound could not be determined however it showed fragmentation peaks much higher than the molecular weight

of **85**. It seemed likely that over silylation had occureed, although the exact structure of this product was not determined.

Several attempts to confirm this suggestion were made by attempting reaction of the compound previously obtained, with *p*-tolyl chlorothionoformate in acetonitrile in order to obtain compound **86**. The lack of reaction supported the suggestion that the 2'-OH was already derivatised.



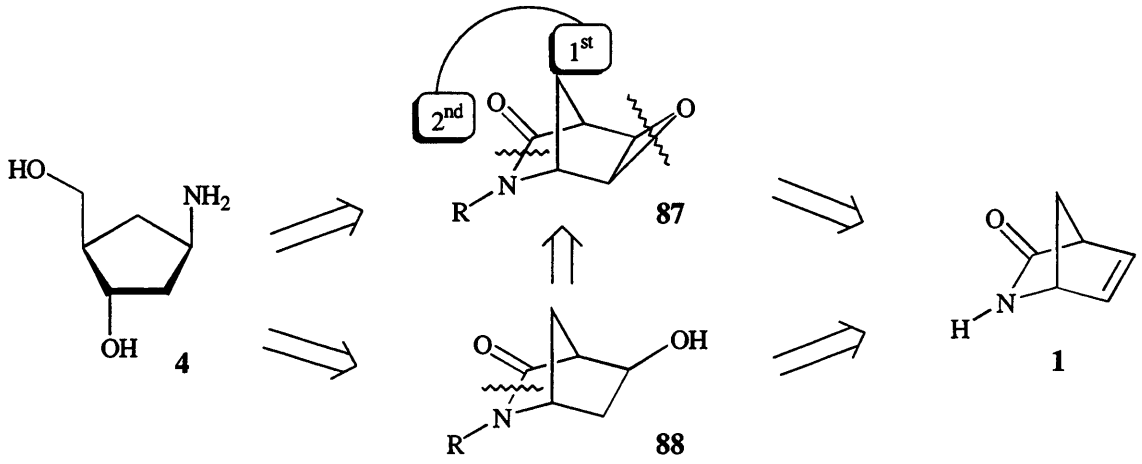
Scheme 68

Considering the importance of achieving successful protection towards the synthesis of compound **4** and the difficulty of handling very polar intermediates, the first synthetic approach (Scheme 65) was abandoned in favour of more efficient strategies.

3.4.3.3 SECOND APPROACH

Two new synthetic routes have been investigated towards the synthesis of the carbocyclic deoxyribose analogue **4** which had in common the use of **1** as source of chirality (Scheme 69). The first one involved formation of a compound **87** which presents apart from the protected nitrogen of the amide, an *exo*-epoxide as new functional groups. That could be the first key intermediate for an innovative synthesis towards the sugar analogue **4**. A regioselective reduction of the epoxide could be attempted at this stage or even a reductive-cleavage of the lactam. Breaking the C-N bond without affecting the epoxide was

considered rather impossible under the acidic conditions described by Oppenheimer.[39] Reductive-cleavage of a secondary lactam would not take place since the carbonyl group has got a higher character of amide rather than ketone. It was reasoned, that this problem might be solved if the nitrogen of the lactam was protected with a "R" group which reduces the participation of the lone pair of electrons on nitrogen in resonance with the carbonyl. This may increase the electrophilic character of the carbonyl towards nucleophilic attack, giving a product which might easily be converted into **4**.



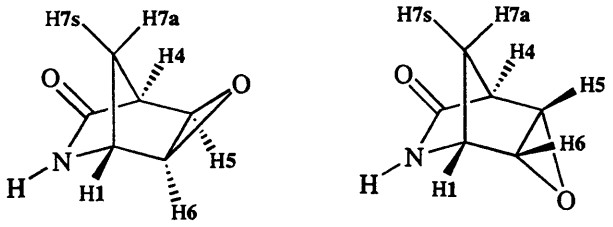
Scheme 69

The second route could be shorter. Thus, **4** might be prepared from compound **88** *via* reductive cleavage or hydrolysis of the lactam. Moreover, **88** may be a product of hydroboration on the olefin in the bicyclic lactam **1** or a product of reduction of the epoxide in **87**.

3.4.3.3.1 EPOXIDATION OF 2-AZABICYCLO[2.2.1]HEPT-5-EN-3-ONE 1 AND DERIVATIVES

Legraverend and Bisagni,[80] claimed that epoxidation of the lactam **1** with oxone (potassium peroxymonosulfate) $2KHSO_5.KHSO_4.K_2SO_4$ as epoxidising agent gives exclusively **89** (Fig. 21), as result of an attack on the top face. The same authors also reported that, when the epoxidation was carried out using MCPBA, yields of the expected compound never exceeded 15%.

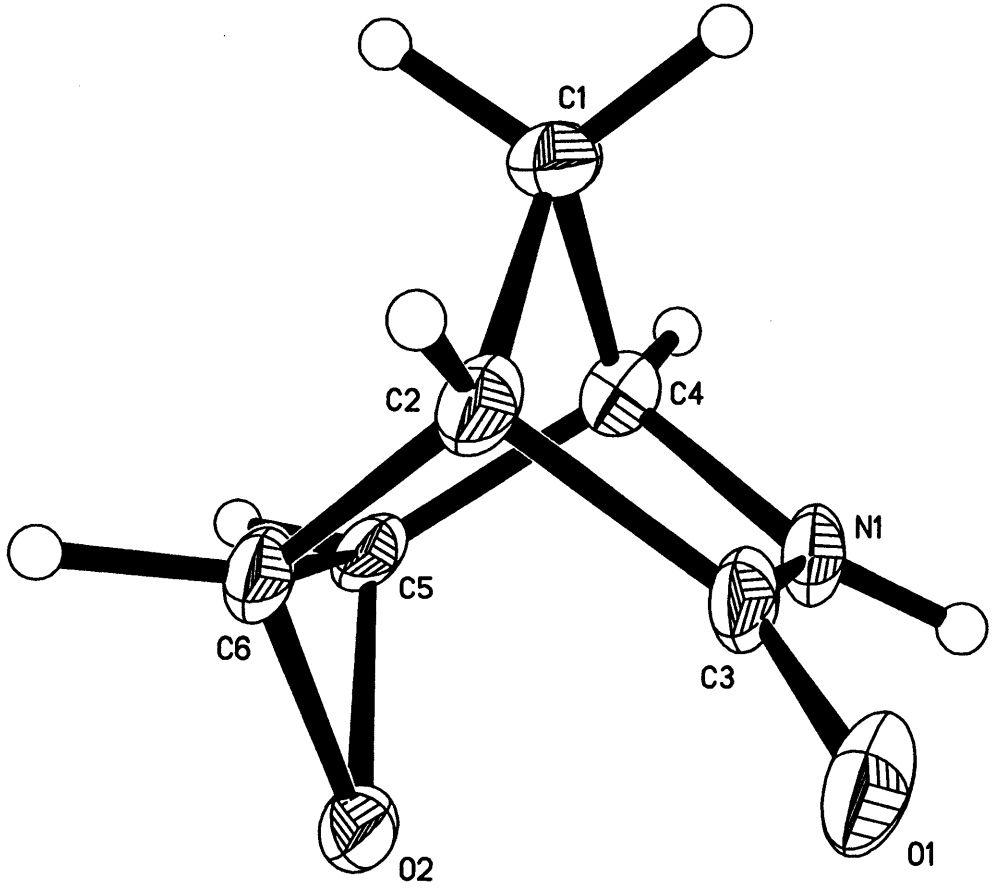
Their experimental conditions were reproduced in this work. Hence, slow co-addition of a solution of KOH (1 M) with the oxone solution in water, to the starting material in MeOH:H_2O, was required in order to maintain a neutral pH and to avoid the opening of the epoxide. Thus, the reaction with oxone described as giving exclusively the *exo*-epoxide **89** in 80% yield, in our experience yields never exceed 45% but more importantly, the product was a mixture of epoxides *exo-endo* **89** and **90**, in a ratio 3:1 respectively, contrary to the literature claims.



89 *exo* **90** *endo*

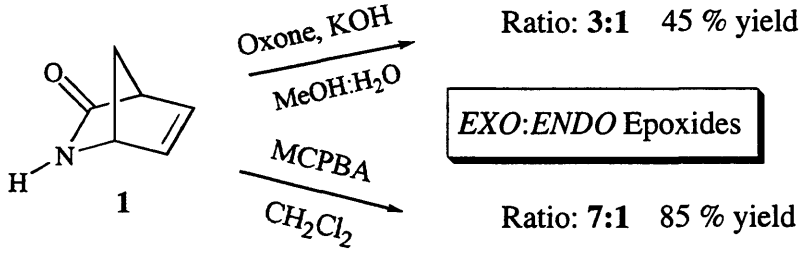
Figure 21

NMR spectroscopy clearly allowed these to be distinguished and confirms their stereochemistry. Thus, on a ^1H NMR study, for the *exo* epoxide, protons H-5 and H-6 were upfield from those of the *endo* epoxide. Moreover, the coupling constant between the protons of the *exo* isomer was 3.7 Hz, showing a doublet for each proton whereas for the *exo* epoxide, these protons appeared as a multiplet. Furthermore, a characteristic coupling constant of 3.7 Hz for the bridgehead H-1 with H-6, was observed for the *endo* isomer whereas there was no coupling between the bridgehead protons and the H-5 and H-6 of the *exo*-epoxide which confirms a dihedral angle of approximately 90°. In addition, the chemical shift of the H-7s proton of the *endo* isomer was downfield from that of the H-7a proton. Nevertheless, the position of the H-7s and H-7a proton signal was reversed in the *exo*-isomer. All these results were in agreement with the ^1H NMR experiments in norbornenes published by Shealy and Clayton.[81] A crystallographic structure of the *endo* epoxide (X-ray structure 1) and further experiments carried out, showed that both stereoisomers **89** and **90** had been obtained.



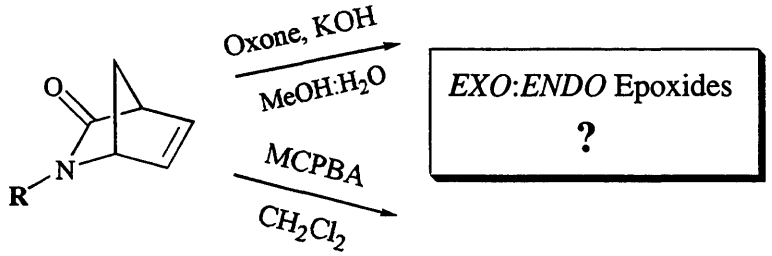
X-Ray structure 1. Compound **90**

It was not clear why epoxidation with MCPBA was reported to be unsuccessful and it was appropriate to reinvestigate this. After several attempts to optimise the reaction conditions as well as the work-up, the best results were obtained with two equivalents of MCPBA per equivalent of starting material. The reaction is rather slow and it should be monitored by ^1H NMR spectroscopy rather than TLC since the starting material **1** appeared to have the same R_f as the *exo*-isomer. After 58 hours the reaction appeared to be complete and the ^1H NMR spectrum showed a ratio *exo-endo* isomers 7:1 (Scheme 70). Extraction of the products with CH_2Cl_2 was not efficient and most of the *endo* epoxide remained in solution. Thus, the best procedure to isolate both products, consisted of the evaporation of the volatile components, followed by purification of the residue by flash chromatography. This afforded 85% of the mixture *exo-endo* stereoisomers.



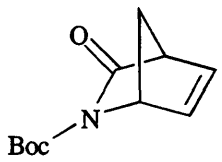
Scheme 70

Alternative epoxidations were investigated to determine the effect that the substitution of the N-H by a suitable protecting group **R** (Scheme 71), would have on the stereoselectivity.



Scheme 71

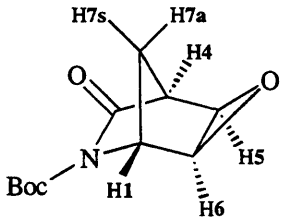
Such a protecting group may also be useful if a further reduction of the lactam is to be carried out, according to Scheme 69. *N*-Protection may also improve the organic solubility of the intermediates. Thus, *tert*-butoxycarbonyl group was chosen as a suitable protecting group. Protection of the nitrogen in the lactam **1** was performed in dichloromethane with di-*tert*-butyl dicarbonate [$(Boc)_2O$], using triethylamine as base and 4-DMAP as catalyst. On complete reaction, after removal of the solvent, purification of the crude product by column chromatography afforded **91** in 85% yield (Fig. 22).[82]



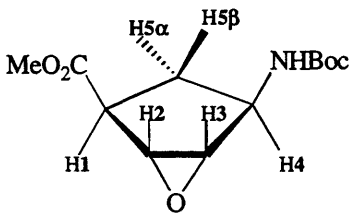
91

Figure 22

Epoxidation of **91** was now attempted using oxone and MCPBA, respectively. Reaction with oxone was carried out following an analogous procedure to that used for the lactam **1**. After purification, two products were isolated and identified as **92** as product of oxidation (85% yield) and **93** as result of participation of the solvent to open the lactam (Fig 23).



92



93

Figure 23

A detailed spectroscopic study allowed the characterisation of both compounds. Thus, the ^1H NMR spectrum for **92**, showed a coupling constant of 3.5 Hz between protons H-5&6. No coupling was observed between H-6&1 and H-5&4 which is consistent with a dihedral angle of 90 ° which would correspond to the formation of the *exo*-isomer. The *endo*-

epoxide would have given a coupling constant to the bridge protons in the order of 3.7 Hz, as was seen for compound **90**. A *W* coupling characteristic of this bicyclic system, was also observed between H-7s&5,6.

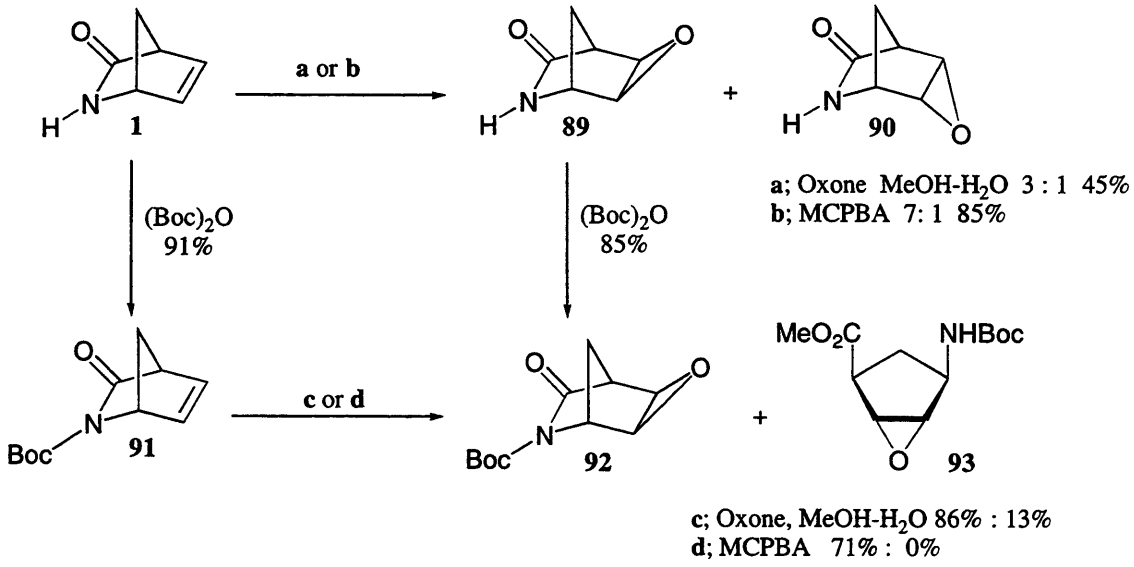
The ^{1}H NMR spectrum for compound **93**, showed a singlet for the OMe at 3.75 ppm. Two signals coupled to each other (*J* 2.7 Hz) and centered at 3.70 and 3.55 ppm, corresponded to H-2 and H-3 respectively. The N-H proton appeared at 4.91 ppm and showed a coupling constant of 7.7 Hz with a proton identified as H-4. In addition H-1 presented a small coupling of 1.5 Hz with H-2 whereas no coupling was observed between H-3&4 which corresponded to a dihedral angle of 90°, which was also observed with other compounds derived from this.

Reaction of **91** with MCPBA, was also monitored by ^{1}H NMR. Despite a slow reaction rate, formation of a single compound was detected. The work-up was performed by addition of $Na_2S_2O_5$ to reduce the excess of MCPBA added, followed by purification of the crude solid obtained by extraction to give compound **92** in 71% yield.

According to these results, it was obvious that the rigid shape of the bicyclic lactam allows the attack of oxone on the top and on the bottom face, and that may be attributed to the small size of this reagent. The reason why, only attack on the top face took place using MCPBA, is presumably due to steric hindrance. An important steric effect on the approach to the bottom face between the Boc group and MCPBA might take place and this would favour the formation of the *exo*-epoxide.

An alternative method to prepare **92** was by treatment of compound **89** with $(Boc)_2O$ (Scheme 72), under similar experimental conditions to the ones used for the formation of **91**. This procedure yielded **92** in 85%, with spectroscopic data that agreed with the data obtained previously.

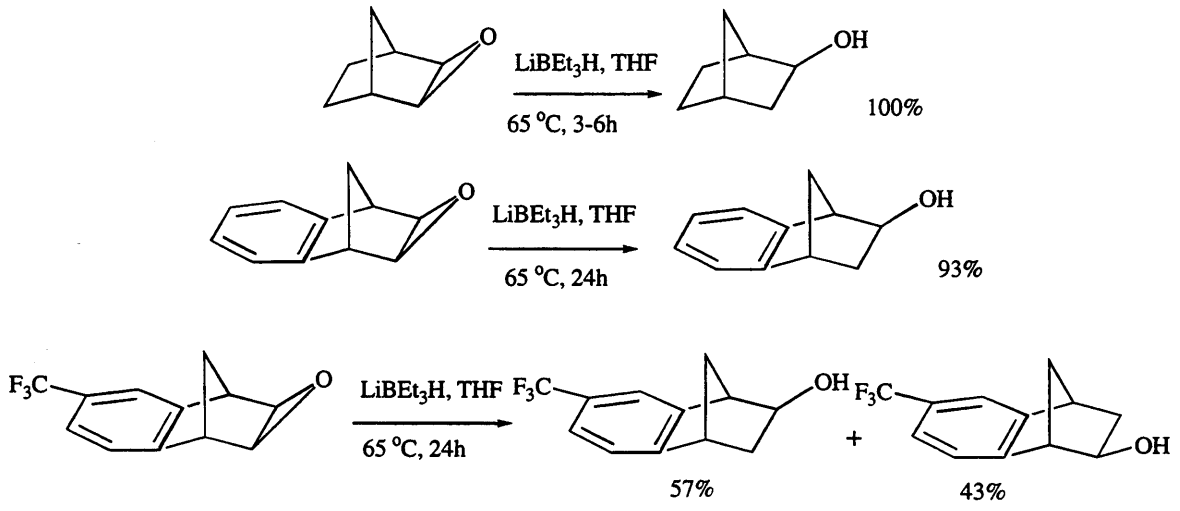
In conclusion, it can be deduced that the most convenient route towards the preparation of compound **92** involved protection of the lactam to give **91** followed by treatment with MCPBA as epoxidising agent (Scheme 72).



Scheme 72: General results obtained using oxone and MCPBA as epoxidising agents.

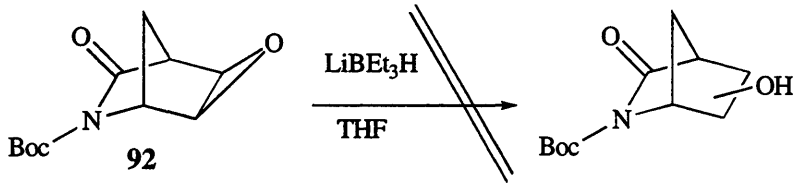
3.4.3.3.2 ATTEMPTS TO REDUCE THE *EXO*-EPOXIDE 92

Reduction of bicyclic epoxides such as norbornanes, which are often, relatively labile to rearrangements have been reported, using "superhydride" ($LiBEt_3H$).[83] This reducing agent showed high *regio* and *stereo* selectivity but also good yields of the expected products (Scheme 73).



Scheme 73

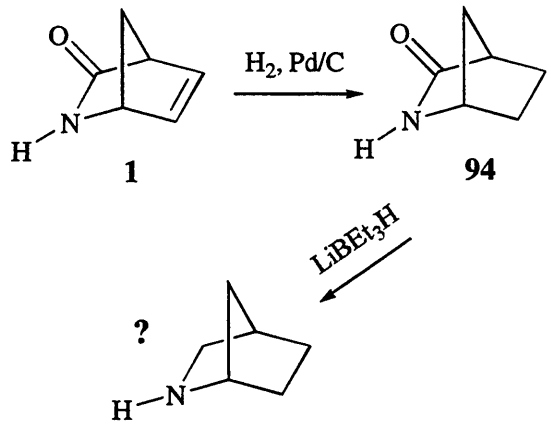
Considering some structural similarities between **92** and these compounds reported, some experiments were undertaken with this reagent. Thus, **92** was dissolved in THF and treated with 1.1 equivalents of superhydride (1 M solution) in THF (Scheme 74). After stirring at room temperature for 14 hours a reaction had taken place since a TLC analysis did not show any starting material, and an egg-shaped spot near the base line was clearly detected. Oxidative work-up with NaOH (3 M) followed a treatment with hydrogen peroxide, was carried out and analysis of the extracted product by ^{1}H NMR spectroscopy, showed peaks at high field which suggested the presence of borane complexes. Attempts to remove these and to isolate any organic material by meaning of stronger work-up conditions (NaOH, 3 or 6 M; H_2O_2 or $NaBO_3.H_2O$) or by chromatography, were unsuccessful.



Scheme 74

Brown and Kim,[84] claimed that tertiary amides undergo C-N bond fusion on treatment with $LiBEt_3H$, to give an alcohol as the reaction product *via* formation of the aldehyde as an intermediate. The basic conditions of reaction might also lead to the condensation of this aldehyde. Thus, it was considered of interest to study the stability of the carbonyl group of the lactam **1** by itself, towards reaction with superhydride. With this purpose the double bond of **1** was first hydrogenated,[85] obtaining the bicyclic lactam **94** (Scheme 75).

Compound **94** was then treated with $LiEt_3BH$ under the same experimental conditions previously described. After work-up, a very small amount of product was isolated by extraction with organic solvent apart from some starting material. This was consistent with superhydride effecting the reduction of the carbonyl group with a consequent formation of the amine salt derivative after the work-up.

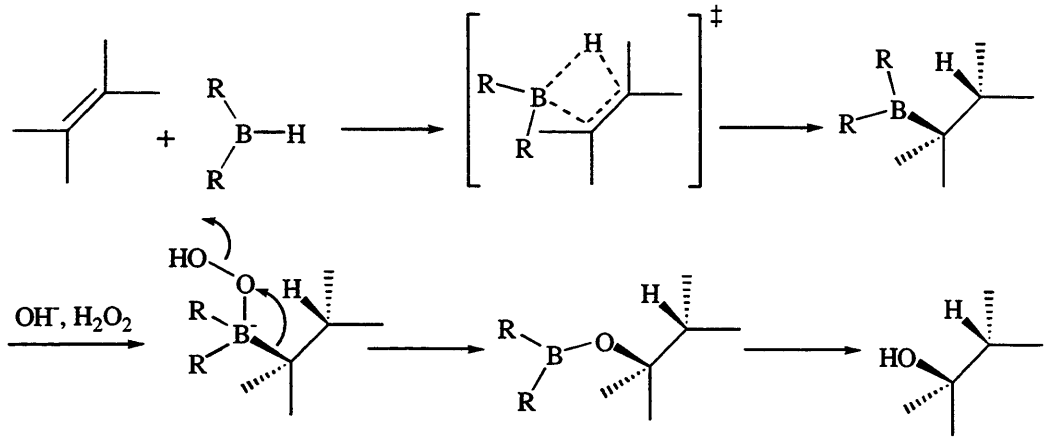


Scheme 75

This would provide an explanation for the problems encountered with substrate **92**. The presence of the Boc group would have increased the electrophilicity of the carbonyl group and therefore made the reduction even more facile.

3.4.3.3.3 HYDROBORATION OF THE LACTAM 91

Hydroboration is one of the most important addition reactions to an alkene. It proceeds *via* a two step sequence, hydroboration plus oxidation of the borane product, the net effect being the hydration of the double bond (Scheme 76). The transition state of the approach undergoes addition of the boron to the less substituted carbon in asymmetric alkenes, which favoured the partial positive charge on the most substituted carbon. Moreover, the C-H and the C-B bonds are formed simultaneously from the same face of the alkene in a *syn* addition. During the oxidation step, the boron is replaced by an hydroxyl group with retention of configuration.

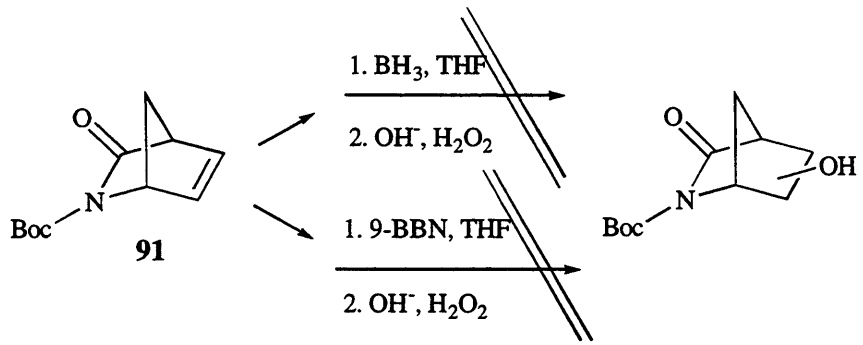


Scheme 76: General mechanism for the hydroboration of an alkene.

It was found that amides and lactams are reduced rapidly and quantitatively by excess of borane in THF.[86] The idea was to study the reactivity of compound **91** with some borane reagents at ambient and low temperatures (Scheme 77).

Several attempts were made treating **91** with a solution of BH_3 in THF, from 0 °C to room temperature followed by the conventional oxidative work-up with NaOH (3 M) and H_2O_2 30%. The result observed was the complete reaction of the starting material but extraction of any organic compound into the organic layer was unsuccessful.

Further experiments were undertaken by treatment of **91** with 9-BBN in THF. A similar result to the use of BH_3 was observed. Reaction had taken place at room temperature, but after work-up only some traces of borane complexes were observed by ^1H NMR spectroscopy.



Scheme 77

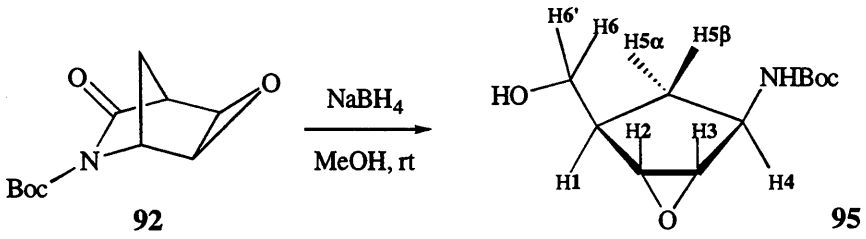
Considering the sensitivity of the carbonyl group in this bicyclic compound to be reduced, the hydroboration was abandoned an a new strategy was devised.

3.4.3.3.4 REDUCTIVE CLEAVAGE OF LACTAM 92

It is well known that $NaBH_4$ is a good reducing reagent for aldehydes, ketones and many other functional groups in the presence of species such as metals.[87] The reduction of tertiary amides does not proceed in alcoholic solvents, secondary amides are inert whereas primary amides are dehydrated to nitriles. Tertiary lactams are expected to undergo reduction to the corresponding cyclic amines. In addition, $NaBH_4$ is considered a mild reducing agent with epoxides.

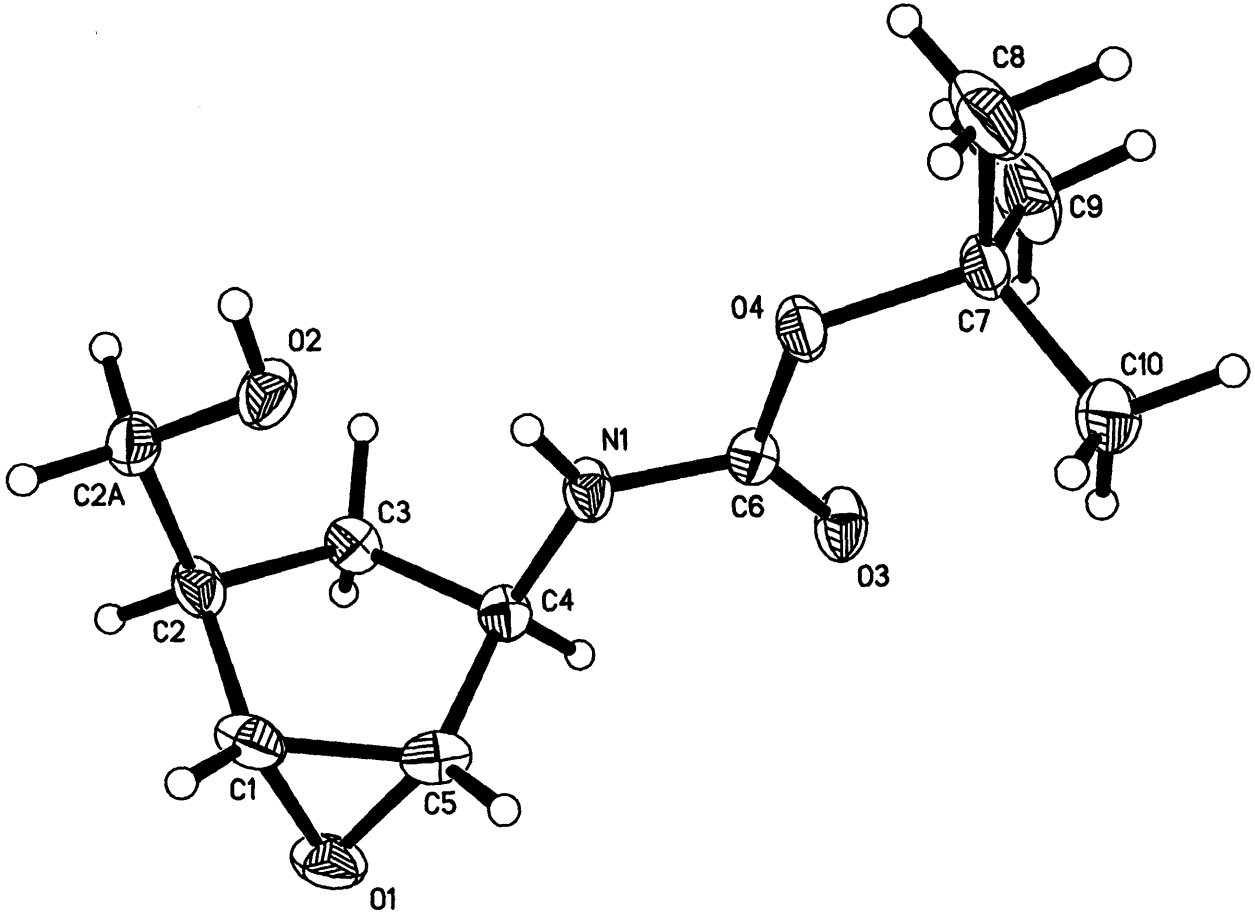
Reducing reagents such as $LiAlH_4$, BH_3 or $LiBEt_3H$, undergo epoxide ring opening under relatively mild conditions. The difficulty remains that amides can be reduced to the corresponding amines, also.

Thus the reactivity of **92** with $NaBH_4$ in methanol was examined as a function of temperature. At 0 °C reaction was complete in almost one hour. TLC analysis showed just one spot with a lower R_f. Characterisation of the product and a crystal structure (X-ray structure 2) showed that what actually had taken place, was the reductive cleavage of the lactam yielding 85% of a compound identified as **95** (Scheme 78).



92 95

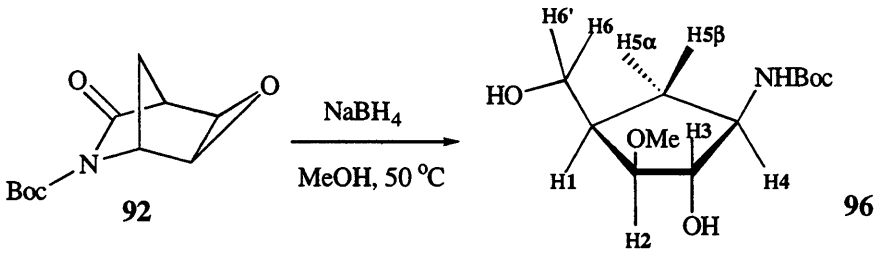
Scheme 78



X-Ray structure 2. Compound **95**

The ^{13}C NMR gave a new CH_2 peak at 63.4 ppm for C-6 and disappearance of the carbonyl group which confirms its reduction. ^1H NMR presented a double doublet for these hydrogens at 3.87 and 3.65 ppm with a geminal coupling of 10.3 Hz. These H-6 protons showed a coupling of 2.0 and 2.4 Hz respectively, with H-1. The chemical shifts of H-2 and H-3 are very close and they appear as an apparent singlet. ^1H COSY spectrum, revealed no coupling between H-1&2 or H-3&4, which corresponded to a dihedral angle of 90 ° between these protons according to the Karplus equation.

Further experiments with substrate **92** at higher temperature were undertaken. Thus, after addition of the hydride at 0 °C the mixture was slowly warmed until 50 °C and then this temperature was held for 16 hours. The first product detected by TLC at room temperature was formation of **95** as previously described. After 15 minutes at 50 °C, TLC showed formation of a new, more polar compound. Acidic work-up and extraction, gave a compound which after complete characterization corresponded to **96** (Scheme 79). Therefore, in addition to a reductive cleavage of the lactam, the epoxide had been regioselectively opened by methanol with complete conversion to **96** in 85% yield.



Scheme 79

The 2-dimensional ^1H NMR spectrum clearly showed that the product obtained had the structure illustrated. The most important evidence of this configuration was the NOEs between H-3 and N-H and between H-5α and H-2.

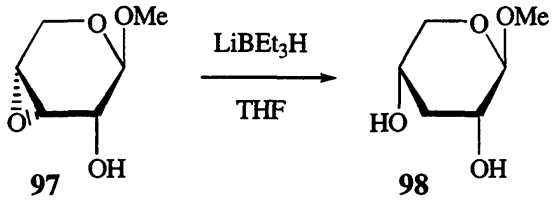
Steric hindrance by the Boc group might direct nucleophilic attack on the epoxide under the basic conditions exclusively to the C-2 of the epoxide to give **96** as a sole product.

3.4.3.3.5 REDUCTION OF THE EPOXIDE 95

3.4.3.3.5.1 USE OF $LiBEt_3H$

The range of suitable reagents to reduce the epoxide **95** was broader than for the bicyclic substrates containing an epoxide as functional group. The problems of reduction of the amide into the corresponding amine no longer exist for **95**, since the carbamoyl moiety was quite stable to reductive conditions. Nevertheless, the control of the regioselectivity of the reductive ring opening of the epoxide remains to be explored.

The first reagent used to test the regioselectivity of the reduction was "superhydride" $LiBEt_3H$, since it was claimed to be a powerful reducing reagent with epoxides as discussed previously (Section 3.4.3.3.2). Moreover, a good example of its application was found in the literature[88] (Scheme 80).



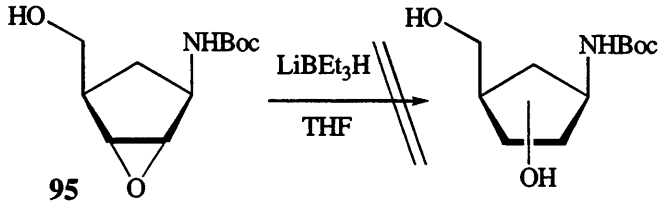
97 98

Scheme 80

On compound **97**, the regiochemistry of the reduction of the epoxide was controlled by the OH-group to give compound **98**. Some similarities between **97** and **95** and the curiosity to find out if a regioselective reduction could also be achieved in our substrate, led us to some experiments with "superhydride". If those results were reproduced, it might be a good method to obtain 3'-deoxyribose analogues.

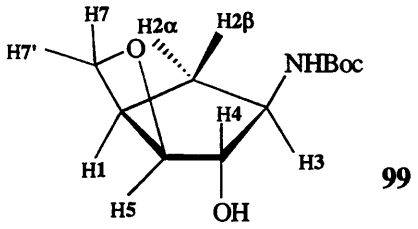
After reaction of **95** with $LiBEt_3H$, a colourless oil was isolated and fully characterised. Mass spectroscopy (FAB), gave a molecular weight of 230 units for a molecular formula $C_{11}H_{20}NO_4$, only one unit higher than the starting material. If reduction had taken place, the molecular weight would have increased by two units. ^{13}C NMR showed the same number of C, CH, CH_2, and CH_3 as for the starting material but at different chemical shifts, which showed that reaction did not proceed as expected. Thus, 4 CH, 2 CH_2, 3 CH_3 and 1

quaternary carbon were observed, with the carbonyl group still present. The presence of 4 C-H clearly showed that reduction had not taken place but an unusual reaction, giving a product whose spectroscopic data differed from the starting material **95** (Scheme 81).



Scheme 81

Infrared spectrum showed characteristic peaks at 3050 cm^{-1} for the N-H and 1700 cm^{-1} for the C=O. ^1H NMR spectroscopy showed integration for 19 protons, similar to the number of hydrogens in the starting material. A singlet at 1.45 ppm integrated for 9 hydrogens which should correspond to the *tert*-butyl group. The N-*H* appears as a doublet at 5.6 ppm with a coupling constant of 8.0 Hz to a proton identified as H-3. H-7 and H-7' appeared downfield (4.9 and 4.1 ppm, respectively) relative to the normal C*HH'*-OH (3.9 and 3.6 ppm, respectively). Moreover, H-3 in **95** was centered at 3.4 ppm as a broad singlet whereas in this case, being identified as H-5, it was more downfield (5.1 ppm). In the COSY spectrum, H-5 did not show coupling with H-4 which corresponded to an dihedral angle of 90°. Furthermore, coupling constant between H-1&5 was 5 Hz, whereas the coupling between H-1&7 was 6.7 Hz, in the same order than the geminal coupling H-6,6'. NOESY experiments also supported the detailed structure **99** (Fig. 24). Thus, on the ^1H NOESY spectrum it was possible to see a cross peak between the NH and H-4; H-5 also presented a cross peak with the OH and between H-3 and H-5. The difference beetwen H-2β and H-2α was also clear. Thus only proton H-2β and not H-2α, showed a cross peak with H-4 and/or H-7.



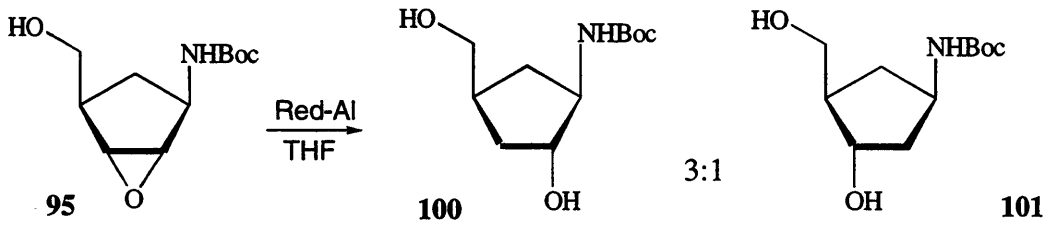
99

Figure 24

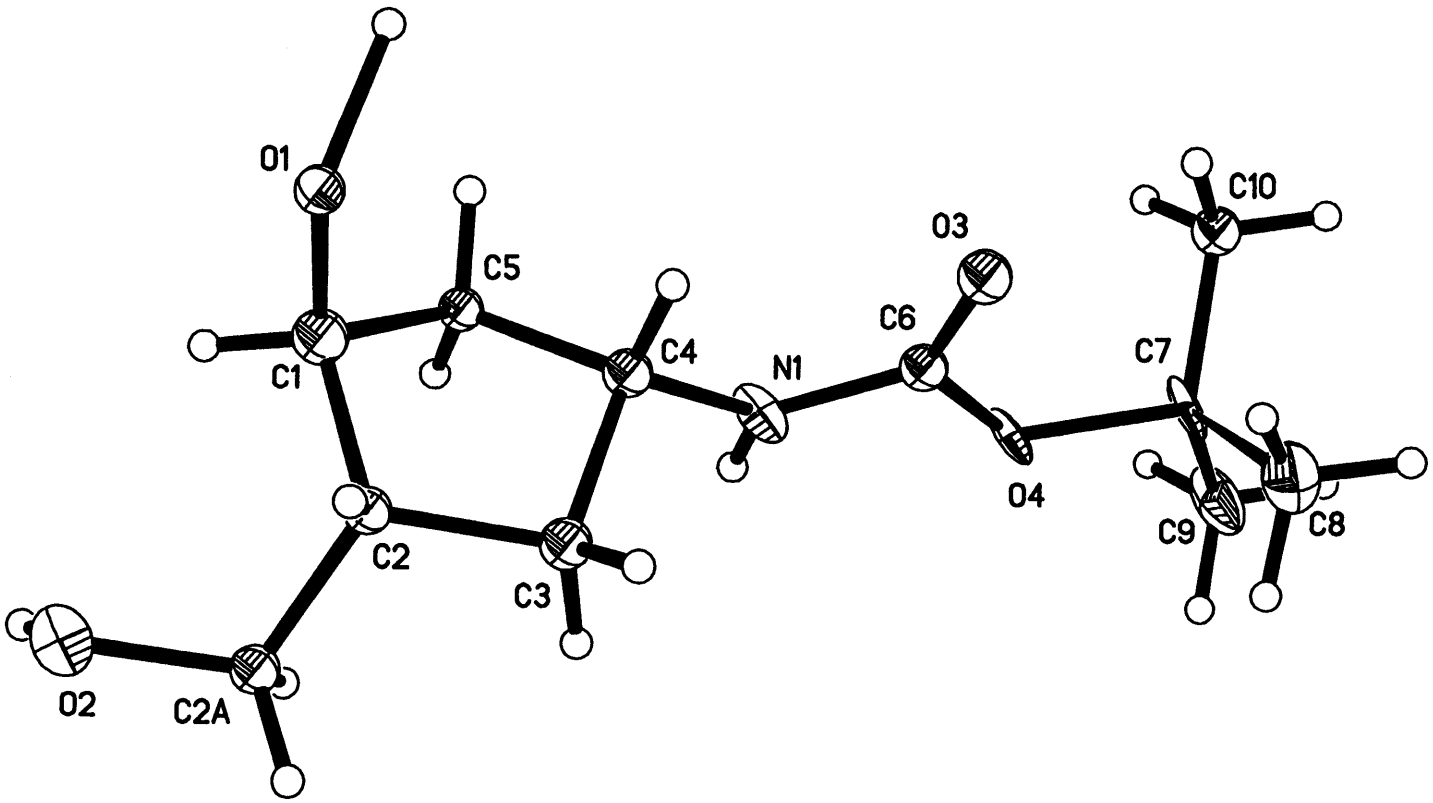
After all those experiments, the only compound which agreed with all the analytical data was the oxetane **99**.

3.4.3.3.5.2 USE OF RED-AL AND DIBAL-H

Further attempts were undertaken first using Red-Al as reducing agent. Thus reaction of **95** with a solution of Red-Al in toluene, led to the formation of two more polar compounds, observed on the TLC plate. After work-up and purification of the crude obtained, two crystalline products were isolated. Complete characterization led to the conclusion that the epoxide had been reduced yielding 71% of the regioisomers **100**:**101** in a ratio 3:1 (Scheme 82).

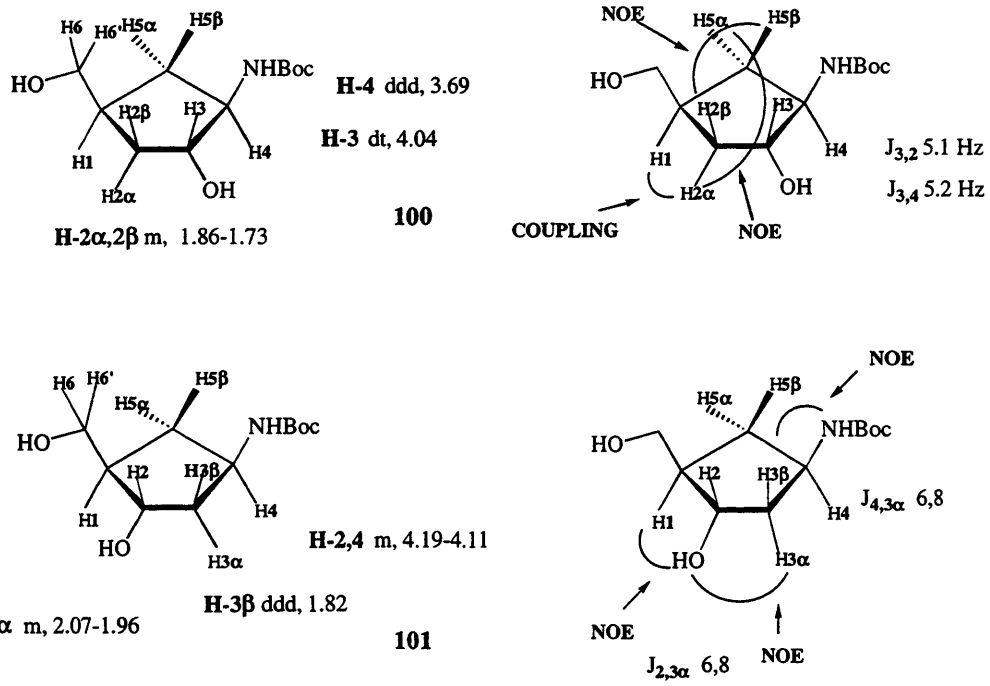


Scheme 82



X-Ray structure 3. Compound **101**

The ^{1}H NMR spectra showed some clear differences between them (Scheme 83). For instance, focusing on compound **100** NOESY allowed the distinction between H-5α and H-5β because a cross peak was observed between H-6,6' and H-5β only. There was an NOE between the H-5α and H-2α and in addition, the ^{1}H COSY spectrum showed a clear coupling between H-2α&1. For compound **101**, a NOE between OH-2 and H-1 was observed as main characteristic for the assignment of that structure and a crystal structure for compound **101** (X-ray structure 3) confirmed the stereochemistry here described.

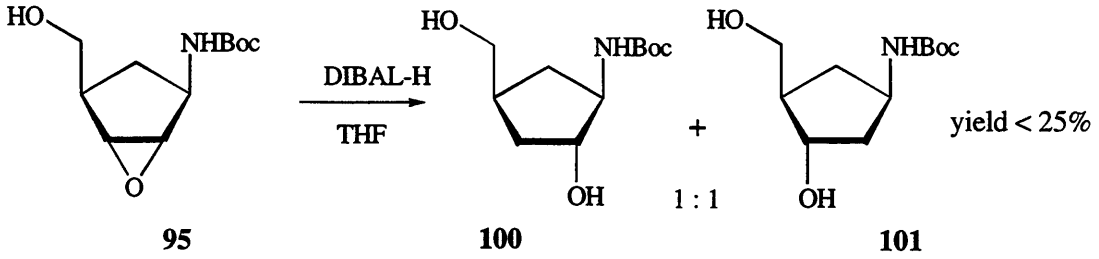


Scheme 83: Some characteristic chemical shifts, coupling constants and NOE, observed for the regioisomers **100** and **101**.

It was reasoned that the ratio of regioisomers **100** and **101** generated in the previous reaction might be inverted if the access at position 2 was more hindered than at position 3. This could be achieved following two different approaches: a) using a different reducing agent and seeing if an inverted regioselectivity could be obtained with a good yield; b) protecting the primary hydroxyl group in **95** with a suitable group that is resistant to the conditions of reduction.

Thus, the first reaction with Dibal-H in THF was performed on a small scale. After an acidic work-up, extraction led to a crude product in low yield which, when characterised by ^{1}H

NMR spectroscopy, corresponded to the two regioisomers **100** and **101** in a ratio 1:1 (Scheme 84).



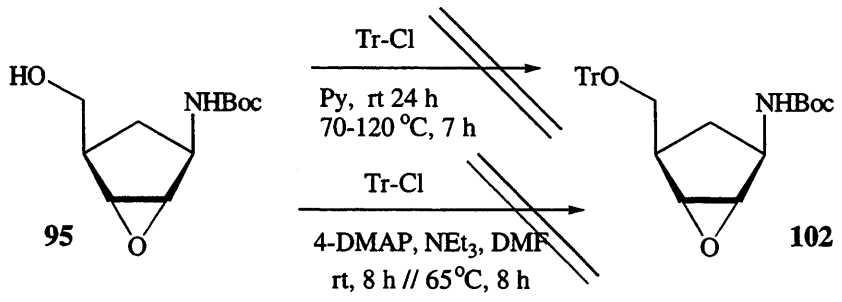
95 **100** **101**

Scheme 84

Because of this low yield, it was decided to explore the protection of the hydroxyl group and see if a further reduction with Red-Al would modify those results.

3.4.3.3.6 PROTECTION OF THE PRIMARY HYDROXYL GROUP IN 95

In order to circumvent the problem of regioselectivity in the reduction of the epoxide, triphenylmethyl chloride (Tr-Cl), one of the most commonly used primary hydroxy protecting groups in synthesis of natural nucleosides, was reacted with **95** under different experimental conditions (Scheme 85). The reaction proceed too slowly and no evidence of **102** was observed.

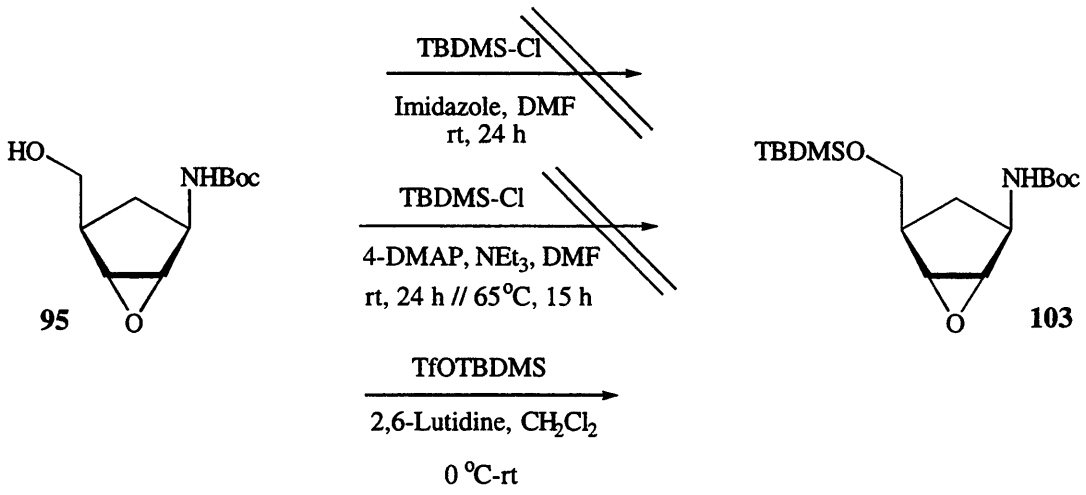


95 **102**

Scheme 85

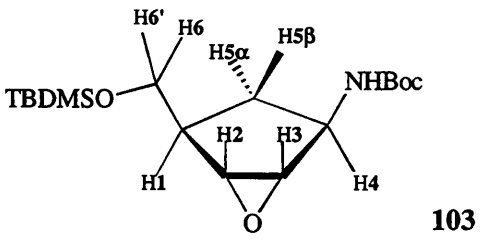
In the chemistry of the protecting groups, the use of *tert*-butyldimethylsilyl chloride was considered also convenient for our purposes. Its resistance towards several reducing reagents and also its bulky size could help promote a regioselective reduction.

Reaction of **95** with the silyl reagent was very slow. Changes in the catalyst (imidazole or 4-DMAP), solvent or temperature did not affect the rate of the reaction (Scheme 86).



Scheme 86

Considering that the reactivity of the triflates (trifluoromethylsulphonyl derivatives) is higher than the respective chlorides, further attempts were made using *tert*-butyldimethylsilyl trifluoromethanesulfonate (TfOTBDMS). Thus, reacting **95** with an equimolar quantitity of the silyl derivative in the presence of 2,6-lutidine, afforded compound **103** as a colourless oil (Fig. 25). Yields after purification, were never lower than 75%. Proof that the hydroxyl group had been protected was made by full characterization. The ^{1}H NMR spectrum showed a slight displacement of H-6,6' downfield (3.89 and 3.66 ppm, respectively) relative to those in the starting material (3.87 and 3.66 ppm).



103

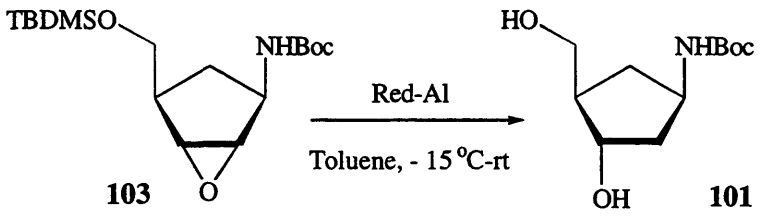
Figure 25

Nevertheless, protons 2 and 3 had been more affected by this protecting group. Thus, the singlet at 3.41 ppm observed for those protons on the starting material **95**, now appeared as a doublet. Moreover, H-2 was centered at 3.36 ppm and H-3 at 3.41 ppm, with a coupling constant H-2&3 of 2.3 Hz. No coupling was observed between H-1&2 or H-3&4 as it was seen with other analogues for protons with a dihedral angle of 90°.

3.4.3.3.7 REDUCTION OF 103 WITH RED-AL

The substrate **103** dissolved in toluene, was treated with an excess of a solution of Red-Al (65% in toluene) at -15 °C. Reaction for 5 hours at room temperature, led to a product which corresponded exclusively to the regioisomer **101** (Scheme 87), previously prepared from **95** also (Section 3.4.3.3.4.2).

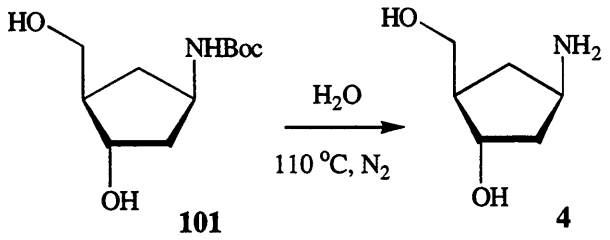
Apart from reduction, deprotection of the primary hydroxyl group had taken place. TLC analysis clearly showed that the new compound formed had an analogous R_f to **101** previously prepared and characterised. That meant that the deprotection of the hydroxyl group had taken place, but after the reduction of the epoxide, otherwise the regioselectivity would not have been observed. Yields of **101**, were highly sensitive to traces of water or other impurities. In general, if the solvent was freshly distilled before use, yields were always over 67%.



Scheme 87

3.4.3.3.8 DEPROTECTION OF THE AMINO GROUP IN 101

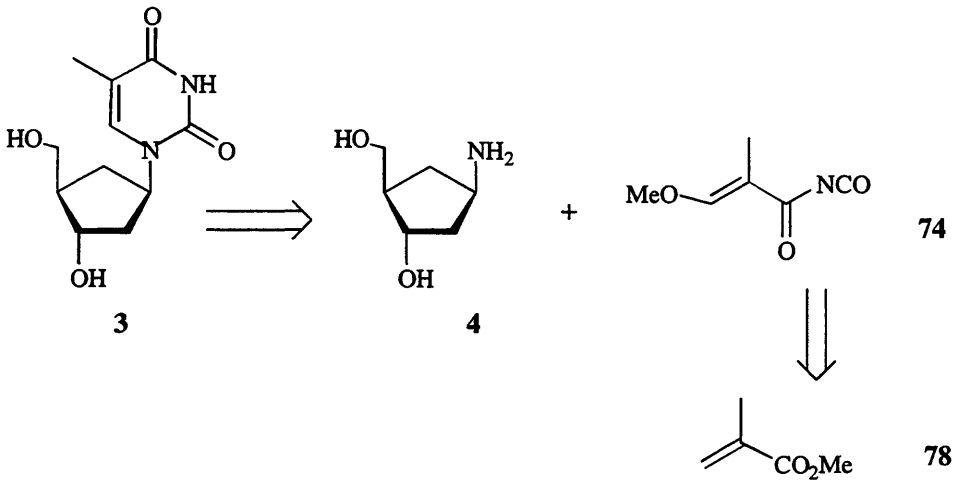
Obtaining compound **101** in a single step from compound **103** meant that we only had to deprotect the primary amine to complete the synthesis of the carbocyclic deoxyribose **4**. Thus, considering the sensitivity of this group to acidic conditions but also to temperature, two alternatives for the deprotection of the primary amine were possible. The first one involved the use of trifluoroacetic acid which would afford the protonated amine derivative and the other was hydrolysis in water under reflux. The latter method was preferred since it would afford a "neutral" primary amine rather than as the amine salt, which is the form of the amine required to build the thymine moiety (Scheme 89). Therefore, compound **101** could be dissolved in distilled water with ultrasonication and refluxed at 110 °C under nitrogen. On complete reaction, evaporation to dryness afforded a quantitative yield of a colourless oil identified as **4** (Scheme 88). This was stored under nitrogen, due to the high sensitivity of the amine towards oxidation. Hence, 1D and 2D-^{1}H and ^{13}C NMR spectroscopy allowed full characterisation of the product obtained.



Scheme 88

3.4.4 PREPARATION OF THE THYMINE MOIETY

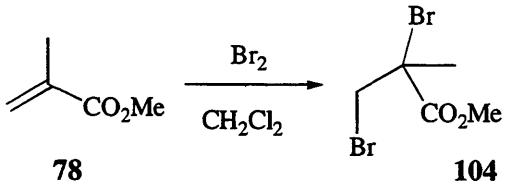
To complete the synthesis of carbocyclic thymidine **3** (Scheme 89) once the carbocyclic deoxyribose had been synthesised, it was only necessary to prepare the isocyanate **74**. That substrate can be prepared from the commercial available methyl methacrylate **78**.



Scheme 89

3.4.4.1 BROMINATION OF METHYL METHACRYLATE 78

Compound **104**[89] was obtained in quantitative yield by treating **78** with bromine (Scheme 90). This reaction, reported using carbon tetrachloride as the solvent, was modified by the use of dichloromethane, to obtain the expected product as a colourless oil in quantitative yield after removal of the excess of bromine by distillation. Mass spectroscopy, revealed the characteristic pattern M+2 for bromine-containing compounds and the ^{1}H NMR spectrum showed a chemical shift of 3.43 ppm for the C*HH* with a geminal coupling constant of 10.1 Hz which proved that addition to the olefin had taken place.

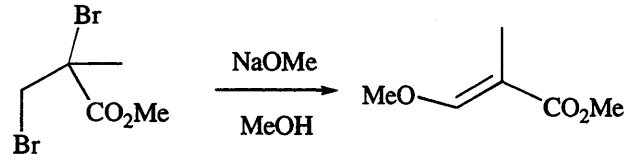


Scheme 90

3.4.4.2 PREPARATION OF 105

Preparation of **105** can be carried out according to literature[71] using sodium methoxide in methanol (Scheme 91). Hence, two equivalents of sodium were carefully dissolved in dry methanol and to this warm solution was added compound **104**. It should be pointed out that

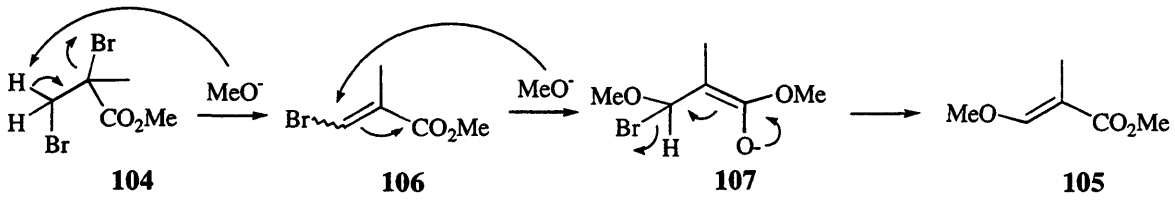
there were some important factors that could significantly decrease the yield because of the formation of by-products.



104 **105**

Scheme 91

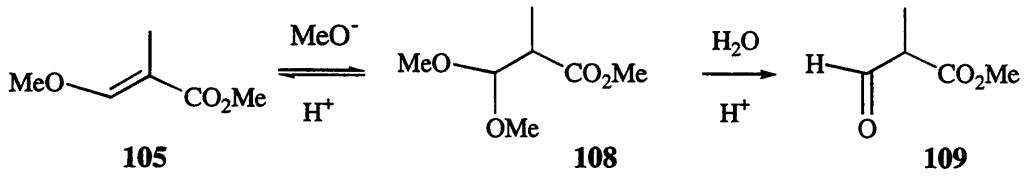
Thus, the formation of **105** proceeds *via* an elimination-addition-elimination mechanism (Scheme 92). Two equivalents of sodium methoxide are required to complete the reaction. The precipitate of NaBr formed should be separated by filtration to displace the equilibrium towards the formation of **105**. Otherwise, the most stable compound seemed to be **106** as was evident from the TLC and ^{1}H NMR analysis of a small sample before work-up.



104 **106** **107** **105**

Scheme 92

When the formation of the monomethoxy-alkene **105** takes place, the presence of MeOH allows a second attack to give the dimethyl acetal **108** (Scheme 93). Therefore, the use of $NaHSO_4$ in catalytic amounts was required to displace the reaction towards the formation of the alkene **105**. To avoid traces of water when the catalyst was added to the equilibrium mixture of **105&108** was rather important, otherwise it would promote hydrolysis to the aldehyde **109**, as observed in some experiments.

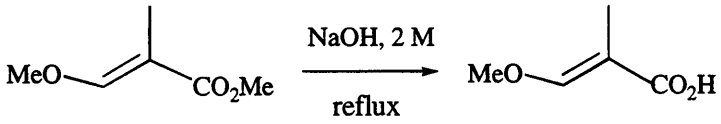


105 108 109

Scheme 93

3.4.4.3 PREPARATION OF 110

Hydrolysis of the ester **105** was performed under basic conditions described in the literature.[71] Reflux for 2 hours followed by an acidic work-up gave the carboxylic acid **110** (Scheme 94). The formation of this product could easily be confirmed by ^{1}H NMR spectroscopy. Disappearance of the signal for the methyl ester and slight displacement of the other signals downfield, proved that the ester had been hydrolysed.

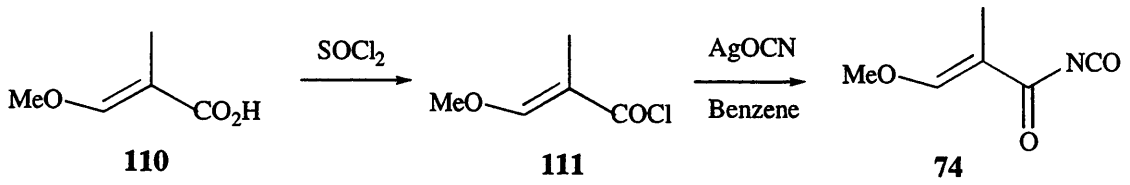


105 110

Scheme 94

3.4.4.4 TOWARDS THE PREPARATION OF THE ISOCYANATE 74

Isocyanates are quite unstable species because they readily hydrolyse in the presence of traces of water, losing carbon dioxide to give the corresponding amine or amide in our case. An analogous chemical behaviour is observed with acid chlorides, since they are easily converted into the carboxylic acid derivative. For all those reasons, compounds **111** and **74** (Scheme 95) were prepared under nitrogen and immediately used in the next step.

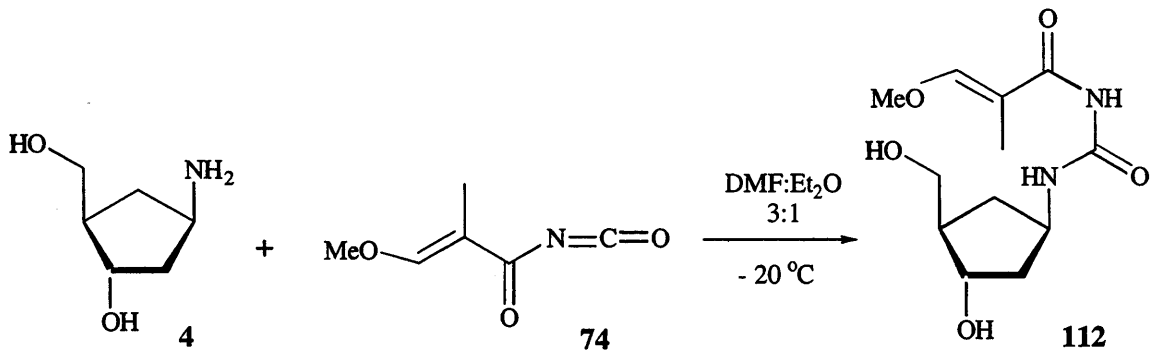


Scheme 95

Preparation of **74** was carried out following a different method to the one reported.[76] Thus, reaction of compound **110** with an excess of thionyl chloride under reflux, gave the corresponding acid chloride **111**. After rapid removal of the excess reagent under reduced pressure, this was treated with a solution of AgOCN in benzene and refluxed for 1.5 hours. Then, the supernatant containing the isocyanate **74** was used for the next step in the coupling reaction with the carbocyclic deoxyribose **4**.

3.4.5 COUPLING REACTION

The attachment of the thymine moiety to the carbocyclic sugar **4** was carried out under two different experimental conditions. The first one involved addition of the isocyanate **74** to a solution of the carbocyclic deoxyribose **4** under an argon atmosphere, dry DMF and - 60 °C (bath with dry CO_2 and $CHCl_3$). After 24 hours at room temperature, TLC analysis showed severe streaking. Some attempts made to isolate some traces of the expected product **112** by chromatography, were unsuccessful.

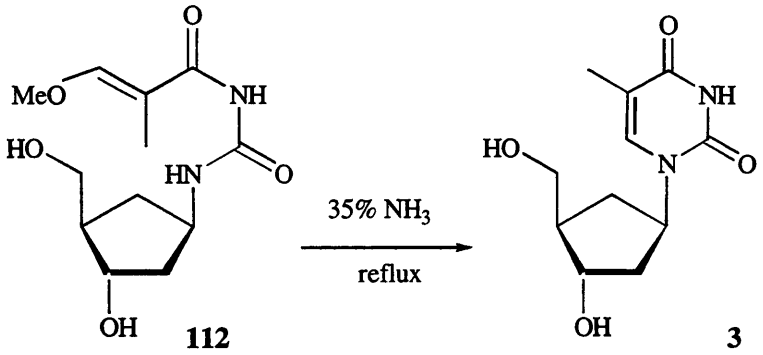


Scheme 96

Further experiments were carried out to optimise the conditions required to obtain an acceptable yield (Scheme 96). Thus, the best conditions were found to be *via* rapid and careful manipulation of compounds **74** and **4**, always under nitrogen atmosphere and in the absence of any trace of water. The addition of the supernatant with **74** (Section 3.4.4.4) to a solution of **4** in DMF:Et_2O at -20 °C, was followed by two hours reaction at room temperature. Evaporation of the volatile materials by co-evaporation with ethanol gave after purification **112**,[74b] in yields that always exceeded 65%.

3.4.5.1 CYCLIZATION TO CARBOCYCLIC THYMIDINE 3

Cyclization of compound **112** can be performed under basic or acidic conditions with reflux.[90] Thus, the first involved treatment with aqueous sulphuric acid, whereas basic conditions involved ammonia (Scheme 97). The later were chosen in this work and the formation of the target molecule **3** was completed in two hours in 75% yield after purification.



Scheme 97

Evidence for successful cyclization could be easily seen in the ^1H NMR spectrum. Key features, among others, being the loss of the peak of the NH and the OMe resonances.

3.5 COMPOUNDS 92 AND 95 AS SYNTHETIC INTERMEDIATES

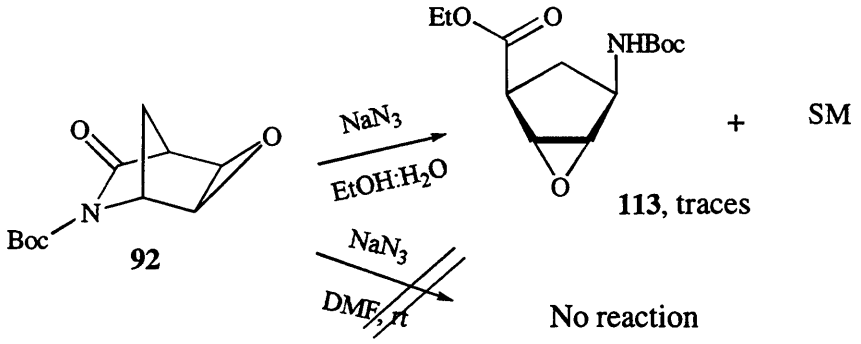
The great interest in the synthesis of a range of carbocyclic analogues of nucleosides and the search for short and efficient alternative routes to prepare them, focused our attention on some key intermediates available from the synthesis of carbocyclic deoxyribose **4**. Hence, the importance of the regioisomer **100** (Scheme 82, Section 3.4.3.3.5.2), is remarkable since studies on stability of hybrid DNA/RNA duplex with 3'-deoxyribose analogues incorporated, have been recently reported[91] opening a new field in research. Moreover, compound **96** (Scheme 79, Section 3.4.3.3.4) might also be an interesting analogue of ribose after deprotection of the amino group.

3.5.1 ATTEMPTS TO OPEN EPOXIDES 92 AND 95 WITH AZIDE AND ACETATE

It was reasoned that some of the intermediates previously obtained might also be used in the preparation of modified analogues of ribose.

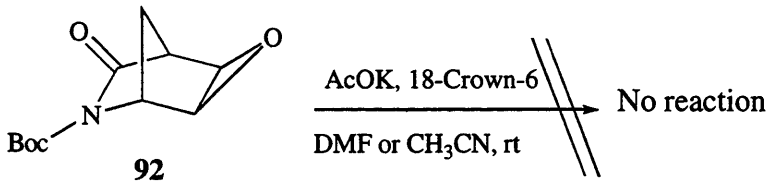
Additional functionality might be introduced into the cyclopentane ring through attack on the epoxide by other different nucleophiles. Our main attention was focused in nitrogen or oxygen nucleophiles, since compounds with these subtituents have been reported to have antiviral and antitumor activity, for instance AZT and aristeromycin (Scheme 55). Thus, the reactivity of **92** and **95** under several experimental conditions was studied.

The first compound examined was **92** using sodium azide and potassium acetate as nucleophiles, respectively. Thus, by treatment with NaN_3 in a mixture of ethanol-water or in DMF no reaction was observed in any of our experiments at room temperature (Scheme 98). Higher temperature only underwent deprotection of the amine without affecting the epoxide. Moreover when the temperature was increased to 70-80 °C in a solution of ethanol:water, a slow reaction, led to formation of compound **113**.



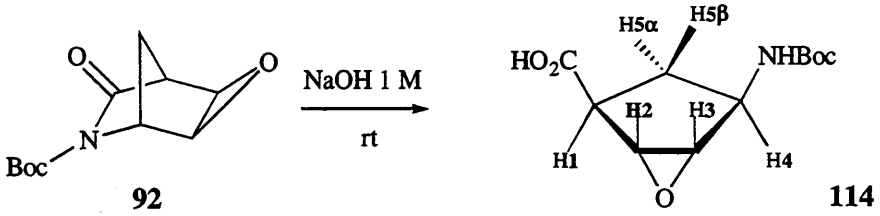
Scheme 98

Acetate was chosen as a possible good oxygen nucleophile for the attack to the epoxide. A similar result to the use of azide as nucleophile was observed (Scheme 99). No reaction occurred even after improving the nucleophilicity of the anion by using dibenzo 18-crown-6 to coordinate the cation, or polar and non-protic solvents, for instance DMF or acetonitrile.

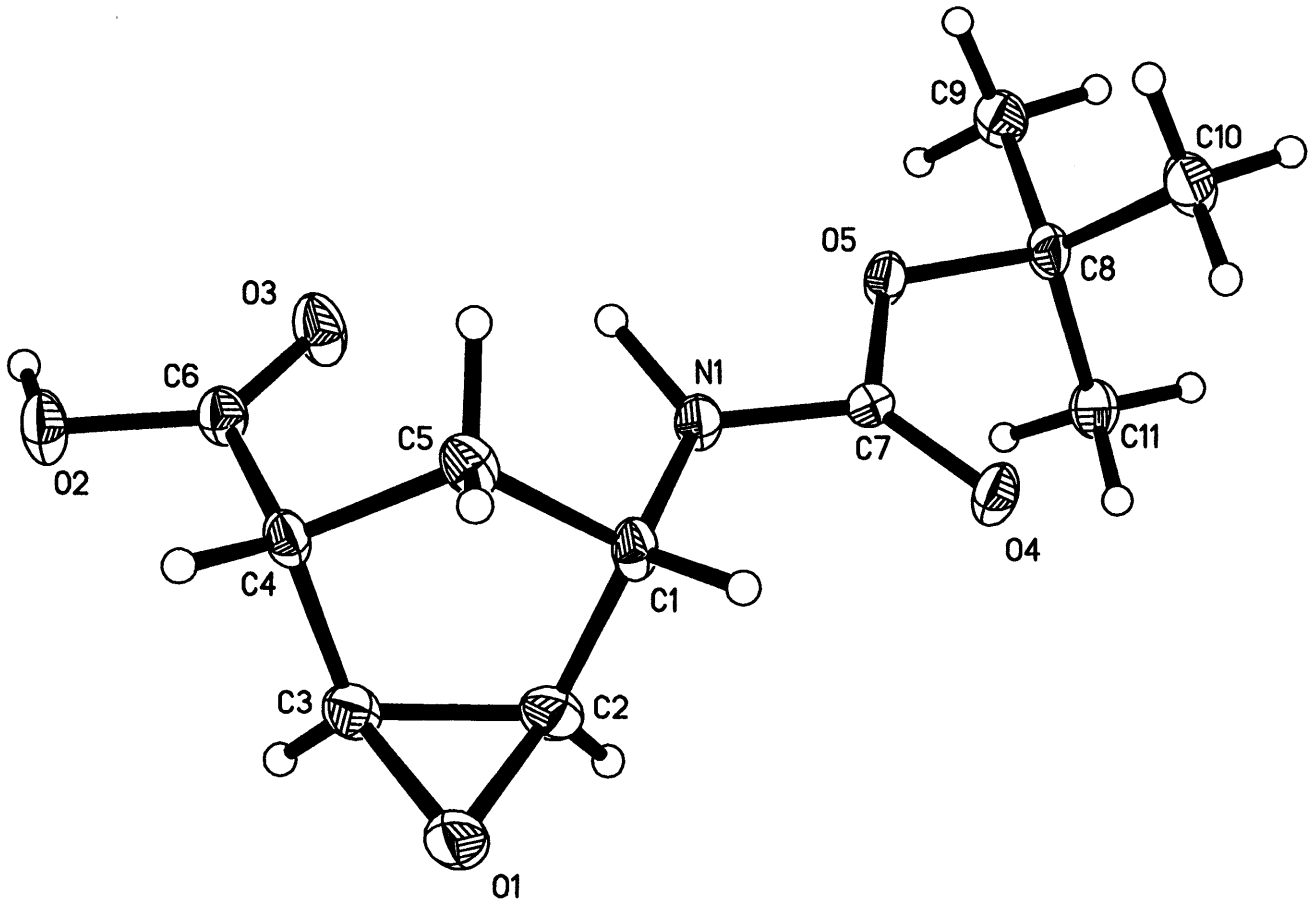


Scheme 99

Treatment with aqueous NaOH, did not seem to affect to the epoxide. However, the lactam had been opened to give compound **114** in 75% yield (Scheme 100). Some attempts with KOH (1 M), and KOH (1 M) in DMF, afforded **114**, also with the only difference that in DMF the opening of the lactam seemed to be slightly faster than in aqueous solution.

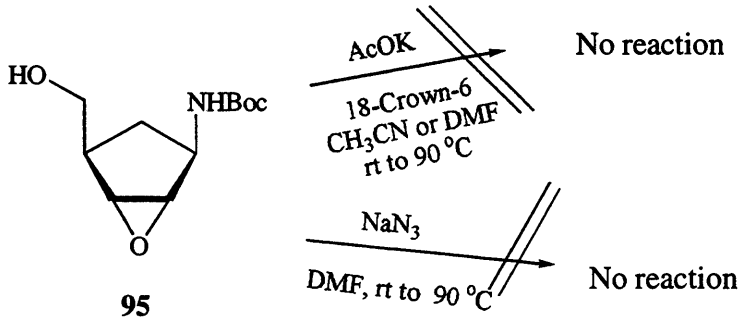


Scheme 100



X-Ray structure 4. Compound **114**

Full characterisation of this product and a crystal structure (X-ray structure 4) confirmed that the epoxide was still intact. The ^1H NMR spectrum showed a coupling constant of 2.3 Hz between protons H-2&3. A very small coupling of 1.6 Hz was observed between protons H-3&4 whereas no coupling was seen between H-1&2. The dihedral angle for H-1&2 is approximately 90 ° as it was observed with similar substrates containing an epoxide. The reactivity of **95** was also examined. Thus, an analogous chemical behaviour was observed by reaction with azide and acetoxide as nucleophiles (Scheme 101). This compound seemed to be very stable under our experimental conditions. Even at higher temperatures the epoxide appeared to be unreactive.

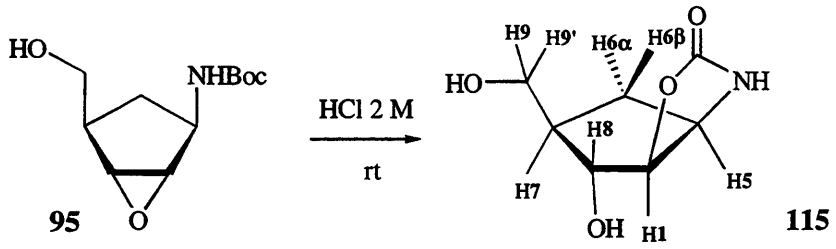


Scheme 101

3.5.2 RING OPENING OF THE EPOXIDE 95, UNDER BASIC AND ACIDIC CONDITIONS

3.5.2.1 FORMATION OF THE URETHANE 115

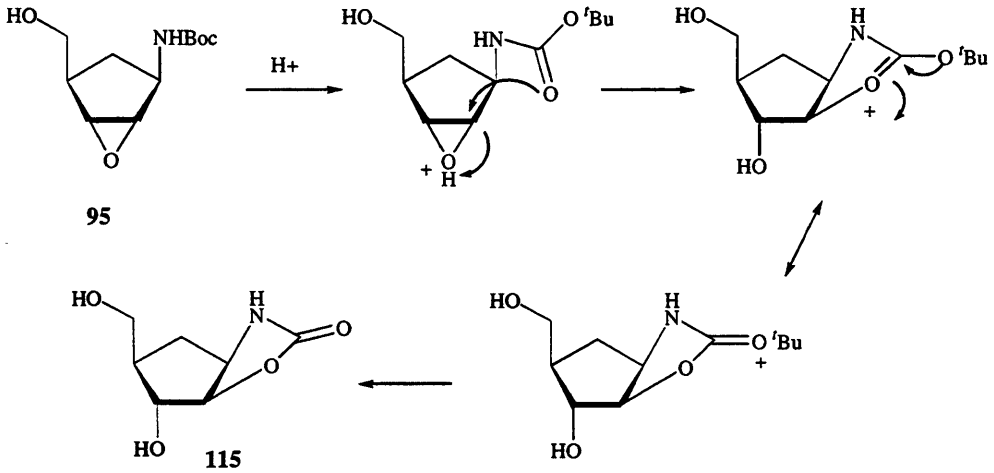
Further experiments were carried out under acidic and basic conditions. By treatment of compound **95** with dilute hydrochloric acid at room temperature, TLC showed formation of a more polar compound than the starting material. On complete reaction, the solution was evaporated to dryness and the crude product purified by chromatography. The product isolated corresponded to the urethane **115** in 75% yield (Scheme 102).



Scheme 102

Spectroscopic techniques clearly confirmed that result. Thus, there was no appearence of a *tert*-butyl group in the ^{1}H NMR spectrum, but ^{13}C NMR and IR spectroscopy still showed the presence of the carbonyl group. The NH signal in the ^{1}H NMR spectrum also proved that the product formed was not an imine and in consequence it was reasoned that the epoxide had been opened. ^{1}H NOESY showed clear evidence of the link to the position 1 and not 8 (Scheme 102). Hence, there was an NOE between H-9,9'&8. Moreover, assuming that it was possible to distinguish between H-6α and H-6β because of an NOE of the latter with H-9,9', there was also an NOE between H-6α&1 and between H-6β&8, which accounted for the structure and the sterochemistry here defined.

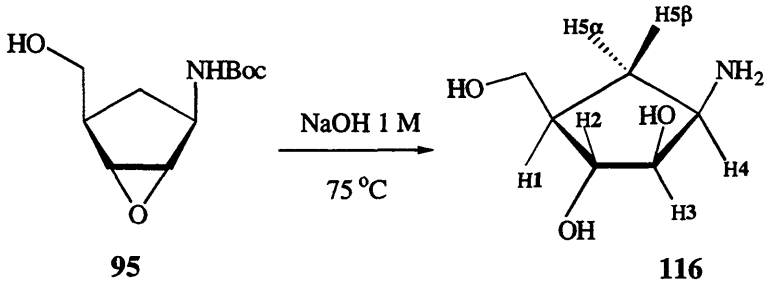
The formation of **115** may be explained as result of the participation of the Boc group in the opening of the protonated epoxide under the acidic conditions of reaction (Scheme 103).



Scheme 103

3.5.2.2 FORMATION OF ARA-RIBOSE 116

Reaction of compound **95** under basic conditions, also affected the epoxide ring opening. Thus, at room temperature TLC analysis showed no changes, and only starting material was detected. Nevertheless, when the temperature was increased to 75 °C, formation of a new compound was observed by TLC. After an acidic work-up to pH=7-8 and evaporation of the solvent to dryness, the ^{1}H NMR spectrum of the crude product seemed to be a single product but ^{13}C NMR spectroscopy, clearly proved that it was a mixture of two. After purification by chromatography, two compounds were isolated in a ratio 4.7:1. Full characterization demonstrated that these two compounds were the amino-alcohol **116** (Scheme 104) in its neutral and protonated forms in 75% total yield.



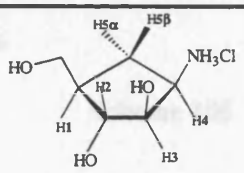
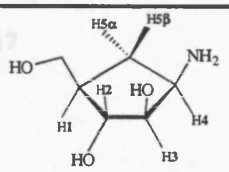
95 **116**

Scheme 104

The spectroscopic data matched with the literature[92] data for the neutral product **116**. Nevertheless, the ^{1}H NMR spectrum for each of the two forms of the compound are very different. Hence, for the protonated species, apart from the expected shift of all the signals more downfield compared to the neutral form, the appearance of the ^{1}H NMR spectrum has also substantially changed. For instance, for the neutral compound (Spectrum 1), five protons H-3, 2,1, 6 and 6' appeared between 3.60 and 4.00 ppm whereas the same protons for the protonated amine (Spectrum 2), suffered a significant change in the positions and the range which now extends from 3.56 to 4.73 ppm. In addition, some important variations have also been observed in the coupling constants. This may be expected considering that coupling constants not only depend on the dihedral angle between protons but also on some electronic effects which can modify conformation of the cyclopentane ring and therefore the dihedral angle, causing important differences between the coupling constant values for

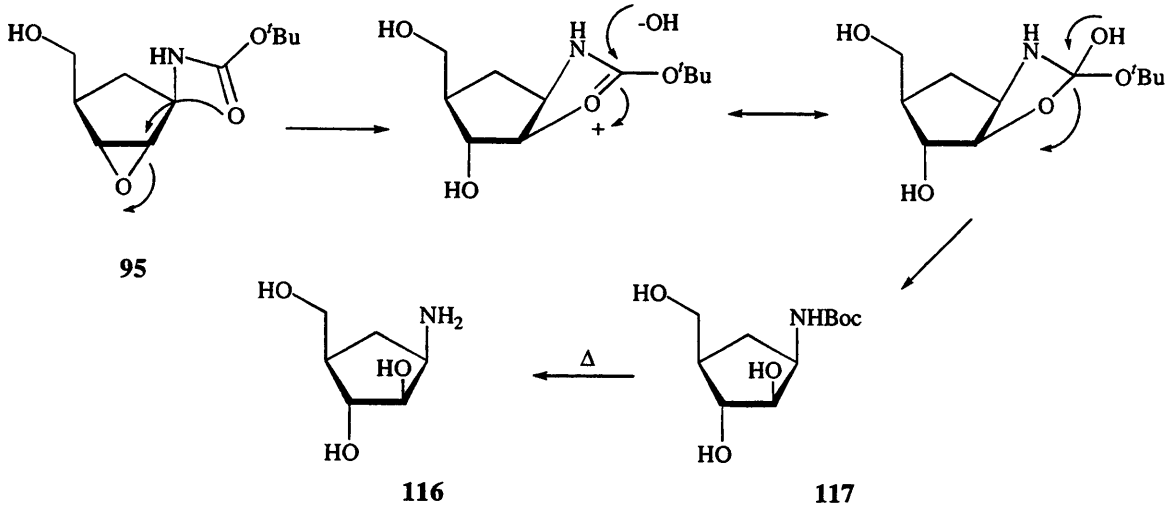
compounds with the same stereochemistry (Table 4). 2D-NOE spectroscopy was crucial for the characterisation of this compound in its protonated and neutral forms, both showing the same NOEs as expected. Hence, the NOE observed between H-2&6,6', H-1&3 and H-2&5β accounted for the stereochemistry of the compound here described.

Table 4: Some characteristic differences between the protonated amino alcohol **116** and the neutral one, on their ^{1}H NMR spectra.*

Compound 116		
δ_{H-4}	4.24 ddd	3.65-3.60 m (H-4,6')
δ_{H-3}	4.71 dd	3.98 dd
δ_{H-2}	4.01 dd	3.84 t
$J_{3,4}$	9.1	5.8
$J_{2,3}$	4.1	3.8

* The ^{1}H NMR spectra were run at 400 MHz, in MeOH-d_4 at 27 °C. The chemical shifts are expresed in ppm and the *J* values in Hz.

The regioselective opening of the epoxide **95** to yield exclusively **116** may be explained considering also the participation of the Boc group, playing an important role in the stereoselectivity of this reaction (Scheme 105). Thus, after the ring opening, attack by the base to the carbonyl group would afford an intermediate **117** which could be deprotected later to give compound **116** (Scheme 105).

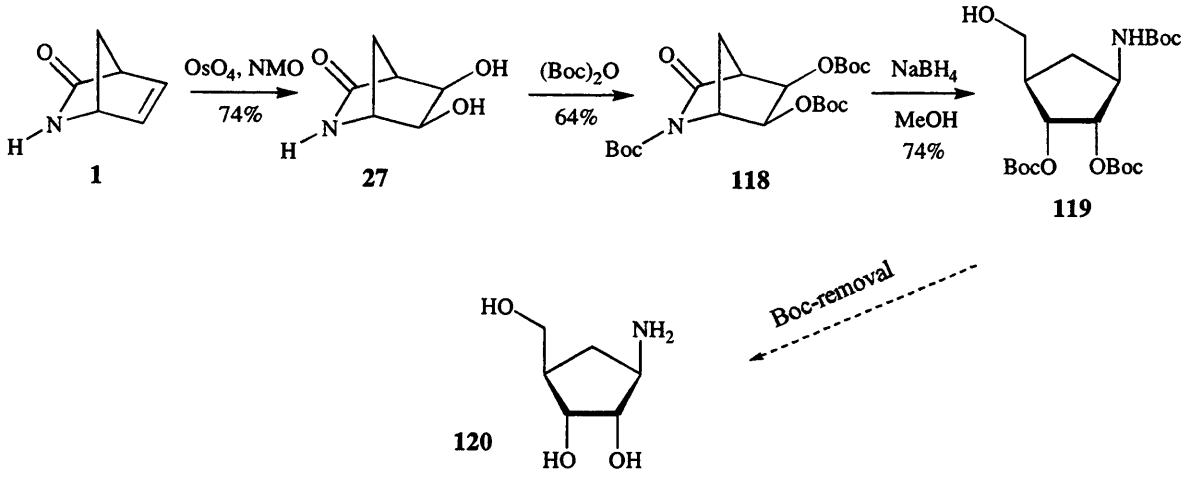


Scheme 105

3.5.3 ALTERNATIVE SYNTHETIC STRATEGIES TOWARDS RIBOSE ANALOGUES

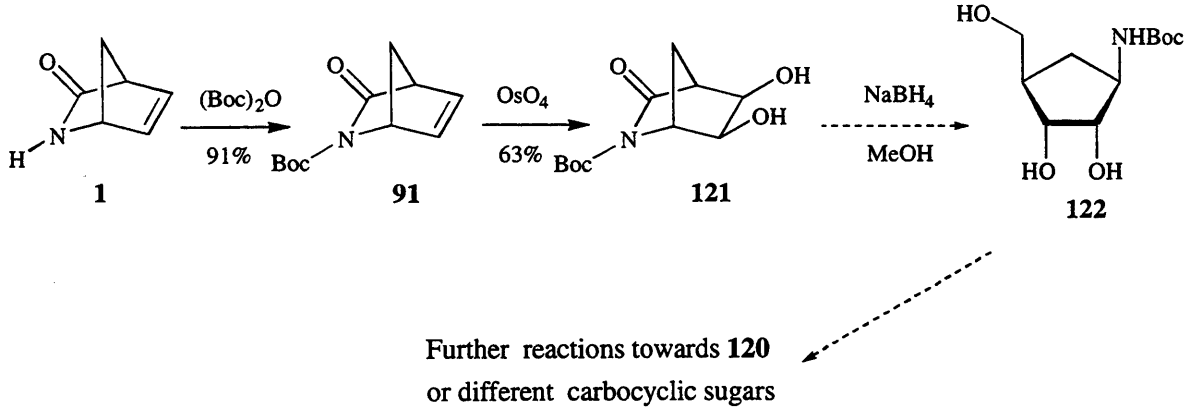
The synthetic strategy developed towards the synthesis of carbocyclic deoxyribose **4** can be adapted for the synthesis of other ribose analogues. The common strategy reported for the preparation of carbocyclic ribose analogues involves the hydrolysis of the lactam **1**, followed by reduction of the carboxylic acid or ester derivative. The methodology developed in this work is shorter and more convenient since the reductive cleavage of the lactam can be done in one step under very straightforward experimental conditions.

Two methods can be followed leading to either the diol in protected form or free. Dihydroxylation of the lactam **1** gives **27** as previously described (Section 3.4.3.2.1). This was followed by treatment with three equivalents of $(Boc)_2O$ at room temperature for 24 hours. This protecting agent firstly considered for the protection of the nitrogen of the lactam, easily reacted with hydroxyl groups also, affording **118** in 64% yield (Scheme 106). Further treatment of **118** with sodium borohydride, reductively cleaved the lactam, as expected. Thus, the cyclopentane ring **119** was isolated in 74% yield after purification. This substrate, can readily afford the carbocyclic ribose **120**, by removal of the Boc groups. In addition, the primary hydroxyl group can be subjected to further reactions if needed, because the problem of selective protection of secondary hydroxyl group in the presence of a primary one is already resolved on that compound.



Scheme 106

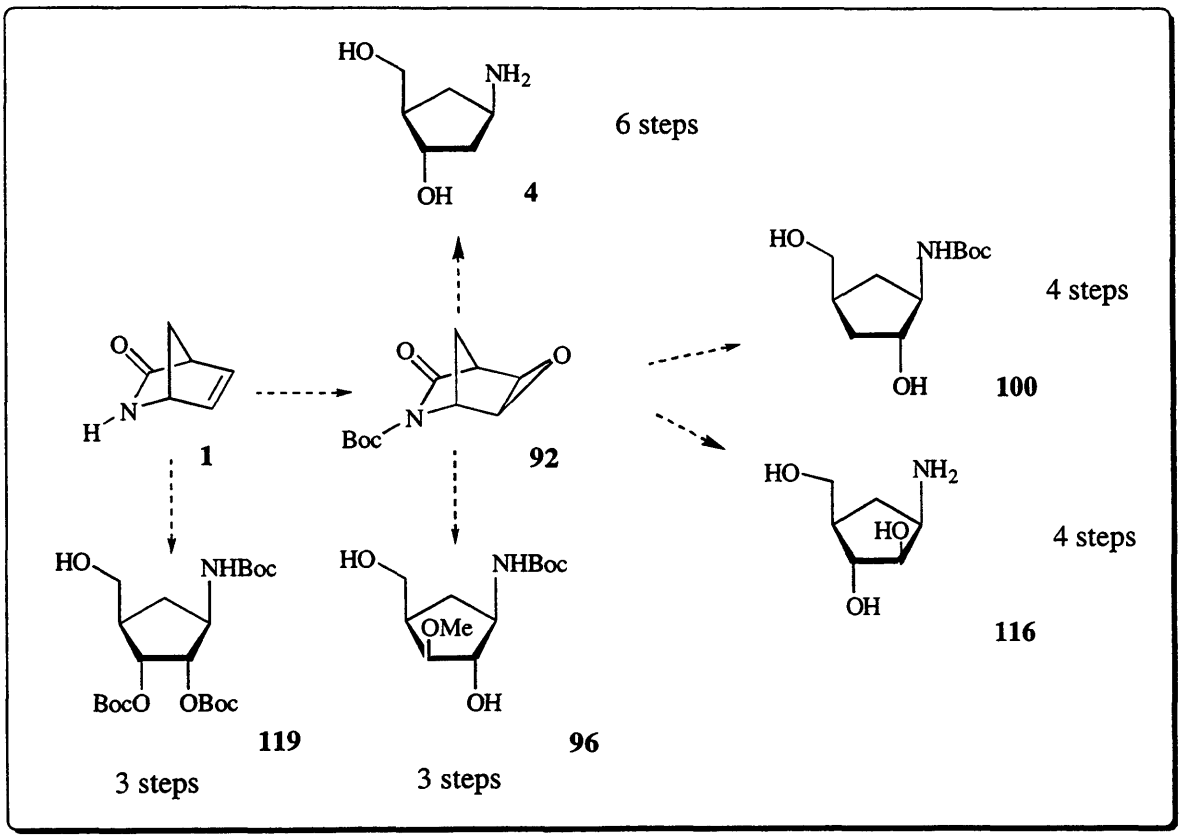
An alternative synthetic route (Scheme 107) can be undertaken by protection of the lactam **1** with Boc group as previously described (Section 3.4.3.3.1). Dihydroxylation of this compound **91** with OsO_4 under similar conditions to the formation of the *exo* diol **27**, gave compound **121** in 63%. A coupling constant of 5.0 Hz between H-5&6 was observed in the ^1H NMR spectrum for this substrate. Following a similar methodology to that previously described, compound **121**, can be reduced with sodium borohydride in methanol, in order to obtain the tri-alcohol **122** which would lead to the carbocyclic ribose analogue **120**. This is a new alternative and shorter synthetic approach planned towards the synthesis of compound **4** (Section 3.4.3.2) or even other carbocyclic sugars.



Scheme 107

3.6 SUMMARY

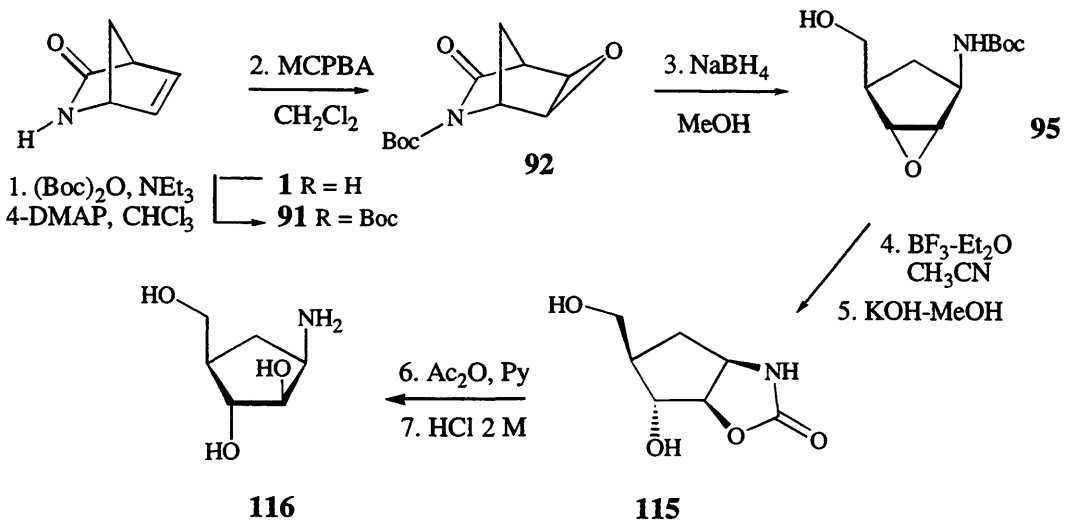
In conclusion to the work described in this chapter, it has been proven that the bicyclic lactam **1** is a very convenient substrate for the synthesis of a wide variety of carbocyclic sugars **4**, **116** or some precursors of carbocyclic sugar **100**, **96** and **119** through short and convenient synthetic strategies (Scheme 108).



Scheme 108: Scope of the different ribose and deoxyribose analogues synthesised.

All these compounds might consequently be used as synthetic intermediates towards their corresponding purine or pyrimidine nucleosides or nucleotides.

During the course of this work, Katagiri and co-workers[93] reported a similar synthesis of compound **116** starting from (±) lactam **1** (Scheme 109) towards the construction of the carbocyclic analogue of Ara-A.



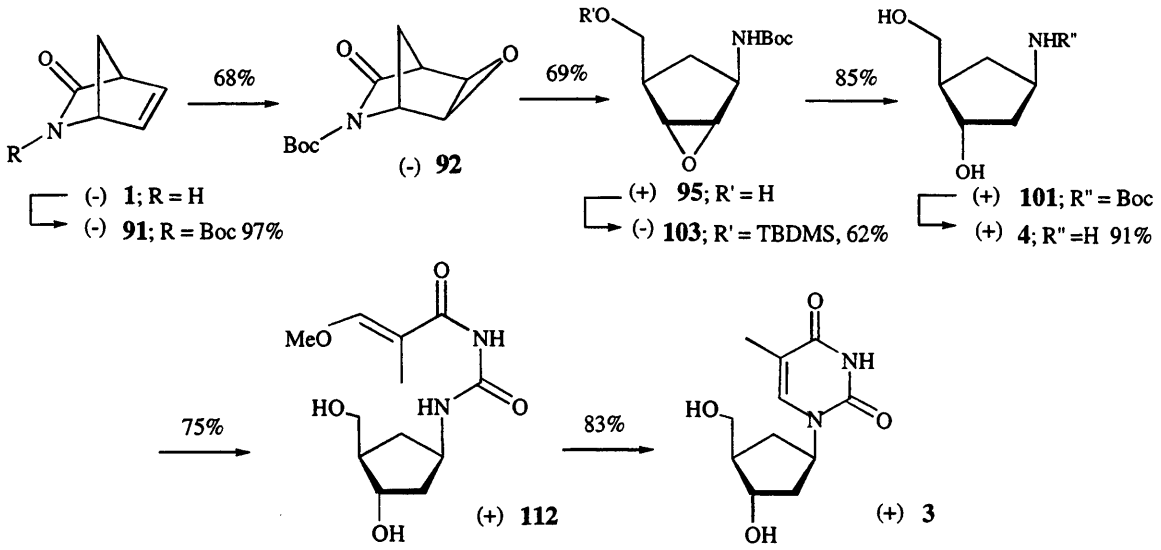
Scheme 109

Their conclusions confirm the results obtained in some common steps described in this Chapter. Our synthetic route is more convenient for the preparation of **116** since it can be completed in four steps rather than in seven, as those authors reported. However, aqueous acidic conditions are sufficient for the opening epoxide **95** leading to the formation of urethane **115**.

3.7 PREPARATION OF ENANTIOMERICALLY PURE CARBOCYCLIC THYMIDINE

Our interest in undertaking some future biological studies with the (±) carbocyclic thymidine **3** previously synthesised, made necessary the preparation of the (+) enantiomer which will have the same absolute configuration as the natural thymidine. The availability of the enantiomerically pure (-)-2-azabicyclo[2.2.1]hept-5-en-3-one **1** allowed the synthesis of (+) **4** following the pathway previously developed with the racemic series (Section 3.4).

(-) Lactam **1** used in this synthetic work was kindly donated by Chiros Ltd, in the U.K and the synthesis of the carbocyclic nucleoside **3** was undertaken in 8 steps, with an overall 15% yield (Scheme 110).



Scheme 110

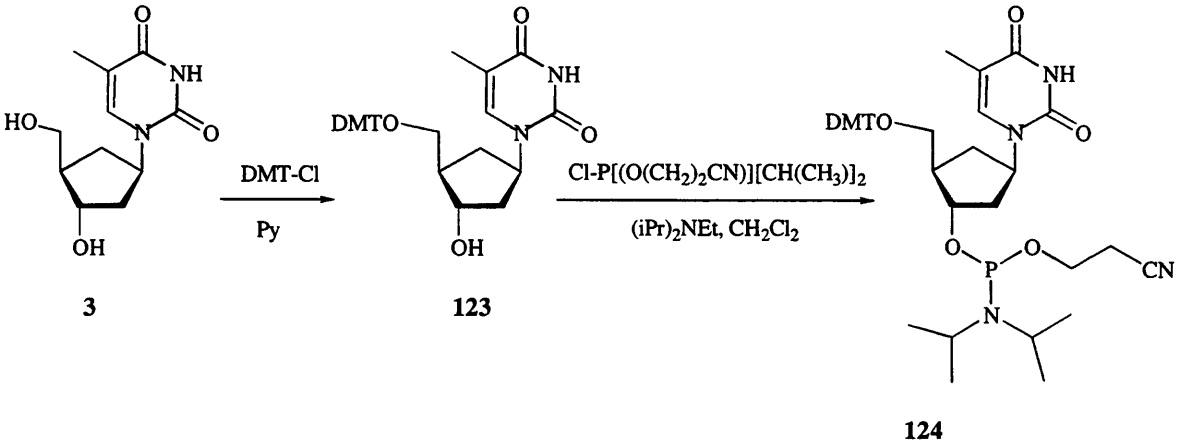
3.8 HYBRID OLIGONUCLEOTIDES: DNA SYNTHESIS

The formation of synthetic DNA involves a standard approach which consists of adding activated mononucleotides to a growing DNA segment linked to a silica support by a covalent bond. The strand of oligonucleotide will be prepared using the phosphoramidite chemistry on an automatic DNA/RNA synthesiser on a μmol scale.

Our biological studies will be undertaken using the self complementary and single strand oligonucleotide of Dickerson-Drew [d(CGCGAATTCGCG)]. The convenience of this specific sequence is because this dodecamer has demonstrated mutation tolerance and its crystallographic structure has been reported. This is relevant for further crystallographic studies with the hybrid oligonucleotide.

Natural thymidine will be replaced by carbocyclic thymidine **3** as the phosphoramidite monomer. With that purpose, **3** was converted into the phosphoramidite derivative **124** *via* two standard chemical steps, firstly with (±) **3** (Scheme 111). Once optimised the experimental conditions needed for the synthesis of the optically active **124** was carried out.

Thus, **3** was treated with dimethoxytrityl chloride (DMT-Cl) in pyridine. The bulky size of this reagent allowed selective protection of the primary hydroxyl group. The high acid-lability of this protecting group required precautions during the purification of the product by chromatography on silica gel. Thus, the protection was undertaken by dissolving compound **3** in pyridine in the presence of activated molecular sieves. At the end of the reaction, the pyridine was removed by evaporation and the product purified by column chromatography on silica with solvent containing 0.2% triethylamine. This afforded the expected **123** in 61% yield (±) and 65% yield (-).



3 123 124

Scheme 111

The last chemical step consisted in the formation of the phosphoramidite derivative. For this purpose, **123** was treated with (±)-chlorodiisopropylamino-β-cyanoethylphosphoramidite and diisopropylethylamine in dichloromethane. After purification, **124** was obtained in 55% yield for the racemic mixture of diastereoisomers and 67% yield for the two enantiomerically pure diastereoisomers **124**.

Commercial oligonucleotide synthesisers use monomers that are all epimeric at phophorus because the phosphorus centres do not end up chiral at the end of the synthesis. Therefore, the two diastereoisomers were not separated, since once prepared, all the protecting groups will be removed from the hybrid DNA segment leading to an enantiomerically pure oligonucleotide.

3.9 CONCLUSIONS

1. A very convenient synthetic route towards carbocyclic deoxyribose **4**, has been developed from the commercial available (±) bicyclic lactam **1**, *via* 6 steps and 15% overall yield. This route is shorter than existing literature methods and does not afford any by-products in any of the steps needed which is an important improvement for the preparation of this sugar analogue.

2. The compound **4** was used as a key intermediate for the preparation of carbocyclic thymidine **3**. Its synthesis was performed *via* a linear approach involving coupling with the isocyanate **74**.

3. The synthetic pathway for the preparation of **4** can be easily adapted for the preparation of a wide range of carbocyclic sugars or derivatives such as **116**, **100**, **96** and **119**.

4. (+) Carbocyclic thymidine **3** was also prepared from the (-) bicyclic lactam **1** in 8 steps *via* the same route as the one used for the preparation of the racemic series.

5. The preparation of (+) thymidine **3** allowed the preparation of compound **124**, *via* phosphoramidite chemistry, which will be used for the future synthesis of hybrid DNA strands and some studies on the effect of the carbocyclic substitution on the structure, chemical stability and resistance to enzymic digestion.

CHAPTER 4

EXPERIMENTAL

4. EXPERIMENTAL

4.1 GENERAL EXPERIMENTAL

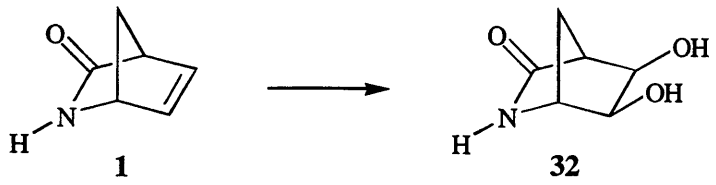
All the reactions involving extraction of the organic products into an organic solvent during the work-up, after combining the organic layers they were dried with anhydrous $MgSO_4$. The reaction solvents used were dried under the following conditions: Tetrahydrofuran and benzene were distilled from sodium-benzophenone, diethyl ether from lithium aluminium hydride, dichloromethane, acetonitrile and toluene from calcium hydride, methanol and ethanol from magnesium, dimethyl formamide was purchased directly from Aldrich, distilled and stored in the dark over molecular sieves. Pyridine and triethylamine were distilled from calcium hydride and then stored over potasium hydroxide pellets, triisopropylethylamine was purchased directly from Aldrich and then distilled from calcium hydride. Flash chromatography was carried out using sorbsil C-60 silica gel, 40-60 μm, TLC analysis was performed using silica gel 60 F_{254} aluminium TLC plates, Merck 5554, PMA dip was employed as TLC stain throughout all the work involved into the preparation of cADPR analogue, described in Chapter 2 whereas $KMnO_4$ dip was utilised during the synthesis of analogues of DNA/RNA nucleosides; TLC stain was observed with UV-lamp 254 nm for all the aromatic compounds prepared. Melting points were measured using a Kofler Hotstage and are uncorrected; elemental analyses were carried out by Butterworth Laboratories, Middlesex. IR spectra were recorded using a Perkin Elmer 298 spectrophotometer; optical rotations were measured using a Perkin Elmer 341 polarimeter and mass spectra were recorded using a Kratos Concept. NMR spectra were recorded on a Bruker ARX 250 (250 MHz ^{1}H, 62.9 MHz ^{13}C, 235 MHz ^{19}F) or a Bruker DRX 400 (400 MHz ^{1}H, 100.6 MHz ^{13}C, 101 ^{31}P). NMR spectra recorded in $CDCl_3$ were calibrated to $CHCl_3$ (^{1}H, δ 7.27), (^{13}C, δ 77.4), all the chemical shifts were taken directly from the spectra and *J* values were given in Hz. Some NMR spectra were run in CD_3OD-d_6, acetone-d_6 or D_2O. The data in these cases was taken directly from the spectra without any prior correction.

(-)-2-Azabicyclo[2.2.1]hept-5-en-3-one was kindly donated by Chiroscience in the UK; FLUKA: (±)-2-azabicyclo[2.2.1]hept-5-en-3-one , 4-dimethylamino-pyridine, *o*-*p*-tolyl-chlorothionoformate. ALDRICH: benzylamine, triethylsilane, 2-(tert-butoxycarbonyloxyimino)-2-phenylacetonitrile-cyclopentylamine, L-phenylalanine, triphenylpyrylium tetrafluoroborate, 2-mesitylenesulfonyl chloride, 2,4,6-triisopropyl-

benzene-sulfonyl-chloride, 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane, osmium tetroxide, *N*-methyl morpholine oxide, oxone, *m*-chloroperbenzoic acid, lithitum triethylborohydride, methyl methacrylate, thionyl chloride, diisobutyl aluminium hydride, Red-Al, silver cyanate, 4,4-dimethoxytrityl-chloride, 2-cyanoethyl-diisopropylchlorophosphoramidite. LANCASTER: di-*tert*-butyl-dicarbonate, chlorotriphenyl methane, *tert*-butyldimethylsilyl trifluoromethanesulfonate.

4.2 ***exo*-(±)-*cis*-5,6-Dihydroxy-2-azabicyclo[2.2.1]heptan-3-one 32** [39,40]

$C_6H_9O_3N$ M.W. 143



1 32

4.2.1 Method A: Oxidation with $KMnO_4$

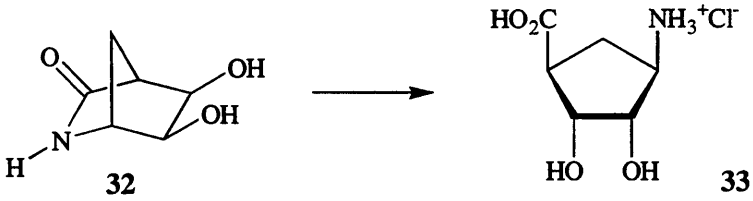
A solution of lactam **1** (206 mg, 1.9 mmol) in acetone (16 cm^3) was vigorously stirred at -78 °C. Potassium permanganate (1.1 M; 2 cm^3) was added dropwise (over a period of 1 h) until the purple colour persisted and the resulting solution was stirred at rt for 1 h. The resulted precipitate was filtered through Celite and washed with acetone (80 cm^3). The combined filtrate was evaporated *in vacuo* and the residue was dissolved in EtOH (40 cm^3) Further MnO_2 was allowed to precipitate overnight at 0 °C. Filtration followed by evaporation of the solvent *in vacuo* afforded a light brown solid corresponding to the title compound **32** (128 mg; 50%), R_f (EtOAc:MeOH 8:2) 0.5; m.p. (from Et_2O:MeOH) 168-169 °C (lit.[39] 169-170 °C). δ_H(250 MHz; D_2O) 8.45 (1 H, br s, NH), 4.15-3.95 (2 H, m, H-5,6), 3.8 (1 H, s, H-4), 2.65 (1 H, s, H-1), 2.05 (2 H, s, H-7,7'); δ_C(63 MHz; D_2O) 181.7 (C-3), 71.4 (C-6), 68.1 (C-5), 59.0 (C-1), 51.6 (C-4), 36.0 (C-7). m/z (FAB) 144.06609 (MH^+), requires 144.06607. ν_{max}(KBr)/cm^{-1} 3600-2700*s* (OH), 1750-1650*s* (C=O), 1470-1390m (CH).

4.2.2 Method B: Oxidation with OsO_4 [40]

To a solution of the (±) lactam **1** (1 g, 9.2 mmol) in ^{t}BuOH (12 cm^3) was added a solution of 4-methyl morpholine *N*-oxide in water (60% w/v; 1.80 cm^3), water (4 cm^3) followed by a solution of osmium tetraoxide in ^{t}BuOH 2.5% (wt/v; 0.42 cm^3). The mixture was stirred for 2 h at rt and then for 15 minutes at 50 °C. Once at rt, $Na_2S_2O_5$ (1 g) was added, and the resulting solution stirred for 15 minutes. The solid was filtered and the filtrate washed with acetone (3 x 25 cm^3). The mixture was evaporated *in vacuo* and azeotroped with water to remove the 4-methyl morpholine *N*-oxide. The residual syrup was dried by addition and evaporation of isopropyl alcohol (2 x 20 cm^3) *in vacuo* to give a white solid (1.1 g; 84%), identified as the title compound **32**, with similar spectral and physical properties to that obtained in method A (Section 4.1.1).

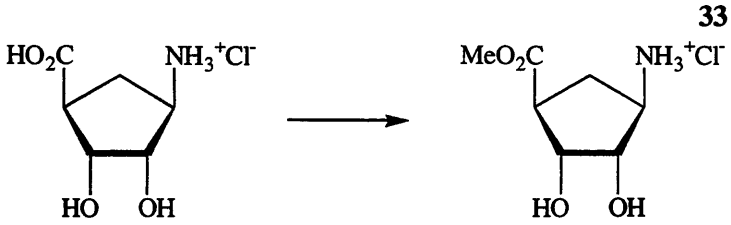
4.3 (±)-Methyl 4β-amino-2α,3α-dihydroxy-1β-cyclopentanecarboxylate hydrochloride 34 [39]

$C_7H_{14}O_4NCl$ M.W. 211



32 **33**

The dihydroxy lactam **32** (155 mg, 1.1 mmol) was heated under reflux in hydrochloric acid (3 M; 0.7 cm^3) for 3 h, followed by rotary evaporation to dryness to give a crude solid identified as **(±)-4β-amino-2α,3α-dihydroxy-1β-cyclopentanecarboxylic acid hydrochloride 33** as a crude solid. δ_H(250 MHz; D_2O) 4.20 (1 H, dd, $J_{1,2}$ 4.5, $J_{2,3}$ 5.7, H-2), 3.95 (1 H, dd, $J_{3,4}$ 7.0, H-3), 3.54-3.40 (1 H, m, H-4), 2.90-2.80 (1 H, m, H-1), 2.50-2.35 (1 H, m, H-5β), 1.75-1.60 (1 H, m, H-5α).

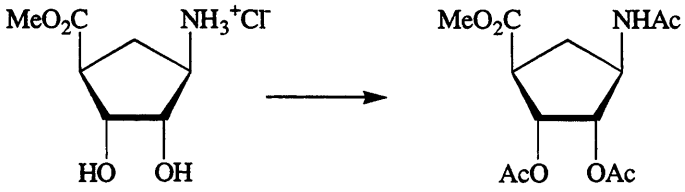


33 34

The crude acid **33** (341 mg, 1.7 mmol) was heated under reflux in a methanolic solution (1 M; 96 cm^3), for 3.5 h and the solvent was evaporated *in vacuo*. Trituration of the residue in Et_2O (25 cm^3) gave an oil which was purified by flash chromatography (EtOAc-CH_3OH 8:2) to afford the title compound **34** (308 mg; 84%) as a white solid; m.p. (from EtAcO:Et_2O) 147-148 °C (lit.[39] 146-147 °C). δ_H(250 MHz; D_2O) 4.25 (1 H, dd, $J_{2,3}$ 5.5, $J_{2,1}$ 7.0, H-2), 4.08 (1 H, dd, $J_{3,2}$ 5.5, $J_{3,4}$ 5.1, H-3), 3.71 (3 H, s, OCH_3), 3.54-3.44 (1 H, m, H-4), 2.80-2.68 (1 H, m, H-1), 2.40 (1 H, dt, $J_{5\beta,\alpha}$ 13.8, $J_{5\beta,1\text{-}4}$ 8.5, H-5β), 1.75 (1 H, dt, $J_{5\alpha,\beta}$ 13.8, $J_{5\alpha,1\text{-}4}$ 9.2, H-5α); δ_C(63 MHz; D_2O) 157 (C-6), 74.7 (C-2), 73.1 (C-3), 54.8 (OMe), 53.0 (C-4), 47.7 (C-1), 27.6 (C-5). m/z (FAB) (176.09226) $(M\text{-}Cl)^+$, requires 176.09226.

4.4 (±)-Methyl 4β-acetamido-2α,3α-diacetoxy-1β-cyclopentanecarboxylate 83 [40]

$C_{13}H_{19}O_{17}$ M.W. 301



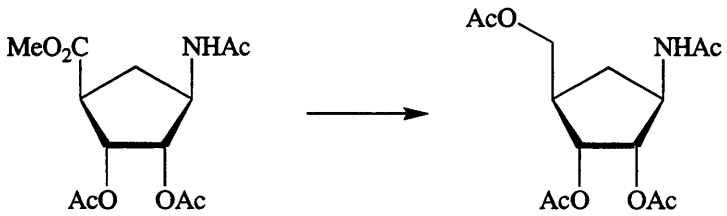
34 83

To a stirred suspension of **34** (1 g, 4.7 mmol) in pyridine (2.5 cm^3) cooled in an ice-bath, was added acetic anhydride (30.5 mmol; 2.9 cm^3), then warmed to rt for 4 h. Ice-water (15 cm^3) was added and this was followed by extraction with CH_2Cl_2, (3 x 20 cm^3). The organic extracts were dried and the solvent evaporated *in vacuo* to give the title compound **83** (1.05 g; 74%) as a colourless syrup, R_f (MeOH:EtOAc 2:8) 0.68; m.p. (from EtOAc:hexane) 115-116 °C (lit.[40] 116 °C). δ_H(250 MHz; $CDCl_3$) 6.30 (1H, br d, $J_{NH,4}$ 7.2, NH), 5.34 (1H, t, $J_{2,1}$ 4.7, $J_{2,3}$ 5.3, H-2), 5.09 (1H, t, J 5.3, H-3), 4.38 (1H, dt, $J_{NH,4}$ 7.2, J 7.5, H-4), 3.66 (3

H, s, CH_3O), 2.91 (1H, ddd, $J_{1,5\beta}$ 9.6, $J_{1,5\alpha}$ 6.9, $J_{1,2}$ 4.7, H-1), 2.59 (1 H, ddd, $J_{5\beta,\alpha}$ 13.8, $J_{5\beta,1}$ 9.4, $J_{5\beta,4}$ 6.3, H-5β), 1.99 (6 H, s, 2CH_3COO), 1.91 (3 H, s, CH_3CON), 1.61 (1 H, dt, $J_{\alpha,\beta}$ 13.8, $J_{1,5\alpha}$ 6.9, H-5α); δ_C(63 MHz; $CDCl_3$) 174.6 (*C*OOMe), 170.8 (O*C*OMe), 170.6 (O*C*OMe), 170.3 (N*C*OMe), 76.1 (C-2), 74.0 (C-3), 53.0 (OCH_3), 52.8 (C-1), 46.0 (C-4), 31.5 (C-5), 23.6 (*C*H_3CON), 21.1 (*C*H_3COO), 21.1 (*C*H_3COO). m/z (EI) 301.11616 (M^+), requires 301.11615.

4.5 (±)-1β-*N*-Acetyl-2α,3α-diacetoxy-4β-acetoxymethylcyclopentylamide 84 [39]

$C_{14}H_{21}O_7N$ M.W. 315



83 **84**

To a mixture of anhydrous calcium chloride (2.67 g, 24.0 mmol) and $NaBH_4$ (1.81 g, 48 mmol) dissolved in THF (52 cm^3) and stirred at rt for 1 h, was added a solution of **83** (2.45 g, 8.1 mmol) in THF (31 cm^3). The mixture was stirred for 19 h at rt and then cooled to 0 °C. Ice-water (20 cm^3) was added followed by HCl (3 M). The solution was concentrated under reduced pressure and coevaporated with MeOH (3 x 45 cm^3) and then with pyridine (2 x 18 cm^3). The residue was dissolved in pyridine (13 cm^3) and the inorganics filtered. The filtrate was cooled to 0 °C and acetic anhydride (0.22 mmol; 20.8 cm^3) was added. After 17 h at rt, the mixture was concentrated and MeOH (37 cm^3) added and refluxed for 15 minutes. The solution was evaporated *in vacuo* to give the title compound **84** (760 mg; 73%) as a colourless oil, R_f (MeOH:EtOAc 2:8) 0.30; m.p. (EtOAc:hexane) 94-95 °C (lit.[40] 94-95 °C). δ_H(250 MHz; $CDCl_3$) 5.96 (1 H, br d, $J_{NH,1}$ 6.6, NH), 5.11-5.01 (2 H, m, H-2,3), 4.46-4.33 (1 H, m, H-1), 4.16-4.03 (2 H, 2dd, $J_{6,6'}$ 11.2, $J_{6,4}$ 5.3, H-6,6'), 2.57-2.39 (2 H, m, H-4,5β), 2.08 (3 H, s, MeCOO), 2.07 (3 H, s, MeCOO), 2.06 (3 H, s, MeCON), 1.25-1.13 (1 H, m, H-5α); δ_C(63 MHz; $CDCl_3$) 169.9 (*C*ON), 169.4 (2*C*OOMe), 74.3 (C-3),

71.6 (C-2), 63.6 (C-6), 51.7 (C-1), 39.1 (C-4), 29.2 (C-5), 22.2 (CH_3CON), 19.8 (2 CH_3CO), 19.7 (CH_3CO). m/z (EI) 315.13181 (M^+), requires 315.13180.

4.6 (±)-1β-Acetamido-2α,3α-dihydroxy-4β-hydroxymethylcyclopentane **82** [94]

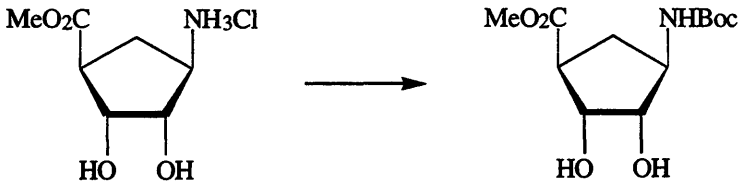
$C_8H_{15}O_4N$ M.W. 189

84 **82**

Compound **84** (1.95 g, 6.2 mmol) was dissolved in MeOH (24 cm^3) and NaOH (1.5 M; 13 cm^3) was added dropwise. The mixture was stirred at rt for 2 h followed by addition of HCl (2 M). Evaporation of the solvent *in vacuo* gave a residue which was purified by flash column chromatography (MeOH:EtOAc 2:1→1:1) to give a white solid identified as the title compound **82** (1.17 g; 96%), R_f (MeOH:EtOAc 1:1) 0.65; m.p. (from EtOH) 116-117 °C (lit.[94] 117-117.5 °C). δ_H(250 MHz; D_2O) 3.98 (1 H, dt, $J_{1,3}$ 8.8, $J_{1,5\alpha}$ 8.8, H-1), 3.78-3.68 (2 H, m, H-2,3), 3.55-3.42 (2 H, 2dd, $J_{6,6'}$ 11.2, $J_{6,4}$ 6.3, H-6,6'), 2.14 (1 H, dt, $J_{5\beta,\alpha}$ 13.2, $J_{5\beta,1\text{-}4}$ 8.5, H-5β), 2.07-1.96 (1 H, m, H-4), 1.89 (3 H, s, CH_3CO), 1.02 (1 H, ddd, $J_{5\alpha,\beta}$ 13.2, $J_{5\alpha,1\text{-}4}$ 8.9, H-5α). m/z (FAB) 190.10793 (MH^+), requires 190.10793.

4.7 (±)-Methyl 4β-*N*-*tert*-butoxycarbonyl-2α,3α-dihydroxy-1β-cyclopentanecarboxylate 35

$C_{12}H_{21}O_6N$ M.W. 275

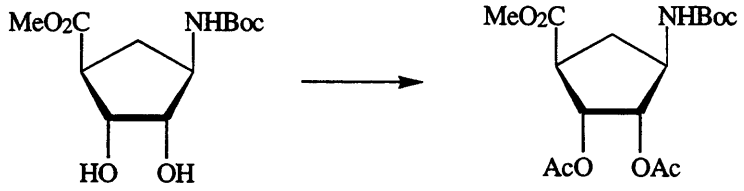


34 35

To a solution of the aminoester **34** (206 mg, 0.9 mmol) in dioxane:water 1:1 (1.4 cm^3) was added triethylamine (0.14 cm^3) and Boc-on (240 mg, 0.9 mmol). After 24 h at rt, the mixture was poured into water (15 cm^3) and extracted with ethyl acetate (3 x 15 cm^3). The combined extracts were washed with citric acid (5% w/v), dried and the solvent evaporated *in vacuo* to obtain a crude product which was purified by flash chromatography (EtOAc:MeOH 9:1) giving the *title compound* **35** (239 mg; 89%) as a white solid, R_f (Et_2O:MeOH 8:2) 0.7; m.p. (from hexane:EtOAc) 78-80 °C. Found: C, 52.05; H, 7.68; N, 5.37. $C_{12}H_{21}0_6N$ requires C, 52.41; H, 7.69; N, 5.09. δ_H(250 MHz; $CDCl_3$) 4.91 (1 H, br s, NH), 4.57 (1 H, br s, OH), 4.27 (1 H, t, $J_{2,3}$ 4.4, H-2), 3.91-3.90 (2 H, m, H-3,4), 3.72 (3 H, s, OCH_3), 3.14 (1 H, br s, OH), 2.94 (1 H, ddd, $J_{1,5'}$ 8.4, $J_{1,5}$ 6.0, $J_{1,2}$ 3.9, H-1), 2.49-2.37 (1 H, m, H-5β), 1.75-1.63 (1 H, m, H-5α), 1.45 (9 H, s, ^{t}Bu); δ_C(63 MHz; $CDCl_3$) 174.48 (HC*C*=O), 157.02 (C=ONH), 80.41 (C), 76.47 (C-3), 74.30 (C-2), 56.33 (C-4), 52.12 (CH_3O), 47.92 (C-1), 29.99 (C-5), 28.17 ($3CH_3$). *m/z* 276.14471 (MH^+), requires 276.14471. ν_{max}(sol.)/cm^{-1} 3420w (OH), 1730*s* (C=O), 1500*s* (OH), 1200*s* (CO).

4.8 (±)-Methyl 4β-*N-tert*-butoxycarbonyl-2α,3α-diacetoxy-1α-cyclopentanecarboxylate 36

$C_{16}H_{25}O_8N$ M.W. 359

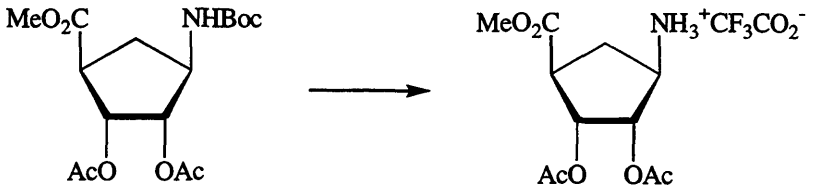


35 **36**

A stirred suspension of **35** (239 mg, 0.9 mmol) in pyridine (0.50 cm^3) and 4-DMAP (214 mg, 1.8 mmol) was cooled in a ice-bath and treated with acetic anhydride (1.8 mmol; 0.17 cm^3). Reaction was complete after 8 h at rt. Ice-water (2 cm^3) was added and the product was extracted with EtOAc (3 x 25 cm^3). The combined organic extracts were washed with saturated aqueous Na_2CO_3, dried and concentrated *in vacuo* to give the *title compound* **36** (265 mg; 85%) as a white solid, R_f (EtOAc:CH_3OH 8:2) 0.9; m.p. (from hexane-EtOAc) 105-106 °C. Found: C, 53.60; H, 7.10; N, 4.17. $C_{16}H_{25}O_8N$ requires C, 53.53; H, 7.02; N, 3.90. δ_H(250 MHz; $CDCl_3$) 5.35 (1 H, t, *J* 5.3, H-2), 5.06 (1 H, t, *J* 5.8, H-3), 4.88 (1 H, br d, $J_{NH,4}$ 5.7, NH), 4.09 (1 H, br t, *J* 7.0, H-4), 3.66 (3 H, s, MeO), 2.88 (1 H, ddd, $J_{1,5\beta}$ 9.8, $J_{1,5\alpha}$ 7.0, $J_{1,2}$ 5.0, H-1), 2.54 (1 H, dt, $J_{5\beta,\alpha}$ 13.8, $J_{5\beta,1\text{-}4}$ 9.3, H-5β), 1.99 (3 H, s, OAc), 1.98 (3 H, s, OAc), 1.62 (1 H, dt, $J_{5\alpha,\beta}$ 13.8, $J_{5\alpha,1\text{-}4}$ 7.0, H-5α), 1.37 (9 H, s, ^{t}Bu); δ_C(63 MHz; acetone-d_6) 174.1 (RC=O), 170.6 (*C*OMe), 170.5 (*C*OMe), 156.5 (*C*ONH), 79.6 (C), 76.8 (C-2), 73.8 (C-3), 54.6 (C-4), 52.8 (CH_3O), 46.5 (C-1), 35.8 (CH_3CO), 31.4 (C-5), 28.9 (CH_3O), 21.0 ($3CH_3$). *m/z* (FAB) 360.16580 (MH^+), requires 360.16584. ν_{max}(sol.)/cm^{-1} 3400*s* (N-H), 2900*s* (C-H), 1750*s* (C=O), 1720*s* (C=O), 1420*s* (C-H), 1280-1250*s* (C-O).

4.9 (±)-2α,3α-Diacetoxy-4β-methoxycarbonylcyclopent-1β-ylammonium trifluoroacetate 31

$C_{13}H_{18}O_8NF_3$ M.W. 373

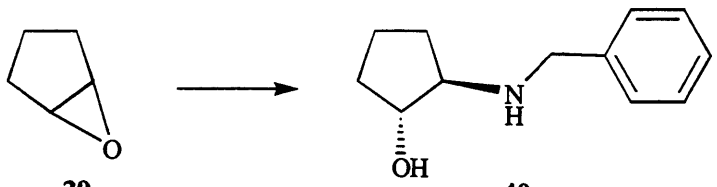


36 **31**

The compound **36** (40 mg, 0.11 mmol) was dissolved in CH_2Cl_2 (0.23 cm^3) and treated with trifluoroacetic acid (1.45 mmol; 0.11 cm^3) and triethylsilane (0.28 mmol; 0.045 cm^3) at rt under nitrogen. After 4 h, the solvent was removed and the oily yellow residue (65 mg) triturated with diethyl ether. The precipitated product was isolated by filtration, washed with more ether and the solvent evaporated *in vacuo* to give the *title compound* **31** (25 mg; 87%) as a white solid, R_f (EtOAc:MeOH 8:2) 0.26; m.p. (from EtOAc) 145-146 °C. Found: C, 41.49; H, 4.83; N, 4.14. $C_{13}H_{18}O_8NF_3$ requires C, 41.82; H, 4.83; N, 3.75. δ_H(250 MHz; D_2O) 5.47 (1 H, t, *J* 5.5, H-2), 5.22 (1 H, t, *J* 7.7, H-3), 3.89 (1 H, q, *J* 8.2, H-4), 3.73 (3 H, s, MeO), 3.26 (1 H, ddd, $J_{1,5\alpha}$ 9.6, $J_{1,5\beta}$ 8.2, $J_{1,2}$ 5.5, H-1), 2.65 (1 H, dt, $J_{5\alpha,\beta}$ 13.5, $J_{5\beta,4\text{-}1}$ 8.2, H-5β), 2.10 (6 H, s, $2CH_3$), 1.94 (1 H, dt, $J_{5\alpha,\beta}$ 13.5, $J_{5\alpha,1\text{-}4}$ 9.6, H-5α); δ_C(100.6 MHz; D_2O) 174.29 (*C*OOMe), 173.31 (CH_3CO), 173.18 (CH_3CO), 74.46 (C-3), 73.00 (C-2), 53.46 (CH_3OCO), 53.00 (C-1), 45.82 (C-4), 30.62 (CH_3COO), 27.72 (C-5), 20.36 (CH_3COO). m/z (FAB) 260.11340 (MH^+), requires 260.11341. ν_{max}(sol.)/cm^{-1} 1750*s* (C=O), 1250*s* (CO), 1100*s* (C-N).

4.10 (±)-*trans*-2-Benzylamino-1-cyclopentanol 40 [43]

$C_{12}H_{17}ON$ M.W. 191

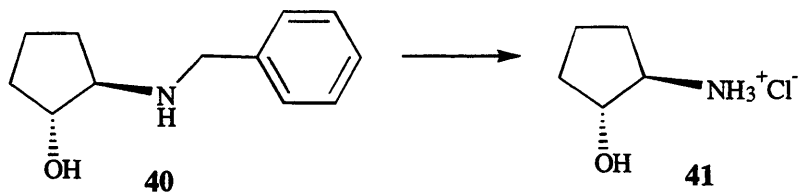


39 40

Cyclopentene epoxide **39** (35.7 mmol; 3.10 cm^3) was dissolved in water (0.6 cm^3) and benzylamine (36.6 mmol; 4.0 cm^3) was added. After 6 h of reflux between 110-120 °C, the solution was allowed to cool to rt and then water (5 cm^3) was added. The aqueous mixture was extracted with Et_2O (3 x 10 cm^3), the organic extracts were combined and dried. The solvent was removed *in vacuo* and the residue was purified by flash chromatography (CH_2Cl_2:NEt_3 9.5:1) to give the title compound **40** (5.50g; 82%) as a white solid, R_f (CH_2Cl_2:NEt_3:MeOH 8.9:1:0.1) 0.56; m.p. (from hexane) 60-61 °C (lit.[96] 60-63 °C). δ_H(250 MHz; $CDCl_3$) 7.45 (5 H, m, C_6H_5), 3.95-3.85 (3 H, m, H-1, NCH_2), 3.0-2.9 (1 H, m, H-2), 2.15-1.90 (2 H, m, H-5,3), 1.85-1.45 (5 H, m, H-5',3,4, NH, OH), 1.40-1.25 (1 H, m, H-4'). *m/z* (EI) 191.13103 (M^+), requires 191.13101.

4.11 (±)-*trans*-2-Hydroxycyclopentylammonium hydrochloride 41 [43]

$C_5H_{12}ONCl$ M.W.137



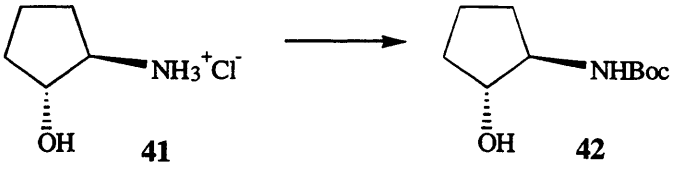
40 41

The product **40** (0.5 g, 2.6 mmol) was dissolved in EtOH (18 cm^3) and palladium-on-charcoal (0.45 g; 5%) added. The mixture was shaken under hydrogen in a Parr hydrogenator at 20 psi and rt for 7 h. The reaction mixture was filtered over Celite under nitrogen atmosphere. The filtrate was acidified with HCl (2 M; 3 cm^3) and the solution

evaporated *in vacuo* to give a white solid identified as **41** (0.33 g; 92%); m.p. (from Et_2O:EtOH) 161-162 °C (lit.[96] 161-163 °C). δ_H(250 MHz; D_2O) 4.13-4.06 (1 H, m, H-1), 3.37-3.26 (1 H, m, H-2), 2.17-1.89 (2 H, m, H-3,5), 1.77-1.45 (4 H, m, H-3', 5',4, 4'). *m/z* (FAB) $(M\text{-}Cl)^+$ 102.09189, requires 102.09189.

4.12 (±)-*trans*-2-*N*-*tert*-butoxycarbonylamino-1-cyclopentanol 42 [95]

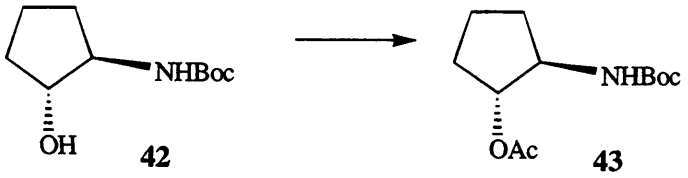
$C_{10}H_{19}O_3N$ M.W. 201



To a solution of **41** (2.3 g, 16.8 mmol) in triethylamine (18.2 mmol; 2.5 cm^3) and water (11.7 cm^3) was added dioxane (11.7 cm^3) and the crystalline Boc-on (4.20 g, 17.0 mmol). The mixture was stirred at rt for 24 h and then refluxed at 80 °C for 1 h. After warming to rt it was poured into water (30 cm^3). The organic product was extracted with EtOAc (3 x 30 cm^3) and the combined extracts were washed with citric acid (5% w/v; 30 cm^3), dried and the solvent removed *in vacuo*. The crude product was purified by flash cromatography (CH_2Cl_2:AcOEt 1:1) to give the solid identified as the title compound **42** (2.5 g; 74%); m.p. (from hexane) 97-98 °C (lit.[95] 96-97 °C), R_f (EtOAc:CH_2Cl_2 1:1) 0.58. δ_H(250 MHz; $CDCl_3$) 4.61 (1 H, br s, NH), 3.96-3.88 (1 H, m, H-1), 3.61-3.50 (1H, m, H-2), 2.04-1.89 (2 H, m, H-5,3), 1.72-1.45 (4 H, m, OH, H-5',3',4), 1.38 (9 H, s, ^{t}Bu), 1.33-1.16 (1 H, m, H-4'). *m/z* (EI) 201.13648 (M^+), requires 201.13649.

4.13 (±)-*trans*-1-Acetoxy-2-*N*-*tert*-butoxycarbonylamino-1-cyclopentane 43

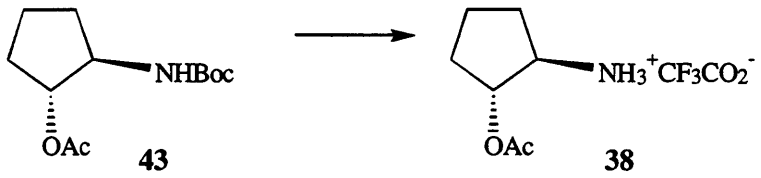
$C_{12}H_{21}O_4N$ M.W. 243



A solution of **42** (568 mg, 3.1 mmol) in pyridine (0.52 cm^3) and 4-DMAP (375 mg, 3.1 mmol) was cooled at 0 °C for 10 minutes and acetic anhydride (6.4 mmol; 0.52 cm^3) was added. The mixture was stirred at rt and after 6 h, it was evaporated to half of the volume. Water (5 cm^3) was added and it was extracted with EtOAc (3 x 15 cm^3). The organic extracts were combined and washed with Na_2CO_3 (2 x 25 cm^3), dried and the solvent evaporated *in vacuo* to give the *title compound* **43** (615 mg; 88%) as a colourless oil, R_f (CH_2Cl_2:EtOAc 9:1) 0.68. δ_H(250 MHz; $CDCl_3$) 4.94 (1 H, dt, $J_{2,1}$ 7.2, $J_{1,5}$ 5.2, H-2), 4.67 (1 H, br s, NH), 3.89 (1 H, dt, $J_{2,1}$ 7.2, $J_{2,3}$ 6.6, H-1), 2.23-1.97 (2 H, m, H-3,5), 2.04 (3 H, s, CH_3), 1.81-1.58 (3 H, m, H-3,4,4'), 1.44 (9 H, s, ^{t}Bu),1.46-1.41 (1 H, m, H-5); δ_C(63 MHz; $CDCl_3$) 171.4 (C=O), 155.8 (NC=O), 80.0 (C-2), 79.9 (C), 57.5 (C-1), 31.0 (C-3), 30.2 (C-2), 28.7 ($3CH_3$), 21.6 (CH_3), 21.2 (C-4). m/z 244.15480 (MH^+), requires 244.15488. ν_{max}(sol.)/cm^{-1} 3040m (N-H), 2980m (C-H), 1730-1710m (C=O), 1420m (C-N), 1100*s* (C-O).

4.14 (±)-*trans*-2-Acetoxy-cyclopentylammonium trifluoroacetate 38

$C_9H_{14}O_4NF_3$ M.W. 257

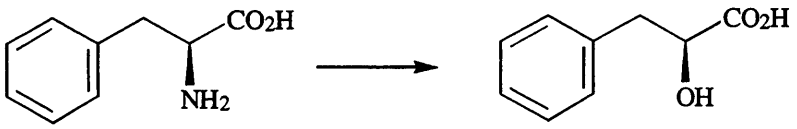


To a stirred solution of **43** (2.28 g, 10.0 mmol) in CH_2Cl_2 (5 cm^3) was added triethylsilane (6.2 mmol; 1 cm^3) and trifluoroacetic acid (25.9 mmol; 2 cm^3) and the mixture was stirred

at rt. After 6 h the solution was evaporated *in vacuo* and the crude product obtained was triturated with Et_2O (15 cm^3). The solid product was isolated by filtration, washed with Et_2O (7 cm^3) and the precipitate dried *in vacuo* to give the *title compound* **38** (1.95 g; 75%) as a white powder, R_f (EtOAc:CH_2Cl_2 9:1) 0.51; m.p. (from C_6H_{12}:EtOAc) 92-93 °C. Found: C, 41.52; H 5.28; N, 5.56. $C_9H_{14}O_4NF_3$ requires C, 42.02; H, 5.44; N, 5.45. δ_H(250 MHz; $CDCl_3$) 8.26 (3 H, br s, NH_3^+), 4.93-4.86 (1 H, m, H-2), 3.32-3.24 (1 H, m, H-1), 2.10-1.99 (2 H, m, H-3,5), 1.93 (3 H, s, CH_3), 1.70-1.55 (4 H, m, H-5',3',4,4'); δ_C(63 MHz; $CDCl_3$) 173 (C=O), 79.5 (C-2), 58.3 (C-1), 31.0 (C-3), 29.8 (C-5), 22.3 (C-4), 21.2 (CH_3). δ_F(235 MHz; $CDCl_3$) - 76.31 (CF_3). *m/z* (FAB) 144.10243 (M^+), requires 144.10245. ν_{max}(sol.)/cm^{-1} 3200m (N-H), 3000-2800*s* (C-H), 1760*s* (C=O), 1530m (N-H), 1400*s* (C-N), 1270*s* (C-O).

4.15 (-)-(2*S*)-Hydroxy-3-phenylpropionic acid **46** [96]

$C_9H_{10}O_3$ M.W. 166



45 **46**

(2*S*)-Amino-3-phenylpropionic acid **45** (40 mg, 0.24 mmol) was dissolved in HCl (0.5 M; 1 cm^3) cooled to 0 °C and stirred with silver nitrite (111 mg, 0.7 mmol) for 6 h. The precipitate was removed by filtration, washed with water (2 cm^3) and the filtrate extracted with Et_2O (3 x 10 cm^3). The organic extracts were dried and the solvent evaporated *in vacuo* to give the title compound **46** (20 mg; 45%) as a white solid, R_f (CH_2Cl_2:MeOH 2:1) 0.26. δ_H(250 MHz; MeOH) 7.17-7.08 (5 H, m, C_6H_5), 4.23 (1 H, dd, $J_{1,2'}$ 8.0, $J_{1,2}$ 4.4, H-1), 2.99 (1 H, dd, $J_{2',2}$ 14.0 and $J_{2',1}$ 8.2, H-2'), 2.79 (1 H, dd, $J_{2,2'}$ 13.8, $J_{2,1}$ 8.2, H-2). *m/z* (FAB) 167.07083 (MH^+), requires 167.07082.

4.16 (-)-(2*S*)-Acetoxy-3-phenylpropionic acid 47 [97]

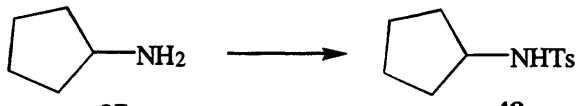
$C_{11}H_{12}O_4$ M.W. 208

45 **47**

To a solution of **45** (80 mg, 0.5 mmol) in glacial acetic acid (1.4 cm^3) stored between 0-10 °C, was added portionwise sodium nitrite (66 mg, 0.07 mmol). After 7 h the mixture was removed from the bath and concentrated *in vacuo*. Water was added (10 cm^3) and the organic product extracted with Et_2O (3 x 10 cm^3). The organic extracts were dried and the solvent removed *in vacuo* to give the title compound **47** (40 mg; 40%) as a colourless oil, R_f (MeOH) 0.85. δ_H(250 MHz; $CDCl_3$) 7.28-7.21 (5 H, m, C_6H_5), 5.18 (1 H, dd, $J_{1,3}$ 8.8, $J_{1,3'}$ 4.4, H-1), 3.18 (1 H, dd, $J_{3',1}$ 4.1 and $J_{3,'3}$ 14.5, H-3'), 3.05 (1 H, dd, $J_{3,1}$ 8.8, $J_{3,3'}$ 14.5, H-3), 2.02 (3 H, s, CH_3). *m/z* (FAB) 209.08137 (MH^+), requires 209.08138.

4.17 *N*-Cyclopentyl toluene-4-sulfonamide 48 [98]

$C_{12}H_{17}O_2NS$ M.W. 239



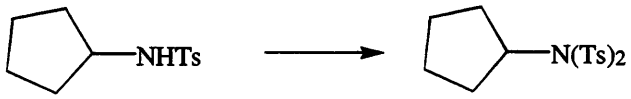
37 **48**

To a solution of cyclopentylamine **37** (1.2 mmol; 0.12 cm^3) in THF (3 cm^3) was added triethylamine (1.2 mmol; 0.112 cm^3). The mixture was cooled for ten minutes and *p*-TsCl (238 mg, 1.2 mmol) was added slowly. After 1.5 h, HCl (1 M; 1 cm^3) was added and the product was extracted with Et_2O (3 x 16 cm^3). The combined extracts were washed with $NaHCO_3$ (5% w/v; 15 cm^3), dried and the solvent removed *in vacuo* to give the title compound **48** (230 mg; 82%) as a white solid, R_f (CH_2Cl_2-EtOAc 9.6:0.4) 0.56; m.p. (from hexane:Et_2O) 82-83 °C (lit.[100] 84-85 °C). δ_H(250 MHz; $CDCl_3$) 7.76 (2 H, d, *J* 8.2, 2H-Ar.), 7.30 (2 H, d, *J* 8.2, 2H-Ar.), 4.61-4.45 (1 H, br s, NH), 3.65-3.52 (1 H, dquint, $J_{1,NH}$ 6.9,

$J_{1,2\text{-}5}$ 6.6, H-1), 2.43 (3 H, s, CH_3), 1.83-1.31 (8 H, m, H-2,2',3,3',4,4',5,5'); δ_C(63 MHz; $CDCl_3$) 134.6 (C-*p*), 138.3 (C-S), 130.0 (2C-*m*), 127.5 (2C-*o*), 55.5 (C-1), 33.8 (C-2,5), 23.5 (C-3,4), 21.9 (CH_3). *m/z* (FAB) 240.10584 (MH^+), requires 240.10583.

4.18 *N,N*-Cyclopentyl di-(toluene-4-sulfonamide) 49

$C_{19}H_{23}O_4NS_2$ M.W. 393

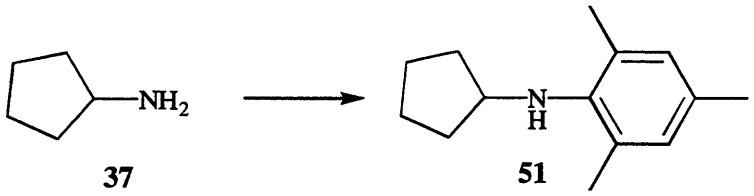


48 **49**

To a solution of **48** (500 mg, 2.1 mmol) in DMF (3 cm^3) was slowly added NaH (60% oil dispersion; 2.1 mmol). A further aliquot of DMF (1.5 cm^3) was added to dissolve the salt completely. After 2 h, *p*-TsCl (450 mg, 2.4 mmol) was added in small portions. The mixture was stirred at rt for 17 h and then poured into water (12 cm^3). The expected product was collected by suction filtration and washed with water (3 cm^3). The cloudy aqueous DMF filtrate was extracted with $CHCl_3$ (2 x 15 cm^3) and the combined extracts washed with water (2 x 10 cm^3). This crude product was added to the solid collected by filtration to yield the *title compound* **49** (638 mg; 78%) as a white solid. R_f (CH_2Cl_2) 0.56; m.p. (from petroleum ether 40-60:Et_2O) 130 °C. Found: C, 58.07; H, 5.68; N, 3.65. $C_{14}H_{21}O_2NS_2$ requires C, 58.01; H, 5.85; N, 3.56. δ_H(250 MHz; $CDCl_3$) 7.85 (4 H, d, *J* 8.2, 2H-Ar.), 7.27 (4 H, d, *J* 8.2, 2H-Ar.), 4.42-4.45 (1 H, quint, $J_{1,2\text{-}5}$ 8.8, H-1), 2.40 (6 H, s, $2CH_3$), 2.10-1.99 (2 H, m, H-2,5), 1.74-1.61 (2 H, m, H-2',5'), 1.39-1.35 (2 H, m, H-3,4), 0.82-0.74 (2 H, m, H-3',4'); δ_C(63 MHz; $CDCl_3$) 144.6 (C-*p*), 138.0 (C-S), 129.6 (2C-*m*), 128.1 (2C-*o*), 63.0 (C-1), 30.0 (C-3,4), 24.0 (C-2,5), 21.7 ($2CH_3$). *m/z* (FAB) 394.11467 (MH^+), requires 394.11468. ν_{max}(sol.)/cm^{-1} 2940-2860m (C-H), 1600m (C=C), 1360*s*, (C-N), 1160*s* (S=O).

4.19 *N*-Cyclopentyl-2,4,6-mesitylenesulfonamide 51

$C_{14}H_{21}NO_2S$ M.W. 267

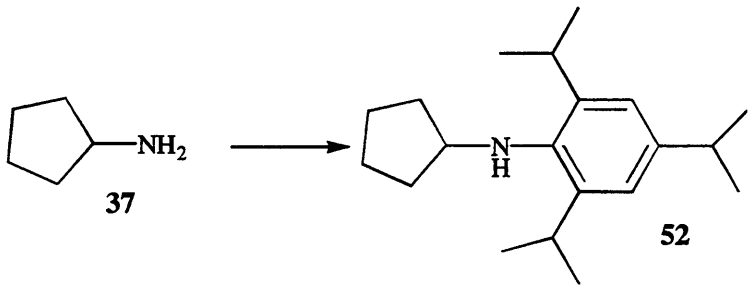


37 51

To a solution of cyclopentylamine **37** (87 mg, 1.0 mmol) in THF (2 cm^3) was added triethylamine (1.1 mmol; 0.15 cm^3). The solution was cooled at 0 °C and 2-mesitylenesulfonyl chloride (234 mg, 1.1 mmol) was slowly added. After stirring for 10 h, on complete reaction, HCl (1 M; 1 cm^3) was added and the solution extracted with Et_2O (3 x 10 cm^3). The organic extracts were combined and washed with $NaHCO_3$ (5% w/v; 2 x 5 cm^3), dried and the solvent evaporated *in vacuo* to give the *title compound* **51** as a white solid (247 mg; 91%), R_f (CH_2Cl_2) 0.43; m.p. (from Et_2O) 115° C. Found: C, 62.82; H 7.29; N, 5.28; S, 11.19. $C_{14}H_{21}O_2NS$ requires C, 62.89; H, 7.91; N, 5.24; S, 11.99. δ_H(250 MHz; $CDCl_3$) 6.88 (2 H, s, H-3,5 Ar.), 4.38 (1 H, d, $J_{NH,1}$ 6.9, NH), 3.55-3.42 (1 H, dt, $J_{1,2}$ 6.6, H-1), 2.57 (6 H, s, $2CH_3$-Ar.), 2.23 (3 H, s, CH_3-Ar.), 1.74-1.66 (2 H, m, H-2,5), 1.65-1.21 (8 H, m, H-2',3,3',4,4',5'); δ_C(63 MHz; $CDCl_3$) 142.4 (*C-p*), 139.3 (*2C-o*), 135.0 (*C*-S), 55.2 (C-1), 33.7 (C-2,5), 23.6 (C-3,4), 23.3 ($2CH_3$-*o*), 21.3 (CH_3-*p*). *m/z* (EI) 267.12930 (M^+), requires 267.12930. ν_{max}(sol.)/cm^{-1} 2960m (C-H), 1600*s* (C=C), 1330m (C-N), 1150*s* (S=O).

4.20 *N*-Cyclopentyl-2,4,6-triisopropyl benzenesulfonamide 52

$C_{20}H_{33}NO_2S$ M.W. 351



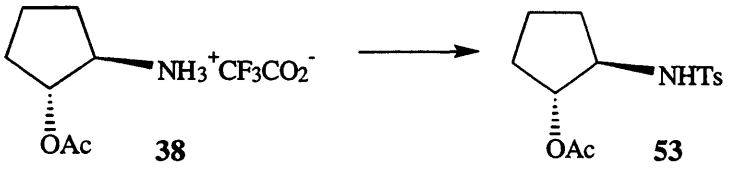
37 52

To a solution of **37** (87 mg, 1.0 mmol) in THF (2 cm^3) was added triethylamine (1.1 mmol, 0.15 cm^3). The solution was cooled to 0 °C and 2,4,6 triisopropylbenzenesulfonyl chloride

(327 mg, 1.1 mmol) was slowly added. After stirring for 10 h, HCl (1 M; 1.5 cm^3) was added and extracted with Et_2O (3 x 10 cm^3). The organic extracts were combined and washed with $NaHCO_3$ (5% w/v; 2 x 5 cm^3), dried and the solvent removed *in vacuo*, to give the *title compound* **52** (256 mg; 72%), as a white solid, R_f (CH_2Cl_2) 0.57; m.p. (from Et_2O) 146° C. Found: C, 68.50; H 8.59; N, 4.00; S, 9.13. $C_{20}H_{33}O_2NS$ requires C, 68.33; H, 9.46; N, 3.98; S, 9.12. δ_H(250 MHz; $CDCl_3$) 7.15 [2 H, s, (H-3,5-Ar.)], 4.30-4.09 (3 H, br s + hept., 2C*H*-isop.), 3.66 (1 H, dt, $J_{1,2}$ 6.6, H-1), 2.90 (1 H, hept, $J_{H,CH3}$ 6.8, CH-isop.), 2.90 (1 H, hept., C*H*-isop.), 1.84-1.77 (2 H, m, H-2,5), 1.66-1.35 (6 H, m, H-2',5',3,3',4,4'), 1.28 (12 H, s, 4CH_3-*o*), 1.25 (6 H, s, 2CH_3-*p*); δ_C(63 MHz; $CDCl_3$) 152.6 (C-*p*), 150.1 (2C-*o*), 133.2 (*C*-S), 123.8 (2C-*m*), 54.8 (C-1), 34.1 (CH-*p*), 33.6 (C-2,5), 29.6 (2C-*o*), 24.8 (4CH_3-*o*), 23.6 (2CH_3-*p*), 23.3 (C-3,4). *m/z* (EI) 351.22321 (M^+), requires 351.22320. ν_{max}(sol.)/cm^{-1} 2960-2860*s* (C-H), 1640*s* (C=C), 1360*s* (C-N), 1150*s*, (S=O).

4.21 (±)-*trans*-1-Acetoxy-2-*N*-*p*-toluene-4-sulfonamido cyclopentane 53

$C_{14}H_{19}O_4NS$ M.W. 297

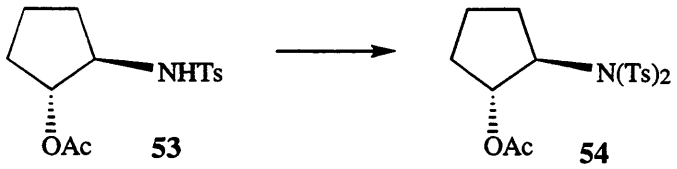


To a solution of **38** (500 mg, 2.0 mmol) in THF (7 cm^3) was added dry triethylamine (4.0 mmol, 0.5 cm^3). The mixture was cooled to 0 °C for 10 minutes and *p*-toluenesulfonyl chloride (37 mg, 2.0 mmol) was added portionwise. After 17 h and it was poured over HCl (2 M; 16 cm^3). The product was extracted with Et_2O (3 x 20 cm^3), the organic extracts combined, dried and the solvent removed *in vacuo*. The crude residue was purified by flash chromatography (CH_2Cl_2:Et_2O 98:2) to give the *title compound* **53** (510 mg; 88%) as a colourless oil, R_f (CH_2Cl_2:Et_2O 9:1) 0.42. δ_H(250 MHz; $CDCl_3$) 7.76 (2 H, d, J_{AB} 8.6, 2H-*o*), 7.30 (2 H, d, J_{AB} 8.2, 2H-Ar), 5.40-4.90 (1 H, d, $J_{NH,1}$ 5.5, NH), 4.85 (1 H, dt, $J_{2,1}$ 5.5, $J_{2,3}$ 7.6, H-2), 3.46 (1 H, ddt, $J_{1,2}$ 5.7, $J_{1.5}$ 6.5, H-1), 2.42 (3 H, s, CH_3 Arom.), 2.07-1.93 (2 H, m, H-3,5), 1.88 (3 H, s, CH_3CO), 1.72- 1.43 (4 H, m, H-3',5',4, 4'); δ_C(63 MHz; $CDCl_3$) 171 (C=O), 143.4 (*C*-Me), 137.4 (*C*-S), 129.6 (2*C*-*m*), 127.2 (2*C*-*o*), 79.6 (C-2), 59.8 (C-1), 31.2 (C-3), 29.4 (C-5), 21.5 (CH_3), 20.9 (C-4), 20.8 (*C*H_3CO). *m/z* (EI)

298.11129 (MH^+), requires 298.11131. ν_{max}(sol.)/cm^{-1} 3040-2960m (C-H), 1730*s* (C=0), 1600w (C=C), 1400*s* (C-N), 1250*s* (C-O), 1160*s* (S=O).

4.22 (±)-*trans*-1-Acetoxy-*N*,*N*-di-toluene-4-sulfonamido cyclopentane 54

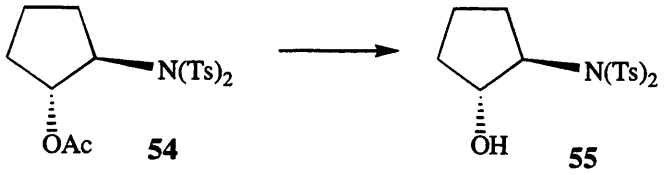
$C_{21}H_{25}O_6NS_2$ M.W. 451



To a solution of **53** (600 mg, 2.0 mmol) in DMF (4.5 cm^3) cooled to 0 °C for 5 minutes, NaH (60% oil dispersion; 2.1 mmol) was added in portions. After 23 h at rt, water (14 cm^3) was added and the product extracted with CH_2Cl_2 (3 x 20 cm^3), dried the organic extracts and the solvent evaporated *in vacuo*. The crude product (773 mg) was purified by flash chromatography (CH_2Cl_2) to give a colourless oil identified as the *title compound* **54** (474 mg; 52%), R_f (CH_2Cl_2) 0.41. δ_H(250 MHz; $CDCl_3$) 7.85 (4 H, d, *J* 8.2, H-4 Ar.), 7.27 (4 H, d, *J* 8.2, H-4 Ar.), 5.61 (1 H, dt, $J_{2,1}$ 5.7, $J_{2,3}$ 3.2, H-2), 4.31 (1 H, dt, $J_{1,2}$ 5.7, $J_{1,5}$ 3.9, H-1), 2.38 (6 H, s, $2CH_3$ Arom.), 2.18-1.98 (2 H, m, H-3,5), 1.82-1.73 (4 H, m, H-4, OAc), 1.57-1.41 (2 H, m, H-3',5'), 0.85-0.75 (1 H, m, H-4'); δ_C(63 MHz; $CDCl_3$) 170.1 (C=0), 144.8 (2C-*p*), 137.4 (2*C*-S), 129.6 (4C-*m*), 128.5 (4C-*o*), 77.8 (C-2), 67.5 (C-1), 31.2 (C-3), 29.9 (C-5), 22.2 (C-4), 21.7 ($2CH_3$), 20.9 (CH_3). *m/z* (FAB) 452.12018 (MH^+), requires 452.12016. ν_{max}(sol.)/cm^{-1} 3000m (C-H), 1730m (C=O), 1600m (C=C), 1370m (C-N), 1240m (C-O), 1170*s* (S=O).

4.23 (±)-*trans*-2-*N*,*N*-Di-*p*-toluene-4-sulfonamido-1-cyclopentanol **55**

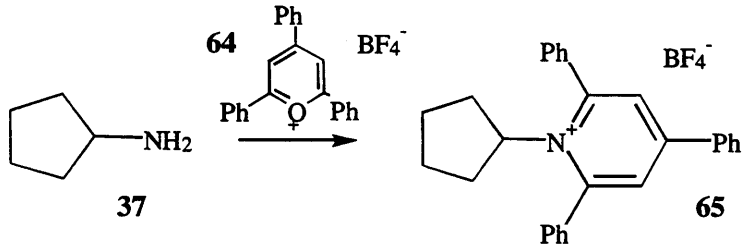
$C_{19}H_{23}O_5NS_2$ M.W. 409



Compound **54** (270 mg, 0.6 mmol) was dissolved in MeOH (2.2 cm^3) and reacted with NaOH (1 M; 0.63 cm^3). After 24 h at rt MeOH was removed *in vacuo*. The residue was diluted with water (10 cm^3) and extracted with CH_2Cl_2 (3 x 15 cm^3). The combined organic extracts were dried, and the solvent removed *in vacuo* to give a white solid identified the *title compound* **55** (239 mg; 95%), R_f (Et_2O) 0.63; m.p. (from Et_2O) 121-122 °C. Found: C, 56.61; H 5.67; N, 3.18. $C_{19}H_{23}O_5NS_2$ requires C, 55.74; H, 5.62; N; 3.42. δ_H(250 MHz; $CDCl_3$) 7.93 (4 H, d, $J_{o,m}$ 8.2, 4H Ar.), 7.34 (4 H, d, *J* 8.2, 4H Ar.), 4.73 (1 H, dt, $J_{2,1}$ 6.3, $J_{2,3}$ 9.4, H-2), 4.17-4.07 (1 H, dt, $J_{1,2}$ 6.3, $J_{1,5}$ 9.1, H-1), 2.45 (6 H, s, $2CH_3$), 2.13-2.01 (2 H, m, H-3,5), 1.98-1.43 (5 H, m, H-3',4,4',5', OH); δ_C(63 MHz; $CDCl_3$) 144.9 (2C-*p*), 137.6 (2*C*-S), 129.7 (4C-*m*), 128.2 (4C-*o*), 75.4 (C-2), 70.8 (C-1), 32.5 (C-3), 28.8 (C-5), 21.7 ($2CH_3$), 20.8 (C-4). *m/z* (FAB) 410.10961 (MH^+), requires 410.10961. ν_{max}(sol.)/cm^{-1} 3000m (C-H), 1600w (C=C), 1360m (C-N), 1170*s* (S=O), 1090m (C-O).

4.24 *N*-Cyclopentyl-2,4,6-triphenylpyridinium tetrafluoroborate **65** [99]

$C_{28}H_{26}NBF_4$ M.W. 463

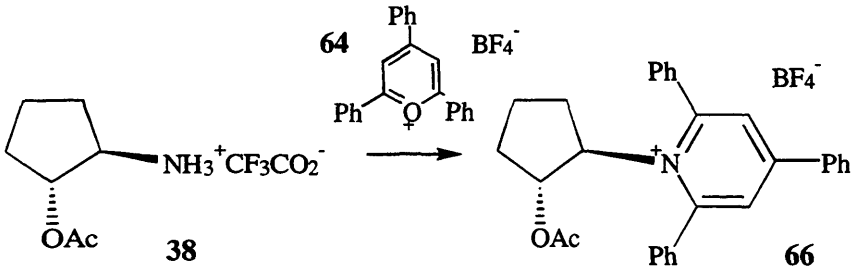


To a solution of 2,4,6-triphenylpyrilium tetrafluoroborate **64** (722 mg, 1.8 mmol) in CH_2Cl_2 (16 cm^3), cyclopentylamine **37** (2.0 mmol; 0.2 cm^3) was added dropwise over a period of 5

minutes, followed by triethylamine (2.0 mmol, 0.27 cm^3) and glacial acetic acid (8.0 mmol; 0.44 cm^3). The colour of the mixture changed inmediately from yellow to orange to brown. After 72 h at rt under nitrogen, the solvent was removed *in vacuo* and the residue triturated with Et_2O (30 cm^3) to give an orange precipitate which was isolated by filtration and identified as the title compound **65** (854 mg; 91%), R_f (EtOAc:CH_2Cl_2 1:1) 0.30; m.p. (from acetone:Et_2O) 178-179 °C (lit.[99] 179-180 °C). δ_H(250 MHz; $CDCl_3$) 7.73- 7.37 (17 H, m, Arom.), 4.97 (1 H, quint, $J_{1,2\text{-}5}$ 9.0, H-1), 2.19-2.01 (2 H, m, H-2,5), 1.98-1.87 (2 H, m, H-2',5'), 1.11-1.06 (2 H, m, H-3,4), 0.90-0.87 (2 H, m, H-3',5'). *m/z* (FAB) 376.20653 (M^+), requires 376.20653.

4.25 (±)-*N*-(*trans*-1-Acetoxycyclopent-2-yl)-2,4,6-triphenylpyridinium tetrafluoroborate 66

$C_{30}H_{28}O_2NBF_4$ M.W. 521

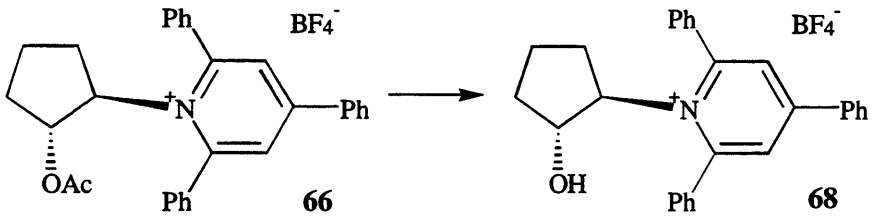


To a solution of 2,4,6-triphenylpyrilium tetrafluoroborate **64** (308 mg, 0.8 mmol) in CH_2Cl_2 (3 cm^3) was slowly added **38** (200 mg, 0.8 mmol) followed by triethylamine (1.5 mmol; 0.2 cm^3) and glacial acetic acid (0.8 mmol; 0.05 cm^3). The colour of the solution during the addition changed from yellow to intense orange. After 26 h at rt, the volatiles were removed *in vacuo* and the resulting yellow gum was shaken with Et_2O (2 x 10 cm^3). The ether solution was filtered and solid residue purified by flash chromatography (CH_2Cl_2) to give the *title compound* **66** (397 mg; 90%) as a white solid, R_f (CH_2Cl_2:EtOAc 1:1) 0.63; m.p. (from CH_2Cl_2:Et_2O) 165 °C. Found: C, 69.07; H 5.20; N, 3.32. $C_{30}H_{28}O_2NBF_4$ requires C, 69.12; H, 5.38; N, 2.69. δ_H(250 MHz; $CDCl_3$) 7.85 (4 H, s, Ar.), 7.80-7.77 (4 H, m, Ar.), 7.61-7.46 (9 H, m, Ar.), 5.52 (1 H, dt, *J* 4.6, *J* 3.3, H-2), 4.87 (1 H, dt, *J* 4.6, *J* 5.1, H-1), 2.67-2.52 (1 H, m, H-5), 2.48-2.34 (1 H, m, H-3), 1.92 (3 H, s, CH_3), 1.41-1.26 (2 H, m, H-5',3'), 1.23-1.11 (1 H, m, H-4'), 0.94-0.80 (1H, m, H-4'); δ_C(63 MHz; $CDCl_3$) 171.4

(C=O), 157.4 (C), 155.4 (C), 133.9 (C), 133.5 (C), 132.1 (CH), 131.0 (CH), 129.8 (CH), 129.6 (CH), 129.0 (CH), 128.8 (CH), 128.3 (CH), 128.0 (CH), 79.54 (C-2), 77.23 (C-1), 34.8 (C-3), 30.5 (C-5), 22.4 (C-4), 20.7 (CH_3). m/z (FAB) 434.21206 $(M\text{-}BF_4)^+$, requires 434.21200. ν_{max}(sol)/cm^{-1} 3000w (C-H), 1730m (C=O), 1670m (C=N), 1620*s* (C=C), 1400m (C-N), 1240*s* (C-O).

4.26 (±)-*N*-(*trans*-1-Hydroxycyclopentyl)-2,4,6-triphenylpyridinium tetrafluoroborate 68

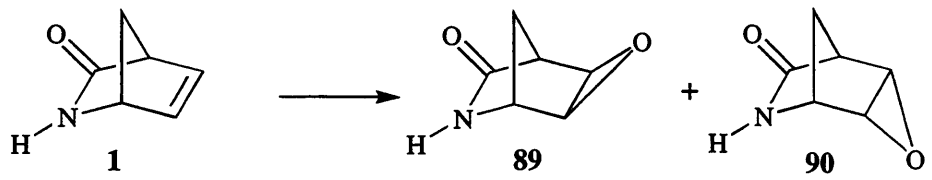
$C_{28}H_{26}ONBF_4$ M.W. 479



The tetrafluoroborate salt **66** (500 mg, 1.0 mmol) was dissolved in MeOH (3.8 cm^3) and treated with NaOH (2 M; 1 cm^3). The colour of the solution changed from light yellow to orange, brown and orange again. After 28 h at rt MeOH was removed *in vacuo* and water (6 cm^3) was added. The aqueous solution was extracted with CH_2Cl_2 (3 x 15 cm^3) and the combined extracts dried and the solvent removed *in vacuo* to give a crude product which was purified by flash chromatography (CH_2Cl_2:AcOEt 1:1). A colourless oil was obtained and identified as the *title compound* **68** (279 mg; 61%), R_f (CH_2Cl_2:EtOAc 1:1) 0.40. δ_H(250 MHz; $CDCl_3$) 7.78 (2 H, s, Ar.), 7.78-7.42 (15 H, m, 3 x C_6H_5), 4.99 (1 H, dt, *J* 5.9, *J* 5.7, H-2), 4.79 (1 H, dt, *J* 5.9, $J_{1,5}$ 5.7, H-1), 4.32 (1 H, br.s, OH), 2.13-2.02 (2 H, m, H-3,5), 1.36-1.12 (2 H, m, H-3',5'), 1.08-0.95 (1 H, tt, $J_{4,4'}$ 6.3, $J_{4,3\text{-}5}$ 6.8, H-4), 0.89-0.76 (1 H, tt, $J_{4',4}$ 6.3, *J* 6.1, H-4'); δ_C(63 MHz; $CDCl_3$) 156.6 (C-2, C-6), 153.8 (C-19), 133.0 (C-4), 132.7 (C-13, C-7), 133.0, 132.7, 130.8, 129.7, 129.1, 128.5, 127.8, 127.2, 77.6 (C-2), 75.6 (C-1'), 32.5 (C-3'), 31.5 (C-5'), 20.4 (C-4'). *m/z* (FAB) 392.20146 M^+, requires 392.20144. ν_{max}(sol)/cm^{-1} 1690w (C-N), 1620*s* (C=C), 1400m (C-N), 1070*s* (C-O).

4.27 **(±)-5,6-Epoxy-*exo*-2-azabicyclo[2.2.1]heptan-3-one 89** [80] **and (±)-5,6-epoxy-*endo*-6-azabicyclo[2.2.1]heptan-3-one 90**

$C_6H_7O_2N$ M.W. 125



4.27.1 Method A: Oxidation with oxone

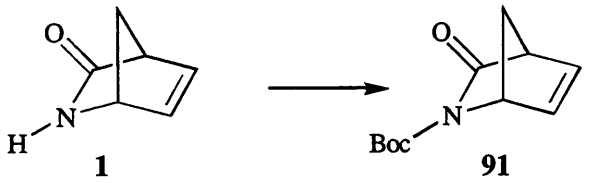
Lactam **1** (300 mg, 2.7 mmol) was dissolved in MeOH (8.2 cm^3) followed by simultaneous addition during 4 h, of a solution of oxone (5 g, 8.1 mmol) in H_2O (20.5 cm^3) and KOH (1 M; 15 cm^3), in order to keep the pH between 6-7. After further 2 h at rt, the mixture was concentrated to half volume and extracted with $CHCl_3$ (3 x 40 cm^3). The combined organic extracts were dried, and the solvent evaporated *in vacuo*. Two white solid compounds were isolated by flash chromatography (EtOAc:Et_2O 2:3) and identified as the epoxides *exo* **89** (131 mg, 38%) and *endo* **90** (16 mg; 5%), R_f (EtOAc:MeOH 8:2) 0.70 and 0.42 respectively. Compound **89**: m.p. (from CH_2Cl_2) 120-121 °C (lit.[100] 120 °C). Found 57.4; H, 4.9; N, 11.1. $C_6H_7O_2N$ requires C 57.5; H, 5.6; N, 11.2. δ_H(400 MHz; $CDCl_3$) 7.21 (1 H, br s, NH), 3.85 (1 H, br s, H-1), 3.63 (1 H, d, $J_{5,6}$ 3.7, H-5), 3.52 (1 H, d, $J_{6,5}$ 3.7, H-6), 2.83 (1 H, br s, H-4), 1.79 (1 H, dd, $J_{7a,s}$ 9.8, $J_{7a,5}$ 1.1, H-7a), 1.62 (1 H, d, $J_{7s,a}$ 9.8, H-7s); δ_C(100.6 MHz; $CDCl_3$) 181.57 (C=O), 55.98 (C-6), 51.89 (C-5), 56.08 (C-1), 47.35 (C-4), 31.65 (C-7); m/z (EI) 125.04768 (M^+), requires 125.04768. ν_{max}(sol.)/cm^{-1} 3420m (N-H), 1725*s* (C=O), 1015m (C-O). Compound **90**: m.p. (from Et_2O:EtOAc) 144-145 °C. Found: C, 57.3; H, 5.1; N, 11.0. $C_6H_7O_2N$ requires C, 57.5; H, 5.6; N, 11.2. δ_H(400 MHz; $CDCl_3$) 6.03 (1H, br s, NH), 3.93 (1 H, br t, $J_{1,6}$ 3.7, H-1), 3.87-3.85 (2 H, m, H-6, 5), 2.79 (1 H, d, $J_{4,7}$ 1.8, H-4), 2.34 (1 H, d, $J_{7s,a}$ 9.2, H-7s), 2.22 (1 H, ddd, $J_{7a,s}$ 9.2, $J_{7a,4}$ 1.8, $J_{7a,1}$ 1.6, H-7a); δ_C(100.6 MHz; $CDCl_3$) 177.06 (C=O), 58.02(C-6), 54.02 (C-1), 53.57 (C-5), 52.01 (C-7), 46.00 (C-4). m/z (EI) 125.04769 (M^+), requires 125.04768. ν_{max}(sol.)/cm^{-1} 3420m (N-H), 3020-2990w (C-H), 1730*s* (C=O), 1030*s* (C-O).

4.27.2 Method B: Oxidation with MCPBA

The lactam **1** (200 mg, 1.8 mmol) was dissolved in CH_2Cl_2 (10 cm^3) and reacted with MCPBA (57-86%; 3.40 mmol). The mixture was stirred at rt for 58 h and the reaction was monitored by ^1H NMR spectroscopy and on complete reaction the spectrum showed a ratio 7:1 *exo-endo* epoxides. The solvent was evaporated *in vacuo* and the crude product was purified by flash chromatography (100% Et_2O, 90% Et_2O:EtOAc→70% Et_2O:EtOAc), obtaining two solid compounds in 85% yield (**89**: 178 mg / **90**: 19 mg), whose spectral and physical properties were similar to that obtained in method A (Section 4.26.1).

4.28 (±)-*N-tert*-Butoxycarbonyl-2-azabicyclo[2.2.1]hept-5-en-3-one **91** [82]

$C_{11}H_{15}O_3N$ M.W. 209



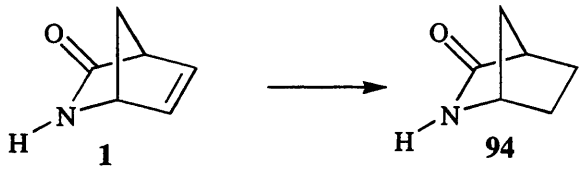
To a solution of the lactam **1** (1.22 g, 11.3 mmol) in CH_2Cl_2 (60 cm^3) was added 4-DMAP (512 mg, 4.2 mmol), triethylamine (1 cm^3) and a solution of $(Boc)_2O$ (3.65 g, 16.7 mmol) in CH_2Cl_2 (5 cm^3). After 18 h at rt, the volatiles were evaporated *in vacuo* and the residue was purified on silica gel (CH_2Cl_2) to yield the title compound **91** (2.14 g; 91%) as a pale yellow solid, R_f (EtOAc:CH_2Cl_2 1:9) 0.68; m.p. (from hexane) 67-68 °C (lit.[82] 68-71 °C). δ_H(250 MHz; $CDCl_3$) 6.90-6.87 (1 H, dd, $J_{6,1}$ 2.2 and $J_{5,6}$ 5.4, H-6), 6.68-6.64 (1 H, m, H-5), 4.97-4.94 (1 H, m, H-1), 3.39-3.37 (1 H, m, H-4), 2.35 (1 H, d, $J_{7s,a}$ 8.5, H-7s), 2.14 (1 H, d, $J_{7a,s}$ 8.5, H-7a); δ_C(63 MHz; $CDCl_3$) 176.2 (C=O, C-3), 150.4 (C=O), 140.0 (C-6), 138.2 (C-5), 82.6 (C), 62.4 (C-1), 54.9 (C-7), 54.5 (C-4), 28.1 ($3CH_3$). m/z (FAB) 210.11302 (MH^+), requires 210.11302. ν_{max}(sol.)/cm^{-1} 3040m (=C-H), 2980m (C-H), 1780*s* (C=O lact), 1710*s* (C=O carbamide), 1420*s* (CH_3), 1340-1360*s* (C-0 ester), 1270-1250*s* (C-N).

4.28.1 (-)-*N-tert*-Butoxycarbonyl-2-azabicyclo[2.2.1]hept-5-en-3-one 91 [101]

Following the experimental procedure described in Section 4.28, (+) lactam **1** (3.13 g, 28.67mmol) was treated with $(Boc)_2O$ (9.7 g, 44.5 mmol), triethylamine (17.3 mmol, 2.2 cm^3) and DMAP (980 mg; 8.0 mmol) in CH_2Cl_2 (150 cm^3) to give the title compound (-) **91** (5.82 g, 97%). $[\alpha_D]^{20}$ - 212.59 (c 1.2, $CHCl_3$), m.p. (from hexane) 85-86 °C (lit.[101] 84-86 °C).

4.29 (±)-2-Azabicyclo[2.2.1]heptan-3-one 94 [85]

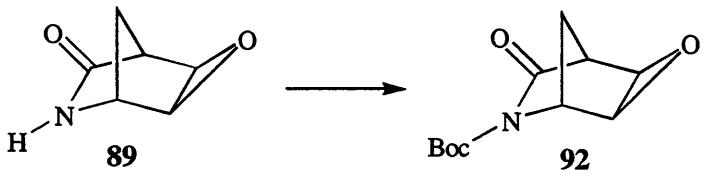
C_6H_9ON M.W. 111



The lactam **1** (125 mg, 1.1 mmol) was dissolved in EtOAc (25 cm^3) and Pd-C 5% (250 mg) was added. The mixture was stirred at rt under hydrogen at atmospheric pressure. After 60 h, on complete reaction, the catalyst was removed by filtration throught a Celite pad and the solid rinsed with some EtOAc (10 cm^3). The filtrate was concentrated *in vacuo* to give a highly hygroscopic white solid **94** (100 mg; 79%) (lit.[85] 71%), R_f (EtOAc:CH_2Cl_2 1:1) 0.52. δ_H(250 MHz; $CDCl_3$) 6.86 (1 H, br s, NH), 3.89 (1 H, br s, H-1), 2.72 (1 H, br s, H-4), 1.94-1.80 (3 H, m, H-6, 6',H-7a), 1.73-1.50 (2 H, m, H-5,5'), 1.41-1.36 (1 H, dd, $J_{7a,s}$ 9.4, $J_{7s,5\text{-}6}$ 1.3, H-7s); δ_C(63 MHz; $CDCl_3$) 181.9 (C=O), 55.7 (C-1), 45.4 (C-4), 41.7 (C-5), 30.4 (C-6), 23.9 (C-7). m/z (EI) 111.06841 (M^+), requires 111.06841. ν_{max}(NaCl)/cm^{-1} 3260*s*, (N-H), 1690m (C=O).

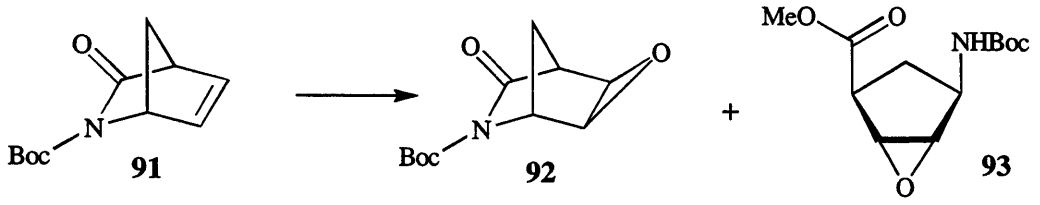
4.30 (±)-*exo*-*N*-*tert*-Butoxycarbonyl-5,6-epoxy-2-azabicyclo[2.2.1]heptan-3-one 92
$C_{11}H_{15}O_4N$ M.W. 209

4.30.1 Method A: From compound 89



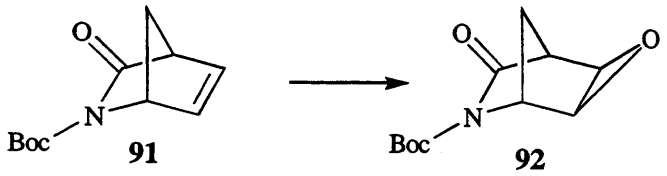
The epoxy-lactam **1** (230 mg, 1.8 mmol) was dissolved in CH_2Cl_2 (16 cm^3) and treated with 4-DMAP (84 mg, 0.7 mmol), triethylamine (0.2 cm^3), followed by a solution of $(Boc)_2O$ (602 mg, 2.8 mmol) in CH_2Cl_2 (5 cm^3). After 2 h at rt, the volatiles were removed *in vacuo* and the residue purified by chromatography (CH_2Cl_2) to yield the *title compound* **92** as a white solid (360 mg; 87%), R_f (CH_2Cl_2-EtOAc 9:1) 0.82; m.p. (from hexane:Et_2O) 115-116 °C (lit.[93] 123-125 °C). Found: C, 58.44; H, 6.56; N, 6.16. $C_{11}H_{15}O_4N$ requires C, 58.66; H, 6.71; N, 6.22. δ_H(400 MHz; $CDCl_3$) 4.63 (1 H, q, *J* 1.6, H-1), 3.79 (1 H, dd, $J_{6,5}$ 3.5, $J_{6,7}$ 1.3, H-6), 3.62 (1 H, dd, $J_{5,7}$ 1.5, $J_{5,6}$ 3.5, H-5), 3.08 (1 H, q, $J_{4,5}$ 1.5, H-4), 1.83 (1 H, ddd, $J_{7a,s}$ 10.3, $J_{1,7a}$ 1.7, $J_{7a,4}$ 1.5, H-7a), 1.65 (1 H, ddd, $J_{7s,a}$ 10.3, $J_{7s,1}$ 1.7, $J_{7s,4}$ 1.8, H-7s), 1.54 (9 H, s, $3CH_3$); δ_C(100.6 MHz; $CDCl_3$) 173.8 (C-3), 150.14 (C=O), 83.75 (C), 59.33 (C-1), 53.51 (C-6), 50.38 (C-5), 48.76 (C-4), 27.48 (C-7), 28.45 ($3CH_3$). m/z (EI) 225.10012 (M^+), requires 225.10011. ν_{max}(sol.)/cm^{-1} 2980*s* (C-H), 1765*s* (C=O lact), 1710*s* (C=O carbamide), 1450m (CH_2), 1370m (CH_3), 1325-1300*s* (C-N), 1210*s* (C-O ether).

4.30.2 Method B: From compound 91 and oxone, as oxidising agent



A solution of oxone (41.4 g, 67.3 mmol) in water (165 cm^3) was added for 2 h to a solution of **91** (1.8 g, 8.6 mmol) in MeOH (25 cm^3). Simultaneous addition of KOH (1 M; 50 cm^3) was required to keep the pH between 6-7. After further 5 h at rt, the mixture was extracted with CH_2Cl_2 (3 x 150 cm^3). The combined organic extracts were dried and the solvent evaporated *in vacuo* to give the crude product. Purification by flash chromatography (EtOAc-CH_2Cl_2 1:9) gave two white solid compounds: the *title compound exo* epoxide **91** as major product (970 mg; 50%) and **(±)-Methyl 4β-N-*tert*-butoxycarbonylamino-2α,3α-epoxy-1β-cyclopentanecarboxylate 93** ($C_{12}H_{19}O_5N$ M.W. 257) as by-product (233 mg, 0.9 mmol), R_f (EtOAc:CH_2Cl_2 9:1) 0.8 and 0.5, respectively. The spectroscopic data for compound **92** matched with the previously described. Compound **93**: m.p. (from hexane:EtOAc) 95° C. Found: C, 55.42; H, 7.07; N, 5.20. $C_{12}H_{19}O_5N$ requires C, 56.03; H, 7.44; N, 5.44. δ_H(400 MHz; $CDCl_3$) 4.91 (1 H, br d, $J_{NH\text{-}1}$ 7.7, NH), 4.19 (1 H, q, J 8.6, H-4), 3.75 (3H, s, OCH_3), 3.70 (1 H, dd, $J_{2,3}$ 2,7, $J_{2,1}$ 1.5, H-2), 3.55 (1 H, br s, H-3), 2.91 (1 H, ddd, $J_{1,2}$ 1.5, $J_{1,5\alpha}$ 8.3, $J_{1,5\beta}$ 10.2, H-1), 2.16 (1 H, ddd, $J_{5\alpha,\beta}$ 13.1, $J_{5\alpha,4}$ 8.3, H-5α), 1.53-1.44 (1 H, m, H-5β), 1.45 (9H, s, $3CH_3$); δ_C(100.6 MHz; $CDCl_3$) 172.19 (C=O, ester), 155.77 (C=O), 80.04 (C), 57.79 (C-3), 56.41 (C-2), 52.48 (OCH_3), 51.6 (C-4), 43.98 (C-1), 28.71 ($3CH_3$), 27.50 (C-5). m/z (FAB) 258.13418 (MH^+), requires 258.13415. ν_{max} (KBr/cm^{-1}) 3380s (N-H), 2960*s* (C-H), 1750*s*, (C=O ester), 1685*s* (C=O carbamide), 1440*s* (CH_3), 1140-1200*s* (C-O).

4.30.3 Method C: From compound 91 and MCPBA as oxidising agent



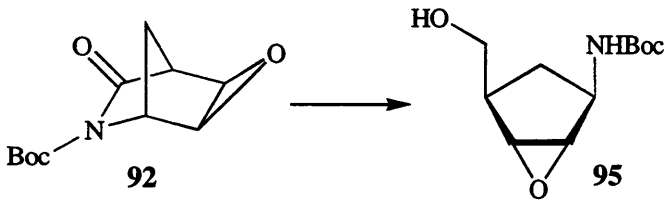
Compound **91** (210 mg, 1 mmol) was dissolved in CH_2Cl_2 (7 cm^3) and reacted with MCPBA (57-86%; 2.0 mmol) for 48 h at rt. $Na_2S_2O_5$, was added and the mixture stirred for ten minutes, followed by addition of saturated $NaHCO_3$ solution (10 cm^3). The aqueous layer was separated and extracted with CH_2Cl_2 (3 x 5 cm^3). The organic extracts were combined and washed with brine (10 cm^3), dried and the solvent evaporated *in vacuo*. The crude product was purified by flash chromatography (Et_2O:petroleum ether 40-60 3:7) to yield a white solid identified as the *exo* epoxide **92** (156 mg; 71%). The spectroscopic data and physical properties agreed with that obtained in the methods above.

4.30.3.1 (-)-*exo*-*N*-*tert*-Butoxycarbonyl-5,6-epoxy-2-azabicyclo[2.2.1]heptan-3-one 92

The experimental procedure described in Section 4.30.3 was applied to (-) **91** (5.32 g, 25.4 mmol), which was treated with MCPBA (17.05 g, 74.2 mmol) in CH_2Cl_2 (200 cm^3) to give the title product (-) **92** (3.8 g; 68%). $[\alpha_D]^{24}$ - 94.58 (c 0.7, CH_2Cl_2), m.p. (from hexane:Et_2O) 122-123 °C.

4.31 (±)-4β-*N-tert*-Butoxycarbonylamino-2α,3α-epoxy-1β-hydroxymethylcyclopentane 95

$C_{11}H_{19}O_4N$ M.W. 229



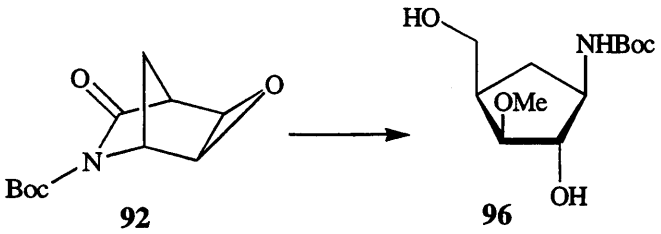
Compound **92** (950 mg, 4.2 mmol) was dissolved in MeOH (40 cm^3), cooled to 0 °C and treated with $NaBH_4$ (638 mg, 17.0 mmol). After 4 h at rt, the mixture was cooled to 0 °C and HCl (1.5 M) was added The solution was concentrated to the half of the volume and then extracted with CH_2Cl_2 (3 x 50 cm^3). The organic extracts were combined, dried and the solvent evaporated *in vacuo*. The crude product was purified by flash chromatography (EtOAc:CH_2Cl_2 3:7) to give the *title compound* **95** (864 mg; 89%) as a white solid, R_f (CH_2Cl_2:EtOAc 1:1) 0.49; m.p. (from CH_2Cl_2:petroleum ether 40-60) 116-117 °C (lit.[93] 118-119 °C). Found: C, 57.81; H, 8.49; N, 6.23. $C_{11}H_{19}O_4N$ requires C, 57.62; H, 8.35; N, 6.11. δ_H(400 MHz; $CDCl_3$) 5.79 (1 H, br d, $J_{NH\text{-}1}$ 7.9, NH), 4.21 (1 H, br t, *J* 7.9, H-4), 3.87 (1 H, dd, $J_{6,6'}$ 10.3, $J_{6,4}$ 2.0, H-6), 3.65 (1 H, dd, $J_{6',6}$ 10.3, $J_{6',4}$ 2.4, H-6′), 3.41 (2 H, s, H-2,3), 2.39 (1 H, br d, $J_{1,5\beta}$ 8.6, H-1), 2.13-2.05 (1 H, m, H-5α), 1.60-1.42 (1 H, m, H-5β), 1.45 (9 H, s, $3CH_3$); δ_C(100.6 MHz; $CDCl_3)_{323K}$ 155.9 (C=O), 79.8 (C), 63.4 (C-6), 60.1 (C-3), 59.7 (C-2), 50.6 (C-4), 40.9 (C-1), 33.2 (C-5), 28.8 ($3CH_3$). m/z (FAB) 230.13924 (MH^+), requires 230.13923. ν_{max}(sol.)/cm^{-1} 3300-3500*s* (O-H), 2980-2860*s* (C-H), 1690*s* (C=O), 1550-1520*s* (C-N), 1465m (CH_2), 1370*s* (CH_3), 1265*s* (C-O).

4.31.1 (+)-(1*S*,2*R*,3*S*,4*R*)-4-*N-tert*-Butoxycarbonylamino-2,3-epoxy-1-hydroxymethylcyclopentane 95

The experimental procedure described in Section 4.31 was followed for (-) **92** (3.53 g, 15.7 mmol), which was treated with $NaBH_4$ (2.3 g, 60.8 mmol) in MeOH (100 cm^3) to yield the expected product (+) **95** after purification (2.37 g; 69%). $[\alpha_D]^{24}$ + 23.13 (c 0.6, CH_2Cl_2), m.p. (from CH_2Cl_2:petroleum ether 40-60) 126-127 °C.

4.32 (±)-4β-*N*-*tert*-Butoxycarbonylamino-3α-hydroxy-2β-methoxy-1β-hydroxymethylcyclopentane **96**

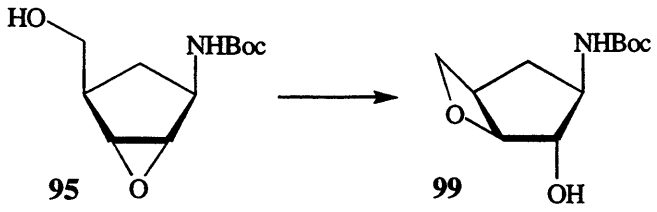
$C_{12}H_{23}O_5N$ M.W. 261



92 **96**

A small sample of **92** (70 mg, 0.3 mmol) was dissolved in MeOH (5 cm^3) and treated with $NaBH_4$ (47 mg, 1.2 mmol) at 0 °C. Then, the mixture was heated at 50 °C for 16 h, cooled to 0 °C and quenched by addition of HCl (1.5 M). Evaporation *in vacuo* to the half of the volume was followed by extraction with EtOAc (3 x 10 cm^3). The combined extracts were washed with brine (10 cm^3), dried and the solvent evaporated *in vacuo* to give a crude product which was purified by flash chromatography (EtOAc:CH_2Cl_2 2:3) leaving a white solid identified as the *title compound* **96** (64 mg; 85%), R_f (EtOAc:CH_2Cl_2 1:1) 0.30; m.p. (from EtOAc:Et_2O) 106 °C. Found: C, 54.50; H, 8.81; N, 5.26. $C_{12}H_{25}O_5N$ requires C, 54.73; H, 9.37; N, 5.32. δ_H(250 MHz; $CDCl_3$) 5.00 (1 H, d, $J_{NH\text{-}1}$ 5.0, NH), 4.39 (1 H, br s, 3-OH), 3.90 (1 H, t, *J* 4.9, H-3), 3.80 (1 H, d, $J_{6,6'}$ 11.6, H-6′), 3.73 (1 H, dd, $J_{2,1}$7.2, $J_{2,3}$ 4.1, H-2), 3.69-3.60 (2 H, m, H-4, 6), 3.44 (3 H, s, CH_3), 2.77 (1 H, br s, OH-6), 2.45-2.31 (1 H, m, H-1), 2.04 (1 H, ddt, $J_{5\alpha,\beta}$ 12.3, $J_{5\alpha,4}$ 7.6, $J_{5\alpha,1}$ 6.1, H-5α), 1.72-1.58 (1 H, m, H-5β), 1.45 (9 H, s, $3CH_3$); δ_C(100.6 MHz; $CDCl_3$)$_{300K}$ 157.7 (C=O), 88.8 (C-2), 84.1 (C-3), 80.0 (C), 62.5 (C-6), 58.1 (C-4), 57.8 (OMe), 41.4 (C-1), 31.0 (C-5), 28.7 ($3CH_3$). m/z (FAB) 262.16545 (MH^+) requires 262.16545. ν_{max}(sol.)/cm^{-1} 3060*s* (N-H), 2800-2300*s* (C-H), 1750*s* (C=O), 1500m (CH_2), 1420*s* (CH_3), 1250*s* (C-O ester), 1080*s* (C-N), 1050*s* (C-O ether).

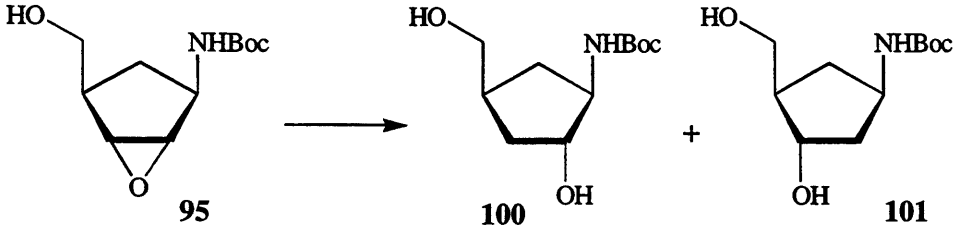
4.33 **(±)-1β,5β,4α-Hydroxy-3β-*N-tert*-butoxycarbonyl]-6-oxabicyclo[3.2.0]heptane 99**
$C_{11}H_{19}NO_4$ M.W. 229



To a cooled solution of $LiBEt_3H$ (0.36 mmol; 0.35 cm^3) in THF (2 cm^3) at 0 °C, was added a solution of **95** (67 mg, 0.29 mmol) in THF (1 cm^3) under nitrogen. The mixture allowed to reach rt. After further 24 h MeOH (4 cm^3) was added ant then NaOH (6 M; 0.8 cm^3). The solution was stirred at rt for 30 minutes and then H_2O_2 (30% v/v; 1.7 cm^3) was added at O °C. After further 2 h, the mixture was extracted with CH_2Cl_2 (3 x 10 cm^3) and the combined extracts washed with $NaHCO_3$ (5% w/v; 15 cm^3) and brine (15 cm^3). The solvent was removed *in vacuo* and the crude product was purified by flash chromatography (EtOAc) to give a colourless oil (25 mg; 37%) identified as the *title compound* **99**, R_f (EtOAc:MeOH 9.5:0.5) 0.68. δ_H(400 MHz; $CDCl_3$) 5.61 (1 H, br d, $J_{NH,3}$ 8.0, NH), 5.11 (1 H, d, $J_{5,1}$ 5.0, H-5), 4.24 (1H, t, J 7.4, H-3), 4.90 (1 H, t, $J_{7,1}$ 6.7, H-7), 4.13-4.10 (1 H, m, H-4,7'), 3.21-3.16 (1 H, m, H-1), 2.79 (1 H, br s, OH), 2.35 (1 H, dt, $J_{2\alpha,3}$ 7.4, $J_{2\alpha,\beta}$ 14.4, H-2α), 1.96 (1 H, d, $J_{2\beta,\alpha}$ 14.4, H-2β), 1.45 (9 H, s, $3CH_3$); δ_C(100.6 MHz; $CDCl_3$) 156.2 (C=O), 92.5 (C-5), 80.31 (C-4), 80.1 (C), 76.5 (C-7), 60.3 (C-3), 38.2 (C-1), 36.9 (C-2). ν_{max}(sol.)/cm^{-1} 3050*s* (NH), 2980*s* (C-H), 1700*s* (C=O), 1420*s*, (C-N), 1250*s* (C-O). m/z (FAB) 230.13926 (MH^+) requires 230.13923.

4.34 (±)-4β-(*N-tert*-Butoxycarbonylamino)-3α-hydroxy-1β-hydroxymethylcyclopentane **100**

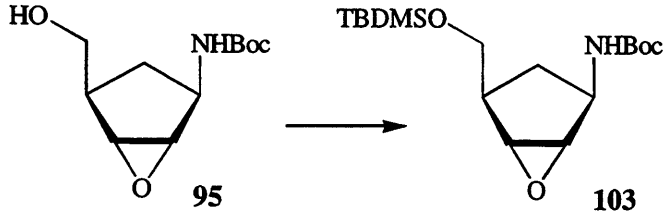
$C_{11}H_{21}O_4N$ M.W. 231



A small sample of the epoxide **95** (60 mg, 0.20 mmol) was dissolved in toluene (1.5 cm^3) and cooled to 0 °C. Red-Al [$(CH_3OCH_2O)AlNa$] 65% wt in toluene (0.2 cm^3, 0.654 mmol) was added dropwise for 15 minutes. After 24 h at rt under nitrogen, the mixture was cooled to 0 °C, quenched very slowly by addition of ice (1 cm^3) followed by HCl (2 M; 0.4 cm^3) and extracted with EtOAc (3 x 3 cm^3). The organic extracts were dried and the solvent evaporated *in vacuo*. The residue was purified by flash chromatography (EtOAc:CH_2Cl_2 1:1→EtOAc). Two compounds in ratio 3:1 were isolated and identified as the title compound **100** as the major product and **(±)-4β-(*N-tert*-Butoxycarbonylamino)-2α-hydroxy-1β-hydroxymethylcyclopentane 101** (synthesis as above described) (65% overall yield), R_f (EtOAc) 0.40 and 0.24 respectively; m.p. (from Et_2O:petroleum ether 40:60) 80 °C. Found: C, 56.91; H, 9.18; N, 6.01. $C_{11}H_{21}O_4N$ requires C, 57.12; H, 9.15; N, 6.06. δ_H(400 MHz; $CDCl_3$) 5.13 (1 H, br d, $J_{NH,4}$ 4.1, NH), 4.04 (1 H, dt, $J_{3,4}$ 5.2, $J_{3,2}$ 5.1, H-3), 3.69 (1 H, dt, $J_{4,3}$ 5.2, $J_{4,5}$ 7.6, H-4), 3.58 (1 H, dd, $J_{6,6'}$ 10.4, $J_{6,1}$ 5.3, H-6), 3.53 (1 H, dd, $J_{6',6}$ 10.4, $J_{6',1}$ 5.5, H-6'), 2.58 (2 H, br s, 2-OH), 2.45-2.34 (1 H, m, H-1), 2.24 (1 H, dt, $J_{5\alpha,\beta}$ 12.9, $J_{5\alpha,1\text{-}4}$ 7.8, H-5α), 1.86-1.73 (2 H, m, H-2,2'), 1.44 (9 H, s, ^{t}Bu), 1.25 (1 H, dt, $J_{5\beta,\alpha}$ 12.9, $J_{5\beta,1\text{-}4}$ 7.1, H-5β); δ_C(100.6 MHz; $CDCl_3$) 157.3 (C=O), 80.26 (C), 78.88 (C-3), 66.28 (C-6), 60.37 (C-4), 37.62 (C-1), 35.07 (C-2), 33.69 (C-5), 28.76 ($3CH_3$). m/z (FAB) 232.15480 (MH^+), requires 232.15488. ν_{max}(sol.)/cm^{-1} 3050m (O-H), 2860m (C-H), 1700*s* (C=O), 1500*s* (CN), 1140*s* (C-O).

4.35 **(±)-1β-(*tert*-Butyldimethylsilyloxymethyl)-2α,3α-epoxy-4β-(N-*tert*-butoxycarbonylamino)cyclopentane 103**

$C_{17}H_{33}O_4NSi$ M.W. 343



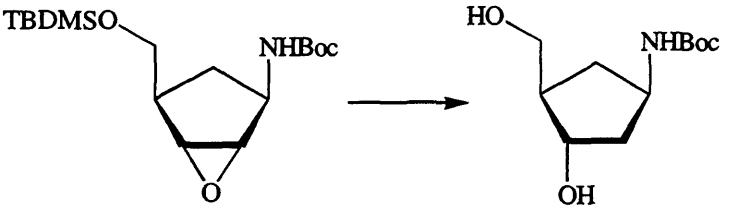
To a solution of the epoxide **95** (223 mg, 0.97 mmol) in CH_2Cl_2 (3 cm^3) cooled to -10 °C was added 2,6-lutidine (2.43 mmol; 0.28 cm^3) followed by slow addition of tert-butyldimethylsilyl trifluoromethanesulfonate (TfOTBDMS) (1.1 mmol; 0.25 cm^3). After stirring for 4 h at rt under nitrogen, the mixture was cooled to O °C, and treated with water (5 cm^3). The organic layer was separated and the aqueous layer extracted with CH_2Cl_2 (3 x 10 cm^3). The organic extracts were combined, washed with $NaHCO_3$ (5%; 10 cm^3) and brine (10 cm^3), dried and the volatiles evaporated *in vacuo*. The crude product was purified by flash chromatography (CH_2Cl_2) to yield a colourless oil identified as the *title compound* **103** (324 mg; 97%), R_f (Et_2O:CH_2Cl_2 1:4) 0.75. δ_H(400 MHz; $CDCl_3$) 5.70 (1 H, br d, $J_{NH\text{-}1}$ 9.5, NH), 4.37 (1 H, br t, *J* 9.0, H-4), 3.87 (1 H, dd, $J_{6,6'}$ 10.5, $J_{6,4}$ 2.0, H-6), 3.66 (1 H, dd, $J_{6',6}$ 10.5, $J_{6',4}$ 2.9, H-6'), 3.41 (1 H, br d, *J* 2.3, H-3), 3.36 (1 H, d, *J* 1.7, H-2), 2.38 (1 H, br d, $J_{1,5\alpha}$ 9.1, H-1), 2.12 (1 H, dt, $J_{5\alpha,\beta}$ 14.3, $J_{5\alpha,1}$ 8.5, H-5α), 1.45-1.38 (1 H, m, H-5β), 1.45 (9 H, s, ^{t}BuO), 0.95 (9 H, s, ^{t}BuSi), 0.16 (3 H, s, CH_3), 0.15 (3 H, s, CH_3); δ_C(100.6 MHz; $CDCl_3$) 155.70 (C=O), 79.5 (C), 65.2 (C-6), 60.2 (C-2), 59.9 (C-3), 50.0 (C-4), 41.2 (C-1), 33.8 (C-5), 28.8 (^{t}BuO), 26.5 (^{t}BuSi), 19.1 (C-Si), -5.0 (Si-CH_3), -5.1 (Si-CH_3). m/z (FAB) 344.22571 (MH^+), requires 344.22571. $\nu_{(film)}$/cm^{-1} 3400m (N-H), 2920-2840*s* (C-H), 1700*s* (C=O), 1500*s* (C-N), 1300*s* (C-O ether), 1890*s* (C-O).

4.35.1 (-)-(1*S*,2*R*,3*S*,4*R*)-1-(*tert*-Butyldimethylsilyloxymethyl)-2,3-epoxy-4-(N-*tert*-butoxycarbonylamino)cyclopentane 103

The experimental conditions described in Section 4.35 were applied to (+) compound **95** (2.16 g, 9.42 mmol) which was treated with TfOTBDMS (10.4 mmol; 2.3 cm^3), 2,6-lutidine (18.6 mmol; 2.1 cm^3) in CH_2Cl_2 (30 cm^3) leading after purification to the expected product (-) **103** (2.1, 62%). $[\alpha_D]^{21}$ - 1.96 (c 0.8, CH_2Cl_2).

4.36 (±)-4β-(*N-tert*-Butoxycarbonylamino)-2α-hydroxy-1β-hydroxymethylcyclopentane 101

$C_{11}H_{21}O_4N$ M.W. 231



103 **101**

Compound **103** (860 mg, 2.5 mmol), was dissolved in toluene (12 cm^3) and cooled to -15 °C. To the solution was slowly added Red-Al, 65% wt solution in toluene (2 cm^3; 6.4 mmol) and stirred for 5 h. To the mixture at 0 °C, was added ice-water (10 cm^3) and HCl (2 M; 3 cm^3).The product was extracted with EtOAc (3 x 30 cm^3) and the combined extracts were dried and the solvent evaporated *in vacuo*. The crude solid, purified by flash chromatography (EtOAc:CH_2Cl_2 9:1) yielded the *title compound* **101** (437 mg; 76%), R_f (EtOAc) 0.24; m.p. (from petroleum ether 40-60:EtOAc) 104-105 °C. Found: C, 56.38; H, 9.08; N, 5.96. $C_{11}H_{21}O_4N$ requires C, 57.12; H, 9.15; N, 6.06. δ_H(400 MHz; $CDCl_3$) 4.70 (1 H, br d, $J_{NH,1}$ 7.34, NH), 4.19-4.11 (2 H, m, H-2,4), 3.79 (1 H, dd, $J_{6,6'}$ 10.4 and $J_{6,1}$ 7.5, H-6), 3.59 (1 H, dd, $J_{6',6}$ 10.4, $J_{6',1}$ 7.9, H-6'), 2.68 (1 H, br s, OH), 2.44 (1 H, br s, OH), 2.28 (1 H, ddd, $J_{5\alpha,\beta}$ 13.0, $J_{5\alpha,4}$ 7.9, $J_{5\alpha,1}$ 7.6, H-5α), 2.07-1.96 (2 H, m, H-1,3α), 1.82 (1 H, ddd, $J_{3\beta,\alpha}$ 13.5, $J_{3\beta,4}$ 6.8 and $J_{3\beta,2}$ 6.8, H-3β), 1.43 (9 H, s, $3CH_3$), 1.12 (1 H, ddd, $J_{5\alpha,\beta}$ 13.0, $J_{5\beta,4}$ 9.6 and $J_{5\beta,1}$ 7.9, H-5β); δ_H(100.6 MHz; $CDCl_3$) 155.5 (C=O), 79.4 (C), 75.1 (C-2), 65.2 (C-6), 49.2 (C-4), 48.7 (C-1), 42.0 (C-3), 34.5 (C-5), 28.4 ($3CH_3$). m/z (FAB) 232.15486

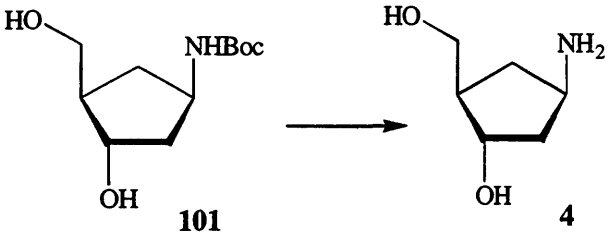
(MH^+), requires 232.15488. ν_{max}(sol.)/cm^{-1} 3040m (O-H), 2980m (C-H), 1700m (C=O), 1420*s* (CH_2), 1250*s* (C-O).

4.36.1 (+)-(1*S*,2*R*,4*R*)-4-(*N*-*tert*-Butoxycarbonylamino)-2-hydroxy-1-hydroxymethylcyclopentane 101

The experimental conditions described in Section 4.36 were applied to (-) compound **109** (2 g, 5.83 mmol), which was treated with Red-Al (15.3 mmol; 4.6 cm^3) in toluene (37 cm^3) to give after purification compound (+) **101** (1.14 g; 85%). $[\alpha_D]^{24}$ + 4.27 (c 0.4, $CHCl_3$), m.p. (from EtOAc:petroleum ether 40-60) 129-130 °C.

4.37 (±)-4β-Amino-2α-hydroxy-1β-hydroxymethylcyclopentane 4 [102]

$C_6H_{13}O_2N$ M.W. 131



101 **4**

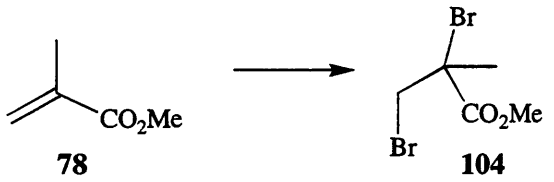
A solution of **101** (427 mg, 1.85 mmol) in distilled water (8.5 cm^3) was stirred at 110 °C under a continuous flow of nitrogen. After 21 h, it was allowed to cool to rt and the solution was evaporated *in vacuo* to give a colourless oil identified as the title compound **4** which was stored under nitrogen (quantitative yield), R_f ($CHCl_3$:MeOH 1:1) 0.17. δ_H(250 MHz; D_2O) 3.94 (1 H, dt, $J_{2,1}$ 7.4, $J_{2,3}$ 4.6, H-2), 3.51 (1 H, dd, $J_{6,6'}$ 11.0, $J_{6,1}$ 6.0, H-6), 3.44-3.31 (2 H, m, H-6',4), 2.16-2.05 (1 H, m, H-1), 1.95-1.73 (2 H, m, H-3,3'), 1.56 (1 H, dt, $J_{5\beta,\alpha}$ 13.0, $J_{5\beta,1\text{-}4}$ 8.0, H-5β), 0.96 (1 H, ddd, $J_{5\alpha,\beta}$ 13.0, $J_{5\alpha,1}$ 9.1, $J_{5\alpha,4}$ 8.3, H-5α); δ_C(100.6 MHz; $CDCl_3$) 73.74 (C-2), 63.85 (C-6), 49.62 (C-4), 49.20 (C-1), 49.02 (C-3), 36.09 (C-5). m/z (EI) 131.09466 (M^+); requires 131.09463. Broad IR. This amine was used inmediately, without any further purification, for the construction of thymidine analogue **3**.

4.37.1 (+)-*(1S,2R,4R)*-4-Amino-2-hydroxy-1-hydroxymethylcyclopentane 4 [73]

The same experimental procedure described in Section 4.36 was applied to (+) compound **101** (1.08 g, 4.7 mmol), which was refluxed in distilled water (22 cm^3). Removal of the solvent gave a colourless oil which was identified as (+) **4** (557 mg; 91.2%). A small sample was purified by flash chromatography ($CHCl_3$:MeOH 1:9). $[\alpha_D]^{17}$ + 20.68 (c 1.0, DMF) (lit.[73] $[\alpha_D]^{26}$ + 34 (c 1.0, DMF).

4.38 (±)-Methyl 2,3-dibromo-2-methylpropanoate 104 [89]

$C_5H_8O_2Br_2$ M.W. 259

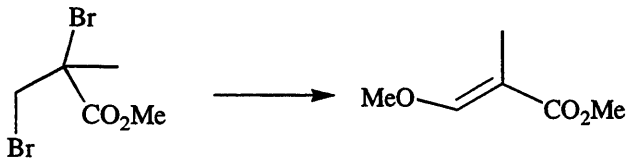


78 **104**

Methyl 2-methylprop-2-enoate **78** (1.40 g, 14.02 mmol) was dissolved in CH_2Cl_2 (50 cm^3) and bromine (145 mmol; 0.75 cm^3) was added dropwise for 0.5 h. After 12 h at rt, most of the solvent was evaporated *in vacuo* and the product distilled under reduced pressure to give a colourless oil identified as the title compound **104** (3.57 g; 98%), R_f (CH_2Cl_2) 0.85. δ_H(250 MHz; $CDCl_3$) 4.23 (1 H, d, $J_{3,3'}$ 10.0, H-3), 3.84 (3 H, s, CH_3O), 3.73 (1 H, d, $J_{3,3'}$ 10.0, H-3´), 2.04 (3 H, s, CH_3); δ_C(63 MHz; $CDCl_3$) 169.6 (C=O), 55.7 (C), 53.9 (CH_3O), 38.5 (CH_2), 26.8 (CH_3). m/z (EI) for $^{79}Br\&^{81}Br$ 259.88706 (M^+), requires 259.88705. ν_{max}(NaCl)/cm^{-1} 3040-2920m (C-H), 1750*s* (C=O), 1450*s* (C-H), 1290*s* (C-O).

4.39 (2*E*)-Methyl 3-methoxy-2-methylprop-2-enoate **105** [71]

$C_6H_{10}O_3$ M.W. 130

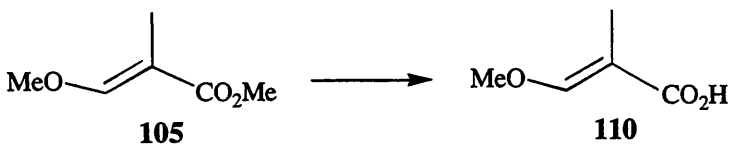


104 **105**

The dibromide **104** (3.27 g, 12.6 mmol) in MeOH (3.5 cm^3) was added to a hot solution from sodium (578 mg, 25.2 mmol) in MeOH (7 cm^3) at a rate enough to keep the mixture boiling. After 7 h at rt the precipitated was filtered and washed with some more MeOH (2 cm^3). The combined filtrates were evaporated *in vacuo* to the half of the volume and more NaBr was removed by filtration. Most of the MeOH was evaporated in vacuo and the residue was treated with water (5 cm^3) followed by extraction with Et_2O (3 x 150 cm^3). The combined extracts were dried and the solvent evaporated *in vacuo*. Fused $NaHSO_4$ (catalytic amount) was added to the crude product until most of the MeOH was distilled. Further distillation *in vacuo* gave the title compound **105** as a colourless oil (868 mg; 53%), R_f (hexane:EtOAc 8:1) 0.55. δ_H(250 MHz; $CDCl_3$) 7.27 (1 H, q, $J_{H,CH3}$ 1.3, HC), 3.81 (3 H, s, CH_3O), 3.71 (3 H, s, CH_3OCO), 1.73 (3 H, d, $J_{CH3,H}$ 1.3, CH_3); δ_C(63 MHz; $CDCl_3$) 169.3 (C=O), 158.5 (CH), 106.2 (C), 61.2 (OCH_3), 51.2 (CH_3OCO), 9.1 (CH_3). m/z (EI) 130.06299 (M^+), requires 130.06299. ν_{max} (sol.)/cm^{-1} 2925m (C-H), 1700*s* (C=O), 1640*s* (C=C), 1295*s* (C-O).

4.40 (2*E*)-3-Methoxy-2-methylpropenoic acid **110** [71]

$C_5H_8O_3$ M.W. 116



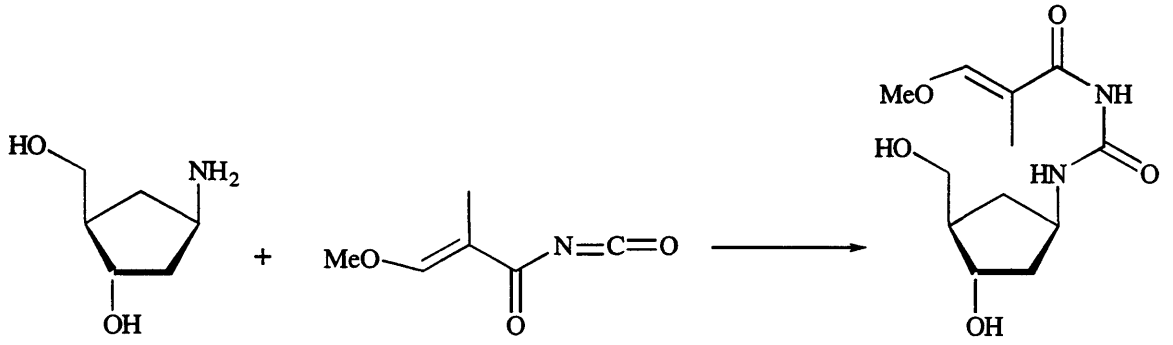
105 **110**

To a round bottom flask with compound **105** (200 mg, 1.5 mmol), provided with a long condenser, was added NaOH (2 M; 1.2 cm^3). The mixture was refluxing for 2 h followed by

treatment with HCl (2 M) at 0 °C until formation of a precipitated was observed. The product was extracted with EtOAc (3 x 10 cm^3) and the combined extracts were dried and the solvent removed *in vacuo* to yield a white solid identified as **110** (123 mg; 69%), R_f (CH_2Cl_2:EtOAc 1:1) 0.62; m.p. (from petroleum ether 40-60:Et_2O) 106-107 °C (lit.[71] 116 °C). δ_H(250 MHz; $CDCl_3$) 7.39 (1 H, q, $J_{3,CH3}$ 1.3, H-3), 3.85 (3 H, s, OCH_3), 1.73 (3 H, d, $J_{CH3,3}$ 1.3, CH_3); δ_C(63 MHz; $CDCl_3$) 174.1 (C=O), 160.4 (C-3), 105.4 (C-2), 61.4 (OCH_3), 8.7 (CH_3). m/z (EI) 116.04734 (M^+), requires 116.04734. ν_{max} (sol.)/cm^{-1} 2940w (C-H), 1700*s* (C=O), 1670m (C=C), 1360m (C-O), 1240m (C-O).

4.41 (±)-3α-Hydroxy-4β-hydroxymethyl-1β-[*N*'-(2*E*)-(3-methoxy-2-methylpropanoyl)ureido]cyclopentane 112 [73,74]

$C_{12}H_{20}O_5N_2$ M.W. 272



4 **74** **112**

3-Methoxy-2-methylpropionic acid **110** (520 mg, 4.5 mmol) was treated with thionyl chloride (5.63 mmol;0.5 cm^3) and the solution was refluxing for 4 h. The volatiles were removed *in vacuo* at rt to yield a residue identified as **3-methoxy-2-methylpropanoyl chloride 111** (not isolated); this crude product was immediately used for the formation of the corresponding isocyanate **74**. Silver cyanate (1.40 g, 59.3 mmol) (dried *in vacuo* over P_2O_5 in the dark at 110 °C for 3 h), was refluxed in benzene (20 cm^3) for 1 h under nitrogen. The mixture was allowed to cool to rt and it was transferred *via* canula to a flask containing the crude acid chloride. The solution was refluxed for 1.5 h and then allowed to cool to rt. After the solid phase had settled, the supernatant containing the isocyanate **3-methoxy-*N*-(aminocarbonyl)-2-methyl-2-propenamide 74** (15 cm^3) previously cooled to

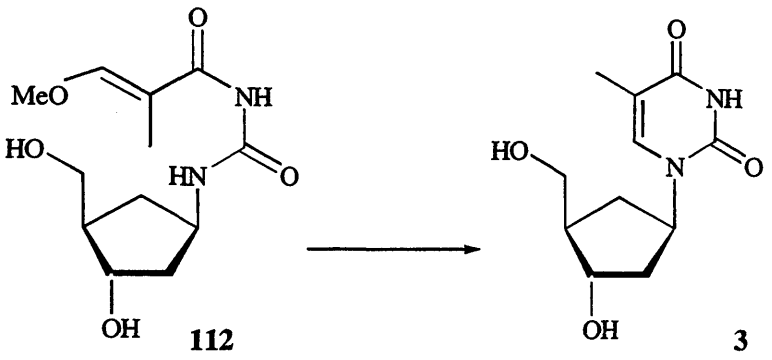
-10 °C, was added via syringe during 30 minutes to a solution of **4** (313 mg, 2.4 mmol) in DMF:Et_2O (3:1, 9 cm^3) at -20 °C, under nitrogen. The mixture was stirred for a further 2 h to complete the coupling. The solvents were evaporated *in vacuo* in codistillation with ethanol (10 cm^3) and the residue was purified by flash chromatography ($CHCl_3$→$CHCl_3$:MeOH 20:1). Further purification on a chromatotron (2 mm silica plate) was necessary to give a solid identified as the title compound **112** (425 mg; 66%), R_f ($CHCl_3$:MeOH 9:1) 0.3; m.p. (from EtOAc) 126 °C (lit.[73] 121-128 °C). δ_H (250 MHz; $CDCl_3$) 8.68 (1 H, d, $J_{NH,CH}$ 7.24, NH), 8.02 (1 H, s, NH), 4.29 (1 H, ddd, $J_{4,NH}$ 7.2, $J_{4,3\text{-}5}$ 7.6, H-4), 4.14 (1 H, dt, $J_{2,3}$ 6.0, $J_{2,2'}$ 9.2, H-2), 3.79 (3 H, s, OMe), 3.71 (1 H, dd, $J_{6,6'}$ 10.4, $J_{6,1}$ 4.9, H-6), 3.53 (1 H, dd, $J_{6',6}$ 10.4, $J_{6',1}$ 4.0, H-6'), 2.44 (1 H, br s, OH), 2.25 (1 H, dt, $J_{5\beta,\alpha}$ 13.2, $J_{5\beta,4\text{-}1}$ 7.6, H-5β), 2.04-1.92 (2 H, m, H-1,3), 1.84 (1 H, dt, $J_{3,3'}$ 13.9, $J_{3',2\text{-}4}$ 6.9, H-3'), 1.69 (3 H, s, CH_3), 1.15 (1 H, ddd, $J_{5\alpha,\beta}$ 12.9, $J_{5\alpha,1}$ 9.4, $J_{5\alpha,4}$ 8.2, H-5α); δ_C(100.6 MHz; $CDCl_3$) 169.55 (C=O), 159.05 (CH=), 153.76 (C=O), 107.16 (Me*C*=), 75.89 (C-2), 65.92 (C-6), 61.98 (OCH_3), 49.15 (C-1), 48.78 (C-4), 42.08 (C-3), 34.65 (C-5), 9.18 (CH_3C=). m/z (FAB) 273.14501 (MH^+), requires 273.14505. ν_{max} (sol.)/cm^{-1} 3200-3400w (O-H), 2500-2800w (C-H), 1750-1650*s* (C=O), 1560-1360m (C-N, N-H), 1250m (C-O).

4.41.1 (+)-3α-Hydroxy-4β-hydroxymethyl-1β-[*N'*-(2*E*)-(3-methoxy-2-methylpropanoyl)ureido]cyclopentane 112

The experimental procedure described in Section 4.41 was applied for the preparation of (+) **112**. Reaction of the acid **110** (853 mg, 7.4 mmol) with $SOCl_2$ (9.0 mmol; 0.8 cm^3) produced the acid chloride derivative **111** (988 mg, 7.4 mmol). Further treatment with silver cyanate (2.4 g, 16.0 mmol) in benzene (30 cm^3) gave the corresponding isocyanate **74** (1.03 g, 7.4 mmol) which was added to a solution of (+) **4** (499 mg, 3.8 mmol) in DMF:Et_2O 3:1 (10.6 cm^3) giving after purification as previously described, the title compound (+) **112** (781 mg; 75%); m.p. (from EtOAc) 149 °C. $[\alpha_D]^{18}$ + 11.44 (c 1.0, MeOH).

4.42 (±)-1'β-[3α-Hydroxy-4β-(hydroxymethyl)-cyclopentyl]-5-methyl-2,4 (1*H*,3*H*)pyrimidinedione 3 [73]

$C_{11}H_{16}O_4N_2$ M.W. 240



A solution of ammonia (35% w/v; 20 cm^3) was added to the compound **112** (209 mg, 0.8 mmol). The mixture was refluxed for 2 h to complete the ring closure. After addition of EtOH (23 cm^3), the volatiles were evaporated *in vacuo* and the crude product was purified by chromatotron chromatography (MeOH:$CHCl_3$ 0.5:9.5; 1 mm silica plate). A white solid identified as the carbocyclic thymidine **3** (126 mg; 67%) was obtained, R_f ($CHCl_3$:MeOH 4:1) 0.54; m.p. (from MeOH) 219-220 °C (lit.[73] 219-221.5 °C). δ_H(400 MHz; MeOD-d_4/$CDCl_3$) 7.74 (1 H, s, NH), 7.46 (1 H, d, $J_{NH,CH3}$ 1.1, CH=), 5.13 (1 H, quint, $J_{1,5\text{-}2}$ 9.0, H-1), 4.24 (1 H, dt, $J_{3,4}$ 6.8, $J_{3,2}$ 4.8, H-3), 3.75 (1 H, dd, $J_{6,4}$ 5.5, $J_{6,6'}$ 10.9, H-6), 3.71 (1 H, dd, $J_{6',6}$ 10.9, $J_{6',4}$ 46.7, H-6'), 2.32 (1 H, dt, $J_{5\beta,\alpha}$ 12.6, $J_{5\beta,4\text{-}1}$ 7.7, H-5β), 2.17-2.03 (3 H, m, 2H-2, 4), 1.96 (2 H, d, $J_{CH3,CH}$ 1.1, CH_3), 1.66 (1 H, dt, $J_{5\alpha,\beta}$ 12.6, $J_{5\alpha,1\text{-}4}$ 10.0, H-5α); δ_C(100.6 MHz; MeOD-d_4/$CDCl_3$) 165.37 (C-4, Thy), 151.95 (C-2, Thy), 138.59 (C-6, Thy), 110.96 (C-5, Thy), 72.77 (C-3), 63.26 (C-6), 54.83 (C-1), 49.11 (C-4), 39.33 (C-2), 32.51 (C-5), 11.95 (*C*H_3, Thy). m/z (FAB) 241.11876 (MH^+), requires 241.11883. ν_{max}(sol.)/cm^{-1} 2500-2900m (C-H), 1680-1630*s* (C=O, C=C), 1330*s* (C-N), 1270*s* (C-O).

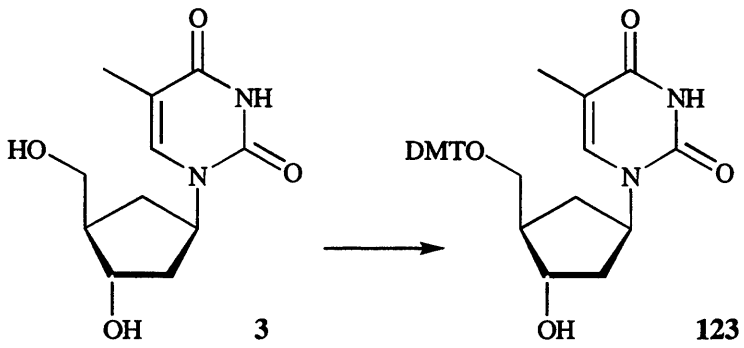
4.42.1 (+)-1'-[(1'*R*,3'*S*,4'*S*)-3'-Hydroxy-4'-(hydroxymethyl)cyclopentyl]-5-methyl-2,4-(1*H*,3*H*)pyrimidinedione 3 [103]

The experimental procedure described in Section 4.42, was applied to (+) compound **112** (678 mg, 2.5 mmol), which was refluxed in ammonia (35% w/v; 65 cm^3) for 2.5 h and led

to the carbocyclic thymidine (+) **3** (496 mg; 83%). $[\alpha_D]^{21}$ + 2.14 (c 1.0, MeOH); m.p. (from MeOH) 167-168 °C (lit.[103] 168-169 °C).

4.43 (±)-1'β-[4'β-(4,4'-Dimethoxytrityloxymethyl)-3'α-hydroxycyclopentyl]-5-methyl-2,4-(1*H*,3*H*)pyrimidinedione **123** [103]

$C_{32}H_{34}O_6N_2$ M.W. 542



Carbocyclic thymidine **3** (44 mg, 0.2 mmol) was dissolved in pyridine (1 cm^3) containning activated molecular sieves 4A and then stirred at rt for 15 minutes. Then 4,4'-dimethoxytrityl chloride (Cl-DMT) (79 mg, 0.2 mmol) was added in one portion and the mixture stirred for 22 h at rt. Evaporation of pyridine *in vacuo* by addition-evaporation with toluene (3 x 5 cm^3) gave a solid residue which was purified by rapid flash chromatography ($CHCl_3$:EtOH:NEt_3 97:3:0.3). Further purification by chromatotron chromatography was neccesary ($CHCl_3$:EtOH:NEt_3 99:1:0.2; 1 mm silica plate) giving the pure title compound **123** (72 mg; 61%), R_f ($CHCl_3$:MeOH 9:1) 0.24; m.p. (from EtOAc:petroleum ether 40:60) 212-213 °C. δ_H(400 MHz; $CDCl_3$) 7.43-6.81 (14 H, m, 2 x C_6H_4, C_6H_5, NH), 6.94 (1H, d, $J_{H,Me}$ 0.9, H-6), 5.12 (1 H, ddd, $J_{1,5}$ 8.7, $J_{1,2}$ 8.3, H-1), 4.23 (1 H, dt, $J_{3,4}$ 9.6, $J_{3,2}$ 6.3, H-3), 3.79 (3 H, s, 2 OMe), 3.40 (1 H, dd, $J_{6,6'}$ 9.1, $J_{6,4}$ 4.7, H-6), 3.12 (1 H, dd, $J_{6',6}$ 9.1, $J_{6',4}$ 6.9, H-6'), 2.26-2.14 (2 H, m, H-4,5β), 2.12-2.02 (2 H, m, H-2,2'), 1.85 (3 H, d, $J_{CH3,H}$ 0.9, CH_3), 1.48-1.26 (1 H, m, H-5α); δ_C(100.6 MHz; $CDCl_3$) 164.02 (C-4, Thy), 158.99 (C-2, Thy), 151.30 (C-6, Thy), 145.12 (C-5, Thy), 137.26 (HC=), 136.33 (C), 136.12 (C), 130.40 (CH), 128.45 (CH), 128.36 (CH), 127.35 (CH), 113.64 (CH), 111.64 (Me*C*=), 86.99 (C), 75.49 (C-3), 65.89 (C-6), 55.64 (2O*C*H_3), 54.20 (C-1), 47.40 (C-4), 39.38 (C-

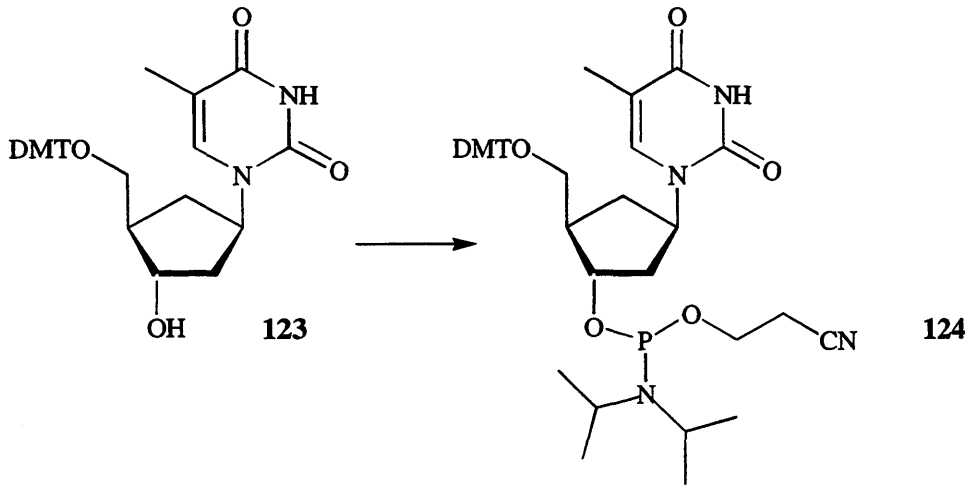
2), 33.43 (C-5), 12.96 (CH_3). m/z (FAB) 543.24957 (MH^+), requires 543.24951. ν_{max}(sol.)/cm^{-1} 3100-2840*s* (N-H, C-H), 1680*s* (C=O), 1420*s* (C=C), 1270-1240 (C-O).

4.43.1 (-)-(1'*R*,3'*S*,4'*S*)-1'-[4'-(4,4'-Dimethoxytrityloxymethyl)-3'-hydroxycyclopentyl]-5-methyl-2,4-(*1H,3H*)-pyrimidinedione 123

The experimental procedure described in Section 4.43 was applied to compound (+) **3** (461 mg, 1.9 mmol)which was treated in pyridine (10.5 cm^3) with activated molecular sieves 4A followed by adition of 4,4'-dimethoxytrityl chloride. This led to the title compound (-) **123** (680 mg; 65%). $[\alpha_D]^{20}$ - 29.04 (c 0.6, CH_2Cl_2), m.p. (from EtOAc:petroleum ether 40:60) 94-95 °C.

4.44 (±)-1'β-[3'α-[2-Cyanoethyl-(diisopropylamino)-phospinoyloxy]-4'β-(4,4'-dimethoxytrityloxymethyl)]-5-methyl-2,4-(1*H*,3*H*)-pyrimidinedione 124

$C_{41}H_{51}O_7N_4P$ M.W. 742



To a solution of **123** (132 mg, 0.2 mmol) in CH_2Cl_2 (1 cm^3) and dry diisopropylethylamine (106 mm^3), was added dropwise over 5 minutes (±)-chlorodiisopropylamino-β-cyanoethylphosphoramidite. The mixture was stirred for 5.5 h, treated with saturated Na_2CO_3 solution (10 cm^3) and extracted with CH_2Cl_2 (3 x 15 cm^3). The combined organic extracts were dried, and the solvent evaporated *in vacuo*, to give a crude product which

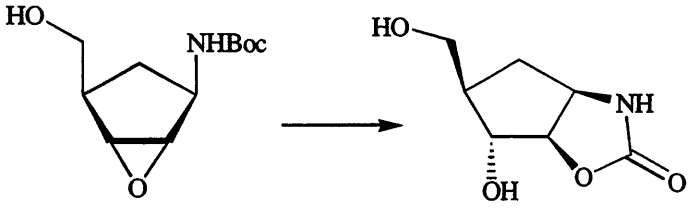
was rapidly purified by flash chromatography (petroleum ether 40-60:EtOAc:NEt_3 44:44:2). Compound **124** was obtained as a racemic mixture of two diastereoisomers) (100 mg, 55%), R_f (EtOAc:petroleum ether 40:60 3:2) 0.76 and 0.64, respectively. Found: C, 65.75; H, 6.38; N, 7.25. $C_{14}H_{21}O_2NS_2$ requires C, 66.31; H 6.87; N, 7.55. δ_H(400 MHz; $CDCl_3$) 8.37 (1 H, br s, NH), 7.35-6.83 (13 H, m, 2 x C_6H_4 and C_6H_5), 7.06-7.05(1 H, m, CH=), 5.21-5.13 (1 H, m, H-1), 4.47-4.33 (1 H, m, H-3), 3.82 (3 H, s, OCH_3), 3.81 (3 H, s, OCH_3), 3.74-3.52 [4 H, m, 2 C*H*$(CH_3)_2$, C*HH*OP], 3.29-3.22 (2 H, m, H-6,6'), 2.64-2.61 (1 H, m, C*H*HCN), 2.50-2.47 (1 H, m, CH*H*CN), 2.44-2.33 (2 H, m, H-4,5), 2.27-2.14 (1 H, m, H-2), 2.12-2.04 (1 H, m, H-2'), 1.86-1.85 (3 H, C*H*$_3$Thy), 1.64-1.44 (1 H, H-5α), 1.20-1.09 (2 H, m, 4 x C*H*$_3$). δ_C(100.6 MHz; $CDCl_3$) 163.91 (C-4, Thy), 158.91 (C), 151.24-151.15 (C-2, Thy), 145.33 (C), 137.29-137.25 (*C*H=), 136.47-136.40 (C), 130.53-130.47 (*C*-H*o*), 128.60-128.54 (*C*-H*o*), 128.24 (*C*-H*m*), 127.22 (*C*-H*m*), 118.15-118.03 (CN), 113.52 (C-H*p*), 111.57-111.50 (C-5,Thy), 86.59 (C), 75.87-75.71 and 75.21-75.04 (C-3), 64.39-63.88 (C-6), 58.66-58.47 and 58.41-58.22 (CH_2-OP), 55.64-55.61 (OMe), 54.42 (C-1), 47.18-46.99-46.93 (C-4), 43.61-43.48 (N*C*H), 38.99-38.84 (C-2), 33.41-33.24 (C-5), 25.02-24.95 (CH_3), 20.90-20.83 and 20.71-20.64 (*C*H_2CN), 12.82 (CH_3,Thy). δ_P(101 MHz; $CDCl_3$) 148.15 and 148.04. m/z (FAB) 743.35764 (MH^+), requires 743.35736. ν_{max} (sol.)/cm^{-1} 3040m (N-H), 2960m (C-H), 2250w (C≡N), 1685m (C=O), 1420m (C-N), 1250*s* (C-O), 690*s* (P-N-C).

4.44.1 1'-[(1'*R*,3'*S*,4'*S*)-[3'-[2-Cyanoethyl-(diisopropylamino)-phospinoyloxy]-4'-(4,4'-dimethoxytrityloxymethyl)]]-5-methyl-2,4 (1*H*,3*H*)-pyrimidinedione 124

The experimental procedure described in Section 4.43 was applied to compound (-) **123** (540 mg, 0.01 mmol) which was dissolved in CH_2Cl_2 (4 cm^3) and treated with diisopropylethylamine (0.45 cm^3) and (±)-chlorodiisopropylamino-β-cyanoethylphosphoramidite (0.25 cm^3). Rapid purification by flash chromatography afforded a mixture of two enantiomerically pure diastereoisomers **124** (498 mg; 67%).

4.45 (±)-1β,5β,8α-Hydroxy-7β-hydroxymethyl-(2-oxa-4-azabicyclo[3.3.0]-3-octanone) 115

$C_7H_{11}O_4N$ M.W. 173

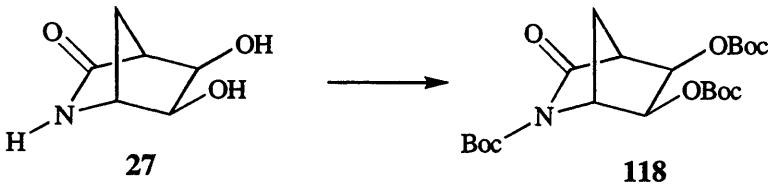


95 **115**

Compound **95** (70 mg, 0.45 mmol) was treated with HCl (2 M; 0.3 cm^3) at rt for 2 h. On complete reaction, the solution was concentrated to dryness and the residue was purified by chromatography (EtOAc:MeOH 1.5:8.5) leading to a solid identified as the title compound **115** (57 mg; 75%), R_f (EtOAc:MeOH 1:1) 0.78; m.p. (from EtOAc:MeOH) 130 °C (lit.[93] 129-130 °C). Found: C, 48.16; H, 6.45; N, 7.68. $C_7H_{11}O_4N$, requires C, 48.56; H, 6.40; N, 8.09. δ_H(250 MHz; D_2O) 4.93-4.87 (1 H, m, H-1), 4.38 (1 H, ddd, *J* 7.9, *J* 7.1 and *J* 7.1, H-5), 4.13 (1 H, dd, *J* 8.2, *J* 4.7, H-8), 3.78 (1 H, dd, $J_{9,9'}$ 11.7, $J_{9,7}$ 5.3, H-9), 3.63 (1 H, dd, $J_{9',9}$ 11.7, $J_{9',7}$ 5.8, H-9´), 2.39 (1 H, ddd, $J_{6\alpha,\beta}$ 13.5, *J* 7.9 and *J* 7.1, H-6α), 2.20-2.05 (1 H, m, H-7), 1.60 (1 H, ddd, $J_{6\beta,\alpha}$ 13.5, *J* 10.3 and *J* 7.9, H-6β); δ_C(63 MHz; D_2O) 161 (C=O), 87.9 (C-1), 78.5 (C-5), 62.0 (C-9), 53.7 (C-8), 45.0 (C-7), 33.9 (C-1). m/z (FAB) 174.07665 (MH^+), requires 174.07663. ν_{max} (KBr)/cm^{-1} 3500-3100*s* (O-H), 2960-2900m (C-H), 1730*s* (C=O), 1400m (C-N), 1240m (C-O), 1050*s* (C-O).

4.46 (±)-*exo-cis-N-tert*-Butoxycarbonyl-5,6-di-O-(*tert*-butoxycarbonyloxy)-2-azabicyclo[2.2.1]heptan-3-one 118

$C_{21}H_{33}O_9N$ M.W. 443

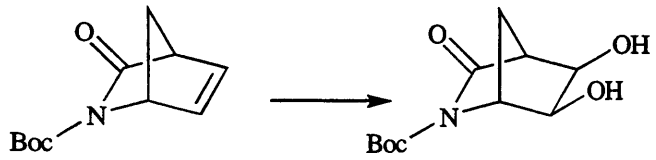


27 **118**

To a suspension of dihydroxy lactam **27** (167 mg, 1.2 mmol) in CH_2Cl_2 (3.5 cm^3), was added triethylamine (1.2 mmol; 0.17 cm^3), 4-DMAP (43 mg, 1.2 mmol) followed by $(Boc)_2O$ (842 mg, 3.9 mmol). After 24 h at rt, the volatiles were evaporated *in vacuo*. Purification of the crude product by flash chromatography (CH_2Cl_2:Et_2O 95:5) led to a solid identified as the *title compound* **118** (330 mg; 64%), R_f (CH_2Cl_2:Et_2O 9:1) 0.72; m.p. (from Et_2O) 163 °C. Found: C, 56.75; H, 7.51; N, 3.12. $C_{21}H_{33}O_9N$ requires C, 56.87; H, 7.50; N, 3.16. δ_H(400 MHz; $CDCl_3$) 5.04 (1 H, dd, $J_{6,5}$ 5.8, $J_{6,1}$ 1.1, H-6), 4.93 (1 H, dd, $J_{5.6}$ 5.8, $J_{5,4}$ 1.7, H-5), 4.46 (1 H, d, $J_{1,4}$ 1.5, H-1), 2.90 (1 H, d, $J_{4,1}$ 1.5, H-4), 2.23 (1 H, dt, $J_{7a,s}$ 10.9, $J_{7a,6\text{-}5}$ 1.3, H-7a), 2.05 (1 H, dt, $J_{7s,a}$ 10.9, $J_{7s,5\text{-}6}$ 1.6, H-7s), 1.50, 1.46, 1.45 (27 H, 3s, 3 ^{t}Bu); δ_C(100.6 MHz; D_2O) 171.04 (C-3), 148.93 (N*C*O), 152.47, 152.37 (2 x O*C*O), 84.22, 83.43, 83.39 (3 x C), 74.10 (C-5), 72.23 (C-6), 60.72 (C-1), 51.74 (C-4), 33.90 (C-7), 28.30 (^{t}*Bu*OCON), 28.00 (2 x ^{t}*Bu*OCOO). m/z (FAB) 466.20524 ($M+Na^+$), requires 466.20533. ν_{max} (KBr)/cm^{-1} 2980m (C-H), 1770*s* (C=O), 1720*s* (C=O), 1460 (C-N), 1250-1300*s* (C-O).

4.47 (±)-*exo-cis-N-tert*-**Butoxycarbonyl-5,6-dihydroxy-2-azabicyclo[2.2.1]heptan-3-one 121**

$C_{11}H_{17}O_5N$ M.W. 243

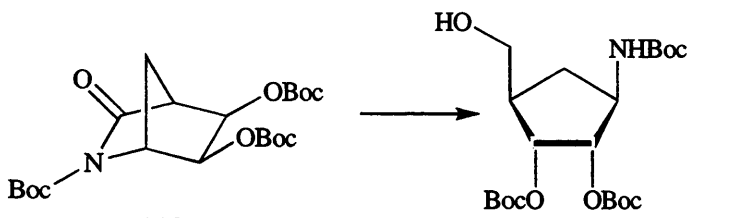


91 **121**

Compound **91** (250 mg, 1.2 mmol) was dissolved in ^{t}BuOH-H_2O 3:1 (2 cm^3) and 4-methylmorpholine *N*-oxide (NMO) solution in water (60% wt; 0.25 cm^3) followed by osmium tetraoxide solution in 2-methyl-2-propanol (2.5% wt; 0.06 cm^3). After 17 h at rt, on complete reaction, the mixture was warmed up to 50 °C for 15 minutes. When the solution reached rt, $Na_2S_2O_5$ (130 mg) was added. The precipitate formed was filtered and washed with acetone. The filtrate was evaporated *in vacuo* and then azeotroped with water to remove the NMO. The residual syrup was dried by addition-evaporation of isopropyl alcohol to give the *title compound* **121** (182 mg; 63%). R_f (CH_2Cl_2:EtOAc 1:1) 0.37; m.p. (from Et_2O:EtOAc) 149 °C. Found: C, 54.00; H, 6.90; N, 5.72. $C_6H_7O_2N$ requires C, 54.31; H, 7.04; N, 5.76. δ_H(100.6 MHz; $CDCl_3$) 4.36 (1 H, dt, $J_{1,7}$ 2.3, $J_{1,4}$ 1.0, H-1), 4.27 (1 H, t, *J* 5.0, H-6), 4.14-4.12 (1 H, m, H-5), 3.47 (1 H, br s, OH-5), 3.45 (1 H, br s, OH-6), 2.82 (1 H, d, $J_{4,7}$ 1.4, H-4), 2.11 (1 H, dt, $J_{7s,a}$ 10.9, $J_{7s,4\text{-}1}$ 1.4, H-7s), 2.0 (1 H, dt, $J_{7a,s}$ 10.9, $J_{7a,5\text{-}6}$ 1.7, H-7a), 1.54 (9 H, s, ^{t}Bu); δ_C(63 MHz; $CDCl_3$) 173.2 (C-3), 150.10 (C=O), 84.14 (C), 70.85 (C-5), 68.41 (C-6), 62.58 (C-1), 54.00 (C-4), 32.26 (C-7), 28.43 (3 x CH_3). m/z (FAB) 244.11849 (MH^+), requires 244.11850. ν_{max} (KBr)/cm^{-1} 3460-3360*s* (O-H), 3000-2940*s* (C-H), 1770*s* (C=O), 1690m (C=O), 1460m (C-N), 1370-1300m (C-O), 1150m (C-O).

4.48 **(±)-4β-(*N-tert*-Butoxycarbonylamino)-2α,3α-(di-*tert*-butoxycarbonyloxy-dihydroxy)-cyclopentan-1β-ylmethanol 119**

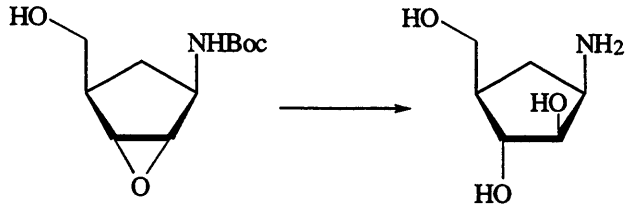
$C_{21}H_{37}O_9N$ M.W. 447



118 **119**

Lactam **118** (100 mg, 0.23 mmol) was dissolved in MeOH (3 cm^3), cooled at 0 °C and treated with $NaBH_4$ (78 mg, 2.0 mmol) at rt for 2.5 h. The mixture was worked-up by addition of HCl (1 M) at 0°C. The organics were extracted with EtOAc (3 x 10 cm^3), the combined exrtracts were dried and the solvent evaporated *in vacuo* and the crude product purified by flash chromatography (CH_2Cl_2:Et_2O 3:2). The white solid obtained was identified as the *title compound* **119** (75 mg; 74%), R_f (Et_2O:CH_2Cl_2 1:1) 0.69; m.p. (from Et_2O) 135 °C. Found: C, 55.99; H, 8.31; N, 3.33. $C_{21}H_{37}O_9N$ requires C, 56.36; H, 8.33; N, 3.13. δ_H(400 MHz; $CDCl_3$) 5.10 (1 H, br s, NH), 5.02 (1 H, t, *J* 5.1, H-2), 4.91 (1 H, t, *J* 5.5, H-3), 4.08 (1 H, br s, H-4), 3.74 (1 H, dd, $J_{6,6'}$ 10.9, $J_{6,1}$ 3.6, H-6), 3.61 (1 H, dd, $J_{6',6}$ 10.9, $J_{6',1}$ 4.3, H-6'), 2.72 (1 H, br s, OH), 2.46-2.28 (2 H, m, H-1,5β), 1.48-1.43 (1 H, m, H-5α), 1.48, 1.48, 1.43 (27 H, 3s, 3 ^{t}Bu); δ_C(100.6 MHz; $CDCl_3$) 155.57, 153.56, 153.29 (3 x C=O), 82.89, 82.84, 79.92 (3 x C), 79.12 (C-3), 76.01 (C-2), 62.69 (C-6), 53.66 (C-1), 42.66 (C-4), 29.93 (C-5), 28.75, 28.12, 28.12 (3 x CH_3). m/z (FAB) 448.25451 (MH^+), requires 448.25466. ν_{max} (KBr)/cm^{-1} 3400m (O-H), 2990m (C-H), 1750-1690*s* (C=O), 1520m (N-H), 1250-1300*s* (C-O), 1170*s* (O-H).

4.49 **(±)-2α,3β-Dihydroxy-4β-amino-1β-hydroxymethylcyclopentane 116** [92]
$C_6H_{13}O_3N$ M.W. 147

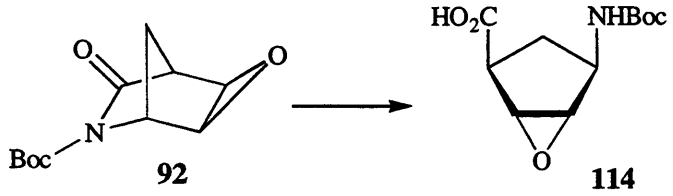


95 **116**

Compound **95** (189 mg, 0.82 mmol) was reacted with NaOH (1 M; 0.96 cm^3) at 75 °C. After 3 h, the solution was cooled to rt and HCl (1 M; 1.5 cm^3) was added. The solution was evaporated *in vacuo* and the crude product purified by flash chromatography (EtOAc:MeOH 8:2→6:4). Two oily compounds were isolated in a ratio 4.7:1 and identified as the title compound **116** (75 mg; 62%) (as the neutral product and the protonated one), R_f (EtOAc:MeOH 4:1) 0.40 and R_f ($MeOH:H_2O$ 1:2) 0.1. Protonated **116**: δ_H(400 MHz; MeOD-d_4) 4.71 (1 H, dd, $J_{3,4}$ 9.1, $J_{3,2}$ 4.1, H-3), 4.24 (1 H, ddd, $J_{4,3}$ 9.1, $J_{4,5\beta}$ 7.7, $J_{4,5\alpha}$ 5.6, H-4), 4.01 (1 H, dd, $J_{2,1}$ 8.2, $J_{2,3}$ 4.1, H-2), 3.70 (1 H, dd, $J_{6,6'}$ 11.1, $J_{6,1}$ 5.2, H-6), 3.58 (1 H, dd, $J_{6',6}$ 11.1, $J_{6',1}$ 6.4, H-6'), 2.29 (1 H, dt, $J_{5\alpha,\beta}$ 13.7, $J_{5\alpha,4\text{-}1}$ 6.8, H-5α), 2.04-1.95 (1H, m, H-1), 1.57 (1 H, ddd, $J_{5\beta,\alpha}$ 13.7, $J_{5\beta,1}$ 9.0, $J_{5\beta,4}$ 7.7, H-5β); δ_C(63 MHz; D_2O) 78.02 (C-1), 75.79 (C-2), 62.84 (C-6), 50.73 (C-5), 44.50 (C-3), 29.36 (C-4). Neutral **116**: δ_H(250 MHz; MeOD-d_4) 3.98 (1 H, dd, $J_{3,4}$ 5.8, $J_{3,2}$ 4.6, H-3), 3.84 (1 H, t, $J_{2,3\text{-}1}$ 4.6, H-2), 3.69 (1 H, dd, $J_{6,6'}$ 10.5, $J_{6,1}$ 5.1, H-6), 3.53-3.60 (1 H, m, H-6',4), 2.33 (1 H, dt, $J_{5\alpha,\beta}$ 13.4, $J_{5\alpha,4\text{-}1}$ 8.4, H-5α), 2.09-2.00 (1 H, m, H-1), 1.61 (1 H, ddd, $J_{5\beta,\alpha}$ 13.4, $J_{5\beta,1\text{-}4}$ 8.4, H-5β); δ_C(63 MHz; D_2O) 87.92 (C-2), 78.56(C-3), 62.02 (C-6), 53.80 (C-1), 45.08 (C-4), 33.08 (C-5). m/z (FAB) 148.09735 (MH^+), requires 148.09737. ν_{max} (sol.)/cm^{-1} (broad peaks) 3450-3000m (OH, NH), 1590m (N-H).

4.50 (±)-4β-(*N-tert*-Butoxycarbonylamino)-2α,3α-epoxy-1β-cyclopentanecarboxylic acid 114

$C_{11}H_{17}O_5N$ M.W. 243



Compound **92** (170 mg, 0.8 mmol) was reacted with NaOH (1 M; 0.80 cm^3) at rt. After 2 h. HCl (1 M) was added and the organic product was extracted with EtOAc (3 x 10 cm^3). The combined extracts were dried and the solvent evaporated *in vacuo*. Purification of the crude product by flash chromatography (CH_2Cl_2:Et_2O 1:1) led to a solid identified as the *title compound* **114** (137 mg; 75%), R_f (EtOAc:MeOH 9:1) 0.49; m.p. (from C_6H_{12}-Et_2O) 124° C. Found: C, 54.79; H, 7.03; N, 5.58. $C_{11}H_{17}O_5N$ requires C, 54.31; H, 7.04; N, 5.76. δ_H(400 MHz; acetone-d_6) 5.73 (1 H, br s, NH), 4.18 (1 H, t, *J* 7.3, H-4), 3.74 (1 H, d, $J_{2,3}$ 2.3, H-2), 3.45 (1 H, d, $J_{3,1}$ 1.6, H-3), 3.18 (1 H, d, $J_{1,5}$ 8.7, H-1), 1.94 (1 H, dd, $J_{5\alpha,\beta}$ 14.4, $J_{5\alpha,4}$ 8.7, H-5α), 1.86 (1 H, dt, $J_{5\beta,\alpha}$ 14.4, $J_{5\beta,4}$ 7.7, H-5β); δ_C(100.6 MHz; $CDCl_3$) 175.07 (C=O, acid), 157.60 (C=O, Boc), 82.30 (C), 58.14 (C-3), 57.84 (C-2), 50.84 (C-4), 44.32 (C-1), 31.06 (C-5), 28.04 ($3CH_3$). m/z (FAB) 244.11842 (MH^+), required 244.11850. ν_{max} (KBr)/cm^{-1} 3400m (N-H), 3200*s* (O-H), 2980m (C-H), 1730*s* (C=O), 1680*s* (C-O), 1520*s* (N-H), 1320*s* (C-O), 1170*s* (O-H).

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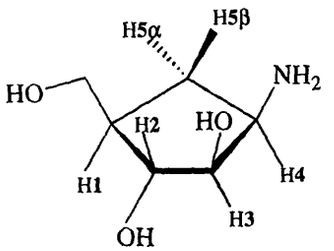
APPENDICES 1, 2, 3

APPENDIX 1

1.- ONE DIMENSIONAL ^{1}H SPECTRA

400 MHz: MeOD-d_4

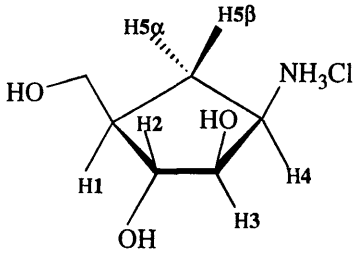
27 °C



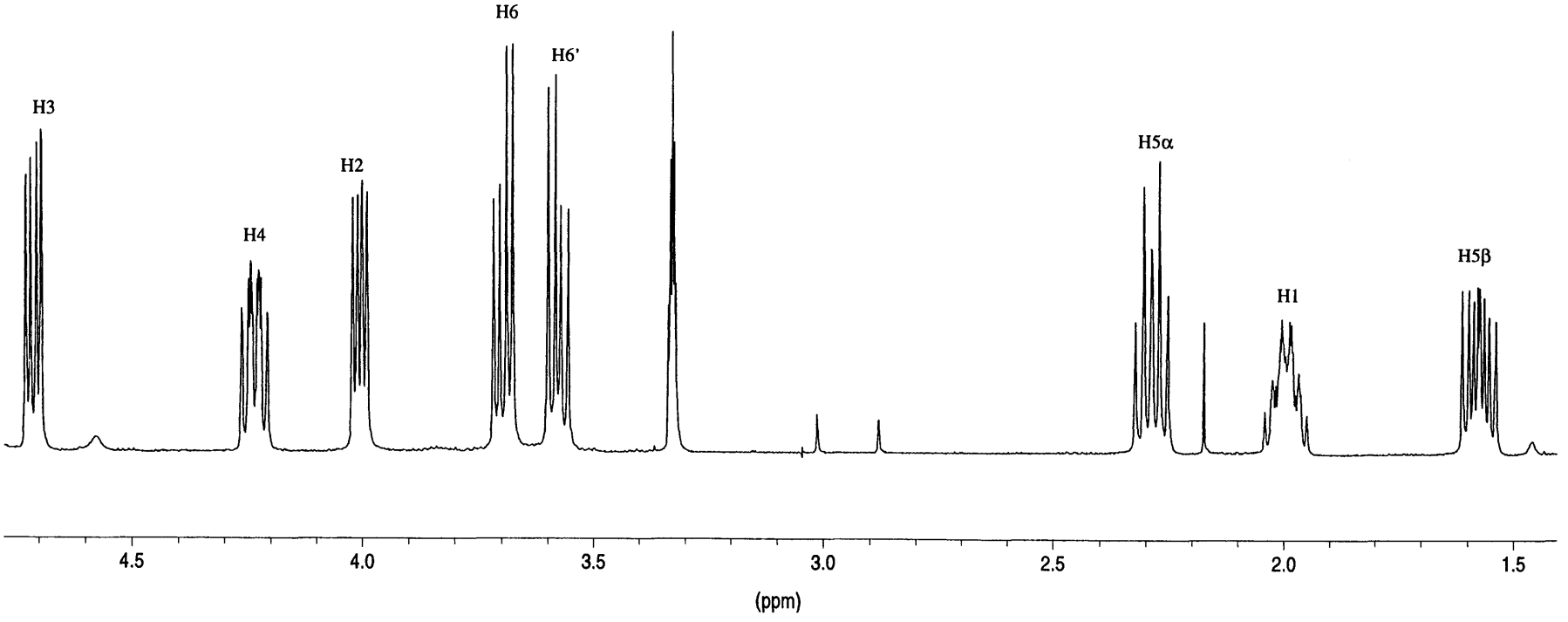
116

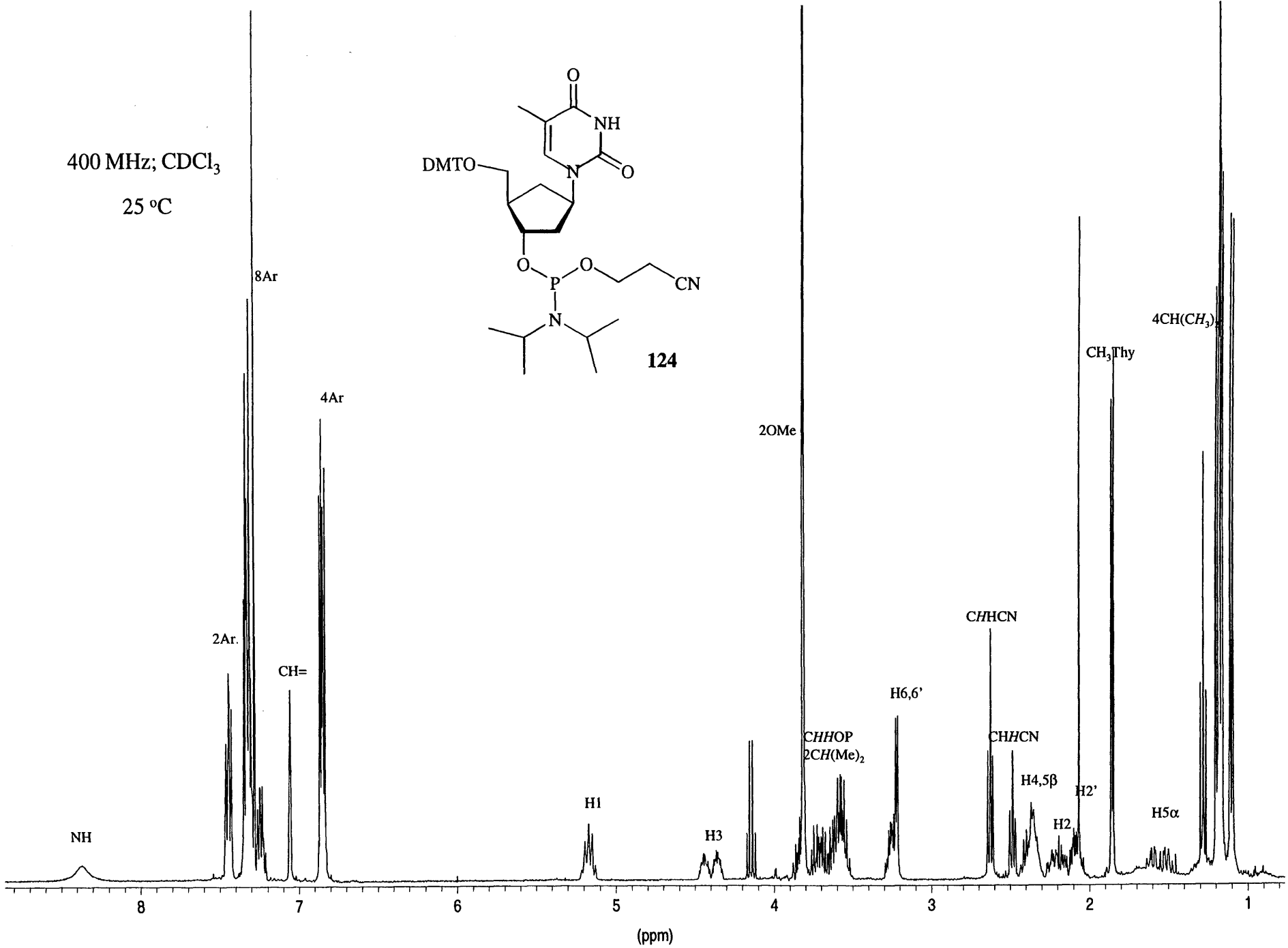
400 MHz: MeOD-d_4

27 °C



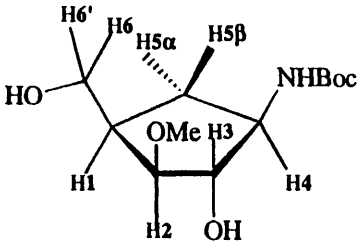
116



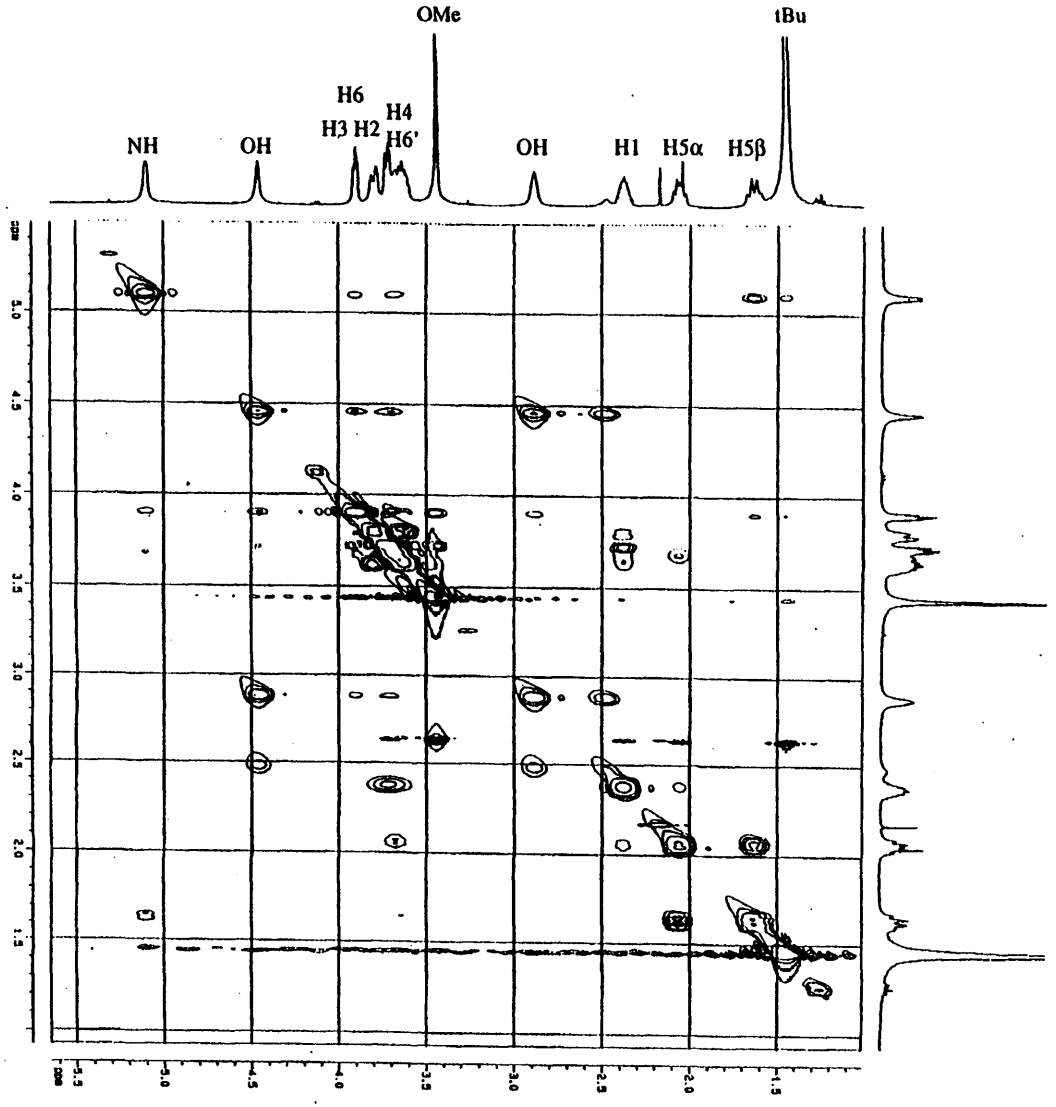

400 MHz; CDCl3
25 °C
DMTO
NH
O
N
O
O
P
O
CN
N
124
8Ar
4Ar
2OMe
4CH(CH3)2
CH3Thy
2Ar.
CH=
H6,6'
CHHCN
CHHOP
2CH(Me)2
CHHCN
H4,5β
H2'
H2
H5α
H1
H3
NH
8
7
6
5
4
3
2
1
(ppm)

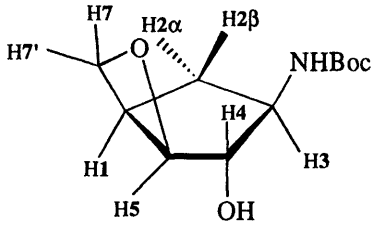
APPENDIX 2

TWO DIMENSIONAL ^{1}H NMR SPECTRA

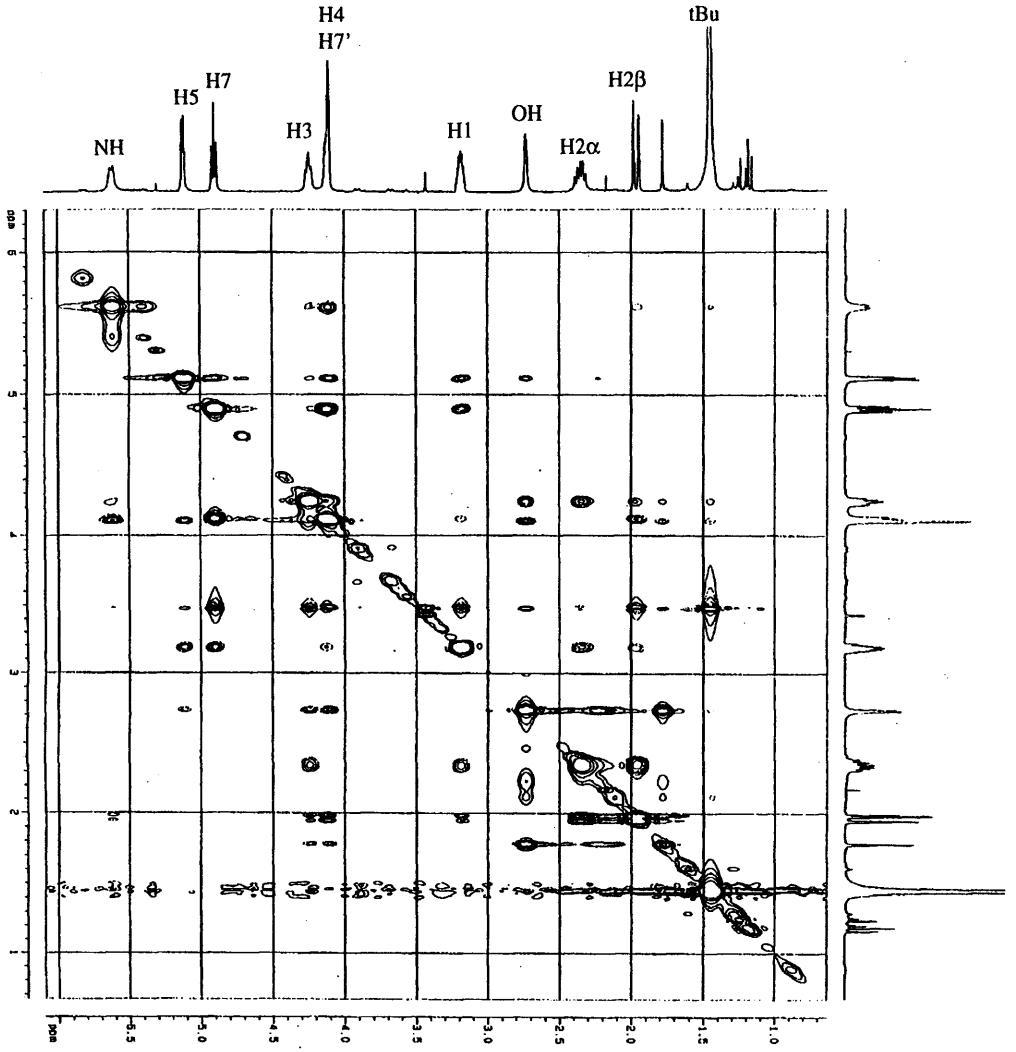


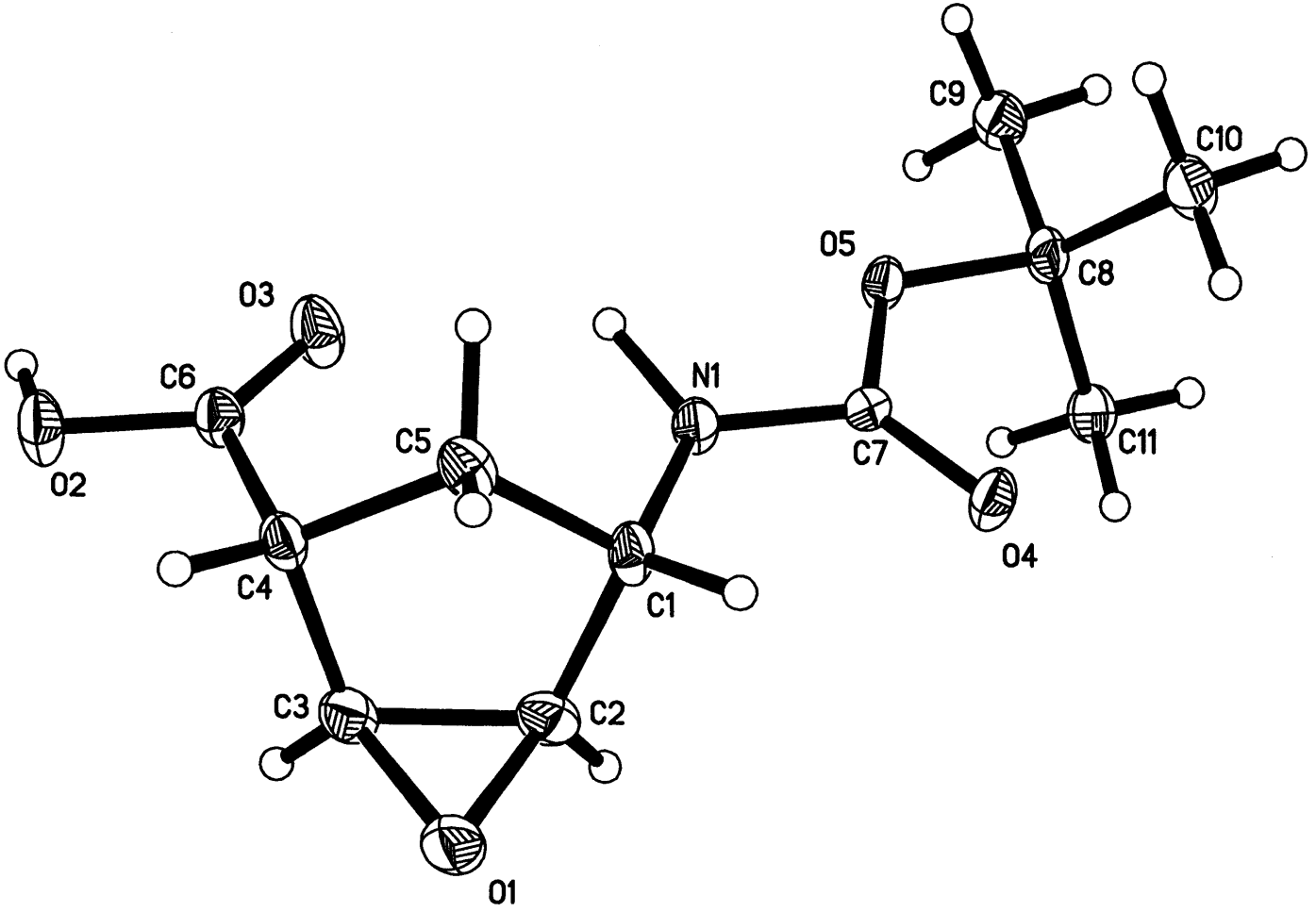
NOESY spectrum
Compound **96**





NOESY spectrum
Compound **99**

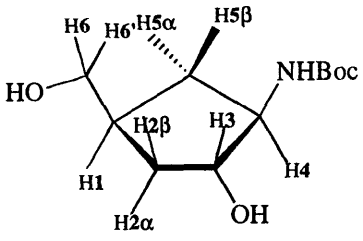




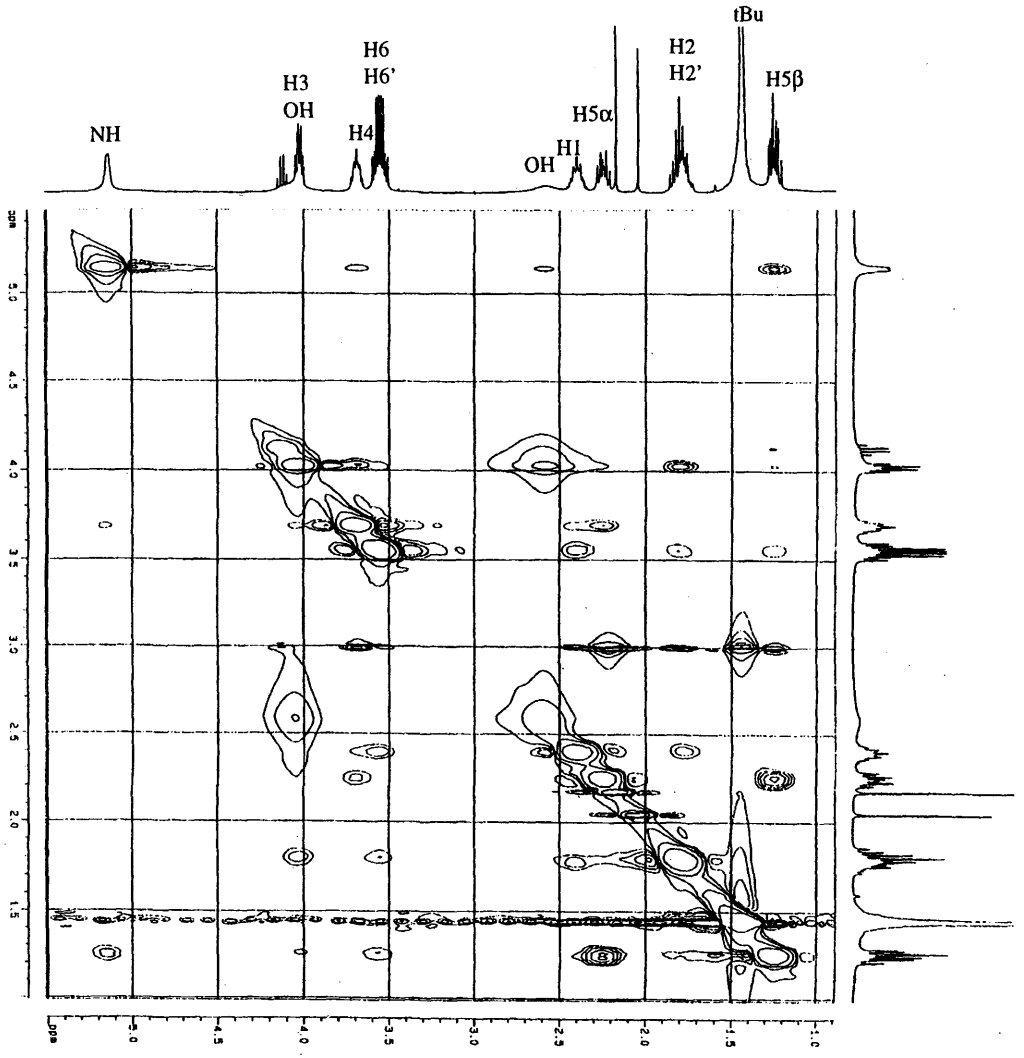
X-Ray structure 4: Compound **114**

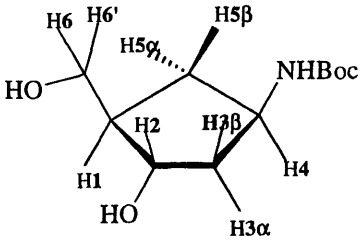
Crystal data and structure refinement for compound **114**

Identification code	bd9749
Empirical formula	$C_{11}H_{17}NO_5$
Formula weight	243.26
Temperature	170 (2) K
Wavelength	0.71073 Å
Crystal system	Triclinic
Space group	P1
Unit cell dimensions	a = 6.081 (2) Å alpha = 86.16 (3) °
	b = 8.713 (3) Å beta = 82.38 (2) °
	c = 11.352 Å gamma = 85.82 (30 °
Volume, z	593.5 (3) $Å^3$, 2
Density (calculated)	1.361 Mg/ m^3
Absorption coefficient	0.108 mm^{-1}
F (000)	260
Crystal size	0.59 x 0.24 x 0.19 mm
θ Range for data collection	3.39 to 25.00 °
Limiting indices	$0 \leq h \leq 7, -10 \leq k \leq 10, -13 \leq l \leq 13$
Reflections collected	2119
Independent reflections	1971 (R_{int} = 0.0410)
Absorption correction	Not applied
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	1971 / 0 / 156
Goodness-on-fit on F^2	1.079
Final R indices [I > 2σ (I)]	R1 = 0.0633, wR2 = 0.1481
R indices (all data)	R1 = 0.0992, wR2 = 0.1728
Largest diff. peak and hole	0.332 and -0.328 $eÅ^{-3}$

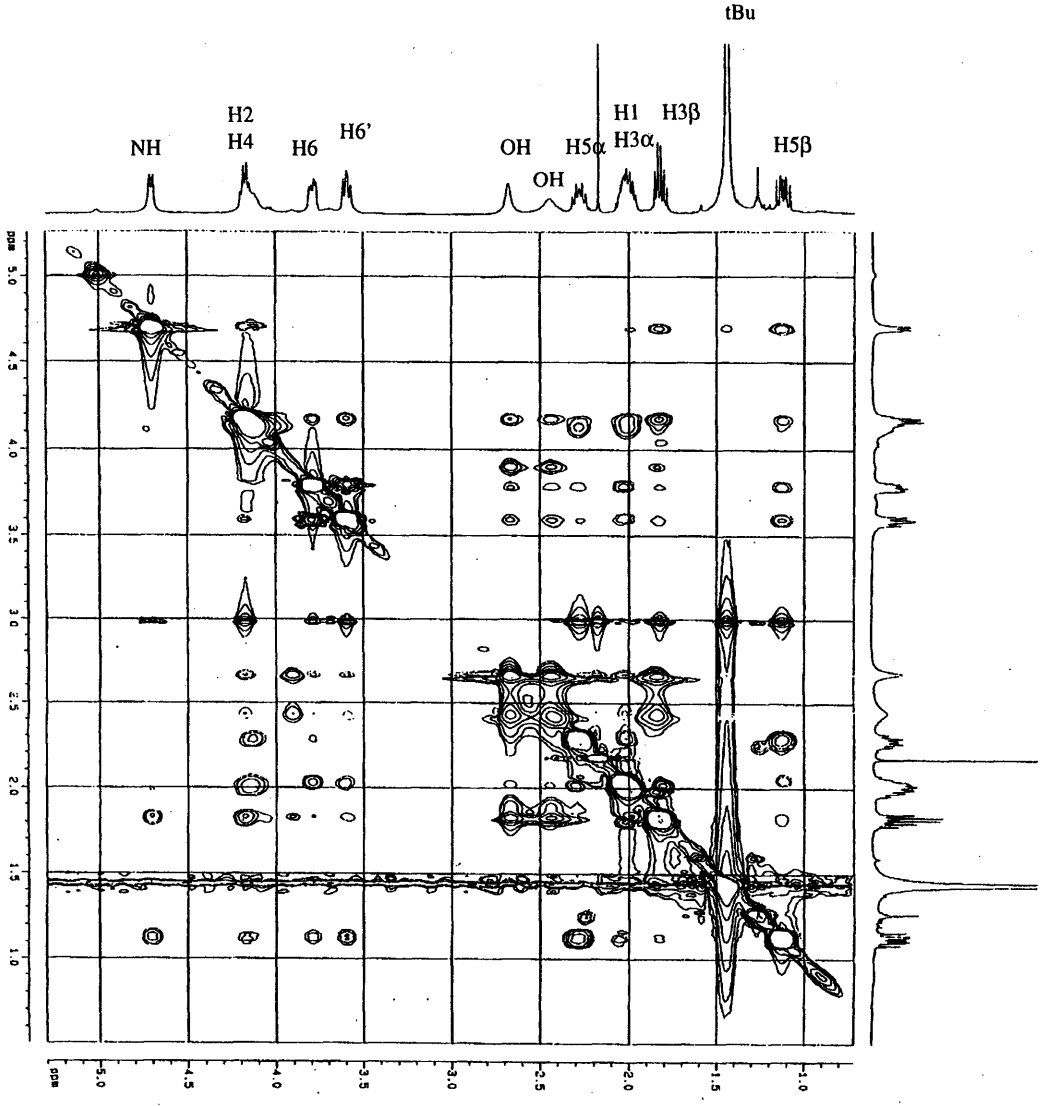


NOESY spectrum
Compound **100**

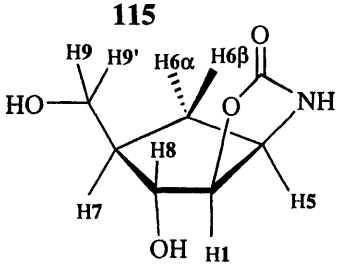




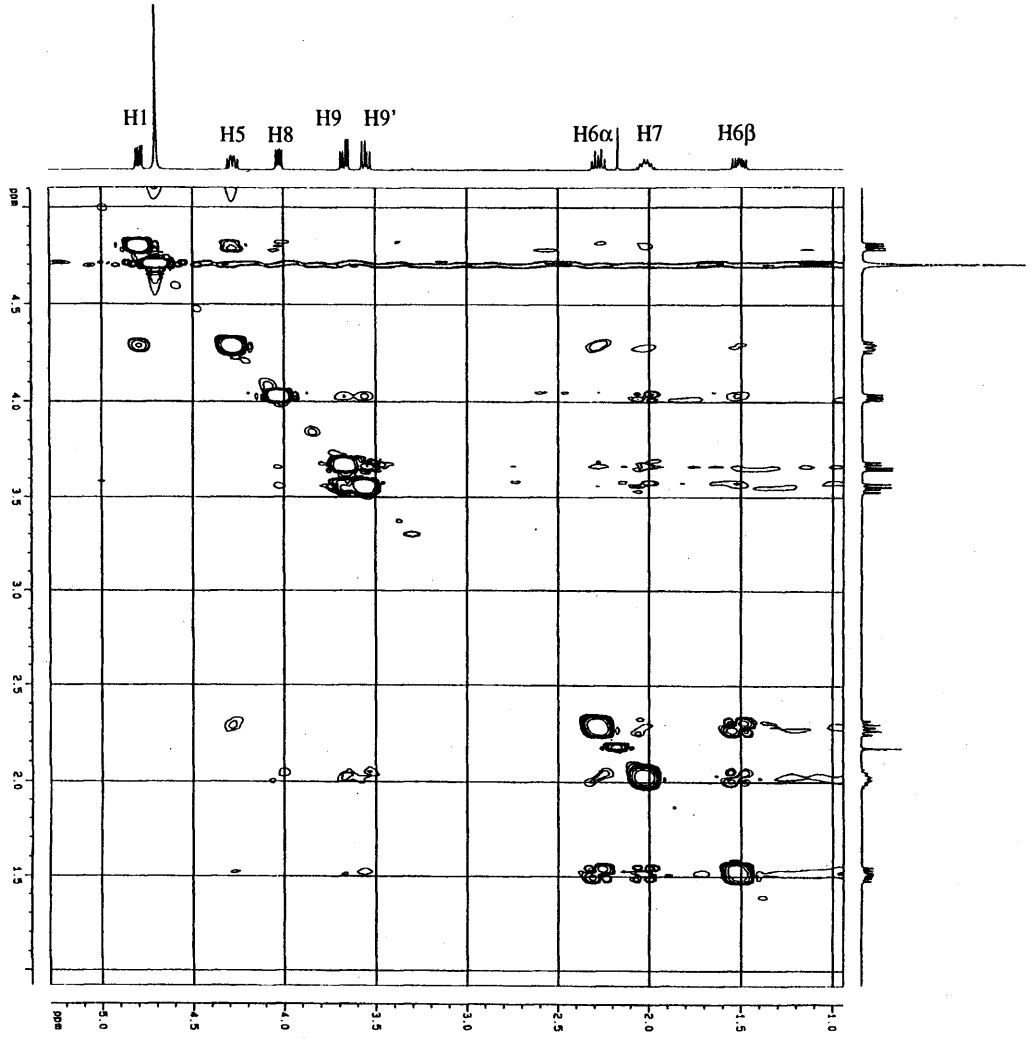
NOESY spectrum
Compound **101**

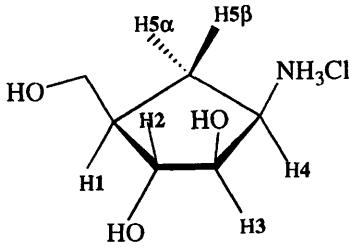


115

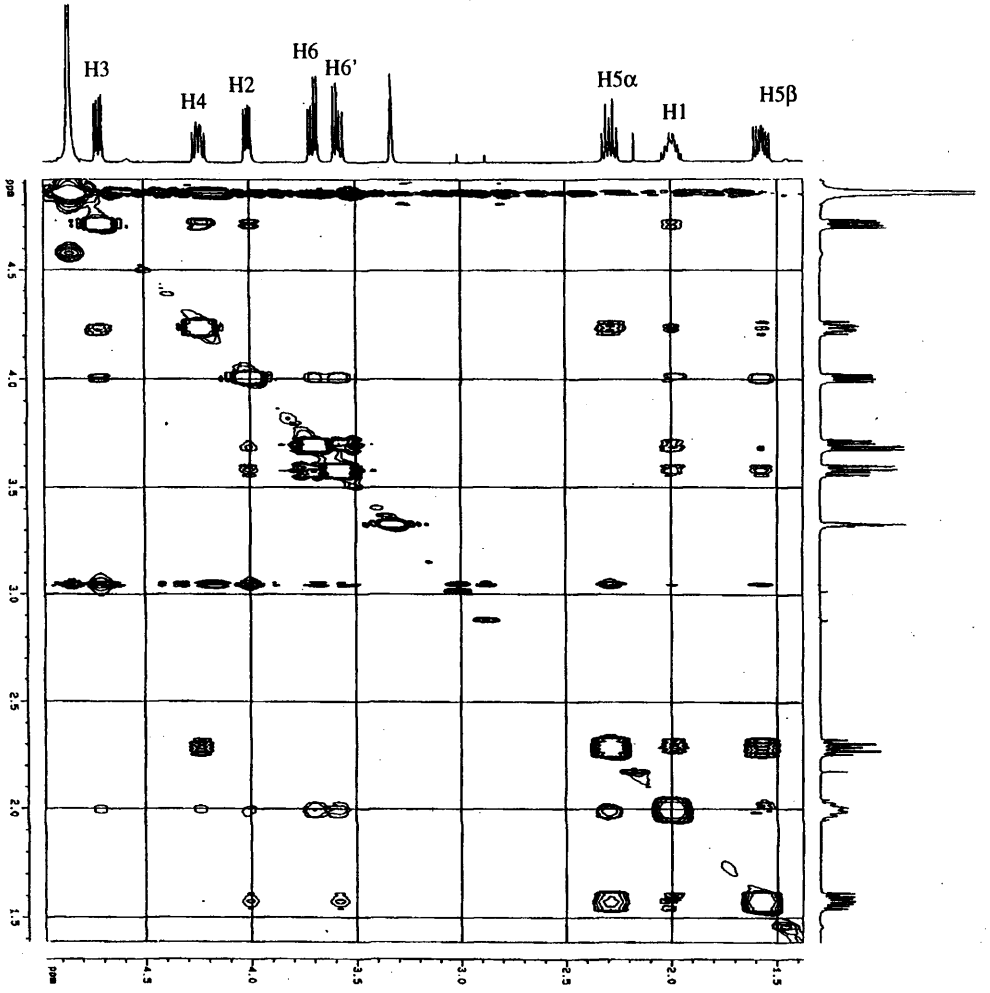


ROESY spectrum
Compound **115**





NOESY spectrum
Compound **116**



APPENDIX 3

X-RAY STRUCTURES

&

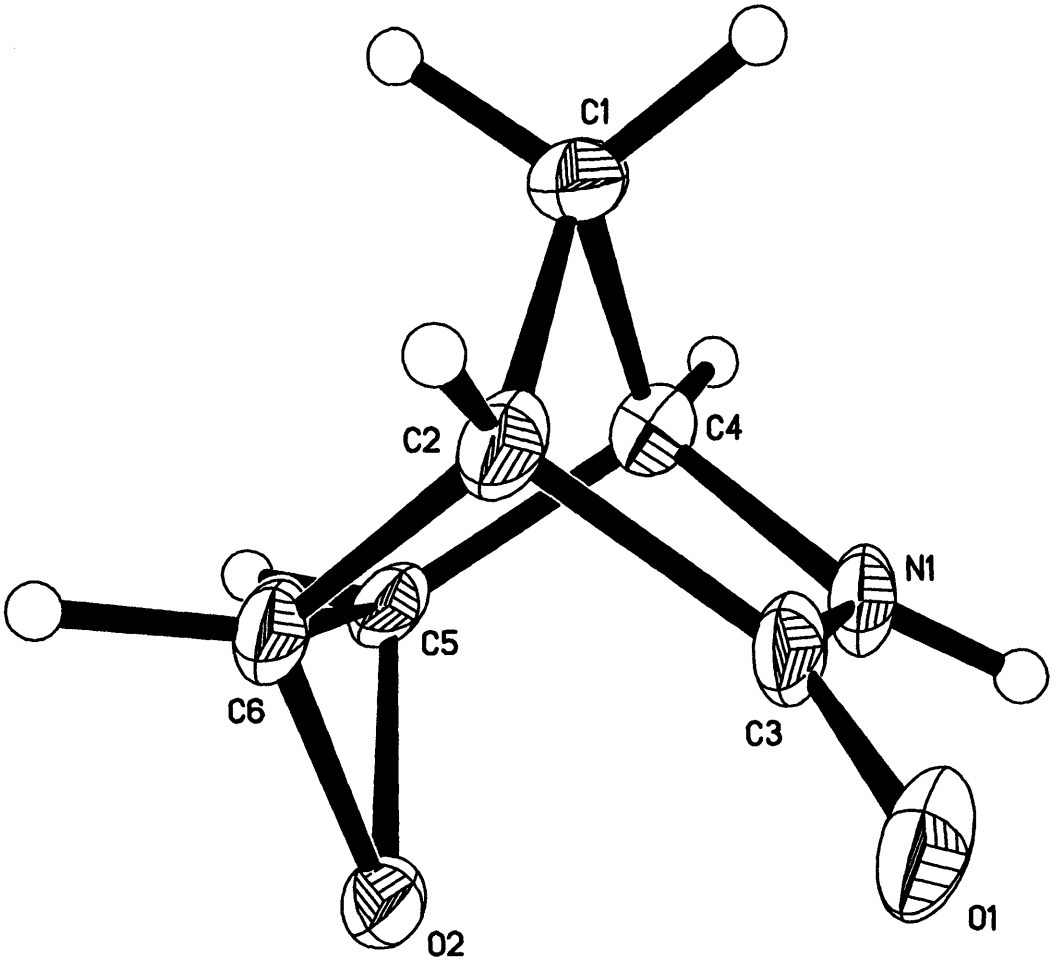
CRYSTALLOGRAPHIC DATA

DATA COLLECTION AND PROCESSING

Data were measured on a Siemens P4 diffractometer at 190 K using graphite monochromated Mo-Kα radiation (λ = 0.7107 Å) using an ω scan technique. Three standard reflections monitored every 100 scans showed no significant variation in intensity, the reflections were corrected for Lorentz and polarisation effects.

STRUCTURE SOLUTION AND REFINEMENT

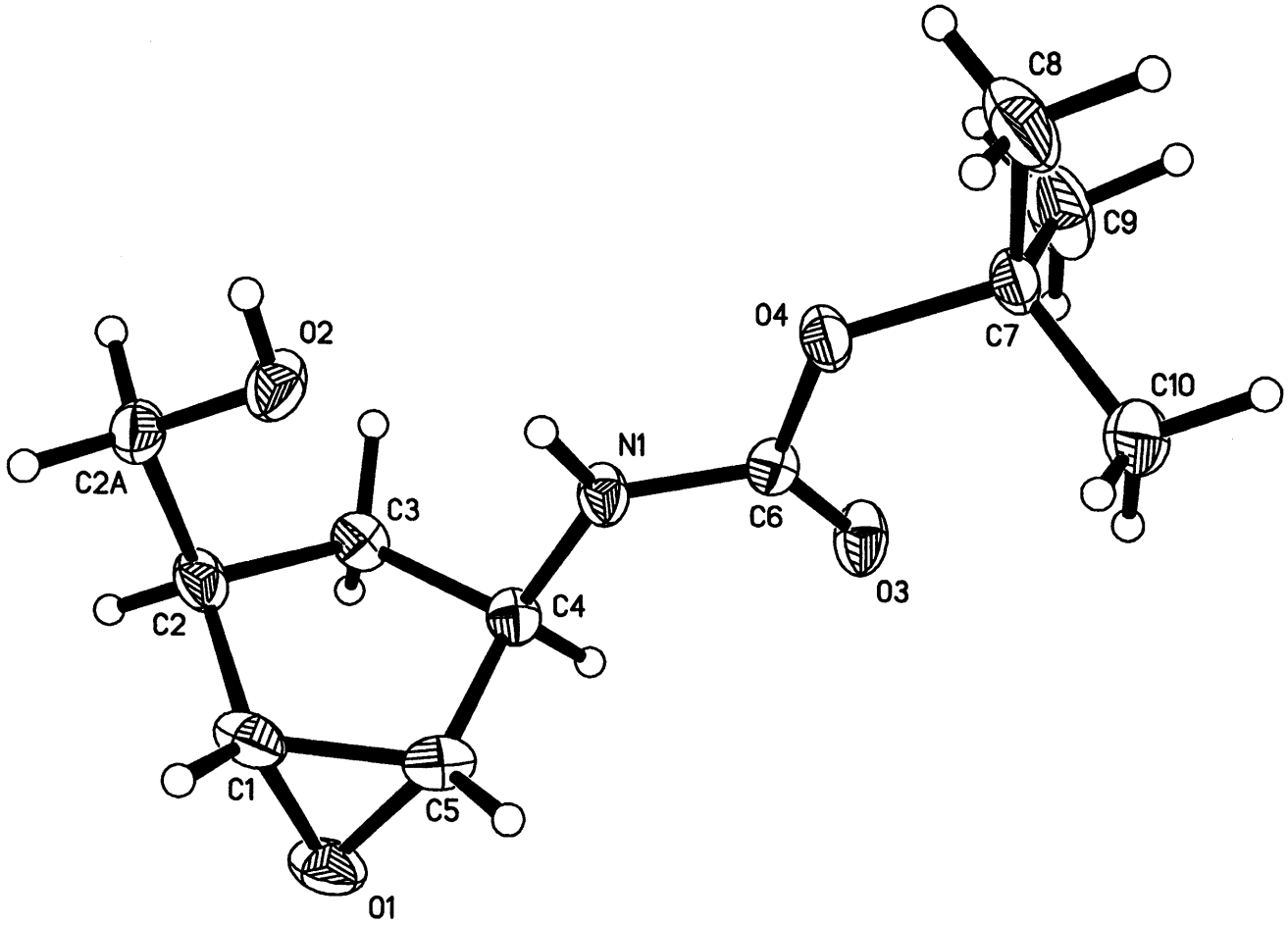
The structures were solved by Direct methods using the program SHELXTL-PC[105] and refined by full matrix least squares on F2 using the program SHELXTL/PC[106] . All hydrogen atoms were included in calculated positions (C-H = 0.96 Å) using a riding model. All non-hydrogen atoms were refined with anisotropic displacement parameters. Full matrix least squares based on F^2.



X-Ray structure 1: Compound **90**

Crystal data and structure refinement for compound **90**

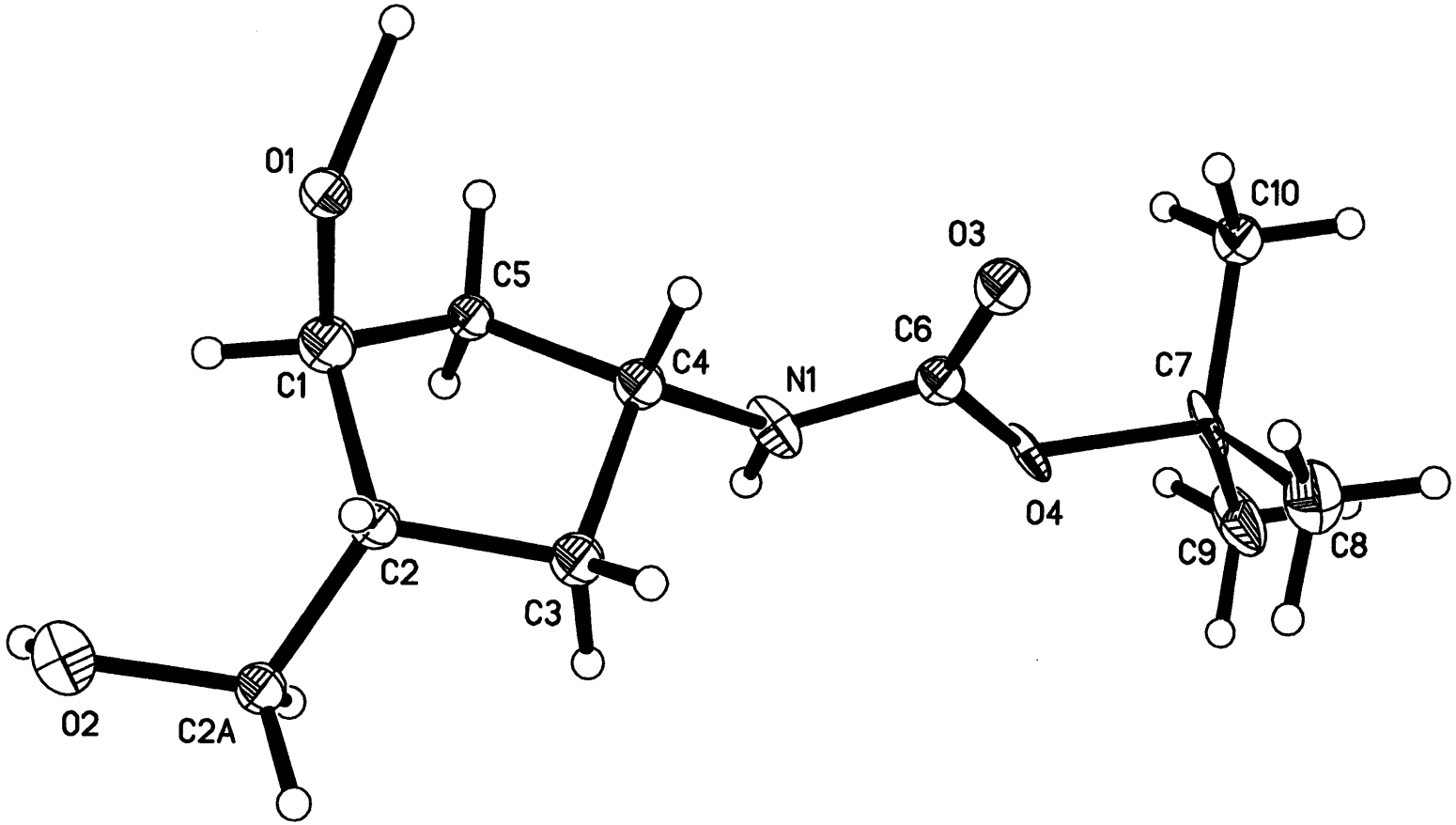
Identification code	9827
Empirical formula	$C_6H_7NO_2$
Formula weight	125.13
Temperature	190 (2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	$P2_1 / n$
Unit cell dimensions	a = 6.28 (5) Å alpha = 90 °
	b = 9.06 (10) Å beta = 90.1 (6) °
	c = 9.91 (6) Å gamma = 90 °
Volume, z	564 (8) $Å^3$, 4
Density (calculated)	1.474 Mg/ m^3
Absorption coefficient	0.112 mm^{-1}
F (000)	264
Crystal size	0.52 x 0.19 x 0.14 mm
θ Range for data collection	3.05 to 26.87 °
Limiting indices	$0 \leq h \leq 7, -1 \leq k \leq 11, -10 \leq l \leq 10$
Reflections collected	1138
Independent reflections	938 (R_{int} = 0.0465)
Absorption correction	Not applied
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	938 / 0 / 82
Goodness-on-fit on F^2	1.114
Final R indices [$I > 2\sigma (I)$]	R1 = 0.1027, wR2 = 0.3047
R indices (all data)	R1 = 0.1420, wR2 = 0.3604
Largest diff. peak and hole	0.704 and -0.430 $eÅ^{-3}$



X-Ray structure 2: Compound **95**

Crystal data and structure refinement for compound **95**

Identification code	1
Empirical formula	$C_{11}H_{19}NO_4$
Formula weight	229.27
Temperature	190 (2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions	a = 9.4295 (14) Å alpha = 90 °
	b = 10.2480 (12) Å beta = 90 °
	c = 12.667 (5) Å gamma = 90 °
Volume, z	1224.0 (5) $Å^3$, 4
Density (calculated)	1.244 Mg/ m^3
Absorption coefficient	0.094 mm^{-1}
F (000)	496
Crystal size	0.57 x 0.56 x 0.48 mm
θ Range for data collection	2.56 to 24.99 °
Limiting indices	$0 \leq h \leq 11$, $-1 \leq k \leq 12$, $-1 \leq l \leq 15$
Reflections collected	1509
Independent reflections	1453 (R_{int} = 0.0125)
Absorption correction	Not applied
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	1452 / 0 / 150
Goodness-on-fit on F^2	1.099
Final R indices [$I > 2\sigma(I)$]	R1 = 0.0388, wR2 = 0.1013
R indices (all data)	R1 = 0.0412, wR2 = 0.1081
Absolute structure parameter	-2 (2)
Extinction coefficientt	0.049 (5)
Largest diff. peak and hole	0.257 and -0.247 $eÅ^{-3}$



X-Ray structure 3: Compound **101**

Crystal data and structure refinement for compound **101**

Identification code	9760
Empirical formula	$C_{11}H_{21}NO_4$
Formula weight	231.29
Temperature	180 (2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions	a = 5.284 (2) Å alpha = 90 °
	b = 6.225 (3) Å beta = 90 °
	c = 38.32 (4) Å gamma = 90 °
Volume, z	1260 (2) $Å^3$, 4
Density (calculated)	1.219 Mg/m^3
Absorption coefficient	0.092 mm^{-1}
F (000)	504
Crystal size	0.51 x 0.22 x 0.04 mm
θ Range for data collection	3.19 to 21.03 °
Limiting indices	$-5 \leq h \leq 1, -6 \leq k \leq 1, -38 \leq l \leq 38$
Reflections collected	2136
Independent reflections	1361 (R_{int} = 0.1910)
Absorption correction	Not applied
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	1360 / 0 / 95
Goodness-on-fit on F^2	1.056
Final R indices [$I > 2\sigma(I)$]	R1 = 0.1225, wR2 = 0.2250
R indices (all data)	R1 = 0.2391, wR2 = 0.2925
Absolute structure parameter	3 (9)
Largest diff. peak and hole	0.360 and -0.371 $eÅ^{-3}$