SYNTHESIS RELATED TO THE
ANTITUMOUR AGENT 593A

A Thesis by
Colin Howes

Submitted in part fulfilment
of the requirements for the
degree of Doctor of Philosophy
at the University of Warwick,
in the Department of Chemistry
and Molecular Sciences.

September 1983
CONTENTS

Page
Acknowledgements 1
Declaration ii
Lists of Figures, Schemes and Tables iii
List of Abbreviations vi
Publications x
Summary xi

CHAPTER 1 Introduction

1.1 The Clinical Evaluation of 593A 1
1.2 Cancer and Chemotherapy 2
1.3 Nomenclature of 2,5-Piperazinediones 8
1.4 Elucidation of the Structure of 593A 9
1.5 Strategies towards the Synthesis of 13
593A
1.6 References 20

CHAPTER 2 Materials, Methods and Instrumentation

2.1 Materials 22
2.2 Methods 22
2.3 Instrumentation 24
2.4 References 26

CHAPTER 3 An Approach to Cyclo(S-aminoalanyl-
S-aminoalanyl) via Cyclo(S-seryl-S-
seryl)
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Introduction</td>
<td>27</td>
</tr>
<tr>
<td>3.2</td>
<td>Methods of Synthesising 2,5-piperazinediones</td>
<td>27</td>
</tr>
<tr>
<td>3.3</td>
<td>Inherent Problems in 2,5-Piperazinedione Formation</td>
<td>31</td>
</tr>
<tr>
<td>3.4.1</td>
<td>Synthesis of Cyclo(S-seryl-S-seryl)</td>
<td>37</td>
</tr>
<tr>
<td>3.4.2</td>
<td>Attempts to Synthesise Cyclo(S-chloroalanyl-S-chloroalanyl)</td>
<td>39</td>
</tr>
<tr>
<td>3.4.3</td>
<td>The Dialkylcarbodiimide Method of Halogenation</td>
<td>41</td>
</tr>
<tr>
<td>3.4.4</td>
<td>The triphenylphosphine/Carbon Tetrachloride Method of Halogenation</td>
<td>42</td>
</tr>
<tr>
<td>3.4.5</td>
<td>The Action of Hydrogen Bromide on Cyclo(S-seryl-S-seryl)</td>
<td>44</td>
</tr>
<tr>
<td>3.4.6</td>
<td>A Modified Phosphorus Pentachloride Method of Halogenation</td>
<td>45</td>
</tr>
<tr>
<td>3.5</td>
<td>Synthesis of (3S,6S)-bis(trimethylsilyloxy)methyl)-2,5-piperazinedione</td>
<td>47</td>
</tr>
<tr>
<td>3.6</td>
<td>Some Reactions of Cyclo(R-aminooxyalanyl-R-aminooxyalanyl)</td>
<td>49</td>
</tr>
<tr>
<td>3.7</td>
<td>Conclusions</td>
<td>51</td>
</tr>
<tr>
<td>3.8</td>
<td>Experimental</td>
<td>51</td>
</tr>
<tr>
<td>3.8.1</td>
<td>Cyclo(S-seryl-S-seryl)</td>
<td>51</td>
</tr>
<tr>
<td>3.8.2</td>
<td>(3S,6S)-bis(trimethylsilyloxy)methyl)-2,5-piperazinedione</td>
<td>52</td>
</tr>
<tr>
<td>3.8.3</td>
<td>Cyclo(R-aminooxyalanyl-R-aminooxyalanyl)</td>
<td>53</td>
</tr>
<tr>
<td>3.8.4</td>
<td>3,6-Bis(methylene)-2,5-piperazinedione</td>
<td>53</td>
</tr>
<tr>
<td>Section</td>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>---------</td>
<td>-------</td>
<td>------</td>
</tr>
<tr>
<td>3.8.5</td>
<td>(3S,6S)-Bis(acetoxymethyl)-2,5-piperazinedione</td>
<td>53</td>
</tr>
<tr>
<td>3.9</td>
<td>References</td>
<td>54</td>
</tr>
</tbody>
</table>

**CHAPTER 4**

The Synthesis and Crystal Structure of *Cyolo* (S-asparagyl-S-asparagyl)

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>Introduction</td>
<td>57</td>
</tr>
<tr>
<td>4.2</td>
<td>The Synthesis of <em>Cyolo</em> (S-asparagyl-S-asparagyl)</td>
<td>58</td>
</tr>
<tr>
<td>4.3</td>
<td>Some Reactions of (S-3-Aminopyrrolidine-2,5-dione</td>
<td>68</td>
</tr>
<tr>
<td>4.4</td>
<td>Antitumour Screening of <em>Cyolo</em> (S-asparagyl-S-asparagyl)</td>
<td>73</td>
</tr>
<tr>
<td>4.5</td>
<td>The Crystal Structure of <em>Cyolo</em> (S-asparagyl-S-asparagyl)</td>
<td>74</td>
</tr>
<tr>
<td>4.6</td>
<td>Attempted Hofmann Rearrangement on <em>Cyolo</em> (S-asparagyl-S-asparagyl)</td>
<td>84</td>
</tr>
<tr>
<td>4.7</td>
<td>The Crystal Structure of Bis(trifluoroacetoxy)iodobenzene</td>
<td>92</td>
</tr>
<tr>
<td>4.8</td>
<td>Experimental</td>
<td>99</td>
</tr>
<tr>
<td>4.8.1</td>
<td>(S)-Nα-Benzyloxy carbonylasparagine Methyl Ester</td>
<td>99</td>
</tr>
<tr>
<td>4.8.2</td>
<td><em>Cyolo</em> (S-asparagyl-S-asparagyl) from (16)</td>
<td>100</td>
</tr>
<tr>
<td>4.8.3</td>
<td>(S)-3-N-Benzyloxy carbonylamino-pyrrolidine-2,5-dione</td>
<td>101</td>
</tr>
<tr>
<td>4.8.4</td>
<td>(S)-3-Aminopyrrolidine-2,5-dione</td>
<td>102</td>
</tr>
</tbody>
</table>
4.8.5  *Cyclo*(S-asparagyl-S-asparagyl)  
from (Z0)  

4.8.6  Fischer's Preparation of *Cyclo*- 
(asparagyl-asparagyl)  

4.8.6 (i)  (S)-Dimethylaspartate Hydrochloride  
4.8.6 (ii)  Dimethyl Ester of *Cyclo*(aspartyl-
 aspartyl)  

4.8.6 (iii)  *Cyclo*(asparagyl-asparagyl)  

4.8.7  *Cyclo*(R-asparagyl-S-asparagyl)  

4.8.8  Reactions of (S)-3-Aminopyrrolidine- 
2,5-dione in Water  

4.8.9  Crystal Data Collection for (Z2)  

4.8.10  Bis(Trifluoroacetoxy)iodobenzene  

4.8.11  Crystal Data Collection for (Z3)  

4.9  References  

CHAPTER 5  Synthesis of (S)-2,3-Diaminopropionic Acid  

5.1  Introduction  

5.2  Synthesis of (S)-N-Z-3-Chloroalanine  
and (S)-N-Z-3-Iodoalanine Methyl 
Esters  

5.3  α,β-Dehydroamino acids  

5.4  The Mitsunobu Reaction  

5.5  Synthesis of (S)-2,3-Diaminopropionic Acid  

5.6  N⁸-Alkylation of (S)-N⁸-Z-2,3-Diamino-
<table>
<thead>
<tr>
<th>Section</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.7</td>
<td>Amino Protecting Groups</td>
<td>144</td>
</tr>
<tr>
<td>5.8</td>
<td>Experimental</td>
<td>152</td>
</tr>
<tr>
<td>5.8.1</td>
<td>(S)-N-Benzylloxycarbonylserine</td>
<td>152</td>
</tr>
<tr>
<td>5.8.2</td>
<td>(S)-N-Z-Serine Methyl Ester</td>
<td>152</td>
</tr>
<tr>
<td>5.8.3</td>
<td>(S)-N-Z-3-Chloroalanine Methyl Ester</td>
<td>153</td>
</tr>
<tr>
<td>5.8.4</td>
<td>(S)-N-Z-3-Iodoalanine Methyl Ester</td>
<td>154</td>
</tr>
<tr>
<td>5.8.5</td>
<td>(S)-N-Z-3-Azidoalanine Methyl Ester</td>
<td>154</td>
</tr>
<tr>
<td>5.8.6</td>
<td>(S)-N&lt;sup&gt;a&lt;/sup&gt;-Z-2,3-Diaminopropionic Acid</td>
<td>155</td>
</tr>
<tr>
<td>5.8.7</td>
<td>(S)-2,3-Diaminopropionic Acid</td>
<td>156</td>
</tr>
<tr>
<td></td>
<td>Hydrochloride</td>
<td></td>
</tr>
<tr>
<td>5.8.8</td>
<td>(S)-N&lt;sup&gt;a&lt;/sup&gt;-Z-2,3-Diaminopropionic Acid Methyl Ester Hydrochloride</td>
<td>157</td>
</tr>
<tr>
<td>5.8.9</td>
<td>N-Z-Dehydroalanine Methyl Ester</td>
<td>157</td>
</tr>
<tr>
<td>5.8.10</td>
<td>(S)-N&lt;sup&gt;a&lt;/sup&gt;-Z-N&lt;sup&gt;β&lt;/sup&gt;-(Benzyloxyethyl)-2,3-Diaminopropionic Acid Methyl Ester</td>
<td>158</td>
</tr>
<tr>
<td>5.8.11</td>
<td>(S)-N&lt;sup&gt;a&lt;/sup&gt;-Z-N&lt;sup&gt;β&lt;/sup&gt;-(Benzyloxyethyl)-N&lt;sup&gt;β&lt;/sup&gt;-(Trifluoroacetyl)-2,3-Diaminopropionic Acid Methyl Ester</td>
<td>159</td>
</tr>
<tr>
<td>5.8.12</td>
<td>(S)-N&lt;sup&gt;a&lt;/sup&gt;-Z-N&lt;sup&gt;β&lt;/sup&gt;-(Benzyloxyethyl)-N&lt;sup&gt;β&lt;/sup&gt;-(Diphenylphosphinyl)-2,3-Diaminopropionic Acid Methyl Ester</td>
<td>160</td>
</tr>
<tr>
<td>5.8.13</td>
<td>(S)-N&lt;sup&gt;a&lt;/sup&gt;-Z-N&lt;sup&gt;β&lt;/sup&gt;-(Benzyloxyethyl)-N&lt;sup&gt;β&lt;/sup&gt;-(Benzyl)-2,3-Diaminopropionic Acid Methyl Ester</td>
<td>161</td>
</tr>
<tr>
<td>5.8.14</td>
<td>(S)-N&lt;sup&gt;a&lt;/sup&gt;-Z-N&lt;sup&gt;β&lt;/sup&gt;-Boc-2,3-Diaminopropionic Acid Methyl Ester</td>
<td>161</td>
</tr>
<tr>
<td>5.8.15</td>
<td>(S)-N&lt;sup&gt;β&lt;/sup&gt;-Boc-2,3-Diaminopropionic Acid Methyl Ester</td>
<td>162</td>
</tr>
</tbody>
</table>
### CHAPTER 6 The β-Lactam Approach to 593A

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.1</td>
<td>Introduction</td>
<td>168</td>
</tr>
<tr>
<td>6.2</td>
<td>Model Studies on the Synthesis of 593A</td>
<td>170</td>
</tr>
<tr>
<td>6.3</td>
<td>(S)-3-Hydroxypiperidine</td>
<td>173</td>
</tr>
<tr>
<td>6.4</td>
<td>Experimental</td>
<td>181</td>
</tr>
<tr>
<td>6.4.1</td>
<td>N-(1'-Nitroacetyl)piperidine</td>
<td>181</td>
</tr>
<tr>
<td>6.4.2</td>
<td>N-(1'-Diazo-1'-Nitroacetyl)piperidine</td>
<td>181</td>
</tr>
<tr>
<td>6.4.3</td>
<td>(S)-γ-Carboxyl-γ-butyrolactone</td>
<td>182</td>
</tr>
<tr>
<td>6.4.4</td>
<td>(S)-γ-Ethoxycarbonyl-γ-butyrolactone and (S)-Diethylhydroxyglutarate</td>
<td>182</td>
</tr>
<tr>
<td>6.4.5</td>
<td>(S)-2-Hydroxypentan-1,5-dicarboxylic acid diamide</td>
<td>183</td>
</tr>
<tr>
<td>6.4.6</td>
<td>γ-Formamido-γ-butyrolactone</td>
<td>183</td>
</tr>
<tr>
<td>6.4.7</td>
<td>(S)-γ-Hydroxymethyl-γ-butyrolactone</td>
<td>183</td>
</tr>
<tr>
<td>6.4.8</td>
<td>(S)-γ-Azidomethyl-γ-butyrolactone</td>
<td>184</td>
</tr>
<tr>
<td>6.4.9</td>
<td>(S)-5-Hydroxypiperidin-2-one</td>
<td>184</td>
</tr>
<tr>
<td>6.4.10</td>
<td>(S)-3-Hydroxypiperidine</td>
<td>185</td>
</tr>
<tr>
<td>6.5</td>
<td>References</td>
<td>185</td>
</tr>
</tbody>
</table>
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Finally, may I thank my wife for tolerating the author during the preparation of this work.

****
DECLARATION

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****
LISTS OF FIGURES, SCHEMES AND TABLES

Figures
1.2.1
1.2.2
1.4.1
1.4.2
1.4.3
2.2.1
3.3.1
3.3.2
3.3.3
3.3.4
3.3.5
3.4.1
3.4.2
4.2.1
4.2.2
4.2.3
4.5.1a
4.5.1b
4.5.2
4.5.3a
4.5.3b
4.5.4
4.5.5
4.5.6
4.7.2
4.7.3
4.7.4
4.7.5
5.2.1
5.7.1

Schemes
1.4.1
1.5.1
1.5.2
1.5.3
3.1.1
3.2.1
3.2.2
3.4.1
3.4.2
3.6.1
4.2.1
4.2.2
4.2.3
4.3.1
4.3.2
4.6.1
5.1.1
5.1.2
5.4.1
5.4.2
5.4.3
5.4.4
5.4.5
5.6.1
5.6.2
5.7.2
6.1.1
6.2.1
6.2.2
6.3.1
6.3.2
6.3.3
6.3.4

Tables
4.4.1
4.5.1
4.5.2
4.6.1
4.7.2
4.7.1
5.4.1
5.6.1
# LIST OF ABBREVIATIONS

## A

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
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<tr>
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<td>aq.</td>
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<td>Asn</td>
<td>asparagine</td>
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<tr>
<th>Abbreviation</th>
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<td>t-butyloxycarbonyl</td>
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<td>(2(t\text{-butoxycarbonyloxyimino})-2\text{-phenylacetonitrile})</td>
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<th>Abbreviation</th>
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</tr>
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</tr>
<tr>
<td>$R_f$</td>
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<tr>
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PUBLICATIONS

Part of the work described in this thesis is being prepared for publication:

1. The synthesis and Crystal Structure of (3S,6S)-Bis(Acetamido)-2,5-piperazinedione
   C. Howes, B. T. Golding and N. W. Alcock

2. Application of the Mitsunobu Reaction for the Preparation of L-2,3-Diaminopropionic Acid for L-Serine
   B. T. Golding and C. Howes

3. The Crystal Structure of Bis(Trifluoroacetoxylodobenzene)

4. Application of the Mitsunobu Reaction for the Preparation of (S)-3-Hydroxypiperidine
   C. Howes and B. T. Golding; in preparation

****
SUMMARY

The natural product antibiotic 593A is useful in the treatment of some forms of cancer. However, like most of the drugs used in the treatment of cancer 593A manifests unpleasant side-effects in patients. The object of this work was to explore syntheses of 593A and its analogues. The results of the initial clinical trials on 593A, and the nature of cancer and chemotherapy are summarised briefly in the first chapter. This chapter also describes the previous attempts to prepare 593A and the synthesis strategies adopted in this work.

Chapter 2 details the technicalities involved in the experimental work.

The third and fourth chapters are concerned with approaches to 593A analogues from piperazinedione starting materials. Chapter 4 describes the first synthesis of the \((S,S)\) isomer of the piperazinedione derived from \((S)\)-asparagine.

The fifth chapter deals with a different synthetic strategy, starting from 2,3-diaminopropionic acid. New syntheses of 2,3-diaminopropionic acid and a number of its derivatives are described.

Chapter 6 deals with a strategy for the preparation of 593A involving a \(\beta\)-lactam intermediate which is popular with other workers in the field. A synthesis of \((S)\)-3-hydroxypiperidine is described which is an important intermediate in this approach to 593A.
CHAPTER 1

Introduction

1.1 The Clinical Evaluation of 593A
1.2 Cancer and Chemotherapy
1.3 Nomenclature of 2,5-Piperazinediones
1.4 Elucidation of the Structure of 593A
1.5 Strategies towards the Synthesis of 593A
1.6 References
CHAPTER 1

Introduction

1.1 THE CLINICAL EVALUATION OF 593A

Piperazine derivatives such as pipobroman and piposulphan were introduced into clinical trials as antitumour agents in the 1950's. The efficacy of these piperazine derivatives led to interest in similar compounds. The antitumour antibiotic 593A is a piperazinedione extracted from the South African soil micro-organism \textit{Streptomyces griseolatus}. The drug shows inhibitory activity against a variety of tumours in rodents including L-1210 lymphocytic leukaemia, Ridgeway osteogenic sarcoma, Walker-256 carcinosarcoma and several neoplastic cell lines. In 1974 the National Cancer Institute of the U.S.A. brought 593A (NSC 135758) to clinical trial. Preliminary results with certain human lymphomas have been very encouraging\textsuperscript{1,2}.

![593A structure](image)
The object of this work was to explore new syntheses of 593A and its analogues. A preclinical toxicological evaluation of 593A in dogs and monkeys demonstrated drug induced injury to the bone marrow, gastrointestinal mucosa, lymphoid tissues and testis. Thus a less toxic, more efficacious, piperazinedione analogue of 593A would be highly desirable. The clinical trials investigated the effect of 593A in patients with advanced cancer, and was found to induce regression in Hodgkin's disease and lymphocytic lymphomas, although no response was observed in patients with renal carcinoma or with other drug-resistant advanced melanomas. However, a response was noted in a small percentage of patients with breast cancer. It was also suggested that the lack of major toxicity of 593A toward organs, could make the drug useful in preparing patients for bone marrow transplantation, and for maintenance therapy in the treatment of leukaemia.

1.2 CANCER AND CHEMOTHERAPY

Cancer, or more precisely malignant growth, is defined as a disorder in which some cell type in the organism begins to increase its population in an apparently unchecked fashion. During this proliferation the cell type, irrespective of its tissue of origin, tends to lose or change some of its normal biochemical characteristics. Also as a result of these changes cancer cells do not form simple, well isolated, tumours,
but infiltrate neighbouring tissue and eventually spread to distant areas of the body, thus forming 'secondary deposits' or metastases.

A tumour cell, whether benign or malignant, is a persistently altered cell that reproduces true to type (i.e. gives rise to cancerous cells). There is no adequate control mechanism in the host to inhibit the growth; the natural immune system of the body is virtually useless. Benign tumours are characterised by the fact that they remain localised in their host. They are classified according to the specific cell type from which they arise. For example, benign tumours arising from skin cells are known as papillomas, keratomas, moles, warts, etc. If they arise from fat tissue they are called lipomas, from connective tissue as fibromas, from bone as osteomas, from muscle as myomas, etc.

In contrast, malignant tumours do not remain localised in their hosts but rather they invade and destroy normal tissues and detach groups of cells which metastasise. Malignant tumours are classified into two broad groups, the carcinomas and the sarcomas. The carcinomas are cancers derived from epithelial cells. The sarcomas on the other hand arise from mesenchymal, that is, from cells found in the connective and supportive tissues such as bone, muscles, tendons, etc. In addition to these two main categories there are tumours that originate from white blood cells known as leukaemias. Certain white blood cells, the granulocytes, give rise to chronic or acute
myelocytic leukaemia and can both be treated with 593A.

Cellular division characteristically proceeds through a sequence of phases. Animal cells only have two apparent phases, these are; a short period of actual cell division called mitosis sandwiched between relatively long interphase or 'resting' periods. A closer examination of the interphase period reveals that DNA synthesis occurs only for a short part of the cycle, leaving two gaps $G_1$ and $G_2$ on either side (Fig. 1.2.1).

![Cell cycle diagram](image)

Fig. 1.2.1 $G_1$, $G_2$ = Gaps 1 and 2  
M = Mitosis  
S = DNA synthesis  
$G_0$ = Resting cell (highly variable time span)

The terms, cell cycle phase specific (CCS) and cell cycle phase nonspecific (CCNS) relate to Fig. 1.2.1 and are often used to describe the action of chemotherapeutic agents\(^6\). Pharmacologically CCS agents show their major cytotoxic activity in a particular phase of the cell cycle. These drugs produce a greater cell kill if the dose is given in multiple, repeated fractions rather than as a
large single dose. In contrast CCNS agents give a degree of cell kill directly proportional to the absolute dose.

Cancer chemotherapeutic agents are commonly classified, by their mechanism of action, into six general categories: alkylating agents, antimetabolites, hormonal agents, immunotherapeutic agents, vinca alkaloids, podophyllotoxins, and miscellaneous agents. The main mechanism of action proposed for 593A is alkylation involving inhibition of the incorporation of several DNA nucleotides. Similar to other alkylating agents, 593A appears to delay progression through the G2 phase of the cell cycle, but it is not truly cell cycle phase specific. The capacity of 593A to act as an alkylating agent was investigated by Brockman et al. Samples of 593A and the bis-aziridine (NSC 201424) derived from 593A were reacted with diethylamine and 4-(p-nitrobenzyl)pyridine (NBP), and the ease and degree of alkylation observed by 1H n.m.r. spectroscopy.

It was considered possible that 593A might be converted to the bis-aziridine (NSC 201424) in the body, and
that this was the active alkylating agent. However, it was found that 593A itself, is a more potent drug, than the bis-aziridine derivative, in the inhibition of DNA synthesis. It is also notable that 593A has been shown to be effective against neoplastic cell lines which are resistant to other alkylating antitumour drugs such as cyclophosphamide, and melphalan. Clearly then, some additional mechanism to alkylation is at work. Some drugs are known to form complexes with DNA, and it is thought that their antitumour activity may be related to this complexation or intercalation between base pairs of the DNA chain. Many of these types of drug are planar, polycyclic quinones, or heterocycles.

An interesting study would be to examine the antitumour activity of a 2,5-piperazinedione which did not bear substituents with alkylating functionality. In Chapter 4 the author describes the first synthesis of cyclo(S-asparagyl-S-asparagyl) [12], which is a 2,5-piperazinedione bearing acetamide substituents in the 3,6-positions. This compound (12) is not expected to act as an alkylating agent. However, it has been shown to have some activity against neoplastic cell lines both sensitive and resistant to the alkylating drug busulphan. This offers a useful insight into the mechanism by which 2,5-piperazinediones can inhibit DNA synthesis. In an article on work carried out by Grafstein, it was reported that 2,5-piperazinediones can fit between DNA base pairs. By using space-filling molecular models Grafstein
Fig. 1.2.2 DNA base pairs in register, with interposing piperazinediones
showed that the 2,5-piperazinedione ring could be inserted between hydrogen bonded nucleic acid base pairs without disrupting the integrity of the DNA code. Indeed, the spatial relationship of the base-pairs is changed, but the insertion allows the hydrogen bonding to remain in-register (Fig. 1.2.2). The crystal structure of cyclo(S-asparagyl-S-asparagyl) [12] which was also established (Chapter 4), revealed (12) to have an extended bowsprit boat conformation. This may be regarded as the most appropriate conformation for insertion into DNA. A surprising lack of intramolecular hydrogen bonding in (12) also favours this postulate. An investigation of the ability of other 2,5-piperazinediones to bind to DNA would be worth pursuing.

1.3 NOMENCLATURE OF 2,5-PIPERAZINEDIONES

A variety of nomenclature has been used in the literature to describe the amino-acid anhydrides including; 2,5-diketopiperazine, 2,5-dioxopiperazine, piperazine-2,5-dione and, 2,5-piperazinedione. The compound may also be named in terms of the cyclic dipeptide, e.g. cyclo(S-asparagyl-S-asparagyl) for the (S)-asparagine derivative. In this thesis such compounds will be referred to as derivatives of 2,5-piperazinediones (generally the 2,5-prefix may be omitted) or as the cyclo-dipeptide.
1.4 ELUCIDATION OF THE STRUCTURE OF 593A

The initial structure deductions for 593A were made by Arison and Beck\(^1\) who carried out a comprehensive spectroscopic analysis of the dihydrochloride salt of 593A. Microanalysis produced the empirical formula \(\text{C}_{7}\text{H}_{11}\text{ClN}_{2}\text{O}\cdot\text{HCl}\), and the infrared spectrum indicated the presence of the following functional groups: \(\text{NH}\) and/or \(\text{OH}\) (3210, 3110, and 3070 cm\(^{-1}\)), \(\text{NH}\) (2700-2400 cm\(^{-1}\)), and ketonic carbonyl or monosubstituted amide (1685, 1665 cm\(^{-1}\)). 593A exhibited only end adsorption in its ultraviolet spectrum.

The 100 MHz \(^1\text{H}\) n.m.r. spectrum of 593A, together with n.m.r. spin-decoupling experiments, indicated a cyclic structure. A significant contribution was made towards the solution by the realisation that the \(^1\text{H}\) n.m.r. spectrum of 5-hydroxypipelicolic acid bore a striking resemblance to the spectrum of 593A (Fig. 1.4.1). It was concluded\(^1\) that 593A was a 2,5-disubstituted piperidine derivative in which both substituents were equatorial. In a partial structure assignment based on all of the data, the chloro group was placed at position C-5 on the piperidine ring since an oxygen was excluded on the basis of i.r. spectrum and empirical formula considerations. The doublet at \(\delta 4.58\) p.p.m. in the \(^1\text{H}\) n.m.r. spectrum was attributed to the C-2 piperidine ring hydrogen, and finally the C-2 substituent postulated as a carbonyl containing function. The structure was completed by Arison and Beck by careful scrutiny of the mass spectral
Fig. 1.4.1  
A 100 MHz $^1$H n.m.r. spectrum of 593A  
B 100 MHz $^1$H n.m.r. spectrum of 5-hydroxy-pipecolic acid
Fig. 1.4.2  E.i. mass spectrum of 593A

Scheme 1.4.1 Proposed fragmentation of 593A
data for 593A. The electron ionisation (e.i.) mass spectrum did not show a molecular ion (Fig. 1.4.2). The highest mass fragment m/z 312 was assigned as a chlorine containing dimer (Scheme 1.4.1). The other major assignments are also shown in Scheme 1.4.1; the base peak at m/z 195 arises via a McLafferty rearrangement. The main deduction to be made from the mass spectrum and microanalytical result was that 593A is a symmetrical dimer with the molecular formula $\text{C}_{14}\text{H}_{22}\text{Cl}_{2}\text{N}_{4}\text{O}_{2}$.

Pettit et al. performed an x-ray crystal structure determination on the disulphate salt pentahydrate of 593A$^{12}$. This confirmed the structure proposed by Arison and Beck, and also allowed the stereochemical assignments to be made. These are:

- $C_1^\alpha$ and $C_2^\alpha$ ($S$)
- $C_1^\beta$ and $C_2^\beta$ ($R$)
- $C_1^\varepsilon$ and $C_2^\varepsilon$ ($S$)

Fig. 1.4.3 Crystal structure of 593A

A detailed discussion of the crystal structures of 2,5-piperazinediones appears in Chapter 4.
1.5 STRATEGIES TOWARDS THE SYNTHESIS OF 593A

The 2,5-piperazinedione 593A is derived from the novel amino-acid named streptolutidine (99) and abbreviated Slt by Pettit et al.12.

Pettit and co-workers also attempted the first synthesis of 593A. This approach was based on the preparation of the hitherto unknown amino-acid (S)-(5-chloro-2-pyridyl)glycine (100) as a key intermediate13. Pettit et al. tried to prepare (100) from 2,5-dichloropyridine but were unable to displace the 2-chloro function with a nucleophile. However they had more success with 2-chloro-5-nitropyridine which reacted with diethylacetamidomalonate carbanion to give (101) [Scheme 1.5.1]. The remainder of the synthesis to amino-acid (100) was fraught with difficulties. For example, the classical Sandmeyer reaction could not be applied to the conversion of the amine (102) to the chloro derivative (103) due to decomposition reactions under the acidic conditions. The principal problem in the rest of the synthesis was the ready decarboxylation of (103) and (104). Amino-acid (100) was eventually obtained by removing the amine protecting group on (104) with the enzyme hog renal acylase I. Even then however, the authors13 were unable to obtain the 2,5-piperazinedione derivative of
Scheme 1.5.1 The first approach to 593A by Pettit et al.\textsuperscript{13}
amino-acid (1Q0) by dimerisation in, for example, ethylene glycol (a standard solvent for this reaction - see Chapter 3). Catalytic reduction of (1Q0) also failed to give streptolutidine (9Q) because hydrogenolysis of the 5-chloro function always persisted.

Another approach to 593A entails a stereospecific synthesis of an (S)-3-hydroxypiperidine derivative involving a regiospecific Baeyer-Villiger oxidation reaction. However, very little progress with this approach was reported.14 In Chapter 6 the author describes the synthesis of (S)-3-hydroxypiperidine (73) and its potential application to a preparation of 593A.

A method of obtaining other stereoisomers of 593A was explored by Ongania and involved the addition of nucleophiles to 3,6-bis(methylene)-2,5-piperazinedione (9).15 This work is discussed fully in Chapter 3.

A total synthesis of rac.593A was recently communicated by Fukuyama et al.16 This synthesis employs a β-lactam intermediate to overcome the problem of controlling the stereochemistry of α,β-diamino-acids, and to avoid the need for drastic dimerisation conditions to obtain the 2,5-piperazinedione ring. Several problems remain in this route, which can give rise to unwanted isomers in a number of the steps. The synthesis is summarised in Scheme 1.5.2. Starting from the imine (1Q5), the cis-β-lactam isomer (1Q6) is exclusively obtained in two steps. This β-lactam (1Q6) can be dimerised readily to give a mixture of isomers of the 2,5-piperazinedione (1Q7). The (S,S) and (R,R) isomers
Scheme 1.5.2 Total synthesis of rac.593A by Fukuyama et al.16.
of (1Q7) were isolated by chromatography. This pair of enantiomers was catalytically reduced and facile double cyclisation effected to give the tricyclic compound (1Q8). Chlorination of (1Q8) gives another diastereomeric mixture which was not purified. Racemic 593A was obtained from (1Q9) by reduction which is presumed to proceed through the tetraquasi-equatorial iminium salt (1Q0).

\[
\text{[1Q0]}
\]

The main criticism of this route must be that most of the steps can produce more than one isomer of each intermediate.

A better approach to 593A which also utilised a \(\beta\)-lactam intermediate was explored by Golding and Smith\textsuperscript{17}. This route is discussed in Chapter 6; it essentially relies upon the formation of a \(\beta\)-lactam (as a precursor to the 2,5-piperazinedione) by photocyclisation of an N-\{(alkyloxycarbonyl)diazoacetyl\}piperidine (72).

\[
\text{(72)}
\]

(See also Scheme 6.1.1)
Scheme 1.5.3 Summary of some strategies to analogues of 593A adopted in this thesis.
The chiral starting material (S)-3-hydroxypiperidine (73) was required for a total synthesis of 593A by this method. A preparation of (73) is described in Chapter 6. In these approaches, the piperazinedione ring is formed at the end of the synthesis.

Alternative strategies to analogues of 593A include building appropriate substituents onto a 2,5-piperazinedione nucleus. Two methods of this type are discussed extensively by the author and are represented in Scheme 1.5.3. In the first case reactions of cyclo(S-seryl-S-seryl) [1] are considered in which efforts to prepare cyclo(S-aminomethyl-S-aminomethyl) [2] via a halide derivative of (1) were explored (Chapter 3). A second route to cyclo(S-aminomethyl-S-aminomethyl) [2] was also investigated starting from cyclo(S-asparagyl-S-asparagyl) [12]. A Hofmann rearrangement reaction of (12) using the reagent bis(trifluoroacetoxy)iodobenzene (23) was examined and the results presented in Chapter 4.

A further, unique, strategy was also adopted as a consequence of the poor solubility and lability of the 2,5-piperazinedione compounds towards bases, resulting in epimerisation. This was the preparation of (S)-2,3-diaminopropionic acid (27) and a number of its derivatives which were applied to the preparation of analogues of 593A (Chapter 5).

Each of these strategies offer new approaches and contribute to the methodology for the synthesis of 593A and its analogues.
1.6 REFERENCES

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CHAPTER 2

Materials, Methods and Instrumentation

2.1 Materials
2.2 Methods
2.3 Instrumentation
2.4 References
CHAPTER 2

Materials, Methods and Instrumentation

2.1 MATERIALS

(i) Solvents. All solvents used were either analytical grade or redistilled laboratory grade. Anhydrous solvents were purified and dried according to a standard method\(^1\), and stored over molecular sieves.

(ii) Chemicals. All commercial chemical reagents were of the highest available purity. Prepared reagents had properties consistent with the best literature data.

2.2 METHODS

(i) Thin layer chromatographic analyses were carried out on aluminium backed plates of silica gel 60 \(F_{254}\) (Merck Art. No. 5554) of 0.2 mm thickness. Liquid column chromatography used silica gel 60, mesh 70-200 (Merck Art. No. 7734). Flash chromatography used silica gel 60, mesh 230-400 (Merck Art. No. 9385), and dry column (suction) chromatography used silica gel 60, mesh < 230 (Fluka No. 60739). The eluting solvents were freshly prepared from analytical grade solvents and are quoted as percentage volume compositions (v/v).

Components on t.l.c. plates were visualised by u.v. light (254 nm), and/or by staining in iodine
vapour.

(ii) All glassware used in moisture sensitive reactions was pre-dried in an oven at 110°C, or flamed and cooled in a dessicator.

(iii) Solutions in organic solvents were dried using anhydrous sodium sulphate. The evaporation of bulk solvents was carried out under reduced pressure (10-15 mmHg) using a Büchi rotary evaporator.

(iv) Solutions of hydrazoic acid in benzene were prepared by adding sulphuric acid to sodium azide according to a standard method, and analysed by titration with 1 mol dm⁻³ sodium hydroxide solution with phenolphthalein as indicator.

(v) Solutions of sodium methoxide in methanol were prepared by adding freshly cut sodium to anhydrous methanol, and analysed by titration with 1 mol dm⁻³ hydrochloric acid with phenolphthalein as indicator.

(vi) Borane solutions were analysed according to a standard method using the apparatus shown in Fig. 2.2.1.

---

**Fig. 2.2.1**

A. Magnetic stirring bar
B. Hydrolysis solution
C. 200-300ml Flask
D. Septum Inlet
E. Spiral condenser
F. Cold Trap (Dry ice/acetone)
G. Flexible polyvinyl tubing
H. Heavy wall glass tubing
I. 250ml Buret
J. Leveling bulb
K. Thermometer
L. 4mm Straight bore stopcock
M. 4mm T-Bore stopcock
N. Water level
2.3 INSTRUMENTATION

(i) Nuclear Magnetic Resonance Spectroscopy

$^1$H n.m.r. spectra were recorded on a Perkin Elmer model R34 spectrometer operating at 220 MHz. Resonances are designated by their chemical shift ($\delta$) in parts per million. The peak multiplicity is given in brackets as:

- s singlet
- d doublet
- t triplet
- q quartet
- dd double doublet
- m multiplet
- br broad unresolved resonance
- q quartet

This is followed by the assignment and the spin-coupling constant (J Hz) where appropriate, and finally the relative intensity (n H).

N.m.r. solutions were prepared using deuterated solvents. Tetramethylsilane was used as an internal standard in organic solvents and trimethylsilylpropane sulphonic acid sodium salt in D$_2$O ($\delta$ 0.00 p.p.m.).

Broad band $^1$H decoupled $^{13}$C n.m.r. spectra and narrow band $^1$H decoupled ($^1$H)$^{13}$C n.m.r. spectra were recorded on a Bruker model WH90 spectrometer operating at 22.63 MHz. Resonances are designated as above. Dioxan was used as the internal standard in D$_2$O ($\delta$ 67.4 p.p.m.).

(ii) Infrared Spectroscopy

Infrared spectra were recorded on a Perkin Elmer model 257 grating spectrophotometer. Samples were either mulls (in Nujol) or the neat liquid, and
were run using sodium chloride plates. Only significant absorption bands are given and are designated by the wave number (cm\(^{-1}\)).

(iii) Melting Points

Melting point values were measured using a Reichert hot stage microscope (Kofler) apparatus, and are uncorrected.

(iv) Optical Rotations

Optical rotations were measured using a Bendix model NPL 143D automatic polarimeter with a cell of 1 cm path length. The instrument was calibrated against a standard sucrose solution ([α]\(_D\)\(^{20}\) 66.5°). Values are expressed as specific rotations, [α]\(_D\)\(^{T}\), at the sodium D line (589 nm) and temperature (T). The solvent and solution concentration (c) in g/100 cm\(^3\) are given.

(v) Mass Spectrometry

Electron ionisation (e.i.) and chemical ionisation (c.i.) mass spectra were recorded on a Kratos model MS80 spectrometer. The reagent gas used for c.i. is given in brackets. Significant peaks are quoted as m/z values followed by the percentage intensity in brackets. The molecular ion is designated as M\(^+\).

(vi) Combustion Analysis

Carbon, hydrogen, nitrogen combustion analyses were carried out by C.H.N. Laboratories, Leicester.
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CHAPTER 3

An Approach to $\text{Cyclo}(S\text{-aminoalanyl}-S\text{-aminoalanyl})$

via $\text{Cyclo}(S\text{-seryl}-S\text{-seryl})$

3.1 Introduction

3.2 Methods of Synthesising 2,5-Piperazinediones

3.3 Inherent Problems in 2,5-Piperazinedione Formation

3.4.1 Synthesis of $\text{Cyclo}(S\text{-seryl}-S\text{-seryl})$

3.4.2 Attempts to Synthesise $\text{Cyclo}(S\text{-chloroalanyl}-S\text{-chloroalanyl})$

3.4.3 The Dialkylcarbodiimide Method of Halogenation

3.4.4 The Triphenylphosphine/Carbon Tetrachloride Method of Halogenation

3.4.5 The Action of Hydrogen Bromide on $\text{Cyclo}(S\text{-seryl}-S\text{-seryl})$

3.4.6 A Modified Phosphorus Pentachloride Method of Halogenation

3.5 Synthesis of $(3S,6S)$-$\text{bis}$(trimethylsilyloxy-methyl)-2,5-piperazinedione

3.6 Some Reactions of $\text{Cyclo}(R\text{-aminoxyalanyl}-R\text{-aminoxyalanyl})$

3.7 Conclusions

3.8 Experimental

3.8.1 $\text{Cyclo}(S\text{-seryl}-S\text{-seryl})$

3.8.2 $(3S,6S)$-$\text{bis}$(trimethylsilyloxymethyl)-2,5-piperazinedione

3.8.3 $\text{Cyclo}(R\text{-aminoxyalanyl}-R\text{-aminoxyalanyl})$

3.8.4 $3,6\text{-bis}$(methylene)-2,5-piperazinedione

3.8.5 $(3S,6S)$-$\text{bis}$(acetoxymethyl)-2,5-piperazinedione

3.9 References
CHAPTER 3

An Approach to \( \text{Cyclo}(S\text{-aminoalanyl}-S\text{-aminoalanyl}) \) via \( \text{Cyclo}(S\text{-seryl}-S\text{-seryl}) \)

3.1 INTRODUCTION

The strategy adopted in this approach to the synthesis of an analogue of 593A firstly requires the conversion of \( \text{cyclo}(S\text{-seryl}-S\text{-seryl}) \) into \( \text{cyclo}(S\text{-aminoalanyl}-S\text{-aminoalanyl}) \). Several possible intermediates were considered for this conversion including \( \text{cyclo}(S\text{-chloroalanyl}-S\text{-chloroalanyl}) \), as well as \( \text{bis}(\text{trimethylsilyloxymethyl}) \), and \( \text{bis}(\text{aminooxymethyl}) \) derivatives of \( \text{cyclo}(S\text{-seryl}-S\text{-seryl}) \). In this Chapter the methods of synthesising 2,5-piperazinediones will be discussed, and some consideration will be given to particular problems associated with these methods. The attempts to prepare the intermediates illustrated in Scheme 3.1.1 will then be discussed. These reactions serve to highlight the problems of epimerisation, and elimination reactions, associated with the chemistry of piperazinediones and which led to the failure of this approach.

3.2 METHODS OF SYNTHESISING 2,5-PIPERAZINEDIONES

2,5-Piperazinediones are amongst the most ubiquitous peptide derivatives occurring in nature and
Scheme 3.1.1  Possible intermediates in the synthesis of cyclo(S-aminoalanyl-S-aminoalanyl) from (1).
were found very early in the development of the chemistry of amino-acids and peptides\textsuperscript{1}. The parent compound \textit{glyclo}(glycyl-glycyl) was first synthesised by Curtius in 1881 and many of the simpler members of the family were prepared by Fischer in the early part of this century. The simplest and most common method of preparation is by dimerisation of amino-acid esters, which are generally liberated from the corresponding amine hydrochloride or hydrobromide salt by the action of methanolic ammonia\textsuperscript{2}. However, in several cases a considerable discrepancy has been recorded in the optical rotation for the synthetic and natural products\textsuperscript{1}. An alternative approach to symmetrical piperazinediones is to heat the appropriate amino acid in ethylene glycol solvent\textsuperscript{1}. Kopple and Ghazarian\textsuperscript{3} have described the preparation of optically pure piperazinediones by heating a dipeptide or its hydrobromide salt in phenol at 140-150°C. This is an improvement on a previous method which employed $\beta$-naphthol\textsuperscript{4}. Also, using phenol rather than ethylene glycol is advantageous in some cases because dark by-products, common when the glycol is used, are not formed.

Leuchs' anhydrides (1,3-oxazolidine-2,5-diones) prepared from \textit{rca} amino-acids are reported to react instantaneously when added to an excess of an aziridine, at room temperature, to give pure 2,5-piperazinediones\textsuperscript{5} (Scheme 3.2.1). A very similar method replaces the aziridine with an amidoxime\textsuperscript{6} (Scheme 3.2.2). The piperazinediones of glutamic and aspartic acids have been prepared by this latter method. A small number of piperazinediones
Scheme 3.2.1 The preparation of piperazinediones from Leuchs anhydrides by aziridine catalysis.

Scheme 3.2.2 The preparation of piperazinediones from Leuchs anhydrides by amidoxime catalysis.
have been prepared from 3-aminopyrrolidones. For example, 3-aminopyrrolidone itself dimerises to 3,6-bis(\(\beta\)-aminoethyl)-2,5-piperazinedione\(^7\). Cycloserine undergoes a similar reaction (see Section 3.6) and we report the dimerisation of (S)-3-aminopyrrolidine-2,5-dione to \(\text{cyclo}(S\text{-asparagyl}-S\text{-asparagyl})\) in Chapter 4. However, probably the simplest method of synthesising symmetrical 2,5-piperazinediones, with the minimum risk of epimerisation, is by hydrogenolysis of N-benzyloxy carbonyl amino-acid methyl esters, which dimerise to afford optically homogeneous piperazinediones.

3.3 INHERENT PROBLEMS IN 2,5-PIPERAZINEDIONE FORMATION

The most troublesome aspects in all methods of 2,5-piperazinedione synthesis are epimerisation and polymerisation. The first step in the mechanism for the synthesis from amino-acid esters is the formation of a dipeptide by a condensation reaction between the amino function of one molecule and the ester function of another molecule. The second step is then a cyclisation by a similar condensation reaction. However, this step requires the dipeptide precursor to adopt the less favoured (Z) conformation from the lower energy (E) conformation (Fig. 3.3.1). The extent of polymerisation arising during the preparation of any particular piperazinedione will be partly dependent upon the magnitude of the rotational energy barrier (\(\Delta E_{\text{rot}}\)) for the HN-CO peptide bond.
Fig. 3.3.1 The (E) and (Z) conformers of a dipeptide precursor.

It could be expected then that dipeptides with a large \( \Delta \text{E}_{\text{rot}} \) would be prone to a greater degree of polymerisation. It is therefore surprising to find that the most commonly employed conditions for synthesis are ambient temperatures and high concentrations (often no solvent is used).

Infrared spectroscopy studies on several dipeptides and model systems by Tsuboi\(^8\) have shown that the ratio of the (E) and (Z) conformers is highly dependent upon temperature and solvent. Also the (E/Z) ratio depends upon the substituent R (Fig. 3.3.1) which influences the extent of intramolecular hydrogen bonding by the N-H grouping to the adjacent carbonyl oxygen. For example in acetylalanine-N-methylamide the infrared evidence\(^8\) suggests that a weak intramolecular hydrogen bonding exists in the (E) conformer, whereas no such bonding occurs in the (Z) conformer of acetylglycine-N-methylamide (Fig. 3.3.2).
Fig. 3.3.2 Hydrogen bonding in the (S') conformer of acetylalanine-N-methylamide (A) is absent in acetylglycine-N-methylamide (B).

A larger R substituent is present in all of the cases under consideration, and so it must be expected that some intramolecular hydrogen bonding exists in the (S) conformation and that this factor, together with the bulkiness of the R substituent, contribute to the magnitude of $\Delta E_{\text{rot}}$. Furthermore, the nature of the R substituent can influence the stability of the (Z) conformation. For example, proline-containing dipeptides are forced to adopt the (Z) conformation to an unusually high degree due to the presence of the pyrrolidine ring. But even when the (Z) conformation has been achieved, steric accessibility is still important. An example of this is the formation of a piperazinedione ring by intramolecular reaction of the cephalosporin (7); the analogous reaction does not occur for the penicillin (8) (Fig. 3.3.3). This observation is explained by the proposal that the gem-dimethyl function at position 2 in (8) prevents attack on the $\beta$-lactam face \textit{cis} to the amide side-chain.
Fig. 3.3.3 The cyclisation by (7) to a piperazinedione does not proceed for (8).
No such interference can occur for the cephalosporin (7).

By far the most persistent problem associated with piperazinedione syntheses is epimerisation. The susceptibility of the preparative methods described to epimerisation depends upon the tendency of the dipeptide precursor or the 2,5-piperazinedione itself to undergo deprotonation at the $C_\alpha$ position. This problem has been scrutinised in several reports\textsuperscript{10}. Fischer was the first to point out that loss of optical activity could occur under the conditions for piperazinedione formation and also noted the relative ease with which piperazinediones are racemised by alkali. Even in the absence of alkali some amino-acid esters suffer loss of optical activity at elevated temperatures.

The fact that dipeptides in the (Z) conformation are more prone to epimerisation than when they are in the (E) conformation was investigated theoretically by Gund\textsuperscript{10} who used substituted acetamides as models for his calculations. The difference in the calculated deprotonation energies for a methylene hydrogen in the two acetamide conformers shown in Fig. 3.3.4 is 27.5 kJ mol$^{-1}$.

\[
\begin{align*}
\text{(E)} & \quad \begin{array}{c}
\text{886.0} \\
\begin{array}{c}
\text{N} \\
\text{H}
\end{array}
\end{array} \quad \rightleftharpoons \\
\text{(Z)} & \quad \begin{array}{c}
\text{858.5} \\
\begin{array}{c}
\text{N} \\
\text{H}
\end{array}
\end{array}
\end{align*}
\]

Fig. 3.3.4 Calculated deprotonation energies (in kJ mol$^{-1}$) for the (E) and (Z) conformers of the acetamide studied by Gund\textsuperscript{10}. 
Gund performed similar calculations for 2,5-piperazinediones and concluded that cyclic dipeptides, especially when N-alkylated, undergo extremely fast epimerisation. Actually, nearly all 2,5-piperazinediones are readily epimerised in dilute sodium hydroxide at 25°C, and if one of the amino-acid residues has a very basic side chain, epimerisation can occur even on standing in solution in water at room temperature, e.g. \textit{cyclo}(S-phenylalanyl S-arginy1). The explanation given for this behaviour is that the \textit{transoid} (E) conformation is the more stable arrangement for the (O=C-N-C') grouping. Thus, a \textit{cis} (Z) dipeptide which gives rise to a \textit{transoid} (E) amido carbanion should epimerise more rapidly than a \textit{trans} (E) dipeptide or indeed the \textit{meso} form of a piperazinedione. This is illustrated by a comparison of the calculated deprotonation energies for the C\textsubscript{4} hydrogens of \textit{cyclo}(S-prolyl-S-alanyl) shown in Fig. 3.3.5; the favoured \textit{transoid} amido carbanion is outlined.

\begin{center}
\begin{figure}[h]
\includegraphics[width=0.5\textwidth]{figure3.3.5.png}
\caption{Deprotonation energies (kJ mol\textsuperscript{-1}) calculated by Gund\textsuperscript{10} for \textit{cyclo}(S-prolyl-S-alanyl).}
\end{figure}
\end{center}

For piperazinediones, the incipient carbanion is held in the more favourable \textit{transoid} conformation. Hyperconjugation effects are also said to influence the speed
of epimerisation, particularly in N-alkylated derivatives.

3.4.1 Synthesis of \textit{Cyelo}(S-seryl-S-seryl)

The method employed for the preparation of \textit{cyelo}(S-seryl-S-seryl) [\textsuperscript{1}] is well known\textsuperscript{11}. (S)-Serine was treated with a saturated solution of hydrogen chloride in dry methanol to give (S)-serine methyl ester hydrochloride in 89\% yield. Upon liberation of the free amine ester and then concentration of the solution to an oil, the piperazinedione (\textsuperscript{1}) crystallised out on standing at room temperature for about 24 hours. The 220 MHz proton nuclear magnetic spectrum (\textsuperscript{1}H n.m.r.) of (\textsuperscript{1}) in deuterated dimethylsulphoxide solvent (\textsuperscript{2}H\textsubscript{6}Me\textsubscript{2}SO) showed \textit{inter alia} two broad resonances: one at \( \delta \) 8.05 ppm and the other (of lower intensity) at \( \delta \) 7.90 ppm. These were assigned to the amide NH resonances of different piperazinediones suggesting that some epimerisation had occurred in the dimerisation process. This conclusion was supported by a discrepancy in the optical rotation values for this product and the literature value\textsuperscript{11} ([\alpha]\textsubscript{D} -56.2\degree and -65.5\degree respectively). Furthermore, the 22.4 MHz carbon-13 nuclear magnetic resonance (n.m.r.) spectrum, in deuterium oxide, also highlighted the presence of the epimer. The chemical shift assignments were made from a carbon-13 off-resonance decoupled n.m.r. spectrum shown in Fig. 3.4.1. The \{\textsuperscript{1}H\}\textsuperscript{13}C chemical shift values are tabulated below.
Fig. 3.4.1 A The 22.4 MHz \(^1\)H\(^{13}\)C n.m.r. (D\(_2\)O) for \(\text{cyclo}(\text{S-seryl-S-seryl})\) showing the presence of epimer.

B The off-resonance \(^{13}\)C n.m.r. for (\(\lambda\)).
<table>
<thead>
<tr>
<th></th>
<th>$\delta_1$</th>
<th>$\delta_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$-CH</td>
<td>57.69</td>
<td>57.52</td>
</tr>
<tr>
<td>$\beta$-CH$_2$</td>
<td>63.6</td>
<td>63.18</td>
</tr>
<tr>
<td>CO</td>
<td>169.1</td>
<td>169.1</td>
</tr>
</tbody>
</table>

$\delta_1$ - S,S isomer

$\delta_2$ - S,R isomer

Table 3.4 Chemical shift values from the 22.4 MHz $^{1}H{^{13}}C$ n.m.r. spectrum of cyclo(S-seryl-S-seryl) and its epimer.

3.4.2 Attempts to Synthesise Cyclo(S-chloroalanyl-S-chloroalanyl)

The expectation that cyclo(S-chloroalanyl-S-chloroalanyl) [3] would serve as a suitable intermediate for the preparation of cyclo(S-aminoalanyl-S-aminoalanyl) [3] was based on a literature precedent$^{12}$, citing the reaction of rac-3,6-bis(β-chloroethyl)-2,5-piperazinedione with various secondary amines to give the corresponding rac-3,6-bis(β-aminoethyl) derivatives. Also, a previous attempt to prepare rac.-(3) has been made$^{13}$. The authors of the report were successful in converting meso-3,6-bis(hydroxymethyl)-2,5-piperazinedione (10) into the meso-3,6-bis(chloromethyl)-2,5-piperazinedione derivative using phosphorus pentachloride in chloroform, but were unable to obtain rac.-(3) from rac.-(1) by the same method. Their account for this failure is based upon the proposition that the hydroxyl functions in rac.-(1) are tightly intra- and inter-molecularly hydrogen bonded to produce a dimeric structure of the type shown in Fig. 3.4.2. This renders the molecule insoluble and unreactive, whereas the meso isomer (10) is free of any such restriction.
Fig. 3.4.2 The dimeric structure proposed for cyclo(S-seryl-S-seryl).
Augustin et al.\textsuperscript{13} also achieved successful reactions of (\text{	extsuperscript{1}O}) with pyrrolidine, piperidine and morpholine in the absence of solvents to afford the corresponding \textit{bis-} (aminomethyl) derivatives, though it is not clear whether the reactions proceeded by an $S_N^2$ mechanism or by an elimination-addition mechanism. However, when methanol was present as the solvent it was noted that (\text{	extsuperscript{1}O}) rapidly eliminated to give 3,6-\textit{bis}(methylene)-2,5-piperazinedione ($\text{	extsuperscript{9}}$). In this respect compound (\text{	extsuperscript{1}O}) mimics the behaviour of rac.-3,6-\textit{bis}(\beta\text{-chloroethyl})-2,5-piperazinedione\textsuperscript{12}.

Augustin's conditions for halogenation were repeated with \textit{cyclo}(S-seryl-S-seryl) [$\text{	extsuperscript{1}}$] and finely divided phosphorus pentachloride, but were not successful. In view of the potential value of (\text{	extsuperscript{3}}) in this strategy for obtaining the key intermediate (\text{	extsuperscript{2}}), the problem was pursued vigorously. Several other methods of halogenation were tried and are outlined below.

\textbf{3.4.3 The Dialkylcarbodiimide Method of Halogenation}

A useful mild method for the conversion of alcohols to alkyl chlorides or bromides involves the reaction of the alcohol with a suitable N,N'-dialkyl-carbodiimide in the presence of a catalytic quantity of anhydrous copper(I) chloride to give an N,N'-dialkyl-O-alkylisourea intermediate\textsuperscript{14}. The isourea can then be converted into an alkyl halide by protonation followed by displacement with a halogen nucleophile (chloride or bromide). The procedure was tested successfully
using benzyl alcohol and N,N'-diisopropylcarbodiimide as
the model system. A sample of the prepared N,N'-
diisopropyl-O-benzylisourea was added to a mixture of
chloroform and hydrogen bromide in glacial acetic acid.
After 2 days at room temperature benzyl bromide was
isolated in 86% yield.

When this procedure was applied to (I) stirring
with N,N'-diisopropylcarbodiimide in the presence of copper(I)
chloride catalyst produced no evidence of reaction after
5 days at room temperature. The mixture was then heated
at 80°C in N,N-dimethylformamide (DMF) solvent and the
reaction was monitored by observing the carbodiimide
(N=C=N) band at 2120 cm\(^{-1}\) in the infrared spectrum.
After 3 hours the band at 2120 cm\(^{-1}\) had completely
disappeared. However, after working up the reaction
mixture only N,N'-diisopropylurea could be isolated from
the complex mixture, indicating that (I) may have
undergone an elimination reaction presumably to give
(II) as the other product. When the reaction was
repeated at 40°C the band at 2120 cm\(^{-1}\) disappeared
slowly over 5 days, but again only the urea could be
isolated from the reaction mixture.

3.4.4 The Triphenylphosphine/Carbon Tetrachloride
Method of Halogenation

The use of triphenylphosphine in carbon
tetrachloride for the preparation of alkyl chlorides
from alcohols was introduced in 1966\(^ {15}\). Since then the
mechanism and kinetics of the reaction have been
extensively examined\(^ {16}\). The generally accepted
Scheme 3.4.1 Mechanism for the triphenylphosphine/carbon tetrachloride halogenation of alcohols.
mechanism for the reaction is given in Scheme 3.4.1. The problem we faced when trying to apply this method to (1) was the very poor solubility of (1) in all solvents except for the most polar ones, e.g. water, DMF, and dimethylsulphoxide. It was therefore necessary to employ a small amount of a co-solvent hoping that this would not interfere with the reaction. Cyclo(S-seryl-S-seryl) was dissolved in a minimum volume of DMF and added to a cooled solution of triphenylphosphine in carbon tetrachloride and then allowed to warm to room temperature and stirred for 24 hours. The solvents were then removed to leave a complex mixture of products. The mass spectrum of the residue produced peaks at m/z 277 (corresponding to Ph₃PO⁺) and m/z 201 (corresponding to Ph₂PO⁺) suggesting that either a halogenation or elimination reaction of (1) had occurred. However, there was no evidence in the mass spectrum for the existence of (2) (M.W. 211), but a peak at m/z 138 suggested the presence of the elimination product (2) (M.W. 138).

The Action of Hydrogen Bromide on Cyclo(S-seryl-S-seryl)

In an effort to convert (1) into its bis(bromo-methyl) derivative, it was treated with hydrogen bromide in glacial acetic acid in the expectation that an acid-catalysed dehydration-halogenation reaction might proceed. The advantage of this method was that it overcame the insolubility problem previously experienced with (1). After 24 hours at room temperature a crop of colourless
crystals was isolated from the reaction mixture and identified by $^1$H n.m.r. as the $(3S,6S)$-bis(acetoxymethyl)-2,5-piperazinedione adduct (I).

This structure was confirmed by comparing (II) with an authentic sample prepared by bis-acetylation of (I) using acetic anhydride in pyridine as described in the literature.

3.4.6 A Modified Phosphorus Pentachloride Method of Halogenation

It was explained in Section 3.4.1 that the reason for the failure by Augustin et al. to obtain rac.-(/) from rac.-(\textcircled{1}), compared with their success in obtaining meso- (\textcircled{2}) from meso-(\textcircled{1}), was the insolubility of rac.- (\textcircled{1}) and that our attempts to halogenate (\textcircled{1}) also failed. Therefore, a modified method was tried employing phosphorus pentachloride in N-methyl-2-pyrrolidone (NMP). As well as being a good solvent for (\textcircled{1}), NMP is reported to have a catalytic effect in the halogenation of alcohols with phosphorus pentachloride (cf. mechanism is shown in Scheme 3.4.2). When phosphorus pentachloride was added to (\textcircled{1}) in NMP an exothermic reaction ensued; after $1^{1/4}$ hours the...
Scheme 3.4.2 Mechanism for the NMP catalysed halogenation of alcohols.
reaction mixture was worked up, but only unchanged starting material (1) could be isolated. The procedure was repeated at 80°C. After 3 hours the reaction mixture was very viscous, probably because of polymerisation of the solvent, and produced only a brown intractable oil. This reaction was not pursued any further.

3.5 THE SYNTHESIS OF (3S,6S)-\textit{bis}-(TRIMETHYLSILYLOXY-METHYL)-2,5-PIPERAZINEDIONE

The use of silicon reagents in organic synthesis has grown rapidly over the past decade and recently two comprehensive reviews have appeared in the literature.\(^{19}\) The versatility of trimethylsilyl iodide is very broad, but the introduction of new and more powerful silylating reagents has expanded the application of this chemistry in organic synthesis.

One method for silylation of alcohols employs trimethylsilyliodide (TMSI) [or trimethylsilylchloride/sodium iodide] in acetonitrile. Evidence has appeared\(^{20}\) showing that the latter, more convenient, reagent is more powerful than TMSI owing to a catalytic effect of free iodide in the medium. However, all efforts to silylate (1) with this reagent or with TMSI were unsuccessful, and we did not obtain the \textit{bis}(iodomethyl) derivative of (1) directly by this method. It therefore became necessary to turn to a more powerful silylation reagent, namely \textit{bis}(trimethylsilyl)acetamide (2).\(^{21}\) Mixing (1) and (2) in dimethylsulphoxide gave the \textit{bis}(trimethylsilyloxyethyl) adduct (4) which crystallised from the
solution almost immediately. Although (12) will reportedly also silylate amides no N-trimethylsilyl products were detected, even when an excess of (12) was used in the reaction.

Two approaches to the target compound \textit{cyclo}(S-aminoalanyl-S-aminoalanyl) [2] from (4) were available. The first involved reacting (4) with TMSI to give (3S,6S)-\textit{bis}(iodomethyl)-2,5-piperazinedione which could then be converted to (5) by reaction with either ammonia or an amine (Scheme 3.1.1). The initial reactions were performed in dry [2H$_6$] dimethylsulphoxide solvent and monitored using a 220 MHz H$^1$ n.m.r. spectrometer. In each case, a singlet resonance quickly appeared at $\delta$ 0.07 p.p.m. corresponding to hexamethyldisiloxane, and the solution developed a deep red colouration due, presumably, to the liberation of iodine. When the reaction was carried out on a preparative scale only \textit{cyclo}(S-seryl-S-seryl) [4] was isolated. Since there was no evidence in the $^1$H n.m.r. spectrum to suggest the occurrence of elimination of (4) to give (5), which would explain the release of iodine into the solution, it must be concluded that traces of moisture trapped in the solvent caused a small degree of hydrolysis of the TMSI reagent. It is unlikely that the \textit{bis}(iodomethyl) derivative, if formed, would be hydrolysed in the aqueous work-up, and so (4) must have arisen from hydrolysis of (4) only.

The second approach to (2) using (4) required the synthesis of (3S,6S)-\textit{bis}(azidomethyl)-2,5-piperazine-
dione (see Scheme 3.1.1). All efforts to make this compound using the reagent trimethylsilylchloride/sodium azide in DMF were also unsuccessful. Either the unchanged starting material (4) or the hydrolysate (1) (when aqueous work-up was used) was isolated from the reaction mixtures.

3.6 SOME REACTIONS OF Cyclo(R-AMINOXYALANYL-R-AMINOXYALANYL)

The preparation of some racemic derivatives of (2) has been reported. D-(R)-Cycloserine is a broad spectrum antibiotic which readily dimerises both in solution and in the solid state to give cyclo(R-aminoxyalanyl-R-aminoxyalanyl) [5] (see Scheme 3.1.1) with 10% epimerisation to the meso isomer (further discussion on this dimerisation is given in Chapter 4). Compound (5) can be easily acylated on both of the aminooxy functions without loss of optical activity. However, when (5) is reacted with thiols or secondary amines, substitution is largely achieved through an elimination-addition mechanism to give a mixture of diastereoisomeric products. Evidence for this mechanism was established by preparing the bis(methylene) elimination product (g) from (5) and carrying out amine and thiol addition reactions under similar reaction conditions (Scheme 3.6.1). Compounds (5) and (g) were prepared as described to serve as spectroscopic references.
Some reactions of cyclo(R-aminooxyalanyl-R-aminooxyalanyl) [5] explored by Ongania23.
3.7 CONCLUSIONS

The work with cyclo(R-aminooxyalanyl-R-aminooxyalanyl) [\(\xi\)] performed by Ongania\(^{23}\) has shown that even if the desired enantiomer were available it would not be possible to carry out substitution reactions with amines to give derivatives of (\(\xi\)), without considerable loss of optical activity. The work of Augustin and others showed that (\(\xi\)), if it could be made, would provide a promising route to derivatives of (\(\xi\)), although elimination and epimerisation are both potential problems. The results we have obtained indicate the propensity of piperazinediones to undergo these reactions, but in addition to these, the lack of solubility of (\(\xi\)) also presented great difficulties.

3.8 EXPERIMENTAL

3.8.1 cyclo(S-seryl-S-seryl) [\(\eta\)]

(S)-Serine (25.0 g, 0.23 mol) was dissolved in a saturated solution of hydrogen chloride in dry methanol (460 cm\(^3\)). The solution was refluxed for 16 hours and then evaporated to dryness and the residue redissolved in a saturated solution of hydrogen chloride in dry methanol (250 cm\(^3\)), and then again refluxed for 5 hours. The solution was evaporated and the residue then dissolved in a minimum volume of dry methanol (~70 cm\(^3\)) and the methyl ester hydrochloride was precipitated by the addition of dry diethyl ether. The solid was filtered and washed with dry methanol and then dried to give
(S)-serine methyl ester hydrochloride (25.0 g, 67% yield), m.p. 162-165°C (dec.) [lit.11 m.p. 163-167°C (dec.)]. 

1H n.m.r. (D2O); δ 4.28 (t, CH, 1H), 4.05 (m, CH2, 2H), 3.87 (s, OCH3, 3H) p.p.m. The hydrochloride was then dissolved in dry methanol and a 3.57 mol dm⁻³ solution of sodium in methanol (40 cm³) was added. After 1 hour dry diethyl ether was added and the mixture cooled and then filtered. The filtrate was evaporated to give an oil which crystallised on standing at room temperature for 24 hours. The solid was recrystallised from water to give the title compound [¶] (4.0 g, 14% yield) m.p. 239-241°C (dec.) [lit.11 m.p. 247°C (dec.)], [α]D²⁰ - 56.2° (c 1.2 in H2O) [lit.11 [α]D - 65.5°], 1H n.m.r. (D2O); δ 4.15 (t, 2 x CH, 2H), 3.85 (m, 2 x CH2, 4H) p.p.m.; ([2H₆]Me₂SO); δ 8.05 (br, 2 x NH, 2H), 7.90 (br, epimeric NH, < 1H), 5.05 (t, 2 x CH, 2H), 3.70 (m, 2 x CH₂, 4H) p.p.m., i.r. (Nujol); 3220, 1670, 1110, 1060 cm⁻¹.

3.8.2 (3S,6S)-Bis(trimethylsilyloxymethyl)-2,5-piperazinedione (¶)

Compound [¶] (0.25 g, 1.43 mmol) was dissolved in dry dimethylsulphoxide (2.5 cm³) and bis(trimethylsilyl)acetamide (0.73 g, 3.58 mmol) was added. White crystals were precipitated almost immediately. After 1 hour the mixture was filtered and the solid washed with dry dichloromethane and dried to give the title compound (¶) (0.35 g, 77% yield) m.p. 245-247°C, 1H n.m.r. (CDCl₃); δ 8.05 (br, 2 x NH, 2H), 3.80 (m, 2 x CHCH₂, 6H), 0.1 (s, 2 x OSi(CH₃)₃, 18H) p.p.m.
3.8.3 **Cyclo(R-aminooxyalanyl-R-aminooxyalanyl)**[^5]

(R)-Cycloserine (1.0 g, 0.01 mol) was dissolved in ethanol (50 cm³) and glacial acetic acid (1.0 cm³) and the solution refluxed for 45 minutes. The solution was then cooled and filtered, and the solid was washed with ethanol and then recrystallised from ethanol-water to give the title compound ([5]) (0.42 g, 42% yield) m.p. 180-181°C (dec.) [lit. 23 m.p. 180-181°C (dec.)] [\(\alpha\)]D\text{20} - 7.0° (c 1.0 in H\text{2}O) [lit. 23 [\(\alpha\)]D - 7.0°],

\(^1\)H n.m.r. (D\text{2}O); \(\delta\) 4.35 (t, 2 x CH, 2H), 4.05-3.90 (m, 2 x CH\text{2}, 4H) p.p.m.; ([\(^2\)H\text{6}]Me\text{2}SO); \(\delta\) 8.05 (br, 2 x NH, 2H), 6.10 (br, 2 x ONH\text{2}, 4H), 4.05 (m, 2 x CH, 2H), 3.70 (m, 2 x CH\text{2}, 4H) p.p.m.

3.8.4 **3,6-Bis(methylene)-2,5-piperazinedione**[^2]

Compound ([5]) (0.20 g, 1 mmol) was dissolved in a 0.008 mol dm⁻³ sodium hydroxide solution (20 cm³). After 30 minutes crystals began to be deposited in the solution after 4 hours the mixture was filtered and the solid washed with water and dried (P\text{4}O\text{10} in \text{vacuo}) to give the title compound ([6]) (0.05 g, 30% yield) \(^1\)H n.m.r. ([\(^2\)H\text{6}]Me\text{2}SO); \(\delta\) 8.05 (br, 2 x NH, 2H), 5.30 and 4.92 (2 x s, 2 x CH\text{2}, 4H) p.p.m.

3.8.5 **(3S,6S)-Bis(acetoxymethyl)-2,5-piperazinedione**[^1]

Compound ([1]) (0.20 g, 1.15 mmol) was dissolved in dry pyridine (15 cm³) with warming and then acetic anhydride (0.35 g, 3.45 mmol) was added and the solution stirred at room temperature for 18 hours. The mixture
was then cooled to -10°C and filtered. The solid was washed with dichloromethane and dried to give the title compound \([1,1]\) (0.15 g, 43% yield) m.p. 234-236°C (lit. \(17\) m.p. 228-230°C), \([\alpha]_D^{20} - 7.1°\ (c 1.0 \text{ AcOH})\) (lit. \(17\) \([\alpha]_D - 7.0°\), \(^1\)H n.m.r. (\([\text{D}_2\text{O}]\text{Me}_2\text{SO}\)); δ 8.45 (br, 2 x NH, 2H), 4.25 (m, 2 x CH, 2H), 3.35 (m, 2 x CH\(_2\), 4H), 2.05 (s, 2 x CH\(_3\), 6H) p.p.m.; i.r. (Nujol); 3200, 1750, 1680 cm\(^{-1}\).

3.9 REFERENCES


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CHAPTER 4

The Synthesis and Crystal Structure of Cyolo(S-asparagyl-S-asparagyl)

4.1 Introduction

4.2 The synthesis of Cyolo(S-asparagyl-S-asparagyl)

4.3 Some Reactions of (S)-3-Aminopyrrolidine-2,5-dione

4.4 Antitumour Screening of Cyolo(S-asparagyl-S-asparagyl)

4.5 The Crystal Structure of Cyolo(S-asparagyl-S-asparagyl)

4.6 Attempted Hofmann Rearrangement on Cyolo(S-asparagyl-S-asparagyl)

4.7 The Crystal Structure of Bis(trifluoroacetoxy)-iodobenzene

4.8 Experimental

4.8.1 (S)-N\(^\alpha\)-Benzyloxycarbonylasparagine Methyl Ester

4.8.2 Cyolo(S-asparagyl-S-asparagyl) from (16)

4.8.3 (S)-3-N-Benzyloxycarbonylaminopyrrolidine-2,5-dione

4.8.4 (S)-3-Aminopyrrolidine-2,5-dione

4.8.5 Cyolo(S-asparagyl-S-asparagyl) from (20)

4.8.6 Fischer's Preparation of Cyolo(asparagyl-asparagyl)

4.8.6.(i) (S)-Dimethylaspartate Hydrochloride

4.8.6.(ii) Dimethyl Ester of Cyolo(aspartyl-aspartyl)

4.8.6(iii) Cyolo(asparagyl-asparagyl)

4.8.7 Cyolo(R-asparagyl-S-asparagyl)

4.8.8 Reactions of (S)-3-Aminopyrrolidine-2,5-dione in Water

4.8.9 Crystal Data Collection for (12)

4.8.10 Bis(trifluoroacetoxy)iodobenzene
4.8.11 Crystal Data Collection for \((2\overline{3})\)

4.9 References
with ammonia in a sealed ginger ale bottle; but the stereochemical identity of the product was not established. It was necessary therefore, to develop a reliable preparative method for optically homogeneous cyclo(S-asparagyl-S-asparagyl) \( \{1^2\} \) before investigating the Hofmann rearrangement of \( \{1^2\} \) to \( \{2\} \) using the \( \text{bis( trifluoroacetoxy)iodobenzene} \) reagent.

4.2 THE SYNTHESIS OF \( \text{Cyclo(S-asparagyl-S-asparagyl)} \)

In Fischer and Koenigs' route\(^3\) to the piperazinedione of \( \text{(S)-asparagine} \) \( \{1^3\} \), the first step was the esterification of \( \{1^3\} \) by reaction in methanol saturated with hydrogen chloride, to give dimethyl aspartate \( \{1^4\} \). Although it would have been more convenient to prepare the monoester derivative of \( \{1^3\} \), and then follow the usual procedure for preparing piperazinediones by neutralising the amine salt of the ester and allowing the free amino-ester to dimerise to \( \{1^2\} \), it is difficult to esterify asparagine without effecting alcoholysis of the amide function. A comparative study of the esterification of amino acids has been carried out\(^5\) in which the mono-methyl ester of \( \text{(S)-asparagine} \) was obtained when a 0.1 to 0.5 mol dm\(^{-3}\) solution of hydrogen chloride in methanol at \(-15^\circ\text{C}\) was used. However, this method was not considered to be satisfactory because to obtain the piperazinedione, it would be necessary to neutralise the amine salt with sodium methoxide in methanol solution. This might produce conditions in which there could occur either methanolysis
Scheme 4.2.1 Fischer's preparation of the piperazinedione of (S)-asparagine.
Page missing in original
Scheme 4.2.2 The synthesis of optically pure \textit{cyclo}(S-asparagyl-S-asparagyl).
at about 2 mol dm$^{-3}$ hydrogen chloride concentrations in methanol and at -15°C with a reaction time of 24 hours could high yields of the monomethyl ester ($\text{I}_6$) be reliably and reproducibly obtained.

The $\text{N}^\text{a}$-benzyloxycarbonyl protecting group was removed easily by hydrogenolysis over 10% palladium on charcoal catalyst in methanol. The oily residue obtained after filtration and evaporation of the solvent crystallised on standing at room temperature for 48 hours to give the derived 2,5-piperazinedione derivative ($\text{I}_2$). $\text{Cyolo}(S$-asparagyl-$S$-asparagyl) ($\text{I}_2$) was recrystallised from water to give a substance with a constant value for its optical rotation of $[\alpha]_D - 26.6^\circ$ (c 1.2 in H$_2$O). No epimerisation product was detected either in the $^1$H or $^1$H$^{13}$C n.m.r. spectra (Fig. 4.2.1). The e.i. mass spectrum for ($\text{I}_2$) illustrates the characteristic diagnostic fragmentation pattern for 2,5-piperazinediones shown in Fig. 4.2.2.

In order to establish the nature of the piperazinedione derivatives obtained from (S)-asparagine ($\text{I}_3$) by Fischer and Koenigs$^3$, and from diethyl fumarate by Fox and Dunn$^4$; their procedures were repeated. By Fischer's method (Scheme 4.2.1) brown crystals of the dimethyl ester of $\text{Cyolo}$($\text{aspartyl-aspartyl}$) ($\text{I}_7$) were produced. No mention was made by Fischer and Koenigs regarding the stereoisomeric composition of their sample of ($\text{I}_7$) and no optical rotation was quoted$^3$. The $^1$H n.m.r. spectrum of ($\text{I}_7$) showed no evidence of diastereoisomers, and ($\text{I}_7$) gave a positive optical rotation. However, this information was no assurance of optical purity. Compound
Fig. 4.2.1 A 220 MHz $^1$H n.m.r. (D$_2$O) and
B 22.4 MHz $^1$H$^{13}$C n.m.r. (D$_2$O) spectra
for cyclo(S-asparagyl-S-asparagyl).
Fig. 4.2.2 The e.i. mass spectrum for cyclo(S-asparagyl-S-asparagyl).

\[ \text{m/z 211} \quad \text{m/z 183} \quad \text{m/z 166} \]

\[ \text{m/z 138} \quad \text{m/z 125} \]
(17) was then treated with liquid ammonia in a sealed glass tube, to give a mixture of stereoisomers of (12). On the two occasions that this synthesis was performed, substances of greatly differing optical purity were obtained, i.e. $[\alpha]_D^{-20.0^\circ}$ and $-5.38^\circ$ (c 1.2 in $H_2O$) respectively. This suggested the possibility that preferential crystallisation of one enantiomer may have occurred in one of the preparations giving rise to differing optical purities.

In the method described by Fox and Dunn (Scheme 4.2.3) diethyl fumarate in ethanol containing ammonia was heated at 100°C.

![Scheme 4.2.3 Fox's preparation of the piperazinedione of asparagine.](image)

The reaction mixture liquors were decanted from the solid crust formed on the walls of the vessel. Fox and Dunn disregarded the solid and obtained a sample of presumably rac. (12) from the liquors. We obtained racemic (12) from the liquors, and recrystallised the solid crust formed in the expectation that it might be enriched with one diastereoisomer. This material gave no measurable optical rotation in water, and was much less soluble in water than cyclo(S-asparagyl-S-
asparagyl) [12] obtained as described above from (16). Its \(^1\)H n.m.r. spectrum (in \([\text{\(^{2}\)H_6}}\] dimethyl sulfoxide) differed from that of the (S,S) isomer. It may therefore be concluded that the solid deposited in the diethyl fumarate ammonia reaction is \(\text{cyclo}(R\text{-asparagyl-S-asparagyl})\).

Hence, both of the published methods\(^3,4\) produce mixtures of stereoisomers of (12), whereas the procedure described in this chapter is the first preparation of optically homogeneous \(\text{cyclo}(S\text{-asparagyl-S-asparagyl})\) [12].

An anomalous feature in the \(^1\)H n.m.r. spectrum of the (S,S)-enantiomer (12) in \([\text{\(^{2}\)H_6}}\] dimethyl sulfoxide solvent was the behaviour of the three different amide hydrogen (NH) resonances at 7.80, 7.42, and 6.93 p.p.m. when D\(_2\)O was added to the n.m.r. solution. The lowest field resonance (at 7.80 p.p.m.) disappeared much more rapidly than the other two NH resonances (Fig. 4.2.3). However, when this experiment was repeated with meso-(12), from Fox and Dunn's method, its three NH resonances disappeared equally quickly. It therefore appears that two of the amide hydrogens in the (S,S) isomer of (12) are more acidic than the other four, possibly because of intramolecular or intermolecular hydrogen bonding. Of course, this may only be the case for the particular conformation of the molecule in solution, but it was decided that an X-ray crystallographic structure determination should be obtained for (12). The results of this study are discussed in Section 4.5.
Fig. 4.2.3 220 MHz $^1$H n.m.r. for cyclo(S-asparagyl-S-asparagyl) in A [2H$_6$] Me$_2$SO and B with D$_2$O added.
SOME REACTIONS OF (S)-3-AMINOPYRROLIDINE-2,5-DIONE

The synthesis of a small number of 2,5-piperazinediones from 3-aminopyrrolidiones have been reported (Section 3.2) and the dimerisation of (R)-cycloserine to cyclo(R-aminoxyalany-R-aminoxyalanyl) \([5]\) has also been mentioned (Section 3.6). The latter reaction has attracted considerable attention and a study of its pH dependence, as well as a kinetic study on the reactions of \([5]\) with acid and alkali, have been carried out. Under basic conditions the cyclic hydroxamic acid group in (R)-cycloserine is converted into its salt which protects the ring from further attack. In aqueous acidic media (pH 1.2 to 2.2) the compound is predominantly hydrolysed to \(\beta\)-aminoxyalanine. However, in 4% acetic acid in ethanol, dimerisation to \([5]\) is the major process. The acid is believed to catalyse the formation of a cyclol structure \([18]\), which is part of a more complex equilibrium involving a second cyclol \([19]\) and the 2,5-piperazinedione \([5]\); this equilibrium is shown in Scheme 4.3.1.

In order to establish whether or not (S)-3-aminopyrrolidine-2,5-dione [(S)-aminosuccinimide] \([20]\) could be dimerised to cyclo(S-asparagyl-S-asparagyl) \([12]\), and to ascertain whether a similar equilibrium to the one described above existed for this process, a study of \([20]\) and its reactions was made. A convenient preparation of \([20]\) was first required.

During the course of an investigation into the synthesis of peptides of (S)-asparagine, Sondheimer and
Scheme 4.3.1 The dimerisation reaction equilibrium for (R)-cycloserine in acid medium.
Holley were surprised to isolate (S)-N-Z-3-aminopyrrolidine-2,5-dione (2) as a significant component from an attempted saponification of (S)-Nα-Z-asparagine methyl ester (\(\text{1}^6\)). Hydrogenolysis of (2) over palladium on carbon catalyst in methanol removed the benzyloxy carbonyl protecting group to give (\(\text{2}^0\)). Sondheimer and Holley also noted that (\(\text{2}^0\)) decomposed in aqueous solution to give asparagine, isoasparagine and a crystalline solid which the authors tentatively identified as the 2,5-piperazinedione of asparagine (\(\text{1}^2\)). The improved esterification of (\(\text{1}^5\)) to (\(\text{1}^6\)), reported in Section 4.2, presented the opportunity to achieve an overall more efficient synthesis of (\(\text{2}^0\)). Compound (2) was prepared as described from the methyl ester (\(\text{1}^6\)).

The hydrogenolysis of (2) to (\(\text{2}^0\)) was initially performed in methanol using hydrogen at atmospheric pressure with palladium/carbon catalyst. However, it was noticed that a degree of methanolysis occurred to produce about 20% of the methyl ester of (S)-asparagine. It was found that this side reaction could be prevented by carrying out the hydrogenolysis more rapidly at 20 p.s.i. hydrogen pressure. On one occasion some pyrrolidinedione (\(\text{2}^0\)) was detected in the reaction mixture from the hydrogenolysis of (\(\text{1}^6\)). This implies that (\(\text{2}^0\)) may be an intermediate in the formation of (\(\text{1}^2\)), or participates in an equilibrium with (S)-asparagine methyl ester. It was found that optically pure piperazinedione (\(\text{1}^2\)) could be obtained in 40% yield by boiling a solution of (\(\text{2}^0\)) in anhydrous acetonitrile for seven days.
Compound (20) was expected to demonstrate pH dependent behaviour in water. At intermediate pH values the formation of piperazinedione (12) might occur from the reaction of the pyrrolidinedione (20) and its conjugate acid. The optimum pH for the production of piperazinedione (12) would then be governed by the pKₐ of the pyrrolidinedione (20). A sample of (20) was dissolved in D₂O and the reaction was monitored by 220 MHz ¹H n.m.r. spectroscopy. After three weeks at room temperature hydrolysis of (20) to (S)-asparagine was complete and no other products were evident in the spectrum. Samples of pyrrolidinedione (20) were then dissolved in 0.5 mol dm⁻³ D₂O phosphate buffer solutions with pD values of 5.61, 6.43, 7.05 and 7.95. These solutions were incubated at 22°C and monitored periodically by ¹H n.m.r. spectroscopy. After about 10 hours some precipitation was visible and this prevented the calculation of the percentage composition of the reaction mixture. It also thwarted our intention to make a kinetic study of the system. All of the reactions were virtually complete after 42 hours. The only products observed were asparagine and the 2,5-piperazinedione (12), which was also believed to be the precipitate. Careful scrutiny of the spectra revealed that in the region of pD 7, the formation of the piperazinedione appeared to be maximised. A preparative scale reaction was then performed so that the precipitate could be identified. A sample of the pyrrolidinedione (20) was dissolved in a 0.5 mol dm⁻³ aqueous phosphate buffer of pH value 7.0 and the solution was maintained at 22°C for two days; during this time a precipitate was again formed. The solution was then
Scheme 4.3.2 The aqueous chemistry of (S)-3-amino-pyrrolidine-2,5-dione.
concentrated and filtered, to give the piperazinedione (12) in a yield of 9.2%. Examination of the filtrate by 
$^1$H n.m.r. spectroscopy showed that it contained asparagine and unchanged (20).

The behaviour of (S)-3-aminopyrrolidine-2,5-dione (20) at both very low and very high pH was examined. Compound (20) was dissolved in a 1 mol dm$^{-3}$ DCl solution (pD - 0.57), and in 1 mol dm$^{-3}$ NaOD solution (pD 13.5) and reactions were monitored by $^1$H n.m.r. spectroscopy. At pD 13.5, rapid hydrolysis to aspartic acid occurred at room temperature; this is in contrast to the behaviour of (R)-cycloserine. The identity of the hydrolysis product was established by addition of asparagine and aspartic acid to the n.m.r. tube. At pD - 0.57, the five-membered ring of (20) survived; no hydrolysis was evident even after five days at room temperature. Hydrolysis did proceed when the solution was heated at 80°C, and was complete after 38 hours at this temperature. The product was again shown to be solely aspartic acid by additions of aspartic acid and asparagine to the n.m.r. tube. A summary of these reactions is given in Scheme 4.3.2.

4.4 ANTITUMOUR SCREENING OF Cyclo(S-ASPARAGYL-S-ASPARAGYL)

A sample of cyclo(S-asparagyl-S-asparagyl) [12] has recently been submitted* for anti-tumour screening. The initial results$^{11}$ appear to be promising. Compound (12) was tested for a differential effect in an in vitro study on the two cell lines Yoshida parental and busulphan

*Christie Hospital and Holt Radium Institute, Manchester
resistant. The ID$_{50}$ (inhibition dose) response is given below.

<table>
<thead>
<tr>
<th>ID$_{50}$ ($\mu g/cm^3$)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Parental</td>
<td>7.5</td>
</tr>
<tr>
<td>Resistant</td>
<td>54.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>(%o)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Busulphan</td>
<td>42</td>
</tr>
<tr>
<td>(%o)</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 4.4 Inhibition responses to busulphan and (\%o)

Thus (1,2) shows a differential effect comparable to the antitumour drug busulphan. An animal study is intended when the present rat toxicity testing is completed.

4.5 THE CRYSTAL STRUCTURE OF Cyclo(S-ASPARGYL-S-ASPARGYL)

The structure and conformation of 2,5-piperazinediones have attracted much attention over the last fifteen years. This interest has arisen because of the suitability of 2,5-piperazinediones as models for the study of certain intramolecular interactions between amide bonds and amino acid side chains. Although 2,5-piperazinediones possess the atypical cis amide bonds, the effects observed are important to the understanding of the conformational constraints present in polypeptides and proteins. X-ray crystallographic studies on several 2,5-piperazinediones (see Table 4.5.1) have revealed that the six-membered ring has a variety of conformational possibilities described as planar, chair, bowsprit boat, flagpole boat, and twist
Fig. 4.5.1a The possible 2,5-piperazinedione ring conformations.

Fig. 4.5.1b The boat (A) and twist boat (B) and the theoretical coupling constants (J/Hz).
boat (Fig. 4.5.1a).

$^1$H n.m.r. studies have played an important role in determining the ring conformation of 2,5-piperazinediones in solution. Koppel and Ohnishi$^{12}$ examined $^1$H n.m.r. spectra of several glycyl 2,5-piperazinediones in $[^2H_6]$ dimethyl sulphoxide and discovered, by measuring H-N-C$_a$-H coupling constants, that the ring takes up a non-planar conformation. The fact that this coupling is non-zero suggested that a twist boat conformation is probable, as opposed to a bowsprit boat, although the flagpole boat conformation could not be reliably excluded on the basis of the size of the coupling constants measured (Fig. 4.5.1b). The observed H-N-C$_a$-H coupling disappeared, as expected, when D$_2$O was added to the solution. The solvent was shown to have an influence on the conformation because in trifluoroacetic acid (TFA) the ring adopts a planar conformation$^{12}$. It is believed that protonation or hydrogen bonding interactions of the amide functions with TFA induces greater double-bond character across the HN-CO bond, thus flattening the ring.

A surprising feature in the structure of 3-substituted and 3,6-disubstituted 2,5-piperazinediones containing an aromatic ring in the side chain, e.g. cyclo(glycyl-S-tyrosyl), is that the molecules adopt a folded flagpole boat conformation in which the aromatic ring faces the piperazinedione ring (Fig. 4.5.2). This is observed in the crystal structure and is supported by aromatic shielding effects recorded in the $^1$H n.m.r. spectra$^{12}$. This effect stabilises the folded conformation.
A study of the chemical shift changes which occur when the temperature is altered has allowed a stabilisation energy of 12 kJ mol\(^{-1}\) to be calculated for the interaction.

![Diagram](image)

Fig. 4.5.2 The folded flagpole boat conformation observed with aromatic substituents

Preference for the folded flagpole boat conformation is less pronounced for \textit{cyclo}(glycyl-S-valyl) which contains a bulky alkyl group instead of an aromatic side chain. Also, in 3,6-disubstituted 2,5-piperazinediones (as opposed to derivatives containing a glycine residue) additional steric considerations become important. If both of the constituent amino acids have the same configuration, i.e. \((S,S)\) or \((R,R)\), then steric interference may prohibit the flagpole boat conformation. However, a dipole-induced dipole interaction with the piperazinedione ring is believed to exert some influence in almost all cases. A bowsprit boat conformation excludes most of this type of interaction. This reasoning could explain the dominance of the planar conformation (elucidated by \(^1\)H n.m.r. studies) in \textit{cyclo}(S-alanyl-S-phenylalanyl). In this compound the steric interaction of the substituents is too great to allow the folded conformation to be attained; even in the planar conformation some aromatic ring/piperazinedione ring interaction is still
possible\textsuperscript{17}. In \textit{cyclo}(S-phenylalanyl-S-phenylalanyl) the aromatic rings do not sit centrally over the piperazinedione ring, but rather each phenyl is associated with just one of the amide groups\textsuperscript{12}. In 2,5-piperazinediones with only alkylmethylene substituents, intramolecular interactions are less important than other factors, such as crystal packing forces. For example, \textit{cyclo}(S-alanyl-S-alanyl) adopts a bowsprit boat conformation because of these packing forces in the crystal; steric interference would not be great enough to prohibit the folded flagpole boat conformation in this molecule\textsuperscript{17}.

The main factors determining the conformation then, are as follows:

(i) Amide resonance; this is maximal for the planar and boat conformations.

(ii) Steric repulsion between 3,6-substituents; this is minimised in the bowsprit boat conformation.

(iii) Hydrophobic interactions between the piperazinedione ring and a 3- or 6-substituent; this stabilises the flagpole boat conformation, particularly with aromatic substituents.

(iv) Inter- or intra-molecular hydrogen bonding; some piperazinediones contain hydrogen bonded water molecules in the crystal structure.

The conformations and stabilising interactions of some 2,5-piperazinediones are summarised in Table 4.5.1.

The 220 MHz \textsuperscript{1}H n.m.r. spectrum of \textit{cyclo}(S-asparagyl-S-asparagyl) \textsuperscript{12} in \textsuperscript{2}H\textsubscript{6} dimethylsulphoxide
<table>
<thead>
<tr>
<th>2,5-Piperazinedione</th>
<th>Conformation of Piperazinedione Ring</th>
<th>Stabilising Interactions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cyclo</strong> (R-leucyl-R-histidyl monohydrate)</td>
<td>Slight deviation from planarity towards flagpole boat</td>
<td>Imidazole faces piperazinedione</td>
<td>13</td>
</tr>
<tr>
<td><strong>Cyclo</strong> (S-histidyl-S-aspartyl) trihydrate</td>
<td>Approximately planar</td>
<td>Imidazole faces piperazinedione; intermolecular H-bond between piperazinedione NH and aspartyl CO₂⁻</td>
<td>14</td>
</tr>
<tr>
<td><strong>Cyclo</strong> (S-threonyl-S-histidyl)</td>
<td>Slight deviation from planarity towards flagpole boat</td>
<td></td>
<td>15</td>
</tr>
<tr>
<td><strong>Cyclo</strong> (S-alanyl-S-alanyl)</td>
<td>Bowsprit boat; 26° angle between amide planes</td>
<td>Extended conformation with methyl groups quasi-equatorial</td>
<td>16</td>
</tr>
<tr>
<td><strong>Cyclo</strong> (S-seryl-S-tyrosyl) monohydrate</td>
<td>Bowsprit boat (or twist-boat because of slight twist in amide units)</td>
<td>Tyrosine faces piperazinedione</td>
<td>17</td>
</tr>
<tr>
<td><strong>Cyclo</strong> (R-alanyl-S-alanyl) Form I</td>
<td>Slight deviation from planarity towards chair (quasi-axial methyl groups)</td>
<td></td>
<td>18</td>
</tr>
<tr>
<td><strong>Cyclo</strong> (R-alanyl-S-alanyl) Form II</td>
<td>Slight deviation from planarity towards chair (quasi-equatorial methyl groups); molecular dimensions identical to those in other modifications</td>
<td></td>
<td>19</td>
</tr>
<tr>
<td>2,5-Piperazinedione</td>
<td>Conformation of Piperazinedione Ring</td>
<td>Stabilising Interactions</td>
<td>Reference</td>
</tr>
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</tr>
<tr>
<td>[Cyclo(H-streptolutyl-H-streptolutyl) bisulphate pentahydrate (593A)]</td>
<td>Twist boat</td>
<td>Extended conformation (quasi-20 equatorial 3,6-substituents) separates bulky piperidine rings as far as possible. Network of hydrogen-bonds, formed from each piperazinedione C=O; one to a water molecule, the other to piperazinedione NH. <em>(cf. Chapter 1).</em></td>
<td></td>
</tr>
<tr>
<td>Cyclo(S-seryl-S-seryl)</td>
<td>Flattened twist-boat with quasi-axial CH₂OH groups</td>
<td>Both serine hydroxyl groups 21 are engaged in intermolecular hydrogen bonds <em>(cf. Chapter 3)</em></td>
<td></td>
</tr>
<tr>
<td>Cyclo(glycyl-glycyl)</td>
<td>Planar</td>
<td></td>
<td>22</td>
</tr>
<tr>
<td>Cyclo(S-seryl-S-histidyl)</td>
<td>Nearly planar</td>
<td>Histidyl ring faces piperazine-23 dione ring; intermolecular hydrogen bonds with water molecules in crystal</td>
<td></td>
</tr>
<tr>
<td>Cyclo(S-methionylglycine)</td>
<td>Nearly planar with the side-chain folded onto the ring</td>
<td>Alternating layers of dipeptide 24 molecules and water molecules of crystallisation</td>
<td></td>
</tr>
<tr>
<td>Cyclo(S-asparagyl-S-asparagyl)</td>
<td>Bowsprit boat; 18° angle between amide planes</td>
<td>No intramolecular hydrogen bonds; intermolecular hydrogen bonds between each piperazinedione C=O and carbamoyl NH₂ (each H of which forms H-bonds to different piperazinediones)</td>
<td></td>
</tr>
<tr>
<td>2,5-Piperazinedione</td>
<td>Conformation of Piperazinedione Ring</td>
<td>Stabilising Interactions</td>
<td>Reference</td>
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<td>-----------</td>
</tr>
<tr>
<td>N,N'Dimethyl-cyclo(glycyl-glycyl)</td>
<td>Chair</td>
<td></td>
<td>25</td>
</tr>
</tbody>
</table>
showed no H-N-C$_{\alpha}$-H coupling, and there was no change in the C$_{\alpha}$-H splitting pattern after adding D$_2$O to the solution (Fig. 4.2.3). This suggests that (12) does not have a twist boat or flagpole boat conformation. A molecular model of (12) shows that several intramolecular hydrogen bonding interactions are possible, depending on the conformation. For example, the folded flagpole boat conformation could be stabilised by intramolecular hydrogen bonding between the side-chain amide NH groups and the ring amide CO groups (Fig. 4.5.3a). The bowsprit boat conformation could be stabilised by intramolecular hydrogen bonding between the ring amide NH groups and the side-chain amide CO groups (Fig. 4.5.3b). Further permutations are also possible.

However, the crystal structure of cyclo(S-asparagyl-S-asparagyl) [12] shows that there is no intramolecular hydrogen bonding, and the conformation of the pipera-zinedione ring is an extended bowsprit boat (Fig. 4.5.4).
Fig. 4.5.4 *Cyclo*(S-asparagyl-S-asparagyl), view showing the extended bowsprit boat conformation.
The atom numbering scheme is shown in Fig. 4.5.5 and the bond lengths and angles are listed in Table 4.5.2 and are normal for 2,5-piperazinediones. The angle between the amide planes C(3)-C(4)-N(2)-C(5) and C(3)-N(3)-C(6)-C(5) is 18° (cf. 26° in cyclo(S-alanyl-S-alanyl)). The molecule contains an approximate two-fold axis, ignoring the differing dihedral angles about C(1)-C(2) [58°] and C(7)-C(8) [9°]. The C(1)-C(2)-C(3)-N(3) dihedral angle is -54° and that for C(8)-C(7)-C(5)-N(2) is -68°.

The crystal packing is dominated by intermolecular hydrogen bonds (Fig. 4.5.6) between N(1) and O(2a), O(2b); N(4) and O(3c), and also N(4) and O(3d) but this is very long (322 nm). The difference between the bond lengths of the C(sp²)-N (av. 134 nm) and the C(sp³)-N (av. 147 nm) is a notable feature in the structure of 2,5-piperazinediones in general.

An unusual property of the crystals of cyclo(S-asparagyl-S-asparagyl) is their high density (1.55 g cm⁻³). This is probably due to the high proportion of nitrogen and oxygen atoms since the crystals do not show a very tightly bound structure.

4.6 ATTEMPTED HOFMANN REARRANGEMENT ON Cyclo(S-ASPARAGYL-S-ASPARAGYL)

The decarboxylative rearrangement of carboxylic acid derivatives, to give initially isocyanates, by Hofmann, Curtius, or Schmidt reactions and their various modifications are well-known. More recently
Fig. 4.5.5  *Cyclo*(S-asparagyl-S-asparagyl) and numbering scheme.
Fig. 4.5.6 *Cyclo*(S-asparagyl-S-asparagyl) crystal packing diagram showing the extended intermolecular hydrogen bonding.
Table 4.5.2 Bond lengths (nm) and Angles (°) [with standard deviations in parenthesis]

<table>
<thead>
<tr>
<th>Bond</th>
<th>Length (nm)</th>
<th>Standard Deviation (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N(1)-C(1)</td>
<td>136(2)</td>
<td>119(4)</td>
</tr>
<tr>
<td>C(1)-O(1)</td>
<td>126(2)</td>
<td>119(4)</td>
</tr>
<tr>
<td>C(1)-C(2)</td>
<td>151(2)</td>
<td>121(1)</td>
</tr>
<tr>
<td>C(2)-C(3)</td>
<td>153(2)</td>
<td>114(1)</td>
</tr>
<tr>
<td>C(3)-C(4)</td>
<td>151(2)</td>
<td>109(1)</td>
</tr>
<tr>
<td>C(3)-N(3)</td>
<td>146(1)</td>
<td>113(1)</td>
</tr>
<tr>
<td>C(4)-N(2)</td>
<td>132(2)</td>
<td>115(1)</td>
</tr>
<tr>
<td>C(4)-O(2)</td>
<td>126(2)</td>
<td>118(1)</td>
</tr>
<tr>
<td>N(2)-C(5)</td>
<td>148(2)</td>
<td>120(1)</td>
</tr>
<tr>
<td>C(5)-C(6)</td>
<td>153(2)</td>
<td>111(1)</td>
</tr>
<tr>
<td>C(6)-O(3)</td>
<td>125(1)</td>
<td>113(1)</td>
</tr>
<tr>
<td>C(7)-C(8)</td>
<td>150(2)</td>
<td>120(1)</td>
</tr>
<tr>
<td>C(8)-N(4)</td>
<td>137(2)</td>
<td>118(1)</td>
</tr>
<tr>
<td>C(8)-O(4)</td>
<td>123(2)</td>
<td>120(1)</td>
</tr>
<tr>
<td>N(1)-H(101)</td>
<td>90(10)</td>
<td>121(1)</td>
</tr>
<tr>
<td>C(1)-O(2a)</td>
<td>118(11)</td>
<td>126(1)</td>
</tr>
<tr>
<td>C(3)-N(3)</td>
<td>132(2)</td>
<td>115(1)</td>
</tr>
<tr>
<td>C(4)-O(2)</td>
<td>126(2)</td>
<td>118(1)</td>
</tr>
<tr>
<td>N(2)-C(5)</td>
<td>148(2)</td>
<td>120(1)</td>
</tr>
<tr>
<td>C(5)-C(6)</td>
<td>153(2)</td>
<td>111(1)</td>
</tr>
<tr>
<td>C(6)-O(3)</td>
<td>125(1)</td>
<td>113(1)</td>
</tr>
<tr>
<td>C(7)-C(8)</td>
<td>150(2)</td>
<td>120(1)</td>
</tr>
<tr>
<td>C(8)-N(4)</td>
<td>137(2)</td>
<td>118(1)</td>
</tr>
<tr>
<td>C(8)-O(4)</td>
<td>123(2)</td>
<td>120(1)</td>
</tr>
<tr>
<td>N(1)-H(101)</td>
<td>90(10)</td>
<td>121(1)</td>
</tr>
<tr>
<td>N(1)-H(102)</td>
<td>118(11)</td>
<td>126(1)</td>
</tr>
<tr>
<td>N(4)-H(401)</td>
<td>78(10)</td>
<td>126(1)</td>
</tr>
<tr>
<td>N(1)-O(2a)</td>
<td>286(1)</td>
<td>138.1(4)</td>
</tr>
<tr>
<td>N(1)-O(2b)</td>
<td>303(2)</td>
<td>138.1(4)</td>
</tr>
<tr>
<td>N(4)-O(3c)</td>
<td>294(2)</td>
<td>138.1(4)</td>
</tr>
<tr>
<td>N(4)-O(3d)</td>
<td>322(1)</td>
<td>138.1(4)</td>
</tr>
</tbody>
</table>
lead tetraacetate has been used successfully to carry out a Hofmann type rearrangement, but the method suffers from the disadvantage of the need to trap and isolate the intermediate isocyanate as the carbamate (by using an alcohol, e.g. t-butanol as the solvent) before conversion to the required amine. A Hofmann-type rearrangement of an amide using an arylidion(III) dicarboxylate reagent was made recently. A kinetic study was carried out using several different aromatic amides and bis(acetoxy)-iodobenzene. A mixture of amine and urea (arising from addition of the amine to the intermediate isocyanate) often resulted from this reaction. When bis(trifluoro-acetoxy)iodobenzene is used instead of bis(acetoxy)-iodobenzene an efficient rearrangement of the amide directly to the amine can be achieved. The release of trifluoroacetic acid in this modification is believed both to catalyse the hydrolysis of the intermediate isocyanate in situ, and also to protonate the product amine thereby preventing formation of the urea by-product. Only primary amides are suitable for use in this reaction; secondary and tertiary amides do not react. Thus (Δ3) appeared to be an ideal reagent for achieving a Hofmann rearrangement of cyclo(S-asparagyl-S-asparagyl) because the secondary amide functions of the piperazinedione should be inert. A mechanism for the reaction is proposed for (Δ3), and has been adopted for (Δ3) (Scheme 4.6.1). The mechanism involves either the formation of an iodine(III)-amide complex or which rearranges, probably in a concerted manner, to the isocyanate. Hydrolysis of
Scheme 4.6.1 Mechanism for the Hofmann rearrangement using bis(trifluoroacetoxyl)iodobenzene (23).
the isocyanate gives the amine.

The procedure\(^1\) for the rearrangement of N-protected-\((S)\)-asparagine allows a number of solvent systems to be used, the most efficient being DMF/water (1:1 v/v) with a two-fold molar excess of pyridine relative to the amount of amide. The reported conditions\(^1\) were repeated exactly with cyclo\((S\)-asparagyl-\(S\)-asparagyl\) \(^{12}\) in an attempt to convert it to cyclo\((S\)-aminoalanyl-\(S\)-aminoalanyl\) \(^{2}\); however, only unchanged starting material \(^{12}\) was isolated. The other recommended solvent systems, i.e. acetonitrile/water and dioxan/water were also investigated but produced the same result. To verify the method \(n\)-butyramide was used as a test substrate. Rearrangement to \(n\)-propylamine did occur, but was only about 40\% complete after the prescribed reaction time. A series of experiments with \(^{12}\) and \(\text{bis}((\text{trifluoroacetoxy})\text{iodo}-\text{benzene}) \(^{23}\) , employing a range of conditions, was then investigated; these are tabulated below (Table 4.6.1).

Only unchanged starting compound \(^{12}\) was isolated from these experiments. The following explanation is proposed: the intermolecular hydrogen bonding, found in the crystal structure of \(^{12}\) and also apparent from the \(^{1}\)\(^{1}\)\(\text{H}\) n.m.r. spectrum in \(^{2}\)\(\text{H}_2\) dimethylsulphoxide solvent, reduces the reactivity of the amide functions, perhaps in a similar manner to the reduction of reactivity of the hydroxyl functions in cyclo\((S\)-seryl-\(S\)-seryl\) \(^{1}\) (and was discussed in Chapter 3). The effect of this intermolecular bonding may decrease the nucleophilicity of the amide nitrogen lone pair, or the amide carbonyl
<table>
<thead>
<tr>
<th>Solvent</th>
<th>Temp./°C</th>
<th>Time/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMF/H₂O/pyridine</td>
<td>20</td>
<td>3½*</td>
</tr>
<tr>
<td>DMF/H₂O/pyridine</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>DMF/H₂O</td>
<td>20</td>
<td>48</td>
</tr>
<tr>
<td>MeCN/H₂O/pyridine</td>
<td>20</td>
<td>2*</td>
</tr>
<tr>
<td>MeCN/H₂O/pyridine</td>
<td>20</td>
<td>4 days</td>
</tr>
<tr>
<td>MeCN/H₂O/pyridine</td>
<td>50</td>
<td>12</td>
</tr>
<tr>
<td>MeCN/H₂O/pyridine</td>
<td>60</td>
<td>4½</td>
</tr>
<tr>
<td>MeCN/H₂O/pyridine</td>
<td>60</td>
<td>18</td>
</tr>
<tr>
<td>MeCN/H₂O/pyridine</td>
<td>80</td>
<td>2</td>
</tr>
<tr>
<td>Dioxan/water</td>
<td>20</td>
<td>24*</td>
</tr>
</tbody>
</table>

*Literature¹ recommended conditions.

Table 4.6.1 Conditions employed in attempts to react (1₂) with (2₃).

Oxygen (depending on whether (2₄) or (2₅) is an intermediate. However, it must then be noted that these effects would not be expected to prevail at the high reaction temperatures tried. On the other hand, \textit{bis}(trifluoroacetoxy)iodobenzene (2₃) is known to lack thermal stability, particularly in basic aqueous media²⁹.

Some effort was made to repeat the reaction of (2₃) with N-protected-(S)-asparagine (1₅) to obtain a 2,3-diaminopropionic acid derivative, but the reported conditions¹ were not successful in the author's hands and did not seem satisfactory. However, an alternative route to derivatives of (S)-2,3-diaminopropionic acid...
was available as described in Chapter 5.

4.7 THE CRYSTAL STRUCTURE OF Bis(TRIFLUOROACETOXY)IODOBENZENE

The chemistry of aryliodine(III)dicarboxylates [ArI(OCOX)₂] is diverse; one of the keenest areas of interest has been the crystal structures of these compounds. A preparation of good crystals of bis(trifluoroacetoxy)iodobenzene (2₃) had so far eluded crystallographers, and so when the author prepared (2₃) for use as a reagent in a Hofmann type rearrangement reaction, the opportunity arose to solve a structure of some interest.

Two distinctly different crystal structures occur for aryliodine(III)dicarboxylates. Both structures have the common slightly distorted T-shaped geometry typical of the dsp³ hybridised trivalent iodine (Fig. 4.7.1), in which the I-C antibonding orbital overlaps with the lone pairs from two oxygen atoms.

![Fig. 4.7.1 The pentagonal plane around iodine(III).](image)

The two different crystal structures are illustrated by bis(acetoxy)iodobenzene (2₂) [Fig. 4.7.2] and bis(1,1-dichloroacetoxy)iodobenzene (2₆) [Fig. 4.7.3]. In (2₂) there are two I-O covalent bonds (of about the
Fig. 4.7.2 Bis(acetoxy)iodobenzene

Fig. 4.7.3 Bis(1,1-dichloroacetoxy)iodobenzene
same length) and two secondary intramolecular I--O bonds (also about the same length) forming a four-membered IOCO ring. However, in (26) the two covalent I-O bond lengths differ significantly. One of the secondary I--O bonds is intramolecular, whereas the other is intermolecular forming an I₂O₂ ring. Thus, the molecular structure is dimeric.

\[
\begin{align*}
\text{PhI(OCCF}_3\text{)}_2 & \quad \text{PhI(OCOCHCl}_2\text{)}_2 & \quad \text{PhI(OCOCH}_3\text{)}_2 \\
(23) & \quad (26) & \quad (22)
\end{align*}
\]

Both (22) and (26) have three strong bonds about iodine [I-C, I-O(1), I-O(3)] and two weak bonds about iodine [I-O(4), I-O(2)].

**Bis(trifluoroacetoxy)iodobenzene (23)** also adopts the primary T-shaped geometry of iodine(III). When the secondary bonding is considered, it is apparent that the structure of (23) more closely resembles that of (26) rather than (22), and it is also dimeric (Fig. 4.7.4). However, there are three secondary I--O bonds in *bis(trifluoroacetoxy)iodobenzene (23)* giving rise to a very irregular six co-ordinate geometry about the iodine (this is clear in Fig. 4.7.5). Unlike (22) or (26) then, there are three strong bonds about iodine [I-C, I-O(1), I-O(3)] and three weak bonds about iodine (I--O(4), I--O(2), I--O(5)). The iodine-oxygen bond lengths for (22), (23) and (26) are compared below.
Fig. 4.7.4 *Bis*(trifluoroacetoxy)iodobenzene view showing the dimeric structure, the I$_2$O$_4$ ring is indicated (---).
Fig. 4.7.5 Bis(trifluoroacetoxy)iodobenzene view showing the six co-ordinate geometry about the iodine.
Table 4.7.2  Bond lengths (nm) and bond angles (°)
for *bis*(trifluoroacetoxy)iodobenzene
(23) [with standard deviations in parentheses)

<table>
<thead>
<tr>
<th>Bond</th>
<th>Length (nm)</th>
</tr>
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<tbody>
<tr>
<td>I-O(3)</td>
<td>218.6(3)</td>
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<td>I-O(1)</td>
<td>213.8(5)</td>
</tr>
<tr>
<td>I-C(5)</td>
<td>207.4(4)</td>
</tr>
<tr>
<td>I-O(4)</td>
<td>300.0(4)</td>
</tr>
<tr>
<td>I-O(5)</td>
<td>313.3(7)</td>
</tr>
<tr>
<td>I-O(2)</td>
<td>303.8(3)</td>
</tr>
<tr>
<td>O(3)-C(1)</td>
<td>127.7(5)</td>
</tr>
<tr>
<td>O(4)-C(1)</td>
<td>119.2(5)</td>
</tr>
<tr>
<td>C(1)-C(2)</td>
<td>154.9(6)</td>
</tr>
<tr>
<td>C(2)-F(2,1)</td>
<td>130.0(7)</td>
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<tr>
<td>C(2)-F(2,2)</td>
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</tr>
<tr>
<td>C(2)-F(2,3)</td>
<td>130.9(7)</td>
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<tr>
<td>O(1)-C(3)</td>
<td>126.5(8)</td>
</tr>
<tr>
<td>O(5)-C(3)</td>
<td>115.9(13)</td>
</tr>
<tr>
<td>C(3)-C(4)</td>
<td>153.8(12)</td>
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<tr>
<td>C(4)-F(4,1)</td>
<td>130.2(13)</td>
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<tr>
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<td>C(4)-F(4,3)</td>
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Table 4.7.2 (Cont.)

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<th>Bond</th>
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<td>O(1)-I-O(5)</td>
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</tr>
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<td>C(5)-I-O(4)</td>
<td>129.8(1)</td>
</tr>
<tr>
<td>C(5)-I-O(5)</td>
<td>86.0(3)</td>
</tr>
<tr>
<td>O(3)-I-O(5)</td>
<td>138.1(2)</td>
</tr>
<tr>
<td>O(1)-I-O(4)</td>
<td>143.4(1)</td>
</tr>
<tr>
<td>O(4)-I-O(5)</td>
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</tr>
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<td>O(2)-I-O(3)</td>
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<td>O(2)-I-O(5)</td>
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<td>C(6)-C(7)-C(8)</td>
<td>119.9(5)</td>
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<td>C(7)-C(8)-C(9)</td>
<td>120.6(5)</td>
</tr>
<tr>
<td>C(8)-C(9)-C(10)</td>
<td>120.5(4)</td>
</tr>
<tr>
<td>C(9)-C(10)-C(5)</td>
<td>119.2(4)</td>
</tr>
<tr>
<td>I-O(3)-C(1)</td>
<td>110.6(2)</td>
</tr>
<tr>
<td>I-O(1)-C(3)</td>
<td>117.9(5)</td>
</tr>
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</table>
\[ X = \begin{array}{ccc}
\text{I-O(1)} & \text{CF}_3 & 213.8 \quad \text{Cl}_2\text{CH} & 213.6 \quad \text{CH}_3 \quad 215.9 \\
\text{I-O(3)} & 218.6 & 216.3 & 215.3 \\
\text{I-C} & 207.4 & 208.3 & 209.0 \\
\text{I-\text{---O(4)}} & 300.0 & 293.6 & 285.0 \\
\text{I-\text{---O(2)}} & 303.8 & 304.9 & 281.0 \\
\text{I-\text{---O(5)}} & 313.3 & - & - \\
\end{array} \]

Table 4.7.1 Comparison of bond lengths (nm) for compounds of the type Phi(OCOX)\(_2\).

4.8 EXPERIMENTAL

4.8.1 (S)-N\(^\alpha\)-Benzyloxycarbonylasparagine Methyl Ester (1\(\_\)\(\_\)\(\_\)\(\_\)\)

(S)-N\(^\alpha\)-Benzyloxycarbonylasparagine (15) was prepared in 94\% yield from (S)-asparagine as described by Boissonas et al.\(^6\): m.p. 163-164\(^\circ\)C (lit.\(^6\) m.p. 163\(^\circ\)C), [\(\alpha\)]\(_D\)\(^20\) 7.5\(^\circ\) (c. 1.5 in AcOH) [lit.\(^6\) [\(\alpha\)]\(_D\) 7.6\(^\circ\)], \(^1\)H n.m.r. (1:1 \([\_\text{H}_6]\)Me\(_2\)SO: \(\text{D}_2\)O); \(\delta\) 7.4 (m, PhH, 5H), 5.12 (s, PhCH\(_2\), 2H), 4.58 (t, CH, 1H), 2.83 (d, CH\(_2\), 2H) p.p.m.

A stirred suspension of this compound (5.00 g, 0.18 mmol) in dry methanol (50 cm\(^3\)) was cooled to -60\(^\circ\)C. Acetyl chloride (7.0 cm\(^3\), 0.1 mol) was added dropwise whilst maintaining the reaction temperature at -60\(^\circ\)C. The temperature was then maintained at -15\(^\circ\)C for 24 hours. The solvent was removed at 0\(^\circ\)C in \(\text{vacuo}\), the residual solid was washed with diethyl ether and dried to give white
crystals of (S)-N\(^\alpha\)-benzyloxycarbonylasparagine methyl ester (\(\mathbf{1}\)) [5.18 g, 98% yield] m.p. 153-153.5\(^\circ\)C (lit. \(^\circ\)m.p. 150\(^\circ\)C), [\(\alpha\)]\(\text{D}\)\(^{20}\) - 2.9\(^\circ\) (c. 4.0 in AcOH) [lit. \(^\circ\)[\(\alpha\)]\(\text{D}\) - 2.0\(^\circ\)], t.l.c. (silica gel F\(_{254}\), 5% v/v MeOH in CH\(_2\)Cl\(_2\)) one spot at R\(_f\) 0.6, \(^1\)H n.m.r. ([\(\text{H}_6\)]Me\(_2\)SO); \(\delta\) 7.3 (m, PhH, 5H), 5.08 (s, PhCH\(_2\), 2H), 4.58 (t, CH, 1H), 3.67 (s, OCH\(_3\), 3H), 2.7-3.0 (m, CH\(_2\), 2H) p.p.m.

4.8.2 \(\text{Cyelo}(S\text{-asparagyl-S-asparagyl})\) [\(\mathbf{12}\)] from (\(\mathbf{1}\))

(S)-N\(^\alpha\)-Benzyloxycarbonylasparagine methyl ester (\(\mathbf{1}\)) [5.00 g, 0.017 mol] in methanol (30 cm\(^3\)) and water (5 cm\(^3\)) containing 10% Pd/C catalyst (0.12 g) was hydrogenated in a Parr apparatus (initial hydrogen pressure 20 p.s.i.) for 4 hours. The mixture was filtered through Celite and the solvent was removed from the filtrate to give (S)-asparagine methyl ester as a yellow oil (2.0 g, 76% yield), \(^1\)H n.m.r. (D\(_2\)O); \(\delta\) 3.88 (t, CH, 1H), 3.75 (s, OCH\(_3\), 3H), 2.72 (d, CH\(_2\), 2H) p.p.m. e.i.m.s. m/z 147 (M\(^+\) + 1, 100%), 115 (25), 102 (6), 87 (42), 43 (15), i.r. (Neat); 3350, 3190, 1720, 1670, 1440 cm\(^{-1}\). Storing this ester for at least 48 hours at room temperature gave a crystalline mass. This was recrystallised from water to give \(\text{Cyelo}(S\text{-asparagyl-S-asparagyl})\) [\(\mathbf{12}\)] as white platelets (1.0 g, 64% yield) which did not melt below 280\(^\circ\)C [\(\alpha\)]\(\text{D}\)\(^{20}\) - 26.6\(^\circ\) (c. 1.2 in H\(_2\)O), \(^1\)H n.m.r. ([\(\text{H}_6\)]Me\(_2\)SO); \(\delta\) 7.80 (br, 2 x NH, 1H, exchanges most rapidly on adding D\(_2\)O), 7.42 (br, 2 x NH, 2H), 6.93 (br, 2 x NH, 2H), 4.16 (t, 2 x CH, 2H), 2.55 (m, 2 x CH\(_2\), 4H) p.p.m.; (D\(_2\)O); \(\delta\) 4.47 (t, CH, 2H), 2.87 (m, 2 x CH\(_2\), 4H) p.p.m., \(^1\)C n.m.r. (D\(_2\)O);
\[ \delta 174.76 \text{ (2 x ring CO)}, 169.76 \text{ (2 x CONH}_2), 52.32 \text{ (2 x CH)}, 38.99 \text{ (2 x CH)} \text{ p.p.m. [cf. } \delta 169.11 \text{ (2 x CO)}, 63.63 \text{ (2 x CH)} \text{ p.p.m. for cyclo(S-seryl-S-seryl) [1]; assignments for CH and CH} _2 \text{ were confirmed by off-resonance decoupling}, \text{ e.i.m.s. m/z 228 (M}^+\text{, 42%)}, 211 \text{ (76), 183 (53), 166 (28), 153 (16), 138 (29), i.r. (Nujol); 3390, 3240, 3120, 1660, 1630, 1300 \text{ cm}^{-1}. \text{ Found C, 42.0; H, 5.25; N, 24.2. C}_8 \text{H}_{12}N_4O_4 \text{ requires C, 42.1; H, 5.25; N, 24.5%. A crystal from this sample was used for the crystallographic determination.}

4.8.3 \text{(S)-3-N-Benzylxycarbonylaminopyrrolidine-2,5-dione (21)}

\text{To a suspension of (S)-N}^\alpha \text{-benzylxycarbonyl-asparagine methyl ester [16] (2.14 g, 7.6 mmol) in water (10 cm}^3\text{) was added 0.5 mol dm}^{-3} \text{ sodium hydroxide solution (15 cm}^3\text{, 7.5 mmol) and the mixture was stirred for 15 minutes. The solution was filtered and the filtrate was acidified with 1 mol dm}^{-3} \text{ aqueous hydrochloric acid. After cooling the mixture for 30 minutes the resulting precipitate was filtered off, washed with ice-cold water and recrystallised from ethyl acetate-petrol (b.p. 40-60°C) to give the title compound (1.40 g, 74% yield) m.p. 80-81°C (lit.}^{10} \text{ m.p. 78-81°C), [\alpha]D^{20} = 42.8^\circ \text{ (c. 3.6 in MeOH) [lit.}^{10} \text{ [\alpha]D = 43^\circ}, 1H \text{n.m.r. (CDCl}_3\text{); } \delta \text{ 9.45 (br, imide HN, 1H), 7.3 (m, PhH, 5H), 6.12 (br, NH, 1H), 5.05 (s, PhCH}_2\text{, 2H), 4.35 (dd, CH, 1H), 2.6-3.05 (m, CH}_2\text{, 2H) p.p.m., i.r. (Nujol); 3430, 3350, 1700, 1640, 1530 \text{ cm}^{-1}.} \]
4.8.4  

(S)-3-Aminopyrrolidine-2,5-dione (20)

(S)-N-3-Benzylxycarbonylaminopyrrolidine-2,5-dione (21) [0.50 g, 2.0 mmol] in methanol (10 cm³) containing 10% Pd/C catalyst (0.15 g) was hydrogenated (Parr, initial hydrogen pressure 10 p.s.i.) for 25 hours. The resulting mixture was filtered through Celite and the filtrate was evaporated to give a white solid. This was recrystallised from aqueous methanol to give

(S)-3-aminopyrrolidine-2,5-dione (0.2 g, 87% yield), m.p. 143-144°C (dec.) [lit.⁠1⁠ m.p. 144°C (dec)], [α]D⁡2⁰  - 77° (c. 2.5 in MeOH) [lit.⁠1⁠ [α]D  - 77°],

1H n.m.r. (D₂O); δ 3.97 (dd, J_vic 5.5 and 10 Hz, H-3, 1H), 3.09 (dd, J_vic 10 and J_gem 18.5 Hz, H-4, 1H), 2.54 (dd, J_vic 5.5 and J_gem 18.5 Hz, H-4, 1H) p.p.m., i.r. (Nujol); 2230, 3280, 1715, 1610, 1200 cm⁻¹.

4.8.5  

Cyclo(S-asparagyl-S-asparagyl) [12] from (20)

(S)-3-Aminopyrrolidine-2,5-dione (20) [0.15 g, 1.3 mmol] was dissolved in dry acetonitrile (20 cm³) and boiled under reflux for seven days. The precipitated solid was filtered off and recrystallised from water to give cyclo(S-asparagyl-S-asparagyl) [12] (0.06 g, 40% yield) 1H n.m.r. (D₂O) and [α]D (in H₂O) were identical to data for the piperazinedione obtained as described in Section 4.8.2 above.

4.8.6  

Fischer's Preparation of Cyclo(asparagyl-asparagyl) [excess of 35,6S isomer]

4.8.6(i)  

(S)-Dimethylaspartate Hydrochloride (14)

(S)-Asparagine (13) [4.00 g, 0.03 mol] was
suspended in dry methanol (15 cm³) and the solution was saturated with dry hydrogen chloride. After refluxing for 3 hours a precipitate of ammonium chloride had appeared. This was removed and the filtrate was evaporated. The residue was redissolved in dry methanol (15 cm³) and again saturated with dry hydrogen chloride. After refluxing for 16 hours, the solvent was removed to afford the diester (14) as a colourless oil (5.5 g, 92% yield), ¹H n.m.r. (CDCl³); δ 3.82 (m, CH, 1H), 3.75 (s, OCH₃, 3H), 3.70 (s, OCH₃, 3H), 2.75 (m, CH₂, 2H), 1.84 (br, NH₃, 3H) p.p.m.

4.8.6(ii) Dimethyl Ester of Cyclo(aspartyl-aspartyl) (S)-Dimethylaspartate hydrochloride (17)

[3.40 g, 0.017 mol] was dissolved in dilute aqueous potassium carbonate solution and the free amino ester was extracted into diethyl ether. The organic layer was dried and evaporated. The amino ester was then heated in a sealed glass tube, at 100°C for 3 days to give brown crystals which were purified by washing with methanol, dilute hydrochloric acid and then methanol to give the title compound (17) [0.5 g, 22% yield] m.p. 242-245°C (dec.) [lit.³ m.p. 145°C (dec.)], [α]D²⁰ + 6.2° (c. 0.97 in 1:1 v/v H₂O/MeOH), ¹H n.m.r. (D₂O); δ 4.52 (t, CH, 1H), 3.73 (s, OCH₃, 3H), 3.00 (m, CH₂, 2H) p.p.m., i.r. (Nujol); 3190, 1735, 1670 cm⁻¹.
4.8.6(iii) **Cyclo**(asparagyl-asparagyl)

Compound (1/7) [1.10 g, 4 mmol] was dissolved in liquid ammonia (2 cm$^3$) and the solution was sealed in a glass tube. After 48 hours at room temperature the tube was cooled and opened and the ammonia allowed to evaporate. The residue was recrystallised from water to give the piperazinedione (0.6 g, 61% yield), [α]$_D^{20}$ - 20° (c. 1.2 in H$_2$O) [second preparation gave material of [α]$_D^{20}$ - 5.3°].

4.8.7 **Cyclo**(R-asparagyl-S-asparagyl)

Dry ammonia gas (5.50 g, 0.32 mol) and diethyl fumarate (11.0 g, 0.06 mol) was dissolved in ethanol (10 cm$^3$). The resulting solution was heated in a sealed glass tube at 100°C for 24 hours. After cooling, the liquor was decanted from the solid crust formed, and evaporated to give a solid which was combined with the solid crust in the vessel and recrystallised from water to give the title compound (4.4 g, 64% yield) which did not melt below 290°C, $^1$H n.m.r. ([$^2$H$_6$]Me$_2$SO); δ 7.84 (br, 2 x NH, 2H), 7.41 (br, 2 x NH, 2H), 6.94 (br, 2 x NH, 2H), 4.10 (t, 2 x CH, 2H), 2.57 (m, 2 x CH$_2$, 4H) p.p.m., e.i.m.s. m/z 228 (M$^+$, 45%), 211 (29), 183 (37), 166 (11), 138 (11), 104 (100), i.r. (Nujol); 3380, 3260, 3190, 1670, 1600, 1335, 1250 cm$^{-1}$.

4.8.8 Reactions of (S)-3-Aminopyrrolidine-2,5-dione (**20** in Water

Samples of (20) [each 0.015 g, 0.13 mmol] were dissolved in 0.5 mol dm$^{-3}$ D$_2$O/phosphate buffers (each
0.5 cm$^3$) with pD values of 5.61, 6.43, 7.05, and 7.95 respectively, and the solutions incubated at 22°C. After approximately 10 hours some precipitation appeared in all of the tubes. After 42 hours the starting compound (2$^0$) was absent from the $^1$H n.m.r. spectra of the solutions. The only products were (S)-asparagine and cyclo(S-asparagyl-S-asparagyl) [the precipitate]. A sample of (2$^0$) [0.15 g, 1.3 mmol] was dissolved in 0.5 mol dm$^{-3}$ H$_2$O/phosphate buffer (3.5 cm$^3$) of pH 7.07 and the solution maintained at 22°C for 48 hours. The mixture was concentrated to about one-third the initial volume, filtered, and the solid washed with water (1 cm$^3$) and dried (P$_4$O$_{10}$, in vacuo) to give (12) 0.015 g, 10% yield. These structural assignments were made by addition of authentic samples to the $^1$H n.m.r. solution.

Samples of compound (2$^0$) [each 0.015 g, 0.13 mmol] were dissolved in 1 mol dm$^{-3}$ DCl (0.5 cm$^3$), pD = 0.97 and in 1 mol dm$^{-3}$ NaOD (0.5 cm$^3$), pD 13.5 and examined by $^1$H n.m.r. spectroscopy. At pD 13.5 hydrolysis to asparagine occurred within two minutes at room temperature. At pD = 0.97 no reaction was evident after 5 days at room temperature. The solution was heated at 80°C, this hydrolysed (2$^0$) completely in 38 hours. The product in both cases was aspartic acid, this was confirmed by addition of authentic aspartic acid to the n.m.r. tubes.

4.8.9 Crystal Data Collection for (12)

C$_8$H$_{12}$N$_4$O$_4$, orthorhombic space group $P2_12_12_1$
Data were collected with a Syntex P21 four circle diffractometer. Maximum 2θ was 50° with scan range ± 1.15° (2θ) around the \( K_a^1-K_a^2 \) angles, scan speed 0.7-29° min\(^{-1}\), depending on the intensity of a 2 sec pre-scan; backgrounds were measured at each end of the scan for 0.25 of the scan time. Three standard reflections were monitored every 100 reflections, and showed only statistical changes during data collection. Unit cell dimensions and standard deviations were obtained by least-squares fit to 15 reflections. 641 observed reflections (\( I/σ(I) > 3.0 \)) (1083 total) were used in refinement, and corrected for Lorentz, and polarisation effects. No absorption correction was made. Systematic absences \( h00, h\neq2n, ok0, k\neq2n, o0l \). \( l\neq2n \) indicate space group \( P2_1^12_1^2 \).

The structure was solved without difficulty using MULTAN-80\(^{33} \). After refinement, hydrogen atoms attached to carbon, N(2) and N(3) were inserted at calculated positions (but not refined). Three of the four attached to N(1) and N(4) could be seen on a difference Fourier synthesis and were inserted and refined (with fixed temperature factors); the unlocated hydrogen atom is that involved in the weakest H-bond.

Final refinement was by full matrix least squares methods, in large blocks. The absolute configuration of the molecule as refined was shown to be correct by comparison
with the known molecular configuration. Unit weights were used and shown to be satisfactory by a weight analysis. The final R-value was 0.075. Computing was with the X-ray 76 system\textsuperscript{34}, on a Burroughs B6700 computer. Scattering factors in the analytical form and anomalous dispersion factors were taken from reference 35.

4.8.10 \textit{Bis(Trifluoroacetoxy)iodobenzene} (23)

40\% Peracetic acid (31 cm\textsuperscript{3}, 0.24 mol) was added dropwise to stirred iodobenzene (20.4 g, 0.1 mol) at 29-31°C over 45 minutes. The solution was then stirred for a further 20 minutes before cooling to 0°C. After about 1 hour a precipitate was filtered from the solution and washed with ice-water and dried (P\textsubscript{4}O\textsubscript{10}, in vacuo) to give \textit{bis(acetoxy)iodobenzene} (22) [28.6 g, 89\% yield] m.p. 160-161°C (lit.\textsuperscript{32} m.p. 159-160°C), \textit{^1H} n.m.r. (CDCl\textsubscript{3}); δ 8.10 (d, 2 x PhH\textsubscript{H}, 2H), 7.50 (m, 3 x PhH\textsubscript{H}, 3H), 2.00 (s, 2 x CH\textsubscript{3}, 6H).

This was then dissolved in 100\% trifluoroacetic acid (50 cm\textsuperscript{3}) at 50-55°C and the solution allowed to cool slowly. The precipitated crystals were filtered and washed with cold 100\% trifluoroacetic acid (20 cm\textsuperscript{3}) and then dried (P\textsubscript{4}O\textsubscript{10}, in vacuo) to give the title compound (33) [30.0 g, 79\% yield] m.p. 119-122°C (dec.) [lit.\textsuperscript{28} m.p. 124-126°C (dec.)], \textit{^1H} n.m.r. (CDCl\textsubscript{3}); δ 8.22 (d, 2 x PhH\textsubscript{H}, 2H), 7.65 (m, 3 x PhH\textsubscript{H}, 3H).

4.8.11 Crystal Data Collection for (23)

C\textsubscript{10}F\textsubscript{6}H\textsubscript{5}IO\textsubscript{4}, Triclinic, space group P\textsubscript{1}
\[ a = 978.7(4) \quad b = 905.5(3) \quad c = 767.4(3) \text{ nm} \]
\[ U = 6700(5) \text{ nm}^3 \quad Z = 2 \]
\[ D_c = 2.13 \text{ g cm}^{-3} \quad D_m = 2.11 \text{ g cm}^{-3} \quad \text{Mo-K}_\alpha \text{ radiation} \]

with graphite monochromator.
\[ \lambda = 71.069 \text{ nm}, \quad \mu (\text{Mo-K}_\alpha) = 24.42 \text{ cm}^{-1}, F(000) = 408 \]

Unit cell dimensions were determined by least squares fit to the positions of 15 reflections using the standard programs of a Syntex P21 four-circle diffractometer. Data were collected with this instrument to \( 2\theta = 50^\circ \) with variable scan rates from 3-29.3° min\(^{-1}\) depending on the intensity of a 2 second prescan; the total background count was half the scan time. The temperature was held at -100°C with the Syntex LT-1 device to prevent decomposition by X-rays. The intensity of three standard reflections measured every 300 reflections showed no significant change. There were no systematic absences and the \( P_1 \) space group was assumed. The position of the iodine atom was located from a Patterson function and the hydrogen atoms found from difference Fourier synthesis. Subsequent cycles of refinement and difference Fourier synthesis revealed that the fluorine atoms were disordered and that two orientations were possible on each \( \text{CF}_3 \) group. The F atoms were given population parameters of 0.8 and 0.2 for the two orientations and refined with fixed temperature factors. Refinement was carried out on 2104 reflections having \( I/\sigma(I) > 3.0 \) (2406 reflections were collected). Refinement converged to \( R = 0.037 \).
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Synthesis of (S)-2,3-Diaminopropionic Acid

5.1 Introduction

5.2 Synthesis of (S)-N-Z-3-Chloroalanine and (S)-N-Z-3-Iodoalanine Methyl Esters

5.3 α,β-Dehydroamino Acids

5.4 The Mitsunobu Reaction

5.5 Synthesis of (S)-2,3-Diaminopropionic Acid

5.6 Nβ-Alkylation of (S)-Nα-Z-2,3-Diaminopropionic Acid

5.7 Amino Protecting Groups

5.8 Experimental

5.8.1 (S)-N-Benzzyloxycarbonylserine

5.8.2 (S)-N-Z-Serine Methyl Ester

5.8.3 (S)-N-Z-3-Chloroalanine Methyl Ester

5.8.4 (S)-N-Z-3-Iodoalanine Methyl Ester

5.8.5 (S)-N-Z-3-Azidoalanine Methyl Ester

5.8.6 (S)-Nα-Z-2,3-Diaminopropionic Acid

5.8.7 (S)-2,3-Diaminopropionic Acid Hydrochloride

5.8.8 (S)-Nα-Z-2,3-Diaminopropionic Acid Methyl Ester Hydrochloride

5.8.9 N-Z-Dehydroalanine Methyl Ester

5.8.10 (S)-Nα-Z-Nβ-(Benzzyloxethyl)-2,3-Diaminopropionic Acid Methyl Ester

5.8.11 (S)-Nα-Z-Nβ-(Benzzyloxethyl)-Nβ-(Trifluoroacetyl)-2,3-Diaminopropionic Acid Methyl Ester

5.8.12 (S)-Nα-Z-Nβ-(Benzzyloxethyl)-Nβ-(Diphenylphosphinyl)-2,3-Diaminopropionic Acid Methyl Ester
5.8.13  (S)-N^α-Z-N^β-(Benzyloxyethyl)-N^β-(Benzyl)-2,3-Diaminopropionic Acid Methyl Ester

5.8.14  (S)-N^α-Z-N^β-Boc-2,3-Diaminopropionic Acid Methyl Ester

5.8.15  (S)-N^β-Boc-2,3-Diaminopropionic Acid Methyl Ester

5.9  References
CHAPTER 5

Synthesis of (S)-2,3-Diaminopropionic Acid

5.1 INTRODUCTION

Some discussion of the difficulties, such as solubility and ready epimerisation, encountered in the chemistry of 2,5-piperazinediones has been given in the previous two chapters. The unsatisfactory results obtained in attempts to cause Hofmann rearrangement of (S)-N\(^\alpha\)-asparagine (14) to a derivative of (S)-2,3-diaminopropionic acid (27) using bis(trifluoroacetox)iodobenzene (23) [Section 4.6], led us to consider an alternative approach to (27).

(S)-2,3-Diaminopropionic acid (27) appeared to be a promising intermediate in the preparation of analogues of 593A since the 3-amino group (with appropriate protecting groups in place in the molecule) could be functionalised before piperazinedione ring formation. This strategy would lead to an analogue of 593A with the dimerisation of the functionalised amino-acid to the piperazinedione as one of the final steps in the synthesis (Scheme 5.1.1).

(S)-2,3-Diaminopropionic acid (27) is an important component of some natural products and of many therapeutically useful compounds. Mokotoff et al.\(^1\) have described the potential value of electrophilic amide analogues of (S)-2,3-diaminopropionic acid (27)
in the inhibition of (S)-asparaginase biosynthesis. Also Fukuyasu et al.\textsuperscript{2} have demonstrated the virucidal activity of derivatives of (27) in humans, land mammals, and fish, Patel et al.\textsuperscript{3} have studied actinomycin-D lactam analogues, containing (S)-2,3-diaminopropionic acid, as potential antibacterial agents. Compound (27) is also a constituent amino-acid of the natural products mimosine\textsuperscript{4} and quisqualic acid\textsuperscript{5} and of some peptide antibiotics including the bleomycins\textsuperscript{6}, edeines\textsuperscript{7}, and tuberactinomycins\textsuperscript{8}. It is also a component of the neurotoxin (S)-N\textsuperscript{B}-oxalyl-2,3-diaminopropionic acid\textsuperscript{9}.

Because of the importance of (S)-2,3-diaminopropionic acid (27), several syntheses have been reported. In addition to the bis(trifluoroacetoxy)-iodobenzene-induced rearrangement discussed earlier, the amino-acid (27) has been prepared by Schmidt\textsuperscript{10} (or Curtius) degradation of aspartic acid, or Hofmann\textsuperscript{11} rearrangement of asparagine-derived precursors. However, these methods mostly require several steps or involve difficult isolation procedures. The approaches to the synthesis of (27) adopted by the author involve novel preparations of the intermediates (31) and (32), and the first preparation of the azide (33) [Scheme 5.1.2]. The syntheses of (31) and (32) both offer some improvement on the literature procedures which are discussed below. The methods of preparing an analogue of 593A from derivatives of (27) are also considered with particular regard to the suitability of amine protecting groups.
Scheme 5.1.1 Generalised route to analogues of 593A from (S)-2,3-diaminopropionic acid derivatives.

Scheme 5.1.2 Routes to (S')-2,3-diaminopropionic acid (27) from (S)-serine (28).
5.2 SYNTHESIS OF (S)-N-Z-3-CHLOROALANINE AND (S)-N-Z-3-IODOALANINE METHYL ESTERS

(S)-N-Benzzyloxycarbonylserine methyl ester [N-Z-Ser OMe] (30) was required as the starting material in each approach to (27). (S)-N-Benzzyloxycarbonylserine (29) was prepared by the simplified Schotten-Baumann method consisting of treating the amino-acid with a sodium bicarbonate solution and adding benzylchloroformate at room temperature. The pH was kept constant by addition of more bicarbonate, instead of by alternate additions of aliquots of the chloroformate and alkali, which is the conventional Schotten-Baumann method. However, it was noted that complications can arise when this method is applied to rac.serine. Furthermore, the product of the reaction depended upon the method used to acidify the reaction mixture during work up. Normally acidification precipitates the product (29) which is then removed and purified. However, when concentrated hydrochloric acid is added dropwise at 10-15°C, the yield of rac.N-Z-serine was reduced and an additional, higher melting, poorly soluble, material was obtained. When the reaction mixture was acidified quickly and without controlling the temperature, good yields (ca. 80%) of rac.N-Z-serine were obtained. When the author prepared (S)-N-Z-serine (29) no insoluble by-product was obtained, although the yield of (29) was variable. The nature of the by-product obtained from rac.serine was investigated. It was
initially thought\(^\text{13}\) that the poorly soluble, high melting substance was a complex consisting of an exactly 1:1 ratio (w/w) mixture of \textit{rac}.benzyloxycarbonylserine and its sodium salt. Other analogous complexes have been reported\(^\text{13}\) but it is unusual to find the components in an exact ratio of 1:1 (w/w). It was speculated that the sodium cation is shared between the (S)- and (R)-isomers, so that an overall symmetry was maintained. However, it was found\(^\text{13}\) that a similar complex could be obtained when (S)-serine was used, which invalidates the proposal that the complex needs a higher degree of symmetry in its crystal packing. The authors\(^\text{13}\) were unable to reach a satisfactory explanation for the stability and insolubility of the complex. Although this specific problem was not encountered in our work with (S)-serine, it may help to explain why, in some instances, the yield of (\text{\textdegree}9) was low.

Compound (\text{\textdegree}9) was converted into the methyl ester (3\text{\textdegree}0) without any difficulty using 2 mol dm\(^{-3}\) hydrogen chloride in methanol at room temperature. In general (S)-N-Z-serine methyl ester (3\text{\textdegree}0) is obtained as a colourless syrup which is suitable for direct use in synthesis, but it can also be obtained as a low melting crystalline solid by pumping the syrup for several days in high vacuum or by N-benzyloxycarbonyl protection of (S)-serine methyl ester\(^\text{14}\) and careful crystallisation from 1% (v/v) diethyl ether in pentane.

(S)-N-Z-3-Chloroalanine methyl ester (3\text{\textdegree}1) has been prepared from (3\text{\textdegree}0) \textit{via} the (S)-N-benzyloxycarbonyl-O-tosylserine methyl ester derivative (3\text{\textdegree}4)\(^\text{15}\). We were able
to obtain (31) directly from (30) by reaction with carbon tetrachloride and triphenylphosphine\(^\text{16}\), followed by chromatographic purification on silica gel. The substance obtained was identical to (31) as prepared by Schwyzer \textit{et al.}\(^\text{15}\) and our method offers the advantages of being shorter and it avoids the risk of racemisation, which is a danger when (S)-serine is esterified before the N-protection step\(^\text{15}\). It was envisaged that (S)-N-Z-3-chloroalanine methyl ester (31) could be converted into an amine derivative by a substitution reaction with a suitable primary amine, followed by conversion into an analogue of 593A by the route generalised in Scheme 5.1.1. However, (31) was very labile towards \(\beta\)-elimination. All of the attempts to convert (31) into an amine derivative led to the \(\beta\)-elimination product N-benzyloxycarbonyl-dehydroalanine methyl ester (35) as the only product; no product of substitution was detected.

\[
\begin{align*}
\begin{array}{c}
\text{NHZH} \\
\text{CO}_2\text{Me}
\end{array}
\end{align*}
\]

(35)

This was surprising because substitution by the \(S_N2\) mechanism is expected to proceed favourably with a primary alkyl halide. Our observations may be compared with a report\(^\text{17}\) in which (S)-3-chloroalanine methyl ester was reacted with cysteine in a strong alkaline solution at 50°C to give a \textit{racemic} thioether derivative. However, Witchek \textit{et al.}\(^\text{18}\) were able to obtain optically active cysteine derivatives of
(S)-N-Z-3-chloroalanylglycyl ethyl ester and other 3-chloroalanyl dipeptide esters by reacting them with various salts of thioacetic acid, thiobenzoic acid and benzylmercaptan. These results are comparable with the reaction of thiols with meso-oyalo(chloroalanyl-chloroalanyl) and the addition of thiols to 3,6-bis-(methylene)-2,5-piperazinedione, discussed in Chapter 3. The application of elimination reactions of 3-chloroalanine derivatives for the preparation of dehydroalanines has been reported. In this case a serine-containing peptide derivative was treated with phosphorus pentachloride to produce a 3-chloroalanyl unit in the peptide. The subsequent elimination reaction was carried out simply by treating the peptide with triethylamine. (S)-N-Z-3-Chloroalanine methyl ester (31) was shown to react in a similar manner. We repeated this elimination reaction to obtain (35) for spectroscopic characterisation and reference purposes. The elimination reactions of amino-acids are discussed more generally in Section 5.3.

The substitution reactions achieved in the 3-chloroalanyl peptide moiety by Wilchek et al. using mercaptans encouraged the author to consider reactions of (31) with the nucleophilic but weakly basic azide anion. If (S)-N-Z-3-azidoalanine methyl ester (33) could be obtained in this fashion from (31) then reduction of the azide function to the amine should be simple to achieve. The role of the solvent in influencing the nucleophilicity of the anion and the rates of the substitution and elimination reactions were also thought to be important.
The marked effect of dipolar aprotic solvents on the rates of nucleophilic substitution reactions is well known. In $S_N^2$ reactions the bond forming process is rate determining and the difference in the energy of the reactants (I.St.) and the transition state (T.St.) is smaller in dipolar aprotic solvents than in protic solvents (Fig. 5.2.1).

![Energy diagram](image)

**Fig. 5.2.1** Effect of protic (p) and dipolar aprotic (a) solvents on the activation energy ($\Delta E$) of $S_N^2$ reactions. --- dipolar aprotic, ——protic solvent.

However, when (31) was treated with sodium azide in benzene $N,N$-dimethylformamide (DMF), or dimethylsulphoxide (DMSO) the only product detected in reactions monitored by $^1H$ n.m.r. spectroscopy, was the product of $\beta$-elimination ($3_5$).

Dipolar aprotic solvents have the negative end of the dipole more fully exposed than protic solvents, and hence are more effective in the solvation of large cations. $n$-Tetrabutylammonium azide is a more effective reagent in substitution reactions with alkyl halides in dipolar aprotic solvents than sodium azide.
When \((3_1)\) was treated with \(n\)-tetrabutylammonium azide in DMF, DMSO or benzene, no trace of \((S)\)-N-Z-3-azidoalanine methyl ester \((3_3)\) was detected; only the \(\beta\)-elimination product \((3_5)\) was obtained.

In an effort to enhance the rate of substitution, \((S)\)-N-Z-3-iodoalanine methyl ester \((3_2)\) was prepared from the chloro compound \((3_1)\) by applying the classical Finkelstein reaction using sodium iodide in acetone. This tactic was frustrated because a similar series of reaction conditions with sodium azide and \(n\)-tetrabutylammonium azide, applied to the iodide \((3_2)\), simply caused elimination to occur approximately sixteen times faster than with \((3_1)\).

Schwyzer and Márki\(^{22}\) have reported the preparation of the iodoalanine derivative \((3_2)\) by a method similar to their synthesis of \((3_1)\) from the \((S)\)-N-Z-0-tosylserine methyl ester\(^{15}\). When the iodo derivative \((3_2)\) was reacted with a salt of dimethylmalonate, rac.-\((3_6)\) was obtained by an elimination-addition sequence.

\[
\begin{align*}
\text{MeO}_2\text{C} & \quad \text{NH}_2 \\
\text{MeO}_2\text{C} & \quad \text{CO}_2\text{Me}
\end{align*}
\]

\((3_6)\)

A reaction in which \((S)\)-N-Z-3-chloroalanine was treated with concentrated aqueous ammonia and heated at 40° C in a pressure bottle for 72 hours to give \((S)\)-N\(^a\)-Z-2,3-diaminopropionic acid \((3_7)\) in low yield has been reported by Moore et al.\(^{23}\). In view of the low yield (ca. 29%) obtained, it may be
supposed that the \((S)\)-isomer was preferentially deposited in the recrystallisation process leaving largely racemised or decomposed material in the liquors. Moore and his coworkers rejected this method (route A Scheme 5.2.1) for preparing \(37\) in favour of another procedure\(^{24}\) (route B Scheme 5.2.1).

\[
\begin{array}{ll}
\text{A} & \text{B} \\
\text{H-Ser-OMe.HCl} & \text{Tos-Asn-OH} \\
\downarrow & \downarrow \\
\text{H-Ala (Cl)-OMe.HCl} & \text{Tos-Dpr-OH} \\
\downarrow & \downarrow \\
\text{H-Ala (Cl)-OH.HCl} & \text{Tos-Dpr (Boc)OH} \\
\downarrow & \downarrow \\
\text{Z-Ala (Cl)-OH} & \text{Z-Dpr (Boc)OH} \\
\downarrow & \downarrow \\
\text{Z-Dpr-OH (37)} & \text{Z-Dpr (Boc)OMe} \\
\downarrow & \downarrow \\
\text{Z-Dpr-OMe.HCl (38)} & \\
\end{array}
\]

Scheme 5.2.1 Routes A and B used by Moore et al.

\((S)\)-N\(^a\)-Tosylasparagine was converted to \((S)\)-N\(^a\)-tosyl-2,3-diaminopropionic acid in 56% yield by the Hofmann rearrangement\(^{24}\) and then transformed to \((S)\)-N\(^a\)-Z-2,3-diaminopropionic acid hydrochloride \((38)\) in four stages in route B (Scheme 5.2.1). The many protection and deprotection steps involved make this synthesis inelegant and cumbersome. In Section 5.5 the preparation of \(38\) from \(30\) in two steps by the application of the Mitsunobu reaction will be described.

5.3 \(\alpha,\beta\)-DEHYDROAMINO ACIDS

Recently, dehydroamino acids and dehydropeptides have been identified as metabolites which have some
antibiotic activity\textsuperscript{25}. The $\alpha,\beta$-double bond in amino-acid and peptide derivatives constitutes, in addition to the amino and carboxyl groups, a third highly reactive function of the molecule. The principal natural source of dehydroamino-acids is through a $\beta$-elimination pathway from a serine or cysteine peptide residue. Several synthetic methods have been developed\textsuperscript{25} for these compounds. $\beta$-Elimination reactions of serine phosphates and $0$-benzoylserine compounds have found occasional application, but the most popular method is the $\beta$-elimination of a serine-0-sulphonic acid\textsuperscript{25}. However, complications can arise including the following:

(i) Oxazoline formation by intramolecular nucleophilic substitution.

(ii) Aziridine formation in derivatives with high $C_{\alpha}$-H acidity and electron donating N-protecting groups such as trityl.

(iii) Hydantoin formation by N-Z-0-tosylamino-acid amides.

(iv) Nucleophilic substitution of the 0-tosyl group.

Thus although $\beta$-elimination was not desired during attempts to carry out substitution reactions on (31) and (32) [see above], the dehydroalanine (35) obtained is a useful compound. One potential application for (35) is for the preparation of the plant growth inhibitor (39).
The plant hormone ethylene is derived from methionine via 1-aminocyclopropanecarboxylic acid (ACC)\(^{26}\). It is thought that the dichloro-derivative (39) might inhibit plant growth as it is a precursor of the plant toxin 1,1-dichloroethylene. An attempt to prepare ACC by reacting (35) in the Simmons-Smith cyclopropanation reaction (using Zn/Cu couple and diiodomethane) was unsuccessful. However, another cyclopropanation method might be more successful. Also, (39) could be prepared by the reaction of (35) with dichlorocarbene generated from potassium hydroxide/chloroform. These routes to ACC and (39) await further investigation.

5.4 THE MITSUNOBU REACTION

Dehydroamino-acids are constituents of certain peptide antibiotics\(^{25}\). In addition to the methods for preparing these compounds mentioned above; a diethyl azodicarboxylate/triphenylphosphine reagent has been used for the intramolecular dehydration of certain hydroxy-compounds\(^{27}\) and for the preparation of dehydroamino-acids from serine and threonine derivatives\(^{28}\). Reactions which employ this reagent are called Mitsunobu reactions. The scope and versatility of the Mitsunobu reaction is vast, and its application to synthetic problems arising in this research project has significantly contributed to its success.

One of the most valuable uses of the diethyl-azodicarboxylate/triphenylphosphine (DEAD/TPP) reagent is
that it enables alcohols to be used as alkylating agents for the replacement of acidic hydrogens. Alkylation by alcohols in the Mitsunobu reaction is applicable not only to conventionally acidic substrates such as carboxylic acids and phenols, but also to weakly acidic materials such as carboximides. It is estimated that a pK\textsubscript{a} of about 11 or below is required for the active hydrogen\textsuperscript{29}. Both C- and O-alkylation can occur in the reaction of alcohols with active methylene compounds. For example ethyl cyanoacetate (pK\textsubscript{a} > 9) reacts with n-propanol in the presence of DEAD/TPP to give ethyl-2-cyanopentanoate by C-alkylation. Similarly malononitrile (pK\textsubscript{a} 11.2) and n-propanol with DEAD/TPP gave n-propylmalononitrile\textsuperscript{29}. However, when ethyl acetoacetate (pK\textsubscript{a} 13.3) was used in the Mitsunobu reaction both C- and O-alkylation took place in the ratio of about 3:1, respectively\textsuperscript{29}. On the other hand, diethyl malonate (pK\textsubscript{a} 13.3) failed to react with n-propanol and DEAD/TPP.

The Mitsunobu reaction has been used for the conversion of alcohols into amines via N-alkylphthalimides\textsuperscript{30}. The reaction is carried out at room temperature and gives an amine with inverted configuration when an optically active alcohol is used. This reaction shows good functional selectivity; the hydroxyl groups of allyl alcohol, 2-chloroethanol, and \textit{rac}.ethyl lactate were replaced by a phthalimide moiety without affecting other functionality\textsuperscript{31}. Thus, the reaction can be seen as a potential general method for converting esters of \(\alpha\)-hydroxy-acids into
α-amino-acids with inverted configuration. However, there is a prospect for β-elimination to occur, presumably via a quasiphosphonium salt intermediate, e.g. diethyl malate reacts to give diethyl fumarate (Scheme 5.4.1). It is not thought that the phthalimide is involved in the elimination reaction, but rather that the proton abstraction is carried out by the diethyl-hydrazodicarboxylate anion formed in the reaction. The mechanism of the Mitsunobu reaction will be discussed fully later.

β-Hydroxycarboxylic acids also undergo elimination to alkenes in the presence of DEAD/TPP, but the mechanism involves a decarboxylative dehydration. Two processes are possible: the reaction can proceed via a phosphonium-hydroxyl oxygen transition complex (path a Scheme 5.4.2), leading to mainly the alkene. Alternatively, when the β-hydroxycarboxylic acid bears bulky alkyl (R) groups, a phosphonium-carboxyl oxygen transition complex is favoured (path b Scheme 5.4.2) and a β-lactone is formed.

The Mitsunobu reaction has also been applied to the preparation of esters (in 80-90% yields), mainly of benzoic acid. The reaction has been shown to proceed with inversion of configuration by reacting (S)-2-octanol with DEAD/TPP and benzoic acid to give (R)-2-octylbenzoate. Again this suggests a phosphonium-hydroxyl oxygen transition complex in the mechanism. However, the reaction is much less successful when aliphatic carboxylic acids are used; yields of only 35-45% are generally obtained.
Scheme 5.4.1 β-Elimination arising in the Mitsunobu reaction with diethyl malate.

Scheme 5.4.2 Decarboxylative dehydration arising in the Mitsunobu reaction with β-hydroxyacids. Path a or b is followed depending upon the size of R.
An improvement in the esterification of aliphatic carboxylic acids was made by the author. Using \( n \)-octanoic acid and oleic acid with triphenylphosphine and the less expensive diisopropylazodicarboxylate (DIAD) in dry methanol solvent, the methyl ester of each of these acids was obtained in 82\% and 62\% yields, respectively. The same procedure gave a 35\% yield of the \( \text{tert} \)-butyl ester of oleic acid by using \( t \)-butanol as the solvent. It is expected that this yield could be improved considerably. The esterification by this method is complete after about 15 minutes at room temperature. The preparation of \( \text{tert} \)-butyl esters is normally quite difficult and often requires \( iso \)-butene as the esterifying agent.

The Mitsunobu method for esterification has been used for inverting the configuration of C-OH asymmetric centres in steroids\(^{35}\) by preparing the inverted steroid-benzoate ester and then saponifying to the inverted hydroxyl. The Mitsunobu reaction also shows a high degree of regioselectivity; for example, it is possible to selectively esterify a diol at a primary hydroxyl in preference to a secondary hydroxyl position\(^{33}\). This selectivity is explained in terms of the steric bulk of the triphenylphosphine moiety.

Finally, an interesting extension has been made to the Mitsunobu procedure by Loibner and Zbiral\(^{33}\). In this case an alkylating agent is used to alkylate the intermediate diethylhydrazodicarboxylate anion, thus releasing an anion which acts as the nucleophile.
Scheme 5.4.3 Loibner-Zbiral modification of the Mitsunobu reaction.
and attacks the carbon atom of the alkoxyphosphonium salt (Scheme 5.4.3). This method can also be used for preparing the inverted alkyl halides or alkyl sulphonates of a chiral alcohol. The intermediate N-alkylated diethylhydrazodicarboxylate (40) in this modification has also found an application in the preparation of hydrazines from alcohols by saponification and decarboxylation of (40). Table 5.4.1 summarises some examples of the applications of the Mitsunobu reaction.

The mechanism proposed for the Mitsunobu reaction by Guthrie and Jenkins is illustrated in Scheme 5.4.4. Initially triphenylphosphine (TPP) and diethylazodicarboxylate (41) react to form the betaine (42); this was demonstrated in a 31P n.m.r. study. The phosphorus resonance for the betaine (42) is seen at 44.9 p.p.m. in tetrahydrofuran (THF) solvent. Notably, this chemical shift shows very little solvent dependence by appearing at 43.9 p.p.m. in benzene, 44.7 p.p.m. in chloroform, and 45.4 p.p.m. in DMF. This implies little or no equilibrium between TPP and (41) with (42), or with a cyclic phosphorane structure analogous to (43).
Table 5.4.1 Further applications of the Mitsunobu reaction

<table>
<thead>
<tr>
<th>Mitsunobu Reaction</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-OH $\xrightarrow{\text{DEAD/TPP, THF}}$ R-NNHCO$_2$Ei $\xrightarrow{\text{CO$_2$Ei}}$ R-NNHN$_2$</td>
<td>36</td>
</tr>
<tr>
<td>$\text{HNO}_2$/CH$_2$OH $\xrightarrow{\text{Carboximide}}$ DEAD/TPP $\xrightarrow{\text{THF}}$</td>
<td>37</td>
</tr>
<tr>
<td>$\text{NaHCO}_3$/H$_2$O $\xrightarrow{\text{DEAD/TPP, THF}}$</td>
<td>38</td>
</tr>
<tr>
<td>$\text{SO}_2$CO$_2$/H$_2$O $\xrightarrow{\text{DEAD/TPP, THF}}$</td>
<td>39</td>
</tr>
<tr>
<td>(+)-gephyrotoxin precursor</td>
<td>41</td>
</tr>
<tr>
<td>$\text{HO}_{2-}^{-}$/CH$_2$OH $\xrightarrow{\text{DEAD/TPP}}$</td>
<td>42</td>
</tr>
<tr>
<td>Me($\text{CH}_2$)$_2$OH $\xrightarrow{\text{1) DEAD/TPP, THF}}$ Me($\text{CH}_2$)$_2$CHO $\xrightarrow{\text{2) $\Delta$ E}Q_C$, C$_2$H$_5$NO$_2$}</td>
<td>44</td>
</tr>
</tbody>
</table>
Scheme 5.4.4 Guthrie and Jenkins' mechanism for the Mitsunobu reaction
Also, (42) has been crystallised from THF by Hilton et al.\textsuperscript{46} and the structure confirmed by mass spectrometry (e.i. 12 eV, \textit{M} \textsuperscript{+} 436) and \textit{13C} n.m.r. spectroscopy. An alcohol was then added to the betaine (42) in the n.m.r. tube\textsuperscript{45} and a major new peak quickly appeared at -55.0 p.p.m. in the \textit{31P} n.m.r. spectrum, corresponding to the formation of the O,N-phosphorane (44). It was noted that immediate formation of the oxyphosphonium salt (45) would have produced a \textit{31P} resonance at about 60.0 p.p.m. When the acid component was added to the n.m.r. tube, the -55.0 p.p.m. peak disappeared and a new major resonance appeared at 65.0 p.p.m. which is consistent with the presence of (45). Some qualification of the assignment of these \textit{31P} n.m.r. resonances needs to be made. The principal peak at -55.0 p.p.m. assigned to the O,N-phosphorane (44) might also be assigned to an O,O-phosphorane structure (46), but this was excluded by Guthrie and Jenkins\textsuperscript{45} on the basis of the stoichiometry of the reaction.

\begin{equation}
\text{Ph}_3\text{P}^\circ\text{OR}_{\text{OR}}
\end{equation}

(46)

(cf. \text{Ph}_3\text{P(OEt)}_2 \text{ -55.0 p.p.m.})

Further, when the betaine (42) was treated with 0.5 mole equivalent of the alcohol, two peaks appeared in the \textit{31P} n.m.r. spectrum at 44.9 p.p.m. and -55.0 p.p.m. corresponding to residual (42) and O,N-phosphorane (44) respectively. However, when Hilton \textit{et al.}\textsuperscript{46}
added 1.0 mole equivalent of an alcohol (3,4-dichlorophenol) to the betaine (42) in an n.m.r. tube, two peaks, of equal intensity, appeared in the $^{31}$P n.m.r. spectrum at 44.8 p.p.m. [betaine (42)] and -63.0 p.p.m. When a further 1 mole equivalent of this alcohol was added, the betaine resonance disappeared and the peak at -63.0 p.p.m. was enhanced, and was therefore attributed to the O,O-phosphorane (47). This evidence suggested that

\[ \text{OAr} \quad \begin{array}{c} \text{Ph} \\ \text{P} \\ \text{Ph} \\ \text{OAr} \end{array} \]

(47)

(42) and (47) are in an equilibrium. Compound (47) was isolated by Hilton et al.\textsuperscript{46} and the O,O-phosphorane structure confirmed by $^1$H and $^{13}$C n.m.r. spectroscopy, although a molecular ion could not be obtained for (47) in the mass spectrometer. The $^1$H n.m.r. spectrum showed no residual alcohol peaks and the coincidence of the ligated aryl resonances indicated that the phenoxide groups in (47) were located apically as shown. Hilton et al. also dispute that an oxyphosphonium salt, such as (45), is an active intermediate\textsuperscript{46}, and state that (47) breaks down when the acid is added to give 0.5 mole equivalent of product and 0.5 mole equivalent of the starting alcohol, which is then recycled to react with the unused TPP and (42). However, if this reasoning is extrapolated, then it can be seen that the maximum yield of product could only be 50% if (47)
were the only intermediate. Greater than 50% yields are of course usually obtained in the Mitsunobu reaction. Evidence against the intermediacy of the 0,0-phosphorane (46) has been established by Guthrie and Jenkins\(^45\). If (46) were an intermediate then treating the Mitsunobu reaction mixture with methyl iodide should give a 50% yield of the oxyphosphonium salt (45) and 50% yield of the methyl ether of the alcohol. However, no methyl ether was detected. An alternative 0,0-phosphorane intermediate (48) is also feasible.

\[
\begin{align*}
\text{Ph}_2P & \underset{\text{OR}}{\overset{\text{OC} = \text{NNHC}O_2\text{Et}}{\text{Ph}_2P}} \overset{\text{Et}}{\text{OEt}} \\
& \text{(48)}
\end{align*}
\]

It is not yet possible to distinguish between (48) and the O,N-phosphorane (44) by n.m.r. spectroscopy, and a consideration of the bond energies provides little differentiation. However, electronically the diethylhydrazocarboxylate (49) would be a much poorer leaving group than its tautomer (50); and generally phosphines with good leaving groups are unstable and exist, at best, as ion pairs.

\[
\begin{align*}
\text{EtO}_2\text{C} \overset{\text{O}}{\overset{\text{CN}}{\text{Et}}} & \overset{\text{O}}{\text{C} = \text{NNHC}O_2\text{Et}} \\
& \text{(49)}
\end{align*}
\]

\[
\begin{align*}
\text{EtO}_2\text{C} \overset{\text{O}}{\overset{\text{CN}}{\text{Et}}} & \overset{\text{O}}{\text{C} = \text{NNHC}O_2\text{Et}} \\
& \text{(50)}
\end{align*}
\]
Since the $^{31}$P chemical shift of the intermediate phosphorane showed no solvent dependence (-55.0 p.p.m. in THF and -55.1 p.p.m. in benzene), an ion pair was ruled out.

On the other hand 0,0-phosphorane intermediates have been detected in Mitsunobu reactions involving vicinal diols, e.g. cis and trans cyclohexane-1,2-diols. In addition to the major resonances observed in the $^{31}$P n.m.r. spectra of these reactions, a minor peak at 20.6 p.p.m. was also usually present. This was assigned to the phosphazine (51) which arises from the betaine (42).

\[
\begin{align*}
\text{Ph}_3\text{P} & \text{N} = \text{N} \quad \text{CO}_2\text{Et} \\
\end{align*}
\]

When triphenylphosphine is replaced in the Mitsunobu reaction by tri-$n$-butylphosphine, phosphoranes were no longer observed in the $^{31}$P n.m.r. spectra. Instead the betaine (51) that was formed ($^{31}$P chemical shift 44.4 p.p.m.) reacted immediately when the alcohol was added to give the oxyphosphonium salt (52) [$^{31}$P chemical shift 103 p.p.m.]. Thus, whereas TPP was transformed into the oxyphosphonium salt via a phosphorane after adding the acid, tri-$n$-butylphosphine gave the salt after adding the alcohol only. This difference is rationalised in terms of Pearson's concept of hard and soft acids and bases. The phosphorane structure is stabilised by harder, i.e. more electronegative, ligands, e.g. phenyl, whereas the
oxyphosphonium salt is stabilised more by softer ligands, such as butyl\textsuperscript{45}.

Finally, Mitsunobu himself has proposed a mechanism for the reaction\textsuperscript{33} (Scheme 5.4.5). The main criticism of the studies carried out by Guthrie and Jenkins\textsuperscript{45} and Hilton \textit{et al.}\textsuperscript{46} is that both of them assumed the order in which the reagents reacted, whereas the Mitsunobu procedure sometimes adds the acid before the alcohol and in any case the reagents are mixed over a short time or simultaneously. Also, the alcohols used in the two studies\textsuperscript{45,46} were very different in terms of pK\textsubscript{a} and steric bulk. Thus, any claim by one author against the other cannot be strictly validated. At best it can be concluded that good evidence has been presented for the intermediacy of both O,N- and O,O-phosphoranes. Which of these phosphoranes predominates in a particular reaction probably depends upon a combination of factors relating to the alcohol and acid components used. Indeed, Mitsunobu does not include either phosphorane in his mechanism\textsuperscript{33}. The final step in each of the mechanisms is unequivocal; nucleophilic attack by the conjugate base of the acid (X\textsuperscript{-}) on the oxyphosphonium salt (\textsuperscript{45}) leading to a product of inverted configuration relative to the starting alcohol.

5.5 \textsc{Synthesis of (S)-2,3-Diaminopropionic Acid}

Loibner and Zbiral\textsuperscript{47} have demonstrated the utility of the Mitsunobu reaction in the preparation
Scheme 5.4.5 Mitsunobu reaction mechanism proposed by Mitsunobu.
of, for example, azides and nitriles from alcohols. This reaction has now been used to prepare (S)-N<sup>a</sup>-Z-2,3-diaminopropionic acid methyl ester hydrochloride (38) from (S)-N-Z-serine methyl ester (30) via the azide (33). The reaction was carried out in dry benzene using the DEAD/TPP reagent with (30) as the alcohol component and hydrazoic acid as the active hydrogen component. The reaction produced (S)-N-Z-3-azidoalanine methyl ester (33) in 20% yield after chromatographic purification. The separation was difficult to achieve. A further amount of the azide (33) was obtained, but was only ca. 50% pure. The main contaminant was the residual DEAD reagent. However, it was possible to improve the procedure considerably by using the less expensive diisopropylazodicarboxylate (DIAD) reagent in place of DEAD, and by performing the azide reduction without isolating (33) from the Mitsunobu reaction mixture. Several methods for reducing azides are available. In this case it was necessary to select a method which would not cleave the N<sup>a</sup>-benzyloxy carbonyl group, or reduce the methyl ester function. The method of choice employed hydrogen sulphide in a pyridine/water solution<sup>48</sup>. Although the reduction was successful, partial ester hydrolysis also occurred. An attempt to replace the solvent system with pyridine/methanol failed, and so the ester hydrolysis from the pyridine/water procedure was taken to completion by working up the reaction in aqueous hydrochloric acid. This allowed the poorly soluble product, (S)-N<sup>a</sup>-Z-2,3-diaminopropionic acid (37) to be
precipitated when the solution was neutralised. A yield of 53% of (37) was obtained from this two-step procedure. Compound (37) could then be easily re-esterified to the methyl ester hydrochloride (38) or converted to (S)-2,3-diaminopropionic acid (27) by hydrogenolysis of the N°-benzyloxycarbonyl group. The compounds (37), (38) and (27) each gave an optical rotation value equal to the best literature values.

Less than 5% w/w of the dehydroalanine elimination product (35) was isolated from the chromatographic purification of the azide (33). A control experiment was carried out in which N-Z-dehydroalanine methyl ester (35) was added to the Mitsunobu reaction mixture (HN3/DEAD/TPP) in benzene, but no azide (33) was detected by either 1H n.m.r. spectroscopy or thin layer chromatography. This result confirmed that the azide (33) did not arise at all via (35) by an elimination-addition pathway from (30).

5.6 N°-ALKYLATION OF (S)-N°-Z-2,3-DIAMINOPROPIONIC ACID METHYL ESTER

The next stage in this strategy to an analogue of 593A required the N°-alkylation of (S)-N°-Z-2,3-diaminopropionic acid methyl ester (53) and the incorporation of a protecting group at the N°-position. The first attempt to alkylate (53) involved reaction with rac. propylene oxide with a view to using (S)-propylene oxide when the correct conditions were established. The trial reactions were monitored by 220 MHz 1H n.m.r. spectroscopy;
the range of conditions investigated are tabulated below.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Temp./°C</th>
<th>Time/hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD₃OD</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>CD₃OD</td>
<td>20</td>
<td>24</td>
</tr>
<tr>
<td>CD₃OD</td>
<td>64</td>
<td>2.5</td>
</tr>
<tr>
<td>CD₃OD</td>
<td>45</td>
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<td>20</td>
<td>96*</td>
</tr>
<tr>
<td>CD₃Cl</td>
<td>40</td>
<td>4</td>
</tr>
<tr>
<td>CD₃Cl</td>
<td>20</td>
<td>24**</td>
</tr>
<tr>
<td>THF</td>
<td>20</td>
<td>4***</td>
</tr>
</tbody>
</table>

Table 5.6.1 Reactions of (5₃) with rac.epoxide

*Added ammonium bromide
**Added magnesium chloride
***Added alumina

In all cases some degree of β-elimination was detected, giving (35), and no clear evidence for the formation of Nβ-alkylated (5₃) was found in the spectra. It appeared that the amine-ester (5₃) was sufficiently basic to induce β-elimination in a second molecule of (5₃). The presence of ammonium bromide as a source of protons seemed to retard the elimination at ambient temperature but still no Nβ-alkylation was detected. The use of anhydrous magnesium chloride was intended as a means of activating the propylene oxide by magnesium-oxygen interaction, but this made no improvement. Also, a procedure in which neutral alumina was used to
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Scheme 5.6.1 The Staudinger reaction and aza-Wittig condensation.

Scheme 5.6.2 Route to a 593A analogue via an amine-aldehyde condensation.
isolated was \((S)-N^a-Z-2,3\text{-diaminopropionic acid methyl ester hydrochloride} (38)\) formed by hydrolysis of the imine intermediate \((55)\) during isolation. It would be possible to reduce the imine intermediate \((55)\) without purification, to give the required \(N^B\)-alkylation derivative. However, the azide \((33)\) was difficult to isolate from the Mitsunobu reaction mixture, and so an alternative procedure using the more easily obtained amine ester \((53)\) was pursued. \((S)-N^a-Z-2,3\text{-Diaminopropionic acid methyl ester} (53)\) was reacted with propanal in dichloromethane in the presence of a desiccant to give the imine intermediate after only 15 minutes. This was immediately reduced without isolation, using sodium borohydride in methanol, to give the \(N^B\)-alkylated derivative \((S)-N^a-Z-N^B\text{-propyl}-2,3\text{-diaminopropionic acid methyl ester} \). The reaction was repeated using \((53)\) and redistilled \(O\text{-benzyloxyethanal} (56)\) to give the \(N^B\)-alkyl derivative \((57)\) via the intermediate imine \((58)\) [Scheme 5.6.2]. Compound \((57)\) is a potential precursor to an analogue of 593A \((59)\). The aldehyde \((56)\)-amine \((53)\) condensation to give \((58)\) was monitored in \(\text{CDCl}_3\) by 220 MHz \(^1\text{H}\) n.m.r. spectroscopy; the reaction was complete after only 10 minutes. On a preparative scale, in dichloromethane solvent, the imine intermediate \((59)\) was not isolated but immediately reduced with sodium borohydride in anhydrous methanol, followed by purification via the hydrochloride salt, to give \((57)\) in 64% overall yield.
The next step towards a 593A analogue was to remove the $N^a$-benzyloxycarbonyl protecting group from (57), but this particular hydrogenolysis proved to be more difficult than usual. When more forcing conditions were employed (higher hydrogen pressure and longer reaction times) some O-benzyl cleavage also occurred. However, it was possible eventually to remove the $N^a$-benzyloxycarbonyl group by treating (57) with 100% trifluoroacetic acid for 4 days at room temperature. The deprotected $\alpha$-amino ester oil (60) showed no indication of crystallising to the 2,5-piperazinedione derivative. Heating (60) in methanol at reflux for

\[
\text{BzO-} \overset{\text{H}}{\text{N}} \overset{\text{NH}}{\text{CO}}_{\text{Me}}
\]

(60)

2 days produced only a complex mixture. It appeared to be necessary to protect the $N^8$-position before attempting $N^a$-deprotection and dimerisation to the 2,5-piperazinedione.

5.7 AMINO PROTECTING GROUPS

The older methods of protecting functionality often relied heavily on deprotection by acid or alkaline hydrolysis, usually at the boiling point of the solvent, although $N$-benzyl and O-benzyl scission by hydrogenation was also established at an early
date. The protection of amino-acid side-chain functional groups is generally regarded as desirable to prevent interference during peptide bond formation. Although many syntheses use a minimum number of protecting groups, the majority tend towards maximum or 'global' protection.52

There are several criteria governing the suitability of components as protecting groups53. These include:

(i) the protection and deprotection steps should proceed in high yield;

(ii) the protecting group should not contain or introduce a chiral centre in the molecule;

(iii) the protecting group should be stable under the conditions for the projected reactions;

(iv) the cleaved protecting group must be easily separable from the product;

(v) deprotection must be done under, preferably mild, specific conditions.

The conflicting requirements of stability and ease of removal of a protecting group are usually met by making the conditions for cleavage as specific as possible. The most popular and most successful amine protecting groups are the benzyloxy carbonyl (abbreviated to Z in honour of L. Zervas), and t-butyloxy carbonyl (Boc) carbamate moieties.

The first amine protecting group chosen to block the N8-position of (57) was the trifluoroacetyl group. This can be introduced by a number of reagents without risk of racemisation.54 The N8-trifluoroacetyl derivative
of (57) was obtained by reaction with ethyl trifluoroacetate to give (61) or, more efficiently, by reaction of (57) with trifluoroacetic anhydride to give (61) in 90% yield.

\[
\text{BzO} \quad \text{CO}_2\text{Me}\quad \text{NHZ}
\]

(61)

The trifluoroacetyl group is usually removed by treatment with 0.01 mol dm\(^{-3}\) sodium hydroxide solution. However, when the \(N^a\)-deprotection was carried out by hydrogenolysis, the \(N^8\)-trifluoroacetyl function was also lost. It is not clear how this arose but \(\text{trans}\)-acylation to the \(N^a\)-amine group may have occurred or promoted solvolysis by the methanol. Nevertheless, rather than investigate the problem fully, an alternative protecting group was selected. After careful consideration, a diphenylphosphinamide was chosen. This functionality has the advantage of being stable to hydrogenation and is cleaved under acidic conditions. This latter feature is particularly desirable because the 2,5-piperazinedione derivative may be too labile toward epimerisation to allow alkaline cleavage of a protecting group to be used. The protecting reagent diphenylphosphinyl chloride (\(\text{Ph}_2\text{POCl}\)) [62] was prepared by bubbling oxygen through a solution of diphenylchlorophosphine (\(\text{Ph}_2\text{PCl}\) in dry benzene). A modification of the literature procedure was then devised for the reaction of (62) with (57). Instead of using \(N\)-methylmorpholine as the solvent,
the reaction was performed in dry acetonitrile with a suspension of poly-4-vinylpyridine to "mop-up" the liberated hydrogen chloride. The reaction was monitored by observing the pH change (from 1 to 6). The reaction was complete after 1 hour giving the N^\text{\textbeta}-phosphinamide (63).

\[
\begin{align*}
\text{Bz} & \quad \text{POPh} \quad \text{NH}_2 \\
\text{N} & \quad \text{CO}_\text{Me}
\end{align*}
\]

Only primary amine groups have previously been protected using this reagent and used 80% glacial acetic acid (3 days) or acetic acid/formic acid in water (24 hours) or some similar acidic medium as deprotection agents\textsuperscript{56,57}. Unfortunately though, (63) was highly susceptible to hydrolysis, and even methanol removed the N^\text{\textbeta}-phosphinamide function of (63). Therefore, the N^\textalpha-benzyloxycarbonyl deprotection was carried out by hydrogenolysis in anhydrous acetonitrile, but attempts to form the 2,5-piperazinedione by heating the unpurified (S)-N^\textbeta-phosphinamide-N^\textbeta-(benzyloxyethyl)-2,3-diaminopropionic acid methyl ester in acetonitrile gave a complex mixture. The mixture was extracted with dilute hydrochloric acid and the extract, which should have contained the N^\textbeta-deprotected 2,5-piperazinedione (59), was examined by mass spectrometry. Although the mass spectrum contained some information diagnostic of a 2,5-piperazinedione (Fig. 5.7.1), a molecular ion for (59) was not observed. This procedure did not offer a
Fig. 5.7.1 Fragmentation diagnostic of a piperazinedione in mass spectra from an attempt to prepare (59).
viable method of preparing (59).

Finally, a third amine protecting group was sought, and to avoid problems of solvolysis, the N-benzyl protecting function was chosen. This group is normally also removed by hydrogenolysis, but should require more forcing conditions than those needed to excise the \( N^a \)-benzyloxycarbonyl group. The \( N^8 \)-benzylolation of (57) was carried out using a procedure suitable for C-, N-, O-, and S-, alkylations reported by Ando and Yamawaki. Compound (57) was added to benzyl bromide in acetonitrile which contained a suspension of diatomaceous earth coated with potassium fluoride (KF-Celite). This gave the \( N^8 \)-benzyl derivative (64) after 24 hours. Unfortunately, the \( N^a \)-benzyloxycarbonyl group could not be removed under the usual hydrogenation conditions (20 p.s.i. of hydrogen), but it was cleaved by treating (64) with 100% trifluoroacetic acid for 4 days. This result is surprising because this \( N^a \)-carbamate function is normally stable under these conditions and would usually require hydrogen bromide in acetic acid to effect its removal.

Only a small sample (ca. 0.01 g) of the oily \( N^8 \)-benzyl-\( N^8 \)-(benzyloxyethyl)-2,3-diaminopropionic acid methyl ester (65) was prepared. No dimerisation to the 2,5-piperazinedione could be detected in this sample.
after 7 days. When a second small amount of (65) was prepared and heated in [\textsuperscript{2}H\textsubscript{3}] acetonitrile at 75°C and monitored by 220 MHz \textsuperscript{1}H n.m.r. spectroscopy, no reaction at all was evident after 16 hours.

Many further permutations and more 'elaborate' amine protecting groups could of course be pursued. However, in view of the difficulties encountered with the \(N^\beta\)-protected derivatives of (57), an approach to \textit{cyclo}(S-aminoalanyl-S-aminoalanyl) \(^2\) was re-adopted. Firstly, the primary \(N^\beta\)-amine function of \((S)-N^\alpha-Z-2,3\)-diaminopropionic acid methyl ester (53) was blocked with the \(t\)-butyloxy carbonyl protecting group using a modification of a procedure described by Itoh \textit{et al.}\(^5\). This method uses the reagent 2(\(t\)-butyloxy carbonyloxyimino)-2-phenylacetonitrile (Boc-ON) \(^6\) which was heated with (53) in dichloromethane (Scheme 5.7.2) and the reaction monitored by t.l.c. The product \((S)-N^\alpha-Z-N^\beta\)-Boc-2,3-diaminopropionic acid methyl ester (67) was isolated by filtration column chromatography on silica gel. The \(N^\alpha\)-carbamate group of (67) was removed by hydrogenolysis without difficulty to give \((S)-N^\beta\)-Boc-2,3-diaminopropionic acid methyl ester (68) as a crystalline solid. Compound (68) showed no inclination to dimerise in solution to give a piperazinedione, possibly because of inaccessibility of the methyl ester function due to steric blocking by the \(t\)-butyl group. This may be compared to the problem discussed in Chapter 3 in which intramolecular cyclisation to a piperazinedione was blocked in a penicillin (8) by the \textit{gem} dimethyl function. Compound (68) was then heated in a sealed tube at 150°C for 3 days but only produced a
Scheme 5.7.2 Reaction of Boc-ON reagent with (53).
brown sticky oil of a complex mixture.

The dimerisation-cyclisation of an \( \text{N}^8 \)-blocked derivative of (53) or (57) may be achieved in a stepwise manner by using a peptide coupling procedure to give a dipeptide, which could then be cyclised to the piperazine-dione (59).

5.8 EXPERIMENTAL

5.8.1 \((S)-N\text{-Benzyloxycarbonylserine} (29)\)

\((S)-\text{Serine (10.00 g, 0.1 mol) was dissolved in a 1 mol dm}^{-3} \text{ solution of sodium bicarbonate (180 cm}^3, 0.18 \text{ mol) and benzylchloroformate (14 cm}^3, 0.105 \text{ mol, } \rho = 1.2 \text{ g cm}^{-3} \text{) added. The mixture was stirred vigorously for 3 hours and the pH maintained by periodically adding more sodium bicarbonate. The mixture was then extracted once with ether and the aqueous phase acidified by adding concentrated hydrochloric acid. The precipitate was then filtered and washed with water and dried (P\text{\textsubscript{4}}\text{O\textsubscript{10}, in vacuo}) to give (29) [14.0 g, 62\% yield] m.p. 113-114^\circ \text{C} \text{ (lit.} 60 \text{ m.p. 119^\circ C}), [\alpha]_D^{20} 5.8^\circ \text{ (c 6 in AcOH) [lit.} 60 \text{ [\alpha]_D 5.9^\circ], } ^1\text{H n.m.r. } ([^2\text{H}_6]\text{Me}_2\text{CO}); \delta 7.4 \text{ (m, PhH, 5H), 6.42 (br, NH, 1H), 5.10 (s, PhCH}_2, 2\text{H), 4.35 (m, CH, 1H), 3.92 (m, CH}_2 2\text{H) p.p.m., e.i.m.s. m/z 239 (M}^+, 25\%), 179 (15), 162 (63), 148 (100), 132 (81), 104 (35), 89 (42).\)

5.8.2 \((S)-N-Z-Serine Methyl Ester (30)\)

\((S)-N-Z-Serine (29) [8.50 g, 0.035 \text{ mol}] \text{ was dissolved in 2 mol dm}^{-3} \text{ hydrogen chloride in methanol}
solution (60 cm³). After 24 hours the solvent was evaporated to give a colourless oil which was triturated with ether and cooled. The mixture was then filtered and the filtrate evaporated and then pumped at high vacuum (0.01 mmHg) for 24-48 hours to give (30) [7.5 g, 83% yield], m.p. 32-34°C (lit.¹₅ m.p. 35-37°C), [α]D²⁰⁻¹₄.₂° (c 0.8 in MeOH) [lit.¹₅ [α]D⁻₁₅°], ¹H n.m.r. (CDCl₃); δ 7.32 (m, PhH, 5H), 5.95 (br, NH, 1H), 5.10 (s, PhCH₂, 2H), 4.42 (m, CH, 1H), 3.9 (m, CH₂, 2H), 3.73 (s, OCH₃, 3H) p.p.m., i.r. (Nujol); 3420, 1740, 1720, 1500 cm⁻¹.

5.8.3  (S)-N-Z-3-Chloroalanine Methyl Ester (31)
(S)-N-Z-Serine methyl ester (30) [6.00 g, 0.023 mol] in CCl₄ (30 cm³) was added to triphenylphosphine (9.32 g, 0.035 mol) in CCl₄ (60 cm³). The mixture was refluxed under nitrogen for 24 hours. The solvent was removed and the residue was subjected to 'flash chromatography' (silica gel 60, elution with dichloromethane). The fractions containing compound (31) were combined and evaporated. The residual solid was recrystallised from ether-petrol (b.p. 40-60°C) to give pure ester (31) [4.15 g, 64% yield] m.p. 54-55°C (lit.¹₅ m.p. 54-55°C), [α]D⁻²²° (c 0.8 in DMF) [lit.¹₅ [α]D⁻₂₁.₈°], ¹H n.m.r. (CCl₄); δ 7.3 (m, PhH, 5H), 5.62 (br, NH, 1H), 5.05 (s, PhCH₂, 2H), 4.67 (m, CH, 1H), 3.88 (m, CH₂, 2H), 3.77 (s, OCH₃, 3H) p.p.m., e.i.m.s. m/z 271 (M⁺, 20%), 212 (15), 108 (100), i.r. (Nujol); 3340, 1750, 1680, 1530, 1250, 1065 cm⁻¹.
5.8.4 (S)-N-Z-3-Iodoalanine Methyl Ester (32)

To the ester (31) [0.10 g, 0.36 mmol] in dry acetone (8 cm³) was added dry sodium iodide (1.0 g, 6.6 mmol). The mixture was boiled under reflux for 4.5 hours, cooled and filtered. The filtrate was concentrated and the residue was taken up in dichloromethane. The solution was washed with 10% aq. sodium thiosulphate until colourless and then with water. The organic layer was dried and evaporated to give a crude product that was recrystallised from ether-petrol (b.p. 40-60°C). Colourless needles of ester (32) were obtained (0.05 g, 38% yield) m.p. 66-67°C (lit. m.p. 67-68°C), [α]D²⁰ -20° (c 0.8 in DMF) [lit. [α]D -19.9°], ¹H n.m.r. (CCl₄);  δ 7.3 (m, PhH, 5H), 5.55 (br, NH, 1H), 5.04 (s, PhCH₂, 2H), 4.46 (m, CH, 1H), 3.75 (s, OCH₃, 3H), 3.55 (d, CH₂, 2H) p.p.m., e.i.m.s. m/z 363 (M⁺, 10%), 304 (15), 260 (31), 236 (10), 217 (18), 108 (100), 91 (61), 77 (48).

5.8.5 (S)-N-Z-3-Azidoalanine Methyl Ester (33)

Triphenylphosphine (13.37 g, 0.05 mol) was added to ester (30) [13.00 g, 0.05 mol] in benzene (230 cm³). To the resulting solution was added a solution of 0.55 mol dm⁻³ hydrazoic acid in benzene (93 cm³, 0.05 mol), followed by diethylazodicarboxylate (8.86 g, 0.05 mol) in benzene (93 cm³) with stirring. After 24 hours the mixture was filtered and concentrated to give a red oil. This was purified by elution with dichloromethane through a column of silica gel (mesh < 230) under suction.
The azide (33) was obtained as a colourless oil (2.89 g, 20% yield) \([\alpha]_D^{21} 28^\circ (c 2.3 \text{ in CH}_2\text{Cl}_2)\), t.l.c. (silica gel F254, dichloromethane) one spot \(R_f 0.3\), \(^1\)H n.m.r. (CDCl\(\text{3}\)); \(\delta 7.3\) (m, PhH, 5H), 5.68 (br, NH, 1H), 5.12 (s, PhCH\(\text{2}\), 2H), 4.52 (m, CH, 1H), 3.77 (s, OCH\(\text{3}\), 3H), 3.73 (d, CH\(\text{2}\) 2H) p.p.m., e.i.m.s. m/z 278.1015 (M\(^+\), 15%, \(\text{C}_{12}\text{H}_{14}\text{N}_4\text{O}_4\) requires 278.1014), 250 (70), 222 (30), 178 (97), 162 (56), 145 (24), 108 (100), i.r. (Neat); 3320, 2105, 1725, 1520, 1220 cm\(^{-1}\). A further fraction (4.1 g) of impure (\(\sim 50\%\)) azide (33) was obtained from the column. This material could be reduced and hydrolysed to (37) as described in Method (ii) below.

5.8.6 \((S)-\text{N}^\alpha-\text{Z}-2,3\text{-Diaminopropionic Acid}\) (37)

Method (i): The pure azide (33) [1.86 g, 6.68 mmol] in water-pyridine (40 cm\(^3\), 1:1 v/v) was bubbled with hydrogen sulphide for 3 hours. The solution was evaporated and the residue was taken up in methanol. After acidification with 1 mol dm\(^{-3}\) hydrochloric acid and filtration, evaporation of the filtrate gave the hydrochloride salt of (37) [1.61 g, 83% yield], \(^1\)H n.m.r. spectrum (in 2 mol dm\(^{-3}\) DCl in D\(\text{2}\)O) was identical to that of (37) prepared in Method (ii) below.

Method (ii): Triphenylphosphine (1.24 g, 4.7 mmol) was added to (30) [1.00 g, 3.94 mmol] in benzene (7 cm\(^3\)). A 0.55 mol dm\(^{-3}\) solution of hydrazoic acid in benzene (8.6 cm\(^3\), 4.7 mmol) was added followed by diisopropylazodicarboxylate (0.95 g, 4.7 mmol) in benzene (5 cm\(^3\)) with stirring. After 24 hours the mixture was evaporated to
give a red oil. This was taken up in water-pyridine (20 cm³, 1:1 v/v) and the solution was bubbled with hydrogen sulphide for 3 hours. The resulting mixture was evaporated and the residue was partitioned between dichloromethane and 1 mol dm⁻³ hydrochloric acid. After 1 hour the aqueous phase was neutralised with saturated sodium bicarbonate solution and the resulting precipitate of \((S)\)-\(N^\alpha\)-Z-2,3-diaminopropionic acid (37) was filtered off, and recrystallised from water and dried (0.51 g, 53% yield) m.p. 243-245°C (dec.) [lit. m.p. 228-230°C (dec.) \(^6\) and 243-245°C (dec.) \(^2\)\(^3\)], \([\alpha]_D^{20} -8.0^\circ\) (c 0.4 in 0.99 mol dm⁻³ aq. sodium hydroxide) [lit. \([\alpha]_D \) -7.8°], \(^1\)H n.m.r. (2 mol dm⁻³ DCl in D₂O); δ 7.45 (m, PhH, 5H), 5.17 (s, PhCH₂, 2H), 4.55 (m, CH, 1H), 3.5 (m, CH₂, 2H) p.p.m., i.r. (Nujol); 3300, 2900, 1690, 1625, 1540 and 1260 cm⁻¹.

Treatment of N-Z-dehydroalanine methyl ester (35) with HN₃/TPP/DIAD in benzene as described for the ester (30) for 24 hours did not afford azide (33) [\(^1\)H n.m.r., infrared and t.l.c. analysis showed unchanged (35)].

5.8.7 \((S)\)-2,3-Diaminopropionic Acid Hydrochloride (27)

\(N^\alpha\)-Z-2,3-Diaminopropionic acid (37) [0.10 g, 0.42 mmol] in 1 mol dm⁻³ aq. hydrochloric acid (5 cm³) containing 10% Pd/C catalyst (10 mg) was hydrogenated (Parr, initial pressure 20 p.s.i.) for 6 hours. The mixture was filtered through Celite and the filtrate

*Ref. 23 reports \([\alpha]_D \) -14.2° (c 1 in AcOH) for this compound. In this solvent we find \([\alpha]_D^{20} \) -1.5°.*
adjusted to pH 7 with triethylamine. After concentration to ca. one-third volume, the solution was cooled and treated with ethanol. Trituration of the mixture caused crystallisation of the title compound which was filtered off and dried (0.045 g, 75% yield) m.p. 237-238°C (dec.) [lit. 61 m.p. 236-237°C (dec.)], \([\alpha]_D^{20} 25^\circ (c 2 \text{ in } 0.5 \text{ mol dm}^{-3} \text{ hydrochloric acid}) [\text{lit. 61 } [\alpha]_D 25.2^\circ],
\]

1H n.m.r. (2 mol dm^{-3} DCl in D_2O); \(\delta 4.48 \ (\text{m, CH, 1H}), 3.60 \ (\text{m, CH}_2, 2\text{H}) \) p.p.m.

5.8.8 \((S)-N^\alpha-Z-2,3\text{-Diaminopropionic Acid Methyl Ester Hydrochloride (38)}\)

\((S)-N^\alpha-Z-2,3\text{-Diaminopropionic acid (37)}\)
hydrochloride (0.56 g, 2.0 mmol) was added to 2 mol dm^{-3} HCl in dry methanol (20 cm^3) and the solution was stirred at room temperature for 17 hours. The resulting mixture was filtered and evaporated to give a white solid which was recrystallised from methanol-ether to give the title compound as white crystals (0.50 g, 86% yield) m.p. 164-165°C (lit. 23 m.p. 163-166°C), \([\alpha]_D^{21} -43^\circ (c 2.5 \text{ in MeOH}) [\text{lit. 23 } [\alpha]_D -43.5^\circ], 1H \text{n.m.r. (D}_2\text{O}); \delta 7.43 \ (m, PhH, 5H), 5.18 \ (s, PhCH}_2, 2H), 4.61 \ (m, CH, 1H), 3.78 \ (s, OCH}_3, 3H), 3.45 \ (m, CH}_2\text{N}^+\text{D}_3, 2\text{H}) \text{p.p.m.,}
\]
c.i.m.s. (NH}_3) m/z 253 (M^+ + 1, 24%), 236 (15), 223 (10), 107 (42), 91 (100), i.r. (Nujol); 3310, 1735, 1670, 1370, 1305 cm^{-1}.

5.8.9 \text{N-Z-Dehydroalanine Methyl Ester (35)}

Method (i): \((S)-\text{N-Z-3-Chloroalanine methyl ester (31)}\) [0.10 g, 0.036 mmol] was added to triethylamine
(0.15 g, 1.4 mmol) in CCl₄ (5 cm³) and the resulting solution was stirred at room temperature for 18 hours. After washing with water the organic layer was dried and evaporated to give the title compound as a colourless oil (0.085 g, 98% yield), ¹H n.m.r. spectrum (in CCl₄) was identical to that of (35) prepared in Method (ii) below.

Method (ii): (S)-N¹-Z-2,3-Diaminopropionic acid methyl ester (53) [0.05 g, 0.2 mmol] was dissolved in methanol and refluxed for 48 hours. The solvent was evaporated and the residue was partitioned between water and dichloromethane. The organic layer was dried and evaporated to give (35) [0.045 g, 98% yield] ¹H n.m.r. (CCl₄); δ 7.28 (m, PhH, 5H), 7.13 (br, NH, 1H), 5.67 and 6.24 (2 x s, =CH₂, 2 x 1H), 5.08 (s, PhCH₂, 2H), 3.78 (s, OCH₃, 3H) p.p.m., e.i.m.s. m/z 235 (M⁺, 1%), 176 (23), 144 (30), 108 (49), 91 (100).

5.8.10 (S)-N¹-Z-N⁸-(Benzzyloxyethyl)-2,3-Diaminopropionic Acid Methyl Ester (57)

The amine hydrochloride (38) [1.17 g, 4.1 mmol] was dissolved in saturated aq. sodium bicarbonate solution (10 cm³) and the solution extracted with dichloromethane (3 x 5 cm³). Anhydrous sodium sulphate (ca. 5 g) was then added to the combined organic aliquots followed by re-distilled benzyloxyethanal (56) [b.p. 66-68°C, 0.35 mmHg] (0.60 g, 4.1 mmol) in dichloromethane (5 cm³). After 15 minutes the solution was filtered and evaporated, and the residue was dissolved in dry methanol (10 cm³). Sodium borohydride (0.40 g,
10 mmol) was then added to the solution over 30 minutes. The mixture was then evaporated and the residue partitioned between water and dichloromethane. The organic layer was dried and evaporated and pumped at higher vacuum (0.01 mmHg) for 48 hours to give the title compound as a colourless oil (1.00 g, 64% yield) 

\( [\alpha]_D^{23} 3.3^\circ \text{ (c 3.4 in CH}_2\text{Cl}_2) \), \( ^1H \text{n.m.r. (CCl}_4) \); \( \delta 7.22 \text{ (m, PhH, 10H)}, 5.90 \text{ (br, NH, 1H)}, 5.01 \text{ (s, PhCH}_2\text{OCO}^-, 2H), 4.41 \text{ (s, PhCH}_2\text{O)}, 4.30 \text{ (m, CH, 1H)}, 3.66 \text{ (s, OCH}_3\text{, 3H)}, 3.42 \text{ (t, OCH}_2\text{, 2H), 2.92 \text{ (m, CH}_2\text{NH, 2H)}^*}, 2.70 \text{ (m, NHCH}_2\text{, 2H)}^*, 2.06 \text{ (br, CH}_2\text{NHCH}_2\text{, 1H) p.p.m., e.i.m.s. m/z 386.1841 (M}^+, 12\% \text{, C}_{21}\text{H}_{26}\text{N}_2\text{O}_5 \text{ requires 386.1841)}, \text{ c.i.m.s. (NH}_3\text{) m/z 387 (M}^+ + 1, 15\%), 279 (23), 236 (32), 253 (18), 152 (21), 108 (100), 91 (45), \text{i.r. (Neat); 3320, 1740, 1070 cm}^{-1}.

5.8.11 \((S)-N^\alpha-Z-N^\beta-(\text{Benzzyloxyethyl})-N^\beta-(\text{Trifluoroacetyl})-2,3-\text{Diaminopropionic Acid Methyl Ester (6)}\)_

Compound (5) \([0.80 \text{ g, 2.0 mmol}]\) was added to a stirred suspension of anhydrous sodium carbonate \([0.24 \text{ g, 2.27 mmol}]\) in dichloromethane \([8 \text{ cm}^3]\). Trifluoroacetic anhydride \([0.55 \text{ g, 2.62 mmol}]\) was then added dropwise over 10 minutes. After 4 hours the mixture was filtered, then washed with water and the organic layer dried and evaporated to give the crude title compound \([0.84 \text{ g, 90% yield}]\). This was purified by elution with dichloromethane on silica gel \(\text{mesh < 230})\) under suction to give \((6) [0.40 \text{ g, 40% yield}]\), \(^1H \text{n.m.r.}

*Assigned by spin decoupling
(CDC\textsubscript{3}); \delta 7.3 (m, PhH, 10H), 5.80 (br, NH, 1H), 5.08 (s, PhCH\textsubscript{2}OCO\textsuperscript{−}, 2H), 4.73 (m, CH, 1H), 4.49 (m, PhCH\textsubscript{2}O, 2H), 3.90 (m, CH\textsubscript{2}, 2H), 3.65 (m, OCH\textsubscript{3} and CH\textsubscript{2}NHCH\textsubscript{2}, 7H) p.p.m., c.i.m.s. (NH\textsubscript{3}) m/z 500 (M\textsuperscript{+} + 18, 5%), 366 (12), 305 (22), 108 (76), 91 (100), i.r. (Neat); 3340, 1735, 1680, 1210 cm\textsuperscript{−1}.

5.8.12 (S)-N\textsuperscript{α}-Z-N\textsuperscript{β}-(Benzyloxyethyl)-N\textsuperscript{β}-(Diphenylphosphinyl)-2,3-Diaminopropionic Acid Methyl Ester (\textsuperscript{63})

Diphenylchlorophosphine (30.0 g, 0.13 mol) was dissolved in dry benzene (100 cm\textsuperscript{3}) and the solution bubbled with dry oxygen and allowed to reflux under its own heat of reaction. After 3 hours the benzene was removed and the residue distilled to give diphenylphosphanyl chloride (\textsuperscript{62}) as a colourless oil (b.p. 144-146\textdegree C, 0.1 mmHg) [31.2 g, 97% yield] \textsuperscript{1}H n.m.r. (CDCl\textsubscript{3}); \delta 7.85 (m, ortho-PhH, 4H, 7.55 (m, meta- and para-PhH, 6H) p.p.m.

Compound (\textsuperscript{57}) [0.18 g, 0.46 mmol] was added to poly-4-vinylpyridine (ca. 1.0 g) in dry acetonitrile (5 cm\textsuperscript{3}) and then diphenylphosphanyl chloride (\textsuperscript{62}) [0.11 g, 0.46 mmol] was added with stirring. The pH of the reaction changed from 1 to 6 (over ca. 15 minutes). After 1 hour the mixture was filtered and then evaporated to give (\textsuperscript{63}) as a colourless oil (0.26 g, 98% yield) \textsuperscript{1}H n.m.r. (CD\textsubscript{3}CN); \delta 7.85-7.20 (m, PhH, 20H), 5.05 (s, PhCH\textsubscript{2}OCO\textsuperscript{−}, 2H) 4.76 (m, CH, 1H), 4.42 (s, PhCH\textsubscript{2}O, 2H), 4.05 (br, NH, 1H), 3.73 (m, CH\textsubscript{2}N\textsuperscript{−}, 2H)\textsuperscript{*}, 3.63 (s, OCH\textsubscript{3}, 3H), 3.45 (m, -NCH\textsubscript{2}, 2H)\textsuperscript{*}, 3.14 (m, CH\textsubscript{2}, 2H) p.p.m., c.i.m.s. (CH\textsubscript{4}) m/z 588 (M\textsuperscript{+} + 2, 10%), 478 (75), 443 (100), 461 (20), 453 (15).

\*Assigned by spin decoupling
5.8.13  
(S)-N^α-Z-N^β-(Benzyloxyethyl)-N^β-(Benzy)-2,3-Diaminopropionic Acid Methyl Ester (64)

Compound (57) [0.40 g, 1.03 mmol] was added to KF-Celite* (0.6 g) in dry acetonitrile (10 cm³), followed by benzyl bromide (0.14 cm³, 1.22 mmol, ρ = 1.4 g cm⁻³) with stirring. After 24 hours the mixture was filtered and evaporated, and the residue was pumped at high vacuum (0.01 mmHg) for 48 hours to give (64) as a colourless oil (0.47 g, 95% yield), [α]D²¹ -2.0° (c 3 in CH₂Cl₂), ¹H n.m.r. (CDCl₃): δ 7.2 (m, PhH, 15 H), 6.05 (br, NH, 1H), 5.00 (q, PhCH₂OCO⁻, 2H), 4.37 (s, PhCH₂O⁻, 2H), 4.28 (m, CH, 1H), 3.60 (s, PhCH₂N⁻, 2H), 3.56 (s, OCH₃, 3H), 3.37 (m, CH₂, 2H), 2.93 and 2.65 (2 x m, CH₂NCH₂, 2 x 2H) p.p.m. c.i.m.s. (NH⁺) m/z 477 (M⁺ + 1, 28%), 369 (22), 254 (25), 108 (10), 91 (40), 18 (100).

5.8.14  
(S)-N^α-Z-N^β-Boc-2,3-Diaminopropionic Acid Methyl Ester (67)

The amine-ester (53) [0.68 g, 2.7 mmol] was added to 2(t-butoxycarbonyloxyimino)-2-phenylacetonitrile (Boc-ON reagent) [0.66 g, 2.7 mmol] in dichloromethane (20 cm³) and the solution refluxed for 4 hours. The solvent was then evaporated and the residue then eluted with dichloromethane on silica gel (mesh < 230) under suction to remove the by-products, and then with methanol to give the title compound as white crystals (0.5 g, 71% yield) m.p. 75-77°C (lit. 74-77°C), [α]D²⁰ -7.0° (c 1.2 in MeOH) [lit.²³ [α]D -6.5°], ¹H n.m.r. (CDCl₃): δ 7.37

*Celite coated with potassium fluoride (1:1 w/w).
(m, PhH, 5H), 5.97 (br, NH, 1H), 5.11 (m, PhCH$_2$OCO$^-$, and NH, 3H), 4.43 (m, CH, 1H), 3.75 (s, OCH$_3$, 3H), 3.50 (m, CH$_2$, 2H), 1.42 (s, t Bu, 9H) p.p.m., i.r. (Nujol); 3350, 1720, 1420, 1250 cm$^{-1}$.

5.8.15 (S)-N$^6$-Boc-2,3-Diaminopropionic Acid Methyl Ester (68)

Compound (67) [0.50 g, 1.4 mmol] was added to 10% Pd/C catalyst (0.20 g) in methanol (80 cm$^3$) and hydrogenated (Parr, initial pressure 25 p.s.i.) for 5 hours. The mixture was then filtered through Celite and evaporated to give (68) as a white solid (0.30 g, 97% yield) m.p. ca. 120°C (dec.), $^1$H n.m.r. (CD$_3$CN); $\delta$ 8.55 (br, NH$_2$ and NH, 3H), 4.20 (m, CH, 1H), 3.79 (s, OCH$_3$, 3H), 3.71 (m, CH$_2$, 2H), 1.41 (s, t Bu, 9H) p.p.m.

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CHAPTER 6

The β-Lactam Approach to 593A

6.1 Introduction

6.2 Model Studies on the Synthesis of 593A

6.3 \((S)-3\)-Hydroxypiperidine

6.4 Experimental

6.4.1 \(N-(1'-\text{Nitroacetyl})\text{piperidine}\)

6.4.2 \(N-(1'-\text{Diazoo-1'-nitroacetyl})\text{piperidine}\)

6.4.3 \((S)-\gamma\text{-Carboxyl-}\gamma\text{-butyrolactone}\)

6.4.4 \((S)-\gamma\text{-Ethoxycarbonyl-}\gamma\text{-butyrolactone and}\n\quad (S)-\text{Diethylhydroxyglutarate}\)

6.4.5 \((S)-2\text{-Hydroxypentan-1,5-dioic acid diamide}\)

6.4.6 \(\gamma\text{-Formamido-}\gamma\text{-butyrolactone}\)

6.4.7 \((S)-\gamma\text{-Hydroxymethyl-}\gamma\text{-butyrolactone}\)

6.4.8 \((S)-\gamma\text{-Azidomethyl-}\gamma\text{-butyrolactone}\)

6.4.9 \((S)-5\text{-Hydroxypiperidin-2-one}\)

6.4.10 \((S)-3\text{-Hydroxypiperidine}\)

6.5 References
6.1 INTRODUCTION

In contrast to the strategy undertaken in the preparation of an analogue of 593A discussed in the previous three chapters, this chapter is concerned with an approach to 593A via a β-lactam intermediate.

The stereochemistry of cyclo(streptolutyl-streptolutyl) [593A] can be correlated with the penicillins, e.g. penicillin G (69) [Fig. 6.1.1]. It was thought that a strategy for the synthesis of β-lactam antibiotics and their nuclear analogues, described by Lowe et al.\(^1\), may be extended to a synthesis of 593A. Lowe et al.\(^1\) prepared β-lactam analogues of piperidine by photolysis of N-[(alkyloxycarbonyl)diazoacetyl]piperidines (72) to give \textit{cis} and \textit{trans} β-lactam stereoisomers (Scheme 6.1.1). This method was first applied to a synthesis of 593A by Golding and Smith\(^2\) who prepared the diastereoisomers (70) and (71) in a model study of the proposed synthesis. The intention was to prepare the 2,5-piperazinedione by dimerisation of the required diastereoisomer-(70).

\((S)-3\text{-Hydroxypiperidine (73)}\) is a vital intermediate in a total synthesis of 593A by this strategy. A convenient synthesis of (73) will be described,
Fig. 6.1.1 Comparison of 593A with a penicillin (see text.)

Scheme 6.1.1 Photocyclisation to obtain a β-lactam by Lowe et al.
involving an application of the Mitsunobu reaction. A possible modification to Golding and Smith's approach has also been explored.

6.2 MODEL STUDIES ON THE SYNTHESIS OF 593A

The approach to 593A adopted by Golding and Smith \(^2\) relied upon a photo-induced insertion reaction of an N-[(alkoxycarbonyl)diazoacetyl]piperidine (Scheme 6.2.1). This reaction was first applied to \(\beta\)-lactam synthesis by Corey and Felix \(^3\) in the preparation of methyl 6-phenylpenicillinate. The ease with which carbenes derived from \(\alpha\)-diazo amides undergo intramolecular C-H insertion, in contrast to the behaviour of non-rigid alkoxy carbonyl carbenes \(^4\), has been attributed to a conformational effect of the planar amide bond forcing the C-H bond close to the divalent centre \(^5\). Since the first report \(^3\), the reaction has been applied to the synthesis of penicillin and cephalosporin antibiotics \(^6\).

In addition to the problem of separating the stereoisomeric mixture produced by the cyclisation, Golding and Smith \(^2\) faced difficulties in converting the ethyl carboxylate function to a primary amine group. This was eventually achieved by reacting the anion of (7\(^4\)) with toluene-\(p\)-sulphonyl azide followed by trimethylsilyl chloride to give a mixture of isomeric azides which were reduced to the amine (7\(^5\)), which was a mixture of isomers. After hydrolysis of the lactam ring, the nitrogen functions of (7\(^0\))/(7\(^1\))
were blocked with trifluoroacetyl protecting group to facilitate separation of the diastereoisomeric aminoacids. In order to obtain 593A by this method it would be necessary to substitute an hydroxyl protected (S)-3-hydroxypiperidine derivative for piperidine in Scheme 6.2.1. After forming the 2,5-piperazinedione, the hydroxypiperidyl moieties could be deprotected and converted to chloropiperidyl groups using thionyl chloride which reacts without inversion at the chiral centre thus preserving the (S)-3-chloropiperidyl configuration.

A proposed modification to this route replaces the diethylmalonate starting material (Scheme 6.2.1) with a nitroacetate ester. A model reaction involving piperidine and methyl nitroacetate to give (76) was perfected by the author. If successful, this modification would avoid the complications involved in converting (74) to (75) via the azide since the nitro group could be reduced directly to the amine, and would also, of course, remove the need to resolve a mixture of stereoisomers. Compound (76) was converted to the diazo derivative (77), but all efforts to form a \( \beta \)-lactam by irradiating (77) failed. The actual outcome of this photolysis is not known; the diazo band (ca. 2200 cm\(^{-1}\)) in the infrared spectrum
Scheme 6.2.1 Approach to 593A by Golding and Smith².

Scheme 6.2.2 An approach to 593A based on Golding and Smith's strategy.
of (77) slowly disappeared during the irradiation using a medium pressure mercury vapour lamp, but β-lactam formation was not evident either in the i.r. spectrum or 220 MHz $^1$H n.m.r. spectrum of the product. An alternative modification using a nitroacetate ester can be proposed. This requires (S)-5-hydroxypiperidin-2-one (78) as a starting material and leads to the amino-acid (79) [Scheme 6.2.2] which is a precursor to 593A. The potential of this route may yet be realised since (78) was prepared as an intermediate in the synthesis of (S)-3-hydroxypiperidine (73) described below.

6.3 (S)-3-HYDROXYPYPERIDINE

In a short communication Inch and Deane described the preparation of (S)-3-hydroxypiperidine (73) from mannitol in eleven stages, (summarised in Scheme 6.3.1), of which some were rather poor. For example, crude (82) was obtained from (81) as the 'principal' product, and the oxidation of alcohol (84) to lactone (85) using chromium trioxide required 'repeated treatments' to effect complete conversion. A later report considered the asymmetric hydroboration of 1-methyl-1,2,3,6-tetrahydropyridine (86) using (-)-di-3-pinanylborane.
Scheme 6.3.1 Preparation of \((S)\)-3-hydroxypiperidine described by Inch and Deane (see text).
The reaction gave a mixture of achiral 4-hydroxy-1-methylpiperidine (20% yield) and the stereoisomers of 3-hydroxy-1-methylpiperidine (49% yield). The latter component was isolated and one isomer, \((R)\)-3-hydroxy-1-methylpiperidine (87) was separated in an unstated yield. The configuration of (87) was established by comparing it with the N-methylated product from a deamination reaction of (S)-arginine hydrochloride\(^9\). This reaction gives \((S)\)-3-hydroxypiperidine (73) via the 3-hydroxypiperidin-2-one (88), and resolution of a racemate.

None of these routes could be regarded as satisfactory for the preparation of \((S)\)-3-hydroxypiperidine (73). A number of possible alternatives were considered (Scheme 6.3.2). In each of these \((S)\)-\(\gamma\)-formamido-\(\gamma\)-butyrolactone (89) is an important intermediate. Compound (89) has been prepared\(^10\) from the mixture of ethyl esters (90) which is prepared\(^11\) in two steps from \((S)\)-glutamic acid (91) [Scheme 6.3.3]. The most direct route to (73) would be the selective reduction of the primary amide group of (89) to give (94) which should easily undergo ring expansion to (73) [path a Scheme 6.3.2]. A literature survey revealed a report by Suzuki et al.\(^12\) in which nitrile, nitro, and primary amide compounds are easily reduced to primary amines using sodium borohydride/transition metal salts systems. The reduction is said to proceed in good yields in hydroxylic solvents as well as non-hydroxylic solvents, examples for four amides are quoted\(^12\). These
Scheme 6.3.2 Possible routes to (S)-3-hydroxypiperidine.

Scheme 6.3.3 The route to the intermediate lactone (89).
include benzamide, and n-butyramide, which were reduced using sodium borohydride and cobaltous chloride hexahydrate in methanol at 30°C to give yields of 60% and 70% respectively. A 'typical' procedure is detailed using benzonitrile as the example, but no spectroscopic analysis was made of the products. Curiously this report also states that the ease of reduction of the amides was primary > secondary > tertiary, with particular difficulty being encountered in reducing 3° amides even at 100°C in dioxan.

Several attempts were made to repeat the procedure, using benzamide and n-butyramide as model systems with NaBH₄/CoCl₂ reducing agent. On each occasion only the starting amide was recovered from the reaction mixture. A number of other transition metal salts were suggested, including cupric chloride, which was tried but again without success. A literature search uncovered nine previous publications that cited the paper by Suzuki et al. Only one of these actually repeated and achieved the conversion of a nitrile into an amine by this method. A number of other reports also discuss the selective reduction of amides, but are mainly concerned with reducing tertiary amides and, by consensus, agree that these are more readily reduced than primary amides. Further, Brown and Rao state that unsubstituted amides are not readily reduced. Others have also been unable to reduce primary amides by Suzuki's method. Heinzman and Ganem have studied the mechanism of sodium borohydride/cobaltous chloride reductions.
They propose that one of the active species in the reduction of benzonitrile is cobalt boride, CoB$_2$, a black solid which is formed by the reagents in methanol, and strongly adsorbs the nitrile onto its surface. However, benzamide was not adsorbed onto cobalt boride to any appreciable extent and no reduction of the amide could be achieved. The full mechanism for this reduction has not yet been elucidated; Heinzman and Ganem were unable to identify the hydride donor in the reaction.

Efforts to reduce the sodium salt derivative (95) of (89) [prepared according to Childers and Struthers' method$^{18}$] were also unsuccessful.

\[
\text{OH}
\]

\[
\text{CONH}_2
\]

(95)

Russa and Caress have noted$^{19}$ that a pentachlorophenyl ester group linked to a tertiary amide was unaffected when the amide was reduced. This might be useful if applied to (89) although primary amides are more resistant to reduction than tertiary amides.

An alternative route to (S)-3-hydroxypiperidine (73) that was considered involves the aziridine intermediate (96) [path b Scheme 6.3.2]. Gassman and Fentiman have described the preparation and some properties of the (S)-isomer of (96) from (S)-proline$^{20}$, and recently Hammer and Weber have shown that the N-ethyl derivative of (96), i.e. (S)-1-azonia-1-ethylbicyclo[3.1.0]hexane
(97) reacts stereospecifically with nucleophiles by an 
$S_N^2$ mechanism. However, the ($R$)-isomer of (96) is 
needed to obtain (73) in the required ($S$)-configuration. 
Hence, the literature procedure to (96) could not be 
used. After encountering difficulties in reducing 
the amide-lactone (89) to (97) {Scheme 6.3.2}, using 
lithium aluminium hydride, the route was rejected in 
favour of a more promising alternative. 

Silverstein et al. have prepared ($S$)-$\gamma$-
hydroxymethyl-$\gamma$-butyrolactone (98) from ($S$)-glutamic 
acid (91) by reduction of the ester (90) with sodium borohydrine 
or more efficiently by reducing the acid (92) 
to (98) with borane-methyl sulphide complex. The lactone-
alcohol (98) was prepared by the latter procedure without 
difficulty, and used in a Mitsunobu reaction (HN$_3$/DIAD/TPP) 
[see Chapter 5] to give the azide (85). Hydrogenation 
of (85) in a Parr apparatus using Pd/C catalyst gave 
($S$)-5-hydroxypiperidin-2-one (78) directly; none of the 
intermediate lactone-amine (94) was detected. The 
lactam (78) had an optical rotation of $[\alpha]_D$ -6.3° compared 
to the literature value of $[\alpha]_D$ 0°, and was easily 
reduced to ($S$)-3-hydroxypiperidine (73) using lithium 
aluminium hydride (Scheme 6.3.4). This method is 
believed to be the simplest and most efficient preparation 
of (73) to date.
Scheme 6.3.4 Preparation of (S)-3-hydroxypiperidine by the author.
EXPERIMENTAL

6.4.1  \textbf{N-(1'-Nitroacetyl)piperidine (76)}

Piperidine (2.00 g, 0.023 mol), triethylamine (2.50 g, 0.024 mol), and methyl nitroacetate (2.00 g, 0.016 mol) were added to acetonitrile (20 cm$^3$) and the mixture boiled under reflux for 62 hours. The solution was evaporated and the residue was partitioned between dilute hydrochloric acid and dichloromethane, the organic layer was dried and then evaporated to give the title compound as a yellow oil (2.15 g, 74% yield), $^1$H n.m.r. (CCl$_4$); δ 5.31 (s, CH$_2$, 2H), 3.60 and 3.30 (2 x m, 2 x αCH$_2$, 2 x 2H), 1.67 (m, 2 x βCH$_2$ + γCH$_2$, 6H) p.p.m., e.i.m.s. m/z 172 (M$^+$, 18%), 138 (10), 126 (100), 112 (8), 97 (35), 84 (29), i.r. (Neat); 1650, 1550, 1465, 1355 cm$^{-1}$.

6.4.2  \textbf{N-(1'-Diazo-1'-nitroacetyl)piperidine (77)}

Compound (76) (2.10 g, 0.012 mol) was added to triethylamine (1.41 g, 0.014 mol) and tosyl azide (2.97 g, 0.015 mol) in acetonitrile (25 cm$^3$) and the reaction monitored by t.l.c. (silica gel F$_{254}^4$), eluted with diethyl ether. After 24 hours the solvent was evaporated and the residue chromatographed on silica gel (mesh < 230) under suction. The silica was first eluted with 10% (v/v) ether in petrol (b.p. 40-60°C) to remove tosyl azide of high $R_f$ and then with 50% (v/v) ether in petrol (b.p. 40-60°C) to give the pure diazo compound (77) (0.1 g, 4% yield), t.l.c. (silica gel F$_{254}^4$, ether) one spot $R_f$ 0.57, $^1$H n.m.r.
(CCl₄); δ 3.60 and 3.54 (2 x m, 2 x αCH₂, 2 x 2H), 1.70 (m, 2 x βCH₂ + γCH₂ 6H) p.p.m., i.r. (Neat); 2940, 2105, 1675, 1450, 1365 cm⁻¹.

6.4.3 (S)-γ-Carboxyl-γ-butyrolactone (92)
A solution of sodium nitrite (70.0 g, 1.01 mol) in water (150 cm³) was added dropwise over 6 hours to a stirred mixture of (S)-glutamic acid (100 g, 0.66 mol), water (266 cm³) and conc. hydrochloric acid (138 cm³) at 0-5°C. The solution was then stirred at room temperature for 18 hours before evaporating the solvent in vacuo. Ethyl acetate (400 cm³) was added to the residue and then filtered and dried and evaporated to give the crude product. The oil was purified by fractional distillation to give a colourless oil (b.p. 139-141°C, 0.1 mmHg) which crystallised to give (92) [65.7 g, 75.4% yield], m.p. 70-72°C (lit.¹¹ m.p. 71-73°C), [α]D²⁰ 15.5° (c 2 in EtOH) [lit.¹¹ [α]D 15.6°], ¹H n.m.r. (D₂O); δ 5.20 (t, CH, 1H), 2.7-2.4 (m, 2 x CH₂, 4H) p.p.m.

6.4.4 (S)-γ-Ethoxycarbonyl-γ-butyrolactone and (S)-Diethylhydroxyglutarate (90)
The lactone-acid (92) [36.0 g, 0.27 mol] was dissolved in ethanol (250 cm³) and conc. sulphuric acid (3 cm³) was added. The solution was refluxed for 45 hours and then evaporated in vacuo. The residue was partitioned between ether and water, and the organic layer dried and evaporated to give the mixture of esters (90) [20.3 g] i.r. (Neat); 3480, 2980, 1785, 1720, 1440, 1370 cm⁻¹.
6.4.5  \((S)-2\text{-Hydroxypentan-1,5-diic acid diamide (93)}\)

The mixture of esters (90) [20.0 g] was stirred in conc. ammonium hydroxide solution for 2 hours. The precipitate was filtered, washed with water, and dried (\(P_{4}O_{10}\), in vacuo) to give the diamide (93) [14.6 g] m.p. 192-193°C (dec.) \(^1H\) n.m.r. (D\(_2\)O); 4.20 (m, CH, 1H), 2.45 (m, CH\(_2\)CH, 2H), 1.95 (m, CH\(_2\), 2H) p.p.m., i.r. (Nujol); 3395, 1695, 1350, 1180 cm\(^{-1}\).

6.4.6  \(\gamma\text{-Formamido-\(\gamma\)-butyrolactone (89)}\)

Diamide (93) [1.85 g, 0.012 mol] was heated in a sublimation apparatus at 160°C and 0.1 mmHg for 4 hours to give crystals of (89) [1.5 g, 91% yield], m.p. 90-91°C, \(^1H\) n.m.r. (D\(_2\)O); \(\delta\) 5.02 (t, CH, 1H), 2.65 and 2.05 (2 x m, 2 x CH\(_2\), 3H and 1H) p.p.m., i.r. (Nujol); 3390, 1775, 1695, 1455, 1185 cm\(^{-1}\).

6.4.7  \((S)\text{-\(\gamma\)-Hydroxymethyl-\(\gamma\)-butyrolactone (98)}\)

2 mol dm\(^{-3}\) Borane-methyl sulphide complex in THF (100 cm\(^3\), 0.2 mol) was added over 1½ hours to the lactone-acid (92) [22.50 g, 0.17 mol] in dry THF (150 cm\(^3\)) under nitrogen with stirring. After a further 3 hours the reaction was quenched by cautious addition of dry methanol (150 cm\(^3\)). Most of the solvent was removed by distillation at atmospheric pressure. Methanol (50 cm\(^3\)) was added and then evaporated (in vacuo) to leave an oil which was fractionally distilled to give the title compound [b.p. 128-130°C, 0.08 mmHg] (17.2 g, 84% yield),
[\alpha]_D^{21} 31^\circ \text{ (c 2.8 in EtOH)} \text{ [lit.}^{11} [\alpha]_D 31.3^\circ], \text{ }^1H \text{ n.m.r. (CDCl}_3); \delta 4.65 \text{ (m, CH, 1H), 3.92-3.61 (m, CH}_2, 2H), 3.5 \text{ (br, OH, 1H), 2.55 (m, CH}_2, 2H), 2.35-2.08 \text{ (m, CH}_2, 2H) \text{ p.p.m., i.r. (Neat); 3420, 1765, 1185 cm}^{-1}.

6.4.8 (S)-\gamma-Azidomethyl-\gamma-butyrolactone (85)

Lactone-alcohol (98) \text{ [3.00 g, 0.025 mol]} \text{ and triphenylphosphine were dissolved in dry benzene (30 cm}^3). \text{ 0.5 mol dm}^{-3} \text{ Hydrazoic acid in benzene (62.0 cm}^3, \text{ 0.031 mol)} \text{ was added followed by diisopropylazodicarboxylate (5.74 g, 0.028 mol) in benzene (20 cm}^3) \text{ with stirring. After 24 hours the solvent was evaporated and the residual red oil chromatographed on silica gel (mesh 70-230) eluting with dichloromethane to give the azide as a colourless oil (1.90 g, 52.1\% yield), [\alpha]_D^{20} 75.6^\circ \text{ (c 1.56 in CH}_2\text{Cl}_2), \text{ }^1H \text{ n.m.r. (CDCl}_3); \delta 4.65 \text{ (m, CH, 1H), 3.5 \text{ (dd, CH}_2\text{N}_3, 2H, J 4.6, 13.6 Hz), 2.58 \text{ (m, CH}_2, 2H), 2.33 and 2.08 \text{ (2 x m, CH}_2, 2 \times 1H) \text{ p.p.m., e.i.m.s. m/z 141.0538 (M}^+, 1\% \text{ [C}_3\text{H}_7\text{N}_3\text{O}_2 \text{ requires 141.0538], 114 (20), 85 (100), 57 (8), i.r. (Neat); 2100, 1775, 1460, 1270, 1180 cm}^{-1}.}

6.4.9 (S)-5-Hydroxypiperidin-2-one (78)

Azide (85) \text{ [1.80 g, 0.012 mol]} \text{ was added to 10\% Pd/C catalyst (0.2 g) in methanol (150 cm}^3) \text{ and hydrogenated (Parr, initial pressure 20 p.s.i.) for 3 hours. The solution was filtered through Celite and evaporated to give an oil which crystallised almost immediately. The
solid was recrystallised from methanol-ether to give the title compound (1.00 g, 73% yield) m.p. 139-142°C (lit.\textsuperscript{7} m.p. 125-127°C), \([\alpha]_D^{20} -6.3^\circ\) (c 1.2 in H\textsubscript{2}O) (lit.\textsuperscript{7} [\alpha]_D^0), \(^1\text{H n.m.r. (D}_2\text{O}); \delta 4.20 \text{ (m, CH, 1H), 3.52-3.20 \text{ (m, CH}_2\text{ND, 2H), 2.90 \text{ (m, CH}_2\text{CO, 2H), 1.97 \text{ (m, CH}_2\text{CH, 2H) p.p.m. e.i.m.s. m/z 115 (M\textsuperscript{+}, 73%), 98 (12), 91 (29), 86 (11), 58 (33), 30 (100), i.r. (Nujol); 3200, 1630, 975, 854 cm}^{-1}).

6.4.10 \((S)-3\text{-Hydroxypiperidine (73)}\)

Lactam (78) [0.16 g, 1.4 mmol] was added to lithium aluminium hydride (0.1 g, 2.63 mmol) in dry 1,4-dioxan (15 cm\textsuperscript{3}) and the mixture was boiled under reflux for 14 hours. Water (0.2 cm\textsuperscript{3}) was added cautiously to the cooled solution, followed by 15% (w/v) sodium hydroxide solution (0.2 cm\textsuperscript{3}) and water (2 cm\textsuperscript{3}). The precipitate was filtered and washed with water. The filtrate was then acidified with 1 mol dm\textsuperscript{-3} hydrochloric acid and evaporated. The residue was recrystallised from water to give the hydrochloride of the title compound (0.07 g, 50% yield), \([\alpha]_D^{20} -7.5^\circ\) (c 1.0 in MeOH) [lit.\textsuperscript{7} [\alpha]_D^0 -7.5^\circ], \(^1\text{H n.m.r. (D}_2\text{O}); \delta 4.10 \text{ (m, CH, 1H), 3.15 \text{ (m, 2 x aCH}_2\text{, 4H), 1.80 \text{ (m, bCH}_2\text{ + yCH}_2\text{, 4H) p.p.m., i.r. (Nujol); 3340, 1145, 980 cm}^{-1}).\]

6.5 REFERENCES

10. B. T. Golding and A. J. Smith unpublished results