SELECTIVE PROTECTION OF POLYAMINES:
SYNTHESIS OF SPERMIDINE DERIVATIVES

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SYNTHESIS OF SPERMIDINE DERIVATIVES

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Aos meus Pais
Aos meus Irmãos
Aos meus Sobrinhos
À memória da minha Irmã Maria de Fátima
ACKNOWLEDGEMENTS

The experimental work described here was carried out at the Institute of Biochemistry, Biomedical Center, University of Uppsala, Sweden, from September/1986 - May/1989, under the supervision of Doctor Ulf Ragnarsson.

I wish to express my sincere gratitude to Doctor Ulf Ragnarsson, who kindly accepted me in his Research Group, for his constant encouragement, support, invaluable insights and guidance throughout the course of this project. His friendship, great enthusiasm and availability for discussions at any time were a constant during my stay.

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I also wish to express my gratitude to The Swedish Institute for a scholarship from September/1986 to May/1989 and to The University of Porto, Portugal, for a leave of absence for a period of three years.

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All my Friends who contributed in some way for the success of my work.
Natural polyamines and their derivatives are important compounds, involved in many processes on the cellular level. Recent progress in this field has shown their potential as antineoplastic agents and in the treatment of parasitic diseases.

From a synthetic point of view, the simultaneous presence of primary and secondary amino groups attracted our interest, particularly so, as in a triamine such as spermidine, with a secondary and two non-equivalent primary groups. Thus, chemically as well as biologically, polyamines are challenging compounds.

Although several methods have been developed for selective functionalization of polyamines, an alternative simple procedure for selective protection of mixed primary/secondary amines seemed desirable.

The novel chemistry based on the exhaustive tert-butoxy-carbonylation of amide type groups, which was developed in Doctor Ragnarsson's group, seemed a promising basis for the development of new methodologies for the synthesis and selective protection of polyamines. Thus, the present work, carried out during nearly three years at the Institute of Biochemistry, Biomedical Center, University of Uppsala, Sweden, under the supervision of Doctor Ulf Ragnarsson, had as its principal objective the study of selective protection of spermidine using the new approach, the DMAP-catalysed N-tert-butoxycarbonylation of urethane groups.

It was possible to synthesize the N\textsuperscript{1},N\textsuperscript{8}-big(tert-butoxycarbonyl)spermidine and N\textsuperscript{1}-benzyloxy carbonyl-N\textsuperscript{8}-tert-butoxy-carbonylspermidine. Then was studied the applicability of these compounds to synthetic work with the preparation of some biologically interesting substrates, such as acetyl and ethyl spermidine derivatives.
In connection with the previous study and aiming at total synthesis of polyamines, several Gabriel type reagents, ZNHCOR, were also prepared. It is expected that in the future these derivatives will be useful for the synthesis of natural polyamine-containing substrates.

The results obtained during this research project are the subject of this dissertation, which also includes an introductory section on the biological aspects of natural polyamines and a review of the methods commonly used in their synthesis.

Part of the results presented in this thesis is the basis of four original articles published in collaboration 109a-d.
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ABBREVIATIONS

Ac  acetyl
Adoc  1-adamantylxycarbonyl
Aloc  allyloxy carbonyl
aq.  aqueous
ATP  adenosine triphosphate
Bz  benzoyl
Bzl  benzyl
Boc  tert-butoxycarbonyl
Boc-ON  tert-butoxycarbonyloxyimino-2-phenylacetoni trile
Bu  n-butyl
Bu  tert-butyl
CDI  N,N'-carbonyldiimidazole
DC  decarboxylase
DEAEA  2-diethylaminoethylamine
DFMO  a-difluoromethylornithine
DMAP  4-dimethylaminopyridine
DMF  N,N-dimethylformamide
DNA  deoxyribonucleic acid
DPP-ox  diphenyl-2-oxo-3-oxazolinylphosphonate
eq.  equivalent
Et  ethyl
Fmoc  9-fluorenlymethoxycarbonyl
h  hour
Hox  2-oxazolone
h.p.l.c.  high-performance liquid chromatography
Me  methyl
m.p.  melting point
MPP  4-(4-methyl-1-piperidinyl)pyridine
NMM  N-methylmorpholine
n.m.r.  nuclear magnetic resonance
ODC  ornithine decarboxylase
Ph  phenyl
Pht  phthaloyl
Ppoc  2-phenylisopropyloxycarbonyl
Pr  iso-propyl
RCO-Im  acylimidazoles
RCO-ox  3-acyl-2-oxazolone
RCO-TT  3-acylthiazolidine-2-thione
Red.  reduction
Red-Al  sodium bis(2-methoxyethoxy)aluminiumhydride
r.t.  room temperature
spd  spermidine
TcBoc  2,2,2-trichloro-tert-butoxycarbonyl
TEA  triethylamine
TFA  trifluoroacetic acid
THF  tetrahydrofuran
t.l.c.  thin layer chromatography
TMG  N,N,N',N'-tetramethylguanidine
TMS  tetramethylsilane
Tos  tosyl
Troc  2,2,2-trichloroethoxycarbonyl
Z  benzyloxy carbonyl
Z(NO₂)  4-nitrobenzyloxy carbonyl
ZOBt²  benzyl benzotriazol-1-yl carbonate
Z(OMe)  4-methoxybenzyloxy carbonyl
Z-TT  3-benzyloxy carbonyl thiazolidine-2-thione

Notes:

a) The nomenclature of the protecting groups is that recommended by IUPAC-IUB as summarized in Pure Appl. Chem., 1984, 56, 595.

b) The bibliographic references are presented in order of their appearance. The abbreviations of the journals are those adopted by the Chemical Society of London.

c) According to the ref. 35a, spermidine is numbered as follows:

\[ \text{NH}_2\text{CH}_2\text{CH}_2\text{NHCH}_2\text{CH}_2\text{CH}_2\text{NH}_2 \]
1 - POLYAMINES

1.1 - Biological aspects

The aliphatic polyamines putrescine 1, spermidine 3, and spermine 4 (Fig. 1) constitute the principal members of a family of natural products present in a relatively high concentration in most living organisms.

\[
\begin{align*}
\text{Putrescine} & : \quad \text{NH}_2(\text{CH}_2)_4\text{NH}_2 \\
\text{Cadaverine} & : \quad \text{NH}_2(\text{CH}_2)_5\text{NH}_2 \\
\text{Spermidine} & : \quad \text{NH}_2(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NH}_2 \\
\text{Spermine} & : \quad \text{NH}_2(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NH}(\text{CH}_2)_3\text{NH}_2
\end{align*}
\]

Figure 1 Structural representation of the naturally occurring polyamines. Spermine is unique to eukaryotes whereas cadaverine is found mainly in prokaryotes.

During the last few years there has been an increasing interest in polyamines and great progress has been made in this field. Although their mechanisms of action are not yet understood in detail, many studies have shown that these substances play a key role in a variety of cellular processes. In this chapter some of the present developments will be briefly outlined whereas detailed aspects of polyamine biochemistry and physiology can be found in recent excellent
reviews\textsuperscript{1-6}. The general metabolic reactions responsible for polyamine biosynthesis and interconversion are outlined in Fig. 2. The initial step is the formation of putrescine \textsuperscript{1}. In organisms lacking arginine decarboxylase, such as mammalian cells and lower eukaryotes, the only route to putrescine is that catalysed by the initial and rate-limiting enzyme ornithine decarboxylase (ODC). On the other hand, plants and some bacteria can initiate this synthetic pathway either directly from ornithine or indirectly from arginine through the activity of arginine decarboxylase via the intermediate agmatine. Putrescine is then converted to spermidine by the enzymatic coupling of an aminopropyl group, derived from decarboxylated S-adenosylmethionine, to one of the terminal amino groups by spermidine synthase. In a reaction catalysed by another aminopropyl transferase, spermine synthase, spermidine is converted to spermine. Prokaryotes lack this last enzyme and therefore spermine. Although the aminotransferase reactions are irreversible, these polyamines can be interconverted via consecutive enzymatic N-acetylation and oxidation reactions. This cyclic process has been explained in terms of the prevention of toxic levels of intracellular polyamines either by their interconversion and degradation or facilitation of their excretion from the cell due to a decrease in the net charge of the polyamine\textsuperscript{7}.

Under physiological conditions the polyamines are largely protonated. Thus, these conformationally mobile polycations
can associate to various extents with anionic binding sites in nucleic acids and membrane phospholipids through electrostatic charge interactions\textsuperscript{4,6,8}. With such macromolecules these interactions have specific structural requirements. For instance, in double-helical regions of DNA the positively charged amino groups of spermine strongly bind to two phosphate groups on one DNA strand and the tetramethylene chain of the polyamine molecule bridges the minor groove to interact with two phosphates on the second DNA strand (Fig. 3). Thus, spermine stabilizes the double helix by binding its two
strands together. This effect is also correlated with the ability of the polyamines to induce the transition from the usually right-handed (B-DNA) to a left-handed (Z-DNA) double helix conformation of DNA. This property has been correlated with a large variety of biological effects such as the stabilization of nucleic acids and the stimulation of RNA, DNA, and protein biosynthesis.

Many findings indicate that these natural polycations have a key role in cell growth and proliferation. These include the observation that: polyamines serve as growth factors for cultured cells; they are found in larger amounts in growing than in non-growing tissues; prokaryotic and eukaryotic mutants deficient in polyamine biosynthesis are auxotrophic for polyamines, and, a more recent and stronger evidence, depletion of intracellular polyamine levels by highly
specific inhibitors of their biosynthesis results in a
cessation of cell growth. The latter feature has received
great attention as it provides biological information
regarding polyamine function and, moreover, it offers
promising prospects for antineoplastic drug action\textsuperscript{1-6}. The
potential of interference with polyamine biosynthesis and/or
function as an anticancer chemotherapeutic strategy is due to
the fact that polyamines are essential for cell
proliferation, the rates of polyamine uptake and biosynthesis
are increased in neoplastic tissues, and the ability of
inhibitors to slow down neoplastic cell growth.

Ornithine decarboxylase (Fig. 2), has been the principal
target for designing irreversible enzyme-activated inhibitors
("suicide inhibitors"). \textit{\alpha}-Difluoromethylornithine (DFMO) is a
potent and specific ODC inhibitor\textsuperscript{1-6}. Its specificity is due
to the fact that only ODC can decarboxylate and thus activate
this substrate analogue. After the enzymatic decarboxylation,
the highly reactive electrophilic intermediate alkylates a
nucleophilic residue within the active site, thus inactivating
the enzyme (Fig. 4)\textsuperscript{6}. By inhibiting the ODC activity, DFMO is
effective in depleting intracellular putrescine and
spermidine. This process can be restored by adding exogenous
polyamines to the cells treated. This inhibitor has proved
important in delineating the effects of polyamine depletion in
animals\textsuperscript{6}. For instance, inhibition of polyamine synthesis by
DFMO treatment in early embryogenesis suppresses protein
synthesis and prevents development. Another important feature
Figure 4 Postulated mechanisms for the irreversible inhibition of ODC by DFMO.

is that when cells in culture are depleted of polyamines with this inhibitor, their progression through the cell cycle is slowed down and sometimes arrested. This cytostatic effect of DFMO has been exploited in the treatment of tumour and cancer cells due to a difference in the response to the inhibitor between normal and transformed cells. Thus, either alone or in combination with other cytotoxic agents, DFMO has been tested clinically as an anticancer agent and encouraging results have been obtained in leukaemia and melanoma. Besides being promising for an anticancer chemotherapy strategy, DFMO has also been exploited in the treatment of the major parasitic diseases, such as African trypanosomiasis (sleeping sickness), malaria, cryptosporidiosis, and more recently, Pneumocystis carinii, an opportunistic protozoan infection in patients with
acquired immune deficiency syndrome (AIDS). Although DFMO is essentially nontoxic it has certain limitations. One of them is the cytostatic rather than cytotoxic response of most tumour systems where the arrest in tumour cell proliferation is rapidly reversed by the removal of the drug or uptake of polyamines. Other limitations are that DFMO is soon cleared from the body and enters into the cells by diffusion rather than by an active amino acid transport. Thus a high concentration is necessary.

Another promising alternative to specific inhibitors of polyamine biosynthetic enzymes is based on the use of polyamine analogues bearing a close structural resemblance to the natural polyamines, such as the ethylated spermidine and spermine derivatives. The basic idea behind the design of these analogues is that they may be taken up into the cell by the polyamine transport system which is more active in proliferating cells. Once inside the cell, polyamine analogues could exert antiproliferative effects by some of the following mechanisms: inhibition and/or regulation of polyamine biosynthetic enzymes; competition for polyamine binding sites and subsequent disruption of critical macromolecular structure and/or function, or as vector molecules for delivering to cancer cells biologically active moieties or small antineoplastic agents. Studies with N\textsuperscript{4}- or N\textsuperscript{1},N\textsuperscript{8}-substituted spermidine analogues showed that the primary amino groups of spermidine are more critical than the secondary one as determinants of cellular uptake and in functions required for
cell proliferation. The central amino group is more important to regulatory activities of spermidine relevant to ODC activity. The alkylated spermidine analogues are more effective derivatives than the corresponding acylated ones because the presence of a positive charge at any of the amino functionalities also plays a very important role in uptake specificity.9d

In accordance with these features, regulation of polyamine biosynthesis by polyamine analogues seems to represent another antiproliferative strategy.9 Generally this approach might offer certain advantages relative to specific enzyme inhibitors (Table I): by utilizing the polyamine transport system, the derivatives should penetrate cells more effectively and at relatively low concentrations; the activity of more than one biosynthetic enzyme may be negatively regulated at the same time; compensatory increases in related enzymes may not occur as they do with enzyme inhibitors and might give substantial decreases in the pools of all polyamines including spermine.

Besides their existence in free form, the most common polyamines, spermidine and spermine, and their homologues are incorporated in many naturally occurring products such as sugars,10 phospholipids,11 peptides,12 alkaloids,13 and siderophores,14 which also have a wide range of important biological activities (Table II, Fig. 4).

In conclusion, natural polyamines seem to have an important role in cell growth and differentiation and, like their
conjugates, have potential as drugs in medicine. These features have prompted the outline of synthetic routes to polyamines and analogues which will be reviewed in the next section.

Table I - Cellular effects by treatment with modulators of polyamine biosynthesis.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DFMO</th>
<th>DEspda</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effective dose</td>
<td>1.5 mM</td>
<td>10-100 µM</td>
</tr>
<tr>
<td>Growth effect</td>
<td>Cytostatic</td>
<td>Cytostatic</td>
</tr>
<tr>
<td>ODC activity</td>
<td>Depleted</td>
<td>Depleted</td>
</tr>
<tr>
<td>S-Adenosylmethionine DC activity</td>
<td>Increased</td>
<td>Decreased</td>
</tr>
<tr>
<td>Putrescine</td>
<td>Depleted</td>
<td>Depleted</td>
</tr>
<tr>
<td>Spermidine</td>
<td>Depleted</td>
<td>Depleted</td>
</tr>
<tr>
<td>Spermine</td>
<td>Increased</td>
<td>Decreased</td>
</tr>
<tr>
<td>S-Adenosylmethionine</td>
<td>Decreased</td>
<td>Unchanged</td>
</tr>
<tr>
<td>Decarboxy-S-adenosylmethionine</td>
<td>Increased</td>
<td>Unchanged</td>
</tr>
<tr>
<td>Spermidine uptake</td>
<td>Increased</td>
<td>Unchanged</td>
</tr>
</tbody>
</table>

aN1,N8-Diethylspermidine.

Table II - Examples of natural polyamine-containing compounds.

<table>
<thead>
<tr>
<th>No</th>
<th>Compound</th>
<th>Biological properties</th>
<th>ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>5a-c</td>
<td>Glycocinnamoyl-spermidines</td>
<td>Broad-spectrum antibiotics</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>Trypanothione</td>
<td>Trypanosomatid metabolite</td>
<td>12</td>
</tr>
<tr>
<td>7</td>
<td>Solapalmitine</td>
<td>Tumour inhibitory</td>
<td>13c</td>
</tr>
<tr>
<td>8</td>
<td>Agrobactin</td>
<td>Iron-chelating</td>
<td>14a</td>
</tr>
<tr>
<td>9a-c</td>
<td>Acarnidines</td>
<td>Antiviral and antimicrobial</td>
<td>15</td>
</tr>
<tr>
<td>10</td>
<td>Spergualin</td>
<td>Antitumour antibiotic</td>
<td>16</td>
</tr>
</tbody>
</table>
Figure 5 Structures of some of the natural polyamine-containing compounds.
1.2 - Synthesis of polyamines and their analogues

1.2.1 - Introduction

The wide application of synthetic derivatives of polyamines for biological purposes has created a demand for efficient synthetic procedures. Thus, an important field in polyamine chemistry is the development of syntheses of such compounds. The remaining part of this chapter provides a comprehensive review of the previous work in this field.

A major problem in the synthesis of naturally occurring polyamines and their analogues is the selective modification of the different amino groups. As simple approaches suffer from problems of regioselectivity, in recent years different procedures have been developed for selective modification and functionalization of various polyamines.

The two general strategies, which are based on total synthesis or on selective protection of preexisting polyamines, will be described below.

1.2.2 - Total synthesis of polyamines

This methodology is of general applicability by which the complete backbone of polyamines is elaborated using adapted preparative methods for amines such as alkylation and reduction reactions.
1.2.2.1- Alkylation methods

A. Alkylation of tosylamides

A key step for the assembly of the carbon-nitrogen framework is the mono- or di-N-alkylation of N-protected-N'-Tos- alkanediamines and N,N'-Tos₂- alkanediamines, respectively, with haloalkanes bearing a latent primary amine group to afford the protected polyamines (Scheme 1)²¹.

\[
\text{PhtN(CH}_2\text{)}_4\text{NHTos} \xrightarrow{\text{i}} \text{PhtN(CH}_2\text{)}_4\text{NTos(CH}_2\text{)}_3\text{NPht}
\]

\[
\text{TosNH(CH}_2\text{)}_4\text{NHTos} \xrightarrow{\text{i}} \text{PhtN(CH}_2\text{)}_3\text{NTos(CH}_2\text{)}_4\text{NTos(CH}_2\text{)}_3\text{NPht}
\]

Scheme 1 Alkylation of tosylamides. Reagents: i, PhtN(CH₂)₃Br, K₂CO₃ (DMF)²¹.

This method has been used for the synthesis of natural polyamine-containing substrates²²,²³. The N-protecting groups most preferred during alkylation are either the phthaloyl²¹ or some of acyl²² or urethane²³ type. The alkylating agents are the N-haloalkylphthalimides²¹,²²b and dihaloalkanes²²a.

The first reported threefold protected spermidine, N⁸-Z-N¹Pht-N⁴-Tos-spermidine, designed by Eugster et al.²³ in their synthetic work with polyamine-containing lactams, is an example of the applicability of this approach (Scheme 2).
PhtN(CH$_2$)$_4$Br $\xrightarrow{i}$ PhtN(CH$_2$)$_4$N$_3$ $\xrightarrow{ii}$ PhtN(CH$_2$)$_4$NH$_2$

15 16 17

TosNH(CH$_2$)$_4$NHZ $\xleftarrow{v}$ NH$_2$(CH$_2$)$_4$NHZ $\xleftarrow{iv}$ PhtN(CH$_2$)$_4$NHZ

19 18

PhtN(CH$_2$)$_3$NTos(CH$_2$)$_4$NHZ

21

Scheme 2  Synthesis of a fully protected spermidine.

Reagents: i, NaN$_3$; ii, H$_2$, Pd-C (EtOH); iii, ZCl (aq. NaHCO$_3$); iv, NH$_2$NH$_2$ (EtOH); v, TosCl (aq. NaOH); vi, PhtN(CH$_2$)$_3$Br, K$_2$CO$_3$ (DMF)\textsuperscript{23}.

More recently, in their studies of water-soluble carbodiimides which mimic the role of ATP/DNA via an autocatalytic pathway, Dörwald et al.\textsuperscript{24} have designed carbodiimides bearing a DNA-binding side chain which, by increasing the relative stability of the mixed complex carbodiimide template increase the rate of autocatalysis. Thus, they have synthesized polyamine-carbodiimides in which the phosphate-activating property of carbodiimide and the DNA-binding property of spermine are combined.

The preparation of the key precursor for the synthesis of the target carbodiimides, the tetramethylated spermine, was based on this methodology as shown in Scheme 3. A suitable terminally differentiated diaminobutane, 22, was subsequently N-alkylated with three alkylating agents of
type \( \text{X(\text{CH}_2)_3R} \) (where \( \text{X} = \text{Br}, \text{I} \) and \( \text{R} = \text{Cl}, \text{N(Me)Tos}, \text{NPht} \)). Two alkylating reagents carry a latent functionality for a primary amino group, the third one a latent functionality for a dimethylamino group. Two routes were available for the assembly of the carbon-nitrogen framework both starting with monoalkylation of tosylamide \( 22 \). The synthetic pathway using the phthalimide group as a primary amine genitor, has proved less effective. This is due to its low stability under the reaction conditions, mainly in the deformylation step and in the cleavage of the tosyl group. Once again, in the final step, the use of the phthalimide derivative was troublesome where the methylated spermine was contaminated with minor amounts of the corresponding alcohol. Instead, replacement of the chlorine atom and the conversion of bromide into azide followed by reduction to afford the spermine derivative \( 29 \) have turned out to be a better alternative.

Although this procedure has allowed the preparation of selectively modified spermidine and spermine, it is important to point out several aspects: the large number of steps involved, the drastic reaction conditions required for the removal of the protecting groups\( ^25 \) and, in some cases, the absence of totally site-specific alkylation\( ^26-28 \), which makes this method less attractive.
Scheme 3  Synthesis of N₁,N₁,N₄,N₈-Me₈-spermine. Reagents: i, Na, MeOH; ii, I(CH₂)₃N(Me)Tos (DMF); iii, conc. HCl (dioxan, reflux), base; iv, TosCl, TEA (CH₂Cl₂); v, NaH (DMF, 60 °C); vi, Br(CH₂)₃NPht; vii, Br(CH₂)₃Cl; viii, 48 % HBr (reflux); ix, 99 % HCOOH, 35 % HCHO (80 °C); x, NaN₃ (aq. MeOH, 60 °C); xi, Na₂S, (NaOH, 60 °C); xii, PhtNK (DMF); xiii, NaOH (reflux), HCl (reflux), NaOH; xiv, 0.48 M CH₃ONa (MeOH).
B. Alkylation of amino groups

Employing neutral alkylation with KF-celite\textsuperscript{30} of amino groups, Samejima et al.\textsuperscript{29} have prepared \textsuperscript{15}N-enriched spermidine and spermine. The key compounds of this method were putrescine or benzylamine as amine reagents and, Br(CH\textsubscript{2})\textsubscript{n}NPht (n = 3 or 4) as an aminoalkyl donor. The Scheme 4 depicts the two general procedures.

\begin{align*}
\textbf{(a)} & \\
\text{NH}_2(\text{CH}_2)_4\text{NH}_2 & \text{BzlNH}_2 \\
1 & 38 \\
\downarrow \text{i} & \downarrow \text{ii} \\
\text{NH}_2(\text{CH}_2)_4\text{NHZ} & \text{PhtN}(\text{CH}_2)_3\text{NHBzl} \\
35 & 39 \\
\downarrow \text{ii} & \downarrow \text{iv} \\
\text{PhtN}(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NHZ} & \text{PhtN}(\text{CH}_2)_3\text{NBzl}(\text{CH}_2)_4\text{NPht} \\
36 & 40 \\
\downarrow \text{iii} & \downarrow \text{v} \\
\text{NH}_2(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NH}_2\cdot 3\text{HCl} & \text{NH}_2(\text{CH}_2)_3\text{NBzl}(\text{CH}_2)_4\text{NH}_2 \\
37 & 41
\end{align*}

\textbf{Scheme 4} Synthesis of spermidine. Reagents: i, ZCl (AcONa, EtOH); ii, PhtN(\text{CH}_2)_3Br (KF-Celite, CH\textsubscript{3}CN, reflux); iii, HCl; iv, PhtN(\text{CH}_2)_4Br "(KF-Celite, CH\textsubscript{3}CN, reflux); v, NH\textsubscript{2}NH\textsubscript{2}; vi, H\textsubscript{2}/PtO\textsubscript{2}.
Procedure (a) involved an alkylation of monobenzyloxy carbonylputrescine in the presence of KF-celite to afford the diprotected spermidine 36 and traces of the dialkylated product. After removal of protecting groups by acid hydrolysis, spermidine was purified on a cation exchange column to afford the trihydrochloride salt of spermidine in 33% overall yield. In procedure (b), benzylamine was successively alkylated with the proper alkylating agents to afford the triprotected spermidine 40. The spermidine could be obtained in 30% yield by successive removal of the protecting groups.

This method was extended to the synthesis of spermine based on the previous procedure (b). The first step involved the synthesis of dibenzylputrescine via reduction of the Schiff base formed from putrescine and benzaldehyde. The secondary amino groups were further alkylated in a similar way to afford the tetraprotected spermine which could be deprotected to afford the tetrahydrochloride salt of spermine.

Recently, Kunesch et al.31, have described the synthesis of N1- and N8-monoacylated spermidines by monoalkylation of diaminoalkanes with the intermediate Br(CH2)nNHCOR32.

The main disadvantages of these approaches are the monoacylation and alkylation steps which afford to some extent the disubstituted derivatives leading to low yield reactions and purification problems. To minimize these by-products it is necessary to use large amounts of amine compound relatively to the acylating or alkylating agent.
1.2.2.2 - Reductive methods

A. Reduction of nitriles

The basic idea of this method is the reduction of nitriles, themselves obtained by cyanoalkylation, to afford part of the C-N polyamine backbone\(^{20,33}\).

\[
\text{red.} \quad \text{RCN} \rightarrow \text{RCH}_2\text{NH}_2
\]

Thus, polyamines can be prepared by mono and dicyanoalkylation of the appropriate diamines followed by catalytic reduction of the resulting nitriles (Scheme 5)\(^{34}\). The mono-cyanoethylated derivatives were prepared by dropwise addition of 1 equivalent of acrylonitrile into the diamine to avoid the possibility of dicyanoethylation which decreases with increase in the methylene chain of the diamine. In a similar way the dinitriles were prepared by 2 equivalents of acrylonitrile. The mononitriles could be purified by vacuum distillation below 160-170 °C. Above this temperature they were either reconverted to starting material by elimination of acrylonitrile or extensively decomposed. On the other hand, although the lower dinitriles could, to some extent, be isolated by distillation, the higher ones underwent extensive decomposition. The final step could then be carried out by catalytic hydrogenation (4 atm) with sponge Raney nickel in \(\text{NH}_3\)-saturated ethanol to suppress the formation of secondary amines.
\[
\begin{align*}
\text{NH}_2(\text{CH}_2)_n\text{NH}(\text{CH}_2)_2\text{CN} & \xrightarrow{\text{ii}} \text{NH}_2(\text{CH}_2)_n\text{NH}(\text{CH}_2)_3\text{NH}_2 \\
\uparrow \text{i} \\
\text{NH}_2(\text{CH}_2)_n\text{NH}_2 \\
\downarrow \text{i} \\
\text{NC}(\text{CH}_2)_2\text{NH}(\text{CH}_2)_n\text{NH}(\text{CH}_2)_2\text{CN} & \xrightarrow{\text{ii}} \text{NH}_2(\text{CH}_2)_3\text{NH}(\text{CH}_2)_n\text{NH}(\text{CH}_2)_3\text{NH}_2 \\
\end{align*}
\]

(n = 2, 3, 4, 5, 6, 9, 10, 12)

**Scheme 5** Synthesis of spermidine, spermine and their homologues. Reagents: i, CH\textsubscript{2}=CHCN; ii, H\textsubscript{2}/Ni (NH\textsubscript{3}, 3-4 atm)\textsuperscript{34}.

Besides, for the polyamines themselves, this methodology has proved useful for preparation of the precursors for synthesis of several polyamine-containing substances\textsuperscript{35-39}.

Tabor et al.\textsuperscript{35} have reported the synthesis of the two monoacetyl spermidines starting from 1,3-diaminopropane or putrescine which were monoacetylated in glacial acetic acid with acetic anhydride (30-50 %). The N\textsuperscript{1}-Ac- or N\textsuperscript{8}-Ac-spermidine was then prepared by reaction of 4-bromo-butynitrile or acrylonitrile, respectively, followed by catalytic reduction (H\textsubscript{2}/PtO\textsubscript{2}) of the mononitrile.

In their synthetic work with alkaloids Quick et al.\textsuperscript{36} have designed a synthesis of N\textsuperscript{4},N\textsuperscript{8}-Boc\textsubscript{2}-spermidine. The mononitrile adduct of putrescine, prepared as usual, was bis-tert-butoxycarbonylated with Boc-ON and, the resulting protected nitrile, was selectively reduced with LiAlH\textsubscript{4} in 70 % yield.
Thus, the $N^1$ nitrogen of spermidine remained free for further functionalization. In principle, this procedure can be extended to other mononitriles and dinitriles to afford the corresponding derivatives of spermine and its homologues. In the mononitrile series, only one primary nitrogen becomes free for selective modification and in the dinitrile one, it is only possible to differentiate the primary amino groups from the secondary ones. Therefore, this approach is of limited value for a general preparation of selectively protected polyamines. Moreover the reduction step may also be a limiting factor in the choice of the type of protective groups.

More recently, based on the above procedure, Bergeron et al.\textsuperscript{19,40} have developed a comprehensive methodology for selective modification of polyamines via their $N$-benzylated derivatives. The preparation of these compounds involved the same starting material, benzylamine, and the three basic reactions (Scheme 6). The first step consisted in consecutive monocyanooalkylation (spermidine derivatives) or dicyanoalkylation (symmetric spermidine homologues) of benzylamine. By selective reduction of the nitriles with LiAlH$_4$/AlCl$_3$ the monobenzylated polyamines 49, 53, and 55 were obtained. The corresponding free polyamines could then be obtained by debenzylation of these derivatives or directly from the benzylated nitriles by hydrogenolysis in acetic acid over a palladium catalyst.

Based on protection / deprotection of the amino groups of
Scheme 6 Synthesis of spermidine, homospermidine, and norspermidine via derivatives N-benzylated on their secondary amino groups. Reagents: i, CH$_2$=CHCN; ii, Cl(CH$_2$)$_3$CN, (butanol, Ca$_2$CO$_3$); iii, LiAlH$_4$/AlCl$_3$; iv, H$_2$/Pd; v, H$_2$/PtO$_2$. These benzylated derivatives, this research group has synthesized the N$_1$,N$_8$-Boc$_2$-spermidine (and its homologues) by direct tert-butoxycarbonylation with Boc-ON followed by hydrogenolysis of the benzyl group. Thus, these two types of polyamine precursors were further used with success as starting material for preparation of N$_1$,N$_8$- and N$_4$-substituted polyamines such as siderophores.

Although this approach is convenient and relatively simple, it is of limited applicability and, therefore, as a
complement to the above precursors, this group designed a triprotected spermidine and its homologues\textsuperscript{43}, which could then be extended to the spermine series\textsuperscript{44}.

Scheme 7 summarizes the different steps for the preparation of the fully protected polyamines. The nitriles of the type 51 (see Scheme 6) were reduced to diamine 57 by the usual procedure. Surprisingly, the authors could selectively tert-butoxycarbonylate the primary amino group using one equivalent of Boc-ON at 0 °C in high yield\textsuperscript{43}. These protected diamines were then cyanoalkylated to give the protected mononitriles which by selective reduction gave the diprotected polyamine 60. The final step involved the protection of the free amino group with the orthogonal protective trifluoroacetyl group\textsuperscript{25}.

Alternatively, the diprotected triamine 60 was cyanoethylated followed by selective reduction of the nitrile group. The resulting diprotected spermine 63 could be selectively trifluoroacylated\textsuperscript{44} at its primary amino function using the active ester N-(trifluoroacetoxy)succinimide\textsuperscript{45}. The final tetraprotected polyamine was obtained by acylation with TcBocCl\textsuperscript{46}.

These fully protected polyamines have been used with success as precursors of the polyamine backbone of siderophores\textsuperscript{47} and alkaloids\textsuperscript{48} and the orthogonality of these protective groups\textsuperscript{43,44} should allow the access to a large number of selectively functionalized polyamines provided they are stable to the reaction conditions.
Scheme 7 Synthesis of selectively, fully protected spermidine and spermine. Reagents: i, H₂/Raney nickel (aq. EtOH, NaOH, 4 atm); ii, Boc-ON (THF, 0 °C); iii, Cl(CH₂)₂CN (BuOH, NaCO₃, KI); iv, (CF₃CO)₂O (TEA, CH₂Cl₂); v, CH₂=CHCN (MeOH); vi, benzene solution of 0.34 M N-CF₃CO-succinimide (CH₂Cl₂, 0 °C); vii, TcBocCl (ether, 0.2 M NaOH).
The main drawback of this procedure is the reduction of the nitriles. Although the authors have previously accomplished the reduction with metal hydrides, the isolated yields were moderate (60 %). Further experiments, carried out by the same group\(^{49}\), have shown that the best procedure for reduction of the nitriles was hydrogenolysis (2.5-3 atm) using Raney nickel in ethanol in the presence of sodium hydroxide. Substituting ammonium hydroxide for sodium hydroxide or pretreating the catalyst with sodium hydroxide gave only 17 % and 33 %, respectively, of the desired amine. Due to these results the authors concluded that the sodium hydroxide must play an active role in this process and not simply preactivate the catalyst. Although this reduction method worked nicely for the different nitriles of \(N^4\)-benzylated polyamines giving high yields of these protected analogues, there are a few limitations. One of these is the low yield if the reaction is carried out on a small scale due to the adsorption of the reactant or product on the catalyst. Another limitation is the strong alkaline conditions of the reduction which restrict the choice of the protecting groups.

B. Reduction of amides

This method is based on the reduction of an amide group\(^{20,33}\) as the key step for assembly of the polyamine framework. The general procedure involves two main steps: the formation of an amide type bond and the reduction of the resulting polyamide as illustrated in Scheme 8\(^{50}\).
Using this approach, Das et al. have synthesized the $N^4,N^8$-Boc$_2$-$N^1$-Pht-spermidine and $N^1,N^8$-Boc$_2$-spermidine as the key intermediates for synthesis of several acylated conjugates of spermidine (Scheme 9). The initial step was the condensation of $N$-Boc-$4$-aminobutyric acid with $3$-amino-$1$-chloropropane using the mixed anhydride as coupling method. The resulting amide could then be selectively reduced to amine by Na(CF$_3$COO)BH$_3$ or first deprotected followed by reduction of the amide group with borane.

The main difficulty of this method is the reduction of the amide function. Although amides can be selectively reduced in the presence of urethane groups, it is of limited application. In some cases the desired amine has been obtained in low yield (30 %) or only the methylated amine was formed.
Scheme 9  Synthesis of two protected spermidines. Reagents: i, ClCO₂Et (TEA, THF, 0-5 °C); ii, Cl(CH₂)₃NH₂ (TEA, CH₃CN-CH₂Cl₂); iii, TFA (ether); iv, BH₂Me₃S (THF, 80 °C, N₂); v, Boc₂O (Na₂CO₃, dioxan-H₂O); vi, PhtNa (DMF, 60 °C, N₂); vii, N₂H₄ (EtOH, 80 °C); viii, Na(CF₃COO)BH₃ (THF).

Although it is a general method for polyamines and their analogues, it is relatively limited in scope when the protected precursors are desired.

C. Reduction of imines

The key step in this method is the formation of a secondary amine by reductive alkylation of the corresponding primary amine.²⁰,³³
Several naturally occurring polyamine derivatives\textsuperscript{55,56} have been synthesized using this general procedure which can be illustrated with the preparation of the N\textsubscript{8}-Ac-spermidine \textsuperscript{85} as shown in Scheme \textsuperscript{10}\textsuperscript{55}. Condensation of monoacetyl putrescine with the protected aldehyde \textsuperscript{82} gave the Schiff base \textsuperscript{83} which was reduced to the protected spermidine \textsuperscript{84}. The final product was then obtained by a simple deprotection step. Virtually, starting from different N-monoprotected diaminoalkanes and N-protected amino aldehydes it is possible to prepare selectively protected polyamines which can be modified at any nitrogen atom.

\[
\begin{align*}
\text{NH}_2(CH_2)_2\text{CH(OEt)}_2 & \xrightarrow{\text{i}} ZNH(CH_2)_2\text{CH(OEt)}_2 \\
80 & \quad \quad 81 \\
\downarrow & \quad \quad \downarrow \text{ii} \\
ZNH(CH_2)_2\text{CH=N(CH}_2)_4\text{NHAc} & \xleftarrow{\text{iii}} ZNH(CH_2)_2\text{CHO} \\
83 & \quad \quad 82 \\
\downarrow & \quad \quad \downarrow \text{iv} \\
ZNH(CH_2)_3\text{NH(CH}_2)_4\text{NHAc} & \xrightarrow{\text{v}} \text{NH}_2(CH_2)_3\text{NH(CH}_2)_4\text{NHAc} \\
84 & \quad \quad 85
\end{align*}
\]

\textbf{Scheme 10} Synthesis of \textsuperscript{8}-Ac-spermidine. Reagents: i, ZCl; ii, 0.06 M HCl (aq. dioxan 1:1); iii, AcNH(CH\textsubscript{2})\textsubscript{4}NH (CH\textsubscript{2}Cl\textsubscript{2}, Na\textsubscript{2}SO\textsubscript{4}); iv, NaBH\textsubscript{4} (MeOH); v, H\textsubscript{2}/10 % Pd-C (Et\textsubscript{2}OH).
The stability of the imine formed is of crucial importance to the success of this approach. The condensation-reduction process can be carried out in two ways: reduction of the isolated Schiff base or reduction of the formed imine in situ. Although in the literature high yields have been found in a "one-pot" synthesis, some authors have reported low yields in the range of 20-50%. This is probably due to further reaction of the formed secondary imine with aldehyde giving rise to tertiary amine. The procedure involving intermediate isolation seems to be more efficient but it is limited to stable imines.

D. Reduction of azides

The azide group can be reduced to primary amines by catalytic reduction or to secondary amines by their reductive alkylation with borane derivatives.

\[ \text{RN}_3 \xrightarrow{\text{H}_2, \text{Pd-C}} \text{RNH}_2 \]

\[ \text{RN}_3 + \text{R}^1\text{BCl}_2 \xrightarrow{} \text{ClBC-N-R} \xrightarrow{} \text{Cl}_2\text{B-NRR}^1 \xrightarrow{\text{MeOH}} \text{R}^1\text{NHR.HCl} \]

Recently, based on this feature, Carrié et al. have developed a method for building the polyamine backbone as outlined in Scheme 11. Thus, the amino azides could be reductively alkylated with the dichloroboranes of the type...
\[
R^1(R^2)N(CH_2)_nN_3 \quad \text{HCl} \quad \xrightarrow{i, \ ii} \quad R^1(R^2)N(CH_2)_nNH(CH_2)_mBr \quad 86
\]

\[
\downarrow \quad \text{iii, iv}
\]

\[
R^1(R^2)N(CH_2)_nNH(CH_2)_mNH_2 \quad \xleftarrow{\nu} \quad R^1(R^2)N(CH_2)_nNH(CH_2)_mN_3 \quad 88
\]

\[
\leftarrow \quad \text{vii, ii}
\]

\[
R^1(R^2)N(CH_2)_nNH(CH_2)_mNH(CH_2)_1Br \quad \text{3HCl} \quad 90
\]

\[
\downarrow \quad \text{vi, ii}
\]

\[
\downarrow \quad \text{iii, iv}
\]

\[
R^1(R^2)N(CH_2)_nNH(CH_2)_mNHR^3 \quad \text{3HCl} \quad 89
\]

\[
R^1(R^2)N(CH_2)_nNH(CH_2)_mNH(CH_2)_1N_3 \quad \ldots \quad 91
\]

\[
n,m = 2, 3; 1 = 3 \quad R^1 = \text{H, alkyl, acyl}; \quad R^2 = \text{H}; \quad R^3 = \text{H, alkyl}
\]

Scheme 11: Synthesis of polyamines by reduction of azides. Reagents: i, Br(\text{CH}_2)_nBCl \quad (\text{CH}_2\text{Cl}_2 \quad \text{or} \quad \text{C}_6\text{H}_6); \quad \text{ii,} \quad \text{MeOH}; \quad \text{iii,} \quad \text{NaN}_3 \quad (\text{H}_2\text{O}, \text{reflux}); \quad \text{iv,} \quad \text{H}_2/\text{Pd}_n\text{C}; \quad \text{v,} \quad R^1\text{BCl}_2; \quad \text{vi,} \quad \text{Br(\text{CH}_2)_nBCl}_2.

\[
\text{Br(\text{CH}_2)_mBCl}_2 \quad 65 \quad \text{to the derivatives 86 after methanolysis.}
\]

They were then transformed to the diamino azides 87 by nucleophilic substitution of bromide with NaN\textsubscript{3}. These compounds could be either hydrogenated or again alkylated by a suitable dichloroborane to give, respectively, the polyamines 88, 89 or 90. If desired, the triamine bearing a bromide atom, could again be treated in the same way to afford higher polyamines.
Although only few alkylated and acylated polyamine derivatives are reported, this approach seems to allow a flexible synthesis not only of polyamines themselves but also of their selectively modified analogues by using appropriately protected starting materials or protecting the intermediate amino azides or bromides.

1.2.3 - Direct selective protection and modification of polyamines

In this section will be described the second strategy for synthesis of polyamine analogues which is based on regioselective acylation and alkoxy carbonylation of primary amino groups or transiently protected polyamine systems masked either as cyclic ureas or as hexahydropyrimidines.

1.2.3.1 - Regioselective acylation and alkoxy carbonylation of primary amines

It is known that secondary amines are more basic and therefore generally more nucleophilic than primary ones towards electrophilic reagents. Nevertheless, the primary amino groups of polyamines such as spermidine show higher reactivity which is explained in terms of an intramolecular hydrogen bonding between the secondary nitrogen atom and the hydrogen of the aminopropyl moiety or steric factors when bulkier agents are employed. This important feature has
been exploited and recently several procedures have been reported where the primary amino groups are selectively functionalized without affecting the secondary ones using different selective reagents.  

A. 2-Oxazolones

Kunieda et al. have developed series of 3-acyl and 3-alkoxycarbonyl-2-oxazolones (RCO-ox) and the corresponding polymers as carbonyl transfer agents to different nucleophiles.

\[
\begin{align*}
\text{RCO-N} & \text{O} \\
\text{O} & \\
\end{align*}
\]

\[
\begin{align*}
\text{92a-c} \\
\text{93a,b} \\
\end{align*}
\]

\( a, R= -\text{CH}_3; b, R= -\text{OCH}_2\text{Ph}; c, R= -\text{OBu}^+ \)

The approach used either the ready-to-use type reagents RCOox or the corresponding carboxylic acids via diphenyl-2-oxo-3-oxazolinylphosphonate (DPP-ox) as carboxyl-activating reagent as depicted in the following scheme.

\[
\begin{align*}
\text{Hox} & \xrightarrow{(\text{PhO})_2\text{PCl}} (\text{PhO})_2\text{POx} \\
& \xrightarrow{\text{TEA}} \text{RCOO}^- \\
& \xrightarrow{\text{RCOOP(PhO)}_2} \text{RCOOF} \\
\xrightarrow{\text{R}^1\text{NHCOR}} & \left[ \begin{array}{c}
\text{R}^1\text{NH-COR} \\
\text{RCOO}\text{(PhO)}_2 \\
\end{array} \right] \\
\end{align*}
\]

(See ref. 124 for preparation of Hox)

**Scheme 12** Aminolysis of DPP-ox.
These reagents have shown several interesting features in the acylation of different amines and aminoalcohols. The selective N-acylation or N,O-diacylation of aminoalcohols could be accomplished in the absence or presence of a catalyst. Another characteristic of these reagents and more relevant to this study, is their reactivity in relation to amines and polyamines which leads to highly preferential acylation of less hindered amino functions. The steric effects were more pronounced with the bulkier polymeric reagent (Table III).

**Table III - Acetylation by polymeric reagent**

<table>
<thead>
<tr>
<th>Nucleophile</th>
<th>yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>PhCH₂NH₂</td>
<td>93</td>
</tr>
<tr>
<td>PhNH₂</td>
<td>10 (91)</td>
</tr>
<tr>
<td>1-Adamantanemethylamine</td>
<td>79 (85)</td>
</tr>
<tr>
<td>1-Adamantylamine</td>
<td>13 (41)</td>
</tr>
<tr>
<td>Cyclohexylamine</td>
<td>61</td>
</tr>
<tr>
<td>Dicyclohexylamine</td>
<td>0</td>
</tr>
<tr>
<td>CH₃CH₂NHCH₃CH₂NH₂</td>
<td>80</td>
</tr>
<tr>
<td>NH₂(C₆H₆)³NH(C₆H₆)⁴NH₂</td>
<td>93</td>
</tr>
</tbody>
</table>

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>Reaction conditions: THF, 6 hr, room temperature.</td>
</tr>
<tr>
<td>b</td>
<td>Acetylation by monomeric 92a.</td>
</tr>
<tr>
<td>c</td>
<td>Only the primary amino groups were acylated.</td>
</tr>
</tbody>
</table>

Thus, the authors have reported the synthesis of the bioactive maytenine by direct simple acylation of the primary amino groups of spermidine with 3-trans-cinnamoyl-2-oxazolone.
Scheme 13  Synthesis of maytenine. Reagents: i, PhCH=CHCO-ox (CH₃CN)₆₆f.

B. Thiazolidine-2-thiones

The 3-acyl- and 3-alkoxycarbonylthiazolidine-2-thione derivatives (RCO-TT)₆⁷ ⁹⁵ have been employed as coupling agents in peptide chemistry and as acylating reagents for amines and alcohols₆⁸.

![Chemical structure of RCO-TT](image)

The general sequence of the aminolysis reaction is shown in Scheme 14 which has a particular characteristic of being monitored by the disappearance of the yellow colour of the starting material (RCO-TT).

![Scheme 14 Aminolysis of 3-acyl-thiazolidine-2-thiones](image)
As in the case of the 2-oxazolones described above, this reagent also exhibited selectivity in relation to several amines. This is, however, affected by the electron density at the nitrogen atom and the sterical environment. Thus, this research group has also reported the syntheses of the spermidine alkaloids maytenine and codonocarpine by direct acylation of the primary amino functions, and the spermidine siderophore parabactin (Scheme 15), by previous direct selective protection of the primary amino groups with Z.

\[
\begin{align*}
H_2N(CH_2)_3NH(CH_2)_4NH_2 & \xrightarrow{i} ZNH(CH_2)_3NH(CH_2)_4NH_2 \\
3 & \xrightarrow{96} \\
H_2N(CH_2)_3NR(CH_2)_4NH_2 & \xrightarrow{iii} ZNH(CH_2)_3NR(CH_2)_4NH_2 \\
98 & \xrightarrow{iv} \\
R_1NH(CH_2)_3NR(CH_2)_4NHR_1 & \xrightarrow{v} R_2NH(CH_2)_3NR(CH_2)_4NHR_2 \\
99 & \xrightarrow{100} \\
\end{align*}
\]

\(R = -CO-C_6H_3-2,3-(OH)_2\) ; \(R_1 = -CO-C_6H_3-2,3-(AcO)_2\); \(R_2 = -CO-C_6H_3-2,3-(OH)_2\)

**Scheme 15** Synthesis of parabactin. Reagents: i, Z-TT (CH₂Cl₂); ii, phenyl-bis(2-thione-1,3-thiazolidinyl)phosphine oxide, \(2(Pr)_2NET (CH₃CN, N₂, reflux)\); iii, 25 % HBr-AcOH; iv, 2,3-diacetoxybenzoyl chloride, Et₃N (THF); v, \(K_2CO_3 (MeOH)\).
C. Imidazoles

In 1984 Scott et al.\textsuperscript{69} reported the synthesis of several N\textsubscript{1},N\textsubscript{8}-diacylspermidine derivatives by direct selective acylation at the primary nitrogen atoms with acylimidazoles (RCO-Im).

\[
\text{RCO-N} \quad \text{(RCO-Im)} \quad 101
\]

\(R=\) aryl

The general approach involves a two-step reaction: formation of the (RCO-Im) by treating the corresponding carboxylic acids with \(N,N'\)-carbonyldiimidazole (CDI) followed by the acylation step as illustrated in Scheme 16 for the synthesis of the natural siderophore \textsuperscript{105}69.

\[
\begin{align*}
2,3-(\text{BzlO})_2\text{-C}_6\text{H}_3\text{-COOH} & \overset{i}{\longrightarrow} 2,3-(\text{BzlO})_2\text{-C}_6\text{H}_3\text{-CO-Im} \\
& \overset{\text{ii}}{\longleftarrow} 2,3-(\text{BzlO})_2\text{-C}_6\text{H}_3\text{-CONH(CH}_2)_3\text{NH(CH}_2)_4\text{NHCO-C}_6\text{H}_3\text{-}2,3-(\text{BzlO})_2 \\
& \overset{\text{iii}}{\downarrow} 2,3-(\text{OH})_2\text{-C}_6\text{H}_3\text{-CONH(CH}_2)_3\text{NH(CH}_2)_4\text{NHCO-C}_6\text{H}_3\text{-}2,3-(\text{OH})_2
\end{align*}
\]

\textbf{Scheme 16} Synthesis of \(N^1,N^8\)-bis(2,3-hydroxybenzoyl)spermidine. Reagents: \textsuperscript{69}i, CDI (CH\textsubscript{2}Cl\textsubscript{2}); ii, spermidine; iii, \(H_2/Pd-C\) (MeOH, 5% AcOH).
Although the procedure is simple and efficient, it has certain limitations. This depends upon the high steric hindrance at the carbonyl group to the attack by the nucleophile. Thus, as the authors have reported, the selectivity was lost and mixtures of products were obtained in the case of cinnamic acid.

More recently, based on this method, Sharma et al. have synthesized spermidine siderophores via primarily protected polyamine precursors which was accomplished with benzyloxycarbonylimidazole in the presence of catalytic amounts of DMAP. The protected spermidine could then be acylated at the secondary nitrogen atom using succinimide esters of various acids. Subsequent removal of the Z groups allowed further symmetrical modification at the primary amino groups to afford for example the siderophore 106.

\[
\text{CH}_3\text{NOH} \\
\text{CO} \\
\text{(CH}_2\text{)}_2 \\
\text{CONH(CH}_2\text{)}_3\text{N(CH}_2\text{)}_4\text{NHCO} \\
\text{HO OH}
\]

**106**

Spermexatol

D. Benzo- and Naphthosultones

Acher et al. have synthesized different strained five-membered benzosultones 107 and naphthosultones 108 and studied their reactivity as coupling agents in peptide
chemistry and as acylating reagents for amines.

\[ \text{107} \]

\[ \text{108} \]

\[ X = Y = H; \quad b: X = \text{NO}_2, Y = H; \quad c: X = Y = \text{NO}_2 \]

Generally, the aminolysis of these aryl esters takes place according to a two-step process involving a tetrahedral intermediate as represented in Scheme 17. The first step, the nucleophilic attack by the carboxylate anion, leads to a mixed anhydride which is then transformed to an activated ester by an intramolecular rearrangement, the seven-membered oxygen $\rightarrow$ oxygen acyl transfer. The second step is the aminolysis of the activated ester to give the acylated amine. This reaction is fast in aprotic solvents which is explained in terms of an anchimeric assistance by the neighbouring $\text{SO}_3^-$ group via an intramolecular general base catalysis.

The benzosultones do not give quantitative yields of the activated esters and, consequently, of amides, due to an incomplete acyl transfer reaction. This equilibrium can favourably be shifted with the naphthosultones because these form more rigid mixed anhydrides. Although the nitro derivatives are more reactive with respect to the nucleophilic attack by carboxylate ions, the aminolysis of the dinitro esters is slower due to the steric hindrance of the o-nitro substituent.
The reaction of primary and secondary amines with these reagents has shown that the acylation of the latter was a much slower process than with the former ones and the activated esters of the benzosultones were more selective than those of the naphthosultones. Thus, these authors have also reported the synthesis of maytenine in 71% yield by direct selective acylation of N¹ and N⁸ of spermidine with 5-nitrobenzosultone and trans-cinnamic acid.

E. 1-Hydroxypiperidine esters

The peptide-coupling agents, the active 1-hydroxypiperidine esters, have also been used as acylating reagents for amines.
The acylation of amines is relatively fast and Young et al.\textsuperscript{72b} have suggested that the transition state for the formation of the adduct (I), and the adduct itself, may be stabilized by hydrogen bonding and the subsequent proton transfer would greatly accelerate the final step (Scheme 18).

\[
\begin{align*}
\text{RC-\text{O-N}} & + R^1\text{NH}_2 \rightarrow \text{RC-\text{O-N}}_1 \\
\text{H} \\
\text{(I)} \\
\text{RCONH}^1 + \text{H-O-N} & \leftarrow \text{RC-\text{O-NH}}_1
\end{align*}
\]

Scheme 18 Acylation of amines with 1-acyloxypiperidine\textsuperscript{72b}.

These reagents have also shown selectivity. For instance, it was reported that the reaction rates of benzoylation of amines with 1-benzoyloxypiperidine decreased in the following general order: n-butylamine $>$ PhCH\textsubscript{2}NH\textsubscript{2} $>$ (CH\textsubscript{3})\textsubscript{2}CHNH\textsubscript{2} $>$ (CH\textsubscript{3})\textsubscript{3}CNH\textsubscript{2} \textsuperscript{72b}. The authors have interpreted these data in such a way that increasing steric effects might prevent the amines from approaching sufficiently close for hydrogen bonding to be effective.

Exploiting these features, Husson et al.\textsuperscript{73} have proposed these active esters for direct selective modification of terminal amino groups of polyamines. Thus, this research group...
has reported the synthesis of several primary diacyl-
spermidine derivatives such as maytenine and other
dihydroxybenzoyl derivatives by reaction of spermidine with
the corresponding active esters.\textsuperscript{73d}

F. Nefkens's reagent

The Nefkens's reagent, N-ethoxycarbonylphthalimide \textsuperscript{110}, has
been used in peptide chemistry for the introduction of
phthaloyl as an N-protective group.\textsuperscript{25}

![Diagram of N-ethoxycarbonylphthalimide](image)

Recently, Sosnovsky et al.\textsuperscript{74} have reported selective
protection of primary amino groups by using this reagent.
Thus, the authors have described the syntheses of several
N,N'-bisphtaloylated polyamines in yields varying between
53-86%. As illustrated in Scheme 19 for the preparation of
N\textsuperscript{4}-Bz-spermidine, these protected precursors were then used
for selective acylation at the secondary nitrogen atom with
acyl chlorides followed by selective deblocking of primary
amino groups by hydrazinolysis.

Although this approach for selective protection is a simple
one-step procedure based on readily available starting mate-
rrial, the main drawback is the strong conditions required for
the removal of the phthaloyl group. This may be a limiting factor with respect to other functional groups present in the target molecule.

G. Nitriles

Two methods have been devised for acylation of amino groups with nitriles\textsuperscript{75,76}. One exploits the ruthenium-catalysed condensation of nitriles with amines in the presence of two equivalents of water at high temperature\textsuperscript{75}.

\[
R^1CN + RNH_2 + H_2O \xrightarrow{\text{RuH}_2(PPh_3)_4} R^1CONHR + NH_3
\]

The second and more recent procedure\textsuperscript{76} uses direct acylation of amines with acyl cyanides\textsuperscript{77} or cyanoformates\textsuperscript{78}.

\[
R^1COCN + RNH_2 \rightarrow R^1CONHR
\]

As in the methods previously described, a selective differentiation between primary and secondary amino groups
has been achieved by acylation with nitriles. For example, N<sup>1</sup>,N<sup>8</sup>-Ac<sub>2</sub>-spermidine and maytenine were synthesized by direct condensation of spermidine with the corresponding nitriles, acetonitrile and trans-cinnamonic acid, in the presence of a Ru catalyst in good yield. It is worth emphasizing that the authors could also introduce selectively the Z group with benzyl cyanoformate (ZCN) to give the known diprotected precursor N<sup>1</sup>,N<sup>8</sup>-Z<sub>2</sub>-spermidine.

The clean acyl cyanide reaction is preferable to the ruthenium-catalysed one because it proceeds under mild conditions, an important requirement for the synthesis of thermally unstable polyamines.

1.2.3.2 - "Transiently protected" polyamines

Ganem et al. have developed a method for selective modification of a polyamine which takes advantage of a temporarily protected polyamine either as a cyclic urea or as a hexahydropyrimidine. The methylene bridge between N<sup>1</sup> and N<sup>4</sup> of polyamines such as spermidine leads to a strain-free six-center cyclic derivative in preference to a seven-membered structure corresponding to an N<sup>4</sup>,N<sup>8</sup>-cyclization.

\[ \text{HN} \xrightarrow{X} \text{N-(CH}_2)_4\text{NH}_2 \]

114  a, X= carbonyl  
      b, X= methylene
A. Cyclic ureas

The synthesis of cyclic urea 114a is depicted in Scheme 20\(^\text{18}\). The first step involves exhaustive methoxycarbonylation of spermidine with methyl chloroformate. In refluxing alkaline media the resulting triprotected spermidine undergoes a preferential hydrolysis and decarboxylation of the terminal urethane groups followed by in situ cyclization via an intramolecular aminolysis between the free amino group of the propyl moiety and the urethane group at the secondary nitrogen atom.

$$\begin{align*}
H_2N(CH_2)_3NH(CH_2)_4NH_2 & \xrightarrow{\text{i}} CH_3OCONH(CH_2)_3N(CH_2)_4NCOOCH_3 \\
& \xrightarrow{\text{ii}} \begin{array}{c}
\text{114a} \\
N(CH_2)_4NH_2
\end{array}
\end{align*}$$

Scheme 20  Formation of spermidine cyclic urea. Reagents: i, ClCO_2CH_3; ii, aq. Ba(OH)_2 (reflux)\(^\text{18}\).

The removal of the carbonyl group can be carried out either by urea exchange or by reduction with LiAlH_4. The first method consists in a transamination by heating the cyclic urea in a large excess of low boiling diamine such as 1,3-propanediamine, which forms the corresponding water-soluble urea.
derivative. The second procedure is less practical because it leads to a mixture of spermidine and the $N^1$-methylated derivative difficult to separate.

These cyclic ureas can be extended to higher polyamines containing a 1,3-diaminopropyl moiety and in the spermine case give the corresponding bis urea 115.

![Chemical Structure](image)

For spermine, however, this approach seems to be of less practical value since all nitrogen atoms are blocked to the most common electrophilic agents. On the other hand, in the cyclic spermidine derivative, the $N^8$ or all three nitrogen atoms can be differentiated by exploiting selective reactions\(^{18}\).

Thus, the synthesis of native spermine 4 was reported where the cyclic urea was monocyanoethylated at $N^8$ followed by selective reduction (borane) of the resulting nitrile. The last step consisted in the removal of the carbonyl group by urea exchange (Scheme 21)\(^{18}\). Although not reported in the literature, the intermediates 116 and 117 may be exploited as potential precursors for selective protection and modification of the different nitrogen atoms.
Scheme 21  Synthesis of spermine. Reagents: i, CH₂=CHCN (C₆H₅); ii, BH₃-THF (room temperature); iii, NH₂(CH₂)₃NH₂ (140°C) 18.

B. Hexahydropyrimidines

As a complement and alternative to cyclic ureas, Ganem et al. 18,79 have also prepared the corresponding hexahydropyrimidine 114b by simple condensation reaction of the polyamine with aqueous formaldehyde.

\[
\begin{align*}
&\text{H}_2\text{N(CH}_2)_3\text{N}(\text{CH}_2)_4\text{NH}_2 + \text{HCHO (0.98 eq.)} \\
&\text{H}_2\text{N(CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NH}_2
\end{align*}
\]

The cleavage of this cyclic gem-diamine can be achieved by acid hydrolysis 80 or by ethyl hydrogen malonate and piperidine in refluxing ethanol (a Knoevenagel type reaction) 79. The latter method is more advantageous for acid-sensitive polyamine analogues.

As in the case of cyclic ureas, spermine also reacts with formaldehyde to give the corresponding bis(hexahydropyrimidines) 114b.
pyrimidine) derivative $\text{118}^{18,81}$.  

\[
\begin{align*}
\text{HN} & \quad \text{N(CH}_2\text{)}_4\text{N} & \quad \text{NH} \\
\text{118}
\end{align*}
\]

In both cases the originally secondary nitrogen atoms are protected against electrophilic reagents and therefore differentiated from the primary ones. The synthesis of terminally diacylated spermidine and spermine derivatives (maytenine and kukoamine A $\text{119}^{1}$) has been reported by direct acylation of $\text{114b}$ and $\text{118}$ with the corresponding acyl chlorides$^{79,81}$.

\[
\begin{align*}
\text{HO} & \quad \text{(CH}_2\text{)}_2\text{CONH(CH}_2\text{)}_3\text{NH(CH}_2\text{)}_4\text{NH(CH}_2\text{)}_3\text{NHCO(CH}_2\text{)}_2\text{OH} \\
\text{119}
\end{align*}
\]

Kukoamine A

The same authors$^{82}$ have also described the synthesis of $\text{N}^4$-acylspermidine derivatives after performing a bis tert-butoxycarbonylation of the hexahydropyrimidine. Selective removal of the methylene bridge affords the diprotected $\text{N}^1,\text{N}^8$-Boc$_2$-spermidine which can be selectively acylated on the secondary amino group.

Another important feature of the hexahydropyrimidine reported by Ganem$^{80,83}$ is the possibility of differentiating between the secondary nitrogen atom and the primary one. For
selective modification at the secondary nitrogen, the described cases are based on the protection of the primary amino group in 114b by generation of the Schiff base 120 (Scheme 22) or by complexation with crown-ether as in the synthesis of N<sup>1</sup>-Ac-spermidine. For the preparation of the N<sup>8</sup>-acetyl isomer, monoacylation was reported at the primary amino group by using a selective acetylating reagent.

Scheme 22 Synthesis of thermospermine. Reagents: i, PhCHO (C<sub>6</sub>H<sub>5</sub>NH<sub>2</sub>, reflux); ii, CH=CH-CN (EtOH); iii, 2M HCl-MeOH (reflux); iv, CoCl<sub>2</sub>·6H<sub>2</sub>O, NaBH<sub>4</sub>.

The synthesis of the cytotoxic spermidine metabolites isolated from the coral Sinularia Brougersma is an example of the usefulness of this approach for selective differentiation of the three nitrogen atoms. After starting from the cyclic urea it proceeds via the hexahydropyrimidine intermediate 125, obtained by selective reduction of the carbonyl group (Scheme 23).
Scheme 23 Synthesis of a cytotoxic spermidine derivative. Reagents: i, HCHO / 88 % HCO₂H; ii, LiAlH₄; iii, aq. Na₂CO₃, CH₂Cl₂; iv, HCO₂H, (heating); v, H₂/Pd-C (ethyl acetate).

In comparison with the cyclic ureas, the hexahydropyrimidines are preferable because they are more easily available, can be deprotected under milder conditions and be useful for diprimary modification of higher polyamines. The main limitation of this approach is that it is only applicable to polyamines containing the aminopropyl moiety.
1.2.4 - Conclusions

The different synthetic methods discussed in this chapter, available for total synthesis and selective protection and modification of polyamines, are summarized in Tables IVa and IVb for the spermidine case.

The methods involving total syntheses are general approaches affording the free polyamines as well as fully or partially protected derivatives. Among these procedures the nitrile approach developed by Bergeron appears to be the most versatile one with which it is possible to obtain protected polyamines in high yield. Moreover, mild conditions are normally employed for the removal afterwards of the remaining protecting groups. The main disadvantage of the total synthesis strategy is the often large number of steps required to afford the target compounds. Nevertheless, for synthetic targets with a new or unusual C,N backbone, total synthesis is the only alternative.

On the other hand, in the spermidine and spermine series, the use of selective protection and modification reagents or Ganem's spermidine-formaldehyde adduct (the hexahydropyrimidine 114b) is generally advantageous. Symmetrical modification at the primary amino groups or selective functionalization of the secondary one can normally easily be accomplished.
Table IVa - Methods for synthesis of spermidine derivatives by a total synthesis approach with selected examples.

<table>
<thead>
<tr>
<th>Method</th>
<th>Protected or modified spermidine</th>
<th>Overall yield %</th>
<th>Conditions for removal of protective groups</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkyl- oxidation of tosyl-amides</td>
<td>PhtN(CH₂)₃N(Tos(CH₂)₄)NH₂</td>
<td>18⁹</td>
<td>NH₂NH₂</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Na⁺NH₂</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>H₂/Pd₂-C</td>
<td></td>
</tr>
<tr>
<td>Alkyl- oxidation of amines</td>
<td>PhtN(CH₂)₃NH(CH₂)₄NH₂</td>
<td>30³</td>
<td>NH₂NH₂</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>H₂/Pd₂-C</td>
<td></td>
</tr>
<tr>
<td>Reduction of nitriles</td>
<td>NH₂(CH₂)₃NBoc(CH₂)₄NHBOc</td>
<td>49³</td>
<td>CF₃COOH</td>
<td>36</td>
</tr>
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</tr>
<tr>
<td></td>
<td>NH₂(CH₂)₃NBzl(CH₂)₄NH₂</td>
<td>36³</td>
<td>H₂/Pd-C</td>
<td>40</td>
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<tr>
<td></td>
<td>BocNH(CH₂)₃NBzl(CH₂)₄NHCOCF₃</td>
<td>60³</td>
<td>CF₃COOH</td>
<td>43</td>
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</tr>
<tr>
<td>Reduction of amides</td>
<td>PhtN(CH₂)₃NBoc(CH₂)₄NHBOc</td>
<td>69³</td>
<td>NH₂NH₂</td>
<td>51a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CF₃COOH</td>
<td></td>
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<tr>
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<td></td>
</tr>
<tr>
<td></td>
<td>BocNH(CH₂)₃NH(CH₂)₄NHBOc</td>
<td>49³</td>
<td>CF₃COOH</td>
<td>51b</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduction of imines</td>
<td>ZNH(CH₂)₃NH(CH₂)₄NHAc</td>
<td>36³</td>
<td>H₂/Pd-C</td>
<td>55</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td>From PhtN(CH₂)₂Br</td>
<td></td>
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<tr>
<td></td>
<td>From NH₂(CH₂)₄NH₂</td>
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<tr>
<td></td>
<td>From PhCH₂NH₂</td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>From NC(CH₂)₂NH(CH₂)₄NH₂</td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>From BocNH(CH₂)₄CO₂H</td>
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</tr>
<tr>
<td></td>
<td>From NH₂(CH₂)₂CH(OEt)₂</td>
<td></td>
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</tr>
</tbody>
</table>

Footnotes:

- From PhtN(CH₂)₂Br
- From NH₂(CH₂)₄NH₂
- From PhCH₂NH₂
- From NC(CH₂)₂NH(CH₂)₄NH₂
- From BocNH(CH₂)₄CO₂H
- From NH₂(CH₂)₂CH(OEt)₂
Table IVb - Some examples illustrating the selective acylation methods described in 1.2.3.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Protected or modified spermidine</th>
<th>Yield %</th>
<th>Conditions for removal of protective groups</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Oxazolones</td>
<td>Maytenine</td>
<td>76</td>
<td>-</td>
<td>66f</td>
</tr>
<tr>
<td>Thiazolidine-2-thiones</td>
<td>96 ZNH(CH₂)₃NH(CH₂)₄NH₂</td>
<td>69</td>
<td>H₂/Pd-C</td>
<td>68j</td>
</tr>
<tr>
<td>Imidazoles</td>
<td>96 ZNH(CH₂)₃NH(CH₂)₄NHZ</td>
<td>76</td>
<td>H₂/Pd-C</td>
<td>70</td>
</tr>
<tr>
<td>Benzosultones</td>
<td>Maytenine</td>
<td>71</td>
<td>-</td>
<td>71</td>
</tr>
<tr>
<td>1-Hydroxy-piperidine esters</td>
<td>94 PhtN(CH₂)₃NH(CH₂)₄NPht</td>
<td>75</td>
<td>NH₂NH₂</td>
<td>74</td>
</tr>
<tr>
<td>Nefkens's reagent</td>
<td>PhtN(CH₂)₃NH(CH₂)₄NPht</td>
<td>75</td>
<td>NH₂NH₂</td>
<td>74</td>
</tr>
<tr>
<td>Nitriles</td>
<td>Maytenine</td>
<td>70</td>
<td>-</td>
<td>75</td>
</tr>
<tr>
<td>Ganem's adduct</td>
<td>Maytenine</td>
<td>85a</td>
<td>-</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>BocNH(CH₂)₃NH(CH₂)₄NHBOc</td>
<td>54a</td>
<td>CF₃COOH</td>
<td>82</td>
</tr>
</tbody>
</table>

*In two steps.*

51
2 - BACKGROUND OF THE PRESENT WORK

2.1 - Introduction

In general, acylations of amines and alcohols can be accomplished with the corresponding anhydride or chloride in pyridine\textsuperscript{86}. However, this method often fails in cases of electronically deactivated or sterically hindered substrates. As recently reviewed\textsuperscript{87-89}, certain 4-dialkylaminopyridines, DMAP \textsuperscript{129} and MPP \textsuperscript{130}, are nowadays commonly used as catalysts to facilitate such difficult acylations.

![DMAP and MPP structures]

Usually only small amounts of the catalyst (typical molar ratio substrate/DMAP 20:1) are required for an efficient acylation. Moreover, in more difficult cases it is often necessary to use at least an equimolar amount of an auxiliary base to remove the acid formed in the reaction. Alternatively, it is possible to use a stoichiometric amount of dialkylaminopyridine to act as both base and catalyst (Table V)\textsuperscript{88}.

Although some DMAP-catalysed reactions such as the formation of urethanes from alcohols and phenylisocyanates can involve general base catalysis\textsuperscript{90}, most of them probably
Table V - Acetylation of 1-methylcyclohexanol (100 mmol) with acetic anhydride.

<table>
<thead>
<tr>
<th>Catalyst (mmol)</th>
<th>Base (150 mmol)</th>
<th>Reaction time /h</th>
<th>Yield of product (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>Pyridine</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>-</td>
<td>TEA</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>DMAP (4.0)</td>
<td>Pyridine</td>
<td>18</td>
<td>66</td>
</tr>
<tr>
<td>DMAP (4.0)</td>
<td>TEA</td>
<td>17</td>
<td>89</td>
</tr>
<tr>
<td>DMAP (1.0)</td>
<td>TEA</td>
<td>16</td>
<td>63</td>
</tr>
<tr>
<td>DMAP (4.0) a</td>
<td></td>
<td>17</td>
<td>39</td>
</tr>
<tr>
<td>DMAP (100.0) a</td>
<td></td>
<td>18</td>
<td>84</td>
</tr>
<tr>
<td>MPP</td>
<td>TEA</td>
<td>17</td>
<td>90</td>
</tr>
</tbody>
</table>

aMethylene chloride was used as solvent.

occur by nucleophilic catalysis via an N-acylpyridinium ion as acylating agent (Scheme 24). It is important pointing out that for DMAP the equilibrium is more shifted to the formation of the N-acyl pyridinium intermediate than for unsubstituted pyridine. This is due to the electron-donating mesomeric effects of the 4-dialkylamino group (see resonance structure II). The formed adducts, which exist as ion pairs in non-polar solvents, are also more stable and in some cases they have been isolated. Thus, their relatively high concentrations facilitate the second step which is subjected to a general base catalysis by the counter ion. Therefore the reactivity of these adducts depends upon the nature of the anion, and in general, acid anhydrides (e.g. X= acetate) are stronger acylating agents than the
corresponding acid chlorides (X = Cl). This second step is also promoted by the presence of strong bases.

Thus, the ability of these reagents to catalyse acylations depends not only on their strongly basic character. They might be considered as "catalytic supports" for an electrophile promoting reaction with a substrate.
2.2 - tert-Butoxycarbonylation of amide type functional groups

In 1983 Grieco et al.\textsuperscript{95} reported for the first time the DMAP-catalysed tert-butoxycarbonylation of secondary amides and lactams with Boc\textsubscript{2}O\textsuperscript{96}. Nevertheless, it was not until recently that Grehn, Gunnarsson and Ragnarsson\textsuperscript{97} began to explore a general procedure for exhaustive tert-butoxycarbonylation of various type of amides using the Boc\textsubscript{2}O/DMAP approach.

Although the first authors used an equimolar amount of DMAP and CH\textsubscript{2}Cl\textsubscript{2} as solvent\textsuperscript{95}, the formation of the Boc\textsubscript{2}O/DMAP adduct turned out to be faster in CH\textsubscript{3}CN and only catalytic amounts of DMAP were required (0.05-0.1 equivalent)\textsuperscript{97}.

\[
\text{RNHR}^1 \quad \text{Boc}_2\text{O (1.1 eq.)} / \text{DMAP (0.1 eq.)} \quad \text{CH}_3\text{CN} \quad \rightarrow \quad \text{RN(Boc)R}^1
\]

for R, R\textsuperscript{1} see Table VI

As shown in Table VI, this acylation reaction has proved to be a very efficient procedure and useful for the conversion of different compounds to the corresponding Boc analogues. However, as pointed out by the authors\textsuperscript{97} and already observed by other research groups\textsuperscript{87-89,95}, steric factors are important. Due to the bulkiness of the activated intermediate when using sterically hindered substrates, long reaction times and an excess of reagent are required (see Table VI, entry 17). In one case, for pivalanilide, the
### Table VI - tert-Butoxy carbonylation of amides $R^1NHR$ with $\text{Boc}_2\text{O}/\text{DMAP}^9$.  

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>Reaction time /h</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ph</td>
<td>HCO</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Ph</td>
<td>CH$_2$CO</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>Ph</td>
<td>PhCO</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>Ph</td>
<td>PhCH$_2$CO</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>Ph</td>
<td>Boc</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>PhCH$_2$</td>
<td>CH$_3$CO</td>
<td>20</td>
</tr>
<tr>
<td>7</td>
<td>Ph(CH$_2$)$_2$</td>
<td>CH$_3$CO</td>
<td>20</td>
</tr>
<tr>
<td>8</td>
<td>PhCH$_2$</td>
<td>Boc</td>
<td>48&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>9</td>
<td>Ph(CH$_2$)$_2$</td>
<td>Boc</td>
<td>50</td>
</tr>
<tr>
<td>10</td>
<td>4-EtOOCOC$_2$H$_4$</td>
<td>CH$_3$CO</td>
<td>2</td>
</tr>
<tr>
<td>11</td>
<td>4-NO$_2$-2-8F$_3$C$_6$H$_3$</td>
<td>CH$_3$CO</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>Ph</td>
<td>4-Me-C$_6$H$_4$-SO$_2$</td>
<td>1</td>
</tr>
<tr>
<td>13</td>
<td>Ph</td>
<td>2-NO$_2$-8F$_3$C$_6$H$_3$</td>
<td>1</td>
</tr>
<tr>
<td>14</td>
<td>Ph</td>
<td>Ph$_2$P(=O)$_2$</td>
<td>1</td>
</tr>
<tr>
<td>15</td>
<td>4-Bu&lt;sup&gt;t&lt;/sup&gt;-C$_6$H$_4$</td>
<td>CH$_3$CO</td>
<td>6</td>
</tr>
<tr>
<td>16</td>
<td>3-Bu&lt;sup&gt;t&lt;/sup&gt;-C$_6$H$_4$</td>
<td>CH$_3$CO</td>
<td>20</td>
</tr>
<tr>
<td>17</td>
<td>2-Bu&lt;sup&gt;t&lt;/sup&gt;-C$_6$H$_4$</td>
<td>CH$_3$CO</td>
<td>50</td>
</tr>
</tbody>
</table>

<sup>a</sup> Prepared in one step from benzylamine and excess of $\text{Boc}_2\text{O}$.  

The desired product was not obtained.

This acylation reaction is by no means restricted to carboxamides. The p-toluenesulfonylanilide and o-nitrobenzenesulfenanilide as well as the diphenylphosphinanilide smoothly afford the corresponding Boc analogues (see Table VI, entries 12-14)<sup>97</sup>.

Furthermore, with reference to the scope of this reaction, in the presence of $\text{Boc}_2\text{O}$ and DMAP, urethane NH groups generally undergo the same substitution as described above for amides. The products are formed in high yields and are of
normal stability. Only few substances of this general type, systematically named imidodicarbonates, had earlier been prepared.

Various urethanes play an important role in synthetic organic chemistry for the protection of amino functions. Judging from this aspect, it is obvious that the chemistry described in the preceding paragraph can be exploited in different ways for double protection of primary amines.

In attempts to extend this approach to other dicarbonate reagents, the stability of the latter to DMAP appeared to be a limiting factor. While Boc₂O forms a rather stable adduct with DMAP, other dicarbonates such as dimethyl dicarbonate and Z₂O decompose rapidly in the presence of DMAP.

2.3 - Selective cleavage of amides

In general, the cleavage of amides requires strong reaction conditions which practically excludes the use of amides as protecting groups.

Grieco et al. have reported a milder method in which the N-Boc derivatives of secondary amides and lactams are selectively hydrolyzed by LiOH or methanolyzed in the presence of NaOMe at the less hindered carbonyl group.

Using a related approach, Grehn, Gunnarsson and Ragnarsson introduced a novel mild procedure in which the previous N-tert-butoxycarbonylated substrates undergo a selec-
Aminolysis or similar base-catalysed methanolysis to give the corresponding acid-labile tert-butyl carbamates.

$$R^1CO(Boc)NR \xrightarrow{\text{aminolysis}} BocNHR \quad \text{or methanolysis}$$

The scope of this cleavage reaction is indicated in Table VII. In general, the rate of DEAEAA-mediated aminolysis is enhanced by electron-withdrawing substituents and decreased for sterically hindered substrates. On the other hand, in the case of compounds containing an aliphatic amide moiety the cleavage proceeds remarkably slowly (see Table VII, entries 7, 8). However, the rate of deacylation can be enhanced by carrying out the reaction in methanol and in the presence of a strong base such as TMG. As the selectivity is retained, this can be an efficient alternative to aminolysis for resistant substrates which do not contain base-labile functions.

As reported by the authors, this procedure is not applicable to substances carrying non-carboxamide groups of tosyl (Tos), 2-nitrophenylsulfenyl (Nps) and diphenylphosphinyl (Dpp) type. The first one is reconverted to Tos-anilide and the latter are almost unchanged even after prolonged reaction times.

The Boc-derivatives of suitable urethanes mentioned in the preceding section, "double-protected amines", can be brought to undergo cleavage to the corresponding Bu carbamates provided the first urethane-protecting group is orthogonal to Boc. In the early work on aminolytic cleavage of
Table VII - Selective deacetylation of CH$_3$CON(Boc)R$^{99}$.

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>Reaction conditions</th>
<th>Time /h</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ph</td>
<td>DEAEA, TMG/MeOH</td>
<td>24</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.2</td>
<td>98</td>
</tr>
<tr>
<td>2</td>
<td>4-Bu$^t$-C$_6$H$_4$</td>
<td>NH$_2$NH$_2$</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>4-EtO$_2$C</td>
<td>DEAEA</td>
<td>2</td>
<td>96</td>
</tr>
<tr>
<td>4</td>
<td>2-Et-C$_6$H$_4$</td>
<td>DEAEA</td>
<td>10</td>
<td>95</td>
</tr>
<tr>
<td>5</td>
<td>4-Bu$^t$-2-NO$_2$-C$_6$H$_3$</td>
<td>DEAEA</td>
<td>2</td>
<td>97</td>
</tr>
<tr>
<td>6</td>
<td>2-Thienyl</td>
<td>DEAEA</td>
<td>2</td>
<td>98</td>
</tr>
<tr>
<td>7</td>
<td>PhCH$_2$</td>
<td>DEAEA, TMG/MeOH</td>
<td>70</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.5</td>
<td>98</td>
</tr>
<tr>
<td>8</td>
<td>Ph(CH$_2$)$_2$</td>
<td>DEAEA, NH$_2$NH$_2$</td>
<td>150</td>
<td>91</td>
</tr>
</tbody>
</table>

DEAEA= 2-diethylaminoethylamine  
TMG= N,N,N',N'-tetramethylguanidinide

Boc-substituted amides, there was evidence for partially selective aminolytic cleavage of Z-groups from the compounds of benzyl tert-butyl imidodicarbonate type$^{99}$.

In conclusion, the novel chemistry outlined in this chapter based on exhaustive tert-butoxycarbonylation of amides and urethanes and subsequent selective deacylation seemed a promising basis for the development of new strategies of synthesis and selective protection of amines such as polyamines which will be the topic of the following chapters.
3 - OBJECTIVES AND SYNTHETIC METHODOLOGY

As previously reviewed, several methods are now available for selective modification of polyamines but there seems to exist no ideal general approach particularly to accomplish full protection of such substrates. In general many steps are required and/or strong conditions for the removal of the protecting groups afterwards. Therefore, an alternative procedure to the reported ones seemed desirable.

The main aim of this project was to explore the possibility of accomplishing selective protection of spermidine by extending to this substrate the novel approach to the use of DMAP-catalysed tert-butoxycarbonylation, described by Ragnarsson et al.\textsuperscript{97-99}, in connection with tactics of protection in simple monoamine compounds.

Thus, the first part of this work consisted of the syntheses of protected spermidine derivatives with application of exhaustive tert-butoxycarbonylation of the previously protected amino groups. As outlined before\textsuperscript{97}, several possibilities were available for the initial protection of these functions. In this context, however, it was necessary to choose a protecting group which was orthogonal in relation to the Boc one. As the well-known benzyloxycarbonyl group (Z) fulfils this requirement and is cleaved under very mild conditions, we decided to use this group for temporary protection of the amino functions. Thus, Schemes 25 and 26 depict the synthetic strategy for the analogues.
Scheme 25 Protection of spermidine. Reagents: i, ZCl (aq. Na₂CO₃); ii, Boc₂O, DMAP (CH₃CN); iii, H₂/Pd-C (MeOH); iv, TMG (MeOH); v, Z₂O (CH₂Cl₂).
Scheme 26 Selective protection of spermidine. Reagents: i, HCHO; ii, Z₂O (CH₂Cl₂); iii, Boc₂O, DMAP (CH₃CN); iv, TMG (MeOH); v, malonic acid, pyridine (MeOH, reflux); vi, NaBH₄, CF₃COOH (THF, 40 °C).

\[
\begin{align*}
\text{NH}_2(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NH}_2 & \xrightarrow{i} \text{HN}(\text{CH}_2)_4\text{NH}_2 \\
\text{ZN} & \xrightarrow{\text{iii}} \text{ZN}(\text{CH}_2)_4\text{N}(\text{Z})\text{Boc} \\
\text{ZN}(\text{CH}_2)_4\text{N}(\text{Z})\text{H} & \xrightarrow{\text{iv}} \text{ZN}(\text{CH}_2)_4\text{NHBoc} \\
\text{ZN}(\text{CH}_2)_4\text{NHBoc} & \xrightarrow{\text{v}} \text{ZN}(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{N}(\text{Z})\text{Boc} \\
\text{ZN}(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NHBoc} & \xrightarrow{\text{vi}} \text{ZN}(\text{CH}_2)_2\text{CONH}(\text{CH}_2)_4\text{NHBoc}
\end{align*}
\]
N^1,N^8-bis(tert-butoxycarbonyl)spermidine 133 and N^1-benzyl-oxycarbonyl-N^8-tert-butoxycarbonylspermidine 138. In the case of the protected derivative 133, the novel methodology would be directly applied to spermidine itself. In the synthesis of 138, the new approach would be accomplished starting with the spermidine-formaldehyde adduct 114b^{18}.

Once these protected precursors were obtained, the analogue 133 would be suitable for selective modification at the secondary nitrogen atom. The derivative 138, by appropriate protection at the secondary amino group, would be advantageous to selective modification on N^1 or N^8. Thus, the second part of this work aimed at studying their scope for synthetic work leading to selectively modified analogues, mainly those of biological interest. Then we decided to synthetize the monoacetylated spermidine analogues which are of importance as metabolites and excretory products^{7}. The synthetic routes leading to all monoacetylated derivatives, 144, 148 and 152, are outlined in Schemes 27 and 28.

The ethyl analogues of spermidine are interesting substrates from a biological point of view^{9}. Surprisingly their prior preparation seems to have been overlooked in the chemical literature where only scarce details regarding their synthesis and properties are available. Therefore, our goal was to prepare them by applying suitable alkylating methods to the previously protected precursors. The results of the attempted syntheses will be discussed in the next chapter.
Scheme 27  Synthesis of N^4-Ac-spermidine. Reagents: i, AcO; ii, BocO, DMAP (CH₃CN); iii, TMG (MeOH); iv, 2.29 M HCl in dioxan.
Scheme 28 Synthesis of $N^1$-Ac- and $N^8$-Ac-spermidine derivatives. Reagents: i, Boc$_2$O (CH$_2$Cl$_2$); ii, H$_2$/Pd-C (MeOH); iii, Ac$_2$O (TEA, CH$_2$Cl$_2$); iv, 2.29 M HCl in dioxan; v, Z$_2$O (CH$_2$Cl$_2$)$_2$. 

Scheme 28

\[
\begin{align*}
\text{ZNH}(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NHBOc} & \rightarrow i \rightarrow \text{ZNH}(\text{CH}_2)_3\text{N(Boc)(CH}_2)_4\text{NHBOc} \rightarrow \text{HN}_2(\text{CH}_2)_3\text{N(Boc)(CH}_2)_4\text{NHBOc} \\
& \downarrow ii \rightarrow \text{AcNH}(\text{CH}_2)_3\text{N(Boc)(CH}_2)_4\text{NHBOc} \rightarrow \text{ZNH}(\text{CH}_2)_3\text{N(Z)(CH}_2)_4\text{NHBOc} \rightarrow \text{ZNH}(\text{CH}_2)_3\text{N(Z)(CH}_2)_4\text{NHBOc} \\
& \downarrow \text{iv} \rightarrow \text{AcNH}(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NH}_2\cdot2\text{HCl} \rightarrow \text{NH}_2(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NHAc}\cdot2\text{HCl} \\
& \downarrow \text{v} \rightarrow \text{HN}_2(\text{CH}_2)_3\text{N(Boc)(CH}_2)_4\text{NHBOc} \rightarrow \text{ZNH}(\text{CH}_2)_3\text{N(Z)(CH}_2)_4\text{NHBOc} \rightarrow \text{ZNH}(\text{CH}_2)_3\text{N(Z)(CH}_2)_4\text{NHBOc} \\
& \downarrow \text{ii, iv} \rightarrow \text{AcNH}(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NH}_2\cdot2\text{HCl} \rightarrow \text{NH}_2(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NHAc}\cdot2\text{HCl}
\end{align*}
\]
Suitable protected NH\textsubscript{3} derivatives, such as Boc\textsubscript{2}NH and BocNHZ, prepared in our laboratory\textsuperscript{100,101}, are potential reagents for a direct synthesis of protected amines\textsuperscript{101} via either the Gabriel\textsuperscript{102} or Mitsunobu\textsuperscript{103} reactions using halides or alcohols, respectively. Being potentially useful also for synthesis of polyamines, a third part of the project was related to the preparation of several new similar reagents. The imidodicarbonates mentioned were prepared by using the Boc\textsubscript{2}O/DMAP-mediated reaction with suitable substrates\textsuperscript{100,101}. As this approach cannot be used with anhydrides others than Boc\textsubscript{2}O\textsuperscript{99}, it was necessary to devise an alternative procedure. For the proposed alkyl benzyl imidodicarbonates 155 the chosen strategy, outlined in Scheme 29, was based on the well-known reaction between isocyanates and alcohols as reported recently by Kocovsky\textsuperscript{104}.

\[
\text{PhCH}_2\text{OCONH}_2 \xrightarrow{i} \text{PhCH}_2\text{OCON}=\text{C}=\text{O} \quad \text{153} \quad \text{154} \\
\text{PhCH}_2\text{OCONHCOOR} \xrightarrow{\text{ii}} 
\]

\[\text{a, } R= \text{C}_2\text{H}_5\text{CH}_2 \]
\[\text{b, } R= 4\text{CH}_2\text{OOC}_2\text{H}_4\text{CH}_2 \]
\[\text{c, } R= 4\text{-NO}_2\text{C}_2\text{H}_5\text{CH}_2 \]
\[\text{d, } R= \text{C}_2\text{H}_5\text{C(\text{CH}_3}_4\text{)Z \quad \text{e, } R= (\text{CH}_3}_3\text{)C} \]
\[\text{f, } R= 1\text{-adamantyl} \]
\[\text{g, } R= \text{CH}_2=\text{CHCH}_2 \]
\[\text{h, } R= \text{CCl}_2\text{CH}_2 \]
\[\text{i, } R= 9\text{-fluorenylmethyl} \]

**Scheme 29** Synthesis of alkyl benzyl imidodicarbonates. Reagents: i, (COCl\textsubscript{2}) (CH\textsubscript{2}Cl\textsubscript{2}); ii, ROH (CH\textsubscript{2}Cl\textsubscript{2}).
4 - RESULTS AND CONCLUSIONS

4.1 - Selective protection of spermidine

4.1.1 - Synthesis of $N^1,N^8$-bis(tert-butoxycarbonyl)spermidine

As shown in Scheme 25 (p. 61), the synthesis of $N^1,N^8$-Boc$_2$-spermidine was based on a three-step sequence starting from spermidine.

The preparation of the $N^1,N^4,N^8$-Z$_3$-spermidine 131 was readily accomplished by using a slight excess of ZCl in aqueous Na$_2$CO$_3$-dioxan to give the crude product as an oil slightly contaminated with benzyl alcohol. The pure compound could then be obtained in high yield after a simple column chromatography.

$$\text{ZCl} / \text{Na}_2\text{CO}_3 \rightarrow \text{NH}_2(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NH}_2 \xrightarrow{\text{dioxan}} \text{NH}(\text{Z})(\text{CH}_2)_3\text{N}(\text{Z})(\text{CH}_2)_4\text{NH}(\text{Z})$$

The key reaction, the exhaustive tert-butoxycarbonylation of the terminal urethane groups in compound 131, using the DMAP-catalysed reaction$^97$, also proceeded essentially quantitatively although, to complete the reaction, it was necessary to add a slight excess of the acylating reagent. Compound 132 was also obtained as an oil which could be purified by column chromatography.
The exhaustively protected derivative 132 could be converted either to product 133 by the removal of all Z groups or to derivative 134 by the selective cleavage of the terminal Z groups.

Thus, the preparation of the diprotected spermidine analogue 133 took advantage of the known orthogonality of the Z/Boc protecting groups. The reaction was easily accomplished by catalytic transfer hydrogenolysis according to a procedure reported by Spatola et al. The hydrogenolysis of compound 132 using ammonium formate as hydrogen donor in the presence of Pd-C in aqueous acetic acid gave the desired product 133, in this case as a white solid in good yield after recrystallization.

A more important finding was that compound 132 could be debenzyloxy carbonylated, essentially selectively, on the originally primary amino groups by the TMG-catalysed methanolysis to afford compound 134 also as an oil in high yield after chromatography. It is worth mentioning
that $^1$H n.m.r. of crude 134 indicated the presence of only trace amounts (< 1 %) of anomalous cleavage products. This high degree of selectivity was somehow unexpected. In earlier experiments was reported a rather low selectivity in the base-catalysed methanolysis of compound Z(Boc)NPh (product ratio BocNHPh : ZNHPh ≈ 6) which was explained in terms of a relative similarity between the Z and Boc groups 99. This cleavage reaction is indeed the key step of the synthesis of the selectively protected spermidine derivative 138 described below.

Compound 134 was also obtained from the diprotected spermidine 133 by simple benzyloxy carbonylation on the secondary amino group.

The yields in the different synthetic pathways aiming at the "symmetrical", diprotected spermidine analogue 133 are summarized in Table VIII.

### Table VIII - Yields and melting points of the compounds isolated during the synthesis of $N^1, N^8$-Boc$_2$-spermidine 133.

<table>
<thead>
<tr>
<th>No</th>
<th>Compounda</th>
<th>Yieldb (%)</th>
<th>m.p. /°C</th>
<th>Thesis, page</th>
</tr>
</thead>
<tbody>
<tr>
<td>131</td>
<td>$N^1, N^4, N^8$-Z$_3$-spd</td>
<td>84</td>
<td>38-40</td>
<td>113</td>
</tr>
<tr>
<td>132</td>
<td>$N^1, N^4, N^8$-Z$_3$-$N^1$,N$^8$-Boc$_2$-spd</td>
<td>92</td>
<td>Oil</td>
<td>114</td>
</tr>
<tr>
<td>133</td>
<td>$N^1, N^8$-Boc$_2$-spd</td>
<td>80</td>
<td>85.5-86.5°C</td>
<td>116</td>
</tr>
<tr>
<td>134</td>
<td>$N^4$-Z-$N^1, N^8$-Boc$_2$-spd</td>
<td>$80^d$</td>
<td>Oil</td>
<td>117</td>
</tr>
</tbody>
</table>

a Characterized by $^1$H and $^{13}$C n.m.r. spectra and elemental analysis (the latter only for 133). b After purification. c Lit. 79-80 °C. d From 132. e From 133.
4.1.2 - Synthesis of $N^1$-benzyloxycarbonyl-$N^8$-tert-butoxy-carbonylspermidine

As shown in Scheme 26 (p. 62), the starting material of this synthesis was the cyclic spermidine derivative 114b, easily prepared in quantitative yield from the triamine and a fresh formaldehyde solution according to the reported procedure$^{80b}$.

As in the previous case, the general strategy worked satisfactorily and only few minor modifications were made.

The attempted benzyloxycarbonylation of the cyclic spermidine 114b with ZCl gave a rather intractable mixture which decomposed further on performing column chromatography on silica. The inspection of the $^1$H n.m.r. spectrum of the crude mixture did not show any correlation with the structure expected. This outcome was probably due to the instability of the cyclic aminal derivative under these reaction conditions$^{107}$. This procedure was therefore abandoned and dibenzyloxycarbonylation was smoothly accomplished with $Z_2O$ under anhydrous conditions. The product 135 contaminated with benzyl alcohol was readily purified by column chromatography.

$$
\begin{align*}
\text{HN} & \text{N(CH}_2)_4\text{NH}_2 \xrightarrow{Z_2O} \text{ZN} & \text{N(CH}_2)_4\text{N(Z)H} \\
114b & & 135
\end{align*}
$$
No problems were encountered in the DMAP-catalysed tert-butoxycarbonylation step which gave product 136 in high yield after chromatography.

\[ \text{ZN} \begin{array}{c} \text{N(CH}_2\text{)}_4 \text{N(Z)H} \\ 135 \end{array} \xrightarrow{\text{Boc}_2\text{O/DMAP}} \text{ZN} \begin{array}{c} \text{N(CH}_2\text{)}_4 \text{N(Z)Boc} \\ 136 \end{array} \]

The synthesis of \( \text{N}^1\text{-Z-N}^8\text{-Boc-spermidine} \) 138 could in principle be achieved by first selective removal of the methylene group followed by selective cleavage of the Z group on \( \text{N}^8 \) or by these procedures in the reversed order.

\[ \text{ZN} \begin{array}{c} \text{N(CH}_2\text{)}_4 \text{N(Z)Boc} \\ 136 \end{array} \xrightarrow{\text{TMG, MeOH}} \text{ZN} \begin{array}{c} \text{N(CH}_2\text{)}_4 \text{NHBoc} \\ 137 \end{array} \]

Malonic acid, pyridine

\[ \text{ZNH(CH}_2\text{)}_3 \text{NH(CH}_2\text{)}_4 \text{N(Z)Boc} \xrightarrow{\text{TMG, MeOH}} \text{ZNH(CH}_2\text{)}_3 \text{NH(CH}_2\text{)}_4 \text{NHBoc} \]

On pursuing the former synthetic pathway, compound 136 being treated with potassium monoethyl malonate and pyridine, no cleavage of the methylene group occurred. Instead, the \( ^1\text{H n.m.r.} \) spectrum of the isolated crude product showed that the Z group on \( \text{N}^8 \) had been selectively cleaved. The model compounds \( \text{N}^1\text{-N}^8\text{-Boc}_2\text{-N}^1\text{-N}^4\text{-methylenespermidine} \) 82 and \( \text{N}^1\text{-N}^2\text{-Z}_2\text{-N}^2\text{-Boc-N}^1\text{-Et-ethylenediamine} \) 109a,b were therefore treated similarly. In the former case the starting material remained unchanged and in the latter the Z group on \( \text{N}^2 \) was cleaved off.
again to give N\textsuperscript{1}-Z-N\textsuperscript{1}-Et-N\textsuperscript{2}-Boc-ethylenediamine\textsuperscript{109a,b}(these experiments are not described in the experimental section). However, the reaction worked nicely when substituting by malonic acid\textsuperscript{82} the potassium salt mentioned above.

Thus, although the final product N\textsuperscript{1}-Z-N\textsuperscript{8}-Boc-spermidine \textsuperscript{138} could be obtained from compound \textsuperscript{136} via derivative \textsuperscript{139} by carrying out the cleavage of the methylene group with malonic acid followed by TMG-catalysed methanolysis, the yields were relatively low. In the light of the previous experiments, this outcome was presumably due to a partial cleavage of the N\textsuperscript{8}-Z group on performing the Knoevenagel type reaction and during the laborious chromatographic procedures.

Nevertheless, good results were obtained via the alternative synthetic pathway. Thus again the key reaction, the TMG-mediated methanolysis, could easily be performed on compound \textsuperscript{136} and the selective cleavage of the Z group on the acyclic moiety gave the compound \textsuperscript{137} in good yield after chromatography. It is worth mentioning that, although longer reaction times were needed, this reaction could also be carried out with a smaller amount of TMG (0.5 eq.) than that originally used (1.5 eq.). No problems were encountered in the removal of the methylene bridge on compound \textsuperscript{137} by performing the reaction with malonic acid. The final product \textsuperscript{138} was obtained as a solid in quite good yield after chromatography.

The structure of this compound was confirmed by an independent synthesis in which the corresponding diprotected
amide 140 was reduced with NaBH$_4$-TFA$^{51b}$ to afford 138 in low yield.

\[
\text{ZNH(CH}_2\text{)}_2\text{CONH(CH}_2\text{)}_4\text{NHBoC} \xrightarrow{\text{NaBH}_4\text{-TFA}} \text{ZNH(CH}_2\text{)}_3\text{NH(CH}_2\text{)}_4\text{NHBoC}
\]

140 138

The yields in the different synthetic pathways leading to the selectively protected spermidine analogue 138 are summarized in Table IX.

<table>
<thead>
<tr>
<th>No.</th>
<th>Compound$^a$</th>
<th>Yield$^b$ (%)</th>
<th>m.p. /°C</th>
<th>Thesis, page</th>
</tr>
</thead>
<tbody>
<tr>
<td>135</td>
<td>N$_1$N$_8$-Z$_2$-N$_1$N$_4$-(CH$_2$)$_4$-spd</td>
<td>76</td>
<td>Oil</td>
<td>119</td>
</tr>
<tr>
<td>136</td>
<td>N$_1$N$_8$-Z$_2$-N$_8$-Boc-N$_1$N$_4$-(CH$_2$)$_4$-spd</td>
<td>90</td>
<td>Oil</td>
<td>120</td>
</tr>
<tr>
<td>137</td>
<td>N$_1$-Z-N$_8$-Boc-N$_1$N$_4$-(CH$_2$)$_4$-spd</td>
<td>88</td>
<td>Oil</td>
<td>121</td>
</tr>
<tr>
<td>139</td>
<td>N$_1$N$_8$-Z$_2$-N$_8$-Boc-spd</td>
<td>48</td>
<td>Oil</td>
<td>123</td>
</tr>
<tr>
<td>138</td>
<td>N$_1$-Z-N$_8$-Boc-spd</td>
<td>78$^c$</td>
<td>63-64</td>
<td>121, 123</td>
</tr>
</tbody>
</table>

$^a$Characterized by $^1$H and $^{13}$C n.m.r. spectra and elemental analysis (the latter only for 138). $^b$After purification. $^c$From 137. $^d$From 139.
4.1.3 - **Attempted synthesis of N⁸-benzyloxy carbonyl-N¹-tert-butoxycarbonylspermidine**

As reviewed in Section 1.2.3.1, many reports have recently appeared in the literature on regioselective acylations of primary amino groups by various reagents. This feature tempted us to undertake some work aiming at the synthesis of N⁸-Z-N¹-Boc-spermidine 161 as an alternative to the previous isomeric substance 138.

Thus, by using the new reagent ZCN⁷⁶, readily available from ZCl and KCN⁷⁸, a preliminary experiment was performed on a model compound (N-ethylethylenediamine) in a "one-pot" procedure according to Scheme 30.

\[
\text{EtNH(CH}_2\text{)}_2\text{NH}_2 \xrightarrow{i, ii} \text{Et(Boc)N(CH}_2\text{)}_2\text{NHZ}
\]

**Scheme 30** Synthesis of N²-Z-N¹-Boc-N¹-Et-ethylenediamine.
Reagents: i, ZCN (CH₂Cl₂); ii, Boc₂O, NMM (CH₂Cl₂).

This reaction worked excellently and compound 157 was obtained in good yield as white crystals after recrystallization. ¹H and ¹³C n.m.r. spectra and t.l.c. (ether) of this compound differed from those of the other isomer Et(Z)N(CH₂)₂NHBoc¹⁰⁹a,b.

Disappointing results were, however, obtained when performing this reaction on the monocyclic spermidine derivative 114b.
T.l.c. and $^1$H n.m.r. of the crude product showed a rather complex mixture containing $\approx 60\%$ of the desired product, $N^8-Z-N^1$-Boc-$N^1,N^4$-methylenepermidine 160, together with considerable amounts of the $N^1,N^8-Z_2-N^1,N^4$-methylenepermidine 135 as well as other impurities. This crude mixture was difficult to separate by column chromatography on silica and afforded only a modest yield of 160. Discouraging results were also obtained in an attempt to isolate the intermediate $N^8-Z-N^1,N^4$-methylenepermidine 159 before tert-butoxycarbonylation of the secondary amino group. After a laborious work-up only 5\% of nearly pure 159 was obtained. These poor results of the selective acylation of derivative 114b were presumably due to its instability to cyanide ions. Although not a strict proof, a t.l.c. experiment showed that when compound 114b was treated with Et$_4$NCN, a rather complex mixture was obtained.
4.2 - Synthesis of modified spermidines

4.2.1 - Synthesis of N\textsuperscript{4}-acetyl spermidine dioxalate

As shown in Scheme 27 (p. 64), the compound N\textsuperscript{4}-Ac-spermidine 144 could be prepared by using the Boc\textsubscript{2}O/DMAP approach\textsuperscript{97} or by direct acylation of the previously prepared intermediate N\textsuperscript{1},N\textsuperscript{8}-Boc\textsubscript{2}-spermidine 133.

No problems were encountered in the acetylation of spermidine with Ac\textsubscript{2}O either in an aqueous or anhydrous reaction system.

\[
\text{AcNH(CH}_2\text{)}_3\text{N(Ac)(CH}_2\text{)}_4\text{NHAc} \quad 141
\]

Under the latter conditions a direct column chromatography of the reaction mixture was preferred in which the triacetylated derivative 141 was obtained in better yield.

Although a moderate decrease in the rate of the Boc\textsubscript{2}O/DMAP-catalysed reaction of aliphatic substrates\textsuperscript{97} was reported earlier, the tert-butoxycarbonylation of compound 141 turned out to be remarkably slow (~ one week reaction) and several additions of Boc\textsubscript{2}O were required to complete the reaction.

\[
\text{BocN(CH}_2\text{)}_3\text{N(Ac)(CH}_2\text{)}_4\text{NBoc} \quad 142
\]
After a usual column chromatography on silica, the fully protected intermediate 142 was obtained in a satisfactory yield.

The next step was easily performed where compound 142 underwent selective deacetylation by the TMG-mediated methanolysis and the desired product 143 was again obtained in good yield after chromatography. In the alternative procedure, this compound was easily prepared by acetylation of the precursor 133 with Ac₂O in the presence of TEA.

\[
\text{Boc(Ac)N(CH}_2\text{)}_2\text{N(Ac)(CH}_2\text{)}_4\text{N(Ac)Boc} \xrightarrow{\text{TMG, MeOH}} \xrightarrow{\text{Ac}_2\text{O, TEA (CH}_2\text{Cl}_2)} \text{BocNH(CH}_2\text{)}_3\text{NH(CH}_2\text{)}_4\text{NHBoc}
\]

142 143

The final step required the removal of the Boc groups and could be accomplished by different reagents. The acidolysis by HCl was chosen in order to obtain directly the compound as the dihydrochloride salt 144.

\[
\text{BocNH(CH}_2\text{)}_3\text{N(Ac)(CH}_2\text{)}_4\text{NHBoc} \xrightarrow{\text{HCl, dioxan}} \xrightarrow{\text{NH}_2(\text{CH}_2)_4\text{N(CH}_2\text{)}_4\text{NH}_2\cdot2\text{HCl}} \text{143 144}
\]

Although the procedure worked well, the resulting product turned out to be rather hygroscopic. Therefore, it was converted to its oxalate salt 144a by passing through an anion exchange column to afford the product as a white solid,
homogeneous by t.l.c. and h.p.l.c. Elemental analysis indicated that the oxalate salt 144a contained two molecules of oxalic acid and half a molecule of crystal water.

Table X summarizes the results for the synthesis of \( \text{N}^4\text{-Ac-spermidine} \).

<table>
<thead>
<tr>
<th>N°</th>
<th>Compound (^a)</th>
<th>Yield (^b) (%)</th>
<th>m.p. /°C</th>
<th>Thesis, page</th>
</tr>
</thead>
<tbody>
<tr>
<td>141</td>
<td>( \text{N}^1,\text{N}^4,\text{N}^8\text{-Ac}_3\text{-spd} )</td>
<td>78</td>
<td>oil</td>
<td>130</td>
</tr>
<tr>
<td>142</td>
<td>( \text{N}^1,\text{N}^4,\text{N}^8\text{-Ac}_3\text{-N}^1,\text{N}^8\text{-Boc}_2\text{-spd} )</td>
<td>68</td>
<td>oil</td>
<td>131</td>
</tr>
<tr>
<td>143</td>
<td>( \text{N}^4\text{-Ac-N}^1,\text{N}^8\text{-Boc}_2\text{-spd} )</td>
<td>88(^c)</td>
<td>oil</td>
<td>133</td>
</tr>
<tr>
<td></td>
<td></td>
<td>74(^d)</td>
<td></td>
<td>134</td>
</tr>
<tr>
<td>144</td>
<td>( \text{N}^4\text{-Ac-spd.2HCl} )</td>
<td>92(^e)</td>
<td>hygroscopic</td>
<td>134</td>
</tr>
<tr>
<td>144a</td>
<td>( \text{N}^4\text{-Ac-spd.2H}_2\text{C}_2\text{O}_4\cdot1/2\text{H}_2\text{O} )</td>
<td>80(^f)</td>
<td>187.5-188.5</td>
<td>134</td>
</tr>
</tbody>
</table>

\(^a\) Characterized by \(^1\text{H}\) and \(^13\text{C}\) n.m.r. spectra and elemental analysis (the latter only for the oxalate salt 144a). \(^b\) After purification. \(^c\) From 142. \(^d\) From 133. \(^e\) From the corresponding HCl salt.
4.2.2 - Synthesis of $N^1$-acetylspermidine dihydrochloride

The synthetic methodology (Scheme 28, p. 65) leading to this compound took advantage of the selectively protected precursor $N^1$-$Z$-$N^8$-Boc-spermidine 138.

This four-step protection-deprotection strategy was easily carried out and no problems arose. The first step was accomplished by tert-butoxycarbonylation at $N^4$ with Boc$_2$O to give intermediate 145 in good yield after purification.

\[
\text{ZNH(CH}_2\text{)}_3\text{NH(CH}_2\text{)}_4\text{NHBOc} \xrightarrow{\text{Boc}_2\text{O, CH}_2\text{Cl}_2} \text{ZNH(CH}_2\text{)}_3\text{N(Boc)(CH}_2\text{)}_4\text{NHBOc}
\]

138 145

The removal of the Z group by hydrogenolysis following the standard procedure afforded compound 146 in high yield. The crude product was essentially pure (t.l.c. and n.m.r.) and could be used for the next step without further purification.

\[
\text{ZNH(CH}_2\text{)}_3\text{N(Boc)(CH}_2\text{)}_4\text{NHBOc} \xrightarrow{\text{H}_2/\text{Pd-C, MeOH}} \text{NH}_2\text{(CH}_2\text{)}_3\text{N(Boc)(CH}_2\text{)}_4\text{NHBOc}
\]

145 146

The acetylation of compound 146 was easily accomplished by performing this reaction under anhydrous conditions and subsequent column chromatography on silica of the reaction mixture afforded compound 147 in high yield.

\[
\text{NH}_2\text{(CH}_2\text{)}_3\text{N(Boc)(CH}_2\text{)}_4\text{NHBOc} \xrightarrow{\text{Ac}_2\text{O, TEA, CH}_2\text{Cl}_2} \text{AcNH(CH}_2\text{)}_3\text{N(CH}_2\text{)}_4\text{NHBOc}
\]

146 147
The final product was obtained as the dihydrochloride salt 148 by removing the Boc groups with HCl.

\[
\text{AcNH(CH}_2)_3N(Boc)(CH}_2)_4NH_2 \xrightarrow{\text{HCl}} \text{dioxan} \quad \text{AcNH(CH}_2)_3NH(CH}_2)_4NH_2.2\text{HCl}
\]

147

Although slightly hygroscopic, the analytical specimen could be obtained by recrystallization to afford the product as a white solid, homogeneous by t.l.c. and h.p.l.c. and its physical data agreed with those previously reported\textsuperscript{35a,110}.

The results for the synthesis of N\textsuperscript{1}-Ac-spermidine are summarized in Table XI.

Table XI - Yields and melting points of the compounds isolated during the synthesis of N\textsuperscript{1}-Ac-spermidine dihydrochloride 148.

<table>
<thead>
<tr>
<th>No</th>
<th>Compound\textsuperscript{a}</th>
<th>Yield\textsuperscript{b} (%)</th>
<th>m.p. /°C</th>
<th>Thesis, page</th>
</tr>
</thead>
<tbody>
<tr>
<td>145</td>
<td>N\textsuperscript{1}-Z-N\textsuperscript{4},N\textsuperscript{8}-Boc\textsubscript{2}-spd</td>
<td>87</td>
<td>oil</td>
<td>135</td>
</tr>
<tr>
<td>146</td>
<td>N\textsuperscript{4},N\textsuperscript{8}-Boc\textsubscript{2}-spd</td>
<td>98</td>
<td>oil</td>
<td>136</td>
</tr>
<tr>
<td>147</td>
<td>N\textsuperscript{1}-Ac-N\textsuperscript{4},N\textsuperscript{8}-Boc\textsubscript{2}-spd</td>
<td>95</td>
<td>oil</td>
<td>137</td>
</tr>
<tr>
<td>148</td>
<td>N\textsuperscript{1}-Ac-spd.2HCl</td>
<td>97</td>
<td>191-193\textsuperscript{c}</td>
<td>137</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Characterized by \textsuperscript{1}H and \textsuperscript{13}C n.m.r. spectra and by elemental analysis (the latter only for the salt 148). \textsuperscript{b}After purification. \textsuperscript{c}Lit. 173-178 °C, 189-191 °C.

80
4.2.3 - Synthesis of N\textsuperscript{8}-acetylspermidine dihydrochloride

This compound was easily obtained starting from the same protected spermidine derivative 138 by an analogous four-step strategy (Scheme 28, p. 65).

In this case the secondary amino group of intermediate 138 was protected by the Z group. Thus, compound 149 was obtained in good yield by using as reagents either Z\textsubscript{2}O\textsuperscript{108} or ZO\textsubscript{2}Bt\textsuperscript{111}.

\[
\text{ZNH(CH}_2\text{)}_3\text{NH(CH}_2\text{)}_4\text{NHBOc} \xrightarrow{\text{Z}_2\text{O/CH}_2\text{Cl}_2} \text{ZNH(CH}_2\text{)}_3\text{N(Z)(CH}_2\text{)}_4\text{NHBOc}
\]

or

\[
\xrightarrow{\text{ZO}Bt/\text{CH}_3\text{CN}} \text{ZNH(CH}_2\text{)}_3\text{N(Z)(CH}_2\text{)}_4\text{NHBOc}
\]

Although the results were similar, the latter reagent required longer reaction times probably due to steric factors\textsuperscript{111}.

The next reaction afforded intermediate 150 by the removal of the Boc group with HCl.

\[
\text{ZNH(CH}_2\text{)}_3\text{N(Z)(CH}_2\text{)}_4\text{NHBOc} \xrightarrow{\text{HCl/dioxan}} \text{ZNH(CH}_2\text{)}_3\text{N(Z)(CH}_2\text{)}_4\text{NH}_2
\]

After a simple extraction step, the product was isolated essentially pure (t.l.c. and n.m.r.) and directly used for acetylation of the free amino group.

Again the acetylated product 151 was obtained in good yield by the same procedure as described in 4.2.2.

\[
\text{ZNH(CH}_2\text{)}_3\text{N(Z)(CH}_2\text{)}_4\text{NH}_2 \xrightarrow{\text{Ac}_2\text{O, TEA}} \text{ZNH(CH}_2\text{)}_3\text{N(Z)(CH}_2\text{)}_4\text{NHAc}
\]
The Z groups were cleaved by hydrogenolysis to give the final product as an oil which was also converted to its dihydrochloride salt 152 in good yield.

\[
\text{ZNH(CH}_2\text{)}_3\text{N(Z)(CH}_2\text{)}_4\text{NHAc} \xrightarrow{1) H_2/Pd-C (MeOH)} \text{NH}_2\text{(CH}_2\text{)}_3\text{NH(CH}_2\text{)}_4\text{NHAc.2HCl}
\]

Recrystallization of the crude material afforded a white powder, homogeneous by t.l.c. and h.p.l.c. Its physical data were also in agreement with those reported in the literature\textsuperscript{35a,110}.

The results for the synthesis of N\textsuperscript{8}-Ac-spermidine are summarized in Table XII.

Table XII - Yields and melting points of the compounds isolated during the synthesis of N\textsuperscript{8}-Ac-spermidine dihydrochloride 152.

<table>
<thead>
<tr>
<th>No</th>
<th>Compound\textsuperscript{a}</th>
<th>Yield\textsuperscript{b} (%)</th>
<th>m.p. /°C</th>
<th>Thesis, page</th>
</tr>
</thead>
<tbody>
<tr>
<td>149</td>
<td>N\textsuperscript{8}-Boc-N\textsuperscript{1},N\textsuperscript{4}-Z\textsubscript{2}-spd</td>
<td>83\textsuperscript{c}</td>
<td>oil</td>
<td>138</td>
</tr>
<tr>
<td>150</td>
<td>N\textsuperscript{1},N\textsuperscript{4}-Z\textsubscript{2}-spd</td>
<td>96</td>
<td>oil</td>
<td>140</td>
</tr>
<tr>
<td>151</td>
<td>N\textsuperscript{8}-Ac-N\textsuperscript{1},N\textsuperscript{4}-Z\textsubscript{2}-spd</td>
<td>85</td>
<td>oil</td>
<td>140</td>
</tr>
<tr>
<td>152</td>
<td>N\textsuperscript{8}-Ac-spd.2HCl</td>
<td>90</td>
<td>202-203\textsuperscript{e}</td>
<td>141</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Characterized by \textsuperscript{1}H and \textsuperscript{13}C n.m.r. spectra and elemental analysis (the latter only for the salt of 152). \textsuperscript{b}After purification. \textsuperscript{c}With ZOBt. \textsuperscript{d}With Z\textsubscript{2}O. \textsuperscript{e}Lit. 204-205 °C\textsuperscript{35a}, 203.5-205 °C\textsuperscript{110}.
4.2.4 - Synthesis of $N^1,N^8$-diethylspermidine

Based on a reported procedure for N-alkylation of urethane type groups with alkyl halides\textsuperscript{28}, the $N^1,N^8$-diethylspermidine trioxalate \textbf{163} was prepared from the tribenzyloxy-carbonylated intermediate \textbf{131} according to Scheme 31.

\[
\text{ZnH(CH}_2\text{)}_3\text{N(Z(CH}_2\text{)}_4\text{NHZ} \xrightarrow{i} \text{Et(Z(N(CH}_2\text{)}_3\text{N(Z(CH}_2\text{)}_4\text{N(ZEt}} \xrightarrow{\text{ii, iii}} \text{EtNH(CH}_2\text{)}_3\text{NH(CH}_2\text{)}_4\text{NHEt.3H}_2\text{C}_2\text{O}_4}
\]

\textbf{Scheme 31} Synthesis of $N^1,N^8$-Et$_2$-spermidine. Reagents: \textit{i}, NaH, EtI (THF-DMF, reflux); \textit{ii}, H$_2$/Pd-C \textit{iii}, oxalic acid.

The first step, the diethylation of compound \textbf{131} required a long reaction time and even so t.l.c. still showed traces of starting material as well as an extra spot, presumably the monoethylated derivatives. Column chromatography on silica of the crude mixture afforded compound \textbf{162} in a satisfactory yield. When the reaction was scaled-up to about 4 mmol, the yield of the pure product decreased to 40-50%.

The final step, the hydrogenolysis of compound \textbf{162}, was readily accomplished using the standard procedure to give the $N^1,N^8$-diethylspermidine in good yield, slightly contaminated with traces of impurities.

As the previous experiments with the acetyl derivatives
showed that their oxalate salts were conveniently handled, the
diethylated analogue was converted to its oxalate salt 163 by
treating an ethanolic solution of the spermidine derivative
with a solution of oxalic acid in ether. As oxalic acid can
form a divalent anion it was originally expected that only 1.5
equivalents of oxalic acid were needed. Elemental analysis
indicated, however, that the salt formed contained three
molecules of oxalic acid. Thus, for further experiments the
salt was always precipitated with a slight excess of three
equivalents of oxalic acid. Recrystallization gave a very pure
salt 163 as shiny white crystals.

The results of this synthesis are summarized in Table XIII.

Table XIII - Yields and melting points of the compounds isolated during the synthesis of $N^1,N^8$-Et$_2$-spermidine trioxalate 163.

<table>
<thead>
<tr>
<th>No.</th>
<th>Compound</th>
<th>Yield $^b$ (%)</th>
<th>m.p. /°C</th>
<th>Thesis, page</th>
</tr>
</thead>
<tbody>
<tr>
<td>162</td>
<td>$N^1,N^4,N^8-Z_3-N^1,N^8$-Et$_2$-spd</td>
<td>65</td>
<td>oil</td>
<td>144</td>
</tr>
<tr>
<td>163</td>
<td>$N^1,N^8$-Et$_2$-spd</td>
<td>92</td>
<td>oil</td>
<td>145</td>
</tr>
<tr>
<td></td>
<td>$N^1,N^8$-Et$_2$-spd $\cdot$ $3H_2C_2O_4$</td>
<td>229.5-230.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$Characterized by $^1$H and $^{13}$C n.m.r. spectra and elemental analysis (the latter only for the oxalate salt 163). $^b$After purification.
4.2.5 - Attempted syntheses of $N^1$-ethyl- and $N^8$-ethyl-spermidine

The synthesis of the title compounds turned out to be a more difficult task than the corresponding to $N^1,N^8$-diethyl derivative 163. In principle it should be possible to make them from proper intermediates previously obtained in this research work by selective alkylation or reduction.

A. Alkylation experiments

In order to synthesize $N^1$-Et-spermidine 164 by the procedure used for the diethyl derivative 163 (Scheme 31, p. 83), $N^1,N^4,N^8,N^8$-Boc$_4$-spermidine 166 was considered a potential intermediate. It should be possible to make 166 in two steps by exhaustive tert-butoxycarbonylation of the key compound 138, followed by selective removal of the Z group, according to the following scheme:

\[
\begin{align*}
ZNH(CH_2)_3NH(CH_2)_4NHBoc & \xrightarrow{\text{Boc}_2O/DMAP} ZN(CH_2)_3N(Boc)(CH_2)_4NBoc \\
138 & \xrightarrow{\text{CH}_3CN} BocNH(CH_2)_3N(Boc)(CH_2)_4N(Boc) \\
& \xrightarrow{\text{H}_2/\text{Pd-C (MeOH)}} BocNH(CH_2)_3N(Boc)(CH_2)_4N(Boc)_2 \\
166
\end{align*}
\]

Before performing the synthesis of derivative 166, it was worthwhile to study the alkylation conditions using simple model compounds. Thus, several attempts were carried out and the results can be summarized as follows:
a) alkylation with EtI/NaH

The model compound Ph(CH₂)₂N(Boc)₂ was treated with NaH and EtI in THF-DMF. Although the compound was stable for about 2 hours at room temperature, it was readily converted (~2 h) to the mono-Boc derivative under refluxing conditions. Thus, the idea of ethylating under these conditions was abandoned.

b) alkylation with EtI/Ag₂O

In the literature it was reported the N-methylation of N-Z and N-Boc amino acids with methyl iodide in the presence of silver oxide. The stability of the model compound mentioned in a) under these conditions prompted us to adopt this procedure.

Preliminary experiments with the model compound N²-Z-N¹-Boc-N¹-Et-ethylenediamine only led to the recovery of the starting material even after long reaction times or heating. It is worth mentioning that methyl iodide also failed to alkylate compound under these conditions.

\[
\text{Boc}(\text{Et})N(\text{CH}_2)_2\text{NHZ} \xrightarrow{\text{EtI (or MeI), Ag}_2\text{O}} \text{no reaction} \quad \text{DMF}
\]

Repeating this experiment with one of the reported amino acids, N-Z-alanine₁¹², showed that the reaction worked nicely with methyl iodide as alkylating reagent but failed with ethyl iodide. The latter reagent afforded only the ester of the N-Z amino acid.
c) alkylation with CF$_3$SO$_3$CH$_2$CH$_3$

The properties of the alkyl perfluoralkanesulfonic esters as highly reactive alkylation agents$^{113}$ prompted us to perform the alkylation reaction using ethyl trifluoromethanesulfonate at room temperature.

The model experiments revealed several interesting features. No alkylation occurred when compound 157 was treated only with CF$_3$SO$_3$CH$_2$CH$_3$ (triflate) in CH$_2$Cl$_2$ according to a described procedure$^{114}$. The presence of a base (NaH) was necessary for the alkylation of a urethane group and the desired product 167 (Scheme 32) was readily obtained ($\approx 4$ h) in quantitative yield when using two equivalents of NaH and triflate. The product was an oil and it agreed by t.l.c. and $^1$H n.m.r. with a product obtained by alkylation with EtI/NaH.

![Scheme 32: Synthesis of N$^2$-Z-N$^1$-Boc-N$^1$,N$^2$-Et$_2$-ethylene-diamine. Reagents: i, CF$_3$SO$_3$CH$_2$CH$_3$, NaH (CH$_2$Cl$_2$) or EtI, NaH (THF:DMF, reflux).](image)
This result seemed promising for the synthesis of 
$N^1$-Et-spermidine as also the compound $Ph(CH_2)_2N(Boc)_2$ referred to in a) was stable to NaH for several hours at room temperature.

However, h.p.l.c. experiments using the model compound 157 and its isomer $N^1-Z-N^2-Boc-N^1$-Et-ethylenediamine 158 surprisingly indicated that the alkylation of derivative 158 was considerably slower than that of 157 (Fig. 6).

![Graph](image)

**Figure 6** Alkylation of $N^2-Z-N^1$-Boc-$N^1$-Et-ethylenediamine 157 (a) and $N^1-Z-N^2$-Boc-$N^1$-Et-ethylenediamine 158 (b) with 1 equiv. of $CF_3SO_2CH_2CH_3$ and 1 equiv. of NaH. (The data were obtained by semiquantitative h.p.l.c.).
While the half-life of compound 157 was about 2 h, only 15% of the desired product 167 was obtained after 24 h following the alkylation of 158 and a by-product was also detected.

d) selective alkylation

In view of the results described in c), it seemed worthwhile to selectively N-alkylate a benzyl carbamate group in the presence of a tert-butyl one. Thus, it was performed an explorative experiment with $N^1-Z-N^4$-Boc-putrescine 168.

\[
\text{ZNH(CH}_2\text{)}_4\text{NHBoc} \xrightarrow{\text{CF}_3\text{SO}_3\text{Et, NaH}} \text{Z(Et)N(CH}_2\text{)}_4\text{NHBoc}
\]

However, rather disappointing results were obtained. It was afforded the product 169 in 35% (by h.p.l.c.) together with three by-products. Moreover, compound 169 was contaminated with one of the by-products which could not be separated either by column chromatography or h.p.l.c.

In summary, the synthesis of $N^1$-Et-spermidine by N-ethylation of a carbamate group of the intermediate $N^1,N^4,N^8,N^8$-Boc$_4$-spermidine 166 seemed to be less practical. First, a compound containing an $N,N$-Boc$_2$ moiety was unstable to NaH in refluxing THF-DMF. Second, the tert-butyl carbamate appeared to have a low reactivity towards triflate in the presence of NaH at room temperature. Third, the selective
N-alkylation of the benzyl carbamate group in \(N^1-Z-N^4\)-Boc-putrescine 168 gave a low yield of the impure product.

B. Reduction experiments

The next series of experiments attempted had as basic idea either the reductive alkylation of amino groups or the reduction of amide groups. The results can be summarized as follows:

a) reductive alkylation

The reductive alkylation of the adducts between the key intermediates 146 or 150 (Scheme 28, p. 65) and acetaldehyde with reduction of the formed imine by NaBH\(_4\), seemed a promising method.

\[
\begin{align*}
\text{NH}_2(\text{CH}_2)_3\text{NBoc}(\text{CH}_2)_4\text{NHboc} & \xrightarrow{1) \text{CH}_3\text{CHO}} \xrightarrow{2) \text{NaBH}_4} \text{EtNH}(\text{CH}_2)_3\text{NBoc}(\text{CH}_2)_4\text{NHboc} \\
\text{ZNH}(\text{CH}_2)_3\text{NZ}(\text{CH}_2)_4\text{NH}_2 & \xrightarrow{1) \text{CH}_3\text{CHO}} \xrightarrow{2) \text{NaBH}_4} \text{ZNH}(\text{CH}_2)_3\text{NZ}(\text{CH}_2)_4\text{NHboc} 
\end{align*}
\]

Preliminary experiments were performed with the model compound \(N^1\)-Boc-\(N^1\)-Et-ethylene diamine 170 and again the results were discouraging. When compound 170 was treated with excess of acetaldehyde in the presence of molecular sieves in benzene followed by isolation of the product, a highly insoluble and reddish material was obtained (probably
due to polymerization of the imine). The alternative way, the reduction in situ of the imine according to a procedure reported by Olsen et al.\textsuperscript{58b}, gave a mixture of starting material, the desired product and the dialkyl derivative, difficult to separate. In view of this outcome this procedure was also abandoned.

b) reduction of the amide group

The reduction of the acetyl derivatives to afford the corresponding ethyl derivatives was one possible alternative (Scheme 33).

\[
\begin{align*}
\text{Boc(Et)N(CH}_2)_2\text{NHAc} & \overset{i}{\longrightarrow} \text{Boc(Et)N(CH}_2)_2\text{NHEt} \\
171 & \text{ } 172 \\
\downarrow & \downarrow \\
\text{EtNH(CH}_2)_2\text{NHAc} & \overset{\text{iii}}{\longrightarrow} \text{EtNH(CH}_2)_2\text{NHEt} \\
173 & \text{ } 174
\end{align*}
\]

\textbf{Scheme 33} Reduction of an N-acetyl to an N-ethyl group. Reagents: i, NaBH\textsubscript{4}, TFA (THF); ii, 2.29 M HCl (dioxan); iii, Red-Al (dioxan, reflux).

Selective reduction of the acetamide group with NaBH\textsubscript{4}/TFA of the model compound \textit{N}^2-\textit{Ac}-\textit{N}^1-\textit{Boc}-\textit{N}^1-\textit{Et-ethylenediamine} 171 afforded a modest yield (35 \%) of \textit{N}^1-\textit{Boc}-\textit{N}^1,\textit{N}^2-\textit{Et}_2-\textit{ethylenediamine} 172 after column chromatography. Another possibility, the reduction of the amide group with Red-Al\textsuperscript{115} after removing the urethane group, gave 52 \% of the reduced compound 174.
Thus, the final syntheses of the title compounds were performed by the action of Red-Al on the deprotected acetyl derivatives 148 and 152 (Scheme 28, p. 65) in refluxing dioxan.

\[
\text{AcNH(CH}_2\text{)}_3\text{NH(CH}_2\text{)}_4\text{NH}_2 \xrightarrow{1) \text{Red-Al}} \text{EtNH(CH}_2\text{)}_3\text{NH(CH}_2\text{)}_4\text{NH}_2\cdot3\text{H}_2\text{C}_2\text{O}_4
\]

\[
\text{148} \xrightarrow{2) \text{oxalic acid}} \text{164}
\]

\[
\text{NH}_2\text{(CH}_2\text{)}_3\text{NH(CH}_2\text{)}_4\text{NHAc} \xrightarrow{1) \text{Red-Al}} \text{NH}_2\text{(CH}_2\text{)}_3\text{NH(CH}_2\text{)}_4\text{NH}_{\text{Et}}\cdot3\text{H}_2\text{C}_2\text{O}_4
\]

\[
\text{152} \xrightarrow{2) \text{oxalic acid}} \text{165}
\]

In both cases t.l.c. of the crude mixtures showed a by-product with the same \(R_f\) as spermidine. This was probably due to a partial hydrolysis of the acetyl group. The work-up was rather laborious as the products were continuously extracted from the aqueous solution. Column chromatography of the crude products gave the compounds as white powders which were homogeneous by t.l.c. but contaminated with inorganic material (elemental analysis gave \(\approx 30\%\) of the product). Thus, it was necessary to carry out another extraction followed by precipitation as oxalate salts. After recrystallization, very pure products were obtained as white shiny crystals in a rather modest yield.
4.3 - Synthesis of alkyl benzyl imidodicarbonates

As shown in Scheme 29 (p. 66), the starting material for the preparation of the alkyl benzyl imidodicarbonates 155a-i was benzyl carbamate 153 which was easily obtained according to a standard procedure reported by Carter et al.116.

\[
\text{NH}_3 \text{ (aq.)} \xrightarrow{ZCl} \text{ZNH}_2
\]

The key intermediate, benzyloxycarbonyl isocyanate 154, was obtained in satisfactory yield from benzyl carbamate and oxalyl chloride according to a known general method117,118.

\[
\text{ZNH}_2 \xrightarrow{(\text{COCl})_2} \text{ZNC} = \text{O}
\]

This intermediate, a colourless liquid, turned out to be very sensitive to moisture, and decomposition to the initial starting material occurred readily. Thus, it should be handled with special precautions and stored below \(-20 \degree C\) in a sealed vessel under nitrogen.

The next step involved the reaction of isocyanate 154 with different alcohols104 and no particular problems were encountered.

\[
\text{ZNC} = \text{O} \xrightarrow{\text{ROH}} \text{ZNHCOOR}
\]

For R see Table XIV
In general the conversion $154 \rightarrow 155$ proceeded smoothly to give the corresponding alkyl benzyl imidodicarbonates in excellent yield and high purity after a simple work up. It is worth pointing out that, in the case of the extremely acid-sensitive PpocNHZ $155d$, the crude product was contaminated with significant amounts of benzyl carbamate $153$. This was probably due to a partial decomposition of $155d$ in the presence of acidic impurities in $154$. Therefore, the isocyanate must be redistilled before the preparation of $155d$.

As mentioned before, these reagents are potential intermediates in amine synthesis$^{101}$ using the Gabriel and Mitsunobu reactions. Thus, they should also be useful for designing new pathways to polyamines. However, their full usefulness in general practice remains to be explored.

By selective removal of one of the blocking groups in such doubly protected amines, subsequent alkylation of the urethane function should be possible. To confirm their usefulness, a preliminary study was performed employing these newly synthesized compounds using conventional deprotection conditions$^{25,120}$. All deblocking reactions displayed an excellent selectivity and in no case even traces of an anomalous deprotection product could be detected in the crude reaction mixtures as judged by t.l.c. and $^1H$ n.m.r. The yields observed were also satisfactory after extractive work-up.

The experimental results obtained in the syntheses and in the deprotection study of these imidodicarbonates are summarized in tables XIV and XV.
Table XIV - Yields and melting points of the alkyl benzyl imidodicarbonates ZNHR 155a-i.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Yield</th>
<th>m.p.</th>
<th>(Lit.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>155a</td>
<td>Z</td>
<td>99</td>
<td>109-109.5</td>
<td>(105.5-106.5-119)</td>
</tr>
<tr>
<td>155b</td>
<td>Z(MeO)</td>
<td>≈100</td>
<td>92.5-93</td>
<td></td>
</tr>
<tr>
<td>155c</td>
<td>Z(NO₂)</td>
<td>97</td>
<td>113.5-114</td>
<td></td>
</tr>
<tr>
<td>155d</td>
<td>Ppoc</td>
<td>91</td>
<td>83.5-84</td>
<td></td>
</tr>
<tr>
<td>155e</td>
<td>Boc</td>
<td>84</td>
<td>oil 101</td>
<td></td>
</tr>
<tr>
<td>155f</td>
<td>Adoc</td>
<td>98</td>
<td>112-112.5</td>
<td></td>
</tr>
<tr>
<td>155g</td>
<td>Aloc</td>
<td>94</td>
<td>79.5-80</td>
<td></td>
</tr>
<tr>
<td>155h</td>
<td>Troc</td>
<td>92</td>
<td>90-90.5</td>
<td></td>
</tr>
<tr>
<td>155i</td>
<td>Fmoc</td>
<td>92</td>
<td>112-113</td>
<td></td>
</tr>
</tbody>
</table>

*Characterized by ¹H and ¹³C n.m.r. spectra and elemental analysis (the latter for 155b, c, d, f-i).*

Table XV - Selective deprotection of alkyl benzyl imidodicarbonates 155.

<table>
<thead>
<tr>
<th>No</th>
<th>Compound</th>
<th>Product</th>
<th>Reaction conditions</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>155b</td>
<td>Z(MeO)NHZ</td>
<td>ZNH₂</td>
<td>TFA/anisole (9:1), 1 h, 0 °C</td>
<td>25</td>
</tr>
<tr>
<td>155c</td>
<td>Z(NO₂)NHZ</td>
<td>Z(NO₂)NH₂</td>
<td>HF/anisole (9:1), 1 h, 0 °C</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>or TFA, reflux, 15 min.</td>
<td></td>
</tr>
<tr>
<td>155d</td>
<td>PpocNHZ</td>
<td>ZNH₂</td>
<td>1.5 % TFA/CH₂Cl₂, 1 h, r.t.</td>
<td>25</td>
</tr>
<tr>
<td>155f</td>
<td>AdocNHZ</td>
<td>AdocNH₂</td>
<td>H₂/Pd-C, MeOH, 1 h, r.t.</td>
<td>25</td>
</tr>
<tr>
<td>155g</td>
<td>AlocNHZ</td>
<td>ZNH₂</td>
<td>(Ph₃P)₂RhCl, 90 % aq. EtOH, 1 h, 70 °C</td>
<td>120b</td>
</tr>
<tr>
<td>155h</td>
<td>TrocNHZ</td>
<td>TrocNH₂</td>
<td>H₂/Pd-C, MeOH, 1 h, r.t.</td>
<td>25, 120a</td>
</tr>
<tr>
<td>155i</td>
<td>FmocNHZ</td>
<td>ZNH₂</td>
<td>20% Piperidine, DMF, 1 h, r.t.</td>
<td>25, 120c</td>
</tr>
</tbody>
</table>
**4.4 - Concluding comments**

The most important spermidine derivatives obtained during this research are listed in Table XVI. The following aspects of the syntheses should be emphasized:

1- The novel protection methodology for amino functions, which differentiates between primary and secondary amino groups, was successful when applied directly to spermidine and gave $N^1,N^8$-Boc$_2$-spermidine 133 as a solid by a simple three-step procedure in quite a satisfactory yield. Therefore, this is a good alternative to the previously reported syntheses. Bergeron et al.$^{41}$ prepared it in five steps from benzylamine. Das et al.$^{51b}$ have also reported a five-step sequence from 4-Boc-aminobutyric acid and 3-amino-1-chloropropane. More recently, Ganem et al.$^{82}$ took advantage of the cyclic spermidine derivative and also obtained a crystalline product in three steps from spermidine. However, for an extension to other polyamines, our approach seems to be of general applicability whereas the Ganem procedure is limited to those containing the aminopropyl moiety. Developments of selective acylation reagents, such as acyl cyanides recently reported$^{76}$, might lead to an efficient one-step alternative. Nevertheless, the access to those reagents is still a restricting factor.

2- A selective protection of spermidine could also be achieved in a four-step route by performing this novel approach on a cyclic spermidine analogue in good overall
<table>
<thead>
<tr>
<th>No.</th>
<th>Compounds (^a)</th>
<th>Overall yield, % (steps)</th>
<th>m.p. /°C</th>
<th>Thesis, page</th>
</tr>
</thead>
<tbody>
<tr>
<td>133</td>
<td>(N^1,N^8)-Boc(_2)-spd</td>
<td>62 (3 from spd)</td>
<td>85.5-86.5c</td>
<td>113</td>
</tr>
<tr>
<td>138</td>
<td>(N^1)-Z-N(^8)-Boc-spd</td>
<td>45 (5 from spd)</td>
<td>63-64</td>
<td>119</td>
</tr>
<tr>
<td>144a</td>
<td>(N^4)-Ac-spd.(2\text{H}_2\text{C}_2\text{O}_4).(1/2\text{H}_2\text{O})</td>
<td>43 (4 from spd)</td>
<td>187.5-188.5</td>
<td>130</td>
</tr>
<tr>
<td>148</td>
<td>(N^1)-Ac-spd.(2\text{HCl})</td>
<td>78 (4 from 138)</td>
<td>191-193d</td>
<td>135</td>
</tr>
<tr>
<td>152</td>
<td>(N^8)-Ac-spd.(2\text{HCl})</td>
<td>65 (4 from 138)</td>
<td>202-203e</td>
<td>138</td>
</tr>
<tr>
<td>163</td>
<td>(N^1,N^8)-Et(_2)-spd.(3\text{H}_2\text{C}_2\text{O}_4)</td>
<td>50 (3 from spd)</td>
<td>229.5-230</td>
<td>144</td>
</tr>
<tr>
<td>164</td>
<td>(N^1)-Et-spd.(3\text{H}_2\text{C}_2\text{O}_4)</td>
<td>20 (2 from (N^1)-Ac-(-N^1,N^8)-Boc(_2)-spd)</td>
<td>218.5-219</td>
<td>146</td>
</tr>
<tr>
<td>165</td>
<td>(N^8)-Et-spd.(3\text{H}_2\text{C}_2\text{O}_4)</td>
<td>22 (2 from (N^8)-Ac-(-N^1,N^4),-Z(_2)-spd)</td>
<td>212.5-213</td>
<td>148</td>
</tr>
</tbody>
</table>

\(^a\)Characterized by \(^1\text{H}\) and \(^13\text{C}\) n.m.r. spectra and elemental analysis. From pure compounds \(^\text{Lit.}\) 79-80 °C. \(^\text{Lit.}\) 173-174 or 189-191 °C. \(^\text{Lit.}\) 204-205 °C.

yield. Although previously referred to by Borowsky et al.\(^{121}\) as an in situ intermediate, this is the first complete synthesis reported for compound \(N^1\)-Z-\(N^8\)-Boc-spermidine 138. Thus, it is an alternative and complement to other derivatives such as \(N^8\)-Z-\(N^1\)-Pht-\(N^4\)-Tos-spermidine\(^{23}\) and \(N^4\)-Bzl-N\(^1\)-Boc-\(N^8\)-(CF\(_3\)CO)spermidine\(^{43}\), which have been prepared for the selective modification of spermidine. The first one requires at least six steps for its preparation and, moreover, rather
drastic conditions for the removal of its protecting groups. The second one, although containing more versatile protecting groups, also requires five-six steps. \(N^1-Z-N^8\)-Boc-spermidine can be easily obtained with the novel procedure. It takes advantage of the properties of the well established \(N\)-protecting groups \(Z\) and Boc which require rather mild orthogonal deprotection conditions. This approach, however, is limited to polyamines containing the aminopropyl moiety.

3- Although selective acylation of the secondary amino groups can be readily accomplished from the \(N^1,N^8\)-Boc\(_2\)-spermidine 133 and similar disubstituted precursors, the Boc\(_2\)/DMAP approach described in this thesis offers an efficient four-step alternative of wider application.

4- Compound \(N^8\)-Boc-\(N^1\)-Z-spermidine 138 turned out to be a good and rather convenient substrate for selective acylation on \(N^1\) and \(N^8\) via the intermediates \(N^4,N^8\)-Boc\(_2\)-spermidine 146 and \(N^1,N^4\)-Z\(_2\)-spermidine 150 (Scheme 28, p. 65), respectively. Thus, the monoacetylated spermidine derivatives were obtained in higher yield and purity compared to the reported ones\(^{35,55,83}\). Tabor et al.\(^{35}\) have first used a simple direct acetylation of spermidine which gave product mixtures and a low yield of the desired product. An improved later synthesis by the same authors\(^{35}\) was based on the nitrile reduction methodology starting from monoacetylated diamines, putrescine and 1,3-propylenediamine, and proper nitriles, acrylonitrile and 4-bromobutyronitrile respectively. Slaich et al.\(^{55}\) also
reported the syntheses of \( N^1 \)- and \( N^8 \)-acetylspermidine derivatives by the imine reduction approach in a relatively low overall yield. Ganem et al.\(^{83}\) took advantage of the selective acetylation of the cyclic spermidine 114b but rather impure products in low yields were obtained.

5- Prior protection with urethane groups (Z or Boc) provides an efficient route for alkylation of amines and in this particular context it worked rather satisfactorily for \( N^1,N^4,N^8-Z_3 \)-spermidine 131. This compound seems to be more convenient in comparison with \( N \)-tosyl-protected polyamines\(^{9k}\) which require drastic conditions for the removal of the tosyl groups and a more laborious work-up.

6- Although the monoethyl spermidine derivatives were formed in rather modest yields by the reduction of the corresponding acetylated intermediates, they were obtained in a very high purity.

In conclusion, the protected spermidine derivatives \( N^1,N^8-Boc_2 \)-spermidine 133 and \( N^1-Z-N^8-Boc \)-spermidine 138, readily obtained by simple three- and five-step routes respectively, are good intermediates for the selective acylation of spermidine. Therefore they are potentially useful for the synthesis of naturally occurring acylated spermidine analogues such as alkaloids and siderophores. On the other hand, the first attempts for selective alkylation of some related spermidine intermediates were not entirely successful.
In this context, a strategy involving a total synthesis may be a better alternative. The Gabriel type reagents prepared, the alkyl benzyl imidodicarbonates \( \text{ZNHCOOR} \, 155a-i \), might also be useful as starting material in such a synthetic approach, leading to new biologically important spermidine analogues.
The syntheses carried out during the course of this work are fully described here in the following order:

1) experiments mainly with N\textsubscript{1}-ethylethylene diamine;
2) experiments with spermidine;
3) experiments related to the syntheses of the imidodicarbonates.

A list of the compounds isolated is presented at the end in order to facilitate the consultation of this experimental section.

Materials. General methods

All solvents applied as reaction media were of analytical grade and dried for several days over a molecular sieve (4A). The spermidine used in this work was obtained from Fluka AG (purum). The content of ZCl in the commercial samples was measured by \textsuperscript{1}H n.m.r. and the quantities applied were corrected accordingly.

Unless otherwise stated, all organic extracts were repeatedly washed in turn with half their volumes of aqueous 1M KHSO\textsubscript{4}, aqueous 1M NaHCO\textsubscript{3}, and saturated aqueous NaCl, and then dried over anhydrous MgSO\textsubscript{4} (for amines Na\textsubscript{2}SO\textsubscript{4}).

T.l.c. analyses were performed on 0.25 mm\textsuperscript{2} thick precoated silica plates (Merck DC-Fertigplatten Kieselgel 60 F\textsubscript{254}) using the following solvent systems: (A) toluene-acetonitrile (2:1); (B) light petroleum-ether (2:1), (1:3); (D), (E) CH\textsubscript{2}Cl\textsubscript{2}-ether (12:1), (20:1); (F), (G), (H), (K) CH\textsubscript{2}Cl\textsubscript{2}-acetone (2:1), (4:1), (9:1), (20:1); (L) CH\textsubscript{2}Cl\textsubscript{2}-acetone-HOAc (5:5:1); (M) EtOAc-acetone-HOAc-water (5:3:1:1); (N), (O), (P) CH\textsubscript{2}Cl\textsubscript{2}-methanol (4:1), (9:1), (20:1); (Q) CH\textsubscript{2}Cl\textsubscript{2}-methanol\textsuperscript{2}HOAc (18:2:1); (R) CHCl\textsubscript{3}-ethanol-water-aqueous 25\% NH\textsubscript{3} (10:50:4:1) and (S) CHCl\textsubscript{3}-methanol-aqueous 25 \% NH\textsubscript{3} (2:2:1). Spots were visualized by inspection under u.v. light at 254 nm or, after brief heating, by exposure to Cl\textsubscript{2} followed by dicarboxidine spray (violet-blue spots).

The analytical h.p.l.c. equipment consisted of two LDC Constametric pumps, an LDC gradient master, a Rheodyne 7125 injector, an LDC Spectromonitor III variable wavelength u.v.-detector, an Altex 400 mixer, and a Shimadzu C-R3A integrator.

Column chromatography was performed on silica gel (Merck,
Kieselgel 60, 70-230 mesh ASTM). The dimensions of the columns were 20 x 2.7 cm, 15 x 5 cm and 20 x 6 cm for 50 g, 160 g and 260 g of silica, respectively.

N.m.r. spectra were routinely recorded for solutions in CDCl₃ on a JEOL FX90Q instrument at 90 MHz (¹H) or 22.5 MHz (¹³C). The chemical shifts are generally reported relatively to TMS as internal standard but for spectra recorded in D₂O, they refer to sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS). The signals, assigned by comparing chemical shifts and peak shapes, are tentative.

Melting points were determined in open capillary tubes on a Gallenkamp melting point apparatus and are uncorrected.

Elemental analyses of selected derivatives were carried out by Mikro Kemi AB, Uppsala, Sweden.
5.1 - Experiments with model compounds

5.1.1 - Synthesis of $N^2$-benzyloxycarbonyl-$N^1$-tert-butoxy-carbonyl-$N^1$-ethylethylenediamine (157)

**One-pot procedure:**

\[
\text{EtNH(CH}_2\text{)}_2\text{NH}_2 \xrightarrow{1) \text{ ZCN}} \xrightarrow{2) \text{ Boc}_2\text{O, NMM}} \text{Boc(Et)N(CH}_2\text{)}_2\text{NHZ}
\]

To a solution of $N^1$-ethylethylenediamine (4.41 g, 50.1 mmol) in dry $\text{CH}_2\text{Cl}_2$ (100 ml), ZCN$^{61}$ (8.22 g, 51.1 mmol), dissolved in dry $\text{CH}_2\text{Cl}_2$ (50 ml), was added dropwise at r.t. with vigorous stirring over a period of 4 h. The resulting colourless solution was stirred for 2 h with the exclusion of atmospheric moisture. A solution of Boc$_2$O (12.0 g, 55.1 mmol) in dry $\text{CH}_2\text{Cl}_2$ (50 ml) was then slowly introduced ($\approx$ 1 h) followed by N-methylmorpholine (5.57 g, 55.1 mmol). The reaction mixture was then stirred overnight. The solvent was removed at reduced pressure below 30 °C. The remaining, almost colourless oil was partitioned between ether (1000 ml) and aqueous 0.2M citric acid (500 ml). The ethereal extract was washed successively with aqueous 0.2M citric acid, aqueous 1M NaHCO$_3$ and saturated aqueous NaCl (3 x 250 ml) and dried (MgSO$_4$). Evaporation to complete dryness left a pale yellow viscous residue which soon solidified upon trituration with cold heptane. The crude, essentially pure product 157 was obtained in quantitative yield. It was recrystallized from
light petroleum (20 ml/g, decolourizing carbon). After seeding and chilling to -20 °C overnight, the precipitated white crystals were collected, rinsed with small portions of cold light petroleum and dried in high vacuo to afford 13.1 g (81 %) of the pure compound 157. T.l.c. (ether) gave one spot, different from the isomeric N²-Boc-N¹-ethyl-N¹-Z-ethylenediamine; m.p. 47.5-48.0 °C; δ H 7.33 (s, 5H, arom. H), 5.35 (broad, ≈ 1H, NH), 5.09 (s, 2H, CH₂Ph), 3.10-3.33 (complex, 6H, CH₂N), 1.44 [s, 9H, C(CH₃)₃], and 1.08 (t, 3H, CH₂CH₃); δ C 156.5 and 155.9 (CO), 136.6, 128.4, and 128.0 (arom. C), 79.7 [C(CH₃)₃], 66.5 (CH₂Ph), 46.0 (CH₃CH₂N), 42.5 (CH₂NEt), 40.3 (CH₂NHZ), 28.4 [C(CH₃)₃], and 13.6 (CH₂CH₃). (Found: C, 63.4; H, 8.1; N, 8.7. C₁₇H₂₆N₂O₄ requires C, 63.3; H, 8.1; N, 8.7%).

5.1.2 - Synthesis of N¹-benzyloxycarbonyl-N²-tert-butoxycarbonyl-N¹,N²-diethylenediamine (167)

A - Alkylation with ethyl iodide

Boc(Et)N(CH₂)₂NH₂ + EtI + NaH → Boc(Et)N(CH₂)₂N(Et)Z + NaI + H₂

157 167

To a solution of compound 157 (500 mg, 1.55 mmol) and EtI (2.20 g, 14.1 mmol) in anhydrous THF:DMF (10:1) (25 ml), NaH (80 % dispersion in oil, 150 mg, 5.00 mmol) was cautiously added at 0 °C with gentle stirring under nitrogen. The resulting suspension was refluxed under nitrogen for 24 h.
The reaction mixture was cooled to r.t. (a white precipitate was formed) and water (2 ml) was added to decompose excess of NaH. The clear yellow solution was evaporated under reduced pressure and the residue partitioned between ether (200 ml) and aqueous 1M KHSO₄ (100 ml). The organic layer was washed and dried as usual. Evaporation to dryness gave a yellow oily residue (498 mg, 92%). This crude material was chromatographed on silica (light petroleum-ether, 2:1). The appropriate fractions were pooled and evaporated under reduced pressure to afford 364 mg (67%) of compound 167 as a pale yellow oil, homogeneous by t.l.c. (B or G); δ_H 7.35 (s, 5H, arom. H), 5.13 (s, 2H, CH₂Ph), 3.21-3.44 (complex, 8H, CH₂N), 1.45 [s, 9H, C(CH₃)₃], and 1.05-1.25 (m, 6H, CH₂CH₃); δ_C 155.8 and 155.2 (CO), 136.7, 128.4, 127.9, and 127.7 (arom. C), 79.3 [C(CH₃)₃], 66.9 (CH₂Ph), 45.6, 45.4, 44.8, and 42.8 (CH₂N), 28.4 [C(CH₃)₃], 13.9 and 13.5 (CH₂CH₃).

B - Alkylation with ethyl trifluoromethanesulfonate

\[
\text{Boc}(\text{Et})\text{N}(\text{CH}_2)_2\text{NH}Z + \text{CF}_3\text{SO}_3\text{Et} + \text{NaH} \\
\text{157} \\
\text{Boc}(\text{Et})\text{N}(\text{CH}_2)_2\text{N(}\text{Et})Z + \text{CF}_3\text{SO}_3\text{Na} + \text{H}_2 \\
\text{167}
\]

A stirred solution of compound 157 (100 mg, 0.320 mmol) in dry CH₂Cl₂ (1 ml) was treated with NaH (20 mg, 0.640 mmol) followed by CF₃SO₃Et (114 mg, 820 µl, 0.640 mmol) at r.t. After 3 h stirring, t.l.c. (G) indicated complete reaction.
The solvent was evaporated under reduced pressure and the yellowish residue chromatographed as under procedure A to afford the pure product 167 (110 mg, 98 %). T.l.c. and $^1$H and $^{13}$C n.m.r. spectra were identical with those given above.

5.1.3 - **Synthesis of N$^1$-tert-butoxycarbonyl-N$^1$-ethylethylene-diamine (170)**

$$\text{Boc}(\text{Et})\text{N(CH}_2)_2\text{NH} + \text{H}_2 \xrightarrow{\text{Pd-C}} \text{Boc}(\text{Et})\text{N(CH}_2)_2\text{NH}_2 + \text{PhCH}_3 + \text{CO}_2$$

Compound 157 (6.00 g, 18.6 mmol) was dissolved in dry methanol (350 ml) and hydrogenolyzed (1 atm, r.t.) in the presence of Pd-C (5 %, 1.00 g).

When the starting material had been consumed (t.l.c. (Q)), the catalyst was filtered off and rinsed with methanol. The colourless filtrate was taken to dryness to leave a light yellow oily residue which was partitioned between aqueous 0.2M citric acid (100 ml) and ether (100 ml). The aqueous phase was again extracted with ether (2 x 100 ml). After making alkaline with solid NaOH (pH ≈ 13), the aqueous phase was extracted with ether (5 x 200 ml). The combined organic phases were washed with saturated aqueous NaCl (2 x 100 ml) and dried ($\text{Na}_2\text{SO}_4$). The extract was carefully evaporated to dryness under reduced pressure (the product was somewhat volatile and excessive drying caused losses) to afford 3.18 g (91 %) of compound 170 as a light yellow oil. T.l.c. (M or Q)
of this crude material showed mainly one spot and was suitable for further synthetic work; δ_H 3.13-3.36 (complex, 4H, CH_2N), 2.82 (t, 2H, CH_2NH_2), 1.98 (s, 2H, amine NH), 1.46 [s, 9H, C(CH_3)_3], and 1.10 (t, 3H, CH_2CH_3); δ_C 155.7 (CO), 79.3 [C(CH_3)_3], 49.7, 42.3, 40.7 (CH_2N), 28.5 [C(CH_3)_3], and 13.7 (CH_2CH_3).

5.1.4 - Synthesis of N^2-acetyl-N^1-tert-butoxycarbonyl-N^1-ethylethylenediamine (171)

\[
\text{Boc(Et)N(CH}_2\text{)}_2\text{NH}_2 + \text{Ac}_2\text{O} + \text{Et}_3\text{N} \quad \downarrow \quad \text{Boc(Et)N(CH}_2\text{)}_2\text{NHAc} + (\text{Et}_3\text{NH})^+ - \text{OAc}
\]

A solution of Ac_2O (1.63 g, 16.0 mmol) was added dropwise to a stirred ice-cold solution of compound 170 (2.15 g, 13.3 mmol) and TEA (2.02 g, 20.0 mmol) in dry CH_2Cl_2 (20 ml). The clear, pale yellow mixture was stirred for 1 h in ice-bath and overnight at r.t. The solvent was evaporated under reduced pressure and the yellow oily residue chromatographed on silica (CH_2Cl_2-acetone 4:1). The appropriate fractions were pooled and evaporated to afford 2.90 g (94 %) of compound 171 as a light yellow oil. This product solidified at -20 °C to a low melting solid, homogeneous by t.l.c. (A, G, or O); δ_H ≈ 6.62 (broad, ≈ 1H, amide NH), 3.12-3.38 (complex, 6H, CH_2N), 1.96 (s, 3H, CH_3CON), 1.47 [s, 9H, C(CH_3)_3], and 1.10 (t, 3H,
5.1.5 - Synthesis of $^1$-tert-butoxycarbonyl-$^1$-$^2$-diethyl-ethylenediamine (172)

A - Reduction of compound 171

The compound 171 (653 mg, 2.84 mmol) in dry THF (15 ml) was treated with NaBH$_4$-TFA, essentially as outlined in ref. 51b. After stirring for 30 h, water (5 ml) was added to the reaction mixture and the solvent evaporated under reduced pressure. The residue was treated with aqueous 0.2 M citric acid (20 ml) and extracted with ether (3 x 20 ml). The aqueous phase was made alkaline with solid NaOH (pH ≈ 13) and extracted with ether (4 x 50 ml). The combined organic layers were washed with saturated NaCl (2 x 20 ml) and dried (Na$_2$SO$_4$). Evaporation of the extract afforded 377 mg (61%) of a yellowish oil. The crude product was chromatographed on silica (CHCl$_3$-EtOH-water-25%aq. NH$_3$, 100:50:4:1) to give a semisolid residue. This material was dissolved in CHCl$_3$ and some white material filtered off. The solvent was evaporated to dryness to afford 227 mg (37%) of compound 172 as a pale yellow oil, essentially pure by t.l.c. (R); $\delta_H$ 3.13-3.37 (m,
$4\text{H, CH}_2\text{NBoc}$, 2.55-2.83 (complex, $4\text{H, CH}_2\text{NH}$), 1.46 [$s, 9\text{H, C(}\text{CH}_3)_3$], 1.40 (broad, $\approx 1\text{H, amine NH}$), and 1.10 ($t, 6\text{H, CH}_2\text{CH}_3$); $\delta_C$ 155.6 (CO), 79.3 [C(CH$_3$)$_3$], 48.2 (CH$_3$CH$_2$NH), 46.7 (CH$_3$CH$_2$NBoc), 44.0 (CH$_2$NHEt), 42.4 [CH$_2$N(Et)Boc], 28.5 [C(CH$_3$)$_3$], 15.4 (CH$_3$CH$_2$NH), and 13.6 [CH$_3$CH$_2$N(Et)Boc].

**B - Hydrogenolysis of compound 167**

\[
\text{Boc(Et)N(CH}_2)_2\text{N(Et)} \xrightarrow{\text{H}_2/\text{Pd-C}} \text{Boc(Et)N(CH}_2)_2\text{NHET + PhCH}_3 + \text{CO}_2
\]

Compound 167 (217 mg, 0.620 mmol) was hydrogenolyzed and worked up as described for compound 170 and the product chromatographed as under procedure A to give 107 mg (80 %) of product 172 as a light yellow oil. T.l.c. and $^1\text{H}$ and $^{13}\text{C}$ n.m.r. spectra were identical with those given above.

*5.1.6 - Synthesis of $\text{N}^2$-acetyl-$\text{N}^1$-ethylethylenediamine (173)*

\[
\text{Boc(Et)N(CH}_2)_2\text{NHAc} \xrightarrow{1) \text{CF}_3\text{COOH}} \text{EtNH(CH}_2)_2\text{NHAc} \xrightarrow{2) \text{aq. K}_2\text{CO}_3} 173
\]

TFA (20 ml) was added to a flask containing compound 171 (505 mg, 2.19 mmol) and the resulting solution was stirred for 20 min. at r.t. The solvent was then quickly evaporated under reduced pressure. The residue was taken up in methanol (10 ml) and concentrated (twice). The remaining oily residue was treated with aqueous 30 % K$_2$CO$_3$ (15 ml) and extracted with
CHCl₃ (4 x 50 ml). The combined organic layers were dried (Na₂SO₄) and evaporated under reduced pressure to afford 242 mg (85 %) of product 173 as a brownish oil. This crude material was essentially pure by t.l.c. (M or Q) and was used without further purification; δ_H ≈ 6.94 (broad, ≈ 1H, amide NH), 3.35 (q, 2H, CH₂NHAc), 2.56-2.83 (complex, 4H, CH₂NHEt), 1.98 (s, 3H, CH₃CON), 1.26 (broad, ≈ 1H, amine NH), and 1.12 (t, 3H, CH₃CH₂N); δ_C 171.1 (CO), 48.4, 43.6 and 38.9 (CH₂N), 23.0 (CH₃CON), and 14.7 (CH₃CH₂N).

5.1.7 - Synthesis of N₁,N₂-diethylethylenediamine dihydrochloride (174)

A - Reduction of compound 173

EtNH(CH₂)₂NHCOC₃H + 2 NaAlH₂(OCH₂CH₂OMe)₂

173

1) Toluene
2) aq. NaOH
3) HCl

EtNH(CH₂)₂NHET.2HCl

174

To an ice-cold solution of compound 173 (109 mg, 0.837 mmol) in dry toluene (10 ml) was cautiously introduced a solution of 3.5M Red-Al in toluene (1.40 ml, 5.02 mmol) with gentle stirring under nitrogen (strong evolution of H₂). The resulting solution was refluxed under nitrogen for 2 h. The coloured reaction mixture was cooled to room temperature and then in an ice-bath. Water (2 ml) was gently added to
decompose excess of Red-Al (strong evolution of $H_2$) and the solvent evaporated under reduced pressure. The remaining residue was treated with aqueous 15% NaOH (1 ml) and water (5 ml) (pH $\approx$ 13) and stirred for 1 h. The yellowish turbid aqueous solution was saturated with NaCl and extracted with CHCl$_3$ (4 x 20 ml). The combined extracts were dried ($Na_2SO_4$) and carefully evaporated under reduced pressure. The remaining oil was precipitated as dihydrochloride salt with excess of a solution of 2.29M HCl in dioxan to afford 82 mg (52%) of product 174. An analytical sample was obtained by recrystallization from ethanol (100 ml/g); m.p. 262-264°C; $\delta_H$ 3.34 (s, 4H, NCH$_2$CH$_2$N), 3.15 (q, 4H, CH$_3$CH$_2$N), and 1.31 (t, 6H, CH$_3$CH$_2$N); $\delta_C$ 46.3 and 45.2 (CH$_2$N), and 13.2 (CH$_3$CH$_2$N).

**B - Acidolysis of compound 172**

\[
\text{Boc} (\text{Et}) N (\text{CH}_2)_2 \text{NH} \text{Et} \xrightarrow{\text{HCl/dioxan}} \text{EtNH} (\text{CH}_2)_2 \text{NH} \text{Et}.2 \text{HCl}
\]

Compound 172 (100 mg, 0.462 mmol) was treated with 2.29M HCl in dioxan (5 ml) with stirring at r.t. for 4 h. The solvent was evaporated under reduced pressure and the white residue was suspended in dry ether (5 ml) and concentrated (twice). The white solid residue was triturated with cold dry ether (2 ml), filtered off and rinsed with small portions of cold dry ether (3 x 1 ml) and dried in vacuo. The yield of crude 174 was 58 mg (66%) and the recrystallized sample was identical with the product prepared according to procedure A.
5.1.8 - **Synthesis of N\(^{1}\)-benzyloxycarbonyl-N\(^{4}\)-tert-butoxy-carbonylputrescine** (168)

\[
\text{ZNH(CH}_2\text{)}_4\text{NH}_2 + \text{Boc}_2\text{O} \rightarrow \text{ZNH(CH}_2\text{)}_4\text{NHBoc} + \text{Bu}^+\text{OH} + \text{CO}_2
\]

To an ice-cold solution of \(\text{N}^{1}\)-Z-putrescine\(^{17b}\) (2.10 g, 9.04 mmol) in dry \(\text{CH}_2\text{Cl}_2\) (10 ml) was added dropwise with stirring a solution of \(\text{Boc}_2\text{O}\) (2.17 g, 9.94 mmol) in dry \(\text{CH}_2\text{Cl}_2\) (10 ml). Evolution of gas started and at the end of the addition of \(\text{Boc}_2\text{O}\) a white precipitate formed. The resulting mixture was then stirred at r.t. and after few minutes a clear, yellow solution was obtained. T.l.c. (O) indicated complete reaction after 2 h. The solvent was removed and the residue partitioned between ether (500 ml) and aqueous 1M \(\text{KHSO}_4\) (200 ml). The yellowish ethereal extract was washed and dried as described before. The extract was evaporated to dryness, leaving 2.89 g (99 %) of crude 168 as a white solid, essentially pure by t.l.c. [ether or (O)]. Recrystallization from ethyl acetate-light petroleum (1:2) (40 ml/g) gave the pure product 168 as a white solid; m.p. 101.5-102.0 °C (lit.\(^{123}\) m.p. 124-126 °C); \(\delta_H\) 7.34 (s, 5H, arom. H), 5.09 (s, 2H, \(\text{CH}_2\text{Ph}\)), \(\approx\) 4.95 and \(\approx\) 4.63 (broad, \(\approx\) 2H, amide NH), 2.98-3.98 (m, 4H, \(\text{CH}_2\text{N}\)), and 1.49-1.56 (m) and 1.43 (s) [together 13H, \(\text{CCH}_2\text{C}\) and \(\text{C(CH}_3\text{)}_3\)]; \(\delta_C\) 156.4 and 160.0 (CO), 136.6, 128.5, and 128.0 (arom. C), 79.1 [\(\text{C(CH}_3\text{)}_3\)], 66.6 (\(\text{CH}_2\text{Ph}\)), 40.7, 40.2, (\(\text{CH}_2\text{N}\)), 28.4 [\(\text{C(CH}_3\text{)}_3\)], and 27.3 (\(\text{CCH}_2\text{C}\)).
5.2- Experiments with spermidine

5.2.1- Synthesis of $N^1,N^8$-bis(tert-butoxycarbonyl)spermidine

$N^1,N^4,N^8$-Tribenzyloxycarbonylspermidine (131)

$$2\text{NH}_2\text{(CH}_2\text{)}_3\text{NH}((\text{CH}_2)\text{)}_4\text{NH}_2 + 6\text{ZCl} + 3\text{Na}_2\text{CO}_3 \rightarrow 2\text{ZNH}((\text{CH}_2)\text{)}_3\text{NZ}((\text{CH}_2)\text{)}_4\text{NH} + 6\text{NaCl} + 3\text{CO}_2 + 3\text{H}_2\text{O} \quad (131)$$

An ice-cold solution of spermidine (1.50 g, 10.0 mmol) in aqueous 10 % $\text{Na}_2\text{CO}_3$-dioxan (4:3, 70 ml) was treated dropwise under vigorous stirring with $\text{ZCl}$ (7.40 g, 35.0 mmol) dissolved in dioxan (30 ml). When the addition was complete (≈ 1 h), the resulting mixture was stirred for 1 h in ice-bath (a precipitation of NaCl occurred) and 16 h at r.t. Most of the dioxan was stripped off under reduced pressure below 30 °C and the remaining suspension partitioned between aqueous 1 M $\text{KHSO}_4$ (100 ml) and ether (200 ml). The colourless, ethereal extract was washed and dried according to the general procedure described earlier. The extract was filtered and evaporated in vacuo to complete dryness to afford a pale yellow oil (4.80 g, 90 %) which, although contaminated with benzyl alcohol and minor impurities, was suitable for further synthetic work. The pure product was readily obtained by column chromatography on silica gel. Benzyl alcohol was eluted with neat $\text{CH}_2\text{Cl}_2$ and further elution with $\text{CH}_2\text{Cl}_2$-acetone (9:1).
gave 4.60 g (84%) of compound 131 as a pale yellow oil, homogeneous by t.l.c. (A or B). The product solidified after several weeks at -20 °C, m.p. 38-40 °C; \( \delta_H = 7.31 \) (s, 15H, arom. H), \( \approx 5.6 \) (broad, \( \approx 2H, \) NH), 5.08 and 5.06 (2s, 6H, CH\textsubscript{2}Ph), 2.98-3.38 (m, 8H, CH\textsubscript{2}N), and 1.48-1.71 (m, 6H, CCH\textsubscript{2}C); \( \delta_C \) 156.4 (CO), 136.0, 128.5, 128.0, 127.9, 127.4, and 126.9 (arom. C), 67.1 and 66.6 (CH\textsubscript{2}Ph), 46.4 and 44.1 (CH\textsubscript{2}NCH\textsubscript{2}), 40.5 and 37.7 (CH\textsubscript{2}NHZ), and 28.9, 28.2, and 25.5 (CCH\textsubscript{2}C).

\[ \text{\(N^1,N^4,N^8\)-Tribenzyloxy carbonyl-N\(^1,\)N\(^8\)-bis(tert-butoxy-carbonyl)spermidine (132)} \]

\[
\text{ZNH(CH}_2\text{)}_3\text{NZ(CH}_2\text{)}_4\text{NHZ} + 2\text{Boc}_2\text{O} \xrightarrow{\text{DMAP}} \text{Boc(Z)N(CH}_2\text{)}_3\text{NZ(CH}_2\text{)}_4\text{N(Z)Boc} + 2\text{Bu}^+\text{OH} + 2\text{CO}_2
\]

A solution of 131 (6.00 g, 11.0 mmol) and DMAP (134 mg, 1.10 mmol) in dry acetonitrile (50 ml) was treated with Boc\textsubscript{2}O (5.00 g, 23.1 mmol) in one portion with agitation at r.t. when evolution of carbon dioxide occurred. After stirring for 2 h, t.l.c. (H) showed that starting material as well as another compound (the mono Boc-derivatives!) still remained in the mixture. More Boc\textsubscript{2}O (2.50 g, 11.0 mmol) was introduced and the brownish mixture was left overnight. A new t.l.c. showed that the starting material was consumed but still indicated the presence of the mono Boc-products. Thus, more Boc\textsubscript{2}O
(2.50 g, 11.0 mmol) was added and after 3 h the reaction was complete (t.l.c. showed one main spot). The solvent was stripped off at r.t. and the brownish syrupy residue was partitioned between ether (300 ml) and aqueous 1M KHSO₄ (150 ml). The brownish aqueous phase was discarded and the yellow ethereal extract worked up as described. After treatment with decolourizing carbon, the extract was taken to dryness under reduced pressure, leaving a dark yellow oil (7.90 g, 96%). The crude product was chromatographed on silica with CH₂Cl₂-ether (20:1) as eluant. The appropriate fractions were pooled and evaporated to afford 7.60 g (92%) of compound 132 as a pale yellow oil, homogeneous by t.l.c. (A or H); δH 7.34 and 7.31 (two s, 15H, arom. H), 5.20 (s, 4H, BocNCO₂CH₂Ph), 5.10 (s, 2H, third CH₂Ph), 3.56-3.62 [m, 4H, CH₂N(Z)Boc], 3.16-3.28 (m, 4H, CH₂NCH₂), and 1.71-1.94 (m) and 1.45 (s) [together 24H, CCH₂C + C(CH₃)]; δC 155.9, 153.8, 153.6, 152.0, and 151.8 (CO), 136.8, 135.5, 128.5, 128.3, 128.2, 127.8, and 127.7 (arom. C), 82.8 and 82.7 [OC(CH₃)₃], 68.2 (BocNCO₂CH₂Ph), 66.9 (third CH₂Ph), 46.4, 46.1, and 44.4 (CH₂N), 27.9 [C(CH₃)₃], and 26.2 and 25.4 (CCH₂C).
\[ \text{N}^1,\text{N}^8-\text{Bis(tert-butoxycarbonyl)spermidine (133)} \]

\[
\begin{align*}
\text{Boc}(Z)N(CH_2)_3NZ(CH_2)_4N(Z)\text{Boc} \\
\text{132} & \quad 1) \text{H}_2/\text{Pd-C, NH}^+ \text{HCOO}^- \\
& \quad (80 \% \text{ HOAc}) \\
& \quad \downarrow 2) \text{aq. K}_2\text{CO}_3 \\
\text{BocNH}(CH_2)_3NH(CH_2)_4\text{NHBoc} + \text{PhCH}_3 + \text{CO}_2 \\
\text{133}
\end{align*}
\]

To a solution of compound 132 (8.70 g, 11.6 mmol) in aqueous 80 \% acetic acid (200 ml) was added ammonium formate (13.0 g, 200 mmol). When all had dissolved, Pd-C (5 \%, 4.00 g) was added in small portions with stirring under nitrogen at r.t. After stirring for 1 h, the catalyst was filtered off, rinsed with aqueous 80 \% HOAc and the colourless filtrate was concentrated under reduced pressure. After the addition of aqueous 30 \% K\text{CO}_3 (250 ml) the product was extracted with ether (3 \times 300 ml). The combined colourless organic layers were washed with saturated aqueous NaCl (2 \times 50 ml) and dried (Na\text{SO}_4). The colourless extract was filtered and evaporated under reduced pressure to give a yellow oil which slowly solidified (3.42 g, 85 \%). Recrystallization of the crude material from light petroleum (100 ml/g) afforded 133 as a white solid (2.9 g, 72 \%), homogeneous by t.l.c. (M), m.p. 85.5-86.5 °C (Lit., 82 79-80 °C); \( \delta_H \approx 5.2 \) and 4.8 (broad, \( \approx 2\text{H} \), amide NH), 3.20 (q, 4\text{H}, CH\text{\_2\text{NHBoc}}), 2.67 (m, CH\text{\_2\text{NCH\_2}}), and 1.52-1.79 (m) and 1.44 (s, together 25\text{H}, C\text{CH\_2\text{C}}, C(CH\text{\_3})_3, and amine NH); \( \delta_C \) 156.1 and 156.0 (CO), 79.0 [OC(CH\text{\_3})_3], 49.4 and 47.7 (CH\text{\_2\text{N}}), 40.5 and 39.2 (CH\text{\_2\text{NHBoc}}),
29.8, 27.9, and 27.3 (CCH$_2$C), and 28.5 [C(CH$_3$)$_3$]. (Found: C, 59.0; H, 10.1; N, 12.2. $C_{17}H_{35}N_3O_4$ requires C, 59.1; H, 10.2; N, 12.2 %).

5.2.2 - Synthesis of $N^4$-benzyloxycarbonyl-$N^1,N^8$-bis(tert-butoxycarbonyl)spermidine (134)

Method A - Methanolysis of compound 132 with excess of TMG

$$\text{Boc(Z)N(CH$_2$)$_3$NZ(CH$_2$)$_4$N(Z)Boc} \xrightarrow{\text{TMG/MeOH}} \text{BocNH(CH$_2$)$_3$NZ(CH$_2$)$_4$NHBoc}$$

Compound 132 (1.41 g, 1.90 mmol) was dissolved in dry methanol (15 ml) and treated with TMG (656 mg, 5.70 mmol) with rapid stirring at room temperature. After 20 h stirring, the solvent was evaporated and the yellowish residue partitioned between ether (100 ml) and aqueous 1M KHSO$_4$ (50 ml). The yellowish extract was washed as usual and taken to dryness affording a pale yellow oil which was dried under high vacuum to remove volatiles. $^1$H n.m.r. and t.l.c. (D) showed that the crude product (820 mg, 90 %) was still contaminated with benzyl alcohol as well as traces of 131. This material was chromatographed on silica with CH$_2$Cl$_2$-acetone (20:1) as eluant to give compound 134 (725 g, 80 %) as a chromatographically pure oil (ether or E); $\delta_H$ 7.34 (s, 5H, arom. H), 5.12 (s, 2H, CH$_2$Ph), 3.08-3.30 (m, 8H, CH$_2$NHBoc and CH$_2$NZCH$_2$), and 1.47-1.74 (m) and 1.43 (s) [together 24H, CCH$_2$C and C(CH$_3$)$_3$]; $\delta_C$ 155.9 (CO), 136.6, 128.5, 128.2, 128.0, and 127.8 (arom. H),

117
79.2 and 79.1 [OC(CH₃)₃], 67.1 (CH₂Ph), 46.4 and 44.3
[CH₂N(Z)CH₂], 40.2 and 37.5 (CH₂NHBoc), 28.4 [C(CH₃)₃], and
28.4, 27.3, and 25.6 (CCH₂C).

Method B - Benzylxycarbonylation of compound 133

\[
\begin{align*}
\text{BocNH(CH₂)₃NH(CH₂)₄NHBoc} & \quad + \quad Z₂O \\
\Downarrow & \\
\text{BocNH(CH₂)₃NZ(CH₂)₄NHBoc} & \quad + \quad \text{PhCH₂OH} \quad + \quad \text{CO₂}
\end{align*}
\]

133

134

To an ice-cold solution of compound 133 (173 mg, 0.50 mmol)
in dry CH₂Cl₂ (2 ml) was added dropwise with vigorous stirring
a solution of Z₂O (157 mg, 0.55 mmol) in dry CH₂Cl₂ (1 ml).
The clear, colourless solution was stirred for 1 h in ice-bath
and overnight at r.t. The solvent was evaporated under reduced
pressure and the crude yellowish residue was directly
chromatographed on silica with ether as eluant to afford the
desired product 134 (186 mg, 77 %) as a pale yellow oil. This
sample was identical with the product obtained by procedure A
(t.l.c. and ¹H and ¹³C n.m.r. spectra).
5.2.3 - Synthesis of \( N^1 \)-benzyloxycarbonyl-\( N^8 \)-tert-butoxy-carbonylspermidine

\[ \text{N}^1,\text{N}^4\text{-Methylenespermidine (114b)} \]

\[ \text{NH}_2(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NH}_2 + \text{HCHO} \rightarrow \text{HN}(\text{CH}_2)_4\text{NH}_2 + \text{H}_2\text{O} \]

This compound was prepared from spermidine according to a previously described procedure, using a fresh formaldehyde solution. The yield of essentially pure 114b, obtained as a colourless oil which soon solidified, was 95%; \( \delta_H \) 3.38 (s, 2H, NCH\(_2\)N), 2.51-2.88 (m, 6H, CH\(_2\)N), 2.17-2.33 (m, 2H, CH\(_2\)NH\(_2\)), and 1.40-1.73 (m, together 8H, CCH\(_2\)C and NH\(_2\)); \( \delta_C \) 69.9 (NCH\(_2\)N), 55.4 and 53.1 (NCH\(_2\)CH\(_2\)CH\(_2\)N), 45.2 and 42.1 (CH\(_2\)N), and 31.8, 27.2, and 24.3 (CCH\(_2\)C).

\[ \text{N}^1,\text{N}^8\text{-Dibenzzyloxycarbonyl-\( N^1 \),N}^4\text{-methylenespermidine (135)} \]

\[ \text{HN}(\text{CH}_2)_4\text{NH}_2 + 2\text{Z}_2\text{O} \rightarrow \text{ZN}(\text{CH}_2)_4\text{NHZ} + 2\text{PhCH}_2\text{OH} + 2\text{CO}_2 \]

A solution of Z\(_2\)O (12 g, 42 mmol) in dry CH\(_2\)Cl\(_2\) (15 ml) was added dropwise to a cooled solution of compound 114b (3.0 g, 19 mmol) in dry CH\(_2\)Cl\(_2\) (15 ml). The resulting clear mixture was stirred for 1 h in ice-bath and 2 h at r.t. The solvent was removed under reduced pressure and the colourless residue was chromatographed on silica using CH\(_2\)Cl\(_2\)-acetone (4:1) as eluant to afford the chromatographically essentially pure (G)
product 135 as a pale yellow oil (6.1 g, 76%); \( \delta_H \) 7.34 (s, 10H, arom. H), 5.12 and 5.08 (2s, 4H, \( \text{CH}_2\text{Ph} \)), 4.14 (s, 2H, NCH\(_2\)N), 3.53 (t, 2H) and 3.13-3.23 (m, 2H, \( \text{CH}_2\text{NZ} \)), 2.71 (t, 2H), and 2.29-2.53 (m, 2H) (CH\(_2\)N), and 1.33-1.74 (m, 6H, CCH\(_2\)C); \( \delta_C \) 156.4 and 155.0 (CO), 136.6, 128.4, and 128.0 (arom. C), 67.0 and 66.4 (CH\(_2\)Ph), 65.0 (NCH\(_2\)N), 52.4 and 52.2 (2 x CH\(_2\)N), 43.8 and 40.8 (2 x ZNCH\(_2\)C), and 27.6, 24.4, and 22.9 (CCH\(_2\)C).

\( N^1, N^8\)-Dibenzylxycarbonyl-\( N^8\)-tert-butoxycarbonyl-\( N^1, N^4\)-methylenespermidine (136)

\[
\begin{align*}
\text{Zn} & \bigcirc N(\text{CH}_2)_4 \text{NHZ} + \text{Boc}_2\text{O} \rightarrow \text{Zn} & \bigcirc N(\text{CH}_2)_4 \text{N(Z)}\text{Boc} + \text{Bu}_t\text{OH} + \text{CO}_2 \\
& \text{135} \rightarrow & \text{136}
\end{align*}
\]

To a stirred solution of compound 135 (4.40 g, 10.3 mmol) in dry CH\(_3\)CN (20 ml) was added DMAP (128 mg, 1.03 mmol), followed by Boc\(_2\)O (2.47 g, 11.3 mmol). After 7 h stirring, t.l.c. (G) showed that starting material still remained in the mixture and more Boc\(_2\)O (1.24 g, 5.65 mmol) was introduced and left overnight. When the reaction was complete the solvent was evaporated under reduced pressure and the brownish residue was chromatographed (silica; ether) to give pure (A and G) compound 136 as a yellow oil (4.9 g, 90%); \( \delta_H \) 7.35 and 7.33 (two s, 10H, arom. H), 5.20 (s, 2H, BocNCO\(_2\text{CH}_2\text{Ph} \)), 5.12 (s, 2H, \( \text{CH}_2\text{Ph} \)), 4.12 (s, 2H, NCH\(_2\)N) 3.35-3.62 (m, together 4H, \( \text{CH}_2\text{NZ} \) and \( \text{CH}_2\text{N(Z)}\text{Boc} \)), 2.67 (t, 2H) and 2.38 (t, 2H) (CH\(_2\)N), and 1.46-1.72 (m) and 1.46 (s) [together 15H, CCH\(_2\)C]
and C(CH$_3$)$_3$); $\delta_C$ 154.8, 153.6, and 151.8 (CO), 136.5, 135.3, 128.3, 128.0, and 127.7 (arom. C), 82.5 [OCC(CH$_3$)$_3$], 68.0 (PhCH$_2$O$_2$CNBoc), 66.9 (CH$_2$Ph), 65.2 (NCH$_2$N), 52.2 and 51.6 (CH$_2$N), 46.2 and 43.6 (CH$_2$NZ), 27.8 [C(CH$_3$)$_3$], and 26.6, 24.2, 22.9, and 22.5 (C$_2$H$_2$C).

$N^1$-Benzyloxycarbonyl-$N^8$-tert-butoxycarbonylspermidine (138)

Method A: via the intermediate 137

a) $N^1$-Benzyloxycarbonyl-$N^8$-tert-butoxycarbonyl-$N^1$-$N^4$-methyl-enedespermidine (137)

\[
\begin{array}{c}
\text{ZN} \\
\text{N(CH$_2$)$_4$} \\
\text{N(Z)Boc} \quad \text{MeOH/TMG} \quad \text{ZN} \\
\text{N(CH$_2$)$_4$} \\
\text{NHBoc}
\end{array}
\]

A stirred solution of compound 136 (3.67 g, 6.98 mmol) in dry methanol (40 ml) was treated with TMG (402 mg, 3.50 mmol) at r.t. for 2 days. The coloured solution was evaporated under reduced pressure and the yellowish residue was chromatographed (silica; CH$_2$Cl$_2$-acetone, 3:1) to afford the chromatographically pure (G) product 137 as a yellow oil (2.42 g, 88 %); $\delta_H$ 7.34 (s, 5H, arom. H), 5.13 (s, 2H, CH$_2$Ph), $\approx$ 4.9 (broad signal, $\approx$ 1H, NH), 4.13 (s, 2H, NCH$_2$N), 3.53 (t, 2H, CH$_2$NZ), 2.96-3.13 (m, 2H, CH$_2$NHBoc), 2.70 (t, 2H) and 2.17-2.40 (m, 2H) (CH$_2$N), and 1.34-1.75 (m) and 1.44 (s) [together 15H, CCH$_2$C and C(CH$_3$)$_3$]; $\delta_C$ 155.9 and 155.0 (CO), 136.6, 128.4, 128.0, and 127.9 (arom. C), 78.9 [OCC(CH$_3$)$_3$], 67.1 (CH$_2$Ph), 65.1 (NCH$_2$N), 52.6 and 52.2 (CH$_2$N), 43.8
(CH₂NZ), 40.4 (CH₂NHBoc), 28.4 [C(CH₃)₃], and 27.7, 24.5, and 22.8 (CCH₂C).

b) ring cleavage of compound 137

\[ \text{ZN} \bigcirc \text{N(CH₂)₄NHBoc} \xrightarrow{1) \text{CH}_2(\text{COOH}) \_ \text{pyridine}} \xrightarrow{2) \text{aq. K}_2\text{CO}_3} \text{ZNH(CH₂)₃NH(CH₂)₄NHBoc} \]

A solution of compound 137 (2.40 g, 6.10 mmol) in dry methanol (50 ml) was refluxed with pyridine (1.50 g, 19.0 mmol) and malonic acid (2.30 g, 22.4 mmol) with stirring for 2 h. The solvent was evaporated under reduced pressure and after the addition of aqueous 30% K₂CO₃ (30 ml) the product was extracted with CHCl₃ (3 x 60 ml). The combined yellowish organic layers were washed with saturated aqueous NaCl (2 x 30 ml), dried (Na₂SO₄), and evaporated to afford a crude oil which was chromatographed on silica (CH₂Cl₂-MeOH-HOAc, 18:2:1). The appropriate fractions were collected and again neutralized as for the crude product to give a yellow oil which was triturated with light petroleum to afford 138 as a white solid (1.81 g, 78%); homogeneous by t.l.c. (M or P). An analytical specimen was obtained by recrystallization from heptane-ether (2:1; 100 ml/g); m.p. 63-64 °C; δH 7.34 (s, 5H, arom. H), ≈ 5.65 (broad, ≈ 1H, amide NH), 5.09 (s, 2H, CH₂Ph), ≈ 4.80 (broad, ≈ 1H, amide NH), 3.06-3.30 (m, together 4H, CH₂NHz and CH₂NHBoc), 2.51-2.74 (m, 4H, CH₂N), and 1.49-1.79 (m) and 1.43 (s) (together ≈ 16H, CCH₂C, C(CH₃)₃, and...
amine NH); $\delta_C$ 156.5 and 156.0 (CO), 136.7, 128.4, and 128.0 (arom. C), 79.0 [OC(CH$_3$)$_3$], 66.4 (CH$_2$Ph), 49.3 and 47.7 (CH$_2$N), 40.9 and 39.9 (CH$_2$NHBOc and CH$_2$NHz), 29.5 (CCH$_2$C), 28.4 [C(CH$_3$)$_3$], and 27.8 and 27.2 (CCH$_3$C). (Found: C, 63.3; H, 8.6; N, 11.3. C$_{20}$H$_{33}$N$_4$O$_4$ requires C, 63.3; H, 8.8; N, 11.1%).

**Method B: via compound 139**

a) $N^1,N^8$-Dibenzyloxy carbonyl-$N^8$-tert-butoxycarbonyl-spermidine (139)

\[
\begin{align*}
\text{ZN} \quad &\quad \text{N(CH$_2$)$_4$N(Z)Boc} \\
\quad &\quad \text{136} \\
1) \quad &\quad \text{CH$_2$(COOH)$_2$, pyridine} \\
\quad &\quad \text{2) \quad ag. K$_2$CO$_3$} \\
\quad &\quad \text{ZNH(CH$_2$)$_3$NH(CH$_2$)$_4$N(Z)Boc} \\
\quad &\quad \text{139}
\end{align*}
\]

To a solution of the $N^1,N^4$-methylene spermidine derivative 136 (2.00 g, 3.80 mmol) in dry methanol (35 ml) were added pyridine (94.1 mg, 1.19 mmol) and malonic acid (1.45 g, 13.9 mmol). The mixture was refluxed with stirring for 2 h, then worked up as described in method A, b) (p. 122) except that the crude product was chromatographed with CH$_2$Cl$_2$-acetone-HOAc (5:5:1) to furnish the essentially pure (L or M) compound 139 as a yellow oil (900 mg, 48 %); $\delta_H$ 7.35 and 7.33 (two s, 10H, arom. H), 5.65 (broad signal, $\approx$ 1H, amide NH), 5.20 (s, 2H, PhCH$_2$O$_2$CNBoc), 5.08 (s, 2H, CH$_2$Ph), 3.65 (t, 2H, CH$_2$N(Z)Boc), 3.24 (m, 2H, CH$_2$NHz), 2.60 (q, 4H, CH$_2$N), and 1.46-1.77 (m) and 1.46 (s) (together $\approx$ 16H, CCH$_2$C, C(CH$_3$)$_3$, and amine NH); $\delta_C$ 156.5, 153.9, and 152.1 (CO), 136.7, 135.5, 128.5, 128.2, and 128.0 (arom. C), 82.7 [OC(CH$_3$)$_3$], 68.2
(PhCH$_2$O$_2$CNBoc), 66.4 (CH$_2$Ph), 49.4 and 47.6 (CH$_2$NCH$_2$), 46.3
[CH$_2$N(Z)Boc], 39.9 (CH$_2$NHZ), 29.6 (CCH$_2$C), 28.0 [C(CH$_3$)$_3$], and
27.0 and 26.6 (CCH$_2$C).

b) methanolysis of compound 139 catalysed by TMG

A stirred solution of compound 139 (281 mg, 0.550 mmol) in dry methanol (2.50 ml) was treated with TMG (32.0 mg, 0.280 mmol) at r.t. for about 2 days. The solvent was removed under reduced pressure and the residue chromatographed and worked up as described in method A, b) (p. 122) to afford compound 138 (135 mg, 65 %); m.p. and $^1$H and $^{13}$C n.m.r. spectra were in agreement with the foregoing data.

Method C: independent synthesis of compound 138

a) N$^1$-(Benzyloxy carbonyl-β-alanyl)-N$^4$-tert-butoxycarbonyl-
tetramethylenediamine (140)

\[
\text{BocNH(CH$_2$)$_4$NH$_2$ + ZNH(CH$_2$)$_2$COOC$_6$H$_4$-NO$_2$-4} \rightarrow \text{ZNH(CH$_2$)$_2$CONH(CH$_2$)$_4$NHBoc + 4-NO$_2$-C$_6$H$_4$OH}
\]

140

To a solution of N$^1$-Boc-tetramethylenediamine$^{17a,121}$ (1.13 g, 6.00 mmol) in dry acetonitrile (20 ml) Z-βAla-ONp (1.72 g, 5.00 mmol) dissolved in dry acetonitrile (20 ml) was added dropwise with vigorous stirring for 15 min. The resulting mixture immediately became bright yellow, and after 1 h agitation at r.t. a precipitate appeared. More
Acetonitrile (10 ml) was added to facilitate stirring, which was continued overnight (20 h). The thick sludge was filtered by suction and the collected white solid was thoroughly triturated repeatedly with cold acetonitrile (3 x 5 ml) and sucked dry. The crude yield of the chromatographically pure product 140 was 1.82 g (92 %). Recrystallization from acetonitrile (30 ml/g) gave, after cooling for a few days, a white fluffy crystalline solid (90 % crystallization yield); t.l.c. (M or Q) gave one spot; m.p. 133-134 °C; δ_H 7.33 (s, 5H, arom. H), 6.10 (broad, ≈1H, CCONH), 5.62 (broad, ≈1H, ZNH), 5.09 (s, 2H, CH_2-Ph), 4.68 (broad, ≈1H, BocNH), 3.46 (perturbed t, 2H), 3.18 (perturbed m, 2H), and 3.13 (perturbed s, 2H) (3 x NCH_2), 2.39 (t, 2H, COCH_2), ≈1.49 (m, 4H, CCH_2CH_2C), and 1.43 [s, 9H, C(CH_3)_3]; δ_C 171.3 (CH_2CONH), 156.5 and 156.1 (2 x CONH), 136.5, 128.4, 128.0, and 127.9 (arom. C), 79.2 [C(CH_3)_3], 66.6 (CH_2-Ph), 40.1 and 39.1 (CH_2NHCO_2), 37.2 and 35.9 (CH_2CONHCH_2), 28.4 [C(CH_3)_3], 27.5 and 26.5 (CCH_2CH_2C).

b) Reduction of the amide 140

\[
\text{ZNH(CH}_2)_2\text{CONH(CH}_2)_4\text{NHBOc} \xrightarrow{1) \text{NaBH}_4, CF}_3\text{COOH} \xrightarrow{2) \text{aq. K}_2\text{CO}_3} \text{ZNH(CH}_2)_3\text{NH(CH}_2)_4\text{NHBOc}
\]

To a suspension of finely ground NaBH_4 (190 mg, 5.0 mmol) in dry THF (8 ml), TFA (385 µl, 5.0 mmol) was added dropwise under vigorous stirring at r.t. for ≈10 min (evolution of 125
gas). The resulting mixture was allowed to stay for 30 min. To this was added the amide 140 (393 mg, 1.00 mmol) in dry THF (10 ml). The suspension was stirred 4 h at r.t. and overnight at 40 °C. Then, 20 % aq. HOAc (3 ml) was carefully added to the reaction mixture under stirring at r.t. After 2 h, the solvent was evaporated and the residue partitioned between CH₂Cl₂ (60 ml) 30 % K₂CO₃-saturated NaCl (1:1) (30 ml). The aqueous phase was again extracted with CH₂Cl₂ (2 x 20 ml). The combined organic layers were washed with saturated NaCl (2 x 20 ml) and dried (Na₂SO₄). Evaporation to dryness afforded 380 mg of a crude mixture which was chromatographed on silica with CH₂Cl₂-MeOH-HOAc (18:2:1). First eluted the amide and some other impurities. The product was then eluted with CH₂Cl₂-MeOH-HOAc (16:4:1). The fractions contained pure compound were pooled and evaporated to dryness. The semisolid residue was worked up as for the crude mixture to give 88 mg (23 %) of a waxy solid. A recrystallized sample was identical with compound 138 as obtained earlier.
5.2.4 - Attempted synthesis of \( N^8 \)-benzyloxy carbonyl-\( N^1 \)-tert-butoxycarbonylspermidine

\( N^8 \)-Benzyloxy carbonyl-\( N^1 \)-tert-butoxycarbonyl-\( N^1 \),\( N^4 \)-methyl-\( N^2 \)-enespermidine (160)

**A - One pot procedure**

\[
\begin{array}{c}
\text{HN} \quad \text{N(CH}_2\text{)}_4\text{NH}_2 \\
114b \\
\frac{1}{2} \xrightarrow{\text{ZCN}} \xrightarrow{\text{Boc}_2\text{O, NMM}} \text{Boc}\text{N} \quad \text{N(CH}_2\text{)}_4\text{NHZ} \\
160
\end{array}
\]

A solution of dried cyclic spermidine 114b (1.19 g, 7.56 mmol) in dry \( \text{CH}_2\text{Cl}_2 \) (30 ml) was treated dropwise over a period of 1 h with ZCN (1.34 g, 8.31 mmol) dissolved in dry \( \text{CH}_2\text{Cl}_2 \) with rapid stirring at r.t. The resulting, slightly turbid reaction mixture was stirred for a further 3 h with exclusion of moisture. A solution of \( \text{Boc}_2\text{O} \) (1.81 g, 8.32 mmol) in dry \( \text{CH}_2\text{Cl}_2 \) (20 ml) was slowly introduced during 20 min at r.t. followed by the dropwise addition of NMM (840 mg, 8.31 mmol) in dry \( \text{CH}_2\text{Cl}_2 \) (25 ml). After stirring overnight, most of the solvent was stripped off and the residue dissolved in ethyl acetate (300 ml). The extract was washed with 1M \( \text{NaHCO}_3 \) and saturated \( \text{NaCl} \) (3 x 100 ml each) and dried (\( \text{Na}_2\text{SO}_4 \)). Evaporation to dryness afforded 2.95 g of a mixture containing \( \approx 60 \% \) of the desired product 160 as well as the \( \text{N}^1\),\( \text{N}^8\)-\( \text{Z}^2 \)-derivative 135 (as judged from t.l.c. (P) and \( ^1\text{H} \) n.m.r.). Column chromatography on silica (\( \text{CH}_2\text{Cl}_2\text{-MeOH, 30:1} \)) gave small amounts of essentially pure product as a pale yellow oil; \( \delta\text{H} \)
7.33 (s, 5H, arom. H), ≈ 5.40 (broad, ≈ 1H, NH), 5.09 (s, 2H, CH₂Ph), 4.06 (s, 2H, NCH₂N), 3.44 (t, 2H, CH₂NBoc), 3.10-3.30 (m, 2H, CH₂NH₂), 2.69 (t, 2H), and 2.33-2.49 (m, 2H) (CH₂N), 1.49-1.62 (m), and 1.45 [s, together 15H, CCH₂ and C(CH₃)₃].

B - With isolation of N⁸-Z-N¹,N⁴-methylenespermidine 159

\[
\text{HN} \left(N(CH₂)₄NH₂ + ZCN \rightarrow \text{HN} \left(N(CH₂)₄NHZ + HCN
\right.\right.\]

114b 159

To a solution of hexahydropyrimidine 114b (4.30 g, 27.0 mmol) in dry CH₂Cl₂ (30 ml) ZCN (4.41 g, 27.0 mmol) in dry CH₂Cl₂ (30 ml) was added dropwise over a period of 3 h with stirring at r.t. After a further 2 h stirring, t.l.c. (CH₂Cl₂-MeOH, 1:1) showed a rather complex mixture. The solvent was evaporated under reduced pressure and the oily residue chromatographed on silica with CH₂Cl₂-MeOH (1:1) as eluant. The fractions containing the almost pure compound were pooled and evaporated to afford 448 mg (5 %) of N⁸-Z-N¹,N⁴-methylenespermidine 159 as a yellow oil. This product (448 mg, 1.54 mmol) in dry CH₂Cl₂ (5 ml) was treated dropwise with Boc₂O in dry CH₂Cl₂ (5 ml) at room temperature. After 2 h stirring, the solvent was evaporated under reduced pressure and the yellowish residue worked up as under procedure A to afford crude 160. This material was chromatographed on silica (CH₂Cl₂-acetone, 3:1) to afford 283 mg (47 %) of a homogeneous yellow oil, identical with
compound 160 as obtained above.

\[
\text{N}^8\text{-Benzyloxy carbonyl-} N^1\text{-tert-butoxycarbonyl spermidine (161)}
\]

\[
\text{BocN} \quad \text{N(CH}_2\text{)}_4\text{NHz} \quad \text{CH}_2\text{(COOH)}_2 \text{pyridine} \quad \text{BocNH(CH}_2\text{)}_3\text{NH(CH}_2\text{)}_4\text{NHz}
\]

Compound 160 was treated and the product worked up as described for compound 138 under method A, b) (p. 122) to afford 167 mg (74%) of 161 as a waxy solid, essentially pure by t.l.c. (M or P); \(\delta_H\) 7.33 (s, 5H, arom. H), \(\approx 5.61\) and \(\approx 5.31\) (broad, \(\approx 2H\), amide NH), 5.08 (s, 2H, \(\text{CH}_2\text{Ph}\)), 3.05-3.27 (m, 4H, \(\text{CH}_2\text{NHBoc, CH}_2\text{NHz}\)), 2.55-2.75 (m, 4H, \(\text{CH}_2\text{NHCH}_2\)), 1.47-1.74 (m) and 1.43 (s, together 16H, \(\text{CCH}_2\text{C, C(CH}_3\text{)}_3\) and amine NH).
5.2.5 - Synthesis of \(N^4\)-acetylsperrmidine dioxalate

\(N^1,N^4,N^8\)-Triacetylsperrmidine (141)

**A - Acetylation in aqueous conditions**

\[
\begin{align*}
\text{NH}_2(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NH}_2 + 3\text{Ac}_2\text{O} + 3\text{NaOH} & \rightarrow \\
3 \rightarrow \text{AcNH}(\text{CH}_2)_3\text{NAc}(\text{CH}_2)_4\text{NHAc} + 3\text{NaOAc} + 3\text{H}_2\text{O}
\end{align*}
\]

Spermidine (1.12 g, 7.7 mmol) was dissolved in 1 M NaOH (20 ml) and, after cooling in ice water, simultaneously treated under stirring dropwise with Ac\(_2\)O (3.17 g, 31.0 mmol) and 1 M NaOH (80 ml) and then left for several hours. The solution was saturated with NaCl and extracted with CHCl\(_3\) (4 x 50 ml). The extract was dried (MgSO\(_4\)) and evaporated to afford 1.63 g (78 %) of a colourless oil. This crude material was chromatographed on silica with CH\(_2\)Cl\(_2\)-MeOH (4:1) to yield 1.36 g (65 %) of compound 141 as a pale yellow oil, homogeneous by t.l.c. (N, L); \(\delta_H \approx 6.98\) and 6.40 (broad, \(\approx 2\text{H}\), amide NH), 3.07-3.46 (m, 8H, CH\(_2\)N), 2.10 and 2.07 (two s, 3H, -N(CH\(_3\))CO-), 1.98 (s, 6H, CH\(_3\)CONH), 1.49-1.87 (m, 6H, CCH\(_2\)C); \(\delta_C\) 171.0 and 170.5 (CO), 48.4, 46.7, 45.2, 42.5, 38.7, 36.9 and 36.1 (CH\(_2\)N), 29.0, 27.7, 27.4, 27.0, 26.5, 25.9, 24.8, 23.3, 23.1, 23.0, 21.4 (other C).
B - Acetylation in anhydrous conditions

\[
\begin{align*}
\text{NH}_2(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NH}_2 + 3\text{Ac}_2\text{O} + 3\text{Et}_3\text{N} \rightarrow \\
\text{AcNH}(\text{CH}_2)_3\text{NAc}(\text{CH}_2)_4\text{NHAc} + (\text{Et}_3\text{NH})_2^+(-\text{OAc})_3
\end{align*}
\]

An ice-cooled solution of spermidine (1.00 g, 6.88 mmol) and TEA (2.16 g, 21.3 mmol) in dry CH\textsubscript{2}Cl\textsubscript{2} (10 ml) was treated dropwise with Ac\textsubscript{2}O (2.18 g, 21.3 mmol) and then stirred overnight at r.t. The solvent was evaporated and the colourless residue was chromatographed as described above to afford 1.37 g (73 \%) of compound 141 as a pale yellow oil, essentially pure by t.l.c. (L, N) which was identical with the product obtained by procedure A.

\begin{align*}
\text{N}_1^\circ\text{N}_4\text{N}_8^\circ\text{Triacetyl-N}_1\text{N}_8\text{bis(tert-butoxycarbonyl)-spermidine (142)} \rightarrow \\
\text{AcNH}(\text{CH}_2)_3\text{NAc}(\text{CH}_2)_4\text{NHAc} + 2\text{Boc}_2\text{O} \rightarrow \text{DMAP} \\
\text{Boc(Ac)N}(\text{CH}_2)_3\text{NAc}(\text{CH}_2)_4\text{N(Ac)Boc} + 2\text{Bu}^\circ\text{OH} + 2\text{CO}_2
\end{align*}

A solution of compound 141 (0.787 g, 2.90 mmol) and DMAP (71 mg, 0.58 mmol) in CH\textsubscript{3}CN (10 ml) was treated with Boc\textsubscript{2}O (1.40 g, 6.40 mmol) in one portion and left with stirring at r.t. After 4 h t.l.c. (N) showed that more than 50 \% of the starting material remained. Additional Boc\textsubscript{2}O was added in six
portions (1 eq. each) at intervals during 5 days. T.l.c. (N) still showed some starting material left and two other major spots. The reaction mixture was therefore evaporated to dryness and the residue was again dissolved in CH$_3$CN (10 ml) and a new batch of Boc$_2$O (1 eq.) and DMAP (0.1 eq.) was added. The reaction was left overnight. This procedure was repeated once more until t.l.c. (N) of the reaction mixture showed one major spot. The solvent was evaporated in vacuo and the dark, brown residue partitioned between 1 M KHSO$_4$ (50 ml) and ether (100 ml). The solution was again extracted with ether (2 x 25 ml) and the combined organic layers were washed in turn with 1 M KHSO$_4$, 1 M NaHCO$_3$ and saturated NaCl (2 x 50 ml each). The yellowish extract was dried (MgSO$_4$) and evaporated.

The brown residue was chromatographed on silica using CH$_2$Cl$_2$-acetone (9:1) to afford 932 mg (68 %) of 142, pure by t.l.c. (N, ether); $\delta_H$ 3.68 [t, 4H, CH$_2$N(Ac)Boc], 3.14-3.40 (m, 4H, CH$_2$NACCH$_2$), 2.46 [s, 6H, CH$_3$CO(Boc)N], 2.07 (s, 3H, -N(CH$_3$CO)-), 1.25-1.73 (m) and 1.54 [s, together ≈ 24H, CCH$_2$C + C(CH$_3$)$_3$]; $\delta_C$ 172.9 (BocNCOCH$_3$), 170.1 and 170.0 (CH$_3$CON), 153.0 and 152.8 (Bu$^t$O-ÇO), 83.5, 83.3, 83.1 and 83.0 [OC(CH$_3$)$_3$], 48.2, 46.3, 45.2, 43.8, 43.6, 43.0, 42.1 and 41.7 (CH$_2$N), 28.1 [C(CH$_3$)$_3$], 26.9, 26.1, 25.9, 25.0 and 21.5 (other C).
**N⁴-Acetyl-N¹,N⁸-bis(tert-butoxycarbonyl)spermidine (143)**

**Method A: Methanolysis of compound 142 in the presence of catalytic amounts of TMG**

\[
\text{MeOH, TMG}
\]

\[
\text{Boc(}\text{Ac})\text{N(}\text{CH}_2\text{)}_3\text{NAC(}\text{CH}_2\text{)}_4\text{N(}\text{Ac})\text{Boc} \rightarrow \text{BocNH(}\text{CH}_2\text{)}_3\text{NAC(}\text{CH}_2\text{)}_4\text{NHBOc}
\]

Compound 142 (547 mg, 1.2 mmol) was dissolved in dry methanol (10 ml) and treated with TMG (30 mg, 0.26 mmol) with stirring at r.t. for 4 h. The reaction mixture was evaporated in vacuo and the yellow residue partitioned between ether (60 ml) and 1M KHSO₄ (30 ml). After further extraction with ether (60 ml), the combined organic layers were washed in turn with 1M KHSO₄, 1M NaHCO₃ and saturated NaCl (2 x 30 ml) and dried (MgSO₄). The extract was evaporated to dryness to afford 418 mg (90 %) of a yellow oil. The crude product was chromatographed on silica (CH₂Cl₂-acetone, 2:1) to furnish 395 mg (88 %) of compound 143 as a pale yellow oil, homogeneous by t.l.c. (F, P): δ_H ≈ 5.4 and 4.7 (broad, ≈ 2H, amide NH), 3.04-3.52 (m, 8H, CH₂N), 2.09 and 2.08 (two s, 3H, CH₃CON), 1.50-1.73 (m) and 1.44 [s, together 24H, CCH₂C + C(CH₃)₃]; δ_C 170.8 and 170.2 (CH₃CO), 156.1 and 156.0 (Bu^t-ÇO), 79.4 and 78.9 [C(CH₃)₃], 48.3, 46.4, 45.3, 42.4, 40.0 and 37.4 (CH₂N), 28.4 [C(CH₃)₃], 29.7, 28.0, 27.6, 26.0, 25.0 and 21.4 (other C).
Method B: acetylation of compound 133

\[
\begin{align*}
\text{BocNH(CH}_2\text{)}_3\text{NH(CH}_2\text{)}_4\text{NHBoc} + \text{Ac}_2\text{O} + \text{Et}_3\text{N} \\
\text{133} & \rightarrow \\
\text{BocNH(CH}_2\text{)}_3\text{NAc(CH}_2\text{)}_4\text{NHBoc} + (\text{Et}_3\text{NH})^+\text{OAc} \\
\text{143}
\end{align*}
\]

A solution of Ac\textsubscript{2}O (123 mg, 1.20 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (5 ml) was added to a cooled solution of 133 (345 mg, 1.00 mmol) and TEA (152 mg, 1.50 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (10 ml) and the reaction was stirred for 4 h. The solvent was evaporated in vacuo and the remaining colourless residue worked up and chromatographed as described in Method A. It was afforded 288 mg (74 %) of compound 143 as a pale yellow oil. \textsuperscript{1}H and \textsuperscript{13}C n.m.r. spectra were identical with those given under Method A.

\[
\begin{align*}
\text{N}^4\text{-Acetylspermidine dioxalate (144a)}
\end{align*}
\]

Compound 143 (324 mg, 0.84 mmol) was treated with 2.29 M HCl in dioxan (2 ml) with stirring at r.t. for 3 h. Most of the solvent was evaporated in vacuo and the sticky residue taken up in ether (20 ml) and evaporated twice. The product was then dissolved in distilled water (40 ml) and the resulting solution extracted with ether (3 x 20 ml). The aqueous layer was flushed with N\textsubscript{2} to remove ether and
lyophilized to afford 202 mg (92 %) of a sticky white residue, essentially pure by t.l.c. (S). This material was converted to its oxalate salt by loading a portion (100 mg), dissolved in water, onto a QAE-Sephadex A-25 column (oxalate form) and eluting with distilled water to afford 111 mg of a white residue after lyophilization. Recrystallization from water-ethanol (1:20, 25 ml) gave the oxalate salt 144a as a white solid; pure by h.p.l.c. (see Figs. 7 and 8, p. 142 and 143 ); m.p. 187.5-188.5 °C; δ <sub>H</sub> (D<sub>2</sub>O) 3.27-3.55 (m, 4H, CH<sub>2</sub>N(Ac)CH<sub>2</sub>), 2.82-3.13 (m, 4H, CH<sub>2</sub>NH<sub>2</sub>), 2.14 and 2.13 (two s, 3H, CH<sub>3</sub>CON), 1.54-2.08 (m, 6H, CCH<sub>2</sub>C); δ <sub>C</sub> 177.2, 176.7 and 176.0 (CO), 51.0, 48.7, 47.9, 45.0, 41.8 and 39.5 (CH<sub>2</sub>N), 28.5, 27.6, 27.5, 26.8, 26.4, 23.3 and 23.1 (other C). (Found: C, 46.1; H, 8.0; N, 14.7. C<sub>9</sub>H<sub>21</sub>N<sub>3</sub>O.C<sub>2</sub>H<sub>4</sub>O<sub>4</sub>.1/2H<sub>2</sub>O requires C, 46.1; H, 8.45; N, 14.7 %).

5.2.6 - Synthesis of <sup>1</sup>N-acetylspermidine dihydrochloride

N<sup>1</sup>-Benzyloxycarbonyl-N<sup>4</sup>,N<sup>8</sup>-bis(tert-butoxycarbonyl)-spermidine (145)

\[
\begin{align*}
ZNH(CH_2)_3NH(CH_2)_4NHBoc + Boc_2O \\
\rightarrow \\
ZNH(CH_2)_3NBoc(CH_2)_4NHBoc + Bu^+OH + CO_2
\end{align*}
\]

To an ice-cold solution of compound 138 (1.90 g, 5.01 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 ml) was added dropwise a solution of Boc<sub>2</sub>O
(1.15 g, 5.26 mmol) in dry CH₂Cl₂ (10 ml). The colourless reaction mixture was stirred for 30 min. in ice and overnight at r.t. The solvent was evaporated and the residue partitioned between 1 M KHSO₄ (100 ml) and ether (500 ml). The extract was washed and dried as described before and evaporated to afford 3.0 g of a pale yellow oil. Column chromatography (silica, ether-light petroleum, 3:1) furnished 2.10 g (87 %) of the product 145, homogeneous by t.l.c. (C,F):

δₜ 7.34 (s, 5H, arom. H), 5.10 (s, 2H, CH₂Ph), ≈ 5.70 and 4.60 (broad, ≈ 2H, amide NH), 3.08-3.32 (m, 8H, CH₂NBoc, CH₂NH₂), 1.51-1.78 (m) and 1.44 [s, together 24H, CCH₂C + C(CH₃)₃]; δC 156.4 and 155.9 (CO), 136.6, 128.4 and 128.0 (arom. C), 79.7 and 79.2 [O(CH₃)₃], 66.4 (OCH₂Ph), 46.6 and 43.7 [CH₂N(Boc)CH₂], 40.2 and 37.8 (CH₂NBoc, CH₂NH₂), 28.4 [C(CH₃)₃], 27.4 and 25.6 (CCH₂C).

N⁴,N⁸-Bis(tert-butoxycarbonylspermidine) (146)

\[
\text{ZNH(CH₂)₃NBoc(CH₂)₄NHBoc + H}_2 \xrightarrow{\text{Pd-C}} \text{NH₂(CH₂)₃NBoc(CH₂)₄NHBoc + PhCH₃ + CO}_2
\]

Compound 145 (1.90 g, 3.96 mmol) was hydrogenolyzed as described for model compound 170 (p. 106). The catalyst was filtered off and rinsed with methanol. The colourless filtrate was evaporated under reduced pressure to give 1.35 g (98 %) of the product 146 as a colourless oil, essentially pure by
t.l.c. \((M, Q)\); \(\delta_H \approx 4.60\) (broad, \(\approx 1\text{H}, \text{amide NH}\)), 3.09-3.46 [m, 6H, \(\text{CH}_2\text{NHBOc}, \text{CH}_2\text{N(Boc)CH}_2\)], 2.69 (t, 2H, \(\text{CH}_2\text{NH}_2\)), 1.53-1.71 (m), 1.45 and 1.44 [two s, together 26H, \(\text{CCH}_2\text{C}, \text{C(CH}_3)_3 + \text{NH}_2\)]; \(\delta_C\) 156.0 and 155.7 (CO), 79.4 and 79.1 [OC(CH\(_3\))\(_3\)], 46.5, 44.2, 40.3 and 39.3 (CH\(_2\)N), 32.3, 27.5 and 25.7 (CCH\(_2\)C), 28.5 [C(CH\(_3\))\(_3\)].

\(N^1\text{-Acetyl-N}^4,N^8\text{-bis(tert-butoxycarbonyl)spermidine (147)}\)

\[
\begin{align*}
\text{NH}_2(\text{CH}_2)_3\text{NBoc}(\text{CH}_2)_4\text{NHBOc} + \text{Ac}_2\text{O} + \text{Et}_3\text{N} & \rightarrow \\
\text{AcNH}(\text{CH}_2)_3\text{NBoc}(\text{CH}_2)_4\text{NHBOc} + (\text{Et}_3\text{NH})^+\text{OAc} & \rightarrow 147
\end{align*}
\]

A solution of compound 146 (1.22 g, 3.53 mmol) was treated and the product purified in a similar manner as described for the derivative 143 (Method B, p. 134): yield 1.30 g (95\%) of the product 147 obtained as an oil; \(\delta_H \approx 6.75\) and 4.60 (broad, amide NH), 3.02-3.33 (m, 8H, CH\(_2\)N), 1.98 (s, 3H, CH\(_3\)CON), 1.53-1.72 (m) and 1.46 and 1.44 [two s, together \(\approx 24\text{H}, \text{CCH}_2\text{C} + \text{C(CH}_3)_3\)]; \(\delta_C\) 170.2 (CH\(_3\)CO), 156.0 (Bu\(_t\)-CO), 79.8 and 79.2 [OC(CH\(_3\))\(_3\)], 46.6, 44.1, 40.1 and 35.9 (CH\(_2\)N), 28.4 (C(CH\(_3\))\(_3\)), 27.7, 27.5 and 25.6 (CCH\(_2\)C), 23.4 (CH\(_3\)CON).

\(N^1\text{-Acetyl spermidine dihydrochloride (148)}\)

\[
\begin{align*}
\text{AcNH}(\text{CH}_2)_3\text{NBoc}(\text{CH}_2)_4\text{NHBOc} & \rightarrow \\
\text{AcNH}(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NH}_2. & \rightarrow 147
\end{align*}
\]

137
Compound 147 (539 mg, 1.39 mmol) was treated with 2.29 M HCl in dioxan (5 ml) at r.t. for 3 h. The solvent was evaporated in vacuo and the white residue suspended in ether (20 ml) and evaporated twice. It was afforded 350 mg (97 %) of the dihydrochloride salt 148. It was recrystallized from ethanol and was pure by h.p.l.c. (see Figs. 7 and 8, p. 142 and 143); m.p. 191-193 °C (lit.35a,110 173-178 °C or 189-191 °C; δH (D2O) 3.28 (t, 2H, J=6.7 Hz, CH2NHAc), 2.98-3.15 (m, 6H, CH2N), 2.00 (s, 3H, CH3CO); δC 177.2 (CO), 49.6, 47.7, 41.4 and 38.7 (CH2N), 28.2, 26.6 and 25.4 (CCH2C), 24.5 (CH3CON). (Found: C, 41.3; H, 8.8; N, 15.9. C9H21N3O.2HCl requires C, 41.54; H, 8.91; N, 16.15%).

5.2.7 - Synthesis of N8-acetylspermidine dihydrochloride

N1,N4-Dibenzyloxy carbonyl-N8-tert-butoxycarbonylspermidine (149)

A - Benzyloxy carbonylation with Z2O

\[
\begin{align*}
&\text{ZNH(CH}_2\text{)}_3\text{NH(CH}_2\text{)}_4\text{NHBoc} + Z_2O \\
&\text{138} \quad \downarrow \\
&\text{ZNH(CH}_2\text{)}_3\text{NZ(CH}_2\text{)}_4\text{NHBoc} + \text{PhCH}_2\text{OH} + \text{CO}_2 \\
&\text{149}
\end{align*}
\]

A solution of compound 138 (380 mg, 1.00 mmol) in dry \(\text{CH}_2\text{Cl}_2\) (2 ml) was treated with \(Z_2O\)108 as described for compound 135 (p. 119). After completion of the reaction
[t.l.c. (L)] the solvent was evaporated under reduced pressure. The oily residue was partitioned between ether (50 ml) and aqueous 1 M KHSO$_4$ (25 ml). The organic layer was washed and dried as usual. Evaporation to dryness afforded the crude product (638 mg) as a pale yellow oil contaminated with benzyl alcohol. The crude material was chromatographed on silica (ether-light petroleum, 3:1) to give 452 mg (88%) of product 149 as a light yellow oil, homogeneous by t.l.c. (ether, C); $\delta_H$ 7.33 and 7.32 (two s, 10H, arom. H), 5.11 and 5.08 (2s, 4H, CH$_2$Ph), 3.04-3.38 (m, 8H, CH$_2$N), 1.51-1.76 (m) and 1.43 [s, together 15H, CCH$_2$C + C(CH$_3$)$_3$]; $\delta_C$ 156.4 and 155.9 (CO), 136.6, 128.5, 128.4 and 128.0 (arom. C), 79.2 [O(CH$_3$)$_3$], 67.1 and 66.5 (CH$_2$Ph), 46.5, 44.1, 40.1 and 37.6 (CH$_2$N), 28.4 [C(CH$_3$)$_3$], 28.2, 27.4 and 25.6 (CCH$_2$C).

B - Benzyloxy carbonylation with ZOBt

$$\text{ZNH(CH}_2\text{)}_3\text{NH(CH}_2\text{)}_4\text{NHBOc} + \text{ZOBt} \rightarrow \text{ZNH(CH}_2\text{)}_3\text{NZ(CH}_2\text{)}_4\text{NHBOc} + \text{HOBt}$$  

To a stirred suspension of ZOBt$^{11}$ (1.30 g, 4.82 mmol) in dry CH$_3$CN (40 ml) was added a solution of compound 138 (1.18 g, 4.77 mmol) in the same solvent (30 ml). The clear solution obtained was left overnight at r.t. The solvent was evaporated and the product worked up and chromatographed as described in procedure A to furnish 2.04 g (83%) of compound 149 as a pale yellow oil. T.l.c. and $^1$H and $^{13}$C spectra were in agreement with those reported for the product obtained.
under procedure A.

$^1\text{N},^4\text{N}-\text{Dibenzyloxy carbonylspermidine (150)}$

\[
\text{ZNH(CH}_2\text{)}_3\text{NZ(CH}_2\text{)}_4\text{NHBoc} \xrightarrow{1) \text{ HCl, dioxan}} \text{ZNH(CH}_2\text{)}_3\text{NZ(CH}_2\text{)}_4\text{NH}_2
\]

Compound 149 (1.84 g, 3.58 mmol) was treated with 2.29 M HCl in dioxan (15 ml) and stirred at r.t. for 3 h. The solvent was evaporated and the white residue treated with 30 % K$_2$CO$_3$ (40 ml) and extracted with CHCl$_3$ (5 x 100 ml). The combined organic layers were dried (Na$_2$SO$_4$) and evaporated to afford 1.42 g (96 %) of product 150 as a pale yellow oil, nearly pure by t.l.c. (M, Q); $\delta_H$ 7.34 and 7.33 (two s, 10H, arom. H), $\approx 5.60$ (broad, $\approx$1H, amide NH), 5.11 and 5.08 (2s, 4H, CH$_2$Ph), 3.05-3.39 (m, 6H, CH$_2$NHz, CH$_2$N(Z)CH$_2$), 2.66 (t, 2H, CH$_2$NH$_2$), 1.25-1.84 (m, 8H, CCH$_2$C + NH$_2$). $\delta_C$ 156.4 (CO), 136.6, 128.5, 128.4, 128.0 and 127.9 (arom. C), 67.1 and 66.5 (CH$_2$Ph), 46.8, 44.1, 41.7 and 37.7 (CH$_2$N), 30.7, 28.1 and 25.8 (CCH$_2$C).

$^8\text{N}-\text{Acetyl-}^1\text{N},^4\text{N}-\text{dibenzyloxy carbonylspermidine (151)}$

\[
\text{ZNH(CH}_2\text{)}_3\text{NZ(CH}_2\text{)}_4\text{NH}_2 + \text{Ac}_2\text{O} + \text{Et}_3\text{N} \xrightarrow{} \text{ZNH(CH}_2\text{)}_3\text{NZ(CH}_2\text{)}_4\text{NHAc} + (\text{Et}_3\text{NH})^+\text{OAc}
\]

A solution of compound 150 (1.18 g, 2.85 mmol) was treated
and the product purified in a similar manner as described for derivative 143 (Method B, p. 134) to give 1.10 g (85 %) of product 151 as an oil; $\delta_H$ 7.33 (s, 10H, arom. H), $\approx$ 6.00 and 5.60 (broad, $\approx$ 2H, amide NH), 5.11 and 5.08 (two s, 4H, CH$_2$Ph), 3.05-3.37 (m, 8H, CH$_2$NZ, CH$_2$NAc), 1.92 (s, 3H, CH$_3$CON), 1.33-1.77 (m, 6H, CCH$_2$C); $\delta_C$ 170.2 (CH$_3$CO), 156.5 (Bz10-CO), 136.5, 128.6, 128.4, 128.0 and 127.9 (arom. C), 67.2 and 66.5 (CH$_2$Ph), 46.5, 44.3, 39.0 and 37.9 (CH$_2$N), 28.4, 26.7 and 25.7 (CCH$_2$C), 23.2 (CH$_3$CON).

N$^8$-Acetylspermidine dihydrochloride (152)

\[
\text{2NH(CH$_2$)$_3$NZ(CH$_2$)$_4$NHAc} \xrightarrow{1) \text{H}_2/\text{Pd-C}} \text{NH}_2(\text{CH$_2$)$_3$NH(CH$_2$)$_4$NHAc}.2\text{HCl}
\]

A solution of compound 151 (497 mg, 1.09 mmol) was hydrogenolyzed in a similar manner as described for model compound 170 (p. 106) to give the free amine (200 mg, 98 %) as a colourless oil. This derivative was converted to its dihydrochloride salt with excess of 2.29 M HCl in dioxan to afford 250 mg (90 %) of the salt 152. It was recrystallized from ethanol and was pure by h.p.l.c. (see Figs. 7 and 8, p. 142 and 143); m.p. 202-203 °C (lit., 35a, 110 204-205.5 °C or 203.5-205 °C); $\delta_H$ (D$_2$O) 3.01-3.24 (m, 8H, CH$_2$N), 1.91-2.26 (m, 2H, CCH$_2$C), 1.98 (s, 3H, CH$_3$CO), 1.53-1.77 (m, 4H, CCH$_2$CH$_2$C). $\delta_C$ 176.1 (CO), 49.8, 46.9, 41.1 and 39.1 (CH$_2$N), 28.0, 26.3 and 25.5 (CCH$_2$C), 24.4 (CH$_3$CON). (Found: C, 40.8; H, 8.8; N, 15.6. C$_{9}$H$_{21}$N$_{3}$O.2HCl requires C, 41.54; H, 8.91; N, 16.15 %).

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Figure 7 Separation of the three monoacetyl spermidine derivatives by reversed-phase ion pair chromatography using a u.v.-absorbing counter ion.

Mobile phase: 0.01 M phosphate buffer, pH = 2.0 / ethanol (94:6),
Flow rate 1.0 ml/min.

Counter ion: Naphthalene-2-sulfonate (0.0004 M).

Support: PLRP-S, 100 A, 5 μm (150 x 4.6 mm I.D.).

Peaks: 144 = N4-Ac-spermidine; 148 = N1-Ac-spermidine; 152 = N8-Ac-spermidine and S = system peak.

Temperature: 25 °C ± 1 °C.
Figure 8 Reversed-phase ion pair chromatography of: 144 = N⁴-Ac-spermidine; 148 = N⁷-Ac-spermidine and 152 = N⁸-Ac-spermidine. Conditions as in Fig. 7.
5.2.8 - Synthesis of $N^1, N^8$-diethylspermidine trioxalate

$N^1, N^4, N^8$-Tribenzyloxycarbonyl-$N^1, N^8$-diethylspermidine (162)

\[
Z\text{NH(CH}_2\text{)}_3\text{NZ(CH}_2\text{)}_4\text{NHZ} + 2\text{EtI} + 2\text{NaH} \quad \downarrow \\
\text{Z(Et)N(CH}_2\text{)}_3\text{NZ(CH}_2\text{)}_4\text{N(Et)Z} + 2\text{NaI} + 2\text{H}_2
\]

A solution of triprotected spermidine 131 (549 mg, 1.00 mmol) and EtI (2.50 g, 16.0 mmol) in anhydrous THF-DMF (10:1) (25 ml), was cautiously treated with NaH (80 % dispersion in oil, 180 mg, 6.00 mmol) at 0 °C under nitrogen. The resulting suspension was refluxed under nitrogen for 24 h. The yellowish reaction mixture was cooled to r.t. (a white precipitate was formed) and water (2 ml) was added. The clear yellow solution was evaporated under reduced pressure and the residue partitioned between ether (200 ml) and 1 M KHSO$_4$ (100 ml). The aqueous solution was again extracted with ether (100 ml). The combined organic layers were washed and dried as usual. Evaporation to dryness gave a crude mixture which was chromatographed on silica with ether-light petroleum (2:1). The fractions containing pure compound were collected and evaporated to afford 392 mg (65 %) of product 162 as a light yellow oil, homogeneous by t.l.c. (G); $\delta_H$, 7.32 (s, 15H, arom. H), 5.11 (s, 6H, CH$_2$Ph), 3.02-3.28 (m, 12H, CH$_2$N), 1.61-1.83 (m, 2H, CCH$_2$C), 1.31-1.58 (m, 4H, CCH$_2$CH$_2$C), and 1.09 (t, 6H, CH$_3$CH$_2$N); $\delta_C$ 160.0 (CO), 136.9, 136.7, 128.4, 127.8 and 127.7
(arom. C), 66.9 and 66.8 (CH$_2$Ph), 46.5, 44.6 and 41.9 (CH$_2$N), 27.4 and 25.6 (CCH$_2$C), and 13.7 (CH$_3$CH$_2$N).

$^{N_1,N^8}$-Diethylspermidine trioxalate (163)

$$\text{Z(Et)N(CH}_2\text{)}_3\text{NZ(CH}_2\text{)}_4\text{N(Et)}Z \xrightarrow{1)} \text{H}_2/\text{Pd-C} \quad \xrightarrow{2)} \text{C}_2\text{H}_2\text{O}_4 \quad \text{EtNH(CH}_2\text{)}_3\text{NH(CH}_2\text{)}_4\text{NHET.}$$

Compound 162 (850 mg, 1.41 mmol) was hydrogenolyzed as described for the model compound 170 (p. 106). The crude product (265 mg, 94 %), essentially pure by t.l.c. (S), was dissolved in ethanol-ether (1:1) (5 ml) and precipitated as its oxalate salt with excess of a solution of 0.1 M oxalic acid in ether (40 ml). The white solid was centrifuged to afford 444 mg (71 %) of the salt. Recrystallization from water-ethanol (1:1) (100 ml/g) gave the pure oxalate salt 163 as light shiny white crystals, chromatographically homogeneous (S); m.p. 229.5-230.0 °C; $\delta_H$ (D$_2$O) 2.83-3.21 (m, 12H, CH$_2$N), 1.83-2.81 (m, 2H, CCH$_2$C), 1.56-1.80 (m, 4H, CCH$_2$CH$_2$C), and 1.27 (t, 6H, CH$_3$CH$_2$N); $\delta_C$ 168.6 (CO, oxalate), 49.7, 48.9, 47.2, 46.5, 45.7 and 45.6 (CH$_2$N), 25.5 (CCH$_2$C), and 13.2 (CH$_3$CH$_2$N). (Found: C, 43.2; H, 7.0; N, 8.8. C$_{11}$H$_{27}$N$_3$·3H$_2$C$_2$O$_4$ requires C, 43.3; H, 7.05; N, 8.91 %).

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5.2.9 - Attempted syntheses of $N^1$-ethyl- and $N^8$-ethyl-spermidines

$N^1$-Ethylspermidine trioxalate (164)

\[
\text{AcNH(CH}_2)_3\text{NH(CH}_2)_4\text{NH}_2 + 3\text{NaAlH}_2(\text{OCH}_2\text{CH}_2\text{OMe})_2 \\
1) \text{Dioxan} \\
2) \text{aq. NaOH} \\
3) \text{C}_2\text{H}_2\text{O}_4
\]

\[
\text{EtNH(CH}_2)_3\text{NH(CH}_2)_4\text{NH}_2 \cdot 3\text{C}_2\text{H}_2\text{O}_4
\]

The crude $N^1$-Ac-spermidine dihydrochloride 148, obtained from acidolysis of the corresponding Boc$_2$-derivative 147 (1.15 g, 2.97 mmol), was treated with aqueous 30 % K$_2$CO$_3$ (20 ml). After saturating with NaCl, the yellowish aqueous phase was extracted with CHCl$_3$ (5 x 50 ml). The combined organic layers were evaporated under reduced pressure to afford 393 mg (71 %) of the free amine as an oil, essentially pure by t.l.c. (S). This crude material was suspended in dry dioxan (40 ml) and cautiously treated with excess of Red-Al as described for model compound 174 under procedure A (p. 110). After refluxing for 4 h, t.l.c. (S) showed a major spot and a minor one with the same $R_f$ as spermidine. The reaction mixture was cooled and worked up as described for compound 174 (p.110) but, in this case, the turbid basic aqueous solution (50 ml) was continuously extracted with CHCl$_3$ (150 ml) for about 6 h. The extract was dried (Na$_2$SO$_4$) and evaporated to afford 498 mg
of a yellow liquid. This crude material was chromatographed on silica (CHCl₃-MeOH-aqueous 25 % NH₃, 2:2:1). The fractions containing the pure product were pooled and evaporated under reduced pressure. Two phases were formed during the evaporation. To avoid bumping, it was necessary to keep the temperature below 25 °C until the chloroform evaporated (only one phase). The temperature was then increased to 40 °C to complete evaporation. A white solid was obtained which was triturated with CHCl₃ (10 ml) and concentrated (twice). The precipitate was again taken up in CHCl₃ (5 ml), filtered and rinsed with small portions of CHCl₃. T.l.c. (S) showed that the yellowish filtrate did not contain the product and the solid (256 mg) was homogeneous. Elemental analysis indicated, however, that this material contained ≈ 30 % of the desired product. This solid was dissolved in 15 % NaOH (20 ml) and after saturating with NaCl, the aqueous solution was extracted with CHCl₃ (5 x 40 ml). The combined organic layers were dried and evaporated to dryness to afford 147 mg of a yellowish liquid. The product was dissolved in ethanol-ether (1:1) (5 ml) and isolated as its oxalate salt (0.1 M oxalic acid in ether, 30 ml). The white precipitate was centrifuged to afford 218 mg (23 %) of the salt. Recrystallization from water-ethanol (1:2) (90 ml/g) afforded 197 mg (21%) of pure oxalate salt as light shiny white crystals, homogeneous by t.l.c. (S); m.p. 218.5-219.0 °C; δH (D₂O) 3.05-3.18 (m, 10H, CH₂N), 2.05-2.14 (m, 2H, CCH₂C), 1.73-1.77 (m, 4H, CCH₂CH₂C), and 1.27 (t, 3H, CH₃CH₂N); δC 168.6 (CO, oxalate), 50.0, 47.4,
46.8, 46.0 and 41.7 (CH$_2$N), 26.9, 25.7 and 25.6 (CCH$_2$C), and 13.4 (CH$_3$CH$_2$N). (Found: C, 40.7; H, 6.7; N, 9.4. C$_9$H$_{23}$N$_3$.3H$_2$C$_2$O$_4$ requires C, 40.63; H, 6.59; N, 9.48 %).

N$^8$-Ethylspermidine trioxalate (165)

\[
\text{NH}_2(CH_2)_3\text{NH}(CH_2)_4\text{NHAc} + 3\text{NaAlH}_2(\text{OCH}_2\text{CH}_2\text{OMe})_2
\]

1) Dioxan
2) aq. NaOH
3) C$_2$H$_2$O$_4$

\[
\text{NH}_2(CH_2)_3\text{NH}(CH_2)_4\text{NHEt}.3\text{C}_2\text{H}_2\text{O}_4
\]

165

The crude N$^8$-Ac-spermidine, obtained from hydrogenolysis of the corresponding Z$_2$-derivative 151 (1.17 g, 2.57 mmol), was treated with Red-Al and the product worked up and purified in a similar manner as described above for compound 164. The yield of the pure oxalate salt 165, as light shiny crystals, was 252 mg (22%); m.p. 212.5-213.0 °C; $\delta_H$ (D$_2$O) 3.04-3.17 (m, 10H, CH$_2$N), 2.05-2.10 (m, 2H, CCH$_2$C), 1.74-1.77 (m, 4H, CCH$_2$CH$_2$C), and 1.26 (t, 3H, CH$_3$CH$_2$N); $\delta_C$ 168.4 (CO, oxalate), 49.7, 48.9, 47.2, 45.6 and 39.2 (CH$_2$N), 26.4 and 25.5 (CCH$_2$C), and 13.2 (CH$_3$CH$_2$N). (Found: C, 40.0; H, 6.7; N, 9.2. C$_9$H$_{23}$N$_3$.3H$_2$C$_2$O$_4$ requires C, 40.63; H, 6.59; N, 9.48 %).
5.3 - Synthesis of alkyl benzyl imidodicarbonates

5.3.1- Benzyloxy carbonyl isocyanate (154)

\[
\text{PhCH}_2\text{OCONH}_2 + (\text{COCl})_2 \rightarrow \text{PhCH}_2\text{OCON}=\text{C}=\text{O} + 2\text{HCl} + \text{CO}
\]

To a suspension of dry, finely ground benzyl carbamate 153, (30.8 g, 0.20 mol) in dry CH\textsubscript{2}Cl\textsubscript{2} (300 ml) was added dropwise with efficient stirring a solution of oxalyl chloride (36.1 g, 0.30 mol) in dry CH\textsubscript{2}Cl\textsubscript{2} (150 ml) over a period of 1 h with ice-cooling under dry nitrogen. The initially clear mixture gradually became turbid after stirring for 1 h at 0 °C. The stirring was continued for 4 h at r.t. and overnight (15 h) under reflux. The mixture was then concentrated to about 2/3 of its original volume and the fine-grained precipitate was filtered off and washed with cold, dry CH\textsubscript{2}Cl\textsubscript{2} (3 x 20 ml). The combined, pale yellow filtrate was evaporated to dryness at r.t. with a minimum exposure to atmospheric moisture. The residual yellowish turbid oil was distilled at reduced pressure under nitrogen. After a forerun, consisting largely of oxalyl chloride and benzyl chloride, product 154 was collected at 78-80 °C/ 0.15-0.20 mm Hg. The yield of 154 was 18.9 g (53 %). This material, a colourless liquid, containing <1% of benzyl chloride, was suitable for further work, except in the synthesis of 155d which required the removal of remaining traces of acidic impurities by a second distillation. This compound being very sensitive to moisture...
was stored below -20 °C in a sealed vessel; δ_H 7.38 (s, 5H, arom. H), 5.20 (s, 2H, CH_2Ph); δ_C 148.6 (PhCH_2OCON), 129.5 (N=C=O), 133.6, 128.5, 128.2 and 128.1 (arom. C), and 70.2 (CH_2Ph).

The white precipitate from above, weighing 4.9 g, consisted largely of N,N'-dibenzyloxy carbonyloxamide (for related derivatives, see Lit.°°); m.p. 211-212 °C (dec., from 1,2-dichloroethane (≈ 1 l/g)). The crystalline, analytical specimen tenaciously retains the solvent, even after drying in high vacuo for several days; δ_H (DMSO-d_6) 11.60 (broad, ≈ 2H, NH), 7.38 (s, 10H, arom. H), and 5.17 (s, 4H, CH_2Ph); δ_C 165.0 (CO-CO), 152.9 (PhCH_2OCON)), 135.3, 128.7 and 128.4 (arom. C), and 67.5 (CH_2Ph).

5.3.2- Alkyl benzyl imidodicarbonates (155a-i)

**General procedure**

![Image](image-url)

To a vigorously stirred solution of the alcohol ROH (20 mmol) in dry CH_2Cl_2 (30 ml), under nitrogen and cooled in ice, a solution of benzyloxy carbonyl isocyanate, 154 (3.10 g, 19 mmol in the preparations of 155a, b, d, e, g, h involving volatile alcohols; 3.43 g, 21 mmol for 155c, f, i), in dry CH_2Cl_2 (30 ml) was added over a period of 30 min. In the preparation of 155d, the crude 154 is redistilled before use.
After the addition, the resulting colourless solution was stirred under nitrogen in the ice-bath for 1 h and overnight at room temperature. The solvent was evaporated and, except for 155e, the residue was thoroughly tritutrated with cold light petroleum (≈ 30 ml, for 155c and 155i cold ether). After several hours in the cold the white crystalline solid was collected by filtration, rinsed with small portions of cold solvent and dried over paraffin chips at reduced pressure.

The analytical samples were obtained by recrystallization (for 155e by chromatography) and the details of the purification and properties of 155a-i are compiled in Tables XVII and XVIII.

Table XVII - Alkyl benzyl imidodicarbonates 155a-i prepared.

<table>
<thead>
<tr>
<th>Compound (%)</th>
<th>Solvent for recrystallization</th>
<th>m.p. °C</th>
<th>Elemental analysis or Lit. m.p. °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>155a 99</td>
<td>CH₂Cl₂-Et₂O (1:7, 50 ml/g)</td>
<td>109-109.5</td>
<td>105.5-106.5¹¹⁹</td>
</tr>
<tr>
<td>155b 100</td>
<td>See 155a</td>
<td>92.5-93</td>
<td>Found: C, 64.8; H, 5.3; N, 4.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C₁₁H₇NO₅ requires: C, 64.75; H, 5.43; N, 4.44 %</td>
</tr>
<tr>
<td>155c 97</td>
<td>CH₂Cl₂-Et₂O (1:4, 60 ml/g)</td>
<td>113.5-114</td>
<td>Found: C, 58.2; H, 4.2; N, 8.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C₁₆H₁₄N₂O₂ requires: C, 58.18; H, 4.27; N, 8.48 %</td>
</tr>
</tbody>
</table>

¹¹⁹ Lit. m.p. 105.5-106.5°C.
155d 91b  Et$_2$O-light petroleum (1:4, 30 ml/g) 83.5-84  C$_{18}$H$_{10}$NO requires:
C, 69.00; H, 6.11; N, 4.47 

155e 97d  purified by oil chromatography  oil

155f 98  Et$_2$O (10 ml/g)  112-112.5  Found: C, 69.2; H, 7.0; N, 4.3  C$_{19}$H$_{23}$NO requires: C, 69.28; H, 7.04; N, 4.25 

155g 94  Et$_2$O-hexane (1:1, 20 ml/g)  79.5-80  Found: C, 61.2; H, 5.5; N, 6.0  C$_{12}$H$_{13}$NO requires: C, 61.27; H, 5.57; N, 5.95 

155h 92  See 155d  90-90.5  Found: C, 40.5; H, 2.9; N, 4.2  C$_{11}$H$_{10}$ClNO requires: C, 40.46; H, 3.09; N, 4.29 

155i 92  CH$_2$Cl$_2$-Et$_2$O (1:10, 70 ml/g)  112-113  C$_{23}$H$_{19}$NO requires: C, 73.98; H, 5.13; N, 3.75 

---

**a** Yield of essentially pure product before recrystallization (<1% of impurities as judged from $^1$H n.m.r. and t.l.c. (A), (G), or ether).

**b** Yield corrected for $\approx 4\%$ of 153 in the crude product. Recrystallized product contained traces of 153. Attempted chromatography on silica caused partial decomposition of the compound.

**c** Crude product contained 1-2% of 153. Yield after chromatography (silica light petroleum-ether, 3:1) 84%.

**d** Softens at $\approx 107^\circ$C.
as previously reported\textsuperscript{101}

\textbf{155f} 1.65 and 2.12 (2 br sign, 15H, H\textsubscript{Halif}); 5.17 (s, 2H, CH\textsubscript{2}); \approx 7.10 (br s, \approx 1H, NH); 7.34 (s, 5H\textsubscript{arom}).

\textbf{155g} 4.60 and 4.67 (2t, 2H, CH\textsubscript{2}CH); 5.17 [s, 2H, CH\textsubscript{2} (Z)]; 5.30 and 5.30 (2m, 2H, =CH\textsubscript{2}); 5.70-6.12 (m, 1H, =CH-); 7.34 (s, 5H\textsubscript{arom}); \approx 7.50 (br s, \approx 1H, NH).

\textbf{155h} 4.78 [s, 2H, CH\textsubscript{2} (Troc)]; 5.22 [s, 2H, (Z)]; 7.37 (s, 5H\textsubscript{arom}); \approx 7.53 (br s, \approx 1H, NH).

\textbf{155i} 4.14-4.28 (4 sign, 1H\textsubscript{Halif}, (Fmoc)); 4.45-4.53 [3 sign, 2H, CH\textsubscript{2} (Fmoc)]; 5.19 [s, 134.8 [C\textsubscript{arom} Z]]; 120.0, 128.5, 128.6, 135.2 (C\textsubscript{arom}); 148.9 (C\textsubscript{Adoc}); 150.9 (CO\textsubscript{Z}).

30.9, 36.0, 41.2 (C\textsubscript{Halif},Adoc); 67.4 (CH\textsubscript{2}); 82.4 (Cquart); 128.4, 128.5, 128.6, 135.2 (C\textsubscript{arom}); 148.9 (CO\textsubscript{Adoc}); 150.9 (CO\textsubscript{Z}).

66.8 (CH\textsubscript{2}-CH); 67.8 [CH\textsubscript{2} (Z)]; 119.1 (=CH\textsubscript{2}); 128.5, 128.6, 134.9 (C\textsubscript{arom}); 131.3 (=CH-); 150.5, 150.6 (CO).

68.3 [CH\textsubscript{2}, (Z)]; 74.7 [CH\textsubscript{2} (Troc)]; 94.3 (CCl\textsubscript{3}); 128.6, 128.7, 128.8, 134.6 (C\textsubscript{arom}); 149.0 (CO\textsubscript{Troc}); 150.4 (CO\textsubscript{Z}).
$2H, CH_2 (Z)]; 7.18-7.80 (m) \quad 124.9, 127.1, 127.9,$

$+ 7.35 (s, together 14H, H_{arom} + NH) \quad 141.2, 142.2 [C_{arom} (Fmoc)]; 150.5, 150.7 (CO).

Z= Benzyloxy carbonyl
Z(OMe)= 4-Methoxybenzyloxy carbonyl
Z(NO$_2$)= 4-Nitrobenzyloxy carbonyl
Ppoc= 2-Phenylisopropyl oxycarbonyl
Adoc= 1-Adamantyl oxycarbonyl
Troc= 2,2,2-Trichloroethoxycarbonyl
Fmoc= 9-Fluorenymethoxycarbonyl
<table>
<thead>
<tr>
<th>Model compounds synthesized</th>
<th>page</th>
<th>ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>N(^2)-Benzyloxycarbonyl-N(^1)-tert-butoxycarbonyl-N(^1)-ethylethlenediamine (157)</td>
<td>103</td>
<td>-</td>
</tr>
<tr>
<td>N(^1)-Benzyloxycarbonyl-N(^2)-tert-butoxycarbonyl-N(^1),N(^2)-diethylethlenediamine (167)</td>
<td>104</td>
<td>-</td>
</tr>
<tr>
<td>N(^1)-tert-Butoxycarbonyl-N(^1)-ethlenediamine (170)</td>
<td>106</td>
<td>-</td>
</tr>
<tr>
<td>N(^2)-Acetyl-N(^1)-tert-butoxycarbonyl-N(^1)-ethyl-ethlenediamine (171)</td>
<td>107</td>
<td>-</td>
</tr>
<tr>
<td>N(^1)-tert-Butoxycarbonyl-N(^1),N(^2)-diethylethylene-diamine (172)</td>
<td>108</td>
<td>-</td>
</tr>
<tr>
<td>N(^2)-Acetyl-N(^1)-ethylethlenediamine (173)</td>
<td>109</td>
<td>-</td>
</tr>
<tr>
<td>N(^1),N(^2)-Diethylethlenediamine dihydrochloride (174)</td>
<td>110</td>
<td>-</td>
</tr>
<tr>
<td>N(^1)-Benzyloxycarbonyl-N(^4)-tert-butoxycarbonyl-putresine (168)</td>
<td>112</td>
<td>123</td>
</tr>
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</table>
Spermidine derivatives synthesized

<table>
<thead>
<tr>
<th>Structure</th>
<th>Page</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N^1, N^4, N^4$-Tribenzyloxy carbonyl spermidine</td>
<td>113</td>
<td>109a, 109b</td>
</tr>
<tr>
<td>$N^1, N^4, N^8$-Tri benzyloxy carbonyl-$N^1, N^8$-bis(tert-butoxy carbonyl)spermidine</td>
<td>114</td>
<td>109a, 109b</td>
</tr>
<tr>
<td>$N^1, N^8$-Bis(tert-butoxy carbonyl)spermidine</td>
<td>116</td>
<td>19, 41, 51b, 82, 109a, 109b</td>
</tr>
<tr>
<td>$N^4$-Benzyloxy carbonyl-$N^1, N^8$-bis(tert-butoxy carbonyl)spermidine</td>
<td>117</td>
<td>109a, 109b</td>
</tr>
<tr>
<td>$N^1, N^4$-Methylene spermidine</td>
<td>119</td>
<td>18, 79, 80, 109b</td>
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<tr>
<td>$N^1, N^8$-Dib enzyloxy carbonyl-$N^1, N^4$-methylene spermidine</td>
<td>119</td>
<td>109b</td>
</tr>
<tr>
<td>$N^1, N^8$-Di benzyloxy carbonyl-$N^8$-tert-butoxy carbonyl-$N^1, N^4$-methylene spermidine</td>
<td>120</td>
<td>109b</td>
</tr>
<tr>
<td>$N^1$-Benzyloxy carbonyl-$N^8$-tert-butoxy carbonyl-$N^1, N^4, N^8$methylene spermidine</td>
<td>121</td>
<td>109b</td>
</tr>
<tr>
<td>$N^1$-Benzyloxy carbonyl-$N^8$-tert-butoxy carbonyl-spermidine</td>
<td>121</td>
<td>121, 109b</td>
</tr>
<tr>
<td>$N^1, N^8$-Dibenzyloxy carbonyl-$N^8$-tert-butoxy carbonyl spermidine</td>
<td>123</td>
<td>109b</td>
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<tr>
<td>$N^1, N^4, N^8$-Tri acetyl spermidine</td>
<td>130</td>
<td>125, 109c</td>
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<tr>
<td>$N^1, N^4, N^8$-Tri acetyl-$N^1, N^8$-bis(tert-butoxy carbonyl)spermidine</td>
<td>131</td>
<td>109c</td>
</tr>
<tr>
<td>$N^4$-Acetyl-$N^1, N^8$-bis(tert-butoxy carbonyl)-spermidine</td>
<td>133</td>
<td>109c</td>
</tr>
<tr>
<td>$N^4$-Acetyl spermidine dioxalate</td>
<td>134</td>
<td>109c</td>
</tr>
<tr>
<td>$N^1$-Benzyloxy carbonyl-$N^4, N^8$-bis(tert-butoxy carbonyl)spermidine</td>
<td>135</td>
<td>121, 109c</td>
</tr>
<tr>
<td>Chemical Name</td>
<td>Page No.</td>
<td>References</td>
</tr>
<tr>
<td>---------------</td>
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</tr>
<tr>
<td>N&lt;sup&gt;4&lt;/sup&gt;,N&lt;sup&gt;8&lt;/sup&gt;-Bis(tert-butoxycarbonyl)spermidine</td>
<td>146</td>
<td>36, 37, 48, 51a, 121, 109c</td>
</tr>
<tr>
<td>N&lt;sup&gt;1&lt;/sup&gt;-Acetyl-N&lt;sup&gt;4&lt;/sup&gt;,N&lt;sup&gt;8&lt;/sup&gt;-bis(tert-butoxycarbonyl)-spermidine</td>
<td>147</td>
<td>109c</td>
</tr>
<tr>
<td>N&lt;sup&gt;1&lt;/sup&gt;-Acetylspermidine dihydrochloride</td>
<td>148</td>
<td>35, 55, 83, 109c</td>
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<tr>
<td>N&lt;sup&gt;1&lt;/sup&gt;,N&lt;sup&gt;4&lt;/sup&gt;-Dibenzyloxy carbonyl-N&lt;sup&gt;8&lt;/sup&gt;-tert-butoxy-carbonyl spermidine</td>
<td>149</td>
<td>109c</td>
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<tr>
<td>N&lt;sup&gt;1&lt;/sup&gt;,N&lt;sup&gt;4&lt;/sup&gt;-Dibenzyloxy carbonylspermidine</td>
<td>150</td>
<td>109c</td>
</tr>
<tr>
<td>N&lt;sup&gt;8&lt;/sup&gt;-Acetyl-N&lt;sup&gt;1&lt;/sup&gt;,N&lt;sup&gt;4&lt;/sup&gt;-dibenzyloxy carbonylspermidine</td>
<td>151</td>
<td>109c</td>
</tr>
<tr>
<td>N&lt;sup&gt;8&lt;/sup&gt;-Acetylspermidine dihydrochloride</td>
<td>152</td>
<td>35a, 55, 83, 109c</td>
</tr>
<tr>
<td>N&lt;sup&gt;1&lt;/sup&gt;,N&lt;sup&gt;4&lt;/sup&gt;,N&lt;sup&gt;8&lt;/sup&gt;-Tri benz yloxy carbonyl-N&lt;sup&gt;1&lt;/sup&gt;,N&lt;sup&gt;8&lt;/sup&gt;-diethyl spermidine</td>
<td>162</td>
<td></td>
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<td>N&lt;sup&gt;1&lt;/sup&gt;,N&lt;sup&gt;8&lt;/sup&gt;-Diethylspermidine trioxalate</td>
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<tr>
<td>N&lt;sup&gt;1&lt;/sup&gt;-Ethyl spermidine trioxalate</td>
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<td>N&lt;sup&gt;8&lt;/sup&gt;-Ethyl spermidine trioxalate</td>
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<td>Alkyl benzyl imidodicarbonates synthesized</td>
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</tr>
<tr>
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# REFERENCES


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Chen. 1976, 1319.


ABSTRACT

A new, simple and efficient preparative procedure of a potentially wide scope for the selective protection of mixed primary/secondary amines is presented. Its applicability is demonstrated on spermidine to make both protected and simple substituted derivatives.

The results obtained during this research are the main subject of this thesis. It also includes an introductory section on the biological aspects of polyamines and a review on the methods commonly used for their synthesis.

The new approach is based on tert-butoxycarbonylation of carbamate groups (exhaustive tert-butoxycarbonylation) derived from the primary amino functions only. In most cases, benzyl polycarbamates are used for this purpose. Subsequent removal of all benzyloxycarbonyl (Z) groups from the resulting intermediates by catalytic hydrogenolysis liberates the secondary amino functions, whereas tert-butoxycarbonyl (Boc) is retained on the primary ones. Alternatively, selective removal of Z only from amino functions, protected by both Z and Boc, which can be accomplished by base-catalysed methanolysis, results in protected polyamines with Boc and Z on their primary and secondary amino groups, respectively. The new reaction has been performed on spermidine to give $N^1,N^6$-Boc$_2$-spermidine. By virtue of the non-equivalence of the two primary amino groups in this molecule, the synthesis of
N⁸-Boc-N¹-Z-spermidine, starting instead with an easily available cyclic derivative (hexahydropyrimidine), is also presented. The yields of most intermediates as well as of the two products were high.

The synthesis of all three monoacetylated spermidines is also reported. The N⁴-acetyl derivative was obtained in four steps from spermidine via the triacetyl intermediate by selective deacetylation after exhaustive tert-butoxycarbonylation as well as directly from N¹,N⁸-Boc₂-spermidine. The N¹-acetyl and N⁸-acetyl derivatives were both obtained in four simple protection/deprotection steps from a common intermediate, N⁸-Boc-N¹-Z-spermidine mentioned above, thus illustrating the versatility of this compound.

The synthesis of a few N-ethylspermidines is also described. The N¹,N⁸-diethylspermidine was obtained in three steps from spermidine via the Z₃-derivative by N¹,N⁸-diethylation followed by removal of the Z groups. The attempted syntheses of the N¹-ethyl and N⁸-ethyl derivatives were performed by reduction of the amide groups of the corresponding acetyl spermidines.

New mixed alkyl benzyl imidodicarbonates were prepared by reaction of benzyloxy carbonyl isocyanate with appropriate alcohols. The compounds are of interest as potential Gabriel reagents. Completely selective removal of one of the alkoxy carbonyl groups from the N-atom of the imidodicarbonates was demonstrated in several instances, giving either benzyl or the alternative carbamate.
Poliaminas naturais (como por ex. espermidina e espermina) e seus derivados constituem um grupo de compostos com grande importância em muitos processos biológicos a nível celular.

Recentemente foi verificado que tais compostos são potenciais agentes antineoplásticos, sendo ainda úteis no tratamento de doenças parasitárias.

Em face da importância biológica referida, a síntese de poliaminas e seus derivados reveste-se de grande interesse. Na maioria dos casos, o êxito da síntese vai depender da modificação selectiva dos diferentes grupos amina. Em 1986, embora na literatura estivessem já descritos alguns métodos para a protecção selectiva dessas aminas, parecia não existir um método ideal, particularmente, no que se refere à protecção total desses compostos. A metodologia usada requeria um grande número de fases e/ou condições drásticas para a remoção dos grupos de protecção (por ex. grupos tosilo e ftaloílo), tornando-se por isso desejável um novo método alternativo mais simples.

Iniciou-se, então, na escola do Doutor Ulf Ragnarsson, Instituto de Bioquímica, Centro de Biomédicas, Universidade de Uppsala, Suécia, um projecto de investigação a fim de explorar um método simples e geral para a protecção selectiva de aminas primárias/secundárias. Deu-se particular atenção ao caso da espermidina, usando como reacção chave a N-tert-butoxi-
carbonilação de grupos uretanos catalisada por 4-dimetilaminopiridina (DMAP). Para este fim, foi usado o grupo benziloxicarboniló (Z) o qual é ortogonalmente removido na presença do grupo Boc e em condições suaves. É de referir que antes de se usar a espermidina como substrato foram sempre realizados estudos preliminares com um composto modelo mais simples, a N-etil-1,2- etanodiamina.

No Esquema I está representado a nova metodologia para o caso da espermidina. Depois de se introduzirem os três grupos Z, efectuou-se a N-tert-butoxicarbonilação nos grupos NH terminais. Em seguida, a protecção Z foi removida por dois métodos alternativos:

a) remoção total por hidrogenólise catalítica, dando origem ao derivado N¹,N⁸-Boc₂-espermidina;

b) remoção selectiva por metanólise catalisada por base, para se obter o composto N⁴-Z-N¹,N⁸-Boc₂-espermidina.

\[
\begin{align*}
\text{NH}_2(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NH}_2 \\
\downarrow \text{ZCl} \\
\text{ZNH}(\text{CH}_2)_3\text{NZ}(\text{CH}_2)_4\text{NHZ} \\
\downarrow \text{Boc}_2\text{O (DMAP)} \\
\text{Boc}(Z)\text{N}(\text{CH}_2)_3\text{NZ}(\text{CH}_2)_4\text{N}(Z)\text{Boc} \\
\xleftarrow{-Z} \\
\text{BocNH}(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NH}\text{Boc} \\
\xrightarrow{-Z} \\
\text{BocNH}(\text{CH}_2)_3\text{NZ}(\text{CH}_2)_4\text{NH}\text{Boc}
\end{align*}
\]

Esquema I
Por outro lado, como a espermidina contém dois grupos -NH₂ não equivalentes, conseguiu-se a partir do derivado cíclico (hexa-hidropirimidina), resultante da reacção entre a triamina e formaldeído, sintetizar o composto chave, ZNH(CH₂)₃NH(CH₂)₄NHBoc.

Uma vez obtidos estes precursores protegidos, foram estudadas as suas potencialidades como reagentes chave na síntese de derivados da espermidina com interesse biológico. Assim, foram sintetizados os seguintes derivados monoacetilados os quais são importantes como metabólitos e produtos de excreção:

- **N⁴-acetilespermidina** [NH₂(CH₂)₃NAc(CH₂)₄NH₂];
- **N¹-acetilespermidina** [AcNH(CH₂)₃NH(CH₂)₄NH₂];
- **N⁸-acetilespermidina** [NH₂(CH₂)₃NH(CH₂)₄NHAc].

A N⁴-Ac-espermidina foi obtida em quatro fases por conversão da espermidina no derivado triacetilado, seguindo-se a N-terr-butoxicarbonilação exaustiva e remoção selectiva dos grupos acetilo. Este derivado também foi obtido directamente a partir do precursor N¹,N⁸-Boc₂-espermidina.

Os derivados N¹-Ac- e N⁸-Ac-espermidina foram obtidos em quatro fases simples de protecção/desprotecção a partir do composto chave N¹-Z-N⁸-Boc-espermidina, ilustrando assim a versatilidade deste composto. No caso do derivado acetilado em N¹, começou-se por proteger o grupo amina secundária com a protecção Boc. Em seguida removeu-se o grupo Z para se obter o intermediário NH₂(CH₂)₃NBoc(CH₂)₄NHBoc. Este derivado foi acetilado no grupo amina, tendo-se depois removido os grupos.
Boc. No caso da N⁸-Ac-espermidina, o grupo amina secundária foi protegido com o grupo Z, seguindo-se a remoção selectiva da protecção Boc. De um modo semelhante, o derivado resultante, ZNH(CH₂)₃NZ(CH₂)₄NH₂, após acetilação seguida de remoção dos grupos Z, deu o composto acetilado em N⁸.

Foram também sintetizados os derivados etilados da espermidina, EtNH(CH₂)₃NH(CH₂)₄NHEt, EtNH(CH₂)₃NH(CH₂)₄NH₂ e NH₂(CH₂)₃NH(CH₂)₄NHEt. Estes compostos podem ser também importantes sob o ponto de vista biológico, nomeadamente em quimioterapia.

A N¹,N⁸-Et₂-espermidina foi obtida em dois passos por N¹,N⁸-dietilação do intermediário N¹,N⁴,N⁸-Z₃-espermidina seguida da remoção dos grupos Z. Os N¹-etil- e N⁸-etil-derivados da espermidina foram sintetizados por redução do grupo amida das correspondentes acetilespermidinas.

Foi possível, paralelamente ao trabalho mencionado, iniciar um estudo que permitisse a síntese total de poliaminas a partir de precursores adequadamente protegidos, tais como, Boc₂NH. Assim, começou-se por sintetizar novos reagentes de Gabriel, imidodicarbonatos de alquilo e benzilo, por reacção do isocianato de benziloxicarbonilo com alcoóis apropriados (Esquema II).

\[
\text{ZNH}_2 \xrightarrow{\text{(COCl)}_2} \text{ZN=C=O} \xrightarrow{\text{ROH}} \text{ZNHCOOR}
\]

Esquema II
A completa remoção selectiva de um dos grupos alcoxicarbonilo do átomo de azoto destes imidodicarbonatos foi demonstrada com vários exemplos, obtendo-se o carbamato de benzilo ou o carbamato alternativo.

Em conclusão, os objectivos do projecto inicial foram amplamente alcançados uma vez que se conseguiu estabelecer um novo método, simples e eficiente, para a protecção selectiva de poliaminas o qual poderá ainda ser relevante no campo dos alcalóides. Por outro lado, os imidodicarbonatos preparados são reagentes promissores para uma estratégia envolvendo a síntese total de poliaminas, usando as reacções de Gabriel ou de Mitsunobu.

Os resultados obtidos durante este projecto de investigação constituem a parte essential e original desta dissertação, que inclui uma breve introdução sobre os aspectos biológicos das poliaminas naturais e ainda uma revisão sobre os métodos mais usados na sua síntese.

Parte dos resultados obtidos nesta investigação deram já origem a quatro artigos publicados em colaboração 109a-c.