SYNTHESIS AND NMR SPECTROSCOPY OF N,N'-BIS(2-THIOETHANOYL)-
2,3-DIAMINOPROPANOIC ACID DERIVED CARRIER CHELANTS FOR
TECHNETIUM RADIOIMAGING

By

RABINDRANATH BANSEE MAHARAJH, B.Sc.

A Thesis
Submitted to the School of Graduate Studies
in Partial Fulfilment of the Requirements
for the Degree
Doctor of Philosophy

McMaster University

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SYNTHESIS OF CARRIER CHELANTS FOR TECHNETIUM RADIOIMAGING
DOCTOR OF PHILOSOPHY (1993)
(Chemistry) McMaster University

Hamilton, Ontario

TITLE: Synthesis and NMR Spectroscopy of \(N,N'\)-bis(2-thioethanoyl)-
2,3-diaminopropanoic Acid Derived Carrier Chelants For Technetium
Radioimaging

AUTHOR: Rabindranath Bansee Maharaj, B.Sc. (McMaster University)

SUPERVISOR: Professor R. A. Bell

NUMBER OF PAGES: xix, 244
Abstract

Because of its availability and favorable disintegration characteristics, there has been interest in recent years to bind technetium-99m ($^{99m}$Tc) to carrier molecules for use as tissue-specific diagnostic radiopharmaceuticals. In contribution to this effort, this thesis describes some investigations into the synthesis and NMR spectroscopy of novel bifunctional chelants for $^{99m}$Tc radioimaging. Using bifunctional chelate strategies, a series of diamide disulfur (DADS) ligand conjugates of biologically active, carrier compounds were prepared for development into prospective myocardial and gastroenterological imaging agents. Carrier molecules of the intended myocardial agents included cardioactive compounds, such as C$_{11}$ and C$_{16}$ long chain fatty acids and the cardenolide aglycone digitoxigenin. DADS ligand derivatives of cholic acid, taurine and β-cyclodextrin were also synthesized in an effort to determine their potential utility as gastroenterological technetium imaging agents. The conjugation of ligands to steroidal molecules represents a new approach towards the production of $^{99m}$Tc-based radiopharmaceuticals.

The 10-membered heterocycle, $N,N'$-[dimethyl-(2,2'-dithiobisethanol)]] ethylenediamine (40), was prepared to aid in the interpretation of the complex NMR spectral characteristics observed in many of the DADS ligands. It exists in solution as a mixture of two $Z,Z$ and one $Z,E$ disulfide and amide ring conformers and has been characterized by NOE, $^1$H-$^1$H, $^1$H-$^{13}$C shift correlated NMR and molecular modelling studies. Among the $Z,Z$ ring conformers, $Z,Z_1$ and $Z,Z_2$, the former predominates and interconverts with the latter isomer by rotation about the S-S bond with an apparent
activation energy of 14.5 ± 1.3 kcal/mol. Coalescence of N-CH$_3$ signals occurred at about 127 °C at 500 MHz, which corresponded to an approximate barrier to amide rotation of 19.3 kcal/mol. Aromatic solvent induced shifts (ASIS) in C$_6$D$_6$ have corroborated molecular mechanics and NOE predictions of amide stereochemistry. The structure of the Z,E stereoisomer of 40 has been determined by single crystal X-ray diffraction at 296 K. A large geminal N-CH$_2$ inequivalence (> 2 ppm in CDCl$_3$) was seen in the Z,Z conformers. Based on empirical calculations of chemical shifts, roughly one-half of the observed splitting was rationalized in terms of nearly equal contributions from magnetic anisotropy and electric field shielding mechanisms of the amide carbonyl groups. An implication of these results is the possibility of using α-CH (and eventually -CH) shifts to probe the local conformational space in structured proteins and other complex amides.
Acknowledgements

This thesis would be incomplete without acknowledging the valuable assistance of several people. I wish to thank my research supervisor, Professor R.A. Bell, for sharing his expert knowledge and guidance throughout the course of this work. His flexibility gave me the intellectual freedom to pursue and implement many ideas.

Special thanks are due to Dr. D.W. Hughes for his guidance at the NMR console and for many enlightening discussions relating to the NMR phenomenon. I am also indebted to Dr. A.D. Bain for his sage advice and review of the dimethyl disulfide (40) manuscript. I am grateful to Dr. J.F. Britten for the X-ray analysis of 40 and Drs. T. Hemscheidt, J.D. Laposa, L. Li, D.B. MacLean, B.E. McCarry, M.J. McGlinchey and R. Smith for very helpful discussions relating to this research. Technical contributions were generously made by B.G. Sayer and G. Timmins. Thanks are also necessary to the members of my thesis committee, Drs. G. Coates, P. Harrison and C.J.L. Lock and to my external examiner, Dr. J.W. Lown, for their interest and input.

And to my fellow inmates and friends with whom I shared ABB 465 - Fred, Chuck, Heinz-Pete, Todd and John - thanks for the good times and laughs. May you all discover the secret of transmuting Pb into Au!

I would like to express my last words of thanks to my wife, Deepika, who patiently endured the many nights and weekends that were spent on the NMR spectrometer and writing this thesis. Your continued confidence and support helped me to laugh at my problems and spurred me to try that much harder.

Finally, I am very grateful to McMaster University for financial assistance.
To my beloved parents whose unfailing support and sacrifices have made this work possible
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<th>Definition</th>
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<tr>
<td>AN</td>
<td>acetonitrile</td>
</tr>
<tr>
<td>Ar</td>
<td>aryl</td>
</tr>
<tr>
<td>bp</td>
<td>boiling point</td>
</tr>
<tr>
<td>br</td>
<td>broad (spectral)</td>
</tr>
<tr>
<td>°C</td>
<td>degrees Celsius</td>
</tr>
<tr>
<td>CAD</td>
<td>coronary artery disease</td>
</tr>
<tr>
<td>CDI</td>
<td>carbonyldiimidazole</td>
</tr>
<tr>
<td>Cl-MS</td>
<td>chemical ionization mass spectrometry (spectrum)</td>
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<tr>
<td>CO&lt;sub&gt;2&lt;/sub&gt;-DADS</td>
<td>diamide disulfur carboxylate; specifically, N,N'-bis (2-thioethanoyl)-2,3-diaminopropanoic acid</td>
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<tr>
<td>COSY</td>
<td>correlated spectroscopy (in NMR)</td>
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<td>Δδ&lt;sub&gt;obs&lt;/sub&gt;</td>
<td>observed chemical shift difference between geminal protons</td>
</tr>
<tr>
<td>δ</td>
<td>chemical shift in parts per million downfield from tetramethylsilane</td>
</tr>
<tr>
<td>d</td>
<td>day(s); doublet (spectral)</td>
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<tr>
<td>DCC</td>
<td>1,3-dicyclohexylcarbodiimide</td>
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<tr>
<td>DCI-MS</td>
<td>desorption chemical ionization mass spectrometry (spectrum)</td>
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<tr>
<td>DCU</td>
<td>1,3-dicyclohexylurea</td>
</tr>
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<td>ddH&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>doubly-distilled, deionized water</td>
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<tr>
<td>dec.</td>
<td>decomposed (in melting point determination)</td>
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<td>DEI-MS</td>
<td>desorption electron ionization mass spectrometry (spectrum)</td>
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<td>dH&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>distilled water</td>
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<td>DIÁRS</td>
<td>o-phenylenebis(dimethylarsine)</td>
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<td>4-dimethylaminopyridine</td>
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<td>DMF</td>
<td>N,N-dimethylformamide</td>
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<td>dimethylphosphinoethane</td>
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<td>dynamic nuclear magnetic resonance</td>
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<td>DTNB</td>
<td>5,5'-dithiobis-(2-nitrobenzoic acid)</td>
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<td>DTPA</td>
<td>diethylenetriaminepentaacetic acid</td>
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<td>EDC</td>
<td>1-(3-dimethylaminopropyl)-3- ethylcarbodiimide hydrochloride</td>
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<td>1-(3-dimethylaminopropyl)-3-ethylurea (salt or free base)</td>
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<td>ethylenediamine tetraacetic acid</td>
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<td>electron impact (in mass spectrometry)</td>
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<td>EtOAc</td>
<td>ethyl acetate</td>
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<td>FAB-MS</td>
<td>fast atom bombardment mass spectroscopy (spectrum)</td>
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<td>FID</td>
<td>free induction decay (in NMR)</td>
</tr>
<tr>
<td>g</td>
<td>gram(s)</td>
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<tr>
<td>G&lt;sup&gt;η&lt;/sup&gt;</td>
<td>Gibbs free energy of activation</td>
</tr>
<tr>
<td>h</td>
<td>hour(s)</td>
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<tr>
<td>HEDP</td>
<td>sodium (or calcium) 1-hydroxyethyl-1,1-diphosphonate</td>
</tr>
<tr>
<td>HIDA</td>
<td>hepatobiliary iminodiacetate(s)</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>HRMS</td>
<td>high resolution mass spectrometry (spectrum)</td>
</tr>
<tr>
<td>Hz</td>
<td>hertz</td>
</tr>
<tr>
<td>i-Pr</td>
<td>isopropyl</td>
</tr>
<tr>
<td>IR</td>
<td>infrared (spectroscopy)</td>
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<tr>
<td>$^nJ$</td>
<td>nuclear spin-spin coupling constant through $n$ bonds (in Hz)</td>
</tr>
<tr>
<td>$^J$-Mod</td>
<td>$J$-modulated $^{13}$C NMR experiment (aka. spin-sort)</td>
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<td>$k$</td>
<td>rate constant</td>
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<tr>
<td>L</td>
<td>liter(s)</td>
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<td>lit.</td>
<td>literature</td>
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<td>$\mu$</td>
<td>micro</td>
</tr>
<tr>
<td>m</td>
<td>multiplet (spectral), meter(s), milli</td>
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<tr>
<td>M</td>
<td>moles per liter, molecular ion (in mass spectrometry)</td>
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<td>Me</td>
<td>methyl</td>
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<tr>
<td>MHz</td>
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<td>$\nu_{\text{MAX}}$</td>
<td>frequency absorbance/transmission (in IR spectrometry)</td>
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<td>NEt$_3$</td>
<td>triethylamine</td>
</tr>
<tr>
<td>NHS</td>
<td>$N$-hydroxysuccinimide</td>
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<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
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<td>Nu</td>
<td>nucleophile</td>
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<td>Pd/C</td>
<td>palladium supported on carbon</td>
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<td>pH</td>
<td>$-\log_{10}[\text{H}^+\text{O}];$ measure of acidity</td>
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<td>Ph</td>
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<td>parts per million</td>
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<td>q</td>
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<td>$R_r$</td>
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<tr>
<td>$t$</td>
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<td>$t_{1/2}$</td>
<td>half-life (of a radionuclide)</td>
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<tr>
<td>TFA</td>
<td>trifluoroacetic acid</td>
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<td>ultraviolet</td>
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<td>v/v</td>
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</tr>
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<td>w</td>
<td>weak (in IR spectroscopy)</td>
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<td>weight per volume</td>
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When asked how his mind operates, Steven Hawking, acclaimed the world’s greatest living scientist, responded:

"I work on intuition, thinking that an idea ought to be right. Then I try to prove it."

"The stream of knowledge is heading toward a nonmechanical reality; the universe begins to look more like a great thought than like a great machine."

Sir James Jean, The Mysterious Universe.
CHAPTER I

INTRODUCTION

In the practice of diagnostic nuclear medicine, a radiopharmaceutical, generally a gamma-ray emitting one, is administered in vivo so that the radioisotope becomes localized in a specific organ or region of the body. The successful targeting of diagnostic radionuclides to diseased tissues and tumors provides not only a tool for the diagnosis of cancer and heart or brain ailments, but also demonstrates the feasibility for therapy where ligand systems can be applied to therapeutic nuclides. For diagnosis, technetium-99m (Tc-99m or $^{99m}$Tc) is at present the most commonly used radionuclide for well-documented reasons (see Section 1.2).

1.1 Anatomical vs Functional Imaging

The importance of radiopharmaceuticals in diagnostic medicine hinges on the fact that the physiological function of an organ, as well as its structure, can be visualized. The availability of this information complements other imaging modalities and has resulted in a better understanding of disease and improved practices in medicine.

Over the years, scintigraphy, ultrasound, computed tomography (CT) and magnetic resonance imaging (MRI) have been used as primary imaging modalities. Because CT and MRI generally provide far superior anatomical detail of tissues, the role of scintigraphy has gravitated more towards the study of organ function using
radioimaging agents. Thus, the general trend in nuclear medicine has been towards more quantitation of function as an integral part of organ imaging. The functional ability of any organ is a prime indicator of its state of health and is clearly important information for the diagnosis and treatment of systemic abnormalities. To illustrate, the introduction of $^{99m}$Tc-labeled iminodiacetate (IDA) analogues (see Figure 3.1) in 1976 has had a major impact on the diagnostic workup of hepatobiliary diseases because of their dual capacity to provide morphologic and physiologic information simultaneously. Specific diagnosis of various hepatobiliary diseases are made by combining morphologic alterations seen on scintigraphy with the physiologic parameters measured quantitatively.\(^\text{1}\)

### 1.2 Physical Properties of $^{99m}$Tc

Technetium has made a major impact in the diagnosis of human diseases, with over six million diagnostic imaging procedures carried out with $^{99m}$Tc compounds annually in the United States.\(^\text{2}\) In 1960, Richards suggested that $^{99m}$Tc, a metastable radionuclide of technetium, possessed decay properties that were ideally-suited for diagnostic imaging.\(^\text{3}\) Summarized schematically in Figure 1.1, these properties include:

1) A short half-life of 6.0 hours, which dramatically reduces the radiation risk to patients in comparison to most conventional radionuclides, while maintaining excellent statistical counting conditions.

2) A 140 keV $\gamma$-ray energy, which is optimal for detection by scintillation cameras and is poorly absorbed by body tissues. Moreover, there is no particulate radiation.\(^\text{4}\)

3) A relatively stable daughter nuclide, which minimizes long-term hazard from particulate radiation. The parent nuclide, $^{99}$Mo, is also relatively stable ($t_{1/2}$ =
Figure 1.1: The preparation and nuclear decay properties of $^{99m}$Tc. Adapted from ref. 4.

67 hours) and has led to the development of a generator which had made $^{99m}$Tc available on demand at any hospital. The generator consists of $^{99}$Mo adsorbed on an alumina column from which $^{99m}$Tc, in the form of pertechnetate ($^{99m}$TcO$_4^-$), could be eluted with a saline solution.

4) The ability of technetium to accept a relatively wide range of donor groups, which allows for the preparation of a large series of different $^{99m}$Tc-labeled radiopharmaceuticals for use in the varied diagnostic problems encountered in routine clinical practice.\(^5\)

Moreover, these attributes allow the routine administration of doses of 30 mCi which allow high photon-flux levels, facilitating lesion detection by single-photon-emission computerized tomography (SPECT).

A typical labeling procedure consists of mixing $^{99m}$TcO$_4^-$, obtained from the $^{99}$Mo generator, with a reducing agent in the presence of the desired ligand. Various reducing agents include stannous ion, ferric chloride plus ascorbic acid, ferrous ion and sodium borohydride and nonmetallic reducers such as hydrogen iodide, concentrated
HCl or hydrazine. The concentration of the labeled material which is sufficient to produce suitable images is usually at the nanomolar level and therefore has very little or no physiological effect.

1.3 Design of $^{99m}$Tc Radiopharmaceuticals

Although $^{99m}$Tc can be used directly as a radioimaging agent, the vast majority of technetium radiopharmaceuticals are chelates of technetium in lower oxidation states. These radiopharmaceutical complexes can be categorized into three general groups (see Table 1.1). In the first group, technetium-tagged radiopharmaceuticals, $^{99m}$Tc functions exclusively as a radiolabel for large substances, such as proteins, colloids and cells. The binding of the radionuclide to such large systems does not significantly perturb the intrinsic biodistribution of the tagged substance; $^{99m}$Tc simply serves as a convenient label to permit the external monitoring of the biological distribution of the tagged compound. In this tagging procedure, the technetium label is bound by donor groups intrinsic to the macromolecule. These Tc-labeled systems generally are not stable chelates or chemically well-defined and are not amenable to detailed investigation by the techniques of inorganic chemistry.

One particularly promising area under active investigation is the use of $^{99m}$Tc-labeled monoclonal antibodies as agents for the detection of localized disease, such as tumours.

Technetium-essential radiopharmaceuticals - the second class of radiopharmaceuticals - comprises those compounds whose biological distribution is primarily determined by the intrinsic physicochemical properties of the Tc complex and the metal (hence its specific epithet). Because of the small size of the chelating moiety, the biodistribution of the chelate is usually significantly different from the
<table>
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<th>Technetium-tagged Radiopharmaceuticals</th>
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ligand molecule. Although many useful (Tc-essential) radiopharmaceuticals have been found, often serendipitously, the alteration in distribution of the parent molecule has limited the predictive ability in radiopharmaceutical design. 

1.3.1 Bifunctional Radiopharmaceuticals

The third category of Tc radiopharmaceuticals - sometimes considered as a subclass of technetium-tagged radiochemicals - includes Tc complexes of "bifunctional ligands". A bifunctional ligand is a ligand system capable of forming a covalent bond with an appropriate (biologically active) substrate, while retaining the ability to bind a metal, such as $^{99m}$Tc. When the bifunctional ligand forms a metal complex with Tc, it

![Diagram](image)

Figure 1.2: Schematic illustration of a receptor-specific, biologically active molecule labeled with $^{99m}$Tc by the bifunctional approach.

is then referred to as a *bifunctional* (technetium) *chelate* (see Figure 1.2). This method
of labeling molecules is called the \textit{bifunctional approach} and leads to the formation of \textit{bifunctional radiopharmaceuticals}.\textsuperscript{7}

In Tc radiochemistry, this dual-function nature of the bifunctional chelate permits the radiolabeling of an appropriate, tissue-specific biological compound (or "carrier") with the formation of \textit{well-defined} chemical bonds to \textit{both} the biomolecule and radionuclide. The ability of the bifunctional ligand to form these stable bonds is an important property for prospective radiopharmaceuticals because non-specific binding of the radioisotope is minimized, leading to optimal image sensitivity and discrimination. The chelate complex, for this reason, must be able to resist acid or cation-promoted dissociation in the low pH conditions of the stomach and liver, and the high cation concentrations in serum, \textit{e.g.}, $[\text{Ca}^{2+}] = 1.25$ mM, $[\text{Zn}^{2+}] = 10^{-5}$ M.\textsuperscript{9}

Generally, the carrier molecule belongs to one of two categories: 1) drugs which elicit some type of pharmacological response in the target organ (\textit{e.g.}, antiarrhythmic drugs for increasing the force of myocardial contraction in heart therapy or hypoglycemic agents which stimulate insulin release); and 2) metabolic substrates (\textit{e.g.}, long chain fatty acids which are used as an energy source in metabolizing heart muscle). Many biochemicals have well established physiological pathways and a rational prediction of their biodistribution can be stated.\textsuperscript{7}

One major problem in bifunctional radiopharmaceutical design is that the conjugation of the ligand (or the Tc-99m chelate) to the biological molecule must not significantly alter its inherent biodistribution, if concentration of the radiolabeled conjugate in targeted tissues is to be successful. As will be discussed in Section 1.3.2, successful localization can be accomplished if the label is attached to a modifiable site\textsuperscript{7} on the carrier molecule. However, the actual radiometal labeling procedure introduces a rather large, charged "foreign" group into the carrier in comparison to, for example,
radiohalogenation with fluorine or iodine. Because of the size and charge of the labeling group, it is important to determine whether the biological properties of the labeled molecule are significantly altered by the foreign substituent. This question must be addressed for each labeled radiopharmaceutical by in vivo monitoring of its pharmacologic properties and rate of metabolism in comparison to the clinically-accepted standard or the C-14 (or H-3) derivative.\textsuperscript{10} While the bifunctional approach has been shown to be useful for some radionuclides, applications to\textsuperscript{99m}Tc have met only limited success.\textsuperscript{11}

1.3.2 Receptor-Binding Radiotracers

Many drugs and biologically active compounds induce physiological effects in the body when given in relatively low doses. These effects are a result of highly specific tissue localization mechanisms. The primary event in the biological action of many peptides, steroid hormones and drugs is thought to be the specific binding to a particular chemical site on the cell’s plasma membrane or in the cytosol. These cellular sites - that are responsible for initiating a specific biological response from the binding of a guest molecule - are called receptors and are generally proteineous in composition.\textsuperscript{12} Guest substrates may be bound by cellular receptors to participate in metabolism (e.g., glucose in the brain or long chain fatty acids in myocardium) or to take part in a specific ligand-substrate interaction (e.g., enzyme catalysis).\textsuperscript{12} The recognition of an antibody by an antigen is yet another illustration of this receptor-mediated binding interaction.

Nuclear medicine could benefit greatly from the high pharmacological activity and molecular specificity of the receptor-binding mechanism, if this potential could be harnessed to build more effective radiopharmaceuticals. Perhaps the most
critical feature of an agent designed for heart, brain or pancreas imaging could be its ability to localize exclusively in these organs, thereby enabling favorable image contrast against the background. In principle, tissue targeting can be achieved if the radiolabel is judiciously attached to a carrier compound. Receptor-binding would then allow the radioactivity to concentrate in the appropriate tissues. The site of attachment of the radioactive tag to the carrier, however, is crucial for tissue specificity, as determined from structure-activity relationships (SAR).

That part of the drug or biomolecule - which is important for receptor recognition and/or binding - is called the "essential" site (see Figure 1.3). Attachment

![Figure 1.3: Schematic illustration of the binding of a receptor-specific molecule to its cellular receptor.](image)

of the technetium chelate in this region of the molecule will invariably reduce or, more likely, eliminate binding affinity. Conversely, the biomolecule may possess another site
at which structural modification will not significantly alter receptor recognition and binding ability. Consequently, for the development of prospective, site-specific bifunctional radiopharmaceuticals, it is important to conjugate the $^{99m}$Tc complex at this "modifiable" region of the biologically active molecule.

The concept of receptor-specific design - which is based on a prior knowledge of the structural requirements of a target receptor protein - is both simple and rational and may constitute the newest, most economical approach to clinically successful radiopharmaceuticals. Stemming from SAR studies of the pharmacological behavior in animals of a series of close structural analogs, or from in vitro binding studies, reasonably accurate stereochemical models are available for the opiate receptor, the estrogen receptor and the dopaminergic receptor, to name a few. The exploitation of receptor properties for the development of imaging agents has been reviewed.

In addition to receptor-binding effects, localization of radiotracers in target tissues is also a function of physical (e.g., lipid solubility or molecular size) or chemical mechanisms (e.g., phagocytosis, particle entrapment, simple and exchange diffusion, space/pool imaging and plasma protein binding). Heindel has reviewed the development of numerous successful radiopharmaceuticals prepared by applications of receptor-binding mechanisms and has summarized the traditional physical partitioning mechanisms as well.

1.4 Steroids as Receptor-Binding Carriers for Bifunctional $^{99m}$Tc Chelates

Previous work has shown that alkylating agents employed in cancer therapy can be successfully delivered to hormone binding receptors in tumors by conjugating the alkylating agent to steroid compounds. Workers in this field correctly
assumed that the lipophilic nature of the steroid molecule—combined with its lock-and-key specificity in receptor recognition and binding—could penetrate membrane barriers and transport the oncolytic agent to targeted tissues.

Steroidal hormone receptors present in target tissues and receptor-positive tumors formed the basis for the development of \(^{18}\text{F}\)-labeled steroid radiotracers as in vivo positron emission tomography (PET) imaging agents. The utility of \(^{18}\text{F}\)-labeled estrogens for imaging the estrogen receptor-positive breast tumors have been demonstrated.\(^{19}\) Hence, it should be likewise possible to develop analogous \(^{99m}\text{Tc}\)-labeled steroids to image other bodily tissues, since many tissues contain steroidal receptors. Moreover, the substitution of \(^{99m}\text{Tc}\), with its excellent imaging characteristics and ready availability, for \(^{18}\text{F}\) in these compounds would remove the synthetic and practical limitations set by the latter nuclide’s short physical half-life of 111 minutes.

1.5 Objectives of this Thesis

Most approaches taken in the development of Tc-based, radiopharmaceutical imaging agents have been confined to either the tagging of macromolecules or cells, according to "technetium-tagged" methodology, or relatively simple molecules (e.g., ligands), via "technetium-essential" strategies. However, the tagging of intermediate-sized molecules with Tc-99m via bifunctional methodology has not been extensively reported, despite several, significant advantages of such a combined approach:

1) the potential for the specific targeting of judiciously-derivatized, receptor-binding radiopharmaceuticals to tissues traditionally considered to be difficult to image (e.g., the non-excretory organs).
2) the ready availability and optimal radioimaging properties of $^{99m}$Tc.

3) the formation of stable, well-defined bonds to both the radiolabel and carrier molecule. This results in higher tissue to background contrast levels and superior images.

4) the potential for radiopharmaceutical design through a more structured and logical approach (in comparison to Tc-essential methods).

5) an accessibility to a relatively inexpensive pool of physiologically active carrier compounds, such as drugs, oligopeptides and metabolic substrates. Other more complex carriers (such as antibodies) are limited by higher cost and development times.

Using bifunctional methodology, the conjugation of a $^{99m}$Tc chelate with relatively small carrier compounds of known tissue specificity (at non-essential, receptor-binding sites) may result in radiopharmaceuticals of marked localization potentials. Steroids, as a unique class of highly-active yet relatively compact biological molecules, seem especially suited for $^{99m}$Tc tagging since steroid target organs - such as the heart, adrenal, mammary, prostate glands, and, to a lesser extent, organs-of-the hepatobiliary system - are not easily studied by conventional radiographic or nuclear medicine procedures. To date, however, no published effort has been made to harness the tissue-specific, receptor-binding mechanism of steroidal carriers for the production of $^{99m}$Tc-based bifunctional radiopharmaceuticals, despite some reported successes of Tc bifunctional radiopharmaceuticals.\textsuperscript{20,21}

A method for diagnosing areas of myocardial ischemia and infarction would be a major achievement in medicine.\textsuperscript{22} Thus, a major objective of our program was to conjugate a suitable technetium bifunctional ligand (see Chapter 2) to inotropic digitoxigenin and long chain fatty acids (see Chapter 3) for subsequent development as
$^{99m}\text{Tc}$-based myocardial imaging agents. Ligand derivatives of taurine, $\beta$-cyclodextrin and cholic acid were also prepared for development as potential ulcer and gastrointestinal radioimaging agents (see Chapter 4). With the possible exception of the taurine bifunctional conjugate (36), all ligand-carrier conjugates were designed to evaluate organ function through receptor-binding and/or physical localization mechanisms. As these ligand compounds often display dauntingly complex NMR spectral profiles, a major portion of this work was devoted to an analysis and discussion of the underlying physical and chemical properties of the diamide disulfide heterocycle (see Chapters 5 and 6).
CHAPTER 2

SYNTHESIS OF THE DADS-CO$_2$ BIFUNCTIONAL LIGAND

2.1 DADS-CO$_2$ as a Bifunctional Ligand for Technetium

Fritzberg et al. have successfully applied Davison’s diamide disulfur (DADS) ligand system to $^{99m}$Tc labeling of antibody fragments in cancer therapy.$^{23,24}$ Unlike other non-specific metal chelants (e.g., EDTA and DTPA), the DADS ligand was designed specifically to coordinate technetium.$^{25}$ As a result, this N$_2$S$_2$ ligand system forms highly stable.$^{24}$ tetradentate complexes with technetium (i.e., $^{99m}$Tc-N$_2$S$_2$, see Figure 2.1, 5a) in the +5 oxidation state. Studies on a variety of

![Chemical structures](figure2_1.png)

Figure 2.1: $^{99m}$Tc complexes of DADS (5a) and DADS-CO$_2$ (5b).

compounds incorporating the DADS donor group showed that high yields of $^{99m}$Tc complexes of predicted structure were obtained.$^{26,27,28}$ Other potential metal-binding
groups, such as the carboxyl group in DADS-CO$_2$ complex (see Figure 2.1.5b), do not participate in binding$^{29}$.

The above characteristics of the $^{99m}$Tc-DADS-CO$_2$ complex epitomize the salient properties of the ideal bifunctional chelate. Moreover, the DADS ligand system is highly amendable to structural modification$^{30}$ using standard synthetic techniques and commercially-available reagents, and as such, represents an attractive chelant to systematically evaluate structure-activity requirements for the preparation of site-specific radioimaging agents. Consequently, the bifunctional DADS-CO$_2$ ligand was chosen for the development of Tc-based radiopharmaceuticals in this research.

A possible drawback of the DADS ligand system, however, may be traced to an insufficient lipid solubility to permit diffusion of the bifunctional radiopharmaceutical through lipoidal barriers.$^{12}$ In many cases, the target binding site is intracellular and the Tc-99m agent must be lipophilic enough to penetrate the capillary wall and the target cell’s lipid bilayer membrane. The polar diamide functions and (negatively) charged nature of the Tc-DADS complex, may render the radiopharmaceutical too hydrophilic for successful localization to cellular binding sites. As earlier stated, experimental testing is the only unequivocal way to determine the success of tissue targeting. However, the issue of adequate chelate lipophilicity is only expected to be important in the development of the proposed imaging agents for myocardium or other non-excretory organs. Those suggested for gastrointestinal or hepatobiliary imaging would necessarily require a hydrophilic character to achieve a similar localization as seen in their polar parent compounds.

If empirical findings show the DADS-CO$_2$ ligand to be too hydrophilic for development as a myocardial imaging agent (as can be inferred from prominent hepatobiliary excretion$^{31}$), it can be modified to the mercaptoamine (BAT) complexing
system of Kung et al., whose technetium chelates have been found to have sufficient lipid solubility to diffuse through the blood-brain barrier.

2.2 Synthetic Strategies toward the Development of $^{99m}$Tc-DADS-CO$_2$ Bifunctional Radiopharmaceuticals (5c)

A retrosynthetic analysis for the synthesis of a general $^{99m}$Tc-DADS-CO$_2$ bifunctional radiopharmaceutical (5c) is presented in Figure 2.2. In general, the synthetic approach taken in the construction of intermediates was based on amide- or ester-forming condensation reactions. The target radiotracer can be realised from the S-protected derivative 5e, following deprotection and subsequent chelation of Tc-99m. Condensation of a S-protected DADS carboxylic acid (3a) and the appropriate carrier molecule, with the formation of an ester or amide conjugative link, should then afford the conjugate 5e. The most straightforward approach towards the synthesis of the DADS-CO$_2$ bifunctional ligand 3a would be to couple two equivalents of a suitably S-protected thioglycolic acid (2-mercaptoacetic acid) with one equivalent of 2,3-diaminopropanoic acid. The synthesis of the ligand system was subsequently investigated from these commercially-available starting materials.

The synthetic sequence is important to the overall yield of the proposed radiopharmaceutical 5c. For example, the convergent procedure of conjugating ligand 3a and a carrier molecule minimizes the number of synthetic transformations which involves the potentially expensive and reactive carrier. A linear synthesis - where a part of, or the entire ligand framework, is sequentially built onto a carrier template (for example, see ref. 32) - would not be cost or time effective and may not be suitable where the carrier molecule possesses reactive or sensitive functional groups. In addition, a convergent strategy is highly amenable to the speedy assembly of
Figure 2.2: Retrosynthetic analysis for the synthesis of a $^{99m}$Tc-DADS-CO$_2$ bifunctional radiopharmaceutical (5c).
prospective tissue-specific radioimaging agents: upon selection of a suitable carrier, a synthesis of 5c can be completed within three steps (i.e., conjugation, deprotection and chelation). The chelation of Tc-99m as the final step ensures that the radiopharmaceutical preparation of 5c is maximally "carrier-free"; this minimizes the saturation of target tissue sites by technetium conjugates incorporating the ineffective $^{99}\text{Tc}$ radionuclide. This approach contrasts with other bifunctional strategies where conjugation to the carrier takes place after ligand complexation with $^{99}\text{mTc}$.

Protection of the thiol group in thioglycolic acid and its derivatives was deemed necessary since the free functionality would interfere with the planned carboxyl activations in subsequent amide or ester-forming reactions. The choice of the S-protecting group and the method for its cleavage was crucial for the efficiency of the proposed synthesis. Thiol groups are normally protected as aryl thioethers, hemithioacetals or thioesters; however, deprotection frequently requires harsh conditions that can lead to decomposition when sensitive functional groups are present. Moreover, the sulfur products of these acidic or basic deprotection schemes can also engage in side reactions, including acid-catalysed N- to S-acyl transfer initiated by SH attack to neighbouring amide groups and disulfide polymerization via thiolate coupling. In the former case for example, decomposition of C$_{11}$ substituted DADS fatty acid amide conjugates has been noted in our lab during S-detritylation using the CF$_3$COOH/Et$_3$SiH method. This is suspected to proceed via nucleophilic attack of the unmasked SH group to the protonated C=O function at the C$_{11}$ amide, resulting in a six-membered thiolactone and the corresponding 11-amino fatty acid (based on analogy to ref. 36). Thus, the high reactivity of unprotected sulfur atoms - compounded with harsh deprotection methods - make this synthetic strategy less desirable for the development of radioimaging precursors.
As the carrier-ligand conjugate 5c must endure the conditions leading to S-deprotection (see Figure 1.5), we wished to develop a deprotection procedure for sulfur which was exceedingly mild in its operation, compatible with the widest range of carrier and ligand substrates, yet afforded relatively stable sulfur derivatives. We noted that iodine had been used in the removal of trityl groups from sulfur with facile, *in situ* oxidation to disulfides.\(^{37}\) Moreover, disulfides are stable, readily prepared and easy to cleave with mild reductants (for example, Sn(II)) to form the dithiol required for coordination with \(^{99m}\)Tc and, as such, represent attractive precursors to Tc chelants. That is to say, reduction of the primary disulfide group and pertechnetate ion by stannous salts and subsequent complexation to afford 5c can be achieved simultaneously in a one-pot chelation step. This convenient labeling process also attractively lends the disulfide form of ligand conjugate 5e (5d) to "instant kit" formulation. The synthesis was therefore undertaken through the "protected" disulfide conjugate 5d, using trityl as the sulfur protecting agent and iodine as its deprotectant.

Radiolabeling and *in vivo* testing of the carrier-ligand conjugates (*i.e.*, compounds 19, 21, 23, 27, 34, 36 and 38) prepared in this study will be accomplished in co-operation with the Department of Nuclear Medicine at the McMaster University Medical Center.

2.3 Synthesis and Characterization of \(N,N'\)-bis[2-(triphenylmethyl)thioethanoyl]-2,3-diamino propanoic acid (3)

The S-protected DADS-CO\(_2\) bifunctional ligand 3 was prepared according to the synthetic pathway outlined in Scheme 2.1. Tritylation of the SH function of thioglycolic acid using triphenylmethanol and boron trifluoride etherate as a Lewis acid catalyst\(^{35}\) readily afforded the protected acid 1. The carboxyl group was subsequently
thioglycolic acid

Scheme 2.1
activated with N-hydroxysuccinimide (NHS) and 1,3-dicyclohexylcarbodiimide (DCC)\textsuperscript{38} to give the succinimidoyl ester \textit{2}. The physical and spectroscopic properties of intermediates \textit{1} and \textit{2} were entirely consistent with \textit{S}-protection and carbonyl activation (see Sections 7.6.1 and 7.6.2).

Concurrently, ethyl 2,3-diaminopropanoate dihydrochloride (6) was prepared from the commercially-available, parent acid monohydrochloride in refluxing ethanolic HCl under standard Fischer esterification conditions. Ester formation was confirmed by the detection of a new ninhydrin positive spot at \textit{R}_f 0.27 (70\% \textit{n}-PrOH, 28\% \textit{d}H_2\text{O}; 2\% HCOOH), as well as a prominent ester carbonyl stretching absorption at 1745 cm\textsuperscript{-1} in its IR spectrum. In addition, an interesting confirmation of the COO\textsubscript{Et} group was revealed by the 200 MHz \textit{1}H NMR spectrum of 6, where two quartets were noted at \textit{\delta} \sim 4.23 and separated by 0.008 ppm. This separation increased at 500 MHz, suggesting that the observed multiplicity was a result of the almost-perfect superimposition of the two different signals and not a coupling effect. An absorption in the range \textit{\delta} 4.1 - 4.3 was highly diagnostic of a methylene group at the \textit{\alpha}-hydroxyl position of an ethyl ester fragment, while the field-dependency of the quartet shift separation indicated that these \textit{CH\textsubscript{2}} protons were diastereotopic. The observed O-\textit{CH\textsubscript{2}}CH\textsubscript{3} diastereotopicity was presumably a result of the group’s proximity to the C-1 stereocenter (see Section 5.2).\textsuperscript{39} Moreover, the \textit{13}C NMR spectrum of 6 revealed signals at \textit{\delta} 13.75 and \textit{\delta} 62.65, corresponding to the methylene and methyl carbon resonances of the COOCH\textsubscript{2}CH\textsubscript{3} moiety.

Treatment of two equivalents of the \textit{N}-hydroxysuccinimidoyl ester \textit{2} with the diaminoo ester \textit{6} in acetonitrile resulted in the acylation of both nitrogens and the formation of the diamide adduct \textit{7}. As evidence for diamide formation, \textit{\textit{1}H} and \textit{\textit{13}C} NMR spectra showed characteristic amide resonances at 6.26 and 6.82 ppm and 168.48
and 168.69 ppm, respectively. In addition, IR spectroscopy indicated strong peaks in the 1520-1660 cm⁻¹ region, which was characteristic of Amide I and II C=O absorptions. Alternatively, compound 7 was synthesized via DCC-mediated coupling of acid 1 and the diamino ester 6. Although the isolated product from this reaction showed significant contamination by dicyclohexylurea (DCU), higher yields of the diamide acid 3 was obtained by the DCC coupling method, providing that crude 7 was used directly without purification in ion-exchange mediated ester hydrolysis.

The ethyl ester 7 was hydrolyzed to the free acid 3 in 92% yield via catalytic assistance from a macroreticular anion-exchange resin in the hydroxide form and a tetraalkylammonium phase transfer reagent. Spectroscopic examination of the residue, obtained from acidic elution of the resin, indicated a structure that was consistent with the diamide acid 3. ¹H NMR spectroscopy of 3 in deuterated dimethylsulfoxide (DMSO-ᵈ) revealed a doublet and triplet at δ 7.61 and 7.75, corresponding to the C₆O-NH and C₄O-NH amide groups, respectively. Moreover, one S-CH₂ group appeared as an AB quartet (²J = -13.9 Hz) at δ 2.79, while the other pair gave a singlet at δ 2.75 (see Section 5.1 for further details). ¹³C NMR spectra corroborated the ¹H NMR findings, showing two signals in the carbonyl amide region (δ 169.28 and δ 170.24). The magnetic non-equivalence of the S-CH₂ groups was also apparent from the closely-separated pair of signals at δ 35.66 and δ 35.84. Positive fast atom bombardment mass spectroscopy (FAB-MS) confirmed a M+1 ion at m/z 737.

2.3.1 Anion-Exchange Chromatography as a Novel Hydrolysis and Purification Method

The macroreticular anion-exchange method of hydrolysis also doubled as an effective purification scheme for the removal of non-anionic impurities, particularly the
troublesome DCU by-product of previous DCC-mediated coupling reactions (see Figure 2.3). These contaminants and the phase-transfer catalyst were easily removed by washing the carboxylate resin with two column volumes of ethanol and acetone. Elution of the free acid from the resin with a weakly acidic solution of aqueous HCl in acetone furnished 3 in high yield after work-up.

Curiously, the activity of the hydroxide form of the ion-exchange resin was found to decrease with time when stored in ethanol. Most notably, a distinctive amine odour was also detected in old preparations, which often required a preliminary "airing" period in a fume-hood after opening the resin storage container. This perplexing observation was rationalized in terms of a first order decomposition of the resin as indicated in Scheme 2.2.

![Scheme 2.2](image)

This suggested decomposition of the resin was based on similar reactions involving trimethylalkylammonium salts and hydroxide ion.\(^{40}\) Note that the volatile trimethylamine by-product can serve to account for the amine smell of old resin preparations. Consequently, with these observations in mind, prewashed resin in the chloride form was converted to the hydroxide form *just prior* to use in ester hydrolysis.

### 2.3.2 Difficulties Encountered in the Synthesis of 3

Initial strategies toward the synthesis of the diamide 3 were aimed at coupling the protected acid 1 directly with the unesterified diamino acid *via* the
Figure 2.3: Hydrolysis of ester R-COOR' and purification of the resulting acid R-COOH from non-anionic impurities. Aliquat 336 = tricaprylylmethylammonium chloride.
carbonyldiimidazole (CDI)\textsuperscript{41,42} and NHS activation of 1.\textsuperscript{35} Although this synthetic plan had the appeal of avoiding cumbersome esterification and hydrolysis steps (as in Scheme 2.1), numerous attempts in this area failed to afford good yields of the diamide. The poor yields were believed to have resulted from the solvolysis of acid-activated intermediates of 1 by the protic solvent components that were required to ensure dissolution of the highly-polar 2,3-diaminopropanoic acid reactant. Protic solvents were necessary components of the reaction mixture since the diamino acid defied solubility in neat aprotic matrices and it was found to be taken up only in water-NaHCO\textsubscript{3} or methanol-NEt\textsubscript{3} mixtures.

Clearly, an aprotic solvent is preferred to a protic one in order to avoid solvolysis of the reactive acyl intermediates generated for subsequent amide formation. It was anticipated, however, that the 10\textsuperscript{6}-fold greater nucleophilicity of the amine relative to water\textsuperscript{43} would ensure faster aminolysis rates and acceptable yields of the desired amide, particularly if a slight excess of CDI reagent was used. Thus, several CDI-mediated condensations were attempted in protic solvent mixtures, typically by adding a mildly-basic, aqueous solution of the amino acid to a solution of the activated imidazolide (1a, Scheme 3.6) in aprotic solvents such as tetrahydrofuran (THF), dimethoxyethane (DME), acetonitrile (AN) and DMSO. Analysis of the reaction mixtures disclosed the incomplete formation of the diamide (3), with reactants comprising the major components. The recovery of starting material from these protic solvent systems hinted that the rate of hydrolysis of the acyl imidazolide intermediate was comparable to that for aminolysis. Clearly, the high moisture-sensitivity of the reactive acyl imidazolide 1a precluded its utility as an efficient coupling reagent in aqueous environments.

The search for an activated acid intermediate of 1 that was sufficiently inert
to solvolysis in the protic environment led to the preparation of the \( N \)-hydroxysuccinimidyld ester of \( 1 \) \(^{25} \) and its subsequent coupling to 2,3-diaminopropanoic acid in dimethylformamide (DMF)-water mixtures. Although improved yields of \( 3 \) were noted relative to the CDI method, regrettably, 20-30% of the activated ester hydrolysis product, \( 1 \), was detected by TLC. This low amide yield arose from the incomplete solubility of the amino acid nucleophile and/or the activated ester in the final reaction mixture. For example, it was possible that the diamino acid formed polar aggregates in the binary solvent, which reduced the effective concentration of the diamine in solution and, ultimately, product yield. Subsequent trials, therefore, involved the substitution of NaHCO\(_3\)-water with NEt\(_3\)-methanol solvent mixtures to ensure homogeneity and a reduction in the solvolysis rate of the activated ester. Although TLC indicated the formation of \( 3 \) \([R_f\ 0.22, 10\% \) MeOH in methylene chloride (CH\(_2\)Cl\(_2\))] and the disappearance of the succinimidoyl ester \( 2 \) \([R_f\ 0.92]\), an estimated 30% of the reaction mixture was contaminated by the methyl ester adduct, Tr-S-CH\(_2\)-COOMe.

Coupling of 2,3-diaminopropanic acid ·HCl and \( 1 \) was also attempted using the water soluble EDC [1-(3-dimethylaminopropyl)-3-ethylcarbodiimide ·HCl] coupling reagent.\(^{44,45} \) In this reaction, a solution of the amino acid in distilled water (dH\(_2\)O) and triethylamine (NEt\(_3\)) was added to a stirred solution of \( 1 \) and EDC in DME after a 10-minute activation period. Turbidity was induced upon slow addition of the amino acid solution. TLC of the reaction mixture after 18 hours revealed starting materials as the major products.

The poor yield of \( 3 \), possibly due to the lack of a suitable aprotic solvent for the diamine and activated ester reagents, prompted the development of ester derivatives of 2,3-diaminopropanoic acid to enhance its lipophilicity in these solvents.
Accordingly, methyl and ethyl esters were prepared and they were found to dissolve in DME and AN, enabling amide coupling to take place in either of these low-boiling, aprotic solvents in the complete absence of water or methanol. As expected, solvolysis products of the activated ester were not observed. Owing to its greater solubility in aprotic solvents, the ethyl ester 6 was employed for the synthesis of 3, as depicted in Scheme 2.1.

2.4 Proposed Mechanism of S-Detritylation and Oxidative Cyclization

S-Detritylation of protected ligand precursors employing 1.1 equivalents of I$_2$ - or 2.1 equivalents in the case of the S-trityl cyclodextrin conjugate 37 (see Section 4.4.4) - occurred quantitatively with concomitant cyclization to the intramolecular disulfide. Though the mechanism of this curious two-step, one-pot reaction has not been reported to our knowledge, it most likely progresses via a sulfenyl iodide intermediate,$^{46,47}$ with the reaction’s driving force hinging on the high resonance stability of the departing trityl cation in the transition state. Two possible pathways for this detritylation-cyclization process are presented in Figure 2.4, with iodine or hydrogen iodide concentrations determining the direction of the reaction paths.

During the initial stages of deprotection when the concentration of iodine is high and that of HI low, Pathway A is favored. In this scheme, the first elementary step involves a donor-acceptor interaction between two iodine molecules and trityl sulfide sulfur atoms, leading to the formation of a 1:1 charge transfer (CT) complex. Experiments have shown that aliphatic sulfides and iodine form strong complexes in solution and in the gas phase. Tideswell and McCullough$^{48}$ have measured the equilibrium constant for complexation, $K_{complexation}$, at 20 °C in CCl$_4$ to be 71 L.mol$^{-1}$ for dimethylsulfide and iodine. In a separate study, Tsubomura and Lang$^{49}$ have
Figure 2.4: Proposed pathways for the deprotection and oxidation of S-trityl ligands with I₂. R = H or CH₃; R' = H or CO-NH(O)-Carrier.
reported \( K_{\text{complexation}}^{20^\circ C} = 210 \text{ L.mol}^{-1} \) for the diethylsulfide-I₂ CT complex. As the differences in these values apparently reflect the electron releasing abilities of the S-alkyl groups and the induced, effective charge on the S atom, a large estimate of \( K_{\text{complexation}}^{20^\circ C} \) can be inferred for the \( \text{CH}_2\text{-S-Tr} \) substructure of protected ligand intermediates. The essence of this comparison is to note that complexation between iodine and the S-protected ligands is highly favored and that both sulfur atoms are likely to complex with iodine molecules and form CT adducts during the early stages of the reaction.

Unlike disubstituted aliphatic sulfide-I₂ complexes (which do not undergo further reaction), the Tr-S-I₂ activated complex readily loses trityl iodide and stabilizes to a diiodosulfenyl intermediate. The driving force for this reaction is presumably linked to the high resonance stability of trityl cation in the transition state. The existence of sulfenyl iodides is well-established\(^{46,47,50}\) and they are the accepted intermediates in the oxidation of organic divalent sulfur by iodine.\(^{50}\)

The conversion of the diiodosulfenyl intermediate to the intramolecular disulfide may proceed through the formation of pentacovalent sulfur intermediate (I), initiated by intramolecular attack of the second S-I function in a S₅N₂ fashion.

\[
\begin{align*}
\text{I} & \quad \text{II} \\
\begin{array}{c}
\text{I} \\
\text{II}
\end{array}
\end{align*}
\]

However, as Ciuffarin and Guaraldi have noted in their work on nucleophilic substitution reactions involving sulfenyl halides and \( n \)-butylamine, sulfur-sulfur coupling through an ion-pair intermediate (II) cannot be ruled out.\(^{51}\) Finally, iodide
combines with iodonium ion to form neutral iodine which can be recycled into Pathway A (or B) to continue the deprotection sequence. Thus, for every two iodine molecules consumed per protected ligand molecule, one molecule is regenerated.

As the reaction proceeds as depicted by Pathway A, the concentration of I₂ drops and that of HI increases through ethanalysis of trityl iodide. In this concentration regime, HI molecules begin to compete with I₂ for basic sulfide sites on the protected ligand via Pathway B. If the concentration of I₂ is sufficiently low, then protonation of both sulfur atoms by HI can occur to afford a dihydroiodide salt intermediate. This S-protonated species may plausibly be stabilized through heterolytic cleavage of the S-trityl bond with the formation of trityl iodide and a dithiol. Subsequently, a SH function of the dithiol can displace iodide from an iodine molecule to generate a thiol-sulfenyl iodide. As in Pathway A, cyclization can occur through displacement of I⁻ by SH attack - by either a SN₂ or ion-pair mechanism - to form the 10-membered disulfide heterocycle and HI.

2.5 Oxidation of the Iodosulfenyl Intermediate of 7 by O₂

Test reactions aimed at deprotecting and oxidizing the S-trityl ether 7 under standard reaction conditions led to the observation of small quantities of M+16 and M+32 ions in the mass spectrum of the products. It would seem that the iodosulfenyl and/or thiol intermediates are sensitive to overoxidation by dissolved oxygen, leading to the formation of thiosulfenates and higher oxidized analogues.⁵² To overcome this side reaction, any reaction mixture containing 7 was degassed for 30 minutes at 15 mm Hg, both before and after the addition of I₂ crystals. This procedure virtually eliminated all product contamination by higher oxidation products. Apart from MS analysis, no attempt was made to characterize or isolate the oxidized products of 7.
CHAPTER 3

SYNTHESIS OF MYOCARDIAL IMAGING AGENTS

3.1 General Introduction

Myocardial perfusion radioimaging with thallium-201 (\(^{201}\)Tl or thallous ion) has become a routine part of the clinical evaluation of patients with coronary artery disease (CAD). While the initial distribution of \(^{201}\)Tl reflects local blood flow (i.e., perfusion), it is not the ideal radiotracer. Rapid serial evaluation of perfusion is not possible because of its long physical half-life (73 hours) and slow myocardial clearance. Its long \(t_{1/2}\) also prevents the use of high injected activities. Image quality is marginal because of its low energy (X-ray) emission which leads to tissue attenuation. Furthermore, it is expensive and its myocardial distribution reflects flow for only a short time after stress because of redistribution.\(^{53}\) These drawbacks can be avoided through a Tc-99m based myocardial imaging agent, since Tc-99m is widely regarded as the optimal emitter for diagnostic imaging applications. Owing to its excellent physical decay properties and its ready availability from \(^{99}\)Mo-\(^{99m}\)Tc generators (see Section 1.2), \(^{99m}\)Tc is the radioisotope of choice for myocardial imaging. Strauss and Pfitz\(^{54}\) have hypothesized that, in relation to \(^{201}\)Tl, a \(^{99m}\)Tc agent would offer a two-fold increase in resolution and allow a four-fold increase in the dose that could be administered. Thus, the development of a routine myocardial perfusion agent based on Tc-99m is an important - albeit to date elusive - goal of cardiovascular nuclear medicine.\(^{55}\)
Several new $^{99m}$Tc complexes for regional myocardial perfusion imaging have been reported. These agents are prospective replacements for $^{201}$Tl as the major radiopharmaceutical for routine nuclear medicine application. The $^{99m}$Tc isonitrile cations (see Figure 3.1), initially developed by Jones and Davison, have reached the final stage of clinical trial and possibly represent the best candidates for the replacement of $^{201}$Tl imaging. The first agent in this series, TBI ($^{99m}$Tc-$t$-butylisonitrile), showed pronounced uptake and retention reflecting regional perfusion. However, its initial lung uptake and subsequent high liver retention clearly indicates the need for further improvement. A new generation of isonitriles, TMIBI ($^{99m}$Tc-2-methoxyisobutylisonitrile) and TCPI ($^{99m}$Tc-2-carboxypropylisonitrile), showed improved liver and lung washout but with the same myocardial uptake and prolonged retention as TBI. TMIBI or "Sestamibi" was chosen for further development and is currently in the advanced stages of clinical trials. Boronic acid adducts of technetium dioximes (BATO) have also been reported. Initial clinical study has indicated that they may be useful as myocardial perfusion tracers. Another group of $^{99m}$Tc-(arene)$_2$ compounds, which are "sandwich" complexes, was reported and initial studies in animal models showed very high myocardial uptake and prolonged retention. Among all of these potential $^{99m}$Tc imaging agents for the heart, TMIBI and CDO-MeB (a neutrally-charged BATO derivative, see Figure 3.2) have been extensively tested in humans and are well on their way to commercialization under the trade names Cardiolite$^\text{®}$ (NEN-Dupont, N. Billerica, MA) and Cardiotec$^\text{®}$ (Squibb, New Brunswick, NJ), respectively.

3.2 Pharmacology of Some Cationic Imaging Agents

The idea of investigating $^{201}$Tl and cationic $^{99m}$Tc isonitriles to localize in
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<th>R</th>
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<tr>
<td></td>
<td>Tc-hexakis-methoxy isobutyl isonitrile</td>
<td>TMIBI (Tc-sostamibi)</td>
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<td></td>
<td>Tc-hexakis-carboxy propyl isonitrile</td>
<td>TCPI</td>
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<td></td>
<td>Tc-hexakis-tertiary butyl isonitrile</td>
<td>TBI</td>
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![Diagram](image_url)

Figure 3.1: Cationic $^{99m}$Tc isonitriles.
the heart in proportion to myocardial blood flow used as the operating principle the observation that many cations have an affinity for the heart. However, studies involving isolated myocytes to explore uptake mechanisms showed that the extraction of thallous ion occurs through the \( \text{Na}^+\text{,K}^+\)-ATPase pump, while that of TCPI and TMIBI resulted through an unknown means. Further tests showed that the uptake of the isonitriles could not be reduced by ouabin inhibition of \( \text{Na}^+\text{,K}^+\)-ATPase; they were mainly associated with the cell membrane. Though these two agents go to the myocardium and remain there for a considerable period of time, the distribution of \( ^{201}\text{Tl} \) constantly changes as it moves toward equilibrium, whereas that of TMIBI is reported to be essentially fixed. Nevertheless, with the actual mechanism for myocardial uptake aside, it is abundantly clear from animal and human imaging studies that thallous ion, the \( ^{99m}\text{Tc} \) isonitriles and the neutral BATO complexes behave as probes for myocardial perfusion.

The concept of heart imaging using "perfusion" agents is based on
measurements of vessel blood flow, which is not necessarily equivalent to tissue perfusion or blood flow through myocardial tissue. Ideally, a measure of the metabolic state of the tissue is required, as this information would furnish directly the functional ability of the heart, as in ischemia. However, at present, no $^{99m}$Tc myocardial agents have been developed as probes of cardiac metabolism. The objective of this research, in part, is to fill this medical need through the development of prospective, $^{99m}$Tc-based imaging agents to assess myocardial function via metabolic uptake mechanisms.

3.3 $^{99m}$Tc Fatty acid-based Agents for Myocardial Imaging

3.3.1 Introduction

Nonesterified, long-chain fatty acids (C$_{14}$-C$_{18}$) serve as an important fuel for normal functioning heart muscle. Evans and associates$^{68}$ proposed that regions of the myocardium with impaired metabolism or diminished arterial blood flow might be imageable through the use of radioiodinated oleic acid. Bilheimer et al. have reported that injection of $^{[11C]}$oleic acid into dogs inflicted with acute myocardial infarction induced localization of the radioactivity in the peripheral and border zones of the lesion.$^{69}$ Reduced uptake of $\gamma$-emitting, radiolabeled fatty acids in ischemic or infarcted myocardium was later demonstrated with $^{[131I]}$oleic acid,$^{70,71,72}$ 16-$^{[123I]}$iodo-9-hexadecenoic acid,$^{73}$ 17-$^{[123I]}$iodoheptadecanoic acid$^{74}$ and 1-$^{[11C]}$palmitic acid.$^{75}$

In patients with CAD, ischemic regions may be clearly recognized in myocardial scintigrams as photo-deficient areas because of the lack of tracer accumulation. A decrease in coronary perfusion is accompanied by a marked diminution of myocardial fatty acid uptake. This decreased uptake is not solely an
expression of decreased delivery of fatty acid due to a decreased blood flow: in ischemic myocardium, there is also evidence to suggest that the extraction rate of the fatty acid is reduced. These effects combine to enhance the effect of reduced perfusion. Thus, in contrast to the situation with glucose, where a decrease of delivery is partially offset by an increase of the extraction rate, myocardial fatty acid uptake has to be regarded as a very sensitive index of myocardial ischemia.\textsuperscript{76} These findings demonstrate the potential of radiolabeled fatty acids as metabolic probes of myocardium, specifically as agents for the detection of infarcted or ischemic areas.

However, the promising radioimaging ability of the above iodinated fatty acids are offset by a number of limitations. The \textit{in vivo} stability of radiiodinating compounds is limited because of dehalogenating enzymes which increase blood activity levels by the release of radioiodide.\textsuperscript{67,77} Moreover, \textsuperscript{131}I has poor physical decay properties for imaging with a \(\gamma\)-scintillation camera, while \textsuperscript{123}I is expensive and not readily available. The short, 20-minute half-life and positron emission (yielding a penetrating \(\gamma\) radiation of 511 keV) of the \textsuperscript{11}C radioisotope restricts \textsuperscript{11}C-labeled imaging agents to medical institutions having cyclotrons and positron cameras.\textsuperscript{67}

The development of a \textsuperscript{99m}Tc-based fatty acid imaging agent - which, like its radiiodinated counterparts, accumulates in proportion to local metabolic activity in impaired myocardium - would be an important complement to existing myocardial perfusion agents. Some researchers have succeeded in developing \textsuperscript{99m}Tc-labeled fatty acids, only to be halted by modest myocardial localization. Livini \textit{et al.} labeled mercapto derivatives of palmitic acid with \textsuperscript{99m}Tc.\textsuperscript{69} A similar approach was attempted by Lobberg and co-workers,\textsuperscript{78} who labeled undecanoic acid derivatives using iminodiacetate (IDA) as the chelating group. In both cases, the myocardial uptakes of these radiochelates were lower than that for \(^3\text{H}\)palmitic acid. This difference was
thought to be due to the formation of high molecular weight, 2:1 fatty acid-technetium complexes and/or the lower lipophilicity of the $^{99m}$Tc-labeled compounds.\textsuperscript{5,67,69,78}

The development of a fatty acid tracer which contains the tetradentate DADS chelating system should result in a 1:1 fatty acid-technetium complex with a higher myocardial localization ability. Moreover, the DADS ligand is structurally amenable to modification and, therefore, represents an attractive chelant system to probe systematically for the optimal lipophilic requirements to achieve good heart localization. Thus, some long chain fatty acids were subsequently modified to accommodate the DADS-CO$_2$ bifunctional ligand.

3.3.2 Synthesis and Characterization of Fatty Acid Ligands

A C$_{11}$ fatty acid DADS ligand (27) was prepared as outlined in Scheme 3.1. The amine methyl ester hydrochloride 24 was prepared from commercially-available 11-aminoundecanoic acid in 98% yield under conditions of typical Fischer esterification. Proton and $^{13}$C NMR confirmed COOH$_3$ resonances at 3.55 and 51.06 ppm, respectively. IR analysis also revealed a strong, presumably H-bonded, carbonyl absorption at 1720 cm$^{-1}$. This fatty ester amine was subsequently coupled with acid 3 using DCC activation to furnish the amide 25 in 93% yield. Amide formation was verified by NMR and IR analyses. The H-11 multiplet had shifted from 2.68 ppm in 24 to 3.08 ppm in the product, while the appearance of a triplet at 6.64 ppm was indicative of the newly-formed amide proton at C$_1$. The corresponding carbon signals were noted in the $^{13}$C NMR spectrum: a paramagnetic shift of the C$_{11}$ resonance to 39.66 ppm and the appearance of the C$_1$ signal in the carbonyl region. Absorbances due to the aromatic groups in 25 could also be noted in NMR and IR spectra. Deprotection and oxidative cyclization to the intramolecular disulfide 26 proceeded in 37% yield.
Scheme 3.1
Cyclization was inferred from the marked broadness of the proton and carbon signals of the ligand in its NMR spectra. This phenomenon was rationalized in terms of slow chemical exchange resulting from ring isomerization and is a characteristic NMR property in these diimide disulfur cycles (see Section 5.3) using CDCl₃ as a solvent. The absence of trityl absorbances from NMR and IR spectra provided additional evidence for successful deprotection. When characterization of 26 was not necessary, as in post-trial syntheses, the disulfide conjugate and impurities were co-isolated by extraction and this crude mixture was used directly for the following hydrolysis. This procedure avoided an excessive loss of the amphiphilic fatty ester disulfide 26 through purification and improved the overall yield of 27.

Ester hydrolysis of 26 to afford the free acid 27 was mediated by a macroreticular anion-exchange resin in the OH⁻ form (see Section 2.3). This novel hydrolytic procedure also doubled as an effective method for the purification of the polar fatty acid product from Tr-OH, Tr-OEt, DCU and other non-anionic impurities generated in previous steps. IR, ¹H and ¹³C NMR analyses of the resin-isolated solid indicated the absence of the methyl ester function at 1740 cm⁻¹, δ 3.6 and δ 51.42, respectively. Moreover, the complexity of the ligand resonances in DMSO-δ₆ (see Section 5.3) suggested that the cyclic ligand structure of 27 was intact and not affected by hydrolysis.

The synthesis of the C₁₆ fatty acid DADS ligand 34 is outlined in Scheme 3.1. This synthesis was similar to that for the C₁₁ fatty acid ligand 27, with a notable exception: the C₁₆ hydroxyl group of the 16-hydroxypalmitic acid starting material must be transformed into the NH₂ function to enable amide conjugation with acid 3. Ligand-fatty acid conjugation via a C₁₆ ester link was considered (to avoid a multistep OH → NH₂ transformation) but the selective ester hydrolysis of the terminal COOCH₃
function in the presence of an internal conjugative ester bond was reasoned to be too
difficult. The most straightforward route to the C_{16} amino fatty acid from the
OH-substituted derivative would involve tosylation of the hydroxyl group,
displacement of the resultant O-tosylate by azide ion and subsequent reduction to
furnish the amine. This approach was adopted to carry out the OH → NH\textsubscript{2}
manipulation.

Commercially-available 16-hydroxypalmitic acid was converted to its
methyl ester 28 in 96% yield under relatively dilute (3.3 mM) Fischer esterification
conditions. TLC using a 10% MeOH in CH\textsubscript{2}Cl\textsubscript{2} mobile phase showed the appearance
of a higher-running component at \( R_f \) 0.64 and the disappearance of the free acid at \( R_f \)
0.39. Spectroscopic analysis of the product was also consistent with the formation of
28, particularly \(^1\text{H} \) and \(^{13}\text{C} \) NMR, which showed the new COOCH\textsubscript{3} resonances at \( \delta \)
3.66 and \( \delta \) 51.40, respectively. Higher concentrations of the hydroxy fatty acid in
methanolic HCl afforded significant amounts of the intermolecular ester,
OH-(CH\textsubscript{2})\textsubscript{15}-COO-(CH\textsubscript{2})\textsubscript{15}COOCH\textsubscript{3}. This product was characterized by TLC, where it
eluted at \( R_f \) 0.73 (10% MeOH in CH\textsubscript{2}Cl\textsubscript{2}), and \(^1\text{H} \) NMR, where it showed a
characteristic COOCH\textsubscript{2} triplet at 4.1 ppm. Its formation was also favored via
transesterification during work-up, if prior removal of dissolved HCl gas from the
methanolic reaction mixture was not performed on a rotary evaporator at \( \text{ca. } 0 ^\circ\text{C} \).

The reaction of tosyl chloride and hydroxy-ester 28 in pyridine to afford
tosylate 29 was complicated by the unexpected formation of by-products. TLC of the
reaction mixture using a 15% EtOAc in hexanes mobile phase revealed three new
components: \( R_f \) 0.0, 0.28 and 0.54, with the former two spots possessing a UV
chromophore. Purification of the worked-up sample by column chromatography and
subsequent NMR and IR spectroscopy of each constituent suggested that the UV
active, \( R_f 0.28 \) component was the desired tosylate 29. A downfield shift of the H-16 triplet from \( \delta 3.60 \) in the hydroxy ester 28 to \( \delta 3.99 \) in the new component was observed, which was consistent with the substitution of the hydroxyl function with the strongly electron withdrawing tosylate group. In contrast, for the UV inactive component at \( R_f 0.54 \), the H-16 protons had moved slightly upfield to 3.50 ppm. In conjunction with its chromatographic properties and the absence of tosyl resonances in the \(^1\)H NMR spectrum, this unknown was most likely the 16-bis ether 29c, formed from the \( S_N2 \) displacement of the tosylate 29 by alcohol 28 (see Figure 3.3). \(^1\)H NMR spectroscopy of the UV-absorbing material at \( R_f 0.0 \) revealed a spectral profile which was remarkably similar to those for quaternerized aliphatic pyridinium salts (such as cetylpyridinium chloride monohydrate\(^{79} \)) sharing characteristic \( N^+\cdot CH_2 \) resonances at ca. 4.8 ppm. Consequently, this component was characterized as the fatty ester-pyridinium adduct 29b. A comparison of H-16 proton integrals for an unpurified sample from the tosylation reaction indicated that the desired tosylate 29 was produced in about 67% yield, while the by-product bis ether 29c and pyridinium salt 29b were formed in 14% and 19%, respectively.

Recognizing the identities of these by-products and that they arose from competing, bimolecular side reactions suggested that the order of reagent addition and concentrations may be useful in optimizing the yield of tosylate. For example, the slow addition of alcohol 28 to a concentrated (1 M) solution of tosyl chloride in pyridine - but ensuring a relatively dilute concentration in alcohol - should favor the reaction kinetics for 16-\( O \)-tosylation while minimizing formation of the bimolecular bis ether. In addition, immediate quenching upon completion of reaction should also minimize the amount of solvolysis product 29b. Regrettably, a few, judiciously-quenched trial reactions - incorporating a constant 1 M concentration of tosyl chloride but a variable
Figure 3.3: Suggested pathway for the formation of tosylate 29, pyridinium fatty ester salt 29b and 16-his ether 29c.
one in the alcohol 28 - indicated no significant reduction of either the ether or alkylpyridinium contaminants.

The displacement of tosylate in 29 by azide ion in DMF readily afforded the low melting, 16-azido ester 30 in 98% yield. A prominent N\textsubscript{2} IR stretch at 2090 cm\textsuperscript{-1} and the \textsuperscript{1}H NMR H-16 triplet at 3.24 ppm assisted in the characterization of this compound.

The azide 30 was subsequently reduced to the primary amine and isolated as its hydrochloride salt 31 by two reduction methods: catalytic hydrogenation (with H\textsubscript{2} gas as the hydrogen source) and catalytic transfer hydrogenation\textsuperscript{80} (with ammonium formate as the hydrogen source). The former, classical reductive method gave a higher yield than the latter, presumably because of the simpler work-up. Evidence of the amine functionality was noted by a ninhydrin positive spot at $R_f$ 0.30 (20% MeOH in CHCl\textsubscript{3}), as well as in \textsuperscript{1}H and \textsuperscript{13}C NMR spectra where the H-16 and C\textsubscript{16} resonances appeared at 2.72 and 40.03 ppm, respectively. Moreover, the positive FAB-MS spectrum showed an intense ion at $m/z$ 286, corresponding to the M+1-HCl structure.

The isolation of amine 31 as its hydrochloride salt avoided the formation of polymeric amides which is possible with the free base (31b, Scheme 3.2).\textsuperscript{81}

Conjugation of the amine fatty ester 31 and the ligand acid 3 using DCC as the condensing agent afforded the amide 32 in 76% yield. \textsuperscript{1}H NMR showed a downfield shift of the H-16 triplet from $\delta$ 2.72 in 31 to $\delta$ 3.16 in the amide product and the appearance of the C\textsubscript{1} amide proton triplet at $\delta$ 6.82. Deprotection and oxidative cyclization to the disulfide cycle 33 occurred in 43% yield. Characterization of this compound was similar to that given for 26, with the exception of a M+1 peak at $m/z$ 518 in the positive NH\textsubscript{3} DCI spectrum.

Methyl ester hydrolysis of 33 to the free acid 34 was accomplished via the
macrocyclic anion-exchange method (Section 2.3) and the product acid was characterized in a similar manner as described for 26.

3.3.3 Requirement for Carboxyl Protection in Fatty Acids

Initial strategies aimed at the synthesis of fatty acid-ligand conjugate 25 entailed the direct N-acylation of 11-aminoundecanoic acid with acid 3. This direct amide coupling method is synthetically attractive in that it avoids the cumbersome protection and deprotection of the carboxylic acid function in the manner illustrated in Scheme 3.1. Thus, in a trial reaction, a slightly-basic solution of 11-aminoundecanoic acid .1HCl was slowly added to a solution of acid 1 and DCC after a 10-minute activation period. TLC of the reaction mixture indicated a mixture of at least five new components, the major one being unreacted acid 1. Although no attempt was made to isolate and characterize these constituents, secondary reactions involving the carboxylate anion of the fatty acid was strongly suspected, given that quantitative yields of amide 25 were obtained when the COOH function of the aminoundecyl nucleophile was protected as its methyl ester (see Section 7.6.28). Therefore, the
presence of these unknown components may be inferred from side reactions involving the following intermediates of 11-aminoundecanoic acid:

\[
\begin{align*}
\text{24a} & \quad \text{24b} \\
\begin{array}{c}
\text{O} \\
\text{O} \\
\text{Tr-S-CH}_2\text{-C-O-C-(CH}_2\text{)}_{10}\text{-NH}_2 \\
\text{R-N-C-O-C-(CH}_2\text{)}_{10}\text{-NH}_2
\end{array} & \\
\text{R} & = \text{C}_6\text{H}_{11}
\end{align*}
\]

Regardless of the mechanistic outcome however, the formation of by-products most likely resulted from reactions of the free carboxylate group of the 11-aminoundecanoic acid nucleophile with DCC. Therefore, protection of the COOH function of the amino acid was carried out as the corresponding methyl ester. An advantage of an ester protecting group was the facility for the purification of the target disulfide acid 27 from "sticky", ambiphilic impurities (e.g., Tr-OEt, Tr-OH and DCU), using the established hydrolysis-separation procedure of the macroreticular anion-exchange resin (see Scheme 2.3).

3.4 A \textsuperscript{99m}Tc Digitoxigenin-based Agent for Myocardial Imaging

3.4.1 Introduction

Cardenolides, otherwise known as cardiac glycosides, are found in several families of plants - most notably the genus \textit{Digitalis} - and comprise an outstanding class of drugs. Digitalis, a preparation made by the extraction of dried seeds and leaves of the common foxglove (\textit{Digitalis purpurea}), is used extensively in heart therapy to cure cardiac failure and to slow the ventricular rate in atrial fibrillation and flutter. The most important pharmacodynamic property of Digitalis, however, is the ability to increase the force of myocardial contraction.\textsuperscript{82}
The active components of *Digitalis* extract are the glycosides of digitoxigenin, digoxigenin and gitoxigenin (see Figure 3.4). Qualitatively, the physiological activity of these cardiac glycosides resides in the steroidal or aglycone component (see Figure 3.4), rather than in the carbohydrate residue. Among the most widely studied aglycones is digitoxigenin [3β, 14β-dihydroxy-5β-card-20(22)-enolide], which is obtained by acid hydrolysis of the glycoside, digitoxin. Digitoxigenin may be considered to possess the minimum structural requirements for cardiotonic activity. Its structure is unique among other classes of steroids, in that both the A/B and C/D ring fusions are *cis*. This geometry places the β or "upward" hydroxyl group at C3 in the axial orientation. The C14 OH group has, likewise, the β configuration. A change in
configuration at C₅ creates a trans A/B fusion and results in a sharp diminution in cardiotonic activity. Epimerization at C₃ (to 3-epidigoxigenin), loss of the C₁₄ hydroxyl group, or saturation of the C₁₇ unsaturated lactone group also leads to a loss in activity.⁸³

3.4.2 Na⁺, K⁺-ATPase as the Digitalis Receptor

Though the mechanistic basis of the inotropic effects of cardiotonic steroids is still uncertain, there is considerable evidence to support the theory that these effects result from the inhibition of myocardial Na⁺, K⁺-ATPase.⁸⁴,⁸⁵ This enzyme is an integral component of the pumping mechanism which transports Na⁺ and K⁺ ions across cell membranes against the natural electrochemical gradient. One important body of evidence which correlates myocardial Na⁺, K⁺-ATPase with the active transport of Na⁺ and K⁺ and with the effects of cardiac glycosides on heart muscle contractility is the analysis of structure-activity relationships (SAR): a persistent connection exists between cardiotonic activity and the inhibition of both Na⁺, K⁺-ATPase and the active transport of Na⁺ and K⁺ ions by cardiac glycosides.⁸⁵ Irrespective of the role of Na⁺, K⁺-ATPase in the mediation of effects on myocardial contractility, the enzyme contains a receptor for Digitalis and allied compounds.

The idea for the development of a myocardial, ⁹⁹ᵐTe-based radioimaging agent was spawned from the high binding affinity of cardiac glycosides to Na⁺, K⁺-ATPase. Although this enzyme is widely distributed in vivo, digitalis glycosides have been shown in distribution studies to concentrate specifically in cardiac tissue at levels 15 to 30 times those in the plasma and over double the quantities found in skeletal muscle.⁸⁶ The marked difference in the distribution between the heart and other areas suggests a special binding affinity of cardiotonic steroids to myocardial Na⁺,
K⁺-ATPase, implying a unique form - and/or *modus operandi* - of the enzyme in cardiac muscle. Nevertheless, the covalent attachment of the bifunctional DADS $^{99m}$Tc chelate at a modifiable site of a cardiotonic steroid (see Figure 1.3) should furnish a promising receptor-binding radiopharmaceutical specific for the heart. The cardenolide aglycone, digitoxigenin, was selected as the biological carrier because of its commercial availability and established binding affinity for Na⁺, K⁺-ATPase, as judged through its potent inotropic activity. Moreover, from SAR studies, derivatization at the 3β hydroxyl position does not appreciably diminish cardiotonic properties and this site thus can be regarded as a "modifiable" site for conjugation with the DADS-CO$_2$ chelate.

3.4.3 Side Reactions of Digitoxigenin

The most straight-forward synthetic strategy toward the development of a digitoxigenin-based DADS ligand would be to directly conjugate the ligand acid 3 to the C$_3$ hydroxyl group of digitoxigenin via an ester linkage, followed by S-deprotection. The successful implementation of this strategy, however, requires an understanding of the limitations imposed by the sensitive character of the aglycone. For example, the C$_{14}$ hydroxyl group is easily dehydrated in acidic environments to form unsaturated products (Scheme 3.3). Moreover, under basic conditions, an internal rearrangement between the cis C$_{14}$ hydroxyl and lactone groups can occur, leading to the formation of an "isogenin" (Scheme 3.4). The reaction is irreversible and the original α,β-unsaturated lactone system cannot be regenerated. Thus, the potential for these undesirable transformations underscores the necessity for mild and neutral chemical manipulations involving derivatives of digitoxigenin.
3.4.4 Cholesterol as a Test Compound for Digitoxigenin

The development of a mild but efficient procedure for esterifying the α-branched ligand-acid 3 to the cardiac aglycone was accurately perceived as
non-trivial: the reacting groups are somewhat hindered, particularly the axial 3β-OH of digitoxigenin (see Figure 3.4). Anticipating the high cost and the potential toxicity that would accompany repetitive usage of digitoxigenin in trial experiments, the synthesis was tested using cholesterol. Cholesterol is inexpensive, non-toxic and somewhat similar to digitoxigenin in structure, except for the difference in the configuration of the C₃ OH group (in the sterol, the OH is equatorial). As such, cholesterol is a reasonable steroidal model to develop the chemistry for coupling and subsequent deprotection steps. After having “fine-tuned” the reaction conditions using the model compound, the synthesis was repeated using digitoxigenin as the carrier. Indeed, this synthetic detour may conveniently result in an effective cholesterol-based ⁹⁹mTc radiopharmaceutical. For example, ¹³¹I-labeled iodocholesterol has been shown to be a valuable radioimaging agent for adrenal tumors.⁶

3.4.4.1 Cholesterol-ligand Conjugation via CDI

With this exploratory strategy in focus, ester coupling was initially attempted through the CDI method (Scheme 3.5). Many variations of imidazolide activation of
the carboxyl function of 3 were performed, of which none proved to be successful (e.g., see 11, Section 7.6.13). A 0.3 fold excess of CDI was used to ensure complete activation of 3, since the purity of the commercially-available reagent is variable due to its extreme sensitivity to water. Moreover, the presence of residual water in the reaction set-up would result in some loss of CDI by hydrolysis. Poor yields of the desired acylated products may be attributed to the considerably faster reaction rate of excess CDI with cholesterol than with the acyl imidazolide 3b.42 This undesirable side reaction between the alcohol and coupling reagent leads to an imidazole-N-carboxylic ester (c) and subsequently either the steroidal dicarbonate (e) or, quite likely, the monoalkylcarbonate (d, see Scheme 3.6). Morton et al. have shown that the yield of the imidazole-N-carboxylic ester may be as high as 90% and that of the desired acyl imidazolide (3b) only 10%, if 1.5 equivalents of CDI was used for acid activation.42 TLC of the final reaction mixture supported this possibility, as many nonpolar components with \( R_f \) values greater than that of cholesterol (\( R_f 0.21 \) in \( \text{CH}_2\text{Cl}_2 \)) were detected. The acylation reaction must therefore be performed in the absence of excess CDI. The method of selectively destroying excess CDI in the presence of the activated imidazolide by incubation with Sephadex LH-2042 was attempted but preliminary results proved to be discouraging. At this point, the decision to employ a different, less water-sensitive coupling reagent was made.

3.4.4.2 Cholesterol-ligand Conjugation via DCC/DMAP

Ester coupling of acid 3 to cholesterol was also attempted using the DCC/DMAP system,90,91 but without encouraging results (see 11, Section 7.6.14). TLC of the reaction mixture after one week revealed unreacted cholesterol (\( R_f 0.21, \text{CH}_2\text{Cl}_2 \)), an unknown aromatic material (\( R_f 0.09, \text{CH}_2\text{Cl}_2 \)) and a minute amount of 3.
\(^1\)H NMR spectroscopy of the CH\(_2\)Cl\(_2\) extracts of the reaction mixture indicated cholesterol to be the major (non-DCU) component (> 60%). A second, UV-absorbing material (~30%) - having the characteristic spectral profile of 14 (see Section 3.4.5.3) - was also determined. The absence of signals in the 4.5-5.2 ppm range (where the C\(_3\) methine proton of cholesteryl esters typically resonate) suggested that the desired cholesterol-ligand ester 11 was not formed.

3.4.5 Evaluation of Coupling Efficiency through Standard Reactions

The results of NMR and TLC indicated that the coupling chemistry for ligand-steroid conjugation was clearly unsatisfactory. The most apparent rationale that can account for these observations would include "steric retardation" and/or an ineffectiveness in the DCC/DMAP activation system. The former effect is related to large steric interactions possible in the transition state stemming from reacting the hindered, secondary hydroxyl group of cholesterol with the \(\alpha\)-branched acid 3. Moreover, solvent effects may also have had a role on the poor ester yield: in the non-polar CH\(_2\)Cl\(_2\) solvent, cholesterol may exist as reverse micelles with their hydrophobic carbon framework oriented toward the solvent matrix while the polar hydroxyl groups are shielded in the interior of a hydrophilic cavity.

Consequently, a number of standard reactions were devised to test: (a) the effectiveness of the DCC/DMAP activating system to promote coupling and; (b) to determine the viability of esterification about the sterically hindered environments of the reacting carboxyl and hydroxyl functionalities of 3 and cholesterol.

3.4.5.1 Synthesis and Characterization of 15

Employing typical coupling conditions with DCC/DMAP reagents,
cholesterol was successfully condensed with hindered diphenyl acetic acid within four hours to afford the ester 15 in 89% yield. $^1$H NMR spectroscopy revealed a downfield shift of the broad multiplet of C₃ methine resonance from 3.49 ppm in cholesterol to 4.69 ppm in the suspected ester. Chemical ionization mass spectroscopy (CI-MS) were also consistent with the formation of 15, showing a M+18 ion at m/z 598. This experiment demonstrated the high coupling efficiency of the DCC/DMAP system and that cholesterol can be quantitatively acylated with hindered acids.

3.4.5.2 Synthesis and Characterization of 12

To probe the steric forces at work in the hindered carboxylic acid 3, the two following reactions were attempted. Cyclohexylamine was allowed to react with 3 in absence of the DMAP catalyst. TLC revealed the complete consumption of the acid at $R_f$0.22 (10% MeOH in CH₂Cl₂) and the appearance of a new component at $R_f$0.32 (2% MeOH in CH₂Cl₂). $^1$H NMR spectroscopy confirmed the product to be the desired cyclohexyl amide 12, through the assignment of the characteristic resonances at 3.56 and 6.69 ppm to the CONH-CH-(CH₂)₅ and CONH-C₆H₁₁ protons. As for 15, the formation of the hindered amide 12 in high yield reinforced the coupling effectiveness of the DCC reagent.

3.4.5.3 Synthesis and Characterization of 13 and 14: Evidence for Rearrangement of the O-Acyl Urea of 3

The next test reaction attempted to esterify 3 with cyclohexanol, an alcohol which closely mimicks the hindered environment of the C₃ hydroxyl group of cholesterol. TLC and NMR spectroscopy of the reaction product supported the formation of two new, UV active components. Using a mobile phase of 2% MeOH in CH₂Cl₂, the major (~50%) component eluted at $R_f$ 0.67, while the minor (~30%) one
occurred at $R_f 0.75$. In the proton NMR spectrum, certain key resonances suggested the presence of the required cyclohexyl ester 13: a quartet at 4.17 ppm implied that the asymmetric C$_2$ methine proton was adjacent to an ester function (compare with 4.15 and 4.20 ppm of esters 7 and 10), while the broad (ca. 40 Hz) multiplet at 4.78 ppm was characteristic of an axial $CH-(CH_2)_5$ resonance alpha to a O-C=O group (compare with methine resonances of 5.00, 4.75 and 4.81 ppm of the respective isopropyl, sec-butyl and cyclohexyl acetates$^{92}$). Proton integration revealed a second set of key resonances which were assigned to a second component in slightly higher composition. This compound was proposed to be the N-acyl urea analog of the acid 3 (14). The formation of N-acyl urea derivatives has been previously noted in DCC-mediated reactions$^{90}$ and is generally believed to result from the rearrangement of the activated O-acyl urea 3c (see Figure 3.5).

As depicted in Figure 3.5, two synthetic outcomes are possible from the reactive acyl agent 3d: cyclohexanol can undergo nucleophilic attack at the polarized carbonyl carbon with rate constant $k_{\text{nucleophilic attack}}$ to form the desired cyclohexyl ester 13; or alternatively, the imino nitrogen of the DCU amidate anion can also react at the carbonyl carbon of 3d, via an (assumed) intramolecular process with rate constant $k_{\text{rearrangement}}$ to give the unreactive N-acyl urea 14. To test the rearrangement proposal, the acid 3 was allowed to react with DCC and DMAP in a control experiment under standard esterification conditions for 4 days, but in the absence of an alcohol nucleophile (see Section 7.6.17). TLC indicated the complete disappearance of 3 and the appearance of the $R_f 0.67$ component observed in the synthesis of 11 and 13. The $^1$H NMR spectrum of the isolated product was consistent with the N-acyl urea derivative of 3 (14) because of the following assignments: the quartet at the characteristic chemical shift of 4.33 can be attributed to the asymmetric H-2 protons
Figure 3.5: Suggested pathway for the formation of cyclohexyl ester 13 and N-acyl urea 14.
proximate to an amide function, while resonances at δ 5.88, 6.10 and 7.00 are ascribed to the amide protons associated with C₂, C₄ and C₆, respectively.

The formation of 14 underscores the background complication of rearrangement when attempting to couple hindered alcohols and acids of type 3 via DCC/DMAP methods. In relatively nucleophilic and unencumbered systems, \( k_{\text{nucleophilic attack}} \) is much greater than \( k_{\text{rearrangement}} \) and the desired amide or ester becomes the major product as seen, for example, in the synthesis of the cyclohexyl amide 12 and the diphenyl acetate 15. However, in certain systems, a combination of large steric and poor nucleophilicity factors can decrease the rate of nucleophilic attack to a level where rearrangement can compete. This latter point was demonstrated through the conversion of 3 to the rearranged product 14. These factors, therefore, are proposed to explain the poor product yields observed for the esterification of cholesterol and cyclohexanol to acid 3.

Since ester formation is a bimolecular process, then halving the reaction volume should increase its rate four-fold. However, experiments where acid and alcohol concentrations were increased showed little or no enhancement in the yield of the cholestryyl ester 11 in relation to N-acyl urea 14, as judged by TLC. Moreover, the solubility of the reactants in a suitable aprotic solvent became limiting at these low reaction volumes.

3.4.6 Modification of 3 to Incorporate a Spacer Chain

Given the failure of the normally effective DCC/DMAP and CDI coupling methods, it is unlikely that another activating system could be applied successfully toward the synthesis of 3-O substituted steroidal esters of acid 3. The problem is evidently related to the high steric conestion about reacting groups and not a
shortcoming of the coupling agents or reaction conditions. With this focus, the synthesis towards these esters was modified to accommodate an unbranched spacer chain which will ultimately link the technetium ligand to the steroidal carrier. A glycyl synthon would suit this purpose, as it can connect acid 3 to the C₃ hydroxyl group of cholesterol (and ultimately digitoxigenin) through amide and ester bonds, respectively. More importantly, however, minimal branching at the α-carbon should ensure quantitative esterification yields because of the reduced crowding adjacent to its carboxyl group.

The choice of the glycyl synthon was based on commercial availability, cost and the ability to be readily incorporated into the existing synthetic method. Methyl glycinate hydrochloride seemed to satisfy all these constraints. The most prudent strategy would involve condensing the amino ester with acid 3 via amide coupling using DCC activation, hydrolysis of the resultant methyl ester adduct 16 and subsequent esterification of the glycyl acid 17 to the C₃ hydroxyl group of cholesterol through DCC/DMAP-mediated acylation to generate the S-protected ligand-steroid conjugate 18 (see Schemes 3.7 and 3.8). Detritylation of 18 and cyclization to the disulfide ligand 19 should readily occur using iodine. A strategic advantage of modifying the ligand acid 3 with the glycyl spacer first and then conjugating the adduct to the steroid - as opposed to the reverse direction - is the benefit of exposing the chemically-sensitive and costly biological carrier to fewer synthetic manipulations. Therefore, the synthesis of a spacer adduct of 3 was undertaken using methyl glycinate .1HCl.

3.4.6.1 Synthesis and Characterization of the Glycylamide of 3 (17)

Acid 3 and Cl·H₂N⁺-CH₂-COOMe were quantitatively converted to the amide derivative 16 by DCC activation in an acetonitrile-chloroform solvent mixture
(Scheme 3.7). Amide formation was confirmed by a number of methods, most notably by $^1$H NMR where the glycyl α-CH$_2$ resonance has shifted from δ 3.80 (DMSO-$d_6$)\(^{33}\) in the amino ester reactant to δ 3.92 in the amide product. Moreover, these methylene protons, like those at C$_3$ and at the O-CH$_2$ position in ester 7 (Section 5.2), have become diastereotopic, adding an interesting dimension to support amide formation. Geminal non-equivalence - which was revealed by their ABX resonance pattern - is a result of the proximity of the CH$_2$ spin system in the glycine residue to the stereocenter at C$_1$.\(^{39}\) In the following transformation, hydrolysis of 16 in 0.1 N NaOH in aqueous ethanol afforded the free acid 17 in 91% yield after an acidic work-up. $^1$H and $^{13}$C NMR spectra showed the absence of the methyl ester function at δ 3.70 and δ 54.75, respectively, confirming hydrolysis.

3.4.7 Synthesis and Characterization of Cholesteryl-ligand Conjugate 19

The key transformation in this linear synthesis was the conjugation of the glycyl acid 17 to the C$_3$ position of cholesterol (Scheme 3.8). Using DCC/DMAP as the activating agent for the carboxyl group, a new, UV-active spot was detected on TLC after the addition of the sterol. This component was isolated and purified from unreacted cholesterol and other aromatic impurities to afford a white solid in 54% yield. TLC and IR spectroscopy of the product indicated a $R_f$ value higher than that for cholesterol (cf. $R_f$ 0.81 and 0.64, respectively; 10% MeOH in CH$_2$Cl$_2$) and a prominent carbonyl absorption at 1740 cm$^{-1}$. However, the assignment of key shifts in $^1$H and $^{13}$C spectra provided unequivocal evidence that the product was the steroid conjugate 18. The downfield shift of the H-3 multiplet, from δ 3.49 in cholesterol to δ 4.62 in the product, suggests it is adjacent to an electron withdrawing group such as an ester. The C$_3$ resonance in $^{13}$C NMR also shows a similar shift from δ 71.9 to δ 75.4, again
supporting ester formation.

The successful synthesis of the glycyl-linked cholesteryl ligand 18 confirmed the existence of large steric effects that disfavored direct esterification of acid 3 and the sterol and the requirement for an unbranched spacer to connect the two domains. However, as only 54% of conjugate 19 was isolated, the method was inefficient: TLC of the reaction mixture revealed the presence of unconsumed cholesterol and a UV-absorbing (i.e., aromatic) impurity at \( R_f 0.55 \) (10% MeOH in \( \text{CH}_2\text{Cl}_2 \)) of combined relative intensity of ca. 30%. The latter component was also noted in greater amounts in the synthesis of the digitoxigenin glycyl ligand conjugate 20 \( (\text{vide infra}) \). Although no attempt was made to isolate and characterize this compound, its TLC polarity characteristics and UV-absorbing properties suggested that it may have been the \( N \)-acyl urea derivative of acid 17. If this is the case, then rearrangement of the \( O \)-acyl urea intermediate of 17 was still relatively significant within the time frame for nucleophilic attack by alcohol, particularly during the latter stages of the reaction when the concentration of nucleophile was low. Trial reactions employing the polar DMSO solvent instead of \( \text{CH}_2\text{Cl}_2 \) were successful in eliminating the \( N \)-acyl urea component at \( R_f 0.55 \) with the complete consumption of acid 17. Acetonitrile and DMSO/\( \text{CH}_2\text{Cl}_2 \) (2:1 v/v) were also investigated, but neither solvent system succeeded in preventing the formation of the \( R_f 0.55 \) component. Neat DMSO, it would seem, was the ideal solvent for the coupling reaction, offering the most efficient conversion of reactants to the ester product 18. However - given the poor solubility of cholesterol in small volumes of DMSO and the difficulty in removing this high-boiling, amphiphilic solvent during work-up - it was felt that the slightly better coupling efficiency in DMSO could be compromised by a simpler work-up using methylene chloride as the reaction solvent. Therefore, the coupling of cholesterol and 17 was carried out in \( \text{CH}_2\text{Cl}_2 \). The greater
esterification efficiency in these solvents can be rationalized in terms of the degree of solvent separation of the ion pair 3d (see Figure 3.5). In the polar DMSO solvent, the amidate and acyl pyridinium ions are more densely solvated and separated than in the non-polar CH₂Cl₂ solvent. This results in a “looser” ion pair association of intermediate 3d, which may lead to a reduction in the rate of the rearrangement reaction because of an increased separation between the reacting ionic entities. In addition, DMSO may favor the formation of "normal" micelles, wherein the reacting C₃ hydroxyl group would be more exposed to the polar solvent matrix for coupling with the acylating reagent 3d. In contrast, employing the non-polar CH₂Cl₂ as the reaction solvent may reduce the rate of nucleophilic attack because the polar OH function would be shielded by the hydrophobic exterior of the steroidal hydrocarbon framework in a reverse micelle scenario.

The final transformation in this model synthesis was the deprotection of the conjugate 18 and intramolecular cyclization to the disulfide 19. The addition of 1.1 equivalents of iodine to a degassed, 5 mM solution of 18 in absolute ethanol resulted in the quantitative disappearance of the starting material at Rf 0.81 and the appearance of a new component at Rf 0.51 (10% MeOH in CH₂Cl₂). This material was isolated in 32% yield following purification and characterized spectroscopically to be the disulfide 19. All protons and most ¹³C resonances associated with the ligand appendage appeared as broad signals: this is a characteristic property of the 10-membered disulfide heterocycle in CDCl₃ indicating slow conformational exchange (see Section 5.3). The absence of the trityl protecting group from IR and NMR spectra confirmed quantitative S-deprotection.

A concern in employing iodine as a deprotectant for the S-tritylated cholesteryl conjugate 18 was the undesired iodination of the C₅-C₆ olefinic bond of the
steroid. However, no unusual shifts were noted for this unsaturated system in the NMR spectra. For example, the $^{13}$C resonances of C$_5$ and C$_6$ are virtually invariant after the deprotection-oxidation of 18 (at $\delta$ 139.3 and $\delta$ 122.9, respectively). Presumably, the rate of C$_5$=C$_6$ dihalogenation is much slower than that of complexation with the S-Tr groups, which resulted in no detectable quantities of the diiodonated product.

3.4.8 Synthesis and Characterization of Digitoxigenin-ligand Conjugate 21

Given that the groundwork chemistry for steroid-ligand conjugation and deprotection had been successfully developed using the cholesterol model, the synthesis was repeated with digitoxigenin (Scheme 3.8). The ligand acid 17 was conjugated at the 3-OH group of the cardiac aglycone using similar conditions as worked out for cholesterol, yielding the conjugate 20 in 59% yield. Spectroscopic data were consistent with the formation of the required 3-O ester conjugate: the H-3 proton of the steroid had shifted downfield to 5.12 ppm, while the $^{13}$C NMR spectrum showed a new C$_3$ resonance at 71.90, shifted from 66.81 ppm in digitoxigenin. The IR spectrum indicated a strong ester carbonyl stretching at 1740 cm$^{-1}$, in addition to aromatic C=C and C-H absorbances at 3050, 1485, 1440, 735 and 690 cm$^{-1}$. The tertiary $^{13}$C$_{14}$ resonance appeared uneffected in both the free and conjugated steroid, confirming the absence of acylation of the C$_{14}$ hydroxyl group. Destritylation of 20 using I$_2$ as the deprotectant readily afforded the disulfide 21 in 24% yield. As for the choleseryl disulfide 19 and other cyclic ligands analyzed in CDCl$_3$-based solvents, deprotection and cyclization was inferred from the broadness of the ligand proton and $^{13}$C resonances and by the absence of aromatic signals in the $^1$H and $^{13}$C NMR spectra. Positive FAB-MS also indicated the presence of a M+$\text{F}^+$ ion at $m/z$ 664. Moreover, $^1$H and $^{13}$C NMR shifts suggested that the sensitive $\alpha,\beta$-unsaturated lactone at C$_{17}$ had not lost its integrity during the previous transformations.
CHAPTER 4

SYNTHESIS OF GASTROENTEROLOGICAL IMAGING AGENTS

4.1 General Introduction

The last 22 years have seen the development of many compounds labeled with $^{99m}$Tc for use primarily as hepatobiliary imaging agents. A summary of these agents in chronological order are: toluidine blue, penicillamine, tetracycline, dihydrothiostic acid, mercaptoisobutyric acid, pyridoxyl derivatives such as pyridoxylidene glutamate, pyridoxylidine phenylalanine and other pyridoxylidine animates, carboxyl-hydroxyquinoline, substituted acetanitride iminodiacetates (IDA ligands), ethylenediamine-$N,N'$-diacetates and orthoiodohippurate analogues. Among this extensive list of hepato(H)-biliary radiopharmaceuticals, the IDAs (or HIDA compounds) have been the most extensively used for basic and clinical research (see Figure 4.1). The clinical utility of IDA cholecintigraphy has been demonstrated for a number of disorders, including intrahepatic and extrahepatic biliary duct obstruction, Caroli’s disease, acute cholecystitis, post-traumatic or post-operative bile leak, organic and functional derangements of the biliary-enteric junction and the possible evaluation of hepatocellular carcinoma metastases.$^{94}$

Despite a respectable list of clinical precedence and impressive diagnostic functions, HIDA derivatives cannot be considered ideal agents for cholecintigraphy. Cardioactive effects and hepatobiliary transport sensitivity rank prominently among
well-documented disadvantages. A review of recent literature indicates that, while imaging procedures have successfully overcome many of the diagnostic challenges posed by the hepatobiliary organs, progress in other relevant areas of the gastroenterological system is lacking. New or improved imaging agents are continually being sought to fill a specific diagnostic application, particularly for the evaluation of organ function in diseased states. Consequently, a major aim in this segment of research was to develop novel gastroenterological agents that may be suitable for these applications. It is hoped that encouraging results in the initial testing of the proposed agents will stimulate the discovery and development of new classes of $^{99m}$Tc-based gastroenterological radiopharmaceuticals.

4.2 A $^{99m}$Tc Cholic Acid-based Agent for Enterohepatic Imaging

4.2.1 Introduction

Bile is a mixture of mono-, di- and tri-hydroxy steroidal acids, which in man are predominantly conjugated with glycine. Conjugated bile acids are produced in the liver, collected in the biliary passages, stored and concentrated in the gall bladder and intermittently discharged into the common bile duct. In the duodenum, bile helps to neutralize the gastric chyme and to emulsify dietary lipids. Their emulsifying action enables intestinal mucosa to absorb fat-soluble nutrients, such as cholesterol and lipophilic vitamins. In the distal ileum, bile acids are reabsorbed and are then returned to the liver through the portal system to complete the enterohepatic circulation.\(^5\)

In general, pathways of gastrointestinal absorption are more specific than those of excretion. Although many substances are rapidly excreted by the liver into the bile following intravenous administration - e.g., $^{99m}$Tc HIDA agents or $^{131}$I-labeled sodium rose bengal - few compounds, however, are reabsorbed and fewer still are
<table>
<thead>
<tr>
<th>X</th>
<th>Y</th>
<th>Name</th>
<th>Abbreviation</th>
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<tr>
<td>CH₃</td>
<td>H</td>
<td>N-(2,6-diisopropyl) IDA</td>
<td>DISIDA</td>
</tr>
<tr>
<td>CH₃</td>
<td>CH₃</td>
<td>N-(2,6-diethyl) IDA</td>
<td>DIDA</td>
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<tr>
<td>H</td>
<td>CH₃</td>
<td>N-(p-isopropyl) IDA</td>
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<tr>
<td>H</td>
<td>C₄H₉</td>
<td>N-(p-n-butyl) IDA</td>
<td>BIDA</td>
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Figure 4.1: $^{99m}$Te-HIDA analogs.
reabsorbed and re-excreted unchanged. This is presumably a consequence of the high molecular specificity built into the active, ionic absorption and transport process of the intestinal wall. Although there are many radioactive tracers suitable for studying the excretory function of the liver and extrahepatic biliary ducts (e.g., see Section 4.1), all of these can be improved upon in some capacity and few - if none at all - are suitable for studying the complete enterohepatic circulation (the gut, liver and biliary tree).

A number of researchers have indicated that a $^{99m}$Tc cholic acid agent could be useful as a hepatobiliary radiopharmaceutical, with the additional advantage of providing a scintigraphic method for assessing ileal absorptive function. Hofmann has emphasized the need for a tracer which behaves like a bile acid and could be employed to investigate enterohepatic circulation, especially ileal reabsorption. Galli and Maini have also pointed out the potential utility of $^{99m}$Tc-labeled bile acids as novel hepatobiliary radiopharmaceuticals. There are a number of reasons to support these unique claims: the hepatocellular transport mechanisms for bile acids differ in part from those of other anions (and presumably $[^{99m}$Tc-HIDA]$^{-1}$ complexes) and their liver extraction coefficient is the highest among all the substances tested to date. In addition, one of the earliest manifestations of hepatocellular dysfunction is a reduction in bile acid transport capacity, which makes these steroidal acids a very sensitive indicator of liver cell function.

To our knowledge, however, the only investigative study in this area involved cholic acid derivatives tagged with selenium-75 at various positions on the C$_{17}$ side chain. These bile acid analogs have demonstrated very similar hepatobiliary excretion and gut absorption properties when compared with that of $[24^{14}$C]cholic acid. Unfortunately, the nuclear properties of $^{75}$Se are not optimal for imaging: this deficiency may have prevented the selenium-tagged cholic acid compounds from
seeing widespread clinical usage. Thus, if the substitution of $^{99m}$Tc - with its excellent
diagnostic imaging characteristics (see Section 1.2) - for $^{75}$Se in these cholic acid
analogs is made, then a practical and novel agent which is suitable for detecting
small-bowel disease may result. On this basis, a major aim of this study was to prepare
a $^{99m}$Tc-tagged, bifunctional derivative of cholic acid for subsequent development as an
enterohepatic radiopharmaceutical.

Based on the $^{75}$Se-tagged cholic acid study, the C$_{17}$ side chain would appear
to be a modifiable area suitable for the attachment of the $^{99m}$Tc-DADS-CO$_2$ chelate,
with receptor recognition and binding being primarily determined by the
hydroxy-steroid skeleton. Moreover, the fact that cholic acid is mainly found in vivo as
its equally-active glycine or taurine conjugate also supports the design strategy that
amide derivatization of the C$_{24}$ carboxylic acid function should not significantly alter
ileal absorption.

4.2.2. Synthesis and Characterization of Cholic Acid Conjugate 23

The cholic acid DADS-CO$_2$ conjugate 23 was prepared as outlined in
Scheme 4.1. Acid 3 was conjugated to 3-cholamidopropylamine$^{102}$ via DCC activation

![Chemical structure](image)

and gave amide 22 in 98% yield. Characterization of 22 by $^{13}$C NMR spectroscopy
revealed the appearance of three new carbonyl resonances corresponding to $C_1$, $C_4$, and $C_6$ of the ligand moiety. These observations - in addition to the newly-created $C_1$-ONH proton resonance at 7.86 ppm - were consistent with the proposed acylation of the $C_27$ amino group. The tetraamide 22 was subsequently deprotected and converted to disulfide 23 in 45% yield, following the established procedure using iodine as the deprotectant-oxidant. Positive FAB-MS of 23 showed a $M+1$ ion, while IR and NMR spectra indicated the absence of trityl absorbances. As in other DMSO-$d_6$ solvated ligand cycles, intramolecular cyclization was inferred from the complex nature of the $C_2$-$H$ $^1H$ and $^{13}C$ resonances (see Section 5.3).

4.3 $^{99m}$Tc Taurine-based Agent for Gastric Ulcer Imaging

4.3.1 Introduction

In the opinion of many surgeons, an imaging agent which can localize in ulcerated tissues of the gastrointestinal tract would be even more valuable than a hepatobiliary tumor-localizing radiopharmaceutical. The failure to detect and localize occult abdominal abscesses is regarded as a major surgical problem. The development of such an imaging agent, therefore, would clearly be a boon to nuclear medical
practitioners.

4.3.2 Sucralfate: A Sulfated Sugar with Ulcer Localizing Abilities

Among the pharmacological properties of sulfated oligosaccharides is their peculiar ability to localize in ulcerative areas of epithelia and to protect these regions from digestive enzymes to promote healing.\textsuperscript{103} Sucralfate (Carafate\textsuperscript{®}, see Figure 4.2),

\[ R = \text{SO}_4\text{Al}_2(\text{CH}_3)_3 \]

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{fig4_2.png}
\caption{Structural formula of sucralfate.}
\end{figure}

a basic aluminium salt of sucrose octasulfate, has been used for over two decades as an effective therapeutic in the treatment of both duodenal and gastric ulcers.\textsuperscript{104} The \textit{in vivo} antiulcer activity of sucralfate has been attributed to its binding with serum proteins exudated by the ulcerous lesion. The resulting protein-sulfate complex is resistant to hydrolysis by harmful agents such as pepsin and gastric acid and it binds selectively to the ulcer site to establish a local protective barrier.\textsuperscript{105} The binding interaction is especially strong: in man, the gel remains adherent to ulcerated epithelium for over 6 hours.\textsuperscript{104} Results of a pH-dependent study\textsuperscript{106} show that this specific binding is electrostatic in nature, with the negatively-charged sulfate groups in sucralfate combining with positively-charged sites on the protein at gastric acidity levels (pH < 4).
The successful localization of $^{99m}$Tc-labeled derivatives of sucrafate and sucrose sulfate in ulcerated epithelia\textsuperscript{103} suggests the possibility of directing technetium-tagged complexes of other sulfated compounds to these areas for ulcer imaging. Based on the electrostatic nature of the sucrafate binding mechanism, the anionic sulfate group - which remains fully ionized at gastric acidity - is essential for target tissue accumulation. Therefore, in the design of a prospective ulcer-avid radiopharmaceutical, carrier candidates must incorporate a strongly acidic group, such as a sulfonic acid or sulfate function. The amino sulfonic acid taurine satisfies this condition. We proposed that a $^{99m}$Tc-DADS taurine agent may prove to have clinical significance in the evaluation of gastrointestinal ulcer diseases and other related dysfunctions that are associated with the loss of mucosal integrity. Therefore, efforts have been directed toward the preparation of a taurine derivative equipped with a DADS-CO$_2$ chelant.

4.3.3 Synthesis and Characterization of Taurine Conjugate 36

The taurine DADS ligand conjugate 36 was synthesized as shown in Scheme 4.1. Taurine was coupled to acid 3 via 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide HCl (EDC) activation to afford the amide conjugate 35 in 96% yield. The extreme
hydrophilic character of the conjugate presented a formidable challenge for its separation from other polar substances, such as DMF solvent, EDCU, NaCl and excess taurine. After bulk removal of DMF under high vacuum, the residue was suspended in hot ethanol and filtered to remove unreacted taurine. The sodium salt 35 was subsequently precipitated from a dilute aqueous solution of the concentrated ethanolic filtrate by salting with NaCl. The aqueous solution was acidified just prior to salt addition to enhance the solubility of the EDCU contaminant in solution through the formation of its hydrochloride salt. The collected residue was suspended in hot ethanol and filtered to remove residual amounts of NaCl. TLC of the solid obtained after the evaporation of ethanol showed excellent homogeneity. Negative FAB-MS revealed an intense peak at m/z 842, corresponding to a loss of Na⁺ from the molecular ion. Amide formation was also evident in ¹H and ¹³C NMR spectra through the appearance of the C₇ONH signal at 7.90 ppm and a third amide signal in the ¹³C carbonyl region. Iodine-effect ed detritylation of 35 led to the formation of the disulfide 36 in 33% yield. The marked broadness and complexity of the proton and carbon signals of the product and the absence of trityl resonances suggested successful deprotection and intramolecular disulfide formation.
4.4 A $^{99m}$Tc Cyclodextrin-based Agent for Lower Bowel Imaging

4.4.1 Introduction

The mucosa of the gastrointestinal tract can be visualized as a semi-permeable membrane which allows passage of water and nutrients but excludes other compounds. Molecules of various sizes are known to cross this barrier by active and passive mechanisms. Molecular size appears to determine the mechanism of transport: small or intermediate-sized compounds can cross the plasma membrane of the microvilli, whereas macromolecules are gradually taken up by endocytosis at the base of the microvilli. In addition, the ends of the villi - where extrusion of mature cells occurs - may present a variable breach in the epithelial barrier and represent yet another gateway for molecular entry. Macromolecules are also known to be taken up by M-cells overlying lymphoid collections.$^{107}$

However, the permeability of healthy intestinal mucosa towards molecules of different molecular size changes when it becomes inflamed or infected.$^{108}$ It seems that in patients afflicted with Crohn’s disease or celiac disease, infected intestinal membranes become more pervious to larger molecules than smaller ones. Thus, the differential permeability of probe molecules through the intestinal wall can be used as a marker for the evaluation of intestinal wall function. Since a better understanding of intestinal permeability (or function) of large molecules might generate further insight into the pathogenesis and therapy of some gastrointestinal and systemic diseases, studies in this area are highly desirable.

4.4.2 In Vivo Measurement of Intestinal Permeation via $^{99m}$Tc Imaging

Intestinal permeation has been measured in man and animals using a variety of probe molecules. Typically, the probe is administered orally and the amount of the
compound excreted in urine is measured with respect to time. Assuming negligible metabolism of the probe, intestinal permeation of the test substance is taken as directly proportional to its urinary excretion. Finally, the probe substance is quantified in the urine by either extraction and derivatization procedures, or by scintigraphy in the case of a radioactive probe. However, these methods of analysis suffer from the disadvantages of being tedious and time consuming procedures, not the least being the need to collect and process urine. A more appealing strategy for the analysis of the test substance might be to employ a $^{99m}$Tc-labeled probe, where the potential for diagnostic imaging and quantitative analysis is possible directly in the in vivo environment without the need for technically demanding, post-excretory, analytical assays. Consequently, the specific diagnosis of dysfunctions and diseases of the intestinal wall can be determined through the combination of morphologic and quantitative physiologic changes as outlined in reference 1.

4.4.3 β-Cyclodextrin as a Carrier Molecule for a $^{99m}$Tc-labeled Intestinal Probe

Clinical measurements of permeation pathways have been carried out using probes in the 100-500 dalton range (e.g., lactulose, mannitol, L-rhamnose, $^{51}$Cr-EDTA and polyethylene glycol (PEG 400)) or on larger molecules with molecular weights greater than 10,000 daltons (e.g., polyvinylpyrrolidine and albumin). However, intermediate-sized molecules have not been studied thus far. A number of conditions must be fulfilled for a compound to find acceptance as a permeability probe. The molecule should be non-toxic and have well characterized properties - such as, hydrodynamic dimensions, molecular weight, charge and polarity.

The unusual shape and size of cyclodextrins (see Figure 4.3 for an illustration of β-cyclodextrin) makes them unique probe candidates with respect to the
Figure 4.3: Structural formula (a) and a schematic illustration (b) of β-cyclodextrin.

above criteria. These torodial-shaped molecules have a molecular weight of about 1300 daltons and are uncharged in aqueous solution at physiological pH. Cyclodextrins are hydrophilic but also display marked lipophilic properties similar to PEG 400 and should therefore pass across the bowel wall as observed for these ethylene glycol polymers and other sugars and alcohols.

The objective of this section of research was to synthetically modify a β-cyclodextrin amine derivative to incorporate a DADS-CO₂ chelant for the technetium-99m nuclide. Such a compound offers the possibility of a simple, novel and aesthetically acceptable method of investigating bowel permeability and certain gastrointestinal diseases.

4.4.4 Synthesis and Characterization of Cyclodextrin Conjugate 38

The β-cyclodextrin DADS-CO₂ bifunctional ligand 38 was synthesized in
16% overall yield as shown in Scheme 4.1. Conjugation of the cyclodextrin diaminopropane 37a (a gift from Dr. Alfredo Capretta) with the S-trityl ligand 3 via

EDC activation in DMF gave the amide adduct 37 in 53% yield. Analysis of the $^1$H NMR spectrum revealed a shift to lower field of the $C_2$-$H$ signal relative to acid 3 ($\delta$ 4.02 in 37 from $\delta$ 3.89 in 3), while the $^{13}$C NMR spectrum showed the appearance of a
third resonance in the amide region between 167.33 and 167.71 ppm, corresponding to the newly-formed C1-O-NH bond. The IR spectrum also revealed the absence of a prominent C=O carboxylic acid stretching frequency in the range 1725-1740 cm−1, providing further confirmation of amide coupling.

Initial attempts to detritylate the β-cyclodextrin ligand conjugate 37 under standard deprotection conditions (employing 1.1 equivalents of I2) resulted in the recovery of ca. 35% of 37 after work-up. This unexpected result can be attributed to the formation of a 1:1 guest-host complex between iodine and the cyclodextrin. In support of this postulate, a crystal structure of the iodine complex of α-cyclodextrin has been reported, clearly showing one molecule of I2 encapsulated by the six-membered carbohydrate ring.111 The cyclodextrin molecule is known to possess a hydrocarbon-like interior, which can dissolve relatively non-polar compounds (like I2) in much the same manner as hydrocarbon solvents.112 Therefore, based on these observations and the well-known affinity of iodine for starch, it is reasonable to propose the existence of a reversible guest-host complex between I2 and β-cyclodextrin. This 1:1 bound complex can serve to rationalize the high recovery of unprotected conjugate 37, which was noted when 1.1 equivalents of I2 was used for deprotection. Consequently, deprotection of 37 was repeated using 2.1 equivalents of I2 and, by maintaining high dilution conditions to preclude intermolecular disulfide formation, in situ intramolecular oxidative cyclization readily afforded the monomeric disulfide 38 in 31% yield. Positive FAB-MS indicated a M+1 peak at m/z 1423 and 1H and 13C NMR spectra confirmed the absence of the trityl group. Cyclization was also inferred from the marked signal complexity of all 13C resonances pertaining to the complexing system (see Section 5.3).
CHAPTER 5

NMR SPECTROSCOPY OF THE DADS LIGAND. PART 1

5.1 General Introduction

NMR spectra of open chain or cyclic derivatives of the N₂S₂ ligand were often markedly complex, inviting interpretations based on product decomposition (e.g., disulfide polymerization) and/or contamination by reaction by-products. The objective of this segment of research was to determine the underlying physical and chemical properties of the DADS system which gave rise to their perplexing NMR spectral profiles.

5.2 Magnetic Non-Equivalence at the C₅ and C₇ Environments in Tritylated Intermediates

Examination of the geminal S-CH₂ protons resonances of ester 7 (ca. 3.0-3.1 ppm) revealed the superimposition of an AB quartet (Δδᵩᵣₛ = 0.05 ppm, ²J = -15.6 Hz) on a singlet (see Figure 5.1). This effect, which was also noted for many other S-tritylated ligand intermediates, implied that one geminal pair of α-thiotrityl methylene protons was magnetically non-equivalent relative to the other at the 500 MHz field strength. The observed selective S-CH₂ non-equivalence could result from: a) the induction of stereochemical non-equivalence - upon the closer C₇ methylene protons - by the C₁ stereocenter; b) chirality-independent, differential shielding by the amide carbonyl anisotropy; or c) restricted rotation, resulting from amide partial
Figure 5.1: 500 MHz $^1$H NMR spectrum of 7 in CDCl$_3$ at 303 K. Inset: expansion between 3.0 and 4.3 ppm.
double bond character, amide hydrogen bonding to oxygen and/or sulfur, or $\pi-\pi$ interactions. If c) is operative, then heating the sample should overcome these relatively small rotational barriers and equilibrate proton environments. Warming 7 to 122.6 °C at 200 MHz did not result in observable change of the S-CH$_2$ proton resonances relative to the room temperature spectrum: an empirical indication that the previously-stated intramolecular bonding interactions were not the source of the observed non-equivalence. Moreover, given the high field $^1$H and $^{13}$C magnetic equivalence of both pairs of the S-CH$_2$ groups in the ethylene diamide 41, the

arguments for differential shielding based on amide carbonyl and prochiral$^{39}$ mechanisms seemed improbable. Therefore, the origins of the observed S-CH$_2$
chemical shift difference in tritylated intermediates such as 7 was most likely due to chirality-induced non-equivalence. In principle, the presence of the C₁ chiral center confers magnetic non-equivalence upon all methylene protons, including those at C₇; however, their non-equivalence cannot be observed at 500 MHz.

A small (0.3 ppm) difference was also noted between C₅ and C₇ shifts in $^{13}$C NMR spectra of most S-tritylated DADS-CO₂ ligands at 126 MHz. Since C₅ and C₇ are not "diastereotopic", their magnetic non-equivalence cannot be rationalized by analogy of the diastereotopic shielding argument used for their attached protons. This differential carbon shift presumably resulted because of the inductive influence of the COOH substituent at C₂; C₇ resonates at lower field than C₅ due to its closer proximity to the electron withdrawing carboxyl group.

5.3 Analysis of Ethyl $N,N'$-(2,2'-dithioacetyl)-2,3-diaminopropanoate (8) in DMSO-$d_6$ and CDCl₃

Compound 8 was synthesized and judged to be a pure sample as evidenced by HRMS, IR, TLC and melting point determinations (see Section 7.6.10). In the high resolution mass spectrum, an excellent correlation was found between calculated and observed mass units for the molecular formula $C₆H₁₄N₄O₄S₂$, with a difference of only
0.0008 μu. IR spectroscopy also disclosed key absorbances which were consistent with ester (1735 cm⁻¹) and amide (1640 and 1550 cm⁻¹) groups. TLC indicated a single component at Rf 0.78 (80% n-PrOH, 18% H₂O, 2% HCOOH). Moreover, the white solid melted over a two-degree range, suggesting excellent purity.

However, like the acid 5 (see Section 7.6.6), ¹H and ¹³C NMR spectra of 8 in DMSO-d₆ were dauntingly complex, indicative of a mixture of compounds (see Figures 5.2 and 5.3; Table 7.2). Examination of the C₂—H methine resonances in ¹H and ¹³C spectra (ca. 3.8-4.9 and 52.1-53.0 ppm, respectively) suggested the presence of at least four different chemical species, while in the carbonyl region, as many as nine separate, ¹³C signals were noted. The NMR data seemed to imply that the disulfide was impure - a conclusion which contradicted the results of the above mentioned characterization methods. The possibility that polymeric disulfides were present was not supported by MS.

Since the prochirality of the ring CH₂ groups or the presence of the chiral center at C₁ cannot fully account for the complexity of the ¹H NMR spectrum in DMSO-d₆, the possibility of slowly exchanging, ring conformers was investigated. Ball and stick models indicated that the 10-membered disulfide heterocycle is large enough to accommodate the rotation of the entire amide group through the ring, provided that the substituent of the N atom was a simple hydrogen atom (as in 8). To test this hypothesis, the C₁ unsubstituted, N-methyl disulfide (40) was prepared. In this model compound, ring interchange would require the passage of the N-CH₃ group through the middle of the ring - a sterically unfavorable process. The ¹H and ¹³C spectra (see Figures 6.1 and 6.2) proved to be complex, suggesting ring interchange by rotation of a complete amide unit through the ring was an unlikely mechanism for rationalizing the NMR spectra of 8 in DMSO-d₆.
Figure 5.2: 500 MHz $^1$H NMR spectrum of 8 in DMSO-$d_6$ at 303 K.

* Tr - OCH$_2$CH$_3$ (impurity)
Another proposal was investigated: restricted rotation about amide and disulfide linkages have been reported\textsuperscript{92,114} to be slow on the NMR time scale, resulting in an equilibrium mixture of discrete geometric isomers at room temperature, where each isomer possesses a unique NMR spectrum. Although the rate of rotational isomerization is temperature-dependent, warming the DMSO-\textit{d}_6 solvated disulfide ester 8 to 120 °C (see Figure 5.4) did not induce an appreciable change in its \textit{1}H NMR spectrum at 250 MHz, unlike that for the dimethyl disulfide 40 (see Figure 6.3). The variable temperature data did not necessarily refute the possibility of restricted rotation; however, they did suggest that, if such a phenomenon was operative, the barriers to isomerization were too high to observe appreciable change at 120 °C (\textit{vide infra}). High barriers to conformational isomerization are possible in strongly polar solvents, such as DMSO-\textit{d}_6, as they can form hydrogen bonds with the amide protons of 8 to confer high conformational stability on conformers (\textit{vide infra}). With the \textit{N}-dialkyl analog 40, hydrogen bonding is not possible as for 8, so conformational exchange can be observed at temperatures below the instrumentally-imposed ceiling temperature of \textit{ca.} 120 °C (see Section 6.1).

Given the aforementioned proposal of solvent stabilization, the effect of non-polar deuterated solvents was investigated, with the \textit{1}H and \textit{13}C spectra in CDCl\textsubscript{3} presented as Figures 5.5 and 5.6. Compared with the DMSO-\textit{d}_6 sample, spectral features have been markedly simplified to permit an unambiguous assignment of all spin systems (see Table 7.2). The findings of the CDCl\textsubscript{3} experiment served to strongly confirm the suspected origins of spectral multiplicity in the DMSO-\textit{d}_6 spectrum of 8: in DMSO-\textit{d}_6, hydrogen bonding and dipole-dipole interactions between the solvent and protic amide functions of 8 resulted in the stabilization of the room temperature, ground-state ring conformers \textit{relative to the transition state for isomerization} (see
Figure 5.4: 500 MHz $^1$H NMR variable temperature spectra of 8 in DMSO-$d_6$. 
Figure 5.5: 500 MHz $^1$H NMR spectrum of 8 in CDCl$_3$ at 303 K.

* Tr - OCH$_2$CH$_3$ (impurity)
Figure 5.7). This "freezing out" of ring conformers into deep potential energy wells -

\[ \Delta G^\ne_{\text{DMSO-d}_6} > \Delta G^\ne_{\text{CDCl}_3} \]

Progress of rotational isomerization

Conformer 1 \[\leftrightarrow\] Conformer 2

Figure 5.7: Effect of solvent [(a) CDCl$_3$ vs (b) DMSO-d$_6$] on the free energy change of activation, $\Delta G^\ne$, for an isomerization process involving bond rotation in 8. Intermolecular solute-solvent bonds in DMSO-d$_6$ stabilize ground state ring conformations relative to the transition state for rotation, thereby increasing $\Delta G^\ne$.

which are separated by high barriers to interconversion (as measured by $\Delta G^\ne$) - effectively reduces the rate of ring conformational interconversion. As a result, the signals in the DMSO-d$_6$ spectrum of 8 became sharp relative to that of the CDCl$_3$ sample, with the absorptions from each of the many ring conformers overlapping to give the observed spectral multiplicity. The barriers to isomerization in DMSO-d$_6$ must be quite high (i.e., $\Delta G^\ne > 20$ kcal/mol) in order to rationalize the negligible effect of high temperature (120 °C) on the $^1$H NMR spectrum of 8. Differential ring conformer stabilization in non-polar CDCl$_3$ was not nearly as pronounced as for DMSO-d$_6$.
because of the inability of the former solvent to form strong hydrogen bonds with \( \text{8} \). This allowed ring isomers to rapidly interconvert over lower rotational barriers (see Figure 5.7 a) and manifested themselves as broader resonances in the CDCl\(_3\) spectrum. This solvent effect was also observed in the \(^{13}\)C NMR spectrum of CDCl\(_3\) and DMSO-\(d_6\) solvated samples of the \(N\)-monoalkylated disulfide \(42\) (see Section 7.6.46) and possibly holds broader application in \(N\)-monoalkylated lactams, where the ring exerts a critical combination of rigidity and flexibility to allow the stabilization of the amide (and/or disulfide) group through solvation with polar solvents. Evidence of conformational isomerism was not noted in NMR spectra of the open chain precursors \(7\) and \(41\), indicating that a ring structure is required for the observation of solvent effects described earlier.

Four, approximately equally-populated, ring isomers, which were identified by their \(C_2\)-H resonances (labeled as H-2\_ISOMER 1, H-2\_ISOMER 2 etc, see Figure 5.2) were detected in the DMSO-\(d_6\), \(^1\)H NMR spectrum of \(8\). The \(^1\)H-\(^1\)H COSY experiment (see Figure 5.8) confirmed that these methine signals belonged to separate spin-systems with connectivities to the \(C_3\)-H protons in the 3.3-3.5 ppm range, thereby constituting different conformations. Ring isomers 1 and 4 (ISOMER 1 and ISOMER 4) had rather broad \(C_2\)-H resonances (small \(T_2\)) and did not show up as diagonal elements, presumably because of the rapid decay of their magnetization from the XY plane of the detector.

The \(C_2\)-H region of the CDCl\(_3\) spectrum of \(8\) (see Figure 5.5) revealed residual amounts of what appeared to be the DMSO-\(d_6\) observed isomers (at \(\delta \sim 4.0\) and \(\delta \sim 4.9\)). This observation spurred the following experiment: a solution of \(8\) in CDCl\(_3\) was cooled to \(-40.4\) °C to decrease the rate of conformational exchange and to determine if the broad proton resonances of the room temperature spectrum could be
Figure 5.8: 500 MHz $^1$H NMR COSY-45 spectrum of 8 in DMSO-$d_6$ at 303 K.
Figure 5.9: 500 MHz $^1$H NMR variable temperature spectra of 8 in CDCl$_3$. 

* Tr - OCH$_2$CH$_3$ (impurity)
sharpened at a low temperature to generate the DMSO-$d_6$ isomers. Figure 5.9 illustrates the beginnings of sharpening and separation of all proton signals as the sample is cooled from room temperature to -40.4 °C. The addition of 5 mg of Ca(ClO$_4$)$_2$ also induced peak sharpening, presumably by locking some or all of the interconverting isomers into a rigid calcium chelate (see Figure 5.10).\textsuperscript{115}

5.4 Conformational Analysis of $N,N'$-[dimethylbis[2-(triphenylmethyl)thioethanovl]]

ethylenediamine 39 in CDCl$_3$

Compound 39 proved to be an interesting NMR spectroscopic example of a chemically exchanging system having conformational preferences around the C-N amide bond. A superficial analysis of the room temperature $^1$H and $^{13}$C NMR spectra in

\[ \text{39} \]

DMSO-$d_6$ (see Figures 5.11 and 5.12), revealed a rather complex resonance profile considering the molecule's symmetrical, non-cyclical structure and isolated spin systems. The NMR results appeared even more puzzling given that spectra taken in CDCl$_3$ were practically identical to the DMSO-$d_6$ ones, which eliminated the possibility for the dramatic solvent effects described for 8 (see Section 5.3).

The most plausible explanation to rationalize the multitude of NMR signals is restricted rotation about the two amide groups; a process which is sufficiently slow at
Figure 5.10: 500 MHz 1H NMR spectrum of $\text{Ca(ClO}_4\text{)}_2$ at 303 K.
Figure 5.11: 500 MHz $^1$H NMR spectrum of 39 in DMSO-$d_6$ at 303 K.
Figure 5.12: 126 MHz $^{13}$C NMR spectrum of 39 in CDCl$_3$ at 303 K.
room temperature on the NMR time scale to allow the observation of distinct stereochemical environments for the alkyl substituents. Restricted amide rotation is attributed to partial double bond character derived from the following delocalized electronic structure:

As a result, the environment of substituents A, B and C are not averaged and, depending on their chemical shifts, these groups can usually be observed as separate signals in the NMR spectrum.\(^9\)

Elevated temperature \(^1\)H NMR studies of 39 showed the temperature dependent coalescence of all aliphatic resonances into the three signals predicted for the symmetrical, averaged compound (see Figure 5.13). Based on peak integration of the averaged, 122.5 °C spectrum and following peak transitions from the room temperature to the high temperature limit, three separate sets of proton resonances - attributable to three geometric isomers - could be identified.

To verify this chemical rate process as restricted amide rotation, the free energy change of activation, \(\Delta G^{\ddagger}\), was estimated to a first order approximation using a modified form of the Eyring formulation\(^{116}\) (Equation 5.1):

\[
\Delta G^{\ddagger} = RT_e \left[ 22.96 + \ln(T_e/\Delta v) \right]
\]  

[5.1]

where \(T_e\) is the coalescence temperature (K), \(R\) is the universal gas constant and \(\Delta v\) is the frequency separation (Hz) between two uncoupled, exchanging nuclei of equal resonance intensity. \(T_e\) was estimated from Figure 5.13 to be about 87 °C at 250 MHz, which afforded \(\Delta G^{\ddagger} = 15.1\) kcal/mol. This value was consistent with typical rotational
Figure 5.13: 200 MHz 1H NMR variable temperature spectra of 39 in DMSO-d$_6$. 

T (°C)
barriers reported for substituted \(N,N'-\)dimethylamides (e.g., dimethylacetamide, \(\Delta G^\circ_{298.2} = 17.4 \text{ kcal/mol}\); dimethylpropanamide, \(\Delta G^\circ_{298.2} = 16.7 \text{ kcal/mol}\)).

Thus, the fluxional process responsible for the complexity of the room temperature spectrum of 39 was most likely a result of restricted rotation about the amide groups. Other possible explanations might have included conformational preferences at the N-C\(\alpha\) bond\(^92\) and/or \(\pi-\pi\) bond interactions\(^118\) between trityl groups; however, these effects are quite likely to be less than 10 kcal/mol in this compound.

Having linked the multi-signal, room temperature spectrum of 39 to rotational isomerism about the amide groups, a total analysis of the \(^1\)H NMR spectrum was developed. Figure 5.14 illustrates the possible amide rotamers of 39 that can co-exist at room temperature. It is known that population distribution among amide stereoisomers in symmetrically substituted \(N,N'\)-dialkylamides can be rationalized in terms of steric competition. In general, the \textit{trans} isomer is energetically favored, except in small ring lactams where the conformation must be \textit{cis} (see Figure 5.15 for a key to nomenclature of amide geometry).\(^92\) Therefore, the following stability trend was expected for amide rotamers of 39: \(Z,Z > Z,E > E,E\), where the \(Z,E\) isomer is
unsymmetric. Correlating peak integrals with the above stability trend, peak integration in the high temperature NMR experiment and symmetry arguments, the three geometric isomers were characterized in the $^1$H and $^{13}$C NMR spectra (see Tables 7.3 and 7.4, Section 7.6.43).

Configurations were also characterized and confirmed through the chemical shift of the N-CH$_3$ group.$^{114}$ In agreement with steric competition arguments, the diamide 39 was found to exist mainly in the Z,Z conformation in which the N-CH$_3$ resonances were always shifted downfield relative to the corresponding N-CH$_3$ signals of the E,E isomer. This observation was supported by the $^1$H NMR resonances of N,N-dimethylacetamide where the N-CH$_3$ signal trans to the carbonyl oxygen resonates at $\sim$0.1 ppm downfield from the cis signal in CDCl$_3$. The same trend was observed to hold for the corresponding $^{13}$C resonances.

The Boltzmann equation was employed to calculate the difference in free energy ($\Delta G$) between the geometric isomers (Equation 5.2). Based on ratio intensities

$$\Delta G = -RT \ln \left( \frac{n_2}{n_1} \right)$$

[5.2]

of appropriate $^{13}$C signals (see Table 7.4), the Z,Z conformer was energetically favored over the Z,E isomer by 0.55 kcal/mol ($\Delta G_{Z,Z \rightarrow Z,E}^{303} = 0.55$ kcal/mol) and the Z,E over the E,E by 0.88 kcal/mol ($\Delta G_{Z,E \rightarrow E,E}^{303} = 0.88$ kcal/mol).
CHAPTER 6

NMR SPECTROSCOPY OF THE DADS LIGAND, PART 2

6.1 General Introduction

The 10-membered heterocycle 40 was prepared to aid in the interpretation of the complex NMR spectral characteristics noted for the cyclic disulfides 5 and 8 in DMSO-d$_6$ (see Section 5.3). However, NMR spectroscopy of this compound proved to be complex in its own right, warranting further investigation.

![Chemical Structure](Image)

6.2 Conformational Analysis

6.2.1 $^1$H and $^{13}$C NMR Spectroscopy

The cyclic disulfide 40 ran as one spot when examined by thin layer chromatography and melted over a 1.0 °C range indicating good sample homogeneity. Infrared, Raman and mass spectra of the product were consistent with its proposed monomeric structure (see Section 7.6.44). The $^1$H and $^{13}$C NMR spectra (see Figures 6.1 and 6.2), however, showed dramatically complex resonance profiles despite the established chemical purity of the sample and its symmetry-related, spin isolated sets of
Figure 6.2: The 126 MHz 1H-modulated 13C NMR spectrum of 40 in CDCl₃ at 303 K.
$^1$H and $^{13}$C nuclei. That this multiplicity of lines arose from the presence of different ring conformers was suggested from the $^{13}$C spectrum which showed 12 lines in the aliphatic region ($\delta$ 30-50) rather than the anticipated three. This increased number of lines was rationalized by noting that each amide group can exist in either Z (trans) or E (cis) stereochemistry due to restricted amide rotation (see Figure 5.15 for a key to Z/E nomenclature in amides): this enables the cyclic disulfide to adopt, in principle, three conformations with the amide groups Z,Z, Z,E and E,E.

6.2.2 $^1$H DNMR Studies

Variable temperature $^1$H NMR spectroscopy was employed to determine the presence of conformational isomers. Studies in the temperature range -61.5 °C to 30.0 °C in CDCl$_3$ indicated that the conformers were in slow exchange at room temperature and below, thereby conferring distinct stereochemical environments of the alkyl substituents. At higher temperatures in DMSO-$d_6$, interconversion rates increased, as evidenced from the gradual coalescence of all resonances (see Figure 6.3). However, it was not possible to observe the three signals expected in the regime of fast exchange at 500 MHz, as this would require heating the sample to an impractically-high level (i.e., $> 127.4$ °C).

To verify this chemical rate process as restricted amide rotation, the free energy of activation, $\Delta G^\ddagger$, was estimated using Equation 5.1. $T_c$ was estimated from Figure 6.3 to be about 127 °C at 500 MHz, based on the coalescence of the singlets at 2.42 and 2.71 ppm (later ascribed to the N-CH$_3$ resonances of the unsymmetrical Z,E conformer). This afforded $\Delta G^\ddagger_{127} = 19.3$ kcal/mol. The measured barrier is consistent with those typically reported for substituted $N,N'$-dimethylamides (e.g., dimethylacetamide, $\Delta G^\ddagger_{25.2} = 17.4$ kcal/mol; dimethylpropanamide, $\Delta G^\ddagger_{25.2} = 16.7$
Figure 6.3: 500 MHz $^1$H NMR variable temperature spectra of 40 in DMSO-d$_6$. 

T (°C)
kcal/mol).\textsuperscript{117} Restricted rotation associated with the S-S linkage and ring inversion presumably contributes to this composite $\Delta G^\ddagger$, thus magnifying it in relation to typical acyclic amide rotational barriers. Therefore, a fluxional process is responsible for the complexity of the room temperature NMR spectra of 40 and it is most likely a result of restricted rotation about the amide groups, which leads to the formation of discrete ring conformers. Other likely restricted rotation-based explanations would include conformational preferences at the disulfide linkage; however, this effect is not likely to be greater than 15 kcal/mol in cyclic systems (e.g., see Section 6.3.2).\textsuperscript{120,121}

A heteronuclear $^1$H-$^{13}$C 2D NMR shift correlated experiment at room temperature identified the geminal pairs of diastereotopic protons for three geometric isomers and, with the aid of standard chemical shifts, permitted the location of their NCH$_2$CH$_2$N groupings. Two of these appeared as sets of A$_2$B$_2$ (or AA′BB′) spin systems in the $F_1$ ($^1$H) domain, consistent with two symmetrical isomers, and the third as a more complex ABCD spin system for an unsymmetrical isomer (see Figure 6.4). Crossections in $F_2$ ($^{13}$C) revealed proton $^2J$ values of normal magnitudes (ca. -13 to -15 Hz, see Figure 6.5 and Table 7.5). The experiment also permitted the assignment of carbon signals associated with the three isomers. The $^1$H-$^1$H connectivity patterns in each isomer were confirmed through a homonuclear 2D COSY-45 NMR experiment (see Figure 6.6).

The findings of the 2D and DNMR experiments, as well as patterns in signal multiplicity, suggested the presence of the three, interconvertible, conformational isomers of 40 at room temperature. These ring conformers have been attributed to two Z,Z and a Z,E amide configurations (see 43 and 44, respectively). Among the Z,Z structures, the designation Z,Z$_1$ refers to the major ring conformer while Z,Z$_2$ indicates the minor one. These geometries have been shown to be consistent with the results of a
Figure 6.4: $^1$H-$^{13}$C chemical shift correlated NMR spectrum of 40 in CDCl$_3$ at 303 K.
Figure 6.5: Crossections in $F_2$ from the $^1$H-$^{13}$C chemical shift correlated NMR spectrum of 40 in CDCl$_3$ at 303 K.
* Folded signal.

Figure 6.6: The 500 MHz $^1$H-$^1$H NMR COSY-45 spectrum of 40 in CDCl$_3$ at 303 K.
number of experimental approaches, including benzene dilution $^1$H NMR shifts, nuclear Overhauser effect (NOE) experiments, single crystal X-ray diffraction studies, molecular modelling and proton shielding calculations (vide infra).

Proton integrals and $^{13}$C resonance intensities (Tables 7.5 and 7.6) suggested the following stability trend among the conformers: $Z,Z_1 > Z,E > Z,Z_2$. This finding can be rationalized through the sterically-driven, conformational preferences governing $N,N'$-dialkylamides: in large-membered lactams, the $Z$ or trans stereochemistry is energetically favored over $E$ (cis). Consequently, the $Z$ configuration in 40 can be expected to be the predominant amide stereochemistry in solution. Based on the ratio of intensities of the N-CH$_3$ $^{13}$C signals (Table 7.6) and, assuming a Boltzmann population distribution at 303 K, the $Z,Z_1$ ring conformer was favored over the $Z,E$ by 0.61 kcal/mol and $Z,E$ over the $Z,Z_2$ by 0.87 kcal/mol. Table 6.1 presents the corresponding percentage compositions in CDCl$_3$, as well as in DMSO-$d_6$ and C$_6$D$_6$ solvents. The results suggested that the isomeric composition of 40 appeared to be relatively independent of solvent.

The most noteworthy NMR feature of 40 is the large geminal shift differences ($\Delta\delta_{OBS}$) between the N-CH$_2$ protons of the $Z,Z$ and, to a lesser extent, $Z,E$ conformers. In CDCl$_3$, the largest $\Delta\delta_{OBS}$ was measured at 2.17 ppm for the minor $Z,Z_2$
Table 6.1: Calculated and observed population distributions for conformers ZZ₁, Δ,E and ZZ₂ at 303 K.

<table>
<thead>
<tr>
<th>Conformer</th>
<th>Calc. E_{Steric} (kcal/mol)</th>
<th>calculated Population Distribution</th>
<th>Observed Population Distributions</th>
<th>CDCl₃</th>
<th>DMSO-d₆</th>
<th>C₆D₆</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZZ₁ (40a)</td>
<td>0</td>
<td>82%</td>
<td>53%</td>
<td>54%</td>
<td>54%</td>
<td></td>
</tr>
<tr>
<td>Δ,E (40c)</td>
<td>2.03</td>
<td>3%</td>
<td>39%</td>
<td>29%</td>
<td>38%</td>
<td></td>
</tr>
<tr>
<td>ZZ₂ (40c)</td>
<td>1.15</td>
<td>15%</td>
<td>8%</td>
<td>6%</td>
<td>8%</td>
<td></td>
</tr>
</tbody>
</table>

a According to the Boltzmann relationship:

\[
\frac{n_2}{n_1} = \exp(-\Delta G/RT), \quad T=303 \text{ K}
\]

b Based on ¹H NMR integrals.

c Small amounts (< 12%) of additional conformers were detected and measured using ¹³C NMR intensities.

isomer, while the ZZ₁ and Δ,E configurations recorded 2.05, 1.15 and 1.29 ppm, respectively (Table 7.5). The chemical shift differences were invariant with the concentration of 40 in CDCl₃, precluding any effects due to intermolecular association. These results suggested that the N-CH₂ (and S-CH₂) groups in the symmetrical ZZ isomers are pairwise equivalent or enantiotopic and that the N-CH₂ protons have markedly different magnetic environments. Large geminal proton chemical shift differences have been observed in a few rigid, polyamide systems.¹²³-¹²⁵ In cyclotetrasarcosyl, 45, the isochronous pairs of the N-methylene protons are separated by about 2 ppm (in CDCl₃),¹²⁴ while one set of those for the tetrathiacyclododecane 46 have a chemical shift difference of 1.78 ppm (in CDBr₃).¹²⁵

Invariably, such large (i.e., > 1.5 ppm) N-carbonyl splittings appear to be associated with the sterically-favored Z amide stereochemistry, which places these protons in or close to the respective amide planes pointing toward the C=O groups.¹²⁶
6.2.3 NOE Studies

To assist in the solution state conformational determination of 40, NOE experiments were carried out in CDCl$_3$ at 303 K (500 MHz) on signals of the two major isomers. Representative difference NOE spectra for these isomers are shown in Figures 6.7 and 6.8. The inserts illustrate NOE interactions for a given geometric isomer, together with their interatomic distances as determined from MM2-calculated structures.

Unfortunately, adequate NOE determinations were not possible for the minor $Z,Z_2$ isomer, largely because of its low abundance and complications involving transfer of saturation. The latter phenomenon results from radiation transfer from the saturated resonance into other signals that are undergoing mutual chemical exchange,$^{127}$ namely the more populated $Z,Z_1$ and $Z,E$ isomers. Saturation transfer effects were also observed for the $Z,E$ and, to a lesser extent, the $Z,Z_1$ conformers; however, useful conformational information can still be obtained from these spectra if some caution is applied in spectral interpretation.

In the major isomer, irradiation of the AB $S-CH_2$ quartet at 3.43 ppm resulted in a large NOE of the $N$-methyl protons at 3.13 ppm [see Figure 6.7(a)], indicative of a pseudoaxial $S-CH$ and $N-CH_3$ proximity about a $Z$ amide configuration.
Figure 6.7: 500 MHz NOE difference spectra (a-c) of the $Z,Z_1$ stereoisomer of 40 in CDCl$_3$ at 303 K. The irradiated peak is indicated at the arrowed (↓) frequency.
This enhancement was not expected for an E stereochemistry, as a larger distance would separate the appropriate protons. The observation of a NOE between the shielded N-CH$_2$ (2.64 ppm) and N-CH$_3$ [see Figure 6.7(b)] was consistent with a Z linkage, where the former protons occupy a pseudoequatorial position syn to the adjacent CH$_3$ group. Irradiation of the N-CH$_3$ resonance caused NOEs at, not only the expected positions of the shielded N-CH$_2$ and S-CH$_2$ protons, but also at the deshielded N-CH at 4.68 ppm [see Figure 6.7(c)]. The weaker enhancement of the deshielded N-CH (relative to the shielded protons) suggested a transannular proximity with its opposite N-CH$_3$ group - an interaction made possible through pseudoaxial configurations for both groups. Thus, a C$_2$ symmetric Z,Z conformation best matched the NOE observations noted for the major solution conformation of 40 (see 40a, Figure 6.14).

In the non-symmetric isomer, irradiation of the S-CH signal at 3.55 ppm induced a strong enhancement of its geminal partner at 3.99 ppm as well as a weaker enhancement of the N-CH$_2$ protons at 4.27 ppm [see Figure 6.8(a)]. Based on the analysis of molecular models, the latter NOE was only possible between the pseudoaxial S-CH [H(3b)] and the pro-R N-CH [H(6b)] about the E amide group. The X-ray structure and molecular mechanics calculations (see Figures 6.11 and 6.14, 40c) supported this result: with an interatomic separation of 1.97 Å, the electron clouds of these protons were overlapping by about 0.4 Å. The positive N-CH$_3$ enhancement at 3.24 ppm was most likely a result of saturation transfer to S-CH [H(10a)] at 4.16 ppm by which it is strongly relaxed [see Figure 6.8(c)]. Barring geminal enhancements, NOEs were not observed for the second E S-CH [H(3a)] signal at 3.99 ppm: this was consistent with a pseudoequatorial position [see Figure 6.8(b)].

Irradiation of the S-CH [H(10a)] resonance at 4.16 ppm [see Figure 6.8(c)]
Figure 6.8: 500 MHz NOE difference spectra (a-d) of the Z,E stereoisomer of 40 in CDCl$_3$ at 303 K. The irradiated peak is indicated at the arrowed (↓) frequency.
induced strong enhancement of the downfield N-CH₃ protons at 3.24 ppm which was indicative of a dipseudoaxial disposition of protons about a Z amide bridge. Analysis of the Z,E computed model revealed that the point of closest approach of these protons was ca. 1.6 Å. Since this NOE was not consistent with an E amide stereochemistry, the enhancement supported the prediction that the downfield N-CH₃ (and N-CH₃ by ¹H-¹³C shift correlation, see Figure 6.4) belonged to the Z amide group in the Z,E conformer.

Irradiating the co-resonating, downfield N-CH₂ protons [H(6b) and H(7a)] at 4.27 ppm created moderate NOEs at 3.55, 3.10 and 3.01 ppm [see Figure 6.8(d)]. The 3.55 ppm enhancement arises from interaction of H(6b) with H(3b) [as in Figure 6.8(a)]. The latter two enhancements were most likely due to geminal interactions with the shielded Z and E N-CH₂ protons [H(7b) and H(6a), respectively]. Thus, a Z,E conformation best correlated with the NOE data obtained for the non-symmetric component of 40 (see 40c, Figure 6.14).

6.2.4 Aromatic Solvent-Induced Shift (ASIS) Studies

The inability of NOE experiments to elucidate structural details for the minor Z,Z₂ component prompted an ASIS investigation to determine its solution state geometry. Studies on a variety of amides have consistently shown that aromatic solvents shift the resonance peaks of the N-Cₓ protons trans to the carbonyl group upfield to a greater extent than those at the cis position. On this basis, these resonances can be assigned to a particular amide stereochemistry.

The accepted model which rationalizes the ASIS effect in N-alkylamides is illustrated in Figure 6.9. The amide molecules associate in an electrostatic manner with the aromatic solvent molecules so as to place the nitrogen atom, with its fractional
positive charge, close to the region of high $\pi$-electron density in the aromatic ring and the negatively-charged carbonyl oxygen positioned far from the electron rich $\pi$-orbitals. In this coplanar arrangement of amide and benzene molecules, the $Z$ methyl group would experience the large diamagnetic anisotropy at the center of the ring and would shift to higher field to a greater extent than the $E$ methyl group. A study of the $^1$H NMR spectrum of 40 (see Figure 6.10) in benzene-$d_6$ revealed that the N-CH$_3$ (and N-CH$_2$) resonances of both major ($Z,Z_1$) and minor ($Z,Z_2$) isomers exhibited very similar upfield aromatic solvent-induced shifts (0.56 and 0.61 ppm, respectively, relative to the CDCl$_3$ shifts). Since the major isomer was confirmed to have the $Z,Z$ geometry, the benzene dilution experiments suggested that the minor isomer also possesses the $Z,Z$ configuration. Their relatively large ASIS values were also consistent with a $Z$ geometry.$^{133}$ Alternatively, an $E,E$ stereochemistry in the minor isomer would have resulted in a smaller ASIS value and an increased separation between the N-CH$_3$ resonances of the major and minor isomers upon benzene-$d_6$ dilution. It is worth noting that the N-CH$_2$ splitting for the $Z,Z_1$ and $Z,Z_2$ ring conformers has increased to 2.58 and 2.69 ppm, respectively, in benzene-$d_6$. 
Figure 6.10: The 500 MHz 1H NMR spectrum of 40 in C₆D₆ at 303 K.
6.2.5 Solid State Structure Studies

An X-ray diffraction study of 40 was undertaken in order to establish unambiguously the geometry which could give rise to the enormous NMR shift differences observed for the geminal methylene protons.

The geometry and numbering of the X-ray structure of 40 is shown in Figure 6.11; crystal data, atomic coordinates, bond lengths and bond angles are listed in Tables A.1 to A.6 in the Appendix. As the structure analysis revealed, the diamide 40 crystallized into the Z,E geometry. This was a surprising result since NMR measurements had shown the Z,Z¹ isomer to be the dominant geometry in solution (Table 6.1). A survey of the bond angles and bond lengths in 40 indicated no unusual deviations from normal values. The disulfide and amide torsional angles (Table A.5) are very close to ground state values (i.e., 90° and planarity, respectively) indicating low torsional strain in the Z,E ring.

A stereodiagram of the unit cell of the Z,E structure of 40 is shown in Figure 6.12. The unit cell contains two Z,E molecules which are related by a center of inversion. Molecules stack in columns with a high degree of packing efficiency given the unsymmetrical nature of the Z,E isomer. The most significant intermolecular contact is O(4)⋯C(8) which is 3.23 Å. Intramolecular non-bonding contacts were observed between O(9) and H(7a), and H(3b) and H(6b). These nuclei - separated by 2.346 Å and 2.007 Å, respectively - are within the sum of the accepted van der Waals radii for oxygen and hydrogen (1.4-1.6 and 1.2 Å, respectively).¹³⁴,¹³⁵ These contacts may contribute, in part, to the rather large NMR shift differences observed for the geminal hydrogen nuclei of the Z,E conformation (see Table 7.5).

An understanding of the proton geometry about the Z and E amide groups - as provided by the crystal structure - allowed for an interpretation of the large Δδ_obs
Figure 6.11: The crystal structure of the Z,E isomer of 40. 30% probability thermal ellipsoids are shown for non-hydrogen atoms.
Figure 6.12: Stereodiagram of the unit cell of the Z,E structure of 40.
noted for the geminal N-CH$_2$ protons (see Table 7.5). In addition, the excellent agreement between the NOE-derived conformational model of the Z,E isomer and the crystal structure established a link to proton shift assignments. For example, the magnitude of $\Delta \delta_{OBS}$ could be qualitatively explained by the positions of the geminal protons relative to their adjacent carbonyl group: in general, a proton will be more deshielded as it approaches the plane and/or midpoint of a carbonyl group.$^{126}$ In the Z amide system, H(7a) and H(7b) (see Figure 6.11) are 10.4° and 13.9° out of the amide plane defined by atoms O(9)-C(9)-N(8) and are located 2.359 and 3.359 Å respectively from the midpoint of the C=O bond. Therefore, H(7a) should resonate downfield from H(7b). Similarly, H(6b) and H(6a) of the E amide are 170.6° and 151.6° removed from the O(4)-C(4)-N(5) plane and are situated at a distance of 3.272 and 3.654 Å respectively from the midpoint of the carbonyl bond. Since H(6b) is "closer" to its adjacent amide group, it should appear at a lower shift than its geminal partner. It is interesting to note that the very similar deshielding influences on H(7a) and H(6b) appears to result from two different orientations of the protons - namely, syn and anti configurations, respectively - with respect to their adjacent C=O group. The above analysis of shielding trends was valuable in the assignment of proton chemical shifts in the Z,Z isomers (see Table 7.5).

If X-ray crystallographic data are used to assist in the structural elucidation of a molecule in solution, it is useful to present some evidence to support similarity between the molecular geometry in solution and in the solid state. This criterion was satisfactorily met through NOE determinations of the Z,E isomer. Support for a comparison of the solution and solid state geometries was primarily based on the characteristic NOE interactions between the H(6b)···H(3b) and the H(10b)···Z N-CH$_3$ protons, which were also noted in the crystal structure. Another method
involves a comparison of $^{13}$C NMR spectra of the molecule in both states. To this end, the cross-polarization, magic angle spinning (CP-MAS) $^{13}$C NMR spectrum of crystalline 40 was determined in the solid state at 25 MHz (see Figure 6.13). Unfortunately, excessive broadening of the $^{13}$C resonances caused by dipolar coupling to $^{14}$N nuclei, as well as signal overlap and sensitivity complications, prevented an unequivocal correlation of characteristic shifts between the solid state and solution state spectra in the 30-50 ppm range. Spectra recorded at a higher field and/or higher sample concentration would probably resolve this inadequacy.

An interesting outcome of the solid state experiment, however, was noted in the carbonyl region: two broad signals of unequal intensity (167.30 and 174.39 ppm) suggested the presence of at least two conformers in the crystal lattice. If the recrystallized sample of 40 consisted only of the Z,E geometric isomer, then only two, equally-intense resonances would be observed in this region. Based on chemical shift and intensity comparisons with the solution state $^{13}$C spectrum (see Figure 6.2), the more intense signal at 167.30 ppm was ascribed to the Z,Z$_1$ structure, while the unresolved resonance at 174.39 ppm was indicative of the expected Z,E conformer. The observation of two solid state species could be explained by the co-crystallization of Z,Z and Z,E structures. The rather high barriers to amide isomerization could promote the crystallization of the Z,Z and Z,E amide ring conformers into separate lattice structures. For example, many acyclic monoamides have been reported to crystallize in either the Z or E geometry, with only one geometric rotamer in each unit cell.$^{136}$

6.3 Computational Studies

The foregoing X-ray structure determination of the Z,E isomer provided a powerful link between $^1$H NMR chemical shift and geometry. This information was
Figure 6.13: The 25 MHz CP-MAS $^{13}$C spectrum of 40 at 303 K.
crucial not only for NMR shift assignments, but also for identifying different shielding mechanisms which have manifested themselves as unusually large $\Delta \delta_{\text{obs}}$ values. Similarly, structural models of the Z,Z isomers were required to assist in NMR signal assignments and to provide an insight into the origins of their large N-CH$_2$ anisochronism. Consequently, a number of computational methods were investigated.

6.3.1 MMX Calculations

Molecular mechanics calculations were performed using PCMODEL to evaluate the conformational geometries and energies of the Z,Z$_1$ and Z,Z$_2$ structures. From over 90 unique starting geometries, a total of five conformers were found for 40 within 5 kcal/mol of the global minimum structure (see Table 6.2, Figure 6.14). Conformers 40a (Z,Z), 40b (Z,Z) and 40c (Z,E) agreed well with the structural models inferred from NOE, thermodynamic, X-ray and NMR symmetry data. Based on this correlation, the observed conformers in the NMR spectra of 40 - Z,Z$_1$, Z,Z$_2$ and Z,E - were proposed to have similar structural characteristics as 40a, 40b and 40c, respectively.

The X-ray determination of 40 confirmed one isomer to have the Z,E configuration (see Figure 6.11) which was presumed to be the source of the unsymmetrical set of NMR resonances. Among the set of Z,E isomers, 40c provided the best correlation between calculated and experimental Boltzmann distributions (Table 6.1). Moreover, when 40c was overlapped with the X-ray structure in PCMODEL, it was evident that they were similar (RMS deviation of atoms = 0.037 Å). To achieve a more realistic comparison, conformational changes that might be imposed on 40 by crystal lattice forces were removed from the X-ray structure by subjecting the molecule to the PCMODEL force field energy minimization program. The observed
Figure 6.14: Stick plots of the five lowest, MMX energy conformers of 40 (40a - 40e). The ethylene moiety is located at the front of each conformation and is aligned along the x-axis for all plots.
Table 6.1: Characterization data for the five lowest energy MMX conformers (40a - 40e) of 40.

<table>
<thead>
<tr>
<th>Conformer</th>
<th>Symmetry</th>
<th>Group</th>
<th>C-S-S-C</th>
<th>C-C_{sp2}N</th>
<th>CH_{3}-N-C=O</th>
<th>Torsional Angle (deg)</th>
<th>Band Angle (deg)</th>
<th>Band Length (\AA)</th>
<th>( \Delta E ) (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40a (Z/E)</td>
<td>C_{2}</td>
<td>R</td>
<td>-104.2</td>
<td>94.6</td>
<td>119.3</td>
<td>119.3</td>
<td>-119.3</td>
<td>2.073</td>
<td>197.2</td>
</tr>
<tr>
<td>40b (Z/E)</td>
<td>C_{2}</td>
<td>L</td>
<td>117.2</td>
<td>171.8</td>
<td>121.5</td>
<td>121.5</td>
<td>-121.5</td>
<td>2.073</td>
<td>177.2</td>
</tr>
<tr>
<td>40c (Z/E)</td>
<td>C_{2}</td>
<td>L</td>
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<td>87.3</td>
<td>120.4</td>
<td>120.4</td>
<td>-120.4</td>
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<tr>
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<td>L</td>
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<td>119.4</td>
<td>-119.4</td>
<td>2.070</td>
<td>0.9</td>
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<tr>
<td>40e (Z/E)</td>
<td>L</td>
<td>L</td>
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<td>29</td>
<td>19.2</td>
<td>19.2</td>
<td>-19.2</td>
<td>2.035</td>
<td>29</td>
</tr>
</tbody>
</table>

\(^a\) Relative steric energy.

\(^b\) R=right-handed chirality, L=left-handed chirality.
change amounted to approximately 0.6 kcal/mol energy loss upon minimization. The resultant "minimized X-ray" structure was then superimposed on 40c and 40d to afford a virtually complete overlay with the former calculated structure (RMS deviation of atoms = 0.021 Å) but gave a poorer alignment with the latter (RMS deviation of atoms = 0.198 Å). Molecular mechanics had thus accurately predicted the solid state geometry of the Z,E conformer. This fact, coupled with the strong similarities between NOE and computed structural models, suggested that the MM2 force field was a reasonable probe for elucidating the solution state conformations of 40. Conformer 40e is the right-handed disulfide isomer of both 40c (and the X-ray structure) and thus inadmissible.

Conformers 40a and 40b possess C₂ symmetry and a Z,Z configuration with both amide functions essentially coplanar and oriented in opposite directions. This antiparallel Z,Z arrangement has been reported for other N-substituted polyamide heterocycles, including cyclic amides 45 and 46. Steric and electronic factors may account for the configurational preference found in these systems. For example, in a parallel conformation, transannular methyl-methyl contacts and a large internal dipole moment (resulting from the sum of the moments from the parallel C=O groups) will undoubtedly be very destabilizing.

Molecular modelling data and mechanical models suggested isomers Z,Z₁ and Z,Z₂ are disulfide ring conformers. At room temperature, interconversion of these rotational isomers was sufficiently slow on the NMR time scale to allow detection by 13C NMR spectroscopy (Table 7.6). Interconversion occurs presumably through the cis transition state by ca. 180° rotation about the asymmetric S-S bond (see Figure 6.15). A 13C DNMR study was carried out with the objective of validating the molecular modelling results through the correlation of a measured barrier to interconversion to
Figure 6.15: Ball and stick plots of the MMX-calculated diastereomers 40a and 40b, illustrating rotational isomerization about the S-S bond.
one for a disulfide rotation.

6.3.2 Measurement of the S-S Rotational Barrier for the \( \text{Z}_1 \leftrightarrow \text{Z}_2 \) Equilibrium via \( ^{13}\text{C} \) DNMR Spectroscopy

The measurement of exchange rate constants as a function of temperature enables the calculation of activation parameters for an underlying chemical or physical process. For chemical systems undergoing conformational exchange, the energy barrier to interconversion has traditionally been measured by the free energy of activation, \( \Delta G^\neq \), which may be calculated at a single temperature by means of the Arrhenius equation:

\[
k = A \exp(-\Delta G^\neq /RT)
\]

One can also estimate the enthalpy of activation \( \Delta H^\neq \) and entropy of activation \( \Delta S^\neq \) from an Eyring plot of \( \ln(k/T) \) vs \( 1/T \). However, most of the careful studies concerned with conformational processes in reasonably inert solvents indicate that \( \Delta G^\neq \) values at different temperatures are equal\textsuperscript{137} within the experimental error (± 0.2-0.5 kcal/mol), suggesting negligible \( \Delta S^\neq \) values and \( \Delta H^\neq \) almost coincident with \( \Delta G^\neq \). Thus, in view of this result and the difficulty in obtaining accurate activation parameters,\textsuperscript{138} the ensuing discussion will be confined to considerations of \( \Delta G^\neq \) for rotation.

In order to acquire rate constants over a large as possible temperature range, the higher boiling DMSO-\( d_6 \) was used in place of CDCl\( _3 \) as a solvent for 40. However, the \( ^{13}\text{C} \) NMR spectrum in DMSO-\( d_6 \) revealed small amounts of of additional isomers, the signals of which created an uncertainty in the assignment of the small \( \text{Z}_1\text{Z}_2 \) N-CH\( _2 \) signal. Moreover, the chance of an inaccurate assignment was compounded by solvent shifts. Consequently, to account for these effects, a high field \( ^1\text{H} - ^{13}\text{C} \) shift correlated
experiment was carried out and confirmed the chemical shifts of the $Z,Z_2$ isomer (see Figure 6.16).

Warming from 25.0 to 56.7 °C resulted in a temperature-dependent broadening and a subtle high-field migration of the $Z,Z_2$ N-C$_\alpha$ signal at 45.4 ppm (see Figure 6.17) - a phenomenon characteristic of a rate process. Rate constants, $k$, for this chemical exchange process were evaluated using the complete bandshape (CBS) method$^{139}$ (Figure 6.17). Based on the two-site exchange of the $Z,Z_1$ and $Z,Z_2$ N-CH$_2$ resonances (43.4 and 45.4 ppm, respectively) between 27.0 and 40.2 °C, the barrier to $Z,Z_1 \rightleftharpoons Z,Z_2$ interconversion ($\Delta G^\ddagger$) was measured to be $14.5 \pm 1.3$ kcal/mol (see Table 6.3, Figure 6.18). The height of this barrier lies close to the range reported for disulfide

<table>
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<th>$T$ (K)</th>
<th>$k$ (s$^{-1}$)</th>
<th>$1/T$ (K$^{-1}$)</th>
<th>ln $k$</th>
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<td>0.00303</td>
<td>1.5261</td>
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rotations in cyclic systems. For example, the barriers to inversion for 1,2-dithiane and 3,3,6,6-tetramethyl-1,2-dithiane have been reported to be 11.6 and 13.6 kcal/mol, respectively.$^{140,141}$

The barrier of 14.5 kcal/mol was too low to be attributed to an amide
Figure 6.16: $^1$H-$^{13}$C chemical shift correlated NMR spectra of 40 in DMSO-$d_6$ at 303 K.
Figure 6.17: Variable temperature, 126 MHz $^{13}$C NMR spectra of the $Z,Z_1$ and $Z,Z_2$ N-CH$_2$ signals of 40 in DMSO-$d_6$, with corresponding simulated spectra.
Figure 6.18: The Arrhenius plot of $\ln k$ vs. $1/T$ for the $Z_1Z_2 = Z_2Z_1$ conformational equilibrium.
rotation, particularly one occurring in a cyclic system (cf. the 19.3 kcal/mol barrier determined for amide rotation in the Z,E ring conformer, Section 6.2.2) and, although it was at the high end for reported disulfide rotations, it was reasonable to attribute the \( Z,Z_1 \rightleftharpoons Z,Z_2 \) chemical exchange process to a disulfide rotational mechanism as illustrated in Figure 6.15. A large component of the observed barrier is reflected in the energy profile of the acyclic disulfide bond, which has a maximum of \( ca. 8-10 \) kcal/mol at a CSSC dihedral angle of 0° (i.e., for the S-cis transition state) and a minimum at 90° (see Figure 6.19).\(^{142}\) The barrier is thought to arise principally from the mutual

![Figure 6.19: The lowest energy conformation of the RS-SR bond.](image)

repulsion between the unshared pairs of 3pz nonbonded electrons on adjacent sulfur atoms.\(^{143}\) In functionalized, cyclic disulfides such as 40 however, the presence of transannular electrostatic and steric interactions will increase the height of the disulfide rotational barrier. Moreover, the amide groups presumably confer additional rigidity to the ring and may also be a contributing factor to the relatively high barrier.
It is interesting to note that S-S rotation generated diastereomeric conformations and not enantiomers that are commonly observed in acyclic, unsymmetric disulfides.\textsuperscript{144} The asymmetric nature of the ring introduces a second source of chirality, which, in combination with asymmetric disulfide rotation, confers a diastereomeric relation between the \(Z,Z_1\) and \(Z,Z_2\) stereoisomers.

Unlike the \(Z,Z_2\) isomer, the S-CH\(_2\) protons in the \(Z,Z_1\) structure did not possess pseudoaxial geometry (see 40a, Figure 6.14). Analysis of space-filling, mechanical models of the \(Z,Z_2\) structure indicated that a pseudoaxial, S-CH\(_2\) configuration brought one set of lone electron pairs on oppositely-related O and S atoms into close proximity. It appeared that the compromised, dipseudoequatorial geometry of the \(Z,Z_1\) conformation defused this transannular interaction by directing the electrons away from each other. Moreover, a pseudoequatorial configuration of S-CH\(_2\) protons was expected to be sterically preferable to a pseudoaxial one as 1,3-diaxial interactions with the methyl groups will destabilize the latter conformation. A combination of these electronic and steric effects may serve to explain the calculated and observed population distributions between the \(Z,Z_1\) and \(Z,Z_2\) ring conformers (Table 6.1).

The S-CH\(_2\) chemical shifts also supported the calculated results which indicated the \(Z,Z_1\) and \(Z,Z_2\) conformations to be disulfide ring conformers. For example, the small chemical shift difference of 0.03 ppm between isochronous S-CH protons of the \(Z,Z_1\) isomer (Table 7.5) is indicative of very similar magnetic environments. This observation was consistent with the dipseudoequatorial geometry of calculated structure 40a. Alternatively, the larger 0.72 ppm difference noted for the corresponding protons of the \(Z,Z_2\) structure correlated well with the magnetically dissimilar, pseudoaxial-pseudoequatorial environments predicted for the calculated 40b
conformer.

MMX-conformational energies agreed moderately well with those of the observed populations (Table 6.1). Since molecular mechanics calculations mimic isolated molecules in their gaseous state, exact agreement between calculated and experimental populations is unattainable given the present level of computational sophistication. This situation arises from the inability to accurately incorporate solvent effects into the calculations. In addition, certain conformations may be differentially stabilized by solvation, particularly in higher energy geometries. Consequently, the more energetic, gas-phase Z,E (40c) conformation may be stabilized to a greater extent than the lower energy Z,Z₂ structure, resulting in a thermodynamic reversal of solution state population levels. The effect may serve to rationalize the differences between calculated and experimental population distributions (Table 6.1), particularly for the Z,E structure.

In analogy to the chemical shift assignments made for protons of the Z,E crystal structure (see Section 6.2.5), the orientation of a MMX-calculated N-CH proton - in relation to its adjacent C=O group - will primarily determine its chemical shift. Consequently, for isomers 40a and 40b (see Figure 6.14), one isoergic N-CH pair occupied a pseudoaxial configuration (denoted as H₉) which was essentially cis coplanar to the C=O group (i.e., 9.2 and 1.4 degrees removed from the amide plane defined by the atoms O, C and N) and, therefore, should be the low-field protons at 4.68 and 4.88 ppm, respectively. An interesting confirmation of this pseudoaxial geometry was noted in the ¹H-¹H COSY spectrum (see Figure 6.6), where the 4.68 ppm signal was weakly correlated to the N-CH₃ resonance at 3.13 ppm. This was indicative of a stereospecific, four-bond coupling originating from the "W" configuration which is often observed between axial protons alpha to C-18 or C-19 methyl groups in
steroids. Four-bond coupling to the methyl protons was not possible for the pseudoequatorial protons in this molecular configuration.

The second, isochronous set of geminal N-CH<sub>eq</sub> protons in the Z,Z<sub>1</sub> and Z,Z<sub>2</sub> ring isomers occupied pseudoequatorial positions (denoted as H<sub>eq</sub>) that were approximately 20.9 and 15.6 degrees out of the O=C-N amide plane. Their respective chemical shifts at 2.64 and 2.71 ppm implied that they were shielded in comparison to the rotationally-averaged, Z,Z N-CH<sub>2</sub> shift of 3.28 ppm for the open chain precursor 39 (Table 7.3). A comparison at a more quantitative level suggested that the H<sub>eq</sub> protons were shielded by ca. 0.64 and 0.57 ppm, while the H<sub>ax</sub> protons were deshielded by 1.40 and 1.60 ppm, respectively. Clearly, the shielding mechanisms - particularly the deshielding forces - at work in the conformational isomers of 40 were quite significant and worthy of further investigation.

6.4 Shielding Calculations

The objective of this section is to investigate the extent to which it is possible to identify the origins of and to quantify the large N-CH<sub>2</sub> nonequivalences observed in the Z,Z<sub>1</sub> and Z,Z<sub>2</sub> conformers, particularly in terms of the neighbouring group effect of the amide groups. Inductive and mesomeric (resonance) through-bond effects of the amide function were not expected to asymmetrically affect the N-CH<sub>2</sub> unit and assumed not to contribute to geminal nonequivalence (i.e., Δδ<sub>DIA</sub> (local) = 0). Consequently, the difference in the N-CH<sub>2</sub> shifts could be primarily attributed to through-space shielding mechanisms.

The observed chemical shift difference, Δδ<sub>OBS</sub>, between a geminal pair of N-CH<sub>2</sub> protons in the rigid heterocycle 40 can be evaluated from Equation 6.2.\textsuperscript{147}
\[
\Delta \delta_{\text{OBS}} = \Delta \delta_{\text{MAG}} + \Delta \delta_{\text{EL}} + \Delta \delta_{\text{SOL}} + \Delta \delta_{\text{VDW}}
\]  

[6.2]

The terms \( \Delta \delta_{\text{MAG}} \), \( \Delta \delta_{\text{EL}} \), \( \Delta \delta_{\text{SOL}} \) and \( \Delta \delta_{\text{VDW}} \) refer to the net chemical shift between N-CH\(_2\) protons ascribed to shielding contributions from magnetic anisotropy, electric field, solvent and van der Waals effects of all neighbouring groups in the molecule. The magnitude of these contributions to \( \Delta \delta_{\text{OBS}} \) will now be examined in turn.

6.4.1 The Solvent Shift Contribution, \( \Delta \delta_{\text{SOL}} \)

The polar character of the amide group can contribute to \( \Delta \delta_{\text{OBS}} \) through the polarization of the solvent via a reaction field. However, this shift contribution from the solvent (\( \Delta \delta_{\text{SOL}} \)) was demonstrated by Clayden and Williams to be unimportant in determining the methylene nonequivalence in cyclotetrasarcosyl, 45\(^{148}\). A plot of the N-CH\(_2\) chemical shift difference versus \((\epsilon - 1)/(\epsilon + 1)\), where \( \epsilon \) is the solvent's dielectric constant, suggested that the splitting remained consistently large. The absence of exchangable protons and a concentration dependence for the magnitude of the N-CH\(_2\) shift difference in 40 indicates that specific solvent-solute and/or solute-solute interactions are not likely shift factors either. Consequently, a \( \Delta \delta_{\text{SOL}} \) component to the N-CH\(_2\) nonequivalence in the conformationally similar Z,Z isomers of 40 is not expected to be significant and was neglected from the \( \Delta \delta_{\text{OBS}} \) calculation.

6.4.2 The van der Waals Shift Contribution, \( \Delta \delta_{\text{VDW}} \)

Hydrogen atoms that are subject to intramolecular steric or van der Waals interactions with proximate nuclei often display shielding effects\(^{149}\) that can be more marked than magnetic anisotropy shifts.\(^{150}\) Qualitatively, the direction of shielding can be associated with the net nuclear charge on the resonant proton which, in turn,
depends on the predominance of either an attractive or repulsive interaction. For example, if the resonant proton feels an attractive van der Waals interaction with a proximate group, its effective nuclear charge will increase to result in an upfield shift (shielding) of the hydrogen nucleus. Conversely, in the repulsive van der Waals regime, electrostatic repulsion of interacting electron clouds will induce charge polarization in the H→C bond direction and produce a downfield shift (desthielding) of the resonant proton. In general, intramolecular separations at or smaller than the sum of the van der Waals radii of the interacting groups usually result in repulsive van der Waals interactions, while attractive interactions will dominate at slightly larger interatomic distances. The attractive interaction is relatively weak compared to its stronger repulsive counterpart, making deshielding effects much larger than the shielding ones. Instances of such deshildings have been observed mostly in rigid systems, where one or more hydrogen atoms were forced too close to other groups because of rigid geometrical contraints. For example, the magnitude of the $\delta_{\text{vdw}}$ shift and N-CH$_2$ nonequivalence in a series of hindered amides has been linearly correlated to the size and number of the interacting groups.

In contrast to steric-based observations reported for 45, a $\Delta\delta_{\text{vdw}}$ contribution to $\Delta\delta_{\text{obs}}$ cannot be ruled out since van der Waals contacts involving the downfield H$_{ax}$ protons were detected in the calculated MMX (see Figure 6.20) and crystal structures of 40. The degree of electron cloud overlap between H$_{ax}$ and interacting groups can be taken as a rough measure of van der Waals compression and the resultant downfield shift that will be experienced by H$_{ax}$. For example, a relatively large, downfield shift is predicted for the H$_{ax}$ protons because of the combined overlap of its electron clouds with those from its adjacent carbonyl oxygen and opposite N-CH$_3$ protons. These through-space contacts can be seen in the CPK
Figure 6.20: Space-filling models of 40b from (a) side and (b) top perspectives. Note that the van der Waals compression of the S-CH pseudoaxial proton with the N-CH₃ protons, and the carbonyl oxygen with Hₐₓ. The N-CH₃ group has been rotated by ca. 60° to illustrate steric compression.
space-filling representation of the Z,Z\textsubscript{2} conformer [see Figure 6.20(a)]. where van der Waals radii (r\textsubscript{VDW}) are used to illustrate an "effective size" for the electron cloud surrounding each atom. Calculations based on this scale model indicated that the oxygen (r\textsubscript{VDW} = 1.5 Å\textsuperscript{152} and methyl (r\textsubscript{VDW} = 2.0 Å) groups protrude into the electron cloud of the H\textsubscript{ax} (r\textsubscript{VDW} = 1.2 Å) protons by at least 0.2 and 0.1 Å respectively. The H\textsubscript{eq} protons were not expected to be affected by a paramagnetic van der Waals shift mechanism given its sterically-unencumbered, pseudo-equatorial geometry. However, small diamagnetic shifts were plausible via through-space, attractive van der Waals interactions and/or inductive, through-bond effects transmitted via the induced H\textsubscript{ax} → C bond dipole (vide supra).

It is worthwhile to note in Figure 6.20(b) that the marked van der Waals compression between the pseudoaxial S-CH and proximate CH\textsubscript{3} groups in the Z,Z\textsubscript{2} conformer may be the dominant shielding mechanism responsible for the large geminal isochrony of the former protons (0.72 ppm). A similar interaction may also serve to rationalize the large geminal Z S-CH\textsubscript{2} splitting (1.13 ppm) in the Z,E isomer, where the electron clouds of H(10a) and H(8) (see Figure 6.11) overlapped by as much as 0.8 Å. Other through-space, steric interactions where paramagnetic van der Waals shifts could be significant include the proximate H(3b) and H(6b) protons of the Z,E conformer.

Unfortunately, in the absence of a general, predictive relationship equating both repulsive and attractive van der Waals interactions to chemical shifts, it was not within the scope of this account to calculate a Δδ\textsubscript{VDW} contribution to Δδ\textsubscript{OBS}. Such determinations await the development of a satisfactory quantitative expression or computative method.

In light of the preceding Δδ\textsubscript{SOL} and Δδ\textsubscript{VDW} discussions, Equation 6.2 can be simplified to:
\[ \Delta \delta_{\text{CALC}} = \Delta \delta_{\text{MAG}} + \Delta \delta_{\text{EL}} \]  

[6.3]

In order to carry out the \( \Delta \delta_{\text{CALC}} \) measurements, the following simplifying assumptions were made: 1) the magnetic and electric dipoles of the amide groups were idealized as point dipoles located at the center of the C=O bonds; 2) the amide groups were approximated to carbonyl groups; 3) the magnitude of the N-CH\(_2\) chemical shift difference was dependent primarily on the through-space shielding effects of the amide C=O bond. Assumptions 1) and 2) were necessary because of a lack of a reasonably simple and exact description of the magnetic susceptibility anisotropy and electric field of an amide group. The carbonyl approximation was validated by the diffraction study of 40, which revealed the C=O amide bond lengths to be very close to C=O lengths in ketone systems (1.23 Å versus 1.22 Å, respectively).

The calculations were carried out using the susceptibility anisotropies and \( \kappa \) value derived by ApSimon and Beierbeck from least-squares analysis of methyl shifts in keto-androstanes in CDCl\(_3\).\(^{153}\) The constants \( \Delta \chi \) and \( \kappa \) were based on induced magnetic and electric point dipoles located 0.60 Å from the carbonyl carbon atom halfway along the C=O bond. This position for the center of the point dipoles was selected since it is reported to afford the best correlation between calculated and observed chemical shifts.\(^{147}\)

6.4.3 Magnetic Anisotropy and Electric Field Shift Contributions, \( \Delta \delta_{\text{MAG}} \) and \( \Delta \delta_{\text{EL}} \)

The magnetic anisotropy shift perturbing a N-CH proton, \( \delta_{\text{MAG}} \), from the anisotropic amide dipole can be estimated from the modified form of McConnell’s equation:\(^{154}\)
\[ \delta_{\text{MAG}} = \frac{1}{(3R^3)} \left( \Delta \chi_{xy}(1 - 3\cos^2 \Theta_y) + \Delta \chi_{xz}(1 - 3\cos^2 \Theta_z) \right) \]  

[6.4]

where \( R \) is the distance between the midpoint of the C=O bond and the proton \( H \) under consideration; \( \Delta \chi_{xy} \) and \( \Delta \chi_{xz} \) are the magnetic susceptibilities between the \( y \)- and \( z \)-axes, respectively, and the axis in the C=O \( \sigma \) bond direction (i.e., \( x \)-axis); \( \Theta_y \) and \( \Theta_z \) are the angles between \( R \) and the \( y \)- and \( z \)-axes, respectively [see Figure 6.21(a)].

The electric field shift of a N-CH proton resulting from the through-space influence of the amide dipole can be estimated from the modified form of Buckingham’s equation:\textsuperscript{155}

\[ \delta_{\text{EL}} = \kappa E_{\text{C-H}} \]  

[6.5]

where \( \kappa \) is an experimentally-determined proportionality constant and \( E_{\text{C-H}} \) is a geometrical factor given by Equation 6.6.

\[ E_{\text{C-H}} = \frac{(3 \cos \alpha \cos \beta - \cos \gamma)}{R^3} \]  

[6.6]

\( R \) is the distance between the midpoint of the C=O bond and the proton \( H \) under consideration; \( \alpha \) is the angle between C=O axis and \( R \); \( \beta \) that between \( R \) and the C-H bond; and \( \gamma \) that between the C=O and the C-H vectors [see Figure 6.21(b)].\textsuperscript{147,148}

The results of the magnetic anisotropy and electric field shift calculations for the \( Z, Z_1 \) and \( Z, Z_2 \) stereoisomers using the carbonyl approximation are presented in Tables 6.4 and 6.5, respectively. According to convention, positive \( \delta \) values correspond to \textit{paramagnetic} or \textit{downfield} shifts, relative to the reference compound (TMS), whereas, negative values to \textit{diamagnetic} or \textit{upfield} shifts.

Carbonyl magnetic anisotropy and electric field shift mechanisms satisfactorily accounted for the large observed \( H_{ax} \) deshielding in conformers \( Z, Z_1 \) and
Figure 6.21: Coordinate systems for (a) the magnetic anisotropy shift and (b) the electric field shift determinations depicting anisotropic susceptibilities and geometrical parameters. The x-axis is taken as the axis of symmetry.
Table 6.4: Calculated magnetic anisotropy shifts for the N-CH$_2$ protons of 40a and 40b.

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<th>CONFORMER</th>
<th>N-CH Proton</th>
<th>Θ$_y$ (Deg)</th>
<th>Θ$_z$ (Deg)</th>
<th>R ($\times 10^{-6}$ cm)</th>
<th>$\delta_{\text{MAG}}^{\text{ADJ}}$ b</th>
<th>Θ$_y$ (Deg)</th>
<th>Θ$_z$ (Deg)</th>
<th>R ($\times 10^{-6}$ cm)</th>
<th>$\delta_{\text{MAG}}^{\text{OPP}}$ b</th>
<th>$\delta_{\text{MAG}}^c$</th>
<th>$\Delta\delta_{\text{MAG}}^d$</th>
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<td>H$_{eq}$</td>
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<td>0.62</td>
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<td>1.00</td>
<td>0.57</td>
</tr>
</tbody>
</table>

$^a$ Based on the carbonyl approximation with the dipole origin located at the midpoint of the C=O bond.

$^b$ $\Delta\chi_{xy} = -3.26 \times 10^{-29}$ cm$^3$/molecule; $\Delta\chi_{xz} = -1.82 \times 10^{-29}$ cm$^3$/molecule.$^{153}$

$^c$ $\delta_{\text{MAG}} = \delta_{\text{MAG}}^{\text{ADJ}} + \delta_{\text{MAG}}^{\text{OPP}}$

$^d$ $\Delta\delta_{\text{MAG}} = \delta_{\text{MAG}}(H_{ax}) - \delta_{\text{MAG}}(H_{eq})$
Table 6.5: Calculated electric field shifts for the N-CH$_2$ protons of 40a and 40b.$^a$

<table>
<thead>
<tr>
<th>CONFORMER</th>
<th>N-CH Proton</th>
<th>ADJACENT C=O</th>
<th>OPPOSITE C=O</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>α (Deg)</td>
<td>β (Deg)</td>
<td>γ (Deg)</td>
</tr>
<tr>
<td>40a (Z,Z$_1$)</td>
<td>$H_{eq}$</td>
<td>97.9</td>
<td>31.3</td>
</tr>
<tr>
<td></td>
<td>$H_{ax}$</td>
<td>66.5</td>
<td>82.4</td>
</tr>
<tr>
<td>40b (Z,Z$_2$)</td>
<td>$H_{eq}$</td>
<td>108.1</td>
<td>31.5</td>
</tr>
<tr>
<td></td>
<td>$H_{ax}$</td>
<td>79.9</td>
<td>82.4</td>
</tr>
</tbody>
</table>

$^a$ Based on the carbonyl approximation with dipole origin located at the midpoint of the C=O bond.

$^b$ $\kappa = -10.6 \times 10^{-30}$ cm$^3$/molecule.$^{153}$

$^c\delta_{EL} = \delta_{EL}^{ADJ} + \delta_{EL}^{OPP}$

$^d\Delta\delta_{EL} = \delta_{EL}(H_{ax}) - \delta_{EL}(H_{eq})$
but they did not seem to be the shielding source for the higher field $H_{eq}$ protons. Based on an averaged $Z, Z$ N-CH$_2$ shift of 3.28 ppm (see 39, Table 7.3), C=O anisotropic and electric field shifts provided a paramagnetic contribution totalling 1.10 and 1.03 ppm respectively to the 1.40 and 1.60 ppm shifts observed for $Z, Z_1$ and $Z, Z_2$. In contrast, the $\delta_{MAG}$ and $\delta_{EL}$ calculations did not indicate any diamagnetic contribution to the $H_{eq}$ protons but, instead, suggested a slight low-field shift. The attractive van der Waals interaction described earlier may account for the missing diamagnetism that apparently influences the $H_{eq}$ protons.

The calculated contributions of anisotropic and electric field shifts to geminal N-CH$_2$ nonequivalences were similar magnitude, in agreement with the findings of Zürcher and ApSimon et al.,$^{147,153}$ though the former mechanism was calculated to have about a 0.1 ppm greater contribution. An important generalization to emerge from these results is that magnetic anisotropy and electrostatic shielding mechanisms should be treated as being equally significant in discussions of deshielding effects in carbonyl systems. For amide systems in particular, the expected contribution of the electric field shift should be even greater than indicated by the calculated $\delta_{EL}$ (Table 6.5) values, since the shielding parameters used in these calculations were based on an approximation of the less polar, ketone carbonyl group.

Magnetic and electric field contributions to the calculated chemical shift difference ($\Delta \delta_{CALC}$) from both amide groups indicated that the shift was largely determined by the adjacent group, which is reasonable for a $R^3$ dependence.

When the combined effects of magnetic anisotropy and electric field shifts were considered, only about one-half of the observed $Z, Z$ N-CH$_2$ splitting was accounted for. Perhaps the most obvious rationale for this chemical shift deficit can be argued from a combination of faulty parameter selection and approximations and a
failure to account for a potentially significant van der Waals shift contribution. At the level of empirical computation, the idea of a simple carbonyl approximating an amide system may be a gross oversimplification, given the amide’s electron delocalization, and that a C=N+ shift contribution from the dipolar resonance form of the amide group should be considered. In addition, for the present treatment, the C=O group was assumed to be the dominant shielding mechanism responsible for the large N-CH₂ shift difference. However, other conceivable shift effects (albeit much smaller) could also contribute to the N-CH₂ anisochrony. Through-space neighbouring group shifts are also possible for other groups, particularly C-C, C-H and S-S bonds. In analogy to sulfide and amine systems, lone pairs of electrons in the disulfide and amide groups may also exert shielding effects.

In addition, the separation of the N-CH₂ protons from the assumed dipole center of adjacent carbonyl groups ranges from about 2.4 Å for the *syn*, Hₐₓ protons to about 3.5 Å for the *anti*, Hₑ𝑞 ones (Tables 6.4 and 6.5). At these relatively small distances, the validity of the dipole approximation in the magnetic anisotropy and electric field shielding calculations is uncertain. Didry and Guy have reported errors in excess of 30% can result using the point dipole approximation at distances of 3 Å from the assumed center of an induced magnetic dipole and which become correspondingly greater at smaller distances. Therefore, caution must be applied (particularly for the shielding effects of the adjacent C=O on Hₐₓ) in the application and interpretation of Equation 6.3 towards a quantitative evaluation of δ_MAG and δ_EL.

As an independent check against the accuracy of the calculated Δδ_MAG and Δδ_EL shifts given in Tables 6.4 and 6.5, visual and semi-quantitative determinations of the total shielding at all N-CH₂ protons were performed on the conventional models for the magnetic anisotropy and electric field shielding mechanisms of the carbonyl group.
The results of this qualitative comparison confirmed the direction (i.e., sign) of shielding and approximate magnitude of the predicted shift contributions. In particular, a semi-quantitative determination for $\delta_{MAG}$ contributions were estimated from Pople's long-range shielding contour plots while, for the $\delta_{EL}$ component, a visual inspection of all C-H orientations relative to the C=O electric field afforded a qualitative confirmation for the direction of shielding at the resonant proton.

Figure 6.22: Models of magnetic anisotropy (a) and electric field (b) shielding mechanisms for the C=O bond. In a), the + and - signs refer to areas of nuclear shielding and deshielding, respectively. In b), the electric field vector along the C-H bond, $E_{C-H}$, determines nuclear shielding; in this case, the $E_{C-H}$ vector is in the H$\rightarrow$C bond direction and the proton is shielded.
CHAPTER 7

EXPERIMENTAL

7.1 Materials

\(N,N\)dimethylformamide was heated at reflux and distilled from BaO prior to use. Acetonitrile was distilled from \(P_2\)\(O_5\) and the distillate was redistilled from CaH\(_2\) discarding the first 5 and the last 10\%. Ethanol (EtOH) was dried and purified via distillation from magnesium turnings and stored over freshly-activated 4Å molecular sieves. Pyridine was distilled from CaH\(_2\) just prior to use. Chloroform-\(d\) (CDCl\(_3\)) was stored over 4Å molecular sieves. All chemical were purchased from Aldrich Chemical Company (Milwaukee, WI, USA) unless otherwise noted.

Melting points (mp) were recorded on a Gallenkamp capillary tube melting point apparatus and are uncorrected.

7.2 Chromatography

Analytical thin layer chromatography (TLC) and column chromatography (according to the "flash" method of Still et al.\(^{161}\)) were carried out on E. Merck silica gel 60 F\(_{254}\) coated Al plates of 0.2 mm thickness and 0.040-0.063 mm (230-400 mesh), respectively. TLC visualization was achieved using a combination of either a UV lamp (254 nm), \(I_2\) vapour or a ninhydrin spray (0.2\% ninhydrin in EtOH) followed by heating and treatment with a phosphomolybdic acid spray (20 g of phosphomolybdic acid and 15 g CeSO\(_4\) dissolved in 1 L of 10\% H\(_2\)SO\(_4\)). A spray solution of 9.2 mL of
m-anisaldehyde in 338 mL of 95% EtOH, 3.75 mL glacial HOAc and 12.5 mL of concentrated H₂SO₄ followed by heating at 100-150 °C was used to visualize the β-cyclodextrin derivatives.

AMBERLYST A-26 anion-exchange resin (Cl⁻) was prewashed and converted to the OH⁻ form just prior to use. Approximately 150 mL of crude, commercial grade resin was purified in a 150 mL coarse sintered glass funnel according to the following wash protocol: 1 N HCl (3 X 100 mL), distilled water (2 X 100 mL), 1:1 v/v aqueous acetone (2 X 100 mL), EtOH (3 X 100 mL), 1 N NaOH (3 X 100 mL), distilled water (until eluate became neutral) and EtOH (3 X 100 mL). Washings were allowed to percolate through the resin under gravity. The washed resin was stored under EtOH until it was ready to be used.

7.3 Spectroscopy

Proton nuclear magnetic resonance (¹H NMR) spectra were recorded on Varian EM 390, Bruker MSL-100, AC-200, WM-250 or AM-500 spectrometers, with the majority of experiments acquired at 500.135 MHz on the latter instrument using a 5 mm dual frequency ¹H-¹³C probe. Sample concentrations were approximately 1-2% w/v. Spectra were typically obtained in 8 scans in 16K data points over a 5.000 KHz spectral width (1.638 s acquisition time). The free induction decay (FID) was processed using exponential multiplication (line broading: 0.2 Hz) and was zero-filled to 32K before Fourier transformation. Sample temperature was maintained at 303 K by a Bruker BVT-1000 variable temperature unit. The abbreviations s=singlet, d=doublet, t=triplet, q=quartet and m=multiplet refer to the multiplicities of the signals observed.

Broad band decoupled, natural abundance carbon thirteen nuclear magnetic resonance (¹³C NMR) spectra were recorded on a Bruker AM-500 spectrometer at
125.759 MHz using the 5 mm dual frequency 1H-13C probe, except in one experiment, which was acquired on a Bruker WP-80 instrument at 20.115 MHz. The spectra were acquired over a 30.0 KHz spectral width in 16K data points (0.279 s acquisition time) using a 1H decoupler power level of 17H. The 13C pulse width was 2.5 μs (35° flip angle). A 0.5 s relaxation delay was used. For spectral edited 13C experiments, the standard J-modulated spin sort pulse sequence was used, incorporating a 7.3 μs 13C 90° pulse width and a relaxation delay of 2.5 s. The FIDs were processed using exponential multiplication (line broadening: 3.5 Hz) and zero-filled to 32K before Fourier transformation.

NOE difference spectra were obtained by subtraction of the off-resonance control FID from the on-resonance FID under non-spinning conditions and employing a sweep rate of 19 Hz. The signal of interest was selectively saturated for 5 s and the decoupler was gated off during acquisition. This saturation period also served as the relaxation delay. Normally 8 scans were acquired for each irradiation with the cycle of irradiation repeated 10 times. Free induction decays were processed using exponential multiplication with a line broadening of 4 Hz before Fourier transformation.

13C dynamic nuclear magnetic resonance (DNMR) spectra were recorded over a 2.066 KHz spectral width in 8K data points (1.98 s acquisition time) incorporating a 5.5 μs pulse width and a 9.0 s relaxation delay to optimize sensitivity and digital resolution. The sample and probe were allowed a 10-minute equilibration period per 5 °C increment. An external digital thermocouple was used to measure the probe temperature to an accuracy of ± 0.5 °C. Rate constants were estimated by visual fitting of experimental to computer-simulated spectra measured for a given temperature. The calculated spectra were generated on an IBM PS/2 Model 30 (286) microcomputer equipped with a HP 7550A graphics plotter using the simulation
program EXCHANGE (available from R.E.D. McClung, Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada, T6G 2G2). The intrinsic linewidth of 0.35 Hz was obtained from a measurement of the peak width at one-half peak height of the S-CH$_2$ resonance at 35.61 ppm at 25.0 °C. The $\Delta G^\circ$ for disulfide rotation was calculated from the slope of the regression line derived from an Arrhenius plot of lnk versus 1/T in Quattro Pro (3.01). The data points corresponding to $T=298.5, 317.6$ and 329.7 K were omitted from the regression analysis. The error in $\Delta G^\circ$ was measured by the standard deviation of the regression coefficient.

Solid state $^{13}$C spectra were obtained at 25.18 MHz on a Bruker MSL-100 spectrometer operating at 2.35 T. The cross polarization magic angle spinning (CP-MAS) spectrum was obtained by using a 1 ms contact time and a recycle time of 10 s. Crystals of 40 - obtained by slow evaporation of a THF solution of the compound - were pulverized and diluted with KBr prior to analysis.

The $^{13}$C-$^1$H 2-D chemical shift correlation spectra were acquired using the standard pulse sequence incorporating the BIRD pulse during the evolution period for the $^1$H - $^1$H decoupling in $F_1$. The spectra in $F_2$ were recorded over a spectral width of 3.76 KHz in 4K data points. The 256 FIDs in $F_1$ were obtained over a $^1$H spectral width of 1.52 KHz. Each FID was acquired in 200 scans. The fixed delays in the pulse sequence were a 1.0 s relaxation delay, BIRD pulse and polarization transfer delays (1/2 $^1J_{CH}$) of 0.003571 s and a refocussing delay (1/4 $^1J_{CH}$) of 0.001786 s. The $^{13}$C 90° pulse width was 6.4 μs, while the $^1$H 90° pulse width through the decoupler channel was 18.6 μs. The data were processed using exponential multiplication (line broadening: 5.0 Hz) in $F_2$ and unshifted sine bell in $F_1$. Zero-filling in $F_1$ resulted in a 2048 X 1024 data matrix.

The compounds analyzed via NMR spectroscopy were dissolved in either
deuterated acetone, deuterated benzene, deuterochloroform or deuterated dimethyl sulfoxide or deuterated methanol (CD$_2$COCD$_3$, C$_6$D$_6$, CDCI$_3$, DMSO-$d_6$ or CD$_3$OD, respectively), or mixtures thereof, obtained from MSD Isotopes. $^1$H and $^{13}$C chemical shifts are reported in parts per million (ppm) using either TMS (0.0 ppm) as an internal reference or the residual signal of the following solvents: CD$_3$COCD$_3$ (2.04; 29.8 ppm), C$_6$D$_6$ (7.15; 128.0 ppm), CDCI$_3$ (7.26; 77.0 ppm) and DMSO-$d_6$ (2.49; 39.5 ppm).

Low resolution mass spectra (MS) and high resolution mass spectra (HRMS) were obtained on either a VG Micromass 7070F or a VG ZAB-E mass spectrometer with sample introduction via a direct inlet system. Infrared (IR) spectra were recorded on a Perkin-Elmer 283 spectrometer using either liquid cells with methylene chloride or chloroform as solvents or potassium bromide pellets. The abbreviations (s)=strong, (m)=medium and (w)=weak are used to to describe the intensity of particular bands in the IR spectra.

7.4 X-ray Diffraction of 40

Crystals of 40 were grown by very slow recrystallization from freshly-distilled tetrahydrofuran at -15 °C. A colorless plate having approximate dimensions of 0.20 X 0.05 X 0.15 mm was mounted on a glass fiber. All measurements were made on a Rigaku AFC6R diffractometer with graphite monochromated CuKα radiation (λ = 1.54178 Å) and a rotating anode generator. The crystal data for 40 is presented in the Appendix (see Tables A.1 to A.6).

Cell constants and an orientation matrix for data collection were obtained from a least-squares refinement using the setting angles of 24 carefully centered reflections in the range 7.0 < 2Θ < 45.0°. Based on packing considerations, a statistical
analysis of intensity distribution and the successful solution and refinement of the structure, the space group was determined to be \( P\bar{1} \) (#2).

The data were collected at a temperature of 23 ± 1 °C using the \( \omega \)-2\( \Theta \) scan technique to a maximum 2\( \Theta \) value of 110.1°. Scans of \((1.21 + 0.30\tan\Theta)^\circ\) were made at a speed of 32.0°/min (in omega). The weak reflections \((I < 10.0\sigma_I)\) were rescanned (maximum of 9 rescans) and the counts were accumulated to assure good counting statistics. Stationary background counts were recorded on each side of the reflection. The ratio of peak counting time to background counting time was 2:1. The diameter of the incident beam collimator was 0.5 mm and the crystal to detector distance was 400.0 mm.

Of the 1479 reflections which were collected, 1362 were unique \((R_{\text{int}} = 0.032)\). The intensities of three representative reflections - which were measured after every 150 reflections - remained constant throughout data collection indicating crystal and electronic stability (no decay correction was applied). The data were corrected for Lorentz and polarization effects.

The structure was solved by direct methods. All non-hydrogen atoms were refined with anisotropic thermal parameters. Hydrogen atoms were placed geometrically and not refined. A correction was made for extinction \((\chi = 0.005(3)\) where \( F^+ = F[1 + 0.002xF^2/(\sin2\Theta)]^{-1/4}\) and an absorption correction was applied using the program DIFABS,165 employing transmission factors ranging from 0.69 to 1.20. Atomic scattering factors for all atoms and corrections for anomalous dispersion for C, O, N and S were taken from the International Tables for Crystallography, Vol. C.166 Refinement minimized the function \( \sum \omega(|F_o| - |F_c|)^2 \), with \( \omega = (\sigma^2F^2 + 0.0008F^2)^{-1} \). The final solution, with \( R = 4.67 \% \) and \( \omega R = 6.80 \% \) \((R = \sum(|F_o| - |F_c|)/\sum F_o)\) for 1006 reflections \((I > 2\sigma_I)\), showed no significant peaks in the final difference map. The
goodness-of-fit was $S = 1.51$. The program SHELXTL was used for all calculations.\textsuperscript{167}

7.5 Molecular Mechanics Studies on 40

Steric energies and conformations were calculated and minimized in PCMODEL V4.0 (available from K. Gilbert, Serena Software, Box 3076, Bloomington, IN 47402-3076, USA) using the MMX force field derived from the MM2 force field of N.L. Allinger (available from Quantum Chemistry Program Exchange, Chemistry Department, Indiana University, Bloomington, IN 47405, USA). The calculations were performed on an IBM PS/2 Model 70 (486) microcomputer. The MMX force field includes the parameters necessary for the minimization calculations. Calculated steric energies were reported relative to the global minimum structure. Vicinal coupling constants measured from energy-minimized structures were calculated using the Altona modification of the Karplus equation which takes into account substituent electronegativity.\textsuperscript{168}

To ensure a comprehensive search of conformational space, initial geometries were generated by either systematic or random methods.\textsuperscript{169} Systematic geometry searches were typically conducted by merging all combinations of accepted ground state geometries of the disulfide, amide and ethyl functional groups of 40; \textit{i.e.}, 90° torsional angle for the disulfide linkage, 180° (\textit{trans}) and/or 0° (\textit{cis}) torsional angles for the amide groups and a staggered configuration for the ethyl fragment. Random geometries were typically generated by either random or pseudorandom atomic variations on either minimized structures or two-dimensionally drawn images. After a crude starting geometry was produced, it was allowed to relax into an optimized geometry by the MMX force field. The search was terminated when new minima
within 5 kcal/mol of the global minimum ceased be to found. Starting geometries were preminimized without hydrogen atoms and then reminimized with added hydrogens. This procedure avoids forcing hydrogens and lone electron pairs into high energy configurations. Minimized structures were finally reminimized to ensure full convergence.

Geometrical parameters (see Figure 6.21) were measured in PCMODEL via atomic distance and bond angle queries on reformatted structure files. To calculate \( R \), a dummy atom was defined as the origin and positioned at the midpoint of the required C=O bond. Dummy atoms were further employed to locate the \( y \) and \( z \) magnetic susceptibility axes for \( \Theta_y \) and \( \Theta_z \) determinations by arbitrarily defining their cartesian coordinates to be \( (0,3,0) \) and \( (0,0,3) \) respectively. Angle \( \gamma \) was measured by adding the C-H difference vector of concern to the appropriate carbonyl carbon coordinates; this ensures a parallel C-H transformation from which \( \gamma \) can be directly measured. All parameters were substituted into the appropriate chemical shift expressions, which were formulated in the spreadsheet format of Quattro Pro (3.01). Since the \( Z,Z_1 \) and \( Z,Z_2 \) isomers of 40 possess \( C_2 \) symmetry, only the chemical shifts for each pair of symmetry-related protons were calculated.

7.6. **Synthesis and Characterization**

7.6.1 2-(Triphenylmethyl)thioethanoic acid (1)

This synthesis was performed according to the method of Brenner et al. A solution of triphenylmethanol (48.0 g, 184.0 mmol), mercaptoacetic acid (12.2 mL, 184.0 mmol) and glacial acetic acid (160 mL) was heated to 70 °C. Boron trifluoride etherate (32.0 mL) was added and the resulting brown solution was
magnetically-stirred at room temperature for 50 minutes. The reaction mixture was poured into ice-cold distilled water, depositing a clear oil which solidified at 5 °C after 18 hours. The solid was broken and washed with distilled water, then a small amount of diethyl ether and dried in vacuo to give 1 as an opaque solid (40.0 g, 65%). A further crop of 1 (4.3 g, 7%) was recovered from the ether washings and purified by recrystallization from toluene. The compound showed:

mp: 155.0-157.0 °C (lit. 158.5-160.0 °C)

TLC: Rf 0.33 (90% CH₂Cl₂:10% MeOH)

IR (KBr pellet):

Δνₐₓₜₐₜ 3000(s)(OH), 1700(s)(C=O),
1600,1490,1445(m)(aromatic C=C),
735,695(s)(monosubstituted aromatic)

MS (FAB-):

m/z(RI%); 333(22)[M-1], 441(11)[M+TG-1],
243(73)[Tr-]

¹H NMR (CD₂COCD₃):

8.20(s,2H,S-CH₂), 7.25(m,15H,aryl),
10.20(s,1H,COOH) (90 MHz)

¹³C NMR (CDCl₃):

834.67(S-CH₂), 67.44(C-Ph₃), 128.05, 144.09(aryl),
175.77(COOH) (20.1 MHz)

![Chemical Structure](image)

1

7.6.2 N-Succinimido 2-(triphenylmethyl)thioethanoate (2)

This synthesis was performed according to the method of Brenner et al.³⁵ To
an ice-cooled solution of 1 (5.01 g, 15.00 mmol) and N-hydroxysuccinimide (1.72 g, 15.00 mmol) in DME (38 mL) was added DCC (3.20 g, 15.50 mmol) such that the temperature remained at approximately 0 °C. The resulting mixture was then stored at 5 °C for 18 hours and then filtered and washed with ice-cold CH₂Cl₂. The filtered solid (DCU) was collected and discarded. The combined CH₂Cl₂ filtrate and washings were pooled and concentrated in vacuo, to afford 2 as a white solid. Residual amounts of DCU were removed via recrystallization from ethyl acetate to give large, opaque prisms (5.43 g, 84%). The compound showed:

mp: 178.5-180.0 °C (lit. 178.5-179.5 °C)

TLC: Rf 0.46 (98% CH₂Cl₂:2% MeOH)

IR (KBr pellet):

ν MAX 3070(w)(aromatic C=C-H), 2940,2860(w)(aliphatic C-H), 1820,1790(s)(N-C=O), 1745(s)(C=O,ester), 1630(w),1580(w),1495(m),1445(m)(aromatic C=C), 735,690(s)(monosubstituted aromatic)

MS (+NH₃ DCI):

m/z(RI%); 449(7)[M+NH₄⁺], 243(100)[Tr+]

¹H NMR (CDCl₃):

δ 6.57[s, 4H,(CH₂-C=O)₂], 3.08(s,2H,S-CH₂), 7.17(m,18H (overlapping residual CHCl₃),aryl) (500 MHz)

¹³C NMR (CDCl₃):

δ 258.71 [CH₂-C=O]₀₂, 31.60(S-CH₂), 127.32-129.94,143.73 (aryl), 165.24(COON), 168.76(CH₂-C=O) (126 MHz)
7.6.3 \textit{N,N'-Bis[2-(triphenylmethyl)thioethanoyl]-2,3- \textit{diaminopropanoic acid (3)}}

To a solution of activated ester 2 (6.00 g, 13.91 mmol) in dry THF (100 mL) was added a solution of 2,3-diaminopropanoic acid monohydrochloride (0.98 g, 6.95 mmol) and \textit{NEt}_3 (12.00 mL) in dry MeOH (160 mL). The methanolic diamino acid solution was added in 1 mL aliquots over a thirty-minute period. The resulting turbid solution cleared after the addition of about three-quarters of the amino acid solution and the mixture was allowed to magnetically stir at room temperature for 60 minutes and then concentrated \textit{in vacuo} to give an opaque oil. This oil was then extracted twice into CH\textsubscript{2}Cl\textsubscript{2} (100 mL) from a 9:1 (v/v) of 1.0 N HCl and saturated NaCl solution (200 mL) and the combined organic solution was backwashed twice with 9:1 (v/v) 1.0 N HCl and saturated NaCl solution (200 mL). The washed CH\textsubscript{2}Cl\textsubscript{2} solution was separated, dried over anhydrous MgSO\textsubscript{4}, filtered and concentrated \textit{in vacuo} to give an amorphous white solid (3.43 g, 67%). TLC and NMR spectra of this crude residue indicate approximately 30% contamination by the methanolysis product, methyl S-tritylthioacetate. Recrystallization from isopropanol gave 3 as a sharp-melting sample after three cycles. The compound showed:

mp: 206.0-207.5 °C
TLC: \( R_f 0.22 \) (90% CH\(_2\)Cl\(_2\):10% MeOH)

IR (KBr pellet):
\( \nu_{\text{MAX}} 3000(\text{s(OH)}), 1725(\text{s(C=O,acid)}, 1665(\text{s(C=O,Amide I)}), 1600,1490,1440(\text{m(aromatic C=C)}), 735,690(\text{s(monosubstituted aromatic)})

MS (FAB+):
\( m/z(\%): 737(19)[\text{M+1}], 493(31), 391(100)[\text{M+1-}C_{22}H_{20}O\text{SN}], 243(71)[\text{Tr+}] \)

\(^1\text{H NMR (DMSO-}d_6\text{):}\n82.75(\text{s,2H,H-5}), 2.79(\text{AB q,} J=13.9 \text{Hz,2H,H-7). } 3.16(\text{m,1H,H-3a(b)}), 3.21(\text{m,1H,H-3a(b)}), 3.89(\text{m,1H,H-2)}, 7.21-7.33(\text{m,30H,aryl}), 7.61(\text{d,} J=7.2 \text{Hz,1H,C}_6\text{O-NH), } 7.75(\text{t,} J=5.2 \text{Hz,1H,C}_6\text{O-NH) (500 MHz) })

\(^{13}\text{C NMR (CDCl}_3\text{):}\n835.66, 35.84(\text{C}_2 \text{ and C}_7), 41.59(\text{C}_3), 55.24(\text{C}_2), 67.72(\text{C-Ph}_3), 127.03-129.66, 143.94, 144.09(\text{aryl}), 169.28, 170.24(\text{br,C}_4 \text{ and C}_6), 177.05(\text{br,C}_1) \text{ (126 MHz) }

![Chemical Structure](attachment:chemistry.png)
7.6.4 Alternative Synthesis of N,N'-Bis[2-(triphenylmethyl)thioethanoyl]-2,3-diaminopropanoic acid (3)

To a solution of ester 7 or 10 (1.61 mmol) in a 2:1 v/v EtOH/CH₂Cl₂ mixture (120 mL) was added EtOH-swollen, freshly-generated AMBERLYST A-26 (OH⁻) anion-exchange resin (100 mL, 100 mmol), Aliquat 336 (5 drops) and ddH₂O (10 mL). After 48 hours at ambient temperature with occasional swirling, the pale-yellow suspension was filtered and the collected resin was washed with EtOH and acetone. The resin was then eluted with a mixture of 0.08 N HCl and acetone (1:1 v/v) until the eluate had a pH of less than 2, then the desorbed resin was washed with CHCl₃. The aqueous and organic eluants were combined and concentrated under reduced pressure at 30 °C to ca. one-third of its original volume, at which point a white precipitate was noted. The suspension was extracted with CHCl₃ and the organic layer was backwashed with 0.5 N HCl and dried over MgSO₄ for two hours. Filtration and rotary evaporation of the filtrate afforded a yellow oil. The oil was triturated with diethyl ether (100 mL) and the suspension was chilled at -15 °C for 18 hours, gravity filtered and the residue washed with cold ether and dried under high vacuum to give 3 as a taupe-colored solid. A further crop was isolated from the ethereal filtrate by dilution with hexanes and collection of the precipitate (total yield: 92%). Recrystallization from isopropanol gave 3 as a sharp-melting, white solid; mp 206.5-207.5 °C.

7.6.5 N,N'-Bis(2-mercaptoethanoyl)-2,3-diaminopropanoic acid (4)

This synthesis was a modification of the procedure of Brenner et al. To an ice-cold, amber-yellow solution of S-protected acid 3 (100 mg, 0.14 mmol) in trifluoroacetic acid (1.3 mL) was added triethylsilane (45 μL, 2.1 eq., 0.29 mmol) with magnetic stirring. Immediate color discharge and the formation of a white precipitate (triphenylmethane) was observed. After 10 minutes, the suspension was diluted with
ddH₂O (10 mL), filtered, with ddH₂O washings (2 X 25 mL), and the filtrate was concentrated in vacuo to give 4 as a pungent-smelling, clear oil (31 mg, 87%). TLC of the product revealed a yellow spot at Rf 0.17 (40% CHCl₃:40% MeOH:20%(17% NH₄OH)) using a 1.98% methanolic-aqueous (9:1 v/v) solution of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) as a thiol detector. The compound showed:

1H NMR (DMSO-d₆):

δ3.15(s,4H,S-CH₂), 3.29,3.48(m,1H each,diastereotopic N-CH₂-CH),
4.30(m,1H,N-CH-COOH), 7.15(br t,~1H,SH),
8.12(t,J=5.8 Hz,1H,H-N-CO-CH₂), 8.27(d,J=7.9 Hz,1H,H-N-CO-CH), 9.45(br s,1H,SH) (500 MHz)

7.6.6 N,N'-(2,2'-Dithioethanoyl)-2,3-diaminopropanoic acid (5)

The dithiol 4 (76 mg, 0.3 mmol) was dissolved in 1 mM ammonium hydroxide (500 mL, [4] = 0.6 mM) with magnetic stirring and the resulting solution was adjusted to pH 10.0 with concentrated ammonium hydroxide. Filtered air was bubbled into the mixture via an air stone and the reaction mixture was left at room temperature for 17 hours. Filtration of the pale-yellow solution and concentration of the filtrate in vacuo gave a taupe-colored, amorphous material (75 mg, 94%). Recrystallization from ddH₂O afforded 5 as an off-white solid. The compound showed:

TLC:

Rf 0.81 [40% CHCl₃:40% MeOH:20%(17% NH₄OH); bromocresol green positive; DTNB negative]

IR (KBr pellet):

νMAX 3150(s)(N-H,OH bonded), 1665(s)(C=O,acid and-Amide I), 1550(w)(C=O,Amide II), 1400(m), 1195(s)(C-O), 1120(m)
MS (FAB-): \textit{m/z}(RI%); 249(30)[M-1], 227(100)

\(^1\text{H} \text{NMR (DMSO-}d_6\text{):}
\begin{align*}
\delta & = 3.2-3.7[m,\sim 10H(overlapping \text{H}_2\text{O}), \text{H-5}, \text{H-7 and H-3}], \\
& 3.82(m,0.4H,\text{H-2 ISOMER 1}), \\
& 4.41(m,0.1H,\text{H-2 ISOMER 2}), \\
& 4.47(m,0.2H,\text{H-2 ISOMER 3}), \\
& 4.77(m,0.3H,\text{H-2 ISOMER 4}), \\
& 7.84-8.45(m,2H,\text{amide})
\end{align*}
(500 MHz)

\(^{13}\text{C NMR:}
\text{See Table 7.1
Table 7.1: $^{13}$C NMR (126 MHz) assignments for 5 in DMSO-$d_6$ at 303 K.

<table>
<thead>
<tr>
<th>CARBON</th>
<th>$\delta$ (ppm)</th>
<th>RI$^a$</th>
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<td>41.25</td>
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<tr>
<td></td>
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<td>42.47</td>
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<td>$C_5, C_7$</td>
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<td>43.71</td>
<td>4.76</td>
</tr>
<tr>
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</tr>
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<td></td>
<td>52.34</td>
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</tr>
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<td>52.49</td>
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<td></td>
<td>53.12</td>
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<tr>
<td>$C_1, C_4, C_6$</td>
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</tr>
<tr>
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<td>0.93</td>
</tr>
<tr>
<td></td>
<td>171.62</td>
<td>0.30</td>
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</tbody>
</table>

$^a$ Relative intensity

7.6.7 Ethyl 2,3-diaminopropanoate .2HCl (6)

This synthesis was performed according to the method of Fritzberg et al.\textsuperscript{171} HCl gas was bubbled, for about 30 minutes, into a suspension of 2,3-diaminopropanoic acid .HCl (504 mg, 3.58 mmol) in absolute EtOH (84 mL) with magnetic stirring and cooling in an ice-bath. The suspension was allowed to reflux for four days. The resulting clear solution was filtered and concentrated in vacuo to afford 6 as an amorphous solid (704 mg, 98%). The compound showed:

mp: 164-166 °C
TLC: 
\[ R_f 0.27 \text{ (70\% CH}_3\text{CH}_2\text{CH}_2\text{OH:28\% H}_2\text{O:2\% HCOOH; ninhydrin positive)} \]

IR (KBr pellet):
\[ \nu_{\text{MAX}} 2900(\text{s})(\text{N-H stretch of salt}), 1745(\text{s})(\text{C=O}), 1580,1505(\text{m})(\text{N-H bend of salt}), 1245(\text{s})(\text{O-C}) \]

MS (FAB+):
\[ m/z(\text{RI\%}); 133(100)[(\text{M-2HCl})+1], 265(10)[2(\text{M-2HCl})+1] \]

\[ ^1\text{H NMR (DMSO-d}_6\text{):} \]
\[ \delta 1.25(t, J=7.1 \text{ Hz, 3H, O-CH}_2\text{-CH}_3), \]
\[ 3.31-3.34(\text{m,2H,diastereotopic H-3}), \]
\[ 4.22(\text{m,2H,diastereotopic O-CH}_2\text{-CH}_3), \]
\[ 4.40(\text{m,1H,H-2}), 8.82(\text{br s,6H,NH}_3^+) \text{ (200 MHz)} \]

\[ ^13\text{C NMR (DMSO-d}_6\text{):} \]
\[ \delta 13.75(\text{O-CH}_2\text{-CH}_3), 38.34(\text{C}_2), \]
\[ 62.65(\text{O-CH}_2\text{-CH}_3), 166.44(\text{C}_1) \text{ (126 MHz)} \]

\[
\text{Cl}^- \quad \text{H}_3\text{N}^+ \quad \text{NH}_3^+\text{Cl}^- \\
\begin{array}{c}
\text{3} \\
\text{2} \\
\text{1} \\
\text{OCH}_2\text{CH}_3
\end{array}
\]

7.6.8 Ethyl N,N'-bis[2-(triphenylmethyl)thioethanoyl]-2,3-diaminopropanoate

(7)

A solution of diamino ester 6 (0.91 mg, 4.41 mmol) in dry AN (40 mL) and NEt\(_3\) (3 mL) was slowly added to a solution of the succinimido ester 2 (3.81 g, 8.82 mmol) in dry AN (160 mL) under a \(\text{N}_2\) atmosphere with magnetic stirring. After 36 hours, the reaction mixture was concentrated \textit{in vacuo} on a rotary evaporator and the
residual white paste was extracted into CH$_2$Cl$_2$ (2 × 100 mL) from 0.5 N HCl (100 mL). The combined CH$_2$Cl$_2$ layer was backwashed with 0.2 N NaHCO$_3$ (200 mL) and saturated brine (200 mL) and dried over MgSO$_4$ for an hour. Filtration and in vacuo concentration of the filtrate afforded 7 as an opaque solid (3.28 g, 97%). The compound showed:

mp: 63-66 °C
TLC: $R_f$ 0.50 (98% CH$_2$Cl$_2$:2% MeOH)
IR (KBr pellet):

$\nu_{\text{max}}$ 3300(s)(N-H bonded), 3060(m)(aromatic C=C-H), 2980,2940(s)(aliphatic C-H), 1740(s)(C=O,ester), 1660(s)(C=O,Amide I), 1520(s)(N-H,Amide II), 1490,1450(s)(aromatic C=C), 1200(s)(C-O), 740,695(s)(monosubstituted aromatic C-H)

$^1$H NMR (CDCl$_3$):

8.127(t,$J$=6.7 Hz,3H,O-CH$_2$-CH$_3$), 3.05(br s,4H,H-5 and H-7), 3.21(m,2H,H-3), 4.15(m,3H,overlapping H-2 and O-CH$_2$-CH$_3$), 6.26(t,$J$=6.4 Hz,1H,C$_4$O-NH), 6.82(d,$J$=6.8 Hz,1H,C$_5$O-NH), 7.19-7.47[m,31H (overlapping residual CHCl$_3$),aryl] (200 MHz).

$^{13}$C NMR (CDCl$_3$):

813.93(O-CH$_2$-CH$_3$), 35.70,35.88(C$_5$ and C$_7$), 41.27(C$_3$), 52.68(C$_2$), 61.84(O-CH$_2$-CH$_3$), 67.57,67.62(C-Ph$_3$), 126.86-129.49, 143.74,143.79(aryl), 168.48,168.69(C$_4$ and C$_6$), 169.46(C$_1$) (126 MHz)
7.6.9 Alternative Synthesis of Ethyl $N,N'$-bis[2-(triphenylmethyl)thioethanoyl]-2,3-diaminopropanoate (7) via DCC Coupling of 1 and 6

A suspension of diamino ethyl ester 6 (0.61 mg, 3.00 mmol), NEt$_3$ (4 drops) in AN (10 mL) was added dropwise to a magnetically-stirred solution of acid 1 (2.00 g, 6.00 mmol), DCC (1.36 g, 1.1 eq., 6.59 mmol) in AN (55 mL) after a 10-minute acid activation period under a N$_2$ atmosphere. After 12 hours, the pale-orange suspension was chilled at -15 °C for three hours, gravity filtered and the residue (DCU) washed with cold CH$_2$Cl$_2$ and discarded. The filtrate was concentrated to 10 mL on a rotary evaporator, diluted with CH$_2$Cl$_2$ (100 mL), washed with 0.5 N HCl (2 X 100 mL) and dried over MgSO$_4$ for two hours. Filtration and rotary evaporation of the filtrate afforded a light-amber oil (2.14 g, 107%) which solidified under high vacuum. This DCU-contaminated crude product was used without further purification for ion-exchange mediated, ester hydrolysis. For characterization purposes, a sample of this crude product was recrystallized from absolute EtOH and the recrystallized material was found to be identical to the ester 7, which was synthesized via the modified procedure of Brenner et al. 35
7.6.10 Ethyl N,N'-((2,2'-dithioethanovyl)-2,3-diaminopropanoate (8)

This synthesis was a modification of the method of Kamber. A solution of the S-trityl ester 7 (400 mg, 0.52 mmol) and resublimed I₂ (145 mg, 1.1 eq., 0.57 mmol) was prepared in a degassed 2:1 v/v mixture (264 mL) of AN and absolute EtOH under an argon atmosphere. The amber-brown solution was further degassed for 30 minutes under aspirator vacuum (15 mm Hg), vented to dry argon gas and allowed to magnetically stir at room temperature. After nine hours, the reaction mixture was concentrated on a rotary evaporator to 20 mL, diluted to 250 mL with ddH₂O and washed with diethyl ether (2 X 200 mL) to remove trityl ethyl ether. After decolorization with Na₂S₂O₃ (2 mL, 0.1 N), the aqueous solution was concentrated on a rotary evaporator until turbidity was induced (30 mL mark). The turbid mixture was chilled at 5 °C for 10 hours, filtered and washed with ice-cold ddH₂O and dried under high vacuum to afford 8 as an opaque solid (80 mg, 55%). The compound showed:

mp: 209.0-211.0 °C
TLC: R₁ 0.78 (80% CH₃CH₂CH₂OH:18% H₂O:2% HCOOH)
IR (KBr pellet): νₓ (s) 1735(s)(C=O,ester), 1640(s)(C=O,Amide I), 1550(s)(N-H,Amide II), 450(w)(S-S)
MS (DEI): m/z(RI%); 278(28)[M⁺], 144(100), 102(80), 85(32), 72(58)
HRMS (DEI): for C₉H₁₄N₂O₄S₂; calculated: 278.0395; observed: 278.0383
¹H and ¹³C NMR: See Table 7.2
Table 7.2: \(^1\text{H} \) NMR (500 MHz) and \(^{13}\text{C} \) NMR (126 MHz) assignments for 8 in DMSO-\( d_6 \) and CDCl\(_3 \) at 303 K.

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<th>SOLVENT</th>
<th>( \delta ) (ppm)</th>
<th>MULTIPLICITY</th>
<th>AREA</th>
<th>ASSIGNMENT</th>
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<td>(^1\text{H} )</td>
<td>DMSO-( d_6 )</td>
<td>1.19(^a) (( J=6.8 ) Hz)</td>
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<td>O-( \text{CH}_2 )-( \text{CH}_3 ) (^b)</td>
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<tr>
<td></td>
<td></td>
<td>3.1-3.6</td>
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</table>

\(^a\) overlapping H\(_2\)O

\(^b\) COSY confirmed

\(^c\) J-modulated confirmed
7.6.11 Methyl 2,3-diaminopropanoate ·2HCl (9)

HCl gas was bubbled, for about 30 minutes, into a suspension of 2,3-diaminopropanoic acid ·HCl (1.03 g, 7.35 mmol) in absolute EtOH (200 mL) with magnetic stirring and cooling in an ice-bath. The suspension was allowed to reflux for two days. The resulting clear solution was filtered and concentrated in vacuo to afford 9 as an amorphous solid (1.38 g, 98%). The compound showed:

mp: 152-155 °C

TLC: 
$R_f$ 0.20 (70% CH$_3$CH$_2$CH$_2$OH:28% H$_2$O:2% HCOOH; ninhydrin positive)

MS (FAB+): $m/z$(RI%); 133(100)[(M-2HCl)+1], 265(10)[2(M-2HCl)+1]

$^1$H NMR (DMSO-$d_6$): δ3.28,3.36(s,2H,diastereotopic H-3), 3.80(s,3H,OCH$_3$), 4.42(m,1H,H-2), 9.00(br s,6H,-NH$_3^+$) (90 MHz)

$^{13}$C NMR (DMSO-$d_6$): δ38.42(C$_3$), 50.17,51.46(C$_2$ and OCH$_3$), 167.48(C$_1$) (126 MHz)
7.6.12 **Methyl N,N'-bis[2-(triphenylmethyl)thioethanoyl]-2,3-diaminopropanoate (10)**

A solution of the diamino methyl ester 9 (1.40 g, 7.34 mmol) in DME (30 mL) and NEt₃ (3 mL) was added to a solution of succinimido ester 3 (6.04 g, 14.01 mmol) in dry DME (40 mL) under a N₂ atmosphere with magnetic stirring. After 24 hours, the reaction mixture was concentrated *in vacuo* on a rotary evaporator and the residual white paste was extracted into CH₂Cl₂ (2 X 200 mL) from 0.5 N HCl (250 mL). The combined CH₂Cl₂ layer was backwashed with 0.3 N NaHCO₃ (200 mL) and saturated brine (200 mL) and dried with MgSO₄ for 18 hours. Filtration and *in vacuo* concentration of the filtrate afforded 10 as an opaque solid (5.17 g, 94%). The compound showed:

**mp:** 66.0-70.0 °C.

**TLC:**

R₇ 0.44 (98% CH₂Cl₂:2% MeOH)

**¹H NMR (CDCl₃):**

δ 3.06 (s, 2H, H-5), 3.07 (AB q, J=-15.0 Hz, 2H, H-7), 3.23 (m, 2H, H-3), 3.72 (s, 3H, OCH₃), 4.21 (m, 1H, H-2), 6.22 (t, J=5.9 Hz, 1H, C₆O-NH), 6.84 (d, J=6.7 Hz, 1H, C₆O-NH), 7.23-7.44 [m, 3H (overlapping residual CHCl₃), aryl] (500 MHz).
13C NMR (CDCl₃): δ35.39, 35.59(C₅ and C₇), 40.87(C₃), 52.34, 52.38(C₂ and OCH₃), 67.35, 67.38(C-Ph₃), 126.58-129.22, 143.49, 143.53(aryl), 168.22, 168.58(C₄ and C₆), 169.71(C₁) (126 MHz)

7.6.13 Attempted Synthesis of Cholesterol N,N'-bis[2-(triphenylmethyl)thioethanoyl]-2,3-diaminopropanoate (11) via CDI Coupling

To a solution of acid 3 (200 mg, 0.27 mmol) in dry THF (5 mL) under a N₂ atmosphere was added carbonyldiimidazole (57 mg, 1.3 eq., 0.35 mmol) and the solution was magnetically stirred for 10 minutes at room temperature. In the meantime, a 0.05 mole % solution of sodium imidazolide - generated in situ by dissolving an excess of sodium metal in a solution of imidazole (58 mg, 0.10 mmol) in dry THF (2 mL) - was added to cholesterol (100 mg, 0.26 mmol) and the resulting mixture was immediately added to the activated imidazolide with dry THF washings (2 X 0.5 mL). The light-amber solution was left at room temperature under a N₂ atmosphere for four hours, concentrated in vacuo and the oily residue was extracted twice into CH₂Cl₂ (200
mL) from a 0.1 M NaH₂PO₄/H₃PO₄ buffer (pH 2.1). The combined organic extracts were backwashed with more buffer (400 mL), brine (400 mL) and then dried for 18 hours over anhydrous MgSO₄. Filtration of the suspension and concentration of the filtrate in vacuo at 35 °C gave a pale-yellow oil which solidified under high vacuum. ¹H NMR spectroscopy and TLC of this solid indicated it to be primarily cholesterol.

7.6.14 Attempted Synthesis of Cholesterol N,N'-bis[2-(triphenylmethyl)]thioethanoyl]-2,3-diaminopropanoate (11) via DCC/DMAP Coupling

To a slightly turbid suspension of acid 3 (100 mg, 0.14 mmol), cholesterol (52 mg, 0.14 mmol) and DCC (52 mg, 1.8 eq., 0.25 mmol) in CH₂Cl₂ (1.0 mL) was added a couple crystals of DMAP with stirring under a N₂ atmosphere. TLC of the reaction mixture after 12 hours showed primarily unreacted cholesterol, 3 and a new UV-active component at Rf 0.09 (CH₂Cl₂). Four cycles - each consisting of DCC addition (~50 mg), a 12-hour reaction period, followed by TLC of the reaction mixture - failed to change the initial TLC profile. Moreover, two cycles of freshly-activated, 4Å molecular sieve additions (~10 beads/cycle) and one cycle of additional DMAP (5 mg), also produced no detectable change. After one week, the reaction mixture was filtered with CH₂Cl₂ washings (4 X 10 mL) and the filtrate was washed with 0.5 N HCl (50 mL) and a saturated brine/water mixture (1:1 v/v, 2 X 50 mL) and dried with MgSO₄ for 18 hours. Filtering the suspension and concentrating the filtrate under reduced pressure afforded a gritty, yellow oil. Chromatographic purification of the oil on silica (20% CH₂Cl₂ in 35-60 °C petroleum ether) gave a yellow oil after solvent removal. TLC of the residue showed the major component eluted at Rf 0.09 (UV active) but with some contamination by DCC and DCU. However, ¹H NMR spectroscopy revealed the major component to be DCC. A second set of signals was detected and subsequently assigned to compound 14.
7.6.15 Cyclohexyl N,N'-bis[2-(triphenylmethyl)thioethanovll-2,3-diaminopropanamide (12)

To a milky-white suspension of acid 3 (80 mg, 0.11 mmol) in CH₂Cl₂ (2.0 mL) under a N₂ atmosphere was added DCC (27 mg, 1.2 eq., 0.13 mmol) with magnetic stirring. After a 15-minute activation period, during which some clearing of the reaction mixture was noted, cyclohexylamine (28 µL, 0.22 mmol) was introduced. The reaction was diluted with CH₂Cl₂ (100 mL) after an hour and washed with 0.5 N HCl (100 mL), 0.5 N NaHCO₃ (100 mL) and then dried over MgSO₄ for an hour. Filtration and concentration of the filtrate in vacuo afforded a granular film. This residue was resuspended in CDCl₃ (1 mL), filtered through glass wool and the filtrate was used for TLC and NMR spectroscopy. The collected solid was determined to be DCU (NMR, TLC). The compound showed:

TLC: \( R_f \) 0.32 (98% CH₂Cl₂:2% MeOH)

\[^1\text{H} \text{NMR (CDCl}_3\text{):}\]
\[ \delta 1.0-1.9 (m, 10H, (CH₂)₅), 2.90-2.93 (m, 4H, H-5 and H-7), 3.56 (br m, 1H, H-1'), 3.88 (m, 1H, H-2), 6.49 (t, J=5.8 Hz, 1H, C₄O-NH), 6.69 (d, J=6.9 Hz, 1H, C₆O-NH), 7.06 (d, J=6.2 Hz, 1H, C₆O-NH), 7.15-7.48 (m, 32H (overlapping residual CHCl₃), aryl) \]

(500 MHz)
7.6.16 Cyclohexyl N,N'-bis[2-(triphenylmethyl)thioethanoyl]-2,3-diaminopropanoate (13)

To a milky-white suspension of acid 3 (80 mg, 0.11 mmol) in CH$_2$Cl$_2$ (2.0 mL) under a N$_2$ atmosphere was added DCC (25 mg, 1.3 eq., 0.12 mmol) with magnetic stirring. After a 15-minute activation period, during which some clearing of the reaction mixture was noted, cyclohexanol (18 µL, 0.18 mmol) and a couple small crystals of DMAP was introduced. The reaction was diluted with CH$_2$Cl$_2$ (100 mL) after 48 hours and washed with 0.5 N HCl (100 mL), distilled water (100 mL) and dried over MgSO$_4$ for an hour. Filtration and concentration of the filtrate in vacuo afforded a granular, oily film (98 mg). This residue was resuspended in CH$_2$Cl$_2$ (1 mL) and filtered through glass wool with CH$_2$Cl$_2$ washings (2 x 1 mL). The collected solid was determined to be DCU (NMR, TLC). Concentration of the filtrate in vacuo and flash chromatography of the yellow residue on silica (step-wise elution with 100% CH$_2$Cl$_2$ then 2% MeOH in CH$_2$Cl$_2$) gave a yellow, oily film (ca. 20 mg). TLC indicated contamination with DCU and a second, more intense, component at $R_f$ 0.67 (14). The compound showed:
TLC:  
$R_f 0.75$ (98% CH$_2$Cl$_2$:2% MeOH)  
$80.88-1.97$[m,38H,(CH$_2$)$_5$], 3.05-3.11(m,5H,H-5 and H-7), 3.22(m,2H,H-3), 4.33(m,1H,H-2), 4.78(br m,1H,H-1’), 6.14(t,$J=6.5$ Hz,1H,C$_3$O-NH), 6.85(d,$J=6.6$ Hz,1H,C$_6$O-NH), 7.1-7.5(m,39H (overlapping residual CHCl$_3$),aryl] (200 MHz)

7.6.17 Dicyclohexylurea $N,N'$-bis[2-(triphenylmethyl)thioethanoyl]-2,3-diaminopropanamide (14)

To a milky-white suspension acid 3 (70 mg, 0.10 mmol) in CH$_2$Cl$_2$ (2 mL) under a N$_2$ atmosphere was added DCC (24 mg, 1.1 eq., 0.10 mmol) with magnetic stirring. After a 15-minute activation period, during which some clearing of the reaction mixture was noted, DMAP (1.2 mg, 10 mol%) was introduced. TLC of the slightly turbid, yellow reaction mixture after 12 hours revealed unreacted acid and DCC (16 mg) was further added. After four days, the reaction mixture was flash chromatographed on silica (step-wise elution with 100% CH$_2$Cl$_2$ then 2% MeOH in
CH₂Cl₂) to afford a yellow, gritty film after *in vacuo* removal of the solvent. The residue was suspended in CDCl₃ (1 mL), filtered through glass wool with CDCl₃ washings (2 x 0.5 mL) and concentrated *in vacuo* to give a yellow foam (56 mg). TLC indicated DCU contamination and a second major component at *R*ₗ 0.8. The compound showed:

**TLC:**

\[ *R*ₗ 0.67 (98% CH₂Cl₂:2% MeOH) \]

**¹H NMR (CDCl₃):**

80.88-1.97[m,61H,(CH₂)₃], 3.05-3.11(m,6.5H,H-5 and H-7), 3.22(m,2H,H-3), 3.30-3.75(m,H-3' and residual methines of DCU), 3.95(m,1H,H-1'), 4.27(m,1H,H-2), 5.88(t,J=5.5 Hz,1H,C₂O-NH), 6.10(t,J=7.2 Hz,1H,C₄O-NH), 7.00(d,J=5.8 Hz,1H,C₆O-NH), 7.1-7.5[m,109H (overlapping residual CHCl₃),aryl] (200 MHz)
7.6.18 Cholesteryl diphenylethanoate (15)

To a magnetically-stirred solution of diphenylacetic acid (500 mg, 2.36 mmol), cholesterol (851 mg, 2.20 mmol) and DCC (535 mg, 1.1 eq., 2.59 mmol) in CH₂Cl₂ (7 mL) at room temperature was added DMAP (29 mg, 10 mol%). After four hours, the suspension was filtered (to remove precipitated DCU) and the filtrate diluted with CH₂Cl₂ (100 mL). The organic phase was washed with 0.5 N HCl (2 × 100 mL), distilled water (100 mL), 0.5 N NaHCO₃ (2 × 100 mL) and dried with MgSO₄ for 18 hours. Filtration and concentration of the filtrate in vacuo afforded 15 as an opaque solid (1.22 g, 89%). The compound showed:

mp: 150.0-155.0 °C

TLC: \( R_f 0.93 \) (98% CH₂Cl₂:2% MeOH)

MS (+NH₃ DCl): \( m/z \) (RI%); 598(23)[M+NH₄⁺],

386(33)[M-(C₁₄H₁₂O₂)+NH₄⁺], 369(100)[M-(C₁₄H₁₂O₂)+H⁺], 230(38)[C₁₄H₁₁O₂+NH₄⁺]

¹H NMR (CDCl₃): 80.70-2.36(m,47H, steroid), 4.69(m,1H,H-3), 5.01(s,1H,H-2'), 5.40(m,1H,H-6), 7.27-7.37(m,11H (overlapping residual CHCl₃),aryl] (200 MHz)
7.6.19 Methyl N-[N',N''-bis(2-(triphenylmethyl)thiopropyl)-2,3-diaminopropanoyl] glycinate (16)

A suspension of glycine methyl ester·HCl (195 mg, 1.55 mmol), NEt₃ (6 drops) in AN (10 mL) was added dropwise to a magnetically-stirred solution of acid 3 (1.14 g, 1.55 mmol) and DCC (352 mg, 1.1 eq., 1.70 mmol) in CH₂Cl₂ (20 mL) after a 10-minute activation period under a N₂ atmosphere. After 12 hours, the suspension was chilled at -15 °C for two hours and filtered (to remove DCC). The filtrate was diluted with CHCl₃ (150 mL) and washed with 0.5 N NaHCO₃ (300 mL) then 0.5 N HCl (2 × 300 mL) and dried over MgSO₄ for two hours. Filtration and rotary evaporation of the filtrate gave 16 as an off-white solid (1.19 g, 95%). The compound showed:

mp: 168.0-171.0 °C

TLC: *R*₂ 0.30 (98% CH₂Cl₂:2% MeOH)

MS (+NH₃ DCl): m/z (RI%); 808(<1)[M+1], 322(10), 262(38), 243(100)[Tr+], 167(48)

IR (KBr pellet): Ψ₉₉₀ 3400,3250(s)(N-H bonded), 3060(w)(aromatic C=C-H), 2930,2850 (w)(aliphatic C-H), 1735(m)(C=O,ester), 1660(s)(C=O,Amide I), 1540(m)(N-H,Amide II), 1490,1440(m)(aromatic C=C), 735,690(s)(monosubstituted aromatic C-H)

¹H NMR (CDCl₃): 8.303[m,5H,H-5,H-7 and H-3a(b)], 3.27[m,1H,H-3a(b)], 3.70(s,3H, COOCH₃), 3.92(ABX,2H,H-8), 3.99(m,1H,H-2), 6.53(t,J=6.3 Hz,1H,C₄O-NH), 7.09(d,J=5.8 Hz,1H,C₄O-NH), 7.16-7.44[m,31H(overlapping residual CHCl₃),aryl and C₄O-NH] (500 MHz)
$^{13}$C NMR (CDCl$_3$):  
83.58, 36.06 (C$_5$ and C$_7$), 41.18 (C$_3$), 52.31 (C$_2$),  
54.75 (OCH$_3$), 67.72 (C-Ph$_3$), 126.98-129.56,  
143.93 (aryl), 169.35, 169.69, 169.80, 170.13 (C$_1$, C$_4$, C$_6$  
and C$_9$) (126 MHz)

![Chemical Structure](image)

16  

7.6.20 $N$-$N^\prime$-$N^\prime$-Bis[2-(triphenylmethyl)thioethanoyl]-2,3- diaminopropanoyl} glycine (17)  

To a magnetically-stirred suspension of ester 16 (1.19 g, 1.47 mmol) in absolute EtOH (25 mL) was added 1 NaOH (2.9 mL). After 12 hours, the suspension  
was rotary evaporated to 10 mL, diluted with 0.5 N NaHCO$_3$ (300 mL) and extracted  
with CHCl$_3$ (200 mL). The organic layer was washed with 0.5 N HCl (2 X 300 mL)  
and dried over MgSO$_4$ for two hours. Filtration and rotary evaporation of the filtrate  
gave a yellow solid. This crude product was dissolved in CHCl$_3$ (3 mL), diluted with  
diethyl ether (60 mL) and filtered to afford 17 as an off-white solid (1.06 g, 91%). The  
compound showed:

mp: 118.5-121.0°C  
TLC: $R_f$ 0.13 (90% CH$_2$Cl$_2$:10% MeOH)  
IR (KBr pellet): $\nu_{\text{MAX}}$ 3300(s)(N-H bonded), 3060(w)(aromatic
C=C-H), 2920(w)(aliphatic C-H).
1730(s)(C=O,acid), 1650,1635(s)(C=O,Amide I),
1530(s)(N-H, Amide II), 1490,1440(m)(aromatic
C=C), 1200(m), 740,695(s)(monosubstituted
aromatic C-H)

$^1$H NMR (CD$_3$COCD$_3$): 8.289,2.92(s,4H,H-5 and H-7), 3.31(m,3H (absorbed
H$_2$O),H-3), 3.89(m,2H,H-8), 4.25(m,1H,H-2),
7.17-7.38(m,29H,aryl) (500 MHz)

$^{13}$C NMR (CD$_3$COCD$_3$): 83.511(C$_5$ and C$_7$), 39.55(C$_5$), 40.24(C$_8$), 52.70(C$_2$),
66(C-Ph$_3$), 125.83-128.51,143.31(aryl), 167.24,
167.87,168.78,169.26(C$_1$,C$_4$,C$_6$ , and C$_9$) (126 MHz)

7.6.21 Cholesteryl N-(N',N''-bis[2-(triphenylmethyl)thioethanoyl]-2,3-

-diaminopropanoyl] glycinate (18)

To an ice-bath cooled suspension of acid 17 (150 mg, 0.19 mmol),
cholesterol (73 mg, 0.19 mmol), DMAP (2 mg, 10 mol%) in CH$_2$Cl$_2$ (2 mL) was added
DCC (59 mg, 1.5 eq., 0.28 mmol) under a N$_2$ atmosphere. After five minutes, the
opaque suspension was allowed to warm to ambient temperature and significant
clearing was noted after 10 minutes. After 48 hours, the reaction suspension was chilled for two hours at -15 °C, filtered and the residue (DCU) was washed with cold CH₂Cl₂ and discarded. The filtrate was diluted with 30-60 °C petroleum ether (50 mL) and stored at -15 °C for 18 hours. The suspension was filtered and the residue washed with cold petroleum ether and dried under high vacuum to afford cholesterol-free crude product as an opaque solid (205 mg). Chromatographic purification of this crude (stepwise elution: CH₂Cl₂ then 1% MeOH in CH₂Cl₂) gave 18 as a pure sample (119 mg, 54%). The compound showed:

**mp:** 92.5-96.0 °C

**TLC:**  
R_f 0.81 (90% CH₂Cl₂:10% MeOH)

**IR (KBr pellet):**  
ν_MAX 3320(s)(N-H bonded), 3050(m)(aromatic C=C-H), 2940(s)(aliphatic C-H), 1740(s)(C=O,ester), 1660(s)(C=O,Amide I), 1510(s)(N-H,Amide II), 1490,1440(s)(aromatic C=C), 1200(s)(C-O), 730,690(s)(monosubstituted aromatic C-H)

**¹H NMR (CDCl₃):**

- 8.0.70-2.32(m,48H,steroid skeleton), 2.93-3.14[m,5H,H-5',H-7' and H-3'a(b)], 3.20-3.34[m,1H,H-3'a(b)], 3.88(ABX,2H,H-8'), 4.01(br s,1H,H-2'), 4.62(m,1H,H-3), 5.36(br s,1H,H-6), 6.55(br t,1H,C₄-O-NH), 7.09(d,J=5.9 Hz,1H,C₆-O-NH), 7.13(br s,1H,C₁-O-NH), 7.18-7.43[m,32H (overlapping residual CHCl₃),aryl] (500 MHz)

**¹³C NMR (CDCl₃):**

- δ11.85(C₁₂), 18.71-39.72(C₅-C₇ and remaining steroid), 41.51(C₃), 41.93(C₈), 50.02(C₉),
54.66(C₂), 56.15(C₁₇), 56.70(C₁₄), 67.70(C-Ph₃), 75.37(C₃), 122.92(C₆), 129.55–126.96, 143.95(aryl), 139.34(C₅), 168.67, 169.25, 169.71, 170.05(C₁⁻, C₄⁻, C₆⁻, and C₉⁻) (126 MHz)

7.6.22 Cholesteryl N-[N',N'-(2,2'-dithiobisethanoyl)-2,3-diaminopropanoyl]glycinate (19)

This synthesis was a modification of the method of Kamber.³⁷ Resublimed I₂ (120 mg, 1.1 eq., 0.47 mmol) was added to a solution of S-tritylated steroid conjugate 18 (500 mg, 0.43 mmol) in degassed absolute EtOH (100 mL) under a N₂ atmosphere. The amber-brown solution was further degassed for 30 minutes under aspirator vacuum (15 mm Hg), vented to dry N₂ gas and allowed to magnetically stir at room temperature. After 12 hours, the amber suspension was rotary evaporated to 1 mL, diluted with ddH₂O (70 mL), decolorized with 0.1 N Na₂S₂O₃ (5 drops) and the resultant suspension was extracted with CHCl₃ (100 mL). The organic layer was washed with a dilute solution of Na₂S₂O₃ and dried over Na₂SO₄ for two hours.
Filtration and concentration of the filtrate in vacuo to 10 mL followed by gradual dilution with cold hexanes afforded an amber solid (221 mg) after filtration. Chromatographic purification of the resultant crude product (stepwise elution: 100% CH₂Cl₂ followed by 5% MeOH in CH₂Cl₂) gave 19 as an off-white solid (94 mg, 32%). The compound showed:

mp: 165.0-167.5 °C
TLC: $R_f$ 0.51 (90% CH₂Cl₂:10% MeOH)
IR (KBr pellet): $\nu_{\text{MAX}}$ 3300(s)(N-H bonded), 3060(w)(C=H)
2940,2869(s)(aliphatic C-H), 1740(s)(C=O,ester),
1655(s)(C=O,Amide I), 1530(s) (N-H,Amide II),
1200(s)(C-O)
MS (+NH₃ DCl):
$m/z$(RI%); 402(10), 383(12), 367(50), 262(100)
$^1$H NMR (CDCl₃): 80.68-2.32(m,48H,steroid skeleton), 3.07[br
s,1H,H-3’a(b)], 3.23-3.82[br overlapping
s,5H,H-5’,H-7’ and H-3’a(b)], 3.82-4.33(br
overlapping s,2H,H-8’), 4.62(br s,1H,H-3), 4.75(br
s,1H,H-2’), 5.36(br s,1H,H-6), 6.80-8.40(br
m,3H,amide) (500 MHz)
$^13$C NMR (CDCl₃): 811.85(C₁₈),18.72-39.74(C₅–C₇ and remaining
steroid), 41.67(br,C₃’), 42.32(C₈’), 50.01(C₉),
56.22(C₁₇), 56.71(C₁₄), 75.47(br,C₃), 122.99(C₆),
139.28(C₅), 169.69(br,C₁’,C₄’,C₆’ and C₉’) (126 MHz)
7.6.23 Digitoxigenin 3-[N-(N',N''-bis[2-(triphenylmethyl)thioethanoyl]-2,3-diaminopropanoyl] glycinate (20)

To an ice bath-cooled suspension of acid 17 (318 mg, 0.40 mmol), digitoxigenin (Sigma Chemical Co.) (150 mg, 0.40 mmol), DMAP (4 mg, 10 mol%) in CH₂Cl₂ (6 mL) was added DCC (91 mg, 1.1 eq., 0.44 mmol) under a N₂ atmosphere. After five minutes, the opaque suspension was allowed to warm to ambient temperature and significant clearing was noted after 10 minutes. After 48 hours, the reaction suspension was chilled for two hours at -15 °C, filtered and the residue (DCU) was washed with cold CH₂Cl₂ and discarded. The filtrate was rotary evaporated to a pale-yellow solid and dissolved in MeOH (10 mL). Five drops of dH₂O was added and the resulting suspension was stored at -15 °C for two hours. The suspension was filtered and the residue washed with cold MeOH/H₂O (98:2 v/v) and dried under high vacuum to afford 20 as an off-white residue (257 mg). The turbid, aqueous-methanolic filtrate was concentrated to 3 mL, diluted with dH₂O (2 mL), filtered and the residue washed with cold MeOH/H₂O (98:2 v/v). After drying under high vacuum, 15 mg of digitoxigenin-free product (total yield = 272 mg, 59%) was recovered. The compound showed:
mp: 115.0-118.0 °C

TLC: 

IR (KBr pellet):

$R_f$ 0.71 (90% CH$_2$Cl$_2$:10% MeOH)

$\nu_{max}$ 3380(s)(N-H,O-H bonded), 3050(m)(aromatic C=C-H), 2930(s)(aliphatic C-H), 1740(s)(C=O,ester), 1660(s)(C=O,Amide I), 1510(s)(N-H,Amide II), 1485,1440(s)(aromatic C=C), 1200(m)(C-O), 735,690(s)(monosubstituted aromatic C-H)

$^1$H NMR (CDCl$_3$):$^{172}$

80.88(s,3H,H-18), 0.95(s,3H,H-19), 1.20-2.14(m,28H,steroid), 2.78(m,1H,H-17), 3.02[m,5H,H-5',H-7' and H-3'a(b)], 3.30[m,1H,H-3'a(b)], 3.92(ABX,2H,H-8'), 4.00(m,1H,H-2'), 4.89(AB q,J=-18.1 Hz,2H,H-21), 5.12(br s,1H,H-3), 5.88(s,1H,H-22), 6.55(t,J=6.3 Hz,1H,C$_4$-O-NH), 7.10(d,J=5.8 Hz,1H,C$_6$-O-NH), 7.18-7.44[m,31H,(overlapping residual CHCl$_3$),aryl] (500 MHz)

$^1$3C NMR (CDCl$_3$):$^{173}$

815.73(C$_{18}$), 21.11,21.19(C$_7$ and C$_{11}$), 23.61(C$_{19}$), 24.96-35.10(remaining steroid), 35.66,36.69(C$_5$ and C$_9$), 35.66,35.81(C$_5$' and C$_7$), 39.94(C$_{12}$), 41.56(C$_3$'), 41.77(C$_9$), 42.04(C$_8$'), 49.55(C$_{13}$), 50.85(C$_{17}$), 54.74(C$_2$'), 67.67(C-Ph$_3$), 71.90(C$_3$), 73.40(C$_{20}$), 85.43(C$_{14}$), 117.69(C$_{22}$), 126.95-129.53,143.91(aryl), 168.71,169.28,169.71,170.06(C$_1$',C$_4$',C$_6$' and C$_9$'), 174.43(C$_{23}$) (126 MHz)
7.6.24 Digitoxigenin \(N'\cdot N''\cdot (2,2'\text{-dithiobisethanoyl})\cdot 2,3\text{-diaminopropanoyl}\) glycinate (21)

This synthesis was a modification of the method of Kamber.\(^{37}\) Resublimed I\(_2\) (77 mg, 1.1 eq., 0.30 mmol) was added to a solution of S-tritylated steroid conjugate 20 (316 mg, 0.27 mmol) in degassed absolute EtOH (80 mL) under a N\(_2\) atmosphere. The amber-brown solution was further degassed for 30 minutes under aspirator vacuum (15 mm Hg), vented to dry N\(_2\) gas and allowed to magnetically stir at room temperature. After 12 hours, the amber-brown suspension was concentrated to 5 mL, diluted with CH\(_2\)Cl\(_2\) (70 mL) and the resultant suspension filtered with CH\(_2\)Cl\(_2\) washings. The tan-brown residue was dissolved in a 15% v/v MeOH in CHCl\(_3\) mixture (60 mL) and decolorized by washing with dilute aqueous Na\(_2\)S\(_2\)O\(_3\) solution (200 mL). A solid precipitated and was separated by decanting the liquid phases. The residue was redissolved in 15% v/v MeOH in CHCl\(_3\) (30 mL), filtered and the filtrate was rotary evaporated to give 21 as an opaque solid (43 mg, 24%) which was homogenous via TLC. The compound showed:
mp: 180 °C (dec.)

TLC: 

\[ R_f 0.34 \ (90\% \ CH_2Cl_2:10\% \ MeOH) \]

IR (KBr pellet): 

\[ \nu_{\text{MAX}} \ 3415(s)(\text{O-H,N-H bonded}), \]
\[ 2920,2850(s)(\text{aliphatic C-H}), 1735(s)(\text{C=O,ester}), \]
\[ 1655(s)(\text{C=O,Amide I}), 1530(s)(\text{N-H,Amide II}), \]
\[ 1200(s)(\text{C-O}) \]

MS (FAB+): 

\[ m/z(\text{RI\%}) \; 664(5)[\text{M}+1], 339(12) \]

\[^1\text{H NMR (CDCl}_3/\text{CD}_2\text{OD):}^{172} \]

\[ 8.01(s,3\text{H},\text{H-18}), 0.90(s,3\text{H},\text{H-19}), \]
\[ 1.11-2.12(m,28\text{H,steroid}), 2.73(m,1\text{H,}\text{H-17}), \]
\[ 3.20-3.80(br \text{ m,7H,}\text{H-5',H-7' and H-3'}), 3.95(br \text{ m,2H,}\text{H-8'}), 4.89(AB \text{ q,J=18.3 Hz,2H,}\text{H-21}), \]
\[ 5.08(br \text{ s,1H,}\text{H-3}), 5.80(s,1\text{H,}\text{H-22}), 7.17(br \text{ m,0.5H,exchanging amide}) \text{ (500 MHz)} \]

\[^{13}\text{C NMR (CDCl}_3/\text{CD}_2\text{OD):}^{173} \]

\[ 61.53(\text{C}_{18}), 20.79(\text{C}_7 \text{ and C}_{11}), 23.14(\text{C}_{19}), \]
\[ 24.58-34.93(\text{remaining steroid}), 35.25,36.70(\text{C}_3 \text{ and C}_9), 39.51(\text{C}_{12}), 40.94(br,\text{C}_3), 41.02(\text{C}_8), \]
\[ 41.15(br,\text{C}_8), 49.51(\text{C}_{13}), 50.66(\text{C}_{17}), 52.39(br)(\text{C}_2), \]
\[ 72.01,72.10(\text{C}_3), 73.63(\text{C}_{20}), 84.64(\text{C}_{14}), \]
\[ 116.74(\text{C}_{22}), 168.95,169.28,169.72,170.29 \text{ (br,} \text{C}_1, \text{C}_4, \text{C}_6, \text{ and C}_9), 175.50,176.03(\text{C}_{23}) \text{ (126 MHz)} \]
7.6.25 3-Cholamido-1-{N,N'-bis[2-(triphenylmethyl)thioethanoyl]-2,3-diaminopropanamido} propane (22)

To a magnetically-stirred solution of acid 3 (316 mg, 0.43 mmol) in DMF (2 mL) was added DCC (97 mg, 1.1 eq., 0.47 mmol) under a N₂ atmosphere. After 10 minutes, a solution of 3-cholamidopropylamine{102 (200 mg, 0.43 mmol), NEt₃ (2 drops) in DMF (2 mL) was added and the mixture was allowed to stir under a N₂ atmosphere. After five hours, the reaction mixture was added to 0.5 N HCl (500 mL) and extracted with CH₂Cl₂ (200 mL). The organic layer was backwashed with 0.5 N NaHCO₃ (500 mL) and dH₂O (500 mL) and dried over MgSO₄ for 48 hours. Filtration and rotary evaporation of the filtrate afforded a solid (497 mg, 98%). Chromatographic purification of this crude material (stepwise elution: CH₂Cl₂ then 10% MeOH in CH₂Cl₂) gave 22 as an opaque solid. The compound showed:

mp: 129.0-131.0 °C

TLC: \( R_f 0.17 \) (90% CH₂Cl₂:10% MeOH)

IR (KBr Pellet): \( \nu_{\text{MAX}} 3380(s)(\text{N-H,O-H bonded}), 3060(m)(\text{aromatic C=C-H}), 2930,2860(s)(\text{aliphatic C-H}), 1655(s)(\text{C=O, Amide I}), 1525(m)(\text{N-H, Amide II}), \)
$^1$H NMR (DMSO-$d_6$):$^{172}$

1490,1445(m)(aromatic C=C), 1380(w), 1075(m), 1030(m), 740,695(s)(monosubstituted aromatic C-H)

$^80.57(s,3H,H-18), 0.80(s,3H,H-19),$

0.83-2.15(m,42H,steroid), 2.72,2.78 (s,4H,H-5’ and H-7’), 2.98(br m,5H,H-25,H-27 and H-3’a(b)),

3.17[br m,3H,H-3 and H-3’a(b)], 3.62(br s,1H,H-7),

3.79(br s,1H,H-12), 3.98,4.07,4.32(br s,3H)

total,-OH), 4.13(m,1H,H-2’), 7.20-7.32(m,36H,aryl),

7.73(br t,1H,C$_{24}$O-NH), 7.86(m,2H,C$_1$-O-NH and C$_4$-O-NH), 8.00(d,$J=7.6$ Hz,1H,C$_6$-O-NH) (500 MHz)

$^{13}$C NMR (DMSO-$d_6$):$^{173}$

612.31(C$_{18}$), 17.10(C$_{21}$), 22.60(C$_{19}$), 22.81(C$_{15}$),

26.21(C$_9$), 27.28(C$_{16}$), 28.54(C$_{11}$), 29.01(C$_{26}$),

30.40(C$_2$), 31.72(C$_{22}$), 32.57(C$_{23}$), 34.37(C$_1$),

34.89(C$_8$), 35.15(C$_{10}$), 35.32(C$_{20}$), 35.89,35.95(C$_5$ and C$_7$),

36.04(C$_{25}$), 36.21(C$_{27}$), 40.67(C$_3’$),

41.35(C$_9$), 41.54(C$_{14}$), 45.73(C$_{13}$), 46.10(C$_{17}$),

54.83(C$_2$’), 65.90(C-Ph$_3$), 66.28(C$_7$), 70.48(C$_3$),

71.06(C$_{12}$), 126.57-129.43,144.05(aryl),

169.12,167.76,167.42 (C$_1’$, C$_4’$ and C$_6’$), 172.78(C$_{24}$)

(126 MHz)
7.6.26 3-Cholamido-1-\{N,N'-(2,2'-dithiobisethanoyl)\}-2,3- diaminopropanamido-
propane (23)

This synthesis was a modification of the method of Kamber.\textsuperscript{37} Resublimed I\textsubscript{2} (71 mg, 1.1 eq., 0.28 mmol) was added to a solution of S-tritylated steroid conjugate 22 (300 mg, 0.25 mmol) in degassed absolute EtOH (60 mL) under a N\textsubscript{2} atmosphere. The amber-brown solution was further degassed for 30 minutes under aspirator vacuum (15 mm Hg), vented to dry N\textsubscript{2} gas and allowed to magnetically stir at room temperature. After 12 hours, the amber solution was rotary evaporated to 1 mL, diluted with ddH\textsubscript{2}O (30 mL), decolorized with 0.1 N Na\textsubscript{2}S\textsubscript{2}O\textsubscript{3} (5 drops) and the resultant light-brown precipitate was vacuum filtered and washed with ddH\textsubscript{2}O and CHCl\textsubscript{3}. The precipitate was dissolved in hot absolute EtOH (10 mL), decolorized with 0.1 N Na\textsubscript{2}SO\textsubscript{3} (3 drops) and, after concentration to 1 mL, diluted with a 1:1 v/v mixture of ddH\textsubscript{2}O and saturated brine. The precipitated solid was vacuumed filtered, washed with ddH\textsubscript{2}O and CHCl\textsubscript{3}, redissolved in hot EtOH (5 mL) and precipitated by the gradual addition of CH\textsubscript{2}Cl\textsubscript{2} (50 mL). The suspension was stored at -15 °C for 18 hours and filtered with cold CH\textsubscript{2}Cl\textsubscript{2} washings to afford 23 as an opaque solid (78 mg, 45%). The compound showed:
mp: 174.0 °C (dec.)

TLC: $R_f$ 0.79 (100% MeOH)

IR (KBr Pellet): $\nu_{\text{MAX}}$ 3400(s)(N-H,O-H bonded), 2930,2860(m)(aliphatic C-H), 1655(s)(C=O,Amide I), 1540(m)(N-H,Amide II), 1075,1040(w)

MS (FAB+): $m/z$(RI%); 698(70)[M+1], 466(100)[(M+1) - C$_7$H$_2$N$_2$O$_3$S$_2$]

$^1$H NMR (DMSO-$d_6$):$^{172}$

$\delta$ 0.57(s,3H,H-18), 0.79(s,3H,H-19), 0.83-2.24(m,31H,steroid), 3.01-3.07 (br m,4H,H-25 and H-27), 3.17(m,2H,H-3\beta), 3.25-3.59[br m,8.4H (overlapping H$_2$O),H-3’,H-5’ and H-7’], 3.60(br m,1H,H-7\beta), 3.76(br m,1H,H-12\beta), 3.96,4.05,4.28(d,$J=3.3$ Hz,1H,=OH), 4.30-4.42(br m,0.5H,H-2’_ISOMER 1), 4.68(br m,0.4H,H-2’_ISOMER 2), 7.95-8.43 (br m,4H,amide) (500 MHz)

$^{13}$C NMR (DMSO-$d_6$):$^{173}$

612.29(C$_{18}$), 17.07(C$_{21}$), 22.57(C$_{19}$), 22.75(C$_{15}$), 26.17(C$_9$), 27.23(C$_{16}$), 28.51(C$_{11}$), 29.07(C$_{26}$), 30.35(C$_2$), 31.70(C$_{22}$), 32.53(C$_{23}$), 34.34(C$_1$), 34.84(C$_6$), 35.13(C$_{10}$), 35.28(C$_{20}$), 36.03,36.26, 36.39(br)(C$_5$;C$_7$;C$_{25}$;C$_2$;\gamma), 40.76,42.00(br)(C$_3$), 41.32(C$_8$), 41.50(C$_{14}$), 45.70(C$_3$), 46.10(C$_{13}$), 47.48(C$_{17}$), 52.18,52.91(br)(C$_2$;\gamma), 66.19(C$_7$), 70.38(C$_3$), 70.96(C$_{12}$), 167.96,168.21,168.33, 168.47,169.16, 169.61(br)(C$_1$;C$_4$;C$_5$), 172.12,172.37,172.51(C$_{24}$) (126 MHz)
7.6.27 Methyl 11-aminoundecanoate .HCl (24)

HCl gas was bubbled for about 30 minutes into a suspension of 11-aminoundecanoic acid .HCl (7.00 g, 34.8 mmol) in absolute MeOH (250 mL) with magnetic stirring. After refluxing for 12 hours and discarding the first 50 mL of distillate, the solution was rotary evaporated to a fluffy, opaque solid (8.59 g, 98%). Recrystallization from absolute MeOH gave 24 as opaque plates. The compound showed:

mp: 136.5-140.0 ºC

TLC: \( R_f \) 0.09 (90% CH\(_2\)Cl\(_2\);10% MeOH; ninhydrin positive)

IR (KBr Pellet): \( \nu_{\text{MAX}} \) 3000(s)(N-H stretch of salt), 2920,2850(w) (aliphatic C-H), 1720(s)(C=O,H-bonded ester), 1555,1500(m)(N-H bend of salt), 1160(s)(O-C)

\(^1\)H NMR (DMSO-\(d_6\)):

8.122(s,12H,H-4 to H-9), 1.49(m,4H,H-3 and H-10), 2.26(t,\(J=7.4\)Hz,2H,H-2), 2.68(m,2H,H-11), 3.55(s, 3H,COOCH\(_3\)), 8.14(br s,3H,-NH\(_3^+\)) (200 MHz)

\(^13\)C NMR (DMSO-\(d_6\)):

824.36,25.84,26.81,28.46-28.69 (C\(_3\) to C\(_{10}\)),
33.20(C_{2}), 38.60(C_{11}), 51.06(OCH_{3}), 173.25(C_{1})

(126 MHz)

![Chemical Structure]

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7.6.28 Methyl 11-\{(N,N'-bis[2-(triphenylmethyl)thioethanoyl]-2,3-diaminopropanamido\} undecanoate (25)

To a magnetically-stirred solution of acid 3 (550 mg, 0.75 mmol) in AN/CH_{2}Cl_{2} (2:1 v/v, 7 mL) was added DCC (170 mg, 1.1 eq., 0.82 mmol) under a N_{2} atmosphere. After 10 minutes, a suspension of amine hydrochloride 24 (189 mg, 0.75 mmol), NEt_{3} (2 drops) in AN (3 mL) was added and the mixture allowed to stir under a N_{2} atmosphere. After 14 hours, the suspension was chilled for 30 minutes at -15 °C, gravity filtered and the residue (DCU) washed with cold CH_{2}Cl_{2} and discarded. The filtrate was diluted to with CH_{2}Cl_{2} (100 mL), washed with 0.5 N HCl (2 X 100 mL) and dried over MgSO_{4} for two hours. Filtration and rotary evaporation of the filtrate afforded a pale-yellow solid (675 mg, 93%). Recrystallization from absolute MeOH gave 25 as opaque plates. The compound showed:

mp: 117.0-119.0 °C

TLC: R_{f} 0.21 (98% CH_{2}Cl_{2}; 2% MeOH)

IR (KBr Pellet): ν_{MAX} 3300(s)(N-H bonded), 3050(m)(aromatic C=C-H), 2920,2850(aliphatic C-H), 1730(m)(C=O,ester), 1650(s)(C=O,Amide I),
1H NMR (CDCl₃):
81.17(s,12H,H-4 to H-9), 1.36(br m,2H,H-3), 1.54(m,4H,H-10 and overlapping H₂O), 2.22(t,J=7.5 Hz,2H,H-2), 2.93[m,5H,H-5',H-7' and H-3'a(b)], 3.08(m,2H,H-11), 3.17[m,1H,H-3'a(b)], 3.59(s,3H,COOCH₃), 3.86(m,1H,H-2'), 6.44(t,J=6.2 Hz,1H,C₄-O-NH), 6.64(br t,1H,C₁-O-NH), 6.94(d,J=5.9 Hz,1H,C₆-O-NH), 7.12-7.33[m,32H (overlapping residual CHCl₃),aryl] (500 MHz)

13C NMR (CDCl₃):
824.94, 26.85, 29.12-29.42(C₃ to C₁₀), 34.11(C₂), 35.84, 36.15(C₅ and C₇'), 39.66(C₁₁), 42.08(C₃'), 51.41(OCH₃), 54.69(C₂'), 67.79(C-Ph₂), 127.01-129.56, 143.96(aryl), 169.03, 169.29, 169.94 (C₁, C₄, and C₆') (126 MHz)
7.6.29 Methyl 11-[N,N'-(2,2'-dithiobisethanol)-2,3-diaminopropanamido]-undecanoate (26)

This synthesis was similar to the general S-detritylation procedure described for compound 8 except that the resultant disulfide conjugate was extracted into CHCl₃ (along with trityl impurities) from a decolorized, NaCl-saturated, aqueous suspension. The crude sample was purified on silica gel (stepwise elution: 100% CH₂Cl₂, 1%, 2% and 4% MeOH in CH₂Cl₂) and 26 was isolated as an opaque solid (37%) from concentration of the 4% MeOH fractions. When purification and characterization were not necessary, the crude sample was used directly in the subsequent ion-exchange mediated hydrolysis so that the yield of the disulfide acid is maximized. The compound showed:

mp: 150.0-152.0 °C

TLC: \( R_f \) 0.39 (90% CH₂Cl₂:10% MeOH)

IR (KBr Pellet): \( \nu_{\text{MAX}} \) 3290(s)(N-H bonded), 2920(s)(C-H), 1740(s)(C=O,ester), 1640(s)(C=O,Amide I), 1550(s)(N-H,Amide II), 430(w)(S-S)

\(^1\)H NMR (CDCl₃): $\delta$ 1.25(s,22H,H-4 to H-14), 1.62(br m,4H,H-3 and H-15), 2.30(t,$J$=6.4 Hz,2H,H-2), 2.72-3.32[br s,3H,H-16 and H-3’a(b)], 3.33-3.95[overlapping br s and m,~8H,COOCH₃,H-3’a(b), H-5 and H-6’], 4.36-5.20(br s,1H,H-2’), 7.61-8.71(br s,~2H,amide) (200 MHz)

\(^{13}\)C NMR (CDCl₃): 824.92-29.37(C₃ to C₁₀), 33.94,34.08(C₂), 39.84, 42.61(br), 49.19, 51.42(OCH₃), 169.33,169.47 (br,C₁’,C₄’ and C₇’), 174.31(C₁) (126 MHz)
7.6.30 11-[(N,N'-dithiobisethanoyl)-2,3-diaminopropanamido] undecanoic acid (27)

This synthesis was similar to the general hydrolytic procedure described for the esters 7 or 10 (Section 7.6.4). Compound 27 was isolated as a pale-yellow, pungent-smelling solid (56% minimum) from rotary evaporation of the aqueous-acetone eluate. The compound showed:

IR (KBr Pellet):

\[ \nu_{\text{MAX}} \] 3404(s)(N-H bonded), 2926,2853(s)(C-H), 1700-1630(s)(C=O,acid and Amide I), 1547(m)(N-H,Amide II), 1261(m), 702(m)

\(^1\)H NMR (DMSO-\(d_6\)):

\[ \delta \] 1.22(s,2H,H-3 to H-14), 1.36(br s,2H,H-3), 1.45(br m,2H,H-15), 2.17(t, J=7.2 Hz,2H,H-2), 3.00(br m,2.6H,ligand), 3.30(br m,~12H (overlapping H\(_2\)O,ligand), 4.31(br m,0.5H,H-2'), 7.99,8.65,8.78(br m,~2H total,amide) (500 MHz)

\(^{13}\)C NMR (DMSO-\(d_6\)):

\[ \delta \] 24.44-28.99(C\(_3\) to C\(_{15}\)), 32.23-33.14(C\(_5\),C\(_7\)), 33.62(C\(_2\), 47.54,47.78C\(_2\'), 169.37, 169.45,169.58,169.68,169.80 (C\(_1\),C\(_4\),C\(_6\)), 174.40(C\(_1\)) (126 MHz)
7.6.31 Methyl 16-hydroxyhexadecanoate (28)

HCl gas was bubbled for about 20 minutes into a solution of 16-hydroxyhexadecanoic acid (900 mg, 3.30 mmol) in absolute MeOH (1 L). After an hour, the solution was degassed in vacuo at 0 °C for 50 minutes on a rotary evaporator and concentrated to give 28 as a light-yellow, opalescent solid (904 mg, 96%). The compound showed:

mp: 52.5-53.5 °C

TLC: \[ R_f 0.64 \text{ (90\% CH}_2\text{Cl}_2;10\% \text{ MeOH)} \]

IR (KBr Pellet): \[ \nu_{\text{MAX}} 3410\text{(s)(O-H bonded),} 2920,2850\text{(w)(C-H),} 1735\text{(s)(C=O),} 1160\text{(m)(O-CH}_3\text{,ester),} 1040\text{(m)(O-CH}_2\text{,alcohol)} \]

$^1$H NMR (CDCl$_3$): \[ 8.134\text{(m,22H,H-4 to H-14),} 1.58\text{(m,4H,H-3 and H-15),} 2.30\text{(t,} J=7.5\text{ Hz,2H,H-2),} 3.60\text{(t,} J=6.6\text{ Hz,2H,H-16),} 3.66\text{(s,3H,COOCH}_3\text{)} (200 \text{ MHz)} \]

$^{13}$C NMR (CDCl$_3$): \[ 824.95,25.73,29.14-29.60\text{(C}_3\text{ to C}_{14}\text{),} 32.81\text{(C}_{15}\text{),} 34.11\text{(C}_2\text{),} 51.40\text{(OCH}_3\text{),} 63.06\text{(C}_{16}\text{),} 174.34\text{(C)} (126 \text{ MHz)} \]
7.6.32 Methyl 16-tosylhexadecanoate (29)

Hydroxy ester 28 (900 mg, 3.14 mmol) was added in equal proportions over 20 minutes to a magnetically-stirred, yellow solution of freshly-recrystallized tosyl chloride (1.20 g, 2 eq., 6.28 mmol) in pyridine (3 mL) with 4Å molecular sieves under a N₂ atmosphere. After 12 hours, the light-brown mixture was pipetted into 0.5 N HCl (300 mL) and extracted into CH₂Cl₂ (200 mL). The aqueous layer was removed and re-extracted with CH₂Cl₂ (50 mL) and the pooled organic layers were backwashed with 0.5 N HCl (300 mL) and dried over MgSO₄ for an hour. Filtration, in vacuo concentration of the filtrate and chromatographic purification of the resultant light-yellow oil (stepwise elution: 40% CH₂Cl₂ then 80% CH₂Cl₂ in hexanes) gave 29 as a crystalline, opaque solid (551 mg, 40%) from the evaporation of the 80% CH₂Cl₂ fractions. The 40% CH₂Cl₂ fractions contained the 16-bis ether as the major component (¹H NMR spectroscopy). The compound showed:

TLC: \( R_f 0.28 \) (85% hexanes:15% EtOAc)

IR (KBr Pellet):

\( \nu_{\text{MAX}} \) 2920,2850(s)(aliphatic C-H), 1740(s)(C=O), 1450(m)(aromatic C=C), 1355(s)(antisymmetric SO₂), 1170(s)(symmetric SO₂), 950(s), 805,830,840(s)(disubstituted aromatic C-H), 660(s)

¹H NMR (CDCl₃):

\( \delta 1.23(m,22H,H-4 to H-14), 1.70(m,4H,H-3 and H-15), 2.27(t,J=7.5 Hz,2H,H-2), 2.42(s,3H,aryl-CH₃), 3.64(s,3H,COOCH₃), \)
3.99 (t, J = 6.5 Hz, 2H, H-16), 7.32 (d, J = 8.1 Hz, 2H, aryl),
7.75 (d, J = 8.3 Hz, 2H, aryl) (200 MHz).

7.6.33 Methyl 16-azidohexadecanoate (30)

To a solution of tosylate 29 (550 mg, 1.25 mmol) in dry DMF (5 mL) under a
\( \text{N}_2 \) atmosphere was added NaN\(_3\) (406 mg, 5 eq., 6.24 mmol) with magnetic stirring.
After 12 hours, the suspension was pipetted into 0.5 N NaHCO\(_3\) (300 mL) and
extracted with CH\(_2\)Cl\(_2\) (150 mL). The aqueous layer was removed and re-extracted
with CH\(_2\)Cl\(_2\) (50 mL) and the pooled organic layers were backwashed with 0.5 N
NaHCO\(_3\) (300 mL) and dried over MgSO\(_4\) for one hour. Filtration, in vacuo
concentration of the filtrate afforded 30 as a clear, non-viscous oil that solidified on
standing after three days at -15 °C (380 mg, 98%). The compound showed:

\[
\text{mp:} \quad 29.0-31.0 ^\circ\text{C}
\]

\[
\text{TLC:} \quad R_f 0.51 (85\% \text{ hexanes}:15\% \text{ EtOAc})
\]

\[
\text{IR (KBr Pellet):} \quad \nu_{\text{MAX}} 2920, 2850(\text{s}(\text{C-H}), 2090(\text{s})(\text{N=N}),
1740(\text{s})(\text{C=O})
\]

\[
\text{\( ^1 \text{H NMR (CDCl}_3\):} \quad \delta 1.24(\text{m,} 22\text{H, H-4 to H-14}), 1.60(\text{m,} 4\text{H, H-3 and H-15}), 2.28(\text{t,} J = 7.6 \text{ Hz,} 2\text{H, H-2}), 3.24(\text{t,} J = 7.0 \text{ Hz,} 2\text{H, H-16}), 3.65(\text{s,} 3\text{H, COOCH}_3) (200 \text{ MHz})
\]

\[
\text{\( ^13 \text{C NMR (CDCl}_3\):} \quad \delta 24.94-29.58(\text{C}_3 \text{ to C}_{15}), 34.09(\text{C}_2),
\]
51.48, 51.40(OCH₃), 174.30(C₁) (126 MHz)

7.6.34 Methyl 16-amino-hexadecanolate·HCl Via Catalytic Hydrogenation (31)

A solution of azide 30 (85 mg, 0.27 mmol) in anhydrous MeOH (2 mL) was added to a suspension of H₂-presaturated 10% Pd/C (10 mg) in MeOH (5 mL). The suspension was allowed to magnetically stir under 1 atmosphere of H₂ gas. After 12 hours, 2 M HCl (5 drops) was added and the suspension was gravity filtered with MeOH washings. The filtrate was concentrated on a rotary evaporator to give an opaque solid (62 mg, 71%). Recrystallization from CHCl₃ gave 31 as opaque plates. The compound showed:

TLC: \[ R_f 0.30 \] (80% CHCl₃; 20% MeOH; ninhydrin positive)

IR (KBr Pellet): \[ ν_{MAX} 3450(s)(N-H bonded), 2920, 2850(s)(C-H), 1740(s)(C=O) \]

MS (FAB+): \[ m/z(RI%) \text{; } 300(28), 286(100)[M+1-HCl] \]

¹H NMR (DMSO-d₆):

\[ δ1.23(m, 22H, H-4 to H-14), 1.49(m, 4H, H-3 and H-15), 2.27(t, j=7.3 Hz, 2H, H-2), 2.72(t, j=8.4 Hz, 2H, H-16), 3.56(s, 3H, COOCH₃), 7.96(br s, 3H, -NH₃⁺) \] (200 MHz)

¹³C NMR (CDCl₃):

\[ δ24.94-29.58(C₃ to C₁₅), 34.11(C₂), 40.03(C₁₆), 51.40(OCH₃), 174.33(C₁) \] (126 MHz)
7.6.35 Alternative Synthesis of 31 Via Catalytic Transfer Hydrogenation

This synthesis was a modification of the method of Gartiser et al.\textsuperscript{174} To a magnetically-stirred solution of azide 30 (60 mg, 0.21 mmol) and ammonium formate (53 mg, 4 eq., 0.84 mmol) in MeOH (2 mL) was added 10% Pd/C (6 mg, 10% by weight). After 12 hours, 2 N HCl (4 drops) was added and the suspension was allowed to stir at room temperature for two hours further. The mixture was gravity filtered with and the filtrate rotary evaporated to about 2 mL then diluted with AN (30 mL) and rotary evaporated to 20 mL. Chilling the solution for two hours at -15 °C induced precipitation of a solid (NH₄OOCH). The suspension was filtered and the filtrate concentrated to an opaque solid (19 mg, 28%) which was TLC and \textsuperscript{1}H NMR characterized to be the required amine hydrochloride 31.

7.6.36 Methyl 16-{N,N'-bis[2-(triphenylmethyl)]thioethanoyl}-2,3-diaminopropanamido}hexadecanoate (32)

To a magnetically-stirred suspension of acid 3 (126 mg, 0.17 mmol) in CH₂Cl₂ (2 mL) was added DCC (54 mg, 1.5 eq., 0.26 mmol) of under a N₂ atmosphere. After 15 minutes, the suspension cleared and a solution of amine hydrochloride 31 (57 mg, 0.17 mmol), 2 drops of NEt₃ in CH₂Cl₂ (1 mL) was added and the mixture allowed to stir under a N₂ atmosphere. After 14 hours, the suspension was chilled for 30 minutes at -15 °C, gravity filtered and the residue (DCU) washed with cold CH₂Cl₂ and discarded. The filtrate was diluted to 100 mL with CH₂Cl₂, washed with 0.5 N
NaHCO₃ (150 mL), 0.5 N HCl (2 X 150 mL) and dried over MgSO₄ for two hours. Filtration and rotary evaporation of the filtrate afforded 32 as a pale-yellow solid (130 mg, 76%). The compound showed:

TLC: \( R_f \) 0.75 (90% CH₂Cl₂:10% MeOH)

IR (KBr Pellet):
\( \nu_{\text{MAX}} \) 3400(s)(N-H bonded), 3060(w)(aromatic C=C-H) 2920,2850(s)(aliphatic C-H), 1740(s)(C=O,ester), 1640(m)(C=O,Amide I), 740,695(s)(monosubstituted aromatic C-H)

MS (+NH₃ DCI):
\( m/z \) (RI%); 554(15), 532(40), 518(100)

\(^1\text{H NMR (CDCl}_3\): \( 8.06-2.04(\text{m,26H,H-3 to H-15}), 2.26(t,J=7.4 \text{ Hz,2H,H-2}), 3.00(s,4H,H-5' and H-7'), 3.16(m,4H,H-3' and H-16), 3.65(s,3H,COOCH}_3\), 3.97(m,1H,H-2'), 6.55(t,J=6.7 Hz,1H,C\(_1\)-O-NH), 6.82(t,J=6.3 Hz,1H,C\(_1\)-O-NH), 7.03(d,J=5.8 Hz,1H,C\(_6\)-O-NH), 7.19-7.55[m,34H (overlapping residual CHCl\(_3\)aryll) (200 MHz)]

\(^{13}\text{C NMR (CDCl}_3\): \( 82.49,29.63(\text{C}_3 \text{ to C}_{15}), 34.12,33.96(\text{C}_2), 35.82,36.15(\text{C}_5' \text{ and C}_7'), 39.67(\text{C}_{16}), 42.06(\text{C}_3'), 51.42(\text{OCH}_3), 54.75(\text{C}_2'), 67.78(\text{C-Ph}_3), 127.00-129.56,143.90 (aryl), 169.03,169.28,169.96 (C\(_1\)'C\(_4\)',C\(_6\)'), 174.32(\text{C}_1) (126 MHz)
7.6.37 Methyl 16-[N,N'-[(2,2'-dithiobisethanol)]-2,3-diaminopropanamido] hexadecanoate (33)

This synthesis was similar to the general S-detritylation procedure described for compound 8. Ten minutes after the addition of I₂, an opaque precipitate was noted and the suspension was stored at -15 °C for 18 hours. The suspension was filtered and the residue washed with cold absolute EtOH and dried under high vacuum. The pale-yellow solid was dissolved in CHCl₃ (100 mL) and washed with 0.1 N Na₂S₂O₃ (2 X 100 mL) and the organic layer dried over MgSO₄ for an hour. Filtration and rotary evaporation of the filtrate afforded 33 as a clear oil (43%). To optimize yields when characterization was not necessary, the disulfide conjugate and trityl impurities were co-isolated, via CHCl₃ extraction or the reaction suspension, and used directly used for the subsequent, ion-exchange mediated hydrolysis. The compound showed:

TLC: \( R_f 0.67 \) (90% CH₂Cl₂:10% MeOH)

IR (KBr Pellet): \( \nu_{\text{MAX}} 3290(\text{s})(\text{N-H bonded}), 2920(\text{s})(\text{C-H}), 1740(\text{s})(\text{C=O,ester}), 1640(\text{s})(\text{C=O,Amide I}), 1550(\text{s})(\text{N-H,Amide II}), 430(\text{w})(\text{S-S}) \)

MS (+NH₃ DCI): \( m/z(\text{RI%}); 518(22)[\text{M+1}], 488(10), 456(7), 286(100), 252(10) \)
$^1$H NMR (CDCl$_3$): $\delta$1.25(s,22H,H-4 to H-14), 1.62(br m,4H,H-3 and H-15), 2.30(t,$J$=7.4 Hz,2H,H-2), 2.72-3.32[br s,3H,H-16 and H-3'a(b)], 3.33-3.95[overlapping br s and m,$\sim$8H,OCH$_3$,H-3''a(b), H-5' and H-7'], 4.36-5.20(br s,1H,H-2'), 7.61-8.71(br s,$\sim$2H,amide) (200 MHz)

![Chemical structure](image)

33

7.6.38 16-[N,N'-[(2,2'-Dithioethanoyl)-2,3-diaminopropanamidol] hexadecanoic acid (34)

To a solution of methyl ester crude 33 (~68 mg, ~0.13 mmol maximum) in EtOH (30 mL) was added EtOH-swollen AMBERLYST A-26 (OH$^-$) (20 mL, 20 mmol) ion-exchange resin, Aliquat 336 (1 drop) and ddH$_2$O (5 drops). After four days at ambient temperature with occasional swirling, the pale-yellow suspension was filtered and the collected resin was washed with EtOH and acetone. The resin then eluted with a mixture of 0.08 N HCl and acetone (1:1 v/v) until the eluate had a pH of less than 2. The clear filtrate was rotary evaporated to give 34 as a pale-amber, pungent-smelling solid (25 mg, 39% minimum). The compound showed:

IR (KBr Pellet): $v_{\text{MAX}}$ 3386(s)(N-H bonded), 2918,2851(s)(C-H),
1730(s)(C=O, acid and Amide I),
1557(m)(N-H, Amide II), 1218(m)

MS (+NH₃ DCl):
m/z(RI%); 397(28), 386(32), 369(100), 351(18),
302(68), 272(82), 77(42)

¹H NMR (DMSO-ᴅ₆):
δ 8.22(s, 2H, H-3 to H-14), 1.36(br s, 2H, H-3), 1.45(br
m, 2H, H-15), 2.17(t, J=7.3 Hz, 2H, H-2), 3.00(broad
m, 2.6H, ligand and H-16), 3.30(br m, ~12H
(overlapping H₂O), ligand), 4.31(broad m, 0.5H, H-2'),
7.99, 8.65, 8.78(br m, ~2H total, amide) (500 MHz)

¹³C NMR (DMSO-ᴅ₆):
24.44-28.99(C₃ to C₁₅), 33.62(C₂), 174.40(C₁) (126
MHz)

7.6.39  N-[N',N''-Bis[2-(triphenylmethyl)thioethanoyl]-2,3-diaminopropanoyl]
taurine, sodium salt (35)

A saturated solution of taurine in 0.5 N NaHCO₃ (1 mL) was added dropwise
over two minutes to a magnetically-stirred solution of acid 3 (150 mg, 0.20 mmol),
EDC (46 mg, 1.2 eq., 0.24 mmol) in DMF (1 mL) after a 10-minute activation period
under a N₂ atmosphere. After 12 hours, the suspension was concentrated under high
vacuum at 35 °C to a viscous semi-solid, suspended in hot EtOH (40 mL) and vacuumed filtered. The filtrate was concentrated on a rotary evaporator to about 5 mL and diluted with dH2O (50 mL). To this clear, soapy solution was added NaCl (2 g) and 2 N HCl (5 drops) and the precipitation of an opaque solid was noted. The milky-white suspension was chilled at -15°C for an hour and vacuum filtered. The filtered residue was suspended in hot EtOH, filtered and the filtrate rotary evaporated to afford 35 as an off-white solid (167 mg, 96%). The compound showed:

TLC: $R_f$ 0.16 (90% CH$_2$Cl$_2$:10% MeOH)

IR (KBr Pellet):

$\nu_{\text{max}}$ 3420(s)(N-H bonded,sulfonate),
3060(w)(aromatic C=C-H), 2920,2850(w)(aliphatic C-H), 1660(s)(C=O,Amide I), 1520(m)(N-H,Amide II), 1490,1440(s)(aromatic C=C), 1040(m),
735,695(s)(monosubstituted aromatic C=C-H)

MS (FAB-):

$m/z$(RI%); 842(100)[M-23], 736(15)[M-23 - C$_2$H$_3$O$_2$S], 622(30), 600(100), 526(50)

$^1$H NMR (DMSO-$d_6$):

8.56(t,$\text{J}=7.1$ Hz,2H,H-1), 2.82(AB q,$\text{J}=13.9$ Hz,2H,H-2), 3.24(m,2H,H-3'), 3.32[m,13H (overlapping H$_2$O),H-5' and H-7'], 4.07(m,1H,H-2'), 7.13-7.38 (m,30H,aryl), 7.85(t,$\text{J}=5.8$Hz,1H, C$_4$O-NH), 7.90(t,$\text{J}=5.5$ Hz,1H,C$_1$O-NH), 8.00(d,$\text{J}=7.8$ Hz,1H,C$_6$O-NH) (500 MHz)

$^{13}$C NMR (DMSO-$d_6$):

83.57,35.90(C$_5$ and C$_7$-), 40.47(C$_1$), 50.09(C$_2$), 52.84(C$_2$), 65.85(C-Ph$_3$),
126.71-129.03,144.04(aryl),
167.35,167.65,168.72(C$_1$C$_7$ and C$_6$) (126 MHz)
7.6.40 N-[N',N''-(2,2'-Dithiobisethanovl)-2,3- diaminopropamidol] taurine.

sodium salt (36)

This synthesis was a modification of the method of Kamber. Resublimed I₂ (71 mg, 1.1 eq., 0.28 mmol) was added to a solution of S-tritylated taurine conjugate 35 (300 mg, 0.25 mmol) in degassed absolute EtOH (60 mL) under a N₂ atmosphere. The amber-brown solution was further degassed for 30 minutes under aspirator vacuum (15 mm Hg), vented to dry N₂ gas and allowed to magnetically stir at room temperature. An opaque precipitate was noted after 10 minutes and after four hours, the suspension was stored at -15 °C for 18 hours. The amber-brown solid was isolated by gravity filtration, washed with cold EtOH, dissolved in warm, ddH₂O (50 mL) and extracted with CH₂Cl₂ (2 x 70 mL) without vigorous mixing of the biphasic emulsion. The aqueous layer was carefully separated and rotary evaporated at 30 °C to an amber-brown, glassy solid. Recrystallization from a water-acetone mixture afforded 36 as an opaque solid (31 mg, 33%). The compound showed:

mp: 282-284 °C

IR (KBr Pellet): \( \nu_{\text{MAX}} \) 3420(s)(N-H bonded,sulfonate),
2920(w)(C-H), 1655(s)(C=O,Amide I)
1530(m)(N-H, Amide II), 1210(m), 1040(m)

MS (FAB-):  
\[ m/z (RI\%) : 251(10), 165(15), 143(30) \]

\(^1\)H NMR (DMSO-\(d_6\)):
82.48(br t, 2H, H-1), 2.57(br m, ~3H), 3.78(br m, ~3H).
2.83(AB q, 2H, H-2), 3.48(br m, ~7H)
(overlapping H2O), 4.0-4.4(br m, ~1H, H-2'),
7.8-8.3(br m, ~3H, amide) (500 MHz)

\(^13\)C NMR (DMSO-\(d_6\)):
835.59, 35.93(C\(_5\) and C\(_7\)), 40.47(C\(_1\)),
42.39, 41.78(br, C\(_3\)), 50.04(C\(_2\)), 52.91(br, C\(_4\)),
169.52, 168.77, 168.21(br, C\(_1\)' s, C\(_4\)' s and C\(_5\)' s) (126 MHz)

7.6.41 3-(Mono-6-amino-\(\beta\)-cyclodextrin)-1-[\(N,N'\)-bis[2-
(triphenylmethyl)thioethanoyl]-2,3-diaminopropanamido] propane (37)

A solution of 3-(mono-6-amino-\(\beta\)-cyclodextrin)propylamine (20 mg, 0.02 mmol), NEt\(_3\) (1 drop) in DMF (1 mL) was added dropwise to a magnetically-stirred solution of acid 3 (13 mg, 0.02 mmol), EDC (6 mg, 1.5 eq., 0.03 mmol) in DMF (1 mL) after a 10-minute activation period under a \(\text{N}_2\) atmosphere. After 48 hours, the clear solution was concentrated under high vacuum to a viscous, clear oil, suspended in ddH\(_2\)O (10 mL) and filtered with ddH\(_2\)O and CHCl\(_3\) washings. The collected solid was
dissolved in hot EtOH, hot-filtered and the ethanolic filtrate was rotary evaporated to
give 37 as an amorphous solid (21 mg, 53%). The compound showed:

TLC: \( R_f 0.85 \) (43% i-PrOH:37% H\(_2\)O:20% EtOAc; 
ninhydrin positive)

IR (KBr Pellet): \( \nu_{\text{MAX}} \)

- 3410(s)(N-H,O-H bonded), 3060(m)(aromatic C=C-H), 2930(s)(aliphatic C-H),
- 1655(s)(C=O,Amide I), 1520(m)(N-H,Amide II),
- 1490,1440(m)(aromatic C=C), 1150(w),
- 1075,1030(m)(C-O), 735,695(s)(monosubstituted aromatic C-H)

\(^1\text{H NMR (DMSO-d}_6\text{):}

- 82.76,2.8(s,~4H,H-5' and H-7'), 3.00(m,~4H,H-8 and H-9), 3.16-3.62(br m,87H (overlapping
  H\(_2\)O)),sugar methines and H-3'), 4.04(br m,1H H-2'),
- 4.35(br s,~4H,H-6 substituted sugar), 4.75(br
  s,~5H,H-6 unsubstituted sugars), 5.70(br
  m,~18H,-OH and -NH), 7.30(m,52H,aryl), 7.91(br
  m,3H,amide) (500 MHz)

\(^1\text{C NMR (DMSO-d}_6\text{):}

- 826.28(C\(_8\)), 35.88,35.98(C\(_5\) and C\(_7\)), 36.67(C\(_9\)),
- 40.00(C\(_3\)), 43.17(C\(_7\)), 52.83(C\(_6\)-NHR), 55.32(C\(_2\)),
- 59.88(C\(_6\) unsubstituted), 65.85(C-Ph\(_3\)), 72.00(C\(_2\)),
- 72.39(C\(_3\)), 73.00(C\(_4\)), 81.51(C\(_3\)), 101.89(C\(_1\)),
- 126.76-129.02,143.98(aryl), 167.33,167.43,167.71
(C\(_1\),C\(_4\) and C\(_6\)) (126 MHz)
7.6.42 3-(Mono-6-amino-β-cyclodextrin)-1-\{N',N'\'-(2,2'-
-dithiobisethanoyl)-2,3-diaminopropanamido\} propane.1HI (38)

This synthesis was a modification of the method of Kamber.\(^\text{37}\) Resublimed I\(_2\) (31 mg, 2.1 eq., 0.12 mmol) was added to a solution of S-tritylated cyclodextrin conjugate 37 (65 mg, 0.06 mmol) in a 4:1 v/v mixture of degassed absolute EtOH and CH\(_2\)Cl\(_2\) (50 mL) under a N\(_2\) atmosphere. The amber-brown solution was further degassed for 30 minutes under aspirator vacuum (15 mm Hg), vented to dry N\(_2\) gas and allowed to magnetically stir at room temperature. After 12 hours, the amber-brown suspension chilled at -15° C for 18 hours and filtered with cold EtOH washings. The amber-colored residue was dissolved in lake-warm ddH\(_2\)O (100 mL) and was further washed with CHCl\(_3\) (2 X 50 mL), filtered through glass wool and rotary evaporated at 30 °C to give 38 as a clear, pale-yellow solid (29 mg, 31%). The compound showed:

mp: 195-200 °C (dec.)

TLC: \(R_f\) 0.0 (43% i-PrOH:37% H\(_2\)O:20% EtOAc; ninhydrin positive))

IR (KBr pellet): \(\nu_{\text{MAX}}\) 3444(s)(O-H,N-H bonded),
2924, 2853(s) (aliphatic C-H), 1652(s) (C=O, Amide I),
1558, 1535(m) (N-H, Amide II),
1156, 1079, 1028(s) (C-O)

MS (FAB+):
ml/z (RI%): 1423(20) [M-1-],
1216(100) [(M-1)-C_6H_11N_2O_2S_2]

^1H NMR (D_2O):
8.347 (br m), 3.75(br m), 4.59(br m), 4.88(br m, H-6
of unsubstituted sugars) (500 MHz)

^13C NMR (CDCl_3):
825.83(C_9), 36.66(br, C_3 and C_7), 37.19, 38.98(C_9),
41.47(br, C_3), 42.57, 43.29, 46.18(C_7), 47.34, 48.58,
53.69(C_6-N^+H_2R), 54.55(br, C_2), 60.72, 61.09(C_6
unsubstituted), 67.94(C_4 substituted), 69.22,
72.21(C_4 unsubstituted), 72.47(C_2 unsubstituted),
73.53(C_3 unsubstituted), 80.85, 81.53(C_5
unsubstituted), 83.56, 101.59(C_1 substituted),
102.28(C_1 unsubstituted), 172.25(br, C_1, C_4 and C_6)
(126 MHz)
7.6.43 \( N,N' \)-[Dimethyl-bis[2-(triphenylmethyl)thioethanoyl]]ethylenediamine

(39)

To a magnetically-stirred solution of succinimido ester 2 (1.00 g, 2.32 mmol) in AN (10 mL) was added a solution of \( N,N' \)-dimethyl ethylenediamine (120 \( \mu \)L, 1.13 mmol) in AN (1 mL) and NEt\(_3\) (2 drops) under a \( N_2 \) atmosphere. After 12 hours, the milky-white suspension was concentrated to 2 mL, diluted with 0.5 N HCl (100 mL) and extracted with CH\(_2\)Cl\(_2\) (200 mL). The organic layer was backwashed with 0.5 N HCl (200 mL), 0.5 N NaHCO\(_3\) (200 mL) and dried over MgSO\(_4\) for an hour. Filtration and concentration of the filtrate \textit{in vacuo} afforded a solid (621 mg, 76%). Chromatographic purification of the resultant crude (stepwise elution: 100% CH\(_2\)Cl\(_2\) followed by 2% MeOH in CH\(_2\)Cl\(_2\)) gave 39 as an opaque solid. The compound showed:

\begin{itemize}
  \item mp: \quad 71.0-72.5 °C
  \item TLC: \quad R\(_f\) 0.79 (90% CH\(_2\)Cl\(_2\):10% MeOH)
  \item IR (KBr Pellet): \quad \nu_{\text{MAX}} 3055(w)(aromatic C=C-H),
  \quad 2930,2850(w)(aliphatic C-H), 1645(s)(C=O, Amide)
\end{itemize}
I), 1485(s), 1440(s)(aromatic C=C), 1400(m),
735, 695(s)(monosubstituted aromatic C-H)

$^1$H NMR:
See Table 7.3

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<th>$^2$Z, E</th>
<th>$^2$E, E</th>
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<td>2.71(s, 3H)</td>
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<td>N-CH$_3$</td>
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<td>2.52(s, 6H)</td>
<td>2.79(s, 4H)</td>
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<td>2.84(s, 2H)</td>
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<td>S-CH$_2$</td>
<td>3.28(s, 4H)</td>
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<td>2.93(s, 4H)</td>
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<td>3.16(t, 2H)</td>
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<td>(N-CH$_2$)$_2$</td>
<td>7.14-7.38(m, 32H$^b$)</td>
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$^a$ Integrals are relative within a geometrical isomer.
$^b$ Overlapping residual CHCl$_3$.

$^{13}$C NMR:
See Table 7.4
Table 7.4: J-modulated $^{13}$C NMR (126 MHz) assignments for 39 in CDCl$_3$ at 303 K.

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<tr>
<td>N-CH$_3$</td>
<td>35.98(-52) 36.67(-10)</td>
<td>34.02(-10)</td>
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<td>2.6</td>
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<td>S-CH$_2$</td>
<td>35.10(55) 34.83(11)</td>
<td>34.64(11) 33.88(5)</td>
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<td>2.5</td>
<td>11.0</td>
<td>4.4</td>
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<td>(N-CH$_2$)$_2$</td>
<td>44.76(52) 47.53(11)</td>
<td>46.94(11)</td>
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<td>Aromatic</td>
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<td>126.64 - 129.73, 143.80(4), 143.92(17), 144.00(18), 144.05(18)</td>
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<tr>
<td>C-Tr</td>
<td>66.71(11) 66.81(3.03)</td>
<td>66.94(2.74)</td>
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<td>CO-NH</td>
<td>168.77(26) 168.24(6)</td>
<td>168.52(7)</td>
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</table>

![Chemical structure of 39](image)
7.6.44 N,N'-[Dimethyl-(2,2'-dithiobisethanoyl)]ethylenediamine (40)

This synthesis was similar to the general S-detritylation procedure described for compound 8, except isopropyl alcohol was used as the quench for trityl cation. After 12 hours, the amber solution was concentrated to 20 mL, diluted with ddH₂O, decolorized with 1.0 N Na₂S₂O₃ and extracted with diethyl ether. An opaque solid was seen at the interface. The organic phase was decanted and, after salting the aqueous layer, the precipitate extracted into CHCl₃ and dried over MgSO₄ for two hours. Filtration and rotary evaporation of the filtrate afforded an off-white, semi-solid (66%). The residue was suspended in isopropyl alcohol, chilled at -15 °C and filtered to separate most of the insoluble trityl carbinol impurity. Recrystallization from peroxide-free THF gave 40 as opaque, non-geometric plates. The compound showed:

mp: 139.0-140.0 °C

TLC: \( R_f 0.49 \) (90% CH₂Cl₂:10% MeOH)

MS (+NH₃ DCI): 252(100)[M+NH₄⁺], 235(48)[M+1], 205(10)

IR (KBr pellet): \( \nu_{MAX} 3445(m)\) (N-H bonded), 2930,2853(m)(C-H), 1629(s)(C=O,Amide I), 1458(m), 1396(m)

Raman: 645(m)(CH₂-S), 510(s)(S-S)

\(^1\)H NMR: See Table 7.5

\(^{13}\)C NMR: See Table 7.6
Table 7.5: $^1$H NMR (500 MHz) assignments for 40 in CDCl$_3$ at 303 K.

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<th>$^{ZZ_1}$ ppm (multiplicity, integral$^a$, amide stereochemistry$^b$)</th>
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<td>3.04(s,~3H,E)</td>
<td>3.15(s,~6H)</td>
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<td>3.24(s,~3H,Z)</td>
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<tr>
<td>N-(CH$_2$)$_2$</td>
<td>2.64(dd,2H)$^c$</td>
<td>3.01(m,~1H,Z)$^c$</td>
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<td>4.68(dd,2H)$^c$</td>
<td>4.27(obs$^d$, m,~1H,E)$^c$</td>
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<tr>
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<td>$^2J_{CH_2}=13.7$ Hz$^e$</td>
<td>$^2J_{CH_2}=-14.41$ Hz</td>
<td>$^2J_{CH_2}=-13.5$ Hz</td>
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<td>$^3J_{ax-ax}=11.2$ Hz$^f$</td>
<td>$^3J_{ax-ax}=11.1$ Hz$^f$</td>
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<td>$^3J_{e_2}=1.4$ Hz</td>
<td>$^3J_{eq-eq}=2.4$ Hz</td>
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<td>S-CH$_2$</td>
<td>3.42(AB d,2H)$^c$</td>
<td>3.55(AB d,1H,E)$^c$</td>
<td>3.19(obs AB d,~2H)$^c$</td>
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<td>3.45(AB d,2H)$^c$</td>
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<td>$^2J_{CH_2}=-12.7$ Hz</td>
<td>$^2J_{CH_2}=-13.2$ Hz</td>
<td>$^2J_{CH_2}=-14.7$ Hz</td>
</tr>
<tr>
<td></td>
<td>3.03(obs AB d,~1H,Z)$^c$</td>
<td>4.16(AB d,1H,Z)$^c$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$^2J_{CH_2}=-12.8$ Hz</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Integrals are relative within a conformer series.

$^b$ Assignment of amide stereochemistry was based on NOE, MM2 and X-ray crystallographic data.

$^c$ Geminally coupled (determined from $^1$H-$^1$C chemical shift correlated experiment).

$^d$ Obscured.

$^e$ All $^2J$ couplings were assumed to be negative.

$^f$ All $^3J$ couplings were measured from MMX-calculated structures.
Table 7.6: \(J\)-modulated \(^{13}\)C NMR (126 MHz) assignments for 40 in CDCl\(_3\) at 303 K.

<table>
<thead>
<tr>
<th>CONFORMER</th>
<th>(ZZ_1)</th>
<th>(Z,E)</th>
<th>(ZZ_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SIGNAL</td>
<td>ppm(relative intensity, amide stereochemistry(^a))</td>
<td>ppm(relative intensity, amide stereochemistry(^a))</td>
<td>ppm(relative intensity, amide stereochemistry(^a))</td>
</tr>
<tr>
<td>N-CH(_3)</td>
<td>36.58(-98)</td>
<td>33.59(-17,E)</td>
<td>37.10(^b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>41.17(-18,Z)</td>
<td></td>
</tr>
<tr>
<td>S-CH(_2)</td>
<td>36.98(144)</td>
<td>37.49(31,Z)</td>
<td>40.69(18)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>42.17(20,E)</td>
<td></td>
</tr>
<tr>
<td>N-(CH(_2))(_2)</td>
<td>44.23(127)</td>
<td>48.20(26)</td>
<td>46.17(16)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48.99(22)</td>
<td></td>
</tr>
<tr>
<td>CO-NH</td>
<td>166.55(14)</td>
<td>168.84(6)</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td></td>
<td>169.46(5)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Stereochemical assignments are based on the analysis of NOE and \(^1\)H-\(^{13}\)C shift correlated data.

\(^b\) This resonance is absent from the \(J\)-modulated spectrum and was determined from the standard \(^{13}\)C experiment.

\[ \text{40} \]

7.6.45 \(N,N'\)-Bis[2-(triphenylmethyl)thioethanoyl] ethylenediamine (41)

To a magnetically-stirred solution of succinimido ester 2 (1.00 g, 2.32 mmol) in AN (20 mL) was added a solution of ethylenediamine (Fisher Scientific) (78 µL, 1.1 mmol) of in AN (1 mL) and NEt\(_3\) (2 drops) under a N\(_2\) atmosphere. After two hours, the milky-white suspension was filtered with cold AN washings and dried under high vacuum to afford 41 as an opaque solid (625 mg, 82%). The compound showed:
mp: 166-168°C

TLC: $R_f$ 0.79 (90% CH$_2$Cl$_2$:10% MeOH)

IR (KBr Pellet): $\nu$$_{\text{MAX}}$ 3400,3280(s)(N-H bonded), 3050(m)(aromatic C=C-H), 2920(w)(aliphatic C-H), 1640(s)(C=O, Amide I), 1550(s)(N-H, Amide II), 1490(m), 1440(s)(aromatic C=C).

735,695(s)(monosubstituted aromatic C-H)

$^1$H NMR (DMSO-$_d_6$): 8.25(s,4H,CH$_2$-S), 2.84(pseudo t,4H,CH$_2$-N), 7.10-7.20(m,30H,aryl), 7.72(s,2H,amide) (500 MHz)

$^{13}$C NMR (CDCl$_3$): 83.83(CH$_2$-S), 39.47(CH$_2$-N), 65.92(C-Ph$_3$), 126.03-129.43,143.95(aryl), 168.78(CO-NH) (126 MHz)

7.6.46 $N,N'$-([2,2'-Dithiobisethanoyl])ethylenediamine (42)

This synthesis was similar to the general $S$-detritylation procedure described
for compound 8. In the work-up, the decolorized, turbid, aqueous suspension was washed with diethyl ether, concentrated to 200 mL, saturated with NaCl and extracted with CHCl₃. The organic phase was dried over MgSO₄ for two hours, filtered and the filtrate concentrated in vacuo to afford 42 as an off-white solid (39%). The compound showed:

mp: 185.0-187.0 °C

MS (+NH₃·DCI): m/z(RI%); 296(20), 224(100)[M+NH₄⁺], 207(20)[M+1], 162(16), 145(40)

¹H NMR (DMSO-d₆): 83.27-3.75[m,15H,(CH₂-N,CH₂-S and overlapping H₂O)], 4.05-4.22(br m,0.5H,CH₂-N of minor isomer), 7.82(br s,1H,amide), 8.17(br s,1H,amide) (500 MHz)

![Chemical Structure](image)
Appendix

X-ray Crystal Data for 40

Table A.1: Crystal Data for 40.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empirical Formula</td>
<td>C₂H₁₄N₂O₂S₂</td>
</tr>
<tr>
<td>Formula Weight</td>
<td>234.33</td>
</tr>
<tr>
<td>Space Group</td>
<td>P1 (#2)</td>
</tr>
<tr>
<td>Crystal System</td>
<td>triclinic</td>
</tr>
<tr>
<td>Crystal Color, Habit</td>
<td>colorless, plate</td>
</tr>
<tr>
<td>Crystal Dimensions</td>
<td>0.20 × 0.05 × 0.15 mm</td>
</tr>
<tr>
<td>a</td>
<td>7.0570(10) Å</td>
</tr>
<tr>
<td>b</td>
<td>8.071(2) Å</td>
</tr>
<tr>
<td>c</td>
<td>11.012(2) Å</td>
</tr>
<tr>
<td>α</td>
<td>106.05(2)°</td>
</tr>
<tr>
<td>β</td>
<td>93.41(1)°</td>
</tr>
<tr>
<td>γ</td>
<td>114.93(1)°</td>
</tr>
<tr>
<td>V</td>
<td>535.3(3) Å³</td>
</tr>
<tr>
<td>Z</td>
<td>2</td>
</tr>
<tr>
<td>D&lt;sub&gt;calc&lt;/sub&gt;</td>
<td>1.454 g cm⁻³</td>
</tr>
<tr>
<td>μ(Cu Kα)</td>
<td>4.342 mm⁻¹</td>
</tr>
<tr>
<td>F&lt;sub&gt;000&lt;/sub&gt;</td>
<td>248</td>
</tr>
<tr>
<td>Final R (obs. data)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>R = 4.67%, ωR = 6.82%&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Weighting scheme</td>
<td>(c²F&lt;sup&gt;2&lt;/sup&gt; + 0.0008F²)⁻¹</td>
</tr>
<tr>
<td>Largest Difference Peak</td>
<td>0.31 eÅ⁻³</td>
</tr>
<tr>
<td>Largest Difference Hole</td>
<td>-0.28 eÅ⁻³</td>
</tr>
</tbody>
</table>

<sup>a</sup> for 1006 reflections with l > 2σ₁
<sup>b</sup> R = Σ(|F<sub>o</sub> - |F<sub>c</sub>|) / Σ F<sub>o</sub>
<sup>c</sup> ωR = [Σ(weight, δ)^2 / Σ(weight, F<sub>o</sub>)²]¹/² where δ = ||F<sub>c</sub>||
Table A.2: Atomic coordinates (X10^4) and equivalent isotropic displacement coefficients (Å^2 X 10^3).

<table>
<thead>
<tr>
<th>Atom</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>U_{eq}^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>S(1)</td>
<td>2354(2)</td>
<td>581(2)</td>
<td>1211(1)</td>
<td>57(1)</td>
</tr>
<tr>
<td>S(2)</td>
<td>1815(2)</td>
<td>2637(2)</td>
<td>2470(1)</td>
<td>56(1)</td>
</tr>
<tr>
<td>C(3)</td>
<td>2612(7)</td>
<td>4613(7)</td>
<td>1793(5)</td>
<td>40(2)</td>
</tr>
<tr>
<td>C(4)</td>
<td>3905(7)</td>
<td>6481(7)</td>
<td>2911(4)</td>
<td>37(2)</td>
</tr>
<tr>
<td>O(4)</td>
<td>3040(6)</td>
<td>6847(6)</td>
<td>3814(3)</td>
<td>55(2)</td>
</tr>
<tr>
<td>N(5)</td>
<td>5926(6)</td>
<td>7663(5)</td>
<td>2905(4)</td>
<td>38(2)</td>
</tr>
<tr>
<td>C(5)</td>
<td>7114(9)</td>
<td>9380(8)</td>
<td>4057(5)</td>
<td>60(3)</td>
</tr>
<tr>
<td>C(6)</td>
<td>7089(7)</td>
<td>7342(7)</td>
<td>1883(4)</td>
<td>37(2)</td>
</tr>
<tr>
<td>C(7)</td>
<td>8656(7)</td>
<td>6622(7)</td>
<td>2219(5)</td>
<td>39(2)</td>
</tr>
<tr>
<td>N(8)</td>
<td>7584(6)</td>
<td>4821(5)</td>
<td>2511(3)</td>
<td>35(2)</td>
</tr>
<tr>
<td>C(8)</td>
<td>7751(9)</td>
<td>4980(8)</td>
<td>3888(5)</td>
<td>55(3)</td>
</tr>
<tr>
<td>C(9)</td>
<td>6531(7)</td>
<td>3148(7)</td>
<td>1506(5)</td>
<td>39(2)</td>
</tr>
<tr>
<td>O(9)</td>
<td>6581(6)</td>
<td>3115(5)</td>
<td>382(3)</td>
<td>50(2)</td>
</tr>
<tr>
<td>C(10)</td>
<td>51177(9)</td>
<td>1295(8)</td>
<td>1766(5)</td>
<td>49(3)</td>
</tr>
</tbody>
</table>

*a Equivalent isotropic U defined as one third of the trace of the orthogonalized U_{ij} tensor.

Table A.3: Bond lengths (Å) with estimated standard deviations in parentheses.

<table>
<thead>
<tr>
<th>Bond</th>
<th>Length</th>
<th>Bond</th>
<th>Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>S(1)-S(2)</td>
<td>2.035(2)</td>
<td>S(1)-C(10)</td>
<td>1.835(6)</td>
</tr>
<tr>
<td>S(2)-C(3)</td>
<td>1.836(6)</td>
<td>C(3)-C(4)</td>
<td>1.517(6)</td>
</tr>
<tr>
<td>C(4)-O(4)</td>
<td>1.229(7)</td>
<td>C(4)-N(5)</td>
<td>1.342(6)</td>
</tr>
<tr>
<td>N(5)-C(5)</td>
<td>1.468(6)</td>
<td>N(5)-C(6)</td>
<td>1.461(7)</td>
</tr>
<tr>
<td>C(6)-C(7)</td>
<td>1.520(9)</td>
<td>C(7)-N(8)</td>
<td>1.466(7)</td>
</tr>
<tr>
<td>N(8)-C(8)</td>
<td>1.479(7)</td>
<td>N(8)-C(9)</td>
<td>1.353(5)</td>
</tr>
<tr>
<td>C(9)-O(9)</td>
<td>1.234(7)</td>
<td>C(9)-C(10)</td>
<td>1.515(8)</td>
</tr>
</tbody>
</table>
Table A.4: Bond angles (deg) with estimated standard deviations in parentheses.

<table>
<thead>
<tr>
<th>Bond</th>
<th>Angle</th>
<th>Bond</th>
<th>Angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>S(2)-S(1)-C(10)</td>
<td>104.0(2)</td>
<td>S(1)-S(2)-C(3)</td>
<td>104.4(2)</td>
</tr>
<tr>
<td>S(2)-C(3)-C(4)</td>
<td>106.6(4)</td>
<td>C(3)-C(4)-O(4)</td>
<td>117.6(4)</td>
</tr>
<tr>
<td>C(3)-C(4)-N(5)</td>
<td>120.5(4)</td>
<td>O(4)-C(4)-N(5)</td>
<td>122.0(4)</td>
</tr>
<tr>
<td>C(4)-N(5)-C(5)</td>
<td>117.2(4)</td>
<td>C(4)-N(5)-C(6)</td>
<td>125.8(3)</td>
</tr>
<tr>
<td>C(5)-N(5)-C(6)</td>
<td>116.9(4)</td>
<td>N(5)-C(6)-C(7)</td>
<td>112.9(4)</td>
</tr>
<tr>
<td>C(6)-C(7)-N(8)</td>
<td>111.4(4)</td>
<td>C(7)-N(8)-C(8)</td>
<td>117.1(3)</td>
</tr>
<tr>
<td>C(7)-N(8)-C(9)</td>
<td>117.7(4)</td>
<td>C(8)-N(8)-C(9)</td>
<td>125.1(5)</td>
</tr>
<tr>
<td>N(8)-C(9)-O(9)</td>
<td>121.3(5)</td>
<td>N(8)-C(9)-C(10)</td>
<td>119.2(5)</td>
</tr>
<tr>
<td>O(9)-C(9)-C(10)</td>
<td>119.4(4)</td>
<td>S(1)-C(10)-C(9)</td>
<td>108.8(5)</td>
</tr>
</tbody>
</table>

Table A.5: Hydrogen atom coordinates (X 10^4) and equivalent isotropic displacement coefficients (Å^2 X 10^3).

<table>
<thead>
<tr>
<th>Atom</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>U</th>
</tr>
</thead>
<tbody>
<tr>
<td>H(3A)</td>
<td>1310</td>
<td>4855</td>
<td>1499</td>
<td>80</td>
</tr>
<tr>
<td>H(3B)</td>
<td>3234</td>
<td>4124</td>
<td>1042</td>
<td>80</td>
</tr>
<tr>
<td>H(5A)</td>
<td>6271</td>
<td>10070</td>
<td>4465</td>
<td>80</td>
</tr>
<tr>
<td>H(5B)</td>
<td>7334</td>
<td>8860</td>
<td>4893</td>
<td>80</td>
</tr>
<tr>
<td>H(5C)</td>
<td>8323</td>
<td>10241</td>
<td>4063</td>
<td>80</td>
</tr>
<tr>
<td>H(6A)</td>
<td>7774</td>
<td>8433</td>
<td>1645</td>
<td>80</td>
</tr>
<tr>
<td>H(6B)</td>
<td>5952</td>
<td>6293</td>
<td>960</td>
<td>80</td>
</tr>
<tr>
<td>H(7A)</td>
<td>9204</td>
<td>6338</td>
<td>1359</td>
<td>80</td>
</tr>
<tr>
<td>H(7B)</td>
<td>9867</td>
<td>7622</td>
<td>2946</td>
<td>80</td>
</tr>
<tr>
<td>H(8A)</td>
<td>8451</td>
<td>4127</td>
<td>3840</td>
<td>80</td>
</tr>
<tr>
<td>H(8B)</td>
<td>6348</td>
<td>4137</td>
<td>3992</td>
<td>80</td>
</tr>
<tr>
<td>H(8C)</td>
<td>8162</td>
<td>6271</td>
<td>4375</td>
<td>80</td>
</tr>
<tr>
<td>H(10A)</td>
<td>5285</td>
<td>1229</td>
<td>2691</td>
<td>80</td>
</tr>
<tr>
<td>H(10B)</td>
<td>5578</td>
<td>231</td>
<td>904</td>
<td>80</td>
</tr>
</tbody>
</table>
Table A.6: Torsional angles with estimated error in parentheses (deg).

<table>
<thead>
<tr>
<th>Bond</th>
<th>Angle</th>
<th>Bond</th>
<th>Angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(10)-S(1)-S(2)-C(3)</td>
<td>85.9(0.3)</td>
<td>S(2)-S(1)-C(10)-H(10A)</td>
<td>-69.2(0.4)</td>
</tr>
<tr>
<td>S(2)-S(1)-C(10)-H(10A)</td>
<td>61.7(0.4)</td>
<td>S(2)-S(1)-C(10)-H(10B)</td>
<td>-170.5(0.3)</td>
</tr>
<tr>
<td>S(1)-S(2)-C(3)-C(4)</td>
<td>-135.8(0.3)</td>
<td>S(1)-S(2)-C(3)-H(3A)</td>
<td>115.4(0.3)</td>
</tr>
<tr>
<td>S(1)-S(2)-C(3)-C(4)</td>
<td>-5.9(0.3)</td>
<td>C(8)-N(8)-C(9)-O(9)</td>
<td>-175.1(0.6)</td>
</tr>
<tr>
<td>C(8)-N(8)-C(9)-H(8B)</td>
<td>-43.3(0.9)</td>
<td>C(7)-N(8)-C(9)-O(9)</td>
<td>4.5(0.8)</td>
</tr>
<tr>
<td>C(9)-N(8)-C(9)-H(8A)</td>
<td>-172.4(0.5)</td>
<td>C(9)-N(8)-C(9)-H(8B)</td>
<td>-43.2(0.9)</td>
</tr>
<tr>
<td>C(9)-N(8)-C(9)-H(8A)</td>
<td>-162.3(0.5)</td>
<td>C(9)-N(8)-C(9)-H(8A)</td>
<td>59.1(0.6)</td>
</tr>
<tr>
<td>C(7)-N(8)-C(8)-H(8B)</td>
<td>137.2(0.6)</td>
<td>C(7)-N(8)-C(8)-H(8C)</td>
<td>18.1(0.8)</td>
</tr>
<tr>
<td>C(7)-N(8)-C(8)-H(8B)</td>
<td>-120.5(0.5)</td>
<td>C(9)-N(8)-C(7)-C(6)</td>
<td>79.8(0.6)</td>
</tr>
<tr>
<td>C(9)-N(8)-C(7)-H(7A)</td>
<td>-32.4(0.7)</td>
<td>C(9)-N(8)-C(7)-H(7B)</td>
<td>-154.5(0.5)</td>
</tr>
<tr>
<td>C(8)-N(8)-C(7)-C(6)</td>
<td>-100.5(0.5)</td>
<td>C(9)-N(8)-C(7)-H(7A)</td>
<td>147.2(0.5)</td>
</tr>
<tr>
<td>C(8)-N(8)-C(7)-C(6)</td>
<td>25.1(0.7)</td>
<td>C(4)-N(5)-C(6)-C(7)</td>
<td>-102.5(0.6)</td>
</tr>
<tr>
<td>C(4)-N(5)-C(6)-H(6A)</td>
<td>131.9(0.6)</td>
<td>C(4)-N(5)-C(6)-H(6B)</td>
<td>20.4(0.8)</td>
</tr>
<tr>
<td>C(5)-N(5)-C(6)-C(7)</td>
<td>74.1(0.6)</td>
<td>C(5)-N(5)-C(6)-H(6A)</td>
<td>-51.5(0.7)</td>
</tr>
<tr>
<td>C(5)-N(5)-C(6)-H(6B)</td>
<td>-163.0(0.5)</td>
<td>C(6)-N(5)-C(4)-O(4)</td>
<td>179.5(0.6)</td>
</tr>
<tr>
<td>C(6)-N(5)-C(4)-C(3)</td>
<td>0.3(0.9)</td>
<td>C(5)-N(5)-C(4)-O(4)</td>
<td>2.9(0.9)</td>
</tr>
<tr>
<td>C(5)-N(5)-C(4)-C(3)</td>
<td>-176.3(0.6)</td>
<td>C(6)-N(5)-C(5)-H(5A)</td>
<td>143.4(0.6)</td>
</tr>
<tr>
<td>C(6)-N(5)-C(5)-H(5B)</td>
<td>-111.0(0.6)</td>
<td>C(6)-N(5)-C(5)-H(5C)</td>
<td>11.4(1.1)</td>
</tr>
<tr>
<td>C(4)-N(5)-C(5)-H(5A)</td>
<td>-39.7(0.9)</td>
<td>C(4)-N(5)-C(5)-H(5B)</td>
<td>65.9(0.7)</td>
</tr>
<tr>
<td>C(4)-N(5)-C(5)-H(5C)</td>
<td>-171.8(0.8)</td>
<td>N(5)-C(6)-C(7)-N(8)</td>
<td>57.5(0.4)</td>
</tr>
<tr>
<td>N(5)-C(6)-C(7)-H(7A)</td>
<td>174.3(0.3)</td>
<td>N(5)-C(6)-C(7)-H(7B)</td>
<td>-67.1(0.5)</td>
</tr>
<tr>
<td>H(6A)-C(6)-C(7)-N(8)</td>
<td>-176.0(0.4)</td>
<td>H(6A)-C(6)-C(7)-H(7A)</td>
<td>-59.2(0.5)</td>
</tr>
<tr>
<td>H(6A)-C(6)-C(7)-H(7B)</td>
<td>59.4(0.6)</td>
<td>H(6B)-C(6)-C(7)-N(8)</td>
<td>-66.0(0.5)</td>
</tr>
<tr>
<td>H(6B)-C(6)-C(7)-H(7A)</td>
<td>50.8(0.5)</td>
<td>H(6B)-C(6)-C(7)-H(7B)</td>
<td>169.4(0.4)</td>
</tr>
<tr>
<td>N(8)-C(9)-C(10)-S(1)</td>
<td>109.0(0.5)</td>
<td>N(8)-C(9)-C(10)-H(10A)</td>
<td>-12.6(1.0)</td>
</tr>
<tr>
<td>N(8)-C(9)-C(10)-H(10B)</td>
<td>-144.2(0.5)</td>
<td>O(9)-C(9)-C(10)-S(1)</td>
<td>-68.1(0.7)</td>
</tr>
<tr>
<td>O(9)-C(9)-C(10)-H(10A)</td>
<td>170.4(0.6)</td>
<td>O(9)-C(9)-C(10)-H(10B)</td>
<td>38.8(0.7)</td>
</tr>
<tr>
<td>O(4)-C(4)-C(3)-S(2)</td>
<td>-58.2(0.7)</td>
<td>O(4)-C(4)-C(3)-H(3A)</td>
<td>59.5(0.7)</td>
</tr>
<tr>
<td>O(4)-C(4)-C(3)-H(3B)</td>
<td>-174.1(0.6)</td>
<td>N(5)-C(4)-C(3)-S(2)</td>
<td>121.0(0.5)</td>
</tr>
<tr>
<td>N(5)-C(4)-C(3)-H(3A)</td>
<td>-121.3(0.6)</td>
<td>N(5)-C(4)-C(3)-H(3B)</td>
<td>5.2(1.0)</td>
</tr>
</tbody>
</table>
Glossary

aglycone: the alcohol (e.g., steroid) with which a sugar is combined with in a glycoside.

antiarrhythmic: capable of suppressing or preventing an arrhythmia (any variation from normal, regular (cardiac) rhythm).

atrial fibrillation: an arrhythmia characterized by total disorganization of atrial electrical activity, in which multiple wavelets course in a chaotic fashion across the atrium.

cardioactive: of or pertaining to a drug or other substance affecting the function of the heart.

cardiotonic: having a tonic or stimulatory effect on the heart.

Caroli’s disease: a congenital condition characterized by cystic dilatation of the intrahepatic bile ducts.

carrier-free: free from cold isotopes of the same element. With respect to $^{99m}$Tc, carrier-free preparations would be relatively devoid of the daughter nuclide, $^{99}$Tc.

cholecystitis: inflammation of the gallbladder.

cholescintigraphy: scintigraphy of the gallbladder.

conjugate: joined in pairs; coupled. In bifunctional radiopharmaceutical chemistry, a combination of a biologically active molecule (e.g., a steroid) with a bifunctional ligand or chelate. In this research, the conjugative bond was either an amide or ester linkage.
Crohn's disease: a chronic inflammatory bowel disease involving the small intestine, the colon, or both, and characterized pathologically by transmural inflammation, deep linear ulceration, and often granulomas.

terohepatic: pertaining to the intestines and liver.

gastric affecting, originating in, or relating to the stomach.

gastroenterological: of or pertaining to the function and disorders of the stomach, small and large intestine, esophagus, pancreas, liver and biliary tract.

gastrointestinal: relating to the stomach and the intestines.

glycoside: any substance consisting of an alcohol (e.g., a steroid) with the hydrogen of its hydroxyl group replaced by a glycosyl group.

hepatobiliary: pertaining to the liver and biliary system.

hypoglycemic agent: a chemical substance acting to lower the level of glucose in the blood.

infarct: an area of coagulation necrosis in a tissue (as of the heart) resulting from obstruction of the local circulation by a thrombus or embolus.

inotropic: affecting the force or speed of muscular contraction.

ischemia: inadequate blood flow to a part or organ.

macroreticular ion-exchange resin: a macroporous, crosslinked polymer containing ionized or ionizable groups such as -SO₂H, -SO₃Na, -COOH, -NH₂, -NH₃Cl or NMe₂Cl.

metastable radionuclide: a radionuclide that remains in an excited state for an appreciable time before decaying by isomeric transition.

myocardium: the intermediate, contractile muscular layer of the heart wall, constituting cardiac muscle, and comprising atrial and ventricular fibers separated by fibrous rings.
myocytes: muscle cells

Na⁺,K⁺-ATPase: an enzyme responsible for the active transport of Na⁺ and K⁺ ions across a cell membrane.

ouabain: 3-[(6-Deoxy-α-L-mannopyranosyl)oxy]-1,5,11α,14,19-pentahydroxy card-20(22)-enolide. A cardiac glycoside obtained from the seed of Strophanthus gratus or the wood or root of Acocanthera schimperi. The pharmacological action of ouabain is similar to that of digitalis but it acts more rapidly and has a shorter duration.

perfusion: the passage of blood or other fluid through the blood or lymph vessels of the body or in part of it.

radionuclide: a nuclide that spontaneously exhibits radioactive decay; a radioactive nuclear species.

radiopharmaceutical: a drug or other administrable agent that is tagged with a radionuclide so that its course can be traced, measured, or imaged.

¹³¹I-sodium rose bengal: a hepatic function, diagnostic agent.
scintigraphy: a clinical diagnostic procedure consisting of the injection, usually intravenously, of a solution containing a radioactive agent with a specific affinity for an organ or tissue of interest, followed by the determination, with the aid of a special external detector (*e.g.*, gamma camera).
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172 Assignments were deduced by correlation with those of the parent steroid in:

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