SYNTHESIS OF SUGAR CONJUGATES: METAL COMPLEXES AND OTHER DERIVATIVES

by

MICHAEL JAMES ADAM

B.Sc., The University of British Columbia, 1975

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY in THE FACULTY OF GRADUATE STUDIES (Department of Chemistry)

We accept this thesis as conforming to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA February 1980

© Michael James Adam, 1980
In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the Head of my Department or by his representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Chemistry

The University of British Columbia
2075 Wesbrook Place
Vancouver, Canada
V6T 1W5

Date MARCH 12/80
ABSTRACT

A number of conjugates of carbohydrates were prepared. Metal conjugates were synthesized in two different ways. Firstly, chelate coordination complexes were synthesized by forming salicylaldimine ligands derived from combinations of amino sugars [methyl-3,4,6-tri-O-acetyl-2-amino-2-deoxy-β-D-glucopyranoside, 1,3,4,6-tetra-O-acetyl-2-amino-2-deoxy-β-D-glucopyranose or 2-amino-2-deoxy-α,β-D-glucopyranose (glucosamine)] and either salicylaldehyde or 3-formyl-2-hydroxy-benzoic acid with subsequent complexation of these to copper (II), cobalt (II), and zinc (II).

A number of physical techniques were used to characterize these complexes including esr spectroscopy, visible absorption spectroscopy, mass spectrometry, nmr spectroscopy and magnetic susceptibility measurements. From the data provided by these techniques the copper-sugar complexes derived from salicylaldehyde were found in general to have the usual bis-bidentate structure. The copper complex derived from the amino-glycoside and 3-formyl-2-hydroxy-benzoic acid was found to be binuclear in structure containing two sugar moieties and two copper atoms.

The second approach to forming metal sugar conjugates consisted of synthesizing organometallic π-complexes: ferrocenyl-sugar conjugates. A variety of organic- and water-soluble compounds were formed by reaction of amino, hydroxyl, or thio sugar groups with suitably
substituted ferrocene derivatives. Thus organic soluble products were obtained from combinations of the sugars [1,3,4,6-tetra-0-acetyl-2-amino-2-deoxy-β-D-glucopyranose, 1-thio-2,3,4,6-tetra-0-acetyl-β-D-glucopyranose, 1,2,5,6-di-0-isopropylidene-α-D-glucopyranose and 1,2,3,4-di-0-isopropylidene-α-D-galactopyranose] with 1- and 1,1'-ferrocenecarbonyl chlorides, N,N-dimethylaminomethylferrocene methiodide, (1-hydroxymethylferrocene)-p-toluenesulphonate and 2,4-dichloro-6-(1-hydroxymethylferrocene)-s-triazine. Water soluble products were prepared by deacetylation of some of the above compounds and by condensation of ferrocene carboxaldehyde with glucosamine to form the corresponding Schiff's base.

Proton spin-lattice relaxation rates were used to assign the substituted cyclopentadienyl rings and to determine the relative spinning rates of the substituted and unsubstituted cyclopentadienyl rings.

The chemistry of cyanuric chloride (2,4,6-trichloro-s-triazine), as a general means of derivatizing carbohydrates was also investigated. Thus, metals, hydrophobic alkyl groups and nitroxide spin labels were attached in various combinations to carbohydrates. A number of monosaccharide derivatives were formed including model glycolipids and a number of polysaccharides, cellulose, agarose, Sephadex, guar gum, xanthan gum and starch were spin labelled using this chemistry. For polysaccharides, information such as extent of derivatization, evidence for a covalent bond, environment of the triazine unit and the distance between triazine units was obtained. This chemistry was also extended to derivatize Bovine Serum Albumin, microporous glass beads and aluminum oxide.
TABLE OF CONTENTS

ABSTRACT ........................................ ii
LIST OF TABLES ................................. vi
LIST OF FIGURES ............................... vii
INTRODUCTION ................................. 1

Chapter
I SYNTHESE OF METAL CHELATE CONJUGATES OF AMINO SUGARS: SCHIFF'S BASE COMPLEXES .......... 7
IA: Introduction ............................. 7
IB: Synthesis .................................. 10
   (i) Schiff's Base Formation ............... 10
   (ii) Schiff's Base Metal Complexes ...... 18
IC: Physical Measurements .................. 26
   (i) Visible Absorption Studies ........... 26
   (ii) Esr Spectroscopy .................... 31
   (iii) Magnetic Moments ................... 40
   (iv) Mass Spectrometry ................... 41
   (v) Nmr Spectroscopy ..................... 44
ID: Other Chemistry ......................... 47
IE: Esr Spectroscopy of Copper (II) ........ 50
   (i) General Esr ......................... 50
   (ii) Copper (II) Esr .................... 51
IF: Summary and Conclusions ............... 57

II SYNTHESE OF SUGAR-ORGANOMETALLIC CONJUGATES: FERROCENYL-MONOSACCHARIDE DERIVATIVES .... 63
IIA: Introduction ........................... 63
IIB: Synthesis ................................ 66
IIC: Proton Nuclear Magnetic Resonance Spectra 74
   (i) Chemical Shifts and Coupling Constants .... 74
   (ii) Proton Spin Lattice Relaxation Rates .... 86
IID: Proton Spin-Lattice Relaxation .......... 94

III CYANURIC CHLORIDE; A GENERAL REAGENT FOR THE CHEMICAL MODIFICATION OF CARBOHYDRATES .... 102
IIIA: Introduction ........................... 102
IIIB: Synthesis ............................. 109
   (i) Nitrogen Nucleophiles ............... 109
   (ii) Oxygen and Sulfur Nucleophiles ...... 115
   (iii) Summary ............................ 122
IIIC: Macromolecule and Surface Modification .... 125
  (i) Polysaccharides ............................ 125
     (a) The General Reaction .................... 125
     (b) Optimization and Quantitation ....... 129
     (c) Evidence for a Covalent Bond ........ 138
     (d) Distance Measurements ................. 140
  (ii) Bovine Serum Albumin .................... 144
  (iii) Aluminum Oxide .......................... 144
  (iv) Controlled Pore Glass ................... 150
  (v) Summary and Conclusions ................. 154

IIID: Nmr Spectroscopy .......................... 157

IIIE: Esr Spectroscopy of Nitroxides ............ 162

IV SUMMARY ...................................... 184

V EXPERIMENTAL .................................. 188

  VA: Electron Spin Resonance .................... 188
  VB: Nmr Measurements ...........................
  VC: General Synthetic Procedures .............
  VD: Chapter I ..................................
     (i) Sources of Materials ...................
     (ii) Literature Preparations .............
     (iii) Synthesis ...........................
  VE: Chapter II ..................................
     (i) Sources of Materials ..................
     (ii) Literature Preparations .............
     (iii) Synthesis of Ferrocenyl-Sugar
            Conjugates ..........................
  VF: Chapter III .................................
     (i) Sources of Materials .................
     (ii) Literature Preparations .............
     (iii) Polysaccharide and Surface
            Derivatization .......................
LIST OF TABLES

Table

CHAPTER I

I-1. Esr parameters for copper (II) complexes 32
I-2. Magnetic moments of copper complexes 41

CHAPTER II

II-1. Chemical shifts (ppm) and multiplet splittings (Hz) for the ferrocenyl monosaccharide compounds 75
II-2. Proton spin lattice relaxation rates 90

CHAPTER III

III-1. Data obtained from elemental analysis and esr double integration of labelled polysaccharides 135
III-2. Distances measurements determined for polysaccharides labelled with [22] 143
III-3. Extent of derivatization of Al₂O₃ with reagent [22] 147
III-4. Correlation times for nitroxides in chloroform solution 173
LIST OF FIGURES

CHAPTER I

I-1. 270 MHz $^1H$ spectrum of compound [8] .............................................. 14
I-2. 270 MHz $^1H$ spectrum of compound [10] .............................................. 15
I-3. 270 MHz $^1H$ spectrum of compound [11] .............................................. 16
I-5. Visible absorption spectra (CHC$_3$) comparing compounds [16] and [19] to previously known alkyl complexes .............................................. 27
I-6. Ambient temperature chloroform solution esr spectra of compounds [16] and [19], n-butyl complex, iso-propyl complex, and t-butyl complex ............. 34
I-7. 77K liquid N$_2$ frozen solution esr spectra of compounds [16] and [19], n-butyl complex, iso-propyl complex, t-butyl complex, and room temperature powder spectrum ......................... 35
I-8. Aqueous room temperature esr spectrum and the 77k frozen aqueous esr spectrum of compound [21] .............................................. 37
I-9. The 77K frozen powder esr spectrum of the mononuclear copper complex impurity within the binuclear complex [23] ......................... 39
I-10. Simulation of the mass spectral isotope pattern for a binuclear copper complex .............................................. 43
I-11. Proton nmr spectrum (270 MHz) of the copper sugar complex [16] in deuteriochloroform .............................................. 46
I-13. Spin state energy level diagram for a copper (II) nucleus in a magnetic field .............................................. 53
I-14. Energy level diagram for two interacting copper (II) nuclei in a magnetic field .............................................. 54
I-15. Schematic representation of the esr spectrum of copper (II) ......................... 56

CHAPTER II

II-1. Some typical aromatic substitution reactions of ferrocene .............................................. 65
II-2. Proton nmr spectra (270 MHz) of compound [7] in deuteriobenzene ......................... 80
II-3. Proton nmr spectra (270 MHz) of compound [6] in deuteriobenzene ......................... 81
| II-4. | Proton nmr spectrum (270 MHz) of compound [8] in deuteriobenzene | 82 |
| II-5. | Proton nmr spectra (270 MHz) of compound [23] in deuterioacetone | 84 |
| II-7. | Proton R$_1$-values for the sugar moiety of compound [6] | 91 |
| II-8. | Rotating reference frame model | 97 |
| II-9. | Plot of magnetization vs $t/T_1$ | 98 |

**CHAPTER III**

| III-1. | Structural formulae of the polysaccharides spin labelled via cyanuric chloride | 127 |
| III-2. | Ambient temperature aqueous esr spectra of spin labelled polysaccharides | 132 |
| III-3. | Comparison of the aqueous esr spectra of labelled Sephadex and Agarose with their chloroform spectra | 134 |
| III-4. | Control esr experiments as evidence for covalent bonding of triazine label to polysaccharides | 141 |
| III-5. | Powder spectrum showing the heights $d_1$ and $d$ and the splitting $2A$ | 142 |
| III-6. | Ambient temperature aqueous esr spectrum of BSA labelled with reagent [22] | 145 |
| III-7(i). | Esr spectrum of labelled alumina | 146 |
| III-7(ii). | Ambient temperature chloroform esr spectra of alumina labelled with [22] and alumina labelled with the non-reactive reagent [11] | 149 |
| III-8. | Ambient temperature chloroform esr spectra of successive spin dilution of nitrooxides on alumina | 151 |
| III-9. | 77K frozen chloroform esr spectra of labelled alumina before and after spin dilution | 152 |
| III-10. | Ambient temperature methanol esr spectrum of silica labelled with reagent [22] | 154 |
| III-11. | Proton nmr spectrum (270 MHz) of compound [21] in dueterioacetone | 158 |
| III-12. | Proton nmr spectrum (270 MHz) of compound [32] | 159 |
| III-13. | Proton nmr spectrum (270 MHz) of compound [36] before and after reduction | 160 |
| III-14. | Energy level diagram for a nitrooxide in a magnetic field | 165 |
| III-15. | Directional dependence of Zeeman and hyperfine interactions | 166 |
| III-16. | Esr spectra of magnetically dilute di-t-butyl nitrooxide showing g and A anisotropies | 167 |
III-19. Effects of the rate of exchange between A and B in magnetic resonance spectra .............................. 175

III-20. 77K infinite dilution chloroform esr spectra of the monoradical [22] and the biradical [25] ............. 180

ACKNOWLEDGEMENT

I am very grateful to Dr. L. D. Hall for his constant supply of encouragement, helpful discussions and moral support, which made the work described in this thesis exciting and rewarding.

Although too numerous to name individually, I would like to thank the graduate students, post doctoral fellows, and visiting professors who were present in this lab during my Ph.D. work, for they, to a large degree, were my teachers. In particular, I would like to thank Kim Wong, John Waterton, John Aplin, Jeremy Saunders, and Mansur Yalpani for many helpful discussions in relation to this thesis work. I would also like to thank Drs. Geoffrey Herring and R. C. Thompson, along with other members of the Chemistry Department, for many stimulating discussions and the National Research Council of Canada for financial support.

Finally, I am especially indebted to the late Professor L. J. Muenster for the generous loan of equipment and for teaching me most of what I know about practical synthetic chemistry.
INTRODUCTION

Carbohydrates are very important and abundant molecules in nature as they play a vital role in the biochemistry of virtually all living things. Both mono- and poly-saccharides are finding an ever increasingly wide range of industrial and biochemical applications; areas of use range from new pharmaceuticals\(^1\) and chromatography materials\(^2\) through to additives for use in the food industry and oil recovery\(^3\) and the development of renewable sources of energy\(^4\). These and other areas would clearly be advanced both by general techniques for changing the physical characteristics of carbohydrates and by the development of methods for studying the structure and function of "natural" and "modified" saccharides.

A basic prerequisite of any of these studies, is the development of a general methodology in which carbohydrates can be chemically modified, preferably in a selective fashion. The main body of work in this thesis consists of the development of chemical procedures whereby a variety of chemical ligands can be attached to both mono- and poly-saccharide families of carbohydrates. At this point it is convenient to discuss the thesis as if it consisted of essentially two parts, the first involves the development of specific chemistry for the attachment of metals to carbohydrates and the second describes the development of a versatile chemistry whereby many different types of chemical groups can be attached.
Due to a long standing interest by our lab in metal-sugar conjugates (the reasons for which will be given later), the synthesis of specific metal sugar compounds was initially undertaken.

Metal complexes of Schiff's bases have occupied a central role in the development of coordination chemistry and have been known for over one hundred years dating as far back as 1840. Since amino sugars are important biological molecules and are constituents of a large number of antibiotics, it might be expected that the combined presence of sugar and metal moieties would give these compounds interesting biological properties. Therefore my first experiments in this area were designed to form Schiff's base ligands by combining amino sugars with suitable aldehydes or ketones, and subsequently complexing these with metals.

Although the chemistry of ferrocene and other aromatic \( \pi \)-complexes is not as old as that of Schiff's-base complexes it still goes back as far as 1948 and is now very well documented. These substances constitute a central role in organometallic chemistry and their applications in chemistry, biochemistry, and industry have grown dramatically over the last ten years. Accordingly, the second round of experiments with regard to sugar metal conjugates involved the formation of organometallic carbohydrate complexes, specifically ferrocenyl-sugar conjugates. The preparation of these substances has involved a variety of monosaccharides including amino- and thio-sugars.

The methods used for the direct conjugation of metals to carbohydrates via these two approaches were, however, thought to be somewhat limited in scope and it seemed logical to try and develop chemistry which would enable one to link a larger variety of organic and inorganic materials to carbohydrates. To achieve this diversity the chemistry
that was investigated involved the versatile coupling reagent cyanuric chloride (2,4,6-trichloro-s-triazine). This substance has been known since 1827 and its chemistry is very well understood. It has been widely used in industry in areas ranging from the dyestuffs industry, where it is used for making reactive dyes for cellulose fibers to its use in the manufacturing of rubber and plastics, and in the preparation of medicinals such as antibacterials and anti-cancer drugs.

Cyanuric chloride is a heterocyclic compound containing three chlorine substituents which can be displaced by many different nucleophiles under a variety of conditions. What makes this reagent so attractive is that unlike most other bridging groups or coupling reagents, which are usually bifunctional, it possesses three points of attachment to which other moieties can be bonded, and hence it can be termed a "trivalent locus". Using this reagent it has been possible to form various combinations of metal compounds, hydrophobic and hydrophilic entities, and spin label nitroxide free radical reporter groups. An example of one such useful combination is illustrated by the synthesis of model glycolipids which can be made with or without a spin label reporter group attached. Such molecules may have uses in probing the functions of "model" and "real" membranes and in studying phenomena.
such as cell-cell interactions\textsuperscript{13}.

It was thought important to demonstrate that the above chemistry can also be applied outside the realm of carbohydrate chemistry, and examples will include the spin labelling of Bovine Serum Albumin (BSA), and the use of cyanuric chloride in the derivatization of other solid support matrices such as aluminum oxide and glass.

Along with the synthetic methodology, suitable spectroscopic tools had to be developed in order to prove the structures of the various products, some of which had rather unusual compositions. Both esr and nmr spectroscopy were used extensively throughout the course of the work. In chapter III, nitrooxide free radical esr will be discussed with particular emphasis on its use in relaying (a) information concerning the environment of the label\textsuperscript{14}, (b) in measuring distances\textsuperscript{15}, and (c) in determining the extent of chemical derivatization\textsuperscript{16}. Using these techniques it will be shown how esr spectroscopy can be used to "debug" the chemistry of cyanuric chloride as it is used in the derivatization of solid support matrices. It is well known that esr is also a good spectroscopic tool for the structure determination of certain transition metal complexes\textsuperscript{17}, and in chapter I it will be discussed in the context of determining structural information about the copper sugar complexes synthesized therein. Nmr spectroscopy will be alluded to throughout the text as a general method for the structural determination of organic compounds. However, a more specialized nmr experiment will be explained in chapter II, where proton spin lattice relaxation will be demonstrated as a technique\textsuperscript{18} for assigning proton resonances and for determining motional correlation times for some of the ferrocenyl-sugar compounds synthesized.
References


13. J. C. Waterton and A. van der Est, manuscript in preparation.


CHAPTER I

SYNTHESIS OF METAL CHELATE CONJUGATES OF AMINO SUGARS: SCHIFF'S BASE COMPLEXES

IA: Introduction

The fact that sugars form complexes with metals has been known for about 100 years\(^1,2\). However, the field of sugar-metal complexes is still largely unexplored. The chemical literature records, since 1973, at least 70 crystalline complexes which contain a sugar and an inorganic salt. These complexes are, however, mainly restricted to group IA and group IIA metals, and the structures of these metal sugar "adducts" is, for the most part, unknown. These complexes are, though, very important in the biology of all living things since it has been shown that sugars are an important factor in the active cellular transport of calcium and other group IIA metals\(^2\). More recently, it has been shown that cyclitols and sugars which contain an axial-equatorial-axial sequence of three hydroxyl groups in a six-membered ring, or a cis-cis sequence in a five-membered ring, form 1:1 complexes with metal cations in a hydroxylic
solvent$^2, 3$. Complex formation has also been found to cause a change in the conformational equilibrium and the anomeric equilibrium of various sugars$^2$. Complex formation also makes possible the separation of some sugars by electrophoresis and ion-exchange chromatography.

Literature on the complexation of sugars with transition metals and metals other than those in group I and IIA is in comparison much more recent and less abundant. Some of these studies have included iron sugar complexes for use as hematinic agents (iron deficiency drugs)$^4$, nickel, cobalt, iron, manganese complexes of glucosaminic acid$^5$, copper, lead, zinc, nickel and cadmium complexes of D-glucosamines for the study of antibiotic functions$^6$, lithium aluminum hydride sugar complexes as asymmetric reducing agents$^7$, interactions of metals with amino-sugar containing antibiotics$^8$, copper capped cyclodextrins for mimicking enzyme binding sites$^9$, copper cobalt and nickel complexes of nitrogen-heterocyclic monosaccharide derivatives as analogues for nucleoside and nucleotide metal complexes$^{10}$, rhodium diphenylphosphine sugar complexes as asymmetric hydrogenation catalyst$^{11}$, rhodium complexes of diphenylphosphine derivatized cellulose$^{12}$, and copper complex formation with celluloses$^{13}$. In our own laboratory, direct carbon-metal bonded complexes of mercury$^{14}$, thallium$^{15}$, tin$^{16}$, and vitamin B$_{12}$, cyclopentadienyl iron, tungsten, and molybdenum triarbonyl$^{17}$ sugar derivatives have also been prepared.

It is clear then that the ability of sugars to sequester metals is of current interest for a wide variety of reasons. Some of the more important applications of this chemistry includes the possible development of novel classes of metal based affinity chromatography materials$^{18}$, chiral homogeneous catalysts$^{11}$, metal chelators for clinical use$^4$, and
of models for biologically important chelates\textsuperscript{10}. Further interests include nmr studies of the interaction of many metals with sugars\textsuperscript{8,19}. These and many other studies would be advanced by the continued development of general methods whereby metals could be readily attached to sugars.

Metal complexes of Schiff's bases have occupied a central role in the development of coordination chemistry\textsuperscript{20} and have been known for over one hundred years, dating as far back as 1840. A tremendous variety of stable chemical species have been synthesized containing both transition and non transition metals. The most important groups of Schiff's base complexes are the complexes of salicylaldimines and $\beta$-ketoamines, and closely related systems.

Schiff's bases are those compounds containing the azomethine group (-RC=N-) and are usually formed by the condensation of a primary amine with an active carbonyl compound. Bases which are effective as coordinating ligands bear a functional group, usually -OH, sufficiently near the site of condensation that a five- or six-membered chelate ring can be formed upon reaction with a metal ion. Because of the great flexibility of Schiff's base formation, many ligands of diverse structural type can be and have been synthesized.

Amino sugars form Schiff's bases readily with salicylaldehyde and other aromatic aldehydes\textsuperscript{21} and have been known since 1922\textsuperscript{22} when Irvine and Earl first showed that sparingly water soluble Schiff's bases of glucosamine could be formed with salicylaldehyde. These Schiff's base derivatives were found to provide a good means for isolating amino sugars from hydrolyzed polysaccharides and proteins\textsuperscript{23}.

In this chapter the formation of metal complexes of amino sugar-
Schiff's base derivatives will be discussed. First the synthesis of salicylaldehyde and 3-formyl-2-hydroxy-benzoic acid Schiff's base ligands derived from both alkylamine and glucosamine derivatives along with their metal complexes will be described. Then the physical techniques of visible absorption spectroscopy, esr, and nmr spectroscopy, mass spectrometry and magnetic moments will be examined as tools for the structure determination of the complexes synthesized. Following this, a section on "other chemistry" will be presented which describes reactions which did not yield the desired product but which do provide some interesting results. Nmr data on the various diamagnetic ligands will be presented throughout the chapter as a routine structural tool. Finally, a section describing some of the basic features of esr spectroscopy as it pertains to studies of copper (II) complexes will be presented. When esr data are given earlier in section IC(ii) the reader not familiar with this technique will be referred ahead to this last section.

IB: Synthesis

(i) Schiff's Base Formation

As mentioned in the introduction, Schiff's bases are those compounds containing the azomethine group which are usually formed by the condensation of a primary amine with an active carbonyl compound. The active carbonyl compounds considered here are 2-hydroxy-benzaldehyde (salicylaldehyde) [1] and 3-formyl-2-hydroxy-benzoic acid (3-aldehydo-salicylic acid) [2]. A typical Schiff's base formation, with cyclohexylamine and salicylaldehyde is shown below ([3] and [4]).

Just as alkylamines readily form Schiff's bases, so do amino sugars. Salicylaldimine compounds (Schiff's bases formed from salicylaldehyde or
substituted salicylaldehyde compounds) from glucosamine (2-amino-2-deoxy-α,β-D-glucopyranose) were first synthesized by Irvine and Earl in 1922. The reaction between glucosamine hydrochloride and salicylaldehyde, as shown below, is very easily accomplished with the water insoluble product being obtained in a high yield. As mentioned in the introduction, this reaction was found to be useful for the isolation of amino sugars, such as glucosamine, from hydrolysis extracts of proteins and
polysaccharides.

As well as "free" glucosamine, "blocked" glucosamine derivatives such as methyl 3,4,6-tri-0-acetyl-2-amino-2-deoxy-β-D-glucopyranoside hydrobromide [7] and 1,3,4,6-tetra-0-acetyl-2-amino-2-deoxy-β-D-glucopyranose [9] also condense with salicylaldehyde to form the Schiff's bases [8] and [10] respectively, as shown below.

Schiff's bases formed from 3-aldehydo salicycic acid [2] have, in comparison to other salicylaldimines, received very little attention. To my knowledge, Schiff's base derivatives formed between amino sugars and 3-aldehydo-salicyclic acid have not before been reported. The formation of the Schiff's base between the blocked glucosamine sugar [7] and this aldehyde is also very easily accomplished giving compound [11] in a high
yield.

The sugar Schiff's base compounds [6], [8], [10] and [11] are crystalline stable compounds as are their alkyl counterparts and are bright yellow in color. These compounds are, however, acid- and base-labile due to their azomethine linkage. Because of this, aromatic aldehydes can be used conveniently as amine blocking groups for amino sugars.

Sugar Schiff's bases are, fortunately, stable to cold anhydrous acylating reagents; thus the amino sugar [9] was prepared by using anisaldehyde (p-methoxy-benzaldehyde) [12] as an amino blocking group with subsequent acetylation and Schiff's base and cleavage as shown below.

Metal complexation using ligands [6], [8], [10] and [11] will be the main focal point of this chapter. In order for metal complexation to occur with these compounds, the hydroxyl function on the aromatic ring must be brought into close proximity with the nitrogen atom of the sugar group. Therefore, the aromatic hydroxyl group should preferably be strongly hydrogen bonded to the nitrogen atom of the sugar ring as shown below for [10]. Evidence for this hydrogen bonding comes from the $^1$H nmr spectra of compounds [8], [10] and [11] which are shown in Figures 1-1, 2 and 3 respectively. The hydroxyl protons for [8], [10] and [11]
Figure I-1. 270 MHz $^1$H spectrum of compound [8].
Figure I-2. 270 MHz $^1$H spectrum of compound [10].
Figure 1-3. 270 MHz $^1$H spectrum of compound [11].
are found at 12.4 ppm, 12 ppm and 14 ppm respectively and such very low field chemical shifts are characteristic of hydrogen bonded protons.

Compound [11] has a very interesting spectrum and additional information for the existence of a strong hydrogen bond in this molecule can be seen. In Figure 1-3 the imine proton and the H$_2$ sugar ring proton, are broader and more poorly resolved than normally expected. This is a result of partial vicinal spin-spin coupling of these protons to the hydrogen bonded hydroxyl proton. By irradiating the hydroxyl
resonance at 14 ppm, the resultant sharpening of the imine and H₂ protons (Figure 1-4) clearly prove that coupling exists and hence proves the existence of hydrogen bonding. The reason the imine and H₂ resonances are only broadened rather than split into a greater multiplicity can be explained by assuming exchange of the hydroxyl proton. If the exchange rate between the hydroxyl proton and the acid function within the molecule or between neighbouring molecules were very rapid, then the hydroxyl proton would be effectively "decoupled" from the imine and H₂ protons; and the hydroxyl and acid proton resonances would collapse to one sharp line. If the exchange rate is slow or non existent the imine and H₂ proton resonances would be split by coupling to the hydroxyl proton and would also have narrow line widths; and the hydroxyl and acid resonances would appear as two individual sharp lines. Therefore, apparently exchange occurs at an intermediate rate thereby broadening the resonances of the imine and H₂ resonances as well as the hydroxyl and acid resonances. In compounds [8] and [10] this broadening effect is not observed, either because hydrogen bonding of the hydroxyl proton to the nitrogen is not strong enough for coupling to be observed, or because the hydroxyl proton is exchanging so rapidly with the hydroxyl group of another molecule that it is effectively decoupled from the imine and H₂ protons.

(ii) Schiff's Base Metal Complexes

As previously noted, of all Schiff's bases, those derived from salicylaldimines have been by far the most thoroughly studied. The particular advantage of the basic salicylaldimine ligand system is the considerable flexibility of the synthetic procedure. As a result, a wide variety of complexes have been formed and by structural variations
Figure 1-4. Decoupling experiment revealing hydrogen bonding in compound [11]. The spectrum in (A) shows the sharpening of the imine and $H_2$ protons upon irradiation of the aromatic hydroxyl proton. Spectrum (B) is the non-decoupled spectrum.
of the ligand systematic changes in properties have been examined.

Salicylaldimine complexes are generally readily prepared and easily purified by recrystallization. Basically two synthetic procedures have been employed.

1. Reaction of the metal ion and Schiff base in a homogeneous alcohol, or aqueous alcohol, solution with the possible addition of a base such as acetate or hydroxide.

2. Reaction of a primary amine with the preformed salicylaldehyde-metal complex. This preformed complex is heated under reflux with the amine in a solvent such as ethanol, or chloroform for a period of 1 h or less and the crude product is obtained by cooling and/or volume reduction. This reaction may be represented as follows.*

\[
\begin{align*}
\text{O} & \quad \text{M} \\
\text{H} & \quad \text{C} = \text{O} \\
\text{R} & \quad \text{NH}_2
\end{align*}
\]

\[
\begin{align*}
\text{O} & \quad \text{M} \\
\text{H} & \quad \text{C} = \text{O} \\
\Theta & \quad \text{NH}_2 \\
\text{R} & \quad \text{H}_2\text{O}
\end{align*}
\]

\[
\begin{align*}
\text{O} & \quad \text{M} \\
\text{H} & \quad \text{C} = \text{N} \\
\text{R} & \quad \text{R}
\end{align*}
\]

*adapted from reference (20).
By removing "electron density," the coordinated metal ion enhances the polarization of the carbonyl group, thereby promoting nucleophilic attack by the amine-nitrogen.

Although the latter synthetic method has been used by other workers more often than the first, it was found in this study that method (1) was more applicable for the formation of sugar complexes.

The most significant complexes of the salicylaldimines are of the structural types (A) and (B) where R, X, and B are generalized nitrogen, ring, and bridging group substituents, respectively. The complexes discussed in this thesis are restricted to the structural type (A) in which X is either a hydrogen or carboxylic acid substituent. Instead of using the cumbersome systematic nomenclature the abbreviated form M(R-sal)\textsubscript{n} will be used.

A wide variety of metals and R groups can be incorporated into complexes of the structural type (A). Typically R can be n-propyl, n-decyl, t-butyl or cyclohexyl, and M, Cu, Ni, Co, Zn, Mn or Cr. The cyclohexyl moiety is the most structurally similar alkyl group to the pyranose sugar ring. It is known\textsuperscript{20} that when n = 2, the bis-complex has the oxygen and nitrogen donor atoms arranged as shown in the structure of the cyclohexyl complex [15].
The cyclohexyl salicylaldimine ligand can complex with several divalent metals to form many complexes of the bis-structure [15]. The analogous sugar salicylaldimine derivatives [8] and [10] were therefore expected to form complexes equally as well (complexation using ligands [6] and [11] will be discussed separately later). Indeed for copper (II) this was the case. When a methanol solution of cupric acetate was added to a methanol solution of [8] at room temperature, the copper sugar complex [16] precipitated immediately as a brown solid in 90% yield.*

*For convenience, from now on, the sugar unit shown in [16], [17] and [18] will be abbreviated as Sug I. In complexes incorporating the sugar units derived from compounds [9] and [5], the sugar moiety will be abbreviated to Sug II and III respectively.
The ir spectrum showed a shift in the -C=N- absorption to lower frequency compared with the ligand ([8], 1630 cm$^{-1}$; [16], 1590 cm$^{-1}$) indicating the coordination of the nitrogen atom to the copper ion$^{26}$. Both zinc and cobaltous acetate also complexed with the ligand [8] in methanol to form the yellow and green bis-complexes [17] and [18] respectively. The yields for these complexes were, however, lower than the copper analogue, being 41% and 51% respectively.

Although stability constants were not determined for these complexes, some evidence suggests that they are not as stable as their alkyl counterparts. Thus the sugar complexes decomposed back to their parent ligands when run on silica gel thin layer chromatography (tlc), whereas alkyl complexes such as [15] are stable on silica gel. Also, the zinc and cobalt complexes [17] and [18] decomposed when dissolved in hot methanol, and had to be recrystallized under cold conditions (see Experimental). The copper complex was, however, stable to this treatment. Thus it seems that the zinc and cobalt complexes are less stable than the copper complex.

The sugar salicylaldimine ligand [10] also forms a complex [19] Cu (Sug II-sal)$_2$ with copper; this was obtained as shiny olive green crystals in 90% yield when a hot ethanol solution of cupric acetate and ligand [10] were mixed and allowed to cool. This complex could be recrystallized from hot ethanol and decomposed only partially on silica gel tlc. However, attempted complexation of [10] with zinc and cobaltous acetate failed to yield any product.

The decomposition mixture of the cobalt complex [18] in hot methanol and the reaction mixture between [10] and cobalt acetate, were both orange in color. This same color was observed for a solution of the
salicylaldehyde cobalt complex [20]. It therefore seems likely that the azomethine bond is being cleaved in both these cases resulting in the formation of the more stable salicylaldehyde complex [20].

Probably the most significant complex prepared from these sugar salicylaldimines, was the copper complex of the free sugar salicylaldimine [6]. This ligand formed a copper complex [21] equally as well as the other ligands using the same reaction conditions. This copper-sugar complex is highly water-soluble and, as mentioned earlier, the combined presence of a naturally occurring carbohydrate and metal ion might have useful applications in the pharmaceutical field. The structure of this complex is, however, less clear cut than the complexes discussed thus far, and the limited evidence which has been obtained will be discussed later. The possible structure of this complex based on data from a combination of elemental analysis, esr and mass spectrometry is shown below.
In contrast to the salicylaldehyde derived salicylaldimine Schiff's base complexes, those formed from the 3-aldehydo-salicylic acid Schiff's bases have not received as much attention. As discussed in IB(i) these ligands can be readily prepared in high yield in the same way as the previously described salicylaldimines. The nitrogen substituent can be quite varied and a number of metals, mostly first row transition, have been incorporated\textsuperscript{27}. The structure proposed for these complexes, where the nitrogen substituent R is non bridging, is shown below.

![Diagram](image)

\[ [22] \quad \equiv \text{M}\{R\text{-salicylic}\} \]

As for the other salicylaldimine complexes, these complexes can be prepared simply by mixing the ligand and metal salt, usually the metal acetate, in an alcohol solution, and then filtering the precipitated product from the reaction mixture.

The reaction of the sugar ligand \[11\] and cupric acetate in ethanol was accomplished in just this way, resulting in a leaf-green product. As will be discussed in more detail later, mass spectrometry and esr spectroscopy strongly suggest that the product has a binuclear copper structure as shown below, with a small percentage of a mononuclear copper complex impurity. This mononuclear complex also contains the sugar moiety, as shown by esr spectroscopy \[\text{IC(ii)}\] and may well be of the same structure as that proposed in \[22\].

Various physical methods can be used to determine the structures of
these complexes and in the next section the use of a variety of these will be described.

IC: Physical Measurements

(1) Visible Absorption Studies

In this section, the use of visible absorption spectroscopy for determining the coordination geometry of the sugar-copper complexes Cu (Sug I-sal)$_2$ [16] and Cu (Sug II-sal)$_2$ [19] and the cobalt complex Co (Sug I-sal)$_2$ [18] will be examined. The data for [16] and [19] will be compared to data obtained for a series of Cu (R-sal)$_2$ complexes of known geometry.

Although the visible absorption spectra of the Cu (R-sal)$_2$ complexes, where R = n-Bu, i-Pr, and t-Bu, have been reported elsewhere$^{28,29}$ they have been measured here for comparison purposes, along with the sugar complexes, in order to obtain systematic information about their coordination geometry; all of the observed spectra are shown in Figure I-5.
Figure I-5. Visible absorption spectra (CHCl$_3$), comparing compounds [16] and [19] to previously known alkyl complexes.
The crystal structure of the complex Cu (R-sal)$_2$, where R = n-Bu, shows that this complex adopts a "normal" planar configuration. In contrast, complexes where R = i-Pr and t-Bu are distorted toward tetrahedral configuration, the inference is that this is due to the presence of bulky R groups because the t-Bu complex has more tetrahedral character than does the isopropyl complex. It has also been shown, by dipole moment measurements and optical absorption spectral studies, that salicylaldimine complexes which are trans-planar in the solid state appear to retain this structure in solution. The solution stereochemistry of those complexes which are pseudotetrahedral as solids is less clear cut and three possibilities exist. The first is that the complexes exist as an equilibrium mixture of planar and "tetrahedral" isomers. The second possibility is that the complexes dissolve without structural change and the third is that the complexes dissolve with a structural change, that is, a change in the dihedral angle $\omega$ upon passing into solution. The t-Bu complex fits into the second category since its spectral parameters do not change upon dissolution. In contrast, complexes with R = i-Pr or cyclohexyl, appear to exist in solution as an equilibrium mixture of square planar and pseudotetrahedral complexes, as revealed by $^1$H nmr and esr studies.
The visible spectra (d-d transitions) in Figure I-5 appear as shoulders on the longer-wave length side of the intense bands in the near-ultraviolet except for Cu (t-Bu-sal)_2 the d-d spectrum of which appears as a broad peak. These spectra do not lend themselves to detailed discussion. However, one important observation concerning the three alkyl complexes, where R = n-Bu, i-Pr, and t-Bu can be made from the spectra in Figure I-5; namely the tendency for the intensities of the d-d spectra to increase in the spectral order n-Bu to t-Bu while the d-d spectra shift to longer wave lengths in the same spectral order. This tendency is consistent with the dependence of the spectral change on a distortion of the coordination geometry towards a tetrahedron \(^28,29\); the greater the tetrahedral distortion, the greater this effect. It is surprising, therefore, that the spectra for the two sugar complexes [16] and [19] are not more similar to the spectrum of the t-Bu complex, since the sugar substituents would appear to be more bulky than the t-Bu group. Perhaps, due to the very different nature of sugar substituents, it is not possible to compare these complexes to the simpler complexes in which R is an alkyl group. However, if the same trend of intensity and wave length shift changes holds true within a series of sugar complexes, then it appears that the complex Cu (Sug II-sal)_2 [19] possesses a more square planar geometry than Cu (Sug I-sal)_2 [16].

Within the series of alkyl Cu (R-sal)_2 complexes, the square planar complexes are always green or brown in color, and the pseudotetrahedral complexes are violet. This violet color is presumably due to the longer wave length shift of the visible absorption maxima. Therefore, the reddish brown color of the Cu (Sug I-sal)_2 complex [16] as compared to the olive green color of the Cu (Sug II-sal)_2 complex [19] may support the
possibility that [19] has a more square planar geometry than does [16]. Caution must be exercised in this interpretation since metal—ligand charge—transfer bands may contribute largely to their color and therefore comparison of the colors of sugar complexes with those of alkyl type complexes may be invalid.

The solid-mull visible spectrum of Cu (Sug I-sal)₂ [16] consists of three bands at 9,500, 13,500, and 20,000 cm⁻¹ which is characteristic of a pseudotetrahedral Cu (R-sal)₂ complex ²⁰,²⁸,²⁹. Therefore, the visible absorption spectra in the solid and solution suggests that, when dissolved, this complex either becomes square planar or loses a significant amount of its tetrahedral character.

The visible spectrum of the cobalt sugar complex Co (Sug I-sal)₂ is straightforward and suggests that it has the tetrahedral geometry expected for a cobalt (II) salicylaldimine complex ²⁰. Three bands are expected for cobalt complexes of this type where ν₁ is generally too far in the infrared region to be easily observed, and ν₂ and ν₃ are often found to be split into several separate components; as a result Co (R-sal)₂ complexes generally show two bands at ~ 7700 and ~ 11,200 cm⁻¹ assigned to components of ν₂, and a well defined shoulder at ~ 17,000 cm⁻¹ ²⁰. The absorption bands obtained for the Co (Sug I-sal)₂ complex [18] were found at 7,300, 11,250, and 17,240 cm⁻¹. Bis-salicylaldimine cobalt complexes have been found to be tetrahedral irrespective of the nature of R and the sugar complex [18] therefore, seems to be no exception.
(ii) Electron Spin Resonance Spectroscopy

Copper II $d^9$ has a $\frac{1}{2}$ electron spin which can be readily detected by esr spectroscopy. A great deal of theoretical and empirical information is known about the esr of copper and much structural data concerning its complexes can be obtained from this technique. The reader unfamiliar with this technique is referred to section IE for a basic discussion of the esr of copper (II). Briefly, though, it is expected that for copper (II) in a square planar or distorted tetrahedral geometry, the room temperature solution spectrum will consist of four lines and the 77K frozen solution spectrum to exhibit a total of eight lines with the four high field lines closely spaced and overlapping. This spectral behavior was observed for most of the copper-sugar complexes synthesized here.

The tabulation for all of the spectral parameters acquired for the copper sugar complexes and the Cu (R-sal)$_2$ complexes, where R = n-butyl, isopropyl and t-butyl, are shown in Table I-1. Here $g_0$ and $a_0$ are parameters from the room temperature solution spectrum and the $g_n$ and $A_n$ values are from either the frozen 77K solution or the spin-dilute powder spectrum.

A classification of the Cu (R-sal)$_2$ complexes can be made on the basis of esr results in the same way as previously described for the visible absorption spectra. It has been shown$^{28}$ that the esr spectral parameters of copper complexes are sensitive to the geometry of coordination about the metal center. As the geometry becomes more tetrahedral in nature, the $A_n$ value becomes smaller, and at the same time $g_n$ and $g_0$ begin to increase while $a_0$ decreases, as does $A_n$. Most of the literature however, uses the changes in $A_n$ as a measure of tetrahedral distortion. The Cu (R-sal)$_2$ complexes with R = n-butyl, isopropyl and t-butyl
TABLE I-1. Esr parameters for copper (II) complexes

<table>
<thead>
<tr>
<th>Complex</th>
<th>$g_0$</th>
<th>$a_0 \times 10^{-4}$ cm$^{-1}$</th>
<th>$g_\parallel$</th>
<th>$A_\parallel \times 10^{-4}$ cm$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Cu (Sug I-sal)$_2$</td>
<td>2.129</td>
<td>64.6</td>
<td>2.246</td>
<td>167.8</td>
</tr>
<tr>
<td>(b) Cu (Sug II-sal)$_2$</td>
<td>2.130</td>
<td>66.6</td>
<td>2.259</td>
<td>177.2</td>
</tr>
<tr>
<td>(c) Cu (n-butyl-sal)$_2$</td>
<td>2.112</td>
<td>73.9</td>
<td>2.223</td>
<td>162.9</td>
</tr>
<tr>
<td>(d) Cu (isopropyl-sal)$_2$</td>
<td>2.119</td>
<td>66.3</td>
<td>&lt;2.23</td>
<td>&lt;166</td>
</tr>
<tr>
<td>(e) Cu (t-Bu-sal)$_2$</td>
<td>2.14</td>
<td>&lt;50</td>
<td>2.271</td>
<td>135.7</td>
</tr>
<tr>
<td>(f) Cu (Sug I-sal)$_2$ 0.5% in Zn (Sug I-sal)$_2$</td>
<td></td>
<td>2.258</td>
<td>129.7</td>
<td></td>
</tr>
<tr>
<td>(g) Cu (Sug III-sal)$_+^+$</td>
<td>2.136</td>
<td>65</td>
<td>2.263</td>
<td>176.4</td>
</tr>
<tr>
<td>(h) Cu (Sug I-salicylic) (impurity)</td>
<td>2.30</td>
<td></td>
<td>2.278</td>
<td>184.0</td>
</tr>
</tbody>
</table>

* CHCl$_3$ solution
* powder
† H$_2$O solution

have esr parameters which clearly show the effect of tetrahedral distortion on these spectral parameters. As $R$ changes from n-Bu to t-Bu the $A$ values decrease and the $g$ values increase [Table I-1 (c), (d), (e)]. The $g_\parallel$ and $A_\parallel$ parameters for the isopropyl complex are only approximate because this complex exists as a mixture of geometrical isomers in solution which broadens the $g_\parallel$ components, making them difficult to measure (more will be said about this later). The spectra for the complexes where $R$ = alkyl groups, were re-measured here as were the visible absorption spectra, so that a more accurate comparison could be made between these complexes and the sugar complexes. As was the case with the visible absorption data, it is not clear just where the sugar complexes Cu (Sug I-sal)$_2$ [16] and Cu (Sug II-sal)$_2$ [19] fall within the series of
alkyl complexes. The A values suggest that the complexes are square planar but g values put them about midway between the n-alkyl planar and t-butyl pseudotetrahedral complexes. When the Zn (Sug I-sal)$_2$ complex [17] was doped with 0.5% of the Cu (Sug I-sal)$_2$ [16], the powder spectrum (f) indicated a highly tetrahedral geometry for the copper center. This can be explained by the fact that the zinc is almost certainly tetrahedral and when it is co-crystallized with a small percentage of the isomorphous copper complex, the latter is forced to adopt a more tetrahedral geometry. Both the A$_n$ and g$_n$ parameters are in agreement with this increased tetrahedral structure and are quite different from the A$_n$ and g$_n$ parameters obtained for the pure Cu (Sug I-sal)$_2$ complex. This experiment implies that the Cu (Sug I-sal)$_2$ complex has only a small tetrahedral distortion, if any, from a square planar geometry. The room temperature solution spectra and the polycrystalline spectra for all of the complexes in Table I-5 are shown in Figures 1-6 and 1-7 respectively.

As well as the data presented thus far, additional information could be obtained about these complexes from direct inspection of their esr spectra. The rate of tumbling of the complex in solution (designated by the correlation time $\tau_c$), can affect the esr spectra dramatically as shown in Figure I-6 (a)-(d). As the molecule tumbles more and more slowly, the low field lines broaden to a greater extent than do the high field lines; this is because incomplete averaging of the g and A anisotropies affect the low field lines before the high field line. This information can then be used to compare the size of copper complexes. From the spectra in Figure I-6, it is clear that the sugar complexes are much "larger" than their alkyl counterparts. The room temperature
Figure I-6. Ambient temperature chloroform solution esr spectra of (a) compound [16], (b) compound [19], (c) the n-butyl complex, (d) the iso-propyl complex, and (e) the t-butyl complex at $< 10^{-4}$ Molar.
Figure 1-7. 77K liquid N₂ frozen solution esr spectra of (a) compound [16], (b) compound [19], (c) the n-butyl complex, (d) the iso-propyl complex, (e) the t-butyl complex, and in (f) the room temperature powder spectrum of 0.5% [16] doped in the zinc complex [17].
spectrum (e) of the t-butyl complex is broad and poorly resolved because the high tetrahedral character of this complex results in a shorter electron relaxation time than for a square planar complex, which in turn results in broadened transitions.

The polycrystalline spectra in Figure I-7 also contain additional information; insofar that they all have an axial type of line shape this implies that the copper center possesses an axial, or near axial, symmetry. Spectrum (d) of the isopropyl complex also reveals that a mixture of geometries are present for this molecule in solution. This deduction is based on the observation of the broad g_v components in this spectrum which have been previously attributed to overlapping lines of a mixture of planar and pseudotetrahedral species. Computer simulation of the spectrum showed that this was a reasonable assignment. This has an important corollary in that it now seems safe to assume from the spectra in Figure I-7 that the other sugar- and alkyl-complexes exist as a single structural species in solution.

Both the room temperature solution spectrum and the 77K spectrum of the water soluble complex Cu (Sug III-sal) [21] are shown together in Figure I-8. The room temperature spectrum (a) reveals that the molecule must be tumbling relatively slowly since the low field lines are very broad. This suggests that the complex is large and therefore implies that the complex does contain the sugar moiety. In this spectrum, however, it appears as though there are two overlapping sets of resonances since the a_0 hyperfine coupling values are not all the same. This suggests that the compound is either a mixture of complexes or a mixture of geometric isomers of the same complex.

The 77K spectrum is an axial type of spectrum but the rather broad
Figure 1-8. (A) the aqueous room temperature esr spectrum and (B) the 77K frozen aqueous esr spectrum of compound [21].
featureless $g_n$ components again suggest a mixture of complexes or complex geometries, as was implied by the room temperature spectrum. Thus although the exact structure of this complex is not unequivocally established by these spectra alone, they are certainly consistent with the structure proposed in IB(ii).

The undiluted powder spectrum of the proposed binuclear copper complex $\text{Cu}_2(\text{Sug I-salicylic})_2$ [23] is shown in Figure I-9. It is believed that this spectrum is not of the binuclear complex itself, but is instead of a trace of a mononuclear copper complex impurity, diluted with the binuclear complex. This interpretation seems reasonable since (a) the powder spectrum of a pure mononuclear copper complex is expected to be highly exchange broadened, which is not observed, and (b) the receiver gain of the instrument had to be set to a very high level in order to see the spectrum, which implies that the molecule giving rise to the spectrum is at a very low concentration. That this spectrum clearly shows nitrogen super-hyperfine structure proves that this mononuclear complex contains the sugar ligand. The reason why the esr spectrum of the binuclear complex itself is not detected is because exchange coupling between the two copper ions produce singlet ($S = 0$) and triplet ($S = 1$) electronic states. Since the singlet state is expected to have zero magnetic moment only molecules in the triplet state can give rise to an esr signal. Two scenarios are then possible to explain the lack of a detectable esr spectrum. The first is that $J > kT$ where $J$ is the energy difference between the singlet and triplet states, $k$ is the Boltzmann constant and $T$ is the temperature. This would ensure that only the $S = 0$ state was populated and the molecule would be diamagnetic. The second possibility is that $J \sim kT$ but that the energy splitting
Figure I-9. The 77K frozen powder esr spectrum of the mononuclear copper complex impurity within the binuclear complex [23].
between the three \( m_s \) states \(+1, 0, -1\), originating from the triplet \( S = 1 \) state, is too large for spin transitions to be induced by microwave energy. The way to distinguish between these two possibilities would be to determine the magnetic moment of the pure compound; this has not been done at this time. The existence of the binuclear copper complex is also strongly supported by the mass spectrum as will be discussed later in I-C(iv).

(iii) Magnetic Moments

Often associated with red spectral shifts, as seen in (i) for the alkyl complexes of Cu (R-Sal)_2 with pseudotetrahedral structures, are larger magnetic moments; a \( \mu_{\text{eff}} \) value of \( \geq 1.89 \) BM has been considered characteristic of non-planar geometry\(^{31} \). This does not, however, appear to be a reliable structural criterion; for example, recent measurements\(^{31} \) of solid Cu (i-Pr-sal)_2 and Cu (t-Bu-sal)_2 which are known to be pseudotetrahedral, have yielded values of 1.84 and 1.83 BM respectively compared to 1.90-1.93 BM obtained earlier\(^{31} \). The magnetic moments calculated from the esr and Faraday methods, for the copper complexes shown previously, are given in Table I-2. Initially it was thought that the change in \( \mu_{\text{eff}} \), for the solid complex Cu (Sug I-sal)_2 (1.91 BM)*, upon dissolution of the solid reflected a change in geometry. However, in light of the recent data cited above for Cu (i-Pr-sal)_2 and Cu (t-Bu-sal)_2 it does not seem wise to be too dogmatic on this conclusion.

The solution magnetic moments were calculated from \( g_0 \) by the relationship

\[
\mu_{\text{eff}} = g_0 \sqrt{S(S+1)}
\]

* Obtained by Dr. R. C. Thompson of this department.
TABLE I-2. Magnetic moments of copper complexes

<table>
<thead>
<tr>
<th>Complex</th>
<th>$\mu_{\text{eff}}$ from esr BM</th>
<th>$\mu_{\text{eff}}$ Faraday BM</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Cu (Sug I-sal)$_2$ [16]</td>
<td>1.84</td>
<td>1.91</td>
</tr>
<tr>
<td>(b) Cu (Sug II-sal)$_2$ [19]</td>
<td>1.84</td>
<td></td>
</tr>
<tr>
<td>(c) Cu (n-Bu-sal)$_2$</td>
<td>1.83</td>
<td></td>
</tr>
<tr>
<td>(d) Cu (i-Pr-sal)$_2$</td>
<td>1.84</td>
<td></td>
</tr>
<tr>
<td>(e) Cu (t-Bu-sal)$_2$</td>
<td>1.85</td>
<td></td>
</tr>
<tr>
<td>(f) Cu (Sug III-sal)$_2$ [21]</td>
<td>1.85</td>
<td></td>
</tr>
<tr>
<td>(g) Mononuclear impurity within binuclear complex [23]</td>
<td>1.99</td>
<td></td>
</tr>
</tbody>
</table>

where S is ½ for copper (II). These data are all very similar (1.83-1.85 BM) although there is a slight trend in going from R = n-Bu to t-Bu. Since it was not possible to obtain $g_0$ for the mononuclear complex impurity within the binuclear complex [23] a value was estimated by substituting a value of $g_1$, (2.03) derived from the spectrum in Figure I-9, in the following equation (see section IE)

$$g_0 = 1/3 (g_n + 2 g_1)$$

$$= 2.30$$

(iv) Mass Spectrometry

Copper has two isotopes ($^{63}\text{Cu}$, 69.09% and $^{65}\text{Cu}$, 30.91%) which confer defined isotope-patterns to the mass spectral fragmentation of copper-containing complexes and thereby make this a useful tool for structure determination.

A simple illustration of how the isotope pattern can be calculated
for a binuclear copper complex is as follows. Firstly, for a single copper atom the M and M + 2 peaks will be in a ratio ca 7:3. With the second copper atom there are three possible combinations: $^{63}\text{Cu}^{63}\text{Cu}$ : $^{63}\text{Cu}^{65}\text{Cu}$ : $^{65}\text{Cu}^{65}\text{Cu}$ which will have relative intensities in the ratio of 49:42:9 respectively because of the same 7:3 ratio. When the carbon isotope effect ($^{13}\text{C}, 1.08\%$) is taken into account for a molecule which has 42 carbon atoms, the isotope pattern is as shown in Figure I-10. This simulation gives exactly the same molecular ion pattern as that obtained experimentally, and this evidence along with the parent mass of 1056, provides concrete evidence for the existence of the binuclear copper complex [23]. Obviously, such isotope patterns are also very useful for characterizing mononuclear copper complexes, for which the peak ratios are approximately 100:20:40:10 for the M, M + 1, M + 2, and M + 3 peaks respectively, depending upon the number of carbon atoms.
Figure I-10. Simulation of the mass spectral isotope pattern for a binuclear copper complex.
involved. The mass spectrum of all the mononuclear copper sugar complexes studied here show this pattern. In the mass spectrum of the water soluble sugar complex [21] there is a parent peak corresponding to the molecular weight with the correct isotope pattern, but some additional peaks are found as high as 420 amu. Since this complex decomposes upon heating to 150°C this may be responsible for the observed high mass peaks. Clearly this complex is not of the bis-type for which a parent peak at 627 amu would be expected.

Zinc has four natural abundance isotopes ($^{64}\text{Zn}$, 48.89%; $^{66}\text{Zn}$, 27.81%; $^{67}\text{Zn}$, 4.11%; and $^{68}\text{Zn}$, 18.57%) which confer well defined isotope-patterns to the mass spectrum of zinc complexes. For a molecule with 40 carbon atoms, using the same method of calculation applied to the copper case, the ratios for the M:M+1:M+2:M+3:M+4:M+5:M+6 peaks are approximately 100:44:65:33:46:18:3. This ratio pattern is indeed seen in the mass spectrum of the zinc-sugar complex [17], thus confirming its structure.

(v) Nmr Spectroscopy

Proton nmr spectroscopy can, very often, be applied to paramagnetic complexes, but the spectra are usually much more difficult to interpret than for diamagnetic complexes. The resolution of such spectra are largely dependent upon the metal involved and the coordination geometry about the metal$^{33}$. The line widths are dependent for the most part upon the relaxation time, $T_{1e}$, of the unpaired electron which in turn is dependent upon the coordination geometry about the copper atom. The shorter $T_{1e}$ the narrower the linewidths become and it is known that copper complexes with a square planar geometry have a longer $T_{1e}$ than complexes with a more tetrahedral coordination. Therefore $^1\text{H}$
spectra of Cu (R-sal)$_2$ complexes where R = t-Bu and the geometry is pseudotetrahedral, have narrower line widths than complexes where R is an unbranched alkyl group and the geometry square planar$^{32}$. This effect has been used to study the possibility of a structural equilibrium between square planar and pseudotetrahedral as proposed for the complex Cu (i-Pr-sal)$_2$.$^{32}$ For this complex it has been found that as the temperature is raised the proton line widths become narrower. This has been attributed to an increased population of the pseudotetrahedral species at high temperature which results in a shorter $T_1e$ and therefore, a narrower line width.

Copper (II) complexes in a square planar or pseudotetrahedral geometry are essentially a borderline case and although the proton resonances are very broad, some of them can be detected. In the case of the sugar complex Cu (Sug I-sal)$_2$ (Figure I-11) only the acetate protons of the sugar rings can be observed clearly, and the other very broad peaks cannot be easily assigned.

Cobalt (II) tetrahedral complexes have the required short $T_1e$ for the observation of well resolved nmr spectra but unfortunately, due to time limitations, the cobalt sugar complex [18] was not examined by nmr.

Nickel (II) complexes of this type have been studied extensively$^{33}$ by $^1$H nmr principally because the spectra of these complexes have narrow line widths and are very well resolved. This can be attributed to the fact that these nickel complexes exist as an equilibrium mixture of diamagnetic (square planar) and paramagnetic (tetrahedral) structures. However, even though sharp resonances can be observed in the spectra of salicylaldimine nickel (II) complexes, assignment is not trivial since the usual chemical shift ordering commonly known for diamagnetic compounds
Figure I-11. Proton nmr spectrum (270 MHz) of the copper sugar complex [16] in deuteriochloroform.
does not hold for these paramagnetic complexes.

The zinc (II) complexes, since they are diamagnetic, can be readily observed by nmr spectroscopy and the $^1$H nmr spectra of the zinc sugar complex Zn (Sug I-sal)$_2$ [17], along with the parent ligand [8] are shown in Figure 1-12. The spectrum of the zinc complex shows some free ligand impurity which could not be removed by recrystallization. It is difficult to say whether this arises from decomposition of the complex in solution, or whether it is intrinsically present in the sample. Since the C:H:N ratios are similar for both the ligand and complex, elemental analysis will not reveal a small percentage (< 10%) of the ligand impurity. The presence of the zinc atom disperses the aromatic region very nicely into two triplets and two doublets and also shifts most of the sugar ring protons to high field compared with the parent ligand. Apart from the observation of these shifts, nothing more can be concluded from the spectrum of the zinc complex.

ID: Other Chemistry

For salicylaldimine complexes in general, by far the most abundant and thoroughly studied are the nickel complexes. All attempts to form nickel complexes from the sugar ligands and nickel acetate, however, failed. Some of these attempted reactions are worth mentioning here, though.

Reaction of the sugar salicylaldimines [6], [8], [10], and [11] in the usual way with nickel (II) acetate in either methanol or ethanol failed to give any product and in all cases the starting sugar ligand could be recovered. If either the amino sugars [7] or [9] were reacted with the preformed salicylaldehyde complex, as shown below, the
Figure 1-12. Proton nmr spectrum (270 MHz) of the zinc complex [17] (A) and the ligand [8] (B).
salicylaldimine Schiff's bases [8] and [10] were isolated from the reaction mixture. Obviously the amino group is condensing with the carbonyl of the salicylaldehyde complex, as it should, but the nickel ion is being expelled, suggesting that these complexes are simply not stable. The analogous cyclohexyl nickel complex, however, can be synthesized readily either by reaction of the salicylaldimine with nickel acetate or by reaction of cyclohexylamine with the preformed salicylaldehyde-nickel complex. It was then reasoned that the sugar substituents might have a repelling effect on the nickel ion and therefore the complexing unit was moved further away from the sugar ring by means of a benzene sulphonamide spacer group, as shown in the following reaction scheme. However, reaction of the salicylaldimine [28] with nickel (II) acetate resulted in cleavage of the Schiff's base C = N double bond and formation of the salicylaldehyde nickel complex along with the free amine.
compound [27]. This cleavage could not be prevented even if sodium acetate was used to make the reaction slightly basic. Reaction of this ligand [28] with cupric acetate was not attempted.

IE: Electron Spin Resonance Spectroscopy of Copper (II)

(i) General Esr

Electron spin resonance spectroscopy occurs in a paramagnetic molecule when transitions between the Zeeman levels, whose degeneracy may be lifted by the application of a magnetic field H, are induced by an
electromagnetic field $H_1$ of frequency $\nu$. The separation of these Zeeman levels is described in (1)

$$E = h\nu = g\beta H$$  \hspace{1cm} (1)

where $h$ is Plank's constant, $\beta$ the Bohr magneton ($e\hbar/2m$), $m$ the mass of the electron, and $g$ the Landé splitting factor, a dimensionless parameter related to the effective magnetic moment of the electron by

$$\mu_e = -g\beta S$$ \hspace{1cm} (2)

where $S$ is the spin angular momentum vector. Differences in the Zeeman energy between different molecules result in changes in $g$ from its free electron spin-only value of 2.00232, as a result of spin-orbit coupling. Thus the $g$ value is used to characterize the position of the resonance in the frequency spectrum.

As shown in equation (1) the resonance frequency is dependent upon the applied field; most experiments, including the ones described herein, are conducted at X-band (about 9.5 GHz), which corresponds to an external field of the order of 2.5-3.5 KG. In the experiments described here, net absorption of microwave energy from $H_1$ occurs at resonance as a result of the greater proportion of spins present in the lower energy state.

(ii) Copper (II) Esr

This section has been adapted in part from the M.Sc. thesis written by Carl Alleyne and from reviews on transition metal esr.\(^{34}\)

For the $d^9$ configuration of copper (II) the effective electron $S = \frac{1}{2}$ and the spin angular momentum $m_S = \pm \frac{1}{2}$ gives rise to a doubly degenerate spin energy state, the degeneracy of which is removed when an external magnetic field is applied. The lower energy state has the spin aligned with the external field corresponding to $m_S = -\frac{1}{2}$, while the high
energy state, \( m_S = + \frac{1}{2} \), has its spin opposed to the field. A transition between the two states occurs upon absorption of microwave energy as given by (1)

\[ E = h\nu = g\beta H \]  

(1)

As will be seen for nitroxides later in section IIIE, the quantity \( g \) is dependent upon the effective orientation of the molecule containing the unpaired electron with respect to the magnetic field. If the copper ion is located in a perfectly cubic crystal site, the \( g \) value is independent of the orientation of the crystal and is said to be isotropic. In a crystal site of lower symmetry, both the \( g \) value and the splitting value \( A \) are orientation dependent and are said to be anisotropic. The \( z \)-direction is defined as coincident with the highest-fold rotation axis.

In axially symmetric systems the \( g \) and \( A \) values are given the notation

\[ g_{xx} = g_{yy} = g \]  

(3)

\[ g_{zz} = g' \]  

(4)

\[ A_{xx} = A_{yy} = A' \]  

(5)

\[ A_{zz} = A'' \]  

(6)

When the unpaired electron of the Cu (II) ion couples with the nuclear spin of \( I = 3/2 \) the absorption is split into \( 2I + 1 \) components. The cause of this "hyperfine" coupling interaction arising mainly from the Fermi contact term. Thus for \( I = 3/2 \) the esr spectrum (Figure I-13) consists of four lines. The selection rules are \( \Delta m_S = \pm 1 \) and \( \Delta m_I = 0 \).

For the special case where there are two copper ions in the same molecule which are exchange coupled, the energy level diagram is quite different (Figure I-14). Now there is a singlet \( S = 0 \) and a triplet \( S = 1 \) electronic state which are separated by an energy difference \( J \). The \( S = 1 \) state is split into three non degenerate \( m_S \) states \(+1, 0, -1\) when a
Figure I-13. Spin state energy level diagram for a copper (II) nuclei in a magnetic field.
Figure 1-14. Energy level diagram for two interacting copper (II) nuclei in a magnetic field.
magnetic field is applied. Although not shown, there are also super-imposed on these $m_S$ states, seven $m_I$ states resulting from the hyperfine interaction of the electron with the two $S = 3/2$ spins of the Cu (II) nuclei. Therefore, only electrons in the $S = 1$ state can give rise to a two line esr spectrum and this mole fraction is dependent upon the Botzeman distribution which is dependent upon the energy difference $J$ and the temperature. No other details will be given here about such binuclear spectra. Hence the room temperature spectrum of a mononuclear copper complex consists of four lines due to the copper nuclear hyperfine interaction. The band shapes are dependent upon the tumbling rate or correlation time $T_c$. If the molecule tumbles rapidly enough the anisotropies in $g$ and $A$ will be averaged, whereas if the rate is slow, line broadening will occur. Since the $g_\|$, low field $m_I = +3/2$ line must average with a distant $g_\perp$, high field $m_I = -3/2$ line (Figure I-15), this component therefore broadens fastest as the rate of tumbling decreases.

In a polycrystalline sample, or 77K frozen solution, the $g$ and $A$ anisotropies are not averaged at all. The molecular axes of symmetry are randomly distributed and the observed resonance line shape represents the superposition of the individual resonances. The derivative spectrum shows a weak set of lines at $g_\|$, corresponding to those molecules with their symmetry axes parallel to the applied field and a set of strong lines at $g_\perp$ corresponding to those molecules with the symmetry axes perpendicular to the applied field. This spectrum is depicted in the stick diagram of Figure I-15. This type of spectrum was seen in Figure I-7 where only three of the four $g_\|$, lines are seen and the $g_\perp$ lines are
Superhyperfine structure can sometimes be seen, as in Figure I-9, which result from the hyperfine interaction of the spin 1 nitrogen nucleus and the electron spin. The $2I + 1$ rule holds here again and therefore the number of superhyperfine lines depends upon the number of nitrogen donor atoms coordinated to the copper ion. Because the copper hyperfine lines are narrower at high field, the nitrogen superhyperfine lines are more clearly resolved on the high field side of the spectrum, although they can sometimes also be seen on the $g_{\|}$ components.

For Table I-1 the $A$ and $g$ values were abstracted directly from the field-corrected spectra. The $A_{\|}$ values were all taken as the separation between the $+\frac{1}{2}$ and $-\frac{1}{2}$ lines and the $a_0$ values were determined from
the $- \frac{1}{2}, - \frac{3}{2}$ separation. Both $g_n$ and $g_0$ were taken as the center point between the $+ \frac{1}{2}$ and $- \frac{1}{2}$ lines. The values of hyperfine constants obtained by this procedure are in units of magnetic field and can be converted into frequency units by the equation
\[
A(\text{cm}^{-1}) = A(\text{gauss}) \times \frac{9.3484 \times 10^{-5}}{g_e}
\]
If desired, $g_1$ and $A_1$ can be obtained from the equations
\[
g_0 = \frac{1}{3}(g_n = 2g_1); \quad a_0 = \frac{1}{3}(A_n + 2A_1)
\]
The esr spectra can also be used to calculate solution magnetic moments for these complexes. The following equations relate $\mu_{\text{eff}}$ and $g_0$
\[
\mu_{\text{eff}} = \mu_{S0}(1 - 2K^2 \lambda_0/10Dq)
\]
where $\mu_{S0}$ is the spin only magnetic moment, $K$ is the orbital reduction factor, and $\lambda_0$ is the spin orbit coupling constant which for the free ion equals $-829$ cm$^{-1}$.
\[
g = g_e(1 - 2K^2 \lambda_0/10Dq)
\]
\[
\mu_{\text{eff}} = g_0 \sqrt{S(S+1)}
\]
Using equation (11) and substituting $S = \frac{1}{2}$ for Cu (II) and the $g_0$ values obtained from the room temperature solution spectra, we can compare $\mu_{\text{eff}}$ calculated from esr data and $\mu_{\text{eff}}$ obtained from bulk magnetic susceptibility measurements as shown earlier in section IC(iii).

IF: Summary and Conclusions

Compounds [6], [8], and [10] have been known for over fifty years and [11] is a new Schiff's base sugar compound; all were prepared readily in high yield. It has been shown by $^1$H nmr spectroscopy that for compounds [8], [10], and [11] hydrogen bonding exists between the aromatic hydroxyl proton and the nitrogen atom from the sugar group.
Hydrogen bonding being a prerequisite for metal-chelation by these ligands, it was not unexpected that these compounds formed sugar metal complexes; however, this only occurred with certain metal ions. Ligand [8] formed copper, cobalt and zinc (II) complexes, whereas ligands [6], [10], and [11] only complexed with copper (II). None of these ligands formed complexes with nickel (II). The zinc and cobalt complexes of [8] were also found to be quite unstable in solution and even the copper complexes of [8] and [10] dissociated to the parent ligand when run on silica gel tlc. It is not understood why nickel complexes of the sugar ligands could not be prepared. Since the tetrahedral zinc and cobalt complexes [17] and [18] were less stable than the copper analogue [16], it could be reasoned that metals which prefer a tetrahedral geometry might not form complexes with these ligands. Nickel (II), however, can readily adopt either a square planar or tetrahedral coordination and therefore this argument does not provide an answer.

A variety of spectroscopic and physical techniques have been used to characterize the sugar-metal complexes synthesized. Visible absorption spectroscopy and electron spin resonance spectroscopy both provide structural information concerning coordination geometry as well as proof of existence. However, neither technique gave unequivocal information with regards to the exact coordination geometry of the copper (II) complexes [16] and [19]. When compared to a series of analogous alkyl complexes of known structure, visible absorption spectroscopy suggested that the copper sugar complexes [16] and [19] had essentially square planar geometry with compound [16] being slightly more distorted towards tetrahedral symmetry than was [19]. The solid mull spectra of [16], however, suggested a pseudotetrahedral structure for [16] in the solid.
It was obvious from the literature that esr spectroscopy was by far the most powerful physical technique which could be used to determine the structure of the copper complexes synthesized here. Thus it can indicate whether there is a copper ion present, how fast it is tumbling in solution from the relative line shapes and hence the approximate size of the molecule, the coordination geometry from $g$ and $A$ parameters, and whether the ligand contains a nitrogen donor atom from superhyperfine interactions. The esr data obtained in this study provide evidence, from $A$ values, of square planar geometry for [16] and [19], but $g$ values gave conflicting evidence for moderate tetrahedral distortion in these complexes. The esr powder spectrum of the copper-doped zinc complex gave the $g_\|$, $A_\|$, $g_\perp$, and $A_\perp$ values expected for a significant tetrahedral distortion. The $A_\|$, value changed from $168 \times 10^{-4}$ cm$^{-1}$ for the pure complex [16] to $129 \times 10^{-4}$ cm$^{-1}$ for the copper-doped zinc complex, while the $g_\|$, values changed from 2.246 to 2.258. The change in $g_\|$, was expected to be much larger than that found and therefore it is felt that changes in $A$ values may be a better criterion for comparing the coordination geometry between similar types of complexes. The zinc-dilution experiment also points out that the copper complex [16] could not have a large tetrahedral distortion in solution. The bulk of the data, therefore, suggests that the copper complexes [16] and [19] possess square planar geometries in solution, or have only a slight tetrahedral distortion.

Magnetic moments, mass spectra, and nmr spectra of the copper complexes are all consistent with the proposed structures. Mass spectrometry was found to be particularly useful in determining the structure of the binuclear complex [23] since the isotope patterns for mono- and binuclear copper complexes are easily distinguished and very diagnostic.

The water soluble glucosamine complex [21] seems to have a different
structure than the normal salicylaldimine copper complexes. Both mass spectrometry and elemental analysis suggest that this complex has only one sugar salicylaldimine unit with the third coordination site taken up by a hydroxyl group from the sugar ring and the fourth by a water molecule.

Clearly, from the standpoint of potential pharmaceutical applications, water soluble sugar complexes such as the one just described [21] are by far the most important. Future continuation of the work in this chapter might best involve attempts to synthesize more of these water soluble sugar complexes, employing a wide variety of sugars, ligand moieties, and metal ions.
References


5. German Patent No. 564437, 1932.


IIA: Introduction

Ferrocene [1] and its derivatives are finding an ever increasingly wide range of industrial and biochemical applications in areas ranging from the development of new iron-containing drugs to the formation of ferrocene-modified polymers and non-toxic "antiknock" fuel additives. Such derivatives are also of interest for a number of other reasons including the development of optically active catalysts of metal compounds for new immunoassay techniques of heavy metal probes for electron microscopy, of the synthesis of radiopharmaceuticals, and the modification of electrode surfaces. It might therefore be anticipated that these, and other, areas would be advanced in a variety of ways by the development of general routes to the synthesis of ferrocenyl-sugar derivatives. And it might also be anticipated that the combined presence
of the sugar and metal moieties, as suggested in chapter I, would give these compounds interesting biological properties. Prompted by this, and by the fact that carbohydrates can be either water soluble, or when suitably substituted, soluble in organic media, we have investigated the synthesis of sugar-ferrocene conjugates in which the sugar moiety is covalently attached to the cyclopentadienyl ligand of the ferrocene complex.

Undoubtedly the most important property of ferrocene is its exceptional aromaticity. Ferrocene undergoes a variety of typical ionic aromatic substitution reactions far more readily than benzene and only the mildest reaction conditions are required. Some of these reactions include Friedel-Crafts acylation, alkylation, metalation, sulfonation and aminomethylation as shown in Figure II-1. Ferrocene is stable to bases and non-oxidizing acids but is readily oxidized to the blue ferrocinium cation when treated with oxidizing agents such as nitric acid.

Ferrocene can be substituted on just one ring or on both and can possess more than one substituent per cyclopentadienyl ring. As well as the initial substitution, the products from these reactions can also undergo a variety of subsequent reactions to form an immense number of organometallic materials with a wide range of properties.

In this chapter, first the synthesis of sugar-ferrocenyl complexes will be described. A variety of carbohydrates containing suitable nucleophilic functionalities have been reacted with a number of ferrocene derivatives which are susceptible to nucleophilic attack. These reactions have resulted in several organic and aqueous soluble ferrocenyl-sugar conjugates.

Since all of these materials are new organometallic compounds they
Figure II-1. Some typical aromatic substitution reactions of ferrocene.
have been fully characterized, as described in the next section, by $^1$H NMR spectroscopy. In the first part of this section, the effects of a proximal chiral substituent on the proton resonances of the cyclopentadienyl ring will be examined along with a complete tabulation of all chemical shifts and coupling constants. Also, the spectra from some of the more interesting compounds will be discussed. This is followed by an account of how proton spin lattice relaxation measurements can be used to assign the proton resonances of the substituted cyclopentadienyl ring and to determine motional correlation times. During this section the reader unfamiliar with this technique, will be referred ahead to the final section where a more detailed explanation of the spin relaxation experiment is given.

IIB: Synthesis

A wide variety of ferrocene derivatives are susceptible to nucleophilic attack by the amino, hydroxyl or thiol functional groups of suitably blocked carbohydrate derivatives. All of the reactions used here to form ferrocenyl sugar conjugates, are of this type. The compounds formed were all crystalline (with exception of [20]) and were usually orange or red in color. This greatly facilitated their detection during thin layer chromatography (TLC) and as a result that technique provided a convenient method for following reactions; for the same reason it proved convenient to purify several compounds by silica-gel chromatography (see Experimental section).

It was already known that the ferrocenyl chlorides [2], [3]$^{11}$, react readily with a variety of nucleophilic reagents, including thiols, amines, and alcohols, in dry organic solvents with a suitable base acceptor and it was a simple matter to react [2] and [3] with a variety
of blocked sugars, each containing one of these three functional groups. Reaction of 2,3,4,6-tetra-O-acetyl-1-thio-β-ᴅ-glucopyranose [4][12], and 1,3,4,6-tetra-O-acetyl-2-amino-2-deoxy-β-ᴅ-glucopyranose [5][13] occurred readily at room temperature in dry chloroform with either pyridine or triethylamine, as the base acceptor, to form the mono and 1,1'-di-thioesters [6], [7], and amides [8], [9].

In like fashion, reaction of the mono ferrocenyl chloride with 1,2:5,6-di-O-isopropylidene-α-ᴅ-glucofuranose [10] in dry pyridine overnight afforded the mono substituted ester [11].
N,N-Dimethylaminomethylferrocene methiodide [12] also reacts with a variety of nucleophilic reagents by virtue of its benzyl-like structure and having the trimethyl amine moiety as a gaseous leaving group. These reactions however, require more vigorous conditions than those involving the acid chlorides. Thus, reaction with the 1-thio sugar [4] in boiling acetonitrile with anhydrous sodium carbonate was successful but a mixture of α and β anomers (40:60) were isolated (as determined by $^1$H nmr), probably because of mutarotation of the 1-thio sugar under the basic reaction conditions. Fortunately, several recrystallizations from ethanol afforded the pure β compound [13].
Reaction of the 2-amino sugar [5] with two equivalents of the methiodide reagent [12] under the same conditions yielded the N,N-bis amine compound [14]. However, this was not a good route to the secondary amine compound [15] since mixtures (as determined by tlc) of both [14] and [15] were always obtained even when a large excess of [5] was employed. All attempts to couple reagent [12] with sugar hydroxyl groups failed.

The ferrocenyl tosylate [16] was next evaluated as an alternative alkylating reagent to [12]. This compound does not appear to have been synthesized previously although its intermediacy was postulated in the formation of the ether [17] during reaction of the alcohol [18] with tosyl chloride in diethyl ether solution. This tosylate [16] proved to have the same high reactivity as other "benzylic" tosylates and as a result could not be prepared by the reaction of [18] with tosyl chloride in pyridine at ambient temperature. Instead it was prepared by the method of Kochi and Hammond where first the sodium alkoxide of [18] was prepared with sodium hydride in ether and then this reacted at low
temperature with tosyl chloride. The tosylate was then used in situ (see Experimental), without isolation. It should be noted here that the alcohol [18] can be readily prepared by reaction of the methiodide [12] with aqueous sodium hydroxide\(^1\).

Reaction of [16] with the 1-thio sugar [4] at ambient temperature for two days gave [13] which was easily isolated since it crystallized readily from ethanol. The yield of [13] was much higher than that for the reaction between [4] and [12] and only the \(\beta\)-anomer was formed. With 1,2:3,4-di-\(\beta\)-isopropylidene-\(\alpha\)-D-galactopyranose [19] reaction of [16] in an ether solution for two days at room temperature was also successful but the yield was much lower than that found for the thio sugar reaction. Since this product [20] was an oil, workup was more difficult and purification was performed on column chromatography.

![Chemical Structures](image)

Reaction of the tosylate with the 2-amino sugar [5] was also low-yielding and the solution had to be heated under reflux for two days in a chloroform/ether solution. The secondary amine product [15] was however, easily isolated since it could be extracted into dilute acid and then extracted back into ether upon neutralization; the starting 2-amino sugar was separated from the product since it was not soluble in ether.
Impressed by the ease with which 1,3,5-triazine derivatives react with carbohydrates (see chapter III) we also evaluated this approach to the synthesis of ferrocenyl-sugar conjugates. Reaction of hydroxymethyl-ferrocene [18] with cyanuric chloride [21] in basic aqueous acetone afforded the ferrocenyl triazine dichloride [22]; although the ether [17] was also formed as a byproduct it could be readily removed by filtration prior to crystallization of [22]. The dichloride [22] was somewhat unstable but could be stored for several weeks in an evacuated desiccator and was suitable for further reactions without purification. Reaction of [22] with two equivalents of the 1-thio glucose sugar [4] occurred readily in acetonitrile with triethylamine to yield the ferrocenyl sugar compound [23]. This reaction will be discussed again in chapter III.
Two approaches were considered for the formation of water soluble ferrocene-sugar conjugates: selective reaction of an unblocked sugar and deblocking of the above described, blocked derivatives.

It is well known that amino sugars form Schiff's bases with aromatic aldehydes\(^{19}\), and reaction of ferrocene carboxaldehyde [24] in ethanol with glucosamine hydrochloride [25] in the presence of triethylamine gave, as expected, the Schiff's base [26] in 70% yield. This reaction should find substantial generality. Unfortunately an initial attempt to stabilize the Schiff's base by reductive amination with sodium cyanoborohydride was unsuccessful; tlc of the reaction mixture indicated the formation of a mixture of compounds. It should be noted here that the aldehyde [24] can be prepared from the methiodide [12]\(^{20}\).

Attempts to deblock the substituted sugar conjugates were very disappointing since only two reactions gave acceptable yields. Thus
de-O-acetylation of compounds [6] and [13] was successfully accomplished using sodium methoxide in methanol\textsuperscript{21}. Compound [13] was cleanly deacetylated and the product [27] crystallized readily from water as the monohydrate. Compound [6], as expected under these conditions, was partially cleaved at the thio-ester linkage but the main product was the unblocked ferrocenyl derivative [28]; the product was easily purified by column chromatography and the methyl ferrocenecarboxylate was also isolated, and subsequently identified by \textsuperscript{1}H nmr. Deacetylation of the 1,1'-di-thio ester [7] proved to be very complex (tlc) and was not pursued further. More surprisingly, deacetylation of both the amides [8] and [9] with sodium methoxide in methanol was complex and no pure product could be isolated. Deacetylation of the amines [14] and [15] also proved disappointing; in each case two compounds were detected by tlc, which had very similar $R_f$ values and attempts to purify these compounds by preparative tlc or by column chromatography failed. The $s$-triazine derivative [23] was also exposed to these deacetylation conditions but tlc showed the major product to be colorless (detection by $H_2SO_4$ charring),
which suggests cleavage of either the thio sugar or ferrocene moieties from the triazine ring.

Removal of the isopropylidene blocking groups from [20] was also unsuccessful. The standard procedure of heating in dilute sulfuric acid could not be used since ferrocene compounds are readily oxidized under these conditions. Heating under reflux in aqueous methanol using IR 120 H\textsuperscript{+} resin was tried but after 1 h the solution turned colorless and a colorless product was detected on tlc having the same R\textsubscript{f} value as the diacetone galactose precursor [19], suggesting that cleavage of the ether linkage was taking place. The resin had turned green also and it was thought that oxidation was occurring under these conditions as well.

IIC: Proton Nuclear Magnetic Resonance Spectra

(1) Chemical Shifts and Coupling Constants

The \textsuperscript{1}H chemical shifts and coupling constants for the ferrocenyl sugar derivatives [6], [7], [8], [9], [11], [13], [14], [15], [20], [23], [26], [27], [28] are summarized in Table II-1.

It is interesting to observe that a proximal chiral group renders all four protons of the substituted cyclopentadienyl ring inequivalent, as previously observed by Kursanov\textsuperscript{22} for cymantrene systems. The chiral group, in our case the sugar, renders the \( \alpha\), \( \alpha'^{\prime} \) and \( \beta\), \( \beta'^{\prime} \) protons
TABLE 1. Chemical Shifts (ppm) and multiplet splittings (Hz) for the ferrocenyl monosaccharide compounds

<p>| Compound | (α+α') | (β+β') | Cp  | H-1 | H-2 | H-3 | H-4 | H-5 | H-6 | H-6' | OAc  | -CH₂ |
|----------|---------|---------|-----|-----|-----|-----|-----|-----|-----|-----|------|------|------|
| 6        | 4.77    | 3.99    | 3.97| 5.72| 5.54| 5.48| 5.32| 3.34| 4.22| 4.0  | 1.70 |      |
|          | 4.68    |         |     | 5.16 | 10.4 | 10.4 | 10.0 | 6.4 | 3.34 | 4.0  | 1.69 |      |
|          |         |         |     |      |       | 10.4 | 10.0 | 6.4 | 3.34 | 4.0  | 1.68 |      |
|          |         |         |     |      |       | 10.4 | 10.0 | 6.4 | 3.34 | 4.0  | 1.65 |      |
| 7        | 4.76    | 4.11    | 4.61| 5.70 | 5.53 | 5.35 | 3.50 | 4.29 | 4.05 | 1.79 |      |
|          |         |         |     | 5.16 | 10.5 | 10.5 | 10.3 | 6.4 | 3.34 | 4.0  | 1.71 |      |
|          |         |         |     | 5.16 | 10.5 | 10.5 | 10.3 | 6.4 | 3.34 | 4.0  | 1.69 |      |
|          |         |         |     | 5.16 | 10.5 | 10.5 | 10.3 | 6.4 | 3.34 | 4.0  | 1.65 |      |
| 8        | 4.71    | 4.01    | 4.68| 5.90 | 4.76 | 5.42 | 5.35 | 3.64 | 4.31 | 4.13 | 1.73 |      |
|          |         |         |     | 5.16 | 10.0 | 10.0 | 10.0 | 6.4 | 3.34 | 4.0  | 1.70 |      |
|          |         |         |     | 5.16 | 10.0 | 10.0 | 10.0 | 6.4 | 3.34 | 4.0  | 1.67 |      |
|          |         |         |     | 5.16 | 10.0 | 10.0 | 10.0 | 6.4 | 3.34 | 4.0  | 1.61 |      |
| 9        | 4.94    | 4.3     | 4.68| 5.99 | 4.48 | 5.43 | 5.23 | 3.94 | 4.35 | 4.16 | 2.34 |      |
|          |         |         |     | 5.16 | 10.0 | 10.0 | 10.0 | 6.4 | 3.34 | 4.0  | 2.14 |      |
|          |         |         |     | 5.16 | 10.0 | 10.0 | 10.0 | 6.4 | 3.34 | 4.0  | 2.12 |      |
|          |         |         |     | 5.16 | 10.0 | 10.0 | 10.0 | 6.4 | 3.34 | 4.0  | 2.10 |      |
|          |         |         |     | 5.16 | 10.0 | 10.0 | 10.0 | 6.4 | 3.34 | 4.0  | 2.09 |      |</p>
<table>
<thead>
<tr>
<th>Compound</th>
<th>(α,α')</th>
<th>(β,β')</th>
<th>Cp</th>
<th>H-1</th>
<th>H-2</th>
<th>H-3</th>
<th>H-4</th>
<th>H-5</th>
<th>H-6</th>
<th>H-6'</th>
<th>OAc</th>
<th>-CH₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>11 b f</td>
<td>4.97</td>
<td>4.07</td>
<td>4.10</td>
<td>5.73</td>
<td>4.36</td>
<td>5.78</td>
<td>4.51</td>
<td>4.51</td>
<td>4.11</td>
<td>4.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.72</td>
<td></td>
<td></td>
<td>J₁₂</td>
<td>3.6</td>
<td>J₃₄</td>
<td>2.0</td>
<td>J₅₆</td>
<td>2.3</td>
<td>J₇₈</td>
<td>3.3</td>
<td>J₉₁₀</td>
</tr>
<tr>
<td></td>
<td>4.15</td>
<td>3.93</td>
<td>3.99</td>
<td>5.37</td>
<td>4.23</td>
<td>5.37</td>
<td>5.25</td>
<td>3.24</td>
<td>4.25</td>
<td>4.06</td>
<td>1.73</td>
<td>3.72</td>
</tr>
<tr>
<td></td>
<td>4.09</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.69</td>
<td></td>
</tr>
<tr>
<td>11 b f</td>
<td>4.23</td>
<td>3.96</td>
<td>4.03</td>
<td>5.99</td>
<td>3.34</td>
<td>5.59</td>
<td>5.26</td>
<td>3.25</td>
<td>4.28</td>
<td>4.0</td>
<td>1.80</td>
<td>3.90</td>
</tr>
<tr>
<td></td>
<td>4.16</td>
<td></td>
<td></td>
<td>J₁₂</td>
<td>8.9</td>
<td>J₃₄</td>
<td>10.6</td>
<td>J₅₆</td>
<td>9.1</td>
<td>J₇₈</td>
<td>10.0</td>
<td>J₉₁₀</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>J₁₂</td>
<td>8.9</td>
<td>J₃₄</td>
<td>10.6</td>
<td>J₅₆</td>
<td>9.1</td>
<td>J₇₈</td>
<td>10.0</td>
<td>J₉₁₀</td>
</tr>
<tr>
<td>11 b f</td>
<td>4.11</td>
<td>3.93</td>
<td>4.02</td>
<td>5.72</td>
<td>2.98</td>
<td>5.20</td>
<td>5.32</td>
<td>3.32</td>
<td>4.31</td>
<td>4.01</td>
<td>1.75</td>
<td>3.64</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>J₁₂</td>
<td>8.5</td>
<td>J₃₄</td>
<td>10.0</td>
<td>J₅₆</td>
<td>9.3</td>
<td>J₇₈</td>
<td>12.4</td>
<td>J₉₁₀</td>
</tr>
<tr>
<td>20 b g</td>
<td>3.98</td>
<td>5.55</td>
<td>4.17</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.51</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>J₁₂</td>
<td>5.0</td>
<td>J₁₂</td>
<td>2.3</td>
<td>J₇₈</td>
<td>2.3</td>
<td>J₉₁₀</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>Compound</td>
<td>(α⁺,α'⁻)</td>
<td>(β⁺,β'⁻)</td>
<td>Cp (Unsub)</td>
<td>H-1</td>
<td>H-2</td>
<td>H-3</td>
<td>H-4</td>
<td>H-5</td>
<td>H-6</td>
<td>H-6'</td>
<td>OAc</td>
<td>-CH₂</td>
</tr>
<tr>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>-----------</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>23³ h</td>
<td>4.59</td>
<td>3.67</td>
<td>3.92</td>
<td>H-1₆ 5.83</td>
<td>H-2₆ 5.64</td>
<td>H-3₆ 5.34</td>
<td>H-4₆ 5.26</td>
<td>H-5₆ 3.26</td>
<td>4.16-4.18</td>
<td>4.03-4.08</td>
<td>1.86</td>
<td>4.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>J₆,₆₄ 10.5</td>
<td>J₆,₆₄ 10.2</td>
<td>J₆,₆₄ 5.2</td>
<td>J₆,₆₄ 10.2</td>
<td>J₆,₆₄ 5.2</td>
<td>J₆,₆₄ 10.2</td>
<td>J₆,₆₄ 5.2</td>
<td>J₆,₆₄ 10.2</td>
<td>J₆,₆₄ 5.2</td>
</tr>
<tr>
<td></td>
<td>4.33</td>
<td></td>
<td></td>
<td>H-1₇ 5.44</td>
<td>H-2₇ 5.34</td>
<td>H-3₇ 5.34</td>
<td>H-4₇ 5.16</td>
<td>H-5₇ 3.34</td>
<td>2.03</td>
<td>4.03-4.08</td>
<td>1.68</td>
<td>4.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>J₇,₇₄ 10.5</td>
<td>J₇,₇₄ 0.1</td>
<td>J₇,₇₄ 4.2</td>
<td>J₇,₇₄ 10.5</td>
<td>J₇,₇₄ 0.1</td>
<td>J₇,₇₄ 4.2</td>
<td>J₇,₇₄ 10.5</td>
<td>J₇,₇₄ 0.1</td>
<td>J₇,₇₄ 4.2</td>
</tr>
<tr>
<td>23³ h</td>
<td>4.67</td>
<td>4.26</td>
<td>4.18</td>
<td>H-1₈ 5.86</td>
<td>H-2₈ 5.25</td>
<td>H-3₈ 5.54</td>
<td>H-4₈ 5.25</td>
<td>2.03</td>
<td>5.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>J₈,₈₄ 10.6</td>
<td>J₈,₈₄ 9.3</td>
<td>J₈,₈₄ 9.3</td>
<td>J₈,₈₄ 9.3</td>
<td>2.03</td>
<td>5.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.40</td>
<td></td>
<td></td>
<td>H-1₉ 5.76</td>
<td>H-2₉ 5.11</td>
<td>H-3₉ 5.47</td>
<td>H-4₉ 5.08</td>
<td>2.02</td>
<td>4.81</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>J₉,₉₄ 10.6</td>
<td>J₉,₉₄ 9.4</td>
<td>J₉,₉₄ 9.4</td>
<td>J₉,₉₄ 9.4</td>
<td>2.02</td>
<td>4.81</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 k</td>
<td>4.27</td>
<td>4.27</td>
<td>2.88</td>
<td>3.61</td>
<td>3.70</td>
<td>3.91</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>J₁,₂ 10.2</td>
<td>J₁,₃ 9.1</td>
<td>J₁,₄ 9.2</td>
<td>J₁,₅ 5.2</td>
<td>J₁,₆ 11.6</td>
<td>J₁,₆' 1.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>4.27</td>
<td>4.12</td>
<td>4.17</td>
<td>4.37</td>
<td>3.27</td>
<td>3.4</td>
<td>3.4</td>
<td>3.4</td>
<td>3.4</td>
<td>3.69</td>
<td>3.89</td>
<td>3.84</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>J₁,₂ 9.6</td>
<td>J₁,₃ 9.4</td>
<td>J₁,₄ 9.4</td>
<td>J₁,₅ 3.6</td>
<td>J₁,₆ 12.2</td>
<td>J₁,₆' 2.2</td>
<td>J₁,₆' 2.2</td>
<td>J₁,₆' 2.2</td>
<td></td>
</tr>
<tr>
<td>27³</td>
<td></td>
<td></td>
<td></td>
<td>J₁,₂ 9.6</td>
<td>J₁,₃ 9.4</td>
<td>J₁,₄ 9.4</td>
<td>J₁,₅ 3.6</td>
<td>J₁,₆ 12.2</td>
<td>J₁,₆' 2.2</td>
<td>J₁,₆' 2.2</td>
<td>J₁,₆' 2.2</td>
<td>J₁,₆' 2.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>J₁,₁ 9.6</td>
<td>J₁,₃ 9.4</td>
<td>J₁,₄ 9.4</td>
<td>J₁,₅ 3.6</td>
<td>J₁,₆ 12.2</td>
<td>J₁,₆' 2.2</td>
<td>J₁,₆' 2.2</td>
<td>J₁,₆' 2.2</td>
<td>J₁,₆' 2.2</td>
</tr>
</tbody>
</table>

TABLE 1. Continued
TABLE 1. Continued

<table>
<thead>
<tr>
<th>Compound</th>
<th>((\alpha,\alpha'))</th>
<th>((\beta,\beta'))</th>
<th>Cp (Unsub)</th>
<th>H-1</th>
<th>H-2</th>
<th>H-3</th>
<th>H-4</th>
<th>H-5</th>
<th>H-6</th>
<th>H-6'</th>
<th>OAc</th>
<th>(-\text{CH}_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>28</td>
<td>4.83</td>
<td>4.49</td>
<td>4.34</td>
<td>4.76</td>
<td>4.87</td>
<td>3.69</td>
<td>3.52</td>
<td>3.46</td>
<td>3.70</td>
<td>3.89</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>J_{3,2} 9.8</td>
<td>J_{2,3} 9.0</td>
<td>J_{3,4} 9.7</td>
<td>J_{4,5} 5.7</td>
<td>J_{5,6} 12.0</td>
<td>J_{6,6'} 2.2</td>
<td></td>
</tr>
</tbody>
</table>

\(a\) assignments were made by comparison to 6 and 7 (22); \(a\) and \(\beta\) resonances are assigned to high field, of their \(\alpha\) and \(\beta\) counterparts.

\(b\) Deuteriobenzene solution.

\(c\) \(\text{H-C-} 5.89, J_{\text{NH, H}_2} = 9.1\) Hz.

\(d\) Deuteriochloroform solution.

\(e\) \(\text{H-C-} 6.90, J_{\text{NH, H}_2} = 9.3\) Hz.

\(f\) \(\text{o, Me 1.39, 1.32, 1.23, 1.04}\)

\(g\) \(\text{o, Me 1.50, 1.42, 1.16, 1.04}\)

\(h\) Since there are two inequivalent sugar rings the notation \(H-1_x H-1_y\) etc. is used.
diastereoptopic and potentially four separate resonances can be observed. This turned out to be the case in the spectrum of compound [7] (Figure II-2), but more usually only the α protons are separated and the β protons remain overlapping as in the spectra of compounds [6] and [8] in Figure II-3 and Figure II-4 respectively. It should be noted that $^{13}$C chemical shifts are more sensitive to this effect.

The spectrum of compound [7] in Figure II-2 reveals the inequivalence of the four cyclopentadieny1 (Cp) ring protons and $^1$H-$^1$H spin decoupling can readily be used, as shown, to assign which protons are on the same side of the ring, that is the pairing of α with β and α' with β'. This spectrum also shows a fairly normal pattern for the sugar ring protons except that the overlap of resonances H$_2$ and H$_3$ result in some second-order effects, observed in the form of "extra" transitions in addition to those expected on a first-order basis.

The spectra of compounds [6] and [8] in Figures II-3, 4, as was stated, both show the more usual pattern of the substituted Cp ring protons, where the α and α' resonances are separated, and the β and β' resonances are overlapping. The unsubstituted Cp ring appears as a sharp singlet at about 4 ppm. The resonances of the sugar ring protons in the spectrum of [8] fit the normal pattern whereas in the spectrum of [7], "virtual coupling" is again evident. The decoupling experiment in Figure II-3 for compound [6] reveals the very small α-α' coupling when the β resonances are irradiated. It should be pointed out here that the beautiful dispersion of all four Cp ring protons in the spectrum of [7] was only obtained in Deuteriobenzene; in contrast, in other solvents such as CDCl$_3$ the β protons were overlapping. While the spectra of most of the compounds in Table II-1 illustrate nothing new from what has just
Figure II-2. Proton nmr spectra (270 MHz) of compound [7] in deuterio-benzene. The upper trace is a decoupling experiment used to assign \( \alpha, \alpha' \) and \( \beta, \beta' \) cyclopentadienyl resonances.
Figure II-3. Proton nmr spectra (270 MHz) of compound [6] in deuteriobenzene. The upper trace is a decoupling experiment showing the $\alpha-\alpha'$ coupling ($\sim 1$ Hz), when $\beta, \beta'$ is irradiated.
Figure II-4. Proton nmr spectrum (270 MHz) of compound [8] in deuteriobenzene.
been discussed, the $^1$H spectrum in Figure II-5 of compound [23] is, however, very interesting indeed and merits separate discussion. The proton resonances of the two sugar rings in this compound are non equivalent in the $^1$H nmr spectrum which implies that the molecule must favour a preferred set of conformations. Although there exists no formal symmetry elements in this molecule, it was argued initially that a pseudo-\(C_2\) rotation axis, as shown below, could be generated if there were free

![Diagram](image)

rotation (fast on the nmr time scale) about the bonds joining the substituents to the central triazine ring. However, the observed non equivalences imply that steric hindrance prevents complete rotation about some of these bonds. In the insert in Figure II-5, of the acetate region, all eight acetate groups are resolved. Since the separation between two acetate resonances, common to both sugar rings, could not be more than 15 Hz, it would therefore be necessary for the bond to rotate slower than \(2\pi \times 15\) revolutions per second in order for separate resonances to be observed. This, however, would correspond to an unrealistically high barrier to rotation; it follows that our original argument is untenable. The following explanation appears to be preferable. It seems likely that the molecule favours a conformation in which the ferrocene moiety is oriented on one side of the triazine ring as shown in the following diagram. This would allow a rapid "cranking" motion about the bonds
Figure II-5. Proton nmr spectra (270 MHz) of compound [23] in deuterioacetone. The top left insert shows the $H_{5x}$ and $H_{5y}$ resonances of [23] in deuteriobenzene and the top right insert shows the eight acetate resonances of [23] in deuterioacetone.
joining the ferrocene group to the triazine moiety which would be reasonable since this motion sweeps out only a small volume. It is then assumed that the bond rotations which would allow the ferrocene unit to tip over to the underside of the ring must be very slow. This seems also to be reasonable since a large inertial moment must be overcome in order for the bulky ferrocene group to make a 360° sweep through solvent molecules; an "absence" of that motion eliminates any symmetry for the molecule. It can then be seen from molecular models that the preferred conformation for the sugar units places one sugar unit above and one below the plane of the triazine ring as shown in the above diagram. These two effects combined would then bring one sugar group much closer to the aromatic ring current of the ferrocene system than the other sugar group and result in a chemical shift difference between resonances of the two sugar moieties. This argument is supported by the spectrum in Figure II-5, since the sugar ring resonances $H_1$-$H_4$ for one sugar group,
are all shifted in one direction with respect to the equivalent resonances of the other sugar group. That is, $H_{1x}$ is to low field of $H_{1y}$, $H_{2x}$ is to low field of $H_{2y}$, etc. The spectrum of this compound should be compared to the similar spin label compound ([36] chapter IIID).

Both sugar rings appear equivalent in this compound because the nitroxide unit is much less bulky than the ferrocene moiety, allowing it to rotate more freely, and also it does not impart the same, large chemical shift perturbation to the system as does the aromatic ferrocene group.

An interesting feature of the deuteriobenzene spectrum of [23] is that the $H_5$ resonances, shown in the insert of Figure II-5, of the two sugar rings, exhibit quite different coupling patterns. The $H_{5y}$ resonance has a much larger $J_{5y,6y} = 5.2$ Hz than does the more usual value for $H_{5x}$ of $J_{5x,6x} = 4.2$ Hz. This suggests that the bond joining $C_{5y}$ and $C_{6y}$, as shown below, is favouring preferred conformations, which

![Diagram](image)

reinforces the previous suggestion that this molecule is sterically crowded.

(ii) Proton Spin Lattice Relaxation Rates

The $^1$H nmr spectrum of an achiral, monosubstituted ferrocene derivative invariably shows two multiplets, each from a pair of equivalent protons \{a,a' and b,b'\}, together with a sharp singlet for the five equivalent protons of the unsubstituted ring. When both rings are
substituted by the same achiral substituent, only two resonances are observed, both rings being essentially identical. And as mentioned in the last section for chiral, mono or 1,1'-di-substituted derivatives, all four proton resonances of these rings are intrinsically inequivalent. Although in some cases a reasonable assignment of such resonances can be inferred by inspection, for many systems not even a tentative assignment can be made; it will now be shown that proton spin lattice relaxation rates (R$_1$-values) provide direct evidence for unequivocal assignments. As an added bonus those same data automatically provide information concerning the relative mobilities of the substituted and unsubstituted cyclopentadienyl rings$^{24}$. Although to our knowledge, no results have previously been reported for organometallic π-complexes, an ample body of data exists for organic molecules which shows that the spin lattice relaxation of the protons of most diamagnetic organic molecules is dominated, generally exclusively, by the dipole-dipole mechanism$^{25}$, which has the general form

$$R_1(D,R) = \frac{\gamma_D^2 \gamma_R^2}{r^6(D\rightarrow R)} \cdot \tau_c(D\rightarrow R)$$

where $R_1(D,R)$ is the specific relaxation contribution between a donor nucleus (D) and a receptor nucleus (R), $\gamma_D$ and $\gamma_R$ are the gyromagnetic ratios of those two nuclides, $r(D\rightarrow R)$ is the distance separating them and $\tau_c(D\rightarrow R)$ is the motional correlation time of the vector joining D and R. For most diamagnetic molecules protons are the only nuclear species with a high gyromagnetic ratio and for that reason the relaxation of each proton generally occurs via the other protons of the system; working at high dilution in a solvent which contains no protons ensures that intramolecular proton relaxation is dominant. Under these circumstances it
is possible to use the experimentally determined values of $R_1(D,R)$ to measure either the relative interproton distances or the relative rates of motion of the various proton-containing moieties.

In the context of ferrocene chemistry, two useful items of information can be obtained from a simple, qualitative interpretation of proton $R_1$ values. Because protons which are $\alpha$ to a substituent have only one neighbouring proton whereas those which are $\beta$ have two, the latter will be characterized by their larger $R_1$ values. And, if it is assumed that all C-H bond lengths and bond-angles are identical, intercomparison of the $R_1$ values can show which cyclopentadienyl ring is rotating about the Cp-Fe-Cp axis more rapidly. For brevity we shall illustrate these points here using the mono- and bis-substituted sugar derivatives [6] and [7].

$$\begin{align*}
[6] & : R = -C - SGlc, R' = H \\
[7] & : R = R' = -C - SGlc \\
HSGlc & = \begin{array}{c}
\text{OAc} \\
\text{OAc} \\
\text{SH}
\end{array}
\end{align*}$$

The 270 MHz proton resonance spectra of compounds [6] and [7] are shown in Figure II-6 and the relevant proton $R_1$ values are summarized in Table II-2. It is clear that although the $R_1$-values of the resonances at 4.77 ppm and 4.68 ppm of [6] are closely similar, both these are relaxing at approximately half the rate of the resonances at 3.99 ppm. Clearly the latter resonances must correspond to $\beta$, $\beta''$, their enhanced
Figure II-6. Partial proton nmr spectra of (A) compound [6] and (B) compound [7], in deuteriobenzene.
TABLE II-2. Proton spin lattice relaxation rates $R_1$ sec$^{-1}$

<table>
<thead>
<tr>
<th>Cp</th>
<th>$\alpha, \alpha'$</th>
<th>$\beta, \beta'$ ( unsub)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.258</td>
<td>0.426</td>
</tr>
<tr>
<td></td>
<td>0.280</td>
<td></td>
</tr>
<tr>
<td>[6] R=-C-SGlc, R'=H</td>
<td>0.483</td>
<td>0.84*</td>
</tr>
</tbody>
</table>

*Due to an accidental overlap with a sugar resonance this rate was estimated from null point determinations.

$R_1$-values being ascribed to the fact that they each have two neighbouring protons. A similar relationship pertains for [7], the interpretation being only marginally complicated by the accidental overlap between the $\beta$ resonance and one of the protons of the sugar ring.

Assignment of which protons are on the same side of the cyclopentadienyl ring, that is the pairing of $\alpha$ with $\beta$ and $\alpha'$ with $\beta'$ in II, is trivially accomplished by homonuclear decoupling as was shown in Figure II-2. Unfortunately, it is not easy to ultimately assign each resonance to a particular proton and we can think of no simple method whereby this can be accomplished.

Although not directly related to the main thrust of the work presented thus far in this section, I would like to illustrate to the reader not familiar with this technique, the conformational information$^{25}$ obtainable from relaxation rates by examining the $R_1$-values for protons of the appended sugar ring in (6) (Figure II-7). As shown earlier in equation [1], the relaxation rate is dependent upon the distance between protons and because
Figure II-7. Conformational structure and proton R$_1$-values for the appended sugar moiety in compound [6].

of the r$^{-6}$ dependence the relaxation contributions drop off sharply as the distance is increased. Due to this, the vicinal trans-diaxial interactions, present between neighbouring protons in this particular sugar ring, are small and contribute little to the R$_1$-values of the ring protons. The R$_1$-value for H$_6$ is the largest observed because there is very efficient relaxation between the closely spaced geminal protons and there is a large contribution from H$_5$ as well. The R$_1$-value of H$_5$ is the next largest since it gets relaxation contributions from both H$_6$ protons and the two syn-diaxial protons H$_1$ and H$_3$. The R$_1$-values for protons H$_4$ and H$_1$ provide an interesting comparison. Since H$_1$ has 1,3-diaxial interactions with both H$_3$ and H$_5$, it relaxes more rapidly than H$_4$ which has only the diaxial interaction with H$_2$. As expected, the R$_1$-value of H$_2$ is the smallest since it gets relaxation only from H$_4$ while H$_4$ gets some
additional relaxation from the H₆ protons. The rate for H₃ seems a little low since it has two 1,3-diaxial interactions with H₅ and H₁ but it is still within reason and, since there may well be a larger systematic error in the R₁-values of H₂ and H₃ because they are partially overlapping and strongly coupled²⁶, it is not worth discussing this point further. It should be clear from the above that conformational information can be readily obtained via the relaxation technique for ferrocene and other classes of organometallic compounds as well.

Information concerning the relative rates of spinning motion of the substituted and unsubstituted cyclopentadienyl rings about the Cp-Fe-Cp axis can be inferred directly from the proton R₁-values. Simply, the observation that the β protons of the substituted ring relax faster than the protons of the unsubstituted ring immediately implies that the latter is rotating faster than the former. A value for this rate difference can be approximated in the following way. Using equation (2) the relaxation

\[ R₁(R) = \frac{3}{2} \sum \rho(D,R) \]  

contribution \( \rho(D,R) \) of the donor nucleus to the receptor, can be calculated from the observed R₁-value of the receptor nucleus. After first calculating \( \rho(α,β) = 0.18 \) this can then be used to calculate a value for \( \rho(β,β) = 0.11 \); and \( \rho(H,H) = 0.62 \) can be calculated separately for the unsubstituted cyclopentadienyl ring. From the relationship

\[ \rho(D,R) = \frac{4/3 I_R(I_R+1)}{8\gamma^2h^2} \frac{\tau^2 \tau^2}{r^6(D+R)} \]  

where I is the nuclear spin and other terms are as for (1), one can calculate the motional correlation time for the two interproton vectors \( α+β(\tau_c = 1.22×10^{-10} \text{ sec-rad.}) \) and \( β+β(\tau_c = 0.71×10^{-10} \text{ sec-rad.}) \), using a
value of the interproton distance \( r = 2.7\,\text{Å} \); this distance need not be accurate since only relative rates are being calculated. An average value for the correlation time of the interproton vectors for the unsubstituted ring is calculated from \( \rho(\text{H,H}) \) to be \( 0.42 \times 10^{-10}\,\text{sec/rad} \). By comparing the average value of \( \tau_c(\alpha\rightarrow\beta) \) and \( \tau_c(\beta\rightarrow\beta) \) for the substituted ring with the average \( \tau_c \) value obtained for the unsubstituted ring it is clear that the unsubstituted ring is rotating about the Cp-Fe-Cp axis approximately 2.3 times more rapidly than the substituted ring. In this calculation it has been assumed that other molecular motions of the molecules contributing to the relaxation rates of these ring protons are approximately the same for both rings, and therefore effectively cancel, leaving the difference in these rates affected mainly by their rotational rates about the Cp-Fe-Cp axis. This rate difference is intuitively reasonable and the differential can be ascribed to the increased size and inertial moment of the substituted ring. It is also worth noting that a two-factor differential exists between the \( R_1 \)-values for the \( \alpha \) protons of [6] and [7] and for the \( \beta \) counterparts. Once again, the sense of this differential indicates that the larger molecule [7] is tumbling more slowly overall than its smaller counterpart [6]; this probably reflects the increased drag associated with the second sugar substituent. The relaxation rates obtained for Table II-2 were also checked by roughly estimating the rates from the null points of individual resonances in the partially relaxed spectra using the relationship

\[
R_1 = \frac{0.69}{t}
\]

where \( t \) is a short delay after the 180° pulse to allow the nuclear spin to partially relax.
Two concluding statements seem to be appropriate. First, that the proton $R_1$ values of many different classes of organometallic substances are likely to be amenable to the same simple, useful interpretations as those given here. Second, that since it is possible to extend the relaxation experiment to a quantitative measurement of interproton distances with an accuracy which under favourable conditions can approach that of a neutron diffraction study, use of this technique to identify the positions of the hydride substituents of certain metal-hydrides clearly merits attention.

IID: Proton Spin-Lattice Relaxation

The following section has been adapted in part from a Ph.D. thesis by Dr. K. F. Wong, and several recent reviews on the subject. Conventionally, three sets of nmr parameters: chemical shifts, coupling constants, and integrated areas, are used as the basis for structural assignment. This, however, neglects two further sets of magnetic resonance parameters; these are spin-lattice relaxation rates ($R_1$-values) and the spin-spin relaxation rates. Recent instrumental developments have made possible the routine measurement of spin-lattice relaxation rates, and this technique is now finding many applications in organic and inorganic chemistry.

Studies of spin-lattice relaxation are basically concerned with the way in which, and the rate at which, magnetic energy is transferred between the magnetic nuclei under study (the "spins") and their surrounding environment (the "lattice"). This energy is transferred by interactions between a rapidly fluctuating magnetic field, generated and located in the lattice, and the rapidly processing nuclei of interest.
There are a number of distinct mechanisms whereby this energy transfer can be effected; fortunately, however, it is often possible to conduct experiments in which one of these mechanisms, the intramolecular dipole-dipole mechanism, dominates the relaxation. In practice, this requires that one be interested in molecules which are moving more or less isotropically in solution. Furthermore, these molecules should be studied as a dilute solution (ca. < 0.1 M) in a magnetically inert solvent, that is, a solvent that contains neither fluorine nor proton atoms; for example, a deuterated organic solvent.

The mathematical form of the intramolecular dipole-dipole mechanism was shown previously in IIC(ii) and is given here again in abbreviated form in (1)

\[ R_1(D,R) \propto \frac{\gamma_D^2 \gamma_R^2}{r^6(D\rightarrow R)} \cdot \tau_c(D\rightarrow R) \] (1)

where \( R_1(D,R) \) is the specific relaxation contribution between a donor nucleus \( D \) and a receptor nucleus \( R \), \( \gamma_D \) and \( \gamma_R \) are the gyromagnetic ratios of those two nuclides, \( r(D\rightarrow R) \) is the distance separating them, and \( \tau_c(D\rightarrow R) \) is the motional correlation time of the vector joining \( D \) and \( R \). If the conditions can be met to ensure a dominance of the dipole-dipole mechanism, which is usually obtainable if one has access to a FT spectrometer, then it can be seen, from equation (1) that both "distance" information and "motional" information (correlation times) can be extracted from spin lattice relaxation rates. For most diamagnetic molecules, protons are the only nuclear species with a high gyromagnetic ratio and for that reason the relaxation of each proton generally occurs via the other protons within the same molecule. Since the relaxation rate \( R_1 \) falls off as the inverse of the sixth power of the internuclear separation of \( D \) and
R then each proton receives most of its relaxation from its nearest neighbour protons. Of course, in most "real" molecules, each individual proton will be relaxed by interactions with several other protons (D-1, D-2, . . .) in the same molecule, providing they are close enough, and the total relaxation rate $R_1(R)$ will have the form given in equation (5).

$$R_1(R) = R_1(D-1) + R_1(D-2) + \ldots$$  \hspace{1cm} (5)

This summation can be stated in another way as shown in equation (2)

$$R_1(R) = \frac{3}{2} \sum \rho(D,R)$$  \hspace{1cm} (2)

where $\rho(D,R)$ is the relaxation contribution of the individual donor nuclei to the receptor, and has the form,

$$\rho(D,R) = \frac{4}{3} I_R (I_R+1) \frac{\gamma_D^2 \gamma_R^2 h^2}{\tau^e(D\rightarrow R)} \cdot \tau_c(D\rightarrow R)$$  \hspace{1cm} (3)

where $I$ is the nuclear spin and other terms are as for equation (1).

Therefore, if $R_1$ is known then it may be possible to calculate these individual relaxation contributions and hence calculate individual proton-proton distances and correlation times for the vectors connecting these protons, as was done in section IIC(ii).

The simplest conceptual model, involving pulse-nmr methods, upon which the basic Spin Lattice Relaxation experiment is based, is the "rotating-reference frame" model.

An ensemble of magnetically equivalent nuclei (spins) subject to the influence of an external magnetic field $B_0$, at thermal equilibrium between the spins and the lattice, will have a net, macroscopic magnetic moment which will be directed along the z-axis, in the rotating reference frame, along with $B_0$ (see Figure II-8). In Figure II-8 this magnetic moment is represented by a vector, whose length is equivalent to the total amount of magnetization present. However, when the vector lies
along the z-axis, no signal is detected by the spectrometer, which is designed to respond only to that component of the magnetization which lies in the x,y-plane. Therefore, to assay the amount of magnetization along the z-axis at any particular time it is necessary to tip the magnetization vector through 90° into the x,y-plane. This is accomplished by applying a suitable amount of radio frequency power at the appropriate frequency, in the form of a short pulse, to tip the magnetization through 90° (a 90° pulse).

In the rotating reference frame, initially the spin system is at thermal equilibrium with its lattice, and the magnetic moment is directed along the +z-axis. The equilibrium is then destroyed by applying a 180° pulse of power which tips the magnetization into the -z direction. Spin lattice relaxation then restores the system to thermal equilibrium and
the amount of magnetization present at any particular time can be assayed by applying a 90° pulse to the residual component into the x,y-plane. If the magnetization along the z-axis is sampled at several known delay times, t, after the initial 180° pulse, then a plot of magnetization, \( M_0 \) versus t, will give the decay curve shown in Figure II-9. Each point on the curve must be obtained individually for progressive increments of t; and a delay time of 5 or more \( T_1 \) (relaxation time = \( \frac{1}{R_1} \)) periods must be left between each 180°-90° pulse sequence, to ensure that thermal equilibrium is restored before the start of the next measurement. The relaxation rate is most conveniently obtained by making a linear plot of \( \ln(M_\infty - M_L) \) vs "t" which gives the relaxation rate from the slope.

The experiment, just described, is termed the "inversion recovery experiment" and although other relaxation experiments exist this was the one

Figure II-9. Plot of magnetization versus \( t/T_1 \) showing exponential recovery of magnetization.
used to obtain the data in section IIC(ii).

If one wants to estimate the relaxation rate without data processing, the delay time $t$ required to null the resonance, that is, the time when $M_0 = 0$, can be used to obtain the relaxation rate from the simple equation (4)

$$R_1 = \frac{0.69}{t}$$  \hspace{1cm} (4)

This null point method is rapid and contrary to much prejudice, it appears to provide rather accurate $R_1$ values.
References


CHAPTER III

CYANURIC CHLORIDE; A GENERAL REAGENT FOR THE CHEMICAL MODIFICATION OF CARBOHYDRATES

IIIA: Introduction

As stated earlier, a more versatile approach to the chemical modification of carbohydrates is clearly needed in order to be able to derivatize carbohydrates with a broader range of organic and inorganic substances, and also to extend the work presented thus far. A future application of this research, and one of the main reasons for the work in this thesis, is shown schematically in the following illustration.

The anchor in this case may be a large macromolecular support matrix such as silica, alumina, a polysaccharide, or even a protein. The probe could be any of a number of spectroscopic probes ("tags" or
"reporter" groups); for example, a spin-label nitroxide used in esr, a fluorescent probe, a metal, or even an nmr probe such as a deuterium or a fluorine atom. The receptor molecule or "hapten" (to use an immunological term) can also vary greatly and access to groups such as dyes, fatty acids, carbohydrates, and proteins would be desirable. The "shark," would represent typically an enzyme with a strong binding affinity for the specific receptor group, but could also represent any less specific interaction or association occurring between two molecules. The rationale underlying an experiment being that the probe molecule would relay back to the experimenter information about such an association. The areas of study which might benefit from this technology would be as varied as the required chemistry and could include the fields of: affinity chromatography for the purification of enzymes and proteins; antibody-antigen interaction studies; substrate-enzyme binding; lipid-bilayer membrane functions; and possibly in the study of cell-cell interactions. It may be advantageous to have the anchor support matrix but this is not a mandatory prerequisite for all of these investigations.

Clearly multivalent reagents are required for these studies in order to combine all of the desired entities. In the present work, the chemistry of one such multivalent reagent, cyanuric chloride, is investigated with particular applications to carbohydrate chemistry.

Cyanuric chloride [1] is a heterocyclic compound containing three chlorine substituents each of which can be displaced by any of a variety of nucleophiles under a wide range of conditions. This reagent has been known since 1827 and is now very fully documented. Owing to its low cost and versatility, this reagent has found uses in an extremely wide range of areas: "medicine," "industry," "chemistry," and "biochemistry."
In "medicine," cyanuric chloride has found uses in the development of bacteriocides and anti-cancer drugs\textsuperscript{1,2}. Quinoline derivatives [2], arsenic and antimony compounds [3] and [4], and triethylene melamine [5] are typical examples; compounds [3] and [5] have been shown to have desirable chemotherapeutic properties. Compound [5] has been subject to a considerable study\textsuperscript{2} and was shown to inhibit growth of certain types of tumors and to be of benefit in treatment of leukemia; (it is also known to cause cancer!).

In "industry"\textsuperscript{1}, substances containing the s-triazine structure have been used as pesticides and preservatives. Compounds of the aryloxyamino sulfonic acid type condensed with cyanuric chloride are useful as moth-proofers and fibrous protectors. In the rubber industry, certain triazine derivatives are useful as antioxidants and vulcanization accelerators. Resins and plastics have also been formed from cyanuric chloride, the most well known of which are the melamine-formaldehyde polymers [6]\textsuperscript{3}; (these form hard plastics and are used in making dinnerware and decorative surface coatings for counter tops, tables, and wall coverings under the brand name of Formica). The melamine necessary for this plastic is formed by the reaction of cyanuric chloride with ammonia. Cyanuric chloride has also been used in the manufacturing of explosives (such as
cyanuric triazide) and in the formation of surface active agents by the reaction of cyanuric chloride with dodecylaminoethylsulfate (C_{12}H_{25}NHCH_{2}CH_{2}O-SO_{3}Na).

Possibly though, the single most important industrial application to date, and also the most important with respect to this chapter, is the use of \(_{6}\)-triazine dyes as reactive dyes for cellulosic fibers.\(^4\)

Replacement of one or two chlorine substituents by the amino group of an aromatic dye gives materials such as the Procion dyes [7] and [8]; these were first introduced by ICI in 1956. It is interesting to note that the \(_{6}\)-triazinyl ring effectively acts as a "chromophoric block" and electronically "insulates" the two chromophoric groups. Therefore if
a blue dye is put on one position and a yellow dye on a second position of the \textit{s}-triazinyl moiety, the resultant reactive dye is green in color. Because they have superior wet fastness the increased use of these dyes has caused a curtailment in the use of other non covalently bonded dyes and in some cases replaced them altogether.

Interestingly, one of these dyes (Cibacron Blue) \cite{9} has recently been shown to be a useful reagent in affinity chromatography\cite{5}, when coupled to Sepharose beads (a cross linked polysaccharide used in
chromatography) it is useful for the purification of certain enzymes.
This is another encouraging result as it relates well to the present
chapter and it is an area to which this thesis work was hoped to advance.
Other chromatographic applications involving cyanuric chloride have
recently appeared. For the most part these have involved the immobi­
lization of enzymes such as trypsin and esterase\(^6\) for use in affinity
chromatography. Other interesting recent work using cyanuric chloride
includes the binding of: NAD to dextran\(^7\); glutathione to cellulose\(^8\);
fluorescent triazine reagents to proteins\(^9\); polyethylene glycol to
proteins\(^10\); antimicrobial compounds to cellulosic fibers; the derivat­
ization of electrode surfaces with ferrocene\(^11\) has already been men­
tioned in chapter (IIA) and will be discussed again later in this one.

Of the very recent work, the most significant, as it applies to
this chapter, was reported by Bishop et al. where polysaccharide-protein
conjugates\(^12\) and monosaccharide-protein conjugates\(^13\), for immunological
studies, were formed using cyanuric chloride. From a historical stand­
point it is noteworthy that the latter represents the first reported
synthesis of a monosaccharide (2-amino-2-deoxy-\(\text{D}\)-glucopyranose)-s-triazine
compound.

In light of the studies mentioned above it will be clear why tria­
zine reagents were chosen as being worthy of further study, and it was
the intention of the present study to develop the use of cyanuric
chloride to form novel conjugates of carbohydrates with interesting
properties and uses. The sequence of the following discussion is as
follows.

First, the reactivity of cyanuric chloride toward nucleophilic
displacement will be discussed in the context of four groups of
compounds; (a) monosaccharides, (b) hydrophobic alkyl molecules, (c) metal compounds, and (d) spin label nitroxides. In each case the reaction conditions and order of addition will be examined. The syntheses in this section will be culminated with an example of the formation of synthetic "glycolipids." Next, the chemical modification of polysaccharides will be discussed and emphasis will be given with regard to spin labelling of these materials; attention will also be given as to how spin labelling can be used in structure-determination of these materials and also as a model "test vehicle" for introducing other substances into polysaccharides via cyanuric chloride. Information as to whether the label is bound or unbound, the quantity attached, the distance between two nitroxides and the mobility of the labels will be discussed.

The two parent spin label nitroxide molecules used in this chapter are shown below. These are heterocyclic pyrrolidine-1-oxyl derivatives

![Nitrooxide Molecules](image)

which contain an unpaired electron making them paramagnetic and therefore detectable by esr spectroscopy. Such nitroxides are amongst the most stable of free radicals and many chemical modifications of the functional groups at position 4 can be effected without destruction of the paramagnetic center. The abbreviations for these labels will be
used throughout this chapter. For background information the reader is referred to section (IIIE) which describes the esr spectroscopy of nitroxides. The spin labelling of Bovine Serum Albumin (BSA), aluminum oxide, and glass will also be demonstrated to illustrate that triazine-nitroxide chemistry is also useful outside the realm of carbohydrate chemistry. Finally, the two spectroscopic techniques, nmr and esr, will be discussed as they provide the necessary structural information for the analysis of these compounds. First, the use of nmr spectroscopy will be briefly described as a structural tool for both the diamagnetic and paramagnetic monomeric compounds synthesized in (IIIB). Then a more detailed discussion of esr spectroscopy of nitroxides will be presented; this will include some of the necessary theory and results needed for the practicing chemist (as distinct from the esr spectroscopist) to understand the results presented previously.

IIIB: Synthesis

(i) Nitrogen Nucleophiles

It is known that replacement of one chlorine substituent in cyanuric chloride by a primary or secondary amine, a hydrazine, or a related compound, deactivates the remaining chlorine substituents; as a result stepwise displacement reactions can be achieved. These displacement reactions are formally analogous to nucleophilic aromatic substitution as shown below. The nitrogen atoms in the heterocyclic ring act as electron withdrawing groups, in much the same fashion as the nitro substituents on a benzene ring, thereby stabilizing the "carbanion" transition state and thus giving the chlorine substituents of cyanuric chloride their initially high reactivity to nucleophilic
displacement (readily hydrolyzed by $\text{H}_2\text{O}$). Substitution of a chlorine atom by an electron donating group such as an amine, destabilizes the transition state and thereby deactivates the remaining chlorine substituents. In contrast, oxygen and sulfur substituents have a very weak deactivating effect and their reactions will be discussed separately later.

The empirical "rule of thumb," usually expressed in the literature, to the stepwise substitution of the chlorine substituent by amines in aqueous media, is shown in the diagram below. This "rule," that the
first chlorine atom is replaced at 0°C, the second at 30-50°C, and the third at 90-100°C cannot be used generally. First, it applies only to aqueous reactions, and other factors such as basicity of the amine, the steric bulk both of the substituents already attached to the triazine moiety and of the approaching amine, the deactivating effect of any amine substituent already attached, and the base acceptor used in the reaction medium, all play key roles in determining reactivity. Thus, this oversimplified "rule of thumb" is at best an inaccurate summary. Some reactions, however, do follow this scheme well; for example, the reaction of cyanuric chloride, first with 4-amino 2,2,6,6-tetramethylpiperidine-1-oxyl [10] at 0°C in aqueous acetone with sodium bicarbonate to form compound [12], and then subsequent reactions with either n-hexylamine or dodecylamine in the same solvent and base acceptor at 45°C and 80°C to form compounds [13], [14], and [15]. Compound [11] had been previously made by Likhtenshtein in 196915, but the present preparation (see Experimental) is much simpler. All of the spin-labelled products were pink or orange in colour due to the presence of the nitroxide moiety; they could be precipitated directly from the reaction mixture and required no further purification except to be dried under vacuum at ~50°C. An interesting point should be made here, concerning the order of addition of substituents to the 5-triazine center. In the above-mentioned sequence of reactions it was found that the spin label nitroxide [10] must be attached to the 5-triazine ring in the first step. Attempts to react the amino spin label [10] with either the mono- or the di-alkyl chloro-triazine derivatives failed. Other examples illustrating the importance of the order of addition will be discussed as they appear.

Substitution of suitably functionalized aromatic π-bonded
\[ \text{Cl} \quad \text{Cl} \quad \text{NH}_2 \quad \text{Cl} \quad \text{Cl} \]

\[ \text{[1]} \quad + \quad \text{[10]} \quad \xrightarrow{\text{H}_2\text{O}/\text{Ace, } 0^\circ\text{C}} \quad \text{[12]} \quad \text{NaHCO}_3 \]

\[ \text{Cl} \quad \text{Cl} \quad \text{NH}_2 \quad \text{Cl} \quad \text{Cl} \]

\[ \text{[12]} \quad \xrightarrow{\text{R.NH, } 45^\circ\text{C}} \quad \text{[13]} \quad \text{R = n-Hexyl} \]

\[ \text{H}_2\text{O}/\text{Ace, NaHCO}_3 \]

\[ \text{Cl} \quad \text{Cl} \quad \text{NH}_2 \quad \text{Cl} \quad \text{Cl} \]

\[ \text{[14]} \quad \xrightarrow{\text{C}_{12}\text{H}_{25}\text{NH}_2, 80^\circ\text{C}} \quad \text{[15]} \quad \text{H}_{25}\text{C}_{12}\text{N-N-C}_{12}\text{H}_{25} \]

\[ \text{H}_2\text{O}/\text{Ace, NaHCO}_3 \]
organometallic complexes are also facile as shown in the following reaction of p-toluidine chromium tricarbonyl [16] with cyanuric chloride to produce [17]. This reaction proceeds as smoothly as the 4-amino spin label reaction and although no subsequent reactions were performed on [17], its derivatization in any of the ways shown in this chapter should pose no problems. Ferrocene can also be attached to cyanuric chloride and this reaction is discussed in section IIIC.

Reactions of amino sugars with cyanuric chloride are also quite facile and typical examples are shown in the following reaction schemes.
As stated before, compound [20] was first prepared by Bishop and the literature synthesis was repeated here successfully. Reaction of the blocked amino sugar [19] was performed in the same way with good results and a yield of 76%. All attempts to react [21] with the 4-aminonitroxide [10] or to react the spin labelled triazine derivative [12] with the blocked amino sugar [19] however, failed. This seems to reflect the low basicity of both the amino sugar and the spin label; however, this same amino sugar reacts readily with the oxygen linked spin label derivative [22] to form [23] as shown below.
This oxygen linked spin labelled triazine derivative [22] also reacts more readily under milder conditions with n-hexylamine than does its nitrogen linked counterpart [12] as shown below:

These examples serve both to illustrate that the difference in reactivity between oxygen and nitrogen substituted chlorotriazines is substantial and also to carry the reader into the next section, in which the reactions of hydroxyl and thiol nucleophiles with cyanuric chloride will be discussed more thoroughly.

(ii) Oxygen and Sulfur Nucleophiles

As mentioned previously, oxygen and sulfur substituents have a much smaller deactivating effect on the reactivity of remaining chlorine atoms of substituted triazine units than do their nitrogen counterparts. Thus one can, as illustrated in the example above, react an amine with an oxygen linked chlorotriazine derivative under conditions similar to those that would be used with cyanuric chloride itself, and not the $\sim 50^\circ C$ needed if substitution by a nitrogen moiety precedes it. This increased level of reactivity has its obvious advantages; however, it also has disadvan-
tages. If this chemistry is to be used to derivatize proteins or other sensitive biological molecules the reaction conditions must be kept mild and temperatures above 50°C will frequently be undesirable. Should the triazine nucleus be already disubstituted with two N-linked groups it is likely that the subsequent coupling reaction could require ~100°C; however, by having one or two of these substituents linked via oxygen or sulfur, the temperature of the subsequent coupling reaction may be kept below 50°C. Even in non-sensitive systems where there are no temperature limitations trisubstitution with an amine or diaminochlorotriazine is sometimes impossible for one reason or another and therefore suitable oxygen or sulfur substituents may provide a viable procedure. The main disadvantage in this chemistry however, is that because there is very little deactivation of the 6-triazine ring by an oxygen or sulfur substituent, reactions of these nucleophiles with cyanuric chloride or dichlorotriazine compounds can sometimes lead to over-substitution and hence to mixtures of compounds from which purification of a single compound can be difficult. This problem is exemplified by the reaction of cyanuric chloride with the 4-hydroxy spin label [11]. While reaction of cyanuric

\[
\begin{align*}
\text{[1]} & + \text{[11]} \\
& \xrightarrow{\text{H}_2\text{O/Ace}, \text{NaOH, } 0^\circ\text{C}} \text{[22]} \\
& \quad \quad \quad \text{[25]} \\
\end{align*}
\]
chloride with one equivalent of the 4-hydroxy nitroxide [11] yields [22] and two equivalents yields [25], reaction conditions must be carefully controlled to prevent mixtures of products from being formed. Both reactions are carried out by the slow dropwise addition (40 min to 1 h) of the 4-hydroxy spin label in sodium hydroxide solution to a stirred solution of cyanuric chloride in ice-cold acetone. Addition must be made slowly in order to keep the concentration of spin label low (it reacts almost immediately) and thereby to suppress the unwanted formation of the biradical (in the case of the monoradical preparation) or the triradical in the case of the biradical preparation. The yields for both these reactions are low (10-16%) probably because both cyanuric chloride and the products are hydrolyzed readily by sodium hydroxide even at 0°C; nevertheless, the products can be filtered directly from the reaction mixture, and only require drying at ~50°C (to sublime away any unreacted cyanuric chloride) for purification. This ease of product isolation and the low cost of the starting materials help to make these low yields tolerable.

The reaction of hydroxymethyl ferrocene [26] (see IIB) with cyanuric chloride discussed earlier in the thesis, is similar to that of the 4-hydroxy spin label. In this reaction a solution of the alcohol [26] in

\[
[1] \quad \text{Fe} \quad \text{CH}_2\text{OH} \quad \text{H}_2\text{O}/\text{Ace} \quad \text{NaOH, 0°C} \quad \text{OFc}
\]

(≡FcCH\text{2OH})

[26]
acetone and an aqueous solution of sodium hydroxide were added separately over a period of 1 h; after filtration of a by-product (see IIB) the filtrate was allowed to become acidic upon standing (because of hydrolysis of cyanuric chloride) and the resulting yellow precipitate filtered. No disubstituted product was detected. In the above, reaction and synthesis of the disubstituted bis-ferrocene derivative was not attempted. Because of a recent interest in coating electrode surfaces with ferrocene, this product may find an immediate application.

Reaction of the free hydroxyl group of a suitably blocked monosaccharide with a chlorotriazine derivative is exemplified by the following reaction:

Reaction of 1,2:3,4-di-0-isopropylidene-α-D-galactopyranose [28] with the oxygen linked spin labelled s-triazine [22] was carried out conveniently in reagent grade benzene with powdered sodium hydroxide, to yield
compound [29]. When sodium hydroxide was replaced by sodium carbonate there was no detectable reaction. Compound [29] was not isolated but instead was reacted directly with n-hexylamine in aqueous acetone to give the trisubstituted derivative [30] in 45% overall yield, without reaction temperatures ever exceeding room temperature! This reaction illustrates the utility of the s-triazine moiety to serve as a "trivalent locus"; being used here to form a synthetic glycolipid complete with spectroscopic "tag". In reality this particular molecule would not be very useful as it stands, since lipids generally have longer alkyl chains (typically C16) and the sugar moieties of glycolipids contain unblocked "free" hydroxyl groups. Meeting these requirements with triazine chemistry is not viewed as a problem and one such more realistic synthetic glycolipid will be discussed after the reactions of sulfur nucleophiles and cyanuric chloride have been presented. Reaction of the blocked galactose [28] directly with cyanuric chloride was also attempted but isolation of the product was not achieved owing to its high reactivity (the product decomposed on silica gel). This, however, does not prevent the subsequent derivatization of this crude product as was done in the case of compound [29].

Thiols also react well with cyanuric chloride and this is exemplified by various reactions of 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranose [31] with chlorotriazines. The -SH group is an extremely good nucleophile and has an advantage over its -OH counterpart in that it does not require a strong base to first remove the attached proton. However, the weak deactivating effect of the sulfur substituent on the s-triazine ring was not discernibly different from the alcohol substituent effect.

Three equivalents of the 1-thio-glucose [31] was reacted with
cyanuric chloride in acetonitrile and triethyl amine to form the symmetrical tri-saccharide derivative [32]. The reaction was immediate

and, after pouring into ice water, the precipitate was filtered and dried to yield the pure product. Reaction of one equivalent of the 1-thio-sugar [31] to form the monosubstituted dichloro compound was unsuccessful owing to the high reactivity of that product and its decomposition similar to that of the mono-galactose preparation. The 1-thio-glucose [31] was
also reacted with the nitrogen and oxygen linked spin labelled s-triazine compounds [12] and [22] to form the mono- and bis-thio sugar derivatives of each. Due to the good nucleophilicity of the thio sugar these reactions all went readily at room temperature, even with the amino spin label derivative [12]. Mixtures of mono- and bis-substitution by the thio sugar did not seem to pose any problems (not detectable on tlc) and the mono-substituted thio glucose products [33] and [34] were isolated easily; this apparent deactivation by the first sulfur substituent may be caused more by steric factors than anything else.

The ferrocene triazine derivative [27] also reacts readily with thio-glucose to form the metal sugar conjugate [37]. More information about this derivative has been given in (IIB).

As mentioned earlier, this particular chemistry involving thio glucose, was extended to form synthetic glycolipids. In a preliminary study, a fourth year undergraduate student, Art van der Est, while working in Dr. Hall's laboratory, prepared one such synthetic glycolipid from the following sequence of reactions. Although elemental analysis was not obtained for the final product, the intermediates up to [33] gave acceptable elemental analysis and compounds [38] and [39] appeared to be homogeneous by tlc; furthermore, esr integration gave a quantitation of spin label to within 10% of the calculated value. This final product was
then incorporated into liposome model membranes.

(iii) Summary

Cyanuric chloride can be readily substituted by the amino, hydroxy and thio groups of many compounds and under a variety of reaction conditions. Both aqueous and non-aqueous media can be used along with a number of base acceptors including: sodium bicarbonate, sodium carbonate, sodium hydroxide, and triethylamine.

Amines add to the triazine ring in a pronounced stepwise addition. Each addition deactivates the ring allowing it to be substituted by three
different amino groups. Alkoxy and thio substituents, however, have a much lesser deactivating effect and both advantages and disadvantages accrue from their use. Due to their low deactivating effect, reactions between cyanuric chloride and hydroxyl and thio-compounds can result in mixtures; however, it was found that this did not occur in most cases and it could be controlled by choosing the appropriate reaction conditions. An obvious advantage of an alkoxy or thio substituted chlorotriazine compound is in its much higher reactivity in subsequent nucleophilic displacement reactions as compared to that of its amino linked analogue.

The order of substitution onto the triazine ring is also quite important. Since many factors contribute to the success or failure of these substitutions, it is difficult, if not impossible, to predict beforehand the correct sequence of addition to prepare di- or tri-substituted triazines. A few general observations can, however, be made: weakly basic amines, such as the spin label [10] and aminosugars do not react well with amine substituted chlorotriazines and should either be added first, or after an alkoxy or thio substitution; when using strongly alkaline conditions to facilitate substitution with hydroxyl containing compounds, substitution also should either be made first, or following an alkoxy or thio substitution, since elevated temperatures may cause competing hydrolysis; relatively basic amines, such as alkylamines, and also thio derivatives, can usually substitute the ring at any stage regardless of the substituents previously attached.

In this study it has been shown that monosaccharides can be linked to the triazine ring through nitrogen, oxygen and sulfur atoms. As well as monosaccharides, a host of interesting groups including alkyl, spin
label and organo-metallic moieties have also been attached to the ring in various combinations. An example of how such a combination might yield useful compounds was shown by the production of "synthetic glycolipids." Other possible applications are numerous and in the next section it will be shown how this chemistry can be applied to chemically modify polysaccharides and other macromolecules and surfaces.

One particularly challenging extension of this work that the author unfortunately was unable to pursue due to time limitations, is the possible formation of chlorotriazine-metal chelate derivatives. Some possible structures are shown schematically in the following illustration. Just as spin labels can be used as "probes," so also can metal derivatives. Such reagents may be useful as: metal probes for biological systems; heavy metal carriers for electron microscopy studies; pharmaceuticals when linked to suitable molecules such as sugars; and even used in affinity chromatography, when linked to supports such as polysaccharides, for the purification of proteins or other metal binding substances. There is considerable interest in the use of metal compounds for these purposes and further work using cyanuric chloride may be fruitful.
IIIC: Macromolecule and Surface Modification

(i) Polysaccharides

(a) The General Reaction

In this section it will be demonstrated how cyanuric chloride can be used to chemically modify polysaccharides in a completely general way. The actual results presented will be restricted to spin labelling via cyanuric chloride; however, the use of spin labelling as a test vehicle or "probe" for the general chemistry of cyanuric chloride mediated derivatization of polysaccharides will be shown. Esr-results and -spectra will be shown and discussed where needed; little explanation is given to the reader not familiar with nitroxide esr, who is instead referred to section IIIE for a more detailed explanation of that particular aspect of nitroxide-esr spectroscopy.

The fact that cyanuric chloride had previously been used successfully in the dyestuffs industry to form reactive dyes for cellulosic fibers was very encouraging and provided a great deal of useful information concerning the reaction and properties of cyanuric chloride-derivatized cellulose.

The present work, as mentioned before, has been solely concerned with the spin labelling of polysaccharides via cyanuric chloride. The chlorotriazine spin labels used in this study were discussed in sections IIIB (i) and (ii) and are summarized below. The underlined abbreviations for these compounds will be used throughout this chapter. All three labels [12], [22] and [25] were used to label various polysaccharides; however, more emphasis was placed on the use of the mono-radicals [12] and [22] since the esr spectra of biradicals are more complicated and less fully understood\(^\text{17}\). The triazine nitroxide [12] was first prepared
by other workers as stated before in IIIB and has been used to label cotton fibers in order to study their structure and conformation\(^\text{18}\).

Although much literature has been published on the spin labelling of proteins and lipids\(^\text{19}\), there has been a general paucity of methodology suitable for labelling polysaccharides until the first systematic study was reported by Aplin and Hall\(^\text{20}\).

The polysaccharides that have been spin labelled using the triazine chemistry were selected from a variety of plant, animal and bacterial sources and their structures are shown in Figure III-1. An abundance of hydroxyl functions is common to all polysaccharides. Since it has been demonstrated (section IIIC) that hydroxyl groups on monosaccharides will react with chlorotriazines, attention has been directed therefore to
Figure III-1. Structural formulae of the polysaccharides spin labelled via cyanuric chloride.
the reactions of the spin labelling reagents [12], [22] and [25] with the hydroxyl groups of polysaccharides.

In a reaction between a polysaccharide and a dichlorotriazine derivative, in aqueous media, five possible products can in principle be obtained, as shown in the following scheme.*

Aqueous conditions are desirable since water swells and penetrates the polymeric fibers and an alkaline pH is required to facilitate the reaction. Mono-alkoxy or amino chlorotriazine derivatives that react readily in the "cold" (ambient temperature) such as the spin label reagents [12], [22] and [25], suffer from loss by hydrolysis (degradation to [44]). One solution to this problem is to stabilize the molecule by substituting the second position with an amino group. However, reaction conditions for

*adapted from reference 4.
the coupling reaction need be more strongly alkaline and temperatures of over 60°C may be necessary for the subsequent reaction of a di-amino-triazine. This approach has been used successfully in the dye industry where premixed dyes may be hydrolyzed to a large degree during long printing runs. This stabilization procedure was however, not undertaken in the present study. It is interesting to note that thickening agents used in the dyestuffs industry cannot be based on starches and other reactive carbohydrates, but sodium alginate (containing only secondary hydroxyl groups) or hydrocarbon emulsions are satisfactory. This indicates that, at least with these dye molecules, the reaction of the triazine moiety is selective for primary alcohols of polysaccharides.

It seems remarkable that heterogeneous reactions with insoluble polysaccharides such as cellulose fibers predominates in competition with the homogeneous reaction with water molecules, even though the latter are present in great excess. A possible reason for this is that the molecules which are absorbed by the fiber have their reactive groups brought into close proximity with the fiber, making the effective concentration of reagent in the fiber much greater than in the aqueous solution.

(b) Optimization and Quantitation

The amount of spin label attached to the polysaccharide depends largely upon the reaction conditions used. Aqueous alkaline reactions proved to be most suitable and highest yields were obtained by the following methods. Water insoluble polysaccharides (cellulose, agarose and Sephadex G25) were first treated overnight with 8% sodium hydroxide and the excess sodium hydroxide solution was removed by decantation; to this pretreated material was then added an aqueous-acetone solution of the triazine spin label. After a suitable reaction time the product was
filtered and washed. In contrast, water-soluble polysaccharides (guar gum, xanthan gum and starch) were dissolved in 4% sodium hydroxide and to this solution was added an acetone solution of the spin label. The guar and xanthan gums were precipitated by adding a large amount of acetone and then were filtered and washed. The starch sample was purified by gel filtration chromatography on Sephadex LH-20. Since the labelling reagents [12], [22] and [25] were insoluble in water their reactions in aqueous media were probably limited by their low effective concentration. The reactions with the soluble polysaccharides did not have the same advantage as the insoluble polysaccharides of effectively concentrating the spin label reagents by adsorption and thereby the degree of competing hydrolysis. Denaturation of agarose, guar and xanthan gums was detected after their reactions with the spin labelling reagents. Guar and xanthan gums, which normally dissolve in water at room temperature were, after reaction, totally insoluble, even in boiling water. Agarose, which normally will dissolve in hot water, would no longer dissolve after reaction. This lack of solubility after derivatization is most probably due to the formation of cross-links between the polysaccharide chains introduced by the dichlorotriazine spin labels [11] and [12], as shown in [42]. This is not an unexpected result since, for example dextran, when cross-linked with epichlorohydrin (Figure III-1), forms an insoluble derivative which is sold for chromatography under the trade name of Sephadex. It is also expected that the NaOH-treatment, especially the overnight treatment of cellulose and agarose, would cause a certain degree of depolymerization of these materials. Sephadex, on the other hand, has been cross-linked and is known to be very stable to base-treatment.
The esr spectra of these spin labelled polysaccharides are shown in Figure III-2. Although it is not the main purpose of this work to analyze in detail the line shapes of these spectra, some important qualitative information can be obtained readily and will be discussed here.*

The mobility of the nitroxide group is dramatically reflected in the spectra of Figure III-2. A relatively small nitroxide molecule such as [10] or [11], tumbling freely and rapidly in solution, shows three sharp lines each of equal intensity (see section IIIE). The spectra in Figure III-2 are however, very different from this and their broad lines reflect for the most part, the much slower motion of the nitroxide as a result of its being affixed at one end, to a very large, slow moving macromolecule. The spectrum of labelled cellulose shows more severe broadening probably due to electron exchange between neighbouring nitroxides (see IIIE).

As will be discussed in more detail, the slower motion of the nitroxide, broadens the high field line to a much greater degree than it does the low field or center line. Various degrees of motional freedom all contribute to the line shapes of these spectra. The motion of the polysaccharide backbone and the rotation about the bonds joining the nitroxide to the polysaccharide provide most of the motional freedom for the appended nitroxide. These motions, however, depend upon the host environment (solvent) in which the polysaccharide is suspended, or

*For a more detailed discussion on line shape analysis of esr spectra of spin labelled polysaccharides, the reader is referred to a report by Aplin and Hall20.
Figure III-2. Ambient temperature aqueous esr spectra of (a) cellulose, (b) agarose, (c) Sephadex G-25, (d) guar gum, (e) xanthan gum, and (f) starch, using from left to right, labelling reagents [12], [22] and [25].
dissolved. A polar solvent which can penetrate the hydrophilic polysaccharide and solvate it, such as water does, imparts much more mobility to a pendant nitroxide than does a non polar organic solvent. Chloroform, for example, which cannot penetrate the polysaccharide matrix, only affects the labels on the outer extremities of the polysaccharide, leaving the inner reaches effectively the same as for a dry powder. The spectra in Figure III-3 of labelled Sephadex and agarose in water and chloroform solvents both show this effect. The esr spectrum in chloroform does indeed look the same as a typical powder spectrum of non interacting (spin dilute), nitroxides (see section IIIE), where the large splitting $2A_m$ is now visible. In aqueous media the spectra reveal a much more mobile nitroxide moiety than for the CHCl$_3$ solution and the $2A_m$ splitting is now partially averaged and is no longer visible. It can be seen from this brief discussion that spin labelling of polysaccharides can provide considerable insight to the way in which solvents interact with polysaccharides.

Determination of the quantity of spin labels on the surface of these polysaccharides was performed both by elemental analysis and by esr double integration of the first derivative spectrum with comparison to a standard. These results are tabulated in table III-1. Unfortun-
Figure III-3. Comparison of the aqueous esr spectra of labelled Sephadex and Agarose in (A) with their chloroform spectra in (B).
TABLE III-1: Data obtained from elemental analysis and esr double integration of labelled polysaccharides

<table>
<thead>
<tr>
<th>Sample</th>
<th>C</th>
<th>H</th>
<th>N</th>
<th>mono-saccharide residues per label from elemental analysis</th>
<th>mono-saccharide residues per label from esr integration</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Cellulose calcd.</td>
<td>44.44</td>
<td>6.17</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(b) Cellulose blank</td>
<td>43.81</td>
<td>6.45</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(c) Cellulose/[22]</td>
<td>43.16</td>
<td>6.39</td>
<td>1.18</td>
<td>26</td>
<td>118-2300</td>
</tr>
<tr>
<td>(d) Cellulose/[12]</td>
<td>44.13</td>
<td>6.31</td>
<td>0.88</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>(e) Cellulose + [22] calcd.</td>
<td>-</td>
<td>-</td>
<td>1.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(f) Cellulose + [22] found</td>
<td>44.07</td>
<td>6.39</td>
<td>1.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(g) Agarose calcd.</td>
<td>47.06</td>
<td>5.88</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(h) Agarose blank</td>
<td>46.40</td>
<td>6.56</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(i) Agarose/[22]</td>
<td>45.35</td>
<td>6.24</td>
<td>2.88</td>
<td>10</td>
<td>2900</td>
</tr>
<tr>
<td>(j) Agarose/[12]</td>
<td>47.43</td>
<td>6.73</td>
<td>0.83</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>(k) Sephadex calcd.</td>
<td>44.44</td>
<td>6.17</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(l) Sephadex blank</td>
<td>37.43</td>
<td>5.45</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(m) Sephadex/[22]</td>
<td>45.31</td>
<td>7.01</td>
<td>1.59</td>
<td>20</td>
<td>1700</td>
</tr>
<tr>
<td>(n) Sephadex/[12]</td>
<td>44.58</td>
<td>7.09</td>
<td>0.25</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>(o) Guar gum/[22]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>900</td>
</tr>
<tr>
<td>(p) Xanthan gum/[22]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1900</td>
</tr>
</tbody>
</table>
ately both methods suffer from important drawbacks. Elemental analysis is satisfactory only for relatively high levels of chemical modification where the elemental ratios are altered significantly from those of the native polysaccharide. Since nitrogen is not present in the native polysaccharides studied here, analysis for nitrogen offers a good probe for the extent of derivatization. To check the validity of the elemental analysis, a sample (f) of cellulose was coated non-covalently with a known amount of the label [22] from a chloroform solution; its analysis was the same as that calculated (e). Other support for the data obtained by elemental analysis comes from the fact that the "blank," untreated samples showed no detectable nitrogen and that carbon and hydrogen analysis were generally acceptable, throughout all samples, after accounting for about 3-5% of tightly bound water. Reactions with the labelling reagent [12] were in all cases lower-yielding than the reactions with the alkoxy analogue [22]. This may be accounted for by its lower solubility in aqueous-acetone or more probably by its lower reactivity. Should derivatization be lower than about one spin label per two hundred saccharide residues, elemental analysis is not sufficiently sensitive to detect this change. Fortunately the esr integration technique is much more sensitive than elemental analysis and derivatization as low as one per several thousand saccharide repeating units can be detected. In
principle, as long as a first derivative spectrum can be obtained, this spectrum can be integrated to give the total number of spins, after comparison with a standard. The following equation can be used to calculate the number of spins in an unknown sample,

$$[X] = \frac{[\text{STD}] A \_G \_\text{STD}}{A \_\text{STD} \ G \_X}$$

where $[X]$ is the unknown concentration, $[\text{STD}]$ is the standard concentration of nitroxide, $A$ is the area under the absorption mode spectrum measured in any arbitrary unit as long as they are the same for both standard and unknown, and $G$ is the relative gain of the spectrum amplifier. For this equation, the microwave power and modulation amplitude must be kept the same for both the standard and unknown. This technique, however, possibly due to its high sensitivity, gave unrepeatable results (see Table III-1) and quite different from those obtained from elemental analysis.

This technique is known$^{21}$ to be subject to many sources of errors. One potentially large source arises from the fact that an esr probe cavity is only sensitive over a very small area. Hence, the total weight of the sample in the cavity cannot be used to calculate the number of spins per gram. Instead, the solid samples have to be treated as "concentrations" and large errors may have resulted from uneven packing densities. In addition, the second integration was carried out by means of peak cutting in the presence of poor baselines.

In general, potentially large sources of errors can come from failure to satisfy the following conditions.

1. The same solvent or host and the same sample geometry should be employed to ensure that the microwave magnetic field $H_1$ is the
same for the unknown and the standard samples.

2. There should be no saturation for either sample or standard and the microwave power level should be the same for both sample and standard.

3. The unknown and standard should be at the same temperature.

4. The unknown and standard sample tubes must be in exactly the same positions in the esr cavity.

5. The standard and unknown should have a similar line shape and concentration.

While conditions 2 and 3 are easily met, 1, 4 and 5 are not. A nitroxide in a solid polysaccharide sample cannot be considered to be in the same host as the solution standard even if both samples are run in the same liquid. Since the instrument used in this study did not have a dual cavity, the sample and standard could not be run simultaneously and equivalent sample positions were difficult to achieve. The line shapes of both standard and sample were also quite different. The polysaccharide samples had very broad esr line widths in comparison to the very narrow lines of the solution standard. This technique, however, even with its drawbacks, has been used successfully by others and it should be possible to obtain at least an accuracy of ±50%. Since the results reported here sometimes carried a much higher deviation than this, even between runs on the same sample, it is felt in retrospect that the performance of the instrument used in this study was simply not compatible with these experiments.

(c) Evidence for a Covalent Bond

One of the assumptions in this work is that a covalent bond exists that joins the triazine moiety to the polysaccharide matrix, and that the
triazine unit is not bound by some other non-covalent association or by entrapment. This is very difficult to prove directly but some evidence for the chemical union between reactive dyes and cellulose is convincing and is summarized below:

1. If cotton is dyed with a Procion M dye (trade name for a chlorotriazine dye) from a neutral bath and then washed with water, most of the color is removed; if, however, the dyed fabric is treated with alkali most of the dye becomes fixed and is not even removed in a boiling soap bath.

2. Procion dyes are not removed from dyed cotton by extraction with boiling pyridine, O-chlorophenol or chloroform. On the other hand direct vat- and azoic-dyes are stripped by these solvents.

3. Cellulose dyed with Procion M dyes are insoluble in cuprammonium solutions, but similar fiber dyed with direct dyes dissolve completely.

4. When cotton dyed with the monoazo dye Procion yellow M-R, is treated with sodium hydrosulphite, the dye is split into two components. The component containing the reactive group remains anchored to the fiber and can be diazotized and coupled with an amine or phenol to give a new dye.

In this thesis work some evidence for the probable occurrence of attachment comes from the fact that monosaccharide triazine spin label compounds can be synthesized as already shown in IIIB (i) and (ii).

Further information concerning the bond between the chlorotriazine derivatives and polysaccharides was obtained by esr spectroscopy; the appropriate esr spectra offering this additional information are shown

*from reference 4.
In Figure III-4. If cellulose, agarose or Sephadex are treated with the spin labelling agent \( \text{Cl}_2\text{TOS} \) [22] under neutral conditions, there is no detectable signal after washing with aqueous-acetone (A). However, if the polysaccharide is first treated with NaOH and then [22] added, the resulting highly "immobile" esr signal in (B) cannot be removed by prolonged washing. To eliminate the hydroxide as having any independent effect, the "non reactive" spin label \( \text{HOS} \) [11] was added to the alkali treated polysaccharides in an identical procedure to that used for the \( \text{Cl}_2\text{TOS} \) reactions, and the product washed. After brief water washings the esr spectra in (C) were obtained. Upon more thorough washing the signals were completely removed. Thus the spectra in (C) show that esr spectroscopy can be used to distinguish between "bound" and "free" nitroxides within the polysaccharides. These "free" signals come from motionally restricted populations of nitroxides temporarily trapped within aqueous voids in the polysaccharides, which are large enough to allow unhindered mobility. Given sufficient time, these unbound nitroxides can diffuse out and be washed away. While these "free" signals in (C) provide proof of a non covalently bound nitroxide, the highly immobile spectrum of (B) does not alone prove the existence of a covalent bond since it does not eliminate the possibility of tightly adsorbed, or trapped labels. The combination of evidence from (A) and (B) however, would seem to rule out this possibility.

(d) Distance Measurements

A very useful piece of information that can be extracted from many esr spectra of labelled materials is the distance between adjacent pairs of free radicals. The parameter \( d_1/d \) shown in Figure III-5 was first used by Kokorin et al.\(^{23} \) on an empirical basis to characterize inter-
Figure III-4. Control esr experiments where (A) shows the esr spectra of the polysaccharides labelled with [22] under neutral conditions, (B) shows the "bound" esr spectra of the polysaccharides labelled with [22] under basic conditions and (C) shows the "free" esr spectra of the three polysaccharides labelled with the non-reactive spin label [11] HOSL under basic conditions with brief washing.
Figure III-5. Powder spectrum showing the heights $d_1$ and $d$ and the splitting 2A.
actions between nitroxides. It has recently been shown in our laboratory by J. D. Aplin and J. C. Waterton\textsuperscript{22} that,

\[
\frac{d_1}{d} = \left(\frac{d_1}{d}\right)_{\text{dil}} + 0.58 \bar{r}^{-3}
\]

where \(\bar{r}\) is the mean nearest neighbour distance and \((d_1/d)_{\text{dil}}\) is the d_1/d value for the labelled material at infinite dilution (spins far enough apart so that no dipolar interactions can occur between neighbouring nitroxides). This value is usually about 0.4 but is dependent upon the material and solvent-host used and therefore it should be checked for each system. The distance values obtained for spin labelled cellulose, agarose and Sephadex are shown in Table III-2. The distance \(\bar{r}\) can also be used to infer relative levels of labelling between samples of the same material but only if the labelling is purely random and no clusters of labels exist.

Distance information is useful in determining the surface topography of polymeric and macromolecular materials and may be especially useful in chromatography and membrane studies. This technique has been used in

<table>
<thead>
<tr>
<th>Sample</th>
<th>((d_1/d)_{\text{dil}})</th>
<th>d_1/d</th>
<th>(\bar{r}) nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose</td>
<td>0.42</td>
<td>0.66</td>
<td>1.33</td>
</tr>
<tr>
<td>Agarose</td>
<td>-</td>
<td>0.48</td>
<td>2.11</td>
</tr>
<tr>
<td>Sephadex</td>
<td>-</td>
<td>0.42</td>
<td>-</td>
</tr>
</tbody>
</table>

\textsuperscript{measured at 77K in chloroform}

\textsuperscript{*a spin dilute labelled cellulose sample was used as \((d_1/d)_{\text{dil}}\) for all samples}
this laboratory, by others, to: determine the outside and inside diameter of spherical liposome vesicles (model membranes); to study the distribution of nitroxides layered on a silica surface [IIIC(iv)] and to determine the "accessible" surface area of cellulose\textsuperscript{22}.

(ii) Bovine Serum Albumin

The amino groups of a variety of proteins are also amenable to modification via the chemistry of cyanuric chloride. Spin labelling of proteins is not new\textsuperscript{19} and Likhtenshtein et al.\textsuperscript{15} have used the triazine spin label reagent [12] to label the lysine ε amino groups of proteins in the hope of investigating their structure and function. In the present work the new spin labelling reagent [22] was used to label bovine serum albumin (BSA), the esr spectrum of which is shown in Figure III-6. BSA is a globular plasma protein, molecular weight approximately 67,000 which contains 55 lysine residues. From esr double integration it was shown that the number of lysine residues labelled with this reagent is about 12 per protein molecule. Although a distance measurement was not done, the spectrum in Figure III-6 indicates a degree of electron exchange between neighbouring nitroxides. This therefore implies that some of the spin labels are in a close proximity (≤ 1 nm). This labelling reagent [22] has an advantage over its amino substituted cousin [12] in that it can be reacted under much milder conditions which are more suitable for proteins.

(iii) Aluminum Oxide

The surface hydroxyl groups of aluminum oxide $\text{Al}_2\text{O}_3$ (alumina) are also susceptible to reaction with chlorotriazine derivatives, and to further illustrate again that the chemistry of cyanuric chloride can be applied to other areas as well as polysaccharides, alumina was labelled
Figure III-6. Ambient temperature aqueous esr spectrum of BSA labelled with reagent [22].
with [22]. In fact this experiment was performed, purely by accident, when the above reagent [22] was being chromatographed on alumina with chloroform in a test to see if unreacted cyanuric chloride could be separated from an alkoxy substituted triazine derivative. It was found that compound [22] became bound by the column and could not be eluted. This initial observation was subsequently followed up with controlled experiments. A 1:1 mixture, by weight, of alumina (Basic Brockman activity 1) and [22] in chloroform was reacted, filtered and then washed with methanol and chloroform to remove any unbound materials. The esr spectrum of this material is shown in Figure III-7(i).

Figure III-7(i). Ambient temperature chloroform esr spectrum of alumina labelled with [22].
This spectrum is again characteristic of a highly immobilized spin label with possibly some degree of exchange broadening. As before, esr double integration and elemental analysis were used to quantitate the extent of labelling (Table III-3). As in the polysaccharide work,

**TABLE III-3: Extent of derivatization of Al₂O₃ with reagent [22]**

<table>
<thead>
<tr>
<th>Sample</th>
<th>C</th>
<th>H</th>
<th>N</th>
<th>Spins per gram from elemental analysis</th>
<th>Spins per gram from esr double integration</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Al₂O₃ calcd.</td>
<td>0.0</td>
<td>-</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(b) Al₂O₃ blank</td>
<td>0.03</td>
<td>0.45</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(c) Al₂O₃ + [22] calcd.</td>
<td>0.80</td>
<td>-</td>
<td>0.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(d) Al₂O₃ + [22] found</td>
<td>1.24</td>
<td>0.77</td>
<td>0.24</td>
<td>2.1 \times 10^{19}</td>
<td>9.0 \times 10^{18}</td>
</tr>
<tr>
<td>(e) Al₂O₃/[22]</td>
<td>0.50</td>
<td>0.57</td>
<td>0.20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

there was a discrepancy between these two techniques although the agreement here is acceptable. The sample for (c) and (d) was run as a control where a known amount of Al₂O₃ and [22], were mixed in chloroform and the mixture evaporated to dryness. The analysis on this material is acceptable and even this low nitrogen percentage could be detected with reasonable accuracy. The difference of about 60% between the elemental analysis and integration values is within experimental error since an uncertainty of about ±50% is expected with the integration and about ±30% for the elemental analysis.

A control experiment was also performed to support the claim of covalent bonding between [22] and the Al₂O₃ surface. The nitroxide HOSŁ [11] was added to alumina in the same way as Cl₂TOSŁ [22] had been and this sample was then filtered and washed overnight with methanol.
and chloroform. After this washing, a small amount of label remained and its esr spectrum, shown in Figure III-7(ii) (B), was characteristic of a highly immobilized nitroxide. This indicates that there is either a strong non covalent bond formed between the surface and the nitroxide or that these nitroxide molecules are trapped in very small pores which restrict their motion. However, assuming that line widths of the [22] treated alumina (A) and of the control spectrum of (B) are roughly the same and that sample volumes are the same (a good assumption since alumina packs evenly and the whole cavity was filled in both cases), then comparison of receiver gain values shows that the alumina labelled with Cl₂TOS⁻ [22] contains almost 250 times more spins than the sample treated with HOS⁻ [11]. Even though these two molecules do not have the same structures and an equivalent adsorption factor cannot be assumed, this result still gives strong evidence that a covalent linkage is formed in the reaction of [22] and alumina. This bond can be illustrated in two possible ways as shown in the following schematic.

![Diagram](image_url)
Figure III-7(ii). Ambient temperature chloroform esr spectra of (A) alumina labelled with [22] (same as Figure III-7(i)) and (B) alumina labelled with the non-reactive reagent [11] HOSi as a control.
The structure represented in (B) is supported partially by the following experiments. When cyanuric chloride was first reacted with alumina and then $\text{Cl}_2\text{TOS}^\text{-}$ [22] was added, no label was incorporated. It therefore seems that cyanuric chloride effectively blocks all of the reactive "binding" sites. When the bound cyanuric chloride was subsequently reacted with an alkali mixture of $\text{HOS}^\text{+}$ [11], no esr signal was detected after washing. This can either mean that structure (B) is correct or that simply, the remaining chlorines are hydrolyzed before or during the attempted reaction with $\text{HOS}^\text{+}$ [11].

Again, as in the case of the polysaccharides, a distance between nitroxides could be calculated from the 77K esr spectrum of the $\text{Cl}_2\text{TOS}^\text{-}$ [22] labelled alumina. To obtain an infinite dilution $(d_1/d)_{\text{dil}}$ value, a series of spin dilution experiments were performed. By reacting samples of alumina with either a 1:1.7 or a 1:4 mole ratio of ($\text{Cl}_2\text{TOS}^\text{-}$ [22]/cyanuric chloride) the spin density was successively diluted. The comparison of these spectra with the undiluted case is shown in Figure III-8. Since alumina packs evenly in CHC$_3$ these spectra represent the same amount of sample within the esr cavity. Relative receiver gain and signal to noise values can be used as a comparison of levels of spin labelling. The $d_1/d$ value for the 1:1.7 dilution gave a value of 0.43 and was used as the infinite dilution value. The $d_1/d$ value for the undiluted sample was 0.739 and the distance $\bar{r}$ calculated [as in IIIC(i)(d)] to be 1.24 nm. The 77K spectra of these two samples are shown in Figure III-9.

(iv) Controlled Pore Glass

In the same fashion as $\text{Al}_2\text{O}_3$, $\text{SiO}_2$ can also be spin labelled with $\text{Cl}_2\text{TOS}^\text{-}$ [22]. Dr. J. C. Waterton, while at UBC, reacted [22] with
Figure III-8. Ambient temperature chloroform esr spectra of (A) alumina labelled with [22] (same as Figure III-7(i)), Receiver Gain ($2 \times 10^3$), (B) alumina labelled with a 1:1.7 molar ratio of [22] to cyanuric chloride, Receiver Gain ($3.2 \times 10^4$), and (C) alumina labelled with a 1:4 molar ratio of [22] to cyanuric chloride, Receiver Gain ($5 \times 10^5$).
Figure III-9. 77K frozen chloroform esr spectra of (A) alumina labelled with undiluted [22] and (B) alumina labelled with a 1:1.7 molar ratio of [22] to cyanuric chloride.
modified controlled pore glass beads as shown below\textsuperscript{22}. First propylamine groups were bonded to the surface by at least one siloxane bond.

\[
\begin{array}{c}
\text{OH} \\
\text{OH} \\
\text{OH} \\
\end{array}
+ \begin{array}{c}
\text{(ETO)}_3 \text{Si(CH}_3\text{)}_3 \text{NH}_2 \\
\end{array}
\rightarrow
\begin{array}{c}
\text{Si} \text{NH}_2 \\
\end{array}
\]

Then the silica bound amino groups was reacted with Cl\textsubscript{2}TOS\textsubscript{2} [22] to give the spin labelled glass surface. The amount of propylamine groups attached to the surface was determined by elemental analysis to be \(6 \times 10^{20}\) ligands/gram. Of these about \(1 \times 10^{20}\) amines were reacted with label [22], as determined by esr double integration, to give an 18\% yield. The esr spectrum of this labelled silica is shown in Figure III-10. In his work, Dr. Waterton spin labelled the propylamino modified glass with a variety of other chemistries to develop techniques for studying modified surfaces.
(v) Summary and Conclusions

Several polysaccharides, cellulose, agarose, Sephadex G-25, guar gum, xanthan gum, and starch, have been spin labelled via the "trivalent" coupling reagent, cyanuric chloride. It was found that aqueous, alkaline conditions were suitable for the coupling procedure and that alkali-pretreatment of the insoluble polysaccharides before addition of the spin label, gave the highest yields. Consistently, the oxygen linked spin label triazine compound gave higher yields than did the nitrogen linked analogue.

From the esr spectra of these nitroxide labelled polysaccharides, a wealth of information concerning both the reaction itself and the resulting labelled materials can be obtained. This information includes: the
quantity of label attached; the mobility of the surface bound nitroxide; the effects of solvents on the environment of the polysaccharide and label; the difference between tightly bound and unbound "free" nitroxide molecules; evidence for covalent bonding between the polysaccharide and the triazine locus; and the average mean nearest neighbour distance between nitroxide labelled sites.

The quantitation of labels, by the esr double integration technique was found to give unacceptable results and instead elmental microanalysis was used. This technique gave reproducible results and control samples were used to check its validity. The number of monosaccharide units between each triazine spin label group, for the three insoluble polysaccharides, (cellulose, agarose, and Sephadex G-25) labelled with C\(^2\)TOS\(^2\) [22], were 26, 10, and 20 units respectively. Labelling with the nitrogen analogue C\(^2\)TNHS\(^2\) [12] gave levels of 37, 40, and 120 units respectively.

From the line shapes of the esr spectra it was shown that water penetrates these polysaccharides to solvate the polysaccharide fibers and the appended nitroxide, whereas chloroform does not penetrate and leaves the polysaccharide essentially as if it were a dry powder.

The esr spectra of these labelled polysaccharides, along with various control experiments, gave strong evidence supporting the existence of a covalent bond and also could be used to distinguish between bound and unbound "free" nitroxide moieties. This latter information can be used to determine correct washing procedures to remove unreacted materials and is of substantial practical importance for the textile industry, for example.

The average mean distance \(\bar{r}\) between neighbouring nitroxides was
found to be 1.3 nm in labelled cellulose and 2.1 nm for labelled agarose. The distance in labelled Sephadex must have been larger than 2.5 nm since no dipolar interaction was detected. The fact that the labels on cellulose are closer together than in the other two polysaccharides and yet out of these three, the quantity of labels on cellulose was the lowest, is a reflection of the smaller accessible surface area of cellulose due to its many highly crystalline regions.

Bovine serum albumin (BSA), aluminum oxide, and controlled pore glass were also derivatized with cyanuric chloride. About 12 out of 55 available ε-lysine residues of BSA were labelled with the spin labelling reagent C\textsubscript{2}TOS\textsubscript{2} [22].

Aluminum oxide was also labelled with this same reagent to the extent of about $2 \times 10^{19}$ spins per gram with a mean average distance between spins of 1.24 nm. Strong evidence, for a control experiment also obtained from esr spectroscopy, supported the existence of a covalent bond between the alumina surface hydroxyl groups and the triazine moiety.

A chemically modified silica surface (propylamine modified controlled pore glass) was also labelled with the same spin label triazine at a level of about $1 \times 10^{20}$ spins per gram.

We suggest that the use of spin labelling via cyanuric chloride offers a powerful test vehicle for the future modification of these and other substrates. Information such as optimum reaction conditions, distances, solvent substrate interactions, evidence for covalent bonds, etc., can then be applied more generally to the understanding of other triazine mediated surface modifications whose products might otherwise be difficult to characterize.
IIID: Nmr Spectroscopy

The nmr spectroscopy in this section is restricted to the spectra obtained from some of the compounds synthesized in section IIIB and C. No background to the technique will be given here because nmr spectroscopy is now used so routinely as a structural tool by organic and inorganic chemists.

Normal $^1$H nmr spectra can be obtained for the diamagnetic compounds of section III B and C as shown in the two example spectra of compounds [21] and [32] in Figure III-11 and Figure III-12 respectively. The protons in the three sugar rings of [32] (Figure III-12) are all equivalent because of the $C_3$ symmetry axis which exists through the center of the triazine ring.

The majority of the compounds in IIIB(i) and (ii) are, however, paramagnetic since they contain the free radical nitroxide group. This broadens the lines in the proton spectrum considerably and makes assignments and hence structural information difficult to extract. This problem can simply be overcome though, by reducing the nitroxide with sodium hydrosulfite (dithionite $Na_2S_2O_4\cdot2H_2O$). Other reducing agents besides dithionite can be used$^{25}$ and also, this step can be reversed by reoxidizing$^{25}$ the reduced label. To illustrate this reduction technique the spectrum of compound [36] is shown in Figure III-13, before and after
Figure III-11. Proton nmr spectrum (270 MHz) of compound [21] in deuterioacetone.
Figure III-12. Proton nmr spectrum (270 MHz) of compound [32].
Figure III-13. Proton nmr spectrum (270 MHz) of compound [36] before and after reduction.
reduction with dithionite. The resulting nmr spectrum of this compound is indeed very interesting. There does not exist a proper symmetry element in this molecule and it is therefore surprising that both sugar rings appear equivalent in the spectrum. The problem here is the same as that encountered earlier for the ferrocene analogue in chapter II. If the bond joining the oxygen atom of the nitroxide to the triazine ring is able to rotate freely (fast on the nmr time scale) then there can be said to exist a pseudo $C_2$ axis of symmetry which can make the two sugar moieties effectively equivalent. This nitroxide group is rather small in size and innocuous in electronic nature in comparison to the aromatic ferrocene group of compound [37]. Due to this, the nitroxide group would not be expected to create as large a chemical shift differential between the two sugar rings and therefore a slower bond rotation is required to create the pseudo $C_2$ symmetry axis needed to make the protons of the two sugar rings appear equivalent.

![Chemical structure](image)

[37]

To prepare the reduced samples, the compound was first dissolved in deuteriochloroform and then washed with aqueous sodium dithionite. As the nitroxide became reduced its orange color disappeared, however, due to rapid decomposition of the dithionite in water, this washing must be repeated a few times. The deuteriochloroform layer was then
separated and dried over sodium sulfate before its nmr spectrum was run.

IIIE: Esr Spectroscopy of Nitroxides

The following section has been adapted, in part, from a Ph.D. thesis written by Dr. J. D. Aplin and a B.Sc. thesis written by Art van der Est.

Electron spin resonance (esr) occurs in a paramagnetic molecule when transitions between the Zeeman levels, whose degeneracy may be lifted by the application of a magnetic field \( H \), are induced by an electromagnetic field \( H_1 \) of frequency \( v \). The separation of these Zeeman levels is described in

\[
hv = g\beta H
\]  

(1)

where \( h \) is Plank's constant, \( \beta \) the Bohr magneton \( (eh/2m) \), \( m \) the mass of the electron, and \( g \) a dimensionless parameter related to the effective magnetic moment of the electron \( (\mu_e) \) by

\[
\mu_e = -g\beta S
\]  

(2)

where \( S \) is the spin angular momentum vector. Differences in the Zeeman energy between different molecules result in changes in \( g \) from its free electron spin-only value of 2.00232, as a result of spin orbit coupling. Thus the \( g \) value is used to characterise the position of the resonance in the frequency spectrum.

The spin Hamiltonian for a nitroxide electron is given by

\[
\hat{H} = \hat{H} \text{(Zeeman)} + \hat{H} \text{(hyperfine)} + \hat{H} \text{(dipolar)} + \hat{H} \text{(exchange)}
\]  

\[
= \beta \cdot H \cdot \hat{S} + \hat{S} \cdot \hat{T} \cdot \hat{I} + \hat{S} \cdot \hat{D} \cdot \hat{S} + J \cdot \hat{S} \cdot \hat{S}
\]  

(3)

where \( \hat{S} \) and \( \hat{I} \) are the electron and nuclear spin operators respectively, and the nuclear Zeeman term has been omitted. The electron g-value, and \( A \), the electron-nuclear hyperfine coupling, are required to be expressed...
as second-rank tensors because they represent direction-dependent quantities which in turn reflect the symmetry of the molecule.

The hyperfine coupling constant consists of a contact term

\[ a_0 \hat{I} \cdot \hat{S} \]  

(4)

where the isotropic coupling constant \( a_0 \) is a scalar defined by

\[ a_0 = \frac{8\pi}{3} g \beta^g N \beta^N \psi(0)^2 \]  

(5)

and \( \psi(0) \) is the unpaired electron wave function at the nucleus: and a dipolar interaction given by

\[ \hat{I} \cdot \hat{S}' = -g \beta^g N \beta^N \frac{(\hat{I} \cdot \hat{S})r^2 - 3(\hat{I} \cdot \hat{r})(\hat{S} \cdot \hat{r})}{r^5} \]  

(6)

where \( r \) is the electron-nucleus distance vector. While the contact term requires \( S \)-orbital character in \( \psi(\mathbf{r})(|\psi(0)|^2 \neq 0) \), the dipolar term disappears if the electron distribution has spherical symmetry. In the present case, as shown below,* the principal hyperfine interaction occurs between the electron, shown in the nitrogen \( 2p_z \) orbital, and the nitrogen nucleus \( (I = 1) \). The transitions obey selection rules \( \Delta m_I = 0, \Delta m_S = \pm 1 \)

*adapted from ref. 21.
as shown in Figure III-14.

The direction-dependence of the Zeeman and hyperfine interactions may most clearly be demonstrated experimentally by the esr spectrum of a diamagnetic host crystal "doped" with nitroxide (Figure III-15). Both the position $g$ and the splitting of the lines $A$ are direction-dependent giving rise to the principal values $g_{xx}$, $g_{yy}$, $g_{zz}$; $A_{xx}$, $A_{yy}$, $A_{zz}$. As might be expected from the molecular symmetry, both tensors are approximately axially symmetric, that is,

$$g_{xx} \neq g_{yy} \neq g_{zz}$$

and

$$A_{xx} \neq A_{yy} \neq A_{zz}$$

These are sometimes given formal axial symmetry by the notation

$$g_{xx} = g_{yy} = g_1$$

$$A_{xx} = A_{yy} = A_1$$

$$g_{zz} = g''$$

$$A_{zz} = A'_z$$

The spectrum obtained from a dilute solution of nitroxide also contains three sharp lines (Figure III-16), but here the $g$ and $A$ anisotropies ($g''$, $g_1$ and $A''$, $A_1$) have been averaged so that only the isotropic splitting constant $a_0$ remains. Figure III-16 also shows the spectrum of a polycrystalline array of nitroxides, as might be obtained by rapid-freezing a dilute solution into a glass. All possible orientations of the nitroxide contribute to the spectrum, which is simply the sum of resonances due to the orientations shown in Figure III-15 together with all others. It is clear, therefore, that while the central maxima contains contributions from all orientations, the outer extrema of the spectrum are due to radicals oriented with the molecular z-axis parallel to the external field,
Figure III-14. Energy level diagram for a nitroxide ($S = \frac{1}{2}$) in a magnetic field with hyperfine coupling to the spin 1 nitrogen nucleus (adapted from reference (21) Swartz, Bolton & Borg).
Figure III-15. Directional dependence of Zeeman and hyperfine interactions demonstrated by the spectrum of a diamagnetic host crystal "doped" with nitrooxide (adapted from reference (27)).
Figure III-16. ESR spectra of magnetically dilute di-t-butyl nitroxide (a) in a polycrystalline solid, (b) a viscous solution, and (c) a non-viscous solution showing g- and A-anisotropies (adapted from reference (21) Wertz & Bolton).
and that at the "rigid limit" the splitting between gives a measure of $2A_n$.

Between the two extremes of non-viscous solution (where $\tau_c \approx 10^{11}$s for a small molecule) and polycrystallinity (where $\tau_c > \approx 10^{-7}$s), partial averaging of anisotropic $g$ and $A$ quantities occurs (Figure III-16). It is clear then, from both Figures III-15 and 16, that as molecular tumbling is slowed, the high field line begins to broaden followed by the low field line and then the central line. Tumbling of a molecule can be both isotropic (tumbling equally about all axes) or anisotropic (restricted motion about some axes), however, contributions by anisotropic motion will not be discussed here.

Figure III-17 shows a series of spectra obtained from a system where the isotropic notion is decreasing and correlation times are increasing. Again, as the motion is reduced, the high field line broadens before the central or low field lines until in (f) the spectrum resembles the polycrystalline spectrum where the splitting $2A$ can clearly be seen.

This line broadening effect, caused by reduced motion, can be a sensitive probe for the size of the molecule containing the nitroxide. In Figure III-18 the solution spectrum of compounds [11] and [36] are compared. Here the difference between molecular weights of 172 and 976 can easily be seen in the broadening and subsequent reduction in the peak-to-peak height of the high field line. From such spectra one can also calculate the correlation time $\tau_c$ for the molecule.

The peaks in the spectra of isotropically tumbling molecules are assumed to be lorentzian. When this is the case the peak-to-peak height and line width are functions of the transverse relaxation time $T_2$. Stone et al. have shown this as given by
Figure III-17. Esr spectra of $[11]$ HOS$^2$ in aqueous glycerol solutions where the viscosity increases and the temperature lowered from (a)-(f) (adapted from reference (21) Swartz, Bolton & Borg).
Figure III-18. ESR spectrum of dilute methanol solutions of the nitroxide [11] HOSl (upper spectrum) and the nitroxide [36] (lower spectrum).
\[
[T_2(m_I)]^{-1} = \tau_c \{[3I(I+1) + 5m_I^2]b^2 + 4(\Delta \gamma H_0)^2 - \frac{4}{15}b \Delta \gamma H_0 m_I \} + X
\]  

(13)

where \( \tau_c \) = correlation time, \( I, m_I \) = nuclear angular momentum quantum numbers, \( H_0 \) = applied field strength and

\[
b = \frac{4}{3} \pi (A_{zz} - A_{xx}) \text{ in } H_z
\]  

(14)

\[
\Delta \gamma = \frac{-\beta e}{h} [g_{zz} - \frac{1}{2}(g_{xx} + g_{yy})]
\]  

(15)

\( X \) is a function which accounts for other contributions to the line width.

This expression is only valid under the following conditions: (1) the hyperfine interaction is axially symmetric, i.e., \( A_{xx} = A_{yy} \); (2) the molecular motion is isotropic and sufficiently slow that \( \omega^2 \tau_c^2 \gg 1 \) (where \( \omega = \frac{\beta e H_0}{h} \) and \( b^2 \tau_c^2 \ll 1 \), so that line widths are influenced; and (3) that \( \tau_c^2 \ll 1 \), which ensures that the three lines do not overlap. Thus at X band the equations are applicable in the range

\[ 5 \times 10^{-11} < \tau_c < 5 \times 10^{-9} \text{ s.} \]

Usually for nitroxides in low viscosity solvents these conditions are met so that if we set \( I = 1 \) for nitrogen in (13) the following expression is obtained:

\[
\frac{T_2(0)}{T_2(m_I)} = 1 - \tau_c T_2(0) \left[ \frac{4}{15} b \Delta \gamma H_0 m_I - \frac{b^2}{8} m_I^2 \right]
\]  

(16)

The ratio \( T_2(0)/T_2(m_I) \) can be expressed in terms of the ratio of the peak-to-peak heights by

\[
\frac{T_2(0)}{T_2(m_I)} = \left( \frac{h_0}{h_{m_I}} \right)^{\frac{1}{2}}
\]  

(17)

where \( h_{m_I} \) is the peak-to-peak height in arbitrary units. \( T_2(0) \) is related to the line widths by

\[
T_2(0) = \frac{1}{\pi \sqrt{3} \Delta v(0)}
\]  

(18)

where \( \Delta v(0) \) is the line width for the central peak in \( H_z \). If we then
insert (17) and (18) into (16) we get

\[
\frac{h_0}{h_{m_1}} = 1 - \frac{\tau_C}{\pi\sqrt{3}\Delta\nu(0)} [C_1m_1 + C_2m_1^2]
\] (19)

where

\[
C_1 = \frac{4}{15}b\Delta\gamma H_0
\] (20)

\[
C_2 = \frac{b^2}{8}
\] (21)

It is now possible to solve for \(\tau_C\) with \(m_1 = +1\) and \(m_1 = -1\) in terms of
\(C_1\) or \(C_2\). Values of \(\tau_C\) derived with \(C_1\) have been found to be very
dependent on the microwave power \(^{21}\) so that it is best to avoid using \(C_1\)
unless the microwave dependence is known. Therefore we solve (19) for
\(\tau_C\) in terms of \(C_2\)

\[
\tau_C = \frac{\frac{h_0}{h_1}}{2} + \frac{\frac{h_0}{h_{-1}}}{2} - 2 \frac{\pi\sqrt{3}\Delta\nu(0)}{2 C_2}
\] (22)

\[
= \frac{\frac{h_0}{h_1}}{2} + \frac{\frac{h_0}{h_{-1}}}{2} - 2 \frac{9\sqrt{3}\Delta\nu(0)}{4\pi(g^2\Delta H(0))^2}
\] (23)

Because it is easier to measure the line widths in gauss than in hertz
we convert \(\Delta\nu(0)\) to \(\Delta H(0)\) and \(A_{zz} - A_{xx}\) from hertz to gauss by Eq. (1)
with \(g\) taken as the averaged \(g\) value. In gauss Eq. (23) is:

\[
\tau_C = \frac{\frac{h_0}{h_1}}{2} + \frac{\frac{h_0}{h_{-1}}}{2} - 2 \frac{9\sqrt{3}h(\Delta H(0))}{4\pi(g^2\Delta H(0))^2}
\] (24)

Then using \(A_{zz} = 31.1\) gauss, \(A_{xx} = 5\) gauss, and \(g = 2.0059^{27}\) in Eq. (24)
values of \(\tau_C\) for compound [11] and [36] in methanol are calculated to be
\(1.4 \times 10^{11}\) s and \(1.1 \times 10^{10}\) s respectively. The assumption of isotropic
tumbling for compound [36] may be unrealistic but these figures, however,
seem intuitively reasonable. Under the author's guidance, a fourth year
student, Art van der Est, calculated the \(\tau_C\) values (Table III-4) for the
following compounds in chloroform. Again, these numbers follow the
TABLE III-4: Correlation times for nitroxides in chloroform solution

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\tau_c$ (s)</th>
<th>MW</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>$2.38 \times 10^{-11}$</td>
<td>171</td>
</tr>
<tr>
<td>12</td>
<td>$6.46 \times 10^{-11}$</td>
<td>319</td>
</tr>
<tr>
<td>39</td>
<td>$9.44 \times 10^{-11}$</td>
<td>711</td>
</tr>
</tbody>
</table>

expected order and approximate magnitudes. The $\tau_c$ value for synthetic glycolipid compound [39] also provides some evidence for the proof of structure since $\tau_c$ clearly indicates [39] is a much larger molecule than its precursor [12].

As well as line broadening due to $A$ and $g$ anisotropies there can also be broadening due to either dipolar or exchange, electron-electron interactions. Both are dependent upon the concentration of mobility of the nitroxide. Dipolar broadening is due to the dipole-dipole interaction between electron spins. Each unpaired electron "feels" a localized field due to the motions of all of the other electrons. In a randomly
oriented sample, where the molecular motion is rapid, the dipolar effects are average to zero; however, in an oriented sample where the motion is slow, each electron experiences a slightly different field due to its local environment. Clearly the magnitude of this effect is dependent upon the separation between adjacent unpaired electrons and hence depends on the concentration. Thus if we can measure the dipolar broadening we can calculate the distance between adjacent radicals. Dipolar broadening is also temperature dependent and as the temperature rises the dipolar effects begin to average.

The other form of electron-electron interaction, that can affect line widths, is electron exchange. This effect can be illustrated clearly in Figure III-19. For simplicity let us consider the radical to exist in two states, A and B, such that the g factor is different in the two states and the separation between the two resonant fields for A and B is $\delta H$. As the electrons begin to exchange between the two states A and B, that is,

$$A \rightleftharpoons B$$

the spectra in (b)-(e) will result. If the exchange rate R is faster than the frequency difference between A and B, that is where $R < \frac{1}{\gamma_e \delta H}$, then we will see an average of the two signals as in (d) and (e) and the line is said to be exchange narrowed. If, on the other hand, the rate is much slower than this splitting, that is where $R > \frac{1}{\gamma_e \delta H}$, then the spectrum in (b) is obtained and the lines are said to be exchange broadened. When $R \approx \frac{1}{\gamma_e H}$ we can no longer distinguish between individual states and the spectrum in (c) results. This model system can also be used to explain the effects of the tumbling rate $\tau_c$ on the line widths of the nitroxide spectra, i.e., averaging or non averaging of g and A anisotropies.
Figure III-19. Effects of the rate of exchange between A and B on the esr spectra (a) no exchange, (b) slow exchange, (c) intermediate exchange, (d) and (e) fast exchange (exchange narrowing) (adapted from reference (21) Swartz, Bolton & Borg).
From the dipolar interaction the distance between nitroxides can be measured, as was shown previously in section IIIC (i)(d).

If we assume a random three dimensional distribution of spins we can relate the mean nearest neighbour distance to the density of spin labels by:

\[ \bar{r} = \left( \frac{4}{3} \pi \rho \right)^{-1/3} \Gamma \left( \frac{4}{3} \right) \]  

where \( \rho \) is the density in \( \text{nm}^{-3} \), \( r \) is the gamma function \( \Gamma(n) = \int_{0}^{\infty} e^{-x} x^{n-1} \, dx \) and \( \Gamma \left( \frac{4}{3} \right) = 0.89261^{29} \), thus

\[ \bar{r} = 0.55373 \rho^{-1/3} \]  

The density is related to the molar concentration by:

\[ \rho = 0.602 [S\%] \]  

where \([S\%]\) is the molar concentration of spin label. Substituting Eq. (26) into (25) we get:

\[ \bar{r} = 0.656 [S\%]^{-1/3} \]

Measuring the effective concentration of spin labels in the labelled sample cannot be done directly, however, since the dipolar broadening is concentration dependent it can be measured indirectly. The spectral parameter which is a measure of the broadening is \( \frac{d_1}{d} \), where \( d_1 \) is the total intensity of the extreme components of the spectrum at 77 K, and \( d \) is the intensity of the central peak as shown in Figure III-5. Using homogeneous solutions of varying concentrations for both 2,2,6,6-tetramethylpiperidine-1-oxyl in methanol and 4-amino-2,2,6,6-tetramethylpiperidine-1-oxyl in aqueous glycerol, Drs. J. D. Aplin and J. C. Waterton found that at 77 K, \( \frac{d_1}{d} \) is related to the concentration by:

\[ \frac{d_1}{d} = \left( \frac{d_1}{d} \right)_{\infty \text{dil}} + 2.04 [S\%] \]  

(27)
Figure III-5. Powder spectrum showing the heights $d_1$ and $d$ and the splitting 2A.
It was found that when the concentration is reduced below a critical value \( \sim 5 \times 10^{-3} \text{ M} \) no dipolar broadening exists and \( d_1/d \) remains constant. This is the value of \( d_1/d \) at infinite dilution \( (d_1/d)_{\infty \text{dil}} \). This number varies somewhat from system to system and is a function of solvent polarity and the amount of residual motion at 77 K. Typical values are close to 0.4. Combining Eq. (27) and (26) gives:

\[
\frac{d_1}{d} = (\frac{d_1}{d})_{\infty \text{dil}} + 0.58 \frac{1}{r^3}
\]

This equation can then be used for any system to calculate distances between nitroxides.

The distance between nitroxides in the biradical (25) can also be calculated using this technique. The distances between nitroxides of a variety of biradicals have previously been determined by Kokorin et al. and Kalikov et al. using, in principal, the same empirical method.

In the distance measurement of (25) a \( (d_1/d)_{\infty \text{dil}} \) value was obtained by using the \( d_1/d \) value (0.467) for \( C\text{Cl}_2\text{TOS} \) [22] at \( 2 \times 10^{-4} \text{ M} \) in chloroform at 77 K. The biradical must also be run at infinite dilution as well so that only dipolar interactions between nitroxides within the same molecule are detected. A value of 0.813 for the \( d_1/d \) of (25) in a 2 \( \times \) 10\(^{-4} \) M chloroform solid solution at 77 K, gave a biradical distance
of 1.19 nm. The frozen spectra of the monoradical [22] and the biradical [25] are shown in Figure III-20.

The room temperature solution spectrum of biradicals can also be very interesting. The solution spectrum of [25] shows (Figure III-21) five lines as opposed to three for a monoradical, because there are two nitrogen spin 1 atoms present and the total spin quantum number takes all integer values from 2 to -2. The five lines are only seen, however, in biradicals that have a high enough frequency of electron exchange between nitroxides. If the frequency of exchange is greater than the hyperfine interaction frequency, the mean residence time of each electron at the two nuclei will be the same and the esr spectrum will consist of five equidistant lines with a ratio of intensities of 1:2:3:2:1. This exchange rate is of course, dependent upon the distance between radicals and, as in the case of compound [25], they are too far apart for complete exchange and as a result the +1 and -1 lines have a very low intensity. Biradicals also have been used as spin probes 17,27.
Figure III-20. 77K infinite dilution chloroform esr spectra of (A) the monoradical [22] and (B) the biradical [25].
Figure III-21. Ambient temperature chloroform esr spectra of (A) the monoradical [11] and (B) the biradical [25].
References


8. J. Danner, H. M. Lenhoff, W. Heagy, J. Solid-Phase Biochem., 1, 177(1976), CA 8821-148369Q.


CHAPTER IV

SUMMARY

Ability to chemically modify carbohydrates, in a variety of different ways, is of great importance and interest. In the context of the present study the term "chemical modification" refers mainly to the attachment of chemical ligands which possess unique specific properties rather than in the sense of blocking-group technology or other purely synthetic carbohydrate procedures. It was the intention of this work to synthesize materials which possess the unique characteristics of carbohydrates, plus the equally unique properties of the appended group of interest. It is hoped that such materials and the underlying chemical technology for their production will have broad applications in a variety of areas of chemistry, some of which have been alluded to in the text of this thesis.

In chapter I the synthesis of metal chelate complexes of sugars was demonstrated by the formation of Schiff's base complexes derived from amino sugars and either salicylaldehyde or 3-formyl-2-hydroxy-benzoic acid. A variety of novel complexes were formed, mostly involving copper (II), including a binuclear complex and a water-soluble, sugar-copper complex. The main impetus behind the work in chapter I, and also that in chapter II, was the hope that the combined presence of sugar and metal moieties would impart to these compounds interesting biological properties, and hence that this chemistry may be useful in the formation of pharmaceuticals. Unfortunately, time did not permit for the
evaluation of those properties, or indeed of the other numerous, potential applications of this chemistry outlined in the introductions to these chapters.

The work in chapter II represents an extension of chapter I to include organometallic π-complexes. Specifically, ferrocene monosaccharide complexes were synthesized to yield both organic- and water-soluble compounds. Here too, it is not naive to suggest numerous applications in the pharmaceutical industry where ferrocene is ideal because of its low toxicity and the ease of substitution of this molecule. Thus, ferrocene derivatives of penicillin and cephalosporin have been prepared; some exhibit high antibiotic activity while others have proven to be potent β-lactamase inhibitors. Some ferrocene derivatives are also known to be useful as hematinic agents. Again, as suggested in the introduction to that chapter, there are also numerous other possible applications of this chemistry. It is also important to note that it was found that proton spin lattice relaxation rates could be used to assign the proton resonances of the substituted cyclopentadienyl ring of ferrocene derivatives. This technique, of course, is not restricted to sugar ferrocenyl compounds but can be applied to any ferrocene derivative. Indeed it should find widespread use in numerous other families of organometallic substances.

In chapter III, some of the potential limitations of the chemistry described in chapters I and II were explored by the investigation of the reactions of cyanuric chloride with carbohydrates. With this reagent it was not only possible to couple metals to monosaccharides, but also to link a variety of other substances, including spin labels and hydrophobic alkyl groups to both mono- and poly-saccharide families of carbo-
hydrates. The main thrust of this chapter involved the nitroxide spin labelling of these carbohydrates via cyanuric chloride. This was shown to be important to future work in this area since the spin label group can relay a great deal of information about the interaction of cyanuric chloride and its derivatives with polysaccharides. It is often particularly difficult to characterize chemical reactions of macromolecular derivatives, especially when the level of derivatization is low, and knowledge gained from spin labelling can be very helpful in this respect. Although ferrocene-triazine derivatives and a chromium organometallic triazine derivative were synthesized in chapter II, no direct link between this chapter and the work in chapter I was made. However, the combination of these two areas, whereby metal chelate ligands can be attached to the triazine ring, leaving at least one displaceable chlorine substituent, could well lead to some very useful compounds. Metal chelate reagents produced from this combination should find many applications in areas such as affinity chromatography and polymer supported heterogeneous catalysis to name just two. As was also demonstrated, this s-triazine chemistry is not limited to carbohydrates but can be extended to the derivatization of other materials such as proteins, alumina and glass. Many extensions of the present studies whether in whole, or in part, may therefore be expected both in the carbohydrate field and in other areas. In retrospect, it is a pity that time did not permit the author to have evaluations made of the properties of some of the substances synthesized here. Those evaluations, together with the extensions alluded to above could well, however, constitute the research work of a considerable army of graduate students; certainly their evaluation far exceeds the scope of one student, even in this laboratory!
References


VA: Electron Spin Resonance

Esr spectra were recorded at X-band using a Varian E-3 instrument in the derivative absorption mode and integrated using a Pacific Precision Co. MO-1012A integrator. The second integration was performed by peak cutting and weighing and comparison with freshly-prepared standard solutions of the 4-hydroxy nitroxide [11]. Spectrometer settings—modulation amplitude, filter time constant and scan rate—were chosen in each case to avoid distortion of the spectral lines, and power levels were non saturating. The ambient temperature was always 25°C ± 1, and the field always increased from left to right. Line widths were measured using the smallest possible scan range (generally not that shown in the diagrams) and at least two measurements were made using different scans in each case. The field was calibrated using a proton nmr magnetometer and the X-band microwave frequency was monitored on a Hewlett-Packard 5245-L electronic counter equipped with a 8-18 GHz frequency converter.

The resonance frequency is dependent upon the applied field: most experiments, including the ones described herein, were conducted at X-band (about 9.1-9.5 HGz), which corresponds to an external field of the order of 3-3.4 KG. In the experiments described here, net absorption of microwave energy from H_1 occurs at resonance as a result of the great proportion of spins present in the lower energy state. Absorption of energy is
monitored with the aid of phase sensitive detection using field modulation at $10^5$ Hz (provided by Helmholtz coils on each side of the cavity and recorded as the first derivative of the absorption signal).

Spectra at 77K were obtained using a Dewar insert containing liquid nitrogen, at 0.16 mW microwave power, the lowest available. Oxygen was prevented from condensing in the sample tube by sealing the top with a rubber septum cap. All low temperature spectra were recorded in 3 mm i.d. quartz tubes when non polar organic solvents were used and in 1 mm i.d. Pyrex tubes for aqueous solutions. At room temperature, both these types of tubes were used along with a flat high-quality quartz cell, capacity 73 µL, with ground glass joints at both ends (J. Scanlon Co.). The 3 mm i.d. tube was preferred for use with the dilute chloroform solutions of the copper complexes since more sample could be placed in the esr cavity thereby giving a better signal to noise ratio. Powder samples of metal complexes were run inside melting point capillary tubes placed inside a 3 mm i.d. quartz tube. A teflon insert designed by Dr. F. G. Herring was used for aqueous slurries of water insoluble polysaccharides. This consisted of a cylinder of diameter 10 mm with a half-cylinder section 30 mm long cut away in the center, forming a flat surface $10 \times 30$ mm upon which the wet polysaccharide was placed beneath a glass cover slip, the latter retained by surface tension. Solutions of nitroxides, whose spectra were to be used for correlation time ($\tau_c$) measurements, were deoxygenated by bubbling nitrogen through for several minutes.
VB: Nmr Measurements

Proton nmr spectra were measured at 270 MHz with a prototype of a home-built spectrometer based on a Bruker WP-60 console, a Nicolet 1180 computer (32K), a Nicolet 293A pulse controller unit, a Diablo Disk, and an Oxford Instruments Superconducting solenoid. Concentrations of normal samples ranged from 0.01 to 0.10 M and $T_1$ measurements were performed in concentrations of $< 0.5$ M, with degassing achieved by five freeze pump thaw cycles. All deuterated solvents were obtained from Merck Sharp and Dohme (Montreal, Canada) and tetramethylsilane was used as a standard.

Relaxation data were obtained using the standard Nicolet software for the phase alternating inversion recovery experiment $(180^\circ-x-t-90^\circ-Acq-Delay-180^\circ-x-t-90^\circ-Acq-Delay)_n/2$ and the $R_1$-values were calculated using a $\ln(M_\infty-M_t)$ vs $t$ plot\(^1\). Only pre-null point relaxation data were used in these plots to accommodate the initial slope approximation\(^2\).

VC: General Synthetic Procedures

All solutions were concentrated using a Buchi rotary evaporator. All melting points were determined using a Thomas Hoover Unimelt instrument (Model 6406-K) and are corrected. All optical rotations were determined using a Perkin-Elmer Polarimeter (model 241-MC).

Thin layer chromatography (tlc) was performed on silica gel plates (Baker-flex Silica gel 182-F) using the following solvents: (A) 1:1, toluene: ethyl acetate; (B) 4:1 toluene: ethyl acetate; (C) 1:5 methanol: chloroform. All compounds were checked for purity by tlc using one of these solvent systems. Column chromatography was performed
using 100-200 ASTM mesh silica (Fischer) packed in columns approximately
2.5 x 50 cm, and eluted with solvents (A), (B), or (C). For reactions
requiring anhydrous (dry) solvents the solvents were dried by standard
methods, distilled and stored under a nitrogen atmosphere. The source
of all chemicals and materials will be given in the experimental section
of each chapter.

All microanalysis was performed by Mr. P. Borda, of this department.
The mass spectra were obtained using an Atlas CH-4B mass spectrometer and
high resolution determinations were obtained using an AEI MS-9 or an
MS-50 mass spectrometer.

VD: Chapter I

(i) Sources of Materials

Sources for the key chemicals used in the synthesis of compounds in
this chapter are as follows: salicylaldehyde was obtained from Fisher and
was vacuum distilled before use; salicylic acid (Malinckrodt); hexam-
ethylenetetramine (Matheson Coleman and Bell); cyclohexylamine,
t-butylamine, n-butylamine and t-propylamine (Eastman); D-glucosamine
hydrochloride (Sigma); carbobenzylxy-chloride (CBZ chloride) (Aldrich);
anisaldehyde (Matheson Coleman and Bell); cupric and cobaltous acetate
(Fisher); zinc acetate (Matheson Coleman and Bell); nickel acetate
(Baker). p-acetamido-benzensulphonyl chloride was prepared by the method
in Vogel$^3$.

(ii) Literature Preparations

A brief summary will now be given for the synthesis of compounds
that were repeated from the literature. Any problems encountered or
changes made in the recipes will be noted.
The compound, 3-formyl-2-hydroxy-benzoic acid [2], was prepared by the method of Duff and Bills\(^4\). A typical preparation involved boiling salicylic acid (40 g) with hexamethylenediamine (27 g) in water. The mixture is then acidified and the yellow precipitate extracted with benzene. This extraction procedure separates the resulting 3-formyl- and 5-formyl-2-hydroxy-benzoic acid mixture since the latter is insoluble in benzene. The barium salt of the title compound is then formed in basic aqueous solution with subsequent hydrolysis by HCl to give the compound in about a (3 g) yield.

The glycoside methyl 3,4,6-tri-O-acetyl-2-amino-2-deoxy-\(\beta\)-D-gluco-pyranoside hydro-bromide [7] was prepared by a combination of recipes from Chargaff\(^5\), Gross\(^6\), and Irvine and Earl\(^7\). First, the carbobenzyloxy derivative of glucosamine was prepared by the method of Chargaff\(^5\). This product was prepared readily by mixing one equivalent of glucosamine hydrochloride and carbobenzyloxy chloride (CBZ chloride) with two equivalents of sodium bicarbonate in water. The product precipitated in a high yield ~90% and was filtered and dried. Next the glycosyl bromide was prepared by the method of Gross et al.\(^6\). A mixture of acetic acid, acetic anhydride, and HBr was added at 0°C to the CBZ glucosamine in portions and after 30 minutes HBr gas was bubbled through the mixture rapidly until the temperature was raised about 10°C. If anhydrous conditions were met the glycosyl bromide usually precipitated during or shortly after the HBr gas was introduced. Ether was then added and after refrigeration for a few hours, the product was filtered. The glycosyl bromide was recrystallized by dissolving it in chloroform at ambient temperature, evaporating this to a small volume with a stream of nitrogen and then adding a small amount of anhydrous ether. The methyl
glycoside was then formed by the method of Irvine and Earl\(^7\) whereby a 5% solution of the glycosyl bromide in anhydrous methanol containing 1% of anhydrous pyridine, was let stand overnight. The mixture was then evaporated under anhydrous conditions to a small volume and ether added. Upon refrigeration the methyl glycoside [7] was obtained.

The tetra-O-acetyl-glucosamine derivative [9] was prepared by the method of Bergman\(^8\), using anisaldehyde (p-methoxy-benzaldehyde) to protect the amino function. First, the Schiff's base was formed by mixing glucosamine hydrochloride [5] and anisaldehyde in water with one equivalent of sodium bicarbonate. The compound formed readily and was filtered from the reaction mixture. This material, when dry, was then acetylated in a 1:1 mixture of acetic anhydride and pyridine to yield the tetra-acetate [13]. The Schiff's base was then cleaved by treating the acetate with a dilute aqueous acetone solution of HCl. The starting acetate sugar [13] and salicylaldehyde were then extracted with chloroform and the aqueous layer neutralized with sodium bicarbonate. Once neutral, the aqueous layer was extracted again with chloroform to give the "free" amino sugar [9]. The product was then recrystallized from an ethanol-water mixture; m.p. 138-139°C. The Schiff's base starting material that was not hydrolyzed initially, was recovered from the first chloroform extraction and treated again with acid.

The sugar salicylaldimines [6], [8], and [10] were all prepared readily by the method of Irvine and Earl\(^9\) by mixing vigorously, the amino sugar and salicylaldehyde together in water or in a methanol water mixture, with one equivalent of sodium bicarbonate added in the preparation of compounds [6] and [8]. The mixtures were then filtered after about 3 h and the products obtained in greater than 80% yields. Recrystal-
lization from either methanol or ethanol gave the pure compounds [6], [8], and [10].

The preparation of the salicylaldimine metal complexes Cu (cyclohexyl-sal)$_2$, Cu (n-Bu-sal)$_2$, Cu (t-Bu-sal)$_2$ and Cu (i-Pr-sal)$_2$ were readily prepared by the original method of Schiff and repeated from a more recent reference by Holm$^{10}$. Both copper (II) and nickel (II) complexes were prepared in yields $\geq 60\%$ by refluxing in ethanol 0.1 mole of the appropriate nickel or copper salicylaldehyde complex with a 10% mole excess of the amine. The products were obtained by cooling the reaction mixture and filtration of the precipitate. Zinc and cobalt complexes can also be prepared by this method.

The salicylaldehyde metal complexes were easily prepared by mixing salicylaldehyde with the appropriate metal acetate in aqueous alcohol.

(iii) Synthesis

To a solution of amino sugar [7] (0.51 g, 1.27 m mol) in methanol (4 ml) was added a solution of sodium bicarbonate (0.25 g, 3 m mol) in H$_2$O (4 ml) and a solution of 3-formyl-2-hydroxy-benzoic acid (0.22 g, 1.28 m mol) in methanol (4 ml). The mixture was stirred at room temperature for 1 h and then evaporated to dryness and a small amount of ethanol added. The resulting precipitate was filtered, washed with cold ethanol and dried under vacuum to give the product in 70% yield. The compound was recrystallized from ethanol; m.p. 251-252°C, $[\alpha]_D^{22} + 245.0^\circ$ (C 2 CHCl$_3$). Anal. calcd. for C$_{21}$H$_{25}$N$_1$O$_{11}$: C 53.94, H 5.39, N 2.99; found: C 53.52, H 5.54, N 2.80.
Preparation of Schiff's Base Metal Complexes from Ligands [6], [8], [10], and [11].

Ligand [6] was dissolved in methanol at room temperature and ligands [8], [10], and [11] were dissolved in hot ethanol. To these stirred mixtures was then added an alcoholic solution of the appropriate metal acetate. The complex then precipitated either immediately or upon cooling and the products were filtered and purified as shown below.

Bis-(N-methyl-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl-2-salicylaldimino) Cu (II) [16] was obtained in 90% yield and was analytically pure directly from the reaction mixture. Slow crystallization from an acetone solution yielded dark brown cubic crystal, m.p. 250-251°C, \([\alpha]^{22}_D + 777° \text{ (C 0.74 CHC}_3\text{)}\). Anal. Calcd. for Cu$_4$H$_4$$_8$CuN$_2$O$_{18}$: C 52.91, H 5.29, N 3.08; found: C 53.08, H 5.21, N 3.02, ms (low resolution) calcd. for Cu$_4$H$_4$$_8$CuN$_2$O$_{18}$: 907 (Cu$^{63}$), 909 (Cu$^{65}$) amu (M:M+1:M+2:M+3; 100:40:50:20); found: 907 and 909 amu (M:M+1:M+2:M+3; 100:40:50:20).

Bis-(N-methyl-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl-2-salicylaldimino) (Zn (II) [17] was obtained in 41% yield from acetone/chloroform, m.p. 272-273°C, \([\alpha]^{22}_D + 240.3° \text{ (C 0.77 CHC}_3\text{)}\). Anal. calcd. for Cu$_4$H$_4$$_8$N$_2$O$_{18}$Zn: C 52.80, H 5.28, N 3.08; found: C 53.00, H 5.40, N 3.07; ms (low resolution) calcd. for Cu$_4$H$_4$$_8$N$_2$O$_{18}$Zn: 908 (Zn$^{64}$), 910 (Zn$^{66}$), 911 (Zn$^{67}$), 912 (Zn$^{68}$) amu (M:M+1:M+2:M+3:M+4; 100:44:65:33:46); found: 908, 910, 911, 912 amu (M:M+1:M+2:M+3:M+4; 100:50:70:30:45).

Bis-(N-methyl-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl-2-salicylaldimino) Co (II) [18] was obtained in 58% yield from chloroform, m.p. 260-261°C, \([\alpha]^{22}_D + 25.6° \text{ (C 0.39 CHC}_3\text{)}\). Anal. calcd. for Cu$_4$H$_4$$_8$CoN$_2$O$_{18}$: C 53.18, H 5.31, N 3.10; found: C 53.06, H 5.39, N 3.20; ms
Bis-(N-1,3,4,6-tetra-O-acetyl-2-deoxy-β-D-glucopyranose-2-salicylaldimino) Cu (II) [19] was obtained in 90% yield from ethanol, m.p. 125-126°C, [α]$_D^{22} + 194°$ (C 0.5 CHCl$_3$). Anal. calcd. for C$_{42}$H$_{48}$CuN$_2$O$_{20}$: C 52.30, H 4.98, N 2.90; found: C 51.90, H 5.03, N 2.84; ms (low resolution) calcd. for C$_{42}$H$_{48}$CuN$_2$O$_{20}$: 963 (Cu$^{63}$), 965 (Cu$^{65}$) amu (M:M+1:M+2:M+3; 100:40:50:20); found: 963, 965 amu (M:M+1:M+2:M+3; 100:40:50:20).

(N-2-amino-2-deoxy-α,β-D-glucopyranose-salicylaldimino) Cu (II) [21] was prepared in 67% yield, m.p. 150°C dec., [α]$_D^{22} + 244.7°$ (C 0.76 H$_2$O). Anal. Calcd. for C$_{13}$H$_{18}$CuN$_7$: C 42.90, H 4.95, N 3.85; found: C 43.50, H 4.82, N 3.68, ms (low resolution) calcd. for C$_{13}$H$_{18}$CuN$_7$: 363 (Cu$^{63}$), 365 (Cu$^{65}$) amu (M+M+2; 10:4); found: 363, 365→420 amu.

Bis-{N-methyl-3,4,6-tri-O-acetyl-2-deoxy-2-(3-carboxyl-salicylaldimino)} Cu$_2$ (II) [23] was obtained in 80% yield, m.p. > 270°C, [α]$_D^{22} + 100.0°$ (C 0.16 CHCl$_3$). Anal. calcd. for C$_{42}$H$_{48}$Cu$_2$N$_2$O$_{22}$: C 47.67, H 4.38, N 2.65; found: C 47.13, H 4.51, N 2.64; ms (low resolution) calcd. for C$_{42}$H$_{48}$Cu$_2$N$_2$O$_{22}$: 1056 (Cu$^{63}$ Cu$^{63}$), 1058 (Cu$^{63}$ Cu$^{65}$), 1060 (Cu$^{65}$ Cu$^{65}$) amu (M+M+1:M+2:M+3:M+4:M+5:M+6; 100:46:96:39:27:8:2); found: 1056, 1058, 1060 amu (M+M+1:M+2:M+3:M+4:M+5:M+6; 100:50:95:40:30:10:2).

Preparation of methyl-2-(p-acetamido-benzenesulphonamido)-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranoside [25].

To a solution of the hydrobromide [7] (0.5 g, 1.25 m mol) and sodium bicarbonate (0.26 g, 3 m mol) in H$_2$O (5 ml) was added a solution of the sulphonyl chloride [24] (0.27 g, 1.17 m mol) in acetone (5 ml). After 1 h at room temperature the mixture was evaporated to dryness and
chromatographed on silica gel using solvent (A) to give the product in 50% yield from ethanol, m.p. 184-185°C, \([\alpha]_D^{22} - 35.50^\circ\) (C 1.55 acetone). Anal. calcd. for C_{21}H_{28}N_2O_7S: C 48.86, H 5.42, N 5.42; found: C 48.88, H 5.48, N 5.31.

Preparation of methyl-2-(p-acetamidobenzene-sulphonamido)-2-deoxy-\(\beta\)-D-glucopyranoside [26].

To a solution of compound [25] (0.2 g, 0.39 m mol) in dry methanol (2 ml) was added a solution of (0.2 N) sodium methoxide (0.7 ml). The mixture was stirred at room temperature for 20 min and then neutralized with IR 120 H\(^+\) ion exchange resin. The mixture was then filtered and evaporated to dryness to give the compound in 75% yield from ethanol. Anal. calcd. for C_{14}H_{20}N_2O_8S: C 46.15, H 5.64, N 7.18; found: C 45.84, H 5.62, N 7.00.

Preparation of methyl-2-(p-amino-benzensulphonamido)-2-deoxy-\(\beta\)-D-glucopyranoside [27].

Compound [26] (0.1 g, 0.26 m mol) was dissolved in NaOH (1N) (10 ml) and heated under reflux for 1.25 h. The mixture was then neutralized with IR 120 H\(^+\) ion exchange resin and the resin then filtered and the solution evaporated to dryness. The product was crystallized from ethanol to give a yield of 60%, m.p. 201-203°C. Anal. calcd. for C_{13}H_{20}N_2O_7S: C 44.83, H 5.75, N 8.04; found: C 44.50, H 5.82, N 7.86.

Preparation of methyl-2-deoxy-2-(p-salicylaldiminobenzenesulphonamido)-\(\beta\)-D-glucopyranoside [28].

Compound [27] (0.1 g, 0.29 m mol) was dissolved in H\(_2\)O (2 ml) and
to this stirred solution at ambient temperature, was added salicylaldehyde (0.05 g, 0.4 m mol). The mixture was stirred vigorously for 3 h and the product filtered to give a 78% yield. The compound was recrystallized from ethanol, m.p. 193-194°C, $[\alpha]_D^{22} = 55.07$ (C 1.42 methanol). Anal. calcd. for $C_{20}H_{24}N_2O_8$: C 53.10, H 5.31, N 6.19; found: C 53.01, H 5.37, N 6.00.

VE: Chapter II

(i) Sources of Materials

Ferrocene was obtained from Sigma Chemical Company, Saint Louis, Missouri. All the ferrocene-derived starting materials were synthesized from known methods (see text) except for 1,1'-ferrocene dicarboxylic acid which was purchased from Strem Chemicals, Inc., Newburyport, (MA). Sodium hydride was purchased from Alfa products (Danvers, MA) as 50% dispersion in oil. p-Toluenesulfonylchloride was purchased from Eastman Organic Chemicals (Rochester, N.Y.), and purified by the method of Pelletier$^{11}$. Cyanuric chloride (97%) was purchased from Aldrich Chemical Co., (Milwaukee, Wisconsin). n-butyllithium (1.6 M) was purchased from Aldrich.

(ii). Literature Preparations

The mono-ferrocene carboxylic acid was prepared from monolithioferrocene by the reaction of Goldberg et al.$^{12}$. Under anhydrous conditions n-butyllithium, in equimolar amounts, was added to solution of ferrocene in ether. Excess amounts of n-butyllithium apparently cause reaction at both cyclopentadienyl rings and using an equimolar amount prevents this mixture. After 6 h the ethereal solution was poured into a slush of ether and dry ice. The ethereal residue was washed with
water and the aqueous extracts acidified with 6 N hydrochloric acid.
After filtration and drying, about 25% yield was obtained as reported.

The acid chlorides [2] and [3] derived from the mono-carboxylic acid
and the di-1,1'-carboxylic acid respectively, were prepared by the method
of Pauson. To a benzene solution of the acid, under a nitrogen atmos­
phere, was added an equimolar amount of PCl₅ and the mixture allowed to
stir at room temperature for 3 h. The benzene solution was then washed
with dilute sodium hydroxide and water, and the benzene layer then dried
and evaporated. The products can be crystallized from pentane but the
crude acid chlorides were always used directly without purification. The
yields were approximately 60%.

The N,N-Dimethylaminomethylferrocene methiodide [12] was prepared
by the method of Lednicer. Under a N₂ atmosphere, ferrocene was added
to a stirred solution of bis (dimethylamino)-methane (prepared by a
recipe within the same reference) and phosphoric acid in acetic acid.
The mixture was then heated on a steam bath for 5 h. The dark amber
mixture was allowed to cool and was diluted with H₂O. Any unreacted
ferrocene was removed by extraction with ether. The aqueous solution
was then made alkaline and the tertiary amine separates as a dark oil.
The mixture was extracted with ether to give the tertiary amine as a
dark red oil. To this oil, dissolved in methanol, was added methyl
iodide. The solution was then heated for 5 min and after cooling,
ether was added. The resulting precipitate was filtered to give the
product in a high yield. Since this reagent is water soluble it can
be easily removed from reaction mixtures by extraction.

The alcohol [18] was easily prepared from the above reagent [12]
by simply boiling the methiodide in sodium hydroxide solution for
3.5 h$^{15}$. The product is then isolated by extraction with ether. Trimethylamine is liberated and therefore the reaction must be done in the fume hood. Holding a moistened piece of pH paper over the top of the condensor is a convenient way of determining the reaction time.

The ferrocene carboxaldehyde [24] was also prepared from the methiodide [12] by the method of Broadhead et al.$^{16}$. The methiodide [12], hexamethylenetetramine and acetic acid were heated under reflux for 1 min and then the mixture was poured into water and extracted with benzene to give the product in 27% yield as compared to the literature value of 37%. The aldehyde was recrystallized from 25% ethanol/water.

(iii) Synthesis of Ferrocenyl-Sugar Conjugates

Reactions of 1-ferrocenecarbonyl chloride [2] and of 1,1'-ferrocene dicarbonyl chloride [3] with sugars [4], [5], and [10].

Either 1 or 2 equivalents of the thio [4] or amino [5] sugars were added to a stirred solution of [2] or [3] (0.25 g) in dry chloroform (15 ml). Then 1 or 2 equivalents of either pyridine or triethylamine was added and the reaction allowed to stir for 15 min at room temperature. The mixture was then extracted twice with each of: water, 5% sodium carbonate, and water again. The chloroform layer was dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The 1,2:5,6-di-O-isoproprylidene glucofuranose sugar [10] was reacted with [2] in dry pyridine overnight and after concentration, chromatographed on a silica gel column using solvent (B).

2,3,4,6-Tetra-O-acetyl-1-deoxy-1-S-(1-ferrocenecarboxylate)-β-D-glucopyranose [6] was obtained in 60% yield from ethanol, m. p. 186-187°C, $[^{[a]}D^{22} + 35.0° (C 1 CHCl₃). Anal. calcd. for C$_{25}$H$_{28}$FeO$_{10}$S: C 52.11, H 4.86, S 5.56; found C 52.02, H 4.92, S 5.80.
1,1'-bis-[S-(2,3,4,6-tetra-O-acetyl-1-deoxy-1-thio-β-D-glucopyranose)] ferrocenecarboxylate [7] was obtained in 56% yield from ethanol, m.p. 213-214°C, [α]_D^{22} = -36.0° (C 1 CHCl₃). Anal. calcd. for C_{40}H_{46}FeO_{20}S₂: C 49.71, H 4.76, S 6.64; found: C 49.56, H 4.76, S 6.55.

{2-N-(1,3,4,6-tetra-O-acetyl-2-amino-2-deoxy-β-D-glucopyranose)}-1-ferrocenecarboxamide [8] was obtained in 50% yield from ethanol, m.p. 182-183°C, [α]_D^{22} = -2.5° (C 1 CHCl₃). Anal. calcd. for C_{25}H_{29}FeNO_{10}: C 53.70, H 5.19, N 2.50; found: C 54.00, H 5.23, N 2.51.

Bis-{2-N-(1,3,4,6-tetra-O-acetyl-2-amino-2-deoxy-β-D-glucopyranose)}-1,1-ferrocenecarboxamide [9] was obtained in 60% yield from ethanol, m.p. 110-110°C, [α]_D^{22} = +20.0° (C 1 CHCl₃). Anal. calcd. for C_{40}H_{48}FeN₂O_{20}: C 51.53, H 5.15, N 3.00; found: C 51.29, H 5.25, N 2.98.

{3-O-(1,2:5,6-di-O-isopropylidene-α-D-glucofuranose)}-1-ferrocene carboxylate [11] was prepared in 50% yield from column chromatography using solvent (B) and crystallization from ethanol/H₂O, m.p. 110-111°C, [α]_D^{22} = -55.5° (C 0.64 CHCl₃). Anal. calcd. for C₂₂H₂₈FeO₆: C 59.50, H 6.31; found: C 59.27, H 6.07.

Preparation of 1,3,4,6-tetra-O-acetyl-2-N,N-bis-(1-aminomethyl-ferrocene)-2-deoxy-β-D-glucopyranose [14].

A mixture of N,N-dimethylaminomethylferrocene methiodide (2.5 g, 6.5 x 10⁻³ mol), the 2-amino sugar [5] (0.9 g, 2.6 x 10⁻³ mol) and sodium carbonate (0.8 g, 7.5 x 10⁻³ mol) in acetonitrile (40 ml) was heated to reflux for 12 h. The mixture was then evaporated to dryness, dissolved in chloroform and extracted five times with water. The chloroform
layer was dried over anhydrous sodium sulfate, filtered, evaporated to dryness and after trituration with hexane, filtered to give 1.28 g (66%). The compound was recrystallized by dissolution in a minimum of benzene, followed by addition of a small amount of hexane and, subsequent addition of petroleum ether (bp 30-60°C) until a slightly cloudy solution was obtained and then cooling; m.p. 97-98°C, $[\alpha]_D^{22} + 77.0^\circ$ (C 1 CHCl$_3$). Anal. calcd. for C$_{35}$H$_{41}$Fe$_2$N$_9$: C 58.19, H 5.52, N 1.88; found: C 57.88, H 5.56, N 1.93.

Preparation of 2,3,4,6-tetra-O-acetyl-l-$\beta$-(1-thiomethylferrocene)-$\beta$-D-glucopyranose [13] from [12].

A mixture of 2,3,4,6-tetra-O-acetyl-l-thio-$\beta$-D-glucopyranose (2 g, $5.5 \times 10^{-3}$ mol), the methiodide [12] (2 g, $5.2 \times 10^{-3}$ mol) and anhydrous sodium carbonate (0.6 g, $5.6 \times 10^{-3}$ mol) in acetonitrile (50 ml) was refluxed for 12 h. The mixture was evaporated to dryness, dissolved in chloroform and extracted five times with water. The chloroform layer was dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The product was then column chromatographed on silica gel using solvent (A) and crystallized from ethanol to give 0.5 g (17%), of [13], m.p. 129-130°C, $[\alpha]_D^{22} + 32.0^\circ$ (C 0.85 CHCl$_3$). This product however was found by $^1$H nmr to be a mixture of $\alpha$- and $\beta$- anomers (40:60). Five repeated recrystallizations from ethanol gave the pure $\beta$ anomer m.p. 139-140°C, $[\alpha]_D^{22} - 39.0^\circ$ (C 1 CHCl$_3$). Anal. calcd. for C$_{25}$H$_{30}$FeO$_9$S: C 53.41, H 5.34, S 5.70; found: C 53.21, H 5.34, S 5.93.
Preparation of (1-hydroxymethylferrocene)p-toluenesulphonyl chloride [16].

Sodium hydride (0.12 g, $2.5 \times 10^{-3}$ mol) was placed in a 50 ml, two necked flask and washed twice with anhydrous ether. Then dry ether was added (15 ml) along with dry 1-hydroxy methyl ferrocene (0.5 g, $2.3 \times 10^{-3}$ mol). The mixture was then heated under reflux overnight under nitrogen. The stirred suspension of the alcoholate was cooled to -23°C and to this was added dropwise, over a period of 0.5 h, a solution of p-toluene-sulphonyl chloride (0.44 g, $2.3 \times 10^{-3}$ mol), in anhydrous ether (15 ml). The mixture was stirred at -10°C for two more hours and allowed to warm to room temperature for another hour. The mixture was then filtered under nitrogen through sintered glass and the resulting solution of the tosylate [16] used directly in subsequent reactions.


1-Hydroxymethylferrocene (0.5 g, $2.3 \times 10^{-3}$ mol) was converted into the tosylate [16], to an ether solution of which was added a solution of the 1-thio sugar [4] (0.5 g, $1.4 \times 10^{-3}$ mol) in dry chloroform (10 ml). The mixture was kept for 40 h at ambient temperature and was then filtered and evaporated to dryness. Upon addition of ethanol, the product crystallized to give 0.67 g (87%) of [13]. One recrystallization from ethanol gave orange plates in 79% yield, m.p. 139-140°C. For further analytical data see the preparation via the methiodide [12].
Preparation of 1,3,4,6-tetra-O-acetyl-2-N-(1-aminomethylferrocene)-β-D-glucopyranose [15].

1-Hydroxymethylferrocene (0.5 g, 2.3 × 10^{-3} mol) was converted into the tosylate; to an ether solution of the tosylate was added a solution of the 2-amino sugar [5] in dry chloroform (20 ml). The mixture was heated under reflux for two days and was then filtered, evaporated to dryness, dissolved in ether and extracted into 5% aqueous HCl. The aqueous layer was then neutralized with sodium bicarbonate and extracted with ether. The ether layer was dried over anhydrous sodium sulfate, evaporated to a small volume and cooled. The compound [15] crystallized as fine needles in 30% yield, m.p. 126-127°C, [α]_D^{22} + 8.0° (C 1 CHCl₃). Anal. calcd. for C_{25}H_{31}FeNO₉: C 55.08, H 5.69, N 2.57; found: C 54.79, H 5.54, N 2.69.

Preparation of 1,2:3,4-di-O-isopropylidene-3-O-(1-hydroxymethylferrocene)-α-D-galactopyranose [20].

1-Hydroxymethylferrocene (0.5 g, 2.3 × 10^{-3} mol) was converted into the tosylate and to the ether solution was added the 6-hydroxy sugar [19] (0.45 g, 1.7 × 10^{-3} mol) in anhydrous ether (5 ml). The mixture was kept for two days and was then filtered, evaporated to dryness and purified on silica gel column chromatography using solvent (A), to give the compound as an oil in 20% yield, [α]_D^{22} - 58.7° (C 0.8 CHCl₃). Anal. calcd. for C_{23}H_{30}FeO₆: C 60.30, H 6.55; found: C 60.16, H 6.59.
Preparation of 2-deoxy-2-N-(1-ferrocenecarboxaldehydeimine)-α,β-D-glucopyranose [26].

A mixture of 1-ferrocenecarboxaldehyde (0.5 g, 2.3 × 10^{-3} mol), 2-amino-2-deoxy-α, β-D-glucopyranose hydrochloride (0.5 g, 2.3 × 10^{-3} mol) and triethylamine (0.24 g, 0.24 × 10^{-3} mol) in absolute ethanol (15 ml) was stirred vigorously for 15 h. The mixture was then evaporated to a small volume and an equal amount of acetone added. The mixture was cooled and filtered to give 0.68 g (71%) product which was recrystallized by dissolving in a minimum of ethanol, evaporating to about half its volume, adding hexane and refrigeration; m.p. 161-162°C, [α]_{D}^{22} + 66.7° (C 0.33 MeOH). Anal. calcd. for C_{17}H_{21}FeNO_{5}: C 54.39, H 5.64, N 3.73; found: C 54.09, H 5.90, N 3.90.

Preparation of 2,4-Dichloro-6-(1-hydroxymethylferrocene)-s-triazine [22].

To an ice cold stirred solution of cyanuric chloride (0.86 g, 4.6 × 10^{-3} mol) in acetone (20 ml), over a period of 45 minutes was added, simultaneously, a mixture of hydroxymethyl ferrocene (1 g, 4.6 × 10^{-3} mol) in acetone (40 ml) and a solution of sodium hydroxide (5 ml of 4%) in water (55 ml). The solution was then filtered to yield a compound which was identified as the ferrocene ether dimer. The filtrate was allowed to become acidic upon standing at 0°C and the resulting orange precipitate filtered after 2 h to give the compound in 50% yield. This product is sufficiently pure for further reactions and should be stored in an evacuated desiccator. Purification was achieved by column chromatography on silica gel using solvent (A) and crystallized from ether/hexane, m.p. 120°C (Dec). Anal. calcd. for C_{14}H_{11}Cl_{2}FeN_{3}O:
C46.21, H 3.02, N 11.54; found: C 46.68, H 3.18, N 11.24; ms (high resolution) calcd. for C_{14}H_{11}C_{2}FeN_{3}O: 362.9615 amu; found 362.9625.

Bis-{1-hydroxymethylferrocene}-ether was obtained in 15% yield, m.p. 113-114°C. Anal. calcd. for C_{22}H_{22}Fe_{2}O: C 63.83, H 5.32; found: C 63.84, H 5.45; ms (low resolution) calcd. for C_{22}H_{22}Fe_{2}O: 414 amu; found: 414.

**Preparation of 2-{1-hydroxymethylferrocene}-4,6-bis-S-(2,3,4,6-tetra-O-acetyl 1-deoxy-1-thio-β-D-glucopyranose)-s-triazine [23].**

Triethylamine (0.2 g, 2.0 \times 10^{-3} mol) in acetonitrile (2 ml) was added to a stirred solution of compound [22] (0.3 g, 8.3 \times 10^{-3} mol) and 1-thio glucose [4] (0.6 g, 1.65 \times 10^{-3} mol) in acetonitrile (25 ml). After 10 min the mixture was poured into ice water (100 ml) and extracted with chloroform. The chloroform layer was dried over anhydrous sodium sulfate, filtered and evaporated to dryness. One recrystallization from isopropanol gave 0.6 g (74%), m.p. 120-121°C, [α]_{D}^{22} + 25.4 (C 1 CHCl_{3}). Anal. calcd. for C_{42}H_{49}FeN_{3}O_{19}S_{2}: C 49.48, H 4.81, N 4.12; found: C 49.51, H 4.72, N 4.12; ms (high resolution) calcd. for C_{42}H_{49}FeN_{3}O_{19}S_{2}: 1019.1749 amu; found: 1019.1769.

**Preparation of 1-deoxy-1-S-(1-thiomethylferrocene)-β-D-glucopyranose [27].**

To a stirred solution of compound [13] (0.1 g) in methanol/chloroform (4 ml 1:1) was added dry 0.2 N sodium methoxide (0.3 ml). After 30 min under nitrogen, the solution was neutralized with IR 120 H⁺ ion exchange resin, filtered, evaporated to dryness and crystallized from H_{2}O to give 52 mg (75%). Recrystallization from H_{2}O gave orange plates of the monohydrate, m.p. 145-146°C, [α]_{D}^{22} - 43.2° (C 0.44 MeOH). Anal.
calcd. for $\text{C}_{17}\text{H}_{22}\text{FeO}_6\text{S}$: C 49.55, H 5.82, S 7.78; found: C 49.84, H 5.65, S 7.70.

Preparation of 1-deoxy-1-$\text{S}$-(1-ferrocenecarboxylate)-$\text{D}$-gluco-
pyranose [28].

To a stirred solution of the thio ester [6] (0.1 g) in methanol/chloroform (4 ml, 1:1) was added 0.2 N sodium methoxide (0.7 ml) and the solution was stirred for 45 min under nitrogen. The solution was then neutralized with IR 120 H$^+$ resin and the mixture filtered, evaporated to dryness and the compound chromatographed on a silica gel column using solvent (C) to give 35 mg (50%) from ethanol, m.p. 145-146°C, $[\alpha]_D^{22} = -3.3^\circ$ (C 0.3 CHCl$_3$). Anal. calcd. for $\text{C}_{17}\text{H}_{26}\text{FeO}_6\text{S}$: C 50.03, H 4.90; found: C 49.75, H 5.08.

VF: Chapter III

(i) Sources of Materials

Cellulose powder (Watman CF11) was obtained as a gift from Drs. S. Chow, P. R. Steiner and J. N. R. Ruddick. Agarose (SK-ME 11335) was obtained as a gift from Marine Colloids. It was a white granular solid containing $\leq 0.5\%$ methoxyl, 0.2-3% pyruvate, 0.65% ash, 0.28% sulfate and had a molecular weight $\sim 10^5$. Sephadex G25 medium was purchased from Pharmacia. Both xanthan and guar gum were gifts from Kelco (San Diego, Cal.), starch was obtained from Allied Chemical Co.

The spin label 4-amino-2,2,6,6-tetramethylpiperidine-2-oxyl [10], n-hexylamine, and n-dodecylamine were purchased from Eastman. Cyanuric chloride was purchased from Aldrich. 1,2:5,6-di-$\text{O}$-isopropylidine-$\text{D}$-glucopyranose [28] was purchased from Koch-Light Laboratories Ltd.
The 1-thio-glucose [31] had previously been prepared by a standard recipe. p-Toluidine chromium tricarboxyl was purchased from Strem (Newburyport, MA, USA). 2,2,6,6-tetramethyl-4-piperidinol was purchased from Aldrich.

(ii) Literature Preparations

4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl [11] was prepared by the method of Rozantsev by oxidizing the secondary amine precursor. 2,2,6,6-tetramethyl-4-piperidinol was mixed with hydrogen peroxide, EDTA, and sodium tungstate in water for five days. Then the mixture was saturated with potassium carbonate and extracted with ether. The residue after evaporating was recrystallized to give large orange crystals of the free radical [11] in high yield. Purity can be checked easily by tlc using methanol or a toluene/ethyl acetate 1:1 mixture. One recrystallization from ether-hexane (2:1) gives the pure compound.

The preparation of 4-[(2,4-Dichloro-s-triazine-6-yl)-2,2,6,6-tetramethylaminopiperidine-1-oxyl [12] has been made before but the present method described is far more convenient. Cyanuric chloride (0.54 g, 2.9 \times 10^{-3} \text{ mol}) was added to acetone (12 ml) and stirred at 0°C. To this stirred solution was added an aqueous solution (20 ml) of 4-amino-2,2,6,6-tetramethylpiperidine-1-oxyl (0.5 g, 2.9 \times 10^{-3} \text{ mol}) and sodium bicarbonate (0.24 g, 2.9 \times 10^{-3} \text{ mol}). The mixture was then stirred at 0°C for 1\frac{1}{2} \text{ h} and then filtered and washed with cold water. The product was heated under vacuum to 50°C in a sublimation apparatus (to remove unreacted cyanuric chloride), giving a 75% yield, m.p. 195°C. Anal. calcd. for C_{12}H_{18}C\&_2N_5O_1: C 45.17, H 5.65, N 21.96; found: C 45.38, H 5.79, N 21.70.
(iii) Polysaccharide and Surface Derivatization

Spin labelling of cellulose, agarose, and Sephadex G25, via cyanuric chloride was achieved by the following method.

The insoluble polysaccharide (0.2 g) was mixed with NaOH (8%) for 1 h and then the excess base was decanted and the wet polysaccharide allowed to stand at room temperature overnight. Then a solution of one of the triazine spin labels [12]), [22] or [26] (0.2 g) in aqueous acetone (1:3) (3 ml) was added and the mixture shaken for 1 h. The products were then filtered on a sintered funnel and washed with water. The products were then shaken in water overnight to remove any traces of unreacted label and then filtered again. After filtration, cellulose and agarose were first washed with methanol and then ether, to remove water, whereas the Sephadex beads were first washed with ethanol and then ether. The products were dried in a drying pistol under 0.01 torr pressure at 56°C.

Spin labelling of the water soluble polysaccharides xanthan gum, guar gum, and starch was performed in the following way. The polysaccharide (0.2 g) was dissolved in 4% NaOH (5 ml) and to this solution was added an acetone solution (3 ml) of the spin label. The mixtures were then shaken for 1 h at room temperature. Guar and xanthan gum were precipitated by adding acetone (∼20 ml) and were then filtered and washed with acetone. The starch sample was purified by gel filtration using Sephadex LH-20.

Labelling of Bovine Serum Albumin (BSA) was achieved by the following method. BSA (0.02 g) was dissolved in phosphate buffer (pH 8, 1 ml) and to this solution was added an ethanol solution (0.2 ml) of the spin label [22] (10 mg). The mixture was then left overnight at room
temperature and was purified by gel filtration using Sephadex G25 eluted with pH 7 phosphate buffer. Uv absorption at 280 nm was used to identify the protein containing fractions. The fractions were then concentrated using dry Sephadex beads before esr spectra were run.

Aluminum Oxide (basic, Brockman activity 1) was labelled very easily by the following procedure. A mixture of alumina (1 g) and the spin label [22] (0.1 g) were mixed in chloroform for 15 min and was then filtered on a sintered funnel. The material was washed with chloroform and methanol and was then shaken in chloroform overnight. After this, the material was filtered again and dried in the drying pistol under 0.01 torr pressure at 56°C.

Controlled pore glass was labelled with reagent [22] by Dr. J. C. Waterton while working at UBC, using the following procedure. 3-amino propyl controlled pore glass was prepared by mixing the controlled pore glass with tri-ethoxy-propylamine-siloxane in water. The glass beads were then filtered. To a dry sample of this material (11.6 mg) was added the label [22] (6.3 mg) in acetonitrile (5 ml) with triethylamine (1 ml). The mixture was shaken for 24 h at room temperature and washed with acetonitrile and dried at 110°C/0.01 torr for 24 h. The yield was 18% based on the level of propylamine modification.

(iv) Synthesis of 8-Triazine Compounds

Preparation of 4-[2-chloro-4(n-hexylamino)-8-triazin-6-yl]-2,2,6,6-tetramethylaminopiperidin-1-oxyl [13].

Compound [12] (0.2 g, 6.3 x 10^{-4} mol) was dissolved in acetone (5 ml) and added with stirring to water (5 ml) at 0°C. To this stirred solution was added an aqueous solution (5 ml) of sodium bicarbonate (0.06 g, 7 x 10^{-4} mol) and an acetone solution (5 ml) of n-hexylamine
(0.064 g, 6.3 × 10^{-4} mol). The mixture was then stirred for 20 min at 45°C and the product filtered, washed with cold water and dried to yield 0.24 g (83%) from ethanol/water, m.p. 160-161°C. Anal. calcd. for C_{18}H_{32}Cl_1N_6O_1: C 56.32, H 8.34, N 21.90; found: C 56.34, H 8.21, N 21.90.

Preparation of 4-[2-chloro-4-(n-dodecylamino)-s-triazin-6-yl]-2,2,6,6-tetramethylaminopiperidin-1-oxyl [14].

Compound [12] (0.25 g, 7.8 × 10^{-4} mol) was dissolved in acetone (10 ml) and added with stirring to water (7 ml) at 0°C. To this stirred solution was added an aqueous solution (10 ml) of sodium bicarbonate (0.07 g, 8.3 × 10^{-4} mol), and a hot acetone solution (10 ml) of dodecylamine (0.145 g, 7.8 × 10^{-4} mol). The mixture was stirred at 45°C for 40 min and the pink compound was then filtered, washed with water and dried. The compound required no further purification and was obtained in 82% yield, m.p. 118-119°C. Anal. calcd. for C_{24}H_{44}Cl_1N_6O_1: C 61.63, H 9.41, N 17.96; found: C 61.66, H 9.41, N 18.16.

Preparation of 4-[4,6-di-(n-dodecylamino)-s-triazin-6-yl]-2,2,6,6-tetramethylaminopiperidin-1-oxyl [15].

Compound [14] (0.2 g, 4.3 × 10^{-4} mol) was dissolved in acetone (5 ml) and added with stirring to water (5 ml). To this stirred solution was added an aqueous solution (5 ml) of sodium bicarbonate (0.04 g, 4.8 × 10^{-4} mol) and a hot acetone solution (10 ml) of dodecylamine (0.08 g, 4.3 × 10^{-4} mol). The mixture was stirred overnight at 80°C and the pink compound was then filtered, washed with water and dried. The product needed no further purification and was obtained in 85% yield, m.p. 78-79°C. Anal. calcd. for C_{36}H_{76}N_7O_1: C 70.15, H 11.36, N 15.90;
Preparation of 2,4-Dichloro-s-triazin-6-p-toluidine chromium tricarbonyl [17].

Cyanuric chloride (0.4 g, $2.2 \times 10^{-3}$ mol) was dissolved in acetone (8 ml) at 0°C and to this stirred solution was added an acetone solution (7 ml) of p-toluidine chromium tricarbonyl (0.53 g, $2.2 \times 10^{-3}$ mol) and an aqueous solution (45 ml) of sodium bicarbonate (0.3 g, $3.6 \times 10^{-3}$ mol). The mixture was stirred at 0°C for 2 h and filtered to give a yield of 85%. The product was recrystallized from ethanol/hexane, m.p. 160-161°C (dec), ir (C=O stretch) 1950 and 1850 cm$^{-1}$. Anal. calcd. for $C_{13}H_8Cl_2Cr_1N_4O_3$: C 39.93, H 2.05, N 14.32; found: C 40.26, H 2.22, N 14.06.

Preparation of 2-(2,4-Dichloro-s-triazin-6-yl) methyl-3,4,6-tri-0-acetyl-2-amino-2-deoxy-glucopyranose [21].

Cyanuric chloride (0.12 g, $6.5 \times 10^{-4}$ mol) was dissolved in acetone (3 ml) and to this ice cold stirred solution was added an aqueous solution (8 ml) of methyl-3,4,6-tri-0-acetyl-2-amino-2-deoxy-glucopyranoside hydrobromide (0.25 g, $6.3 \times 10^{-4}$ mol) and sodium bicarbonate (0.12 g, $1.4 \times 10^{-3}$ mol). The mixture was stirred for 1 h at 0°C and then filtered to give the product in a 76% yield, m.p. 190-191°C, $[\alpha]^2_22 - 30.0^\circ$ (C 1 CHCl$_3$). Anal. calcd. for $C_{16}H_{20}Cl_2N_4O_8$: C 41.13, H 4.28, N12.00; found: C 41.36, H 4.50, N 11.90.
Preparation of 4-[2,4-Dichloro-s-triazin-6-yloxy]-2,2,6,6-tetramethylpiperidin-1-oxyl [22].

4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (2.0 g, 1.2 x 10^-2 mol) was dissolved in a mixture of water (50 ml) and 4% NaOH (12 ml) and was added dropwise to a stirred ice cold acetone solution (40 ml) of cyanuric chloride (1.8 g, 9.8 x 10^-3 mol) over a period of 1 h. The orange product was then filtered, washed with water and heated to 50°C in a sublimation apparatus to remove unreacted cyanuric chloride. The product required no further purification and was obtained in a 16% yield, m.p. 108-109°C. Anal. calcd. for C_{12}H_{17}Cl_{2}N_{4}O_{2}: C 45.05, H 5.31, N 17.50; found: C 44.74, H 5.42, N 17.56.

Preparation of 4-[4-chloro-6-(methyl-3,4,6-tetra-O-acetyl-2-deoxy-β-D-glycopyranosyl) amino-s-triazin-2-yl]-2,2,6,6-tetramethylaminopiperidin-1-oxyl [23].

A mixture of [22] (0.1 g, 3.1 x 10^-4 mol) methyl-3,4,6-tri-O-acetyl-2-amino-2-deoxy-β-D-glycopyranose hydrobromide (0.125 g, 3.1 x 10^-4 mol) and sodium carbonate (0.1 g, 9.4 x 10^-4 mol) was stirred in acetonitrile (30 ml) at room temperature overnight. The mixture was then filtered and the filtrate evaporated to dryness. The product was then purified silica column chromatography using solvent (A). The orange compound was crystallized from ethanol/water to give a 53% yield, m.p. 90-91°C, [α]_D^{22} = 39.0° (C 1 CHCl_3). Anal. calcd. for C_{25}H_{37}Cl_7N_5O_{10}: C 49.82, H 6.14, N 11.61; found: C 49.69, H 6.35, N 11.32.
Preparation of 4-[2-chloro-4-(n-hexylamino)-s-triazin-6-yloxy]-
2,2,6,6-tetramethylpiperidin-1-oxyl [24].

Compound [12] (0.1 g, $3.2 \times 10^{-4}$ mol) was dissolved in acetone
(3 ml) and added to an acetone (2 ml), water (5 ml) solution of n-hexyl-
amine (0.032 g, $3.2 \times 10^{-4}$ mol) and sodium bicarbonate (0.03 g, $3.6 \times 10^{-4}$
 mol) and the mixture was then stirred at room temperature for 20 min.
The product was extracted with chloroform and the chloroform layer
washed with water and dried over sodium sulfate. The orange product
was then filtered, evaporated to dryness and passed down a short silica
gel column using solvent (A) to give an orange syrup in 75% yield. Anal.
calcd. for $C_{18}H_{31}Cl_N_{5}O_{2}$: C 56.20, H 8.06, N 18.20; found: C 56.22,
H 7.85, N 17.90.

Preparation of 4,4-[2-chloro-s-triazin-4,6-yloxy]-di-2,2,6,6-
tetramethylpiperidin-1-oxyl [25].

4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (2 g, $1.2 \times 10^{-2}$
mol) was dissolved in a mixture of H$_2$O (50 ml) and 4% NaOH (13 ml) and
added dropwise, over a period of 40 min to an ice cold stirred acetone
solution (40 ml) of cyanuric chloride (1 g, $5.4 \times 10^{-3}$ mol). The
reaction was left to stir for 1½ h at room temperature and the orange
product then filtered and washed with water. The product was then dried
at 50°C, to remove any unreacted cyanuric chloride, to give the compound
in 10% yield, m.p. 194-195°C. Anal. calcd. for $C_{21}H_{34}Cl_N_{5}O_{4}$: C 55.36,
H 7.46, N 15.36; found: C 55.10, H 7.31, N 15.08.
Preparation of 4-[4-(1,2:3,4-di-α-isopropylidene-galactopyranosyl)oxy-6-(n-hexylamino)-s-triazin-2-yloxy]-2,2,6,6-tetramethylpiperidin-1-oxyl [30].

A mixture of compound [12] (0.1 g, 3.1 × 10⁻⁴ mol), 1,2:3,4-di-α-isopropylidene-α-D-galactopyranose (0.08 g, 3.1 × 10⁻⁴ mol), and one crushed sodium hydroxide pellet were stirred at room temperature in benzene (10 ml) overnight. The mixture was then filtered and evaporated to dryness. To this syrup was added an ice cold aqueous/acetone solution (10 ml 25:75) of sodium bicarbonate (0.03 g, 3.6 × 10⁻⁴ mol) and n-hexylamine (0.03 g, 3 × 10⁻⁴ mol). The mixture was allowed to warm to room temperature and then stirred for an additional 2 h. The pink compound was purified by silica gel column chromatography using solvent (A) and crystallized from ethanol/water to give a 40% overall yield, m.p. 170-171°C, [α]D₂₂ ± 55.0° (C 1.4 CHC₂). Anal. calcd. for C₃₀H₅₀N₅O₈: C 59.23, H 8.22, N 11.51; found: C 59.44, H 8.25, N 11.40.

Preparation of 2,4,6-tri-(2,3,4,6-tetra-α-acetyl-β-D-glucopyranosyl)thio-s-triazine [32].

Thio-glucose (0.4 g, 1.1 × 10⁻³ mol) was dissolved in acetonitrile (6 ml) and added to a stirred acetonitrile solution (6 ml) of cyanuric chloride (0.067 g, 3.6 × 10⁻⁴ mol). To this stirred mixture was added triethylamine (0.11 g, 1.1 × 10⁻³ mol) and the mixture then allowed to stir at room temperature for ½ h. The mixture was poured into ice water (50 ml) and the precipitate filtered, washed with water and dried. The compound required no further purification and was obtained in 66% yield, m.p. 122-124°C, [α]D₂₂ + 9.37° (C 0.32 CHC₂₃). Anal. calcd. for C₄₅H₅₇N₃O₂₇S₃: C 46.29, H 4.88, N 3.60, S 8.24; found: C 46.10, H 4.82,