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Synthesis of biologically active molecules: Novel macrocycles and guanine nucleotides

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SYNTHESIS OF BIOLOGICALLY ACTIVE MOLECULES: NOVEL MACROCYCLES AND GUANINE NUCLEOTIDES.

by

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A dissertation submitted to the Graduate Faculty in Chemistry in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

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ABSTRACT

SYNTHESIS OF BIOLOGICALLY ACTIVE MOLECULES: NOVEL MACROCYCLES AND GUANINE NUCLEOTIDES.

by

Cunxiang Chen

Advisor: Professor Valeria Balogh-Nair

The [3+2] condensation of terephthaldicarboxaldehyde with tris(2-aminoethyl)amine and subsequent oxidation of the resulting imino macrocycle afforded a macrobicyclic oxaziridine, the first example of a macrocycle containing the oxaziridine functionality. The potential of this novel class of macrocycles as oxidizing agents and oxygenase mimics was demonstrated. The [3+2] condensation of terephthaldicarboxaldehyde with tris(4-hydroxylaminobenzoyloxy-ethyl)amine yielded a macrobicyclic nitrone, the first example of a macrocycle containing the nitrone group. Light-induced ring closure of the six nitrone moieties to the corresponding oxaziridines was investigated. [1+1] and [2+1] Condensations followed by ring closure and/or oxidations led to a series of podands containing nitrone or sulfonyloxaziridine moieties.

Syntheses of a series of isotopically labelled and otherwise modified GDP and GTP analogs were undertaken to serve as model compounds in Raman spectroscopic studies of G protein - guanine nucleotide interactions. Deuterated analogs, [8-^{2}H]GDP, [8-^{2}H]GTP, and [8-^{2}H]GMPPCP were obtained by high yield exchange reactions.

An efficient synthesis was developed to obtain [6-^{18}O]GDP in three steps, from (-)-2-amino-6-chloropurine riboside by its conversion to the [6-^{18}O]guanosine followed by chemical and enzymatic phosphorylations. [^{18}O]-Labelled analogs, [β-^{18}O]GDP and [β-$^{18}O_4$]GDP, were obtained by chemical phosphorylation of an activated GMP derivative, and by conversion of [β-S]GDP to [β-$^{18}O_4$]GDP. The [6-H]GDP and 9-butylguanine were prepared *via* multistep syntheses.

Dedicated to my wife Xinpei and my daughter Vicki

Acknowledgments

I would like to express my thanks to Professor Valeria Balogh-Nair for her guidance and inspiration. The freedom that she created, the counsel and support that she gave, made it possible for me to be able to devote all my attention to the research project during these years.

I would also like to thank Dr. Claude Brathwaite for his constant help in my laboratory work.

I also wish to thank Mr. R. Pal and Mr. H. Schimatz for mass spectroscopy and glass blowing services respectively.

Finally, I would like to thank my parents, and my wife for their encouragement, patience and tolerance over the years, partly due to which I worked so hard to finish my thesis work.

Table of Contents

PART II. Synthesis of Isotopically labelled Guanine Nucleoside and Nucleotide Analogs

List of Illustrations

1. INTRODUCTION

18-Crown-6, the first member of a series of cyclic polyethers reported by Pedersen in 1967,[1] opened the door to the field of synthetic macrocyclic chemistry. Since then, various macrocyclic compounds have been synthesized, and many investigations on the structure of their complexes, their specific characteristics as well as applications were developed.[2-7] Early recognition of the importance of Pedersen's discovery by Lehn[8] and Cram[9] and their stimulating and innovative contributions to supermolecular complexation, chiral recognition, and catalysis, led to the expansion of the crown ether concept to the establishment of the entirely new fields of supramolecular and host-guest chemistry. The importance of these discoveries was recognized in 1987, when the Nobel prize in chemistry was awarded to Pedersen, Cram, and Lehn for their pioneering contributions.

Early work focused on the synthesis of crown ethers of various sizes containing oxygen and/or other donor heteroatoms. Cyclic amino ethers in which nitrogen replaces some of the oxygen donor atoms of a crown ether are known as azacrown ethers, and cyclic polyether sulfides in which sulfur replaces some of O donors are called thiacrown ethers. Macrocyclic compounds with three kinds of donor atoms, O, N, and S, are called azathiacrown ethers. Macrocyclic polyamines bearing only N atoms as donors are named azacrowns and macrocyclic polysulfides bearing only S atoms as donors are called thiacrowns.

Lehn and coworkers were the first to conceive the idea of macrobicyclic polyether ligands,[8] and named them "cryptands", that complexed cations or anions with great selectivity in their three-dimensional spherical cavities. Besides cryptands, there are many other polymacrocyclic compounds containing intramolecular cavities and clefts of specific sizes, shapes and electronic properties that are very effective complexing agents.

Cram and coworkers developed the concept of "host-guest chemistry" defined as " the field of chemistry consisting of syntheses and applications of highly structural molecular complexes formed by recognition and incorporation of the matched guest by the host molecule having a designed cavity".[7] The synthesis of a series of chiral crown ethers bearing binaphthyl groups, and studies of their complexation properties, optical resolution, optically selective transport, and asymmetric reactions led to important advances in chiral recognition fundamental to the understanding of complexation in living organisms.[7,9]

Lehn *et al.*[6,11] introduced the term "supramolecular chemistry" as the extension of host-guest chemistry. According to Lehn, supramolecular chemistry is "the structures and functions of supermolecules that result from binding substrates to molecular receptors" or "the new field of the chemistry concerning the higher-order molecular interaction between two or more molecules, that is, chemistry which is beyond the concept of the molecules."

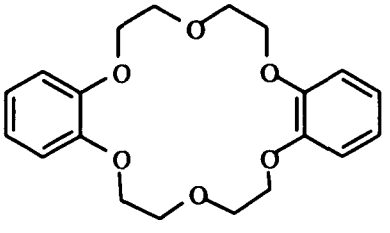
In 1972, when the term "biomimetic chemistry" was proposed by Breslow,[10] the field it represented was aimed at reproducing the

rate and excellent stereospecificity of an enzymatic reaction in a chemical reaction using smaller molecules. At present the scope of biomimetic chemistry has expanded not only to simulate biological processes using molecular recognition in host-guest/supramolecular chemistry, and molecular self-assembly to yield highly organized systems, but also to generate new materials such as molecular photonic, electronic, and ionic devices, that can perform signal and information processing at the molecular level.

1.1. Macrocyclic Ethers

1.1.1 Crown ethers.

Pedersen isolated the crown ether, dibenzo[18]crown-6, **1**, by reacting catechol with bis(2-chloroethyl) ether in the presence NaOH.[2] Reinhoudt *et al.*[12] synthesized benzocrown ether derivatives in good yields (52-67%) from catechol and polyethylene glycol ditosylates by using metal fluorides as nucleophiles, and the metal ions (K, Rb, or Cs) acting as templates. Czech *et al.*[13] effected the intramolecular cyclization of oligoethylene glycol containing aromatic moieties such as benzene, naphthalene, and binaphthyl units, and obtained high yields of the corresponding crown compounds.



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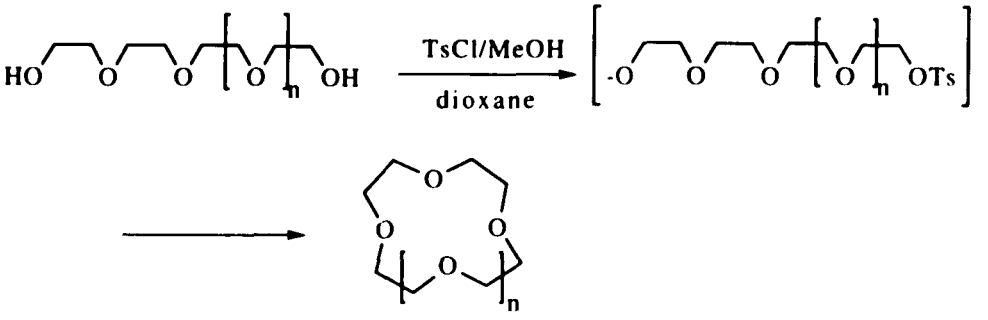
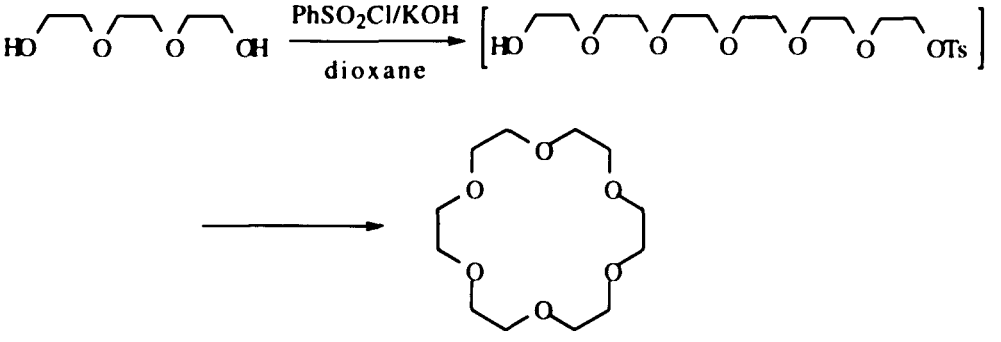
A one-pot intramolecular cyclization procedure for preparing crown ethers was developed by Okahara's group[14-16] (Scheme 1).



Scheme 1.

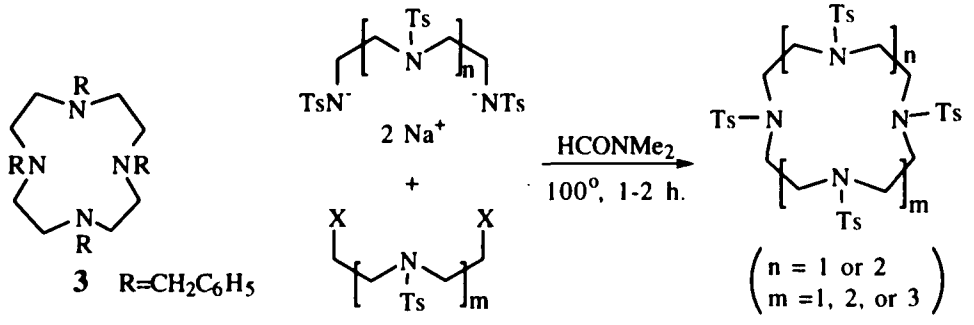


Scheme 2.

Treatment of oligoethylene glycols with arenesulfonyl or alkanesulfonyl chlorides in an aprotic solvent in the presence of powdered alkali metal hydroxide gave the corresponding crown ethers in moderate to high yields. The template effect of alkali metal cations was found to depend on the reaction temperature.[15] In the case of tri- and tetraethylene glycols, dimerization/trimerization of the glycol occurred prior to the cyclization reaction to yield 18-crown-6 and 24-crown-8, respectively[16] (Scheme 2).

1.1.2 Azacrowns and azacrown ethers.

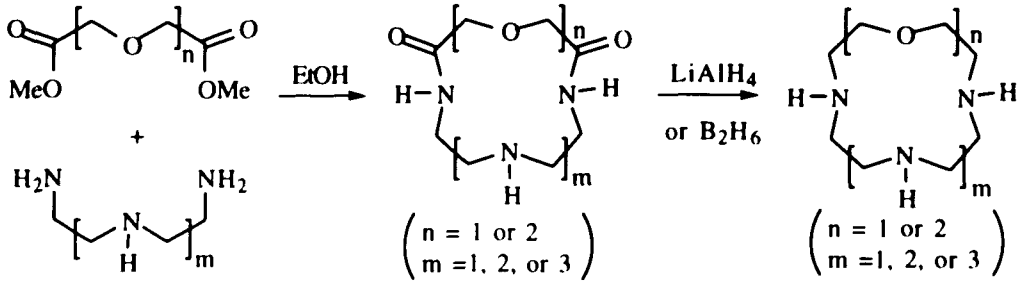
A wide variety of azacrowns have been synthesized to date by intermolecular cyclocondensation of commercially available or readily prepared starting materials.[17] A unique procedure, the reaction between N-benzylaziridine and *p*-toluenesulfonic acid in refluxing aqueous ethanol afforded the tetraaza-12-crown-4 derivative, **3**, in 96% yield.[18] However, a more generally applicable route, suitable to synthesize whole series of azacrowns (Scheme 3), is based on the condensation of N-tosyldiethanolamine ditosylates (or dimesylates, dichlorides, dibromides, diiodides) with a tritosylamine salt, followed by deprotection procedures to remove the tosylates.[17]



Scheme 3.

An efficient synthetic route to obtain azacrown ethers, also applicable to the synthesis of thiacrown ethers, that requires neither high-dilution techniques nor protection of the secondary amine in the polyethylenepolyamine starting materials has been reported.[19] It consists of the cyclization of readily available α,ω-dimethylesters

with commercially available polyethylenepolyamines, and subsequent reduction of the macrocyclic amides obtained as shown in Scheme 4.

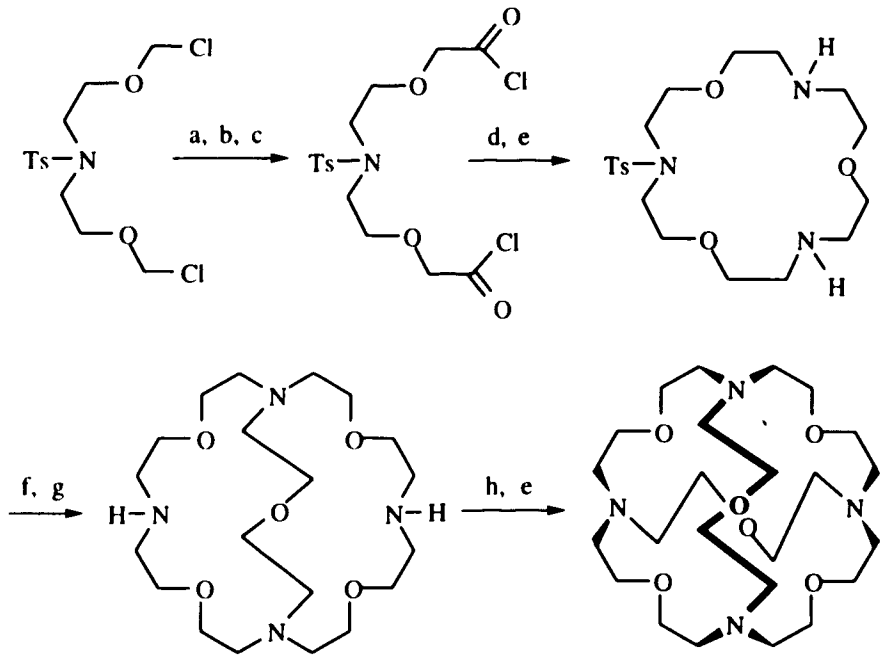


Scheme 4.

1.2. Macrobicyclic, macrotricyclic and macropolycyclic compounds.

Graf and Lehn[20,21] were the first to report the synthesis of spherical macrocyclic polyether ligands (Scheme 5) which can encapsulate metal cations in spherical cavities with high selectivity, forming very strong complexes. Using these ligands, called cryptates, one can effectively control the electrochemical, photophysical, and other properties of both the included and the external species. A further important feature of the polycyclic crown compounds is that it can combine several kinds of binding subunits resulting in "polytopic hosts". Whereas monotopic hosts possess functions of recognition, catalysis, and transport, the cooperative participation of several binding subunits in a polytopic hosts may also initiate higher forms of molecular functions such as cooperativity, allostery, and regulation. Of special interest are combinations of different kinds of

binding subunits in a heterotopic host molecule, e.g., crown and polyamine or crown and cyclophane.



a: NaCN, $HCONMe_2$; b: $Ba(OH)_2$, H_2O then HCl; c: $(COCl)_2$, C_6H_6
d: $H_2NCH_2CH_2OCH_2CH_2NH_2$, C_6H_6; e: B_2H_6;
f: $TsN(CH_2CH_2OCH_2COCl)_2$, C_6H_6; g: $LiAlH_4$;
h: $ClOCCH_2OCH_2COCl$, C_6H_6

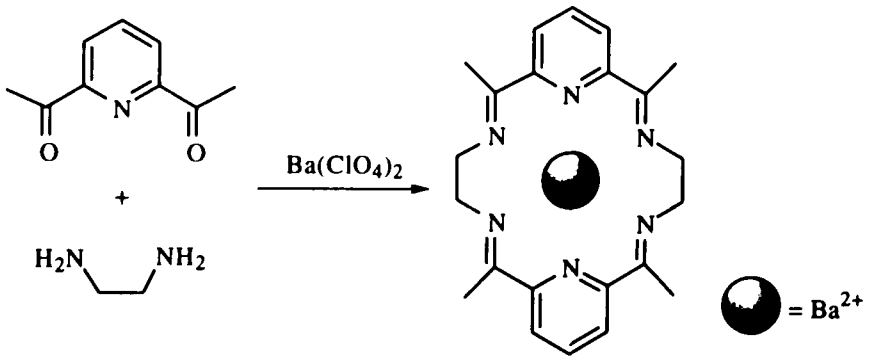
Scheme 5.

1.3. Macrocyclic imines.

Macrocyclic imines have been widely employed as ligands in transition metal chemistry, their metal complexes were used as models for metal centers in biologically important molecules,[22] and binucleating macrocyclic imines have been studied to probe the unique properties of the two metal centers in the active sites of

metalloproteins, e.g., hemocyanin, tyrosinase and dopamine-β-hydroxylase.[23]

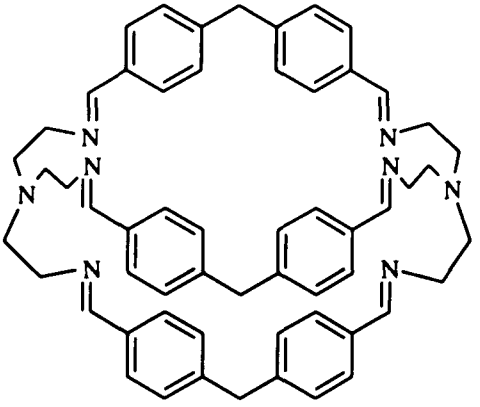
Macrocyclic imines are polydentate ligands containing at least three donor atoms either incorporated in or, less commonly, attached to a cyclic framework. In the early studies, synthesis of macrocyclic imines was accomplished by template-directed condensation of aldehydes (or ketones) with amines to afford metal containing macrocycles (Scheme 6). In 1987 ditopic macrobicyclic imines were



Scheme 6.

synthesized employing amine + aldehyde, [2+3], condensations to obtain the metal-free ligands.[24] Thus, macrocyclic cryptands **4** and **5** were obtained in a one-pot reaction without using a metal template. Nelson and MacDonnel[25] extended the work of Lehn by preparing three analogs of **5**, using [2+3] condensations, in which phenyl, furyl and thiophene units replaced the pyridine headgroup. When **4** or **5** was treated with $[Cu(CH_3CN)_4]ClO_4$, both formed Cu^I cryptates in high yields.[24] X-ray analysis of **6**, the cryptate formed from **4**, showed that each copper atom is coordinated to three weakly

basic imino nitrogens and one more basic tertiary bridgehead nitrogen, and that the two metallic centers do not interact. Further, complexation induced considerable changes in the conformation of the metal-free macrobicycle. While the length of the macrobicyclic cage remained the same, macrobicycle **4** had an elongated ellipsoidal shape containing staggered bridgehead C-N bonds, but these were eclipsed in the dicopper complex, **6**.



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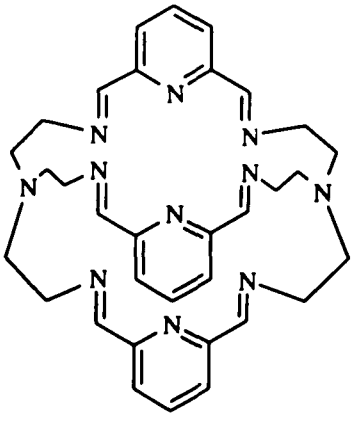

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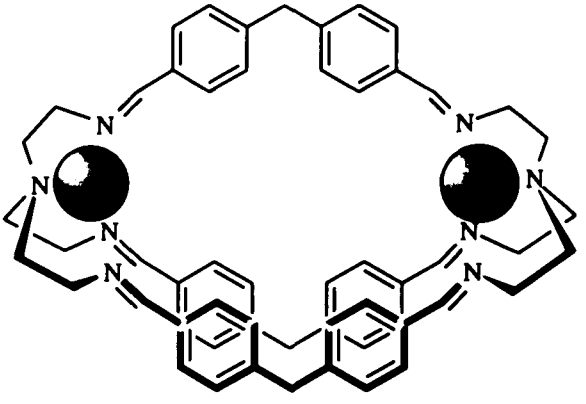

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1.4. Molecular modeling in Hosts-Guest chemistry.

In most of the host-guest systems it is difficult to visualize the structure of the host in three dimensions, hence space-filling molecular models were employed to aid in the design of novel hosts. Host selectivity for various ions,[8] diastereo- and enantioselectivity by chiral hosts[26], allosteric effects, and features of many crystal structures have been successfully predicted or were confirmed using these models.

Computational methods are more powerful tools for model building, and for energy calculations. Thus, appropriate software packages allow model building and manipulation, e.g., direct import of structures from crystallographic data bases, or structure entry using templates and builders followed by docking operations for the manipulation of intermolecular positioning. Calculation techniques comprise molecular orbital calculations and molecular mechanics techniques.[27] The former allows to determine parameters such as partial charges on relevant atoms, and the latter permits to generate an energy minimized conformation of a molecule, that can be used as input into a molecular orbital calculations program. Molecular mechanics calculations employ a database of empirical heats of formation and bond lengths, angles and force constants to find optimal conformations of molecules. Most molecular mechanics studies involving macrocyclic compounds have used energy minimization programs that have the following steps in common: Selection of a starting conformation, calculation of the conformational energy using the potential energy expression, modification of the

independent variables using an appropriate algorithm, recalculation of the conformational energy, determination of the direction toward lower energy, adjustment of the independent variables in the direction of lower energy, and repetition of the last three steps until the minimum energy structure is obtained.

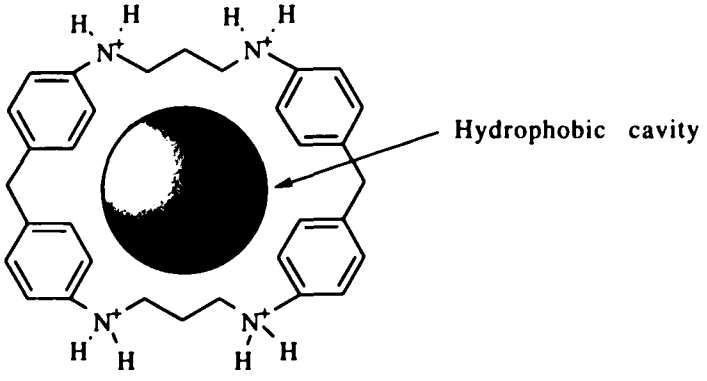
1.5. Enzyme modeling with macrocyclic compounds.

Complexation studies of synthetic polyether macrocycles have contributed greatly to the development of the concept of molecular recognition, and have facilitated greater understanding of selective molecular interactions in both natural and artificial systems.[6,28] One major application of the synthetic macrocycles is the design and synthesis of artificial enzymes which can catalyze a useful synthetic reaction in an enzyme-mimetic manner, through non-covalent complexes.[29] Thus, the ultimate goal of the organic chemist is to develop catalysts to achieve reactions with high rates and selectivity without the bulky peptide structures that orient the reactive sites of enzymes. Successful efforts in this direction include the use of complexes formed between metals and organic ligands, hydrogen-bonded complexes, and preorganized cation/anion complexing systems that mimic enzyme's active sites.[30] A few selected examples are given below:

Crown ethers have been used as binding sites for cationic guests, such as metal, ammonium, or sulfonium ions, etc., or neutral guests which have acidic hydrogens. Anionic guests can be accommodated when the binding site is cationic or when such guests

behave as a single guest because of tight ion pairing with the counter cation. Thus, crown ether type hosts possess a polar binding cavity in organic media. Such complexes have a significant advantage in that a guest occupies a specific position with a particular geometry in the complex, and provide a reliable basis for the design of enzyme mimics.

Macrocyclic polyethers or polyamines incorporating aromatic groups, the cyclophanes, provide a hydrophobic cavity surrounded by aromatic rings. These hosts, illustrated by the tetracationic receptor **7**,[31] bind organic molecules, which then become soluble in water by virtue of the polar groups introduced into the backbone of the host.



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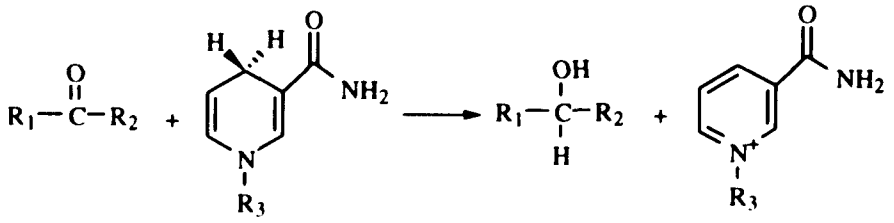
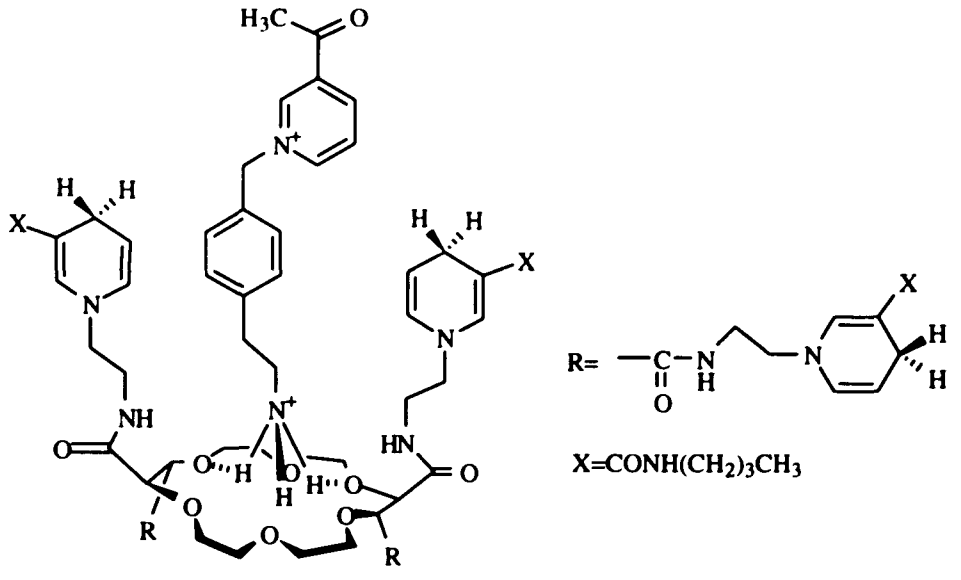
The hydride transfer properties of 1,4-dihydro-pyridine derivatives (Scheme 7), make them good mimics of the coenzymes, NADH/NADPH, and it has been suggested that molecular complex formation precedes the hydride transfer.[33] Therefore, it seems reasonable to assume that the close proximity of the hydride donor



Scheme 7.

and acceptor should accelerate the reaction. Such rate enhancement has been observed in the reaction between the host as a hydride donor and the substrate with an ammonium group as a hydride acceptor, the complex shown in **9**.[33] Complete inhibition by KBF_4 suggested that hydride transfer occurred through prior formation of a complex between the crown ring and the ammonium ion.



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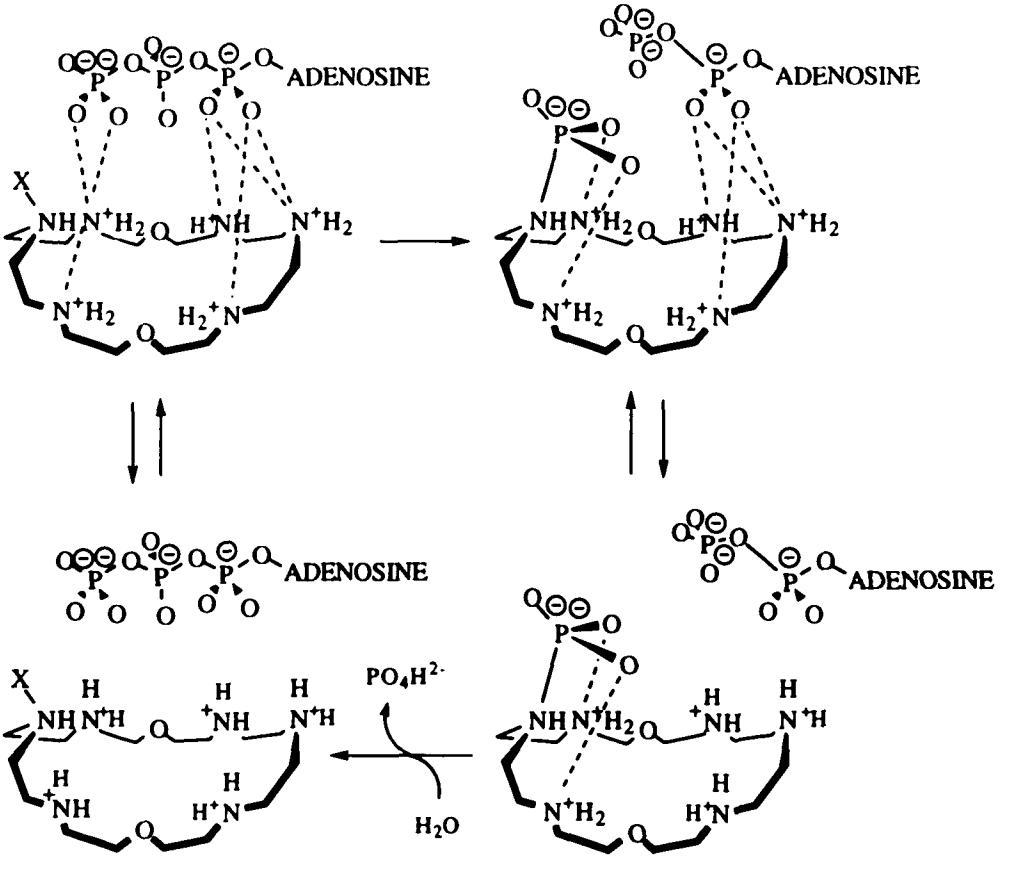
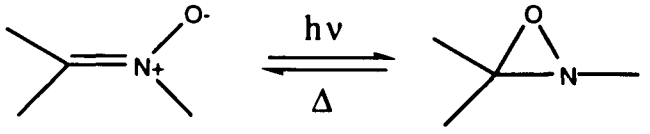
In their study on anion receptor molecules, Lehn and co-workers[34,35] found that cyclic polyamines and derivatives used in ATP hydrolysis mediated the formation of pyrophosphate. This type of macrocycles can bind with mono- or dinucleotide polyphosphates by interaction between the macrocyclic polycationic moiety and the polyphosphate chain as demonstrated by ^{1}H and ^{31}P NMR (Scheme 8). The protonated cyclic polyamines work as an anion receptor and strongly binds ATP and markedly accelerates its hydrolysis to yield ADP and inorganic phosphate.



Scheme 8.

2. RESULTS AND DISCUSSION

Oxaziridines, by virtue of the unique properties of their strained ring, are extensively employed in organic synthesis as oxygen transfer agents.[36] In fact, they are among the most effective oxidizing agents currently available for chiral epoxidations.[37] Nitrones, that yield oxaziridines by electrocyclic ring closure (Scheme 1), are versatile building blocks employed in constructing heterocycles *via* 1,3-additions to the nitrone dipole.[38]



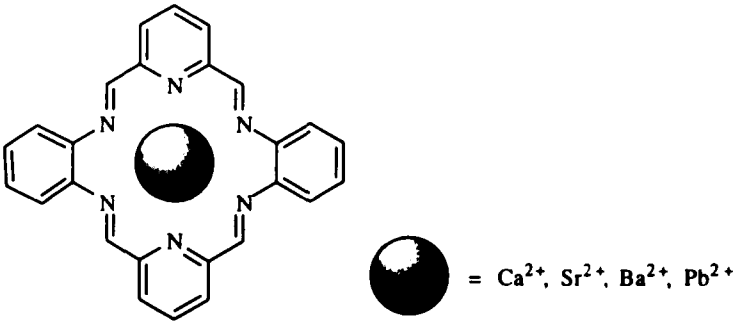
Scheme 9.

Despite the high potential of the oxaziridines and nitrones in effecting organic syntheses, and the abundance of host-guest systems containing a variety of functionalities, the chemistry of oxaziridine and nitrone groups has never been explored in macrocyclic chemistry. The objective of this work was to close this gap by synthesizing novel families of macrocycles, containing oxaziridine and nitrone functions. While these macrocycles are bound to display interesting reactivities, they also could serve as mimics of biological oxidizing agents, the oxygenases. Further, the photochromism displayed by nitrones/oxaziridines, could permit the construction of oxygenase mimics whose action might be triggered by light. To achieve the afore mentioned objective, condensation of aldehydes

with hydroxylamines or oxidations of the hydroxylamines to obtain nitrones,[38] and oxidation of imines, or ring closure of nitrones to synthesize the oxaziridines,[39] were considered appropriate.

2.1 Podands.

Nitrogen atoms in macrocyclic imines[40] are excellent ligands for cation complexation, and hence are widely used in coordination chemistry. The majority of their syntheses involve the use of template-directed [2+2] condensations. Thus, Nelson's group[41] prepared macrocyclic imine **10** by using metal ions as templates in the condensation of 2,6-pyridinedicarboxaldehyde with *o*-phenylenediamine. The first reports on the synthesis of metal-free



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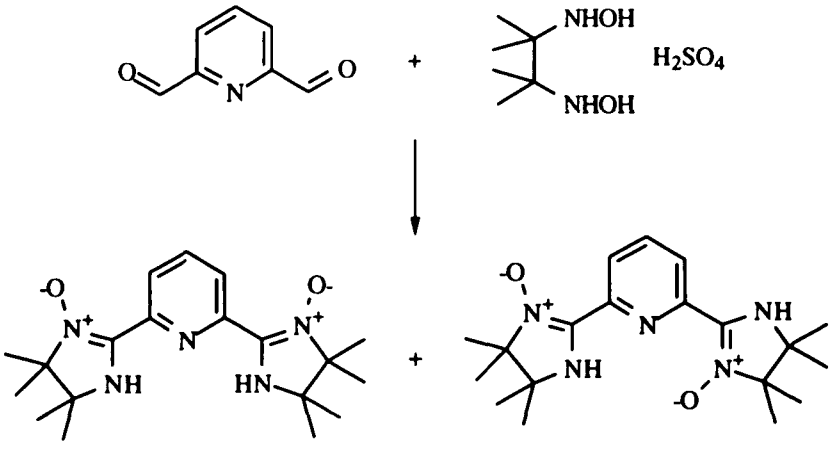
macrocyclic imines appeared as late as 1986, and these also involved template-directed [2+2] cyclizations as the initial step; exchange of the templates for potassium ion and subsequent removal of the latter, by competitive complexation with a crown ether, allowed the preparation of metal-free macrocycles.[42] Recently, a better

understanding of conformational requirements led to the development of methods that yield metal-free macrocyclic imines via facile [2+3][43] or [2+2][44] condensations of the appropriate dialdehydes and diamines, without the intermediary of templates.

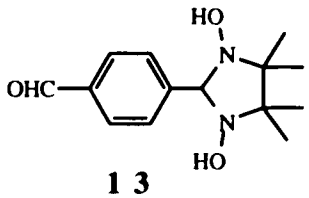
Our attempts to use [2+2] condensations to prepare the metal-free macrocyclic imine, **10**, that could serve as appropriate starting material for the synthesis of the corresponding oxaziridine, or to use [2+2] condensations of dialdehydes with bishydroxylamines to obtain macrocyclic nitrones, led only to [1+1] and [1+2] addition products. Thus, [1+2] condensation of 2,6-pyridine-dicarboxaldehyde with 2,3-bis(hydroxylamino)-2,3-dimethylbutane sulfate in the absence of a metal ion template (Scheme 10) gave a mixture of podands, **11** and **12**, in 90% overall yield. Separation of this mixture showed that podand **11** was the major product (85%) in agreement with MM2



11 **12**

Scheme 10.

calculations that confirmed the higher stability of this podand compared to its conformer, podand **12**. Using La^{3+} as the template in the above condensation, also yielded the same podands, instead of the desired [2+2] macrocyclization product. The same difficulty was encountered when terephthaldicarboxaldehyde or 2,5-furan-dicarboxaldehyde were employed instead 2,6-pyridinedicarbox-aldehyde as the substrates. Condensation of terephthal-dicarboxaldehyde with 2,3-bis(hydroxylamino)-2,3-dimethylbutane sulfate gave a [1+1] adduct, **13,** as the intermediate, and podand **14,** as the final product. Condensation of 2,5-furandicarboxaldehyde with 2,3-bis(hydroxyl-amino)-2,3-dimethylbutane sulfate afforded the [1+1] adduct, **15.** However, despite of the various conditions employed, including the template-directed route, nitrone **16**, our first macrocyclic nitrone target, could not be obtained.



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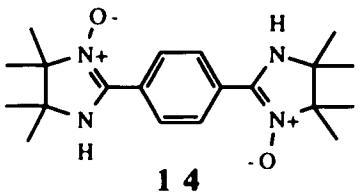

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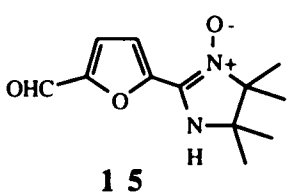

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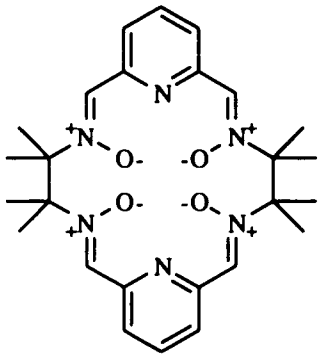

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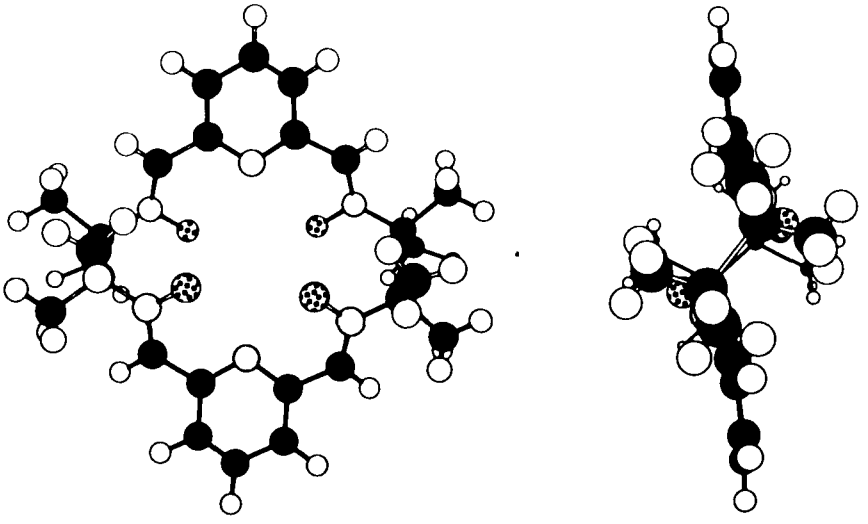
In order to ascertain why the macrocyclic nitrone, **16**, can not be obtained in a one-step condensation, molecular mechanics calculations were carried out first using the PCMODEL program on an IBM-PC, and then on a Silicon Graphics IRIS INDIGO workstation employing the MACROMODEL program. The 3D structures generated by MACROMODEL (Figure 1), show that macrocycle **16** is twisted, because the four nitrone oxygens each with two lone-pairs cannot exist in the same plane. Thus, there is not only a lack of space in the center of the macroring to complex a templating ion, but there is also severe lone-pair repulsion in the untemplated form. Therefore, macrocycles with larger cavities and/or of different symmetry properties need to be designed (See section 2.2 and 2.4) to obtain macrocyclic nitrones/oxaziridines.



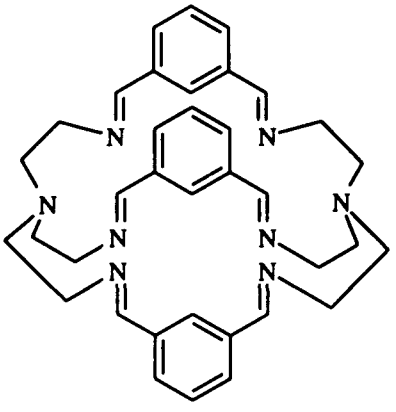
16 (top view) **16** (side view)

Figure 1.

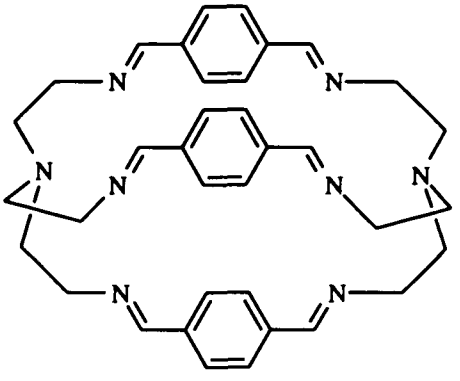
2.2 Macrocyclic oxaziridines.

The oxaziridine ring system has been known for close to forty years, and the ability of oxaziridines to transfer oxygen was almost immediately recognized by Emmons,[45] and Horner,[46] but it was only recently that their potential as chiral oxidizing agents has been fully appreciated.[36,37] For instance, unfunctionalized alkenes could be oxidized to afford epoxides with excellent enantioselectivities[47] and nonfunctionalized sulfides could be converted to sulfoxides with high enantioselectivity employing the more reactive, chiral N-sulfonyloxaziridines.[48]

Since N-alkylimines are readily converted to N-alkyl-substituted oxaziridines by oxidation with peroxyacids,[36] we decided to employ macrobicyclic imines **17** and **18** as precursors to obtain macrobicyclic oxaziridines. Employing a modified form of the literature procedures,[43,49] the [3+2] condensations of isophthal-aldehyde or terephthaldicarboxaldehyde with tris(2-amino-ethyl)amine afforded imines **17** and **18** respectively, in high yields.



17



18

The ^{1}H nmr spectrum of the macrobicyclic imine **17** was unresolved at high field (CH_2 protons), but the protons attached to the aromatic rings appeared as three well-resolved signals, at δ 5.30 (s), 7.5 (t), and 8.15 (d) ppm (Figure 2). By lowering the temperature to -20°C, thereby reducing conformational mobility, as expected, the high field region (CH_2 groups) also became well-resolved, displaying four signals, at δ 2.64 (d), 2.86 (t), 3.20 (t) and 3.76 (d) ppm (Figure 3). To explain why one of the aromatic protons appears at unusually high field (5.30 ppm), the 3D structure of **17** was generated using MACROMODEL (Figure 4). It showed that one proton at each aromatic ring points directly towards the center of another aromatic ring, causing considerable shielding of this proton, resulting in a signal at 5.30 ppm. The ^{13}C nmr spectrum (Figure 5), consisted only of a total of seven peaks, confirmed the highly symmetric structure of **17**, a consequence of its 3-fold axis of symmetry.

17 (side view) **17** (top view)

Figure 4.

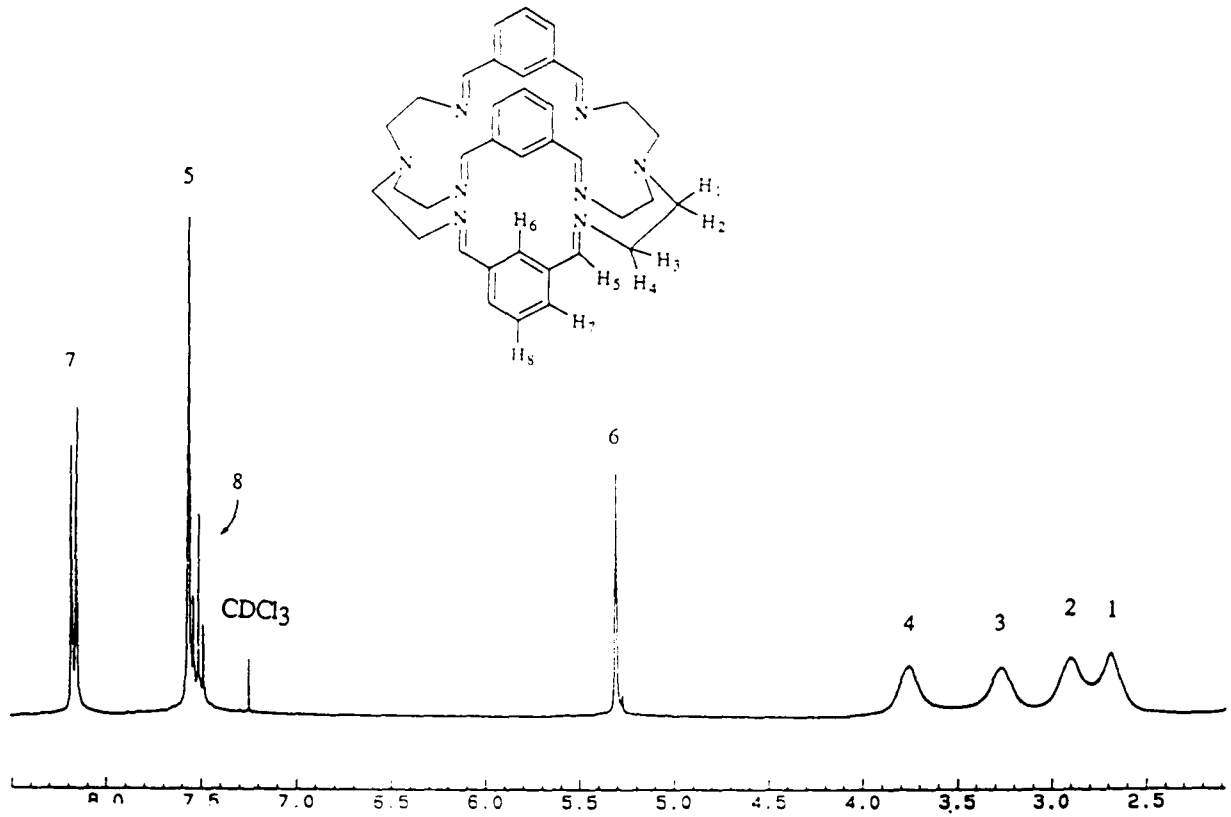


Figure 2. The ^{1}H nmr spectrum of the macrobicyclic **imine 17** at rt.

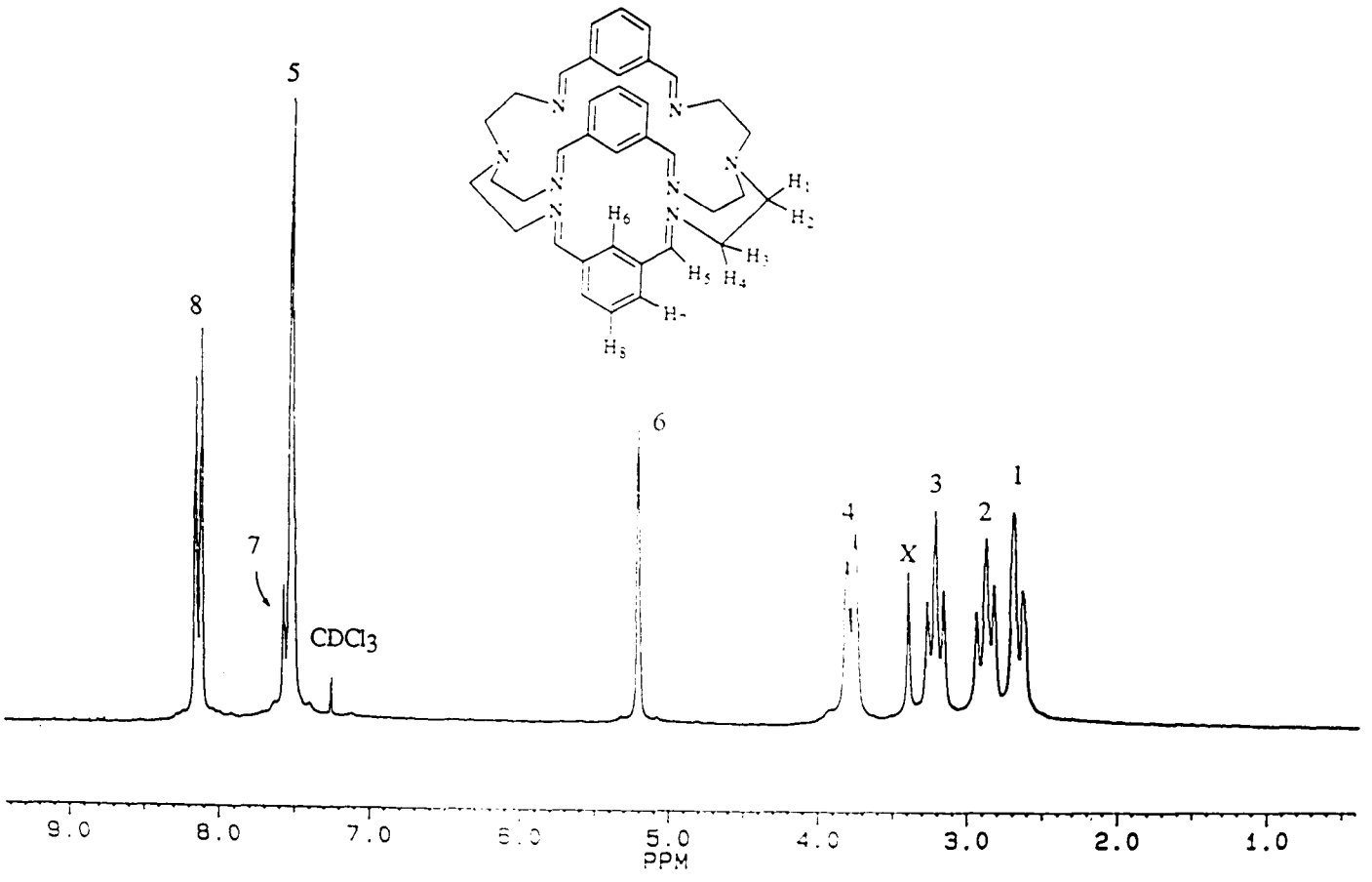


Figure 3. The ^1H nmr spectrum of the macrobicyclic imine **17** at -20°C.

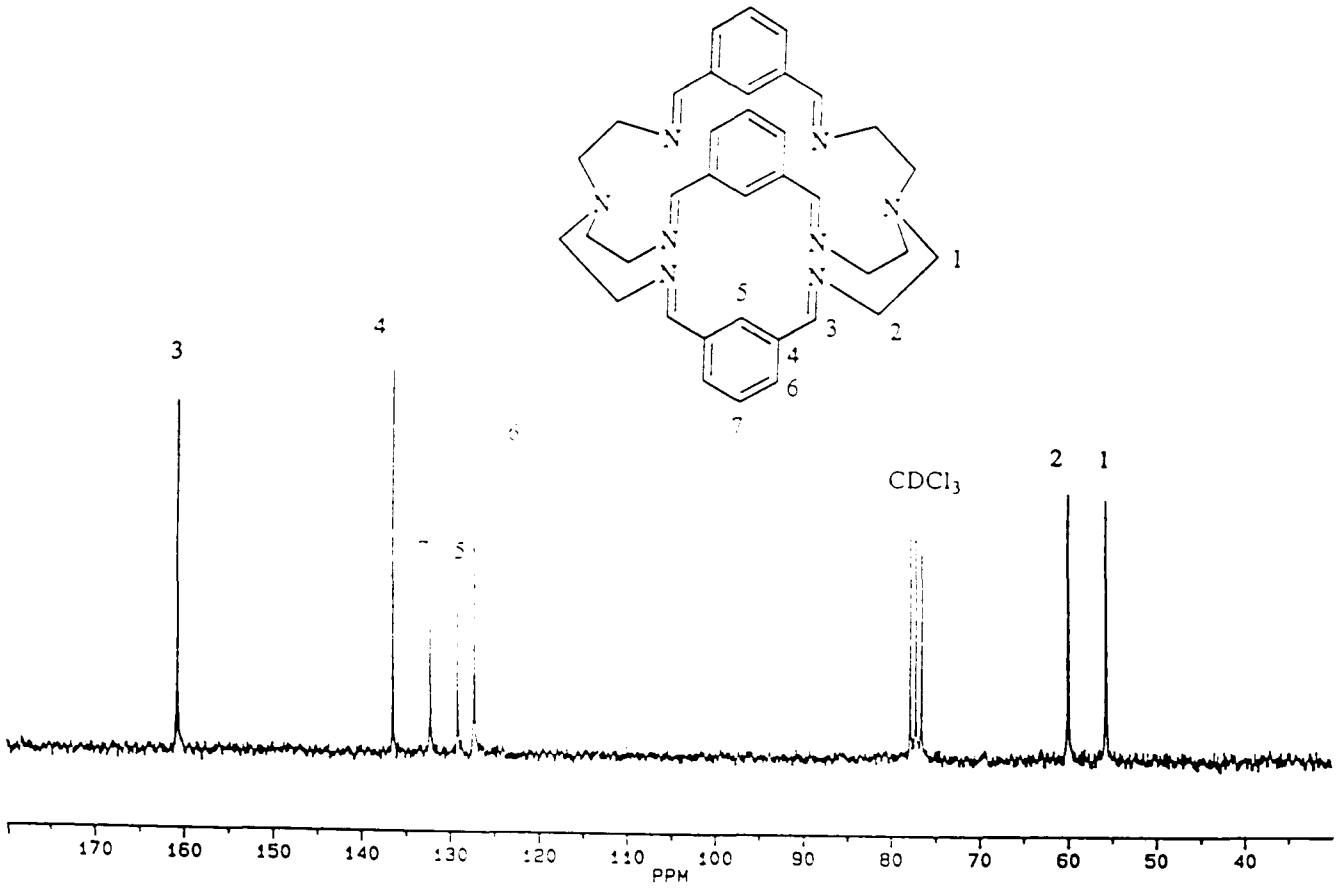
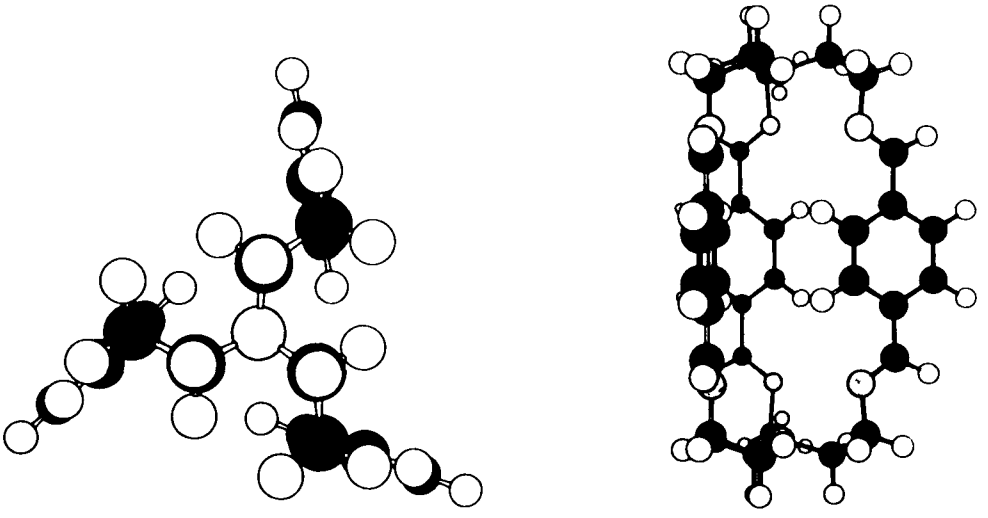


Figure 5. The ^{13}C nmr spectrum of the macrobicyclic **imine 17.**

Both the ^{1}H and the ^{13}C nmr spectrum of the macrobicyclic imine **18** was much simpler than that of the imine **17**, consisting only of four and five signals respectively (Figure 6). The energy minimized 3D structure of the macrobicyclic imine **18**, generated using MACROMODEL, shown in Figure 7, clearly indicates the geometrical constraints imposed by the 1,4-disubstituted benzene headgroups. Thus, macrobicyclic imine **18**, constructed employing 1,4-disubstituted headgroups (instead of the 1,3-disubstituted ones as in **17**), which confer lesser conformational mobility to it, force the molecule to adopt a highly symmetric and rigid structure around the 3-fold axis of symmetry.



18 (top view) **18** (side view)

Figure 7.

The "soft" ligands of macrobicyclic imine **18**, complex Cu(I) in CH_2Cl_2/CH_3CN solution to yield orange colored crystals of the dinuclear copper complex, **19**. The UV spectrum of compound **19** has

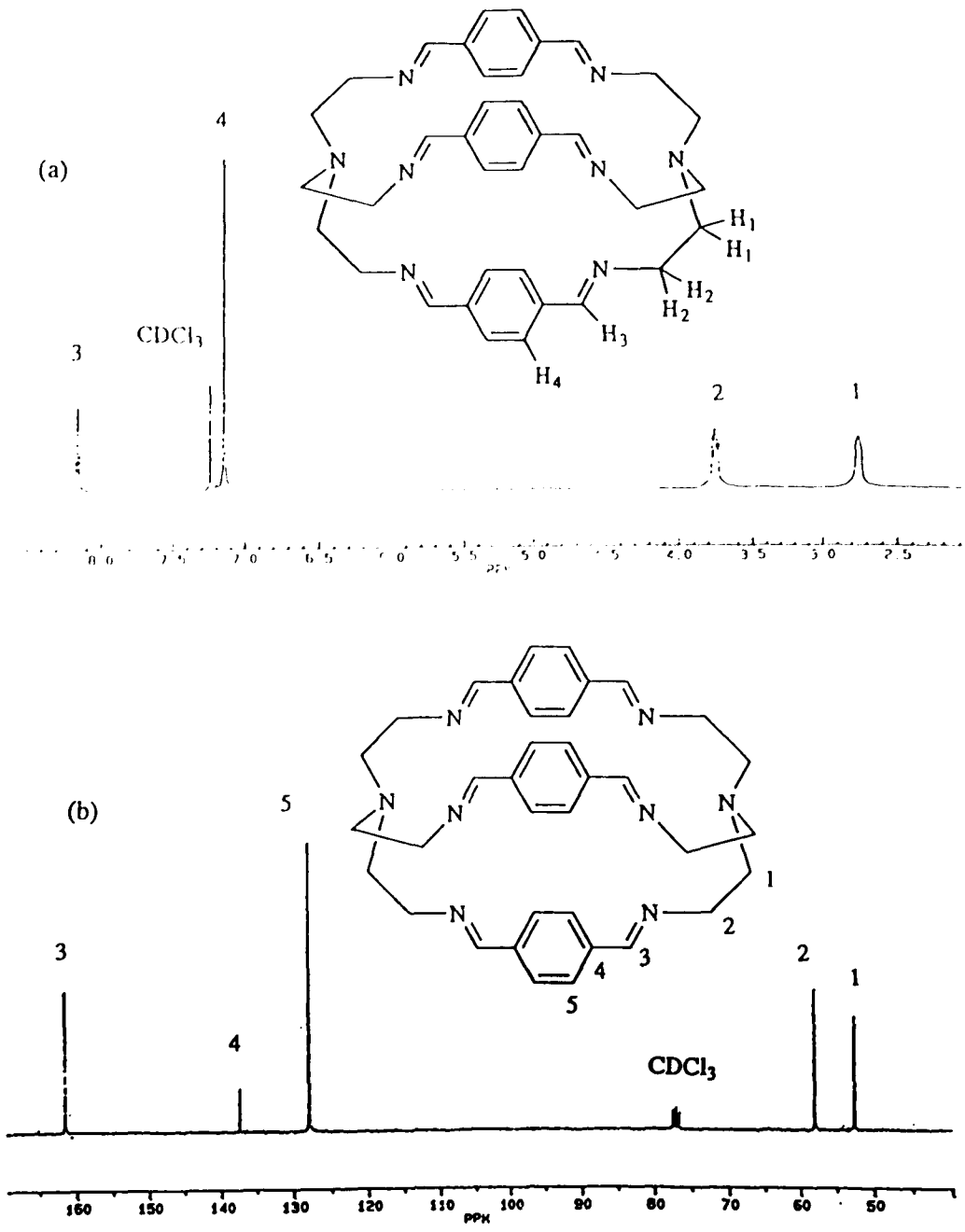
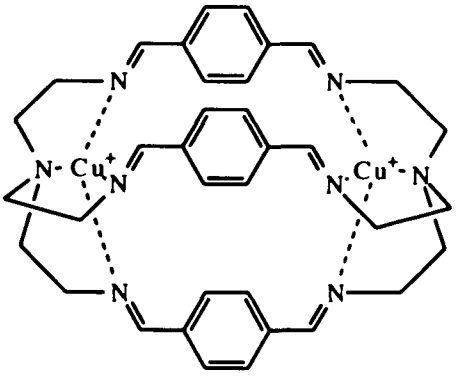


Figure 6. (a). The ^{1}H nmr spectrum of macrobicyclic imine **18**.
(b). The ^{13}C nmr spectrum of macrobicyclic imine **18**.

a new, red-shifted absorption at 364 nm, assigned to the MLCT (metal-to-ligand charge transfer) state (Figure 8).



19

A generally applicable synthetic route, the oxygen atom transfer from peroxyacids to imines that yield oxaziridines, was applied to obtain macrocyclic oxaziridines as follows: Biphasic buffered oxidation of macrobicyclic imines **17** and **18** with *m*-chloroperbenzoic acid (*m*-CPBA) was carried out at 0-2°C in the presence of benzyltriethylammonium chloride (BTEAC) as phase transfer catalyst. Reaction of the imine **17** led to formation of an oxaziridine, as part of a complex mixture (^{1}H & ^{13}C nmr) consisting of unreacted starting materials and other unidentified products. The desired oxaziridine could not be isolated from this mixture probably because like other N-alkyl substituted oxaziridines, **17** is prone to decomposition. However, reaction of macrobicyclic imine **18** with *m*-CPBA under the same conditions as for **17**, afforded a more stable macrobicyclic oxaziridine, **20**, that could be isolated as white crystals in 18% yield (Scheme 11). Compound **20** was stable in the solid state

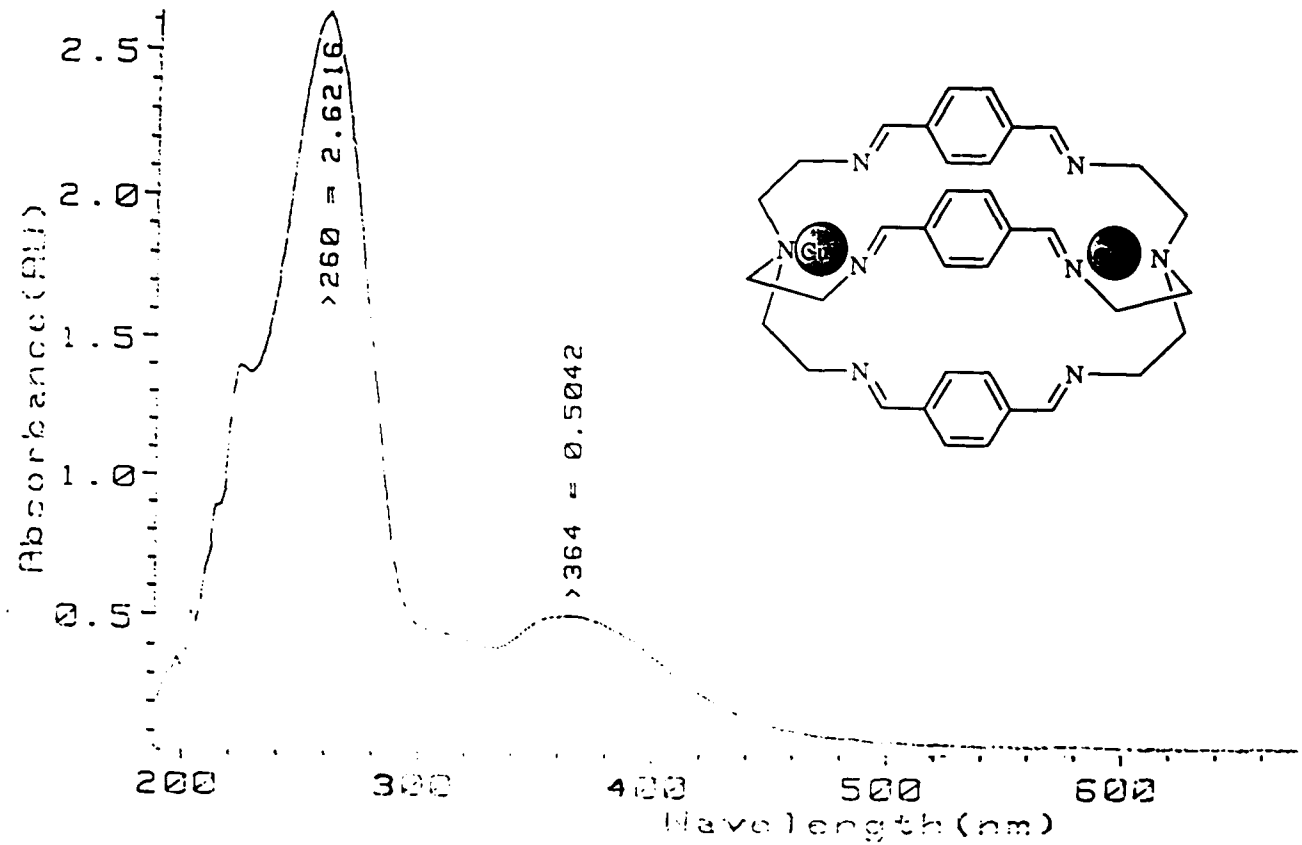
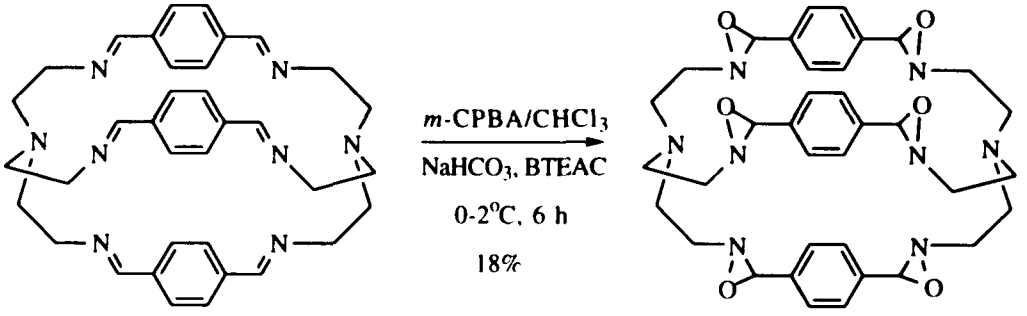


Figure 8. The UV spectrum of compound **19**.

at rt, but decomposed slowly in most organic solvents, e.g., $CHCl_3$ and CH_3CN.



18 **20**

Scheme 11

The structure of **20** was confirmed by elemental analysis, MS, ^1H and ^{13}C nmr spectral data. The ^1H nmr (Figure 9a) showed only six signals, and the ^{13}C spectrum (Figure 9b) consisted of five peaks, indicating that oxaziridine **20** is a highly symmetric molecule, which was further confirmed by the identical chemical shifts (6.95 ppm) seen for all of its aromatic protons. All the six protons that are attached to the oxaziridine rings also have the same chemical shifts (4.67 ppm), and the signals of all the six oxaziridine carbons appear at 80.19 ppm. Oxaziridines can exist as *E* or *Z* isomers, with different chemical shifts for the oxaziridine protons. In the *Z* isomer,

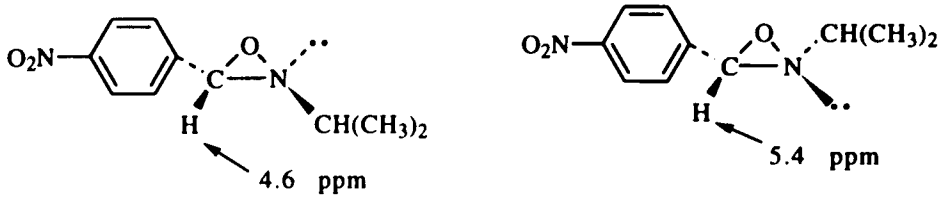


Figure 10. Chemical shifts of oxaziridine protons in the *E* and *Z* isomers.[51]

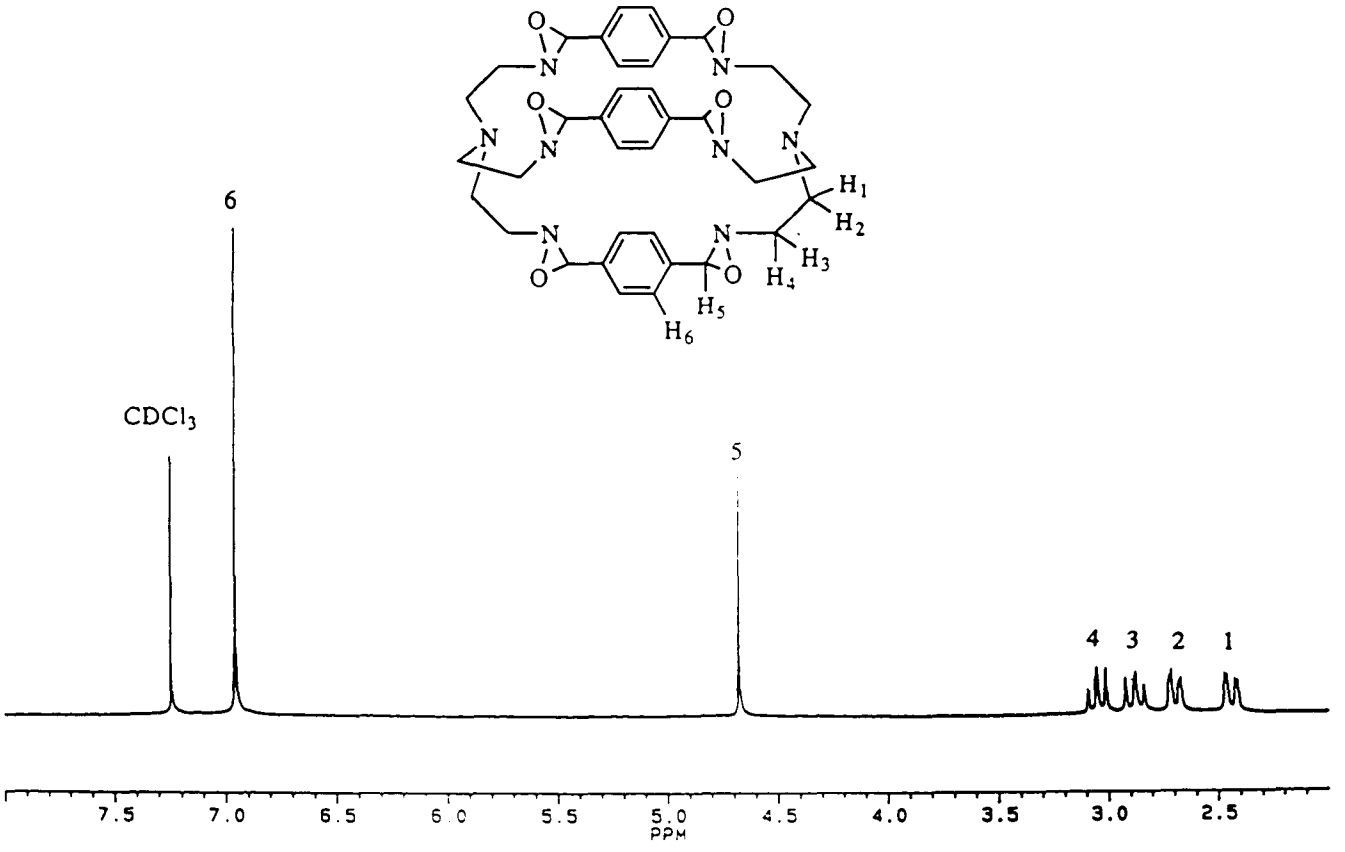


Figure 9a. The ^{1}H nmr spectrum of macrobicyclic oxaziridine **20.**

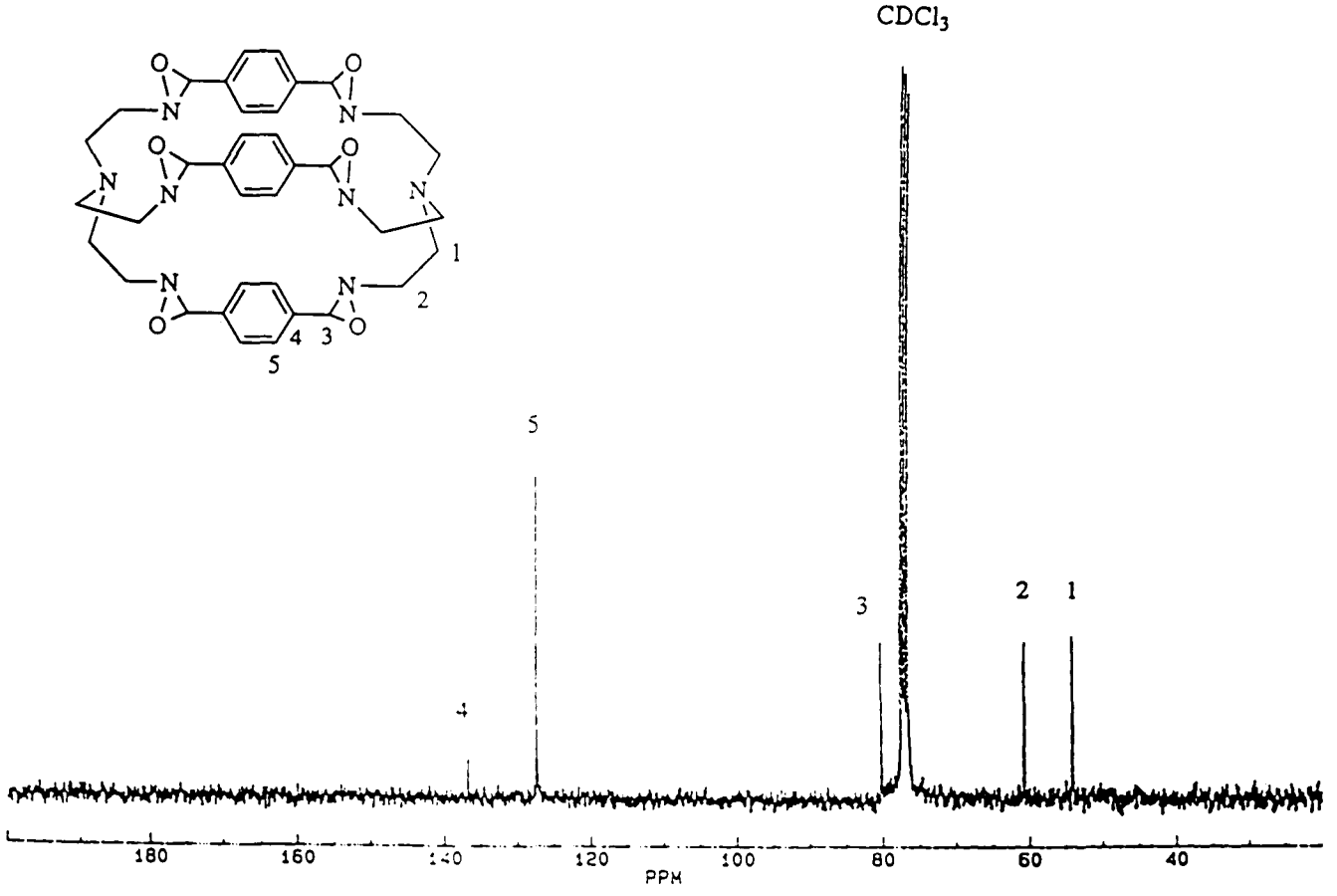
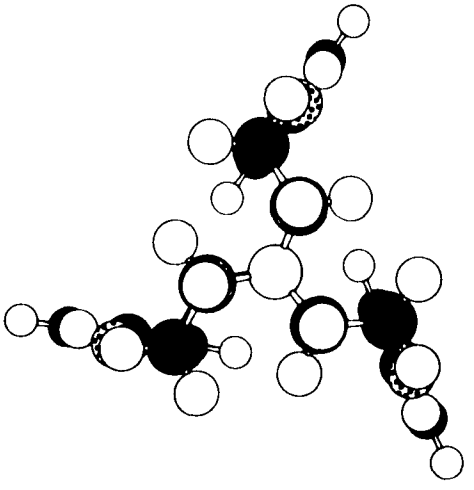
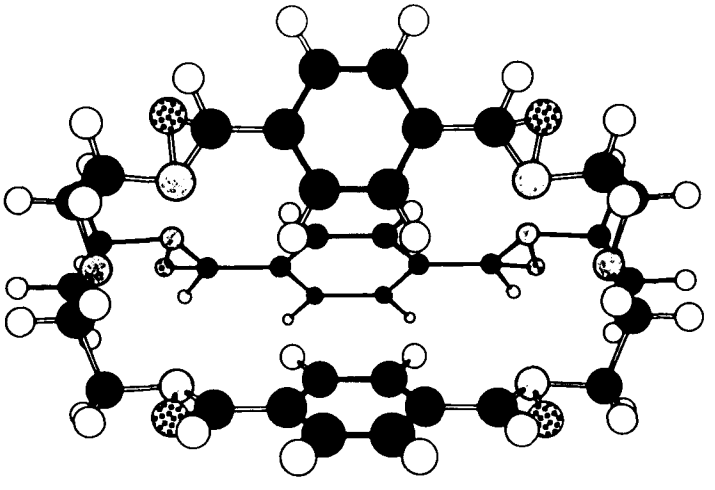


Figure 9b. The ^{13}C nmr spectrum of macrobicyclic oxaziridine **20**.

this proton being on the same side as the nitrogen lone pair, it absorbs at a lower field (5.4 ppm in $CDCl_3$) than in the *E* isomer (Figure 10).[51] Since in **20** all six oxaziridine protons absorb at 4.67 ppm, all should be *E* isomers. Figure 11 shows the most stable conformation of macrobicyclic oxaziridine **20** generated by MACROMODEL on a Silicon Graphics workstation, supports this assignment.



20 (top view)



20(side view)

Figure 11a.

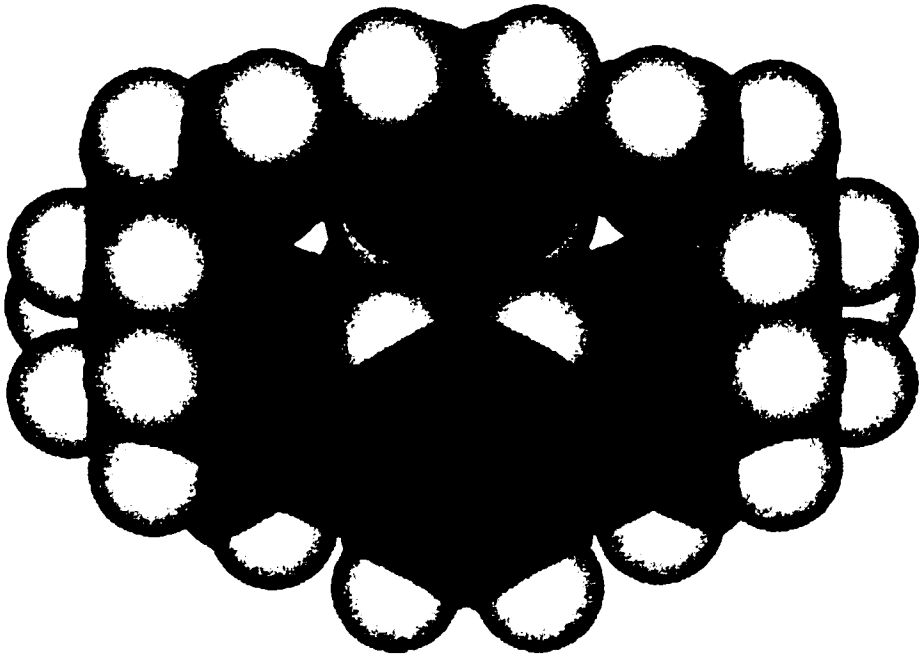


Figure 11b. CPK model of **20**.

It also explains why all the protons attached to the aromatic rings have identical chemical shifts (6.95 ppm), indicating that the molecule has a perfect three-fold axis of symmetry. That the oxidation by *m*-CPBA should yield a single oxaziridine isomer is understandable from the structures generated by MACROMODEL (Figures 7 and 11). These suggest that the attack by the peracid is blocked from the "endo" face of the C=N double bond favoring the formation of the thermodynamically more stable, *trans* isomer.

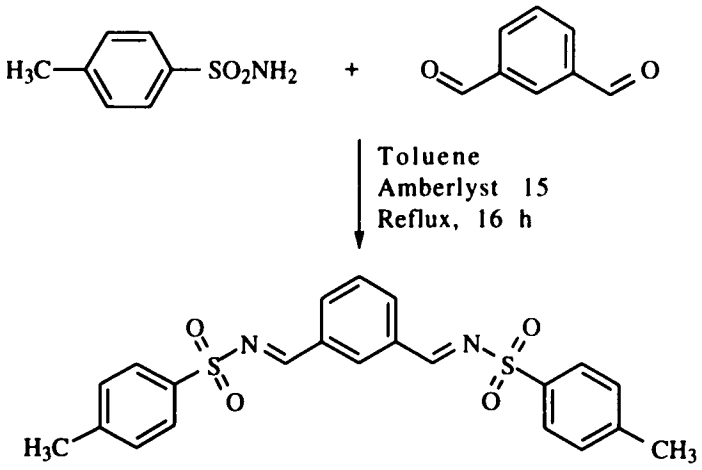
It is significant to note that the broad range of stability displayed by oxaziridines depends strongly on their substitution pattern.[36] Therefore, precautions must be taken when isolating novel oxaziridines for the first time. In the case of **20**, if after reaction the solvent is removed using a rotary evaporator, the initially white residue rapidly changes to a yellow material. The ^1H nmr spectrum shows in addition to the oxaziridine, aldehyde peaks resulting from decomposition. Therefore, to isolate **20** in pure form, a crystallization technique was used: After reaction was over, instead of removing the solvent, $CHCl_3$, the mixture was diluted with ether and was allowed to stand overnight at rt to yield **20** as white needles.

2.3 N-sulfonyloxaziridines.

Chiral N-sulfonyloxaziridines, Davis' reagents,[36] are generally not only more stable than oxaziridines, they are also more powerful oxidizing agents, due to the enhanced electrophilicity of their oxygen atom. Currently, they are the most suitable reagents to effect

asymmetric oxidation of nonfunctionalized substrates. Therefore, synthesis of macrocyclic N-sulfonyloxaziridines would be of particular interest, since further steric bias could be built into the macrocyclic framework. We intended to prepare macrocyclic N-sulfonyloxaziridines by oxidation of the corresponding macrocyclic N-sulfonylimines with *m*-CPBA or potassium peroxymonosulfate (OXONE).

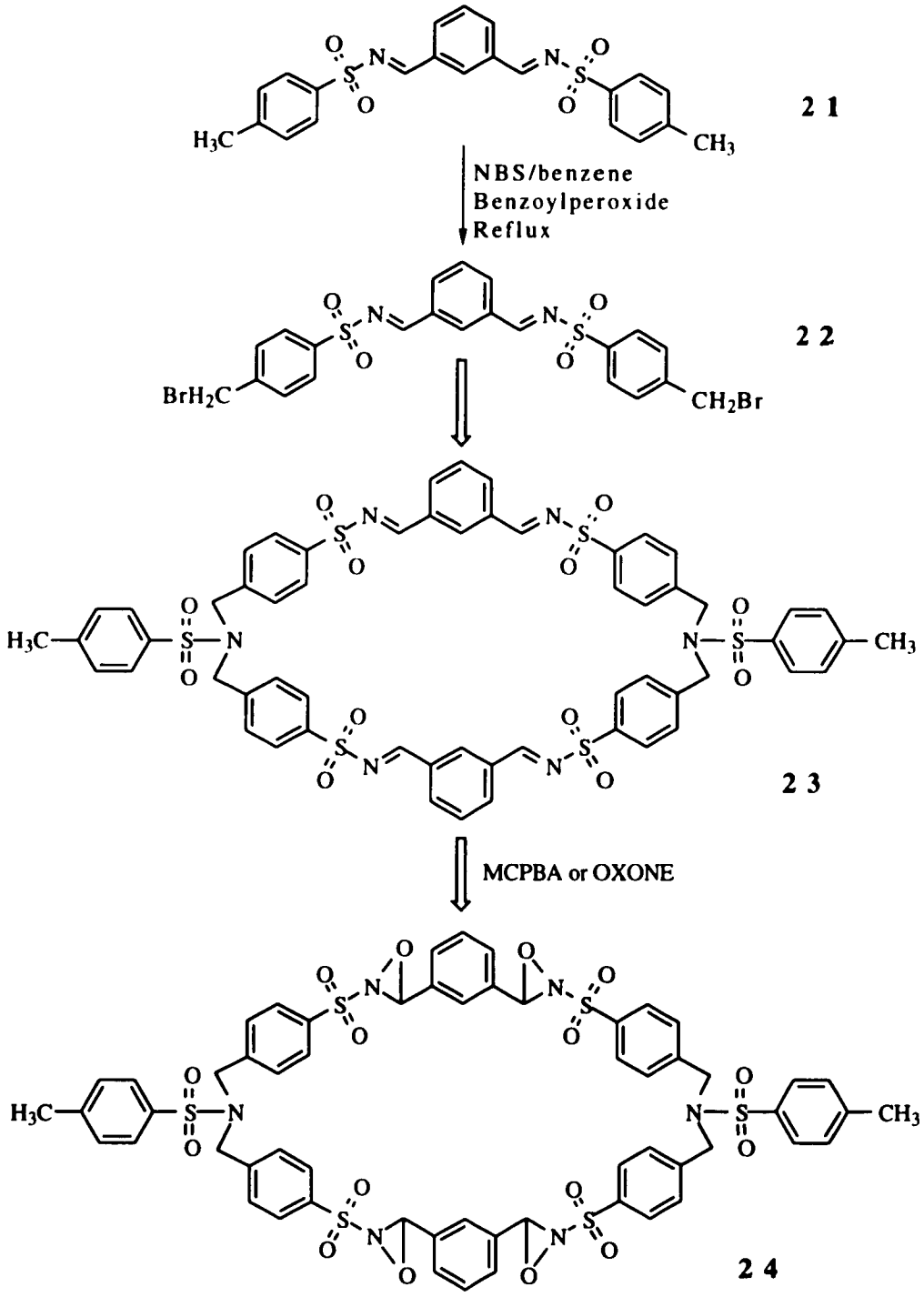
To obtain the macrocyclic N-sulfonylimines, we first prepared N-sulfonylimine **21** by condensation of isophthalaldehyde and *p*-toluenesulfonamide in the presence of an ion-exchange resin as catalyst (Scheme 12). Then we tried to convert **21** to bromosulfonyl-



21

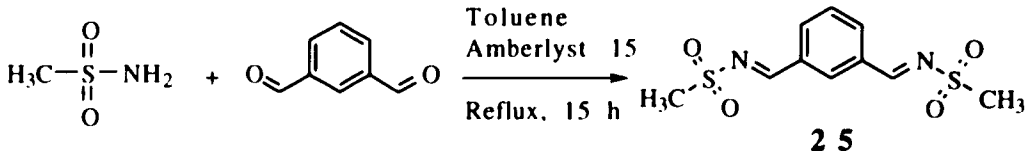
Scheme 12.

imine **22** by benzylic bromination using NBS[52] (Scheme 13). ^1H nmr analysis of the reaction mixture showed a ca. 50% conversion to the desired bromination product, but isolation and purification of it was not possible due to the rapid decomposition during chromatographies on both alumina and silica gel. Due to the small solubility difference,


21
NBS/benzene
Benzoylperoxide
Reflux
22
23
MCPBA or OXONE
24

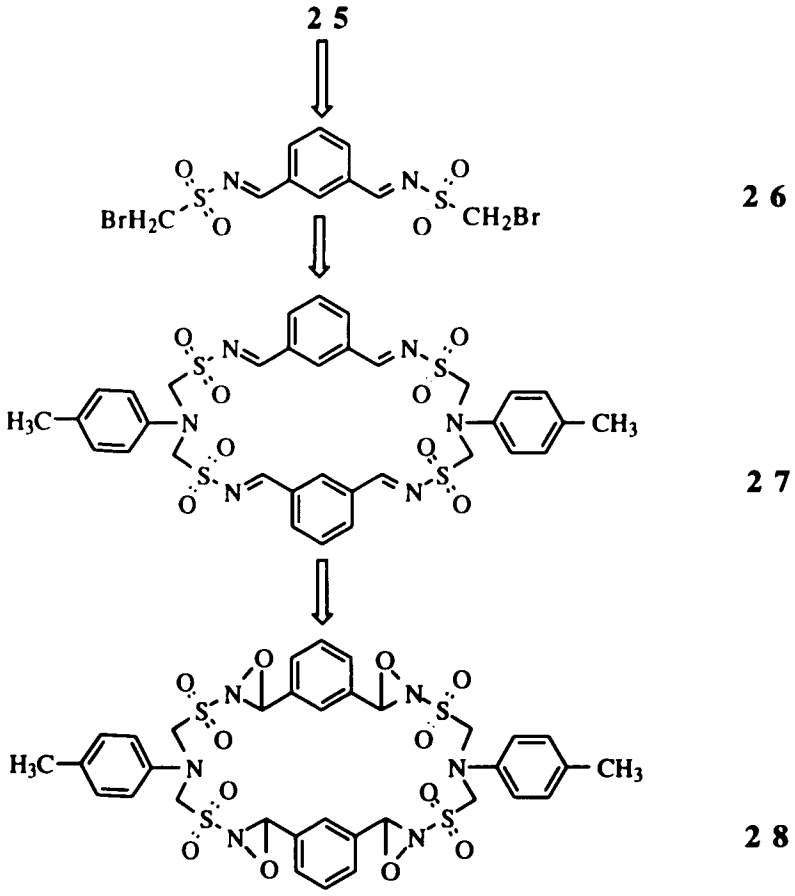
Scheme 13.

separation by crystallization was also unsuccessful thwarting synthesis of **24** via Scheme 13. We then prepared the more soluble sulfonylimine, **25** (Scheme 14), but were unable to brominate it; the NBS reaction resulted mostly in unreacted starting material.



25

Scheme 14.



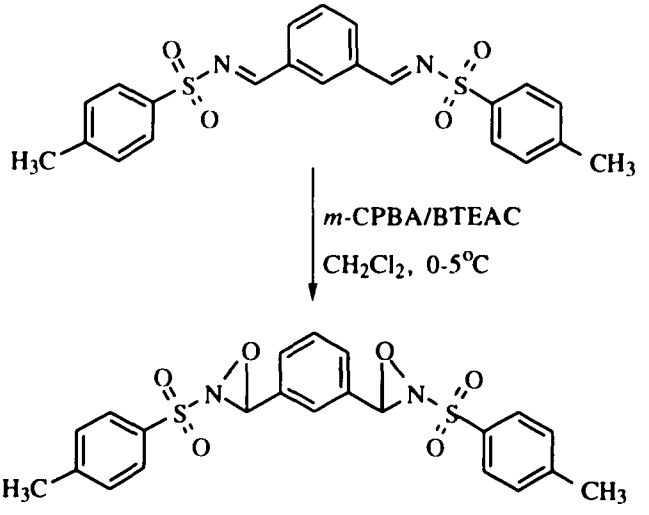
26

27

28

Scheme 15.

Although we were not successful in preparing the macrocyclic sulfonyloxaziridines according to Schemes 13/15, we could however convert bis-sulfonylimine **21** to the podand **29** in 92% yield (Scheme 16). The ^1H nmr of **29** showed only one type of oxaziridine proton at 5.40 ppm, therefore this podand has two sulfonyloxaziridine groups of identical stereochemistry, and interestingly, it is the first compound that has two of these functionalities incorporated into the same molecule.



21

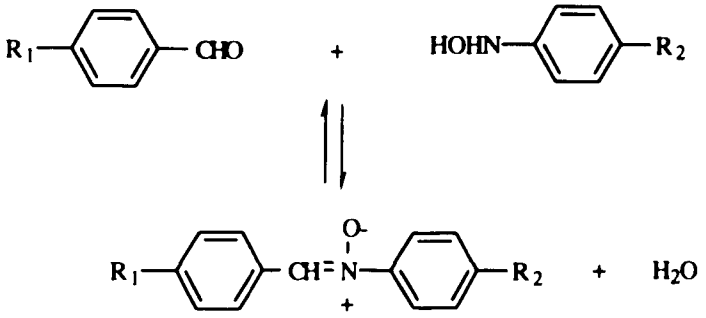
29

Scheme 16.

2.4 Macrobicyclic nitrones.

Nitrones are usually prepared either by the condensation of aldehydes with hydroxylamines or by the oxidation of hydroxylamine precursors.[37] The condensation of arylhydroxyl-

amines with substituted benzaldehydes offers the most direct route to the preparation of diarylnitrones (Scheme 17).

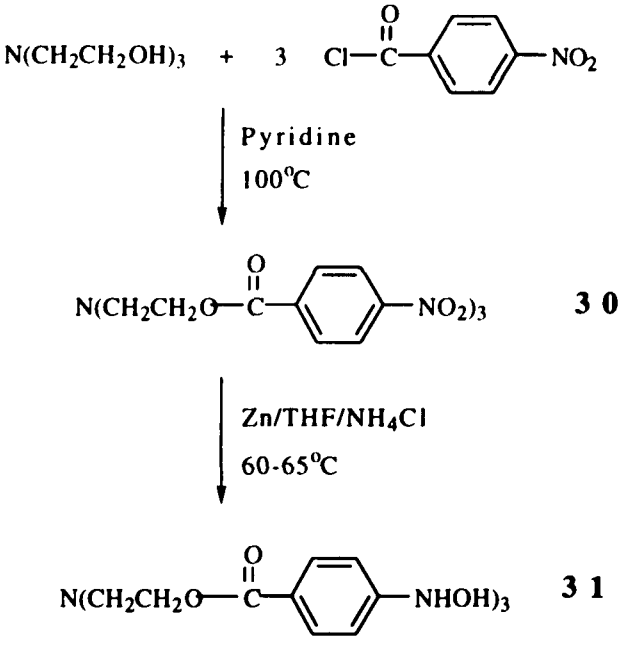


Scheme 17.

Electron withdrawing and electron donating substituents provide the push-pull effect necessary to make the hydroxylamines less nucleophilic and the aldehydes less electrophilic. These substituents can also reduce the equilibrium constant for the condensation reaction to such an extent that the formation of the nitrone becomes impossible.[53]

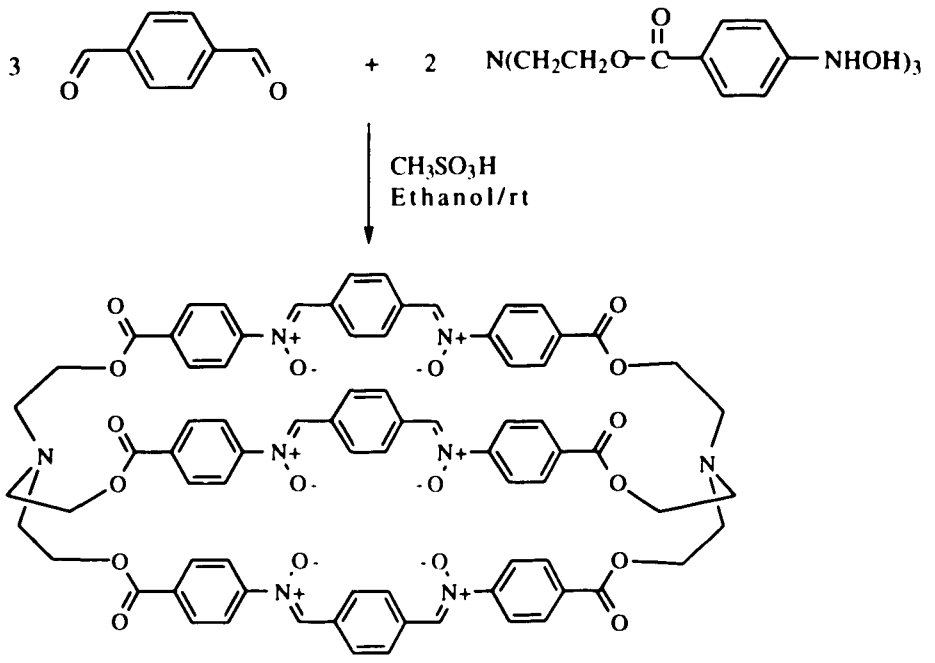
The major problem met in the syntheses of nitrones is the preparation of the hydroxylamine precursors. Thus, if the desired tris(arylhydroxylamine) precursors can be prepared, then a [2+3] condensation, similar to the one we employed in the synthesis of macrobicyclic imines, could yield the desired macrobicyclic nitrones. Arylhydroxylamines are prone to easy decomposition, and hence are notoriously difficult to prepare. They can be made either by dissolving metal reductions or by catalytic hydrogenation of the

corresponding nitro compounds. Introduction of electron-withdrawing groups would make the nitro compounds less reactive, but the hydroxylamines derived from them more stable. Electron-donating groups in contrast would favor the formation of arylamines. Therefore, we prepared the tris(4-nitrobenzoyloxyethyl)amine and the tris(4-hydroxyl-aminobenzoyloxyethyl)amine precursors required for the synthesis of nitrones according to Scheme 18.



Scheme 18.

Condensation of tris(4-hydroxylaminobenzoyloxyethyl)amine, **31** using methanesulfonic acid as catalyst (Scheme 19) afforded a brownish, polymer-like powder, only very slightly soluble in organic solvents. However, the FAB-MS of this product, was in agreement with a macrobicyclic nitrone structure, **32**.



3 2

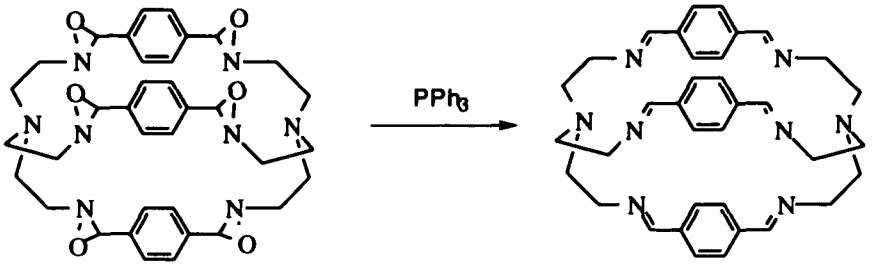
Scheme 19.

2.5 Preliminary study of the properties of macrobicyclic oxaziridines and macrobicyclic nitrones.

Among the many reactions of oxaziridines,[36] such as thermal rearrangement to nitrones, thermal and photochemical isomerization to amides, cycloadditions, and oxygen-transfer reactions, the last is particularly interesting both from chemical and biological points of view. From the chemical point of view, since oxaziridines have their active oxygen in a three-membered ring, they are useful model systems to study the mechanism of oxygen transfer in other similar, though much less stable systems such as metal peroxides and

dioxiranes. Sulfonyloxaziridines being neutral aprotic agents, are uniquely suited to oxidize enolates and carbanions, sulfides, selenides, amines, etc., they are also the best chiral oxidizing agents known. From the biological point of view, oxaziridines can be employed as models for biological oxygen-atom transfer reactions catalyzed by mono-oxygenase enzymes. Nitrones[38] are highly versatile synthetic intermediates and spin-trapping agents. A particularly interesting aspect of their chemistry is the facile light-induced electrocyclic ring closure that yields oxaziridines. Two important properties of the novel macrobicyclic compounds synthesized here was explored: 1) The ability of the macrobicyclic oxaziridine to transfer oxygen, and 2) The light sensitivity of the macrobicyclic nitrone.

The macrobicyclic oxaziridine **20** transferred all of its six oxygens to triphenylphosphine readily and quantitatively to yield triphenylphosphine oxide and macrobicyclic imine **18** (Scheme 20, Figure 12). The macrobicyclic oxaziridine, **20**, could be readily regenerated from the latter by oxidation with *m*-CPBA.



20 **18**

Scheme 20.

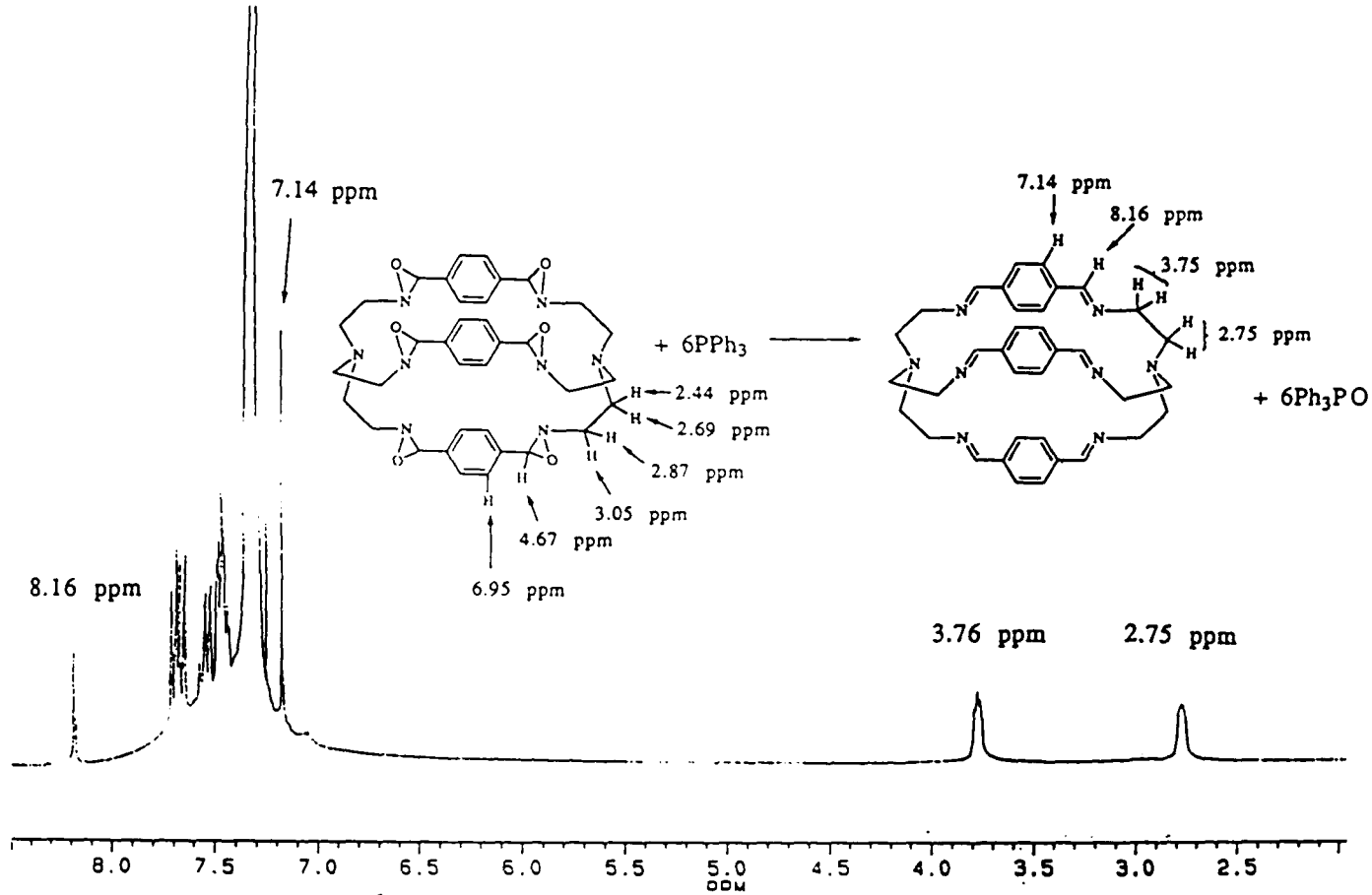
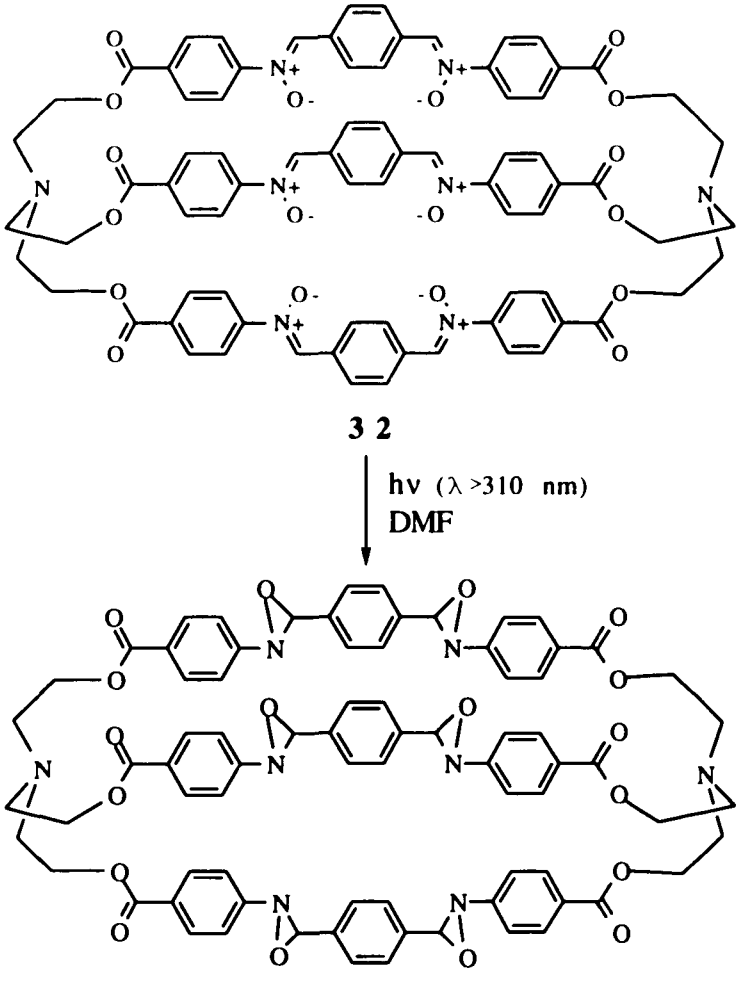


Figure 12. The ^{1}H nmr spectrum of the reaction mixture after treatment of **20** with triphenylphosphine in $CDCl_3$.

As expected, macrobicyclic nitrone **32** was light-sensitive. On irradiation by UV light[54] in dilute DMF solution it underwent ring closure that is expected to yield a macrobicyclic oxaziridine (Scheme 21). Indeed, the disappearance of the characteristic absorption of the macrobicyclic nitrone at ca. 370 nm and concomitant increase in absorption at 298 nm *via* an isosbestic point was observed (Figure 13). However, similar to many N-aryloxaziridines, this oxaziridine was too unstable precluding its isolation to ascertain the structure.



Scheme 21.

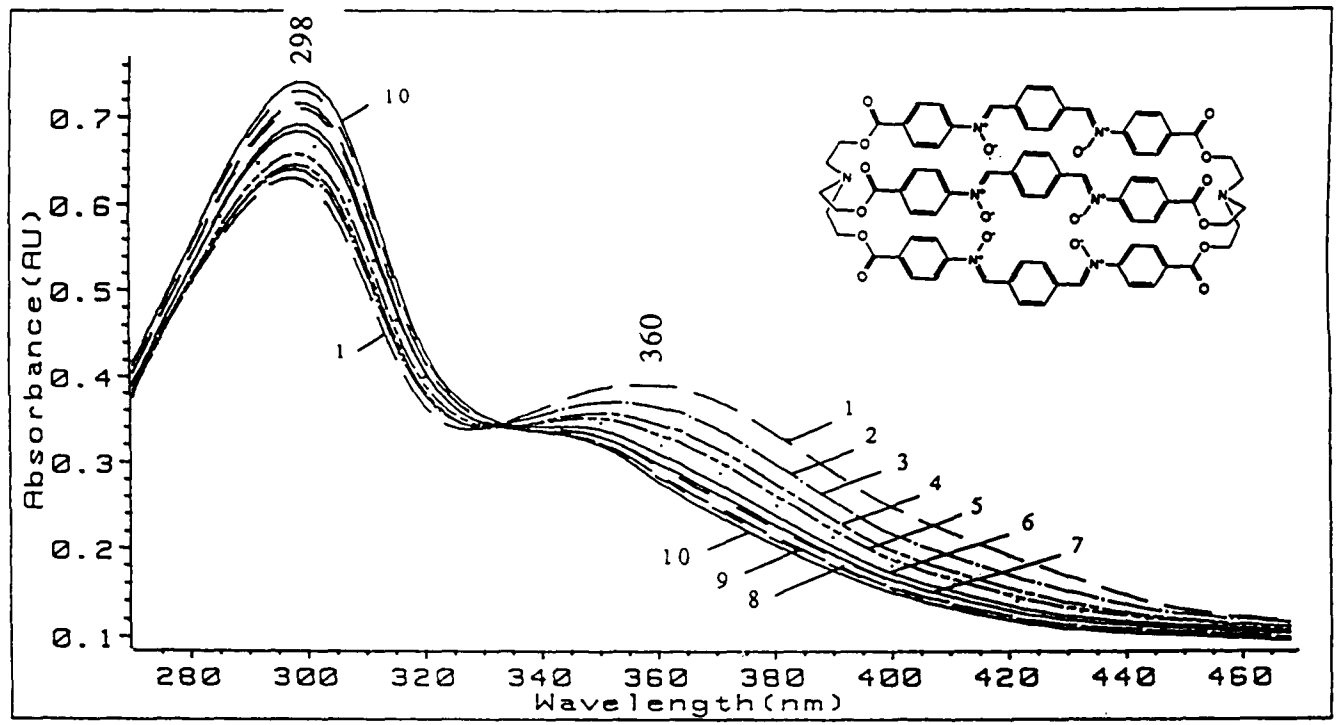


Figure 13. Time-dependent UV spectra showing the conversion of the macrobicyclic nitrone 32 to an oxaziridine by irradiation at>320 nm in DMF solution. Curves 1-10: 0, 10, 20, 30, 40, 50, 60, 70, 80, and 90 min.

2.6 CONCLUSION

Initial studies employing space-filling molecular models in the design of novel types of macrocyclic compounds failed to predict accurately their conformation and stability, hence a series of [1+1] and [1+2] adducts, podands, were obtained instead of the desired macrocycles expected from [2+2] additions. Molecular modeling programs using PCMODEL and the more powerful MACROMODEL to aid molecular design predicted more accurately the structural prerequisites of the starting materials that were likely to yield the target macrocycles. Extensive use of MACROMODEL led to the selection of two hexaimino macrocycle precursors, that by oxidation were likely converted to macrocyclic oxaziridines with reasonable stability. The oxaziridine obtained by *m*-CPBA oxidation of the imino macrocycle, derived from [3+2] addition of isophthalaldehyde with tris(2-aminoethyl)amine, was not sufficiently stable for isolation, demonstrating the stringent stereoelectronic requirements in the macrocyclization step. The corresponding macrocyclic imine derived form terephthaldicarboxaldehyde, however, afforded a crystalline macrocyclic oxaziridine. This macrobicyclic oxaziridine is the first example of a novel class of macrocycles that contain oxaziridine moieties, capable of transferring all its six oxygen atoms to triphenylphosphine, rapidly and quantitatively. Thus, this type of macrocycles containing oxaziridine moieties have a high potential as oxidizing agents and oxygenase mimics.

Computer-aided synthesis using PCMODEL and MACROMODEL afforded the first example of macrocyclic compound containing nitrone functionalities. This macrobicyclic nitrone underwent light-induced ring closure to the corresponding oxaziridine macrocycle. It is expected that MACROMODEL guided synthesis will afford congeners of the first macrocyclic nitrone, specifically designed to undergo 1,3-additions leading to the formation of whole arrays of novel materials with interesting optic and/or electronic properties.

3. EXPERIMENTAL

General techniques.

^{1}H and ^{13}C Nuclear magnetic resonance spectra (nmr) were recorded on a Bruker NR-300 MHz instrument. Chemical shifts are given as δ values from tetramethylsilane. Ultraviolet-visible spectra were recorded on a Hewlett-Packard HP UV 8452A fast scan UV/VIS diode array spectrophotometer. Electron impact, chemical ionization and fast atom bombardment mass spectra (EI-, CI- and FAB-MS) were obtained on a Finnigan Mat SSQ-70 instrument. High performance liquid chromatography (HPLC) was performed by using a Waters 6000A liquid chromatography equipped with Model 440 Absorbance UV/VIS detector. Molecular mechanics calculations were carried out using the PCMODEL program on an IBM-PC, and on a Silicon Graphics IRIS INDIGO workstation employing the MACROMODEL program of W.C. Still (Columbia Univ.). Reagents, solvents and buffers were obtained unless otherwise stated from Aldrich/Sigma Chemical Companies.

2,6-(4,4,5,5-tetramethylimidazoline-3-oxide)-pyridine (podand-11) and 2-(4,4,5,5-tetramethylimidazoline-3-oxide)-6-(4,4,5,5-tetramethylimidazoline-6-oxide)-pyridine (podand-12).

To a stirred solution of 2,6-pyridinedicarboxaldehyde (50 mg, 0.36 mmol) in *n*-butanol (15 ml), 2,3-bis(hydroxylamino)-2,3-dimethylbutane sulfate (1 g, 4 mmol) in H_2O (5 ml) and sodium

bicarbonate (1 g) were added in small portions over a period of 1 h at ca. 20°C. Stirring was continued for 24 h at rt, then the organic layer was separated and the aqueous layer was extracted with *n*-butanol (3 x 10 ml), and the organic layers were combined. The solvent was removed under reduced pressure and the residue was taken in *n*-butanol (30 ml) and was passed through a nylon syringe filter (0.45 μm) to remove insoluble materials. The solvent was removed under reduced pressure at rt, the residue was dissolved in methanol (50 ml), and the yellow solution was stirred overnight at rt in contact with air. The methanol was removed, the mixture was separated by preparative TLC on Si gel eluting with methanol to give two compounds, podand-**11** (110 mg, 85%) and podand-**12** (6 mg, 5%), respectively as yellow crystals.

podand-11

Mp: 263-265°C

λ_{max} (CH_3OH): 370 nm (ε=11,000) and 270 nm (ε=8,200).

^1H nmr ($CDCl_3$): δ 1.34 (s, 12 H, C*H*3), 1.40 (s, 12 H, C*H*3), 4.72 (s, 2 H, N*H*), 7.97 (t, 1 H, J=8.0 Hz, pyridine-4*H*), 9.44 (d, 2 H, J=8.0 Hz, pyridine-3&5*H*).

^{13}C nmr (CD_3OD): δ 19.4 (**C**H3), 23.7 (**C**H3), 62.8 (>**C**(CH_3)2), 76.5 (>**C**(CH_3)2), 127.4 (pyridine-**C3&C5**), 139.7 (pyridine-**C4**), 144.5 (pyridine-**C2&C6**) and 147.4 (-**C**=N^+-O^-).

podand-12

Mp: 263-265°C

λ_{max} (CH_3OH): 354 nm and 270 nm.

^1H nmr($CDCl_3$) δ 1.34 (s, 12 H, C*H*3), 1.40 (s, 12 H, C*H*3), 4.89 (s, 2 H,

N*H*), 7.87 (dd, 1 H, J=8 Hz, pyridine-4*H*), 8.21 (d, 1 H, J=8 Hz, pyridine-5*H*), 9.45 (d, 1 H, J=8.0 Hz, pyridine-3*H*).

4-(N,N'-dihydroxy-4,4,5,5-tetramethylimidazoline)-1-benzaldehyde (13).

To a solution of 2,3-bis(hydroxylamino)-2,3-dimethylbutane sulfate (246 mg, 1 mmol) in water (5 ml), Na_2CO_3 (106 mg, 1 mmol) was added, followed by terephthaldicarboxaldehyde (67 mg, 0.5 mmol). The resulting slurry was stirred for 24 h in a stoppered flask and the precipitate was filtered off, washed with cold 1% sodium metabisulfite, followed by water and then was dried in vacuum, to give 105 mg of **13** as a white amorphous powder (80% yield).
Mp: 174-175°C
^{1}H nmr (DMSO-d6) δ 1.03 (s, 6 H, C*H*3), 1.06 (s, 6 H, C*H*3), 4.59 (s, 1 H, C*H*), 7.68 (d, 2 H, J=8.1 Hz, Ar-3*H*), 7.86 (d, 2 H, J=8.1 Hz, Ar-2*H*), 7.86 (s, 2 H, NO*H*), 9.98 (s, 1 H, *H*C=O).
^{13}C nmr (DMSO-d6): δ 17.2 (CH_3), 24.2 (CH_3), 66.4 (-C(CH_3)$_2$), 89.6 (Ar-CH<), 128.9 (ArC-CH<), 135.6 (ArC-H), 148.9 (ArC-CHO), and 192.9 (HC=O).

1,4-Bis(4,4,5,5-tetramethylimidazoline-3-oxide)-benzene (podand-14).

A 100 ml two-necked round-bottomed flask equipped with a dry ice condenser was charged with a solution of terephthaldicarboxaldehyde (50 mg, 0.36 mmol) in *n*-butanol (15 ml)

and saturated aqueous $NaHCO_3$ (15 ml). To this mixture under stirring 2,3-bis(hydroxylamino)-2,3-dimethylbutane sulfate (1 g, 4 mmol) and sodium bicarbonate (1 g) were added in small portions over a period of 1 h, and stirring was continued for 24 h at rt. The organic layer was separated, the aqueous layer was extracted with *n*-butanol (3 x 10 ml), and the organic layers were combined. Butanol was removed under reduced pressure, the residue was re-dissolved in *n*-butanol (30 ml) and was passed through a nylon syringe filter (0.45 μm) to remove insoluble material. The solvent was removed under reduced pressure at rt, the residue was dissolved in methanol (50 ml) to give an yellow solution that was stirred for 48 h at rt in contact with air. The methanol was removed, and the mixture was separated by preparative TLC on Si gel developing with methanol to give 65 mg of the podand-**3** (49% yield).

Mp: >250°C (dec.)

$\lambda_{max}(CH_3OH)$: 370 nm. 256 nm.

^1H nmr ($CDCl_3$): δ 1.32 (s, 24 H, C*H3*), 8.35 (s, 4 H, Ar-*H*).

5-(4,4,5,5-tetramethylimidazoline´-3-oxide)-2-furanaldehyde (15).

To a solution of 2,5-furandicarboxaldehyde (20 mg, 0.16 mmol) in $CHCl_3$ (15 ml), a solution of 2,3-bis(hydroxylamino)-2,3-dimethylbutane sulfate (200 mg, 0.81 mmol) in water (15 ml) was added, followed by $NaHCO_3$ (100 mg), and the mixture was stirred for 40 h at rt. The organic layer was separated, the aqueous layer was extracted with $CHCl_3$ (3 x 10 ml). The solvent was removed from

the combined organic phases, and the product was separated by preparative TLC on Si gel eluting with methanol, to give 9 mg of **15** (23% yield).

^{1}H nmr ($CDCl_3$): δ 1.31 (s, 6 H, C*H3*), 1.35 (s, 6H, 4.22 (s, 1 H, N*H*), 7.35 (d, 1 H, J=5.7 Hz, furan-*H*), 7.92 (d, 1 H, J=5.7 Hz, furan-*H*) and 9.65 (s, 1 H, *H*C=O).

^{13}C nmr ($CDCl_3$): δ 19.3 (CH_3), 24.2 (CH_3), 62.8 (-C<), 73.7 (-C<), 115.9 (Furan-CH), 123.0 (Furan-CH), 145.2 (-C=N^+-O^-), 151.7 (Furan-CH-CHO) and 177.2 (HC=O).

Macrobicyclic imine (17).

In a 100 ml, three-necked, round-bottomed flask, equipped with a magnetic stirrer, a condenser, and a nitrogen inlet, 292 mg (2 mmol) of tris(2-aminoethyl)amine and anhydrous methanol (50 ml) were introduced. Then, isophthalaldehyde (402 mg, 3 mmol) was added in one portion with stirring. Stirring of the reaction mixture was continued at reflux under an inert atmosphere for 3 h. The reaction mixture was allowed to stand overnight at rt without stirring and the insoluble materials were filtered off through a sintered-glass funnel of medium porosity. The solvent was removed using a rotary evaporator to yield 500 mg of the crude product, as a white crystalline solid, which was recrystallized from CH_3CN/CH_2Cl_2 (1:1) to yield 427 mg white crystals (73% yield).

Mp: >300°C (dec.)

^{1}H nmr ($CDCl_3$) at rt : δ 2.68-3.74 (broad, 24 H, -C*H2*C*H2*-), 5.30 (s, 3 H, Ar*H*), 7.50 (t, 3 H, J=7.8 Hz, Ar*H*) and 8.16 (d, 6 H, J=7.8, Ar*H*)

^{1}H nmr ($CDCl_3$) at -20°C: δ 2.64 (d, 6 H, J=12.0 Hz, C*H*$_2$), 2.86 (t, 6 H, J=10.6 Hz, C*H*$_2$), 3.20 (t, 6 H, J=10.8 Hz, C*H*$_2$), 3.78 (d, 6 H, J=10.6 Hz, C*H*$_2$), 5.19 (s, 3 H, Ar*H*), 7.50 (s, 6 H, -C*H*=N), 7.54 (d, 6H, J=7.8 Ar*H*) and 8.13 (d, J=7.7 Hz, Ar*H*).
^{13}C nmr ($CDCl_3$): δ 56.1 (-**C**H$_2$-N<), 60.0 (-**C**H$_2$-N=), 127.4 (**Ar**), 128.9 (**Ar**), 132.4 (**Ar**), 137.0 (**Ar**) and 160.7 (-**C**=N-).

MCPBA oxidation of macrobicyclic imine 17.

To a solution of 0.1 g (0.17 mmol) of macrobicyclic imine **17** in 10 ml $CHCl_3$, 300 mg (1.3 mmol) of benzyltriethylammonium chloride (BTEAC) and saturated solution of $NaHCO_3$ (5 ml) were added. To this mixture, cooled to 0-2°C, a solution of 400 mg (1.3 mmol) of *m*-CPBA (50-60%) in 10 ml $CHCl_3$ was added dropwise with stirring over a period of 30 min. The mixture was stirred at 0-2°C for an additional 6 h. The organic layer was separated, and was washed successively with 10% sodium bisulfate solution (5 ml), 5% K_2CO_3 solution, and finally twice with H_2O. Removal of the solvent from the pale yellow solution gave a yellow precipitate. Analysis, by ^{1}H and ^{13}C nmr showed the precipitate is a complex mixture of an oxaziridine, unreacted starting materials, as well as other unidentified products.

Macrobicyclic imine (18).

A 100 ml, three-necked, round-bottomed flask was equipped with a magnetic stirrer, a condenser, and a pressure equalized adding funnel attached to a nitrogen inlet. The flask was charged with

tris(2-aminoethyl)amine (292 mg, 2 mmol) and anhydrous methanol (50 ml), then terephthaldicarboxaldehyde (402 mg, 3 mmol) was added in one portion with stirring, and the mixture was refluxed under nitrogen atmosphere for 3 h. The reaction mixture was allowed to stand overnight at rt without stirring, and the insoluble materials were filtered through a sintered-glass funnel of medium porosity. The filtrate was reduced to half of its volume, then water was added (10 ml) slowly with stirring until a white precipitate formed. The mixture was allowed to stand for 1 h at rt. The precipitate was filtered through a sintered-glass funnel of medium porosity and was washed with water (2 x 5 ml) then dried in vacuum for 8 h, at rt to give 515 mg of the macrobicyclic imine, **18**, as a white powder (88% yield).

Mp: >300°C (dec.)

^{1}H nmr ($CDCl_3$): δ 2.75 (t, J=4.2 Hz, 12 H, CH_2), 3.75 (t, J=4.2 Hz, 12 H, CH_2), 7.14 (s, 12H, Ar*H*) and 8.16 (s, 6H, *H*C=N).

^{13}C ($CDCl_3$): δ 52.5 (CH_2), 57.9 (CH), 127.8 (**Ar**), 137.3 (**Ar**) and 161.4 ppm (C=N).

FAB-MS (glycerol): *m/z* 587 $[M+1]^+$

Macrobicyclic imine-copper(I) complex (19).

Macrobicyclic imine, **18**, (58.5 mg, 0.1 mmol) was dissolved in CH_2Cl_2 (3 ml) then CuCl (19.8 mg, 0.2 mmol) in acetonitrile (5 ml) was added with stirring in a dark room under red light (λ >600 nm). Evaporation of the solvent gave 71 mg of an orange colored solid, **19** (90.5% yield).

$\lambda_{max}(CH_3CN/CH_2Cl_2=1/1)$: 364 nm.

FAB-MS (glycerol): *m/z* 714 $[M+1]^+$, 651 $[M+1-Cu]^+$.

^1H nmr (CD_3OD): δ 3.21 (t, 12 H, J=5.4 Hz, C*H*$_2$), 3.96 (t, 12 H, J=5.2 Hz, C*H*$_2$), 7.81 (s, 12 H, Ar*H*) and 8.70 (s, 6 H, -C*H*=N).

Macrocyclic oxaziridine (20).

A 100 ml round-bottomed flask was equipped with a magnetic stirrer and a pressure equalized dropping funnel. The flask was charged with the macrobicyclic imine **18** (100 mg, 0.17 mmol) dissolved in $CHCl_3$ (10 ml), saturated aqueous $NaHCO_3$ (5 ml) and BTEAC (300 mg). The mixture was cooled to 0-2°C and a solution of *m*-CPBA (50-60%) (400 mg, 1.25 mmol) in $CHCl_3$ (10 ml) was added dropwise with stirring over a period of 30 min. The reaction mixture was stirred for an additional 6 h at 0-2°C, then the layers were separated. The organic layer was washed successively with 10% aqueous sodium bisulfite (5 ml), 5% aqueous K_2CO_3 (5 ml) and then H_2O (2 x 5 ml). After addition of ether (10 ml), the organic layer was allowed to stand at rt overnight. Macrobicyclic oxaziridine, **20**, was separated from solvent as a white crystalline product, (2 mg, 18% yield).

Mp: >250 (dec.)

Elemental analysis:

63.30% (C), 6.20% (H), 16.37% (N) and 13,78% (O); $C_{36}H_{42}N_8O_6$ requires 63.33% (C), 6.20% (H), 16.41% (N) and 14.06% (O).

λ_{max} (CH_3CN, 0°C): 224 nm (ε=33,000).

^{1}H nmr ($CDCl_3$): δ 2.44 (dd, 6 H, J=12.8 Hz, C*H*$_2$), 2.69 (dd, 6 H, 12.8 Hz, C*H*$_2$), 2.87 (t, 6 H, J=12 Hz, C*H*$_2$) and 3.05 (t, 6 H, J=12 Hz, C*H*$_2$), 4.67 (s, 6 H, oxaziridine-*H*) and 6.95 (s, Ar*H*).

^{13}C nmr ($CDCl_3$): δ 54.1 (NCH_2), 60.7 (O-NCH_2), 80.2 (oxaziridine-C), 127.3 (aromatic-C) and 136.7 (aromatic-CH).

Sulfonimine (21).

In a flame-dried, 100 ml three-necked round-bottomed flask equipped with magnetic stirrer, condenser, and pressure equalized adding funnel with a nitrogen inlet, was placed isophthalaldehyde (335 mg, 2.5 mmol), *p*-toluenesulfonamide (850 mg, 5 mmol), Amberlyst 15 ion-exchange resin (200 mg), and anhydrous toluene (40 ml); the reaction mixture was heated at reflux under an inert atmosphere for 3 h. Heating was discontinued, and while the mixture was still warm, CH_2Cl_2 (40 ml) was added to dissolve the solid imine formed. The warm solution was filtered and the filtrate was evaporated on a rotary evaporator. The crude product was crystallized from absolute ethanol to give 520 mg of white needles of sulfonimine **21** (47% yield).

Mp: 274-275°C

^{1}H nmr($CDCl_3$): δ 2.43 (s, 6 H, C*H*$_3$), 7.34 (d, 4 H J=8.1 Hz, Ar*H*), 7.61 (dd, 1 H, J=7.8 Hz, Ar*H*), 7.87 (d. 4 H, J=8.1 Hz, Ar*H*), 8.12 (d, 2 H, J=7.8 Hz, Ar*H*), 8.40 (s, 1 H, Ar*H*) and 9.01 (s, 2 H, *H*C=N).

^{13}C nmr ($CDCl_3$): δ 21.6 (CH_3), 128.3 (**Ar**), 129.9 (**Ar**), 130.9 (**Ar**), 133.2 (**Ar**), 133.6 (**Ar**), 134.7 (**Ar**), 136.3 (**Ar**), 145.0 (**Ar**) and 168.2 (-C=N-).

Bromination of sulfonimine 21.

A mixture of sulfonimine **21** (100 mg, 0.23 mmol) and N-bromosuccinimide (82 mg, 0.46 mmol) in 50 ml benzene was refluxed for 30 minutes, and then benzoyl peroxide (30 mg) was added. The reaction mixture was refluxed for another 3 h and the benzene was removed. The solid residue was washed with a small amount of absolute ethanol and dried in vacuum. ^1H nmr showed a peak at 4.46 ppm (CH_2Br) in addition to the peak at 2.43 ppm (CH_3). Conversion to the benzylbromide was about 50% based on ^1H nmr.

Sulfonimine (25).

In a flame-dried 100 ml three-necked round-bottomed flask equipped with magnetic stirrer, condenser, pressure equalized adding funnel and nitrogen inlet was placed isophthalaldehyde (335 mg, 2.5 mmol), methanesulfonamide (475 mg, 5 mmol), Amberlyst 15 ion exchange resin (200 mg), anhydrous toluene (40 ml) and the reaction mixture was heated at reflux under an inert atmosphere for 15 h. Heating was stopped, and while still warm, 40 ml of CH_2Cl_2 was added to solubilize the solid imine that formed. The warm solution was filtered and the solvents from the filtrate were removed on a rotary evaporator. The crude product was crystallized from absolute ethanol to give 388 mg of sulfonimine **25** (54% yield).

Mp: 248-249°C

^1H nmr($CDCl_3$): δ 3.17 (s, 6H, C*H*3), 7.72 (t, 1H, J=7.8 Hz. Ar*H*), 8.21 (d, 2 H, J=7.8 Hz, Ar*H*), 8.56 (s, 1H, Ar*H*) and 9.10 (s, 2H, *H*C=N).

Bromination of N-sulfonimine 25.

A mixture of sulfonimine **25** (100 mg, 0.35 mmol) and N-bromosuccinimide (125 mg, 0.7 mmol) in 50 ml benzene was refluxed for 30 min., and then benzoyl peroxide (30 mg) was added. The reaction mixture was refluxed for another 6 h and benzene was removed. The solid was washed with a small amount of absolute ethanol and dried in vacuum. No reaction occurred based on ^1H nmr.

Sulfonyloxaziridine (29).

In a three-necked round-bottom 1000 ml flask equipped with a magnetic stirrer and a pressure equalized dropping funnel, saturated aqueous $NaHCO_3$ (50 ml), sulfonimine **21** (1.0 g, 2.3 mmol), and benzyltriethylammonium chloride (BTEAC, 1.15 g, 5 mmol) in CH_2Cl_2 (400 ml) were introduced. To this mixture cooled to 0-2°C in an ice-water bath, a solution of *m*-chloroperbenzoic acid (50-60%) (1.1 g, 6.3 mmol) in CH_2Cl_2 (10 ml) was added dropwise over a period of 30 min. The reaction mixture was stirred for an additional 30 min, the CH_2Cl_2 layer was separated, washed with water (50 ml), 10% aqueous sodium sulfite (50 ml), 10% aqueous sodium carbonate (50 ml) and finally with saturated aqueous NaCl (100 ml). After drying over anhydrous Na_2SO_4 and filtering, the CH_2Cl_2 was removed using a rotary evaporator at rt. The solid residue on drying gave 0.99 g of sulfonyloxaziridine **29** in 92% yield.

Mp: 62-63°C

^{1}H nmr($CDCl_3$): δ 2.46 (s, 6H, C*H3*), 5.40 (s, 2H, oxaziridine-*H*), 7.3-7.5 (m, 8 H, Ar*H*), 7.8-8.0 (m, 4 H, Ar*H*).

^{13}C nmr ($CDCl_3$): δ 21.74 (**C**H_3), 75.53 (oxaziridine-**C**), 127-134 (**Ar**) and 146.52 (**Ar**).

Tris(4-nitrobenzoyloxylethyl)amine (30).

Triethanolamine (1.49 g, 10 mmol) was mixed with 4-nitrobenzoyl chloride (5.57 g, 30 mmol) in anhydrous pyridine (5 ml) with stirring and the mixture was heated on a water bath for 30 min. After it was allowed to reach rt, saturated aqueous $NaHCO_3$ (30 ml) was added, the solid ester was broken up with a stirring rod, and was filtered. The residue was washed with saturated $NaHCO_3$ (10 ml), followed by distilled water by filtering under suction to as dry as possible; finally drying in a vacuum pistol gave 5.05 g of **30** as a pale yellow powder (85% yield).

Mp: 125°C.

λ_{max} (CH_3OH): 258 nm.

^{1}H nmr (DMSO-d_6): δ 3.01(t, 6 H, J=5.1 Hz, CH_2N), 4.39 (t, 6 H, J=5.1 Hz, CH_2O), 7.97 (d, 6 H, J=8.7 Hz, Ar*H*) and 8.11(d, 6 H, J=8.7 Hz, Ar*H*).

Tris(4-hydroxylaminobenzoyloxyethyl)amine. (31)

To a 100 ml three-necked round-bottomed flask, equipped with a condenser, thermometer, adding funnel and magnetic stirring bar, tris(4-nitrobenzoyloxyethyl)amine (596 mg, 1 mmol), 50% aqueous THF (30 ml) and ammonium chloride (190 mg) were added.

The mixture was heated to 60-65°C on a water bath, then activated zinc powder (392 mg, 6 mmol) was added over a period of 30 min. The mixture was stirred for a additional 6 h at the same temperature. The warm reaction mixture was filtered under vacuum to remove the zinc oxide, and the residue was washed with THF (10 ml). After removal of THF using a rotary evaporator, the aqueous portion was filtered to obtain a white precipitate which was crystallized from ethanol to obtain 440 mg of **31** (80% yield).

λ_{max} (THF): 284 nm.

^{1}H nmr (DMSO-d$_6$): δ 2.97 (t, 6 H, J=5.2 Hz, C*H*$_2$N), 4.25 (t, 6 H, J=5.2 Hz, C*H*$_2$O), 6.78 (d, 6 H, J=8.5 Hz, Ar*H*), 7.38 (d, 6 H, J=8.5 Hz, Ar*H*), 5.94 (s, N*H*), 8.62 (s, NO*H*) and 8.93 (s, NO*H*).

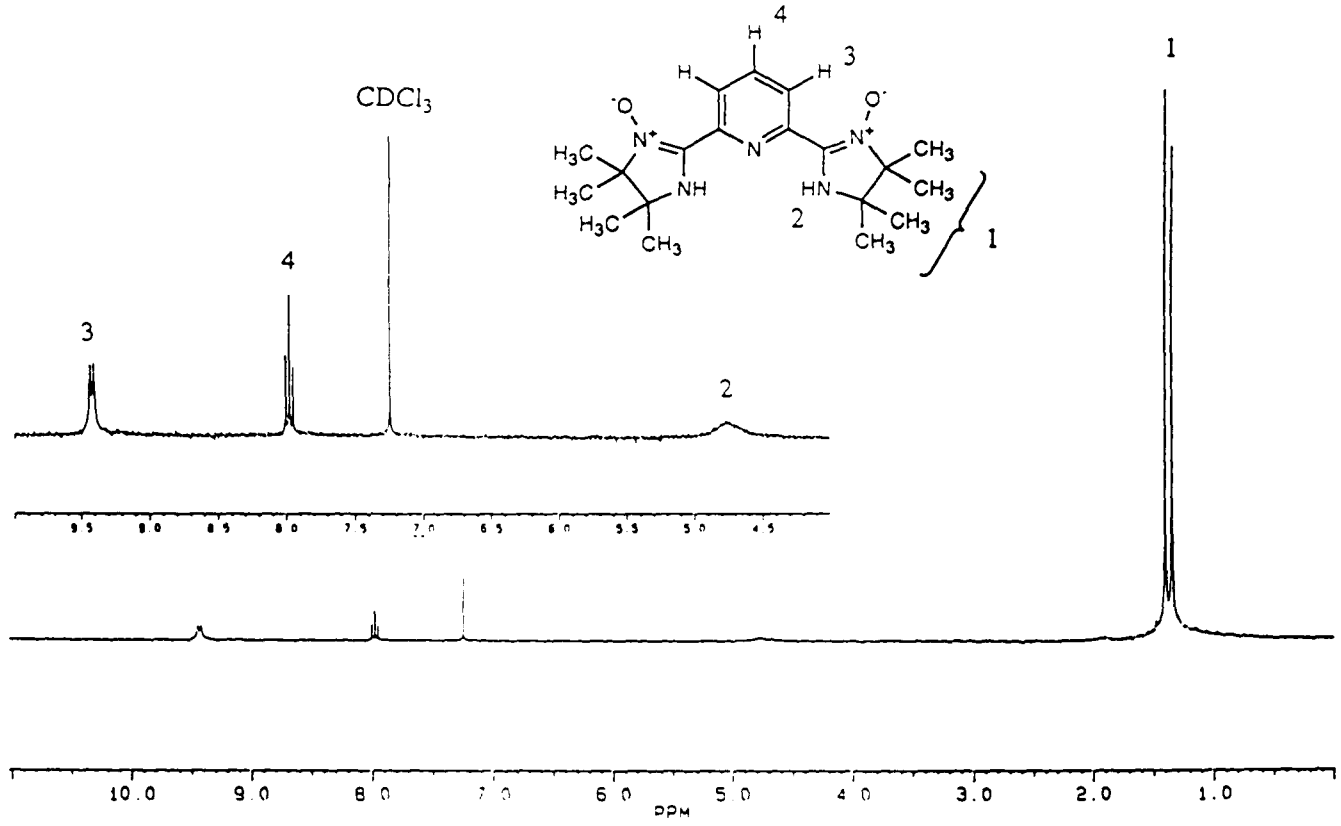
Macrobicyclic nitrone (32).

A mixture of tris(4-hydroxylaminobenzoyloxyethyl)amine (280 mg, 0.5 mmol) and terephthaldicarboxaldehyde (50 mg, 0.37 mmol) was stirred in ethanol (20 ml) for 15 min at rt, then methanesulfonic acid (20 µl) was added. A yellow precipitate was formed immediately; stirring was continued for another 15 min. Filtration, washing with ethanol (3 x 5 ml) and drying in vacuum gave 260 mg of the macrobicyclic nitrone **32** as a yellow powder (33% yield).

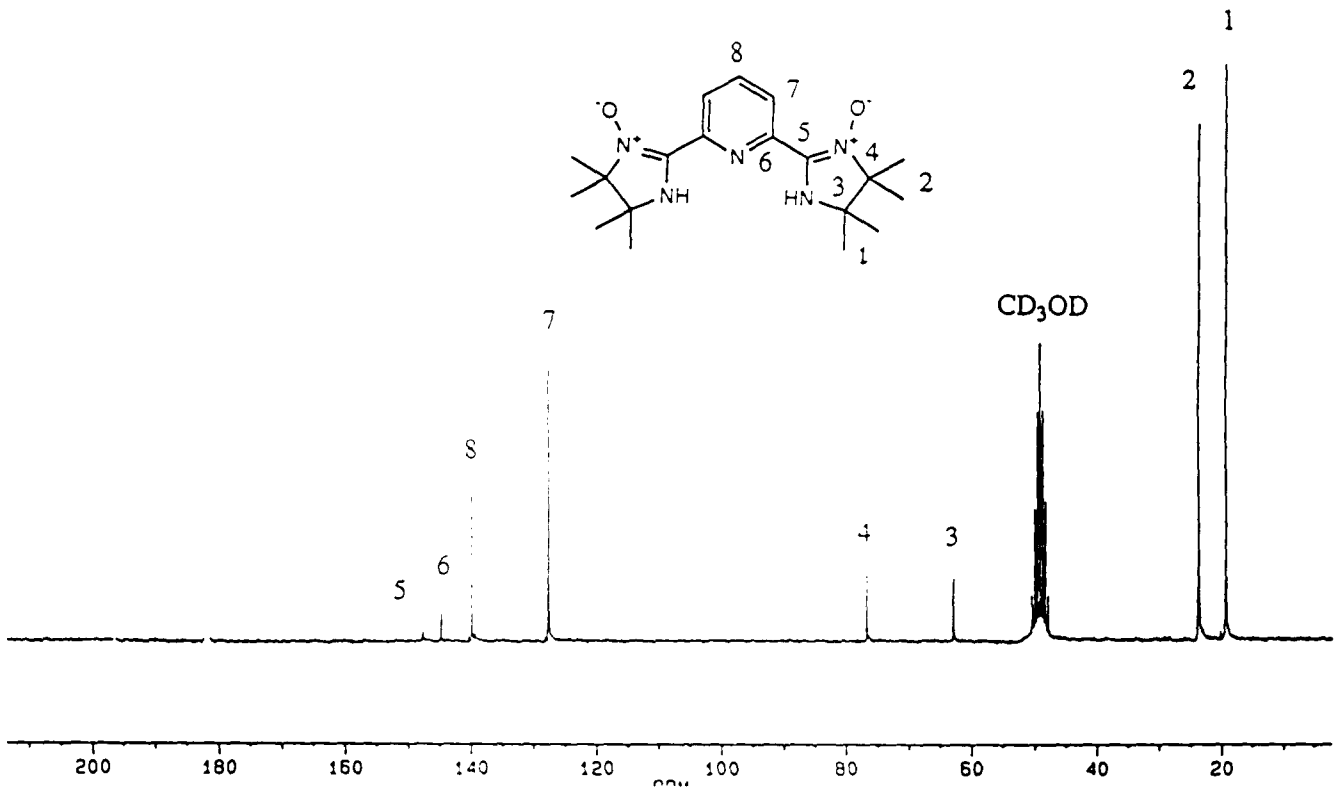
Mp >350°C (dec.)

λ_{max} (DMF): 360 (broad), 298 nm.

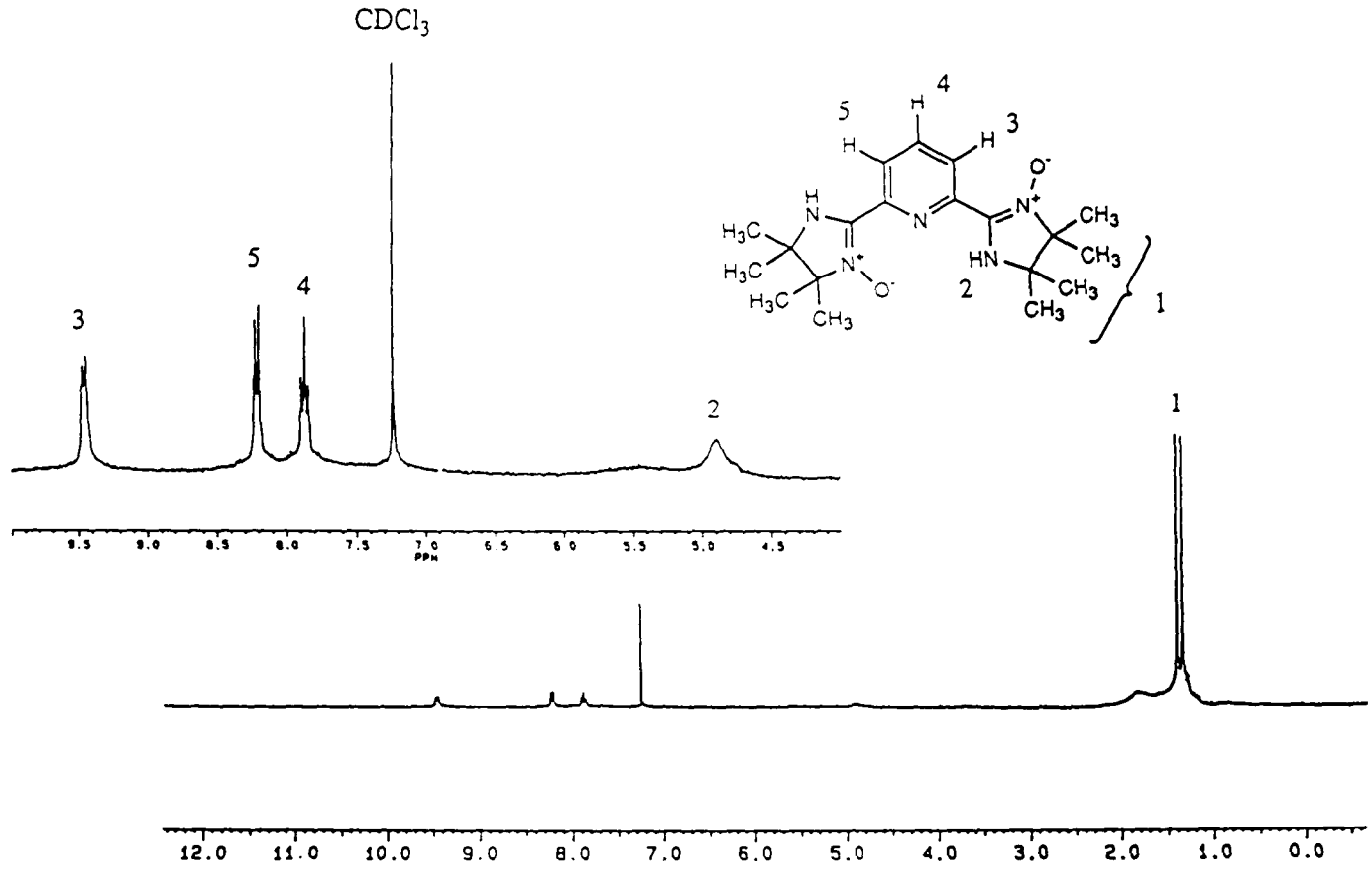
FAB-MS (glycerol): *m/z* 1405 $[M+2]^+$.



^{1}H nmr spectrum of **11** in $CDCl_3$.



^{13}C nmr spectrum of **11** in CD_3OD.



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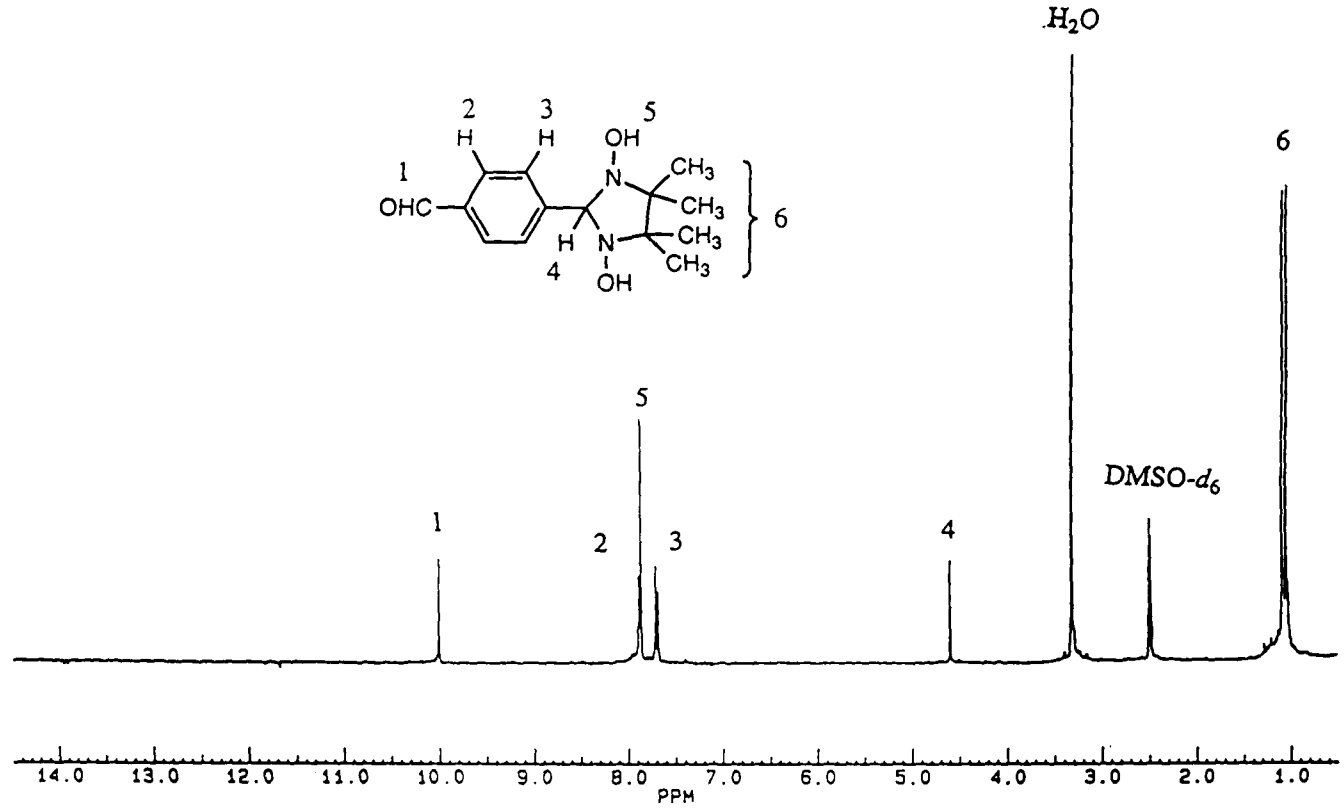
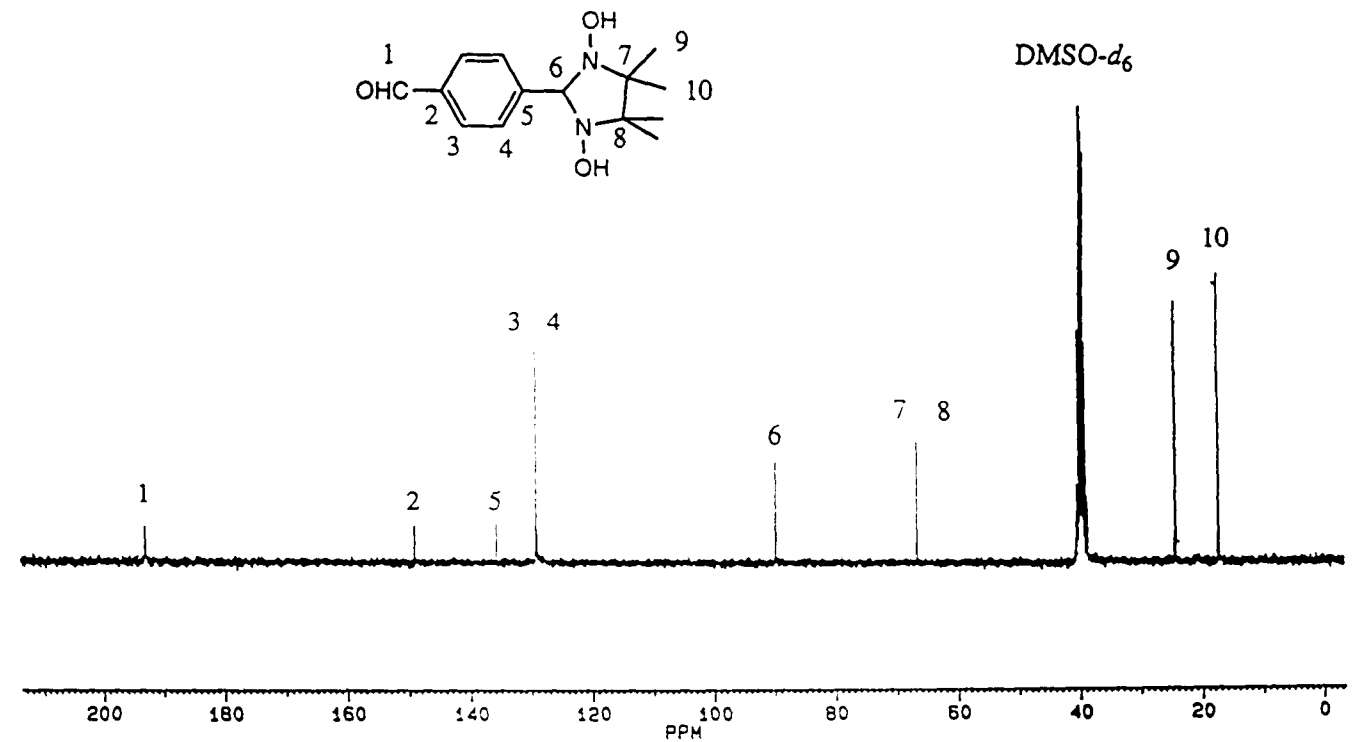
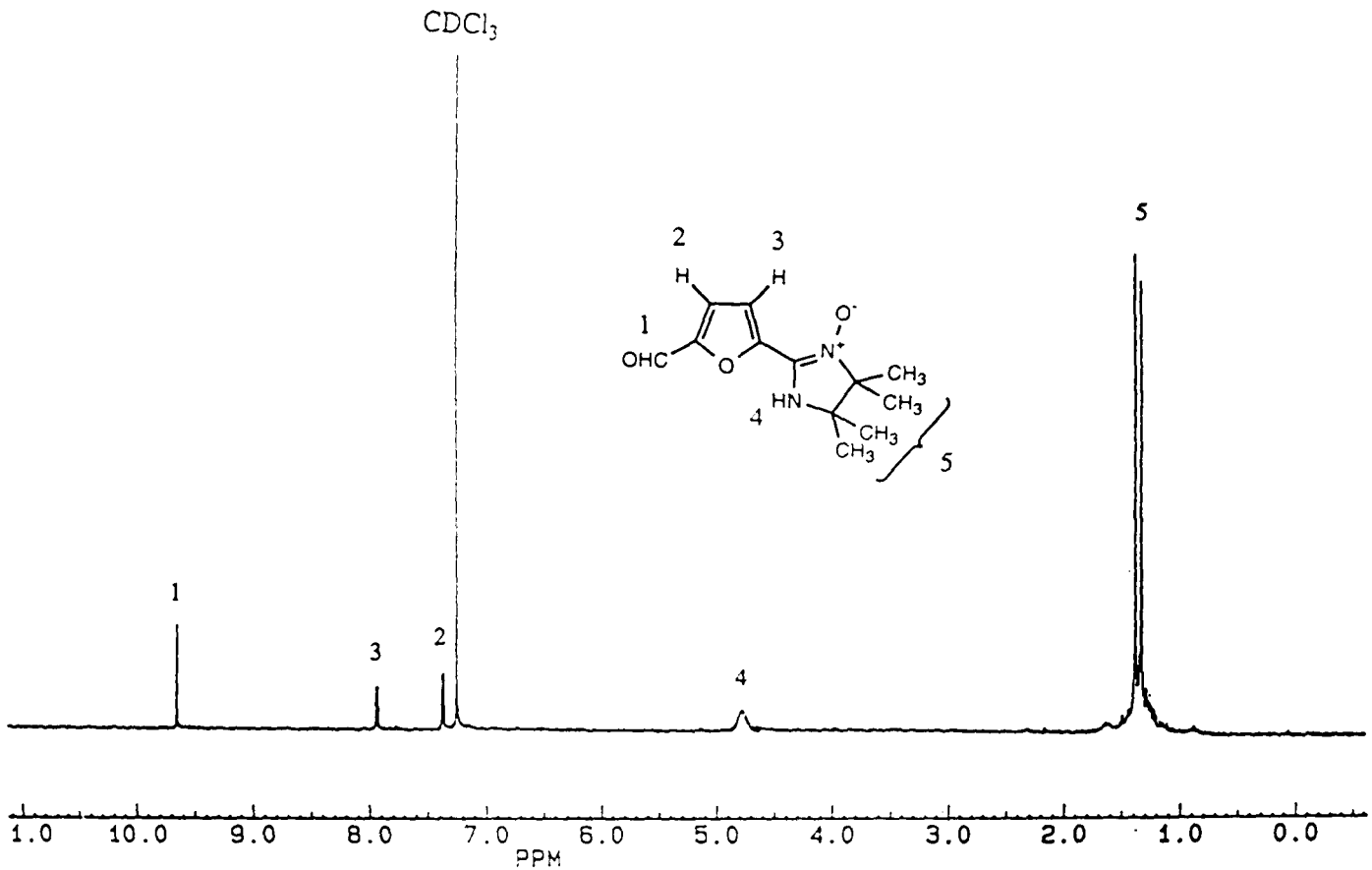


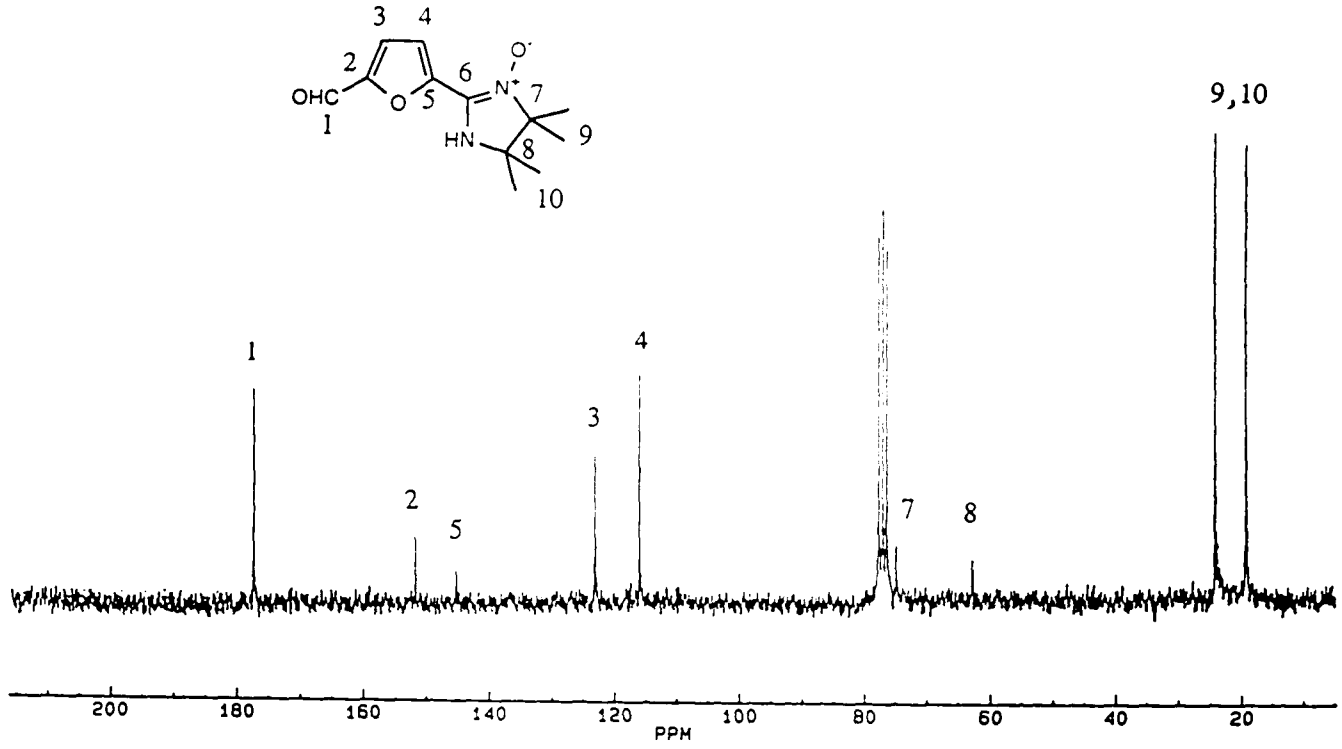
^{1}H nmr spectrum of **13** in DMSO-d_6.



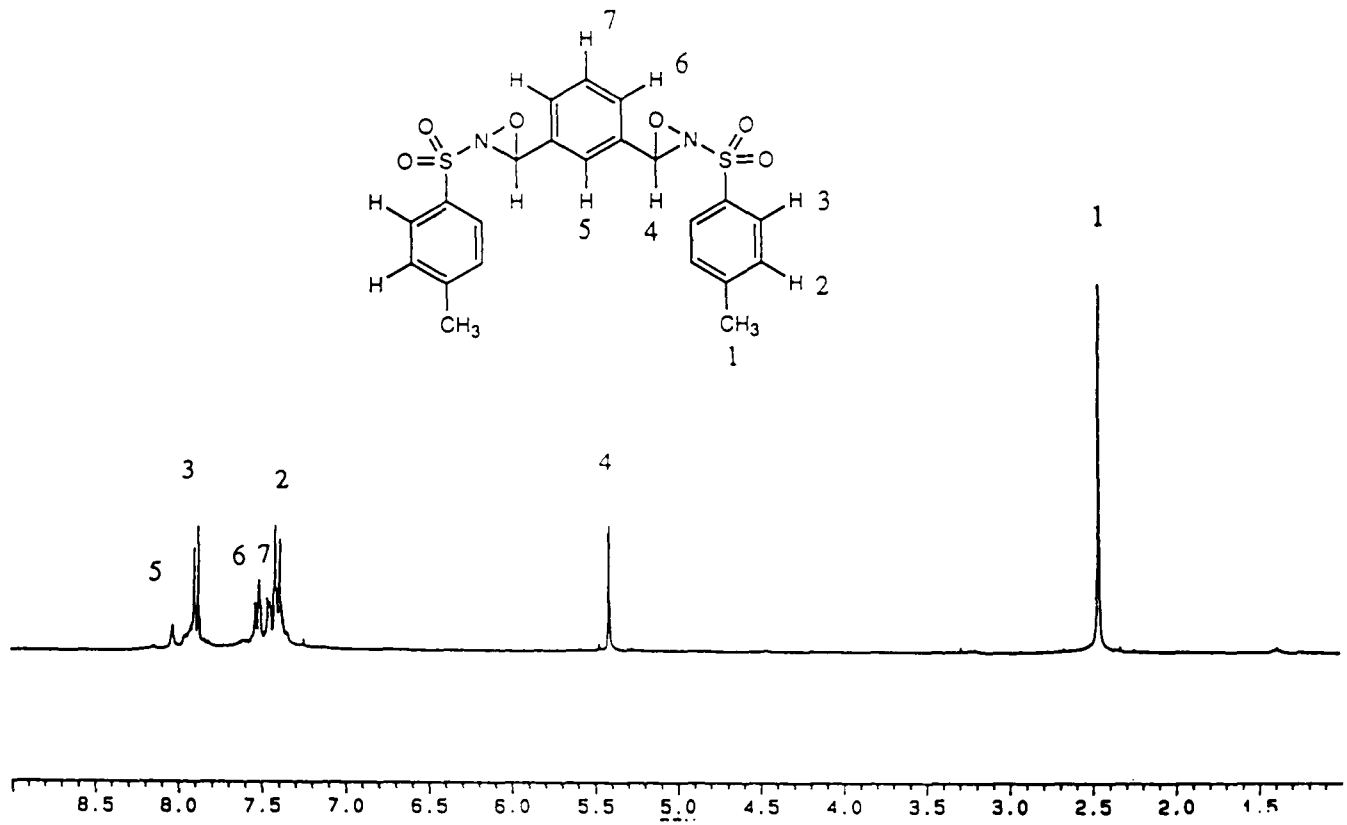
^{13}C nmr spectrum of **13** in DMSO-d_6.



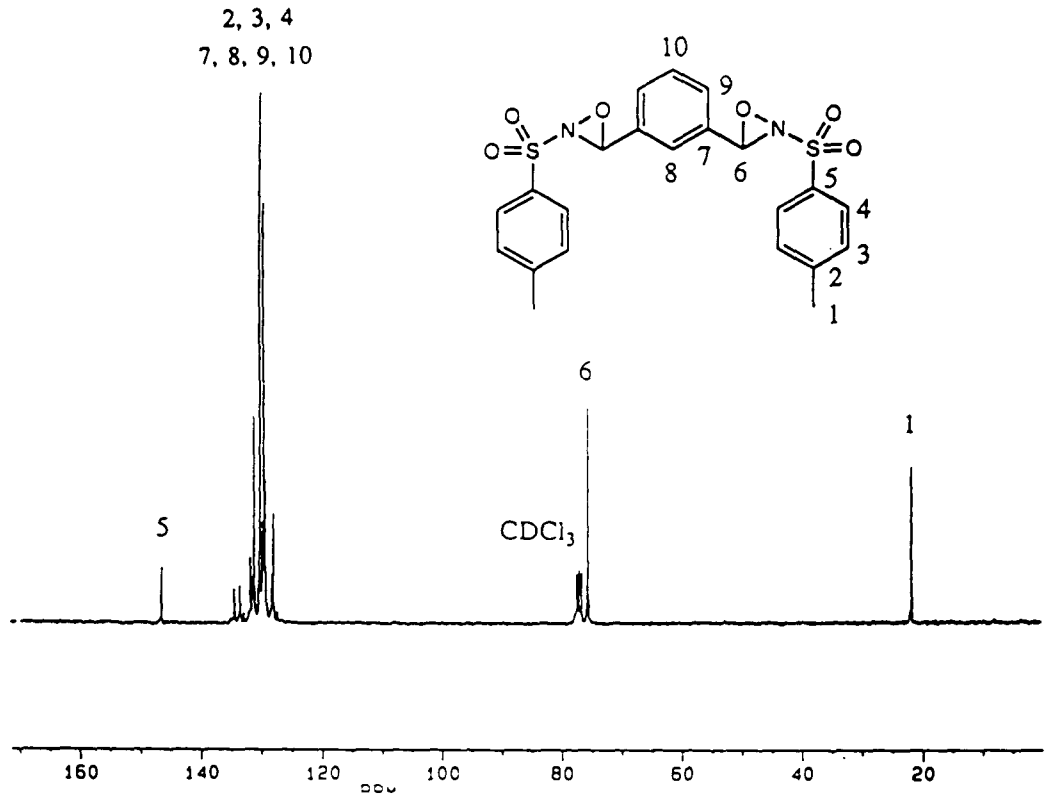
^{1}H nmr spectrum of **15** in $CDCl_3$.



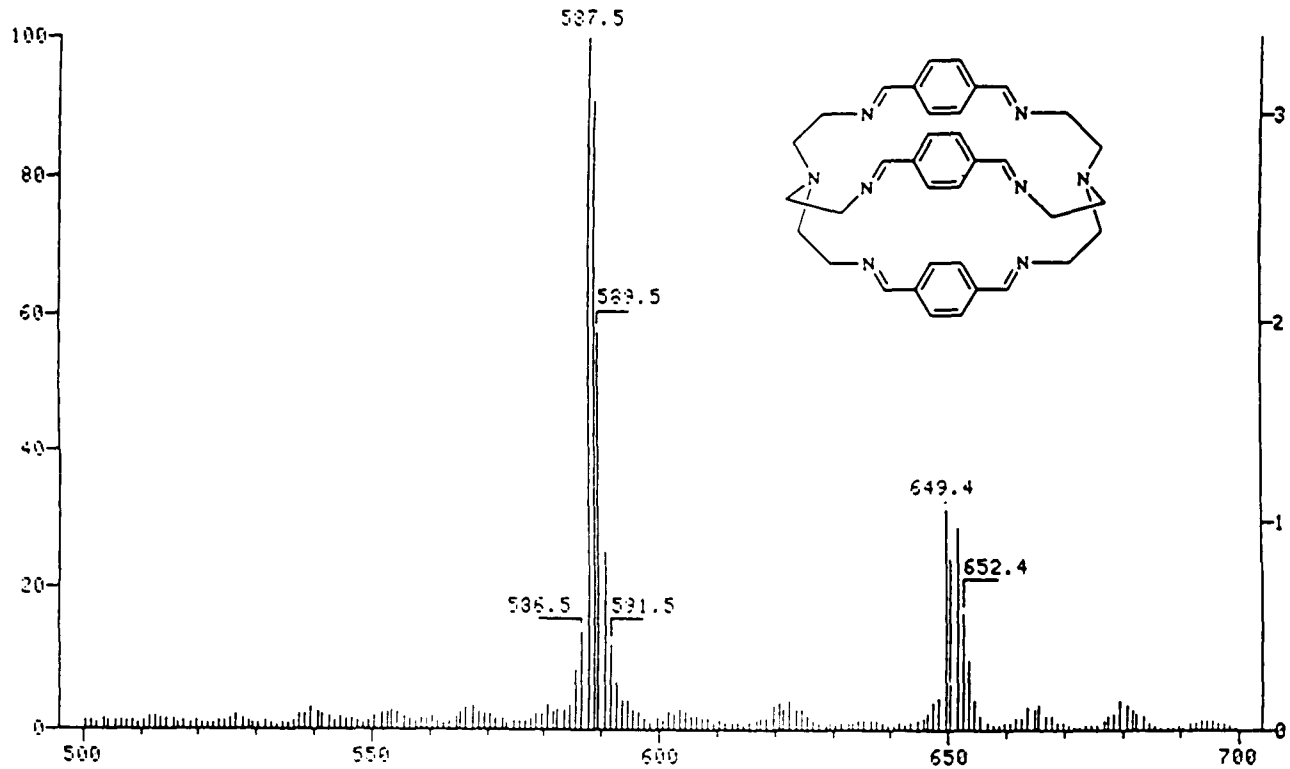
^{13}C nmr spectrum of **15** in $CDCl_3$.



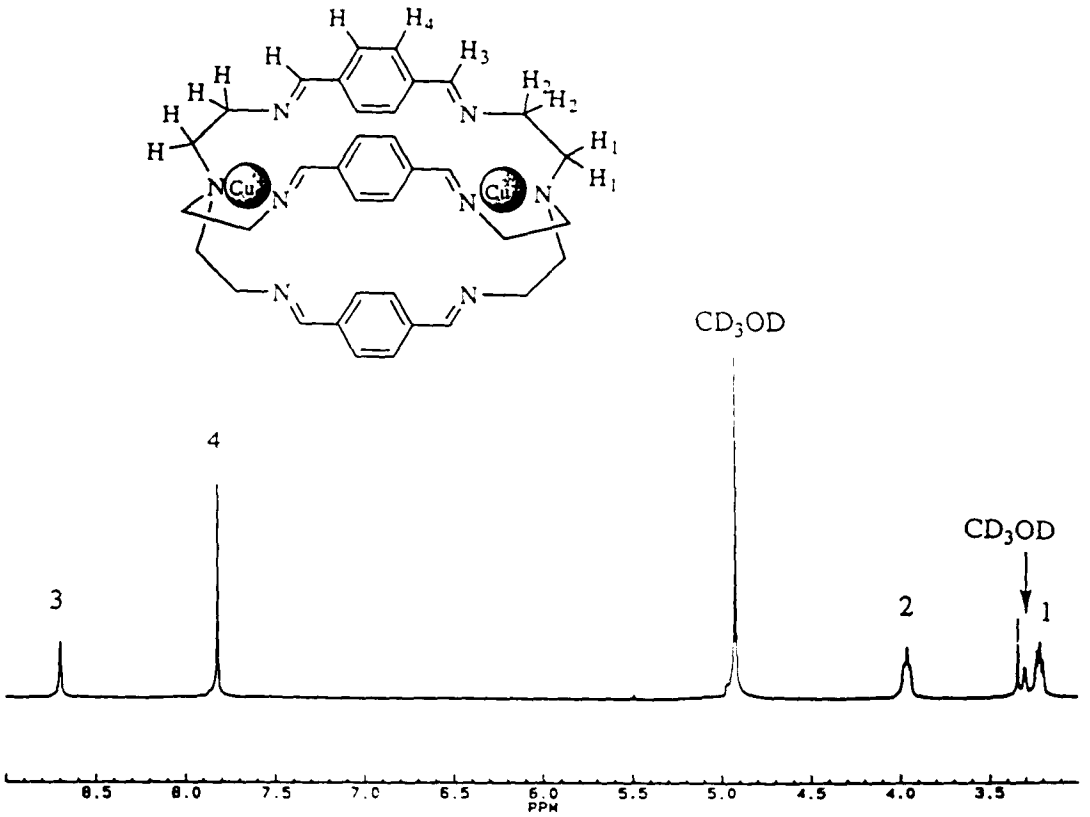
^{1}H nmr spectrum of **16** in $CDCl_3$.



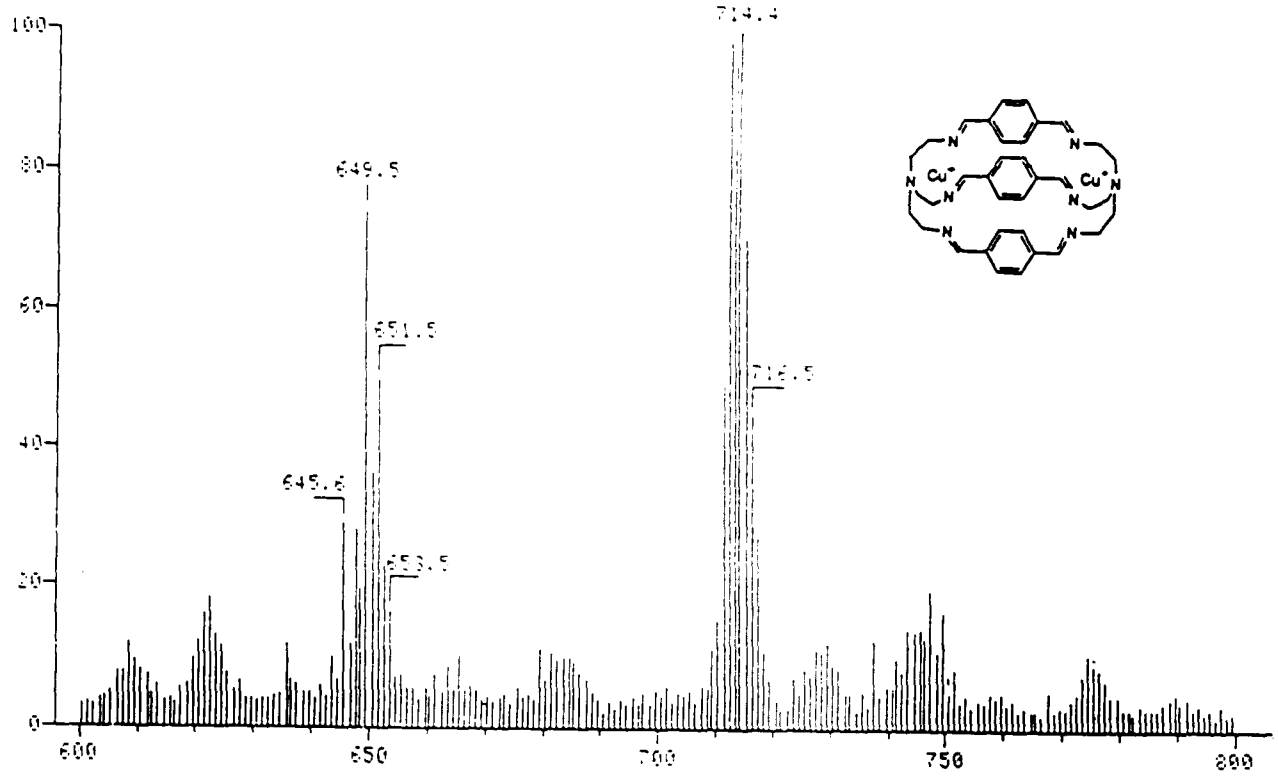
^{13}C nmr spectrum of **16** in $CDCl_3$.



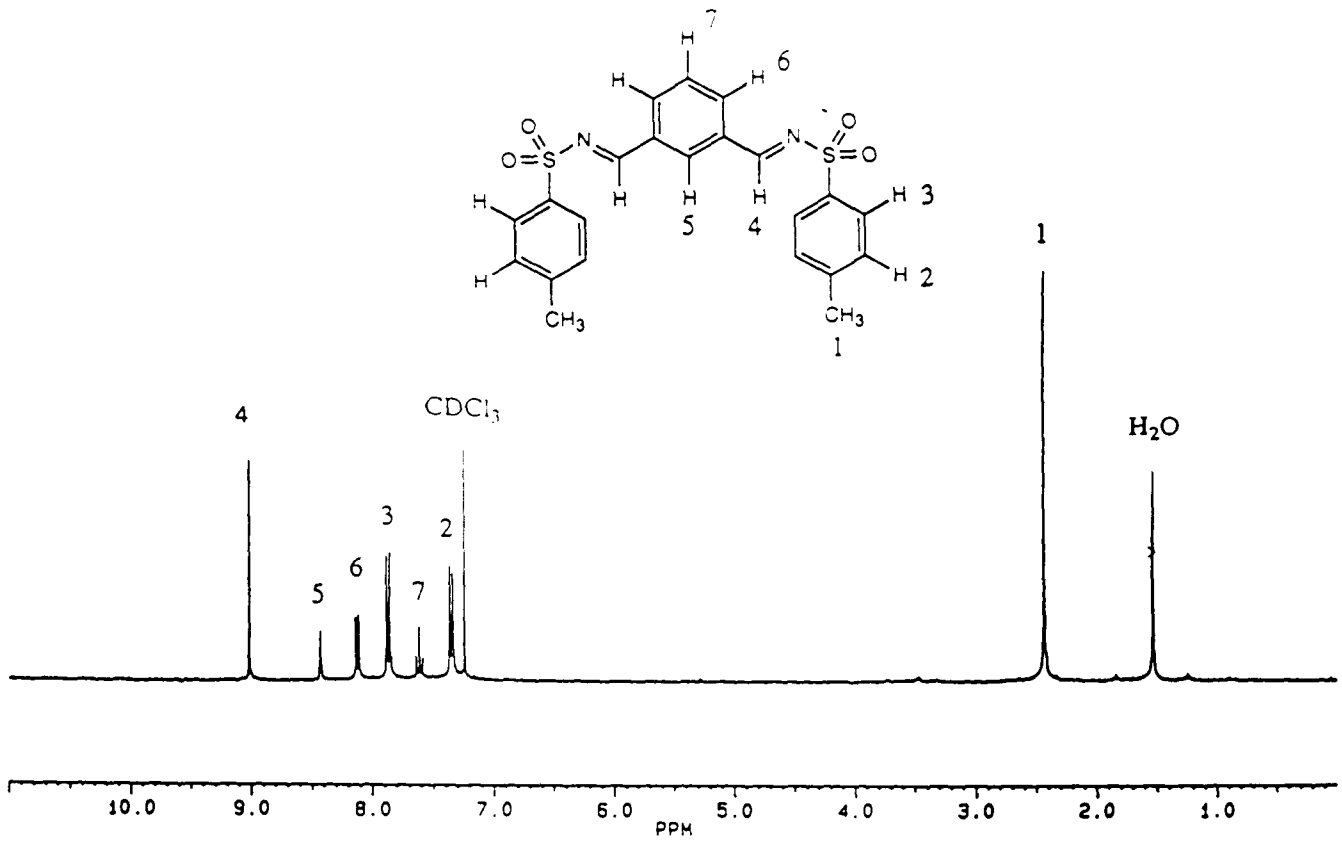
FAB-MS (glycerol) of **18**.



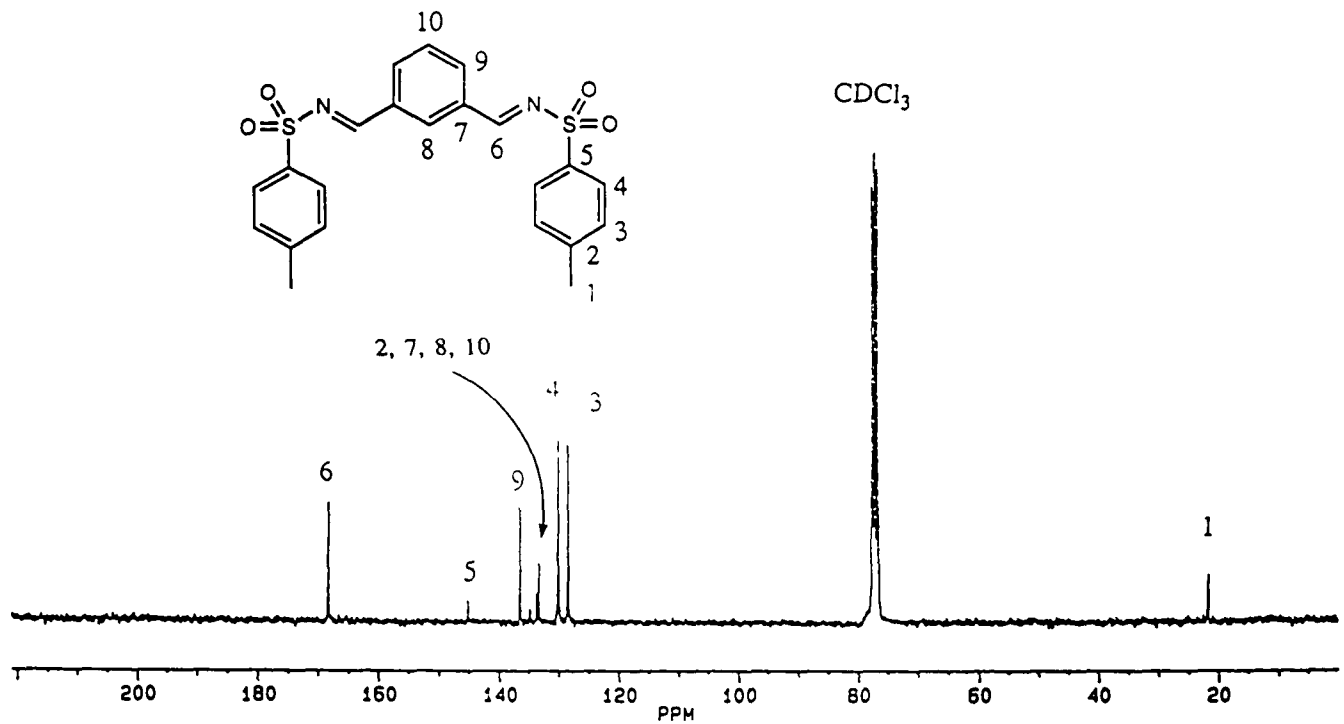
^{1}H nmr spectrum of **19** in CD_3OD.



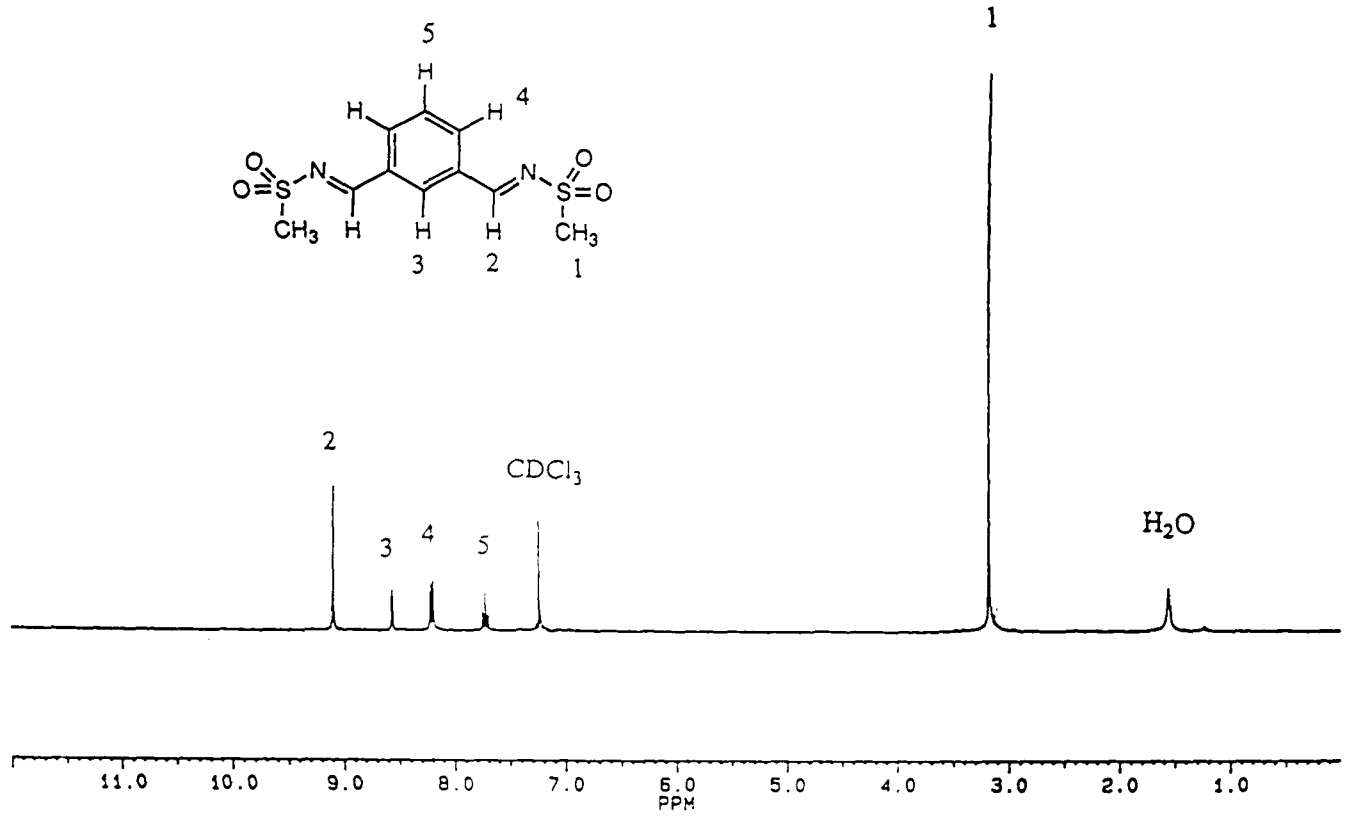
FAB-MS (glycerol) of **19**.



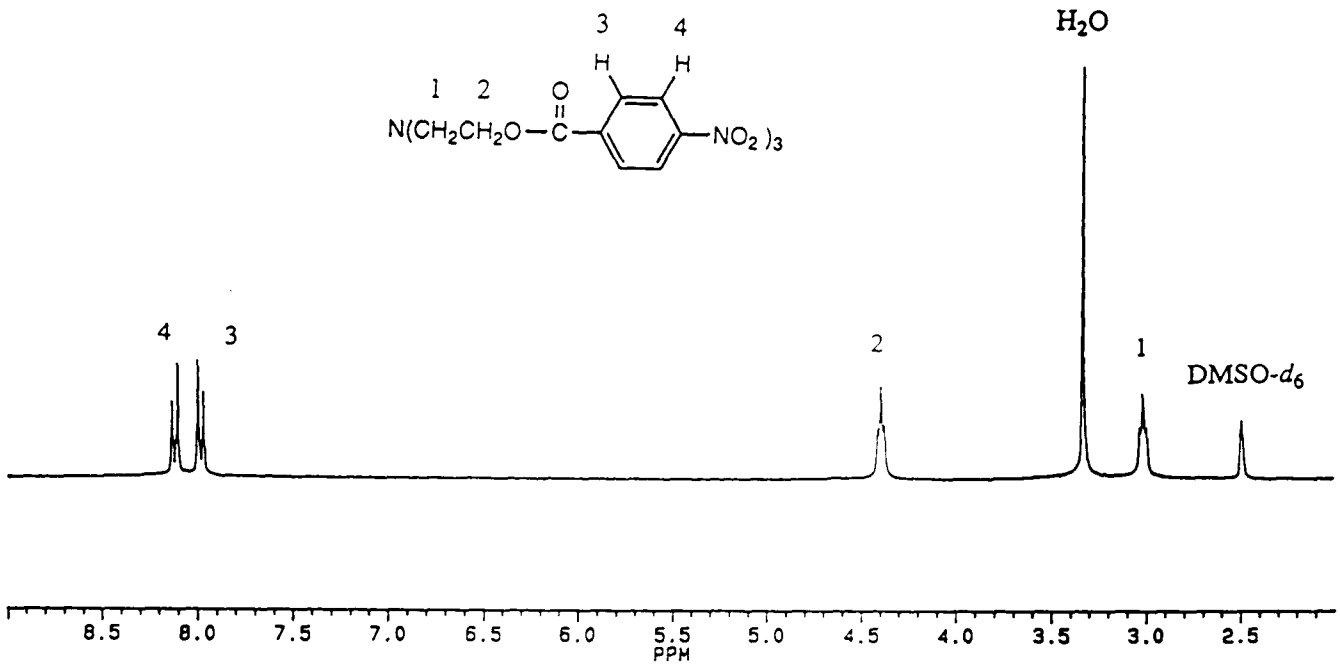
^{1}H nmr spectrum of **21** in $CDCl_3$.



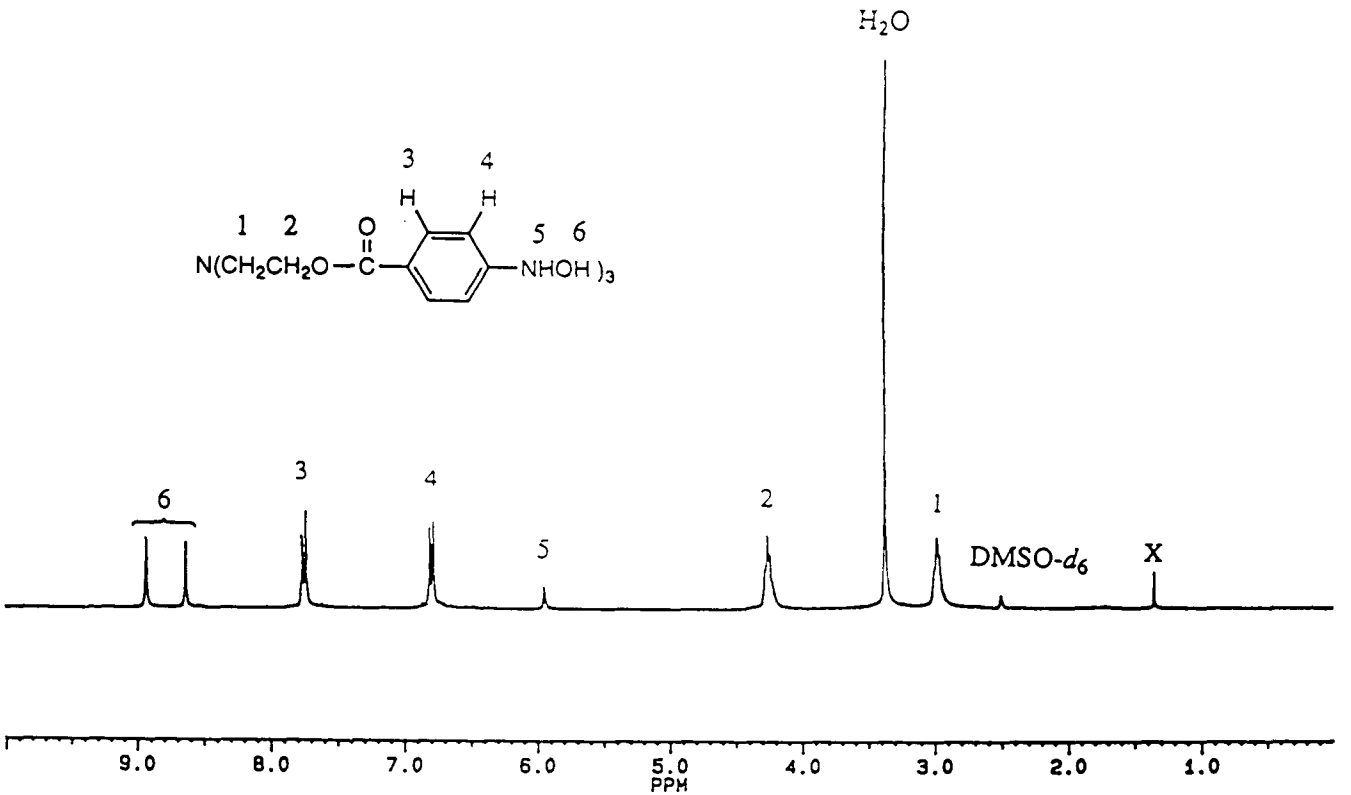
^{13}C nmr spectrum of **21** in $CDCl_3$.



^{1}H nmr spectrum of **25** in $CDCl_3$.



^{1}H nmr spectrum of **30** in DMSO-d_6.



^{1}H nmr spectrum of **31** in DMSO-d_6.

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Part II. Synthesis of Isotopically Labelled Guanine Nucleoside and Nucleotide Analogs.

1. INTRODUCTION

1.1 G-PROTEINS

Within the past decade, there have been considerable efforts to understand the workings of the family of guanine nucleotide binding regulatory proteins, the G proteins.[1-5] These proteins share many functional and structural features. G-proteins act as molecular switches that turn on and off several biological processes.[6,7] These switching functions are strictly controlled by the nature of bound nucleotides. Normally, they contain GDP in a specialized binding site, forming a G•GDP binary complex. A particular stimulus causes the release of GDP at the binding site and its replacement by GTP, forming G•GTP. The guanine nucleotides act as allosteric effectors to control protein conformation and activity, with no activity associated with G•GDP. In the active, G•GTP conformation, the protein promotes some chemical reaction often acting enzymatically. The phosphate of the bound GTP is eventually hydrolyzed, and the G protein reverts to its normal "dormant" state. There are several members of this family and its subfamilies, that are involved in a rather diverse sets of biological functions. A large number of them are intermediaries in a variety of transmembrane signaling processes. For example in sensory processes, the absorption of a photon by the visual pigment rhodopsin yields excited rhodopsin, which thermally decays to a meta-II intermediate which in turn effects the exchange of GDP by GTP in transducin, the G protein of the visual system.[8] Activated transducin stimulates a cGMP phosphodiesterase. The hydrolysis of

cGMP then results in the closure of sodium channels in the outer segments of rod cells, leading to cell hyperpolarization and subsequent visual excitation. An example of a hormone regulated system is the adenylyl cyclase which affects a wide range of cAMP-dependent biochemical reactions and is modulated by both stimulatory and inhibitory G proteins. In addition to the functional similarities, the G protein family also share common structural patterns; their sequence homology and tertiary structure similarities are one of the closest found in biology.[9] Intensive research has been carried out to clarify how the GTP and GDP forms can cause such dramatically different biochemical processes. Thus, it is of considerable interest to investigate the interactions between the bound nucleotides and these proteins.

The two G-proteins that were chosen for our studies are the bacterial elongation factor protein EF-Tu and the *ras* p21 protein. We chose these proteins since they are relatively easy to obtain in high purity and large quantities, and because much is already known concerning their biochemical and biophysical properties. High resolution X-ray structures have been obtained for EF-Tu,[10-13] and *ras* p21,[14,15] that provide useful indicators for the spectroscopic studies of their molecular interactions.

The elongation factor is abundant in *E. coli* with some 5-10% of its soluble proteins being EF-Tu depending on growth conditions. The growth factor plays an essential role in bacterial protein biosynthesis as illustrated by the following simplified Figure1.[16-18]

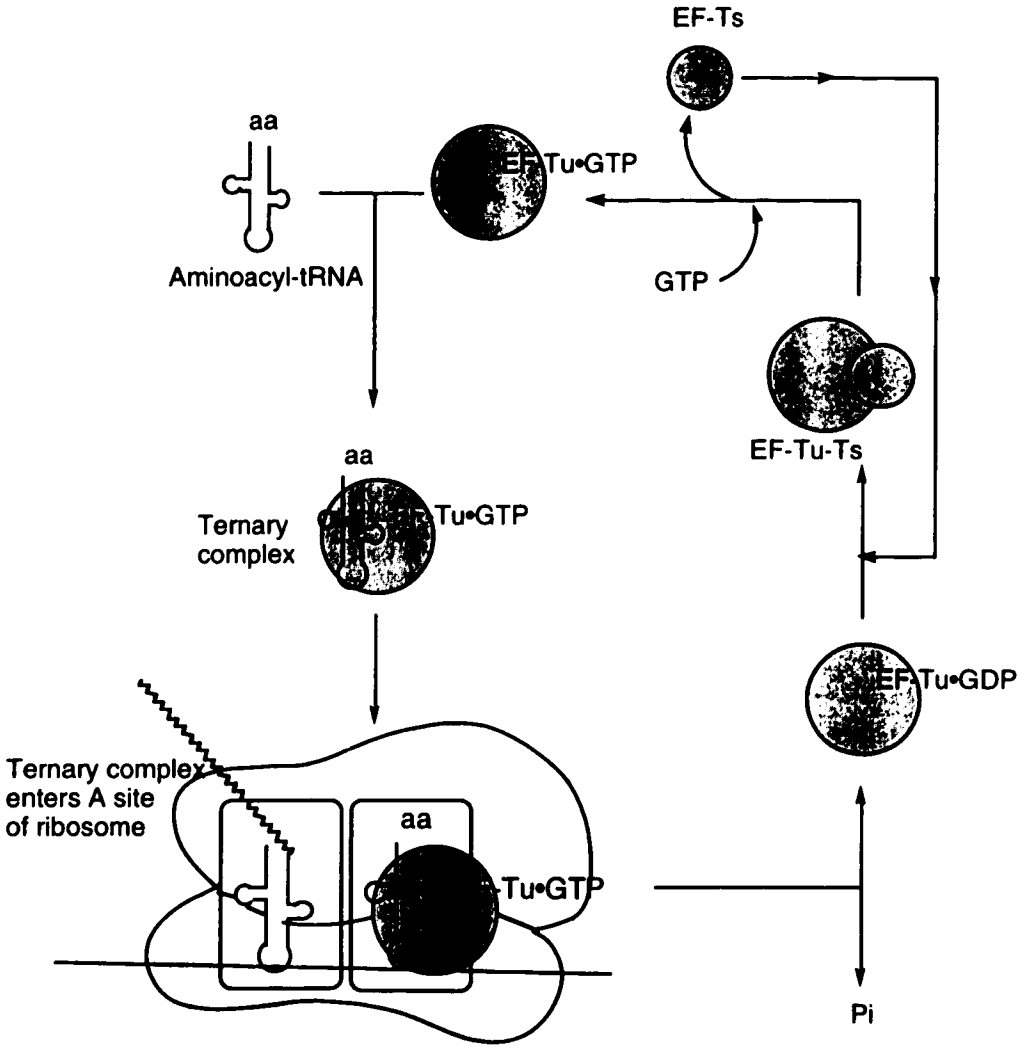


Figure 1. EF-Tu•GTP places aminoacyl-tRNA on the ribosome and then is released as EF-Tu•GDP, which requires EF-Ts to mediate the replacement of GDP by GTP.

EF-Tu is a critical molecule in the protein biosynthesis, since it carries aminoacyl-tRNA in the form of a ternary complex with GTP to the ribosomal A-site, in order that the tRNA can decode the mRNA. EF-Tu is apparently released from the ribosome in the form of a binary complex with GDP, since this complex has a low affinity for the ribosome and does not recognize aminoacyl-tRNA and is,

therefore, the "dormant" state of the molecule. Activation is effected by the exchange of the bound GDP for GTP, a step catalysed by another protein, EF-Ts.[19]

A schematic representation of the nucleotide binding site of EF-Tu is given in Figure 2. This diagram is based on the X-ray crystallographic studies[19] and upon binding studies with mutated EF-Tu. While the general outlines of the schematic are essentially correct, the interactions shown have been hypothesized rather than

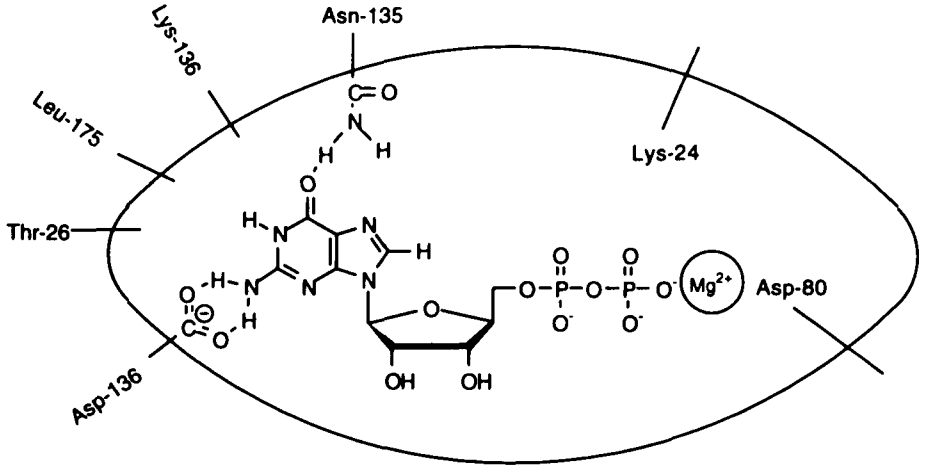


Figure 2. A sketch of the GDP binding site of EF-Tu.

proven. Most of the conserved amino acid residues seem to play an important structural role in the binding of GDP. The N-terminal domain (residues 1-200) binds GDP, the middle and C-terminal domains play essential roles in regulating the activity of the N-terminal domain of the intact molecule, as well as in the interactions of EF-Tu with aminoacyl-tRNA, elongation factor Ts, and the antibiotic kirromycin.[20] The GDP molecule binds to the surface of N-

terminal domain of EF-Tu. The guanine ring is partly buried in a cavity defined by the residues Leu-175, Thr-26 and the side chain of Lys-136. With an acceptor to donor distance of 2.7 Å, the main chain nitrogen of Asn-135 can form hydrogen bond to O6 of guanine. Asp-138 can form hydrogen bonds to the proton on the endocyclic N1 and a proton on the exocyclic 2-amino group of guanine, both of which are located 2.9 Å away from the carboxyl oxygens of Asp-138.

Ras-p21's are found in about 40% of human colon cancers and in many other tumors. The proteins encoded by these genes are called p21 as they have a molecular weight of about 21,000 D. Three types are known, c-H-*ras*, c-K-*ras*, and N-*ras*.[21] Often, the difference between a normal *ras* gene and one that is associated with a cancer is a single base pair mutation. This results in one amino acid change in the activated (cancer transforming) p21 protein; this change is very often associated with the GDP-GTP binding site. In the active GTP-bound form, *ras*-p21 interacts with the GTPase activating protein, GAP,[22] which is either an effector of *ras* action or its negative regulator. The interaction between p21 and GAP ensures that p21 is maintained in the GDP bound conformation. Single mutations at certain positions (i.e., positions 12, 13, 35 or 61) of p21 reduce its intrinsic and GAP-induced GTPase activities, thereby allowing these mutants to stay in the active GTP-bound state over a prolonged time[23] which lengthens the transmission of the growth signal, resulting in unregulated cell growth. Two other cytosylic proteins, *ras*-guanine nucleotide-release protein,[24] and an inhibitor of GTPase activity,[25] were also found recently to interact with *ras*-p21.

There is a great deal of homology between EF-Tu and p21 with respect to the nucleotide binding site[23,26,27] (Figure 3). The roles of Ala-146 and Asp-119 in p21 are similar to those of Asn-135 and Asp-138 in EF-Tu. The carboxylate group of Asp-119, located in the

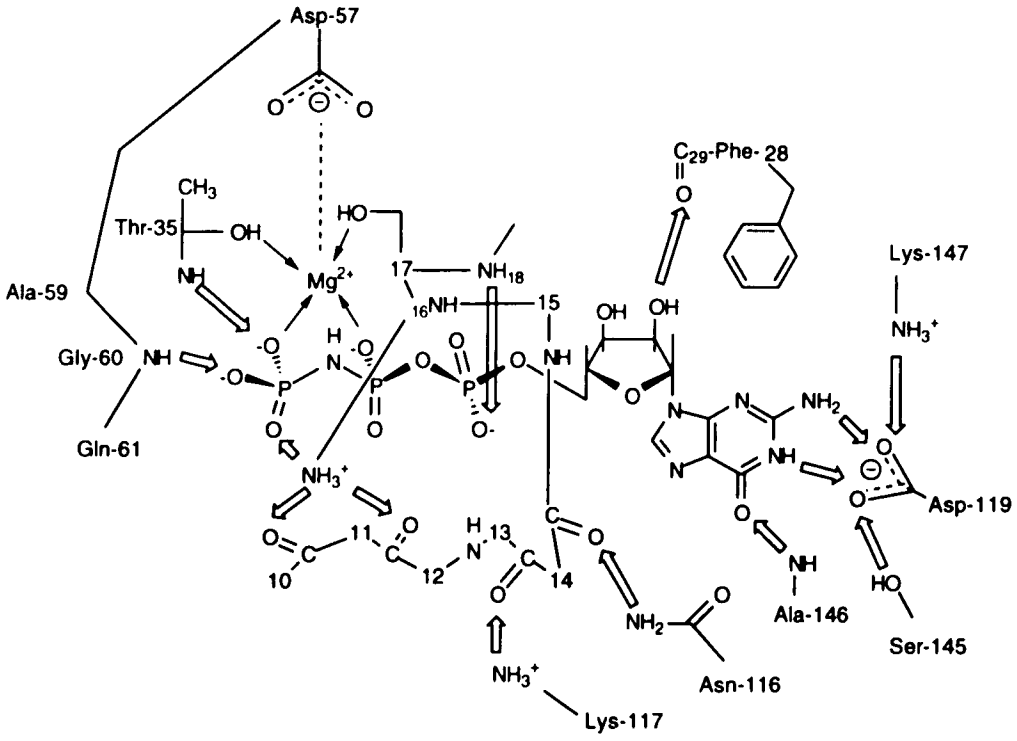


Figure 3. The three-dimensional structure of the GTP binding domain in p21, established by X-ray crystallography of a nonhydrolyzable analog, guanosine-5'-(β,γ-imidotriphosphate) (GppNHp). Open arrows indicate hydrogen bonds, and the bonds between the Mg^{2+} ion and its ligands are indicated by solid arrows.[23]

guanine-specificity region, contacts the exocyclic 2-amino group and the endocyclic N1 of guanine. Asp-119 is also close enough to interact with the side chain hydroxyl of Ser-145 and with the amino group of Lys-147. As in the case of Asp-138 in EF-Tu, mutation of

Asp-119 drastically reduces the affinity for the guanine nucleotides.[23] The sequence motifs Asn-Lys-X-Asp and Ser-Ala-Lys/Ser-Ala-Leu are the structurally most conserved regions of the guanine-nucleotide binding pocket.[28] X-ray studies[29] also show that there are several hydrogen bonds between phosphate groups and the conserved consensus sequence Gly-X-X-X-X-Gly-Lys-Ser/Thr motif of the phosphate-binding loop. As generally is the case for nucleotide binding proteins, the phosphate binding site is stabilized by the positive dipole moment of the helix, of which the carbonyl oxygen atoms in the loop all point in a direction away from the β-phosphate.

The vibrational properties of a bound ligand are sensitive indicators of its microenvironment within the protein's binding site. The difficulty in employing a ligand as the reporter group to probe the dynamic structure of active sites by vibrational spectroscopy lies in the disproportionately large protein signals relative to those of the ligand. This problem can best be solved by a judicious combination of sophisticated instrumentation, capable of accurately measuring differences in signal intensities as small as 0.1%, and the use of synthetically modified ligands.

We plan to employ a three-pronged approach to use synthetically modified ligands to achieve these proposed goals:

1) To obtain the vibrational spectra of GDP/GTP when bound to EF-Tu and p21 and compare them with the data obtained from isotopic labelling studies, a prerequisite for their interpretation.

These spectra will be obtained using the classical Raman difference spectroscopy.

2) To examine how the spectra of the bound nucleotides vary when key specific residues of EF-Tu and p21 are modified by site specific mutagenesis, and

3) To determine the Raman spectra of key residues themselves, within EF-Tu and p21.

We plan to realize these objectives employing the following strategies:

1) Synthesis of isotopically labelled or otherwise modified GTP/GDP analogs.

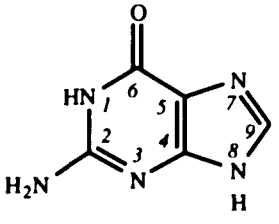
2) Preparation of EF-Tu and p21 in a nucleotide-free form that is sufficiently stable for our measurements.

3) Measurement of the classical Raman difference spectra of protein•ligand complex vs apoprotein.

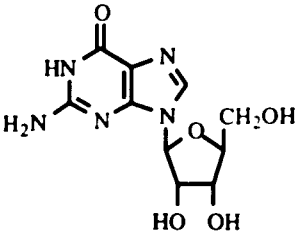
4) In cases when the nucleotide-free G protein is unstable, we plan to use the alternative strategy of "isotope editing." In this technique, the difference spectrum is obtained between two protein•nucleotide complexes, where only in one the nucleotide is labelled with a stable isotope. Vibrational modes affected by the presence of the label (e.g., ^{2}H, ^{18}O, ^{15}N, etc.), or other substitutions (e.g., O→S) in the nucleotides will appear in the difference spectra, whereas all other bands that do not involve the isotopic tag, will cancel out.

1.2. Synthesis of isotopically labelled guanine nucleosides and nucleotides.

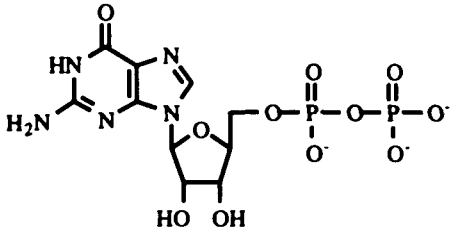
Despite the central role they play as ligands in signal transducing G proteins associated with cell growth and differentiation, and their high potential as therapeutic antiviral/antitumor agents, there is a dearth of adequate synthetic routes to obtain isotopically labelled (e.g., ^{2}H, ^{13}C, ^{15}N and ^{18}O) guanine nucleosides and nucleotides, and guanine-type synthetic analogs.[30-33] With very few exceptions, e.g., the inexpensive and commercially available 6-thioguanine and 6-thioguanosine, there is also a paucity of derivatives suitable for conducting spectroscopic studies. A brief summary of the presently available synthetic routes to obtain ligands of the type **1-4** are given below:



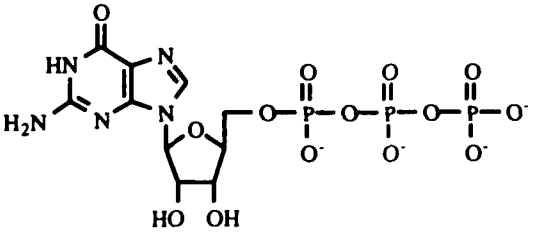
1 (Guanine)



2 (Guanosine)



3 (Guanosine 5'-diphosphate) (GDP)



4 (Guanosine 5'-triphosphate) (GTP)

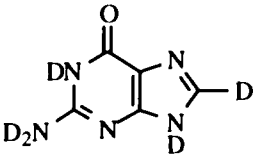
A. Deuterated analogues.

Isotopic hydrogen exchange studies in purines[34] were initiated over 40 years ago employing a biosynthetic approach in which tritium from [$C^3H_3CO_2Na$] was introduced into yeast DNA.[35] Although the position of the label in the hydrolysis products, the labelled purine nucleosides/bases, could not be established, it was assumed that the C-8 positions in adenine and guanine were labelled, since other labelled positions would have been too labile to survive under the conditions of hydrolysis. In subsequent studies,[36] exchange at the 8-position was effected employing a reduced platinum catalyst and HTO or D_2O at 100°C to afford tritiated and deuterated adenine and guanine. This exchange could also be effected in the absence of any platinum catalyst by heating the mixture for a longer period of time.[37] The deuterated product of this exchange reaction was shown to be identical to that obtained by the desulfurization of 8-mercaptopurine with deuterated Raney nickel. Further confirmation of the site of exchange was provided by the unambiguous synthesis of [8-^{2}H]purine by ring closure of 4,5-diaminopyrimidine with [$^2H_2$]-formic acid. In addition, hypoxanthine, inosine, adenine, adenosine, and 6-chloropurine were found to exchange the C-8 proton on heating in D_2O at 100°C for 10-20 min.[38]

In preliminary labelling studies of nucleic acids, it was found that the C-8 proton of adenosine 5'-monophosphate exchanged in D_2O at 92°C with a half-life of 90 min.[39] Ostermann *et al.*[40] prepared tritiated nucleoside diphosphates by heating the unlabelled

compounds in HTO at 100 °C. Tsang and co-workers[41] obtained [8-^{2}H]guanosine 5'-monophosphate by heating a D_2O solution of GMP at pD 7-9 for up to 8 h at 100 °C in a sealed container. Kinetic studies provided a mechanistic explanation that accounts for the facile exchange of the C-8 proton of purines, which also explains the exceptionally fast exchange rate observed in the case of guanosine at elevated pHs.[42]

The following fully and selectively deuterated analogues, guanine-d_5, **5**, guanine-d_4, **6**, guanine-d, **7**, and the corresponding labelled guanosines, GDPs and GTPs **8**, **9**, **10** were prepared by employing a combination of known and modified methodologies (see Discussion/Eperimental).



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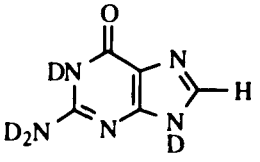

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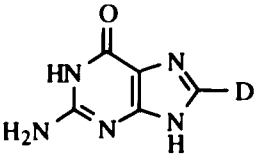

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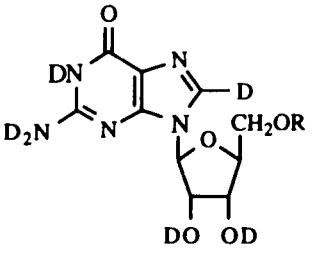

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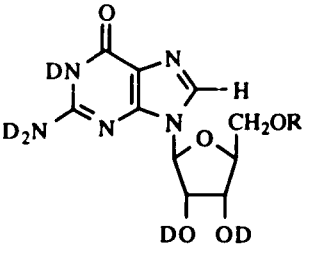

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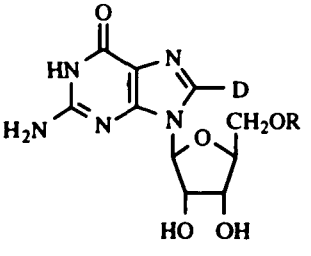


10

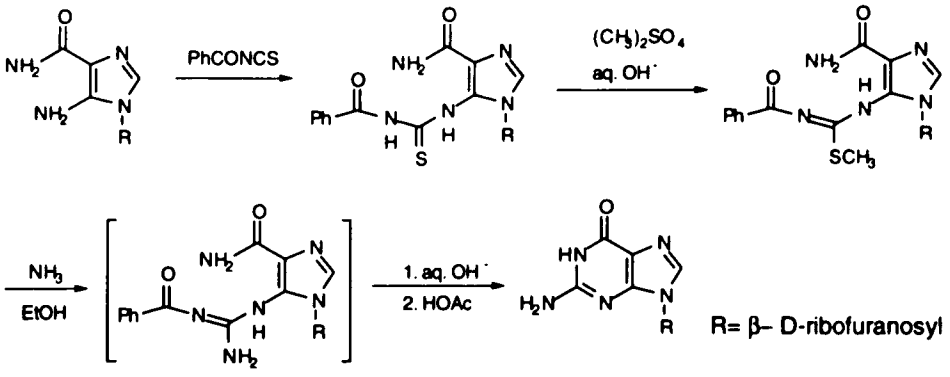
8a: R=D **9a:** R=H **10a:**R=D
8b: R=5'-diphosphate **9b:** R=5'-diphosphate **10b:**R=5'-diphosphate
8c: R=5'-triphosphate **9c:** R=5'-triphosphate **10c:**R=5'-triphosphate

B. ^{18}O-Labelled derivatives.

For structural elucidation of the binding sites in EF-Tu and p21, the oxygen at position 6 of the guanine ring and the oxygens of the phosphate groups are particularly relevant, therefore isotopic labelling at these positions will be discussed:

1. [6-^{18}O]Guanosine.

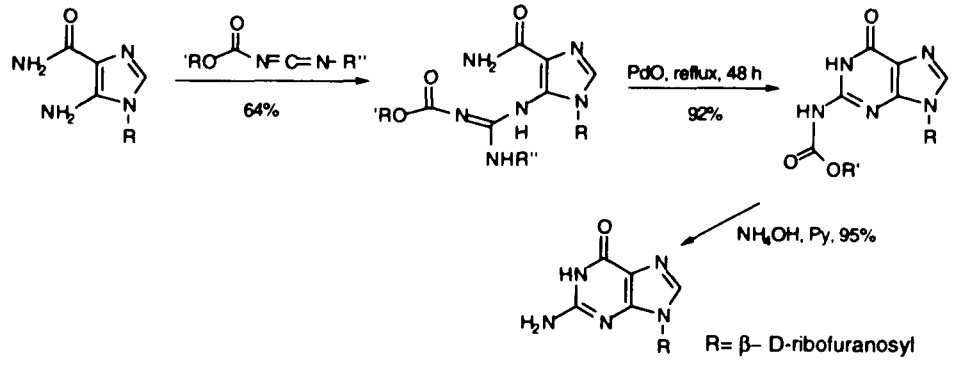
The standard Yamazaki ring closure methodology[43] (Scheme 1) to prepare guanosine is not suitable for the synthesis of isotopically labelled guanosines, not only because of the multiple steps, but also because of the low overall yields of 15-20%. Recently, a more efficient route to prepare guanosine-type nucleosides was reported



Scheme 1.

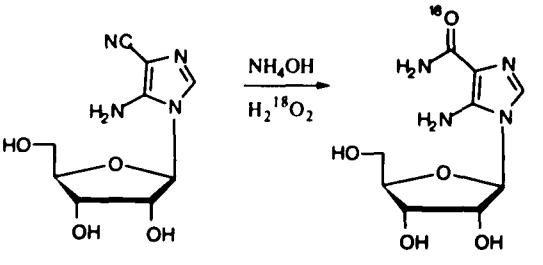
which can be adapted to synthesize our target compound, [6-^{18}O] guanosine. This three-step synthesis[33] in high overall yield, similar to Yamazaki's method, employs an *o*-amino carbamyl nucleoside precursor, 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (AICA-riboside), but effects the ring closure more efficiently by employing an acyl(arylmethyl)carbodiimide (Scheme 2).

Introduction of the [^{18}O]-label is based on the observation of Townsend *et al.*[44] that treatment of AICA-riboside with methoxycarbonyl isothiocyanate followed by cyclo-desulfurization yields a nitrile, instead of a purine ring. This nitrile is a suitable



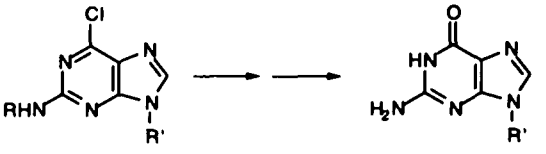
Scheme 2.

intermediate because its hydrolysis with alkaline $H_2{}^{18}O_2$ yields an [^{18}O]-labelled AICA-riboside (Scheme 3); the latter can then be converted to [6-^{18}O]-labelled guanosine (Scheme 2).



Scheme 3.

Commercially available (-)-2-amino-6-chloropurine riboside could also serve as a useful starting material to prepare [6-^{18}O]-labelled guanosine because 6-chloropurine derivatives[45-48] could be converted to guanosines in moderate to good yields (Scheme 4).



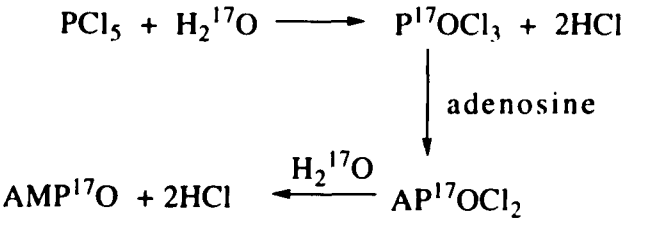
Scheme 4.

2. Nucleotides enriched with ^{17}O or ^{18}O on the phosphate group.

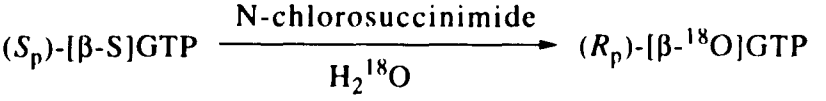
Goody and Leberman[49] studied the interaction of phosphorothioate analogs, the diastereomers of GDP[α-S] and GDP[β-S], with polypeptide elongation factor (EF-Tu) from *E. coli* and concluded that an α,β-bidentate Mg•GDP complex is the substrate recognized by EF-Tu and that the interaction of GDP with the enzyme at the β-phosphate might involve the two non-bridging oxygens. In contrast, ESR studies on EF-Tu isolated from *Bacillus stearothermophilus* employing not only the phosphorothioate analogs but also GDPs selectively labelled with ^{17}O at the α- or β-phosphate groups led to a very different conclusion: only the β-phosphate of GDP is coordinated to the metal ion in the EF-Tu•Mg•GDP complex, and the metal ion is coordinated only to the enzyme and to one oxygen atom of the phosphate. Raman spectroscopic studies employing GDPs selectively labelled on the phosphate, should clarify the nature of the phosphate/metal binding site, thus resolving this controversy.

A simple procedure[51] for the synthesis of a nucleoside 5'-monophosphate enriched with ^{17}O (or ^{18}O) that does not require isolation of the intermediates $P^{17}OCl_3$ (or $P^{18}OCl_3$) has been reported (Scheme 5), and specifically labelled nucleoside diphosphates, [α-$^{17}O_3$]GDP and [β-$^{17}O_4$]GDP/[β-$^{18}O_4$]GDP, were also prepared, the former by phosphorylation[50] of the labelled GMP, the latter by phosphorylation[52] of GMP employing $^{17}O/^{18}O$-enriched phosphates.[53-55] GTP analogs stereospecifically labelled with ^{18}O on

the phosphate group have been synthesized[56,57] from the corresponding phosphorothioates as shown in Scheme 6.



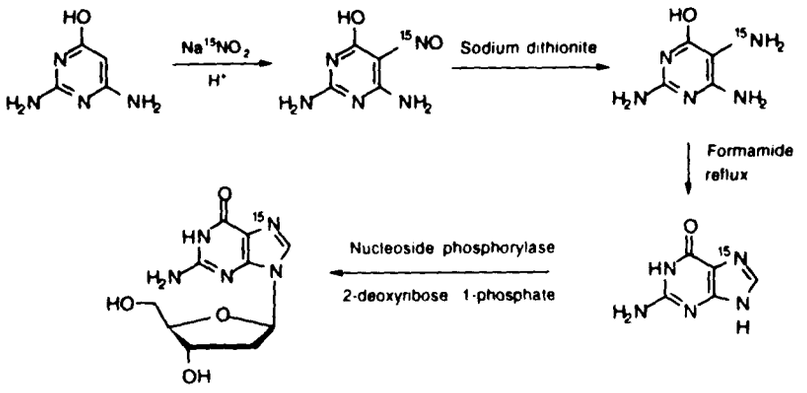
Scheme 5.



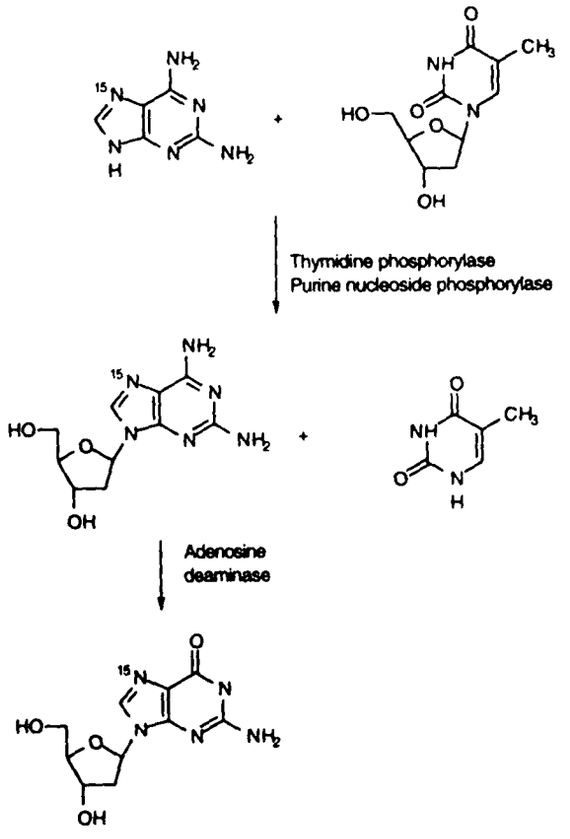
Scheme 6.

C. ^{15}N-labelled derivatives.

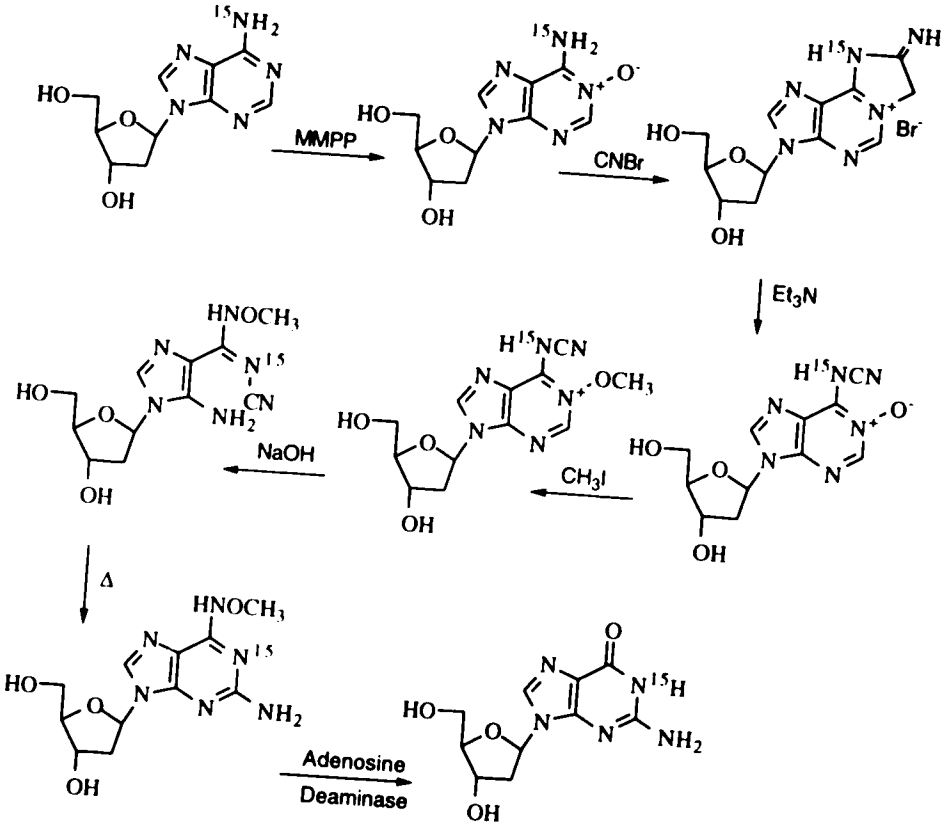
The synthesis of ^{15}N-labelled nucleotides, which can be incorporated into oligonucleotides that serve as models of DNA, was spurred by the importance of spectroscopic studies in elucidating DNA-protein interactions, and interactions of DNA with small molecules such as antibiotics, antiviral, and antineoplastic agents. Thus, adenine and cytosine were labeled at specific positions and the ^{15}N-labelled analogs were incorporated into oligonucleotides.[58-63] In the case of guanine, however, the first systematic study to label positions 1-, 2-, 3-, 7-, and 9- with ^{15}N that was reported was only of limited use to us due to the lack of any experimental details.[64] Subsequently, a few successful routes have been reported to synthesize guanosine analogs labelled at the 1-, 2- and 7-positions (Schemes 7,[65] 8,[66] and 9-10[67]).



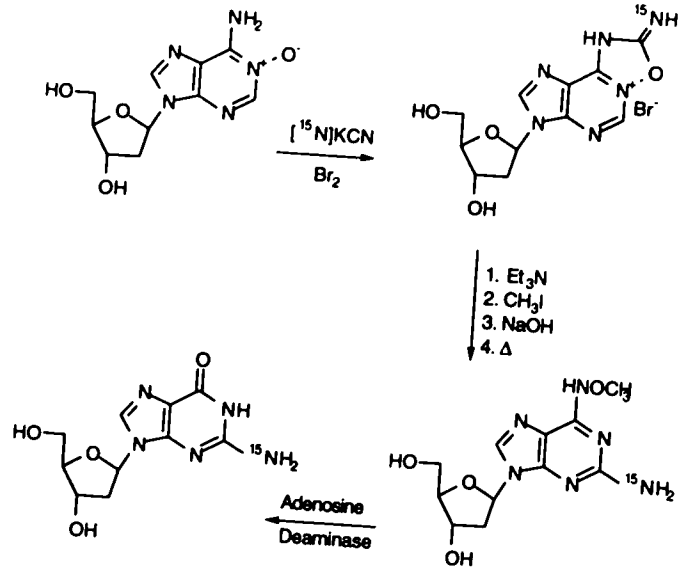
Scheme 7.



Scheme 8.



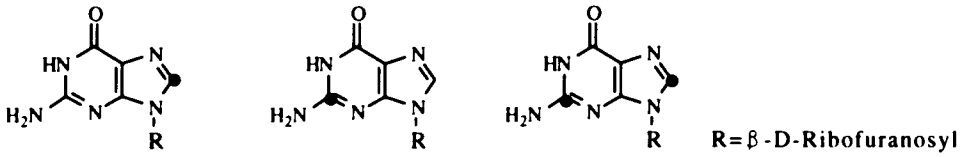
Scheme 9.



Scheme 10.

D. ^{13}C-Labelled analogues.

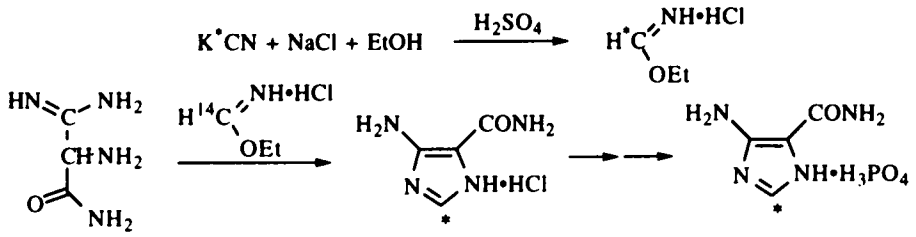
Guanosines **11**, **12**, and **13** are the only ^{13}C-labelled analogs that can be synthesized in a straightforward manner, employing known procedures outlined below:



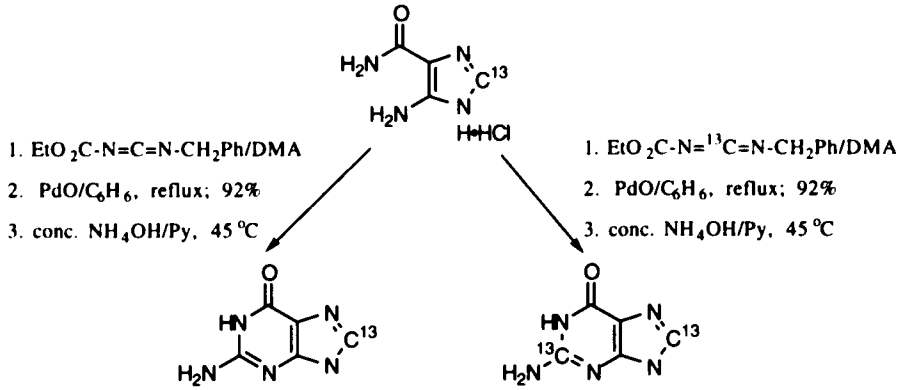
R=β-D-Ribofuranosyl

11 **12** **13**

4(5)-Amino-5(4)-imidazolecarboxamide (AICA) phosphate, prepared by an efficient two-step procedure[68] from [^{14}C]-ethylformimidate hydrochloride[69] (Scheme 11), or the corresponding [^{13}C]-labelled compound can be converted to [8-^{13}C]guanine or [2-, 8-^{13}C]guanine[33,70] as shown in Scheme 12.

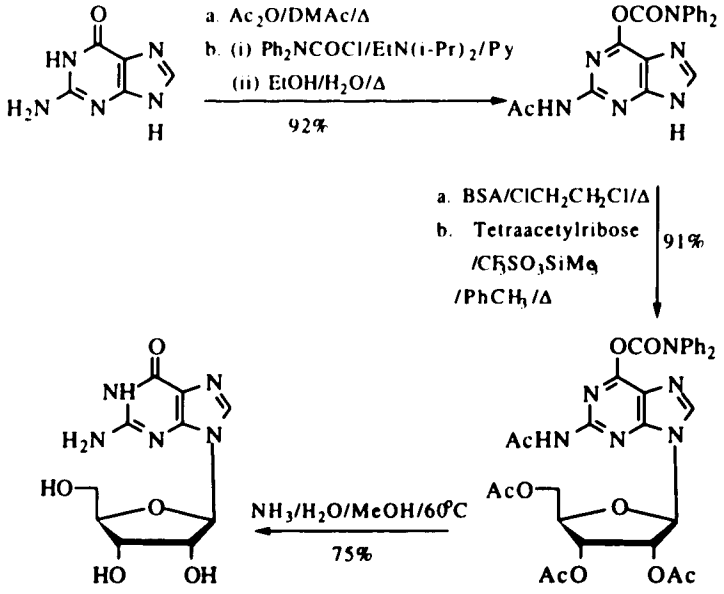


Scheme 11.



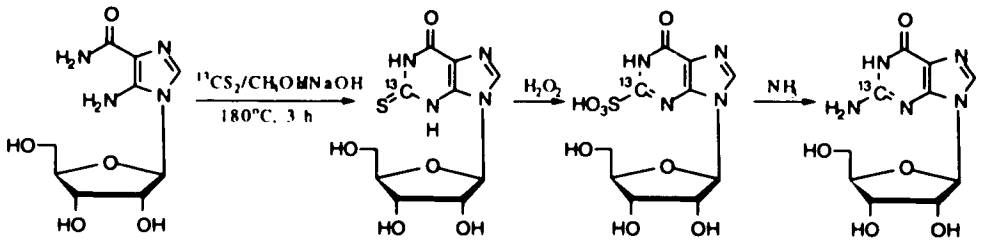
Scheme 12.

Direct glycosylation of the guanine produces N7/N9 isomeric mixtures that are difficult to separate. Changes in experimental conditions affect these isomer ratios,[71,72] but they do not eliminate contamination of desired thermodynamic N9 product by the kinetic N7 isomer. Zou and Robins[73] reported regioselective coupling of bis-trimethylsilylated 2-N-acetyl-6-O-diphenyl-carbamoylguanine with glycosyl acetates or α-haloethers as a convenient new route that affords N9 glycosylated products exclusively (Scheme 13).



Scheme 13.

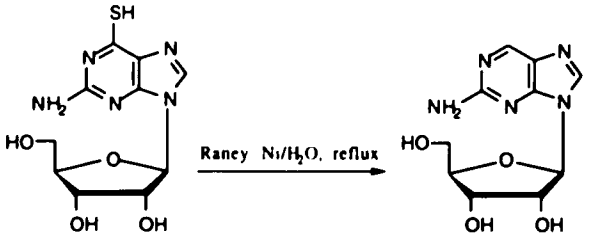
An alternate route to obtain [2-^{13}C]guanosine would employ the commercially available 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (AICA-riboside) and sodium methylxanthate (prepared *in situ* from $^{13}CS_2$) in the key, high yield ring closure step[74] (Scheme 14).



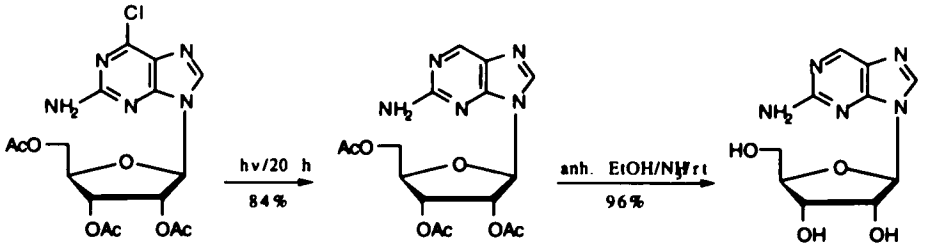
Scheme 14.

E. 6-H and 6-SH guanine nucleosides and nucleotides.

2-Amino-9-β-D-ribofuranosylpurine (6-H-guanosine) that can be prepared efficiently (*via* Schemes 15[75] or 16[76]), is a potent inhibitor of adenosine deaminase and other purine-metabolizing enzymes,[77] and it can be incorporated into *E. coli* and T4-DNA.[78,79] In



Scheme 15.

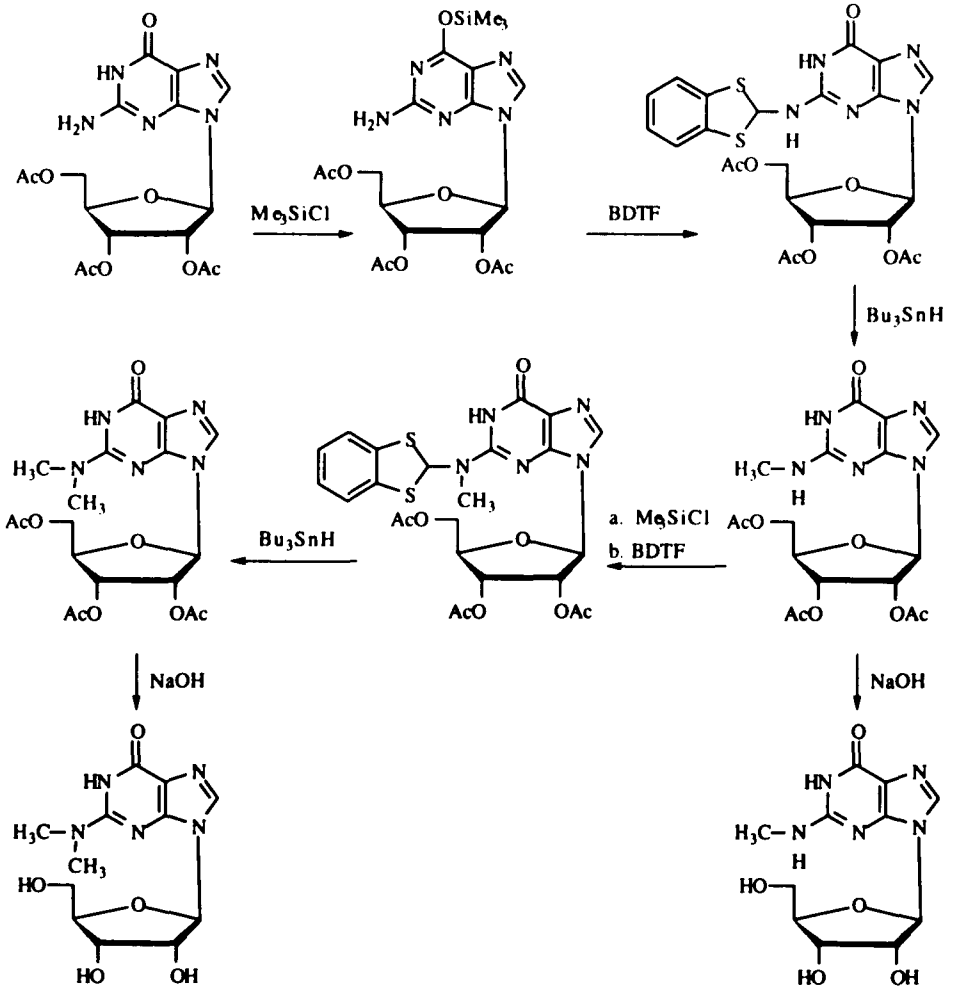


Scheme 16.

the present studies, 6-H-guanosine together with the commercially available 6-thioguanosine and the alkylated analogs (see below) helped to clarify the nature of the hydrogen bonding interactions of the guanine moiety with the binding site of two G proteins, EF-Tu and p21.[80]

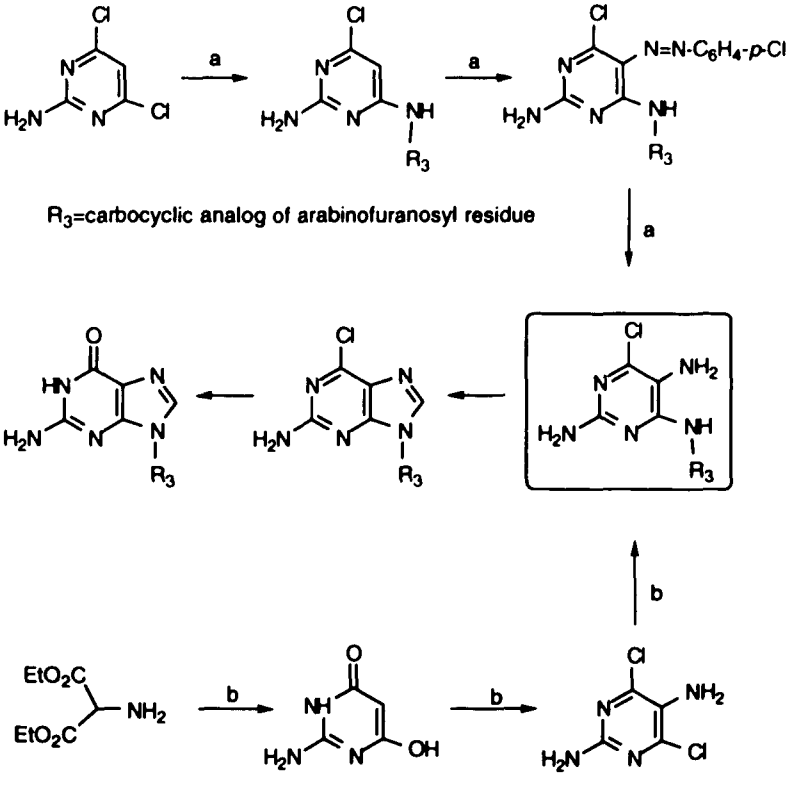
F. Alkylated analogs.

The key steps in a novel N-alkylation method[81] of guanosine (Scheme 17) involve the conversion of the 2-amino- to a 1,3-benzodithiol-2-yl group which can then be reductively cleaved at the C-S bond to afford the desired N-alkylation product. Repetition of this sequence then yields N,N-dialkylated guanosine derivatives.



Scheme 17.

Among the many methods that can be employed to synthesize 9-substituted guanines,[82-87] the route outlined in Scheme 18, from a commercially available precursor, via the diazo intermediate (route "a") is the one most commonly used.[82] The overall yield of the key intermediate via route "a" ranges between 20-69%. An alternative route employs 2,5-diamino-4,6-dichloropyrimidine as the precursor (route "b"), because this pyrimidine, obtained by condensation of diethyl hydroxyiminomalonate with guanidine in 31% yield, can be converted to the key intermediate in 72% yield.[82]



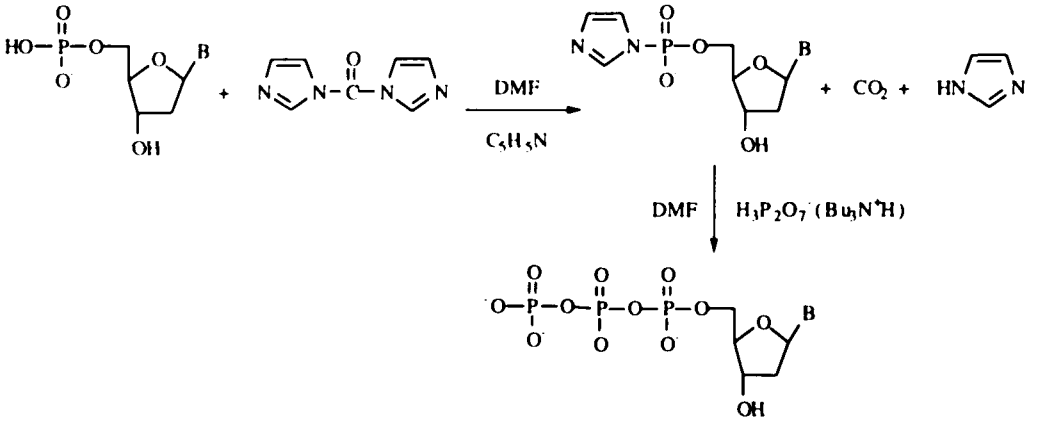
Scheme 18.

G. Phosphorylation of guanosine.

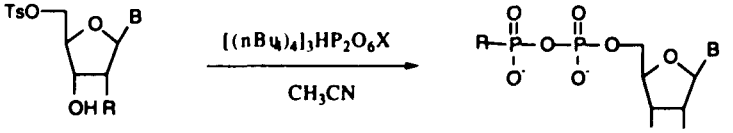
There are two main types of methods to phosphorylate nucleosides, chemical methods[88-91] and enzyme catalyzed syntheses.[92-97]

The early phosphorylation procedures of nucleosides involved the use of diphenyl or dibenzyl phosphorochloridates,[98] but in the case of guanosine, that has a more inert 5'-hydroxyl group, tetra-p-nitrophenyl pyrophosphate was employed.[99] Because of the low yields obtained, phosphoryl chloride was seldom used until Yoshikawa *et al.*[100] employed it to convert 2',3'-O-isopropylidene nucleosides to the 5'-nucleotides in trialkylphosphate solvents containing trace amounts of water. A modification of this method allowed the conversion even of unprotected nucleosides to the 5'-nucleotides in good yields and high selectivity. For instance, phosphorylation of guanosine afforded 5'-GMP in 85% yield, along with only a total of 9% of 2',5'- or 3',5'-diphosphates and 2',3',5'-triphosphate.[30,89,100] Phosphorylation with high selectivity of the primary hydroxyl group in unprotected nucleosides has been also achieved using phosphoryl chloride or pyrophosphoryl chloride in organic solvents to produce 5'-monophosphates in good yields[90,101] The latter can be converted to 5' triphosphates according to Scheme 19, a sequence particularly suitable to microscale preparation of 5'-triphosphates of mono- and oligodeoxyribonucleotides.[88] An elegant, single-step procedure that utilizes a nucleophilic displacement of 5'-O-tosylnucleosides by the tris(tetra-*n*-butylammonium) form of

pyrophosphoric acid at room temperature (Scheme 20) permits the syntheses of 5'-diphosphates, methanediphosphonates, and difluoromethanediphosphonates in 43-93% yields. A modification of this procedure was also found to be suitable for the preparation of triphosphates. Thus, reacting tetra-*n*-butylammonium hydrogen triphosphate with 5'-tosyladenosine afforded adenosine triphosphate in 55% yield.



Scheme 19.



B = Adenine
Uracil
Cytosine
Thymine

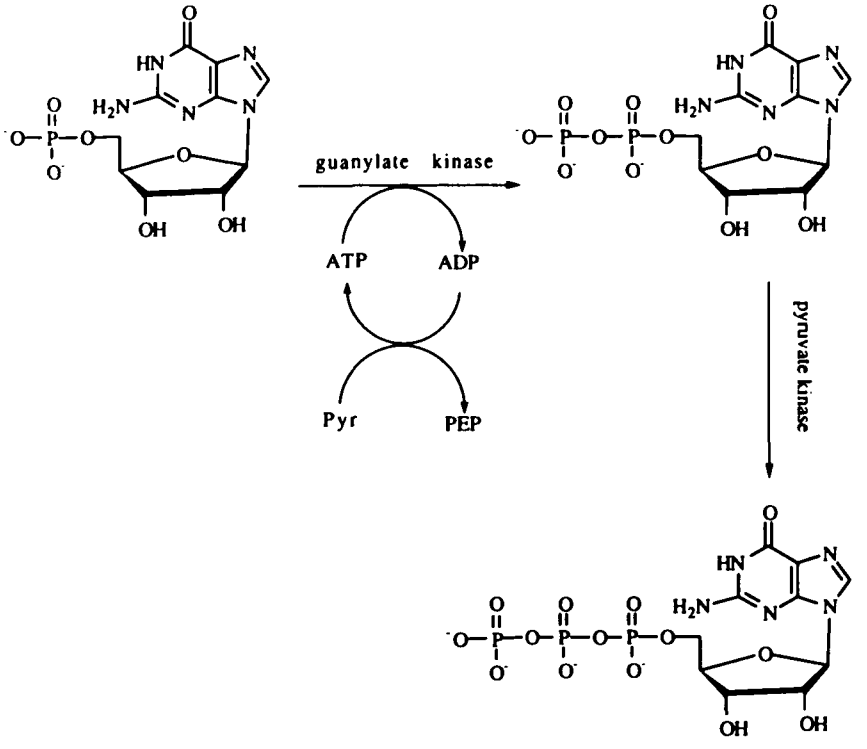
R = H, OH

X = O, CH_2, CF_2

Scheme 20.

Recent success with the enzyme-catalyzed syntheses of cytidine 5'-triphosphate, guanosine 5'-triphosphate, and uridine 5'-triphosphate from the corresponding nucleoside monophosphates by

Whitesides *et al.*[93,94] afforded the enzymatic route that is also suitable for the large scale syntheses of nucleoside triphosphates. The nucleoside monophosphates are readily available commercially, and the reaction can easily be scaled up. The route to GTP involves phosphorylation of GMP using guanylate kinase which catalyzes the phosphate transfer from ATP to GMP leading to the formation of GDP and ADP.[97-101] Regeneration of ATP is achieved by the action of pyruvate kinase using phosphoenolpyruvate (PEP) as phosphoryl donor. This enzyme also phosphorylates GDP to GTP. Thus, a combination of these two enzymatic reactions allows the synthesis of GTP starting from cheap GMP and ATP (Scheme 21).



Scheme 21.

2. RESULTS AND DISCUSSION

Purine nucleosides and nucleotides are important synthetic targets because of their usefulness as model compounds in the structure elucidation of G proteins and as tools for studying the structure and function of nucleic acids. Synthetic efforts to obtain them, especially the guanine derivatives, were further stimulated by the discovery of nucleoside antibiotics and the antivirals such as 3'-azido-3'-deoxythymidine (Zidovudine or AZT), clinically useful in the treatment of AIDS.

Here we report the development of synthetic routes leading to the purine derivatives [8-^{2}H]GDP, [8-^{2}H]GTP, [8-^{2}H]GMPPCP, [6-^{18}O]GDP, [6-H]GDP, GDP ^{18}O labelled at the phosphate group, and 9-butylguanine. The information obtained on the active site's structures of the G proteins, *ras* oncogen and the elongation factor Ef-Tu, employing these compounds in conjunction with Raman spectroscopic studies will be discussed briefly at the end of this section.

2.1 Deuterated analogs.

Infrared spectroscopy distinguishes between ^{1}H and ^{2}H isotopes bound to carbon, oxygen and nitrogen; this difference in the spectra had been employed in the early experiments to study the slow exchange of hydrogen in DNA. However, these studies, in which nucleosides, including guanosine, dissolved in D_2O and incubated for several hours at 95°C were employed as model compounds,[102] could

lead to the assignment of only a few vibrational frequencies. A combination of infrared and Raman spectroscopic studies of guanines labelled with ^{15}N and ^{2}H at selected positions allowed the assignment of a few more of the fundamental vibrational frequencies of the purine ring system.[103] However, these studies reported only scant experimental data for the syntheses of the isotopically labelled guanines, and provided no independent confirmation of the positions of the labels, hence these assignments of the vibrational modes reported have only limited value. On the other hand, the structures of guanosine and 5'-GMP, selectively deuterated at position 8 (>90% ^{2}H), prepared by re-exchange of the fully deuterated compounds, were unambiguously established by ^{1}H and ^{2}H nmr.[104] Even though these compounds were employed as models in solid state nmr studies to characterize internal motions in DNA and RNA, they were not used to assign infrared or Raman vibrational frequencies.

Our objective to investigate of the dynamic structures of G proteins using Raman spectroscopy, required selectively deuterated guanosine diphosphates (GDPs) and guanosine triphosphates (GTPs). The paucity of information on the syntheses of labelled guanines and guanosines, and the fact that the few previous labelling studies were conducted in D_2O at ~100°C, where our target compounds were not expected to be stable, required a systematic study to develop novel methodologies.

Preliminary experiments in which GDP was heated with D_2O at different temperatures to determine the extent of the exchange reaction, indicated that the proton at position 8 is not exchanged at

37°C even after 39 hours; 90% exchanged at 95°C. However, this was accompanied by a 60% decomposition of GDP. Taking into account the known pH-dependence of the exchange rate,[105,106] addition of various amounts of ND_4OD to increase the pH was tested, but still no exchange was observed at 37°C. However, addition of various amounts of K_2DPO_4 followed by heating of the solution at different temperatures, allowed us to establish the optimum conditions to prepare GDP deuterated at position 8 (Table 1, last row).

Table 1. Exchange of the proton at position 8 of GDP.

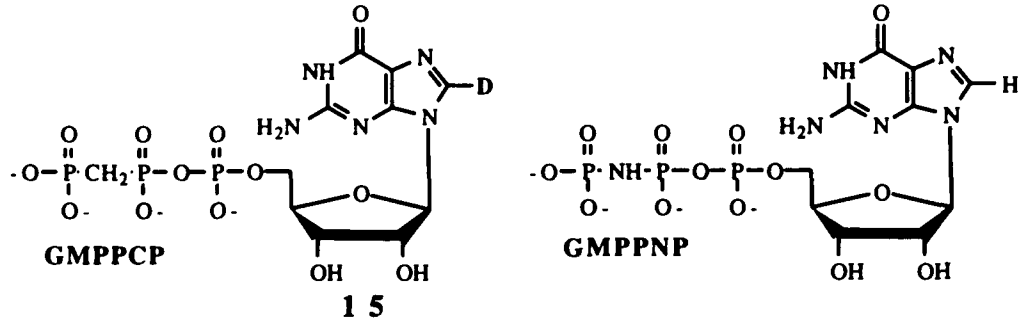
GDP Concentration (mg/ml)	K_2DPO_4 Concentration (mg/ml)	Temperature (°C)	Time (hour)	8-^{2}H (%)	GDP decomposition (%)
10	0	37-38	39	0	5
	3	95	5	90	50
	5	95	5	>95	30-40
	10	95	5	~100	20
	20	95	5	~100	10
	25	80-85	5	~90	10
	30	90-95	5	~100	10

When the above procedure was employed to effect the exchange of the proton at position 8 in GTP (Table 2), in addition to deuterated GTP (60%), deuterated GDP (~35%) and deuterated GMP (~5%) were also obtained. However, a facile and effective separation of the mixture was achieved by ion exchange chromatography at 4°C on a DEAE-Sephadex A-25 column (Figure 4) that provided labelled GTP as well as GDP of high purity.

Table 2. Exchange of the proton at position 8 of GTP.

GTP Concentration (mg/ml)	K_2DPO_4 Concentration (mg/ml)	Temperature (°C)	Time (hour)	8-^{2}H (%)	GTP decomposition (%)
10	0	95	5	95	~55
	7.5	95	5	97	~40
	15	95	5	98	~40
	25	95	5	98	~40

We tested the applicability of this exchange reaction at the 8 position to the GTP analogs modified at the phosphate groups (Figure 5). Deuterated GMPPCP, **15**, could be prepared in excellent yield, but the method was not suitable to exchange the proton at position 8 of GMPPNP. Thus, even in the presence of high concentrations of K_2DPO_4, instead of deuterated GMPPNP, only the decomposition products, GDP and GMP, were isolated after five hours reaction at 95°C. This can be explained by the fact that GMPPNP is more prone to hydrolysis than GMPPCP, the P-N bond being less strong than the P-C or P-O bonds.



15

Figure 5.

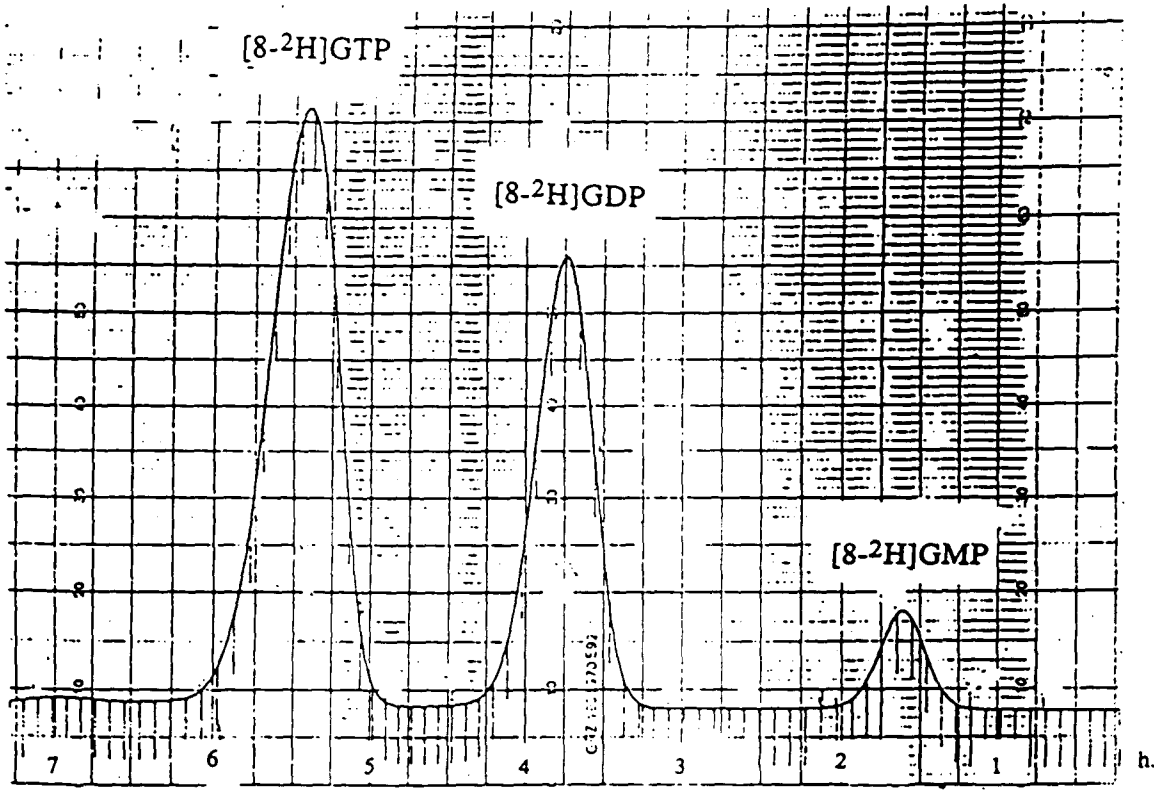


Figure 4. Separation of [8-^{2}H]GTP (**10c**), [8-^{2}H]GDP (**10b**), and [8-^{2}H]GMP (**14**) using ion exchange chromatography on DEAE Sephadex A-25, monitored at 254 nm.

The method we developed to prepare deuterated guanosine phosphates is straightforward and inexpensive: The phosphate, K_2DPO_4, added during the exchange reaction plays a dual role; it not only acts as a buffer, but also as a base. Thus, adjustment of the pH, to achieve the fastest possible proton exchange rate without concomitant decomposition of the labelled nucleotide phosphates was accomplished. This is in contrast to the case of purine ribosides,[3 4] where fast exchange rates could be achieved simply by increasing the pH or the temperature since decomposition of the target compounds is not a concern. Our results also account for the failure of the ammonium hydroxide to achieve optimal exchange rates, which instead led to the decomposition of the nucleotides.

2.2 [^{18}O]-Labelled derivatives.

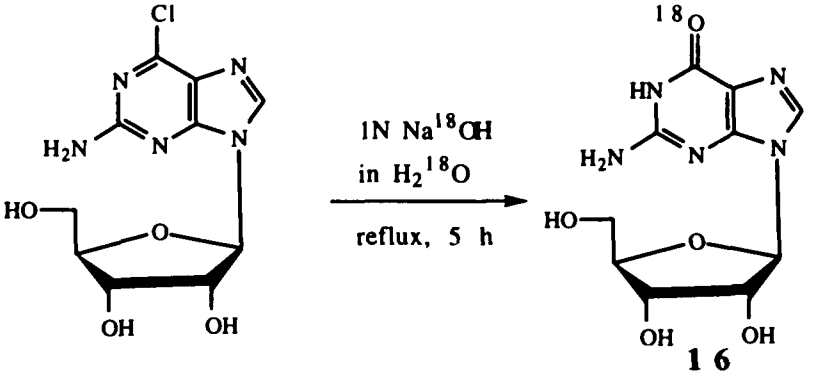
In defining the interactions between the G proteins and the guanosine phosphates using Raman spectroscopy, two types of oxygen interaction sites are the most relevant; the oxygen of the guanine ring (6-O) and the phosphate oxygens. Therefore, the syntheses of both types of ^{18}O-labelled nucleosides and nucleotides were undertaken.

2.2.1 [6-^{18}O]-Labelled guanine nucleoside and nucleotides.

The known synthesis of [6-^{18}O]guanosine from a commercially available starting material 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide (AICA-riboside) involves conversion of the AICA-riboside to the nitrile, hydrolysis of the latter with alkaline ^{18}O-

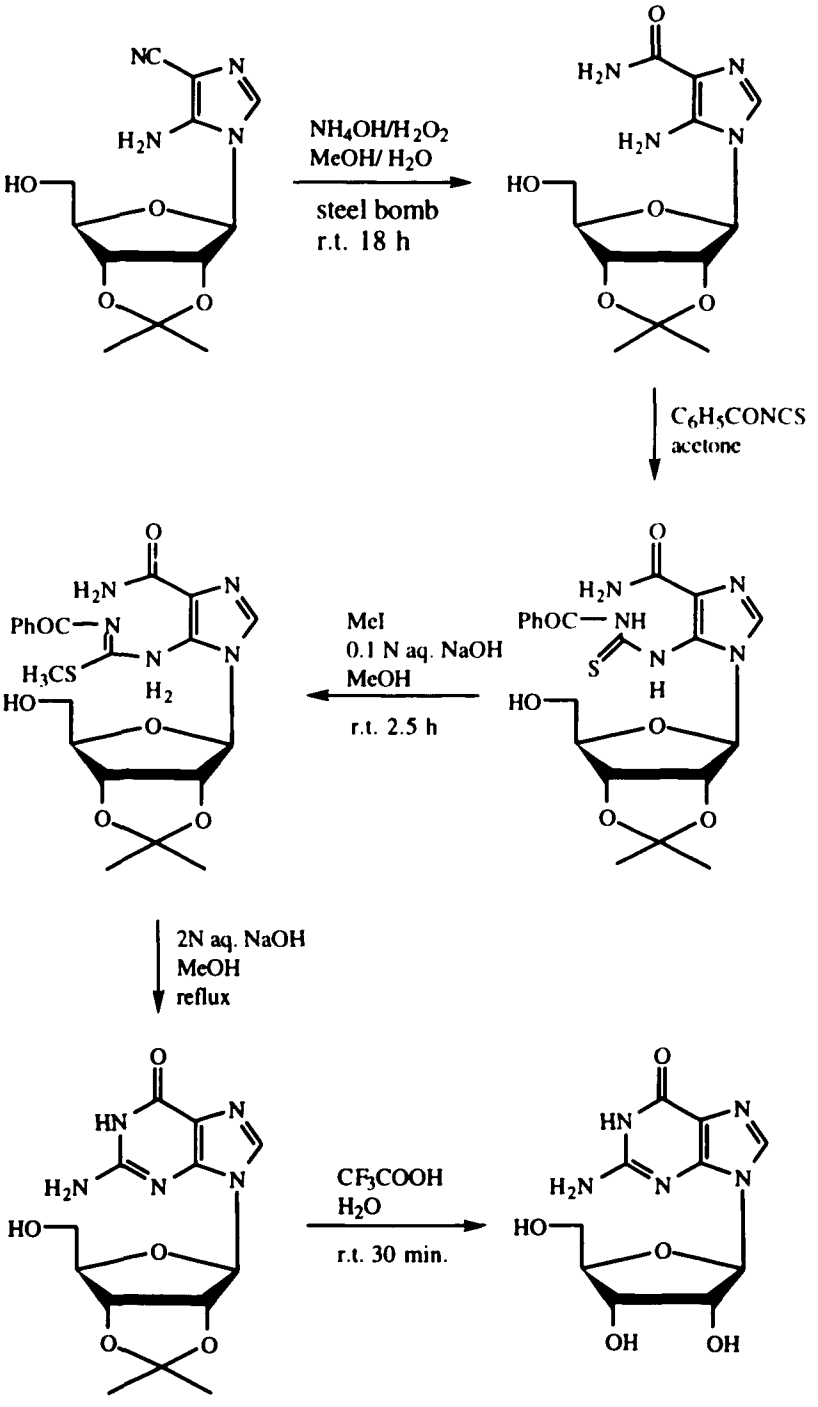
labelled H_2O_2, and subsequent ring closure to obtain the guanine ring (Scheme 22).[107,108] This synthesis, as well as other multistep routes that have as the key step the ring closure reaction, give rather low overall yield (ca. ~20%), and since $H_2{}^{18}O$ is expensive and is no more available commercially, it was not practical to prepare the target compound, [6-^{18}O] guanosine, by these methods.

An alternate approach is to convert the commercially available (-)-2-amino-6-chloropurine riboside to [6-^{18}O]guanosine in a one step reaction by treatment with either acid or base.[109,110] Treatment with 1N HCl was not suitable because acid will easily open the riboside ring. Hydrolysis using sodium methoxide as base,[111] in refluxing methanol in presence of one equivalent of $H_2{}^{18}O$ led only to unlabelled guanosine indicating that the oxygen came from the methanol-methoxide not come from $H_2{}^{18}O$. However, when the reaction was carried out with $Na^{18}OH$ as base in $H_2{}^{18}O$ (Scheme 23) monitoring its progress by the disappearance of the 310 nm absorption (Figure 6), [6-^{18}O]guanosine, **16**, was obtained in 53% yield. CI-MS (NH_3) showed an $[M+1]^+$ ion at *m/z* 286 confirming the product was [6-^{18}O]guanosine (Figure 7).



16

Scheme 23.


NC
N
H2N
N
HO
O
O
O
NH4OH/H2O2
MeOH/ H2O
steel bomb
r.t. 18 h
O
H2N
N
H2N
N
HO
O
O
O
C6H5CONCS
acetone
O
H2N
N
PhOC
N
H3CS
N
N
H2
HO
O
O
O
MeI
0.1 N aq. NaOH
MeOH
r.t. 2.5 h
O
H2N
N
PhOC
NH
S
N
N
H
HO
O
O
O
2N aq. NaOH
MeOH
reflux
O
HN
N
H2N
N
N
HO
O
O
O
CF3COOH
H2O
r.t. 30 min.
O
HN
N
H2N
N
N
HO
O
OH
OH

Scheme 22.

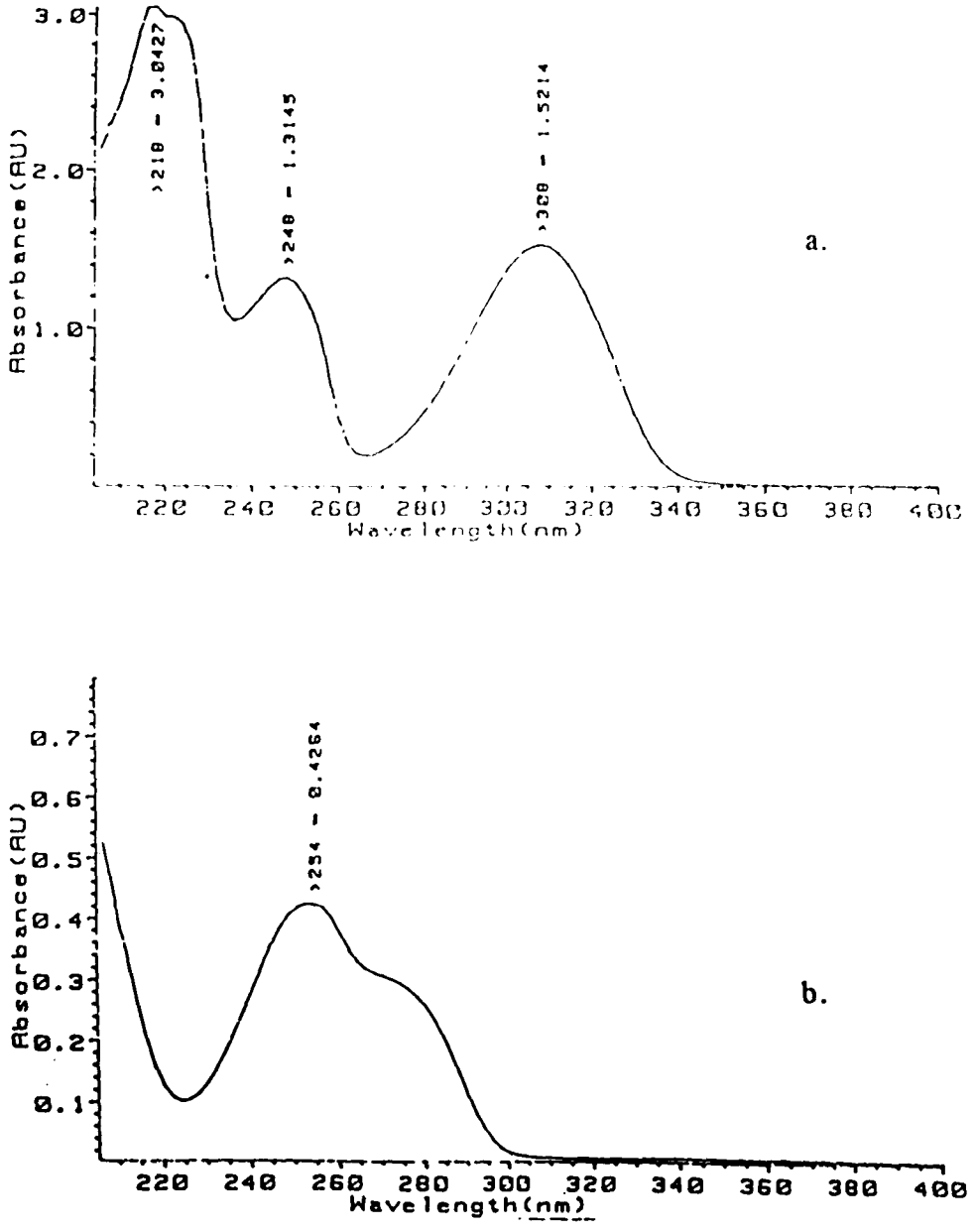


Figure 6. UV absorption of (a) (-)-2-amino-6-chloropurine riboside and (b) [6-^{18}O]guanosine (**16**).

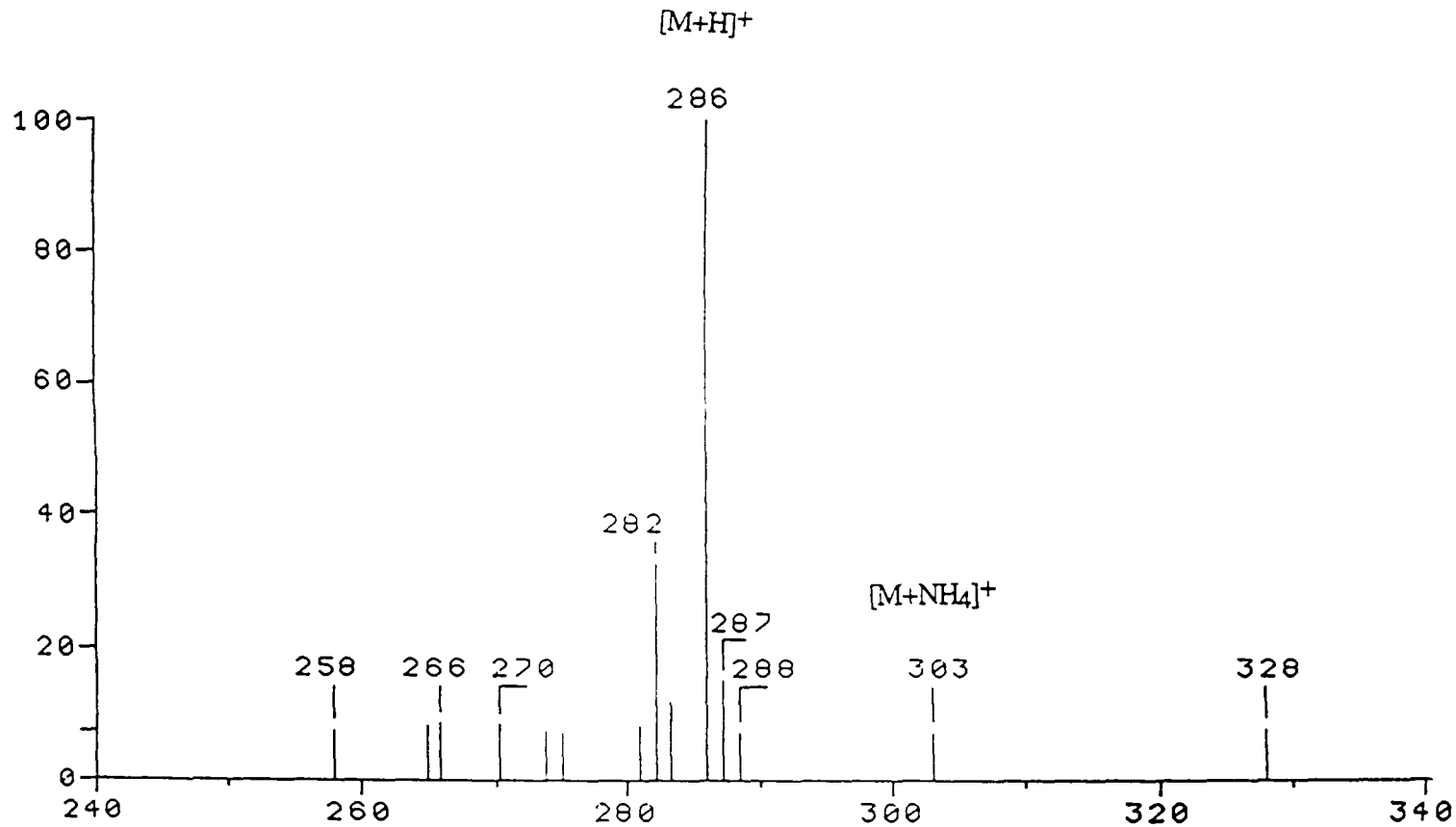
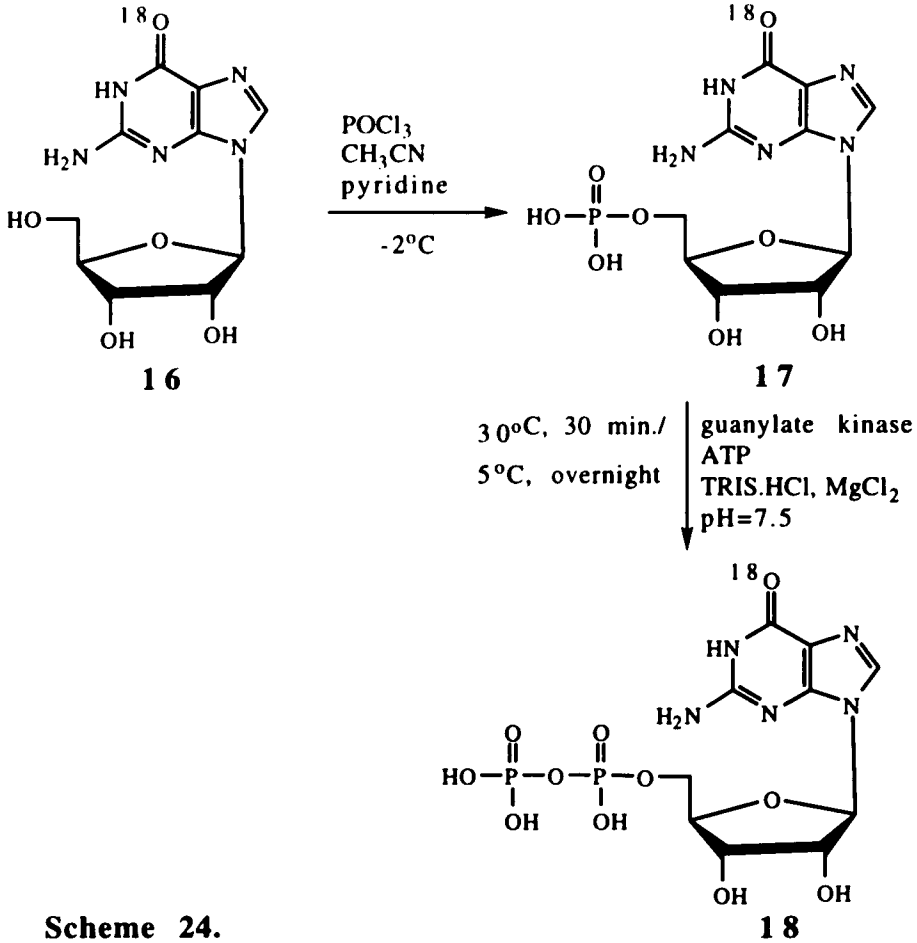


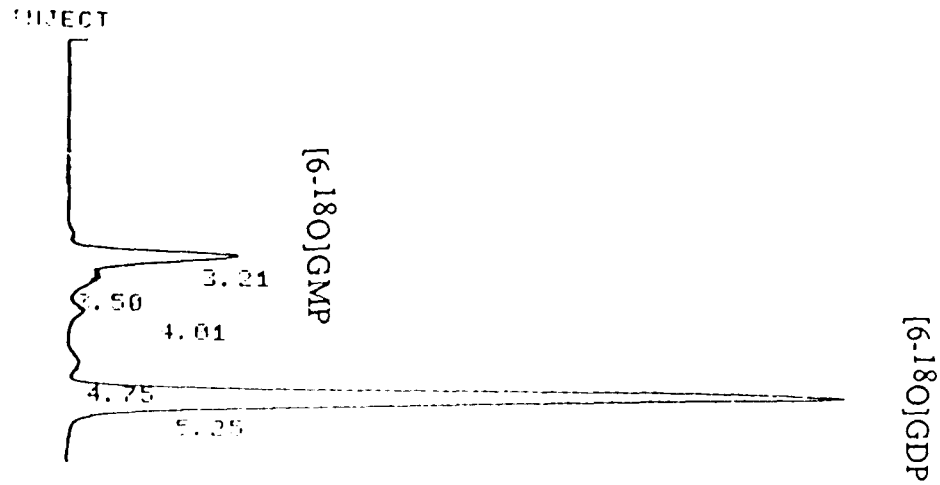
Figure 7. CI-MS (NH_3) of [6-^{18}O]guanosine **(16).**

[6-^{18}O]Guanosine was converted to [6-^{18}O]GMP, **17**, according to a known procedure,[101] and the latter to the [6-^{18}O]GDP, **18**, by using an enzymatic phosphorylation method developed in our laboratory (Scheme 24).[112] The enzymatic step, catalyzed by guanylate kinase, is highly specific for the formation of GDP and ADP. It was necessary to monitor the progress of the reaction by HPLC (Figure 8) in order to establish conditions that result in 80% conversion to [6-^{18}O]GDP. The almost quantitative recovery of the product from the reaction mixture was easily accomplished by ion exchange chromatography (Figure 9).



16 **17** **18**

Scheme 24.



PEAK#	AREA%	RT. min.	AREA BC
1	15.224	3.21	3044 02
2	2.376	3.5	475 02
3	1.31	4.01	262 03
4	1.335	4.75	267 02
5	79.755	5.25	15947 03
TOTAL	100.		19995

Figure 8. HPLC analysis of the reaction mixture obtained from enzymatic phosphorylation of [6-O]GDP (17).

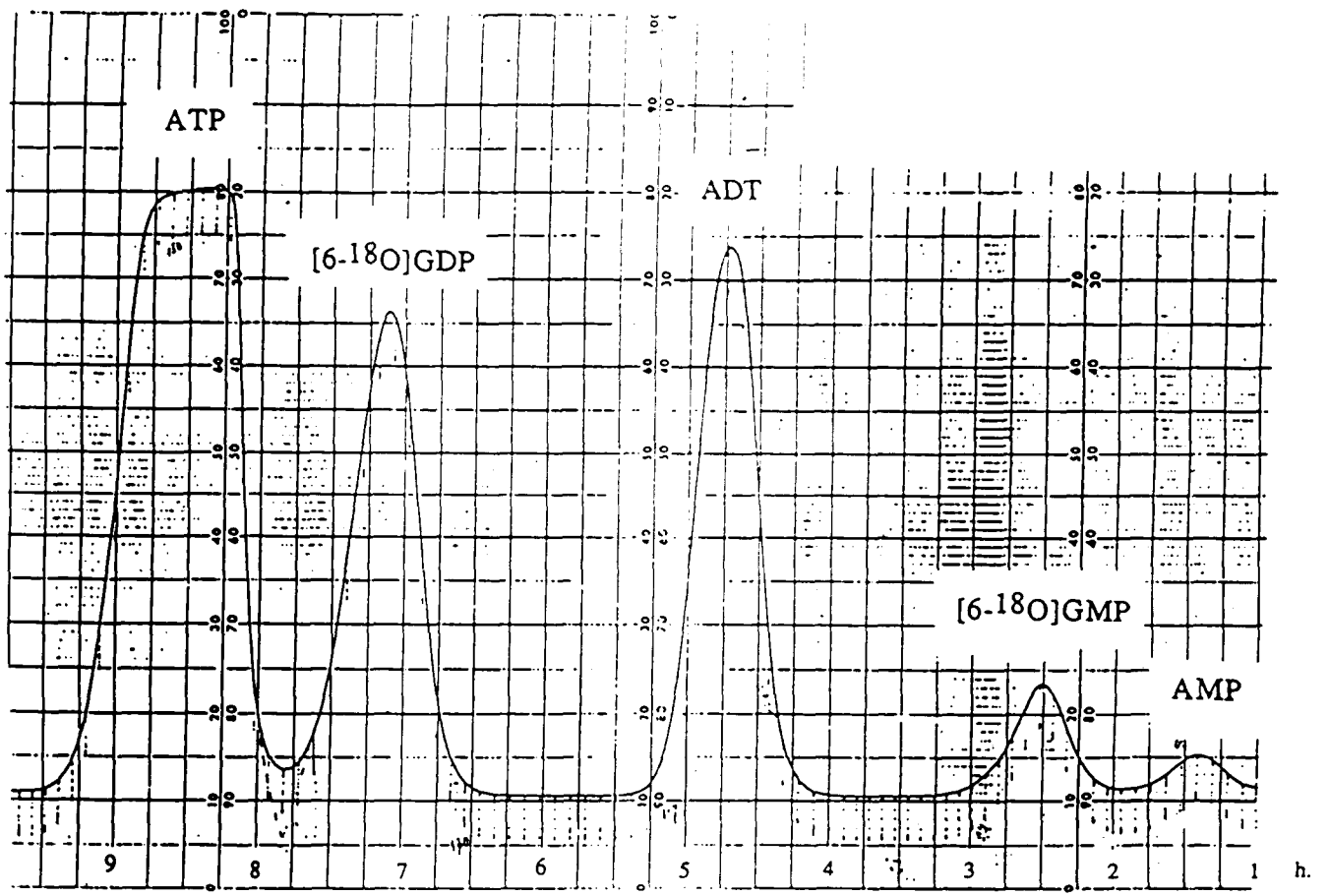


Figure 9. Ion exchange chromatographic separation of the products of the enzymatic phosphorylation of [6-^{18}O]guanosine monophosphate (**17**).

2.2.2 GDP Enriched with ^{18}O on the phosphate group.

We selected two target compounds to be synthesized for our initial studies since these would be particularly relevant to investigate the interaction of the nucleotide with the protein at the β-phosphate's binding site: one fully labelled [β-$^{18}O_4$]GDP (**19**), and one specifically labelled on one oxygen of the β-phosphate only, [β-^{18}O]GDP (**20**).

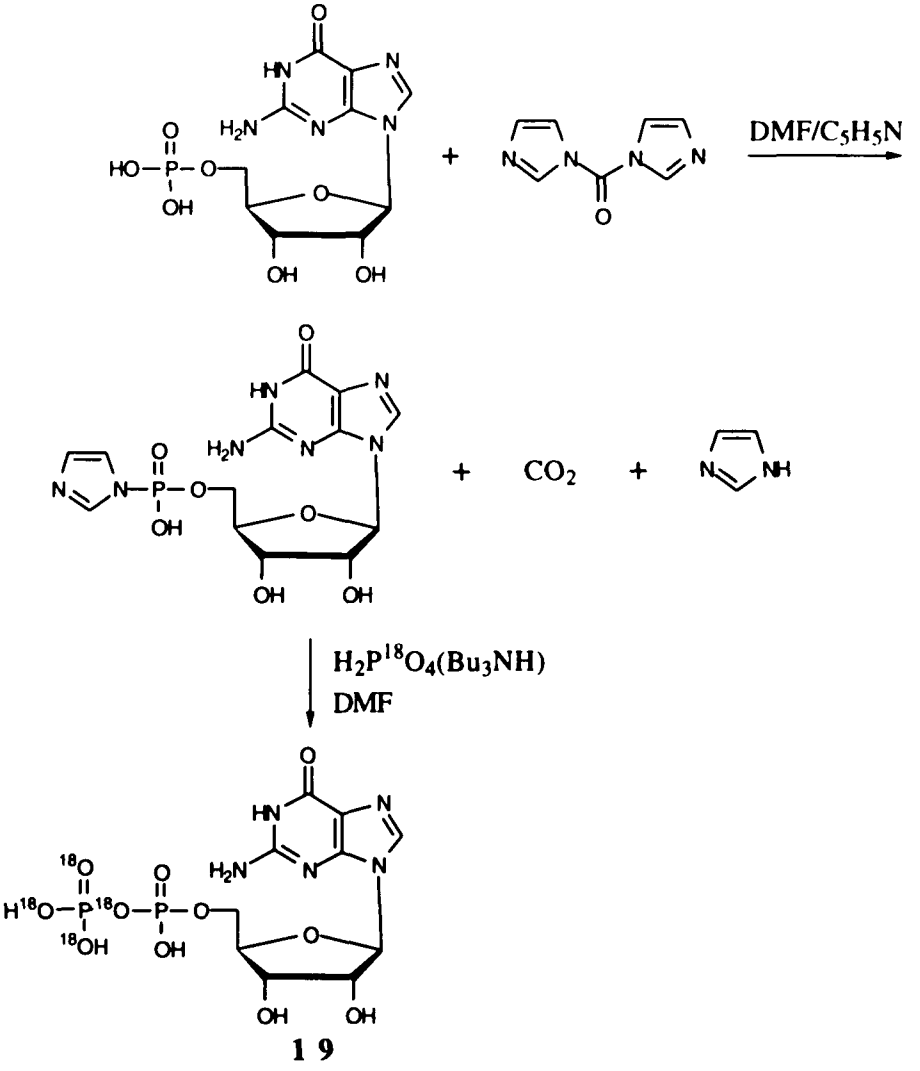
Two methods to prepare guanosine diphosphates labelled on the phosphate oxygen(s), that are suitable for small scale preparations were considered:

1) Phosphorylation of guanosine with oxygen labelled phosphate[113] group(s) will lead only to the synthesis of [α-$^{18}O_3$]GDP, or [α,β-$^{18}O_7$]GDP. But phosphorylation of the unlabelled GMP can be employed to obtain GDP labelled fully at the β-phosphate, [β-$^{18}O_4$]GDP.

2) Conversion of commercially available [β-S]GDP to [β-^{18}O]GDP by adaptation of the method of Wittinghofer *et al.*[57]

To synthesize the [β-$^{18}O_4$]GDP, **19**, it is not practical to employ the enzyme-catalyzed phosphorylation method we developed to obtain [6-^{18}O]GDP, **18**, in excellent yield since synthesis of **19** would require [$^{18}O_4$]labelled nucleotide (ATP) for the reaction. Therefore, we chose a method that involves condensation of an activated GMP derivative, i.e., we prepared tributylammonium [$^{18}O_4$]phosphate using PCl_5 and $H_2{}^{18}O$ at low temperature,[114] which was then used to

phosphorylate the activated GMP derivative (Scheme 25). This method worked well for the preparation of labelled GTP, and we were also successful in obtaining [β-$^{18}O_4$]GDP by using this method (Figure 10). Even though GTP was also obtained, along with the desired GDP, the former could be converted to GDP by using the same method that we developed to prepare deuterated GTP (**10c**) followed by a facile separation step.



19

Scheme 25.

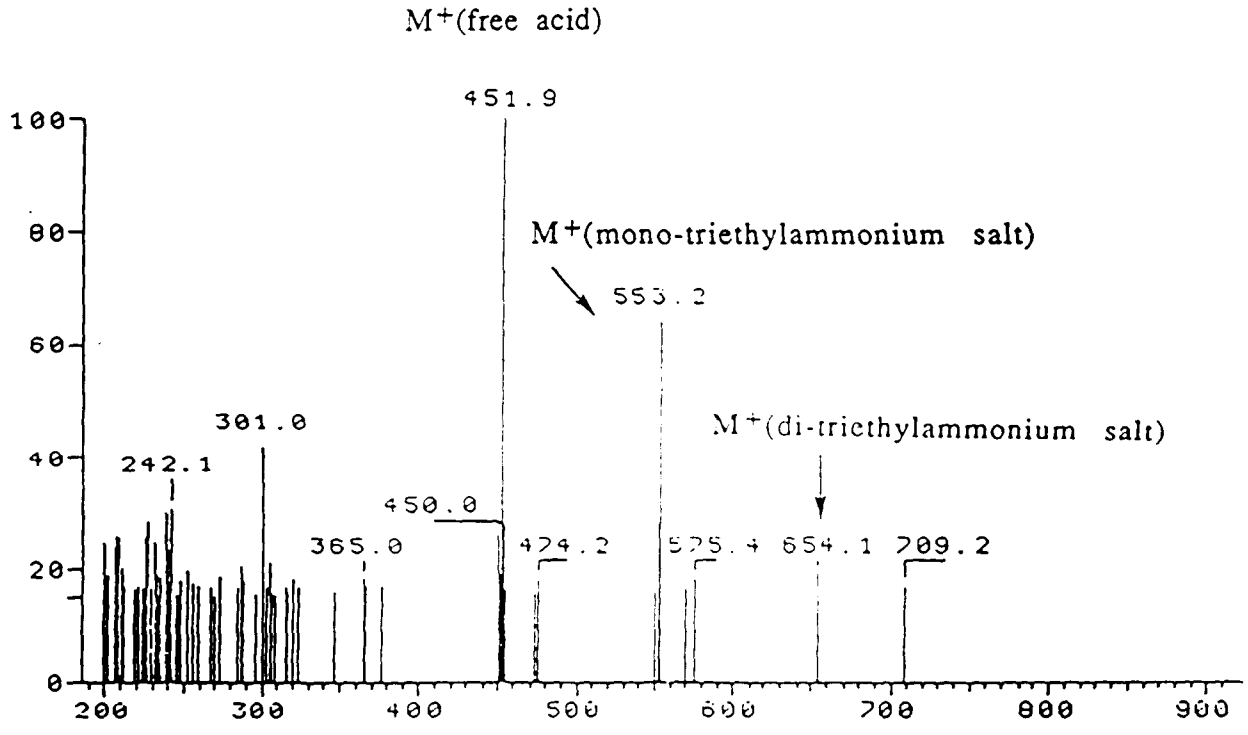


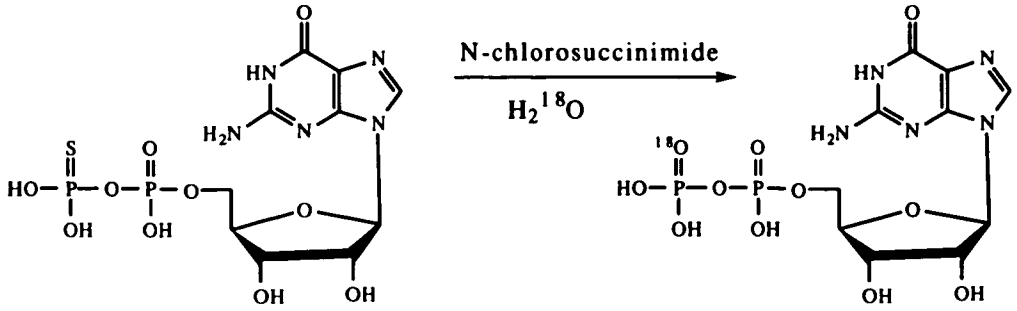
Figure 10. FAB-MS (glycerol) of [β-$^{18}O_4$]GDP (**19**).

GTP analogs which are specifically labelled with ^{17}O on the β-phosphate group have been synthesized from the corresponding phosphorothioates according to Scheme 26.[115]

$$(S_p)\text{-}[\beta\text{-S}]NTP \xrightarrow[H_2{}^{17}O]{\text{N-bromosuccinimide}} (R_p)\text{-}[\beta\text{-}^{17}O]NTP$$

Scheme 26.

Wittinghofer *et al.*[57] reported that this method worked well with adenine nucleotides, however, in the case of guanine nucleotides they found that bromination of the 8-position of guanine ring was always a major side reaction, and this led to serious difficulties in isolating the desired product. However, when they used N-chlorosuccinimide instead of N-bromosuccinimide [β-^{17}O]GTP was obtained in 57% yield. To prepare the [β-^{18}O]GDP, **20**, needed for our studies we adapted this method (Scheme 27) to convert the commercially available diphosphate, [β-S]GDP to [β-^{18}O]GDP (**20**) in 54% yield. The small amount of GMP impurity (Figure 11) did not affect the Raman studies.



20

Scheme 27.

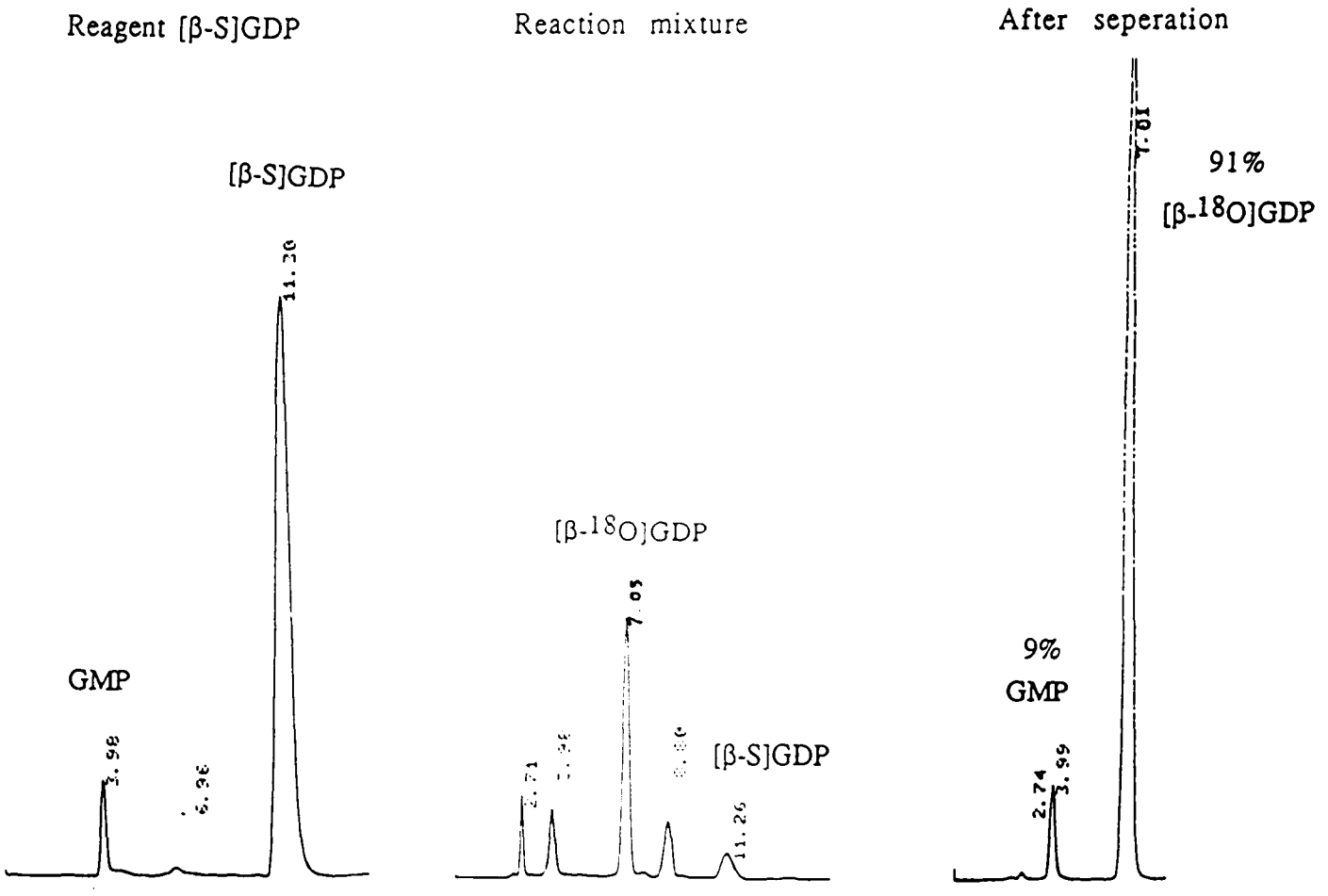
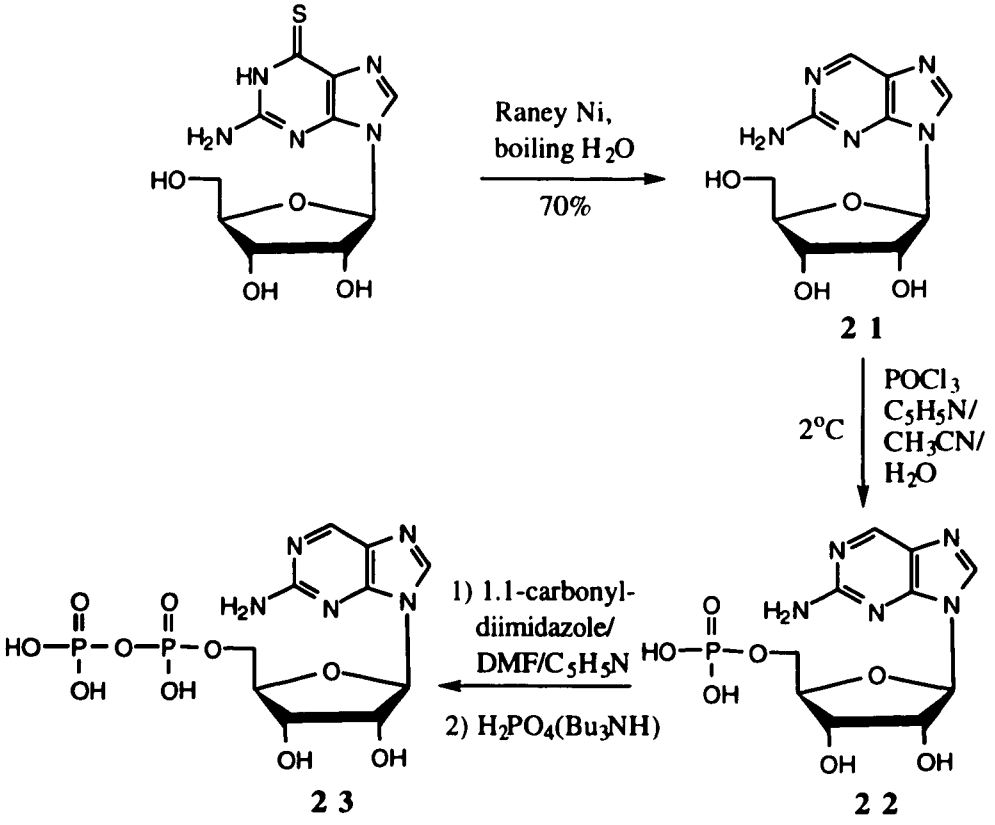


Figure 11. HPLC analysis of the [β-S]GDP and the reaction mixture containing **20** obtained from the N-chlorosuccinimide/$H_2{}^{18}O$ reaction according to Scheme 27, and purified **20.**

2.3 6-H-GDP.

Raman studies[116] using compound [6-^{18}O]GDP (**18**) and [6-S]GDP permitted to assign crucial vibration modes of the protein-bound nucleotide. In order to complement the information obtained concerning the crucial H-bonding locus at position 6 of the guanine ring, synthesis of [6-H]GDP was also necessary. Since recently 2-amino-6-mercapto-9-β-ribofuranosylpurine ("6-thioguanosine") became commercially available, its reduction with Raney nickel to the [6-H]guanosine,[117] **21**, monitored by UV (Figure 12) and subsequent phosphorylations afforded a simple route to the [6-H]GDP, **23**, (Scheme 28).



Scheme 28

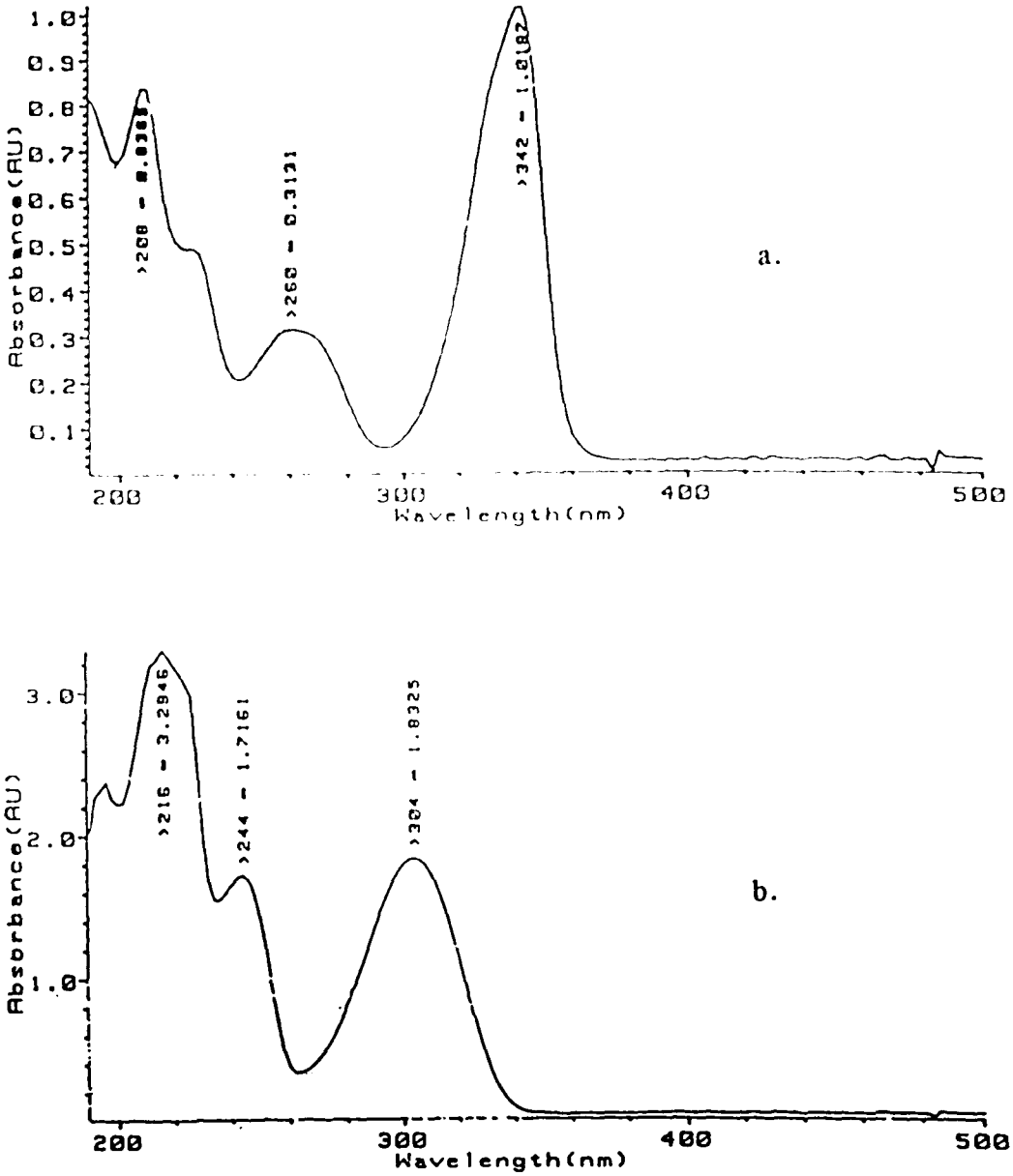
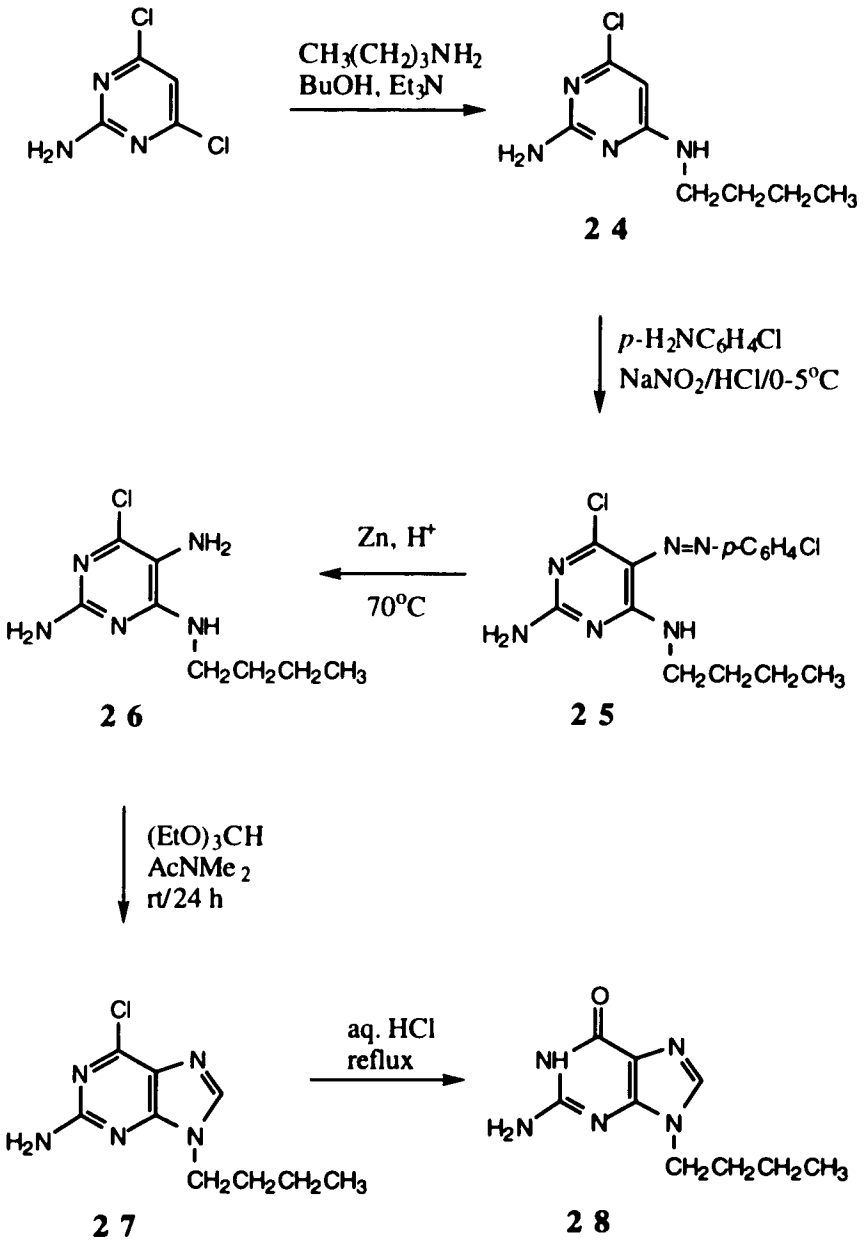


Figure 12. UV spectrum (a) of 6-thio-guanosine and (b) [6-H]guanosine, **21.**

2.4 9-Alkyl substituted guanines.

N-9 alkylation of purines serving as guanine precursors is the principal route to pharmaceutically important acyclic nucleoside analogues, e. g. the guanine based antiviral compounds acyclovir, gancyclovir and pencyclovir,[118] but these reactions are rarely regiospecific. In particular the alkylation of 2-aminopurines usually gives rise to a mixture of N-9 and N-7 derivatives[119], the separation of which requires tedious chromatography or fractional crystallization.

The method that we chose to prepare N-9-alkyl guanine involved guanine ring synthesis. Condensation of 2-amino-4,6-dichloropyrimidine and butylamine in refluxing 1-butanol in presence of triethylamine[120-122] afforded 2-amino-4-(butylamino)-6-chloro-pyrimidine, **24**. The intermediate 2-amino-4-(butylamino)-5-(*p*-chlorophenylazo)-6-chloropyrimidine, **25**, was obtained in good yield (90%) by reaction of *p*-chlorobenzenediazonium chloride with 2-amino-4-(butylamino)-6-chloropyrimidine.[123,124] Subsequent reduction of **25** with zinc in acetic acid afforded the 2,5-diamino-4-(butylamino)-6-chloropyrimidine, **26**. Condensation of the latter with triethyl orthoformate in dimethylacetamide afforded the 9-butylguanine derivative **27** that by treatment with aqueous hydrochloric acid was converted to target compound **28** (Scheme 29).


Cl
N
H2N
N
Cl
CH3(CH2)3NH2
BuOH, Et3N
NH
CH2CH2CH2CH3
24
p-H2NC6H4Cl
NaNO2/HCl/0-5°C
NH2
Zn, H+
70°C
N=N-p-C6H4Cl
26
25
(EtO)3CH
AcNMe2
rt/24 h
aq. HCl
reflux
O
27
28

Scheme 29.

2.5 CONCLUSION

Among all the synthetic methodologies available to obtain nucleoside and nucleotide analogs, those of the guanine-based compounds have least been investigated. Further, despite the importance of the guanine ring both from the synthetic and biological points of view, the syntheses which were available were lengthy and overall yields low. The procedures we report here for the preparation of selectively deuterated and ^{18}O-labelled compounds, [8-^{2}H]GDP, [8-^{2}H]GTP, [8-^{2}H]GMPPCP, and [6-^{18}O]GDP, are short and efficient. Our method of phosphate catalyzed exchange of the 8-proton was accomplished in high yields, and [6-^{18}O]GDP was synthesized in three steps with excellent overall yield. Thus, the commercially available (-)-2-amino-6-chloropurine riboside was converted to [6-^{18}O]guanosine followed by chemical and enzymatic phosphorylations. The enzymatic step converted the mono- to the diphosphate in 80% yield. Syntheses of [β-^{18}O]GDP and [β-$^{18}O_4$]GDP, [6-H]GDP, and 9-butylguanine were accomplished with equal efficiency by employing a combination/adaptation of known methodologies.

The Raman data obtained using these labelled compounds/analogs allowed unambiguous assignment of several important vibrational modes, leading to complete characterization of the vibrational frequencies of the guanine, and guanosine and its phosphates. Further, the labelled compounds incorporated in the proteins allowed important insights on the dynamic binding structures in EF-Tu and p21 proteins[80].

3. EXPERIMENTAL

General techniques.

Nuclear magnetic resonance (^1H and ^{13}C) spectra were recorded on a Bruker NR-300 MHz instrument. Chemical shifts are given as δ values from tetramethylsilane. Ultraviolet-visible spectra were recorded on a Hewlett-Packard HP UV 8452A fast scan UV/VIS diode array spectrophotometer. Infrared spectra were measured on a Perkin-Elmer 247 grating infrared spectrophotometer as KBr pellets. Electron impact, chemical ionization and fast atom bombardement mass spectra (EI-MS, CI-MS and FAB-MS) were obtained with a Finnigan Mat SSQ-70 instrument. High performance liquid chromatography (HPLC) was performed by using a Waters 6000A liquid chromatography equipped with Model 440 Absorbance UV/VIS detector. Reagents, solvents and buffers were obtained from Aldrich and Sigma Chemical Companies, unless otherwise stated. Raman spectra were run by Dr. R. H.. Callender's group in the Department of Physics at City College. The isotopic purity of the deuterated materials was determined by the intensity of the expected proton resonance signals.

[8-^{2}H]Guanosine 5'-diphosphate (10b).

A solution of GDP (trisodium salt, 10 mg) and Na_2DPO_4 (25 mg) in D_2O (1 ml) was heated for 5 h at 90-95°C in a capped reactivial with stirring. After cooling to rt, the solution was taken to dryness at rt under reduced pressure, and the residue was dissolved in H_2O. The

last two steps were repeated 4 times to exchange the imino, amido, and hydroxyl deuteriums with protons, after which the products were dried at rt *in vacuo*. The mixture obtained contained 90% GDP and 10% GMP with each over 90% deuterium at the 8-position, as determined by HPLC analysis and ^1H nmr respectively. The product was purified by passing it through a P-2 column (Bio-Rad, Richmond, CA; 1.5 cm x 35 cm) in distilled H_2O. The purity of the product was ascertained by HPLC on a Waters Radial-Pak C_{18} reverse phase column using a mobile phase consisting of 5 mM tetrabutylammonium bromide and 5% CH_3CN in 50 mM potassium phosphate buffer (pH 6.5).

[8-^{2}H]Guanosine 5'-triphosphate (10c).

A solution of GTP (trisodium salt, 10 mg) and Na_2DPO_4 (10 mg) in D_2O (1 ml) was heated for 5 h at 90-95°C in a capped reactivial with stirring. After cooling to rt, the reaction mixture was analyzed by HPLC (Waters RCM Radial-Pak C_{18} cartridge column), and was shown to contain 60% GTP, 35% GDP and 5% GMP. The mixture was separated on a DEAE-Sephadex A-25 (2.5 x 15 cm) column eluting with a linear gradient of 100-600 mM triethylammonium bicarbonate (1 L each), at 4°C, after which the deuterated products were dried at rt under reduced pressure. The extent of deuteration at the 8-position was determined to be >95% based on the ^1H NMR spectrum. The purity of the products was ascertained by HPLC on a C_{18} reverse phase column employing a mobile phase consisting of 5

mM tetrabutylammonium bromide and 5% CH_3CN in 50 mM potassium phosphate buffer (pH 6.5).

[8-^{2}H]Guanosine 5'-monophosphate (14).

A solution of GMP (disodium salt, 10 mg) in D_2O (1 ml) was heated for 8 h at 95-100°C in a capped reactivial with stirring. The solution was evaporated to dryness at rt under reduced pressure; the residue was redissolved in H_2O. The last two steps were repeated 4 times to re-exchange the imino, amido, and hydroxyl deuteriums with protons, after which the product was dried at rt under vacuum. The extent of deuteration at the 8-position was essentially complete based on the ^{1}H NMR spectrum. The purity of the compound was confirmed by HPLC (Waters Radial-Pak C_{18} reverse phase column, using a mobile phase consisting of 5 mM tetrabutylammonium bromide in 50 mM potassium phosphate buffer containing 5% CH_3CN adjusted to pH 6.5).

[8-^{2}H]-β,γ-Methyleneguanosine-5'-triphosphate (15).

A solution of 5'-GMPPCP (lithium salt, 20 mg) and Na_2DPO_4 (50 mg) in D_2O (2 ml) was heated for 5 h at 90-95°C in a closed reactivial with stirring. After cooling to rt, analysis by HPLC (Waters RCM Radial-Pak C_{18} cartridge column eluting with a buffer of 5mM tetrabutylammonium bromide, 50mM phosphate and 5% acetonitrile at pH=6.4) showed that the reaction mixture contained 67% GMPPCP and 33% GMP. The mixture was separated on a DEAE-Sephadex A-25

(2.5 x 15 cm) column eluting with a linear gradient of 100-500 mM triethylammonium bicarbonate buffer (pH 8.5) (1 L each), at 4°C. The deuterated products were dried at rt under reduced pressure. The extent of deuteration at the 8-position was determined to be ca. 95% based on the ^1H NMR spectrum. The purity of the [8-^2H]5'-GMPPCP was confirmed by HPLC on a Waters RCM Radial-Pak C_{18} reverse phase column with a mobile phase consisting of 5 mM tetrabutylammonium bromide and 5% CH_3CN in 50 mM potassium phosphate buffer (pH 6.5).

[6-^{18}O]Guanosine (16).

(-)-2-Amino-6-chloropurine riboside (80 mg, 0.265 mmol) was mixed with 1N $Na^{18}OH$ (2 ml, prepared from sodium metal and 97% $H_2{}^{18}O$). The mixture was refluxed for 5 h, cooled to rt, and then was neutralized with dry HCl gas. The precipitate was filtered off and the water was recovered. The crude product was dissolved in 1N HCl and was treated with activated charcoal. After filtration, the solution was neutralized with 6N NaOH. The white precipitate was filtered off, washed three times with cold water, and dried under reduced pressure at 110°C to give 40 mg of **16** (53% yield).

λ_{max} (H_2O): 254 nm.

^1H nmr (DMSO-d_6):

δ3.55 (m, 2 H, $C_{5'}CH_2$), 3.85 (q, 1 H, $C_{4'}H$), 4.06 (q, 1 H, $C_{3'}H$), 4.38 (q, 1 H, $C_{2'}H$), 5.03 (t, 1 H, $C_{5'}OH$), 5.11 (d, 1 H, $C_{3'}OH$), 5.39 (d, 1 H, $C_{2'}OH$), 5.68 (d, 1 H, J=4.18Hz, $C_{1'}H$) 6.45 (br s, 2 H, NH_2), 7.92 (s, 1 H, C_3H), 10.61 (br s, 1 H, NH).

CI-MS (NH_3): *m/z* 286 $[M+H]^+$, 303 $[M+NH_4]^+$.

[6-^{18}O]Guanosine 5'-monophosphate (17).

To a mixture of freshly distilled phosphoryl chloride (50 µl 0.54 mmol), water (62 µl, 0.34 mmol), pyridine (47 µl, 0.59 µmol), and CH_3CN (122 µl, 2.30 mmol), maintained at 2°C, [6-^{18}O]guanosine (29 mg, 0.1 mmol) was added, and the mixture was stirred for 4 h. The reaction mixture was diluted with ice water (5 ml) and stirred for 1 h at 5°C. Analysis of an aliquot of the solution by HPLC (Waters RCM Radial-Pak C_{18} cartridge column eluting with a buffer of 5mM tetrabutylammonium bromide, 50mM phosphate buffer and 5% acetonitrile at pH=6.4) showed that about of the 67% [6-^{18}O]guanosine (**16**) was converted to [6-^{18}O]GMP (**17**).

The solvents were removed under reduced pressure at rt, the residue was dissolved in 1 ml of water, and the crude product was purified on a DEAE-Sephadex A-25 (2.5 x 25 cm) column eluting with a linear gradient of 100-500 mM triethylammonium bicarbonate buffer (1 L each) to give 21.9 mg of [6-^{18}O]GMP (54% yield).

λ_{max} (H_2O): 254 nm.

FAB-MS (glycerol): *m/z* 409 $[M^+]$(disodium salt), 410 $[M+1]^+$, 411 $[M+2]^+$; 386 $[M^+]$(monosodium salt); 365 $[M^+]$(free acid form).

[6-^{18}O]Guanosine 5'-diphosphate (18).

[6-^{18}O]GMP (20 mg, 0.049 mmol), ATP (117 mg, 0.21 mmol) and 6 ml buffer (50 mM Tris.HCl, 50 mM $MgCl_2$) were mixed, and the

pH was adjusted to 7.5. Guanylate kinase (0.35 ml, 5 units, from Sigma) was then added, and the solution was incubated for 30 min at 30°C, followed by 18 h at 5°C. The solvent was evaporated to dryness under reduced pressure. The crude product, dissolved in 0.1 M triethylammonium bicarbonate buffer (2 ml) pH 8.5, was purified on a DEAE-Sephadex A-25 (2.5 x 40 cm) column (bicarbonate form) eluting with a linear gradient of 100-700 mM triethylammonium bicarbonate buffer (2 L each) to give 29 mg of **18** in 80% yield. The purity of the product was ascertained by HPLC on a Waters RCM Radial-Pak C_{18} reverse phase column with a mobile phase consisting of 5 mM tetrabutylammonium bromide and 5% CH_3CN in 50 mM potassium phosphate buffer, pH 6.5.

FAB-MS (glycerol): *m/z* 511 ([M^+] (trisodium salt).

Guanosine 5'-diphosphate-β-$^{18}O_4$ (19).

GMP (free acid, 181 mg 0.5 mmol) was evaporated with pyridine (2 ml), then with tributylamine (150 µl), followed by evaporation with dry pyridine (2 x 2 ml). The sample was then dissolved in anhydrous DMF (5 ml) and was added to 1,1'-carbonyldiimidazole (100 mg, 0.62 mmol). After stirring at rt for 22 h under inert atmosphere, tributylammonium [$^{18}O_4$]phosphate (prepared from 1 mmol of PCl_3, $H_2{}^{18}O$, and tributylamine, see below) was added the to activated guanosine 5'-monophosphate, and the reaction mixture was stirred at rt for 17 h. The reaction mixture was diluted with methanol (20 ml) and evaporated to dryness under reduced pressure. The product was purified on a DEAE-Sephadex A-

25 (2.5 x 28 cm) column by eluting with a gradient of 2 L each of 100 and 600 mM triethylammonium bicarbonate buffer.

Tributylammonium [$^{18}O_4$]phosphate.

To a sample of $H_2{}^{18}O$ (0.2 ml, 1 mmol) in a reactivial, frozen with dry ice, PCl_5 (208 mg, 1 mmol) was added and the mixture was warmed up to rt in 2 min. The HCl was pumped off and tributylamine (357 ml, 1.5 mmol) was added. Excess solvents were removed at reduced pressure, and the sample was dried further by evaporation with anhydrous DMF (2 x 1 ml) under reduced pressure.

Guanosine 5'-diphosphate-β-^{18}O (20).

To a solution of GDP-β-S (trilithium salt, 20 mg, 42 μmol) in $H_2{}^{18}O$ (200 μl) and anhydrous dioxane (100 μl), a solution of N-chlorosuccinimide (26 mg, 200 μmol) in anhydrous dioxane (500 μl) was added, and the mixture was stirred for 20 min at rt. The reaction was quenched by adding 30 μl of triethylamine and 30 μl of 2-mercaptoethanol. The mixture was evaporated to dryness under reduced pressure, and the residue dissolved in H_2O (1 ml) was analyzed by HPLC (Waters RCM Radial-Pak C_{18} cartridge column eluting with 5 mM tetrabutylammonium bromide and 5% CH_3CN in 50 mM potassium phosphate buffer, pH 6.5). The HPLC assay showed the absence of the starting material, but the products also contained GMP (from the decomposition of GDP), and some additional unidentified impurities. Separation of the mixture on a DEAE-

Sephadex A-25 (2.5 x 15 cm) column by eluting with a linear gradient of 100-500 mM triethylammonium bicarbonate buffer (1 L each) gave 10.8 mg of pure **19** (52% yield).

FAB-MS (glycerol): *m/z* 489 [M^+] (disodium salt); 467 [M^+] (monosodium salt).

2-Amino-9-β-D-ribofuranosylpurine (21).

2-Amino-6-mercapto-9-β-ribofuranosylpurine ("6-thio-guanosine", 1.0 g, 3.3 mmol) dissolved in boiling water (50 ml) was treated with Raney nickel (ca. 2 g). After ca. 2 h, the reaction was complete as shown by the absence of an absorption maximum at 340 nm. The nickel catalyst was removed by filtration then was washed repeatedly with boiling water. The washings and the filtrate were combined, treated with charcoal, filtered, and concentrated in vacuum to a syrup which was then dissolved in hot ethanol and was reconcentrated. The syrupy residue was azeotroped with benzene repeatedly until 0.59 g of **21** a yellow amorphous solid, was obtained (65% yield).

λ_{max} (H_2O): 304 nm.

^1H nmr (DMSO-d_6): δ 3.59 (m, 2 H, C5'CH_2), 3.88 (q, 1 H, J=3.6 Hz, C4'H), 4.10 (t, 1 H, J=4.2 Hz, C3'H), 4.49 (t, 1 H, J=5.4 Hz, C2'H), 5.0-5.5 (m, 3 H, C5'OH, C3'OH and C2'OH), 5.82 (d, 1 H, J=6.0 Hz, C1'H), 6.56 (br s, 2 H, NH_2), 8.28 (s, 1 H, C3H), 8.57 (s, 1 H, C6H).

Guanosine 5'-monophosphate-6-H (6-H-GMP) (22).

To a stirred mixture of freshly distilled phosphoryl chloride (192 µl, 2.2 mmol), water (25 µl, 1.4 mmol), pyridine (194 µl, 2.4 mmol), and CH_3CN (500 µl, 9.45 mmol), 2-Amino-9-β-D-ribofuranosylpurine ("6-H-guanosine", 133.5 mg, 0.5 mmol) was added at 2°C, and stirring was continued for 4 h. The reaction mixture was poured into ice water and stirred for another 1 h at 5°C. Analysis of an aliquot of the resulting solution by HPLC (Waters RCM Radial-Pak C_{18} reverse phase column) showed that almost all of the starting material was converted to 6-H-GMP. The product was separated on a DEAE-Sephadex A-25 (2.5 x 15 cm) column eluting with a linear gradient of 2 L each of H_2O and 500 mM triethylammonium bicarbonate buffer. After solvent removal, 247 mg of 6-H-GMP was obtained as the triethylammonium salt (90% yield).

Guanosine 5'-diphosphate-6-H (23).

6-H-GMP (triethylammonium salt, (247 mg, 0.45 mmol) was evaporated with pyridine (2 ml), tributylamine (100µl), and finally with dry pyridine (2 ml). Then it was dissolved in anhydrous DMF (5 ml) and 1,1'-carbonyldiimidazole (100 mg) was added. The reaction mixture was stirred for 20 h at rt under an inert atmosphere. Tributylammonium phosphate (402 mg, 1 mmol) was added and the mixture was stirred for another 16 h at rt. The solvents were removed at reduced pressure, and the crude product was purified on a DEAE-Sephadex A-25 (2.5 x 15 cm) column eluting with a linear gradient of 100-500 mM triethylammonium bicarbonate buffer (1 L

each) to give 118 mg (0.16 mmol) of **23** in 36% yield. The purity of 6-H-GDP was checked by HPLC on a Waters RCM Radial-Pak C_{18} reverse phase column with a mobile phase consisting of 5 mM tetrabutylammonium bromide and 5% CH_3CN in 50 mM potassium phosphate buffer, pH 6.5.

FAB-MS (glycerol): *m/z* 493 $[M]^+$ (trisodium salt); 471 $[M]^+$ (di-sodium salt); 450 $[M+H]^+$ (monosodium salt).

2-Amino-4-(butylamino)-6-chloropyrimidine (24).

A solution of 2-amino-4,6-dichloropyrimidine (Sigma, 9.3 g, 57 mmol), butylamine (4.24 g, 58 mmol), and triethylamine (6 g, 59 mmol) in 1-butanol (130 ml) was heated under reflux for 48 h. The volatile materials were removed under reduced pressure and the brownish oily residue was washed with water (80 ml), and then was dissolved in MeOH (200 ml), water was added until a white precipitate was formed. This mixture was allowed to stand overnight at rt, the white solid which had formed was filtered off under suction and was washed with cold water (3 x 5 ml), dried under vacuum, to give the product, 2-amino-4-(butylamino)-6-chloropyrimidine, **24**, (9.4 g, 77% yield).

Mp. 137-138°C.

^1H nmr (DMSO-d_6): δ 0.87 (t, 3H, J=7.3 Hz, CH_3), 1.27 (m, 2H, J=7.4 Hz, CH_2), 1.43 (m, 2H, J=7.3 Hz, CH_2), 5.70 (s, 1H, Ar*H*), 6.36 (s, 2H, NH_2), 7.07 (s 1H, N*H*).

2-Amino-4-(butylamino)-5-(*p*-chlorophenylazo)-6-chloro-pyrimidine (25).

A solution of 2-amino-4-(butylamino)-6-chloropyrimidine (4.8 g, 23 mmol) in acetic acid (105 ml) and water (100 ml), containing sodium acetate trihydrate (42 g), was cooled to 5-10°C and a cold solution (5-10°C) of *p*-chlorobenzenediazonium chloride [prepared by diazotation from *p*-chloroaniline (3.2 g, 25 mmol), conc. HCl (7.2 ml), water (25 ml), and a solution of sodium nitrite (1.9 g, 28 mmol) in water (25 ml)] was added dropwise in 30 min. The mixture was stirred at rt for 48 h, the yellow crystalline product was vacuum filtered, and washed with cold water (3 x 5 ml), and dried at 50°C under reduced pressure to give **25** (7.3 g, 90% yield).

Mp. 199-201°C

^{1}H nmr (DMSO-d$_6$): δ 0.88 (t, 3H, J=7.3 Hz, C*H*$_3$), 1.26 (m, 2H, C*H*$_2$), 1.44 (m, 2H, C*H*$_2$), 3.28 (m, 2H, NHC*H*$_2$-), 6.70 (s, 2H, N*H*$_2$), 7.80-8.40 (m, 4H, C$_6$*H*$_4$).

2,5-Diamino-4(butylamino)-6-chloropyrimidine (26).

To a suspension of 2-amino-4-(butylamino)-5-(*p*-chlorophenyl azo)-6-chloro-pyrimidine (3.8 g, 0.011 mol) in 50% aqueous EtOH (200 ml), containing glacial acetic acid (10 ml) heated to 75°C under an inert atmosphere, activated zinc dust (8.0 g) was added in small portions over a period of 30 min. A light yellow solution resulted and the stirring was continued at 75°C for an additional 4 h. The solid material was removed by filtration, the filtrate combined with the

washings was evaporated under reduced pressure. The residue was chromatographed on a Si gel column eluting with CH_2Cl_2:EtOH (95:5) to afford the title compound **26** (1.7 g, 67% yield).

Mp. 176-179°C.

^1H nmr (DMSO-d_6): δ 0.97 (t, 3H, J=7.3 Hz, C*H*$_3$), 1.30 (m, 2H, C*H*$_2$), 1.55 (m, 2H, C*H*$_2$), 3.80 (t, 2H, J=7.3 Hz, NHC*H*$_2$), 5.90 (s, 2H, N*H*$_2$), 6.95 (s, 2H, N*H*$_2$).

2-Amino-6-chloro-9-butyl-purine (27).

To a solution of 2,5-diamino-4-(butylamino)-6-chloropyrimidine (400 mg, 1.7 mmol) in anhydrous N,N-dimethylacetamide (20 ml) cooled to 0°C, anhydrous triethyl orthoformate (20 ml, 120 mmol) and conc. HCl (1.2 ml) were added and the mixture was stirred overnight at rt. After evaporation to dryness under reduced pressure, the residue was stirred in 50% aqueous acetic acid (50 ml) for 4 h. After evaporation to dryness and several coevaporations with methanol (50 ml each), the syrup obtained was stirred in 5% ammonia in methanol (50 ml) for 14 h and was again evaporated to dryness. The product was purified by column chromatography on Si gel eluting with CH_2Cl_2:EtOH (95:5) to yield 374 mg of **27** (90% yield).

M.p. 143-145°C.

^1H nmr ($CDCl_3$): δ 0.93 (t, 3H, J=7.3 Hz, C*H*$_3$), 1.32 (m, 2H, C*H*$_2$), 1.80 (m, 2H, C*H*$_2$), 4.05 (t, 2H, J=7.2 Hz, N-C*H*$_2$), 5.36 (s, 2H, N*H*$_2$), 7.77 (s, 1H, purine C*H*).

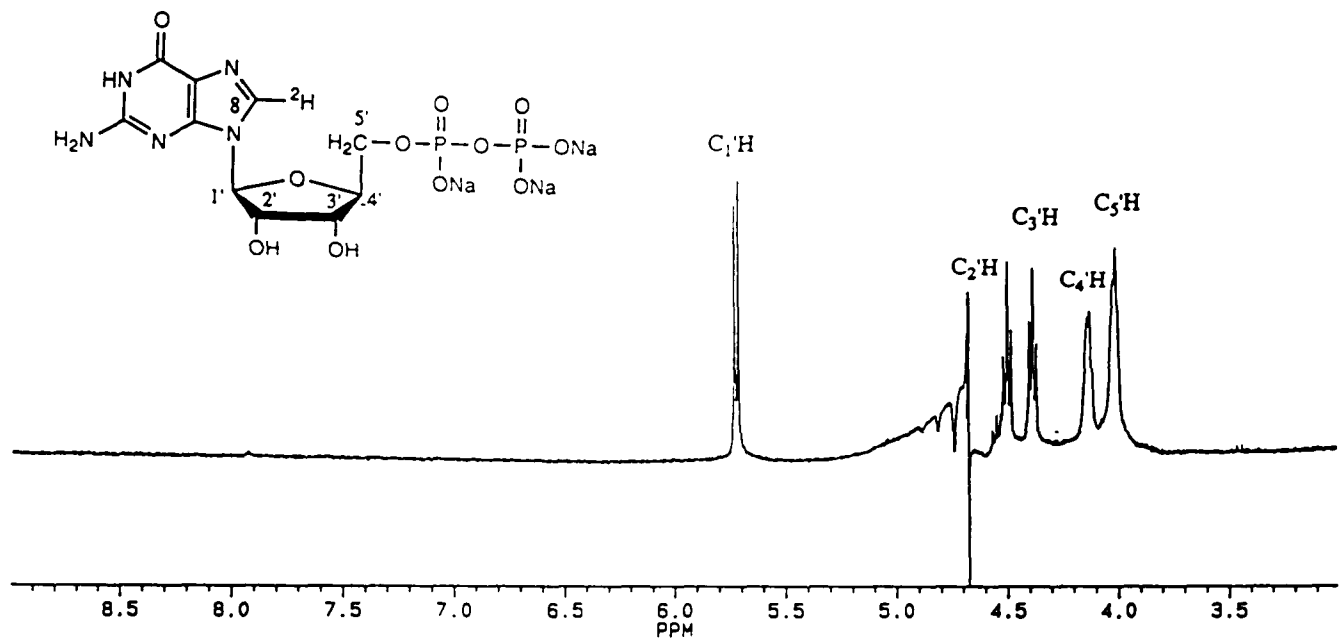
9-Butylguanine (28).

A solution of 2-amino-6-chloro-9-butyl-purine (150 mg, 0.8 mmol) in 1 N HCl (15 ml) was refluxed for 6 hours. Evaporation to dryness followed by coevaporation with ethanol gave a residue, which was dissolved in water (3 ml) and neutralized with 6N NaOH. The crystalline precipitate was filtered off, washed three times with cold water, and dried under reduced pressure at rt to yield 116 mg of **30** (77% yield).

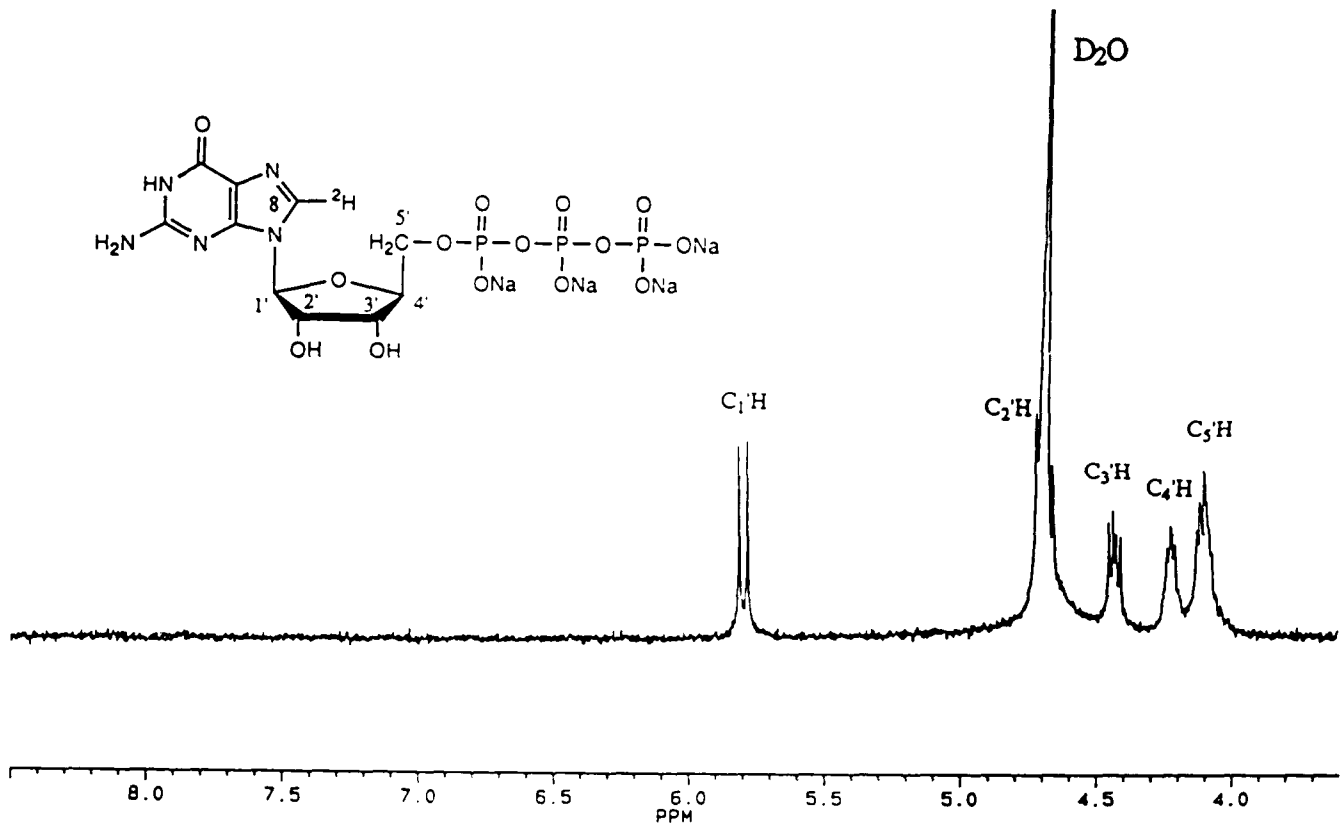
M.p. >300°C

^{1}H nmr (DMSO-d$_6$): δ 0.87 (t, 3H, J=7.3 Hz, C*H*$_3$), 1.22 (m, 2H, J=7.4 Hz, CH$_2$), 1.68 (m, 2H, J=7.3 Hz, C*H*$_2$), 3.90 (t, 2H, J=7.1 Hz, NC*H*$_2$), 6.46 (s, 2H, N*H*$_2$), 7.67 (s, 1H, purine C*H*) 10.52 (s, 1 H, N*H*).

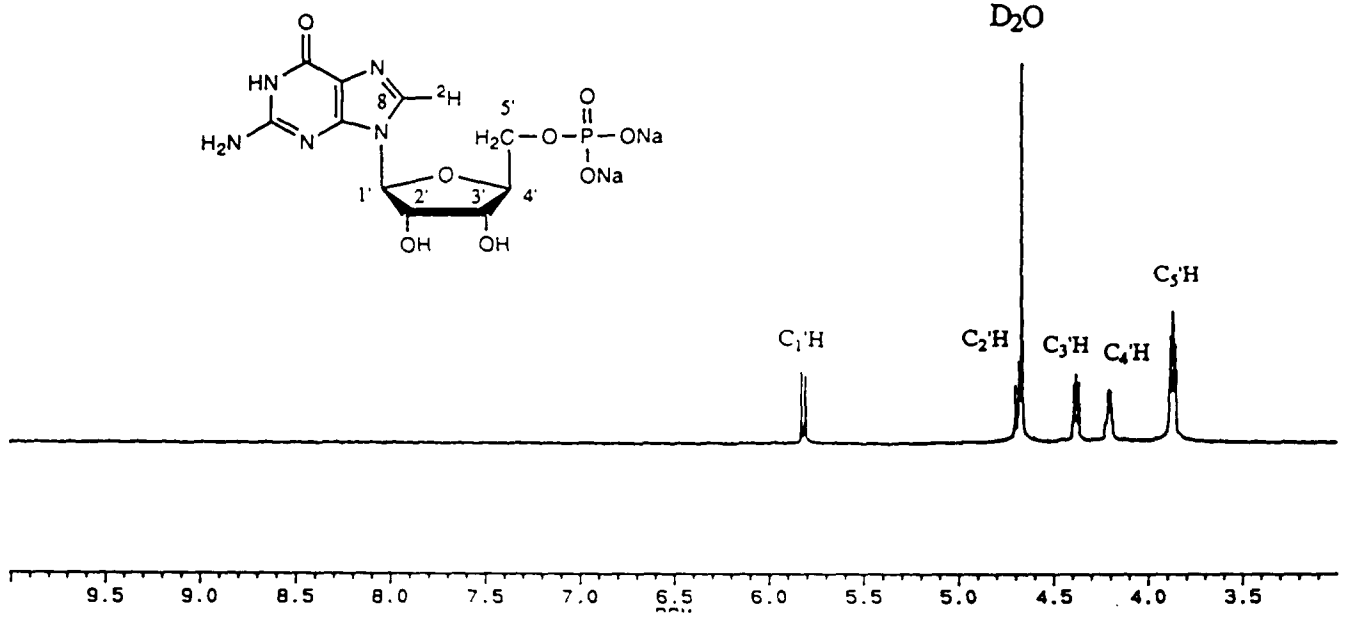
EI-MS: *m/z* 207[M]$^+$, 164[M-CH$_2$CH$_2$CH$_3$]$^+$, 151[M- CH$_2$CH$_2$CH$_2$CH$_2$]$^+$.



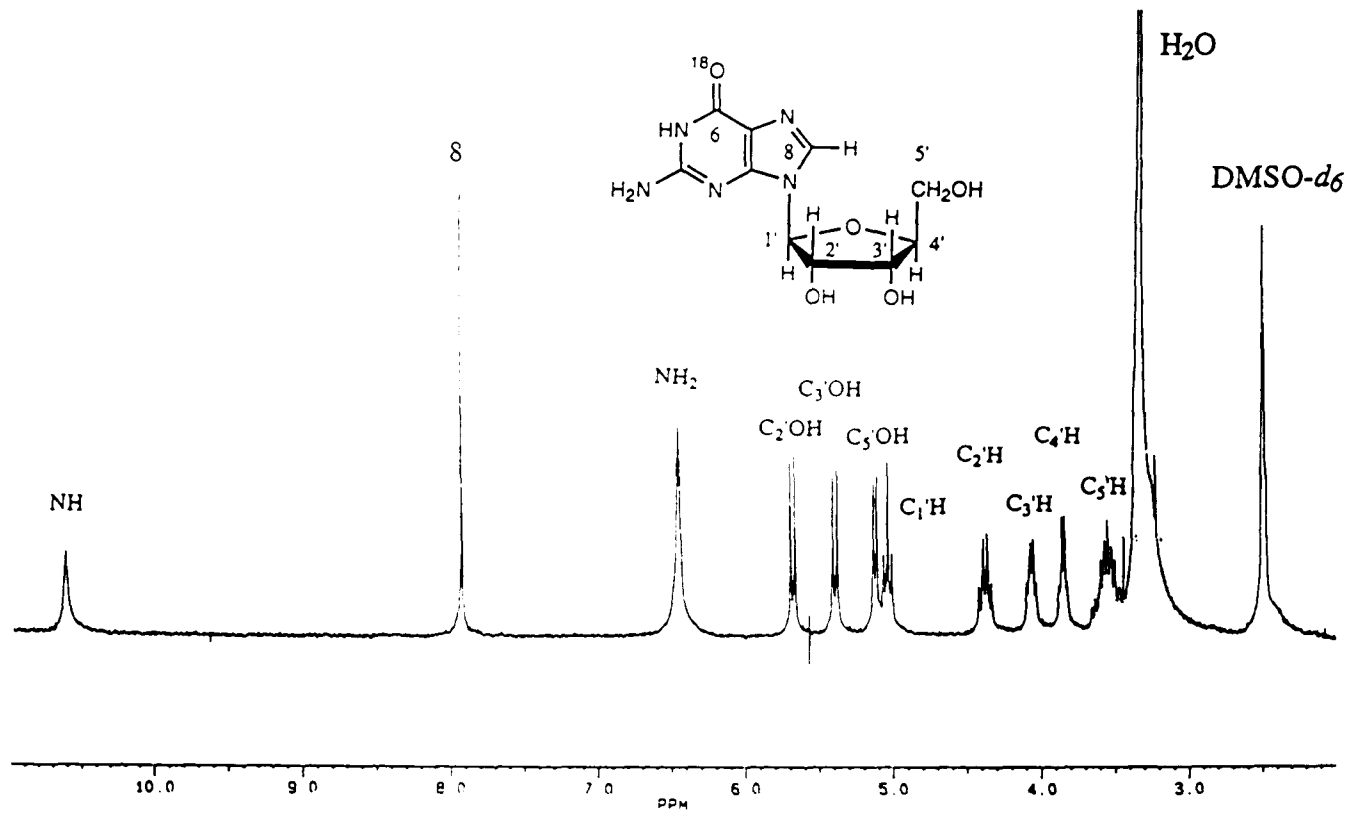
^{1}H nmr spectrum of [8-^{2}H]GDP (**10b**) in D_2O.



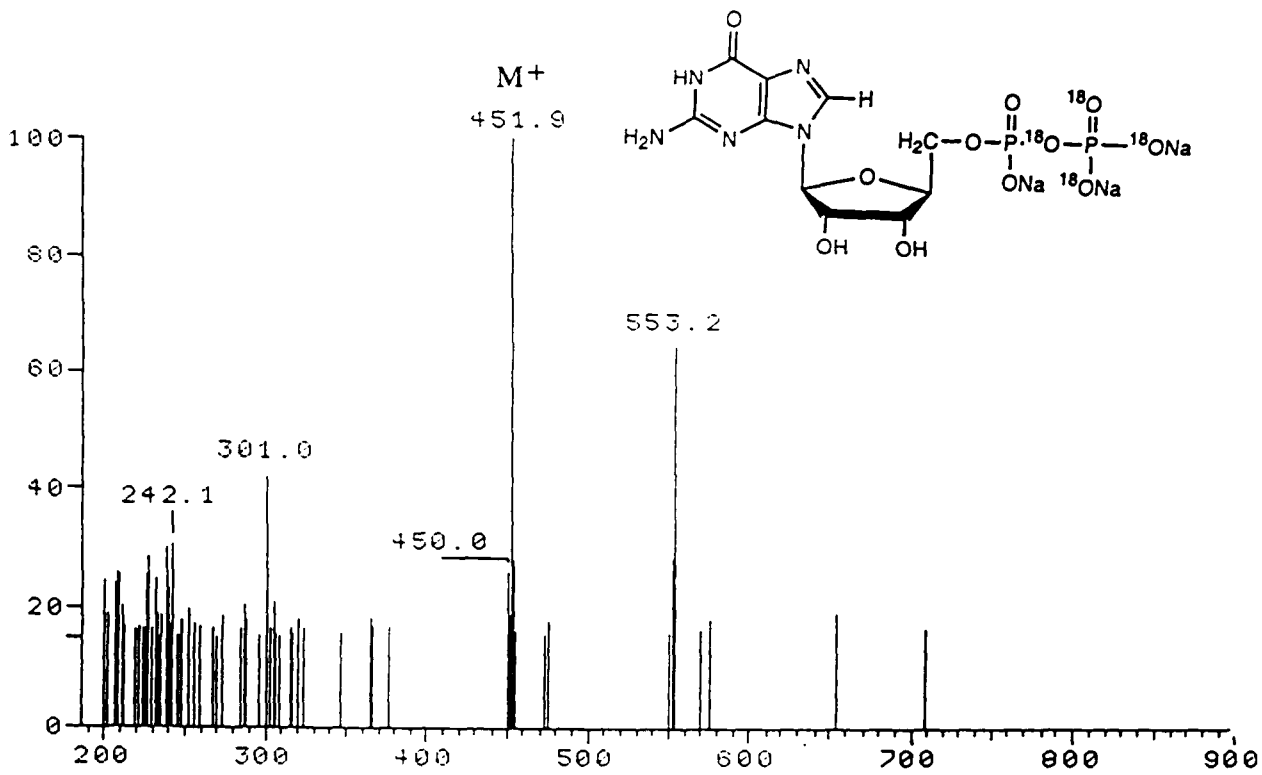
^{1}H nmr spectrum of [8-^{2}H]GTP (**10c**) in D_2O.



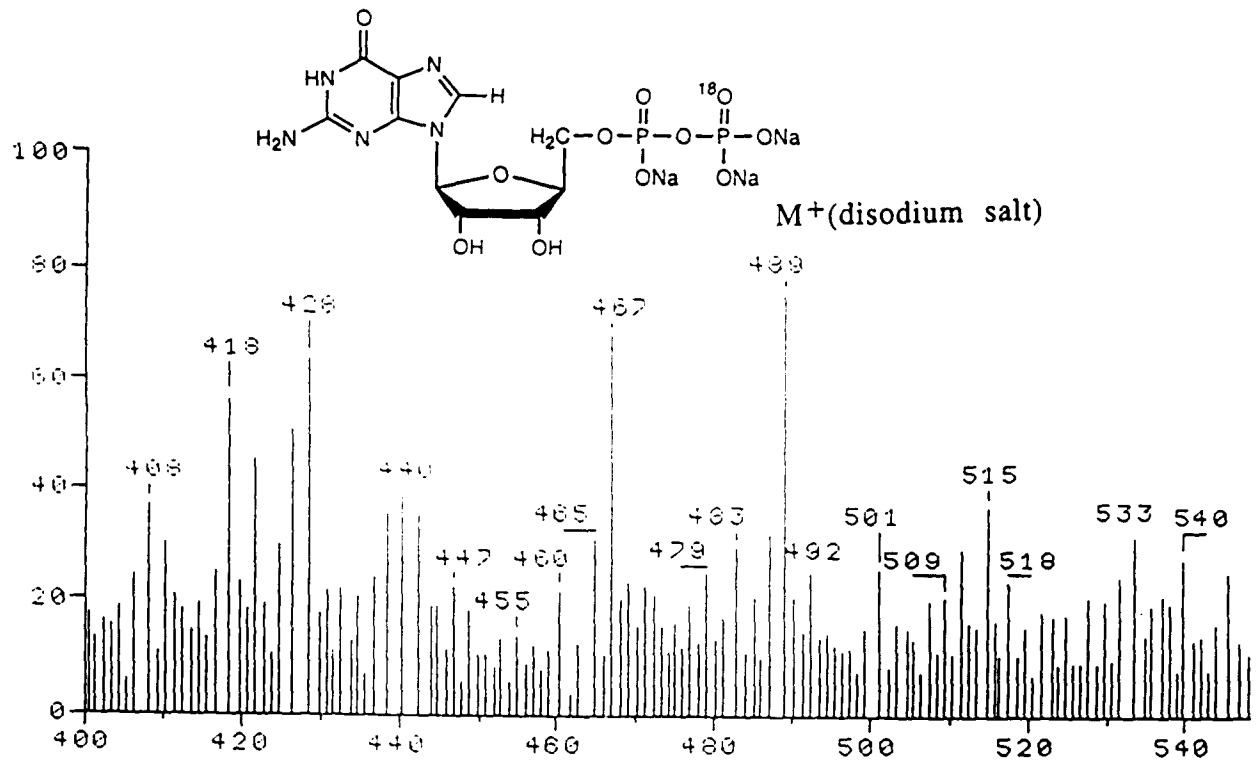
^{1}H nmr spectrum of [8-^{2}H]GMP (**14**) in D_2O.



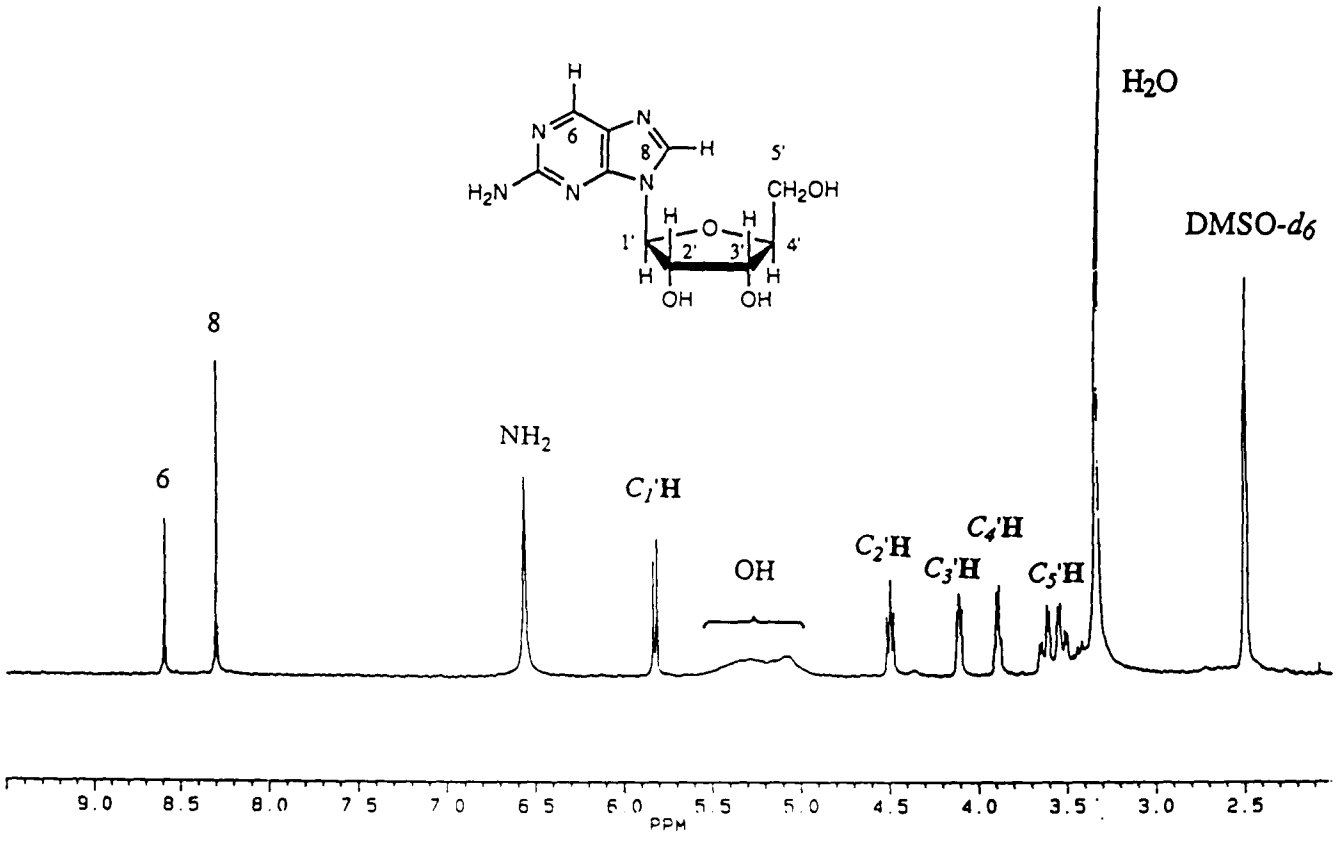
^1H nmr spectrum of [6-^{18}O]Guanosine (**16**) in DMSO-d_6.



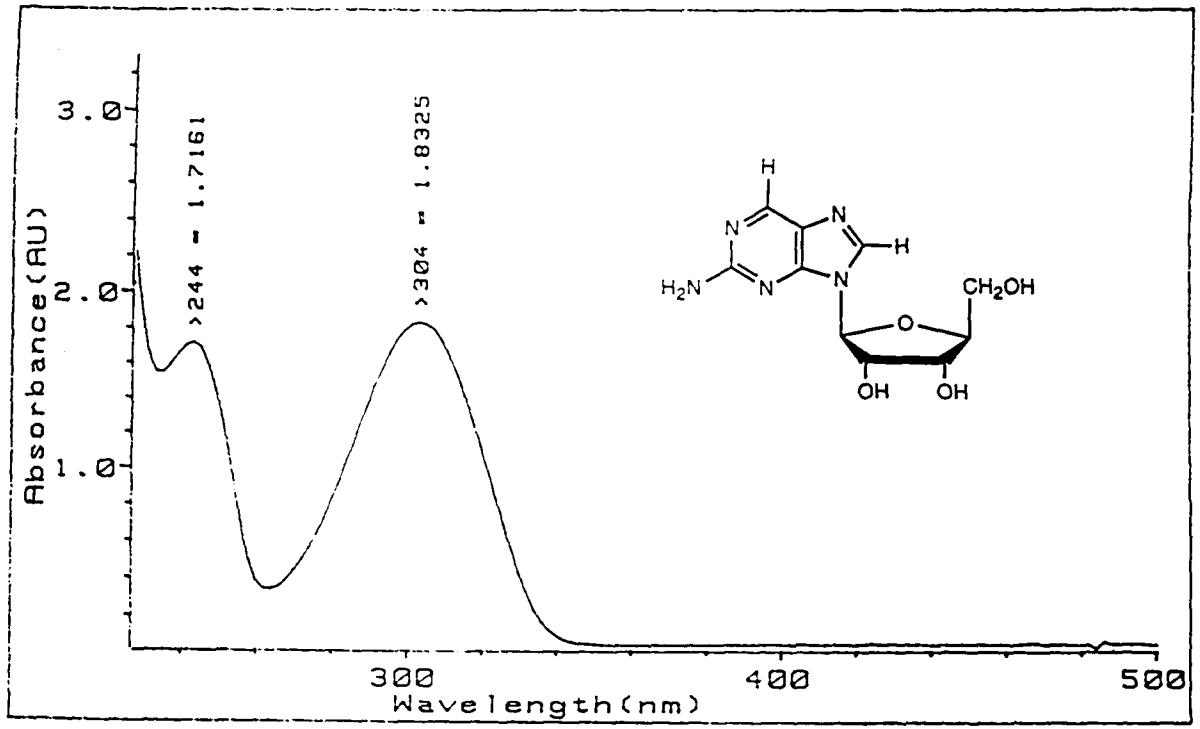
FAB-MS (glycerol) spectrum of [β-$^{18}O_4$]GDP (**19**).



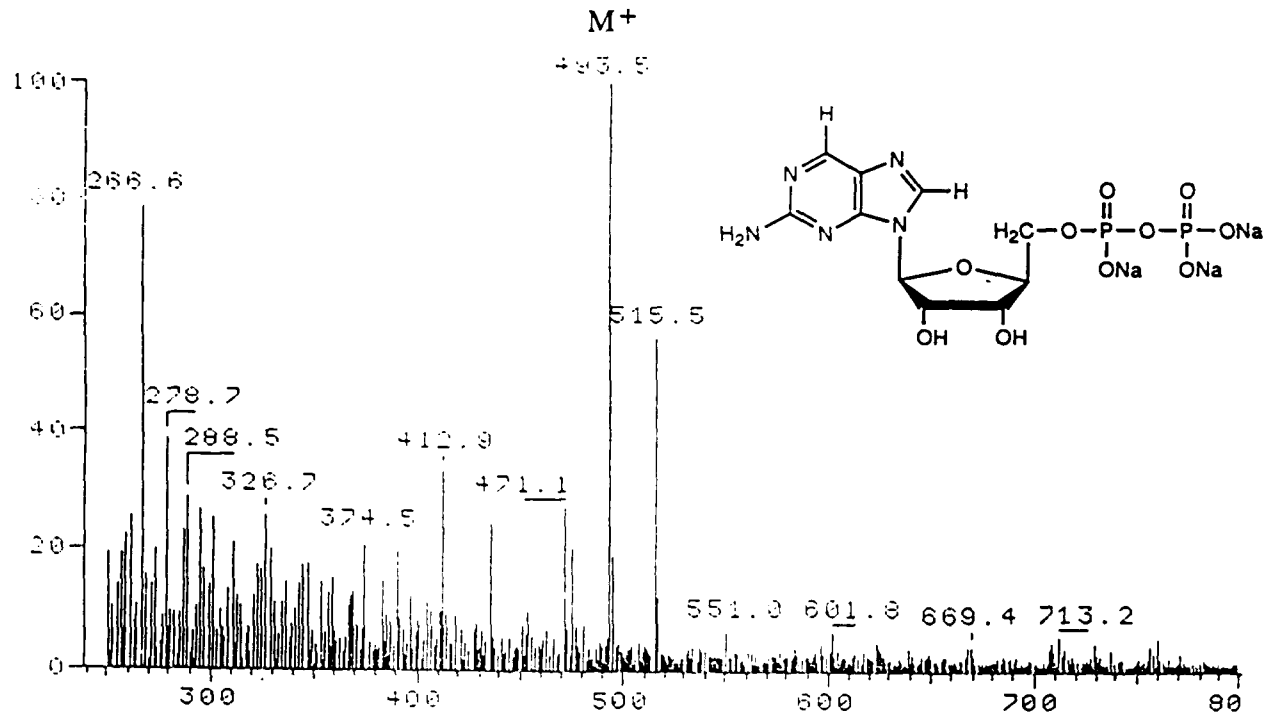
FAB-MS (glycerol) spectrum of [β-^{18}O]GDP (**20**).



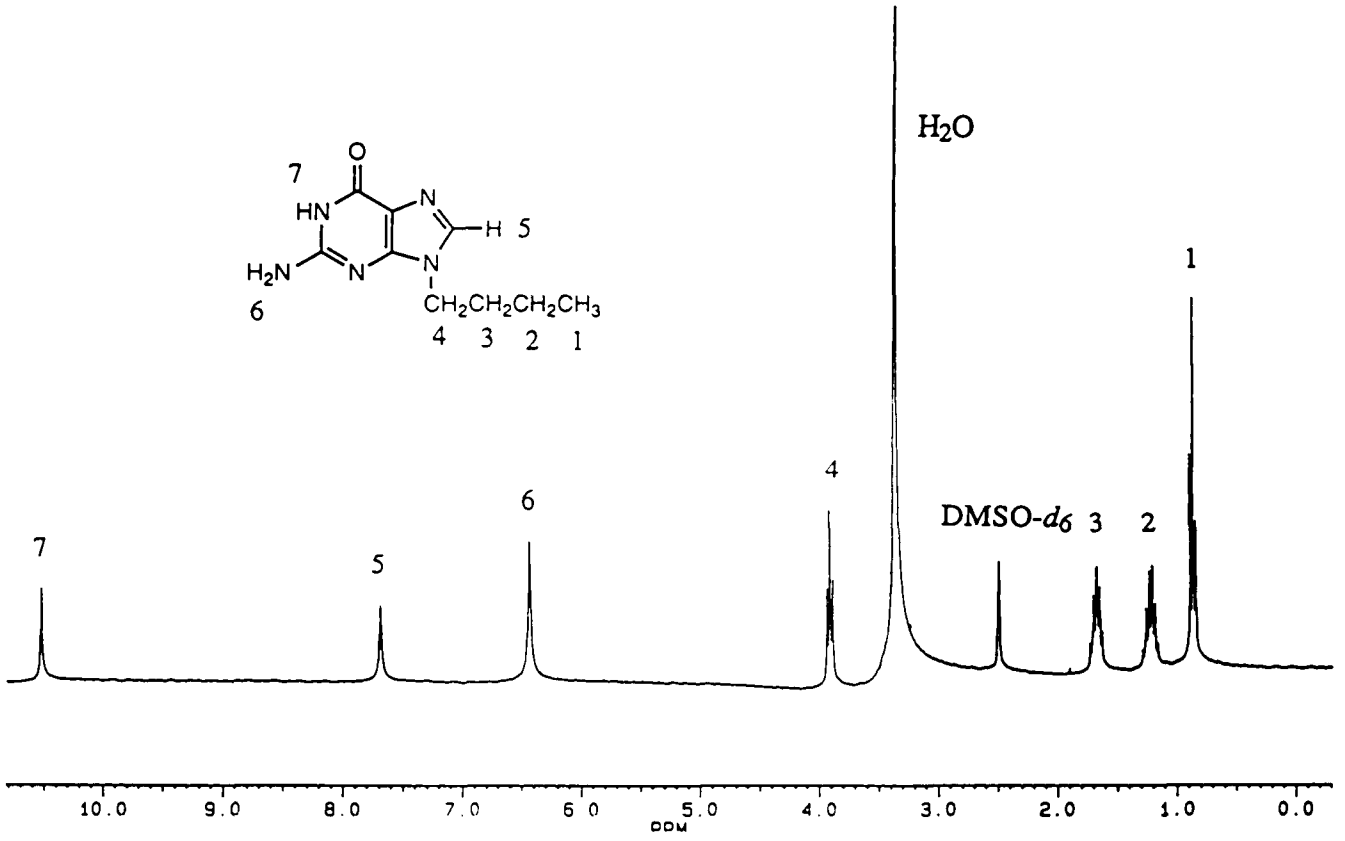
^{1}H nmr spectrum of 2-amino-9--D-ribofuranosylpurine (**21**) in DMSO-d_6.



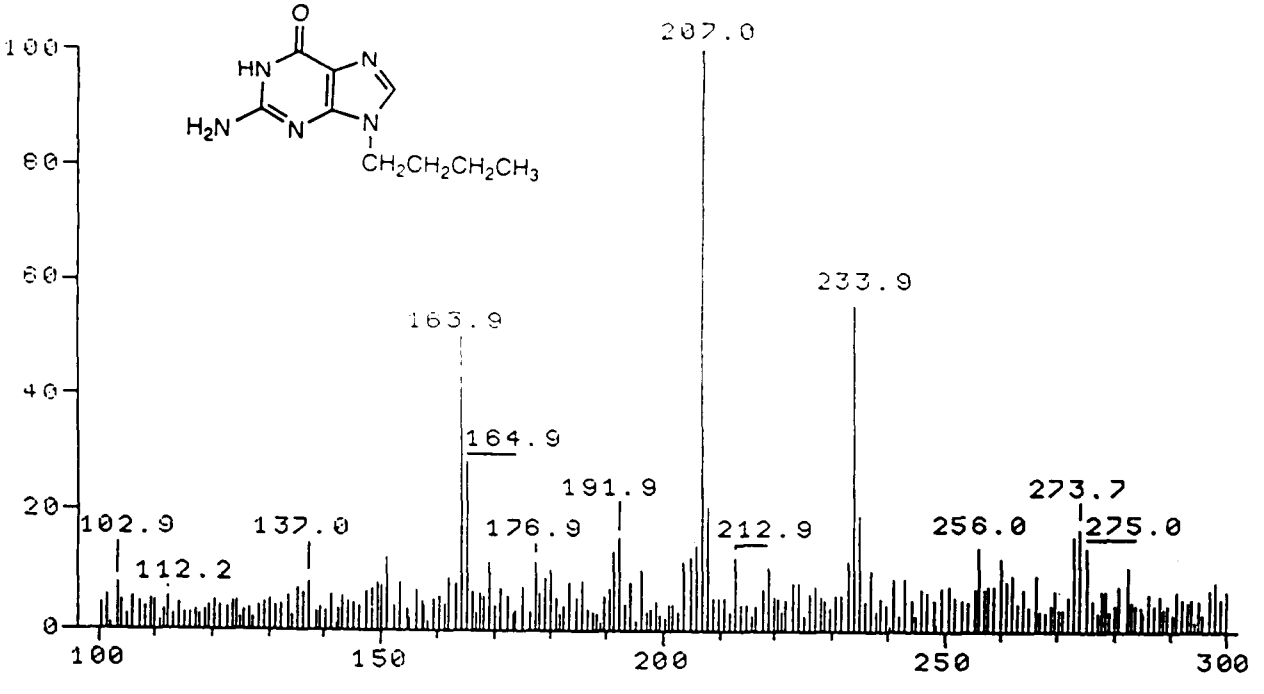
UV spectrum of 2-Amino-9-β-D-ribofuranosylpurine **(21)** in H_2O.



FAB-MS spectrum of [6-H]GDP (**23**).



^{1}H nmr spectrum of 9-butylguanine (**28**) in DMSO-d_6.



EI-MS spectrum of 9-butylguanine (**28**).

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