STUDIES TOWARD A CHIRAL, CHARGE SEPARATED, WATER SOLUBLE CYCLOPHANE

By

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ABSTRACT

TRZEBINSKA-YAGER, DANUTA Studies Toward a Chiral, Charge Separated, Water Soluble Cyclophane.

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It has been suggested that the key issue in the structure-function correlation in enzyme catalysis is electrostatic stabilization of the ionic transition states[7]. In order to mimic receptors in biological systems we designed a novel hydrophilic macrocycle, **10**, with an electrostatic dipole across its hydrophobic cavity. The dipole moment would be established between a positively charged Fe(II)-terpyridyl complex on one end of the macrocycle and the two carboxylate substituents at the opposite end of the receptor.

An experimental strategy based on a convergent synthesis is presented. The negative end of the macrocycle, designed "CAP" (**16**), is generated in three steps from salicylic acid (**12**) in a 10% overall yield. The positive end, an Fe(II) bound in a terpyridine ligand is generated in four steps from 2-acetyl-pyridine (**17**) in a 10% overall yield. The reaction of **16** with the terpyridine moiety **22** in the presence of NaH (in THF) failed to generate the expected ether linkages. Suggestions are presented for a different method of making the desired ether linkage between the **16** and the terpyridine moiety. The final macrocyclization step using Fe(II) is suggested. Potential substrates for binding and orientation studies are suggested.

Acknowledgment

I would like to thank Professor James C. Adrian for his guidance and support throughout my research work in his laboratory.

I am deeply thankful to the entire Chemistry Department at Union College which made my undergraduate study a challenging, but enjoyable experience.

I am very thankful to my parents, husband and friends who gave me all support and love that I needed.

" Our life must contain mountains or marathons or their equivalents, else we will not be sure we have reached our potential. The person who descends from a mountain is not the same person who began the ascent."

George Sheehan

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1. Introduction to Bioorganic Chemistry.

Bioorganic chemistry is a discipline essentially concerned with the application of the tools of organic chemistry to the understanding of biochemical processes. This understanding can often be achieved with the aid of molecular models chemically synthesized in the laboratory. Biomodels have several advantages over biological systems. One of them refers to the fact that they are structurally simpler, and therefore allow studying one type of interaction at time ("sorting out" of many variable parameters simultaneously operative within the biological system).[1] Biomimetic chemistry is not limited only to the mimicry of living systems for its better understanding.[1] It can potentially lead to new structures, new types of catalysts, and to synthetic transporters capable of functions other than those seen in biological systems, but with comparable selectivity and efficiency.

A general strategy that scientists use to construct bioorganic models of biological systems combines two distinct but interconnected chemical levels. On one level, a bioorganic specific receptor is constructed from classical organic synthesis.[1] Such a receptor will be used to recognize and interact specifically with a given substrate and reach the second level of molecular interaction, the supramolecular level[1] (Figure 1). Molecular recognition in the design of supermolecules seeks an understanding of processes like replication, retrieval, immunological response, ion transfer, enzyme-substrate interactions to name a few.[1]

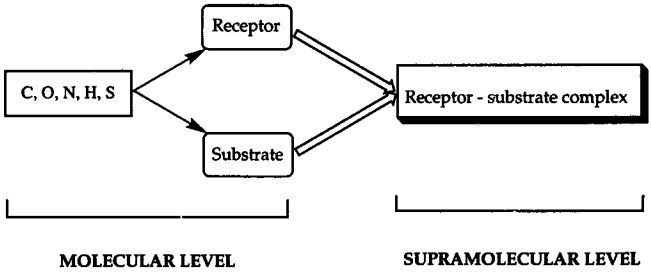


Figure 1. Levels of chemical interactions.

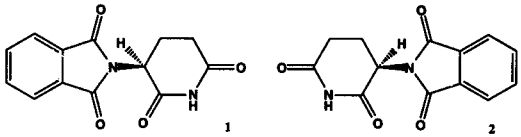
2. Selectivity in Chemical Reactions.

One of the fundamental problems facing organic chemists today is the design of highly selective reactions. In chemical reactions there are many forms of the selectivity. At the simplest level, the chemist is concerned with ensuring that, as far as possible, only the desired reaction takes place (chemoselectivity).[2] One of the first areas of research in this context was peptide synthesis.[2] The selective synthesis of peptides has been possible because protecting or blocking groups were devised to permit the sequential construction of the required peptide.[2]

The next level of control occurs when a reaction can take place at two or more positions of similar reactivity (regioselectivity).[2] There are different methods of controlling regioselectivity. For instance, it has usually been

found that the less reactive a reagent, the more selective its action.[2] Bromine atoms, for example, are more selective in alkane halogenation compared with more reactive chlorine.[2] Also, it is sometimes possible to control a reaction by choosing the conditions such that either the kinetically or the thermodynamically favored product predominates (e.g. ketone enolate formation).[2]

The last level of control is concerned with the stereochemistry and in particular with the formation of only one desired optical isomer (stereoselectivity).[2] There are several reasons for which chemists are strongly interested in stereoselectivity. One of them is due to the fact that many naturally occurring compounds, which are common synthetic targets, are biologically active in only one stereoisomeric form.[2] Suckling gives an example of the failure to take account of chilarity when the drug thalidomide, a mild analgesic was prescribed to women in 1960's.[2] Chilarity proved to be crucial to the biological effects of the drug. Two enantiomers of thalidomide (Figure 2) form two complexes with their receptor and those complexes have a diastereoisomeric relationship.[2] Unfortunately in the case of thalidomide, the potential consequences of this relationship were not taken into account and the drug was manufactured as a racemic mixture.[2] Tragically, women who had taken the drug gave birth to deformed offspring. Further research showed that only the S isomer **1** was teratogenic and the R isomer **2** was safe.[2]



a) S teratogenic b) R safe analgesic

Figure 2. Stereoisomers of thalidomide.

Suckling points out that it is more a rule than an exception to expect a difference in biological activity between enantiomers.[2] It should be clear then that it becomes essential to produce pure enantiomers. One effective route is to employ enzymatic catalysis.

In designing highly selective reactions chemists often direct their interest toward biological systems. Biological systems (enzymes) are capable of carrying out the reactions in living organisms with high selectivity. It has been thought that if chemists could imitate and generalize such biological reactions with simpler organic systems by the use of artificial host-guest complexes, it could enable to run highly selective reactions in organic laboratory.[3]

3. Enzymes

3.1 Structure of Enzymes.

It has been estimated that an average living cell contains some 3,000 different enzymes.[5] Enzymes have complex and compact structures consisting of chains of amino acids folded to form a globular macromolecule with a well defined cavity (active site) to accommodate the substrate. The polar amino acids are at the surface of the molecule directed toward the solvent, while the non-polar ones are generally oriented toward the interior of the molecule away from the solvent.[6] While some enzymes are simple proteins, others can have more complex structure. They have a non-protein group more or less associated with the protein group and the total complex is termed the holoenzyme[6] (Figure 3).

Some enzymes require the presence of ions for their proper function. Metal cations, for instance, are bound to specific oxygen, nitrogen, and/or sulfur ligands of the protein.[7] A few examples include : K^{+}, Mg^{2+},Fe^{2+}, Fe^{3+}, Co^{3+}, Mo^{6+}. Other enzymes have anions associated with them. The enzyme amylase, for example, present in saliva, is activated by chloride ions.[6]

Enzymes are present in cells in active or inactive form. Inactive state, zymogen, is known for some proteolytic enzymes.[4] One of them, trypsinogen, can be activated to trypsin by a number of enzymes, including trypsin itself. The activation process requires the removal of a short peptide, followed by some structural changes in the protein molecule.[4]

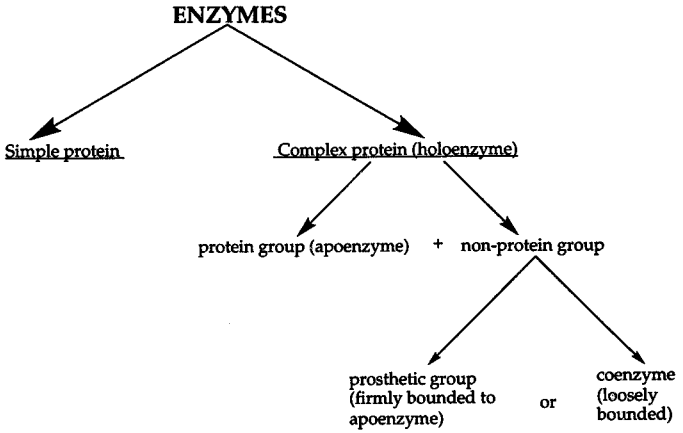


Figure 3. Enzyme structure.

3.2 Enzyme-Substrate Complexes.

The extent to which substrate is bounded to the enzyme is measured by the binding energy which is the sum of all the interaction energies and is measured by the value of the equilibrium dissociation constant between the enzyme and the substrate.[4] There are several different forces involved in these attractive interactions:

1. Ionic interactions (or electrostatic interactions) between the oppositely charged groups (Figure 4). Those interactions are greatly weakened by water. This is because water molecules orient themselves around ions forming hydration shells[4] (Figure 4b). The hydration shells, stabilized by ion-dipole interactions, screen out the electrostatic interactions between ions. This in turn reduces their energy of interaction, compared with the interaction in a vacuum, by a factor of 80, the value of the dielectric constant of water.[4]

Strong electrostatic interactions are present, on the other hand, in nonaqueous environments (Figure 4a). For example in a hydrophobic cavity of an enzyme, ionic interactions can be of a great importance in stabilizing the enzyme-substrate complex.

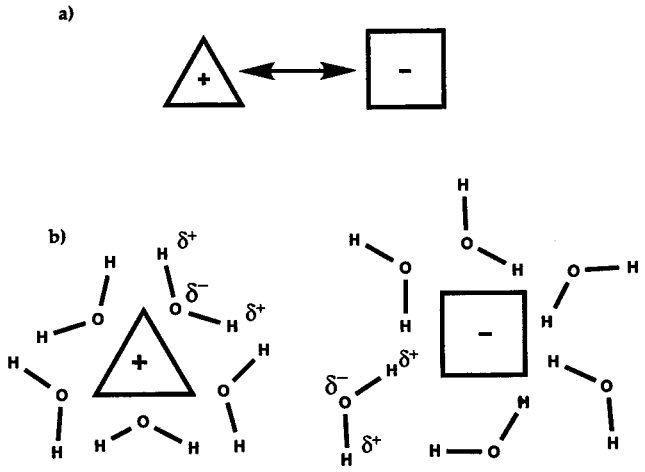


Figure 4. Electrostatic Interactions. (a) In non-aqueous environment; (b) In aqueous environment

2. Hydrogen bonds are also greatly weakened in water. This is due to the fact that water forms hydrogen bonds with enzyme and substrate competing with the formation of hydrogen bonds between enzyme and substrate.

3. Van der Waals interactions are the weakest interactions but can be numerous and therefore add significant binding energy.

4. Hydrophobic effects in excluding water from hydrophobic cavities of active sites of enzymes provide a large entropic contribution to the free energy of binding.

Water molecules in bulk solution form three-dimensional ice-like structures through the formation of hydrogen bonds with each other. Each oxygen of the water molecule can act as both a donor and an acceptor of hydrogens (Figure 5). At any moment, the member of a pair of hydrogen-bonded oxygen atoms that is closer to the hydrogen can be considered the donor while the other the acceptor. However, the small relative rotation of hydrogens is sufficient to reverse the roles of donor and acceptor. Hydrogen nuclei (protons) are then delocalized in the ice-like structure in the same sense that electrons are delocalized in molecular orbitals.[4]

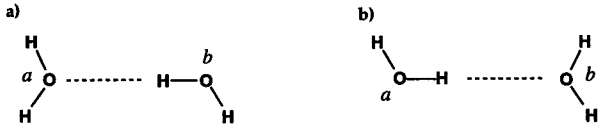


Figure 5. Delocalization of hydrogen nuclei in ice-like structure of water. (a) Oxygen acts as a hydrogen acceptor in molecule *a* and as hydrogen donor in molecule *b*; (b) Oxygen acts as a hydrogen donor in molecule *a* and as hydrogen acceptor in molecule *b*

The presence of the receptor disrupts the ice-like structure of water, because water molecules inside and outside of the enzyme cavity cannot form hydrogen bonds to the same extend as when the receptor was absent. Water molecules in the presence of the receptor are of higher energy (and lower entropy) than when the receptor is absent (Figure 6a). Removing the water molecules from the receptor cavity and replacing them with the guest molecule is energetically favorable. The removed water molecules can participate again in high entropy ice-like structure (Figure 6b). This energetically favorable hydrophobic effect is one of the driving forces for enzyme-substrate formation.

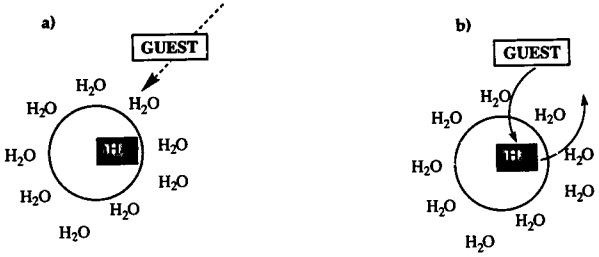


Figure 6. Entropy effect in hydrophobic ineractions. (a) High energy (low entropy) water; (b) Increase in entropy of water molecules when they are removed from the receptor cavity to the outside.

5. Covalent bonds occur in some enzyme-substrate interactions and, once formed, add to the strength of binding.[4]

3.3 Specificity of Enzymes.

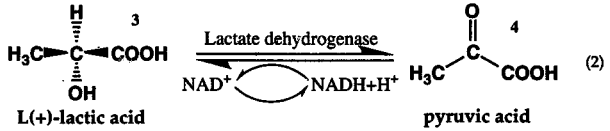
Enzymes vary in the kind and degree of specificity they exhibit.

(a) Absolute specificity occurs when one enzyme binds only one substrate e.g. urease catalyses hydrolysis of urea and no any other compound (equation 1).[6]

$$NH_2CONH_2 + H_2O \longrightarrow CO_2 + 2NH_2 \qquad (1)$$

(b) Stereochemical specificity is when enzymes catalyze only one of the possible stereochemical isomers (cis or trans, R or S, etc.).

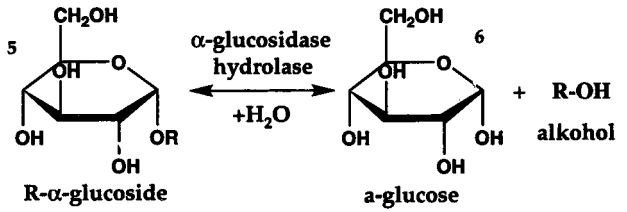
For instance, lactate dehydrogenase oxidizes only L (+)-lactic acid (it does not affect D -lactic acid) (equation 2).[6]



L(+)-lactic acid pyruvic acid (2)

More strikingly, perhaps, when acting in reverse, it reduces pyruvic acid (**4**) only to L (+)-lactic acid (**3**), whereas chemical synthesis by addition of hydrogen to the carbonyl double bond always gives the racemic mixture.[6]

(c) Group specificity is exhibited when enzymes catalyze reactions involving a series of substrates which have in common one identical group but differ in some other way. α-glucosidase which hydrolyses several α-glucosides can serve as one example (equation 3).[6] In this case the product consists of α-glucose (**6**) and an alcohol (methanol, ethanol, etc.) or only glucose in case of hydrolysis of maltose.



(3)

4) Low specificity occurs when enzyme binds any substrate that possesses a particular linkage, e.g. certain esterases will attack a wide range of compounds of the type R-COO-R', where R and R' can be varied considerably. Lipase, for instance, will hydrolyze (at different rates) most of natural fats.[6]

3.4 Efficiency of Enzymes.

The other function of enzymes, besides regulation of reactions, is acceleration of those same reactions. A large number of reactions in living organisms must be carried out at a speed that is seldom matched in the organic chemistry laboratory.[3] At the same time cell must perform chemical reactions within a relatively narrow range of physical conditions: high temperature and extremes of pH are incompatible with the existence of the living cells. Thus there is the necessity to speed up reactions without increasing the temperature. Enzymes enable reactions to occur under physical conditions which would otherwise be unacceptably slow. For instance, under standard conditions urea will not react with water at an appreciable rate even though the reaction is highly exothermic.

$$NH_2CONH_2 + H_2O \longrightarrow CO_2 + 2NH_2 \qquad \Delta G^{\circ}(1) = -57\ kJ/mole \quad (1)$$

In the presence of urease, however, the rate of this reaction increases by as much as 10^{14}.[4]

For a reaction to be possible, it is necessary that the reacting molecules possess a certain minimum energy, the activation energy E_A. Mathematically, the rate of reaction is related to the activation energy by the Arrhenius equation (equation 4):[4]

$$\text{Rate constant} = PZe^{-E_a/RT} \qquad (4)$$

where e is R is the gas constant (8.3 J/K·mol), T the absolute temperature, Z the frequency of molecular collision, and P is the probability of successful collision (max. value, 1.0). P tends to rise when orientation improves and Z is related, among other things, to the concentration of reactants.[4] It is easy to see that even a small decrease in activation energy has a large effect on the reaction rate.

Enzymes enable reactions by re-routing reactions (Figure 7); those re-routed reactions are essentially different reactions with their own activation energies, even that the substrates and products remain the same as if no enzyme was present.[4]

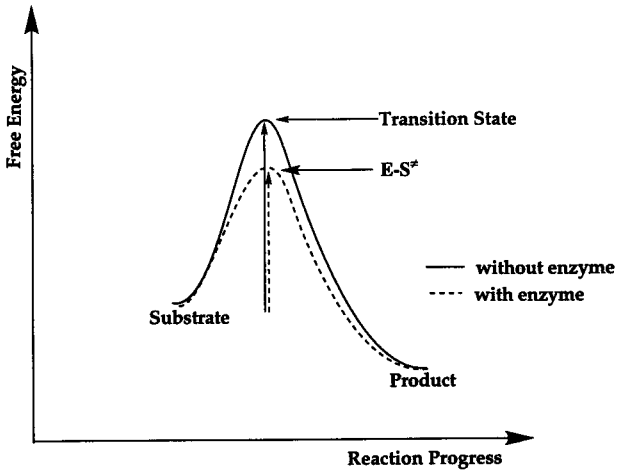


Figure 7. Activation energy of a reaction.

In the course of the reaction molecules have to go through a transition state (Figure 7). In the case of an enzyme-catalyzed reaction, the structure of a transition state is energetically favorable relative to the transition state in the absence of the enzyme. One should bear in mind that enzymes do not shift equilibria, but merely increase the rate at which the equilibrium is reached.[4]

There are several theories which attempt to explain the formation of transition states in reactions where appropriate enzymes are present:

1. Induced fit theory suggested by Koshland assumes that enzymes change their conformation when they complex with substrate.[5] It may bring the

corresponding catalytic sites and reacting groups into the proper position and by doing so enhances the reaction rate.

Although induced fit may contribute in a small way to the enhancement of the rate of enzymatic reaction, the studies suggest that by itself it cannot account for such a large acceleration of enzymatic reactions.[7] The induce-fit theory was examined recently by the "adiabatic maping" procedure where one of the substrate internal coordinates is fixed at various points along the reaction coordinate, while the energy of the enzyme-substrate complex is minimized with respect to all its Cartesian coordinates.[7] In this way the least energetic reaction pathway can be found and the flexibility of an enzyme can be tested. Then, comparing the steric energy of the system for the adiabatic mappings with and without the enzyme gives the strain energy contribution to the activation energy of the reaction.[7]

Such calculations have been performed in the cases of lysozyme and hemoglobin. It was found that the shifts in substrate or enzyme geometry during the reaction are less than 1.0 Å for any atom. This decreases the activation energy by less than 1-2 kcal/mol.[7] Thus, it seems that steric strain by itself cannot account for a significant reduction of ΔG(cat) relative to ΔG(solv.) which can amount to as much as 10 kcal/mol).[7]

2. Steric strain theory suggests that the enzyme, which is rather a rigid molecule, may be built so that the steric interaction with the substrate will "squeeze" the ground state into a geometry close to that of the transition state

(by stretching or by a change in angle of some relevant bonds in the substrate).[7] This in turn will result in a reduction of the activation energy and increase in the rate of the reaction.

Although it seems at first that the concepts of strain and induced fit contradict each other, it does not have to be necessary so. It is possible that some parts of the active site are relatively rigid and that binding substrate to those portions brings about a stress in the substrate, while other parts of the active site become distorted by the binding of the substrate bringing about a conformational change in the active site of the enzyme.[4]

3. Electrostatic Stabilization theory argues that the key point in structure-function correlation in enzyme catalysis is the electrostatic stabilization of the ionic t: on states.[7] Warshel describes an enzyme active site as a "supersolvent" for a particular transition state, with enzyme dipoles preoriented to create an electric field complementary to the charge distribution of the transition state.[7] Enzymes, by interaction in an optimal way with the charge distribution of the ionic resonance forms at the transition state would decrease ΔG(cat.) relative to ΔG(solv).[7] Such conclusions have emerged from the studies of the catalysis of peptide hydrolysis by serine proteases where the enzyme reduces the energy of the ionic resonance form [His+tet-] of the ionized histidine and the negatively charged tetrahedral intermediate relative to their energy in aqueous

solution.[7] These studies suggest that the reduction of the activation energy is almost linearly related to the stabilization of the ionic resonance forms.[7]

4. Host-Guest Complexation Chemistry.

Several groups of artificial hosts have been developed. Examples include: cycloamyloses (cyclodextrins)[8], cycloalkanes[9], cyclophanes[10], cyclic peptides[10], cyclic neutral polyligands[11, 12-16] (crown ethers[11,12,14,15,16], cryptans[12-16], etc.), cyclic polyions[18-20] and acyclic neutral polyligands.[21] All of them with the exception for the last one are composed of macrocyclic compounds.

4.1 Cyclophanes: General Information.

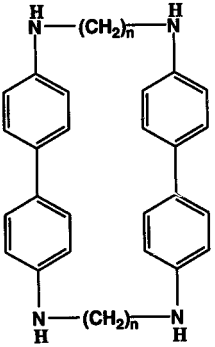
Cyclophanes or "bridged aromatic compounds" are macrocycles with well defined cavity which can form inclusion complexes with guest molecules.[22] Aromatic rings of cyclophanes may act as rigid structural units, as hydrophobic and van der Waals binding sites.

Cyclophanes seem to be a perfect group for enzyme-substrate interactions study for several reasons. They can be made water soluble. They possess well defined hydrophobic cavities. They are completely synthetic and thus subject to a wide range of structural modifications.[22]

4.2 Preliminary Study in Cyclophanes.

First studies done on cyclophanes go back to 1950's and were initiated by Cram.[23,24] During this time large cyclophanes were used for the first time as host molecules for organic guests.

A series of paracyclophanes **7** containing two benzidine skeletons bridged by two alkyl chains.[25]

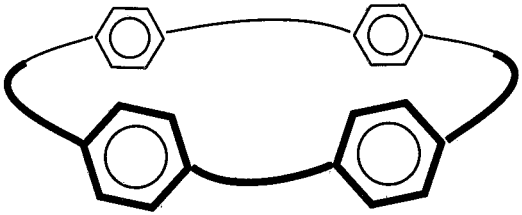


7a n = 2
7b n = 3
7c n = 4

7

Paracyclophanes can adopt two different conformations: face and lateral (or edge) (Figure 8).[26,27] The face conformation (Figure 8a) allows inclusion cavities with a sufficient depth to adopt a potential substrate whereas the lateral conformation (Figure 8b) fills up the cavities and therefore the inclusion of a guest is not favored.[22]

a)



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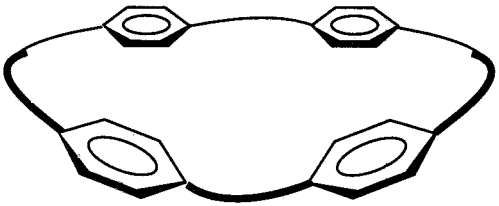
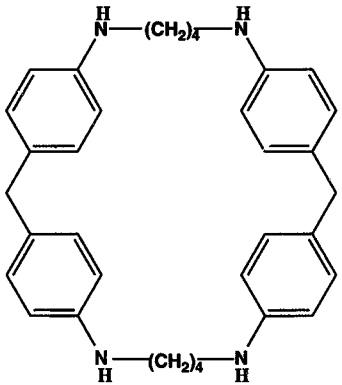


Figure 8. Conformations of paracyclophanes. (a) Face conformation; (b) Lateral conformation

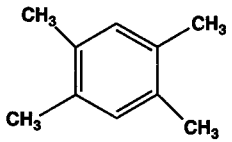
It has been shown that the size of the cavity affects the formation of inclusion complexes. For instance, recrystallization of **7b** and **7c** from dioxane or benzene yielded crystalline molecular inclusion complexes with the solvents.[25] The complex of **7c** with dioxane was so stable that solvent could not be removed after drying *in vacuo* at 150 °C for 30 hours.[25] In contrast such molecular complexes could not be obtained for **7a**. The cavity is too small to accommodate a foreign molecule.[25]

At this point it reminded to be confirmed that cyclophanes indeed form inclusion complexes instead of simple stacking complexes with certain guest molecules. The direct evidence was obtained from 1,6,20,25-Tetraaza[6.1.6.1]paracyclophane (**8**) which has two diphenylmethane skeletons bridged by two methylene chains.[28]



8

X-ray crystallographic study confirmed formation of 1:1 complex of **8** with durene (**9**).[28]



9

Durene (**9**) is fully included in the cavity of the host molecule 8.[28] The hydrophobic cavity has a rectangularly shaped open ends (3.5 x 7.9 Å) and a depth of 6.5 Å.[28] The benzene ring of durene is nearly parallel to the inner wall and it adopts a face conformation. It has been suggested that the primary reason for this good fit is the close correspondence between the thickness of the aromatic ring (3.4 Å) and the shorter width of the cavity open ends (3.5 Å).[28] Cram also demonstrated a transannular electronic coupling between the π electrons of the two benzene rings of the diphenylmethane unit.[28]

The similar results were obtained for naphthalene as a guest.[29] This study seems to confirm the importance of hydrophobic interactions since neither durene or naphtalene possess a dipole moment which would be necessary for any electrostatic binding.

Based on many similar studies it can be concluded that the structure of the complex formation is directly related to the goodness of fit between receptor and substrate: the better the fit, the stronger the complex formation.[22] Also, the increase in the hydrophobic area of a host improves the complex formation.[22]

5. Aim of Our Proposal.

It has been suggested that the key point in structure-function correlation in enzyme catalysis is the electrostatic stabilization of the ionic transition states.[7] If the ionic stabilization is indeed important in biological systems, then it should be of the equal importance in simple synthetic systems. The question we would like to ask is how the electrostatic interactions can orient a guest molecule within the hydrophobic cavity of the receptor.

II. SYNTHESIS

1. General Requirements For Designing a Receptor-Substrate Model.

We propose to synthesize a macrocyclic receptor. This macrocycle should be hydrophilic, but with a well defined hydrophobic cavity (since the active sites of most enzymes are hydrophobic clefts) (Figure 9a). Since we want to test for the importance of electrostatic interactions in molecular recognition phenomena, a dipole moment must be established across the cavity. This can be accomplished by creating a positive charge on one end of the macrocyle and a negative charge on another (Figure 9b).

Different compounds can be used as a guest, but they all should be of a right size and geometry to allow for a good fit with the receptor. A guest molecule can be charged or neutral but it must possess a dipole moment. It should have a considerable hydrophobic surface area and yet be water soluble.

So designed model should allow us to see whether electrostatic interactions will affect the orientation of the guest molecule within the receptor's cavity. According to Warshel, a guest molecule should orient itself in a very specific way within the host cavity, so that the dipole moments of the receptor and the guest are antiparallel[7] (Figure 9c). Figure 10 shows the macrocyle **10** that we propose to synthesize.

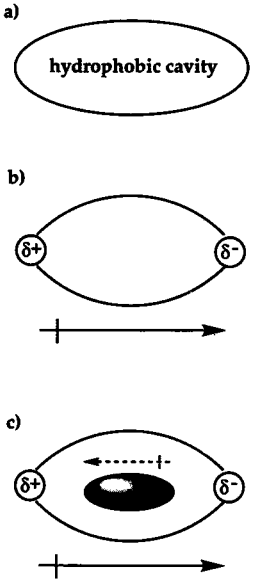


Figure 9. Receptor-Substrate model (a) Hydrophobic cavity of a macrocycle; (b) Dipole moment across the receptor's cavity; (c) Recepor-Substrate complex

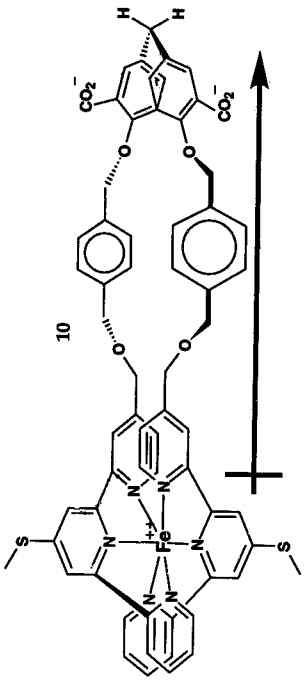


Figure 10. Macrocycle

2. General Experimental Strategy.

A convergent synthesis is proposed to produce the macrocycle **10** (Figure 11). The negative end of **10**, designed "CAP" (**16**) and the positive end of **10**, an Fe(II) bound in a terpyridine ligand ("TERPYRIDINE- unit") are synthesized. Those two ends will be then connected together to form "CAP-TERPYRIDINE" complex (**26**). The last step of the synthesis would be a macrocyclization. To do that we would like to take an advantage of the fact that terpyridine can act as a ligand and form the coordination complexes with the transition metal ions. For instance, a reaction of two terpyridine units with one equivalent of Fe(II) results in a bis-terpyridi. with two terpyridine molecules positioned pependicular to one another, 90° out of plane (Figure 12).[30] The complex **11** is of a high kinetic and thermodynamic stability.[30] Running a macrocyclization reaction in a highly diluted solution (in order to avoid polymerization) should create a desired macrocycle with a positively charged "Fe(II)-TERPYRIDYL" unit and negatively charged "CAP" unit. At this point a guest molecule can be introduced and the host-guest complex studied.

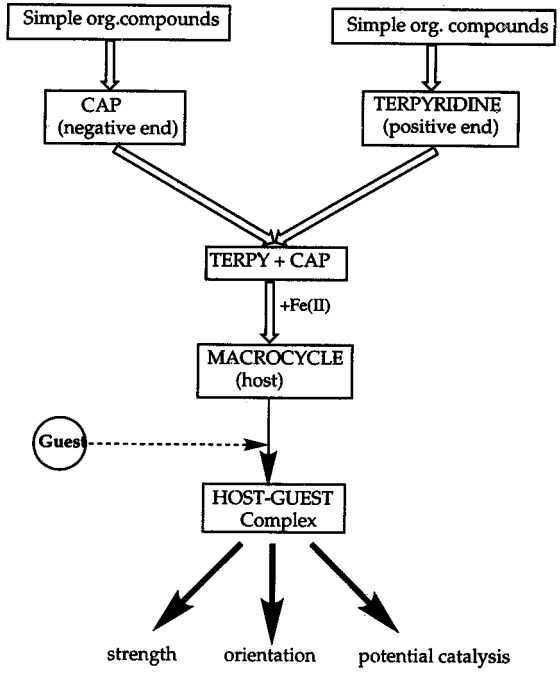
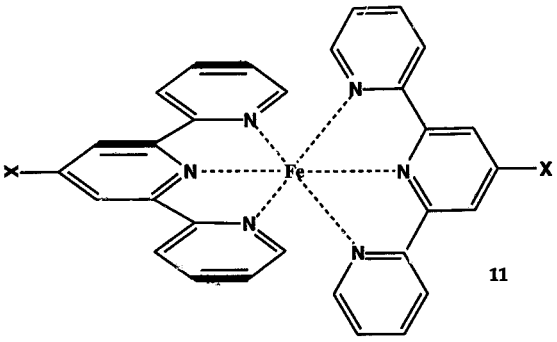


Figure 11. General experimental strategy.



11

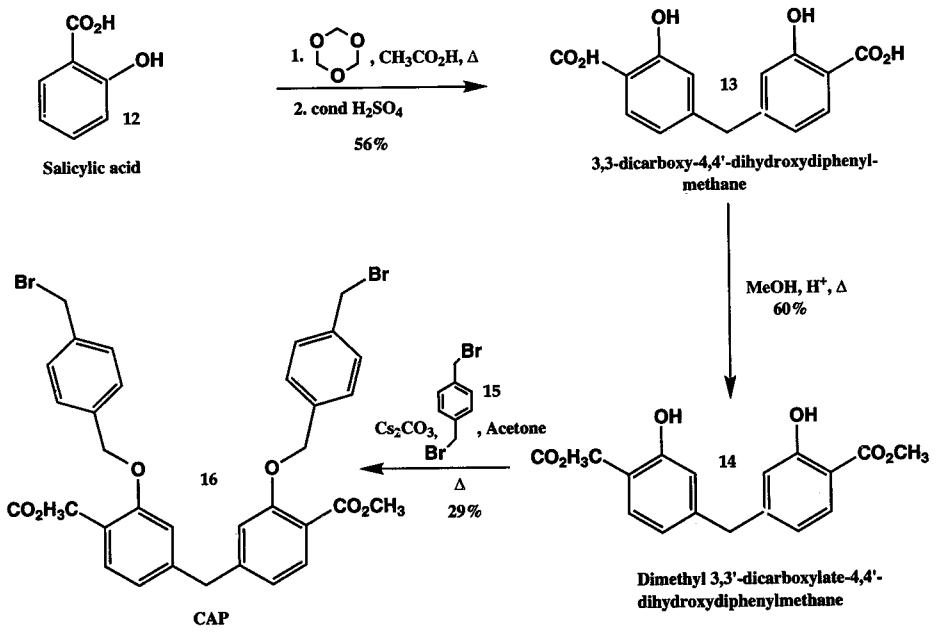
Figure 12. Distorted octahedral coordination of 2,2':6',2"-Terpyridine with Fe(II).

3. Results and Discussion.

3.1 Synthesis of the "CAP" (16) (Scheme 1).

In the first step 3,3-Dicarboxy-4,4'-Dihydroxydiphenylmethane (**13**) was prepared from salicylic acid (**12**) by condensation with trioxane in the presence of concd sulfuric acid in 56% yield. The methylene disalicylic acid (**13**) was converted into dimethyl 3,3'-dicarboxylate-4,4'-dihydroxydiphenylmethane (**14**) by reaction with methanol in the presence of a catalytic amount of concd sulfuric acid in a 60% yield. The ester **14** was reacted with α,α-dibromo-*p* -xylene (**15**) in the presence of ceasium carbonate in refluxing acetone to produce [Dimethyl 3,3'-dicarboxylate-4,4'-(bis(4-bromomethyl-benzoxy)) diphenyl] methane (**16**) in a 29% yield. The relatively low yield of the reaction can be explained by the complexity of the purification process.

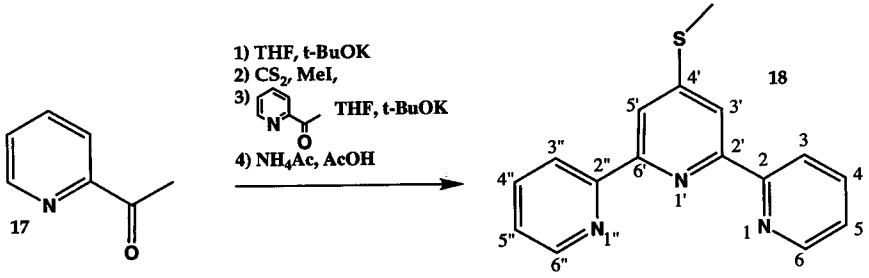
Scheme 1


CO2H
OH
12
Salicylic acid
1. , CH3CO2H, Δ
2. cond H2SO4
56%
OH
OH
CO2H
CO2H
13
3,3-dicarboxy-4,4'-dihydroxydiphenyl-methane
MeOH, H+, Δ
60%
OH
OH
CO2H3C
CO2CH3
14
Dimethyl 3,3'-dicarboxylate-4,4'-dihydroxydiphenylmethane
Br
15
Cs2CO3, , Acetone
Br
Δ
29%
Br
Br
O
O
16
CO2H3C
CO2CH3
CAP

3.2 Synthesis of the Terpyridine-moiety 22.

One pot synthesis of terpyridine was established and successfully used by Potts (Scheme 2), to produce the unsubstituted 4'(methylthio)-2,2':6',2''-terpyridine (**18**).[31] Pott's method was used by us in order to get a terpyridine substituted in the fourth position (Scheme 3). In the third step of the Pott's synthesis 1 equivalent of methyl 2-acetyl-pyridine-4-carboxylate (**19**) instead of 1 equivalent of 2-acetylpyridine (**17**) was used. This synthesis did not generate the expected 4-methylcarboxyl-4'-methylthio-2,2':6'2''-terpyridine (**20**) but an unsubstituted terpyridine (**18**). At some point in the synthesis decarboxylation must have taken place to produce clean **18**. At this point we turned our attention to Pott's two step synthesis of terpyridine.[30,32] In this method the diathio-acetal intermediate formed in the synthesis, 3,3'-bis(methylthio)-1-(2-pyridinyl)-2-propen-1-one (**21**) was isolated and purified before adding methyl 2-acetyl-2-pyridine-4-carboxylate(**19**) (Scheme 4). Scheme 5 shows the mechanism for the synthesis of terpyridine. The crude product consisted of the mixture of the ester (**20**) and its carboxylic acid. The crude mixture was reacted with methanol in the presence of a catalytic amount of concd sulfuric acid in order to convert any of the carboxylic acid to the ester **20**. The ester **20** was reduced to the alcohol **22** by reaction with $LiAlH_4$ in EtO_2 in a 50% yield (Scheme 6). The use of THF instead of Et_2O resulted in only a 10% yield.

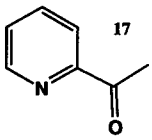
Scheme 2



2-acetylpyridine

4'(methylthio)-2,2':6',2"-terpyridine

Scheme 3



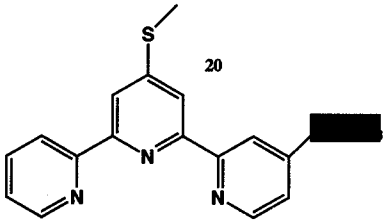
17

2-acetylpyridine

1) THF, t-BuOK,
2) CS_2, MeI
3)  , THF, t-BuOK

19

4) NH_4Ac, AcOH

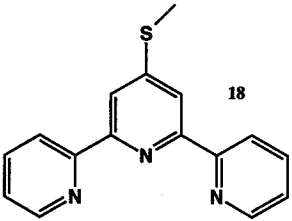


20

4-methylcarboxyl-4'-methylthio-2,2':6'2"-terpyridine

expected

generated

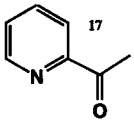


18

4'(methylthio)-2,2':6',2"-terpyridine

Scheme 4

Step 1

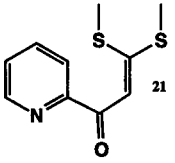


2-acetylpyridine

1) THF, t-BuOK

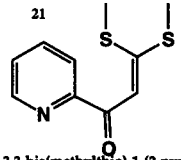
2) CS_2, MeI

80%

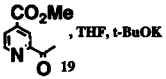


3,3-bis(methylthio)-1-(2-pyridinyl)-2-propen-1-one

Step 2



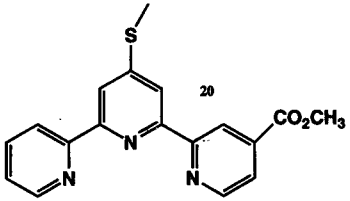
3,3-bis(methylthio)-1-(2-pyridinyl)-2-propen-1-one

1) 

, THF, t-BuOK

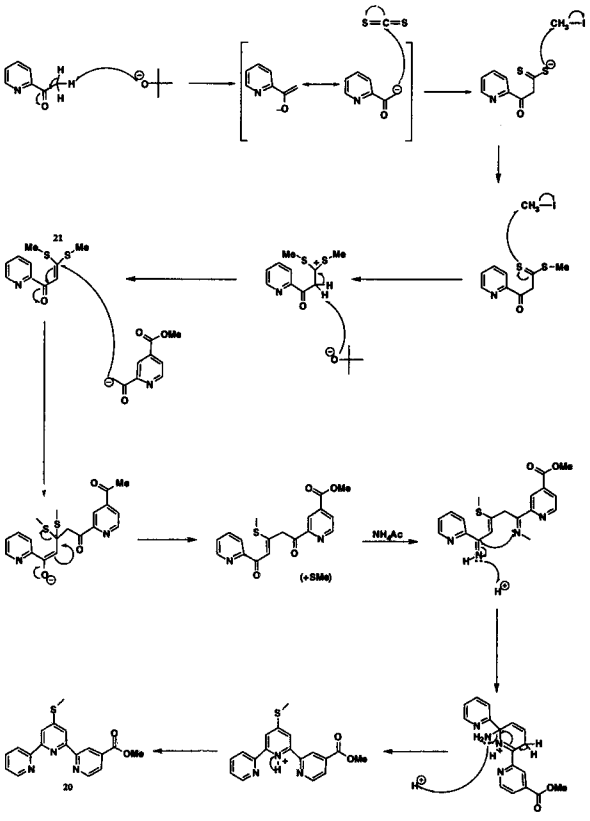
2) NH_4Ac, AcOH

45%

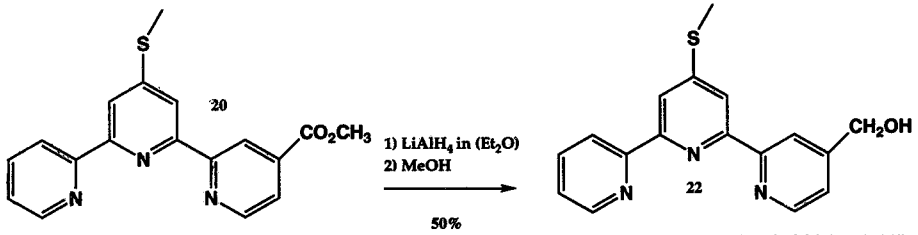


4-methylcarboxyl-4'-methyltio-2,2':6',2''-terpyridine

Scheme 5



Scheme 6



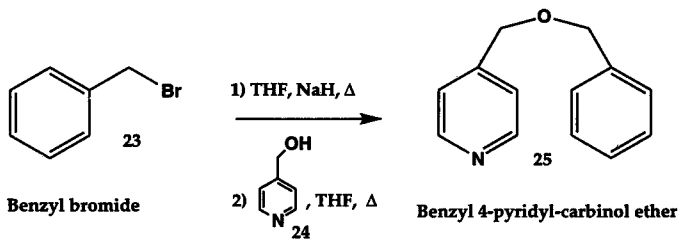
4-methylcarboxyl-4'-methyltio-2,2':6',2"-terpyridine

4-(Hydroxymethyl)-4'-(methylthio)-2,2':6',2"-terpyridine

3.3 Reaction of the "CAP" (16) with the"TERPYRIDINE-unit" (22).

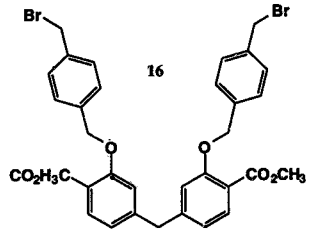
Before reacting the "CAP" (16) with 22 the model study for ether linkage formation has been done. Benzyl bromide (23) was reacted with 4-pyridyl-carbinol (24) in the presence of NaH (in THF) to generate benzyl-4-pyridyl carbinol ether (25) in quantitative yield (Scheme 7).[33]

Scheme 7

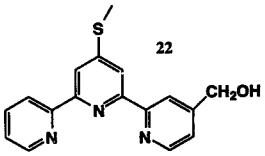


At this point we were ready to react 1 equivalent of 16 with 2 equivalents of 22 into one unit by making two ether linkages (Scheme 8). The reaction of 16 with 22 in the presence of NaH (in THF) did not generate, however, the expected product 26. It is not clear to us why the ether linkages were not formed. It has been suggested that sodium reacts with the terpyridine to produce insoluble salts.[34]

Scheme 8

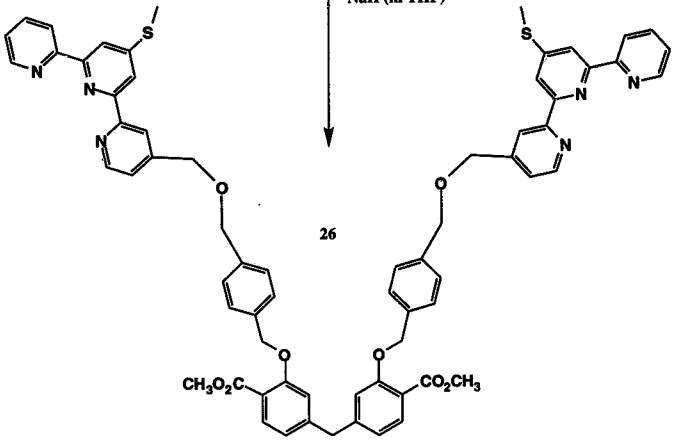


CAP



4-(Hydroxymethyl)-4'-(methylthio)-2,2':6',2"-terpyridine

NaH (in THF)



III. CONCLUSIONS

Although we were not able to generate the ether linkage between the "CAP" (**16**) and the terpyridine (**22**) up to this point, we still believe that the general experimental strategy for our study is a good one. At the present moment there are studies under way in our laboratory to find a method of generating the desired ether linkage. Should they turn out to be unsuccessful, we might consider making a carbon-carbon bond instead of a carbon-oxygen bond. It should not affect the size of the macrocycle's cavity significantly, so that the receptor will still be able to accommodate an aromatic guest.

The proposed macrocycle (**10**) (Figure 13) would have a well defined box shaped cavity which could accommodate, for instance, p-toluensulfonate (**27**) as a guest. Based on computer modeling studies, the rectangular cavity of **10** opens up when **27** is introduced (Figure 14 and Figure 15).

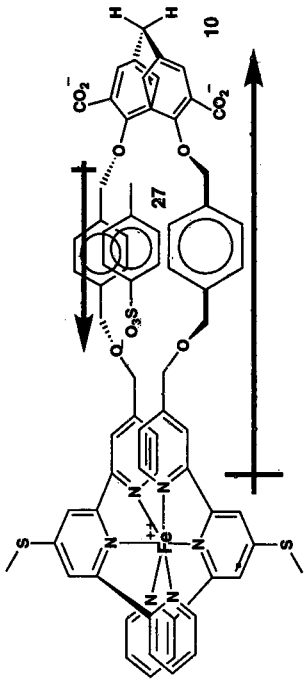
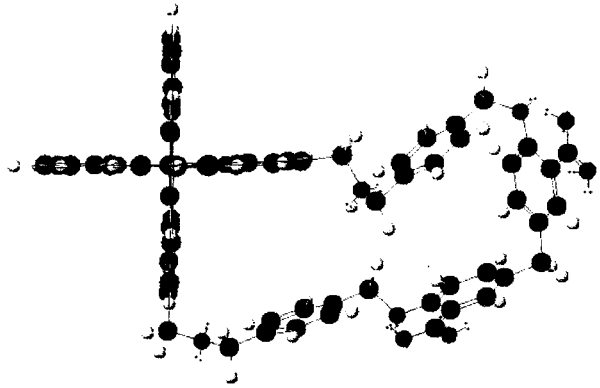


Figure 13. Macrocycle + Substrate.

a)



b)

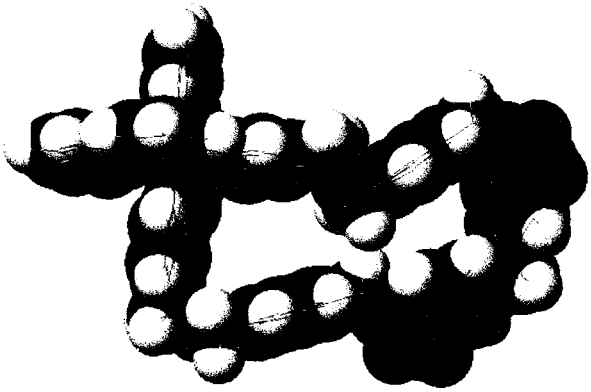


Figure 14. Macrocycle. (a) Ball and stick model; (b) CPK model (corresoponds to 85% Van der Waals radii)

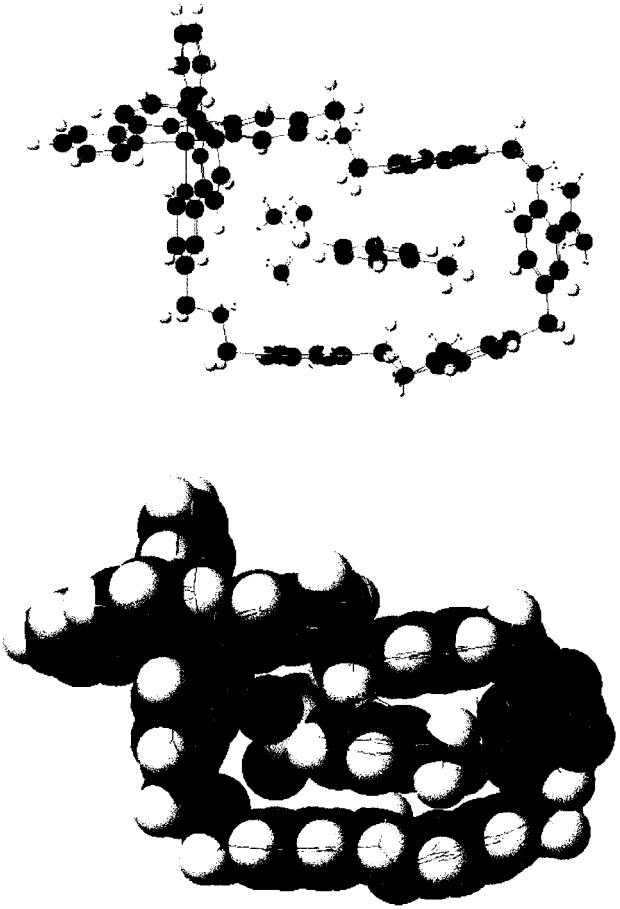


Figure 15. Macrocycle plus substrate **27.**

IV. EXPERIMENTAL

1. General Experimental.

Melting points were determined on Mel-Temp capillary melting point apparatus and are uncorrected. Proton and carbon magnetic resonance spectra were obtained on a Varian Gemini-200 spectrometer. Chemical shifts (^{1}H-NMR and ^{13}C-NMR) are expressed in parts per million (δ units) downfield from tetramethylsilane (TMS) used as an internal reference. The following abbreviations are used: s=singlet, d=doublet, t=triplet, m=multiplet. Yields are reported based on the amount of isolated material obtained after the indicated procedure.

Thin layer chromatography (TLC) was performed using Ether Whatman KG Silica Gel or Alumina Plates as appropriate. Fischer basic alumina activity I or Fischer neutral alumina activity I were used for open column chromatography.

Tetrahydrofuran (THF) and diethyl ether (Et_2O) were distilled from sodium and benzophenone under a dry nitrogen atmosphere respectively. All other commercially available reagents and solvents were used without further purification.

Where indicated, reactions run under nitrogen atmosphere were arranged with a mercury or oil bubbler so that the system could be alternately evacuated and filled with nitrogen and left under positive pressure. Syringes

were dried in an oven and cooled in a desiccator over calcium sulfate prior to use. "Room temperature" refers to ambient laboratory conditions: T=20-27 °C, P=720-770 mm Hg. "Concentration in vacuo" refers to concentration on a rotary evaporator equipped with a heating bath (T< 80°C) sometimes followed by further concentration using a high vacuum (< 2 mm Hg) pump.

2. Preparations

3,3'-Dicarboxy-4, 4'-dihydroxydiphenylmethane (13).[35] A stirred solution of 80 g (0.58 mol) of salycylic acid (**12**) and 7.0 g (78 mmol) of trioxane in 100 mL of glacial acetic acid was heated to 95 °C. After cooling to RT, a solution of 1 mL of concd H_2SO_4 in 5 mL of glacial acetic acid was added to the mixture. After 5 min the mixture was poured into 4 L of water, allowed to remain until a clear supernatant liquid formed and the precipitate was isolated by filtration. The isolated solid was added to 400 mL of a solution of equal volumes of glacial acetic acid and water and filtered again. The filtrate was poured into 500 mL of water and allowed to stand. The precipitate was separated and washed with 5 x 200 mL portions of water, and air dried to afford 43 g (56%) of **13** as white powder: mp 240--242 °C (Lit: m.p. 238 °C).[35]

Dimethyl 3,3-dicarboxy-4,4'-dihydroxydiphenylmethane (14). A stirred solution of 30 g (0.10 mol) of the diacid **13** and 4 mL of concd H_2SO_4 in 500 mL of CH_3OH was heated at reflux overnight. After 15 hours the heat was removed and the reaction mixture was allowed to cool. Next it was diluted

into 500 mL of CH_2Cl_2 and extracted with 2 x 200 mL portions of $NaHCO_3$. The organic phase was washed with 4 x 100 mL portions of water, dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford 23 g of yellow solid. The crude material was crystallized from 100 mL of ethanol (95%) to afford 20 g (60%) of **14** as white microcrystals : mp 91-92 °C; R_f = 0.6 (SiO_2, 15/85%, ethyl acetate/hexane); 200 MHz ^{1}H-NMR ($CDCl_3$) δ 3.84 (s, 2H), 3.92 (s, 6H), 6.92 (d, 2H, J = 9Hz), 7.25 (dd, 2H, J = 6Hz, J = 1Hz), 7.62 (d, 2H, J = 1Hz), 10.65 (s, 2H). (Appendix 1).

[Dimethyl-3,3′ dicarboxylate-4,4′-(bis(4-bromomethyl-benzoxy)) diphenyl] methane (16).[36] To a stirred solution of 1.0 g (3.2 mmol) of dimethyl-3,3′-dicarboxylate-4,4′-dihydroxydiphenylmethane (**14**) in 61 mL of dry acetone were added: 5.2 g (16 mmol) of ceasium carbonate and 8.3 g (32 mmol) of α–α -dibromo-*p* -xylene (**15**). The solution was gently refluxed in the dark. After 20 hours the ceasium salts were removed by filtration and washed with 20 mL of acetone. The filtrate was concentrated *in vacuo* , recrystallized from acetone, and then from toluene which afforded 1.49 g of crude material. Purification by flash chromatography (SiO_2, eluted with 30% ethyl acetate/hexane) afforded 0.63 g (29%) of **16** as white crystals: mp 102-106 °C; R_f = 0.23 (SiO_2, 30% ethyl acetate/CH_2Cl_2); 200 MHz ^{1}H-NMR ($CDCl_3$) δ 3.99 (s, 8H), 4.51 (s, 4H), 5.15 (s, 4H), 6.91 (d, 2H, J = 9Hz), 7.20 (dd, 2H, J = 9Hz, J = 2Hz), 7.41 (d, J = 8Hz), 7.47 (d, 9Hz), 7.62 (d, 2H, J = 1Hz) (Appendix 2); ^{13}C-NMR ($CDCl_3$) δ 174.0, 156.5, 137.0, 133.8, 133.0, 131.9 ,129.0, 126.8, 113.9, 65.0, 50.9, 39.8, 32.5.

4-Methylcarboxyl-4'-methylthio-2,2':6',2"-terpyridine (20)

(one pot synthesis).[31] To a stirred solution of 1.4 g (12 mmol) of tert-butoxide in 10 mL of anhydrous THF was added, dropwise, over the course of 10 min, 1.0 mL (8.9 mmol) of 2-acetylpyridine (**17**) in 3.0 mL of THF. The resulting dark red mixture was stirred for 10 min and then 0.5 mL (8.3 mmol) of carbon disulfide was added to it (the mixture turned orange) followed by 0.5 mL (8.0 mmol) of methyl iodide. The resulting brown mixture was stirred at RT for 4 hours and then 13 mL of THF was added, followed by 1.4 g (12 mmol) of tert-butoxide and 1.0 mL (5.6 mmol) of methyl 2-acetyl-2-pyridyl-4-carboxylate (**19**). The resulting dark red mixture was stirred at RT for 18 hours and then 4.4 g (57 mmol) of ammonium acetate and 6.5 mL (110 mmol) of glacial acetic acid were added to it. The resulting dark yellow mixture was stirred at RT for 10 min and then the excess of THF was distilled off. The mixture was filtered and concentrated *in vacuo* which afforded 2.7 g of a dark green solid.

4-Methylcarboxyl-4'-methylthio-2,2':6',2"-terpyridine (20)

(two step synthesis).[32] To a stirred solution of 3.7 g (33 mmol) of tert-butoxide in 83 mL of anhydrous THF was added 2.0 g (11 mmol) of methyl 2-acetyl-2-pyridine-4-carboxylate (**19**). The resulting dark brown mixture was stirred for 10 min and then 3.7 g (16 mmol) of 3,3-bis(methylthio)-1-(2-pyridinyl)-2-propen-1-one (**21**) was added to it and the mixture (dark red) was stirred at RT for 22 hours (during this time the mixture turned bright red). Then, 19 g (0.25 mmol) of ammonium acetate and 41 mL of glacial acetic acid were added. The resulting yellow mixture was stirred for 15 min at RT (the color changed first to brown and then to black). The reaction mixture was

then cooled to 0 °C for 4 hours. The mixture was concentrated on a rotavaporator and poured into a separatory funnel containing 200 mL of CH_2Cl_2. The organic layer was washed with 3 x 100 mL portions of water, followed by 100 mL of sat. $NaHCO_3$ and 50 mL of sat. NaCl. The organic layer was dried (Na_2SO_4), filtered and concentrated *in vacuo*. The solid was redissolved in 100 mL of CH_3OH and 1.5 mL concd H_2SO_4 and heated at reflux. After 15 hours the heat was removed and the reaction mixture was allowed to cool. Next, it was neutralized with $NaHCO_3$, filtered and concentrated *in vacuo*. The residue was redissolved in 40 mL of CH_2Cl_2 and 5 g of neutral alumina was added to it. The mixture was filtered and the concentrated *in vacuo* . The black solid was recrystallized from 20% ethyl acetate/hexane (80 mL) to afford 1.7 g (45%) of **20** as yellow fluorets: mp 130-132 °C; R_f = 0.75 (SiO_2, acetone); 200 Mhz ^{1}H-NMR ($CDCl_3$) δ 2.57 (s, 3H), 4.02 (s, 3H), 7.35 (ddd, 1H, J = 7Hz, J = 5Hz, J = 1Hz), 7.89 (m, 2H), 8.33 (d, 1H, J = 2Hz), 8.35 (d, 1H, J = 2Hz), 8.68 (m, 2H), 8.82 (dd, 1H, J = 5Hz, J = 1Hz), 9.10 (dd, 1H, J = 2Hz, J = 1Hz) (Appendix 3).

4-(Hydroxymethyl)-4′-(methylthio)-2,2′:6′,2″-terpyridine (22).[30] To a stirred solution of 1.6 g (4.8 mmol) of 4-methylcarboxylate-4′-methylthio-2,2′: 6′,2″-terpyridine (**19**) in 100 mL of dry THF was added 4.8 mL of 1M $LiAlH_4$ in THF. After 5 min, 32 mL of CH_3OH was added and the reaction mixture was stirred for 2 hours (the color changed from green into yellow). The mixture was

filtered and the supernatant concentrated *in vacuo* which afforded 1.5 g of yellow solid. Threefold purification by open column chromatography (neutral alumina, activity I; 5% CH_3OH/CH_2Cl_2) afforded 130 mg (0.42 mmol) of the pure product **22** as a yellow oil, which solidifies on standing[21]; 200 Mhz ^{1}H-NMR ($CDCl_3$) δ 2.62 (s, 3H), 3.20 (s, 1H), 4.85 (s, 2H), 7.35 (m, 2H), 7.85 (m, 1H), 8.27(d, 1H, J = 2Hz), 8.29 (d, 1H, J = 2Hz), 8.50-8.70 (m, 4H) (Appendix 4).

Benzyl-4-pyridyl carbinol ether (25).[33] To a stirred solution of 1.00 mL (8.40 mmol) of benzyl bromide (**23**) in 10 mL of THF was added 2.70 g (66.9 mmol) of NaH (60%) oil dispersion in 1.0 mL of THF. The mixture was stirred at RT for 15 min and refluxed for 15 hours. Next, 1.00 g (9.00 mmol) of 4-Pyridyl carbinol (**24**) in 10 mL of THF was added to the mixture and it was refluxed. After 15 hours the heat was removed and the reaction mixture was allowed to cool. It was quenched then by the addition of 10 mL of water. Next, it was diluted into 100 mL of methylene chloride and washed with 2 x 50 mL portions of water followed by 50 mL of sat. NaCl. The organic phase was dried (Na_2SO_4), filtered and concentrated *in vacuo* to afford **25** as an oil. ^{1}H-NMR indicates the quantitative formation of the benzyl-4-pyridyl carbionol ether (**23**); 200 Mhz ^{1}H-NMR ($CDCl_3$) δ 4.57 (s, 2H), 4.64 (s, 2H), 7.32 (m, 7H), 8.57 (d,2H, J = 2Hz).

4-(Hydroxymethyl)-4'-(methylthio)-2,2':6',2''-terpyridine (22) +[Dimethyl-3,3'-dicarboxylate-4,4'-(bis(4-bromomethyl-benzoxy)) diphenyl] methane (16).[37]

62.8 mg (0.20 mmol) of terpyridine (**20**) in 0.50 mL of THF was added to 32 mg (1.3 mmol) of NaH (60%) oil dispersion in 0.50 mL of THF. The mixture was stirred at RT for 15 min and then refluxed for 15 hours (it turned dark brown). Next, 68.2 mg (0.10 mmol) of the CAP (**16**) in 1.0 mL of THF was added to the mixture. The resulting dark yellow mixture was refluxed for 5 hours, then concentrated *in vacuo* and water was removed by sublimation. It afforded 155 mg of a yellow solid (Appendix 5).

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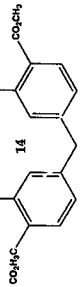
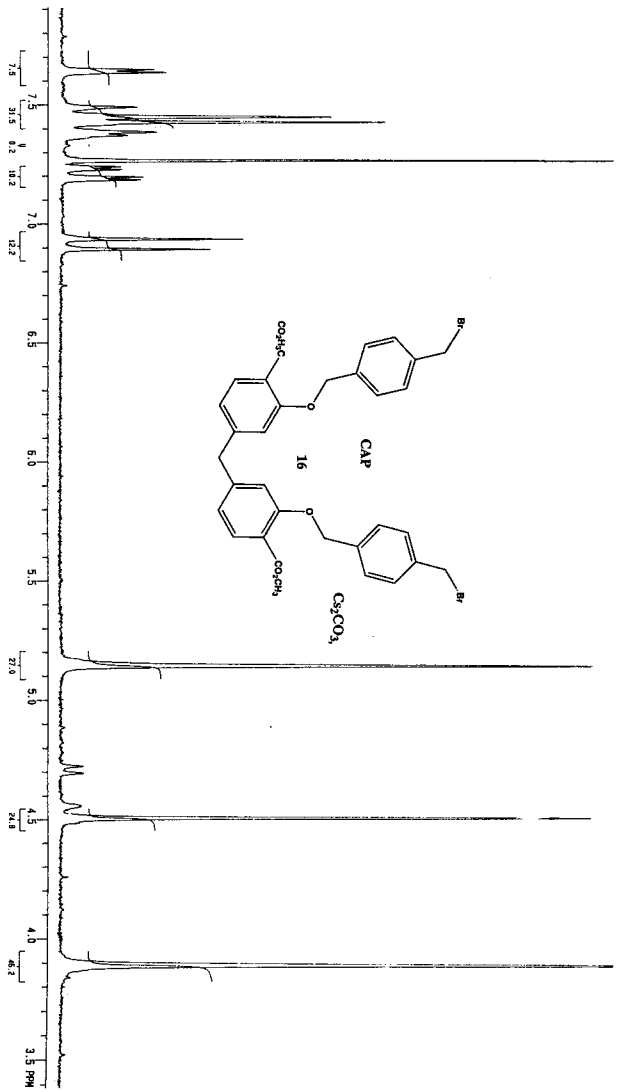
Appendix 1

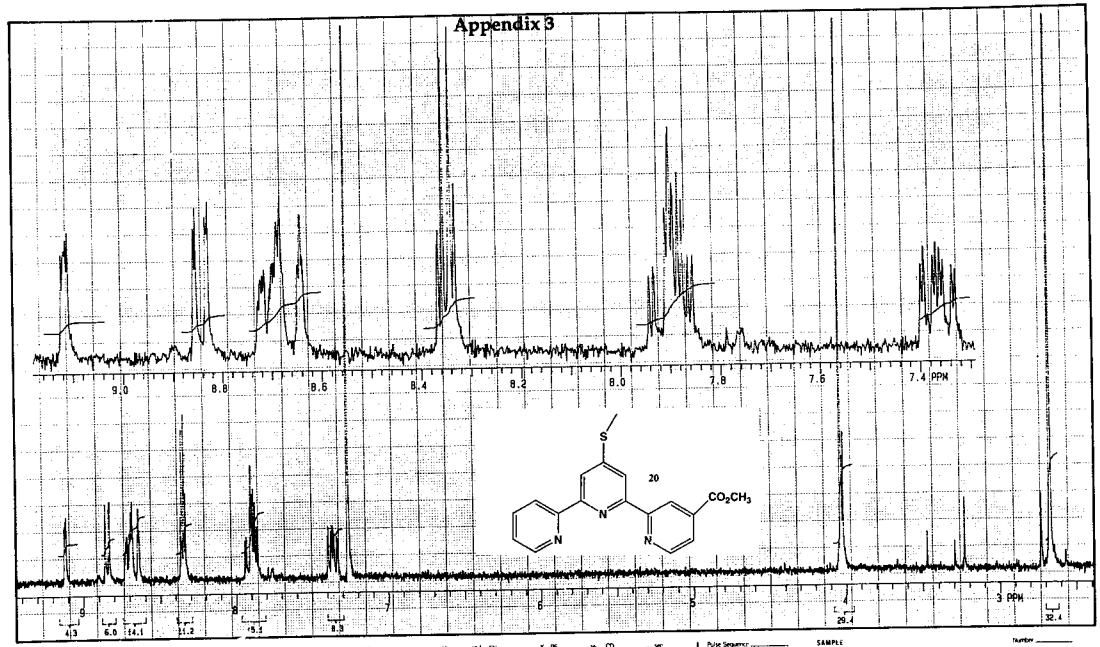


Dimethyl 3,3'-dicarboxylate-4,4'-dihydroxydiphenylmethane

Appendix 2



Appendix 3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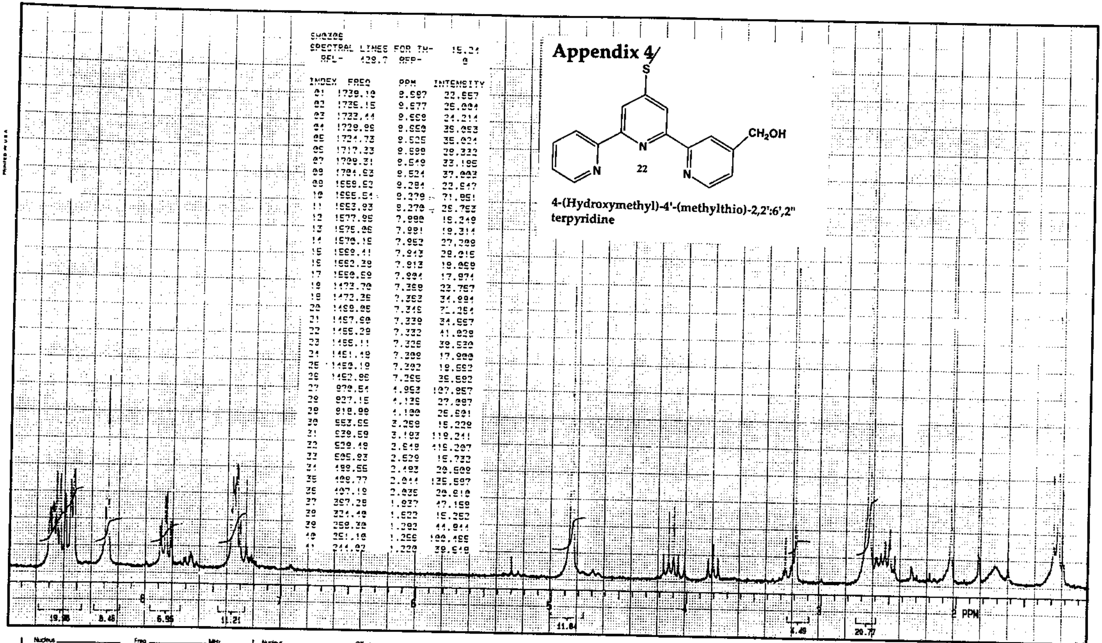
8.6
8.4
8.2
8.0
7.8
7.6
7.4 PPM
TMS
3 PPM
20
S
N
N
N
CO_2CH_3
4.3
6.0
14.1
11.2
15.1
8.3
29.4
32.4

Appendix 4

22

4-(Hydroxymethyl)-4'-(methylthio)-2,2':6',2" terpyridine



SH0308
SPECTRAL LINES FOR TH- 15.24
REF- 429.7 RFP- 0

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03	1733.44	8.668	24.214
04	1729.89	8.650	39.053
05	1724.73	8.625	35.024
06	1717.33	8.588	28.332
07	1709.31	8.548	33.195
08	1704.53	8.524	37.003
09	1658.53	8.294	22.547
10	1655.54	8.279	71.851
11	1653.93	8.270	25.753
12	1577.95	7.890	15.249
13	1575.86	7.881	19.314
14	1570.16	7.852	27.289
15	1568.41	7.843	28.015
16	1562.29	7.813	19.059
17	1560.59	7.804	17.874
18	1473.70	7.369	23.757
19	1472.35	7.363	34.994
20	1468.05	7.341	71.254
21	1467.60	7.339	34.557
22	1465.29	7.332	41.829
23	1465.11	7.326	39.530
24	1461.49	7.308	17.980
25	1460.19	7.302	19.552
26	1453.05	7.266	35.592
27	979.54	4.898	107.957
28	827.15	4.136	27.097
29	819.98	4.100	25.691
30	653.55	3.268	15.229
31	639.59	3.198	119.241
32	529.49	2.648	415.207
33	505.93	2.529	15.732
34	498.55	2.493	20.609
35	408.77	2.044	135.597
36	407.19	2.036	29.818
37	387.28	1.937	47.159
38	324.48	1.622	15.252
39	258.38	1.292	44.944
40	251.18	1.256	100.455
41	244.97	1.225	39.549

OBSERVE: Nucleus ____ Freq ____ MHz; Spec Width ____ Hz; Offset ____ Hz; Acq Time ____ sec; Delay ____ sec; Pulse Width ____ μsec; Transients ____

DECOUPLE: Nucleus ____ Offset ____ Hz; Mode ____ Power ____ db; Modulation Mode ____ Freq ____ Hz; Pulse Width ____ μsec; Power Mode ____

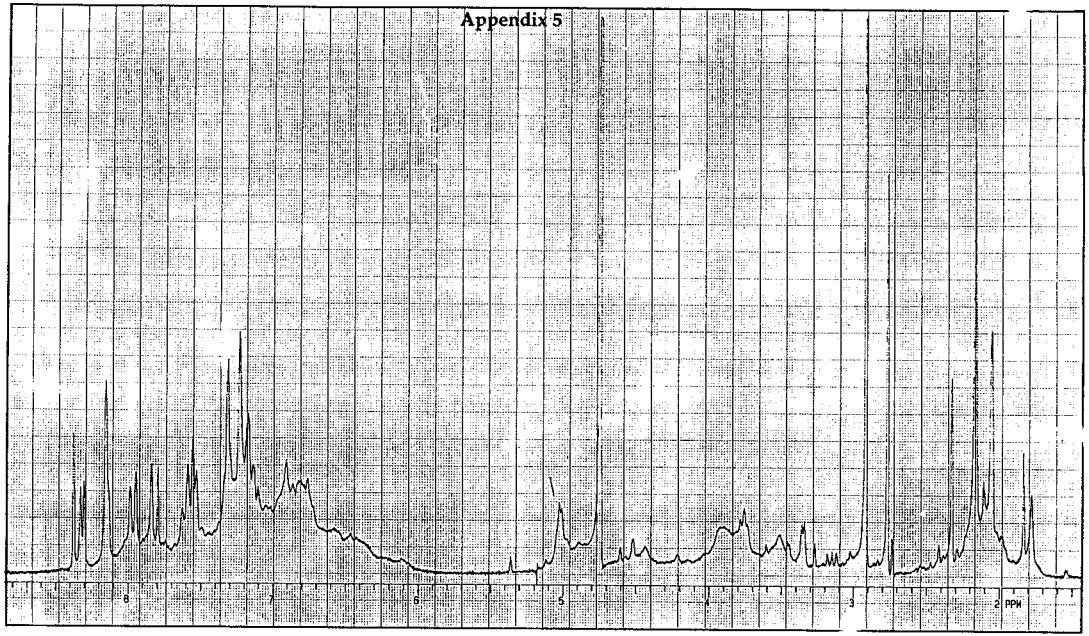
PLOT/PROCESSING: FN ____ K RE ____ sec CD ____ sec; LB ____ Hz AF ____ sec CCD ____; Width ____ Hz/ppm Start ____ Hz/ppm; Reference ____

EXPERIMENT: Pulse Sequence ____; Tube OD ____ mm; Temp ____ °C; Solvent ____

SAMPLE

Number ____; File ____; Date ____; XL ____

Appendix 5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EXPERIMENT
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SOL. P120.