

LINKED BETA-CYCLODEXTRINS

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A Thesis

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The Faculty of the Department of Chemistry  
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In Partial Fulfillment

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by

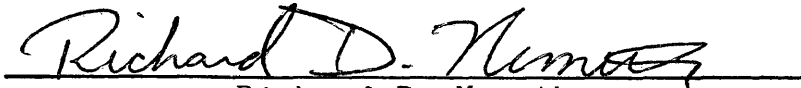
Richard Desider Nemeth

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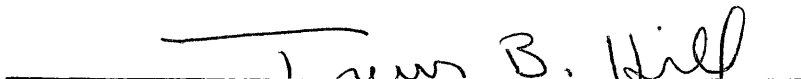
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Richard D. Nemeth

Approved, August 1988

  
Christopher J. Abelt, Ph.D.

  
Randolph A. Coleman, Ph.D.

  
Trevor B. Hill, Ph.D.

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## ABSTRACT

The syntheses of two covalently linked cyclodextrin molecules were developed. First (3-deoxy-3-[(4-thiomethyl)benzylthio])-beta-cyclodextrin was synthesized and then (6-deoxy-6-[(4-thiomethyl)benzylthio])-beta-cyclodextrin was synthesized. The 2,3-epoxide was reacted with the dithiol compound to form the 3-deoxy compound. The C(6) tosylate was reacted with the dithiol compound to form the 6-deoxy compound. Both products should be useful in the photochemical dimerization of certain active molecules.

LINKED BETA-CYCLODEXTRINS

## INTRODUCTION

There are certain organic molecules which have the ability to form cyclic inclusion complexes with other molecules. The cyclodextrins belong to this group. More commonly though, this feature is known as a host-guest relationship.<sup>1</sup> In this situation, cyclodextrin acts as a host to another molecule which is the guest. The guest molecule is then included within the cavity of the cyclodextrin. Because of this unique quality, these molecules can be used as enzyme models, and thus, other uses as potential catalysts can be explored.

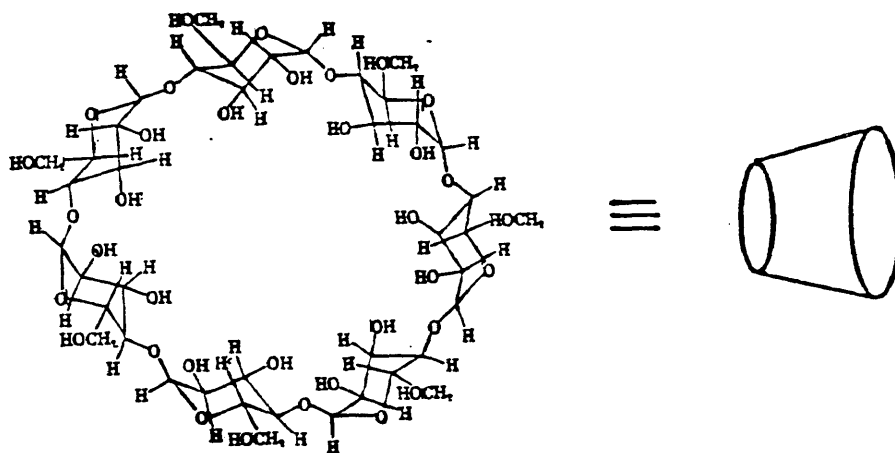
As a result of the structure of cyclodextrin, it possesses the capability of binding to other molecules at both its primary and secondary face. When a molecule attempts to enter the cyclodextrin cavity, it must possess the proper shape and size in the same manner in which an enzyme and its substrate works. Since selectivity occurs here, a regioselective functionalization may result in many of these reactions, and cyclodextrins will function to optimize the inclusion

during functionalization. Thus, cyclodextrins can be a great insight to the workings of enzyme systems.

There are many ways in which cyclodextrin can be used as an inclusion compound, some of which include thermally regulated reactions<sup>1</sup> and also photochemical reactions.<sup>2</sup> In addition, cyclodextrin derivatives have been synthesized and used in various linking reactions which mimic the actions of enzymes.<sup>3</sup>

Figure 1

beta-cyclodextrin



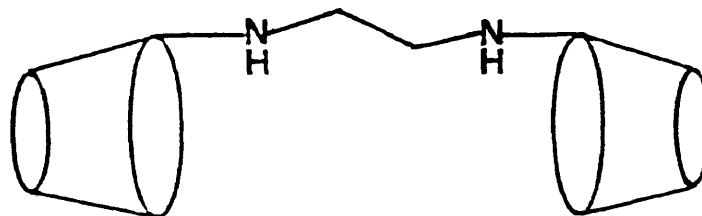
The research we have undertaken pertains to the synthesis of two hosts, so that they may later be utilized in photochemical dimerizations. One of these hosts occurs through linking on the primary side (narrower side) of beta-cyclodextrin, while the other one occurs through linking on the secondary side (wider side). The target structure

attempted with primary hydroxyl group binding is (6-deoxy-6-[(4-thiomethyl)benzylthio])-beta-cyclodextrin, while the structure attempted with secondary hydroxyl group binding is (3-deoxy-3-[(4-thiomethyl)benzylthio])-beta-cyclodextrin.

The basic idea in both target structures is to prepare a bis-beta-cyclodextrin using a linking bridge composed of a certain compound which will covalently bond to the cyclodextrin at both the primary and secondary sites. Therefore, the guest molecule will bind to this host molecule via non-covalent interactions. This bis-beta-cyclodextrin will be a more effective catalyst which will provide a greater rate enhancement over a catalyst with only one beta-cyclodextrin present<sup>4</sup> (Figure 2).

Figure 2

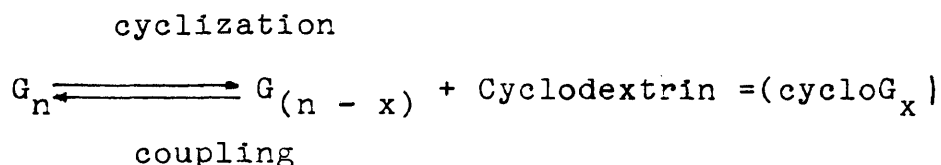
A linked beta-cyclodextrin



## BACKGROUND ON CYCLODEXTRINS

The history of cyclodextrins dates back nearly 100 years. Discovered by Villiers in 1891, cyclodextrins are a group of oligosaccharides which are produced by the action of amylase of *Bacillus macerans* on starch and other similar compounds. However, it wasn't until several years later that Schardinger was able to isolate and describe the preparation of these cyclodextrins as being cyclic oligosaccharides composed of anywhere from six to twelve alpha - 1,4 linked glucose units.<sup>5</sup> The most common cyclodextrins though, have six, seven, or eight glucose units and are designated alpha, beta, and gamma respectively. The other cyclodextrins are not as common and are therefore much more difficult to isolate. Cyclodextrins with less than six glucose units, though, have not been detected, probably because of the large degree of steric hindrance which would be present.<sup>6</sup> Regardless of steric hindrance, the degradation of starch is still the most common method of obtaining cyclodextrins because it is a fairly simple and direct process.

Starch is a linear polysaccharide made up of alpha(1,4) linked glucose units, and when degraded by amylase or other glycosyl transferases, the glucose units are detached and then joined together at the ends to produce the cyclic molecules.<sup>8</sup> These glycosyl transferases all act in the same manner following the scheme



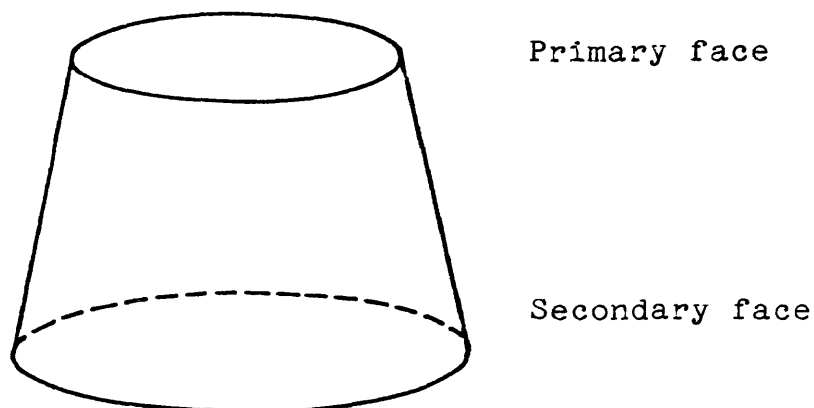
and are thus usable on many oligo- and polysaccharides.<sup>8</sup> The varying number of glucose molecules is a result of the enzymes never detaching a specific length of glucose units from the starch and also because different enzymes have a tendency to detach different lengths especially when influenced by the addition of organic compounds.<sup>9</sup> In the case of producing industrial beta-cyclodextrins, the most profitable method has been to use the glycosyl transferase from *Bacillus* No. 38-2. Even at 80° C and at pH 6 - 10, conditions which would cause severe damage to other enzymes, it is unaffected and is successful in converting 80% of the starch into beta-cyclodextrin while only trace amounts of other cyclodextrins are produced. In addition, no organic compound need be added to obtain high yields, and this is very fortunate as they are often toxic.<sup>10</sup>

Cyclodextrins have a toroidal shape (Figure 3) with all the glucose units in basically unaffected C1 chair confor-

mations, with the 3 hydroxyl hydrogen being hydrogen bonded to the 2 hydroxyl oxygen of the neighboring ring. From NMR studies it has been shown that the C1 conformation persists in solution also and that the hydrogen bonding is also unaffected in solution.<sup>5</sup> It is this apparently strong intramolecular hydrogen bonding which may very well be responsible for the conical shape of cyclodextrins. They possess a hydrophobic cavity and inner surface, relatively apolar compared to water, and a hydrophilic face. As a result of a lack of free rotation in the glycosidic bond, the cyclodextrins are not perfectly cylindrical but rather have a more cone-like shape. The narrow side is on the 6-hydroxyl face, while the wider face has the 2,3-hydroxyls on it.

Figure 3

Cyclodextrin



The interior of the torus consists of only a ring of C-H groups, a ring of glucosidic oxygens and one more ring of C-H groups. On the primary hydroxyl side, the hydroxyl groups can

rotate slightly so as to block the cavity a small amount, whereas on the secondary hydroxyl side the hydroxyl groups are on fairly rigid chains which do not allow for any rotation.<sup>1</sup>

From <sup>13</sup>C-NMR, the chemical shifts of alpha, beta, and gamma cyclodextrins can be compared with other similar compounds to show the similarities between these cyclic oligosaccharides and other linear chain analogs like alpha-maltose and amylose. From the values shown in Table 1,<sup>7</sup> the conformational restrains of cyclodextrins can be readily understood. Basically, because of strain, the shifts are downfield when compared to amylose and alpha-maltose by about 2-3 ppm at C1 and about 3-5 ppm at C4.

Table 1

<sup>13</sup>C Chemical shifts for alpha, beta, and gamma-cyclodextrin and some similar compds.

Compound	Solvent	C1	C2	C3	C4	C5	C6
alpha-cyclo	D <sub>2</sub> O	90.5	118.6	120.0	110.7	120.3	131.6
	DMSO	89.7	118.3	119.5	109.5	119.5	131.5
beta-cyclo	D <sub>2</sub> O	90.1	118.8	119.8	110.8	120.0	131.6
	DMSO	89.7	118.5	119.2	110.1	119.6	131.6
gamma-cyclo	D <sub>2</sub> O	90.3	119.1	119.7	111.5	120.2	131.7
	DMSO	89.9	118.3	118.9	110.6	119.4	131.5
amylose	DMSO	91.6	118.4	119.8	113.8	120.0	131.2
6-deoxy-beta-cyclo	DMSO	89.4	118.5	119.1	103.4	125.1	174.3

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Peaks are downfield from a standard CS<sub>2</sub>.

These results show just how strong the intramolecular

hydrogen bonding is within these molecules. It is so strong that even in dimethyl sulfoxide the hydrogen bonding stays intact, whereas in most cases DMSO breaks down hydrogen bonds between two solute molecules.<sup>11</sup> The strength of these hydrogen bonds is directly resultant from the limited rotation of the glucose units in the cyclodextrins. Overall, this makes cyclodextrins very stable molecules. Interestingly, the free energy of alpha, beta, and gamma- cyclodextrins are higher than those for their respective length linear glucose chains. At 25° C they are higher by +2.29, +1.71 and +1.99 kcal/mole for alpha, beta, and gamma-cyclodextrin. Therefore, the cyclization of linear glucose chains into cyclodextrins is not favorable energetically and when done the enthalpies increase by +6.60, +4.41, and +4.40 kcal/mole for alpha, beta, and gamma-cyclodextrin. This is clear proof of the instability given to the molecule when cyclization is forced upon it. However, the entropy values, which are +14.4, +9.1, and +8.1 e.u. respectively for alpha, beta, and gamma-cyclodextrin, give a favorable indication to the cyclization process, but this is probably a result of the water being stabilized and reorganized about the glucose chain during the cyclization process.<sup>12</sup>

The most important characteristic that cyclodextrins possess is the ability to form inclusion complexes with various other compounds, in which these "guest" compounds are included into the cavity of the cyclodextrin. Thus, they are given the name of guest-host complexes.<sup>13</sup> These guest compounds range from polar substances like acids, amines,

small ions like  $\text{ClO}_4^-$ ,  $\text{SCN}^-$ , and halogen anions, to very apolar aliphatic and aromatic hydrocarbons and even noble gases.<sup>14,15</sup> Inclusion complexes can be formed in both a crystalline state or in solution, with water being the most commonly used solvent, but with DMSO and DMF also being used.<sup>16</sup> Stability of the complexes depends upon the size of both the guest and the host molecules (Table 2), with a very large substrate not being able to penetrate the cavity resulting in no complex formation occurring. Yet, if the substrate is too small, it will pass in and out of the cavity without proper binding occurring, and once again no complex will form. Basically, the substrate must be within certain limits of size to properly bind with the cyclodextrin molecule.<sup>17</sup> For this reason, certain solvents may cause one type of cyclodextrin to precipitate out of solution while leaving other cyclodextrins in solution because the insoluble complex could only be formed where the substrate made a proper fit.

Table 2

Cyclodextrin Parameters			
Cyclodextrin	# of glucose units	Cavity measurements (Å)	
		Diameter	Depth
alpha	6	4.5	6.7
beta	7	7.0	7.0
gamma	8	8.5	7.0

The ratio of guest to host molecules in most complexes is 1:1 on a molar scale when formed in solution. There are some exceptions, though, when the guest molecules are certain long chain aliphatic carboxylic acids. The detection of these

inclusion complex formations can be done by nuclear magnetic resonance, absorption, fluorescence, and optical rotation. However, the most effective method of verification of an inclusion complex is by  $^1\text{H-NMR}$ .<sup>1</sup> In addition, thin layer chromatography can be employed for a quick verification of whether the inclusion complex has formed, provided a solvent can be found in which both the cyclodextrin alone and the inclusion complex are stable. In contrast, IR spectroscopic methods are not very useful for detecting inclusion complexes because the bands from the cyclodextrin often mask the bands which should be assigned to the guest molecule.

It is the ability of cyclodextrins to form inclusion complexes in aqueous solution which enables the use of spectroscopic methods for analysis. Therefore, NMR has been able to contribute the most to the understanding of guest-host interactions. Whereas X-ray methods of analysis gave structural details about these complexes, NMR spectroscopy allows the dynamic properties of the complexes in solution to be studied.<sup>8</sup>

In solution, inclusion complexes more often than not form at a 1:1 ratio of host to guest. Yet, this does not hold true for the majority of inclusion complexes in the crystalline state where the ratios of host to guest are usually nonstoichiometric. This is related to the three dimensional structure of the crystalline inclusion complexes, and has been verified by X-ray crystallography which has been performed on a large number of inclusion complexes.<sup>18,19</sup> In addition, within the crystalline state, there are two dif-

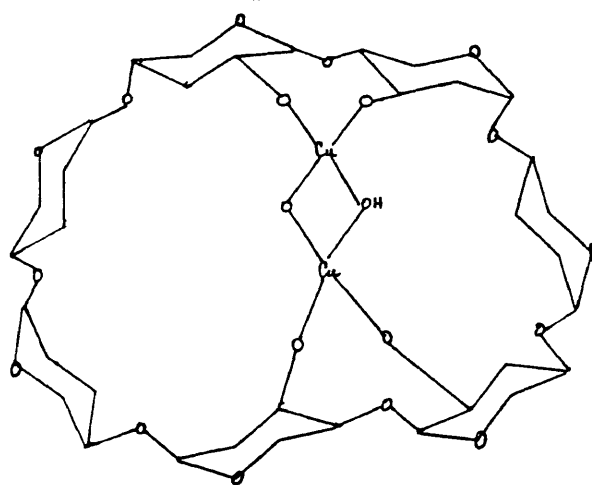
ferent types of inclusion complexes. One is called channel, while the other is known as a cage-type structure. The channel structures form when cyclodextrins stack themselves on top of each other to produce an endless channel, inside of which of course the guest molecules are included. Cage structures come about when the arrangement of cyclodextrins are displaced and the guest molecules end up being located in small cavities within the structure.<sup>20</sup> An example of the cage-type structure is the alpha-cyclodextrin-iodine tetrahydrate where the iodine molecule is included in the cavity and is co-axial with the alpha-cyclodextrin.<sup>21</sup> On the other hand, the complex of alpha-cyclodextrin with methyl orange sodium salt or with potassium salt possesses a 2:1 guest-host ratio in the crystalline state and is constructed with a typical channel structure. The azo group and the benzene ring are both located within the cyclodextrin cavity, while the dimethylamino and sulfonate groups remain on the exterior of the cavity and are hydrogen bonded to the primary hydroxyl groups of the adjacent alpha-cyclodextrin molecules. The structures of the complexes with the methyl orange sodium salt and with the potassium salt were the same in all areas.<sup>22</sup>

When these inclusion complexes form, those substrates which are large enough to fill the cavity in the cyclodextrin all occupy the center of the cyclodextrin cavity. However, when a substrate is too small to fully fill the cyclodextrin cavity, then the cavity is filled in an unorganized manner. An example is that only one methanol molecule will occupy the cavity in alpha-cyclodextrin but it is divided over two sites

with an equal 50% division. At one site, 50% of the population is near to the O(6) side of the cavity where the methanol is hydrogen bonded to the O(6) hydroxyl group. The other 50% of the population is held within the cavity but is not involved in any hydrogen bonding. This has also been shown for krypton and n-propanol.<sup>23,24,25</sup> From this information, Saenger et al. have proposed that the motivating force behind inclusion complex formation is the release of strain energy which occurs when the high energy conformation of the cyclodextrin-water complex is converted to the lower energy conformation of the cyclodextrin-guest complex.<sup>26</sup>

In addition to inclusion complexes, cyclodextrins can also form coordination complexes with  $\text{Cu}^{2+}$  by using the hydroxyl groups on the cyclodextrins. In beta-cyclodextrin, two pairs of C2 and C3 secondary hydroxyl groups on the glucose units are cross-linked by  $\text{Cu}(\text{OH}^-)(\text{O}^{2-})\text{Cu}$  ion bridge. (Figure 4)

Figure 4<sup>1</sup>



Another function of cyclodextrin is not as a host but as a competitive inhibitor. Beta-cyclodextrin binds in a 3:1 ratio to the active center of pancreatic amylase. In this case, the

cyclodextrin comfortably fits into a well on the amylase molecule, where the substrate amylose usually binds, and therefore, the two, cyclodextrin and amylose compete for the active site.<sup>27</sup>

In general, the formation of inclusion complexes can be broken down into several parts. 1) The approach of the substrate and cyclodextrin together. 2) The removal of water molecules from the cavity of the cyclodextrin and from the areas nearest to the cyclodextrin ring. 3) Assimilation of these water molecules into the water surrounding the cyclodextrin --- a gain in entropy. 4) Attraction of the substrate and cyclodextrin together because of van der Waals forces that are present and the possible formation of hydrogen bonds. 5) The hydrated structure being reconstituted around the final complex.<sup>8</sup> With beta-cyclodextrin, when the substrates are very small ions, then there is no steric hindrance and the complex formation can occur much more quickly. The entire rate seems to be dependent more upon substrate size than anything else, and is therefore, not diffusion controlled.<sup>28</sup> (Table 3)

Table 3<sup>28</sup>

Thermodynamic and kinetic parameters in complex formation with beta-cyclodextrin

Guest	$K_D$ mol/l	$k_R$ 1/molx1/s	$k_D$ 1/s
Cl <sup>-</sup>	0.39	$5.4 \times 10^7$	$2.1 \times 10^7$
Br <sup>-</sup>	0.15	$4.5 \times 10^7$	$6.9 \times 10^6$
SCN <sup>-</sup>	0.10	$4.4 \times 10^7$	$4.4 \times 10^6$
NO <sub>3</sub>	0.18	$4.5 \times 10^7$	$8.2 \times 10^6$

Many different crystal structures of alpha and beta-cyclodextrin have been studied. For alpha-cyclodextrin, complexes with water,<sup>29</sup> methanol,<sup>30</sup> polyiodide,<sup>31</sup> iodine,<sup>32</sup> krypton,<sup>33</sup> n-propanol,<sup>34</sup> m-nitrophenol,<sup>35</sup> and methyl orange,<sup>36</sup> and potassium acetate,<sup>37</sup> plus some others have been analyzed. With beta-cyclodextrin, the crystal structures of the complexes with water, n-propanol, n-iodophenol, and p-nitroacetanilide<sup>38,39,40</sup> have all been studied. In comparing all these crystal structures, the toroidal shape of the cyclodextrins remains intact in the inclusion complexes and the glucose units remain in the C1 chair conformation.<sup>8</sup>

The X-ray data on alpha, beta, and gamma-cyclodextrins show that the average distances between O(2) and O(3) are definitely different (Table 4). The values show that in alpha-cyclodextrin the interactions are weaker and it therefore possesses greater flexibility than does beta or gamma-cyclodextrin. This data is also confirmed by theoretical studies and spectroscopic data.<sup>41,42</sup>

Table 4

Cyclodextrin	O(2) - O(3) Distance
alpha	3.00 A
beta	2.86 A
gamma	2.81 A

The driving force behind the formation of complexes with cyclodextrins has been debated for as long as the presence of inclusion complexes have been known to exist.<sup>43,44</sup> The fact that more than one force is involved is well known, but how

extensive each one is depends upon which substrate happens to be involved in the complex. Basically van der Waals forces are the most prevalent during inclusion complex formation but the formation of hydrogen bonds also has a large effect on the complex formation. In addition, hydrophobic interactions are also involved since during inclusion the guest molecule must expel water molecules already present from the cyclodextrin cavity, plus remove its own water surroundings. The freed water molecules then join with the water medium surrounding everything, where they become freer and thus add stability to the entire complex which in turn leads directly to the increase in entropy of the system.<sup>8</sup> It is also possible that the water within the empty cavity of the cyclodextrin molecule may provide its own inherent energy to the cyclodextrin since this water is in a very unfavorable and hydrophobic environment. It might cause the water to be forced out from the cavity because it cannot hold on to its bonding comfortably. So, by being forced out of the cavity, the complex formation is favored since it is accompanied by an increase in the entropy in addition to a gain in the potential energy.<sup>45,46</sup>

With alpha-cyclodextrin, the empty molecule is in a strained state, so when complex formation occurs, this strain is relieved as hydrogen bonds are formed between the O(2)H and O(3)H groups of neighboring glucose units. Thus, a gain in energy occurs. This holds only for alpha-cyclodextrin since the empty molecules of beta and gamma-cyclodextrin are not under strain.<sup>47</sup>

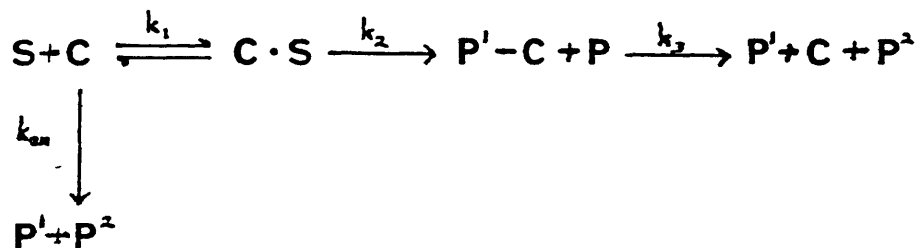
Though more recent studies have shown that van der Waals forces and hydrophobic interactions are the most prominent forces contributing to inclusion complex formation, the inherent forces of the cyclodextrin molecule may also play some role. Once again, the amount which each force provides to the whole is still dependent upon the specific substrate which is going to be the included guest molecule. The ring structure of cyclodextrins is very important, though, as inclusion complexes will only form if the physical fit between host and guest is proper and within certain limits which depend on the size of the cyclodextrin. In Table 5, some of the thermodynamic parameters in the formation of inclusion complexes are shown.<sup>1</sup>

Table 5<sup>95,96,97</sup>

Thermodynamic parameters of inclusion formation				
Guest	Cyclo-dextrin	H kcal/mol	S (e.u.)	Determined
p-nitrophenol	alpha	-4.2	-2.8	spectrophotometric
p-nitrophenolate ion	alpha	-7.2	-8.7	spectrophotocalorimetry
	beta	-3.4	+1.2	
m-ethylphenyl acetate	beta	-4.6	-3.0	kinetic method
Benzoylacetic acid	beta	-5.7	-8.6	kinetic method
p-methylbenzoyl acetic acid	beta	-6.6	-9.8	kinetic method
m-chlorobenzoyl acetic acid	beta	-5.2	-6.0	kinetic method
Diisopropyl phosphorofluoridate	alpha	-7.3	-21.0	kinetic method

Definitely, the most interesting use of cyclodextrins is their application as enzyme active site models. In these applications the cyclodextrins are not just forming inclusion complexes, but are actually breaking and making covalent bonds. In the case of racemic mandelic acid esters, they are hydrolyzed at a rate that is 1.38 times faster when cyclodextrins are present than when they are absent. In this reaction, a partial chiral induction occurs as the cyclodextrins act as enzymes and exhibit specific catalytic properties.<sup>48</sup> Thus, cyclodextrins are very useful as catalysts for certain reactions, and these catalyses may be broken

down into two parts. One type is where the cyclodextrin forms a covalently bonded intermediate, and the other type is where no covalently bonded intermediate is formed. If a covalently bonded intermediate does form, then it can be designated by this general formula,<sup>45,46</sup>



where  $k_m$  is the rate constant for the uncatalyzed reaction without cyclodextrin present, and  $k_1$  is the rate of enzyme-substrate complex formation,  $k_2$  is the formation of the covalent intermediate  $P^1-C$ , which is in turn hydrolyzed at a rate of  $k_3$  to the final end product and cyclodextrin. Several reactions which are cyclodextrin catalyzed, and by what method, are shown in Table 6.<sup>8</sup>

Table 6<sup>98</sup>

## Cyclodextrin catalyzed reactions with or without intermediates

Reaction	Guest	Acceleration factor	intermediate
Oxidation	alpha-hydroxyketones	3	no
Ester hydrolysis	phenyl esters	300	yes
	mandelic ester	1.4	?
Amide hydrolysis	penicillins	89	yes
	acetanilides	16	yes
Carbonate cleavage	aryl carbonates	19	no
Decarboxylation	glyoxylate ion	4	no
	cyanoacetate ion	44	no

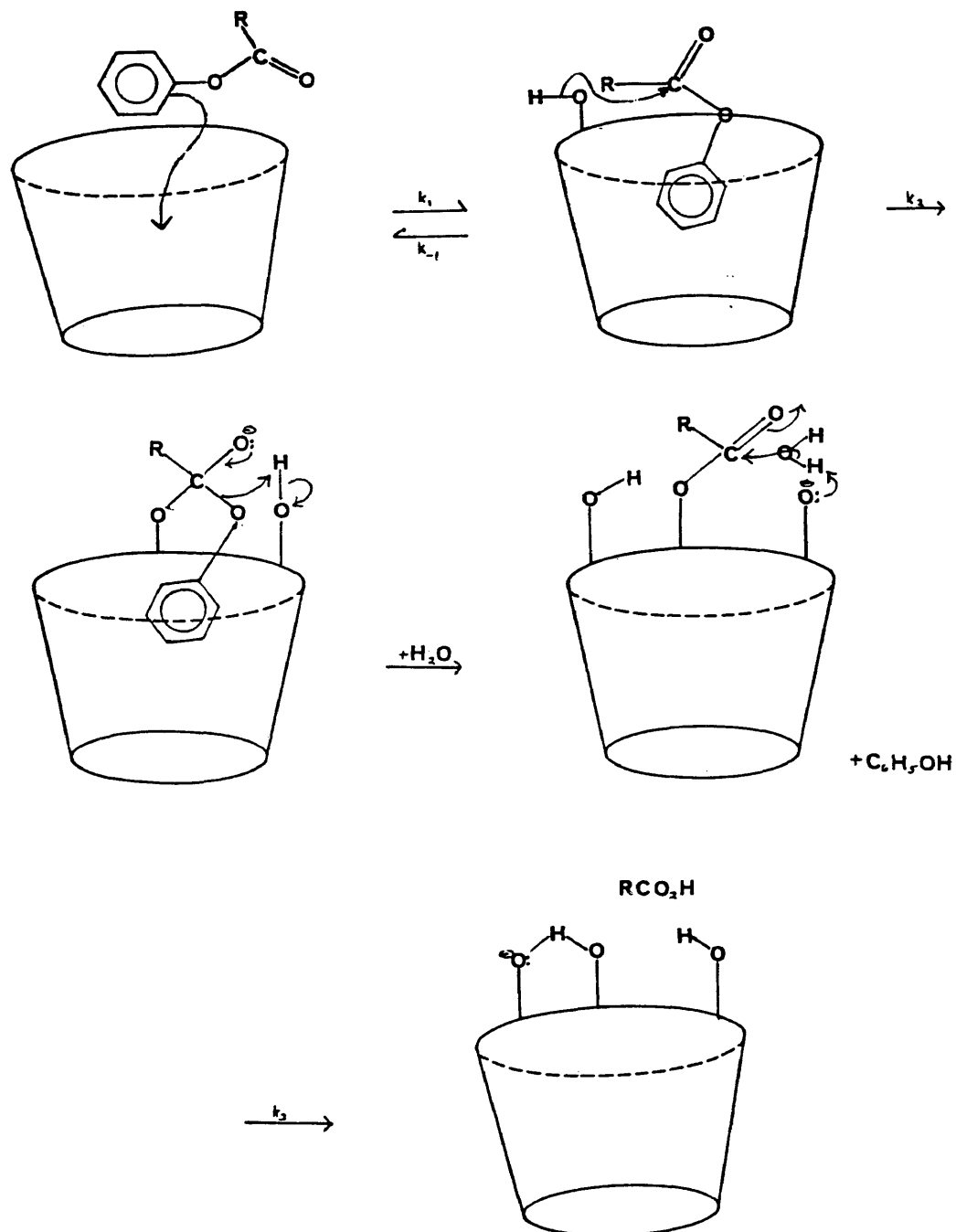
In the case where no covalently bonded intermediate is formed, the catalysis is a result of a "microsolvent effect of the microheterogeneous cavity" within the cyclodextrin.<sup>49</sup> Thus, an inhibition must occur if a foreign molecule is added to the reaction as the substrate must compete with it to bind with the cyclodextrin.<sup>45</sup> These phenomena have been well studied to give conclusive proof that cyclodextrin catalyzed reactions act very much like basic enzymatic reactions and, therefore, serve very well as models for enzyme behavior.

One reaction pathway that has been investigated in great detail is the hydrolysis of phenyl esters since much is known about an analogous biochemical pathway, the cleavage of

esters by chymotrypsin.<sup>50</sup> Phenyl acetates can be hydrolyzed most efficiently at pH=11<sup>46</sup> with cyclodextrin present. The cyclodextrin nucleophilically attacks the carboxy group on the substrate with the deprotonated secondary hydroxyl groups functioning as the active species.<sup>51</sup> (Figure 5) From experiments on meta and para-substituted phenyl acetates, it has been found that the ratio of  $k_2/k_{un}$  is extremely dependent upon the geometry of the substrate. The values for para and meta-toluy1 at a pH=10.6 at 25°C were 3.3 and 95 respectively, showing that the substrate enters the cyclodextrin cavity in an axial manner and with the para form the orientation remains axial. However, when the substituent is in the meta position, the acetate group is nearer to the ring that is formed by hydrogen bonds between O(2)H and O(3)H, which in turn allows for a more sterically favorable interaction to take place in the reaction.<sup>8</sup>

Figure 5

## Phenyl acetate hydrolysis



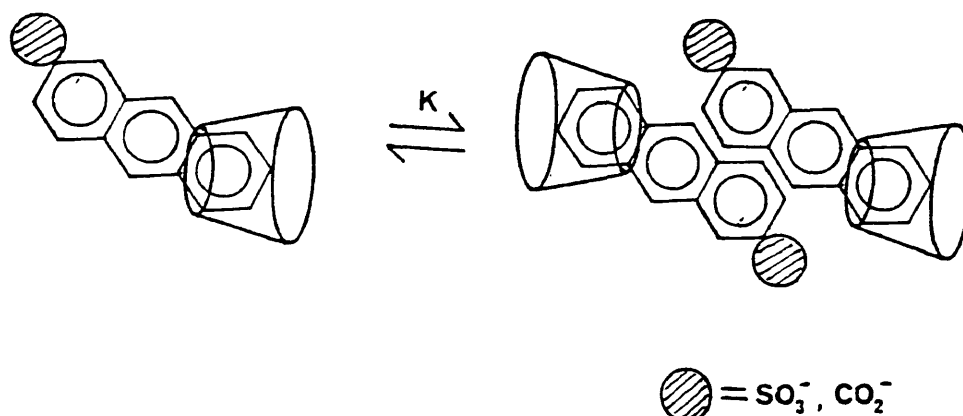
Cyclodextrins in this case, though, are not perfect enzyme mimics. The first catalytic step is approximately

equal for both cyclodextrin and chymotrypsin, but it is necessary to keep in mind that cyclodextrin performs at its optimal level in a strongly basic environment, whereas chymotrypsin works best in a near neutral environment of pH=8. In addition, step  $k_3$  is very quick for chymotrypsin, but slow for cyclodextrin. However, through the many years chymotrypsin has specialized itself as an enzyme to function especially well in the hydrolysis of esters and amides, where peptide bonds are present, and cyclodextrin only accelerates these reactions nominally.<sup>46,52</sup> Some of the amide hydrolyses which cyclodextrins catalyze are penicillin derivatives,<sup>53</sup> acetanilide,<sup>54</sup> and N-acylimidazole.<sup>52</sup> The microsolvent effect and the steric properties of the inclusion complex only play a role if there is no intermediate involved, so the cyclodextrin doesn't determine as much of the reaction proceedings as if there was an intermediate.

Recently, research has been done in an area which is very near to our work. This is the finding that some water-soluble anthracene derivatives, like anthracenesulfonate and anthracenecarboxylate, are included in aqueous solution with beta and gamma-cyclodextrin by up to two molecules. This results in a double guest host (2:2) complex and a two guest-one host (2:1) complex which can then be photodimerized more rapidly by the complex formation.<sup>83,84</sup> (Figure 7)

Figure 7<sup>85</sup>

Anthracene inclusion complexes with beta-cyclodextrin



The specific inclusion pattern of anthracene in these complexes is significantly dependent upon the substituent group which is attached to the aromatic ring. In the cases of 2 - anthracenesulfonate (2AS) and 2 - anthracenecarboxylate (2AC), the hydrophilic substituent groups at the C-2 position greatly increase the overall stability of the inclusion complex, since the entire aromatic portion of the guest can

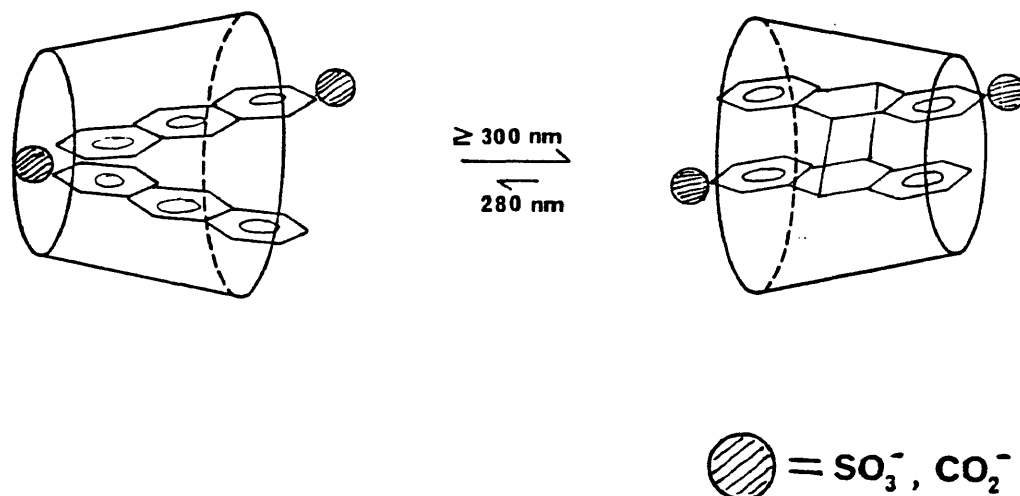
be totally immersed into the hydrophobic cavity of the host, while the hydrophilic group remains outside of the host in the aqueous surroundings. However, the meso-substituted (C-9) derivative severely reduces the stability of the inclusion complex because it hinders the aromatic portion from being totally engulfed into the hydrophobic cavity. The substituent residing at the C-1 position, though, only causes a small change in behavior of the inclusion complex.<sup>85</sup>

Two anthracene molecules will fit into the cavity of gamma-cyclodextrin since its diameter is 8.5 Å, but two anthracene molecules will not fit into the beta-cyclodextrin cavity with a diameter of approximately 7 Å. As a result, only 1:1 complexes form and the stability of those complexes with 2AS and 2AC is greater than that of complexes with 1AS and 1AC. With gamma-cyclodextrin the 2:1 (Figure 8) inclusion complex will form, yet it was not detected with beta-cyclodextrin because of the smaller cavity size.

In the instances of 2AS and 2AC in the presence of beta-cyclodextrin, the photodimerization is strictly regioselective, whereas this is not the case in solutions which contain gamma-cyclodextrin. There is only one isomer produced in the presence of beta-cyclodextrin (Figure 7), where this selectivity is most probably a result of the specific configuration of the guest molecules within the host cavity, where two guest molecules end up solely in a head to tail configuration. The joining of two 1:1 complexes together in such a fashion can thus produce the 2:2 complex.<sup>86</sup>

Figure 8<sup>85</sup>

Anthracene inclusion complexes with gamma-cyclodextrin



It is believed that the chirality associated with cyclodextrin, which is composed of D(+) - glucopyranose units, may account for the stereospecific recognition and stereoselective reaction that finally causes the 2:2 complexes to form.<sup>87</sup> Further research must be performed in this area, however, to provide more conclusive evidence as to whether or not this is true.

In the end, cyclodextrins can be utilized in a great many ways simply because of the fact that they can form inclusion complexes with just about any small molecule having a diameter between 5 and 8 Å. Most applications of cyclodextrins will probably be in the micro-encapsulation area in an effort to find new uses in industry and for biological purposes. Currently, cyclodextrins are too expensive to be used on a large scale, but with research increasing, it may

soon be needed in more applications and possibly its manufacture may be made profitable on an industrial scale. As research continues, only time will tell.

## CYCLODEXTRINS AND THEIR APPLICATIONS

Over the past several years, the practical application of cyclodextrins has been expanded into many areas. Since cyclodextrin inclusion is a micro-encapsulation, cyclodextrins can be used in the stabilization of sensitive substances. Stabilization in turn helps to improve handling and utilization of these substances. Cyclodextrins will covalently bind to polymers and are useful in column chromatography. In addition, their application in agriculture has recently expanded and opened new areas to explore.<sup>8</sup>

In the inclusion of guest molecules within cyclodextrins, each guest is individually surrounded by a cyclodextrin molecule. This is seen as encapsulation on a microscopic level, and it can lead to beneficial changes in the chemical and physical properties of the guest molecule. For this reason, research has been performed on cyclodextrins with the idea of making inclusion a useful process in the manufacture of many household products. In industry, the improvements which cyclodextrin inclusion complexes have

brought about are very numerous.<sup>55-60</sup>

Among the improvements incurred are the stabilization of light and oxygen-sensitive substances. The chemical activity of guest molecules have been altered, thereby allowing for dangerous unstable substances to be made safe and workable through inclusion complexes. Reactions can be made more selective and rates can be manipulated to better suit the products. In addition, very volatile substances can be made controllable through inclusion complexes with cyclodextrin. They can be handled better and their tendency towards vaporization reduced so smaller quantities are needed to perform the same tasks. Also, the physiochemical properties of the guest molecules can be modified. Only moderately soluble substances can be transformed into extremely soluble ones, and once complexed, they can be powdered or freeze-dried so that they may be easily worked with and measured. Finally, pigmentation and color of certain substances may be changed, and the unpleasant tastes of edible substances can be cover over or even improved upon.<sup>8</sup>

One industrial use of cyclodextrins has been in insecticides. The active parts of "Dalmatian insect powder" from *flores pyrethri* are the pyrethins --- derivatives of chrysanthamum monocarboxylic acid ester and their synthetic analogues.<sup>61</sup> They are extremely toxic towards insects but totally harmless towards warm-blooded animals. These pyrethins are yellowish, light-sensitive oils and because of this their usefulness is not very broad. Yet, on inclusion in beta-cyclodextrin, a powder is formed which is very easy to

handle and is also totally stable and its toxicity lasts for a long time after its application.<sup>58,62</sup> (Table 7)

Table 7<sup>62</sup>

Pyrethrin activity decline against pine caterpillar.

Compound	Type	Dose%	Caterpillars killed % after # of weeks		
			0	1	2
Pyrethrins	Emulsified concentrate	0.1	90	10	0
		0.05	50	0	0
	Inclusion complex	0.1	90	100	90
		0.05	100	100	40

Like *flores pyrethri*, the insecticide DDVP (O -(2,2 - dichlorovinyl) -O,0 - dimethylphosphate) is another example which is unstable in its pure form but can be used very effectively after inclusion with beta-cyclodextrin.<sup>63</sup>

There are many other substances which are unstable when exposed to light or air, and which like these insecticides, can be stored after inclusion in cyclodextrin. One such beta-cyclodextrin complexed substance is the very unstable vitamin D<sub>3</sub> (cholecalciferol). After beta-cyclodextrin complexation and thorough study,<sup>64</sup> the thermal stability was found to be much higher than for pure vitamin D<sub>3</sub>, and its oxygen uptake very low even after a few weeks. Simultaneously, its sensitivity to light was found to be substantially lower when complexed, than when in the uncomplexed form. The cyc-

lodextrin complexes of prostaglandins have shown similar results and advantages, and these results have been passed on to production, as prostaglandin E<sub>1</sub> is now available commercially in the form of an alpha-cyclodextrin complex.<sup>65,66,67</sup>

In addition, beta-cyclodextrin has also had stabilizing effects on various household substances like aspirin.<sup>68,69,70,71</sup> Indomethazine is stabilized by beta-cyclodextrin, but alpha-cyclodextrin has no effect on it.<sup>72</sup> Azopropazone and trichlorophone, both insecticides, are more rapidly deactivated by beta-cyclodextrin than by alpha-cyclodextrin and in a related manner beta-cyclodextrin causes the isomerization of prostaglandin A<sub>1</sub> to prostaglandin B<sub>1</sub> and the dechlorination of chlorpromazine. Also, cyclodextrins have some small effects, through inclusion, on the stability of phenothiazines.<sup>73,74,75</sup>

The area of foodstuff production and the toiletry industry is where the real significance of the stabilization of aromatic substances comes into play for cyclodextrins. Anethole, a food additive, is usually a very oxidation sensitive substance, but after inclusion in beta-cyclodextrin, it is almost totally immune to oxidation. On top of this, the oily aromatic substances which exist in camomile, onions, dill, garlic, caraway, horseradish, and mustard are all very hard to handle until they are included in beta-cyclodextrin, after which they become very easy-to-handle powders.<sup>58,59,60</sup> Since all of their components are complexed to the same extent, the taste and smell of these substances remain totally unchanged. There are also some additional uses for

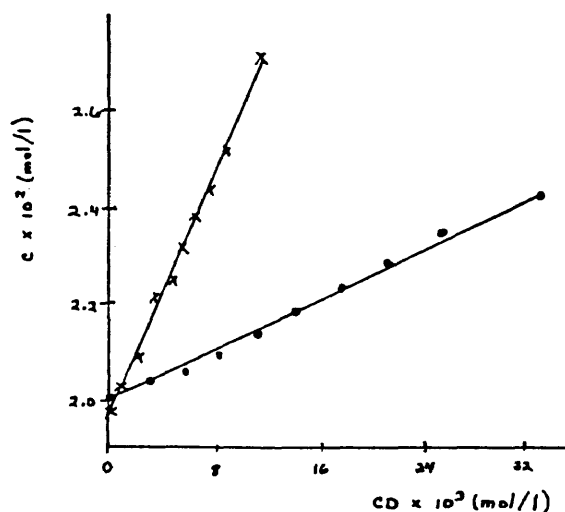
cyclodextrins in soaps and toothpastes.<sup>8,76</sup>

One unique effect beta-cyclodextrin has is in the stabilization of nitroglycerin when an inclusion complex is formed. Once complexed, the resulting crystals can be made into nice convenient tablets which are no longer explosive.<sup>77</sup> Similarly, the inclusion of ethylene into cyclodextrin provides another easy-to-work-with powder which is effective in controlling the growth of certain plants. Also, emulsions become more stable from cyclodextrins which allows for the manufacture of mayonnaise from an oil which can be beaten without the addition of eggs when beta-cyclodextrin is added. Other examples are butter and margarine which are infinitely more stable after the addition of beta-cyclodextrin.<sup>78</sup> Egg whites are more stable too, when complexed with cyclodextrin, and when frozen, their preservability is greatly enhanced up to a period of several months.

The increase in solubility of only sparingly soluble substances is accomplished with cyclodextrin complexation. This increase in solubility occurs in a very linear fashion in relation to the addition of cyclodextrin, and will eventually reach a maximum after which increasing cyclodextrin levels have no effect. (Figure 6)<sup>71,79</sup>

Figure 6

Increase in solubility of aspirin after addition  
of (X) alpha-cyclodextrin and (\*) beta-cyclodextrin



As a general rule, a cyclodextrin complex in the powdered form will dissolve much more easily and more quickly than the guest component would on its own, and even if the complexes do not dissolve, they can be more easily absorbed by living organisms. This is a direct result of the very fine dispersion which is present in the powdered form of a cyclodextrin complex.<sup>80</sup> Thus, the inclusion of drugs with cyclodextrin can allow for more rapid and more potent action when administered orally in comparison to the drugs being administered by themselves. Indomethazine is an anti-rheumatic agent which has ulcer producing capabilities, but when it is included with beta-cyclodextrin, its ulcer producing abilities are

almost totally hindered.<sup>60,81</sup> One fairly recent application of cyclodextrin is in peptide analysis, where the limits of detection are much better with cyclodextrins present since they produce a large increase in the fluorescence intensity of dansyl derivatives.<sup>82</sup>

## EXPERIMENTAL

beta-cyclodextrin-2-tosylate

The tosylate was prepared according to Murakami<sup>88</sup> et al. as follows: Stir 11.35 g (0.01 mol) beta-cyclodextrin into 130 ml of DMF and 25 ml of benzene. Using a Dean-Stark trap, heat an oil bath to 155° - 160° C to distil the water/ benzene azeotrope. After removing approximately 10 ml of water, add 6.25 g (0.025 mol) of dibutyltin oxide to the solution and continue heating at 155° - 160° C for 2.5 hours under a nitrogen atmosphere to remove water formed from the condensation reaction. Cool to 0° C. Add dropwise a solution of 3.15 g (0.031 mol) triethylamine and 5.15 g (0.027 mol) toluenesulfonyl chloride dissolved in 50 ml of DMF. Let this mixture stir at room temperature for approximately 18 hours. Distill the DMF/benzene mixture under aspirator pressure to almost complete dryness. Add 800 ml acetone to the yellow syrup to precipitate the beta-cyclodextrin and its derivatives. Let

stir for about 30 minutes and then filter the acetone and collect the precipitate by vacuum filtration. Wash the precipitate with acetone, and then dry **in vacuo**. Dissolve the dry powder in water and purify by reverse phase column chromatography (Silica Gel RP-18, 20 g). Elution gradient is 5% to 100% with aqueous acetonitrile with collection done in 8 ml fractions. Locate the tosylate by thin layer chromatography (silica gel on glass plates, 5:4:3 solvent with n-butanol, ethanol, water, and vanillin stain,  $R_f = 0.7$ ). Then collect all fractions (21 -34) with tosylate and concentrate **in vacuo**. The remaining crystals are collected and weighed. The yield of pure tosylate is approximately 10% (0.98 g). A small amount of beta-cyclodextrin-2,3-epoxide is also formed by this procedure.

#### beta-cyclodextrin-2,3-epoxide

Dissolve 1.40 g (0.0177 mol) of ammonium bicarbonate in 10.0 ml of water, add 720 mg (0.5 mmol) of beta-cyclodextrin-2-tosylate and let stir at 60°C for 5-6 hours. Perform an ion exchange using Cl ion exchange resin to remove excess ammonium bicarbonate and then purify by reverse phase RP-18 chromatography with a gradient elution of 10% to 80% aqueous acetonitrile. Verification of the epoxide is by thin layer chromatography (silica gel on glass plates, 5:4:3 solvent with n-butanol, ethanol, water, and vanillin stain,  $R_f = 0.6$ ).

Yield by this procedure is approximately 35 - 40% (280 mg).

3-deoxy-3-[(3-thiopropyl)thio]-beta-cyclodextrin

To the above epoxide, add 500 mg (4.62 mmol) of propanedithiol and stir at 60° C for 24 hours under a nitrogen atmosphere. The solution is then cooled and washed with three 50 ml portions of diethyl ether to remove the majority of the remaining unreacted propanedithiol. Add 50 ml of water and distill under aspirator pressure to remove all remaining dithiol until about 10 ml of fluid remains. Verify product formation by thin layer chromatography (silica gel on glass plates, 5:4:3 solvent with n-butanol, ethanol, water, and vanillin stain,  $R_f = 0.3$ ). Perform an ion exchange using Cl ion exchange resin and then concentrate the product **in vacuo**. Purify the product by reverse phase RP-18 chromatography with a gradient elution of 10% to 80% aqueous acetonitrile. The eluent is collected in 8 ml fractions and the product located by thin layer chromatography. All fractions with product (21 - 40) are then collected and concentrated **in vacuo**. The yield was less than 1% with 2 mg product isolated.

3-O-(4-hydroxyphenyl)-beta-cyclodextrin

To 50 ml of water, add 440 mg (0.004 mol) of hydroquinone and 60 mg of sodium bicarbonate (0.0007 mol). Next, add 500 mg (0.0004 mol) of the beta-cyclodextrin-2,3-epoxide dissolved in 25 ml of water. Add the epoxide solution dropwise to the hydroquinone solution and let stir overnight at 60°C. Neutralize the solution with ca. 5 ml of dilute 3% HCl after the reaction is complete and then wash with 250 ml of diethyl ether. After three attempts the solution continually ended up black instead of clear. No yield was obtained.

para-xylylene dibromide

Place 6.4 g (0.024 mol) of p-xylene, 22.0 g (0.124 mol) of N-bromosuccinimide, and 200 mg (0.0008 mol) of benzoyl peroxide in 60 ml of carbon tetrachloride. Reflux at 100°C until the vigorous boiling has subsided. Let the solution cool for a few minutes and then filter while still hot. Concentrate the solution **in vacuo** at 50°C. Cover the precipitate in 30 ml of petroleum ether (bp 35° - 60° C) and let stand overnight. The p-xylylene bromide crystallizes out slowly overnight. Recrystallize the residue crystals in ethanol and filter under aspirator pressure. Purity is verified by melting point (mp 140° C, literature value), and by thin layer chromatography (silica gel on glass plates, 5:4:3 solvent with n-butanol, ethanol, water, and vanillin stain,  $R_f = 0.4$ ). The yield for this reaction is 68%.<sup>89</sup>

1,4-bis(thiomethyl)benzene

Mix 1.46 g (0.0056 mol) of p-xylylene bromide with 6.00 ml of 2-propanol. Add a mixture of 1.59 g (0.021 mol) of thiourea in 2.5 ml of water while stirring. After the exothermic reaction has subsided, heat the mixture and stir while refluxing for 30 minutes. Let the mixture cool to room temperature. Add 2.10 ml of ammonium bicarbonate and heat once more to reflux. Let reflux continue for another 20 - 30 minutes. Recrystallize the resulting precipitate from hexane and 2 ml of chloroform. Filter the hexane/chloroform mixture and allow to stand overnight. Collect the crystals (0.28 g) by vacuum filtration and dry *in vacuo*. Verify the product by melting point (mp 155° C) and by thin layer chromatography (silica gel on glass plates, 3:1 solvent with methylene chloride, hexane, and vanillin stain,  $R_f = 0.45$ ). Yield is 5%.<sup>90</sup> Physical data is as follows: <sup>1</sup>H NMR: (DMSO-d<sub>6</sub>) δ4.69 (s, 4H), δ7.42 (s, 4H).

(3-deoxy-3-[(4-thiomethyl)benzylthio]-beta-cyclodextrin

Combine 500 mg (0.43 mmol) of the epoxide and 350 mg (2.06 mmol) of the dithiol compound in 40 ml of water. The dithiol compound must be three-fold molar ratio excess of the epoxide used. Heat the mixture to 60° C and stir for 19 hours

under nitrogen. Dilute the solution to 200 ml with water and extract it three times with 75 ml portions of diethyl ether. Concentrate the water layer **in vacuo** to dryness. Perform thin layer chromatography (silica gel on glass plates, 5:4:3 solvent with n-butanol, ethanol, water, and vanillin stain,  $R_f = 0.7$ ) to verify the compound. Approximately 0.55 g (0.43 mmol) of the crude adduct is retrieved from the water layer. Purify the product by reverse phase RP-18 chromatography using a gradient elution of 2% to 100% aqueous acetonitrile in sixteen 50 ml fractions. The product is located by thin layer chromatography and fractions 6 - 14 collected and concentrated **in vacuo**. Dissolve the resulting crystals in 100 ml of water and 15 ml of acetonitrile. Concentrate once again **in vacuo**. Collect the crystals and weigh. The adduct retrieved was 80 mg (0.062 mmol). The yield is 15% of still impure material. The  $R_f$  of the adduct is similar to that of beta-cyclodextrin-2-tosylate and is consistent with all results up to this point. Physical data for the adduct is as follows:  $^1\text{H}$  NMR: (DMSO- $d_6$ ) in addition to beta-cyclodextrin resonances  $\delta$ 4.47 (s, 4H),  $\delta$ 7.23 (s, 4H). There is a slight upfield shift for the 1,4-bis(thiomethyl)benzene peaks on the adduct. The NMR is consistent, however, with product formation and the NMR resonances give evidence for covalent bonding between beta-cyclodextrin and 1,4-bis(thiomethyl)benzene. The product was formed in such low yield, though, that further characterization was impossible.

beta-cyclodextrin-6-tosylate

Beta-cyclodextrin, reagent grade, was dried overnight **in vacuo** at 110° C, and pyridine was distilled under calcium sulfate drying immediately before use. Dissolve 29.60 g (0.026 mol) of dry beta-cyclodextrin in 300 ml of dry pyridine. Cool to 5° C. Add with stirring a solution of 3.65 g (0.019 mol) of toluenesulfonyl chloride in 30 ml of dry pyridine. After mixing, let stir overnight at room temperature. Distill off all the pyridine under reduced pressure at 50° C to complete dryness. A white residue remains in the flask. Let the residue cool and add 700 ml of diethyl ether and let stir at room temperature for about 5 hours. Filter off the ether and verify the product by thin layer chromatography (silica gel on glass plates, 5:4:3 solvent with n-butanol, ethanol, water, and vanillin stain,  $R_f = 0.6$ ). Cover the precipitate in 400 ml of acetone to remove any pyridinium hydrochloride which may have formed as a by-product. Let stir for 3 - 4 hours and filter. Concentrate the product using a Soxhlet extraction with 600 ml of isopropanol and 30 ml of water. Let the extraction continue overnight, and then remove the isopropanol and verify the product by thin layer chromatography. Wash the product in 100 ml acetone and concentrate **in vacuo** to isolate the tosylate. Purify the solid by reverse phase RP-18 flash chromatography using a gradient elution of 2% to 80% aqueous acetonitrile in sixteen 50 ml fractions. Locate the tosylate by thin layer chromatog-

raphy (fractions 9 - 12) and concentrate **in vacuo**. The crystals are then collected and weighed. Yield is only 3% pure C(6) tosylate based on tosyl chloride.<sup>91</sup>

(6-deoxy-6-[(4-thiomethyl)benzylthio])-beta-cyclodextrin

Using the C(6) tosylate directly, dissolve 81 mg (0.476 mmol) of the 1,4-bis(thiomethyl)benzene in 14 ml of water. Carefully add 140 mg (0.0018 mol) of ammonium bicarbonate and then add 300 mg (0.233 mmol) of the C(6) tosylate and 8 ml of DMSO to help dissolve all reactants. Stir while heating at 60° C for 16 hours under nitrogen. Let cool to room temperature and verify the product by thin layer chromatography (silica gel on glass plates, 5:4:3 solvent with n-butanol, ethanol, water, and vanillin stain,  $R_f = 0.6$ ). Wash the solution with three 40 ml portions of diethyl ether to extract any unreacted dithiol. Concentrate the water layer **in vacuo** to 5 ml and then add 100 ml acetone to precipitate out the product. Let stir for 2 hours and filter. Purify the product by reverse phase RP-18 chromatography with a gradient elution from 2% to 80% aqueous acetonitrile in 50 ml fractions. Locate the product by thin layer chromatography and concentrate the fractions (2 - 5) with the product **in vacuo**. The crystals are then collected and weighed (40 mg). The yield is 13%. Physical data for the product is as follows: <sup>1</sup>H NMR: DMSO-d<sub>6</sub> δ3.60 (s, 4H), δ4.20 (s, 4H). The NMR is not

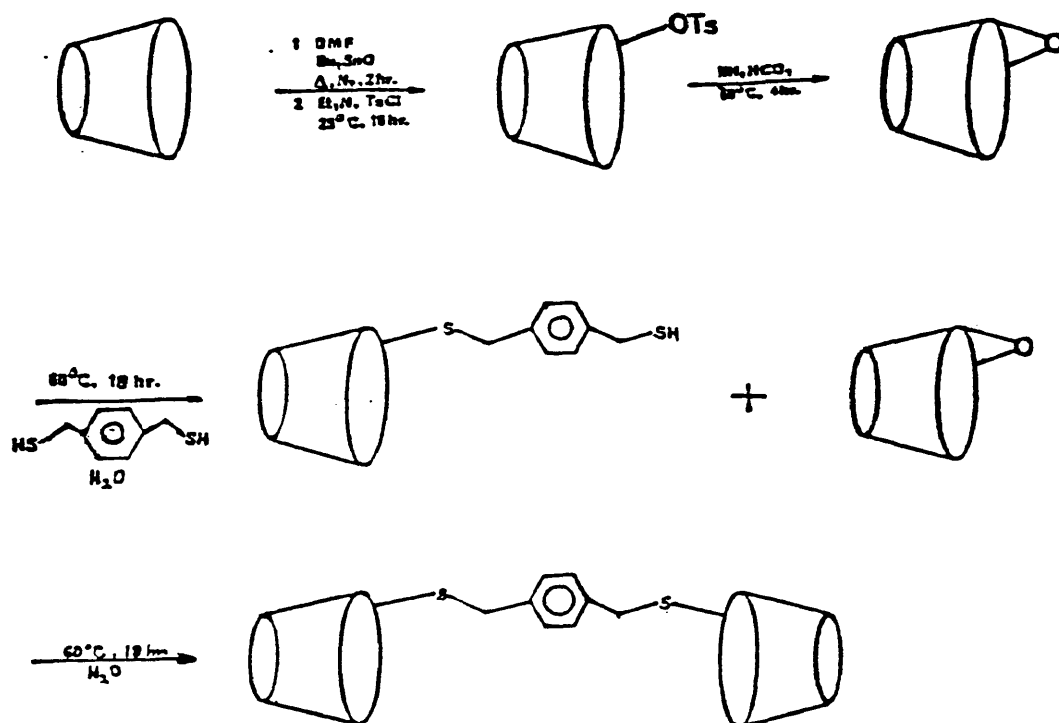
consistent with formation of the product. The low yield allowed for no further characterization.

## RESULTS AND DISCUSSION

Before discussing the synthesis of the linked beta-cyclodextrins, it is useful to recount the overall goal in the research. The singly-linked beta-cyclodextrin we are intending to synthesize may have far reaching effects in being able to control and catalyze the photochemical dimerization of certain organic reactions. Since the cyclodextrins are covalently linked they have the potential to perform like enzymes with cyclodextrin acting as the host. Thus, the inclusion process is controlled mostly by the cyclodextrin and accounts for some regioselectivity.

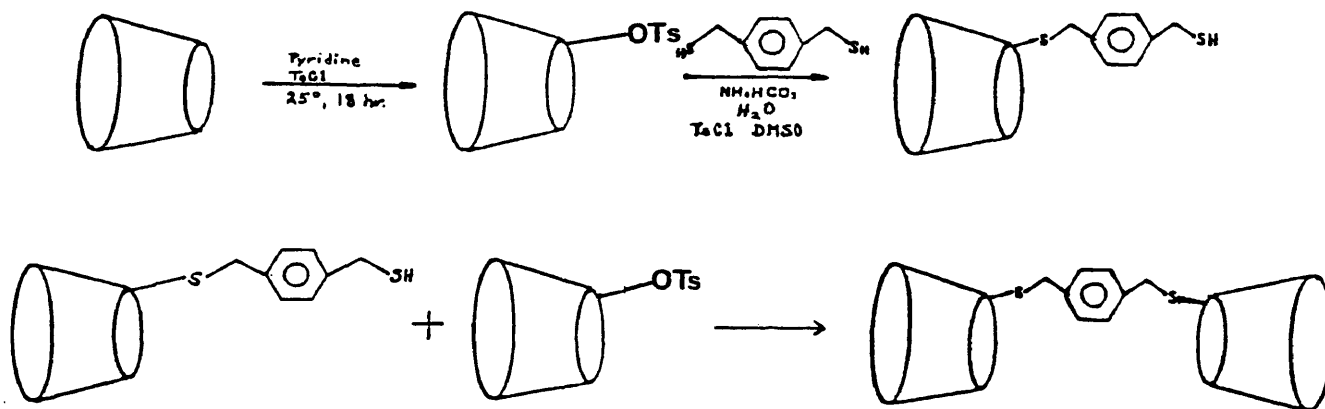
The method by which we prepared the linked 3-beta-cyclodextrin is shown below in Scheme 1.

## Scheme 1



Our proposed method to prepare a linked 6-beta-cyclodextrin is shown below in Scheme 2.

## Scheme 2



The first step involves synthesizing the beta-cyclodextrin-2-tosylate using Murakami's synthesis. This

reaction utilizes dibutyltin oxide to produce the regioselective sulfonylation. It has been effective in reactions with 1,2-diols and is very successful here in providing for an easy pathway to the sulfonation of cyclodextrins. The C(2) hydroxyl group is much preferred over the C(3) group due to easier attack by the tosyl chloride.<sup>88</sup> As a result, Murakami, et al. have succeeded in producing a much higher yield of C(2) tosylate than was previously possible. In the first step of Murakami's synthesis, water is lost as a nucleophilic addition takes place with the Sn. Upon adding  $\text{Et}_3\text{N}$ , ionization occurs with Sn acquiring a positive charge and suffering subsequent attack by  $\text{Et}_3\text{N}$  which then allows TsCl to react with the C-2 alkoxide.

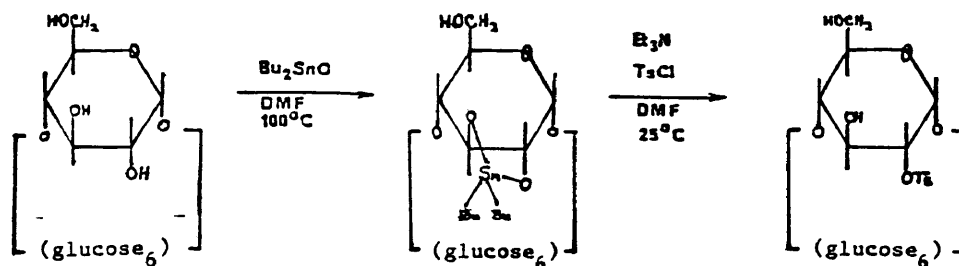
By this procedure, we were able to obtain a 10% yield of pure beta-cyclodextrin-2-tosylate. Though less than the 30% reported by Murakami, et al., our lower yield is probably due to a difficulty in separating and purifying the C(2) tosylate from the unreacted cyclodextrin and the very small amount of di-tosylate present. Product verification through thin layer chromatography demonstrated that the C(2) tosylate was present ( $R_f = 0.7$ ) along with the trace amounts of di-tosylate ( $R_f = 0.8$ ). This compares to pure beta-cyclodextrin with an  $R_f = 0.5$ .

As mentioned above, purification and separation of the C(2) tosylate from beta-cyclodextrin and di-tosylate was our major problem. Using reverse phase RP-18 silica gel, with a gradient elution of 5% to 100% aqueous acetonitrile, we obtained the best separation and the easiest method of

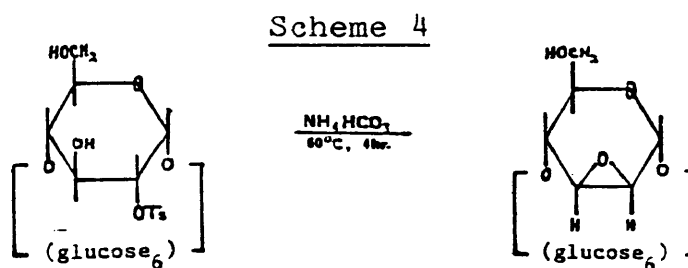
isolating the C(2) tosylate from the beta-cyclodextrin. Scheme 3 shows the Murakami synthesis for beta-cyclodextrin-2-tosylate.

Scheme 3

Murakami synthesis for beta-cyclodextrin-2-tosylate



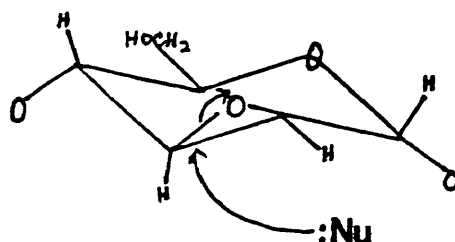
The tosylate was produced so that we could continue with it and synthesize the beta-cyclodextrin-2,3-epoxide. Proceeding with the procedure outlined by Breslow, et al., we were able to take the C(2) tosylate and transform it in the presence of a weak base to the 2,3-epoxide. Scheme 4 shows the process.



Once the epoxide has been formed, it is then susceptible to nucleophilic attack. Since the attack occurs axially, which requires the nucleophile to enter the cyclodextrin cavity, the expected product will be a C(3) derivative of cyclodextrin. (Scheme 5) The epoxide was verified by thin layer chromatography ( $R_f = 0.6$ ), but due to the similarity of the epoxide to beta-cyclodextrin, it was almost impossible to isolate it.

Scheme 5

Axial attack



Initially, our main attempts in research were in preparing and isolating 3-deoxy-3-[(3-thiopropyl)thio]-beta-cyclodextrin. This would then be reacted with another 2,3-epoxide molecule to form the bis-beta-cyclodextrin. Isolation was very difficult with this compound, however. The unreacted cyclodextrin which was present after completion of the reaction made it very difficult to isolate the product. The ion exchange was successful in removing any unreacted sulfonate ion, but gradient elution with 10% to 80% aqueous acetonitrile through reverse phase did not sufficiently separate the product from unreacted beta-cyclodextrin. Attempts at

isolating the product were not overly successful, but small amounts of product that could be isolated were indicated by thin layer chromatography ( $R_f = 0.3$ ). Unfortunately, the product was still slightly contaminated with beta-cyclodextrin and some dithiol.

After several unsuccessful attempts with the dithiol, the synthesis of 3-O-(4-hydroxyphenyl)-beta-cyclodextrin was attempted which would then be used in exactly the same manner as the propanedithiol derivative. This compound is a singly-linked cyclodextrin derivative. Once again the epoxide was reacted directly, however, through three consecutive attempts, the solution turned black and no singly-linked product was formed. The exact reasons for the failed reaction are not known. Thin layer chromatography showed only streaking and no indentifiable spots.

Our next linking group was to be 1,4-bis(thiomethyl)benzene. It was first necessary to synthesize this molecule. Para-xylylene bromide was made from p-xylene, N-bromosuccinimide and benzoyl peroxide. The p-xylene reacts with the N-bromosuccinimide, losing a hydrogen and picking up a bromide. The benzoyl peroxide functions as a catalyst. Then using the p-xylylene bromide directly with 2-propanol and thiourea, the desired product, 1,4-bis(thiomethyl)benzene, was isolated. Again, purification was a hindrance in obtaining high yields. The initial crystals were examined by thin layer chromatography and found to be impure. Recrystallization purified the thiomethyl compound, but it gave low yields. Thin layer chromatography ( $R_f = 0.45$ ) corroborated the

purity of the product.

In synthesizing (3-deoxy-3-[(4-thiomethyl)benzylthio])-beta-cyclodextrin, we used the epoxide directly in the presence of our thiomethyl compound. The thiomethyl compound had to be used in a three-fold excess molar ratio to assure formation of the derivatized beta-cyclodextrin. The possible presence of the product was indicated by a spot on thin layer chromatography ( $R_f = 0.7$ ). Beta-cyclodextrin and some unreacted thiomethyl compound were still in the product so further purification was done by reverse phase RP-18 chromatography with an aqueous acetonitrile gradient elution from 2% to 100%. For this a 15% yield of product was obtained, and its formation supported by  $^1\text{H}$  NMR. The product unfortunately could not be further characterized because of the low yield.

It may have been that the major problem with this reaction stems from the difficulty in making all reactants soluble in the media used. Due to less than perfect solubility, the yield of the reaction may have been less than optimal. Thus, whereas the product did form, better solubility in the reaction would possibly have given greater yield.

The other aspect of our research centered around producing beta-cyclodextrin-6-tosylate. Following the method established by Fujita, et al., dry beta-cyclodextrin was reacted with toluenesulfonyl chloride in dry pyridine. There was some difficulty in dissolving both reactants in the pyridine, but once accomplishing this, the reaction proceeded

readily. Once having isolated the product in crude form, pyridinium hydrochloride was removed by stirring the precipitate in acetone and filtering. Fujita, et al. report isolation of the product by recrystallization, but this did not work very well. In an attempt to recover as much tosylate as possible, the tosylate was concentrated by Soxhlet extraction with isopropanol and water (20:1). The tosylate was separated from the beta-cyclodextrin by reverse phase RP-18 flash chromatography.

Isolating the C(6) tosylate was the most troublesome step in this reaction. Even after concentration by Soxhlet extraction, it was only possible to successfully recrystallize 20 mg of product. Reverse phase chromatography was then successful and gave a good clear separation between C(6) tosylate and beta-cyclodextrin. The 15% yield we attained was pure and enabled us to continue with our research and link the thiol group onto the C(6) tosylated beta-cyclodextrin.

The next step was done according to a procedure established by Breslow, et al. where the tosylate is reacted with the bis-thiol in water. DMSO was added to completely dissolve the reactants. Ammonium bicarbonate was added as a base catalyst for the reaction. After slowly dissolving at room temperature, the reaction proceeded steadily at 60°C under a nitrogen atmosphere. Thin layer chromatography showed some possibility that the derivative may have formed. <sup>1</sup>H NMR did not confirm the formation of product, and again a low yield made it impossible for any further characterization.

## CONCLUSION

Overall, this research project has been successful. A singly linked beta-cyclodextrin derivative was synthesized and isolated. The next step is the reaction of this with beta-cyclodextrin to form the bis-beta-cyclodextrin derivative. The basic route outlined earlier was successful in producing the correct product. While the course of this work was not always problem free, the end result was always achieved through practical and repeatable methods.

All research involves some difficulties, but in our case one of the major problems encountered was in purifying and isolating the various compounds synthesized. Where simple methods of recrystallization should have sufficed, it was necessary to use more elaborate reverse phase RP-18 chromatographic methods to purify and isolate the desired products. Even with reverse phase, the product was sometimes still impure and often the yield was severely decreased. An important improvement would be the discovery of a technique for purification and isolation which is better able to tot-

ally isolate the product from any impurities like unreacted starting material, and which will provide a higher yield of return in the product. Beyond this, careful preparation was helpful in producing a desired product. In some instances the reaction proceeded very well and gave a large yield, as in the synthesis of p-xylylene dibromide, while in others, like 1,4-bis(thiomethyl)benzene, the yield was very small.

Finally, with the preparation of the linked beta-cyclodextrin derivative, its further use can now be worked upon. Research can be continued with the product to catalyze photochemical dimerizations once the bis-beta-cyclodextrin derivative has been isolated. Therefore, the study of photochemically active reactions can continue in hopes of better understanding their functions and perhaps using them to advance our knowledge of their applications.

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## VITA

### Richard Desider Nemeth

Born in Toronto, Ontario, Canada, February 11, 1965. Received B.S. in Chemistry from the College of William and Mary in Virginia, May, 1987. The author entered the M.A. program in Chemistry at the College of William and Mary in June, 1987.

After graduation, the author will be residing in Arlington, Virginia.