Inclusion Processes of Cyclodextrins and their Polymers: A Molecular Modeling Study

by

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Submitted in partial fulfillment
of the requirements for
Honors in the Department of Chemistry

Union College

June, 1996

ABSTRACT

FELLOWS, STACEY L. Inclusion processes of cyclodextrins and their polymers: A molecular modeling study. Department of Chemistry, June 1995.

Cyclodextrins (CDs) and cyclodextrin polymers (CDPs) are molecules capable of creating host/guest complexes, where a host molecule allows another molecule, a guest, to bind to it without either molecule losing its molecular identity. CDs are hydrophilic but, contain a unique hydrophobic cavity that can attract organic guest molecules. CDP is a derivative of CD and consist of two or more CD units connected by chains of glyceryl linkages ($-CH_2-CHOH-CH_2-O-)_n$. CDPs are typically more hydrophilic than the CD monomers making them more useful for applications. However, behavior of the CDP is not as well understood as that of the monomer. Computer modeling can predict the possible conformation preferences of CD and CDP when a guest molecule is in the CD cavity. Six CD and CDP complexes are examined through energy minimization calculations using MM2 force field parameters in a water solvent in order to determine the lowest energy structure from various starting conformations. The two starting conformations investigated are "clam shell" and "open" binding. Clam shell binding conformation has the guest molecule encapsulated by two CD units. Open binding conformation has the guest molecule complexed with only one CD unit. For CDPs, the role of the glyceryl linkages must also be considered.

This investigation rationalizes the observed changes in guest molecule fluorescence after it binds into the CD cavity indicating a change in its environment. The complexes examined include β-CD with pyrene, β-CDP with pyrene, α-CD and α-CDP with p-nitrophenol, and α-CD and α-CDP with p-hydroxylmethyl benzoate.

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Acknowledgment

I would like acknowledge the Union College Chemistry Department as it provided the necessary equipment and resources to complete my research. For without their investment, my work would have never been made possible. I would also like to recognize the Internal Education Fund for its partial funding the Cambridge Data Base.

I would also like to thank Professor Janet Anderson for all of her advice, support, and patience. It was really appreciated and valuable to my work.

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INTRODUCTION

Models are primary tools we use to understand phenomenon not readily observed. Molecular modeling, in particular, is useful in defining structures at the molecular level.[1] Recent advances in computers have expanded the role of molecular modeling to the point where they are able to solve complicated theoretical equations for large molecules. Thus, the prediction of molecule geometry, conformation and physical properties is possible for large molecules in a reasonable amount of computational time. Computer modeling is particularly useful in the investigation of molecular inclusion processes. It helps to visualize the orientation and geometry of the host and guest.[1] Experimentally, CDs have been shown to include various molecules into the cavity.[1,2-13] However, there is little conclusive evidence that shows the CDP behaves similarily.[4,10] Therefore, this investigation hopes to provide insight into the behavior of the CD and CDP using computational methods.

Cyclodextrin's inclusion ability has fascinated many and inspired many investigations into its structure. The CD is a toroidal molecule formed from the enzyme cyclodextrin transglycosylase (CTG) with starch.[2,14] See Figure 1 for CD structure. It consists of D(+)-glucopyranose units joined by α-(1-4)-linkages to form a cyclic ring with an internal cavity. An example of the glucopyranose unit is presented in Figure 2 and labeled with position notations used throughout this paper. The number of glucopyranose units in the ring are typically six, seven, or eight units and are denoted as α-CD, β-CD, and γ-CD respectively.[2-6,13,14] The

CD structure has two openings into its internal cavity. One is narrower in diameter and characterized by the presence of primary hydroxyl groups. The other opening is slightly wider in diameter and has secondary hydroxyl groups.[7,8,14] Figure 1 gives the enzymatic synthesis, structure, molecular dimensions and other various properties of α, β, and γ-CDs.[3] The glucopyranose unit structure in Figure 2 furnishes an explanation of CDs unique properties. The hydroxyl groups populate the two openings into the cavity are located at the top and bottom of the glucopyranose unit giving its overall hydrophilic nature. However, the region between these hydroxyl groups contains cyclic carbons and oxygens with their hydrogens projecting into the cavity. It is this unique structure that creates the hydrophobicity of the cavity.[3,5,8,8]

It is the hydrophobic cavity of the CD that gives it the possibility to form inclusion complexes. An inclusion complex is defined as "...two substances intimately linked but, not through covalent bonds."[3] Non polar organic molecules from inclusion complexes with CDs by binding into the hydrophobic cavity because "... it is less polar that the surrounding aqueous medium."[4,5,13,14] The forces involved with the binding of a guest molecule into the cavity are hydrophobic interactions, van der Waals or London dispersion forces, hydrogen bonding, the release macromodel ring strain energy[8] and dipole-dipole interactions.[9] Several inclusion complexes investigated include pyrene[4,10,11], ibuproxan[7], naproxen[12], suronacrine[8], and nootropic drugs[1] with β-CD, and anthracycline antibiotics with γ-CD.[6]

It is possible for CD inclusion complexes to occur in two stoichiometric ratios of 1:1 and 2:1 host/guest. The 1:1 host/guest ratio is commonly referred to as "open" binding and the 2:1 host/guest ratio is referred to as "clam shell" binding. In more descriptive terms, clam shell binding is where guest molecule is "doubled capped" by two CD hosts.[4,10,11,12] See Figures 3,5 and 7 for examples of clam shell binding. Open binding conformation is where the guest molecule only binds into one CD cavity.[4,10,11,12] See Figures 4,6, and 8 for examples of open binding conformations.

The ability of CD to bind to other molecules grants it many diverse applications. CDs can modify the guest molecule to increase its stability and solubility in aqueous medium.[1,13] The CD is also very useful in the separation of structural, geometric and optical isomers.[1,5,13] Another application is the separation of mixtures of aromatic hydrocarbons in the petrochemical industry. CDs can also reduce the volatility and smell of insecticides to provide safer handling. In food science applications, CDs help to remove cholesterol from egg and dairy products. CDs also protects the active ingredient in perfume.[3] CDs are also used as mobile phases in HPLC, a modifier of photochemical behavior, to control dye aggregation equilibrium, and for models of protein complexes.[2,13]

However, the most useful CD, the β-CD, has limited solubility in water (0.014-0.016 M)[2,3,13] while the α- and γ-CDs are not. Therefore, a highly water soluble derivative, the β-CDP, was synthesized.[2,3,13] The β-CDP synthesis involves the use epichlorohydrin. This reaction yields a polydisperse mixture containing β-CD units joined by repeating glyceryl linkages (-(CH_2-CHOH-CH_2-

O-)$_n$).[2,3,13] The glyceryl linkages attach themselves to the β-CD at any one of hydroxyl groups at position 2',3', or 6' on the glucopyranose ring. The solubility of the β-CDPs varies in a ratio of 2:1:1 for the various glyceryl linkage attachments of 3':2':6' positions respectively.[15] Therefore, β-CDPs where glyceryl units are attached at position 3' are twice as soluble than those attached at the other positions on the glucopyranose ring. The CDPs are commercial available with α- and γ-CDPs as well.[4] See Figures 9 through 26 for examples of α- and β-CDPs with various glyceryl linkage lengths and attachment positions.

Based on the results from β-CD monomer, it has been proposed that the β-CDP forms inclusion complexes in clam shell binding and open binding conformations.[4,10] The β-CDP clam shell binding conformation is where two neighboring CD units on a glyceryl linkage encapsulates a guest molecule. For the β-CDP open binding conformation, the guest molecule binds into one β-CD cavity or binding occurs on the linkage unit.[4,10] It is not experimentally conclusive that clam shell binding actually happens with β-CDP.[4,10] Therefore, it is the aim of this study to determine if the clam shell binding conformation for β-CDP is energetically favorable and if it can be applied for the α-CDP. It is also hoped that the factors affecting conformation of the host/guest complex can be better understood.

EXPERIMENTAL

β-Cyclodextrin Monomer and Polymer

All the structures are built on a Silicon Graphics Indigo^2 work station. All modeling and calculations are done using the MacroModel program version 5.0.[16] All structural minimization calculations are performed using MM2 force field parameters with simulation in a water solvent. Energy minimization calculations on the structures are repeated until gradients dropped to about 1 kJ/mol with convergence being 0.05 kJ/mol.[17] The gradients are calculated using PRCG routine (conjugate gradient minimization by using the Polak-Ribiere first derivative method) to allow for a full relaxed optimization of structure.[1,16] The β-CD structure is from the Cambridge Structure Database on CD-ROM.[18] The β-CDP structure is visually built using the β-CD in clam shell and open binding conformations. The guest molecule, pyrene, is visually built and minimized. See Figure 27.

To create a host/guest complex, the guest molecule is visually placed into the β-CD cavity using the Macromodel DOCKING option. Based on previous modeling work, the guest molecule is docked into the cavity using the wider opening.[1,9] "At the narrower rim, there is a large energy barrier that is not likely to passed at room temperature."[9] Consistency of guest orientation is achieved using the BUMP CHECK option in order to place the guest into the center of the cavity to have least amount of contact with the cavity wall. This forces the major axis of the guest to be approximately parallel to the symmetry axis of the β-CD

unit. The complex structures are built in two conformations; clam shell and open binding. In Figures 9-11, the clam shell conformation is shown. Figures 18-20 shows the open binding conformation. The unique symmetry of pyrene eliminates further criteria on its orientation in the cavity.

Three versions of the β-CDPs are built in two conformations with the various glyceryl linkage lengths of four (Figures 9 and 18), eight (Figures 10 and 19), and ten (Figures 11, and 20). The three versions of β-CDPs vary by the placement of the glyceryl linkage attachment to the β-CD. Attachment can occur at the any of the three hydroxyl groups on the D-glucopyranose ring. See Figure 2. The β-CDP typically exists with multiple β-CD units between the glyceryl linkage with other dangling glyceryl linkages. For the sake of simplicity, a single glyceryl linkage with β-CD on each end is considered to represent the β-CDP. For simplicity sake, only the 2' position is shown for the β-CDP.

In the clam shell binding conformation, a decision must be made as to how to orient the glyceryl linkage attachments to the D-glucopyranose rings of the two β-CD units. Preliminary modeling shows that when the glyceryl linkage attachments are directly across from one another, the β-CD units are forced apart. Thus, this eliminates complete encapsulation and guest isolation from the hydrophilic environment. The problem is solved by attaching the glyceryl linkage to the neighboring D-glucopyranose rings where the glyceryl linkage extends the longest. For example, if attached to the 2' position, then the glyceryl linkage attachment is attached to the d-glucopyranose unit to the right of attachment. If attached to the 3' position, then glyceryl linkage attachment is attached to the D-

glucopyranose unit to the left of attachment. At the 6' position, this problem is irrelevant because both neighboring d-glucopyranose units are equal in distance.

Once all the β-CD and β-CDP complexes are built, the structures are minimized in a simulated water solvent. Energy minimization of a structure predicts the lowest energy conformation from a specific starting conformation. For the clam shell binding conformation, the glyceryl linkages are built to create a loop whose size depends on the glyceryl linkage length. For open binding conformations, the glyceryl linkages are built creating a roughly straight line in order to place the β-CD units as far apart as possible. It is important to realize that "...inclusion molecules in solution are not static species. Substrates (guests) included in the cavity, not only exchange rapidly with free substrate molecules but, also re-accommodate themselves by presenting several molecular orientations."[3] Therefore, the lower energy structures are only a favored to exist and are not the only structure that exists in solution.

Calculations run on molecules of this scale occupy a lot of computer time. Therefore, Macromodel furnishes two methods of minimization, either interactively or as background, using BATCHMIN. For example in the Macromodel program, a molecule is built and saved as a Macromodel data file, molecule.dat. In the interactive method, there are four buttons in the ENERGY menu needed to commence the minimization calculation: ECALC, PRCG, SOLVT and then START. With the ECALC function, the minimization calculation is the option chosen. Choosing the PRCG function calculates the energy

gradient. The SOLVT function gives a choice between various solvents. However, running interactively ties up the computer and nothing else can run. Alternatively, a calculation can be run in the background using the BATCHMIN program. To run a calculation in the background, a command file must be created and the command line typed into the SGI. See below.

chandler%bmin < molecule.com > molecule.log &

See Figure 28 for the file used in this investigation. Once minimization is finished, the program creates several other files. The files containing the output information are in the molecule.out, molecule.m1, and molecule.m2. Molecule.out is the visual of the minimized molecule.dat structure, molecule.m2 is the coordinates of the minimized structure, and molecule.m1 records the minimized energy and its components. See Figure 29 for example of molecule.m1.

Included with the total energy of the molecule is the breakdown of all the contributing factors. These factors are stretch, bend, proper torsion, improper torsion, electrostatic interactions, cross terms, van der waals forces, and solvation energies. Typical for these molecules is that the electrostatic interactions and the van der Waals forces are the major contributors to the total energy of the complex.

The minimized energy of each complex can be thought of as the sum of the energies of all the structural components making up each structure. The

complex energy is partitioned into two parts: the two β-CD units with pyrene and the glyceryl linkages. Equation (1) shows how the total complex energy, E_{total}, depends on the number of glyceryl linkage units.

$$E_{total} = E_{glyceryl\ linkages} * (\text{number of glyceryl units}) + E_{CD/pyrene} \qquad \text{(Eq 1)}$$

Graphing the total energy on the y-axis versus the number of glyceryl linkages on the x-axis, the glyceryl linkage energy per linkage is determined from the slope and the CD/pyrene complex energy from the y-intercept. See Figure 30 for sample calculation.

To determine the conformation preference for different linkage lengths, the total complex energy of the open binding conformation must be related to the clam shell binding conformation. To accomplish this, the glyceryl linkage energy per linkage and the β-CD/pyrene complex energies are first averaged over the all three variations in position of glyceryl linkage attachments. The values of β-CD/pyrene complex energy and glyceryl linkage energy per linkage are substituted back into Eq1 and the number of glyceryl linkages graphed versus the total complex energy for both conformations. The intersection point of the lines is where they are equal in energy. This plot also shows where the energy of each conformation falls in relation to other for various other lengths. This graphically represented in Figure 31.

α-Cyclodextrin Monomer and Polymer

All α-CD structures are built from X-ray coordinates manually entered directly into the computer.[19] The α-CDP is visually built from the α-CD structure. The α-CD and α-CDP guest molecules are benzene derivatives p-nitrophenol (pNP) and p-hydroxylmethyl benzoate (pHMB) and are also visually built. All structures are energetically minimized with MM2 force field parameters simulated water solvent and gradients calculated using PRCG function. Procedures are similar to those described for β-CD and the β-CDP.

Because pyrene is symmetric, its orientation in the cavity is only simple to describe. However, p-nitrophenol and p-hydroxylmethyl benzoate are not as symmetrical. See Figures 32 and 33. Both molecules are para substituted benzene rings, but the substituents are not the same. Therefore, insertion is dependent on which substituent is inserted first into the cavity. For clam shell binding, both substituents are encapsulated by the α-CD units and orientation is again simple. However, for open binding only one substituent can be inserted into the cavity. Thus, there are two conformations that are possible for binding into the α-CD. After some preliminary calculations, it is found that hydroxyl substituent insertion for both molecules is lower in energy than the alternate. Thus, for all the open binding conformations of the α-CD and α-CDP, the hydroxyl substituent is inserted first into the cavity with the other groups hanging out of the α-CD unit.

RESULTS AND DISCUSSION

β-Cyclodextrin Monomer and Polymer with Pyrene

Experiments have been done to determine the behavior of the β-CD monomer and β-CDP with the guest molecule, pyrene.[4,10,11] Results of energy minimizations of the β-CD monomer structure with pyrene in clam shell and open binding conformations are shown in Figure 3 and 4. It is clear that the lower energy conformation is clam shell binding. This is due to the increased solvent exclusion in the hydrophobic cavity[11] making the environment around pyrene to be more hydrophobic. This trend correlates with previous experimental results showing clam shell binding occurring between β-CD monomer and pyrene.[4,10,11]

The minimized β-CDP/pyrene complexes for the various glyceryl linkage lengths are presented in Figures 9-11 for clam shell and in Figures 18-20 for open binding conformations. Using the method described in the experimental, the total energy for the β-CDP/pyrene complex is divided into the β-CD/pyrene complex energy and the glyceryl linkage energy per linkage. The β-CD/pyrene complex energy is compared between clam shell and open binding conformations in Figure 34 for all positions of glyceryl linkage attachment to the β-CD unit. Clearly, the clam shell binding conformation is lower in energy than the open binding conformation for all the positions.

The structural difference between the monomer and the polymer is the glyceryl linkages attaching the CD units together. It is possible that the attachment position as well as the glyceryl linkages play a role in conformation

preference. The β-CD/pyrene complex energy indicates that when the glyceryl linkages are attached at position 6' the energy is lower than when positioned at positions 3' and 2'. Position 3' ends up being highest in energy. (Figure 34) Clam shell binding conformation is defined as encapsulation of a guest molecule by two CD units. (Figure 3) To accomplish this, the two larger openings, populated with the secondary hydroxyl groups of positions 2' and 3', must face each other as they surround the guest molecule. Figures 9, 10, and 11 show the effect of glyceryl linkage attachment at the 2' position. This is specially clear in Figures 9 and 11 because there is increased exposure versus the clam shell bound pyrene in the CD monomer.

The second half of the total complex energy is shown in Figure 35. It compares the glyceryl linkage energy per linkage for the various attachment positions of glyceryl linkages to the β-CDs in clam shell and open binding conformations. The glyceryl linkage energy per linkage is lower in the open binding than in clam shell binding conformation. This is probably due the variations in the glyceryl linkage structure and orientation when in the different complex conformations. The clam shell binding causes the glyceryl linkages to be folded which restricts their movement. (See Figures 9,10 and 11) On the other hand, the glyceryl linkages in an open binding position are extended out into a rough straight line. (See Figures 18, 19, and 20) This allows the glyceryl linkages to have more freedom of movement. This trend is shared by all three positions of glyceryl linkage attachment. Due to the large variation in possible positioning of the glyceryl linkages, it is not reliable to compare the glyceryl

linkage energy per linkage energies between the different glyceryl linkage attachment positions.

The β-CD/pyrene complex and glyceryl linkage per linkage energies are then used to find the number of glyceryl linkages where clam shell and open binding conformations prefer to exist. In Figure 31, the total complex energy is plotted versus the number of glyceryl linkages for both clam shell and open binding conformations producing a linear relationship for both. The clam shell and open binding conformation lines intersect one another at four linkages. Thus, at this point the conformations are equal in energy. Other information that is taken from Figure 31 is the energetic behavior with longer and shorter linkage lengths. When the linkage length is shorter than four linkages, the clam shell binding drops below the open binding line. Thus, polymers with four or less linkages will be lower in energy when in a clam shell binding conformation. However, when the linkage length is greater than four linkages, the open binding line drops below the clam shell line. Thus, for polymers with four or more linkages will be lower in energy when in an open binding conformation.

α-Cyclodextrin Monomer and Polymer with p-Nitrophenol

The structures resulting from the energy minimization of the α-CD monomer and p-nitrophenol (pNP) are presented in Figures 5 and 6. The α-CD monomer is shown in clam shell and open binding conformations with pNP inserted into the cavity. Surprisingly, the open bound α-CD/pNP complex

conformation is the lower energy conformation as compared with the results from the β-CD monomer results. In contrast to the pyrene molecule that is very hydrophobic and active in seeking out the most hydrophobic environments; the pNP is an alcohol derivative of benzene and is slightly polar. See Figure 32. Therefore, pNP will be more soluble in a hydrophilic solvent and the increased hydrophobicity of the clam shell environment could increase the pNP energy in the cavity. This will ultimately increase the total complex energy. Thus, it is less likely that a second CD unit forming the clam shell conformation will create a lower energy complex.

The resulting complexes from minimization calculations on the α-CDP with pNP at various glyceryl linkage lengths are displayed in Figures 12-14 for clam shell and in Figures 21-23 for open binding conformations. The energy trends, however, do not follow the trend of the β-CDPs. See Figure 36. In the 2' position, the α-CD/pNP complex energy for clam shell binding is lower than for open binding conformation. This follows the previous β-CDP results. However, an opposite trend is observed at the 3' and 6' position. It is possible that the results from position 2' are erroneous. The α-CD units that encapsulate the pNP do not cover as well as with positions 3' and 6'. Good encapsulation depth adequately covers the pNP promoting isolation from the solvent.

Glyceryl linkage attachment position can affect complex conformation preference for the α-CDP with p-NP, similar to β-CDP with pyrene. The attachment on the secondary hydroxyl groups at positions 2' and 3' again interferes with complete encapsulation . Figures 12, 13, and 14 shows the

glyceryl linkage attachment at the 3' position. Especially for the longer length linkages, the completeness of encapsulation is less pronounced. Energetically, neither glyceryl linkage attachment position reduces glyceryl linkage energy per linkage more than the others. See Figure 37.

Two distinct glyceryl linkage orientations are created in order to limit the extreme variations involved with random placement. For clam shell binding conformations, the glyceryl linkages are built in a loop conformation while in open binding the glyceryl linkages are built in a line. However, for the clam shell binding the α-CDP and pNP complex the glyceryl linkage orientations are not as pronounced. The linkages for position 2' does not show a loop of glyceryl linkage similar to other complexes built and are shown in Figures 9, 10 and 11. Therefore, the structures are not consistent as needed to show these relationships between clam shell and open binding conformations. Figure 37 shows the glyceryl linkage energy per linkage. The trends are not distinct and it is reasonable to assume that more work is needed to correct the inconsistencies.

Using the α-CD and pNP complex and the glyceryl linkage per linkage energy, the number of glyceryl linkages where clam shell and open binding conformations are preferred can be obtained. In Figure 38, the total complex energy is plotted versus the number of glyceryl linkages for both the clam shell and the open binding conformations producing linear relationships for both. These lines are intended to intersect one another at a point indicating the length of the glyceryl linkages where the conformations are equal in energy.

Unfortunately, the clam shell and open binding conformation lines for α-CDP and pNP complex do not cross. Therefore, there is no point where the conformations are equal in energy. Instead the graph shows that clam shell binding is always higher in energy than the open binding conformation. This is due to the fact that there is no difference between the glyceryl linkage energy per linkage for the clam shell and open binding conformations.

α-Cyclodextrin Monomer and Polymer with p-Hydroxylmethyl Benzoate

The structures resulting from the energy minimizations of the α-CD monomer and pHMB are shown in Figures 7 and 8. The α-CD monomer is shown in both clam shell and open binding conformations with pHMB inserted with the hydroxyl group first. Just as with the previous pNP, the open bound α-CD/pHBM complex is the lower energy conformation. The pHMB is also an alcohol derivative of benzene and is slightly polar. Therefore, like the pNP it will be less inclined to form clam shell binding complexes.

The resulting α-CDP/pHMB complexes from minimization calculations are shown in Figures 15, 16, and 17 for clam shell binding and in Figures 24, 25, and 26 for open binding conformations. These figures show the α-CDP with glyceryl linkage attachment at the 6' position.

Like the α-CD/pNP complex energy, α-CD/pHMB complex energy trends also do not follow the trends of the β-CD/pyrene complex energy. See Figure 39. The α-CD/pHMB complex energy is clearly lower in energy for the open binding conformation at all glyceryl linkage attachment positions. The glyceryl

linkage attachment position that is lowest in energy is position 3'. In agreement with the α-CD monomer results, it is reasonable that the open binding complex is lower in energy because the pHMB is in a partially hydrophobic and hydrophilic environment.

For the β-CDP with pyrene and α-CDP with p-NP complexes, the 6' position provides very good encapsulation since the glyceryl linkage attachment is not on the opening facing the other CD unit so that it almost completely covers the guest molecule. However, the α-CDP does not completely surround the pHMB. See Figures 15, 16 and 17. The clam shell binding α-CD units of the α-CDP places the para substituents into the cavity while exposing some of the benzene base because the p-HMB structure contains a very bulky ester substituent. See Figure 33. As the length of the glyceryl linkages grows, more of the benzene base shows.

The α-CDP with pHMB glyceryl linkage energy per linkage energy does not show the trend seen previously with the β-CDP and pyrene. See Figure 40. For all positions of glyceryl linkage attachment, the glyceryl linkage energy per linkage for the clam shell binding conformation is lower in energy while the open binding conformation is higher in energy.

Using the α-CD/pHMB complex energy and the glyceryl linkage energy per linkage, the number of glyceryl linkages where clam shell and open binding conformations are favored can be determined. In Figure 41, the total complex energy is graphed versus the number of glyceryl linkages for both clam shell and open binding conformations producing linear relationship for both conformations.

The clam shell and open binding lines intersect one another approximately at twelve linkages indicating that the conformations are equal in energy. This plot is more useful as it shows what is happening above and below this point. When the linkage length is shorter than twelve linkages, the clam shell binding line is below the open binding conformation line. Thus, clam shell is lower in energy for linkages of this length. However, when the linkage length is greater than twelve linkages, the open binding line is below the clam shell binding line. Thus, it is lower in energy for linkages at these lengths. However, it is important to state the intersection point is found by extrapolation of the data and may be uncertain.

CONCLUSIONS

β-Cyclodextrin Monomer and Polymer with Pyrene

The glyceryl linkage length dependence shown in this study is a possible explanation for the experimental disagreement in existence of clam shell binding conformation for CDPs.[5,11] Energy dependence is due to the higher glyceryl linkage energy per linkage for the clam shell than for the open binding conformation. Therefore, the energy difference between the conformations of the β-CD/pyrene complex is filled with the increasing energy of longer linkages.

In Xu's study, the number of glyceryl linkages was varied from one to eight. This range includes the glyceryl linkage lengths seen in Figure 31 where the molecule energy of clam shell and open binding conformations are equal. As a result, clam shell binding is postulated to occur for the β-CDPs.[11] However, in Werner and Colwell's study commercial β-CDPs with 11-14 glyceryl linkages were used and the results were not consistent with Xu.[5] Therefore, computer modeling provides an explanation as to this discrepancy in experimental results.

α-Cyclodextrin Monomer and Polymer with p-Nitrophenol and p-Hydroxylmethyl Benzoate

It was hoped that the glyceryl linkage attachment dependence seen for β-CDP and pyrene would also apply to the α-CDP with pNP and pHMB. However, the results did not agree. For the pHMB, the glyceryl linkage length where the

clam shell and open binding conformations are equal in energy is twelve linkages. The number of linkages could not be determined for pNP.

It is possible to spectulate that the difference in linkage lengths where the energy of clam shell and open binding are equal for the α-CDP and β-CDP is caused by the variation in guest molecules. The β-CDP complex involves a very nonpolar, symmetrical molecule that fits snugly into the β-CD cavity. The α-CDP complex involves the inclusion of polar and symmetrical molecules. The pHMB contains a bulky ester substituent which does not fit well into the α-CD cavity. Thus, it need more room to create a better clam shell conformation.

ENDNOTES

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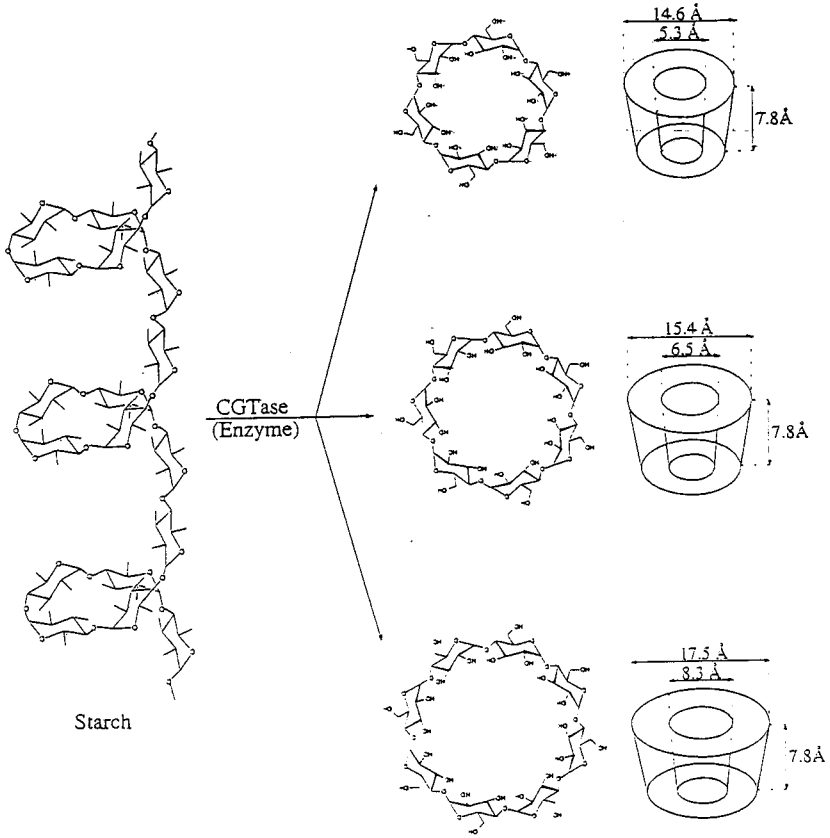
Figures



α-cyclodextrin (top)	
Molecular Weight	973
Glucose Units	6
Specific Rotation $[\alpha]_{D25}$	150.5
Cavity Volume mL/g	0.10
H_2O molecules included (in solution)	6

β-cyclodextrin (center)	
Molecular Weight	1135
Glucose Units	7
Specific Rotation $[\alpha]_{D25}$	162.5
Cavity Volume mL/g	0.14
H_2O molecules included (in solution)	11

γ-cyclodextrin (bottom)	
Molecular Weight	1297
Glucose Units	8
Specific Rotation $[\alpha]_{D25}$	177.4
Cavity Volume mL/g	0.20
H_2O molecules included (in solution)	17

Figure 1. Enzymatic synthesis, structure, molecular dimensions, and other properties of cyclodextrins.[3]

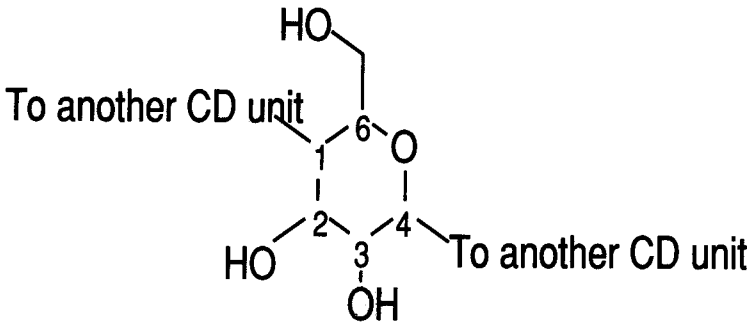


FIGURE 2: The D-glucopyranose unit of the cyclodextrin molecule.

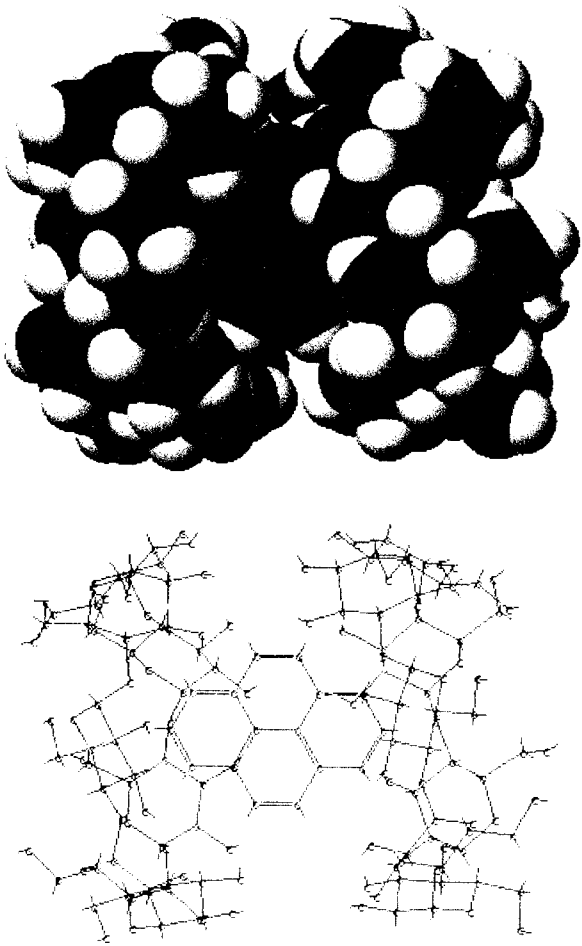


FIGURE 3: Beta Cyclodextrin with Pyrene in Clam Shell Binding Conformation. The minimization energy of this complex is -4670 kJ/mol.

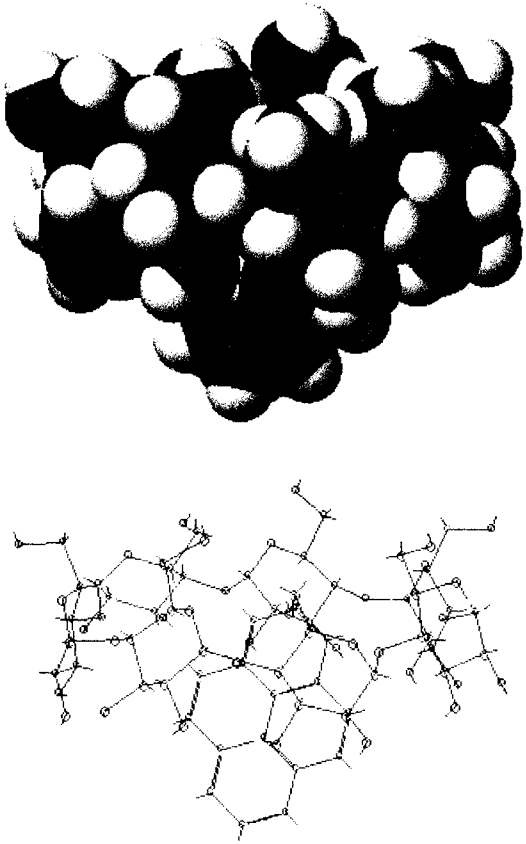


Figure 4: Alpha Cyclodextrin with Pyrene in Open Binding Conformation. The minimized energy for this complex is -4660 kJ/mol.

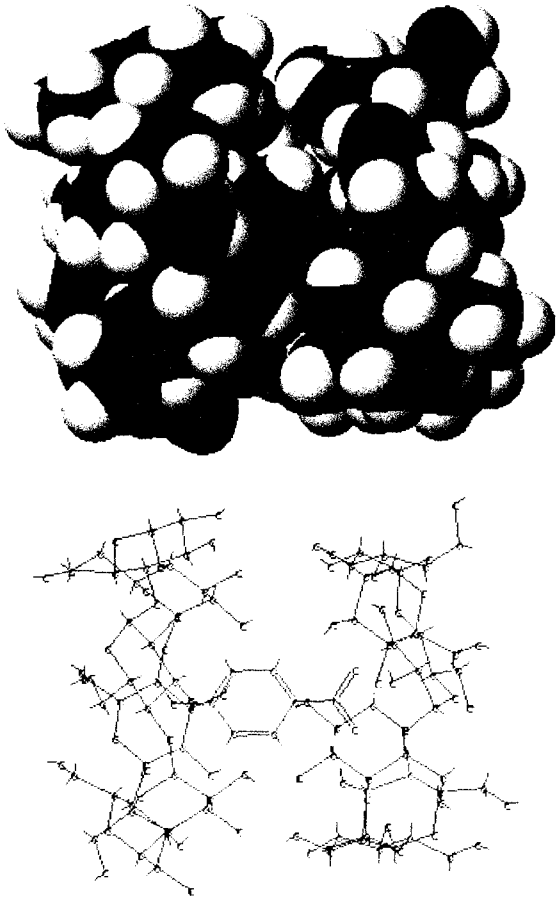


FIGURE 5: Alpha Cyclodextrin with p-Nitrophenol in Clam Shell Binding Conformation. The minimization energy of this complex is -3970 kJ/mol.

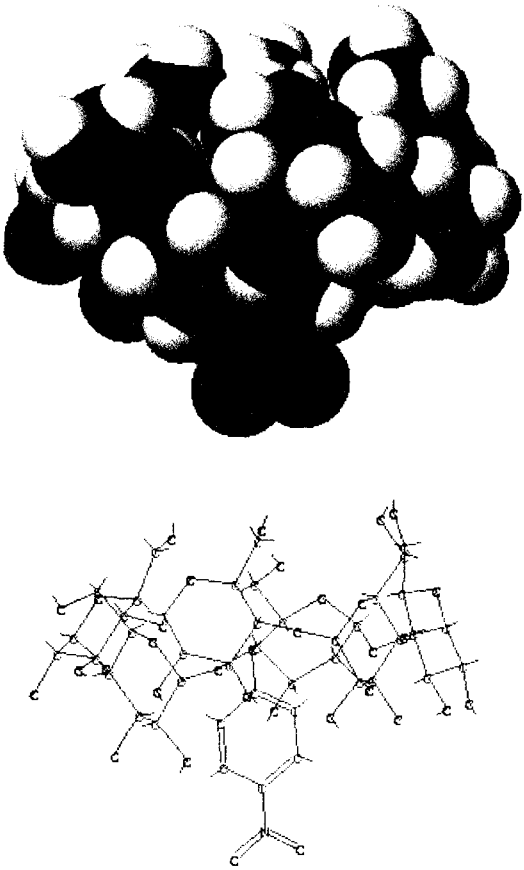


FIGURE 6: Alpha Cyclodextrin with p-Nitrophenol in Open Binding Conformation. The minimized energy for this complex is -3991 kJ/mol.

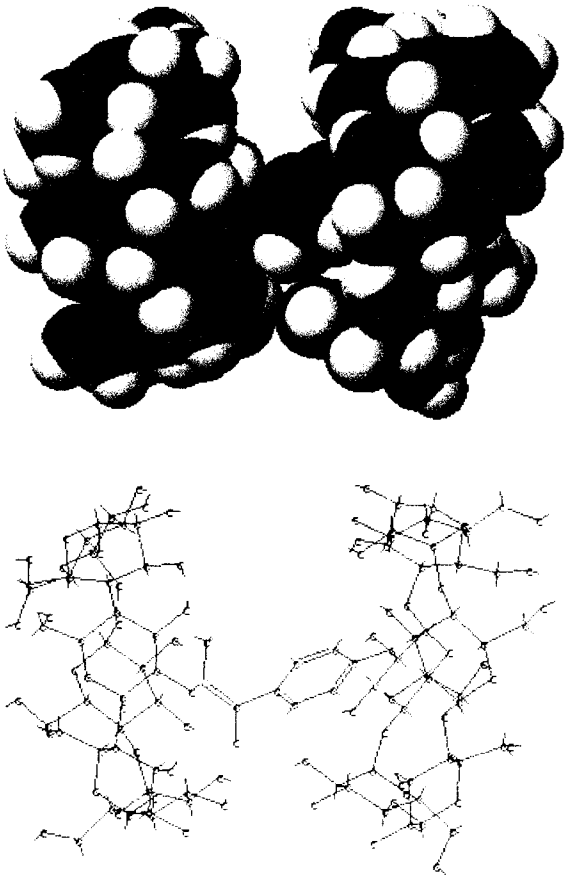


FIGURE 7: Alpha Cyclodextrin with p-Hydroxylmethyl Benzoate in Clam Shell Binding Conformation. The minimization energy for this complex is -3783 kJ/mol.

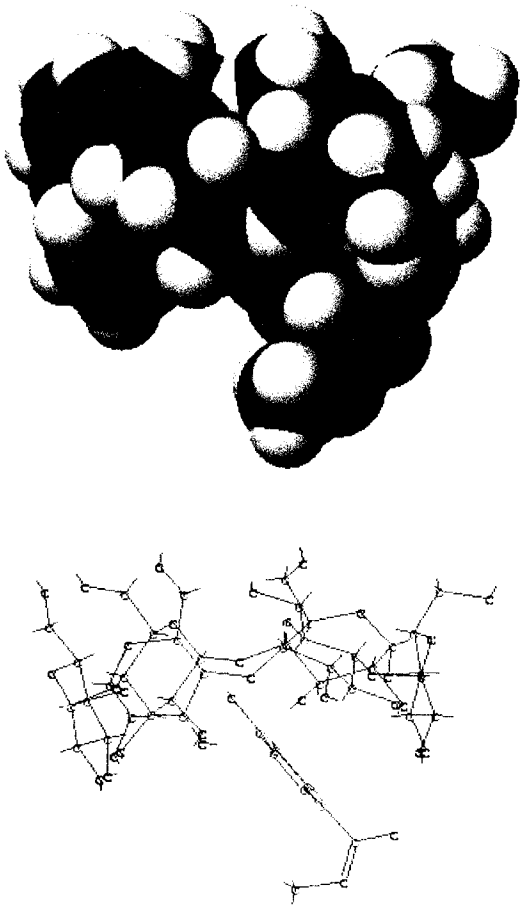


FIGURE 8: Alpha Cyclodextrin with p-Hydroxylmethyl Benzoate in Open Binding Conformation. The minimization energy of this complex is -3789 kJ/mol.

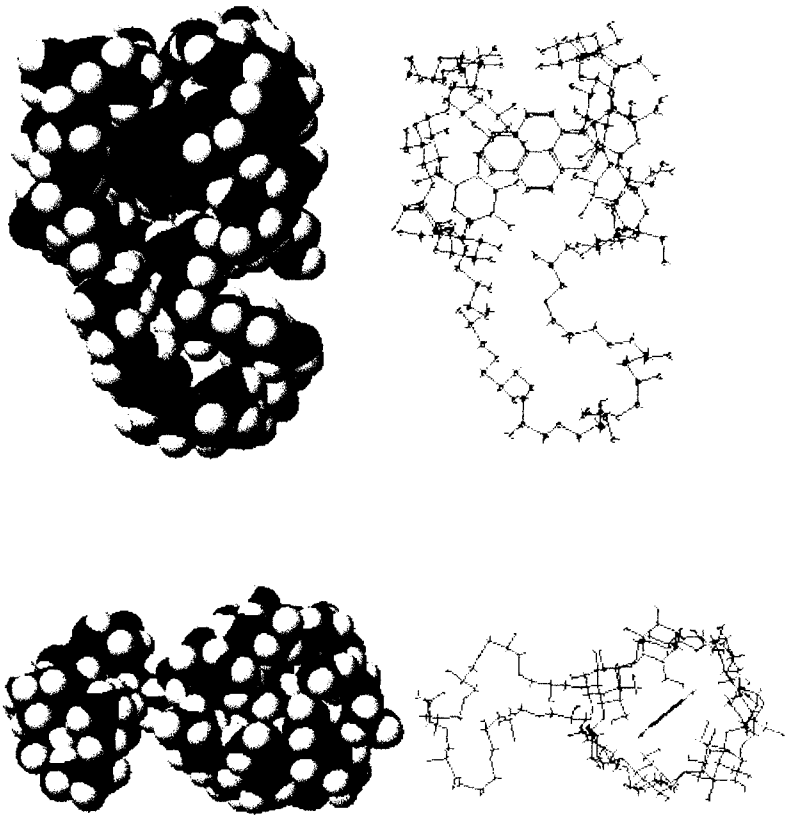


FIGURE 9: The Beta Cyclodextrin Polymer with Pyrene in Clam Shell Conformation. The Beta-CDP has ten glyceryl linkages attached at the 2' position. (Perspectives are approximately 90 degrees of one another)

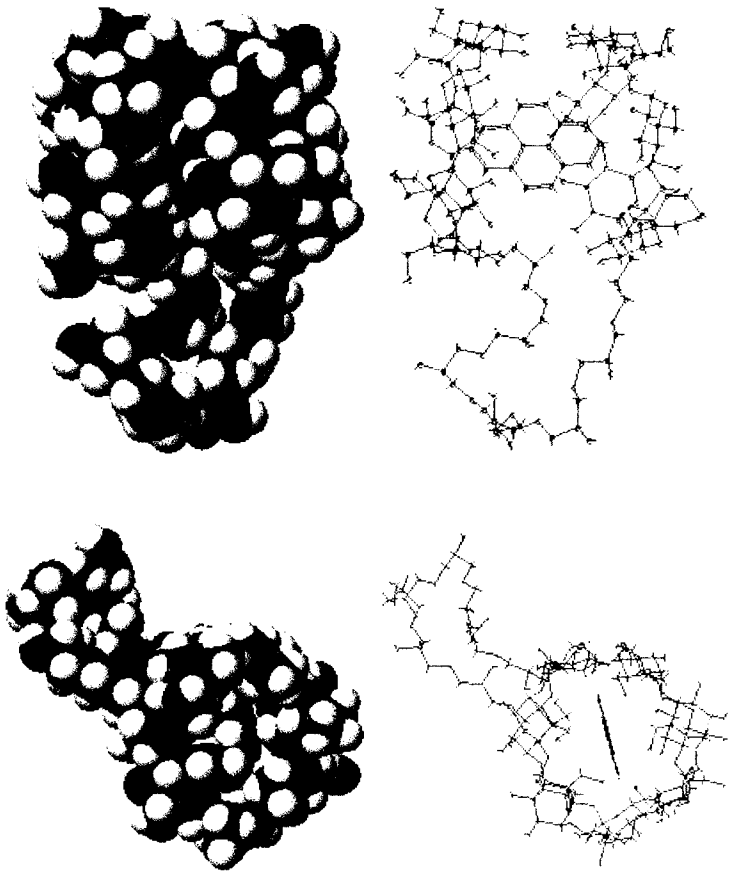


FIGURE 10: Beta Cyclodextrin Polymer with Pyrene in Clam Shell Binding Conformation.
The Beta-CDP has eight glyceryl linkages attached at the 2' position.
(Perspectives differ by approximately 90 degrees)

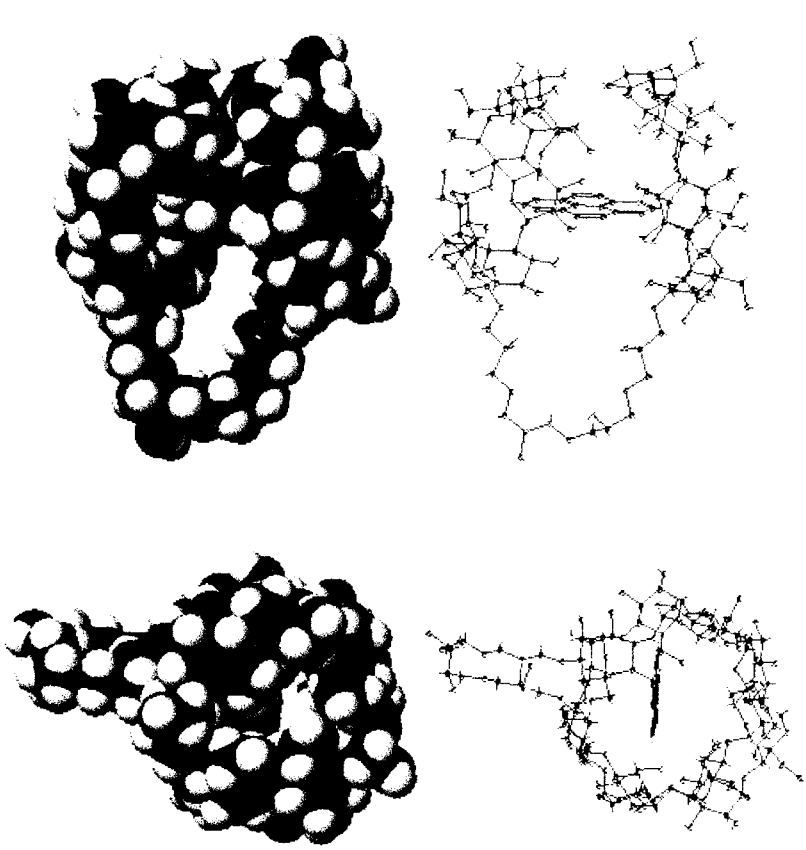


FIGURE 11: Beta Cyclodextrin Polymer with Pyrene in Clam Shell Binding Conformation. The Beta-CDP has four glyceryl linkages attached at the 2' position (Perspectives differ by approximately 90 degrees)

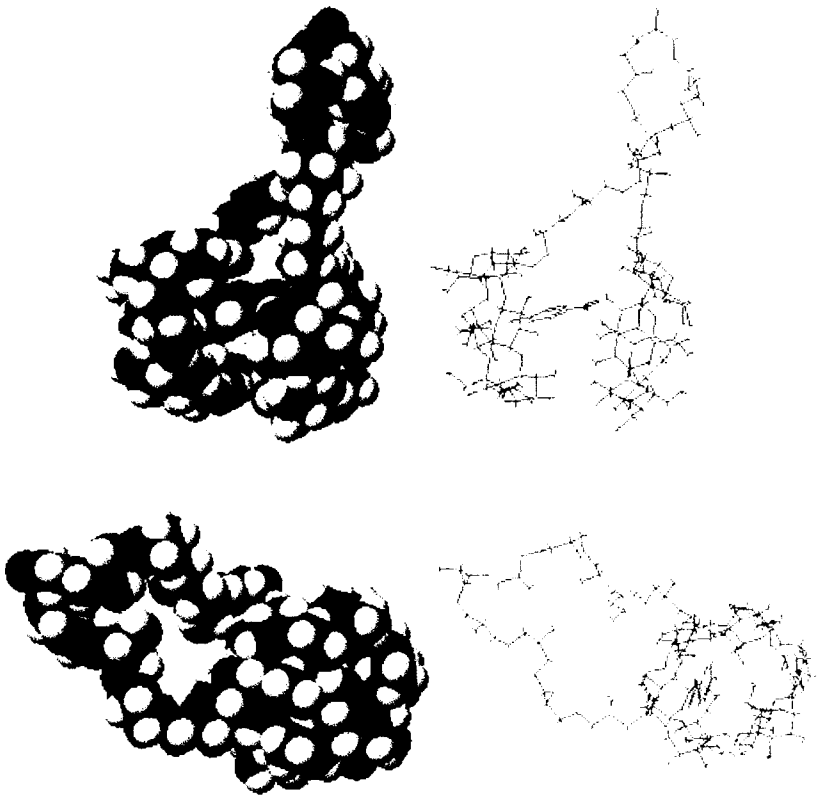


FIGURE 12: Alpha Cyclodextrin Polymer with p-nitrophenol in Clam Shell Binding Conformation. The Alpha-CDP has ten glyceryl linkages attached at the 3' position. (Perspectives differ by approximately 90 degrees)

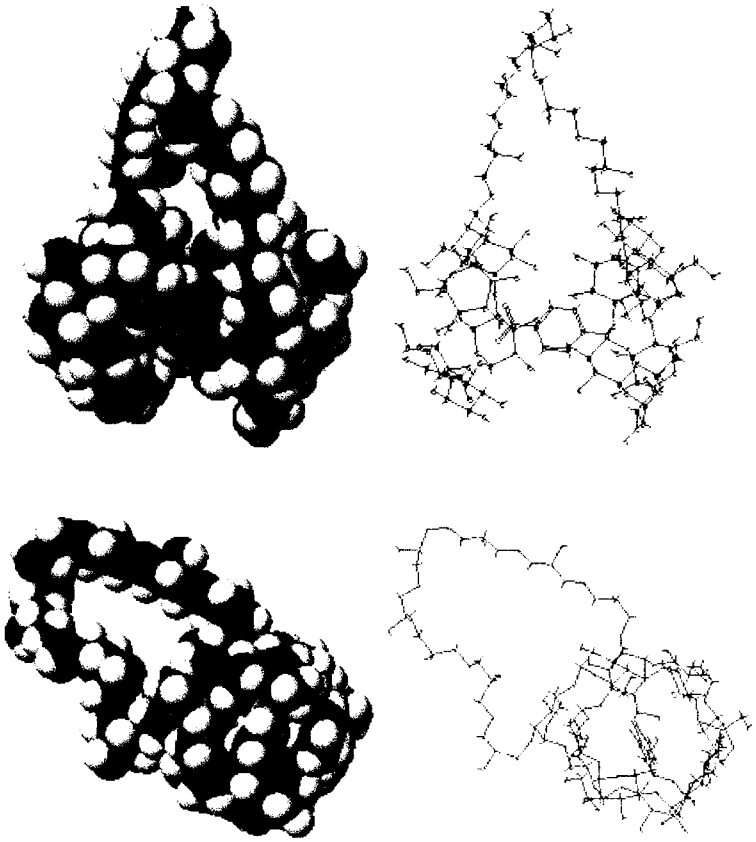


FIGURE 13: Alpha Cyclodextrin Polymer with p-nitrophenol in Clam Shell Binding Conformation.
The Alpha-CDP has eight glyceryl linkages attached at the 3' position.
(Perspectives differ by approximately 90 degrees)

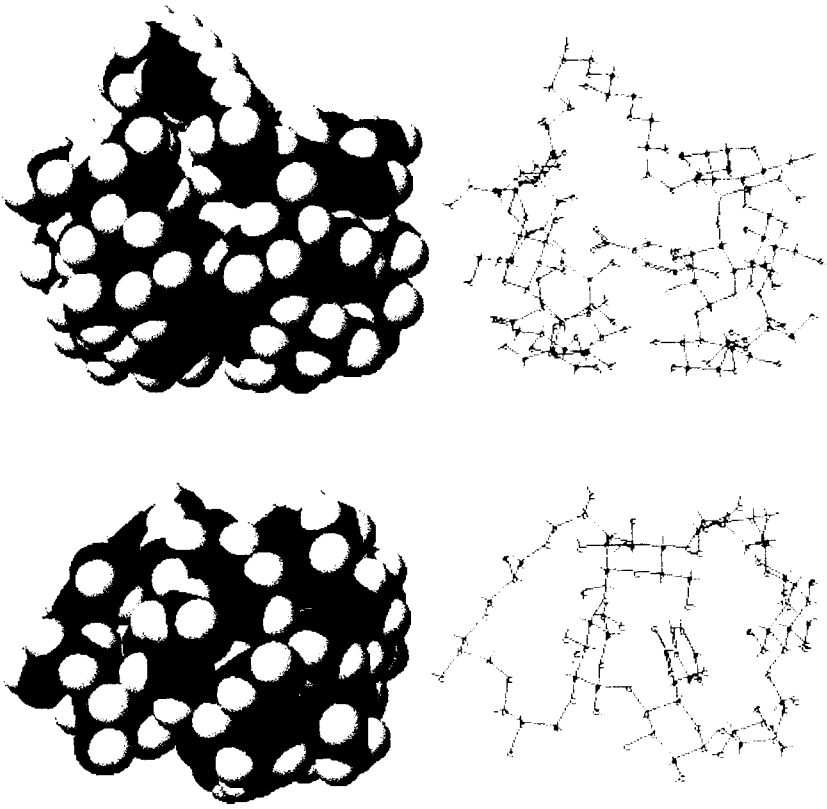


FIGURE 14: Alpha Cyclodextrin Polymer with p-nitrophenol in Clam Shell Binding Conformation.
The Alpha-CDP has four glyceryl linkages attached at the 3' position.
(Perspectives differ by approximately 90 degrees)

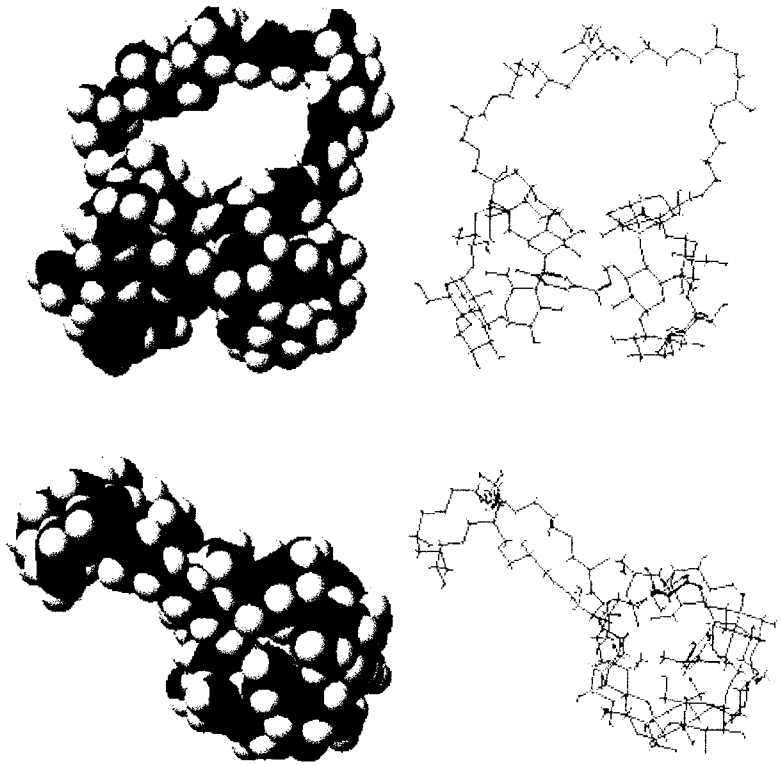


FIGURE 15: Alpha Cyclodextrin Polymer with p-hydroxylmethyl benzoate in Clam Shell Binding Conformation.
The Alpha-CDP has ten glyceryl linkages attached at the 6' position.
(Perspectives differ by approximately 90 degrees)

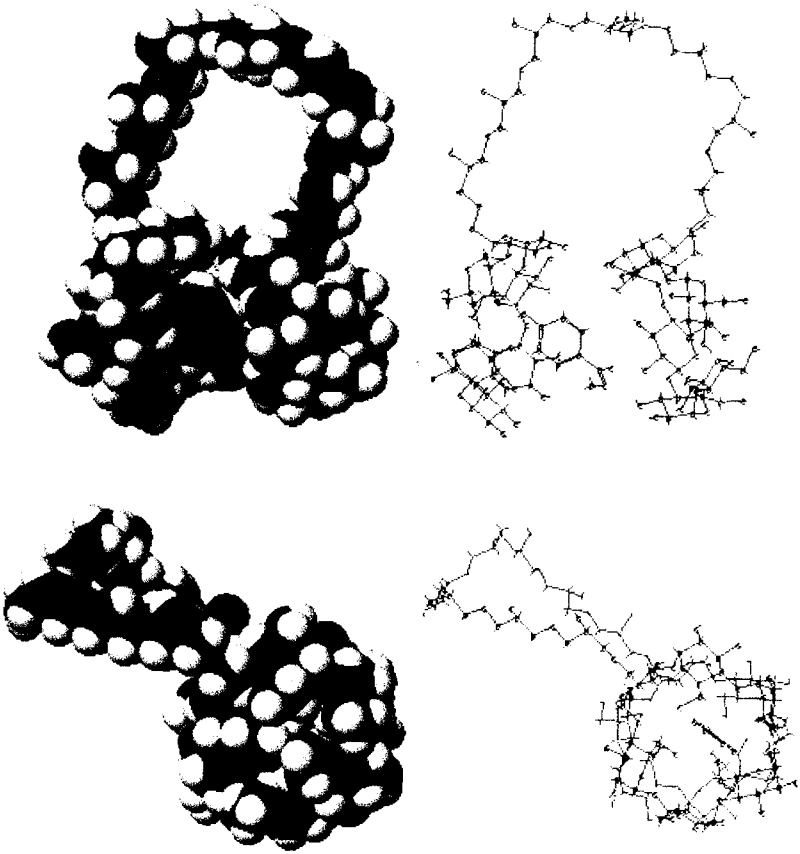


FIGURE 16: Alpha Cyclodextrin Polymer with p-hydroxymethyl benzoate in Clam Shell Binding Conformation.
The Alpha-CDP has eight glyceryl linkages attached at the 6' position.
(Perspectives differ by approximately 90 degrees)

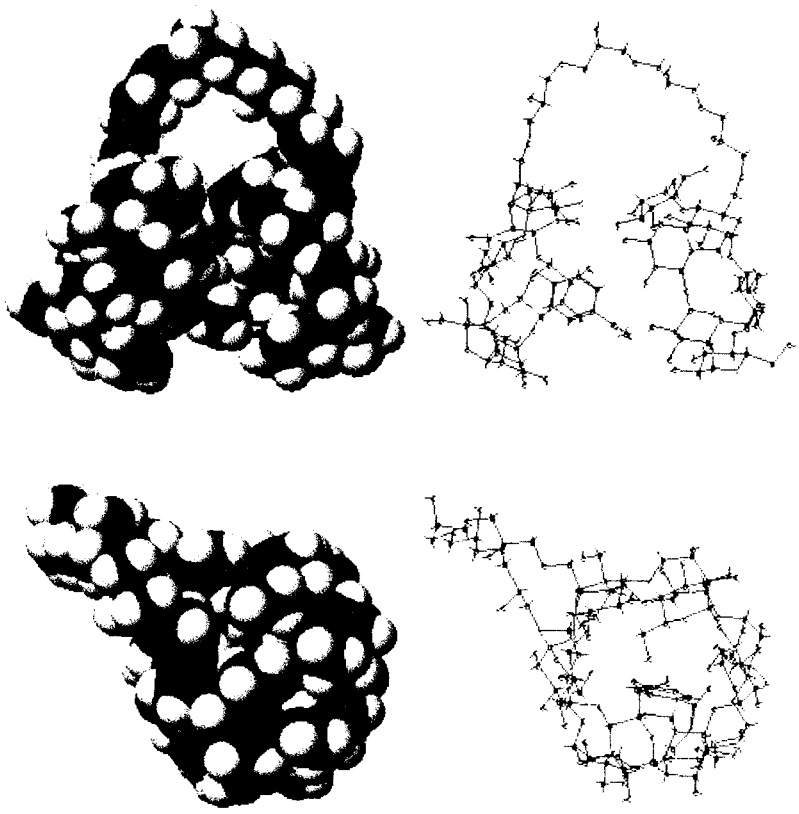


FIGURE 17: Alpha Cyclodextrin Polymer with p-hydroxylmethyl benzoate in Clam Shell Binding Conformation.
The Alpha-CDP has four glyceryl linkages attached at the 6' position.
(Perspectives differ by approximately 90 degrees)

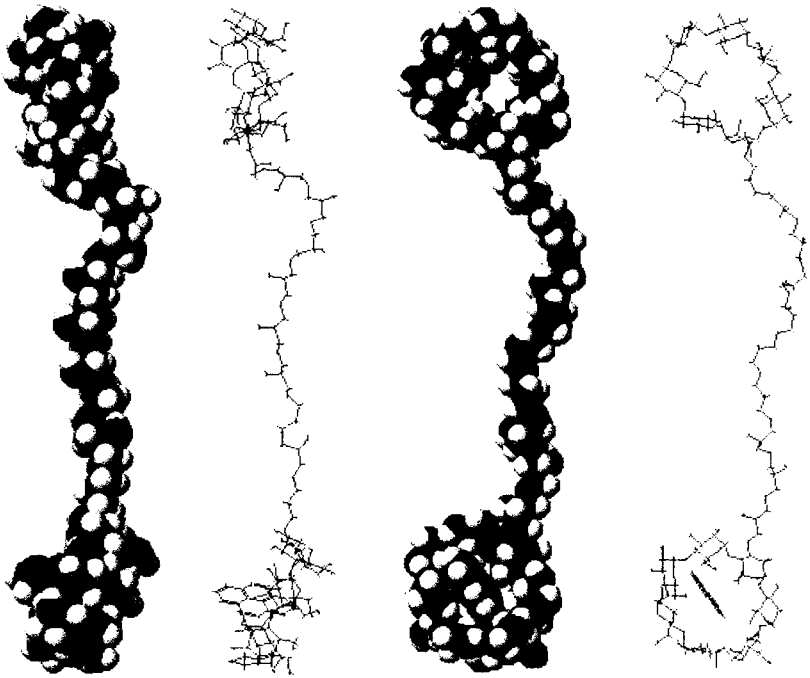


Figure 18: Beta Cyclodextrin Polymer with Pyrene in an Open Binding Conformation. The Beta-CDP has ten glyceryl linkages attached at the 2' position. (Perspectives differ by approximately 90 degrees)

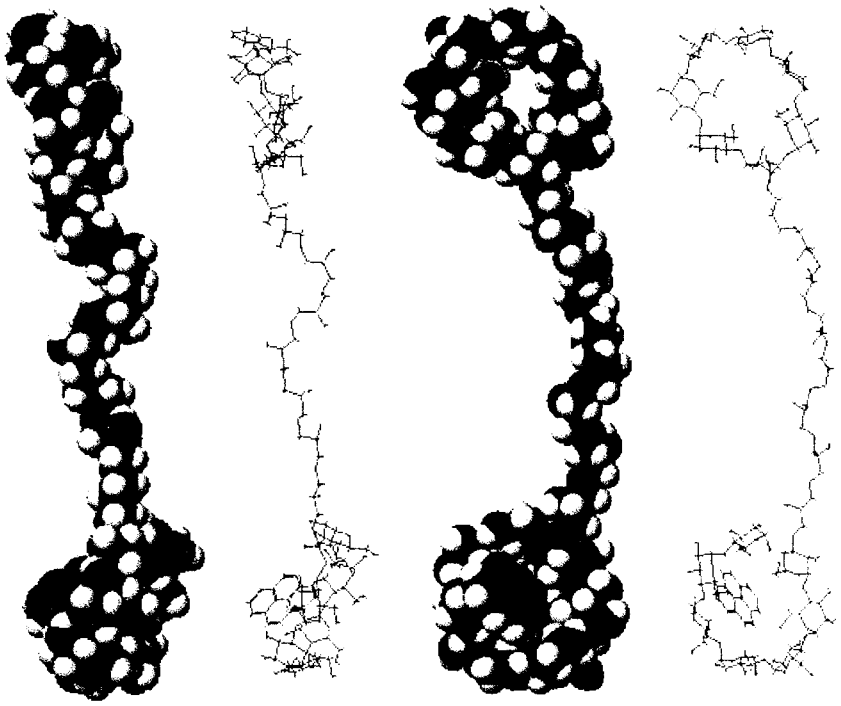


FIGURE 19: Beta Cyclodextrin Polymer with Pyrene in an Open Binding Conformation. The Beta-CDP has eight glyceryl linkages attached at the 2' position. (Perspectives differ by approximately 90 degrees)

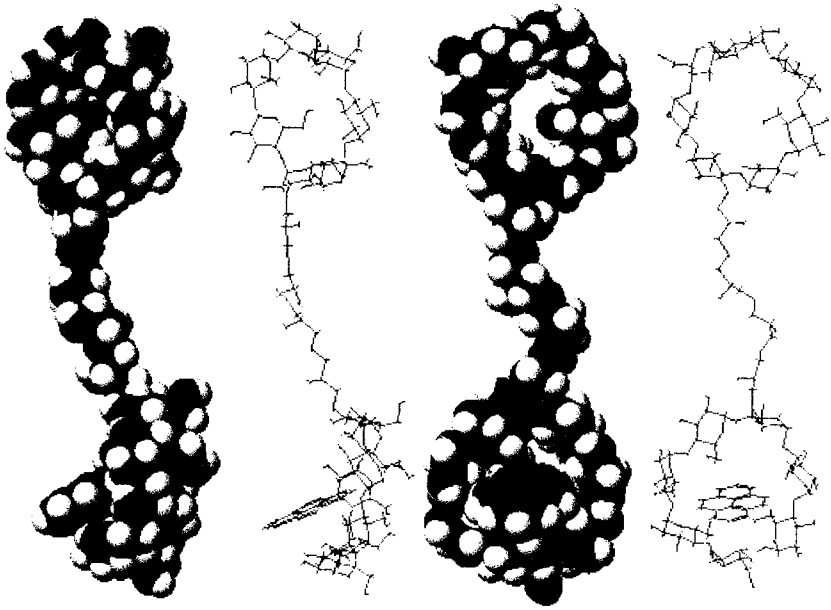


FIGURE 20: Beta Cyclodextrin Polymer with Pyrene in an Open Binding Conformation. The Beta-CDP has four glyceryl linkages attached at the 2' position. (Perspectives differ approximately 90 degrees)

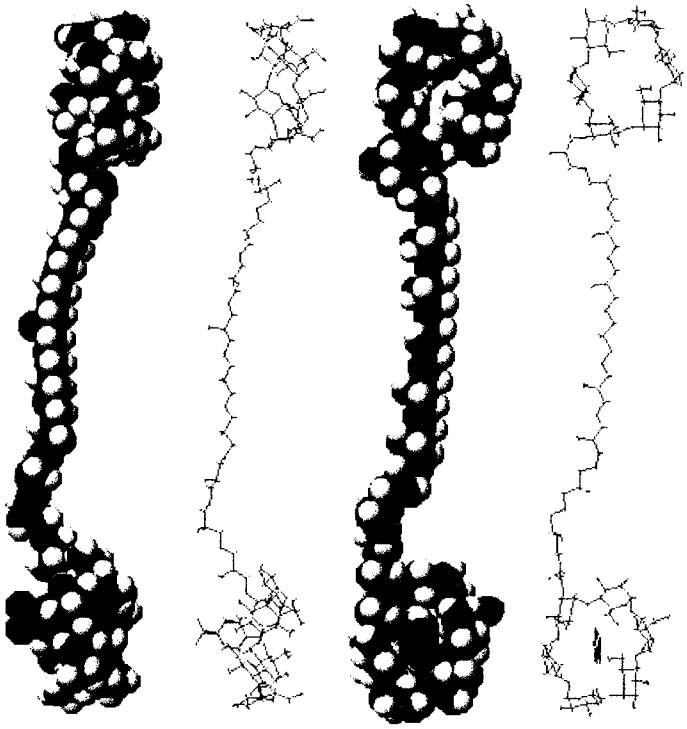


FIGURE 21: Alpha Cyclodextrin Polymer with p-nitrophenol in an Open Binding Conformation.
The Alpha-CDP has ten glyceryl linkages attached at the 3' position.
(Perspectives differ by approximately 90 degrees)

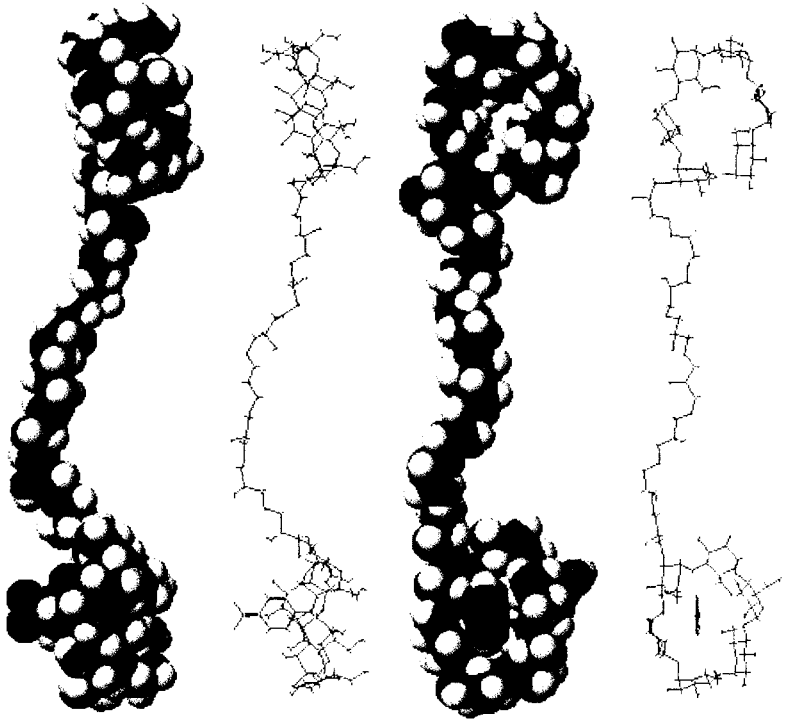


FIGURE 22: Alpha Cyclodextrin Polymer with p-nitrophenol in an Open Binding Conformation. The Alpha-CDP has eight glyceryl linkages attached at the 3' position. (Perspectives differ by approximately 90 degrees)

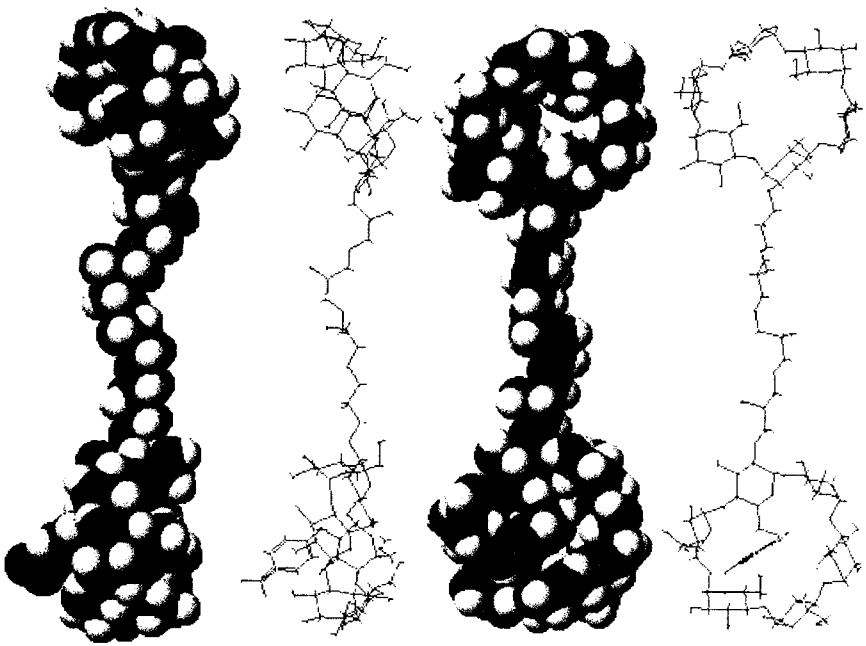


FIGURE 23: Alpha Cyclodextrin Polymer with p-nitrophenol in an Open Binding Conformation. The Alpha-CDP has four glyceryl linkages attached at the 3' position. (Perspectives differ by approximately 90 degrees)

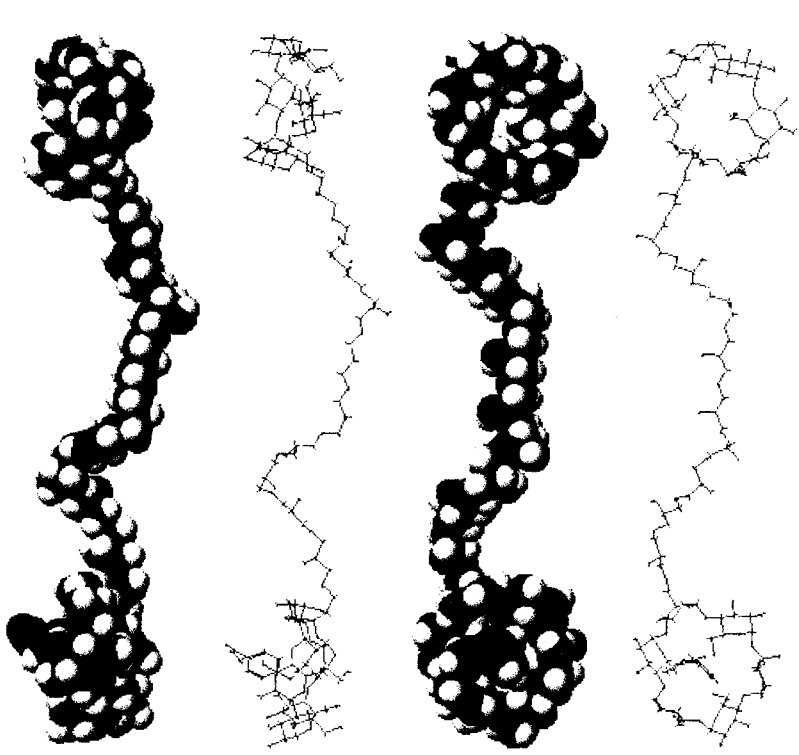


FIGURE 24: Alpha Cyclodextrin Polymer with p-hydroxylmethyl benzoate in an Open Binding Conformation.
The Alpha-CDP has ten glyceryl linkages attached at the 6' position.
(Perspectives differ by approximately 90 degrees)

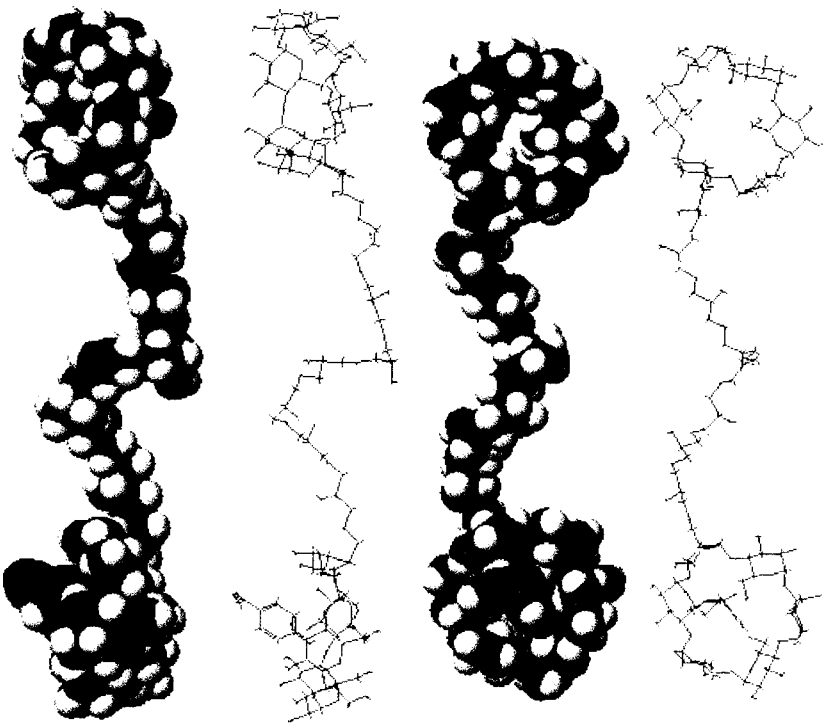


FIGURE 25: Alpha Cyclodextrin Polymer with p-hydroxylmethyl benzoate in an Open Binding Conformation.
The Alpha-CDP has eight glyceryl linkages attached at the 6' position.
(Perspectives differ by approximately 90 degrees)

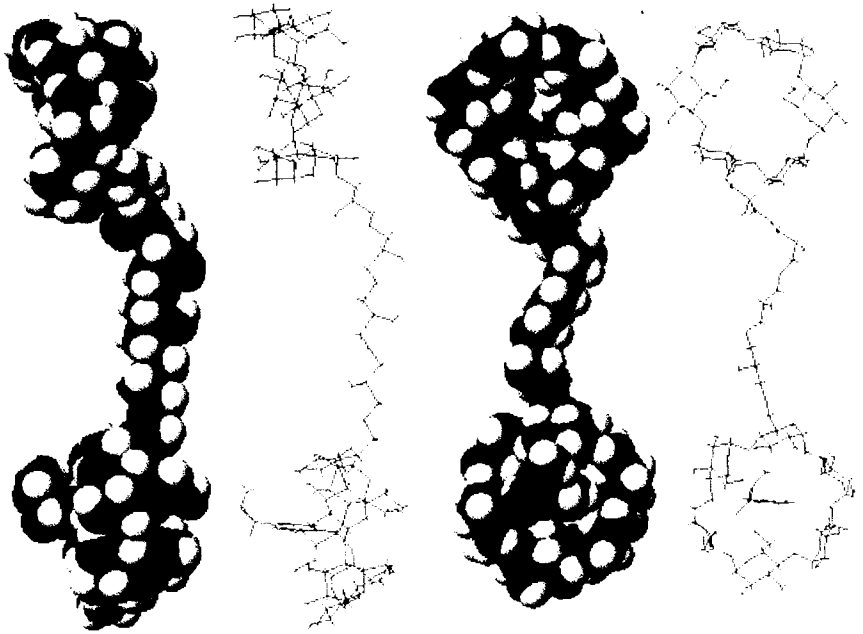


FIGURE 26: Alpha Cyclodextrin Polymer with p-hydroxylmethyl benzoate in an Open Binding Conformation.
The Alpha-CDP has four glyceryl linkages attached at the 6' position.
(Perspectives differ by approximately 90 degrees)

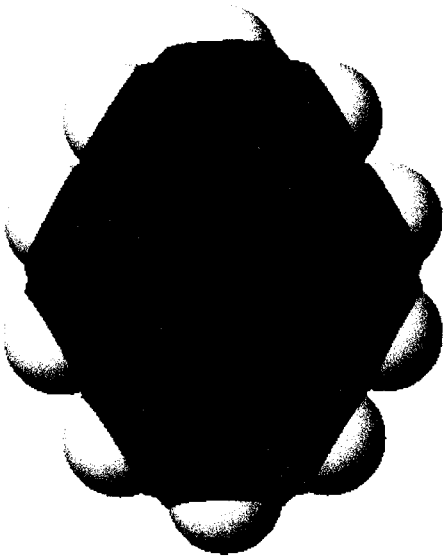


FIGURE 27: The guest molecule for Beta-Cyclodextrin Monomer and Polymer, Pyrene.

```
/usr/people/fellows/acdgly102es.dat
/usr/people/fellows/acdgly102es.out
 MMOD        0       1
 SOLV        3       1       0       0       0.0000      0.0000      0.0000      0.0000
 EXNB        0       0       0       0       0.0000      0.0000      0.0000      0.0000
 FFLD        1       1       0       0       1.0000      0.0000      0.0000      0.0000
 READ
 ELST        0       0       0       0       0.0000      0.0000      0.0000      0.0000
 MINI        1       0     500       0       0.0000      0.0000      0.0000      0.0000
```

Figure 28: A sample command file for a BATCHMIN calculation.

```
MINI   008 Computing final energy, standby...
MINI   009 Final Gradient =  0.796E+00 kJ/A-mol    CPU Time =  1068.0 sec
MINI   010              Total Energy =-0.4285178E+04 kJ/mol
MINI   011                   Stretch = 0.7476354E+02 kJ/mol
MINI   012                      Bend = 0.5510919E+03 kJ/mol
MINI   013                   Torsion =-0.1035025E+03 kJ/mol
MINI   014          Improper Torsion = 0.1586184E-01 kJ/mol
MINI   015                       VDW = 0.5203550E+03 kJ/mol
MINI   016            Electrostatic =-0.5218019E+04 kJ/mol
MINI   017 Explicit Hydrogen Bonds =-0.1378052E+02 kJ/mol
MINI   021                Cross Terms = 0.3371167E+02 kJ/mol
MINI   031                 Solvation =-0.1298149E+03 kJ/mol
MINI   040  No more updates
DONE   001
```

Figure 29: A sample output file for BATCHMIN calculation as background

Clam Shell Binding Position

Glyceryl Units	Total Energy
4	-4820.07
8	-5016.05
10	-5074.88

Glyceryl Linker Energy (kJ/mol)
-43.401
CD/Inclusion Molecule Complex (kJ/mol)
-4652.1

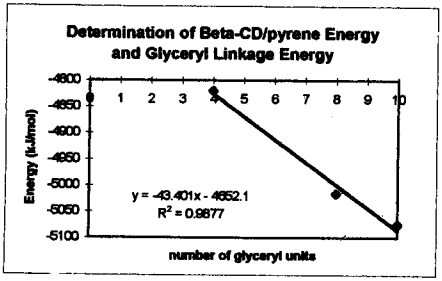


Figure 30: A sample calculation to determine the CD/guest molecule energy and the glyceryl linkage energy per linkage.

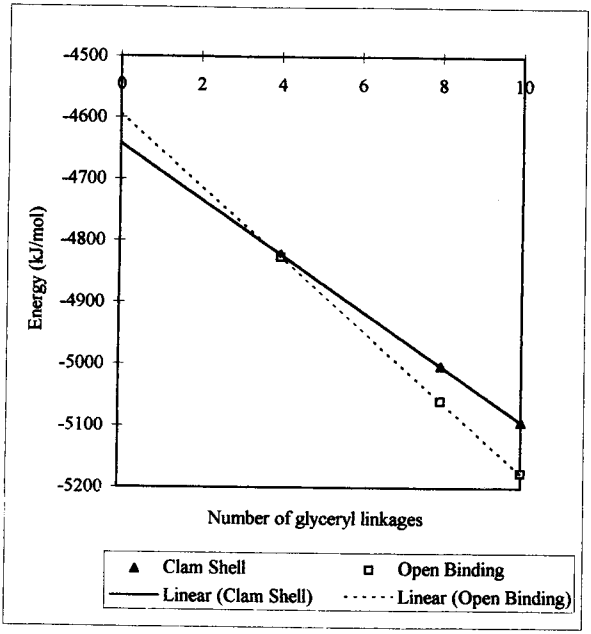


Figure 31: A Linear Plot of Clam Shell Conformation Energy versus the Open Binding Conformation Energy for β-CDP with pyrene

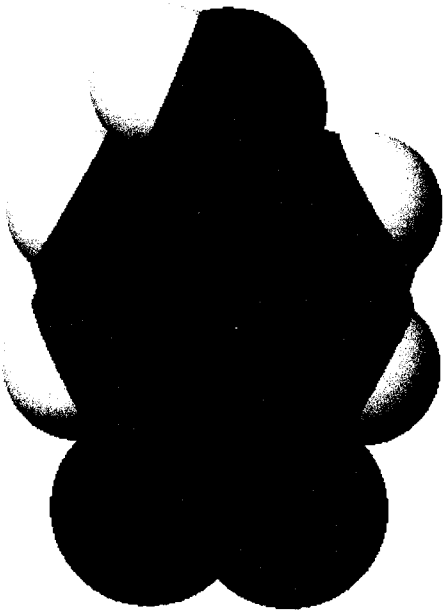


Figure 32: The guest molecule for Alpha Cyclodextrin Monomer and Polymer, p-nitrophenol

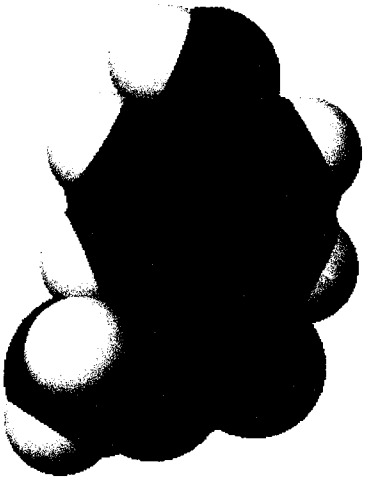


FIGURE 33: The Guest Molecule for the Alpha Cyclodextrin monomer and polymer, p-hydroxylmethyl benzoate

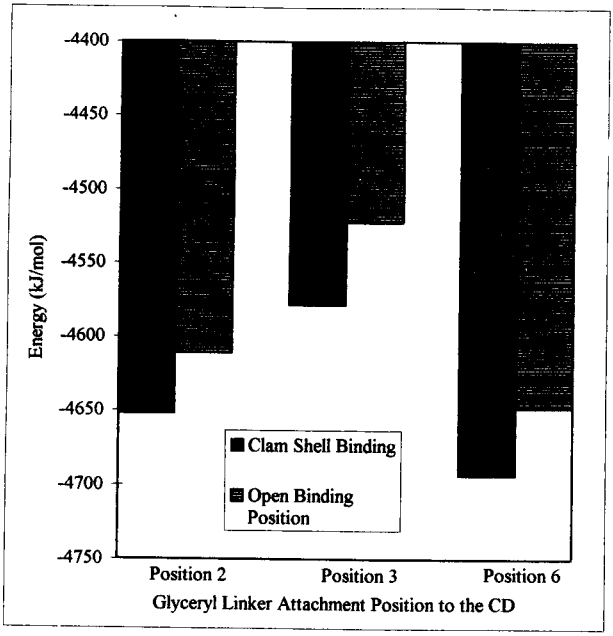


Figure 34: Comparison of the β-CD/Pyrene Complex Energy between Clam Shell and Open Binding Conformations

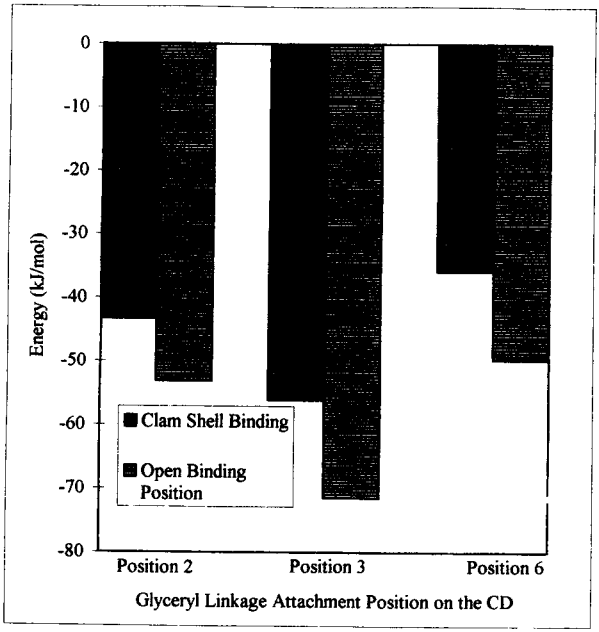


Figure 35: Comparison of the Glyceryl Linkage Energy per Linkage between Clam Shell and Open Binding Conformations of the β-CDP and Pyrene

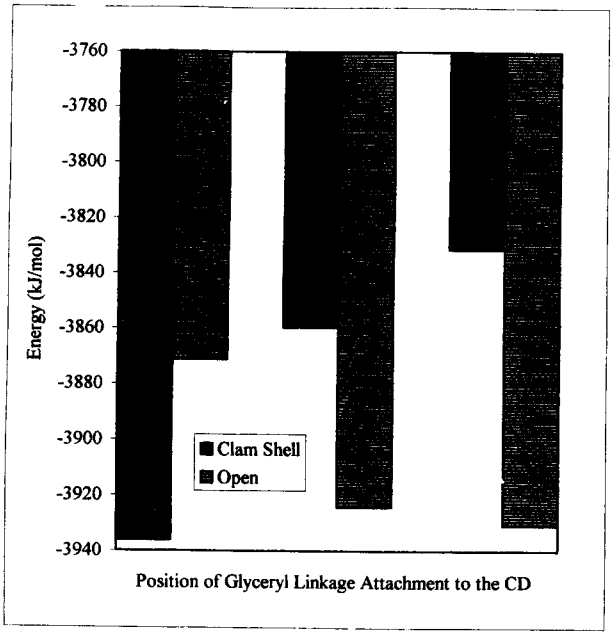


Figure 36: Comparison of the α-CD/p-nitrophenol Complex Energy between Clam Shell and Open Binding Conformations

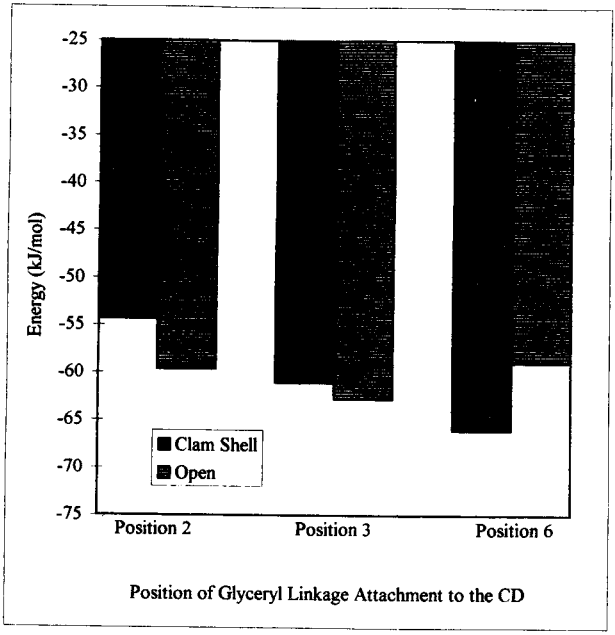


Figure 37: Comparison of the Glyceryl Linkage Energy per Linkage between Clam Shell and Open Binding Conformations of the α-CDP and p-nitrophenol

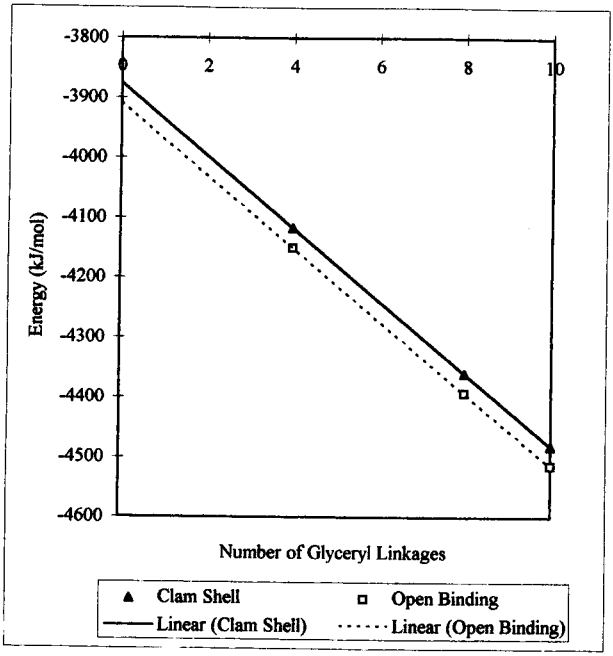


Figure 38: A Linear Plot of the Clam Shell Conformation Energy versus Open Binding Conformation Energy for α-CDP with p-nitrophenol

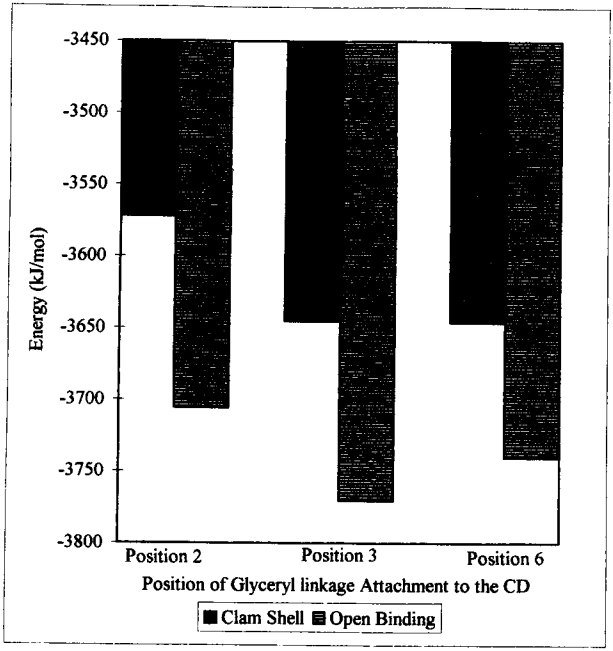


Figure 39: Comparison of the α-CD/p-hydroxylmethyl benzoate Complex Energy between Clam Shell and Open Binding Conformations

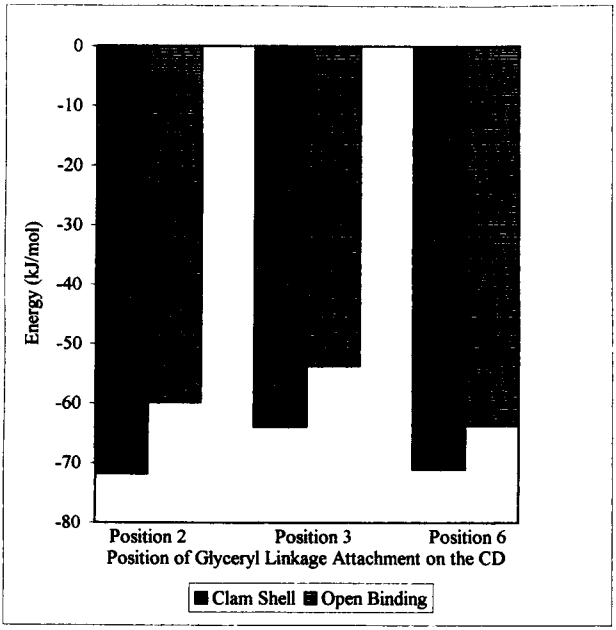


Figure 40: Comparison of the Glyceryl Linkage Energy per Linkage between Clam Shell and Open Binding Conformations of the α-CDP and p-hydroxylmethyl benzoate

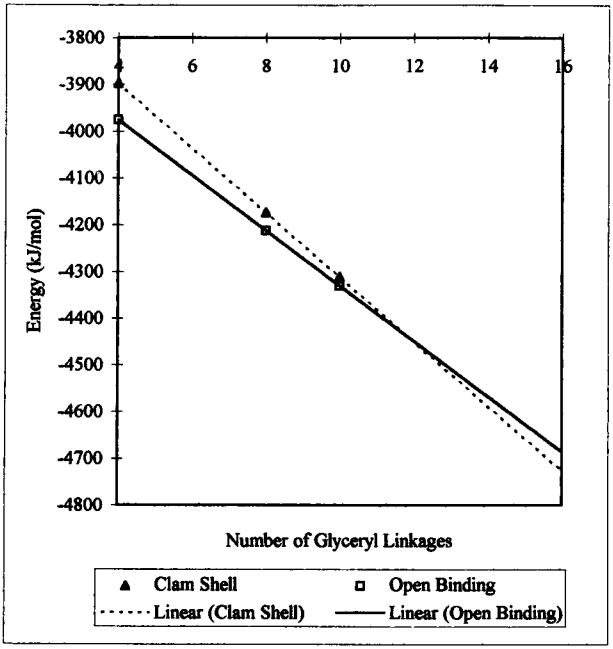


Figure 41: A Linear Plot of Clam Shell Conformation Energy versus the Open Binding Conformation Energy for α-CDP with p-hydroxylmethyl benzoate