CHAPTER 2

CRYSTALLIZATION AND X- RAY CRYSTAL STRUCTURE DETERMINATION

2.1 INTRODUCTION

X-ray crystallography is an important tool to reveal the three-dimensional structure of a molecule. The crystal and molecular structures of the compounds play an important role in determining their physical, chemical and biological properties. Knowledge of the conformations of molecules plays a vital role in relating the structures to the biological activities.

Single crystal X-ray diffraction technique is the most powerful method for the structure determination and it gives information about the conformation adopted by the molecules, the packing of the molecules and how the molecules are getting stabilized by inter and intramolecular forces etc. The conformational studies of biomolecules, play a major role in the field of drug design. The drug actions are very essential to understand the mechanisms of the cell damage (or) repair. The mechanism of the drug action can be understood by its three-dimensional molecular structure. The major part of this thesis deals with the crystal structure analysis of some compounds of biological interest.

This chapter provides a brief outline of the processes involved in crystallization and X-ray crystal structure analysis such as, data collection,
data reduction, structure solution, structure refinement, conformation of the molecules and inter and intramolecular interactions.

2.2 CRYSTALLIZATION

In the process of crystallization, randomly arranged ions, atoms or molecules in the gas or liquid phase take up into regular position in the solid state. This process involves the phenomenon of nucleation. Once nucleus is formed the crystallite faces start growing by deposition (Pamplin 1979). Several methods are available for crystallization and they are classified as follows:

(i) Melt growth method
(ii) Vapour growth method
(iii) Solution growth method

2.2.1 Melt growth method

Melt growth is process of crystallization by fusion and resolidification of the pure material. The rate of growth of this method is higher than other methods. This method is widely used for growing large size single crystals. The melt growth method can be classifieds as follows:

(i) Bridgman Technique    (ii) Czochralski Technique
(iii) Verneuil Technique   (iv) Zone Melting Technique
(v) Heat Exchanger method  (vi) Skull melting process and
(vii) Shaped Crystal growth Technique
2.2.2 Vapour growth method

Crystal grown from the vapour phase are solvent free and have well defined face, which aid in orientation. They can also have low dislocation contents. The vapour grown crystals possesses perfectly flat external surface. In general, single crystals can be grown from vapour phase system by following two methods, namely, physical vapour deposition and chemical vapour deposition.

2.2.3 Solution growth method

There are two methods in solution growth depending upon the solvent and the solubility of the solute. They are, (a) low temperature solution growth and (b) high temperature solution growth. The low temperature solution growth can be done by in two ways viz. aqueous solution growth and gel growth. High temperature solution growth can be done by two ways, (a) flux growth and (b) hydrothermal growth. The growth of a single crystal by low temperature aqueous solution growth technique can be achieved by the slow cooling method, slow evaporation method or temperature gradient method.

2.2.3.1 Slow evaporation method

Among the various methods of growing single crystals, slow evaporation method occupies a predominant place owing to its versatility and simplicity. It is also yielding good quality single crystals.

The mechanism of crystallization from solution by this method is governed in addition to other factors, which is based on solubility of the substance in the solution. A solution is a homogeneous mixture of solute in a
solvent. Solute is the component, which is present in a smaller quantity and that one which gets dissolved in the solution. For a given solute, there may be different solvents.

In this method, an excess of given solute is established by utilizing the difference between the rate of evaporation of solvent and the solute. The solution loses particles, which are weakly bound to the other components, and therefore the volume of the solution decreases. In almost all cases, the vapour pressure of the solvent above the solution is higher than the vapour pressure of the solute and therefore, the solvent evaporates more rapidly and the solution becomes supersaturated (Myerson 1993). Supersaturation is an important parameter for the solution growth process. Usually, it is sufficient to allow the vapour formed above the solution to escape freely into the atmosphere.

If the saturated solution is free from mechanical vibration and allowed to evaporate without disturbance, the best quality crystals can be obtained. A good quality crystal possesses uniform internal structure, pure at the atomic, molecular level, proper size and shape and it should not be twined or composed of microscopic sub crystal. It should not be grossly fractured, bent or physically distorted. A proper solvent and constant temperature also play an important role to produce good quality crystals (Stout and Jensen 1989).

All compounds presented in this thesis were obtained by slow evaporation method at room temperature. The crystallization details such as the solvents used and time of crystallization are presented in Table 2.1.
Table 2.1 Crystallization details for the compounds

<table>
<thead>
<tr>
<th>Compound name</th>
<th>Solvent system</th>
<th>Ratio of solvent</th>
<th>Time of crystallization (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Indole</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BPSI-BD</td>
<td>Hexane- Chloroform</td>
<td>2:1</td>
<td>70</td>
</tr>
<tr>
<td>BPSI-DB</td>
<td>Hexane- Chloroform</td>
<td>2:1</td>
<td>70</td>
</tr>
<tr>
<td>BPSI-BMA</td>
<td>Hexane- chloroform</td>
<td>2:1</td>
<td>70</td>
</tr>
<tr>
<td>BPSI-PY</td>
<td>Ethyl acetate -Hexane</td>
<td>2:1</td>
<td>70</td>
</tr>
<tr>
<td>BPSI-BZ</td>
<td>Hexane- chloroform</td>
<td>2:1</td>
<td>70</td>
</tr>
<tr>
<td>AMBI</td>
<td>Methanol</td>
<td>-</td>
<td>60</td>
</tr>
<tr>
<td>BOPSI</td>
<td>Ethyl acetate -Hexane</td>
<td>4:1</td>
<td>70</td>
</tr>
<tr>
<td><strong>Chalcone</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPHPP</td>
<td>Ethanol</td>
<td>-</td>
<td>60</td>
</tr>
<tr>
<td>CPHPP</td>
<td>Methanol- Chloroform</td>
<td>1:1</td>
<td>60</td>
</tr>
<tr>
<td>MPBPP</td>
<td>Methanol- Chloroform</td>
<td>1:1</td>
<td>60</td>
</tr>
<tr>
<td><strong>Cyclophane</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DCTD-I</td>
<td>Hexane- Chloroform</td>
<td>4:1</td>
<td>48</td>
</tr>
<tr>
<td>DCTD-II</td>
<td>Hexane- Chloroform</td>
<td>4:1</td>
<td>48</td>
</tr>
<tr>
<td><strong>Cyclohexanol</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPC</td>
<td>Acetonitrile</td>
<td>-</td>
<td>70</td>
</tr>
<tr>
<td>BMPC</td>
<td>Chloroform-Methanol</td>
<td>1:1</td>
<td>70</td>
</tr>
</tbody>
</table>
2.3  X- RAY CRYSTAL STRUCTURE DETERMINATION

Crystal structure determination means the finding of the positions of atoms in the unit cell and expanding the lattice by the knowledge of symmetries present in it. The determination of the positions of the atoms in the unit cell requires the measurement of reflection intensities and the solution of the phase problem. The number of reflections that can be measured for a given structure is determined by the volume of the limiting sphere, which in turn depends on the volume of the unit cell and the wavelength used. Although the intensity symmetry may reduce the number of symmetry independent reflections to one half (or) one quarter (or) even less than the total number of possible reflections, few thousands of reflections have to be measured for determining the structure of medium size. Intensity data collection process was carried out by means of a SMART CCD area detector equipped with MoK$\alpha$ radiation with $\omega$ and $\phi$ scans mode.

2.3.1  Intensity Data Collection using CCD

Over the recent years significant technological advances have been achieved in intensity data collection. Scintillation detector which measured single reflection have been replaced by multi-wire, fast television and imaging plate area detectors capable of measuring many reflections at a time.

In 1994, Siemens introduced the SMART (Siemens Molecular Analysis Research Tool) system, the first commercially made available charged coupled device (CCD) based detector system, the latest development in data collection technology, which made major impact on the field of small molecule crystallography.
A CCD is an electrical device that is used to create images of objects and store the information. It receives input light from an object and converts it into an electronic signal. The electronic signal is then processed to produce an image or user valuable information. The CCD based detector system has high dynamic range and fast data readout.

Siemens SMART CCD area-detector is fitted in a 3-circle diffractometer equipped with graphite monochromated MoK\(\alpha\) radiation (\(\lambda = 71073\text{Å}\)). The data collection covered over a hemisphere of reciprocal space by a combination of three sets of exposures and each set had a different \(\phi\) angle (0, 88 and 180°) for the crystal and each exposure of 30s covered 0.3° in \(\omega\). The distance between the crystal and detector was 4 cm and the detector swing angle was -35°. Crystal decay was monitored by repeating thirty initial frames, at the end of the data collection and analyzing the duplicate reflections. The decay was found to be negligible for all the crystals studied. Data collection, cell refinement and data reduction were carried out using SMART (Bruker 1998) and SAINT (Bruker 1998a) programs. The intensities were corrected for the Lorentz and polarization effects, and absorption by using the SADABS (Sheldrick 1996) program.

### 2.3.2 Structure Solution

If magnitude and phase of a structure factor are known, the electron density distribution in the unit cell can be calculated, and hence the three dimensional structure of the molecule can be elucidated.

The general expression for the structure factor is given by

\[
F_{hk\ell} = \sum_{j=1}^{N} f_j \exp \left[ 2\pi i (hx_j + ky_j + lz_j) \right]
\]  

(2.1)
where \((x_j, y_j, z_j)\) are the fractional coordinates of the \(j^{th}\) atom and \(n\) is the number of atoms in the unit cell.

\(F_{hkl}\) is a complex quantity, so we can rewrite the \(F_{hkl}\) as,

\[
F_{hkl} = |F_{hkl}| \exp (i \phi_{hkl})
\]

(2.2)

where \(|F_{hkl}|\) is the structure amplitude and \(\phi_{hkl}\) is the phase of the reflection hkl. In order to locate the positions of atoms, one usually computes the electron density at various points. The position at which the electron density is maximum represents the position of the atom. The general expression for the electron density function \(\rho(x,y,z)\),

\[
\rho(x,y,z) = \frac{1}{V} \sum_{-\infty}^{\infty} \sum_{-\infty}^{\infty} \sum_{-\infty}^{\infty} F_{hkl} \exp \left[-2\pi i (hx+ky+lz)\right]
\]

(2.3)

where \(V\) is the volume of the unit cell and \((x, y, z)\) are the fractional coordinates of any point in the unit cell.

It appears from the above equation that we have simply to map \(\rho(x,y,z)\) and locate the maxima in it, in order to get the structure. But the process is not so straightforward: to sum the series \(\rho(x, y, z)\), we have to find out the complex structure factor \(F_{hkl}\)

\[
\rho(x,y,z) = \frac{1}{V} \sum_{h} \sum_{k} \sum_{l} F_{hkl} \exp \left[-2\pi i (hx+ky+lz)\right]
\]

(2.4)

The Fourier synthesis of equation (2.4) requires the exact values of the complex quantities. \(F_{hkl}\) has both magnitude and phase. The experimental data however contains only the structure amplitude \(|F_{hkl}|\) as the square root of the observed intensity. There is no way to experimentally get the phase values, in order to compute the electron density and hence to locate the positions of atoms. This is called ‘phase problem’ (Stout and Jensen 1989) in crystallography. The phase problem is generally overcome by any one of the
following methods: Direct methods, Heavy atom method, Isomorphous replacement method and Anomalous dispersion method. If the molecule consists of limited number of light atoms, the direct methods can be used for the structure determination. We have used only the direct methods to solve all the structures presented in this thesis. The computer program used for the structure solution is SHELXS97 (Sheldrick 1997).

2.3.3 Direct Methods

The term ‘direct methods’ is applied to that class of methods, which seek directly to solve the phase problem by the use of phase relationships based on the observed intensities. From equation (2.1), one can see that a large $F_{hkl}$ will be found if $(hx_j+ky_j+lx_j)$ mod 1 is approximately constant for all $j$, or, in other words, if all the atoms lie near one of the (hkl) planes. The phase $\phi_{hkl}$ depends on the value of the constant and changes with respect to the origin.

The electron density in (2.3) can be thought of as a superposition of density waves parallel to lattice planes, the amplitudes of which are the $|F_{hkl}|$ values, the relative phases being given by the $\phi_{hkl}$ values.

The direct methods procedures work on three important basic assumptions. They are (i) positivity (ii) atomicity and (iii) randomness. The electron density is assumed to be positive everywhere. A real crystal with continuous electron density is replaced by an idealized one, the unit cell of which consists of N discrete, non-vibrating point atoms. Atoms are assumed to be of point type (F now being replaced by E, the normalized structure factors, which do not vary with $\sin\theta/\lambda$). Although these methods have some limitations in their applications when the number of non-hydrogen atoms in a
molecule is > 50, for smaller structures the success rate of these methods is close to 100%. With user intervention even more complex structures could be solved. But this needs the user’s intuition and expertise.

2.3.3.1 Steps in Direct Methods

The following steps are involved in the direct methods.

a) From the given intensity value, normalized structure amplitude $|E_{hkl}|$ for each reflection is calculated using the formula,

$$|E|^2 = |F|^2 / (\varepsilon \Sigma f_j^2)$$

(2.5)

The factor $\varepsilon$ in the denominator takes account of the fact that reflections in certain reciprocal lattice zones or rows may have an average intensity, which is greater than that for the general reflections. The $\varepsilon$-factor depends upon the crystal class.

b) When symmetry elements are present, the origin cannot be chosen arbitrarily. For example when a two fold rotation axis is present, the origin has to lie on this axis; when there is a 2₁ screw axis, the origin has to lie on this axis; when two 2₁ screw axes intersect each other, the origin has to be at that intersection point and so on. The phase of the normalized structure factors changes with respect to shifts in origin. Although individual phase changes with respect to shift in origin, there are some special linear combinations of phases whose values do not change with respect to shift in origin and these are called the structure invariants. A simple example is the triplet, (or a three phase structure invariant) $\phi_H + \phi_K + \phi_L$ where $H+K+L = 0$. Probabilistic estimates for these types of invariants are already available in
the literature. Those linear combinations of phases whose values remain unchanged when the origins are moved within those permitted by space group symmetry are called the *structure semi-invariants*.

**c)** The structure invariants, structure semi-invariants and the origin problem in every space group are all well documented in the Tables published by the *International Union of Crystallography* (*International tables for crystallography 1992*). These structure invariants and semi-invariants play major roles in direct methods procedures for phase determinations. The structure invariants serve to link the observed magnitudes $|E|$ with the desired phases $\phi_{hkl}$. This is the fundamental principle of direct methods.

In SHELXS97, (Sheldrick 1997), the computer program used to solve crystal structures, triplets are followed by negative quartets estimation. An option exists to generate the two-phase semi-invariants from the quartets, but these relationships are not automatically used in subsequent phasing steps, Sigma-1 type formulae are always used to estimate the one-phase semi-invariants.

**d)** Phase relations are setup using three phases or four phase invariants. This is called convergence procedure. In this procedure only reflections with strong $|E|$ values are chosen, as the reliability of the probabilistic estimates has a direct relation to the product of the normalized structure factor magnitudes of the reflections entering into the triplets or quartets. The bottom of the convergence list has few reflections, which are common in many of the invariants. They are called the well-linked reflections.
e) The phases of the above small set of reflections (well-linked ones) are now assumed (for the choice of origin and enantiamorph) and knowing the values of two phases and the value of the sum of the three phases, the value of the unknown phase can be found. This is called phase propagation or phase extension. Magic integers are used in this process.

f) The above procedure for phase extension is well documented (Karle and Karle 1966) and the phase refinement is carried out using the tangent formula of Karle and Hauptman (1950).

g) Depending upon the choices of the phase values for the reflections chosen for the origin and enantiomorph, the direct methods procedures become multi-solution in nature. Before doing an E-map (a Fourier synthesis with E’s as coefficients), the set with the lowest value for the Combined Figure Of Merit (CFOM) is selected as the correct one.

h) Since |E| and $\phi_H$ are now known, an E-map could now be calculated for this set. This map will reveal mostly the entire structure. In case of incompleteness, the existing model is refined for a few cycles (isotropic refinement) and a difference Fourier will reveal the rest of the atoms.

2.3.3.2 Structure solution

The structure solution of all the compounds were presented in this thesis carried out using SHELXS97 program written by G.M.Sheldrick. SHELXS97 is primarily designed for the solution of ‘small moiety’ (1-200 atoms in the unit cell) structures at atomic resolution. The program is a general one and is efficient for all space groups in all settings, and there are effectively no limits on the number of reflection data, atoms, phases refined in
direct methods, scattering factor types etc. The direct methods routine of SHELXS97 is based on the random start multisolution technique. The program is more accurately described as a multiple-permutation single-solution procedure, since it tries hard to identify the correct solution, which is then ‘improved’ by the E-Fourier or partial structure extension procedure. The program starts by sorting all reflections with \( E > E_{\text{min}} \) into groups. This program uses some selected one-phase seminvariants with known phases. The initial phase refinements are normally performed using a reflection subset (which include the fixed phases). The best 10\% of the subset phase permutation as judged by \( R_\alpha \) (defined below) is then refined using the full set of reflections. The best set with lowest combined figure of merit (defined below) is taken as the correct structure. For the best set the residual R factor for the E-values is calculated by the program and for a correct solution the R-values range from 20-30\%.

The combined Figure of Merit (CFOM) is defined as,

\[
\text{CFOM} = R_\alpha + [0 \text{ or } (\text{NQUAL} - \text{wn}), \text{whichever is larger}]^2 \tag{2.6}
\]

where wn is a structure dependent constant which should be about 0.1 more negative than the expected value of NQUAL. CFOM should be minimum for the best solution and only this solution is retained for calculating the E-map.

\( R_\alpha \) is defined as,

\[
R_\alpha = -\sum w (\alpha - \alpha_{\text{est}})^2 / \sum w (\alpha_{\text{est}})^2 \tag{2.7}
\]

where the weight w is \( 1/(\alpha_{\text{est}} + 5) \) (to avoid largest \( \alpha \)’s dominating) and \( \alpha \) is the reliability coefficient. NQUAL is defined as:
\[
NQUAL = \frac{\sum[\sum(E_1^*E_2^*)*\sum(E_3^*E_4^*E_5^*)]}{\sum[\sum(E_1^*E_2^*)]*\sum[\sum(E_3^*E_4^*E_5^*)]} \tag{2.8}
\]

where the outer summations are performed over all refined reflections and inner summations are over the triplets and negative quartet reflections involving a given reflection. NQUAL approaches \(-1\) for the correct solution.

### 2.3.3.2 Structure Refinement

The structure refinement consists in obtaining the best fit between a set of observed measurements and quantities calculated from a model postulated to explain them. Difference between the observed and calculated values can arise from random errors (statistical fluctuation) in the observations and defects in the model (systematic errors). The trial structure obtained from the structure solution is refined in order to get the accurate atomic positions and the associated displacement parameters.

Several structure refinement processes such as least-squares method, rigid-body method, energy minimization method, simulated annealing method, maximum entropy method and maximum likelihood method are used in structure determination. Full matrix least-squares refinement technique is the conventional one and is widely used in small molecular structure determination. The least-squares refinement consists in using the squares of the difference between the observed and calculated values as a measure of their disagreement and adjusting the parameters so that the total disagreement is a minimum. The refinement is based on \(F_0^2\) because it is impossible to refine on \(F\) using all the data, which would involve taking the square root of a negative number for reflections with negative \(F_0^2\) (i.e. background higher than the peak as a result of statistical fluctuation).
The refinement on $F_0^2$ using all the data provides a good result for weakly diffracting crystal and in particular for pseudo symmetry problems.

The residual factor of the reliability index, $R$ defining the correctness of the model is given by

$$R = \frac{\sum \left|F_0^2 - |F_c|^2\right|}{\sum |F_0|^2}$$

where the summation is made over all observed reflections [$F_0 > 4\sigma(F_0)$].

Lower the $R$ value, greater is the accuracy of the molecular model. A suitable weighting scheme is applied at the end of the refinement procedure and the weighted $R$ factor $wR$ (intensity based) is given as

$$wR = \left[ \frac{\sum w_i \left(|F_0^2| - |F_c|^2\right)^2}{\sum w_i |F_0|^2} \right]^{1/2}$$

where $w = 1/\left[\sigma^2(F_0^2) + (aP)^2 + bP\right]$ a and b are the constants and

$$P = \frac{[2F_c^2 + \text{Max}(F_0^2, 0)]}{3}$$

The Goodness of the Fit ($S$) is always based on $F^2$

$$S = \left[ \frac{\Sigma (w(F_0^2 - F_c^2))^2}{(n - p)} \right]^{1/2}$$

where $n$ is the number of reflection and $p$ is the total number of parameters refined.
2.4  CALCULATION OF GEOMETRICAL PARAMETERS

Crystal and molecular structure determination provide us the unit cell constants and fractional atomic coordinates of all the atoms and their associated displacement parameters. The geometrical parameters such as bond lengths, bond angles and torsion angles can be calculated from the coordinates of the relevant atoms. For a triclinic lattice, the distance between the two points with fractional atomic coordinates \((x_1, y_1, z_1)\) and \((x_2, y_2, z_2)\) is given by the law of cosines in three dimensions as

\[
L = \left\{ (\Delta x a)^2 + (\Delta y b)^2 + (\Delta z c)^2 - 2ab\Delta x\Delta y \cos \gamma \\
- 2ac\Delta x\Delta z \cos \beta - 2bc\Delta y\Delta z \cos \alpha \right\}^{\frac{1}{2}}
\]

where \(a, b, c, \alpha, \beta, \) and \(\gamma\) are the unit cell parameters and

\[
\Delta x = x_1 - x_2, \quad \Delta y = y_1 - y_2, \quad \Delta z = z_1 - z_2.
\]

The above equation can be applied for any crystal system to calculate the bond lengths. Bond length values are useful to identify the nature of chemical bonds (triple, double, partially double or single bond) present in the molecule.

Bond angle formed by the three atoms A, B, C where the angle is subtended by the bonds AB and AC can be calculated using the formula.

\[
\theta = \cos^{-1}\left( \frac{(AB)^2 + (AC)^2 - (BC)^2}{2(AB)(AC)} \right)
\]

Bond angles are useful to find the type of hybridization of a particular atom. Torsion angle is the angle of inclination between the two planes defined as ABC and BCD of the four atoms A, B, C and D.
Torsion angles are calculated as
\[ \chi = \frac{\mathbf{N}_1 \times \mathbf{N}_2}{|\mathbf{N}_1||\mathbf{N}_2|} \] (2.16)
where \( \mathbf{N}_1 \) and \( \mathbf{N}_2 \) are normal to the ABC and BCD planes respectively.

2.4.1 Ring conformations

The possible conformations of a five membered ring are planer, envelope and half-chair. Possible conformations of the six membered ring is as shown in Figure 2.1. Ring conformations can be determined with the help of mirror and two-fold symmetries in the ring as depicted in Figure 2.2. The maximum symmetry i.e., six two-fold and six mirror symmetries are present for a planar six numbered ring and for a chair conformation, three mirror and three two-fold symmetries are present. In case of boat conformation, two mirror symmetries and for twist boat, two two-fold symmetries are present. The other two are the sofa and half-chair conformations. The former one possesses only one mirror and the later one possesses only one two-fold symmetry. Many rings are found to be distorted, without having any defined conformations.

The seven membered ring adopts chair, half-chair, boat, twist-boat and distorted boat conformations. Possible conformations of the seven membered rings are as shown in Figure 2.3. In practice, conformations are described from the asymmetry parameters, which give the extent of deviation of the ring from the ideal conformations (Cremer and Pople 1975, Duax et al 1976 and Nardelli 1983). Program PARST (Nardelli 1995) and PLATON (Spek 2003) was used for the calculations of geometrical parameters.
\[
\Delta C_S = \left[ \frac{\sum_{i=1}^{m} (\phi_i + \phi'_i)^2}{m} \right]^{\frac{1}{2}}
\]
Mirror asymmetry

\[
\Delta C_2 = \left[ \frac{\sum_{i=1}^{m} (\phi_i - \phi'_i)^2}{m} \right]^{\frac{1}{2}}
\]
Two-fold asymmetry

\( \phi, \phi' \rightarrow \) symmetry related torsions

\( m \rightarrow \) number of individual comparisons

Figure 2.1 Possible conformations of the five membered ring
\[ \Delta C_s = \left[ \frac{\sum_{i=1}^{m} (\phi_i + \phi'_i)^2}{m} \right]^{\frac{1}{2}} \]

\[ \Delta C_2 = \left[ \frac{\sum_{i=1}^{m} (\phi_i - \phi'_i)^2}{m} \right]^{\frac{1}{2}} \]

Mirror asymmetry  Two-fold asymmetry

\( \phi, \phi' \rightarrow \) symmetry related torsions

\( m \rightarrow \) number of individual comparisons

Figure 2.2 Possible conformations of the six membered ring
Figure 2.3 Possible conformations of the seven membered ring

Mirror asymmetry

Two-fold asymmetry

\[
\Delta C_s = \left( \frac{\sum_{i=1}^{m} (\phi_i + \phi'_i)^2}{m} \right)^{\frac{1}{2}}
\]

\[
\Delta C_2 = \left( \frac{\sum_{i=1}^{m} (\phi_i - \phi'_i)^2}{m} \right)^{\frac{1}{2}}
\]

\(\phi, \phi' \rightarrow\) symmetry related torsions

\(m \rightarrow\) number of individual comparisons
2.4.2 **Intermolecular interactions**

In crystalline state, the molecular packing is stabilized by intermolecular interactions like hydrogen bonds and van der Waals forces. Hydrogen bonding is the specific type of non-bonded interaction between two electronegative atoms (donor and acceptor), where the hydrogen atom is bonded to one of them. The attractive interaction between the protons with other electronegative atoms of the neighbouring molecules will stabilize the molecular packing. The usual convention for the representation of the hydrogen bond is D-H…A where D is the donor and A is the acceptor. The hydrogen bonds are highly directional and the D–H…A angle should be 180° for an ideal one. The crystal structures presented in this thesis are found to have C–H…N, N–H…O, C–H…Br, O–H…O, N–H…N and C–H…O types of hydrogen bonds. The most important geometrical characteristics of hydrogen bonds are that the distance between the proton and acceptor atom is shorter than sum of their van der Waals radii (Taylor and Kennard 1982). The van der Waals radii used were as follows: C = 1.70, H = 1.20, Cl = 1.75, Br = 1.85, N = 1.55, O = 1.52,. The existence of C–H…O hydrogen bonds in crystals is evident from the study of Desiraju and Steiner (1999), Steiner (1996), Desiraju (1991), Jeffrey and Saenger (1991) and Berkovitch-Yellin and Leiserowitz (1984). A long C–H…O contact (D > 3.6Å) may be acceptable as a hydrogen bond if the angle tends towards linearity (θ > 150°).

Very short C–H…O contacts (D < 3.2 Å) with very bent angles (90-110°) should be carefully examined and angular considerations should take precedence over length considerations. The following criteria are generally used to identify a hydrogen bond:

\[
\begin{align*}
    d (D…A) &< R(D) + R(A) + 0.50 \, \text{Å}, \\
    d (H…A) &< R(H) + R(A) - 0.12\, \text{Å} \quad \text{and} \quad D–H…A > 100°
\end{align*}
\]
where D is the Donor, A is the Acceptor, R(D) is the van der Waals radii of donor atom, R(A) is the van der Waals radii of acceptor atom, R(H) is the van der Waals radii of hydrogen atom, d (D…A) is the distance between the donor and acceptor atom and d (H…A) is the distance between the hydrogen and acceptor atom.

Weak attractive forces between uncharged atoms or molecules are collectively referred to as van der Waals forces. These forces arise from the electrostatic attraction for the nuclei of one molecule by the electrons of different molecules, as well as the nuclei of two molecules counteract the electrostatic attractions, but there is always a small net attractive force. The van der Waals forces are short-range forces i.e., they are significant only when the molecules are very close to one another.

The van der Waals forces are used for non-specific attractions between two atoms that are close to each other. These interactions depend on the distance between the respective atoms (or atom groups or molecules). At too close distance, repulsive forces are dominating (overlapping of electron shells). van der Waals attractions are additive and have thus a much greater impact on macro than on small molecules. Table 2.2 shows some properties of very strong, strong and weak hydrogen bonds (Desiraju and Steiner 1999).

2.5 DETERMINATION OF THE ABSOLUTE STRUCTURE

Absolute structure is a crystallographer’s term and applies to non-centrosymmetric crystal structures. The definition of absolute structure according to IUCr nomenclature is that it is the spatial arrangement of the atoms of a physically identified non-centrosymmetric crystal and its description by way of unit-cell dimensions, space group and representative coordinates of all atoms (Flack and Bernardinelli 1999). Absolute structure
<table>
<thead>
<tr>
<th></th>
<th>Very strong</th>
<th>Strong</th>
<th>weak</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bond energy (-Kcal/mol)</td>
<td>15-40</td>
<td>4-15</td>
<td>&lt; 4</td>
</tr>
<tr>
<td>Examples</td>
<td>[F…H…F]^-</td>
<td>O–H…O=C</td>
<td>C–H…O</td>
</tr>
<tr>
<td></td>
<td>[N…H…N]^+</td>
<td>N–H…O=C</td>
<td>O–H…π</td>
</tr>
<tr>
<td></td>
<td>P–OH…O=P</td>
<td>O–H…O–H</td>
<td>Os–H…O</td>
</tr>
<tr>
<td>Bond lengths</td>
<td>H–A ≈ X–H</td>
<td>H…A &gt;X–H</td>
<td>H…A &gt;&gt; X–H</td>
</tr>
<tr>
<td>Lengthening of X–H(Å)</td>
<td>0.05-0.2</td>
<td>0.01-0.05</td>
<td>≤ 0.01</td>
</tr>
<tr>
<td>D(X⁻⁻⁻⁻⁻ A) range (Å)</td>
<td>2.2-2.5</td>
<td>2.5-3.2</td>
<td>3.0-4.0</td>
</tr>
<tr>
<td>d(H⁻⁻⁻⁻⁻ A) range (˚)</td>
<td>1.2-1.5</td>
<td>1.5-2.2</td>
<td>2.0-3.0</td>
</tr>
<tr>
<td>Bonds shorter than vdW</td>
<td>100%</td>
<td>Almost 100%</td>
<td>30-80%</td>
</tr>
<tr>
<td>θ(X—H…A) range (˚)</td>
<td>175-180</td>
<td>130-180</td>
<td>90-180</td>
</tr>
<tr>
<td>kT (at room temperature)</td>
<td>&gt; 25</td>
<td>7-25</td>
<td>&lt; 7</td>
</tr>
<tr>
<td>Effect on crystal packing</td>
<td>Strong</td>
<td>Distinctive</td>
<td>Variable</td>
</tr>
<tr>
<td>Utility in crystal engineering</td>
<td>Unknown</td>
<td>Useful</td>
<td>Partly useful</td>
</tr>
<tr>
<td>Covalency</td>
<td>Pronounced</td>
<td>Weak</td>
<td>Vanishing</td>
</tr>
<tr>
<td>Electrostatics</td>
<td>Significant</td>
<td>Dominant</td>
<td>Moderate</td>
</tr>
</tbody>
</table>
describes the spatial arrangement of atoms in a non-centrosymmetric crystal structure for which the structure has been clearly distinguished from its inverse in a point. This is in contrast to an ambiguous structure determination where the results of the analysis are a choice between the two centrosymmetrically related images. The inversion-distinguishing power of a diffraction experiment measures its capacity to distinguish between a crystal structure and its inverse. An experiment with high inversion-distinguishing power has the capacity to lead to an absolute-structure determination, whereas a low inversion-distinguishing power permits only an ambiguous structure determination. Inversion-distinguishing power may be increased in an X-ray diffraction experiment either by the inclusion of dispersive scatterers in the crystal structure, there being very few restrictions on their position within the molecule or crystal structure, or by suitably tuning the wavelength. A measure of the inversion-distinguishing power of a diffraction experiment is afforded by the reciprocal of the standard uncertainty of the Flack (1983) parameter.

### 2.5.1 Flack parameter

It is the parameter $x$ in the squared structure-amplitude equation

$$G^2(h,k,l,x) = (1-x)|F(h,k,l)|^2 + x|F(\bar{h},\bar{k},\bar{l})|^2. \quad (2.17)$$

when $x$ takes a value of zero, the atomic arrangement of the model and the crystal are identical; when $x$ takes a value of one, the atomic arrangement of the model and that of the crystal are inverted one with respect to the other. For a twinned crystal, $x$ gives a measure of the relative amounts of the structure and its inverse in the crystal. The reciprocal of the standard uncertainty of $x$ is a direct measure of the inversion-distinguishing power of the diffraction measurement, while $x$ itself is a way of indicating what has been distinguished.