The application of chiral liquid crystal solvents to the NMR of polymers

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The Application of Chiral Liquid Crystal Solvents to the NMR of Polymers

Thesis submitted for the Degree of Master of Science

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CONTENTS

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Summary

In recent years there has been a significant amount of research into the uses of chiral liquid crystal polymers as solvents in solution state Nuclear Magnetic Resonance (NMR).

The NMR spectra of molecules in liquid crystalline solvents can be markedly different to the spectra observed for the isotropic solution. In these anisotropic environments which are neither isotropic liquid phase or crystalline, some of the NMR parameters do not average to zero. Chemical shift anisotropy, dipolar couplings and quadrupolar interactions become a feature of the spectra and in certain circumstances can be measured. Dipolar couplings, if introduced to a spectrum, can make the results very complicated as there can be a great many interactions. Quadrupolar couplings are often larger than dipolar couplings and therefore easier to observe, however this is dependent on the nuclei under investigation having a spin >1/2.

In these studies, it was the observation of chemical shift anisotropy which was to be the aim of the investigation and in particular the hypothesis that the technique could be used for enantiomeric purity determination. It is assumed that the *R* and *S* species will be oriented slightly differently in the solvent and so produce slightly different spectra.

Once the method of sample preparation had been perfected the aim was to apply this technique to the elucidation of the stereochemistry of polymer samples. The purpose being to establish a protocol to assign polymer tacticity without the need for isotopic substitution, such as deuterium labelling.

Introduction

1.1 The Problem with Polymers and Tacticity Studies.

By definition polymers are molecules of high molecular mass and are composed of large numbers of repeating units. Naturally occurring polymers include latex, proteins, starch, cellulose and of course DNA. One of the first known uses of a processed natural latex, adapted for a specific purpose, can be found in the archives relating to ancient Mesoamerican civilization[1]. It is believed that by 1600BC these ancient peoples were processing latex (a soft, sticky substance) from the native *Castilla elastica* tree with the sap from the 'Morning Glory' vine *Ipomoea alba.* This produced a tough but pliable material which was used to make human figurines, bindings and most importantly for these peoples, rubber balls. These rubber balls were central to the sacred and religious ball games which are believed to have revolved around human sacrifice and fertility rituals and also symbolized the battle of good against evil. The Morning Glory vine is known to contain sulphurous compounds which are capable of crosslinking the latex polymer. This is a similar mechanism to that used by Charles Goodyear who has traditionally been credited with inventing the rubber making process.

The mechanisms and processes involved in creating polymers from natural sources which have specific, desired properties can be time-consuming and expensive to ascertain and it is now increasingly common to make wholly synthetic polymers from small monomer units which are added together. There are already a large number of synthetic polymers and as there are many different types of polymers there are also a large range of methods of synthesis, including condensation reactions and more simple addition reactions.

The most basic classification of polymers would divide the group into, for example: rigid plastics and fibres which are resistant to deformation; elastomers which exhibit elastic properties and in between these two categories would fall flexible plastics[2]. This is rather simplistic however, as the range of materials covered by the name polymer is enormous.

Polymers are now used to replace more traditional materials in more and more situations. Metals and wood are often replaced by rigid polymers as are natural fibres by the more flexible polymers in the textiles manufacturing industry. As the characteristic mechanical and physical properties of polymers are controlled by the structure and methods of synthesis, it is necessary to gain as full an understanding of these topics as possible.

The structure-property relationship of polymers falls into two main areas. Viewing the chain as a whole, either branched, cross-linked or linear, can be seen as the gross architectural properties of the polymer. The finer details of the structure of the polymer at the chemical level, for example the particular monomer used, existence of co-polymers etc., is fundamental to the physical properties of the larger molecule. These parameters ultimately relate to sample crystallinity, elasticity and the thermal properties of the three-dimensional aggregate.

While the general chemical composition (eg. monomer residue unit) confers the physical behaviour of a polymer, it is also true that more subtle aspects of the chemistry at this level have a bearing. The configuration of monomer residues at a microstructure level has a considerable influence on the final polymer characteristics.

The configuration or stereochemistry of polymers can be extremely complicated. A

simple example of polymer stereochemistry or tacticity can be demonstrated from examining the case of polystyrene. Styrene itself is not chiral. However, each repeat unit of the chain acquires a pseudo-chiral atom when it is polymerized to form polystyrene.

In cases such as polystyrene, it is the sequence of absolute configurations along the backbone of the chain which become important. Two consecutive absolute configurations in the sequence are usually the shortest distinguishing portion and have been termed a diad.

A meso diad is formed when the two consecutive configurations in a chain have the same absolute configuration eg *RR* or *SS*. If the configurations in the pair are different eg. *RS* or *SR* then the diad is designated a racemic diad. If you consider three or more consecutive residues (triad, tetrad etc.) the level of complication increases dramatically[3].

An isotactic polymer consists entirely of meso diads. This means that the entire polymer chain absolute configuration sequence consists of either *RRRRRR* etc. or *SSSSSS* etc. For practical purposes these will be indistinguishable from each other. A syndiotactic polymer, in contrast, consists entirely of racemic diads ie *RSRSRS* etc. Syndiotactic polymers usually have very different properties from their isotactic counterparts.

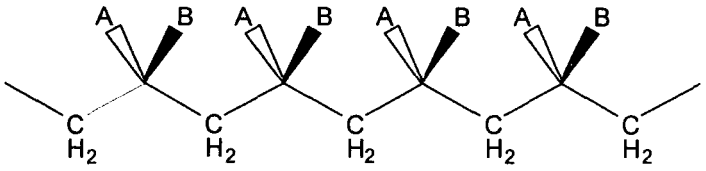
There are a huge range of possibilities for the sequencing of R and S monomers throughout a polymer chain. One commonly encountered case is where the sequence is completely random. The polymers in which this occurs and most polymers in which the sequence of configurations is not highly ordered are termed atactic polymers. (Fig1.)

Relative orientations of monomer units in a homopolymer are complicated by the presence of double bonds in the polymer backbone. It is possible for the double bonds to have either *cis* (Z) or *trans* (E) configurations. In some polymers only one configuration will be present and the backbone will comprise entirely of *cis* or *trans* isomers, whereas in others there will be a random mixture of the two forms. This is referred to as 'geometrical isomerism'.

Directional isomerism also occurs in homopolymers where the monomer in question can be thought of as having a "head" and a "tail" if it is not symmetrical about the centre of the molecule. In these circumstances it is possible for a monomers in a polymer chain to be oriented head-tail-head-tail etc., head-tail-tail-head etc. or a mixture of the two. This directional isomerism is a possibility for many types of polymers including vinylic polymers in which the head-tail-head-tail configuration is predominant. The head-tail-tail-head inversions are also found in vinylic polymers, but are less common.

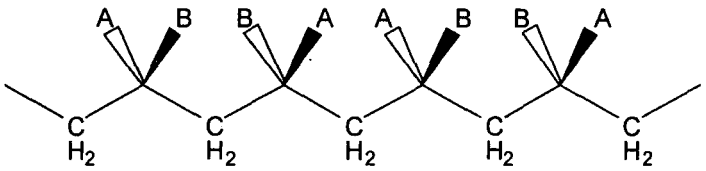
Relative stereochemistry of neighbouring substituents, however, can show variation despite a polymer being entirely head-tail in directional terms. Complicated forms of tacticity arise when the monomer produces true chiral centres on polymerization or when a vinylic monomer is present in which the two backbone carbons are differently substituted.

As these seemingly small changes have such an impact on the nature of the polymer, it is crucial to investigate their precise configuration and research syntheses which control this aspect of the process of polymerization.



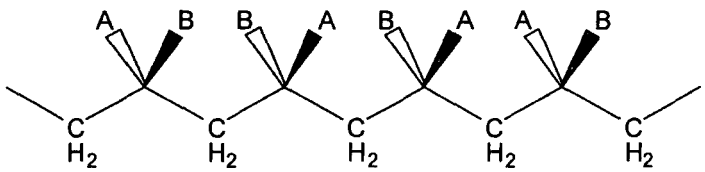
Isotactic Configuration.

All groups are of the same configuration.



Syndiotactic Configuration.

The configurations alternate along the polymer chain.



Atactic Configuration.

The configurations are irregular and can be entirely random.

Fig 1. Schematic representation of the differing polymer configurations.

Identification of the tacticity of polymers is particularly difficult. In certain circumstances polymers which crystallise can be studied by X-ray Crystallography and diffraction patterns acquired which could be used to identify elements of the polymer's stereochemistry. Unfortunately a large number of polymers do not fall into this category because they do not form crystals.

Polymer microstructure in solution can be studied by high resolution NMR techniques. This is due to the fact that in relatively dilute solution the molecular motion is sufficient to reduce the effect of long range interactions. Consequently, it is the short range effects which dominate. NMR spectra of polymers are notoriously difficult to assign, however, as the signals are often broad and encompass the resonances from many equivalent or near equivalent environments.

Careful preparation of polymers to give specific isotactic, heterotactic and syndiotactic samples has, in the past, been useful in the assignment of the spectra of mixtures. This is difficult and not always possible for a given system. The process of assigning configurations to residues of monomers in polymers is further complicated by repeating vinylic regions which will confer *cis* or *trans* (or a mixture of the two) character on the polymer. All of which correspond directly to the characteristics of the polymer.

Before launching into the investigation of the tacticity of polymers, which is inherently very complicated, it is useful to assess the methods which have been used previously to establish the enantiomeric composition of more moderately sized organic molecules.

1.2 Previously Used Methods

Enantioselective synthesis of smaller organic molecules has been of great interest and importance for several years now. The enantiomeric composition of organic compounds yield differing properties, in particular, differing pharmacological effects. A particularly extreme example of this phenomenon is the differing therapeutic uses of the *R* and *S* forms of the compound Propranolol (Fig2). While the (*S*)-enantiomer of Propranolol is used in the treatment of heart disease as an antihypertensive and antiarhythmic drug, the (*R*)-enantiomer, on the other hand, is a contraceptive.

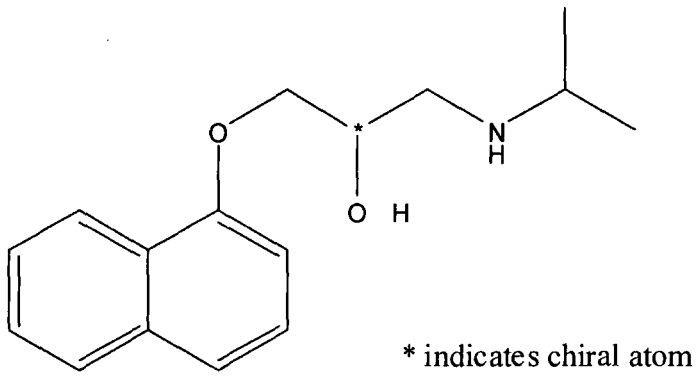


Fig.2 Structure of Propranolol indicating the chiral centre (*).

The pharmaceutical industries especially must pay great attention to the synthesis of any chiral compounds and their subsequent quality control. Enantiomeric purity of compounds is now, therefore, of extreme importance to manufacturers as the consequences of contamination with an undesirable enantiomer can be extremely damaging. One of the most famous cases of the differences in enantiomeric drug efficacy being the use in the 1960's of thalidimide as an anti-nausea treatment for pregnant women. The resultant deformities of some of the children being caused by only one enantiomer of the drug which is now known to racemise spontaneously invivo.

As a consequence of the obvious importance of enantiomeric purity, a great deal of research has been undertaken into methods of synthesizing enantiomerically pure compounds, termed enantioselective synthesis. Equal importance has also been placed on the subsequent determination of the enantiomeric purity of the compounds once they have been produced.

Originally, the method of establishing enantiomer content of a compound was by the measurement of optical rotation. This generally involved measurement of the rotation of plane polarized light using a polarimeter and under strict conditions of solvent purity, concentration, temperature and also the wavelength of the incident light. Once a figure in degrees was obtained for the sample, it was compared with the figures in the literature for the known rotation of the optically pure compound.

There are, however, several problems with this measurement of optical purity as a direct indication of the enantiomeric purity of a compound or mixture. It has been discovered that enantiomeric composition does not necessarily vary linearly to optical rotation. There have also been examples of incorrect literature values for the pure compound, as well as incorrect interpretations of the literature values. As a consequence of these problems there has been a move away from this method as a means of enantiomer analysis.

Despite advances in HPLC and GC analytical techniques, a large number of synthetic organic chemists regularly use NMR techniques for enantiomeric composition analysis of synthetic compounds.

There is a major problem associated with solution state NMR and the differentiation of enantiomers. Under normal circumstances, in an isotropic solution, the resonances of nuclei from each of the enantiomers will be indistinguishable. As it is impossible to distinguish between enantiomers in isotropic solution, it is also impossible to determine if one enantiomer is present in excess of the other. This is due to the fact that the magnetic properties of each enantiomer, as observed by conventional solution state techniques, is

fundamentally equivalent. This is in contrast to the effect seen in diastereotopic nuclei which display differences in magnetic properties and are, therefore, distinguishable in an isotropic environment.

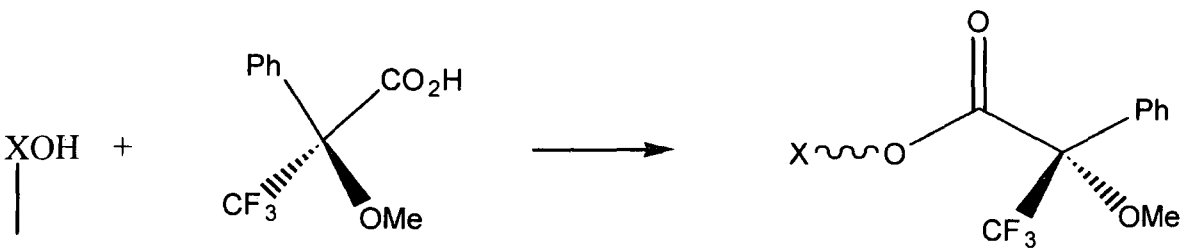
Diastereotopic nuclei in diasteroisomers have been demonstrated to show chiral shift inequivalence. This occurs when the stereogenic centres are covalently linked within the molecule. The method of enantiomeric discrimination would, therefore rely on the conversion of a mixture of enantiomers into a mixture of diasteroisomers. In practice, this requires the use of chiral auxilliaries to convert the mixture. This is generally performed in one of three ways using:

Chiral Derivatizing Agents
Chiral Lanthanide Shift Reagents
or Chiral Solvating Agents.

These methods have been fully described and evaluated by Professor D. Parker[4] and will only be described briefly here:

Chiral Derivatizing Agents

The use of chiral derivatizing agents for chiral discrimination requires the derivatization of the enantiomers under investigation using an enantiomerically pure compound. One of the most commonly used being Mosher's acid [5].



Mosher's Acid Mosher's Ester

Fig 3.General scheme of the reaction of Mosher's Acid with a subject molecule (X) producing a Mosher's ester.

This technique produces discrete diastereoisomeric derivatives which lead to chiral shift inequivalence. This method has been used for enantiomeric analysis of compounds using not only proton NMR shift inequivalence, but also ^{19}F, ^{31}P and ^{29}Si. The normal analysis of the derivatized compounds is via conventional solution state NMR, however this method has also been applied successfully to the solid state technique observing the ^{31}P resonances of the following compound.(Fig 4.)

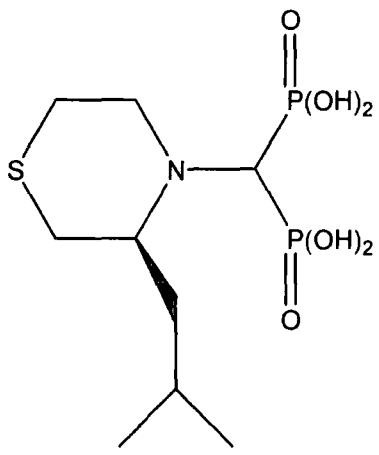


Fig. 4 Compound investigated by solid state ^{31}P spectroscopy

Chiral Lanthanide Shift Reagents

Chiral paramagnetic lanthanide complexes produce species whose proton or occasionally

Carbon-13 resonances are either moved to a higher or lower frequency. These lanthanide complexes form addition compounds with nucleophilic functional groups of organic compounds in solution and these are in fast exchange with the unbound species on the NMR timescale.

This association induces movement of the peaks in the NMR spectra and this lanthanide-induced shift can be useful in that it may help to simplify spectra where there are overlapping signals. As a consequence, assignment of the signals of a complicated spectrum can in certain circumstances be achieved more easily using shift reagents. Since the shifting of NMR signals depends on the structure and geometry of the complex formed between the organic substrate and the shift reagent, this method can also be applied to the conformational analysis of organic compounds in solution.

The most frequently used lanthanide shift reagents are Ytterbium (III), Praseodymium (III) and Europium (III) chelates. Generally speaking Praseodymium complexes induce an upfield shift and the Europium complexes induce a downfield shift. In conjunction with shifting NMR signals and their possible simplification of spectra with overlapping signals, another useful element in using these complexes is found with optically active reagents. One such reagent is tris[(3-trifluoromethylhydroxymethylene)-(+)-camphorato]Europium(III) (which is commonly abbreviated to [Eu(tfmc)$_3$]) which can be used for the determination of optical purity of chiral organic compounds.

This technique has previously been applied to polymer NMR, in particular to Polymethylmethacrylate (PMMA) and it was determined that the induced shifts were dependent on the tacticity of the sample[6]. The lanthanide complex used in these investigations was tris[1,1,1,2,2,3,3-heptafluoro-7,7-dimethyloctanedionato(4,6)]Europium(III) (or [Eu(fod)$_3$]). This complex was found to move signals from the α-methylprotons in the syndiotactic (rr) triad more than in the isotactic (mm) triad and the isotactic methoxy proton peak shifted more than the other peaks.

Although there is obviously merit in this method of tacticity determination of polymers there is a general problem with this technique. Apart from difficulties with selecting lanthanide complexes which produce significant shifts and solubility issues, a major problem is that with fast exchange during the NMR experiment a certain amount of linebroadening is introduced. This impediment is exacerbated at higher magnetic fields due to the broadening effect on the signals being directly proportional to $\mathbf{B_0}$ squared. ($\mathbf{B_0}$ is the applied external magnetic field.)

Chiral Solvating Agents

Chiral Solvating agents use yet another method of chiral discrimination. In this case the chiral solvating agent forms a diastereomeric solvation complex with the solute enantiomers. This is in competition with the bulk solvent and is in rapid reversible equilibrium. The degree of chemical shift anisotropy will be determined by a great many factors in this case, including the concentrations involved, choice of solvent, temperature and the relative sizes of the complexation constants.

All of the methods of chiral discrimination described here rely on the fairly tight control of experimental conditions. Which method is chosen for each compound being investigated will depend on physical and chemical properties and the method considered the best to maximize the chiral anisotropy while minimizing undesirable elements such as line broadening. It is obvious, therefore, that the best results for discrimination for each method are found with different compounds.

Each of these methods relies on the chemical shift inequivalence being large enough and also the lines produced being sufficiently well resolved that integration of the peaks will give an accurate value for the diastereoisomeric composition of the mixture. This in turn

should provide a figure for the enantiomeric composition of the original mixture of enantiomers.

These techniques, although largely successful are a little limiting (particularly if the molecules whose stereochemistry you wish to investigate are polymeric). In the 1960's, however, the idea of using chiral liquid crystal compounds as NMR solvents was formulated and the possibility of using these solvent systems to distinguish between enantiomers was proposed.

1.3 Chiral Liquid Crystal Solvent Systems and Their Application to NMR Elucidation of Mixtures of Enantiomers.

As there is no observable difference in the NMR spectra of enantiomers in isotropic solution attention turned to other media to facilitate the differentiation of mixtures. In the 1960's the idea that liquid crystalline solvent systems might hold the key became popular. It was subsequently established that for a cholesteric liquid crystalline phase the response to an external magnetic field is dependent upon the sign of the molecular anisotropic magnetic susceptibility or $\Delta \chi m$. [7]

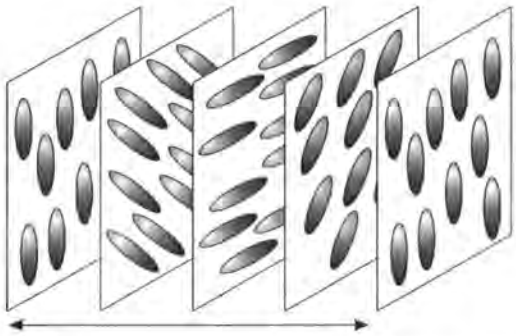


Fig. 5 Alignment of layers of a liquid crystal at slight angle.

Fig. 6 General helical form produced by the rotating layers.

A cholesteric or chiral nematic liquid crystal phase is one in which nematic mesogenic molecules containing a chiral centre produce intermolecular forces which favour alignment between molecules at a slight angle to one another (Fig.5). This leads to the formation of a structure which can be visualized as a stack of very thin 2-D nematic-like layers with the

director in each layer twisted with respect to those directly above and below. This forms a continuous helical pattern. An important characteristic of the cholesteric mesophase is the pitch which is defined as the distance taken for the director to rotate one full turn in the helix (Fig.6).

If the sign of the molecular anisotropic magnetic susceptibility is positive, then the tendency is for the helix to orient perpendicular to the external magnetic field, $\mathbf{B_0}$. In this situation, the director of the liquid crystal becomes parallel to $\mathbf{B_0}$. Under these circumstances the NMR spectra produced contain broad lines and no significant improvements in spectral resolution can be attained.

If the sign of $\Delta \chi \mathbf{m}$ is negative then the helix aligns parallel to $\mathbf{B_0}$ and the director of the liquid crystal is therefore perpendicular to $\mathbf{B_0}$. It has been found that in these circumstances the director does not align with sufficient homogeneity to provide high resolution spectra.

In certain circumstances, when the magnetic field is large enough[7] (which is specific to each liquid crystal), the effect is to unwind the supramolecular cholesteric helix. The unwinding of the helix creates a chiral nematic phase. This chiral nematic phase is sufficiently homogeneous to provide high resolution NMR spectra whilst still providing an oriented environment for chiral discrimination.

When dissolved in chiral anisotropic material, it is known that it is possible for enantiomers to produce differing NMR spectra, depending upon the nucleus observed. The difference in spectra produced by *R* and *S* enantiomers occurs due to enantioselective interactions with the chiral liquid crystal (CLC). These generate Differential Ordering Effects (DOEs) which are observable using NMR.

Several chiral liquid crystal molecules have been investigated for these purposes, principally by Jacques Courtieu et. al.[10] as has much of the groundwork on chiral discrimination

techniques. There are some important criteria which a chiral liquid crystal molecule must fulfill in order to be of use in the NMR study of chiral discrimination of enantiomers. Primarily, the NMR spectrum of the molecule should not overlap the regions of interest for the solute molecule being investigated. The liquid crystal should provide a low viscosity and homogeneous anisotropic mesophase to produce high resolution spectra. In essence, the combination of chiral liquid crystal and solute should provide a near isotropic environment whilst still facilitating the observance of the DOE.

The experiments used can therefore be based upon solution state experiments with reasonable degree of confidence that parameters will be sufficiently close to optimal for initial spectra and also attain a similar signal to noise ratio.

Amongst the chiral liquid crystals found to exhibit these characteristics was polybenzyl-L-glutamate (PBLG) and the enantiomer polybenzyl-D-glutamate (PBDG). Both are solid, fibrous molecules at room temperature and require the assistance of a helicogenic co-solvent to function as a chiral discrimination tool. The poly-benzyl-L-glutamate molecules form an alpha-helix which is a rod-like structure. The tightly coiled polypeptide main chain forms the inner core of the rod and the side chains extend outward in a helical array or outer more flexible alpha-helix. (Fig.7)

As with other naturally occurring polypeptide helices, the helix is stabilized by hydrogen bonds between the NH and the CO groups of the main chain[19]. The CO group of each amino acid is hydrogen bonded to the NH group of the amino acid that is situated four residues ahead in the linear sequence. All the main chain CO and NH groups are hydrogen bonded. Each residue is related to the next one by a translation of 1.5 Angstroms along the helix axis and a rotation of 100 degrees, which gives 3.6 amino acid residues per turn of the helix. Amino acids which are three or four apart in the chain are spatially very close whereas amino acids two apart linearly are on opposite sides of the helix[8].

The pitch of the helix is approximately 5.4 Angstroms, the product of translation and the number of residues per turn. The screw-sense of a helix can be right-handed or left-handed. In the case of naturally occurring proteins the helices are right-handed. Both PBLG and PBDG are synthetic and therefore not necessarily right or left-handed[8].

Organic solutions of PBLG, within a certain concentration range display liquid crystalline properties. The PBLG exhibits the characteristics of cholesteric liquid crystals forming a supramolecular helical structure of directors in the mesophase. Under the influence of a strong external magnetic field (such as that produced by a superconducting high field NMR magnet) however, the helical structure unwinds. This effectively produces a chiral liquid nematic phase. This changed phase displays a positive molecular anisotropic magnetic susceptibility and consequently the director aligns homogeneously parallel to the external magnetic field. This was described previously as an essential characteristic for this particular investigation (Fig. 8.).

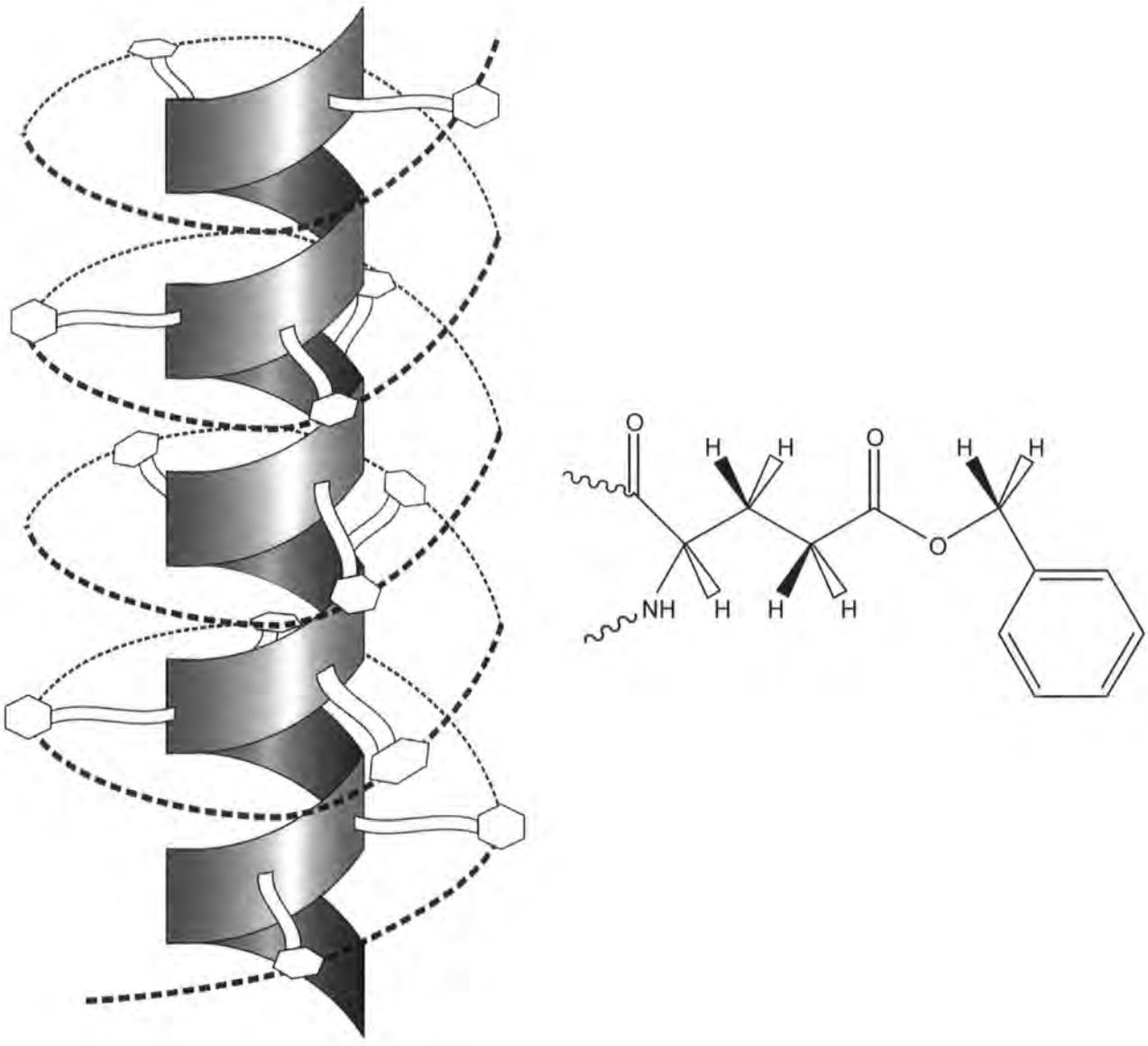


Fig.7 Alpha helix form of the poly-benzyl-L-glutamate molecule with expansion showing the stereochemistry of the individual benzyl-L-glutamate residue. The diagram is not to scale as the inner alpha helix has a diameter of 5.6 Å and the distance from the outside of the inner core to the outer most part of the benzene ring of the glutamate residue is 12-13 Å.

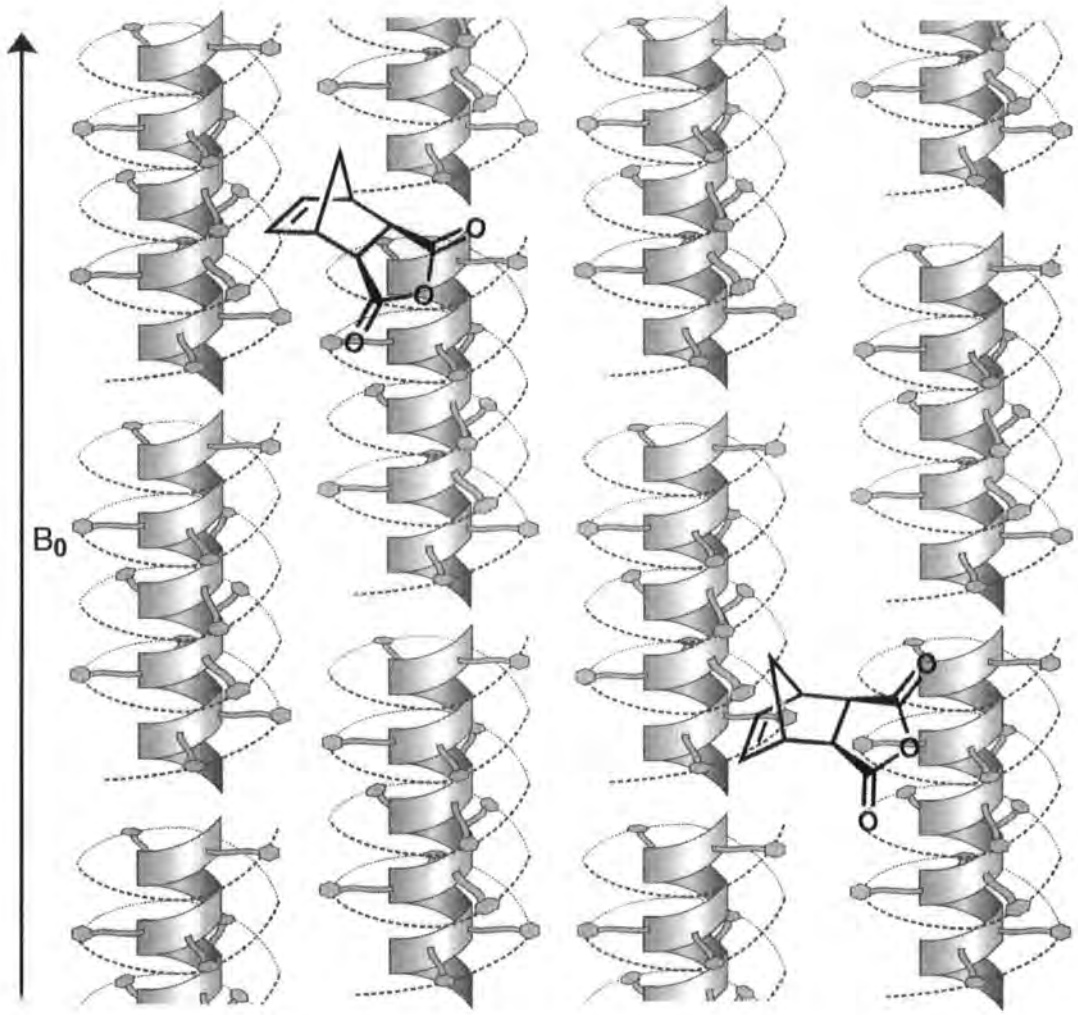


Fig. 8. Schematic representation of the chiral liquid crystal poly-benzyl-L-glutamate matrix of alpha helices aligned in the magnetic field. The PBLG fibres are aligned in a head to tail fashion Molecules of *cis*-5-norbornene-anhydride monomer represent molecules which are oriented in the matrix. The diagram is not to scale but serves to represent the theory behind the orientation of the substrate molecules.

The NMR spectra of chiral molecules which are dissolved in this chiral liquid crystal medium have the potential to provide an enormous amount of information. Not only are they affected by the usual chemical shifts and scalar couplings, but also by anisotropic interactions. These can be dipolar couplings, chemical shift anisotropy (CSA) and in the case of nuclei with spin >1/2, by quadrupolar interactions. These are more commonly seen in solid state NMR spectroscopy and it should be noted that the introduction of all possible dipolar couplings would provide very complicated spectra.

It is the differences in these anisotropic interactions which it is thought will provide the required discrimination. It is the fact that the different enantiomers are oriented slightly differently in the liquid crystal solvent system which provides the measurable difference between the two forms. In the case of chemical shift anisotropy, the differing orientation of the two enantiomers provides NMR spectra in which the isotropic shift of a particular signal is split and the R and S forms appear at differing chemical shift(Fig 9). The shift in Hertz of the new, separate signals can be very small depending on the substrate molecule and the reliance on the method to produce spectra of sufficient resolution is therefore high.

In the case of, for example a proton-decoupled C-13 resonance in an isotropic environment, the NMR spectrum displays a chemical shift of one line. This line is centred on the isotropic shift, υ^{iso} . In the chiral nematic solvent environment, this resonant frequency will be different as the chemical shift anisotropy will no longer be averaged to zero.

$$\upsilon^{aniso} = \upsilon^{iso} + \Delta\upsilon$$

where

$$\Delta\upsilon = \gamma B_0 \Delta\sigma/2\Pi$$

γ = magnetogyric ratio of the nucleus
B_0 = applied external magnetic field

In this case , $\Delta\upsilon$ depends on the order parameters and consequently if the two enantiomers

are not ordered identically we can have $\Delta \upsilon_R$ and $\Delta \upsilon_S$ which are different. This splits the signal into two lines, one for each enantiomer.

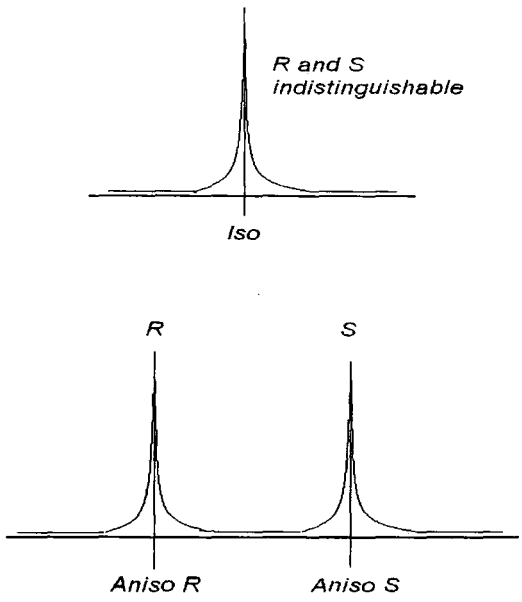


Fig. 9 Representation of the chiral discrimination principle in Chiral Liquid Crystal (CLC) media based on the difference in chemical shift anisotropy.

It is also possible to use dipolar spin-spin couplings to discriminate between enantiomers as these interactions are also sensitive to ordering in the liquid crystal systems, however, inter proton dipolar couplings, because of their complexity, can make spectra difficult to interpret. The ^{13}C-^{1}H couplings are usually larger, however, the couplings observed are likely to be due to both scalar and dipolar interactions and to obtain a value for the dipolar coupling you must subtract the scalar element.

Quadrupolar couplings are usually large and more easily observed in these systems, however, the existence of these interactions is dependent on the presence of a nucleus which has spin >1/2 such as deuterium[11].

1.4 Sample Preparation

The liquid crystal solvent system requires careful preparation. The reason for this is to maintain the liquid crystal properties of the mixture and to ensure that the sample is spatially homogeneous. Often liquid crystals are composed of moderate size organic molecules which tend to be elongated and roughly cigar shaped. Their elongated shape, under appropriate conditions exhibits orientational order such that all the axes line up in a particular direction.

In this case the γ-poly-benzyl-L-glutamate forms an alpha-helix which is common in many proteins and of course to DNA. The PBLG is also termed a lyotropic liquid crystal as it requires the presence of a co-solvent to form a liquid crystalline phase. As with other liquid crystals the bulk order has profound influences on the way light and electricity behave in the material. If the direction of the orientation varies in space, the orientation of light passing through the medium (ie. the polarization) can follow this variation. Under a range of conditions these liquid crystal systems can exhibit a variety of physical properties and phase transitions.

As the PBLG being used here is a lyotropic liquid crystal, transitions occur not by changes in temperature (thermotropic) as with other species, but with the addition of co-solvents, as previously mentioned.

To achieve a birefringent liquid crystal system, careful attention must be paid to the quantity and type of the co-solvent used. The lyotropic mesophases occur as the solvent encourages formation of micellar structures. Lyotropic mesogens are usually amphiphilic in that they contain lyophilic and lyophobic regions. As the lyophobic areas reject the solvent and turn inwards, the lyophilic turn outwards towards the

solvent molecules. In the case of PBLG in a helicogenic solvent, the main chain of the synthetic homo-polypeptide acquires a rigid alpha-helical conformation. The glutamate side chains which branch from the alpha helix form another outer or secondary helix.

In a specific concentration range liquid crystal phases form. The chiral strands of PBLG orientate to form a macroscopic, supramolecular helical structure of directors in the mesophase. This behaviour is typical of cholesteric liquid crystals and the primary function of the solvent for chiral discrimination.

Concentration of PBLG is, therefore, a critical element in the production of the solvent system as well as the concentration and physical properties of the co-solvent. Following the guidelines of Jacques Courtieu et al.[7], the co-solvents chosen for the investigations here came from a surprisingly short list. Common solvents which form successful liquid crystal systems with PBLG are listed below:

Chloroform,

Dichloromethane, dichloroethane, trichloropropane,

Tetrahydrofuran, dioxane,

Benzene, toluene, dimethylformamide (these give a gel phase)

The following common solvents do not form a liquid crystal system with PBLG:

Acetone, ether, trifluoroacetic acid

Methanol, ethanol,

Dimethylsulphoxide,

Carbontetrachloride,

Hydrocarbons such as pentane, hexane etc.

These solvents either do not dissolve PBLG (which is solid and string-like at room temperature) fibres or they force random coiling of the PBLG instead of the desired alpha-helical form. In the case of trifluoroacetic acid and dimethylsulphoxide the unsuitable nature of the solvents arises because they form strong intermolecular hydrogen bonds with the polypeptide chain. This in turn produces a randomised coil formation which consequently destroys any liquid crystalline properties.

In our experiments the co-solvents chosen were chloroform, dichloromethane and tetrahydrofuran. All, of course were the deuterated analogues of the solvent and were chosen because they are easily accessible, relatively cheap and primarily because they dissolved the target molecules successfully.

Average molecular weight of PBLG is another important factor to be considered in the preparation of samples as viscosity plays a very important role in the success or otherwise of chiral discrimination of solutes. Both concentration of the polymer and the nature of the co-solvent determine the viscosity of the sample. If the molecular weight of the polymer is reduced and the concentrations of both polymer and co-solvent are kept constant the viscosity of the sample decreases dramatically. Improved spectral resolution and signal to noise ratios of the solute spectra are achieved with more fluid liquid crystalline samples. These produce longer apparent transverse relaxation times for solutes, however, below a certain level of polymer molecular weight the liquid crystalline phase disappears. If the average molecular weight is too high, then spectral resolution will be insufficient to obtain worthwhile data.

According to Courtieu et.al.[10] the optimal values for the Degree of Polymerization (DP) of PBLG for these purposes are between 300-600. As the stock of PBLG used for all these experiments was purchased from Aldrich, the DP was checked to ensure that the correct range of molecular weights was in use from the start.

Following lengthy investigations, optimal concentrations for solutes in the PBLG solvent system were found to be approximately 40% by weight. In practice, using a 5mm NMR tube and 0.5ml of co-solvent (in the vast majority of cases this was chloroform) the quantities adopted were 50mg of solute and 120mg of PBLG. Obviously the molecular weights of solutes varied even if the PBLG average molecular weight did not, however, this sample preparation method usually established a stable liquid crystal system without interference from dissolved solute molecules. It is known that certain solutes can disrupt the liquid crystal properties of the solvent rendering the system useless and the PBLG effectively irretrievable.

The importance of homogeneity within the ternary mixture, chiral solute/PBLG/co-solvent, cannot be overstated. The mixture is extremely viscous and the quality of the NMR spectra observed are dependent on how carefully the samples are prepared. A concentration gradient of solute within the liquid crystal or a concentration gradient of PBLG within the co-solvent for example would have detrimental consequences to the resultant spectra, especially the linewidths achieved. Also, the chance of observing chiral shift anisotropy will increase if the solute is distributed evenly throughout the mixture. It is a phenomenon which is observed due to a very slight difference in the orientation of the enantiomers involved which in turn is a very small effect in an otherwise tumbling molecule.

After several attempts to prepare samples with varying levels of success, a procedure for consistent sample preparation was devised. This involved weighing 120mg of the fibrous PBLG directly into a 5mm NMR tube. The PBLG was then gently pushed to the bottom of the tube. Separately, the 50mg of solute was dissolved in 0.5ml of co-solvent (usually $CDCl_3$) and this homogeneous mixture was added to the PBLG in the NMR tube. The tube was then sealed with a tube top and parafilm to slow down the evaporation of the solvent. This mixture was then, ideally, left to stand for a couple of hours or overnight to allow the PBLG to dissolve in the co-solvent.

The preparation method of Prof.Jacques Courtieu[9] recommends centrifuging the sample in both directions until an optically homogeneous birefringent phase is obtained. Unfortunately we are not in possession of a centrifuge and because of the high speeds attained, these are time consuming to operate. Balancing, speeding up and allowing time for the centrifuge to stop would make sample preparation a lengthy business.

An alternative was constructed (Fig 11). This consists of a domestic cooling fan with the blades removed and situated on the spindle attached to the motor, a Perspex tube. The Perspex tube being a little longer than the original blades of the fan, but not long enough to restrict rotation and gaining as much centrifugal force as possible. The bore of the tube runs the entire length and is little more than the diameter of the 5mm NMR tube. At either end a plastic screw cap has been fitted for easy access.

Placing an NMR tube in the spinner, a cap pushed on to the bottom for cushioning, a sample can be 'centrifuged' rapidly from one end of the tube to the other. The 'Fan Spinner' works at far slower speeds than a conventional centrifuge, however, this was

found to be more than sufficient to encourage the mixture from one end of the tube to the other. It is very quick to stop and start while the Perspex tube ensures a clear view of the progress of the sample. Reversing the centrifuge involves merely tipping the Perspex tube until the NMR tube falls gently to the opposite end where the process can begin again.

The final step is to centrifuge the mixture to the bottom of the NMR tube. Centrifuging the sample from end to end twenty times is sufficient to produce a homogeneous mixture and used in this fashion a liquid crystal solvent system can be ready for experimentation within a few minutes, rather than several hours.

It was thought that thorough mixing of the samples would be aided by the addition of small, spherical glass beads to the NMR tube for the centrifuging process. The size of the glass beads used for this purpose were a fraction less than the diameter of the NMR tube. The theory being that smaller beads would not effect efficient mixing and that heavier beads would be propelled through the mixture more readily.

The glass beads however, were not easily retrievable from the tubes and leaving them in the bottom of the NMR tube created some difficulty in shimming the sample. This was not the principle reason for abandoning their use. None of the experiments conducted following the use of the beads produced evidence of chemical shift anisotropy and it was concluded that it was possible that there had been some damage to the liquid crystalline system. Aids to mixing were also considered unnecessary as the centrifuging regime produced reliable chiral liquid crystal mixtures.

There are many factors which affect the quality of NMR spectra and shimming is one

of the major considerations. As the information we were looking for in the spectra required as high a resolution of lines as possible, everything which has a bearing on magnetic field homogeneity had to be investigated.

The temperature stability of the experiments was the first to be considered and experiments were initially carried out at fixed, slightly elevated temperatures. The hypothesis being that the resolution would improve with slight lowering of viscosity and stabilization of the temperature to prevent fluctuations in temperature leading to gradients within the sample or at worst convection.

The spectra obtained were found to be no better in quality than those obtained at room temperature and so forced stabilization of the temperature was abandoned. In reality the probe temperature was found to be very stable all year round at 22 °C +/- 0.5°C due to the efficiency of the laboratory air conditioning.

Shimming is more usually performed using the deuterium lock signal from the deuterated co-solvent. In this case, however, it was found that the signal from the deuterium in the co-solvent was not strong enough to provide a reliable shimming method. Choosing co-solvents which contained a greater number of deuterons per molecule did not affect this finding and it was thought shimming using the Free Induction Decay (FID) of the sample the only method. In effect, this involves adjusting the shims to elongate the FID, as the longer the FID lasts the greater the resolution will be. Also, the closer the shape of the FID to a perfect exponential decay, the better the lineshape achieved in the final spectrum.

This method of shimming is facilitated using computers which integrate the total area of the FID and produce a number which is indicative of the resolution. In this way the

sample can be shimmed in a similar way to the conventional lock shimming method which relies upon the intensity of the lock signal to indicate resolution. There are advantages to FID shimming over the lock shimming method and it is now considered superior.

There is, however, a problem with the lack of a deuterium lock signal when you consider the acquisition of the data for the experiment. The frequency lock feedback mechanism as it is more properly referred to, is an essential element to the stability of an experiment, especially for any experiment which will run for any considerable length of time. A spatially homogeneous and temporally constant magnetic field is necessary to produce a high resolution NMR spectrum of any sample. This is affected by temperature fluctuations, movement of metal object in the vicinity of the magnet, but more usually by the variation in field strength of magnets over time, otherwise referred to as 'magnet drift'.

The field frequency lock circuit within a spectrometer is specifically designed to compensate for this and constantly monitors the lock resonance frequency of the deuterium signal in the solvent. If the resonance moves then small changes are made to the $\mathbf{B_0}$ magnetic field to keep the resonance constant.

The only way we found to establish a reliable lock signal in the samples was by means of a coaxial insert placed in the NMR tube containing a solvent possessing large numbers of deuterons per molecule. The most easily available solvent meeting these criteria was acetone and as it would have no contact with the liquid crystal solvent system, would not interfere with the liquid crystal properties.

The coaxial insert was placed in the NMR tube after the centrifugation and this was found to be a particularly difficult process as the bore of the tube was very close to

the outside diameter of the insert and inevitably there was some LC residue on the insides of the tube. Consequently a vacuum seal was created on attempting to locate the insert and rotating the insert until the trapped air made its way past was the only way found to overcome this problem. After assembly, the tube and insert were stoppered and sealed with parafilm to slow down the rate of solvent evaporation.

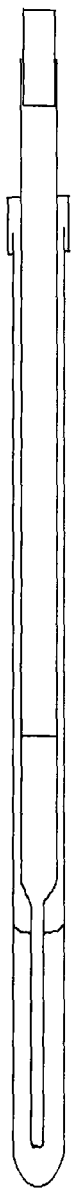


Fig 10. Diagram of 5mm NMR tube with capillary insert.

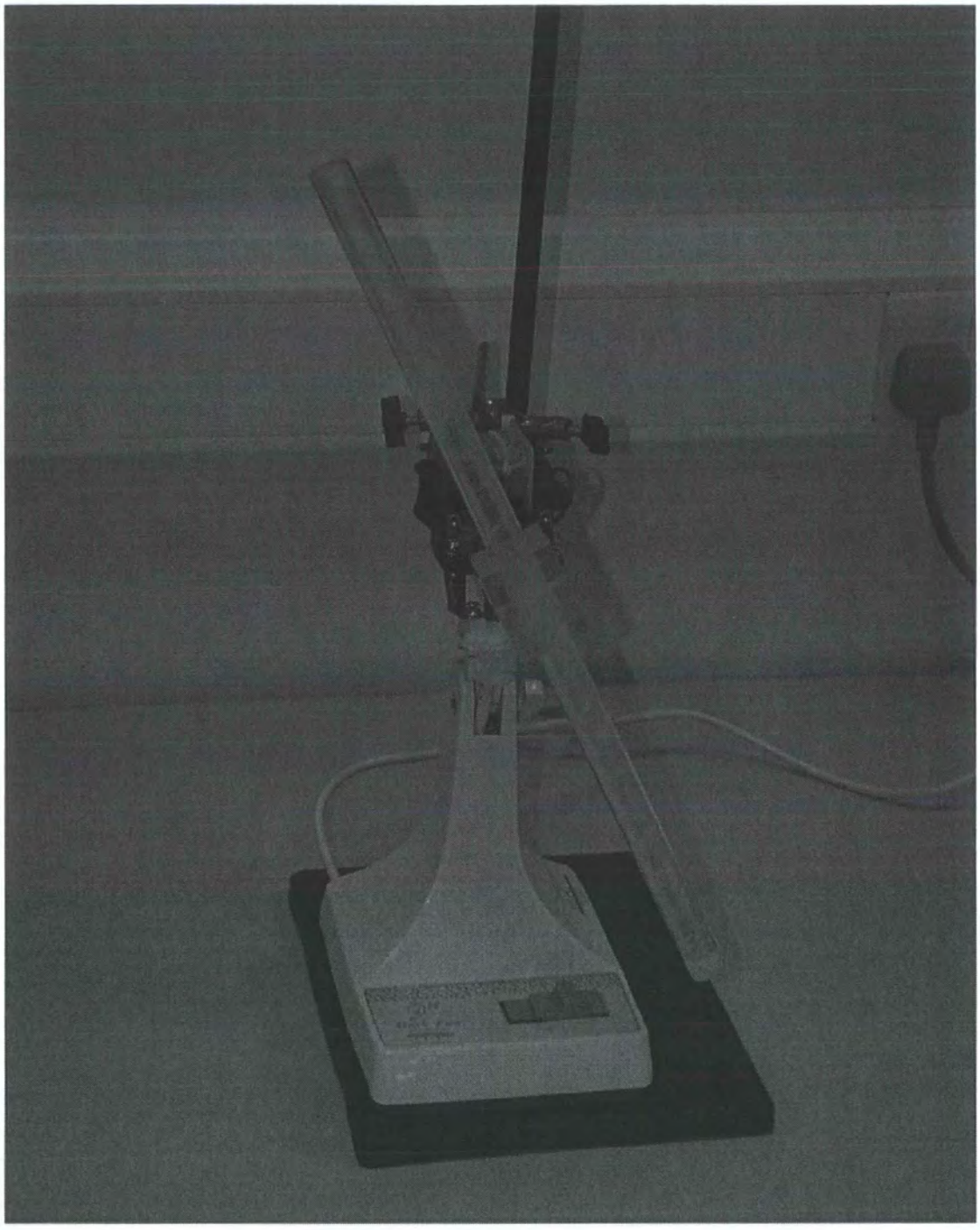


Fig. 11. Photograph showing the 'Sample spinner' (designed by Dr Alan Kenwright) on which can be seen the perspex tube designed to hold a single NMR tube. As the tube is hollow all the way through, it is a simple matter to change direction of the flow of NMR tube contents.

CHAPTER 2

2.1 Results from Small Molecule test samples.

Following the work of Courtieu et al.[8] on a range of small molecules which gave some interesting results, it was thought useful to further practice the technique of sample preparation and try to establish criteria for success or otherwise on simple, small molecules. As with isotropic samples, broadband proton decoupling 13C spectra of samples in a PBLG solvent system usually shows well-resolved peaks. All of which can normally be assigned to the non-equivalent carbon atoms within a given molecule.

If the difference in chemical shift anisotropy between enantiomers is large enough, then it is possible to see two distinct resonances. This is described here as a split in the isotropic carbon resonance signal. It is described in this way primarily because the ^{13}C chemical shift anisotropy values in PBLG are somewhat small and consequently the chemical shifts of each carbon are very close to those seen for the isotopic equivalent.

Despite the fact that the difference in hertz of the resonances are comparatively small, it is a feature which aids in the identification of carbons which are differentiated. It is also an aid to quick assignments of signals from the spectra. It is anticipated that the difference in hertz of resonances will only be in the region of 3-15Hz as this is a typical value. Only in rare circumstances has a value of 40Hz been recorded which applied to an sp carbon. It was anticipated that the probability of finding chiral discrimination for sp and sp_2 hybridized carbons is greater, although there was some hope that sp_3 hybridized carbons might also show some differentiation especially as a magnet of 500MHz (for proton resonance) was to be used. As the sensitivity of the

chemical shift anisotropy (CSA) to the Differential Ordering Effect (DOE), generated by the Chiral Liquid Crystal increases with $\mathbf{B_0}$.

This is in contrast to both the homonuclear and heteronuclear spin-spin coupling interactions which are independent of the magnitude of the magnetic field used.

Success of the technique here is being described as splitting of all or at least one 13C signal in a standard one dimensional experiment. A selection of molecules were used for this exercise and the results were varied and informative.

+/- Butyn-2-ol (Figs.12 and 13)

The first molecule used for this purpose was +/- 3-butyn-2-ol, a relatively simple molecule with only four, well spaced carbon signals. As can be seen from the carbon spectrum there is splitting of two carbon signals corresponding to the alkyne carbons (sp) of the molecule at 71ppm and 85ppm. The splitting in Hertz of the signals was 20.91Hz and 30.65Hz respectively and obviously a large enough splitting to be seen even if the signals were broadened due to the viscous nature of the liquid crystalline solvent system. It was assumed that this was due to different signals separated in chemical shift resulting from each of the enantiomers present. As the results for the butyn-2-ol were clear, it was thought a slightly longer chain molecule of similar chemistry may prove to give similar results, however, this was not found to be the case.

+/- 4-Pentyn-2-ol (Figs. 14 and 15)

The next in the series to be used was +/- 4-pentyn-2-ol. At only one carbon longer in chain length, the results were disappointing. After a couple of transients we briefly

observed a split in the alkyne signals at 71ppm and 85ppm, however, this was found to disappear on further acquisition and the signals returned to the singlet appearance. Despite efforts to repeat the experiment, the split was thought to be transitory and attempts at temperature stabilisation and further shimming to maximise conditions proved fruitless.

The results for +/- 4-pentyn-2-ol were particularly disappointing especially given the presence of an sp hybrized carbon in a virtually equivalent position to that of the +/- 3-butyn-2-ol. It appeared that it was not quite as simple to predict chiral discrimination as previously thought.

+/- 2, 3-Dibromopropionic Acid (DPBA) (Figs. 16 and 17)

It became obvious that further study with other molecules was necessary to attempt to establish which compounds would show splitting under this particular set of conditions, as the results thus far were not giving immediately obvious criteria.
The next molecule investigated was +/- 2,3-dibromopropionic acid (DBPA), containing only three carbon signals the theory was to return to a simple system to evaluate the method. The results for this molecule were rather more complicated than anticipated and as you can see from the carbon spectrum of the sample in the liquid crystal environment every signal was split. The carbonyl signal was split simply into a doublet with a splitting of 55.72Hz, but the other signals are far more complicated and it was thought that there had been a far more significant reintroduction of the dipolar coupling element evident within this molecule. It can probably be assumed from these signals that although both enantiomers would be visible, there had been further splitting due to the introduction of dipolar coupling. Consequently, establishing and accurately evaluating the splitting pattern is no simple matter and the decision was made to investigate a molecule which, being slightly larger would perhaps bind

less tightly to the helices of the liquid crystal and ultimately exhibit a less dramatic reintroduction of dipolar couplings. Dipolar couplings are traditionally a major feature of solid state NMR spectra and in this case are a source of unwelcome complexity in the results.

+/- Ibuprofen (Figs 18 and 19)

The choice of +/- Ibuprofen was determined largely by the presence in the molecule of an aromatic ring. As the previous observations of splittings had mainly involved carbons with a degree of unsaturation, (sp giving the larger differences) it was assumed that either the electronic or geometric (or both) properties of these carbons had some bearing on whether there was some slight orientation in the liquid crystal solvent system. A chemical shift anisotropy (CSA) leading to the splitting of the corresponding signals into two, one for each enantiomer would be the ideal outcome. As can be seen from the spectrum of Ibuprofen in the PBLG system, there are a significant number of split signals in the carbon spectrum and not all the signals displaying a splitting are aromatic. As can been seen from the spectrum, there is a range of splittings across the molecule and the largest of these do belong to carbons of the aromatic ring, suggesting that perhaps the planar nature of the ring has some influence in the probability of one orientation being dominant in an otherwise tumbling, if not truly isotropic environment. The other major splitting observed in the molecule involves the signal corresponding to the carbonyl carbon which is another nucleus in a planar environment. The difference in Hertz between the signals even within the aromatic ring are not identical.

The next step was to establish whether the technique could be useful in a quantitative analysis of enantiomers. This was thought to be a practical element which could be

brought to the technique. Although many molecules produced in the laboratory will be present in a racemic mixture, this is not normally the case in nature and may not always be desirable from a scientific standpoint. Consequently identification with attendant quantification of enantiomeric mixtures must be the ultimate goal of the exercise.

Menthol (Figs. 20-23)

The next molecule to be investigated was menthol and as can be seen from the carbon spectra of the racemic and the natural (-) form of the molecule it is virtually impossible to distinguish between the two spectra despite one being only one form of the compound. The sample used for the liquid crystal experiment was, therefore, made up of a mixture of racemic and natural menthol which would have an excess of the one form (the natural (-) form). The results were disappointing and as can be seen from the expansion of the spectrum there is only one signal with a small splitting of 5.69Hz. There is information to be extracted from this apparently unhelpful result, however, which is that there is an obvious difference in peak height which may suggest that the lower peak is that of the minor enantiomer. This is only a suggestion as the conditions under which the spectrum was obtained would not lead to a strictly quantitative result. The conditions for quantitative carbon spectra being somewhat different to those necessary to merely observe signals. The relaxation time of carbon nuclei can differ substantially and therefore the recovery delay and consequently the proton decoupling are usually adjusted in the pulse sequence to allow for full relaxation of all carbons within a given molecule. The results did indicate that perhaps menthol was not an ideal candidate for this particular investigation.

Menthyl Anthranilate (Figs. 24 and 25)

A sample of menthyl anthranilate was prepared and spectra obtained using the established method. The results obtained showed no splitting of any carbon signals. This was the largest molecule, except for polymeric material, to be tested in this manner.

+/- Terpinene-4-ol (Figs. 26-28)

Following this result a smaller molecule was used and interestingly one which had been isolated from a natural source as an unequal mixture of enantiomers. The compound tested was terpinene-4-ol, (4-isopropyl-1-methyl-1-cyclohexen-4-ol) a naturally occurring terpinene and present in many plants including Tea Tree (*Melaleuca alternifolia)* Oil in which it is present in the following ratio of enantiomers: (+/-) 1.8-2.4:1 which is indicative of the origin of the terpinene as in other species the ratio of isomers is different. Effectively, the presence of this ratio in the mixture is a test for the origin of the oil. This is especially important as this component is generally considered to be the key anti-microbial active compound in Tea Tree oil and has also been found to be effective as an anti-inflammatory. The results for this mixture were very encouraging as it was apparent that the signals for the carbons in the double bond were split and further investigation including quantitative analysis gave an isotopic ratio of 2:1. Further investigations followed, including the preparation of a mixture of terpinen-4-ol which had the ratio of enantiomers adjusted to be roughly 50:50 . This was a check to see if the technique was viable if the mixture was changed in any way. The results were successful as the mixture was similarly treated and quantitatively analysed to give a mixture of 50:50 of the (+) and (-) forms. This was an exciting result, as it meant that mixtures of

enantiomers (and therefore origin) of the main active ingredient of a popular therapeutic oil can be established using NMR under mild and relatively simple experimental conditions.

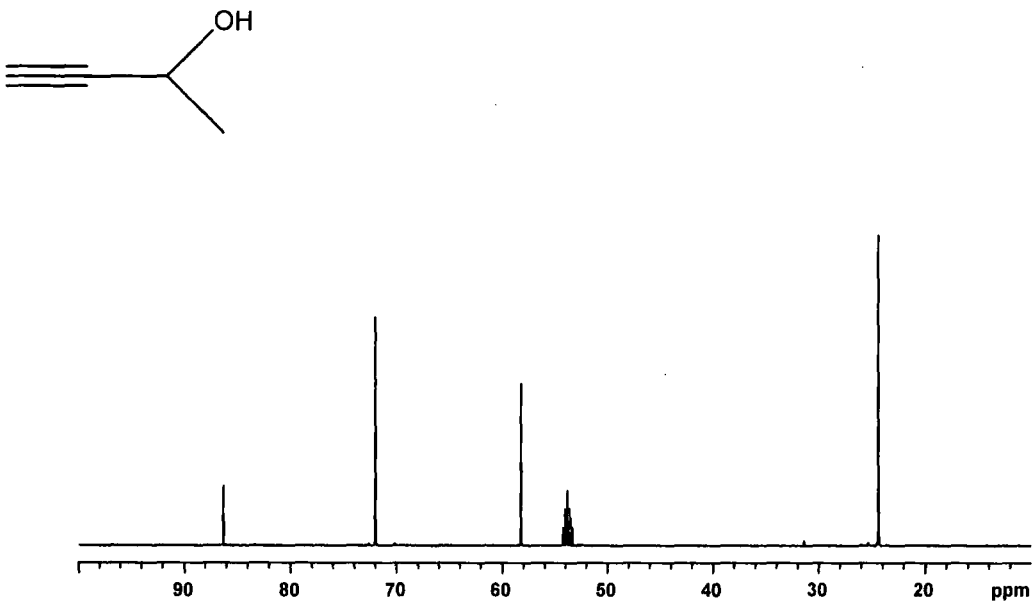


Fig. 12 +/- Butyn-2-ol Carbon-13 solution state NMR spectrum

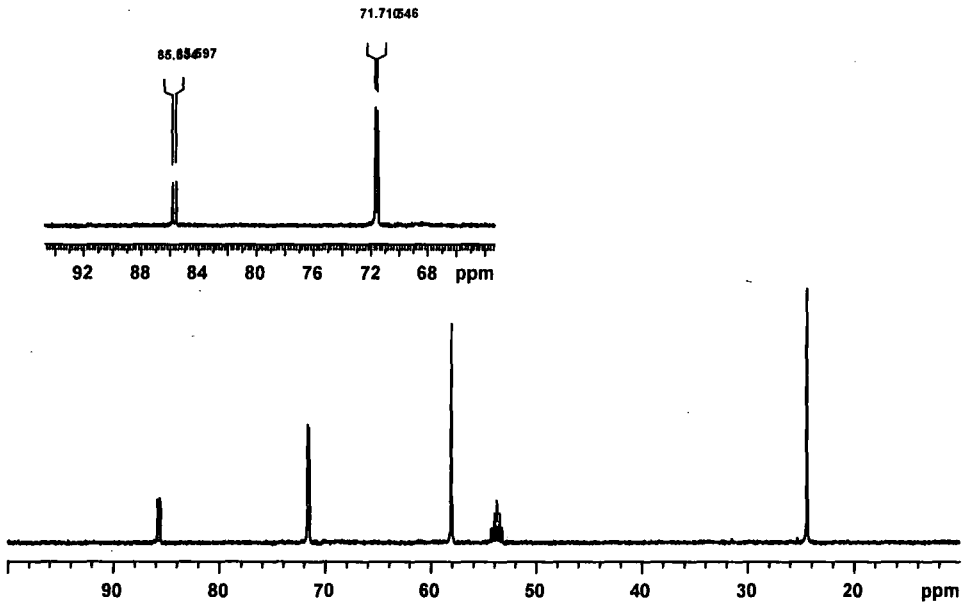


Fig. 13 +/- Butyn-2-ol Carbon-13 Spectrum in PBLG solvent system

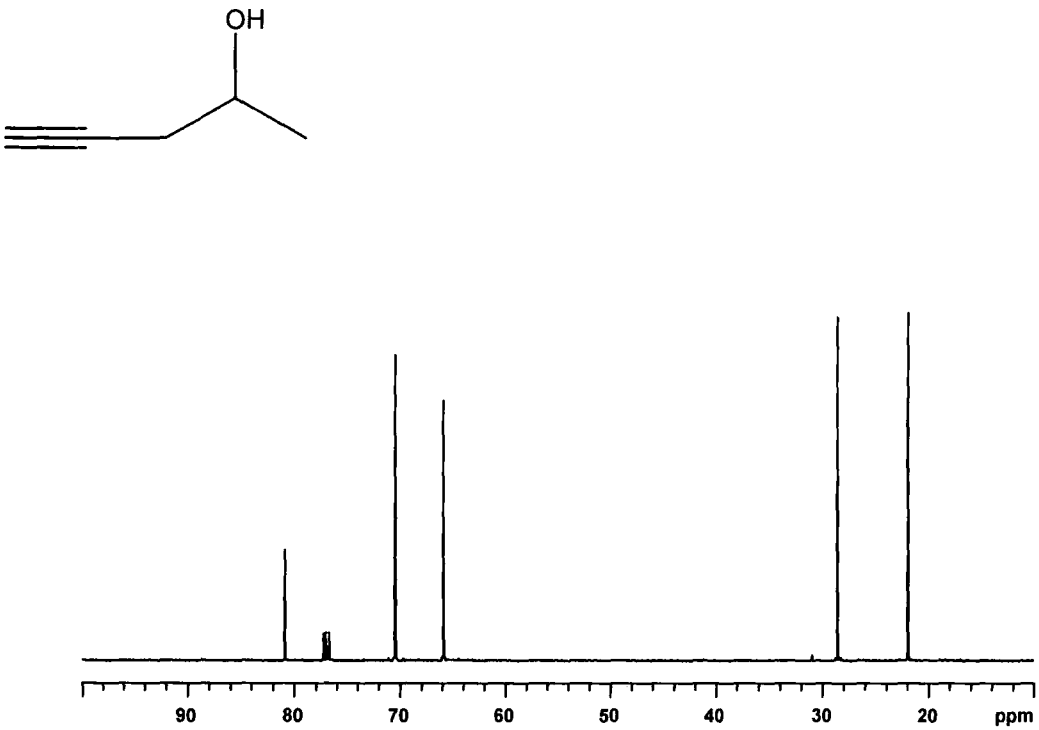


Fig.14 +/- Pentyn-2-ol Carbon-13 NMR spectrum in solution

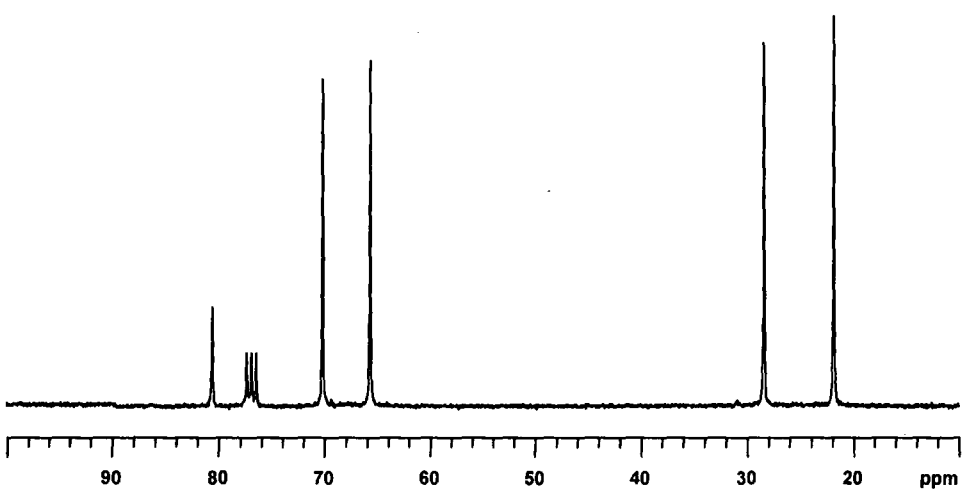


Fig.15 +/- Pentyn-2-ol Carbon-13 NMR spectrum in PBLG solvent system

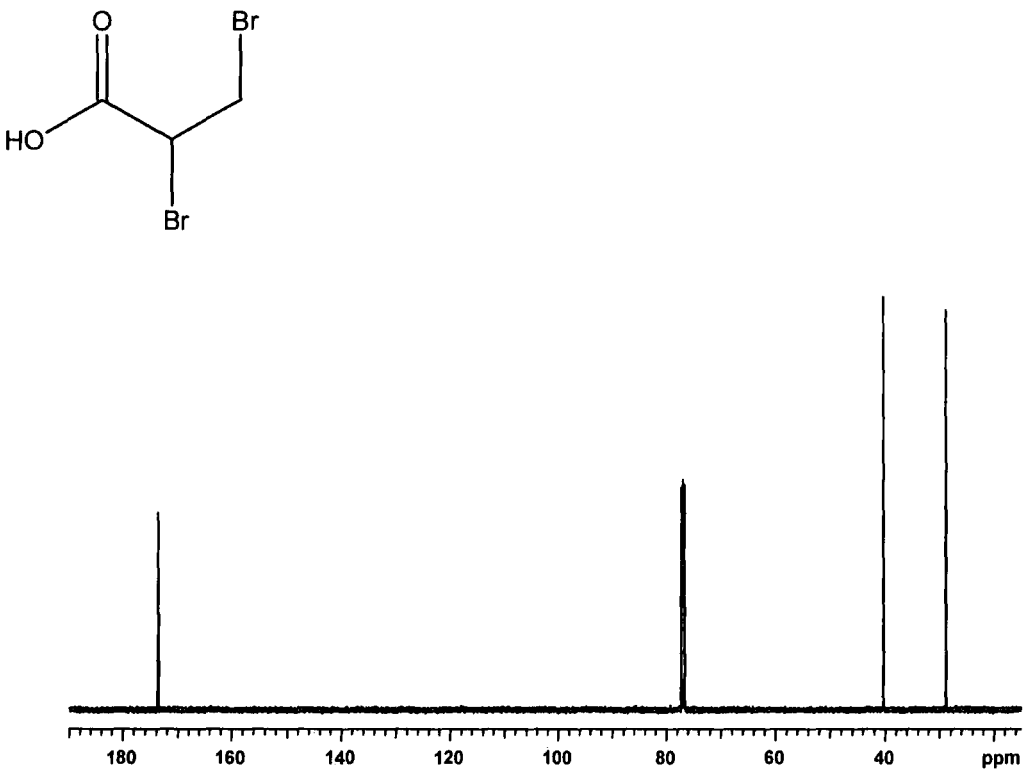


Fig.16 +/- 2,3-Dibromopropionic acid Carbon-13 NMR spectrum in solution

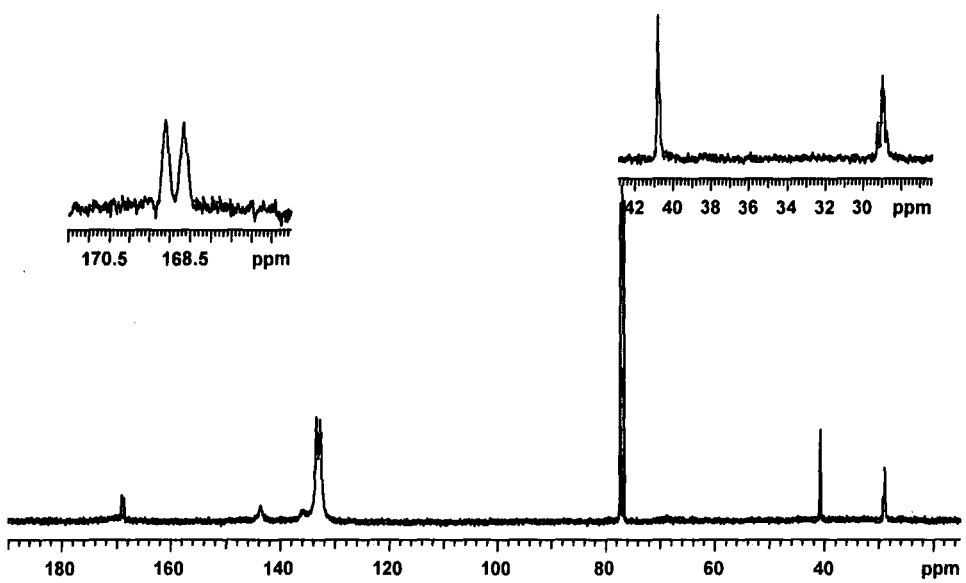


Fig.17 +/- 2,3-Dibromopropionic acid Carbon-13 NMR spectrum in PBLG solvent system.

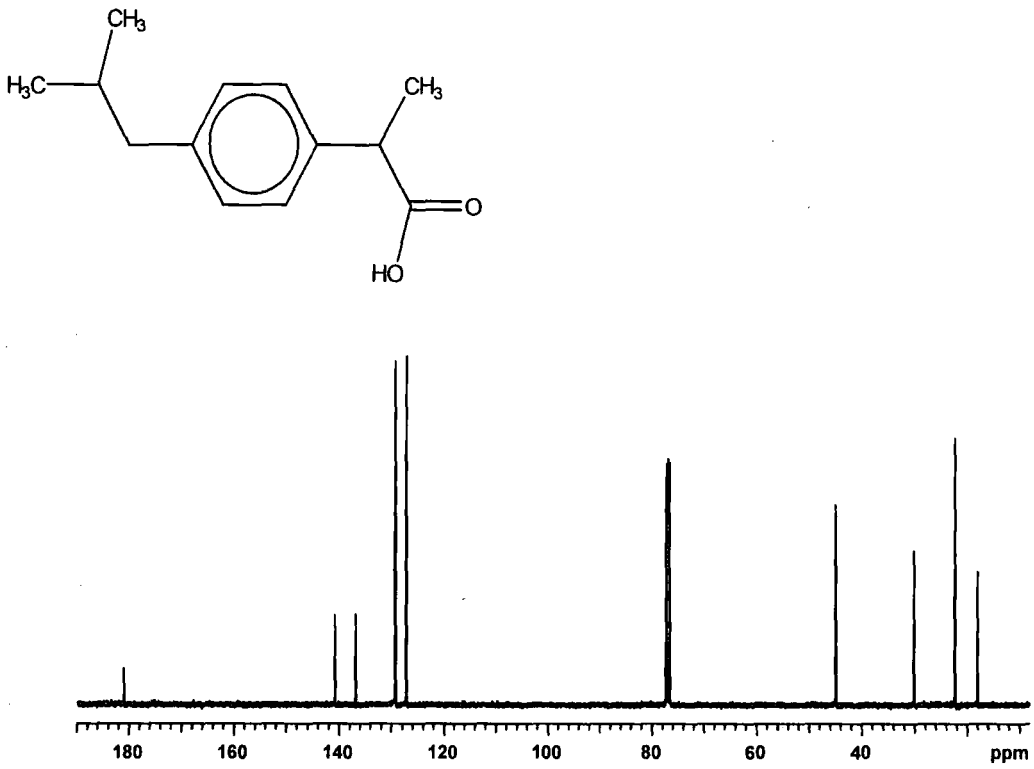


Fig.18 +/- Ibuprofen Carbon-13 NMR spectrum in solution

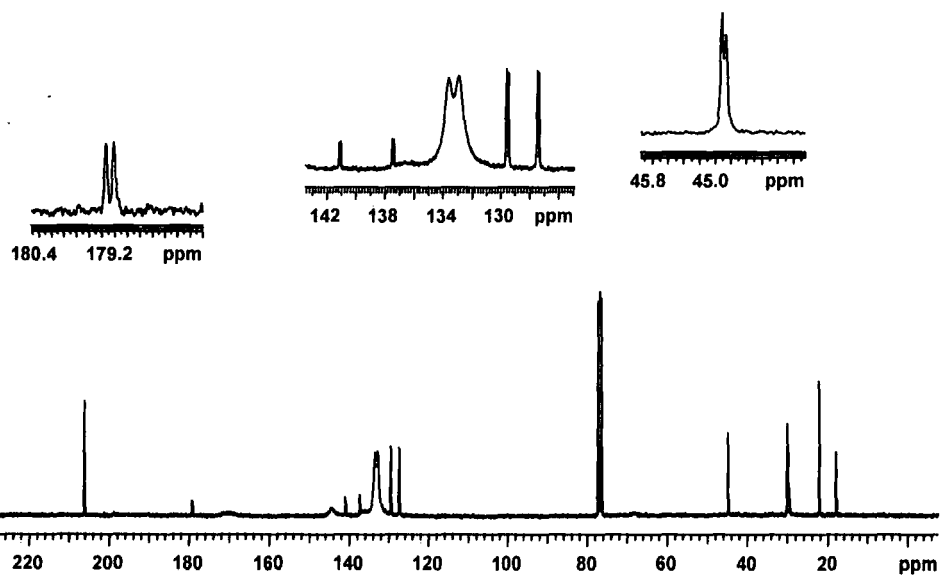


Fig. 19 +/- Ibuprofen Carbon-13 NMR spectrum in PBLG solvent system.

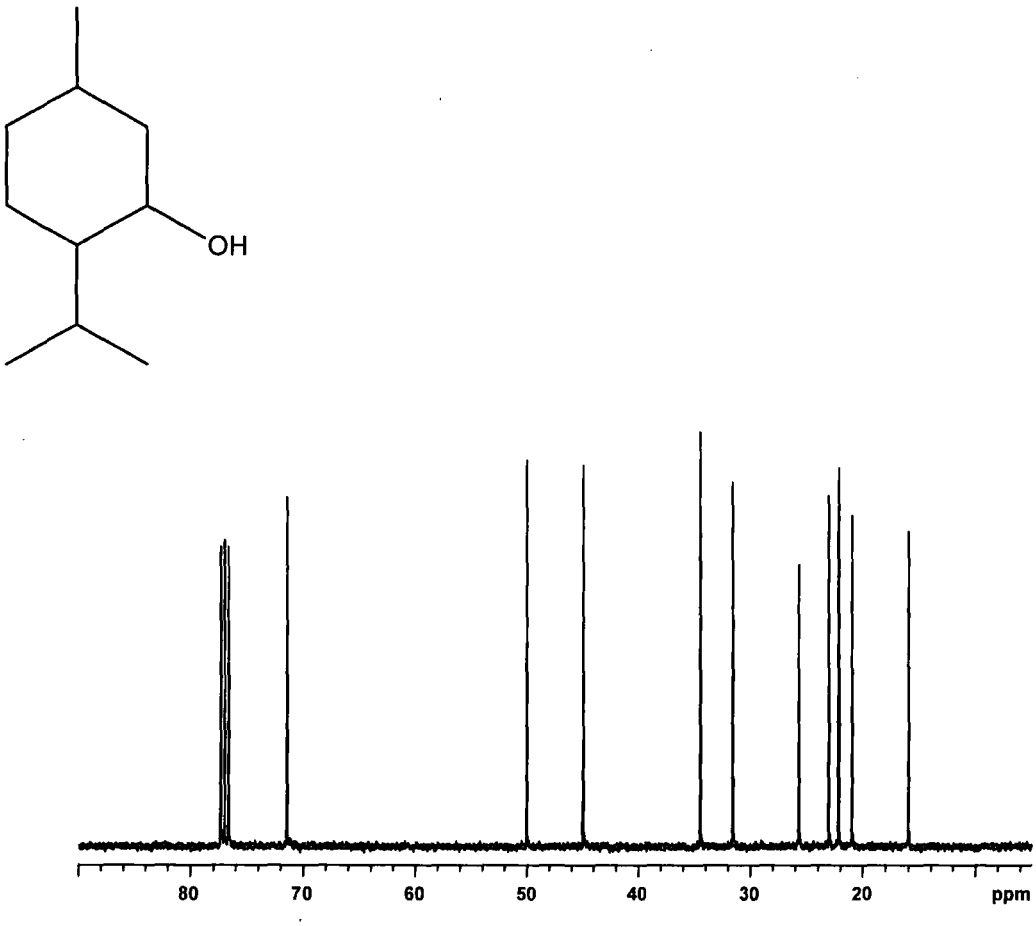


Fig. 20 Racemic Menthol Carbon-13 NMR spectrum in solution

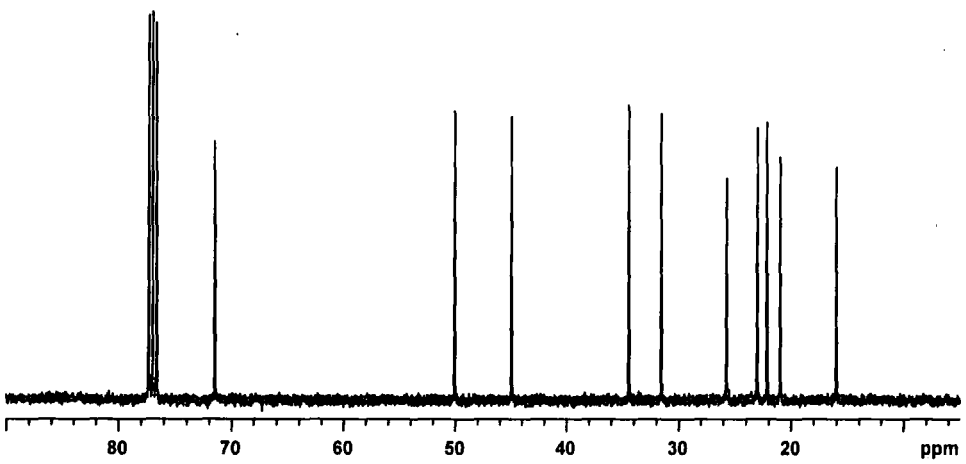


Fig.21 Natural (-) Menthol Carbon-13 NMR spectrum in solution which is identical to the racemic spectrum above.

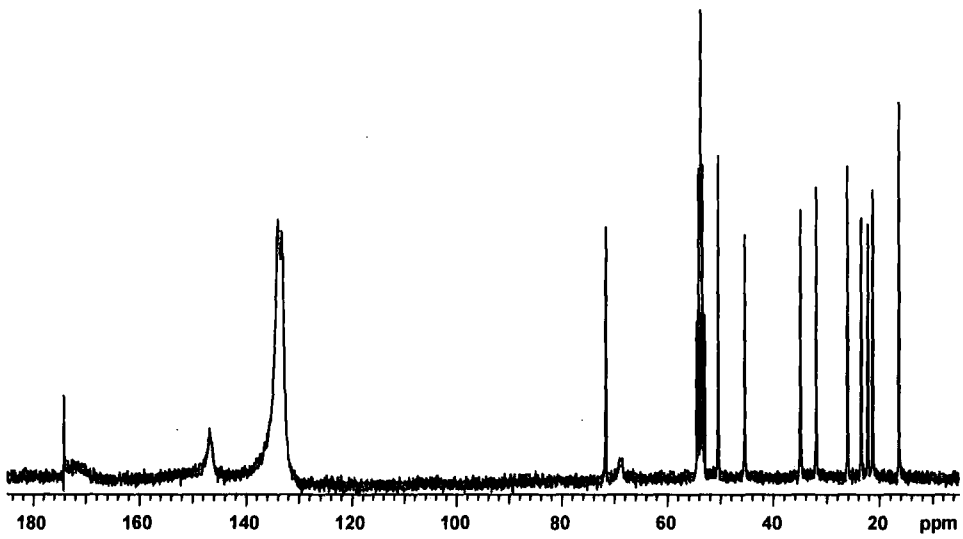


Fig. 22 Mixture of Racemic and Natural (-) Menthol (in excess) Carbon-13 NMR spectrum in PBLG solvent system.

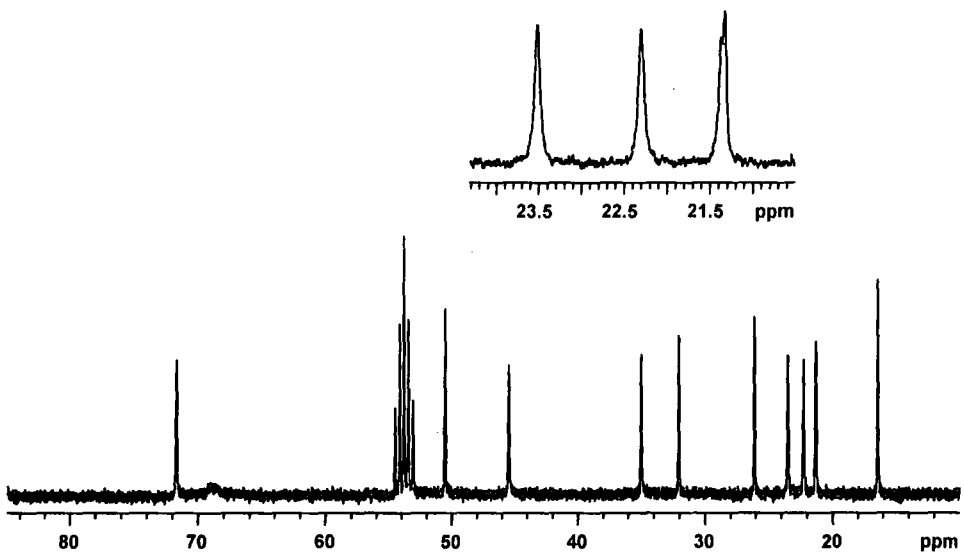


Fig.23 Expanded Carbon-13 NMR spectrum of Racemic and Natural Menthol in PBLG solvent system.

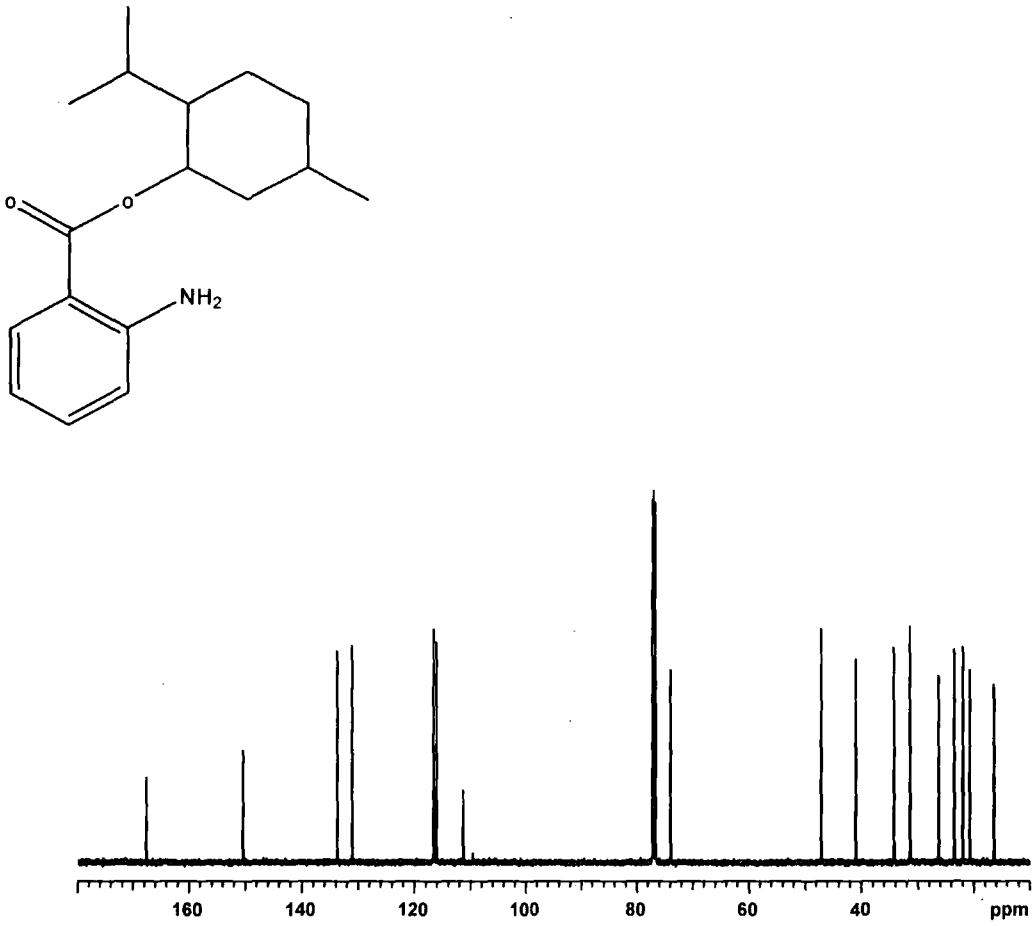


Fig. 24 Menthyl Anthranilate Carbon-13 NMR spectrum in solution.

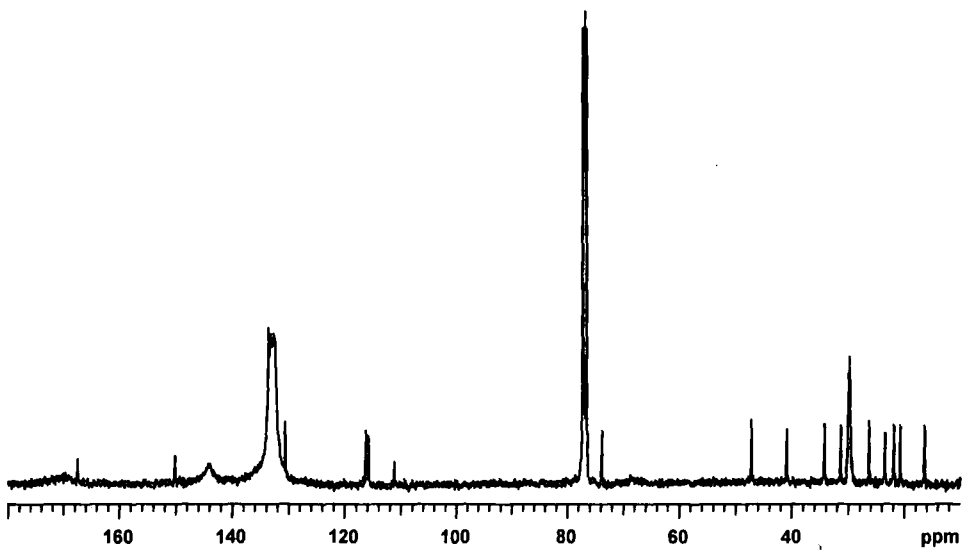


Fig. 25 Menthyl Anthranilate Carbon-13 NMR spectrum in PBLG solvent system.

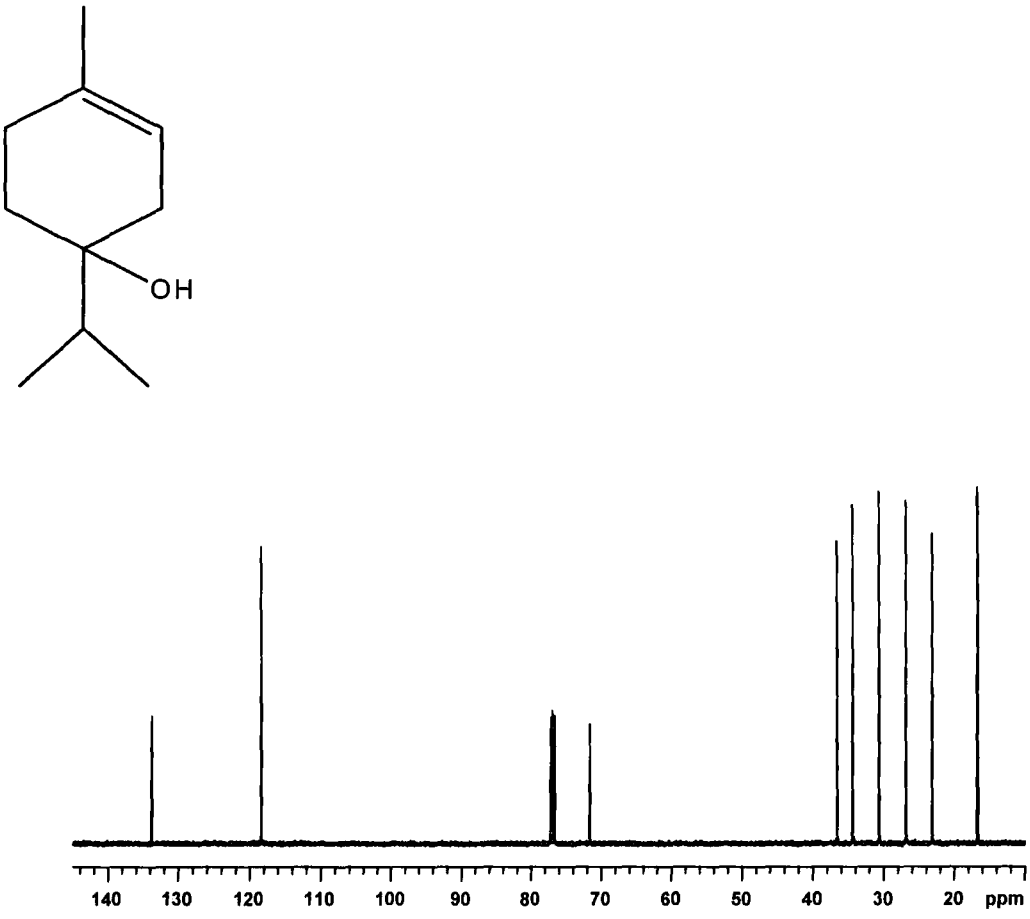


Fig.26 Mixture of +/- Terpinene-4-ol Carbon-13 NMR spectrum in solution.

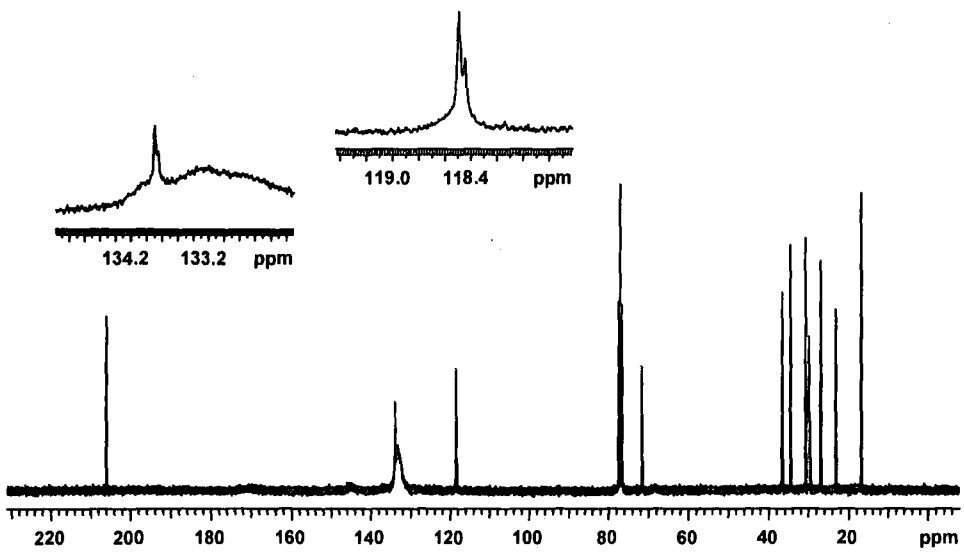


Fig. 27 Mixture of enantiomers of Terpinene-4-ol (ratio of 2:1) in PBLG solvent system.

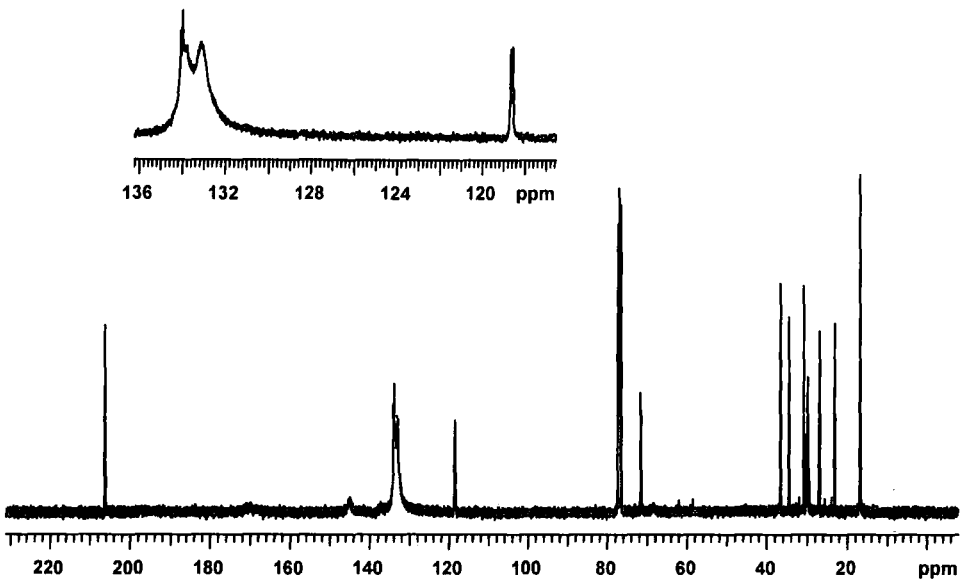
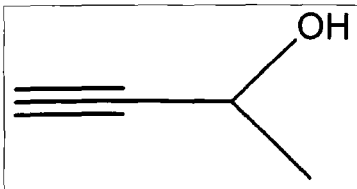
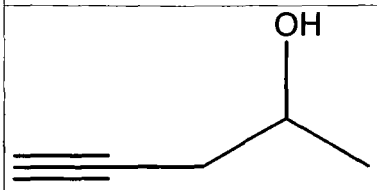
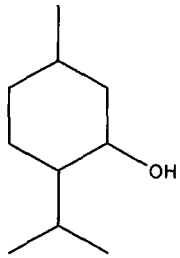
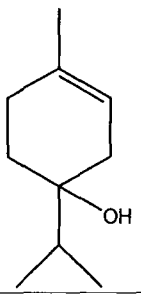
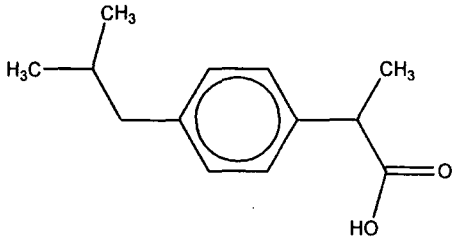
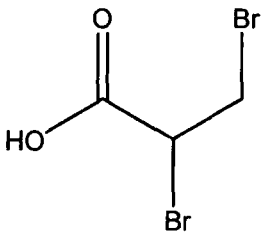
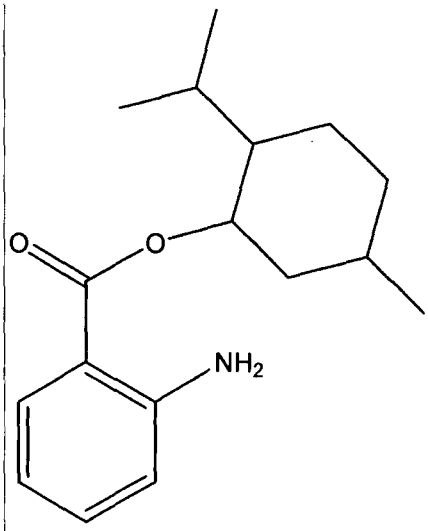


Fig. 28 Mixture of enantiomers of Terpinene-4-ol (ratio 50:50) in PBLG solvent system. The enantiomeric ratio is measured by integration of the signals from each enantiomer.

	**+/- Butyn-2-ol** Splittings observed in the alkyne region of the carbon spectrum.
	**+/- Pentyn-2-ol** Partial splitting observed briefly, however, this did not persist.
	**D and L forms of Menthol** One small split signal observed in the aliphatic region of the carbon spectrum, however, this was not well resolved.
	**+/- Terpinene-4-ol** Reasonably well resolved split in the alkene region of the carbon spectrum
	**Ibuprofen** Well resolved splittings of the signals throughout the carbon spectrum
	**Racemic Dibromopropionic Acid** Complicated splittings of carbon signals which are thought at least partly due to the reintroduction of dipolar couplings. Although possibly also to inadequate decoupling.



Racemic Menthyl Anthranilate

No splittings were observed in the carbon spectrum.

CHAPTER 3

3.1 Monomer Investigations

Having spent some time investigating the necessary criteria for successful enantiomeric differentiation in the previously described molecules, it was thought that the assessment of monomers should be the next obvious step. The monomers used were chosen from a relatively short list as the end goal was to ascertain if the technique is viable for studying polymer tacticity. The monomers were, therefore, those which are susceptible to ring opening metathesis polymerization. Ring Opening Metathesis Polymerization (ROMP)[12] is a method which is used to produce linear chain polymers containing unsaturated sites from, primarily, cycloalkenes or bicycloalkenes. In the case of bicycloalkenes, not only are sites of unsaturation created, but also ring structures are incorporated.

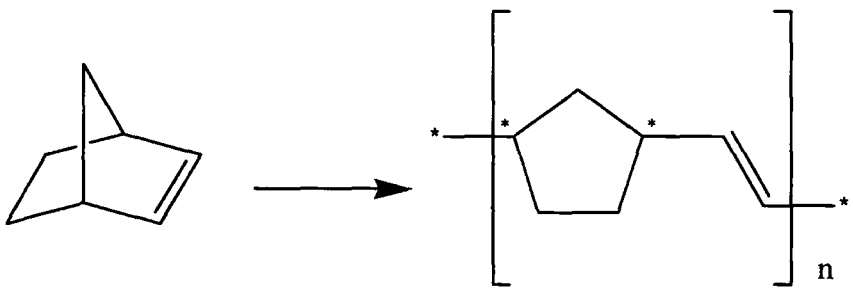
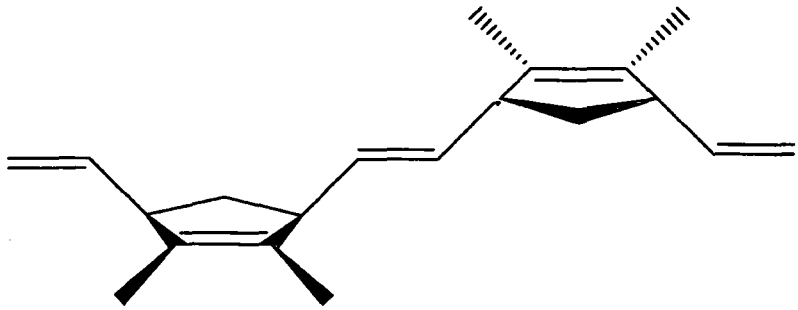
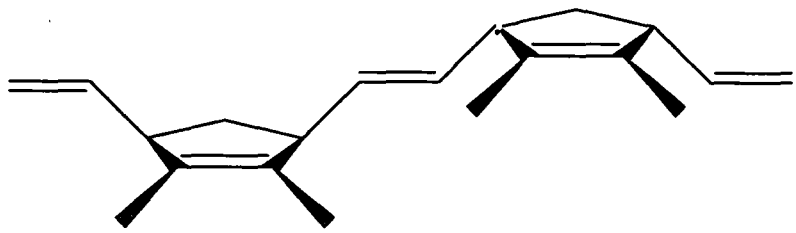


Fig. 29 The basic outline for Ring Opening Metathesis Polymerization.

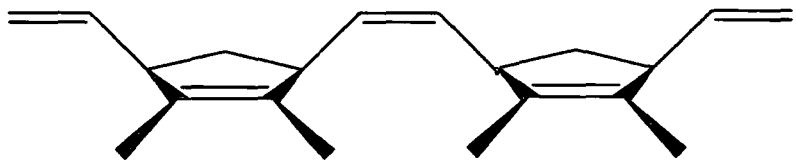
These polymers are, therefore, ideal for our purposes as they contain both a *cis* and/or *trans* element within the chain and also have chiral centres in the rings. In general, if the chirality of the centres on either side of the double bond is the same, then a racemic diad is formed. If, however, the chiral centres have opposite chiralities, then a meso diad is formed. Norbornene (bicyclo[2.2.1]hept-2-ene) is one of the better known monomers which is capable of this reaction with metathesis catalysts. A variety of the derivatives of norbornene were prepared in the PBLG solvent system to investigate the possibility of differentiating any chemical shift anisotropy created by the chiral centres within the molecules.



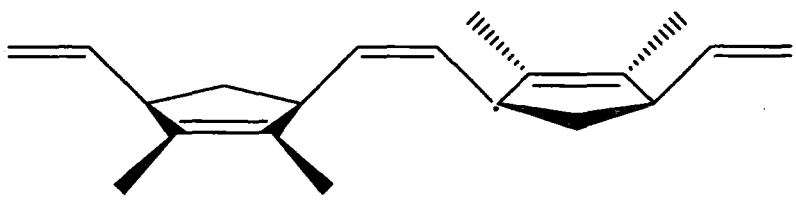
Trans Isotactic Configuration



Trans Syndiotactic Configuration



Cis Isotactic Configuration



Cis Syndiotactic Configuration.

Fig. 30 Possible diads formed from the Ring opening Metathesis Polymerization Reaction of norbornene. All of which have the potential to produce polymers of differing physical characteristics.

As discussed previously, the norbornene derivatives are ideal candidates for liquid crystal investigations. They are the only structures which are readily available, have chiral centres, even if they are not chiral molecules and perform ROMP reactions to give polymers of interest. Experiments were carried out on seven of these monomers and the results varied substantially.

It also became apparent during the course of the experiments that another problem sometimes occurred. In some 13C spectra, although not all, there was evidence of incomplete proton decoupling. This generally manifest itself as split carbon signals which were not always a problem for every carbon with a proton attached within a molecule. This adds another dimension of complexity to the correct assignment of effects causing splitting. It may be that when a signal is split, supposedly due to chemical shift anisotropy or dipolar coupling or both, what you are observing is nothing more than incomplete proton decoupling. The logical extension to this argument is that you may be observing all three effects: proton coupling, chemical anisotropy and dipolar coupling. There is then a question as to how you can tell these different causes apart. On closer inspection of the spectra it becomes more obvious which ones display incomplete proton decoupling as the intensity of the peaks is usually significantly reduced as they are broadened markedly. The spectra also have a lower signal to noise ratio and the frequency in Hertz of the splitting is more typical of H-C couplings as observed in ordinary solution state spectra.

The results for several of the monomers were very unexpected, including 5-norbornene-2-*endo*,3-*endo*-dichloromethane, n-hexyl-5-norbornene-2,3-dicarboxylic imide, 1,4-methanonaphthalene-5;8-diacetate. None of the carbon spectra of these

monomers showed any split signals.

The results of the following monomers were rather more promising: *cis*-5-norbornene-*exo*-2,3-dicarboxylic anhydride, n-(phenyl)-5-norbornene-2,3-dicarboxylic acid imide, 5-Norbornene-2-*exo*,3-*exo*-dimethanol, and 5-norbornene-2-*endo*, 3-*exo*-dicarboxylic acid. All of them displayed some splitting of carbon signals, although in the case of n-(phenyl)-5-norbornene-2,3-dicarboxylic acid imide this was a rather complicated spectrum, possibly involving dipolar coupling and/or incomplete proton decoupling. As can be seen from the carbon spectrum of n-(phenyl)-5-norbornen-2,3-dicarboxylic acid imide the signals are very complicated. Deciphering exactly the cause of the splittings was not considered to be of immediate benefit and other monomers were considered.

A monomer showing a splitting, although not particularly well resolved, was 5-norbornene-2-*exo*,3-*exo*-dimethanol. The signal displaying the split is that assigned to the methylene carbons. This is significant as it underlines the fact that although one might expect splittings in the signal from the alkene carbons due to their electronic environment, the observed splittings indicate that the interactions with the solvent are somewhat more complicated.

5-Norbornen-2-*endo*,3-*exo*-dicarboxylic acid also displays splitting in the 13C signals. These are far better resolved than for 5-norbornene-2-*exo*, 3-*exo*-dimethanol as can be seen from the spectra. Again, it is the alkene carbon signals which are split giving more weight to the theory concerning unsaturation. As the molecule's substituents are *exo* and *endo* to the bicyclic ring it is possible that the added degree of asymmetry (*R* and *S*) has some affect on the orientation of the molecule. One of the most interesting of the monomers to be investigated was the *cis*-5-norbornene-*exo*-2,3-dicarboxylic anhydride. In the carbon spectra of this molecule there are two signals which are split, however, one of these is from the aliphatic region of the spectrum. A result which until now had proved to be somewhat elusive.

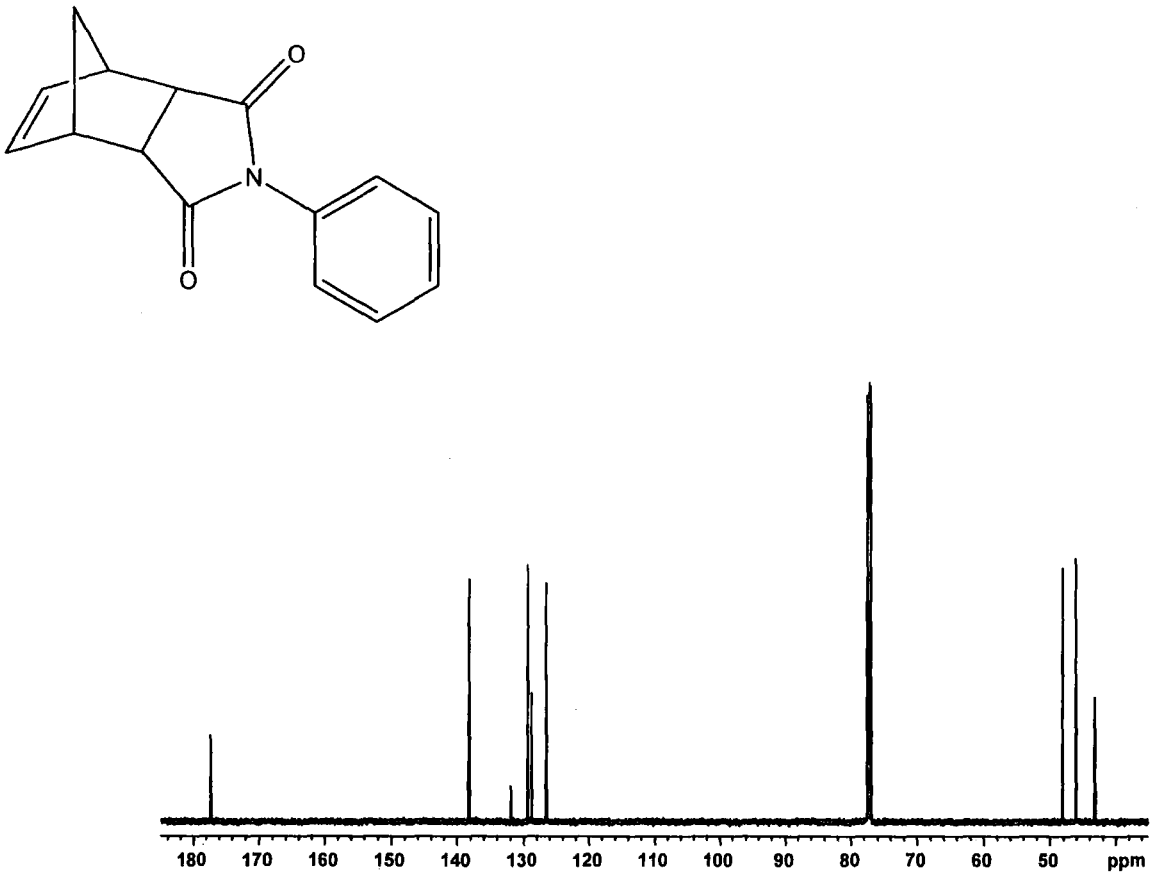


Fig.31 Solution state 13C Spectrum of N-(phenyl)-5-norbornene-2,3-dicarboxylic acid imide.

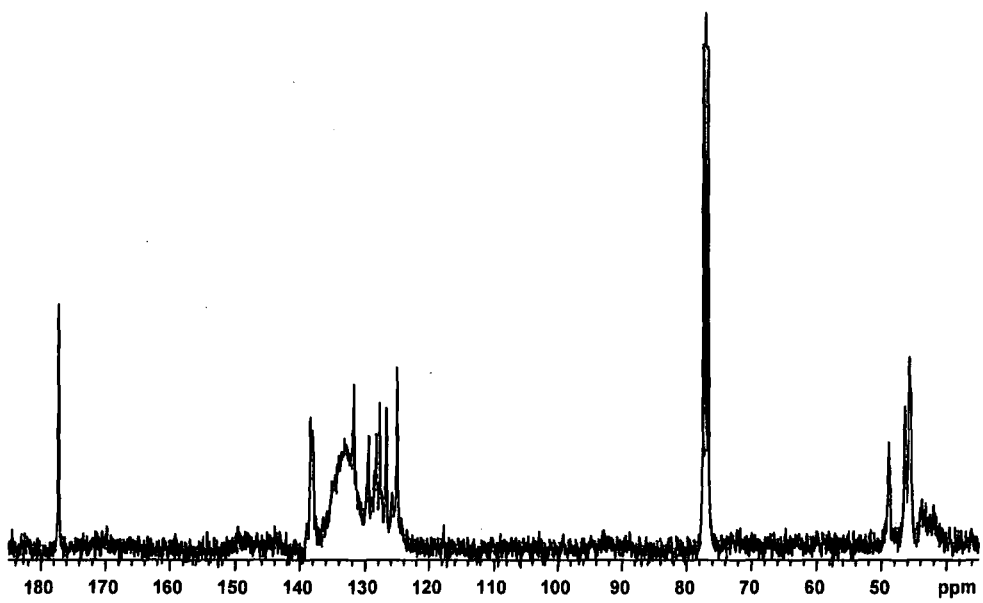


Fig.32 N-(phenyl)-5-norbornene-2,3-dicaboxylic acid imide 13C spectrum in PBLG solvent system showing complex splitting of the carbon signals.

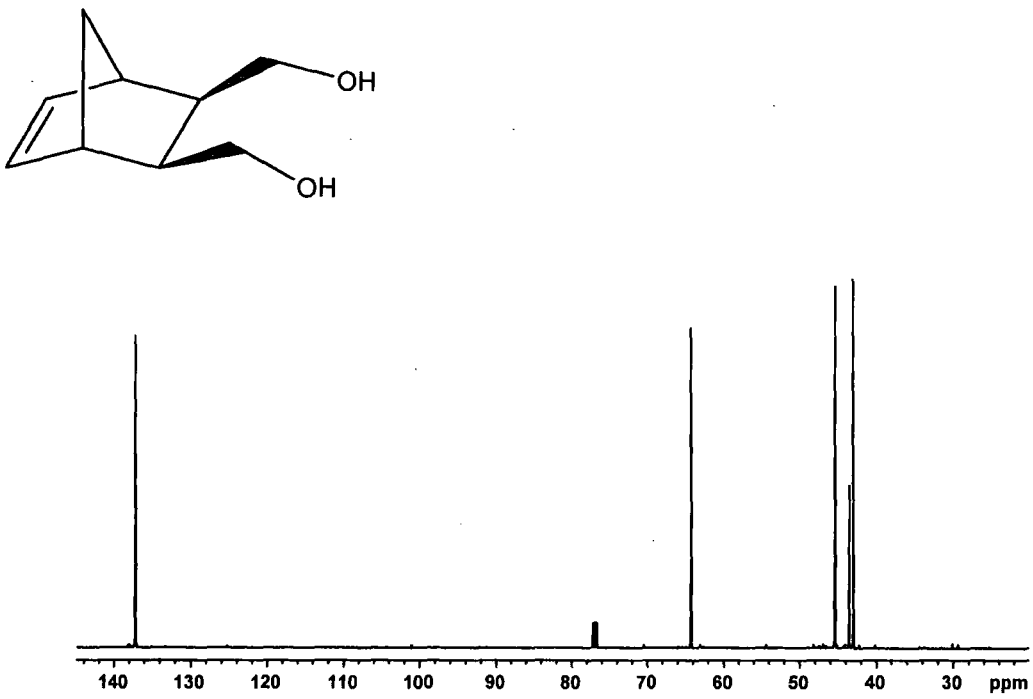


Fig.33 Solution state 13C spectrum of 5-Norbornene-2-*exo*,3-*exo*-dimethanol

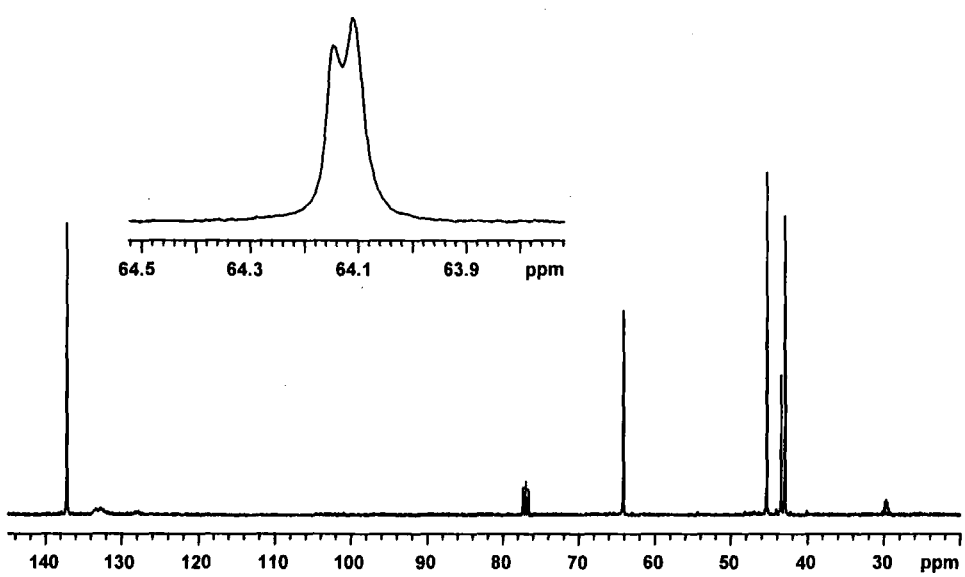
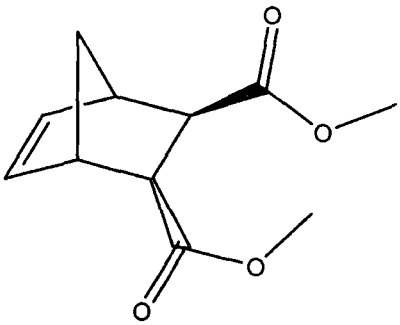


Fig.34 5-Norbornene-2-*exo*,3-*exo*-dimethanol 13C spectrum in the PBLG solvent showing a split in the methylol signal which is not particularly well resolved.



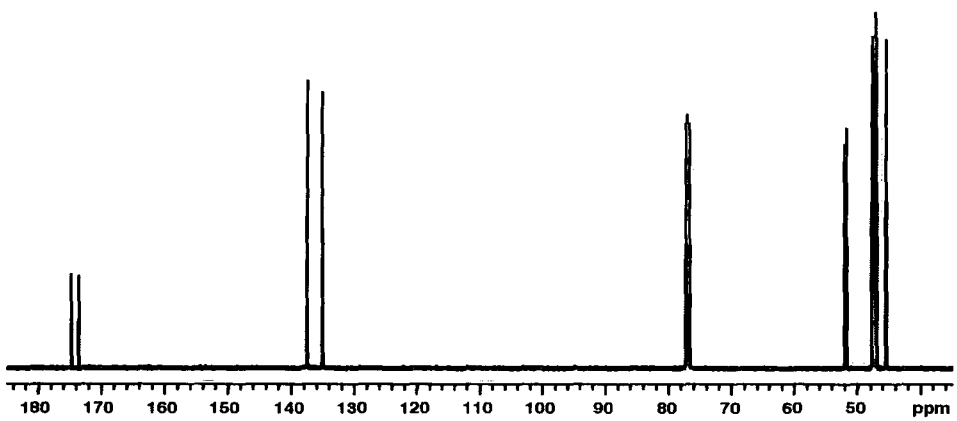


Fig. 35 Solution state 13C spectrum of 5-Norbornene-2-*endo*,3-*exo*-dicarboxylic acid.

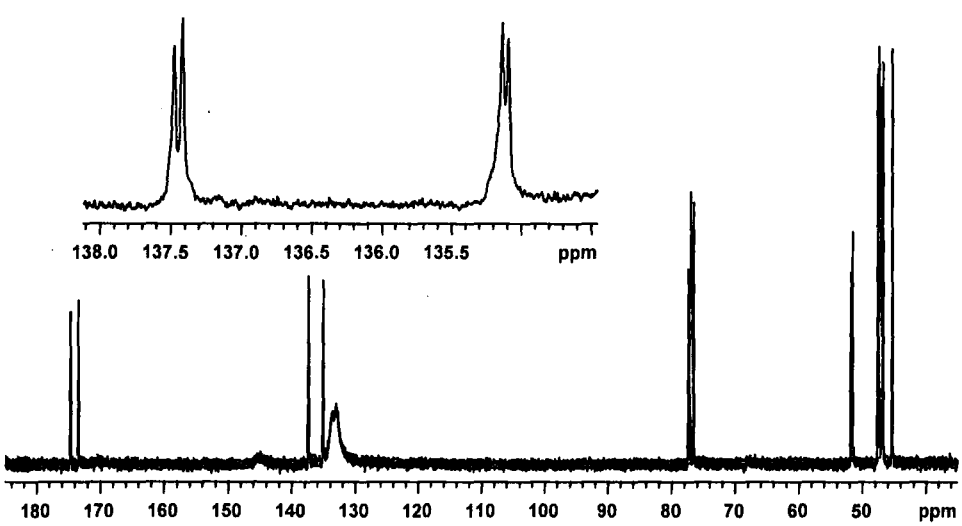


Fig. 36 5-Norbornene-2-*endo*,3-*exo*-dicarboxylic acid 13C spectrum in the PBLG solvent system displaying fairly well resolved splitting of the alkene carbon signals.

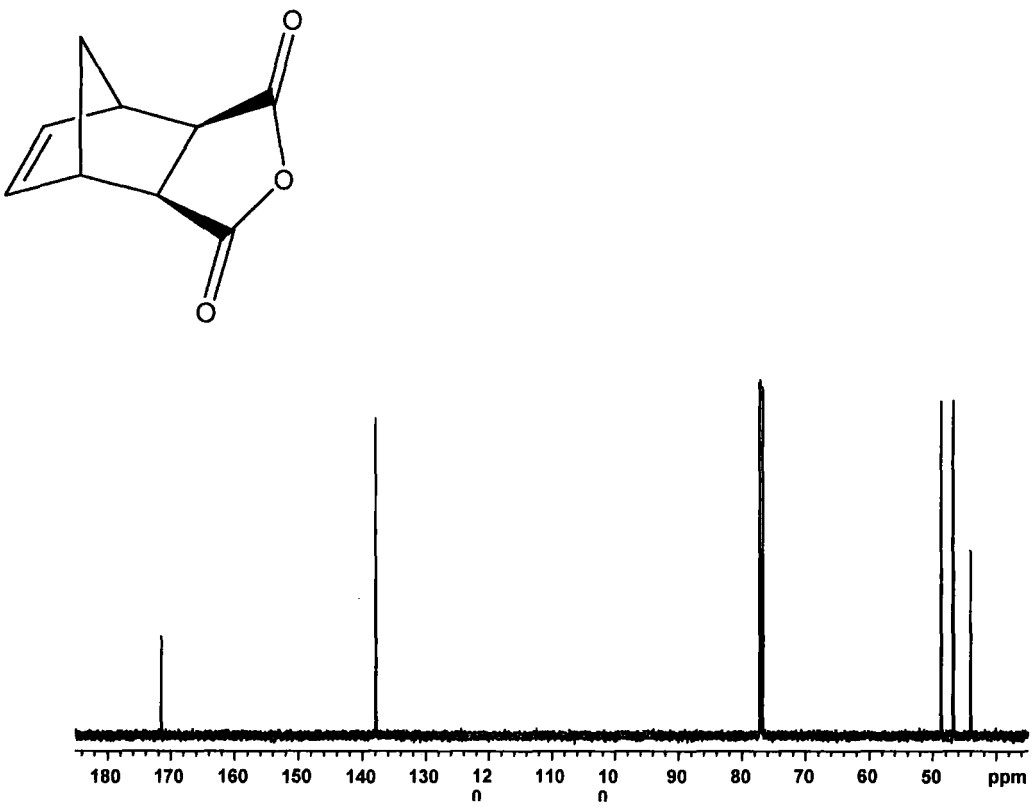


Fig. 37 Solutions state 13C spectrum of *Cis*-5-Norbornene-*exo*-2,3-dicarboxylic anhydride

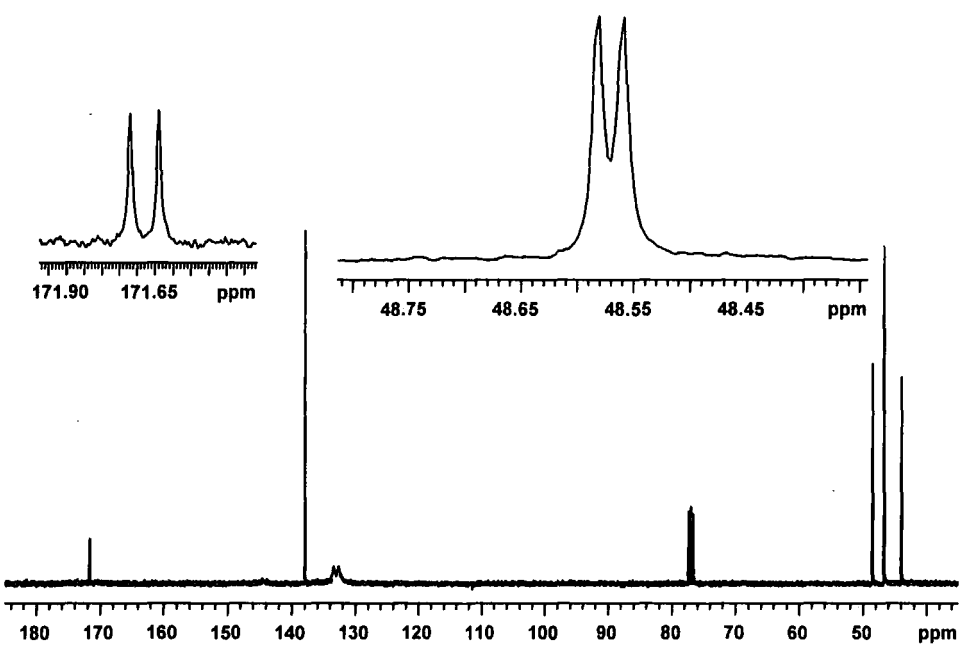


Fig. 38PBLG solvent system displaying very well resolved splittings in the carbon signals including one which is present in the aliphatic region of the spectrum.

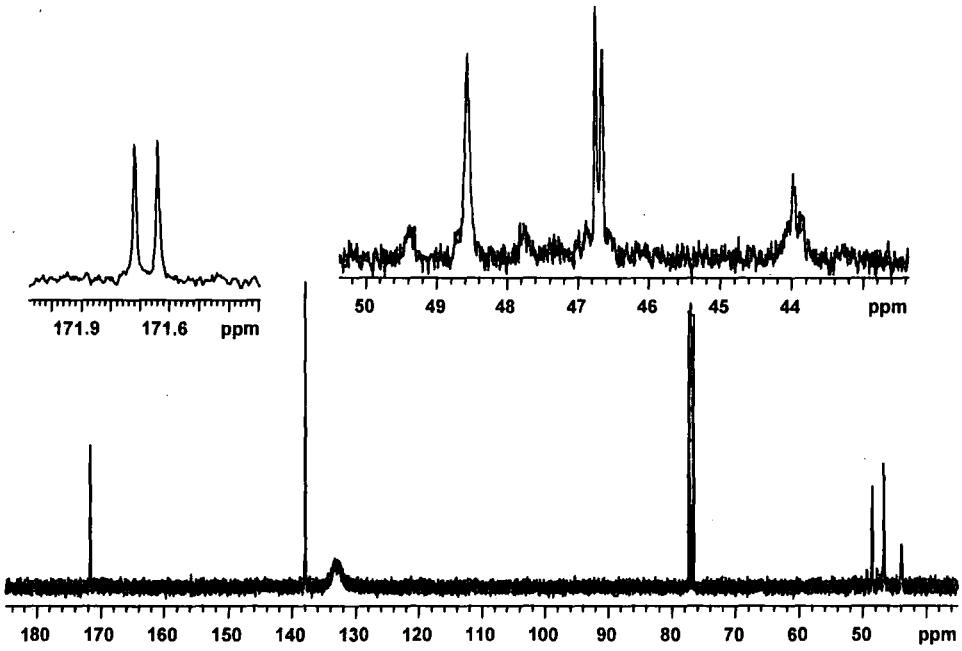
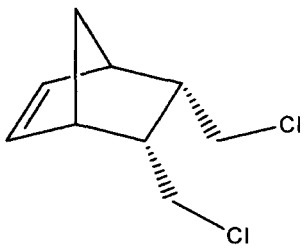
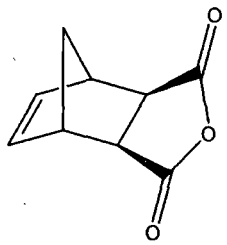
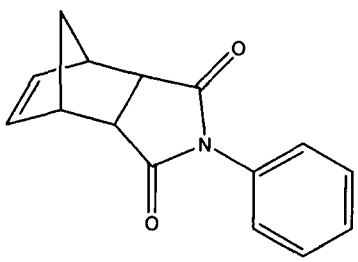
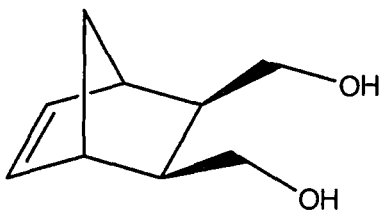
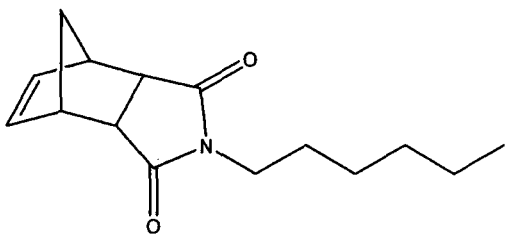
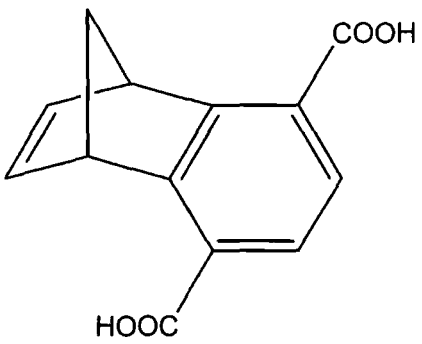
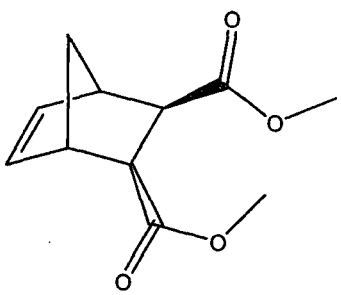


Fig 39 *Cis*-5-Norbornene-*exo*-2,3-dicarboxylic anhydride 13C spectrum in thePBLG solvent system. This spectrum displays some of the problems which were encountered with incomplete proton decoupling. Note the expansion of the signals from the aliphatic region, specifically the methlyene signal which is reduced to a broad split signal of greatly decreased intensity.

	**5-Nornornen-2-*endo*,3-*endo*-dichloromethane** No splitting observed in the carbon spectrum. The proton spectrum gave broad signals only.
	**5-norbornene-*exo*-dicarboxylic acid anhydride** Two signals split in the carbon spectrum, depending on the decoupling accuracy. The proton spectrum displayed broad complicated signals.
	**N-(phenyl)-5-norbornene-2,3-dicarboxylic acid imide** Very complicated splittings in the carbon spectrum possibly caused by incomplete proton decoupling. The methylene signals are somewhat diminished.
	**5-norbornene-2-exo, 3-exo-dimethanol** One peak in the carbon spectrum is split although it is not at all well resolved.
	**N-(hexyl)-5-norbornen-2,3-dicarboxylic acid imide** No signals in the carbon spectrum appear split.

	**1,4-methanonaphthalene-5,8-diacetate** No signals in the carbon spectrum are split
	**5-norbornene-2-endo, 3-exodicarboxylic acid** Two signals in the carbon spectrum are split.

CHAPTER 4

4.1 Application of Further 1D and 2D Techniques

It is obvious from the investigations previously performed that not all carbons-13 resonances assigned to solutes in the chiral liquid crystal solvent system show differentiation of enantiomers. Many factors contribute to the likelihood of chemical shift separation of enantiomers and probably the major of these is the Differential Ordering Effect (DOE). To be able to see far more, if not all of the 13C enantiomer resonances for a given molecule separated in chemical shift the NMR spectrometer would have to incorporate a magnet which is greatly increased in field magnitude. This would be necessary as chemical shift anisotropy is sensitive to DOE proportionally with increases in $\mathbf{B_0}$ magnetic field. Unfortunately, a magnetic field double in magnitude is not readily available to test this particular theory and therefore other NMR experiments were studied to investigate whether an enhancement of chiral discrimination was possible by alternative means.

All the carbon-13 spectra previously described involved broadband proton decoupling. This is normally applied to simplify carbon spectra usually giving single resonances for each of the inequivalent carbons within a molecule. Problems were encountered with incomplete broadband proton decoupling with some of the samples prepared in the chiral liquid crystal solvent system and the spectra which displayed this problem were easily identified.

The signal to noise ratio for a given sample and number of data points acquired is greatly reduced as the resonances are significantly broadened in the proton coupling pattern. Not all of the incompletely proton decoupled spectra showed coupling patterns which were easily distinguished and readily attributable to the attached protons. The possibility of dipolar interactions in conjunction with ordinary scalar spin-spin coupling must therefore be considered.

The proton coupling of the carbon spectra could however, provide us with another route to chiral discrimination of enantiomers. Not only might it be possible to distinguish two sets of splitting patterns in a proton coupled spectrum, indicating the presence of two enantiomers, but comparison with the solution state spectrum could also provide information. Assuming that the normal isotropic carbon spectrum with no proton decoupling provides a spectrum displaying splitting patterns which are solely attributable to proton-carbon spin-spin coupling, then a proton coupled carbon spectrum in the liquid crystal solvent provides both scalar and residual dipolar couplings.

Comparing the two spectra should give a value for the residual dipolar coupling in the chiral liquid crystal solvent, although it must be remembered that this is a residual dipolar coupling only and full reintroduction of dipolar interactions is undesirable as it would provide a far more complicated spectrum. It would also be advantageous to obtain values for proton-carbon coupling constants for the accurate setting of parameters in two dimensional experiments such as heteronuclear correlation studies (the heteronuclei in this case being protons and carbons).

The main aim of two dimensional experiments is usually to aid in the elucidation of structures. It is often the case even for fairly simple molecules that the proton spectra are crowded and signals overlap. Sometimes this is also true of carbon spectra, but it is more unusual. This can make assignment of the spectra difficult or essentially impossible. Performing heteronuclear correlation experiments in two dimensions is viewed as a means of dispersing the data so that the resonances from the complex proton spectrum can be more easily interpreted.

The greater dispersion of the X nucleus (in this case carbon) chemical shifts effectively disperses the proton data making it simpler to interpret. With either the Heteronuclear

Single Quantum Correlation (HSQC) experiment or the Heteronuclear Multiple-Quantum Correlation (HMQC) experiment there is one more benefit regarding structural elucidation. This is that you gain confirmation of which protons are directly attached to which carbon nuclei. The Heteronuclear Multiple Bond Correlation (HMBC) experiment on the other hand provides correlations between carbons and neighbouring protons over more than one bond. It is termed a 'long-range' correlation as this generally provides information for proton-carbon connectivities through couplings over two or three bonds. Couplings between protons and carbons over greater distances than three bonds are usually too small to be observed.

The HMQC[22] experiment has been used for the determination of relative stereochemistry of six-membered ring structures in a series of experiments reported by J.Yan et al.[13]. In this case one of the systems in question was 4,6-*O*-ethylidene-D-glucopyranose. The HMQC experiments were used to collect data on the residual dipolar couplings in the molecules. Residual dipolar couplings were first observed in solution by NMR in the 1960's and these studies were initially focused on organic liquid crystals and produced very large dipolar couplings which were difficult to analyze. The residual dipolar couplings in these studies were used to ascertain structural information on small magnetically anisotropic molecules and these techniques have been extended to measure residual dipolar couplings on biomolecules which have been partially aligned using liquid crystals.

The measurement of residual dipolar coupling has been applied to structural problems regarding proteins, nucleic acids and biopolymers, however, it's application to the structural problems associated with smaller organic molecules has not yet been fully explored. In the experiments reported by Yan et al. the HMQC experiment was modified slightly to detect the carbon-13 satellites in the proton dimension. The experiments were also tailored to reduce the spectral widths of both f1 and f2 dimensions in order to gain as much data as

possible over the required range.

Of the experiments mentioned here, it was considered that the proton-carbon one bond correlations would prove to be the most useful. If the correlations between protons and carbons of different enantiomers could be dispersed in a two dimensional experiment, then it may be possible to view correlations otherwise hidden in a normal one dimensional spectrum.

The following is a brief description of Heteronuclear Single Bond Correlation Spectroscopy.[23]

As with all two dimensional spectroscopic techniques in NMR, the two dimensions referred to are both frequency dimensions. In the case of homonuclear 2D techniques they cover the same range of frequencies for the nucleus involved. In the heteronuclear experiments two differing frequency dimensions are represented, one for each of the nuclei and the third dimension is one of intensity. The plotted spectra comprising of a two dimensional graph with two axes representing the two frequency dimensions (f1 and f2)and the contoured plots on the graph being indicative of regions of through space, through bond etc. coupling.

All NMR spectra can be described as the production of a frequency domain spectrum which is acquired from the regular sampling of magnetization as it varies as a function of time. In the case of two dimensional spectra the production of a spectrum with two frequency domains requires that data is sampled as a function of two separate time variables. The frequency domains being referred to as f1 and f2 and the time variables as t1 and t2. (This should not be confused with T1 and T2 which refer to relaxation of spins). The general pattern of two-dimensional pulse sequences is as follows:

Preparation → Evolution (t1) → 'Mixing' → Detection (t2)

Evolution during t1 modulates the signal at the start of the mixing period. The signal is

sampled as a function of t1 and the Fourier Transform with respect to t1 gives the frequency dimension of f1.

During the HSQC[24] experiment transverse (single quantum) magnetization of the heteronuclear spin (13C) evolves during the t1 period. This is unlike the HMQC experiment where multiple quantum magnetisation is involved. The multiple quantum coherence may be described as the accumulation of the transverse magnetization of coupled spins, in particular the proton and its directly bonded carbon, which evolves coherently but cannot be directly observed. In the case of the HSQC experiment the transverse heteronuclear magnetization is generated by polarization transfer from the attached protons via the 'Insensitive Nuclei Enhancement By Polarisation Transfer' (INEPT[25]) sequence of pulses. (Its purpose being to enable non-selective polarization transfer between spins.)

The carbon nucleus magnetisation evolves during t1 with the proton 180 degree pulse at its midpoint refocusing proton-carbon coupling evolution. In this way proton-carbon interaction is decoupled so that only carbon shifts remain in f1. The carbon magnetization is transferred back to the protons, following t1, by the reverse of the INEPT sequence. This produces proton magnetization for detection in the presence of carbon decoupling. As only the carbon magnetization evolves during t1 it is not affected by homonuclear proton couplings. This results in improved resolution in the f1 dimension and is an improvement on HMQC when the carbon spectrum is not very well dispersed.

There is however, a disadvantage to using the HSQC experiment which is that there is an increase in the number of pulses within the sequence particularly X-Nucleus 180 degree pulses, resulting in potential problems with Rf inhomogeneity, off-resonance excitation and pulse miscalibration errors. Despite these potential problems it was thought a useful experiment to utilize and consequently the pulse widths were recalibrated for the PBLG liquid crystal samples. Unsurprisingly they were found to be different to the normal

solution state standard parameters for the probe used.

The Multiple Bond Correlation Spectroscopy Experiment.

The HMBC sequence (Fig 44) is effectively the same as the HMQC sequence (Fig 45), but with the preparation period altered to allow evolution of long range proton-carbon couplings. Long range proton-carbon couplings are at least an order of magnitude smaller than the one-bond couplings. One-bond ^{1}H-^{13}C scalar couplings in isotropic solutions are typically in the range of 100-200Hz, whereas the longer range couplings over two or three bonds are more typically in the range of 0-10Hz. The preparation period in the pulse sequence would then probably be about 100ms (1/2nJCH), however, it is common for shorter delays to be used in order to reduce the possibility of relaxation losses.

Generally speaking, modern HMBC sequences are optimized to eliminate ^{1}H-^{12}C correlations, ^{1}H-^{1}H correlations and in the main ^{1}H-^{13}C one-bond correlations. This is done by various means including the use of pulsed field gradients, phase cycling, low pass filters and 'tuning' of the pulse sequence to allow only the required correlations to present.

HMBC was considered not to be the most useful experiment for our purposes as the spectra are usually more crowded as there are more correlations to consider and the possibility of reintroducing the couplings in the proton dimension is unworkable without reintroducing unwanted correlations. Also, the increased linewidths (reduced T2's) in PBLG could cause sensitivity problems

An interesting result was achieved by performing a standard HMBC experiment on a sample of *cis*-5-norbornene-exo-2,3-dicarboxylic anhydride in the liquid crystal solvent system. All the anticipated correlations appeared in the spectrum, however, an unexpected correlation was also observed. (Figs 40 and 41)

This correlation was between one of the bridgehead protons and one of the

carbonyl carbons. This correlation would not be observed in isotropic solution as the J-coupling involved would be far too small. In the liquid crystal environment, however, this coupling manifests as an offset doublet. It is probable that the coupling is present due to the reintroduction of some dipolar coupling element and that the offset nature of the doublet would indicate some problems with refocusing in the pulse sequence. The apparent dipolar couplings between carbon and proton are usually combined with the scalar couplings, however, in this case it would be reasonable to expect the scalar coupling to represent a very small part of the equation.

The spectra obtained from the gHMBC experiment on *cis*-5-norbornene-exo-2,3-dicarboxylic anhydride in the PBLG solvent system proved to be very interesting and informative. This was especially true of the interaction between the bridgehead protons and the carbonyl carbons of the adjacent ring (Fig.42) The rest of the spectrum was complicated by the shear number of interactions involved and attention was therefore, refocused onto the gHSQC experiment. The anticipation was that refining the gHSQC experiment (Fig 46) through changes to the pulse sequence would provide evidence of J-couplings from both enantiomers. It was thought that in the 2D experiment, given the extra dimension could provide an opportunity to see interactions which are not clear or present in the 1D spectra. Although the dipolar couplings are interesting in the gHMBC experiment, they are an added complication and the aim of the exercise remained to provide evidence of the existence of both enantiomers in a sample and if possible their relative proportions.

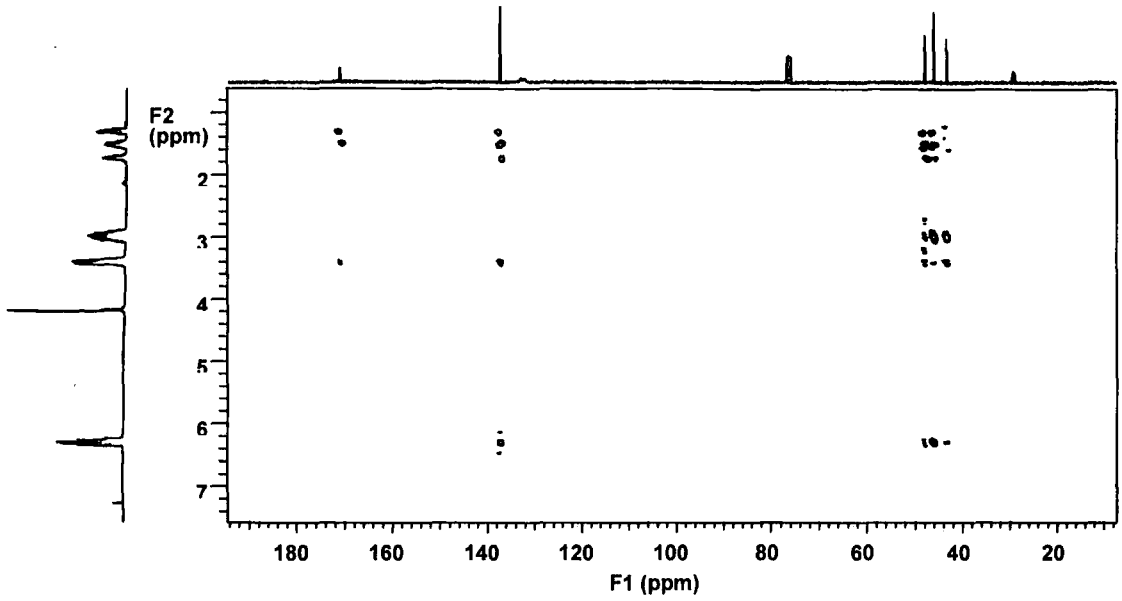


Fig.40 gHMBC Spectrum of norbornene anhydride in PBLG solvent system

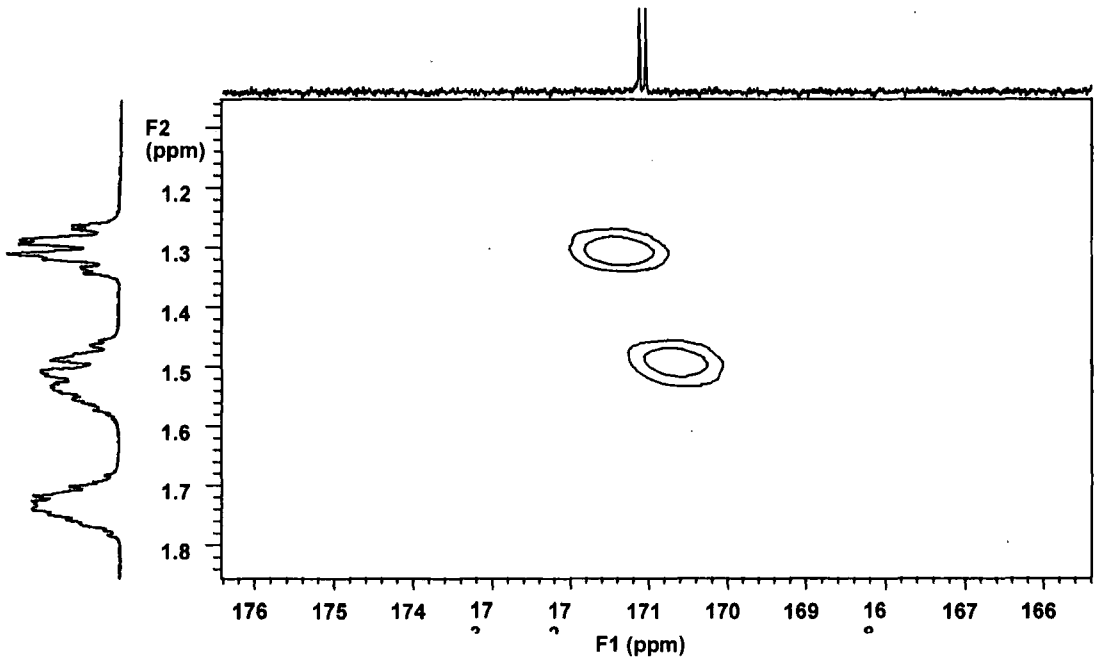


Fig.41 Expansion of the gHMBC spectrum of norbornene anhydride showing correlation between one of the methylene bridge protons and one of the carbonyl carbons on the adjacent ring.

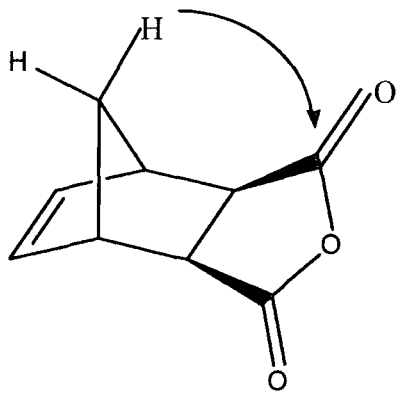


Fig.42 Cis-**5-norbornene-exo-2,3-dicarboxylic anhydride showing the dipolar interaction between the methylenebridge proton and the carbonyl carbon in the adjacent ring.**

The pulse sequences for many of the more common heteronuclear 2D pulse sequences were studied and various modifications attempted. Below is a list of those experiments attempted:

HET2DJ (Heteronuclear J-resolved Experiment)

(Generally used to provide measurements of heteronuclear coupling constants and/or ascertain the multiplicity of the heteroatom)

HETCOR (Heteronuclear Correlation)

Uses the lower γ nuclide (in this case 13C) which significantly lowers the sensitivity, but does benefit from high resolution in the 13C dimension.

Various forms of this experiment were investigated including HETCORPS which is phase sensitive and absolute value. A modification to this experiment was tried which was the long range coupling version, called the lrHETCOR.

FLOCK

So called because of the number of BIRD* pulses present in the sequence and predecessor pulse sequence to the HETCOR. Its long range version the lrflock was also investigated.

* BIRD representing Bilinear Rotation Decoupling and is a cluster of pulses which is used

to provide selective inversion of the 12C-bound protons effectively eliminating them from the experiment.

Modification of the gHSQC Pulse Sequence

The pulse sequence, provided by Varian Ltd. for the Unity 500AS spectrometer which includes gradients, was studied closely (for a pictorial representation of the pulse sequences see Fig. 44). Careful analysis of the sequence, establishing exactly what amendments were required and which elements of the sequence were absolutely essential followed. The sequence as shown broadly follows the description previously given although due to the number of gradient pulses and some BIRD pulses it appears rather more complicated. In practice however, the principles remain the same and it was established that the modification which would be of greatest benefit would be the reintroduction of J-coupling in the proton dimension. In theory, even if there was no apparent splitting of a particular signal in the original one dimensional proton spectrum, it might be possible to see a difference between the J-couplings of the attached carbon and each of the individual proton from each of the enantiomers. Using the VNMR 6.1C software provided with the spectrometers a portion of the original gHSQC experiment was removed and the subsequent new pulse sequence was renamed as CCOUP (for Coupled spectrum). A pictorial representation of the new CCOUP pulse sequence is depicted in Fig 47. The modification to the gHSQC pulse sequence can be seen along the TX (proton transmitter line). Following the two BIRD pulse combinations, the first of two 180 degree pulses has been removed. The effect of this is to reintroduce J-coupling in the proton dimension of the spectrum.

This experiment was attempted on several of the chosen monomers, however, the most promising results came from *cis*-5-norbornene-exo-2,3-dicarboxylic anhydride and efforts to perfect the experiment were concentrated on this molecule. CCOUP experiments which were conducted on the full sweepwidths of the proton and carbon spectra were found to have insufficient resolution in the subsequent 2D spectrum and a method of running the

experiment on a greatly reduced spectral width in both dimensions was established. The signals corresponding to the vinylic region in both the proton and the carbon spectra were chosen for the experiment and the details of the relevant spectral widths were used to adjust the parameters of the 2-dimensional experiment. The results obtained can be seen in Fig 43.

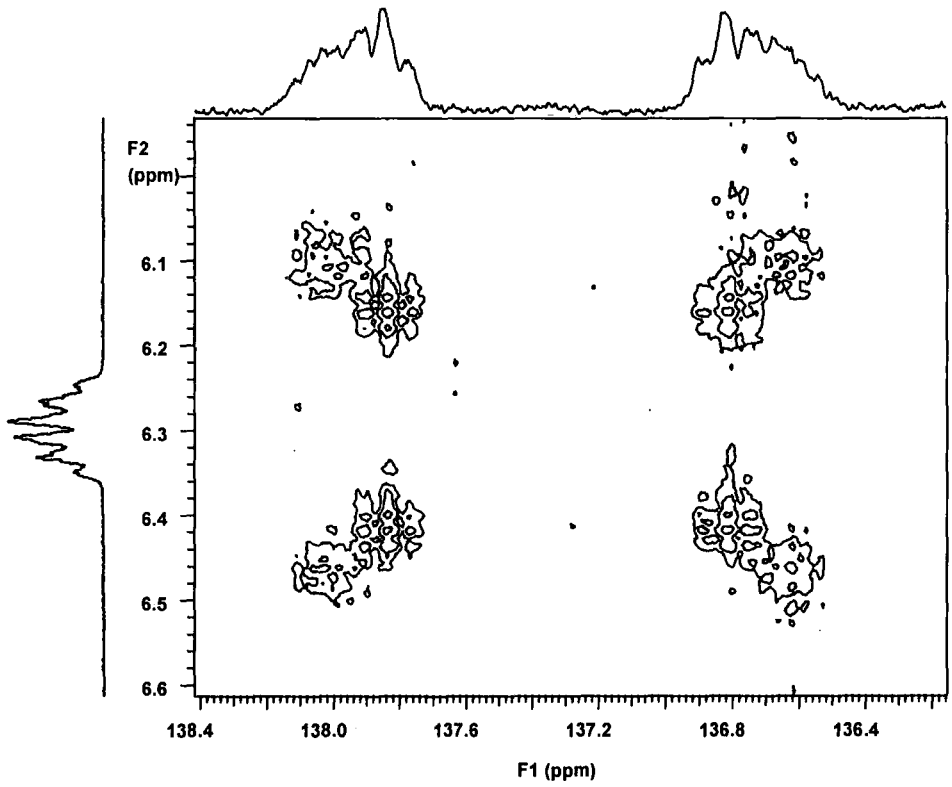


Fig.43 CCOUP Experiment results performed on the *cis*-5-norbornen-exo-2,3-dicarboxylic anhydride monomer displaying the proton coupled carbon-13 spectrum instead of the usual proton decoupled spectrum.

The interesting features which can be seen in this spectrum relate to the correlations between the protons and the carbon nuclei of the vinylic region of the *cis*-5-norbornene-exo-2,3-

dicarboxylic anhydride. The 1D carbon-13 spectrum which can be seen across the top of the diagram is the carbon spectrum with no proton decoupling. Ordinarily a carbon-13 spectrum is produced with the proton coupling to the carbon nuclei removed. This is done in ordinary 1D carbon spectra because it simplifies what might otherwise be a rather complicated spectrum. This is especially important for molecules which contain a large number of carbon atoms with hydrogen atoms attached.

In this case the modification of the pulse sequence which suppresses the proton coupling is effectively removed and these normally unwanted couplings are reintroduced. Reintroducing the proton couplings in the carbon spectrum has the effect of neatly identifying the two main vinylic resonances. The 2D spectrum displays an interesting pattern which is offset at either side of the complicated proton resonance. Effectively there are eight correlation spots and this is a rather neat representation of the variation in couplings exhibited by the two forms with an absence of chemical shift difference.

If there were greater solid-state character to these spectra the introduction of inter proton dipolar couplings would render the spectrum too complicated to be of use in the identification or quantification of separate enantiomers.

In this case the chiral liquid crystal solvent system produces an environment which orients the substrate molecules only very slightly while maintaining sufficient isotropic character to produce NMR spectra of reasonable resolution.

The CCOUP experimental result for the Norbornene Anhydride molecule was acquired over a very long time (approximately thirteen hours) by today's standards, especially for modern 2D experiment. The results would have been more even more useful, however, if the resolution achieved had been sufficient to allow for the accurate measurement of the volumes of the correlation spots. These would have been an indication of the relative concentrations of the enantiomers in the sample.

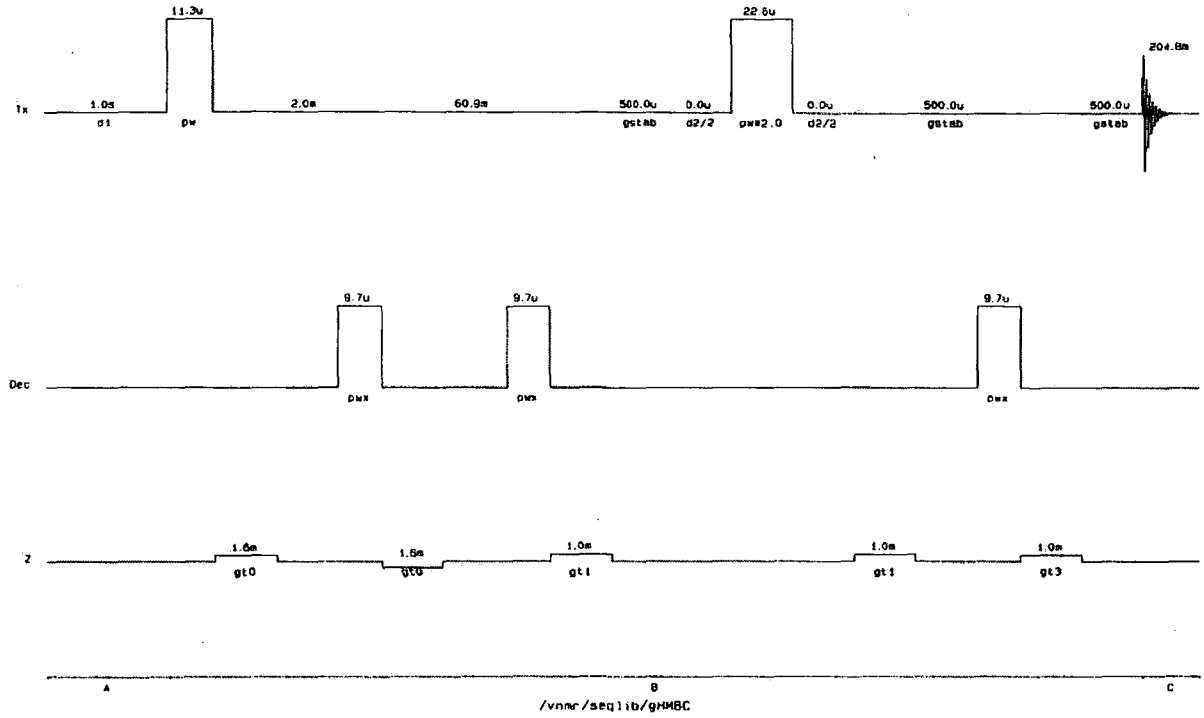


Fig. 44 Standard gHMBC Pulse Sequence

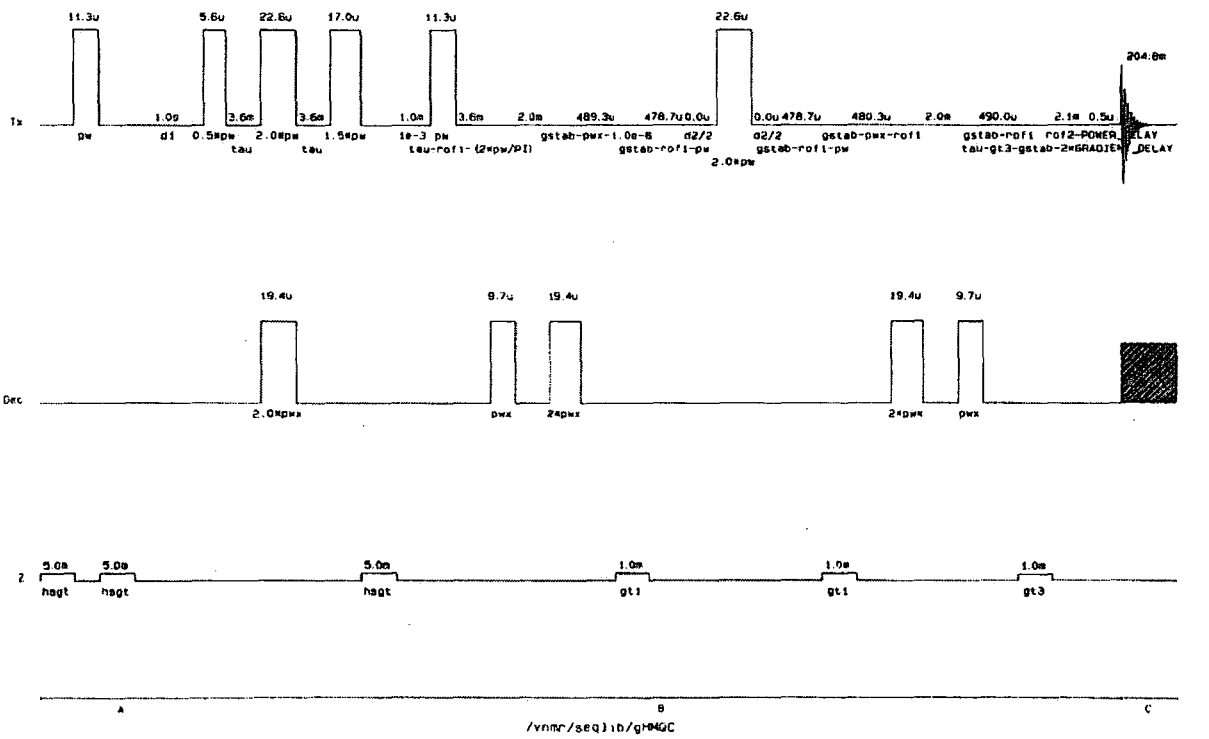


Fig. 45 Standard gHMQC Pulse Sequence

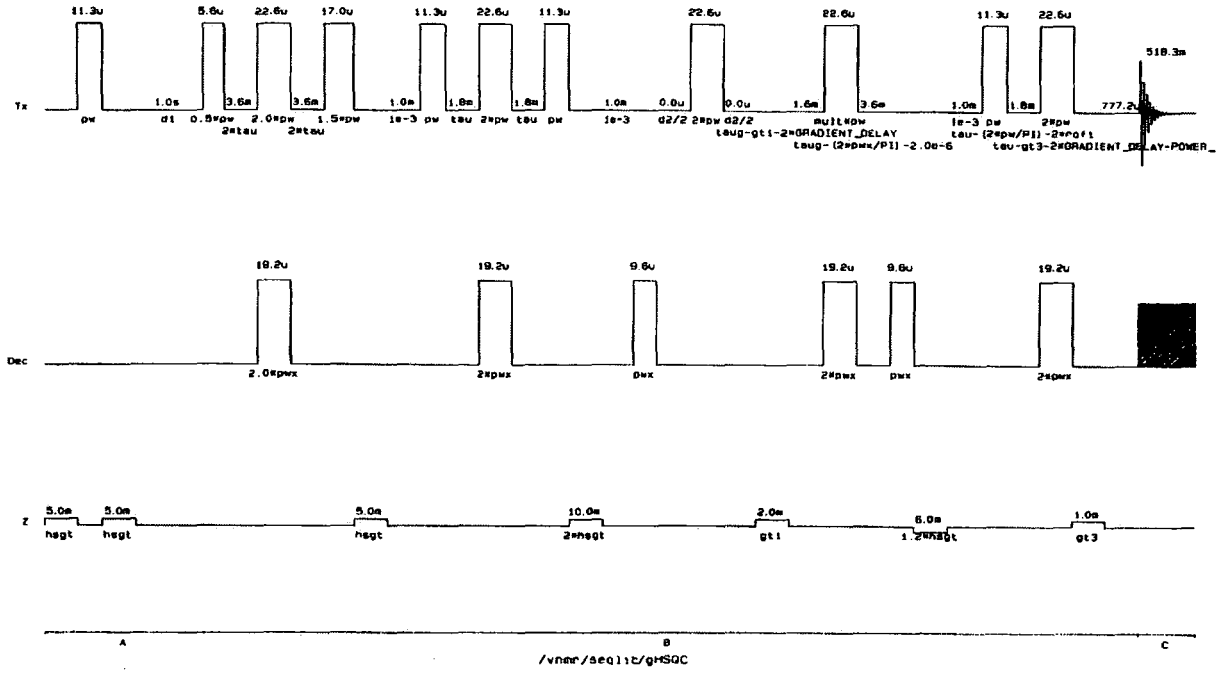


Fig. 46 Standard gHSQC Pulse Sequence

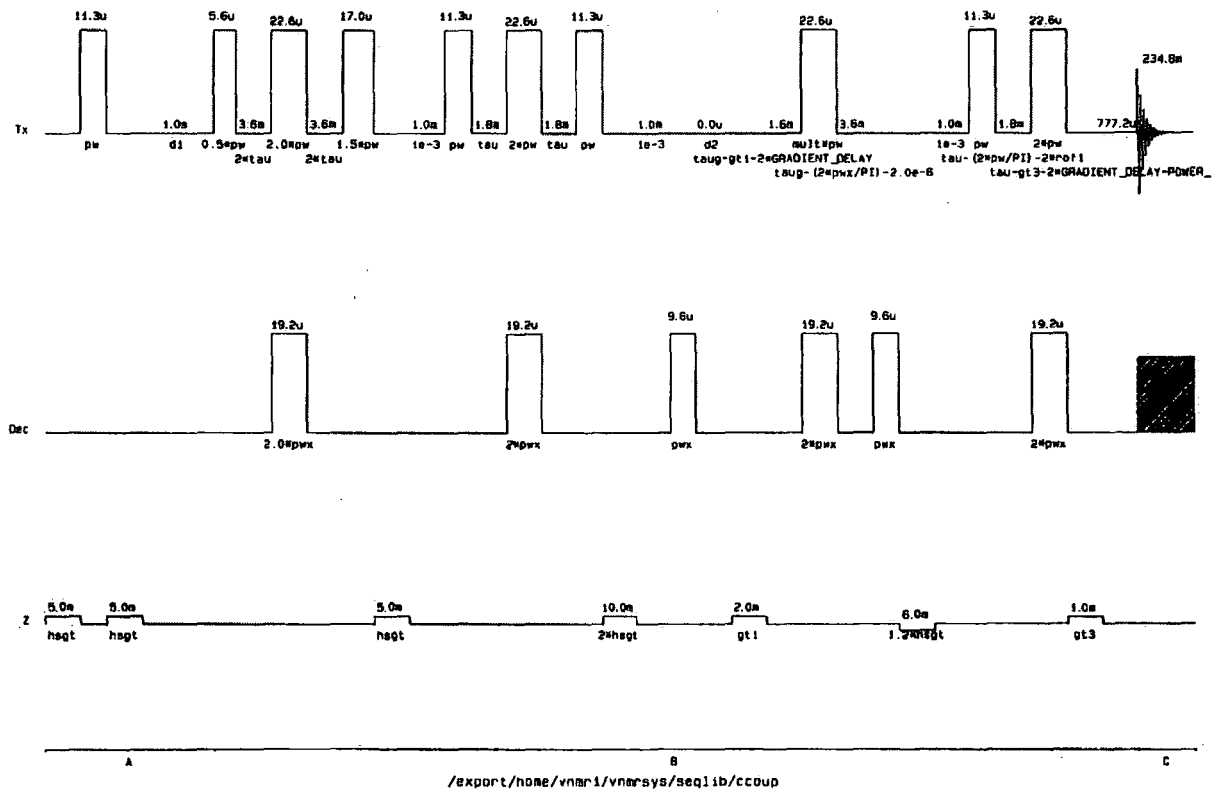


Fig. 47 New CCOUP Pulse Sequence which is a modification of the above HSQC sequence.

CHAPTER 5

5.1 Polymer Investigations using the Chiral Liquid Crystal Solvent System.

The purpose of the investigations into the use of the chiral liquid crystal solvent systems in NMR is ultimately to aid in the elucidation of the finer structures of polymers, in particular, the stereochemistry or tacticity. This is an area which is notoriously difficult to assess and is usually very time-consuming. The theory behind this technique is that differing chiral environments within the polymer structure which would be indistinguishable from an NMR standpoint in an isotropic environment will be oriented slightly differently in the chiral liquid crystal solvent system.

Providing that the difference between differing chiral environments displays a differential ordering effect (DOE) translating to a chemical shift anisotropy which is sufficiently well resolved, then identification of these differing configurations will be facilitated. Furthermore, if the spectrum produced is quantitative and the signals are sufficiently well resolved then a far more useful picture of the stereochemical composition of the polymer will be produced. The proportions of differing stereochemical configurations within a sample can be the key to explaining the polymerization processes which have taken place and tailoring them in the future.

The search for suitable polymers on which to perform these experiments was refined following the investigations into monomer molecules. The monomers investigated were all derivatives of norbornene and exhibited surprisingly different levels of success regarding orientation within the chiral liquid crystal solvent system. In the case of polynorbornene a certain amount of information can be elucidated from the isotropic solution state 13C NMR spectrum.[26] Each of the four carbon atoms (Fig.48) in the repeating unit is sensitive to the configuration of the adjacent carbon-carbon double bond. For the carbon which has been

labelled number four, two resonances can be distinguished, one for carbons situated in the trans RHC=CHR groups and one for carbons situated in cis RHC=CHR groups. Peak fitting of 13C NMR spectra allows us to determine accurately the relative amounts of cis and trans C=C double bonds in the polynorbornene.

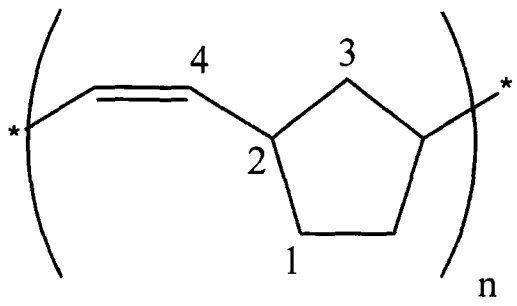


Fig. 48 Polynorbornene structure

To investigate any enantiomeric discrimination between chiral carbon atoms, it is useful to select polymers for analysis which already have the cis/trans content established. In an ideal situation, before embarking on any new analytical technique for the identification of polymer tacticity, it would be useful to start with a polymer whose stereochemical structure had been previously determined. It would then be a easier to assess whether the technique provided any advantage in terms of accuracy or speed of analysis or in fact produced further corroborative data.

In these circumstances we are limited to polymers which are available, soluble in the chiral liquid crystal solvent system and are of a suitable molecular weight range. If the polymer is too large, the probability of it's being sufficiently soluble decreases as does the likelihood that identification of an oriented chiral atom will be distinguishable. Most isotropic solution state spectra of high molecular weight polymers contain characteristically broad signals. In this case, the resolution of the signals from differing chiral atoms would be extremely difficult to achieve.

Using two-dimensional NMR experimental techniques such as the CCOUP experiment would be a possiblity in the case of a high molecular weight polymer, however, it is likely that these would also be of limited success. If the signal from the relevant nucleus decays too quickly, then the probability of a two dimensional experiment being successful is remote. In these cases, the timing of pulses is tightly controlled and the appearance of cross-peaks dependent, up to a point, on the longevity of the signals involved.

Polymer samples which largely met these criteria were kindly provided by Dr Ezat Khosravi and were variations of polybis(trifluoromethyl)norbornene.(Figs. 49 and 50)

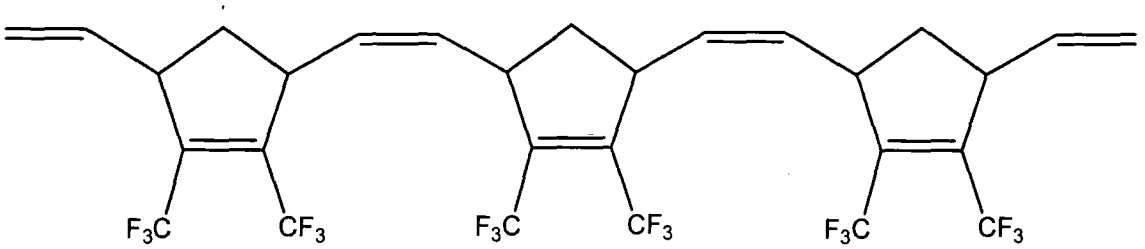


Fig 49 Cis Poly Bis(trifluoromethyl)norbornadiene

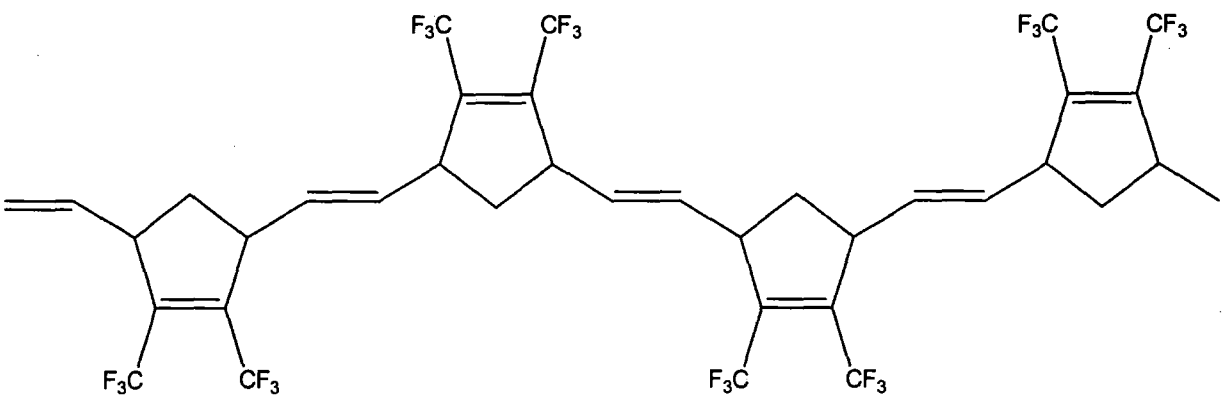


Fig. 50 Trans Poly Bis(trifluoromethyl)norbornadiene

The possibility of a particular sample of the polymer containing only one of the described forms is questionable, however, samples were provided which were mainly *cis* and mainly *trans*. The tacticity of the samples, however, was unknown although they were both thought to be highly tactic. The fact that the geometry of the double bonds is known gives an advantage in that we are already aware of some of the structure of the polymer and it is interesting to compare the results for both forms.

There have been many investigations into the tacticity of polymers produced by ring-opening metathesis polymerization[27], in particular those which are formed from derivatives of the norbornene monomer. On ring opening these monomers produce polymers containing the cyclopentylene-vinylene repeating unit (Fig 49 and 50). In this case tacticity occurs due to the relative orientations of neighbouring cyclopentylene rings. In this way racemic or meso junctions can be formed along the whole length of a given polymer molecule. NMR spectroscopy has been used in most literature reports to describe the cis or trans nature of the double bond, however, the finer details of tacticity are often lost in solution state NMR spectra.

In the case of symmetric polymers made from norbornadiene derivatives one approach has been to prepare polymers which have attached pendant groups containing chiral centres of single, defined handedness[28] In these circumstances, there are, in principle two kinds of vinylic proton which in the case of isotactic polymers occur on the same double bond, but in the case of syndiotactic polymers they occur on different double bonds[30]. It is therefore a simple matter of distinguishing whether these protons are coupled to each other or not to establish tacticity. The only major criterion being sufficient resolution in the proton spectra.

In these investigations we wish to establish the tacticity of polynorbornene-type molecules without the addition of chiral pendant groups or the deuteration of the polymer[29]. The aim is to provide the differentiation of the forms within the microstructure of the polymer by

orienting the polymer in the chiral liquid crystal solvent system. Success would mean a quick and direct method of establishing tacticity .

The only difference between the preparation of the polymer samples and the monomer molecules is the co-solvent used. In the case of the monomer samples the co-solvent used was deutero-chloroform. The Bis(trifluoromethyl)norbornadiene polymers, however, are insoluble in chloroform. The other solvent which had been used successfully as a PBLG co-solvent is dichloromethane and this was also found to be incompatible with the polymers under investigation.

The only solvent which is known to produce the desired liquid crystalline environment and which was known to dissolve the polymers was tetrahydofuran. The samples were therefore prepared according to the standard protocol whilst still using the acetone insert for the lock signal maintenance. The appearance of the samples, however, was somewhat different to those prepared previously and there was some suspicion that the required liquid crystalline state had not been achieved. It is normal that the prepared samples produce a particular iridescent affect in daylight which is the human eye's perception of the birefringence which occurs with liquid crystals. This served as a quick, qualitative assessment of samples prior to insertion in the magnet.

The samples were all placed in the magnet and 1D proton and 1D carbon experiments were performed. The results indicated that the solvent system had not achieved the desired liquid crystalline environment and the subsequent spectra were broadened and uninformative. Following these results the sample preparation method was altered slightly on the assumption that it is perhaps a function of the size of the polymer molecule that the liquid crystal environment had not formed.

1D proton and carbon spectra, together with a 2D gHSQC experiment were performed on a more dilute sample of the mainly *trans* polymer in the hope that this would provide the

required environmental conditions for differential orientation. These results also showed broad amorphous peaks and it was clear that the solvent system was not producing the required chiral liquid crystal system despite the reduction in concentration of solute.
The combinaton of tetrahydrofuran, PBLG and polymer did not produce a stable solvent system as the PBLG appeared to be separating out, forming white strands within the NMR tube. Alternative solvents were investigated for the polymer, however, none were found which would provide the liquid crystalline environment.

It is difficult to ascertain if the size of the polymer molecule being studied is the limiting factor in the formation of the chiral liquid crystal system or whether it is the combination of the size of the polymer molecules and the increased size of the co-solvent molecule involved. Chloroform and dichloromethane are both significantly smaller molecules than tetrahydrofuran. It is also possible that the size of the molecules involved is not the only factor and purely chemical considerations come into play. It is possible that strong intermolecular hydrogen bonds had formed between the polymer and the PBLG. This would produce a randomized coil formation which consequently destroys any liquid crystal properties.The geometry of the component elements is obviously critical and further studies would have to be performed to ascertain optimal conditions for these polymers systems.

CHAPTER 6

6.1 Conclusions

The aim of this investigation was to provide a method of distinguishing, quantitatively the tacticity in polymers of unknown stereochemistry. The ideal circumstances being in solution state and with the need for only small amounts of polymer sample and requiring little in the way of preparation and specialist equipment.

The fundamental premise of the studies was that a solute molecule, when dissolved in the chiral liquid crystal solvent system (Poly-benzyl-L-glutamate/co-solvent) and exposed to a magnetic field would exhibit a very slight orientation with respect to $\mathbf{B_0}$. In the case of chiral solute molecules this orientation would differ very slightly for each enantiomer and this Differential Ordering Effect (DOE) would be the basis of the differentiation between the two forms.

These experiments were achieved using a standard 500MHz magnet and spectrometer, using a readily available chiral liquid crystal and common organic solvents.
Perfection of the sample preparation technique was probably the single most important element in the development of this method as the concentration of the substrate, quantity and average molecular weight of the chiral liquid crystal and volume of co-solvent were discovered to be critical to the formation and stability of the birefringent liquid crystal phase.

Once optimal composition of the samples was established a method of mixing the resultant viscous mixture was perfected to ensure homogeneity. Homogeneity was essential to ensure that shimming of the samples once in the magnetic field would yield spectra of sufficient resolution for our purposes. In the case of these somewhat unusual samples shimming was not a trivial matter, but was critical to the success of the experiment.

The techniques used for sample preparation were perfected on the small organic molecules mentioned and these experiments were also used to establish which molecules produced the best results and to formulate a theory as to why. As with many of the results produced by Courtieu et al.[7,11,14,15], the more promising results were usually obtained from the **sp** and **sp2** hybridized carbon atoms within a molecule, although this was by no means the sole determining factor governing orientation within the system. Geometry and hydrogen bonding must also play a role.

The results obtained from the small organic molecules were varied, with the most promising results coming from the +/- Ibuprofen and the +/- terpinen-4-ol samples. The splittings in what would be the isotropic signals corresponding to the sp_2 carbon nuclei in the molecules. In the case of the +/- terpinen-4-ol the results were of sufficient resolution to be able to identify the two enantiomers quantitatively without the introduction of all possible dipolar couplings which would be undesirable due to their increased complication of the spectra. In the case of +/- terpinene-4-ol, the experiment was tested further with samples containing known and differing proportions of each enantiomer. The results were confirmed as being quantitatively accurate after measurement of the integrals of the peaks involved.

This is an interesting result as the varying proportions of the + and – forms of terpinene-4-ol are particular to different biological specimens. Logically, therefore, this technique could be used to establish the biological origin of the compound. It is not unthinkable, therefore that this method of enantiomeric quantification could be applied to other molecules from other species and used as a means of identification and/or quality control.

The synthetic analgesic compound +/- Ibuprofen was also found to exhibit splittings in the 1D spectra and as the quantification of enantiomers was measured in the biological sample of Terpinen-4-ol, so can the enantiomeric composition of the synthetic drug. As has been mentioned previously the Pharmaceutical Industry spends huge amounts of time and money controlling the production of drugs to ensure that they are enantiomerically pure. This

technique, if it could be applied to the quality control of suitable materials, would be another method in the armoury of chemists to ensure the production of stereochemically pure drugs.

Although this technique was found to be very useful in the enantiomeric determination of the relatively simple molecules which were investigated, it is possible that a molecule containing a large number of different chiral atoms would have stereochemistry which is difficult to establish.

Having achieved some success in the technique the next step was to identify monomer molecules which would display some orientation in the chiral liquid crystal medium. The monomers studied were all norbornene derivatives as they would all perform the Ring Opening Metathesis Polymerisation (ROMP) and ultimately the resultant polymers would be of type of most interest in this investigation.

The norbornene anhydride monomer was found to exhibit the most promising results including an unusual correlation between the one of the methylene bridge protons and one of the carbonyl carbon atoms in the adjacent ring. This was seen in the 2D gHMBC or multiple bond experiment and is unusual in that it is assumed to be a dipolar coupling instead of an ordinary long range scalar coupling although there is not a large scale introduction of dipolar couplings which would make the spectra very complicated. Although this does not give any direct information regarding the stereochemistry of the molecule, it does provide information about the proximity of certain atoms in space. This type of information is usually provided by 2D experiments such as NOESY (Nuclear Overhauser Effect Spectroscopy) which give correlations between protons which are in close proximity but not having an immediate through-bond connections. In this case, however, the information acquired regards the spacial proximity of differing nuclei and as such could be of use in studies of the structural geometry of many systems in solution. This would be a very useful method although it is reliant on the introduction of only certain dipolar couplings and not all

of the possible dipolar couplings.

It is clear from this result that although it is not the main result being sought in the study it does give valuable information regarding the restriction of movement of the molecule. Dipolar couplings are seen most commonly in solid-state NMR spectra.

Following on from the results for norbornene anhydride monomer, the investigation now centred on the experiments used to gather the data. Standard 2D experiments, although useful were found to be too restrictive and it was assumed that modification of the gHSQC experiment would provide more detailed information on a small region of the spectrum. This was to be achieved by reducing the spectral width examined in both the proton and carbon dimensions and by reintroducing couplings in the proton dimension. The modified 2D pulse sequence was named CCOUP.

The vinylic region of the norbornene anhydride molecule which contains sp2 carbon nuclei was chosen for the investigation using the new CCOUP pulse sequence. In the 1D proton and carbon spectra there is no evidence of splittings in the vinylic regions and it was thought that it would be interesting to perform the experiment to see if the 'hidden' splittings in the vinylic region would appear in the 2D experiment. In this way, the assumption that there is no orientation of the molecule in the liquid crystal solvent system because there is no evidence in the 1D spectra can be challenged. This could apply to many molecules including the polymer systems which were the target of the study.

The CCOUP experiment was performed on the norbornene anhydride monomer in the restricted region of the vinyl resonances and to acquire sufficient data for this area the parameters of the experiment were adjusted leaving an experiment which took approximately thirteen hours to run. The results were interesting and informative regarding the exposure of the different couplings which were previously undetected although the length of time the experiment took was surprising.

Following the success experienced with the norbornene anhydride monomer, the technique was applied to the problem of polymer elucidation. The only polymers available on which to try the method were variations of the Bis(trifluoromethyl)norbornadiene. Mainly *cis* and mainly *trans* samples of the polymer were prepared according to the established protocol and the 1D proton and carbon experiments and 2D correlation experiments were performed.

The results for the Bis(trifluoromethyl)norbornadiene samples were inconclusive as the solvent system employed did not produce a stable chiral liquid crystalline phase. Although it would have been interesting to attempt these experiments on the polymeric form of the Norbornene Anhydride monomer, this is not possible. The need for a stereo regular polymer is paramount if you are to be able to ascertain any useful information and as such poly norbornene anhydride is unsuitable. The method by which it is polymerized includes the use of a Mo (Molybdenum) initiator. The Mo initiators have a low tolerance for functional groups and it is unlikely that a suitable polymer would be produced to satisfy the required criteria in these circumstances.

The conclusion of these studies is fourfold:

1. There is enormous scope to employ the chiral liquid crystal solvent system in a method of enantiomeric elucidation of racemic mixtures of certain molecules. The most successful being those molecules of relatively small size and containing sp or sp2 hybridised carbon atoms. This is different to some of the techniques described in the literature[7,17,18] which rely on the presence of deuterium and hence quadrupolar interactions in order to facilitate differentiation. It is also clear that this method, once the sample preparation technique is perfected, is a reliable and relatively robust experimental technique.

2. The use of this method can in certain circumstances provide valuable information about the proximity of otherwise unconnected atoms via the reintroduction of some dipolar

couplings.

3. The modification of the 2D one bond carbon-proton correlation experiment to give carbon couplings in the proton dimension can yield information regarding the enantiomeric content of some samples even when there is no evidence of such in the 1D proton and carbon spectra.

4. There is now evidence to suggest that this technique could be useful in the future to provide details of polymer stereochemistry. Although this was not achieved in this particular investigation due to the lack of stability of the chiral liquid crystal solvent system, it is not unreasonable to assume that further work could establish optimal conditions for the purpose. It is also possible that only certain types of polymers would be suitable for this technique and more work would have to carried out to establish which systems would be acceptable.

6.2 Further Studies

There is potential for this technique to be used with stereoregular polymers to produce results similar to those experienced with the norbornene anhydride molecule. As there is no requirement for resolution in the 1D proton or carbon spectra, the CCOUP experiment is ideal for use with polymer samples whose spectra are usually broad compared to smaller organic molecules in isotropic solutions.

Providing that a suitable organic solvent is found which readily dissolves the polymer under investigation and that as a co-solvent it produces a stable birefringent chiral liquid crystal solvent system, then the CCOUP experiment could be performed. There are many variables to be considered in the preparation of the samples including the type of polymer, whether it is branched, contains co-polymers, the average molecular weight and average chain length all have a bearing on the results which are possible. The liquid crystal used can also be varied as there are a range of readily available liquid crystal systems which can be used for this purpose.

It would be interesting to test this theory further, starting with the preparation of the chiral liquid crystal solvent system as this is the primary requirement of the method. There are a range of chiral liquid crystals available today and it is possible that optimal results for any one substrate molecule may be achieved by only one liquid crystal system.

Another element to any investigation which might provide valuable data is the substitution of deuterium atoms in polymer molecules in place of hydrogen atoms. This could provide important information about the polymer stereochemistry without altering the gross chemical properties of the polymer. The technique of using isotopes as a means of differentiation in chiral liquid crystals media has also been studied by Courtieu et al.
In particular to establish the existence of enantiomers in molecules which are only chiral

by virtue of isotopic substitution. This method has not as yet been applied to the study of polymer molecules, although it is an area which could provide a great deal of information.

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Appendix I

Summary of general experimental conditions:

All NMR experiments were performed on a Varian Inova AS500 NMR Spectrometer operating at 500MHz for proton nuclei and 125MHz for carbon nuclei. One-dimensional and two-dimensional experiments were optimized for each solute/solvent system by adjusting the sweepwidth, number of transients and other standard acquisition parameters. Where there have been major changes to the standard, supplied experiments these have been specified.

Software used for the acquisition of spectra and the processing of subsequent data was provided by the instrument manufacturers (VNMR Version 6.1C).

All PBLG samples and deuterated solvents used were obtained from commercial suppliers and used as supplied (~99% deuterated or above) All small molecules used were obtained by commercial manufacturers except for the norbornene derivatives which were supplied by Dr Ezat Khosravi et al. Polymers tested were also supplied by Dr Khosravi.