SYNTHESIS AND BARRIERS TO ROTATION IN AXIALLY CHIRAL BARBITURIC AND THIOBARBITURIC ACID DERIVATIVES

by

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ABSTRACT

SYNTHESIS AND BARRIERS TO ROTATION IN AXIALLY CHIRAL BARBITURIC AND THIOBARBITURIC ACID DERIVATIVES

In this study, 5,5-dimethyl-1-(*o*-bromophenyl)-2-thiobarbituric acid has been synthesized by the reaction of the corresponding *N*-*o*-arylthiourea with the dimethylmalonic acid in the presence of acetylchloride. The *o*-fluoro and the *o*-chloro substituted 5,5-dimethyl-1-(*o*-aryl)barbituric acids have been synthesized by the direct conversion reaction of the thiocarbonyl in the 5,5-dimethyl-1-(*o*-fluorophenyl)-2-thiobarbituric and 5,5-dimethyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid, respectively, into a carbonyl group by treatment with bromine in 90 per cent acetic acid. In all of the trials the yielded products were composed of the target compounds and the thiobarbiturate precursor. However, the two components could not be separated from each other neither by crystallization nor by chromatographic methods.

The studied barbituric and -2-thiobarbituric acids are chiral due to nonplanar ground states. The aim of this project is to determine the activation energies for the racemization of these compounds. In this respect, the activation barrier for the 5,5-dimethyl-1-(*o*-fluorophenyl)barbituric acid has been determined by using dynamic NMR spectroscopy, whereas the barrier for the 5,5-dimethyl-1-(*o*-bromophenyl)-2-thiobarbituric has been determined by thermal racemization subsequent to the separation of the enantiomers of this compound on the chiral sorbent, cellulose tris(3,5-dimethylphenyl) carbamate. The required separation of the enantiomers for the thermal racemization of 5,5-dimethyl-1-(*o*-chlorophenyl)barbituric acid could not be achieved by liquid chromatography and therefore the barrier to rotation for this compound could not be determined.

The obtained barrier value for the 5,5-dimethyl-1-(*o*-bromophenyl)-2-thiobarbituric acid has been compared to the literature values for the *o*-chloro and the *o*-fluoro derivatives of 5,5-dimethyl-1-(*o*-aryl)-2-thiobarbituric acid and to the literature values for the *o*-

halogen substituted 5,5-dimethyl-3-(*o*-aryl)-2,4-oxazolidinedione derivatives and a similar relationship between the size of the *o*-substituents and the barrier values has been observed. Using this relationship, the yet uncalculated barrier values for the *o*-halogen substituted 5,5-dimethyl-1-(*o*-aryl)barbituric acids have been estimated.

ÖZET

KİRAL BARBİTÜRİK VE TİYOBARBİTÜRİK ASİT TÜREVLERİNDE SENTEZ VE DÖNME BARİYERLERİ

Bu çalışmada 5,5-dimetil-1-(*o*-bromofenil)-2-tiyobarbitürik asit, uygun *N*-*o*-ariltiyoürenin dimetilmalonik asitle asetilklorür varlığında tepkimesinden elde edilmiştir. *Orto*-floro ve *orto*-kloro sübstitüentli 5,5-dimetil-1-(*o*-aril)barbitürik asitler, sırasıyla 5,5-dimetil-1-(*o*-florofenil)-2-tiyobarbitürik ve 5,5-dimetil-1-(*o*-klorofenil)-2-tiyobarbitürik asitlerdeki tiyokarbonil grubunun direkt olarak karbonile, yüzde 90 asetik asit içinde brom yardımıyla dönüşümü tepkimesinden üretilmiştir. Bütün denemelerde çıkan ürünlerin hedef bileşikler ile tiyobarbitürat giriş maddelerinden oluştuğu belirlenmiştir. Fakat bu bileşenler ne kristalizasyon ile ne de kromatografik yöntemlerle birbirinden ayrılabilmişlerdir.

Çalışılan barbitürik ve -2-tiyobarbitürik asitler düzlemsel olmamaları nedeniyle kiraldirler. Bu projede amaç, bu bileşiklerdeki engelli dönme aktivasyon enerjilerinin belirlenmesidir. Bu bağlamda, 5,5-dimetil-1-(*o*-florofenil)barbitürik asitin aktivasyon bariyeri dinamik NMR spektroskopisi kullanılarak bulunmuştur. 5,5-Dimetil-1-(*o*-bromofenil)-2-tiyobarbitürik asitin aktivasyon bariyeri ise engelli dönme enantiyomerleri likit kromatografide optikçe aktif, selüloz tris(3,5-dimetilfenil) karbamat dolgu maddesinde ayrıştırıldıktan sonra uygulanan termal rasemizasyon yöntemiyle bulunmuştur. 5,5-Dimetil-1-(*o*-klorofenil)barbitürik asitin enantiyomerleri, termal rasemizasyon için gerekli olan likit kromatografiyle ayrıştırılamadığından, bu bileşiğin dönme bariyeri belirlenememiştir.

5,5-Dimetil-1-(*o*-bromofenil)-2-tiyobarbitürik asitin bulunan enerji bariyeri 5,5-dimetil-1-(*o*-aril)-2-tiyobarbitürik asitlerin *o*-floro ve *o*-kloro türevlerinin literatürde geçen bariyer değerleriyle ve *o*-halojen sübstitüentli 5,5-dimetil-3-(*o*-aril)-2,4-oksazolidindiyon türevlerinin literatürde geçen bariyer değerleriyle karşılaştırılmış ve *o*-sübstitüentlerin

büyüklüğü ile bariyerler arasında benzer bir ilişki bulunmuştur. Bu durumdan yola çıkılarak henüz deneysel olarak belirlenmemiş olan *o*-halojen sübstitüentli 5,5-dimetil-1-(*o*-aril)barbitürik asitlere ait aktivasyon bariyerleri tahmin edilmiştir.

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LIST OF SYMBOLS/ABBREVIATIONS

A_m	Amount of the compound in the mobile phase
A_s	Amount of the compound in the stationary phase
C_m	Concentration of the compound in the mobile phase
C_s	Concentration of the compound in the stationary phase
F	Flow rate
h	Planck's constant
Hz	Hertz
J	Joule
k	Rate constant for the equilibrium
K	Kelvin
k'	Capacity factor
k_b	Boltzman constant
k_c	Rate constant at coalescence
k_f	Rate constant for forward reaction
k_r	Rate constant for reverse reaction
s	second
t	Time
T	Temperature
T_c	Coalescence temperature
t_R	Retention time
V_0	Dead volume
V_n	Net retention volume
V_R	Retention volume
α	Separation factor
δ	Chemical shift
$\Delta G^{\#}$	Free energy of activation
$\Delta\nu$	Chemical shift difference in Hertz
$CDCl_3$	Deuterated chloroform
Chiralcel OD-H	Tris-(3,5-dimethyl) phenylcarbamate
DMSO-d_6	Hexadeuterated dimethylsulfoxide

DNMR	Dynamic Nuclear Magnetic Resonance
HPLC	High Pressure Liquid Chromatography
NMR	Nuclear Magnetic Resonance
UV	Ultraviolet

1. INTRODUCTION

The first indications that rotation around a single bond was not always free but could be restricted were provided by Bischoff in 1891. The experimental proof of this phenomenon was produced for the first time in 1922 when Christie and Kenner resolved 6,6'-dinitrophenic acid (Figure 1.1) into optically active forms [1].

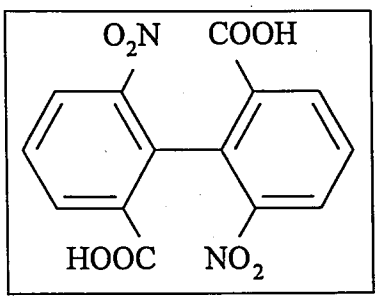


Figure 1.1. The structure of 6,6'-dinitrophenic acid

The resolvability of this biphenyl derivative into optically active forms was attributed to the fact that the two bulky groups present on each ring are in close proximity to each other, which in return prevents the free rotation around the central C-C single bond [2].

This finding led to the introduction of the term 'atropisomerism' or 'rotational isomerism' into the field of chemistry. The term is used to denote any kind of stereoisomerism due to restricted rotation about single bonds where the isomers can actually be isolated [3].

Chemical literature bears numerous papers reporting on the optical resolution of rotational isomers of biphenyl derivatives carried out since the aforementioned work done by Christie and Kenner in 1922. These studies serve as examples to the investigation of restricted rotation around C-C single bonds. The research into the restricted rotation in *N*-heterocyclic compounds, which are analogous to biphenyls and exemplify the restricted rotation around C-N single bond, started only after 1931 when Adams and coworkers reported on the resolution of *N*-phenylpyrroles and *N,N'*-dipyrryls [4-6].

In 1967, Mislow *et al.* studied *N*-aryl cyclic amides and held the restricted rotation around C_{Aryl}-N bond responsible for the observed chemical shift nonequivalence in these compounds. The work also covered the estimation of kinetic data on hindered rotation [7].

In 1970, Colebrook *et al.* published a paper reporting on the existence of high rotational barriers about C-N bonds in aryl substituted heterocyclic compounds that they worked with. The course of equilibration in rotational isomers was followed by integration of their NMR signals and the resultant high values of energy of activation was attributed to the steric interference between a bulky *ortho*-substituent on the aryl group and the heterocyclic moiety [8].

Shortly after this work, Colebrook *et al.*, in 1972, reported on another study revealing the existence of high barriers to rotation in some aryl substituted heterocyclic compounds lacking bulky *ortho*-substituents. The large values of barrier height in the compounds studied were suggested to be due to the buttressing effect of the substituent adjacent to the *ortho*-position. Also the necessary severity of steric interaction between the two cycles in the transition state in order for a high barrier to exist was reported to be provided by the bulk and geometry of the heterocyclic moieties, compensating for the small effective size of the aryl groups [9].

A comparative study on the influence of the effective sizes of the *o*-chloro and the *o*-methyl substituents on hindered rotation observed in 1-arylhydantoins , 3-arylhydantoins, and 3-aryl-2-thiohydantoins was presented, again by Colebrook *et al.*, in 1973. They reported on the existence of a familiar, expected steric influence pattern in 1-arylhydantoins that the *o*-methyl substituent induces higher barriers than the *o*-chloro substituent. But in 3-arylhydantoins and their thio analogues, this effect was observed to be the reversal of the previous case. The latter situation was attributed to the fact that the electrostatic repulsion between the electronegative chlorine on the phenyl and the oxygen of the carbonyl on the heterocyclic moiety rendered the effective size of the chlorine higher than that of the methyl, increasing the transition state energy of the chloro derivative and thus causing higher rotational barrier. The rotational barrier values involved in this study were estimated by dynamic NMR method [10].

Later on, in 1980, Kashima and Katoh working on restricted rotation in *ortho*-substituted 1-aryl-4,6-dimethylpyrimidine-2(1H)-ones and the corresponding thiones succeeded in separating the related rotational isomers by recrystallization of the salts formed with D-camphor-10-sulfonic acid. They calculated the rotational barriers and unexpectedly, the barrier for the *o*-tolyl substituted compound resulted to be larger for the oxo derivative than for the thioxo analogue in spite of the lower standard bond length of the carbonyl bond than the thiocarbonyl and the larger van der Waals radius of sulfur than oxygen. This behavior was explained to be due to the greater single bond character of the thiocarbonyl group (Figure 1.2), which prompted bond bending and rendered the rotation easier [11].

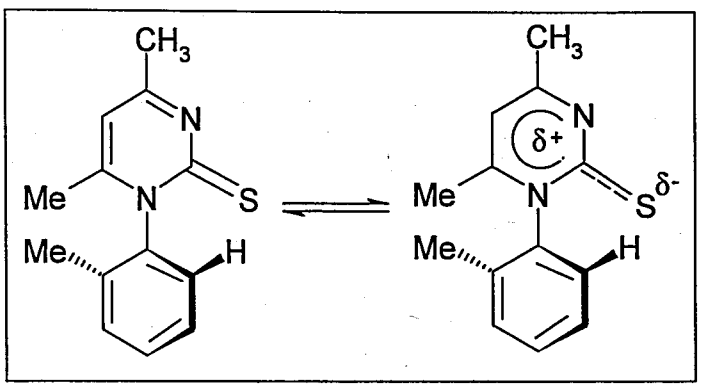


Figure 1.2. The structure of 1-(*o*-tolyl)-4,6-dimethylpyrimidine-2(1H)-thione studied by Kashima *et al.* and the polarized form, as induced by the single bond character of the carbon-sulfur double bond

Another example to investigation of restricted rotation around C-N bond was presented in 1985 by Mannschreck *et al.*, who worked with sterically hindered *N*-aryl-4-pyridone derivatives. In this study, they achieved the enrichment of the related rotamers by liquid chromatography on triacetylcellulose and estimated the barriers to rotation by thermal racemization [12].

In 1988, Roussel *et al.* declared an opposition to the suggestion made by Kashima *et al.* [11] that the greater single bond character of the thiocarbonyl group in 1-(*o*-tolyl)-4,6-dimethylpyrimidine-2(1H)-thione induces an easier rotation than in the oxygen analogue. In their study Roussel *et al.* also observed the unexpected larger magnitude of rotational barrier in the same pyrimidine thione derivative than in the oxo analogue. However, their

X-ray analyses of the cited compounds revealed that the length of carbon-sulfur bond in the thiocarbonyl group was shorter enough to pass for an actual double bond. Moreover, the bond length between the carbon of the thiocarbonyl group and the *N*-aryl nitrogen was observed to be longer than expected in the X-ray analyses. This finding was thought to be indicative of a weak link between the involved atoms in the molecule. In the light of these results, Roussel *et al.* proposed a ring opening-ring closure mechanism (Figure 1.3) for racemization of these compounds instead of internal rotation around the pivot bond [13].

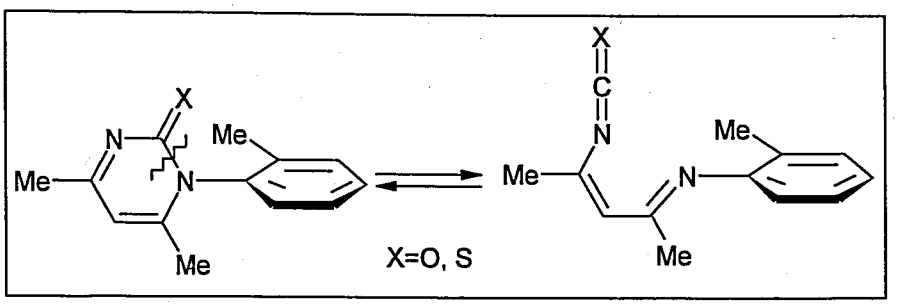


Figure 1.3. The ring opening-ring closure mechanism for the racemization of 1-(*o*-tolyl)-4,6-dimethylpyrimidine-2(1H)-one and the corresponding thione, as suggested by Roussel *et al.*

In 1990, Mintas *et al.* investigated the barriers to racemization in enantiomeric *N*-aryl-2(1H)-quinolones and *N*-aryl-6(5H)-phenanthridinones by separation on chiral triacetylcellulose sorbent and performing thermal racemization [14].

In 1992, Mintas, Kastner, and Mannschreck published a paper on *N*-aryl and *N*-heteroaryl substituted 2,5-dimethylpyrrole-3-carbaldehydes, in which they reported the estimation of the related rotational barriers obtained either by means of enrichment on triacetylcellulose, followed by thermal racemization or by way of dynamic NMR [15].

In 1993, Doğan *et al.* investigated the enantiomers of *N*-aryl-2-thioxo-4-oxazolidinones and *N*-arylrhodanines for the first time analytically on liquid chromatography on triacetyl and tribenzoylcellulose. The barriers to rotation about C-N bond were determined by thermal racemization subsequent to chromatographic semi-preparative enrichment [16].

In 1998, Karataş and Doğan reported on the synthesis of *N*-(*o*-tolyl) and *N*-(*o*-chlorophenyl)-2,4-thiazolidinediones by a direct conversion from the corresponding 2-thioxo-4-thiazolidinone derivatives. The conversion method involved the treatment of the parent compound with Br_2 in 90 per cent acetic acid solution at reflux temperature (Figure 1.4). The work also covered the investigation of the rotamers in the presence of the optically active auxiliary, (S)-(+)-1-(9-anthryl)-2,2,2-trifluoroethanol by NMR [17].

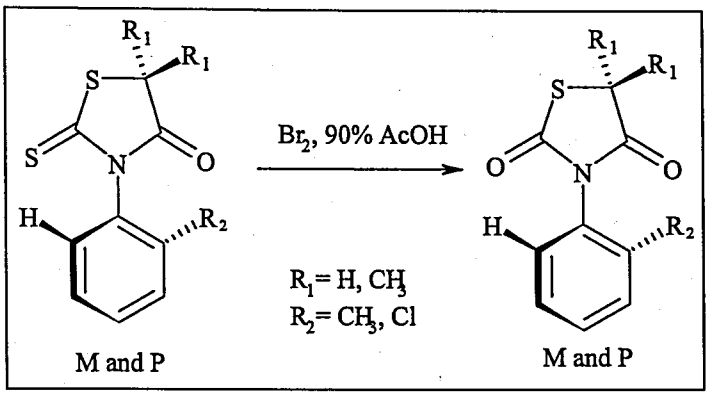


Figure 1.4. The direct conversion of the thiocarbonyl to the carbonyl group, as carried out by Karataş and Doğan

In 2003, Oğuz and Doğan presented a paper reporting on the investigation of barriers to enantiomerization of 5,5-dimethyl-1-(*o*-aryl)barbituric and 2-thiobarbituric acid derivatives (Figure 1.5). The study covered the separation of the related enantiomers by micropreparative liquid chromatography and estimation of the activation barriers for the conversion of one enantiomer to its counterpart upon thermal racemization of the separated enantiomers [18].

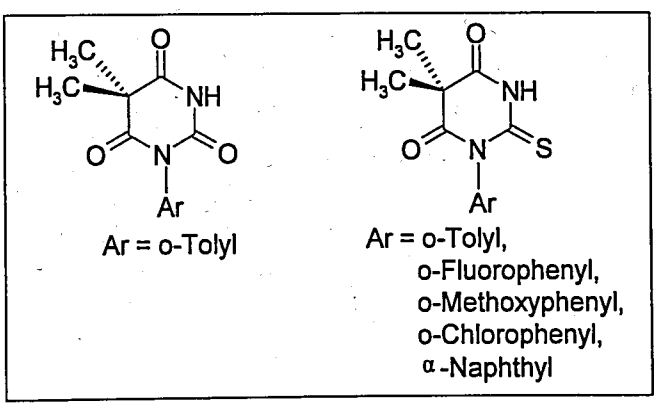


Figure 1.5. The 1-(*o*-aryl)barbituric and -2-thiobarbituric acids studied by Oğuz *et al.*

More recently, Demir Ordu and Doğan published a paper reporting on the estimation of rotational barriers in 5,5-dimethyl-3-(*o*-aryl)-2,4-oxazolidinedione enantiomers (Figure 1.6) by way of thermal racemization and dynamic NMR. An examination of the obtained barrier values for the *o*-halogen substituted aryl derivatives, based on the van der Waals radii of the halogen substituents, yielded a linearly increasing relationship between the barriers and the sizes, as going from fluorine to iodine. The work also covered the assignment of absolute conformation of the enantiomers [19].

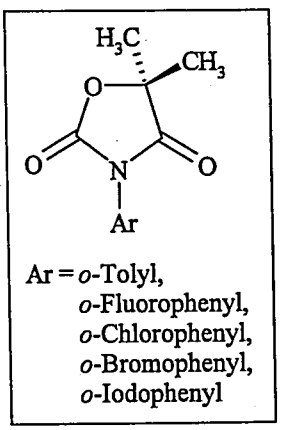


Figure 1.6. The 5,5-dimethyl-3-(*o*-aryl)-2,4-oxazolidinedione derivatives studied by Demir Ordu and Doğan

This study covers the investigation of restricted rotation in novel derivatives of 5,5-dimethyl-1-(*o*-aryl)barbituric and 2-thiobarbituric acids (Figure 1.7), and thus, is complementary to the earlier research carried out in our group by S. Funda Oğuz, Ph.D. [20].

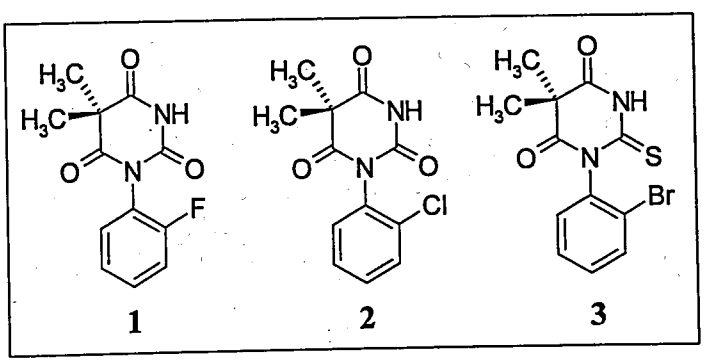


Figure 1.7. The structure of novel 5,5-dimethyl-1-(*o*-aryl)barbituric and -2-thiobarbituric acids

In this previous research, the synthesis of 5,5-dimethyl-1-(*o*-aryl)barbituric acid derivatives from their corresponding starting materials, namely *N*-(*o*-aryl)urea and 1,1-diethyl-2,2-dimethyl malonate, was revealed to be very problematic. All attempts to synthesize 5,5-dimethyl-1-(*o*-aryl)barbituric acid derivatives failed, except for the *ortho*-tolyl derivative, which was obtained with a very low yield. Therefore, in this project a different procedure for the synthesis of 5,5-dimethyl-1-(*o*-aryl)barbituric acids has been experimented. This procedure involves the direct conversion of the thiocarbonyl group in parent 5,5-dimethyl-1-(*o*-aryl)-2-thiobarbituric acid derivatives to a carbonyl group. The method has been utilized by Karataş and Doğan in the conversion of *N*-(*o*-aryl)rhodanines to their corresponding *N*-(*o*-aryl)-2,4-thiazolidinediones and reported to give successful results [17]. In this study, it has been observed that the conversion has worked only partially for the synthesis of 5,5-dimethyl-1-(*o*-fluorophenyl)barbituric acid and 5,5-dimethyl-1-(*o*-chlorophenyl)barbituric acid. In all of the trials, the barbituric acid was found to be present together with the corresponding thiobarbituric acid and it was not possible to seperate the two neither by crystallization nor by chromatographic methods. For the synthesis of 5,5-dimethyl-1-(*o*-bromophenyl)-2-thiobarbituric acid, the reaction of the corresponding starting materials, *N*-arylthiourea and 2,2-dimethylmalonic acid has been carried out.

Barbituric acid derivatives are a well-known class of compounds many of which are widely-used drugs having such disparate pharmacological activities as depressants, hypnotics and stimulants. Besides their medicinally important benefits, these compounds closely resemble several nitrogeneous bases found in nucleic acids and some bacteria metabolize certain pyrimidines to barbituric acid derivatives [21]. These features have made them one of the focal points in chemical research.

The compounds, which this study has focused upon (Figure 1.7), are also barbituric acid derivatives. The names of the compounds are 5,5-dimethyl-1-(*o*-fluorophenyl) barbituric acid, **1**, 5,5-dimethyl-1-(*o*-chlorophenyl)barbituric acid, **2**, and 5,5-dimethyl-1-(*o*-bromophenyl)-2-thiobarbituric acid, **3**. These derivatives are optically active and exist as a pair of thermally-interconvertable enantiomers due to the presence of restricted rotation around the central C-N axis (Figure 1.8). The restriction to rotation is induced by

the steric interaction of bulky *o*-substituents on the phenyl moiety with the carbonyl and the thiocarbonyl functional groups on the barbituric acid heterocycle.

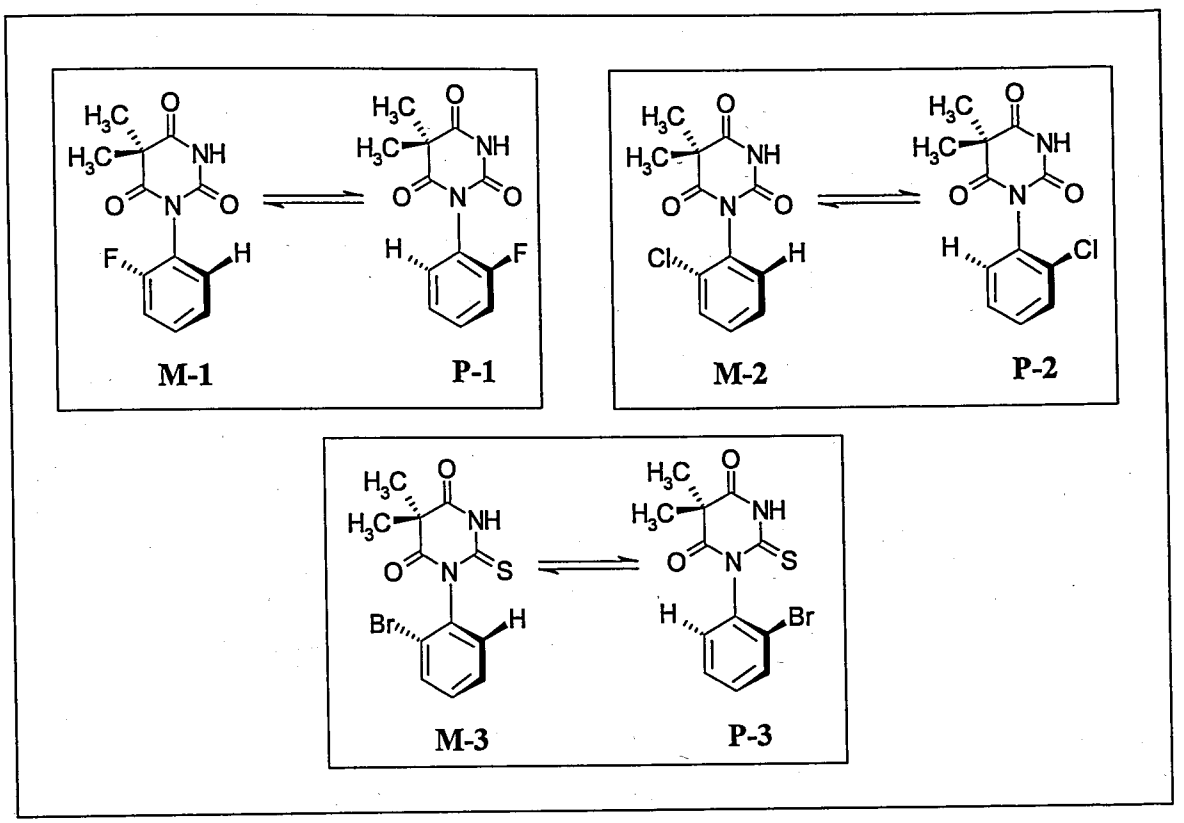


Figure 1.8. The thermally-interconvertable enantiomers resulting from the restricted rotation in compounds **1**, **2**, and **3**

The quantitative aid which is used in debating about the possibility of optical resolution in biphenyl derivatives and analogues is the thermodynamic entity called 'rotational barrier'. If the rotational barrier is more than about 23 kcal/mol (*ca.* 100 kJ/mol) at room temperature, it should be possible to resolve the two rotational isomers [11]. Therefore, determination of rotational barriers in the compounds subject to this study will be useful in giving an idea about the resolvability of the enantiomers. Also, an insight into the mechanism of enantiomerization in the compounds could be gained from the rotational barriers.

In this study, the barrier to rotation in compound **1** has been determined by temperature-dependent ^{1}H NMR. Since, the existence of compound **1** in the form of a mixture with its thiobarbiturate precursor would not violate the DNMR experiment, it was

used as such. For compound **2** and **3**, the rotational barrier has been attempted to be determined by thermal racemization, for their more largely sized *ortho*-halogen substituents would not allow rapid rotation at ordinary NMR probe temperatures. However, the required separation of enantiomers for compound **2**, could not be achieved by liquid chromatography on the optically active sorbent, cellulose tris(3,5-dimethylphenyl) carbamate. Therefore, only the rotational barrier for compound **3**, the enantiomers of which were separated efficiently on cellulose tris(3,5-dimethylphenyl) carbamate, has been determined by thermal racemization.

The obtained rotational barrier for compound **3** has been compared with the previously determined energy barrier values for 5,5-dimethy1-1-(*o*-fluorophenyl)-2-thiobarbituric and 5,5-dimethyl-1-(chlorophenyl)-2-thiobarbituric acid [18] and a graph of the energy barriers versus van der Waals radii of *ortho*-substituents has been plotted. From this graph, existence of a linearly increasing relationship between the size of the *ortho*-substituents and the barriers has been detected. Previously, the existence of a similar relationship between the size of *ortho*-halogen substituents and the rotational barriers in the 5,5-dimethyl-3-(*o*-aryl)-2,4-oxazolidinedione derivatives has been reported by Demir Ordu and Doğan [19]. The graph of the barriers versus van der Waals radii, for the *ortho*-halogen substituted 5,5-dimethyl-1-(*o*-aryl)-2-thiobarbituric acids has been compared to the reported graph for the *ortho*-halogen substituted 5,5-dimethyl-3-(*o*-aryl)-2,4-oxazolidinedione derivatives and the slopes have been observed to agree with each other, perfectly. Using the slopes of these graphs and the determined barrier value for compound **1**, a similar graph has been plotted and from this graph the barriers for the, yet uncalculated, *o*-chloro, *o*-bromo and *o*-iodo derivatives in the series of 5,5-dimethyl-1-(*o*-aryl)barbituric acids have been predicted.

2. THEORY

2.1. Chirality and Chiral Molecules

The property of non-identity of an object with its mirror image is termed 'chirality', and molecules having such a property are said to be 'chiral'. The possession of chirality for a molecule necessitates asymmetric occupation of three-dimensional space about either a center, an axis, or a plane, each of which is defined as a 'stereogenic unit'. A chiral molecule and its non-superimposable mirror image, which is also a chiral molecule, are described as 'enantiomers' of each other. Enantiomers exhibit identical physical and chemical properties in the absence of an external chiral influence. That is, they have identical melting points, identical boiling points, and identical solubilities in the same solvent, etc. However, they do differ in their response to a chiral agent such that they show different reactivity towards the same chiral substance and they rotate the plane of plane-polarized light, which is also a chiral entity in a sense, into different directions. The latter is defined as 'optical activity' and is described by 'specific rotation', which is a measurable quantity used to determine the enantiomeric excess of an optically active mixture.

Central chirality is imposed on a molecule when it has an atom having a molecular geometry of a tetrahedron, the corners of which are occupied by four different groups. Such an atom is, then, called a 'stereocenter' and the most common example is a carbon atom having four different groups attached to it. However, there are atoms other than carbon which can be a stereocenter in a molecule. As examples to such atoms, tetrahedral nitrogen, sulfur, and phosphorus would be given (Figure 2.1).

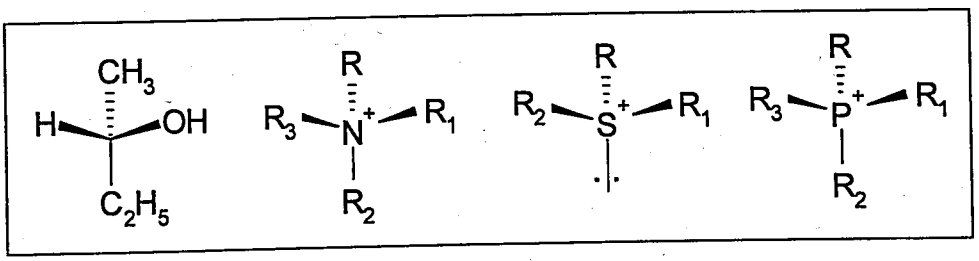


Figure 2.1. Examples of chiral molecules with different type of stereocenters

Rotational isomerism resulting in axial chirality is caused by inhibition of the free rotation around a certain single bond in an asymmetrically substituted molecule, leading to enantiomeric configurations. Such a bond is, then, called a 'chiral axis', and this situation is most commonly observed for biphenyl derivatives (Figure 1.1) and its analogues. The inhibition of free rotation is brought about by the bulkiness of the substituents in the vicinity of the chiral axis.

The last type of chirality, which is called 'planar chirality', is possible in a molecule when the groups about a hypothetical plane are arranged in an asysmmetric fashion.

2.1.1. Configurational Stability and Barriers to Interconversion

Possibility of asymmetric occupation of three-dimensional space around a stereogenic unit in a molecule would not suffice for chirality. Sometimes, a certain chiral configuration may exist only in theory. For example, if a tertiary amine has four different groups attached to the nitrogen, this will make a chiral configuration. However, in practice the resolution of the relevant enantiomers is usually impossible due to the rapid interconversion (Figure 2.2). This interconversion occurs by way of nitrogen inversion and the barrier to the process is about 25 kJ mol^{-1} for most simple amines, low enough to occur readily at room temperature [22]. Therefore, stability of a certain chiral configuration is an important issue to be taken into consideration when discussing about the chirality of a molecule.

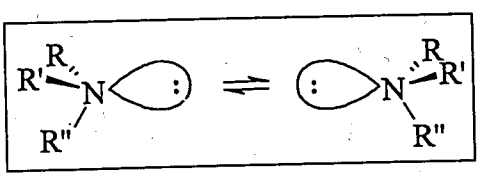


Figure 2.2. Interconversion of amine enantiomers

Stability of a chiral molecule can be inferred from a thermodynamic parameter called 'barrier of interconversion', which is simply defined as the free energy of activation that must be overcome by an enantiomer for interconversion to its counterpart. The barriers of interconversion may be termed as 'rotational barriers' for axially chiral molecules, for

which the interconversion occurs through rotation around the pivot bond. The axially chiral 5,5-dimethyl-1-(*o*-aryl)barbituric and -2-thiobarbituric acid enantiomers studied in this project are thermally-interconvertible to one another, through rotation around the central C-N bond. The values for the free energy of activation to enantiomerization ($\Delta G^{\#}$ in Figure 2.3) in these molecules are expected to be high enough for configurational stability. Individual values of rotational barriers can be used to judge about the possibility of optical resolution at room temperature [11]. On the other hand, comparison between the rotational barriers of structurally related compounds may contribute to the disclosure of the nature of the steric effects of substituents exerted upon racemization.

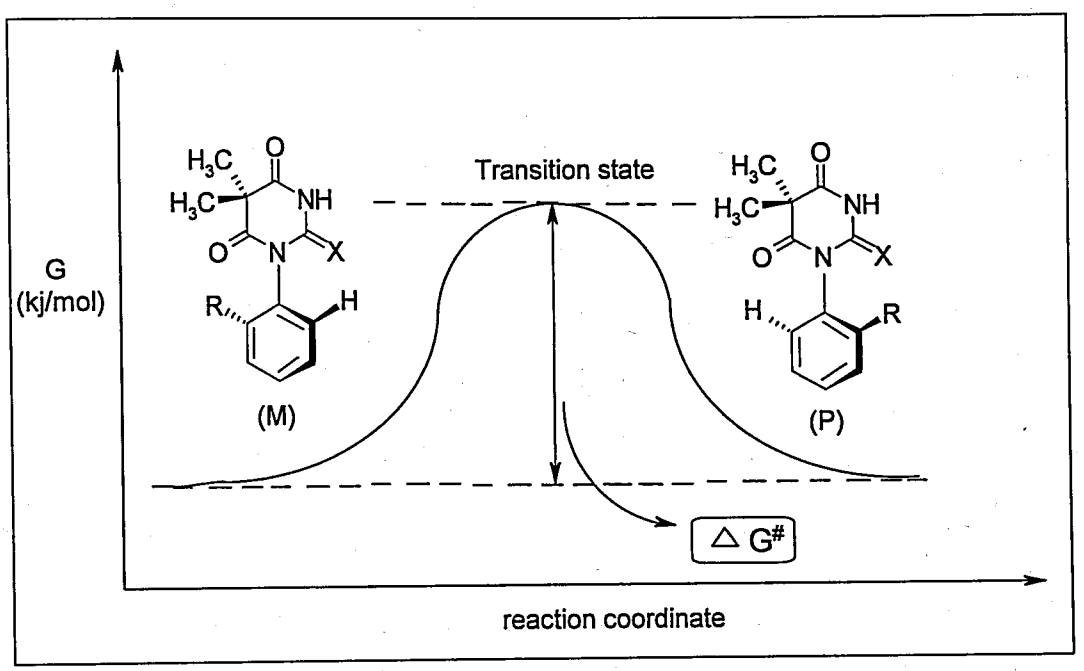


Figure 2.3. Graphical illustration for the free energy of activation for the interconversion of the rotamers

2.2. Axial Chirality and Configurational Stability in 5,5-Dimethyl-1-(*o*-aryl)barbituric and -2-thiobarbituric Acids

The molecules of 5,5-dimethyl-1-(*o*-aryl)barbituric and -2-thiobarbituric acids investigated in this project are heterocyclic analogues of biphenyls and they exhibit rotational isomerism due to restricted rotation of the molecule around the central C-N single bond adjoining the two rings. It is a well-known theoretical fact that every molecule

must assume possibly the most stable conformation in their ground states. These compounds meet this criterion in such a way that the two ring systems do not lie in a coplanar arrangement in their ground state. Consequently, the molecules are sufficiently relieved from the steric interactions between the *ortho* substituent on the phenyl and the exocyclic oxygen or the sulfur of the heterocyclic moiety, and hence the stability is attained. Moreover, the *ortho* substituent on the phenyl ring brings dissymmetry to the molecule leading to the chirality of the ground-state conformations, and thus to the existence of enantiomers M and P (Figure 1.8).

2.3. Nomenclature

The chirality of axially chiral molecules is defined in terms of their helicities M and P in the Cahn, Ingold, Prelog system [23]. In this system, first an axis is drawn through the single bond around which the conformation is defined and the smaller torsion angle formed between the two carbon atoms bearing the group of the highest priority is used to define the helix. A resulting clockwise rotation is denoted as "P" (plus) whereas the counter clockwise rotation is denoted as "M" (minus). In accord with this naming procedure, the configurations of 5,5-dimethyl-1-(*o*-aryl)barbituric and -2-thiobarbituric acids are denoted as shown in Figure 2.4.

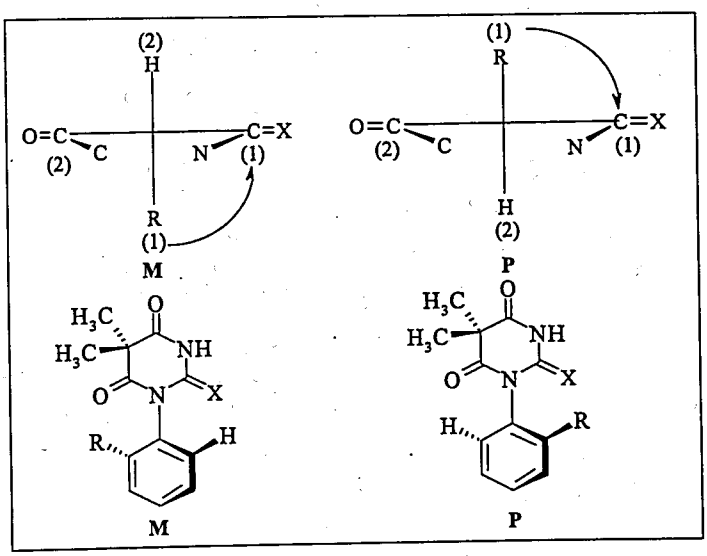


Figure 2.4. Descriptors for the axially chiral 5,5-dimethyl-1-(*o*-aryl)barbituric and -2-thiobarbituric acids

2.4. Dynamic NMR Spectroscopy

2.4.1. A General Review of Dynamic NMR Technique

It is a well-known fact that the appearance of a broad signal on a ^{1}H NMR spectrum is an indication of an exchange process taking place in the sample molecule [24]. This situation is frequently observed when samples with OH and NH functional groups are being analyzed by means of ^{1}H NMR. For instance, when ordinary ethanol is to be analyzed by ^{1}H NMR, the proton bonded to oxygen gives a broad singlet due to the rapid exchange of places with the protons of the surrounding water molecules. This process is so fast on the NMR time scale that it can not be clearly detected, and hence a broad peak, instead of a sharp one, appears on the spectrum. For a better visualization of this phenomenon in mind, an analogy between the NMR spectrometer and a camera with a relatively slow shutter speed, have been suggested by J. D. Roberts, a pioneer in the application of NMR spectroscopy to problems of organic chemistry. According to this analogy, just as a camera with a slow shutter speed blurs photographs of objects that are moving rapidly, the NMR spectrometer 'blurs' its picture of molecular processes that are ocurring rapidly [22].

Apart from the case of proton exchange in alcohols described above, one can occasionally come across a situation where two diastereotopic nuclei showing distinct sharp signals on the spectrum exhibit a broad singlet as the analysis temperature is raised. The reason for this is that, at lower temperatures, there exists an exchange of places between these nuclei, which happens to be in a slow manner. Consequently, the NMR spectrometer is capable of distinguishing between the exchanging sites of these nuclei. However, when the temperature is raised, the rate of exchange may increase to such an extent that the NMR spectrometer is no longer able to distinguish between the sites that the exchange is going on, and thus, a broad band is observed. This feature of NMR spectroscopy has been exploited so well that a new analysis technique called 'dynamic NMR spectroscopy' has been developed.

Dynamic NMR spectroscopy, or shortly DNMR, is a tool for analyzing the exchange behavior of certain nuclei, varying with the temperature. Therefore, DNMR is also called 'temperature-dependent NMR spectroscopy'. In this technique, the NMR spectra of the sample at different temperatures are taken until no observable valley exists between the two signals under analysis, which are originally resonating at chemical shift values of δ_A and δ_B. The temperature at which this occurs is called 'coalescence temperature' and it is denoted as 'T_c'. The determination of this temperature is quite informative about the kinetic parameters of the exchange process. Further increase in temperature would result in the appearance of one sharp peak of double intensity with the average shift $(\delta_A+\delta_B)/2$.

Theoretically, the NMR spectrum of a pair of diastereotopic nuclei that undergo an exchange process is a function of the difference in the resonance frequencies of the two signals, $\Delta\nu$, and of the rate of exchange, k. If the exchange is slow and $k << \Delta\nu$, one can observe two distinct signals for the diastereotopic nuclei. On the other hand, if the rate of exchange is high, we may observe no two separate signals but one broad signal, or even a sharp singlet if the exchange rate is far much higher, i.e. $k >> \Delta\nu$. The difference in resonance frequencies are known to depend on the applied magnetic field strength. For example, at a 60 MHz instrument, if the chemical shift difference between certain diastereotopic nuclei is one ppm, this corresponds to 60 Hz. Therefore, at a given temperature, if the rate of exchange between these nuclei far exceeds 60 s^{-1}, then a sharp singlet may be observed. However, if we are to analyze the same nuclei at a 400 MHz instrument at the same temperature, we may observe two distinct signals provided that the exchange rate, which has been said to be higher than 60 s^{-1}, does not exceed 400 s^{-1}. This means that an NMR spectrometer operating at 400 MHz for ^1H nuclei may detect two sites that are not detected by a machine operating at 60 MHz for ^1H, at a given temperature [24]. Another implication of this fact is that at a higher applied magnetic field strength, the coalescence takes place at a higher temperature. Thus, T_c is not a constant, but is a quantity which depends on the observing magnetic field strength [25].

Dynamic processes are most commonly investigated using ^1H NMR spectroscopy, but resonances of other nuclides such as ^{19}F, ^{31}P, and especially ^{13}C, can also be used. Such measurements are often carried out as an extension of ^1H NMR studies, as the greater

range of chemical shifts for these nuclides allows peak coalescences to be observed at higher temperatures than for protons [25].

2.4.2. Determination of the Activation Energies for Hindered Rotation by DNMR

The restricted rotation around the central C-N bond in 5,5-dimethyl-1-(*o*-aryl)barbituric and -2-thiobarbituric acid derivatives, studied in this project, renders the C-5 methyl groups diastereotopic in NMR spectroscopy. That is, they are observed as differently shielded singlets of the same intensity (Figures 4.2, 4.3 and 4.4). The coalescence of these two singlets into one broad peak could be observed if the observing temperature is raised, by which the restriction to the rotation would weaken in strength and eventually will disappear causing the diastereotopicity to be lost. The coalescence temperature thus obtained could be used in order to estimate the activation energy for restricted rotation or the rotational barrier, by incorporating it into a simple calculation process, the details of which are given below:

For the coalescence temperature, the rate constant, k_c can be calculated by:

$$k_c = 2.22\ \Delta\nu \qquad (2.1)$$

where $\Delta\nu$ is the difference in Hz between the resonance frequencies of the two signals in the absence of interconversion. This equation preserves its validity as long as:

- the exchange process follows first-order kinetics,
- the two singlets have equal intensities, and
- there is no coupling between the diastereotopic nuclei.

After estimation of the rate of rotational interconversion, k_c, it is substituted into the Eyring Equation, which expresses the exponential decrease of k_c with the free molar activation energy, $\Delta G^{\#}$, as:

$$k_c = \left(k_b.T_c/h\right).e^{-\Delta G^{\#}/R.T} \qquad (2.2)$$

where R is the gas constant, k_b is the Boltzmann constant, and h is the Planck's constant. Upon substitution of the numerical values of these constants, Equation (2.2) takes the following form:

$$\Delta G^{\#} = 19.1\ T_c\ [10.32 + \log (T_c / k_c)]\ .\ 10^{-3} \qquad (2.3)$$

which gives the free molar activation energy $\Delta G^{\#}$, in kJ / mol, for the interconversion of the rotational isomers, where a first-order rate law is being obeyed.

In this study, the activation barrier for hindered rotation around the central C-N bond in 5,5-dimethyl-1-(*o*-fluorophenyl)barbituric acid, **1**, was calculated by following the temperature-dependent ^{1}H NMR spectra of the protons on the diastereotopic C-5 methyl groups and subsequently performing the calculations described above. Since, the existence of compound **1** in the form of a mixture with its thiobarbiturate precursor would not violate the DNMR experiment, it was used as such. This method could not be applied to the determination of the rotational barriers for compounds **2** and **3**, because the high energy barriers, due to the presence of the chlorine substituent in **2** and of the thiocarbonyl and the bromine substituent in **3**, would not allow rapid rotation at ordinary NMR probe temperatures.

2.5. Chromatographic Separation of Stereoisomers

2.5.1. A Review of HPLC Technique and Basic Chromatographic Theory

In general terms, chromatography is a separation and identification technique that relies on the different partition coefficients of the components of a mixture for two different phases, the mobile phase and the stationary phase [26]. There are many types of chromatography, which are distinguished from one another depending on the nature of the mobile phase and the stationary phase.

In the particular technique called liquid chromatography, the stationary phase has a solid nature and the mobile phase is liquid. Depending on how the chromatographic

process is developed, there exists different modes for liquid chromatography, one of which is the high-performance or high-pressure liquid chromatography (HPLC).

HPLC has numerous applications and has been the key element in the solution of very important analytical problems, ever since it first became available for analyses, which took place in about 1970 [27]. In HPLC, the chromatographic process is carried out in such a way that the liquid mobile phase is forced through a thin column under high pressure. The column contains a special solid packing material, which serves as the stationary phase. Separation is achieved by injecting the analyte mixture dissolved in a suitable solvent, to the column, through which the mobile phase runs and carries the analyte mixture. As the analyte travels through the column aided by high-pressure pumping of the mobile phase, each component is retained to different extents by the solid packing material. The components that are strongly retained by the stationary phase move slowly with the flow of the mobile phase. On the other hand, those that are weakly held by the stationary phase travel rapidly. This difference in retentions of components is the exploited phenomenon for separation in HPLC, as well as in other chromatographic techniques.

In theoretical terms, the retention of a component on a column can be expressed by its retention time (t_R), retention volume ($V_R = t_R F$, where F is the flow rate) or the capacity ratio (k'), which is directly related to its equilibrium distribution constant (K) in the stationary-mobile phase system. The capacity ratio is defined by:

$$k' = A_s/A_m \tag{2.4}$$

where A_s and A_m denote the amount of the compound in the stationary and the mobile phase, respectively. Let V_s and V_m be the volumes of the respective phases; then

$$k' = C_s V_s/C_m V_m = KV_s/V_m \tag{2.5}$$

V_m is commonly written as V_0 and represents the dead volume in the column, which does not contribute to the separation. Consequently, the net retention volume, V_n, can be written as $V_n = V_R - V_0$, and since $K = V_n/V_s$, combination with equation (2.5) gives:

$$k'=(V_R-V_0)/V_0 \quad (2.6)$$

This expression permits the determination of the capacity factor from the chromatogram. The chromatographic separation of two components (1 and 2) depends on the separation factor (α) of a column. It can be written as $k_2'/k_1' = K_2/K_1 = \alpha$. From Equation (2.6), α can be formulated as :

$$\alpha=(V_{R2}-V_0)/(V_{R1}-V_0) \quad (2.7)$$

Thus the separation factor is simply the ratio of the net retention volumes of the two components. If the dead volume V_0, has been determined, the separation factor is easily calculated from Equation (2.7) [28].

2.5.2. Separation of Enantiomers by chiral HPLC

Enantiomers are known to behave chemically and physically in an identical manner. Therefore, it would be impossible to separate enantiomers by ordinary HPLC as they would be retained by the stationary phase to the same extent. However, enantioselective separation could be possible when separation system used in HPLC is dissymmetric. This can be ensured in two ways. In one way, the mobile phase is chiral but the stationary phase is not. In the other way, the reverse situation is the case. That is, the stationary phase is chiral but the mobile phase is non-chiral. The latter method is quite easy to perform and for this purpose, HPLC columns packed with chiral stationary phases are available on the market. The chiral stationary phase could also be synthesized on-site. However, this takes a good deal of time and requires experience [29].

The stereochemical resolution of enantiomers on chiral stationary phases is accomplished through the formation of transient diastereomeric complexes between the analyte enantiomers and the stationary phase, which has an optically pure chiral selector incorporated into its structure. The energy differences between these temporary diastereomeric complexes are responsible for the different extents with which the enantiomers are retained.

There are many different types of chiral stationary phases available to the modern chemist. Some of them are based on synthetic or natural polymers and are totally and intrinsically chiral. Others consist of chiral selectors of low molecular weight which are bound to a hard, incompressible matrix, usually silica [28].

Among the natural polymers that are used in chiral column construction for HPLC, polysaccharides, especially cellulose and its derivatives, have been found to exhibit an excellent ability of chiral recognition. Cellulose consists of conformationally stable cyclic sugar residues connected through glycosidic linkages. Therefore, cellulose and its derivatives possess a certain degree of rigidity and assume extended helical structures (Figure 2.5). This rigid and regular structure of the cellulose derivatives must be responsible for their excellent ability of stereochemical recognition [30].

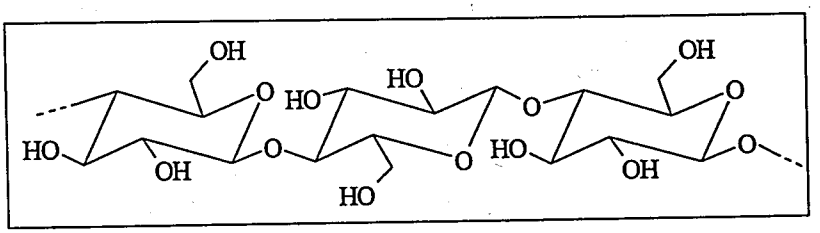


Figure 2.5. The chemical structure of cellulose

In this project, the chiral stationary phase that have been used for chromatographic enantioseparation is a cellulose derivative named as cellulose tris-(3,5-dimethyl)phenyl carbamate (Figure 2.6). The mechanism of chiral recognition by cellulose carbamates has not been clarified yet, but it is believed that the chiral attractive interaction results from the urethane linkages.

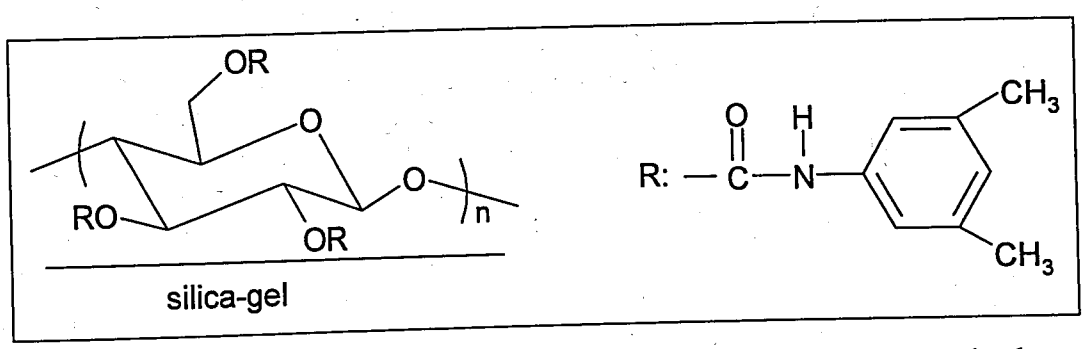


Figure 2.6. Cellulose tris(3,5-dimethylphenyl) carbamate on a 5 μm silica-gel substrate

2.6. Determination of the Kinetic and Thermodynamic Constants of the Internal Rotation Process for 5,5-Dimethyl-1-(*o*-aryl)-2-thiobarbituric acids

The interconversion of the either enantiomer of 5,5-dimethyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid and of 5,5-dimethyl-1-(*o*-bromophenyl)-2-thiobarbituric acid to its counterpart may be achieved by thermal means. At a sufficiently high temperature, the hindered free rotation in one of the enantiomers, separated from the other by liquid chromatography, gains speed and after a certain amount of time enough for half the molecules to overcome the free energy of activation elapses, an equilibrium mixture, comprising equal compositions of M and P conformations, is yielded. The process follows reversible first-order kinetics and the relevant activation barrier at a constant temperature can be estimated by keeping track of the concentration of the uninterconverted enantiomer in certain time intervals up to the point when the equilibrium composition has been reached and by subsequently carrying out the kinetic calculations for a reversible first-order process, the theory of which is given below:

The reversible reaction M⇌P is first order in both the forward (f) and the reverse direction (r), so that $r_f = k_f[M]$ and $r_r = k_r[P]$. If $(d[M]/dt)_f$ denotes the rate of change of [M] due to forward reaction, then $-(d[M]/dt)_f = r_f = k_f[M]$. The rate of formation of [M] by the reverse reaction is $(d[M]/dt)_r = r_r = k_r[P]$. Then,

$$(d[M]/dt) = -k_f[M] + k_r[P] \tag{2.8}$$

We have $\Delta[P] = -\Delta[M]$, so $[P] - [P]_0 = -([M] - [M]_0)$. Substitution of $[P] = [P]_0 + [M]_0 - [M]$ into Equation (2.8) gives

$$d[M]/dt = k_r[P]_0 + k_r[M]_0 - (k_f + k_r)[M] \tag{2.9}$$

At equilibrium, the rates of the forward and reverse reactions become equal, the concentration of each species being constant, thus d[M]/dt is 0. Let $[M]_{eq}$ be the equilibrium concentration of M. Setting d[M]/dt=0 and $[M]=[M]_{eq}$ in Equation (2.9), we get

$$k_r[P]_0 + k_r[M]_0 = (k_f + k_r)\,[M]_{eq} \qquad (2.10)$$

The use of Equation (2.10) in Equation (2.9) gives $d[M]/dt = (k_f + k_r)\,([M]_{eq} - [M])$. Using the identity $\int (x+s)^{-1}dx = \ln(x+s)$ to integrate this equation, we get,

$$\ln ([M]-[M]_{eq}/[M]_0-[M]_{eq}) = -(k_f + k_r)t \qquad (2.11)$$

Since $k_f = k_r$ for the racemization of enantiomers, Equation (2.11) could be written as Equation (2.12) for the racemization of enantiomers [31].

$$\ln ([M]-[M]_{eq}/\,[M]_0-[M]_{eq}) = -2kt \qquad (2.12)$$

By using Equation (2.12), a plot of $\ln ([M]-[M]_{eq}/\,[M]_0-[M]_{eq})$ versus time gives a straight line, the slope being equal to $-2k$. Having determined k, the free energy of activation can be calculated using the Eyring Equation (2.13),

$$\Delta G^{\#} = RT\ln(k_b\,.T/\,k\,.h) \qquad (2.13)$$

where R= 8.3143 J/mol.K, T= temperature (Kelvin) at which the interconversion takes place, k_b (Boltzmann constant) = $1.3805.10^{-23}$ J/K, h (Planck constant)=$6.6256.10^{-34}$ J.s, k=the rate constant for the racemization reaction.

In this study, the required separation of enantiomers for compound **2**, could not be achieved by liquid chromatography on the optically active sorbent, cellulose tris(3,5-dimethylphenyl) carbamate. Therefore, only the rotational barrier for compound **3**, the enantiomers of which were separated efficiently on cellulose tris(3,5-dimethylphenyl) carbamate, has been determined by thermal racemization.

3. ORGANIC SYNTHESES

3.1. Synthesis of 5,5-Dimethyl-1-(*o*-aryl)-2-thiobarbituric Acids

3.1.1. General Procedure

The 5,5-dimethyl-1-(*o*-aryl)-2-thiobarbituric acids were synthesized by the reaction of the corresponding *N*-(*o*-aryl)thiourea and 2,2-dimethylmalonic acid in acetylchloride (Figure 3.1).

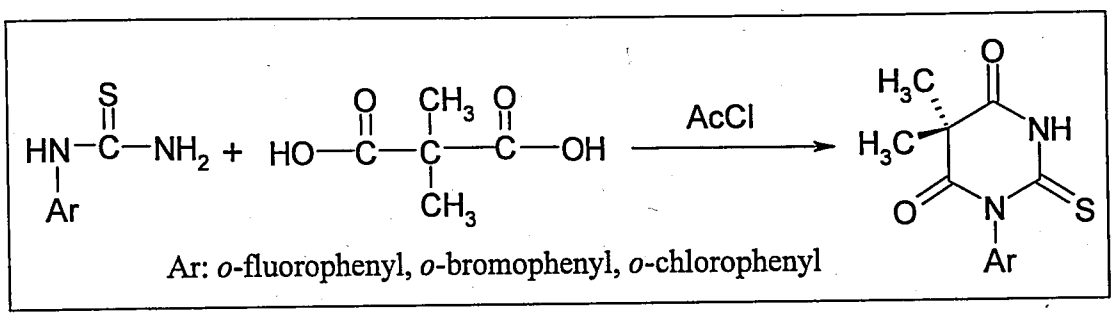


Figure 3.1. Synthesis of 5,5-dimethyl-1-(*o*-aryl)-2-thiobarbituric acids

In a 100 ml round bottom flask, fitted with a reflux condenser and a magnetic stirrer, the appropriate *N*-(*o*-aryl)thiourea and 2,2-dimethylmalonic acid were combined and large excess amount of acetylchloride was added. The resulting mixture was heated at reflux for 24 hours. At the end of this period, the reaction was stopped and the flask was let to cool a bit. Then, the mixture was treated with ice cubes in small pieces, and cautiously, up to the point when the resulting effervescence stops. After that, the mixture was concentrated by evaporating the solvent in a water bath and the precipitated crystals were collected by filtration. The crude product, thus obtained, was purified by successive recrystallizations from ethanol. The yields were not optimized.

3.1.1.1. 5,5-Dimethyl-1-(*o*-fluorophenyl)-2-thiobarbituric Acid. This compound had been synthesized by Oğuz *et al.* [18], for the first time. During the present study, it was synthesized for the purpose of conversion to the oxo analogue.

Starting materials:

o-Fluorophenylthiourea: 1.70 g (0.01 mole)

2,2-Dimethylmalonic acid: 1.32 g (0.01 mole)

The compound was synthesized according to the general procedure. At the end of the 24 hours of reflux period, a dark red colored solution was obtained. After successive recrystallizations from ethanol, 5,5-dimethyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid was obtained as yellow powder and identified by its ^{1}H NMR spectrum.

Yield: 0.73 g, 27.4 %

Melting point: 148°C

Spectral data:

^{1}H NMR (400 MHz) data (ppm):

Solvent: $CDCl_3$

Diastereotopic methyl protons at C-5: 1.67, 1.71 (s)

Aromatic protons: 7.17-7.49 (m)

-NH proton: 9.28 (broad s)

3.1.1.2. 5,5-Dimethyl-1-(*o*-bromophenyl)-2-thiobarbituric Acid. This compound was synthesized in this study, for the first time.

Starting materials:

o-Bromophenylthiourea: 1.88 g (0.008 mole)

2,2-Dimethylmalonic acid: 1.07 g (0.008 mole)

The compound was synthesized according to the general procedure. At the end of the 24 hours of reflux period, a dark red colored solution was obtained. After recrystallization from ethanol, 5,5-dimethyl-1-(*o*-bromophenyl)-2-thiobarbituric acid was obtained as brown powder and identified by its ^{1}H NMR and ^{13}C NMR spectrum.

Yield: 0.20 g, 7.51 %

Melting point: 226°C

Spectral data:

^{1}H NMR (400 MHz) data (ppm):

Solvent: $CDCl_3$

Diastereotopic methyl protons at C-5: 1.68 (s), 1.76 (s)

Aromatic protons: 7.23-7.46 (m)

-NH proton: 9.03 (broad s)

^{13}C NMR (400 MHz) data (ppm):

Solvent: $CDCl_3$

Diastereotopic methyl carbons: 23.43, 26.29

Carbonyl carbons of the heterocyclic ring: 169.58, 170.69

C-5 carbon of the heterocyclic ring: 48.69

Thiocarbonyl carbon of the heterocyclic ring: 177.11

Aromatic carbons: 122.88-136.93

IR data:

$\overline{\nu}$ of N-H stretching: 3254 cm^{-1}

$\overline{\nu}$ of N-C=O stretching:1733, 1699 cm^{-1}

$\overline{\nu}$ of C-N stretching: 1333 cm^{-1}

$\overline{\nu}$ of C=S stretching: 1211 cm^{-1}

3.1.1.3. 5,5-Dimethyl-1-(*o*-chlorophenyl)-2-thiobarbituric Acid. This compound had been synthesized by Oğuz *et al.* [18], for the first time. During the present study, it was synthesized for the purpose of conversion to the oxo analogue.

Starting materials:

o-Chlorophenylthiourea: 4.08 g (0.022 mole)

2,2-Dimethylmalonic acid: 2.90 g (0.022 mole)

The compound was synthesized according to the general procedure. At the end of the 24 hours of reflux period, a dark red colored solution was obtained. During treatment with ice cubes, the color turned from clear dark red to clear orange and when the effervescence had stopped, precipitation occured with every new addition of ice cube. At this point, treatment with ice cold water was terminated. After recrystallization from ethanol, 5,5-

dimethyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid was obtained as light yellow powder and identified by its ^{1}H NMR spectrum.

Yield: 1.237g, 20 %

Melting point: 218°C

Spectral data:

^{1}H NMR (400 MHz) data (ppm):

Solvent: $CDCl_3$

Diastereotopic methyl protons at C-5: 1.67 (s), 1.74 (s)

Aromatic protons: 7.21-7.54 (m)

-NH proton: 9.07 (broad s)

3.2. The Conversion of 1-(*o*-Aryl)-2-thiobarbituric Acids to Their Corresponding Oxo Analogues

3.2.1. Synthesis of 5,5-Dimethyl-1-(*o*-fluorophenyl)barbituric Acid

5,5-dimethyl-1-(*o*-fluorophenyl)barbituric acid was synthesized by the reaction of 5,5-dimethyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid with bromine in 90 per cent acetic acid (Figure 3.2).

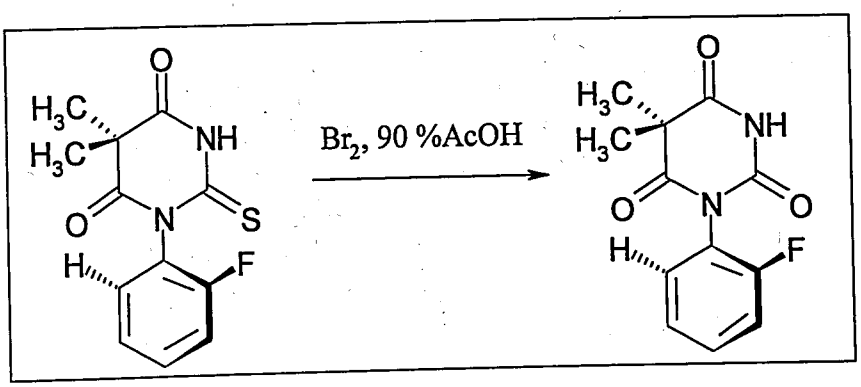


Figure 3.2. Conversion reaction of 5,5-dimethyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid

The procedure carried out for converting the thiocarbonyl in 5,5-dimethyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid to a carbonyl group so as to produce 5,5-dimethyl-1-(*o*-fluorophenyl)barbituric acid, has been successfully applied by Karataş and Doğan [17] to the synthesis of *N*-(*o*-aryl)thiazolidinediones from the corresponding rhodanines. In this study, the following steps were taken during the conversion of 5,5-dimethyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid to its oxo analogue.

In a 25 ml round bottom flask, fitted with a reflux condenser and a magnetic stirrer, 0.27 g 5,5-dimethyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid (1 mmol) was dissolved by 2.5 ml, 90 % acetic acid solution. Then, 0.32 g bromine (2 mmol) was added and the reaction mixture was kept under reflux, for eight hours. At the end of this period, a clear orange colored solution of crude product was obtained. The mixture was treated with charcoal and then filtered. The charcoal on the filter paper was washed several times with small portions of ice-cold diethyl ether and the washings were combined with the filtrate. Acetic acid and ether were evaporated away in water bath. The residue was dissolved in chloroform and the chloroform insoluble part was filtered off. The soluble part was in light yellow color. After evaporating the chloroform away from the soluble part, in a water bath, the residue was recrystallized from ethanol and then identified by its ^{1}H NMR spectrum. It has been observed that 84.4 % of the thiobarbituric acid was converted to the barbituric acid. However, it was not possible to separate the 5,5-dimethyl-1-(*o*-fluorophenyl)barbituric acid from the thiobarbiturate precursor, which were present together in the final product.

Relative per cent amount in the final product: 84.4% (oxo), 15.6% (thioxo)

Spectral data:

^{1}H NMR (400 MHz) data (ppm):

Solvent: $CDCl_3$

Diastereotopic methyl protons at C-5: 1.59 (s), 1.62 (s)(of oxo and thioxo)

Aromatic protons: 7.12-7.43 (m; the range of oxo and thioxo superimposed)

-NH proton: 7.92 (broad s; -NH of the oxo), 9.02 (broad s; -NH of the thioxo)

^{13}C NMR (400 MHz) data (ppm):

Solvent: $CDCl_3$

Diastereotopic methyl carbons: 23.40 and 25.68 (of thioxo), 23.80, 25.97 (of oxo)

Carbonyl carbons of the heterocyclic ring of the thioxo: 169.41 and 171.03
Carbonyl carbons of the heterocyclic ring of the oxo:148.52, 171.67, 172.34
C-5 carbon of the heterocyclic ring: 48.30 (of oxo), 48.83 (of thioxo)
Aromatic carbons: 116.72-131.59 (the range of oxo and thioxo superimposed)

3.2.2. Synthesis of 5,5-Dimethyl-1-(*o*-chlorophenyl)barbituric Acid

For the synthesis of 5,5-dimethyl-1-(*o*-chlorophenyl)barbituric acid, an attempt to convert the thiocarbonyl in 5,5-dimethyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid to a carbonyl group was made by performing the method of Karataş and Doğan. [17]. However, in all of the trials, where different conditions were maintained, a mixture of the desired product and the unreacted parent compound was obtained.The product mixtures were identified by their ^{1}H NMR spectra. The details of each trial are given below.The results of this synthesis will be more elaborately discussed in section 4.5.

3.2.2.1. Trial 1 In a 100 ml three necked flask, fitted with a reflux condenser and a magnetic stirrer, 0.26 g 5,5-dimethyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid (1 mmol) was dissolved by 30 ml, 90 % acetic acid solution. Then, 0.32 g bromine (2 mmol) was added and the reaction mixture was kept under reflux, for eight hours. Then, the mixture was treated with charcoal and then filtered. The charcoal on the filter paper was washed several times with small portions of ice-cold diethyl ether and the washings were combined with the filtrate. Acetic acid and ether were evaporated away in water bath. The residue was dissolved in chloroform and the chloroform insoluble part was filtered off. After vacuum distillation of the chloroform from the soluble part, the residue was recrystallized from ethanol, and yellow powder resembling to the original 5,5-dimethyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid was obtained. The ^{1}H NMR spectrum showed no appreciable amount of conversion.

3.2.2.2. Trial 2 In this synthesis, only the amount of 90 per cent acetic acid solvent was changed to 3 ml. The reaction mixture was kept under reflux, for eight hours. After vacuum distillation of the chloroform from the soluble part, the residue, which was orange colored, was recrystallized from ethanol. The filtrate of recrystallization was light yellow colored. The obtained crystals were observed to be a mixture of the desired oxo and the

unreacted thioxo compound, from the ^{1}H NMR spectrum (see section 4.5). The yield was calculated to be 30.48 %.

3.2.2.3. Trial 3 In a 50 ml three necked flask, fitted with a reflux condenser and a magnetic stirrer, 0.28 g 5,5-dimethyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid (1 mmol) was dissolved by 3 ml, 90 % acetic acid solution. Then, 0.32 g bromine (2 mmol) was added and the reaction mixture was kept under reflux, for 16 hours. Then, the mixture was treated with charcoal and then filtered. The charcoal on the filter paper was washed several times with small portions of ice-cold diethyl ether and the washings were combined with the filtrate. Acetic acid and ether were distilled off by vacuum. The residue was dissolved in chloroform and the chloroform insoluble part was filtered off. After vacuum distillation of the chloroform from the soluble part, the residue was recrystallized from ethanol. The obtained crystals were observed to be a mixture of the desired oxo and the unreacted thioxo compound, from the ^{1}H NMR spectrum (see section 4.5). The yield was calculated to be 36.74 %.

3.2.2.4. Trial 4 In a 50 ml three necked flask, fitted with a reflux condenser and a magnetic stirrer, 0.28 g 5,5-dimethyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid (1 mmol) was dissolved by 5 ml, 90 % acetic acid solution. Then, 0.32 g bromine (2 mmol) was added and the reaction mixture was kept under reflux, for 24 hours. Then, the mixture was treated with charcoal and then filtered. The charcoal on the filter paper was washed several times with small portions of ice-cold diethyl ether and the washings were combined with the filtrate. Acetic acid and ether were evaporated away in water bath. The residue, which was a mass of needle like crystals, was dissolved in chloroform and the chloroform insoluble part was filtered off. After evaporation of the chloroform from the soluble part, the residue, which was light yellow colored, was recrystallized from ethanol. The obtained crystals were observed to be a mixture of the desired oxo and the unreacted thioxo compound with very tiny percentage of the desired oxo, from the ^{1}H NMR spectrum (see section 4.5). The yield was calculated to be 9.92 %.

3.2.2.5. Trial 5 In a 25 ml round bottom flask, fitted with a reflux condenser and a magnetic stirrer, 0.20 g 5,5-dimethyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid (0.7 mmol) was dissolved by 3 ml, 90 % acetic acid solution. Then, 0.32 g bromine (2 mmol) was

added and the reaction mixture was kept under reflux, for 19 hours. Then, the mixture was treated with charcoal and then filtered. The charcoal on the filter paper was washed several times with small portions of ice-cold diethyl ether and the washings were combined with the filtrate. Acetic acid and ether were evaporated away in water bath. The residue was dissolved in chloroform and the chloroform insoluble part was filtered off. After vacuum distillation of the chloroform from the soluble part, the residue was recrystallized from ethanol. The obtained crystals were observed to be a mixture of the desired oxo and the unreacted thioxo compound, from the ^{1}H NMR spectrum (see section 4.5). The yield was calculated to be 36.74 %.

3.3. Synthesis of *N*-*o*-arylthioureas

3.3.1. General Procedure

N-*o*-Arylthioureas, which were used as starting materials to synthesize 5,5-dimethyl-1-(*o*-aryl)-2-thiobarbituric acids, were prepared according to the reaction shown in Figure 3.3.

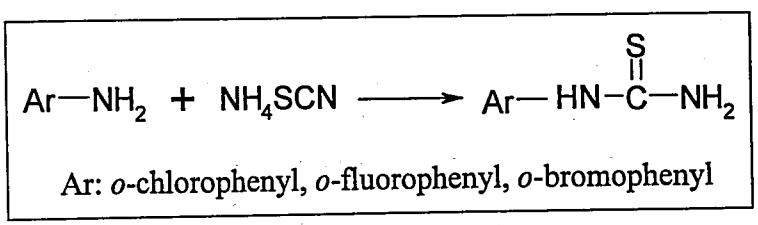


Figure 3.3. Synthesis of *N*-*o*-Arylthioureas

In 300 ml warm water, 0.1 moles of suitable *ortho*-substituted aniline was suspended and concentrated hydrochloric acid was added to break up the suspension, and hence, to obtain a clear solution. The resulting solution was transferred into a 500 ml porcelain evaporating dish, 0.1 moles of ammoniumthiocyanate was added, and the mixture was heated on steam bath for one hour. The liquid was allowed to cool and set aside at room temperature for one hour. After that it was again put in steam bath and evaporated slowly to dryness. The resulting residue was finely crushed and again 300 ml water was added and evaporated slowly on the steam bath. After evaporation had gone to completion, the

obtained residual powder was finally heated on the steam bath for 4-5 hours. The final mixture of crude *N*-*o*-arylthiourea and ammonium chloride was powderized finely, and suspended in 300 ml of water. The mixture was warmed slowly to 70°C with mechanical stirring, then allowed to cool down to 35°C, and the solid was filtered with suction.

The crude product was dissolved in 30 ml of absolute ethanol and then the solution was filtered as hot. The filtrate was diluted with 50 ml of hot benzene and 10 ml of light petroleum ether. The mass of *N*-*o*-arylthiourea crystals was deposited out from the filtrate gradually on cooling and standing and it was separated by vacuum filtration. At the last step, the obtained crystals were washed with light petroleum ether and dried.

3.3.1.1. *N*-*o*-Chlorophenylthiourea. *N*-*o*-Chlorophenylthiourea was prepared according to the general procedure.

Starting materials:

o-Chloroaniline: 38.3 g (0.3 mole)

HCl: 27.5 ml (0.3 mole)

Ammoniumthiocyanate: 25 g (0.3 mole)

Yield: 16.78 g, 30 %

Melting point: 132-135°C

Spectral data:

IR data:

$\bar{\nu}$ of N-H stretching: 3267 cm^{-1}, 3170 cm^{-1}

$\bar{\nu}$ of C-H out-of-plane bending of *o*-disubstituted benzene ring: 754 cm^{-1}

3.3.1.2. *N*-*o*-Fluorophenylthiourea. *N*-*o*-Fluorophenylthiourea was prepared according to the general procedure.

Starting materials:

o-Fluoroaniline: 11.1 g (0.1 mole)

HCl: 9.12 ml (0.1 mole)

Ammoniumthiocyanate: 8.30 g (0.1 mole)

Yield: 5.70 g, 33.53 %

Melting point: 138-142°C

Spectral data:

IR data:

$\bar{\nu}$ of N-H stretching: 3258 cm^{-1}, 3155 cm^{-1}

$\bar{\nu}$ of C-H out-of-plane bending of mono substituted benzene ring: 764 cm^{-1}

3.3.1.3. *N-o*-Bromophenylthiourea. *N-o*-Bromophenylthiourea was prepared by carrying out the general procedure up to the step where heating to 70°C and cooling down to 35°C is performed.

Starting materials:

o-Bromoaniline: 17.68 g (0.1 mole)

HCl: 9.12 ml (0.1 mole)

Ammoniumthiocyanate: 8.3 g (0.1 mole)

During every evaporation step, a large mass of pasty suspension was observed in evaporating dish. However, the contents of the evaporating dish were allowed to dry thoroughly. At the end, a light brown colored mass of solid material, which was firmly 'glued' to the evaporating dish, was obtained. Although this material was very hard to crush, we achieved to obtain yellow-brown colored powder, eventually. During the final suspending in 300 ml water, this powder again yielded a pasty suspension. Nevertheless, it was heated up to 70°C, and maybe a little more, and then let to cool down to room temperature. After setting aside for a long time, maybe overnight, white crystals were observed to deposit at the bottom of the beaker. These were filtered and, without further treatment, given to NMR. The resulting spectrum indicated the presence of pure *N-o*-bromophenylthiourea, which was white colored. The filtrate of the last filtration was again heated up to 70°C and cooled to room temperature, and after a time additional crops of *N-o*-bromophenylthiourea were observed to deposit. In the end, both fractions were combined.

Yield: 1.88 g, 8.14 %

Melting point: 118°C

Spectral data:

^{1}H NMR (400 MHz) data:

Solvent: $CDCl_3$

-NH proton:7.83 (b)

Aromatic protons:7.18-7.69 (m)

-NH_2 protons: 6.14 (broad s)

3.3.1.4. *N-o*-Iodophenylthiourea. We also aimed at synthesizing 5,5-dimethyl-1-(*o*-iodophenyl)-2-thiobarbituric acid and for this purpose tried to synthesize *N-o*-Iodophenylthiourea according to the general procedure and using the following amounts of reactants. However, the synthesis did not yield the desired thiourea, presumably due to the steric effect induced by the large size of the iodine substituent.

Starting materials:

o-Iodoaniline: 21.3 g (0.1 mole)

HCl: 9.12 ml (0.1 mole)

Ammoniumthiocyanate: 8.3 g (0.1 mole)

3.4. Apparatus

^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian 400 NMR spectrometer.

Melting points were recorded using a Bibby Stuart Scientific melting point apparatus.

The IR analyses were performed on a Perkin Elmer 1600 FTIR using KBr windows.

Liquid chromatography analyses were performed using Chiralcel OD-H (Daicel Ltd., particle size: 5μm, column size: 250 x 4.6 mm) column, Lab Alliance Series III pump and Waters Assoc. UV absorbance detector.

3.5. List of Chemicals

Table 3.1. Reagents

Name	Formula	Supplier	% Purity
o-F Aniline	$FC_6H_4NH_2$	Merck	>98
o-Cl Aniline	$ClC_6H_4NH_2$	Merck	>98
o-Br Aniline	$BrC_6H_4NH_2$	Merck	>98
Ammoniumthiocyanate	NH_4SCN	Merck	98,5
Hydrochloric acid	HCl	Merck	37
Acetylhloride	CH_3COCl	Merck	>98
Dimethylmalonic acid	$C(CH_3)_2(COOH)_2$	Fluka	98
Bromine	Br_2	Merck	>99
Acetic acid	CH_3COOH	Merck	99-100
Hexane for HPLC	$CH_3(CH_2)_4CH_3$	J.T. Baker	95
Ethanol for HPLC	CH_3CH_2OH	J.T. Baker	99,5
Ethanol	C_2H_5OH	Akkimya	99,5
Chloroform	$CHCl_3$	Akkimya	99,5

4. RESULTS AND DISCUSSION

4.1. ^{1}H NMR Spectra of the Compounds

The barriers to rotation around the C-N axis of 5,5-dimethyl-1-(*o*-aryl)barbituric and -2-thiobarbituric acids are expected to be high enough for the observation of their non-planar configurations on the NMR time scale. The presence of the *ortho* substituents in the phenyl groups makes the molecules dissymmetric, and hence, the stable non-planar configurations become chiral. The absence of a stereogenic unit other than the chiral C-N axis in the molecules means that the compounds **1**, **2**, and **3** are enantiomeric, and thus exist in two enantiomeric forms of M and P (Figure 1.8). Enantiomers can not be differentiated by NMR spectroscopy, in achiral media. That is to say, the protons of M and P enantiomers give only one single spectrum in an achiral solvent, regardless of the relative ratio with which they are mixed. Consequently, one may not be able to know whether a certain molecule is chiral or not. However, if a diastereotopic group is present in a chiral molecule, evidence of chirality can be obtained from the ^{1}H NMR spectrum. Diastereotopic groups can be described as chemically equivalent but magnetically non-equivalent nuclei. The protons of the methyl groups attached to the C-5 of the heterocyclic ring of compounds **1**, **2**, and **3** are of such nature, and as a result, they have appeared as two different singlets in the ^{1}H NMR spectra (Figures 4.2, 4.3, and 4.4). Hence, the molecules studied in this project are chiral. Although compounds **1** and **2** have been obtained as a mixture of their thio analogues, there appears only one pair of diastereotopic methyl signals in the ^{1}H NMR spectra, which have been recorded in $CDCl_3$. This shows that the signals of C-5 methyl protons of both compound **1** and its thio analogue, and of both compound **2** and its thio analogue, coincide in the ^{1}H NMR specrum. The ^{1}H NMR spectral assignments for the compouns are given in Table 4.1.

In the ^{1}H NMR spectra of compounds **1** and **2**, which are mixed with the corresponding thio analogues, there are two broad singlets located at around 8 ppm and 9 ppm. Based on these signals, the presence of the thio and the oxo compound in the mixtures were detected. The singlets at around 9 ppm can be assigned to the N-H protons of the thioxo precursors, namely the 5,5-dimethyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid

and 5,5-dimethyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid, based on a comparison with the previous assignments on the pure 1-(*o*-aryl)-2-thiobarbituric acids, reported by Oğuz and Doğan [18]. Therefore, the singlets at around 8 ppm should belong to the N-H protons of the 5,5-dimethyl-1-(*o*-fluorophenyl)barbituric acid and 5,5-dimethyl-1-(*o*-chlorophenyl)barbituric acid derivatives. The broad singlet at around 9 ppm, observed in the ^{1}H NMR spectrum of compound **3**, can be assigned to the N-H proton of the heterocyclic ring.

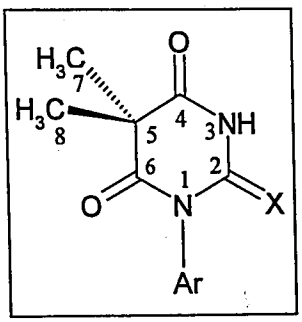


Figure 4.1. The general structure of the 5,5-dimethyl-1-(*o*-aryl)barbituric and -2-thiobarbituric acids, with the numbering of the atoms on the heterocyclic ring

Table 4.1. 400 MHz ^{1}H NMR spectral data for the 5,5-dimethyl-1-(*o*-aryl)barbituric and -2-thiobarbituric acids in $CDCl_3$

Compound No	X	Ar	δ (ppm) of 5-CH_3	δ (ppm), aromatic H	δ (ppm), 3-NH
1	O	*o*-fluorophenyl	1.58[a] and 1.62[a]	7.02-7.43[c]	7.92[d] 9.02[e]
2	O	*o*-chlorophenyl	1.68[a] and 1.75[a]	7.23-7.56[c]	7.81[d] 8.98[e]
3	S	*o*-bromophenyl	1.68[b] and 1.76[b]	7.23-7.46	9.03

[a] : superimposed diastereotopic protons of the barbiturate and the thio analogue.

[b] : diastereotopic methyl protons

[c] : superimposed aromatic proton range of the barbiturate and the thio analogue.

[d] : NH protons of the barbiturate. [e] : NH protons of the thio analogue.

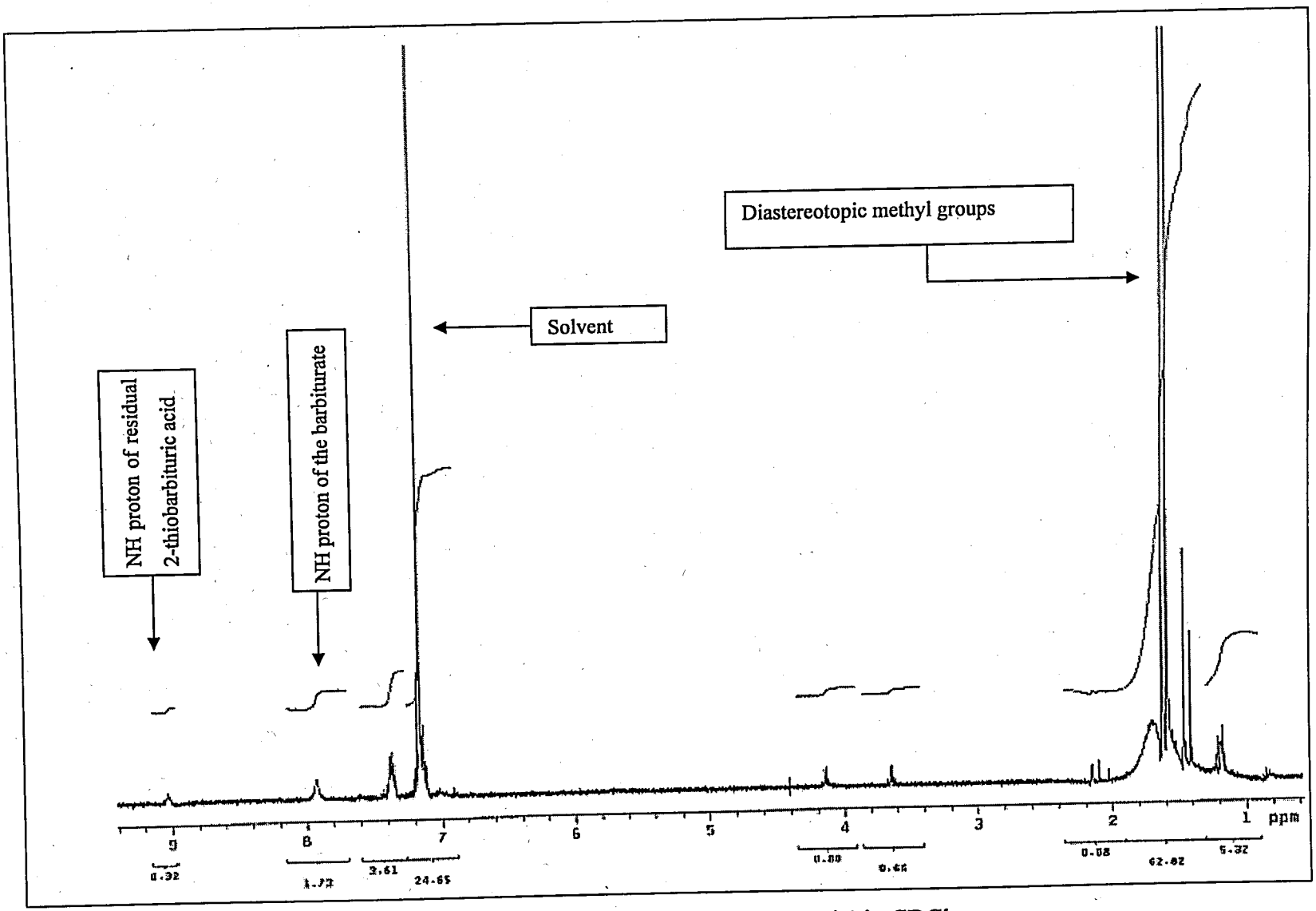


Figure 4.2. ^{1}H NMR spectrum of compound **1** in $CDCl_3$

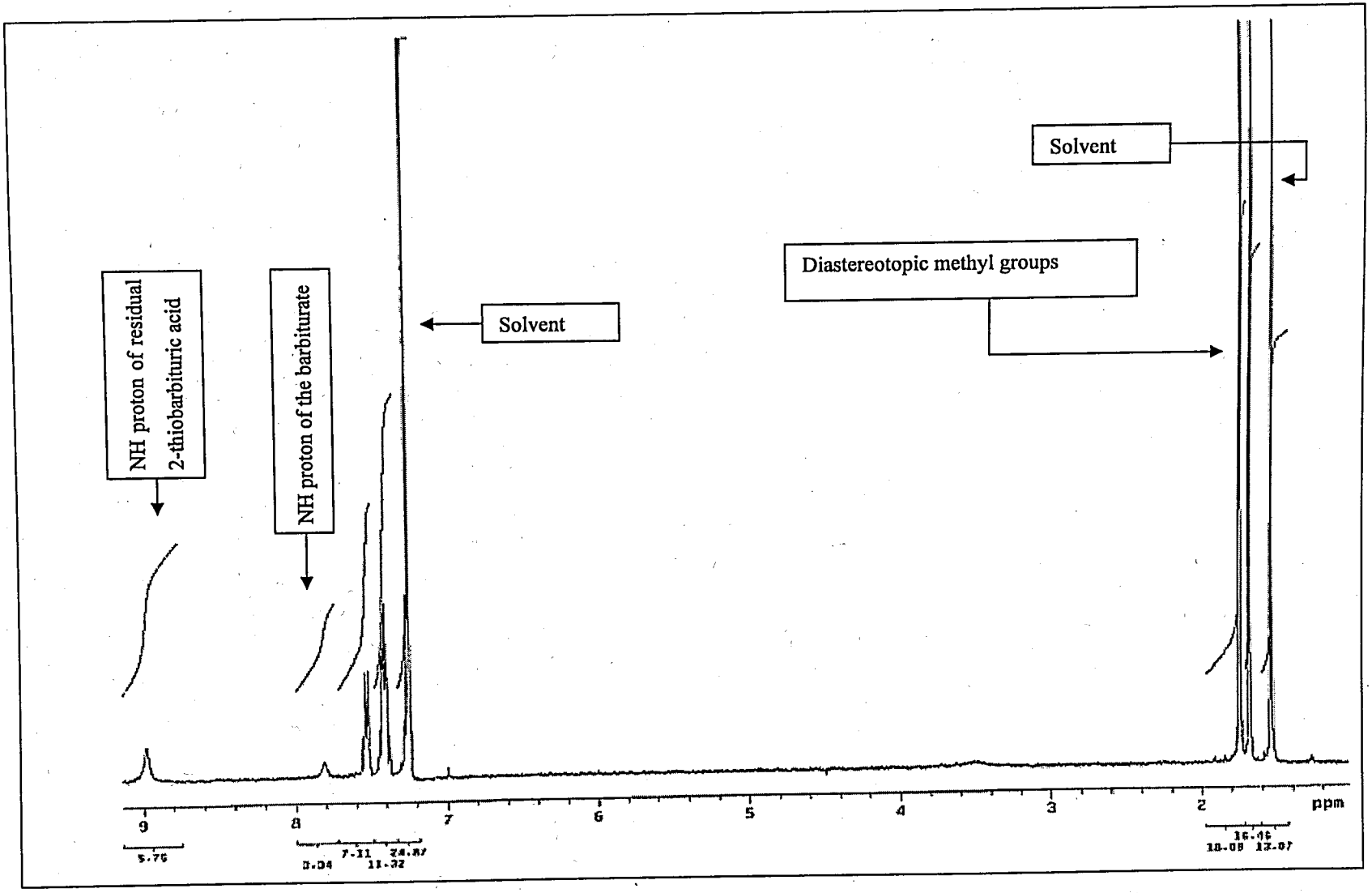


Figure 4.3. ^{1}H NMR spectrum of compound **2** in $CDCl_3$

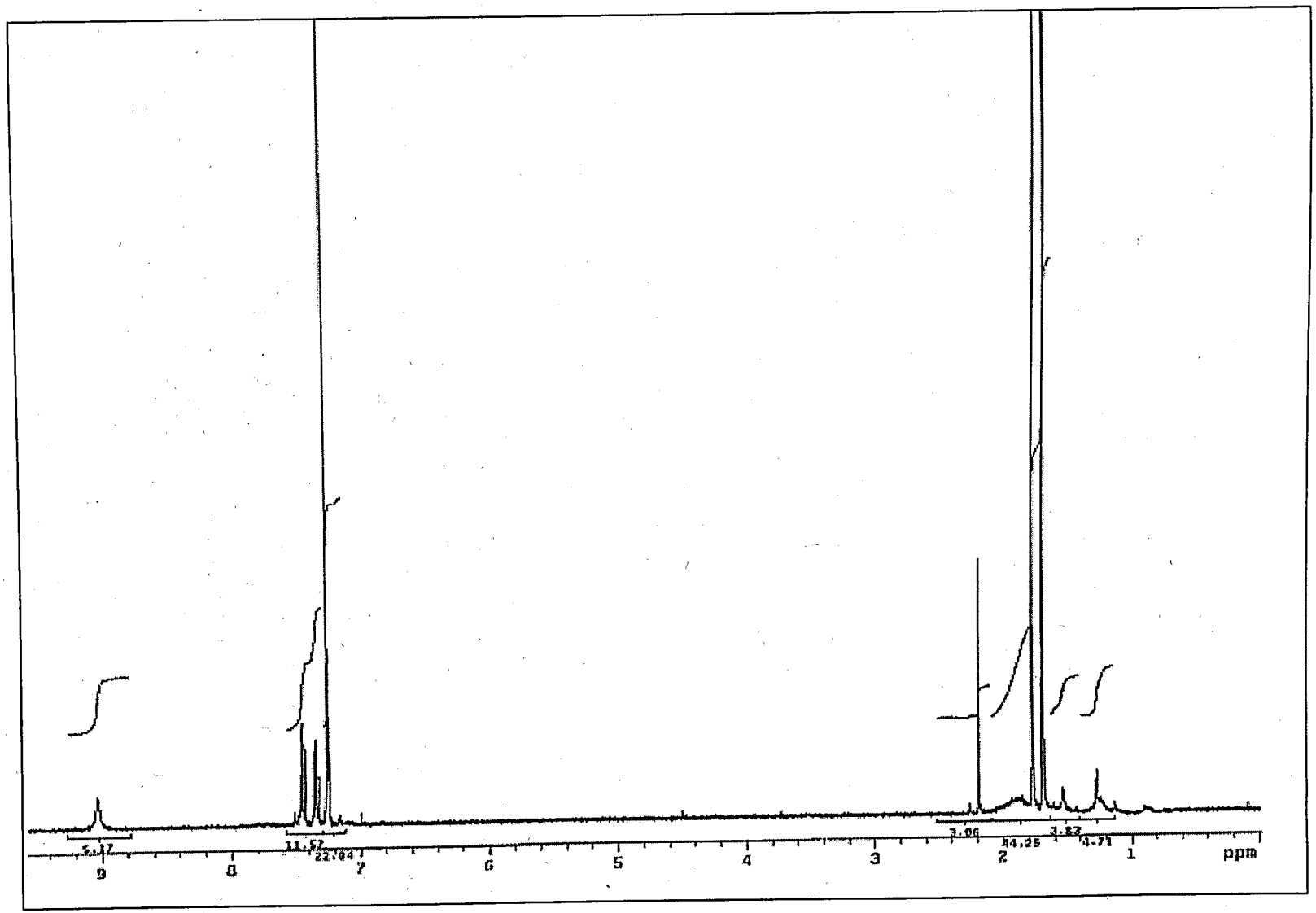


Figure 4.4. ^{1}H NMR spectrum of compound **3** in $CDCl_3$

4.2. ^{13}C NMR Spectra of the Compounds

Further evidence for the chirality of the compounds studied in this project can be obtained from the examination of their ^{13}C NMR spectra. The methyl group carbons attached to the C-5 of the heterocyclic ring are diastereotopic and they give two different signals in the ^{13}C NMR spectra (Figures 4.5, 4.6, and 4.7). Since, compounds **1** and **2** are in mixed form with their thio analogues, there appear two distinguishable, though very closely located, signals for each carbon atom, except for the C-2. For the C-2, there appear two distantly located signals at around 148 ppm and 178 ppm, which belongs to the carbonyl carbon of the oxo compound and to the thiocarbonyl carbon of the thioxo analogue, respectively. The ^{13}C NMR spectral assignments of the compounds **1-3** are given in Table 4.2. The chemical shift values for the carbons of compounds **1** and **2** have been distinguished from that of the corresponding thio precursors, based on the previously reported ^{13}C NMR data of the pure thiobarbiturates [18].

Table 4.2. ^{13}C NMR chemical shifts (ppm) of the 5,5-dimethyl-1-(*o*-aryl)barbituric and -2-thiobarbituric acids in $CDCl_3$

Carbon No[a]	Compound **1**	Compound **2**	Compound **3**
2	148.52	*	177.12
4, 6	171.67, 172.34	169.45, *	169.57, 170.69
5	48.30	48.07	48.69
7, 8	23.80, 25.97	23.62, 26.42	23.43, 26.29
aromatic	116.72-131.59	128.09-135.26	122.88-136.93

[a]: see Figure 4.1 for the numbering

*: very weak; it could not be assigned.

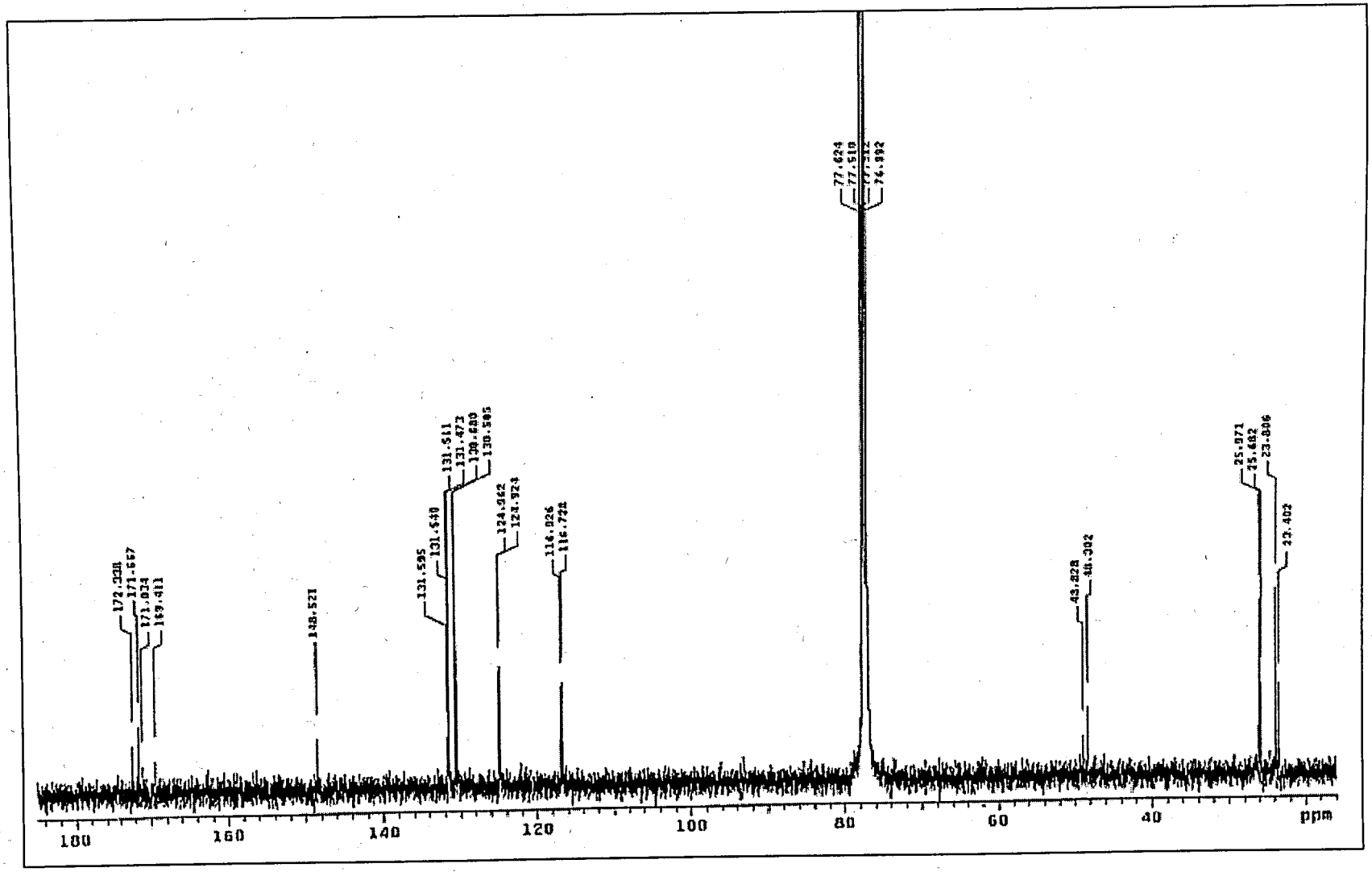


Figure 4.5. ^{13}C NMR spectrum of compound 1 in $CDCl_3$

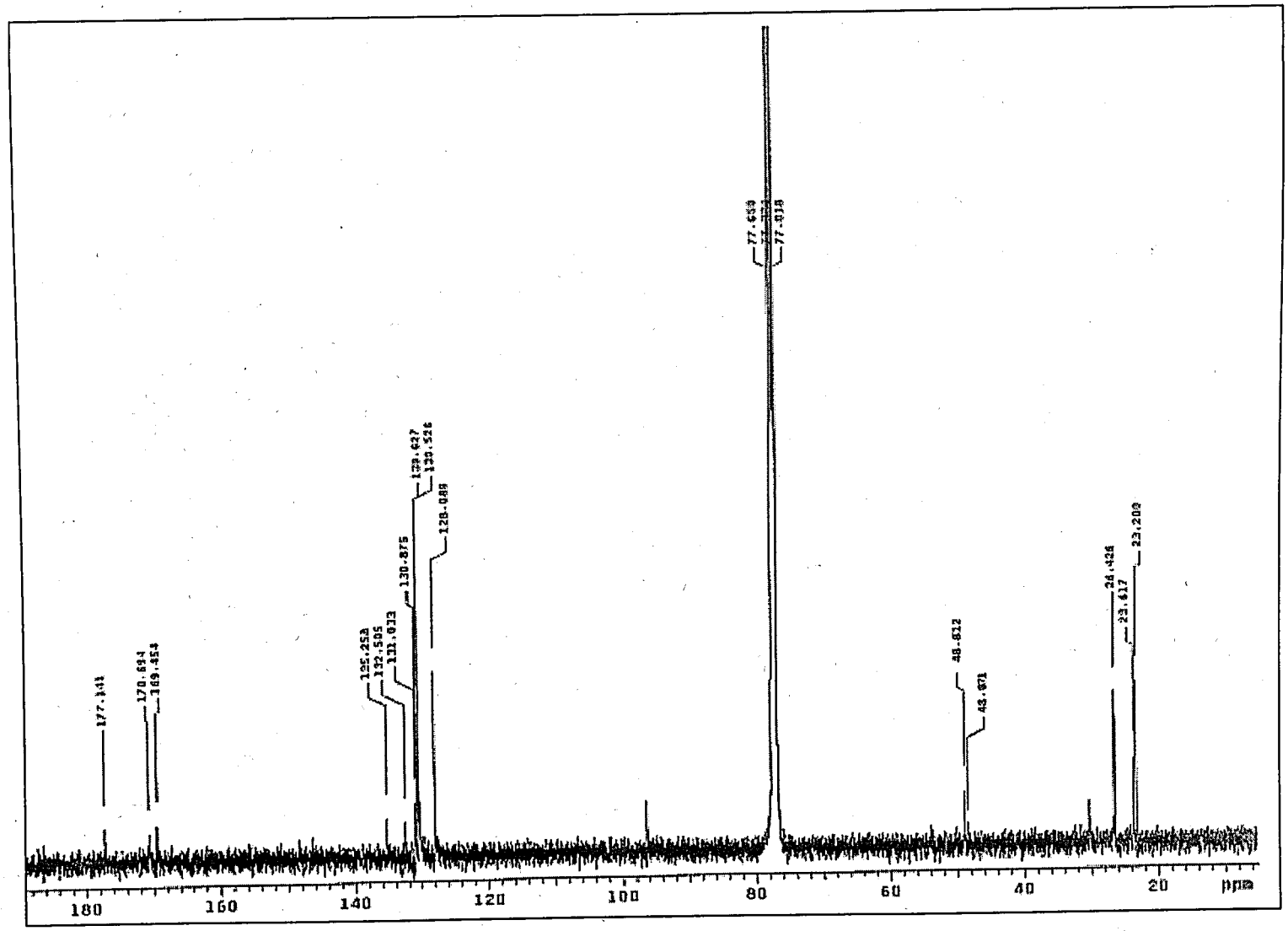


Figure 4.6. ^{13}C NMR spectrum of compound **2** in $CDCl_3$

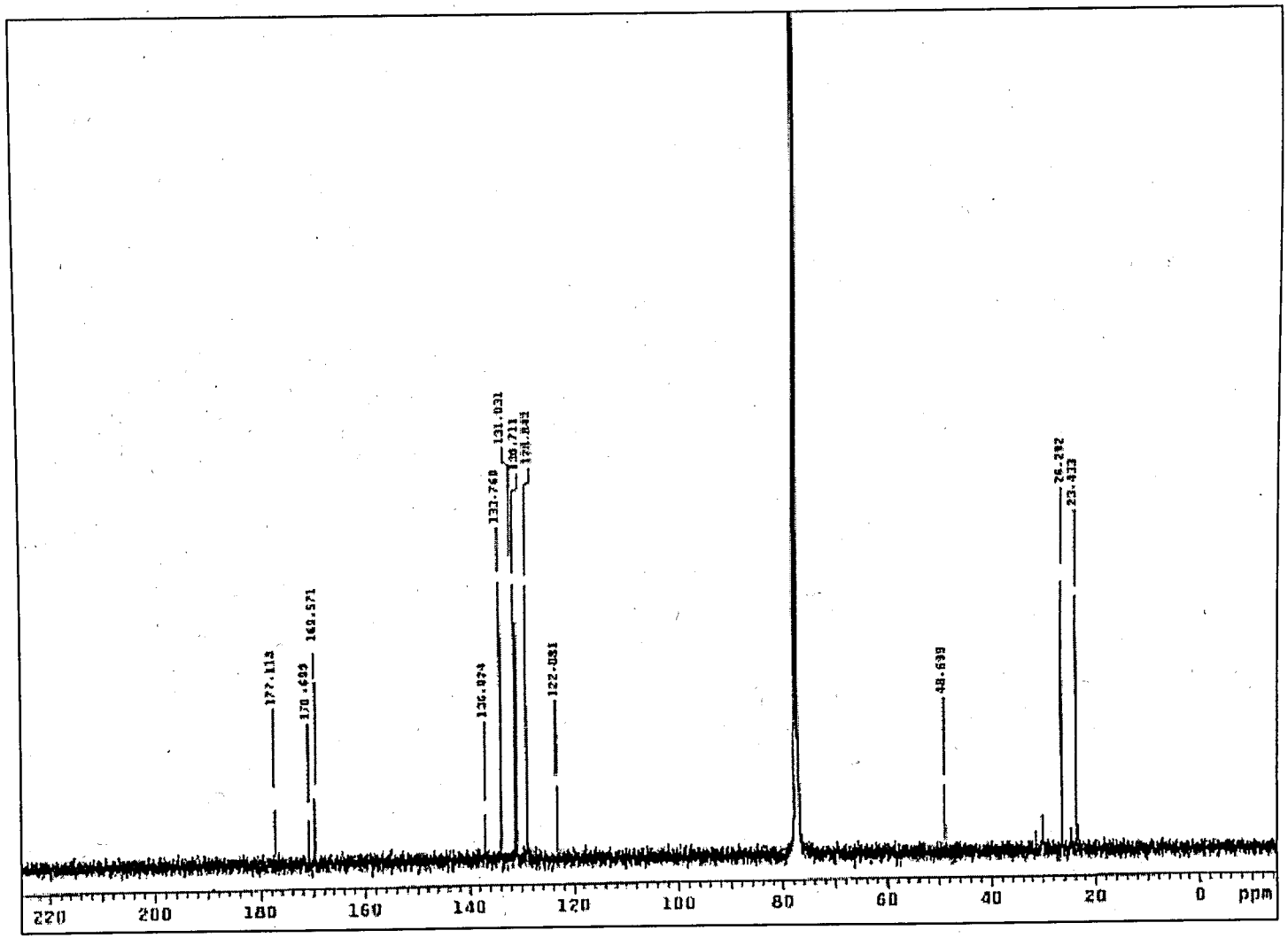


Figure 4.7. ^{13}C NMR spectrum of compound **3** in $CDCl_3$

4.3. Determination of the Activation Barrier for Hindered Rotation in 5,5-Dimethyl-1-(*o*-fluorophenyl)barbituric Acid by DNMR

Due to the small size of the fluorine atom and the absence of a thiocarbonyl group, the rotational barrier in 5,5-dimethyl-1-(*o*-fluorophenyl)barbituric acid was expected to be low enough to be determined by temperature-dependent NMR. Thus, the energy of activation for rotation of this compound has been determined by using its temperature-dependent ^{1}H NMR spectrum in DMSO-d_6 (Figure 4.8). The coalescence of the diastereotopic C-5 methyl peaks occured at 82°C (355 K). Although compound **1** is present in the form of a mixture with its thiobarbiturate precursor, this does not violate the calculations. The calculations performed for determination of the rotational barrier of this compound are as follows:

The rate constant at coalescence temperature, T_c :

$k_c = 2.22\ \Delta\nu$

$k_c = 2.22(7.332\ \text{Hz}) = 16.28\ \text{sec}^{-1}$

Therefore, $\Delta G^{\#}$:

$\Delta G^{\#} = 19.1\ T_c\ [10.32 + \log (T_c / k_c)].10^{-3}$

$\Delta G^{\#} = 19.1\ (355)[10.32+\log (355/16.28)].10^{-3}$

$\Delta G^{\#} = 79.05 \pm 0.02$ kJ/mol

Compound **2** and **3**, due to the size of the chlorine and the bromine substituent, respectively, were expected to have such high rotational barriers that coalescence would not be reached at ordinary NMR probe temperatures. Therefore, their rotational barriers were not to be determined by using DNMR.

The value of rotational barrier for compound **1** is in good agreement with the trend that the activation barriers of 1-(*o*-aryl)barbituric acids are lower than their thio analogues, which has been previously postulated by Oğuz and Doğan [18]. The rotational barrier for the thio analogue of compound **1**, namely, 5,5-dimethyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid, has been reported to be 93.1 kJ/mol [18]. The rotational barrier for the compound **1**, itself, has been determined to be 79.05 kJ/mol, in this study. This latter value

being lower than the former one, once again, verifies that the suggestion made by Kashima *et al.* [11], which states that the greater single bond character of the thiocarbonyl group causes lower energy barriers for the heterocycles with the thioamide group than their oxo analogues, is not applicable to the series of heterocyclic 1-(*o*-aryl)barbituric and -2-thiobarbituric acid compounds we work with.

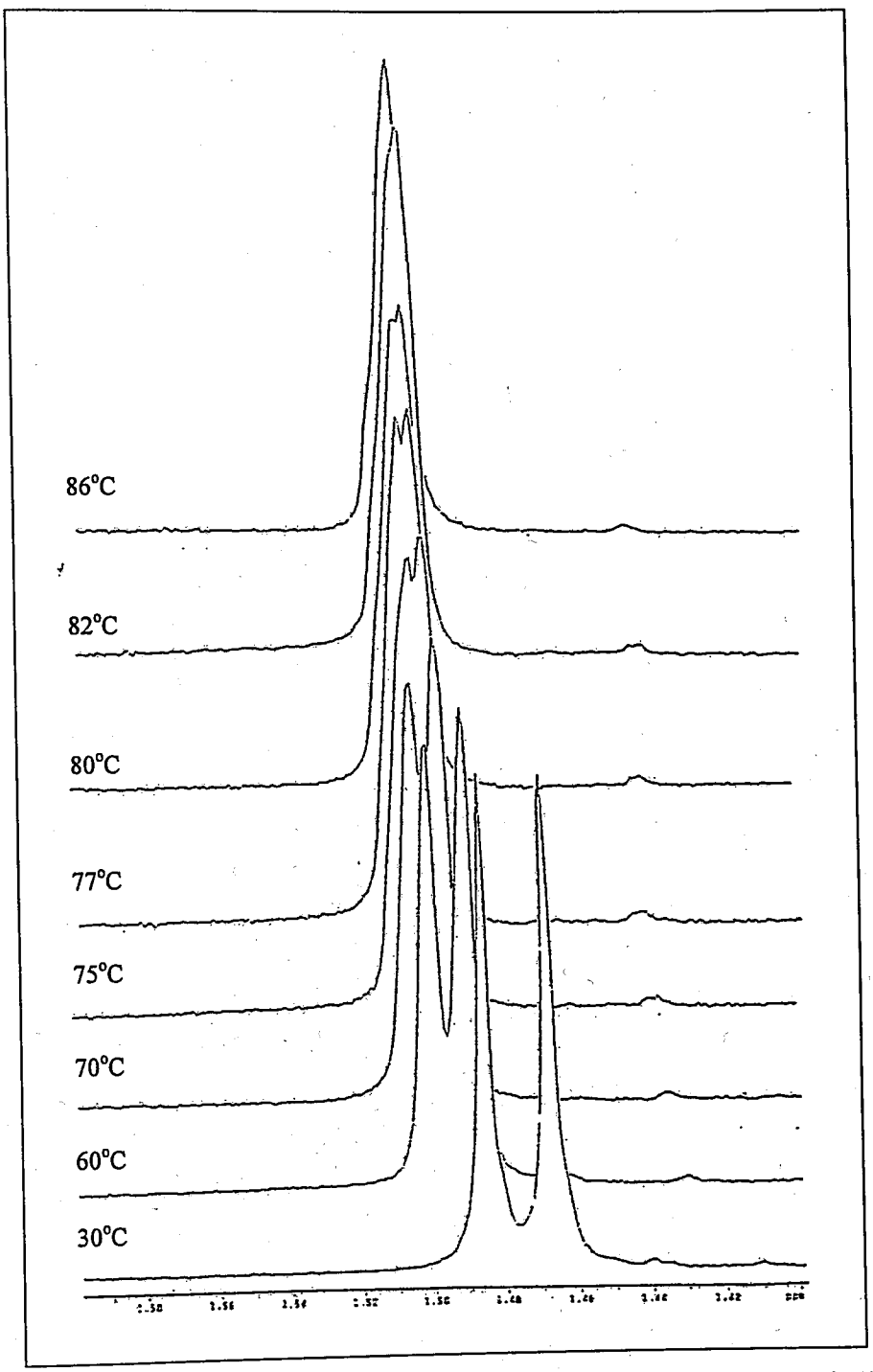


Figure 4.8. The temperature dependent ^{1}H NMR spectrum of compound **1** in DMSO-d_6

4.4. Determination of the Rotational Barriers by Thermal Racemization

The rotational barriers for compounds **2** and **3** have been attempted to be determined by thermal racemization method. This method requires the separation of the racemic mixtures into pure enantiomers, in advance. Although compound **2** had been obtained as a mixture of its thio precursor from the conversion reaction, it was thought that, by enantioselective liquid chromatography, separation of both of the racemic 5,5-dimethyl-1-(*o*-chlorophenyl)barbituric acid and its thio analogue into their corresponding enantiomers could be possible, and thus, the thermal racemization experiment could be carried out with the pure enantiomeric fraction of compound **2**. However, in all of the trials, separation could not be achieved due to the same extent with which the enantiomers of compound **2** and its thio analogue were retained by cellulose tris(3,5-dimethylphenyl) carbamate.

The thermal racemization experiment for compound **3** was performed successfully in the following way: The enantiomers of the compound were separated by liquid chromatography on cellulose tris(3,5-dimethylphenyl) carbamate. The eluent used was 60:40 hexane:ethanol mixture. The column temperature was 7°C and the flow rate was 0.4 ml/min. Having collected 0.4 mg of the first eluted enantiomer of compound **3**, the solid was dissolved in 200 µl of absolute ethanol, and 20 µl of the solution was injected into the column so as to record the initial concentration of the enantiomer. The solution left was kept in a constant temperature oil bath at 72°C. From then on, at certain time intervals, 20 µl of the sample was taken from the solution after the ongoing racemization, for that time interval, had been quenched by immersing the flask into an ice bath, and it was injected to the column. This process was repeated until no more sample was left, and at the end, the relative per cent composition of each enantiomer (Table 4.3), which had been recorded by following the integral ratio of the peaks (Figure 4.9), was used in a reversible first-order kinetical calculation. The relative per cent composition values of the first eluted enantiomer, which had undergone the racemization experiment, were substituted into Equation 2.12 and a graph of the obtained results versus time (Figure 4.10) was plotted.

$$\ln ([M]-[M]_{eq}/\ [M]_0-[M]_{eq})=-2kt \qquad (2.12)$$

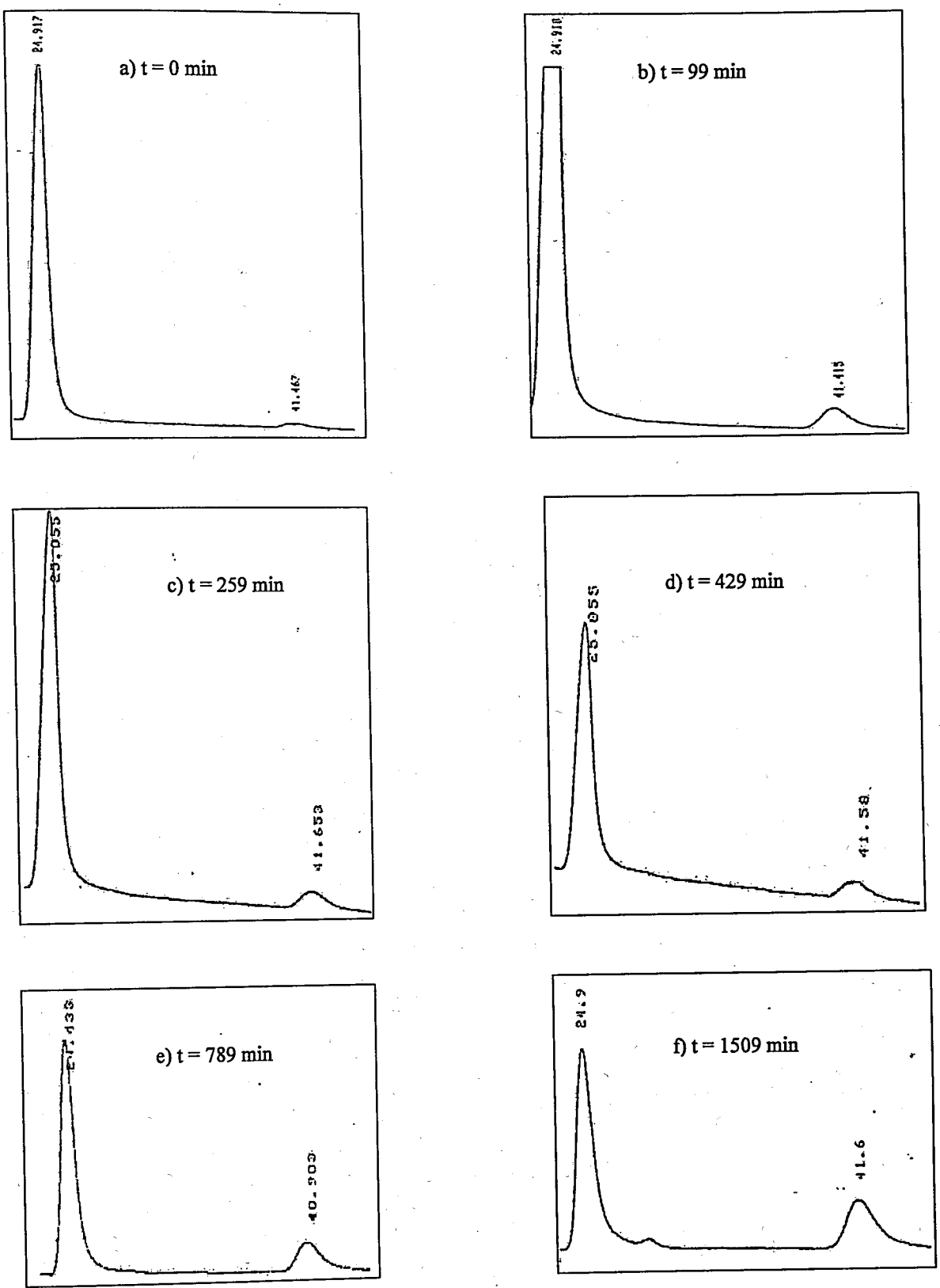


Figure 4.9. The course of thermal racemization of compound **3**, as followed by HPLC

Table 4.3. The change in the relative per cent composition of each enantiomer of compound **3** versus time, followed by HPLC[a] at 345 K

Time (min)	The relative per cent composition of **A**[b], (%)	The relative per cent composition of **B**[c], (%)
0	97.728	2.272
99	95.092	4.908
259	92.398	7.602
429	89.668	10.332
789	81.997	8.003
1509	72.168	27.832

[a] : Column: Cellulose carbamate OD-H, eluent: hexane:ethanol (60:40), flow rate = 0.4 ml/ min, k_A'=2.43, k_B'=4.74, α =1.95, t_A=24.9 min, t_B= 41.6 min, detection: UV at 240 nm

[b] : 'A' represents the first eluted enantiomer. Axial chirality has not been specified.

[c] : 'B' represents the second eluted enantiomer. Axial chirality has not been specified.

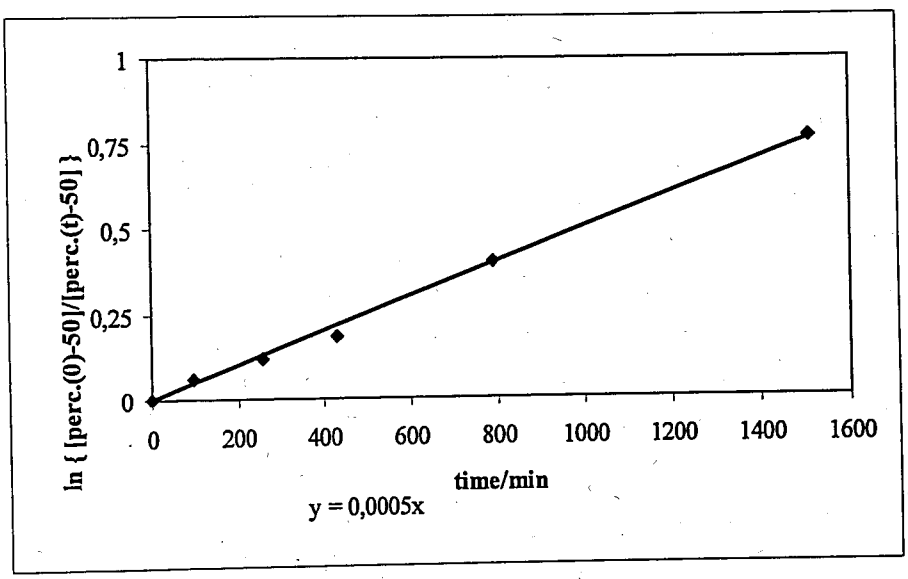


Figure 4.10. The plot of ln ($[M]-[M]_{eq}/ [M]_0-[M]_{eq}$) versus time at 345 K for **3**

From slope of the graph shown in Figure 4.10, the first-order rate constant, k was calculated to be 0.00025 min^{-1}. This value, which corresponds to $0.42.10^{-5}$ sec^{-1}, was substituted into Equation 2.13:

$$\Delta G^{\#} = RT\ln(k_b .T/ k .h) \qquad (2.13)$$

where R= 8.3143 J/mol.K, T= temperature (Kelvin) at which the interconversion takes place, k_b (Boltzmann constant) = $1.3805.10^{-23}$ J/K, h (Planck constant)=$6.6256.10^{-34}$ J.s, k=the rate constant for the racemization reaction.

After these calculations the rotational barrier for compound **3** was found to be 120.5±2.45 kJ/mol. When compared with the previously calculated barriers of 5,5-dimethyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid and of 5,5-dimethyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid derivatives, which are 93.1 kJ/mol and 115.8±0.3 kJ/mol [18], respectively, this value seems to be the largest of all. This is consistent with the expectation that the larger size of the bromine substituent in compound **3**, induces a higher steric effect at the rotational transition state as compared to that of the fluorine and chlorine, in 5,5-dimethyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid and 5,5-dimethyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid, respectively. When a graph of the barriers versus the van der Waals radii (Table 4.4) of the substituents is plotted for this series of compounds, a linearly increasing relationship is observed (Figure 4.11).

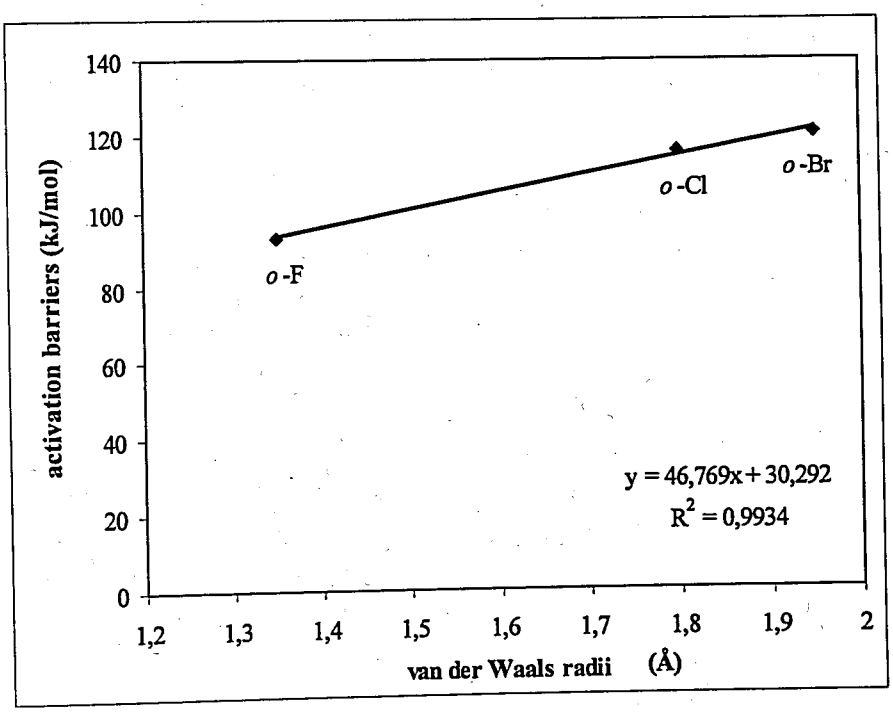


Figure 4.11. The plot of activation barriers versus van der Waals radii of the *o*-substituted halogens for the series of 5,5-dimethyl-1-(*o*-aryl)-2-thiobarbituric acid derivatives

Table 4.4. Van der Waals radii of the hydrogen atom and the halogen atoms [32]

Table 4.4. Van der Waals radii of the hydrogen atom and the halogen atoms

Atom	Van der Waals radii (Å)
H	1.2
F	1.35
Cl	1.80
Br	1.95
I	2,15

In the literature, existence of a linear relationship between the size of the *ortho*-halogen substituents and the rotational barriers has also been reported for 5,5-dimethyl-3-(*o*-aryl)-2,4-oxazolidinedione derivatives (Figure 1.6), by Demir Ordu and Doğan [19]. The rotational barriers for this series of compounds (Table 4.5) have been plotted against the van der Waals radii of the *ortho*-halogen substituents and the graph shown in Figure 4.12 has been obtained.

Table 4.5 The rotational barriers of *ortho*-halogen substituted 5,5-dimethyl-3-(*o*-aryl)-2,4-oxazolidinediones, as reported by Demir Ordu and Doğan

N-Aryl substituent[a]	$\Delta G^{\#}$(kJ/mol)
o-Fluorophenyl	57.33±0.05
o-Chlorophenyl	83.24±0.05
o-Bromophenyl	86.62±0.05
o-Iodophenyl	94.16±1.31
[a]: see Figure 1.6	

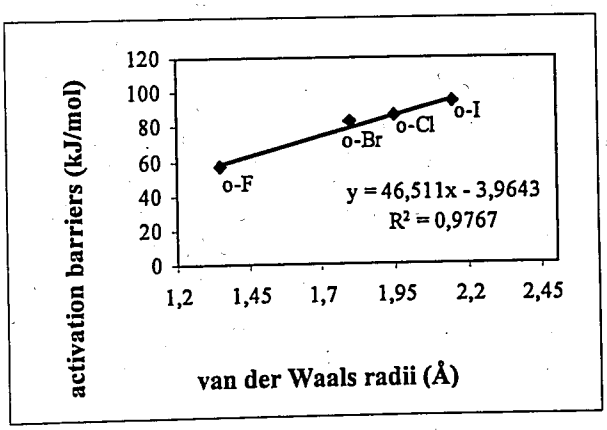


Figure 4.12. The plot of activation barriers versus van der Waals radii of the *o*-substituted halogens for the series of 5,5-dimethyl-3-(*o*-aryl)-2,4-oxazolidinediones

The slopes of these graphs, which are 46,769 and 46,511, are in perfect agreement with each other. This may indicate that the overall effect of *ortho*-halogen substituents in the rotational barriers around the C-N bond are pronounced to the same extent in *N*-aryl substituted heterocycles, regardless of the ring size of the heterocycle and the nature of the substituents neighboring to the *N*-aryl nitrogen on both sides. With this idea at hand, one can draw another graph using the slope and the value of rotational barrier of compound **1**, which have been calculated to be 79.05 kJ/mol. When the average of the two slopes is used along with this value of rotational barrier, the graph shown in Figure 4.13 is obtained:

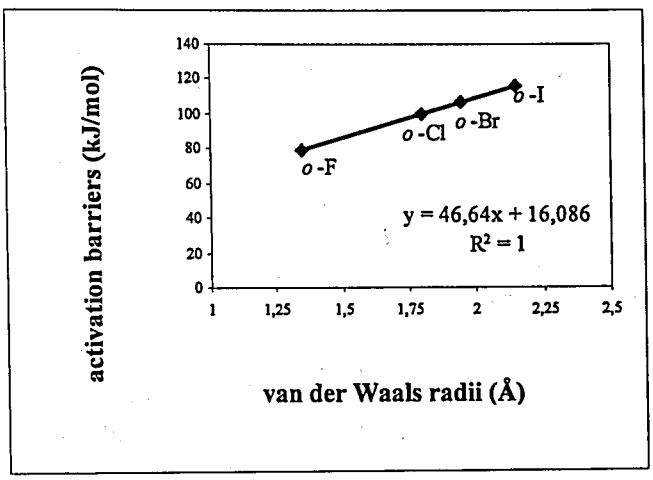
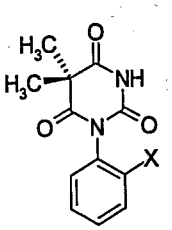


Figure 4.13. The predicted plot of activation barriers versus van der Waals radii of the *o*-substituted halogens for the series of 5,5-dimethyl-1-(*o*-aryl)barbituric acids

From this graph, the rotational barriers for the series of *ortho*-halogen substituted 5,5-dimethyl-1-(*o*-aryl)barbituric acids can be predicted to be the values shown in Table 4.6.

Table 4.6. The predicted rotational barriers for the series of *ortho*-halogen substituted 5,5-dimethyl-1-(*o*-aryl)barbituric acids



X	$\Delta G^{\#}$ (kJ/mol)
F	79.05*
Cl	100.0
Br	107.0
I	116.4

* : Experimentally determined

The previously determined [18] barrier value for the 5,5-dimethyl-1-(*o*-tolyl)-2-thiobarbituric acid, 115.8±0.3 kJ/mol and the value for the 5,5-dimethyl-3-(*o*-tolyl)-2,4-oxazolidinedione, 73.49±0.05 kJ/mol [19], do not fall on the line of their respective graphs of barriers versus van der Waals radii. This is due to the lower energy value for the *o*-tolyl derivative of oxazolidinedione than the *o*-chlorophenyl derivative and the similar energy value for the *o*-tolyl derivative of thiobarbituric acid and the *o*-chlorophenyl derivative. The barrier for the *o*-tolyl derivative being lower than that of the *o*-chlorophenyl derivative of oxazolidinedione, despite the larger van der Waals radius of the methyl group (2.0 Å), compared to the chlorine atom, suggests that the electrostatic repulsion between the lone pairs of carbonyl oxygen and chlorine atom can be expected to increase the free energy of the rotational transition state and thus to cause larger energy barrier than the *o*-tolyl derivative.

The energy value for the 5,5-dimethyl-1-(*o*-tolyl)barbituric acid, which has previously been determined as 102.8±0.3 kJ/mol [18], when compared to the predicted energy barrier for the 5,5-dimethyl-1-(*o*-chlorophenyl)barbituric acid, 100.0 kJ/mol (Table 4.6), is observed to be larger by about 2.8 kJ/mol. However, the fact that the predicted energy value for the *o*-bromophenyl derivative, 107 kJ/mol, is larger than the previously determined barrier value for the *o*-tolyl derivative despite the lower van der Waals radii for the bromine (Table 4.4.) than the methyl (2.0 Å), does not let the value for the *o*-tolyl derivative on the line of the hypothetical graph shown in Figure 4.13.

In conclusion, it can be suggested that the difference in the steric effects of the methyl and the halogen substituents in the rotational barriers for the cited compounds depends on the rotational transition state geometries. The tetrahedral geometry of the methyl group may allow an ease with which the two rings pass each other in the transition state and thus cause a lower barrier than the spherical chlorine atom.

4.5. The Conversion of Compounds 1 and 2 to Their Corresponding Oxo Analogues

The conversion reaction carried out for the synthesis of compound **1** and **2**, mechanistically, occurs in the way shown in Figure 4.14.

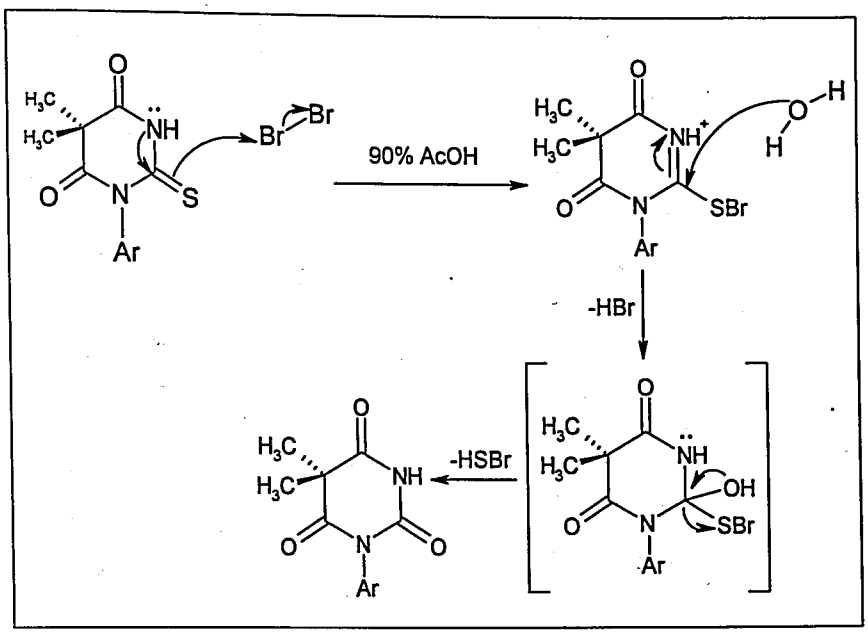


Figure 4.14. The mechanism of the conversion reaction

The final product obtained from the conversion reaction of 5,5-dimethyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid to its oxo analogue, compound **1**, was composed of a mixture of both the parent -2-thiobarbituric acid and the target compound in relative per cent amount of 15.6 and 84.4, respectively. This quite high amount of 84.4 per cent for the target compound in the final mixture was thought to be sufficient for our further investigations, and thus, the product was used as such.

Compound **2** was also obtained in mixed form with its thiobarbiturate precursor in all of the trials, where different conditions were maintained. The conditions for each trial are given in Table 4.7.

Table 4.7. The conditions of the trials for the conversion reaction of compound **2**

Trial No[a]	Thiobarbiturate amount (mmol)	Br_2 amount (mmol)	90% Acetic acid amount (ml)	Refluxing period (hours)
1	1.000	2.000	30	8
2	1.000	2.000	3	8
3	1.000	2.000	3	16
4	1.000	2.000	5	24
5	0.700	2.000	3	19

[a] : see section 3.2.2. for the details.

The relative percent composition of the final products obtained from all of these trials are given in Table 4.8.

Table 4.8. The per cent composition of the final products of the conversion trials for **2**

Trial No[a]	Relative amount[b] of **2** (%)	Relative amount[b] of the thiobarbiturate (%)
1	-	100
2	30.48	69.52
3	36.74	63.26
4	9.92	90.08
5	36.74	63.26

[a] : see section 3.2.2. for the details.

[b] : calculated from the integral ratio of NH peaks.

As seen from the Table 4.8 , no more than about 36 per cent of the desired product could be obtained. The fact that the first trial yielded no appreciable amount of the target compound may be due to the large amount of acetic acid used, in which case the bromine reagent concentration became very dilute. After rationalization of this fact, the remaining synthetic trials were performed using lesser amount of the solvent. Comparison between the yields of the second and the third trials suggested that increasing the duration of reflux increased the final composition of **2** by about six per cent. Therefore, in the next trial, the contents of the reaction vessel were kept refluxing for 24 hours. However, in this case, only about 10 per cent of the desired compound was present in the final mixture.This may be due to the possibility that during the addition of the bromine reagent, which was performed after the reactant had been observed to dissolve, or during the 24 hours of reflux period, some of the bromine perhaps escaped from the reaction vessel. If the latter situation occured, it may have been needed to refreshen the amount of bromine in the reaction vessel, from time to time. The last trial was performed with 0,7 mmol thiobarbiturate precursor, which was all of the obtained amount from the prior thiobarbiturate synthesis. The final relative composition of the last trial did not differ from that of the third trial. In this last trial, during the 19 hours of reflux period, from time to time, amounts of bromine and solvent were refreshened.

Depending on our trials, it can be concluded that for the conversion reaction of 5,5-dimethyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid to compound **2**, the best results are obtained by using about 3 ml of solvent and refluxing for about 16 hours. The better yield from the conversional synthesis of compound **1** as compared to that of the compound **2**, could be attributed to the less steric effect exerted by the smaller size of fluorine substituent with respect to the chlorine substituent. A parallel relationship between the thiobarbituric acid and the *N*-*o*-arylthiourea yields and the size of the *ortho*-halogen substituents has been observed (see section 3). In fact, the failed synthesis of the *N*-*o*-iodophenylthiourea is a result of the steric effect caused by the largest size of the iodine.

5. CONCLUSIONS

In this project, 5,5-dimethyl-1-(*o*-aryl)barbituric and 5,5-dimethyl-1-(*o*-aryl)-2-thiobarbituric acids (Figure1.7), where the hindered molecular rotation around the C-N single bond causes axial chirality, have been investigated. The novel compound of 5,5-dimethyl-1-(*o*-bromophenyl)-2-thiobarbituric acid has been synthesized by the reaction of *N*-*o*-bromophenylthiourea with dimethylmalonic acid. The compounds of 5,5-dimethyl-1-(*o*-fluorophenyl)barbituric acid and the 5,5-dimethyl-1-(*o*-chlorophenyl)barbituric acid have been partially synthesized by the direct conversion of the thiocarbonyl group in 5,5-dimethyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid and 5,5-dimethyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid ,respectively, into a carbonyl group, by treatment with bromine in 90 per cent acetic acid.

The racemization barriers in these compounds are affected by the size of the *ortho* aryl substituent and by the presence of carbonyl or thiocarbonyl moiety in the *N*-heterocycle. The barrier to rotation in 5,5-dimethyl-1-(*o*-fluorophenyl)barbituric has been determined to be 79.05 kJ/mol, by using temperature-dependent ^{1}H NMR spectra. The activation barrier for 5,5-dimethyl-1-(*o*-bromophenyl)-2-thiobarbituric acid, on the other hand, has been determined to be 120.5 kJ/mol, by thermal racemization experiment using HPLC with Chiralcel OD-H column. The rotational barrier for the *o*-fluoro barbituric acid derivative, 79.05 kJ/mol, is lower than the previously calculated value for its thio analogue [18], 93.1 kJ/mol. This shows that the suggestion made by Kashima *et al.* [11], which states that the greater single bond character of the thiocarbonyl group causes lower energy barriers for the heterocycles with the thioamide group than their oxo analogues is not applicable to the series of heterocyclic 1-(*o*-aryl)barbituric and -2-thiobarbituric acid compounds we work with.

The rotational barrier for 5,5-dimethyl-1-(*o*-bromophenyl)-2-thiobarbituric acid, which has been found to be 120.5 kJ/mol, has been compared to the previously calculated [18] barrier values of the *o*-fluorophenyl and the *o*-chlorophenyl derivatives in the same series and the barriers have been observed to increase linearly with the van der Waals radii of the *ortho*-halogen substituents. A similar pattern was observed for the *ortho*-halogen

substituted 5,5-dimethyl-3-(*o*-aryl)-2,4-oxazolidinedione derivatives, previously [19]. The graphs illustrating these relationships have been compared and the slopes have been observed to agree with each other, perfectly.Using this slope and the determined energy barrier value for the 5,5-dimethyl-1-(*o*-fluorophenyl)barbituric acid derivative, a hypothetical graph representing the relation between the barriers and the sizes for the series of *ortho*-halogen substituted 5,5-dimethyl-1-(*o*-aryl)barbituric acids have been obtained. From this graph the, yet uncalculated, barriers for the *o*-chloro, *o*-bromo, *o*-iodo derivatives have been predicted.

The conversion reactions yielded mixtures of the target compound and the thiobarbiturate precursor for both 5,5-dimethyl-1-(*o*-fluorophenyl)barbituric acid and 5,5-dimethyl-1-(*o*-chlorophenyl)barbituric acid. Due to the less steric effect exerted by the smaller size of the fluorine, the former one could be obtained in more relative amounts than the latter one.

The resolution of enantiomers of 5,5-dimethyl-1-(*o*-chlorophenyl)barbituric acid and of the corresponding thiobarbiturate precursor, which are mixed together, have been attempted on Chiralcel OD-H column and no separation of the the enantiomers of the 5,5-dimethyl-1-(*o*-chlorophenyl)barbituric acid from those of the thiobarbiturate precursor could be achieved. Therefore, thermal racemization could not be performed for 5,5-dimethyl-1-(*o*-chlorophenyl)barbituric acid.

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