CHAPTER III

RESULTS AND DISCUSSION

The objective of this study is to prepare enantiomerically pure or enriched 4,6-diamino-1,2-dihydro-1,3,5-triazines. There are several potential methods to achieve the goal including:

- Resolution by formation of diasteromeric salts followed by recrystallization
- Resolution by chromatography using chiral stationary or mobile phase
- Asymmetric synthesis and/or resolution by means of covalently attaching chiral auxiliary to the racemic mixture followed by separation of the diastereomeric mixture formed

Our attempts to resolve dihydrotriazines by each method will be discussed below.

3.1 Resolution by formation of diastereomeric salts followed by recrystallization

Resolution of the racemic acids or bases by using optically active bases or acids to form of diastereomeric salts is the one of the oldest method which is still commonly used.[30, 34-36] The standard technique for the resolution of a racemate involves addition of one chiral resolving agent to the racemate followed by recrystallization of the diastereomeric salts. The success of this method depends on the differential solubility and crystallization ability of the two diastereomeric salts. The suitable resolving agent must be chosen by trial and error and is one important factor to determine the success of the resolution. Good chiral resolving agent should be cheap, readily available in an optically pure state and easily recovered after the resolving operation.[27]

In the experiment, attempts have been made to separate the enantiomer of dihydrotriazine by formation of diastereomeric salts. In this method, the racemic dihydrotriazine free base was reacted with a suitable chiral resolving agent, which, in this case is an optically active acid. Subsequent separation of the mixture of

diasteromeric salts could be potentially achieved by fractional crystallization. Initially, (+)-camphorsulfonate salt of 1-(4′-chlorophenyl-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine **(ID 12)** was prepared by mixing equimolar amounts of the racemic dihydrotriazine free base with (+)-camphorsulfonic acid in ethanol. The product before recrystallization must be racemic, which was confirmed by ^{1}H NMR using (+)-camphorsulphonic acid as chiral solvating agent.* Thus while camphorsulfonate salt of racemic **(ID 12)** gave only one ^{1}H NMR signal of C_2-H as singlet at 5.89 ppm. Addition of excess (+)-camphorsulphnic acid split this C_2H into two peaks at 6.28 and 6.37 ppm in $CDCl_3$ (Figure 3.2). It should be noted that the splitting was not observed in more polar solvent such as DMSO while chiral Lanthanide shift reagents gave poor resolution. Disappointingly, repeated recrystallization from a variety of solvents including ethanol, water and chloroform gave the product with 1:1 ratio of both enantiomer, *ie*, no enrichment was observed according to ^{1}H NMR analysis as described above.

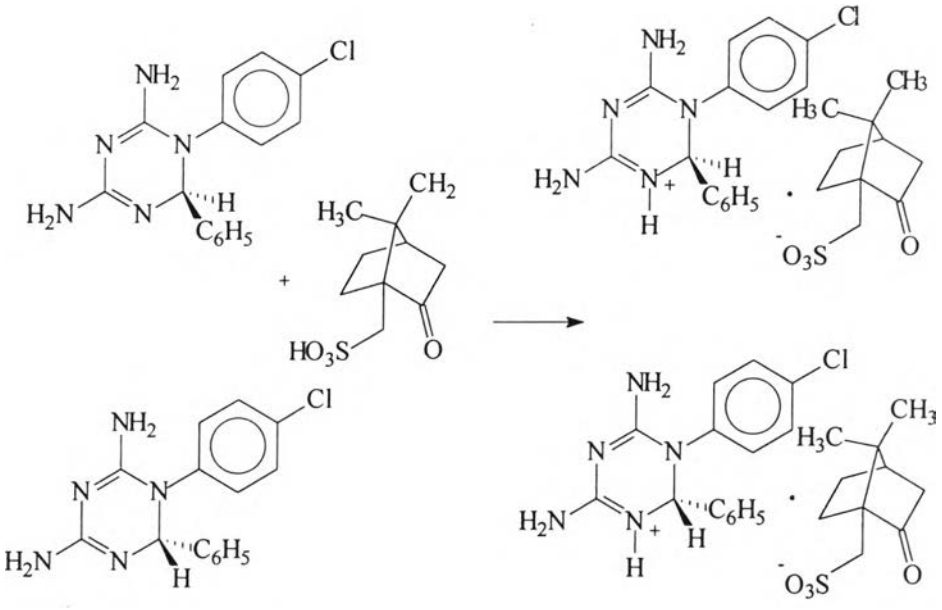


Figure 3.1 Diastereomeric-(+)-camphorsulfonate salt of 1-(4′-chlorophenyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine **(ID 12)**

*camphorsulfonic acid has been used in determination optical purity of optically active amino nitrile[37]

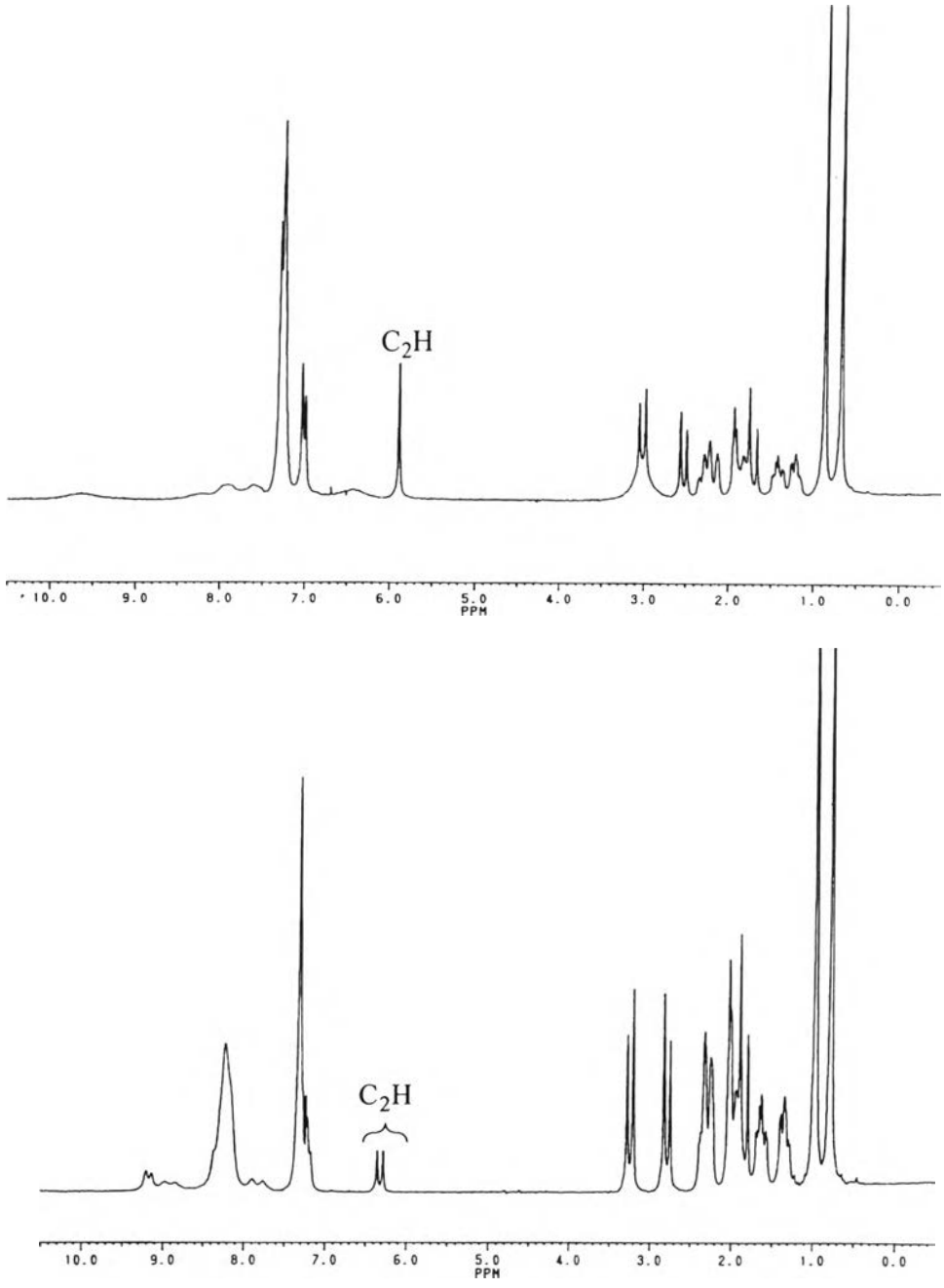


Figure 3.2 ^{1}H NMR spectra ($CDCl_3$, 200 MHz) of (+)-camphorsulfonate salt of 1-(4′-chlorophenyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine (**ID 12**) from recrystallization before (upper) and after (lower) addition of more (+)-camphorsulfonic acid as a chiral solvating agent

The same result was obtained in an attempt to separate (+)-hydrogentartrate salt of racemic 1-(4′-chlorophenyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine **(ID 16)**. ^{1}H NMR in Figure 3.4 revealed 1:1 ratio of the mixture of (+)-hydrogentartarate salt (6.30 and 6.37 ppm) even after recrystallization twice from ethanol.

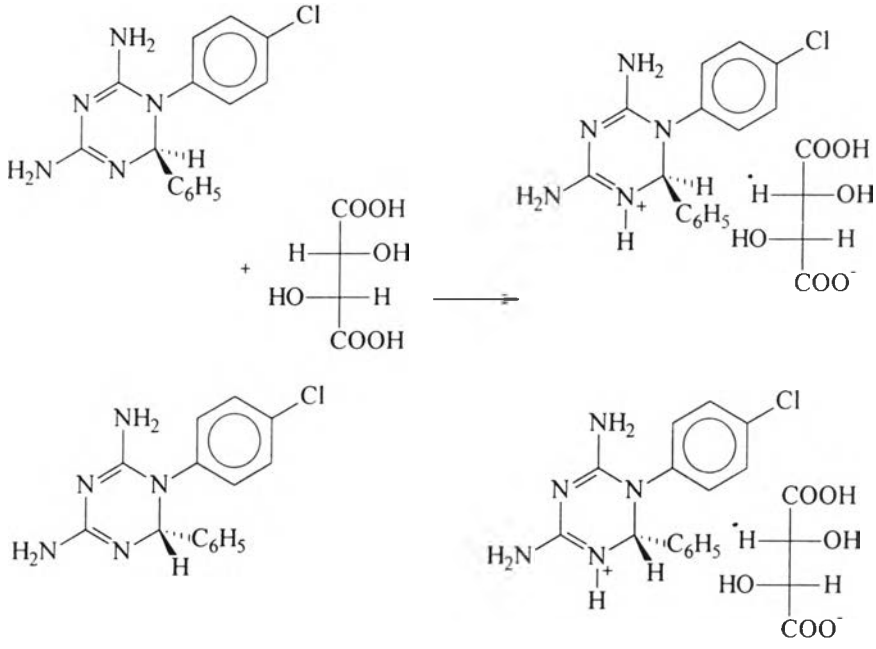


Figure 3.3 Diastereomeric-(+)-hydrogentartrate salt of 1-(4′-chlorophenyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine **(ID 16)**

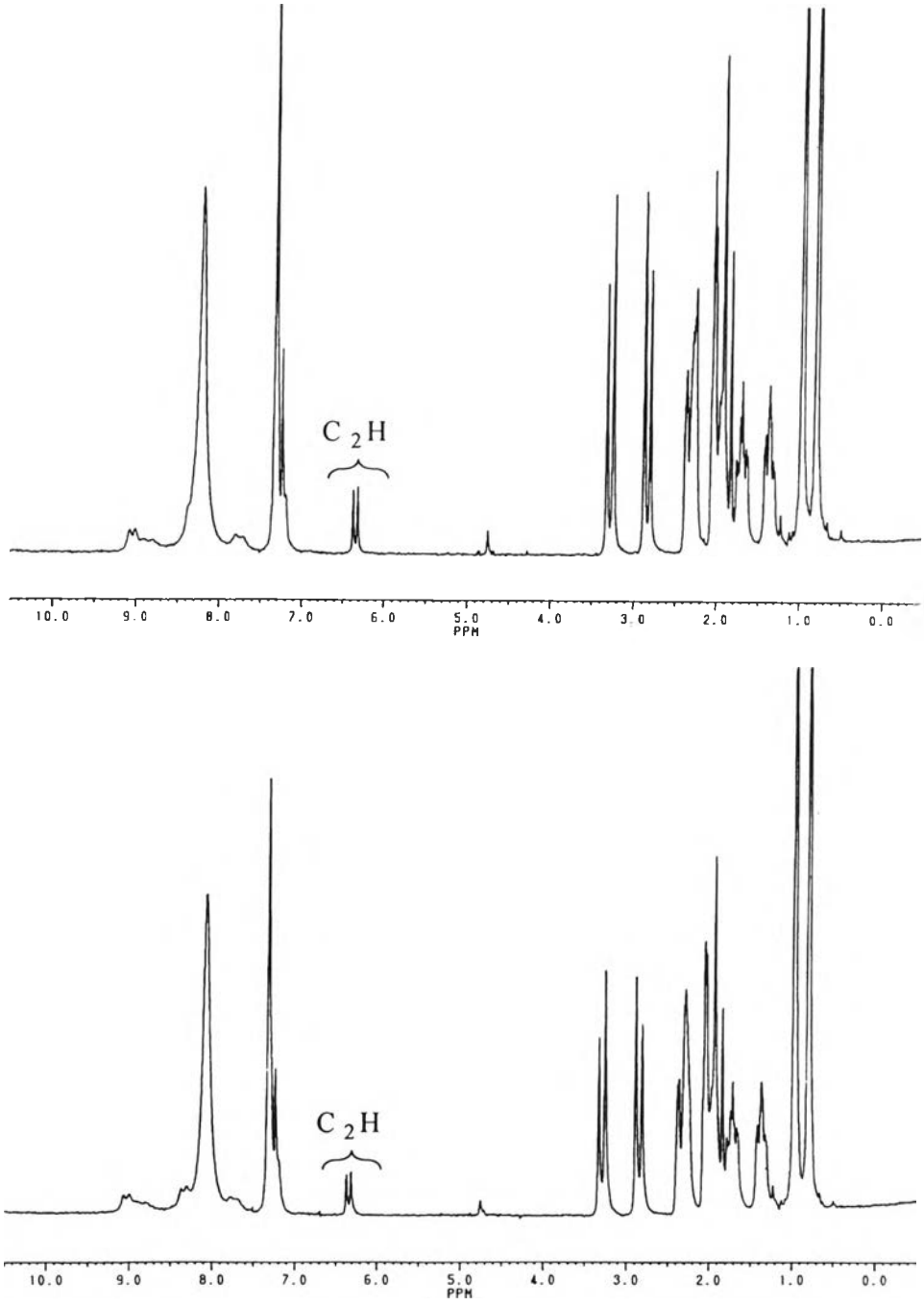
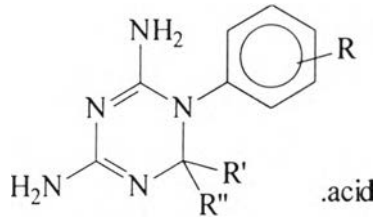


Figure 3.4 ^{1}H NMR spectra ($CDCl_3$, 200 MHz) of (+)-hydrogentartrate salt of 1-(4′-chlorophenyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine (**ID 12**) (after adding more (+)-camphorsulfonic acid as a chiral solvating agent) before (upper) and after (lower) recrystallization

Many other dihydrotriazine salts with optically active acids were similarly pre pared (Table 3.1). All diastereomeric salts were obtained nearly quantitative yield (over 95%).

Table 3.1 Diastereomeric salts of dihydrotriazine



ID	R	R′	R″	Acid	%yield
10	4-Cl	H	-C_6H_5	(-)-Camphanic acid	97
11	4-Cl	H	-C_6H_5	(+)-Camphoric acid	99
12	4-Cl	H	-C_6H_5	(1*S*)-(+)-Camphor-10-sulfonic acid	99
13	4-Cl	H	-C_6H_5	(-)-*O*,*O*′-Dibenzoyl-L-tartaric acid	100
14	4-Cl	H	-C_6H_5	*R*(-)-α-Methoxyphenyl acetic acid	98
15	4-Cl	H	-C_6H_5	(-)-Menthyloxyacetic acid	97
16	4-Cl	H	-C_6H_5	(+)-Tartaric acid	96
17	4-Cl	H	-C_6H_5	*R*(-)-1,1′-Binaphthalene-2,2′-diyl hydrogenphosphate	96
18	4-Cl	H	-CH_3	(-)-Camphanic acid	99
19	4-Cl	H	-CH_3	(+)-Camphoric acid	98
20	4-Cl	H	-CH_3	(1*S*)-(+)-Camphor-10-sulfonic acid	98
21	4-Cl	H	-CH_3	(-)-*O*,*O*′-Dibenzoyl-L-tartaric acid	98
22	4-Cl	H	-CH_3	(-)-Menthyloxyacetic acid	99
23	4-Cl	H	-$CH_2CH_2CH_3$	(-)-*O*,*O*′-Dibenzoyl-L-tartaric acid	97
24	4-Br	H	-C_6H_5	(1*S*)-(+)-Camphor-10-sulfonic acid	96
25	4-CH_3	H	-C_6H_5	(1*S*)-(+)-Camphor-10-sulfonic acid	96
26	3-Cl	H	-$CH_2CH_2CH_3$	(+)-Camphoric acid	97
27	3-Cl	H	-CH_2CH2CH_3	(1*S*)-(+)-Camphor-10-sulfonic acid	98
28	3,4-Cl_2	H	-$CH_2CH_2CH_3$	(-)-Camphanic acid	97
29	3,4-Cl_2	H	-$CH_2CH_2CH_3$	(1*S*)-(+)-Camphor-10-sulfonic acid	96
30	3,4-Cl_2	H	-CH_3	(-)-Camphanic acid	96

ID	R	R′	R″	Acid	% yield
31	3,4-Cl_2	H	-CH_3	(+)-Camphoric acid	99
32	3,4-Cl_2	H	-CH_3	(1*S*)-(+)-Camphor-10-sulfonic acid	99
33	3,4-Cl_2	H	-CH_3	(-)-*O*,*O*′-Dibenzoyl-L-tartaric acid	98
34	3,4-Cl_2	H	-CH_3	(-)-Menthyloxyacetic acid	96
35	3,4-Cl_2	H	-CH_3	(+)-Tartaric acid	98
36	3,4-Cl_2	H	-C_6H_5	*R* (-)-1,1′-Binaphthalene-2,2′-diyl hydrogenphosphate	96
37	3,4-Cl_2	H	-C_6H_5	(+)-3-Bromocamphor-10-sulfonic acid hydrate	97
38	3,4-Cl_2	H	-C_6H_5	(-)-Camphanic acid	98
39	3,4-Cl_2	H	-C_6H_5	(+)-Camphoric acid	97
40	3,4-Cl_2	H	-C_6H_5	(1*S*)-(+)-Camphor-10-sulfonic acid	96
41	3,4-Cl_2	H	-C_6H_5	(-)-*O*,*O*′-Dibenzoyl-L-tartaric acid	96
42	3,4-Cl_2	H	-C_6H_5	*R* (-)-α-Methoxyphenyl acetic acid	98
43	3,4-Cl_2	H	-C_6H_5	(-)-Menthyloxyacetic acid	96
44	3,4-Cl_2	H	-C_6H_5	*R* (-)-2-phenylpropionic acid	96
45	3,4-Cl_2	H	-C_6H_5	(+)-Tartaric acid	98

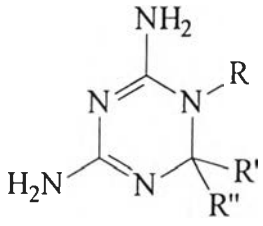
Recrystallization of all diastereomeric salts gave no observable enrichment according to ^{1}H NMR analysis, therefore this proved not a viable route. The reason to this is unclear. One possibility is that the suitable combination of acid/ dihydrotriazine and solvent was not yet found. The other explanation is that 1,2-dihydrotrizine is configurationally unstable under the crystallization condition. Support to this proposal will be discussed in the next session. In addition, we have attempted to co-crystallize racemic 1-(4′-chlorohphenyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydro-chloride with β-cyclodextrin with the hope that one enantiomer may form more stable or more easily crystallized inclusion complex. Unfortunately ^{1}H NMR analysis of the crystals formed revealed that they were pure cyclodextrin indicating that no inclusion complex had formed.

3.2 Resolution by chromatography using chiral stationary or mobile phase

This technique involves formation of transient diastereomeric complexes between the racemic compound to be resolved and the chiral stationary phase or mobile phase.[27] The mobility of such diastereomeric complex will not be equal, therefore the enantiomer pass through the column at different rates.

W. K. Chui and his team from the National University of Singapore, has claimed that they could separate the enantiomers of dihydrotriazine by chiral HPLC technique and by recrystallization of (+)-camphorsulfonate salts. However, their resolution by recrystallization was not successful in our hand and their works have never been published. As resolution using chiral HPLC appeared to be straightforward, we have attempted to resolve 1-aryl-4,6-diamino-1,2-dihydro-1,3,5-triazine by chiral reverse phase HPLC. A Merck's LiChro CART® HPLC cartridge 250-4 chiraDex1 (5μm) which contain β-cyclodextrin coated silica was used. Peaks were monitored by measuring UV-absorbance at 254 nm. Triethylammonium acetate or ammonium acetate buffer (0.1 M, pH 7.0) and methanol were used as mobile phase in the experiment. By varying the ratio of triethylammonium acetate and methanol, the best separation condition was found to be the isocratic system of 15% MeOH and 85% 0.1 M triethylammonium acetate. Since structure will definitely influence the adsorption ability of the enantiomers of dihydrotriazines, many of these enantiomers were tested to find the best separated structure. From 21 compounds analyzed (Table 3.2), 1-(4′bromophenyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(4)** (entry 1) and 1-(4′-methylphenyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(5)** (entry 17) gave maximum t_R difference between the first and second peak of their enantiomers (Figure 3.5). Both of them therefore were selected for preparative chiral reverse phase HPLC.

Table 3.2 4,6-Diamino-1,2-dihydro-1,3,5-triazine analyzed by chiral reverse phase HPLC



Entry	R	R′	R″	t_R (1) (min)	t_R (2) (min)
1	4-BrC_6H_4-	H	-C_6H_5	13.6	15.7
2*	4-BrC_6H_4-	Me	-$CH_2(CH_2)_4CH_3$	22.6	22.6
3*	2,4-$Cl_2C_6H_3$-	H	-$CH(CH_3)_2$	3.6	3.6
4*	3-ClC_6H_4-	H	-$CH_2(CH_2)_2CH_3$	6.7	6.7
5	3-ClC_6H_4-	H	-C_6H_5	5.5	6.4
6	3,4-$Cl_2C_6H_3$-	H	-$CH_2(CH_2)_2CH_3$	15.1	16.4
7*	3,4-$Cl_2C_6H_3$-	CH_3	-$CH_2(CH_2)_3$	10.9	10.9
8*	3,5-Cl_2-C_6H_3-	H	-C_6H_5	6.6	6.6
9*	4-ClC_6H_4-	H	-CH_3	4.9	4.9
10*	4-ClC_6H_4-	H	-$CH_2(CH_2)_2CH_3$	8.6	8.6
11	4-ClC_6H_4-	H	-C_6H_5	6.2	7.4
12*	4-ClC_6H_4-	Me	-$CH(CH_3)_2$	10.3	10.3
13*	4-ClC_6H_4-	Me	-$CH_2CH_2(CH_3)_2$	7.2	7.2
14*	4-EtC_6H_4-	H	-$CH(CH_3)_2$	13.6	13.6
15	4-EtC_6H_4-	H	-C_6H_5	18.4	21.6
16*	4-MeC_6H_4-	H	-C_6H_{11}	6.6	6.6
17	4-MeC_6H_4-	H	-C_6H_5	6.5	8.4
18	4-MeC_6H_4-	Me	-$CH(CH_3)_2$	11.9	12.8
19	4-MeC_6H_4-	Me	-$CH_2(CH_2)_2CH_3$	8.2	8.8
20*	4-MeC_6H_4-	Me	-$CH_2CH_2(CH_3)_2$	10.6	10.6
21	3-NO_2,4-ClC_6H_3-	H	-C_6H_5	11.3	12.7

*no separation was observed

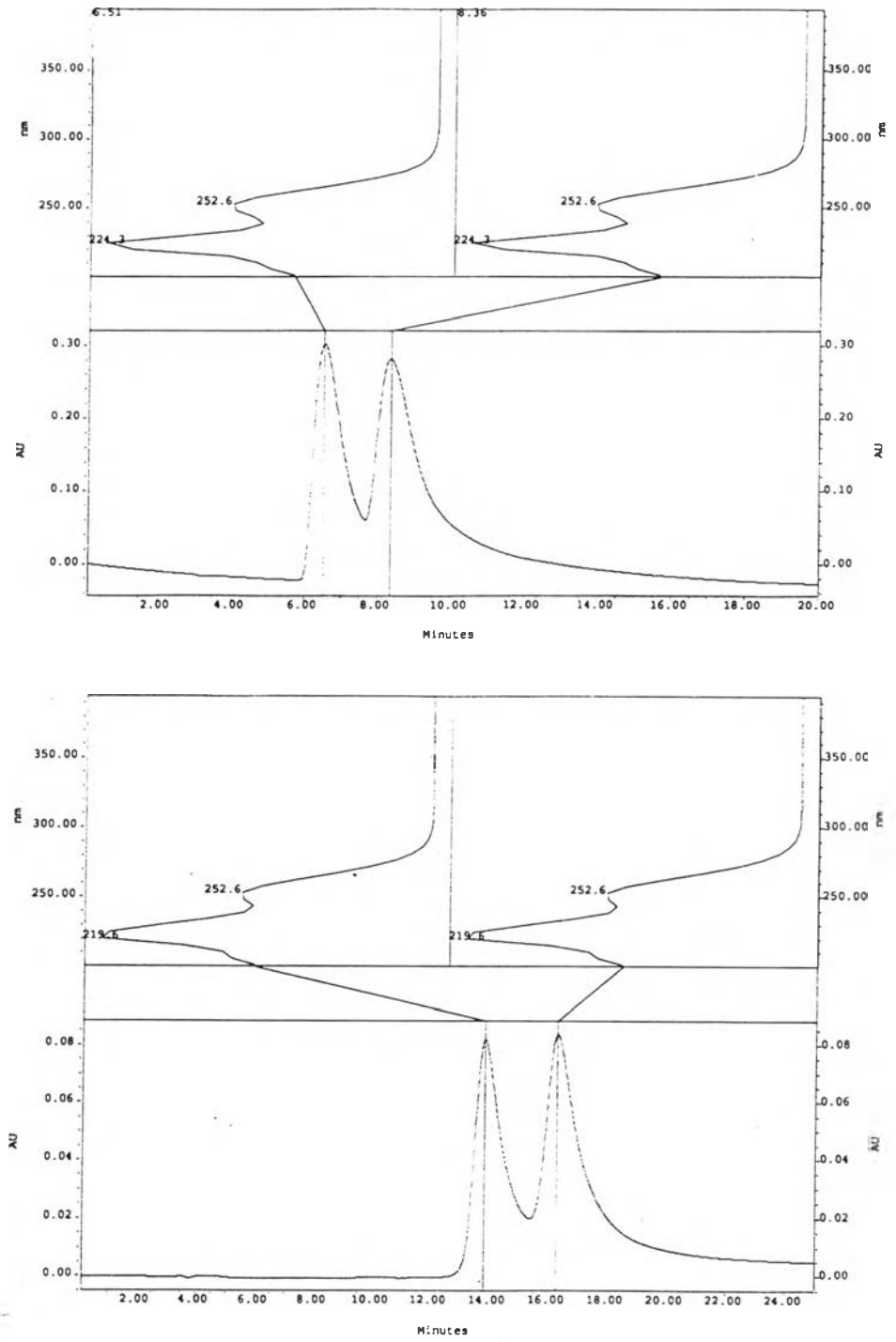


Figure 3.5 Chiral HPLC chromatogram of 4,6-diamino-1,2-dihydro-1,3,5-triazine **(4)** ; entry 1(upper) and **(5)**; entry 17 (lower), (mobile phases: 15% MeOH and 85% 0.1 M triethylammonium acetate)

Since triethylammonium acetate can caused damage to the chiral phase β-cyclodextrin coating in the column for long-term usage, ammonium acetate buffer was used for preparative scale instead although the resolution was poorer.

Two fractions containing each enantiomer of 1-(4′-bromophenyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine **(4)** were collected and the eluents and buffer removed by freeze drying to obtain the product as acetate salt. Each fraction was purified again by second HPLC on the same column. The samples after freeze-drying were stored in the freezer for bioactivity test. The success of the separation if enantiomers of dihydrotriazine **(4)** was confirmed by HPLC analysis (Figure 3.6). The first peak **(4a)** in chromatogram, (t_R = 12.0 min) was obtained in enantiomerically pure, while the second peak **(4b)**, (t_R = 18.2 min) was obtained in only 67% enantiomerically excess. CD spectroscopy displayed almost opposite CD signals for each peak as shown in Figure 3.7 confirming that the two peaks were indeed enantiomers.

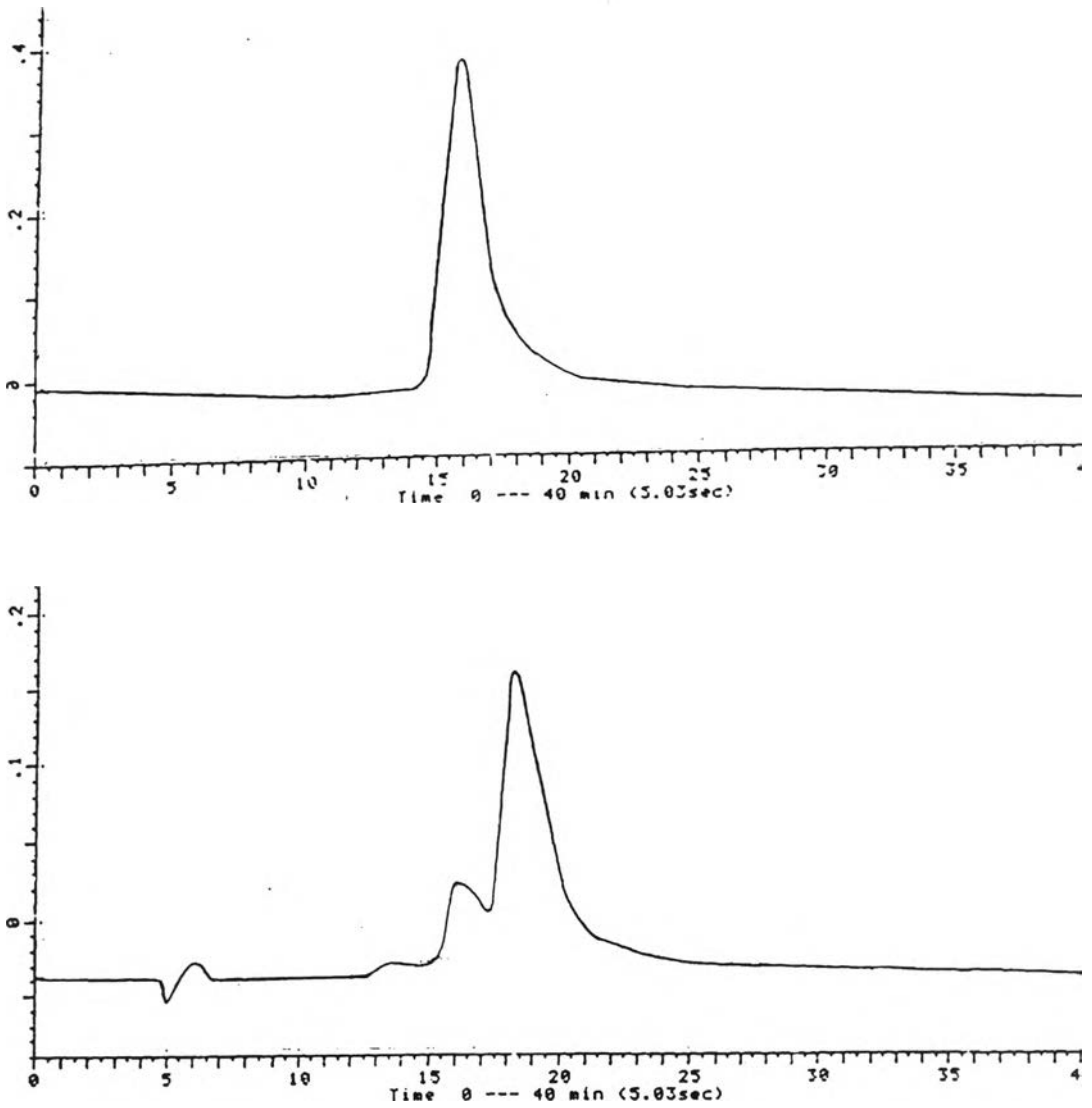


Figure 3.6 Chromatogram of peak 1 **(4a)** (upper) and peak 2 **(4b)** (lower) of compound **(4)** after separation by chiral reverse phase HPLC

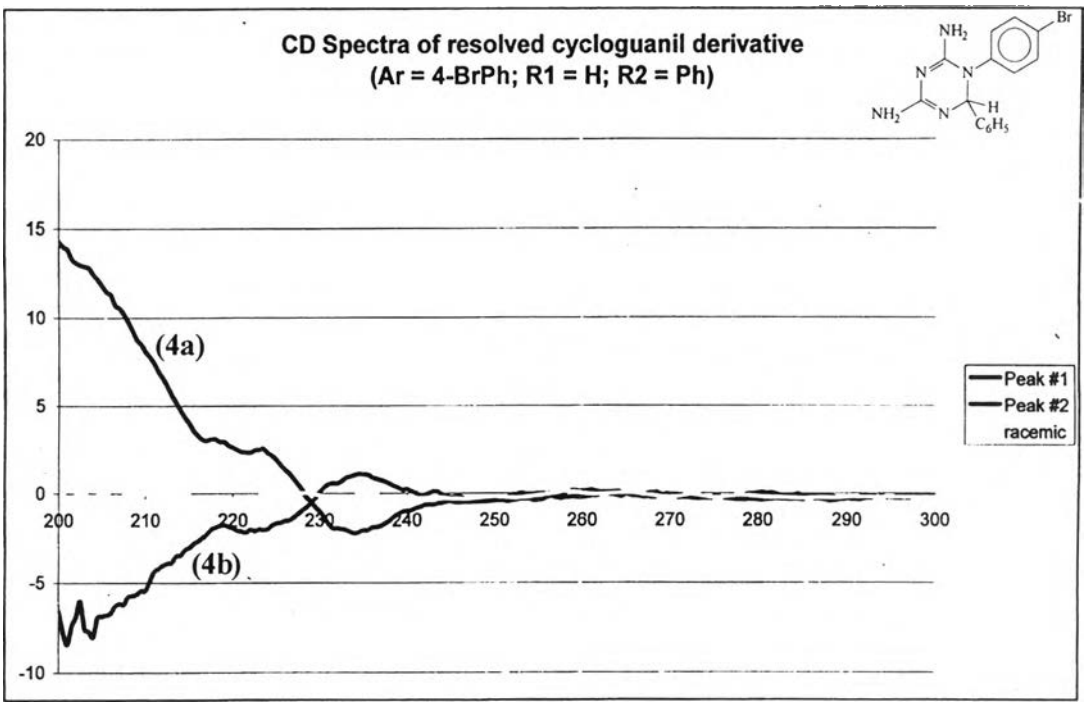


Figure 3.7 CD spectra of **(4a)** and **(4b)** of compound **(4)** after separation by chiral reverse phase HPLC

1-(4′-Methylphenyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine **(5)** was also similarly separated by chiral reverse phase HPLC to give **(5a)** and **(5b)** with comparable success. The results were shown in Figure 3.8 and 3.9.

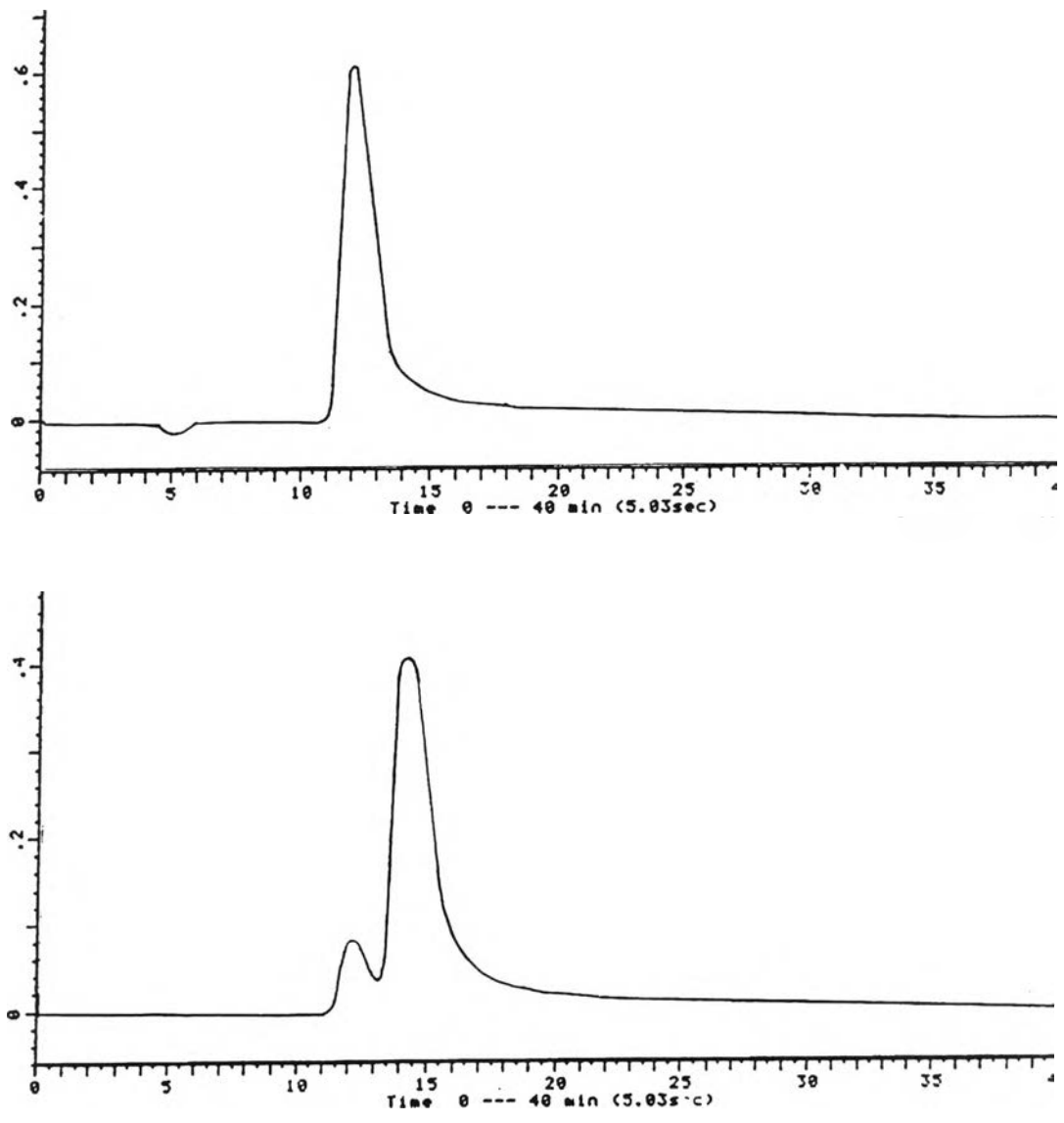


Figure 3.8 Chromatogram of peak 1 **(5a)** (upper) and peak 2 **(5b)** (lower) of compound **(5)** after separation by chiral reverse phase HPLC

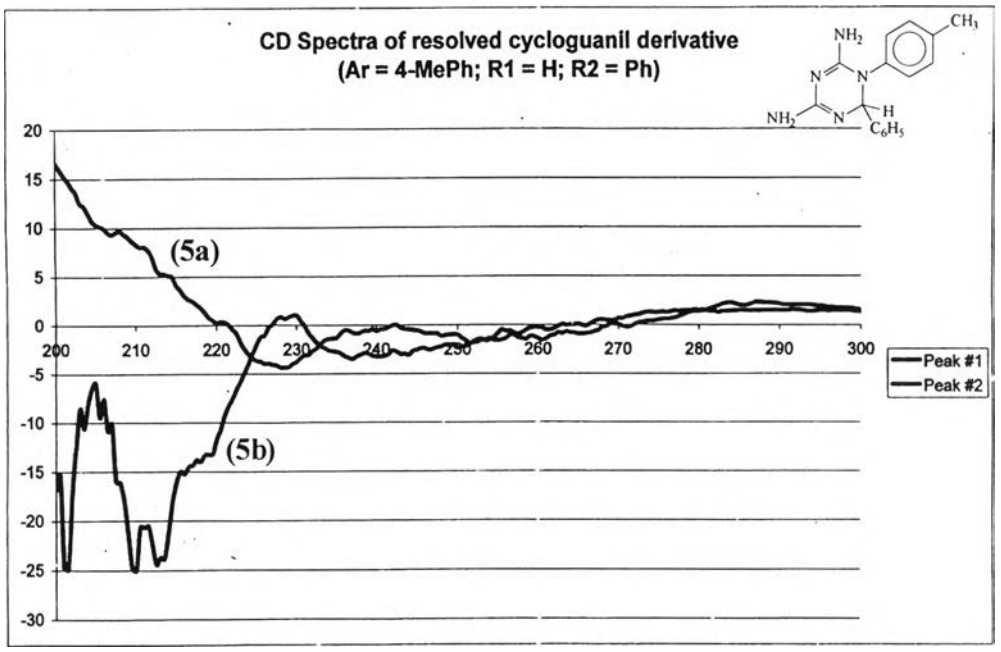
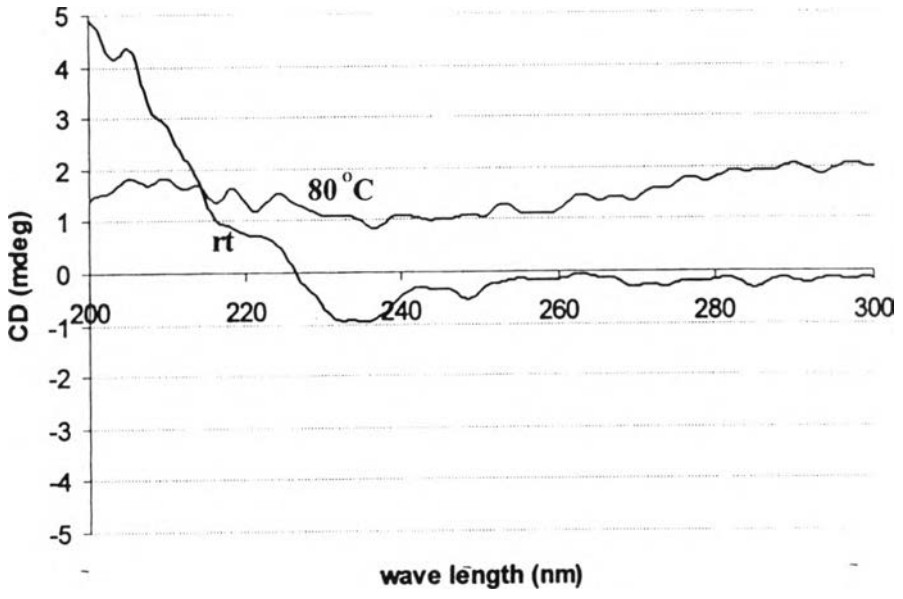


Figure 3.9 CD spectra of sample **(5a)** and **(5b)** of compound **(5)** after separation by chiral reverse phase HPLC

Although dihydrotriazine **(4)** and **(5)** were successfully separated by reverse phase chrial HPLC, this method was laborious and time-consuming. Only sub-milligram quantities of each sample were obtained which was just enough for testing bioactivity but not for determination of absolute configuration by X-ray-crystallography. The facile racemization of dihydrotriazines was another complicating problem in separation of these compounds. CD analysis in Figure 3.10 revealed that the signal of pure enantiomer disappear rapidly and irreversibly upon heating in H_2O at 80 °C for 30 minutes including that it has already transformed into a racemic mixture.



Half-time for racemisation : ~24 hr at 40 °C
< 30 min at 80 °C

Figure 3.10 CD spectrum: racemization of pure enantiomer

Furthermore, it was found that the triethylammonium acetate and ammonium acetate buffers used as an eluent caused deterioration of the chiral column and its efficiency leading to non-reproducible separation (Figure 3.11). From all above reasons, resolution of dihydrotriazine compounds was attempted by different methods.

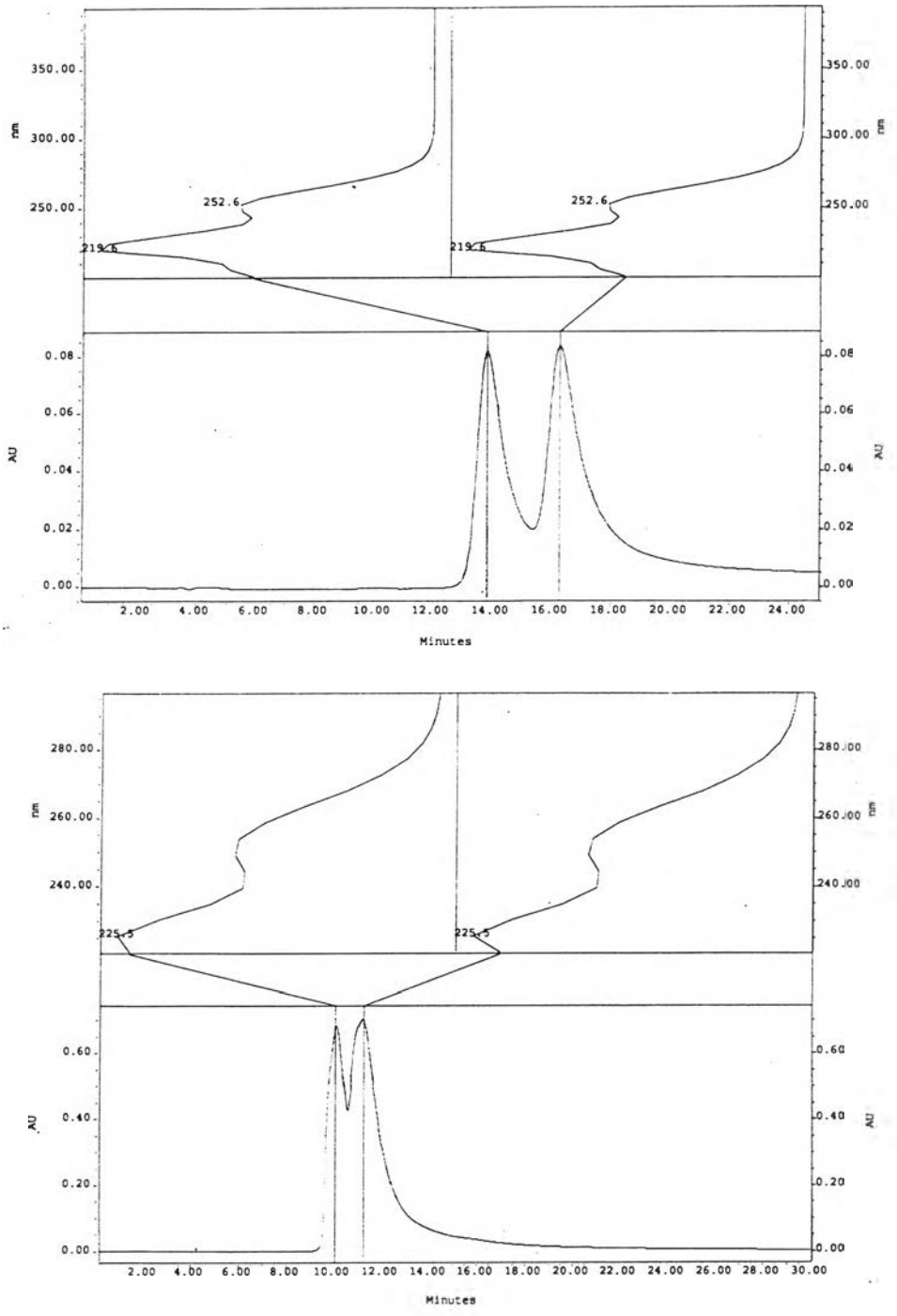
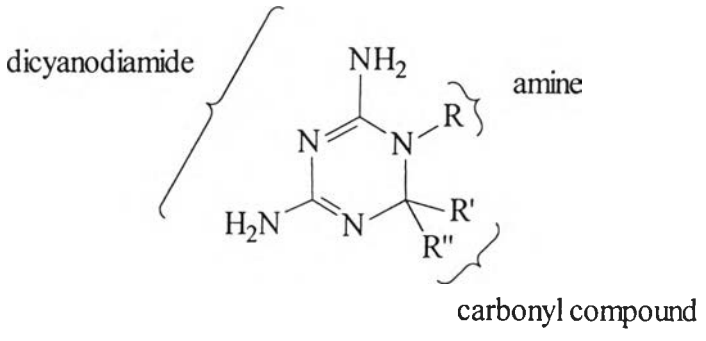


Figure 3.11 Chromatogram of 1-(4′-bromophenyl)-2-phenyl-4,6-diamino-1,2dihydro-1,3,5-triazine **(4)** before (upper) and after (lower) deterioration of the chiral reverse phase column under the same conditions

3.3 Asymmetric synthesis and resolution by means of covalently attaching chiral auxiliary to the racemic mixture of enantiomer followed by separation of the diastereomeric mixture formed

The principle of this method is to covalently attaching of a chiral auxiliary to the racemic mixture to obtain diastereoisomers which may be separated by conventional techniques such as crystallization, column chromatography etc.



4,6-diamino-1,2-dihydro-1,3,5-triazine

The structure of 4,6-diamino-1,2-dihydro-1,3,5-triazine consists of three variable groups: R, R′ and R″. The R part comes from an aromatic amine and R′, R″ are from a carbonyl compound. As a result, there are two possibilities to attach the chiral auxiliary. One is adding to the part of amino compound (*ie*, use R group which is chiral), the other is adding to the carbonyl compounds (*ie*, use R′, R″ group which is chiral).

Attachment of the chiral moiety *via* the carbonyl compound was the first experiment attempted in this study. The commercially available and inexpensive (1*R*,2*S*,5*R*)-(-)-2-isopropyl-5-methylcyclohexanol ((-)-menthol) and *p*-hydroxybenzal-dehyde were used to prepare the required chiral carbonyl compound (Figure 3.12).

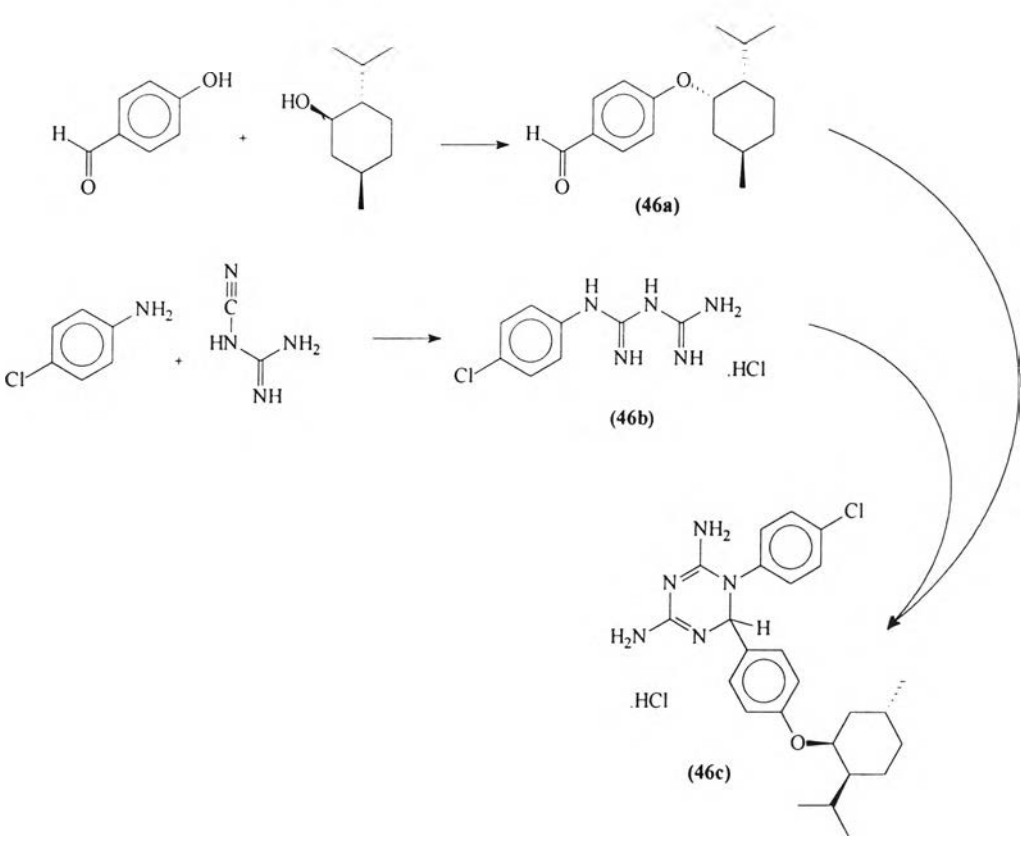


Figure 3.12 Synthesis of 1-(4′-chlorophenyl)-2-[4′-(2″*S*-isopropyl-5″*R*-methyl-1″*S*-cyclo-hexyloxy)phenyl]-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(46c)**

We have attempted to find out a suitable method to produce the 4-(2′*S*-isopropyl-5′*R*-methyl-1′*S*-cyclohexyloxy)benzaldehyde **(46a)** as the starting material. The first method was to introduce the (-)-menthyl moiety into *p*-hydroxybenzaldehyde by alkylation (Figure 3.13). The OH group of menthol was converted to a good leaving group by tosylation or mesylation followed by nucleophilic displacement *via* S_N^2 reaction. Consequently, inversion at the 1-position is expected. Synthesis of *O*-mesyl derivative of (-)-menthol was achieved by using $MsCl/Et_3N$ in dichloromethane. However, reaction of *O*-mesyl derivative of (-)-menthol with *p*-hydroxybenzaldehyde in the presence of K_2CO_3/DMF gave no expected product even after prolonged heating. One possible explanation is that the *p*-hydroxybenzaldehyde might be too weak nucleophile to react with the relatively sterically hindered *O*-mesyl derivative of (-)-menthol.

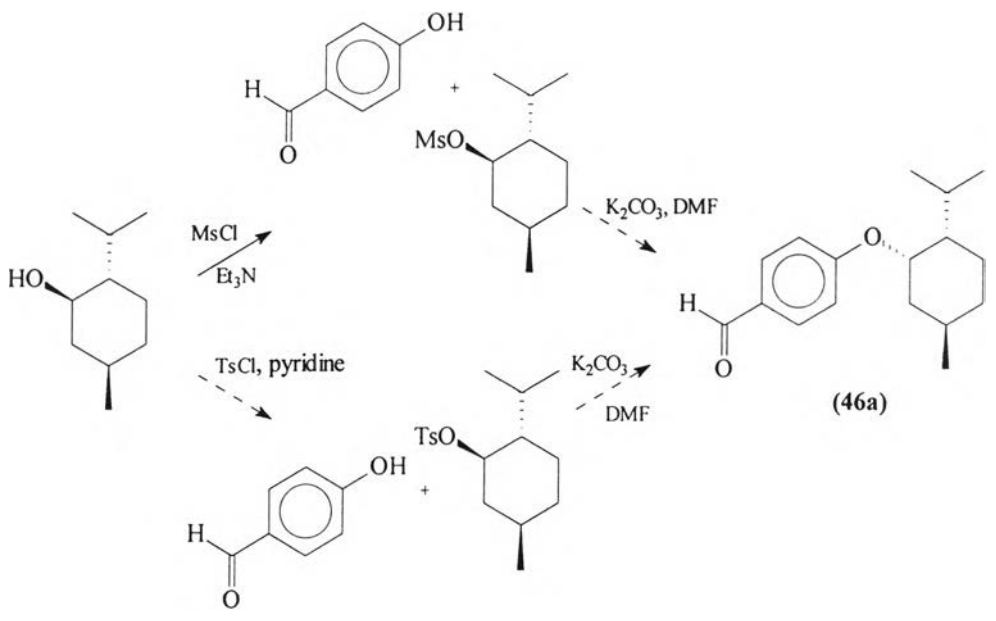


Figure 3.13 Attempted synthesis of 4-(2′*S*-isopropyl-5′*R*-methyl-1′*S*-cyclohexyloxy)-benzaldehyde **(46a)**

The other method to synthesize **(46a)** was Mitsunobu reaction between *p*-hydroxybenzaldehyde, (-)-menthol and Ph_3P/DIAD. The success of this methods depended on the quality of the starting material especially the THF solvent which had to be free from water. The mechanism is proposed to involve the OH group of *p*-hydroxybenzaldehyde (pKa ~ 7.66) that was deprotonated by PPh_3-DIAD complex and became phenoxide ion. The resulting protonated Ph_3P-DIAD complex was then reacted with menthol to give a phosphonium salt. Subsequent S_N^2 displacement of the phosphonium salt by anion of *p*-hydroxybenzaldehyde gave the required product and by products which were separated by column chromatography to give pure **(46a)** as a colorless oil in 26% yield. Inversion at the 1-position is again expected (Figure 3.14).

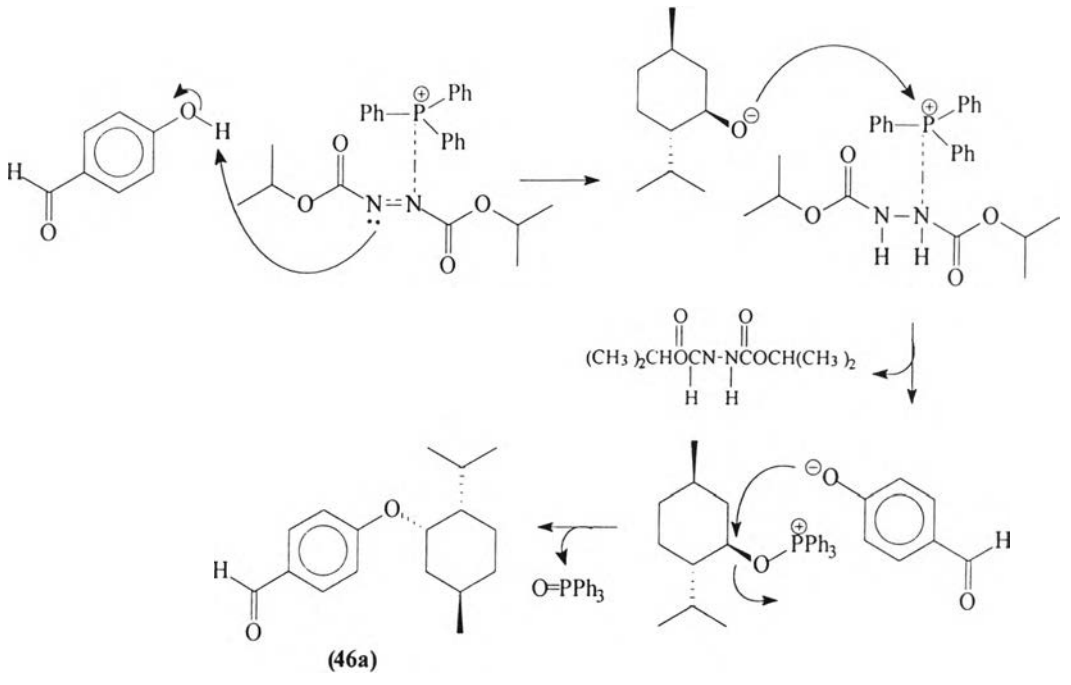


Figure 3.14 Synthesis of 4-(2′*S*-isopropyl-5′*R*-methyl-1′*S*-cyclohexyloxy) benzaldehyde **(46a)** *via* Mitsunobu reaction

Having obtained the optically active aldehyde **(46a)**, synthesis of the target compound 4,6-diamino-1,2-dihydro-1,3,5-triazine **(46c)** was attempted by the two-component method.[38] This reaction involved condensation of an aryl biguanide and a ketone or an aldehyde under acidic condition. The rate of reaction was found to be dependent to the temperature employed. Moreover, this reaction was best carried out under anhydrous condition. In this respect, Vilaivan and Saesaengseerung has shown that addition of a miscible water scavenger such as triethyl orthoacetate (TEOA) is advantageous in forcing the reaction to occur under mild condition.[39] By the way, the rearrangement product which may be caused by strong acids and high temperature used might also be occurred. For this reason, the reaction condition need to be carefully controlled in order to avoid complicating side-reactions.

The desired dihydrotriazine product **(46c)** was sucecssfully synthesized by a reaction between chiral aldehyde **(46a)** and aryl biguanide **(46b)** prepared from *p*-chloroaniline and dicyanodiamide (Figure 3.15) by stirring together in the presence of concentrated HCl and TEOA at room temperature.

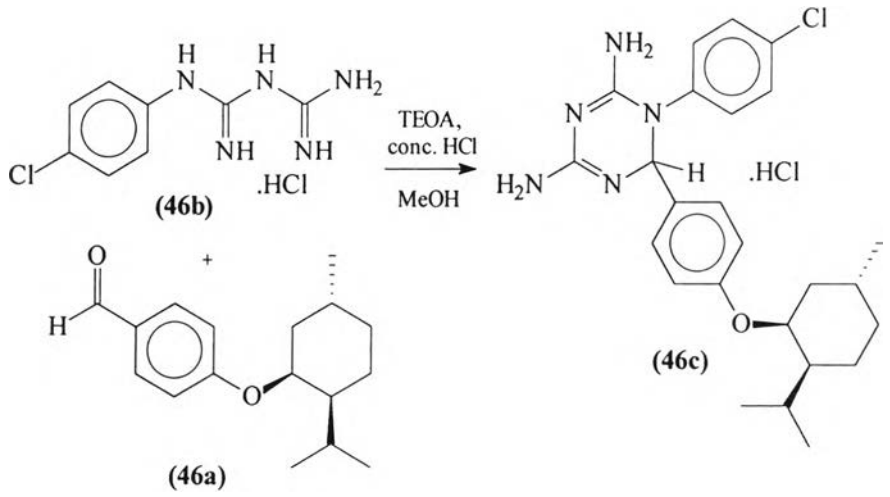


Figure 3.15 Two component condensation of 1-(4′-chlorophenyl)-2-[4′-(2″*S*-isopropyl-5″*R*-methyl-1″*S*-cyclohexyloxy)phenyl]-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(46c)**

^{1}H NMR suggested that the product contained only one diasteromer, *ie*, the menthyl group had induced stereoselective formation and/or preferentially crystallization of only one out of the two possible diastereomers. (Figure 3.16)

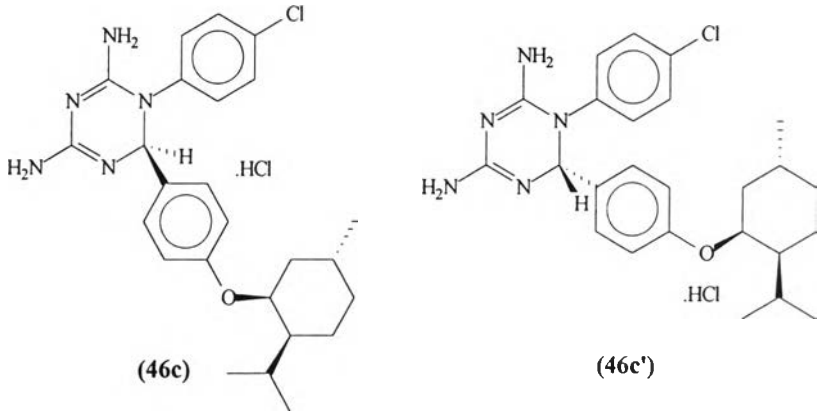
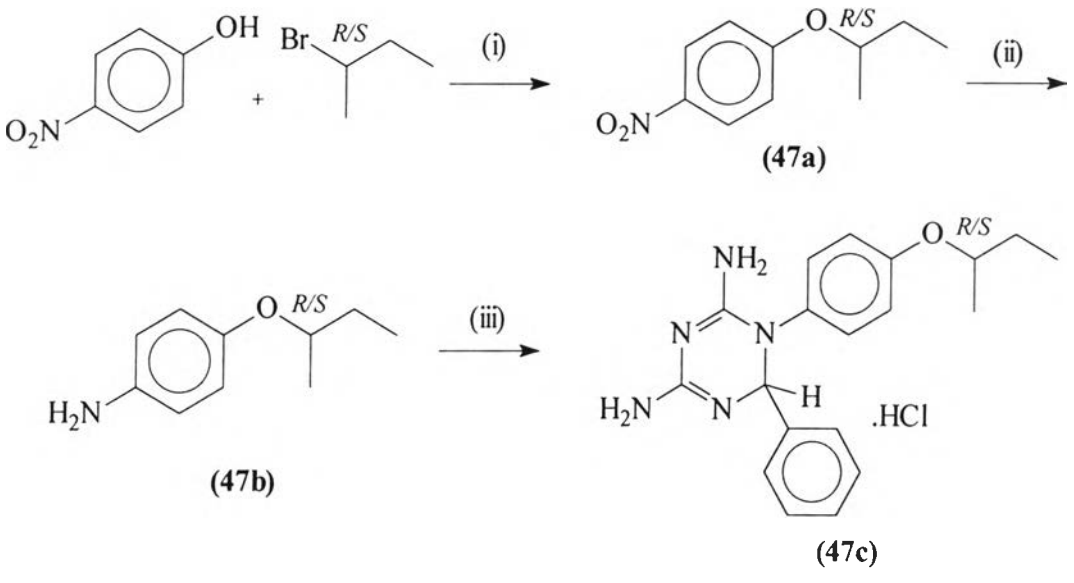


Figure 3.16 Two possible structure of diastereoisomers **(46c)** and **(46c′)**

Unfortunately, attempts to prove the absolute configuration of **(46c)** by X-ray crystallography was not successful because no good quality crystals could be obtained. Furthermore, the condition for synthesis of this compound was not reproducible. Therefore, the reaction was not further pursued.

There was the other idea to attach the chiral moieties to the dihydrotriazine ring through the N_1-aryl substituent. The chiral N_1-aryl substituent might be obtained by using an appropriate chiral aromatic amine. Preparation and cyclization of which will be fully discussed below.



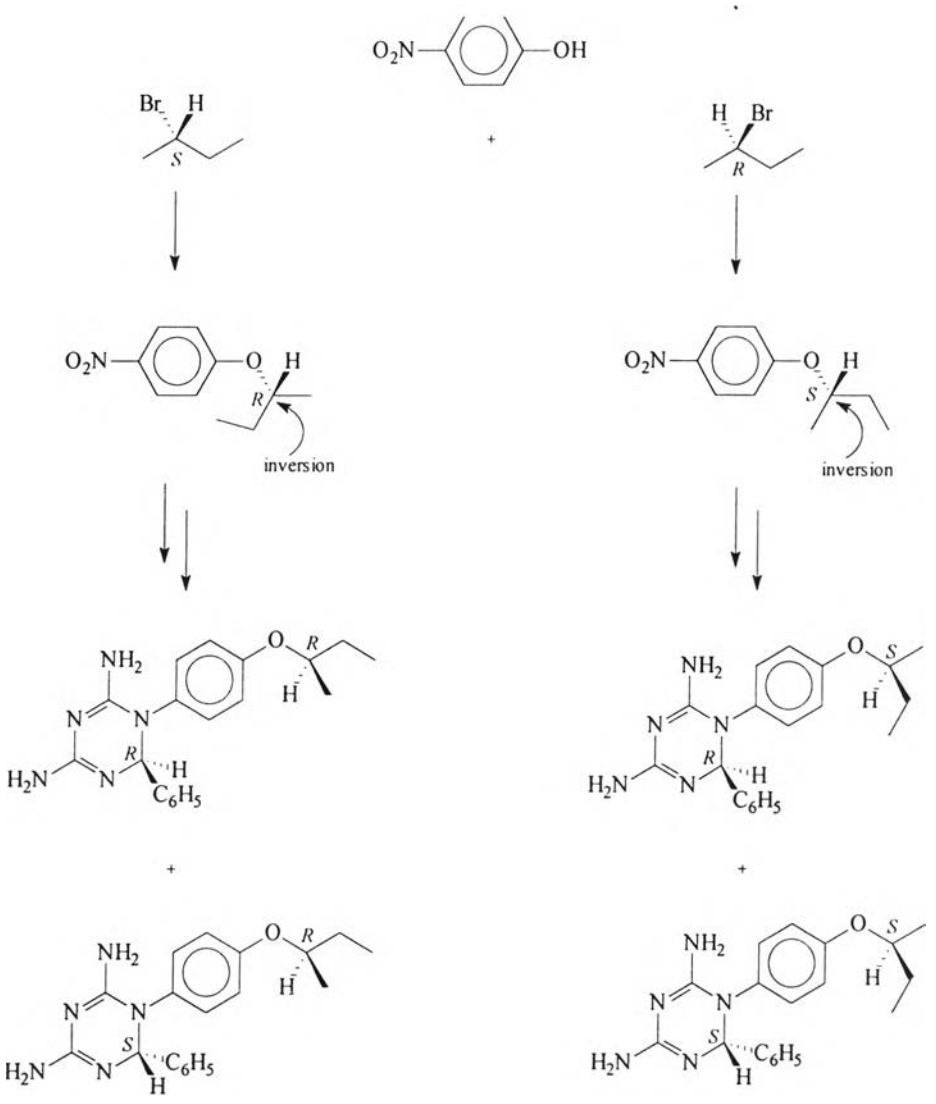
i) K_2CO_3, DMF, 70 °C

ii) $SnCl_2.H_2O$, EtOH, 70°C

iii) dicyanodiamide, benzaldehyde, EtOH, conc. HCl

Figure 3.17 Synthesis of 1-(4′-butoxyphenyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(47c)**

The first idea is to steroselectively alkylate 4-aminophenol or 4-nitrophenol with a suitable optically active alkyl halides. The simplest chiral alkyl halide available is 2-bromobutane. Since optically active 2-bromobutane is very expensive, it was decided to do the model reaction with racemic 2-bromobutane (Figure 3.17). If stereodifferentiation by the 2-butyl moiety is successful then we expected to obtain only one enantiomer pair of **(47c)** with racemic 2-bromobutane and one enantiomer of **(47c)** with optically active 2-bromobutane.



Scheme 3.1 Synthesis of diasteroisomer **(47c)** starting from racemic (2)-bromobutane

Alkylation of 4-nitrophenol was thus carried out using K_2CO_3/DMF/(±)-2-bromobutane at 70 °C to give 1-sec-butoxy-4-nitrobenzene **(47a)**. Reduction of the nitro group was accomplished by $SnCl_2.2H_2O$ gave the racemic amine **(47b)**. Synthesis of **(47c)** was carried out by three component synthesis[40] which involved the condensation of the arylamine, dicyanodiamide and ketone or an aldehyde, with loss of one-molecule of water (Scheme 3.1). This method produced the **(47c)** in moderate yield (37%). However, the X-ray analysis in Figure 3.18 could not clearly reveal the stereochemistry of the 2-butyl moiety due to its high degree of flexibility. It is quite likely that all 4 stereoisomers were present after crystallization. This implied that stereodifferentiation by the 2-butyl group, which is small and far from the stereocenter to be differentiated at C_2, is not possible. It can be further conferred that

even when optically active 2-bromobutane was used, no stereodifferentiation would be possible. For this reason, we continued to find another suitable chiral molecule to reach the target.

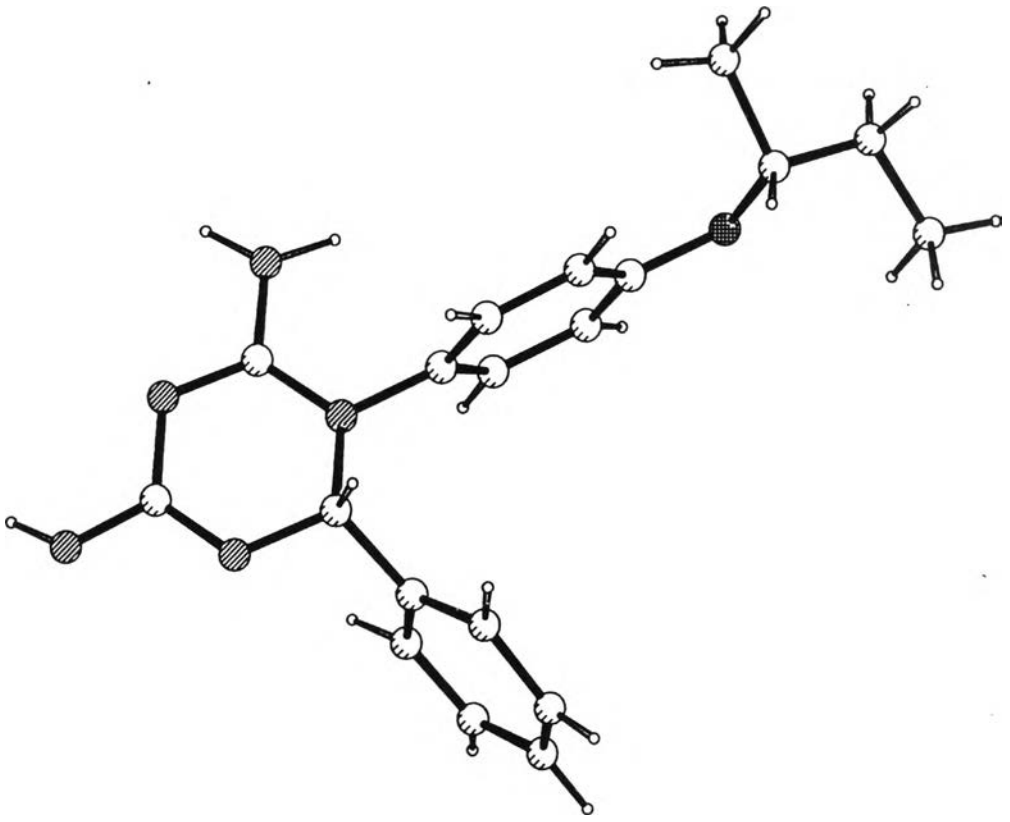
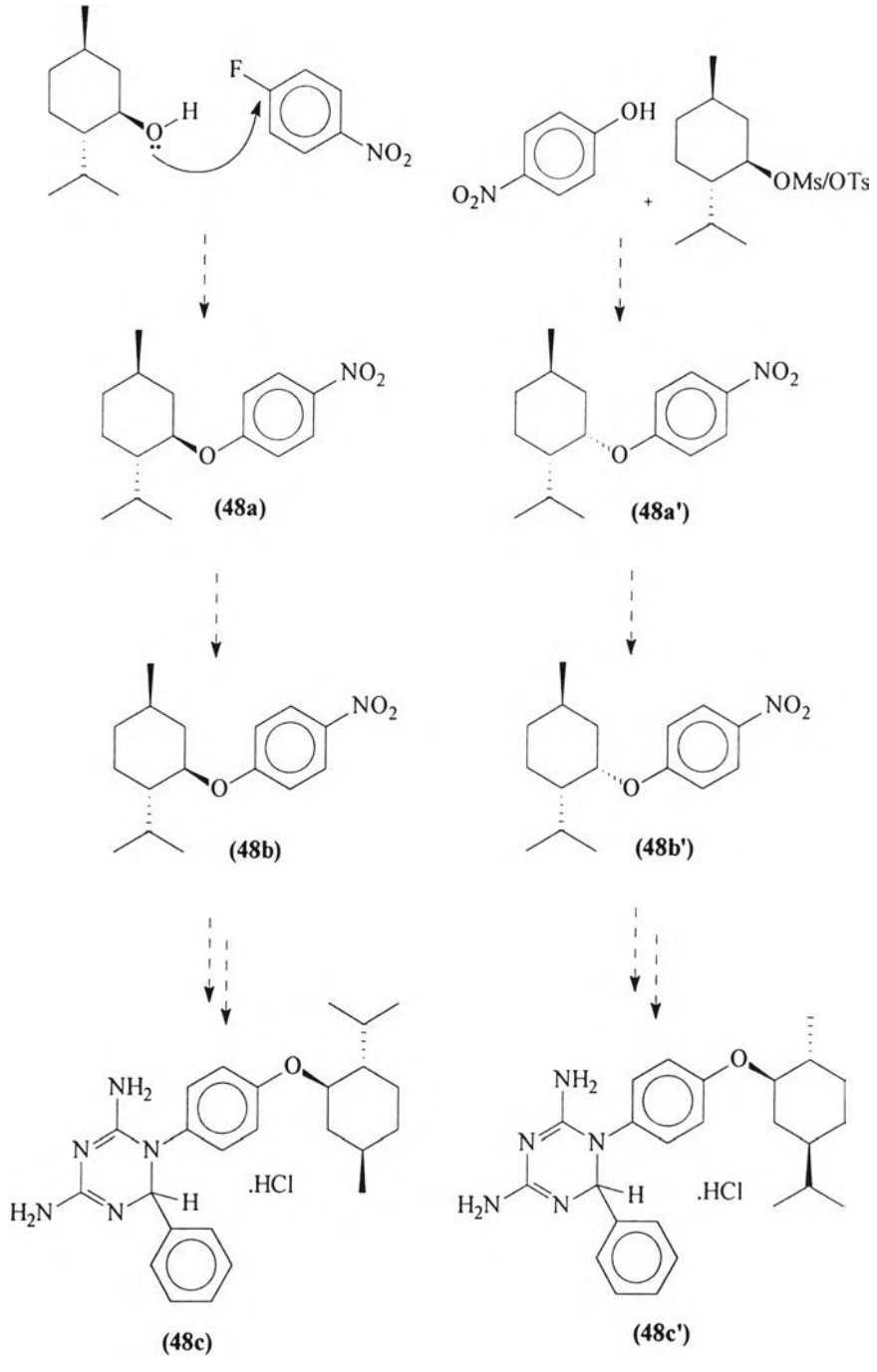


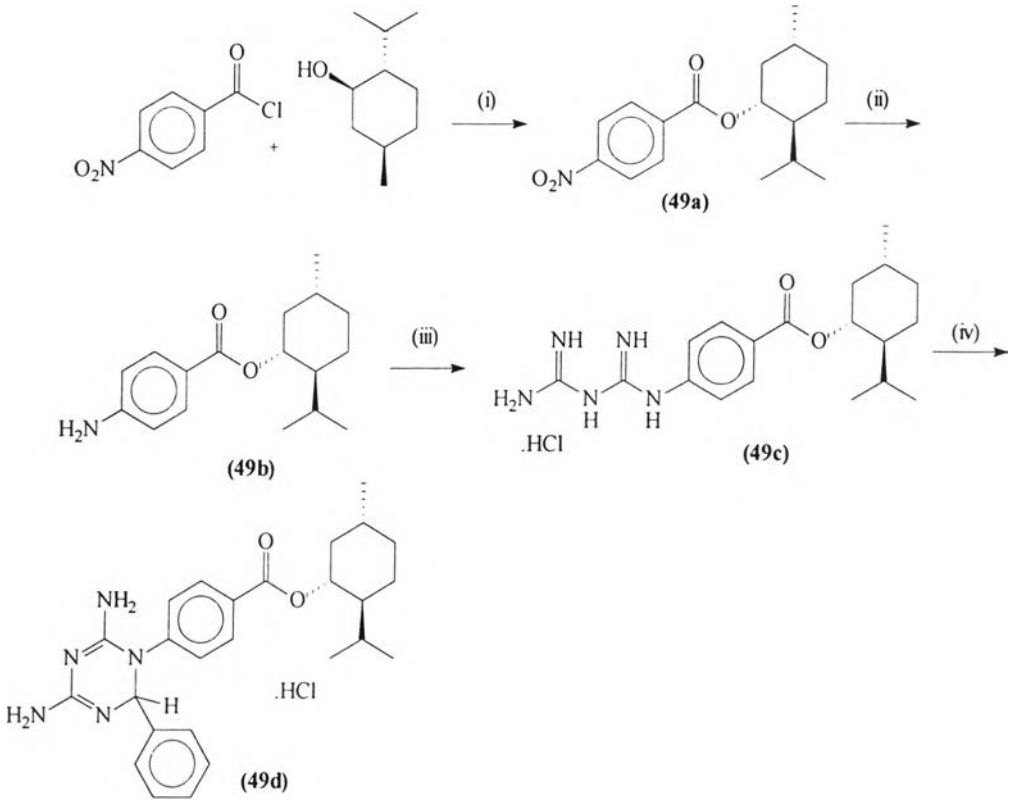
Figure 3.18 X-ray structure of 1-(4′-butoxyphenyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(47c)**



Scheme 3.2 Attempted synthesis of chiral amino compound **(48b)**

Reduction of the nitro compound **(48a)** or **(48a′)** gives the optically active amine **(48b)** or **(48b′)** which may be further converted to the optically active dihydrotriazine **(48c)** or **(48c′)** (Scheme 3.2). Alkylation of 4-nitrophenol with (-)-menthyl tosylate or (-)-menthyl mesylate under a variety of conditions was not successful. An alternative S_NAr-type alkylation of (-)-menthol by 4-fluoronitrobenzene in the presence of K_2CO_3/18 Crown-6/DMF at 140 °C also gave

no desired product even after heating for 5 days. Therefore it was proved impossible to prepare **(48c)** by this route.



i) Et_3N, CH_2Cl_2

ii) cyclohexene, Pd/C, abs. MeOH, N_2, reflux

iii) dicyanodiamide, abs. EtOH, conc. HCl, reflux

iv) benzaldehyde, TEOA, abs. MeOH, conc. HCl

Figure 3.19 Synthesis of 1-['4(2''*S*-isopropyl-5''*R*-methyl-1''*R*-cyclohexyloxy-carbonyl)phenyl]-2phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(49d)**

It was proposed that the optically active menthyl moiety could be introduced through an ester bond. The amino ester **(49b)** was thus required as starting material (Figure 3.19). Synthesis of (-)-menthyl 4-nitrobenzoate **(49a)** was achieved by a reaction of 4-nitrobenzoyl chloride and (-)-menthol in the presence of triethylamine. The nitro compound **(49a)** was then reduced by catalytic transfer hydrogenation using the cyclohexene and Pd/C catalyst to give (-)-menthyl 4-aminobenzoate **(49b)** which was purified by column chromatography to give a yellow oil (80% yield). Synthesis of the dihydrotriazine **(49d)** was succeeded by the two-component condensation

between the biguanide **(49c)** and benzaldehyde under the condition successfully employed for the synthesis of **(46c)**. ^{1}H NMR suggested that only one out of two possible diastereoisomers had formed. Unfortunately, all attempts to crystallize **(49d)** for X-ray crystallography analysis failed therefore the configuration at C_2 could not be conclusively determined this way. Replacement of benzaldehyde with isobutyraldehyde, cyclohexanecarboxaldehyde and methyl isobutyl ketone in order to synthesize the analogues compounds **(49e)**, **(49f)**, **(49g)** failed to give the desired products (Figure 3.20). In all case the cyclization step did not proceed to completion

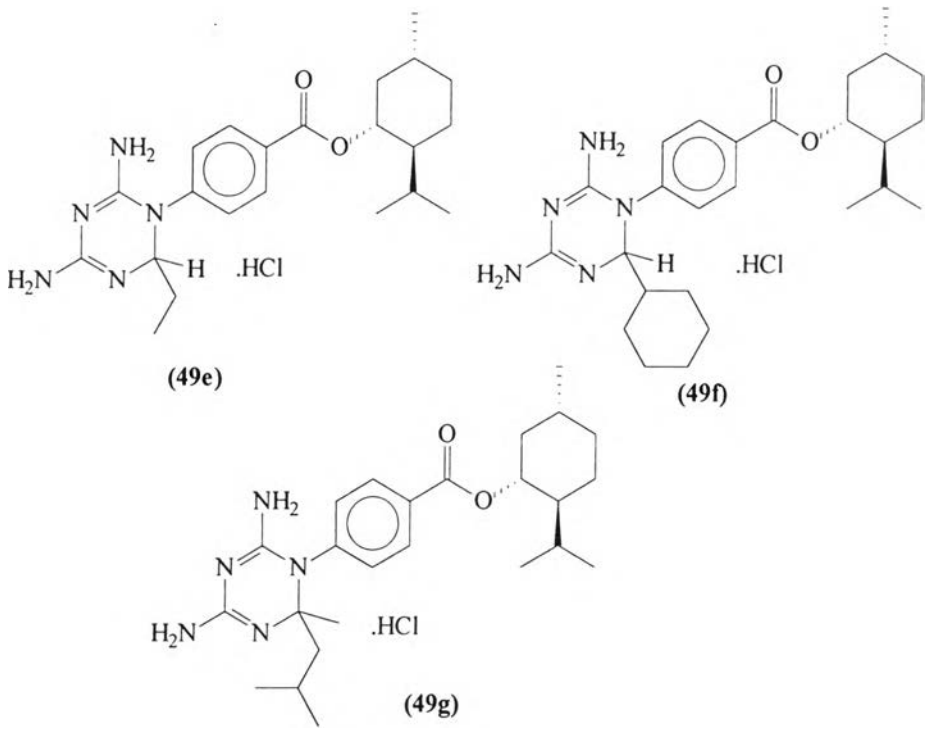
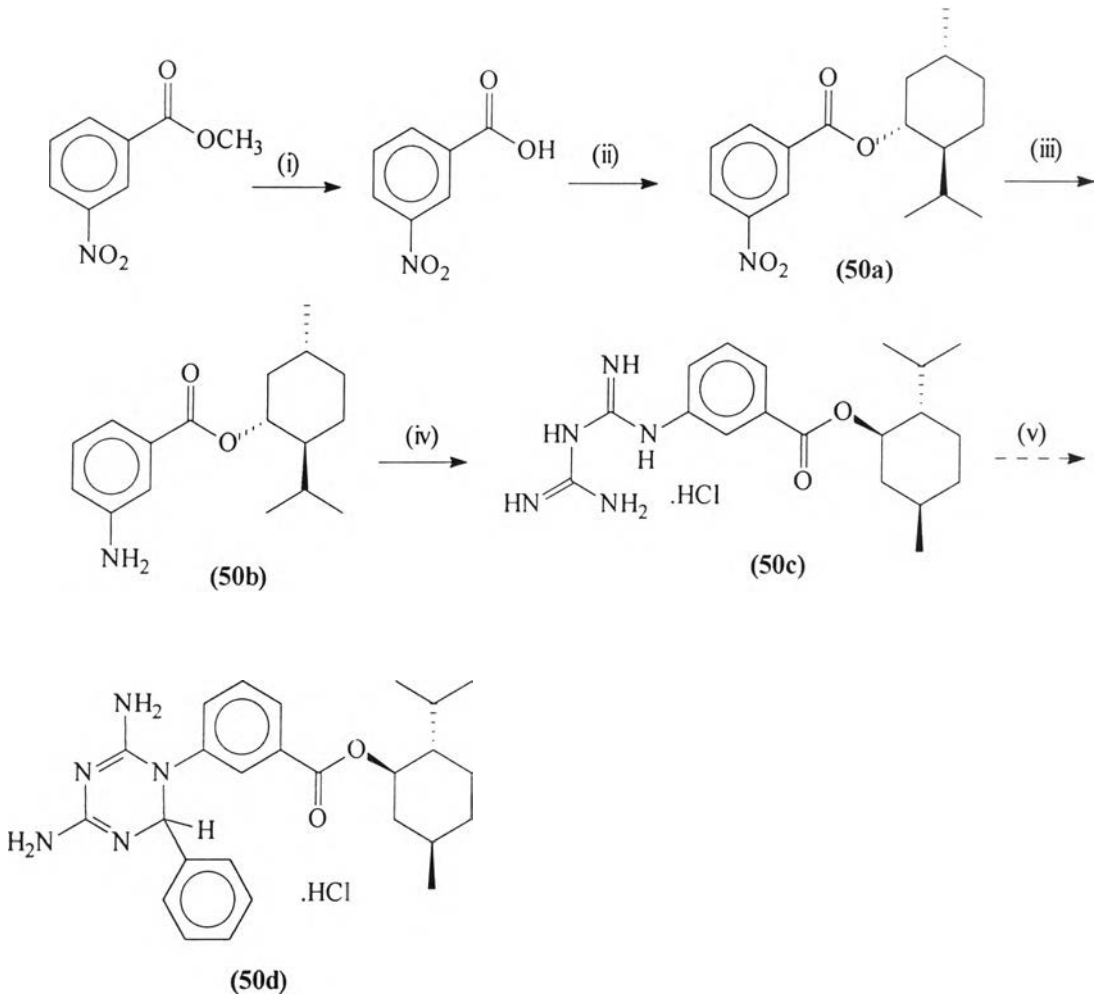


Figure 3.20 Structure of diastereomers **(49e)**, **(49f)** and **(49g)**



i) NaOH, then conc. HCl

ii) oxalyl chloride, DMF, CH_2Cl_2

iii) (-)-menthol, Et_3N

iv) cyclohexene, Pd/C, abs. MeOH, N_2, reflux

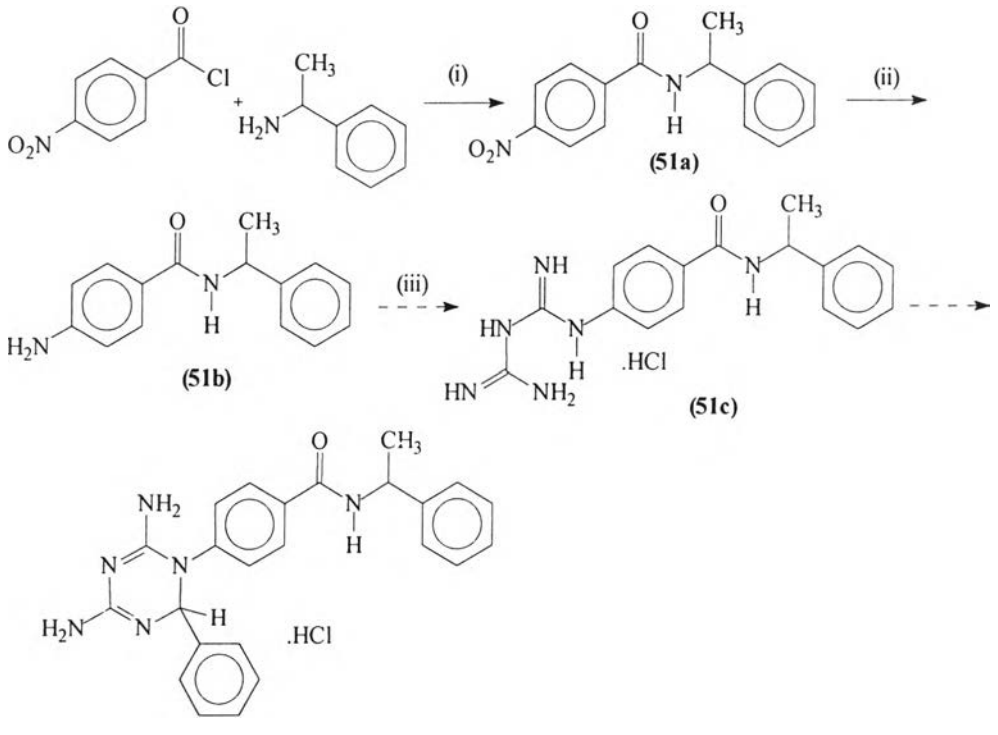
v) dicyanodiamide, abs. EtOH, conc. HCl, reflux

vi) benzaldehyde, TEOA, abs. MeOH, conc. HCl

Figure 3.21 Attempted synthesis of 1-[3′-(2″*S*-isopropyl-5″*R*-methyl-1″*R*-cyclohexyl oxycarbonyl)phenyl]-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(50d)**

Synthesis of the analogous dihydrotriazine **(50d)** has also been investigated (Figure 3.21). The procedure of preparation of compound **(50d)** was analogous to that of **(49d)** but 3-nitrobenzoyl chloride was synthesized from methy-3-lnitro benzoate. Disappointingly, **(50b)** failed to give biguanide **(50c)** when allowed to condense with dicyanodiamide in the presence of concentrated HCl.

Another attempt to find out alternative optically active amino compounds was carried out by attaching α-methylbenzylamine to the aromatic ring *via* an amide bond. (±)-α-Methyl benzylamine was initially used to explore the chemistry.



i) Et_3N, CH_2Cl_2

ii) Cyclohexene, Pd/C, MeOH, N_2, reflux

iii) Dicyanodiamide, conc. HCl, abs. EtOH

Figure 3.22 Attempted synthesis of 4-(α-methylbenzylcarbamoylphenyl)biguanide hydrochloride **(51c)**

Reaction of (±)-methyl benzylamine and *p*-nitrobenzoyl chloride in the presence of triethylamine gave racemic *N*-(α-methyl benzyl)-4-nitrobenzamide **(51a)** (Figure 3.22). It was then reduced to give the chiral amino compound **(51b)**. However, no biguanide **(51c)** was formed in the next step. This route was therefore not investigated further.

The two or three component condensation gave good yield only with aromatic amines and aromatic biguanides as starting material. Analogous reaction with aliphatic amines gave much lower yield.[41] So construction of 1-alkyl-4,6-diamino-1,2-

dihydro-1,3,5-triazine from aliphatic amine must be done using a different method. Another literature[41] route to synthesize this type of compound involves a condensation of dicyanodiamide and a protonated Schiff base as shown in Figure 3.23

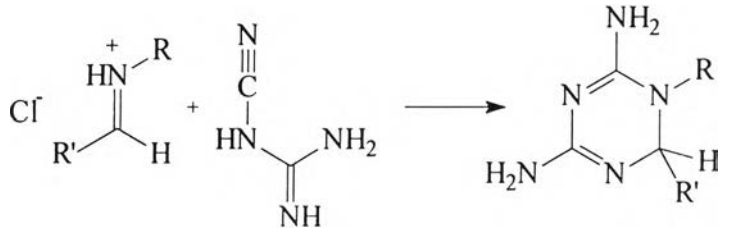


Figure 3.23 Synthesis of dihydrotriazine *via* reaction of Schiff base with dicyanodiamide

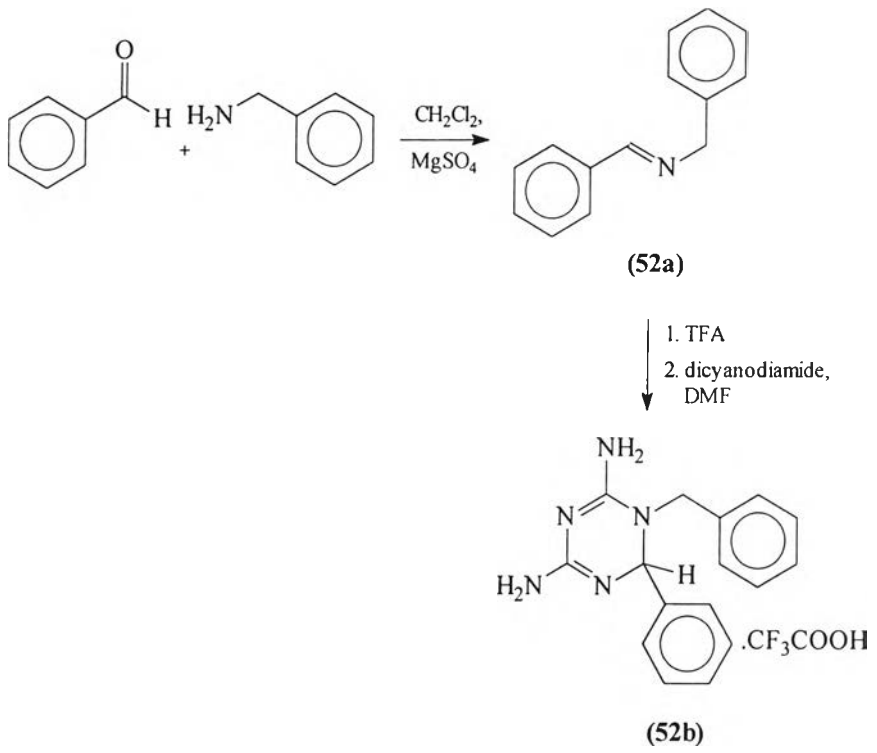


Figure 3.24 Synthesis of 1-benzyl-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine **(52b)** *via* Schiff base

Benzylamine was the first aliphatic amine to be used as a model reaction to synthesize 1-alkyl-4,6-diamino-1,3,5-triazine (Figure 3.24). 1-Benzyl-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine **(52b)** has been previously prepared by reacting the

hydrochloride of Schiff base **(52a)** and dicyanodiamide in dimethylformamide at room temperature. The literature conditions were adjusted to provide more convenient method. It was found that the use of trifluoroacetic acid, a relatively strong organic acid as an acid catalyst, gave the condensation product in acceptable yield without the need to use anhydrous hydrogen chloride as described in the original literature. After stirring at room temperature in a mixture of DMF/CH_3CN for 2 days, TLC indicated formation of a news product. Addition of diethyl ether caused precipitation of **(52b)** as a white solid. As evidenced by H^1 NMR spectra and X-ray analysis (Figure 3.25), it was confirmed that the target structure was successfully synthesized and that the alkyl group was at N_1 position. It was therefore decided to try to the reaction with other optically active aliphatic amines in order to synthesize optically active dihydrotriazine.

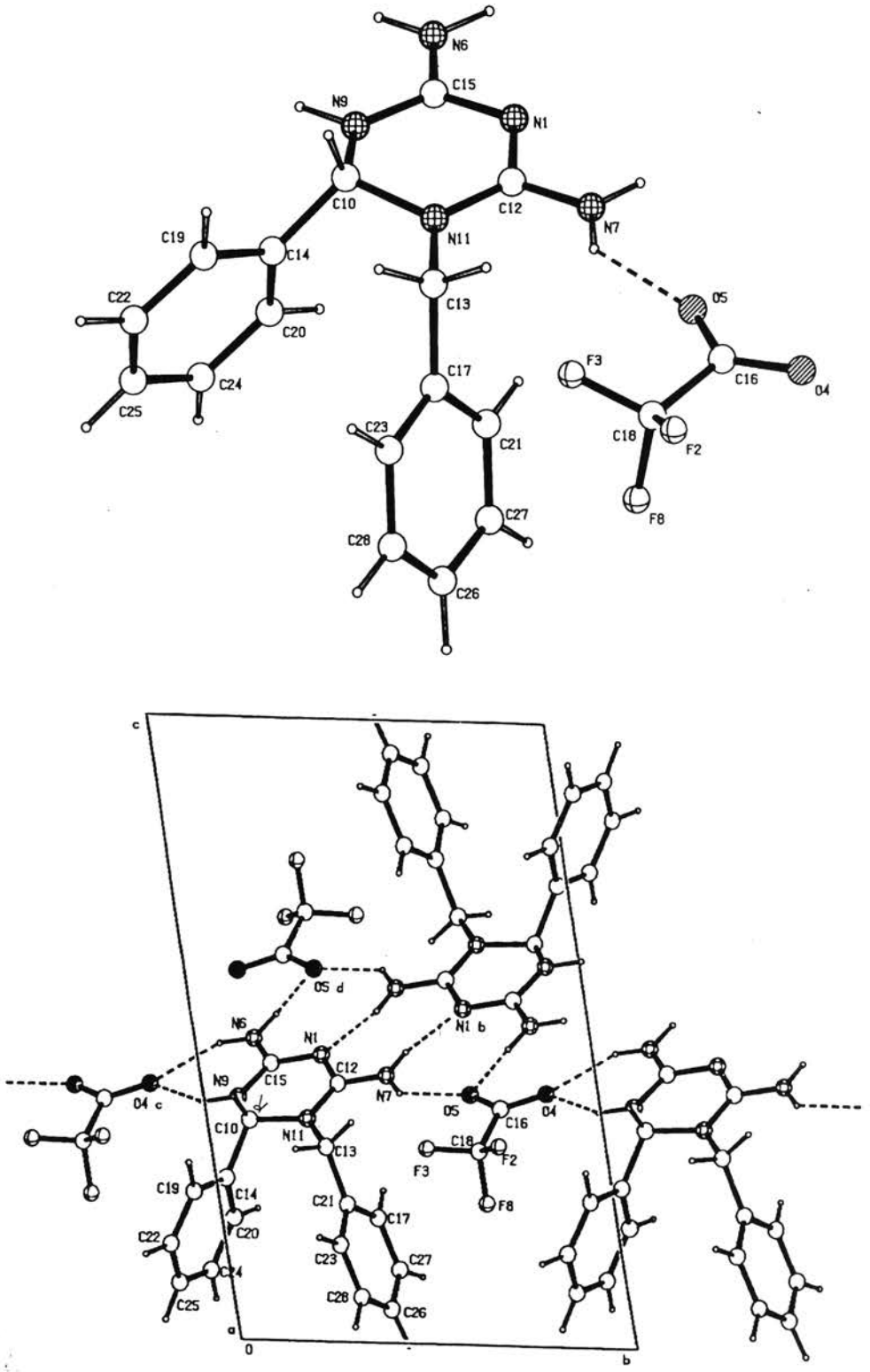
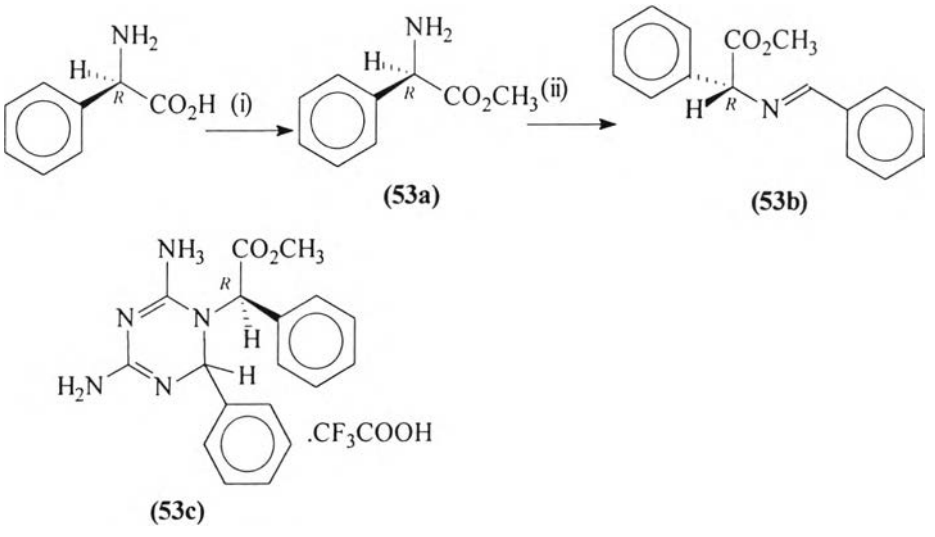


Figure 3.25 X-ray structure of dihydrotriazine **(52b)**



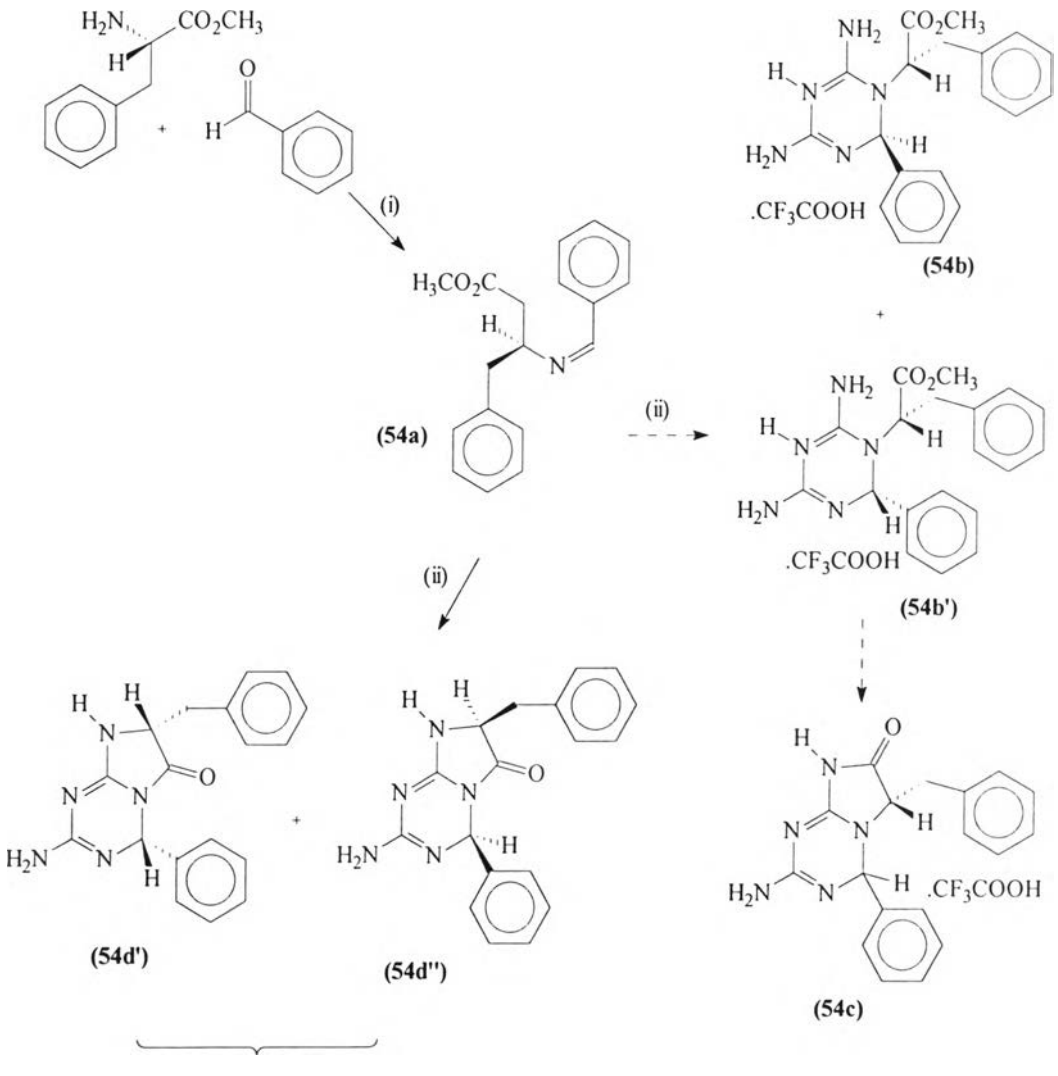
i) CH_3COCl, CH_3OH, reflux

ii) benzaldehyde, CH_2Cl_2, $MgSO_4$

iii) TFA, dicyanodiamide, DMF

Figure 3.26 Attempted synthesis of diasteroisomer **(53b)**

D(-)-Phenylglycine methyl ester was employed as optically active aliphatic amine to synthesize the dihydrotriazine diastereomers **(53c)** (Figure 3.26). Thus the Schiff base formed from benzaldehyde and D-phenylglycine methyl ester was treated with dicyanodiamide in the presence of trifluoroacetic acid in MeCN/DMF. Unfortunately, TLC analysis indicated that a mixture of products had formed.



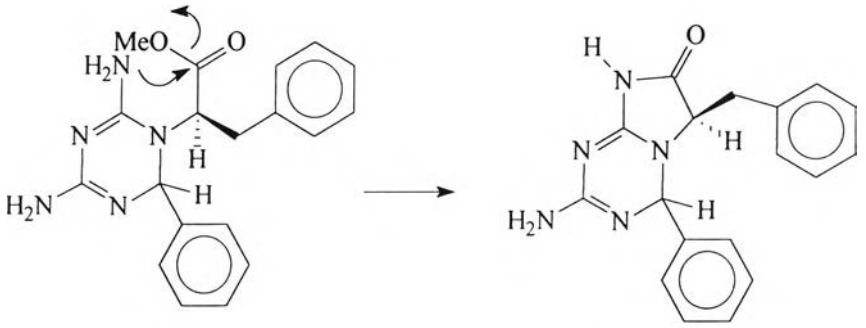
i) $MgSO_4$, CH_2Cl_2

ii) dicyanodiamide, TFA, DMF

Figure 3.27 Attempted synthesis of 2*S*-(2′phenyl-4′,6′-diamino-1′,2′-dihydro-1′,3′,5′-triazin-1′-yl)-2-phenylacetic acid methyl ester trifluoroacetate diastereomers **(54b)** and **(54b′)**

When L-Phenylalanine methyl ester was used as the optically active amine, diastereomeric 2*S*-(2′phenyl-4′,6′-diamino-1′,2′-dihydro-1′,3′,5′-triazin-1′-yl)-2-phenylacetic acid methyl ester trifluoroacetate diastereomers **(54b)** and **(54b′)** were the expected products (Figure 3.27). Reaction of the protonated Schiff base **(54a)**, obtained from reaction of phenylalnine methyl ester with benzaldehyde, and dicyanodiamide in trifluoroacetice acid gave a white crystalline solid **(54d)** after addition of diethyl ether and prolonged standing. ^{1}H NMR indicated that it contained

only one stereoisomer, but the methyl ester peak at 3.75 ppm was missing. Initially, it was believed that the product was **(54c)** resulting from cyclization between the ester group and the amino group at the 4-position as shown in Figure 3.28



(54b) **(54c)**

Figure 3.28 Cyclization of **(54b)** to **(54c)**

However, X-ray crystallographic analysis of the picrate salt of **(54d)** conclusively showed that in fact **(54d)** was an isomeric product with the structure shown in Figure 3.29. Surprisingly, it was found that **(54d)** was a recemic mixture in spite of the fact that optically pure L-phenylalanine methyl ester was used at the beginning.

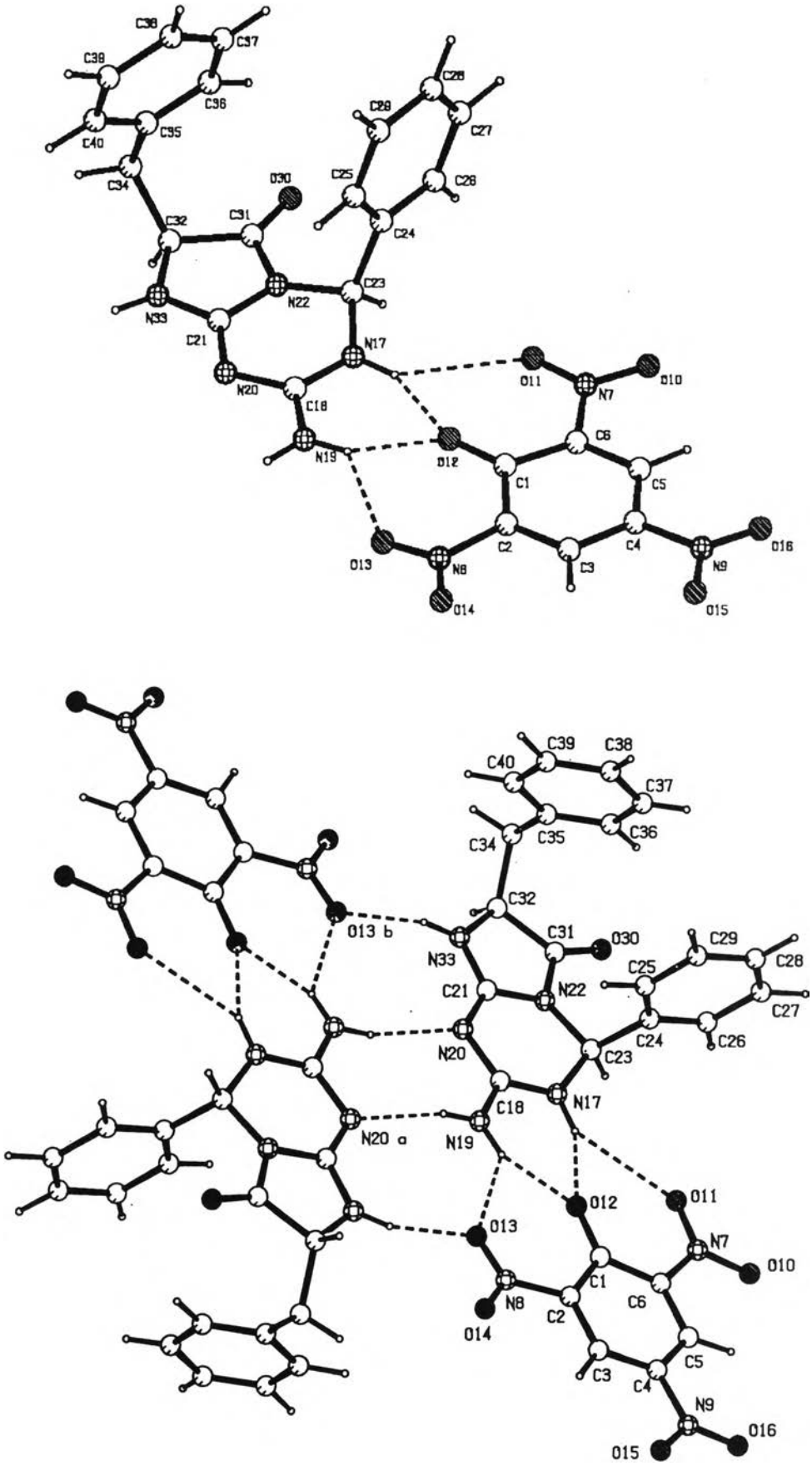


Figure 3.29 X-ray structure of dihydrotriazine **(54d)**

In order to find the explanation for racemization of the phenylalanine residue, a deuterium exchange experiment was performed. A solution of **(54d)** in DMSO-d_6 was treated with a few drops of D_2O. ^1H NMR revealed complete disappearance of the α-CH at 4.77 ppm after standing overnight at 23 °C, which suggested that this proton is quite acidic, presumably due to resonance stabilization of the planar anion (Figure 3.30). This can explain why the α-carbon could recemize easily. From these results, formation of **(54d′)** and **(54d″)** could be explained in Figure 3.31 and 3.32.

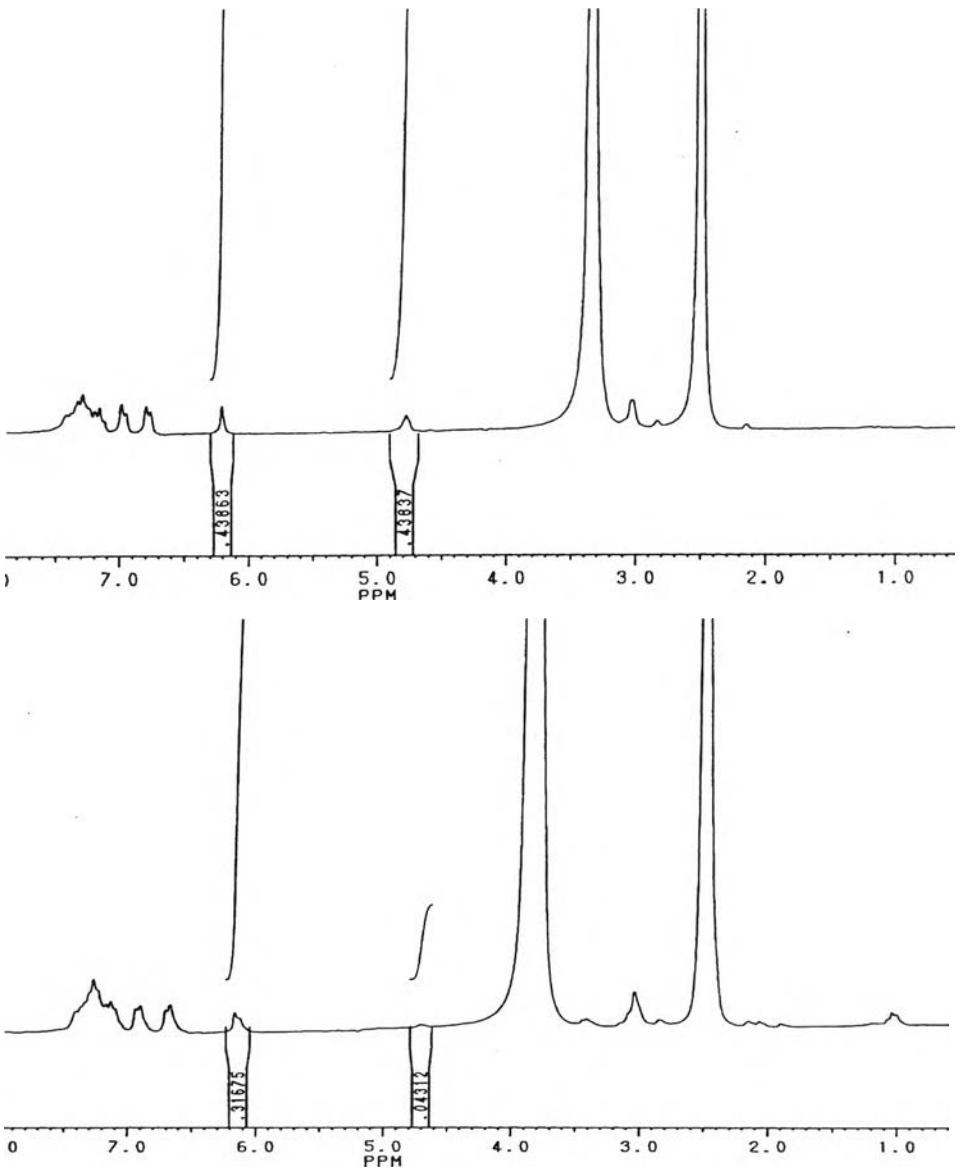


Figure 3.30 ^1H NMR (DMSO, 200 MHz) spectra of the phenylalanine residue before (upper) and after (lower) racemization of the phenylalanine residue

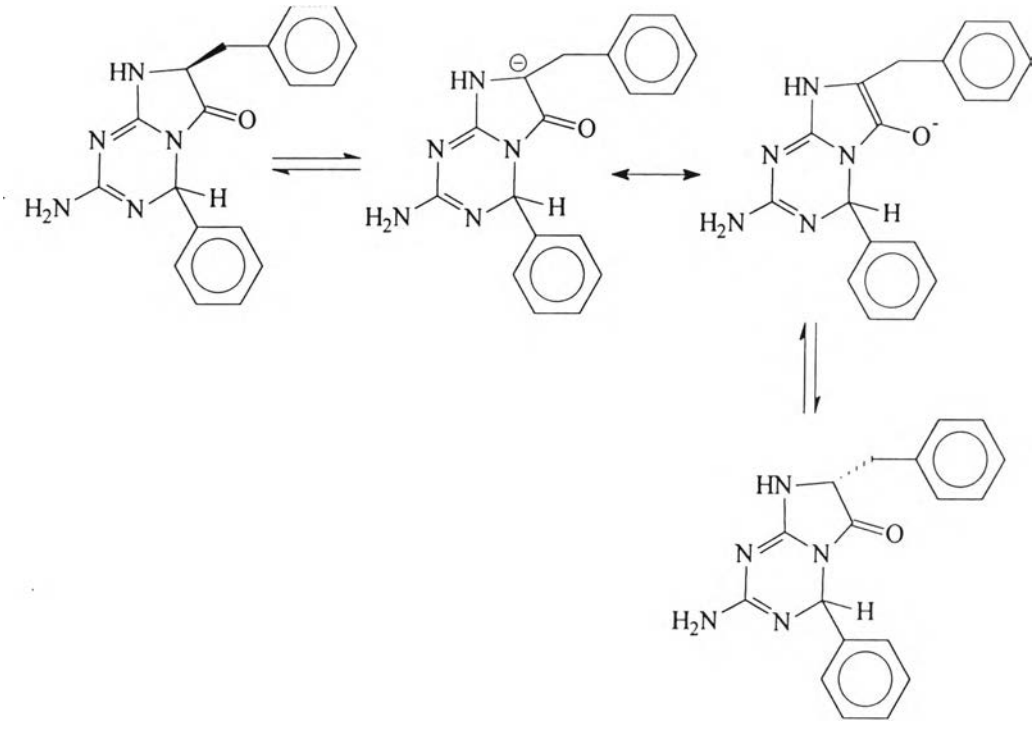


Figure 3.31 Resonance stabilization of the planar anion

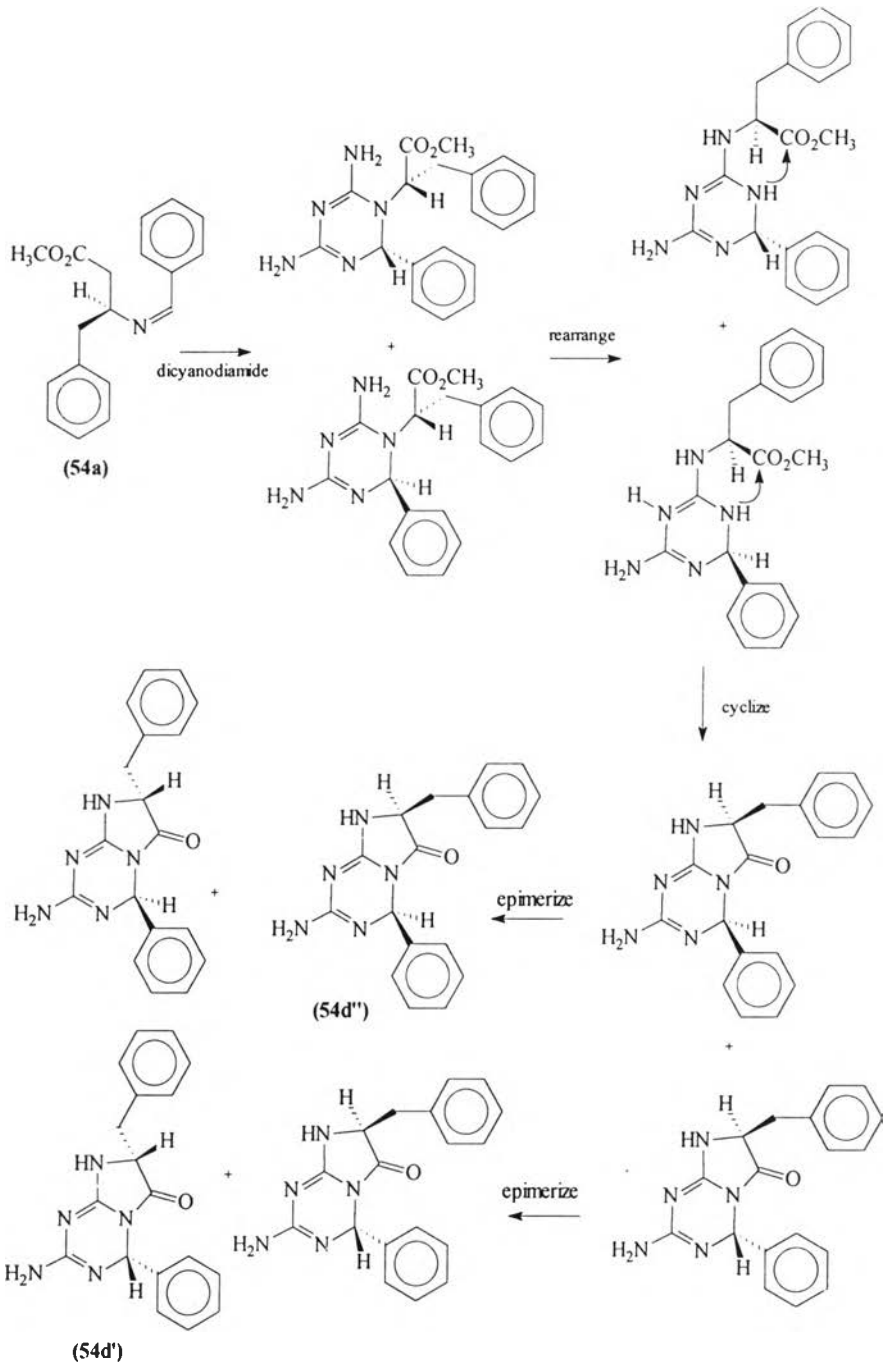


Figure 3.32 Mechanism of formation of all four possible stereoisomers of **(54d)**

According to this mechanism all four possible stereoisomers should form. The fact that only one enantiomeric pair **(54d′)** and **(54d′′)** was obtained indicated that they are the more thermodynamically stable diastereomers, or they may simply crystallize more readily. We have not enough information on relative stability of each stereoisomer of **(54d)**, but no doubt they can crystallize more readily than the other two stereoisomers.

Finally, optically active α-methyl benzylamines which are readily available in both enantiomers were selected as the chiral aliphatic amine component.

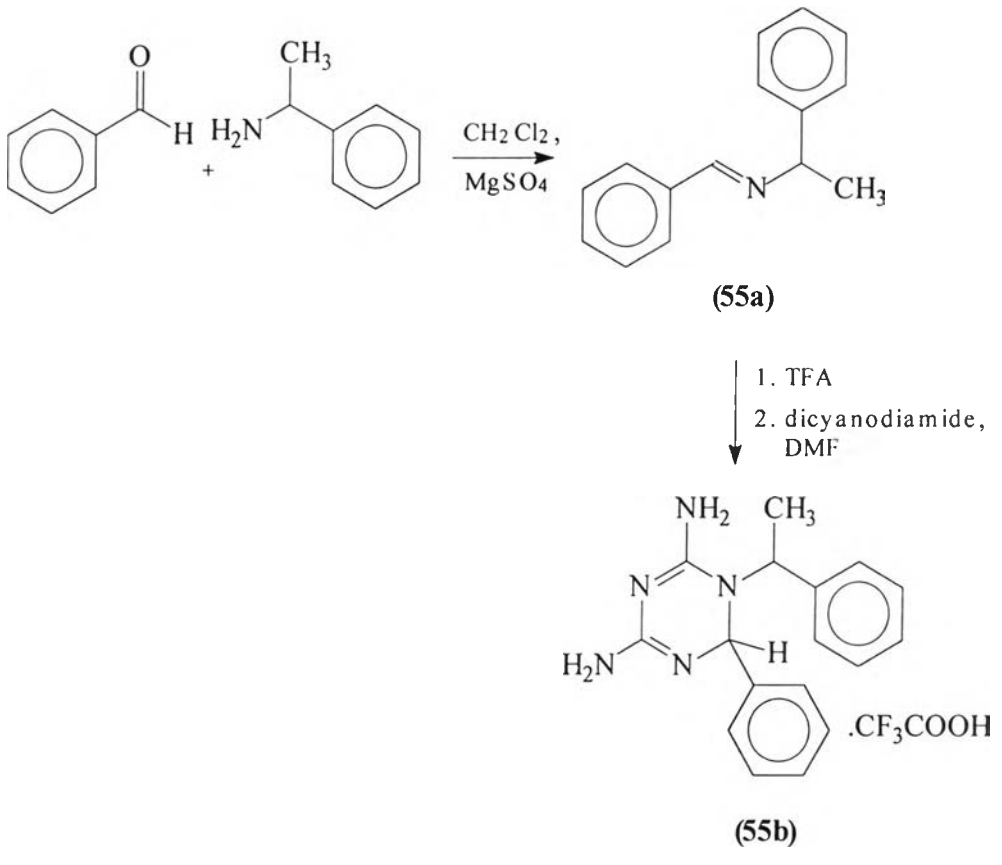


Figure 3.33 Synthesis of 1-(1′*RS*-phenylethyl-2*SR*-phenyl-4,6-diamino-1,2dihydro-1,3,5-triazine trifluoroacetate **(55b)**

Racemic (±)-α-methylbenzylamine was initially used as the starting material for synthesis of **(55b)** (Figure 3.33). The procedure was similar to preparation of **(52b)**. Thus when a Schiff base **(55a)** formed from α-methyl benzylamine and benzaldehyde was reacted with dicyanodiamide in the presence of trifluoroacetic acid, a product **(55b)** was obtained. ^{1}H NMR of **(55b)** indicated that only one out of two possible enantiomeric pairs was formed. X-ray analysis of picrate salt of **(55b)** indicated that it was the enantiomeric 1′*R*, 2*S* and 1′*S*, 2*R* pair (Figure 3.34).

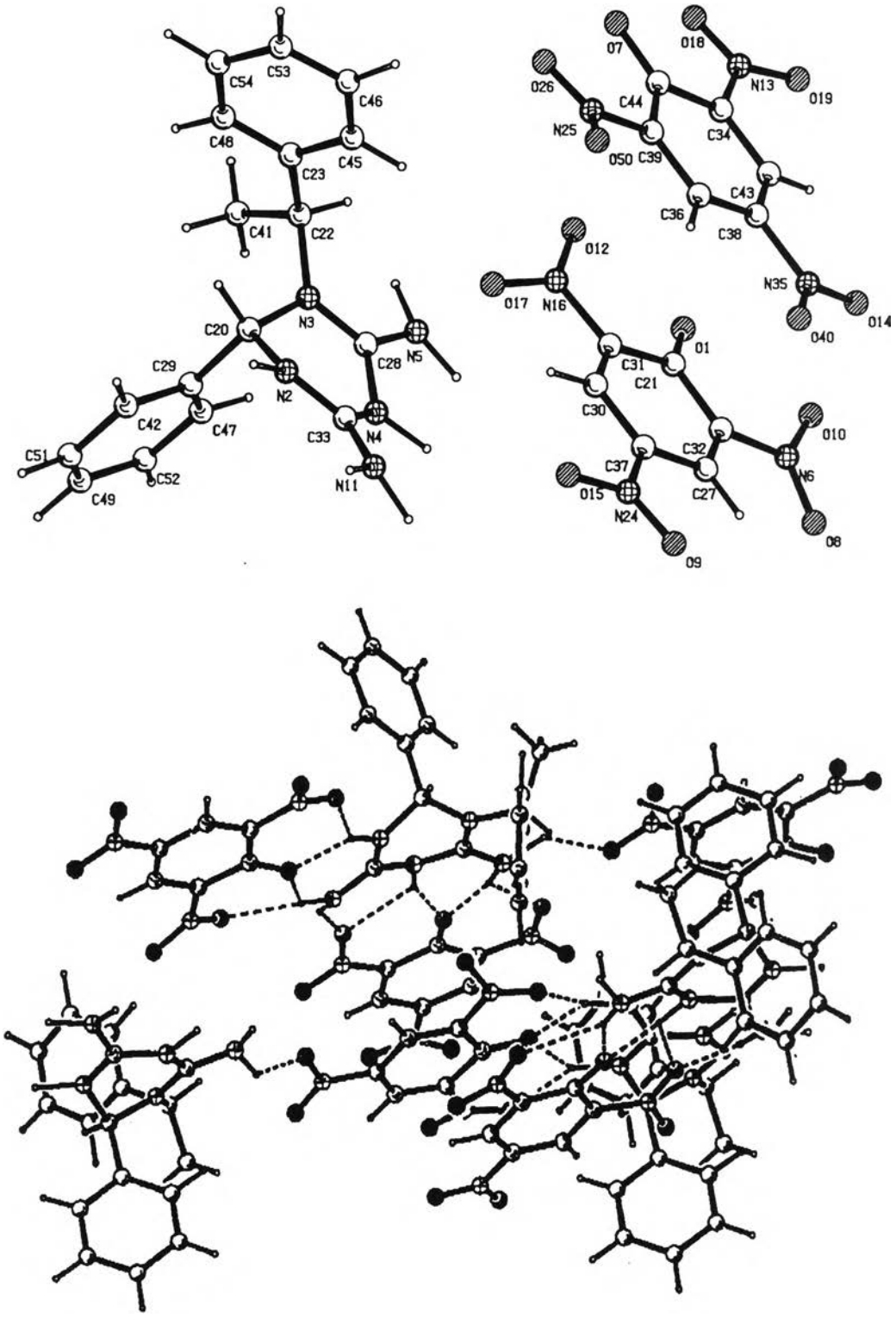


Figure 3.34 X-ray structure of synthesis of 1-(1′*RS*-phenylethyl)-2*SR*-phenyl-4,6-diamino-1,2dihydro-1,3,5-triazine picrate **(55b)**

From the X-ray analysis, it can be inferred that if one enantiomer of optically active α-methyl benzylamine was used, the product obtained would be single

enantiomer with predictable configuration at C_2. Starting from the *R* enantiomer of α-methylbenzylamine, the C_2 configuration would be *S*. In contrast, if the *S* enantiomer was used, C_2 position would have the opposite *R* configuration. Further experiments were therefore performed using optically active α-methylbenzylamines.

Synthesis of **(55b′)** and **(55b″)** were carried out in the analogous manner to the racemic compound starting from optically active *R* and *S* α-methylbenzylamines respectively (Figure 3.35).

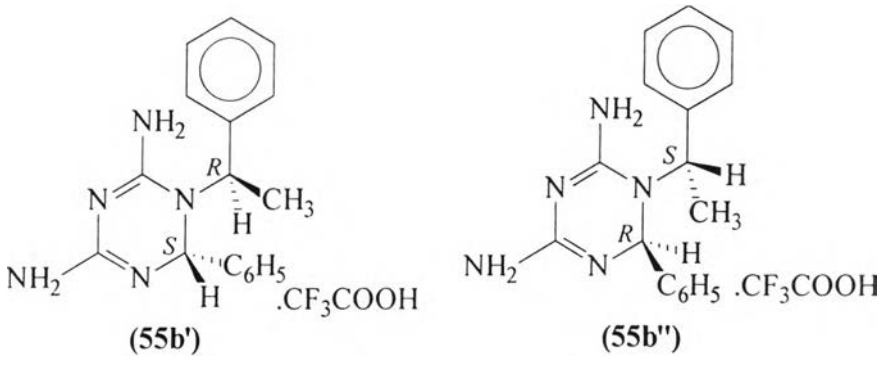


Figure 3.35 The configuration structure of 1-(1′*RS*-phenylethyl)-2*SR*-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine trifluoroacetate **(55b′)** and **(55b″)**

^{1}H NMR of **(55b′)** and **(55b″)** were identical and indistinguishable from the racemic form **(55b)**. Attempts to crystallize either **(55b′)** or **(55b″)** for X-ray analysis failed. However, CD and $[\alpha]_D$ indicated that both compound are optically active and are mirror images to each other. Furthermore, the NOE analysis of **(55b″)** in Figure 3.36 revealed that when the proton of methyl group at 1.29 ppm was irraidated, NOE enhancement was obserbed at 5.50 ppm ($C\underline{H}CH_3$) and 5.65 ppm (C_2-H) which is in good agreement with the assumption that the C_2 has an *R* configuration based on the conformation obtained from X-ray analysis of the racemic compound. From these results, it may be concluded that the first optically active 1-alkyl-4,6-diamino-1,2-dihydro-1,3,5-triazines with defined configuration at C_2 have been successfully prepared by asymmetric synthesis. Careful examination of the crude reaction mixture revealed that **(55b′)** was preferentially formed compared to its diastereoisomer possessing *R*-configuration at C_2 (dr ~ 4.8:1.0). After crystallization, only single stereoisomer was obtained.

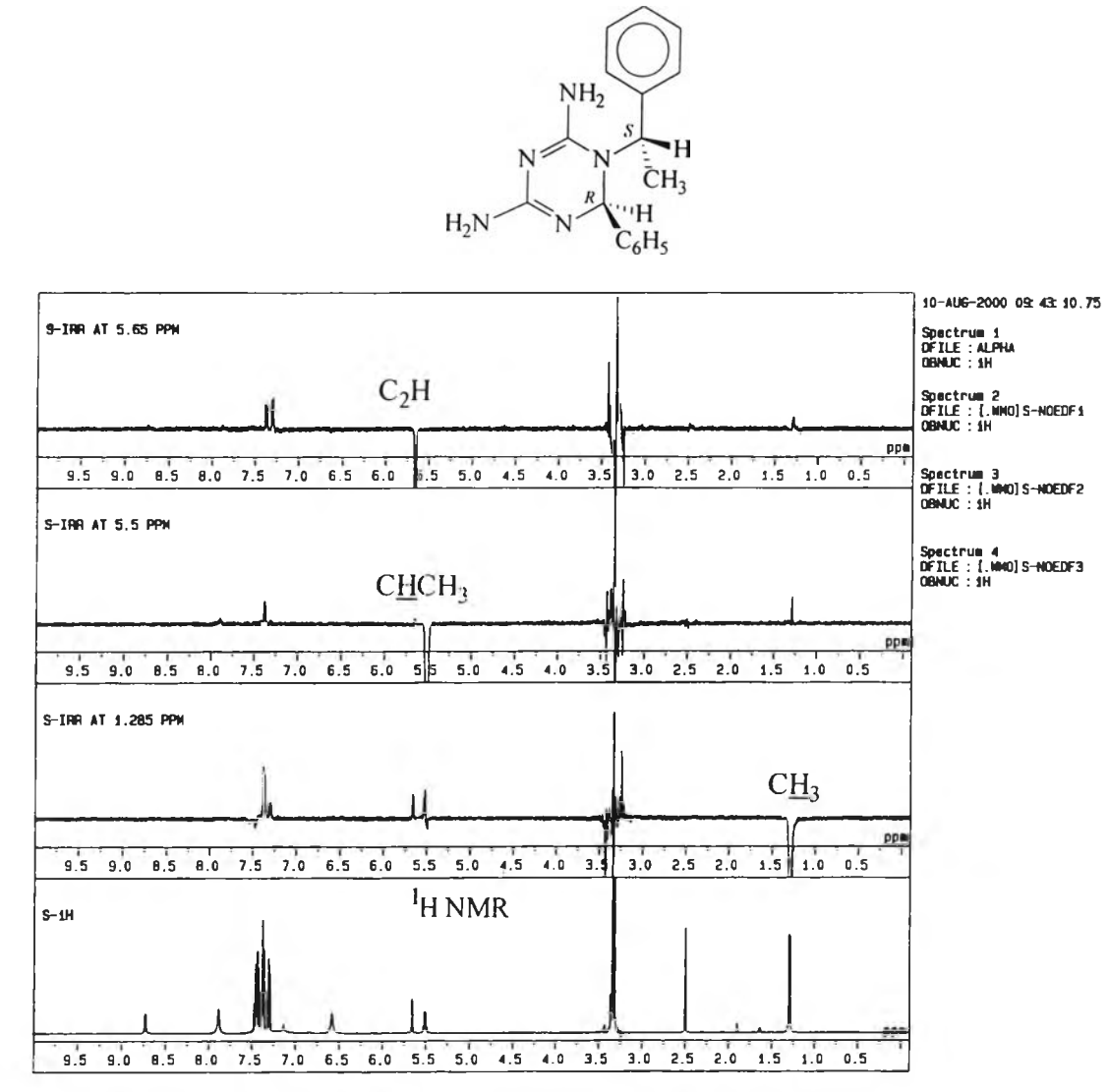


Figure 3.36 NOE difference spectra of 1-(1′*S*-phenylethyl)-2*R*-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine trifluoroacetate **(55b′′)**

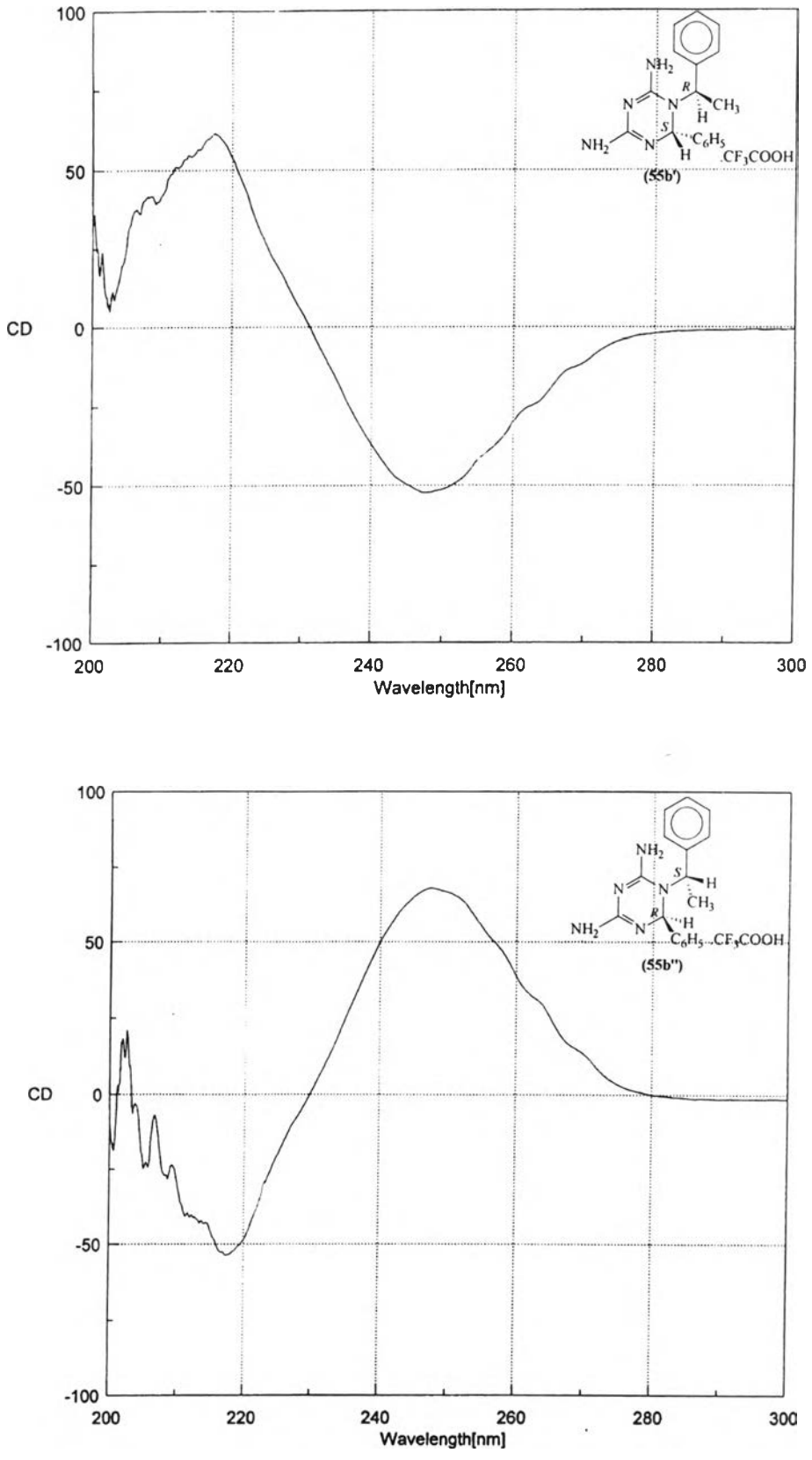
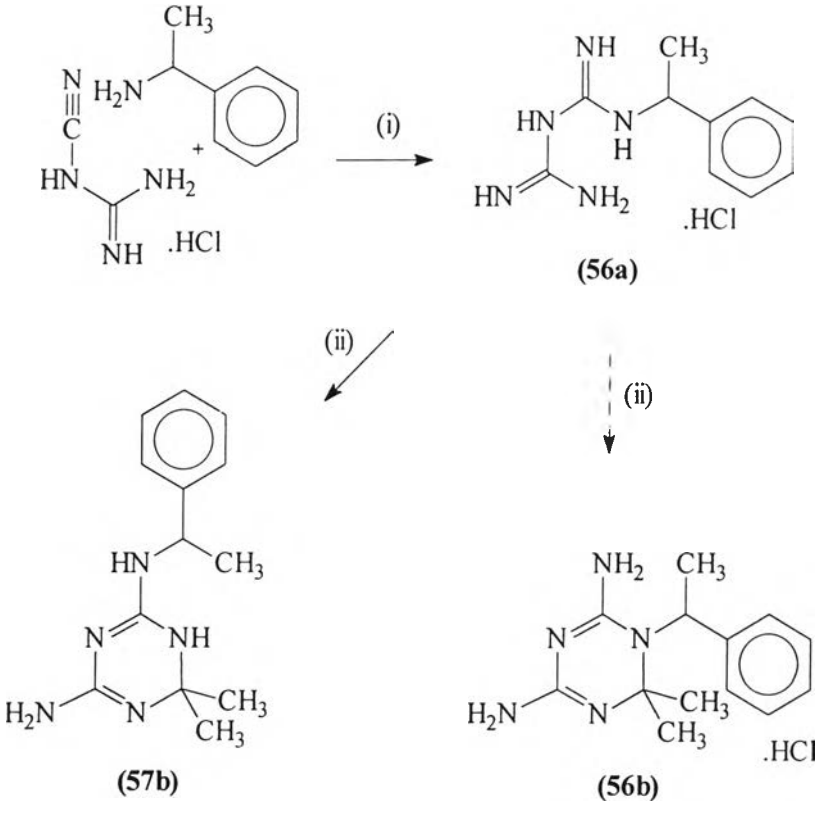


Figure 3.37 CD spectra (MeOH) of diastereomers **(55b')**; 49.1 μM and **(55b'')**; 51.5 μM

Although the N_1-substituent of **(55b′)** was similar to **(55b″)**, their configuration were different leading to the opposite CD signals (Figure 3.37). The different configuration at N_1 position might have unpredictable effect to the bioactivity. For this reason, we have also attempted synthesize **(56b)** which contained two methyl group at C_2 position to investigate the effect of configuration of the N_1-substituent.



i) dicyanodiamide, fuse 160 °C

ii) TEOA, acetone, conc. HCl

Figure 3.38 Attempted synthesis of (*RS*)-1-α-methylbenyl-2,2-dimethyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(56b)**

(±)-Methylbenzylamine hydrochloride was fused with dicyanodiamide for 6 hours to give the biguanide hydrochloride **(56a)**. Unfortunately, **(56b)** was not successfully synthesized when the biguanide hydrochoride was cyclized with acetone in the presence of HCl/TEOA but **(57b)** was obtained instead (Figure 3.38). NOE analysis in which NOE enhancement at the C_2-CH_3 resonance at 5.13 ppm was

observed on irradiation of the N_1-methyl proton at 1.40 ppm and methyl at C_2-CH_3 (5.13 ppm) confirmed that it is the rearrangement product **(57b)** (Figure 3.39).

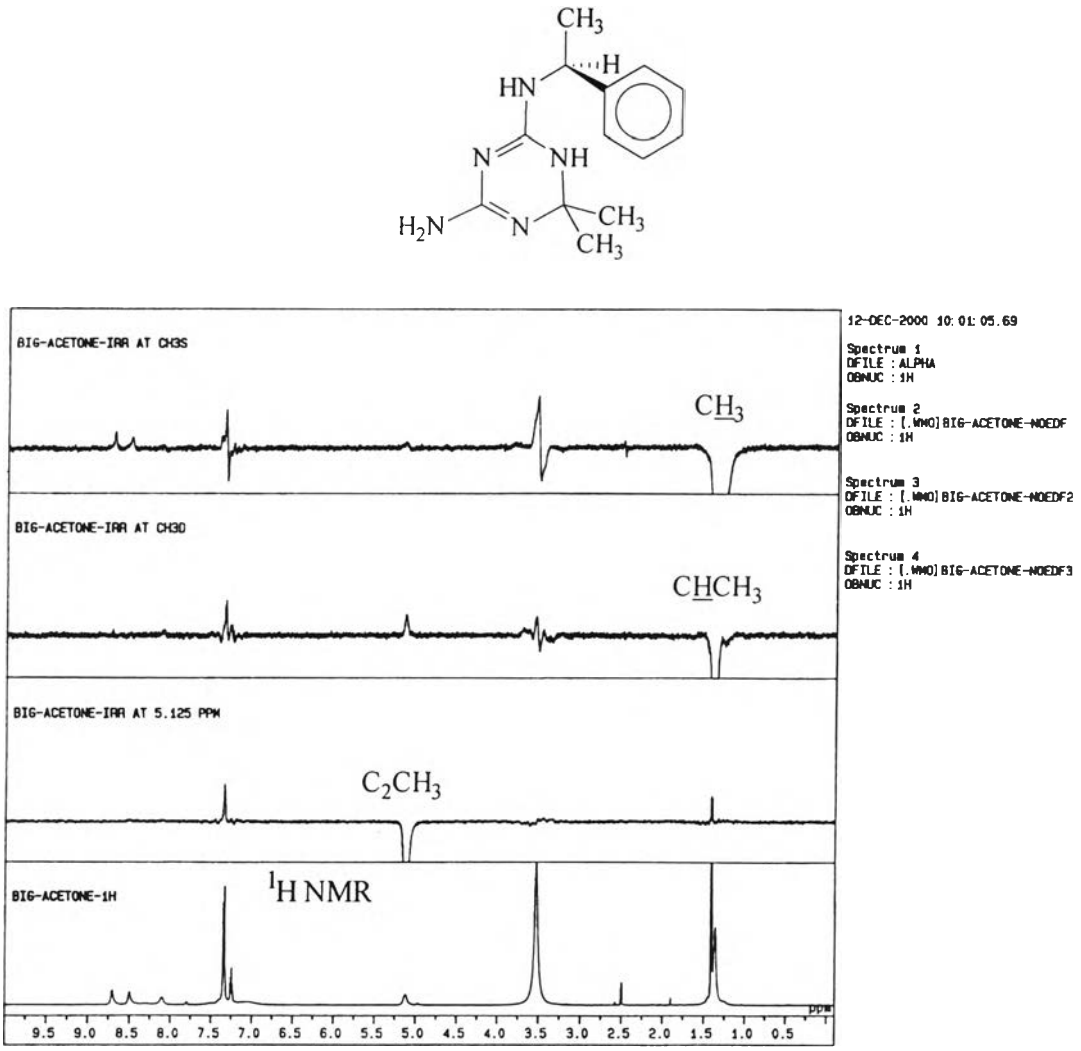


Figure 3.39 NOE spectra of rearrangement product **(57b)**

There was another way to study the influence of N_1-optically active methylbenzyl moiety to bioactivity by synthesizing compound **(55b‴)** which possessed the same *S*-configuration at N_1 but the configuration at C_2 position was different. Comparing the bioactivity of **(55b′)**, **(55b″)** and **(55b‴)** might provide some useful information (Figure 3.40).

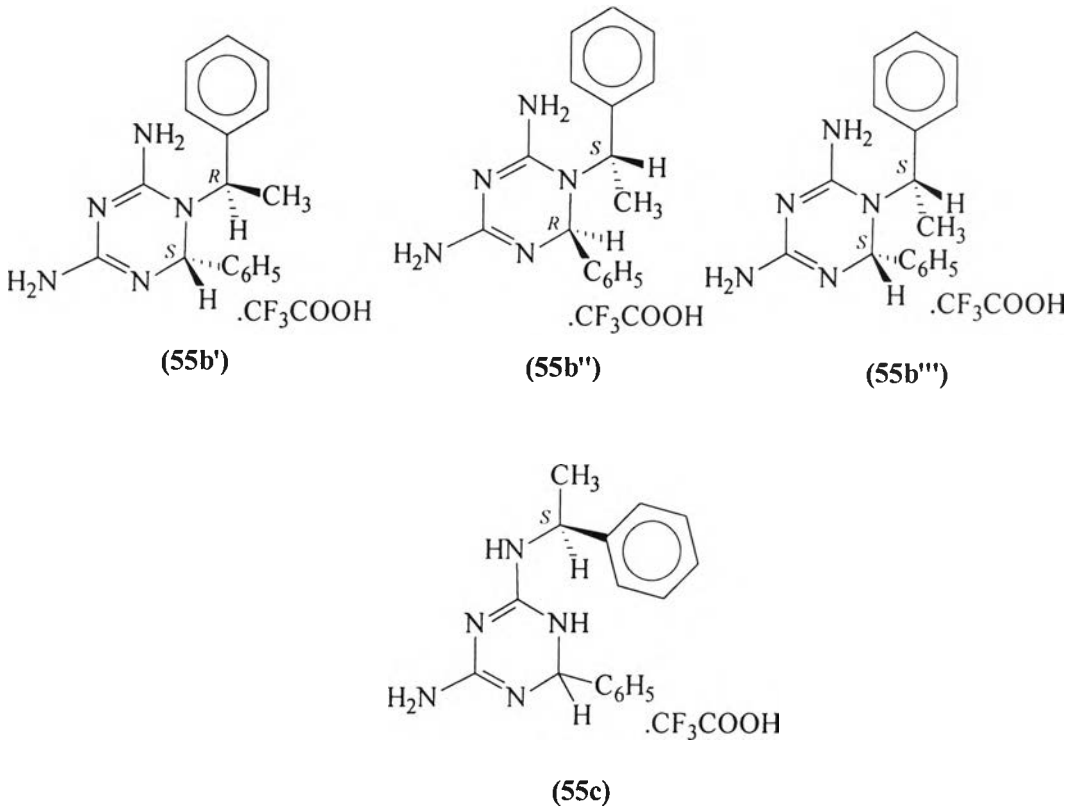


Figure 3.40 Structure of optically active dihydrotriazines **(55b′)**, **(55b″)**, **(55b‴)** and **(55c)**

We have previously shown that the C_2 position of dihydrotriazine readily racemize on heating (section 2.2). Consequenly, synthesis of the enantiomer **(55b‴)** was accomplished by heating **(55b″)** in acetonitrile for 45 hours which gave an equilibrium mixture of **(55b″)** and **(55b‴)** (7:3) (see section 3.4). Prolonged heating caused complete rearrangement to give **(55c)** which complicated the separation process therefore over-heating should be avoided. Separation of **(55b‴)** was first attempted by preparative TLC. But this was not successful therefore reverse phase HPLC was attempted using 15% acetonitrile and 85% 0.02 M triethyl ammonium acetate as mobile phase. Chromatogram in Figure 3.41 revealed 2 peaks due to two isomers. The first major peak (t_R = 49.1 min) was the unchanged **(55b″)** and the second peak (t_R = 60.1 min) was the desired **(55b‴)** which was confirmed by spiking with the pure diasteroisomer **(55b″)**. **(55b‴)** was collected from the reverse phase column and was purified again by HPLC. After the second purification about 0.4 mg of pure **(55b‴)** was obtained. It was kept in the freezer before sending to bioactivity tests.

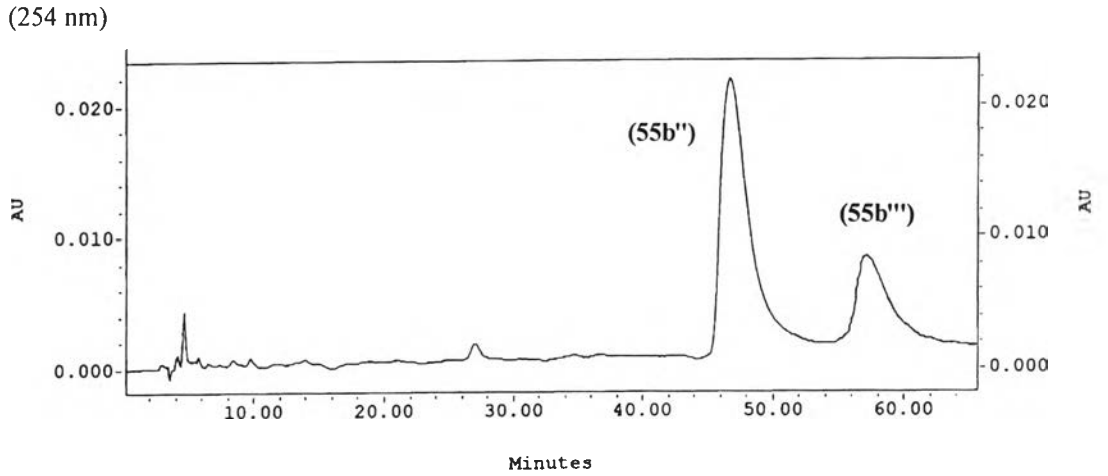


Figure 3.41 Chromatogram of dihydrotriazines **(55b'')** and **(55b''')**

Optically active Shiff bases derived from the reaction of benazaldehyde and L-leucinol, L-phenyl alaninol were also prepared for synthesis optically active dihydrotriazine **(58)** and **(59)** respectively (Figure 3.42). However, all attempts to cyclize these hydroxy group-containing Schiff base failed. The explanation could be due to side reactions with the hydroxy group.

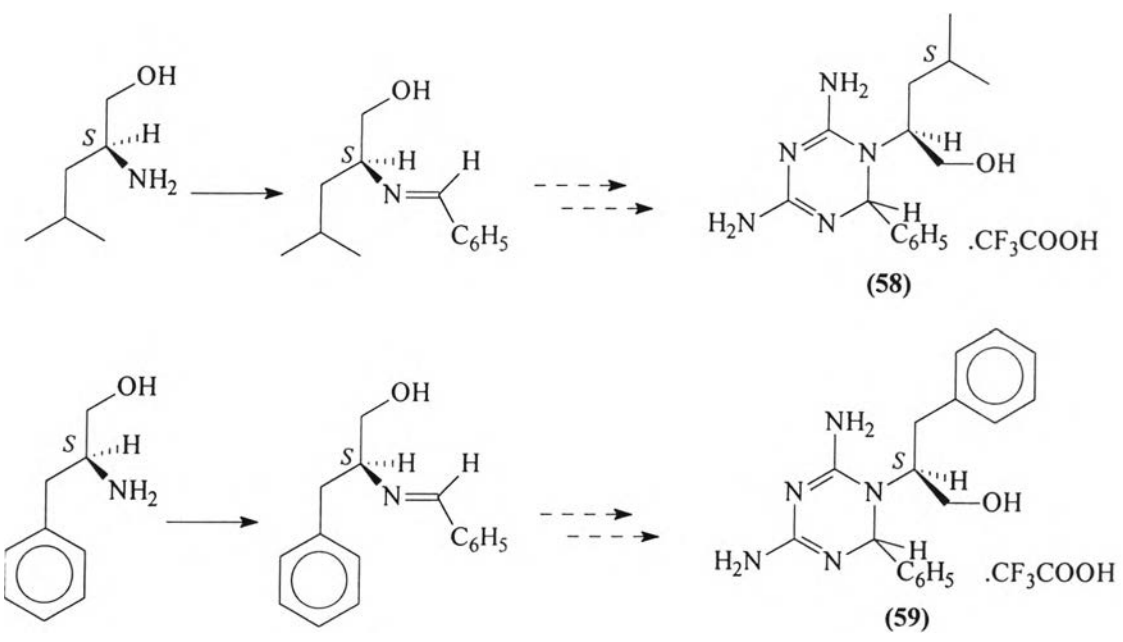


Figure 3.42 Attempted synthesis of optically active dihydrotriazines **(58)** and **(59)**

Since all attempts to synthesize optically active dihydrotriazine based on N_1-aryl-4,6-diamino-1,2-dihydro-1,3,5-triazine system were unsuccessful, and those based on 1-allyl-4,6-diamino-1,2dihydro-1,3,5-triazine although gave the required optically active products, it proved difficult to study the biological activities due to the interference from the non-removable chiral auxiliary, it was therefore desirable to find way to attach a removable chiral auxiliary to the dihydrotriazine which could be removed once the stereochemistry at C_2 has been set up.

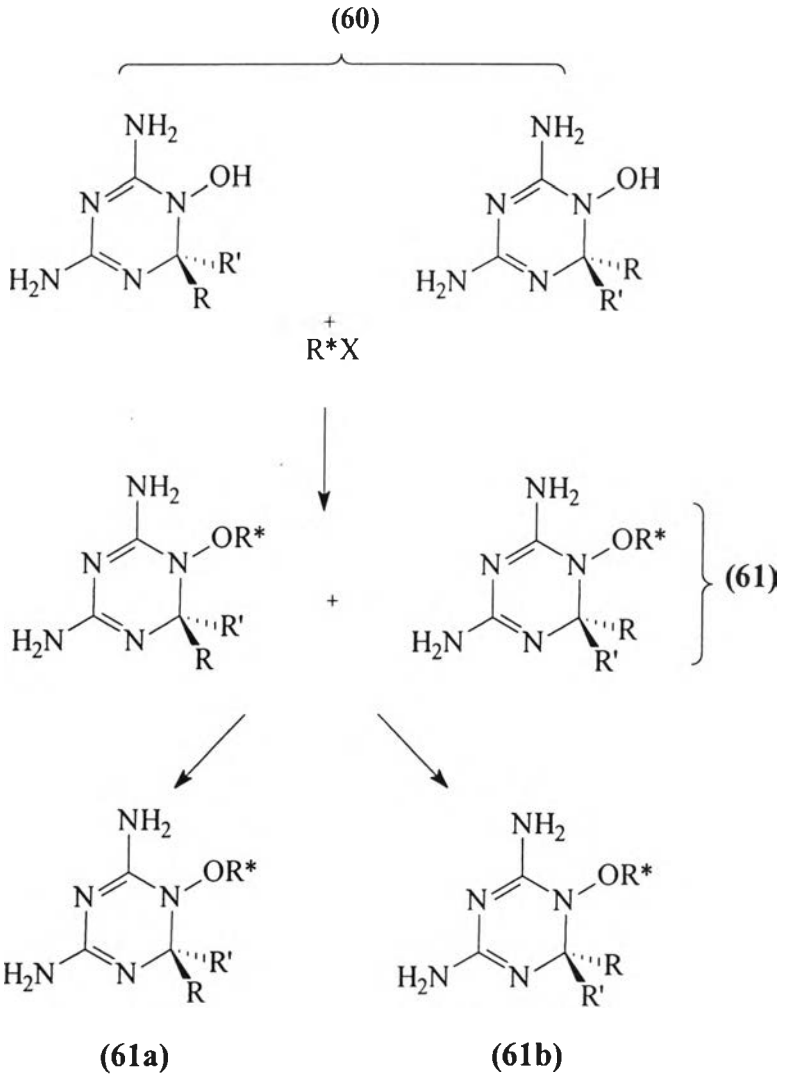


Figure 3.43 Alkylation of N-OH bond in dihydrotriazine of N_1-hydroxy using optically active alkyl halides

One attractive possibility is to attach the chiral auxiliary *via* an N-OH bond in dihydrotriazine of type **(60)** (Figure 3.43). Mamalis[42-43] has been shown that *N*-

hydroxy triazine **(60)** readily obtained from *N*-*O*-benzylderivative could be alkylated with alkyl halide to give product such as **(61)**, shown in Figure 3.44. If one use an optically active benzylic-type alkyl bromide, the resulting diastereomeric dihydrotriazine formed could then be separated in a conventional way. After that, the chiral auxiliary then removed by hydrogenolysis and the optically active *N*-hydroxy triazine further alkylated with a suitable alkyl halide for further biological testing.

In the first experiment, a model reaction between alkyl halide **(61a)**, obtained from a reaction of D(-)-phenylglycine with aqueous $HBr/NaNO_2$ and *N*-hydroxytriazine **(60b)** using KOH as a base. TLC analysis indicated that **(61b)** had not formed. We proposed that the carboxyl group at the alkyl part in **(61a)** might be interfering the reaction. Hence the carboxyl group should be protected.

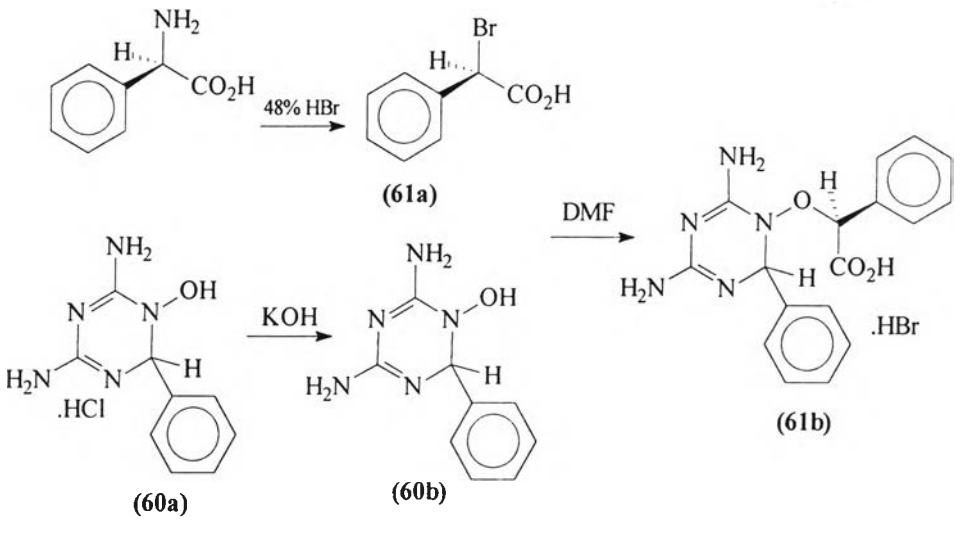
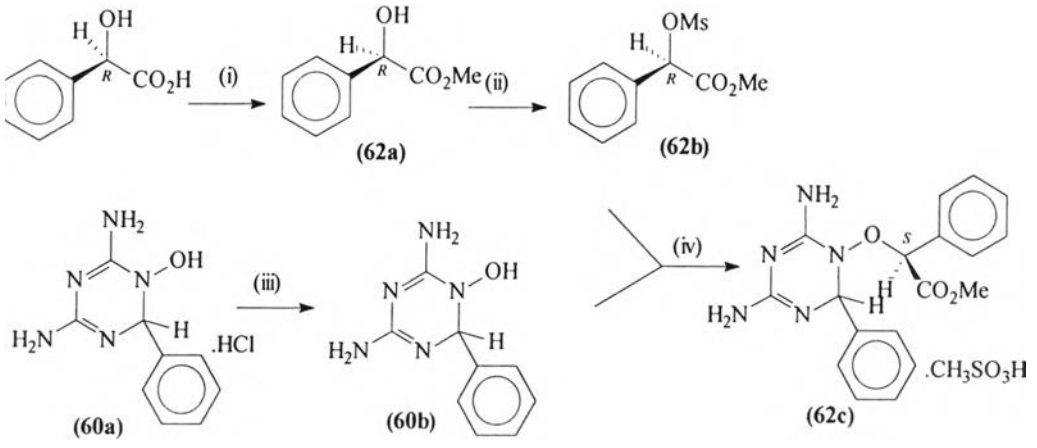


Figure 3.44 Attempted synthesis of *S*-α-(2′-phenyl-4′,6′-diamino-1′,2′-dihydro-1′,3′,5′-triazine-1′-yloxy)phenylacetic acid hydrobromide **(61b)**

From the proposal above, the experiment was repeated (Figure 3.45). The carbonyl group of D (-)-mandelic acid was protected as a methyl ester **(62a)** by reaction with $MeOH/H_2SO_4$. Mesylation of **(62a)** gave **(62b)** in 98% yield. Reaction of **(62b)** with **(60b)**, however, gave no desired product. The reaction was therefore not pursued further.



i) conc. H_2SO_4, MeOH

ii) MsCl, Et_3N, CH_2Cl_2

iii) KOH, MeOH

iv) DMF

Figure 3.45 Attempted synthsis of *S*-α-(2′-phenyl-4′,6′-diamino-1′,2′-dihydro-1′,3′-5′-triazin-1′-yloxy)phenylacetic acid methyl ester methanesulfonate **(62c)**

3.4 Racemization and rearrangement of 4,6-diamino-1,2-dihydro-1,3,5-triazine

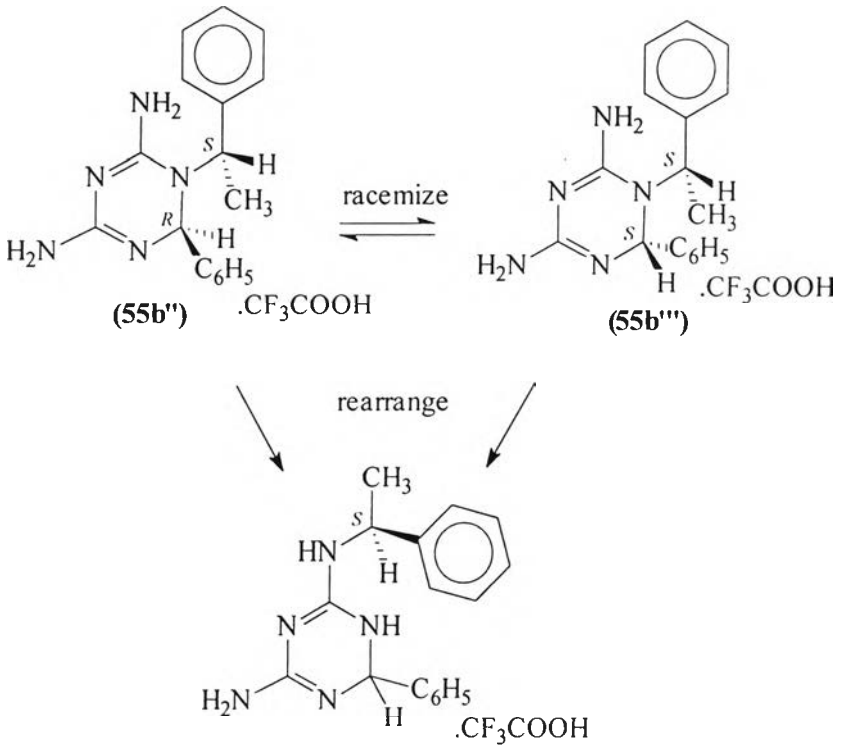


Figure 3.46 Racemization and rearrangement of **(55b'')**

Since **(55b'')** is a dihydrotriazine bearing two chiral centres, one at C_2 and the other at N_1, racemization of this compound at C_2 will give diastereomeric products (Figure 3.46) which should be easily distinguished by ^1H NMR. It was therefore chosen as a model compound for racemization studies. In the experiment, **(55b'')** was dissolved in DMSO and heated at 100 °C overnight. It was found that rearrangement of **(55b'')** proceeded rapidly under this condition. ^1H NMR in Figure 3.47 revealed complete disappearance of the methyl signal of compound **(55b'')** at 1.29 ppm and a new methyl signal of the rearrangement product was observed at 1.44 ppm. The rate of rearrangement was slower under milder condition. Therefore when **(55b'')** was heated at 50 °C, another methyl peak (1.62 ppm) which was neither **(55b'')** nor the rearrangement product **(55c)** was observed. Upon increasing the temperature or prolonged heating, this peak disappeared to give the rearrangement product as previously observed. It was believed that this intermediate peak was the other one diastereomer of **(55b''')** as evidence from the similar NMR and UV-absorption. The

result led to the synthesis and isolation of the C_2-epimer of **(55b″)** which was described in section 3.3.

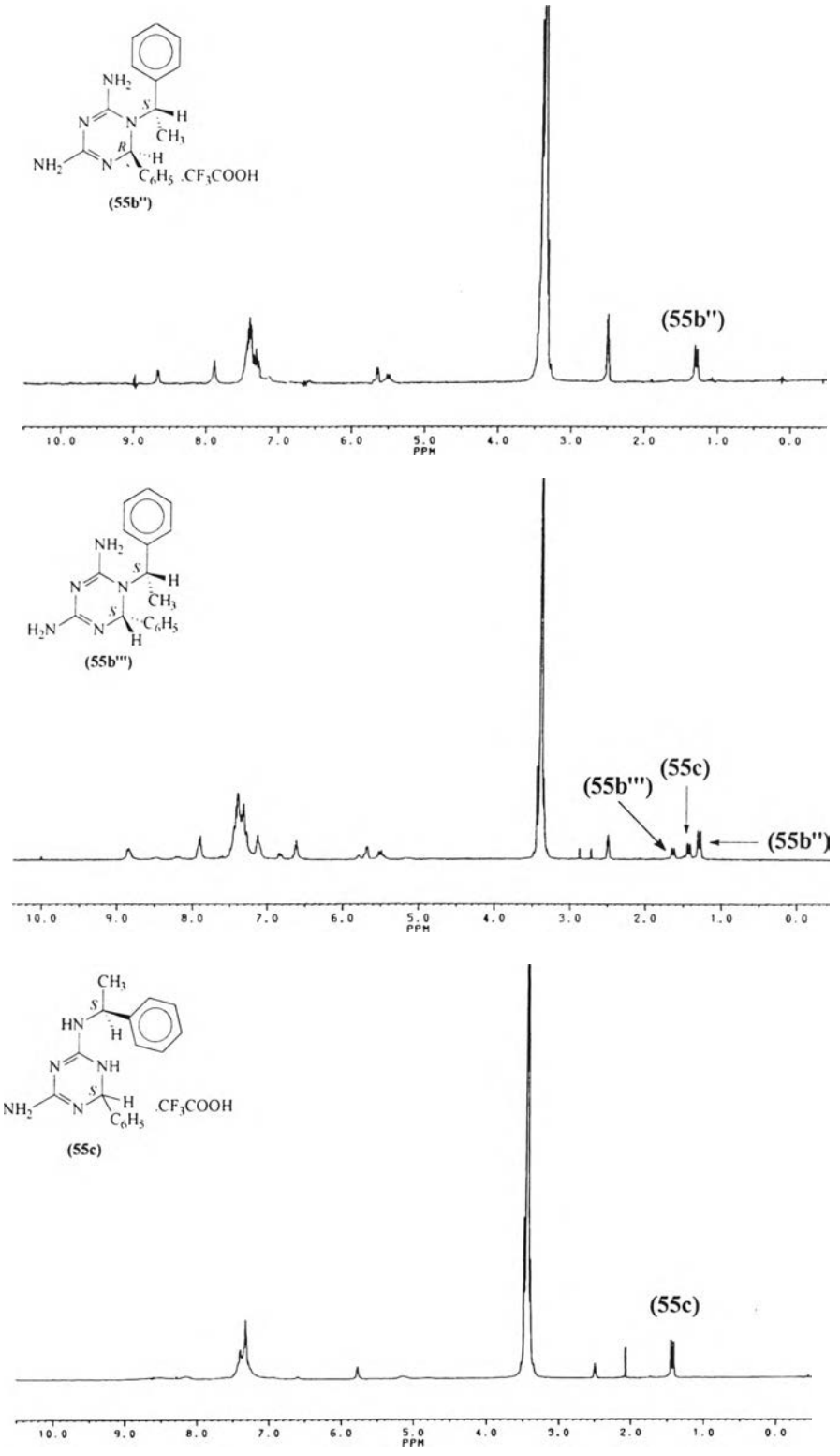


Figure 3.47 ^{1}H NMR spectra (DMSO, 200 MHz) of **(55b″)** before, between, and after rearrangement respectively

From the literature,[39-40] it was known that the triazine compound undergoes a thermodynamically favorable rearrangement. The rearrangement process was believed to proceed through the ring-opened intermediate resulting from N_1-C_2 cleavage (Figure 3.48). Racemization of dihydrotriazine had not been previously reported, the mechanism was therefore unknown. The study of rearrangement of dihydrotrizine **(55b'')** above lead to the assumption that the racemization may occur *via* the same intermediate of rearrangement (Figure 3.48). As a result, the further experiment was made to understand the mechanism of the rearrangement and racemization. The first experiment was to study the effect of substituents to the rate of rearrangement/ racemization.

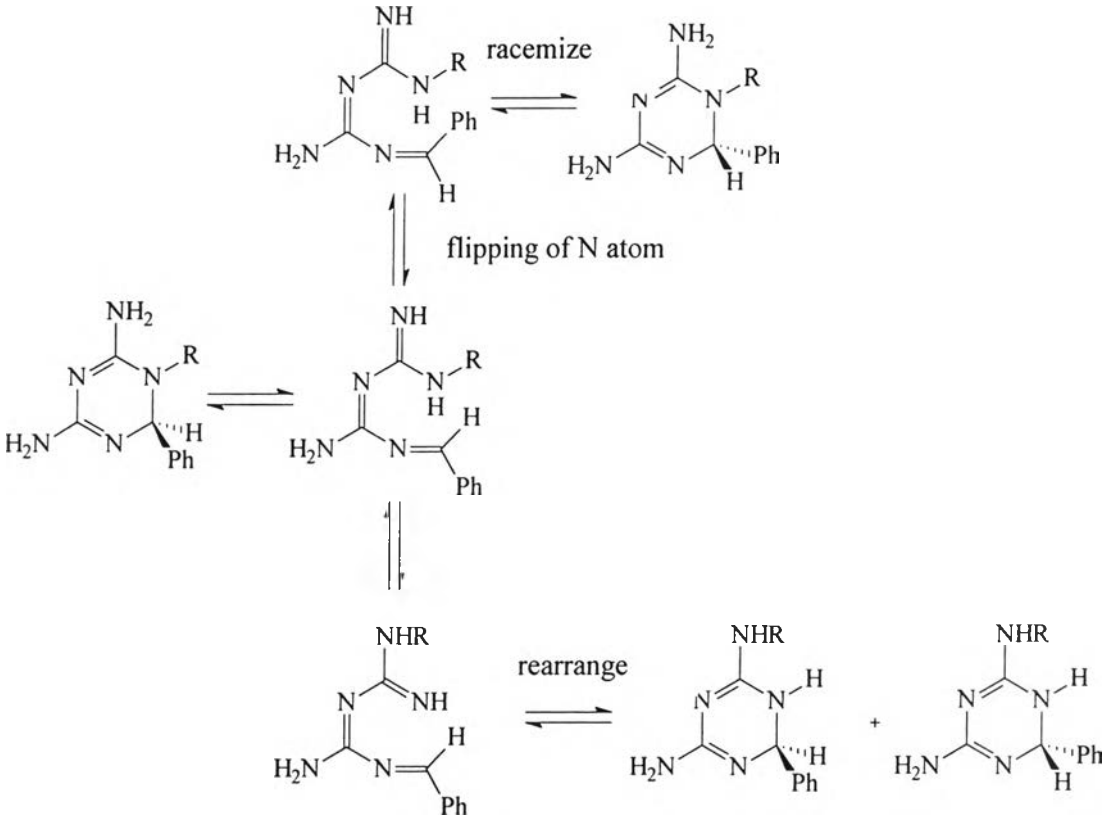


Figure 3.48 The assumption rearrangement/racemization mechanism of dihydrotriazine

1-(4-chlorophenyl-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(1)**, 1-benzyloxy-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(63)**, 1-benzyl-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(64)**, 1-benzyl-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine trifluoro-

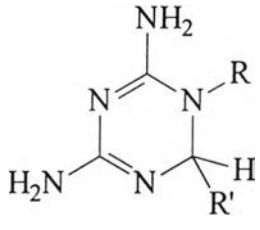
acetate **(65)**, 1-benzyl-2-(4′-nitrophenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine trifluoroacetate **(66)**, 1-benzyl-2-(4′-chlorophenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine trifluoroacetate **(67)**, 1-benzyl-2-(4′-methoxyphenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine trifluoroacetate **(68)** were used in the experiment. The compounds were heated at 100 °C in DMSO and extent of rearrangement was monitored by ^{1}H NMR spectroscopy. In all cases the C_2-H signal of the starting dihydrotriazine and its rearranged product appeared at different chemical shift. The reaction was stopped when no signal of the starting material was observed. It was found that the rearrangement in each case followed the first order kenetics.[44] In addition, the first order rate constants have been calculated (Table 3.3).

For first order reaction

$$\ln [A] = -kt + \ln [A] \qquad (1)$$

$$t_{1/2} = \frac{\ln 2}{k} \qquad (2)$$

Table 3.3 The k values of the reaction of rearrangement of dihydrotriazine



ID	R	R'	k (sec^{-1})	k(rel) (sec^{-1})	half-life (hr)	Salt
(1)	4-ClC_6H_4	C_6H_5	0.03	-	23.11	hydrochloride
(63)	$C_6H_5CH_2O$	C_6H_5	0.04	-	17.33	hydrochloride
(64)	$C_6H_5CH_2$	C_6H_5	<0.01	-	-	hydrochloride
(65)	$C_6H_5CH_2$	C_6H_5	0.74	1.00	0.94	trifluoroacetate
(66)	$C_6H_5CH_2$	4-$NO_2C_6H_4$	0.02	0.03	34.66	trifluoroacetate
(67)	$C_6H_5CH_2$	4-ClC_6H_4	0.69	0.93	1.00	trifluoroacetate
(68)	$C_6H_5CH_2$	4-$OCH_3C_6H_4$	1.56	2.11	0.44	trifluoroacetate

Dihydrotriazines **(1)**, **(63)** and **(64)** contained different substituents group at N_1 position which were aromatic, alkyloxy and alkyl group respectively. The rate constants for the rearrangement of **(64)** < **(1)** < **(63)**, It was concluded that dihydrotriazine which N_1-substitued group was alkyl group was less likely to rearrange than dihydrotriazine carrying N_1-aryl and N_1-alkyloxy group. Dihydrotriazines **(64)** and **(65)** were identical in the structure but the counter ions are different, being hydrochloride and trifluoroacetate respectively. The rate of rearrangement **(64)** was very slow compared to **(65)** which suggested that the rate of rearrangement and possibly racemization of dihydrotriazine also dependent to the influence of counter ion. The more basic trifluoroacetate ion gave rise to higher rate of rearrangement.

Dihydrotriazines **(65)**, **(66)**, **(67)** and **(68)**, were different in the C_2-substituents which were the phenyl, 4-nitrophenyl, 4-chlorophenyl and 4-methoxyphenyl respectively. The k(rel) are in the following order **(68)** > **(65)** > **(67)** > **(66)**, *ie*, the rearrangement was faster in the presence of electron donating substituent at C_2. On the other hand, the rearrangement was slower in the electron withdrawing C_2 substituents.

The dependency of rate of rearrangement to electron density of the substituent provides supporting evidence that the rearrangement of dihydrotriazine was not occurred through pericyclic or radical pathway. It is difficult, however, to extrapolate this to the racemization process. Nevertheless, in the absence of other evidences, the mechanism of racemization shown in Figure 3.48 seems probable. And if the assumption is correct, the presence of electron withdrawing substituent at C_2 and less basic counter-ion may slow down the racemization process.

3.5 Biological studies of optically active 4,6-diamino-1,2-dihydro-1,3,5-triazine

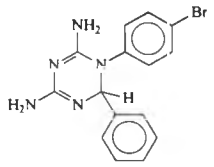
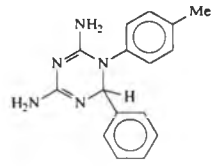
Enantiomers of dihydrotriazines successfully separated were sent to test for inhibition activity against DHFR from *P. falciparum*. Table 3.4 summarizes the binding affinity (K_i) values for the wild-type and A16VS108T mutant DHFR.

The results from Table 3.4, showed that the second peak **(4b)** of dihydrotriazine **(4)** inhibited the wild-type pfDHFR 40.7 times better than the first peak **(4a)** which was the other enantiomer of dihydrotriazine **(4)** while the difference in K_i values of **(4b)** and racemic **(4)** was not significant. **(4b)** also inhibited A16VS108T 15.4 times better than the **(4a)**. The same result observed in the case compound **(5)** and its two enantiomers **(5a)** and **(5b)** (first and second peak respectively). The K_i values for **(5b)** was 20.7 times higher than **(5a)** but not significantly different from dihydrotriazine **(5)**. The results support the hypothesis that one enantiomer of optically active dihydrotriazine can bind with DHFR better than the other. From the study of interaction between dihydrotriazine and DHFR both wild type and A16VS108T by molecular modeling, decreasing in binding affinity to A16VS108T mutant of DHFR caused by the steric effect at A16V position. It was further proposed that the dihydrotriazine would interact DHFR by turning the smaller substituent at C_2 to the bulkier side chain of A16V. The best binding activity was predicted to be the enantiomer possessing the *S* configuration at C_2 position.[24] According to this model, **(4b)** and **(5b)** should be the C_2 *S* configuration enantiomer. The prediction would be confirmed if we know the absolute configuration of some or all of the separated enantiomers. Unfortunately, the limited amount of pure enantiomers obtained chiral reverse phase HPLC and in tendency to recemize brought about difficulty in determination of the absolute configuration. Disappointingly still, K_i values of **(55b′)**, **(55b″)** and **(55b‴)** with known absolute configuration were very high both against wild type and A16VS108T mutant DHFR inhibition. The difference in K_i values of **(55b″)** and **(55b‴)** which were enantiomer were too close to draw any sensible conclusions. It was possible that the methylbenzyl group at N_1 substitution be responsible in the loss of binding activity of these compounds to DHFR.

In conclusion we have successfully resolved some dihydrotriazine to their respective enantiomers and have confirmed that they bind to both wild and A16VS108T pfDHFR with different binding constants. Unfortunately, absolute

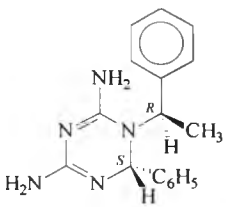
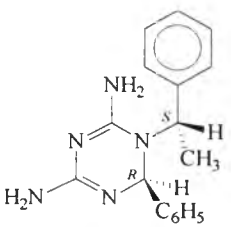
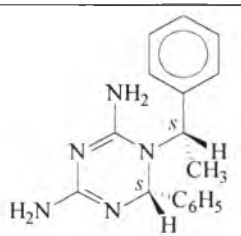
configuration could not be determined, therefore the ultimate goal of this research to validate the model has not yet been achieved. We have also synthesized optically active dihydrotriazines with known absolute configuration by asymmetric syntheses. However, all compounds showed very poor inhibition constant and no significant in binding affinity to wild type and mutant enzymes were observed.

Table 3.4 Inhibition constats (K_i) of dihydrotriazine and its enantiomers against the Wild-Type and A16VS108T Mutant of *P. falciparum*

ID	Structure	Salt	K_i-wt(nm)	Rel.cyc	K_i-A16VS108T	rel.cycmut	mut/wt	%ee
(4)		HCl	2.90 ± 1.20	1.90	90.30 ± 11.40	0.07	31.10	0
(4a)		Acetate	122.00 ± 4.90	81.30	999.20 ± 157.40	1.76	8.10	100
(4b)		Acetate	3.00 ± 0.20	2.00	64.70 ± 6.10	0.05	21.60	67
(5)		HCl	7.70 ± 2.20	5.13	170.40 ± 13.80	0.13	22.10	0
(5a)		Acetate	149.30 ± 5.50	99.50	375.20 ± 44.60	0.28	2.51	100
(5b)		Acetate	7.20 ± 1.20	4.80	68.80 ± 6.80	0.01	9.56	75

*(4a) and (5a) gave CD signal, therefore they should posses the same configuration at C_2

Table 3.4 (continued)

ID	Structure	Salt	K_i-wt(nm)	Rel.cyc	K_i-A16VS108T	rel.cycmut	mut/wt	%ee
(55b′)		Acetate	6702.70 ± 1260.20	4468.50	15001.96 ± 2237.90	11.40	2.20	-
(55b″)		Acetate	17864.50 ± 801.70	11909.70	12567.05 ± 460.00	9.60	0.70	-
(55b‴)		Acetate	>10000	666.6	8179.2 ± 2055.9	6.22	<0.8	-

3.6 Further prospects

According to the literature,[37] optically active but configurationally unstable α-amino nitrile was successfully synthesized by asymmetric transformation. In a special case of this method, a mixture of two solid chiral stereoisomers $\mathbf{A_s}$ and $\mathbf{B_s}$ being in equilibrium with a solution of the equilibrating stereoisomer, is completely transformed into a single solid stereoisomer. This method is referred to as asymmetric transformation according to the a principle of Otto Dimroth, which is not restricted to chiral compounds but applies generally to couple equilibria $\mathbf{1_s}$, $\mathbf{1_l}$, $\mathbf{3_s}$, and $\mathbf{3_l}$ (Figure 3.49).

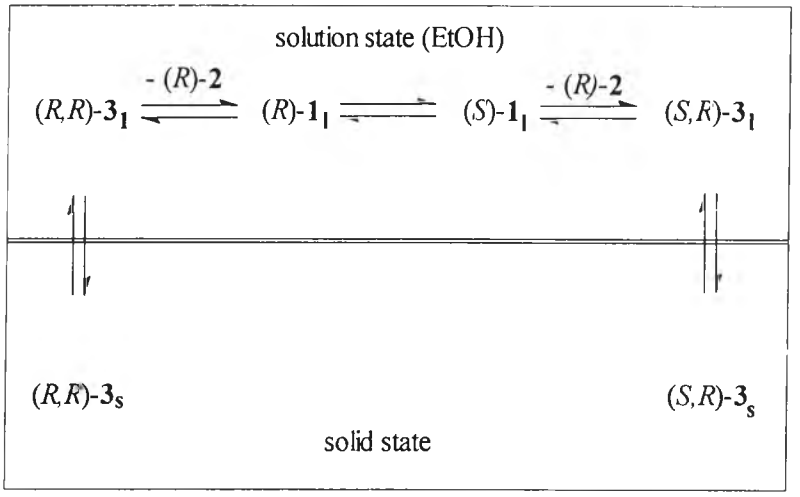


Figure 3.49 Transformation of diasteriomeric compound **(3)** by application of Dimroth's principle

Since we have demonstrated that dihydrotriazine can readily racemize, it should be possible to apply the principle of asymmetric transformation in preparation of optically active dihydrotriazines.

Based on the asymmetric transformation principle shown above, racemic 1-(3′,4′-dichlorophenyl-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine free bases were dissolved together with an equivalent of D(-)-mandelic acid in ethanol. The reaction suspension was stirred for a week. The white precipitated solid formed was filtered off and analyzed by ^{1}H NMR spectroscopy, using (+)-camphorsulfonic acid as a solvating shift reagent as described in section 3.1. Gratifyingly it was found that D (-)-mandelate salt of 1-(3′,4′-dichlorophenyl)-2-phenyl-4,6-diamino-1,2-dihydro-

1,3,5-triazine showed a significant enrichment of one diasterioisomer (dr = 1.5:1.0) (Figure 3.50).

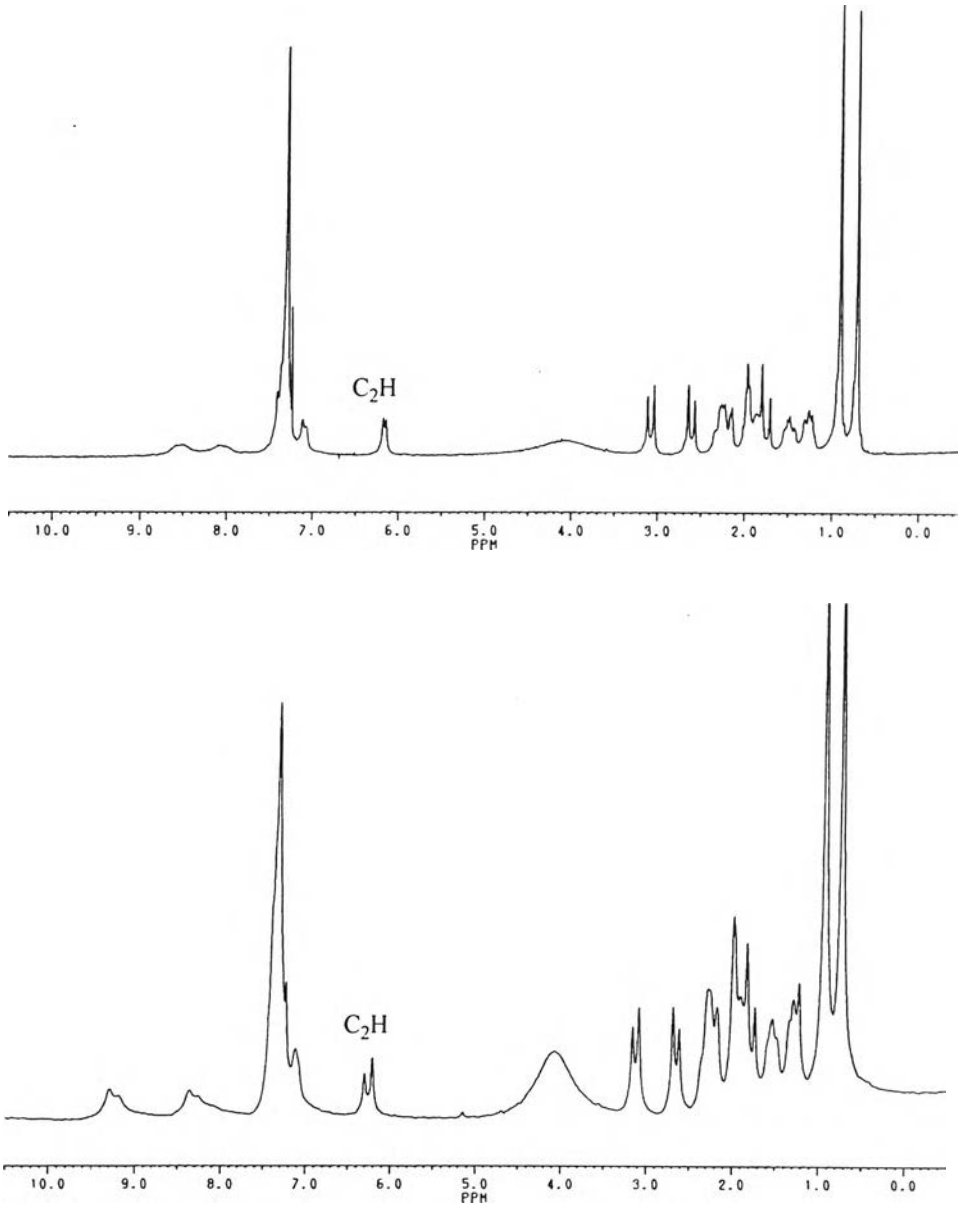


Figure 3.50 ^{1}H NMR spectrum ($CDCl_3$, 200 MHz) of the racemic (upper) and the asymmetrically transformed D(-)-mandelate salt of 1-(3′,4′-dichloro-phenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine (lower)

According to this encouraging preliminary results, the asymmetric transformation might provide a way to obtain the optically active dihydrotriazine in sufficient quantity for determination of absolute configuration and further biological study in the future.