CHAPTER II

EXPERIMENTAL

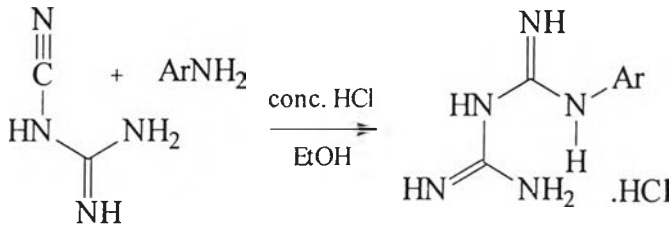
2.1 General

Thin layer chromatography (TLC) was performed on Merck silica gel 60 F_{254} 0.2 mm pre-coated aluminium plates. Flash column chromatography was carried out on Merck silica gel 60 (0.040-0.063 mm). Evaporation refers to the rotary evaporation of solvent under aspirator pressure. A Hypersil™ 5 μm BDS C_{18} HPLC column particle size 4.6x250 mm and A Merck's LiChro CART® HPLC cartridge (No. 716617) 250-4 ChiraDex® (5 μm), column were used for both analytical and preparative HPLC purpose. Peak monitoring and data processing were performed on Compaq Prolinca 486 compatible computer operating a Millennium Version 2.1 software. Specific rotations were measured on a Perkin-Elmer 341 polarimeter and $[\alpha]_D$-values are given in units of 10^{-1} deg.cm^2.g^{-1}. CD spectra were obtained from Model ORDE-307W ORD Unit spectropolarimeter (JASCO Coporation Tokyo). NMR spectra were recorded in an appropriate deuterated solvent at 200 MHz (^{1}H), unless otherwise noted. Chemical shifts are in part per million (ppm,δ) down field relative to the internal standard tetramethylsilane. Spectral patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. MALDI-TOF mass spectra of all cycloguanil derivatives were analysed by Ms. Nathiga Panchan on Bruker BIFLEX™ mass spectrometer using doubly recrystallized 2-cyano-4-hydroxy cinnamic acid (CCA) as matrix, and calibrated with human angiotensin II (M+H, 1047). 0.1% Trifluoroacetic acid in acetonitrile : water (70 : 30) was used as diluting agent for MALDI-TOF samples. The weight of all substances was determined on a Metler AT 200 electrical balance. Elemental Analyses were performed on a Perkin Elmer Elemental analyzer 2400 CHNS/O at the Research Equipment Centre, Chulalongkorn University. X-ray crystallography was analyzed on Enraf-Nonius CAD 4 diffractometer with MoK α radiation (λ = 0.71073 A°) in θ-2θ mode.

Distilled water was used for all chemical experiments. All chemicals and solvents were obtain from commercial suppliers (Aldrich Fluka and Merck) and were purified according to the literature. Reactions involving air-and moisture-sensitive reagents were executed under an atmosphere of dry nitrogen. Analytical samples were dried under vacuum at room temperature for several hours.

2.2 Resolution by formation of dihydrotriazine salts followed by recrystallization

2.2.1 Synthesis of biguanide hydrochloride



General procedure

To a mixture of dicyanodiamide (27.0 mmol) and the aryl amine (25.0 mmol) in absolute ethanol (10.0 mL) was added concentrated HCl (27.5 mmol). The reaction mixture was refluxed for 5 hours. The precipitated product was filtered, washed with cold ethanol and air dried.

3-Chlorophenylbiguanide hydrochloride

White crystalline solids (23.3 mmol) (3.86 g, 67% yield). ^{1}H NMR (D_2O) δ_H 6.97-7.24 (5H, m, aromatic C-H).

4-Chlorophenylbiguanide hydrochloride

White crystalline solids (25.2 mmol) (3.86 g, 62% yield). ^{1}H NMR (D_2O) δ_H 7.06-7.22 (2x2H, AB doublet, J = 8.0 Hz, aromatic C-H).

4-Bromophenylbiguanide hydrochloride

White crystalline solids (25.0 mmol) (5.83 g, 80 % yield). ^{1}H NMR (D_2O) δ_H 7.0-7.37 (2x2H, AB doublet, J = 8.0 Hz, aromatic C-H).

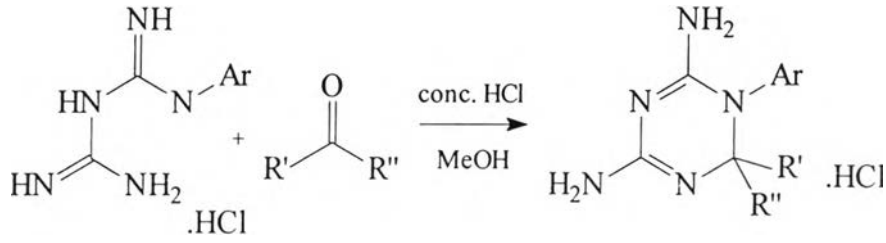
4-Methylphenylbiguanide hydrochloride

White crystalline solids (32.1 mmol) (5.83 g, 80% yield). ^{1}H NMR (D_2O) δ_H 7.0-7.37 (2x2H, AB doublet, J = 8.0 Hz, aromatic C-H).

3, 4-Dichlorophenylbiguanide hydrochloride

White crystalline solid (24.0 mmol) (5.40 g, 80% yield). ^{1}H NMR (D_2O) δ_H 6.97 (1H, dd, J = 8.5 Hz, 2.6 Hz, aromatic C-H) and 7.31 (2H, m, aromatic C-H).

2.2.2 Synthesis of 1-aryl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride



1-(4′-Chlorophenyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(1)**

Concentrated HCl (0.42 mL, 4.8 mmol) was added to a mixture of 4-chlorophenylbiguanide hydrochloride (1.20 g, 5.0 mmol) and benzaldehyde (1.00 mL, 10.0 mmol) in absolute ethanol (3.40 mL). The reaction mixture was refluxed for 5 hours. After cooling, the precipitate formed was filtered and washed with cold ethanol, to give the product as a white crystalline solid (1.42 g, 85% yield). Anal. Calcd. for $C_{15}H_{15}N_5Cl_2$: C, 53.6; H, 4.5; N, 20.8%. Found; C, 53.4; H, 4.4; N, 20.9%. ^{1}H NMR (D_2O) δ_H 5.95 (1H, s, H-2), 6.98 (2H, part of AB doublet, J = 8.0 Hz, aromatic C-H) (Figure 1); *m/z* (MALDI-TOF) 300 ($M.H^+$).

1-(4′-Chlorophenyl)-2-methyl-4,6-diamino-1,2-dihydro-1, 3, 5-triazine hydrochloride **(2)**

The same procedure as for 1-(4′-chlorophenyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride (5.77 g, 23.4 mmol) was followed but acetaldehyde (1.41 mL, 25.0 mmol) was used instead of benzaldehyde to give the product as a white crystalline solid (3.00 g, 47% yield). Anal. Calcd. for

$C_{10}H_{13}N_5Cl_2$: C, 43.8; H, 4.8; N, 25.6%. Found; C, 43.8; H, 4.9; N, 25.4%. ^{1}H NMR (D_2O) δ_H 1.20 (3H, d, J = 6 Hz, H-2), 7.22 and 7.40 (2x2H, AB doublet, J = 8.0 Hz, aromatic C-H) (Figure 2); *m/z* (MALDI-TOF) 238 ($M.H^+$).

1-(4′-Chlorophenyl)-2-ethyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(3)**

The same procedure as for 1-(4′-chlorophenyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride was followed but propioraldehyde (0.73 mL, 10.0 mmol) was used instead of benzaldehyde to give the product as a white crystalline solid (2.10 g, 86% yield). Anal. Calcd. for $C_{12}H_{17}N_5Cl_2+H_2O$: C, 45.0; H, 6.0; N, 21.9%. Found; C, 45.6; H, 5.7; N, 21.5%. ^{1}H NMR (D_2O) δ_H 0.65 (3H, t, J = 6.5 Hz, $CH_3CH_2CH_2$-2), 1.15 (2H, m, $CH_3CH_2CH_2$-2), 1.55 (2H, m, $CH_3CH_2CH_2$-2), 4.94 (1H, dd, J = 6, 4 Hz, H-2), 7.25 and 7.42 (2x2H, AB doublet, J = 8.0 Hz, aromatic C-H) (Figure 3); *m/z* (MALDI-TOF) 266 ($M.H^+$).

1-(4′-Bromophenyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(4)**

The same procedure as for 1-(4′-chlorophenyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride but 4-bromophenylbiguanide hydrochloride was used instead of 4-chlorophenylbiguanide (4.99 g, 17.2 mmol) to give the product as a white crystalline solid (3.00 g, 47% yield). Anal. Calcd. for $C_{15}H_{15}N_5Cl_2Br$: C, 47.3; H, 4.0; N, 18.4%. Found; C, 47.4; H, 4.0; N, 18.4%. ^{1}H NMR (D_2O) δ_H 5.88 (1H, s, H-2), 6.88 and 7.34 (2x2H, AB doublet, J = 8.0 Hz, aromatic C-H) (Figure 4); *m/z* (MALDI-TOF) 344, 346 ($M.H^+$).

1-(4′-Methylphenyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(5)**

The same procedure as for 1-(4′-chlorophenyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride was followed but 4-methylphenylbiguanide hydrochloride (2.68 g, 11.8 mmol) was used instead of 4-chlorophenylbiguanide to give the product as a white crystalline solid (3.30 g, 89% yield). Anal. Calcd. for $C_{16}H_{18}N_5Cl$: C, 60.8; H, 5.8; N, 22.2%. Found; C, 60.9; H, 5.5; N, 22.1%. ^{1}H NMR (D_2O) δ_H 2.05 (3H, s, CH_3), 5.87 (1H, s, H-2), 6.82 and 6.98 (2x2H, AB doublet, J =

8.0 Hz, aromatic C-H), 7.16 (5H, m, aromatic C-H) (Figure 5); *m/z* (MALDI-TOF) 280, 346 ($M.H^+$).

1-(3′-Chlorophenyl)-2-propyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(6)**

The same procedure as for 1-(4′-chlorophenyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride was followed but 3-chlorophenylbiguanide hydrochloride (4.20 g, 17.0 mmol) and butyraldehyde (1.71 mL, 19.0 mmol) was used instead of 3-chlorophenylbiguanide hydrochloride and benzaldehyde to give the product as a white crystalline solid (2.10 g, 86% yield). ^1H NMR (D_2O) δ_H 0.68 (3H, t, J = 6.8 Hz, $CH_3CH_2CH_2$-2), 1.18 (2H, m, $CH_3CH_2CH_2$-2), 1.60 (2H, m, $CH_3CH_2CH_2$-2), 4.96 (1H, dd, J = 6.5, 4 Hz, H-2), 7.22 and 7.38 (4H, m, aromatic C-H) (Figure 6); *m/z* (MALDI-TOF) 266 ($M.H^+$).

1-(3′,4′-Dichlorophenyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(7)**

The same procedure as for 1-(4′-chlorophenyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride was followed but 3,4-dichlorophenylbiguanide hydrochloride (2.25 g, 8.0 mmol) was used instead of 4-chlorophenylbiguanide hydrochloride to give the product as a white crystalline solid (3.00 g, 98% yield). Anal. Calcd. for $C_{15}H_{14}N_5Cl_3$: C, 48.6; H, 3.8; N, 18.9%. Found; C, 48.7; H, 4.0; N, 18.7%. ^1H NMR (D_2O) δ_H 5.89 (1H, s, H-2), 6.90 (1H, dd, J = 8.0 Hz, aromatic C-H), 7.19-7.33 (7H, m, aromatic C-H) (Figure 7); *m/z* (MALDI-TOF) 334 ($M.H^+$).

1-(3′,4′-Dichlorophenyl)-2-methyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(8)**

The same procedure as for 1-(4′-chlorophenyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride was followed but 3,4-dichlorophenylbiguanide hydrochloride (6.02 g, 21.4 mmol) and acetaldehyde (1.36 mL, 24.0 mmol) were used instead of 4-chlorophenylbiguanide hydrochloride and benzaldehyde to give the product as a white crystalline solid (5.00 g, 76% yield). Anal. Calcd. for $C_{10}H_{12}N_5Cl_3$+HCL+H_2O: C, 33.1; H, 4.2; N, 19.3%. Found; C, 33.4; H, 4.3; N, 19.0%. ^{1}HNMR (D_2O) δ_H 1.18 (3H, d, J = 6.5 Hz, CH_3-2), 5.04 (1H, q, J = 6.5 Hz, H-

2), 7.15 (1H, dd, J = 8.5, 2.6 Hz, aromatic C-H), 7.50 (2H, m, aromatic C-H) (Figure 8); *m/z* (MALDI-TOF) 272 ($M.H^+$).

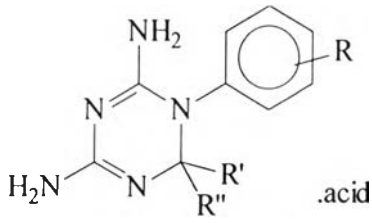
1-(3′,4′-Dichlorophenyl)-2-propyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(9)**

The same procedure as for 1-(4′-chlorophenyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride was followed but 3,4-dichlorophenyl-biguanide hydrochloride (5.73 g, 20.3 mmol) and butyraldehyde (2.00 mL 22.0 mmol) were used instead of 4-chlorophenylbiguanide hydrochloride and benzaldehyde to give the product as a white crystalline solid (4.91 g, 72%). ^1H NMR (D_2O) δ_H 0.70 (3H, t, J = 7.0 Hz, $CH_3CH_2CH_2$-2), 1.20 (2H, m, $CH_3CH_2CH_2$-2), 1.58 (2H, m, $CH_3CH_2CH_2$-2), 4.91 (1H, dd, J = 8.4, 2.4 Hz, aromatic C-H) and 7.56 (2H, m, aromatic C-H) (Figure 9); *m/z* (MALDI-TOF) 300, 302, 304 ($M.H^+$).

2.2.3 Formation of diastereomeric salts

General procedure

The 4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride (1.0 mmol) was dissolved in water with warming. The solution was cooled to room temperature. An aqueous NaOH (1.1 mmol) was then added to the solution with stirring at room temperature until pH ~ 10. After being stirred for 6 minutes, the precipitated solid was filtered and washed with cold water and diethyl ether and air-dried to give the unstable free-base as white solids. The free base and the optically active acid (1:1) were dissolved together in methanol. After stirring for a few minutes at room temperature, the solvent was removed by evaporation. The diastereomeric salt was then recrystallized from a range of solvents such as acetone, acetonitrile, ethanol, methanol and water.



ID	R	R′	R″	Acid
10	4-Cl	H	$-C_6H_5$	(-)-Camphanic acid
11	4-Cl	H	$-C_6H_5$	(+)-Camphoric acid
12	4-Cl	H	$-C_6H_5$	(1*S*)-(+)-Camphor-10-sulfonic acid
13	4-Cl	H	$-C_6H_5$	(-)-*O*,*O*′-Dibenzoyl-L-tartaric acid
14	4-Cl	H	$-C_6H_5$	*R*(-)-α-Methoxyphenyl acetic acid
15	4-Cl	H	$-C_6H_5$	(-)-Menthyloxyacetic acid
16	4-Cl	H	$-C_6H_5$	(+)-Tartaric acid
17	4-Cl	H	$-C_6H_5$	*R*(-)-1,1′-Binaphthalene-2,2′-diyl hydrogenphosphate
18	4-Cl	H	$-CH_3$	(-)-Camphanic acid
19	4-Cl	H	$-CH_3$	(+)-Camphoric acid
20	4-Cl	H	$-CH_3$	(1*S*)-(+)-Camphor-10-sulfonic acid
21	4-Cl	H	$-CH_3$	(-)-*O*,*O*′-Dibenzoyl-L-tartaric acid
22	4-Cl	H	$-CH_3$	(-)-Menthyloxyacetic acid
23	4-Cl	H	$-CH_2CH_3$	(-)-*O*,*O*′-Dibenzoyl-L-tartaric acid
24	4-Br	H	$-C_6H_5$	(1*S*)-(+)-Camphor-10-sulfonic acid
25	$4-CH_3$	H	$-C_6H_5$	(1*S*)-(+)-Camphor-10-sulfonic acid
26	3-Cl	H	$-CH_2CH_2CH_3$	(+)-Camphoric acid
27	3-Cl	H	$-CH_2CH_2CH_3$	(1*S*)-(+)-Camphor-10-sulfonic acid
28	$3,4-Cl_2$	H	$-CH_2CH_2CH_3$	(-)-Camphanic acid
29	$3,4-Cl_2$	H	$-CH_2CH_2CH_3$	(1*S*)-(+)-Camphor-10-sulfonic acid
30	$3,4-Cl_2$	H	$-CH_3$	(-)-Camphanic acid
31	$3,4-Cl_2$	H	$-CH_3$	(+)-Camphoric acid
32	$3,4-Cl_2$	H	$-CH_3$	(1*S*)-(+)-Camphor-10-sulfonic acid
33	$3,4-Cl_2$	H	$-CH_3$	(-)-*O*,*O*′-Dibenzoyl-L-tartaric acid
34	$3,4-Cl_2$	H	$-CH_3$	(-)-Menthyloxyacetic acid

ID	R	R′	R″	Acid
35	3,4-Cl_2	H	-CH_3	(+)-Tartaric acid
36	3,4-Cl_2	H	-C_6H_5	*R*(-)-1,1′-Binaphthalene-2,2′-diyl Hydrogenphosphate
37	3,4-Cl_2	H	-C_6H_5	(+)-α-Bromocamphor-10-sulfonic acid hydrate
38	3,4-Cl_2	H	-C_6H_5	(-)-Camphanic acid
39	3,4-Cl_2	H	-C_6H_5	(+)-Camphoric acid
40	3,4-Cl_2	H	-C_6H_5	(1*S*)-(+)-Camphor-10-sulfonic acid
41	3,4-Cl_2	H	-C_6H_5	(-)-*O*,*O*′-Dibenzoyl-L-tartaric acid
42	3,4-Cl_2	H	-C_6H_5	*R*(-)-α-Methoxyphenyl acetic acid
43	3,4-Cl_2	H	-C_6H_5	(-)-Menthyloxyacetic acid
44	3,4-Cl_2	H	-C_6H_5	*R*(-)-2-Phenylpropionic acid
45	3,4-Cl_2	H	-C_6H_5	(+)-Tartaric acid

2.3 Resolution by chromatography using chiral stationary or mobile phase

(Separation of 4,6-diamino-1,2-dihydro-1,3,5-trizine hydrochloride by HPLC technique)

2.3.1 1-(4′-Bromophenyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(4)**

The sample for chiral reverse phase HPLC was prepared by dissolving in mobile phase (15% methanol and 85% 0.1 M ammonium acetate). The solution was filtered through a nylon membrane filter (0.45 μm), the two enantiomers were separated by chiral reverse phase HPLC A Merck's LiChro CART® HPLC cartridge 250-4 ChiraDex® (5 μm) column monitoring by UV-absorbance at 254 nm eluting with an isocratic system of 15% acetonitrile-0.1M ammonium acetate. Each enantiomer was separately collected. After freeze drying, it was confirmed to be the desired product by HPLC analysis and by CD-measurement.

2.3.2 *1-(4′-Methylphenyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride* (5)

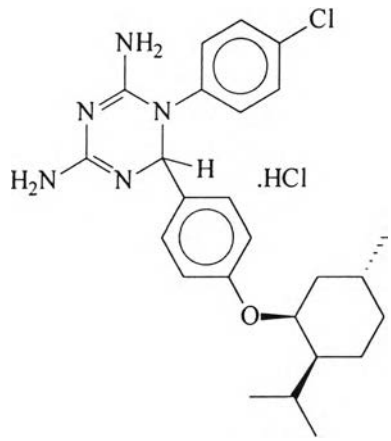
The same procedure as above was followed but 1-(4′-methylphenyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride was used instead of 1-(4′-bromophenyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride.

2.3.3 *1′S-phenylethyl-2S-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine trifluoroacetate* (55b′′′)

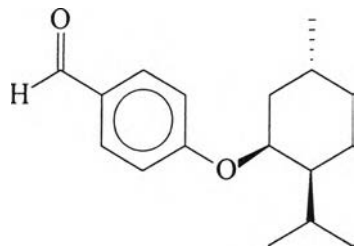
The solution of 1′*S*-phenylethyl-2*R*-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine trifluoroacetate (0.10 g, 0.2 mmol) in acetonitrile (2 mL) was heated at 50 °C for 45 hours. The solvent was removed by evaporation to give the sample as a white solids. Preparation of sample was similar to 1-(4′-bromophenyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride above. The compound was separated by using reverse phase HPLC on a with 15% acetonitrile-0.02 M triethyl ammonium acetate as eluting solvent. After finishing separation, the samples were dried at room temperature and kept in freezer.

2.4 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

2.4.1 Attempted synthesis 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)**

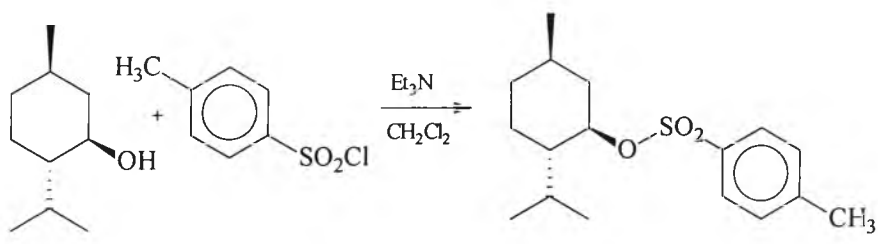


(1) 4-(2'*S*-Isopropyl-5'*R*-methyl-1'*S*-cyclohexyloxy)benzaldehyde **(46a)**



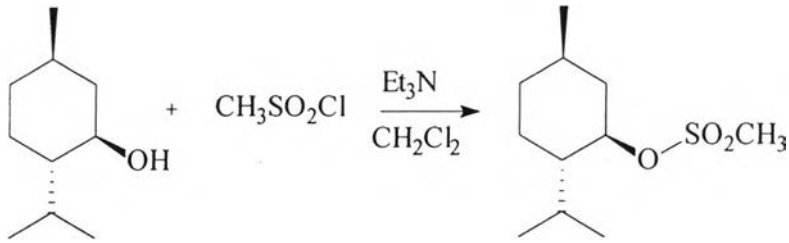
method A:

Attempted synthesis of toluene-4-sulfonic acid 2*S*-isopropyl-5*R*-methyl-1*R*-cyclohexyl ester



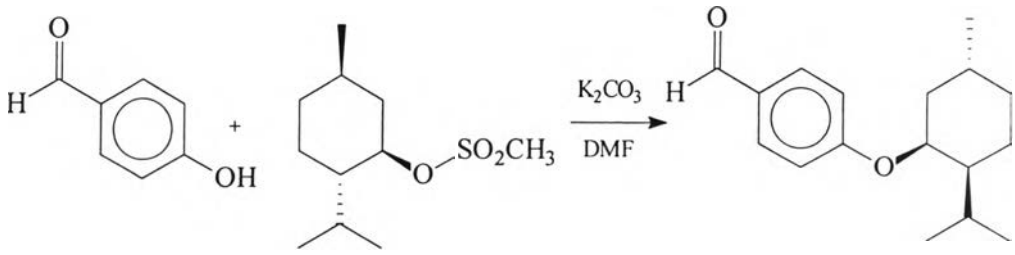
p-toluenesulfonyl chloride (0.24 g, 1.0 mmol) was added to a solution of (-)-menthol (0.16 g, 1.0 mmol) in dichloromethane (5 mL) with stirring at room temperature. Triethylamine (0.17 mL, 1.2 mmol) was then added. After stirring overnight, TLC indicated no reaction had occurred.

Synthesis of methanesulfonic acid 2*S*-isopropyl-5*R*-methyl-1*R*-cyclohexyl ester



The same procedure as for toluene-4sulfonic acid 2*S*-isopropyl-5*R*-methyl-1*R*-cyclohexyl ester was followed but methanetoluenesulfonyl chloride (1.0 mmol) was used instead of *p*-toluene sulfonylchloride. After stirring overnight, The solution was washed with 5% HCl, 5% $NaHCO_3$ and water. It was then dried with $MgSO_4$, filtered, concentrated under reduced pressure to give the crude product as a yellow oil (0.22 g, 90% yield). ^{1}H NMR ($CDCl_3$) δ_H 0.81 (3H, d, J = 7.2 Hz, CH_3), 0.90 and 0.94 (2x3H, d, J = 7.2 Hz, $CH(CH_3)_2$), 0.98-1.75 and 1.89-2.30 (9H, m, menthol CH, CH_2), 3.00 (3H, s, S-CH_3) 4.51 (1H, dt, J_d = 7.2, J_t = 14.1 Hz, -OCH).

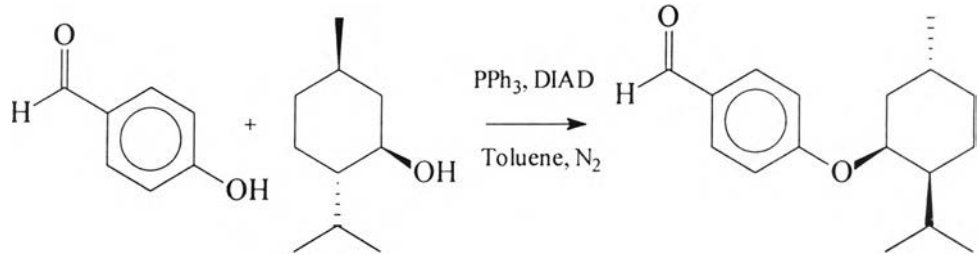
Attempted synthesis of 4-(2′*S*-isopropyl-5′*R*-methyl-1′*S*-cyclohexyloxy)benzaldehyde **(46a)**



methanesulfonic acid 2*S*-isopropyl-5*R*-methyl-1*R*-cyclohexyl ester (0.22 g, 0.9 mmol) was added to a mixture of *p*-hydroxybenzaldehyde (0.15 g, 2.3 mmol) and K_2CO_3 (0.32 g, 2.3 mmol) in dimethylformamide. The reaction mixture was heated at

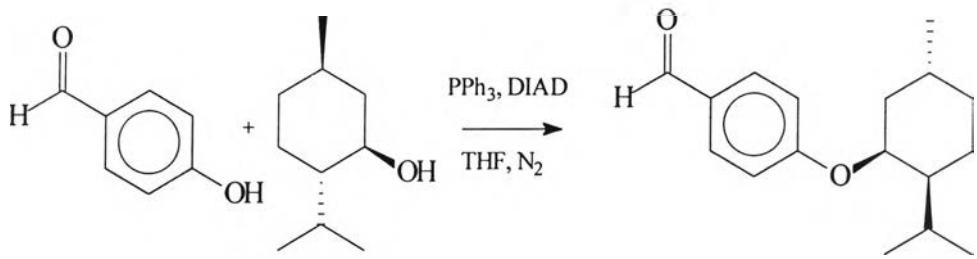
90 °C overnight. It was then diluted with dichloromethane, washed with 10% NaOH, dried with $MgSO_4$ and evaporation. ^1H NMR indicated that no desired product was obtained by this method.

Method B:



Diisopropylazodicarboxylate (0.35 mL, 1.6 mmol) was added dropwise to a solution of (-)-menthol (0.18 g, 1.2 mmol), *p*-hydroxybenzaldehyde (0.17 g, 1.4 mmol) and triphenylphosphine (0.42 g, 1.6 mmol) in toluene (5 mL) at room temperature under N_2. The initially orange colored solution faded to yellow and the solution was stirred overnight. The reaction occurred about 5% completion to give the desired product according to TLC analysis.

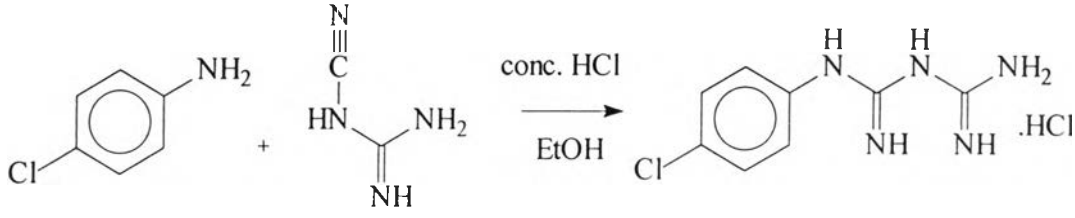
Method C:



The same as above was used but THF was used as a solvent instead of toluene at 0°C with an ice bath under N_2 and the reaction was stirred overnight. The solvent was then removed by evaporation. The residue was purified by flash column chromatography on siliga gel using 20% ethyl acetate-hexane to give the desired product as a yellow oil (1.1 mmol) (0.08 g, 26% yield). ^1H NMR ($CDCl_3$) δ_H 0.81 (3H, d, J = 7.0 Hz, $C\underline{H}_3$), 0.85 and 0.91 (2x3H, d, J = 7.2 Hz, $CH(C\underline{H}_3)_2$), 0.94-1.21

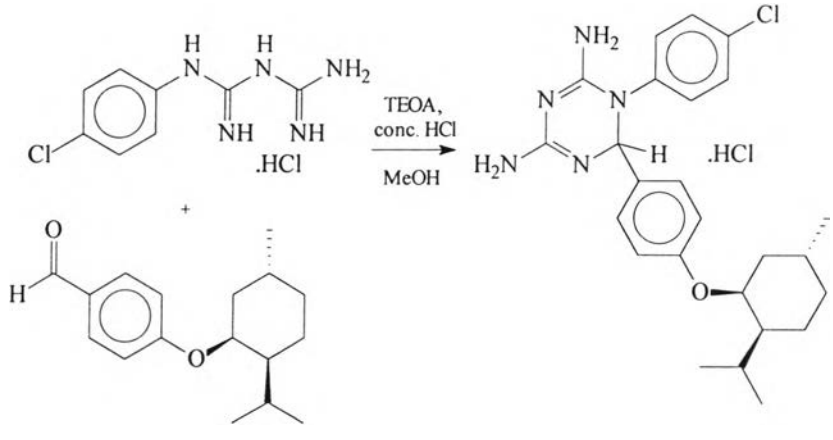
and 1.23-1.82 (9H, m, menthol CH, CH_2), 6.97 and 6.81 (2x2H, AB doublet, J = 8.0 Hz, aromatic C-H) (Figure 10). $[\alpha]_D^{20}$ +60.7 (c=3.0, MeOH).

(2) 4-Chloropheynylbiguanide hydrochloride **(46b)**



Dicyanodiamide (2.31 g, 27.5 mmol) was added to a suspension of *p*-chloroaniline (3.20 g, 25.0 mmol) in absolute ethanol (10 mL). Concentrated HCl (2.4 mL, 27.4 mmol) was then added and the reaction mixture was refluxed for 5 hours. The precipitated solid was filtered and washed with cold ethanol to give the desired product as a white solid (3.86 g, 62% yield). ^{1}H NMR (D_2O) δ_H 7.06-7.22 (2x2H, AB doublet, J = 8.0 Hz, aromatic C-H).

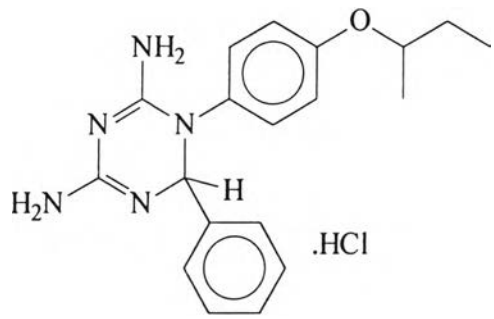
(3) 1-(4′-Chlorophenyl)-2-[4′-(2″*S*-isopropyl-5*R*″-methyl-1″*S*-cyclohexyloxy)phenyl] 4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(46c)**



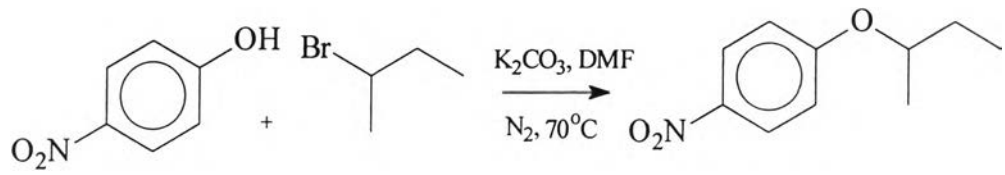
Triethyl orthoacetate (0.75 mL, 4.1 mmol) was added to a mixture of 4-chlorophenylbiguanide hydrochloride (0.14 g, 0.6 mmol) and 4-(2′*S*-isopropyl-5′*R*-methyl-1*S*″-cyclohexyloxy)benzaldehyde (0.16 mg, 0.6 mmol) in absolute methanol (5 mL). Concentrated HCl (0.02 mL, 0.2 mmol) was then added to the reaction with

stirring at room temperature. The solution was stirred until TLC showed complete disappearance of the biguanide hydrochloride (2-4 days). The precipitated solid was filtered and washed with cold methanol to give a product as a white solid (0.07 g, 25% yield). ^{1}H NMR (DMSO) δ_H 0.75 (3H, d, J = 7.1 Hz, C<u>H</u>$_3$), 0.91 (2x3H, d, J = 7.2 Hz, CH(C<u>H</u>$_3$)$_2$), 0.92-1.11 and 1.42-1.99 (9H, m, menthol C<u>H</u>, C<u>H</u>$_2$), 4.66 (1H, s, CO<u>H</u>), 5.99 (1H, s, <u>H</u>-2), 6.91-7.18 and 7.28-7.44 (2x2H, 2xAB doublet, J = 8.0 Hz, aromatic C-H) (Figure 11). $[\alpha]_D^{20}$ +24.7 (c=1.70, MeOH).

2.4.2 Attempted synthesis of 1-(4'-sec-butoxyphenyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(47c)**

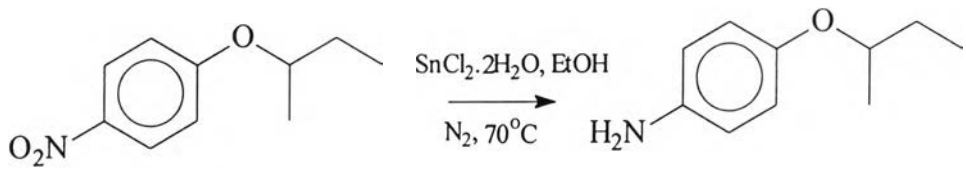


(1) <u>1-sec-Butoxy-4-nitrobenzene **(47a)**</u>



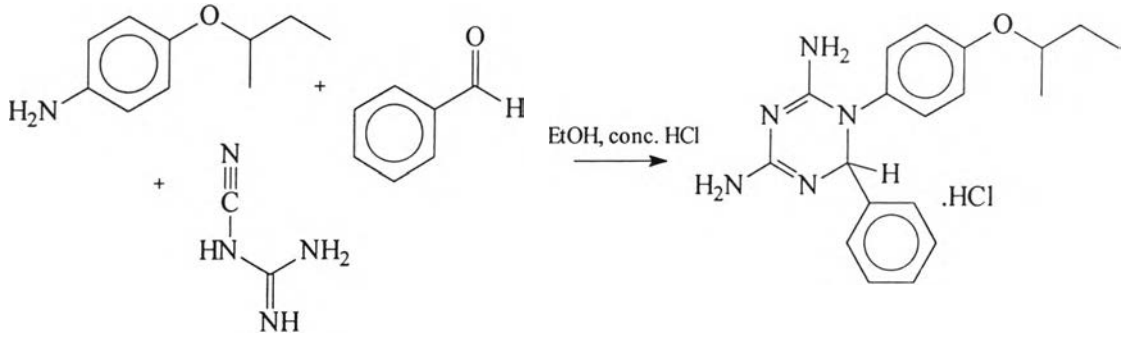
Racemic 2-bromobutane (1.36 g, 9.5 mmol) was added to a mixture of *p*-nitrophenol (1.38 g, 9.9 mmol) and anhydrous potasssium carbonate (1.40 g, 10.6 mmol) in DMF (20 mL). The mixture was stirred at 70 °C under N_2 for 5 days. The reaction mixture was diluted with dichloromethane, washed with 5% HCl, 5% $NaHCO_3$ and water. It was then dried with $MgSO_4$, filtered, concentrated under reduced pressure to give the crude product as a yellow oil (1.75 g, 90% yield).

(2) 4-sec-Butoxyaniline **(47b)**



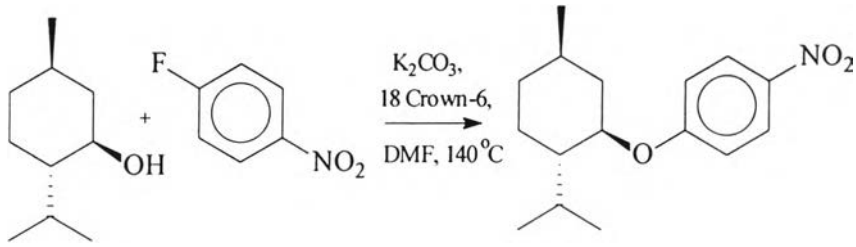
$SnCl_2.2H_2O$ (2.23 g, 9.9 mmol) was added to a solution of 4-sec-butoxy-4-nitrobenzene (0.40 g, 2.1 mmol) in absolute ethanol (20 mL) with stirring under N_2 at 70 °C for 3 hours. Water was added to the mixture and 5% $NaHCO_3$ was added until pH = 7. The solution was extracted with ethyl acetate, washed with brine and water. It was then dried with $MgSO_4$, and concentrated under reduced pressure. The residue was purified by flash column chromatography using 10% ethyl acetate-hexane as eluent to give the product as a yellow oil (0.33 g, 98% yield).

(3) 1-(4′-sec-Butoxyphenyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(47c)**



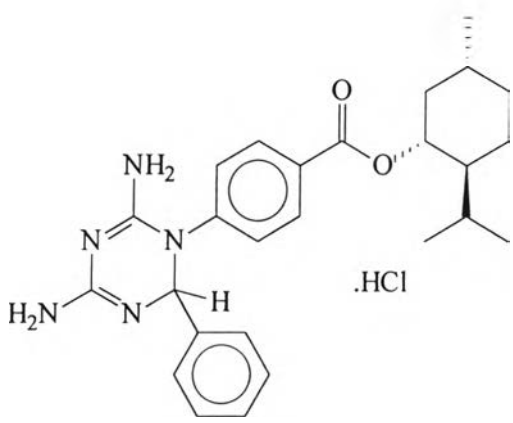
Concentrated HCl (0.28 mL, 3.2 mmol) was added to a mixture of 4-sec-butoxyaniline (0.33 g, 2.0 mmol) dicyanodiamide (0.22 g, 2.6 mmol) and benzaldehyde (0.40 mL, 3.9 mmol) in absolute ethanol (5 mL). The reaction mixture was refluxed for 5 hours and the precipitated solid was collected by filtration to give the product as a white solid (0.28 g, 37% yield). ^{1}H NMR (DMSO) δ_H 0.84 (3H, t, J = 7.1 Hz, $CH_2\underline{CH_3}$), 1.54 (2H, m, $C\underline{H}CH_3$), 4.32 (1H, m, C$\underline{H}$), 5.95 (1H, s, $\underline{H}$-2), 6.86 and 7.01 (2x2H, AB doublet, J = 7.9 Hz, aromatic C-H), 7.35 (5H, m, aromatic C-H) (Figure 12).

2.4.3 Attempted synthesis of 1-(2'S-isopropyl-5'R-methyl-1'R-cyclohexyloxy)-4-nitrobenzene **(48a)**

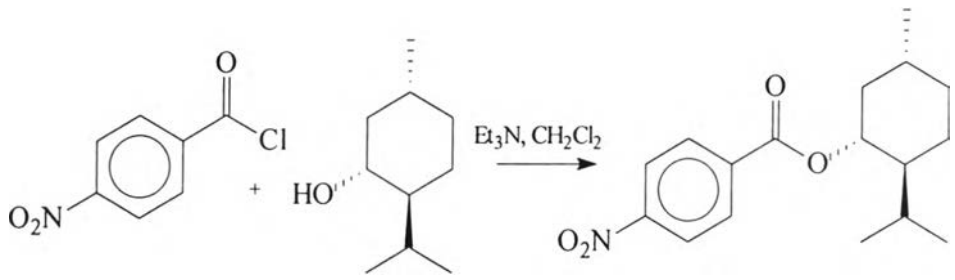


p-Fluoronitrobenzene (0.13 mL, 1.2 mmol) was added to a mixture of (-)-menthol (0.18 g, 1.2 mmol), potassium carbonate (0.11 g, 0.8 mmol) and 18 crown-6 (0.05 g, 0.2 mmol) in DMF. The mixture was stirred at 140 °C for 5 days. The reaction mixture was diluted with dichloromethane, washed with 5% HCl, 5% $NaHCO_3$ and water. It was then dried with $MgSO_4$, filtered, concentrated under reduced pressure. The residue was purified by column chromatography using 10% ethyl acetate-hexane as eluent to give the crude product as a yellow oil (1.75 g). ^1H NMR showed that it was an undesired product.

2.4.4 Attempted synthesis of 1-[4'-(2''S-isopropyl-5''R-methyl-1''R-cyclohexyloxy-carbonyl)phenyl]-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine-hydrochloride **(49c)**

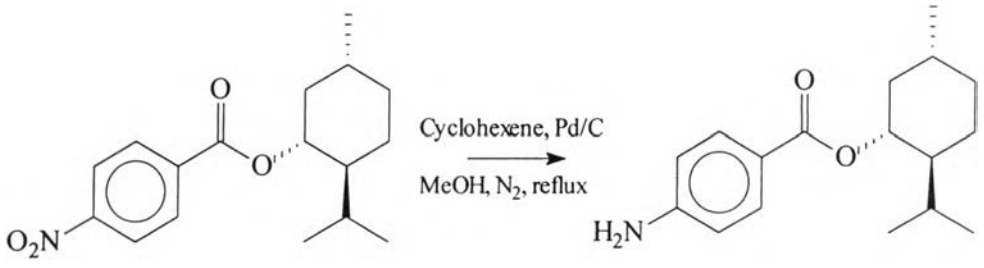


(1) 4-Nitrobenzoic acid (-)-menthyl ester **(49a)**



(-)-Menthol (1.57 g, 10.0 mmol) was added to a mixture of *p*-nitrobenzoyl chloride (1.86 g, 10.0 mmol) and triethylamine (1.39 mL, 10.0 mmol) in dichloromethane (12 mL) with stirring at room temperature for 5 days. The reaction mixture was diluted with dichloromethane, washed with 5% $NaHCO_3$ and water. It was then dried with $MgSO_4$, filtered, concentrated under reduced pressure. The residue was purified by flash column chromatography using 10% ethyl acetate-hexane as eluent to give the product as a yellow oil (1.86 g, 61% yield). ^{1}H NMR ($CDCl_3$) δ_H 0.75 (3H, d, J = 7.0 Hz, CH_3), 0.71 and 0.92 (2x3H, 2xd, J = 7.0 Hz, $CH(CH_3)_2$), 1.01-1.25 and 1.55-2.20 (9H, m, CH, CH_2), 4.95 (1H, dt, J = 8.0 Hz, OCH), 8.20 and 8.40 (2x2H, AB doublet, J = 8.0 Hz, aromatic C-H) (Figure 13). $[\alpha]_D^{20}$ –76.0 (c=2.0, MeOH).

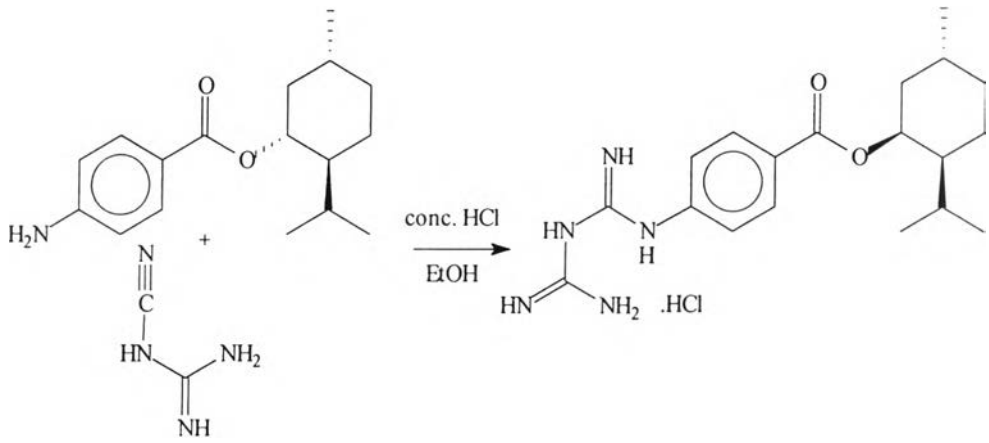
(2) 4-Aminobenzoic acid (-)-menthyl ester **(49b)**



Cyclohexene (1.20 mL, 11.8 mmol) was added to a mixture of 4-nitrobenzoic acid (-)-menthyl ester (1.80 g, 5.9 mmol) and palladium/charcoal as catalyst in absolute methanol (5 mL). The reaction mixture was refluxed under N_2 overnight. The catalyst was removed by filtration with celite, washed with methanol. The solution was then evaporated to give the crude product which was purified by column

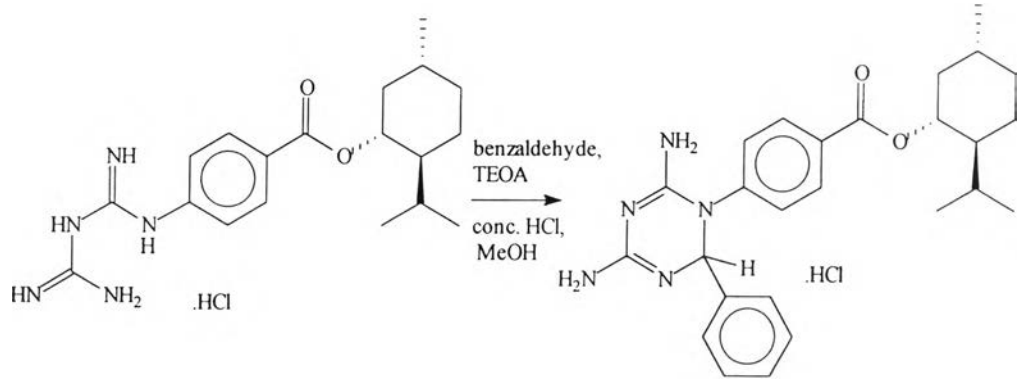
chromatography using 10% ethyl acetate-hexane as eluent to give the product as a yellow oil (1.30 g, 80% yield). ^{1}H NMR (DMSO) δ_H 1.01 (2x3H, d, J = 7.6 Hz, CH(CH_3)$_2$), 1.06 (3H, d, J = 7.0 Hz, CH_3), 1.25-1.65 and 1.82-1.96 (9H, m, menthol CH, CH_2), 3.67 (1H, dt, J_t = 14.2, J_d = 7.2 Hz, OCH), 6.35 and 6.52 (2x2H, AB doublet, J= 8.0 Hz, aromatic C-H).

(3) 4-(2′*S*-Isopropyl-5′*R*-methyl-1′*R*-cyclohexyloxylcarbonyl)phenylbiguanide-hydrochloride **(49c)**



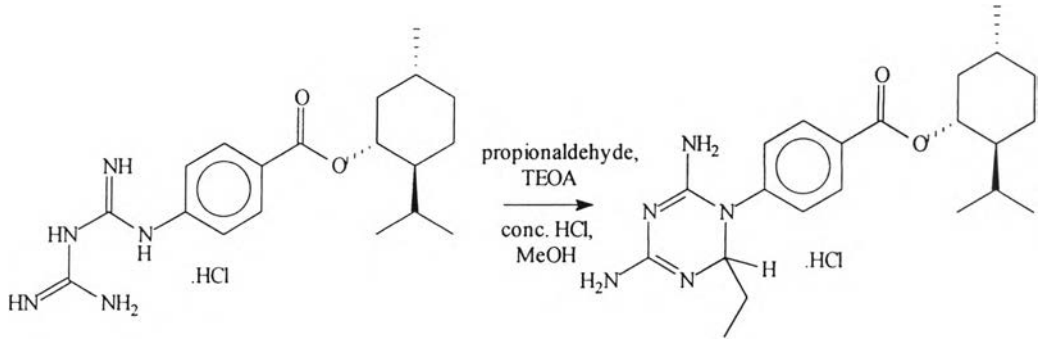
Dicyanodiamide (0.18 g, 2.2 mmol) was added to a solution of 4-aminobenzoic acid (-)-menthyl ester (0.50 g, 1.8 mmol) in absolute ethanol (8 mL). Concentrated HCl (0.15 mL, 1.7 mmol) was then added and the reaction mixture was refluxed for 5 hours. The solvent was removed under reduced pressure. The crude reaction product was washed with acetone and ethanol to give a white solid (0.52 g, 73% yield). ^{1}H NMR (DMSO) δ_H 0.74 (3H, d, J = 7 Hz, CH_3), 0.85 and 0.96 (2x3H, d, J = 7.6 Hz, CH(CH_3)$_2$), 1.05-2.05 (9H, m, menthol CH, CH_2), 4.80 (1H, dt, J = 14.2, 7.2 Hz, OCH), 7.45 and 8.45 (2x2H, AB doublet, J = 8.0 Hz, aromatic C-H) (Figure 14).

(4) 1-[4′-(2″*S*-isopropyl-5″*R*-methyl-1″*R*-cyclohexyloxycarbonyl)phenyl]-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(49d)**



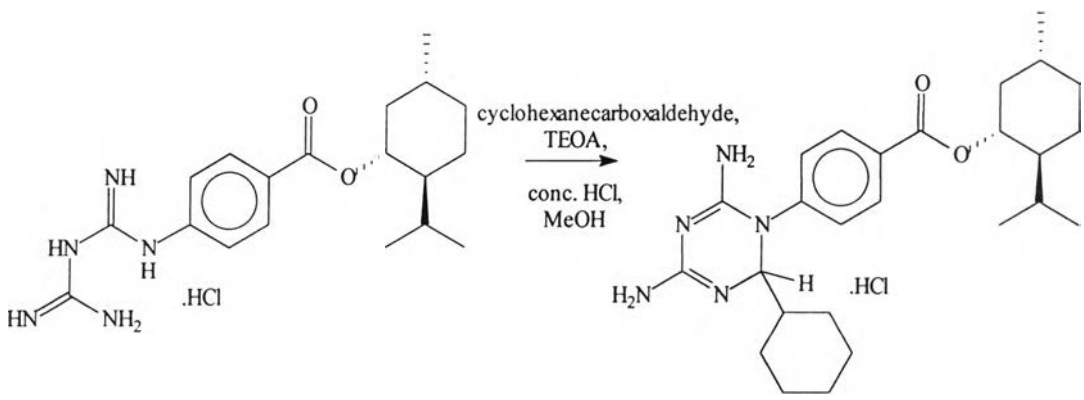
The same procedure as for 1-(4′-chlorophenyl)-2-[4′-(2″*S*-isopropyl-5″*R*-methyl-1″*S*-cyclohexyloxy)phenyl]-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride (2.4.1-(3)) was followed but 4-(2′*S*-isopropyl-5′*R*-methyl-1′*R*-cyclohexyloxycarbonyl)phenylbiguanide hydrochloride (8.3 mmol) was used instead of 4-chloropheynylbiguanide hydrochloride to give the product as a white solid (0.2 g, 50% yield). ^{1}H NMR (DMSO) δ_H 0.7 (3H, d, J = 7 Hz, C$\underline{H}_3$), 0.81 and 0.89 (2x3H, d, J = 7.6 Hz, CH(C$\underline{H}_3$)$_2$), 0.99-1.19 and 1.4-2.1 (9H, m, menthol C$\underline{H}$, C$\underline{H}_2$), 4.82 (1 H, dt, J = 14.2, 7.2 Hz, OC$\underline{H}$), 6.15 (1H, s, $\underline{H}$-2), 7.38-7.41 and 7.85-7.99 (10H, m, aromatic C-H), 9.18 (1H, br m, N$\underline{H}$) (Figure 15). $[\alpha]_D^{20}$ –11.8 (c=1.1, MeOH); *m/z* (MALDI-TOF) 447 (M.H$^+$).

2.4.5 Attempted synthesis of 1-[4'-(2''S-isopropyl-5''R-methyl-1''R-cyclohexyloxy-carbonyl)phenyl]-2-ethyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(49e)**



The method described in the foregoing preparation of 1-[4'-(2''*S*-isopropyl-5''*R*-methyl-1''*R*-cyclohexyloxy)phenyl]-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride (2.4.4-(4)) above was followed with benzaldehyde being replaced by butyraldehyde. TLC indicated that no triazine hydrochloride had formed by this method.

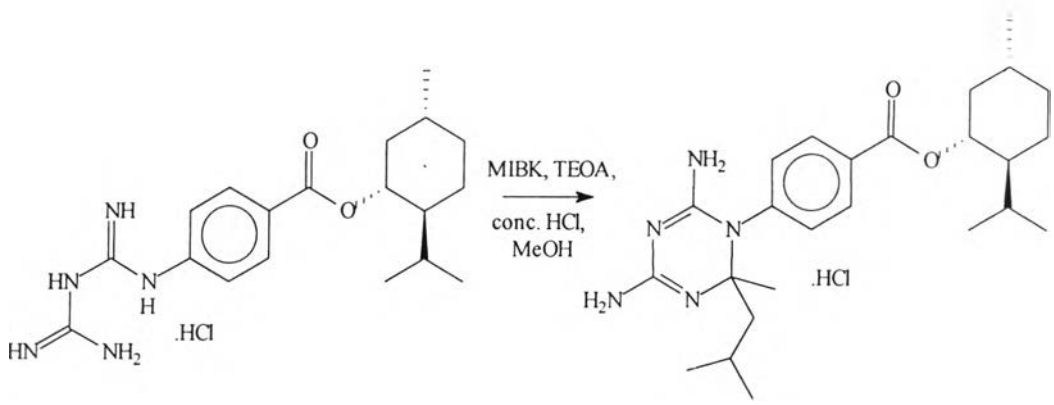
2.4.6 Attempted synthesis of 1-[4'-(2''S-isopropyl-5''R-methyl-1''R-cyclohexyloxycar-bonyl)phenyl]-2-cyclohexyl-4,6-diamino-1,2-dihydro-1,3,5-triazin hydrochloride **(49f)**



The method described in the foregoing preparation of 1-[4'-(2''*S*-isopropyl-5''*R*-methyl-1''*R*-cyclohexyloxycarbonyl)phenyl]-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride (2.4.4-(4)) above was followed with benzaldehyde being

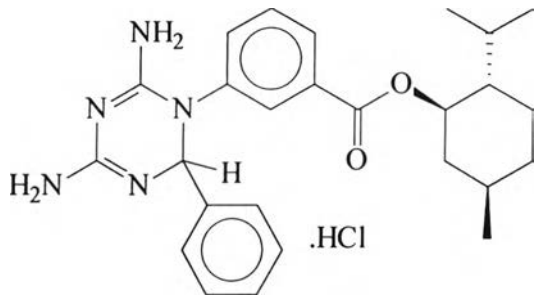
replaced by cyclohexanecarboxaldehyde. TLC indicated that no triazine hydrochloride had formed by this method.

2.4.7 Attempted synthesis of 1-[4'-(2''S-isopropyl-5''R-methyl-1R''-cyclohexyloxy-carbonyl)phenyl]-2-methyl-2-isobutyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(49g)**

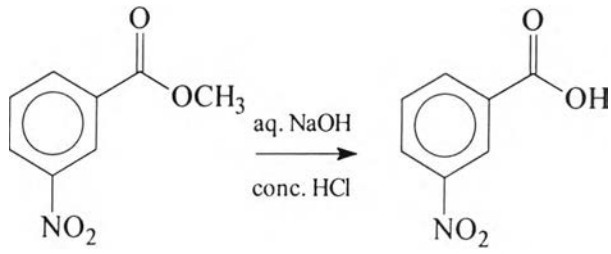


The method described in the foregoing preparation of 1-[4'-(2''*S*-isopropyl-5''*R*-methyl-1''*R*-cyclohexyloxycarbonyl)phenyl]-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride (2.4.4-(4)) above was followed with benzaldehyde being replaced by methyl isobutyl ketone. TLC indicated that no triazine hydrochloride had formed by this method.

2.4.8 Attempted synthesis of 1-[3'-(2''S-isopropyl-5''R-methyl-1''R-cyclohexyloxycarbonyl)phenyl]-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(50d)**

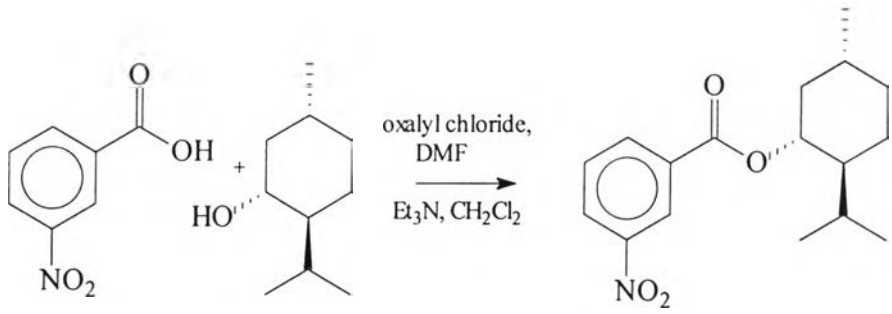


(1) 3-Nitrobenzoic acid



Methyl *m*-nitrobenzoate (2.72 g, 15.0 mmol) was added to an aqueous NaOH (0.60 g, 15.0 mmol) with stirring at room temperature. The solution was acidified with concentrated HCl until a white crystalline solid formed. The mixture was filtered and washed with water to give 3-nitrobenzoic acid as a white crystalline solid (2.45 g, 98% yield). ^{1}H NMR (D_2O) δ_H 7.73 (1H, dd, J = 8.5, 2.6 Hz, aromatic C-H), 8.53 (2H, m, aromatic C-H), 9.06 (1H, s, aromatic C-H).

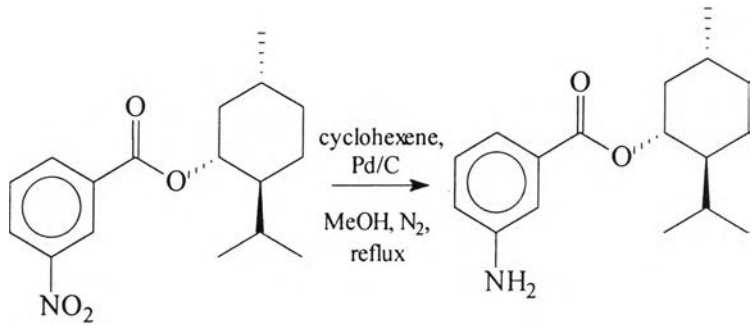
(2) 3-Nitrobenzoic acid (-)-menthyl ester **(50a)**



DMF (0.02 mL) was added to a mixture of 3-nitrobenzoic acid (1.67 g, 10.0 mmol) and oxalyl chloride (0.88 mL, 10.1 mmol) in dichloromethane (20 mL). (-)-Menthol (1.56 g, 10.0 mmol) and triethylamine (1.39 mL, 10.0 mmol) were then added and the reaction mixture was stirred for 5 days. The mixture was diluted with dichloromethane, washed with 5% $NaHCO_3$ and water. It was then dried with $MgSO_4$, filtered, concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel using 10% ethyl acetate-hexane as eluent to give a product as a yellow oil (1.83 g, 60% yield). ^{1}H NMR ($CDCl_3$) δ_H 0.77 (3H, d, J = 7.0 Hz, CH_3), 0.95 and 0.97 (2x3H, 2xd, J = 7.0 Hz, $CH(CH_3)_2$), 1.06-1.21 and 1.52-

2.07 (9H, m, C<u>H</u>, C<u>H</u>$_2$), 4.98 (1H, dt, J = 8.0 Hz, OC<u>H</u>), 7.67 (1H, t, J = 7.2 Hz, aromatic C-H), 8.35 and 8.39 (2x1H, d, J = 7.8 Hz, aromatic C-H) 8.83 (1H, s, aromatic C-H) (Figure 16). $[\alpha]_D^{20}$ –65.0 (c=3.0, MeOH).

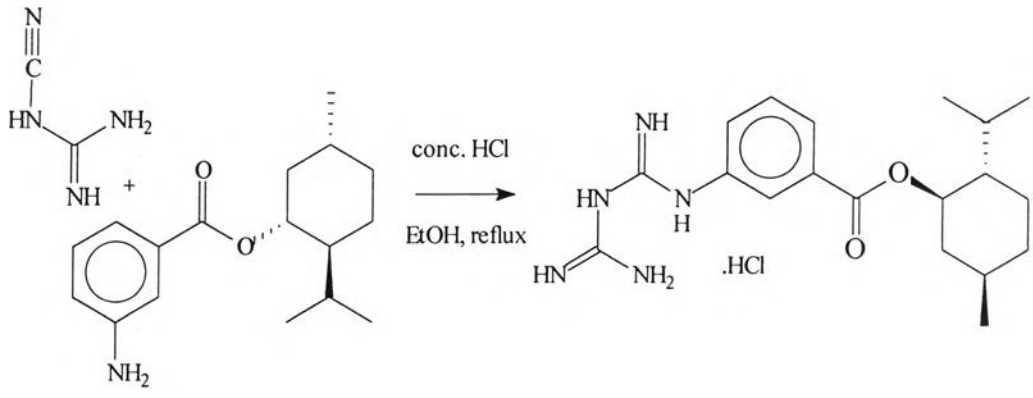
(3) <u>3-Aminobenzoic acid (-)-menthyl ester</u> **<u>(50b)</u>**



The same procedure as for 4-aminobenzoic acid (-)-menthyl ester (2.4.4-(2) was followed but 3-nitrobenzoic acid (-)-menthyl ester (3.5 mmol) was used instead of 4-nitrobenzoic acid (-)-menthyl ester to give the product as a yellow oil (0.80 g, 75% yield).

(4) <u>3-(2′*S*-5″*R*-methyl-1″*R*-cyclohexyloxycarbonyl)phenylbiguanide hydrochloride</u>

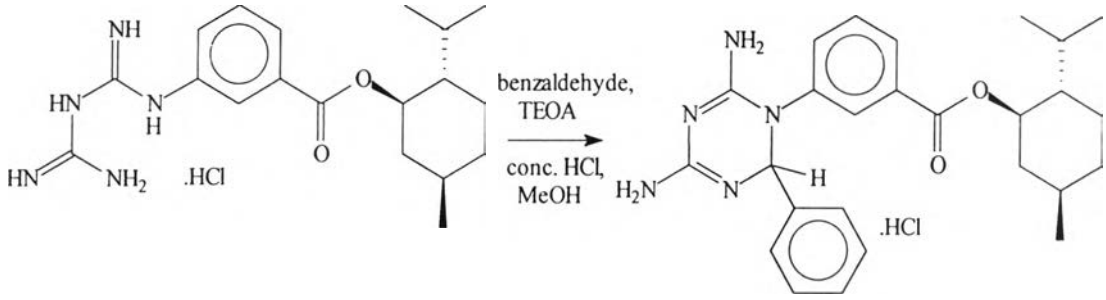
<u>(50c)</u>



The same procedure as for 4-(2′*R*-isopropyl-5′*R*-methyl-1′*R*-cyclohexyloxy-carbonyl)phenylbiguanide hydrochloride (2.4.4-(3)) was followed but 3-aminobenzoic acid (-)-menthyl ester (3.8 mmol) was used instead of 4-aminobenzoic acid (-)-

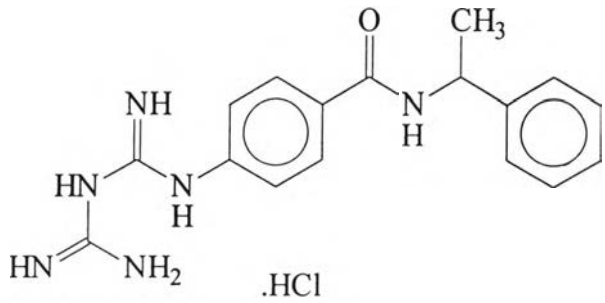
menthyl ester. The reaction was refluxed for 5 hours. The solvent was then removed under reduced pressure to give a crude reaction as a semisolid (0.12 g).

(5) 1-[3′-(2″*S*-isopropyl-5″-*R*-methyl-1″*R*-cyclohexyloxycarbonyl)phenyl]-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(50d)**

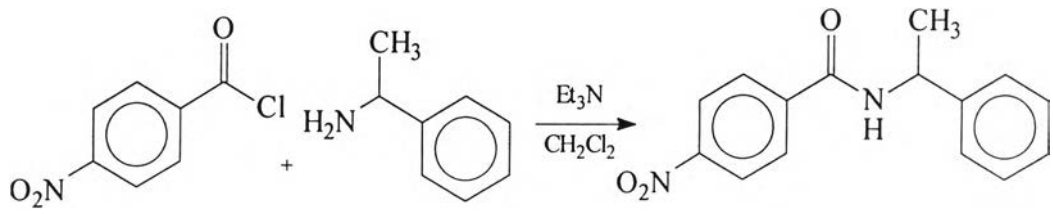


The method described in the foregoing preparation of 1-[4′-(2″*S*-isopropyl-5″-*R*-methyl-1″*R*-cyclohexyloxycarbonyl)phenyl]-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride (2.4.4-(4)) above was followed with 4-(2′*R*-isopropyl-5′*R*-methyl-1′*R*-cyclohexyloxycarbonyl)phenylbiguanide hydrochloride being replaced by 3-(2′*R*-isopropyl-5′*R*-methyl-1′*R*-cyclohexyloxycarbonyl)phenylbiguanide hydrochloride (1.0 mmol). TLC indicated that no triazine hydrochloride had formed by this method.

2.4.9 Attempted synthesis of (±)-4-(α-Methylbenzylcarbamoylphenyl)biguanide hydrochloride **(51c)**

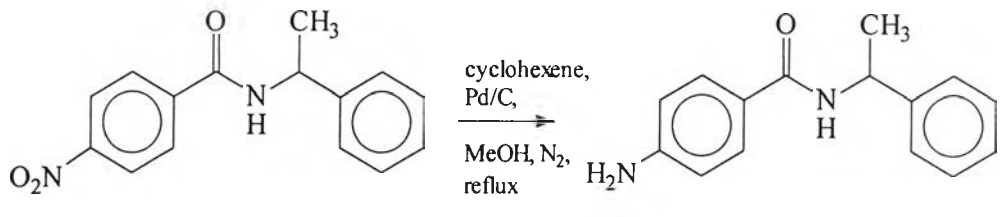


(1) (±)-*N*-(α-Methylbenzyl)-4-nitrobenzamide



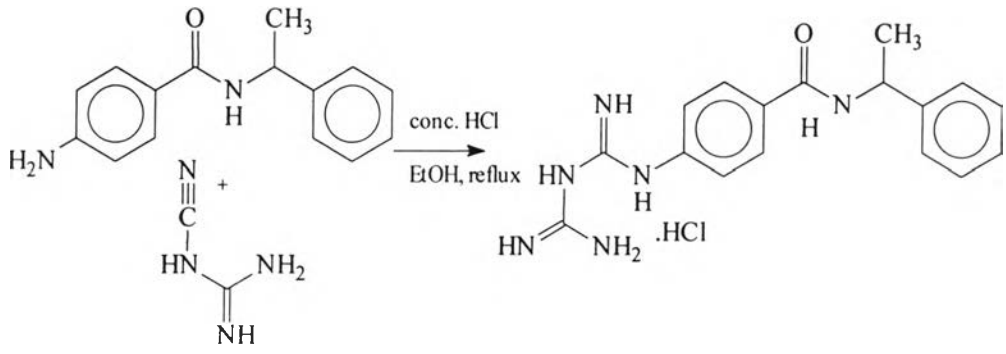
Triethylamine (0.14 mL, 1.0 mmol) was added to a mixture of *p*-nitrobenzoyl chloride (0.19 g, 1.0 mmol) and (±)-α-methylbenzylamine (0.13 mL, 1.0 mmol) in dichloromethane (5 mL). After stirring at room temperature overnight, the reaction was diluted with dichloromethane, washed with 5% $NaHCO_3$ and water. It was then dried with $MgSO_4$, filtered and evaporation to give the product as a yellow oil (0.26 g, 96% yield) which was pure enough for practical purposes.). ^{1}H NMR ($CDCl_3$) 1.58 (3H, d, J = 2.8 Hz, C<u>H</u>$_3$), 5.05 (1H, m, C<u>H</u>), 7.08-7.21 (5H, m, aromatic, C-H), 8.21 and 8.37 (2x2H, AB doublet, J = 8.2 Hz, aromatic C-H).

(2) (±)-*N*-(α-Methylbenzyl)-4-aminobenzamide **(51b)**



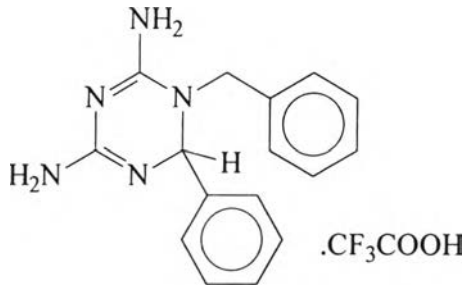
The same procedure as for 4-aminobenzoic acid (-)-menthyl ester (2.4.4-(2)) was followed but (±)-*N*-(α-methylbenzyl)-4-nitrobenzamide (1.2 mmol) was used instead of 4-nitrobenzoic acid (-)-menthyl ester. The crude product was purified by column chromatography eluting with 50% ethyl acetate-hexane to give the product as a yellow oil (0.20 g, 70% yield). ^{1}H NMR ($CDCl_3$) 1.54 (3H, s, C<u>H</u>$_3$), 5.32 (1H, q, J = 7.0 Hz, C<u>H</u>), 6.51-6.77 and 7.54-7.69 (2x2H, AB doublet, J = 8.0 Hz, aromatic C-H), 7.19-7.41 (5H, m, aromatic C-H) (Figure 17).

(3) (±)-4-(α-Methylbenzylcarbamomylphenyl)biguanide hydrochloride **(51c)**

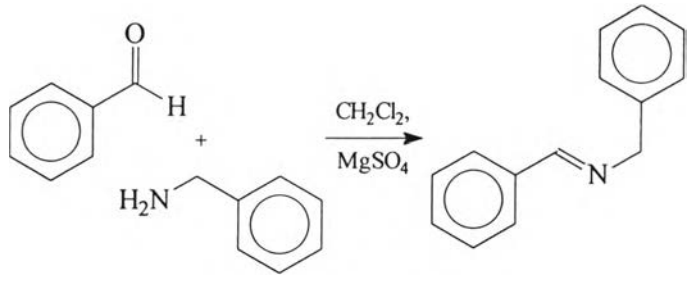


The same procedure as for 4-(2′*S*-isopropyl-5′*R*-methyl-1′*R*-cyclohexyloxy-carbonyl)phenylbiguanide hydrochloride (2.4.4-(3)) was followed but *N*-(α-methyl-benzyl)-4-aminobenzamide (0.9 mmol) was used instead of 4-aminobenzoic acid (-)-menthyl ester. The precipitated solid was filtered and washed with cold ethanol. ^{1}H NMR indicated that it was an undesired product.

2.4.10 Synthesis of 1-benzyl-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine trifluoroacetate **(52b)**

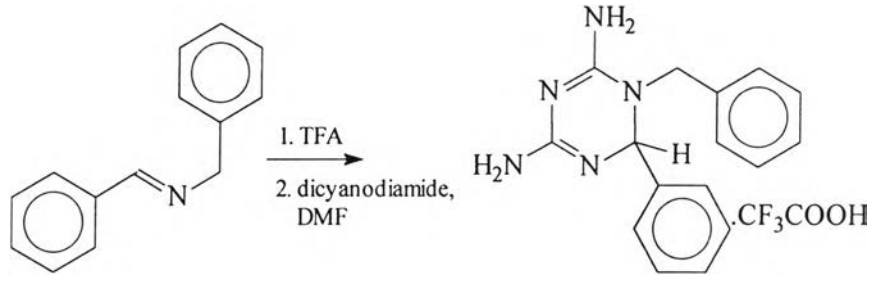


(1) *N*-Benzylidenebenzylamine **(52a)**



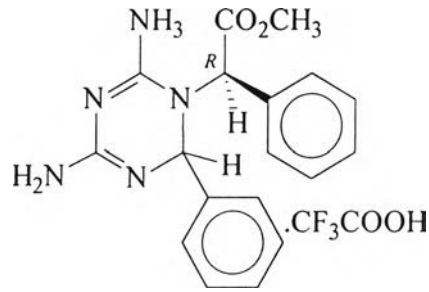
benzylamine (1.00 mL, 10.0 mmol) and benzaldehyde (1.00 mL, 10.0 mmol) were dissolved in dichloromethane (10.0 mL) with stirring at room temperature. Anhydrous $MgSO_4$ (200 mg) was then added and the reaction was stirred until TLC showed disappearance of the starting materials. The reaction was filtered and concentrated the solution under reduced pressure to give the desired product as a yellow oil (1.95 g, 100% yield). ^1H MNR ($CDCl_3$) δ_H 4.89 (2H, s, $CH_2(C_6H_5)$, 7.19-7.44 and 7.78-7.90 (10H, m, aromatic C-H), 8.38 (1H, s, =CH) (Figure 18).

(2) 1-Benzyl-4,6-diamino-1,2-dihydro-1,3,5-triazine trifluoroacetate **(52b)**

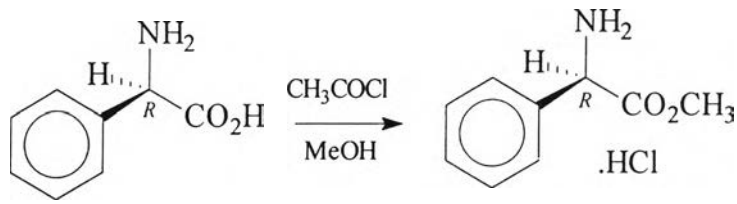


Trifluoroacetic acid (0.39 mL, 5.1 mmol) was add to a round bottom flask containing *N*-benzylidenebenzylamine (0.97 g, 5.0 mmol) The reaction was stirred for 10 minutes and dimethyl formamide (2.50 mL) was added followed by dicyanodiamide (0.42 g, 5.0 mmol). The reaction was stirred at room temperature until TLC showed complete disappearance of the starting materia! (1-2 days). Excess diethyl ether was added to precipitate the product, which was collected by filtration and washed with diethyl ether to give the crude product. Recrystallization from methanol in ether gave a white crystalline solid (0.67 g, 34% yield). ^1H NMR (DMSO) δ_H 4.10 and 5.00 (2x1H, 2xd, J = 17.0 Hz, CH_2), 5.65 (1H, s, H-2), 7.15-7.50 (10H, m, aromatic C-H) (Figure 19); *m/z* (MALDI-TOF) 279 ($M.H^+$).

2.4.11 Attempted synthesis of 2-(2′-phenyl-4′,6′-diamino-1′,2′-dihydro-1′,3′,5′-triazin-1′-yl)-2-phenyl acetic methyl ester trifluoroacetate **(53c)**

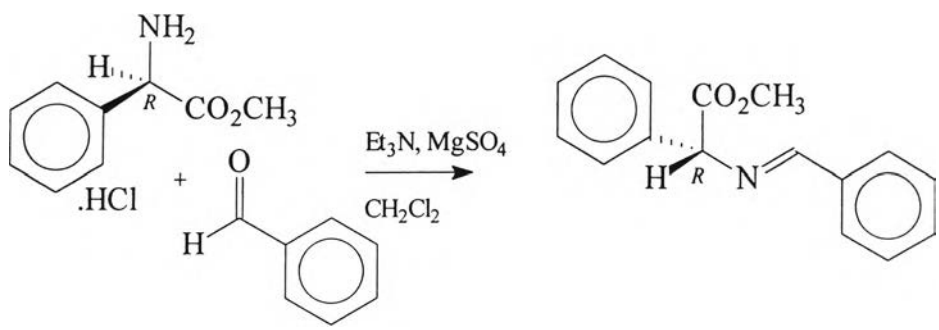


(1) *R*-Phenylglycine methyl ester hydrochloride **(53a)**



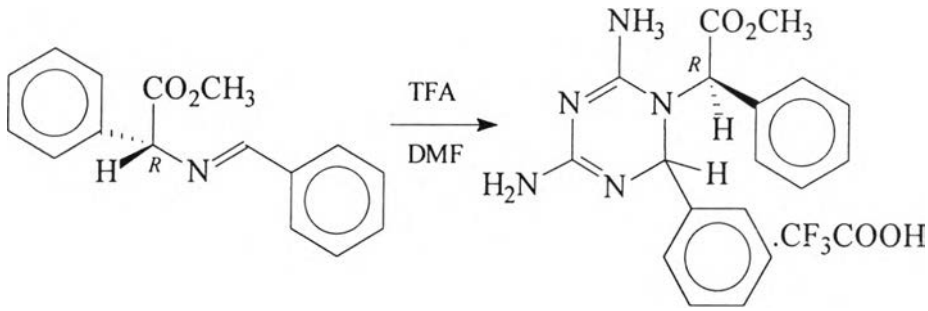
Acetyl chloride (0.89 mL, 12.5 mmol) was added to a suspension of D(-)-phenyl glycine (0.76 g, 5.0 mmol) in methanol (20 mL). The mixture was refluxed for 6 hours and the solvent was removed under reduced pressure. The residue was washed with diethyl ether and collected by filtration to give the product as a white solid (0.91 g, 90% yield). ^{1}H NMR (DMSO) δ_H 3.34 (3H, s, C<u>H</u>$_3$), 3.72 (1H, s, C<u>H</u>(C_6H_5)) 7.42-7.58 (5H, m, aromatic C-H), (2H, br m, N<u>H</u>).

(2) *N*-benzylidene-*R*-phenylglycine methyl ester **(53b)**



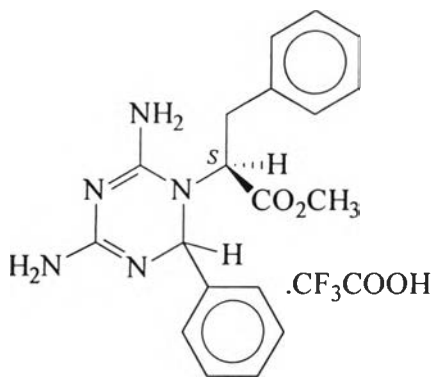
The same procedure as for *N*-benzylidenebenzylamine (2.4.10-(1)) was followed but D(-)-phenylglycine methyl ester hydrochloride (0.74 g, 3.7 mmol) and triethylamine (0.62 mL, 4.4 mmol) was used instead of benzylamine to give the product as yellow oil (0.93 g, 100% yield). ^{1}H NMR ($CDCl_3$) δ_H 3.74 (3H, s, OCH_3), 5.21 (1H, s, $CHCO_2CH_3$), 7.30-7.45 (5H, m, aromatic C-H), 7.50 and 7.81 (5H, 2xm, aromatic C-H), 8.33 (1H, s, N=CH) (Figure 20).

(3) 2-(2′-phenyl-4′,6′-diamino-1′,2′-dihydro-1′,3′,5′-triazin-1′-yl)-2-phenyl acetic methyl ester trifluoroacetate **(53c)**

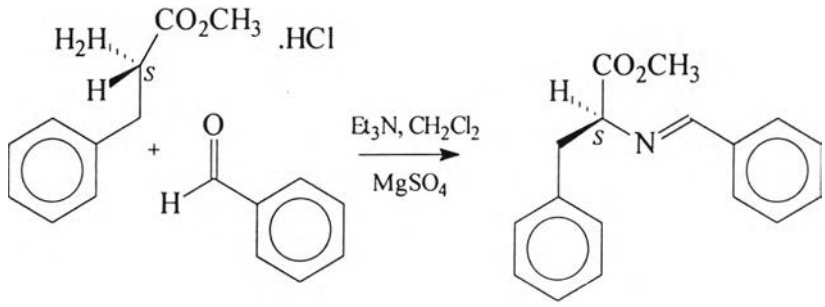


The same procedure as for 1-benyl-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine trifluoroacetate (2.4.10-(2)) was followed but *N*-benzylidene-*R*-phenylglycine methyl ester (3.0 mmol) was used instead of *N*-benzylidenebenzylamine. TLC showed the presence of complex mixture in the reaction product.

2.4.12 Attempted synthesis of 2S(2′-phenyl-4′,6′-diamino-1′,2′-dihydro-1′,3′,5′-triazin-1-yl)-3-phenylpropionic acid methyl ester trifluoroacetate
(54b and 54b′)

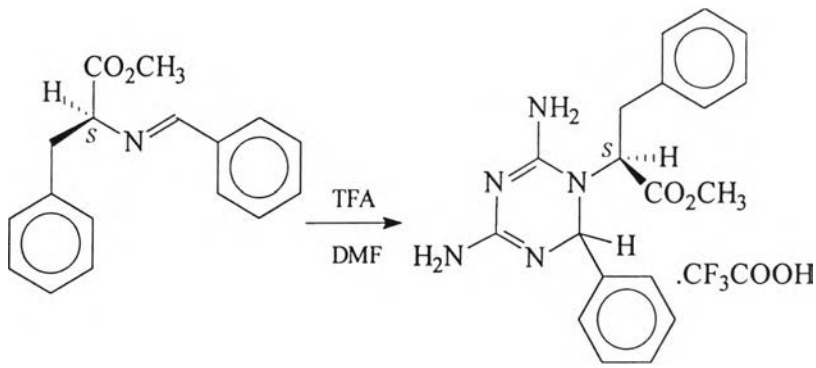


(1) *N*-benzylidene-*S*-phenylalanine methyl ester **(54a)**



The same procedure as for *N*-benzylidenebenzylamine (2.4.10-(1)) was followed but but L-phenylalanine methyl ester hydrochloride (0.64 g, 3.0 mmol) and triethylamine (0.44 mL, 3.2 mmol) was used instead of benzylamine to give the product as a yellow oil (0.76 g, 96% yield). ^{1}H NMR ($CDCl_3$) δ_H 1.25 (1H, y, J = 7.8 Hz, C<u>H</u>CO_2CH_3) 3.75 (3H, s, O<u>C</u>H_3), 4.20 (2H, dd, J = 2.2, 2.6 Hz, <u>C</u>$H_2C_6H_5$), 7.07-7.25 (5H, m, aromatic C-H), 7.36 and 7.69 (5H, 2xm, aromatic C-H), 7.90 (1H, s, N=C<u>H</u>) (Figure 21). $[\alpha]_D^{20}$ –10.8 (c=1.2, MeOH).

(2) 2*S*-(2′-phenyl-4′,6′-diamino-1′,2′-dihydro-1′,3′,5′-triazin-1′-yl)-3′-phenylpropionic acid methyl ester trifluoroacetate 1,3,5- triazine trifluoroacetate **(54b and 54b′)**



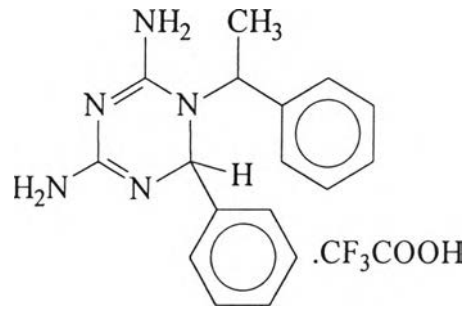
The same procedure as for 1-benzyl-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine trifluoroacetate (2.4.10-(2)) was followed but *N*-benzylidene-*S*-phenylalanine methyl ester (10.0 mmol) was used instead of *N*-benzylidenebenzylamine. The reaction was left for a long time to precipitate a white solid **(54d)** which was filtered and washed with acetone (0.20 g, 4% yield). From ^{1}H NMR it can be concluded that **(54d)** not the desired product. (see section 3.3, Figure 3.27). ^{1}H NMR (DMSO) δ_H

3.03 (2H, d, J = 8.0, $CH_2(C_6H_5)$, 4.77 (1H, t, J = 7.7, $CHCH_2C_6H_5$), 6.30 (1H, s, H-2), 6.74-6.81 and 6.92-7.01 (5H, 2xm, aromatic C-H), 7.11-7.45 (5H, m, aromatic C-H) (Figure 22).

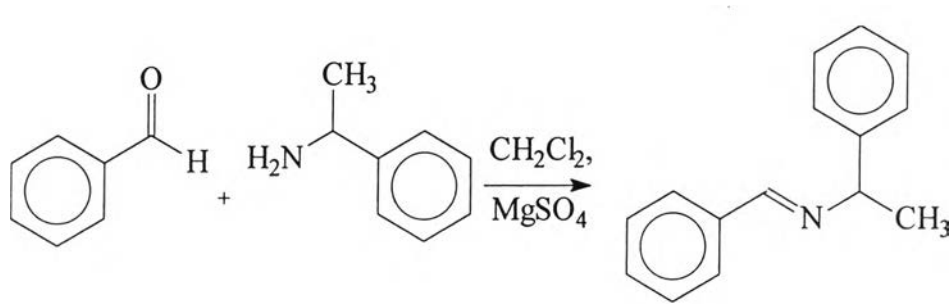
Measurement the racemization of rate of deuterium exchange of **(54d)**

(54d) (4.20 mg) was dissolved in DMSO-d_6 (0.5 mL) with 3 drops of D_2O. The rate of deuterium exchange was monitored by ^{1}H NMR spectroscopy.

2.4.13 Synthesis of racemic 1-(1'RS-phenylethyl)-2SR-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine trifluoroacetate **(55b)**

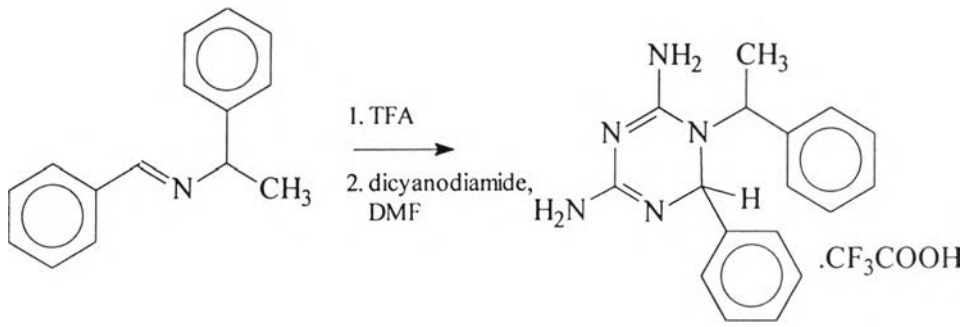


(1) racemic *N*-Benzylidene-2-methylbenzylamine**(55a)**



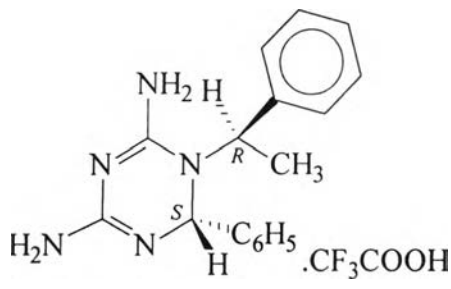
The same procedure as for *N*-benzylidenebenzylamine (2.4.10-(1)) was followed but (±)-methylbenzylamine (1.30 mL, 10.0 mmol) was used instead of benzylamine to give the product as a yellow oil (1.93 g, 95% yield). ^{1}H NMR ($CDCl_3$) δ_H 1.59 (3H, d, J = 7.9 Hz, CH_3), 4.51 (1H, q, J = 7.9 Hz, CH), 7.19-7.48 and 7.79-7.83 (10H, m, aromatic C-H), 8.39 (1H, s, =CH) (Figure 23).

(2) racemic 1-(1′*RS*-Phenylethyl)-2*SR*-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine-trifluoroacetate **(55b)**

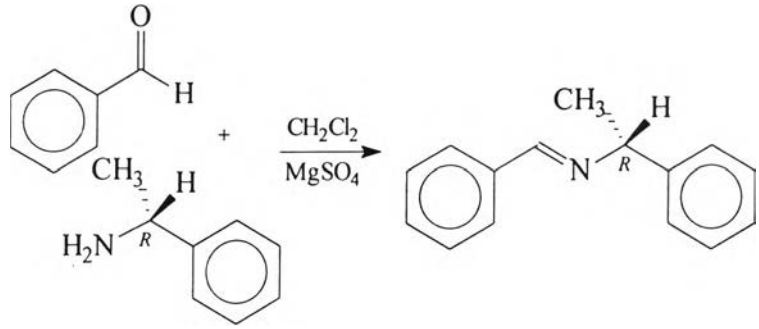


The same procedure as for 1-benzyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine trifluoroacetate (2.4.10-(2)) was followed but racemic *N*-Benzylidene-2-methylbenzylamine (1.05 g, 5.0 mmol) was used instead of *N*-benzylidene-benzylamine to give the product as a white solid (3.25 g, 80% yield). ^{1}H NMR (DMSO) δ_H 1.30 (3H, d, J = 7.6 Hz, C<u>H</u>$_3$), 5.50 (1H, q, J = 7.6 Hz, C<u>H</u>), 5.65 (1H, s, <u>H</u>-2), 7.25-7.50 (10H, m, aromatic C-H), 8.61 and 8.63 (3H, br m, N<u>H</u>) (Figure 24); *m/z* (MALDI-TOF) 294 (M.H$^+$).

2.4.14 Synthesis of 1-(1′R-phenylethyl)-2S-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine trifluoroacetate **(55b′)**

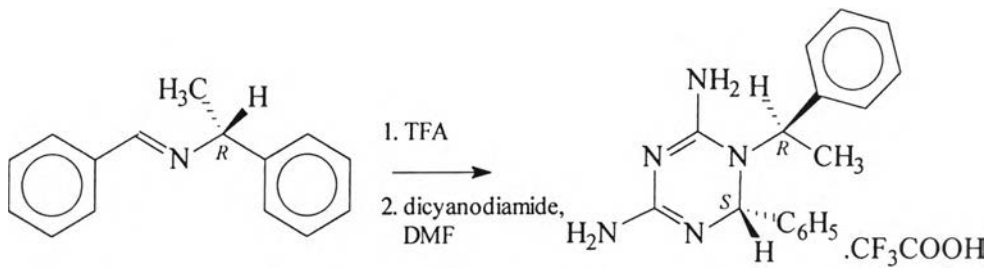


(1) (*R*)-*N*-Benzylidene-2-methylbenzylamine **(55a')**



The same procedure as for *N*-benzylidenebenzylamine (2.4.10-(1)) was followed but *R*-(+)-methylbenzylamine (0.65 g, 5.0 mmol) was used instead of benzylamine to give the product as a yellow oil (1.05 g, 100% yield). ^{1}H NMR ($CDCl_3$) δ_H 1.55 (3H, d, J = 8.0 Hz, CH_3), 4.55 (1H, q, J = 8.0 Hz, CH), 7.25-7.50 and 7.75-7.80 (10H, m, aromatic C-H), 8.40 (1H, s, =CH) (Figure 25). $[\alpha]_D^{20}$ –60.5 (c=2.6, EtOH).

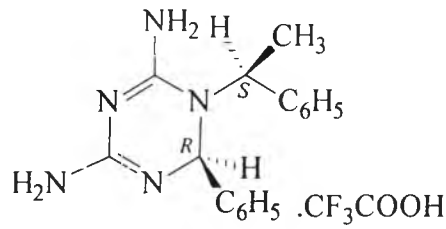
(2) 1-(1'*R*-phenylethyl)-2*S*-phenyl-4,6-diamino-1,2-dihydro-1,3,5- triazine trifluoroacetate **(55b')**



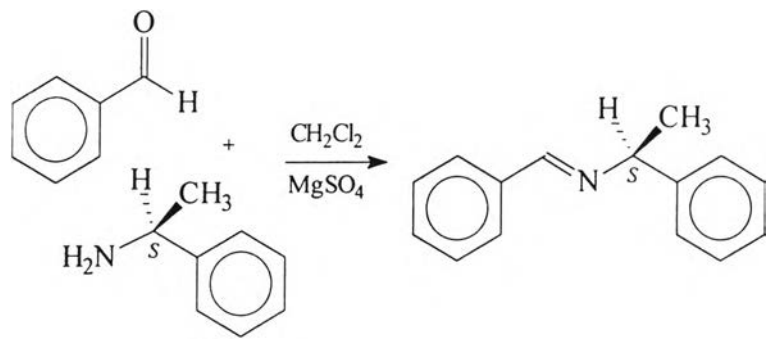
The same procedure as for 1-benzyl-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine trifluoroacetate (2.4.10-(2)) was followed but (*R*)-*N*-Benzylidene-2-methylbenzylamine (2.09 mg, 10.0 mmol) was used instead of *N*-benzylidene-benzylamine to give the product as a white solid (3.13 g, 77% yield). Anal. Calcd. for $C_{57}H_{60}F_9N_{15}O_6$+2CH_3CN+H_2O: C, 55.4; H, 5.2; N, 18.0% Found; C, 55.0; H, 5.0; N, 18.1%. ^{1}H NMR (DMSO) δ_H 1.31 (3H, d, J = 6.9 Hz, $CHCH_3$), 5.50 (1H, q, J = 6.9 Hz, $CHCH_3$), 5.66 (1H, d, J = 3.8 Hz, H-2), 7.28-7.50 (10H, m, aromatic C-H), 7.92

and 8.96 (3H, br m, NH) (Figure 26). $[\alpha]_D^{20}$ –121.5 (c=2.0, EtOH); *m/z* (MALDI-TOF) 294 ($M.H^+$).

2.4.15 Synthesis of 1-(1'S-phenylethyl)-2R-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine trifluoroacetate **(55b'')**

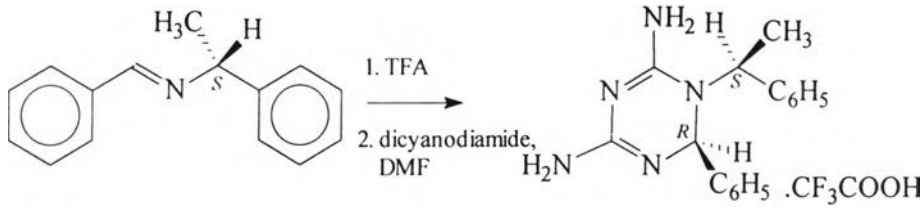


(1) (*S*)-*N*-Benzylidene-2-methylbenzylamine **(55a'')**



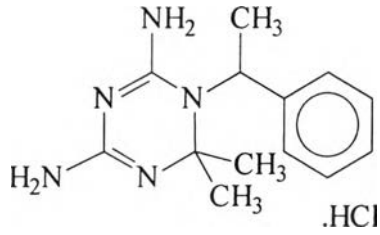
The same procedure as for *N*-benzylidenebenzylamine (2.4.10-(1)) was followed but (*S*)-(-)-methylbenzylamine (0.65 g, 5.2 mmol) was used instead of benzylamine to give the product as a yellow oil (1.09 g, 100% yield). ^{1}H NMR ($CDCl_3$) δ_H 1.65 (3H, d, J = 7.9 Hz, CH_3), 4.55 (1H, q, J = 7.9 Hz, CH), 7.25-7.50 and 7.75-7.80 (10H, m, aromatic C-H), 8.40 (1H, s, =CH) (Figure 27). $[\alpha]_D^{20}$ +70.0 (c=2.2, EtOH).

(2) 1-(1′*S*- phenylethyl)-2*R*-phenyl-4,6-diamino-1,2-dihydro-1,3,5- triazine trifluoroacetate **(55b″)**

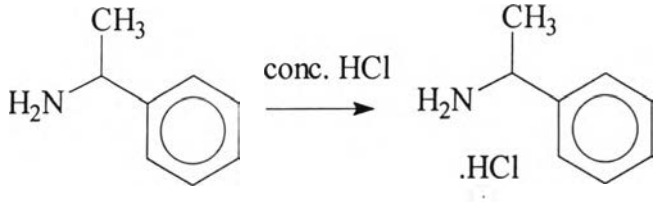


The same procedure as for 1-benyl-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine trifluoroacetate (2.4.10-(2)) was followed but (*S*)-*N*-benzylidene-2-methylbenzylamine (2.00 g, 9.9 mmol) was used instead of *N*-benzylidene-benzylamine to give the product as a white solid (2.59 g, 64% yield). Anal. Calcd. for $C_{57}H_{60}F_9N_{15}O_6$+2CH_3CN+H_2O: C, 55.4; H, 5.2; N, 18.0% Found; C, 55.2; H, 4.6; N, 18.1%. ^{1}H NMR (DMSO) δ_H 1.29 (3H, d, J = 6.9 Hz, $CHCH_3$), 5.50 (1H, q, J = 6.9 Hz, $CHCH_3$), 5.66 (1H, d, J = 3.8 Hz, H-2), 7.27-7.49 (10H, m, aromatic C-H) and 86.9 (3H, br m, NH) (Figure 28). $[\alpha]_D^{20}$ +132 (c=1.0, EtOH); *m/z* (MALDI-TOF) 294 ($M.H^+$).

2.4.12 Attempted Synthesis of (RS)-1-(α-Methylbenzyl)-2,2-dimethyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(56b)**

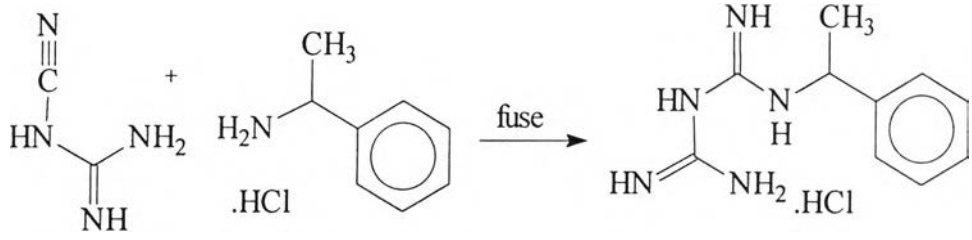


(1) *(RS)-α*-methylbenzylamine hydrochloride



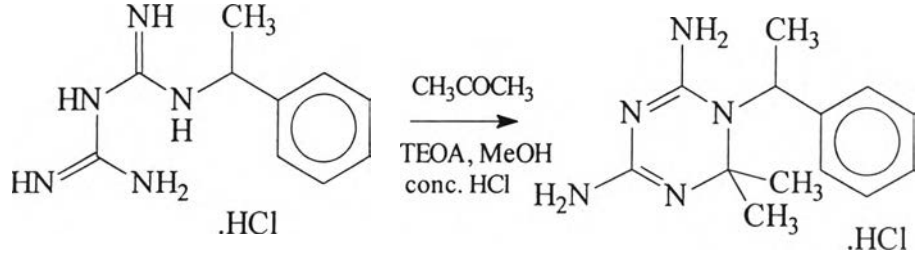
Concentrated HCl (0.87 mL, 10.0 mmol) was added to *(RS)*-α-methylbenzylamine (0.95 mL, 10.0 mmol) with stirring at room temperature for 4 hours. After that the excess concentrated HCl was removed by evaporation. The crude reaction was washed with diethyl ether and filtered to give a white crystalline solid (1.23 g, 78% yield).

(2) *(RS)*-α-Methylbenzylbiguanide hydrochloride **(56a)**



(RS)-α-Methylbenzylamine hydrochloride (0.79 g, 5.0 mmol) and dicyanodiamide (0.42 g, 5.0 mmol) were mixed together in a round bottomed flask and heat at 160 °C for 6 hours. The crude reaction mixture was washed with ether and filtered to give the product as a white crystalline solid (0.62 g, 51% yield). ^{1}H NMR (D_2O) δ_H 1.29 (4H, d, J = 7.9 Hz, C<u>H</u>, C<u>H</u>$_3$), 7.11-7.20 (5H, m, aromatic C-H) (Figure 29).

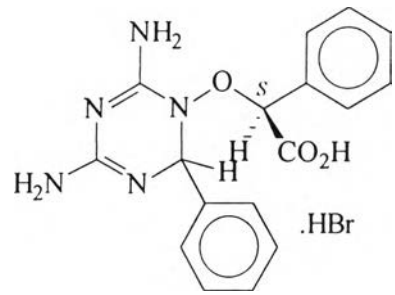
(3) <u>*(RS)*-1-(α-Methylbenzyl)-2,2-dimethyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(56b)**</u>



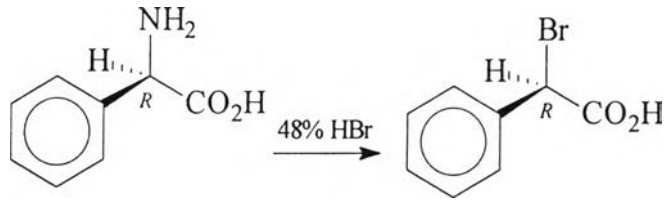
Concentrated HCl (0.02 mL, 0.2 mmol) was added to a mixture of triethyl orthoacetate (0.75 mL, 4.1 mmol), acetone (0.50 mL, 8.6 mmol) and (*RS*)-α-methylbenzylbiguanide hydrochloride (0.24 mL, 1 mmol) in absolute methanol. The reaction mixture was stirred at room temperature for 2-3 days. The solvent was then

removed by evaporation to give the white solid (0.23 g). ^{1}H NMR (D_2O) 1.36 (6H, s, 2xCH_3), 1.44 (3H, d, J = 7.2 Hz, CH_2CH_3), 5.09 (1H, q, J = 8 Hz, $CHCH_3$), 7.29 (5H, m, aromatic C-H), 8.49 and 8.70 (2H, br m, NH). ^{1}H NMR indicated that it was not the desired product (Figure 30).

2.4.17 Attempted synthesis of S-α-(2'-phenyl-4',6'-diamino-1',2'-dihydro-1',3',5'-triazin-1'-yloxy)phenylacetic acid hydrobromide **(61b)**

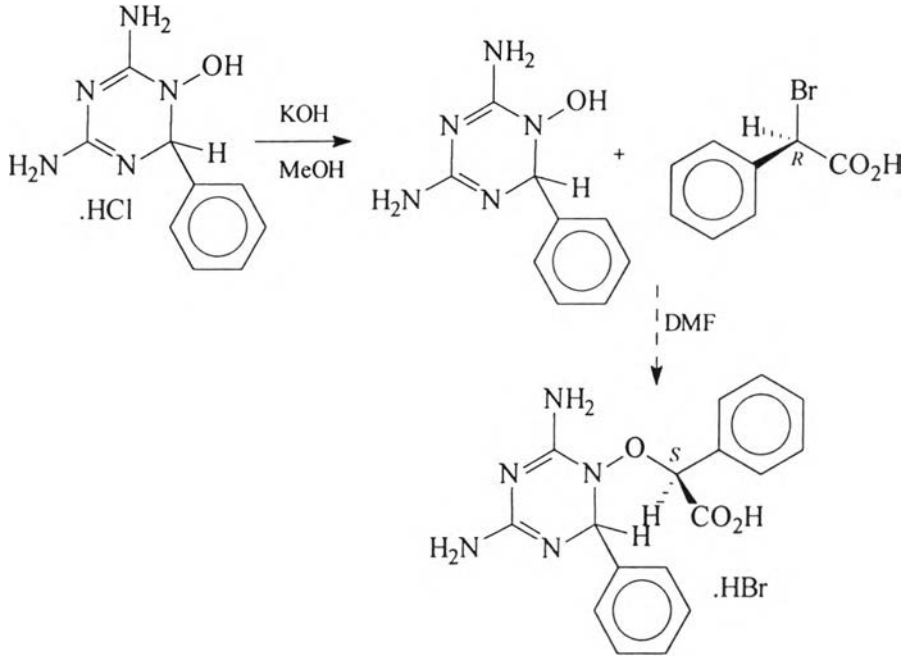


(1) *R*-α-Bromophenylacetic acid **(61a)**[32]



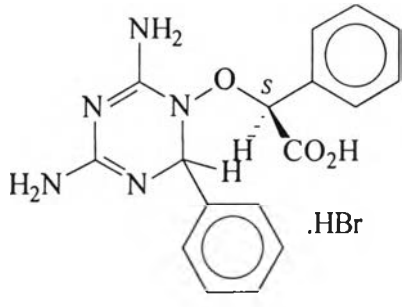
Aqueous $NaNO_2$ (0.22 g, 3.2 mmol) was added to a mixture of D(-)-α-phenyl glycine (0.15 g, 1.0 mmol) and 48% HBr (3 mL) with stirring at room temperature. After being stirred for 3 hours, the reaction mixture was extracted (ethyl acetate) and evaporated under reduced pressure. The crude reaction was dissolved in diethyl ether, washed with 10% $Na_2S_2O_5$, water containing 2-3 drops of concentrated HCl and dried with $MgSO_4$. The solvent was then removed by evaporation to give the product as a pale yellow oil (0.14 g, 68% yield). ^{1}H NMR ($CDCl_3$) 5.38 (1H, s, CH), 7.30 and 7.45 (5H, 2xm, aromatic C-H) (Figure 31).

(2) *S*-α-(2′-phenyl-4′,6′-diamino-1′,2′-dihydro-1′,3′,5′-triazin-1-yloxy)phenylacetic acid hydrobromide **(61b)**

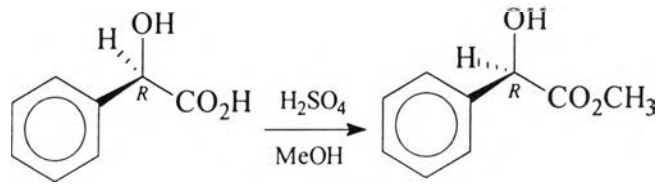


KOH (0.03 g, 0.6 mmol) was added to a mixture of 1-hydroxy-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride (0.10 g, 0.5 mmol) in absolute methanol (5 mL). After stirring for 6 hours, the solvent was removed by evaporation. *R*-α-bromophenylacetic acid (0.18 g, 1 mmol) and DMF (2.0 mL) were then added to the crude reaction with stirring at room temperature. After being stirred for 6 hours, the reaction had not occurred (according to TLC).

2.4.18 Attempted synthesis of S-α-(2′-phenyl-4′,6′-diamino-1′,2′-dihydro-1′,3′,5′-triazin-1′-yloxy)phenyl acetic acid methyl ester methansulfonate **(62c)**

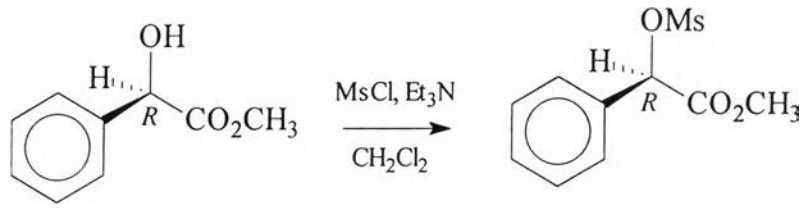


(1) D(-) Methyl mendalate



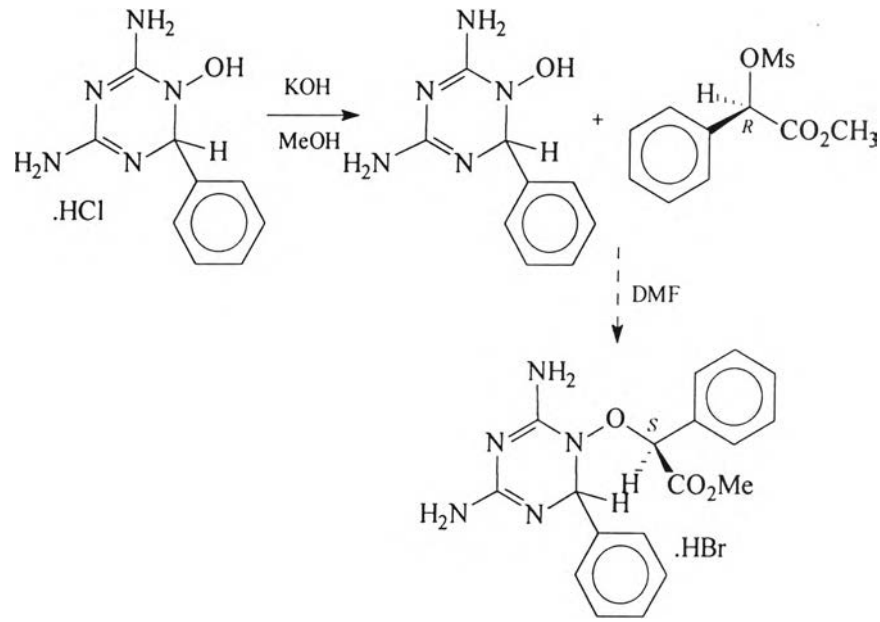
Concentrated H_2SO_4 (3 mL) was added to a solution of D(-) mandelic acid (3.98 g, 26.1 mmol) in methanol (50 mL). The reaction mixture was refluxed for 6 hours. After that the solvent was removed by evaporation water was added and aqueous $NaHCO_3$ was added to the solution until pH = 7. The solution was extracted with ethyl acetate and the solvent was removed by evaporation to give the product as a white solid (3.62 g, 83% yield).). ^1H NMR ($CDCl_3$) 3.79 (3H, s, CH_3), 5.94 (1H, s, CH), 7.35-7.49 (5H, m, aromatic C-H).

(2) *(R)*-α-Methanesulfonyloxyphenylacetic acid methyl ester **(62b)**



p-Methanesulfonyl chloride (1.35 mL, 14.7 mmol) was added to a solution of D(-) methyl mandelate (2.64 g, 15.9 mmol) in dichloromethane (15 mL). Triethylamine (2.44 ml, 17.5 mmol) was then added to the reaction mixture with stirring at 0 °C. After being stirred for 6 hours, the reaction mixture was diluted with dichloromethane, washed with 5% HCl, 5% $NaHCO_3$ and water. It was then dried with $MgSO_4$, filtered. The solvent was removed by evaporation to give the product as a white solid (3.81 g, 98% yield). ^1H MNR ($CDCl_3$) δ_H 3.79 (3H, s, CH_3), 5.94 (1H, s, CH), 7.35-7.49 (5H, m, aromatic C-H) (Figure 32).

(2) *S*-α-(2′-phenyl-4′,6′-diamino-1′,2′-dihydro-1′,3′,5′-triazin-1′-yloxy)phenylacetic-acid methyl ester methansulfonate **(63c)**



The same procedure as for α-*S*-(2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine-1-yloxyl)phenylacetic acid hydrobromide (2.4.17-(2)) was followed but *(R)*-α-methanesulfonyloxy phenylacetic acid methyl ester (0.8 mmol) was used instead of *(R)*-α-bromophenylacetic acid. TLC indicated that no desired product had formed by this method.

2.5 Study of rearrangement of 4,6-diamino-1, 2-dihydro-1,3,5-triazine

General procedure

The 4,6-diamino-1,2-dihydro-1,3,5-triazine was dissolved in to the NMR tube containing DMSO-d_6. The solution was incubated in a water bath at the controlled temperation. The rearrangement of compound was monitored by ^{1}H NMR spcectroscopy.

2.5.1 1-(4′-Chlorophenyl)-2-phenyl-4, 6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(1)**

4.1 mg in DMSO 0.47 mL (0.3M), temperature = 100 °C (Figure 37)

2.5.2 1-(Benzyloxy)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(63)**

3.5 mg in DMSO 0.54 mL (0.03 M), temperature = 100 °C (Figure 38)

2.5.3 1-(Benzyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(64)**

3.4 mg in DMSO-d_6 0.4 mL (0.03 M), temperature = 100 °C (Figure 39)

2.5.4 1-(Benzyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine trifluoroacetate **(65)**

4.1 mg in DMSO 0.43 mL (0.02 M), temperature = 100 °C (Figure 40)

2.5.5 1-(Benzyl)-2-(4′-nitrophenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine trifluoroacetate **(66)**

4.2 mg in DMSO-d_6 0.46 mL (0.02 M), temperature = 100 °C (Figure 41)

2.5.6 1-Benzyl-2-(4′-chlorophenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine trifluoroacetate **(67)**

8.0 mg in DMSO-d_6 0.40 mL (0.04 M), temperature = 100 °C (Figure 42)

2.5.7 1-Benzyl-2-(4′-methoxyphenyl)-4, 6-diamino-1, 2-dihydro-1, 3, 5-triazine trifluoroacetate **(68)**

5.8 mg in DMSO 0.41 mL (0.03 M), temperature = 100 °C (Figure 43)

2.6 Enzyme assays and inhibition by cycloguanil analogues

The activities of wild-type and A16VS108T mutant pfDHFRs were determined spectrophotometrically according to the method previously described.[22] The reaction (200 μL) contained 1x DHFR buffer (50 mM TES, pH 7.0, 75 mM β-mercaptoethanol, 1 mg/mL Bovine Serum Albumin), 100 μM each of the substrate H_2folate and cofactor NADPH, and appropriate amount (0.001-0.005 units) of the affinity-purified enzymes. The inhibition of the enzymes with cycloguanil analogues and combinatorial libraries was investigated in a 96 well plate with 200 μL reaction of the above mixture, in the presence of antifolate. The kinetic reaction was followed by a microplate reader (Labsystems, Finland). The K_i values of the inhibitors for the enzymes were then determined by fitting to the equation $IC_{50} = K_i\ (1+([S]/K_m))$,[33] where IC_{50} is the concentration of inhibitor which inhibits 50% of the enzyme activity under the standard assay condition and K_m is the Michaelis constant for the substrate H_2folate.