# DBU-Assisted Cyclorelease Elimination: Combinatorial Synthesis and γ-Glutamyl Cysteine Synthetase and Glutathione-S-Transferase Modulatory Effect of C-Nucleoside Analogs

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# DBU-Assisted Cyclorelease Elimination: Combinatorial Synthesis and γ-Glutamyl Cysteine Synthetase and Glutathione-S-Transeferase Modulatory Effect of C-Nucleoside Analogs

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**Abstract**: A combinatorial library of 60 C- nucleoside analogs was synthesized by sequential coupling of building blocks followed by cyclative cleavage with DBU in an efficient manner. Only DMSO soluble compounds were tested for their modulatory effect against filarial γ-glutamyl cysteine synthetase (γ-GCase) and glutathione-S-transeferases (GSTs). Several compounds were found to be weak inhibitors of filarial γ-GCase, whereas, most of them stimulated filarial GSTs.

**Key Words**: Cyclorelease elimination, C- nucleosides, combinatorial synthesis, diazabicyclo (5.4.0) undec-7-ene, γ-glutamyl cysteine synthetase, glutathione-S- transeferase, and dihydropyrimidinones.

## INTRODUCTION

Combinatorial chemistry has precipitated a paradigm shift in the drug discovery process and has dramatically changed the search for new biologically active compounds [1-3]. The parallel synthesis of numerous single compounds has become popular and can be accomplished by sequential coupling of building blocks to solid phase substrates with suitable protecting and anchoring groups followed by chemical manipulations [4]. Screening a library of such compounds permits identification of lead molecules in a very short span of time.

The availability of effective treatment regimens to decrease microfilaraemia have been primarily responsible for the declaration by World Health Organization that lymphatic filariasis can be eliminated globally as public health and socioeconomic problem [5, 6]. Novel therapeutic targets are being identified from among the myriad of enzymes, receptors, metabolic pathways, and genomic data and are being utilized during drug development against parasitic diseases [7]. Glutathione, a tripeptide that is unusually abundant in parasites, facilitates the detoxification of electrophilic endogenous and xenobiotic substances. The role of glutathione-S- transferase in protection of filarial worms from oxidant stress and eicosanoid production (the immunopathogenic components) makes it an attractive target for chemotherapy. The discovery of trypanothine, which is a spermidine containing analogue of GSH and the natural substrate of GST in some parasites, emphasizes the need for more basic research aimed at the identification of substrate/inhibitors of this multifunctional enzyme in filariids [8, 9].

Because of their structural diversity and biological significance, carbohydrates provide a relatively unexplored dimension of drug design and development [10]. In our ongoing studies towards the design and development of novel mechanism based antifilarial agents we have observed that certain sugar derivatives posses very good activity against filarial glutathione metabolizing enzymes *in vitro* [11,12]. In addition to enzyme inhibition, immunomodulatory activity [13] would be another beneficial property of this class of compounds in the treatment of parasitic infections. In particular, the importance of dihydropyrimidinones in organic and biological chemistry has stimulated scientists to synthesize and test these compounds as anti-HIV and antimicrobial substances [19].

C- nucleosides having C-C linkage instead of carbon to nitrogen linkage between the aglycon and the sugar moiety are known mainly for their anticancer [14], antiviral [15] and antileukemic [16] activities. A C-C bond between the sugar and aglycon makes them more stable towards enzymatic degradation, and therefore the half-lives of these compounds in the body are far greater than the corresponding N-nucleosides. In addition, sugar derivatives are known to offer better stability, better pharmacokinetic parameters, facilitate the transport of drugs, and at the same time are less toxic [17, 18]. Recently we identified a novel glycoconjugate as a lead for parasite chemotherapy [19]. Since glycoconjugates having β-amino acid peptide analogs have shown GST modulatory activity in our antifilarial screening programme, we intended to manipulate the peptide bond to yield a molecule which might modulate the enzyme activity in a better way.

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A variety of organic transformation reactions [20] have been carried out effectively in the presence of the organic soluble amidine base, 1, 8-diazabicyclo [5, 4, 0] undec-7-ene (DBU), under relatively mild conditions. Several reports exist for the synthesis of C- nucleoside analogs [21], but the reported procedures are difficult to perform and are unsuitable for the large-scale synthesis of such compounds. In addition, there is no report of solid phase synthesis of dihydropyrimidinone nucleosides having C-C linkages using DBU as cyclative cleavage agent. In this paper we report the synthesis of a combinatorial library of 60 C- glycosylated dihydropyrimidinones as possible modulators of filarial γ-GCase and GSTs.

## EXPERIMENTAL

The library was synthesized on Wang resin (Nova biochem) using a standard method of ester linkage formation with standard coupling reagents (DCC, HOBt, DMAP). The progress of the reaction was checked using IR spectrometry, where appearance of a band at ~ 1720 $\mu m^{-1}$ (ester linkage) and disappearance of the –OH band at 3400 $cm^{-1}$ indicated complete loading. Loading of the scaffolds was determined to be 79% by taking the initial and final weights of the resin. Aromatic substituted isocyanates were purchased from Lancaster, Sigma Aldrich. All other reagents and solvents were of standard quality and used after further purification. The scaffolds **3a** and **3b** were synthesized by 1,4-conjugate addition of ammonia on olefinic ester (**1**) derived from sugar followed by hydrolysis of amino ester (**2**) with aqueous ethanolic triethylamine at room temperature or using $LiOH.H_2O$ in $THF/H_2O$. The free amino group in precursors of scaffolds **2a** and **2b** were protected with an Fmoc protecting group. Deprotection was carried out with piperidine/DMF. NMR spectra were recorded on a Bruker 300 MHz instrument. HPLC was carried out using a Shimadzu LC 10 AS using a $C_{18}$ column (4.6 X 250 mm) with acetonitrile-water as mobile phase. Reductive alkylation/arylation with different aldehydes with sodium cyanoborohydride/ trimethylorthoformate in the amino glycosylated resin bound compounds (**4**) afforded the N-substituted resin bound glycosylaminoester derivatives (**5**) having at least one secondary amine functionality. The reaction of (**5**) with different isocyanates at room temperature gave resin-bound glycosylated urea derivatives (**6**), which on treatment with DBU at 60°C afforded the desired compounds (**7-66**) in 50-90 % yield. The structures of all the compounds were determined on the basis of IR, $^1$HNMR and FAB MS spectral data.

### Combinatorial Synthesis of Dihydropyrimidinone Nucleosides

The synthesis of a library of C-nucleoside analogues was carried out in parallel on Wang resin (1.13 mmol/g substitution) using anhydrous DMF and DCM and an Advanced Chemtech Vantage Millenium synthesizer. The coupling reagents DCC, DMAP and HOBt were dried over KOH in a desiccator under vacuum. Loadings of Fmoc-3-amino- [(1'R, 2'R, 3'S, 4'R)-3'-O-methyl-1', 2'-O-isopropylidene-1',4'-tetrahydrofuranos-4'-yl)]-propanoic acid and Fmoc-3-amino- [(1'R, 2'R, 3'S, 4'R)-3'-O-benzyl-1', 2'-O-isopropylidene-1',4'tetrahydrofuranos-4'-yl)]-propanoic acid derivatives 3a and 3b on Wang resin were carried out in a sintered funnel specially designed for solid phase synthesis.

### Fluorenyl methoxy carbonyl -3-amino- [(1'R, 2'R, 3'S, 4'R) -3'-O-methyl-1', 2'-O-isopropylidene-1', 4'-tetrahydro-furanos-4'-yl)-propanoyl resin (4): General procedure.

Wang resin (1.2 g, 1.356 mmol) was placed in a solid phase reaction vessel and treated with DMF (10 ml) two times for 5 min at room temperature under nitrogen agitation. The resin was again swelled in DCM (15 ml) two times for 5 min at room temperature under nitrogen agitation. Fmoc-3-amino- [(1'R, 2'R, 3'S, 4'R)-3'-O-methyl-1', 2'-O-isopropylidene -1', 4'tetrahydrofuranos-4'-yl)]-propanoic acid (**3a**, 3.93 g, 8.14 mmol) was then added to the reaction vessel followed by addition of DCC (1.68 g, 8.14 mmol), DMAP (0.33g, 2.71 mmol), and HOBt (1.1 g, 8.14 mmol) in DMF (15 ml). and the reaction mixture was agitated with a slow stream of nitrogen gas for 12 h at room temperature. The solvents and excess reagent were removed and the process of loading compound **3a** was repeated in order to have complete loading. The resin was washed with DMF (3 x 2 min), methanol (3 x 2 min) and DCM ((3 x 2 min) and dried in vacuo to give acylated Wang resin **4a** (1.802g).

Fmoc-3-amino-[(1'R, 2'R, 3'S, 4'R)-3'-O-benzyl-1', 2'-O-isopropylidene-1', 4'-tetrahydrofuranos-4'-yl)-propanoyl resin (**4b**) was prepared in a specially designed sintered funnel as described above.

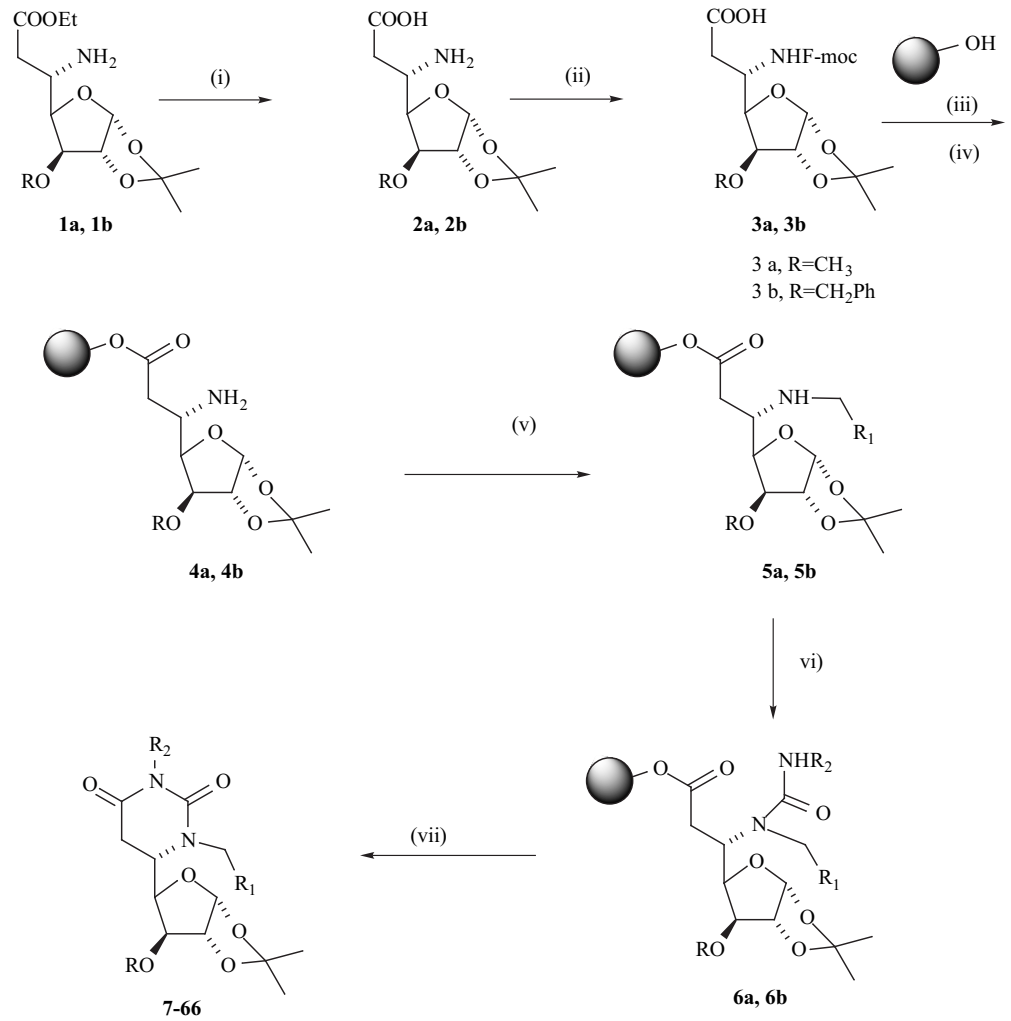
### Synthesis of Library

Aldehydes and isocyanates were used as monomers to build the library in a parallel format. The aldehydes were anisaldehyde, benzaldehyde, 4- bromobenzaldehyde, vaniline, furfuraldehyde, and salicyldehyde, and the isocyanates were 3-acetyl phenyl-, 4-acetyl phenyl-, benzyl-, 3-cyanophenyl-, 3,5-dimethyl phenyl-, 2,4-dichlorophenyl-, 3,4-dichlorophenyl-, 3,5-dichloro phenyl-, and 4-chloro phenyl- isocyanates.

Synthesis of 5,6-dihydro -1,3-disubstituted- [(1'R, 2'R, 3'S, 4'R)-3-O-methyl (benzyl)-1', 4'-tetrahydrofuranos-4-yl]-pyrimidin-2, 4-diones (7-66).

The resin bound 3-fluorenylmethoxycarbonyl-amino-[(1'R, 2'R, 3'S, 4'R) -3'-O-methyl(benzyl)-1', 2'-O-isopropylidene-1', 4'-tetrahydrofuranos-4'-yl)]-propanoyl resin (0.05 g, 0.0565 mmol) was placed in 60 wells of the reaction block and treated with 20% piperidine – DMF (1.0 ml) twice for 5 min each. After 25 min. the resin in each well was washed with DMF (3 x 2 min), MeOH (2 x 2 min) and DCM (3 x 2 min).

1. Reductive amination: A solution of each aldehyde (1.13 mmol) from above in anhydrous trimethyl orthoformate




**Scheme-1**

**Reagents & conditions:**

(i) $Et_3N$, EtOH and $H_2O$. (ii) Fmoc-osu, $Na_2CO_3$, THF/$H_2O$. (iii) DCC, DMAP, HOBT; (iv) 20% Piperidine/ DMF. (v) $R_1CHO$ in TMOF, $NaCNBH_3$, AcOH. (vi) $R_2NCO$, DCM (vii) DBU, $CHCl_3$, 60ºC.

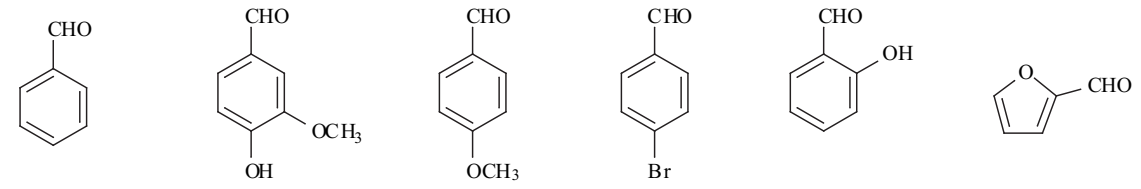
(TMOF, 1.5 ml) was transferred to separate reaction wells and mixed for 2 h. Next, a suspension of $NaCNBH_3$, (1.13 mmol) in TMOF (0.5 ml) was added to each well and mixed for 30 min. This was followed by addition of glacial acetic acid (20 µl) to each well in TMOF (80 µl) and mixing for 15 min. The resin bound compounds were washed with DMF (3 x 2 min), MeOH (2 x 2 min) and DCM (3 x 2 min).

2. Reaction with aromatic substituted isocyanate monomer: A solutions of each isocynate (0.565 mmol) in anhydrous DCM (2.0 ml) were dispensed into targeted wells and mixed at 25°C under 50-psi $N_2$ pressure (inside reaction well) for 12 h. The resin bound compounds were washed with DMF (3 x 2 min), MeOH (2 x 2 min) and DCM (3 x 2 min).

3. DBU Assisted Cyclorelease Elimination: DBU (0.5% solution in anhydrous $CHCl_3$, 2.0 ml) was added to each reaction well, and the wells were agitated at 60°C under 50-psi nitrogen pressure for 12 h (all mixing were carried out at 600 rpm ). The reaction block was cooled to 15°C by passing liquid $N_2$ through the block. Cleavage vials were placed below the reaction block, and the compounds were obtained in chloroform by emptying the reaction wells. The chloroform was evaporated under $N_2$, and the compounds were dissolved in t-butanol/water (4:1) and lyophilized to give compounds **7-66**. The compounds were characterized using FTIR (neat), HPLC, FAB mass spectroscopy and $^1$HNMR spectroscopy. The C-nucleoside analogues were obtained in 50-90 % purity, which was sufficient for *in vitro* screening.

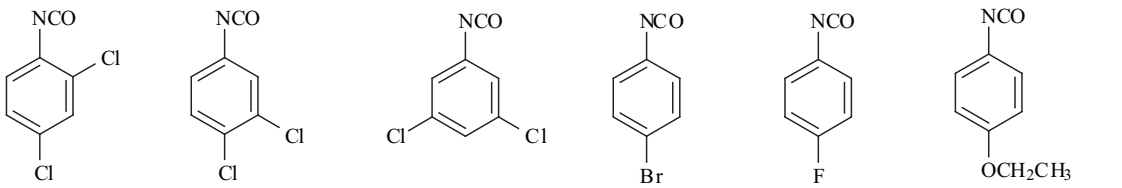
## 5-Amino- 5,6-dideoxy- 1,2-O-isopropylidene-3-O-methyl-β-L-ido-heptofuranuronic acid (2a)

To a magnetically stirred solution of the ethyl-[5-amino-3-O-methyl- 5,6-dideoxy-1, 2-O-isopropylidene]-β-L-*ido*-heptofuranuronate (2.82 gm, 9.757 mmol) in aqueous ethanol (50 %, 57 ml), triethyl amine (14.5 ml) was added, and the contents were stirred for 50 h. The solvent was evaporated under reduced pressure to give a residue which

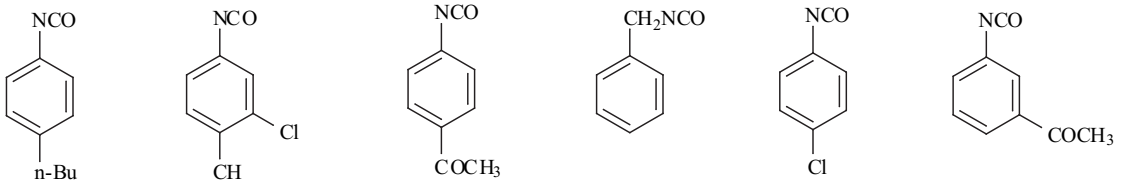



**1a** **1b** **1c** **1d** **1e** **1f**

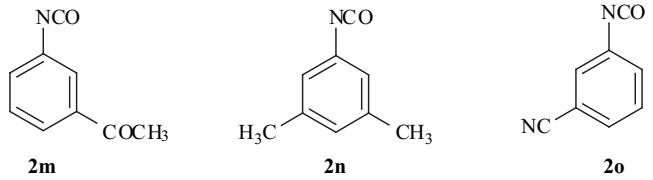
**Fig. (1a).** Aldehydes used as monomers.




**2a** **2b** **2c** **2d** **2e** **2f**

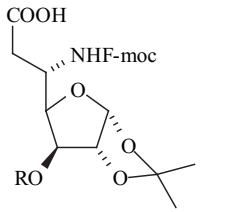



**2g** **2h** **2i** **2j** **2k** **2l**




**2m** **2n** **2o**

**Fig. (1b).** Isocyanates used as monomers.



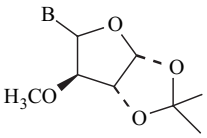

**3a**, R=$CH_3$; **3b**, R=$CH_2C_6H_5$

**Fig. (1c).** Glycosylated amino acids used as scaffolds.

was subjected to column chromatography over $SiO_2$ using $CHCl_3$:MeOH (9:1) as eluant to give the desired compound as colourless solid, m.p. = 200°C (2.23gm, 91% yield), IR (KBr); $\nu_{max}$ $cm^{-1}$ 2900,2800($CH_3CH_2$ stretching); MS (FAB): 262 $[M+1]^+$; $^1H$ NMR ($CDCl_3$): δ5.94 (d, J=3.66 Hz, 1H, H-1); 4.57 (d, J=3.66 Hz, 1H, H-2); 4.37 and 4.33 (dd, J=2.8 Hz and 7.86Hz, H-4); 3.77 (d, J=2.87 Hz, 1H, H-3); 3.68 (m, 1H, H-5); 3.39 (s, 3H, -$OCH_3$); 2.48 (m, 2H, H-6); 1.49 and 1.29 (each s, each 3H, >C ($C\underline{H}_3)_2$; $^{13}C$ NMR ($CDCl_3$): δ168.5 (>C=O), 111.5 [>$\underline{C}$ ($CH_3$) $_2$], 105.19 (C-1), 83.77 (C-2), 82.57 (C-4), 81.93 (C-3); 47.81 (C-5), 38.62 (C-6), 27.13 and 26.69 [>C ($\underline{C}H_3$) $_2$] Anal. $C_{11}H_{19}O_6N$ (C, H, N) .

## 5-AMINO- 5,6-DIDEOXY- 1,2-O-ISOPROPYLIDENE-3-O-BENZYL-β-L-IDO-HEPTOFURANURONIC ACID (2B)

Compound **2b** was obtained by reaction of amino ester **1b** with aqueous triethyl amine as in case of **2a** to produce a colourless solid, m.p. 164 °C , yield 90 %; IR (KBr); $\nu_{max}$ $cm^{-1}$ 2980, 2930, 2880,1730; MS (FAB): 338 $[M+1]^+$;



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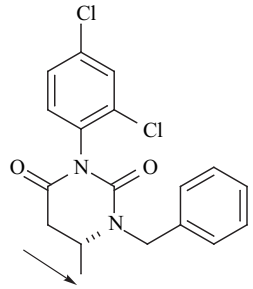

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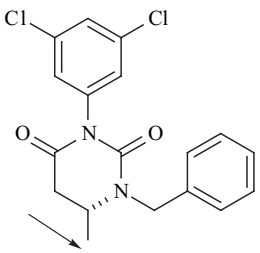

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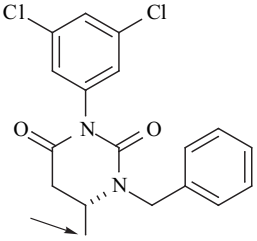

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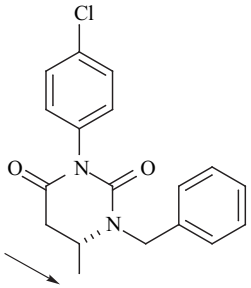

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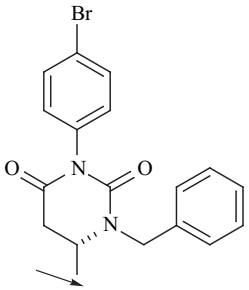

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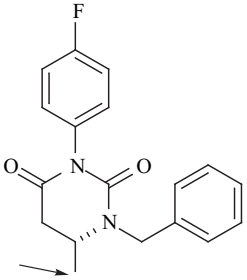

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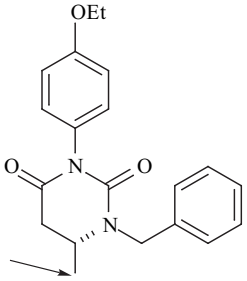

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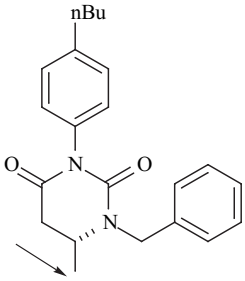

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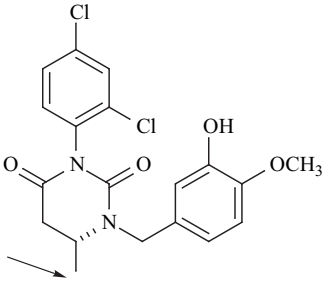

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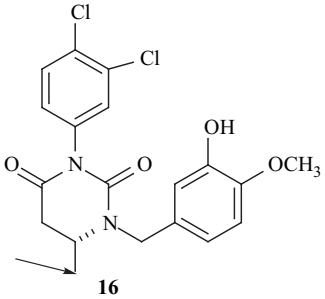

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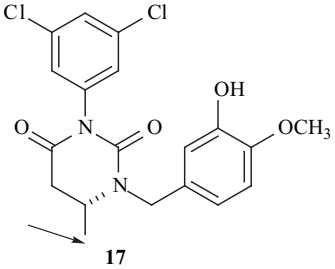

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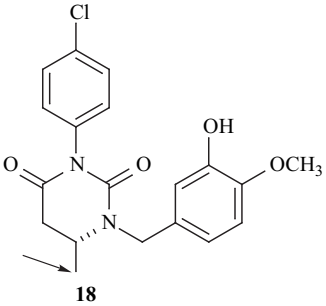

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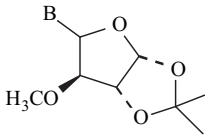
**(Fig. 2) contd....**



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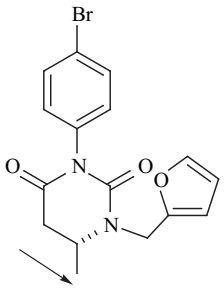

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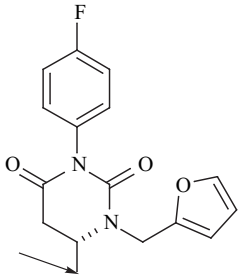

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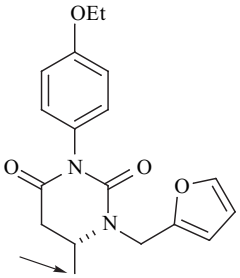

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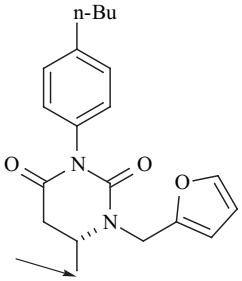

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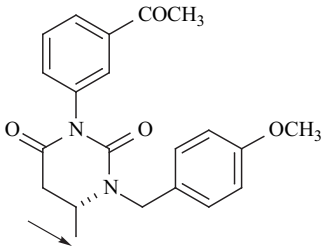

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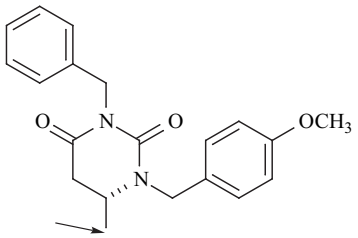

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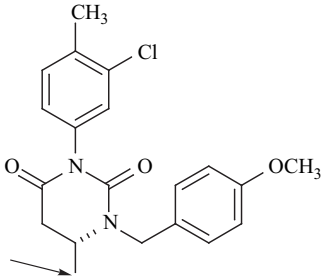

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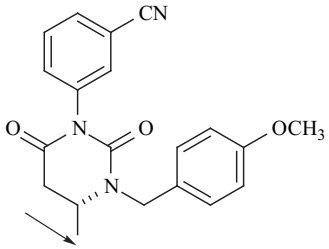

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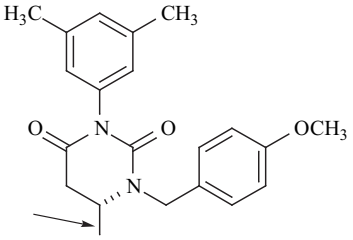

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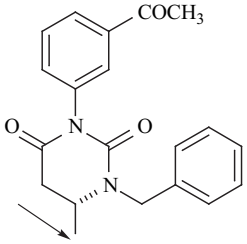

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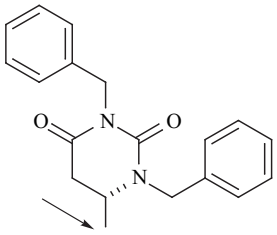

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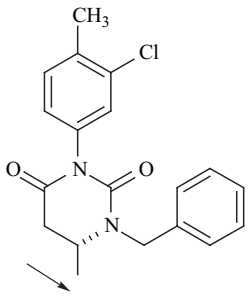

**46**

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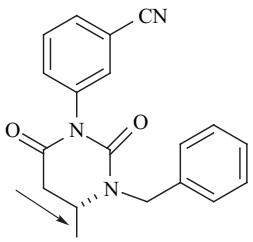

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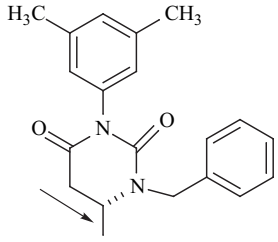

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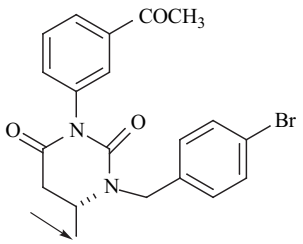

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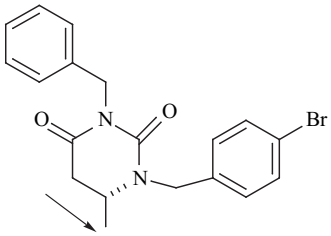

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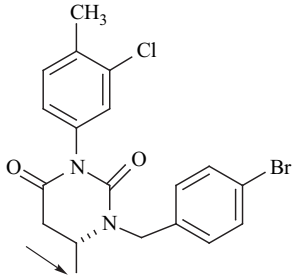

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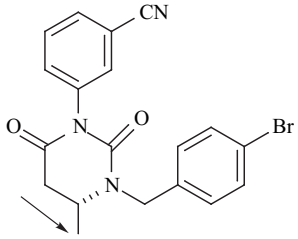

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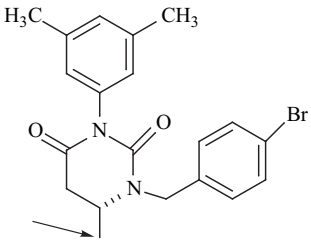

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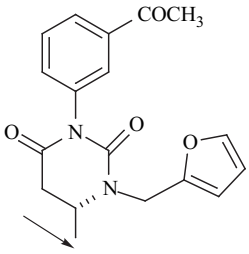

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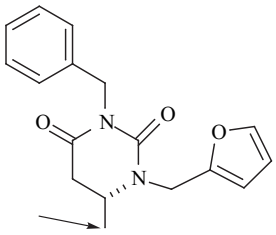

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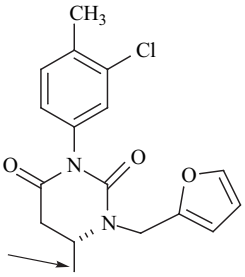

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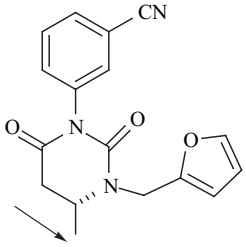

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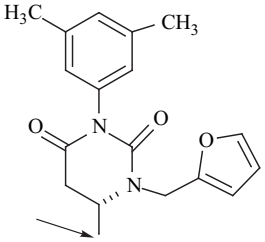

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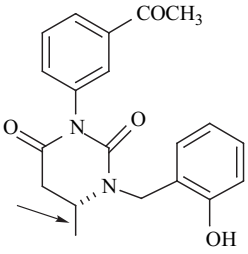

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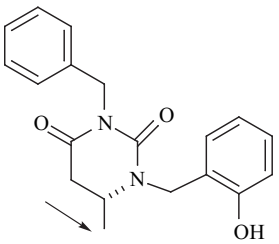

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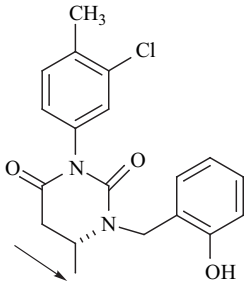

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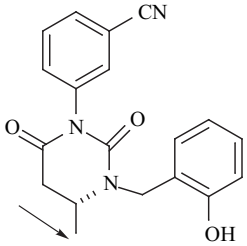

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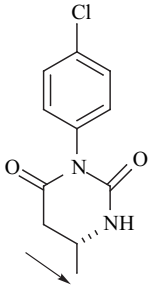

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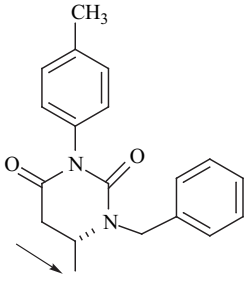

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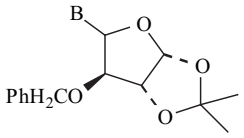
**(Fig. 2) contd....**



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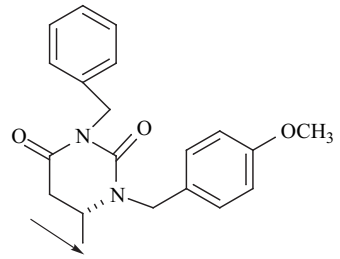

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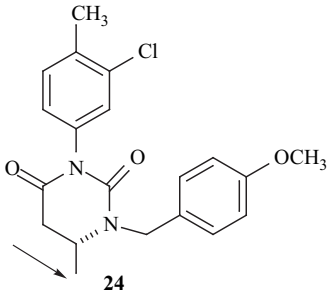

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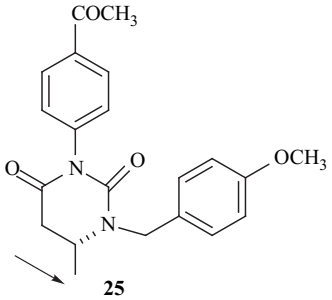

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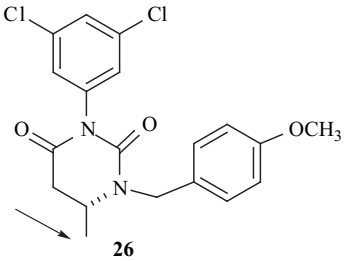

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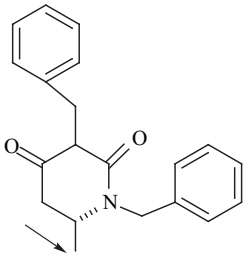

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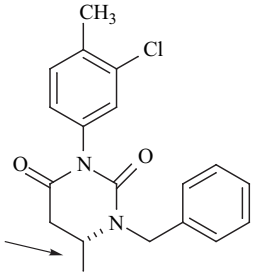

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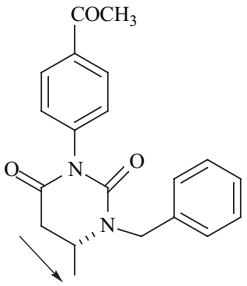

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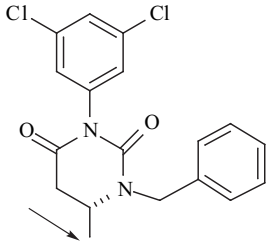

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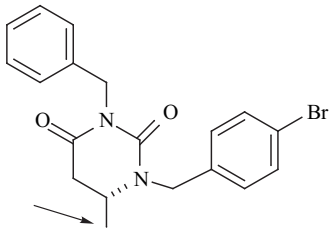

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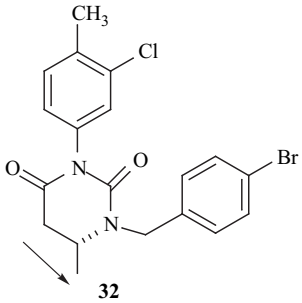

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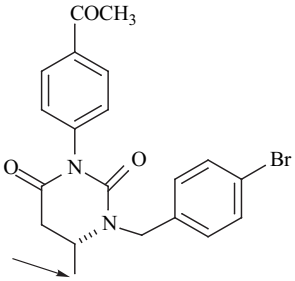

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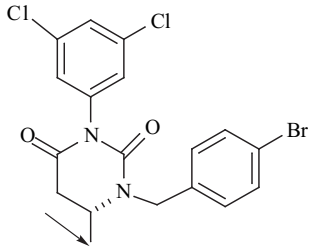

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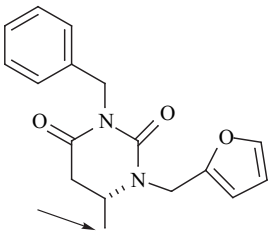

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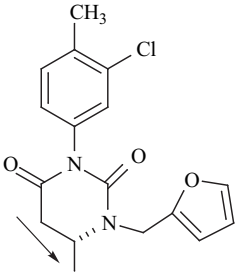

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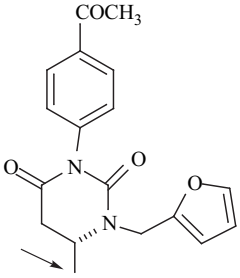

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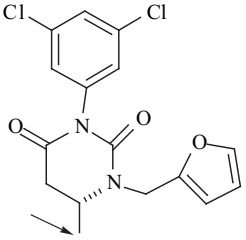

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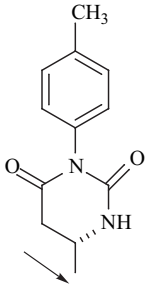

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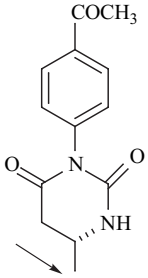

**65**

$^1$H NMR ($CDCl_3$): δ 7.35 (m, 5H, ArH), 5.99 (d, J=3.64 Hz, 1H, H-1), 4.73 (m, 2H, H-2 and -$OCH_APh$), 4.59 (d, J=11.5 Hz, 1H, -$OCH_BPh$), 4.30 (dd, J= 7.8 and 3.2 Hz, 1H, H-4); 4.10 (d, J=3.24 Hz, 1H, H-3); 3.71 (m, 1H, H-5); 3.34 (s,1H, exchanges with $D_2O$); 2.43 (m, 1H, H-6), 1.43 and 1.33 [each s, each 3H, >C ($C\underline{H}_3$) $_2$]. $^{13}$C NMR ($CDCl_3$): δ 171.36 (COOH), 135.84, 126.73, 126.20, (ArC), 109.58 [$\underline{C}(CH_3)_2$ ], 1O2.86 (C-1), 79.51, 78.55, 77.60 (C-2,C-4,C-3), 69.24 (-O$\underline{C}H_2$Ph), 34.68 (C-6), 46.98 (C-5), 26.91 and 26.47 [>C ($\underline{C}H_3$) $_2$]. Anal. $C_{17}H_{23}O_6N$ (C, H, N)

**Fluorenyl methoxy carbonyl -3-amino- [(1'R, 2'R, 3'S, 4'R) 3'-O-methyl-1', 2'-O-isopropylidene-β-L-1', 4'-pentofuranos-4'-yl)- propanoic acid (3a).**

The amino acid derivative **2a**, (5.0 gm, 19.16 mmol) was dissolved in a 25 ml aquous soln. of $Na_2CO_3$ (2.12 g, 20 mmol) and stirred magnetically at 0° C. A solution of the protecting reagent *N*-(9-fluorenylmethoxycarbonyloxy) succinimide, (7.1g, 21 mmol) in THF (25 ml) was added, and the reaction mixture was stirred at 0° C for 1 h. The stirring was continued at 30° C overnight, the solvent was evaporated, the crude product was dissolved in water (50 ml) and then extracted with ether. The aqueous layer was acidified with $KHSO_4$ solution and extracted with ethyl acetate (3 x 100 ml), dried over $Na_2SO_4$ and concentrated at reduced pressure to give **3a** as a colourless foam. The yield was 96%; melting sinters at 80 °C and completely melts at 90 °C.

**FABMS**: *m/z* = 484 $[M+H]^+$; **$^1$H NMR** (200 MHz, $CDCl_3$): δ1.30 and 1.47 (each s, each 3H, >$C(CH_3)_2$); 2.66 (m, 2H, $C\underline{H}_2COOH$); 3.35 (s, 3H, -$OCH_3$); 3.71 (d, J=3.0Hz,1H, H-3'); 4.21-4.26 (m, 5H, H-4', H-5',-$OC\underline{H}_2$ and –$OCH_2C\underline{H}$); 4.58 (d, J= 3.5 Hz, 1H, H-2'); 5.60 (bs, 1H, -NH); 5.91 (d, J= 3.5 Hz, 1H, H-1'); 7.25-7.75 (m, 8H, fluorenyl H).

**Fluorenyl methoxy carbonyl-3-amino-[(1'R, 2'R, 3'S, 4'R)-3'-O-benzyl-1', 2'-O-isopropylidene-β-L-1', 4'-tetrahydrofuranos-4'-yl)- propanoic acid (3b)**

Compound 3b was obtained by protection of the amino group of 2b according to the procedure for 3a. Yield 98 %, FABMS: *m/z* = 560 $[M+H]^+$. $^1$HNMR, 300 MHz ($CDCl_3$): δ 1.32 and 1.48 (two s, each 3H, >$C(CH_3)_2$ ); 2.66 (m, 2H, $CH_2CO_2H$); 4.01 (dd, J=6Hz, J=3Hz, 1H, H-4') 4.19 (m, 4H, -$OCH_2$, H-5'and –$OCH_2C\underline{H}$); 4.43 (d,1H, J=3Hz, H-3'); 4.47 (d, J=12Hz, 1H, -$OCH_APh$);4.64 (d, J=3.0 Hz, 1H, H-2'); 4.69 (d, J=12Hz, 1H, -$OCH_BPh$); 5.29 (bs, 1H, -NH); 5.97 (d, J= 3.0 Hz, 1H, H-1'); 7.34 (m, 9H, ArH,); 7.59 (m, 3H, ArH,); 7.76 (m, 2H, ArH,).

## PHYSICAL DATA OF SOME SELECTED NEW COMPOUNDS

**1-Benzyl-3-[{3, 5-di-chloro-} phenyl-5, 6-dihydro-6-{(1'R, 2'R, 3'S, 4'R)-1', 2'-O-isopropylidene-3'-O-methyl-1', 4'-tetrahydrofuranos-4'-yl}]-pyrimidin-2, 4-dione (9)**

FABMS: *m/z* =521 $[M+H]^{+\cdot}$ IR (Neat): 1678 $cm^{-1}$ (CONRCO). $^1$HNMR(300 MHz, $CDCl_3$): δ 7.63 (d, J=1.8 Hz, 1H, ArH); 7.51 (d, J=1.5 Hz, 2H, ArH); 7.28(m, 5H, ArH); 5.89(d, J=3.9Hz, 1H, H-1'); 4.87 and 4.20(each d, J=15.6 Hz, each 1H, -$NCH_A$ and -$NCH_B$); 4.56(d, J=3.9 Hz, 1H, H-2'); 4.43 (dd, J=9.6 Hz and3.0 Hz, 1H, H-4'); 3.91(m, 1H, H-6); 3.59(d, J=3.0 Hz, 1H, H-3'); 3.38(s, 3H, -$OC\underline{H}_3$); 2.59(m, 2H, H-5); 1.44 and1.30 (each s, each 3H, >$C(CH_3)_2$).

**1- Benzyl- 3-[(4-chloro)-phenyl-5, 6-dihydro-6- {(1'R, 2'R, 3'S, 4'R)-1', 2'-O-isopropylidene-3'-O-methyl-1', 4'-tetrahydrofuranos-4'yl}]-pyrimidin-2, 4-dione (10)**

FABMS: *m/z* = 487 $[M+H]^+$. IR (Neat): 1680 $cm^{-1}$(CONRCO). $^1$HNMR(300 MHz, $CDCl_3$): δ 7.43 (d, J=8.4 Hz, 2H, Ar-H); 7.28 (m, 5H, ArH); 7.20(d, J=8.4 Hz, 2H, ArH); 5.88(d, J=3.6Hz, 1H, H-1'); 4.81 and 4.26(each d, J=15.9 Hz, each 1H, -$NCH_A$ and -$NCH_B$); 4.55(d, J=3.6 Hz, 1H, H-2'); 4.17 (m, 2H, H-4'and H-6); 3.94(d, J=3.0 Hz, 1H, H-3'); 3.42(s, 3H, -$OCH_3$); 2.54(m, 2H, H-5); 1.29 and1.25(each s, each 3H, >$C(CH_3)_2$).

**1- Benzyl- 3-[(4-bromo)-phenyl-5, 6-dihydro-6- {(1'R, 2'R, 3'S, 4'R)-1', 2'-O-isopropylidene-3'-O-methyl-1', 4'-tetrahydrofuranos-4'yl}]-pyrimidin-2, 4-dione (11)**

FABMS: *m/z* = 531$[M+H]^+$. IR (Neat): 1675 $cm^{-1}$ (CONRCO). $^1$HNMR(300 MHz, $CDCl_3$): δ 7.60 (d, J=8.4 Hz, 2H, Ar-H); 7.36 (m, 5H, ArH); 7.06 (d, J=8.4 Hz, 2H, ArH ); 5.98(d, J=3.9Hz, 1H, H-1'); 4.80 and 4.28(each d, J=15.3 Hz, each 1H, -$NCH_A$ and -$NCH_B$); 4.58(d, J=3.9 Hz, 1H, H-2'); 4.44 (dd, J=9.6 Hz and3.0 Hz, 1H, H-4'); 3.85(m, 1H, H-6); 3.70(d, J=3.0 Hz, 1H, H-3'); 3.38(s, 3H, -$OC\underline{H}_3$); 2.52(m, 2H, H-5); 1.49 and1.37(each s, each 3H, >$C(CH_3)_2$).

**1-Benzyl-3-[{4-ethoxy}-phenyl-5, 6-dihydro-6- {(1'R, 2'R, 3'S, 4'R)-1', 2'-O-isopropylidene-3'-O-methyl-1', 4'-tetrahydrofuranos-4'-yl}]-pyrimidin-2, 4-dione (13)**

FABMS: *m/z* = 497 $(M+H)^+$. IR (Neat): 1682 $cm^{-1}$(CONRCO). $^1$HNMR(300 MHz, $CDCl_3$): δ 7.33 (m, 5H, ArH); 7.07 and 6.95(each d, J=8.7 Hz, each 2H, ArH); 5.98(d, J=3.9Hz, 1H, H-1'); 5.42 and 4.28 (each d, J=15.0 Hz, each1H, benzylic protons); 4.60 (d, J=3.9 Hz, 1H, H-2'); 4.47(dd, J=9.6 Hz and3.3 Hz, 1H, H-4'); 4.04 (q, J=6.9 Hz, 2H, -$C\underline{H}_2CH_3$); 3.82 (m, 1H, H-6); 3.70(d, J=3.3 Hz, 1H, H-3'); 3.45(s, 3H, -$OC\underline{H}_3$); 2.51(m, 2H, H-5) 1.50 and1.34 (each s, each 3H, >$C(CH_3)_2$); 1.41(t, J=6.9 Hz, 3H, -$OCH_2C\underline{H}_3$).

**1-{(3-hydroxy, 4-methoxy)-phenyl}-methyl}-3-[{3, 5-di-chloro-} phenyl-5, 6-dihydro-6- {(1'R, 2'R, 3'S, 4'R)-1', 2'-O-isopropylidene-3'-O-methyl-1', 4'-tetrahydrofuranos-4'-yl}]-pyrimidin-2, 4-dione (17)**

FABMS: *m/z* = 567 $[M+H]^+$. IR (Neat): 1676 $cm^{-1}$ (CONRCO). $^1$HNMR (300 MHz, $CDCl_3$): δ 8.11 (bs, 1H, Ar-OH); 7.51 (d, J=1.5 Hz, 1H, ArH); 7.42 (d, J=1.8 Hz, 2H, ArH); 6.94 (m, 3H, ArH); 5.90 (d, J=3.6Hz, 1H, H-1'); 4.73 and 4.23 (each d, J=15.9 Hz, each1H, -$NCH_A$ and

-$NCH_B$); 4.58 (d, J=3.6 Hz, 1H, H-2'); 4.27 (m, 1H, H-4'); 4.10(m, 1H, H-6); 3.89(d, J=3.0 Hz, 1H, H-3'); 3.46(s, 3H, Ar-$OC\underline{H}_3$); 3.42 (s, 3H, -$OC\underline{H}_3$); 2.56 (m, 2H, H-5); 1.49 and1.30 (each s, each 3H, $>C(CH_3)_2$).

**1- (furylmethyl)- 3-[(4-bromo-phenyl) -5, 6-dihydro-6-{(1'R, 2'R, 3'S, 4'R)-1', 2'-O-isopropylidene-3'-O-methyl-1', 4'-tetrahydrofuranos-4'yl}]-pyrimidin-2, 4-dione (19)**

FABMS: *m/z* = 521 $[M+H]^+$. IR (neat): 1685 $cm^{-1}$(CONRCO). $^1$HNMR (300 MHz, $CDCl_3$): δ 7.36 (s, 1H, furyl ring-H); 7.31 (d, J=8.4 Hz, 2H, ArH); 7.20 (d, J=8.4 Hz, 2H, ArH); 6.12 (m, 2H, furyl ring-H); 5.92 (d, J=3.7 Hz, 1H, H-1'); 4.56 (d, J=3.7 Hz, 1H, H-2'); 4.12 (dd, J=9.6 Hz and3.0 Hz, 1H, H-4'); 3.85 (d, J=3.0 Hz, 1H, H-3'); 3.81 (m, 1H, H-6); 2.54 (m, 2H, H-5); 1.35 and 1.27 (each s, each 3H, $>C(CH_3)_2$).

**1-(Furylmethyl)-3-[4-{n-butyl} phenyl-5, 6-dihydro-6-{(1'R, 2'R, 3'S, 4'R)-1', 2'-O-isopropylidene-3'-O-methyl-1', 4'-tetrahydrofuranos-4'-yl}]-pyrimidin-2, 4-dione (22)**

FABMS: *m/z* = 499 $[M+H]^+$. IR (neat): 1680 $cm^{-1}$ (CONRCO). $^1$HNMR (300 MHz, CDCl3): δ 7.39 (1H, furan ring proton); 7.24 and 6.99 (each d, J=8.4 Hz, each 2H, ArH); 6.35-6.25 (m, 2H, furan ring proton); 5.97 (d, J=3.6Hz, 1H, H-1'); 5.28 and 4.34 (each d, J=15.9Hz, -$NCH_A$ and -$NCH_B$); 4.62 (d, J=3.6 Hz, 1H, H-2'); 4.45 (dd, J=6.9 Hz and 3.0 Hz, 1H, H-4'); 4.01 (m, 1H, H-6); 3.72 (d, J=3.3 Hz, 1H, H-3'); 3.39 (s, 3H, -$OC\underline{H}_3$); 2.98 (m, 2H, H-5); 2.61 (m, 2H, Ar-$CH_2$); 1.59 (m, 4H, -$C\underline{H}_2C\underline{H}_2CH_3$); 1.50 and 1.33 (each s, each 3H, $>C(CH_3)_2$); 0.91 (t, J=7.2 Hz, 3H, -$CH_2C\underline{H}_3$).

**1-{(4-bromo)-phenyl}-methyl)- 3-[benzyl-5, 6-dihydro-6-{(1'R, 2'R, 3'S, 4'R)-1', 2'-O-isopropylidene-3'-O-benzyl-1', 4'-tetrahydrofuranos-4'yl}]-pyrimidin-2, 4-dione. (31)**

FABMS: *m/z* = 621 $[M+H]^+$. IR (neat): 1679 $cm^{-1}$ (CONRCO). $^1$HNMR (300 MHz, $CDCl_3$): δ 7.45 (d, J=8.1 Hz, 2H, Ar-H); 7.33 (m, 10H, ArH); 7.18 (d, J=8.4 Hz, 2H, ArH); 5.91 (d, J=3.6Hz, 1H, H-1'); 5.32 and 4.96 (each d, J=15.3 Hz, each1H, -$OCH_A$ and -$OCH_B$); 4.64 and 4.38 (each d, J=15.3 Hz, each 1H, -$NCH_A$ and -$NCH_B$); 4.62 (s, 2H, -$NCH_2$); 4.58 (d, J=3.6 Hz, 1H, H-2'); 4.10 (dd, J=9.9 Hz and3.0 Hz, 1H, H-4'); 3.94 (d, J=3.0 Hz, 1H, H-3'); 3.75 (m, 1H, H-6); 2.52 (m, 2H, H-5); 1.28 and1.25 (each s, each 3H, $>C(CH_3)_2$).

**1-(4-bromophenylmethyl)-3-[3-{cyano}-phenyl-5, 6-dihydro-6- {(1'R, 2'R, 3'S, 4'R)-1', 2'-O-isopropylidene-3'-O-methyl-1', 4'-tetrahydrofuranos-4'-yl}]-pyrimidine-2, 4-dione (52)**

FABMS: *m/z* = 557 $[M+H]^+$. IR (neat) 1682 $cm^{-1}$(CONRCO).

$^1$HNMR (300 MHz, $CDCl_3$): δ 7.86 (d, J=8.4 Hz, 2H, ArH); 7.68 (d, J=7.5 Hz, 2H, ArH); 7.11- 7.58 (m, 4H, ArH); 5.98 (d, J=3.9Hz, each 1H, H-1'); 5.30 and 4.88 (each d, J=15.0 Hz, each 1H, -$NCH_A$ and -$NCH_B$); 4.62 (each d, J=3.9 Hz, 1H, H-2'); 4.41 (dd, J=12.6 Hz and 3.3 Hz, 1H, H-4'); 3.81 (m, 1H, H-6); 3.70 (d, J=3.3 Hz, 1H, H-3'); 3.39 (s, 3H, -$OC\underline{H}_3$); 2.55 (m, 2H, H-5); 1.41 and 1.37, (each s, each 3H, $>C(CH_3)_2$).

**1-(2-hydroxyphenylmethyl)-3-[{3-acetyl} phenyl-5, 6-dihydro-6- {(1'R, 2'R, 3'S, 4'R)-1', 2'-O-isopropylidene-3'-O-methyl-1', 4'-tetrahydrofuranos-4'-yl}]-pyrimidin-2, 4-dione (59)**

FABMS: *m/z* = 511 $[M+H]^+$. IR (neat): 1680 $cm^{-1}$ (CONRCO). $^1$HNMR (300 MHz, $CDCl_3$): δ 8.39 (bs, 1H, Ar-OH); 7.99 (d, J=8.1 Hz, 1H, ArH); 7.58 (d, J=8.1 Hz, 1H, ArH); 7.26 (m, 5H, ArH); 6.96 (d, J=8.4 Hz, 1H, ArH); 6.02 (d, J=3.6Hz, 1H, H-1'); 5.17 and 4.37 (each d, J=15.3 Hz, each 1H, -$NCH_A$ and -$NCH_B$); 4.67 (d, J=3.6 Hz, 1H, H-2'); 4.49 (dd, J=9.6 Hz and3.0 Hz, 1H, H-4'); 4.08 (m, 1H, H-6); 3.76 (d, J=3.0 Hz, 1H, H-3'); 3.46 (s, 3H, -$OC\underline{H}_3$); 2.60 (m, 2H, H-5); 2.59 (s, 3H, $COC\underline{H}_3$); 1.50 and 1.36 (each s, each 3H, $>C(CH_3)_2$).

**1- {(2-hydroxy)-phenyl}-methyl)- 3-[benzyl-5, 6-dihydro-6- {(1'R, 2'R, 3'S, 4'R)-1', 2'-O-isopropylidene-3'-O-methyl-1', 4'-tetrahydrofuranos-4'-yl}]-pyrimidin-2, 4-dione (60)**

FABMS: *m/z* = 483 $[M+H)^+$; IR (neat): 1676 $cm^{-1}$ (CONRCO). $^1$HNMR (300 MHz, $CDCl_3$): δ 8.57 (bs, 1H, Ar-OH); 6.91 and 7.36 (two m, 9H, Ar-H); 5.92 (d, J= 3.72 Hz, 1H, H-1'); 5.14 (d, J = 15.7 Hz, 1H,$CH_APh$); 4.97 (d, J = 4.4 Hz, 2H, -$NC\underline{H}_2$Ar-OH); 4.57 (d, J= 3.72 Hz, 1H, H-2'); 4.27 (d, J = 15.7 Hz, 1H, $CH_BPh$); 4.11 (dd, J= 6.5 Hz and 3.0 Hz, 1H, H-4'); 3.98 (m, 1H, H-6); 3.65 (d, J = 3.0Hz, 1H, H-3'); 3.37 (s, 3H, $OCH_3$); 2.64 (dd, J=16.6Hz, 6.6Hz, 1H, H-$5_A$); 2.45 (d, J = 16.6Hz, 1H, H-$5_B$); 1.27 (s, 6H, $>C(CH_3)_2$).

**1-{(2-hydroxy)-phenyl}-methyl)-3-[{3-chloro, 4-methyl}-phenyl-5, 6-dihydro-6- {(1'R, 2'R, 3'S, 4'R)-1', 2'-O-isopropylidene-3'-O-methyl-1', 4'-tetrahydrofuranos-4'-yl}]-pyrimidin-2, 4-dione (61)**

FABMS: *m/z* = 517 $[M+H]^+$. IR (neat): 1678 $cm^{-1}$(CONRCO). $^1$HNMR (300 MHz, $CDCl_3$): δ 7.52 (d, J=2.1 Hz, 1H, ArH); 7.23 (m, 5H, ArH); 7.07 (d, J=8.4Hz, 1H, ArH); 6.01 (d, J=3.9Hz, 1H, H-1'); 5.16 and 4.36 (each d, J=15.0 Hz, each1H, -$NCH_A$ and -$NCH_B$); 4.65 (d, J=3.9 Hz, 1H, H-2'); 4.44 (dd, J=9.6 Hz and3.3 Hz, 1H, H-4'); 3.86 (m, 1H, H-6); 3.75 (d, J=3.3 Hz, 1H, H-3'); 3.41 (s, 3H, -$OC\underline{H}_3$); 2.55 (m, 2H, H-5); 2.38 (s, 3H, Ar-$C\underline{H}_3$); 1.52 and 1.33 (each s, each 3H, $>C(CH_3)_2$).

Filarial γ-glutamylcysteine synthetase (γ GCase) assay: A 10% homogenate of the actively moving female bovine filarial worms (*Setaria cervi*) was prepared in 50 mM Tris-HCl buffer (pH 7.4) containing glutamate, $MgCl_2$ and

PMSF. The homogenate was then centrifuged at 10,000 rpm for 60 min, and the supernatant was saved and used as the source of γ-glutamyl cysteine synthetase. (γ-GCase) activity was determined at 37 $^0$C in reaction mixtures (final volume 1.0 ml) containing Tris HCl buffer, KCl, ATP, PEP, L-glutamate, L-α–aminobutyrate, $MgCl_2$, $Na_2EDTA$, NADH, pyruvate kinase, and lactate dehydrogenase. The reaction was initiated by the addition of NADH. The absorbance at 340 nm was monitored for 5 min at 30 sec intervals.

### *Filarial Glutathione-S- Transeferases (GSTs) assay*

Batches of actively moving *S. cervi* females (approx. 2.0 g) were homogenized in 10 volumes of 50 mM Tris–HCl buffer, pH 7.4, containing 250 mM sucrose and 0.2 mM EDTA (buffer A) in a Potter Elvejhem homogeniser fitted with a Teflon pestle. The *S. cervi* worm homogenates were centrifuged at 1000 x g for 10 min., 10,000 x g for 30 min and 100,000 x g for 60 min. to obtain nuclear, mitochondrial and cytosolic fractions, respectively. Each fraction was dialysed against 100 volumes of 20 mM potassium phosphate, pH 7.4, containing 0.2 mm EDTA. GST activity in dialysed fractions of *S. cervi* females was determined as follows. The reaction mixture contained 100 mM phosphate, pH 6.5 or 7.4, 1.0 mM 1-chloro-2, 4-dinitrobenzene (CDNB) in 20µl ethanol, 1.0 mM GSH, and enzyme. Enzyme was omitted from the reference cuvette. Product formation was measured at 345 nm in a Schimadzu double beam spectrophotometer maintained at 25°C.

### *Enzyme–Inhibition Studies*

The effect of the compounds on γ-GCase and GST activity of *S. cervi* females was studied by adding them directly to each assay system (200 µM and 67 µM respectively) 10 min prior to the addition of the substrate. The percentage inhibition/stimulation of the enzyme activity by the compounds was calculated by comparison with control tubes. Protein in the sample was determined according to Lowry *et al.* [23].

## RESULTS AND DISCUSSION

Out of 60 C-nucleoside analogs synthesized only 50 could be screened for their ability to modulate γ-GCase and GST activities. The remaining compounds could not be tested because of their insolubility in DMSO. Congeners exhibited low to good modulatory activity (Table-**1**). A careful analysis of the enzyme modulatory activity revealed that substitutions at N-1 and N-3 with functionalised phenyl or aryl rings having substituents (**22, 23, 26, 31, 32, 49, 50, 52, 59 and 62**) were more active than unfunctionalised analogues (**27** and **45**). A+ the N-1 position, substitution with a hydroxyl (**62**) or halogen (**52**) group showed greater activity than methoxy group substitution (**39-42**). Substitution of the aryl ring with electron withdrawing groups such as halogen (**31** and **32**) or a cyano group (**52**) resulted in an increase in enzyme modulatory activity. Among the halogen substituents (F, Cl and Br) chlorine produced the highest modulatory activity both at the N-1 and N-3 positions, and substitution with a cyano group accounted for the greatest activity. In general 3-O- benzylated sugar derivatives were found to be more active than the corresponding methyl derivatives.

Compounds **26**, **50** and **54** were found to be weak inhibitors of filarial γ-GCase (Table-**1**). BSO also showed weak inhibition of filarial γ-GCase in our study, which confirms most [24] but not all [25] literature reports that BSO is a relatively poor inhibitor of bacterial γ-GCase, particularly in the presence of physiological levels of L-glutamate. Therefore, studies on the interaction of filarial 5-Amino- 5,6-dideoxy- 1,2-O-isopropylidene-3-O-methyl-β-L-ido-heptofuranuronic acid. (2a)γ–GCase with *S*-BSO, its amino acid substrates and compounds such as **26**, **50** and **54** are needed to elucidate binding interactions that might be exploited in designing novel, more potent inhibitors.

In contrast to the weak inhibitory effect of these few compounds, nearly all the compounds investigated showed a stimulatory effect on filarial GSTs, which is the major detoxification enzyme of the helminth parasite [26]. GSTs have also been associated with the development of drug resistance in mammalian tumors, insects, plants, and malarial parasites [27,28]. GSTs in mammals can also be stimulated by a variety of compounds, including food additives and chemotherapeutic agents [29]. Since the results in Table-**1** provide evidence that filarial GSTs can be stimulated by the above series of compounds, the synthesis of more powerful agents of this class might be useful during chemotherapy of filarial infections in human beings.

## CONCLUSION

We have developed a new method for the synthesis of C-nucleoside analogs. These low molecular weight compounds have a modulatory effect on filarial γ-GCase and GST and might be useful for lead optimization in filarial chemotherapy. This application is in addition to potential uses as anticancer and antimicrobial agents.

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**Table-1. Effect of Library Compounds on Gamma-Glutamyl Cysteine Synthetase (γ-GCase) and Glutathione-S-Transferases (GSTs) from Bovine Filarial Worms**

| S.No. | Compound No. | Percent Inhibition (*S. cervi*) | |
|---|---|---|---|
| | | γ-GCase$^{+}$ | GSTs$^{++}$ |
| 1. | Enzyme+DMSO | NIL | NIL |
| 2. | Enzyme+BSO (32.0mM) | 27.5 | -- |
| 3. | Enzyme+hemin (0.06mM) | 82.7 | 85.9 |
| 4. | Enzyme+7 | 21.0(+) | 17.2(+) |
| 5. | Enzyme+8 | NIL | 3.31(+) |
| 6. | Enzyme+9 | NIL | 3.92(+) |
| 7. | Enzyme+10 | NIL | 45.2(+) |
| 8. | Enzyme+11 | 19.0(+) | 24.1(+) |
| 9. | Enzyme+12 | 4.00 | 39.2(+) |
| 10. | Enzyme+13 | NIL | 51.15(+) |
| 11. | Enzyme+14 | 25(+) | 80.9(+) |
| 12. | Enzyme+15 | 6.00 | 17.47(+) |
| 13. | Enzyme+16 | NIL | 45.5(+) |
| 14. | Enzyme+17 | 3.40 | 47.3(+) |
| 15. | Enzyme+18 | 8.20 | 18.3(+) |
| 16. | Enzyme+19 | NIL | 80.9(+) |
| 17. | Enzyme+20 | NIL | 74.39(+) |
| 18. | Enzyme+21 | NIL | 57.3(+) |
| 19. | Enzyme+22 | 20.2(+) | 121.9(+) |
| 20. | Enzyme+23 | NIL | 102.4(+) |
| 21. | Enzyme+24 | Not sol. In DMSO | Not sol. In DMSO |
| 22. | Enzyme+25 | NIL | 88.4(+) |
| 23. | Enzyme+26 | 15.8 | 117.7(+) |
| 24. | Enzyme+27 | NIL | 71.3(+) |
| 25. | Enzyme+28 | Not sol. In DMSO | Not sol. In DMSO |
| 26. | Enzyme+29 | Not sol. In DMSO | Not sol. In DMSO |
| 27. | Enzyme+30 | Not sol. In DMSO | Not sol. In DMSO |
| 28. | Enzyme+31 | NIL | 117(+) |
| 29. | Enzyme+32 | NIL | 101.2(+) |
| 30. | Enzyme+33 | Not sol. In DMSO | Not sol. In DMSO |
| 31. | Enzyme+34 | Not sol. In DMSO | Not sol. In DMSO |
| 32. | Enzyme+35 | Not sol. In DMSO | Not sol. In DMSO |

(**Table 1**) **contd….**

| S.No. | Compound No. | Percent Inhibition (*S. cervi*) | |
|---|---|---|---|
| | | γ-GCase[+] | GSTs[++] |
| 33. | Enzyme+36 | Not sol. In DMSO | Not sol. In DMSO |
| 34. | Enzyme+37 | Not sol. In DMSO | Not sol. In DMSO |
| 35. | Enzyme+38 | Not sol. In DMSO | Not sol. In DMSO |
| 36. | Enzyme+39 | **NIL** | 4.70(+) |
| 37. | Enzyme+40 | NIL | 46.9 |
| 38. | Enzyme+41 | NIL | 26.8 |
| 39. | Enzyme+42 | NIL | 24.0(+) |
| 40. | Enzyme+43 | NIL | 15.6 |
| 41. | Enzyme+44 | **NIL** | 41.6 |
| 42. | Enzyme+45 | NIL | 94.0(+) |
| 43. | Enzyme+46 | NIL | 45.9 |
| 44. | Enzyme+47 | NIL | 54.9(+) |
| 45. | Enzyme+48 | NIL | 21.3(+) |
| 46. | Enzyme+49 | NIL | 112.0(+) |
| 47. | Enzyme+50 | 57.2 | 109.3(+) |
| 48. | Enzyme+51 | NIL | 73.2(+) |
| 49. | Enzyme+52 | NIL | 165.0(+) |
| 50. | Enzyme+53 | NIL | -- |
| 51. | Enzyme+54 | 25.0 | 10.2(+) |
| 52. | Enzyme+55 | NIL | 5.60 |
| 53. | Enzyme+56 | NIL | 41.0(+) |
| 54. | Enzyme+57 | NIL | 36.0(+) |
| 55. | Enzyme+58 | NIL | 24.0 |
| 56. | Enzyme+59 | NIL | 102.0(+) |
| 57. | Enzyme+60 | 22.3(+) | 21.0(+) |
| 58. | Enzyme+61 | NIL | 38.0(+) |
| 59. | Enzyme+62 | NIL | 120.0(+) |
| 60. | Enzyme+63 | 23.0(+) | 34.9(+) |
| 61. | Enzyme+64 | NIL | 86.6(+) |
| 62. | Enzyme+65 | NIL | 59.7(+) |
| 63. | Enzyme+66 | NIL | 8.70 |

+ Conc. of the test sample in the assay system 200 μM
++ Conc. of the test sample in the assay system 67 μM

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