ABSTRACT

(As required under clause 13 of ordinance VI of the University of Delhi)

Title of the thesis entitled “Biocatalytic Acylation Studies on Novel Sugar Precursors & UNA-U and Synthesis of 2-Alkylamino-arabinofuranosyl Pyrimidines & 5’-Thionucleoside Disulfides”

The thesis is divided into four chapters, i.e. Chapter I, Chapter II, Chapter III and Chapter IV. A brief account of each chapter is given below:

The work presented in Chapter I entitled “Synthesis and Selective Biocatalytic Acylation Studies on Novel Carbohydrate Monomers” describes the synthesis and development of efficient lipase mediated selective acylation of one of the primary hydroxyl groups of the 4-C-hydroxymethyl-1,2-O-arylidene-β-L-threo-pentofuranose.

The application of biocatalysis in organic synthesis is a well-defined area of research. The selective protection of only one out of the several similar hydroxyl functional groups present in the sugar derivatives and nucleosides has always been a challenge for the organic chemists. Among the use of different biocatalysts in organic synthesis, lipases are the most frequently used ones. In particular, this class of enzymes is able to perform stereoselective hydrolytic reactions and catalyzes the formation of a wide range of ester and amide bonds. Because of this role, they are most frequently employed as biocatalysts for organic transformations. Lipases are being recognized as efficient catalysts for many stereospecific, regioselective and chemoselective reactions on carbohydrates and nucleosides. In this chapter, we have described the synthesis of novel sugar precursors, which can be used for the synthesis of sugar-PEG based polymeric materials for drug delivery applications. Further we have developed an efficient lipase mediated selective acylation reaction on sugar precursors.

In view of the importance of 4-C-hydroxymethyl-1,2-O-isopropylidene-β-L-threo-pentofuranose as a precursor for the synthesis of sugar-PEG based copolymers for drug delivery applications, we have synthesized three novel sugar monomers, 4-C-hydroxymethyl-1,2-O-benzylidene-β-L-threo-pentofuranose, 4-C-hydroxymethyl-1,2-O-biphenylidene-β-L-threo-pentofuranose and 4-C-hydroxymethyl-1,2-O-α-naphthylidene-
$\beta$-L-threo-pentofuranose which can be used for PEGylation to afford novel Sugar-PEG based copolymers for study as drug delivery agents. Further, we have developed an efficient and green chemoenzymatic methodology for the diastereoselective monoacylation of the novel sugar precursors.

The 4-C-hydroxymethyl-1,2-O-isopropylidene-$\beta$-L-threo-pentofuranose was synthesized starting from D-glucose diacetonide by following the procedure described by Moffatt et al. It was then converted into triacylated 1,2-diol in two steps, i.e. by peracetylation using acetic anhydride and DMAP, followed by the cleavage of 1,2-isopropylidene moiety of the resulted triacetate sugar derivative to afford the desired product in an overall yield of 90%. The triacetyl 1,2-diol sugar derivative thus obtained was used for 1,2-O-arylidene protection using arylaldehyde dimethyl acetals in the presence of camphor sulfonic acid (CSA) as catalyst under vacuum on rotary evaporator to afford 4-C-acetoxymethyl-3,5-di-O-acetyl-1,2-O-arylidene-$\beta$-L-threo-pentofuranoses in 75 to 80% yields. The triacetylated sugar compounds thus obtained were completely deacetylated using saturated MeOH/NH$_3$ to obtain 4-C-hydroxymethyl-1,2-O-benzylidene-$\beta$-L-threo-pentofuranose, 4-C-hydroxymethyl-1,2-O-biphenylidene-$\beta$-L-threo-pentofuranose and 4-C-hydroxymethyl-1,2-O-$\alpha$-naphthylidene-$\beta$-L-threo-pentofuranose in 85 to 90% yields.

To study the diastereoselective monoacylation 4-C-hydroxymethyl-1,2-O-arylidene-$\beta$-L-threo-pentofuranose, screening of lipases in different organic solvents was performed in order to find out the best lipase and solvent system which can carry out an efficient selective acylation of one of the two primary hydroxyl groups in the compounds. The lipases screened for diastereoselective acylation study on 4-C-hydroxymethyl-1,2-O-arylidene-$\beta$-L-threo-pentofuranose were *Candida antarctica* lipase-B (Novozyme®-435), *Thermomyces lanuginosus* lipase immobilized on silica (Lipozyme® TL IM), Amano PS lipase, *Candida rugosa* lipase (CRL) and procine pancreatic lipase (PPL) in four sets of organic solvents, viz. acetonitrile (CH$_3$CN), tetrahydrofuran (THF), toluene and dichloromethane (DCM), using vinyl acetate at different reaction temperatures, viz. 40, 45, 50 and 55 °C in an incubator shaker at 180 rpm.

On the basis of screening of different lipases, using vinyl acetate as acyl donor we found that Novozyme®-435 in acetonitrile at 40-42 °C was the best condition for
carrying out the selective acylation. Further, another set of lipase catalyzed regio-selective acylation reactions were carried out with an aim to study the effect of different acyl chain length on the selectivity of lipase Novozyme®-435. For the synthesis of these different ester analogues, different acylating agents, such as vinyl acetate, vinyl propanoate, vinyl butanoate and vinyl benzoate & the benzoic-, acetic-, propanoic-, butanoic, isobutanoic-, pentanoic- and hexanoic anhydrides were used for the selective manipulation of the C-5-OH over the C-5′-OH and C-3-OH groups in the synthesized sugar monomers under above mentioned standardized condition. The results revealed that the lipase Novozyme®-435 mediates the transfer of acyl function selectively onto C-5-OH group of the sugar monomers from the active esters resulting in the formation of 4-C-hydroxymethyl-5-O-acyl-1,2-O-benzylidene-β-L-threo-pentofuranose, 4-C-hydroxymethyl-5-O-acyl-1,2-O-biphenylidene-β-L-threo-pentofuranose and 4-C-hydroxymethyl-5-O-acyl-1,2-O-α-naphthylidene-β-L-threo-pentofuranose in 85-95% yields. In case of vinyl benzoate and acid anhydrides there was no reaction observed even after longer incubation time and higher temperatures. The turnover in the reactions involving transfer of acetyl function from vinyl acetate was slightly higher than other acyl transfer reactions.

The same set of the enzymes were further screened for selective deacetylation reactions on novel sugar triacetate precursors, i.e. 4-C-acetoxyethyl-3,5-di-O-acetyl-1,2-O-arylidene-β-L-threo-pentofuranoses using small amount of n-butanol as the acyl acceptor, but there was no conversion observed even at higher temperature and over longer reaction time. The structures of all the synthesized compounds were unambiguously established on the basis of their IR, ¹H NMR, ¹³C NMR spectra and HRMS data analysis. Further the structures of the three mono acylated compounds were confirmed by single crystal X-ray data analysis.

The work presented in Chapter II entitled “Biocatalytic Acylation Studies on 5′-O-(4,4′-Dimethoxytrityl)-2′,3′-secouridine (UNA-U)” describes the biocatalytic selective and environmentally friendly methodology for the synthesis of 2′-O-acyl-5′-O-DMT-2′,3′-secouridine nucleosides.
Synthesis of monomeric building blocks of UNA, i.e. phosphoramidites of acyclic nucleosides guanosine, adenosine, cytidine and uridine requires selective manipulation of one of the two primary hydroxyl groups at the C-2'- and C-3'-positions in the corresponding 5′-O-(4,4′-dimethoxytrityl)-seconucleosides. The chemical methods available for the preparation of 2′-O-benzoylated 5′-O-DMT-seconucleosides requires the use of unfriendly chemicals or extremely low temperature and often leads to the formation of all possible benzoylated products, which reduces the yield of the desired compound and makes the protocol inefficient.

We herein report a high yielding, selective and environment friendly enzymatic methodology for the synthesis of 2′-O-acetyl-5′-O-DMT-2′,3′-secouridine nucleosides from its corresponding dihydroxy acyclic nucleoside and conversion of the resulted mono-benzoylated compound into phosphoramidite building block of UNA U. The acyclic nucleoside monomer of UNA U, i.e. 5′-O-DMT-2′,3′-secouridine was obtained from RiboTask, Denmark as a gift, which can also be obtained from uridine following the literature procedure. For selective acylation of 2′-hydroxyl function in 5′-O-DMT-2′,3′-secouridine with different acyl donors such as vinyl carboxylates and acid anhydrides, different lipases, viz. Candida antarctica lipase-B immobilized on polyacrylate (Novozyme®-435 or CAL-B), Thermomyces lanuginosus lipase immobilized on silica (Lipozyme® TL IM), Amano PS lipase, Candida rugosa lipase (CRL) and porcine pancreatic lipase (PPL) were screened in organic solvents, such as tetrahydrofuran (THF), acetonitrile, toluene, diisopropyl ether (DIPE) and acetone at 40-42 °C and 150 rpm in an incubator shaker. Among all the lipases, Lipozyme® TL IM (50% w/w of compound) in toluene showed an efficient selectivity towards the C-2′-OH group over the C-3′-OH group of acyclic nucleoside in moderate to good yields. Among all acyl donors in toluene, vinyl benzoate was found to be the best acyl donor.

Thus in a typical biocatalytic reaction, a solution of 5′-O-(4,4′-dimethoxytrityl)-2′,3′-secouridine in dry toluene containing 1.2 equivalent of vinyl esters / acid anhydride was agitated with Lipozyme® TL IM in an incubator shaker at 40-42 °C and 150 rpm. On completion of the reaction, as indicated by analytical TLC examination, enzyme was filtered off and the solvent was removed under reduced pressure. The crude product thus
obtained was passed through a silica gel column to afford the pure acylated compound, with higher R$_f$ value than the starting compound in 58 to 92% yield.

The Lipozyme® TL IM catalyzed acylation reaction on UNA, revealed that the rate of butanoylation was 3.0 times faster than the rate of propanoylation and acetylation of nucleoside and 5.0 times faster than the isobutanoylation and pentanoylation of nucleoside. The rate of acetylation / benzoxylation with activated esters i.e. vinyl benzoate and vinyl acetate as acyl donor was found to be 1.3 and 1.5 times faster than the benzoxylation / acetylation with benzoic anhydride and acetic anhydride, respectively.

Further, 2′-O-benzoyl-5′-O-DMT-2′,3′-secouridine was converted into corresponding phosphoramidite, a building block for the synthesis of UNA, by reaction with 2-cyanoethyl-$N,N$-diisopropylchloro-phosphoramidite [P(-Cl)(-OCH$_2$CH$_2$CN){-N(iPr)$_2$}] in acetonitrile in the presence of diisopropylethylamine (DIPEA) following the literature procedure. The structures of all the acylated nucleosides, 2′-O-acyl-5′-O-(4,4′-dimethoxytrityl)-2′,3′-secouridine and phosphoramidite were unambiguously established on the basis of IR, $^1$H-NMR, $^{13}$C-NMR and $^{31}$P-NMR spectral data and HRMS analysis.

The work presented in Chapter III entitled “Synthesis of 2-Alkylamino-arabinofuranosyl Pyrimidines” describes the synthesis of base modified nucleosides, i.e. 2-alkylamino-1-(1′-β-D-arabinofuranosyl)-5-methyl-pyrimidine-4-ones.

The last two decades have witnessed an upsurge in the synthesis of several modified nucleosides and nucleic acid derivatives. Modified nucleosides and nucleotides have broad spectrum of applications and large number of antiviral drugs belongs to this class. Chemically altered nucleosides have found various applications ranging from chemical biology to nanotechnology. A common synthetic approach for modified nucleosides and nucleic acids is a challenge for synthetic organic chemists.

As a part of our continued interest in nucleoside chemistry, we described herein the synthesis of new base modified nucleoside analogs, i.e. 2-alkylamino-arabinofuranosyl thymine via 2,2′-anhydro ring opening reaction. Anhydro nucleoside derivatives are versatile precursors in chemical transformations. We selected D-xylose as starting
compound which was converted into 1,2,3,5-tetra-O-acetyl-D-xylofuranose in three steps according to the reported literature procedure. Then, synthesis of 2',3',5'-tri-O-acetyl-β-D-xylofuranosyl thymine was carried out by using Vorbruggen nucleoside coupling of anomic mixture of 1,2,3,5-tetra-O-acetyl-D-xylofuranose with thymine base as a white solid in 80% yield. The acetylated nucleosides, thus obtained were stirred in saturated methanolic ammonia solution leading to the formation of β-D-xylofuranosyl thymine in 90% yield. Further, hydroxynucleoside was reacted with diethyl carbonate (DEC) / diphenyl carbonate (DPC) in the presence of catalytic amount of sodium bicarbonate at 150 °C to afford the formation of 2,2'-anhydro-β-D-arabinofuranosyl thymine in 70% yield. The formation of 2,2'-anhydro-derivative with epimerisation at C-3' is unusual in the field of nucleoside chemistry. The mechanism for the above rearrangement could be explained by considering the initial formation of 3',5'-carbonate derivative which on subsequent rearrangement forms the epoxide intermediate. Further, this compound underwent ring opening reaction to form the 2,2'-anhydro-derivative.

Finally 2,2'-anhydro compound with different amines resulted in selective opening of the anhydro ring through C-2 position over the C-2' position leading to the formation of 2-alkylamino-1-(1'-β-D-arabinofuranosyl)-5-methyl-pyrimidine-4-ones in 68-85% yields. The structures of all the compounds were unambiguously established on the basis of their IR, 1H-NMR, 13C-NMR spectra and HRMS data analysis.

The work presented in Chapter IV entitled “Synthesis of 2′,5′-Dideoxy-5′-thionucleoside Disulfides” describes the synthesis of 2′,5′-dideoxy-5′-thionucleoside disulfides by Mitsunobu reaction conditions followed by reaction with saturated methanolic ammonia.

Thionucleosides and their thioesters can be readily produced from alkyl halides or alkene derivatives, and their conversion into symmetrical disulfides can be performed without any problem. Deprotection of the thiol group by removal of the acyl group can occur under basic, acidic or neutral conditions. The formation of symmetrical disulfides, instead of the expected thiol has been observed very frequently when
deprotection is performed under basic conditions, in open atmosphere or when dissolved in an oxygen-containing solvent.

In view of the wide range of applications of thionucleosides, we have synthesized four new 5′-S-benzoyl-3′-O-levulinyl-2′,5′-dideoxy-5′-thionucleosides i.e. 5′-S-benzoyl-3′-O-levulinyl-2′,5′-dideoxy-5′-thiorymidine, N4,5′-S-dibenzoyl-3′-O-levulinyl-2′,5′-dideoxy-5′-thiocytidine, N6,5′-S-dibenzoyl-3′-O-levulinyl-2′,5′-dideoxy-5′-thioadenosine and N2-isobutyryl-5′-S-benzoyl-3′-O-levulinyl-2′,5′-dideoxy-5′-thioguanosine and their corresponding disulfides i.e. 2′,5′-dideoxy-5′-thiorymidine disulfide, 2′,5′-dideoxy-5′-thiocytidine disulfide, 2′,5′-dideoxy-5′-thioadenosine disulfide and 2′,5′-dideoxy-5′-thioguanosine disulfide, respectively. In our approach, deoxy ribonucleoside was employed as starting material, which is ideal for diversity-oriented synthesis of dinucleoside disulfides containing different nucleobases.

5′-O-(4,4′-Dimethoxytrityl)-2′-deoxy nucleosides, were obtained as gift from Rasayan inc., USA. They can also be synthesized using D-ribose which can be peracetyled followed by introduction of the nucleobase using Vorbrüggen protocol. Then complete deprotection of acetyl protecting groups in the deoxyribonucleosides in methanolic ammonia followed by protection of 5′-OH group using DMTrCl can afford the desired compound. Further, 5′-O-DMT-2′-deoxy nucleosides, have been selected as starting compounds in the synthetic protocol for the synthesis of 5′-S-benzoyl-3′-O-levulinyl-2′,5′-dideoxy-5′-thionucleosides and their disulfides. Reaction of 5′-O-(4,4′-dimethoxytrityl)-2′-deoxy nucleosides with levulinic acid in the presence of DCC/DMAP in anhydrous dioxane under nitrogen atmosphere afforded 5′-O-(4,4′-dimethoxytrityl)-3′-O-levulinyl-2′-deoxy-nucleosides. After completion of the reaction as indicated by TLC examination, solvent was removed under reduced pressure and the compounds were purified by flash column chromatography. 5′-O-4,4′-Dimethoxytrityl protecting group was then removed in the presence of trichloroacetic acid (Cl3CCOOH) in dry dichloromethane (DCM) over 30min-1hr at 0 °C-RT and the compounds were purified by flash column chromatography to afford 3′-O-levulinyl-2′-deoxy-nucleosides in quantitative yields. The 5′-OH group of the compounds were selectively converted into 5′-S-benzoyl-3′-O-levulinyl-2′,5′-dideoxy-5′-thionucleosides in 50-95% yields under Mitsunobu reaction condition using PPh3,
DIAD and thiobenzoic acid in dry THF and DMF. 5′-S-Benzoyl-3′-O-levulinyl-2′,5′-dideoxy-5′-thionucleosides thus obtained were reacted with saturated MeOH/NH3 at RT over 6-12hrs to afforded the dinucleoside disulfides, i.e. 2′,5′-dideoxy-5′-thionucleoside disulfides in 85-90% yield. The structures of all the synthesized compounds were unambiguously established on the basis of their IR, ¹H NMR, ¹³C NMR spectra and HRMS data analysis.

The work presented in the thesis entitled “Biocatalytic Acylation Studies on Novel Sugar Precursors & UNA-U and Synthesis of 2-Alkylamino-arabinofuranosyl Pyrimidines & 5′-Thionucleoside Disulfides” by Mr. Chandra Shekhar Reddy Lokasani for the award of the degree of Doctor of Philosophy from the University of Delhi, has been carried out in the Laboratories of the Department of Chemistry, University of Delhi, under the supervision of Professor Ashok K Prasad and Professor V. S. Parmar. The work is original and has not been submitted in part or in full, for any other Diploma or Degree of this or any other University. The extent of information derived from the existing literature has been indicated in the body of the thesis at appropriate places giving the source(s) of information.

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