Chapter IV

Synthesis of 2',5'-Dideoxy-5'-thionucleoside Disulfides
4. Introduction

Nucleosides containing 5'-thio-substitution found in a number of natural products are responsible for several important functions in the cell. The most prominent representative is the cofactor S-adenosyl-L-methionine (1, AdoMet), which is the major methyl group donor in a myriad of methyltransferase catalysed reactions (Scheme 1). The transmethylation reaction yields the cofactor product S-adenosyl-L-homocysteine (2, AdoHcy), which is a potent inhibitor of methyltransferases and is removed by S-adenosyl-L-homocysteine hydrolase. In addition, 1 is involved in the polyamine biosynthesis. Decarboxylation of 1 by S-adenosyl-L-methionine decarboxylase leads to decarboxylated AdoMet 3, which serves as aminopropyl donor for the synthesis of spermidine and spermine. Due to the biological importance and a variety of S-adenosyl-L-methionine metabolism, a great number of nucleosides containing 5'-sulfur atoms have been synthesized. They serve as pharmacologically valuable inhibitors for S-adenosyl-L-homocysteine hydrolase, S-adenosyl-L-methionine decarboxylase, aminopropyl transferases and spermidine & spermine synthases (polyamine transferase I and II). Structurally related analogues of AdoHcy 2 have shown significant inhibitory effects on S-adenosyl-L-methionine-dependent ethyltransferases. Recently, the human C5-cytosine DNA methyltransferase was suggested as a potential target for drug design because hypermethylation of upstream regulatory sequences leads to an inactivation of tumor suppressor genes in numerous cancers. Thus, inhibitors of the human DNA methyltransferase could reverse the effects of DNA methylation and could have therapeutic value in the treatment of cancer. Efficient synthetic routes have been developed for the synthesis of AdoHcy. Elmar Weinhold et al. described the synthesis of compounds 6, 7 in which the 5'-OH group is converted into good leaving groups and 5'-thionucleoside 8 by using Mitsunobu reaction conditions. Further these compounds were converted into adenosine 5'-thioether 9 (Scheme 2).
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**Scheme 1**: Biological transformations of S-adenosyl-L-methionine (1)

R. Petrelli *et al.*\(^{11}\) have described the synthesis of dinucleoside disulfides\(^{12a}\) 12, 13 and 14 from 5′-thionucleoside monomers 10 and 11 in the presence of saturated methanolic ammonia (Scheme 3), and these compounds were found moderate inhibitors of *M. Tuberculosis*. Diadenosine disulfide 14 was reported to inhibit NAD kinase from *Lysteria monocytogenes* and the crystal structure of the enzyme-inhibitor complex was solved. Synthesized ditiazofurin disulfide 12 and the tiazofurin adenosine disulfide 13 were found to be moderate inhibitors of human NAD kinase (IC\(_{50} = 87\) µM and IC\(_{50} = 110\) µM, respectively) and *Mycobacterium tuberculosis*.
NAD kinase (IC$_{50}$ = 45 µM and IC$_{50}$ = 80 µM, respectively) (Scheme 3). Introduction of bromine at the C-8 of the adenine ring restricted the adenosine moiety of diadenosine disulfides to the syn conformation making it more active, i.e. 3-15-fold increase of potency against *Mycobacterium* and human enzyme.

![Scheme 3: Synthesis of ditiazofurin disulfide 12, tiazofurin adenine disulfide 13, and dithioadenosine 14 (DTA).](image)

4.1. **Natural thionucleosides and their disulfides**

Marine sponge is known as prolific source of biologically active and structurally unique metabolites. W. Hassan *et al.*$^{12b}$ isolated new alkaloids from the Mediterranean Sponge *Hamigera hamigera*. In the search for biologically active substances from marine sponges especially the family Anchinoidae was shown to be rich source for secondary metabolites which include tripeptide and dimeric peptide alkaloids, sulfur-containing compounds,$^{13-15}$ chlorine-containing phenolic compounds,$^{16}$ and antifungal and cytostatic macrolides.$^{17,18}$ Biologically active brominated compounds have previously been isolated from *Hamigera tarangensis*.$^{19,20}$ The Mediterranean sponge *Hamigera hamigera* (family Anchinoidae) was studied since its total extract showed
deterrent activity in a fish feeding assay. Eight compounds were isolated from the biologically active fractions and four of them, i.e. compounds 15-18 proved to be new natural products (Figure 1).

During a systematic investigation into cytotoxic metabolites from marine organisms, C. Peng et al.\textsuperscript{21} described a novel family of indole alkaloids, from a specimen of a marine sponge, \textit{Trachycladus laevispirulifer}.\textsuperscript{22} The trachycladindole pharmacophore exhibited selective sub-\textmu M toxicity to human lung (A549), colorectal (HT29) and breast (MDAMB- 231) cancer cell lines, and remains the subject of ongoing synthetic and mode of action investigations. During the course of study, they also detected a minor non-cytotoxic co-metabolite believed to be a novel nucleoside, identified as 9-(5'-deoxy-5'-thio-\textbeta-D-xylofuranosyl)adenine disulfide (19) (Figure 2). This compound is the first reported naturally occurring nucleoside disulfide and was first isolated from an Australian marine sponge, \textit{Trachycladus laevispirulifer}, by C. peng and coworkers in 2010.\textsuperscript{21} This compound is the third natural xylo-nucleoside, while the other two are 9-(5'-deoxy-5'-thiomethyl-\textbeta-D-xylofuranosyl)adenine (20)\textsuperscript{23} and 4-amino-7-(5'-deoxy-\textbeta-D-xylofuranosyl)-5-iodopyrrolo-[2,3-d]pyrimidine (21) (Figure 2).\textsuperscript{24}

Though nucleoside disulfide 19 exhibited little cytotoxic effect against human breast and cervical cancer cell lines in biological assays, its chemical ecology role in enhancing survival of the producing organism remained undetermined.\textsuperscript{25} Nucleoside
is an important natural regulatory compound, produced from enzymatic processing of the ubiquitous biosynthetic methylation agent S-adenosyl-L-methionine. Demethylation of 20 could plausibly lead to the free 5'-thiol, which would in turn undergo ready oxidation and dimerization to the disulfide 19.

H. X. Ding et al.\textsuperscript{26} described the first total synthesis of natural nucleoside disulfide 19 from D-xylose (Scheme 4),\textsuperscript{27a} which is an ideal synthesis for diversity oriented nucleoside disulfides containing different nucleobases. The crystalline 1,2-O-isopropylidene-\(\beta\)-D-xylofuranose 23 was prepared by acid-catalyzed acetonide protection of D-xylose 22, followed by partial hydrolysis with aqueous sodium carbonate. Then 5-OH was selectively converted into tosylate with tosyl chloride (TsCl) and triethylamine (TEA) as base to afford 24. The protection of 3-OH using benzoyl chloride gave compound 25. Substitution of the tosyl group with potassium thioacetate in anhydrous DMF at 80 °C afforded compound 26.\textsuperscript{27b} The 1,2-O-isopropylidene group was removed in acetic acid/acetic anhydride mixture with catalytic amount of sulfuric acid to give nucleoside precursor 27.\textsuperscript{27c} In the key step Vorbruggen glycosylation\textsuperscript{27d} was utilized to attach adenine to xylose moiety to afford the desired nucleoside 29. Then removal of all protecting groups with ammonia and \textit{in situ} oxidation in open air afforded the target nucleoside disulfide 19 (Scheme 4).

Scheme 4: Reagents and conditions: (a) Acetone, \(\text{H}_2\text{SO}_4\), r.t., 3hrs, (b) TsCl, Py, r.t., 4hrs, (c) BzCl, Py, r.t., 5hrs, (d) potassium thioacetate, DMF, 80 °C, 3hrs, (e) AcOH, \(\text{Ac}_2\text{O}\), \(\text{H}_2\text{SO}_4\), 24hrs, (f) \(N^6\)-benzoyladenine (28), BSA, TMSOTf, dichloroethane, 0 to 80 °C, 2hrs, (g) aq. ammonium hydroxide, methanolic ammonia, air, overnight.
4.2. Synthetic dinucleoside disulfides

Synthetic oligonucleotides with favourable antisense properties are few in number. Whilst many nuclease-resistant analogues have been prepared, relatively few of these have displayed favourable hybridization properties with their target RNA. Recently, backbone modifications which confer structural rigidity have attracted interest as they may provide the necessary preorganization requirements for optimal duplex formation. Solution conformation studies have shown that disulfide bonds exist as rigid systems which are conformationally restricted.\(^{28}\) Thus oligonucleotides 30 and 31 in which the phosphodiester bonds are replaced with neutral, achiral disulfide linkages,\(^{29}\) are attractive antisense candidates since they are not only expected to be nuclease resistant but they may also display good hybridization properties (Figure 3).

![Figure 3](image)

Incorporation of a thiol function in nucleosides, nucleotides, or oligonucleotides has led to a number of analogues possessing interesting biological properties.\(^{30}\) For example, 2\(^{-}\),3\(^{-}\)-dideoxy-3\(^{-}\)-mercaptop nucleoside 5\(^{-}\)-triphosphates (T, C, A, G) irreversibly stopped DNA chain elongation by modulation of AMV and HIV reverse transcriptase, and the corresponding nucleosides displayed antiviral activities.\(^{31a-b}\) Recently, S. Chambert \textit{et al}.\(^{32}\) demonstrated that another nucleotide possessing a thiol function on the sugar, 2\(^{-}\)-deoxy-2\(^{-}\)-mercaptopuridine 5\(^{-}\)-diphosphate strongly inactivates \textit{in vitro} \textit{E. coli} ribonucleoside diphosphate reductase (RDPR).\(^{33}\) This enzyme catalyzes the reduction of the four natural ribonucleotides in the corresponding 2\(^{-}\)-
deoxyribonucleotides and thus is a key enzyme in the synthesis of DNA.\textsuperscript{34} The thiol function of the modified nucleotide interacts with a cysteine residue at the active site to lead to a perthiy radical formed on the enzyme. Numerous modified oligonucleotides containing a sulfur atom on the base, sugar or phosphoryl group were synthesized and used as tools in studies of nucleic acid structure and function, protein-nucleic acid interactions, and antisense therapy.\textsuperscript{35} For example, 4-thiouridine, a natural constituent of t-RNA is used as a photochemical probe in the study of nucleic acids.\textsuperscript{36} 2′-Deoxy-6-thioguanosine is used for the treatment of leukemias,\textsuperscript{37} and more recently, 2′-deoxy-2′-mercaptoctydine were incorporated in oligonucleotides. The developments of new method for incorporating a thiol function in nucleosides, nucleotides, and oligonucleotides for preparing their stable useful precursors of interest in search of bioactive compounds or biological tools have gained importance. In this regard, mixed disulfides can be interesting precursors.

3′-Thiothymidine 5′-triphosphate was reported as a highly effective terminator of DNA synthesis that is catalyzed by HIV reverse transcriptase.\textsuperscript{31b,c} Owing to its antiviral activity, synthesis of 3′-deoxythymidin-3′-yl methyl disulfide (\textbf{34 Scheme 5}), which is also a potent antiviral agent and a precursor of 3′-thiothymidine has been reported. S. Chambert et al.\textsuperscript{32} developed the synthesis for methyl disulfides \textbf{38} and \textbf{39} (\textbf{Scheme 6}). 5′-Protected derivatives of 3′-thiothymidine were prepared previously by reaction of 2,3′-anhydro-5′-tritylthymidine or 1-(2-deoxy-3-mesyl-5-(4-monomethoxytrityl)-β-D-lyxosyl)thymine with potassium or sodium thiobenzoate; further quick oxidation of 3′-thiothymidine to the corresponding symmetrical disulfide had been observed.\textsuperscript{38a,b}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{Scheme5}
\caption{Scheme 5: (a) 2-(Trimethylsilyl)ethanethiol, NaH, DMF, 90 °C, 1hr, (b) 2% DCA, CH\textsubscript{2}Cl\textsubscript{2}, aqueous NaHCO\textsubscript{3}, (c) dimethyl(methylthio)-sulfuniumtetrafluoroborate, THF, r.t.}
\end{figure}
Scheme 6: (a) 2-(Trimethylsilyl)ethanethiol, K₂CO₃, DMF, 120 °C, (b) dimethyl(methylthio)sulfoniumtetrafluoroborate/THF, r.t, (c) Ac₂O/Py, (d) 1,2,4-triazole/POCl₃/Et₃N/CH₂CN, (e) ammonia, dioxane, (f) saturated aqueous ammonia, ethanol.

Meena et al.³⁹ have described the synthesis of 3'-thio nucleoside derivatives 42 and 43 (Scheme 7) and 49a,b (Scheme 8). 2,3'-Anhydro thymidine 40 and N⁴-benzoyl cytidine 41 on ring opening reaction with thiobenzoic acid led to the formation of 42 and 43, respectively (Scheme 7). Similarly compounds 44a and 44b on epimerization of C-3' position followed by nucleophilic substitution reaction and then deprotection of DMT group afforded compounds 49a and 49b, respectively (Scheme 8).
Further, 3’-thio nucleosides 49a, 49b, 51a and 51b were converted into their corresponding 2’,3’-dideoxy-3-thionucleosides triphosphates (ddtNTPs) 50a, 50b, 54a and 54b, respectively (Scheme 9 and 10). The thiol functional group in these compounds can be used as the nucleophile in the formation of a phosphorothiolate linkage when primer extension is catalyzed by Y410F mutant of the Deep Vent polymerase. On the basis of previous reports, polymerase-I and HIV appear to be less accommodating in their use of such substrates. Although different polymerases are likely to vary in the details of substrate recognition, there does not appear to be any fundamental prohibition of using a thiol nucleophile on a phosphate anhydride electrophile, while corresponding catalyzed reactions on phosphodiesters, appear to be much less effective.41,42 The successful elongations suggest that it may be possible to synthesize longer phosphorothiolate sequences in enzymatic reactions by using these monomers.
If sufficiently long and accurate elongations can be achieved with ddtNTPs, it might be possible to use \textit{in vitro} selection methods to generate functional thiolate-DNA molecules such as aptamers and DNA thio-enzymes. DNA linkages containing 3'-phosphorothiolates have been used to stabilize structural motifs,\textsuperscript{43} to probe enzymatic processes,\textsuperscript{44} and to enhance antisense complexes.\textsuperscript{45}

A. A. El-Barbary \textit{et al.}\textsuperscript{46} described a convergent synthesis of 2',3'-dideoxy-3'-mercapto nucleosides as potential anti HIV agents and 3'-mercapto-3'-deoxy thymidine which suppresses HIV viruses as efficiently as AZT. R. Cosstick \textit{et al.}\textsuperscript{47} have described that the analogs of DNA and RNA are essential tools for probing structural and mechanistic aspects of nucleic acid biochemistry. Nucleic acid analogs in which one of the phosphoryl oxygen atoms is replaced with sulfur are particularly useful as they are isoelectronic and iso-steric with the natural structures and in many cases act as substrates for DNA/RNA-processing enzymes. Replacement of the 3'-bridging oxygen atom gives rise to an achiral 3'-S-phosphorothiolate (3'-PS) linkage \textsuperscript{55} (Figure 4), which has been incorporated by chemical synthesis into both DNA\textsuperscript{48a-d} and RNA.\textsuperscript{49a,b} A key feature of the 3'-PS linkage is its ability to probe the role of metal ions in the enzyme-catalyzed cleavage of a phosphodiester bond through metal specificity switch experiments. These experiments can highlight direct interactions between a metal ion and an oxyanion leaving group exploiting the difference in affinity that a metal center displays for an oxo \textit{versus} a thiolate group. Thus, the use of a phosphorothiolate linkage can provide detailed
information on the mechanism of the phosphodiester bond cleavage process, particularly with regard to the involvement of metal ions.\textsuperscript{48b,50} Oligodeoxynucleotides containing this linkage have, therefore, been used for in-depth mechanistic analysis into a number of enzymatic manipulations of DNA including phosphodiester cleavage catalyzed by the restriction endonuclease EcoRV,\textsuperscript{48a,c,d} exonuclease activity of E. coli DNA polymerase I,\textsuperscript{51,52} the resolution of Holliday junctions by RuvC\textsuperscript{53} and DNA repair by E. coli DNA T:G mismatch endonuclease.\textsuperscript{54} In terms of their effect on the structure of DNA, this sugar conformation is associated with RNA, and thus oligodeoxynucleotides containing 3'-PS linkages adopt an RNA-like conformation locally at the site of modification and in this respect 3'-PS oligodeoxynucleotides can be considered as good mimics of RNA. More recently, the 3'-PS linkage has been shown to stabilize an unusual intercalated DNA conformation that is associated with telomeric regions of DNA.\textsuperscript{55} Potential new applications for the 3'-PS linkage are stabilization of other multistranded nucleic acid structures (such as triplexes) and incorporation into RNA for use in RNA interference. M. Olesiak et al.\textsuperscript{56} described a new type of internucleotide phosphorodithioate linkage, wherein one of the sulfur atoms occupies a 5'-bridging position 56a-d (Figure 4). The 5'-S-phosphorodithioate linkage in dinucleotide analogues was found to be resistant toward nucleolytic degradation with snake venom PDE and nuclease P1.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Figure4.png}
\caption{3'-S-Phosphorothiolate Linkage 55 and 5'-S-Phosphorodithioate Linkages 56a-d.}
\end{figure}

Modified oligodeoxynucleotides are versatile tools to solve a variety of problems in molecular biology. The cleavage of oligodeoxynucleotides with restriction enzymes is selective and quantitative. For an equivalent chemical cleavage, the oligodeoxynucleotides must be modified beforehand. K. Jahn-Hofmann et al.\textsuperscript{57} selected the thiomodification because of its electronic and steric similarity with the natural congener and the possibility for later derivatization. These backbone modified oligodeoxynucleotides can be cleaved selectively and quantitatively at the P-S or C-S bonds by Ag\textsuperscript{+} and Hg\textsuperscript{2+} ions or by a
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concentrated I$_2$ solution under mild conditions. For this study one need all the four modified amidites 61 and 66a-c were synthesized as described in Scheme 11 and 12. The synthetic procedures according to Schemes 11 could not be applied to all the four amidite building blocks, but only to the compound 61. Thus, the four 5'-thionucleosides were synthesized by introducing the (MeO)$_2$Tr group with the thiol reagent 63 leading to 3'-phosphoramidite building blocks 61 and 66a-c (Schemes 12).

Scheme 11

Scheme 12: Synthesis of 5'S-(4,4'-dimethoxytrityl)-2'-deoxy-5'-thionucleoside-3'- (2-cyanoethyl diisopropylphosphoramidites) 61 and 66a-c.
4.3. Present work

Thionucleosides and their thioesters can be readily produced from alkyl halide or alkene derivatives, and their conversion into symmetrical disulfides can be performed without any problems. 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 application 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′-thiouridine (74a), \( N^4,5′-S\)-dibenzoyl-3′-O-levulinyl-2′,5′-dideoxy-5′-thiocyctidine (74b), \( N^6,5′-S\)-dibenzoyl-3′-O-levulinyl-2′,5′-dideoxy-5′-thioadenosine (74c) and \( N^2\)-isobutyryl-5′-S-benzoyl-3′-O-levulinyl-2′,5′-dideoxy-5′-thioguanosine (74d) and their corresponding disulfides i.e. 2′,5′-dideoxy-5′-thiothymidine disulfide (75a), 2′,5′-dideoxy-5′-thiocyctidine disulfide (75b), 2′,5′-dideoxy-5′-thioadenosine disulfide (75c) and 2′,5′-dideoxy-5′-thioguanosine disulfide (75d), respectively. In our approach, deoxy ribonucleoside was employed as starting material, which is ideal for diversity-oriented synthesis of dinucleoside disulfides containing different nucleobases.

D-ribose was peracetylated followed by introducing nucleobase using Vorbrüggen protocol.\(^{27d}\) Then complete deprotection of acetyl protecting groups in the deoxyribonucleosides 69a-d in methanolic ammonia followed by protection of 5′-OH group using DMT\(\text{TrCl}\) afforded compound 70a-d. These compounds have been obtained as gift from Rasayn inc., USA. 5′-O-DMT-2-Deoxy nucleosides 70a-d, have been selected as starting compound in the synthetic protocol for the synthesis of 5′-S-benzoyl-3′-O-levulinyl-2′,5′-dideoxy-5′-thionucleosides 74a-d and their disulfides 75a-d. Reaction of 5′-O-DMT-2-Deoxy nucleosides 70a-d 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 71a-d.\(^{58a,b}\) After completion of the reaction as indicated by TLC examination, solvent was removed under reduced pressure and the compounds 71a-d were purified by flash column chromatography. 5′-O-(4,4′-Dimethoxytrityl protecting group was then removed in the presence of trichloro acetic acid (\(\text{Cl}_3\text{C COOH}\)) in dry dichloromethane (DCM) over 30min-1hr at 0 °C- RT followed by flash column chromatography to afford 3′-O-levulinyl-2′-deoxy-nucleosides 72a-d in...
quantitative yields. The 5'-OH group of the compounds 72a-d were selectively converted into 5'-S-benzoyl-3'-O-levulinyl-2',5'-dideoxy-5'-thionucleosides 74a-d in 50-95% yields under Mitsunobu\textsuperscript{59,60} reaction condition using PPh\textsubscript{3}, DIAD and thiobenzoic acid in dry THF and DMF. Thus obtained 5'-S-benzoyl-3'-O-levulinyl-2',5'-dideoxy-5'-thionucleosides 74a-d were then reacted with saturated MeOH/NH\textsubscript{3} at RT over 6-12 hrs to afforded dinucleoside disulfides, \textit{i.e.} 2',5'-dideoxy-5'-thionucleoside disulfides 75a-d in 85-90% yield.

The structure of all the compounds 70a-d, 71a-d, 72a-d, 74a-d and 75a-d synthesized were unambiguously established on the basis of their spectral (IR, \textsuperscript{1}H-, \textsuperscript{13}C- and mass spectra) data analysis. The structure of known compounds 70a-d, 71a-d and 72a-d were further confirmed by the comparison of their physical and spectral data with those reported in the literature.\textsuperscript{32,58a,b}
4.4. Results and Discussion

4.4.1. 5′-S-Benzoyl-3′-O-levulinyl-2′,5′-dideoxy-5′-thiothymidine (74a)

Compound 74a was synthesized by Mitsunobu reaction on 3′-O-levulinyl-2′-deoxy thymidine (72a) using thiobenzoic acid, PPh₃ and DIAD in dry THF and DMF as solvents under inert condition as white solid in 95% yield (Scheme 14). Its high resolution mass spectrum showed [M+Na]⁺ peak at m/z 483.1256 which confirmed its molecular formula to be C₂₂H₂₄N₂O₇S. The absorption peaks in the IR spectrum at 3419, 1715, 1701, 1664 cm⁻¹ were assigned to NH, and carbonyl groups in the molecule. The ¹H NMR (Figure 5) and the ¹³C NMR spectra (Figure 6) showed all the proton and carbon peaks at appropriate chemical shift values, such as C-6H, C-1'H, C-4'H, C-3'H, C-5'H, C-2'H, -COCH₃ and C-5CH₃, appeared at δ 7.28, 6.26, 5.13-5.15, 4.26-4.27, 3.53-3.54, 2.21-2.24, 2.20 and 1.82 ppm, respectively in the ¹H NMR spectrum. Similarly the characteristic carbon peaks assigned for C-5″, C-2, C-4, C-6, C-5CH₃ and sugar carbons C-1′, C-4′, C-3′, C-2′, C-5′ appeared at δ 190.28, 150.10, 163.35, 136.26, 12.36 and 84.21, 82.41, 75.76, 37.78, 30.93, ppm, respectively in the ¹³C NMR spectrum. Based on the complete spectral data (IR, ¹H NMR, ¹³C NMR spectra and HRMS) analysis, structure of the compound was unambiguously established as 5′-S-benzoyl-3′-O-levulinyl-2′,5′-dideoxy-5′-thiothymidine (74a).
Figure 5: $^1$H NMR spectrum of compound 74a (400 MHz, CDCl$_3$)

Figure 6: $^{13}$C NMR spectrum of compound 74a (100 MHz, CDCl$_3$)
4.4.2. \( N^4,5'S\)-Dibenzoyl-3'-O-levulinyl-2',5'-dideoxy-5'-thiocytidine (74b)

Compound 74b was synthesized by Mitsunobu reaction on \( N^4\)-benzoyl-3'-O-levulinyl-2'-deoxy-cytidine (72b) by using thiobenzoic acid, PPh\(_3\) and DIAD in dry THF as solvent under inert condition, as white solid in 80% yield (Scheme 14). Its high resolution mass spectrum showed [M+Na]\(^+\) peak at \( m/z \) 572.0385 which confirmed its molecular formula to be C\(_{28}\)H\(_{27}\)N\(_3\)O\(_7\)S. The absorption peaks in the IR spectrum at 3448, 1746, 1712, 1675 cm\(^{-1}\) appeared for the NH and carbonyl groups in the molecule. The \(^1\)H NMR (Figure 7) and the \(^{13}\)C NMR spectra (Figure 8) showed all the proton and carbon peaks at appropriate chemical shift values, such as NH, C-6H, C-1'H, C-4'H, C-5'H, C-2'H and -COCH\(_3\) appeared at \( \delta \) 8.67, 8.08, 6.24, 4.40-4.41, 3.53-3.55, 2.19-2.20 and 2.17 ppm, respectively in the \(^1\)H NMR spectrum. Similarly the carbon peaks assigned for C-5'', C-4, C-2, C-6 and sugar carbons C-1', C-4', C-3', C-2', C-5' appeared at \( \delta \) 190.34, 162.08, 154.51, 143.79 and 87.01, 83.42, 75.81, 38.53, 31.06 ppm, respectively in the \(^{13}\)C NMR spectrum. Based on the complete spectral data (IR, \(^1\)H NMR, \(^{13}\)C NMR spectra and HRMS) analysis, structure of the compound was unambiguously established as \( N^4,5'S\)-dibenzoyl-3'-O-levulinyl-2',5'-dideoxy-5'-thiocytidine (74b).
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Figure 7: $^1$H NMR spectrum of compound $74b$ (400 MHz, CDCl$_3$)

Figure 8: $^{13}$C NMR spectrum of compound $74b$ (100 MHz, CDCl$_3$)
4.4.3. \( N^6,5'-S\)-Dibenzoyl-3'-O-levulinyl-2',5'-dideoxy-5'-thioadenosine (74c)

Compound 74c was synthesized by mitsunobu reaction on \( N^6\)-benzoyl-3'-O-levulinyl-2'-deoxy-adenosine (72c) by using thiobenzoic acid, PPh\(_3\) and DIAD in dry THF as solvent under inert condition as white solid in 60% yield (Scheme 14). Its high resolution mass spectrum showed [M+Na]\(^+\) peak at \( m/z \) 596.6190 which confirmed its molecular formula to be \( C_{29}H_{27}N_5O_6S \). The absorption peaks in the IR spectrum at 3315, 2927, 1735, 1717, 1669, 1610, 1582 cm\(^{-1}\) appeared for the NH and carbonyl groups in the molecule. The \(^1\)H NMR (Figure 9) and the \(^{13}\)C NMR spectra (Figure 10) showed all the proton and carbon peaks at appropriate chemical shift values, such as C-8H, C-2, C-1'H, C-4'H, C-3'H, C-5'H, C-2'H and -COCH\(_3\) appeared at \( \delta \) 8.77, 8.38, 6.44, 5.38-5.39, 4.38-4.41, 3.54-3.55, 2.92-3.12 and 2.17 ppm, respectively in the \(^1\)H NMR spectrum. Similarly the carbon peaks assigned for C-5'', C-2, C-6, C-8 and sugar carbons C-1', C-4', C-3', C-2', C-5' appeared at \( \delta \) 190.64, 152.23, 151.41, 141.89 and 85.02, 83.60, 76.18, 37.83, 31.12 ppm, respectively in the \(^{13}\)C NMR spectrum. Based on the complete spectral data (IR, \(^1\)H NMR, \(^{13}\)C NMR spectra and HRMS) analysis, structure of the compound was unambiguously established as \( N^6,5'-S\)-dibenzoyl-3'-O-levulinyl-2',5'-dideoxy-5'-thioadenosine (74c).
Figure 9: $^1$H NMR spectrum of compound 74c (400 MHz, CDCl$_3$)

Figure 10: $^{13}$C NMR spectrum of compound 74c (100 MHz, CDCl$_3$)
4.4.4. \(N^2\)-Isobutyryl-5'-S-benzoyl-3'-O-levulinyl-2',5'-dideoxy-5'-thioguanosine (74d)

Compound 74d was synthesized by mitsunobu reaction on \(N^2\)-isobutyryl-3'-O-levulinyl-2'-deoxy-guanosine (72d) using thiobenzoic acid, PPh\(_3\) and DIAD in dry THF as solvent under inert condition as white solid in 50% yield (Scheme 14). Its high resolution mass spectrum showed \([M+Na]^+\) peak at \(m/z\) 578.1965 which confirmed its molecular formula to be \(C_{26}H_{29}N_5O_7S\). The absorption peaks in the IR spectrum at 3220, 2971, 1735, 1717, 1683, 1608 cm\(^{-1}\) appeared for the NH and carbonyl groups in the molecule. The \(^1\)H NMR (Figure 11) and the \(^{13}\)C NMR spectra (Figure 12) showed all the proton and carbon peaks at appropriate chemical shift values, such as NH, C-8H, C-1'H, C-4'H, C-3'H, C-5'Ha, C-5'Hb, C-2'H and -CO\(CH_3\) appeared at \(\delta\) 12.10, 7.75, 6.12, 5.35-5.36, 4.25-4.27, 3.88-3.94, 3.39-3.90, 3.14-3.26 and 2.09 ppm, respectively in the \(^1\)H NMR spectrum. Similarly the carbon peaks assigned for C-5"\(\), C-2, C-6, C-8 and sugar carbons C-1', C-4', C-3', C-2', C-5' appeared at \(\delta\) 192.14, 147.77, 155.57, 138.35 and 85.39, 82.93, 76.07, 37.70, 30.52 ppm, respectively in the \(^{13}\)C NMR spectrum. Based on the complete spectral data (IR, \(^1\)H NMR, \(^{13}\)C NMR spectra and HRMS) analysis, structure of the compound was unambiguously established as \(N^2\)-isobutyryl-5'-S-benzoyl-3'-O-levulinyl-2',5'-dideoxy-5'-thioguanosine (74d).
Figure 11: $^1$H NMR spectrum of compound 74d (400 MHz, CDCl$_3$)

Figure 12: $^{13}$C NMR spectrum of compound 74d (100 MHz, CDCl$_3$)
4.4.5. 2',5'-Dideoxy-5'-thiothymidine disulfide (75a)

Compound 75a was synthesized by debenzoylation of 5'-S-benzoyl-3'-O-levulinyl-2',5'-dideoxy-5'-thiothymidine (74a) using saturated methanolic ammonia at RT as white solid in 90% yield (Scheme 14). Its high resolution mass spectrum showed [M+Na]⁺ peak at m/z 537.1484 which confirmed its molecular formula to be C₂₀H₂₆N₄O₈S₂. The absorption peaks in the IR spectrum at 3419 cm⁻¹ and 1701, 1664 cm⁻¹ appeared for the OH, NH and carbonyl groups in the molecule. The ¹H NMR (Figure 13) and the ¹³C NMR spectra (Figure 14) showed all the proton and carbon peaks at appropriate chemical shift values, such as NH, C-6H, C-1'H, C-4'H, C-3'H, C-5'H, C-2'H and C-5CH₃ appeared at δ 11.32, 7.46, 6.19, 4.20-4.21, 3.94-3.97, 3.03-3.09, 2.20-2.22 and 1.79 ppm, respectively in the ¹H NMR spectrum. Similarly the carbon peaks assigned for C-4, C-2, C-6, C-5CH₃, and in sugar C-1', C-4', C-3', C-2', C-5' appeared at δ 163.67, 150.46, 136.01, 12.14 and 84.50, 84.01, 72.46, 40.12, 38.95 ppm, respectively in the ¹³C NMR spectrum. Based on the complete spectral data (IR, ¹H NMR, ¹³C NMR spectra and HRMS) analysis, structure of the compound was unambiguously established as 2',5'-dideoxy-5'-thiothymidine disulfide (75a).
Figure 13: $^1$H NMR spectrum of compound 75a (400 MHz, DMSO-$d_6$)

Figure 14: $^{13}$C NMR spectrum of compound 75a (100 MHz, DMSO-$d_6$)
4.4.6. 2',5'-Dideoxy-5'-thiocytidine disulfide (75b)

Compound 75b was synthesized by debenzoylation of N4,5'-S-dibenzoyl-3'-O-levulinyl-2',5'-dideoxy-5'-thiocytidine (74b) using saturated methanolic ammonia at RT as white solid in 89% yield (Scheme 14). Its high resolution mass spectrum showed [M+Na]+ peak at m/z 507.1602 which confirmed its molecular formula to be C18H24N6O6S2. The absorption peaks in the IR spectrum at 3402, 1654 cm⁻¹ appeared for the OH, NH₂, and carbonyl groups in the molecule. The ¹H NMR (Figure 15) and the ¹³C NMR spectra (Figure 16) showed all the proton and carbon peaks at appropriate chemical shift values, such as NH, C-6H, C-5H, C-1'H, C-3'OH, C-4'H and C-5'H appeared at δ 7.19, 7.56, 5.75, 6.18, 5.42, 4.16-4.17 and 2.95-3.04 ppm, respectively in the ¹H NMR spectrum. Similarly the carbon peaks assigned for C-4, C-2, C-6, C-5 and sugar carbons C-1', C-4', C-3', C-2', C-5' appeared at δ 165.55, 155.10, 140.97, 94.39 and 85.17, 84.52, 72.64, 41.07, 36.07 ppm, respectively in the ¹³C NMR spectrum. Based on the complete spectral data (IR, ¹H NMR, ¹³C NMR spectra and HRMS) analysis, structure of the compound was unambiguously established as 2',5'-dideoxy-5'-thiocytidine disulfide (75b).
Figure 15: $^1$H NMR spectrum of compound 75b (400 MHz, DMSO-$d_6$)

Figure 16: $^{13}$C NMR spectrum of compound 75b (100 MHz, DMSO-$d_6$)
4.4.7. 2′,5′-Dideoxy-5′-thioadenosine disulfide (75c)

Compound 75c was synthesized by debenzoylation of N^6,5′-S-dibenzoyl-3′-O-levulinyl-2′,5′-dideoxy-5′-thioadenosine (74c) using saturated methanolic ammonia at RT as white solid in 87% yield (Scheme 14). Its high resolution mass spectrum showed [M+Na]^+ peak at m/z 555.1389 which confirmed its molecular formula to be C_{20}H_{24}N_{10}O_{4}S_{2}. The absorption peaks in the IR spectrum at 3220, 2971 cm^{-1} appeared for the OH, NH₂ groups in the molecule. The \(^{1}H\) NMR (Figure 17) and the \(^{13}C\) NMR spectra (Figure 18) showed all the protons and carbon peaks at appropriate chemical shift values, such as C-8H, C-2, C-1′H, C-4′H, C-3′H, C-5′H and C-2′H, appeared at δ 8.30, 8.13, 6.34, 4.38-4.39, 4.02-4.06, 2.90-3.16 and 2.26-2.28 ppm, respectively in the \(^{1}H\) NMR spectrum. Similarly the carbon peaks assigned for C-6, C-2, C-4, C-8 and sugar carbons C-1′, C-4′, C-3′, C-2′, C-5′ appeared at δ 156.07, 152.59, 149.13, 139.73 and 85.17, 83.65, 72.90, 41.11, 37.96 ppm, respectively in the \(^{13}C\) NMR spectrum. Based on the complete spectral data (IR, \(^{1}H\) NMR, \(^{13}C\) NMR spectra and HRMS) analysis, structure of the compound was unambiguously established as 2′,5′-dideoxy-5′-thioadenosine disulfide (75c).
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Figure 17: $^1$H NMR spectrum of compound 75c (400 MHz, DMSO-$d_6$)

Figure 18: $^{13}$C NMR spectrum of compound 75c (100 MHz, DMSO-$d_6$)
4.4.8. 2′,5′-Dideoxy-5′-thioguanosine disulfide (75d)

Compound 75d was synthesized by debenzylation of N²-isobutyryl-5′-S-benzoyl-3′-O-levulinyl-2′,5′-dideoxy-5′-thioguanosine (74d) using saturated methanolic ammonia at RT as white solid in 85% yield (Scheme 14). Its high resolution mass spectrum showed [M+Na]⁺ peak at m/z 587.1205 which confirmed its molecular formula to be C₂₀H₂₄N₁₀O₆S₂. The absorption peaks in the IR spectrum at 3412, 2256, 2129 cm⁻¹ appeared for the OH and NH₂ groups in the molecule. The ¹H NMR (Figure 19) and the ¹³C NMR spectra (Figure 20) showed all the proton and carbon peaks at appropriate chemical shift values, such as C-8H, C-1'H, C-4'H, C-3'H, C-5'H and C-2'Ha & C-2''Hb appeared at δ 7.87, 6.12, 4.29-4.30, 3.98-4.01, 2.96-3.03 and 2.63-2.67 & 2.17-2.22 ppm, respectively in the ¹H NMR spectrum. Similarly the carbon peaks assigned for C-6, C-2, C-4, C-8, and sugar carbons C-1', C-4', C-3', C-2', C-5' appeared at δ 156.92, 153.66, 151.05, 135.54 and 87.64, 85.04, 72.78, 40.18, 38.15 ppm, respectively in the ¹³C NMR spectrum. Based on the complete spectral data (IR, ¹H NMR, ¹³C NMR spectra and HRMS) analysis, structure of the compound was unambiguously established as 2′,5′-dideoxy-5′-thioguanosine disulfide (75d).
Figure 19: $^1$H NMR spectrum of compound 75d (400 MHz, DMSO-$d_6$)

Figure 20: $^{13}$C NMR spectrum of compound 75d (100 MHz, DMSO-$d_6$)
4.5. Conclusion

In conclusion, we have synthesized novel 5′-S-benzoyl-3′-O-levulinyl-nucleosides 74a-d and their disulfides 75a-d (T, C, A and G) in moderate to good yields. Disulfides are stable and these disulfides can be cleaved to free thiol. The free 5′-thiols can be further converted into the useful monomeric building blocks (5′-S-DMT-monomers) which are useful for synthesis of oligonucleotides.

4.6. Experimental results

4.6.1. General

Reactions were conducted under an atmosphere of nitrogen when anhydrous solvents were used. Column chromatography was carried out using silica gel (100-200 mesh) for purification of compounds. Melting points are recorded by using BUCHI melting point apparatus M-560. Analytical TLCs were performed on pre-coated Merck silica gel 60F254 plates; the spots were detected either using UV light or by charring with 4% sulfuric acid in alcoholic solution. The IR spectra were recorded on a Perkin-Elmer model 2000 FT-IR spectrophotometer. The 1H and 13C NMR spectra were recorded on JEOL Delta spectrometer operating at 400 MHz and 100 MHz, respectively. The chemical shift values are reported as δ ppm relative to TMS used as internal standard and the coupling constants (J) are measured in Hz. The FAB-HRMS spectra of all the compounds were recorded on a JEOL JMS-AX505W high-resolution mass spectrometer in positive ion mode using the matrix HEDS (bishydroxyethylsulphide) doped with sodium acetate. And microTOF-Q instrument from Bruker Daltroics, Bremen and were run in ESI positive mode.

4.6.2. Materials

Chemicals were obtained from commercial suppliers and were used without any further purification unless otherwise noted. Organic solvents THF, chloroform, methanol, petroleum ether and ethyl acetate were distilled over Na wire, CaCl2, CaO, P2O5 and K2CO3, respectively.
4.6.3. General procedure for synthesis of 5′-S-benzoyl-3′-O-levulinyl-2′,5′-dideoxy-5′-thionucleosides (74a-d)

Triphenyl phosphine (2 eqv., 1.17 mmol) was dissolved in 5 ml THF and cooled to 0 °C and then added to DIAD (2 eqv., 1.17 mmol). The solution was stirred for 30 min. at 0 °C and then for another 30 min. at RT. This solution was then added to a solution of 3′-O-levulinyl-2′-deoxy-thymidine (73a-d, 1 eqv., 0.58 mmol) and thiobenzoic acid (1.5 eqv., 0.87 mmol) dissolved in THF:DMF (1:1v/v) at 0 °C and the solution was stirred for 2hrs at 0 °C and 2hrs at RT. After completion of the reaction as indicated by TLC examination (50% Ethyl acetate in Hexane), the solvent was removed under reduced pressure. Water was added to the reaction mixture and was extracted with DCM. Organic phase was dried over Na₂SO₄, solvent was removed under reduced pressure and the obtained crude products were purified by flash column chromatography over silica gel (100-200 mesh) by eluting with 1:1 ethyl acetate hexane followed by 1% methanol in chloroform to afford pure product 74a-d in 50-95% yield.

4.6.3.1. 5′-S-Benzoyl-3′-O-levulinyl-2′,5′-dideoxy-5′-thiothymidine (74a)

It was obtained as white solid (0.642 g) in 95% yield; Rₓ = 0.45(1% methanol in chloroform, v/v); m.p. 154-155 °C; IR (cm⁻¹, KBr) νmax: 3419, 1715, 1701, 1664, 1265; ¹H NMR (CDCl₃, 400 MHz): δ 8.68 (1H, s, NH), 7.98-8.00 (2H, m, ArH), 7.60-7.65 (1H, m, ArH), 7.49-7.46 (2H, m, ArH), 7.28 (1H, s, C-6H), 6.26 (4H, t, J = 4.4 Hz, C-1'H), 5.13-5.15 (1H, m, C-4'H), 4.26-4.27 (1H, m, C-3'H), 3.53-3.54 (2H, m, C-5'H), 2.77-2.80 (2H, m, -CH₂-CO-), 2.59-2.62 (2H, m, -O-CO-CH₂-), 2.21-2.24 (2H, m, C-2'H); 2.20 (3H, s, -CO-C₃H₃); 1.82 (3H, s, C-5CH₃); ¹³C NMR (CDCl₃, 100 MHz): δ 206.32 (-CO-CH₃), 190.28 (C-5'S-CO-Ph), 172.26 (-O-CO-CH₂-), 163.35 (C-4), 150.10 (C-2), 136.26 (C-6), 134.94 (ArC), 133.98 (p-ArC), 128.80 (m-ArC), 127.39 (o-ArC), 111.51 (C-5), 84.21 (C-1'), 82.41 (C-4'), 75.76 (C-3'), 37.78 (C-2'), 37.05 (-CH₂-CO-), 30.93 (C-5'), 29.75 (-CO-CH₃), 27.84 (-O-CO-CH₂-), 12.36 (C-5-CH₃); HR-ESI-TOF-MS: m/z 483.1256 ([M+Na]⁺), calcd. for [C₂₂H₂₄N₂O₇S+Na]⁺ 483.1304.
4.6.3.2. \( N^4,5'\)-S-Dibenzoyl-3'-O-levulinyl-2',5'-dideoxy-5'-thioctydine (74b)

It was obtained as white solid (0.19 g) in 80% yield; \( R_f = 0.42 \) (1% methanol in chloroform, v/v); m.p.133-134 °C; IR (cm\(^{-1}\), KBr) \( v_{\text{max}} \): 3448, 3187, 1746, 1712, 1675, 1478, 1299, 1080 and 912; \(^1\)H NMR (CDCl\(_3\), 400 MHz): \( \delta \) 8.67 (1H, s, NH), 8.08 (1H, d, \( J = 7.32 \) Hz, C-6H), 8.00-7.98 (2H, m, ArH), 7.87 (2H, d, \( J = 5.12 \) Hz, C-1'H), 6.24 (1H, t, \( J = 5.12 \) Hz, C-1'H), 5.16 (1H, d, \( J = 6.81 \) Hz, C-5H), 4.40-4.41 (1H, m, C-3'H), 3.53-3.55 (2H, m, C-5'H), 2.77-2.78 (2H, m, -CH\(_2\)-CO), 2.60-2.61 (2H, m, -CO-C\(_\text{H}_2\)-), 2.19-2.20 (2H, m, C-2'H), 2.17 (3H, s, -CO-C\(_\text{H}_3\)); \(^{13}\)C NMR (CDCl\(_3\), 100 MHz): \( \delta \) 206.24 (-CO-CH\(_3\)), 190.34 (C-5''S-CO-Ph), 172.12 (-O-CO-CH\(_2\)-), 166.22 (-N-CO-Ph), 162.08 (C-4), 154.51 (C-2), 143.79 (C-6), 136.27 (ArC), 133.89 (p-ArC), 133.17 (ArC), 128.99 (m-ArC), 128.77 (m-ArC), 127.52 (o-ArC), 127.39 (o-ArC), 96.66 (C-5), 87.01 (C-1'), 83.42 (C-4'), 75.81 (C-3'), 38.53 (C-2'), 37.35 (-CH\(_2\)-CO-), 31.06 (C-5'), 29.73 (-CO-CH\(_3\)), 27.83 (-OCO-CH\(_2\)-); HR-ESI-TOF-MS: \( m/z \) 572.0385 (\([\text{M}+\text{Na}]^+\)), calcd. for \([\text{C}_{28}\text{H}_{27}\text{N}_3\text{O}_7\text{S}+\text{Na}]^+\) 572.1030.

4.6.3.3. \( N^6,5'\)-S-Dibenzoyl-3'-O-levulinyl-2',5'-dideoxy-5'-thioadenosine (74c)

It was obtained as white solid (0.146 g) in 60% yield; \( R_f = 0.54 \) (1% methanol in chloroform, v/v); m.p. 153-154 °C; IR (cm\(^{-1}\), KBr) \( v_{\text{max}} \): 3315, 2927, 1735, 1717, 1669, 1610, 1582, 1457, 1250, 1208, 1156, 1074, 911 and 689; \(^1\)H NMR (CDCl\(_3\), 400 MHz): \( \delta \) 8.77 (1H, s, C-8H), 8.38 (1H, s, C-2H), 7.90-8.02 (4H, m, ArH), 6.44 (4H, t, \( J = 5.88 \) Hz, C-1'H), 5.38-5.39 (1H, m, C-4'H), 4.38-4.41 (1H, m, C-3'H), 3.54-3.55 (2H, m, C-5'H), 2.92-3.12 (2H, m, C-2'H), 2.79-2.85 (2H, m, -CH\(_2\)-CO-), 2.59-2.77 (2H, m, -O-CO-CH\(_2\)-); 2.17 (3H, s, -CO-C\(_\text{H}_3\)); \(^{13}\)C NMR (CDCl\(_3\), 100 MHz): \( \delta \) 206.45 (-CO-CH\(_3\)), 190.64 (C-5''S-CO-Ph), 172.09 (-OCO-CH\(_2\)-), 164.99 (-N-CO-Ph), 152.23 (C-2), 151.41 (C-6), 149.36 (C-4), 141.89 (C-8), 136.33 (ArC), 133.74 (ArC), 133.23 (ArC), 132.89 (ArC), 128.78 (ArC), 128.68 (ArC), 128.06 (ArC), 128.04 (ArC), 127.36 (ArC), 122.89 (C-5), 85.02 (C-1'), 83.60 (C-4'), 76.18 (C-3'), 37.83 (C-2'), 36.92 (-CH\(_2\)-CO-), 31.12 (C-5'), 29.76 (-CO-CH\(_3\)), 27.87 (-OCO-CH\(_2\)-); HR-ESI-TOF-MS: \( m/z \) 596.6190 (\([\text{M}+\text{Na}]^+\)), calcd. for \([\text{C}_{29}\text{H}_{27}\text{N}_3\text{O}_7\text{S}+\text{Na}]^+\) 596.6196.
4.6.3.4. N²-Isobutyryl-5'-S-benzoyl-3'-O-levulinyl-2',5'-dideoxy-5'-thioguanosine (74d)

It was obtained as white solid (0.12 g) in 50% yield; R_f = 0.5 (1% methanol in chloroform, v/v); m.p. 142-143 °C; IR (cm⁻¹, KBr) ν_max: 3220, 2971, 1735, 1717, 1683, 1608, 1560, 1363, 1207, 1156, 1026, 911 and 689; ¹H NMR (CDCl₃, 400 MHz): δ 12.10 (1H, brs, NH), 10.10 (1H, brs, NH), 7.84 (2H, d, J = 7.99 Hz, Ar H), 7.75 (1H, s, C-8H), 7.53 (1H, t, J = 7.67 Hz, Ar H), 7.38 (2H, m, C-4'H), 4.25-4.27 (m, 1H, C-3'H), 3.88-3.90 (m, 1H, C-5'H), 3.39-3.40 (m, 1H, C-5'H), 3.14-3.26 (1H, d, J = 5.87 Hz, C-2'H), 2.76 (1H, m, -CH-(CH₃)₂), 2.67-2.68 (2H, m, -CH₂-CO-), 2.40-2.48 (2H, m, -O-CO-CH₂-), 2.09 (3H, s, -CO-CH₃), 1.19 (3H, d, J = 7.32 Hz, -CH₃); ¹³C NMR (CDCl₃, 100 MHz): δ 206.53 (-CO-CH₃), 192.14 (-CO-Ph), 179.29 (-CO-CH₂-C), 171.99 (-CO-CH₂-C), 155.57 (C-6), 147.77 (C-2), 147.66 (C-4), 138.35 (C-8), 136.19 (Ar C), 133.93 (Ar C), 128.69 (Ar C), 127.14 (Ar C), 122.07 (C-5), 85.39 (C-1'), 82.93 (C-4'), 76.07 (C-3'), 37.70 (C-2'), 36.03 (-CH₁-CO-), 35.74 (-CH(CH₃)₂), 30.52 (C-5'), 29.64 (-CH₃), 27.76 (-O-CO-CH₂-), 18.99 (-CH₃), 18.72 (-CH₃); HR-ESI-TOF-MS: m/z 578.1965 ([M+Na]⁺), calcd. for [C₂₆H₂₉N₅O₇S+Na]⁺ 578.1788.

4.6.4. General procedure for synthesis of 2',5'-Dideoxy-5'-thionucleoside disulfide (75a-d)

5'-S-Benzoyl-3'-O-levulinyl-2',5'-dideoxy-nucleosides 74a-d (0.19 mmol.) was dissolved in saturated methanolic ammonia (5ml) and the solution was stirred for 6-12hrs at room temperature. After completion of the reaction as indicated by TLC examination (50% Methanol in chloroform), methanol was removed under reduced pressure and crude product thus obtained was washed with 1:1 water -methanol (5-10ml) on suction pump to afford the products 75a-d as white solid in an overall 85-90% yield.

4.6.4.1. 2',5'-Dideoxy-5'-thiothyomidine disulfide (75a).

It was obtained as white solid (0.9 gr.) in 90% yield; R_f = 0.45 (50% Methanol in chloroform, v/v); m.p. 255-256 °C; IR (cm⁻¹, KBr) ν_max: 3419, 1715, 1701, 1664, 1265, 1037 and 960; ¹H NMR (DMSO-d₆, 400 MHz): δ 11.32 (1H, s, NH), 7.46 (1H,
m, C-6H), 6.19 (1H, t, J = 4.4 Hz, C-1'H), 5.42-5.43 (1H, m, C-3'OH), 4.20-4.21 (1H, m, C-4'H), 3.94-3.95 (1H, m, C-3'H), 3.03-3.09 (2H, m, C-5'H), 2.20-2.22 (1H, m, C-2'Hα), 2.08-2.10 (1H, m, C-2'Hβ), 1.79 (3H, s, -C3H3);
\[ ^{13}C \text{ NMR (CDCl}_3, 100 \text{ MHz): } \delta 163.67 (\text{C-4}), 150.46 (\text{C-2}), 136.01 (\text{C-6}), 109.77 (\text{C-5}), 84.50 (\text{C-1'}), 84.01 (\text{C-4'}), 72.46 (\text{C-3'}), 40.12 (\text{C-2'}), 38.95 (\text{C-5'}), 12.14 (-\text{C3H3}); \]
HR-ESI-TOF-MS: m/z 537.1484 ([M+Na]+), calcd. for [C20H26N4O8S2+Na]"}^{+} 537.1192.

4.6.4.2. 2',5'-Dideoxy-5'-thiocytidine disulfide (75b)

It was obtained as white solid (0.8 g) in 89% yield; Rf = 0.57 (50% methanol in chloroform, v/v); m.p. 222-223 °C; IR (cm\(^{-1}\), KBr) \( \nu_{\text{max}} \): 3402, 2256, 2129, 1654, 1025, 1003, 826 and 765; \(^1\)H NMR (DMSO-\(d_6\), 400 MHz): \( \delta \) 7.56 (2H, d, \( \text{J} = 7.32 \text{ Hz, C-6H} \)), 7.19 (2H, brs, NH), 6.18 (1H, t, \( \text{J} = 6.95 \text{ Hz, C-1'H} \)), 5.75 (1H, d, \( \text{J} = 7.32 \text{ Hz, C-5H} \)), 5.42 (1H, brs, C-3'OH), 4.16-4.17 (1H, m, C-4'H), 3.95-3.96 (1H, m, C-3'H), 2.95-3.04 (2H, m, C-5'H), 1.99-2.11 (2H, m, C-2'H); \( ^{13}C \text{ NMR (DMSO-}d_6, 100 \text{ MHz): } \delta 165.55 (\text{C-4}), 155.10 (\text{C-2}), 140.97 (\text{C-6}), 94.39 (\text{C-5}), 85.17 (\text{C-1'}), 84.52 (\text{C-4'}), 72.64 (\text{C-3'}), 41.07 (\text{C-2'}), 36.07 (\text{C-5'}); \) HR-ESI-TOF-MS: m/z 507.1602 ([M+Na]+), calcd. for [C18H24N6O6S2+Na]+ 507.1199.

4.6.4.3. 2',5'-Dideoxy-5'-thioadenosine disulfide (75c)

It was obtained as white solid (0.84 g) in 87% yield; Rf = 0.64 (50% methanol in chloroform); m.p. 210-211 °C; IR (cm\(^{-1}\), KBr) \( \nu_{\text{max}} \): 3220, 2971, 1608, 1560, 1363, 1207, 1156, 1026, 911, 689; \(^1\)H NMR (DMSO-\(d_6\), 400 MHz): \( \delta \) 8.30 (1H, s, C-8H), 8.13 (1H, s, C-2H ), 6.34 (1H, t, \( \text{J} = 6.59 \text{ Hz, C-1'} \) ), 5.49 (1H, d, \( \text{J} = 3.68 \text{ Hz, C-3'OH} \) ), 4.38-4.39 (1H, m, C-4'H), 4.02-4.06 (1H, m, C-3'H), 2.90-3.16 (2H, m, C-5'H), 2.26-2.28 (2H, m, C-2'H); \( ^{13}C \text{ NMR (DMSO-}d_6, 100 \text{ MHz): } \delta 156.07 (\text{C-6}), 152.59 (\text{C-2}), 149.13 (\text{C-4}), 139.73 (\text{C-8}), 128.92 (\text{C-5}), 85.17 (\text{C-1'}), 83.65 (\text{C-4'}), 72.90 (\text{C-3'}), 41.11 (\text{C-2'}), 37.96 (\text{C-5'}); \) HR-ESI-TOF-MS: m/z 555.1389 ([M+Na]+), calcd. for [C20H24N10O4S2+Na]+ 555.1423.
4.6.4.4. 2′,5′-Dideoxy-5′-thioguanosine disulfide (75d)

It was obtained as white solid (0.9 g) in 85% yield; m.p. 204-203 °C; R_f = 0.35 (50% methanol in chloroform, v/v); IR (cm⁻¹, KBr) v_max: 3412, 2256, 2129, 1655, 1368, 1217, 1026, 1004, 826 and 764; ¹H NMR (DMSO-d₆, 400 MHz): δ 7.87 (1H, s, C-8H), 6.50 (2H, brs, NH₂), 6.12 (1H, t, J = 5.83 Hz, C-1'H), 5.43 (1H, brs, C-3'OH), 4.29-4.30 (1H, m, C-4'H), 3.98-4.01 (1H, m, C-3'H), 2.96-3.03 (2H, m, C-5'H), 2.63-2.67 (1H, m, C-2'Ha), 2.17-2.22 (1H, m, C-2'Hb); ¹³C NMR (DMSO-d₆, 100 MHz): δ 156.92 (C-6), 153.66 (C-2), 151.05 (C-4), 135.54 (C-8), 116.81 (C-5), 87.64 (C-1'), 85.04 (C-4'), 72.78 (C-3'), 40.18 (C-2'), 38.15 (C-5'); HR-ESI-TOF-MS: m/z: 587.1205 ([M+Na]+), calcd. for [C₂₀H₂₄N₁₀O₆S₂+Na]+ 587.1322.
4.7. References


