Synopsis of the Thesis

Design, Synthesis and Applications of Novel Thiosugars & Amino acid Derivatives

Glycosidases are carbohydrate processing essential enzymes necessary for the growth and development of all organisms such as intestinal digestion, post-translational processing of glycoproteins and the lysosomal catabolism of glycoconjugates. The function of these glycosidases is limited and studies are still in progress to understand their function at cellular level. In recent years, biological role of carbohydrates has resulted in various carbohydrate-based therapeutics. These carbohydrates serve as a tool to study the function of glycosidases by inhibiting their active site. The concept of inhibition is yet another approach for the discovery of drugs.

Glycosidase inhibitors studied are often sugar analogs and a wide range of such inhibitors are reported in the literature. Thiosugars, in particular, have gained new perspectives owing to their electronic, geometric, conformational and flexibility differences, as sulfide moiety being less electronegative and more polarizable than the oxygen counter-part. These differences make the thiosugars distinct from their oxygen analogs and hence can mimic the active site of the enzyme. Many molecules are reported to be promising glycosidase inhibitors but are not easily accessible due to difficulties in their synthesis. Hence, the chemical synthesis of thio-analogs of carbohydrates, by synthetic routes, remains a major challenge. To address the complexity of synthesis and to make available new strategies, we envisioned the use of benzyltriethylammonium tetrathiomolybdate \([\text{BnEt}_3\text{N}]_2\text{MoS}_4\), a versatile and efficient sulfur transfer reagent.
Objectives of the study:

a. Design novel thiosugars as glycosidase inhibitors.
b. Devise strategy for the synthesis of novel thiosugars through a simple, practical approach.
c. Evaluate the synthesized molecules as glycosidase and HIV-1 protease inhibitors, *in silico*.
d. Study miscellaneous applications of the novel thiosugar-derived thialactones.

The thesis is divided into five sections:

Section A entitled “Synthesis of deoxythiosugars and thiosugar-based lactones” is divided into two parts, Part A and Part B.

Part A – “An introduction and background on thiosugars and sulfur transfer reagents” has been provided. A brief discussion of sulfur transfer reagents in carbohydrate synthesis and earlier work related to the use of benzyltriethylammonium tetrathiomolybdate, [BnEt$_3$N]$_2$MoS$_4$, as an efficient sulfur transfer reagent have been provided.

Part B – “Design of inhibitors of glycosidases and HIV-1 protease” deals with the design of inhibitors of glycosidase and HIV-1 protease. The designed thiosugar molecules exhibit the characteristics of sugars and will act as planar molecules to mimic the active site conformation of a good inhibitor. Synthetic methodologies devised and adopted for the synthesis of constrained sugar-derived thialactones include: (a) Double displacement, (b) Displacement-*cum*-intramolecular thia-Michael addition, (c) Epoxide ring-opening-*cum*-intramolecular thia-Michael addition, and (d) Displacement-*cum*-epoxide ring opening in an intramolecular fashion. In all the above mentioned strategies, sulfur transfer step is the crucial step which was achieved by the use of benzyltriethylammonium tetrathiomolybdate [BnEt$_3$N]$_2$MoS$_4$ as the key reagent.

(a) Various constrained thialactones synthesized by double displacement strategy using tetrathiomolybdate as the sulfur transfer reagent are shown in Scheme – 1.

(b) A number of constrained thialactones were synthesized following nucleophilic displacement-*cum*-intramolecular thia-Michael addition strategy as shown in Scheme – 2.

(c) Synthesis of bicyclic thiolactones was achieved using the strategy of epoxide ring-opening-*cum*-intramolecular thia-Michael addition. (Scheme – 3)

(d) A few bicyclic thialactones were synthesized through displacement- epoxide ring opening-cyclization as shown in Scheme – 4.
Scheme 1

1-Deoxy-5-thio-D-glucopyran-3,6-lactone
1-Deoxy-4-thio-arabino-1,4-lactone
1-Deoxy-5-thio-D-mannopyran-3,6-lactone
1-Deoxy-4-thio-D-lyxono-2,5-lactone
1-Deoxy-5-thio-D-glucopyran-2,6-lactone
1-Deoxy-5-thio-D-galactopyran-2,6-lactone
1-Deoxy-5-thio-D-talopyran-3,6-lactone
1-Deoxy-D-glycer-D-galacto-[(2,3)-(4,5)]-bis-isopropylidene-6-methyl carboxylate thioseptanose
Thiosugar carboxylate

Scheme 2

Scheme 3

1-Deoxy-4-thio-L-ribo-3,5-lactone
1-Deoxy-5-thio-D-gulono-4,6-lactone

Scheme 4
The methodology was also utilized for the synthesis of thiosugar derivatives and azido-thialactones. (Fig. 1)

**Figure 1**

![Chemical structures](image)

**Synthesis of deoxythiosugars:** The bicyclic thialactones (designed as inhibitors) on reduction with borohydride exchange resin (BER) easily furnished the deoxythiosugars (Fig. 2). It is worth mentioning that the synthesis of these thiosugars as reported in the literature involved lengthy procedures whereas the present methodology turns out to be short and concise.

**Figure 2**

![Chemical structures](image)

**Section B** entitled “Synthesis of amines, β-amino acids and novel thiosugar-based dehydroamino acids” comprises a brief introduction on the importance of amines, β-amino acids and dehydroamino acids. In this section the effective utilization of benzyltriethylammonium tetrathiomolybdate as a key reagent for reductive transformations and its application in the synthesis of amines, β-amino acids and dehydroamino acids have been presented.
A one pot reduction of azides to amines followed by intermolecular aza-Michael addition employing tetrathiomolybdate was achieved to furnish a number of different $\beta$-amino esters as shown in Scheme - 4:

**Scheme 4**

![Scheme 4](image)

The study was further extended to the reduction of a few anomeric azides to afford the corresponding anomeric amines and derivatives. (Fig. 3)

**Figure 3**

A one-pot thia-Michael addition-vinyl azide reduction in a tandem fashion employing benzyltriethylammonium tetrathiomolybdate was studied and was shown to be effective for the synthesis of thiosugar derived dehydroamino acid derivatives. (Scheme – 5)

**Scheme 5**

![Scheme 5](image)

**Section C** entitled “Molecular docking studies of deoxythiosugar probes” gives an overview of different glycosidases, HIV-1 protease and their inhibitors. This section also deals with a brief introduction on active site conformations of potent inhibitors. In this connection we have studied the crystal conformations of the synthesized molecules whose conformations
were the same as that of the existing inhibitors in the active site. (Fig. 4) With this background in silico study of the synthesized deoxythiosugar probes was conducted on human glycosidases: \(\alpha\)-mannosidase, \(\alpha\)-galactosidase, \(\beta\)-glucosidase and HIV-1 protease, respectively.

**Figure 4**

Molecular docking was carried out using Autodock suite, molecular modeling simulation. Separate docking procedures were employed for the four different receptors. The PDBs representing the four enzyme targets were 2V3D, 3H53, 1X9D and 3I8W for \(\beta\)-glucosidase, \(\alpha\)-galactosidase, \(\alpha\)-mannosidase and HIV-1 protease respectively.

The control compounds used for \(\alpha\)-mannosidase were mannostatin and kifunensine. NMB, THK, and BED were the positive controls for HIV-1 protease. Similarly, NBV and cyclophellitol were the controls used for \(\beta\)-glucosidase and NOJ, \(N\)-methyl calystegine \(B_2\) for \(\alpha\)-galactosidase. (Fig. 5) Ligands TGSB68 and TGSB482 had the energy value of \(-6.49\) kcal/mol comparable to that of the average reference value of the positive control, and thus, the potent candidate as identified by molecular docking to HIV-1 protease. (Fig. 6a) The control compounds used for \(\alpha\)-mannosidase were mannostatin and kifunensine, which bind
with mean binding energy of -9.11 and -5.56. In the case of $\alpha$–mannosidase, the same compounds TGSB68 and TGSB482 were selected due to comparable energy and a good cluster size with that of positive control. (Fig. 6b) For $\beta$–glucosidase, ligands TGSC108 and TGSC236, which had comparable values to that of positive control was identified as the

**Figure 5**

![Figure 5](image)

**Figure 6**

![Figure 6](image)
potent candidate. (Fig. 6c) In the case of \( \alpha \)-galactosidase, again the ligands TGSB68 and TGSB482 were selected based on binding energies. (Fig. 6d)

In conclusion, the concept analogy (deoxy nature, planarity, thiosugar framework, lactone moiety) for the design of inhibitors indeed worked positively. The results are really encouraging. An \textit{in vivo} study of the synthesized novel thiosugar probes will certainly provide a potent inhibitor.

Section D entitled “Research methodology” provides experimental procedures adopted with details of synthesis.

Section E entitled “Bibliography” provides the references cited in this work.

References:


List of Publications:


4. "An expeditious synthesis of deoxythiosugars with Furano- and Pyrano- motif from L-(+)-Gulono-1,4-lactone" T. Gunasundari and S. Chandrasekaran. (To be submitted)

5. "Thiosugars- A perspective" T. Gunasundari and S. Chandrasekaran. (Review article)


8. "One-pot synthesis of $\beta$-amino acids through tandem aza-Michael addition" T. Gunasundari and S. Chandrasekaran. (Article)


**Work presented (recent):**

- **JCF-Frühjahrssymposium Essen, GDCh-JCF 2009** in Essen, on 11-14th Mar-2009 and delivered an oral presentation entitled “*Deoxythio-sugar carboxylic acid derivatives: Synthesis and biological significance*” and was awarded a cash prize of 800 €.

- Fourth Junior-National Symposium organized by National Organic Symposium Trust (NOST) in Madurai, on 6-9th Dec-2008 and presented poster entitled “*TOWARDS THE SYNTHESIS OF THIO-SUGAR BASED AMINO ACIDS*”.

- Tenth National Symposium in Chemistry, NSC-10 organized by Chemical Research Society of India (CRSI) in Bangalore, on 1-3rd Feb-2008 and presented poster entitled “*SYNTHETIC STUDIES ON THIO-SUGAR BASED AMINO ACIDS*”. 

ix