CHAPTER II

An Asymmetric Synthesis of Clopidogrel Hydrogen Sulfate
2.1 Introduction

Acute coronary syndromes (ACS) represent a life-threatening symptom of atherosclerosis. It is usually precipitated by acute thrombosis, induced by a ruptured or eroded atherosclerotic plaque, with or without concomitant vasoconstriction, causing a critical reduction in coronary blood flow.\(^1\)

The plaque prone to instability are those with a large lipidic core, a high concentration of inflammatory cells, a low density of smooth muscle cells, and a thin fibrous cap.\(^2\) Thrombosis plays a key role in the development of ACS. This has been widely demonstrated by autopic data and angiographic detections of thrombi at the site of the culprit lesion.\(^3\)-\(^5\) In both plaque rupture and erosion, the lipid-rich core exposed is highly thrombogenic and has a high concentration of the tissue factor.\(^6\) In the case of erosion, the thrombus adheres to the surface of the plaque, whereas when rupture occurs, the thrombus involves the deeper layers down to the lipid core. As a consequence, the plaque has a rapid growth and progression, if the thrombus is not accommodated by a positive remodeling, thus leads to a sudden change in the severity of the stenosis.\(^1\)

The thrombus is fibrin-rich and totally occlusive in patients with ST elevation myocardial infarction (STEMI), whereas it is platelet-rich and partially or intermittent occlusive in patients with non-ST elevation myocardial infarction (NSTEMI).\(^1\) Thus, platelet activation and aggregation which are crucial in the transformation of a stable atherosclerotic plaque to an unstable lesion, play a key role in the setting of ACS.

Antiplatelet therapy is essential in the treatment of patients with ACS and aspirin still represents a cornerstone in the modern therapy of those patients.\(^7\) Because of the unique role of platelets in coronary thrombosis, the need for inhibition of other platelet activation pathways has led to the development of various other antiplatelet drugs, such as clopidogrel.\(^8\) Clopidogrel in combination with aspirin is the current “gold standard” for reducing cardiovascular events in such patients.\(^9\)
2.1.1 P2Y₁₂ Receptor antagonists

Adenosine-5'-diphosphate (ADP) plays a key role in platelet function, although ADP itself is a weak platelet agonist, when secreted from the platelet dense granules where it is stored, it amplifies the platelet responses induced by other platelet agonists. The transduction of the ADP signal involves both transient rise in free cytoplasmic calcium mediated by the G_q-coupled P2Y₁ receptor, and inhibition of adenylyl cyclase mediated by the G_i-coupled P2Y₁₂ receptor. Concomitant activation of both the G_q and G_i pathways by ADP is necessary to elicit normal ADP-induced platelet aggregation. Activation of the G_q pathway through P2Y₁ leads to platelet shape change and rapidly reversible aggregation, whereas the activation of the G_i pathway through P2Y₁₂ elicits a slow progressive and sustained platelet aggregation not preceded by shape change. In addition to its role in ADP-induced platelet aggregation, P2Y₁₂ mediates the potentiation of platelet secretion induced by strong agonists and the stabilization of thrombin-induced platelet aggregates. P2Y₁₂ has a more selective tissue distribution than P2Y₁, making it an attractive molecular target for therapeutic intervention. Indeed, P2Y₁₂ is the target of effective antithrombotic agents like ticlopidine and clopidogrel, which are already used in clinical practice either alone or in combination with other antithrombotic drugs.

Ticlopidine and clopidogrel are structurally related compounds, belonging to the thienopyridine family of ADP receptor antagonists and they are pro-drugs that are inactive in vitro and need to be metabolized in vivo by the hepatic cytochrome P-450 1A enzymatic pathway to active metabolites, which have very short half-lives. They irreversibly and specifically inhibit the function of the platelet P2Y₁₂ receptor, reproducing the platelet function abnormalities that are observed in patients who are congenitally deficient in P2Y₁₂ and in P2Y₁₂ knock-out mice. The ability of thienopyridines to inhibit platelet aggregation induced by several platelet agonists (such as thromboxane A₂ analogues, collagen, and low concentrations of thrombin) is accounted by the suppression of the amplifying effect on platelet aggregation of ADP secreted from platelet dense granules. Treatment with these thienopyridines renders the
thrombin-induced platelet aggregates more susceptible to deaggregation and inhibits shear-induced platelet aggregation.

2.1.2 Development history of Clopidogrel hydrogen sulfate and its properties

Clopidogrel hydrogen sulfate is a potent and orally active, thienopyridine class antiplatelet agent used to inhibit blood clots in coronary artery disease, peripheral vascular disease and cerebrovascular disease. It is marketed by Sanofi-Aventis and Bristol-Myers Squibb under the trade name Plavix®. The drug works by irreversibly inhibiting a receptor called P2Y_12, an adenosine diphosphate (ADP) chemoreceptor on platelet cell membranes. Adverse effects include hemorrhage, severe neutropenia, and thrombotic thrombocytopenic purpura (TTP). Clopidogrel works by preventing harmful blood clots. Studies shown that clopidogrel is more effective in blocking platelet aggregation than aspirin and ticlopidine even at much lower dosage. A dosage of 75 mg of clopidogrel has been shown to be more effective than a dosage of 325 mg of aspirin. Clopidogrel is widely used in combination with aspirin after placement of intravascular stents. Plavix, (Clopidogrel hydrogen sulfate) is the second best-selling drug in the world, with global sales of $6.4 billion in 2006 and $7.3 billion in 2007.

Clopidogrel is a prodrug, the action of which may be related to an ADP receptor on platelet cell membranes. The drug specifically and irreversibly inhibits the P2Y_{12} subtype of ADP receptor, which is important in activation of platelets and eventual cross-linking by the protein fibrin.

Clopidogrel is a pro-drug activated in the liver by cytochrome P450 enzymes, including CYP2C19. Due to opening of the thiophene ring, the chemical structure of the active substance has three sites that are stereochemically relevant, making a total of eight possible isomers. These are: a stereocentre at C4 (attached to the -SH thiol group), a double bond at C3-C16, and the original stereocentre at C7. Only one of the eight structures is an active antiplatelet drug. This has the following configuration: Z-
configuration at the C3-C16 double bond, the original $S$-configuration at C7,$^{18}$ and although the stereocenter at C4 can’t be directly determined, as the thiol group is too reactive, work with the active metabolite of the related drug prasugrel suggests that $R$-configuration of the C4 group is critical for P2Y$_{12}$ and platelet-inhibitory activity. The active metabolite has an elimination half-life of about eight hours and acts by forming a disulfide bridge with the platelet ADP receptor. Patients with a variant allele of CYP2C19 are 1.5 to 3.5 times more likely to die or have complications than patients with the high-functioning allele.$^{19-21}$

Following an oral dose of $^{14}$C-labeled clopidogrel in humans, approximately 50% is excreted in the urine and approximately 46% in the feces after five days from dosing.

2.1.2.1 Effect of food
Administration of clopidogrel bisulfate with meals did not significantly modify the bioavailability of clopidogrel as assessed by the pharmacokinetics of the main circulating metabolite.

2.1.2.2 Absorption and distribution
Clopidogrel is rapidly absorbed after oral administration of repeated doses of 75 mg clopidogrel (base), with peak plasma levels (approx. 3 mg/L) of the main circulating metabolite occurring approximately one hour after dosing. The pharmacokinetics of the main circulating metabolite are linear (plasma concentrations increased in proportion to dose) in the dose range of 50 to 150 mg of clopidogrel. Absorption is at least 50% based on urinary excretion of clopidogrel-related metabolites. Clopidogrel and the main circulating metabolite bind reversibly in vitro to human plasma proteins (98% and 94%, respectively). The binding is nonsaturable in vitro up to a concentration of 110 μg/mL.
2.1.2.3 Metabolism and elimination

In vitro and in vivo, clopidogrel undergoes rapid hydrolysis into its carboxylic acid derivative. In plasma and urine, the glucuronide of the carboxylic acid derivative is also observed.

The enantioselective synthesis of the individual enantiomer is necessary because their (S)- or (R)-isomers probably display different pharmacological properties. Of the possible two stereoisomers of clopidogrel, only the S-enantiomer is suitable for pharmaceutical use, as the R-enantiomer shows no antithrombotic activity and causes convulsions in animal experiments. This has led to different proposals from many groups for the preparation of enantiomerically pure clopidogrel by separating racemic mixture through enzymatic or chemical resolutions, and the asymmetric synthetic methods with chiral building blocks were also developed.

2.1.3 Biological activity and mechanism of Clopidogrel hydrogen sulfate

Clopidogrel hydrogen sulfate is an anti-platelet drug, which inhibits the ability of platelets to clump together as part of a blood clot. Clopidogrel prevents blood clots by irreversibly binding the P2Y_{12} receptor on platelets, preventing adenosine diphosphate (ADP) from activating platelets. It belongs to a class of drugs called P2Y_{12} inhibitors. Other drugs in this class include ticagrelor (Brilinta) and prasugrel (Effient). Clopidogrel is similar to ticlopidine (Ticlid) in chemical structure and in the way it works. Unlike ticlopidine, clopidogrel hydrogen sulfate does not cause serious reductions of white cells in the blood and, therefore, routine blood testing to determine if the white blood cell count is low is not necessary during treatment. The risk of heart attacks and strokes (which are usually caused by blood clots) is increased in patients with a recent history of stroke or heart attack, and patients with peripheral vascular disease (peripheral vascular disease is the same as atherosclerotic arterial disease or "hardening" of the arteries in which the arteries become narrowed. It frequently occurs in the legs and often...
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causes claudication or pain in the legs upon walking). Clopidogrel hydrogen sulfate is used to reduce the risk of heart attacks and strokes in these patients.

Clopidogrel is used for preventing strokes, heart attacks, and deaths in individuals who had a previous stroke, unstable angina, heart attack or have peripheral arterial disease (PAD). The combination of clopidogrel and aspirin is better than aspirin or clopidogrel alone in preventing another heart attack but the risk of bleeding is higher.

Table 2.1 Overview of pharmaceutical properties of Clopidogrel hydrogen sulfate

<table>
<thead>
<tr>
<th>Generic Name</th>
<th>Clopidogrel hydrogen sulfate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brand Name</td>
<td>Plavix®</td>
</tr>
<tr>
<td>Active Ingredient</td>
<td>Clopidogrel</td>
</tr>
<tr>
<td>Innovator</td>
<td>Sanofi</td>
</tr>
<tr>
<td>Chemical Name</td>
<td>Methyl-((+)-(S)-\alpha-(2\text{-}chlorophenyl)-6,7\text{-}dihydrothieno [3,2-\text{c}]pyridine)-5(4\text{H})-acetate hydrogen sulfate</td>
</tr>
<tr>
<td>Chemical Formula</td>
<td>\text{C}<em>{16}\text{H}</em>{16}\text{ClNO}_{2}\text{S. H}_2\text{SO}_4</td>
</tr>
<tr>
<td>Molecular Weight</td>
<td>419.90 g/mol</td>
</tr>
<tr>
<td>Melting Point</td>
<td>184 °C</td>
</tr>
<tr>
<td>Chemical Structure</td>
<td><img src="image" alt="Chemical Structure" /></td>
</tr>
<tr>
<td>CAS Registry No.</td>
<td>[135046-48-9]</td>
</tr>
<tr>
<td>Physical Description</td>
<td>White crystals</td>
</tr>
<tr>
<td>Solubility</td>
<td>Soluble in water, methanol</td>
</tr>
</tbody>
</table>
2.2 Previously reported synthetic approaches

D. Aubert et al.\textsuperscript{23} reported the synthesis of racemic clopidogrel 4 in Scheme 2.1, in which esterification of (2-chlorophenyl)hydroxy acetic acid 2 in the presence of methanol and hydrochloric acid to give methyl-2-chloro-(o-hydroxyphenyl)acetate 3 which upon chlorination with thionyl chloride gave methyl-2-chloro-(o-chlorophenyl) acetate 4. Condensation of methyl-2-chloro-(o-chlorophenyl) acetate 4 with 4,5,6,7-tetrahydrothieno[3,2-c]pyridine 5 in the presence of potassium carbonate afforded clopidogrel 6 as a hydrochloride salt.

\begin{equation}
\text{Scheme 2.1 Reagents and Conditions:} (a) \text{HCl, MeOH, reflux, 5 h, 84%;} \ (b) \text{SOCl}_2, \ \text{reflux, 83%;} \ (c) \ K_2\text{CO}_3, \ \text{DMF, 90 °C, 4 h, 45%}.
\end{equation}

Burgos et al.\textsuperscript{24} reported (Scheme 2.2) the synthesis of racemic clopidogrel ([benzene-U\textsuperscript{13}C]-rac-SR25990C) 6 using [benzene-U\textsuperscript{13}C]-2-chlorobenzaldehyde 11 which in turn obtained from the commercially available [benzene-U\textsuperscript{13}C] benzoic acid 7. In this, [benzene-U\textsuperscript{13}C] benzoic acid 7 is converted into oxazoline 8 using Shono et al.\textsuperscript{25} method. Lithiation followed by chlorination of 8 in the presence of s-BuLi and hexachloroethane to give 9. N-alkylation of 9 with methyl iodide followed by reduction with sodium borohydride in ethanol afforded 10. Hydrolysis of 10 with 2N hydrochloric acid gave [benzene-U\textsuperscript{13}C]-2-chlorobenzaldehyde 11. Strecker synthesis of 11 with
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4,5,6,7-tetrahydrothieno [3,2-c] pyridine 5 in the presence of acetone cyanohydrin in toluene to afford 12 which up on hydrolysis with hydrochloric acid yielded cyano compound 13 in quantitative yield. Hydrolysis followed by salt formation of 13 with sulphuric acid to give racemic clopidogrel ([benzene-U-\textsuperscript{13}C]-rac-SR25990C) 6 as hydrogen sulfate.

Scheme 2.2 Reagents and Conditions: (a) SOCl\textsubscript{2}, NH\textsubscript{2}CMe\textsubscript{2}CH\textsubscript{2}OH, SOCl\textsubscript{2}; (b) (i) s-BuLi, toluene, -78 °C, 30 min; (ii) C\textsubscript{2}Cl\textsubscript{6}, -40 °C to rt, 58%; (c) (i) MeI, reflux, 16 h; (ii) NaBH\textsubscript{4}, EtOH, rt, 2 h; (iii) 2N HCl, rt, 30 min; (d) acetone cyanohydrin, MgSO\textsubscript{4}, toluene, 45 °C, 48 h, 31%; (e) HCl, MeOH, rt, 48 h, 94%; (f) (i) H\textsubscript{2}SO\textsubscript{4}, MeOH, reflux, 114 h, 50%; (ii) H\textsubscript{2}SO\textsubscript{4}, Et\textsubscript{2}O, rt.
Use of multicomponent procedures\textsuperscript{26-35} for the synthesis of racemic clopidogrel 6 has been only hardly reported. For instance, this compound can be regarded as the ester derivative of a non-natural $\alpha$-amino acid. Some well-established multicomponent procedures allowing the formation of this core unit have been used as the key step of its preparation. Accordingly, the synthesis of 6 has been reported (Scheme 2.3 & 2.4) by Kalinski et al.\textsuperscript{36} either by using an Ugi\textsuperscript{37} or Petasis\textsuperscript{38} three component reaction with better overall yields compared to the original patented Sanofi\textsuperscript{23} multi-step synthesis.

**Ugi-3CR**

Reaction of 4,5,6,7-tetrahydrothieno [3,2-c] pyridine 5, 2-chlorobenzaldehyde 14 and 1-isocyanocyclohexene 15 in methanol and formic acid under microwave irradiation to give amide 16 which upon hydrolysis with aqueous hydrochloric acid afforded carboxylic acid 17. Esterification of 17 in the presence of methanol and sulfuric acid gave racemic clopidogrel 6.

\[
\begin{align*}
\text{5} & \quad \text{14} & \quad \text{15} & \quad \text{16} \\
\text{b} & \quad \text{c} \\
\end{align*}
\]

**Scheme 2.3 Reagents and Conditions:** (a) MeOH, Et$_3$N, HCOOH, 60 °C, MW, 1 h, 83%; (b) THF-HCl$_{aq}$ (9:1), rt, 1 h, 98%; (c) MeOH, H$_2$SO$_4$, reflux, 3 h, 90%.
Petasis-3CR

Reaction of 4,5,6,7-tetrahydrothieno[3,2-c]pyridine 5, glyoxyl acid 18 and 2-chlorophenylboronic acid 19 in dimethylformamide to afford carboxylic acid 17. Esterification of 17 in the presence of methanol and sulfuric acid afforded racemic clopidogrel 6.

\[
\begin{align*}
\text{5} & \quad + \quad \text{18} & \quad + \quad \text{19} & \quad \xrightarrow{a} \quad \text{17} \\
\text{b} & \quad \rightarrow & \quad \text{6}
\end{align*}
\]

**Scheme 2.4 Reagents and Conditions:** (a) DMF, rt, one week, 49%; (b) MeOH, H₂SO₄, reflux, 3 h, 90%.

Mannich-related multicomponent synthesis of racemic clopidogrel 6 has been reported (Scheme 2.5) by Aillaud et al.³⁹ Microwave irradiation of methyl glyoxylate 20 with 2-chlorophenylzinc bromide 21 and 4,5,6,7-tetrahydrothieno[3,2-c]pyridine 5 in acetonirile afforded (±)-clopidogrel 6.

\[
\begin{align*}
\text{20} & \quad + \quad \text{BrZn} & \quad + \quad \text{5} & \quad \xrightarrow{a} \quad \text{6} \\
\end{align*}
\]

**Scheme 2.5 Reagents and Conditions:** (a) MeCN, MW 50W, 20 min, 32%.
Furthermore, synthesis of (±)-clopidogrel 6 also been reported in Scheme 2.6 through trans-esterification of ethyl analogue of clopidogrel. In this, 2-bromochlorobenzene 22 was converted into 2-chlorophenylzinc bromide 21 in the presence of zinc and cobalt bromide. Multicomponent reaction of 21 with ethyl glyoxylate 23 and 4,5,6,7-tetrahydrothieno[3,2-c]pyridine 5 in acetonitrile to afford (±)-clopidogrel ethyl ester analog 24. Finally trans-esterification of 24 in the presence of methanol yielded (±)-clopidogrel 6.

\[
\begin{align*}
\text{ bromochlorobenzene 22 } & \xrightarrow{\text{a}} \text{ chlorophenylzinc bromide 21 } \\
\text{ ethyl glyoxylate 23 } & \xrightarrow{\text{b}} \text{ ethyl ester analog 24 } \\
\text{ methyl ester 24 } & \xrightarrow{\text{c}} \text{ clopidogrel 6 }
\end{align*}
\]

**Scheme 2.6 Reagents and Conditions:** (a) Zn, CoBr₂, MeCN, rt, 30 min, ~75%;
(b) MeCN, toluene, 60 °C to rt, ~1 h, 78%; (c) MeOH, Na, reflux, 30 min, 52%.

Asymmetric synthesis of (S)-(+-)clopidogrel hydrogen sulfate 1 through kinetic resolution method has been reported (Scheme 2.7) by Badorc et al. in which resolution of racemic clopidogrel 6 with camphor-10-(L)-sulfonic acid monohydrate to yield camphor-10-(L)-sulfonic acid salt of methyl-(α)-5(4,5,6,7-tetrahydro(3,2-c)thienopyridyl)-(2-chlorophenyl)acetate 25. Hydrolysis of 25 with saturated sodium bicarbonate solution to afford methyl-(α)-5(4,5,6,7-tetrahydro(3,2-c)thienopyridyl)-(2-chlorophenyl)acetate.
followed by salt formation with sulphuric acid in the presence of acetone afforded (S)-(+)clopidogrel hydrogen sulfate 1.

\[ S \text{NClCOOCH}_3 \quad \text{a} \quad S \text{NClCOOCH}_3 \quad \text{b} \]

\[ \text{H}_2\text{CCH}_3\text{SO}_3\text{H} \]

1

**Scheme 2.7 Reagents and Conditions:** (a) Camphor-10-(L)-sulfonic acid monohydrate, acetone, rt, 48 h, 48%; (b) (i) saturated aq. NaHCO\(_3\) solution, CH\(_2\text{Cl}_2\), rt; (ii) acetone, H\(_2\text{SO}_4\), rt, 92%.

L. Yin *et al.*\(^{42}\) reported (Scheme 2.8) a nice asymmetric approach towards the synthesis of (S)-(+)clopidogrel 1 from methyl-(R)-o-chloromandelate 27 via Ru-catalyzed asymmetric hydrogenation and transfer hydrogenation. In which asymmetric hydrogenation of \(\text{o-}\)chlorophenyl-oxo-acetic acid methyl ester 26 with Ru-(\(R,R\))-2,4,6-triisopropyl Ts-DPEN and HCOOH-Et\(_3\)N afforded methyl-(\(R\))-o-chloromandelate 27. Esterification of 27 with 4-nitrobenzenesulfonyl chloride 28 in the presence of DMAP and triethylamine to yield (\(R\))-4-nitrobenzenesulfonxyloxy-2-(2-chlorophenyl)acetate 29. Condensation of 29 with 4,5,6,7-tetrahydrothieno[3,2-c]pyridine 5 in the presence of aqueous potassium carbonate in dichloromethane to give (S)-(+)clopidogrel 1.
Scheme 2.8 Reagents and Conditions: (a) (i) Ru-(R,R)-2,4,6-triisopropyl Ts-DPEN, 50 °C, 20 h; (ii) HCOOH/Et$_3$N (5:2), 25 °C, 40 min; (b) DMAP, Et$_3$N, CH$_2$Cl$_2$, 0 °C, 3 h, 78%; (c) 30% aq. K$_2$CO$_3$, CH$_2$Cl$_2$, reflux, 2.5 h, 70%.

Attrition-enhanced deracemization in the synthesis of (S)-(+-)clopidogrel 1 has been reported (Scheme 2.9) by van der Meijden et al.$^{43}$ which involves deracemization of 2-(benzylideneamino)-2-(2-chlorophenyl)acetamide 30 in the presence of DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) in acetonitrile to give (S)-(E)-2-(benzylideneamino)-2-(2-chlorophenyl)acetamide 31 followed by treatment with hydrochloric acid in acetone yielded (S)-2-chlorophenylglycinamide hydrochloride$^{44}$ 32. The amide 32 was converted to methyl (S)-2-(2-chlorophenyl)glycinate 33 in the presence of sulfuric acid in methanol.$^{45}$ Condensation followed by cyclization of 33 with 2-(2-bromoethyl)-3-(bromomethyl)thiophene$^{46}$ 34 in the presence of diisopropylethylamine in acetonitrile afforded (S)-(+-)clopidogrel 1 in 88% yield with ee of > 99%.
Scheme 2.9 Reagents and Conditions: (a) DBU, MeCN, 70 °C to 20 °C, overnight, 80%; (b) HCl, acetone, rt, 1 h, 95%; (c) H₂SO₄, MeOH, reflux, 4 h, 94%; (d) DIPEA, MeCN, reflux, overnight, 88%.

Ferraboschi et al.⁴⁷ reported (Scheme 2.10) an asymmetric synthesis of (S)-(+) -clopidogrel 1 via enzyme-catalyzed resolution method, in which resolution of (RS)-N-Boc-2-chlorophenylglycine methyl ester 35 in the presence of cross-linked enzyme aggregate (Alcalase-CLEA®)⁴⁸-⁵⁰ followed by hydrolysis to afford (S)-N-Boc-2-chlorophenylglycine 36. Esterification of 36 with methanol in the presence of DCC and DMAP in dichloromethane afforded N-Boc-(S)-2-chlorophenylglycine methyl ester 37 which upon hydrolysis with TFA gave methyl-(S)-2-(2-chlorophenyl)glycinate 33. Condensation⁵¹ of 33 with 2-(2-thienyl)ethyl-1-p-tolylsulfonate 38 in the presence of sodium bicarbonate and potassium iodide to give methyl-(S)-2-(2-chlorophenyl)-2-[2-(thien-2-yl)ethylamino]acetate 39. Ring formation of 39 with paraformaldehyde⁵² in 1,2-dichloroethane afforded (S)-(+) -clopidogrel 1.
Scheme 2.10 Reagents and Conditions: (a) Alcalase-CLEA®, 2M NaOH, THF, H₂O, 30 °C, 14 h, 34%; (b) MeOH, DCC, DMAP, CH₂Cl₂, rt, 2 h, 95%; (c) TFA, CH₂Cl₂, rt, 3 h, 95%; (d) NaHCO₃, KI, MeCN, reflux, 20 h, 70%; (e) paraformaldehyde, 1,2-dichloroethane, HCl in DMF, reflux, 6 h, 50%.
2.3 Objective of the research work

Various methods have been reported for the synthesis of this potent antithrombotic drug, most of which involve either the ortho-chloromandelate or 2-(2-chlorophenyl)glycine derivatives as starting materials. The final product is usually in racemic form and resolved through fractional crystallization with a resolving agent such as tartaric acid\textsuperscript{43} or camphorsulfonic acid.\textsuperscript{41,54} Economically, the use of an enantiomerically pure reagent at the start of a synthetic sequence is more cost-effective and less polluting. For this reason, several strategies have recently been developed for the preparation of enantiomerically pure ortho-chloromandelate by enzymatic routes, such as kinetic resolution with hydrolases\textsuperscript{55,56} or enantioselective reduction of a prochiral ketoacid.\textsuperscript{57} Although enantioselective hydrolysis of 2-(2-chlorophenyl)glycine amide by microbes possessing amidase activity has been reported,\textsuperscript{58,59} the low solubility of the amide in water and necessity of fermenting a specific bacterial strain limits the application of this process on large scale.

Although numerous examples of successful enzyme-catalyzed resolutions of phenylglycine have recently been reported in the literature,\textsuperscript{60-64} only a few instances of resolution of 2-(2-chlorophenyl)glycines have been described. D-Phenylglycine and several of its analogs, variously substituted on the aromatic ring [including 2-(2-chlorophenyl)glycine], that are useful as starting material for semisynthetic pencillins and cephalosporins, have been prepared by using D-hydantoinase.\textsuperscript{65} (R,S)-2-(2-chlorophenyl)glycine has been successfully resolved by Fadnavis and co-workers,\textsuperscript{66} who used an immobilized penicillin G acylase, and by Ferraboschi and co-workers, who adopted a chemo-enzymatic approach.\textsuperscript{47} Although the synthetic approaches were appreciable in terms of the strategies adopted to address the chemistry, these methodologies suffers few drawbacks. Such as (i) using expensive reagents and chiral ligands like acetone cyanohydrins, CoBr\textsubscript{2}, Ru-(R,R)-2,4,6-triisopropyl Ts-DPEN, Alcalase-CLEA\textsuperscript{®} etc. (ii) hazardous reagents like s-BuLi, etc. (iii) half the material is
lost as the unwanted isomer which greatly diminishes their synthetic utility when scaled up.

Recently, Perez-Fuertes and co-workers\textsuperscript{67} prepared enantiopure \(\alpha\)-arylglycines using asymmetric Strecker reaction. This asymmetric synthesis encouraged us to attempt a preparation of enantiomerically pure \((S)\)-clopidogrel \textbf{1} by a similar stereoselective route. This chapter is dedicated to an asymmetric synthesis of clopidogrel hydrogen sulfate \textbf{1}.

The retro synthetic analysis for \((S)\)-(+) -clopidogrel hydrogen sulfate \textbf{1} shown in Scheme 2.11. Methyl \((S)\)-2-(2-chlorophenyl)glycinate \textbf{33} and 2-(2-bromomethyl)-3-(bromomethyl) thiophene \textbf{34} are precursors of \((S)\)-(+) -clopidogrel \textbf{1}. Glycinate \textbf{33} might be obtained from \((2S)\)-(2-chlorophenyl){\[(1S)-1-(4-methoxyphenyl)ethyl]amino} acetonitrile hydrochloride \textbf{41}, which in turn, could be prepared from 2-chloro benzaldehyde \textbf{14}.

\textbf{Scheme 2.11 Retrosynthetic analysis for Clopidogrel hydrogen sulfate 1}
2.4 Results and discussion

Here we report a strategy involving a chiral auxiliary based Strecker reaction as the key step for the synthesis of (2S)-2-(2-chlorophenyl)glycine hydrochloride 42, followed by esterification to give the methyl ester 33, a key intermediate for clopidogrel 1.

\[
\begin{align*}
14 & \quad \text{CHO} + \text{ClI-H}_2\text{N} \quad \text{CH}_3 & \quad \text{c} & \quad \text{c} \\
& \quad \text{14} & \quad \text{40} & \quad \text{COOH} & \quad \text{33} & \quad \text{34} & \quad \text{COOCH}_3 \\
& \quad \text{CHO} + \text{ClI-H}_2\text{N} \quad \text{CH}_3 & \quad \text{H}_2\text{SO}_4 & \quad \text{H}_2\text{SO}_4
\end{align*}
\]

Scheme 2.12 Reagents and Conditions: (a) NaCN, MeOH:H₂O (1:1), rt, 16 h, 75%; (b) 6M HCl, Et₂O, CHCl₃, rt to 90 °C, 4 h, 80%; (c) (i) MeOH, H₂SO₄, 0 °C to reflux, 5 h; (ii) 20% Na₂CO₃, CH₂Cl₂, rt, 85%; (d) (i) DIPEA, MeCN, EtOAc, reflux; (ii) H₂SO₄, acetone, rt to 0 °C, 6 h, 75%.

As outlined in Scheme 2.12, addition of 2-chlorobenzaldehyde 14 to a solution of sodium cyanide and (1S)-1-(4-methoxyphenyl)ethanamine hydrochloride 40 gave diastereometrically pure (2S)-(2-chlorophenyl)[[(1S)-1-(4-methoxyphenyl)ethyl]amino] acetonitrile hydrochloride 41. In this step, cyanide undergoes preferential nucleophilic attack at the re-face of the imine intermediate formed from 2-chlorobenzaldehyde 14 and chiral auxiliary 40 to give the diastereomerically pure nitrile 41. The NMR spectrum of product 41 confirmed that it was obtained with a high diastereoselectivity (> 95%).
In the mass spectrum (Figure 2.1) peak at 301.5 corresponds to free base molecular ion (M+H)+. The IR spectrum (Figure 2.2) displayed a peak at 3312 cm⁻¹ corresponds to aromatic –NH stretching, peak at 3064 cm⁻¹ belongs to aromatic –CH stretching, peak at 2983 cm⁻¹ corresponds to aliphatic –CH stretching, the characteristic peak at 2226 cm⁻¹ belongs to nitrile (-CN) stretching, peak at 1584 cm⁻¹ corresponds to aromatic C=C stretching, and peak at 1243 & 1036 cm⁻¹ belongs to aralkyl –COC stretching. The 1H NMR spectrum (Figure 2.3, DMSO-d6) displayed a multiplet at δ 7.76-7.75 (m, 1H) corresponds to aromatic proton adjacent to chlorine atom, a signal at δ 7.52-7.49 (m, 3H) belongs to aromatic protons, a signal at δ 7.39-7.31 (m, 2H) corresponds to aromatic protons, and a signal at δ 6.94-6.84 (m, 2H) corresponds to aromatic protons adjacent to methoxy group. Signal at δ 4.56 (d, 1H) belongs to –CH proton adjacent to nitrile and –NH group, a signal at δ 3.99 (q, 1H) corresponds to –CH proton flanked between benzene and methyl group, a signal at 3.74 (s, 3H) belongs to methyl (CH₃) protons of methoxy group, a signal at δ 3.63 (d, 1H) corresponds to –NH proton, and a signal at δ 1.32 (d, 3H) corresponds to –CH₂ protons respectively. The proton decoupled 13C NMR spectrum (Figure 2.4) showed 15 signals. The aromatic carbon adjacent to methoxy group appeared at δ 159.1, where as the aromatic carbon attached to chlorine atom appeared at δ 135.5 and remaining aromatic carbons appeared at δ 132.5, 131.0, 130.3, 129.7, 128.7, 128.4, 119.1 respectively. Signal at δ 114.4 corresponds to nitrile carbon, a signal at δ 56.2 belongs to methoxy carbon, a signal at δ 55.3 corresponds to carbon atom flanked between benzene and methyl group, a signal at δ 49.3 belongs to carbon atom adjacent to nitrile and –NH group, and a signal at δ 24.8 corresponds to methyl carbon respectively.

Removal of the chiral auxiliary and concomitant hydrolysis of the nitrile group in aqueous hydrochloric acid to give enantiopure (2S)-2-(2-chlorophenyl)glycine hydrochloride 42. The pure compound 42, washed with chloroform, had a specific optical rotation of [α]D²⁵ = +88.2 (c 1, 1M aq. HCl).
The compound 42 was characterized on the basis of its spectral data. In the mass spectrum (Figure 2.5) peak at 185.9 corresponds to free base molecular ion (M+H)^+. The IR spectrum (Figure 2.6) displayed a peak at 3415 cm\(^{-1}\) corresponds to acid –OH stretching, a peak at 3171 cm\(^{-1}\) belongs to –NH stretching, peak at 3033 cm\(^{-1}\) corresponds to aromatic –CH stretching, the characteristic peak at at 1639 cm\(^{-1}\) corresponds to acid carbonyl stretching, and a peak at 1506 cm\(^{-1}\) belongs to aromatic C=C stretching. The \(^1\)H NMR spectrum (Figure 2.7, D\(_2\)O) displayed a doublet at \(\delta\) 7.5 (d, 1H) corresponds to aromatic proton adjacent chlorine atom, a signal at \(\delta\) 7.4-7.3 (m, 3H) belongs to remaining aromatic protons, and a signal at \(\delta\) 5.15 (s, 1H) corresponds to –CH proton flanked between amine and acid group. The proton decoupled \(^13\)C NMR spectrum (Figure 2.8) showed 8 signals. The carbonyl carbon appeared at \(\delta\) 172.4, where as the aromatic carbon adjacent to chlorine atom, amine group appeared at \(\delta\) 133.7, aromatic carbon attached to chlorine atom appeared at \(\delta\) 131.7, and remaining aromatic carbons appeared at \(\delta\) 130.3, 130.2, 128.0 respectively and the signal at \(\delta\) 55.6 corresponds to carbon atom flanked between amine and acid group.

The degree of asymmetric induction was assessed by \(^1\)H NMR analysis of the diastereomeric mixtures, and the absolute stereochemistry of the (2S)-(2-chlorophenyl){[(1S)-1-(4-methoxyphenyl)ethyl]amino}acetonitrile hydrochloride 41 was determined by conversion into its methyl ester 33 by treatment with methanol and sulfuric acid.\(^5\) The absolute configuration was confirmed by comparison of the measured optical rotations with that of a known sample of methyl (2S)-2-(2-chlorophenyl)glycinate\(^4\) 33.

The compound 33 was characterized on the basis of its spectral data. In the mass spectrum (Figure 2.9) peak at 199.9 corresponds to molecular ion (M+H)^+. The IR spectrum (Figure 2.10) displayed doublet at 3383 & 3319 cm\(^{-1}\) corresponds to primary –NH stretching, a peak at 3061 cm\(^{-1}\) belongs to aromatic –CH stretching, a peak at 2952 cm\(^{-1}\) corresponds to aliphatic –CH stretching, the characteristic peak at 1747 cm\(^{-1}\)
corresponds to methyl ester carbonyl stretching, and a peak at 1574 and 1463 cm\(^{-1}\) belongs to aromatic C=C stretching. The \(^1\)H NMR spectrum (Figure 2.11, DMSO-\(d_6\)) displayed a multiplet at \(\delta 7.64-7.61\) (m, 2H) and \(\delta 7.50-7.46\) corresponds to aromatic protons, a signal at \(\delta 5.5\) (s, 1H) belongs to –CH proton flanked between amine and ester group and a signal at \(\delta 3.72\) (s, 3H) corresponds to methyl protons of ester. The proton decoupled \(^13\)C NMR spectrum (Figure 2.12) showed 9 signals. The ester carbonyl carbon appeared at \(\delta 168.5\), where as the aromatic carbon adjacent to chlorine atom and amine group appeared at \(\delta 133.7\), aromatic carbon attached to chlorine atom appeared at \(\delta 131.9\), and remaining aromatic carbons appeared at \(\delta 130.9\), 130.5, 130.3, 128.5 respectively. Signal at \(\delta 53.9\) corresponds to carbon atom flanked between amine and ester group and a signal at \(\delta 52.9\) belongs to methyl carbon of ester.

Condensation of 33 with 2-(2-bromomethyl)-3-(bromomethyl)thiophene 34 in the presence of \(N,N\)-diisopropylethylamine\(^{46}\) to give clopidogrel, followed by salt formation with sulfuric acid in acetone gave enantiopure (\(S\))-\((+)-\)clopidogrel hydrogen sulfate 1.

The compound 1 was characterized on the basis of its spectral data. In the mass spectrum (Figure 2.13) peak at 322.0 corresponds to free base molecular ion (M+H\(^+)\). The IR spectrum (Figure 2.14) displayed a characteristic peak at 3446 cm\(^{-1}\) corresponds to tertiary amine stretching, a peak at 2956 cm\(^{-1}\) belongs to aliphatic –CH stretching, a peak at 1752 cm\(^{-1}\) corresponds to methyl ester carbonyl stretching, and a peak at 1439 and 1463 cm\(^{-1}\) belongs to aromatic C=C stretching. The \(^1\)H NMR spectrum (Figure 2.15, DMSO-\(d_6\)) displayed a multiplet at \(\delta 7.70-7.68\) (m, 2H), a signal at \(\delta 7.57-7.53\) (m, 2H) corresponds to aromatic protons of benzene, a signal at \(\delta 7.45-7.44\) (m, 1H) belongs to aromatic proton adjacent to sulphur atom, and a signal at \(\delta 6.90-6.88\) (d, 1H) corresponds to aromatic proton of thiopyridine ring. Signal at \(\delta 5.62\) (br s, 2H), \(\delta 4.2\) (br s, 2H) & \(\delta 3.08\) (br s, 2H) corresponds to methylene protons of thiopyridine ring. \(\delta 4.93\) (br s, 1H) and \(\delta 3.76\) (s, 3H) belongs to –CH proton flanked between ester and thiopyridine ring and methyl protons of ester respectively.
The physical and spectroscopic data of all the compounds were in good agreement with the proposed structures and with literature data.

2.5 Conclusions

In conclusion, we achieved an asymmetric synthesis of (S)-(+) clopidogrel hydrogen sulfate 1 by means of an asymmetric Strecker reaction.
2.6 Experimental section

**General:** The $^1$H and $^{13}$C spectra were recorded in DMSO-$d_6$ & D$_2$O at 400 MHz on Varian Gemini 400 MHz FT NMR spectrometer. Chemical shifts are relative to TMS. The FT-IR spectra were recorded in the solid state as KBr dispersions by using a Perkin-Elmer 1650 FT-IR spectrometer. Mass spectra (70 eV) were recorded on an Agilent-6310 LC-MS spectrometer. Optical rotations were measured using Rudolph Autopol-V polarimeter and $[\alpha]_D^{25}$ values are given in units of $10^{-1}$ deg. cm$^2$ g$^{-1}$. Organic solvents were distilled before use or, if otherwise noted, dried according to the usual procedures.

2.6.1 (2S)-(2-Chlorophenyl)\{[(1S)-1-(4-methoxyphenyl)ethyl]amino\}acetonitrile hydrochloride 41. (1S)-1-(4-methoxyphenyl)ethanamine hydrochloride 40 (6.76 g, 36 mmol) and sodium cyanide (1.85 g, 38 mmol) were dissolved in a mixture of water (5 mL) and methanol (5 mL). 2-Chlorobenzaldehyde 14 (5.0 g, 36 mmol) was added to above solution and stirred for 16 h at 25-30 °C. On completion of reaction (TLC), the mixture was diluted with water (12.5 mL) and stirred for 30 min at 25-30 °C. The separated solid was filtered, washed with water (5 mL) and dried under vacuum at 70 °C to afford (2S)-(2-chlorophenyl)\{[(1S)-1-(4-methoxyphenyl)ethyl]amino\}acetonitrile hydrochloride 41 as a cream colored powder (9.0 g, 75%, > 95% de). mp: 112-114 °C; $[\alpha]_D^{25} = -129$ (c 0.5, MeOH); IR (KBr, cm$^{-1}$): 3312, 3064, 2983, 2226, 1613, 1584, 1243, 1036, 756, 559; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 7.76-7.75 (m, 1H), 7.52-7.49 (m, 3H), 7.39-7.31 (m, 2H), 6.94-6.84 (m, 2H), 4.56 (d, $J = 11.6$ Hz, 1H), 3.99 (q, $J = 4.0$ Hz, 1H), 3.74 (s, 3H), 3.63 (d, $J = 2.5$ Hz, 1H), 1.32 (d, $J = 6.4$ Hz, 3H); $^{13}$C NMR (400 MHz, DMSO-$d_6$): $\delta$ 159.1, 135.5, 132.5, 131.0, 130.3, 129.7, 128.7, 128.4, 127.9, 119.1, 114.4, 56.2, 55.3, 49.3, 24.8; MS: m/z = 301.5 [M+H]$^+$ (free base); Anal. Calcd for C$_{17}$H$_{18}$Cl$_2$N$_2$O: C, 60.54; H, 5.38; N, 8.31; Found: C, 60.66; H, 5.55; N, 8.32.
2.6.2 (2S)-2-(2-Chlorophenyl)glycine hydrochloride 42. (2S)-(2-chlorophenyl)\{[(1S)-1-(4-methoxyphenyl)ethyl]amino\}acetonitrile hydrochloride 41 (5 g, 15 mmol) and 6M aq. HCl (160 mL) were stirred for 4 h at 90 °C. On completion of reaction (TLC), the mixture was cooled to 25-30 °C and extracted with ether (2x30 mL). The organic extracts were discarded and the aqueous layer was concentrated under reduced pressure to give crude product, which was stirred with chloroform (10 mL) for 30 min at 25-30 °C. The separated solid was filtered, washed with chloroform (2.5 mL) and dried under vacuum to afford (2S)-2-(2-chlorophenyl)glycine hydrochloride 42 as a white solid (2.66 g, 80%, 95.5% ee). mp: 200-202 °C; [α]D 25 = +88.2 (c 1, 1M aq. HCl); IR (KBr, cm⁻¹): 3415, 3171, 3033, 1679, 1639, 1506, 750; ¹H NMR (400 MHz, D₂O): δ 7.5 (d, 1H), 7.4-7.3 (m, 3H), 5.15 (s, 1H); ¹³C NMR (400 MHz, D₂O): δ 172.4, 133.7, 131.7, 130.3, 130.2, 128.0, 55.6; MS: m/z = 185.9 [M+H]⁺ (free base); Anal. Calcd for C₈H₉Cl₂NO₂: C, 43.27; H, 4.08; N, 6.31; Found: C, 43.3; H, 4.12; N, 6.41.

2.6.3 Methyl (2S)-2-(2-Chlorophenyl)glycinate 33. Sulphuric acid (11.27 g, 115 mmol) was slowly added to methanol (30 mL) at 0-5 °C. To this solution was added (2S)-2-(2-chlorophenyl)glycine hydrochloride 42 (5 g, 23 mmol) and the resulting mixture was refluxed for 5 h. On completion of reaction (TLC), methanol was distilled completely under reduced pressure and added the water (20 mL). The resulting mixture pH adjusted to 7.0-8.0 with 20% aq. Na₂CO₃ and the product was extracted with chloroform (3x10 mL). The organic extracts were combined, dried (Na₂SO₄) and concentrated under reduced pressure to afford methyl (2S)-2-(2-chlorophenyl)glycinate 33 as a pale oil (3.78 g, 85%, 99% ee). [α]D 25 = +122 (c 1, MeOH), lit.¹² [α]D 25 = +123; IR (neat, cm⁻¹): 3383, 3061, 2952, 1747, 1593, 1574, 1463, 1169, 751; ¹H NMR (400 MHz, DMSO-d₆): δ 9.25 (s, 3H), 7.64-7.61 (m, 2H), 7.50-7.46 (m, 2H), 5.5 (s, 1H), 3.72 (s, 3H); ¹³C NMR (400 MHz, DMSO-d₆): δ 168.5, 133.7, 131.9, 130.9, 130.5, 130.3, 128.5, 53.9, 52.9; MS: m/z = 199.9 [M+H]⁺.
2.6.4 (S)-(+) - Clopidogrel hydrogen sulfate 1. 2-(2-Bromomethyl)-3-(bromomethyl)-
thiophene 34 (5 g, 18 mmol) was added to a mixture of (2S)-2-(2-chlorophenyl)glycinate
33 (4.31 g, 21.6 mmol) and MeCN (50 mL) and the resulting mixture was stirred for 15
min at 25-30 °C. Diisopropylethylamine (3.28 g, 32 mmol) dissolved in acetonitrile
(5 mL) was added, and the resulting mixture was refluxed for overnight. On completion
of reaction (TLC), the solvents were completely evaporated under reduced pressure.
Ethyl acetate (100 mL) was added to the residue and washed with water (3x75 mL). The
combined organic extracts were washed with sat. aq. NaCl (75 mL), dried (Na₂SO₄) and
concentrated under reduced pressure to give clopidogrel as a yellow oil. The oil was
mixed with acetone (50 mL), carbon (0.25 g) and hyflow filter aid (0.3 g), and the
mixture was stirred for 15 min at 25-30 °C. The solids were collected by filtration and
washed with acetone (5 mL). The filtrate was mixed with water (0.7 mL) and cooled to
0-5 °C. Sulphuric acid (1.94 g, 19.8 mmol) was added slowly over a period of 1.5 h and
the mixture was stirred for 6 h at 0-5 °C. The separated solid was filtered, washed with
acetone (5 mL) and dried under vacuum to afford (S)-clopidogrel hydrogen sulfate 1 as
an off-white powder (5.6 g, 75%, 99.9% ee). [α]D²⁵ = +55 (c 1, MeOH), lit.¹² [α]D²⁵ =
+54.8; IR (KBr, cm⁻¹): 3446, 3121, 2956, 1752, 1439, 1188, 750, 569; ¹H NMR (400
MHz, DMSO-d₆): δ 7.70-7.68 (m, 2H), 7.57-7.53 (m, 2H), 7.45-7.44 (m, 1H), 6.90-6.88
(d, J = 5.0 Hz, 1H), 5.62 (br s, 2H), 4.93 (br s, 1H), 4.2 (br s, 2H), 3.76 (s, 3H), 3.08 (br s,
2H); MS: m/z = 322.0 [M+H]^+ (free base).
Figure 2.1 Mass spectrum of 41

Figure 2.2 IR spectrum of 41
Figure 2.3 400 MHz $^1$H NMR spectrum of 41 in DMSO-$d_6$

Figure 2.4 400 MHz $^{13}$C NMR spectrum of 41 in DMSO-$d_6$
Figure 2.5 Mass spectrum of 42

Figure 2.6 IR spectrum of 42
Figure 2.7 400 MHz $^1$H NMR spectrum of 42 in D$_2$O

Figure 2.8 400 MHz $^{13}$C NMR spectrum of 42 in D$_2$O
Figure 2.9 Mass spectrum of 33

Figure 2.10 IR spectrum of 33
Figure 2.11 400 MHz $^1$H NMR spectrum of 33 in DMSO-$d_6$

Figure 2.12 400 MHz $^{13}$C NMR spectrum of 33 in DMSO-$d_6$
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Figure 2.13 Mass spectrum of 1

Figure 2.14 IR spectrum of 1
Figure 2.15 400 MHz $^1$H NMR spectrum of 1 in DMSO-$d_6$
2.7 References


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