PART B

Chapter 3

Approach towards the synthesis of Penmacric acid
3.1. Introduction

Unnatural and non-proteinogenic amino acids have become very important and attractive synthetic targets due to their intrinsic biological activities and applications as conformational modifiers for physiologically active peptides. Pyroglutamic acid (1), due to its unique structural features, is utilized as a versatile chiral building block for the synthesis of several natural products such as pyrrolidine alkaloids,¹ kainoids,² (-)-bulgecinine,³ (-)-domic acid,⁴ enantiomerically pure proline derivatives⁵ and a wide variety of non-proteinogenic amino acids.⁶ Derived from glutamic acid, derivatives of pyroglutamic acids are an inexpensive source of chirality and have been used as chiral auxiliaries in Diels-Alder reactions⁷, alklylation and aldol reactions⁸ of chiral enolates, kinetic resolution of amino acids and Michael addition processes (Fig. 1).

![Figure 1. Applications of pyroglutamic acid (1)](image)

In the area of amino acids, differently substituted prolines⁹ as well as γ-amino acid derivatives¹⁰ have been synthesized from pyroglutamic acid (1). Chiral saturated heterocyclic compounds such as pyrrolidines¹¹, (+)-adalinine¹², indolizidines¹³ and fused lactams¹⁴ have also been prepared. Alkaloids such as (-)-stemoamide¹⁵ and (+)-ipalbidine¹⁶, (+)-lentiginosine¹⁷, PKC modulators¹⁸, ACE and esterase inhibitors¹⁹ and
Approach towards the synthesis of Penmacric acid

Chapter 3

gastro protective substances such as A1-77-B\textsuperscript{20} are representative compounds synthesized using pyroglutamic acid derivatives. Other significant natural products such as dihydro-kikumycin B\textsuperscript{21}, anthelvencin\textsuperscript{22}, heliotridane\textsuperscript{23}, several alexines\textsuperscript{24}, swainsonine derivative\textsuperscript{25}, manzamine alkaloids\textsuperscript{26}, calyculins\textsuperscript{27}, bulgaine\textsuperscript{28} and carzinophin A\textsuperscript{29} are representative examples which have been synthesized employing pyroglutamic acid as the source of chirality.

In the recent years, C-C bond forming reactions at various positions of pyroglutamates have attracted attention from a number of research groups and considerable thought has been paid on the stereochemical outcome of such reactions, and consequently in recent years, a number of total syntheses of several complex natural products and bioactives have been reported (Fig. 2).

\begin{tikzpicture}
% Drawing code...
\end{tikzpicture}

\textit{Figure 2.} Natural products synthesized using pyroglutamate template
The advantage of pyroglutamic acid in asymmetric synthesis lies in a rigid five-membered skeleton with strong stereo-electronic influence of two different carbonyl entities in the molecule which can be differentially functionalized. Thus, due to their importance of substituted pyroglutamates have been the favored targets of many groups and need of more simpler and safe route is still to exist (Fig. 3).

![Functionalisation diagram](image)

**Figure 3. Possible sites of functionalisation on pyroglutamate skeleton**

### 3.2. Basis of work

Penmacric acid (2) (Fig. 4) is an unusual amino acid isolated in 1975 independently by Welter et al.\textsuperscript{31} and Mbadiwe\textsuperscript{32} from the seeds of the leguminous tree *Pentaclethra macrophylla*, commonly known as “pauco nuts” or “owala seeds” in less than 0.5% w/w of the dry bean endosperm; these legumes are indigenous to the humid lowlands of West Africa, the seeds of which are used both as staple food in the local diet, and also find application for their medicinal value as anti-inflammatories.\textsuperscript{33,34} The absolute configuration of penmacric acid was initially assigned from \textsuperscript{1}H NMR studies in association with CD measurements and this was later supported by a single crystal X-ray
A number of chemical studies were carried out on penmacric acid by the Belgian workers who originally reported its isolation; thus, the lactam of penmacric acid is hydrolysed under a variety of acidic conditions to the substituted adipic acid 3 which recloses in dilute acid solution to give either of two possible substituted pipecolic acids 4 and 5 (Scheme 1). Despite detailed chemical studies nothing is known about the biological origin or role of penmacric acid.

**Scheme 1. Chemical studies on penmacric acid**

The impressive biological properties, low natural abundance, and the distinctive architecture have made penmacric acid and its analogs attractive target for the synthetic efforts. From a structural perspective, assembling the glycine motif at beta position of C-4 of pyroglutamate poses a significant synthetic challenge, and to date, only three research groups have documented strategies to address the synthetically demanding C-4 stereocenter in the context of its synthesis.

**Moloney's approach (2003)**

The first synthetic approach for 2 was attempted by Moloney and co-workers in 2003 (Scheme 2). They have utilized the bicyclic lactams 6a and 6b which is derived from pyroglutaminol following a simultaneous protection of the hydroxyl and amide functionalities by a single benzylidene protecting group. Lithium enolate of bicyclic lactam 6a react with various aldehydes gave a diastereomeric mixture of exo and endo products (7a:7a′=2:1, PhCHO; 1:2.7, chloral). With this outcome, they anticipated that
required stereochemistry at C-4 for penmacric acid would be available from the reaction of bicyclic lactam with an activated imine. However these additions proved not to be so stereoselective as the aldehyde since the addition of N-tosyl imines derived from furfural to lithium enolate of 6a gave exo-adduct 8a only but diastereomeric at C-1' position (dr = 2:1). However, the outcome of the reaction of lactam 6b with same imine gave exo-adduct (1'R)-8b as single diastereomer. The undesired stereochemistry at C-7 was then corrected by epimerization of lactam enolate generated with NaH (condition A) or LDA (condition B) followed by quenching with hindered phenol (di-tert-butylphenol) in arbitrarily low yields. Epimerization of exo-lactam 8b with NaH in refluxing THF gave 26% of the desired product 9b, but also 45% of recovered starting
material and 22% of the elimination product 9b'. Compound 9b was then converted to ester 10 in low yield. Deprotection (TFA, 45%) was followed by the usual oxidation-protection sequence, gave lactam 12 in 8% yield over the three steps, a compound with the correct relative configuration for penmacric acid (2).

Naito’s approach (2007)\(^\text{40}\)

\[
\begin{align*}
\text{HO}_2 & \xrightarrow{\text{i)} \text{CbzCl, NaHCO}_3} \text{N} \xrightarrow{\text{ii)} \text{H}_2\text{SO}_4, \text{MeOH}} \xrightarrow{\text{iii)} \text{MeI, DEAD, PPh}_3} \\
\text{13} & \xrightarrow{\text{i)} \text{DBU, PhMe}} \xrightarrow{\text{ii)} \text{mCPBA}} \xrightarrow{\text{iii)} \text{MgI}_2, \text{PhMe}} \\
\text{14} & \xrightarrow{\text{N} \xrightarrow{\text{NOBn}} \text{MeO}_2\text{C}} \text{Et}_3\text{B} \\
\text{15} & \xrightarrow{\text{3M HCl, AcOEt}} \text{NBO} \xrightarrow{\text{MeO}_2\text{C}} \text{17a} \\
\text{16} & \xrightarrow{\text{4 steps}} \\
\text{18a} & \xrightarrow{\text{3M HCl, AcOEt}} \\
\text{18b} & \xrightarrow{\text{4 steps}}
\end{align*}
\]

\text{Scheme 3. Naito’s approach to 2}

In 2007, Naito and co-workers described the first total synthesis of 2 in 12 steps (5.4% overall yield) as well as the synthesis of the (1'-epi)-2 (3.0% overall yield). The key step of Naitos approach involves the Et\(_3\)B-induced radical reaction of oxime ether 16 and iodoproline 15 via an iodine atom-transfer process (Scheme 3). 3-hydroxy-4-iodoproline (15) required for the Et\(_3\)B-induced radical reaction was prepared by a Mitsunobu reaction on protected trans-4-hydroxy-L-proline (13) to convert hydroxyl group to iodide.
Approach towards the synthesis of Penmacric acid

Chapter 3

to give 14 following the elimination of HI using DBU to obtain 3,4-dehydroporphline which was epoxidated with mCPBA in the presence of 4,4'-thiobis(6-ter-butyl-o-cresol) as a radical scavenger, the epoxide ring of which was regioselectively opened with magnesium iodide to afford the expected 3-hydroxy-4-iodoprolone (15) via nucleophilic attack of the iodide ion on less hindered carbon of the epoxy ring. Radical addition of hydroxyproline 15 to oxime ether 16 using Et$_3$B as the radical indicator gave inseparable 1:1 diastereomers, 17a and 17b which was converted into separable fully protected penmacric acid 18a and epi-penmacric acid 18b via number of steps involving removal of Cbz group, introduction of Boc group, removal of hydroxyl group using Barton-McCombie radical deoxygenation and oxidation of the corresponding pyrrolidine into separable pyrrolidinone. Finally, deprotection of two Boc groups and hydrolysis of two methyl esters 18a and 18b gave penmacric (2) and (1'-epi)-2 acid respectively.

Pelloux-Leon & Minassian approach (2009)

Penmacric acid (2) has also been synthesized by Minassian and co-workers starting from N-triisopropylsilylpyrrole (19) (Scheme 4). The key-steps of the Minassians route are the addition of the pyrrole nucleus onto a chiral nitrone and the formation of the pyroglutamic acid moiety by reductive hydrogenation of the pyrrole followed by oxidation of the corresponding pyrrolidine into pyrrolidinone. The chiral glycinyl substituent was introduced by the addition of 19 onto the cyclic chiral nitrone (5)-20 followed by methanolsysis to give intermediate 21, which on treatment with palladium black as catalyst in formic acid gave amino ester, the free amino group of which was then protected with Boc followed by the removal of bulky silyl group using TBAF/acetic acid to give 22. The carbemethoxy group at the α-position was introduced by subsequent trichloroacetylation and treatment of the crude mixture with sodium methoxide to give corresponding methyl ester and protection of nitrogen of the pyrrole nucleus with Boc following typical procedures gave 23. The later was hydrogenated using Rh/Al$_2$O$_3$ as catalyst at 60 bars pressure in methanol to give (1:1) mixture of inseparable pyrrolidines.
24a & 24b which on subsequent oxidation with ruthenium tetraoxide was converted into fully protected separable diastereomers 18a and 25, which were finally deprotected in two step sequence to give free 2 and (5-epi)-2.

Scheme 4. Minassian's approach to 2

3.3. Retrosynthesis

Pyroglutamic acid based strategies for the chiral synthesis of several complex molecules primarily take advantage of differential nature of the two carbonyls, the enolization of which is directed by the substitution on ring nitrogen. Lithium enolates of N-urethane protected pyroglutamates react with various aldehydes to give a mixture of 4α- and 4β-
products, with 4α- adduct predominating due to facial preference induced by the carboalkoxy group.\textsuperscript{49} Titanium enolates, however, give almost exclusively the 4α- aldol adducts.\textsuperscript{50} Similar facial preferences have been reported by Moloney and co-workers\textsuperscript{37} for the aldol reaction of bicyclic lactam (Fig. 5). Extension of this strategy to penmacric acid synthesis using N-Tosyl benzaldehyde\textsuperscript{51} however proved unsuccessful as only 4α-products were obtained.\textsuperscript{37} These results were in consonance with an earlier report that the reaction of pyroglutamate derived enolates with N-Tosyl benzaldehyde provides only two diastereomeric 4α- products in 4:1 ratio.\textsuperscript{52} We hypothesized that exclusive formation of 4α- adducts in the reaction might be due to steric bulk of N-Tosyl group and its replacement with N-Boc might reduce this facial selectivity to provide direct access to penmacric acid (Fig 5).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5.png}
\caption{Retrosynthetic analysis}
\end{figure}

3.4. Results and discussion

We initiated our efforts with the preparation of N-Boc benzaldehyde (27)\textsuperscript{53} by reacting benzaldehyde, benzenesulfonic acid sodium salt and t-butyl carbamate in presence of formic acid resulted in α-amido benzenesulfone 26 as white solid which was filtered,
dried and then treated with K$_2$CO$_3$ in THF under refluxing condition to give benzaldimine 27 in 90% yield for two steps (Scheme 5). Imine 27 was fully characterized by comparison of its spectral data with that of the reported data. The MS spectrum of 27 showed M+1 peak at m/z 198. The IR spectrum of 27 showed characteristic absorptions at 1717 (s, C=O), 1633 (m, C=N) cm$^{-1}$ whereas the $^1$H-NMR showed characteristic peaks at δ 7.48-7.94 (5H, m, ArH), 8.88 (1H, s, CH=N), 1.61 (9H, s, C(CH$_3$)$_3$) (Fig. 6).

**Scheme 5. Synthesis of N-Boc benzaldimine (27)**

Figure 6. $^1$H-NMR of compound 27
The common methodology used for the functionalisation of pyroglutamate at C-4 position is the regioselective deprotonation at C-4 of N-carbamate protected pyroglutamate using LiHMDS followed by quenching with electrophiles. The deprotonation at C-4 is facilitated by the urethane protecting group at nitrogen as the ring carboxyl becomes imidic in nature thus facilitating the deprotonation at C-4. In addition, the carbamate group may also stabilize the resulting enolate by coordinating with the lithium (Fig. 7).

![Figure 7. C-4 lithium enolate](image)

Thus, Methyl N-Boc pyroglutamate (28), prepared from glutamic acid following literature procedures, on treatment with LiHMDS at -78 °C afforded the ester enolate which reacted with N-Boc benzaldimine (27) at same temperature to give a diastereomeric mixture of three products (29, 30 and 31) in 5:2:3 ratio as judged by the integration of signals in the $^1$H-NMR spectrum (Scheme 6).

![Scheme 6. Reaction of imine 27 with Li enolate of 28](image)
Approach towards the synthesis of Penmacric acid

Chapter 3

A mixture of two compounds 29 and 30 (83:17) could be separated from the third 31 by flash column chromatography. Repeated recrystallisations of the mixture of 29 and 30 from diethyl ether gave a pure sample of 29 as distinct crystalline white solid (29%) and 31 as clear viscous oil (7.0%). All the three diastereomers were fully characterized by their spectral data ie MS, IR and NMR (Table 1).

Table 1. Spectral data of compounds 29, 30 and 31

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>IR cm(^{-1})</th>
<th>(^1)H NMR (CDCl(_3))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\delta)</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>1791</td>
<td>7.30, 5.65, 9.78</td>
</tr>
<tr>
<td></td>
<td>1753</td>
<td>4.47, 3.75, 3.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.2, 2.06, 1.48, 1.39</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1717</td>
<td>6, 4.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.6, 7.2</td>
</tr>
<tr>
<td>30</td>
<td>1787</td>
<td>7.30, 6.64</td>
</tr>
<tr>
<td></td>
<td>1751</td>
<td>4.8, 4.2</td>
</tr>
<tr>
<td></td>
<td>1710</td>
<td>3.74, 3.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.09, 1.95, 1.45, 1.39</td>
</tr>
<tr>
<td>31</td>
<td>1788</td>
<td>7.29, 6.50</td>
</tr>
<tr>
<td></td>
<td>1751</td>
<td>4.94, 3.57</td>
</tr>
<tr>
<td></td>
<td>1709</td>
<td>3.16, 2.41, 1.65*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.46, 1.38</td>
</tr>
</tbody>
</table>

\(^*\)H-3S and H-3R were not assigned; \(^*\)unresolved dd

With all the isomers in hand after chromatography and crystallisation, a series of two dimensional NMR experiments were performed in order to assign the relative stereochemistry at C-4 and C-6. The stereochemistry at C-4 of the major isomer 29 was judged to be trans with respect to C-2 ie (4S) using NOE experiments in CDCl\(_3\) (Fig. 8).
First we irradiated the hydrogen at C-2 in order to determine the spatial orientation of the hydrogens at C-3. The proton at 2.2 ppm was assigned as H-35 by its enhancement of 6.7% on irradiating the signal for H-2 at 4.47 ppm, and irradiation of H-4 at 2.83 ppm gave an enhancement of 5.1% to H-3R at 2.06 ppm.

Figure 8. Key NOESY correlations for compound 29
The isomer 30 was deemed to have the same (4S) stereochemistry as the transfer of magnetization was observed between H-2 proton and H-3S and H-4 and H-3R in its NOESY spectrum (Fig. 9). Thus both isomers exhibited trans stereochemistry at C-4 with respect to C-2.

Figure 9. Key NOESY correlations for compound 30
Careful crystallization of 29 enabled the isolation of crystals suitable for single-crystal X-ray analysis and confirmed its (6R) stereochemistry at C-6 (Fig. 10). Hence 30, the other 4α- product, was assigned 2S,4S,6S configuration.

**Figure 10. X-ray structure of compound 29.**

The stereochemistry at C-4 of 31 was thus deductively assigned as (4R). In order to establish its relative stereochemistry at C-4 and C-6, phenyl group in 31 was oxidatively transformed using *in situ* generated RuO₄ from RuCl₃/NaIO₄ to carboxylic

![Chemical Structures](image)

**Scheme 7. Synthesis of epimers of penmacric acid**
function and esterified to 32 using diazomethane in overall 35.6% yield (Scheme 7). 32 was found to be C6-epimer of penmacric acid ester having spectral data identical with that of methyl ester of epi-penmacric acid reported by Takeaki Naito et al.40 Similarly, 4,6-epi-, and 4-epi-penmacric acids (33 and 34) were prepared from 29 and 30 respectively following the same protocol. All the three epimers of penmacric acid were fully characterized by their spectral data ie MS, IR and NMR. The C-4 trans relationship of compound 33 and 34 was reconfirmed by NOE experiments in deuterobenzene (Fig. 11 and 13) and the absolute configuration [(2S,4S,6R)-33 and (2S,4S,6S)-34] was later supported by single crystal X-ray structure of compound 33 (Fig. 12).

**Table 2. Spectral data of compounds 32, 33 and 34**

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>IR cm⁻¹</th>
<th>¹H NMR*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NH</td>
<td>H-6</td>
</tr>
<tr>
<td>32</td>
<td>1789</td>
<td>5.67</td>
</tr>
<tr>
<td></td>
<td>1750</td>
<td>br</td>
</tr>
<tr>
<td></td>
<td>1717</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>1790</td>
<td>5.38</td>
</tr>
<tr>
<td></td>
<td>1750</td>
<td>d</td>
</tr>
<tr>
<td></td>
<td>1717</td>
<td>J₅ NH,6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.9</td>
</tr>
<tr>
<td>34</td>
<td>1789</td>
<td>5.76</td>
</tr>
<tr>
<td></td>
<td>1749</td>
<td>br</td>
</tr>
<tr>
<td></td>
<td>1718</td>
<td>J₆,6 9.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>J₆ NH 4.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Internal standard for 32 (CDCl₃), 33 (C₆D₆) and 34 (C₆D₆), b H-3S and H-3R were not assigned.

6-epi, 4-epi, 4,6-epi corresponds to 1'-epi, 3-epi, 3,1'-epimers of penmacric acid (2) respectively according to usual pyroglutamate numbering.
Figure 11. Key NOESY correlations for compound 33

Figure 12. X-ray structure of compound 33
As titanium enolates are known to enhance the diastereoselectivity in aldol reactions, an addition of chlorotitanium enolate of 28 with imine 27 was also studied. Thus, pyroglutamate derived titaniumtrichloro enolate, prepared by dropwise addition of TiCl₄ (1M in CH₂Cl₂) to a solution of 28 in CH₂Cl₂ at -78 °C followed by slow addition of DIPEA after 5 min resulted in deep violet enolate solution, reacted with N-Boc
benzaldimine (27) at same temperature for 2 h. In contrast to the results obtained with lithium enolate, only a single diastereomer 29 was obtained in excellent yield (Scheme 8).

\[
\text{Scheme 8. Reaction of imine 27 with Ti enolate of 28}
\]

Since the structure of penmacric acid consists of pyroglutamic acid residue with glycine motif at C-4 and also carboxylate group is expected to be sterically less hindered than phenyl group, in order to achieve one step synthesis of penmacric acid we next studied the reaction of lithium enolate of 28 with Methyl N-Boc-α-iminoacetate (37) prepared in situ from Methyl N-Boc-2-bromoglycinate (36).

\[
\text{Scheme 9. Synthesis of Methyl N-Boc-α-iminoacetate (37)}
\]

2-bromoglycine ester 36 required for the synthesis was prepared by irradiating a solution of Methyl N-Boc-glycinate (35) and N-bromosuccinimide (NBS) in CCl₄, keeping the reaction mixture between 10-30 °C using a water bath. Subsequent elimination with TEA was achieved at -78 °C and the cold crude imine 37 was rapidly filtered and transferred into a pre-prepared lithium enolate solution of 28, prepared with 1.2 equiv of LiHMDS in THF at -78 °C for 30 minutes. No aldol product was obtained and the starting pyroglutamate 28 was recovered unchanged. This may be due to inefficiency of the transfer and filtration procedure which allowed the sensitive imine 37
to warm considerably above -78 °C. To overcome this problem, the cold lithium enolate solution of pyroglutamate 28 was rapidly transferred via cannula to the crude imine 37 solution at -78 °C without isolating the later. This method gave a mixture of two diastereomeric products, 33 and 34 (Scheme 10) in 71% yield in a ratio of 7:3 as judged by integration of signals in the $^1$H NMR spectrum.

\[
\begin{align*}
&\text{O~C}^2\text{Me}^* \\
&\text{Boc} \\
&\text{N} \\
&\text{CO}_2\text{Me} \\
&\text{28}
\end{align*}
\]

\[
\begin{align*}
&\text{LiHMDS, THF, -78 °C} \\
&\text{37, 2 h, -78 °C}
\end{align*}
\]

High stereoselectivity (exclusive 6S) was also observed in reaction of titanium enolate of 28 with imine 37, where 34 was obtained as the sole product (Scheme 10). The attack of Ti-enolate to the si-face of imine in reaction could be rationalized through a more chelated intermediate (Fig. 14).

\[
\begin{align*}
&\text{O} \\
&\text{N~H~Boc} \\
&\text{MeO}_2\text{C}^\text{65°, 45°} \\
&\text{32} \\
&\text{O~N} \\
&\text{CO}_2\text{Me} \\
&\text{33} \\
&\text{MeO}_2\text{C}^\text{65°, 45°} \\
&\text{Boc} \\
&\text{O~N} \\
&\text{CO}_2\text{Me} \\
&\text{34} \\
&\text{MeO}\text{C}^\text{65°, 45°} \\
&\text{37}
\end{align*}
\]

\[
\begin{align*}
&\text{MeO}_2\text{C}^\text{65°, 45°} \\
&\text{Boc} \\
&\text{O} \\
&\text{N} \\
&\text{H} \\
&\text{CO}_2\text{Me} \\
&\text{34}
\end{align*}
\]

**Scheme 10. Reaction of imine 37 with Li & Ti enolate of 28**

In an effort to establish our proposed chelated transition state assembly, we next investigated the reaction of Ti-enolate of 28 with activated N-tosyl benzaldimine (38) derived from benzaldehyde (absence of chelation due to carbmethoxy group).
Approach towards the synthesis of Penmacric acid

Chapter 3

\[
\text{PhCHO} + \text{TsNH}_2 + \text{TEA} \xrightarrow{\text{TiCl}_4, \text{CH}_2\text{Cl}_2, 0^\circ\text{C}} \text{38 (72%)}
\]

**Scheme 12. Synthesis of N-tosyl benzaldimine (38)**

N-tosyl benzaldimine (38)\(^{51}\) was prepared following the method reported in literature by treating TiCl\(_4\) to a ice-cooled solution of benzaldehyde, tosylamide and TEA in CH\(_2\)Cl\(_2\) for 30 min to give 38 in 72% yield. Imine 38 was fully characterized by comparison of its melting point and spectral data with that of the reported data. The MS spectrum of 38 showed M+1 peak at m/z 260 whereas its \(^1\)H-NMR showed characteristic peaks at \(\delta\) 7.33-7.94 (9H, m, ArH), 9.03 (1H, s, CH=N), 2.44 (3H, s, PhCH\(_3\)) (Fig. 15).

**Figure 15. \(^1\)H-NMR of compound 38**
It is pertinent to note that in absence of such chelation in N-Tosyl imine 38, its reaction with titanium enolate of 28 afforded a diastereomeric mixture of 4α-products (39 and 40) in a ratio of 65:35 (by $^1$H-NMR) similar to the results observed in case of Li-enolate as reported by Young et al.$^{52}$ (Scheme 12).

![Scheme 12. Reaction of imine 38 with Ti enolate of 28](image)

The structure and stereochemistry of both the diastereomers 39 and 40 were assigned by comparison of their delta and coupling values with the reported analogues (41a and 41b).$^{52}$ Chromatography and recrystallisation gave the pure major isomer 39 in 23% yield and impure 40 but the spectrum of minor isomer was obtained by subtraction (Table 3 and 4).

![Table 3. Comparison of $^1$H-NMR data b/w 41a and 39](image)

<table>
<thead>
<tr>
<th>Type of proton</th>
<th>reported (40a)</th>
<th>observed (39)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\delta$</td>
<td>multiplicity</td>
</tr>
<tr>
<td>NH</td>
<td>6.37</td>
<td>d</td>
</tr>
<tr>
<td>H-2</td>
<td>4.46</td>
<td>dd</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 4. Comparison of $^1$H-NMR data b/w 41b and 40

<table>
<thead>
<tr>
<th>Type of proton</th>
<th>reported (41b)</th>
<th>observed (40)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\delta$</td>
<td>multiplicity</td>
</tr>
<tr>
<td>NH</td>
<td>6.78</td>
<td>d</td>
</tr>
<tr>
<td>H-2</td>
<td>4.17</td>
<td>dd</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-6</td>
<td>4.61</td>
<td>dd</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-4</td>
<td>3.21</td>
<td>m</td>
</tr>
<tr>
<td>H-3S</td>
<td>1.90</td>
<td>m</td>
</tr>
<tr>
<td>H-3R</td>
<td>2.05</td>
<td>m</td>
</tr>
</tbody>
</table>

The high exo-stereoselectivity observed in the reaction of imine 37 with lithium enolate of 28 is indeed surprising and hence it appeared interesting to study the condensation of unsubstituted N-Boc imine 43 (Scheme 13) with lithium enolate of 28.

**Scheme 13.** Unsuccessful efforts to tert-butyl methylenecarbamate (43)
We attempted to prepare the N-Boc imine 43 employing the same strategy as described for N-Boc benzaldimine (27) ie synthesis of sulfone corresponds to formaldehyde followed by its elimination with base (Scheme 13). Treatment of 30% aq. formaldehyde solution with benzene sulfinic acid sodium salt, tert-butyl carbamate in the presence of formic acid for 3 d gave sulfone 42 as white solid in 86.4% yield which was fully characterized by MS, NMR and IR. The MS spectrum of 42 showed \([M+NH_4]^+\) peak at \(m/z\) 298 whereas \(^1\)H-NMR showed characteristic peaks at \(\delta\) 7.53-7.94 (5H, m, ArH), 5.42 (1H, br, NH), 1.26 (9H, s, C(CH\(_3\)_3)) (Fig. 16).

Elimination of sulfone with K\(_2\)CO\(_3\) however does not give the desired imine 43 and the reaction was ended up with complex mixture which was difficult to separate. Having met with failure with K\(_2\)CO\(_3\), we have switched to other bases like NaH and LiHMDS (Scheme 13). Thus 45 was treated with NaH or LiHMDS in THF at different temperatures, but in both the cases no product could be observed. Thus all our
approach towards the synthesis of Penmacric acid Chapter 3

Attempts to isolate the imine 43 were unsuccessful. Intrigued by the instability of 43 it was decided to generate the imine 43 in situ from the sulfone 42 with LiHMDS. The addition of Lithium enolate of pyroglutame 28, generated by treatment with 2.3 equiv of LiHMDS at -78 °C with the sulfone 42 (1.1 equiv) at same temperature, expectedly found to be non-stereoselective, giving an almost 1:1 ratio of 4α- and 4β-products 44 and 45, respectively (Scheme 14) as judged by integration of signals in the 1H-NMR spectrum.

Scheme 14. Reaction of sulfone 42 with Li enolate of 28

Both the diastereomers were fully characterized by their spectral data ie MS, IR and NMR (Table 5).

Table 5. Spectral data of compound 44 and 45

<table>
<thead>
<tr>
<th>compound No.</th>
<th>IR cm⁻¹</th>
<th>mp °C</th>
<th>1H NMR (CDCl₃)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>NH</td>
</tr>
<tr>
<td>44</td>
<td></td>
<td></td>
<td>δ</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>br</td>
</tr>
<tr>
<td></td>
<td>1772</td>
<td>1707</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>multi-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>plicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>J (Hz)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>J₂,₃₅</td>
<td>8.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>J₂,₃₈</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td></td>
<td></td>
<td>δ</td>
</tr>
<tr>
<td></td>
<td>1786</td>
<td>1784</td>
<td>106</td>
</tr>
<tr>
<td></td>
<td>multi-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>plicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>br</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>dd⁵</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>s</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>m/m</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>m</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>m, m</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>s</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>s</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*unresolved dd
With both the isomers in hand after chromatographic separation, 4-β stereochemistry of the isomer 45 was assigned using NOE experiments in CDCl₃, by the irradiation of H-2, H-3 and H-4 (Fig. 17). This experiment was similar to the one on intermediate 29. First we determined the spatial orientation of hydrogens at C-3. The proton at 2.51 ppm was assigned as H-3S by its enhancement of 5.8% on irradiating the signal for H-2 at 4.5 ppm, and irradiation of H-4 at 2.75 ppm gave an enhancement of 3.5% to H-3S at ppm. Also, an 2.3% enhancement of H-4 was also observed on
Irradiation of H-2. All these results indicated that N-Boc aminomethyl moiety and β-oriented H-3R have a cis-relationship.

In contrast to the results obtained with Li enolate, reaction of chlorotitanium enolate of 28 with imine 42 gave predominantly 4α-isomer 44 in 67% yield (Scheme 15). Incidentally, this also constitutes a one step synthesis of 44 and 45 as intermediates for (4S) and (4R) 4-aminomethyl glutamic acid respectively serving as glutamate transport inhibitors which were otherwise have been synthesized in multisteps.58

![Scheme 15. Reaction of sulfone 42 with Ti enolate of 28](image)

3.5. Conclusion

In conclusion, we have accomplished a short formal synthesis of three epimers of penmacric acid, and have shown dependency of the stereochemical outcome in the reaction of imines with Li enolates of pyrogluatmate, to the steric bulk of substituents. On the other hand, chelation seems to be predominant factor in reaction with titanium enolates.
3.6. Experimental

3.6.1. General methods

All enolate reactions were done using flame-dried glassware under nitrogen atmosphere. THF was distilled from sodium/benzophenone ketyl immediately before use. Triethylamine, \(N, N\)-diisopropylethylamine and dichloromethane were distilled over calcium hydride. \(N\)-bromosuccinimide was recrystallised from water prior to use. Reactions were monitored by thin layer chromatography (TLC) using 0.25 mm E. Merck pre-coated (Merch 60 F\(_{254}\)) silica gel plates using ninhydrine as visualizing agent. Purification was performed by flash chromatography using silica gel (230-400 mesh). NMR spectra were recorded either on a Bruker Advance-300 or Advance-400 spectrometers. Chemical shifts are reported as ppm (delta) relative to TMS as internal standard. Mass spectra were recorded on LCQ Advantage MAX (ESI), JOEL JMS-600H (EI/HRMS) and Accu. TOF JMS T100 LC (DART/HRMS) mass spectrometer. IR spectra were recorded on a Perkin-Elmer FT-IR RXI spectrometer. Optical rotations were determined on an Autopol III polarimeter. Unit cell determination and intensity data collection \((2\theta = 50^\circ)\) was performed on a Bruker Smart Apex diffractometer with CCD area detector at 293 K. Structure solutions by direct methods and refinements by full-matrix least-squares methods on \(F^2\), \textit{XSCANS} [Siemens Analytical X-ray Instrument Inc.: Madison, Wisconsin, USA 1996] was used for data collection and reduction. \textit{SHELXL-NT} [Bruker AXS Inc.: Madison, Wisconsin, USA 1997] was used for structure refinement.

3.6.2. Experimental details

Two-step preparation of N-tert-butoxycarbonyl benzaldimine (27)

\textbf{Step 1:} tert-Butyl carbamate (2.93 g, 25 mmol) and benzenesulfonic acid sodium salt (8.2 g, 50 mmol) were taken into a 250 ml round bottom flask and dissolved in a mixture of methanol (25 ml) and water (50 ml). Benzaldehyde (50 mmol) was then added, followed
by formic acid (1.9 ml) and the reaction mixture was stirred for 3 days at room temperature. The resulting white solid was filtered off and washed with water and ether, redissolved in dichloromethane, dried over Na₂SO₄, and concentrated under reduced pressure to afford the respective α-amido benzenesulphone (26) as white solid.

**Step 2:** An oven dried round bottom flask was charged with anhydrous potassium carbonate (16 g) and dry THF (10 ml/mmol) under nitrogen atmosphere. Then, the α-amido benzenesulphone 26 (19.4 mmol) was added and the mixture was heated to reflux with stirring under a nitrogen atmosphere for 17 h. The mixture was then cooled to room temperature, filtered through a short pad of celite and concentrated under reduced pressure. The residue was redissolved in dichloromethane, dried over Na₂SO₄, filtered and concentrated under reduced pressure to afford the corresponding N-Boc aldimine 27 in 90% yield (for two steps) which was kept under high vacuum at rt for several hours before use.

Colorless oil; IR (thin film, cm⁻¹) 2983, 1713, 1627, 1263, 1217, 1157; ¹H NMR (300 MHz, CDCl₃) δ 8.88 (1H, s, CH=N), 7.91-7.94 (2H, m, ArH), 7.54-7.57 (1H, m, ArH), 7.48-7.50 (2H, m, ArH), 1.61(9H, s, (CH₃)₃CO); ¹³C NMR (75 MHz, CDCl₃) δ 169.8, 162.7, 134.1, 133.6, 130.3, 128.9, 82.4, 28.0; MS (ESI): m/z 205, found 205[M+H]⁺, 106[M-C₂H₆O₂]⁺.

**Methyl(25,4RS)-N-tert-butoxycarbonyl-4-[(6RS)-N-tert-butoxycarbonylamidobenzyl]pyroglutamate (29, 30, 31)**

**Lithium enolate**

Under nitrogen atmosphere and at -78 °C, LiHMDS (1.0 M in THF, 7.63 ml, 7.64 mmol) was added slowly to the magnetically stirred solution of Methyl (25)-N-tert-butoxycarbonypyroglutamate 28 (1.54 g, 6.36 mmol) in freshly distilled THF (30 ml), and the reaction mixture was stirred for 1 h at -78 °C. A solution of N-Boc aldimine 27 (1.69 g, 8.27 mmol) in tetrahydrofuran (20 ml) was added dropwise to the reaction mixture and stirring was continued for additional 2 h at -78 °C. The reaction mixture was
quenched with saturated aqueous ammonium chloride (15 ml), allowed to warm to room temperature, diluted with water (25 ml), and extracted with ethyl acetate (3 x 25 ml). The combined organic layer was washed with brine (25 ml), dried (Na₂SO₄), and concentrated in vacuo to give a mixture of three diastereoisomers 29, 30 and 31 (2.48 g, 87%) as white foam which was flash chromatographed on a silica gel column using ethyl acetate-hexane (30:70) as eluant to give a mixture of 29, 30 (83:17) and pure 31 as clear viscous oil (0.68 g, 23.8%). Repeated recrystallisations of the mixture of 29 & 30 from diethyl ether gave a pure sample of 29 as distinct crystalline white solid (0.82 g, 29%) and 30 as clear viscous oil (0.19 g, 7.0%).

**Titanium enolate**

A solution of TiCl₄ (1.0 M in CH₂Cl₂, 1.16 ml, 1.16 mmol,) was added dropwise to a stirred solution of 28 (0.26 g, 1.07 mmol) in anhydrous CH₂Cl₂ (10 ml) at -78 °C under nitrogen, giving a yellow slurry. After 5 minutes, N,N-diisopropylethylamine (0.37 ml, 2.14 mmol) was added slowly over a period of 15 min. The resulting violet colored solution was stirred at -78 °C under nitrogen for 1.5 h, a solution of N-Boc aldimine 27 (0.26 g, 1.28 mmol) in CH₂Cl₂ (5 ml) was added to it over a period of 10 mins, and the stirring was continued at -78 °C for 2 h. The reaction was quenched by addition of saturated aqueous ammonium chloride (5 ml), allowed to warm to room temperature, diluted with water (15 ml), and extracted with CH₂Cl₂ (3 x 10 ml). The combined organic extracts were washed with brine (2x10 ml), dried (Na₂SO₄) and concentrated in vacuo to give a white foam which was purified using flash chromatography over a silica gel column using ethyl acetate-hexane as eluant (30:70) to give pure 29 as white crystalline solid (0.34 g, 72%).

**Methyl(2S,4S)-N-tert-butoxycarbonyl-4-[(6R)-N-tert-butoxycarbonylamidobenzyl]pyroglutamate (29)**

Colorless crystalline solid; mp 172-175 °C; [found: C, 61.51; H, 7.12; N, 6.32. C₂₃H₃₂N₂O₇ requires C, 61.59; H, 7.19; N, 6.25%];
Approach towards the synthesis of Penmacric acid Chapter 3

$R_f$ (30% EtOAc/hexane) 0.35; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.26-7.35 (5H, m, ArH), 5.64-5.67 (1H, d, $J_{6,\text{NH}}$ 7.2 Hz, NH), 4.87-4.91 (1H, dd, $J_{6,4}$, 4.9 Hz, H-6), 4.46-4.49 (1H, d, $J_{2,3}$ 8.6 Hz, H-2), 3.75 (3H, s, CO$_2$CH$_3$), 3.13-3.21 (1H, m, H-4), 2.14-2.26 (1H, m, H-3S), 2.03-2.10 (1H, m, H-3R), 1.48 (9H, s, (CH$_3$)$_3$CO); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 172.6, 171.6, 155.7, 149.1, 140.3, 128.7, 127.0, 84.0, 80.0, 56.9, 54.9, 52.7, 46.9, 28.3, 27.9, 26.1; IR (thin film, cm$^{-1}$) 2980, 1791, 1753, 1717, 1500, 1456, 1370, 1314, 1156, 1047; MS (ESI): m/z 448, found 471 [M+Na]$^+$; HRMS (DART): m/z calcld. for C$_{23}$H$_{33}$N$_2$O$_7$ [M+H]$^+$: 449.2287; found: 449.2271; $[^\alpha]_{D}^{20}$ = -10.3 (c 0.81, CHCl$_3$).

Methyl(2S,4S)-N-tert-butoxycarbonyl-4-{[(6S)-N-tert-butoxycarbamimidobenzyl]pyroglutamate (30)

Clear viscous oil; $R_f$ (30% EtOAc/hexane) 0.35; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.27-7.34 (5H, m, ArH), 6.64 (1H, br, NH), 4.91-4.94 (1H, dd, $J_{6,4}$, 4.9 Hz, H-6), 4.09-4.12 (1H, d, $J_{6,\text{NH}}$ 9.1 Hz, $J_{6,NH}$ 4.8 Hz, H-6), 4.09-4.12 (1H, d, $J_{6,\text{NH}}$ 9.1 Hz, H-6), 3.74 (3H, s, CO$_2$CH$_3$), 3.18-3.24 (1H, m, H-4), 2.06-2.12 (1H, m, H-3R), 1.91-2.00 (1H, m, H-3S), 1.45 (9H, s, (CH$_3$)$_3$CO), 1.39 (9H, s, (CH$_3$)$_3$CO); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 173.8, 171.4, 155.0, 148.8, 138.3, 128.7, 128.0, 127.9, 83.9, 79.7, 57.0, 54.6, 52.7, 46.1, 28.3, 27.8, 25.1; IR (thin film, cm$^{-1}$) 3021, 2366, 1787, 1751, 1700, 1456, 1370, 1313, 1217, 1157; MS (ESI): m/z 448, found 471[M+Na]$^+$; HRMS (DART): m/z calcld. for C$_{23}$H$_{33}$N$_2$O$_7$ [M+H]$^+$: 449.2287; found: 449.2271; $[^\alpha]_{D}^{20}$ = -10.3 (c 0.81, CHCl$_3$).

Methyl(2S,4R)-N-tert-butoxycarbonyl-4-{[(6R)-N-tert-butoxycarbamimidobenzyl]pyroglutamate (31)

Clear viscous oil; [found: C, 61.45; H, 7.26; N, 6.22. C$_{23}$H$_{32}$N$_2$O$_7$ requires C, 61.59; H, 7.19; N, 6.25%]; $R_f$ (30% EtOAc/hexane) 0.31; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.26-7.32 (5H, m, ArH), 6.50 (1H, br, NH), 4.92-4.97 (1H, dd, $J$ 8.8, 4.9 Hz, H-6), 4.39-4.45 (1H, dd (unresolved), H-2), 3.57 (3H, s, CO$_2$CH$_3$), 3.12-3.20 (1H, m, H-4), 2.36-2.47 (1H, m, H-3),
Approach towards the synthesis of Penmacric acid

Chapter 3

1.64-1.67 (1H, b m, H-3), 1.46 (9H, s, (CH$_3$)$_3$CO), 1.38 (9H, s, (CH$_3$)$_3$CO); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 173.4, 171.1, 155.1, 148.8, 138.4, 128.7, 127.9, 127.0, 84.1, 79.7, 57.0, 54.6, 52.5, 46.9, 28.3, 27.9, 24.0; IR (thin film, cm$^{-1}$) 2923, 1788, 1751, 1709, 1497, 1455, 1370, 1313, 1217, 1157, 1028; MS (ESI): m/z 448, found 471 [M+Na]$^+$; HRMS (ESI): m/z calcd. for C$_{23}$H$_{33}$N$_2$O$_7$ [M+H]$^+$: 449.2287; found: 449.2315; [α]$_D^{29}$ = +22.5 (c 0.88, CHCl$_3$).

General procedure for Methyl (2S,4RS)-N-tert-butoxycarbonyl-4-[(6RS)-methoxycarbonyl-N-tert-butoxycarbonylaminomethyl]pyroglutamate (32, 33, 34)

To a stirred solution of 31 (0.22 g, 0.51 mmol) in a mixture of CH$_3$CN (4 ml) and CCl$_4$ (4 ml) was added a solution of NaIO$_4$ (1.14 g, 5.34 mmol) in water (7 ml) and the stirring was continued for additional 15 min. RuCl$_3$.3H$_2$O (10 mg, cat.) was added to it and the reaction mixture was vigorously stirred for 12 h. CH$_2$Cl$_2$ (15 ml) was added and the aqueous layer was extracted with CH$_2$Cl$_2$ (3 × 10 ml) and dried (Na$_2$SO$_4$). The residue obtained after concentration was redissolved in THF (5 ml) to which a solution of diazomethane in ether was added with swirling. Water (15 ml) was added to it and extracted with ethyl acetate (3 × 15 ml). The combined organic phases were dried (Na$_2$SO$_4$) and concentrated in vacuo to give a colorless oil (0.094 g, 43%); purified by flash column chromatography on silica gel using ethyl acetate-hexane as eluant (45:65) to give 32 as viscous oil (0.078 g, 35.6%) which was crystallised from diethyl ether to white solid after standing overnight.

Methyl(2S,4R)-N-tert-butoxycarbonyl-4-[(6R)methoxycarbonyl-N-tert-butoxycarbonylaminomethyl]pyroglutamate (32)

White solid; mp 122-125 °C; [found: C, 53.11; H, 7.09; N, 6.57. C$_{19}$H$_{30}$N$_2$O$_9$ requires C, 53.02; H, 7.02; N, 6.51%]; R$_f$ (50% EtOAc/hexane) 0.4; $^1$H NMR (300 MHz, CDCl$_3$) δ 5.67 (1H, br, NH), 4.50-4.61 (2H, m, H-6+H-2), 3.80 (3H, s, CO$_2$CH$_3$), 3.77 (3H, s, CO$_2$CH$_3$), 3.04-3.12 (1H, m, H-4), 2.51-
Approach towards the synthesis of Penmacric acid

Chapter 3

2.61 (1H, m, H-3), 2.04-2.13 (1H, b m, H-3), 1.50 (9H, s, (CH\(_3\))\(_3\)CO), 1.45 (9H, s, (CH\(_3\))\(_3\)CO); 
\(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 171.1, 170.3, 169.5, 154.2, 147.9, 83.0, 79.3, 56.0, 51.9, 51.6, 51.5, 44.4, 27.1, 26.7, 23.0; IR (thin film, cm\(^{-1}\)) 2960, 2923, 1789, 1750, 1717, 1501, 1457, 1396, 1259, 1156, 1027; MS (ESI): m/z 430, found 453 \([\text{M}+\text{Na}]^+\); HRMS (DART): m/z calcld. for C\(_9\)H\(_{15}\)N\(_2\)O\(_5\) [M-C\(_8\)H\(_{15}\)O\(_4\)]\(^+\): 231.0981; found: 231.1008; \([\alpha]\)\(^{29}\)D = -7.7 (c 0.31 CHCl\(_3\)).

Methyl(2S,4S)-N-tert-butoxycarbonyl-4-[(6R)-methoxycarbonyl-N-tert-butoxycarbonylaminomethyl] pyroglutamate (33)

Colorless crystalline solid; 36.2%; mp 137-139 °C; [found: C, 53.09; H, 7.12; N, 6.48. C\(_{19}\)H\(_{30}\)N\(_2\)O\(_9\) requires C, 53.02; H, 7.02; N, 6.51%]; \(R_f\) (50% EtOAc/hexane) 0.62; \(^1\)H NMR (400 MHz, C\(_6\)D\(_6\)) \(\delta\) 5.37-5.39 (1H, d, \(J_{\text{NH},5} \) 8.9 Hz, NH), 4.65-4.57 (1H, dd, \(J_{\text{NH},6} \) 2.6 Hz, H-6), 4.27-4.30 (1H, dd, \(J_{2,3R} \) 9.8 Hz, H-2), 3.41-3.47 (1H, m, H-3), 3.26 (3H, s, CO\(_2\)CH\(_3\)), 1.98-2.07 (1H, m, H-3), 1.63-1.68 (1H, m, H-3R); 1.36 (9H, s, (CH\(_3\))\(_3\)CO), 1.34 (9H, s, (CH\(_3\))\(_3\)CO); \(^{13}\)C NMR (100 MHz, C\(_6\)D\(_6\)) \(\delta\) 171.5, 171.3, 170.7, 156.7, 150.2, 83.3, 80.0, 56.9, 52.6, 52.3, 51.9, 45.6, 28.2, 27.8, 25.0; IR (thin film, cm\(^{-1}\)) 3021, 2981, 1790, 1759, 1750, 1717, 1501, 1370, 1316, 1216, 1154; MS (ESI): m/z 430, found 453 \([\text{M}+\text{Na}]^+\); HRMS (EI): m/z calcld. for C\(_{19}\)H\(_{30}\)N\(_2\)O\(_9\) [M]: 430.1951; found: 430.1977; \([\alpha]\)\(^{29}\)D = -65.0 (c 0.32 CHCl\(_3\)).

Methyl(2S,4S)-N-tert-butoxycarbonyl-4-[(6S)-methoxycarbonyl-N-tert-butoxycarbonylaminomethyl] pyroglutamate (34)

White foam; 23%; \(R_f\) (50% EtOAc/hexane) 0.53; \(^1\)H NMR (400 MHz, C\(_6\)D\(_6\)) \(\delta\) 5.78 (1H, br, NH), 4.65-4.69 (1H, dd, \(J_{6,4} \) 9.4 Hz, \(J_{5,\text{NH}} \) 4.2 Hz, H-6), 4.42-4.45 (1H, dd, \(J_{2,3R} \) 9.8 Hz, \(J_{2,3R} \) 1.4 Hz, H-2), 3.26 (3H, s, CO\(_2\)CH\(_3\)), 3.20 (3H, s, CO\(_2\)CH\(_3\)), 2.95-2.99 (1H, m, H-4), 2.03-2.11 (1H, m, H-3S), 1.66-1.72
(1H, ddd, J_{3R,3S} 10.5 Hz, J_{3R,4} 9.2 Hz, J_{3R,2} 1.4 Hz, H-3R); $^{13}$C NMR (100 MHz, CD$_3$OD) δ 172.1, 171.6, 171.1, 155.4, 150.3, 83.2, 79.8, 57.1, 53.3, 52.08, 52.05, 45.3, 28.3, 27.9, 25.0; IR (thin film, cm$^{-1}$) 3020, 2979, 1789, 1749, 1718, 1501, 1370, 1316, 1216, 1154; MS (ESI): m/z 430, found 453 [M+Na$^+$]; HRMS (EI): m/z calcd. for C$_{19}$H$_{30}$N$_2$O$_9$ [M$^+$]: 430.1951; found: 430.1984; [α]$^D$ = +3.3 (c 0.56, CHCl$_3$).

Methyl(25,45)-N-tert-butoxycarbonyl-4-[(6RS)-methoxycarbonyl-N-tert-butoxycarbonylaminomethyl] pyroglutamate (33 and 34) [Scheme 10]

Lithium enolate

$N$-bromosuccinimide (0.56 g, 3.16 mmol) was added to a stirred solution of Methyl $N$-tert-butoxycarbonylglycinate (35)$^{27}$ (0.57 g, 3.02 mmol) in CCl$_4$ (10 ml) under nitrogen atmosphere. The stirred solution was illuminated with 500 W lamp for 1 h, keeping the reaction mixture between 10-30 ºC using a water bath. The resulting orange reaction mixture was then filtered and the filtrate was concentrated in vacuo to afford the crude Methyl 2-Bromo-$N$-tert-butoxycarbonylglycinate (36) as yellow oil. To the crude bromide 36 in THF (6 ml) at -78 ºC under nitrogen was added anhydrous triethylamine (0.46 ml, 3.30 mmol) with stirring and the reaction mixture was stirred for 45 min at -78 ºC to afford a crude imine 37$^{25}$ solution. Meanwhile to a stirred solution of pyroglutamate 28 (0.67 g, 2.75 mmol) in THF (12 ml) was slowly added LiHMDS (1.0 M in THF, 3.44 ml, 3.44 mmol) at -78 ºC and the reaction mixture was stirred for 1 h. The cold crude enolate solution was then quickly added to crude imine solution via cannula and stirred for another 2 h at -78 ºC. Saturated aqueous ammonium chloride (6 ml) was added and the reaction mixture was allowed to warm to room temperature; water (15 ml) was added and was extracted with ethyl acetate (3 × 15 ml). The combined organic extracts were washed with brine (15 ml), dried (Na$_2$SO$_4$) and concentrated in vacuo to give a mixture of two diastereoisomers (33 and 34) as yellow foam (0.84 g, 71%) which was purified by flash column chromatography on silica gel, using ethyl acetate-hexane.
Titanium enolate

28 (0.29 g, 1.21 mmol) was dissolved in anhydrous CH₂Cl₂ (10 ml) under nitrogen with stirring, cooled to -78 °C and TiCl₄ (1.0 M in CH₂Cl₂, 1.32 ml, 1.32 mmol) was added dropwise followed by slow addition of DIPEA (0.25 ml, 1.46 mmol) after 5 min, and the resultant deep violet enolate solution was stirred for 1.5 h at -78 °C. Meanwhile a fresh imine 37 solution (-78°C) was prepared in anhydrous CH₂Cl₂ (6 ml) as described above, quickly added to enolate solution via cannula and the reaction mixture was stirred for another 2 h at -78°C. The reaction mixture was then quenched with saturated aqueous ammonium chloride (10 ml), allowed to warm to room temperature, diluted with water (15 ml) and extracted with dichloromethane (3 x 15 ml). The combined organic extracts were washed with brine (15 ml), dried (Na₂SO₄), and concentrated in vacuo to give the crude product as yellowish foam (0.37 g, 71.7%) which was purified by flash column chromatography on silica using ethyl acetate-hexane as eluant (40:60) to give 34 as a white foamy solid (0.32 g, 62.3%).

N-tert-para-toluenesulfonylamidobenzyl benzaldimine (38)

A solution of TiCl₄ (1.0 M in CH₂Cl₂, 9.48 ml, 9.5 mmol) was added dropwise to a stirred, ice cooled solution of benzaldehyde (1.92 ml, 19 mmol), p-toluenesulfonamide (3.25 g, 19 mmol) and triethylamine (7.94 ml, 57 mmol) in anhydrous CH₂Cl₂ (40 ml) and the mixture was stirred for 30 min. The titanium dioxide was removed by suction filtration through celite and washed with CH₂Cl₂ (20 ml). Evaporation of the solvent under reduced pressure afforded a white solid which was broken up by refluxing in anhydrous diethyl ether (75 ml) for 15 min. The residual triethylamino hydrochloride salt was removed by filtration and the filtrate was concentrated under reduced pressure to afford the crude imine. Purification by crystallization (hexane/CH₂Cl₂) afforded 38 as white solid (1.76 g, 72%).
White solid, mp 110 °C; \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 9.03 (1H, s, CH=N), 7.87-7.94 (4H, m, ArH), 7.59-7.64 (1H, t, \(J = 7.5\), ArH), 7.46-7.51 (2H, t, \(J = 7.3\), ArH), 7.33-7.36 (2H, d, \(J = 8.2\), ArH), 2.44 (3H, s, PhCH\(_3\)); \(^13\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 170.2, 144.7, 135.0, 131.3, 129.8, 129.2, 128.1, 21.7; IR (thin film, cm\(^{-1}\)) 1602, 1320, 1158, 1088; MS (ESI): \(m/z\) 259, found 260[M+H]+.

**Methyl(2S,4S)-N-tert-butoxycarbonyl-4-[(6RS)-N-para-toluenesulfonylamidobenzyl]pyroglutamate (39 and 40)**

Compounds 39 and 40 were obtained following the general procedure for 29, 30 & 31.

Titanium enolate

Purification by flash column chromatography on silica gel, using ethyl acetate-hexane (40:60) as eluant separated mixture of 39 and 40 (0.526 g, 85%) together with unreacted tosylamide. The major diastereoisomer 39 was separated and purified by repeated recrystallisation from diethyl ether which provided distinct crystalline white solid 39 (0.142 g, 23%). The minor isomer 40 could not be obtained pure but the spectrum was obtained by subtraction from the mixture.

**Methyl(2S,4S)-N-tert-butoxycarbonyl-4-[(6R)-N-para-toluenesulfonylamidobenzyl]pyroglutamate (39)**

White crystalline solid; mp 171-175 °C; [found: C, 59.70; H, 5.99; N, 5.61. C\(_{25}\)H\(_{30}\)N\(_2\)O\(_7\)S requires C, 59.75; H, 6.02; N, 5.57%]; \(R_f\) (40% EtOAc/hexane) 0.32; \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 7.46-7.49 (2H, d, \(J = 8.1\) Hz, ArH), 7.09-7.17 (7H, m, ArH), 6.45-6.47 (1H, d, \(J_{NH} = 4.0\) Hz, \(NH\)), 4.40-4.44 (1H, d, \(J_{2,3S} = 9.5\) Hz, H-2), 4.33-4.36 (1H, dd, \(J_{6,NH} = 4.0\) Hz, \(J_{6,4} = 6.9\) Hz, H-6), 3.71 (3H, s, CO\(_2\)CH\(_3\)), 3.01-3.10 (1H, m, H-4), 2.35 (3H, s, ArCH\(_3\)), 2.00-2.12 (1H, m, H-3S), 1.78-1.85 (1H, m, H-3R), 1.74 (9H, s, (CH\(_3\))\(_3\)CO); \(^13\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 173.0, 171.3, 148.8, 143.2, 137.2, 136.8, 129.3, 128.5, 128.1, 127.7, 127.4, 84.2, 59.0, 56.7,
Approach towards the synthesis of Penmacric acid

Chapter 3

52.7, 46.7, 27.9, 25.6, 21.5; IR (thin film, cm\(^{-1}\)) 2993, 1749, 1720, 1457, 1328, 1215, 1157, 1092; MS (ESI): m/z 502, found 525 [M+Na]\(^{+}\); HRMS (DART): m/z calcld. for C\(_{20}\)H\(_{23}\)N\(_{2}\)O\(_{5}\)S: 403.1327; found: 403.1281; [\(\alpha\)]\(_D\)\(^{28}\) = +77.5 (c 0.6, CHCl\(_3\)).

Methyl(25,45)-N-tert-butoxycarbonyl-4-[[65]-N-para-toluenesulfonylamidobenzyl]pyroglutamate (40 from 39+40)

\(R_f\) (40% EtOAc/hexane) 0.32; \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 7.42-7.45 (2H, d, J 8.2 Hz, ArH), 7.00-7.17 (7H, m, ArH), 6.75-6.78 (1H, d, J\(_{\text{NH}}\), 9.1 Hz, NH), 4.63-4.68 (1H, dd, J\(_{\text{NH}}\), 9.1 Hz, J\(_6\), 4.8 Hz, H-6), 4.13-4.17 (1H, dd, J\(_{2,3}\), 9.1 Hz, J\(_{2,3}\), 2.3 Hz, H-2), 3.73 (3H, s, CO\(_2\)CH\(_3\)), 3.16-3.24 (1H, m, H-4), 2.30 (3H, s, ArCH\(_3\)), 1.79-2.12 (2H, m, H-3S+H-3R), 1.43 (9H, s, (CH\(_3\))\(_3\)CO); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 173.6, 171.2, 148.6, 142.9, 137.7, 136.4, 129.2, 128.6, 128.0, 127.9, 126.9, 84.1, 57.7, 57.0, 46.5, 27.8, 24.7, 21.4; IR (thin film, cm\(^{-1}\)) 2933, 1749, 1720, 1457, 1327, 1263, 1214, 1156, 1092; MS (ESI): m/z 502, found 525 [M+Na]\(^{+}\).

tert-butyl phenylsulfonylmethylcarbamate (42)

A mixture of tert-butyl carbamate (1.5 g, 12.8 mmol) and benzenesulfinic acid sodium salt (4.20 g, 25.6 mmol) were suspended in a solution of methanol in water (1:2, 45 ml) followed by the addition of 30% aqueous formalin (2.56 ml, 25.6 mmol) and formic acid (98%, 1.5 ml). The reaction was allowed to stir for 3 days at room temperature during which time the product precipitated as a white solid. The crystalline sulfone 42 was filtered off with suction, washed with water and diethyl ether, redissolved in dichloromethane, dried over Na\(_2\)SO\(_4\), and concentrated under reduced pressure to afford the sulfone 42 (3.0 g, 86.4%).

White solid; mp 155-157 °C; \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 7.91-7.94 (1H, d, J 7.4 Hz, ArH), 7.53-7.68 (3H, m, ArH), 5.42 (1H, br, NH), 4.52-4.54 (2H, d, J 7.0 Hz, CH\(_2\)), 1.26 (9H, s, (CH\(_3\))\(_3\)CO); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 154.0, 137.0, 134.2, 129.3, 129.1, 81.2, 62.2, 28.1; IR (thin film, cm\(^{-1}\))
Approach towards the synthesis of Penmacric acid

Chapter 3

1703, 1477, 1448, 1421, 1364, 1291, 1142, 1087, 1007; MS (ESI): m/z 271, found 289

Methyl (2S,4RS)-N-tert-butoxycarbonyl-4-(N-tert-butoxycarbonylaminomethyl)pyroglutamate (44 and 45)

Lithium enolate
LiHMDS (1.0 M in THF, 6.14 ml, 6.14 mmol) was added dropwise to a solution of 28 (0.65 g, 2.672 mmol) in anhydrous THF (15 ml) at -78 °C under nitrogen atmosphere with stirring. The reaction mixture was stirred for 1.2 h, a solution of sulfone 42 (0.79 g, 2.93 mmol) in THF (15 ml) was added to it, and the stirring was continued for a further 2 h at -78 °C. The reaction was quenched with aqueous saturated ammonium chloride solution (15 ml), allowed to warm to room temperature, diluted with water (25 ml), and extracted with ethyl acetate (3 x 25 ml). The combined organic extracts were washed with brine (15 ml) and dried (Na₂SO₄). Removal of the solvent in vacuo gave the product as a pale yellow foam (0.73 g, 74%) which was purified by flash column chromatography on silica gel using ethyl acetate-hexane (45:65) as eluant to give 44 (0.34 g, 35%) and 45 (0.33 g, 33.2%) as viscous oils which crystallised to white solid on standing overnight.

Titanium enolate
TiCl₄ (1.0 M in CH₂Cl₂, 3.3 ml, 3.3 mmol) was added dropwise to a solution of 28 (0.35 g, 1.43 mmol) in anhydrous CH₂Cl₂ (10 ml) at -78 °C under nitrogen atmosphere with stirring followed after 5 mins by dropwise addition of DIPEA (0.62 ml, 3.59 mmol) resulting into a deep violet colored solution. The reaction mixture was stirred for 1.5 h at -78 °C and a solution of sulfone 42 (0.42 g, 1.58 mmol) in CH₂Cl₂ (10 ml) was added (warm to dissolve). After stirring for 2 h at the same temperature, the reaction was quenched with aqueous saturated ammonium chloride solution (12 ml) and the mixture was allowed to warm to room temperature. Water (15 ml) was added and the product was extracted with dichloromethane (3 x 20 ml). The combined organic extracts were
washed with brine (10 ml), dried (Na₂SO₄) and concentrated in vacuo to yield the crude product 44 (0.43 g, 82%) which was purified by flash column chromatography on silica gel, using ethyl acetate-hexane (45:65) as eluant to give 44 (0.358 g, 67%) as clear oil which crystallised on standing overnight.

Methyl (2S,45)-N-tert-butoxycarbonyl-4-(N-tert-butoxycarbonylaminomethyl)pyroglutamate (44)

White solid; mp 95-97 °C; [found: C, 54.88; H, 7.45; N, 7.61. C₂₃H₃₂N₂O₇ requires C, 54.83; H, 7.58; N, 7.52%]; Rₐ (70% EtOAc/hexane) 0.5; ¹H NMR (300 MHz, CDCl₃) δ 5.15 (1H, br, NH), 4.56-4.60 (1H, dd, J₂,3.5 8.9 Hz, J₂,3.1 1.7 Hz, H-2), 3.78 (3H, s, CO₂CH₃), 3.46-3.52 (1H, m, H-6), 3.30-3.36 (1H, m, H-6), 2.77-2.85 (1H, m, H-4), 2.06-2.23 (2H, m, H-3R+H-35), 1.50 (9H, s, (CH₃)₃CO), 1.42 (9H, s, (CH₃)₃CO); ¹³C NMR (75 MHz, CDCl₃) δ 174.4, 171.6, 156.3, 83.9, 79.5, 57.0, 52.7, 42.7, 39.5, 28.3, 27.9, 25.6; IR (thin film, cm⁻¹) 2928, 1729, 1707, 1520, 1459, 1374, 1312, 1258, 1217, 1158; MS (ESI): m/z 372, found 395 [M+Na]⁺; HRMS (DART): m/z calcd. for C₁₇H₂₅N₂O₇ [M+H]⁺: 373.1974; found: 373.2010; [α]₂² = -40.2 (c 0.28, CHCl₃).

Methyl (2S,4R)-N-tert-butoxycarbonyl-4-(N-tert-butoxycarbonylaminomethyl)pyroglutamate (45)

White solid; mp 105-107 °C; [found: C, 54.91; H, 7.53; N, 7.49. C₂₃H₃₂N₂O₇ requires C, 54.83; H, 7.58; N, 7.52%]; Rₐ (70% EtOAc/hexane) 0.43; ¹H NMR (300 MHz, CDCl₃) δ 5.17 (1H, br, NH), 4.47-4.53 (1H, dd (unresolved), H-2), 3.77 (3H, s, CO₂CH₃), 3.46-3.52 (1H, m, H-6), 3.30-3.38 (1H, m, H-6), 2.70-2.80 (1H, m, H-4), 2.46-2.56 (1H, m, H-35), 1.74-1.84 (1H, m, H-3R), 1.49 (9H, s, (CH₃)₃CO), 1.42 (9H, s, (CH₃)₃CO); ¹³C NMR (75 MHz, CDCl₃) δ 174.2, 171.7, 156.3, 149.2, 84.1, 79.6, 57.4, 52.6, 43.4, 40.3, 28.4, 27.9, 25.2; IR (thin film, cm⁻¹) 3020, 1786, 1748, 1710, 1511, 1369, 1308, 1216, 1156; MS (ESI): m/z 372, found 395 [M+Na]⁺; HRMS (DART): m/z calcd. for C₁₇H₂₅N₂O₇ [M+H]⁺: 373.1974; found: 373.1982; [α]₂² = -44.1 (c 0.2, CHCl₃).
3.7. **Spectral data**

**Figure 18.** 1H-NMR of compound 29

**Figure 19.** 13C-NMR of compound 29
Approach towards the synthesis of Penmacric acid

Figure 20. $^1$H-NMR of compound 30

Figure 21. $^{13}$C-NMR of compound 30
Approach towards the synthesis of Penmacric acid

Chapter 3

Figure 22. $^1$H-NMR of compound 31

Figure 23. $^{13}$C-NMR of compound 31
Approach towards the synthesis of Penmacric acid

Chapter 3

Figure 24. $^1$H-NMR of compound 32

Figure 25. $^{13}$C-NMR of compound 32
Approach towards the synthesis of Penmacric acid

Chapter 3

Figure 26. \(^1\)H-NMR of compound 33

Figure 27. \(^{13}\)C-NMR of compound 33

162
Approach towards the synthesis of Penmacric acid

Chapter 3

Figure 28. $^1$H-NMR of compound 34

Figure 29. $^{13}$C-NMR of compound 34
Approach towards the synthesis of Penmacric acid

Figure 30. $^1$H-NMR of compound 39

Figure 31. $^{13}$C-NMR of compound 39
Approach towards the synthesis of Penmacric acid

Figure 32. $^1$H-NMR of compound 39+40

Figure 33. $^{13}$C-NMR of compound 39+40
Approach towards the synthesis of Penmacric acid

Chapter 3

Figure 34. DEPT-135 of compound 39+40

Figure 35. $^1$H-NMR of compound 44

166
Approach towards the synthesis of Penmacric acid

Figure 36. $^{13}$C-NMR of compound 44

Figure 37. $^1$H-NMR of compound 45
Approach towards the synthesis of Penmacric acid

Chapter 3

\[ \text{Figure 38. } ^{13} \text{C-NMR of compound 45} \]
3.8. References


13. (a) Marchalin, S.; Szemes, F.; Bar, N. and Decroix, B. *Heterocycles* 1999, 110, 8696  


24. (a) Ikota, N. *Tet. Lett.* 1992, 33, 2533  


30. For a review, see: (a) Nájera C.; Yus M.; *Tetrahedron: Asymmetry* 1999, 10, 2245;  
Approach towards the synthesis of Penmacric acid


