Synthesis and characterization of N-9-fluorenyl amino acid derivatives
Chapter 4

Synthesis and Characterization of N-9-fluorenyl amino acid derivatives

1. Introduction

Amines and amino acid compounds are found to have significant amount of applications in various pharmaceutical and synthetic applications. It is noted from the literature that 9-fluorenyl ligands derived from amino acid esters are of great importance and are studied in detail for the diethyl zinc\(^1\) addition to aldehyde. The preparation of amino alcohol synthesis from fluorenyl substituted amino ketones using sodium borohydride or the L-selectride at \(-78^\circ\text{C}\)\(^2\) are evidenced. The preparation of N-[9-(9-phenyl fluorenyl)-L-alaninal]\(^3\) is also described. The brief procedure for the preparation of N-alkylation of L-phenyl alanine methyl ester with 9-bromofluorene are reported\(^4\). The use of titanium tetra isopropoxide as a reagent for the Schiff base in dichloromethane is reported\(^5\) and as well the use of titanium tetrachloride for the Schiff base preparation using 2, 7 dibromo-9-(phenylethyl imino) fluorene are evidenced\(^6\). When the carbonyl moiety is used for the preparation of ketimines, forcing conditions like high reaction temperatures, non-stoichiometric amounts of reagents, protic or Lewis acids, long reaction times are generally required. More recent studies have shown that it is often possible to prepare ketimines by a room temperature condensation in the presence of drying reagents such as TiCl\(_4\)\(^7\). Such procedures have not proven successful in the case of hindered ketone especially when the method is extended to the preparation of ketimines using 9-fluorenone and L-amino acids.
The present work describes a convenient method to prepare the synthesis of N-9-fluorenyl amino acid methyl ester derivatives and subsequently reduced to obtain the corresponding amino alcohol. Synthesis of N-alkylation of different amino acid methyl ester listed in table 4.1 using organic base in aprotic solvent are studied. N-9-fluorenyl amino acid derivatives are further studied for their biological activity using different microorganism and molecular docking using P-glycoprotein in the chapter 5 and 6.
2. Reaction scheme

![Reaction scheme diagram]

Where in R is described as isopropyl, isobutyl, phenyl, methyl, secondary butyl which are listed in below table 4.1.

Table 4.1: List of L-amino acid methyl ester

<table>
<thead>
<tr>
<th>S. No</th>
<th>Amino ester compound</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>L-valine methyl ester</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>L-leucine methyl ester</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>L-isoleucine methyl ester</td>
<td>25</td>
</tr>
<tr>
<td>4</td>
<td>L-alanine methyl ester</td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td>L-phenyl alanine methyl ester</td>
<td>25</td>
</tr>
</tbody>
</table>

Reaction condition: Step-1 NaBH₄/ethanol at 0°C; step-2 POCl₃/N,N dimethyl aniline at 100°C Step-3: Hunig’s base/Acetonitrile at 80°C Step-4: 2.0M LAH in THF at -40°C to -50°C
3. Experimental

3.1 Material and methods

The key starting material 9-fluorenone, L-amino acids were obtained from catalogue suppliers and the other reagents used in the synthesis were procured from catalogue chemicals. L-amino acid methyl esters using L-amino acid, methanol and thionyl chloride were prepared in-house for the present study. The characterizations of the isolated product were done using Bruker Spin NMR instrument 300/400MHz for the structural elucidation. Mass spectral data were obtained using LC-MS by Agilent.
3.2 General procedure-1: Preparation of L-amino acid methyl ester

In a 500.0 mL reaction flask methanol (250.0 mL, 10.0 Vol) followed by L-amino acid (0.2 moles) was added and cooled the reaction mass to 0°C. Gradually thionyl chloride (0.25 moles) was added to the reaction mass and stirred at 0°C. The reaction mass was stirred at 25°C for 2 hrs and the progress of the reaction was monitored by TLC (Mobile phase: chloroform: methanol 9:1) using ninhydrin as staining agent. The reaction mass was distilled to remove most of the solvent and triturated with diethyl ether. The product was filtered under nitrogen atmosphere. The isolated yield was found to be quantitative. The L-amino acid methyl esters listed in table 4.1 were characterized by $^1$HNMR and used further.

3.2.1 Procedure for the preparation of L-valine methyl ester

In a 500.0 mL reaction flask methanol (250.0 mL, 10.0 Vol) followed by L-valine (23.4 g, 0.2 moles) was added and cooled the reaction mass to 0°C. Gradually thionyl chloride (18.2 mL, 0.25 moles) was added to the reaction mass and stirred at 0°C. The reaction mass was stirred at 25°C for 2h and the progress of the reaction was monitored by TLC. The reaction mass was distilled to remove most of the solvent and triturated with diethyl ether. The product was filtered under nitrogen atmosphere. The isolated yield was found to be quantitative.
3.3 General Procedure-2: N-alkylation of L-amino acid ester

In 500 mL reaction flask acetonitrile (250.0 mL, 10.0 Vol) followed by 9-chlorofluorene (25.0 g, 0.125 moles, 1.0 eq.) was added to get a clear solution at 25°C. L-amino acid methyl ester compound (0.137 moles, 1.1eq.), N,N-diisopropyl ethyl amine (40.45 g 0.313 moles, 2.5eq.) was added in one lot and stirred. The reaction mass slowly heated to reflux at 80°C and the reaction was maintained for 16 h. The progress of the reaction was monitored by TLC (Mobile phase: ethyl acetate: n-hexane 1:9). The reaction mass gradually cooled to 0-5°C and stirred for 30 min. The product was crystallized from the reaction mass and the slurry was filtered. The product was washed with chilled acetonitrile. The isolated product was dissolved in ethanol (125.0 mL, 5.0 Vol) at 60°C to get the clear solution. The solution was gradually cooled to 0-5°C to get the pure product. The product was dried under vacuum at 35-40°C and was characterized by $^1$HNMR, $^{13}$C NMR and LC-MS. The details of the compound prepared were listed in table 4.2.
Table 4.2: List of N-9fluorenyl derivatives of amino acid ester and amino alcohols

<table>
<thead>
<tr>
<th>Structure of the compound</th>
<th>Name of the compound</th>
<th>Designated compound</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Structure of the compound" /></td>
<td>2-(9H-fluoren-9-ylamino)-3-methyl-butyric acid methyl ester</td>
<td>Compound 5a</td>
<td>70.6</td>
</tr>
<tr>
<td><img src="image2.png" alt="Structure of the compound" /></td>
<td>2-(9H-fluoren-9-ylamino)-4-methyl-pentanoic acid methyl ester</td>
<td>Compound 5b</td>
<td>71.0</td>
</tr>
<tr>
<td><img src="image3.png" alt="Structure of the compound" /></td>
<td>2-(9H-fluoren-9-ylamino)-3-methyl-pentanoic acid methyl ester</td>
<td>Compound 5c</td>
<td>73.8</td>
</tr>
<tr>
<td><img src="image4.png" alt="Structure of the compound" /></td>
<td>2-(9H-fluoren-9-ylamino)-3-phenyl-propionic acid methyl ester</td>
<td>Compound 5d</td>
<td>70.0</td>
</tr>
<tr>
<td><img src="image5.png" alt="Structure of the compound" /></td>
<td>2-(9H-fluoren-9-ylamino)-propionic acid methyl ester</td>
<td>Compound 5e</td>
<td>69.0</td>
</tr>
<tr>
<td>Structure of the compound</td>
<td>Name of the compound</td>
<td>Designated compound</td>
<td>Yield %</td>
</tr>
<tr>
<td>---------------------------</td>
<td>----------------------</td>
<td>---------------------</td>
<td>---------</td>
</tr>
<tr>
<td><img src="image" alt="Structure of compound" /></td>
<td>2-(9H-fluoren-9-ylamino)-3-methyl-butanol</td>
<td>Compound 6a</td>
<td>28.0</td>
</tr>
<tr>
<td><img src="image" alt="Structure of compound" /></td>
<td>2-(9H-fluoren-9-ylamino)-4-methyl-pentanol</td>
<td>Compound 6b</td>
<td>25.3</td>
</tr>
<tr>
<td><img src="image" alt="Structure of compound" /></td>
<td>2-(9H-fluoren-9-ylamino)-3-methyl-pentanol</td>
<td>Compound 6c</td>
<td>28.6</td>
</tr>
<tr>
<td><img src="image" alt="Structure of compound" /></td>
<td>2-(9H-fluoren-9-ylamino)-3-phenyl-propanol</td>
<td>Compound 6d</td>
<td>32.6</td>
</tr>
<tr>
<td><img src="image" alt="Structure of compound" /></td>
<td>2-(9H-fluoren-9-ylamino)-propanol</td>
<td>Compound 6e</td>
<td>33.0</td>
</tr>
</tbody>
</table>
3.3.1 Procedure for the preparation of 2-(9H-fluoren-9-ylamino)-3-methyl-butyric acid methyl ester (Compound 5a)

In a 500 mL reaction flask, acetonitrile (250.0 mL, 10.0 Vol) followed by 9-chlorofluorene (25.0 g, 0.125 moles, 1.0 eq.) was added to get a clear solution at 25°C. L-valine methyl ester (22.97g, 0.137 moles, 1.1eq.), N,N-diisopropyl ethyl amine (40.45 g, 0.313 moles, 2.5eq.) was added in one lot and stirred. The reaction mass slowly heated to reflux at 80°C and the reaction was maintained for 16 h. The progress of the reaction was monitored by TLC (Mobile phase: ethyl acetate: n-hexane 1:9). The reaction mass gradually cooled to 0-5°C and stirred for 30mins. The product was crystallized from the reaction mass and the slurry was filtered. The product was washed with chilled acetonitrile. The isolated product was dissolved in ethanol (125.0 mL, 5.0 Vol) at 60°C to get the clear solution. The solution was gradually cooled to 0-5°C to get the pure product of 26.0 g with 70.6% yield. The product was dried under vacuum at 35-40°C and was characterized by $^1$HNMR, $^{13}$C NMR and LC-MS.

Note: Similar procedure was followed to prepare the analogous compounds 5b to 5e.

3.3.1.1 Characterisation data of the compounds

2-(9H-fluoren-9-ylamino)-3-methyl-butyric acid methyl ester (Compound 5a)

26.0g off-white solid, 70.6% yield; $[\alpha]_{D}^{20}=-113.43^\circ$ (C1.0, CHCl$_3$)
\[\text{\textsuperscript{1}H-NMR (300MHz, DMSO-}{d_6}) \ \delta \ 0.64-0.67 \ (d, J=6.72\text{Hz}, \ 3H), \ 0.82-0.85 \ (d, J=6.69\text{Hz}, \ 3H), \ 1.54-1.61(m, \ 1H), 2.22-2.28 \ (m,1H), \ 3.19 \ (s,3H), \ 3.23-3.28 \ (m,1H), \ 4.91- \ 4.93(d, J=3.9\text{Hz}, \ 1H),7.21-7.80 \ (m,8H).\]

\[\text{\textsuperscript{13}C-NMR (75 MHz, DMSO-}{d_6}) \ \delta \ 19.27,19.46, \ 32.17, \ 51.18, \ 62.15, \ 62.65, \ 120.27, \ 120.29, \ 125.7, \ 126.32, \ 127.02, \ 127.60, \ 127.86, \ 128.23, \ 128.53, \ 140.59, \ 140.91, \ 145.22, \ 176.33\]

m/z by LC-MS found to be 295.6 (+ve mode)

**2-(9H-fluoren-9-ylamino)-4-methyl-pentanoic acid methyl ester (Compound 5b)**

27.5g off-white solid, 71% yield; \([\alpha]^{20}_{D} \ -108.19^{\circ}\) (c 1.0, CHCl\(_3\));

\[\text{\textsuperscript{1}H-NMR (300MHz, DMSO-}{d_6}) \ \delta \ 0.5 \ (d, J=6.71\text{Hz}, \ 3H), \ 0.68 \ (d, J=6.69\text{Hz}, \ 3H), \ 1.06-1.34 \ (m, \ 3H), \ 1.59-1.66 \ (m, \ 1H), \ 2.61 \ (\text{broad s, 1H}), \ 3.20 \ (s, \ 3H), \ 4.89 \ (s, \ 1H), \ 7.22-7.80 \ (m,8H)\]

\[\text{\textsuperscript{13}C-NMR (75 MHz, DMSO-}{d_6}): \ \delta \ 22.19, \ 22.52, \ 23.17, \ 24.26, \ 43.82, \ 51.06, \ 51.38, \ 53.15, \ 53.75, \ 54.82, \ 62.47, \ 120.29, \ 120.35, \ 125.48, \ 126.25, \ 127.08, \ 127.65, \ 128.27, \ 128.54, \ 140.66, \ 140.84,145.43\]

m/z by LC-MS found to be 309.6 (+ve mode)

**2-(9H-fluoren-9-ylamino)-3-methyl-pentanoic acid methyl ester (Compound 5c)**

28.5g off-white solid, 73.8% yield \([\alpha]^{20}_{D} \ -96.86^{\circ}\) (c 1.0, CHCl\(_3\))

\[\text{\textsuperscript{1}H-NMR: (400MHz, DMSO-d6) \ \delta \ 0.61-0.64 \ (t, J=6.8\text{Hz}, \ 3H),0.66-0.68 \ (d, J=7.2\text{Hz}, \ 3H), \ 0.99-1.06 \ (m, \ 1H), \ 1.34-1.57 \ (m, \ 2H), \ 2.33-2.37 \ (m, \ 1H), \ 3.19 \ (s, \ 3H), \ 3.26-3.29 \ (m,1H),7.23-7.80 \ (m, \ 8H)\]
C-NMR (75 MHz, DMSO-\(d_6\)): \(\delta\) 11.35, 15.57, 25.19, 38.87, 51.13, 60.63, 62.65, 120.25, 120.27, 125.63, 126.28, 127.02, 127.58, 128.51, 140.62, 140.91, 145.21, 145.47, 176.23.

m/z by LC-MS found to be 309.6 (+ve mode)

### 2-(9H-fluoren-9-ylamino)-3-phenyl-propionic acid methyl ester (Compound 5d)

30.0g off-white solid, 70.0% yield \([\alpha]^{20}_D -53.61^\circ (c 1.0, CHCl_3)\)

\(^1\)H-NMR: (400MHz, DMSO-\(d_6\)) \(\delta\) 2.68-2.70 (d, \(J=7.08\) Hz, 2H), 2.84-2.90 (m, 1H), 3.17 (s, 3H), 3.33-3.39 (m, 1H), 4.86-4.87(d, \(J=4.36\)Hz, 1H), 6.98-7.77 (m, 13H)

\(^{13}\)C-NMR (75 MHz, DMSO-\(d_6\)): \(\delta\) 51.36, 58.73, 62.52, 120.23, 120.30, 125.31, 126.03, 126.71, 127.13, 127.56, 128.28, 128.44, 128.59, 128.69, 128.68, 138.08, 140.56, 140.70, 145.23, 145.49, 175.61.

m/z by LC-MS found to be 344.8 (+ve mode)

### 2-(9H-fluoren-9-ylamino)-propionic acid methyl ester (Compound 5e)

23.0g off-white solid, 69.0% yield; \([\alpha]^{20}_D -95.90^\circ (c 1.0, CHCl_3)\)

\(^1\)H-NMR:(300MHz, DMSO-\(d_6\)) \(\delta\) 1.03-1.05 (d, \(J=6.93\) Hz, 3H), 2.93-2.95 (broad m, 1H), 3.34 (s, 3H), 4.88 (s, 1H), 7.23-7.79 (m, 8H);

\(^{13}\)C-NMR (75 MHz, DMSO-\(d_6\)): \(\delta\) 20.49, 51.60, 52.45, 62.40, 120.34, 125.36, 126.02, 127.23, 127.71, 128.30, 128.47, 140.60, 140.62, 145.57, 145.95, 176.42.

m/z by LC-MS found to be 267.2 (+ve mode)
4.0 General Procedure-3: Synthesis of N-alkylated L-amino alcohol

![Chemical Structure]

In 250 mL reaction flask under nitrogen blanket dry tetrahydrofuran (40.0 mL, 20.0 Vol) followed by N-(9- fluorenyl)-L-amino acid ester (0.01 moles) was added. The reaction mass was cooled to -40°C and a 2.0M solution of lithium aluminium hydride in tetrahydrofuran (0.005 moles) obtained from Sigma Aldrich was added slowly at -40°C during 10 min. The reaction mixture was stirred for additional 60 min at -40°C. The progress of the reaction was checked by TLC (Mobile Phase: ethyl acetate: n-hexane 1:9). Ethyl acetate (20.0 mL) was added slowly to decompose the unreacted lithium aluminium hydride. The reaction mixture was diluted with ethylacetate and washed with water. The organic layer was dried over anhydrous magnesium sulphate and distilled to remove most of the solvent. The concentrate was slurried with 60-120 mesh silica gel and was placed on the column of silica gel (230-400 mesh, 50.0 g). The column was eluted with ethyl acetate and n-hexane with gradient elution. The product fraction was collected and distilled to remove most of the solvent. The product was isolated and characterized by $^1$H NMR, $^{13}$CNMR and LC-MS. The details of the compound prepared were listed in table 4.2.
4.1. Procedure for the preparation of 2-(9H-fluoren-9-ylamino)-3-methyl-butanol (Compound 6a)

In 250 mL reaction flask under nitrogen blanket dry tetrahydrofuran (40.0 mL, 20.0 Vol) followed by 2-(9H-fluoren-9-ylamino)-3-methyl-butyric acid methyl ester (0.01 moles) was added. The reaction mass was cooled to -40°C and a 2.0M solution of lithium aluminium hydride in tetrahydrofuran(0.005 moles) obtained from Sigma Aldrich was added slowly at -40°C during 10 min. The reaction mixture was stirred for additional 60 min at -40°C. Ethyl acetate (20.0 mL) was added slowly to decompose the unreacted lithium aluminium hydride. The reaction mixture was diluted with ethyl acetate and washed with water. The organic layer was dried over anhydrous magnesium sulphate and distilled to remove most of the solvent. The concentrate was slurried with 60-120 mesh silica and the same was placed on the silica gel (230-400 mesh, 50.0 g) column. The column was eluted with ethyl acetate/n-hexane with gradient elution. The product fraction was collected and distilled to remove most of the solvent. The product was isolated and characterized by $^1$H NMR, $^{13}$CNMR and LC-MS.

4.1.1 Characterization data of the compounds

2-(9H-fluoren-9-ylamino)-3-methyl-butanol (Compound-6a)

0.5g pale yellow solid, 28.0 % yield;
$^1$H-NMR: (400MHz, DMSO-$d_6$) $\delta$ 0.83 (d, J=6.92Hz, 3H), 0.88 (d, J=6.88Hz, 3H), 1.74-1.80 (m, 1H), 2.60-2.64 (m, 1H), 3.17-3.33 (m,2H), 4.36 (broad s, 1H), 4.89 (s, 1H), 7.28-7.79 (m, 8H).

$^{13}$C-NMR (75 MHz, DMSO-$d_6$): $\delta$ 18.71, 28.80, 61.37, 61.90, 62.06, 120.26, 120.31, 125.69, 127.49, 127.55, 128.15, 128.21, 140.03, 140.26, 147.31, 147.88.

m/z by LC-MS found to be 268.2 (+ve mode)

2-(9H-fluoren-9-ylamino)-4-methyl-pentanol (Compound-6b)

0.46 g pale yellow solid, 25.3 % yield

$^1$H-NMR: (400MHz, DMSO-$d_6$) $\delta$ 0.68 (d, J=6.56Hz, 6H), 1.10-1.22 (m, 2H), 1.58-1.64 (m, 1H), 2.39 (broad s, 1H), 2.62 (t, J=5.6Hz, 1H), 3.08-3.20 (m, 2H), 4.36 (t, J=5.82Hz ,1H), 4.90 (s, 1H), 7.28-7.63 (m, 8H).

$^{13}$C-NMR (75 MHz, DMSO-$d_6$): $\delta$ 23.36, 23.38, 24.53, 42.73, 54.71, 61.82, 64.76, 120.26, 125.65, 127.49, 127.55, 128.14, 128.18, 140.16, 140.36, 147.24, 147.76.

m/z by LC-MS found to be 282.4 (+ve mode)

2-(9H-fluoren-9-ylamino)-3-methyl-pentanol (Compound-6c)

0.52g pale yellow solid, 28.6 % yield

$^1$H-NMR: (300MHZ, DMSO-$d_6$) $\delta$ 0.7-0.77 (m, 3H), 0.81-0.84 (m, 6H), 1.05-1.1 (m, 2H), 2.72-2.77 (m, 1H), 3.14-3.20 (m, 1H), 4.38 (broad s, 1H), 4.87 (s, 1H), 7.26-7.80 (m, 8H).

$^{13}$C-NMR(75 MHz, DMSO-$d_6$): $\delta$ 14.89, 22.55, 25.78, 26.55, 29.16, 29.46, 31.75, 36.02, 60.5, 61.01, 61.76, 120.24, 125.74, 127.46, 127.51, 128.12, 128.16, 128.49, 129.93, 135.82, 140.10, 140.20, 147.57, 147.60.
2-(9H-fluoren-9-ylamino)-3-phenyl-propanol (Compound-6d)

0.6g pale yellow solid, 32.6 % yield;

$^1$H-NMR (400MHz, DMSO-$d_6$) δ 2.46-2.62 (m, 3H), 2.77-2.80 (m, 1H), 3.09-3.14 (m, 2H), 4.48-4.51 (t, J=5.16Hz, 1H), 4.93 (s, 1H), 7.01-7.80 (m, 13H)

$^{13}$C-NMR (75 MHz, DMSO-$d_6$): δ 58.71, 61.98, 63.81, 120.26, 120.30, 125.63, 125.66, 126.21, 127.51, 128.19, 128.51, 129.72, 140.10, 140.15, 140.26, 147.21, 147.28.

Mas by LC-MS was found to be 317.2 (+ve mode)

2-(9H-fluoren-9-ylamino)-propanol (Compound-6e)

0.6g pale yellow solid, 33.0 % yield

$^1$H-NMR: (300MHz, DMSO-$d_6$) δ 0.79 (d, J=6.3Hz, 3H), 2.68-2.72 (m, 2H), 3.10-3.13 (broad t, J=5.07Hz, 2H), 4.42-4.46 (broad t, J=5.34Hz, 1H), 4.89 (s, 1H), 7.26-7.80 (m, 8H).

$^{13}$C-NMR (75 MHz, DMSO-$d_6$): δ 19.06, 52.03, 61.70, 66.84, 120.30, 120.36, 125.65, 125.69, 127.48, 127.52, 128.14, 128.18, 140.25, 147.11, 147.71.

m/z by LC-MS found to be 240.2 (+ve mode)
Figure-25: $^1$H-NMR of 2-(9H-fluoren-9-ylamino)-3-methyl-butyric acid methyl ester

(Compound 5a)

Figure-26: $^1$H-NMR of 2-(9H-fluoren-9-ylamino)-3-methyl-butyric acid methyl ester

(Compound 5a)
Figure-27: $^{13}$C-NMR of 2-(9H-fluoren-9-ylamino)-3-methyl-butyric acid methyl ester (Compound 5a)

Figure-28: $^{13}$C-NMR of 2-(9H-fluoren-9-ylamino)-3-methyl-butyric acid methyl ester (Compound 5a)
Figure-29: LC-MS of 2-(9H-Fluoren-9-ylamino)-3-methyl-butyric acid methyl ester

(Compound 5a)

Figure-30: $^1$H-NMR of 2-(9H-fluoren-9-ylamino)-4-methyl-pentanoic acid methyl ester

(Compound 5b)
Figure-31: $^1$H-NMR of 2-(9H-fluoren-9-ylamino)-4-methyl-pentanoic acid methyl ester (Compound 5b)

Figure-32: $^{13}$C-NMR of 2-(9H-fluoren-9-ylamino)-4-methyl-pentanoic acid methyl ester (Compound 5b)
Figure-33: $^{13}$C-NMR of 2-(9H-fluoren-9-ylamino)-4-methyl-pentanoic acid methyl ester (Compound 5b)

Figure-34: LC-MS of 2-(9H-fluoren-9-ylamino)-4-methyl-pentanoic acid methyl ester (Compound 5b)
Figure-35: $^1$H-NMR of 2-(9H-fluoren-9-ylamino)-3-methyl-pentanoic acid methyl ester (Compound 5c)

Figure-36: $^1$H-NMR of 2-(9H-fluoren-9-ylamino)-3-methyl-pentanoic acid methyl ester (Compound 5c)
Figure-37: $^{13}$C-NMR of 2-(9H-fluoren-9-ylamino)-3-methyl-pentanoic acid methyl ester (Compound 5c)

Figure-38: $^{13}$C-NMR of 2-(9H-fluoren-9-ylamino)-3-methyl-pentanoic acid methyl ester (Compound 5c)
Figure 39: LC-MS of 2-(9H-fluoren-9-ylamino)-3-methyl-pentanoic acid methyl ester

(Compound 5c)

Figure 40: $^1$H-NMR of 2-(9H-fluoren-9-ylamino)-3-phenyl-propionic acid methyl ester

(Compound 5d)
Figure-41: $^1$H-NMR of 2-(9H-fluoren-9-ylamino)-3-phenyl-propionic acid methyl ester

(Compound 5d)

Figure-42: $^{13}$C-NMR of 2-(9H-fluoren-9-ylamino)-3-phenyl-propionic acid methyl ester

(Compound 5d)
Figure-43: $^{13}$C-NMR of 2-(9H-Fluoren-9-ylamino)-3-phenyl-propionic acid methyl ester
(Compound 5d)

Figure-44: LC-MS of 2-(9H-fluoren-9-ylamino)-3-phenyl-propionic acid methyl ester
(Compound 5d)
Figure 45: $^1$H-NMR of 2-(9H-fluoren-9-ylamino)-propionic acid methyl ester
(Compound 5e)

Figure 46: $^1$H-NMR of 2-(9H-fluoren-9-ylamino)-propionic acid methyl ester
(Compound 5e)
Figure-47: $^{13}$C-NMR of 2-(9H-fluoren-9-ylamino)-propionic acid methyl ester (Compound 5e)

Figure-48: $^{13}$C-NMR of 2-(9H-fluoren-9-ylamino)-propionic acid methyl ester (Compound 5e)
Figure-49: LC-MS of 2-(9H-fluoren-9-ylamino)-propionic acid methyl ester (Compound 5e)

Figure-50: $^1$H-NMR of 2-(9H-fluoren-9-ylamino)-3-methyl-butanol (Compound 6a)
Figure-51: $^1$H-NMR of 2-(9H-fluoren-9-ylamino)-3-methyl-butanol (Compound 6a)

Figure-52: $^{13}$C-NMR of 2-(9H-fluoren-9-ylamino)-3-methyl-butanol (Compound 6a)
Figure-53: $^{13}$C-NMR of 2-(9H-fluoren-9-ylamino)-3-methyl-butanol (Compound 6a)

Figure-54: LC-MS of 2-(9H-fluoren-9-ylamino)-3-methyl-butanol (Compound 6a)
Figure-55: $^1$H-NMR of 2-(9H-fluoren-9-ylamino)-4-methyl-pentanol (Compound 6b)

Figure-56: $^1$H-NMR of 2-(9H-fluoren-9-ylamino)-4-methyl-pentanol (Compound 6b)
Figure-57: $^{13}$C-NMR of 2-(9H-fluoren-9-ylamino)-4-methyl-pentanol (Compound 6b)

Figure-58: LC-MS of 2-(9H-fluoren-9-ylamino)-4-methyl-pentanol (Compound 6b)
Figure-59: $^1$H-NMR of 2-(9H-fluoren-9-ylamino)-3-methyl-pentanol (Compound 6c)

Figure-60: $^1$H-NMR of 2-(9H-fluoren-9-ylamino)-3-methyl-pentanol (Compound 6c)
Figure-61: $^{13}$C-NMR of 2-(9H-fluoren-9-ylamino)-3-methyl-pentanol (Compound 6c)

Figure-62: $^{13}$C-NMR of 2-(9H-fluoren-9-ylamino)-3-methyl-pentanol (Compound 6c)
Figure-63: LC-MS of 2-(9H-fluoren-9-ylamino)-3-methyl-pentanol (Compound 6c)

Figure-64: $^1$H-NMR of 2-(9H-fluoren-9-ylamino)-3-phenyl-propanol (Compound 6d)
Figure-65: $^1$H-NMR of 2-(9H-fluoren-9-ylamino)-3-phenyl-propanol (Compound 6d)

Figure-66: $^{13}$C NMR of 2-(9H-fluoren-9-ylamino)-3-phenyl-propanol (Compound 6d)
Figure-67: LC-MS of 2-(9H-fluoren-9-ylamino)-3-phenyl-propanol (Compound 6d)

Figure-68: $^1$H-NMR of 2-(9H-fluoren-9-ylamino)-propanol (Compound 6e)
Figure-69: $^1$H-NMR of 2-(9H-fluoren-9-ylamino)-propanol (Compound 6e)

Figure-70: $^{13}$C-NMR of 2-(9H-fluoren-9-ylamino)-propanol (Compound 6e)
Figure-71: $^{13}$C-NMR of 2-(9H-fluoren-9-ylamino)-propanol (Compound 6e)

Figure-72: LC-MS of 2-(9H-fluoren-9-ylamino)-propanol (Compound 6e)
5.0 Results and Discussion

Synthesis of N-9-fluorenyl amino acid alkyl ester was attempted using different methods such as

- 9-fluorenimine derivatives using acid mediated condensation
- Reductive amination using titanium tetrachloride mediated imine formation followed by the reduction
- N-alkylation of amino acid ester using 9-chlorofluorene in aprotic solvent

The various amino acids ester were prepared from L-valine, L-leucine, L-isoleucine, L-alanine and L-phenyl alanine using the literature procedure and the L-amino acid methyl ester hydrochloride were characterized. The obtained amino acid methyl ester was used to prepare various 9-fluorenimine derivative of amino acid methyl ester using the conventional acid catalyst mediated azeotropic removal of water using toluene as solvent. The reaction did not proceed in the high boiling solvent. The imine formation using ammonia under pressure or the use of ammonia or the L-amino acid methyl ester using titanium tetrachloride as dehydrating agent to obtain 9-fluorenimine derivative have not been successful.

The alternate approach of N-alkylation of amino acid methyl ester was reported by use of lead nitrate as acid scavenger in dichloromethane for the coupling of 9-phenyl bromofluorene with L-phenyl alanine ethyl ester. However, this procedure involves the use of lead salt as scavenger which is a heavy metal and poisonous. In the present study the use of organic base namely N, N-diisopropyl ethyl amine in aprotic solvents was attempted to replace the lead nitrate and the same was found to be successful.
Various N-9-fluorenyl amino acid methyl esters were prepared using the organic base with good yield and the same was characterized by $^1$HNMR, $^{13}$CNMR and LC-MS. The isolated products are optically active. The synthesis is a new approach to couple the amino acid derivative with 9-chlorofluorene wherein Hunig’s base was facilitating the coupling reaction and the same can be used for preparing the various N-9-fluorenyl amino acid ester derivatives.

The preparations of various N-9-fluorenyl amino alcohols were attempted by the reduction of N-9-fluorenyl amino acid derivative using lithium aluminium hydride at different temperature in tetrahydrofuran solvent. The results and observations of the reduction reactions are tabulated in table 4.3.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Temperature during reaction (°C)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-60</td>
<td>No reaction and starting material is present</td>
</tr>
<tr>
<td>2</td>
<td>-40</td>
<td>Product formation with other side reaction observed</td>
</tr>
<tr>
<td>3</td>
<td>0-5</td>
<td>Less product formation significant amount of side products formation observed</td>
</tr>
</tbody>
</table>

It is noted from the above table that the reduction reaction is sensitive to temperature and it is observed that the product formation is significant at -40°C compared to 0-5°C. The lower temperature is not favoring the reduction reaction. Various N-9-fluorenyl amino alcohols are prepared and characterized by $^1$HNMR, $^{13}$CNMR and LC-MS.
The prepared N-9-fluorenyl amino acid methyl ester and N-9-fluorenyl amino alcohol are further investigated for its biological activity and docking using different microorganism and P-glycoprotein in the subsequent chapter 5 and chapter 6.
REFERENCES


6. KengoAsai, Gen-ichiKonishi et al, Polymer Chem., 2010, 1,321-325