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Reductive amination of (alpha) - amino acids: Solution - Phase synthesis

Rohini D'Souza

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REDUCTIVE AMINATION OF α-AMINO ACIDS: SOLUTION – PHASE SYNTHESIS

Rohini D’Souza

July 2001

A thesis submitted in partial fulfillment of the requirements for the degree of Masters of Science in Chemistry

Approved: Kay Turner
Thesis Advisor

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Reductive Amination of $\alpha$ - Amino Acids :
Solution-Phase Synthesis

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To My Parents
With Gratitude
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ABSTRACT

Preparation of a small library of secondary amino acids was achieved by solution-phase organic synthesis using reductive amination reactions with selected $\alpha$-amino acids and aromatic aldehydes. Reductive amination employing sodium triacetoxyborohydride instead of sodium cyanoborohydride was found to give shorter reaction times and much safer byproducts. With less sterically hindered $\alpha$-amino acids, direct reductive amination generally yielded the bisalkylated product. However, monoalkylation was achieved by adopting an indirect reductive amination route. Reaction mixtures were characterized by HPLC and LC-MS, resulting in the synthesis of a 21 compound library.
1. INTRODUCTION

1.1 PHILOSOPHY OF COMBINATORIAL CHEMISTRY

Combinatorial chemistry can be defined as a branch of chemistry that involves a reaction scheme using a small number of chemical reagents in all combinations, to yield a large number of well-defined products that can be easily screened for properties of interest. In combinatorial chemistry, a synthesis experiment is designed in a manner leading to a large collection of new chemical substances (molecular diversity) and this collection is presented in a format, which allows the selection of substances possessing a property of interest.\(^1\)

According to Pasteur, “Chance favors the prepared mind. As the number of chances increases, so increases the effect of favor.” Several discoveries of interest in the field of chemistry have come about as a consequence of chance rather than one of careful design. If we were to carry out experiments with design as well as increase the chance level in them, we could increase the probability of success - this is the basic philosophy of combinatorial chemistry.

A combinatorial chemist could probably be considered a rebel when compared with chemists of the previous generation, who used reasoned intellectual efforts to design an experiment to synthesize a single compound of high yield and purity. A combinatorial chemist aims at synthesizing a mixture of organic compounds for the purpose of screening for properties of interest. Recent strategies for discovery and optimization of drugs, ligands and catalysts rely on combinatorial chemistry. This branch of chemistry has been widely applied in academics as well as in the industry in order to satisfy the increasing
need for new chemical entities.\textsuperscript{2} Even though combinatorial chemistry has its origins in solid-phase synthesis, the solution-phase route has widely been adopted.

### 1.2 HISTORY OF COMBINATORIAL CHEMISTRY

#### Table 1.1: Time Line Leading up to Modern Molecular Diversity\textsuperscript{3}

<table>
<thead>
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<tr>
<td>First human beings</td>
<td>5,000,000</td>
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<tr>
<td>Hippocrates' birth</td>
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<td>Medicinal value of Aspirin</td>
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Solid–supported peptide synthesis was developed in the early 60's by R. Bruce Merrifield,\textsuperscript{4} for which he received the Nobel prize in 1984. Henry Rappoport and Clifford Leznoff continued work on organic solid-phase synthesis in the 70's. Mario Geysen, an Australian, developed the Geysen pin method in 1984. He synthesized peptides on a rack of functionalized polypropylene pins and tested their binding abilities while the peptides were attached chemically onto the pins.\textsuperscript{5}

In 1985, Richard Houghten discovered the tea-bag method of synthesis. In this method, an inert mesh similar to a tea-bag was used to encapsulate the polymer used for synthesis resulting in a tea-bag reactor. Addition of an amino acid to all the reactors was carried out simultaneously. The bags were then washed to remove any excess of reagent. Next they were distributed to new
beakers, followed by the addition of another amino acid. The peptide could be recovered by chemical cleavage from the polymeric support.\textsuperscript{6}

In 1991, Furka and Lam developed the split and mix method of synthesis to generate mixtures of peptides. Here, individual reaction vessels containing similar sized portions of the resin were reacted with different monomers. On completion of the reactions, the resin was pooled together and mixed well. A common protecting group could be removed or a common transformation could be carried out in a single reaction vessel. The resin is split again for the coupling of the second monomer and the process is continued till the desired combinatorial synthesis has taken place. Lam realized that in this technique, every individual bead carried a different peptide giving rise to the \textit{“one bead - one compound”} technique.\textsuperscript{7,8} One bead one compound creates a diverse library of potential compounds and is the principle behind the library of libraries.

Janda et al. developed libraries using soluble polymers as carriers instead of utilizing solid-phase synthesis.

The solid-phase synthesis technique invented by Merrifield was used only for the synthesis of peptides and nucleotides till 1993. Clifford Leznoff first applied Merrifield’s method using polymer supports in organic synthesis in 1993. In 1997, extensive work done by Bunin et al. showed that several organic reactions could be carried out using the solid-phase techniques. This led to an explosion in the field of combinatorial chemistry.\textsuperscript{9}
1.3 SOLID-PHASE SYNTHESIS

The emergence of solid-phase synthesis answered the need for fast, efficient, reproducible and reliable methods of synthesizing high purity peptides. Prior to Merrifield's work, peptide synthesis involved preparation of peptides in solution using laborious and time-consuming methods. Intermediate purification steps had to be carried out and the solubility characteristics of the intermediates had to be taken into consideration. Solution-phase methods are still in use today.

In solid-phase synthesis, a linker is covalently coupled to an insoluble support or resin, which contains a suitable functional group X. This is followed by the covalent anchoring of the first building block to the end of the linker. The first building block may contain other functional groups that could be covalently attached to the linker. In order to allow only the desired coupling reaction to occur, undesired functional groups are blocked by temporary and/or permanent protecting groups. In the next step, a deprotecting agent is used to deprotect the group where the anchoring of the next building block is desired, followed by the covalent coupling of the second building block. A series of treatments with deprotecting and coupling reagents is continued until the desired product is achieved. The last step is the deprotection of the protected groups, followed by cleavage of the product from the resin. The stepwise schematic diagram of solid-phase synthesis is shown in Figure 1.1.
Figure 1.1: Schematic Diagram of Solid-Phase Synthesis

Solid-phase or solution-phase synthesis can be carried out in two ways: Parallel synthesis or Split pool synthesis.

1.31 Parallel Synthesis

In this method several reactions are carried out in the wells of a microtitre plate. All the wells contain the same resin, and although the reactions take place simultaneously, each well generates a unique compound. First each row is
treated with a different compound followed by the addition of a different reagent into each column. The scheme is illustrated in Figure 1.2, where the columns are treated with compounds A, B, C, D, and E respectively, while the rows are treated with reagents V, W, X, Y, and Z. This results in a library consisting of 25 products. The advantages of this method are, since each well contains only one product, time need not be spent on isolation and the product will be of higher purity. More importantly, structure-activity relationships can be easily determined. The libraries synthesized however have less number of products as compared to the split-pool method.

**Figure 1.2 : Parallel Synthesis**

<table>
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<tr>
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1.32 **Split Pool Synthesis**\(^{10}\)

Figure 1.3: Split Pool Synthesis

This method is an excellent way of synthesis of equimolar mixtures. In this method as shown in Figure 1.3, the resin is divided into three equal parts, each part being treated with a synthetic unit of type A resulting in equimolar amounts.
of three product resins A1, A2 and A3. The product resins are then pooled together and further divided equally into three groups.

The groups are then treated with B1, B2, or B3 respectively resulting in 9 different products. These products are then pooled together and equally divided into three groups. The groups are then treated with C1, C2, or C3 respectively resulting in 27 different products. The total number of synthesized products that can be obtained is given by the formula \( N = n^m \). Where \( N \) is the total number of compounds synthesized, \( n \) is the number of building blocks that have been added onto the resin and \( m \) is the number of chemical steps that have been carried out. In this case that would be \( 3^3 \).

The most obvious advantage of this method is that large libraries can be produced. One major disadvantage of this method is that identification of key compounds would be time consuming. However, this can be overcome by using deconvolution tools such as tagging and encoding. Much lower yields are obtained since the reaction mixture is sub-divided.

1.4 FACTORS TO BE CONSIDERED FOR SOLID-PHASE SYNTHESIS

1. Choice of solid support

The general requirements of a good support are:

- Compatibility with protic and aprotic solvents like DMF, alcohols, THF, acetonitrile, dichloromethane, etc.

- Capable of use in a temperature range from -78 °C to 155 °C.
Compatibility with a wide range of reagents such as acids, bases, Lewis acids, reducing agents, oxidizing agents, and transition metal complexes.

- Resin should be capable of swelling in both organic and aqueous media.
- Size and substitution homogeneity.
- Resin resistance to the formation of clusters (cluster formation can prevent the statistical distribution of resin beads and can decrease the number of structures that can be formed).

Polystyrene crosslinked with 1 or 2% divinylbenzene is the most commonly used polymer backbone. The disadvantages of polystyrene are that it has a large inner surface area, which is readily accessible to solvents and regents, hence severe washings have to be given, in order to remove excess reagents and high boiling solvents from the inner spaces. Extensive mechanical stirring can also cause damage to the resin.12

The search for better resins led to the discovery of TentaGel resins and soluble resins like PEG resins. TentaGel resins are polyoxyethylene grafted polystyrenes and are preferred for the following reasons:

- Swell better in polar solvents like methanol and water though they have a lower loading capacity compared to polystyrene based resins.
- Uniform in size and are non-sticky.
- Have a functionalizable group at the end of the polyethylene chains, which is far from the hydrophobic polystyrene chain.
2. Choice of Cleavable linkers

- The linker should be stable to all chemical reactions that are carried out.
- The desired end product should be readily cleaved from the resin bead.
- The cleavage conditions should not degrade any of the library products.
- It should not leave any residual functional groups on the compounds of the library.
- An ideal linker would be one that is capable of yielding the release of different functional groups depending upon the reagent used for cleavage.

There are two approaches to attach the first building block to the resin via a linker. In the first approach, the hybrid of the first building block along with the linker is synthesized in solution and then attached to the functionalized resin. In the second approach, the building block links onto the resin-linker complex. The most widely used linkers are acid labile and can be considered as a modification of the benzyl-type linkage.

The library compounds are usually cleaved from the resin beads in either one or two steps. Linker cleavage is enhanced by the presence of substitutions on the aromatic ring.

Hodges et al. have recently developed a new resin known as the Rasta resin. Rasta resins are similar to TentaGel resins. While the TentaGel resin has a cross linked polystyrene bead with the reactive functional group only at the end, the Rasta resin has the reactive functional groups all along the polymeric chain resulting in a fuzzy or hair-like bead. The latter is capable of higher loading, and may also react with impurities and/or reagents.\footnote{13}
3. Selection of Protecting Groups

The most commonly used amino protecting groups are BOC and FMOC. Due to the easy handling and simple deprotection, the FMOC group is preferred over the BOC group.

BOC (t-butylcarbonate) \[ \text{C-O-CH}_3 \text{CH}_3 \]

FMOC (9-fluorenylmethoxycarbonyl group) \[ \text{FMOC} \]

FMOC is stable against acids and catalytic hydrogenation, but is easily cleaved under mildly basic non-hydrolytic conditions. The FMOC group is introduced by treating the amine with 9-fluorenymethylchloroformate. The amine is deprotected by deblocking of the FMOC group by using liquid ammonia or with piperidine, ethanolamine or morpholine at room temperature.\(^{14}\)

In comparison the BOC group has to be deprotected using strong acids like HF or TFMSA (trifluoromethyl sulphonic acid) in trifluoroacetic acid solution, thus making FMOC the preferred choice of a protecting group.

The most widely used protecting group for alcohols and phenols is \(t\)-butyl ethers, which allows the simultaneous final deprotection of the functional group together with the cleavage of the substrate from the linker. Likewise, acid functional groups can be protected as methyl or \(t\)-butyl esters or carbamates. Often acid-labile linkers are chosen depending on the nature of the protecting groups used to allow for concurrent deprotection and cleavage.
4. Choice of Reagents

In solid-phase synthesis, coupling reactions have been thoroughly studied and optimized, particularly with respect to the influence of solvents and temperature. A common problem with optically active $\alpha$-amino acids is racemization. However, several coupling reagents have been developed to minimize these undesired side reactions. An excellent review summarizes the current status of coupling conditions for solid-phase synthesis.$^{15}$

One such reagent is hydroxybenzotriazole (HOBt).

HBTU (2-1H-benzotriazoyl-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate is a commonly used activating agent that requires basic conditions. It is preferred to other activating agents since the uronium and phosphonium activating agents react with faster rates than other agents.$^{16}$

1.5 SOLUTION-PHASE SYNTHESIS

The main limitation of solution-phase synthesis is the isolation and purification of synthesized products. If the advantages of sample isolation characteristics of solid-phase synthesis can be incorporated into solution-phase synthesis then this major disadvantage can be eliminated. If the reaction can be
carried out in a manner wherein the intermediates are soluble and the final product is insoluble, then the steps required for isolation and purification can be eliminated. The lack of requirements for linkers, attachment and development of cleavage conditions would make solution-phase combinatorial synthesis an especially attractive complement to solid-phase synthesis. In each step of the scheme, the reactants, un-reacted starting materials, reagents and their byproducts are removed by simple extraction procedures resulting in intermediates and final products of high purities. The extraction procedures in the scheme are used not only to isolate the intermediates and product but also to purify the products and intermediates. A solution-phase protocol can be used to synthesize a single compound, a small mixture of compounds ranging from 5 - 50 compounds or a large mixture of compounds (100 - 2500). Deconvolution of these libraries can be carried out which is a powerful and rapid tool for lead identification. The solution-phase approach is extremely valuable for both lead discovery and lead optimization.
1.6 COMPARISON OF SOLID-PHASE SYNTHESIS AND SOLUTION-PHASE SYNTHESIS

A comparison of the two methods is shown in Tables 1.2 and 1.3.

Table 1.2: Pros of Solid-Phase versus Solution-Phase Combinatorial Organic Synthesis

<table>
<thead>
<tr>
<th>Solid-phase Synthesis</th>
<th>Solution-phase Synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Has the advantage of split and pool approach</td>
<td>• Ample literature on various reactions that can be used</td>
</tr>
<tr>
<td>• Widely adopted after 5 years of use</td>
<td>• Presence of solid support does not hinder reaction rates</td>
</tr>
<tr>
<td>• Reactions can be driven to completion by use of excess reagents due to mass action effect</td>
<td>• Cleavage step not required</td>
</tr>
<tr>
<td>• Solvents and excess reagents can be removed by washing and filtration</td>
<td>• Choice and development of linker not required</td>
</tr>
<tr>
<td>• Handling of liquids is not required</td>
<td>• Any chemical reaction can be carried out even with the use of complex organometallic reagents</td>
</tr>
<tr>
<td>• Products can be obtained without any evaporation</td>
<td>• High temperature reactions can also be carried out without any difficulties</td>
</tr>
<tr>
<td>• Protecting groups can be used</td>
<td>• Protecting groups can be used only if their removal gives volatile byproducts</td>
</tr>
<tr>
<td></td>
<td>• Product can be isolated in high yields</td>
</tr>
</tbody>
</table>
Table 1.3: Cons of Solid-phase versus Solution-phase Combinatorial Organic Synthesis

<table>
<thead>
<tr>
<th>Solid-phase Synthesis</th>
<th>Solution-phase Synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Extensive literature not readily available</td>
<td>• Stoichiometric amounts of reagents have to be used unless one or more of the products of the reaction is volatile or a solid-phase resin is used to remove the excess of reagent</td>
</tr>
<tr>
<td>• Different solid-phase supports display different reaction rates</td>
<td>• Excess reagent cannot be recovered</td>
</tr>
<tr>
<td>• Care should be taken to prevent swelling or deformation of beads at high temperatures</td>
<td>• Liquid handling is required</td>
</tr>
<tr>
<td>• Due to the high cost of reagents product yields are very low in 2-10 mg quantities.</td>
<td>• Product isolation is associated with solvent evaporation</td>
</tr>
</tbody>
</table>

The choice of method depends on the desired library and the reaction sequence that takes place as both methods have their pros and cons.

1.7 ANALYTICAL EVALUATION OF SOLID-PHASE SYNTHESIZED LIBRARIES

In combinatorial solid-phase synthesis, compounds are linked to polymer resin beads to generate intermediate and final products. Monitoring the progress of these reactions would involve the analysis of the compound while it was still attached to the resin. Combinatorial synthesis also involves the generation of a large number of compounds in microgram and nanogram quantities, which also require screening. This increases the demand for nondestructive analytical methods for in situ monitoring and analysis. Conventional analytical techniques like TLC, HPLC, GC and MS require the cleavage of the compound of interest.
from the resin. Hence these techniques had to be modified in order to fulfill the criteria. Near and middle infrared spectroscopy is the most commonly used technique. A peak at 1529 nm is characteristic of an amine. When an amine reacts with benzoyl chloride, it results in the formation of an amide. A peak at 1476 nm is characteristic of an amide. Monitoring of the reaction between aminomethylstyrene resin beads and benzoyl chloride, shows the decrease in the amine band at 1529 nm and the simultaneous increase in the amide band at 1476 nm (Figure 1.4).

**Figure 1.4**: Changes in the Absorption Spectra during Loading of Benzoyl Chloride onto Aminomethylstyrene Beads.

![Absorption Spectra](image)

The presence and efficiency of removal of the FMOC protecting group can also be determined by monitoring the amine band at 1529 nm.¹⁹

Several other methods have been developed for the evaluation of solid-phase libraries. One commonly used method is the Magic Angle Spinning
$^1$H NMR. In this technique, the sample is spun at the magic angle (MA) at a relatively high speed. Line broadening, which is caused due to magnetic susceptibility mismatches with the sample, is eliminated by the use of magic angle spinning. This is used as a valuable tool for monitoring the progress of a solid-phase synthesis reaction.

One of the latest tools being developed is the Direct Injection NMR (DI NMR). This non-destructive technique uses an NMR flow probe, which injects the sample into the probe through an inlet tube, and the flow is stopped in order to obtain an NMR spectrum of the sample. On obtaining the spectrum, the sample is pulled out of the probe through the container.

1.8 ANALYTICAL EVALUATION OF SOLUTION-PHASE SYNTHESIZED LIBRARIES

Solution-phase libraries are relatively easier to evaluate. Classical techniques like TLC, GC, MS and HPLC are used as tools for monitoring the progress of the reaction and analysis of the final isolated product. Hyphenated instruments like LC-MS are the tools of the 21st century. These libraries are more cost effective and can be synthesized in larger quantities as compared to solid-phase libraries making their analytical evaluation much easier.
1.9 REDUCTIVE AMINATION / REDUCTIVE ALKYLATION

Reductive amination is an extremely important and widely used tool in organic synthesis. In this process, a carbonyl group reacts with a primary amine, followed by reduction to yield a secondary amine. This amine alkylation is a stepwise process. In the first step, the aldehyde or ketone reacts with a primary or secondary amine to form an imine or iminium ion respectively. The next step involves the reduction of this intermediate by the reducing agent via hydride reduction (Scheme 1.1).^{21}

**SCHEME 1.1 REDUCTIVE AMINATION / REDUCTIVE ALKYLATION**

![Diagram of reductive amination/alkylation](image-url)
Reductive amination can be carried out in two ways: direct reductive amination and indirect reductive amination. In direct reductive amination, the amine, carbonyl compound and the appropriate reducing agent are all reacted together without prior formation of the intermediate enamine, imine or iminium salt. Indirect reductive amination involves the preformation of the imine, enamine or iminium salt followed by reduction in a separate step.

In some cases, the aldehyde may be generated from a carboxylic acid prior to reductive alkylation. This would require the transformation of the carboxylic acid into derivatives like acid chlorides, esters or amides that can then be selectively reduced to the corresponding aldehyde. An alternative to this pathway would be the reduction of the carboxylic acid to the primary alcohol and then its oxidation to the corresponding aldehyde.\textsuperscript{22}

The most commonly used reducing agents for direct reductive amination are hydrogen in the presence of catalytic amounts of palladium, nickel or platinum, sodium borohydride and sodium cyanoborohydride.

Catalytic hydrogenation is an effective reducing agent for reductive amination and is economical; hence it is used in scale up reactions. The main drawback with this method is that selective reduction is not achieved especially in the presence of other reducible functional groups like carbon-carbon double bonds, nitro or cyano groups. Catalytic activity maybe inhibited in the presence of compounds containing divalent sulfur. Lower yields and side reactions are also observed.\textsuperscript{23}
Sodium borohydride is also an effective reducing agent, but it is also not compatible with compounds containing double or triple bonds and other reducible functional groups.

The quest for selective reducing agents continued. Wittig decided to modify the reducing power of complex metal hydrides by using substituted borohydrides. The reactivity of the borohydride ion was influenced to a great extent by the steric and electronic effects of the substituents. In 1951, Wittig synthesized sodium cyanoborohydride and lithium cyanoborohydride. Due to the strong electron withdrawing cyano group these agents proved to be much milder and more selective reducing agents over sodium borohydride.24 The stability of sodium and lithium cyanoborohydride in an acidic medium of pH 3 enhances the reducing ability of this reagent.25 The low pH aids in the exchange of hydride ions. The reagent is also soluble in hydroxylic solvents. Borch et al. also discovered that at higher pH ranges between 6-8, the imines are preferentially protonated and are reduced much faster than aldehydes or ketones, thereby minimizing the possibility of unwanted side products.26 The reagent is selective and a very good choice for direct reductive amination.

There are however limitations to the use of this reagent. In certain cases a five-fold excess of the amine is required. The reduction is slow and sluggish in the case of aromatic ketones and weakly basic amines. Also, the products can be easily contaminated with cyanide.27 Extreme care needs to be administered while handling the reagent as it is highly toxic. Hydrogen cyanide and sodium cyanide are also highly toxic byproducts of the reaction upon workup.28
With the increasing concern and awareness for the environment, the need for environment friendly reducing agents increased. Gribble et al.\textsuperscript{29,30} initially explored the potential of sodium triacetoxyborohydride as a reducing agent for reductive amination. The reasons for the mildness of the reagent could be steric shielding of the boron-hydrogen bond and electron withdrawing effects of the acetoxy groups. Aldehydes were reduced in preference to ketones.

Abdel-Majid et al.\textsuperscript{31} further explored the use of sodium triacetoxyborohydride as an effective reducing agent in the reductive amination of primary and secondary amines with aldehydes and saturated aliphatic ketones. The reagent proved to be an excellent alternate to sodium cyanoborohydride in hydride induced reductive amination reactions. The main advantage of this reagent is the elimination of possible cyanide contamination in the product and toxic byproducts making it an environment and health friendly reducing agent. Unlike sodium cyanoborohydride, the pH need not be controlled for the desired reaction to take place.

Several other reducing agents like borane-pyridine,\textsuperscript{27} borohydride exchange resin,\textsuperscript{32} zinc-acetic acid,\textsuperscript{33} and silica gel and zinc borohydride\textsuperscript{34} have been developed. However, the most widely used reducing agent for reductive amination in recent years is sodium triacetoxyborohydride.
2. OBJECTIVE

Amino acids are a very essential part of the human body. They form a solid basis for the discovery of new drugs in the pharmaceutical industry. Reductive amination is a very popular route for the synthesis of new drug molecules.

The goal of our research was to synthesize a solid-phase as well as a solution-phase library of amine derivatives of amino acids with the intention of comparing these two methods of combinatorial synthesis. We also wanted to determine the efficiency of the two most widely used reducing agents for reductive amination - sodium cyanoborohydride and sodium triacetoxyborohydride.

2.1 SELECTION OF REDUCING AGENT FOR REDUCTIVE AMINATION.

The reducing agents sodium cyanoborohydride and sodium triacetoxyborohydride would be evaluated and the better reducing agent will be used for all further studies.

2.2 COMPARISON OF SOLID-PHASE SYNTHESIS VERSUS SOLUTION-PHASE SYNTHESIS.

We devised two schemes, one using solid-phase and the other using solution-phase. The solid phase scheme utilized Wang hydroxy resin onto which L-phenylalanine was to be loaded prior to reductive amination using 7 different aldehydes and one ketone. The same aldehydes and ketone were to be used for the solution-phase library.
2.3 LIBRARY OF DERIVATIVES OF L-VALINE AND L-ISOLEUCINE.

Depending on the efficiency of the two combinatorial methods, one of the methods would then be chosen to synthesize a library of derivatives of L-valine and L-isoleucine using the same aldehydes and ketones.
3. EXPERIMENTAL

All chemicals that were used were from Sigma-Aldrich and all the solvents used were HPLC grade solvents. The Wang Hydroxy resin was obtained from Argonaut Technologies Inc.

3.1 COMPARISON OF REDUCING AGENTS

Reductive amination of 3-aminophenol with 2,4-dimethoxybenzaldehyde using Sodium cyanoborohydride (NaBH$_3$CN) and sodium triacetoxyborohydride (NaBH(OAc)$_3$).

3.1.1 Solution-Phase Studies - Reductive Amination using Sodium Cyanoborohydride.

A charge of 3-aminophenol (0.0211g, 0.193 mmol) was placed in a scintillation vial. To this was added 2,4-dimethoxybenzaldehyde (0.102 g, 0.61 mmol). This was followed by the addition of 2 mL of 1% acetic acid in DMA. The mixture was shaken for 5 mins. This was followed by the addition of (0.215 g, 3.42 mmol) of NaBH$_3$CN over a 2 hour period. The reaction mixture was stirred at room temperature for 24 hours. An aliquot of this sample was used as the analytical sample.

3.1.2 Solution-Phase Studies - Reductive Amination of 3-Aminophenol using Sodium Triacetoxyborohydride.

A charge of 3-aminophenol (0.067 g, 0.61 mmol) was placed in a scintillation vial. To this was added 2,4-dimethoxybenzaldehyde (0.102 g, 0.61 mmol). This was followed by the addition of 3 mL of THF. The mixture was
shaken for 5 minutes. This was followed by the addition of NaBH(OAc)$_3$ (0.032 g, 0.15 mmol). The reaction mixture was stirred at room temperature for 24 hours.

### 3.1.3 Analytical Evaluation of the Alkylated Product.

A HPLC method was developed on HPLC Hewlett Packard series 1050, with the following conditions.

HPLC column used: Vydac 215MR5414 monomeric C$_{18}$, 5 µm, 300 °A, 4.6 mm i.d. x 150 mm.

Mobile Phase: Methanol : 0.05 M aqueous sodium acetate (50:50)

Flow Rate: 1 mL/min

Detection Wavelength: 254 nm

### 3.2 SOLUTION-PHASE STUDIES – DIRECT REDUCTIVE AMINATION OF L-PHENYLALANINE USING SODIUM TRIACETOXYBOROHYDRIDE

L-Phenylalanine (0.825g, 5 mmol) was added into a scintillation vial, followed by triethylamine (0.556 g, 5.5 mmol). To this was added 5.5 mmol of aldehyde/ketone and 10 mL of dichloromethane. This was followed by the addition of sodium triacetoxyborohydride (1.59 g, 7.5 mmol). The reaction was stirred for 24 hours at room temperature. The product was then filtered, washed with dichloromethane and air dried. This is a general procedure for all amino acids and aldehydes/ketones.
3.3  SOLID-PHASE STUDIES – DERIVATIVES OF L-PHENYLALANINE USING SODIUM TRIACETOXYBOROHYDRIDE

Scheme 3.1 Solid–Phase Synthesis of Derivatives of L-Phenylalanine

APPARATUS: A Biorad fritted polypropylene vial was used to carry out the reaction. The vials were placed on a Labquake Rotor with clips for continuous end over end mixing.

3.3.1. Resin Pretreatment.

ArgoGel Wang Hydroxy resin (200 mg) was transferred into a Biorad fritted polypropylene reaction vial. Pretreatment of the resin was carried out by sequentially washing the resin with a series of solvents to remove impurities as well as to swell the resin – 1:1 acetic acid: methylene chloride (4 X 5 mL),
methylene chloride (4 X 5 mL) and DMF (4 X 5 mL). The resin was finally stored in 2 mL of DMF.

3.3.2. **Loading of L-Phenylalanine N-FMOC onto ArgoGel Wang Hydroxy Resin.**

L-phenylalanine N-FMOC (181 mg, 6 equivalents) and dimethylaminopyridine (10 mg, 1 equivalent) was taken in a scintillation vial and dissolved in 2 mL of DMF. This was followed by the addition of diisopropylcarbodiimide (100 μL, 6 equivalents). This solution was then transferred to the reaction vial containing the pretreated resin. The reaction was allowed to proceed end over end for 4 hours at room temperature. The excess solvents and reagents were then drained off. The loaded resin was rinsed sequentially rinsed again with DMF (4 X 5 mL), anhydrous THF (4 X 5 mL), methylene chloride (4 X 5 mL) and DMF (4 X 5 mL).

3.3.3. **Deprotection of the FMOC Protecting Group.**

5 mL of piperidine in DMF (1:1) was added to the loaded resin and the reaction was allowed to proceed end over end for 2 hours at room temperature. The excess solvents and reagents were drained off. The resin was then rinsed with DMF (4 X 5 mL), anhydrous THF (4 X 5 mL), methylene chloride (4 X 5 mL) and DMF (4 X 5 mL).

3.3.4. **Reductive Amination.**

2,4-dimethoxybenzaldehyde (0.102 g, 0.61 mmol) was transferred into the reaction vial followed by the addition of 5 mL of DMF. The mixture was shaken
for 5 mins. This was followed by the addition of NaBH(OAc)$_3$ (0.032 g, 0.15 mmol). The reaction was allowed to proceed end over end at room temperature for 24 hours. The excess solvents and reagents were drained off. The resin was then rinsed with DMF (4 X 5 mL), anhydrous THF (4 X 5 mL) and methylene chloride (4 X 5 mL).

3.3.5. Cleavage.

TFA (50%) in CH$_2$Cl$_2$ (4 mL) was added to the resin, and the reaction was allowed to proceed end on end for 2 hours. The resin was washed with 50% TFA in CH$_2$Cl$_2$ (2 x 1.5 mL) and CH$_2$Cl$_2$ (2 x 1 mL). An aliquot of the combined filtrate was used as the analytical sample.

3.4 SOLUTION-PHASE STUDIES – INDIRECT REDUCTIVE AMINATION OF L-VALINE USING SODIUM TRIACETOXYBOROHYDRIDE

L-Valine (0.825 g, 5 mmol) was added into a scintillation vial, followed by triethylamine (0.556 g, 5.5 mmol). To this was added 5.5 mmol of aldehyde/ketone and 10 mL of dichloromethane. The reaction was stirred for 24 hours at room temperature. This was followed by the addition of sodium triacetoxyborohydride (1.59 g, 7.5 mmol). The reaction was stirred for another 24 hours at room temperature. The product was then filtered, washed with dichloromethane and air dried. This general procedure was used for L-isoleucine as well.
3.5 SOLUTION-PHASE STUDIES – INDIRECT REDUCTIVE AMINATION WITH A SOLID-PHASE APPROACH FOR DERIVATIVES OF L-VALINE

L-valine (0.825 g, 5 mmol) was transferred into a Biorad fritted polypropylene reaction vial, followed by the addition of triethylamine (0.556 g, 5.5 mmol). To this was added 5.5 mmol of aldehyde/ketone and 5 mL of dichloromethane. The reaction was allowed to proceed end on end for 24 hours at room temperature. The filtrate was collected in a scintillation vial and the residue was washed with CH$_2$Cl$_2$ (5 x 2 mL). To the combined filtrate, sodium triacetoxyborohydride (1.59 g, 7.5 mmol) was added. The reaction was stirred for 1 hour at room temperature. The product was then filtered, washed with dichloromethane and air dried. This general procedure was used for L-isoleucine as well.

3.6 ANALYTICAL EVALUATION OF SOLUTION-PHASE AND SOLID-PHASE SYNTHESIZED LIBRARIES OF L-PHENYLALANINE BY HPLC AND LC-MS.

3.6.1. HPLC Conditions

HPLC column used: Vydac 219TP5415, Diphenyl, 300 A, 5 μm, 4.6 mm i.d. x 150 mm.

Mobile phase: Methanol : 0.05 M aqueous sodium acetate (pH 4.0)
Gradient conditions:

<table>
<thead>
<tr>
<th>Time (mins)</th>
<th>%B Methanol</th>
<th>% D 0.05M sodium acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>10</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>15</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>18</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>21</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>25</td>
<td>5</td>
<td>95</td>
</tr>
</tbody>
</table>

Run time: 25 minutes.
Flow rate: 1 mL / min
Detection wavelength: 254 nm
Temperature: 30°C

3.6.2. LC-MS Conditions

HPLC column used: Hypersil BDS-C18, 3.0 mm i.d. x 50 mm.
Mobile phase: A: 0.1M aqueous ammonium acetate (pH 4.65)
B: 1:1 Acetonitrile : isopropyl alcohol

Gradient Conditions:

<table>
<thead>
<tr>
<th>Time (mins)</th>
<th>%A 0.1M ammonium acetate</th>
<th>% B 1:1 (acetonitrile: IPA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>11</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>21</td>
<td>90</td>
<td>10</td>
</tr>
</tbody>
</table>

Flow rate: 2 mL / min
Detection wavelength: 254 nm
4. RESULTS

4.1 COMPARISON OF REDUCING AGENTS

4.1.1 Solution-Phase Studies - Reductive Amination using Sodium Cyanoborohydride.

Figure 4.1: Chromatogram of Reductive Amination of 3-Aminophenol using Sodium Cyanoborohydride as the Reducing Agent.

4.1.1 Solution-Phase Studies - Reductive Amination using Sodium Triacetoxyborohydride.

Figure 4.2: Chromatogram of Reductive Amination of 3-Aminophenol using Sodium Triacetoxyborohydride as the Reducing Agent.
4.2 SOLUTION-PHASE STUDIES – DIRECT REDUCTIVE AMINATION OF L-PHENYLALANINE USING SODIUM TRIACETOXYBOROHYDRIDE

Table 4.1: Library of Derivatives of L-Phenylalanine.

<table>
<thead>
<tr>
<th>Aldehyde/Ketone</th>
<th>Product</th>
<th>Aldehyde/Ketone</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td><img src="image1" alt="Image of molecule A" /></td>
<td>F.W = 285</td>
<td><img src="image2" alt="Image of molecule E" /></td>
</tr>
<tr>
<td>p-methoxy benzaldehyde</td>
<td><img src="image1" alt="Image of molecule A" /></td>
<td></td>
<td><img src="image2" alt="Image of molecule E" /></td>
</tr>
<tr>
<td>B</td>
<td><img src="image3" alt="Image of molecule B" /></td>
<td>F.W = 285</td>
<td><img src="image4" alt="Image of molecule F" /></td>
</tr>
<tr>
<td>o-methoxy benzaldehyde</td>
<td><img src="image3" alt="Image of molecule B" /></td>
<td></td>
<td><img src="image4" alt="Image of molecule F" /></td>
</tr>
<tr>
<td>C</td>
<td><img src="image5" alt="Image of molecule C" /></td>
<td>F.W = 285</td>
<td><img src="image6" alt="Image of molecule G" /></td>
</tr>
<tr>
<td>m-methoxy benzaldehyde</td>
<td><img src="image5" alt="Image of molecule C" /></td>
<td></td>
<td><img src="image6" alt="Image of molecule G" /></td>
</tr>
<tr>
<td>D</td>
<td><img src="image7" alt="Image of molecule D" /></td>
<td>F.W = 254</td>
<td><img src="image8" alt="Image of molecule H" /></td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td><img src="image7" alt="Image of molecule D" /></td>
<td></td>
<td><img src="image8" alt="Image of molecule H" /></td>
</tr>
<tr>
<td>E</td>
<td><img src="image2" alt="Image of molecule E" /></td>
<td></td>
<td>2,4-dimethoxy benzaldehyde</td>
</tr>
<tr>
<td>F.W = 315</td>
<td></td>
<td>3,4-dimethoxy benzaldehyde</td>
<td>F.W = 315</td>
</tr>
<tr>
<td>G</td>
<td><img src="image6" alt="Image of molecule G" /></td>
<td>2,3-dimethoxy benzaldehyde</td>
<td><img src="image6" alt="Image of molecule G" /></td>
</tr>
<tr>
<td>H</td>
<td><img src="image8" alt="Image of molecule H" /></td>
<td>Acetophenone</td>
<td><img src="image8" alt="Image of molecule H" /></td>
</tr>
</tbody>
</table>
Figure 4.3: Chromatogram of Reductive Amination of L-Phenylalanine with p-Anisaldehyde.

Chromatogram of Reductive Amination of L-Phenylalanine with p-Anisaldehyde.
Figure 4.4: LC-MS Chromatogram of L-Phenylalanine with p-Anisaldehyde.

F.W = 285
R2001-

F.W = 285
R2001-2222-1 113 (4.150) Cm

F.W = 285
R2001-2222-1 112 (4.132) Cm

R2001-2222-1 225 (8.789) Cm

R2001-2222-1 275 (10.090) Cm
Figure 4.5: Chromatogram of Reductive Amination of L-Phenylalanine with Benzaldehyde.

Figure 4.6: Chromatogram of Reductive Amination of L-Phenylalanine with 2,3-Dimethoxybenzaldehyde.
Figure 4.7: Chromatogram of Reductive Amination of L-Phenylalanine with Acetophenone.
### Table 4.2: HPLC Data of L-Phenylalanine Library

<table>
<thead>
<tr>
<th></th>
<th>Total area</th>
<th>Area of Phe</th>
<th>Area of imine</th>
<th>Area of product</th>
<th>other imp.</th>
<th>% Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylalanine + p-anisaldehyde</td>
<td>47506.56</td>
<td>5567.86</td>
<td>9562.50</td>
<td>30811.90</td>
<td>1564.30</td>
<td>84.99</td>
</tr>
<tr>
<td>%</td>
<td>11.72</td>
<td>20.13</td>
<td>64.86</td>
<td>3.29</td>
<td>84.99</td>
<td></td>
</tr>
<tr>
<td>Phenylalanine + o-anisaldehyde</td>
<td>39631.20</td>
<td>3933.80</td>
<td>15404.30</td>
<td>14626.70</td>
<td>5666.40</td>
<td>75.78</td>
</tr>
<tr>
<td>%</td>
<td>9.93</td>
<td>38.87</td>
<td>36.91</td>
<td>14.30</td>
<td>75.78</td>
<td></td>
</tr>
<tr>
<td>Phenylalanine + m-anisaldehyde</td>
<td>33635.05</td>
<td>2362.95</td>
<td>12398.70</td>
<td>17856.10</td>
<td>1017.30</td>
<td>89.95</td>
</tr>
<tr>
<td>%</td>
<td>7.03</td>
<td>36.86</td>
<td>53.09</td>
<td>3.02</td>
<td>89.95</td>
<td></td>
</tr>
<tr>
<td>Phenylalanine + benzaldehyde</td>
<td>36073.80</td>
<td>4572.60</td>
<td>1242.70</td>
<td>23441.90</td>
<td>6816.60</td>
<td>68.43</td>
</tr>
<tr>
<td>%</td>
<td>12.68</td>
<td>3.44</td>
<td>64.98</td>
<td>18.90</td>
<td>68.43</td>
<td></td>
</tr>
<tr>
<td>Phenylalanine + 2,4-dimethoxybenzaldehyde</td>
<td>68082.70</td>
<td>3918.30</td>
<td>24136.10</td>
<td>32031.60</td>
<td>7996.70</td>
<td>82.50</td>
</tr>
<tr>
<td>%</td>
<td>5.76</td>
<td>35.45</td>
<td>47.05</td>
<td>11.75</td>
<td>82.50</td>
<td></td>
</tr>
<tr>
<td>Phenylalanine + 3,4-dimethoxybenzaldehyde</td>
<td>7992.68</td>
<td>0.00</td>
<td>1086.88</td>
<td>6039.30</td>
<td>866.50</td>
<td>89.16</td>
</tr>
<tr>
<td>%</td>
<td>0.00</td>
<td>13.60</td>
<td>75.56</td>
<td>10.84</td>
<td>89.16</td>
<td></td>
</tr>
<tr>
<td>Phenylalanine + 2,3-dimethoxybenzaldehyde</td>
<td>30649.90</td>
<td>6447.70</td>
<td>11101.40</td>
<td>11817.90</td>
<td>1282.90</td>
<td>74.78</td>
</tr>
<tr>
<td>%</td>
<td>21.04</td>
<td>36.22</td>
<td>38.56</td>
<td>4.19</td>
<td>74.78</td>
<td></td>
</tr>
<tr>
<td>Phenylalanine + acetophenone</td>
<td>5578.10</td>
<td>5273.00</td>
<td>305.10</td>
<td>0.00</td>
<td>0.00</td>
<td>5.47</td>
</tr>
<tr>
<td>%</td>
<td>94.53</td>
<td>5.47</td>
<td>0.00</td>
<td>0.00</td>
<td>5.47</td>
<td></td>
</tr>
</tbody>
</table>
Table 4.3: Summary of Library Results of L-Phenylalanine

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>YIELD * % (CRUDE)</th>
<th>% PURITY (HPLC)</th>
<th>MELTING POINT</th>
</tr>
</thead>
</table>
| F.Wt = 285  
[HOOC]  
[MeO]  
[CH2CH2NH]  
[A1]  
[97.4]  
[85.0]  
[>320] |
| F.Wt = 285  
[COOH]  
[OMe]  
[NH]  
[CH2CH2]  
[B1]  
[76.6]  
[75.6]  
[>320] |
| F.Wt = 285  
[HOOC]  
[OMe]  
[NH]  
[CH2CH2]  
[C1]  
[93.5]  
[90.0]  
[>320] |
| F.Wt = 254  
[HOOC]  
[CH2CH2NH]  
[D1]  
[96.6]  
[93.2]  
[>320] |
| F.Wt = 315  
[COOH]  
[OMe]  
[NH]  
[CH2CH2]  
[G1]  
[99.2]  
[82.5]  
[>320] |

* All calculations have been performed with the amino acid as the limiting reagent.
Table 4.3: Summary of Library Results of L-Phenylalanine (contd)

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>% YIELD (CRUDE)</th>
<th>% PURITY (HPLC)</th>
<th>MELTING POINT</th>
</tr>
</thead>
<tbody>
<tr>
<td>F.Wt 315 HOOC \CH_2-\CH</td>
<td>98.0</td>
<td>89.2</td>
<td>&gt; 320</td>
</tr>
<tr>
<td>F.Wt 315 COOH \CH_2-\CH</td>
<td>91.8</td>
<td>74.8</td>
<td>&gt; 320</td>
</tr>
<tr>
<td>F.Wt 269 COOH \CH_2-\CH</td>
<td>113.4</td>
<td>5.5</td>
<td>&gt; 320</td>
</tr>
</tbody>
</table>

*All calculations have been performed with the amino acid the limiting reagent.*
### 4.3 SOLUTION-PHASE STUDIES – REDUCTIVE AMINATION OF L-VALINE USING SODIUM TRIACETOXYBOROHYDRIDE

Table 4.4: Library of Derivatives of L-Valine.

<table>
<thead>
<tr>
<th>Aldehyde/Ketone</th>
<th>Product</th>
<th>Aldehyde/Ketone</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong>&lt;br&gt;CHO&lt;br&gt;OMe&lt;br&gt;p-methoxy benzaldehyde</td>
<td><img src="image1.png" alt="Image" /> F.W = 237</td>
<td><strong>E</strong>&lt;br&gt;CHO&lt;br&gt;OMe&lt;br&gt;2,4-dimethoxy benzaldehyde</td>
<td><img src="image2.png" alt="Image" /> F.W = 267</td>
</tr>
<tr>
<td><strong>B</strong>&lt;br&gt;CHO&lt;br&gt;OMe&lt;br&gt;o-methoxy benzaldehyde</td>
<td><img src="image3.png" alt="Image" /> F.W = 237</td>
<td><strong>F</strong>&lt;br&gt;CHO&lt;br&gt;OMe&lt;br&gt;3,4-dimethoxy benzaldehyde</td>
<td><img src="image4.png" alt="Image" /> F.W = 267</td>
</tr>
<tr>
<td><strong>C</strong>&lt;br&gt;CHO&lt;br&gt;OMe&lt;br&gt;m-methoxy benzaldehyde</td>
<td><img src="image5.png" alt="Image" /> F.W = 237</td>
<td><strong>G</strong>&lt;br&gt;CHO&lt;br&gt;OMe&lt;br&gt;2,3-dimethoxy benzaldehyde</td>
<td><img src="image6.png" alt="Image" /> F.W = 267</td>
</tr>
<tr>
<td><strong>D</strong>&lt;br&gt;CHO&lt;br&gt;Benzaldehyde</td>
<td><img src="image7.png" alt="Image" /> F.W = 207</td>
<td><strong>H</strong>&lt;br&gt;H3C&lt;br&gt;Acetophenone</td>
<td><img src="image8.png" alt="Image" /> F.W = 221</td>
</tr>
</tbody>
</table>
4.3.1 Direct Reductive Amination

Figure 4.8: Chromatogram of Direct Reductive Amination of L-Valine with Benzaldehyde.
Figure 4.9: LC-MS Chromatogram of Direct Reductive Amination of L-Valine with Benzaldehyde.
### Table 4.5: HPLC Data - Direct Reductive Amination of L-Valine Library

<table>
<thead>
<tr>
<th></th>
<th>L-Valine + p-anisaldehyde</th>
<th>L-Valine + o-anisaldehyde</th>
<th>L-Valine + m-anisaldehyde</th>
<th>L-Valine + m-anisaldehyde (Double reduction)</th>
<th>L-Valine + benzaldehyde</th>
<th>L-Valine + 2,4-dimethoxybenzaldehyde</th>
<th>L-Valine + 3,4-dimethoxybenzaldehyde</th>
<th>L-Valine + 2,3-dimethoxybenzaldehyde</th>
<th>L-Valine + 2,3-dimethoxybenzaldehyde</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Area</strong></td>
<td>2054.55</td>
<td>1262.80</td>
<td>65768.00</td>
<td>8065.50</td>
<td>19224.10</td>
<td>33419.20</td>
<td>4173.20</td>
<td>10701.90</td>
<td>8008.60</td>
</tr>
<tr>
<td><strong>Area of Val</strong></td>
<td>300.65</td>
<td>324.90</td>
<td>0.00</td>
<td>0.00</td>
<td>555.70</td>
<td>115.40</td>
<td>286.60</td>
<td>75.80</td>
<td>1119.80</td>
</tr>
<tr>
<td><strong>Area of Intermediate</strong></td>
<td>131.50</td>
<td>53.50</td>
<td>39912.20</td>
<td>145.70</td>
<td>1508.40</td>
<td>1025.20</td>
<td>696.10</td>
<td>400.20</td>
<td>26.60</td>
</tr>
<tr>
<td><strong>Area of Product</strong></td>
<td>1603.60</td>
<td>792.60</td>
<td>21772.00</td>
<td>6076.80</td>
<td>16119.60</td>
<td>28092.50</td>
<td>3179.20</td>
<td>6772.40</td>
<td>6210.60</td>
</tr>
<tr>
<td><strong>Other Imp.</strong></td>
<td>18.80</td>
<td>91.80</td>
<td>4083.80</td>
<td>1843.00</td>
<td>1040.40</td>
<td>4186.10</td>
<td>11.30</td>
<td>3453.50</td>
<td>651.60</td>
</tr>
<tr>
<td><strong>%</strong></td>
<td>14.63</td>
<td>25.73</td>
<td>0.00</td>
<td>0.00</td>
<td>2.89</td>
<td>0.35</td>
<td>6.87</td>
<td>0.71</td>
<td>13.98</td>
</tr>
<tr>
<td><strong>% Product</strong></td>
<td>84.45</td>
<td>67.00</td>
<td>93.79</td>
<td>77.15</td>
<td>91.70</td>
<td>87.13</td>
<td>92.86</td>
<td>67.02</td>
<td>77.88</td>
</tr>
</tbody>
</table>
Table 4.6: Summary of Results of Direct Reductive Amination of L-Valine

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>YIELD * % (CRUDE)</th>
<th>% PURITY (HPLC)</th>
<th>MELTING POINT</th>
</tr>
</thead>
<tbody>
<tr>
<td>F.W. = 357</td>
<td><img src="image" alt="Structure A2A" /></td>
<td>32.2</td>
<td>84.5</td>
</tr>
<tr>
<td>F.W. = 357</td>
<td><img src="image" alt="Structure B2B" /></td>
<td>45.0</td>
<td>67.0</td>
</tr>
<tr>
<td>F.W. = 357</td>
<td><img src="image" alt="Structure C2C" /></td>
<td>64.5</td>
<td>77.1</td>
</tr>
<tr>
<td>F.W. ≈ 297</td>
<td><img src="image" alt="Structure D2D" /></td>
<td>55.6</td>
<td>91.7</td>
</tr>
<tr>
<td>F.W. = 417</td>
<td><img src="image" alt="Structure E2E" /></td>
<td>32.8</td>
<td>87.1</td>
</tr>
</tbody>
</table>
Table 4.6: Summary of Results of Direct Reductive Amination of L-Valine (contd)

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>YIELD * % (CRUDE)</th>
<th>PURITY % (HPLC)</th>
<th>MELTING POINT</th>
</tr>
</thead>
<tbody>
<tr>
<td>F.Wt = 417</td>
<td>40.8</td>
<td>92.9</td>
<td>Charred at 310 C</td>
</tr>
<tr>
<td>F.Wt = 417</td>
<td>96.5</td>
<td>67.0</td>
<td>Charred at 320 C</td>
</tr>
<tr>
<td>F.Wt = 221</td>
<td>136.1</td>
<td>0.0</td>
<td>230 C</td>
</tr>
</tbody>
</table>

* All calculations have been performed with the aldehyde as the limiting reagent.
4.3.2 Indirect Reductive Amination

Figure 4.10: Chromatogram of Indirect Reductive Amination of L-Valine with Benzaldehyde.

![Chromatogram of Indirect Reductive Amination of L-Valine with Benzaldehyde](image-url)
Figure 4.11: LC-MS Chromatogram of Indirect Reductive Amination of L-Valine with Benzaldehyde.
### Table 4.7: HPLC Results of Indirect Reductive Amination of L-Valine

<table>
<thead>
<tr>
<th>Aldehyde</th>
<th>Area of L-Val</th>
<th>Area of Impurity</th>
<th>Area of Mono Alk. Product</th>
<th>Area of Bis Alk. Product</th>
<th>Total Area</th>
<th>% Mono Alk. Product</th>
<th>% Bis Alk. Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzaldehyde</td>
<td>1286.5</td>
<td>759.5</td>
<td>8645</td>
<td>4100.3</td>
<td>14791.3</td>
<td>58.5</td>
<td>27.7</td>
</tr>
<tr>
<td>p-anisaldehyde</td>
<td>3257.7</td>
<td>8807</td>
<td>48033</td>
<td>20206</td>
<td>80303</td>
<td>59.8</td>
<td>25.2</td>
</tr>
<tr>
<td>2,4-dimethoxy benzaldehyde</td>
<td>0</td>
<td>3334</td>
<td>26175.5</td>
<td>15841</td>
<td>45350.9</td>
<td>57.7</td>
<td>34.9</td>
</tr>
</tbody>
</table>

### Table 4.8: Time Related Study for Monitoring Indirect Reductive Amination.

<table>
<thead>
<tr>
<th></th>
<th>Time in Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>m-anisaldehyde</td>
<td></td>
</tr>
<tr>
<td>% Mono Alkylated Product</td>
<td>99.0</td>
</tr>
<tr>
<td>% Bis Alkylated Product</td>
<td>0</td>
</tr>
<tr>
<td>2,3-dimethoxybenzaldehyde</td>
<td></td>
</tr>
<tr>
<td>% Mono Alkylated Product</td>
<td>99.0</td>
</tr>
<tr>
<td>% Bis Alkylated Product</td>
<td>0</td>
</tr>
<tr>
<td>benzaldehyde</td>
<td></td>
</tr>
<tr>
<td>% Mono Alkylated Product</td>
<td>98.0</td>
</tr>
<tr>
<td>% Bis Alkylated Product</td>
<td>0</td>
</tr>
</tbody>
</table>
4.3.3 Indirect Reductive Amination with a Solid-Phase Approach for Derivatives of L-Valine.

Figure 4.12: Chromatogram of Indirect Reductive Amination with a Solid-Phase Approach of L-Valine with Benzaldehyde.

Table 4.9: HPLC Results of Indirect Reductive Amination with a Solid-Phase Approach for Derivatives of L-Valine.

<table>
<thead>
<tr>
<th>Aldehyde</th>
<th>% Mono Alkylated Product</th>
<th>% Bis Alkylated Product</th>
<th>% Yield * (crude)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzaldehyde</td>
<td>95.2</td>
<td>0</td>
<td>84.5</td>
</tr>
<tr>
<td>p-anisaldehyde</td>
<td>94.8</td>
<td>0</td>
<td>87.1</td>
</tr>
<tr>
<td>2,4-dimethoxy benzaldehyde</td>
<td>95.7</td>
<td>0</td>
<td>87.3</td>
</tr>
</tbody>
</table>

* All calculations have been performed with the aldehyde as the limiting reagent.
4.4 SOLUTION-PHASE STUDIES – L-ISOLEUCINE USING SODIUM TRIACETOXYBOROHYDRIDE

Table 4.10: Library of Derivatives of L-Isoleucine

<table>
<thead>
<tr>
<th>Aldehyde/ Ketone</th>
<th>Product</th>
<th>Aldehyde/ Ketone</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>A CHO p-MeO</td>
<td>F.W = 251</td>
<td>E CHO OMe</td>
<td>F.W = 281</td>
</tr>
<tr>
<td>p-Methoxy benzaldehyde</td>
<td>CH₃CH₂CH HOOC-CH MeO-CH₂NH-CH₂</td>
<td>2,4-dimethoxy benzaldehyde</td>
<td>CH₃CH₂CH HOOC-CH MeO-CH₂CH₂</td>
</tr>
<tr>
<td>B CHO OMe</td>
<td>F.W = 251</td>
<td>F CHO OMe</td>
<td>F.W = 281</td>
</tr>
<tr>
<td>o-Methoxy benzaldehyde</td>
<td>CH₃CH₂CH CHCOOH MeO-CH₂NH-CH₂</td>
<td>3,4-dimethoxy benzaldehyde</td>
<td>CH₃CH₂CH HOOC-CH MeO-CH₂CH₂</td>
</tr>
<tr>
<td>C CHO OMe</td>
<td>F.W = 251</td>
<td>G CHO OMe</td>
<td>F.W = 281</td>
</tr>
<tr>
<td>m-Methoxy benzaldehyde</td>
<td>CH₃CH₂CH MeO-CH₂NH-CH₂</td>
<td>2,3-dimethoxy benzaldehyde</td>
<td>CH₃CH₂CH HOOC-CH NH-CH₂</td>
</tr>
<tr>
<td>D CHO</td>
<td>F.W = 221</td>
<td>H</td>
<td></td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td>CH₃CH₂CH HOOC-CH MeO-CH₂NH-CH₂</td>
<td>Acetophenone</td>
<td>CH₃CH₂CHHOOC-CH NH-CH₂CH₃</td>
</tr>
<tr>
<td></td>
<td>D3</td>
<td></td>
<td>H3</td>
</tr>
</tbody>
</table>

- A3, B3, C3, F3
- A, B, C, D

- 4-methoxy benzaldehyde
- Ome = OCH₃
- CH₃ = methyl group
- COOH = carboxylic acid group
- CH₂NH = amino group
- CH₂ = methylene group
- MeO = methoxy group
4.4.1 Direct Reductive Amination

Figure 4.13: Chromatogram of Direct Reductive Amination of L-Isoleucine with 2,4-Dimethoxybenzaldehyde.
Figure 4.14: LC-MS Chromatogram of Direct Reductive Amination of L-Isoleucine with 2,4-Dimethoxybenzaldehyde.
Table 4.11: HPLC analysis of Library of Derivatives of L-Isoleucine

<table>
<thead>
<tr>
<th></th>
<th>Total Area</th>
<th>Area of Ileu</th>
<th>Area of Intermediate</th>
<th>Area of product</th>
<th>Other Imp.</th>
<th>% Product</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>L-isoleucine + p-anisaldehyde</strong></td>
<td>24265.30</td>
<td>0.00</td>
<td>5781.60</td>
<td>10253.50</td>
<td>8230.20</td>
<td>66.08</td>
</tr>
<tr>
<td>%</td>
<td>0.00</td>
<td>23.83</td>
<td>42.26</td>
<td>33.92</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>L-isoleucine + o-anisaldehyde</strong></td>
<td>196939.90</td>
<td>0.00</td>
<td>85155.20</td>
<td>39763.70</td>
<td>72021.00</td>
<td>63.43</td>
</tr>
<tr>
<td>%</td>
<td>0.00</td>
<td>43.24</td>
<td>20.19</td>
<td>36.57</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>L-isoleucine + m-anisaldehyde</strong></td>
<td>114068.80</td>
<td>0.00</td>
<td>12517.10</td>
<td>56625.00</td>
<td>44926.70</td>
<td>60.61</td>
</tr>
<tr>
<td>%</td>
<td>0.00</td>
<td>10.97</td>
<td>49.64</td>
<td>39.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>L-isoleucine + benzaldehyde</strong></td>
<td>36373.90</td>
<td>0.00</td>
<td>4185.80</td>
<td>25036.10</td>
<td>7152.00</td>
<td>80.34</td>
</tr>
<tr>
<td>%</td>
<td>0.00</td>
<td>11.51</td>
<td>68.83</td>
<td>19.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>L-isoleucine + 2,4-dimethoxybenzaldehyde</strong></td>
<td>29208.70</td>
<td>0.00</td>
<td>897.30</td>
<td>28311.40</td>
<td>0.00</td>
<td>100.00</td>
</tr>
<tr>
<td>%</td>
<td>0.00</td>
<td>3.07</td>
<td>96.93</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>L-isoleucine + 3,4-dimethoxybenzaldehyde</strong></td>
<td>30969.20</td>
<td>0.00</td>
<td>3461.40</td>
<td>23511.40</td>
<td>3996.40</td>
<td>87.10</td>
</tr>
<tr>
<td>%</td>
<td>0.00</td>
<td>11.18</td>
<td>75.92</td>
<td>12.90</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>L-isoleucine + 2,3-dimethoxybenzaldehyde</strong></td>
<td>38698.30</td>
<td>5.28</td>
<td>29.57</td>
<td>51.14</td>
<td>14.01</td>
<td>80.71</td>
</tr>
<tr>
<td>%</td>
<td>0.00</td>
<td>29.57</td>
<td>51.14</td>
<td>14.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>L-isoleucine + acetophenone</strong></td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.0</td>
</tr>
</tbody>
</table>
Table 4.12: Summary of Results of Direct Reductive Amination of L-Isoleucine

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>YIELD * % (CRUDE)</th>
<th>% PURITY (HPLC)</th>
<th>MELTING POINT</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Structure A3A" /></td>
<td>35.4</td>
<td>66.1</td>
<td>200°C</td>
</tr>
<tr>
<td><img src="image" alt="Structure B3B" /></td>
<td>63.2</td>
<td>67.0</td>
<td>Charred at 310°C</td>
</tr>
<tr>
<td><img src="image" alt="Structure C3C" /></td>
<td>53.1</td>
<td>60.6</td>
<td>Charred at 340°C</td>
</tr>
<tr>
<td><img src="image" alt="Structure D3D" /></td>
<td>59.0</td>
<td>80.3</td>
<td>Charred at 330°C</td>
</tr>
<tr>
<td><img src="image" alt="Structure E3E" /></td>
<td>53.1</td>
<td>100.0</td>
<td>Charred at 340°C</td>
</tr>
</tbody>
</table>

* All calculations have been performed with the aldehyde as the limiting reagent.
Table 4.12: Summary of Results of Direct Reductive Amination of L-Isoleucine (contd)

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>YIELD* % (CRUDE)</th>
<th>PURITY % (HPLC)</th>
<th>MELTING POINT</th>
</tr>
</thead>
<tbody>
<tr>
<td>F.Wt = 431</td>
<td>54.1</td>
<td>87.1</td>
<td>Charred at 310°C</td>
</tr>
<tr>
<td>F.Wt = 431</td>
<td>51.9</td>
<td>95.4</td>
<td>Charred at 320°C</td>
</tr>
<tr>
<td>F.Wt = 235</td>
<td>94.8</td>
<td>0.0</td>
<td>163°C</td>
</tr>
</tbody>
</table>

* All calculations have been performed with the aldehyde as the limiting reagent.
4.4.2 Indirect Reductive Amination

Figure 4.15: Chromatogram of Indirect Reductive Amination of L-Isoleucine with 2,4-Dimethoxybenzaldehyde.

L-Isoleucine with 2,4-dimethoxybenzaldehyde
Indirect reductive amination
Figure 4.16: LC-MS Chromatogram of Indirect Reductive Amination of L-Isoleucine with 2,4-Dimethoxybenzaldehyde.
Table 4.13: HPLC Results of Indirect Reductive Amination of L-Isoleucine

<table>
<thead>
<tr>
<th>Aldehyde</th>
<th>Area of L-isoleu</th>
<th>Area of Imp.</th>
<th>Area of Mono Alk. Product</th>
<th>Area of Bis Alk. Product</th>
<th>Total Area</th>
<th>% Mono Alk. Product</th>
<th>% Bis Alk. Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzaldehyde</td>
<td>0</td>
<td>83418</td>
<td>1077.5</td>
<td>86835</td>
<td>18690</td>
<td>5.8</td>
<td>46.5</td>
</tr>
<tr>
<td>p-anisaldehyde</td>
<td>0</td>
<td>7297</td>
<td>24906.9</td>
<td>12153</td>
<td>44356.5</td>
<td>56.2</td>
<td>27.4</td>
</tr>
<tr>
<td>2,4-dimethoxybenzaldehyde</td>
<td>0</td>
<td>2470</td>
<td>18819.3</td>
<td>12015</td>
<td>33304.1</td>
<td>56.5</td>
<td>36.1</td>
</tr>
</tbody>
</table>

4.4.3 Indirect Reductive Amination with a Solid-Phase Approach for Derivatives of L-Isoleucine.

Figure 4.17: Chromatogram of Indirect Reductive Amination with a Solution-Phase Approach of L-Isoleucine with 2,4-Dimethoxybenzaldehyde.

VWD1 A, Wavelength=254 nm (6-2516-270007.D) mAU

L-valine with 2,4-dimethoxybenzaldehyde for 24 hrs + sodium triacetoxyborohydride for 30 mins
Table 4.14: HPLC Results of Indirect Reductive Amination with a Solid-Phase Approach for Derivatives of L-Isoleucine.

<table>
<thead>
<tr>
<th>Aldehyde</th>
<th>% Mono Alkylated Product</th>
<th>% Bis Alkylated Product</th>
<th>% Yield * (crude)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzaldehyde</td>
<td>95.6</td>
<td>0</td>
<td>93.7</td>
</tr>
<tr>
<td>p-anisaldehyde</td>
<td>96.1</td>
<td>0</td>
<td>61.4</td>
</tr>
<tr>
<td>2,4-dimethoxy benzaldehyde</td>
<td>95.3</td>
<td>0</td>
<td>86.5</td>
</tr>
</tbody>
</table>

* All calculations have been done with the aldehyde as the limiting reagent.

NOTE: All HPLC analysis has been carried out under the assumption that all molar responses are equal.
5. DISCUSSION

5.1 COMPARISON OF REDUCING AGENTS

Sodium cyanoborohydride and sodium triacetoxyborohydride are the two most commonly used reducing agents for reductive amination. Initial studies were carried out to decide which one of these reducing agents would be most effective in our research. A model reaction was carried out using 3-aminophenol and 2,3-dimethoxybenzaldehyde. The reactions were monitored over a time period of 24 hours. Figure 4.1, clearly reveals that even after 24 hours there is still some unreacted amine and aldehyde in the reaction mixture. While Figure 4.2, reveals that all the amine and aldehyde have reacted to give the desired product. This clearly indicates that sodium triacetoxyborohydride is a better choice for reductive amination in addition to the other advantages that have been discussed earlier. Based on the above results, we decided to use sodium triacetoxyborohydride in all our further work.

5.2 SOLUTION-PHASE LIBRARY OF DERIVATIVES OF L-PHENYLALANINE BY DIRECT REDUCTIVE AMINATION

Reductive amination on the esters of amino acids\(^{21}\) has been carried out in the past at room temperature as well as at accelerated temperatures. In our research, the amino acids were used directly. The first amino acid that was used was L-phenylalanine (1). L-phenylalanine was chosen mainly because of its easy identification by HPLC. Compounds A-H were used as the carbonyl compounds and the synthesized library is depicted in Table 4.1. The chromatograms are
presented in Appendix A. Figure 4.3 shows the first synthesized compound of this library. This compound was also sent for an LC-MS analysis, which is shown in Figure 4.4. The LC-MS chromatogram revealed that the product had a formula weight of 285.1. This is the formula weight of the desired product, indicating that the reaction was successful. Representative chromatograms of this library are seen in Figures 4.5 and 4.6. Separate reactions were carried out only with amino acid, triethylamine and aldehyde to determine the retention time of the imine. All the chromatograms show small amounts of unreacted L-phenylalanine. The total HPLC data analysis of this library is depicted in Table 4.2, and the summary of results for this library is shown in Table 4.3. Figure 4.6, shows, that there was incomplete reduction of the intermediate imine, which was commonly observed in other cases too. In order to overcome this problem, a second reduction probably needs to be carried out. The ketone acetophenone did not undergo reductive amination at all as can be seen from Figure 4.7. This was not completely surprising as sodium triacetoxyborohydride is known to react somewhat sluggishly with ketones.31 Abdel-Majid et al also found that ketones require much longer times to undergo reductive amination, in some cases even upto 10 days.31

With the knowledge that this reaction is successful in solution, we decided to carry out the synthesis on solid-phase.
5.3 SOLID-PHASE LIBRARY OF DERIVATIVES OF L-PHENYLALANINE BY DIRECT REDUCTIVE AMINATION

Before any reaction is carried out on solid-phase the reaction is usually carried out in solution, since the reaction can be carried out on a slightly larger scale making analytical evaluation a lot easier.

The next reaction that was tried in solution was the deprotection of the FMOC protecting group. The deprotection procedure proved to be very effective and required less than two hours.

With the knowledge that the major steps in scheme 3.1, namely reductive amination and the deprotection of the FMOC group works in solution, the only other hurdles that we would have to face would be the loading of L-phenylalanine N-Fmoc onto the resin and the final cleavage of the product from the resin.

The resin that was chosen was Argogel Wang Hydroxy resin. L-phenylalanine N-FMOC was to be loaded onto the resin via an ester linkage. The loading step could not be monitored by conventional analytical techniques hence the sample has to be sent for nano-probe NMR and ATR-IR for confirmation of loading. The analytical results revealed that the resin was not loaded at all. The possible reason for unsuccessful loading could be that hindered carboxylic acids like L-phenylalanine N-FMOC may require stronger activating conditions other than DIC & DMAP.\textsuperscript{35} Other activating agents that have been suggested are 1-(Mesitylene-2-sulfonyl)-3-nitro-1H-1,2,4-triazole (MSNT),\textsuperscript{36} and PyBOP at low temperatures less than – 20 C.\textsuperscript{37}

The solid-phase synthesis was a much more laborious process and with inadequate facilities for monitoring the steps of the reaction, the focus was
shifted back to solution-phase synthesis. Solution-phase libraries for two more α-amino acids – L-valine and L-isoleucine were attempted.

5.4 SOLUTION-PHASE LIBRARY OF DERIVATIVES OF L-VALINE

5.4.1. Direct Reductive Amination

The same carbonyl compounds A-H were used for the synthesis of this library as well. The desired library is shown in Table 4.4. A reaction was carried out with L-valine, triethylamine and aldehyde to determine the retention time of the imine. The HPLC analysis of the results is shown in Table 4.5. The reaction between L-valine and m-anisaldehyde, resulted in 60.7% of unreacted intermediate in the product. This led us to believe that reduction was incomplete in this case.

The product obtained from the reaction between L-valine and m-anisaldehyde was subjected to a further reduction for 24 hours. After this second reduction, only traces of intermediate (1.8%) were observed.

Figure 4.8 depicts a typical chromatogram of the library indicating that the reaction was successful. For confirmation of results the product of the reaction between L-valine and benzaldehyde was also sent for LC-MS analysis, the results of which are shown in Figure 4.9. Contrary to the results of the L-phenylalanine library, it was observed that the bisalkylated product was found as the major product and the monoalkylated product was found as mere traces in some of the cases. The summary of results of direct reductive amination, are
given in Table 4.6. No reductive amination products were observed with acetophenone. The chromatograms are presented in Appendix B1.

In this reaction, only 5.5 mmoles of the carbonyl compound was used instead of 10 mmoles, which would have been required for complete bis-alkylation. Yields were recalculated to reflect results for the bisalkylated product hence the yields are considerably low.

5.4.2. Reaction of Reductive Amination Revisited

In light of the above finding we decided to reexamine at the stepwise reaction of reductive amination with respect to alpha amino acids (Scheme 5.1). A common problem faced with amino acids is that these compounds are not freely soluble in organic solvents mainly because they exist in their zwitterionic form.

Scheme 5.1 Reductive Amination Revisited
Steps 1 & 2 in the reaction Scheme 5.1 are reversible and due to the poor solubility of the amino acid, it would be justifiable to assume that these steps are the slow steps or the rate-determining steps in the reaction. In order to confirm our hypothesis indirect reductive amination was carried out in which sufficient time (24 hours) was given for complete imine formation prior to reduction.

5.4.3. Indirect Reductive Amination.

Figure 4.10 shows the HPLC chromatogram for D2 obtained by indirect reductive amination. This chromatogram appears different from the one obtained with direct reductive amination. There are two major peaks visible here indicating that the first peak may be due to the monoalkylated product while the second peak, which matched in retention time with that in Figure 5.8 could be due to the bis alkylated compound. In order to confirm these suspicions, the sample was subjected to an LC-MS analysis, the results of which are seen in Figure 4.11.

Figure 4.11 clearly reveals the presence of two peaks. The peak at a retention time of 6.19 minutes is the major peak with a formula weight of 207, which corresponds to the monoalkylated product while the peak at 10.22 minutes is the minor peak with a formula weight of 297 which corresponds to the bis alkylated product (D2D).

F.W. = 207

\[
\begin{align*}
\text{F.W.} &= 207 \\
\text{D2 Mono alkylated Product} \\
\end{align*}
\]

\[
\begin{align*}
\text{F.W.} &= 297 \\
\text{D2D Bis alkylated Product} \\
\end{align*}
\]

This reaction was repeated along with two other aldehydes - A and E. The
results of which are seen in Table 4.7. With this procedure, the monoalkylated product can be synthesized as the major product, but the undesired bisalkylation still occurs. The chromatograms are presented in Appendix B2.

The question that was now raised in our minds was that how was the bisalkylated compound formed. In order to answer this question a closer look at Scheme 5.1 was taken. The carbonyl compounds used in these reactions were taken in a 0.5 mmole excess to the amino acid. This excess of carbonyl compound still existed in solution even after all the imine had been formed. With the addition of the reducing agent, the imine is preferentially reduced to the monoalkylated compound. This monoalkylated product could possibly react with the excess aldehyde, resulting in a second reductive amination. This is possible since the reducing agent is present in 2.5 mmole excess to the amino acid. Scheme 5.2 predicts the formation of the bis alkylated product.
Scheme 5.2: Formation of Bisalkylated Product

Aldehyde/Ketone + Primary amine (alpha amino acid) → Bis alkylated product

Step 1: Formation of Hydroxylamine (addition product)

Step 2: Elimination of water to form Imine (elimination product)

Step 3: Reduction of Imine to form Mono alkylated Product

Step 4: Reduction of Mono alkylated Product to form Hydroxylamine (addition product)

Step 5: Hydroxylamine reacts with Aldehyde/Ketone to form Imine (elimination product)

Step 6: Reduction of Bis alkylated product to form Aldehyde/Ketone
The next step was to test the predicted scheme. This was achieved by monitoring the reaction as a function of time.

5.4.3.1 Monitoring the Reaction Progress Between L-Valine and m-Anisaldehyde, 2,3-Dimethoxybenzaldehyde and benzaldehyde Respectively.

Indirect reductive amination was carried out in which the amino acid and the carbonyl compound were allowed to react for 4 hours prior to addition of the reducing agent for sufficient imine formation. The results of this study are depicted in Table 4.8. Within 30 minutes of addition of the reducing agent, we saw 99% of monoalkylated product was formed with no traces of the bisalkylated product in any of the cases. After 4 hours of reduction, a small amount of the bisalkylated product was observed which only increased with time. At the end of 24 hours, a mixture of mono and bisalkylated products were observed in the case of m-anisaldehyde only. In the other two cases only bisalkylated products were obtained. A further reduction for 24 hours in the case of m-anisaldehyde resulted in the complete conversion of the monoalkylated product into the bisalkylated product. The chromatograms are presented in Appendix B3.

A graphical representation of these results are shown in Figures 5.1, 5.2 and 5.3 respectively.
Figure 5.1: Reaction Progress of L-Valine with m-Anisaldehyde.

![Graph showing reaction progress of L-Valine with m-Anisaldehyde.](image)

Figure 5.2: Reaction Progress of L-Valine with 2,4-Dimethoxybenzaldehyde.

![Graph showing reaction progress of L-Valine with 2,4-Dimethoxybenzaldehyde.](image)
This above time related study proved our hypothesis that the monoalkylated product undergoes a second reductive amination to yield the bisalkylated product.

From this study it was also evident that the reduction step was a very fast one and did not require a 24 hour time period as complete reduction took place within 30 minutes.

Our goal was to synthesize the monoalkylated product as the only product of this reaction.

5.4.4. Indirect Reductive Amination with a Solid-Phase Approach.

We then decided to incorporate the advantages of the solid-phase technique and apply it to our reaction. The reactants were taken in a Bio Rad
polypropylene vial, which is normally used for solid-phase analysis. The idea behind using this technique is that in solid-phase synthesis methods, the synthesized compound is linked onto the resin and the excess reagents and side products of the reaction are washed away, thereby increasing the purity of the synthesized compound.

So, this advantage was used in the reverse way. The reaction between L-valine and benzaldehyde was allowed to proceed in the vial for 24 hours. This was followed by washings of the residue in order to extract the imine. The filtrate now contained the imine, TEA and traces of the carbonyl compound. The residue left behind in the vial would be similar to the resin after the synthesized product was cleaved off the resin. The obtained filtrate would now be of higher purity as all the unreacted amino acid was eliminated from the reaction mixture.

So, far all the reactions that were carried out were with a 0.5 mmole excess of the carbonyl compound. If the carbonyl compound was used as the limiting reagent in the reaction, then bisalkylation could probably be controlled. An experiment was carried out by using 5 mmoles of L-valine and 4.5 mmoles of the carbonyl compound (benzaldehyde) and this reaction was monitored. A representative chromatogram is seen in Figure 4.12 and the data for this reaction is presented in Table 4.9. This data clearly indicates that even after 24 hours the monoalkylated product is predominant with a purity greater than 96%. The bis alkylated product is present in traces ranging from 2 to 4%. Graphical representation of this data is shown in Figure 5.4 and the chromatograms are presented in Appendix B4.
5.5 SOLUTION-PHASE LIBRARY OF DERIVATIVES OF L-ISOLEUCINE

The same carbonyl compounds A-H were used to synthesize this library. The desired derivatives are shown in Table 4.4.

5.5.1. Direct Reductive Amination

The compounds of this library were extremely hygroscopic, so these samples were dried at 100°C. The HPLC analysis of the synthesized library is shown in Table 4.10. Insufficient aldehyde was used in all these reactions for complete bisalkylation. Like in the case of L-valine, yields were recalculated to reflect results for the bisalkylated product hence the yields are considerably low.

All the products seemed to contain varying amounts of the intermediate. A typical chromatogram of the library is shown in Figure 4.13. The same sample
was also analyzed by LC-MS. The LC-MS chromatogram is shown in Figure 4.14. Instead of seeing one peak we observed six peaks. Table 5.1 shows the possible interpretation of these results.

**Figure 4.14:** LC-MS Chromatogram of Direct Reductive Amination of L-Isoleucine with 2,4-Dimethoxybenzaldehyde.
Table 5.1: Interpretation of LC-MS Results

<table>
<thead>
<tr>
<th>Peak</th>
<th>Possible Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>![Structure A] F.Wt = 150</td>
</tr>
<tr>
<td>B</td>
<td>![Structure B] F.Wt = 281</td>
</tr>
<tr>
<td>C</td>
<td>![Structure C] F.Wt = 150</td>
</tr>
<tr>
<td></td>
<td>![Structure C'] F.Wt = 237</td>
</tr>
<tr>
<td>D</td>
<td>![Structure D] F.Wt = 317</td>
</tr>
<tr>
<td>E</td>
<td>![Structure E] F.Wt = 431</td>
</tr>
<tr>
<td>F</td>
<td>![Structure F] F.Wt = 387</td>
</tr>
<tr>
<td></td>
<td>![Structure F'] F.Wt = 181</td>
</tr>
<tr>
<td>G</td>
<td>![Structure G] F.Wt = 150</td>
</tr>
</tbody>
</table>

It is a possibility that the four extra fragments that are seen are due to product decomposition as a result of heating the sample at 100°C. Peak B is the monoalkylated product while peak E is the bisalkylated product. The summary of results of this library is shown in Table 4.11. The reaction with acetophenone did not result in any reductive amination products. Chromatograms are presented in Appendix C1.
In the L-valine library, only traces of the monoalkylated product were seen while in this library, a mixture of monoalkylation and bisalkylation is seen.

5.5.2. Indirect Reductive Amination

Indirect reductive amination was carried out in which a 24 hour time period was given for complete imine formation. The product was vacuum dried instead of drying at 100°C. Figure 4.15 shows the HPLC chromatogram, which reveals that the product is a mixture of mono and bis alkylated compounds. This was confirmed by LC-MS, as shown in Figure 4.16. We do not see several fragments in Figure 4.16 as compared to Figure 4.14. Hence we can conclude that the extra fragments in Figure 4.14 might be due to thermal decomposition of the product.

The experiment was repeated again with two more carbonyl compounds p-anisaldehyde and 2,4-dimethoxybenzaldehyde for confirmation of results. These results are tabulated in Table 4.12. Reactions with p-anisaldehyde and 2,4-dimethoxybenzaldehyde were terminated after 24 hours while that with benzaldehyde was allowed to proceed for 48 hours. The monoalkylated product seems to predominate in the cases of p-anisaldehyde and 2,4-dimethoxybenzaldehyde. While in the case with benzaldehyde, the bisalkylated product seems to predominate with traces of the monoalkylated product. This again confirms Scheme 5.2, indicating that with time, the monoalkylated product undergoes a second reductive amination to yield the bisalkylated product. The chromatograms for this section are presented in
Appendix C2.

5.5.3. Indirect Reductive Amination with a Solid-Phase Approach

In order to synthesize only monoalkylated products the solid-phase approach was adopted as used in the case of L-valine. The reactions were terminated after 30 minutes of reduction time. Only the monoalkylated product was obtained as can be seen from Figure 4.17. Table 4.13 gives the HPLC results and the corresponding yields. The corresponding chromatograms are present in Appendix C3. It can be seen that the purity of the monoalkylated products are around 96% and not even traces of bisalkylation is observed.

After having carried out indirect reductive amination with L-valine and L-isoleucine we now have a better understanding as to what was possibly happening in the one-pot reaction or in direct reductive amination.

5.6 A CLOSER LOOK AT DIRECT REDUCTIVE AMINATION

In direct reductive amination, the carbonyl compound, amino acid and the reducing agent are all added simultaneously and the reaction is allowed to proceed. The solubility of the amino acid is a hindrance to the reaction progress. As the amino acid slowly begins to dissolve in solution, the aldehyde then attacks the amino acid to form the imine, which subsequently gets reduced to form the monoalkylated product. As only a small amount of the aldehyde has reacted with the amino acid, the remaining aldehyde probably prefers to attack the monoalkylated product instead of the amino acid resulting in the formation of
the bisalkylated product. The monoalkylated product is a better nucleophile as compared to the amino acid and will hence react preferentially with the carbonyl compound.\textsuperscript{38} It is possibly for this reason that we do not see a substantial amount of monoalkylated product in the case of \( \alpha \) aliphatic amino acids like L-valine and L-isoleucine.

In the case of the library of derivatives of L-phenylalanine, which is an aromatic \( \alpha \)-amino acid, the monoalkylated product predominates. The first explanation for this is steric hinderance. D1 is one of the products of this library. The presence of the bulky aromatic - \( R \) group on the amino acid most definitely would prevent a second reductive amination.

\[
\begin{align*}
\text{HOOC} & \quad \text{CH} \quad \text{CH}_2 \\
\text{D1} & \quad \text{NH} \\
& \quad \text{CH}_2
\end{align*}
\]

The presence of the bulky aromatic \( R \) group also decreases the nucleophilicity of the monoalkylated product thereby reducing its reactivity and preventing bisalkylation.
5.7 A BIRD'S EYE VIEW OF RESULTS

Here is a graphical representation of results and yields obtained in the libraries indicating that the products of all the libraries were in fairly good yields and excellent purity.

Figure 5.5: Purity and Yields of the L-Phenylalanine Library

Figure 5.6: Purity and Yields of the L-Valine and L-Isoleucine Library by Indirect Reductive Amination with a Solid-Phase Approach.
6. CONCLUSION

It is possible to reductively aminate \(\alpha\) - amino acids using both direct and indirect reductive amination. Sodium triacetoxyborohydride was found to be a superior reducing agent to sodium cyanoborohydride and had the added advantage of being environment friendly.

Bisalkylation has been reported as an undesired side reaction of reductive amination using sodium triacetoxyborohydride and several other reducing agents.\(^{31,38,39}\) In this study, bisalkylation was a minor reaction product when L-phenylalanine was used as the substrate. But, with the less sterically hindered aliphatic \(\alpha\) - amino acids L-valine and L-isoleucine, bisalkylation was initially the major product.

Using indirect reductive amination, reaction of aliphatic \(\alpha\) - amino acids using the carbonyl component as the limiting reagent and shorter reaction times (30 minutes) yielded primarily, the monoalkylated product.

All products precipitated from the reaction medium and were isolated in 75-95% purity by HPLC. These secondary amines can be further derivatized by alkylation or acylation to yield an expanded library of new derivatives.

Using these reaction conditions, ketones were found to be unreactive and would require alternate methods such as silica gel / zinc borohydride\(^{34}\), and or borohydride exchange resin\(^{32}\) which have been previously reported.

In terms of future work, the reductive amination of other amino acids can be carried out, in addition to expanded library of these secondary amines by alkylation or acylation. Substitution effects of carbonyl compounds can be
studied on the reductive amination of soluble amines as well as the determination of suitable conditions for reductive amination of ketones.
7. REFERENCES


APPENDIX A – DIRECT REDUCTIVE AMINATION OF L-PHENYLALANINE.

Figure A. 1: Product A1. ................................................................. 85
Figure A. 2: Product B1. ................................................................. 85
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Figure A. 2 : Product B1.
=ure A. 3 : Product C1.

Figure A. 4 : Product D1.
Figure A. 5: Product E1.

Figure A. 6: Product F1.
Figure A. 7 : Product G1.

L-phe with 2,3-dimethoxybenzaldehyde

Phe
21.0%

Imine
36.2%

Product
38.6%

Figure A. 8 : Product A8.

Phe
94.5%

Imine
5.5%
APPENDIX B1 – DIRECT REDUCTIVE AMINATION OF L-VALINE

Figure B1. 1: Product A2A ................................................................. 90
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Figure B1. 3: Product C2C ................................................................. 91
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Figure B1. 3 : Product C2C.

Figure B1. 4 : Product D2D.
Figure B1. 5 : Product E2E.

Figure B1. 6 : Product F2F.
Figure B1. 7: Product G2G.

L-val with 2,3-dimethoxybenzaldehyde

Figure B1. 8: Product H2.

L-val with acetophenone
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Figure B2. 2: Product of L-Valine with p-Anisaldehyde.
Figure B2. 3: Product of L-Valine with 2,3-Dimethoxybenzaldehyde.
APPENDIX B3 – TIME RELATED STUDIES

1. Monitoring the Reaction Between L-Valine and p-Anisaldehyde
   - Figure B3.1: After 30 Minutes
   - Figure B3.2: After 4 Hours
   - Figure B3.3: After 18 Hours
   - Figure B3.4: After 24 Hours
   - Figure B3.5: After 48 Hours (Double Reduction)

2. Monitoring the Reaction Between L-Valine and 2,3-Dimethoxybenzaldehyde
   - Figure B3.6: After 30 Minutes
   - Figure B3.7: After 4 Hours
   - Figure B3.8: After 18 Hours
   - Figure B3.9: After 24 Hours

3. Monitoring the Reaction Between L-Valine and Benzaldehyde
   - Figure B3.10: After 30 Minutes
   - Figure B3.11: After 4 Hours
   - Figure B3.12: After 18 Hours
   - Figure B3.13: After 24 Hours
1. Monitoring the Reaction Progress between L-valine and m-anisaldehyde.

Figure B3.1: After 30 Minutes.

Figure B3.2: After 4 Hours.
Figure B3.3: After 18 Hours.

Figure B3.4: After 24 Hours.
2. Monitoring the Reaction Progress between L-valine and 2,3-Dimethoxybenzaldehyde.

Figure B3.5: After 48 Hours (Double Reduction).

Figure B3.6: After 30 Minutes.
Figure B3.7: After 4 Hours.

Figure B3.8: After 18 Hours.
3. Monitoring the Reaction Progress between L-valine and benzaldehyde.

Figure B3. 9: After 24 Hours.

Figure B3. 10: After 30 Minutes.
Figure B3.11: After 4 Hours.

Figure B3.12: After 18 Hours.
Figure B3.13: After 24 Hours.

VWD1 A, Wavelength=254 nm (980918A/WAL00049.D)

24 Hours

Bar Alkylated Product

69.7%

9.7%
APPENDIX B4 – INDIRECT REDUCTIVE AMINATION WITH A SOLUTION PHASE APPROACH

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Figure B4. 1 : L-Valine with Benzaldehyde After 25 Minutes.

Figure B4. 2 : L-Valine with Benzaldehyde After 4 Hours.
Figure B4. 3 : L-Valine with Benzaldehyde After 24 Hours.

Figure B4. 4 : L-Valine with p-Anisaldehyde.
Figure B4. 5: L-Valine with 2,4-Dimethoxybenzaldehyde.

L-valine with 2,4-dimethoxybenzaldehyde for 24 hrs + sodium triacetoxyborohydride for 30 mins
APPENDIX C1 – DIRECT REDUCTIVE AMINATION OF

L- ISOLEUCINE

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Figure C1.2: L-Isoleucine with o-Anisaldehyde.
Figure C1.3: L-isoleucine with m-Anisaldehyde.

Figure C1.4: L-Isoleucine with Benzaldehyde.
Figure C1. 5: L-Isoleucine with 2,4 Dimethoxybenzaldehyde.

Figure C1. 6: L-Isoleucine with 3,4-Dimethoxybenzaldehyde.
Figure C1.7: L-Isoleucine with 2,3-Dimethoxybenzaldehyde.
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Figure C3. 1 : L-Isoleucine with Benzaldehyde.

Figure C3. 2 : L-Isoleucine with p-Anisaldehyde.
Figure C3. 3: L-Isoleucine with 2,4-Dimethoxybenzaldehyde.

L-Isoleucine with 2,4-dimethoxybenzaldehyde for 24 hours + sodium triacetoxyborohydride for 30 mins

Mono Alkylated Product

95.3%