

Dipeptide Derivatives Synthesis

Abstract

In this series of base catalyzed reactions, the methyl ester N-dipeptide was successfully synthesized. The reaction was performed in two experiments. The first experiment used Schotten-Baumann conditions to synthesize (p-toluoylamino) acetic acid using anhydrous glycine and p-toluoyl chloride. The melting point of this product was determined to be 156.6-159°C. The second experiment was also base catalyzed using the dehydrating agent DCC. The [5] was synthesized using glycine methyl ester chloride and p-toluoylamino acetic acid. The melting point of the final product was determined to be 147.3-148.0°C. The products from both experiments were also scanned using Fourier-transform infrared spectroscopy (FTIR) to confirm the presence of desired functional groups. This experiment yielded 0.02590 g of [5] with a percent yield of 5.578%.

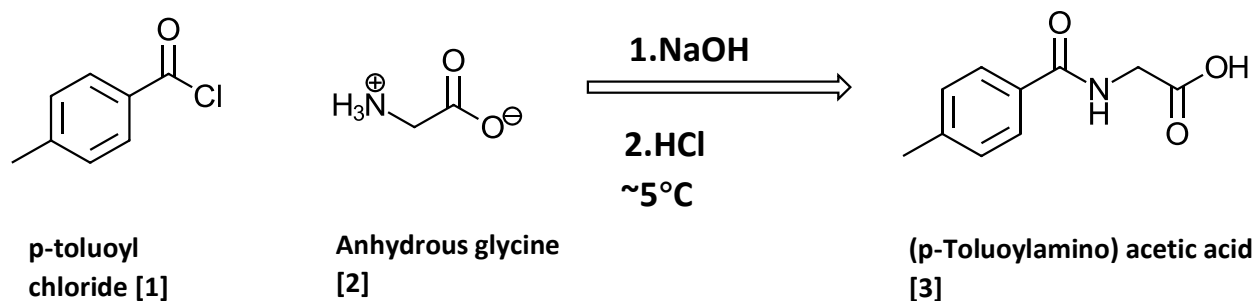
Introduction

This report discusses the preparation of Methyl [(p-toluoylamino)methyl]carbonylamino} acetate. Both reaction schemes and the full mechanism can be found below. Reagents, reaction conditions and deviation from experimental literature will be discussed. The results will be presented including product, FTIR, NMR, melting points, summary FTIR and NMR tables, and a sample yield calculation will be presented. The results will be discussed, and the literature expectation will be rationalized.

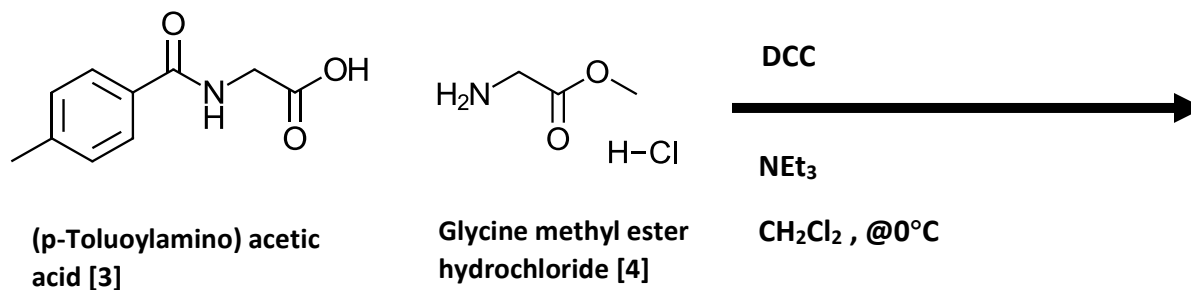
Experiment one involves the simple base catalyzed addition-elimination reaction of p-toluoyl chloride and anhydrous glycine. The reaction utilizes Schotten-Bauman conditions. This reaction produces p-toluoylamino acetic acid which is used in the following reaction.

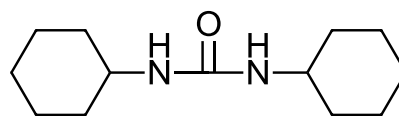
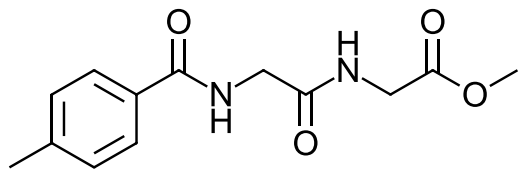
In this reaction N,N'-Dicyclohexylcarbodiimide (DCC) is used as a dehydrating coupling agent. DCC is a common reagent used to synthetically link amino acids in a laboratory. This reagent promotes the hydrolysis reaction which creates a peptide bond between two amino acids. The reaction is kept at moderate temperatures such as 0°C to prevent the formation of the inactive N-Acylisourea. The addition of heat promotes the nitrogen of DCC to act as a nucleophile and attack the carbonyl carbon of [3]. This would decrease yield significantly. ¹

Reaction Scheme Experiment 1



Reaction Scheme Experiment 2

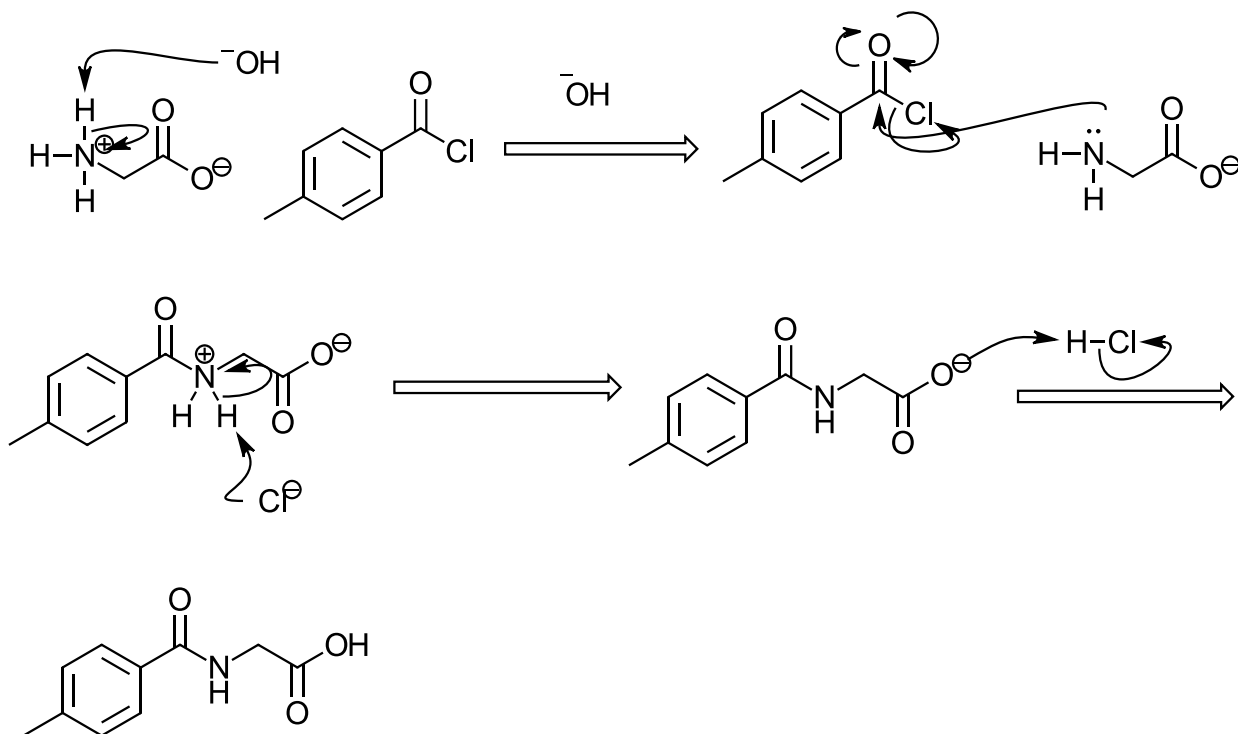


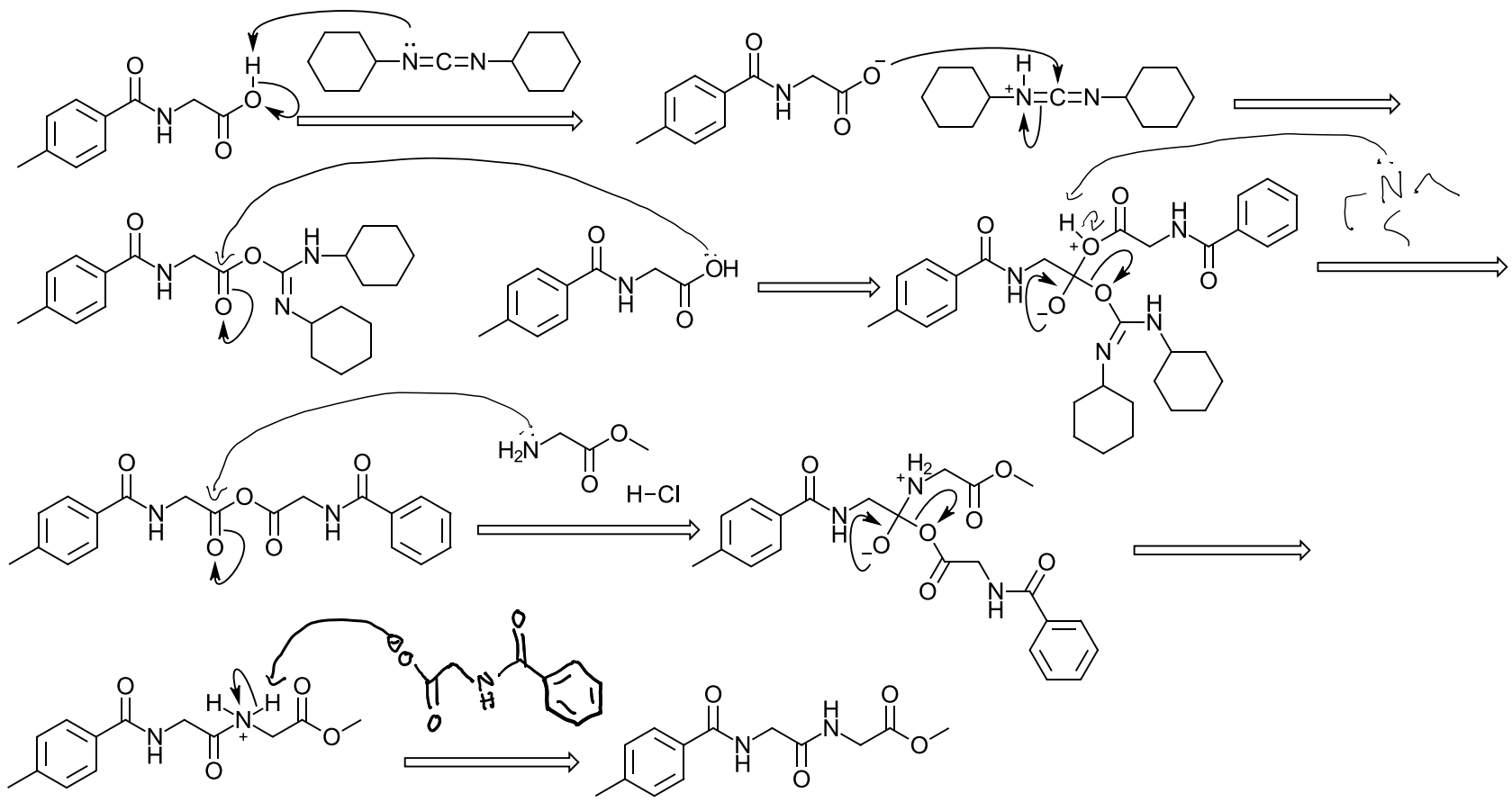


Methyl [[(p-toluoylamino)methyl]carbonylamino]acetate
[5]

DCU

Reaction Mechanism Experiment 1





Formation of DCU and Regeneration of Base

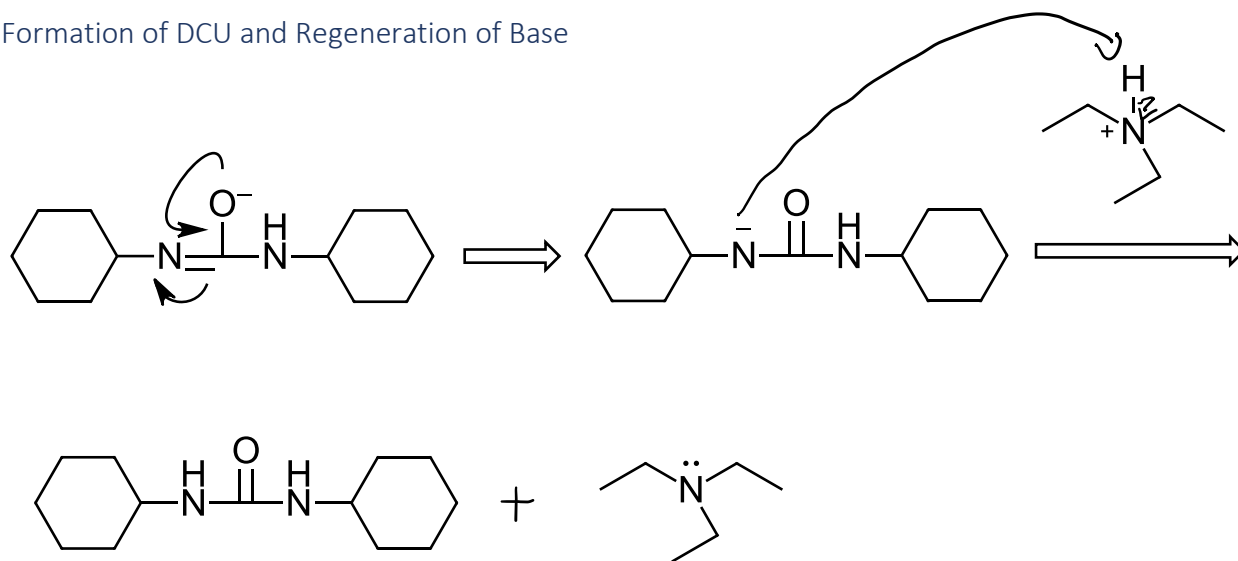


Figure 1. Mechanism of the formation of DCU and the regeneration of triethylamine

Experimental

The experiment was prepared according to the literature method^{2,3}, although there were a few exceptions. The unknown used in the experiment was #3 and it was a clear and colourless liquid. After the solution was acidified to an approximate pH of 2 the solution precipitated instantly and only required a small amount of hydrochloric acid (~5mL) compared to the recommended 10-15 mL. Once this product was washed with water the precipitate remained a yellow powder. The dry, crude product was then recrystallized using acetate and hexanes. During the recrystallization process there was difficulty getting the product to recrystallize. Hot gravity filtration was used to separate the solvent from the remaining precipitate. A significant of product was lost in the filtration process as there was only one filtration performed. The remaining filtrate was then recrystallized, and the product was a fine white powder.

A 100mL round bottom flask (RBF) was used instead of the recommended 100mL RBF as a smaller scale experiment was performed. After the addition of the DCC from the dropping funnel the solution was white and cloudy. The DCU filtrate was a very fine white powder while removing it by vacuum filtration. The washed filtrate was transferred to a 50mL Erlenmeyer flask and an appropriate amount of anhydrous sodium sulfate was added until the powder was able to flow freely in the liquid without clumping. The solution was corked and left until next lab period.

Next lab period, all the solvent in the Erlenmeyer flask had evaporated. Approximately 20 mL of dichloromethane was added to the Erlenmeyer flask to redissolve the crude product. The crude product was transferred to an RBF and placed on the Roto-vap to remove remaining solvent. DCU was still present after the first Roto-vap. Then 20 mL of dichloromethane was added to the RBF again and the solution was gravity filtered to remove the DCU. The crude product was

placed on Roto-vap again and less DCU was present. The crude product was recrystallized then washed with little amount of water. The precipitate was not washed with ethanol/pet. Ether to prevent further product loss. The final product was a white powder with a slight yellow tint.

Table 1. Table of Reagents used

Chemical Name and Formula	MW ⁴ (g/mol)	Mass (g) or Volume (mL)	# of moles	Mole Ratio	Physical Data ⁴
Anhydrous glycine C ₂ H ₅ NO ₂	75.067	3.0016 g	0.03998	1	MP=290°C D=1.607 g/mL
p-toluoyl chloride C ₈ H ₇ ClO	154.59	-	-	1.1	MP= -1.5°C D= 1.186 g/mL
Sodium hydroxide NaOH	39.997	~24 mL	-	-	MP= 323°C BP= 1388°C D= 2.13 g/mL
Diethyl ether C ₄ H ₁₀ O	74.121	40 mL	-	-	MP= -116.23°C BP= 34.45°C D= 0.7135 g/mL
Hydrochloric acid HCl	36.461	~15 mL	-	-	MP= -114.17°C BP= -85°C D= 1.187 g/mL
Ethyl acetate C ₄ H ₈ O ₂	88.106	-	-	-	MP= -83.8°C BP= 77.1°C D= 0.9006 g/mL
Hexane C ₆ H ₁₄	86.175	--	-	-	MP= -95.27°C BP= 68.72°C D= 0.6593 g/mL
Dichloromethane CH ₂ Cl ₂	84.933	-	-	-	MP= -94.91°C BP= 39.81°C D= 1.3232 g/mL
Triethylamine C ₆ H ₁₅ N	101.190	0.4 mL	-	-	MP=-114.7°C BP= 88.8°C D=0.7275 g/mL
DCC C ₁₃ H ₂₂ N ₂	206.327	3 mL	-	-	MP= 34.5 BP= 155
Acetic acid C ₂ H ₄ O ₂	60.052	0.5 mL	-	-	MP= 17°C BP= 117.9°C D=1.0510 g/mL
Sodium bicarbonate NaHCO ₃	84.007	10 mL	-	-	MP= 527°C D= 2.20 g/mL
Sodium sulfate Na ₂ SO ₄	142.043	-	-	-	MP= 884°C D= 2.7 g/mL
Ethanol	46.068	-	-	-	MP= -114.14°C

C ₂ H ₆ O					BP= 78.24°C D= 0.7893 g/mL
Petroleum ether		-	--	-	
Glycine methyl ester hydrochloride C ₃ H ₇ NO ₂ · HCl	125.55	0.3676 g	0.002928	1	MP= 175
P-toluoyl amino acetic acid C ₉ H ₄ NO ₂	165.19	0.6024 g	0.003647	1	-

Results

Table 2. Table of Products

Product Name and Formula	MW (g/mol)	Properties	Theoretical yield	Actual yield	% Yield
p-toluoylamino acid C ₉ H ₁₁ NO ₂	165.19 ⁴	Exp MP= 156.6-159° C ⁴ Lit MP= 163-165°C ¹ Appearance= fine white powder	6.6052 g	0.6634 g	10.04%
Methyl {(p-toluoylamino methyl) carbonylamino} acetate C ₁₃ H ₁₆ N ₂ O ₄	264.28 ⁴	Exp MP=147.3-148.0°C Appearance= white powder with slight yellow tint	0.4643 g	0.02590 g	5.578%

Analyst
Date

FTIR3
Thursday, November 3, 2022 2:39 PM

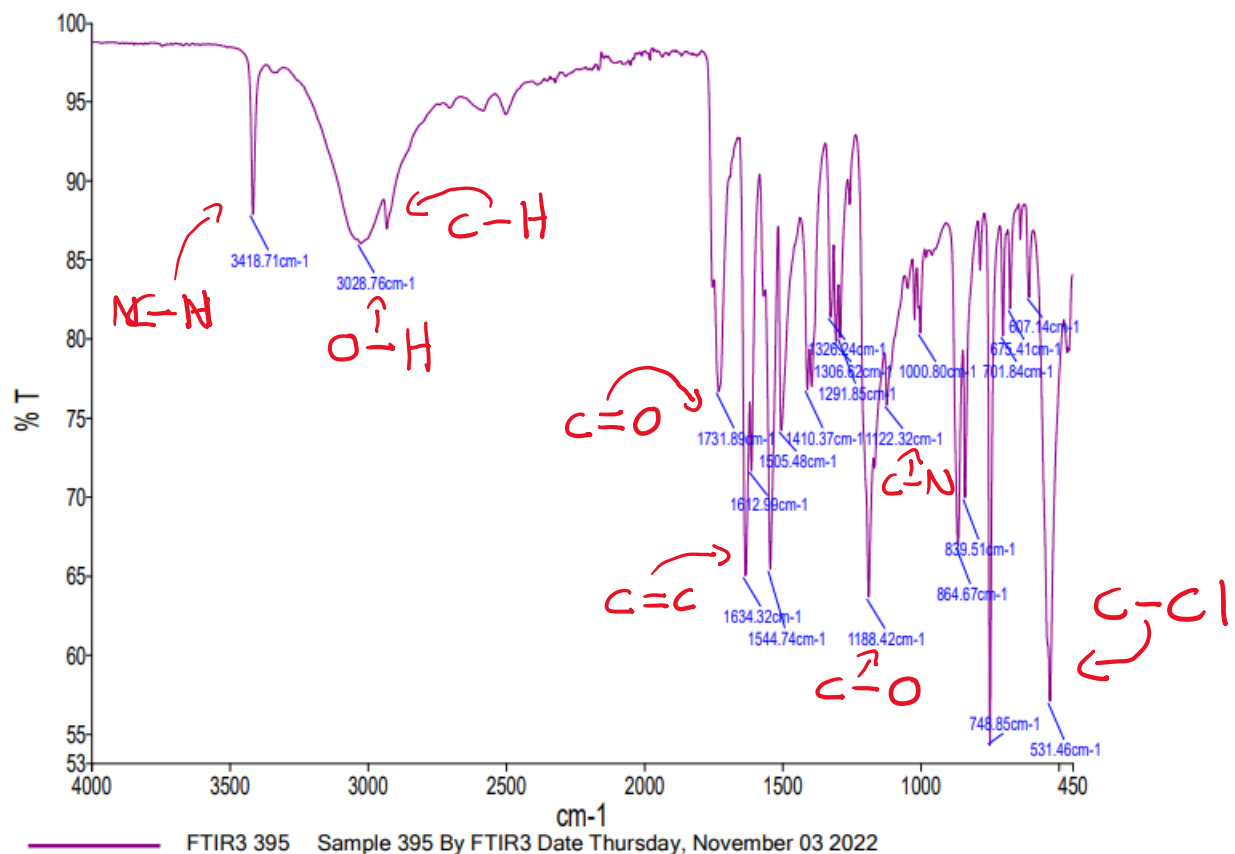


Figure 2. IR spectra of the isolated product [3] from Experiment 3.

Table 3. Analysis of IR spectra for [3]

Experimental Frequency (cm ⁻¹)	Appearance	Group
3418.71	medium	N-H Stretch
3028.76	Strong, Broad	O-H Stretch
~2900	Medium	C-H Stretch
1731.89	Strong	C=O Stretch
1634.32	medium	C=C Stretch
1188.42	Strong	C-O Stretch
1122.32	Strong	C-N Stretch
531.46	Strong	C-Cl Stretch

Analyst
Date

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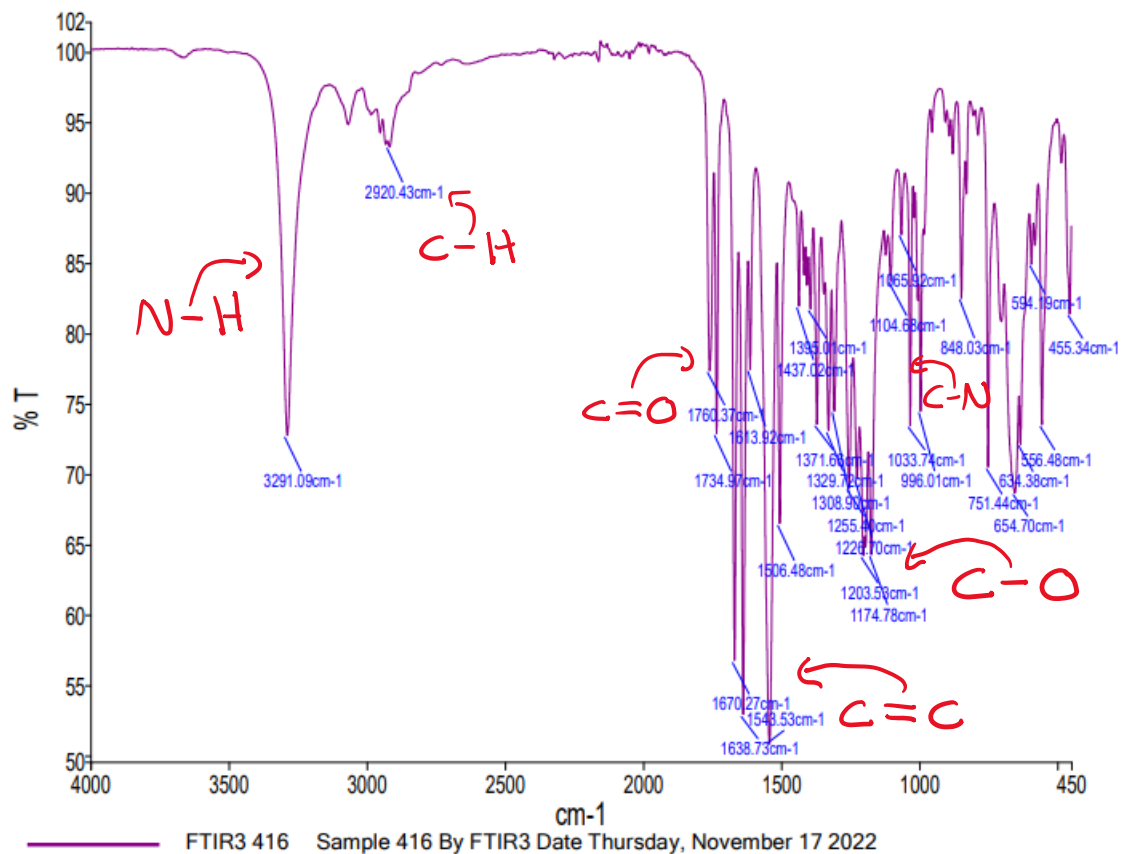


Figure 3. IR spectra of the isolated product [5] from experiment 4

Table 4. Analysis of IR spectra for [5]

Experimental Frequency (cm ⁻¹)	Appearance	Group
3291.09	strong	N-H Stretch
2920.43	Medium	C-H Stretch
1760.37	Strong	C=O Stretch
1638.73	Strong	C=C Stretch
1203.58	Strong	C-O Stretch
1033.74	Medium	C-N Stretch

NMR

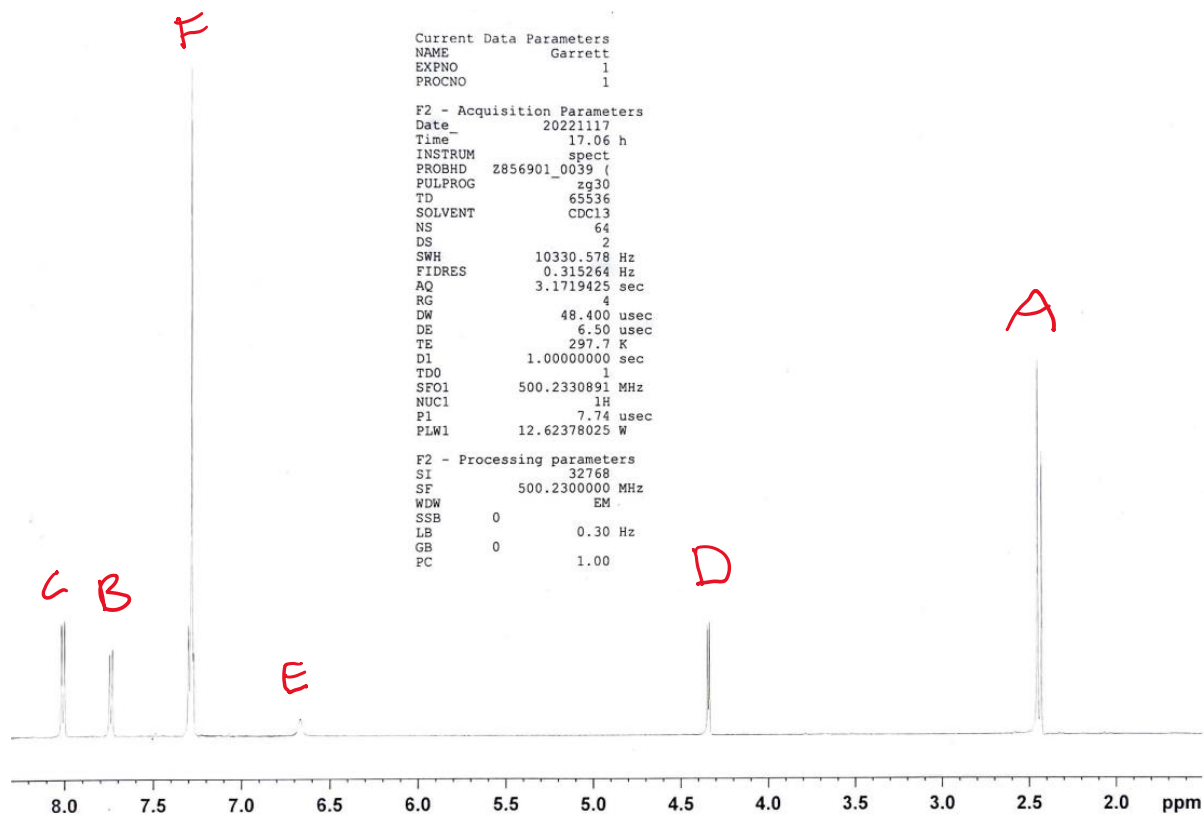


Figure 4. NMR spectra of [3]

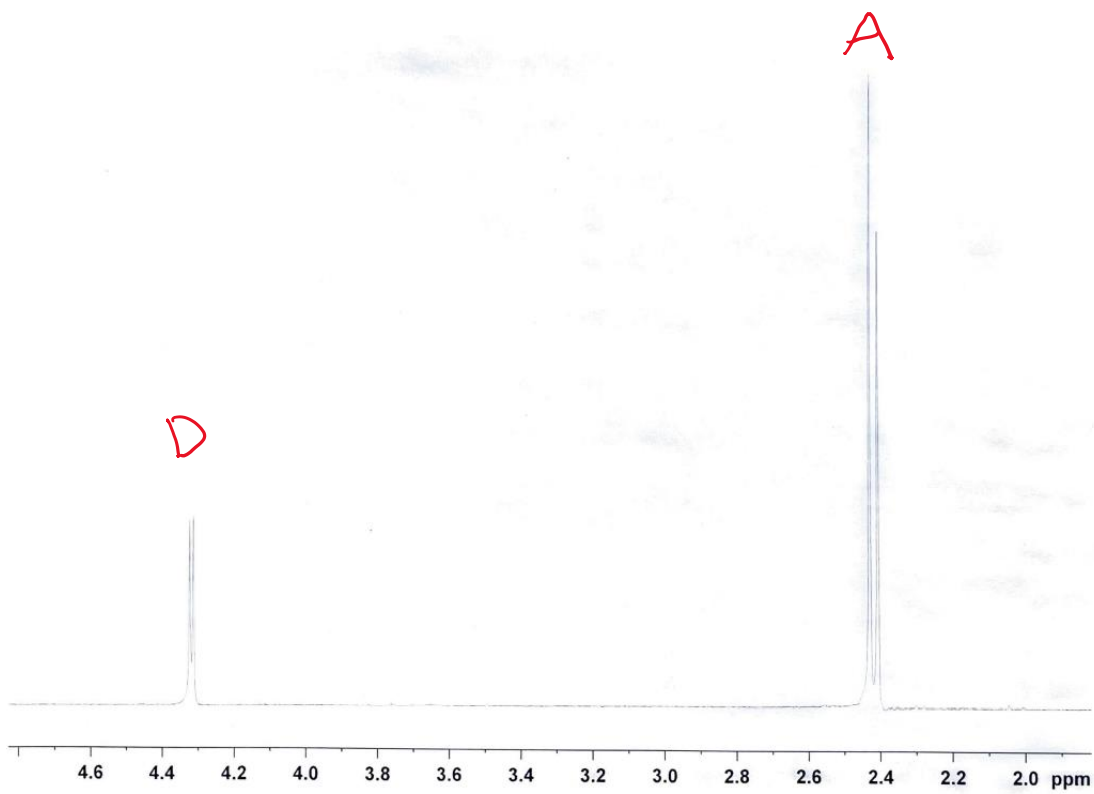


Figure 5. Increased Zoom NMR of [3]

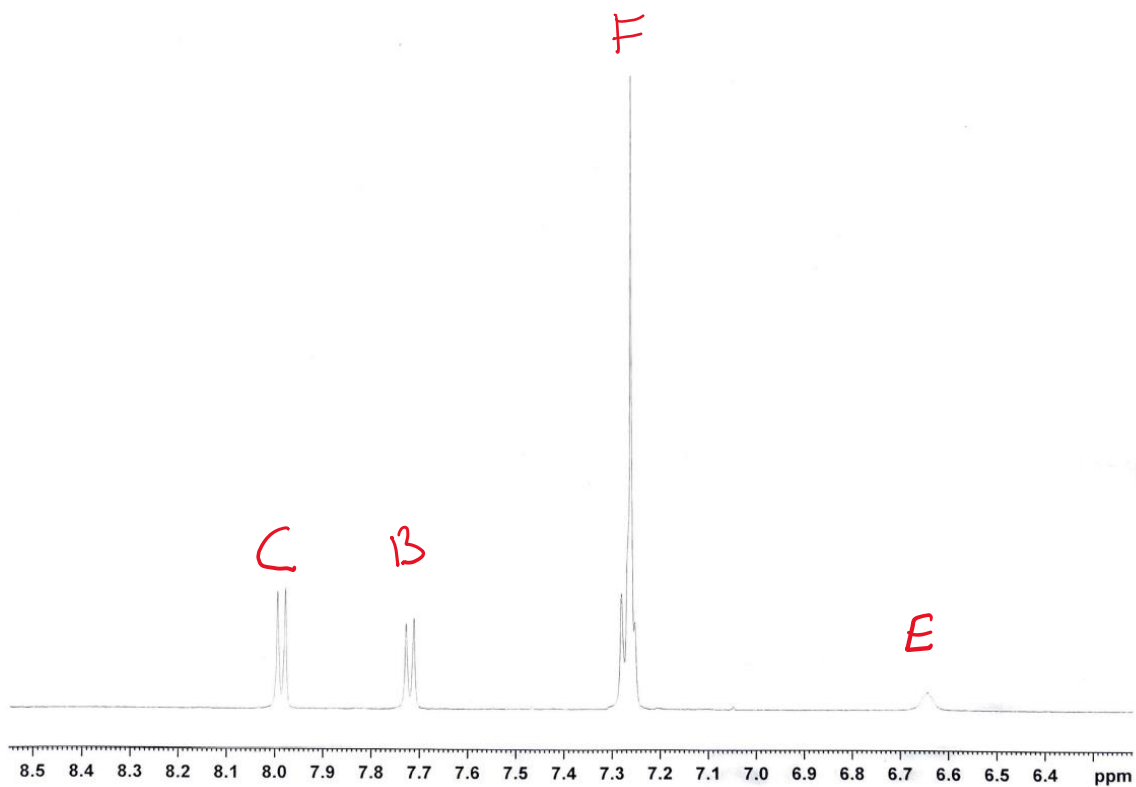


Figure 6. Increased Zoom NMR of [3]

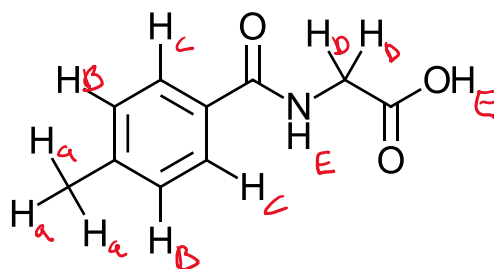


Table 5. Summary for the NMR spectra of [3]

Peak Label	Chemical Shift (ppm)	Integration/Ratio	Splitting	Identity of signal and neighbors
A	2.4	3	Singlet	C-CH ₃
B	7.72	2	D of D	H _a -C=C-H _b
C	7.98	2	D of D	H _b -C=C-H _c
D	4.3	2	singlet	C-CH ₂
E	6.65	Exchange with D ₂ O	Broad singlet	NH, OH
F	7.26	1	singlet	CHCl ₃

Percent Yield Calculation Sample

Glycine is the limiting reagent in experiment 1 because it was in a 1:1.1 molar ratio to the unknown acid chloride.

Glycine

$$3.0016 \text{ g glycine} \times \frac{1 \text{ mol glycine}}{75.067 \text{ g}} = 3.9985 \times 10^{-2} \text{ mol glycine}$$

Theoretical Yield

1:1 mole ratio to [3], glycine contains the least moles therefore it is the limiting reagent.

$$3.9985 \times 10^{-2} \text{ mol glycine} \times \frac{1 \text{ mol [3]}}{1 \text{ mol glycine}} \times \frac{165.19 \text{ g}}{1 \text{ mol [3]}} = 6.6052 \text{ g [3]}$$

Percent Yield

$$\% \text{ Yield} = \frac{\text{Actual Yield (g)}}{\text{Theoretical Yield (g)}} \times 100\%$$

$$\% \text{ Yield} = \frac{0.6634 \text{ g}}{6.6052 \text{ g}} \times 100\% = 10.04 \%$$

Discussion

In this experiment, [5] was successfully synthesized through two successive experiments. Experiment 1 uses Schotten-Bauman reactions conditions to synthesize [3] from p-toluoyl chloride and anhydrous glycine. The product of this reaction was identified using the FTIR spectra of Figure 2. The experimental melting point of 156.6-159°C was slightly depressed compared to the literature value of 163-165°C. Although depressed the melting point was very close to literature indicating the product was most likely formed. The experimental melting point range observed was 2.4°C indicating the product is not very pure. This was determined because pure substances melt at a very small temperature range of 0.5-1.0°C⁵. This was also confirmed as chlorine was present in the IR spectrum indicating starting material is present.

In the second experiment, [5] was successfully synthesized from [3] and [4]. The products of this reaction were determined by comparing FTIR of Figure 2 and Figure 3 this will be discussed below. The experimental melting point of this product was determined to be 147.3-148.0°C. the melting point range small indicating a pure product.

The FTIR spectra in Figure 2 and Figure 3 were successful in distinguishing the product. Only one factor was used to determine the product which included the disappearance of the alcohol group. The disappearance of the alcohol group can be seen in Figure 3 around 3000 cm⁻¹ where it is expected. All other data identified in the FTIR scans of Table 3 and Table 4 are present in both molecules, therefore cannot be used to determine identity of the product. Except for the presence of chlorine in Figure 2 which only indicated starting material is present.

The NMR scans of Figure 4, Figure 5 and Figure 6 were able to determine the unknown R group on the benzene ring. The R group was determined to be a methyl group instead of a methoxy group because the peak which integrated for 3 hydrogens was found at 2.4 ppm instead of downfield at ~4.0 ppm. This is due to the inductive effects of the oxygen. The large singlet at 7.3 ppm was CDCl₃ which was the solvent used. The small peak at 6.6 ppm was due to the D₂O exchange of the N-H and the O-H. The methyl group was determined to be in the para position because there were two symmetrical doublet of doublet peaks in the 7.5-8.0 ppm range. Having the methyl in the para position on the aromatic ring causes two of the four hydrogens to feel the same magnetic effects.

The mechanism of experiment 1 begins with the hydroxide base deprotonating the amine of the glycine. This activates the nitrogen nucleophile which proceeds to attack the carbonyl carbon of [1] and the chlorine leaves as a leaving group. The negative chlorine returns to deprotonate the positive nitrogen which was the nucleophile. This forms the by product of

hydrochloric acid. The acid workup ensures the terminal oxygen is protonated to a carboxylic acid. Product [3] is now formed.

The mechanism of experiment 2 begins with DCC deprotonating the carboxylic acid of [3]. The now negative oxygen on [3] attacks the center carbon of DCC and the pi electrons are pushed to the nitrogen containing the hydrogen in DCC. Another molecule of [3] returns and the terminal oxygen electrons attack the central carbonyl carbon forming a tetrahedral intermediate. The electrons of the attacked carbonyl carbon come back down and the DCC derivative is kicked off as a leaving group. Triethylamine now comes to deprotonate the central oxygen which has a positive charge. Then nitrogen of [4] attacks one of the central carbonyl carbons forming another tetrahedral intermediate. The carbonyl carbon electrons come back down and kick off one of the [3] as a leaving group. The negative charge of this leaving group returns to deprotonate the positive nitrogen. The final product of [5] is then formed.

This reaction yielded 0.02590 g of [5] with a percent yield of 5.578. An area of improvement for the experiment includes the first gravity filtration which was done during the recrystallization in experiment 1. There was a lot of filtrate which was undissolved in the filter after the gravity filtration. This could have contributed to the small %yield of the experiment. To improve this the filtrate should be washed multiple times and efforts should be made to dissolve as much filtrate as possible. Another area of improvement could be the substitution of DCC for another dehydrating reagent. The DCC is a dangerous reagent and required many precautions during the lab, potentially there is another available dehydration reagent available for use.

DCC is a very common reagent for the coupling reactions of amino acids. These amino acids are able to make up proteins which also include inhibitors. Inhibitors are very important in the medical field such as pharmaceutical research as they are able to inhibit certain reactions. The Dengue Virus is the most geographically distributed mosquito borne flavivirus.⁶ They include the Japanese encephalitis virus and the Zika virus. In this study, the glycyrrhizic acid derivatives were investigated and synthesized as they have been studied as dengue virus inhibitors. In the procedure they use DCC as the dehydrating coupling reagent to link the amino acids. Using DCC they synthesized a library of 20 amino acid derivatives would contribute to the inhibition of the Dengue Virus.⁶

Conclusions

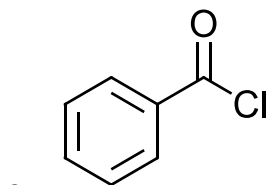
In this experiment, Methyl $\{[(p\text{-toluoylamino)methyl]carbonylamino\}$ acetate was successfully synthesized in the base catalyzed reactions of reaction scheme 1 and 2. Experiment 1 yielded 0.6634 g of *p*-tolylamino acetic acid with a percent yield of 10.04%. The melting point of this product was measured to be 156.6-159°C. The NMR spectra of this product also confirmed the identity of unknown #3. The second experiment yielded 0.02590 g of [5] with a percent yield of 5.578%. The melting point of this product was 147.3-148.0°C. The final product was confirmed from FTIR by the absence of the alcohol group in the scan (Figure 2). The literature melting point of 163-165°C for product [3] was very close to the experimental melting point of 156.6-159°C. This helped indicate the identity of this product. A literature melting point for the final product [5] was not found. Although the small melting point range indicates a pure product.

References

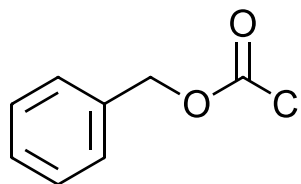
- ¹Hermanson, G. Dicyclohexylcarbodiimide or by reacting a carboxylate with benzyl halide, Science Direct, 2013, <https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/dicyclohexylcarbodiimide>
- ² Huttunen-Hennelly, H et al., Experiment #3 Preparation of a Glycine N-amine Derivative Using Schotten-Baumann Conditions, TRU Chemistry Lab Manual, Electronic copy, 2022,
- ³Huttunen-Hennelly, H et al., Experiment #4 Preparation of a Methyl Ester N-dipeptide Compound by the N,N'-Dicyclohexylcarbodiimide (DDC) Method, TRU Chemistry Lab Manual, Electronic copy, 2022,
- ⁴Physical Constants of Organic Compounds. In CRC Handbook of Chemistry and Physics. 103rd ed;[Online];RN <http://hbcpc.chemnetbase.com> (accessed December 11, 2022).
- ⁵Mettler Toledo, n.d. mt.com. https://www.mt.com/ca/en/home/applications/Application_Browse_Laboratory_Analytics/Thermal_Values/melting-pointdetermination.html#:~:text=Melting%20points%20are%20often%20used,exhibit%20a%20large%20melting%20interval. (Accessed October 19, 2022)
- ⁶Hour, M,J . Chen, Y. , Lin, S. Baltina, A. Et al. Glycyrrhizic Acid Derivatives Bearing Amino Acid Residues in the Carbohydrate Part as Dengue Virus E Protein Inhibitors:Synthesis and Antiviral Activity, JOMS, 2022 <https://doi.org/10.3390/ijms231810309>

Questions

1. If you were investigating a new compound to determine physical properties it would be important to ensure that the product is pure. Multiple recrystallizations would ensure the product is pure by removing any impurities such as water or leftover solvent. This would depreciate the melting point and the reported melting point would be incorrect. Melting points are done after each recrystallization to compare to the last one. Once the melting points start to become very similar the product can be determined to be pure. If the melting point has a small range it is also considered to be very pure.
2. Sodium hydroxide was added alternatively instead of in one portion to ensure the reaction conditions remained basic. When the glycine amine attacks the acid chloride the chlorine is kicked off. The chlorine then comes back and takes a hydrogen from the positive nitrogen. This forms hydrochloric acidic as a by-product. Because the reaction continues to proceed we need to ensure the base neutralizes the hydrochloric acid. The solution needs to remain basic so the hydroxide can continue to deprotonate the amine and drive the reaction.



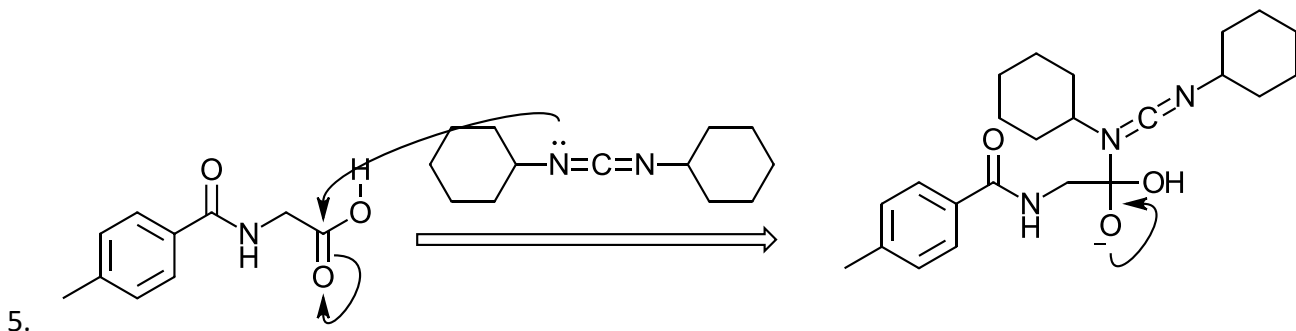
Acid chloride



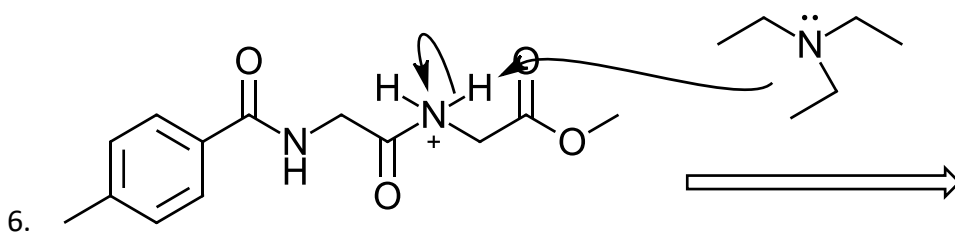
carbonyl chloride

I would expect the carbonyl chloride to be more reactive than the acid chloride. This is due to extra oxygen which is electronegative and pulls electron density away from the carbonyl carbon through induction. This makes the carbonyl carbon slightly more positive making it more likely for nucleophilic attack to occur.

4. If any other amino acid was used I would use polarimetry to determine the absolute characterization of the product. Since glycine does not contain a chiral carbon, polarimetry can not be used to determine composition.



The reaction temperature is kept moderate to ensure the DCC deprotonate the carboxylic acid instead of the nitrogen nucleophilically attacking the carbonyl. Adding heat to the reaction would promote the nitrogen attack since this attack requires energy.



One equivalent of triethylamine is used to deprotonate the last positive oxygen in the reaction mechanism. The triethylamine then donates its hydrogen to the negative nitrogen of the leftover DCC which ultimately forms the by-product of the reaction DCU. The triethylamine base is then regenerated in this process. This is seen in Figure 1.