January 01, 2010

Exploring new technologies in synthesis using flow chemistry

Andrea Lebed
Northeastern University

EXPLORING NEW TECHNOLOGIES IN SYNTHESIS USING FLOW CHEMISTRY

A thesis presented

by

Andrea Lebed

to
The Department of Chemistry and Chemical Biology

In partial fulfillment of the requirements for the degree of
Master of Science

in the field of

Chemistry

Northeastern University
Boston, Massachusetts
May, 2010

EXPLORING NEW TECHNOLOGIES IN SYNTHESIS USING FLOW CHEMISTRY

by

Andrea Lebed

ABSTRACT OF THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Chemistry
in the Graduate School of Arts and Sciences of
Northeastern University, May, 2010

ABSTRACT

Increases in efficiency, reduction of turnaround time, and many other factors have long influenced the pursuit of new technologies in chemistry. With the goal of effective synthesis of previously unattainable compounds and the more efficient synthesis of libraries of compounds, these methods continue to evolve and have grown to include tandem orientation of various tools.[1] Through the use of flow systems, many significant advances have been made in the field of organic synthesis: for example, unattended automation, resin tethered reagents, and extensive library production.[2-3] New methodology and instrumentation of flow systems are constantly evolving and adapting to new developing research. The goal of this research project was to apply flow chemistry to various projects, produce novel syntheses of various compounds that, in the end, demonstrate the versatility of flow methodology.

The synthesis of amide libraries was explored in a project executed at Merck & Co., Inc. The goal of this project was to use the Syrris AFRICA system to decrease the turnaround time between synthesis and screening of libraries of compounds. With the improvement on the turnover time of libraries, the efficiency and throughput of the drug discovery process would largely improve by allowing the screening many additional extended libraries with ease. Also, the method included unattended synthesis of these libraries, with a carboxylic acid tethered to a macroporous (MP) resin via an activated tetrafluorophenol (TFP) ester linkage.[2] In this project, a library of 20 pure amides was synthesized consecutively, and in dimethyl sulfoxide (DMSO), which provided the molecules a straight route to assay. The TFP-MP resin column was re-used over 36 times, with reproducible efficiency, in a coupling time of an hour.

A project was then initiated that extended this resin-bound reaction technology further to include methodology for efficient, high-throughput radiolabeling of many different substrates, ranging from small molecules to proteins and other biomolecules. Rapid and direct fluorination of biologically sensitive molecules is not possible.[4-6] The use of a linker between the molecule and the radiolabel is needed and had to be chosen carefully. After exploring the use of 4-fluorobenzaldehyde as a fluorine source and boc-aminooxyacetic acid as a linker, a N-succinimidyl-4-fluorobenzoate moiety was chosen to fulfill both requirements. A synthetic route was developed to rapidly radiolabel proteins and peptides with ease. These labeled molecules may be used to develop new imaging agents for positron emission tomography (PET) imaging.

DEDICATION

This work is dedicated to my family, specifically my sister, Arianna, and my parents, Orest and Alexandra, who have given me love and support that knows no bounds and without them I would not be the person I am today.

ACKNOWLEDGEMENTS

I would like to thank my research advisor, Dr. Graham Jones, for his guidance and support, not only throughout my research in his lab, but also through my five years at Northeastern University. He has taught me to work through the many problems that I had encountered in my career in chemistry and yet, still taught me to enjoy chemistry through it all, which I believe will help bring me success in this field. I thank the members of the Jones group, Paul LaBeaume, Amy Kallmerten, Rhiannon Thomas, Mike Placzek, and Sara Sadler for their constant support, advice, and humor.

I would also like to thank the members of my thesis committee, Dr. Graham Jones, Dr. David Forsyth, and Dr. Michael Pollastri, for their suggestions, advice, and patience in the review process.

I would like to thank Paul Tempest, Scott Berk, and the whole Parallel Medicinal Chemistry group at MRL Boston for all their help and encouragement throughout my project at Merck & Co, Inc.

Finally, I would like to thank my family for their constant support, guidance, patience, and encouragement during my years at Northeastern University.

TABLE OF CONTENTS

GLOSSARY OF ABBREVIATIONS

AFRICA	Automated Flow Reaction, Incubation, Control Apparatus
Boc	tert-butyloxycarbonyl
CT	computed tomography
DCM	methylene chloride
DIC	diisopropylcarbodiimide
DIEA	diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DMSO	dimethylsulfoxide
DSC	disuccinimidyl carbonate
eq	equivalents
FDG	fluorodeoxyglucose
FLLEX	flow liquid-liquid extractor
Fmoc	fluorenylmethyloxycarbonyl
LC	liquid chromatography
M	molar
MP	macroporous
MRI	magnetic resonance imaging
MS	mass spectrometry
NMR	nuclear magnetic resonance
PBS	phosphate buffer solution
PET	positron emission tomography

ppm	parts per million
QC	quality control
RIM	reagent injection module
TFA	trifluoroacetic acid
TFP	tetrafluorophenol
TLC	thin layer chromatography
UV	ultraviolet

LIST OF FIGURES

LIST OF SCHEMES

Chapter 1

INTRODUCTION

1.0.1 Flow Chemistry

The chemical industry is constantly developing new technologies to increase the ease of syntheses. In the past five years, technologies such as microwaves and flow chemistry for small scale reactions have emerged and have been widely implemented in synthesis and other applications. Flow chemistry has long been employed in manufacturing, but a need for implementation on the small scale was needed. There are many types of flow chemistry, however, the three primary performed on a small scale include micro flow (Figure 1), meso flow (Figure 2), and flow using tethered reagents (Figure 3).[1,4] Micro flow technology is when there are a series of interconnecting channels in a planar surface, usually a chip. The reagents are brought together, mixed, and allowed to react for a specific period of time. Meso flow is similar to micro flow, but instead of the two reagents mixing in channels in micrometer sized channels in a chip, they mix in a tube reactor, therefore these reactions can be done on a larger scale. Flow chemistry using tethered reagents can be performed in two fashions. First the reagent could flow through the column, displace the tethered reagent C to combine and make product B or reagent A could flow through the column, couple to reagent C to make B and then have to be cleaved off of the resin, as can be seen in Figure 3.[1] These types are not only seen as an alternative route to perform syntheses, but are now slowly becoming the preferred route for certain types of reactions, particularly in those reactions where unstable intermediates are formed.

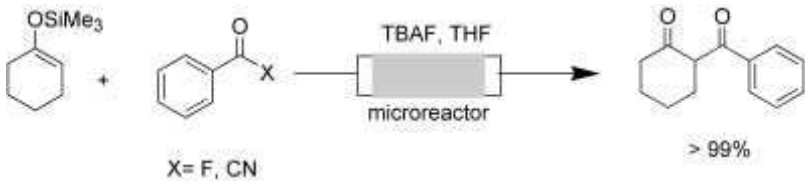


Figure 1. Micro flow reaction[1]

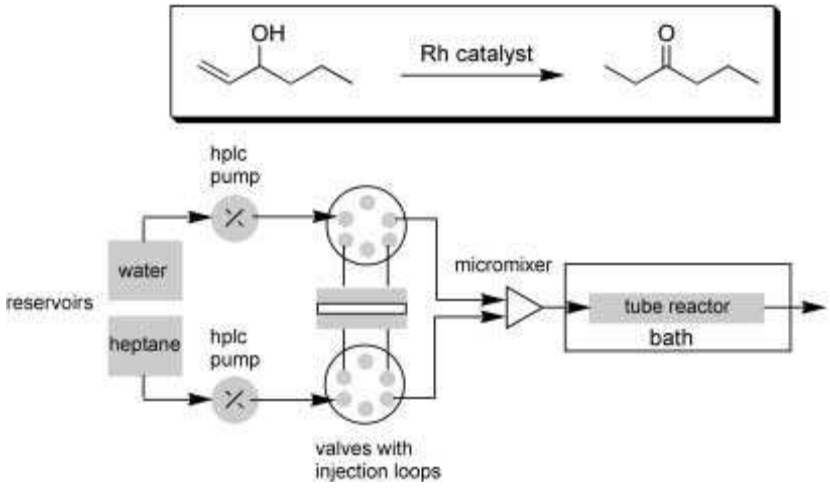


Figure 2. Meso flow reaction[1]

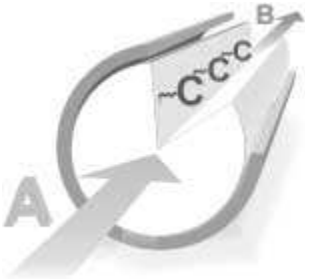


Figure 3. Flow reaction using tethered reagents[1]

Flow chemistry has many advantages over conventional batch chemistry. One advantage is that reactions can be run in a temperature that is higher than the boiling point of the solvent that the reaction is in, by pressurizing the vessel that the reaction is flowing through. This can especially be done in micro flow reactions, where the reaction flows through a chip that can easily and safely be pressurized because of its small size and design.[5] Another advantage to flow chemistry is that multi-step reactions can be set

up in-line and be performed in a continuous sequence, as seen in Figure 4.[1] This can be done with all three types of flow chemistry mentioned above, by using a resin tethered catalyst or reagent, by flowing a new reagent further down the line, or even by heating at certain points of the sequence to perform a reaction. This process can be very helpful when the products from one step are very unstable and need to be reacted in the next step immediately.[3] Another reaction that can be easily performed with flow chemistry, and can be used in-line in a multi-step reaction, is one that incorporates a gaseous reagent, such as hydrogen which can be bubbled through the flow line to hydrogenate reactants.[1]

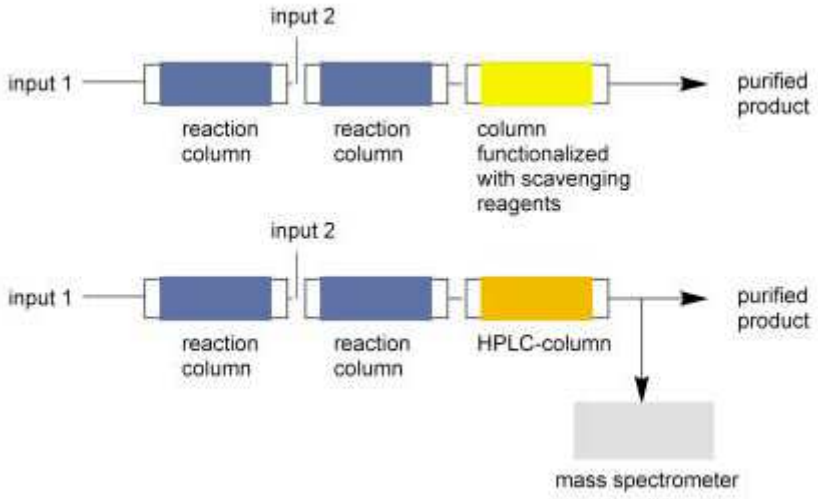


Figure 4. Multi-step reactions using flow methodology[1]

In general, flow chemistry can increase efficiency, reproducibility, and safety. First, the efficiency of a scientist may be increased significantly when using flow chemistry for a number of reasons, the primary being automation.[1] After the preparation of the system, automation can do a majority of the manipulations, while the scientist is freed to do other tasks. Also, if the flow reactions being performed are using a resin tethered reagent or catalyst, the column can often be easily recharged or recoupled.[3] This allows for a quick turnaround time in between each reaction which, in turn, reduces the

cycle time of each reaction. In addition, efficiency can be increased because flow chemistry reactions are generally very easily scaled up to make larger batches. Among other ways, this can be achieved by flowing through more reagents or making a larger column with tethered reagents. Second, reproducibility is often increased through the use of flow chemistry because consistent conditions of mixing and heat transfer are applied to the reaction. With automation, reactions can also have improved reproducibility, since all the parameters are set exactly each time the reaction is run, often removing the aspect of human error Lastly, safety of the scientist is greatly increased when using flow chemistry. The pressure in the reactor can be controlled, further reducing the risk of injury due to exploding reaction vessels. In addition, since the reaction is occurring in a relatively small volume, the impact of a dangerous runaway reaction is significantly reduced. In non-automated mode, the use of a calibrated manual injector can improve reproducibility.

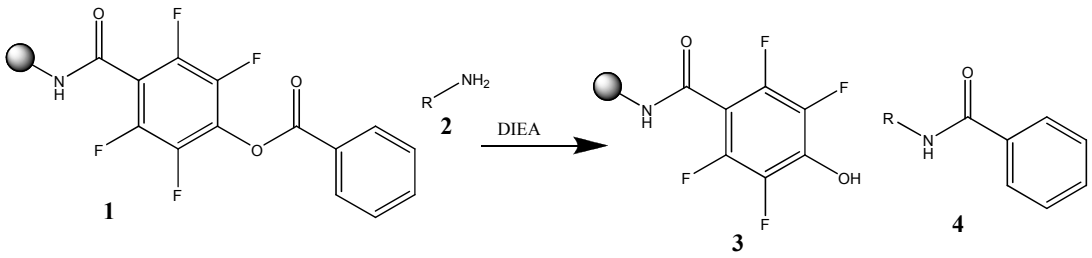
Although flow chemistry has many advantages over conventional chemistry, one disadvantage with it is that the method development is quite tedious because of the multitude of variables that must be taken into account. Also, when the chemistry methodology is understood, the instrumentation and method often need to be optimized with each new synthesis. However, once the method and instrumentation are optimized, the efficiencies generated can greatly compensate for the time used for optimization, and the method can also be used for various other reactions with little additional modification. Another disadvantage of flow chemistry is that only homogeneous reactions can be performed. Suspensions cannot be used with this methodology because this solution could easily clog the tubing of the system. Lastly, a significant disadvantage to flow

chemistry is the cost of the equipment. Whether a complete instrument is bought or a custom system is built, the cost of all the components is large when compared to standard laboratory glassware (roundbottom flasks, etc). This can limit the applications of flow chemistry methodology.

1.0.2 TFP-Resin

As a proof-of-concept library, it was decided to synthesize libraries of amides from amines and a carboxylic acid. This is a simple reaction that has few side products other than unreacted reagents. It was determined to tether one of our reagents to a resin column for convenience, fast turnover, and increased efficiency.

Tetrafluorophenol (TFP) can be tethered to a bead of resin, and are known to form an activated ester bond with carboxylic acids.[2] There are many different forms of solid support. Two, silica and macroporous resin, were used when starting this project, but the macroporous resin was chosen as it produced higher yields. This could be contributed to the particle size of the macroporous bead. The carboxylic acid, benzoic acid, was coupled onto the TFP resin and formed a column of a known amount of the resin and the activated acid (**1**). As reagents (in this case amines (**2**)) flowed through the column, the carboxylic acid was displaced, producing the amide (**4)** and leaving behind the uncoupled phenolic resin (**3),** as seen in Scheme 1.[2]



Scheme 1. Amide Library Synthetic Methodology

With the use of a column of a constant quantity of resin, the number of members of the library being synthesized determined the amount of each compound to be synthesized. For example, if a large library was to be synthesized, smaller amounts of each product were produced, and vice versa. Also, the method allowed for flexibility in varying the amounts of each product synthesized.[2]

1.0.3 Instrumentation

The Syrris AFRICA (Automated Flow Reaction, Incubation, Control Apparatus) system was the platform upon which this project was built. This instrument is a micro flow reactor, made up of up to 10 modules, which can be added or removed out of the flow line as needed.

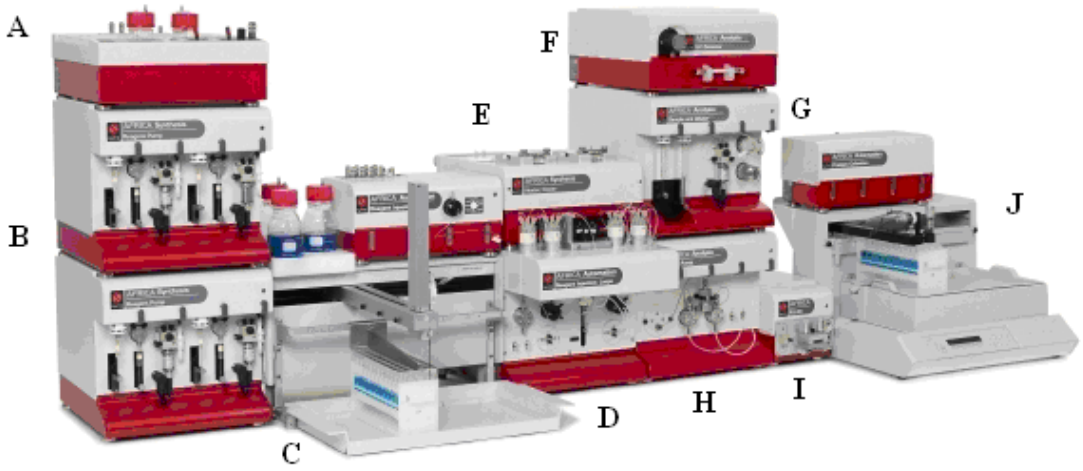


Figure 5. Syrris AFRICA (Automated Flow Reaction, Incubation, Control Apparatus)[7]

A – Solvent Store
B – Reagent Pumps
C – Reagent Injector
D – Injection Loops
E – Heater/Cooler
F – UV Detector
G – Sampler and Diluter
H – HPLC Pump
I – FLLEX (Flow Liquid-Liquid Extractor)
J – Product Collector

Since a column of solid supported reagent was used for our library synthesis, only the core of the instrument was needed, which simplified our system. The core of the instrument consists of pumps (**B**), the reagent injector modules and loops (**C,D**), and the product collection module (**J**), as seen in Figure 5. Figure 6 illustrates a schematic of this flow line with using only the core of the AFRICA system. The selection valve gave control to choose any solvent or the coupling mixture. This solvent then flowed through the pumps and into the Reagent Injection Module (RIM). The RIM was where each amine (**2**) was injected, from the automated reagent

injector, through the RIM: Feed and into the RIM: Loops. Once the injection valve opened, the solvent would flow through the RIM: Loops, introducing the reagent into the flow stream. The reagent and solvent then flowed through the activated resin column, reacted, and the resulting products flowed into the Product Collection Module, where each reagent was collected for 7.5 minutes. Using this flow line, all the syntheses and coupling runs were run at a flow rate of 1 mL/min. At this flow rate, the residence time of each amine through to product was 7 minutes.

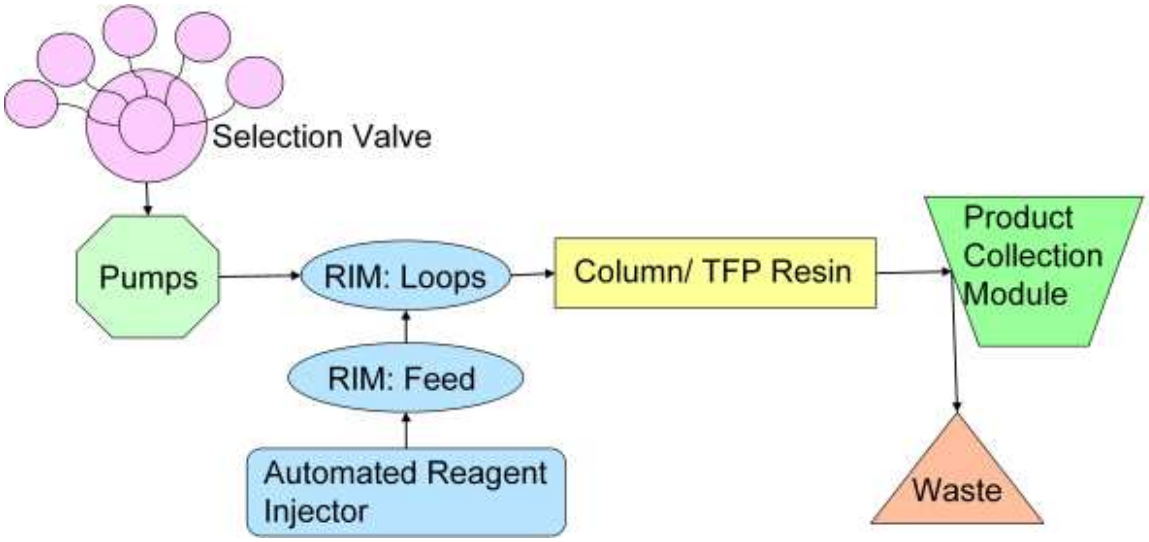


Figure 6. A Schematic of the Flow Line using the Syrris AFRICA

RESULTS AND DISCUSSION

1.1.0

When starting this project and working on the proof-of-concept study, it was decided to operate the system in manual mode. In place of the automated injector, a manual injector and loop were used, and were installed in the flow line. This avoided the challenge of the simultaneous development of both the synthetic method and the method for automation. Once the column was packed and installed, the resin was activated with the carboxylic acid (**6**) and quality control experiments were performed before synthesizing any libraries. Quality control (QC) was completed on the column after every fifth library was synthesized, throughout the whole project. These results were compared to the first QC results, and demonstrated that the column could be coupled with the same efficiency over 36 times.

As the syntheses of the libraries began, carryover was discovered between all our products, which were observed in the NMR spectra of each product. Upon seeing this problem, the thought that the wash time of 15 minutes was not sufficient but, as the wash time was prolonged to over an hour, no improvements were seen. Other causes for this problem needed to be explored. In any flow experiments, the problem of dispersion and tailing is common. Dispersion is when the reagent disperses into the solvent in which it's flowing. This causes the reagent band to broaden, causing dispersed product elution. Therefore, to collect all the product, a large fraction of solvent must be collected, which adversely impacts the efficiency of library synthesis. Tailing is related to dispersion, and is observed when the reagent disperses behind the slug (Figure 7). As seen, this results in the amine reagents tailing into the following reagent, through the wash. As the wash gets longer, the tailing is not eliminated, the tail just becomes thinner.[6]

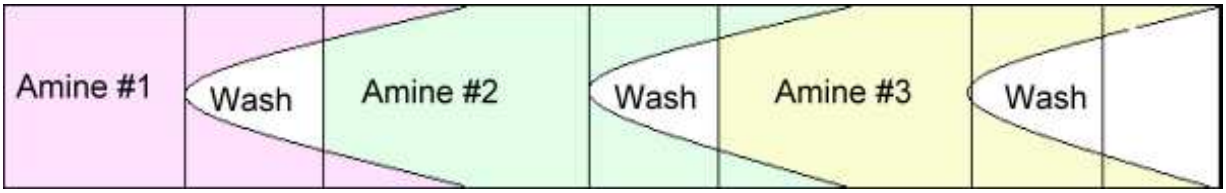


Figure 7. A Schematic of Tailing

To see if the carryover was really occurring from a dispersion or tailing problem, the same conditions were used as ones used for library synthesis, but instead of injecting a limited amount of an amine, a limited amount of tri-tert-butylbenzene (**11**) was injected. Tri-tert-butyl benzene (**11**) does not react with our column, so by injecting this reagent into the system with the same library synthesis conditions, it would show whether tailing/dispersion or a reaction with the column cause the carryover problem. The tri-tert-butylbenzene (**11**) did not show the same characteristics of tailing that the amines did, as can be seen in Figure 8, where the tri-tert-butylbenzene (**11**) is the control and isobutylamine (**7**) is the amine. This suggested that an interaction between the amine and the column was causing the carryover.

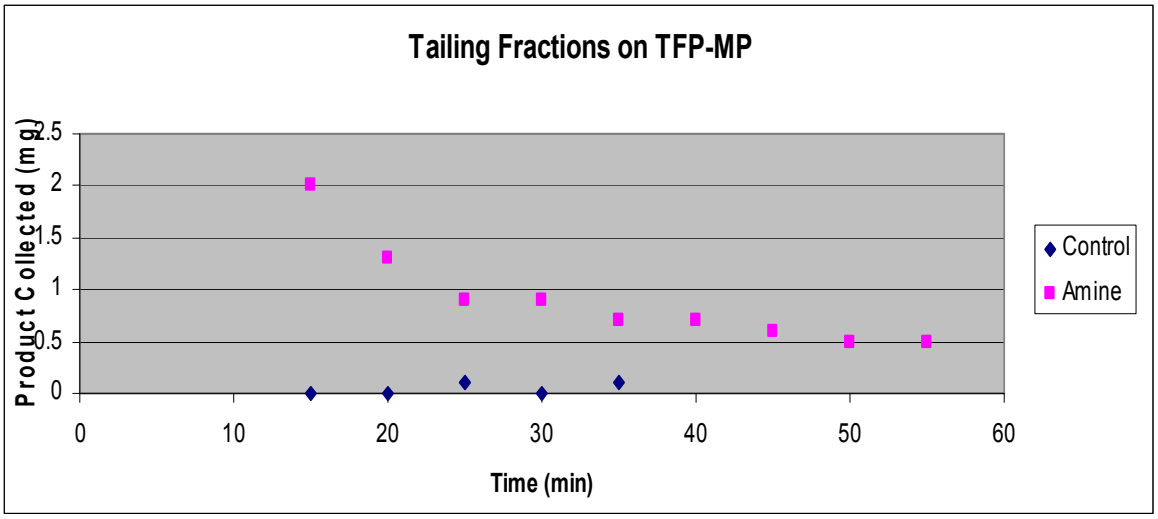


Figure 8. Tailing Comparison

The possibility of an acid-base interaction with the column was then explored as a possible cause of the carryover. The reasoning that was suggested was that the amine was

flowing through the column and, instead of reacting with the coupled column and displacing the carboxylic acid, it was interacting with the uncoupled (acidic) phenolic resin. And, as the library synthesis continued, the first amine eventually flowed through the column and resulted in contamination in another product. To explore the possibility of such interaction, three batches of the uncoupled resin were reacted with these amines: phenethylamine (**12**), N-methylphenethylamine (**13**), and 3-(aminomethyl)-pyridine (**14**). The resins were then washed with solvent, which was subsequently evaporated. These fractions did have product in them, but the yields were not 100%. The resins were then washed with 0.1 M DIEA in solvent, and the solvent was removed. These fractions included the rest of the product, which suggested that the carryover was caused by an acid-base interaction. Henceforth, all the amines were injected with in a solution of DIEA in DMSO and the wash also contained the same concentration of DIEA. This solved the carryover problem and allowed us to start synthesizing larger libraries of more diverse amides.

Twenty pure amides were synthesized in flow in DMSO. Only five amides had a yield under 50%. The amides that had the low yields were mostly towards the end of the run, except for aniline, which is less nucleophilic and was the likely source of low reaction conversion. Low yields at the end of the run were likely due to the presence of less active TFP ester for reaction. Indeed, as these specific amines were run in smaller libraries, their yield was improved.

METHODS

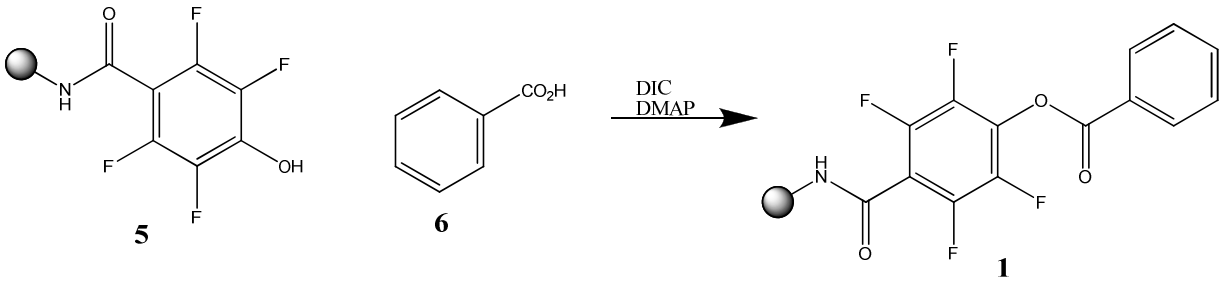
1.2.1 Objective

The objective of this project was to provide a proof of concept that libraries of pure products can be made in a quick efficient manner using the AFRICA system. For this proof of concept experiment, libraries of amides were synthesized from amines. The overall goal was to develop an in-line synthesis and screening of compounds to reduce cycle time and to improve the feedback loop. To accomplish this the parameters needed were synthesizing our libraries using a fast reaction with a short retention time so the synthesis was efficient, producing pure products so that no purification was needed, and to run the synthesis in DMSO so that the product solutions could potentially be sent straight to biological assays.

All of the libraries were synthesized on the Syrris AFRICA system. During method development, these methods were performed using manual injection, but were further optimized using the automation line described above. After each synthesis, the products were collected in tared vials and evaporated in a Genevac. All the yields reported are based on weight. Purity was based on product NMR spectra.

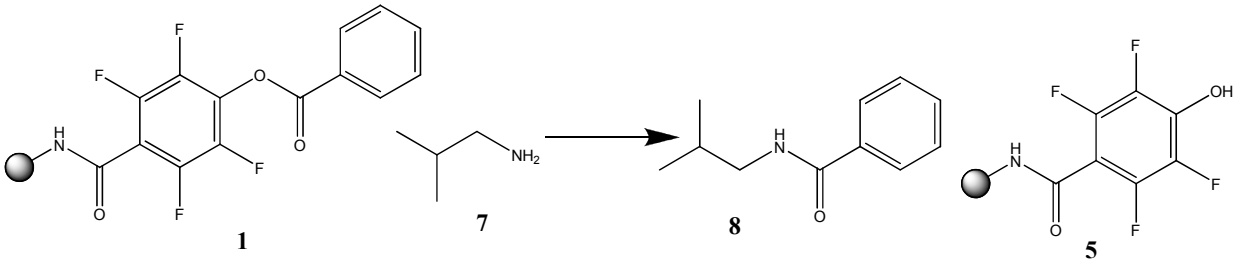
All the work on this project was performed at Merck & Co., Inc. (Merck Research Laboratories Boston). The resin used was purchased from Argonaut Technologies and all other reagents were purchased from Sigma Aldrich and used as received. All solvent evaporation was done in a Genevac centrifugal solvent evaporator. ^{1}H NMR spectra were recorded on a 500 MHz Varian instrument and were presented in ppm downfield relative to trimethylsilane as an internal standard.

1.2.2 Column Coupling[2]



A solution of 25% DMF was run through the column (1.5 g of resin, 0.55 mmol/g) (**5**) in DCM for 15 minutes at a flow rate of 1 mL/min. A solution of 40 mL of 25% DMF in DCM with the benzoic acid (488 mg, 0.1 M, 4.00 mmol) (**6**) and DMAP (293 mg, 0.6 eq, 2.40 mmol) was made. This solution was run through the column for 10 minutes at 500 μL/min. Then DIC (2.43 mL, 4.5 eq, 15.5 mmol) was added and the rest of the solution was allowed to run through the column at 500 μL/min, until the solution was consumed. This was followed by a wash of 25% DMF in DCM for 15 minutes, then DCM for 15 minutes, and finally another 15 minutes of the reaction solvent, all at a flow rate of 1 mL/min.

1.2.3 QC of Column[2]

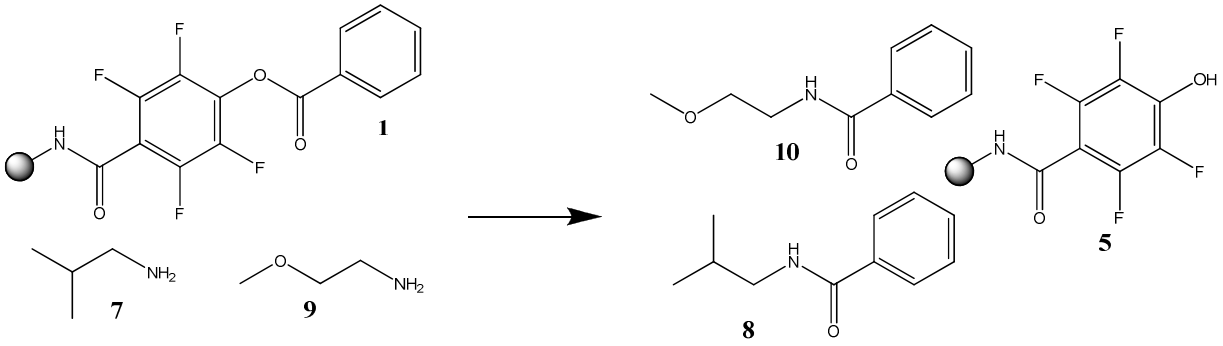


1 **7** **8** **5**

The activated TFP-MP resin (200 mg, 0.55 mmol/g) (**1**) was added to a capped filter vessel. DCM (2 mL) and the amine (0.361 mL, 0.2 M, 3.63 mmol) (**7**) were added and allowed to react for 1.5 h on a shaker. The reaction was filtered into a 25 mL roundbottom flask. The TFP-MP resin was then washed three more times with DCM, which was also collected into a roundbottom flask. The solvent was evaporated and the residue was dried under vacuum for 10 minutes. N-isobutyl-benzamide (**8**) was isolated

Compound **8** was isolated with a yield of 19 mg (0.107 mmol) which confirms the column loading of 0.54 mmol/g. This result is comparable to previously made TFP-MP columns.

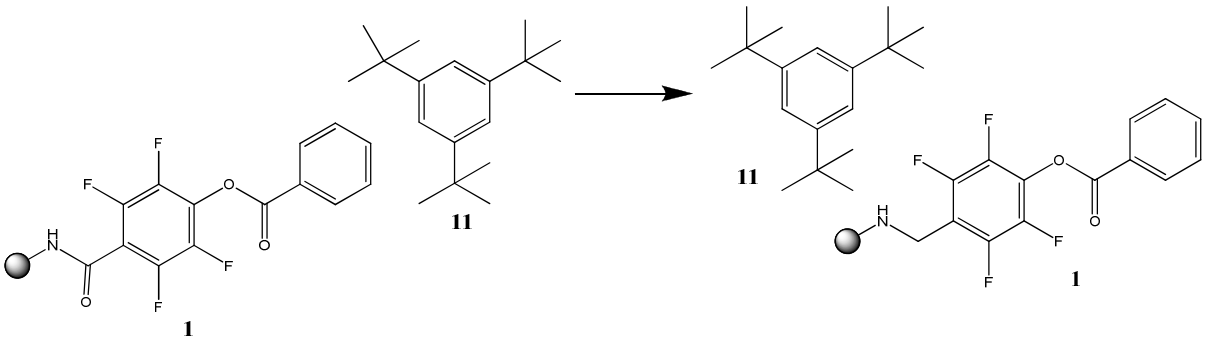
1.2.4 Multiple Amide Synthesis: Method Development[2]



Solutions of each amine were made with 0.1 mmol of the amine (7.51 mg of 2-methoxyethylamine (**9**) and 7.31 mg of isobutylamine (**7**)) in 1 mL of DCM. The activated TFP-MP resin (0.54 mmol/g) (**1**) was purged with DCM for 15 minutes at a flow rate of 1 mL/min. Then the 2-methoxyethylamine (**9**) solution was injected at 500 µL/min. An eluent of approximately 10 mL was collected when elution of the amide was observed on the UV detector. The column was then purged for approximately 30 minutes at 1 mL/min with DCM. Then the isobutylamine (**7**) solution was injected at 500 µL/min, followed by DCM. Again an eluent of approximately 10 mL was collected when the amide was observed on the UV detector. The eluents were dried down in the Genevac, each were weighed, and a NMR was taken of each. Compound **10** was isolated in 40% yield (7.2 mg, 0.04 mmol) and compound **8** was isolated in 33% yield (5.8 mg, 0.03 mmol).

Tailing of 2-methoxyethylamine is evident, as **10** can be seen in the NMR of the **8**.

1.2.5 Control Tailing Experiment



A 0.25M solution of 1,3,5 tri-tert-butylbenzene (**11**) in 8 mL of DCM was made. This solution was run through the column at a flow rate of 500 µL/min for 7 minutes. This was followed by DCM. Once the start of the peak was seen on the UV detector, eight fractions of the product were collected into tared vials, each for 5 minute intervals. These fractions were then dried by vacuum overnight. An NMR was taken of the product in vial #2 and analysis was done by taking the weight in each of the aliquots.

The NMR showed that the 1,3,5 tri-tert-butylbenzene (**11**) did not react with the column and the weights did not show any tailing.

Vial	Collection Started (min)	Product (mg)
1	0	152.6
2	5	57.2
3	10	0.0
4	15	0.0
5	20	0.0
6	25	0.1
7	30	0.0
8	35	0.0

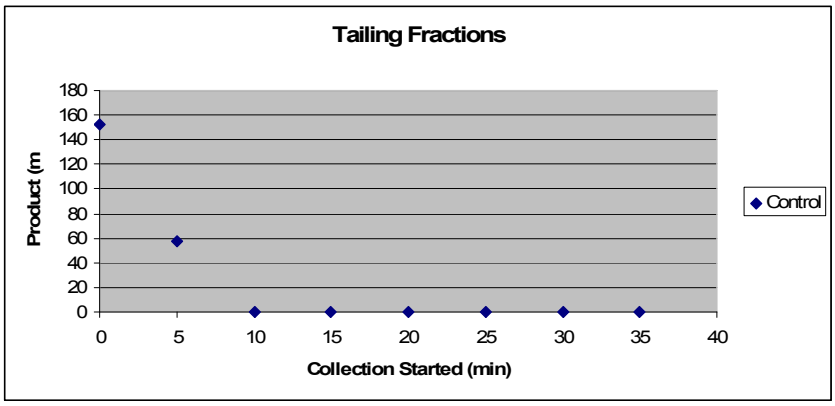
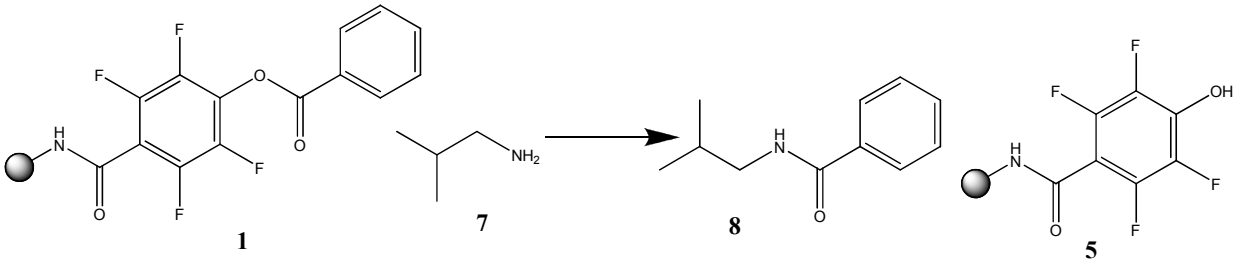


Figure 9. Tailing fractions of 1,3,5 tri-tert-butylbenzene (**11**)

1.2.6 Experiment to Analyze Tailing



1 **7** **8** **5**

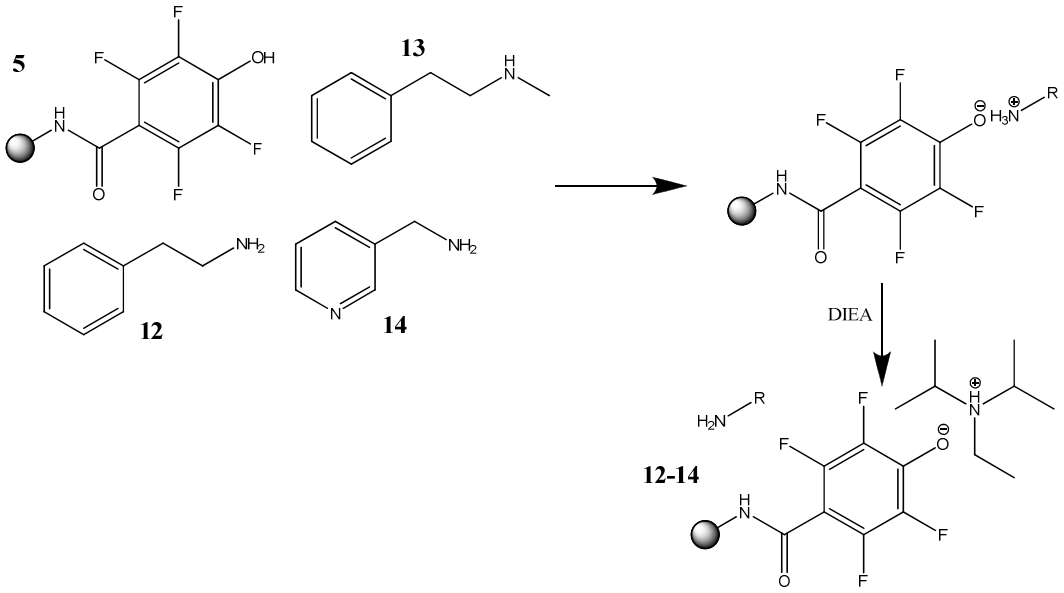
A solution of 0.25 M isobutylamine (**7**) in DCM was made and run through the column (0.54 mmol/g) (**1**) at a flow rate of 500 µL/min for 7 minutes. This was followed by DCM. Once the start of the peak was seen on the UV detector, twelve fractions of the product were collected into tared vials, each for 5 minute intervals. The fractions were dried by vacuum. Analysis was done by taking the weight of each of the aliquots, summarized below in the table. A NMR was taken of aliquot #12.

By weight, the amide was seen to tail off the column compared to the 1,3,5 tri-tert-butylbenzene (**11**). Also, although the UV trace was negative when the aliquot of vial #12 was flowing through the detector, a NMR confirmed that there was still product washing off the column.

Vial	Collection Started (min)	Product (mg)
1	0	18.2
2	5	48.9
3	10	11.3
4	15	2.0
5	20	1.3
6	25	0.9
7	30	0.9
8	35	0.7

Vial	Collection Started (min)	Product (mg)
9	40	0.7
10	45	0.6
11	50	0.5
12	55	0.5

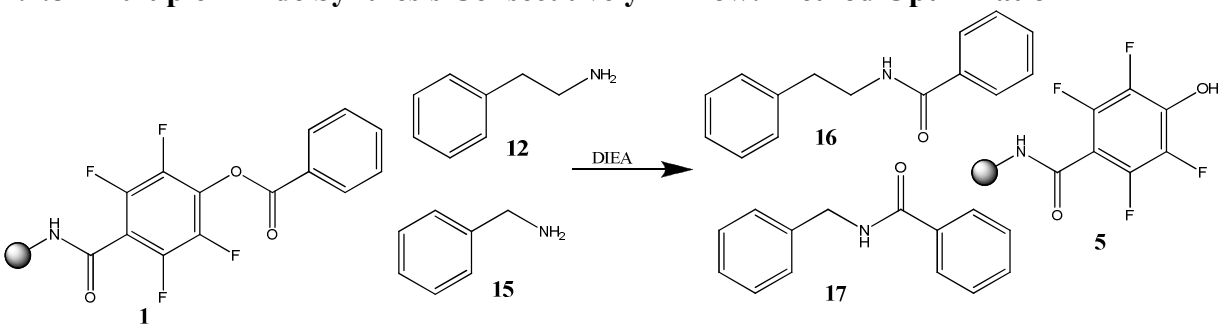
1.2.7 Detecting Salt Formation and Interaction of Base



In three vials, 0.5 mmol of TFP-MP resin (0.54 mmol/g) was weighed out in each. 10 mL of DCM was added to each, followed by 0.1 mmol of each amine, one in each vial. The mixture was allowed to react for 1.5 h on a shaker. The mixture was then filtered through filter paper into a tared roundbottom flask. The resin was then washed with 0.1 M diisopropylethylamine (DIEA) in DCM and the wash was collected in another tared vial. This was done to the other two vials as well. All the vials were dried in the Genevac, weighed, and then a NMR was taken of each.

Product	Yield after DCM wash (dried again)	Yield after 0.1 M DIEA in DCM wash
Phenethylamine (**12**)	10.4 mg 0.05 mmol (86%)	1.8 mg 0.008 mmol (15%)
N-methylphenethylamine (**13**)	11.7 mg 0.05 mmol (89%)	1.0 mg 0.004 mmol (7%)
3-(aminomethyl)-pyridine (**14**)	19.2 mg 0.09 mmol (177%)	1.9 mg 0.009 mmol (17.5 %)

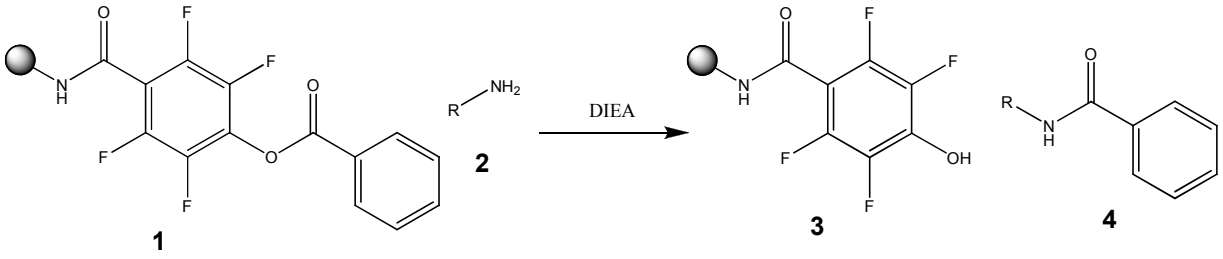
1.2.8 Multiple Amide Synthesis Consecutively in Flow: Method Optimization[2]



Solutions of each amine were made with 0.01 mmol of the amine and 125 µL of DCM and 0.01 mmol (1.74 µL) of DIEA. The column (0.54 mmol/g) (**1**) was purged with DCM for 15 minutes at a flow rate of 1 mL/min and then with 0.1 M DIEA in DCM for 2 minutes at a flow rate of 1 mL/min. Then 125 µL of phenethylamine (**12**) was injected at 1 mL/min, followed by 0.1 M DIEA in DCM, and an aliquot of approximately 10 mL was collected 3.5 minutes after it was injected. The column was then purged for approximately 15 minutes at 1 mL/min with 0.1 M DIEA in DCM. Then the 125 µL of benzylamine (**15**) was injected at 1 mL/min, followed by 0.1 M DIEA in DCM. Again an aliquot of approximately 10 mL was taken 3.5 minutes after it was injected. The column was then flushed with DCM. The aliquots were then dried down in the Genevac, then were weighed, and NMR spectra was taken of each.

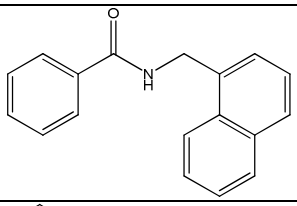
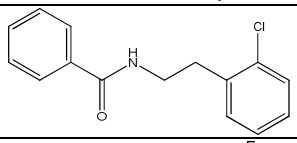
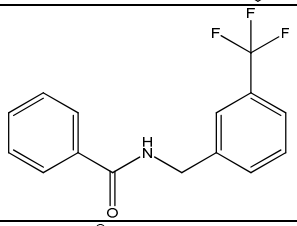
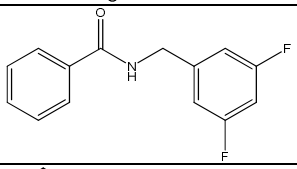
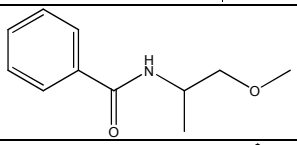
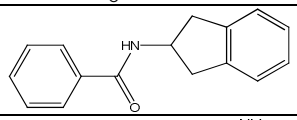
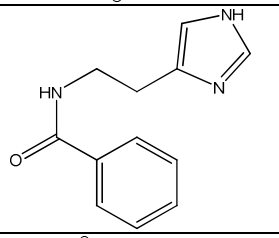
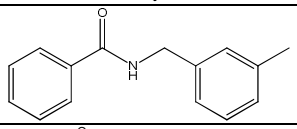
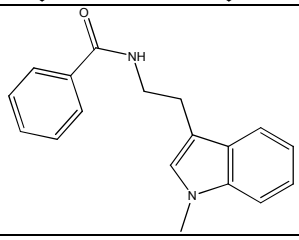
Compound **17** was isolated with a yield of 11 mg (20%, 0.05 mmol) and compound **16** was isolated with a yield of 8 mg (25%, 0.04 mmol). NMR spectra showed no unreacted amines and no contamination from the previous product. There was a lot of residual base and some unknown peaks.

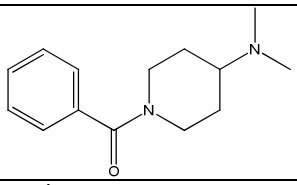
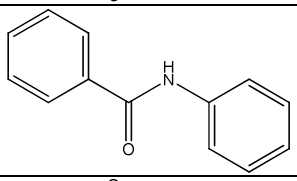
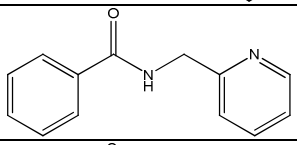
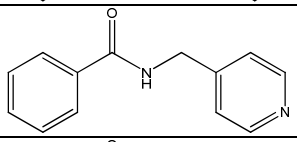
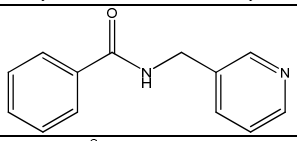
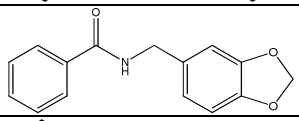
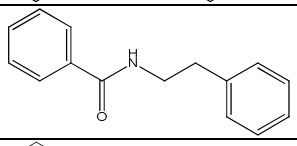
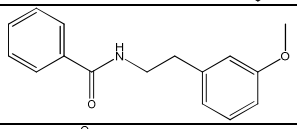
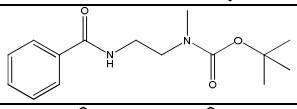
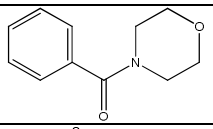
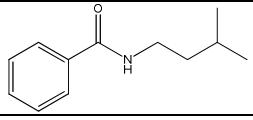
1.2.9 Multiple Amide Synthesis Consecutively in Flow: 20 Member Library[2]



Solutions of each amine were made with 0.3 mmol of the amine and 3000 μL of DMSO and 0.3 mmol of DIEA. The column was purged with 0.1 M DIEA in DMSO for 15 minutes at a flow rate of 1 mL/min. The amines were injected automatically at the same flow rate. The amines were collected for 7.4 minutes into tared vials 2.5 minutes after injection and the volume in between slugs was 10mL. Throughout the whole run, the solvent was 0.1 M DIEA in DMSO. After the amines were collected, they were put in the Genevac to dry. They were then weighed and NMR spectra were taken of each vial.

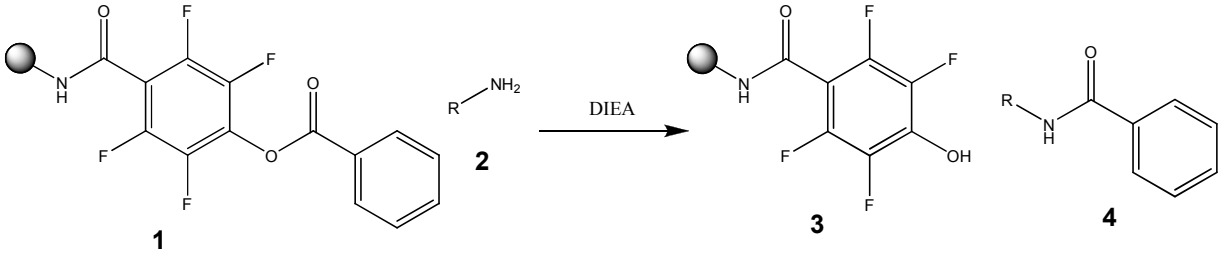
The yields of the products are listed in the table below. Only 6 amides had a yield < 50%. The lower yielding amines displayed a trend where the yields were lower towards the end of the run, except aniline. Carryover is less that 5% in all of the products. The only problem is that DIEA can be seen in the NMRs, but if the products were dried longer in the Genevac, the DIEA should be eliminated.

#	Structure	Theoretical Yield (mg)	Actual Yield (mg)	Yield by weight(%)	Yield of Purity by NMR (%)
18		2.61	2.6	100	90
19		2.60	2.5	96	89
20		2.79	2.8	100	92
21		2.47	2.0	81	74
22		1.93	0.3	16	5
23		2.37	2.7	114	98
24		2.15	2.1	98	86
25		2.25	2.2	98	96
26		2.78	2.8	101	92

27		2.32	2.3	99	85
28		1.97	0.5	25	10
29		2.12	2.3	108	96
30		2.12	2.3	108	94
31		2.12	2.3	108	97
32		2.55	1.9	74	65
16		2.25	1.3	58	45
33		2.55	1.0	39	22
34		2.78	2.5	90	85
35		1.91	0.6	31	15
36		1.91	0.3	16	8

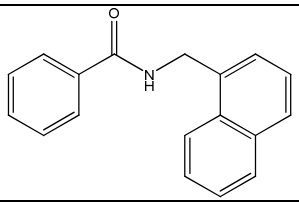
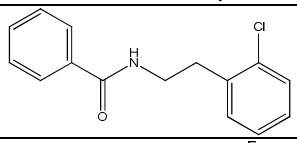
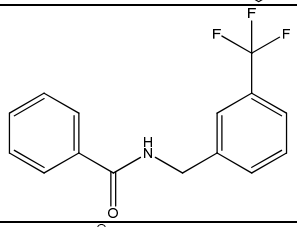
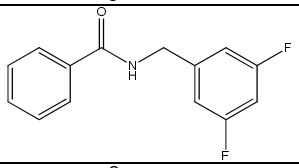
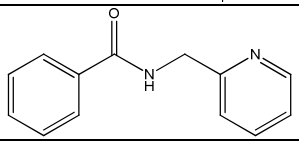
21 ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.61 (s, 2H), 6.89 (s, 3H), 7.41-7.58 (m, 3H), 7.82 (d, 2H, J = 7.0 Hz).

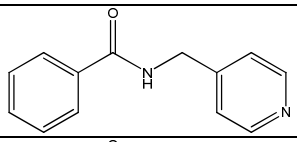
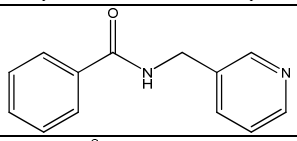
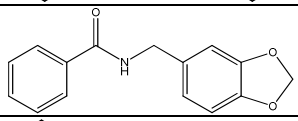
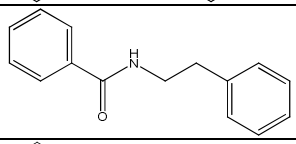
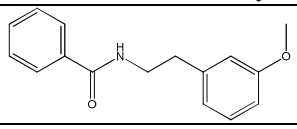
1.2.10 Multiple Amide Synthesis Consecutively in Flow: 10 Member Library[2]



The same procedure as listed above was followed.

Yield – All the yields are high. Only one, the last, amide had a yield < 50%. The trend was again seen that the yields lowered throughout the run.

#	Structure	Theoretical Yield (mg)	Actual Yield (mg)	Yield by weight (%)	Yield of Purity by NMR (%)
18		2.61	2.3	88	79
19		2.60	2.7	104	95
20		2.79	2.6	93	87
21		2.47	2.0	81	74
29		2.12	2.1	99	93

30		2.12	2.4	113	91
31		2.12	2.2	104	91
32		2.55	1.9	74	68
16		2.25	1.7	75	64
33		2.55	1.2	47	35

CONCLUSIONS

1.3.0

In the end, the largest library that was synthesized using the in-line flow method was a twenty member amide library. These products all were synthesized in DMSO, which provided an easy route straight to assay, since compounds are introduced into most biological assays as DMSO solutions. Our column was recoupled over 36 times with the same efficiency as the first time it was coupled and was constantly quality control tested. The regeneration of the column provided a quick turnover between library syntheses. In addition, to provide a scope of this method, with 1 mmol of TFP-MP resin and injecting 0.01 mmol of each amine, it has become possible to synthesize 100 compounds per column per coupling. This method can easily be adapted to synthesize many libraries of different compounds. It provides a straightforward automated route, which can greatly increase the efficiency of library synthesis.

Chapter 2

INTRODUCTION

2.0.1 Positron Emission Tomography

Positron Emission Tomography (PET) is an *in vivo* imaging technique with excellent sensitivity and quantitation capabilities. The scans produce a 3-dimensional real time image of the body. The system works by detecting gamma emissions from radionuclides. These radionuclides are introduced into the body on biologically active molecules that show the movement of these molecules through the body.[8] One of the most common biologically active molecules that used for PET imaging is fluorodeoxyglucose (FDG), which is an analog of glucose. The concentration of this tracer in tissues is then quantified and subsequently shows regional glucose uptake.[9] Other biological molecules can be labeled and used for PET imaging to image different *in vivo* systems.[10]

The imaging actually occurs in the living subject as the radionuclide undergoes beta decay and emits a positron. This positron is then annihilated when it collides with an election, producing a pair of gamma photons that travel in opposite directions. These are simultaneously detected by the instrument, which can determine the location of the radionuclide by extrapolating the center of the line the gamma photons traveled.[8]

PET scans are now often used in tandem with computed tomography (CT) and magnetic resonance imaging (MRI) imaging techniques. By using these techniques in combination, a 4-dimensional image can be constructed, where the fourth dimension is time, thus PET imaging is a very valuable technique for imaging various diseases and disorders not only to diagnose them, but to also determine the stage that the disease is in and if treatments are being successful.[8]

2.0.2 Fluorine Tethering

Fluorine is one of the more readily available and versatile radionuclides. As mentioned previously, the most common use of PET imaging uses the biological molecule FDG, which is radiolabeled with ^{18}F.[10] As PET imaging evolves, so must the biologically active molecules used for scanning. With a half life of 110 minutes, which is the longest of all the routinely available positron emitters, the difficulty of working against the clock is eased somewhat. This is not the only advantage of using ^{18}F as a radionuclide. In contrast to other positron emitters, ^{18}F decays are not associated with abundantly high energy gamma emissions, which can lead to image degradation.[11] A significant issue with using ^{18}F is that it is not possible to rapidly and directly fluorinate such biologically sensitive molecules as proteins or peptides. However, such molecules are well-suited for PET imaging, because they are not rapidly expelled from the body and their use images real, *in vivo* interactions. These molecules cannot be radiolabeled rapidly and directly, firstly, because they do not have a leaving group that could be displaced rapidly by fluoride anion under conditions that these biomolecules can tolerate. Under forcing conditions, these biologically sensitive molecules would degrade or become inactive.[12,13]

Since proteins and peptides cannot be rapidly and fluorinated directly, a linker must be used. These must be chosen carefully, as to not complicate the imaging. First, the ligand cannot take a long time to be synthesized or take a long time to react with the molecule that is being radiolabeled. It also cannot take multiple synthetic steps to synthesize this ligand, because of the half life of ^{18}F precludes such long processes. A second qualification of a ligand is that it cannot change the *in vivo* properties of the molecule that is being radiolabeled. This can lead to imaging off target binding or excretion of the molecule before imaging can occur. The final qualification for a ligand is that the reaction conditions needed to couple the linked radiolabel cannot be harsh.

Just as with the direct fluorination, harsh reaction conditions could inactivate or destroy these molecules.

2.0.3 N-succinimidyl 4-fluorobenzoate

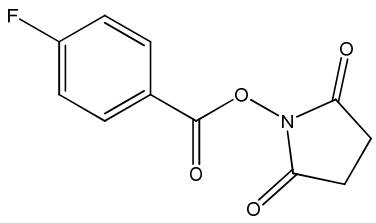


Figure 10. N-succidinimidyl 4-fluorobenzoate (**40**)

N-succinimidyl 4-fluorobenzoate was chosen as the ligand to radiolabel the chosen biologically sensitive molecules. This ligand looked very ideal in the pursuit to label these molecules firstly because it is small, and therefore is less likely to change the *in vivo* behavior of the molecule. This linker is also ideal in the fact that it quickly reacts with any molecule with a free amine moiety because it has a very good leaving group, the N-hydroxy succinimidyl group. Not only this, but these coupling reactions take place in mildly basic solutions, such as phosphate buffer solution (PBS), where proteins, peptides, and other biologically sensitive molecules are very stable. This molecule is also ideal because it can be synthesized in a very rapid and easy manner, with both hot and cold fluorine.[14,15]

DISCUSSION AND RESULTS

2.1.0

At the start of this project, the N-succinimidyl 4-fluorobenzoate (**40**) was not used as the ligand for the ^{18}F radionuclide. A method was being developed for using 4-fluorobenzaldehyde with an additional linker (Figure 11).[13,19-20] As this method was being optimized, it proved not to be successful in providing an easier route to synthesizing radiolabeled proteins and peptides. But through this method optimization, much was learned about what was needed in finding the right method that would fulfill the goals of this project.

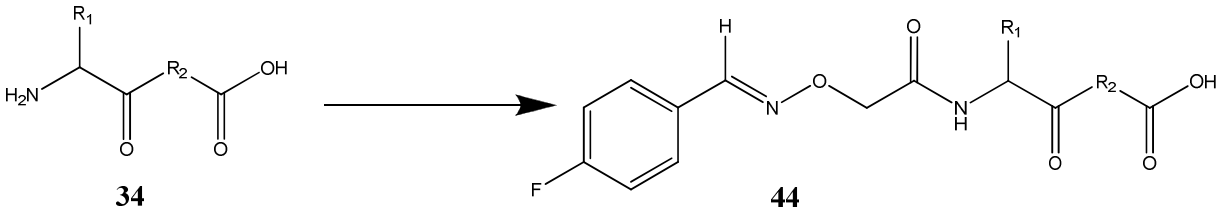


Figure 11. Aminooxy-imine tether

For example, it was determined that the amine protecting group of tert-butyloxycarbonyl (Boc) could not be used at all when the reagents are tethered to the resin. Acid can cleave the reagent from the resin, which also effects deprotection of the Boc group.[17] This greatly compromised all the yields during the synthetic process and, therefore, needed to be changed or eliminated. Since other problems existed with the linker, such as the length, the thought of looking into another a ligand that did not need the use of a linker was suggested.

As the ligand of N-succinimidyl 4-fluorobenzoate was chosen, a protecting group strategy was needed. Since it was known that the Boc protecting group could not be used, Fmoc was explored because this protecting group is cleaved by base, which was favorable towards this method.[17,21] The protection reaction is performed in ether, which does not dissolve the peptide, so additional water must be added. Problems arose, firstly, when abundant water was added, as

even with vigorous stirring was unable to mix the two solvent layers sufficiently. Also, if insufficient water was added, little to no protection was achieved.[16] Using the method described here for the protection reaction, peptides were protected at various yields. DL-leucine-glycine-glycine for instance, reacted very differently versus glycine-glycine-glycine, where the degree of discrepancy is surprising for the fact that an alkyl chain is the only substituent difference between these peptides. The tri-glycine peptide never fully dissolved in the reaction conditions as the other peptide did, and 50% conversion could only be achieved, whereas with the other peptide, ~50% conversion could be readily achieved. Optimization of this method is discussed in the future work of this thesis.

Although Wang resin, which was chosen for this project, differed from the previous flow project, the same coupling reaction could be used.[22] Wang resin has been readily found in solid phase synthesis of proteins and peptides.[23] When exploring a coupling reaction, the coupling conditions from the amide library project were seen as ideal because the conditions are not harsh, therefore, a peptide would be stable in them. Optimization and QC was performed in order to assure that the coupling reaction worked just as efficiently with the new reagents.[22]

In literature, N-succinimidyl 4-fluorobenzoate has been known to easily react in basic solutions, such as phosphate buffer solution (PBS) or in organic solvent with an added base.[14,15] PBS is known to stabilize proteins and peptides so this was seen as the obvious first choice as a solvent for this coupling reaction. Unfortunately, this was not the most ideal solvent for the resin. The reaction was impeded by the resin clumping up and floating in the solvent, even with a vigorous stir. With the resin clumping up in the solvent, the reagent tethered to the resin is impervious to the ligand in the PBS. Therefore, there was little, if any product formed.

Since the resin has been observed to react better in organic solvents, the next choice in

solvent was an organic solvent with an added base. The first solvent chosen was dichloromethane (DCM) with diisopropylethylamine (DIEA) as the added base. This reaction was allowed to go overnight at room temperature. Although product was observed in the NMR spectra, the reaction did not go to completion and the product was not pure. To improve yields and purity, another polar aprotic solvent, dimethylformamide (DMF) was chosen as the next solvent for possible optimization of this step.[24] To the DMF, DIEA was added as well and was allowed to stir at room temperature. The reaction seemed to stall at the same point that the reaction in DCM did, so the reaction was warmed to 50°C to obtain more conversion. After 5 more hours, the reaction looked like it stalled again, so more base was added to the solvent and the reaction was allowed to go overnight. After this reaction, followed by the cleavage reaction, the pure product was found with residual DMF.

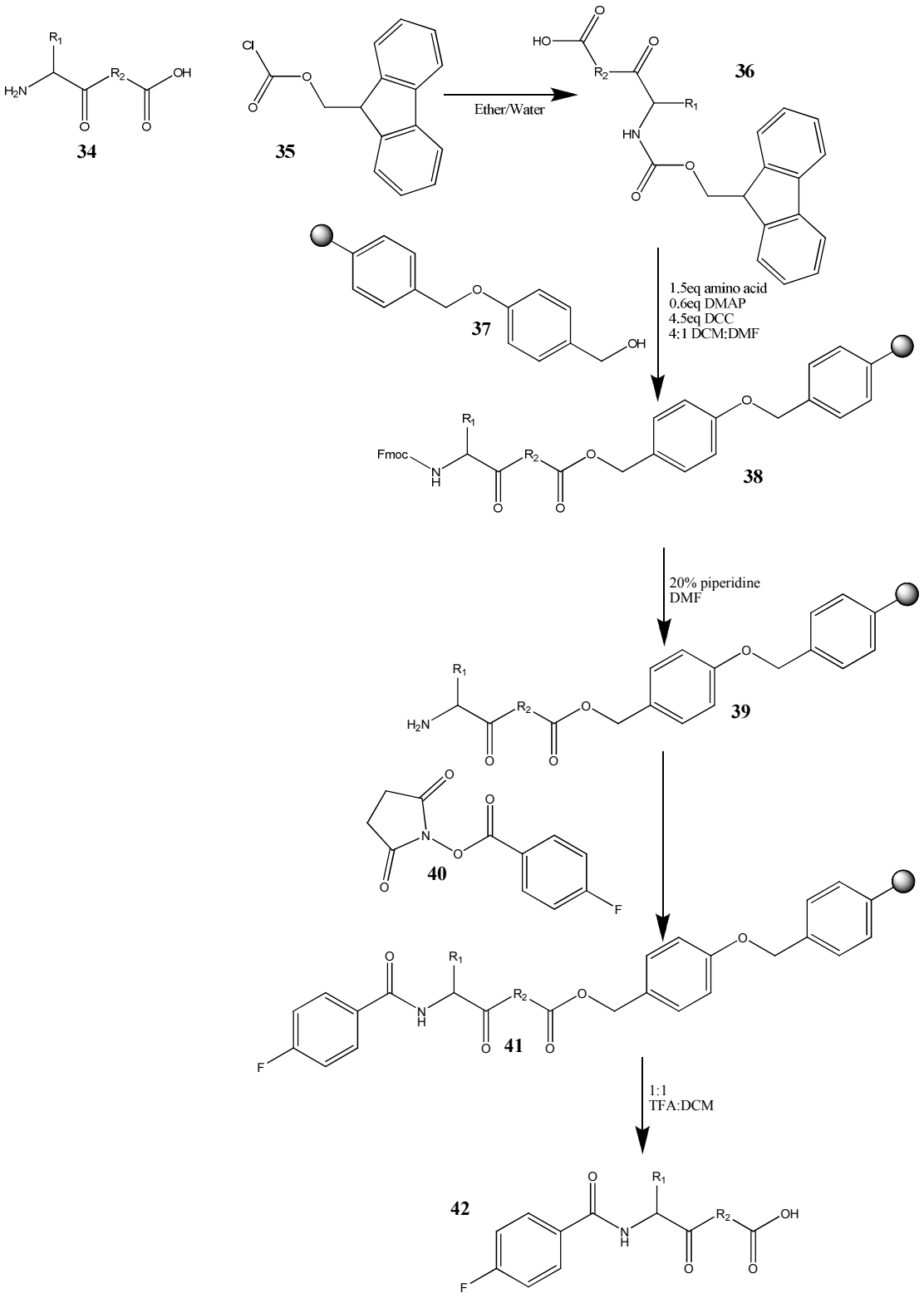
METHODS

2.2.1 Objective

The objective of this project was to radiolabel proteins, peptides, and other biologically sensitive molecules with ^{18}F, using N-succinimidyl 4-fluorobenzoate as a coupling ligand, since it is not possible to directly and rapidly fluorinate these molecules. When synthesizing these molecules with radioactive ^{18}F, the half life of the radionuclide must be kept in mind. Because of this, the synthesis of these molecules is limited to a few steps, with no need for a final purification step. This is the reason it was decided to tether our analyte molecule on a resin and then couple the linker to the molecule as it is tethered, so that there was a possibility of washing away any contaminants or side products. This improves the likelihood of synthesizing pure products. During this project, Wang resin was used because forms ester bonds with the molecule and it is well-precendented for its use in protein synthesis.[25] Scheme 2 illustrates the multi-step synthesis used to radiolabel the proteins. The protein (**34**) is first protected with Fmoc-Cl (**35**) and is then tethered onto the Wang resin (**37**). It is subsequently deprotected and then coupled with the linker (**40**). Lastly, the molecule is cleaved off of the resin. All of these experiments were done with cold fluorine to develop on the methodology.

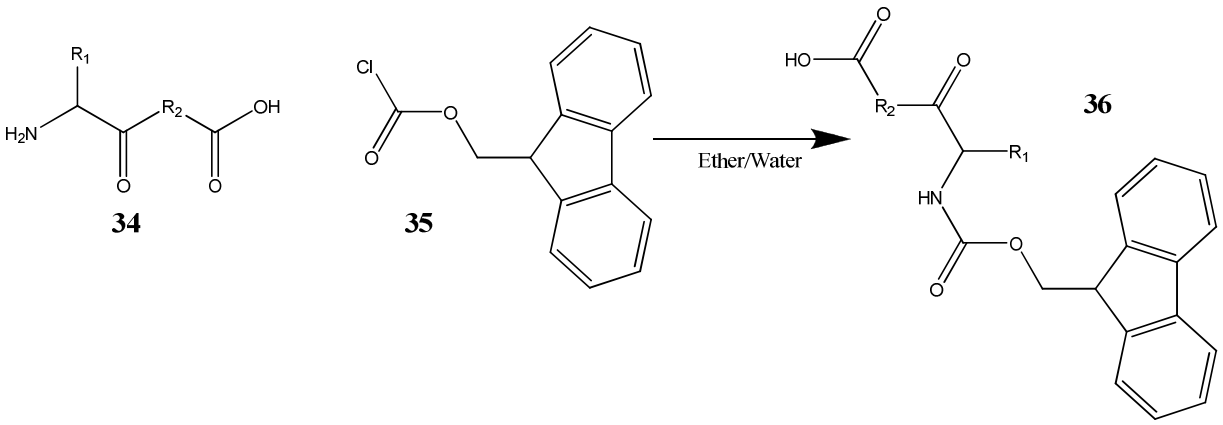
The resin was purchased from Fisher scientific. All the other reagents were purchased from Sigma Aldrich, including the tripeptides. The ^{1}H NMR were recorded on a 500 MHz Varian instrument and are reported in ppm down field relative to tetramethylsilane as an internal standard. TLC analyses were carried out on general-purpose silica gel on polyester TLC plates and were visualized under UV.

2.2.2 Radiolabeling Synthetic Scheme[3,15-18]



Scheme 2. Synthesis of ^{18}F-radiolabeled proteins

2.2.3 Fmoc Protection[16]

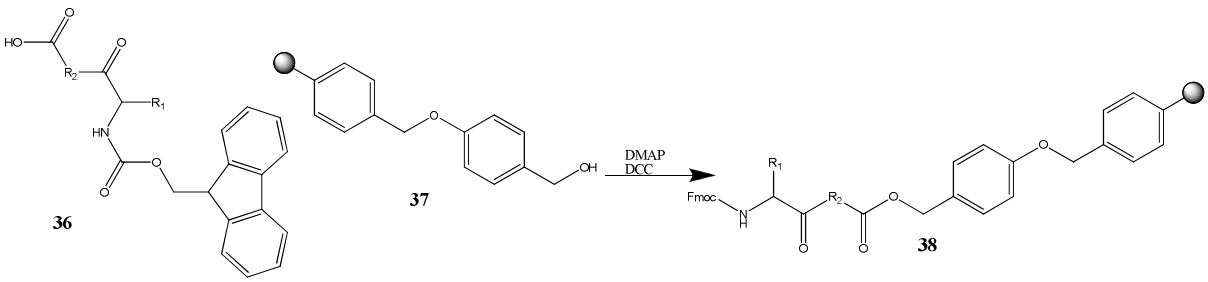


The peptide (1 mmol) (**34**) was put in a 25 mL roundbottom flask with 7 mL ether. Then at 0°C, fluorenylmethyloxycarbonyl chloride (Fmoc-Cl) (0.27 g, 1 mmol, 1 eq) (**35**) in 4 mL ether was added to the flask. This mixture was stirred at 0°C for 5 minutes and then 2 mL of deionized water was added. This was stirred at 0°C for 20 minutes. The reaction warmed to room temperature and stirred at room temperature overnight. The reaction was then evaporated to dryness and compound **36** was isolated.

Fmoc-DL-Leu-Gly-Gly-OH (**36a**)[26] was isolated in 99% yield (0.456 g, 0.98 mmol) with a purity of 50% by NMR, but the crude product was taken onto the next step.

Fmoc-Gly-Gly-Gly-OH (**36b)**[27] was isolated in 150% yield (0.611 g, 1.49 mmol) with a purity of 50% by NMR, but the crude product was taken onto the next step.

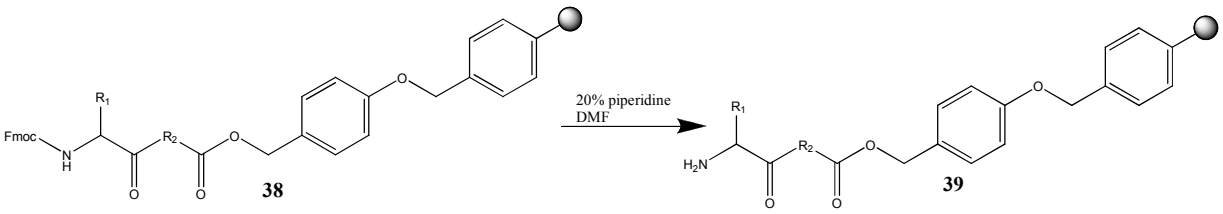
2.2.4 Wang Resin Coupling[2]



The Wang resin (1.5 g, 0.5-1.3 mmol/g) (**37**) was placed in a 250 mL roundbottom flask with 100 mL of 4:1 DCM/DMF, the Fmoc-protected peptide (1 mmol) (**36**), and DMAP (0.223g, 0.6eq, 1.0 mmol). This mixture was stirred for 15 minutes at room temperature, then DCC (2.12g, 4.5eq, 10 mmol) was added. This reaction stirred overnight at room temperature. The resin was then filtered and washed with 4:1 DCM/DMF, DMF, DCM. The reaction solution and wash were monitored by TLC and no Fmoc peptide was observed in either reaction, meaning the Fmoc-protected protein was linked to the resin (**38**).

This reaction was performed with with Fmoc-DL-Leu-Gly-Gly-OH (**36a**) and Fmoc-Gly-Gly-Gly-OH (**36b**), and Fmoc-L-Ala-OH (Commercially available, **36c**).

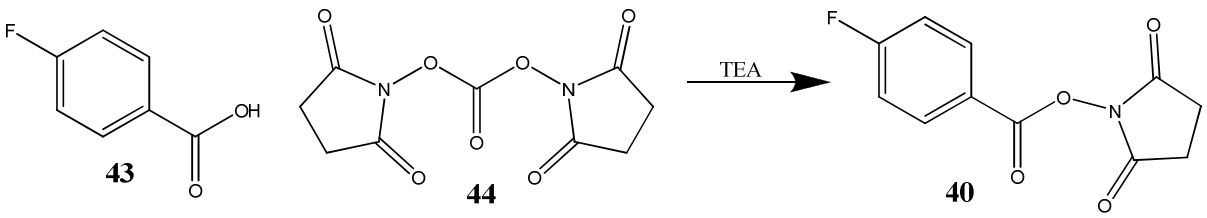
2.2.5 Fmoc Deprotection[17]



The resin tethered product (1.5 g, 0.5-1.3 mmol/g) (**38**) was placed in a 100 mL roundbottom flask with 30 mL of 20% piperidine in DMF. This was stirred overnight at room temperature. The reaction was filtered and the resin bound product was washed with DMF, DCM, deionized water, and DCM. When the reaction and wash were analyzed by TLC, the Fmoc cleavage product was observed in both, which demonstrates the cleavage of the protecting group from resin-tethered product (**39**).

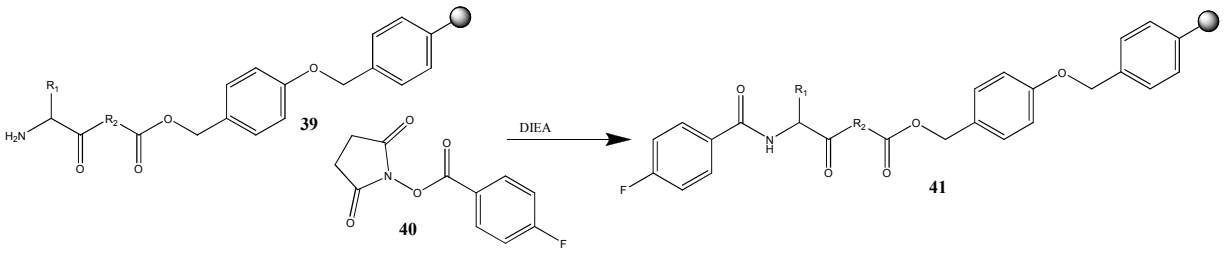
This reaction was done with Fmoc-DL-Leu-Gly-Gly, Fmoc-Gly-Gly-Gly, and commercially available Fmoc-L-Ala.

2.2.6 Synthesis of N-succinimidyl 4-fluorobenzoate[15]



To a 250 mL roundbottom flask was added 4-fluorobenzoic acid (1.12 g, 8.2 mmol) (**43**) and N,N-disuccinimidyl carbonate (DSC) (2.5 g, 1.1 eq, 9.8 mmol) (**44**) under argon. Then dried acetonitrile (45 mL) and triethylamine (3.2 mL, 3eq, 23.0 mmol) were added. The solution was stirred at 80°C for 2.5 hours. The reaction was concentrated and diluted with ethyl acetate (200 mL). This was washed twice with saturated sodium bicarbonate. The aqueous layers were back-extracted with ethyl acetate. The combined organic layers were washed with brine, dried with magnesium sulfate, and then the solvent was evaporated. The residue was purified on silica gel with 70% hexanes/ethyl acetate. After evaporation, compound **40** was obtained in 56% yield (1.08 g, 4.6 mmol), pure white crystals (mp = 111°C). ^{1}H NMR (500 MHz, DMSO) δ 2.91 (s, 4H), 7.20 (t, 2H, J = 11.5 Hz), 8.17 (d(d), 2H, J = 9.0, 5.0 Hz).

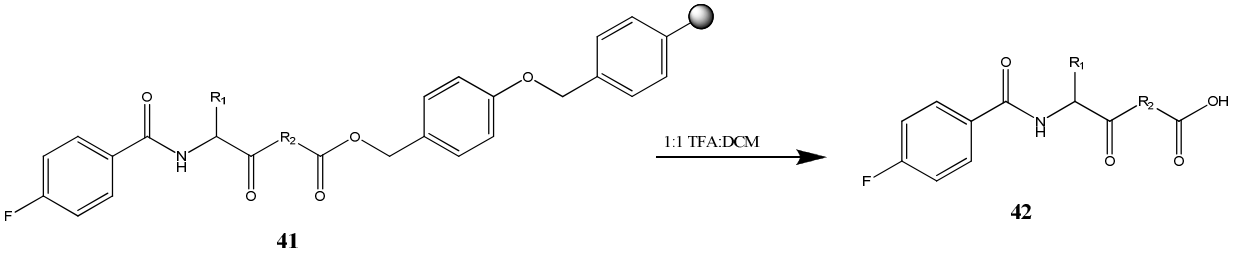
2.2.7 Fluorine Tethering[14,15]



The resin tethered product (1.5 g, 0.5-1.3 mmol/g) (**39**) was placed in a 100 mL roundbottom with N-succinimidyl 4-fluorobenzoate (0.5g, 2mmol) (**40**) in DMF (40 mL) and DIEA (2 mL, 11 mmol). After stirring the reaction for an hour at room temperature, the reaction was heated to 50°C for 5 hours. At this point, DIEA (1 mL, 5.5mmol) was added and the reaction stirred overnight. The resin was then filtered and washed by stirring the resin with DMF, DCM, water, and DCM separately.

This reaction was done with DL-Leu-Gly-Gly, Gly-Gly-Gly, and L-Ala, all tethered to Wang resin.

2.2.8 Resin Cleavage[18]



41 42

The resin tethered reagent (1.5g, 0.5-1.3 mmol/g) was placed in a 100 mL roundbottom flask with 1:1 TFA:DCM (40 mL). This reaction was stirred at room temperature overnight. The resin was then filtered and washed sequentially with DCM, methanol, ethyl acetate, and DCM. The washes were collected in a roundbottom flask and the solvent was evaporated.

No product was collected with Gly-Gly-Gly.

With DL-Leu-Gly-Gly, 50% (0.171 g, 0.47 mmol) was isolated. The NMR spectra was very dirty and showed <5% product.

With L-Ala, 70% (0.200 g, 0.95 mmol) was isolated. The NMR does include excess DMF and some excess peaks, product purity is 60%.

FUTURE WORK

2.3.0

Through all the research, the methods found for this project were not fully optimized for the specific goals of this project. Although proof-of-concept was achieved, by being able to fluorinate the amino acid alanine with the N-succinimidyl 4-fluorobenzoate ligand, most of the steps of the synthesis could be optimized further to make the synthesis more efficient.

Within the first protection step, the first optimization that could be explored is the solvent. By observing the difference in the two tri-peptides in these reaction conditions, one can hypothesize how a more complex protein or peptide would react. Most of these conditions would have to include some kind of aqueous or two phase system, though a two phase system would be most ideal because of the work-up afterwards. The possibility of exploring another Fmoc precursor could be explored as well.

Neither the resin coupling nor the deprotection steps needed further optimization. The only aspect that may be explored in the resin coupling step would be the use of DIC instead of DCC. Since DIC forms a more soluble urea and therefore can be washed away with ease, thus reducing contamination of the products.[2]

As previously mentioned, the coupling of the N-succinimidyl 4-fluorobenzoate ligand and the peptide could be optimized further. The reaction was optimized with the use of solvent, but the possibility of further optimization with another solvent could be explored. This could be performed in tandem with probing optimization with the use of a different base. Since base needed to be added twice in the experiment in which the best yield was obtained, the possibility of using more of the base upfront or even using a stronger base may provide better results. With a stronger base, though, side reactions may result. Also, to translate this procedure to hot

radiosynthesis, the method must be optimized to provide improved yields, and more rapidly, in order to ensure enough of the half life of the radionuclide for imaging. This goal can be explored with the use of the microwave. Instead of heating the product conventionally at 50°C overnight, the idea of quickly heating the reaction with the microwave can be explored. Also, many microwaves have a simultaneous cooling system. Therefore when the microwave irradiates the reaction, it simultaneously cools the reactions, so that it does not reach exorbitant temperatures and cause the peptides to decompose or to become inactive.

The wash systems of each step needs to be optimized. Since the goal is to provide pure products, the wash systems need to wash away any side products, unreacted reagents, and any other contaminants. Throughout the various synthetic steps various reagents are used, which then can lead to various contaminants. Therefore, a thorough wash protocol must be devised, that would fully wash the resin and product after each step. If one cannot be devised, the use of scavenger columns could also be implemented. These columns are filled with tethered reagents that scavenge different classes of contaminants from the products. Multiple different scavengers could also be used in tandem, to scavenge multiple contaminants. The use of these columns can be very helpful as a last resort to clean up the product.

After the method is optimized, a future project would be to radiolabel more complex proteins with the use of this method. When radiolabeling these proteins, the method can be examined to see if selective fluorination is possible. Proteins that have amino acids such as arginine, lysine, glutamine, etc. have at least two amino groups present; the one the N-terminus and one on the amino acid side chain. These amino groups have different pKa values, therefore we can explore if our ligand selectively reacts with one amino group over the other based on the pKa values, the solvent, and the base used.[24,28]

Lastly, the method could be used in radiolabeling proteins of interest with the use of hot ^{18}F. This method would ideally allow us to radiolabel proteins that were previously unable to be radiolabeled with ^{18}F, and furthermore could perform *in vivo* imaging studies with these proteins.[29]

REFERENCES

1. Jas, G.; Kirschning, A. Continuous Flow Techniques in Organic Synthesis *Chem. Eur. J.* **2003**, 9, 5708-5723

2. Salvino, J.W.; et.al Polymer-Supported Tetrafluorophenol: A New Activated Resin for Chemical Library Synthesis *J.Comb.Chem.* **2000**, 2,691-697

3. Baxendale, I.R.; Ley, S.V. Solid Supported Reagents in Multi-Step Flow Synthesis *New Avenues to Efficient Chemical Synthesis: Emerging Technologies. Ernst Schering Foundation Symposium Proceedings 2006-3*, Eds P.H. Seeberger, T. Blume, Springer-Verlag: Berlin, Heidelburg, **2007**, *Chp 3*, pages 151-185

4. Shi, B.; Scobie, M.; Boyle, R.W. Parallel Synthesis of Unsymmetrically Substituted Tetraphenyl Porphyrins on Wang Resin *Tetrahedron Lett.* **2003**, 44, 5083-5086.

5. Lee, Y.S.; et.al. An Improved Method of 18F Peptide Labeling: Hydrazone Formation with HYNIC-conjugated c(RGDyK) *Nucl. Med. Bio.* **2006**, 33, 677-683.

6. Poethko, T.; et al. Two-Step Methodology for High-Yield Routine Radiohalogenation of Peptides: 18F-Labeled RGD and Octreotide Analogs. *J. Nuc. Med.* **2004,** 45, 892-902.

7. http://www.syrris.com/AFRICA.aspx

8. Sutcliffe-Goulden, J.L.; O'Doherty, M.J.; Bansal, S.S. Solid Phase Synthesis of [18F] Labeled Peptides for Positron Emission Tomography *Biorg. Med. Chem. Lett.* **2000**, 10, 1501-1503.

9. Vaidyanathan, G.; Zalutsky, M.R.; Synthesis of N-succinimidyl 4-[18F] fluorobenzoate, an Agent for Labeling Proteins and Peptides with 18F. *Nat. Protoc.* **2006**, 4, 1655-1661.

10. Miller, P.W.; Long, N.J.; Vilar, R.; Gee, A.D. Synthesis of ^{11}C, ^{18}F, ^{15}O, and ^{13}N Radiolabels for Positron Emission Tomography *Angew. Chem. Int. Ed.* **2008**, 47, 8998-9033.

11. Stanger, K.H.; Krchnak, V. Incorporation of the Wang Linker upon Cleavage from Polystyrene-based Resin to form O-(4-Hydroxy)benzyl Derivatives *J. Comb. Chem*. **2006**, 8, 652-654.

12. Arimitsu, K.; Ichimura, K. Nonlinear Organic Reaction of 9-fluorenylmethyl Carbamates as Base Amplifiers to Proliferate Aliphatic Amines and the Application to a Novel Photopolymer System *J. Mater. Chem.* **2004**, 14, 336-343.

13. Baumann, M.; Baxendale, I.R.; Martin, L.J.; Ley, S.V. Development of Fluorination Methods using Continuous-Flow Microreactors *Tetrahedron* **2009**, 65, 6611-6625.

14. Sutcliffe-Goulden, J.L.; O'Doherty, M.J.; Marsden, P.K.; Hart, I.R.; Marshall, J.F.; Bansal, S.S. Rapid Solid Phase Synthesis and Biodistribution of ^{18}F-labelled Linear Peptides *Eur. J. Nucl. Med.* **2002**, 29, 754-759.

15. Greene, T.W.; Wuts, P.G.M. *Protective Groups in Organic Synthesis*, 3rd ed.; Wiley & Sons: New York, 1999; pg 507.

16. Rigo, P.; Paulus, P.; Kaschten, B.J.; Hustinx, R.; Bury, T.; Jerusalem, G.; Benoit, T.; Foidart-Willems, J. Oncological Applications of Positron Emission Tomography with Fluorine-18 Fluorodeoxyglucose *Eur. J. Nucl. Med.* **1996**, 23, 1641-1674.

17. Duléry, V.; Renaudet, O.; Dumy, P. Ethoxyethylidene Protecting Group Prevents N-Overacylation in Aminooxy Peptide Synthesis *Tetrahedron*, **2007**, 63, 11952-11958.

18. McMurry, J.S. Solid Phase Synthesis of a Cyclic Peptide using Fmoc Chemistry *Tetrahedron Lett.* **1991**, 32, 7679-7682.

19. Dispersion of Soluble Matter in Solvent Flowing through a Tube. *Proceedings of the Royal Society of London. Series A, Mathematical, Physical, and Engineering Sciences*; The Royal Society: London, 1954; Vol. 219, pp 446-448.

20. Shreve, P.D.; Anzai, Y.; Wahl, R.L. Pitfalls in Oncologic Diagnosis with FDG PET Imaging: Physiologic and Benign Variants *RadioGraphics* **1991**, 19, 61-77.

21. Muehllehner, G.; Karp, J.S. Positron Emission Tomography *Phys. Med. Biol.* **2006**, 51, R117-R137.

22. Ley, S.V.; Baxendale, I.R. New Tools and Concepts for Modern Organic Synthesis *Nat. Rev. Drug Discovery* **2002,** 1, 573-586.

23. Watts, P.; Wiles, C. Recent Advances in Synthetic Micro Reaction Technology *Chem. Comm.* **2007**, 443-467.

24. Okarvi, S.M. Recent Progress in Fluorine-18 Labelled Peptide Radiopharmaceuticals *Eur. J. Nucl. Med.* **2001**, 28, 929-938.

25. Hall, D.G.; Manku, S.; Wang, F. Solution- and Solid-Phase Strategies for the Design, Synthesis, and Screening of Libraries Based on Natural Product Templates: A Comprehensive Survey. *J. Comb. Chem.* **2001**, 3, 125-150.

26. Schlummer, S.; Vetter, R.; Kuder, N.; Henkel, A.; Chen, Y.; Li, Y.; Kuhlmann, J.; Waldmann, H. Influence of Serine *O*-Glycosylation or *O*-Phosphorylation Close to the vJun Nuclear Localisation Sequence on Nuclear Import. *ChemBioChem* **2005**, 7, 88-97.

27. Shaikh, H.A.; Sönnichsen, F.D.; Lindhorst, T.K. Synthesis of Glycocluster Peptides. *Carbohydr. Res.* **2008**, 343, 1665-1674.

28. Vaidyanathan, G.; Zalutsky, M.R. Improved Synthesis of N-Succinimidyl 4-[^{18}F]Fluorobenzoate and Its Application to the Labeling of a Monoclonal Antibody Fragment *Bioconjugate Chem.* **1994**, 5, 352-356.

29. Chen, X.; Park, R.; Shahinian, A.H.; Tohme, M.; Khankaldyyan, V.; Bozorgzadeh, M.H.; Bading, J.R.; Moats, R.; Laug, W.E.; Conti, P.S. ^{18}F-labeled RGD Peptide: Initial Evaluation for Imaging Brain Tumor Angiogenesis *Nuc. Med. Biol.* **2004**, 31, 179-189.