Development of an ^{18}F Radiolabeling Method Using Solid Phase Chemistry

A thesis presented to The Faculty of Graduate Studies of Lakehead University by

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In partial fulfilment of the requirements for the degree of

Master of Science

April 8, 2023

Abstract

New radiotracers are constantly being developed to expand the clinical applications of positron emission tomography (PET). One of the challenges in the development of PET tracers is the incorporation of a positron emitting isotope. Short half-lives combined with added safety measures when working with radioactive materials require a fast and simple synthesis.

This project describes a solid phase fluorine-18 ($t_{1/2}$ = 110 min) radiolabeling procedure that allows removal of impurities through filtration. The synthesis takes an indirect approach by first labeling a small molecule with F-18 and then coupling this molecule to an amine containing compound using solid phase dichlorotriazine (DCT).

This procedure has been used to label various compounds containing primary and secondary amine groups without the need for an additional purification step. The total synthesis time required is approximately 80 min from the from delivery of the [^{18}F]fluoride, with a decay corrected radiochemical yield of 3% for the example tested (yield will likely vary depending on the substrate). This demonstrates the potential of solid phase reagents like DCT to be used in radiolabeling, but further development is required to improve yields for this reaction.

Acknowledgments

I would like to thank Dr. Campbell for the continued guidance throughout the length of this project and many hours spent at the Thunder Bay cyclotron. I would also like to thank Dr. Gottardo and Dr. Hou for being on my committee. Finally, I'd like to thank D. Puumala for her assistance with obtaining many of the chemicals required for this project.

Table of Contents

List of Tables

List of Figures

List of Schemes

List of Abbreviations

APCI	Atmospheric pressure chemical ionization
C18	Octadecylsilane
CT	Computed tomography
DCM	Dichloromethane
DCT	Dichlorotriazine
DIPEA	N,N-Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
EDC	Ethyl-dimethylaminopropyl carbodiimide
EtOAc	Ethyl acetate
FDG	Fluorodeoxyglucose
Hex	Hexanes
HPLC	High-performance liquid chromatography
IR	Infrared radiation
K_{222}	Kryptofix 222
MeCN	Acetonitrile
MeOH	Methanol
MRI	Magnetic resonance imaging
MS	Mass spectrometry
NMM	N-Methylmorpholine
Nosylate	p-Nitrophenylsulphonate
PET	Positron emission tomography
QMA	Quaternary methyl ammonium
RBF	Round bottom flask
Rf	Retention factor
SPE	Solid-phase extraction
TBRHSC	Thunder Bay Regional Health Sciences Center
TMS	Tetramethylsilane
TLC	Thin-layer chromatography
Ts-Cl	p-Toluenesulfonyl chloride

CHAPTER 1 – Introduction

1.1 PET and PET tracers

Positron emission tomography (PET) is a functional imaging technique that uses radiotracers labeled with a positron emitting isotope. A PET tracer will target a specific biological pathway or function, accumulate at the target site, and then decay at this location. Coupled with an anatomical imaging method like X-ray computed tomography (CT) or magnetic resonance imaging (MRI), PET can provide useful information allowing for diagnosis and analysis of the disease state [1,2].

The most commonly used radiotracer in PET imaging is the glucose analogue 2-deoxy-2-[^{18}F]-fluoro-D-glucose ([^{18}F]FDG). The uptake of FDG is correlated with glucose consumption, which can give information about inflammation, infection, cancer, and brain function [3]. FDG is used for imaging in 90% of cancers but does not give any information that is not dependent on glucose metabolism, ex: cancer proliferation or aggression [3]. Furthermore, the non-specificity of glucose-metabolic changes and lack of contrast between physiological and pathological uptake limits its sensitivity [4]. New PET tracers are constantly being developed to address these limitations and to give more specific information used for personalized medicine [3-4].

PET's clinical relevancy is not only limited to cancer diagnosis; it also has use in fields including general internal medicine, infectious diseases, cardiology, neurology, surgery, traumatology, orthopedics, endocrinology, psychiatry, neuropsychology, and cognitive neuroscience [5]. Most applications require their own unique tracers, so the development of new more specific PET tracers is essential to expand PET's range of clinical relevance [6-7].

1.2 Fluorine-18 as a PET Radionuclide

Some commonly used positron emitting nuclides used in PET imaging are ^{15}O, ^{13}N, ^{11}C, and ^{18}F, with half-lives of 2.037, 9.965, 20.39, and 109.8 min, respectively. ^{18}F is the most used radioisotope for PET. It has a more useful half life (110 minutes) compared to the other positron emitting nuclides (Table 1.1) which allows for multistep synthesis and time for transportation [3]. It also has a relatively low positron energy, which leads to a short positron range allowing higher spatial resolution. It also has relative ease of production, most commonly produced using the ^{18}O(p,n)^{18}F reaction. ^{18}O enriched water ([^{18}O]H_2O) is normally used as the ^{18}O source, which is irradiated with a proton beam produced by a cyclotron [3]. This produces nucleophilic [^{18}F]F^- in an aqueous solution. A common method for producing electrophilic ^{18}F is through the ^{20}Ne(d,α)^{18}F reaction by accelerating deuterons into a ^{20}Ne target at 14 MeV. A small amount of nonradioactive F_2 gas is added to the ^{20}Ne gas and ^{18}F-^{19}F ([^{18}F]F_2) is produced with a low specific activity. The ^{18}O(p,n)^{18}F using $^{18}O_2$ as the target is also commonly used for producing [^{18}F]F_2 with a low specific activity.

Table 1.1: Nuclear properties of commonly used positron emitters [3]

Isotope	Maximum Positron Energy (MeV)	Maximum and (Average) Range in Water (mm)
^{15}O	1.72	8.2 (2.7)
^{13}N	1.19	5.4 (1.5)
^{11}C	0.96	4.1 (1.0)
^{18}F	0.635	2.4 (0.6)
^{68}Ga	1.9	9.0 (3.7)

Along with the favorable nuclear properties discussed, F-18 has versatile chemistry allowing various direct and indirect methods of introducing ^{18}F into a molecule of interest [8].

1.3 Radiofluorination

Radiolabelling with fluorine-18 can be done through electrophilic and nucleophilic methods. Electrophilic fluorinations (using $[^{18}F]F_2$) generally result in a lower specific activity because fluorine-19 gas must be added as a carrier to extract the $[^{18}F]F_2$ from the target. There are also often issues with regioselectivity [3]. Nucleophilic fluorination can result in better yields and is normally the method of choice. However $[^{18}F]F^-$ is produced in aqueous solution and the hydrogen bonds it forms with water inhibits its ability to act as a nucleophile. Therefore the $[^{18}F]F^-$ must be dried before use and the fluorination must be done in anhydrous conditions. This is normally done by passing the aqueous $[^{18}F]F^-$ through an anion exchange cartridge where it is trapped, allowing the water to flow through. It is then eluted with a mixture of small amounts of water in MeCN or MeOH containing a base like K_2CO_3. The base also prevents the formation of volatile $[^{18}F]HF$ which can lead to the loss of ^{18}F. The $[^{18}F]KF$ salt can be further dried through azeotropic drying with MeCN.

1.4 Direct and Indirect Radiolabeling

One of the challenges in developing a PET tracer is designing a synthesis to incorporate the positron emitting isotope. The radioisotopes have short half-lives, so the synthesis and purification must be completed quickly. The two general strategies of incorporating ^{18}F onto a molecule are through direct or indirect methods. For direct labeling the ^{18}F is incorporated directly on the compound of interest. In indirect labeling ^{18}F is first added to a prosthetic group, which is then attached to the compound of interest (Figure 1.1).

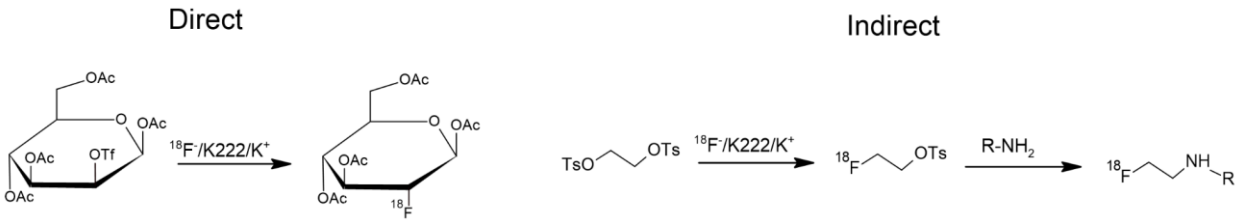


Figure 1.1: Direct labeling: A step in FDG synthesis. ^{18}F is directly attached to the sugar ring [9]. Indirect labeling: A prosthetic group is radiolabeled and is then conjugated to the compound of interest R.

Direct radiolabeling requires fewer reaction steps, which leads to a simpler and faster synthesis involving fewer purification steps. The disadvantages of direct radiolabeling are the high temperatures and the basic conditions often required for fluorination which are not suitable for some biomolecules. For example, peptides and proteins often have a large number of diverse functional groups present that make conventional nucleophilic fluorine-18 labeling difficult [10,11]. When a molecule is not compatible with the conditions required for fluorination, an indirect method must be used. The disadvantage of indirect labeling is that the multiple steps required also increase the number of purification steps, leading to a more complex and time-consuming synthesis often requiring HPLC separations [12].

1.5 Hot Cell Chemistry

Radiochemistry has an added risk to the chemist of exposure to ionizing radiation, so there are additional safety precautions that must be taken. Most radiochemical synthesis procedures are carried out in a lead-shielded hot cell (Figure 1.2) which allows chemistry to be controlled remotely from a safe area [3]. A hot cell contains an interconnected system of vials, reaction vessels, and places for inserting SPE cartridges (Ex: C-18 cartridges) used for purification. Using this system reduces radiation exposure to an acceptable level [3], but also makes more complex syntheses difficult and limits the variety of techniques available to the chemist. Low activity manual radiosynthesis can also be performed, which gives the chemist more control, and allows the reaction to be more closely monitored (Ex: TLC). This can be useful when developing a radiosynthesis procedure.

(A) (B)

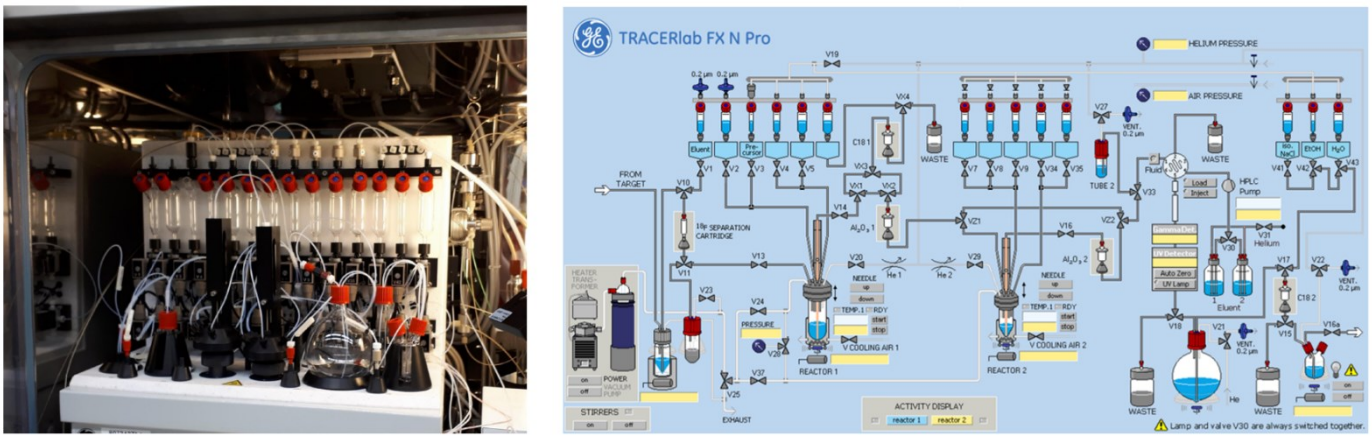


Figure 1.2: (A) The hot cell at the TBRHSC cyclotron radiochemistry lab. (B) Software used to control the hot cell.

1.6 Research Objectives

The goal of this project is to design a simple radiolabeling procedure applicable to a variety of compounds. The method developed in this study uses indirect F-18 radiolabeling to label compounds containing a primary or secondary amine group. Many pharmaceuticals and PET imaging agents that are currently being used or are in development contain amines [13,14], and indirect labeling is often the method of choice when labeling peptides [15]. Developing a simple indirect labeling method that targets a wide range of amines has the potential to be useful to many radiochemists.

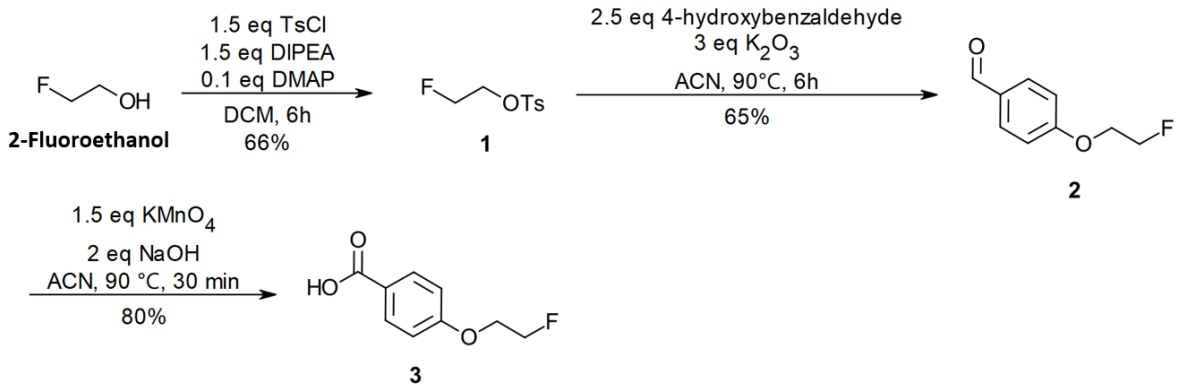
CHAPTER 2 – The Prosthetic Group

2.1 Introduction

The objective for this project is to use a solid phase amide coupling reagent to couple an F-18 labeled prosthetic group to a variety of amines. To achieve this, the precursor for the intended prosthetic group must have a site for incorporating F-18 and a method of forming a carboxylic acid group for amide coupling. The carboxylic acid must be formed after radiofluorination so that it does not inhibit the [^{18}F]fluoride through hydrogen bonding. The F-18 source for this project will be [^{18}F]F^- produced at the TBRHSC medical cyclotron, which can be added the prosthetic group through nucleophilic substitution. Common leaving groups for aliphatic nucleophilic substitution are -OTf, -ONs, -OTs, -OMs, -I, -Br, or -Cl in order of highest to lowest leaving group ability [3]. A better leaving group may result in better yield but will also have a higher tendency for E2 Elimination. Common leaving groups for aromatic nucleophilic substitution are -NMe_3^+, -NO_2, -F, -Cl, -Br, -I in order of highest to lowest leaving group ability. NMe_3^+ is a good leaving group that often results in good radiochemical yield, but side products from demethylation are common [3]. Polar aprotic solvents are normally best for nucleophilic S_N2 and S_NAr substitution, so a phase transfer catalyst must be used or the majority of [^{18}F]KF will not go into solution. The most commonly used phase-transfer catalyst for K^+ is 4,7,13,16,21,24-Hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane (Kryptofix, K_{222}) [3] which is what was used in this project.

2.2 The prosthetic group

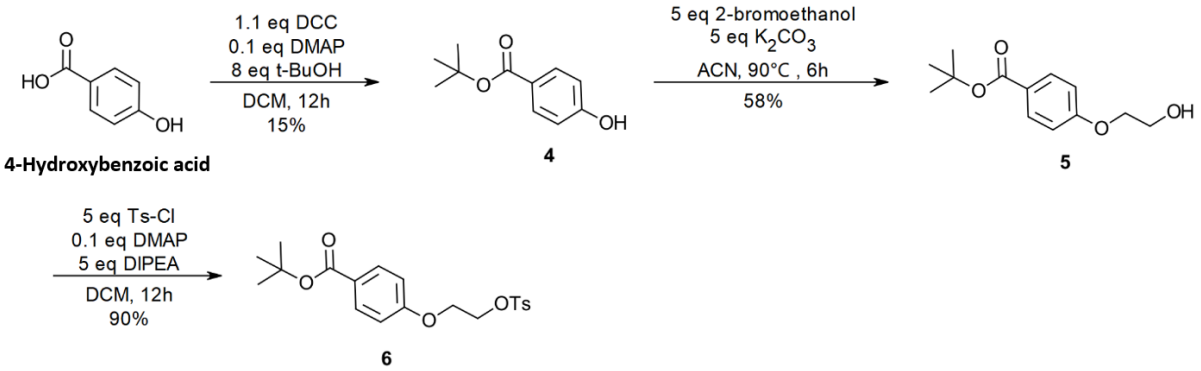
Since the demethylation side product of NMe_3^+ can be significant under the conditions required for nucleophilic aromatic fluorination and nitro groups have a lower leaving group tendency [3] compound **3** (Scheme 2.1) was chosen as the prosthetic group which will allow the fluoride to be added using aliphatic nucleophilic substitution. Furthermore, this is a novel prosthetic group that adds to the library available to radiochemists. The ^{19}F version can be synthesized through Scheme 2.1.



Scheme 2.1: The synthesis of [^{19}F] **3**

2.3 Developing a synthesis strategy for [^{18}F]3

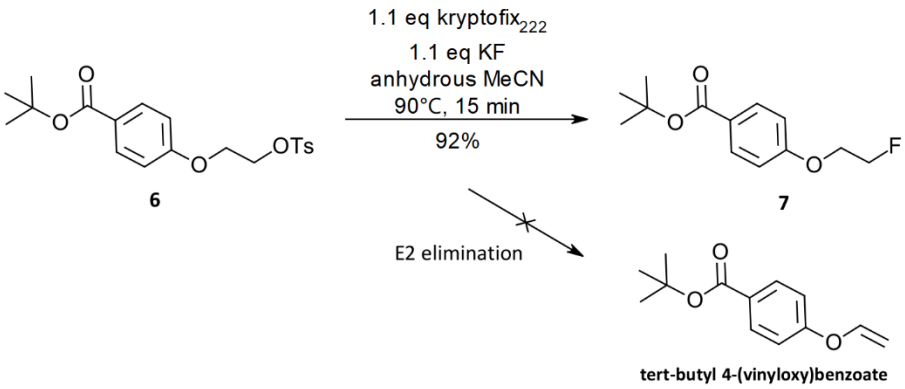
Compound **6** was chosen as a precursor for radiofluorination; it contains a tosylate leaving group for nucleophilic fluorination and a t-butyl ester protecting group that can be removed to form the acid. Chapters 2.3.1 to 2.3.4 will discuss why this compound was chosen and how a method was developed to use it as a starting material to synthesize and purify [^{18}F]**3**. Compound **6** can be synthesized through Scheme 2.2.



Scheme 2.2: The synthesis of **6**

2.3.1 A leaving group for fluorination

A tosylate leaving group was chosen due to its easy synthesis and good leaving group ability. The cold fluorination reaction (Scheme 2.3) is complete in under 15 minutes yielding a pure product on TLC after workup suggesting that minimal E2 elimination product is formed. The relatively fast reaction time and clean product is ideal for radiosynthesis.



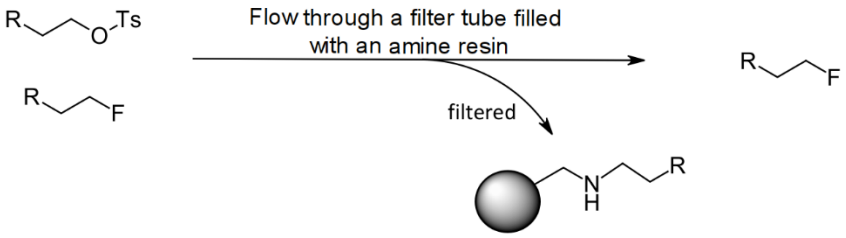
Scheme 2.3: Nucleophilic fluorination of **6**

2.3.2 Purification of the fluorinated product

In a radiosynthesis only nanogram quantities of [^{18}F]F$^-$ will be reacting with **6.** After the reaction, the unreacted starting material will need to be separated from the [^{18}F]**7** generated before it is carried over to the next step of the synthesis. The TracerLab FXN-Pro synth box used in this project is designed with slots for SPE cartridges, which is one of the only purification methods available if HPLC is to be avoided.

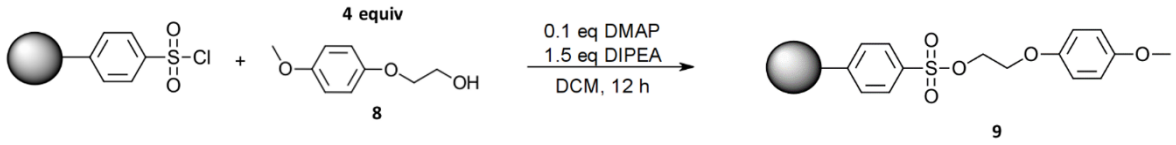
The SPE cartridges used in this project were Sep-Pak Plus C18 Cartridges (Waters, SKU: WAT020515) and Sep-Pak plus Alumina B Cartridges (Waters, SKU: WAT020505). To determine an appropriate eluent, 5 mg of **6** and 1 mg of **7** were dissolved into various ratios of hexane:DCM for Alumina B separations and MeCN:water for C18 separations. Unfortunately, no solvent combination attempted yielded complete separation. A nosylate and mesylate leaving group version of compound **6** were then synthesized to alter the polarity of the molecule, but this had little effect and separation was still not possible.

Another method attempted was to remove the tosylate **6** from solution using a Si-triamine resin (1.34 mmol/g, Silicycle, Product number: R48030B) and aminomethyl polystyrene resin (2.9 meq/g, Chem-Impex, catalog number: 04252). This method would bind the unreacted starting material to the resin which could be removed through filtration (Scheme 2.4). To test this 0.2 eq of **6** in 82°C MeCN were flowed through 1 mL filter tubes filled with the amine resins (~0.53 g). The tosylate was still present in solution for both (according to TLC).



Scheme 2.4: Separating unreacted tosylate from the fluorinated product by reacting the tosylate with an amine resin and filtering.

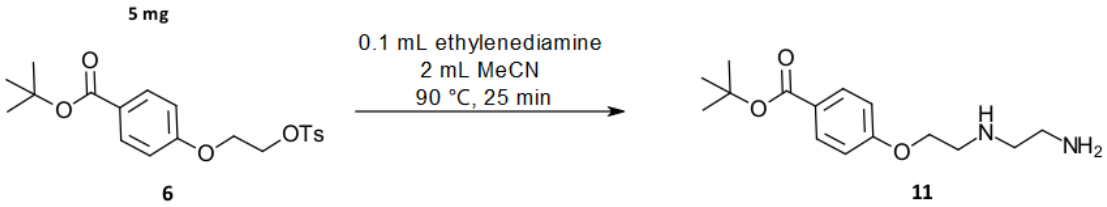
An alternative strategy explored was using tosyl chloride resins which would allow the unreacted tosylate to be filtered out of solution. Solid phase tosylates were synthesized through Scheme 2.5 using a polymer bound sulfonyl chloride resin (1.5-2.0 mmol/g, Sigma Aldrich, SKU: 498211).



Scheme 2.5: Synthesis of the solid phase tosylate **9**

A radiofluorination was performed using the fluorination conditions described in chapter 4. The dried $[^{18}F]F^-/K222/K_2CO_3$ solution was sent to a vial containing **9** outside of the hotcell in dry MeCN (3 mL). This mixture was heated on a hotplate for 30 minutes at 90°C. This resulted in a ~30% yield of **10** according to radio-TLC. When this reaction was repeated several times, it was found that it often yielded little to no fluorinated product in radiofluorination. This may be from an increased sensitivity to moisture since it became less reliable during the more humid summer months. To ensure the radiosynthesis is reliable, it was decided that it would be better to use a solution phase tosylate.

The final strategy attempted was reacting **6** with ethylenediamine (Scheme 2.6) to yield compound **11**. The goal was to create a polar product that could be easily separated from **7** using SPE cartridges. To test this, 1 mg of **7** was added to the reaction mixture of Scheme 2.6 and the reaction progress was monitored on TLC. TLC showed the reaction was complete in ~25 minutes without affecting compound **7** in solution.



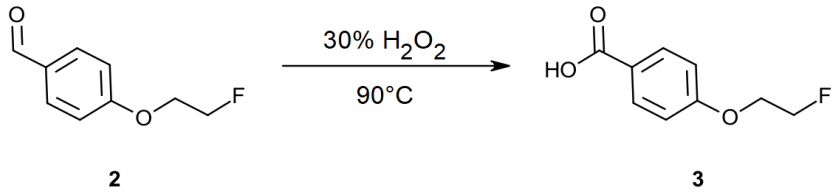
Scheme 2.6: Reacting **6** with ethylenediamine to form the polar product **11**

To separate **7** from **11** using SPE, the reaction solution was diluted with water (4 mL) and run through a C18 cartridge (preconditioned with MeCN (10 mL) and water (20 mL)). The C18 cartridge was then rinsed with a 1:4 MeCN:water mixture (5 mL) which was effective at removing ethylene diamine and any other contaminants. Approximately 1 to 1.5 mL of MeCN was then required to completely elute the trapped compound **7**. This yields pure **7,** according to TLC and APCI-MS, with negligible loss of product.

2.3.3 Forming the Carboxylic Acid

To form the carboxylic acid an aldehyde can be oxidized, or an acid protecting group can be used which can be removed. It is ideal to use reagents that can be removed by evaporation for simple purification when using an automated synthesis.

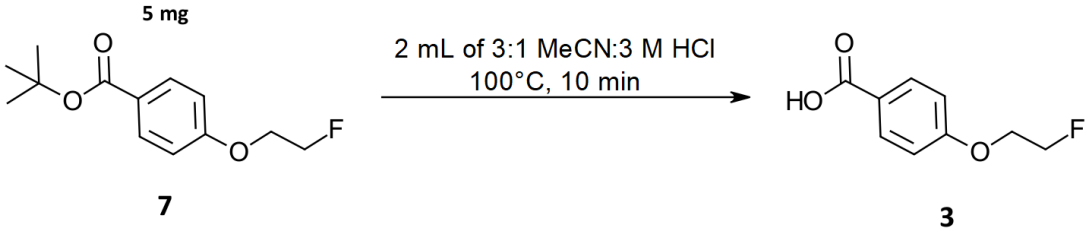
In initial attempts, the aldehyde (**2**) was oxidized using a 30% H_2O_2 solution (Scheme 2.7) which could be easily removed by evaporation after the reaction. After a relatively long 45 minute reaction time there was still a small amount of aldehyde remaining according to TLC.



Scheme 2.7: The oxidation of **2** using hydrogen peroxide

A radiofluorination was performed to produced [^{18}F]**2** using a tosylate leaving group and fluorination conditions described in chapter 4. The resulting solution was diluted with water (8 mL) and sent through a C18 cartridge. The cartridge was rinsed with water (5 mL) and the product was eluted with MeCN (2 mL) and collected outside of the hotcell. The solvent was removed by heating to 90°C on a shielded hot plate. 5 mL of H_2O_2 was added and the solution was stirred at 90°C. Complete conversion of [^{18}F]**2** to the acid was not seen and the reaction yielded side products according to radio-TLC.

The t-butyl ester version (**7**) was then synthesized and could be deprotected using a mixture of MeCN and HCl (scheme 2.8). Under these conditions there was 100 % conversion to the acid in under 10 minutes as determined by TLC.



Scheme 2.8: Deprotecting compound **7** to form the carboxylic acid

In a hot synthesis, 100% conversion of [^{18}F]**7** to the acid product was seen in 10 minutes according to radio-TLC.

2.4 Conclusion

In this chapter, a method was determined for generating a fluorinated prosthetic group that contains a carboxylic acid. In Chapter 2.3.1 it was found that using a tosylate leaving group and Kryptofix 222 in a nucleophilic fluorination generated a pure fluorinated product in under 15 minutes (Scheme 2.3). In Chapter 2.3.2 a method was determined for removing unreacted tosylate using ethylenediamine and a C18 SPE cartridge (Scheme 2.6). Finally, the carboxylic acid can be generated through an acid deprotection of a t-butyl ester (Scheme 2.8). The generates compound **3** using methods that are suitable for radiosynthesis. The next step in the synthesis will be attaching **3** to compounds that need labeling through amide coupling. This amide coupling step will be discussed in Chapter 3.

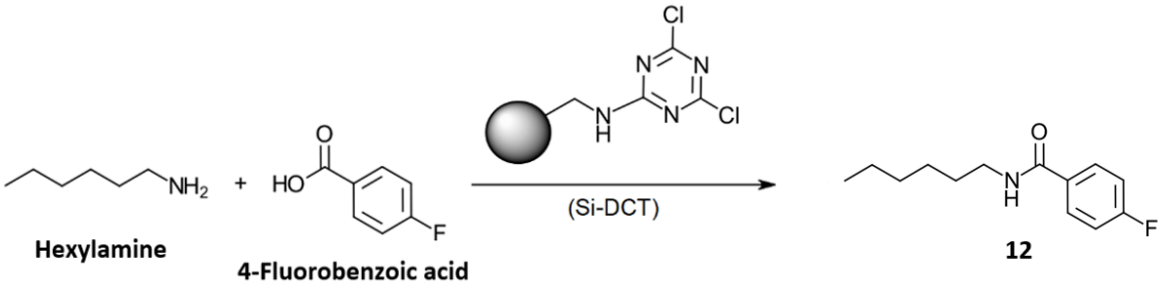
CHAPTER 3 – Amide Coupling

3.1 Introduction

Once the prosthetic group is labeled with fluorine-18 it must be attached to the compound that needs labeling. In this project, this will be done through an amide coupling reaction. Amide bonds are often formed by reacting carboxylic acids with amines using a coupling reagent that converts the –OH of the acid into a good leaving group. Without the help of a coupling reagent, the necessary elimination of water will only occur at very high temperatures (e.g. >200°C) [16]. An ideal procedure for this project would be an amide coupling reaction that uses mild conditions suitable for a wide range of compounds and occurs very quickly. Furthermore, it should be a solid phase reagent that can be loaded into a filter tube and works fast enough that the reaction will occur in the time it takes to flow the reagents through. If a clean product is generated this would avoid the need for further purification.

3.2 Choosing a coupling reagent

Two commercially available solid phase amide coupling reagents at the time of this project were SiliaBond Ethyl-Dimethylaminopropyl Carbodiimide (Si-EDC, Silicycle, product number: R70630B) and SiliaBond dichlorotriazine (Si-DCT, Silicycle, discontinued). To couple 4-fluorobenzoic acid to hexylamine, the resins were loaded into 1 mL filter tubes and the appropriate reagents were flowed through in DCM. The Si-EDC did not show product formation while the Si-DCT was successful (Scheme 3.1). The amide product **12** was clean after workup according to ^{1}H NMR (<5 % impurities).

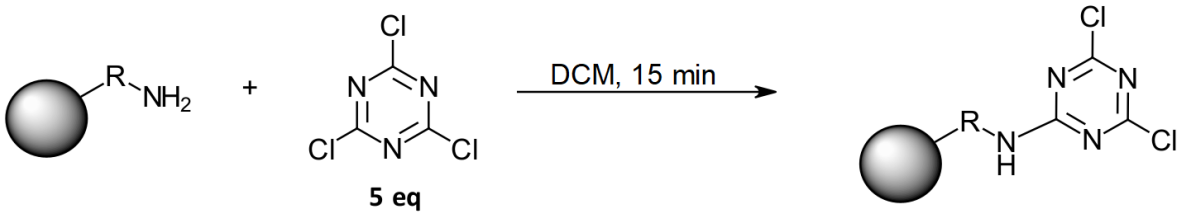


Scheme 3.1: Coupling hexylamine to 4-fluorobenzoic acid using Si-DCT

3.3 Polymer Bound DCT

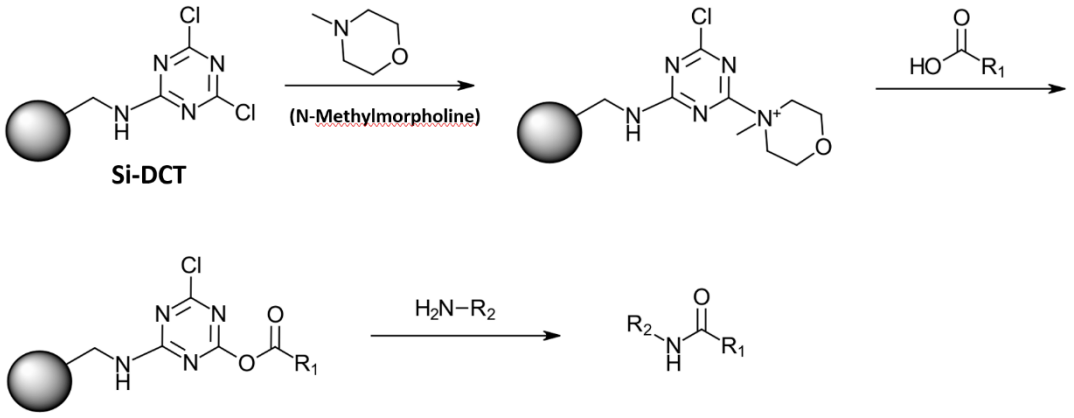
3.3.1 Synthesis and general amide coupling scheme

Unfortunately, Siliabond Si-DCT is no longer commercially available, but polymer bound DCT can be easily synthesized as described in Scheme 3.2 [17].



Scheme 3.2: A general procedure for the synthesis of polymer bound DCT from amine resins. The amine resin is stirred in a DCM solution of cyanuric chloride (5 equiv) for 15 minutes and is then filtered and rinsed with DCM.

To perform the amide coupling reaction (Scheme 3.3), the resin is first primed with N-methylmorpholine (NMM) and the carboxylic acid is loaded. The resin is then rinsed, and the amine is added to form the amide. The use of polymer bound Si-DCT as an amide coupling reagent is well covered but typically reaction times of 1-3 hours have been used [17].



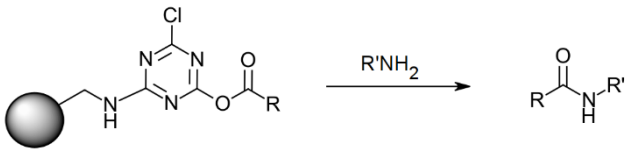
Scheme 3.3: A general amide coupling scheme using polymer bound DCT

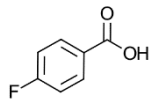
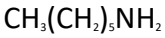
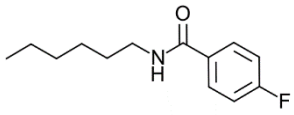
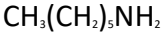
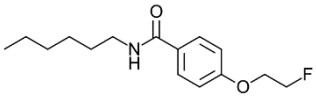
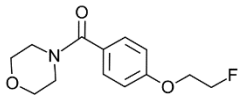
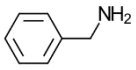
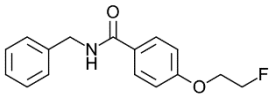
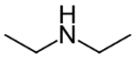
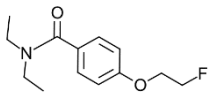
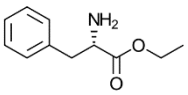
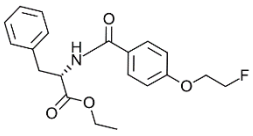
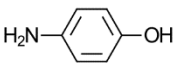
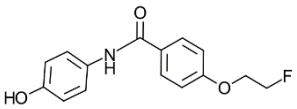
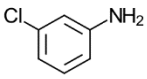
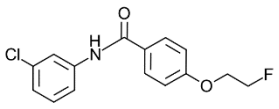
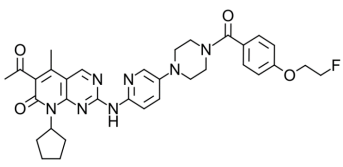
3.3.2 Flow through method for the synthesis of an amide library

To determine if polymer bound DCT can work for a range of compounds in a flow through reaction it was used to couple compound **3** to the amines in Table 3.1. Polymer bound DCT was generated through Scheme 3.2 using aminomethyl polystyrene resin (2.9 meq/g, Chem-Impex, catalog number: 05066) and was loaded into filter tubes (~0.50 g per tube). A solution of NMM (4 equiv.) in MeCN (2 mL) was flowed through followed by a 90°C solution of **3** (1 equiv.) in MeCN (2 mL). The filter tubes were rinsed with MeCN (4 mL) and a solution of the amine (1 equiv) in MeCN (2 mL) was flowed through and collected. Exceptions to this were L-phenylalanine ethyl ester which was flowed through in MeCN (1.9 mL) and water (0.1 mL) solution due to its insolubility in MeCN. Palbociclib (0.11 equiv.) was flowed through in 100°C DMF (3 mL) since product did not form unless the solution was heated and palbociclib had

low solubility. All formed clean products on TLC. The Yields shown in Table 3.1 are a corrected yield based on a theoretical maximum 20% loading of cyanuric chloride for this resin [17].

Table 3.1: Amides synthesized through amide coupling in a flow through reaction using polymer bound DCT.



Acid	Amine (R')	Product	Yield
 **4-Fluorobenzoic acid**	 **Hexylamine**		(**12**) 30%
3	 **Hexylamine**		(**13**) 21%
3	 **Morpholine**		(**14**) 21%
3	 **Benzylamine**		(**15**) 25%
3	 **Diethylamine**		(**16**) 15%
3	 **L-Phenylalanine ethyl ester**		(**17**) 10%
3	 **4-Aminophenol**		(**18**) 10%
3	 **3-Chloroaniline**		(**19**) 4%
3	**Palbociclib**		(**20**) 18% *Palbociclib was the limiting reagent

The yields in Table 3.1 show that the most reactive amines were hexylamine, morpholine and benzylamine with 21%, 21% and 25% yields respectively. Diethylamine showed a slightly lower yield of 15%, possibly due to increased steric hindrance from the ethyl groups that are not bound in a ring as they are in morpholine. When L-phenylalanine ethyl ester was used it resulted in a 10% yield, which is also likely due to steric hinderance and potentially from the small amount of water that was used to increase its solubility. Using 4-aminophenol and 3-chloroaniline resulted in a 10% and 4% yield respectively. The lone pair on the amine may be less available for nucleophilic attack due to delocalization into the benzene ring through resonance. The electron withdrawing effect of the *meta*-chlorine through induction may increase this effect resulting in the low yield. The least reactive compound was Palbociclib, which did not generate product when it was flowed through in a room temperature solution. This is likely due to steric hinderance from the large size of the compound.

3.3.3 Optimizing flow reaction conditions

In a flow through type reaction there is very little time for the reagents to interact, making it difficult achieve a high yield. In a radiosynthesis, the coupling resin and amine will be in large excess with respect to the amount of ^{18}F generated, but to be certain the best radiochemical yield is achieved, the optimal conditions for the flow through reaction were found. To do this, 4-fluorobenzoic acid was coupled to hexylamine using the procedure described in Chapter 3.3.2 while varying resins, solvent temperatures, and reaction times.

Amine Resin:

The ability of an Si-triamine resin (1.34 mmol/g, Silicycle, Product number: R48030B) was compared to the ability of an aminomethyl polystyrene resin (2.9 meq/g, Chem-Impex, catalog number: 05066) to generate the DCT resin. The Si-triamine resin has three active sites per molecule giving a loading of 4.02 mmol/g. The acid was flowed through at room temperature in MeCN. Both produced similar amounts of amide product (~ 14-16 mg of **12**) with the amount of coupling resins that could fit into a 1 mL filter tube (~0.53 g for each).

Preparing the resin:

Stirring the amine resin longer in cyanuric chloride (10 min vs 3 h) did not increase the yield. Stirring the resin in a solution of NMM for 30 minutes rather than using a flow through method did not improve the yield.

The carboxylic acid flow through:

Flowing through the 4-fluorobenzoic acid in a heated MeCN solution (82°C) increased the yield to ~20 mg of **12**.

Since the deprotection step will occur before the amide coupling step, the 4-fluorobenzoic acid was flowed through in 3:1 MeCN:3 M HCl (2 mL) heated to 82°C. This yielded only 1 mg

of product. It was found that neutralizing the acid by adding NMM (0.25 mL) before flowing the mixture through did not significantly improve the yield, only yielding 2 mg of product. This suggests the MeCN:HCl solution will need to be removed and the carboxylic acid redissolved in MeCN before flowing through the DCT resin in a radiosynthesis.

The amine flow through:

Amines are easily removed from solution as will be discussed in Chapter 3.4 which means a large excess can be used in a radiosynthesis. It was found palbociclib had to be flowed through in a heated solution to see the formation of product, but this was not required for any of the other amines used in Table 3.1. In another experiment, hexylamine was flowed through in a 1:1 water:MeCN mixture which generated 17 mg of **11** (with 4-fluorobenzoic acid flowed through in 82°C MeCN). This suggests that the reaction is not particularly sensitive to water so it can be used when solubility is an issue.

3.4 Removing the excess amine

To remove excess amine, a DOWEX sulfonic acid resin (50WX2 hydrogen form, Sigma Aldrich, SKU: 217441) and Si-tosic acid resin (0.62 meq/g, Silicycle, Product number: R60530B) were used. Each was packed into 1 mL filter tubes and various amounts of hexylamine was flowed through in MeCN. It was found that the Si-tosic acid resin completely removed the hexylamine (according to TLC and APCI-MS) when less than 0.75 equiv of hexylamine were used relative to the loading of the resin. The 1 mL volume of DOWEX resin removed a similar amount of amine but a small amount always made it through. This is likely because the DOWEX beads are larger and do not pack as tightly into the filter tubes. These resins were effective at removing all of the amines used in Table 3.1 except for Palbociclib since the amide also stuck to the resin. In a radiosynthesis, there is no limit to the amount of acid resin that can be added to the line leading to the final collection vial, so as much amine as needed can be used for the synthesis.

3.5 Conclusion

In this chapter, a solid phase amide coupling method was determined that works for a range of amine compounds and is suitable for radiosynthesis. In Chapter 3.2-3.3 it was found that polymer bound DCT is an amide coupling reagent that works quickly and generates a clean product (Scheme 3.3). Furthermore, it is easily generated in-lab by stirring an amine resin in a DCM solution of cyanuric chloride (Scheme 3.2). In the procedure used in this chapter, the coupling resin is loaded into a filter tube and the acid and amine is flowed through in MeCN, generating the amide. In Chapter 3.3 it was found that flowing the carboxylic acid through in 90°C MeCN increased the yield by ~30% compared to a room temperature solution. A library of amides was synthesized (Table 3.1) using this method which demonstrates the versatility of this reaction. In Chapter 3.4, it was found that the unreacted amine can be removed from solution by flowing it through a filter tube filled with

a tosic acid resin. This is better suited for radiosynthesis than a normal acid workup since it minimizes exposure to radiation.

In Chapter 4, this amide coupling procedure will be used in a radiosynthesis to couple [^{18}F]**3** to amine containing compounds.

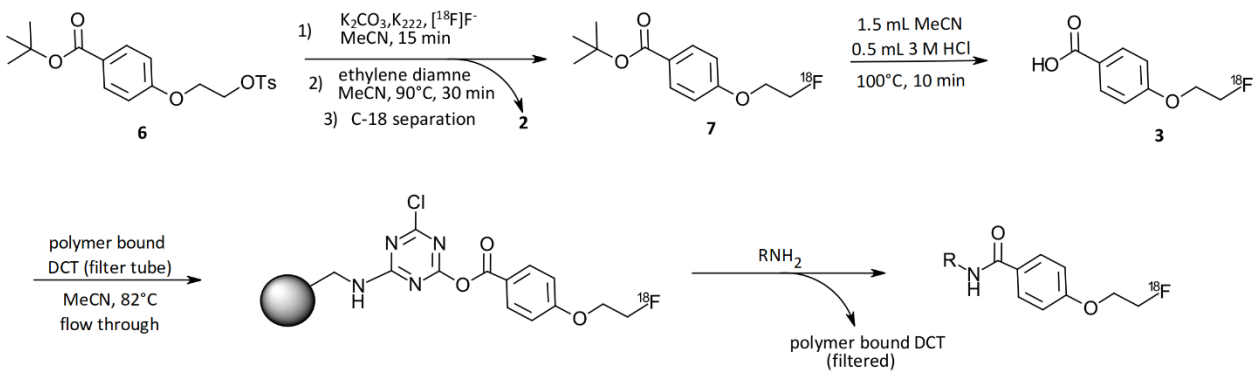
CHAPTER 4 – Radiosynthesis

4.1 Introduction

The objective of the work done in this chapter is to produce [^{18}F]**3** and couple it to various amines using the solid phase amide coupling procedure described in Chapter 3. The hot cell used contains a General Electric Healthcare TracerLab FXN-Pro synth box (Figure 1.2) and [^{18}F]F^- supplied by the Thunder Bay Regional Health Sciences Centre's ACSI TR-24 PET Cyclotron. This hot cell is designed for use in F-18 nucleophilic substitution reactions and provides two reactors and many reagent vials which allow for multistep synthesis.

4.2 Radiosynthesis: labeling amines with 3 through solid phase amide coupling

Scheme 4.1 summarizes the radiosynthesis method used in this project for coupling [^{18}F]**3** to amines.



Scheme 4.1: Radiosynthesis method for the indirect labeling of amine containing compounds with [^{18}F]**3** using solid phase coupling.

4.2.1 [F-18]fluoride anion exchange and drying

To begin the radiosynthesis, the aqueous [^{18}F]F^- solution from the cyclotron is sent through a quaternary methyl ammonium (QMA) anion exchange cartridge (Sep-Pak® Light QMA cartridge, Waters, SKU: 186004051) preconditioned with 0.5 M K_2CO_3 (10 mL) and water (20 mL). The [^{18}F]F^- is then eluted into the first reactor using a 1 mL mixture of K_2CO_3 (2.1 mg/mL in water) and Kryptofix 222 (11.8 mg/mL in MeCN) (15/85, v/v). Water was then removed from the reaction vessel through azeotropic distillation by applying a vacuum (~15-20 kPa) and heating to 70°C for 4 minutes. Anhydrous MeCN (2 mL) was then added, and the reaction vessel was dried again under the same conditions.

4.2.2 Radiofluorination and C18 Separation

The reaction vessel was cooled to 50°C and 5 mg of **6** dissolved anhydrous MeCN (1.5 mL) was added. Lowering the temperature prevents solvent loss when using a vacuum to pull the reagents into the reactor. The mixture was stirred at 90°C for 15 minutes, cooled to 60°C, and a solution of ethylene diamine (0.1 mL) in MeCN (0.4 mL) was added. The solution was stirred for 30 minutes at 90°C, the reactor was cooled to 30°C and water (4 mL) was added. This mixture was then sent through a C18 cartridge (preconditioned with MeCN (10 mL) and water (20 mL)) to waste. The C18 was then rinsed with 4:1 water:MeCN (5 mL) which was sent to waste. The C18 was then eluted into the second reactor with MeCN (1.5 mL) which yielded pure [^{18}F]**3** according to radio-TLC.

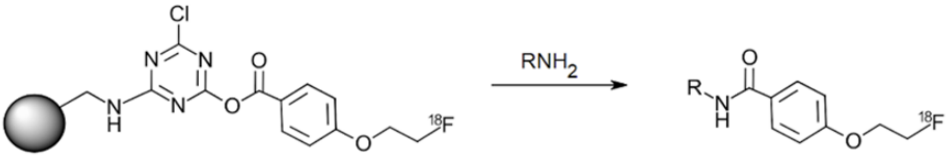
4.2.3 Deprotection

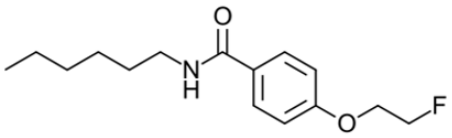
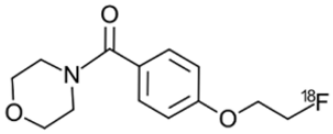
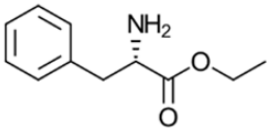
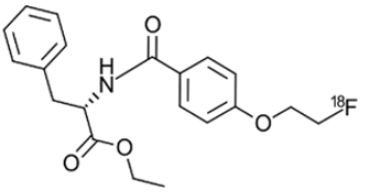
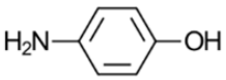
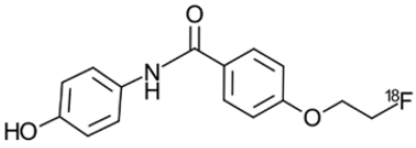
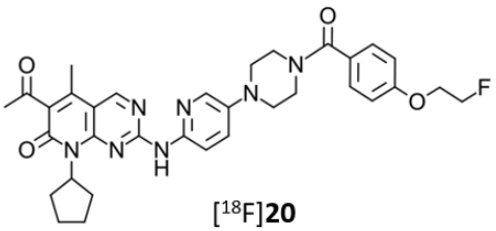
Deprotection was carried out by stirring[^{18}F]**7** in 3:1 MeCN:3 M HCl (2 mL) for 10 minutes which yielded 100% conversion to [^{18}F]**3** according to radio-TLC. The acidic solution was removed under vacuum (~10 kPa) at 50°C for 5 minutes. The lower temperature is used to minimize the amount of product lost due to evaporation.

4.2.4 Amide coupling

The [^{18}F]**3** was dissolved in anhydrous MeCN (2 mL) and heated to 82°C. This solution was then sent through a 1 mL filter tube filled with ~0.53 g of silica bound DCT preconditioned with a MeCN solution of NMM (4 equiv). The coupling resin used was synthesized through scheme 3.2 using Si-triamine resin (1.34 mmol/g, Silicycle, Product number: R48030B)**.** MeCN (2 mL) was added to the reactor, which was then heated to 82°C and sent through the DCT resin to waste. The resin was rinsed again with room temperature MeCN (6 mL). It was found that if the resin is not rinsed sufficiently [^{18}F]**3** will be present in the final product. A solution of the amine was then flowed through the coupling resin to produce the amides in table 4.1. The resulting solution was then run through two 1 mL filter tubes filled with DOWEX sulfonic acid resin (50WX2 hydrogen form, Sigma Aldrich, SKU: 217441) and then one filter tube filled with Si-tosic acid resin (0.62 meq/g, Silicycle, Product number: R60530B). This procedure was used to generate the amides in Table 4.1 and yielded pure amide products as determined by radio-TLC (some examples shown in Figure 4.1).

Table 4.1: Amines labeled with [^{18}F]**3** through solid phase coupling. Products were confirmed using radio-TLC and comparing the Rf to the cold product.



Amine solution	Amide
$CH_3(CH_2)_5NH_2$ **Hexylamine** 50 µL in MeCN (2 mL)	 [^{18}F]**13**
 **Morpholine** 50 µL in MeCN (2 mL)	 [^{18}F]**14**
 **L-Phenylalanine ethyl ester** 50 mg in 1.9 mL MeCN, 0.1 mL water	 [^{18}F]**17**
 **4-Aminophenol** 50 mg in MeCN (2 mL)	 [^{18}F]**18**
Palbociclib 20 mg in 90°C DMF (4 mL)	 [^{18}F]**20**

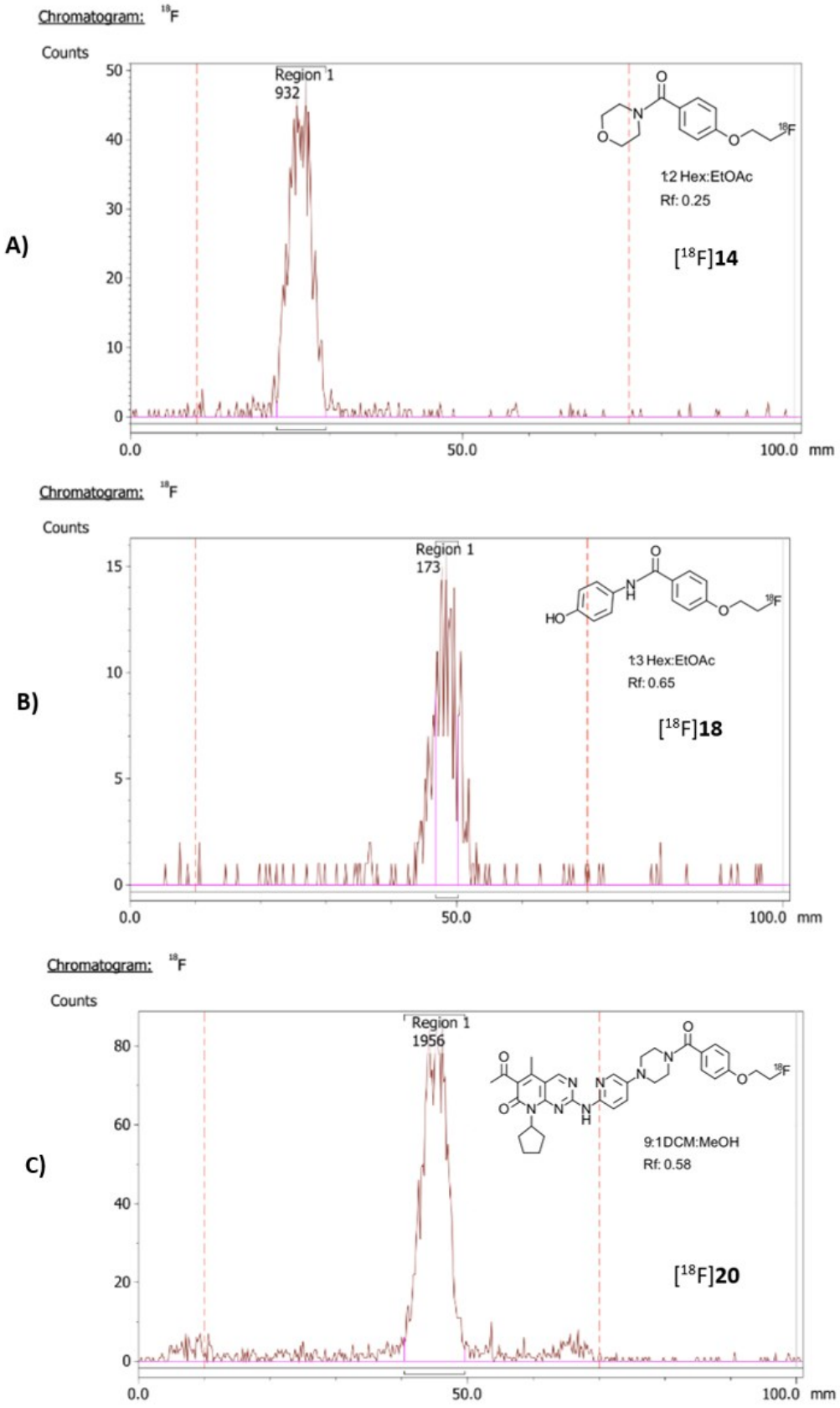


Figure 4.1: Radio-TLC of [^{18}F]**14** (**A**), [^{18}F]**18** (**B**), and [^{18}F]**20** (**C**).

4.3 Radiochemical yield

The a mount of ^{18}F used in the synthesis of the amides in Table 4.1 was approximately 0.5 GBq, which was too low to get a precise activity reading from the meters in the hot cell. To help determine the yield, a synthesis was performed starting with 3 GBq of activity and using a solution of morpholine (100 µL) in MeCN (3 mL) for the amide coupling reaction. The radiochemical yield was calculated using the activity reading in reactor 1 (Figure 4.2) after the F-18 was eluted from the QMA cartridge as the initial activity. The final product was collected in a vial outside of the hotcell, and the activity of the vial and total synthesis time were recorded. The decay corrected yield was approximately 3% showing a significant loss of ^{18}F. It is possible a significant amount of product is lost during the drying step after the deprotection (Chapter 4.2.3) and during the acid flow through. To determine whether eliminating the drying step would improve the yield, another run was done where the deprotection was performed before the C18 separation. The deprotected product was eluted from the C18 with MeCN and run through a filter tube of anhydrous $MgSO_4$ before being used in the coupling reaction. A similar yield of approximately 3% was achieved.

Through cold experiments it was found that if the HCl solution was not completely removed before the coupling reaction, it would reduce the yield by ~90% (when coupling 4-fluorobenzoic acid to hexylamine). This was seen when there was only a small drop of the acid solution remaining in the vial before redissolving in MeCN. This suggests that remaining acid could be the cause of the low yield. A significant amount may also be lost during due to incomplete fluorination and/or during the C18 separation.

4.4 Radiosynthesis procedure using TracerLab FXN-Pro synth box

To perform this coupling reaction the TracerLab FXN-Pro synth box was set up as shown in figure 4.2. Note that vial 2 in this figure is the container labeled tube 2 in the Tracerlab software (Figure 1.2). The full procedure is given in Figure 4.3.

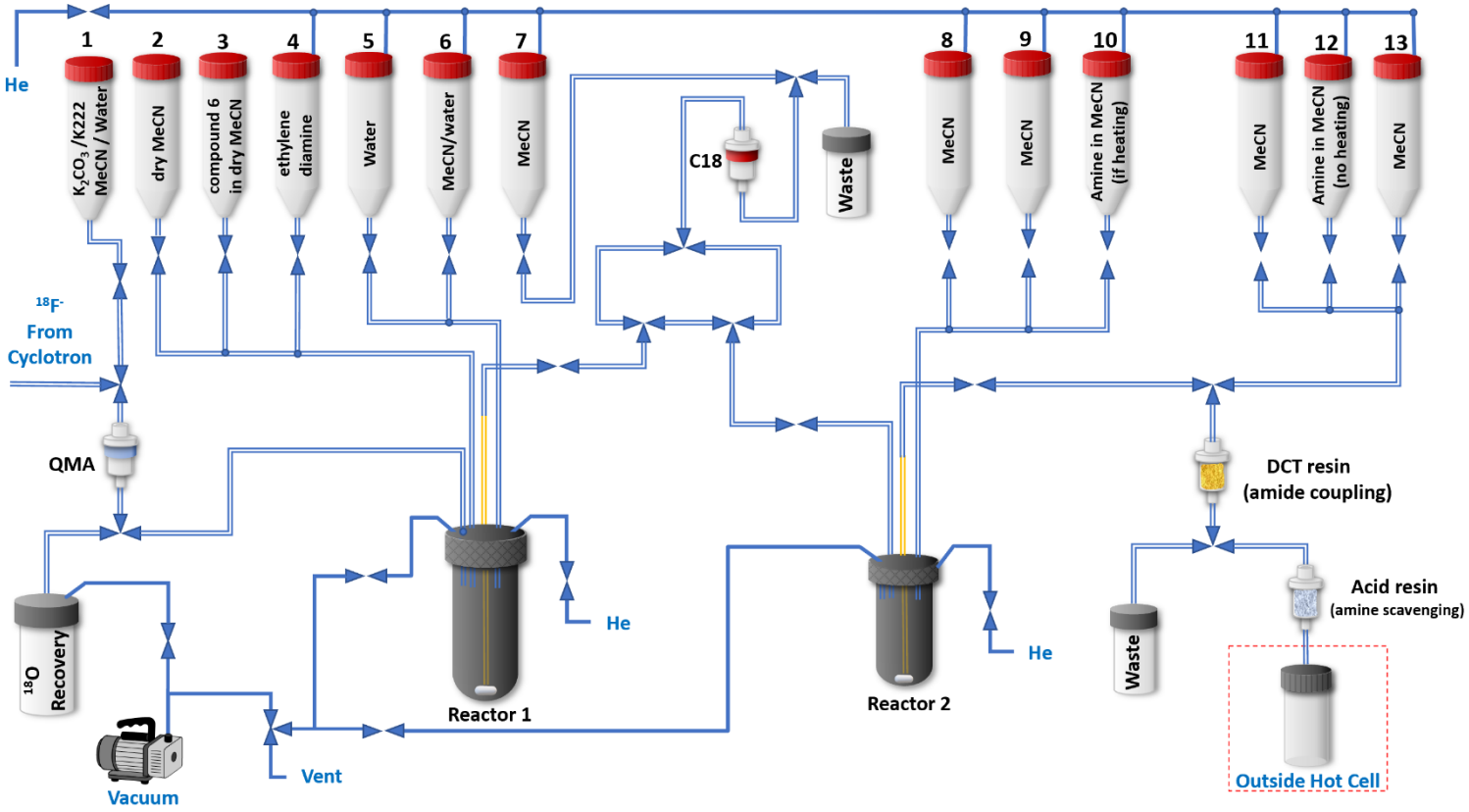


Figure 4.2: A simplified diagram of a Tracerlab FXN-Pro synth box set up for the indirect labeling of amines through solid phase amide coupling.

Figure 4.3: GUIDE FOR LABELING AMINES WITH [^{18}F]3 THROUGH SOLID PHASE AMIDE COUPLING
Synth box: GE Healthcare TracerLab FXN-Pro (figure 4.2)

Preparing the synth box

Load the vials:

1-	10 mg K_{222}, 0.32 mg K_2CO_3 in 1 mL MeCN/water (85/15, v/v)	**5-**	4 mL water	**10-**	amine solution (if heating)
		6-	4 mL water, 1 mL MeCN	**11-**	6 mL MeCN
2-	2 mL dry MeCN	**7-**	1.5 mL MeCN	**12-**	amine solution (if not heating)
3-	Tosylate (**6**) in 1.5 mL dry MeCN	**8-**	2 mL dry MeCN	**13-**	4 mL MeCN
4-	0.1 mL ethylenediamine in 0.4 mL dry ACN	**9-**	2 mL dry MeCN	**Reactor 2-**	0.5 mL 3.5 M HCl

Precondition SPE and resin cartridges:

QMA- 10 mL 0.5 M K_2CO_3, 20 mL water **C18-** 10 mL MeCN, 20 mL water
Polymer bound DCT (1 mL filter tube)- 3 mL MeCN solution of NMM (4 equiv).

Load cartridges into the synth box and attach acid resin filled filter tubes to the line connecting to the collection vial outside of the hot cell.

Synthesis

1. Send the aqueous [^{18}F]F^- solution from the cyclotron through the QMA to the ^{18}O recovery vial (vacuum).
2. Elute the [^{18}F]F^- into **reactor 1** using the solution in **vial 1** (vacuum).
3. Dry **reactor 1** under vacuum at 70°C for ~4 minutes.
4. Add the dry MeCN from **vial 2** to **reactor 1** (vacuum) and dry under vacuum at 70°C for ~4 minutes.
5. Cool the reactor to 50°C and add the compound **6** solution (**vial 3**) to **reactor 1** (vacuum). Stir at 90°C for 15 minutes.
6. Add the ethylenediamine solution in **vial 4** to **reactor 1** (He) and stir for 30 minutes at 90°C.
7. Cool **reactor 1** to 25°C and add the 4 mL of water (**vial 5**) to **reactor 1**.
8. Send this solution from through the **C18** cartridge to waste (He).
9. Add the water/MeCN solution from **vial 6** to **reactor 1** and then send it through the **C18** to waste (He).
10. Elute the **C18** into **reactor 2** using the 1.5 mL of MeCN in **vial 7** (He).
11. Stir the MeCN/3.5 M HCl solution in **reactor 2** for 10 minutes at 100°C.
12. Dry **reactor 2** under vacuum at 50°C.
13. Add the dry MeCN from **vial 8** to **reactor 2** (He), heat it to 82°C and send through the DCT tube to waste.
14. Add the dry MeCN from **vial 9** to **reactor 2** (He), heat it to 82°C and send through the DCT tube to waste.
15. Send the 6 mL MeCN from **vial 11** through the DCT tube to waste.
16. Room temperature flow through: Send the amine solution (**vial 12**) through the DCT tube and collect in the vial outside the hotcell. Hot flow through: Add the amine solution (**vial 10**) to **reactor 2** and heat to the desired temperature. Then send the solution through the DCT tube and collect outside the hot cell.

TIMING
Steps 1-4, 10 min; Steps 5 and 6, 45 min; Steps 7-10, 5 min; Steps 11 and 12, 15 mins; Steps 13-16, 5 min.
Total: 80 min.

4.5 Conclusion

In this chapter, the solid phase amide coupling procedure developed in Chapter 3 was used to couple [^{18}F]**3** to the amines in Table 4.1. The synthesis was complete in ~80 minutes, and the products produced clean peaks on radio-TLC. The issue with this procedure is the low radiochemical yield achieved of ~3% (decay corrected). Through cold experiments, it was found that the loss is likely due to any remaining HCl inhibiting the carboxylic acid from binding to the amide coupling resin. Adding a base did not improve the yield. Future work is required to improve the procedure to increase the radiochemical yield.

CHAPTER 5 – Conclusions and future work

The procedure developed in this project has been successfully used to indirectly radiolabel a variety of primary and secondary amines with ^{18}F through solid phase amide coupling. The synthesis is complete in 80 minutes and generates a clean product without the need for further purification. The ability of this method to be used for a range of compounds and produce pure products demonstrates the potential for polymer bound DCT to be used as an amide coupling reagent in radiosynthesis. The problem with the procedure presented here is the low radiochemical yield achieved (~3%). Through cold experiments it was found that any acid remaining from the deprotection step would significantly decrease the loading of the carboxylic acid onto the DCT resin (~90% lower yield). This is likely where a significant amount of product is being lost, with other loses coming from incomplete fluorination and potentially from the C18 separation. Future work is required to improve the radiochemical yield of this method. The yield of each step of the radiosynthesis should be found to determine with more certainty the source of the loss. A method should be developed to improve the loading of the acid onto the coupling resin after acid deprotection, or the synthesis should be redesigned to avoid this acid step. A coupling resin with a higher loading may improve yields, so other amine resins could also be attempted to achieve a higher DCT loading.

CHAPTER 6 – Experimental Methods

6.1 General Instrumentations and Methods

Reagents were purchased from Sigma-Aldrich, Fisher Scientific or Chem Impex and used without further purification.

Infrared (IR) spectra were obtained using a Perkin Elmer 1320 IR Spectrometer with a resolution of 1 cm^{-1}. All spectra were determined without solvent (neat) in the transmission mode with samples loaded onto NaCl plates and are reported as wavenumbers.

NMR spectra were collected on a 500 MHz Bruker Advance Neo spectrometer with SmartProbe at room temperature and processed using Bruker Topspin 4 software. Chemical shifts are reported in parts per million (ppm) from an internal standard of tetramethylsilane (TMS). The NMR data are reported as follows: chemical shift multiplicity, coupling constant in Hertz, integration).

Mass spectral data was obtained using an Advion Expression CMS spectrometer operated in atmospheric pressure chemical ionization mode (APCI) unless otherwise specified.

6.2 Chapter 2 Experimental Procedures

6.2.1 General procedures

SPE cartridge separations

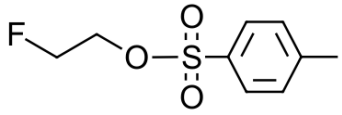
Using Sep-Pak Plus C18 Cartridges (Waters, SKU: WAT020515)

The C18 cartridges were preconditioned by flowing through MeCN (10 mL) and DI water (20 mL). Solutions ranging from 4:1 MeCN:water to 1:9 MeCN:water were used to dissolve the desired compounds (usually less than 10 mg). The solution was flowed through and collected. The cartridge was then rinsed with the same or different solvent ratio and collected. Any product remaining on the cartridge could then be eluted with MeCN (normally ~2 mL).

Using Alumina B Cartridges (Waters, SKU: WAT020505)

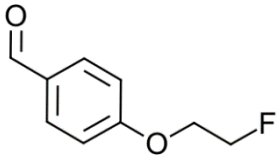
The Alumina B cartridges were preconditioned by flowing through hexanes (4 mL). Solutions ranging from pure hexane to 1:4 hexane:DCM were used as eluent to dissolve the desired compounds (usually less than 10 mg). The solution was flowed through and collected. The cartridge was then rinsed with an appropriate ratio and collected. Any product remaining on the cartridge could be eluted with DCM (normally ~2 mL).

6.2.2 Synthesized Compounds



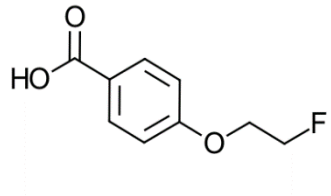
2-Fluoro-1-(4-methylphenylsulfonyloxy)ethane (1)

2-Fluoroethanol (1.82 mL, 31.2 mmol), Ts-Cl (9.70 g, 50.9 mmol) and DMAP (0.545 g, 4.45 mmol) were added to a 200 mL RBF and dissolved in DCM (100 mL). DIPEA (9 mL, 51.7 mmol) was then added, and the mixture was stirred at room temperature for 6h. The solution was then washed with 10% HCl (50 mL x1), water (50 mL x1), and sat. NaCl (50 mL x1). The DCM layer was dried with Na_2SO_4 (anhydrous) and the solvent was removed using a rotary evaporator. The product was then purified using column chromatography (1:2 hex:EtOAc) yielding **1** (4.535 g, 20.8 mmol, 66.6% yield) as a yellow oil. IR (neat) ν_{max}/cm^{-1}: 2960, 1598, 1359, 1179; ^1H NMR (500 MHz, $CDCl_3$) δ_H 7.80 (d, J = 8.4 Hz, 2H), 7.35 (d, J = 8.0 Hz, 2H), 4.63 (dt, $J_{H,F}$ = 47.0 Hz, J = 4.0 Hz, 2H) , 4.30 (dt, $J_{H,F}$ = 27.1 hZ, J = 4.0 Hz, 2H), 2.45 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 145.2, 132.7, 130.0, 128.0, 81.2 (d, $J_{C,F}$ = 173.6 Hz), 68.6 (d, $J_{C,F}$ = 21.1 Hz), 21.7; APCI-MS m/z $[M + H]^+$ calc'd for $C_9H_{11}FO_3S$+H: 219.0; found: 218.9 (93%), 236.0 ($[M+NH_4]^+$, 100%).



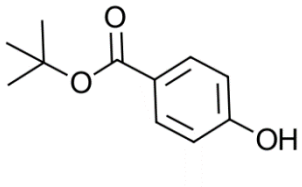
4-(2-Fluoroethoxy)benzaldehyde (2)

1 (2.07g, 9.48 mmol), 4-hydroxybenzaldehyde (2.97g, 24.3 mmol) and K_2CO_3 (3.96 g, 28.7 mmol) were added to a 100 mL RBF. Acetonitrile (50 mL) was then added, and the resulting slurry was refluxed for 6h. The solvent was removed using a rotary evaporator and redissolved in EtOAc (60 mL). The mixture was washed with water (40 mL x1), 10% NaOH (40 mL x3), 10% HCl (40 mL x1), water (40 mL x1) and the EtOAc layer was dried with anhydrous Na_2SO_4. The product was then purified using column chromatography (2:1 hex:EtOAc), yielding **2** (1.02 g, 6.07 mmol, 64 % yield) as a white solid. m.p. 53-54°C; IR (neat) ν_{max}/cm^{-1}: 2937, 1678, 1608; ^1H NMR (500 MHz, $CDCl_3$) δ_H 9.90 (s, 1H), 7.85 (d, J = 9.0 Hz, 2H), 7.05 (d, J = 8.7 Hz, 2H), 4.79 (dt, $J_{H,F}$ = 47.3 Hz, J = 4.2 Hz, 2H), 4.31 (dt, $J_{H,F}$ = 27.5 Hz, J = 4.0 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 190.8, 163.4, 132.0, 130.4, 114.9, 81.5 (d, $J_{C,F}$ = 171.7 Hz), 67.3 (d, $J_{C,F}$ = 19.8 Hz). APCI-MS m/z $[M + H]^+$ calc'd for $C_9H_9FO_2$+H: 169.1; found: 169.1.



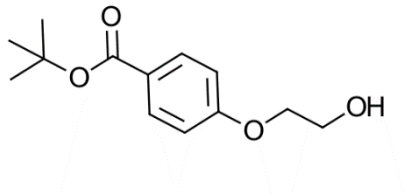
4-(2-Fluoroethoxy)benzoic acid (3)

2 (0.409 g, 2.43mmol), $KMnO_4$ (0.682 g, 4.32 mmol) and NaOH (0.242 g, 6.05 mmol) were added to a 20 mL vial and dissolved in water (16 mL). The vial was capped and stirred at 90°C for 30 minutes. The solution was poured into a separatory funnel and 10% HCl was added (50 mL). The product was extracted with EtOAc (40 mL x3). The EtOAc layer was then washed with 10% HCl (20 mL x1) and the solvent was removed using a rotary evaporator yielding **3** (0.349 g, 1.90 mmol, 78% yield) as white crystals. m.p. 202.8-203.8°C; IR (neat) ν_{max}/cm^{-1}: 2966, 2940br, 1672, 1607 ^1H NMR (500 MHz, $(CD_3)_2SO$) δ_H 12.65 (br, 1H), 7.89 (d, J = 9.1 Hz, 2H), 7.04 (d, J = 9.0 Hz, 2H), 4.77 (dt, $J_{H,F}$ = 47.6 Hz, J = 3.7 Hz, 2H), 4.33 (dt, $J_{H,F}$ = 30.1 Hz, J = 4.1 Hz, 2H); ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ_C 167.4, 162.2, 131.8, 123.8, 114.8, 82.5 (d, $J_{C,F}$ = 166.6 Hz), 67.7 (d, $J_{C,F}$ = 18.9 Hz); APCI-MS m/z $[M + H]^+$ calc'd for $C_9H_9FO_3$+H: 185.1; found: 185.0.



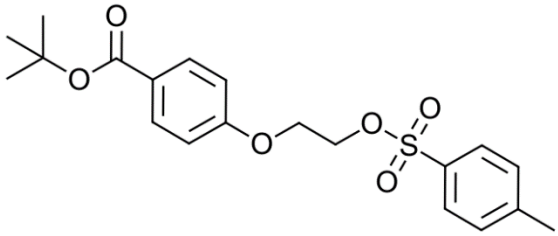
tert-Butyl 4-hydroxybenzoate (4)

4-Hydroxybenzoic acid (2.506 g, 18.14 mmol), N,N'-Dicyclohexylcarbodiimide (4.304 g, 20.86 mmol), DMAP (0.301 g, 2.46 mmol) and tert-butanol (10.805 g, 145.8 mmol) were added to a 100 mL RBF and dissolved in DCM (50 mL). The resulting slurry was stirred at room temperature overnight (~12 h). Solids were filtered out using gravity filtration and rinsed with DCM (15 mL x3). The solvent was removed using a rotary evaporator, and the product was purified using column chromatography (2:1 hex:EtOAc) yielding **4** (0.4274 g, 2.20 mmol, 12.1% yield) as a white solid. m.p. 133-135°C; IR (neat) ν_{max}/cm^{-1}: 3310br, 2980, 1677, 1608; ^1H NMR (500 MHz, $CDCl_3$) δ_H 7.90 (d, J = 8.9 Hz, 2H), 6.82 (d, 8.9 Hz, 2H), 5.21 (s, 1H), 1.58 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 165.6, 159.2, 131.7, 124.8, 114.9, 80.7, 28.3; APCI-MS m/z $[M + H]^+$ calc'd for $C_{11}H_{14}O_3$+H: 195.1; found: 139.0 ($[M + H]^+ - C_4H_8$).



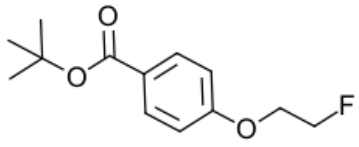
tert-Butyl 4-(2-hydroxyethoxy)benzoate (5)

4 (0.784 g, 4.04 mmol), 2-bromoethanol (1.60 mL, 22.6 mmol), and K_2CO_3 (3.501 g, 25.3 mmol) were added to a 100 mL RBF. Acetonitrile (60 mL) was then added, and the resulting slurry was refluxed for 6 h. The solvent was removed using a rotary evaporator, and EtOAc (60 mL) was added to the flask. The EtOAc was washed with 10% HCl (50 mL x1), 10% NaOH (50 mL x3), water (50 mL x1) and the EtOAc layer was dried with anhydrous Na_2SO_4. The solvent was removed by a rotary evaporator and the product was purified using column chromatography (2:1 hex:EtOAc) yielding **5** (0.5604 g, 2.35 mmol, 58% yield) as a clear viscous oil. IR (neat) ν_{max}/cm^{-1}: 3446, 2976, 1697, 1606; ^1H NMR (500 MHz, $CDCl_3$) δ_H 7.93 (d, J = 9.1 Hz, 2H), 6.90 (d, J = 9.1 Hz, 2H), 4.13 (t, J = 4.6 Hz, 2H), 3.98 (dt, J = 4.9, 4.9 Hz, 2H), 2.10 (t, J = 6.1, 1H), 1.58 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 165.5, 162.0, 131.4, 124.9, 113.9, 80.6, 69.3, 61.3, 28.3. APCI-MS m/z $[M + H]^+$ calc'd for $C_{13}H_{18}O_4$+H: 239.128; found: 183.0 ($[M + H]^+$ - C_4H_8).



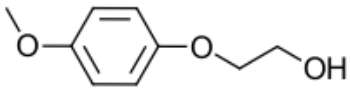
tert-Butyl 4-[2-(4-methylphenylsulfonyloxy)ethoxy]benzoate (6)

5 (0.519 g, 2.18 mmol), TsCl (2.32 g, 12.2 mmol) and DMAP (0.029 g, 0.24 mmol) were added to a 50 mL RBF and dissolved in DCM (30 mL). DIPEA (2.20 mL, 12.6 mmol) was then added, and the solution was stirred at room temperature overnight (~12h). The mixture was then washed with 10% HCl (40 mL x2), 10% NaOH (40 mL x1), sat. NaCl (40 mL x1) and then dried with anhydrous Na_2SO_4. The solvent was removed using a rotary evaporator and the product was purified using column chromatography (3:1 hex:EtOAc) yielding **6** (0.772 g, 1.97 mmol, 90.3% yield) as a white solid. m.p. 82-85°C; IR (neat) ν_{max}/cm^{-1}: 2977, 2931, 1705, 1606, 1362, 1176; ^1H NMR (500 MHz, $CDCl_3$) δ_H 7.89 (d, J = 9.1 Hz, 2H), 7.80 (d, J = 8.4 Hz, 2H), 7.33 (d, J = 8.1 Hz, 2H), 6.76 (d, J = 9.0 Hz, 2H), 4.38 (t, J = 5.0 Hz, 2H), 4.18 (t, J = 4.7 Hz, 2H), 2.45 (s, 3H), 1.58 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 165.4, 161.26, 145.0, 132.8, 131.4, 129.9, 128.0, 125.2, 113.9, 80.7, 67.8, 65.4, 28.2, 21.7. APCI-MS m/z $[M + H]^+$ calc'd for $C_{20}H_{24}O_6S$+H: 393.1; found: 319.2 ($[M-C_4H_9O]^+$, 100%), 337.2 ($[M + H]^+$ - C_4H_8, 17%), 355.2 ($[M + NH_4]^+$ - C_4H_8, 22%), 410.3 ($[M+NH_4]^+$, 15%).



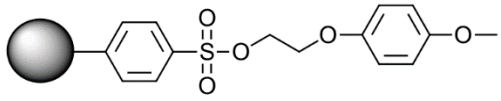
tert-Butyl 4-(2-fluoroethoxy)benzoate (7)

6 (0.082 g, 0.209 mmol), Kryptofix 222 (0.086 g, 0.230 mmol), and KF (0.021 g, 0.360 mmol) were added to a 20 mL vial and dissolved in anhydrous MeCN (6 mL). The solution was heated to 90°C and stirred for 15 min. The solvent was then removed using a rotary evaporator and the mixture was redissolved in EtOAc (10 mL). The mixture was then washed with 10% HCl (10 mL x1), 10% NaOH (10 mL x1), sat. NaCl (10 mL x1) and then dried with anhydrous Na_2SO_4. The product was purified using column chromatography (3:1 hex:EtOAc) yielding **7** (0.46 g, 0.192 mmol, 92% yield) as a white solid. m.p. 53-55°C; IR (neat) ν_{max}/cm^{-1}: 2977, 1703, 1607; ^1H NMR (500 MHz, $CDCl_3$) δ_H 7.96 (d, J = 8.8 Hz, 2H), 6.93 (d, J = 8.8 Hz, 2H), 4.77 (dt, $J_{H,F}$ = 47.4 Hz, J = 4.1 Hz, 2H), 4.26 (dt, $J_{H,F}$ = 27.6 Hz, J = 4.3 Hz, 2H), 1.58 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 165.5, 161.7, 131.4, 125.1, 114.0, 81.8 (d, $J_{C,F}$ = 171.1 Hz), 80.6, 67.1 (d, $J_{C,F}$ = 20.2 Hz), 28.2; APCI-MS m/z $[M + H]^+$ calc'd for $C_{13}H_{17}FO_3$+H: 241.1; found: 241.0 (20%), 184.9 ($[M + H]^+$ - C_4H_8, 100%).



2-(4-Methoxyphenoxy)ethanol (8)

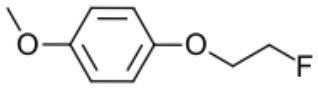
4-methoxyphenol (0.155 g, 1.24 mmol), 2-bromoethanol (0.50 mL, 7.04 mmol), and K_2CO_3 (0.341 g,2.48 mmol) were added to a 20 mL vial and dissolved in MeCN (12 mL). The vial was sealed, and the mixture was stirred at 90°C for 5 h. The solvent was then removed, and the mixture was redissolved in EtOAc (12 mL). The EtOAc was washed with 10% HCl (8 mL x1), 10% NaOH (8 mL x1), water (8 mL x1) and dried with anhydrous Na_2SO_4. The product was purified using column chromatography (2:1 hex:EtOAc) yielding **8** (0.059 g, 0.35 mmol, 28% yield) as a white solid. m.p. 67-68.5°C; IR (neat) ν_{max}/cm^{-1}: 3283br, 2953, 2930, 1513; ^1H NMR (500 MHz, $CDCl_3$) δ_H 6.85 (m, 4H), 4.04 (t, J = 4.5 Hz, 2H), 3.94 (dt, J = 5.1, 5.1 Hz, 2H), 3.77 (s, 3H), 2.02 (t, J = 6.3 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 154.1, 152.7, 115.6, 114.7, 69.9, 61.6, 55.7; APCI-MS m/z $[M + H]^+$ calc'd for $C_9H_{12}O_3$+H: 169.1; found: 168.9.



Polymer bound 4-methoxy-1-[2-(phenylsulfonyloxy)ethoxy]benzene (9)

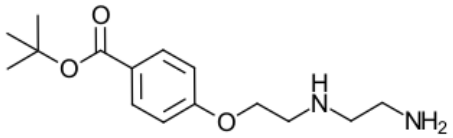
To a 20 mL vial, were added 0.532 g of polymer bound sulfonyl chloride resin (1.5-2.0 mmol/g, Sigma Aldrich, SKU: 498211)(~1 mmol), and **8** (1.020 g, 6 mmol). This mixture was then

dissolved in DCM (3 mL) and pyridine was added (2 mL, 24.8 mmol). The solution was stirred overnight (~10 h). The resin was then filtered and rinsed with DCM (4 x 10mL).



1-(2-Fluoroethoxy)-4-methoxybenzene (10)

4-Methoxyphenol (0.452 g, 3.64 mmol), **1** (0.257 g, 1.18 mmol), and K_2CO_3 (0.605 g, 4.38 mmol) were added to a 100 mL RBF and dissolved in MeCN (40 mL). The solution was refluxed for 5 h. The solvent was then removed, and the mixture was redissolved in EtOAc (60 mL). The EtOAc was washed with 10% HCl (50 mL x1), 10% NaOH (50 mL x3), water (50 mL x1) and dried with anhydrous Na_2SO_4. The solvent was removed using a rotary evaporator and the product was purified using column chromatography (2:1 hex:EtOAc) yielding **8** (0.126 g, 0.74 mmol, 63% yield) as a white solid. m.p. 55-57°C; IR (neat) ν_{max}/cm^{-1}: 2959, 2927, 2840; ^1H NMR (500 MHz, $CDCl_3$) δ_H 6.86 (m, 4H), 4.74 (dt, $J_{H,F}$ = 47.5 Hz, J = 4.2 Hz, 2H), 4.16 (dt, $J_{H,F}$ = 27.9 Hz, J = 4.2 Hz, 2H), 3.77 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 154.3, 152.6, 115.8, 114.7, 82.1 (d, $J_{C,F}$ = 170.2 Hz), 67.9 (d, $J_{C,F}$ = 20.2 Hz), 55.7; APCI-MS m/z $[M + H]^+$ calc'd for $C_9H_{11}FO_2$+H: 171.1; found: 171.0.

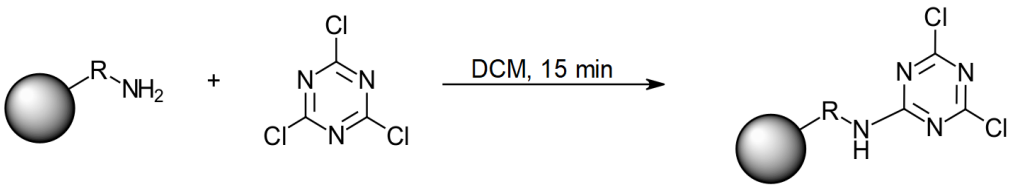


tert-Butyl 4-[2-(2-aminoethylamino)ethoxy]benzoate (11)

Compound **6** (15 mg, 0.054 mmol) and ethylene diamine (0.10 mL, 1.85 mmol) were added to a 20 mL vial and dissolved in MeCN (2 mL). The vial was sealed, and the mixture was stirred at 90°C. The reaction was complete at 25 minutes according to TLC. The solvent was then removed using a rotary evaporator and the mixture was redissolved in EtOAc (10 mL). The product was extracted using 10% HCl (1x10 mL). The HCl was then basified using NaOH pellets to pH ~12 (using pH paper). The product was then extracted with EtOAc (3x 10 mL), and the solvent was removed. This yielded **11** (4 mg, 0.014 mmol, 26% yield) as a clear viscous oil; IR (neat) ν_{max}/cm^{-1}: 3303br, 2976, 2932, 1706, 1606; ^1H NMR (500 MHz, $CDCl_3$) δ_H 7.94 (d, J = 8.7 Hz, 2H), 6.90 (d, J = 9.0 Hz, 2H), 4.12 (m, 2H), 3.04 (t, J = 5.1 Hz, 2H), 2.86 (t, J = 5.9 Hz, 2H), 2.77 (t, J = 5.7 Hz, 2H), 1.97 (br, 3H), 1.58 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 165.6, 162.2, 131.4, 124.7, 113.9, 80.5, 67.6, 52.0, 48.5, 41.5, 28.3.

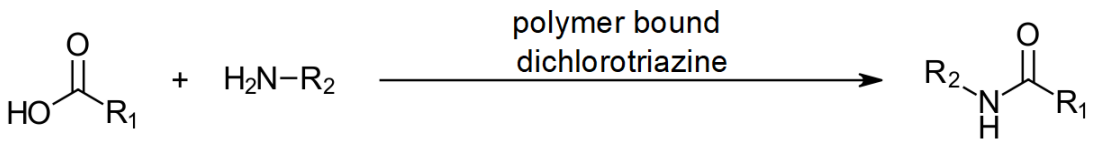
6.3 Chapter 3 Experimental Procedures

6.3.1 General Procedures



Polymer bound dichlorotriazine (DCT)

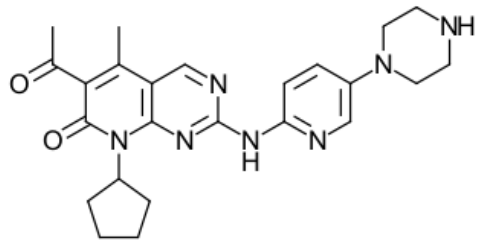
Amine resin (1 eq) and cyanuric chloride (4 eq) were added to an RBF and stirred in DCM (10 mL per gram of cyanuric chloride) for 15 minutes. The resin was filtered and washed several times with DCM. Once dry, the resin could be stored for several days at 4°C.



Amide synthesis using polymer bound dichlorotriazine

Polymer bound DCT was generated using aminomethyl polystyrene resin (2.9 meq/g, Chem-Impex, catalog number: 05066) and was loaded into filter tubes (0.50 g per tube, 0.29 mmol assuming 20% DCT loading [17]). A solution of NMM (0.148 mL, 1.16 mmol) in MeCN (2 mL) was flowed through followed by a 90 °C solution of **3** (50 mg, 0.29 mmol) in MeCN (2 mL). The filter tubes were rinsed with MeCN (4 mL). Finally, a solution of the amine (0.29 mmol) in MeCN (2 mL) was flowed through and collected. This solution was washed with 10% HCl (2 mL x2), 10% NaOH (2 mL x1), and water (2 mL x1) using a Pasteur pipette to remove the aqueous layer from the vial each time. The solution was dried with anhydrous Na_2SO_4 and the solvent removed by a rotary evaporator. If necessary, the compound was purified using column chromatography.

*THF, DMF, ACN were also successfully used as solvents for this reaction, and ACN:water mixtures (up to 1 : 1) were successfully used as a solvent for the amine flow through step.

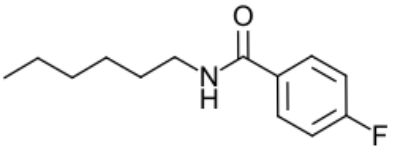


Palbociclib

IUPAC name: 6-acetyl-8-cyclopentyl-5-methyl-2-{[5-(1-piperazinyl)-2 pyridinyl]amino}pyrido[2,3-d]pyrimidin-7(8H)-one

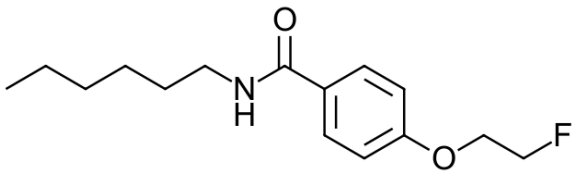
One capsule of palbociclib (Pfizer Ibrance 125 mg capsule) was cut open. The capsule contents were poured into a 100 mL beaker containing 2.5% HCl (40 mL) and stirred. The mixture was gravity filtered, washing with 2.5% HCl (40 mL). The filtrate was basified to pH = 10 using 10% NaOH and extracted with DCM (5 x 20 mL). The combined DCM layer was washed with sat. NaCl (30 mL), dried (Na_2SO_4) and concentrated under vacuum to give the free base of palbociclib as a yellow solid (0.110 g, 0.25 mmol). m.p. 252-255°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.81 (s, 1H), 8.17 (d, J = 8.9 Hz, 1H), 8.11 (br, 1H), 8.05 (d, J = 2.8 Hz, 1H), 7.34 (dd, J = 9.2, 2.9 Hz, 1H), 5.91 (quint, J = 8.9 Hz, 1H), 3.15 (m, 4H), 3.08 (m, 4H), 2.55 (s, 3H), 2.37 (s, 3H), 2.36 (m, 2H), 2.07 (m, 2H), 1.88 (m, 2H), 1.70 (m, 2H), 1.59 (br s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 202.7, 161.4, 158.1, 157.2, 155.5, 144.8, 144.2, 141.8, 136.6, 130.7, 125.8, 113.6, 107.7, 54.0, 50.7, 46.0, 31.5, 28.1, 25.8, 14.0. APCI-MS m/z calc'd for $C_{24}H_{29}N_7O_2$+H: 448.2; found: 448.2.

6.3.2 Synthesized Amides



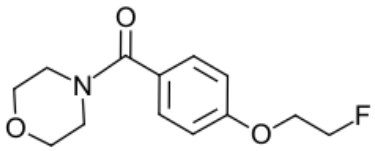
N-Hexyl-4-fluorobenzamide (12)

Refer to chapter 6.3.1 for the full procedure. 4-fluorobenzoic acid was used as the acid and hexylamine was used as the amine. Obtained **12** (19 mg, 0.087 mmol, 30% yield) as a pale-yellow solid. m.p. 40-43°C; IR (neat) ν_{max}/cm^{-1}: 3315br, 2930, 2858, 1638; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.77 (dd, J = 8.6 Hz, $J_{H,F}$ = 5.4 Hz, 2H), 7.09 (dd, $J_{H,F}$ = 8.8 Hz, J = 8.8 Hz, 2H), 6.17 (br, 1H), 3.43 (dt, J = 12.9, 7.2 Hz, 2H), 1.60 (quint, J = 7.4 Hz, 2H), 1.40-1.30 (m, 6H), 0.89 (t, J = 7.2 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.5, 164.6 (d, $J_{C,F}$ = 251.3 Hz), 131.1 (d, $J_{C,F}$ = 2.9 Hz), 129.1 (d, $J_{C,F}$ = 8.6 Hz), 115.5 (d, $J_{C,F}$ = 22.5 Hz), 40.2, 31.5, 29.6, 26.7, 22.6, 14.0; APCI-MS m/z $[M + H]^+$ calc'd for $C_{13}H_{18}FNO$+H: 224.1; found: 224.0.



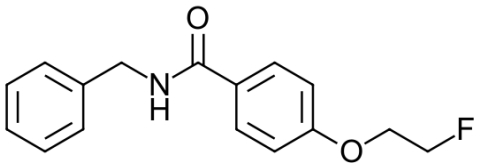
N-Hexyl-4-(2-fluoroethoxy)benzamide (13)

Refer to chapter 6.3.1 for the full procedure. Compound **3** was used as the acid and hexylamine was used as the amine. Purified using column chromatography (1:2 hex:EtOAc) to yield **13** (16 mg, 0.061 mmol, 21% yield) as a white solid. m.p. 98-100°C; IR (neat) v_{max}/cm^{-1}: 3289, 2958, 2926, 2855, 1635; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.72 (d, J = 8.9 Hz, 2H), 6.96 (d, J = 8.8 Hz, 2H), 6.02 (br, 1H), 4.78 (dt, $J_{H,F}$ = 47.3 Hz, J = 4.2 Hz, 2H), 4.26 (dt, $J_{H,F}$ = 27.5 Hz, J = 4.2 Hz, 2H), 3.43 (dt, J = 13.3, 7.0 Hz, 2H), 1.59 (quint, J = 7.4 Hz, 2H), 1.41-1.30 (m, 6H), 0.89 (t, J = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.8, 160.8, 128.7, 127.8, 114.3, 81.8 (d, $J_{C,F}$ = 171.2 Hz), 67.1 (d, $J_{C,F}$ = 20.7 Hz), 40.1, 31.5, 29.7, 26.7, 22.6, 14.0; APCI-MS m/z $[M + H]^+$ calc'd for $C_{15}H_{22}FNO_2$+H: 268.2; found: 268.0.



[4-(2-Fluoroethoxy)phenyl](morpholin-4-yl)methanone (14)

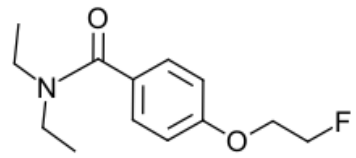
Refer to chapter 6.3.1 for the full procedure. Compound **3** was used as the acid and morpholine was used as the amine. Purified by running through a column of silica (12:1 DCM:MeOH) to yield **14** (15 mg, 0.061 mmol, 21% yield) as a white solid. m.p. 71-73°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.39 (d, J = 8.8 Hz, 2H), 6.94 (d, J = 9.0 Hz, 2H), 4.77 (dt, $J_{H,F}$ = 47.5 Hz, J = 4.0 Hz, 2H), 4.25 (dt, $J_{H,F}$ = 27.6 Hz, J = 4.1 Hz, 2H), 3.70 (br, 8H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.3, 159.7, 129.3, 128.0, 114.5, 81.7 (d, $J_{C,F}$ = 171.4 Hz), 67.2 (d, $J_{C,F}$ = 20.8 Hz), 69.9. APCI-MS m/z $[M + H]^+$ calc'd for $C_{13}H_{16}FNO_3$+H: 254.1; found: 254.0.



N-Benzyl-4-(2-fluoroethoxy)benzamide (15)

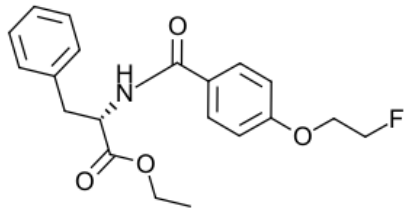
Refer to chapter 6.3.1 for the full procedure. Compound **3** was used as the acid and benzylamine was used as the amine. Purified by running through a column of silica (1:2 hex:EtOAc) to yield **15** (20 mg, 0.073 mmol, 25% yield) as a white solid. m.p. 123-125.5°C; IR (neat) v_{max}/cm^{-1}: 3254br, 2922, 1635; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.75 (d, J = 9.0 Hz, 2H), 7.34 (m, 4H), 7.29 (m, 1H), 6.93 (d, J = 8.9 Hz, 2H), 6.38 (br, 1H), 4.77 (dt, $J_{H,F}$ = 47.5 Hz, J = 4.1 Hz, 2H), 4.62 (d, J = 5.7 Hz, 2H), 4.25 (dt, $J_{H,F}$ = 27.7 Hz, J = 4.2 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.7, 161.0, 138.3, 128.85, 128.77, 127.9, 127.6, 127.3, 114.4, 81.8 (d, $J_{C,F}$ = 172.2

Hz), 67.2 (d, $J_{C,F}$ = 19.8 Hz), 44.1; APCI-MS m/z $[M + H]^+$ calc'd for $C_{16}H_{16}FNO_2$+H: 274.1; found: 274.3.



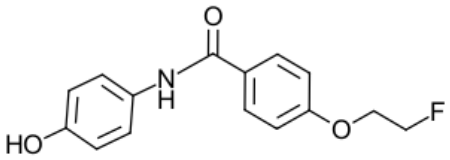
N,N-Diethyl-4-(2-fluoroethoxy)benzamide (16)

Refer to chapter 6.3.1 for the full procedure. Compound **3** was used as the acid and diethylamine was used as the amine. Purified by running through a column of silica (1:2 hex:EtOAc) to yield **16** (10 mg, 0.042 mmol, 15% yield) as a clear oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.34 (d, J = 8.8 Hz, 2H), 6.92 (d, J = 8.7 Hz, 2H), 4.77 (dt, $J_{H,F}$ = 47.5 Hz, J = 4.1 Hz, 2H), 4.25 (dt, $J_{H,F}$ = 27.8 Hz, J = 4.2 Hz, 2H), 3.34 (br, 4H), 1.18 (br, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 171.1, 159.1, 130.2, 128.3, 114.3, 81.8 (d, $J_{C,F}$ = 168.2 Hz), 67.1 (d, $J_{C,F}$ = 21.3 Hz), 36.6, 24.7. APCI-MS m/z $[M + H]^+$ calc'd for $C_{13}H_{18}FNO_2$+H: 240.1; found: 239.9.



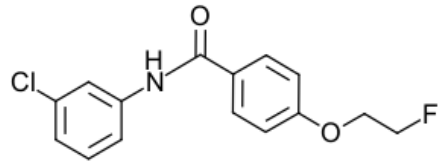
Ethyl (S)-2-[4-(2-fluoroethoxy)benzylamino]-3-phenylpropionate (17)

Refer to chapter 6.3.1 for the full procedure, with the exception that the amine was flowed through in a MeCN (1.9 mL) and water (0.1 mL) mixture. Compound **3** was used as the acid and amine L-phenylalanine ethyl ester hydrochloride was used as the amine. Purified by running through a column of silica (1:2 hex:EtOAc) to yield **17** (10 mg, 0.029 mmol, 10% yield) as a white solid. m.p. 136-139°C. IR (neat) ν_{max}/cm^{-1}: 3340, 2926, 1740, 1632, 1609; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.71 (d, J = 8.9 Hz, 2H), 7.30-7.23 (m, 3H), 7.15 (d, J = 8.2 Hz, 2H), 6.95 (d, J = 9.0 Hz, 2H), 6.51 (d, J = 6.5 Hz, 1H), 5.07 (dt, J = 7.5, 5.7 Hz, 1H), 4.77 (dt, $J_{H,F}$ = 47.4 Hz, J = 4.2 Hz, 2H), 4.26 (dt, $J_{H,F}$ = 23.5 Hz, J = 4.2 Hz, 2H), 4.21 (m, 2H), 3.29 (m, 2H), 1.29 (t, J = 7.1 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 171.7, 166.2, 161.2, 136.0, 129.4, 128.9, 128.5, 127.1, 126.9, 114.4, 81.8 (d, $J_{C,F}$ = 171.2 Hz), 67.2 (d, $J_{C,F}$ = 19.8 Hz), 61.6, 53.5, 38.0, 14.2; APCI-MS m/z $[M + H]^+$ calc'd for $C_{20}H_{22}FNO_4$+H: 360.2; found: 360.3.



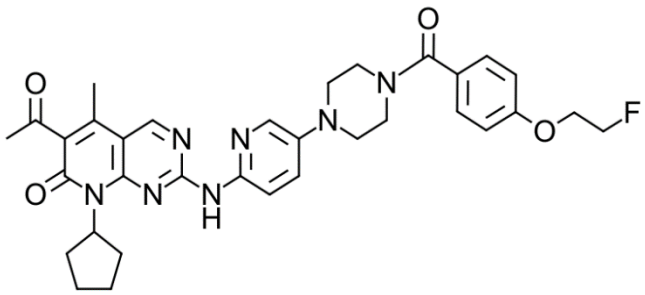
N-4-Hydroxyphenyl-4-(2-fluoroethoxy)benzamide (18)

Refer to chapter 6.3.1 for the full procedure. Compound **3** was used as the acid and 4-aminophenol was used as the amine. Purified by running through a column of silica (1:2 hex:EtOAc) to yield **18** (8 mg, 0.029 mmol, 10% yield) as a white solid. m.p. 211-214.5°C. ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ_H 9.87 (s, 1H), 9.25 (br, 1H), 7.94(d, J = 8.8 Hz, 2H), 7.51(d, J = 8.7 Hz, 2H), 7.08(d, J = 8.7 Hz, 2H), 6.74(d, J = 8.7 Hz, 2H), 4.76(dt, $J_{H,F}$ = 47.8 Hz, J = 3.8 Hz, 2H), 4.32 (dt, $J_{H,F}$ = 30.1 Hz, J = 3.7 Hz, 2H); ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ_C 164.7, 161.0, 154.0, 131.3, 129.9, 128.0, 122.7, 115.4, 114.5, 82.5 (d, $J_{C,F}$ = 166.5 Hz), 67.7 (d, $J_{C,F}$ = 19.1 Hz); APCI-MS m/z $[M + H]^+$ calc'd for $C_{15}H_{14}FNO_3$+H: 276.1; found: 276.1.



N-3-Chlorophenyl-4-(2-fluoroethoxy)benzamide (19)

Refer to chapter 6.3.1 for the full procedure. Compound **3** was used as the acid and 3-chloroaniline was used as the amine. Purified by running through a column of silica (1:2 hex:EtOAc) to yield **19** (3 mg, 0.00116 mmol, 4% yield) as a yellow solid. m.p. 134-135°C. IR (neat) ν_{max}/cm^{-1}: 3280, 2925, 1645; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.85 (d, J = 9.2 Hz, 2H), 7.77 (dd, J = 2.0, 2.0 Hz, 1H), 7.70 (br, 1H), 7.48 (dm, J = 8.1 Hz, 1H), 7.30 (dd, J = 8.1, 8.1 Hz, 1H), 7.13 (dm, J = 7.8 Hz, 1H), 7.0 (d, J = 8.5 Hz, 2H), 4.85 (dt, $J_{H,F}$ = 47.4 Hz, J = 4.1 Hz, 2H), 4.33 (dt, $J_{H,F}$ = 27.7 Hz, J = 4. 1 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 165.0, 161.5, 139.2, 134.8, 130.0, 129.0, 127.3, 124.4, 120.2, 118.0, 114.7, 81.1 (d, $J_{C,F}$ = 171.5 Hz), 67.2 (d, $J_{C,F}$ = 20.8 Hz); APCI-MS m/z $[M + H]^+$ calc'd for $C_{15}H_{13}ClFNO_2$+H: 294.1; found: 294.1.



6-Acetyl-8-cyclopentyl-2-(5-{4-[4-(2-fluoroethoxy)benzoyl]piperazin-1-yl}pyrid-2-ylamino)-5-methyl-1,3,8-triazanaphthalen-7(8H)-one (20)

Refer to chapter 6.3.1 for the full procedure with the exception that 0.11 eq of the amine Palbociclib dissolved in 100°C DMF (3 mL) was used, and only an acid workup was done. Compound **3** was used as the acid. The product was purified by running it through a column of silica (12:1 DCM:MeOH) to yield **20** (3.5 mg, 0.0058 mmol, 18% yield) as a yellow solid. m.p. (decomposed 100°C); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.82 (s, 1H), 8.28 (br, 1H), 8.22 (d, J = 8.8 Hz, 1H), 8.06 (d, J = 2.8 Hz, 1H), 7.45 (d, J = 8.6 Hz, 2H), 7.37 (dd, J = 9.2, 2.8 Hz, 1H), 6.98 (d, J = 8.7 Hz, 2H), 5.91 (quint, J = 8.9 Hz, 1H), 4.79 (dt, $J_{H,F}$ = 47.6 Hz, J = 4.0 Hz, 2H), 4.27 (dt, $J_{H,F}$ = 27.8 Hz, J = 4.0 Hz, 2H), 3.82 (br, 4H), 3.19 (br, 4H), 2.55 (s, 3H), 2.38 (s, 3H), 2.36 (m, 2H), 2.08 (m, 2H), 1.92 (m, 2H), 1.71 (m, 2H), 1.62 (br, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 202.6, 170.3, 161.4, 159.8, 158.0, 157.2, 155.5, 145.7, 141.7, 137.2, 130.9, 129.3, 128.1, 126.8, 114.5, 113.6, 107.9, 81.8 (d, $J_{C,F}$ = 170.5 Hz), 67.2 (d, $J_{C,F}$ = 20.2 Hz), 54.0, 50.1, 31.5, 29.7, 28.1, 25.8, 14.2, 14.0; APCI-MS m/z $[M + H]^+$ calc'd for $C_{33}H_{36}FN_7O_4$+H: 614.3; found: 614.4.

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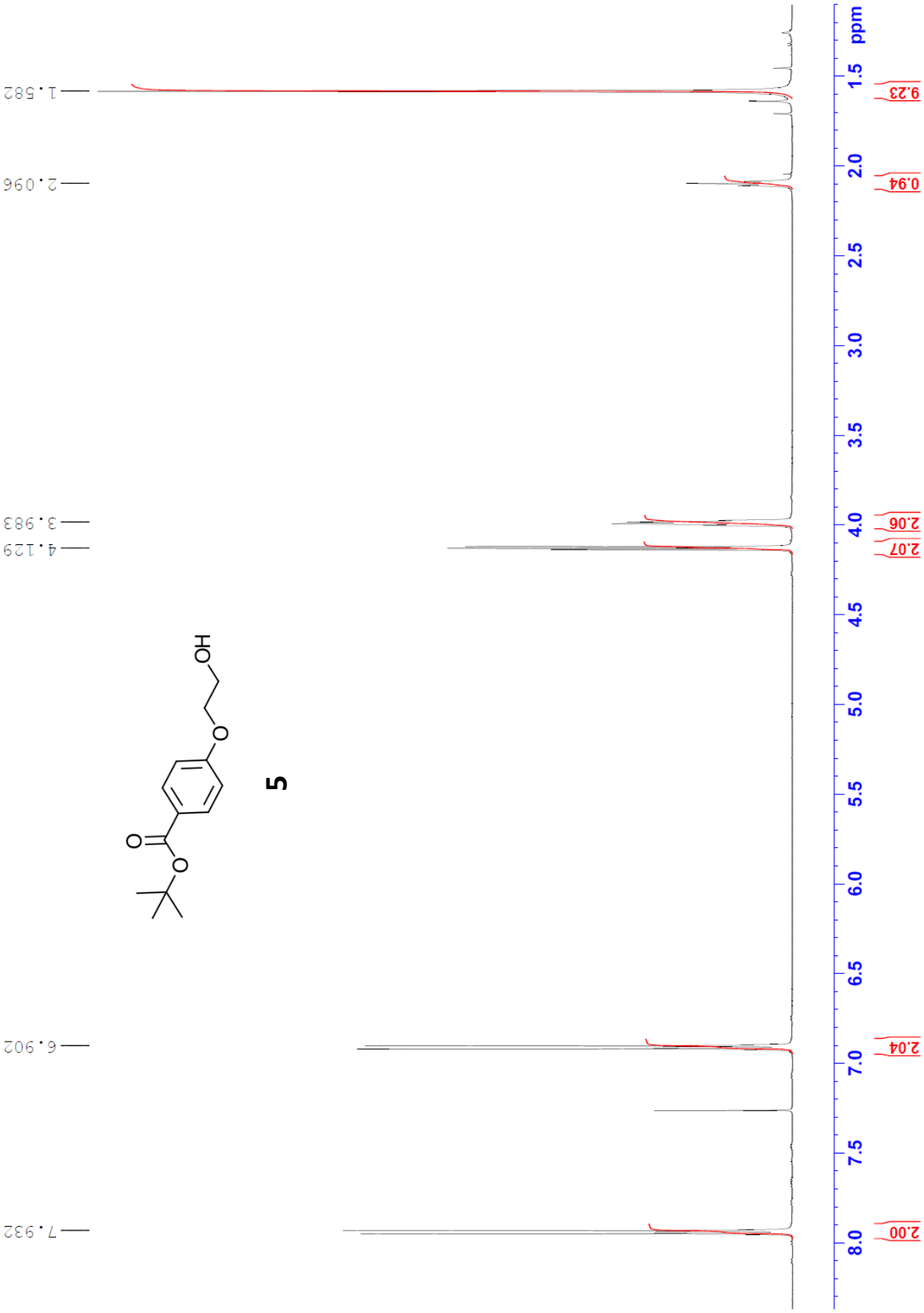
Appendix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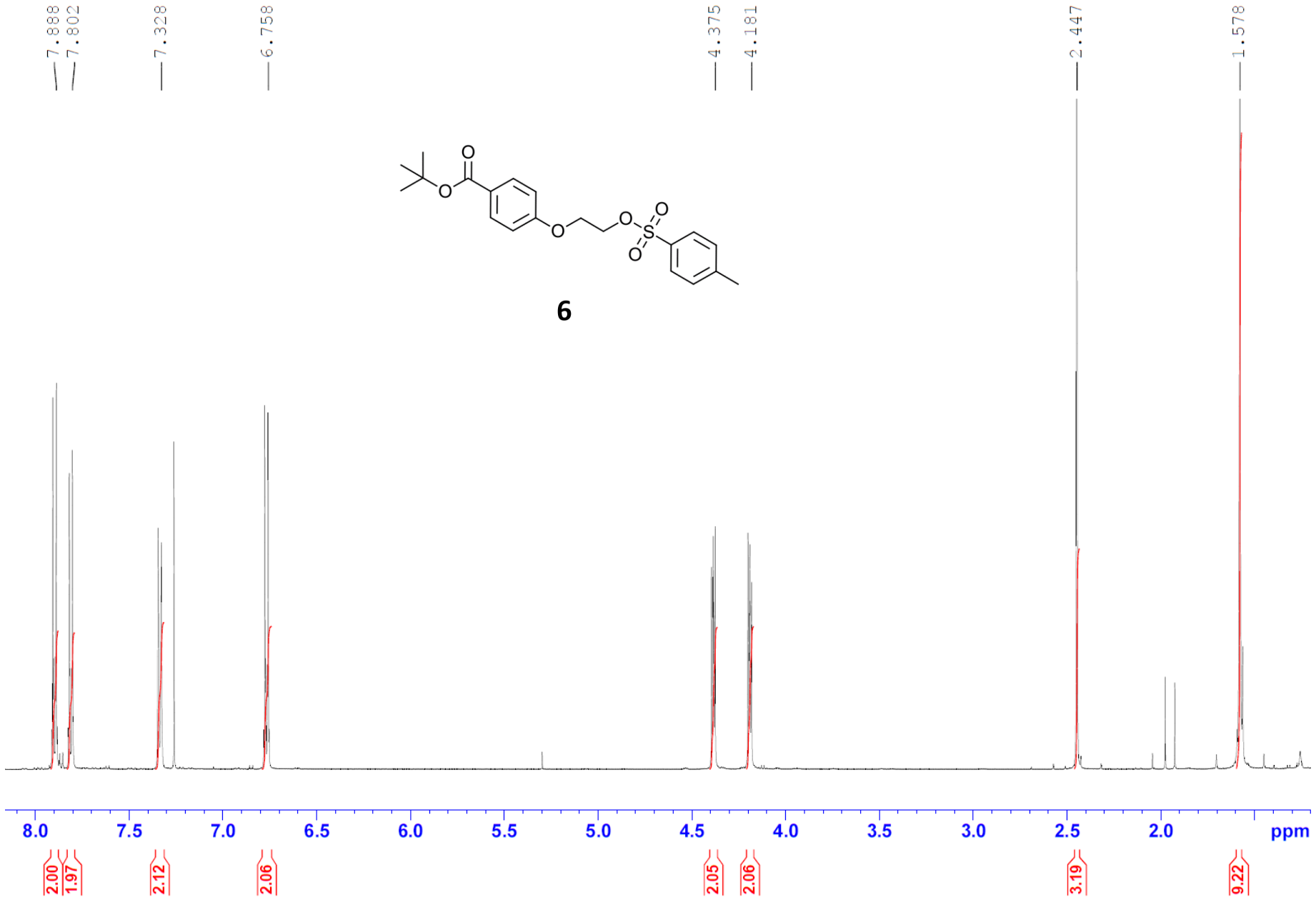
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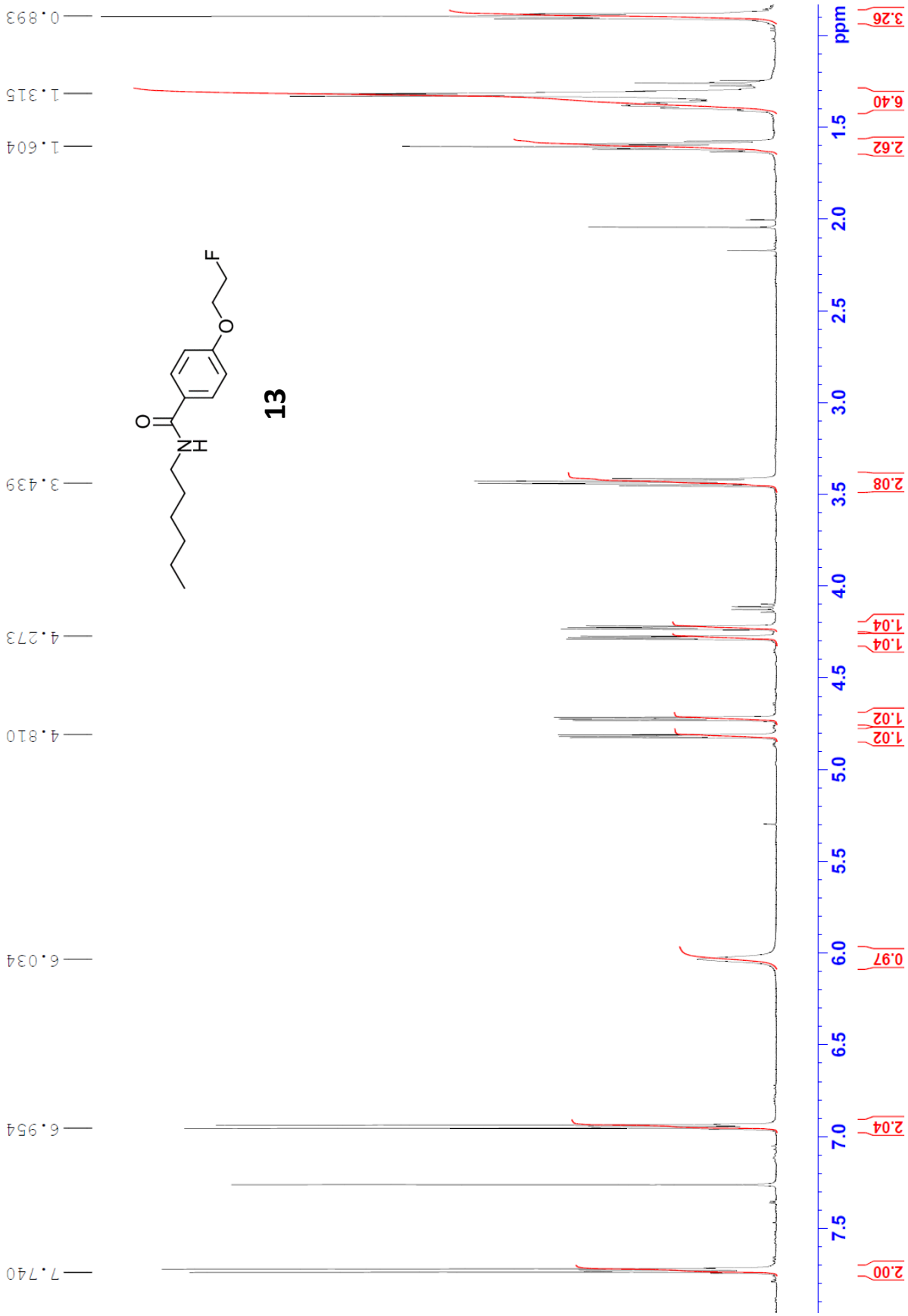
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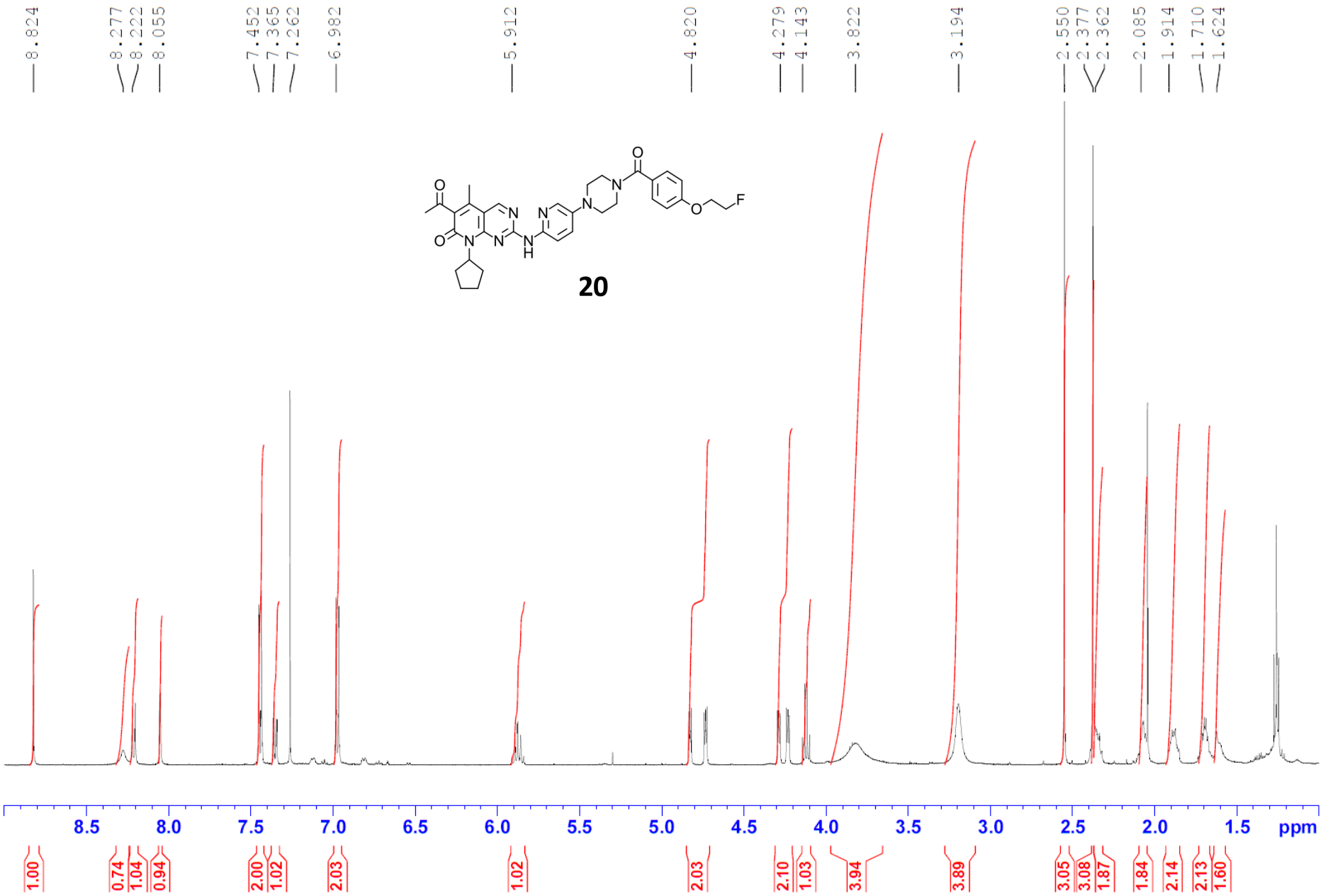
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8.277
8.222
8.055
7.452
7.365
7.262
6.982
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4.820
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3.822
3.194
2.550
2.377
2.362
2.085
1.914
1.710
1.624
20
8.5
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5.0
4.5
4.0
3.5
3.0
2.5
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ppm
1.00
0.74
1.04
0.94
2.00
1.02
2.03
1.02
2.03
2.10
1.03
3.94
3.89
3.05
3.08
1.87
1.84
2.14
2.13
1.60