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POSITRON EMISSION TOMOGRAPHY IMAGING INSIDE THE BODY BY USING BIOLOGICALLY ACTIVE MOLECULES

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***Abstract*— To radiolabel proteins, peptides, and other biologically sensitive molecules with 18F, 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 18F, 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.Scheme 2 illustrates the multi-step synthesis used to radiolabel the proteins. The protein is first protected with Fmoc-Cl and is then tethered onto the Wang resin. It is subsequently deprotected and then coupled with the linker. 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 1H NMR was 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.

***Index Terms*— DIEA-diisopropyl ethyl amine, DMF-DiMethylFormamide, FDG-Flurodeoxy Glucose, NSF-N-Succinimidyl-Fluorobenzoate, PET-positrone mission tomography, PBS-Phosphate Buffer Solution.**

I. INTRODUCTION

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. 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. Other biological molecules can be labeled and used for PET imaging to image different in vivo systems.

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.

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.

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 18F. 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 18F as a radionuclide. In contrast to other positron emitters, 18F decays are not associated with abundantly high energy gamma emissions, which can lead to image degradation. A significant issue with using 18F 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 placed rapidly by fluoride anion under conditions that these biomolecules can tolerate. Under forcing conditions, these biologically sensitive molecules would degrade or become inactive.

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 18F 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.

N-succinimidyl 4-fluorobenzoate

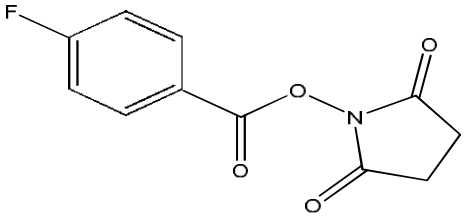


Figure 1: N-succidinimidyl 4-fluorobenzoate (40)

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.

II. DISCUSSION AND RESULTS

At the start of this project, the N-succinimidyl 4-fluorobenzoate (40) was not used as the ligand for the 18F radionuclide. A method was being developed for using 4-fluorobenzaldehyde with an additional linker (Figure 2). 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.

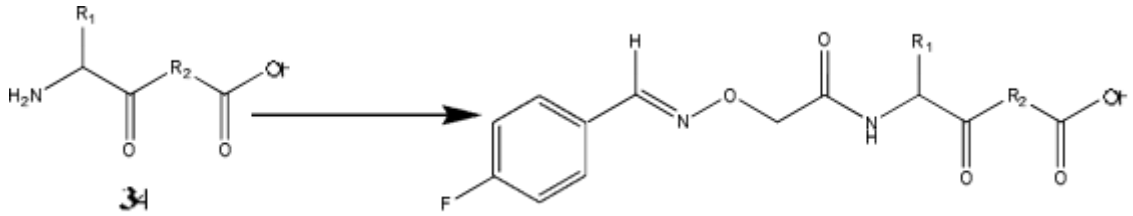


Figure 2: Aminoxy-imine tether

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.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.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. 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. 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.

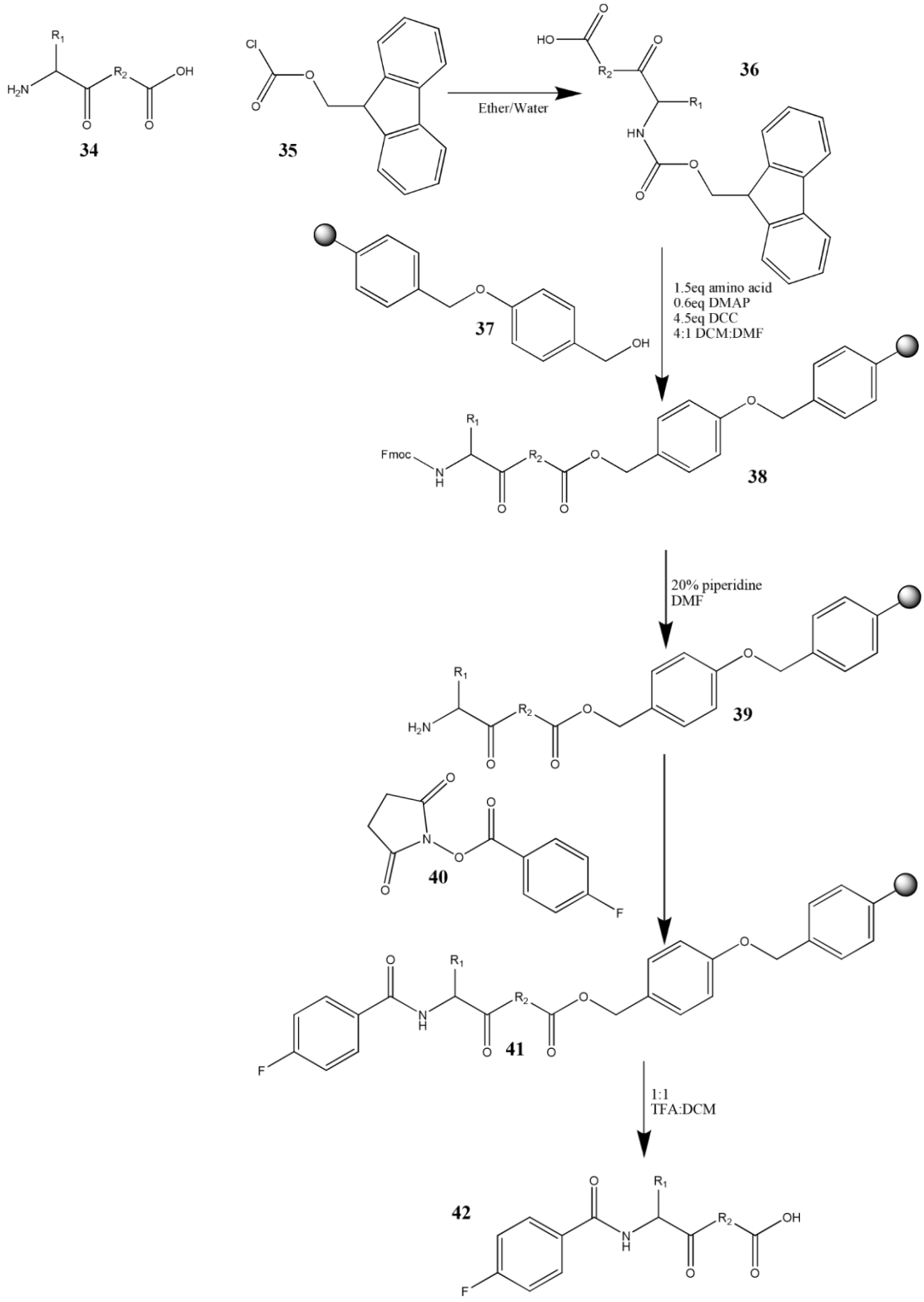
In literature, N-succinimidyl 4-fluorobenzoate has been known to easily react in basic solutions, such as phosphate buffer solution (PBS) or in organic solvent with an added base.

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. 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.

III. RADIOLABELING SYNTHETIC SCHEME



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

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