'Click for PET'

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Summary

'Click' for PET: 'Click' chemistry as a tool for [^{18}F]-radiolabelling

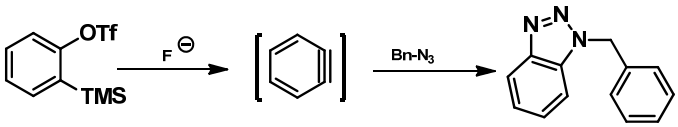
The work described in this thesis focuses on adding the 1,3-dipolar cycloaddition of azides and alkynes to the toolbox of reactions used for [^{18}F]-radiolabelling of positron emission tomography probes. Positron emission tomography is a nuclear imaging technique which produces a quantitative, three-dimensional image of various functional processes within a living being. These images are achieved by radiolabelling a biological molecule with a short lived radionuclide, which is injected into a patient, and produces image as the radionuclide decays and emits positrons.

The discipline of radiochemistry involves a set of unique challenges that the chemist must consider: short synthetic time frames (as the radionuclide is constantly decaying), very small amounts of radioactive reagents, and limited available methods of characterization. Superimposed on these challenges is the stringent requirement that the products be consistently pure and that the results be reproducible as ultimately the protocols may be used to prepare compounds for injection in patients.

The copper-catalyzed 1,3-dipolar cycloaddition of alkynes and azides (CuAAC) and its copper-free counterpart promoted by ring strain have both proven to be reliable and orthogonal ligations. The CuAAC in particular is rapid, amenable to a wide range of conditions, selective and robust. In a field where simplicity and speed of reaction are crucial, it is only natural that 'click' chemistry began to emerge as an excellent radiolabelling technique.

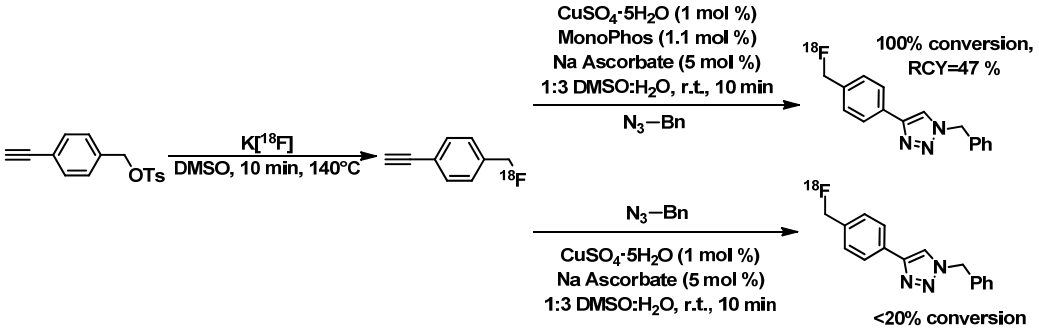
Throughout this thesis, two main goals were pursued; first to accelerate the rate of reaction to make it appropriate for radiolabelling using [^{18}F] and secondly to explore possible ways to avoid the use of copper in the 'click' reaction to open up the possibility of *in vitro* and *in vivo* labeling.

The research project started with the investigation of alternative means to activate the alkyne that would preclude the use of copper. In Chapter 2, an alternative to the copper-catalyzed azide-alkyne cycloaddition was explored. By generating the very reactive species, benzyne, *in situ*, it was possible for the cycloaddition with azides to proceed in the absence of copper, yielding a variety of benzotriazole products.



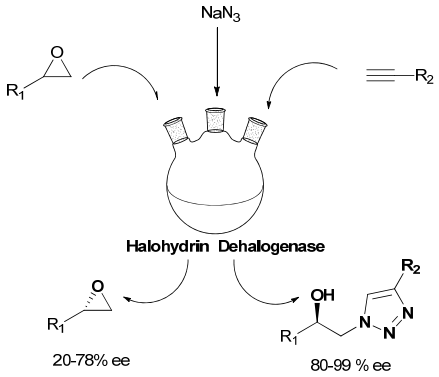
Scheme 1 Fluoride-induced benzyne generation and in situ cycloaddition with azides.

In Chapter 3 the other goal of the project is explored: to accelerate the CuAAC to make it amenable to rapid radiolabelling. Chapter 3 describes the work done toward the acceleration of the CuAAC by the addition of a ligand. Using standard 'click' conditions, a wide variety of ligands were screened to determine the effect of their presence on the rate of the reaction, and it was discovered that MonoPhos, a simple phosphoramidite ligand, had the effect of dramatically reducing reaction times. Comparing a ligand free reaction with the MonoPhos accelerated reaction using small [^{18}F] probes demonstrated that MonoPhos could yield an increase in conversion of a 10 min reaction more than 80% (Scheme 2).



Scheme 2 Demonstration of the acceleration of the CuAAC by MonoPhos

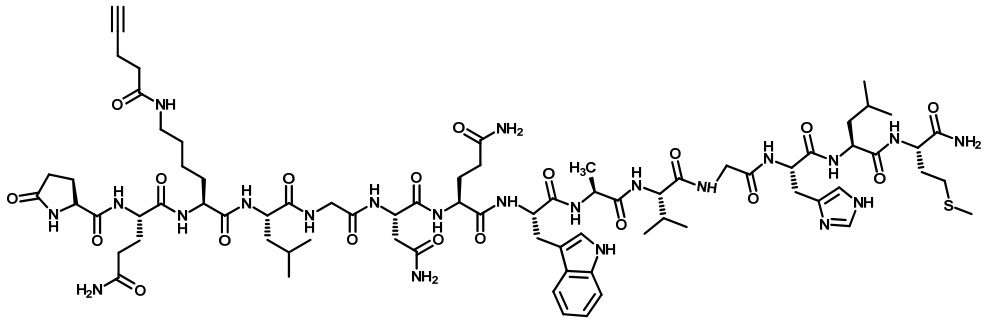
Chapter 4 describes work that centered on exploiting the selectivity of the CuAAC by combining it with an enzymatic transformation. Using the enzyme halohydrin dehalogenase (HheC), a racemic epoxide can be stereoselectively and enantioselectively ring opened with an azide anion producing optically pure azidoalcohols. The goal of this part of the project was to combine a selective biotransformation in one pot with the CuAAC to yield β-hydroxytriazoles. Following the optimization of this two-step, one-pot transformation, a tracer based upon the β-hydroxytriazole motif was synthesized with the intention of using it for imaging cerebral β-adrenergic receptors. Although it was not possible to synthesize the compound enzymatically, it was possible to achieve a high yielding synthesis chemically and the compound demonstrated promising *in vitro* affinities for the target receptor.



Scheme 3 One-pot transformation from a racemic epoxide to an optically pure β-hydroxytriazoles

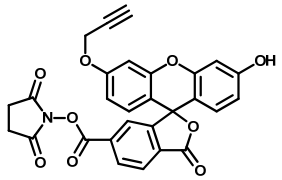
Chapters 5 and 6 describe the modification and labeling of bombesin, a peptide which has a high affinity for the gastrin releasing peptide receptor (GRPr) which is massively overexpressed on certain kinds of tumor cells. The goal of these two chapters was to develop a method, using 'click' chemistry, to label bombesin selectively with [^{18}F] without altering its binding affinity for the target receptor.

In Chapter 5, the labeling of bombesin was performed by CuAAC. Bombesin was modified with an alkyne (Scheme 4). An azido [^{18}F]-bearing prosthetic group was prepared and 'clicked' on to bombesin yielding the modified radiotracer.



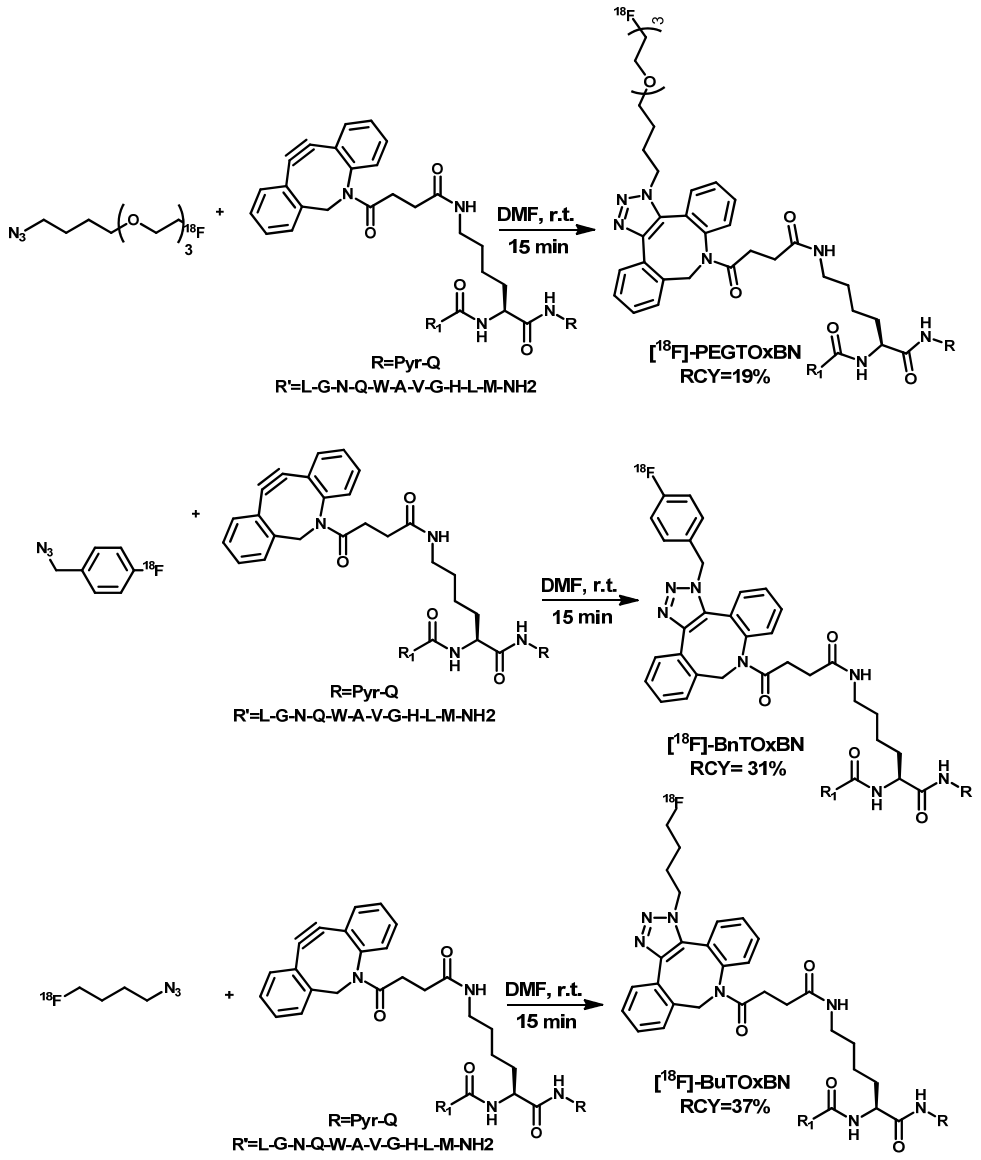
Scheme 4 Alkynyl-modified bombesin

The *in vitro* binding affinity of the new tracer was tested on the target receptors, and proved to have retained high affinity for the receptor. Subsequently, a scaffold was designed which incorporated both a fluorophore allowing for optical imaging as well as an alkyne serving as a handle for labeling with [^{18}F]. Several designs were investigated before settling on a motif that incorporated the fluorescein structure as part of the scaffold (Scheme 5). The scaffold was designed to be versatile in its application. In theory it could be attached to any amino containing target biomolecule for labeling.



Scheme 5 Multi-modal scaffold design

In Chapter 6, an alternative way of labeling bombesin was explored. A new synthetic route to a known strained aza-dibenzocyclooctyne was developed. After determining that the strained cyclooctyne was sufficiently reactive to yield [^{18}F]-containing triazoles on the appropriate radiolabelling time scale, the aza-dibenzocyclooctyne was attached to bombesin. The modified peptide was then labeled with three different [^{18}F] azides. The labelling occured rapidly in under 15 min, and produced triazole compounds with various lipophilicities (Scheme 6). The binding affinity of the tracers for the target receptors was tested, and in spite of the large modification that was introduced, they still maintained affinity for the target receptor.



Scheme 6 Aza-dibenzycyclooctyne modified bombesin labeled with various [^{18}F]-bearing azides