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18F radiolabelling of carbohydrates using click chemistry for the development of new anticancer imaging agents for positron emission tomography

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^{18}F Radiolabelling of carbohydrates using click chemistry for the development of new anticancer imaging agents for positron emission tomography

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List of Abbreviations:

ACN:	Acetonitrile
APF:	4-Azidophenacyl fluoride
$CDCl_3$:	Chloroform
^{13}C NMR:	Carbon nuclear magnetic resonance
Cu(I):	Copper I
Cu(II):	Copper II
CT:	Computed tomography
DCM:	Dichloromethane
DMF:	Dimethylformamide
DMSO:	Dimethyl sulfoxide
DIPEA:	Diisopropylethylamine
EC:	Electron capture
ESI:	Electrospray ionization
FBA:	4-Fluorobenzoic acid
FDG:	2-Fluoro-2-deoxy-D-glucose
FET:	Fluoroethyltyrosine
FPH:	4-Fluorophenyl hydrazine
^{1}H NMR:	Proton nuclear magnetic resonance
HPLC:	High performance liquid chromatography
HRMS:	High resolution mass spectrometry
IT:	Isomeric transition
MRI :	Magnetic resonance imaging
MS:	Mass spectrometer
m/z:	Mass to charge ratio

Na ascorbate	Sodium ascorbate
NPFP:	4-Nitrophenyl-2-fluoropropionate
PET:	Positron emission tomography
Rf:	Retention factor
RGD:	Arg-Gly-Asp
RT:	Room temperature
SAA:	Sugar amino acid
SDA:	Sugar diamio acid
SFB:	N-succinimidyl-4-fluorobenzoate
SPECT:	Single photon emission computed tomography
TFA:	Trifluoroacetic acid
TLC:	Thin layer chromatography
TMS:	Tetramethylsilane
^{o}C:	Degree Celsius
^{18}F:	Fluorine-18

Abstract

The objective of this study was to utilise click chemistry to develop novel types of ^{18}F labelled carbohydrates for use in positron emission tomography imaging through the coupling of [^{18}F]-fluoroalkynes with various 1-azido sugars and 2-azido sugars. In total, eight novel compounds were synthesized in this study.

The products of the click reaction between various types of alkynes and 1-azido sugars, the 1,2,3-triazole sugars, were afforded in good yields. Two hydroxy triazole sugars prepared in this manner were fluorinated and used as cold standards for HPLC analysis and identification of the analogous radiolabelled material. It was subsequently demonstrated that 1-azido glucosamine could be succesfully labelled with fluorine-18 in just 8 mins via a click reaction using 4-[^{18}F]-fluoro-1-butyne.

The above work was then extended to the preparation of 2-azido sugar triazoles, which widens the scope of click reactions on 2-azido sugars as only a few examples have been reported in the literature. Further extension of the work to the conjugation of 1-azido glucosamine and 1-azido glucuronide methyl ester with an acetylenic phenol, provided new examples of sugar triazole compounds which might be labelled with fluorine-18 using the same methods as those used to radiolabel the well known imaging agent fluoroethyltyrosine.

In summary, the click reaction is a very useful method for radiolabelling carbohydrates, which can be achieved in short reaction times and in good yield. The methodology that was developed here can be used to modify various bioactive peptides with radiolabelled carbohydrates to generate new types of cancer imaging agents.

CHAPTER 1

INTRODUCTION

1.1 Cancer and angiogenesis

Cancer is a major disease in many developed countries and accounts for one in every fours deaths in the United States.[1] Cancer is a complex disease composed of a varied population of rapidly growing and dividing cells that have lost the ability to divide and grow in a controlled process. However, this rapid rate of tumour cell growth and proliferation cannot grow beyond a certain size (generally 1-2 mm^3), primarily due to the lack of oxygen, essential building blocks and energy. To facilitate their growth, the tumours secrete various biochemicals called growth factors such as Vascular Endothelial Growth Factor (VEGF) and basic Fibroblast Growth Factor (bFGF) to induce blood vessel growth and thus sustain and nourish the rapidly growing tumour mass. This induction of blood vessel growth by the tumours is called angiogenesis.

Angiogenesis is also required for the spread or metastasis of a tumour. Single cancer cells can break away from an established solid tumour, enter the blood vessel, and be carried to a distant site, where they can implant and begin the growth of a secondary tumour. The subsequent growth of such metastases also requires a supply of nutrients, oxygen and a waste disposal pathway. Although angiogenesis is an essential step in the transition from a small harmless cluster of cells, to a large tumour, it is also necessary for normal organ growth, wound healing and repair, and embryonic development inside the womb and after birth.[2]

The process of angiogenesis occurs when stimulators and chemicals (which are naturally synthesized by the human body) that prevent angiogenesis are not balanced. A fluctuating balance between these pro- and anti-angiogenic stimuli results in an 'angiogenic switch'.[2] This term refers to the progression of the tumour and metastasis, where the tumour changes from a small and localized tumour to a growing, spreading cancer. The tumour sends the signals to the cells lining nearby blood vessels

(endothelial cells). Once the switch is turned on, the endothelial cells will grow and multiply, enzymes will be directed to clear a pathway to the tumour and blood vessels will form new stems to reach the tumour. Above all, there are a series of events involved in the angiogenesis process (Fig.1.1). Ever since it was proposed in the 1970s by Dr. Judah Folkman, inhibition of angiogenesis is considered to be one of the best and most effective strategies to treat and save a cancer patient.[3]

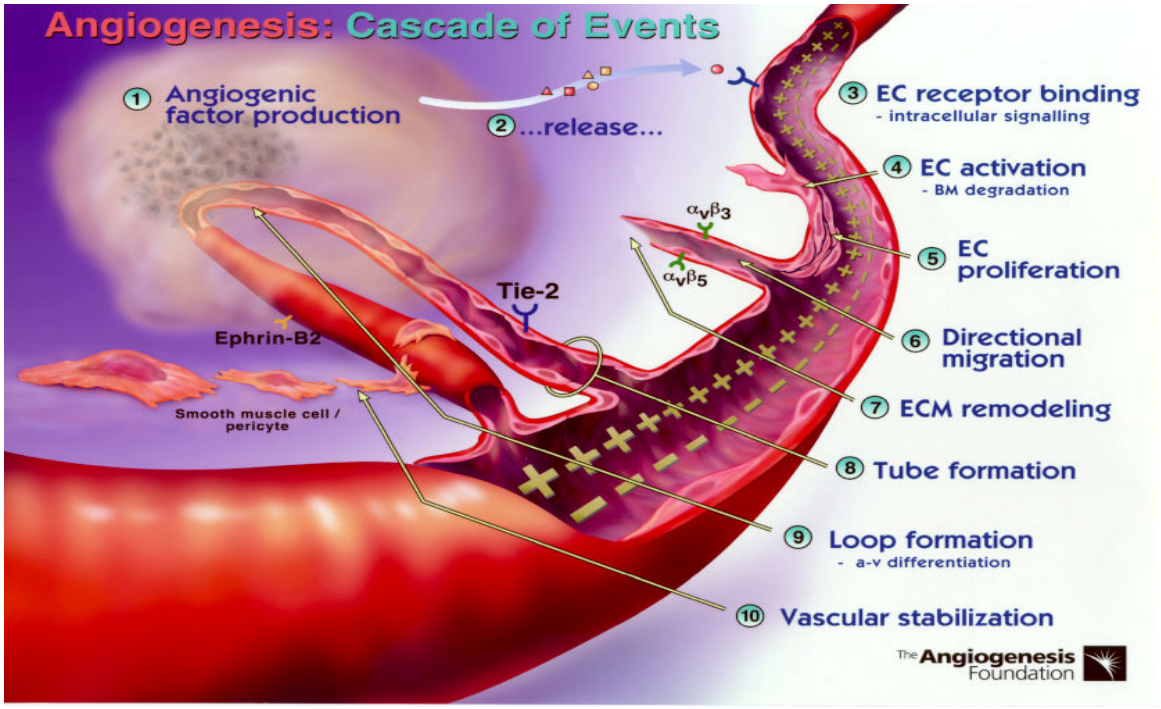


Fig. 1.1: An orderly series of events in the angiogenesis process.[4]

Endothelial cells have long been considered genetically more stable than cancer cells. This genomic stability confers an advantage to targeting endothelial cells using anti-angiogenic therapy, compared to chemotherapy directed at cancer cells, which rapidly mutate and acquire 'drug resistance' to treatment. For this reason, endothelial cells are thought to be an ideal target for therapies directed against them. Angiogenesis-based tumour therapy relies on natural and synthetic angiogenesis inhibitors like angiostatin, endostatin and tumstatin.

1.2 Integrins

Integrins are cell surface receptors that interact with the extracellular matrix and mediate various intracellular signals. They are very important in cell adhesion and cell-cell communication in the human body. Integrins are non-covalent heterodimers consisting of α and β subunits.[5] Integrins have been widely studied, and to date 18 α and 8 β glycoprotein integrin subunits have been discovered. They are expressed on the cell surface in 24 different heterodimeric combinations and named as integrins $\alpha_x\beta_y$.[6]

The size of the α subunit is between 120-180 kDa with around 1000 amino acids, while the β subunit is between 90-110 kDa with around 700 amino acids.[7, 8] Each subunit has a large extracellular domain, a single membrane and a short non-catalytic cytoplasmic tail, except for integrin β_4 (Fig.1.2). The integrin α subunits contain 4 domains and there are 8 domains in the β subunits.[8, 9] Some α subunits, especially α_v have been shown to associate with more than one β subunit and both the extracellular domains of the α and β chain have been identified as ligand binding sites.[10, 11]

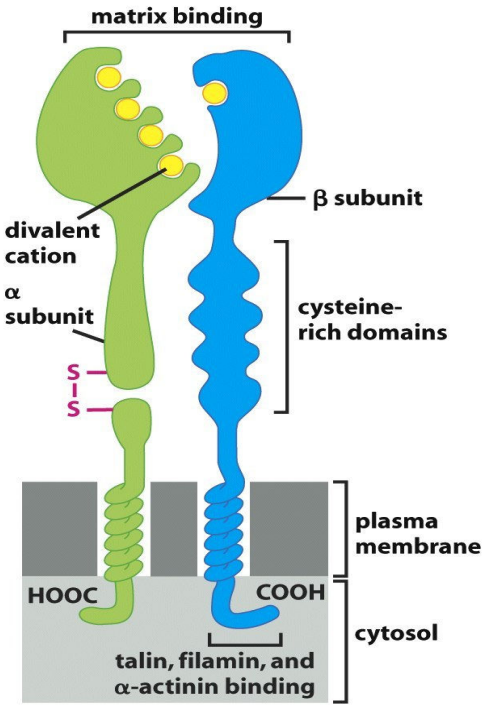


Fig.1.2: General structure of integrin.[12]

Integrins function in signal transduction events and play a major role in cell adhesion, proliferation, apoptosis, polarity, shape, gene expression and differentiation.[13] Particular integrins are known to play a critical role in lymphangiogenesis ($\alpha_9\beta_1$), thrombus formation ($\alpha_{IIb}\beta_3$) and the integrity of skin ($\alpha_6\beta_4$). β_3-Integrins also have a role in enhancing angiogenesis, wound healing, inflammation and atherosclerosis.[13] In 2002, an interaction between α_v-integrin and the matrix proteins vitronectin and tenascin in brain tumours was found. [14]

Integrins also play a key role in cancer metastasis,[15] with recent evidence showing that $\alpha_v\beta_3$ integrin is an important regulator of angiogenesis.[16] Most of the integrin recognition sequences, including those of $\alpha_5\beta_1$, $\alpha_v\beta_1$, $\alpha_v\beta_3$ and $\alpha_v\beta_5$, share features with the ligand Arg-Gly-Asp (RGD).[13] Therefore, angiogenesis antagonists based on RGD peptides have been extensively investigated both as new types of anticancer agents and as cancer imaging agents.

1.3 Radiopharmaceuticals for imaging cancer

In the field of nuclear medicine, tracers used for diagnosis and therapy are known as radiopharmaceuticals,[17, 18] and they consist of two parts; the compound and the radioisotopic label.[19] A bioactive molecule is labelled with a radionuclide forming the radiopharmaceutical ligand, which then binds to a specific receptor or protein that is connected to the target, for example a tumour cell. Radiopharmaceuticals can be administered to the patients by injection, orally, or placed directly into the organs or tissues such as the bladder, eye and even a solid tumour. The initial biodistribution of the radiopharmaceuticals generally reflects the blood flow to areas of the major organs. Over time the radioactivity will accumulate in the targeted organs and wash out from

areas of the body which do not represent specific targeting. The radioactivity will then be detected and an image produced by the respective imaging equipment.

Today there is much interest in developing radiopharmaceutical ligands that are highly selective and bind to specific sites on the tumour, thus providing critical metabolic and biochemical information about the tumour. An important aspect of radiopharmaceutical development is the pharmacokinetic optimisation of the compound that will ultimately provide the required contrast of the image. There are many radiopharmaceutical ligands that have been developed and are already in clinical use, and others still in preclinical studies, which bind to specific receptors[20, 21] and target diseases such as proliferation, angiogenesis, apoptosis and metastasis (Fig. 1.3).

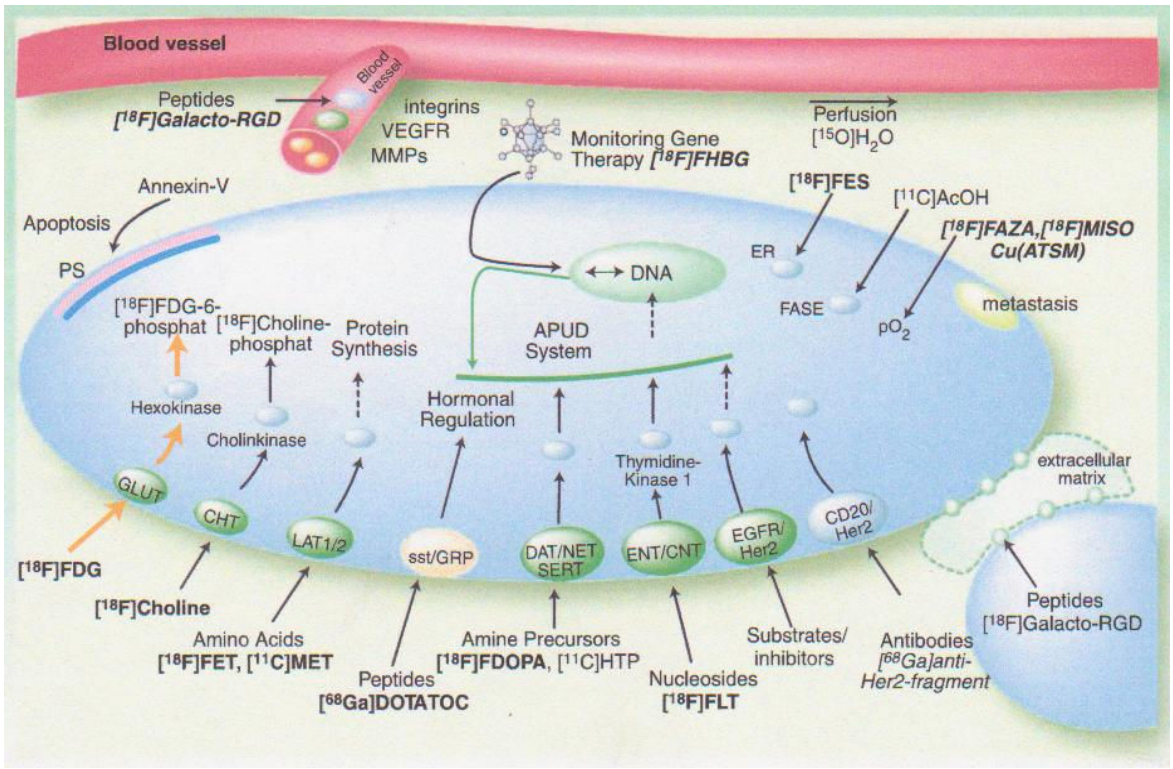


Fig. 1.3: Radiopharmaceutical ligands already in the clinic (bold) or in clinical studies (italic).[20]

In general, radiopharmaceuticals must fulfil common criteria such as high specificity and affinity to the target molecule, rapid blood clearance, low plasma binding, good in vivo stability and low radiation dose to critical organs. In addition, the

labelling process should include a minimum of radiosynthetic steps, be produced in high radiochemical yield and purity, and high specific activity.[20, 21]

1.4 Radionuclides

Radionuclides are unstable isotopes that undergo radioactive decay to achieve stability.[22, 23] The radioactivity comes from a spontaneous decay process that emits electromagnetic radiation such as gamma(γ)-rays or charged alpha (α) and beta (β) particles. The type of emission that is emitted is very important in determining whether the radionuclide is suitable for imaging or treatment of patients.[23]

The penetrating power of gamma radiation is more than that of charged particles.[22] It can pass through the body and is ideal for use in external imaging.[24] In contrast, for therapeutic uses, the objective is to destroy the tumour mass, hence a low penetration is needed to minimize harm to surrounding healthy tissue.[22] As alpha and beta radiation consists of particles that can't penetrate far into the body, they are restricted to the target tissue and are suitable for therapeutic purposes.[25]

There are several factors that should be considered in selecting radionuclides for medical use such as the type of radiation emitted, the half life, energy of the radiation, dose minimisation to the patient and detection characteristics.[22, 23, 26] Ideally, the synthesis and imaging of the radiopharmaceuticals must be within a time frame compatible with the radioisotope's half life.[27] If the half life is too short, the compound will not reach a maximum target to background ratio as most of the radioisotope would have already decayed. In contrast, a too long half life makes the normal tissue exposed to an unnecessary radiation dose.[26] Some of the common radioisotopes used in nuclear medicine are listed in Table 1.1.

Table 1.1: Radionuclides of interest in nuclear medicine (adapted from Ref.[22]).

Radionuclide	Mode of decay	Principal Photons (KeV)[a]	Half-life
Used clinically, mainly for diagnosis			
^{99m}Tc	IT	140	6 hrs
^{123}I	EC	159	13 hrs
^{201}TI	EC	167	73 hrs
^{11}C	β^+	511	20 mins
^{18}F	β^+, EC	511	110 mins
^{68}Ga	β^+, EC	511	68 mins
Used clinically, mainly for therapy			
^{32}P	β^-	None	14.3 days
^{89}Sr	β^-	None	52 days
^{177}Lu	γ, β^-	β^- 0.5 MeV	6.7 days
^{131}I	β^-	γ, 208 keV 364	8.1 days

[a]Values in parentheses are % emission frequency. IT = isomeric transition; EC = electron capture.

Radiopharmaceuticals incorporating the positron emitting radioisotopes such as fluorine-18, carbon-11, nitrogen-13 and oxygen-15 have been used to study specific biochemical functions in the human body. Furthermore, as carbon, nitrogen and oxygen constitute the basic building blocks of most physiologically important biochemical compounds, carbon-11, nitrogen-13, and oxygen-15 can be conveniently incorporated directly into biomolecules.[22, 27] However, except for fluorine-18, the use of these radionuclides is confined to clinical research centres with in-house cyclotrons due to their very short half lives.

1.5 Positron emission tomography

Medical imaging techniques provide invaluable information on the status of disease in the body. There are several different techniques used such as ultrasound, computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET) and single photon emission computed tomography (SPECT).

Ultrasound, CT and MRI are anatomical imaging methods that provide good anatomical information on the status of disease, however, they do not have the ability to provide biochemical information such as tumour growth, response to therapy, and early response to biochemical treatments.[28] PET and SPECT on the other hand are functional imaging modalities that have the ability to measure the regional biochemistry especially in early detection of the disease based on molecular and biochemical processes.[28, 29]

PET imaging has become an important technique to study physiological, biochemical and pharmacological functions in humans. It is a non-invasive imaging technique that can measure the concentration of the tracer in tissues accurately due to its high sensitivity and high spatial resolution.[30, 31] SPECT has a problem in collimation and attenuation resulting in a reduced ability to quantitate the distribution of radiotracers compared to PET.[19] In addition, several important positron emitters (carbon, nitrogen and oxygen) are also the physiologically important elements.[32] Therefore, PET is a very useful method for both treating and monitoring cancer.

In PET, once the radiopharmaceutical is given to the patient, the positron, or positive electron, travels no more than a few millimetres in the tissue and then collides with a negative electron.[19] The resultant annihilation process creates two gamma rays of 511 keV each, which travel in opposite directions[33] (Fig.1.4). These rays that are 180 degrees apart will be detected by a scintillator in the camera, which after computer processing can produce an image of the physiology and biochemistry in vivo.

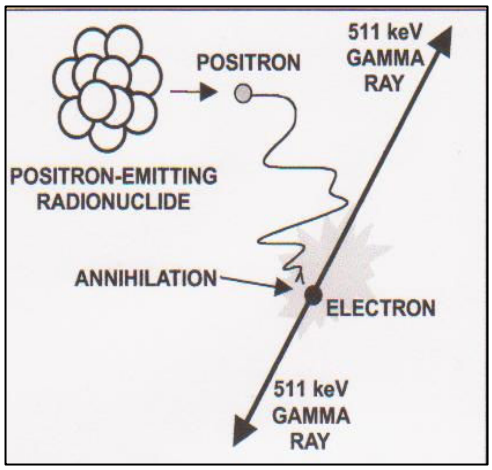
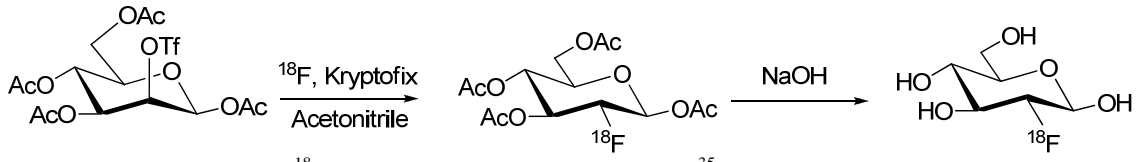


Fig. 1.4: Mechanism of annihilation.[34]

The most common positron emitters are ^{15}O ($t_{1/2}$= 2.07 min), ^{13}N ($t_{1/2}$= 9.96 min), ^{11}C ($t_{1/2}$= 20.4 min) and ^{18}F ($t_{1/2}$= 109.7 min) which are produced on particle accelerators called cyclotrons. By far, the most common radiopharmaceutical used in PET imaging is 2-[^{18}F]-fluoro-2-deoxy-D-glucose, or [^{18}F]FDG.

1.5.1 [^{18}F]-Fluoro-2-deoxy-D-glucose, [^{18}F]FDG

[^{18}F]FDG is one of the most widely used PET radiopharmaceuticals and is commercially available in most advanced countries. [^{18}F]FDG is a glucose analogue whereby the hydroxyl group on carbon-2 is replaced by a fluorine atom. [^{18}F]FDG is prepared by nucleophilic substitution of a triflate precursor (Scheme 1.1). Based on glucose, [^{18}F]FDG is transported across cell membranes and is then phosphorylated by hexokinase.[22] The absence of the hydroxyl group of [^{18}F]FDG in the carbon 2-position prevents further metabolism, hence it is retained in the cell for a longer time allowing imaging of the tumour to take place.[22, 35]



Scheme 1.1: Synthesis of [^{18}F]FDG by nucleophilic substitution.[35]

[^{18}F]FDG is not only very useful in evaluating many malignancies but also for the staging and detection of recurrence for many tumour types.[36] However, its specificity in cellular dynamics in regards to energy requirements has limited its use in molecular imaging.[37] Normal fast growing cells, such as brain and pancreatic cells can also take up [^{18}F]FDG making it a non-cancer-specific tracer. Furthermore, slow growing tumours have a low [^{18}F]FDG uptake, making them difficult to image. Nanni et al. have addressed the issue that there is a need for new PET radiopharmaceuticals other than [^{18}F]FDG as there are still many biological aspects of cancer that cannot be measured by [^{18}F]FDG alone.[38]

1.6 Radiolabelled peptides

Amino acids are the building blocks of peptides and are linked together through peptide bonds. Proteins or peptides are found in all living things. More than 80 polypeptide drugs have made it into the market and are approved for usage such as teriparatide which is used for osteoporosis treatment and etanercept for the treatment of rheumatoid arthritis.[39] Rationally, since many of the cell surface receptors for peptides are overexpressed in cancer, the interest in developing peptide radiopharmaceuticals has risen.[40] Peptides are chosen because of their size, with up to about 50 amino acids and 10,000 Da in molecular mass, which is considerably smaller than monoclonal antibodies resulting in more rapid blood clearance thus providing superior imaging characteristics.[31, 40, 41]

To date, several peptides were developed such as octreotide, bombesin, annexin V and endothelin analogues that have been used for diagnosis and therapy in oncology, neurology, cardiology and infection/inflammation. Various techniques have been developed in labelling these peptides using radionuclides such as ^{99m}Tc, ^{123}I, ^{111}In and ^{18}F, with ^{111}In–labelled octreotide the first radiopeptide approved for diagnostic tumour imaging.[31] A comparison of the advantages and limitations of the various radionuclides used for peptide labelling is listed in Table 1.2.

Table 1.2 : Characteristics of different radionuclides used for peptide labelling [42]

Advantages	Limitations
Technetium-99m	
▪ Readily available, low cost ▪ Easy to handle ▪ Reasonable half life-6 h ▪ 140 keV γ-emission ▪ Excellent imaging characteristics ▪ Absence of α and β radiation ▪ Favourable dosimetry	▪ Relatively more difficult to incorporate into small drugs
Iodine-123	
▪ 159 KeV γ-emission ▪ 13 h half life ▪ Good imaging characteristics ▪ Cyclotron-produced radionuclide	▪ High cost ▪ Limited availability
Indium-111	
▪ 171 and 245 KeV γ-emission ▪ Half life of 2.8 days ▪ Useful for acquiring delayed images	▪ High cost ▪ Limited availability ▪ Relatively high radiation burden to patients ▪ Suboptimal nuclear characteristics
Fluorine-18	
▪ Can be produced in high quantity ▪ Low positron energy of 0.635 MeV ▪ High resolution and sensitivity ▪ Low radiation dose to patients ▪ The half-life of 110 min permits both radiopharmaceutical preparation and imaging studies	▪ Relatively short half-life

As described before, integrins especially those of the $\alpha_v\beta_3$ subtype play important roles in many intracellular signalling pathways. Therefore, integrins are an effective target of radiopeptides as some of the integrins such as $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_v\beta_1$ and $\alpha_{IIb}\beta_3$ are

recognised by the Arg-Gly-Asp (RGD) sequence.[40, 43] The first generation of RGD radiotracers were the cyclo(-Arg-Gly-Asp-D-Tyr-Val-) and cyclo(-Arg-Gly-Asp-D-Phe-Tyr-) that when labelled with iodine-125, showed high affinity and selectivity to $\alpha_v\beta_3$ in vitro and receptor specific tumour uptake in vivo.[44, 45] But, these tracers have high concentrations in the liver due to their lipophilic character. To overcome this problem, the peptide was modified and conjugated with a sugar amino acid based on glucose. As a result, the radio-iodinated gluco-RGD showed high tumour uptake with an improved pharmacokinetic profile.[46]

Based on these results, radiofluorination of the cycloRGD peptide was developed by attaching a sugar amino acid based on galactose to the cyclic RGD peptide followed by conjugation with [^{18}F]fluoro propionic acid resulting in the generation of [^{18}F]galacto-RGD (Fig. 1.5). The [^{18}F]galacto-RGD displayed reduced lipophilicity and enhanced renal excretion compared to the non-glycosylated analogue,[45] and ultimately advanced into clinical imaging of $\alpha_v\beta_3$ integrins in melanoma patients.[20]

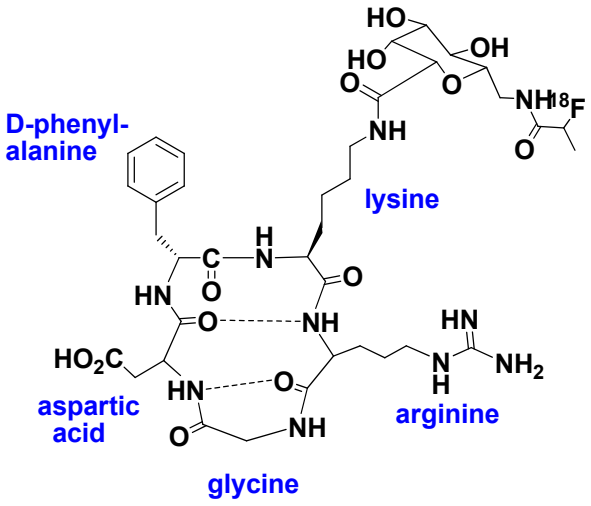


Fig.1. 5: The structure of [^{18}F]galacto-RGD.[45]

PET imaging often uses ^{18}F due to its favourable physical and nuclear characteristics.[47] To date, several radiofluorinated conjugates have been developed for

the radiofluorination of peptides and proteins including N-succinimidyl 4-[^{18}F]fluorobenzoate ([^{18}F]SFB), 4-nitrophenyl-2-[^{18}F]-fluoropropionate ([^{18}F]NPFP) and 4-[^{18}F]fluorobenzoic acid ([^{18}F]FBA) (Fig.1.6). Peptide labelling is achieved by conjugation to three functional groups: the amino group, the carboxylic acid group and the sulfhydryl group.[47, 48] [^{18}F]NPFP was used in the labelling of [^{18}F]galacto-RGD resulting in both high radiochemical yield and purity.[45] Above all, [^{18}F]SFB was reported to be the most useful for radiofluorination and peptide labelling.[49]

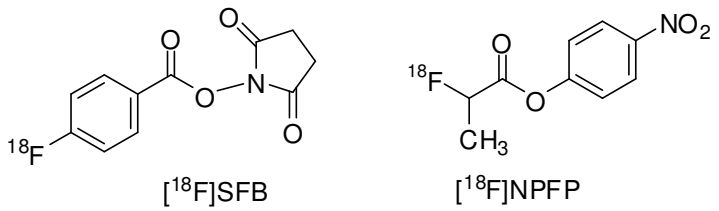


Fig. 1.6: Structures of N-succinimidyl 4-[^{18}F]fluorobenzoate (SFB) and 4-nitrophenyl-2-[^{18}F]-fluoropropionate (NPFP).

1.6.1 Click chemistry

The most disadvantageous aspect of labelling with ^{18}F is the use of time consuming multi-step reactions, and there is still great need for method improvement in this area. Table 1.3 shows some of the different approaches for labelling biomolecules with ^{18}F.

Click chemistry is a recent discovery which uses fewer chemical reactions and milder conditions to generate labelled substrates compared to other current methods.[50] It has become a powerful and selective reaction employed in various fields of chemistry.[51] The term "click chemistry" was introduced by Sharpless et al., who followed nature's lead to join small units together with heteroatomic links (C-X-C). The click reaction is high yielding and easy to perform using readily available reagents and starting materials. It is also tolerant to water, and the subsequent work-up and product isolation are straight-forward.[52, 53] An attractive feature of the click reaction is

that it can be performed without protecting groups due to the chemoselectivity in the reaction.[54]

Table 1. 3: Different approaches for labelling biomolecules with ^{18}F. (Adapted from ref.[47, 55])

Method	^{18}F labelling agent	Preparation time (min)	Radiochemical yield (%)
Acylation	4-Nitrophenyl-2-[^{18}F]fluoropropionate [NPFP]	90	60
	N-Succinimidyl-4 [^{18}F]fluorobenzoate [SFB]	35-100	25-60
Imidation	3- [^{18}F]Fluoro-5-nitrobenzimidate	45	20-23
Alkylation	4- [^{18}F]Fluorophenacyl bromide	75	28-40
Photochemical conjugation	4-Azidophenacyl- [^{18}F]fluoride	15	71
Click reaction	[^{18}F]fluoroalkynes	10-15	36-81

Click chemistry utilises the Huisgen 1,3-dipolar cycloaddition reaction of terminal alkynes and organo azides to form 1,2,3-triazoles (Fig.1.7). The most widely used click reaction is a Cu(I)-mediated reaction that produces only the 1,4-regioisomer. In contrast the thermal reaction is non-regiospecific.[56]

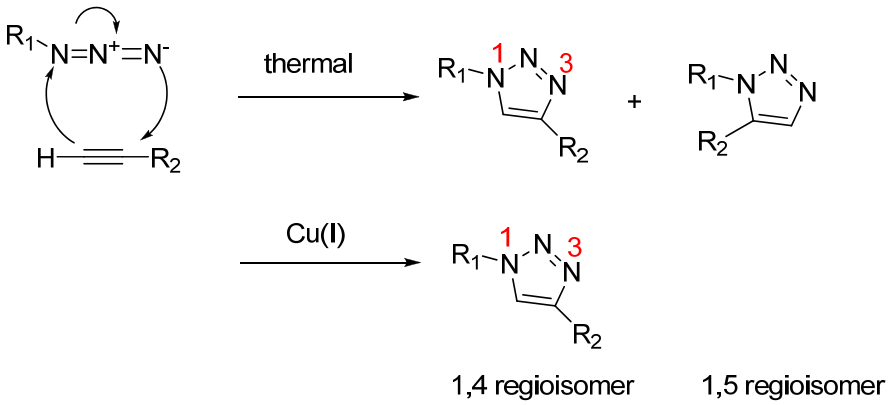


Fig. 1.7: The 'click reaction' of an azide and terminal alkyne to give a 1,2,3-triazole.[56]

A variety of Cu(I) sources have been used in the Huisgen cycloaddition reaction including CuI salts such as copper iodide[57] and copper bromide,[58] however, this type of reaction needs a large of excess of copper and ligand to work efficiently.[59] The use of metallic copper has also been employed, however, the reaction with copper turnings took a long time to form the desired triazole in good yield.[51] Despite this, the most

common click reactions use an in situ reduction of a Cu(II) salt system to produce Cu(I),such as Cu(II) sulfate with sodium ascorbate as reducing agent. The advantages of this system is it is cheap, does not require an inert atmosphere, can be perfomed in the presence of water and eliminates the need for a base.[51, 60]

Generally, cycloadditions proceed through a concerted mechanism.[60] However, density funtional theory calculations showed that copper catalysed reaction proceeds in a stepwise mechanism (**A→B→C→D**) rather than a concerted process (**A→D**) (Fig.1.8).[51, 61] The Huisgen 1,3-dipolar cycloaddition begins with formation of a Cu(I) acetylide via π-complexation, then deprotonation to form the σ-acetylide Cu(I) complex **A**, followed by generation of the copper-azide-acetylide complex **B**, cyclisation and dissociation to yield the desired triazole product and the copper catalyst ligand (Fig.1.8).[51, 61]

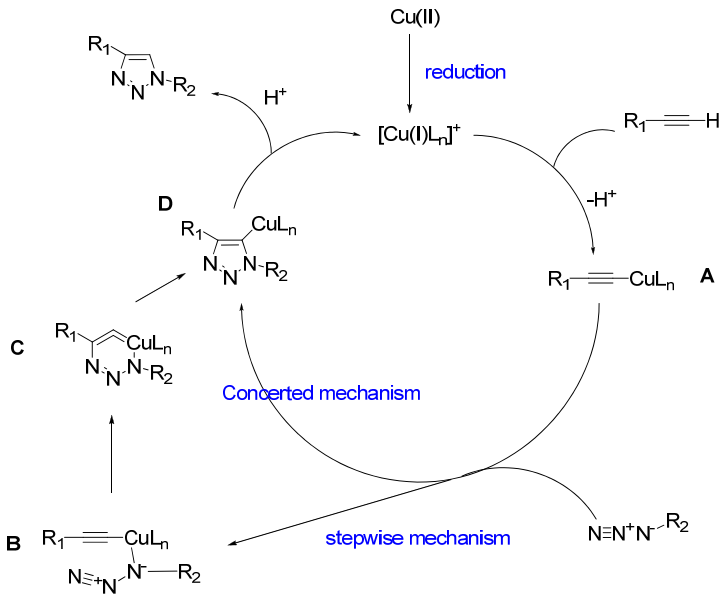


Fig.1.8: Catalytic cycle of the Huisgen cycloaddition.[61]

The use of click chemistry has now expanded into the radiochemistry field, where the Cu(I)-mediated coupling of an azide and alkyne has been used to form labelled triazole containing compounds. The first peptide labelling using click chemistry was introduced by Marik and Sutcliff and demonstrated that peptides could be efficiently

labelled with [^{18}F]alkynes in high yield, under mild conditions, and with rapid preparation times of 30 min.[62] Other labelled compounds using click chemistry which have been developed are 4-[^{18}F]fluoro-1-butyne that was used in the first radiolabelling of a sugar azide,[63] 2-[^{18}F]fluoroethylazide in terminal alkyne labelling,[64] [^{11}C]methyl azide in the generation of ^{11}C-labelled 1,2,3-triazoles[65] and metal chelated ^{99m}Tc labelled biomolecules[66] (Fig. 1.9).

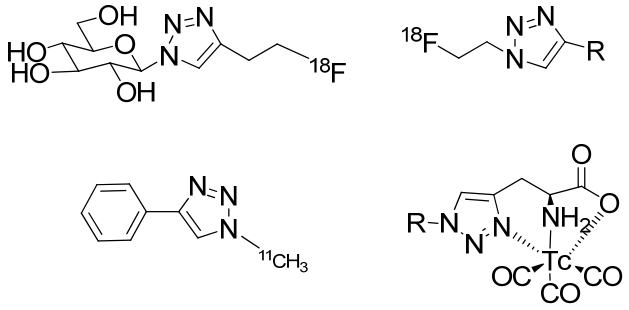


Fig. 1.9: Radiolabelled compounds produced using "click chemistry'.

1.6.2 Carbohydrates

Carbohydrates are widely represented in biological systems and the most abundant group of natural products.[67] Recently, carbohydrate-containing derivatives have attracted much interest in drug development. This is because the incorporation of a sugar moiety is often found to improve the pharmacokinetic profile of a drug. It has been reported that conjugation of a sugar into the peptide backbone and / or side chains, can improve its pharmacological properties including bioavailability, stability of the drug toward enzymatic degradation and solubility under physiological conditions.[45, 68]

Carbohydrate derivatives bearing an amino acid and a carboxylic acid moiety, also known as sugar amino acids (SAAs), are of particular interest.[69] SAAs are easy to synthesise from commercially available monosaccharides such as glucose, glucosamine and galactose. The amino functionality of the SAA can be introduced as an azide, cyanide or nitromethane, while the carboxylic function can be introduced directly as

CO_2 or as a hydrolysable cyanide.[70] SAAs can be employed as structural analogues in mimics of Leu-enkephalin integrin antagonists (RGD), somatostatin, cyclodextrins and as pharmacophore mapping libraries.[71] SAAs have been incorporated into cyclic peptides not only to improve the pharmacokinetic profile of the drug but also to introduce radioisotope labelling in order to allow imaging.[70]

In addition to SAAs, sugar diamino acids (SDAs) have recently emerged as an interesting new building block in peptidomimetic chemistry (Fig. 1.10). SDAs have the potential to be even more useful building blocks than SAAs, as they have both an amino and carboxylic acid moiety available for peptide coupling and an additional amino moiety available for labelling or other types of modification. For example, protected SDAs have been used in the development of RNA ligands based on the incorporation of the binding motifs from naturally occurring aminoglycosides into nonnatural synthetic analogues by combinatorial methods.[71] Although SDAs hold great potential, their synthesis is quite challenging and there is much room for method development in this area. To date, only two types of SDAs have been reported.[69, 72] Fig.1.10 shows examples of some of the known SAAs and SDAs.

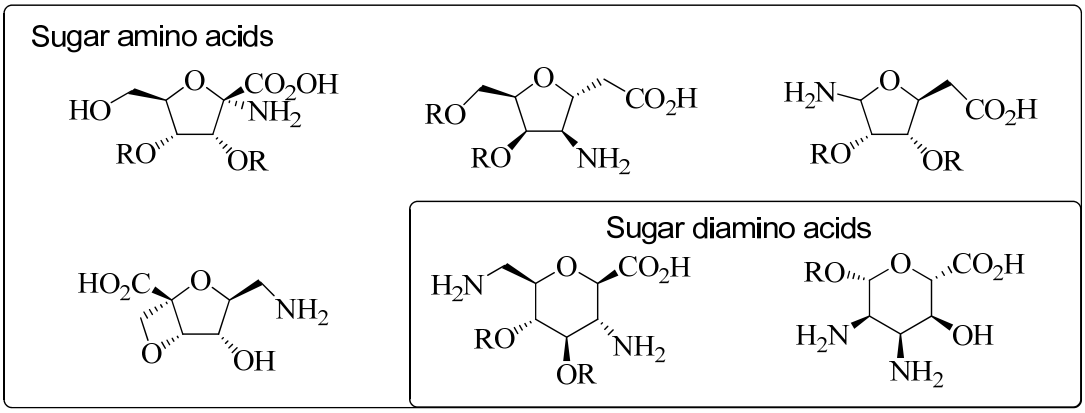


Fig. 1.10: Examples of sugar amino and sugar diamino acids.

1.7 Project aims

Carbohydrates such as FDG have been widely used in cancer imaging. Recently, it has also been found that the incorporation of certain carbohydrate moieties into peptide based tumour targeting and imaging agents such as cyclo-RGD improves their biodistribution and pharmacokinetic profile. We were interested in generating novel analogues of cyclo-RGD through the attachment of radiolabelled carbohydrates to the available lysine residue (see Fig. 1.5). There is also much scope for the inclusion of radiolabelled carbohydrate moieties into a whole range of existing pharmaceutical agents. Furthermore, new methods for labelling carbohydrates and new types of labelled carbohydrates are much needed in this area. Thus the aims of the project are:

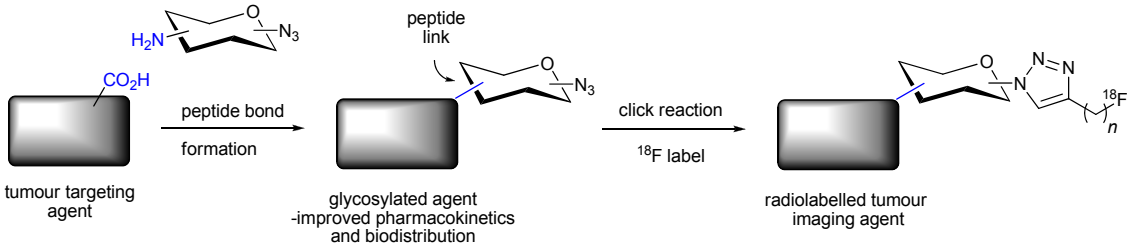
- To utilise click chemistry to develop novel types of ^{18}F labelled carbohydrates for use in PET imaging through the coupling of [^{18}F]-fluoroalkynes with various 1-azido sugars and 2-azido sugars.
- To extend the methodology developed above to the labelling of sugar amino and sugar diamino acids, which have a free carboxylic acid or amino moiety for coupling to tumour targeting agents such as the peptide cyclo-RGD.

CHAPTER 2

RESULTS AND DISCUSSION (PART I)

This chapter details the utilisation of click chemistry in the development of novel types of ^{18}F labelled carbohydrates for use in PET imaging on their own and/or attached to tumour targeting agents. The development of methodology for the coupling of alkynols with 1-azido glucosamine derivatives via click chemistry, followed by fluorination of hydroxyl triazole sugars will be presented in the first part. The chapter closes with the successful generation of an [^{18}F]-radiolabelled sugar derivative.

2.1 Labelling of 1-azidoglucosamine (2) with aliphatic alkynes



Scheme 2.1: General scheme for glycosylating and subsequent radiolabelling of tumour targeting agents.

Carbohydrates are often used to modify the properties of bioactive peptides including their biodistribution and pharmacokinetic profile. The project described here involves the use of click chemistry to prepare radiolabelled carbohydrates that could readily be coupled to bioactive peptides such as cyclo RGD to generate novel imaging agents with improved properties. The carbohydrate starting materials for this project require both an azido group for click chemistry and either a carboxylic acid or an amino functionality to attach to the peptide of interest using standard peptide coupling conditions. An example of this type of glycosylation and subsequent radiolabelling of a tumour targeting agent is shown in Scheme 2.1.

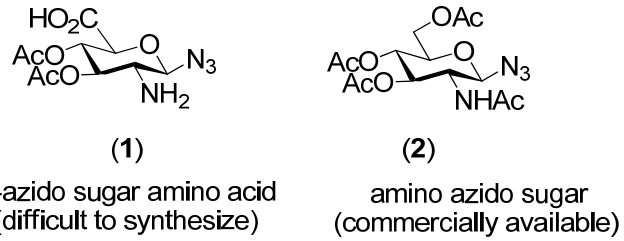


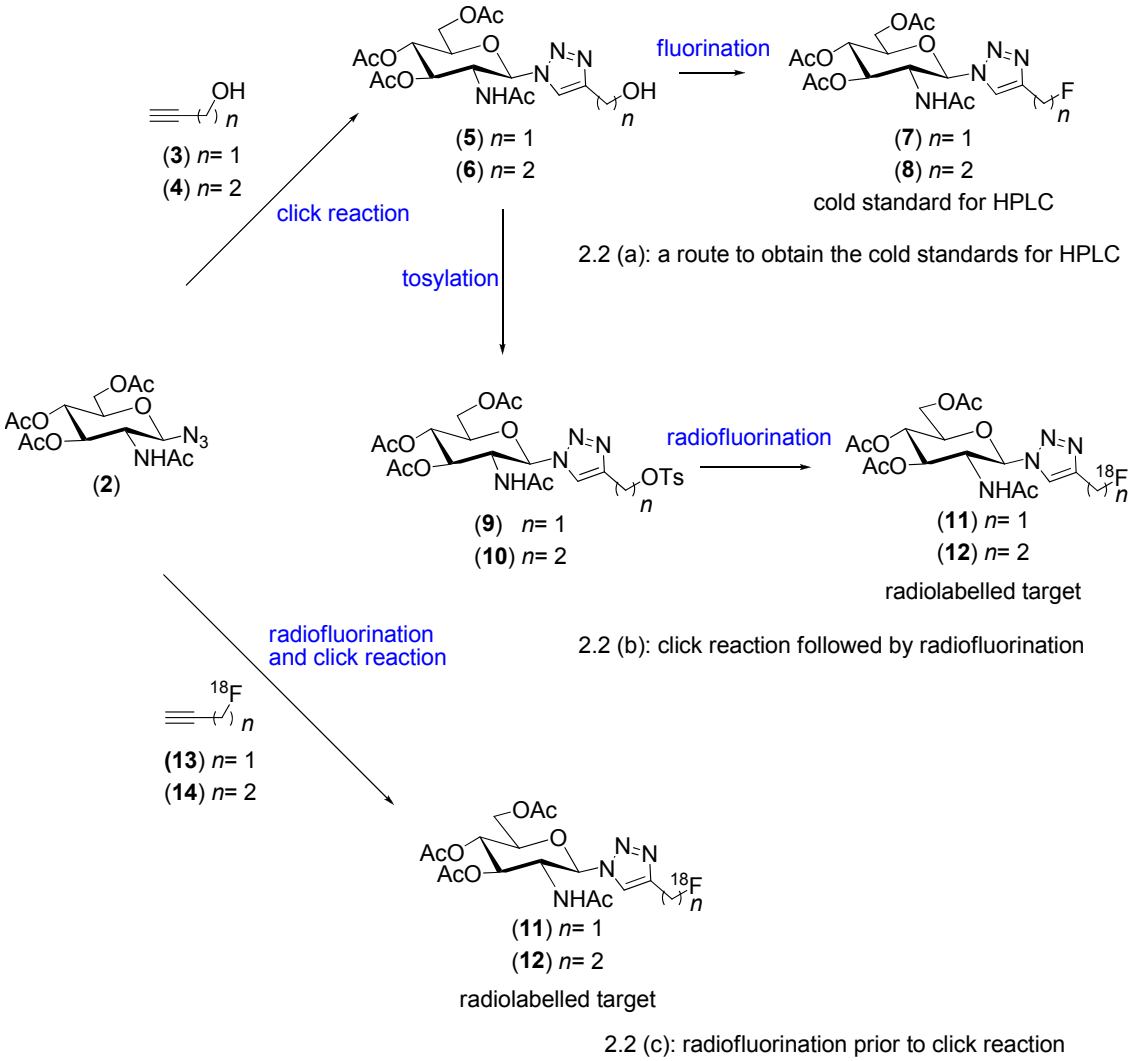
Fig. 2.1: A sugar amino azido acid (**1**) and the model amino azido sugar (**2**).

Sugar diamino acids (SDAs) bearing two amino groups (or an amino and azido group) and a carboxylic acid residue would be the ideal candidates for this type of chemistry ((**1**), fig.2.1). However, there are few reported SDAs in the literature and their synthesis is still largely in the developmental stage. SDAs such as (**1**) are difficult to synthesize so the commercially available 1-azidoglucosamine (**2**), was used as a simplified model of a SDA for this project. This sugar still has a free amino group for coupling to bioactive peptides and/or tumour targeting agents along with an azido group for click reaction (Fig. 2.1).

The general strategy behind the first part of the work is shown in scheme 2.2 and involves a click reaction between the commercially available 1-azidoglucosamine (**2**) with the alkynols (**3**) and (**4**), to give the hydroxy triazoles (**5**) and (**6**), which are converted to their fluoro derivatives (**7**) and (**8**). These latter compounds are used as cold standards for the HPLC purification and identification of the analogous radiolabelled material (Scheme 2.2a).

There were two routes then used to obtain the radiolabelled target (**12**). The first route involved subjecting the sugar (**2**) to a click reaction followed by radiofluorination (Scheme 2.2b). The second route involved radiofluorination prior to the click reaction (Scheme 2.2c). That is, the alkynols (**3**) and (**4**) could be converted to the [^{18}F]-fluoroalkyne derivatives (**13**) and (**14**) and coupled to (**2**) to give the radiolabelled targets (**11**) and (**12**) as shown in Scheme 2.2c.

Radiolabelling of 1-azido sugars via click chemistry using the fluoroalkyne (**14**) has been reported in the literature.[63] However, the use of the homologous fluoroalkyne (**13**) is novel and would expand the existing library of fluorination precursors for radiolabelling. The radiolabelling of (**2**), as shown below is described in detail in the following section.



Scheme 2.2: Various strategies to label the 1-azidoglucosamine (**2**).

There are several examples in the literature of 1-azido sugars undergoing click reactions to give a range of products including (**15**)[73], (**16**)[74], (**17**)[75], (**18**)[76] and (**19**)[77] in good yields as shown in Fig 2.2. The click reaction has been found to be a useful method for the glycosylation of peptides, for example to produce the alanine-type

derivative (**17**)[75] through the click reaction of an azido amino sugar and amino acid. However, most of the reported work in this area is not involved with the radiolabelling of sugars and therefore the proposed work using click chemistry for the radiolabelling of carbohydrates is novel. This work will also be a useful expansion to the existing research on sugar-click chemistry and potentially generate a new class of drugs based on glycosylated peptides.

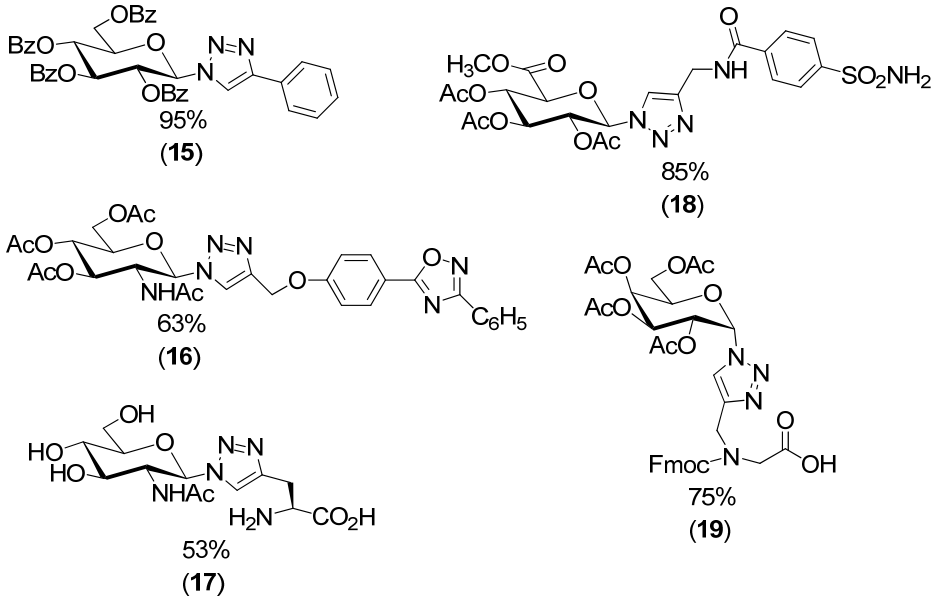
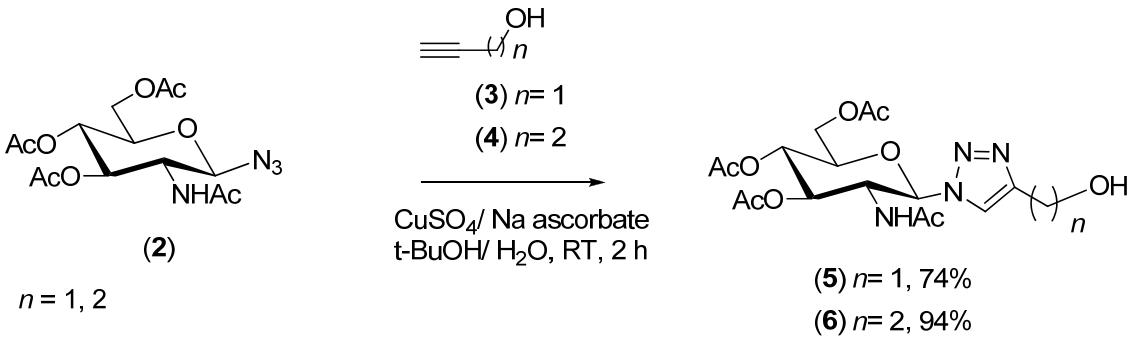


Fig 2.2: Selected examples of the products from reported click reactions on 1-azido sugars.

2.1.1 Click reaction of 1-azidoglucosamine (2) with alkynols (3) and (4)



Scheme 2.3: The click reaction of 1-azidoglucosamine (**2**) with alkynols (**3**) and (**4**).

Reported methods utilising click conditions, e.g., $CuSO_4$ and sodium ascorbate in a *tert*-butanol / water mixture (1:1), were used to synthesise both the known hydroxy triazole (**5**) and the novel derivative (**6**) (Scheme 2.3).[63] Analysis of the ^1H NMR spectra of the known derivative (**5**) is in accordance with the literature data for this compound.[76] The ^1H NMR spectra of the novel derivative (**6**) is similar to that of the homologue (**5**), as shown in Table 2.1. The full ^1H and ^{13}C NMR assignments of the novel compound (**6**) were based on 2D NMR data derived from gHSQC and gHMBC experiments, while 2D gCOSY experiments were done on the known compound (**5**). The structure of (**6**) was confirmed by assignment of the additional two protons of the methylene carbon to a multiplet peak at δ 2.99 with an integration of two protons. Further key ^1H and ^{13}C spectral assignments of compound (**6**) are shown in Fig. 2.3.

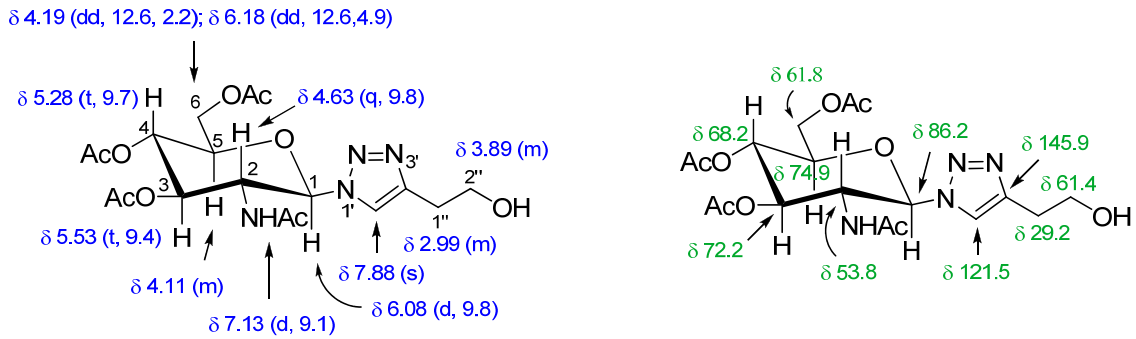


Fig. 2.3: Key ^1H and ^{13}C NMR spectral assignments for tetrazole (**6**) shown in blue and green respectively.

In addition, a molecular ion peak at, *m/z* 465 in the ESI mass spectrum was assigned to the MH^+ ion of the hydroxyl sugar (**6**), with the $(M + Na)^+$ formula of $C_{18}H_{26}N_4O_9Na$ confirmed by HRMS with a peak at, *m/z* 465.1596 compared to the calculated value of 465.1597. The optimisation of the reaction conditions afforded 74% of the known compound (**5**) and 94% of the novel compound (**6**), both as single products not requiring chromatographic purification. This is evidence, as proposed by

Kolb and co-workers, that click reactions have simple work-up procedures and are highly selective for a single product.[52]

Table 2.1: ^{1}H, ^{13}C and selected 2D NMR ($CDCl_3$, 400 MHz) data for the novel triazole sugar (**6**) and the known triazole sugar (**5**).

	(**6**)			(**5**)		
Position	δ_C	δ_H [m, *J* (Hz)]	*g*HMBC[a]	δ_C[b]	δ_H [m, *J* (Hz)]	*g*COSY
1	86.2	6.08 (d, 9.8)	2	90.0	6.03 (d, 9.9)	2
2	53.8	4.63 (q, 9.8)	1, 3	57.4	4.62 (q, 10.1)	3, 1, N*H*
3	72.2	5.53 (t, 9.4)	2, 4, *C*(O)CH_3	77.9	5.47 (t, 10.0)	2, 4
4	68.2	5.28 (t, 9.7)	3, *C*(O)CH_3	67.3	5.28 (t, 9.8)	3, 5
5	74.9	4.11 (m)	*C*(O)CH_3	73.4	4.01 (m)	4, 6a,6b
6a	61.8	4.19 (dd, 12.6, 2.2)	*C*(O)CH_3	78.7	4.14 (dd, 12.6, 2.0)	6b
6b	61.8	4.33 (dd, 12.6, 4.9)	*C*(O)CH_3	78.7	4.29 (dd, 12.6, 4.9)	6a
1’	-	-	-	-	-	-
2’	-	-	-	-	-	-
3’	-	-	-	-	-	-
4’	145.9	-	1”	148.0	-	
5’	121.5	7.88 (s)	1”, 2”, 5’	121.3	7.83	
1”	29.1	2.98 (m)	2”, 4’, 5’	56.1	4.80 (s)	
2”	61.4	3.89 (m)	1”	-	-	
OH	-	-[e]	-	-	2.67 (bs)	
O*C*(O)CH_3	169.4[d]	-	4	174.8[d]	-	
	170.7[d]	-	3, 6a, 6b	174.9[d]	-	
	170.8[d]	-	3, 6a, 6b	175.0[d]	-	
OC(O)*CH_3*	20.6[c]	2.07 (s)	*C*(O)CH_3, C(O)*C*H_3	25.7[c]	2.04 (s)	
	20.7[c]	2.08 (s)	*C*(O)CH_3, C(O)*C*H_3	25.9[c]	2.07 (s)	
	20.7[c]	2.09 (s)	*C*(O)CH_3, C(O)*C*H_3	26.0[c]	2.10 (s)	
N*H*C(O)CH_3	-	7.13 (d, 9.1)	*C*(O)CH_3	-	6.23 (d, 9.0)	2
NH*C*(O)CH_3	171.2[d]	-	-	175.5[d]	-	
NHC(O)*CH_3*	22.7	1.74 (s)	*C*(O)CH_3	27.8	1.76 (s)	

[a]Protons correlated to carbon resonances in the δ_C column. [b]Literature values for δ_C, ref.[76] [c]Assignments may be switched. [d]Assignments may be switched.

2.1.2 Fluorination of the hydroxy triazole sugars (**5**) and (**6**)

n = 1, 2 **(5)** *n*= 1
(6) *n*= 2

(7) *n*= 1, 10-33%
(8) *n*= 2, 17-30%

Scheme 2.4: Fluorination of the hydroxyl compounds (**5**) and (**6**) using diaminosulfur trifluoride(DAST).

The fluorination of the hydroxy triazole sugars (**5**) and (**6**) was carried out using diethylaminosulfur trifluoride (DAST), which is a common fluorinating agent for the conversion of aliphatic alcohols into alkyl fluorides.[78, 79] The synthesis of the methyl- and ethylfluoride derivatives (**7**) and (**8**) was based on procedures in the literature (Scheme 2.4).[63, 78] The preparation of these compounds was performed in order to obtain a cold (i.e. non-radioabelled) standard to be used in authenticating the ^{18}F-radiolabelled derivatives and in developing HPLC conditions for radiolabelling, purification and quality control (see Section 2.1.7).

In the analysis of the ^{1}H NMR spectra of the methyl fluoride derivative (**7**), a peak at δ 5.44-5.56 ppm, which appeared as a broad doublet with an integration of two protons and a coupling constant of 48 Hz, clearly showed the splitting of two protons by the fluorine atom in the fluoromethyl moiety. The splitting is due to the coupling of hydrogens with a fluorine atom which has a spin of ½, and typically gives short range coupling constants between protons and fluorine of about 47 Hz and long range coupling constants of around 25 Hz.[80] For the ethyl fluoride derivative (**8**), the structure of the product was confirmed by the presence of two mutually coupled, doublet of triplet peaks in the ^{1}H NMR spectrum at δ 3.10-3.19 and δ 4.63-4.78 with an integration of two protons each, which could be assigned to the four protons in the two methylene carbons.

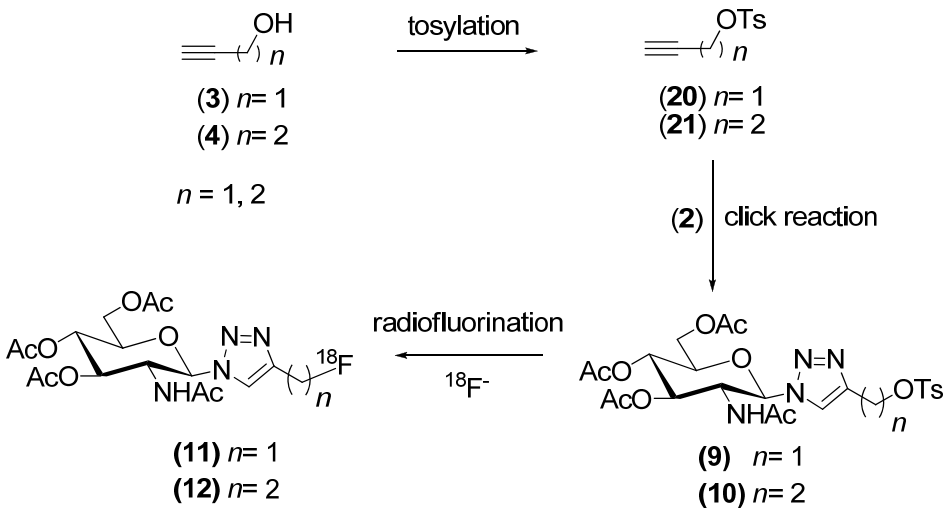
In addition, molecular ion peaks at, *m/z* 453.1 and 467.1 in the ESI mass spectra of the above compounds was assigned to the MH^+ ion of the fluoro compounds (**7**) and (**8**) respectively. Furthermore, the $(M + Na)^+$ formulae of $C_{17}H_{23}N_4O_8NaF$ and $C_{18}H_{25}N_4O_8NaF$ were confirmed by HRMS with peaks observed at, *m/z* 453.1393 and 467.1577 compared to the calculated values of 453.1398 and 467.1554 respectively.

The low yields obtained in the synthesis of these two compounds, (**7**) and (**8**), may be due to some decomposition occurring prior to, and after, purification. This is based on observations from thin layer chromatography (TLC) that was performed 30-45 min after the reaction, and then again before and after the purification step. Furthermore, preparative TLC was required to isolate the fluoromethyl derivative (**7**) after initial fractionation by gravity column chromatography was not successful. To minimise any further decomposition, the fluorinated derivatives (**7**) and (**8**) were kept at -18 ^oC in the freezer. Although the yields for these compounds were low, they were adequate for the required purpose, which was to obtain a cold standard for HPLC. The next step after this reaction would be the deprotection/removal of the acetyl groups, whereupon the product could also be used as a cold standard for HPLC.

2.1.3 Synthesis of propargyl tosylate (**20**) and butynyl tosylate (**21**)

The radiolabelled target systems for this study are the ^{18}[F]-fluoro triazoles (**11**) and (**12**), which will be prepared using ^{18}[F]-fluoride. DAST can be used for the fluorination of the triazole alcohols (**5**) and (**6**), however, it is not available as a radiolabelled reagent, and therefore a good leaving group (such as the tosyl group) needs to be introduced so that the radiofluorination can be performed by a nucleophilic substitution reaction. There are two options to prepare the tosylated compounds (**9**) and (**10**), the first is via a click reaction of the 1-azido glucosamine (**2**) with the alkynols (**3**)

and (**4**) then tosylation of the hydroxyl triazole (**5**) and (**6**) (Scheme 2.2b). The second option involves the tosylated alkynes (**20**) and (**21**) which can be clicked with (**2**) to form the tosylated derivatives (**9**) and (**10**).

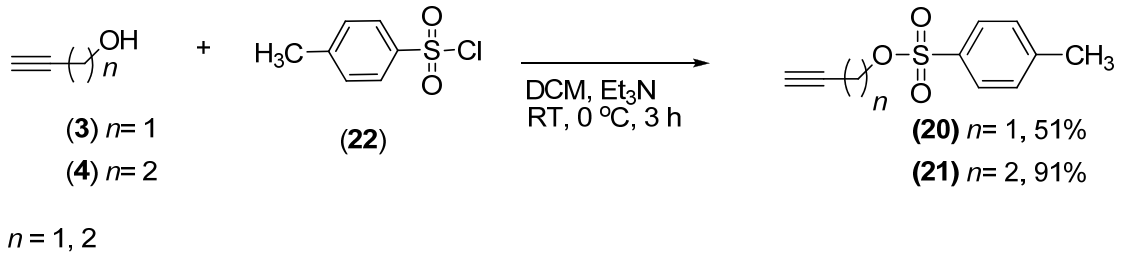


Scheme 2.5: A strategy to radiolabel the target system (**12**) by employing a click reaction with the tosylated alkynes (**20**) and (**21**).

Tosylation of propargyl alcohol (**3**) and butynyl alcohol (**4**), in the presence of triethylamine as a base to remove the hydrochloric acid formed from the reaction with tosyl chloride (**22**), was performed in order to prepare the tosylated alkynes (**20**) and (**21**) (Scheme 2.6).[63] These alkynes could then be used either in a click reaction with 1-azido glucosamine (**2**), to prepare the tosylated triazoles (**9**) and (**10**) as precursors for the radiofluorination step (Scheme 2.5), or in the preparation of the [^{18}F]-fluoroalkynes (**13**) and (**14**) (section 2.17) for the direct click attachment of the radiolabel to the glucosamine (**2**) (Scheme 2.2c).

The tosylated products (**20**) and (**21**) were confirmed by analysis of their MS and ^{1}H NMR spectra. All signals were identical with those for the same compounds reported in the literature.[63, 81] The tosylated compounds (**20**) and (**21**) were obtained in 51% and 91% yield respectively, with an improved yield for (**21**) compared to the yield

in the literature (72%).[63] In contrast, the yield of (**20**) was lower than the reported yield (96%).[81]



Scheme 2.6: Tosylation of the propargyl- and butynyl alcohols (**3**) and (**4**)**.**

2.1.4 Attempted click reaction of 1-azido glucosamine (2) with various alkynes

Several different conditions were used to optimize the click reaction on the 1-azido glucosamine (**2**) with different alkynes as shown in Table 2.2.

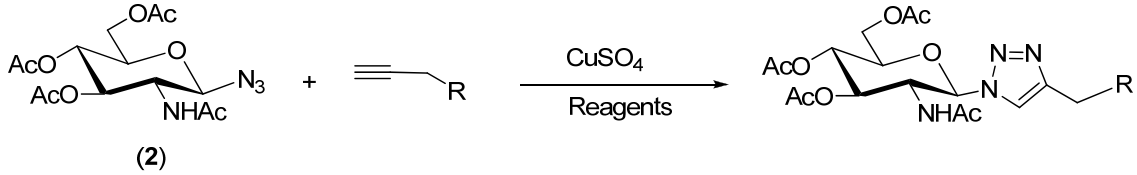


Table 2.2: Attempted click reaction on 1-azido glucosamine with different alkynes.

Entry	R	Reagents	Temp. (°C)	Time	Yield (%)
1	OH	Ascorbic acid, H_2O/*Tert*-butanol	50	O/N	10
2	OH	Ascorbic acid, Sodium hydrogen carbonate, H_2O/*Tert*-butanol	RT	2h	67
3	OH	Sodium ascorbate, H_2O/*Tert*-butanol	RT	2h	74
4	CH_2OH	Ascorbic acid, Sodium hydrogen carbonate, H_2O/*Tert*-butanol	RT	2h	80
5	CH_2OH	Sodium ascorbate, H_2O/*Tert*-butanol	RT	2h	94
6	OTs	Ascorbic acid, Sodium hydrogen carbonate, H_2O/*Tert*-butanol	50	O/N	0
7	OTs	Sodium ascorbate, H_2O/*Tert*-butanol	RT	2h	0
8	CH_2OTs	Sodium ascorbate, H_2O/*Tert*-butanol	RT	2h	0

The click reaction on 1-azido glucosamine (**2**) was successful with the propargyl- (**3**) and butynyl alcohol derivatives (**4**) as shown in Entries 1-5. The use of sodium ascorbate with H_2O/ *tert*-butanol gave the best results and the highest yield of the

desired compounds (Entries 3 and 5), while the combination of ascorbic acid and hydrogen carbonate to form the sodium ascorbate under 50 ^{o}C and in an overnight reaction, gave the lowest yields (e.g. Entry 1).

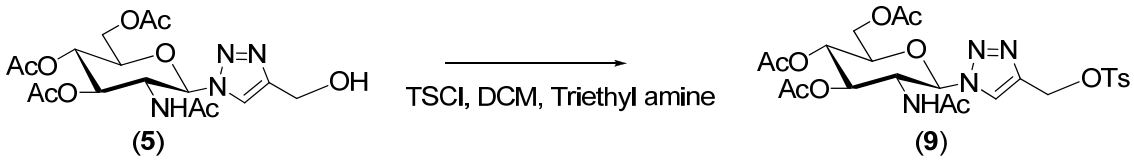
The attempted click reaction of 1-azidoglucosamine (**2**) with the tosylated alkynes (**20**) and (**21**) (Entries 6-8) was carried out in order to prepare the tosylated triazoles (**9**) and (**10**) which can be labelled directly with ^{18}F (Scheme 2.2b and 2.5). Unfortunately, the click reaction with the tosylated alkynes was unsuccessful (Entries 6-8). Based on TLC analysis of the reaction mixtures, they appeared to go to completion with the formation of a new product, however ^{1}H NMR analysis of the crude reaction product showed that the tosylated alkynes (**20** and **21**) had not "clicked" with the 1-azido glucosamine (**2**), as there were no signals for a triazole proton (normally at δ7.78- 8.03 ppm) present. The nature of the product obtained is unclear at this stage and this reaction was not pursued further.

2.1.5 Attempted tosylation of the hydroxy triazole sugar (**5**)

As the click reaction of the 1-azido sugar (**2**) with the tosylated alkynes (**20**) and (**21**) did not afford the tosylated triazoles (**9**) and (**10**), the preparation of these compounds was attempted by tosylation of the corresponding hydroxyl triazoles (**5**) and (**6**) (Scheme 2.7). This reaction was performed in order to compare the synthesis time and yield of the radiolabelled targets (**11**) and (**12**) using two different routes (see Schemes 2.2b and 2.2c).

An investigation into whether the click reaction followed by radiofluorination (Scheme 2.2b), or radiofluorination prior to the click reaction (Scheme 2.2c), would give higher yields and shorter synthesis times (as reported in the literature[63]) was planned. The product of the reaction below (Scheme 2.7) could potentially be

fluorinated directly with ^{18}F, however, the tosylation of the hydroxy triazole sugar (**5**) was unsuccessful. This reaction was attempted two times.



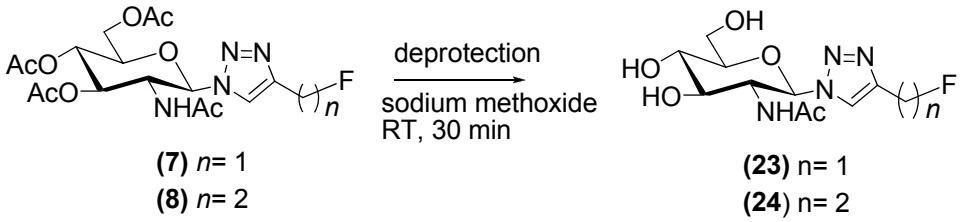
(**5**) (**9**)

Scheme 2.7: Attempted tosylation of the hydroxy triazole sugar (**5**).

The reaction was set up in 100 mg scale of (**5**) for the first attempt and 50 mg the second time. In the first attempt the reaction was stirred at 0 ^{o}C for 3 h. The ^{1}H NMR of the crude product after the first work up showed only the triethylamine signal and aromatic ring of the tosylate group but none of carbohydrate signal even though the extraction afforded a large amount of crude material. Then re-extraction of the aqueous layer gave an additional 20 mg of crude product which consisted of some of the desired compound (**9**) as detected by LRMS (ESI^{+}).

In the second attempt the reaction was stirred at 0 ^{o}C for 3 h then left overnight at 4 ^{o}C. The tosylated compound (**9**) should be less polar than the starting material (**5**), however there was no such compound evident on the TLC. Instead, TLC analysis showed the formation of several polar by-products and therefore the work-up was not performed. The LRMS (ESI^{+}) on the reaction mixture also did not show the presence of peaks of matching those of the desired compound (**9**). Neither this reaction, nor the attempt to tosylate the hydroxyl sugar (**6**) was investigated any further. In addition, the literature showed that the route involving radiofluorination prior to the click reaction (Scheme 2.2c) should give higher yields and shorter synthesis times for the radiolabelled targets (**11**) and (**12**), and therefore we decided to change our strategy.[63]

2.1.6 Deprotection of the acetyl groups of the fluorinated compounds (7) and (8)



(7) *n*= 1
(8) *n*= 2

(23) n= 1
(24) n= 2

Scheme 2.8: Deprotection of the fluorinated compounds (**7**) and (**8**) using sodium methoxide.

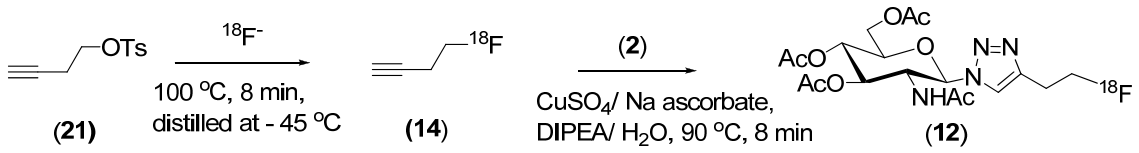
This reaction was carried out in order to investigate whether the click reaction could be performed without the need for protecting the sugar hydroxyls as stated in the literature.[54, 63, 82] Once again, the products of this type of reaction, (**23**) and (**24**), could be used as cold standards for developing the HPLC conditions for the radiolabelled analogues. Unfortunately, the preparation of (**23**) and (**24**) using this approach was not successful in obtaining the pure product.

Due to the low yield obtained for the preparation of the fluorinated starting materials (**7**) and (**8**) (see section 2.1.2), the attempted deprotection of the acetyl groups described above was performed on just 100 mg and without purification of the starting materials (Scheme 2.8). This resulted in the production of numerous side products based on TLC analysis. The ^{1}H NMR spectra of the crude product also did not readily show the presence of the desired product, although some of it was detected by LRMS (ESI^{+}). The attempted extraction of (**23**) and (**24**) from the reaction mixture gave a low crude yield (~30 mg) and due to time constraints, the purification and repetition of the reaction was not performed.

2.1.7 Preparation of radiolabelled target (12)

The radiolabelling work described here was performed at ANSTO under the direction of the radiopharmaceutical chemists (Dr Tien Pham and Dr Thomas Bourdier) to show that labelling of carbohydrates with ^{18}F can be readily achieved through click chemistry. The method used follows the route described in section 2.1.5, i.e. radiofluorination prior to the click reaction, and utilizes the fluorinated substrate (**8**) as the cold standard for HPLC identification of the target (**12**) via peak matching.

The labelling of the 1-azido glucosamine (**2**) began with radiofluorination of the tosylated precursor (**21**) to give 4-[^{18}F]-fluoro-1-butyne (**14**) via nucleophilic substitution (Scheme 2.9). To a dried K[^{18}F]fluoride/Kryptofix$_{222}$ complex the tosylated alkyne (**21**) in acetonitrile was added and the reaction facilitated by heating, after which micro-distillation was used to obtain the desired compound (**14**)[62, 63] (Scheme 2.9). The fluorobutyne (**14**) has a boiling point of 45 °C and was chosen as the preferred alkyne to label the 1-azido glucosamine (**2**) as it is already used in the literature. Furthermore, the lower boiling point of propargyl fluoride (15 °C) would make it more difficult to handle.[62, 63]



Scheme 2.9: Labelling of 1-azido glucosamine (**2**) with ^{18}F via a click reaction with the fluorobutyne (**14**).

With the radiolabelled alkyne in hand, the next step was the click reaction with the 1-azido glucosamine (**2**) (Scheme 2.9). Diisopropylethylamine (DIPEA, m.p. -50 °C) was used for the click reaction during the radiofluorination instead of *tert*-butanol (m.p. 23-26 °C), as the latter would freeze during the distillation at -45 °C. DIPEA also gave

the best results of the click reaction during the distillation to produce 4-[^{18}F]fluoro-1-butyne (**14**).[50, 62, 63] The reagents were combined and the reaction stirred for 8 min after which, the reaction mixture containing the desired radiolabelled product (**12**) was filtered to remove any precipitate. The reaction mixture was then directly injected onto a Bondclone 300 x 7.8 mm column, with a flow rate of 4 mL/min. A fraction was collected between 7.0-13.4 min (Fig. 2.4), which showed 741 µCi of radioactivity.

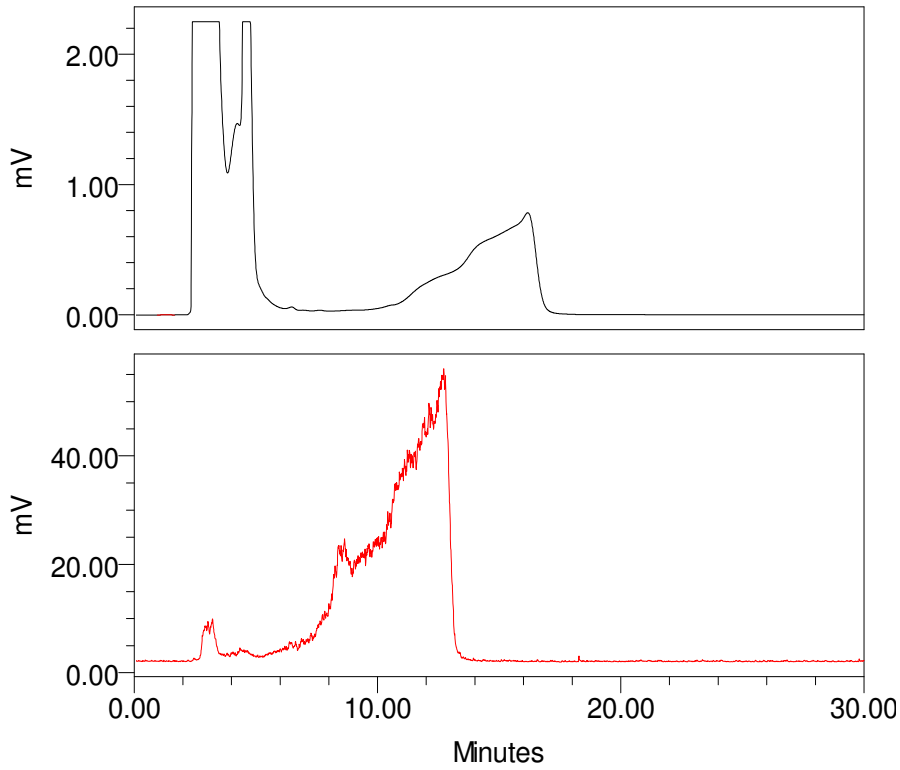


Fig. 2.4: HPLC traces of the reaction mixture from the preparation of (**12**) using a Bondclone 300 x 7.8 mm column (solvent: 20% ACN, 80% H_2O, 0.1% TFA at 4 mL/min, λ= 226 nm). UV detector shown in black and radioactivity detector shown in red.

An aliquot from this fraction, which contained 500 µCi, was co-injected with the cold fluorine standard (**8**), with co-elution of the peaks between 12.7-13.6 min confirming the presence of the radiolabelled target (**12**) (Fig. 2.5). The 4-[^{18}F]fluoro-1-butyne (**14**) was successfully synthesized in acetonitrile at 100 ^{o}C in 50%

radiochemical yield (unoptimised), while the click reaction to form the radiolabelled compound (**12**) was successful at 90 °C for 8 min with a radiochemical yield of 22% (unoptimised) and radiochemical purity of 80%.

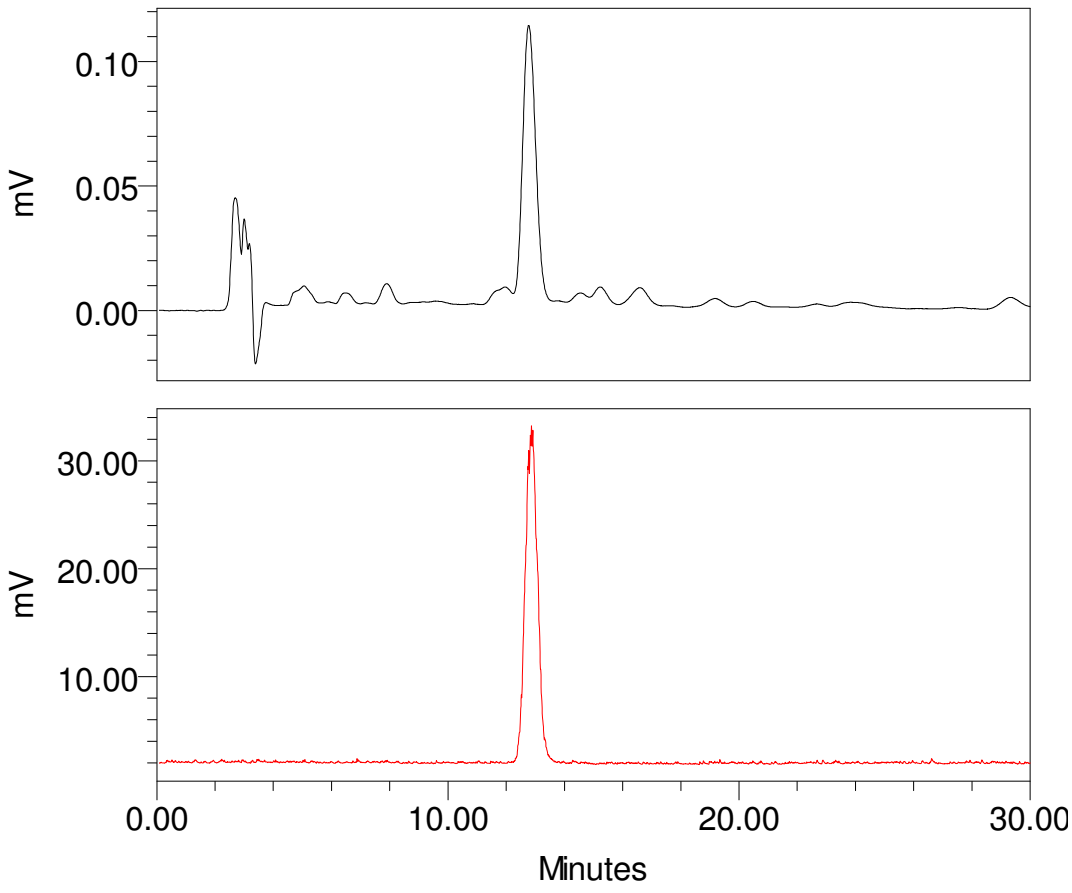


Fig. 2.5: Co-injection of the fluoro standard (**8**) (black) and the radiolabelled product (**12**) (red) on a Bondclone 300 x 7.8mm HPLC column (solvent: 20% ACN, 80% H_2O, 0.1% TFA at 4 mL/min, λ= 226 nm).

The 4-[^{18}F]-fluoro-1-butyne (**14**) and the corresponding click reaction with the azido sugar to give (**12**) was successfully synthesized in 50% and 22% radiochemical yield respectively, however the reaction (temperature, time, solvent and concentrations of reagents) and the microdistillation parameters were not yet optimised. The volatility and efficiency of the 4-[^{18}F]-fluoro-1-butyne (**14**) from a Wheaton microreactor using

peak tubing during the reaction conditions (heating at 100 ^{o}C in acetonitrile) poses further challenges with respect to yield losses and efficiency in transfers.

The radiochemical yield (RCY) obtained here which is 22%, via the click chemistry are lower than the use of acylation method (RCY 25-60%) (Table 1.3). But it is comparable using the imidation method (RCY 20-23%) (Table 1.3). In terms of time, the use of the click reaction is superior which only took 8 min while the acylation and imidation method took 35-100 min and 45 min respectively to complete.[47]

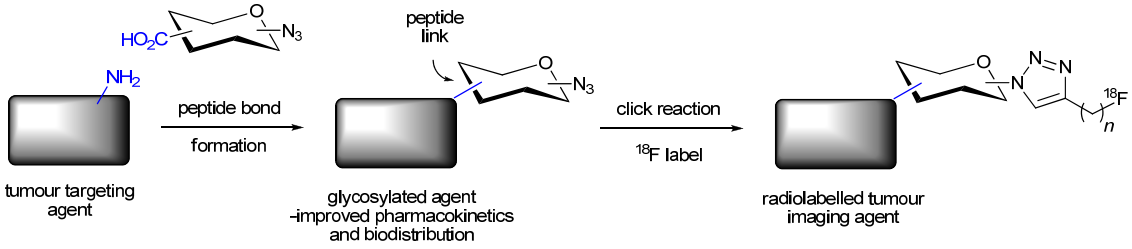
In summary, this chapter has detailed the preparation of the radiolabelled sugar (**12**) derived from the commercially available 1-azido glucosamine (**2**). Deacetylation at C-2 of (**12**) would give a free amine which could be coupled to an available carboxylic acid on bioactive peptides to potentially generate new imaging agents. The next chapter will present the extension of the work on the click reaction to the preparation and radiolabelling of 1-azido glucuronic acid derivatives, 2-azido sugar derivatives and the use of other alkyne reagents such as 3-ethynylphenol.

CHAPTER 3

RESULTS AND DISCUSSION (PART II)

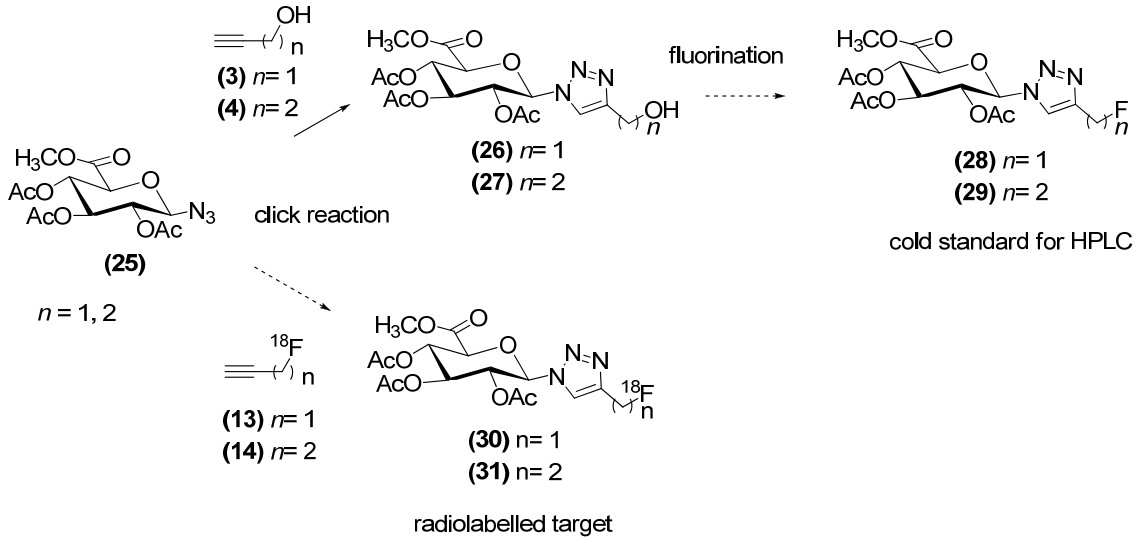
The radiolabelling methodology described in Chapter 2 was extended to the labelling of 1-azido glucuronic acid derivatives, which can be coupled via the carboxylic acid to the free amino moiety of appropriate tumour targeting agents such as the peptide cyclo-RGD. The work was then further extended to the labelling of 2-azido sugars and to the use of other alkyne reagents such as 3-ethynylphenol.

3.1 Labelling of 1-azidoglucuronic acid methyl ester (25) with aliphatic alkynes



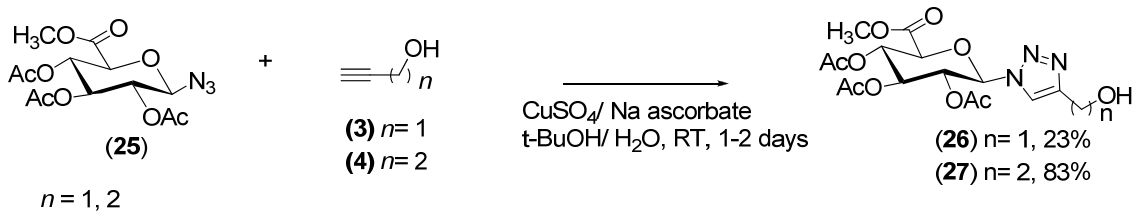
Scheme 3.1: General scheme for glycosylating and subsequent radiolabelling of tumour targeting agents with a free amino moiety.

In the first part of the study, an azido sugar bearing an amino group (protected as NHAc) was employed, where the amino group could be made available for coupling to a carboxylic acid-containing bioactive peptide and/or tumour targeting agent (Scheme 2.1). In the second part of the study, the aim was to extend the labelling methodology described earlier to the labelling of 1-azido glucuronic acid methyl ester (**25**) as shown in Scheme 3.2. Removal of the ester group by saponification would give a free carboxylic acid at C-6, which could be readily coupled to a free amino group on a tumour targeting peptide (Scheme 3.1). A similar strategy to that employed in Chapter 2 could be used to label the glucuronic acid derivative (Scheme 3.2).



Scheme 3.2: Strategy for labelling1-azidoglucuronic acid methyl ester with fluoroalkynes.

3.1.1 Click reaction of 1-azidoglucuronic acid methyl ester (25) with alkynols (3) and (4)



Scheme 3.3: Click reaction of glucuronic acid derivative (**25**) with propargyl (**3**) and butynyl (**4**) alcohol.

The click reaction of 1-azidoglucuronic acid methyl ester (**25**) with alkynols (**3**) and (**4**) was performed according to Scheme 3.3. Analysis of the ^{1}H NMR spectra of the product obtained from the reaction with propargyl alcohol, showed a singlet peak at δ 4.79 with an integration of two protons, which was assigned to the two protons in the methylene carbon, while another singlet peak at δ 3.74 was assigned to the three protons of the methyl ester and a one proton singlet at δ 7.86 was assigned to the triazole proton of (**26**), confirming that the click reaction was successful. The product from the reaction with butynyl alcohol, that is compound (**27**), was confirmed by

assignment of the additional two protons of the methylene carbon to a broad singlet (likely to be an unresolved triplet) at δ 2.96 with an integration of two. In addition, the $(M + Na)^+$ formulae of $C_{16}H_{22}N_3O_{10}$ and $C_{17}H_{24}N_3O_{10}$ were confirmed by HRMS with peaks at 416.1305 and 430.1451, compared to the calculated values of 416.1305 and 430.1462 respectively. 2D NMR experiments including gCOSY, gHSQC and gHMBC were used in assigning the ^1H and ^{13}C NMR spectra of compound (**26**), which then assisted in the assignment of peaks in ^1H and ^{13}C NMR spectra of (**27**).

The products (**26**) and (**27**) were obtained as white solids in 23% and 83% yield respectively. The low yield of (**26**) is because lots of starting material remained in the reaction after 48 h based on TLC analysis. This reaction did not proceed further even with the addition of aliquot of $CuSO_4$/ Na ascorbate , and the product required purification by gravity column chromatography to separate it from the starting materials. In contrast, compound (**27**) was obtained in high yield after extraction from the reaction mixture. Due to time constraints, the remaining steps in Scheme 3.2, i.e. the fluorination of (**26**) and (**27**) with DAST and the radiolabelling step, were not yet performed.

3.2 Labelling the 2-azido sugar (32) with aliphatic alkynes

There are several examples in the literature of click reactions of azido sugars where the azido group is at the anomeric position, however, there are only three reported examples of a 2-azido-2-deoxy sugar undergoing click chemistry (Fig. 3.1).

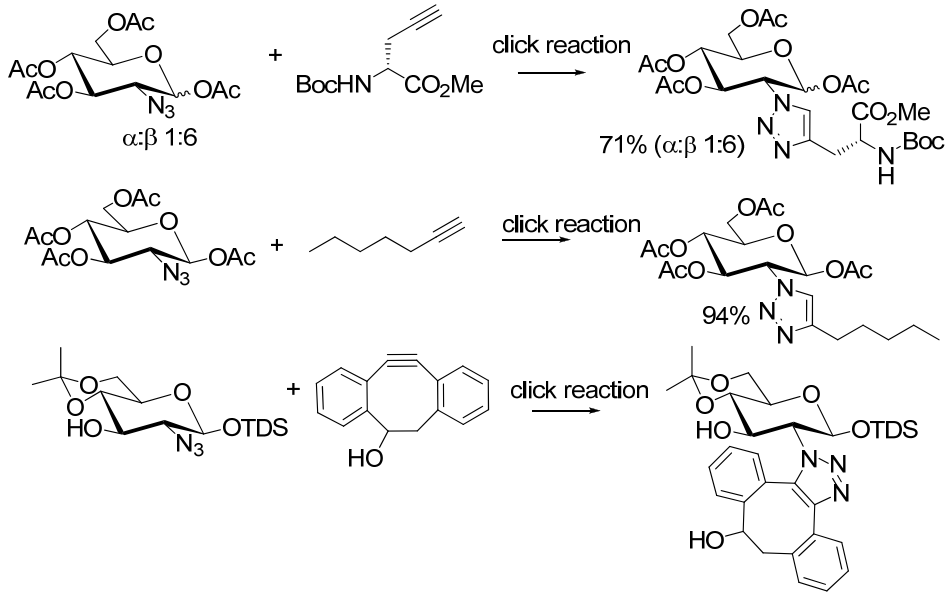
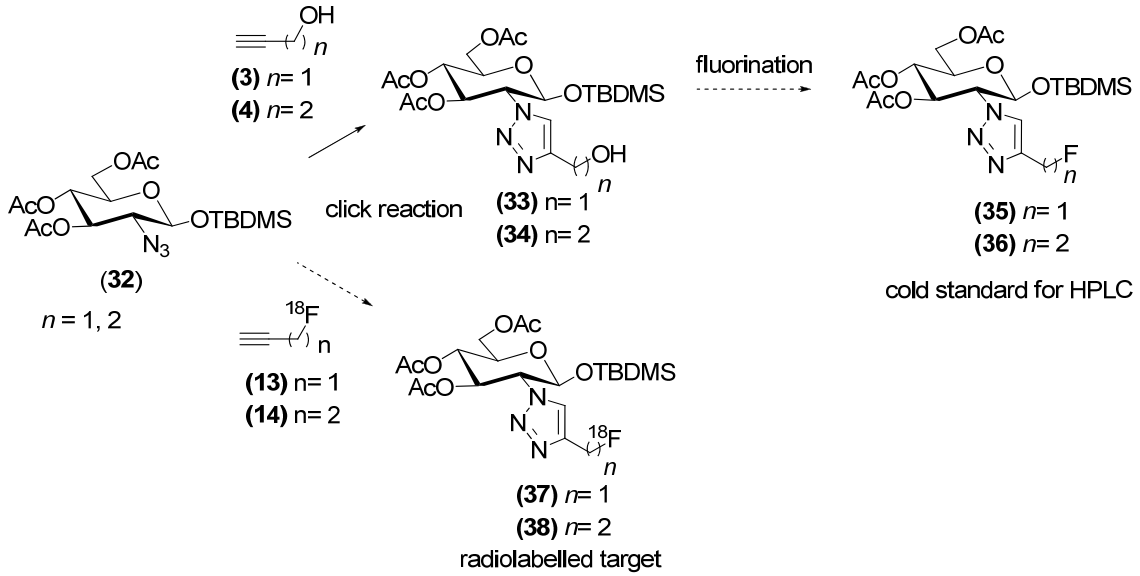


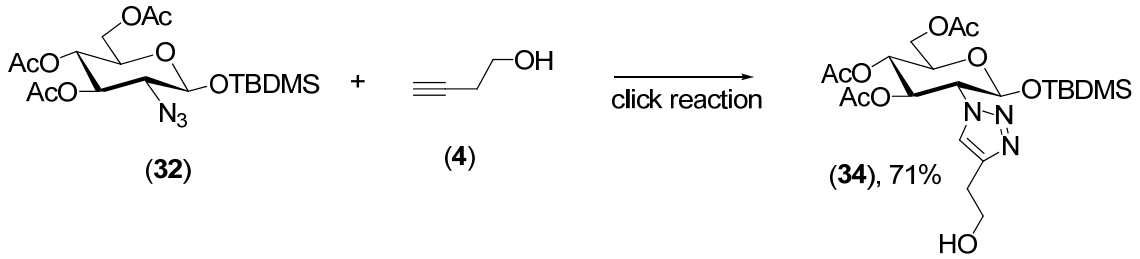
Fig. 3.1: Examples of 2-azido sugars undergoing click chemistry.[83, 84, 85]

Another project within our research group, involved the development of sugar amino acids (SAAs) bearing an azido group at C-2. Therefore, it was of interest to extend these investigations on sugar-click chemistry to the reaction of 2-azido sugars. As 2-azido SAAs are time-consuming to prepare, it was desirable to model the reaction using the commercially available and simplified 2-azido sugar (**32**). Not only would this be of use to other projects in the research group, but it would also add to the limited repertoire of this type of reaction in the literature. The strategy to label the TBDMS-protected 2-azido sugar (**32**) is shown in Scheme 3.4.



Scheme 3.4: A strategy to label the 2-azido sugar (**32**) with aliphatic alkynes**.**

3.2.1 Click reaction of the 2-azido sugar (32) with butynyl alcohol (4)



Scheme 3.5: The click reaction of the 2-azido sugar (**32**) with butynyl alcohol (**4**), see Table 2.2 for reaction conditions.

Four sets of different conditions were investigated on a small-scale to optimise the click reaction on the 2-azido sugar (**32**) (Scheme 3.5, Table 3.1). Reactions performed under 80 °C and using *tert*-butanol proceeded but still had lots of starting material remaining as judged by TLC (Entries 1 and 3), whereas the reaction in dichloromethane did not proceed at all (Entry 2). The desired product was present as determined by LRMS (ESI^+) under the conditions described for Entry 1 and 3, however due to the small scale of the reactions (around 20 mg), isolation and purification of the product was not performed. It appears that the reaction proceeds in the presence of *tert*-butanol but not in the presence of dichloromethane.

Table 3.1: Conditions for the attempted click reaction on the 2-azido sugar (**32**) with butynol (**4**).

Entry	Solvent	Temp. (^{o}C)	Time (h)	Product detected (LRMS)	Isolated yield (%)
1	H_2O/ t-BuOH (1:1)	40-80	24	Yes	-†
2	H_2O / CH_2Cl_2 (1:1)	RT	24	No	-†
3	H_2O/ t-BuOH / CH_2Cl_2 (1:1:1)	RT	24	Yes	-†
4	H_2O/ t-BuOH (1:1)	80-120	72	Yes	71

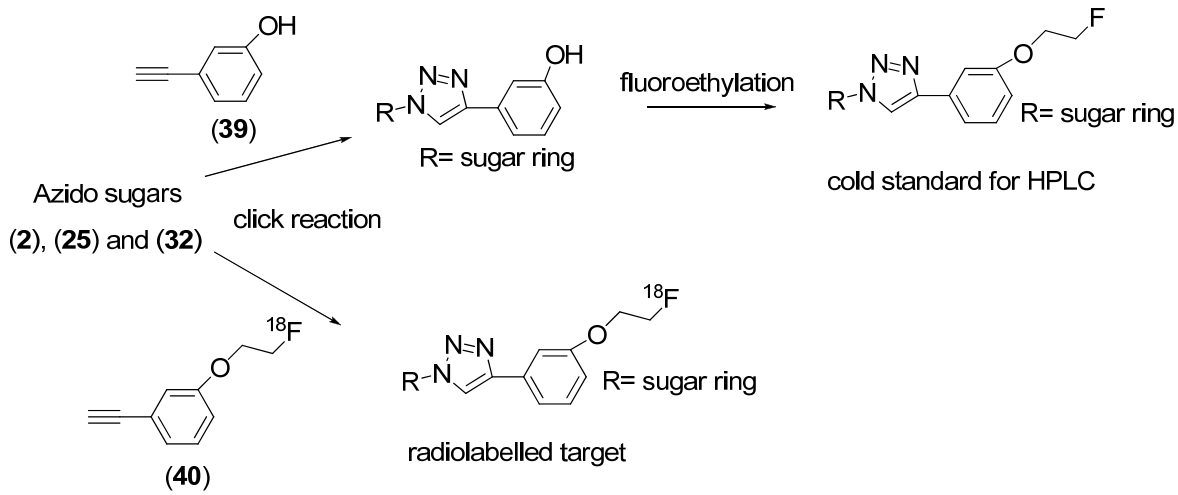
† Reaction performed on small scale (< 30 mg) and therefore isolation of product not attempted.

The scale up reaction (Entry 4) afforded the novel compound (**34**) in a rewarding 71% yield. The product was confirmed by analysis of the ^{1}H NMR spectrum, which showed a new peak at δ 7.49 as a singlet with an integration of one proton that was assigned to the triazole proton of (**34**). The formation of the triazole confirmed that the 2-azido sugar underwent a click reaction with butynyl alcohol (**4**). The assignment of the ^{1}H and ^{13}C NMR spectra of (**34**) was assisted by 2D NMR (gCOSY, gHSQC and gHMBC). In addition, a molecular ion at, *m/z* 534 in the ESI mass spectrum was assigned to the $(M + Na)^+$ ion of (**34**), whereas the $(M + H)^+$ formula, $C_{22}H_{38}N_3O_9Si$ confirmed by HRMS with a peak observed at 516.2371, compared to the calculated value of 516.2377. Due to time constraints, the remaining synthetic steps in Scheme 3.4 were not pursued at this stage, but are planned for future work.

3.3 Labelling 1- and 2-azidosugars with aromatic alkynes

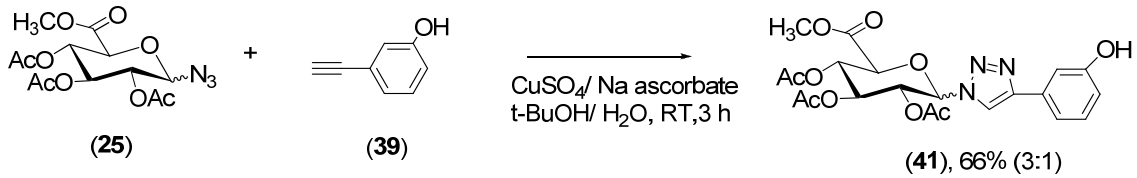
Up until now, the focus has been on attaching the ^{18}F label through 3-fluoropropynyl (**13**) and 4-fluorobutynyl (**14**) type residues, which both have low boiling points of 15 ^{o}C and 45 ^{o}C respectively.[63] In this section, the work was extended to the development of other radiolabelling precursors using commercially available alkynes of greater molecular weight and higher boiling points, such as the acetylenic phenol (**39**). The proposed route for labelling 1- and 2-azido sugars with aromatic alkynes is shown in Scheme 3.6. The acetylenic phenol (**39**) could be converted to the ^{18}F derivative using the same type of chemistry as that used in the production of the well known imaging agent, [^{18}F]-fluoroethyltyrosine ([^{18}F]FET).[86] The acetylenic phenol (**39**) would be fluorinated using 1-tosyloxy-2-[^{18}F]fluoroethane, then the [^{18}F]fluoro acetylenic phenol could be clicked to the 1- and 2-azido sugars.

The novel radiolabelled precursor (**40**) could then be "clicked" to 1- and 2-azido sugars as described earlier, providing a new series of labelled compounds that are easier to handle. The stability, ease of synthesis, yields and other parameters of the various radiolabelled compounds could be compared to one another in order to determine the best radiolabelling strategy for azido sugars.



Scheme 3.6: A strategy for the labelling of azido sugars via aromatic alkynes.

3.3.1 Click reaction of 1-azidoglucuronic acid methyl ester (25) with the acetylenic phenol (39)



Scheme 3.7: The click reaction of 1-azidoglucuronic acid methyl ester (**25**) with aromatic alkyne (**39**).

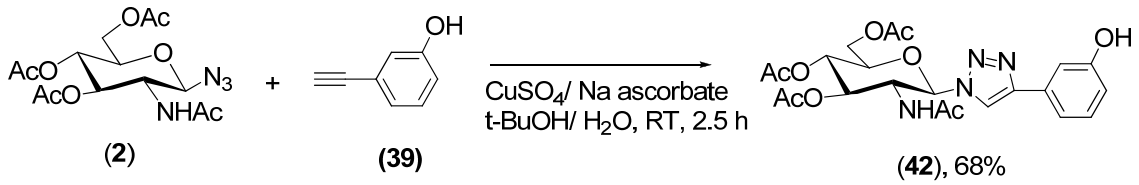
The click reaction was performed as shown in Scheme 3.7, resulting in a good yield of (**41**). The reaction took around 3 h to reach completion as judged by TLC. The product obtained after extraction appeared as a single spot on TLC, however, the NMR spectrum of the crude product showed there were impurities. Consequently, HPLC was used to purify the compound. During the HPLC runs the reaction mixture precipitated several times, which could be avoided by adding dimethylformamide.

The product (**41**) was obtained as an isomeric mixture in the ratio 3:1 under the conditions described in Scheme 3.7. Presumably these are α/β-anomers rather than 1,4/1,5-regiosiomers due to the large chemical shifts differences observed for the protons on the sugar ring (e.g. 6.43 and 6.21 ppm for the major and minor H1 proton, respectively). In contrast, under the conditions described in Scheme 3.3, the same starting material (**25**), gave a single product. Analysis of the ^{1}H NMR spectra of the product showed a peak at δ 7.28 as a multiplet with an integration of three protons and another at δ 6.75 as a multiplet with an integration of one proton, which were assigned to the four protons in the aromatic ring. A one proton singlet at δ 8.94/ 8.82* was assigned to the triazole proton, confirming the production of compound (**41**). The 2D NMR derived from gCOSY and gHSQC were used in assisting the assignment of the ^{1}H and ^{13}C NMR spectra of the desired compound.

* Belongs to the minor isomer.

In addition, the ESI^+ mass spectrum of the phenol product (**41**), a peak at, *m/z* 500.3, was assigned to the $(M + Na)^+$ ion. Furthermore, the $(M + H)^+$ formula of $C_{21}H_{24}N_3O_{10}$ was confirmed by HRMS with a peak at, *m/z* 478.1481 compared to the calculated value of 478.1462. The fluoroethylation of (**41**) could not be performed due to time constraints, but is planned for future work.

3.3.2 Click reaction of 1-azidoglucosamine (2) with the acetylenic phenol (39)



Scheme 3.8: Click reaction of 1-azido glucosamine with the aromatic alkyne (**39**).

Preparation of the phenolic triazole sugar (**42**) provides another useful example to add to the existing library of click reactions on anomeric azido sugars. The click reaction of the 1-azidoglucosamine (**2**) with the aromatic alkyne (**39**) was performed as shown in Scheme 3.8. The reaction took 2.5 h to reach completion, after which the reaction mixture was filtered and purified by HPLC. Analysis of the ^1H NMR and ^{13}C NMR spectra confirmed the isolation of the product (**42**), where peaks at δ_H 8.74 and δ_C 120.3 were assigned to the triazole proton and triazole carbon respectively. The four protons of the aromatic ring were assigned to peaks at δ 7.25, which appeared as a multiplet with an integration of three, and a peak at δ 8.11 as a doublet with an integration of one proton. The broad singlet at δ 9.60 with an integration of one proton was assigned to the hydroxyl proton. The 2D NMR on related compounds (e.g. gCOSY data of (**5**) and gHSQC of (**41**) were used to assist in the assignment of the ^1H and ^{13}C NMR spectra of (**42**).

In addition, a base peak at, *m/z* 513 in the ESI^+ mass spectrum was assigned to the $(M + Na)^+$ ion of the phenol product (**42**), with the $(M + Na)^+$ formula of $C_{22}H_{26}N_4O_9Na$ confirmed by HRMS with a peak at, *m/z* 513.1586 compared to the calculated value of 513.1597. Again, due to time constraints, the remaining steps in Scheme 3.6, i.e. fluoroethylation of (**42**) and the click reaction of 2-azido sugar (**32**) with the aromatic alkyne (**39**), remain for future work.

In conclusion, this chapter has described the development of a new method for radiolabelling various carbohydrates for use in PET imaging through the utilisation of click chemistry. We have shown that the click reaction is very useful and easy to perform under mild conditions to conjugate various alkynes with 1- and 2-azido sugars. The method developed here could be used to radiolabel various azido sugars, including the SAAs and SDAs, which are in development within our research group. These radiolabelled sugars have the potential to become key building blocks in the development of improved peptidomimetic imaging agents.

CHAPTER 4

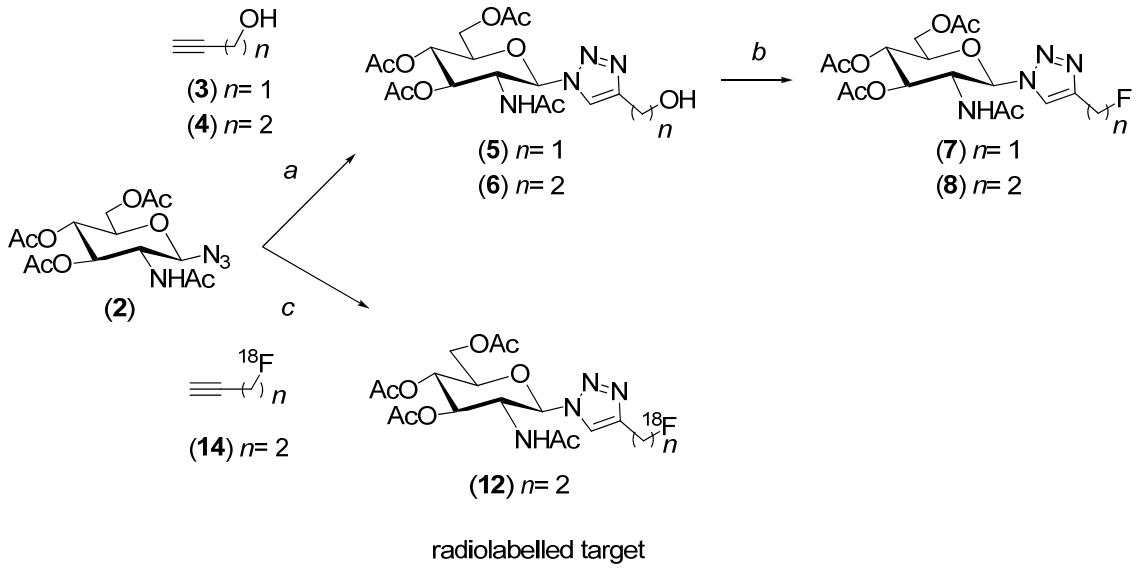
CONCLUSIONS AND FUTURE DIRECTIONS

4.1 Conclusions

PET imaging of tumours is important because it is a non-invasive functional imaging modailty which can provide information not only about the location of the disease but also about how the target area (organ) is functioning. PET imaging offers an advantage by employing positron emitting isotopes of carbon, oxygen and nitrogen, which are the main elements of bioorganic molecules.

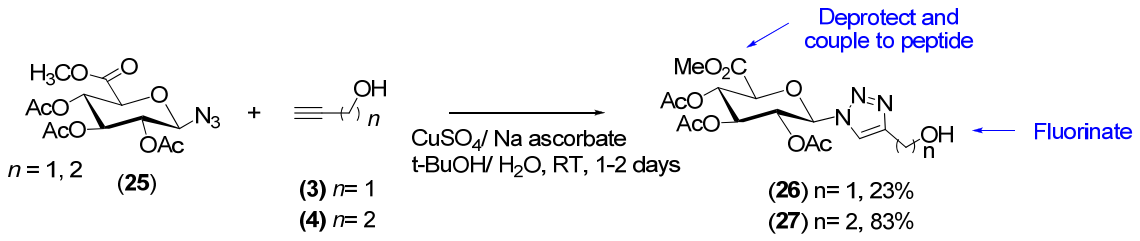
Herein we have described our development of a new method for radiolabelling various carbohydrates for use in PET imaging through the utilisation of click chemistry. Our work resulted in the generation of eight new carbohydrate derivatives including the novel [^{18}F]-labelled glucosamine derivative (**12**)**.** This radiolabelled sugar contains a NHAc group at C-2, which upon liberation of the free amine can be coupled via standard peptide coupling methods to various bioactive peptides used in cancer imaging such as the integrin antagonist cyclo-RGD (see Fig. 2. 1).

As described in Chapter 2, the click reaction using $CuSO_4$/ Na ascorbate as catalyst, in the *tert*-butanol/water gave a good yield of the desired hydroxymethyl triazole sugar (**5**) and novel hydroxyethyl triazole sugar (**6**). The synthesis of the known hydroxymethyl compound (**5**) afforded the compound in 74% yield after 2 hours, a significant improvement over the corresponding literature reaction, which resulted in 62% yield after 3 hours. The hydroxy triazoles (**5**) and (**6**) were then successfully fluorinated to afford the novel fluoromethyl and fluoroethyl triazole sugars (**7**) and (**8**) respectively, as standards for the radiolabelling and purification of the target compound [^{18}F]-labelled glucosamine (**12**). The radiolabelling of [^{18}F]-labelled glucosamine (**12**) using 4-[^{18}F]fluoro-1-butyne was successfully accomplished in 8 min, therefore we demonstrated that click chemistry can be used to prepare the radiolabelled ^{18}F-compounds efficiently and rapidly (Scheme 4.1).



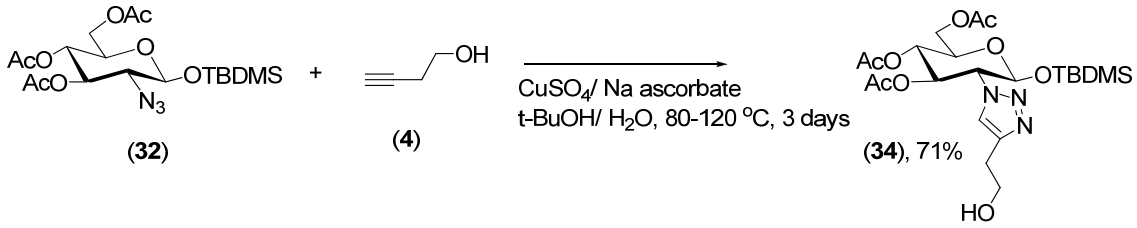
Scheme 4.1: *Reagents and conditions:* a) $CuSO_4.H_2O$, Na ascorbate, t-BuOH/ H_2O, RT, 2h, 74% yield (*n* = 1), 94% yield (*n* = 2); b) DAST, DCM, 30- 45 mins, 33% yield (*n* = 1), yield 30% (*n* = 2); c) $CuSO_4.H_2O$, Na ascorbate, DIPEA / H_2O , 90 ^{0}C, 8 min, 22% radiochemical yield, 80% radiochemical purity.

As described in Chapter 3, this work was then extended to the click reaction of 1-azidoglucuronic acid methyl ester (**25**) to afford the glucuronide hydroxymethyl and hydroxyethyl triazole sugars (**26**) and (**27**) in moderate to good yields. The two novel glucuronide triazoles (**26**) and (**27**) added yet more examples of novel sugar-click derivatives. Furthermore, after deprotection, they can be potentially conjugated to peptides through the carboxylic acid at C-6 (Scheme 4.2).



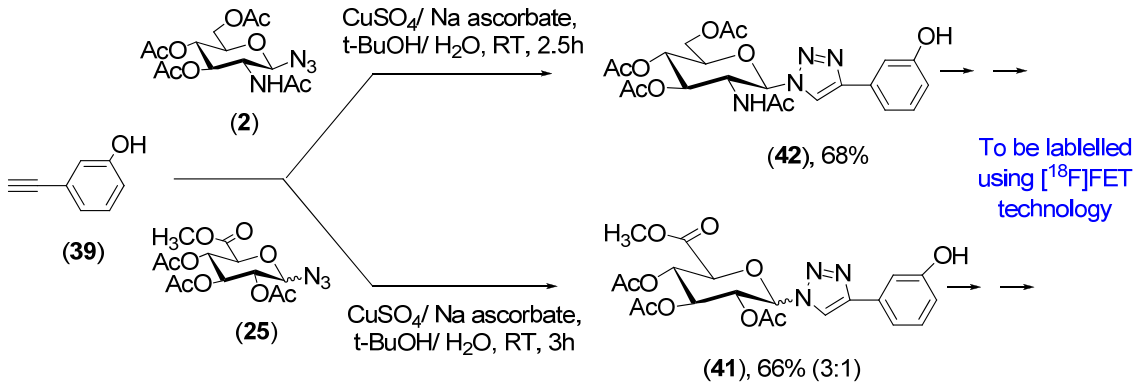
Scheme 4.2: The click reaction of 1-azido glucuronide (**25**) with alkynols (**3**) and (**4**).

The click reaction of the 2-azido sugar (**32**) gave a high yield of the novel 2-azido triazole sugar (**34**) and widened the scope of click reactions on 2-azido sugars, of which only a few examples have been reported in the literature (Scheme 4.3).



Scheme 4.3: Click reaction on 2-azido sugar.

The click reaction on 1-azido glucosamine (**2**) and 1-azidoglucuronic acid methyl ester (**25**) with the acetylenic phenol (**39**) gave the novel glucuronic and glucosamine phenol triazole sugars (**41**) and (**42**) respectively in good yields. These compounds containing an aromatic phenol can be potentially F-18 labelled using the same method as in the radiolabelling of the well known imaging agent [^{18}F]FET (Scheme 4.4).



Scheme 4.4: A strategy for the radiolabelling of 1-azido sugar (**2**) and 1-azido glucuronide (**25**) using [^{18}F]FET technology.

The work described in this thesis is part of an on-going project in our research group aimed at improving existing imaging agents as well as the development of new types of imaging compounds. The click-based methodology developed and described here has significant potential for use in labelling bioactive peptides in the future.

4.2 Future directions

There is considerable scope for more research work to be undertaken in this project. The hydroxymethyl and hydroxyethyl triazole sugars (**5**) and (**6**) could be coupled to bioactive peptides such as cyclo RGD and radiolabelled using the methods described previously (see Section 2.1). After which, binding studies to $\alpha_v\beta_3$-integrin in U87 cells then could be performed to assess how the addition of the sugar triazole affects integrin binding. There is potential here for the generation of a new integrin antagonist type imaging agent. Preparation of the radiolabelled fluoromethyl triazole sugar (**11**) could also be undertaken, however, the low boiling point (15 ^{o}C) properties of the propargyl fluoride (**13**) do present challenges.

In order to complete the preparation of the radiolabelled 1-azidoglucuronic derivatives (**30**) and (**31**), fluorination of the compounds (**26**) and (**27**) could be performed to obtain the cold standards for HPLC, followed by the radiolabelling of (**25**) using the method described previously. Likewise, for the preparation of the radiolabelled 2-azido sugar derivatives (**34**), fluorination could be performed on compound (**34**) in order to obtain the cold standard. The click reaction also could be performed on the 2-azido sugar (**32**) with propargyl alcohol (**3**) to provide another example of a 2-azido sugar triazole derivative.

In order to complete the preparation of the radiolabelled 1- and 2-azido sugar derivatives with the acetylenic phenol (**39**), fluoroethylation is needed to obtain the cold standards for compounds (**41**) and (**42**). After that, the preparation of the radiolabelled phenol could be attempted using the same methods as in the preparation of [^{18}F]FET, and the radiolabelled aromatic alkyne 'clicked' with the various azido sugars. Considerable scope still remains in the optimisation of the parameters (such as time and temperature) for all of the reactions to obtain even better results.

CHAPTER 5

EXPERIMENTAL

5.1 General comments

Chemicals and solvents were obtained from Sigma – Aldrich Chemical Co. unless otherwise noted and used without further purification. All reactions were performed in standard glassware. Solvent removal was performed using a Buchi rotary evaporator at temperatures not greater than 60 ^{o}C. The 1-azido sugars (**2**) and (**25**) were purchased from Carbosynth Limited. The 2-azido sugar (**32**) was purchased from Sigma Aldrich. Melting points were recorded on an Optimelt-Automated Melting Point System, Digital Image Processing Technology apparatus. Temperatures are reported in degrees Celsius (^{o}C) and are uncorrected. Fluorine-18 [^{18}F]HF was produced on a GE PET trace Cyclotron via the ^{18}O(p, n)^{18}F nuclear reaction (Cyclotek, Australia). F-18 fluorination reactions were carried out in the presence of potassium carbonate and the amino polyether Kryptofix$_{2.2.2}$. in acetonitrile under nitrogen.

Nuclear magnetic resonance (NMR) spectroscopy

Proton (^{1}H) nuclear magnetic resonance (NMR) spectra were recorded at 400 MHz on a Bruker NMR spectrometer. Spectra were recorded in deuterated chloroform ($CDCl_3$), using chloroform (δ 7.26), TMS (δ 0.00) as the internal standards and deuterated dimethyl sulfoxide (DMSO-d_6, δ 2.50). Chemical shifts (δ) were measured in parts per million (ppm). Multiplicities were reported as singlet (s), doublet (d), doublet of doublets (dd), triplet (t), doublet of triplets (dt), quartet (q), and multiplet (m). Coupling constants (J) are reported in Hertz. Hydrogen and carbon assignments were also made using standard gradient correlation spectroscopy (gCOSY), gradient heteronuclear single quantum correlation (gHSQC) and gradient heteronuclear multiple bond correlation (gHMBC) spectroscopic techniques.

Mass spectrometry

Low resolution mass spectra (LRMS) were obtained using a Waters Micromass ZQ quadropole Mass Spectrometer and high resolution mass spectra (HRMS) were obtained on a Micromass QTOF2 ultima mass spectrometer.

Chromatography

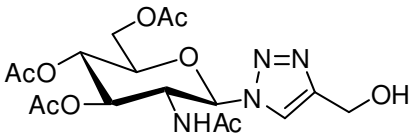
Thin layer chromatography (TLC) was performed on Merck F_{254} silica plates. Detection was done under UV light and with 5% H_2SO_4 in ethanol for sugar containing compounds. Gravity column chromatography was performed using Merck silica gel, grade 60, 70-230 mesh, 60Å. The solvent used in individual chromatographic experiments is indicated and eluents are expressed as volume: volume (v:v) proportions.

The [^{18}F] radiolabelled products were assessed and purified by high performance liquid chromatography (HPLC), consisting of a Waters 600 Controller, Waters 515 HPLC pump and Waters in line degasser AF, λ= 226 nm. A Linear UV is detector (λ = 254 nm) was used in series with a Carroll and Ramsey model 105S gamma detector, on a Phenomenex Bondclone C18 column (300 x 7.8 mm, 10 µm) at 4 ml/min with 20% ACN, 80% H_2O and 0.1% TFA as the mobile phase. The identity of the labelled compounds was confirmed by co-injection with the authentic compounds on the above HPLC system. Radioactivity was measured with a Capintec R15C dose calibrator.

For compound (**40**) and (**41**), HPLC was performed on a semi-preparative RP Altima C18 250 x 22 mm column using a Waters 771 plus Auto sampler, Waters pump Prep LC Controller and Waters^{TM} 486 Tunable Absorbance detector set at 210 nm.

5.2 Chemical synthesis

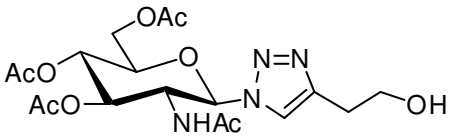
1-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-$_D$-glucopyranosyl)-4-(hydroxymethyl)-1,2,3-triazole (**5**)



2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-$_D$-glucopyranosyl azide (400 mg, 1.08 mmol) and propargyl alcohol (63.0 µl, 1.08 mmol) were added to a solution of $CuSO_4.5H_2O$ (55.0 mg, 0.22 mmol) and sodium ascorbate (86.0 mg, 0.43 mmol) in a 1:1 mixture of tert-butanol / water (6 ml). The reaction mixture was stirred for 2 h at RT and then extracted with ethyl acetate (3 x 20 ml). The organic extracts were combined, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The extraction afforded **5** as a white solid (340 mg, 74%), m.p. 146-148 ^oC, (note that the melting point of this known compound is not reported in the literature [76]). R_f 0.56 (silica, 9:1, DCM : MeOH). Spectral and physical data for compound **5** were identical to those reported.[76]

^{1}H-NMR ($CDCl_3$) δ1.76 (s, 3H, NHAc), 2.04 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.67 (O*H*), 2.10 (s, 3H, OAc), 4.01 (m, 1H, H-5), 4.14 (dd, 1H, J = 12.5 Hz, 2.0 Hz, H-6a), 4.28 (dd, 1H, J = 12.6 Hz, 4.9 Hz, H-6b), 4.62 (q, 1H, J = 10.1 Hz, H-2), 4.80 (s, 2H, C*H*$_2$OH), 5.28 (t, 1H, J = 9.8 Hz, H-4) 5.47 (t, 1H, J = 10.0 Hz, H-3), 6.03 (d, 1H, J = 9.9 Hz, H-1), 6.23 (d, 1H, J = 9.0 Hz, N*H*Ac), 7.83 (s, 1H, *H*-triazole). **MS: LRMS** *m/z* (ES$^+$) 451.1 (M + Na)$^{+\cdot}$

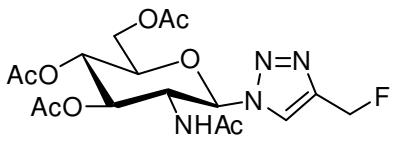
1-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-$_D$-glucopyranosyl)-4-(hydroxyethyl)-1,2,3-triazole (**6**)



2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-$_D$-glucopyranosyl azide (400 mg, 1.08 mmol) and

butynyl alcohol (82.0 µl, 1.08 mmol) were added to a solution of $CuSO_4.5H_2O$ (55.0 mg, 0.22 mmol) and sodium ascorbate (86.0 mg, 0.43 mmol) in a 1:1 mixture of tert-butanol/water (6 ml). The reaction mixture was stirred for 2 h at RT and then extracted with ethyl acetate (3 x 20 ml) The organic extracts were combined, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The extraction afforded **6** (450 mg, 94%) as a white solid, m.p. 180-184 ^oC, R_f 0.6 (silica, 20:1, DCM : MeOH). **^{1}H-NMR** ($CDCl_3$) δ 1.74 (s, 3H, NHAc), 2.07 (s, 3H, OAc), 2.08 (s, 3H, OAc), 2.09 (s, 3H, OAc), 2.98 (m, 2H, C*H*$_2$CH$_2$OH), 3.89 (m, 2H, C*H*$_2$OH), 4.11 (m, 1H, H-5), 4.18 (dd, 1H, J = 12.6 Hz, 2.2 Hz, H-6a), 4.33 (dd, 1H, J = 12.6 Hz, 4.9 Hz, H-6b), 4.63 (q, 1H, J = 9.8 Hz, H-2), 5.28 (t, 1H, J = 9.7 Hz, H-4) 5.53 (t, 1H, J = 9.4 Hz, H-3), 6.08 (d, 1H, J = 9.8 Hz, H-1), 7.13 (d, 1H, J = 9.1 Hz, N*H*Ac), 7.88 (s,1H, *H*-triazole). (1H, O*H* is not observed in the spectrum). **^{13}C-NMR** ($CDCl_3$) δ 20.6 (OAc), 20.7 (OAc), 20.7 (OAc), 22.7 (NHAc), 29.2 (*C*H$_2$CH$_2$OH), 53.8 (C-2), 61.4 (*C*H$_2$OH), 61.8 (C-6), 68.2 (C-4), 72.2 (C-3), 74.9 (C-5), 86.2 (C-1), 121.5 (*C*H-triazole), 145.9 (C triazole), 169.4 (C=O), 170.7 (C=O), 170.8 (C=O), 171.2 (C=O). **MS: LRMS** *m/z* (ES^+) 465 (M + Na)$^{+\cdot}$. **HRMS** calculated for $C_{18}H_{26}N_4O_9Na$: 465.1597, found 465.1596 .

1-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-$_D$-glucopyranosyl)-4-(fluoromethyl) 1*H*-1,2,3-triazole (7)

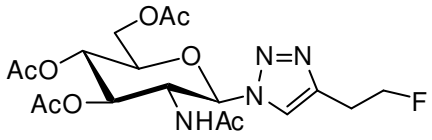


DAST (69 µl, 0.52 mmol) was added to hydroxyl sugar **5** (150 mg**,** 0.35 mmol) dissolved in 4.5 ml of dichloromethane and the reaction mixture was stirred for 30 min at 0 ^oC. Sodium hydrogen carbonate was added and then the reaction mixture was extracted with dichloromethane (3 x 20 ml). The organic extracts were combined, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The

product was purified using gravity column chromatography on silica gel (DCM: MeOH, 18:1) to afford **7** (50 mg, 33%) as a light brown solid, m.p. 198-203 ^{o}C, R_f 0.5 (silica, 10:1, DCM : MeOH).

^{1}H-NMR ($CDCl_3$) δ 1.79 (s, 3H, NHAc), 2.08 (s, 6H, OAc), 2.09 (s, 3H, OAc), 4.06 (m, 1H, H-5), 4.16 (dd, 1H, J = 12.5 Hz, 2.1 Hz, H-6a), 4.30 (dd, 1H, J = 12.6 Hz, 5.0 Hz, H-6b), 4.66 (q, 1H, J = 10.1 Hz, H-2), 5.28 (t, 1H, J = 9.5 Hz, H-4), 5.51 (t, 1H, J = 9.2 Hz, H-3), 5.49 (d, 2H, J = 48 Hz, CH_2F), 6.09 (d, 1H, J = 9.8 Hz, H-1), 6.63 (bs, 1H, N*H*Ac), 8.03 (s, 1H, *H*-triazole). **^{13}C-NMR** ($CDCl_3$) δ 20.5 (OAc), 20.5 (OAc), 20.6 (OAc), 22.7 (NHAc), 53.4 (C-2), 61.6 (C-6), 67.9 (C-4), 72.1 (C-3), 74.6 (*C*H_2F), 76.1 (C-5), 85.8 (C-1), 123.1 (*C*H-triazole), 143.1 (C triazole), 143.3 (C=O), 169.2 (C=O), 170.5 (C=O) 170.7 (C=O) **MS: LRMS** *m/z* (ES^+) 453.1 $(M + Na)^+$. **HRMS** calculated for $C_{17}H_{23}N_4O_8NaF$: 453.1398, found 453.1393.

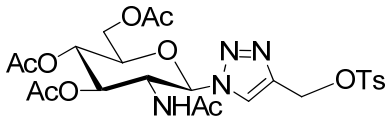
1-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-$_D$-glucopyranosyl)-4-(2-fluoroethyl)-1*H*-1,2,3-triazole (**8**)



Compound **8** was synthesized using the same procedure to produce **7**. DAST (45 µl, 0.34 mmol) was added to the hydroxyl sugar **6** (100 mg, 0.23 mmol) which was dissolved in 3 ml of dichloromethane and the reaction mixture was stirred for 45 min at 0 ^{o}C. Sodium hydrogen carbonate was added and then the reaction mixture extracted with dichloromethane (3 x 20 ml). The organic extracts were combined, dried over Na_2SO_4, and concentrated under reduced pressure. The product was purified using gravity column chromatography on silica gel (DCM : MeOH, 99 :1) to afford **8** (30 mg, 30%) as a light brown solid, 155-161 ^{o}C, R_f 0.6 (silica, 12:1, DCM: MeOH).

^{1}H-NMR ($CDCl_3$) δ 1.76 (s, 3H, NHAc), 2.06 (s, 6H, OAc), 2.07 (s, 3H, OAc), 3.15 (dt, 2H, J = 25.0 Hz, 6.1 Hz, C*H*$_2$CH$_2$F), 4.03 - 4.07 (m, 1H, H-5), 4.15 (dd, 1H, J = 12.6 Hz, 2.1 Hz, H-6a), 4.29 (dd, 1H, J = 12.6 Hz, 4.8 Hz, H-6b), 4.62 (q, 1H, J = 9.8 Hz, H-2), 5.26 (t, 1H, J = 10.0, H-4) 5.54 (t, 1H, J = 9.4 Hz, H-3), 4.70 (dt, 2H, J = 46.9 Hz, 6.1 Hz, C*H*$_2$F) 6.1 (d, 1H, J = 10.0 Hz, H-1), 6.8 (d, 1H, J = 9.3 Hz, N*H*Ac), 7.88 (s,1H, *H*-triazole). **^{13}C-NMR** ($CDCl_3$) δ 20.5 (OAc), 20.6 (OAc), 20.6 (OAc), 22.7 (NHAc), 27.3 (*C*H$_2$CH$_2$F), 53.2 (C-2), 64.9 (*C*H$_2$F), 72.3 (C-6), 74.7 (C-4), 81.3 (C-3), 83.0 (C-5), 85.7 (C-1), 121.3 (*C*H-triazole), 143.8 (C triazole), 143.9 (C=O), 169.3 (C=O), 170.5 (C=O), 170.6 (C=O). **MS: LRMS** *m/z* (ES^+) 467.1 $(M + Na)^+$, **HRMS** calculated for $C_{18}H_{25}N_4O_8NaF$: 467.1554, found 467.1577.

Attempted synthesis of (1-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-$_D$-glucopyranosyl)-1*H*-1,2,3-triazol-4-yl)methyl 4-methylbenzenesulfonate (9)



Method A - Tosylation of hydroxyl sugar (5)

1-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-$_D$-glucopyranosyl)-1*H*-1,2,3-triazol-4yl)methanol (**5**) (100 mg, 0.23 mmol) was dissolved in anhydrous dichloromethane (3ml) and triethyl amine (190 µl, 1.35 mmol) was added to the solution. The solution was stirred for 1 hr before the addition of tosyl chloride (90.0 mg, 0.45 mmol) and then stirred for another 3 h at 0 ^{o}C. The reaction mixture was extracted with dichloromethane (3 x 20 ml) and the organic extracts were combined, dried over Na_2SO_4 and the solvent evaporated to yield a white powder (190 mg). TLC analysis indicated the formation of a single product for which ^{1}H NMR spectral analysis showed the presence of the aromatic

ring of the tosylate group and triethyl amine peaks, but none of the carbohydrate protons were detected. Re-extraction of the aqueous layer with ethyl acetate and washing with sodium bicarbonate and brine afforded 30 mg of crude product, which was found by LRMS (ESI^+) to produce a peak at 605 ($M + Na^+$) consistent with that of the desired compound (**9**). Due to the low yield, the product was not further isolated and purified.

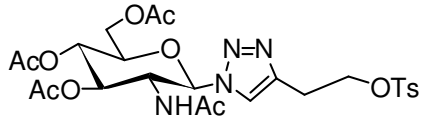
Method B - Tosylation of hydroxyl sugar (5)

1-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-$_D$-glucopyranosyl)-1*H*-1,2,3-triazol-4-yl)methanol (**5**) (50 mg, 0.12 mmol) was dissolved in anhydrous dichloromethane (1.5ml). Triethyl amine (17.0 µl, 0.12 mmol) was added to the solution, which was stirred for 1 h before tosyl chloride (46.0 mg, 0.24 mmol) was added. The reaction mixture was stirred for a further 3 hrs at 0 ^oC and then left in the fridge overnight. TLC analysis of the reaction mixture showed several by-products and LRMS (ESI^+) did not show the presence of the desired compound (**9**).

Method C – Click reaction of azido sugar (2) with tosylated alkyne (20)

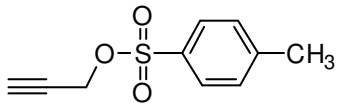
1-Azido glucosamine (**2**) (150 mg, 0.40 mmol) and propargyl tosylate (**20**) (84.1 mg, 0.40 mmol) were added to a solution of $CuSO_4.5H_2O$ (20.0 mg, 0.08 mmol) and sodium ascorbate (31.7 mg, 0.16 mmol) in a 1:1 mixture of tert-butanol/water (3 ml). The reaction mixture was stirred at RT for 2 h and then left overnight in the fridge. The reaction mixture was extracted with ethyl acetate (3 x 20 ml) and the combined organic extracts dried over Na_2SO_4, and the solvent evaporated. TLC analysis showed the starting material was completely consumed but the NMR of the crude product showed that the azide (**2**) and the alkyne (**3**) had not undergone a click reaction.

Attempted synthesis of 2-(1-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-$_D$-glucopyranosyl)-1*H*-1,2,3-triazol-4-yl)ethyl 4-methylbenzenesulfonate (10)



The attempted synthesis of (**10**) utilised the same conditions as those described in the attempted synthesis of (**9**) (method C). 1-Azido glucosamine (**2**) and butynyl tosylate (**4**) (89.8 mg, 0.40 mmol) were added to a solution of $CuSO_4.5H_2O$ (20.0 mg, 0.08 mmol) and sodium ascorbate (31.7 mg, 0.16 mmol) in a 1:1 mixture of tert-butanol/water (3 ml). The reaction mixture was stirred at RT for 2 h and was left overnight in the fridge. The reaction mixture was then extracted with ethyl acetate (3 x 20 ml), the combined organic extracts dried over Na_2SO_4, and the solvent evaporated. TLC analysis of the crude material showed that the starting material was completely consumed but the NMR of the crude product showed that the azide (**6**) and alkyne (**4**) had not undergone a click reaction.

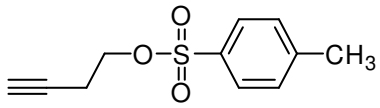
2-Propynyl-4-methylbenezene sulfonate (**propargyl tosylate**) (**20**)



To a solution of propargyl alcohol (2.10 g, 0.03 mol) in dry dichloromethane (150 ml), triethylamine (3.03 g, 0.03 mol) was added and the mixture stirred for 1 h at RT. Tosyl chloride (11.4 g, 0.06 mol) was then added and the mixture stirred for a further 3 h at 0 ^oC. After the addition of water (50 ml), the mixture was extracted with dichloromethane (5 x 30 ml), washed with water, dried over Na_2SO_4, filtered and evaporated. The product was purified using gravity column chromatography on silica gel (ethyl acetate: petroleum ether, 20:80) to afford **20** (4.04 g, 54%) as light dark oil. R_f 0.13 (silica, petroleum ether: ethyl acetate, 9:1). Spectral and physical data for compound **20** were identical to those reported.[81]

^{1}H-NMR ($CDCl_3$) δ 2.46 (s, 3H, C*H*$_3$), 2.52 (s, 1H, C*H*), 4.70 (s, 2H, C*H*$_2$O), 7.36 (d, J = 8.4 Hz, 2H, Ar*H*), 7.80 (d, J = 8.4 Hz, 2H, Ar*H*). **MS: LRMS** *m/z* (ES^+) 232.9 $(M + Na)^+$.

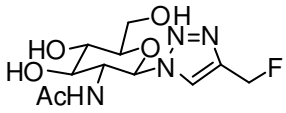
3-Butynyl-4-methylbenezene sulfonate (**butynyl tosylate**) (**21**)



The 3-butynyl-4-methylbenezene sulfonate (butynyl tosylate) **21** was synthesized according to the same procedure used to produce **20**. A solution of 3-butyn-1-ol (2.00 g, 0.04 mol) and triethylamine (3.61 g, 0.04 mol) dissolved in dry dichloromethane (150 ml), was stirred for 1 h at RT. Tosyl chloride (13.53 g, 0.07 mol) was added and the mixture stirred for another 3 h at 0 ^oC. After the addition of water (50 ml), the mixture was extracted with dichloromethane (5 x 30ml), washed with water, dried over Na_2SO_4, filtered and evaporated. The product was purified using gravity column chromatography on silica gel (ethyl acetate: petroleum ether, 20:80) to afford **21** (6.13 g, 91.07%) as an oil. R_f 0.36 (silica, petroleum ether: ethyl acetate, 9:1). Spectral and physical data for compound **21** were identical to those reported.[63]

^{1}H-NMR ($CDCl_3$) δ 1.97 (s, 1H, C*H*), 2.46 (s, 3H, C*H*$_3$), 2.56 (m, 2H, C*H*$_2$CH$_2$O), 4.13 (m, 2H, CH$_2$C*H*$_2$O), 7.35 (d, J = 8.6 Hz, 2H, Ar*H*), 7.81 (d, J = 8.4 Hz, 2H, Ar*H*). **MS: LRMS** *m/z* (ES^+) 247.1 $(M + Na)^+$.

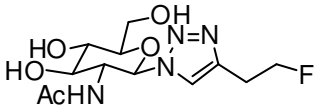
Attempted synthesis of 1-(2-acetamido-2-deoxy-β-$_D$-glucopyranosyl)-4-(fluoromethyl)-1*H*-1,2,3-triazole (**23**)



This reaction was performed according to a literature method.[63] To the crude fluoromethyl triazole sugar (**7**) (100 mg, 0.23 mmol) in acetonitrile (2 ml), sodium methoxide (0.5 M in ACN, 120 µl,

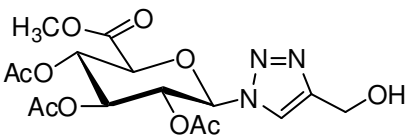
0.06 mmol) was added and the reaction mixture stirred at RT for 30 min. The reaction mixture was then neutralized by stirring with Amberlite IR-120 acidic ion-exchange resin for 30 min. Filtration of the resin followed by removal of the solvents under reduced pressure gave 30 mg of crude product. The formation of the desired product was observed by LRMS (ESI+) with a peak at, *m/z* = 327 corresponding to $(M + Na)^+$ for (**23**), however the isolation and further characterisation of the compound was not performed due to the low yield of product and complex mixture of side products.

Attempted synthesis of 1-(2-acetamido-2-deoxy-β-$_D$-glucopyranosyl)-4-(2-fluoroethyl)-1*H*-1,2,3-triazole (**24**)



As described above, the crude fluoroethyl triazole sugar (**8**) (100 mg, 0.23 mmol) was dissolved in ACN (2ml), and to this was added sodium methoxide (0.5 M in ACN, 120 µl, 0.06 mmol). The reaction mixture was worked up as described for (**7**) to give 30 mg of the crude product. Again, the TLC analysis showed the presence of several products. Analysis of the crude mixture by LRMS (ESI^+) revealed the presence of a peak at, *m/z* = 341 $(M + Na)^+$ consistent with that of the desired compound (**24**), however, the product isolation and further characterisation were not performed due to the low yield of product and complex mixture of side products.

(1-(Methyl 2,3,4-tri-*O*-acetyl- β-$_D$-glucuronide)-1*H*-1,2,3-triazole-4-yl)methanol (**26**)

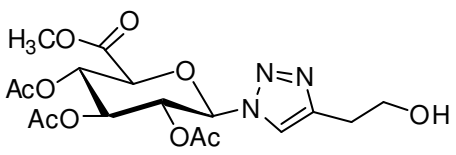


1-Azido-2,3,4-tri-O-acetyl-β-$_D$-glucuronide methyl ester (150 mg, 0.42 mmol) and propargyl alcohol (24.4 µl, 0.42 mmol) were added to a solution of$CuSO_4.5H_2O$ (21.0 mg, 0.08 mmol) and sodium ascorbate (33.3 mg, 0.17 mmol) in a

1:1 mixture of tert-butanol/water (3 ml). The reaction mixture was stirred for 48 h at RT. TLC (10: 90, MeOH: $CHCl_3$) showed minimal product had formed so another 0.2 equivalents of $CuSO_4.5H_2O$ (21 mg, 0.08 mmol) was added to accelerate the reaction and the mixture was stirred at RT for another 24 h. The reaction was then extracted with ethyl acetate (2 x 20 ml). The organic extracts were combined, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The resulting solid was purified by gravity column on silica gel (1-5% MeOH: DCM) to yield **26** (40 mg, 23%) as a white solid, m.p.187-190 ^oC, R_f 0.4 (silica, 90:10, CH_2Cl_3: MeOH).

^{1}H-NMR ($CDCl_3$) δ 1.87 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.50 (bs, 1H, O*H*), 3.74 (s, 3H, CO_2CH_3), 4.34 (d, 1H, J = 9.9 Hz, H-5), 4.79 (s, 2H, CH_2OH), 5.37 (m, 1H, H-2), 5.46-5.49 (m, 2H, H-3, H-4), 5.93 (d, 1H, J = 9.1 Hz, H-1), 7.86 (s, 1H, H-triazole). **^{13}C-NMR** ($CDCl_3$) δ 20.3 (OAc), 20.5 (OAc), 20.6 (OAc), 53.3 (CO_2CH_3), 56.6 (CH_2OH), 69.1 (C-4), 70.2 (C-2), 72.0 (C-3), 75.0 (C-5), 85.6 (C-1), 120.4 (*C*H-triazole), 147.6 (C-triazole), 166.3 (C=O), 169.1 (C=O), 169.5 (C=O), 169.8 (C=O). **MS: LRMS** *m/z* (ES^+) 438.3 $(M + Na)^+$. **HRMS** calculated for $C_{16}H_{22}N_3O_{10}$: 416.1305 found 416.1305 $(M + H)^+$.

(1-(Methyl 2,3,4-tri-*O*-acetyl-β-$_D$-glucuronide)-1*H*-1,2,3-triazole-4-yl)ethanol (27)

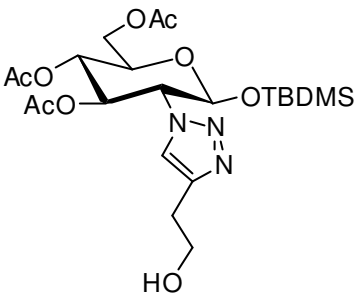


1-Azido-2,3,4-tri-O-acetyl-β-$_D$-glucuronide methyl ester (150 mg, 0.42 mmol) and butynyl alcohol (31.8 µl, 0.42 mmol) were added to a solution of $CuSO_4.5H_2O$ (21.0 mg, 0.08 mmol) and sodium ascorbate (33.3 mg, 0.17 mmol) in a 1:1 mixture of tert-butanol/water (3 ml). The reaction mixture was stirred overnight at RT and then extracted with ethyl acetate (2 x 20 ml). The organic extracts were combined, dried over anhydrous Na_2SO_4, and concentrated under reduced

pressure. The extraction afforded **27** (150 mg, 83%) as a white solid, m.p. 179-181 ^{o}C, R_f 0.4 (silica, 90:10, CH_2Cl_3: MeOH).

^{1}H-NMR ($CDCl_3$) δ 1.88 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.96 (bs, 2H, C*H*$_2$CH$_2$OH), 3.76 (s, 3H, CO_2CH_3), 3.92 (bs, 2H, C*H*$_2$OH), 4.35 (d, 1H, J = 9.8 Hz, H-5), 5.34-5.44 (m, 2H, H-4, H-3) 5.47-5.52 (m, 1H, H-2) 5.92 (d, 1H, J = 9.1 Hz, H-1), 7.74 (s, 1H, *H*-triazole), (1H, O*H* is not observed in the spectrum). **^{13}C-NMR** ($CDCl_3$) δ 20.1 (OAc), 20.4 (OAc), 20.5 (OAc) 28.8 (*C*H$_2$CH$_2$OH), 53.1 (CO_2CH_3), 61.4 (*C*H$_2$OH), 69.0 (C-4), 70.3 (C-2), 71.7 (C-3), 74.9 (C-5), 85.5 (C-1), 120.4 (*C*H-triazole), 146.4 (C-triazole), 166.2 (C=O), 169.1 (C=O), 169.3 (C=O), 169.7 (C=O). **MS: LRMS** *m/z* (ES^+) 452.2 $(M + Na)^+$. **HRMS** calculated for $C_{17}H_{24}N_3O_{10}$: 430.1462 found 430.1451 $(M + H)^+$.

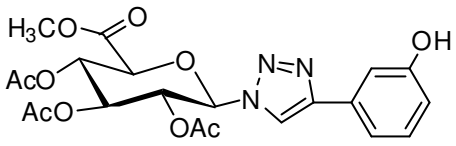
(1-(1-*O*-*Tert*-butyldimethylsilyl-2-deoxy-β-$_D$-glucopyranosyl-3,4,6-tri-*O*-acetyl)-1*H*-1,2,3-triazol-4-yl)ethanol (34)



1-O-T*ert*-butyldimethylsilyl-2-azido-2-deoxy-β-$_D$-glucopyranoside-3,4,6-triacetate (200 mg, 0.44 mmol) and butynyl alcohol (40 µl, 0.53 mmol) were added to a solution of $CuSO_4.5H_2O$ (22.0 mg, 0.09 mmol) and sodium ascorbate (35.7 mg, 0.18 mmol) in a 1:1 mixture of tert-butanol/water (3 ml). The reaction mixture was stirred for 3 days at 80-120 ^{o}C and then extracted with ethyl acetate (2 x 20 ml). The organic extracts were combined, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The product was purified using gravity column chromatography on silica gel (1-5% MeOH: DCM) to afford **34** (160 mg, 71%) as a brown thick oil, R_f 0.4 (silica, 16: 1, DCM: MeOH).

^{1}H-NMR ($CDCl_3$) δ -0.14 (s, 3H, $SiCH_3$), -0.05 (s, 3H, $SiCH_3$), 0.67 (s, 9H, ($SiC(CH_3)_3$), 1.76 (s, 3H, OAc) 1.98 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.86 (t, 2H, J = 6.1 Hz, CH_2CH_2OH) 3.84 (t, 2H, J = 6.0 Hz, CH_2OH), 3.90-3.93 (m, 1H, H-5), 4.10 (dd, 1H, J = 12.1 Hz, 2.2 Hz, H6a), 4.22-4.32 (m, 2H, H-6b, H-2), 5.06 (t, 1H, J = 9.6 Hz, H-4), 5.15 (d, 1H, J = 7.7 Hz, H-3) 5.81 (t, 1H, J = 9.2 Hz, H-1), 7.41 (s, 1H, H-triazole), (1H, O*H* is not observed in the spectrum). **^{13}C-NMR** ($CDCl_3$) δ -4.7 ($SiCH_3$), -5.8 ($SiCH_3$), 17.7 (($SiC(CH_3)_3$), 20.3 (OAc), 20.6 (OAc), 20.7 (OAc), 25.2 ($SiC(CH_3)_3$), 28.6 (CH_2CH_2OH), 61.5 (CH_2OH), 62.3 (C-6), 66.1 (C-2), 69.3 (C-4), 71.9 (C-3), 72.0 (C-5), 95.8 (C-1), 123.4 (*C*H-triazole), 144.9 (C-triazole), 169.4 (C=O), 169.8 (C=O), 170.6 (C=O). **MS: LRMS** *m/z* (ES^+) 538.4 $(M + Na)^+$. HRMS calculated for $C_{22}H_{38}N_3O_9Si$: 516.2377 found 516.2371 $(M + H)^+$.

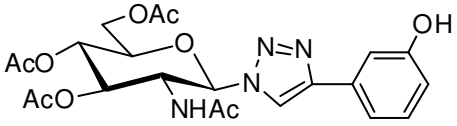
3-(1-(Methyl 2,3,4-tri-*O*-acetyl-β-$_D$-glucuronide)-1*H*-1,2,3-triazol-4-yl)phenol (41)



1-Azido-2,3,4-tri-O-acetyl-β-$_D$-glucuronide methyl ester (200 mg, 0.60 mmol) and phenol **39** (56.3 µl, 0.86 mmol) were added to a solution of $CuSO_4.5H_2O$ (27.5 mg, 0.11 mmol) and sodium ascorbate (45.6 mg, 0.23 mmol) in a 1:1 mixture of tert-butanol/water (3 ml). The reaction mixture was stirred 3 h at RT and then extracted with ethyl acetate (2 x 20 ml). The organic extracts were combined, dried over anhydrous Na_2SO_4, concentrated under reduced pressure and then purified using HPLC (RP Altima C18 column, flow rate = 50-300 mL/min, isocratic run 40% ACN, 50% H_2O, 10% NH_4HCO_3, t_R = 18.4 min) to afford **41** (180 mg, 66%) as a white solid, m.p. 198-200 ^{o}C, R_f 0.4 (silica, 1:20, MeOH : DCM).

^{1}H-NMR (DMSO-d_6) δ 1.81 (s, 3H, OAc), 2.00 (s, 3H, OAc), 2.03 (s, 3H, OAc), 3.65 (s, 3H, CO_2CH_3), 4.84/4.1* (d, 1H, J = 10.0 Hz, H-5), 5.23 (m, 1H, H-2), 5.67/5.43* (t, 1H, J = 9.4 Hz, H-4), 5.78/5.58* (t, 1H, J = 9.3 Hz, H-3), 6.43/6.21* (d, 1H, J = 9.2 Hz, H-1), 6.75 (m, 1H, ArH), 7.26 (m, 3H, ArH), 8.94/8.82* (s, 1H, *H*-triazole). (1H, O*H* is not observed in the spectrum). **^{13}C-NMR** (DMSO-d_6) δ 19.9 (OAc), 20.2 (OAc), 20.3 (OAc), 52.7 (CO_2CH_3), 68.5 (C-4), 69.9 (C-2), 71.5 (C-3), 72.9 (C-5), 83.9 (C-1), 112.1 (Ar*C*H), 115.5 (ArC), 116.1 (Ar*C*H), 120.5 (*C*H-triazole), 130.1 (ArCH), 131.1 (Ar*C*H), 147.2 (*C*-triazole), 157.9 (Ar*C*OH), 166.6 (C=O), 168.9 (C=O), 169.4 (C=O), 169.6 9 (C=O). **MS: LRMS** *m/z* (ES^+) 500.3 $(M + Na)^+$. **HRMS** calculated for $C_{21}H_{24}N_3O_{10}$: 478.1462 found 478.1481 $(M + H)^+$.

3-(1-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-$_D$-glucopyranosyl)-1*H*-1,2,3-triazol-4-yl)phenol (42)



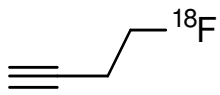
2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-$_D$-glucopyranosyl azide (100 mg, 0.27 mmol) and the phenol **39** (21.2 µl, 0.32 mmol) were added to $CuSO_4.5H_2O$ (13.5 mg, 0.05 mmol) and sodium ascorbate (21.4 mg, 0.11 mmol) in a 1:1 mixture of tert-butanol/water (4 ml). The reaction mixture was stirred for 2.5 h at RT and then extracted with ethyl acetate (2 x 20 ml). The organic extracts were combined, dried over anhydrous Na_2SO_4, concentrated under reduced pressure and then purified using HPLC (RP Altima C18 column, flow rate = 50-300 mL/min, isocratic run 40% ACN, 50% H_2O, 10% NH_4HCO_3, t_R = 7.7 min) to afford **42** (90 mg, 68%) as a white solid, m.p. 243-245 ^{o}C, R_f 0.5 (silica, 80:20, $CHCl_3$: MeOH).

* Denotes the minor isomer (ratio of isomers is 3:1 based on ^{1}H NMR integrals).

^{1}H-NMR (DMSO-d_6) δ 1.59 (s, 3H, NHAc), 1.96 (s, 3H, OAc), 2.00 (s, 3H, OAc), 2.02 (s, 3H, OAc), 4.09 (bd, 1H, J = 12.0 Hz, H-6a), 4.17 (dd, 1H, J = 12.4 Hz, 4.7 Hz, H-6b), 4.24 – 4.27 (m, 1H, H-5), 4.65 (q, 1H, J = 9.8 Hz, H-2), 5.11 (t, 1H, J = 9.9 Hz, H-4), 5.38 (t, 1H, J = 10.1 Hz, H-3), 6.13 (d, 1H, J = 9.8 Hz, H-1), 6.76 (d, 1H, J = 7.2 Hz, N*H*Ac), 7.25 (m, 3H, ArH), 8.11 (d, 1H, J = 9.0 Hz, ArH), 8.74 (s, 1H, *H*-triazole), 9.60 (s, 1H, O*H*). **^{13}C-NMR** (DMSO-d_6) δ 20.4 (OAc), 20.5 (OAc), 20.6 (OAc), 22.4 (NHAc), 52.4 (C-2), 61.7 (C-6), 68.2 (C-4), 72.5 (C-3), 73.5 (C-5), 85.0 (C-1), 112.1 (Ar*C*H), 115.4 (ArC), 116.2 (Ar*C*H), 120.3 (*C*H-triazole), 130.2 (Ar*C*H), 131.5 (Ar*C*H), 146.7 (C-triazole), 157.9 (Ar*C*OH), 162.6 (C=O), 169.6 (C=O), 169.7 (C=O) 170.3 (C=O). **MS: LRMS** *m/z* (ES^+) 513.3 $(M + Na)^+$. **HRMS** calculated for $C_{22}H_{26}N_4O_9Na$: 513.1597 found 513.1586.

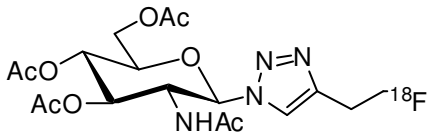
5.3 Radiochemical synthesis

[^{18}F]-4-Fluorobutyne (**14**)



Aqueous H[^{18}F]fluoride approximately 30 mCi, was added to a 2.5 ml round bottom vial containing a solution of Kryptofix (K_{222}) (130 µl in ACN) and K_2CO_3 (30 µL in H_2O). The solvent was evaporated under a stream of nitrogen at 100 °C with a reducing vacuum. This azeotropic drying was repeated twice by further addition of anhydrous acetonitrile (2 x 1 ml). The precursor, butynyl tosylate **21** (10 mg) was dissolved in CH_3CN (2 ml) and added to the dried $K222.K_2CO_3.K^{18}F$ complex. The reaction mixture was heated at 100 °C for 8 min, during which the 4-[^{18}F]fluoro-1-butyne (bp 45 °C) was distilled with acetonitrile into another vial via an inert peak transfer line for use in the click reaction. After the distillation, measurement of radioactivity indicated 12.3 mCi into the second vial corresponding to a radiochemical yield of 50%.

[^{18}F]-1-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-$_D$-glucopyranosyl)-4-(2-fluoroethyl)-1*H*-1,2,3-triazole (12)



The 4-[^{18}F]fluoro-1-butyne **14** was distilled with acetonitrile into a 2.5 ml round bottom vial containing a solution of the 2-azido sugar (**2**) (2.00 mg, 5.36 mmol), $CuSO_4.5H_2O$ (3.1 mg, 16 µmol), sodium ascorbate (21 mg, 106 µmol) and CH_3CN (500 µl) at -45 ^oC. After completing the distillation, DIPEA (21 µl, 120 µmol) and H_2O (500 µl) was added to the reaction mixture. The reaction mixture was heated at 90 ^oC for 8 min and the acetonitrile was evaporated for 5 min. The reaction mixture was filtered using a Waters 0.45 µm GHP Acrodisc filter then analysed by HPLC (Bondclone column 300 x 7.8 mm, flow rate = 4 ml/min, isocratic run 20% ACN, 80% H_2O and 0.1% TFA, t_R = 7.0-13.4 min) to afford **12** with 741 µCi corresponding to 22% radiochemical yield and 80% radiochemical purity. Co-injection of the reaction mixture with the cold standard **8** confirmed the identity of the product (**12**).

CHAPTER 6

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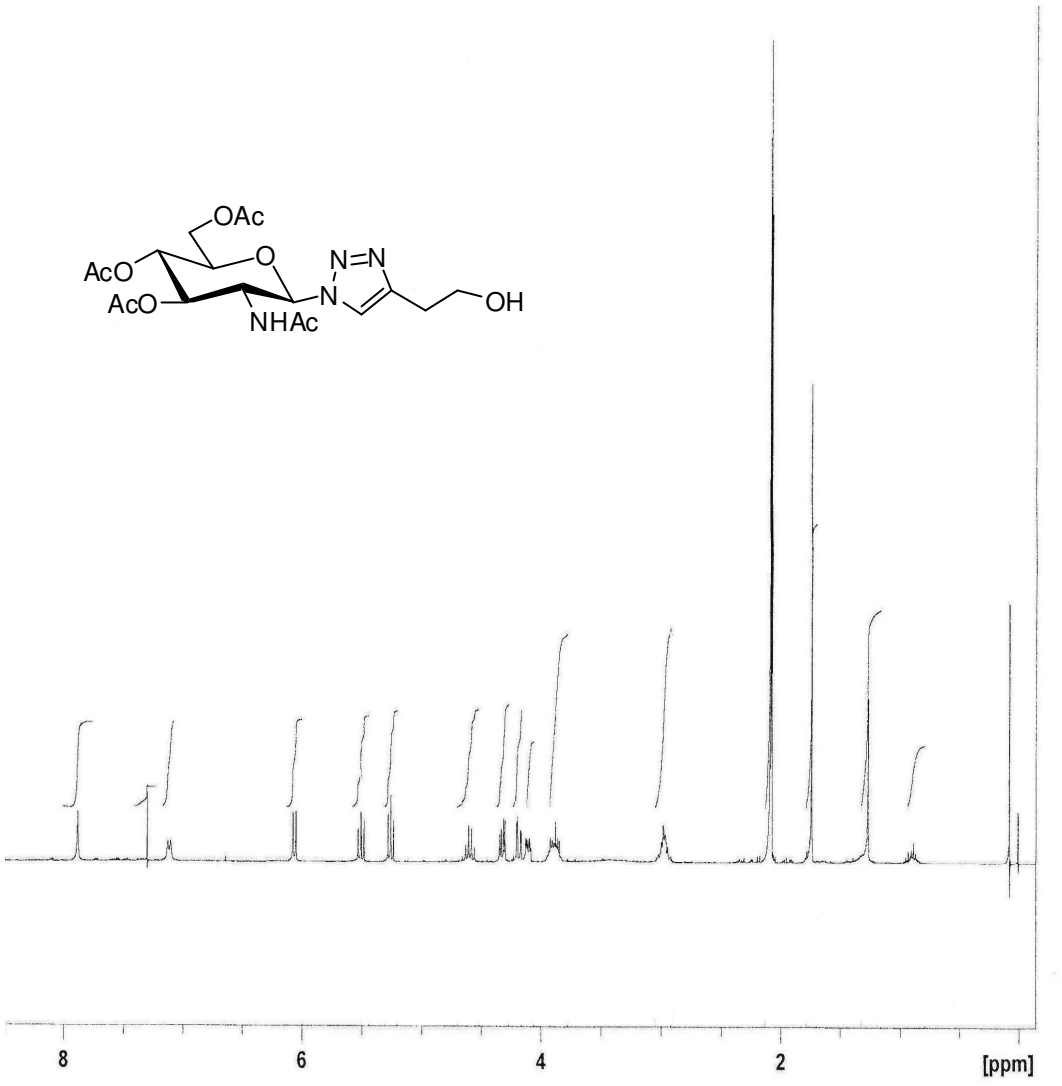
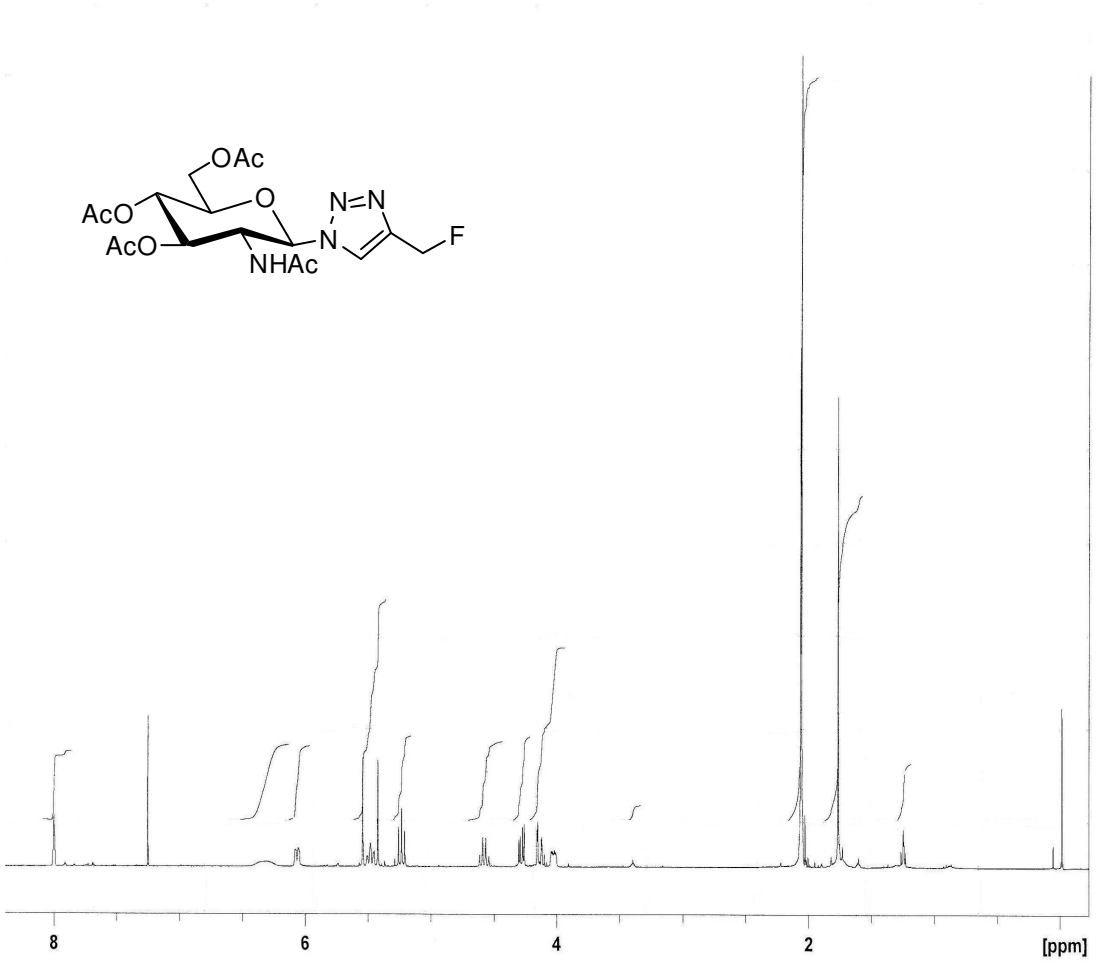
CHAPTER 7

APPENDICES

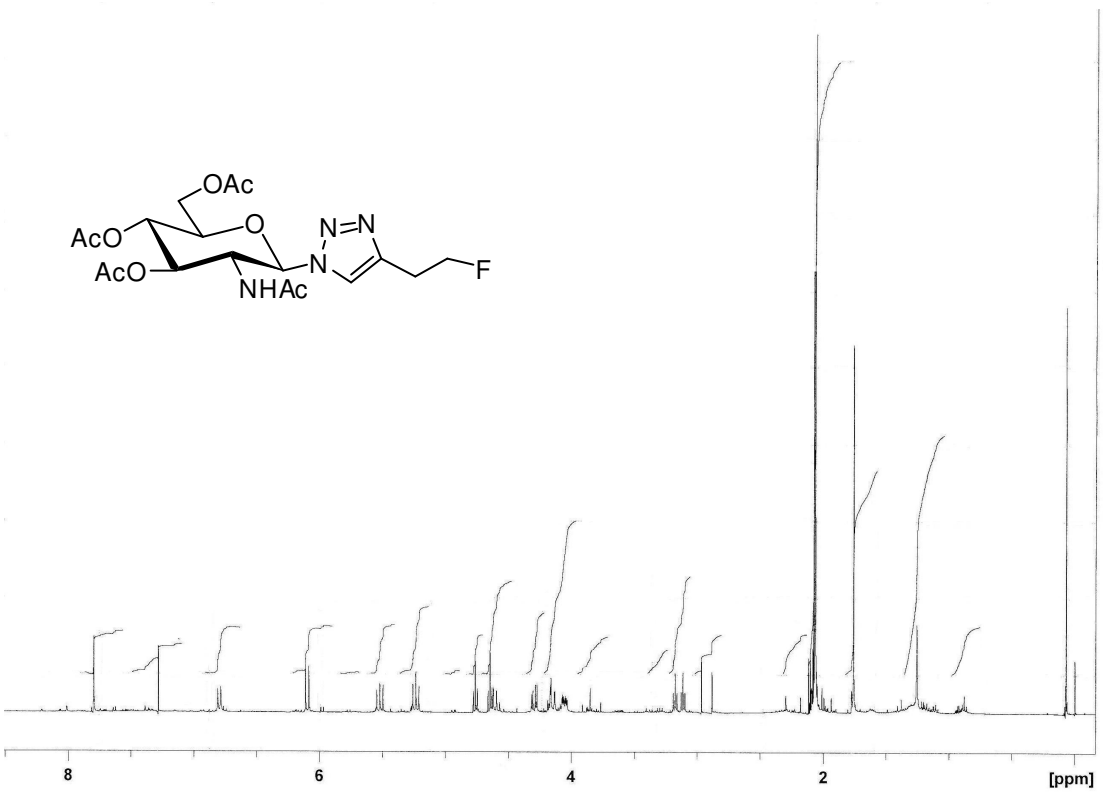
Appendix 1: ^{1}H-NMR spectra (400 MHz, $CDCl_3$) of 1-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-$_D$-glucopyranosyl)-4-(hydroxyethyl)-1,2,3-triazole (**6**).



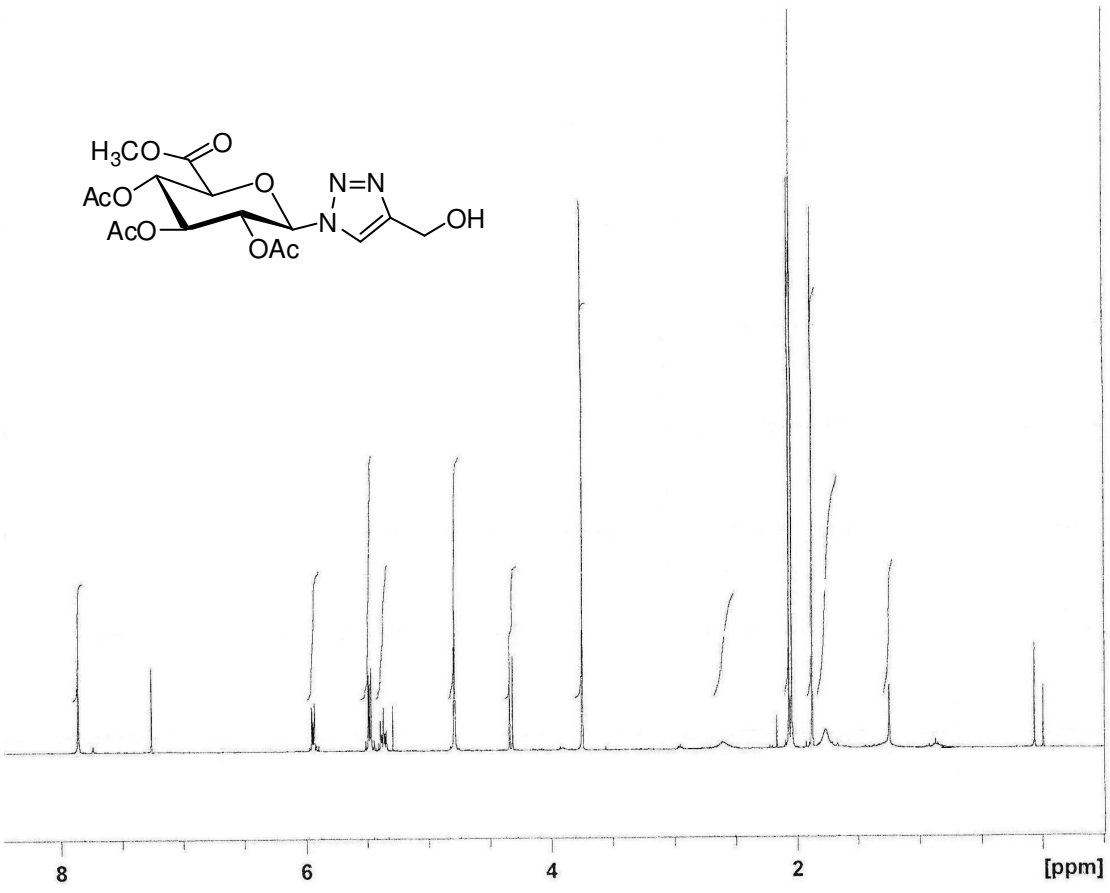
Appendix 2: ^{1}H-NMR spectra (400 MHz, $CDCl_3$) of 1-(2-Acetamido-3,4,6-*O*-acetyl-2-deoxy- β-$_D$-glucopyranosyl)-)-(4-fluoromethyl) 1*H*- 1*H*-1,2,3-triazole (**7**)



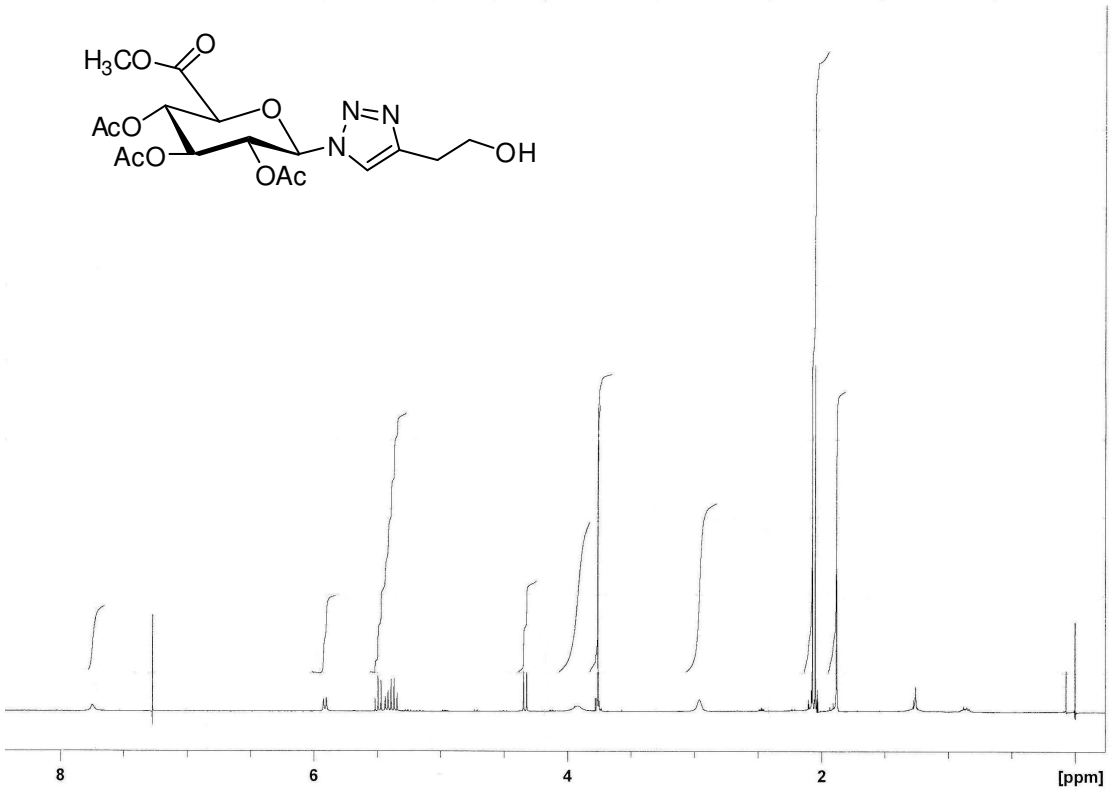
Appendix 3: ^{1}H-NMR spectra (400 MHz, $CDCl_3$) of 1-(2-Acetamido-3,4,6-*O*-acetyl-2-deoxy- β-$_D$-glucopyranosyl)-)-(4-fluoroethyl) 1*H*- 1*H*-1,2,3-triazole (**8**)



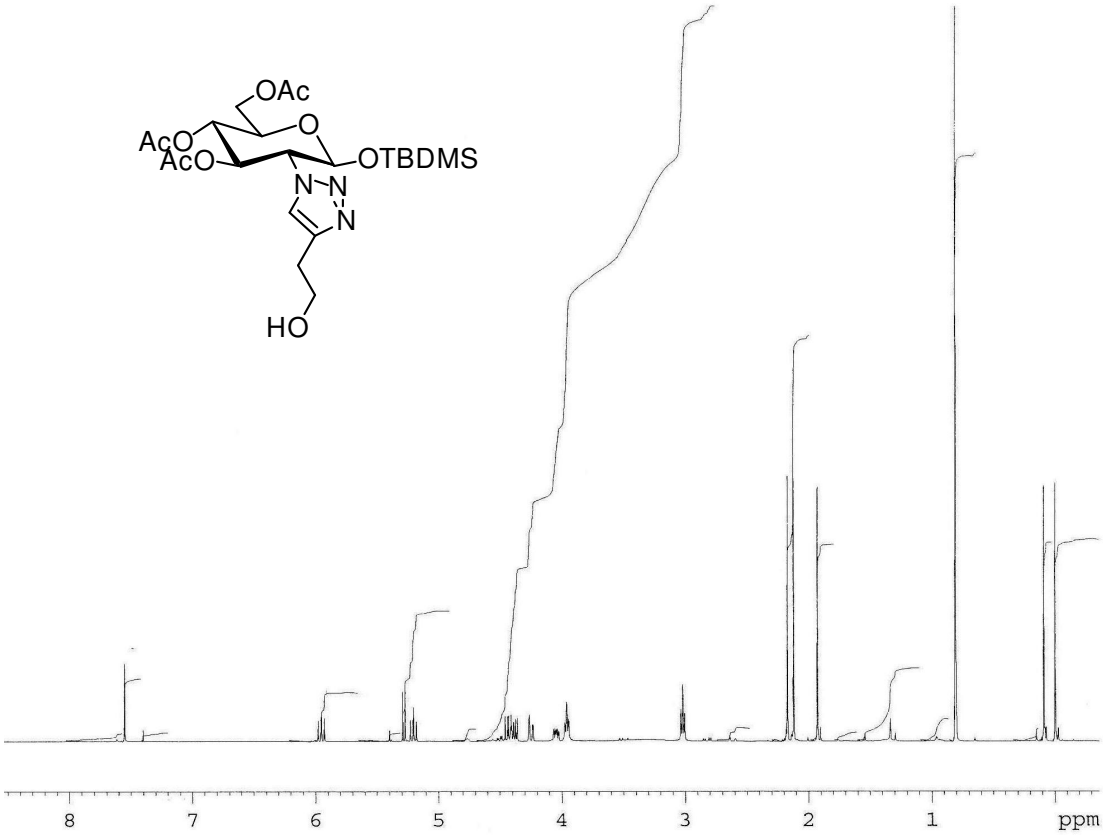
Appendix 4: ^{1}H-NMR spectra (400 MHz, $CDCl_3$) of (1-(Methyl 2,3,4-tri-*O*-acetyl- β-$_D$-glucuronide)-1*H*-1,2,3-triazole-4-yl)methanol (**26**)



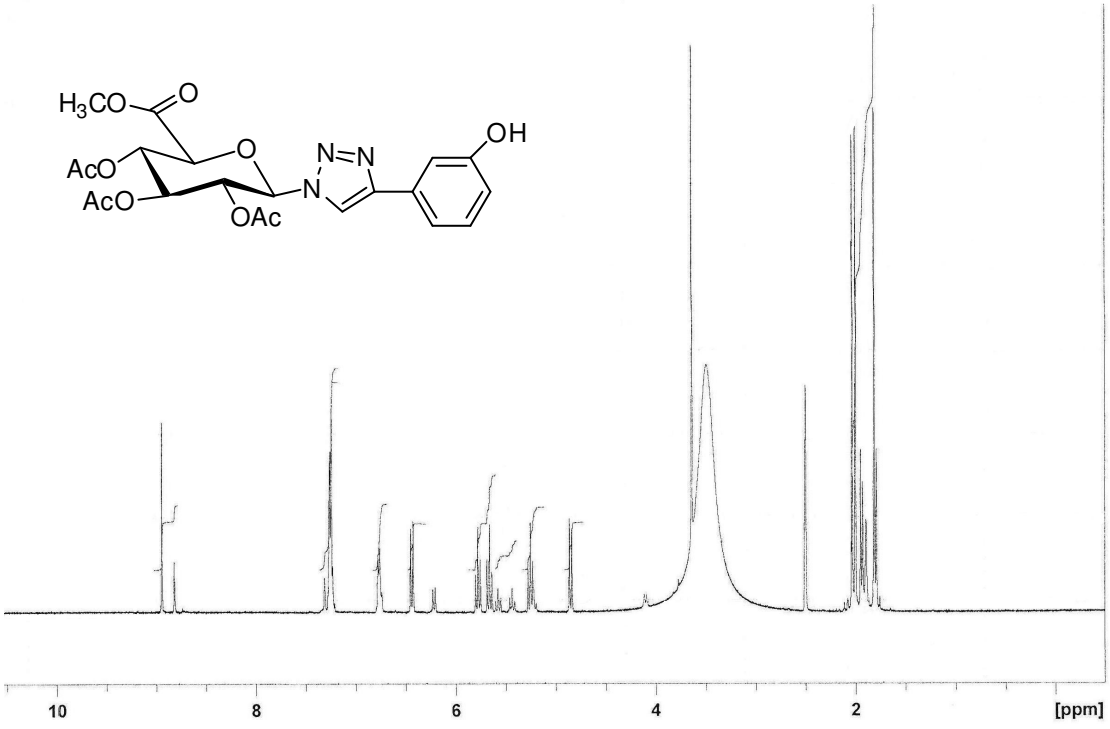
Appendix 5: ^{1}H-NMR spectra (400 MHz, $CDCl_3$) of (1-(Methyl 2,3,4-tri-*O*-acetyl- β-$_D$-glucuronide)-1*H*-1,2,3-triazole-4-yl)ethanol (**27**)



Appendix 6: ^{1}H-NMR spectra (400 MHz, $CDCl_3$) of ((1-(1-*O*-*Tert*-butyldimethylsilyl-2-deoxy-β-$_D$-glucopyranosyl-3,4,6-tri-*O*-acetyl)-1*H*-1,2,3-triazol-4-yl)ethanol (**34**)



Appendix 7: ^{1}H-NMR spectra (400 MHz, DMSO-d_6) of 3-(1-(Methyl 2,3,4-tri-*O*-acetyl-β-$_{D}$-glucuronide)-1*H*-1,2,3-triazol-4-yl)phenol (**41**)



Appendix 8: 1H-NMR spectra (400 MHz, DMSO-d_6) of 3-(1-(2-Acetamido-3,4,6-*O*-acetyl-2-deoxy- β-$_D$-glucopyranosyl)-1*H*-1,2,3-triazol-4-yl)phenol (**42**)