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Synthesis and application of β -configured [$^{18/19}\text{F}$]FDGs

Novel prosthetic CuAAC click chemistry
fluoroglycosylation tools for amyloid PET imaging
and cancer theranostics

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Cover: Depicting the fluorescence emission from melanoma cells incubated with [19F]fluoroglycosylated curcumin.

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“You are urgently warned against allowing yourself to be influenced in any way by theories or by other preconceived notions in the observation of phenomena, the performance of analyses and other determinations.” - **Emil Fischer** (1852-1919)

(Laboratory Rules at Munich. Quoted by M. Bergmann, 'Fischer', in Bugge's Das Buch der Grosse Chemiker. Trans. Joseph S. Froton, Contrasts in Scientific Style: Research Groups in the Chemical and Biomedical Sciences (1990), 172.

Till Pelle som väckte min passion för kemi och ständigt förser den med nytt bränsle.

Abstract

Positron emission tomography (PET) is a non-invasive imaging method that renders three-dimensional images of tissue that selectively has taken up a radiolabelled organic compound, referred to as a radiotracer. This excellent technique provides clinicians with a tool to monitor disease progression and to evaluate how the patient respond to treatment. The by far most widely employed radiotracer in PET is called 2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG), which is often referred to as the golden standard in PET. From a molecular perspective, [^{18}F]FDG is an analogue of glucose where a hydroxyl group has been replaced with a radioactive fluorine atom (^{18}F). Historically, [^{18}F]FDG was utilized for diagnosis of neurological disorders e.g., Alzheimer's disease but have now largely been replaced by more specific radiotracers. [^{18}F]FDG is a metabolic radiotracer, meaning that after an intravenous injection, the tracer will, just as glucose, predominantly accumulate in tissue with a high glycolytic rate e.g., tumors but also in the brain and heart. Despite its prominent role, [^{18}F]FDG-PET investigations are associated with some severe drawbacks. Since [^{18}F]FDG imaging depends on the preferential uptake of the tracer in tissue with elevated glucose metabolism, there is a risk of accidentally image healthy, active cells (e.g., involved in inflammation- and infection processes) which then can be falsely diagnosed as cancer. Conversely, certain types of cancer (e.g., certain types of prostate- and lung cancer) have a low glucose metabolism, on the same order as benign tissue, and can therefore not be detected. Clearly, there is a great demand for new and significantly more selective tracers with high affinity for cancer and other diseases. This in turn forces chemists to develop new, widely applicable and mild methods for radiolabelling of sensitive, albeit high-affinity ligands with a defined biological target. It is well known that covalent attachment of carbohydrates (i.e., glycosylation) to biomolecules tend to improve their properties in the body, in terms of; improved pharmacokinetics, increased metabolic stability and faster clearance from blood and other non-specific tissue. It is therefore natural to pursuit the development of a [^{18}F]fluoroglycosylation method where [^{18}F]FDG is chemically conjugated to a ligand with high affinity for a given biological target (e.g., tumors or

disease-associated protein aggregates). This would not only facilitate radiolabelling but also, simultaneously, provide a tracer with improved properties in the human body. This concept has been pursued by chemists for almost 20 years but the existing methods are often too limited in scope or too complicated to apply.

This thesis describes a novel [^{18}F]fluoroglycosylation method that in a simple and general manner facilitate the conjugation of [^{18}F]FDG to a significantly larger set of ligands than being available with pre-existing methods. In this thesis, the utility of the developed [^{18}F]fluoroglycosylation method is demonstrated by radiolabelling of curcumin, thus forming a tracer that may be employed for diagnosis of Alzheimer's disease. Moreover, a set of oligothiophenes were fluoroglycosylated for potential diagnosis of Alzheimer's disease but also for other much rarer protein misfolding diseases (e.g., Creutzfeldt-Jakob disease and systemic amyloidosis). In addition, the synthesis of a series of ^{19}F -fluoroglycosylated porphyrins is described which exhibited promising properties not only to detect but also to treat melanoma cancer. Lastly, the synthesis of a set of ^{19}F -fluorinated *E*-stilbenes, structurally based on the antioxidant resveratrol is presented. The *E*-stilbenes were evaluated for their capacity to spectrally distinguish between native and protofibrillar transthyretin in the pursuit of finding diagnostic markers for the rare but severe disease, transthyretin amyloidosis.

Populärvetenskaplig Sammanfattning

Positronemissionstomografi (PET) är en icke-invasiv medicinsk avbildningsteknik som återger en tredimensionell bild av vävnad som selektivt har ackumulerat en injicerad radioaktiv organisk förening, en så kallad radiomarkör. Denna teknik förser därmed läkare med ett utmärkt verktyg för att följa sjukdomsförlopp och för att se hur den undersökta patienten svarar på behandling.

Den mest kända radiomarkören är 2-deoxy-2-[^{18}F]fluor-D-glukos ([^{18}F]FDG) vilken ofta benämns som den gyllene standarden inom PET-fältet. Molekylärt sett så är [^{18}F]FDG en analog till glukos där en hydroxylgrupp har ersatts med en radioaktiv fluoratom (^{18}F). [^{18}F]FDG används rutinmässigt i klinik för att diagnosticera, utvärdera dos-respons svar och i synnerhet för att lokalisera metastaser hos cancerpatienter. Historiskt sett så har [^{18}F]FDG också använts frekvent inom neurologin för att studera demenssjukdomar men har numera i huvudsak ersatts med mer specifika radiomarkörer. [^{18}F]FDG är en metabolisk radiomarkör vilket innebär att efter en intravenös injektion så kommer [^{18}F]FDG (precis som glukos) företrädesvis ansamlas i vävnad som har en hög glykolytisk ämnesomsättning, ex., tumörer men även i hjärna och hjärta.

Trots sin dominerande roll så är [^{18}F]FDG-PET avbildning behäftad med vissa allvarliga tillkortakommanden. Eftersom avbildning med [^{18}F]FDG är baserad på selektivt upptag i vävnad med förhöjd glukosmetabolism så finns det en risk att friska, aktiva celler (t.ex., celler involverade i infektion- och inflammationsprocesser) avbildas och misstas för cancer. Dessutom så kan inte tumörer som har en relativt låg glukosmetabolism, såsom vissa typer av prostata- och lungecancer, särskiljas från frisk vävnad. Därmed finns det ett stort behov av att framställa nya och betydligt mer selektiva radiomarkörer, vilket i sin tur ställer krav på kemister för utvecklandet av en praktisk, generell och mild metod för radioinmärkning av ligander med hög selektivitet mot cancer och andra sjukdomar.

Det är väl känt att kovalent förankring av kolhydrater (s.k. glykosylering) till biomolekyler tenderar till att markant förbättra deras egenskaper i kroppen såsom; förbättrad farmakokinetik, ökad metabolisk stabilitet och snabbare utrensning från blod och icke-specifik vävnad. Det är därför naturligt att eftersträva utveckling av en ^{18}F -fluorglykosyleringsmetod där kopplandet av $[^{18}\text{F}]\text{FDG}$ till en given ligand med hög affinitet för ett specifikt biologiskt mål (ex., tumörer eller sjukdomsalstrande proteinaggregat) inte bara medför radioinmärkning, vilket möjliggör PET undersökningar, utan även förbättrar radiomarkörens egenskaper i kroppen. I snart 20 år har kemister utvecklat olika ^{18}F -fluorglykosyleringsmetoder för just detta ändamål men de är tyvärr många gånger allt för begränsade eller för komplicerade att tillämpa.

I denna avhandling beskrivs utvecklingen av en ny ^{18}F -fluorglykosyleringsmetod vilket möjliggör koppling av $[^{18}\text{F}]\text{FDG}$ till ett betydligt större utbud av biologiska ligander än vad som tidigare har varit möjligt. Vidare så beskrivs även hur denna ^{18}F -fluorglykosyleringsmetod använts för att radioinmärka; (1) kurkumin (det gula färgpigmentet i gurkmeja) för möjlig diagnos av Alzheimers sjukdom, (2) oligotiofener för möjlig diagnos av Alzheimers sjukdom och andra betydligt mer sällsynta proteinaggregeringssjukdomar såsom Creutzfeldt-Jakobs sjukdom och transtyreтин-amyloidossjukdomar. Dessutom behandlas syntes av en serie icke-radioaktiva ^{19}F -fluorglykosylerade porfyriner vilka uppvisade goda egenskaper för att både påvisa men även behandla hudcancer. Slutligen så behandlas också syntes av en serie fluorerade *E*-stilbener vilka strukturellt sett är baserade på resveratrol, en antioxidant som bland annat återfinns i rött vin. Dessa stilbener utvärderades för deras förmåga att spektralt särskilja nativt från protofibrillärt transthyretin-protein vilket är av stor vikt för att hitta diagnostiska markörer för systemisk amyloidos.

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Papers Included in the Thesis

- I. **β -Configured clickable [18F]FDGs as novel 18F-fluoroglycosylation tools for PET**
Mathias Elgland, Patrik Nordeman, Timmy Fyrner, Gunnar Antoni, K. Peter R. Nilsson and Peter Konradsson. *New J. Chem.*, 2017, **41**, 10231–10236.
- II. **Synthesis and evaluation of a [¹⁸F]fluoroglycosylated curcumin derivative for amyloid PET imaging**
Mathias Elgland, Alexander Säberg, Alexander Sandberg, Hanna Appelqvist, Patrik Nordeman, Gunnar Antoni, K. Peter R. Nilsson, Peter Konradsson and Per Hammarström (in manuscript)
- III. **Synthesis of ¹⁸F-fluoroglycosylated oligothiophenes for multimodal imaging of protein aggregates**
Mathias Elgland, Marcus Bäck, Hanna Appelqvist, Patrik Nordeman, Gunnar Antoni, Peter Konradsson, Per Hammarström, and K. Peter R. Nilsson (in manuscript)
- IV. **Synthesis and characterization of novel fluoro-glycosylated porphyrins that can be utilized as theranostic agents**
Katriann Arja, Mathias Elgland, Hanna Appelqvist, Peter Konradsson, Mikael Lindgren and K. Peter R. Nilsson (submitted to ChemistryOpen)
- V. **Novel Trans-Stilbene-based Fluorophores as Probes for Spectral Discrimination of Native and Protofibrillar Transthyretin**
Raúl. I. Campos Melo, Xiongyu Wu, Mathias Elgland, Peter Konradsson and Per Hammarström, *ACS Chem. Neurosci.*, 2016, **7**, 924–940.

Contribution to Included Papers

- I. Planned and performed all the non-radioactive synthetic work and chemically characterized all synthesized compounds. Took part in planning the radioactive syntheses. Wrote most of the paper.
- II. Planned and performed all the non-radioactive synthetic work and chemically characterized all synthesized compounds. Planned the radioactive synthetic procedure. Wrote most of the paper.
- III. Planned all the non-radioactive synthetic work. Synthesized and characterized most of the compounds. Planned the radioactive synthetic sequence. Wrote most of the paper.
- IV. Actively participated in the planning of the project. Performed the synthesis and characterization of all the required azidosugars. Took part in planning the biological analyses. Wrote parts of the paper.
- V. Participated in the planning of the project. Performed the synthesis and characterization of the fluorinated *trans*-stilbenes. Wrote parts of the paper.

Papers Not Included in the Thesis

Red junglefowl have individual body odors

A. Karlsson, P. Jensen, M. Elgland, K. Laur, T. Fyrner, P. Konradsson and M. Laska, *J. Exp. Biol.*, 2010, **213**, 1619–1624.

In vivo polymerization and manufacturing of wires and supercapacitors in plants

E. Stavriniidou, R. Gabrielsson, K. P. R. Nilsson, S. K. Singh, J. F. Franco-Gonzalez, A. V. Volkov, M. P. Jonsson, A. Grimoldi, M. Elgland, I. V. Zozoulenko, D. T. Simon and M. Berggren, *Proc. Natl. Acad. Sci. U.S.A.*, 2017, **114**, 2807–2812.

Conference Contributions

Synthesis of Glycosylated Porphyrin-Oligothiophene Conjugates

Mathias Elgland, Katriann Arja, Peter Konradsson and K. Peter R. Nilsson.

XVth Conference on Heterocycles in Bio-organic Chemistry, **2014**, Riga, Latvia.

Synthesis of an azide-functionalized β -mannosyl triflate:

An FDG precursor for PET imaging

Mathias Elgland, Peter Konradsson and K. Peter Nilsson.

Organikerdagarna, **2014**, Stockholm, Sweden.

Synthesis of β -configured CuAAC clickable FDGs as novel [18F]fluoroglycosylation tools for PET *in vivo* imaging

Mathias Elgland, K. Peter R. Nilsson and Peter Konradsson

Organikerdagarna, **2016**, Umeå, Sweden.

Synthesis of β -configured clickable [¹⁸F]FDGs as novel ¹⁸F-fluoroglycosylation tools for PET *in vivo* imaging

Mathias Elgland, Patrik Nordeman, Timmy Fyrner, Gunnar Antoni, K. Peter R. Nilsson and Peter Konradsson.

ACS national meeting, **2017**, San Francisco, USA.

¹⁸F-fluoroglycosylated LCOs - PET tracers for systemic amyloidosis

Mathias Elgland, Marcus Bäck, Patrik Nordeman, Gunnar Antoni, Peter Konradsson and K. Peter R. Nilsson

6th Amyloid Disease Annual Meeting, **2017**, Kolmården, Sweden.

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Abbreviations

A β	Amyloid beta fragment from Amyloid β precursor protein
AD	Alzheimer's disease
ASCT2	Sodium-dependent neutral amino acid transporter type 2
ATP	Adenosine triphosphate
ATTR	transthyretin amyloidosis
BBB	Blood-brain barrier
CSF	Cerebral spinal fluid
CuAAC	Copper (I) catalyzed azide-alkyne [3 +2] cycloaddition
DCM	Dichloromethane
DIPEA	N, N-Diisopropylethylamine
DMF	<i>N,N</i> -Dimethylformamide
EtOAc	Ethyl acetate
[18F]FDG	2-deoxy-2-[18F]fluoro-D-glucopyranose
[18F]FDM	2-deoxy-2-[18F]fluoro-D-mannopyranose
K[2.2.2]	1,10-diaza-4,7,13,16,21,24-hexaoxabicyclo[8.8.8]hexacosane
LAT1	L-type amino acid transporter 1
LCO	Luminescent Conjugated Oligothiophene
NADH	Nicotinamide adenine dinucleotide
NFT	Neurofibrillary tangles
NGP	Neighboring group participation
NiAAC	Nickel catalyzed azide-alkyne [3 +2] cycloaddition
NMR	Nuclear magnetic resonance
PBS	Phosphate-buffered saline
PDT	Photodynamic therapy
PET	Positron emission tomography
PET-CT	Positron emission tomography-computed tomography
Pfp ₂ O	Pentafluoropropionic anhydride
PPTS	Pyridinium <i>p</i> -toluenesulfonate

PrP	Prion protein
RCC	Radiochemical conversion
RCY	Radiochemical yield
R_f	Retention factor
RGD	Arginylglycylaspartic acid
RuAAC	Ruthenium catalyzed azide-alkyne [3 +2] cycloaddition
TBAB	Tetrabutylammonium bromide
Tf ₂ O	Trifluoromethanesulfonic anhydride
TLC	Thin-layer chromatography
TTR	Transthyretin

Table of Contents

Abstract	I
Populärvetenskaplig Sammanfattning	III
Acknowledgements.....	V
Papers Included in the Thesis	VIII
Contribution to Included Papers	IX
Papers Not Included in the Thesis	X
Conference Contributions.....	XI
Thesis Committee	XII
Abbreviations	XIII
Preface	XIX
1. Introduction	1
1.1. The Chemistry of Carbohydrates.....	1
1.2. Metabolism of Carbohydrates	4
1.2.1. Transportation of glucose over cell membranes.....	4
1.2.2. Glycolysis	5
1.2.3. Cancer Metabolism.....	6
1.3. Positron emission tomography	7
1.3.1. The positron-emitting nuclide fluorine-18.....	9
1.4. [18F]FDG	11

1.4.1.	Synthesis of [18F]FDG.....	13
1.4.2.	Drawbacks with [18F]FDG.....	15
1.5.	Peptide-based imaging agents.....	16
1.6.	[18F]fluoroglycosylation for generation of high-affinity PET tracers with improved <i>in vivo</i> properties.....	17
1.7.	Click chemistry	20
1.8.	[18F]fluoroglycosylation using CuAAC click chemistry	23
1.8.1.	The Maschauer approach	23
1.8.2.	Applications of the [18F]fluoroglycosylation method by Maschauer	26
1.9.	Protein Misfolding and Associated Diseases	28
1.9.1.	Alzheimer's disease.....	30
1.9.2.	Miscellaneous protein misfolding diseases.....	31
2.	Research Aim	32
3.	Synthesis of β-configured [18/19F]FDGs.....	33
3.1.	Synthetic rational and protection group strategy	33
3.2.	Early work – FDG synthesis via acid-catalyzed rearrangement of a 1,2-di-<i>O</i>-glycoorthoester	36
3.3.	FDG synthesis via 1,2-anhydrosugars (Paper I)	40
3.3.1.	Synthetic rational	40
3.3.2.	Synthesis.....	41
3.3.3.	Synthesis of α -configured clickable FDGs.....	45
3.4.	Radiofluorination of α- and β-[18F]FDG precursors	46
3.4.1.	Results	46

3.5.	Configurational dependence of [18F]FDG precursors to undergo radiofluorination	47
3.5.1.	The Richardson-Hough rules governing S _N 2 displacements on pyranosides.....	49
4.	[18F]fluoroglycosylation of amino acids.....	51
5.	[18F]fluoroglycosylation of curcumin (Paper II).....	54
5.1.	Curcumin	54
5.2.	Curcumin as an Aβ-imaging agent (Paper II).....	56
5.3.	Synthesis.....	58
5.4.	Radiochemistry	59
5.5.	Cell uptake in a melanoma cell-line and toxicity studies	60
6.	[18F]fluoroglycosylation of LCOs	62
6.1.	PET tracers for AD imaging.....	62
6.2.	Luminescent Conjugated Oligothiophenes.....	63
6.3.	Synthesis.....	65
6.4.	Staining of human brain slides displaying AD pathology	68
6.5.	Conclusions	70
7.	[19F]fluoroglycosylation of glycoporphyrins (Paper IV).....	71
7.1.	Porphyrins in photodynamic therapy	71
7.2.	Synthetic rational.....	73
7.3.	Synthesis.....	74
7.3.1.	Porphyrin synthesis	74
7.3.2.	Synthesis of 2-azidoethyl β-D-glycopyranosides.....	76
7.4.	Flow cytometry assay.....	79

8.	Synthesis of fluorinated <i>trans</i>-stilbenes for spectral discrimination of native and protofibrillar transthyretin	82
8.1.	Synthesis rational.....	82
8.2.	<i>E</i> -stilbene synthesis	84
8.3.	Results	85
9.	Conclusions and Future Perspectives.....	86
10.	References	88

Preface

Chemistry has been a long-term passion of mine. When I first was introduced to the spectacular phenomena of chemistry I was fully amazed. It turned out that these phenomena could fascinatingly be demystified and rationalized by comprehensible laws given by my enthusiastic high-school chemistry teacher. I knew already then that I would pursue a career as a chemist. Some years later as an undergraduate student at Linköping university, I found myself particularly interested in the field of organic synthesis, i.e., the field where, predominately, carbon-based molecules are synthesized and assembled in different manners to produce new compounds with novel properties for instance for use as pharmaceuticals. Later, during my graduate work, I came in contact with the intriguing sub-category of organic synthesis - carbohydrate synthesis.

In a sense, carbohydrate synthesis can be viewed as an intricate and highly stimulating board game with an extensive list of rules to follow. To synthesize the desired carbohydrate compound a multi-step sequence is required where each step may prove to be a pitfall that will terminate the whole synthetic sequence, if the rules have not been adequately met. In addition, new rules are continuously added to the deck and there is an ever-increasing demand for custom made carbohydrate-based compounds which ensures that the game will remain challenging and engaging for years to come.

During my PhD studies, I have come to realize that finishing a round in this game may come with a great reward as it may provide novel diagnostic markers for some of our most terrible and challenging diseases, e.g., cancer and Alzheimer's disease, thus setting an admirable goal to pursue.

The results underlying this thesis adds a brick to the wall of knowledge in terms of synthesizing and exploiting fluorinated sugars for diagnostic and therapeutic applications. However, I am convinced that the interdisciplinary field glycobiology, especially in the context of radiochemistry, is only in its infancy where major discoveries are still waiting to be unveiled.

With this, I hope you will enjoy reading my thesis!

Yours sincerely,

Mathias Elyland Linköping, March, 2018

1. Introduction

1.1. The Chemistry of Carbohydrates

The simplest of carbohydrates, monosaccharides, can be described as organic compounds adhering to the formula $C_m(H_2O)_n$, where m and n are integers. Carbohydrates can therefore be considered as “hydrated carbon atoms”. More specifically, carbohydrates are aliphatic polyhydroxy aldehydes – or ketones, or derivatives thereof formed either by reduction of the carbonyl group (alditols) or by oxidation (uronic or aldarcic acids). Derivatives formed by replacing one or several hydroxyl groups with functional groups such as amino (glycosyl amines), thiol (thioglycosides), or hydrogen (deoxysugar) are also considered to be carbohydrates (Figure 1.1).¹

Figure 1.1 Examples of different forms of carbohydrates.

Carbohydrates contain many stereogenic centers and therefore exist as a great number of stereoisomers. A convenient way of depicting the stereochemical information is by drawing the structures in a Fischer projection (Figure 1.2). In a Fischer projection, the terminal carbon at the reducing end is given the highest priority, i.e., the lowest number. The number then increases as one progresses towards the other end of the sugar. The

stereogenic center with the highest number, referred to as the center of reference, determines the absolute configuration of the sugar. If the hydroxyl group is placed on the right-hand side the sugar is given the prefix D (*dexter*, latin for right) or the prefix L (*laevus*, latin for left) if the hydroxyl group is pointing to the left in the Fischer projection. Acyclic monosaccharides readily undergo an intramolecular ring closing between a hydroxyl group and the carbonyl functional group resulting in the formation of a hemiacetal. The ring closing can either form a five-membered ring (furanose) or a six-membered ring (pyranose). In aqueous solution, these forms exist in an equilibrium which is heavily shifted towards the cyclic forms, in particular towards the pyranoses. The ring closing creates an additional stereogenic center called the anomeric center which gives rise to two diastereomers (or epimers) often referred to as anomers in carbohydrate nomenclature. If the newly formed hydroxyl group bears a cis-relation to the center of reference (C-5 in D-glucose) then the anomer is denoted with the prefix α . Conversely, if the two hydroxyl groups bear a trans-relation then the configuration is assigned with the prefix β . In aqueous solution, the acyclic form of D-glucose constitutes 0.002%, the α - and β -anomer of the furanose 0.5 % each, whereas the predominant species, the α - and β -anomer of the pyranose, constitute 38.0% and 62.0% respectively.²

Figure 1.2 a) Depicting the acyclic form of D-glucose and its intramolecular cyclization to give either D-glucopyranose or D-glucopyranose. b) Illustrating the equilibrium between the acyclic-, furanose- and pyranose form of D-glucose.

1.2. Metabolism of Carbohydrates

1.2.1. Transportation of glucose over cell membranes

Glucose, synthesized from CO₂ and H₂O in plants by photosynthesis is charged with energy from the sun and serves as a primary source of energy for most organisms, including mammals.³ Due to the hydrophilic nature of glucose, cell uptake over the lipophilic plasma membrane in eukaryotes is facilitated by specific glucose transporter membrane proteins (GLUT). To date, 14 members of the GLUT-family are known, although GLUT 1-4 (referred to as class I) were the first to be described and are also the most well-characterized members.⁴

GLUT-1 is expressed in virtually all tissue and has a high affinity for glucose (K_m 3 mmol/L), but also allows passage of the related monosaccharides galactose (K_m 17 mmol/L), mannose (K_m 20 mmol/L) and glucosamine (K_m 2.5 mmol/L).⁵ The brain, amounting to only 2 % of our body mass, consume approximately 20 % of our glucose reserves, thereby making it the main consumer of glucose in mammals.⁶ To meet the high energy demands of the brain, GLUT-1 is expressed on the tightly joined epithelial cells making up the highly discriminating blood-brain barrier (BBB) thus allowing glucose to enter the brain from our circulating blood stream. Well on the inside, glucose is primarily taken-up in neurons by another glucose transporter called GLUT-3. In contrast to GLUT-1, GLUT-3 is essentially only expressed in the brain and in the testis. With its high affinity for glucose (K_m 1.6 mmol/L)⁷, GLUT-3 ensures that the high energy demand of the brain is met. In particular, it has been shown that GLUT-3 is expressed to a larger extent in the grey matter of the brain which suggests that GLUT-3 may preferentially facilitate glucose uptake in regions of the brain that have a particularly high metabolic rate.⁸

1.2.2. Glycolysis

In living organisms, the stored energy in glucose is released in a process called glycolysis that takes place in the cytosol.⁹ In a series of enzymatic steps, glucose is converted to two 3-carbon units of pyruvate, producing two molecules of adenosine triphosphate (ATP) as well as two molecules of nicotinamide adenine dinucleotide (NADH) (Scheme 1.1). ATP itself is an energy-rich compound that is used to fuel many biological processes.

Scheme 1.1 Glycolysis. In the glycolysis, glucose is broken down to two units of pyruvate in a chain of enzymatic reactions. Although energy in terms of two ATP units has to be expended in the early phosphorylation steps, formation of each pyruvate is accompanied with the production of two ATP units. Overall, one glucose unit generates two ATP and two NADH units.

However, the ancient process of releasing energy from glucose via the anaerobic metabolic process – glycolysis, only releases a fraction of the stored energy of glucose. In eukaryotes, the generated pyruvate then enters an aerobic metabolic pathway called the citric acid cycle where pyruvate is eventually metabolized to CO₂ producing another ATP molecule per cycle. Furthermore, NADH (and FADH₂) generated along the way go on to the oxidative phosphorylation where it is estimated that in total about 30 ATP molecules can be produced from one molecule of glucose following these metabolic pathways.¹⁰

1.2.3. Cancer Metabolism

One of the hallmarks of cancer is its uncontrolled capacity for proliferation which renders the cancer cells in increased demand for sugars, fatty acids and amino acids, that provide energy and serve as key building blocks for important biomolecules. The rate-limiting step in the glycolysis is considered to be the transport of glucose over the cell membrane.¹¹ In one study the rate of glycolysis in a cancer cell-line was more than 30 times faster compared to benign cells.¹² In order to uphold the high glycolytic rate, cancer cells tend to up-regulate the expression of GLUT proteins on the cell surface in order to increase the up-take of glucose. GLUT-1, being ubiquitously distributed, has been shown to become up-regulated in most types of cancer.^{12,13} On the other hand, protein expression of GLUT-3 has for instance been found in lung¹⁴-, breast¹⁵- and bladder cancer¹⁶, i.e., in tissue where GLUT-3 is not normally expressed. GLUT-3 has also been found to be abnormally up-regulated in various brain cancers.¹⁷⁻¹⁹

Similar to the consumption of glucose, many amino acid transporters are also up-regulated in cancers to meet the increased demand for nutrients. The amino acid transporter LAT-1 (large neutral amino acid transporter) preferentially transports bulky, branched amino acids (valine, leucine, isoleucine) and aromatic amino acids (tryptophan, tyrosine). Interestingly, LAT-1 expressed on the BBB has a much higher affinity for amino acids than for those expressed in peripheral tissue.²⁰ Up-regulation of LAT-1 has been observed in a number of cancers, e.g., human bladder carcinoma²¹ and human glioma carcinoma²².

Another important amino acid transporter is the sodium-coupled ASCT2 that accept all neutral amino acids, in particular the essential amino acid glutamine, as substrates for transport in both directions.²³ Glutamine is not only a source of energy but also an important building block for proteins and DNA and is therefore particularly important for the proliferation of cancer cells. In one study, it was shown that the need for glutamine was 10-fold higher in a cancer cell-line compared to healthy cells.²⁴ Up-regulation of ASCT2 has also been shown to play a key role in colon²⁵ and liver cancer²⁶.

The elevated and abnormal expression of both glucose and amino acid transporters makes them very promising biomarkers in cancer diagnostics. Furthermore, since essentially the same transportation mechanism applies for passage of nutrients over the BBB, glycosylation or amino acid conjugation of drugs may facilitate delivery across the BBB for both therapeutic and diagnostic purposes.

1.3. Positron emission tomography

Positron emission tomography (PET) is a non-invasive *in vivo* imaging method that is widely used in neuroimaging²⁷ and in oncology²⁸, both for research and clinical use. PET imaging enables clinicians to accurately diagnose and monitor disease-progression for instance in cancer and neurodegenerative diseases such as Alzheimer's disease.

PET utilizes radiolabelled (positron-emitting) biologically active molecules, i.e., radiotracers, that are administered intravenously in animals and humans. The radiotracer is then distributed in the body and selectively accumulates in a certain tissue (e.g., tumor). Abnormal accumulation may then indicate a pathological condition.

The radionuclide inserted into the organic carrier (together making up the radiotracer) decay by β^+ -emission, i.e., by emitting a positron (the positively charged anti-particle of the electron). The emitted positron will travel a short distance, called the positron range, through the surrounding tissue before it encounters an electron from normal matter. The positron range depends on the actual radionuclide being used. The higher energy of the

positron the longer becomes the positron range which in turn decreases the spatial resolution that can be retrieved. Being their respective anti-particles, the positron and the electron will annihilate, whereby their mass is converted to electromagnetic energy manifested as two gamma-rays, with 511 keV in energy each, that set off with a 180 degrees trajectory with respect to each other.²⁹ The gamma-rays, forming a line, are then registered in a circular photodetector. The precise location of the annihilation event is unknown but it must have occurred somewhere on the line. With a great number of these annihilation events, a computer can produce an accurate, three-dimensional image of the tissue (Scheme 1.2). Modern PET scanners are, in addition, equipped with an X-ray computed tomography scanner (PET-CT) that has revolutionized the nuclear imaging field. The CT-component of PET-CT complements with images of the anatomy, i.e., the dense matter of the patient. The images from both scanners are then superposed which lead to far more precise images.³⁰

Scheme 1.2 The principle of PET imaging. a) A cancer patient is intravenously injected with a radiotracer, exemplified with $[^{18}\text{F}]\text{FDG}$. b) The radiotracer is allowed to accumulate in the cancer tissue, revealing a primary tumor and metastases. c) The ^{18}F -nuclide incorporated in $[^{18}\text{F}]\text{FDG}$ releases a positron that briefly travels through the surrounding tissue a short distance (i.e. the positron range) before encountering an electron from normal matter. Being their respective anti-particles, they annihilate, giving rise to two gamma rays that set off in opposite directions and are recorded in a surrounding photodetector that ultimately produces a 3D image of the tumor.

1.3.1. The positron-emitting nuclide fluorine-18

Common positron emitting nuclides are the proton-rich carbon-11, nitrogen-13, oxygen-15 and fluorine-18. Their physical data are given in Table 1.1.³¹ The incorporation of the positron-emitting isotopes of carbon, nitrogen and oxygen into biomolecules has the benefit that their physicochemical and biochemical properties are unaltered and indistinguishable from their non-radioactive counterparts. Fluorine however, is not a naturally occurring element in biomolecules but is often used as a bioisosteric replacement for a hydrogen atom or a hydroxyl group. Fluorine is approximately the same size as hydrogen (van der Waal's radii of fluorine and hydrogen are 1.47 and 1.20 Å, respectively) and does not impose any significant steric constraints on substitution.³²

Moreover, fluorine, being the most electronegative element (3.98 on the Pauling electronegativity scale compared to 2.20 for H, 3.44 for O, and 2.55 for C) induces a strong dipolar moment that alters the lipophilicity of the organic carrier. Accordingly, fluorine incorporation is now routinely applied in drug development to modulate, not only the lipophilicity, but also the metabolic stability, pK_a and other pharmacokinetic properties.³³

Fluorine-18 has almost optimal properties as a PET radionuclide. The relatively low positron emission energy (0.64 MeV) lead to a short positron range and thus to higher spatial resolution compared to the other common nuclides. Moreover, the comparably long half-life (110 min) provide ample time for the production of more complex PET tracers that require multi-step synthesis, and also allows for longer *in vivo* investigations. Ideally, the synthesis and purification of a PET tracer should not exceed 2-3 half-lives of the radionuclide in use. Another benefit with fluorine-18 is that it can be transported to clinical PET facilities that lack their own cyclotron equipment for radionuclide synthesis.³¹

Nuclide	Half-life (/min)	Maximum energy (/MeV)
¹⁸ F	110	0.64
¹¹ C	20.3	0.97
¹³ N	10	1.20
¹⁵ O	2	1.74

Table 1.1 Physical properties of common positron-emitting radionuclides.

1.4. [18F]FDG

In PET, the most common carrier of fluorine-18 is 2-deoxy-2-[18F]fluoro-D-glucose ([18F]FDG, Figure 1.3). [18F]FDG is, by far, the most widely used radiopharmaceutical in clinics, both in oncology and neurology, where approximately 90 % of all PET scans are performed using ([18F]FDG.³⁴ It is therefore not surprising that [18F]FDG often is referred to as the golden standard in PET. There seems to be a consensus within the PET community that the PET field would not exist if not for the discovery and easy access to [18F]FDG. Structurally, [18F]FDG is a derivative of D-glucose where the 2-OH has been replaced with ¹⁸F.

Figure 1.3 Comparison between the chemical structure of D-glucose and [18F]FDG. In [18F]FDG, the hydroxyl group at C-2 has been substituted with ¹⁸F.

Figure 1.4 Principle of metabolic trapping of [18F]FDG [18F]FDG is able to enter cells via glucose transporters. In the cytoplasm, [18F]FDG is phosphorylated at the 6-OH position by hexokinase II forming [18F]FDG-6-phosphate. However, in contrast to D-glucose, [18F]FDG cannot undergo the subsequent enzymatic reaction i.e., isomerization to fructose-6-phosphate mediated by phosphohexose isomerase, due to the fluorine substitution at C-2. Consequently, [18F]FDG-6-phosphate, now being negatively charged, can no longer exit the cell through the glucose transporters and becomes metabolically trapped in the cell. Therefore [18F]FDG constitutes an ideal tracer for probing the glucose demand and/or the hexokinase activity in the investigated tissue. [Extracted from M. L. James and S. S. Gambhir, *Physiological Reviews*, 2012, 92, 897–965. Copyright © 2012 American Physiological Society]

Just as D-glucose, [18F]FDG is able to enter cells via glucose transporters. However, in contrast to D-glucose, [18F]FDG only takes part in the first step of the glycolysis (Scheme 1.1), i.e., the phosphorylation of 6-OH by hexokinase II forming [18F]FDG-6-phosphate. [18F]FDG-6-phosphate, now being negatively charged, can no longer exit the cell through the glucose transporters. As a consequence, [18F]FDG becomes metabolically trapped in the cell (Figure 1.4).³⁵ The subsequent enzymatic reaction; isomerization to fructose-6-phosphate mediated by phosphohexose isomerase, is prohibited due to the fluorine substitution at C-2.

The aforementioned properties of [18F]FDG therefore make it an excellent biomarker for glucose metabolism. Since an accelerated glycolytic rate is one of the main hallmarks of cancer (section 1.2.3), [18F]FDG is well suited for cancer imaging. Once injected intravenously into a cancer patient, [18F]FDG will preferentially accumulate in the

glucose demanding tumor tissue, thus providing oncologists with a tool to diagnose and monitor the staging and therapy response.

As covered earlier (section 1.2), another area of high glucose consumption is the brain. [18F]FDG has previously been employed to diagnose and monitor the decreased metabolic activity associated with neurodegenerative diseases, in particular Alzheimer's disease. However, nowadays [18F]FDG has largely been replaced with more specific PET tracers (eg. [11C]PIB) that bind directly to the characteristic protein aggregates.

1.4.1. Synthesis of [18F]FDG

[18F]FDG (**4**) was first prepared by Ido et al. in 1978 at the Brookhaven National Laboratory, New York (Scheme 1.3).³⁶ They produced [18F]F₂ gas that underwent an electrophilic addition to tri-acetyl-*D*-glucal (**1**), generating 3,4,6-tri-*O*-acetyl-2-deoxy-2-[18F]fluoro- α -*D*-glucopyranosyl fluoride (**2**) and 3,4,6-tri-*O*-acetyl-2-deoxy-2-[18F]fluoro- β -*D*-mannopyranosyl fluoride (**3**) that were isolated with preparative HPLC in 17.6% and 4.8% radiochemical yield (RCY) respectively. Subsequent acidic deprotection using HCl provided [18F]FDG (**4**) in 8% RCY.

Scheme 1.3 The original synthesis of [18F]FDG by Ido et al.

However, the low yield and poor stereoselectivity, along with the difficult handling of $[^{18}\text{F}]\text{F}_2$ prevented this method from gaining widespread use. Significant improvements were later made in 1986 by Hamacher et al.³⁷ who synthesized $[^{18}\text{F}]\text{FDG}$ using nucleophilic $[^{18}\text{F}]\text{F}^-$ displacement, assisted with the cryptand kryptofix (K[2.2.2]), on the precursor 1,3,4,6-tetra-*O*-acetyl-2-*O*-trifluoromethanesulfonyl- β -D-mannopyranose³⁸ **5** (Scheme 1.4a). Mechanistically, the triflate group at C-2 on the precursor **5** undergoes an $\text{S}_{\text{N}}2$ reaction with the nucleophile $[^{18}\text{F}]\text{F}^-$, with accompanying inversion of configuration, yielding acetylated $[^{18}\text{F}]\text{FDG}$ (**6**). Acidic deacetylation by refluxing in 1M HCl at 130 °C for 15 minutes afforded $[^{18}\text{F}]\text{FDG}$ (**4**) in approximately 50% RCY. As the acidic deprotection of $[^{18}\text{F}]\text{FDG}$ is the most time-consuming step in the synthesis of the short-lived $[^{18}\text{F}]\text{FDG}$, considerable efforts to optimize the reaction were made during the 1990's. Generally, in organic synthesis, esters are typically hydrolysed under alkaline conditions, since the reaction often proceeds both faster and under milder conditions compared to acidic conditions. However, in the case of ester hydrolysis of reducing carbohydrates (i.e., aldoses and ketoses) such as $[^{18}\text{F}]\text{FDG}$, alkaline conditions can lead to an unwanted epimerization at C-2 of the deacetylated $[^{18}\text{F}]\text{FDG}$, yielding 2-deoxy-2- $[^{18}\text{F}]\text{fluoro-D-mannopyranose}$ ($[^{18}\text{F}]\text{FDM}$) through the Lobry de Bruyn-Alberda van Ekenstein transformation.³⁹

In 1996, Füchtner et al. carefully studied de-acetylation of *per*-acetylated $[^{18}\text{F}]\text{FDG}$ under alkaline conditions and they found that ideal conditions for the hydrolysis were using 0.3 M NaOH for 1 minute at room temperature.⁴⁰ Under these conditions, $[^{18}\text{F}]\text{FDG}$ was obtained in essentially quantitative yield, thus not only avoiding the formation of $[^{18}\text{F}]\text{FDM}$ but also significantly shortening and simplifying the procedure for $[^{18}\text{F}]\text{FDG}$ synthesis (Scheme 1.4b). Interestingly though, in their investigation they also found that prolonged reaction times, or increased concentration of NaOH, led to significantly lower RCYs. This effect was predominantly caused by abstraction of $[^{18}\text{F}]\text{fluorine}$ from tri-acetylated $[^{18}\text{F}]\text{FDG}$ (but not from deacetylated $[^{18}\text{F}]\text{FDG}$) regenerating $[^{18}\text{F}]\text{F}^-$ in the process, but also due to the accompanying epimerization.

To date, the later protocol still forms the basis for routine synthesis of [^{18}F]FDG using automatic systems at PET centres world-wide.

Scheme 1.4 Synthesis of [^{18}F]FDG employing the precursor **5** developed by Hamacher et al. followed by the (a) original acidic de-acetylation at elevated temperatures or (b) the improved de-acetylation under alkaline conditions at ambient temperature by Füchtner et al.

1.4.2. Drawbacks with [^{18}F]FDG

[^{18}F]FDG is currently the only FDA approved oncological PET tracer. Despite its prominent role and almost ideal properties as a biomarker of glucose metabolism, [^{18}F]FDG is not a target-specific PET tracer. As a consequence, certain benign processes are imaged by [^{18}F]FDG-PET scans as well leading to the risk of false-positives in cancer diagnostics. For instance, it is well known that infections or inflammations give rise to an increased uptake of [^{18}F]FDG in the afflicted tissue. Moreover, brown fat tissue and lymphoid lesions can also exhibit a high glycolytic rate.⁴¹ For this reason, [^{18}F]FDG-PET images have to be carefully reviewed in order to avoid false-positives. Conversely, certain cancer forms (e.g., most prostate cancers, bronchoalveolar-cell carcinoma in the lung or renal-cell carcinoma) have a glycolytic rate on the same magnitude as benign tissue and can therefore not be detected in an [^{18}F]FDG-PET scan. Despite the shortcomings of [^{18}F]FDG, as an oncological PET tracer, there are hardly any available approved alternative tracers.

Clearly, there is a great need for new PET tracers with high affinity for a specific biomolecular target. This in turn requires chemists to develop new methods for simple and practical radiolabeling of a diverse set of ligands.

1.5. Peptide-based imaging agents

A promising class of oncological imaging agents are peptide-based PET tracers. Certain peptide binding cell-membrane receptors are (similar to glucose- and amino acid receptors) up-regulated in tumors, and therefore serve as a potential biomolecular targets for cancer diagnostics. The advantage of using small peptide tracers (compared to antibodies and proteins) is that they often have rapid pharmacokinetic properties with fast clearance from the blood and other non-target tissues. Moreover, they have a very high affinity for their receptor target and are fairly easy to synthesize (e.g., with a solid-phase peptide synthesizer) and chemically modify. However, the major drawback with native (or minimally modified) peptides is that the biological half-life is often very short, effectively preventing them from reaching their target in time. One approach is to modify the peptide with for instance the inclusion of unnatural amino acids with altered side chains, or making isosteric substitutions e.g., by employing aminoalcohol replacements for the amide bonds. The challenge, however, lies in performing these chemical modifications while still retaining high affinity for the corresponding receptor.⁴²

Several classes of peptide-based imaging agents have been developed for cancer imaging. For neuroendocrine tumors, the target is mainly the somatostatin receptor II for which several radiolabeled somatostatin analogues have been reported, including the ⁶⁸Ga-DOTATOC.⁴³ Another class of peptide-based imaging agents are analogues of the bombesin peptide, which is a 14 amino acid peptide that bind to bombesin receptors⁴⁴, but also to human gastrin-releasing peptide receptors which has been found to be overexpressed in several types of cancer, including prostate, breast, gastrointestinal and small cell lung cancer.⁴² RGD (Arginylglycylaspartic acid) is a tripeptide that binds to the integrin receptor $\alpha\beta3$ that, for instance, play a key role in tumor-induced angiogenesis⁴⁵ and metastasis and has been found to be overexpressed, among others, in gastric-, glioma-, lung- and prostate cancer.⁴⁶ Radiolabelled RGD peptides are therefore promising candidates for imaging of fast growing and metastasis prone tumors. In 2003, Haubner et al. developed a glycosylated [¹⁸F]galacto-RGD tracer where the

sugar unit was found to significantly improve the pharmacokinetic profile, increase the metabolic stability and provided a good tumor-to-background ratio.^{47,48}

1.6. [18F]fluoroglycosylation for generation of high-affinity PET tracers with improved *in vivo* properties

Peptides are highly selective ligands with high affinity for a given receptor but they often suffer from poor metabolic stability and short retention in the targeted tissue. On the other hand, [18F]FDG has an excellent metabolic stability and tissue retention due to the metabolic trapping property, imparted by the fluorine substitution at C-2, but lack target specificity and is restricted to detect tumors with a high glycolytic rate. It therefore seems that the shortcomings of peptides are remedied by the properties of [18F]FDG and vice versa making the ligation of the two highly desirable. The concept of employing [18F]FDG as a prosthetic group for radiolabelling of sensitive biomolecules e.g., peptides and proteins was introduced by Prante et al. in 1999. In their initial study they used a chemoenzymatic approach where *per*-acetylated [18F]FDG was converted to [18F]FDG-1-phosphate that acts as a substrate for the enzyme UDP-glucose-pyrophosphorylase, yielding the glyconucleotide, uridine-5'-diphospho-2-deoxy-2-[18F]fluoro- α -D-glucopyranose (UDP-[18F]FDG) (Figure 1.5a). The synthesized UDP-[18F]FDG was designed in order to act as glycosylation agent mediated by glycosyltransferases for selective [18F]fluoroglycosylation of biomolecules.⁴⁹ Since then, a number of different chemical approaches have been employed for [18F]FDG conjugation, e.g., Lewis acid promoted glycosylation (using $\text{Hg}(\text{CN})_2$ and SnCl_4) with *per*-acetylated [18F]FDG as a glycosyl donor to 2-nitroimidazole. Subsequent deacetylation yielded the tracer 1-(2-deoxy-2-[18F]fluoro- β -D-glucopyranosyl)-2-nitroimidazole that was evaluated for imaging of hypoxia (Figure 1.5b).⁵⁰ In 2007, the group of Prante reported on the thiol-reactive agent 3,4,6-tri-O-acetyl-2-deoxy-2-[18F]fluoroglucopyranosyl phenylthiosulfonate that chemoselectively forms disulphide linkages to cysteine containing peptides in a site-specific manner. This was effectively demonstrated by [18F]fluoroglycosylation of the cyclic cysteine-linked RGD-peptide (cRGDfC) forming the [18F]FDG-RGD peptide in quantitative radiochemical yield

within 15 minutes (Figure 1.5c).⁵¹ Another ingenious approach to [18F]fluoroglycosylation is that of oxime formation. Oxime formation is a chemoselective and high-yielding reaction that takes place between an aldehyde (or a ketone) and hydroxylamine in a condensation reaction. In aqueous solution, [18F]FDG undergoes mutarotation (section 1.1) and transiently equilibrates to its acyclic form, revealing the aldehyde functional group that in turn can react with a hydroxylamine-bearing ligand. The group of Gambhir and Hultsch simultaneously reported on the use of this approach to form [18F]FDG-RGD conjugates for potential imaging of integrin receptors (Figure 1.5d)^{52,53} while the group of Pietzsch used this approach to radiolabel the human protein annexin V for imaging of apoptosis *in vivo* (Figure 1.5e).⁵⁴

While [18F]fluoroglycosylation by oxime formation offers a fairly straightforward way to radiolabel peptides, the reaction often has to proceed at elevated temperatures and under acidic conditions, in order to facilitate a fast equilibration to acyclic [18F]FDG, which may not be tolerated by sensitive biomolecules. Furthermore, it is improbable that the linear [18F]FDG moiety is still recognized as a glucose substrate by GLUT transporters, or for phosphorylation by hexokinases, thus potentially abolishing many of the key properties that are sought in [18F]FDG conjugates.

a)

UDP-[18F]FDG

for chemoenzymatic [18F]fluoroglycosylation utilizing glycosyltransferases.

Prante et al. *J. Label Compd. Radiopharm*, 2007, **50**, 55–63.

b)

[18F]FDG-2-nitroimidazole targeting hypoxia

Patt et al. *Applied Radiation and Isotopes*, 2002, **57**, 705–712.

c)

[18F]FDG-cRGD targeting integrin receptors

Prante et al. *Bioconjugate Chem.*, 2007, **18**, 254–262.

d)

[18F]FDG targeting integrin receptors

Namavari et al. *Bioconjugate Chem.*, 2009, **20**, 432–436.

e)

[18F]FDG-annexin V targeting apoptosis

Wuest et al. *Bioconjugate Chem.*, 2008, **19**, 1202–1210.

Figure 1.5 Literature examples of different [18F]fluoroglycosylations methods.

Prepared via a) Chemoenzymatic synthesis from [18F]FDG-1-phosphate catalysed by UDP-glucose-pyrophosphorylase. b) Lewis acid promoted Koenig-Knorr glycosylation. c) Site-selective disulphide coupling d) Oxime formation e) Oxime formation (PDB ID: 1AVR)

1.7. Click chemistry

The concept of “click chemistry”, as coined by Sharpless in 1999, is defined as a class of reactions that ligate two molecular units under the following criteria: the reaction must be high-yielding, producing only non-offensive by-products that can easily be removed without the need for chromatography. Furthermore, the reaction must be modular and stereospecific (but not necessarily enantioselective), and have a wide scope.

Several reactions meet these criteria as outlined by Kolb et al.⁵⁵ :

- Cycloadditions of unsaturated species, e.g., predominantly 1,3-dipolar cycloaddition reactions but also Diels-Alder reactions.
- Nucleophilic ring-opening of strained heterocyclic electrophiles e.g., epoxides and aziridines.
- Certain carbonyl reactions, including the formation of ureas, oximes and hydrazones.
- Additions to carbon-carbon multiple bonds e.g., epoxidation, dihydroxylation, aziridination but also Michael additions.

Although many reactions satisfy these rules, without question, the by far most utilized and the most important click reaction is the copper (I) catalyzed azide-alkyne [3 +2] cycloaddition (CuAAC). The CuAAC reaction was independently discovered by the group of Sharpless⁵⁶ and Meldal⁵⁷ in 2002.

The CuAAC reaction was rapidly and successfully applied in a great number of research areas spanning drug discovery, biochemical applications (e.g., site-specific labelling of cells and proteins, labelling and sequencing of DNA)⁵⁸ and material design. The CuAAC reaction has also been readily adopted in the field of glycoscience where a tremendous amount of glycoconjugates (derived from peptides, proteins, lipids and pharmaceuticals etc.) have been prepared.⁵⁹ It is therefore with good reason that the CuAAC reaction often is referred to as the “quintessential click reaction”.⁶⁰

The uncatalyzed reaction between organic azides and terminal alkynes was reported back in the 1960's by Huisgen.⁶¹ Without a catalyst, the reaction is slow, requires elevated temperatures and often yields a regioisomeric mixture of 1,4- and 1,5-substituted 1,2,3-triazoles (Scheme 1.5), thus disobeying the criteria to be considered a click reaction. In contrast, the CuAAC click reaction exclusively forms the 1,4-substituted 1,2,3-triazole and is estimated to proceed about 10^7 times faster than the uncatalyzed reaction. In 2005, a ruthenium catalyzed azide-alkyne cycloaddition reaction (RuAAC) was reported by Fokin and collaborators that regioselectively provided the complementary 1,5-substituted 1,2,3-triazole.^{62,63} Interestingly, the RuAAC reaction also accept internal alkynes as substrates thus broadening the scope of the reaction. However, the complex and sensitive ruthenium catalysts, often requiring inert and anhydrous conditions, have prevented wide application of the RuAAC reaction. Recently, a corresponding nickel catalyzed azide-alkyne cycloaddition (NiAAC) was discovered by Woo Gyum Kim et al. that, similarly to the RuAAC, provides the 1,5-substituted 1,2,3-triazole with exceedingly high regioselectivity. In addition, in contrast to the RuAAC reaction, the NiAAC reaction can be performed in aqueous solution at ambient temperature. This property may potentially allow for click coupling to sensitive biomolecules. Towards that end, they were able to demonstrate the successful NiAAC reaction of an azido-equipped β -D-glucopyranoside to an alkyne-equipped phenylalanine, providing the glucosyl amino acid in 65 % yield and with > 99:1 regioselectivity, favoring the 1,5-substituted 1,2,3-triazole.

Scheme 1.5 Depicting the regiochemistry of 1,2,3-triazoles obtained from azide-alkyne [3+2] cycloadditions depending on the particular catalyst employed. The uncatalyzed reaction gives a regioisomeric mixture of 1,4- and 1,5-triazoles, the Cu (I) catalyzed reaction provides exclusively the 1,4-regioisomer whereas the Ru/Ni catalyzed reaction exclusively affords the 1,5-triazole.

1.8. [^{18}F]fluoroglycosylation using CuAAC click chemistry

1.8.1. The Maschauer approach

In 2009, CuAAC click chemistry was for the first time employed for [^{18}F]fluoroglycosylation by the pioneering work of Maschauer in the group of Olaf Prante. In their study, they synthesized a set of azide- and alkyne equipped 2-*O*-trifluoromethylsulfonyl substituted *D*-mannopyranosides as precursors for clickable [^{18}F]FDGs.⁶⁴ Since the substitution of triflate-for- ^{18}F fluorine is accompanied with an inversion of configuration ($\text{S}_{\text{N}}2$ reaction) the precursor, by necessity, has to be a *D*-mannopyranoside.

Their synthesis commenced from 1,3,4,6-tetra-*O*-acetyl- β -*D*-mannopyranose (**7**, Scheme 1.6) that was acylated at *O*-2 with pentafluoropropionic anhydride (Pfp₂O) yielding compound **8**. Bromination with HBr-HOAc subsequently afforded the corresponding α -bromosugar **9** that was used as a glycosyl donor in a AgOTf-promoted

Scheme 1.6 The synthetic route to clickable [^{18}F]FDG precursors by Maschauer et al. in 2009. Reagents and conditions: (i) Pfp₂O, py, CH₂Cl₂, 1 h, 0 °C, quant.; (ii) HBr-AcOH, CH₂Cl₂, 0 °C to rt, 20 h, 90%; (iii) AgOTf, propargyl alcohol, CH₂Cl₂, rt, 40 min, 65%; (iv) EtOH, py, rt, 2 h, quant.; (v) Tf₂O, py, CH₂Cl₂, 20 °C to 0 °C, 1 h, 74% for **12**, 36% for **16**, 40% for **18 β** , 55% for **18 α** ; (vi) AgOTf, 2-bromoethanol, CH₂Cl₂, rt, 40 min, 68%; (vii) NaN₃, DMF, rt, 20 h, 39%; (viii) NaN₃, DMF, rt, 20 h, then EtOH, py, rt, 1 h, 79%.

Koenig-Knorr glycosylation to propargyl alcohol yielded compound **10**. Deprotection of the Pfp-group was accomplished under slightly basic conditions using pyridine in ethanol to provide mannopyranoside **11**, that was triflated using triflic anhydride (Trf_2O) in pyridine and DCM to afford the alkyne equipped $[18\text{F}]\text{FDG}$ precursor **12**. In a similar manner, bromosugar **9** was reacted with 2-bromoethanol providing the mannopyranoside **13** that was further elaborated on to provide the azido equipped $[18\text{F}]\text{FDG}$ precursor **16**. Direct azidation of bromosugar **9**, with Pfp-removal performed in one-pot, provided an anomeric mixture of the mannosyl azide, i.e., $[18\text{F}]\text{FDG}$ precursor **18** ($\alpha/\beta \sim 1:1$) that could be separated by column chromatography.

The set of $[18\text{F}]\text{FDG}$ precursors was then radiolabelled using $[18\text{F}]\text{F}^-$, kryptofix ($\text{K}[2.2.2]$) and a $\text{K}_2\text{CO}_3\text{-KH}_2\text{PO}_4$ buffer in anhydrous acetonitrile (MeCN) at 85°C for 5 min. The results showed that none of the α -configured precursors (**12**, **16** or **18 α**) were radiolabelled to a significant degree (RCY 7-14%, Table 1.2) and these were therefore deemed unsuitable for PET. However, in stark contrast, the β -azido equipped precursor **18 β** provided the corresponding clickable $[18\text{F}]\text{FDG}$ **22** in 71% RCY.

Entry	Precursor	[18F]FDG product	RCY	Anomeric configuration
1			7%	α
2			8-9%	α
3			14%	α
4			71%	β

Table 1.2 Radiochemical yields for clickable [18F]FDGs as reported by Maschauer et al. The results strongly suggests that β -configured [18F]FDG precursors are required in order to obtain favourable RCYs.

Evidently, the choice of anomeric configuration plays a crucial role in the radiolabelling of these precursors. The authors attributed the low RCYs exhibited from the α -configured precursors to steric hindrance or electronic effects elicited from the α -aglycon, but did not clarify it to any more detail.

1.8.2. Applications of the [18F]fluoroglycosylation method by Maschauer

The β -azide equipped [18F]FDG (**22**) has successfully been utilized as a ^{18}F -fluoroglycosylation tool to provide [18F]FDG conjugates from a diverse set of ligands, e.g., a RGD peptide targeting the integrin receptor (Figure 1.6a),⁶⁵ for site-specific ^{18}F -labeling of the sex hormone-binding globuline (SsbG protein, Figure 1.6b),⁶⁶ folic acid targeting the folate receptor (Figure 1.6c)⁶⁷ and also to a cyanoquinoline for imaging of epidermal growth factors (Figure 1.6d)⁶⁸ See the review by Maschauer et al. for a more thorough account on synthesized [18F]FDG conjugates.⁶⁹

Figure 1.6 Literature examples where the [18F]fluoroglycosylation approach using CuAAC click chemistry developed by Maschauer et al. has been employed to radiolabel a) a cyclic RGD peptide b) the sex hormone-binding globuline (SsbG protein) c) folate d) cyanoquinoline.

The β -azido clickable [18F]FDG (**22**) offers a hitherto unparalleled approach for chemoselective [18F]fluoroglycosylation. Still, the method is associated with the following drawbacks; (1) Most notably, the corresponding alkyne-equipped [18F]FDGs are not readily accessible (in sufficient RCY) by their synthetic procedure, thus considerably diminishing the number of potential click couplings to biological ligands for tracer development. (2) Spacers (i.e., aglycons) between the [18F]FDG moiety and the azide group could not be introduced without compromising efficient radiolabeling

of the precursor. Moreover, the spacing should be modular to ensure that no steric interactions between the [18F]FDG and the ligated ligand takes place which may affect their affinity for their biomolecular target.

Another related [18F]-fluoroglycosylation was reported by Kim et al. in 2008, almost simultaneously as the [18F]-fluoroglycosylation method by Maschauer.⁷⁰ Their intent was to synthesize an analogue of [18F]FDG with improved *in vivo* properties. The synthesis was realized by preparation of β -D-glucosyl azide **26** that was click coupled to a pre-labelled [18F]-butyne spacer (**25**) forming the [18F]FDG analogue **27** (Scheme 1.7). Although the synthesis was rapid and effective, the radiolabeled triazole product was found not to act as a substrate for phosphohexokinase. Moreover, the product did not enter cells expressing GLUT-1 transporters. Therefore, it seems the [18F]FDG analogue did not retain any of the desired properties of [18F]FDG. The authors concluded that the C-1 triazole prevented the product from being recognized as a glucose substrate. Recall that the C-1 triazole motif is present in all of the [18F]FDG conjugates reviewed in this thesis so far. The [18F]-fluoroglycosylation by Kim et al. is severely limited in scope since the click reaction occurs under quite harsh conditions, thus ruling out labelling of biomolecules.

The approach by Kim et al. to radiolabel sugars was more recently extensively elaborated on by Consolato James Cara et al.^{71,72} James synthesized a range of azido sugars (featuring uronic acid or amine handles) and further demonstrated their conjugation to amino acids, peptides and neopeptides for future PET-studies. While this approach is indeed very clever, it is still restricted by the fact that the C-1 triazole may compromise the biological effect of the sugar. Furthermore, only simple alkyne-bearing radioactive ligands can be attached. But foremost, the synthesis of the glycopeptides are challenging and time-consuming thus hampering its wide application. To the best of our knowledge, no PET-studies have so far been conducted using this approach.

Scheme 1.7 Fluoroglycosylation method by Kim et al. in 2008.

Reagents and conditions: (i) TsCl, Et₃N, CH₂Cl₂, 0 °C, 3 h, 72% (ii) K[¹⁸F]F/K[2.2.2], CH₃CN, 100 °C, 20 min (iii) CuI, sodium ascorbate, 2,6-lutidine, 90 °C, 10 min

1.9. Protein Misfolding and Associated Diseases

Several of the compounds described in this thesis are designed to bind to protein aggregates for detection, either by fluorescence or by positron emission. It is therefore appropriate to here give a brief introduction to protein misfolding and the accompanying diseases that result thereof.

Proteins are heavily involved in most of the processes that are essential for life. In the human body, the proteins in our skin and nails provide us with a mechanical defence whereas the proteins in cartilage provide the body with mechanical support. Our ability to see would not be possible without the aid from the protein rhodopsin. As mentioned in previous sections (1.2.3), proteins embedded in our cell membrane are involved in cell-cell recognition and ensure that the cell receives nutrients, e.g., glucose and amino acids, from the blood stream. The nutrients are then processed by enzymes - a class of proteins that catalyze many of the chemical reactions that constantly occur in our body, for instance to harvest energy or to synthesize new proteins.

Proteins are composed of amino acids that are joined by consecutive peptide bonds forming long polymeric chains. Under physiological conditions, these strings of amino acids fold in a specific manner to adopt a particular three-dimensional structure. This structure, referred to as the native state, is necessary in order for the protein to perform its natural task. However, sometimes, either spontaneously or under external influence,

the folding event goes wrong or the native state is disrupted - either way leading to a misfolded conformation that lead to loss of function. The folding process can be represented by an energy landscape (Figure 1.7) with Gibb's free energy on the y-axis and the width of the funnel representing entropy.^{71,72} Initially, the unfolded protein has a lot of conformational freedom but as the folding process proceeds, the molecule becomes more restricted giving rise to a conformational volume with the appearance of a funnel (green). Both the native state and the corresponding amyloid fibrils can form spontaneously and are therefore low in free energy.

If the folding process towards the native state goes wrong, the protein may become trapped in the neighboring aggregation funnel leading to the disease-associated amyloid formation.

Figure 1.7 The protein folding energy landscape. The green area depicts the folding of native proteins, whereas the red area depicts the misfolding of proteins leading to amyloid fibrils. [Illustration - courtesy of Maria Jonson, PhD thesis "Investigating Amyloid β toxicity in *Drosophila melanogaster*", 2017.]

The body is equipped with many mechanisms to avoid misfolding or to discard the proteins that become misfolded. For instance, molecular chaperones (themselves being proteins) bind to certain proteins during the folding process and aid the protein to fold to the native conformation.⁷³ In some instances, the misfolded protein is able to induce misfolding in the neighboring native protein thus setting off a cascade of misfolding and aggregation events. Interestingly, the misfolded proteins are not randomly ordered but rather tend to associate to form highly ordered assemblies that are rich in β -sheet structure, referred to as amyloid fibrils, where the β -strands are aligned perpendicular to the fibril axis (Figure 1.8). Depending on the particular associated amyloid disease, these amyloid aggregates are either deposited in the tissue where the protein was originally located, or are transported systemically in the body and deposited in another organ. There, the amyloid aggregate, or the intermediate oligomeric species, interfere with the normal biological role of the surrounding tissue eventually leading to regional cell death or organ failure.

1.9.1. Alzheimer's disease

Figure 1.8 Depicting the structure of the native α -helical A β (1-42) peptide in aqueous hexafluoropropanol (left; NMR structure; PDB ID: 1Z0Q and the β -sheet rich structure of a A β (1-42) fibril that result from misfolding and concomitant aggregation of the native A β (1-42) peptide (right; NMR structure; PDB ID: 2MXU).

Alzheimer's disease (AD) is the most common form of dementia. There is no available cure for AD. In 2016, about 47 million people worldwide lived with dementia, and this number is expected to increase dramatically over the coming decades. In 2050, it is

projected that more than 137 million people will suffer from dementia, mainly due to the increase in life expectancy worldwide. This will put an enormous toll on the healthcare system worldwide.⁷⁴ Consequently, continued research to develop a drug towards AD and/or diagnostic markers for early detection of the disease is of utmost importance. AD is pathologically characterized by insoluble extracellular deposits of amyloid- β (A β) protein and by intracellular deposits from neurofibrillary tangles (NFTs) resulting from the misfolding of hyperphosphorylated tau protein, in the brain.⁷⁵ In AD, the oligomeric species eventually leading to mature fibrils are believed to be the toxic species that induce neuronal cell death with accompanying disruption of the cognitive ability.⁷⁶

1.9.2. Miscellaneous protein misfolding diseases

The misfolding of proteins is associated with many other diseases apart from AD. For instance; misfolding of α -synuclein is involved in Parkinson's disease⁷⁷ whereas misfolding of the cerebral prion protein (PrP) give rise to the invariably fatal disease known as Creutzfeldt-Jakob disease in humans or bovine spongiform encephalopathy, i.e., the "mad cow disease" in cattle.⁷⁸ Upon misfolding, the protein transthyretin (TTR) forms amyloid deposits most often in the heart and peripheral nerves. In addition, all the more research indicates that amyloid formation of the protein IAPP is associated with the death of β -cells in the pancreas leading to type II diabetes.⁷⁹

The development of amyloidophilic probes, preferably for non-invasive *in vivo* imaging, is therefore of great need for adequate and early diagnosis of a plethora of diseases.

2. Research Aim

The prospects of a practical and general [^{18}F]fluoroglycosylation method hold great promise. The research aims underlying this thesis are:

- Develop a synthetic route to β -configured [$^{18}/^{19}\text{F}$]FDGs, and their corresponding precursors, amenable for CuAAC click chemistry.
- Evaluate how the particular anomeric configuration of the [^{18}F]FDG precursors affects the radiochemical yield.
- Ligate [18 and/or ^{19}F]FDGs by CuAAC click couplings to a set of biologically relevant ligands (e.g., amino acids, amyloidophilic oligothiophenes (LCOs), curcumin and to porphyrins) and evaluate their potential as PET radiotracers or fluorescent probes for diagnosis and, in the case of porphyrins, treatment of melanoma cancer.
- Study the impact of [^{19}F]fluoroglycosylation on LCOs, curcumin and porphyrins, in terms of cell uptake in a malignant vs benign cell-line (melanoma vs fibroblasts).

Moreover, disclosed in this thesis is also the synthesis of a library of fluorinated and non-fluorinated resveratrol-based stilbenes as fluorescent probes for the spectral discrimination of native and protofibrillar transthyretin protein (TTR). These compounds may be utilized as diagnostic markers for TTR-mediated systemic amyloidosis.

3. Synthesis of β -configured [18/19F]FDGs

3.1. Synthetic rational and protection group strategy

The synthesis of β -configured [18/19F]FDGs with click-functionalized aglycons, as we shall come to see, is very challenging due to the pronounced effects imparted by the choice of protection groups. As mentioned *vide supra*, the PET precursor to [18F]FDG is by necessity a β -D-mannopyranoside, due to the accompanying S_N2 inversion during the fluorination, with a good leaving group (e.g., triflate, tosylate, mesylate etc.) installed at C-2. This is unfortunate since β -D-mannopyranosides are exceptionally difficult to synthesize because of the simultaneous occurrence of the α -directing anomeric effect⁸⁰, the repulsion between the axial C-2 substituent and the approaching nucleophile⁸¹ and neighboring group participation for 2-acyl mannosyl donors⁸². These three effects all direct the stereochemical outcome to the α -anomer (For instance, as seen in the Koenig-Knorr glycosylations employed in Maschauer's FDG synthesis, section 1.8.1). For this reason, when aiming for the β -anomer, most direct approaches employing a mannosyl donor in a conventional glycosylation method is bound to fail. A notable exception has been provided by the excellent work of David Crich and co-workers who developed a work-around to this predicament. At the end of the 1990's they discovered that 2,3-*O*-di-benzyl-4,6-*O*-benzylidene protected mannosyl triflates (**29**, generated from the corresponding thioglycoside or sulphoxide) provide the elusive β -D-mannopyranoside with excellent stereoselectivity (**30**) (Scheme 3.1). Fascinatingly, the epimeric 2,3-*O*-di-benzyl-4,6-*O*-benzylidene protected glucosyl triflate (**32**) furnished the corresponding α -D-glucopyranoside (**33**), so the stereoselectivity of these donors are inverse to that of conventional donors.^{83,84}

Scheme 3.1 Stereoselectivity observed for 4,6-*O*-benzylidene protected manno- or glucosyl donors upon glycosylation to a general alcohol (ROH).

The glycosylation, as discovered by David Crich and co-workers, provide the elusive β -D-mannopyranoside and the corresponding α -D-glucopyranoside, respectively.

J. Org. Chem., 1999, 64, 4926–4930.

Unfortunately, this method is not compatible with clickable FDG synthesis due to the presence of the benzyl ethers (and the benzylidene acetal) on the mannosyl donor that have to be removed under highly reducing conditions (e.g., Pd/C and H_2). These conditions are not compatible with either azides or alkynes. Deprotection of the benzylidene acetal can of course also be achieved under acidic conditions, (for instance in aqueous solution) but is not a viable option, since it may compromise the glycosidic linkage. Another synthetic challenge lies in the fact that only the substituents on C-1 and C-2 of the original FDG should be modified (in order to attach click aglycons at C-1 and appropriate leaving groups at C-2). Thus, ideally requiring no, or a minimal set of chemical manipulations on the remaining monosaccharide sites, in order to find a short and practical route to the desired target compounds (see the summarized criteria in Table 3.1).

Criteria for clickable FDG synthesis

1	Final deprotection of [^{18}F]FDG has to be rapid and proceed under non-reducing conditions.
2	Installation of click aglycons at C-1 must proceed in a regio- and stereoselective manner.
3	Fluorine, and appropriate leaving groups, should regio- and stereoselectively be installed at C-2.
4	A minimal set of reactions should be performed on the remaining monosaccharide sites.

Table 3.1 Criteria for clickable FDG synthesis.

Since a direct β -mannosylation can be ruled out, in this thesis - two different approaches (section 3.2 and 3.3) have been pursued that both rely on the epimerization at C-2 of a β -configured glucopyranoside to the corresponding mannopyranoside.

3.2. Early work – FDG synthesis via acid-catalyzed rearrangement of a 1,2-di-*O*-glycoorthoester

Scheme 3.2 Reagents and conditions: i) BzCl, *cat.* DMAP, pyridine, 0 °C → r.t. ii) HBr/HOAc (33 wt %), Ac₂O, DCM, 0 °C → r.t. iii) 2,6-lutidine, 2-chloroethanol, *cat.* TBAI, DCM, 50 °C iv) *aq.*, 1.6 M NaOH, H₂O/dioxane 1:1 v) NaH, PMBCl, DMF, 0 °C → r.t. vi) *cat.* CSA, 2-chloroethanol (20%), DCM, 50 °C vii) NaN₃, *cat.* TBAB, DMF, 110 °C viii) *aq.* 2M LiOH, H₂O/dioxane 1:1, 60 °C ix) DMSO/Ac₂O 2:1, r.t., overnight x) NaBH₄, DCM/MeOH 1:1, 0 °C → r.t. xi) Tf₂O, py, DCM, -20 → 10 °C xii) KF, K[2.2.2], MeCN, 85 °C, 20 min

Alkyl 1,2-di-*O*-glycoorthoesters are known to rearrange to the corresponding 2-*O*-acyl glycopyranosides under catalytic acidic conditions with accompanying transfer of the alkoxy group from the orthoester to the anomeric center in a highly stereoselective manner. The stereoselectivity is dictated by the NGP-effect induced from the forming 2-*O*-acyl sugar, thus exclusively providing 1,2-*trans* glycosides.⁸⁵

In my first approach, I sought to exploit this elegant approach that, only in a few steps, provides a suitable protection group pattern and simultaneously establishes the β -configuration.

The synthesis of the 1,2-di-*O*-orthoester and the acid rearrangement was performed by slight modifications of the literature procedure.^{86,87} D-glucose (**34**) was routinely *per*-benzoylated and further elaborated on to its corresponding bromosugar (**35**) using HBr

in HOAc (Scheme 3.2). The bromosugar was then treated with 2,6-lutidine, 2-chloroethanol, and a catalytic amount of TBAI in DCM at 50 °C to provide orthoester **36** in 83% overall yield. The esters were then removed using 1.6 M *aq.*, NaOH/1,4-dioxane (v/v 1:1) providing the triol **37** in 54 %. Alkylation with PMBCl and NaH in DMF gave the *per*-PMB protected orthoester **38** in 93 % yield. The para-methoxy substituted benzyl ether, PMB, was chosen as a suitable protecting group since it, in contrast to unsubstituted benzyl ethers, can be removed under oxidizing conditions (classically by DDQ⁸⁸) and therefore offers a compatible non-reducing deprotection method. Initially, the rearrangement was attempted with the Lewis acid, TIPSOTf (10 mol%), in DCM containing 2-chloroethanol (10 mol%) at room temperature. TIPSOTf has previously been found to reduce the reaction time to minutes at room temperature instead of hours at elevated temperatures compared to the more conventional Bronstedt acid, CSA, by the Fraser-Reid group. However, in contrast to our expectations, TIPSOTf triggered a rapid and efficient deprotection of the PMB-ethers, instead of the intended rearrangement, despite the fact that only catalytical amounts of the acid was employed. While it is well known that PMB-ethers can be cleaved under acidic conditions, it had just recently been reported that silyl triflates and triflic acid have this catalytic property.⁸⁹ The rearrangement was instead attempted using the more conventional acid, CSA, that along with 2-chloroethanol (20 mol %) in DCM at 50 °C, provided the desired β -D-glucopyranoside **39** in an acceptable yield of 63 %. Azidation of the aglycon was readily achieved using NaN₃ and a catalytic amount of TBAB in DMF at 110 °C, providing the azidosugar **40** in 94 % yield. The next step, hydrolysis of the 2-*O*-benzoate, proved to be very challenging. A typical ester removal under Zemplén conditions⁹⁰ (i.e., NaOMe in MeOH), in either catalytical or stoichiometric amounts, did not affect the ester at all. Nor did stirring in *aq.*, 1M NaOH/1,4-dioxane (v/v 1:1), either at room temperature or at 60 °C, overnight. The issue was however resolved by turning to the more Lewis acidic LiOH, that under the following conditions; 2M LiOH/1,4-dioxane (v/v 1:1) at 60 °C for 4 h readily provided the 2-OH glucoside **41** in 67 % yield. The stage was now set for the two-step epimerization, i.e., oxidation to a ketone followed by a stereoselective reduction to the axial mannopyranoside. A choice of route

that was greatly inspired by the excellent work of Danishefsky and co-workers during the 1990's.⁹¹

The oxidation was achieved using the simple method by Albright and Goldman⁹² (that is particularly applicable to sterically hindered alcohols) by stirring the 2-OH sugar in DMSO/Ac₂O (2:1) overnight. After a simple work-up, to remove the solvents, the ketone was subjected to reduction using NaBH₄ in DCM/MeOH (v/v 1:1), providing only the *manno* sugar **42** in 80 % yield. The success of the epimerization was confirmed by ¹H-NMR that clearly revealed a distinct ³J_{H-1,H-2} = 1.0 Hz for the *manno* epimer, whereas the corresponding signal for the starting *gluco* epimer was found to be 7.8 Hz. The high stereoselectivity was in good agreement with the results by Lichtenthaler et al. for a similar 2-ketosugar which provided a *manno*/*gluco* ratio of 50:1.⁹³

The results of many stereoselective reductions of ketohexuloses with sodium borohydride have been summarized and rationalized in great detail by Chang et al.⁹⁴ They concluded that the pivotal criterion to obtain the axial 2-OH (i.e., *manno* sugar) is *the presence of a vicinal equatorial alkoxy-substituent*. This criterion is suitably met for the ketuloses at hand. In contrast, participating vicinal acyl groups generally seems to decrease or inverse the selectivity.

The next step, triflation of the free 2-OH using Tf₂O in pyridine and DCM at -20 to 10 °C, formed the [18F]FDG precursor **43** which, after work-up, was directly evaluated in a fluorination reaction mimicking the conditions used for [18F]FDG synthesis at PET facilities.^{64,95} Reaction of the triflate **43** with KF and K[2.2.2] in refluxing dry MeCN at 85 °C for 20 minutes, under a nitrogen atmosphere, provided the desired PMB-protected FDG-derivative **44** in 72 % yield over two steps. The success of the fluorination could be confirmed with ¹⁹F-NMR, where the 2-F resonates with a characteristic signal at -195.72 p.p.m. (ddd, ²J_{2-F,H-2} = 50.8 Hz, ³J_{2-F,H-3} = 15.3 Hz, ³J_{2-F,H-1} = 3.2 Hz).

Unfortunately, as it turned out, the triflation to give the precursor was difficult to reproduce and drive to completion. Moreover, prolonged reaction times or increasing the amount of Tf₂O often led to unwanted deprotection of the PMB ethers. This was

presumably caused by catalytic amounts of adventurous TfOH (formed from hydrolysis of Tf₂O) before an adequate yield could be obtained.

In the end, although the [19F]FDG derivative, and its triflate precursor, could be obtained through this route, the total synthetic route became rather long. In addition, the synthetic route was not modular in the sense that the nature of the aglycon could not be replaced without having to repeat most of the synthetic steps. For these reasons, a fundamentally different and shorter route was called for.

3.3. FDG synthesis via 1,2-anhydrosugars (Paper I)

3.3.1. Synthetic rational

Learning from the shortcomings of the first synthetic approach to clickable FDGs, what needed to be addressed was:

- Reduce the number of synthetic steps.
- Simplify the process of installing alternative aglycons.
- Utilize carbohydrate protecting groups that offer a practical, ideally, with one final deprotection step.

Accordingly, I turned to exploit the multi-faceted chemical properties of the unsaturated carbohydrate precursors, glycals, more specifically tri-acetyl-*D*-glucal. Glycals are an incredibly versatile class of compounds that undergo a plethora of useful reactions.⁹⁶ Indeed, we have already seen how tri-acetyl-*D*-glucal was employed in the first synthesis of [18F]FDG by Ido et al. where the glucal underwent an electrophilic [18F]fluorination (section 1.4.1). The benefit of using an ester protected glucal is that it can be readily deprotected under alkaline or acidic conditions. Furthermore, esters, being a very common protection group, is expected to be encountered in the majority of potential ligands to be [18F]fluoroglycosylated, thus facilitating a one-step final deprotection. Interestingly, in some instances, glycals can be generated by pyrolysis of the corresponding orthoester, so the two FDG synthetic approaches presented herein (section 3.2 and 3.3) are related to each other.

Glucals are readily converted to 1,2-anhydrosugars (i.e., epoxides) by epoxidation using DMDO. 1,2-anhydrosugars are in turn excellent glucosyl donors that stereoselectively form β -D-glucopyranosides⁹⁷ or β -D-mannopyranosides⁹¹ by the oxidation-reduction mentioned *vide supra*. The beauty of this particular glycosylation method is *that the 2-OH is created during the glycosylation* which obviates the need for laborious protection group manipulations prior to the glycosylation. The work on glycals and their

corresponding 1,2-anhydrosugars have most predominantly been pursued by the Danishefsky group.

In their work in 1989, they mainly employ *per*-benzylated D-glucal as precursor to the corresponding 1,2-anhydrosugar because the epoxidation quantitatively furnishes the α -epoxide (since the bulky 3-*O*-substituent blocks the β -face). This in turn exclusively leads to the formation of β -D-glucopyranosides. However, DMDO epoxidation of tri-acetyl-*D*-glucal resulted in an undetermined mixture that was deemed unsuitable for glycosylation purposes in their hands. They therefore concluded that glycals should be equipped with nonparticipating protection groups in order to act as suitable glycosyl donors. It was not until 2006 that the Dondoni group managed to resolve the composition of the presumed 1,2-anhydrosugar mixture and found it to simply consist of the expected 1,2- α -anhydrosugar **45 α** (sometimes referred to as Brigl's anhydride that was prepared almost a 100 years ago albeit with a more lengthy route)⁹⁸ and the diastereomeric 1,2- β -anhydrosugar **45 β** in a α/β ratio of approx., 7:1.⁹⁹

We hypothesized that the relatively low stereoselectivity observed for DMDO epoxidation of tri-acetyl-*D*-glucal actually could work to our advantage since it would provide us, not only with the desired β -D-glucopyranoside, but also with a direct access to click-enabled α -D-mannopyranosides. Since the 2-OH is readily exposed it is easily converted to its corresponding triflate, i.e., the [18F]FDG precursors reported by Maschauer.¹⁰⁰ This would therefore allow us to do a side-by-side comparison of α - vs β -[18F]FDG precursors in terms of their propensity to undergo the radiofluorination.

3.3.2. Synthesis

The synthesis of clickable FDGs, and their corresponding precursors, are depicted in Scheme 3.3. For the first step, DMDO was prepared by cryogenic distillation on a rotary evaporator to give a yellow 0.07 M DMDO-acetone solution according to the procedure by Murray.¹⁰¹ The DMDO-acetone solution was then directly titrated to a solution of glucal **1** which was rapidly consumed – forming a polar product as judged by TLC.

Scheme 3.3 General conditions and reagents: i) oxone, acetone, TBAHSO₄, DCM, satd. *aq.* NaHCO₃, 0 °C to rt. ii) Cl(CH₂)₂OH, ZnCl₂, DCM, 0 °C to rt. iii) propargyl alcohol, ZnCl₂, DCM, 0 °C to rt. iv) NaI, acetone, 70 °C v) NaN₃, DMF, rt. vi) Tf₂O, py, DCM, -15 to 10 °C. vii) TBANO₂, toluene, 50 °C. viii) DAST, diglyme, 100 °C, 10 min. ix) TBANO₂, toluene, rt. Reproduced from *New J. Chem.*, 2017, 41, 10231–10236 with permission from the Centre National de la Recherche Scientifique (CNRS) and the Royal Society of Chemistry.

However, to our dismay, any attempts at using the 1,2-anhydrosugar for glycosylation to 2-chloroethanol using 1M ZnCl₂ in diethyl ether as promotor failed. We speculate that the highly reactive glycosyl donor was hydrolysed by traces of adventitious water that accompanied the distillation of DMDO, instead forming the nonreactive *vic*-1,2 diol.

The chosen route requires access to multi-gram quantities of 1,2-anhydrosugar. Furthermore, the scale-up of the peroxide, DMDO, is associated with a risk for explosion. In addition, DMDO production is highly laborious, and the DMDO-acetone solutions obtained has an inherently low concentration and short shelf-life. Instead, we decided to explore a slightly different approach – namely to generate DMDO *in situ*. *In situ* produced DMDO epoxidations of glycals were first described by Cheshev et. al in 2006.⁹⁹

In their epoxidation protocol, DMDO is formed *in situ* from a biphasic buffered solution containing the oxidant oxone, acetone, saturated NaHCO₃ and DCM. This protocol was further elaborated on by Lafont et al. in 2011 by the simple but clever addition of the phase transfer catalyst, tetrabutylammonium hydrogen sulphate (TBAHSO₄) that significantly increased the reaction speed as well as the reproducibility, according to themselves and confirmed by us. The oxone-acetone-TBAHSO₄ epoxidation protocol reliably and consistently allowed us to prepare the 1,2-anhydrosugar **45** (α/β mixture) in essentially quantitative yield.

With the glucosyl donor at hand, the subsequent glycosylation of 2-chloroethanol or propargyl alcohol, promoted by ZnCl₂ in DCM, provided, as expected, a diastereomeric mixture of glycosides (*gluco/manno* ~ 9:1). The β -D-glucopyranosides **46** and **51** could efficiently be isolated by crystallization from ethyl acetate/heptane in 39 % and 29 %, respectively over two steps. The modest yield was acceptable since it would require several additional steps to reach this product by other means (e.g., via 1,2-di-*O*-glycoorthoesters). The predominant by-product formed was the corresponding *vic*-1,2-diol hydrolysis product as determined by LCMS. In contrast to the azidation of the aglycon in the orthoester approach (section 3.2), utilizing NaN₃ in DMF at elevated temperatures (60-110 °C), with or without the additives TBAI or TBAB, consistently resulted in a complex mixture of products due to acetate migration to the unprotected 2-OH. In contrast, no reaction occurred at ambient temperature. We therefore reasoned that installing a better leaving group might make the reaction proceed under milder conditions and thus avoid the accompanying migration. This was realized by converting the chloro compound **46** to the corresponding iodo compound by means of the classical Finkelstein reaction¹⁰² utilizing NaI (2 eq.) in acetone at 80 °C for 48 h. The azidation, using NaN₃ in DMF, now proceeded cleanly at ambient temperature to afford the azidoethyl glucoside **47** in good yield (77 %) over two steps.

Now, having acetylated instead of PMB-ether protected substrates for the pending epimerisation, the high stereoselectivity observed for the ketosugar reduction with NaBH₄ in the orthoester approach was predicted to be lost due to the lack of a sterically

demanding 3-*O*-alkoxy substituent blocking the β -face (as supported by the results summarized in the literature review by Chang et al.⁹⁴)

Fortunately, a complementary epimerisation method is available, namely the nitrite-mediated Lattrell-Dax inversion, that act by displacing a triflate group at the inversion center with NO_2^- in an $\text{S}_{\text{N}}2$ reaction. The nitrite-compound spontaneously decompose to the sought hydroxy-functional group. The Lattrell-Dax inversion has been extensively studied by the Ramström group who concluded that the inversion proceeds reliably - and even stereospecifically - *if and only if a vicinal equatorial ester is present*.^{103,104}

This criterion is suitably met for the glucosides at hand. Standard triflation of glucoside **47** or **51** using TiF_4 followed by inversion using TBANO_2 in toluene at ambient temperature overnight yielded the β -D-mannopyranosides **48** and **52** as the only isolated product, albeit in moderate yields of 46 % and 48 %, respectively. The β -anomeric configuration was confirmed by evaluating the $J_{\text{C-1,H-1}}$ coupling constant in a coupled ^1H - ^{13}C NMR experiment and was found to be 157.6 Hz and 159.0 Hz respectively for β -D-mannopyranosides **50** and **54**.¹⁰⁵ With the desired β -D-mannopyranosides at hand, standard triflation afforded the β -D-mannopyranosyl triflates, i.e., the [18F]FDG precursors, **49** and **53** in 48% and 60%, respectively after silica gel chromatography. Subsequently, to afford the [19F]FDG analogues (i.e., the non-radioactive references) the same fluorination protocol as for the orthoester approach (section 3.2), using KF and $\text{K}[2.2.2]$ in MeCN at 85 °C was attempted. Unfortunately, only trace amounts of the expected fluorosugar could be observed, most likely as a result of the known poor nucleophilicity of the fluoride ion.¹⁰⁶ Instead, we choose to directly fluorinate the β -D-mannopyranosides bearing a free hydroxyl-group in the second position, **48** and **52**, using DAST.¹⁰⁷ At first the reaction was run in DCM at -25 °C to room temperature over night to give the fluorosugars **50** and **54** in 31 % and 19 %, respectively. However, the poor yield rendered us to instead perform the reaction as reported by Kovac et al.¹⁰⁸ in diglyme at 100 °C for 10 min, which afforded the fluorosugars **50** and **54** in a significantly increased yield of 66 % and 35 %, respectively. The fluorination proceeded as expected with inversion of configuration, as verified by the characteristic $J_{\text{H-1,H-2}}$

coupling constant of 7.6 Hz for **50** and 7.7 Hz for **54**, thus confirming the re-established *gluco*-configuration. Furthermore, 1D ^{19}F -NMR revealed a characteristic FDG signal for fluorosugar **50** at -199.7 p.p.m. (ddd, $^2J_{\text{H-2,F-2}} = 50.5$ Hz, $^3J_{\text{H-3,F-2}} = 14.3$ Hz, $^3J_{\text{H-1,F-2}} = 2.7$ Hz), and a similar signal for **54**.

3.3.3. Synthesis of α -configured clickable FDGs

To test our hypothesis that β -configured $[^{18}\text{F}]\text{FDG}$ precursors are more prone to undergo radiofluorination than their α -anomeric counterpart, the minor α -D-mannopyranoside products had to be isolated. Accordingly, in a separate glycosylation experiment, the diastereomeric 2-chloroethyl glycosides were directly subjected as a mixture for standard azidation providing glycosides **47** and **15** (Scheme 3.4). Similarly, glycosylation to propargyl alcohol provided the glycosides **51** and **11**. The resulting α -D-mannopyranosides could then be isolated with preparative HPLC. The isolated α -D-mannopyranosides were then readily triflated, or fluorinated with DAST, to furnish the required α -precursors (**16** and **12**) and the corresponding α - $[^{19}\text{F}]\text{FDGs}$ (**19** and **20**).

Scheme 3.4 General conditions and reagents: i) oxone, acetone, TBAHSO₄, DCM, satd. aq. NaHCO₃, 0 °C to rt. ii) Cl(CH₂)₂OH, ZnCl₂, DCM, 0 °C to rt. iii) propargyl alcohol, ZnCl₂, DCM, 0 °C to rt. iv) NaI, acetone, 70 °C. v) NaN₃, DMF, rt. vi) Tf₂O, py, DCM, -15 to 10 °C. vii) DAST, diglyme, 100 °C, 10 min. *Reproduced from New J. Chem.*, 2017, 41, 10231–10236 with permission from the Centre National de la Recherche Scientifique (CNRS) and the Royal Society of Chemistry.

3.4. Radiofluorination of α - and β -[^{18}F]FDG precursors

3.4.1. Results

With the α - and β -[^{18}F]FDG precursors at our disposal, they could finally be subjected to radiofluorination under typical conditions for conventional [^{18}F]FDG synthesis. An aliquot was taken from the reaction mixture and analyzed with an HPLC, equipped with a radiodetector. The results clearly revealed that β -[^{18}F]FDG precursors (**49** and **53**) indeed readily undergo the radiofluorination since the expected β -[^{18}F]FDGs were produced in 77 % and 88 %, respectively (Table 3.2 and Figure 3.1). In stark contrast, none of the α -[^{18}F]FDG precursors provided any detectable α -[^{18}F]FDG product, thus performing even poorer than in Maschauer's study.

$\text{Precursor} \xrightarrow[85^\circ\text{C}, 30\text{ min}]{^{18}\text{F}/\text{K}[2.2.2], \text{MeCN}} \text{Product}$

Entry	Precursor	Product	RCY(%)
1	49	 [^{18}F] 50	77 $\pm$ 2
2	16	 [^{18}F] 20	0
3	53	 [^{18}F] 54	88 $\pm$ 5
4	12	 [^{18}F] 19	0

Table 3.2 ^{18}F -radiolabeling of α - and β -D-mannopyranoside precursors to yield the corresponding [^{18}F]FDGs.

The β -configured precursors readily underwent the radiofluorination in high RCY whereas the corresponding α -configured precursors did not react at all under these conditions.

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Figure 3.1 HPLC analysis of the reaction mixture to provide [18F]FDG ([18F]50). The radiodetector (red trace) detects residues of F⁻ at 0.38 min but predominately the [18F]FDG product at 5.6 min.

3.5. Configurational dependence of [18F]FDG precursors to undergo radiofluorination

What is the explanation for the vast difference observed for α vs β -configured precursors, and first of all, has this effect been previously documented? Fortunately, the answer is yes. To put this into context, there has been a renewed interest in using [18F]FDM, the *manno* epimer of [18F]FDG, for PET investigations. In 2014, the Japanese group led by Fukuda¹⁰⁹ evaluated two different precursors (**55** and **57**, Scheme 3.5) for [18F]FDM synthesis. Since conventional [18F]FDG is easily obtained in high RCY from the β -mannosyl triflate **5** by the Hamacher protocol (section 1.4.1), the authors reasoned that synthesizing the corresponding precursor with inverted stereochemistry at C-2 (and C-1, precursor **55**) would then provide the desired [18F]FDM in a similar manner. To their surprise, this did not happen. In fact, precursor **55** was radiolabelled to less than 1 %! In contrast, the β -configured 4,6-di-*O*-benzylidene protected precursor **57** provided a RCY of 50-68 %. On the same note – in 1985, Haradahira et al. evaluated the non-radioactive fluorination of two 4,6-di-*O*-benzylidene protected mannosyl triflates, with different anomeric configuration, for [19F]FDG synthesis (Scheme 3.6).¹¹⁰ They discovered that the more synthetically accessible α -configured precursor **59** did not undergo fluorination with TBAF even at

elevated temperatures, whereas the epimeric β -configured mannosyl triflate **61** readily proceeded to the expected fluorosugar **62** at room temperature in 64 % yield.

Scheme 3.5 Radiofluorination of [18F]FDM precursors by Furumoto et al.¹⁰⁹

Radiolabelling of the α -configured glucoside (precursor **55**) provided the corresponding [18F]fluorosugar **56** in <1 % RCY, whereas the β -configured glucoside (precursor **57**) provided the corresponding [18F]fluorosugar **58** in 50-68 % RCY.

Scheme 3.6 Fluorination of α - and β methyl 4,6-di-*O*-benzylidene protected mannosyl triflates. The α -configured precursor **59** did not undergo fluorination with TBAF even at elevated temperatures (top) whereas the epimeric β -configured precursor **61** (bottom) proceeded to the expected fluorosugar **62** at room temperature in 64% yield. Haradahira et al. in 1985.¹¹⁰

3.5.1. The Richardson-Hough rules governing S_N2 displacements on pyranosides

The literature is filled with similar examples suggesting that β -D-pyranosides generally undergo clean S_N2 displacements at C-2 in good yield, whereas the corresponding α -pyranosides only react poorly, or not at all. A set of empirical rules governing this class of carbohydrate reactions was jointly dictated by Richardson and Hough in 1979¹¹¹, and was recently updated to encompass another 30 years of empirical data in support of this theory.¹¹² Nucleophilic attack from the [18F]F⁻ proceeds in an S_N2 manner leading to the penta-coordinated transition states depicted in Scheme 3.7. Both the approaching [18F]F⁻ and the departing triflate group are highly electron withdrawing, with a large associated dipole moment of opposite direction. The difference in reactivity between α - and β -configured precursors lies in how these dipoles align with the ones already present in the pyranoside structure. In the α -case (Scheme 3.7a), the C-1-axial aglycon dipole (green) is perfectly aligned with the C-2-¹⁸F dipole (green), and the C-1-O (i.e. the endogenous oxygen) dipole (blue) is partially aligned with C-2-OTf dipole (blue). As a result, the two pairs of dipoles (green and blue, respectively) give rise to electrostatic repulsion that impart a high activation energy which impedes the reaction.

In contrast, in the β -case (Scheme 3.7b), the dipole directed in the aglycon direction (green) is no longer aligned with the C-2-¹⁸F dipole (green). In fact, they now have opposite directions thus providing a much lower activation energy that allow the reaction to proceed. It can thus be concluded that the propensity of the precursors at hand to undergo the S_N2 displacement with [18F]F⁻ *is predominantly a stereoelectronic effect*. However, bulky aglycons, particularly in the α -case, further sterically inhibits the reaction. Interestingly, by switching the position of [18F]F⁻ and the OTf-group, essentially the same transition state applies. The Richardson-Hough rules therefore successfully predicts that β -D-glucopyranosides (in contrast to the α -anomers) readily undergo S_N2 displacements - for instance with [18F]F⁻ to afford [18F]FDM.

Scheme 3.7 The Richardson-Hough theory explaining the difference in reactivity between α- and β-[18F]FDG precursors. At the transition state, the β-anomer experience a lower electrostatic repulsion from the illustrated green and blue pairs of dipoles which lowers the activation energy, thus making the reaction more favourable. R = CH₂CH₂N₃ or CH₂CCH.

4. [¹⁸F]fluoroglycosylation of amino acids

With ready access to clickable [¹⁸F]FDGs, we next turned to investigate their performance in a click reaction. As a model system, we choose to use the click-functionalized Fmoc-protected amino acids, Fmoc-N-(propargyl)-glycine (**63**) and Fmoc-3-azido-L-alanine (**65**) (Scheme 4.1). After radiolabelling of the [¹⁸F]FDG precursors for 30 minutes, the MeCN was evaporated with a stream of helium. The protected [**18F**]**50** or [**18F**]**54** was then dissolved in dry DMF. To this solution *aq.* CuSO₄ (50 μ L, 0.20 M), *aq.* (+)-L-sodium ascorbate (50 μ L, 0.60 M) along with amino acid **63** or **65** (2.5 μ mol) were added. The reaction was then heated to 60 °C for 20 min, whereby an aliquot was taken from the reaction mixture and analyzed by HPLC. The click reaction worked very well under these conditions with full conversion of the [¹⁸F]FDG to the desired [¹⁸F]fluoroglycosylated amino acid in 75 $\pm$ 2% (Figure 4.1) and 83 $\pm$ 2% radiochemical conversion (RCC) for compound [**18F**]**64** and [**18F**]**66** respectively. Both compounds had a radiochemical purity > 99 %, as deducted by HPLC. The total synthesis time, including preparative HPLC purification, was 2 hours. The corresponding non-radioactive FDG amino acid conjugates were synthesized using microwave irradiation at 80 °C for 5 min (Scheme 4.2). The click reaction between FDG **50** (1.2 eq.) and amino acid **63** (1.0 eq.) and between FDG **54** (1.2 eq.) and amino acid **65** (1.0 eq.) yielded FDG conjugates **64** and **66** in 64 % and 86 %, respectively.

Scheme 4.1 [18F]fluoroglycosylation of amino acids using a thermal click coupling.
General conditions and reagents: i) amino acid (0.25 μmol) aq., CuSO_4 (50 μL , 0.20 M), aq., (+)-sodium L-ascorbate (50 μL , 0.60 M), 60 $^\circ\text{C}$, 20 min, DMF. *Reproduced from New J. Chem.*, 2017, 41, 10231–10236 with permission from the Centre National de la Recherche Scientifique (CNRS) and the Royal Society of Chemistry.

Scheme 4.2 [19F]fluoroglycosylation of amino acids.

[19F]fluoroglycosylation of amino acids under microwave irradiation provided the non-radioactive fluoroglycosylated amino acids in 64 % and 86 %, respectively. *Reproduced from New J. Chem.*, 2017, 41, 10231–10236 with permission from the Centre National de la Recherche Scientifique (CNRS) and the Royal Society of Chemistry.

Figure 4.1 HPLC analysis of the [18F]fluoroglycosylation of glycine. HPLC analysis of the click reaction above at 20 minutes. The radiodetector (red trace) detects residual $^{18}\text{F}^-$ (left peak) and the expected [18F]fluoroglycosylated amino acid [18F]64 (right peak). The identity of the product was confirmed since it co-elutes with the corresponding cold reference.

The radiochemistry results obtained so far states that:

- β -configuration is a pivotal criterion for efficient radiofluorination of [18F]FDG precursors.
- The clickable β -[18F]FDGs readily undergo the CuAAC click reaction to both alkyne and azide-equipped amino acids in high radiochemical yield. The scope of the described [18F]fluoroglycosylation method is thus wider than previously reported [18F]fluoroglycosylation methods.

Encouraged by the successful results in this assay, we were now in a position to evaluate the [18F]fluoroglycosylation of ligands relevant for our research interests.

5. [18F]fluoroglycosylation of curcumin (Paper II)

5.1. Curcumin

Curcumin is a natural product responsible for the yellow color of the south-asian spice turmeric, which is isolated from the plant *Curcuma longa*. Turmeric typically contains the three curcuminoids – curcumin (77 %), demethoxycurcumin (17 %) and bis-demethoxycurcumin (3 %) of which the most prevalent curcumin is also the most well-studied (Figure 5.1). Apart from being a dietary supplement, curcumin has been used for centuries in traditional indian ayurvedan medicine for treatment of many different ailments.¹¹³ This has spurred modern research to investigate the therapeutic effects of this intriguing polyphenol. To date, curcumin has been shown to be associated with a plethora of therapeutic properties, e.g., antioxidative¹¹⁴, anti-inflammatory¹¹⁵ and anticancer activities¹¹⁶. The scientific interest in curcumin is immense, as testified by more than a hundred clinical studies performed with curcumin in various medicinal contexts.¹¹⁷

Figure 5.1 The composition of curcuminoids in turmeric.

Epidemiological studies show that the prevalence of AD in Asia, most notably in India, is considerably lower than in the western world. This effect has been attributed to the consumption of curcumin, for instance in curry dishes.^{118,119}

Curcumin seems to be able to combat AD through many different mechanisms. The protein misfolding of the A β peptide and tau protein and the accompanying amyloid deposition of A β plaques and neurofibrillary

tau tangles (NFTs), are characteristic hallmarks of AD disease. The intermediate oligomeric species are postulated to be the main causative agents for the neuronal cell death. Several reports claim that curcumin, or derivatives thereof, can modulate the fibrillation, and possibly depolymerize pre-formed amyloids of A β (1-40 and 1-42) and the tau protein *in vitro*, typically in the micromolar range.^{120,121} In contrast, the Hammarström group found that curcumin rather promotes fibrillation of pre-fibrillar/oligomeric species of A β (1-42) which alleviated the neurotoxic effect in *Drosophila*.¹²² Derivatives of curcumin have also been shown to inhibit amyloid formation of α -synuclein, which is associated with Parkinson's disease, suggesting that curcumin may have general anti-amyloidogenic properties.¹²³ In addition, the anti-oxidative property of curcumin is believed to alleviate oxidative stress-induced protein misfolding caused by radical oxygen species (ROS).¹²⁴ and the central 1,3-diketo functionality is known to sequester metal ions (e.g., Fe²⁺ and Cu²⁺) that can induce protein misfolding via ROS formation (Figure 5.2).¹²⁵

Figure 5.2 Structure-activity relationship for the anti-AD properties of curcumin.

Despite the many promising therapeutic properties, the inherently poor water solubility, low bioavailability and metabolic stability of curcumin has prevented its use as a therapeutic drug. In order to remedy these shortcomings, various chemical modifications have been investigated. Typically, the central 1,3-diketo functional group is converted to a five-membered heterocyclic motif (e.g., pyrazole or isoxazole) in order to prevent decomposition of curcumin via a retro-aldol condensation.¹²³

5.2. Curcumin as an A β -imaging agent (Paper II)

Curcumin is well-studied for potential therapeutic applications but much less so for potential diagnostic applications. In fact, curcumin is a fluorescent dye that can stain A β plaques and NFTs, located in tissue slides from AD patients, with similar binding affinities to that of Thioflavin S.^{120,126} Curcumin can therefore be put in the same class as conventional fluorescent dyes such as Congo Red¹²⁷, X-34¹²⁸ and Thioflavin T¹²⁹. However, in contrast to the charged Congo Red or Thioflavin S, curcumin is highly hydrophobic, and also neutral and nontoxic. In addition, curcumin has been found to pass the BBB, albeit moderately.¹²⁰ For this reason, curcumin is a suitable candidate as a PET tracer for A β imaging.

Only a few reports have been made with curcumin-based PET tracers. In 2006, Eun Kyoung Ryu et al. synthesized a *O*-[18F]fluoropropylated curcumin (**67**, Figure 5.3a) through three different approaches. The most practical and high-yielding was found to be [18F]fluoropropylation of a phenolic benzaldehyde, followed by a B₂O₃-promoted aldol condensation using the Pabon protocol¹³⁰ to form the desired curcumin. The yield was, however, quite low (0-25% RCY, decay-corrected,). The [18F]fluoropropylated curcumin **67** displayed favourable A β plaque affinity, acceptable initial passage over the BBB as well as good metabolic stability in the brain of healthy mice.¹³¹

Figure 5.3 Examples of curcumin-based PET tracers in the literature.

a) The [18F]fluoropropylated curcumin tracer reported by Eun Kyoung Ryu et al. in 2006 b) The pyrazole-curcumin tracer reported by Rokka et al. in 2014.

In 2014, Johanna Rokka et al. reported on the synthesis of a pyrazole derivative of curcumin where a ^{18}F -labelled PEG-linker was attached using click chemistry (compound **68**, Figure 5.3b).¹³² This promising tracer was synthesized in a radiochemical yield of $21 \pm 11\%$ and exhibited selective affinity for A β aggregates. Unfortunately, the tracer had a negligible BBB penetrance, thus hampering its use for *in vivo* A β -amyloid imaging. Moreover, despite the installation of a pyrazole moiety, the chemical handling of the precursor, and preceding intermediates, required the presence of the additive L-(+)-ascorbate in order to remain intact. More recently, the group tried to employ a nanoliposomal formulation of this radiotracer but it did not significantly improve the brain uptake of the curcumin tracer.¹³³

In 2011, Dolai et al. used click chemistry to glycosylate curcumin on the phenolic site (via a PEG-spacer) with galactose.¹³⁴ The glycocurcumin exhibited approximately a 1000-fold improvement in the inhibition of A β peptide and tau protein aggregation over curcumin itself (inhibition at nM instead of μM concentrations). The improvement was most likely due to the increase in water solubility. Surprisingly, the glycocurcumin was a worse inhibitor of A β peptide fibrillation at μM concentrations compared to nM concentrations, suggesting a competing micelle formation of the amphiphilic glycocurcumin, as reasoned by the authors. The beneficial properties that glycosylation imparts on curcumin encouraged us to attempt our [^{18}F]fluoroglycosylation protocol on this polyphenolic ligand.

[^{18}F]fluoroglycosylation of curcumin has the potential to simultaneously yield a metabolically stabilized curcumin PET tracer with improved amyloid affinity. Furthermore, the attached [^{18}F]FDG moiety may facilitate passage over the BBB by exploiting the GLUT-mechanism (section 1.4). In addition, metabolic trapping of [^{18}F]FDG may ensure retention in the brain and simultaneously improve the metabolic stability of the tracer to a larger extent than a non-fluorinated sugar would.

5.3. Synthesis

Curcumin (**69**, Scheme 5.1) was synthetically prepared following the procedure by Pabon.¹³⁰ Propargylation of curcumin **69** using propargyl bromide (1 eq.) and K₂CO₃ (1 eq.) in DMF was achieved according to the protocol by Dolai et al.¹³⁴ Purification by preparative HPLC provided the mono-propargyl curcumin **70** and di-propargyl curcumin **71** in 30 % and 7.5 % yield, respectively. The alkyne equipped curcumins were then ligated with azido-[19F]FDG **50** or the corresponding azido-glucoside **72**¹³⁵ by means of the CuAAC click reaction which was effectively realized using aq., CuSO₄ (0.3 eq/alkyne), aq. and (+)-sodium L-ascorbate (0.6 eq/alkyne) under microwave irradiation at 80 °C for 5 min in DMF.¹³⁶ Preparative HPLC subsequently provided the *per*-acetylated glycocurcumin **73** and **74**. Lastly, Zemplén methanolysis (0.3 M NaOMe in MeOH) gave the deacetylated glycocurcumins **75** and **76** in quantitative yield.

Scheme 5.1 Synthesis of non-radioactive glycocurcumins

5.4. Radiochemistry

Curcumin is prone to undergo a retro-aldol condensation in alkaline aqueous medium. We therefore devised a protocol where deacetylation of [^{18}F]FDG occurs prior to the click reaction. The clickable [^{18}F]FDG ([**18F**]50, Scheme 5.2) was prepared as described in **Paper I**. The solvent was then evaporated with a stream of nitrogen gas under heating. The subsequent deacetylation was rapidly realized using 0.5 M NaOH for 2 minutes at 50 °C, in accordance with the results by Füchtner et al.^{137,138}, effectively providing the clickable [^{18}F]FDG [**18F**]77. The reaction mixture was then neutralized with acetic acid. To this solution, propargyl curcumin **70** (in 100 μL DMF), aq. CuSO_4 (100 μL , 0.20 M) and aq. (+)-sodium L-ascorbate (100 μL , 0.60 M) was added. Heating the solution at 60 °C for 15 min resulted in the formation of the [^{18}F]fluoroglycosylated curcumin [**18F**]73 in a yield of 45.5 ± 6.5 % (as confirmed by co-elution with the non-radioactive reference **73**).

The click protocol thus offers a simple, practical and high-yielding means to [^{18}F]fluoroglycosylate curcumin and most likely other sensitive substrates.

Scheme 5.2 [^{18}F]fluoroglycosylation of propargyl curcumin (**70**).

5.5. Cell uptake in a melanoma cell-line and toxicity studies

The cell uptake of curcumin derivatives was evaluated in normal human fibroblasts as well as a human melanoma cell-line. The acetylated glucocurcumin (**74**, Figure 5.4) displayed a positive, albeit low fluorescence signal suggesting a successful uptake, whereas the deacetylated analogue (**76**) did not give rise to any fluorescence signal at all. However, turning to the fluorinated glycocurcumins (**73** and **75**), the seemingly minor chemical modification of replacing the second substituent from $R = \text{OAc}$ or OH to $R = \text{F}$ had a tremendous effect on the apparent cell uptake. The resulting fluorescence signal was now intense, and also localized in a distinct punctuate pattern.

With the given results at hand, we hypothesize that in fact, all of the glycocurcumins are effectively taken up by the cells. However, they are most likely metabolized at different rates and to different degrees by general lipases and glycosidases. The deacetylated glycocurcumin (**76**), may directly be subjected to the cascade of enzymes involved in the glycolysis, cleaving the sugar unit from the curcumin thus rendering the easily metabolized curcumin subject for further degradation, which in turn extinguishes the fluorescence signal. The acetylated glycocurcumin (**74**) however, would first have to become deacetylated by lipases in order to be recognized as substrates for glycolysis. This may explain the greater fluorescence intensity. Finally, the excellent fluorescence signal observed for the fluoroglycosylated curcumins, both acetylated and non-acetylated (**73** and **75**), suggest that these compounds are metabolically stabilized, presumably via metabolic trapping. Further studies are required to elucidate the exact mechanism of cell uptake and the subsequent metabolic processes. However, it can be concluded that fluoroglycosylation improves the biochemical properties of curcumin.

Figure 5.4 A) Fibroblast and B) melanoma cells incubated with 20 μ M of the synthesized curcumin derivatives for 24 h. Confocal microscopy images of cells stained with curcumins (green) and DAPI (nuclei staining; seen in blue). Scale bar 20 μ m.

6. [18F]fluoroglycosylation of LCOs

6.1. PET tracers for AD imaging

The most prominent PET tracer for AD diagnosis is the Pittsburgh compound B ([¹¹C]PIB) which is a small, neutral and lipophilic benzothiazole derivative that was reported in 2004 (Figure 6.1a).¹³⁹ [¹¹C]PIB displays excellent binding affinity to human derived A β aggregates. The main drawback of [¹¹C]PIB is the short half-life of C-11 ($t_{1/2}$ = 20 min). In order to prepare [¹¹C]PIB, a cyclotron in conjunction to the medical facility is required, which restricts its widespread use in clinical applications. For this reason, PIB analogues labelled with F-18 has since then been developed, e.g., ¹⁸F-flutemetamol¹⁴⁰ and 18F-AZD4694¹⁴¹, that essentially display unaltered imaging properties to that of the parent [¹¹C]PIB molecule, but are more long-lived. There is also a set of stilbene based A β amyloid imaging agents, where the first described was the C-11 methylated SB-13.¹⁴² F-18 labelled stilbene analogues have also been prepared, most notably florbetapir F-18 (amyvid)¹⁴³ and [18F]AV-1¹⁴⁴ (Figure 6.1b). Despite the number of available A β amyloid imaging agents, development of new and more selective PET tracers that may distinguish between the polymorphic nature of amyloid diseases is highly desirable, not only for AD, but also for other proteinopathies.

Figure 6.1 Examples of AD imaging agents for PET. a) Benzothiazole based tracer [¹¹C]PIB and ¹⁸F-analogues thereof. b) The ¹¹C-labelled stilbene SB-13 and ¹⁸F-analogues thereof.

6.2. Luminescent Conjugated Oligothiophenes

Luminescent conjugated oligothiophenes (LCOs), developed in the Nilsson group at Linköping university, have successfully been employed as novel tools for fluorescence imaging of protein aggregates. In comparison to conventional ligands, LCOs have been shown to detect a wide range of disease associated protein aggregates e.g., A β or NFT deposits in Alzheimer's disease.^{145,146,147} Furthermore, LCOs can also optically differentiate between different prion strains associated with prion diseases,¹⁴⁸ and have even been found to elicit a pronounced prophylactic and therapeutic effect in prion infected mice.¹⁴⁹ The flexible backbone of LCOs, exemplified by the pentameric LCO p-FTAA (Figure 6.2), adopts a different conformation depending on the polymorphic nature of the protein aggregate that it binds to. As a consequence, the effective conjugation length differs and thereby change the color of the emitted fluorescence signal.¹⁵⁰ One probe can therefore be applied to optically distinguish between different protein aggregates in the same sample.

Figure 6.2 Twisting of the oligothiophene backbone affect the color of the emitted fluorescence light.

Although there are several reported PET tracers for AD imaging available, efficient tracers for other amyloid associated diseases are much rarer. Recently, it was discovered that [^{11}C]PIB also bind to amyloid deposits, resulting from ATTR and AL, located in the heart.¹⁵¹ This finding prompted the Nilsson group to investigate the utility of LCOs for PET imaging of systemic amyloidosis. For this reason, three radiolabelled LCOs (Figure 6.3a) were evaluated as PET tracers for the study of local and systemic amyloidosis *ex vivo* using tissue from patients with amyloid deposits in the heart, spleen and liver.¹⁵² The three LCOs efficiently visualized the amyloid deposits on the tissue slides, which were further confirmed by fluorescence staining with the corresponding non-radioactive LCOs. Moreover, it was found that the tetrameric oligothiophene, [^{11}C]q-FTAA-CN ([^{11}C]79) bound very well to cardiac amyloids. However, a biodistribution evaluation in a healthy monkey revealed that the LCO PET tracers were limited in passing the BBB and as such are not suitable for the detection of cerebral amyloidosis. They also exhibited a relatively poor blood clearance, presumably due to the hydrophobic nature of the ligands. Nevertheless, the promising results suggest that LCOs can be refined to highly selective amyloid PET tracers. We reasoned that [^{18}F]fluoroglycosylation of the LCO scaffold, forming a second generation of LCO-based PET tracers (Figure 6.3b), could potentially solve these shortcomings and enable passage over the BBB by exploiting the GLUT-mechanism. It could simultaneously increase the plasma solubility and blood clearance by increasing the hydrophilicity of the tracer. The synthesis and evaluation of the second generation of LCO PET tracers is further detailed in **Paper III**.

Figure 6.3 Depicting the (a) first and (b) second generation of LCO-based PET tracers for β -amyloid imaging.

6.3. Synthesis

For the synthesis of the non-radioactive reference compounds, the azide equipped [19F]FDG (**50**) was efficiently ligated to the propargylamide functionalized LCOs (**83** or **86**, Scheme 6.1a), by means of the CuAAC reaction using aq. CuSO_4 (0.3 eq.) and aq. (+)-sodium L-ascorbate (0.6 eq.) in DMF under microwave irradiation at 80 °C for 5 minutes. The reaction provided compound **84** and **87** in 89 % and 79 % yield, respectively. Global deesterification using aq. LiOH in 1,4-dioxane furnished the desired [19F]FDG-LCO conjugates **85** and **88**. Interestingly, the *tert*-butyl ester was efficiently removed under these conditions but was not affected at all using NaOH under the same conditions.

For the radiolabelling experiment, the azido-[18F]FDG (**[18F]50**) was prepared as described in **Paper I**, (section 3.4) and the click reaction was attempted in DMF at 60 °C for 20 min. Unfortunately, the reaction consistently failed to provide the desired [18F]FDG conjugate (**[18F]85**, Scheme 6.2). We reason that poor solubility and/or stereoelectronic effects elicited from the propargylamide functional group on the LCO scaffold prevented the reaction from occurring. Apart from this, considering the quite

long reaction time required for the alkaline ester deprotection, where prolonged alkaline conditions at elevated temperatures can lead to fluorine abstraction from the [18F]FDG moiety according to the study by Füchtner et al. (described in section 5.4) along with the potential hydrolysis of the labile cyano-group. Taking the above mentioned into consideration, we were forced to reconsider the radiosynthetic procedure. For the same reason, [18F]fluoroglycosylation of the tetrameric propargylamide LCO **86** was abolished. Therefore, we chose to employ the click procedure developed for [18F]fluoroglycosylation of curcumin utilizing deacetylated [18F]FDG (section 5.4). This time, two azido-PEGylated pre-deprotected LCOs (**89** and **90**) were prepared thus requiring the complementary alkyne equipped [18/19F]FDG **54** (Scheme 6.1 [19F]fluoroglycosylation of a) propargylamide equipped LCOs and b) azide equipped LCOs.b). The [19F]FDG-LCO reference compounds were readily prepared using the same procedure as for the propargylamide equipped LCOs.

Scheme 6.1 [19F]fluoroglycosylation of a) propargylamide equipped LCOs and b) azide equipped LCOs.

ascorbate (100 μ L, 0.60 M). Heating the solution at 60 $^{\circ}$ C for 15 min resulted in the formation of the [18F]fluoroglycosylated LCOs **[18F]81** and **[18F]82** in 63.5 ± 6.5 % and 56 ± 6 %, respectively. Their identity was confirmed by co-elution with the corresponding non-radioactive reference compound (**81** or **82**, respectively).

6.4. Staining of human brain slides displaying AD pathology

The [19F]FDG-LCOs (**81** and **82**, Scheme 6.1), the corresponding azido-PEGylated LCOs (**89** and **90**) and the parent unsubstituted LCOs (i.e., featuring a terminal carboxylic acid functional group), were selected for evaluating their binding to protein deposits located in human brain tissue sections with AD pathology using fluorescence microscopy. As seen in Figure 6.4, the substituted LCOs (either [19F]fluoroglycosylated or azidoPEGylated) are clearly able to stain A β deposits, similar to that of the parent LCO. Fluoroglycosylation can thus be made without compromising the LCO binding affinity to protein aggregates. Strikingly, the pentameric LCOs also bound to NFTs (Figure 6.4C), whereas the tetrameric oligothiophene derivatives only identified A β deposits. The [19F]fluoroglycosylated LCOs also retained their ability to selectively identify protein aggregates.

Figure 6.4 Fluorescent images of protein deposits in human brain tissue sections with AD pathology stained by either the parent, the azidoPEGylated or the [19F]fluoroglycosylated LCO. A) Staining of A β deposits using the parent tetrameric LCO (CN-q-FTAA) and its azido and [19F]FDG derivative (**79** and **82**, respectively). B) Staining of A β deposits using the parent pentameric LCO (p-FTAA) and its azido and [19F]FDG derivative (**89** and **81**, respectively). C) Staining tau neurofibrillary tangles (NFTs) using the parent pentameric LCO (p-FTAA) and its azido and [19F]FDG derivative (**89** and **81**, respectively).

6.5. Conclusions

In **Paper III** we demonstrated that oligothiophene precursors can be readily [^{18}F]fluoroglycosylated in a rapid, mild and practical three-step, one pot reaction using click chemistry to provide the tracers in high overall radiochemical yield. These LCO based PET tracers hold great promise as multimodal imaging agents for protein aggregates, as the non-radioactive [^{19}F]fluoroglycosylated LCOs displayed excellent binding towards protein deposits in human AD tissue slides. Future *in vivo* investigations in animal models will hopefully reveal the biological impact of the ligated [^{18}F]FDG moiety in terms of BBB penetrance, metabolic stability and pharmacokinetic properties. We foresee that the modular nature of the click reaction may allow for screening of other thiophene based radiotracers and expand the tool box of ligands for multimodal imaging of a variety of disease-associated protein aggregates.

7. [19F]fluoroglycosylation of glycoporphyrins (Paper IV)

7.1. Porphyrins in photodynamic therapy

Porphyrins have numerous interesting photophysical and biochemical properties that have led to their application in many research fields e.g., medicinal chemistry and material science. Porphyrins were early on recognized for their photodynamic properties and have since the early 1990's been used in clinic as photosensitizers to treat cancer using photodynamic therapy.^{123,124} Photodynamic therapy (PDT) is a minimally invasive therapy that employs a photoactive drug, i.e., a photosensitizer that is administered and selectively accumulated in the malign tissue to be treated. Photofrin was one of the first photosensitizers to become commercially available and have been the main reason why PDT has gained wide-spread use in oncology world-wide (in strong analogy with the PET protagonist, [18F]FDG) (Figure 7.1).¹⁵³

Figure 7.1 Structure of photofrin - the most well-used photosensitizer for PDT. Photofrin is sold as a mixture of oligomeric porphyrins, here illustrated as a dimer. The dashed bonds illustrate the aromatic delocalization in each porphyrin ring system.

The photosensitizer is non-toxic on its own, however, upon irradiation with light of a specific wavelength, the photosensitizer is excited to a triplet state and transfers its energy to molecular oxygen (present in the surrounding tissue). In this process, the molecular oxygen, existing in a triplet state, is converted to cytotoxic singlet oxygen that eventually terminate the malign tissue (e.g., cancer cells) from within. PDT can

therefore be made selective by restricting the light exposure only to the area of the body that needs to be treated (Figure 7.2). Nevertheless, photosensitizers still need to display a sufficient selectivity for malign cells over healthy cells. Porphyrins show inherent propensity of tumor tissue enrichment that has been ascribed to their amphiphilic nature, which facilitates binding to various blood-circulating proteins and the subsequent transportation and uptake into malignant cells.¹⁵⁴ However, glycosylation of porphyrins has been shown to significantly enhance uptake and selectivity in cancer cells due to over-expression of GLUT and certain carbohydrate binding receptors (e.g., lectins).

Figure 7.2 Principle of photodynamic therapy. a) The radiolabelled photosensitizer is administered intravenously. b) The photosensitizer is allowed to accumulate in the tumor to be treated, which is made visible with PET imaging. c) The malign tissue is irradiated with light thus inducing the photocytotoxic effect. d) The tumor has been destroyed, as verified with PET.

Moreover, glycosylation is a convenient approach to synthesize amphiphilic, yet neutral, porphyrins. For this reason, the synthesis of glycoporphyrins has been extensively explored and recently reinvigorated by the advent of click chemistry.^{155,156 157,158}

7.2. Synthetic rational

In **Paper IV** we synthesized a set of [^{19}F]fluoroglycosylated glycoporphyrins that, apart from a [^{19}F]FDG moiety, featured tri-substitution of either glucose (**92**), galactose (**93**) or N-acetyl glucosamine (**94**) (Figure 7.3).

Figure 7.3 Glycoconjugated porphyrins featuring tri-substitution of glucose (**92**), galactose (**93**) or N-acetyl glucosamine (**94**) moieties together with [^{19}F]FDG.

The rational for [^{19}F]fluoroglycosylation of porphyrins is that the FDG-property of metabolic trapping could ensure retention of the photosensitizer in the targeted tissue during PDT treatment. Furthermore, the non-radioactive fluorine isotope ^{19}F has excellent NMR¹⁵⁹ and MRI¹⁶⁰ properties being second only to that of the hydrogen nucleus. For instance, ^{19}F has a 100 % natural abundance and its high gyromagnetic ratio provides a very high signal sensitivity that is only 17 % less than the hydrogen nucleus. Furthermore, the chemical shift of ^{19}F (spanning ~ 400 ppm) is highly sensitive to its chemical surroundings. For that reason, ^{19}F -labelled compounds have gained considerable attention in recent years as a promising class of probes to study biological events, mainly due to technical improvements of NMR hardware and the wider availability of fluorine incorporation methods.^{161,162} ^{19}F -NMR has for instance been utilized to study the misfolding of the prion protein associated with Creutzfeldt-Jacob's disease¹⁶³, the A β (1-40) peptide associated with AD¹⁶⁴ and other biological events.¹⁶⁵

Moreover, the [^{18}F]fluoroglycosylation method described in this thesis could be used for future synthesis of ^{18}F -labelled glycoporphyrins for PET investigations. Porphyrins are typically radiolabelled by insertion of positron emitting transition metals (e.g., ^{64}Cu , ^{55}Co) in the chelating porphyrin centre. However, it has been shown that the biodistribution can be altered and the photocytotoxicity compromised by the metalation.¹⁵⁴ Therefore, non-metallic ^{18}F -labelling is an attractive option for PET-PDT molecular imaging, but the reports on ^{18}F -labelled porphyrins are scarce in the literature and the few examples are accompanied by low RCYs.^{166,167} Fluorine incorporation into porphyrins may thus provide new avenues for evaluating the pharmacokinetics and pharmacodynamics of a photosensitizer by combined PDT-PET, -MRI or -NMR, thus facilitating the development of new photosensitizers.

In **Paper IV** we report on the synthesis of [^{19}F]fluoroglycosylated glycoporphyrins and evaluate their cell uptake in a melanoma/fibroblast system. In addition, their photophysical and singlet oxygen generation properties were evaluated.

7.3. Synthesis

7.3.1. Porphyrin synthesis

The parent porphyrin, Me-pcTPP (**95**), was obtained via the Lindsey method in an one-pot two-step reaction of pyrrole, benzaldehyde and methyl 4-formylbenzoate promoted by $\text{BF}_3\cdot\text{OEt}_2$ catalysis followed by DDQ oxidation.¹³⁷ Porphyrin **95** was then treated with chlorosulfonic acid that, in an electrophilic aromatic substitution, readily provided the *tri*-chlorosulfonated porphyrin **96** that immediately was reacted with propargylamine, in the presence of DIPEA, to yield *tris*-alkyne functionalized porphyrin with one ester site (compound **97**, Scheme 7.1). Next, the porphyrin was metallated with zinc, by using $\text{Zn}(\text{OAc})_2$ in DCM/MeOH at room temperature, in order to avoid the insertion of copper during the upcoming click reactions. The zincylated porphyrin **98** was obtained in 40 % yield over three steps. Removal of the methyl ester using conventional hydrolysis with a slight excess of sodium hydroxide in THF-water mixture unfortunately resulted in partial hydrolysis of the sulfonamide bonds. Therefore, the

Krapcho demethylation was employed using lithium chloride in DMF under microwave irradiation¹⁶⁸ which successfully provided compound **99** in 77 % yield without any indication of side reactions.

Scheme 7.1 General conditions and reagents: i) HSO_3Cl , r.t.; ii) propargylamine, DIPEA, DCM, r.t.; iii) $\text{Zn}(\text{OAc})_2 \cdot (\text{H}_2\text{O})_2$, DCM, MeOH, r.t., 40 % over 3 steps; iv) LiCl, DMF, MW, 160 °C, 77 %.

7.3.2. Synthesis of 2-azidoethyl β-D-glycopyranosides

Scheme 7.2 Synthesis of 2-azidoethyl glycosides of glucose, galactose and N-acetyl glucosamine.

2-azidoethyl β-D-gluco- and galactopyranoside, **72** and **113** respectively, were synthesized according to a literature procedure by Chernyak et al.¹³⁵ *Per*-acetylated D-glucose or D-galactose was subjected to a boron-trifluoride dietherate-promoted glycosylation to 2-chloroethanol followed by an azidation using NaN₃ in DMF at 80 °C which provided the 2-azidoethyl β-D-gluco- and galactopyranoside, **72** and **113** in 49 % and 75 % yield, respectively over two steps (Scheme 7.2). The azide-equipped N-acetyl glucosamine **117** was synthesized starting from *per*-acetylated D-glucosamine that was converted to its corresponding oxazoline by the method described by Nakabayashi et al.¹⁶⁹ Next, a PPTS-promoted glycosylation to 2-chloroethanol furnished the corresponding chloroethyl glycoside **116**.¹⁷⁰ Lastly, azidation using NaN₃ and TBAB in DMF at 80 °C provided azidosugar **117** in 75 % yield.

The azidosugars were then click coupled to porphyrin **99** using CuSO₄, sodium L(+)-ascorbate in THF/*t*-BuOH/H₂O (v/v 6:2:1) under microwave irradiation¹⁵⁷ at 85 °C for 3 min thus providing the *tri*-glycosylated porphyrins **100**, **101** and **102**, with full conversion according to LCMS analysis albeit in the isolated yield of 59 % (**100**), 89 % (**101**), 84 % (**103**) (Scheme 7.3). The free carboxylic acid at **100**, **101**, and **102** underwent EDC-NHS-facilitated amide coupling reactions to propargyl amine

providing the corresponding alkynes **103**, **104** and **105**. The subsequent [19F]fluoroglycosylation using [19F]FDG **50** provided the *per*-acetylated *tetra*-glycosylated porphyrins **106**, **107** and **108** in 62 %, 55 % and 78 %, respectively. Global deprotection using aqueous NaOH in MeOH readily provided the target [19F]fluoroglycosylated porphyrins **92**, **93** and **94** in 99 %, 80 % and 100%, respectively.

Scheme 7.3 General conditions and reagents: i) CuSO_4 , sodium L-(+)-ascorbate, THF, *t*-BuOH, H_2O , MW, 85°C , 59 % (**100**), 89 % (**101**), 84 % (**102**), 62 % (**106**), 55 % (**107**), 78 % (**108**); ii) 1. NHS, EDC, DMF, r.t.; 2. Propargylamine, DIPEA, r.t., 85 % (**103**), 61 % (**104**), 52 % (**105**); iii) NaOH, MeOH, H_2O , r.t., 99 % (**92**), 80 % (**93**) and 100 % (**94**).

7.4. Flow cytometry assay

The cell uptake of the glycoporphyrins was quantitatively evaluated in fibroblasts and melanoma cell cultures by flow cytometry. The cells were incubated with 20 μ M of the respective porphyrin derivatives for 24 h. Then, an aliquot of the cell sample was injected into the flow cytometer and each individual cell was forced to pass a fluorescence detector that registers the intensity of the emitted fluorescence light (upon excitation) from the cells which have taken up porphyrin. The method allows for the discrimination of stained vs unstained cells, but also a quantitative estimate of the relative uptake for each porphyrin in the respective cell line. To probe the effect of the [19F]FDG moiety on the porphyrin scaffold, a corresponding non-fluorinated *tetra*-glucose analogue (**118**) and a non-glycosylated *tetra*-sulphonic acid porphyrin (**119**) was synthesized in order to probe the impact of glycosylation on the porphyrin scaffold (Figure 7.4). The combined results of four replicate flow cytometry measurements are depicted as a histogram in Figure 7.5.

Interestingly, the non-glycosylated porphyrin **119** displayed a preferential up-take in fibroblasts compared to melanoma cells, thus ruling it out as a plausible PDT agent. Moreover, no significant difference between fibroblasts and melanoma cells could be observed for the N-acetyl glucosamine equipped porphyrin **94**, nor for the *tetra*-glucosylated porphyrin **118**. However, in strong contrast, the fluorinated analogue **92** displayed a significant preferential uptake in melanoma cells. So did the galactosylated porphyrin **93**, and the intensity of the fluorescence signal did not differ significantly between the two (**92** and **93**).

In this assay, both the glucose- and galactosylated porphyrin bearing [19F]FDG (i.e., **92** and **93** respectively) exhibited excellent selectivity and high cell uptake favoring melanoma. The unexpectedly promising properties observed for the galactosylated porphyrin **93** could possibly be explained by the finding that certain galactose binding lectins, particularly Gal-9, have been found to be overexpressed in some melanoma cell-lines. This may explain why the galactosylated porphyrin is on par with the glucosylated porphyrin **92**.¹⁷¹ From a therapeutic perspective, since glucose transporters are almost

ubiquitously expressed (and to a large magnitude) in the human body, the more modest expression of galactose transporters may represent a much more attractive target for drug delivery, e.g., for PDT. This concept has been pursued by Olof Solin and his co-worker, Satish Jadhav, who recently prepared a dendrimeric galactosylated porphyrin for this reason.¹⁷² In conclusion, the [19F]FDG auxiliary impart a pronounced biological effect favoring uptake in melanoma cells suggesting its use in PDT applications. The result seem to suggest that [19F]FDG metabolically stabilizes the glycoporphyrin, presumably via metabolic trapping, however more experiments are required to unveil the cause of this effect.

Figure 7.4 Structure of reference porphyrins for the flow cytometry assay. Porphyrin **118** is *tetra*-glucosylated whereas porphyrin **119** is equipped with four sulphonic acids.

Figure 7.5 Flow cytometry evaluation of fibroblast and melanoma cells incubated with glycoporphyrins.

8. Synthesis of fluorinated *trans*-stilbenes for spectral discrimination of native and protofibrillar transthyretin

8.1. Synthesis rational

TTR is a 55 kDa homotetrameric protein that circulates the human plasma and cerebrospinal fluid (Figure 8.1).¹⁷³ TTR is charged with several different tasks: in human plasma, TTR act as a distributor of thyroid hormones, particularly L-thyroxine (T₄). Furthermore, TTR has anti-amyloid properties and has been found to be the main sequester of the A β peptide in human CSF (K_d of 28 ± 5 nM) resulting in a decrease of aggregation and toxicity of A β .^{174, 175} TTR therefore seems to act as a natural chaperone to prevent amyloid formation associated with AD. Somewhat ironic, TTR itself is an amyloidogenic protein that upon misfolding forms amyloid fibrils that are predominately deposited in peripheral nerves and and heart, leading to loss of function and eventually to the death of the inflicted organ. This condition is referred to as transthyretin amyloidosis (ATTR).

In order for the fibrillation to occur, the native tetrameric conformation dissociates to form non-native misfolded monomers that propagate the fibrillation, eventually forming amyloid fibrils.¹⁷⁶ It has been found that certain hydrophobic ligands, that are able to bind to a hydrophobic pocket on the native homotetrameric protein, can inhibit the initial dissociation thus preventing the misfolding event.¹⁷⁷ One of these ligands is resveratrol (Figure 8.1).¹⁷⁸ This mechanism opens up a potential window for therapeutic treatment of the disorder. Currently, there is only one drug that is approved for use as an aggregation inhibitor, namely the small molecule, tafamidis.^{179,180}

Figure 8.1 Resveratrol binding to the hydrophobic pocket in human transthyretin.¹⁷⁸ (PDB-doi:[10.2210/pdb1dvs/pdb](https://doi.org/10.2210/pdb1dvs/pdb)).

In **paper V**, we synthesized a small library of *trans*-stilbenes (22 compounds), based on the resveratrol scaffold. The compounds were screened using a fluorescence microscopy assay (Figure 8.2). The aim was to find a stilbene ligand that effectively binds to both native and protofibrillar TTR, but gives rise to spectrally distinguishable fluorescence emission profiles for the two TTR forms. Three of the stilbenes (**120**, **121** and **122**) were fluorinated to enable future PET applications for ATTR diagnosis, as motivated by the success of the ¹⁸F-labelled stilbene, florbetapir, and the ¹¹C-labelled stilbene, [11C]-BF-227, for cardiac ATTR imaging.^{181,182} In addition, fluorine incorporation could also enable ¹⁹F-NMR investigations for ATTR.¹⁶⁵ The fluorinated *trans*-stilbenes also featured a benzimidazole motif since it is a remarkably common pharmacophore in a wide variety of therapeutic applications.¹⁸³

Figure 8.2 Selection of synthesized *trans*-stilbenes found in the small library in **paper V**. The stilbenes are structurally based on the resveratrol scaffold.

8.2. *E*-stilbene synthesis

The three fluorinated stilbenes (**120**, **121** and **122**, Scheme 8.1), along with the non-fluorinated dimethoxy stilbene analogue, **123**, were prepared using the *E*-selective Horner-Wadsworth-Emmons modification of the Wittig reaction¹⁸⁴, according to the literature procedure by Lee et al.¹⁸⁵ The required fluorinated benzyl phosphonates were in turn synthesized from the corresponding benzyl bromide in a microwave mediated Michaelis-Arbuzov reaction (Scheme 8.2).¹⁸⁶

Scheme 8.1 Horner-Wadsworth-Emmons reaction between substituted benzyl phosphonates and the aldehyde (1H-benzo[d]imidazole-5-carbaldehyde) providing the desired *E*-stilbenes.

Scheme 8.2 Synthesis of benzyl phosphonates by means of a microwave mediated Michaelis-Arbuzov reaction using trimethyl phosphite under neat conditions.

8.3. Results

Unfortunately, for the aim of the paper, the fluorinated stilbenes were found to be poorly fluorescent. Interestingly, the subtle modification of replacing the trifluoromethyl substituents in stilbene **122** with methoxy groups (stilbene **123**) significantly increased the fluorescence signal for native TTR but not for protofibrils. The fluorinated stilbenes were therefore discarded from further selection trials using fluorescence. However, two *trans*-stilbenes (**124** and **125**), both bearing a naphthyl moiety, emerged from the library that passed the selection criteria: they exhibited an increase in fluorescence intensity and provided different spectral peaks upon binding to native or protofibrillar TTR. Upon binding to protofibrils, stilbene **124** had a blue-shifted emission peak at 395 nm. The emission difference are clearly visible by eye (Figure 8.3). Similarly, stilbene **125** displayed a blue-shifted emission peak at 395 nm and gave rise to an additional emission peak at 490 nm.

Figure 8.3 The different fluorescence emission profiles as deduced by eye for *trans*-stilbene **124** upon binding to either native or protofibrillary TTR.

9. Conclusions and Future Perspectives

In this thesis, a [^{18}F]fluoroglycosylation method to attach the widely used radiopharmaceutical [^{18}F]FDG, to a ligand of choice with a given biological target using click chemistry, has been described. The developed [^{18}F]fluoroglycosylation method is performed in three steps in the same reaction vessel: first radiofluorination of the precursor, then deacetylation followed by a click coupling to the ligand of choice in an aqueous buffer solution. This prosthetic group methodology offers a practical and mild approach to radiolabel sensitive ligands (e.g., biomolecules), since the harsh reaction steps are performed prior to the click coupling. Moreover, the substrate scope of the described [^{18}F]fluoroglycosylation method has been significantly extended, compared to previously reported methods, since it encompasses click coupling to ligands bearing either azide or alkyne linkers.

Moreover, in this thesis it was systematically shown that β -configuration is a pivotal criterion for efficient radiolabelling of clickable [^{18}F]FDG precursors. We demonstrated the utility of the [^{18}F]fluoroglycosylation method by forming [^{18}F]FDG conjugates to the highly amyloidophilic ligands, LCOs, thus forming PET tracers for potential imaging of both systemic and cerebral amyloidosis. [^{18}F]FDG was also conjugated to curcumin, providing a metabolically stabilized glycocurcumin for potential use as a PET tracer for AD imaging.

Furthermore, a set of [^{19}F]fluoroglycosylated glycoporphyrins were synthesized. Two of the conjugates exhibited significantly increased uptake and selectivity for melanoma cancer, compared to their non-glycosylated counterparts and may be used for both diagnosis and photodynamic therapy treatment of cancer and other disorders.

In addition to offer a practical method to generate PET tracers, with improved *in vivo* properties for a plethora of diseases, fluoroglycosylation may open new avenues for multimodal imaging using ^{19}F -NMR or MRI to study biological processes.

The results underlying this thesis adds a brick to the wall of knowledge in terms of synthesizing and exploiting fluorinated sugars for diagnostic and therapeutic applications. However, so far, focus has mainly been directed at glycosylations using simple fluorinated D-glucose. In the future, I am convinced that we will have a toolbox of radioactive sugars at our disposal for molecular imaging of a plethora of diseases.

10. References

- (1) Levy, D., and Fügedi, P. (Eds.). (2009) The Organic Chemistry of Sugars. CRC Press.
- (2) Lindhorst, T. K. (2007) Essentials of carbohydrate chemistry and biochemistry.
- (3) Singhal, G. S., Renger, G., Sopory, S. K., and Irrgang, K. D. (2012) Concepts in Photobiology: Photosynthesis and Photomorphogenesis.
- (4) Barron, C. C., Bilan, P. J., Tsakiridis, T., and Tsiani, E. (2016) Facilitative glucose transporters: Implications for cancer detection, prognosis and treatment. *Metab. Clin. Exp.* 65, 124–139.
- (5) Uldry, M., Ibberson, M., Hosokawa, M., and Thorens, B. (2002) GLUT2 is a high affinity glucosamine transporter. *FEBS Letters* 524, 199–203.
- (6) Erbslöh, F., Bernsmeier, A., and Hillesheim, H. R. (1958) Der Glucoseverbrauch des Gehirns und seine Abhängigkeit von der Leber. *Archiv für Psychiatrie und Zeitschrift f. d. ges. Neurologie* 196, 611–626.
- (7) Colville, C. A., Seatter, M. J., Jess, T. J., Gould, G. W., and Thomas, H. M. (1993) Kinetic analysis of the liver-type (GLUT2) and brain-type (GLUT3) glucose transporters in *Xenopus* oocytes: substrate specificities and effects of transport inhibitors. *Biochem. J.* 290, 701–706.
- (8) Haber, R. S., Weinstein, S. P., O'Boyle, E., and Morgello, S. (1993) Tissue distribution of the human GLUT3 glucose transporter. *Endocrinology* 132, 2538–2543.
- (9) Nelson, D. L., and Cox, M. M. (2008) PRINCIPLES OF BIOCHEMISTRY 528–538.
- (10) Berg, J. M., Tymoczko, J. L., Gregory J Gatto, J., and Stryer, L. (2015) Biochemistry 8 ed., pp 552–553.
- (11) Hatanaka, M. (1974) Transport of sugars in tumor cell membranes. *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer* 355, 77–104.
- (12) Ganapathy, V., Thangaraju, M., and Prasad, P. D. (2009) Nutrient transporters in cancer: Relevance to Warburg hypothesis and beyond. *Pharmacology & Therapeutics* 121, 29–40.
- (13) Airley, R. E., and Mobasher, A. (2007) Hypoxic regulation of glucose transport, anaerobic metabolism and angiogenesis in cancer: novel pathways and targets for anticancer therapeutics. - PubMed - NCBI. *Chemotherapy* 53, 233–256.
- (14) Suzawa, N., Ito, M., Qiao, S., Uchida, K., Takao, M., Yamada, T., Takeda, K., and Murashima, S. (2011) Assessment of factors influencing FDG uptake in non-small cell lung cancer on PET/CT by investigating histological differences in expression of glucose transporters 1 and 3 and tumour size. *Lung Cancer* 72, 191–198.
- (15) Krzeslak, A., Wojcik-Krowiranda, K., Forma, E., Jozwiak, P., Romanowicz, H., Bienkiewicz, A., and Brys, M. (2012) Expression of GLUT1 and GLUT3 Glucose Transporters in Endometrial and Breast Cancers. *Pathol. Oncol. Res.* 18, 721–728.
- (16) Conde, V. R., Oliveira, P. F., Nunes, A. R., Rocha, C. S., Ramalhosa, E., Pereira, J. A., Alves, M. G., and Silva, B. M. (2015) The progression from a lower to a higher invasive stage of bladder cancer is associated with severe alterations in glucose and pyruvate metabolism. *Experimental Cell Research* 335, 91–98.

- (17) Boado, R. J., Black, K. L., and Pardridge, W. M. (1994) Gene expression of GLUT3 and GLUT1 glucose transporters in human brain tumors. *Molecular Brain Research* 27, 51–57.
- (18) Nagamatsu, S., Sawa, H., Wakizaka, A., and Hoshino, T. (1993) Expression of Facilitative Glucose Transporter Isoforms in Human Brain Tumors. *Journal of Neurochemistry* 61, 2048–2053.
- (19) Nishioka, T., Oda, Y., Seino, Y., Yamamoto, T., Inagaki, N., Yano, H., Imura, H., Shigemoto, R., and Kikuchi, H. (1992) Distribution of the Glucose Transporters in Human Brain Tumors. *Cancer Res* 52, 3972–3979.
- (20) Boado, R. J., Li, J. Y., Nagaya, M., Zhang, C., and Pardridge, W. M. (1999) Selective expression of the large neutral amino acid transporter at the blood-brain barrier. *Proceedings of the National Academy of Sciences* 96, 12079–12084.
- (21) Kim, D. K., Kanai, Y., Choi, H. W., Tangtrongsup, S., Chairoungdua, A., Babu, E., Tachampa, K., Anzai, N., Iribe, Y., and Endou, H. (2002) Characterization of the system L amino acid transporter in T24 human bladder carcinoma cells. *Biochimica et Biophysica Acta (BBA) - Biomembranes* 1565, 112–122.
- (22) Kobayashi, K., Ohnishi, A., Promsuk, J., Shimizu, S., Kanai, Y., Shiokawa, Y., and Nagane, M. (2008) ENHANCED TUMOR GROWTH ELICITED BY L-TYPE AMINO ACID TRANSPORTER 1 IN HUMAN MALIGNANT GLIOMA CELLS. *Neurosurgery* 62, 493–504.
- (23) Kanai, Y., and Hediger, M. A. (2004) The glutamate/neutral amino acid transporter family SLC1: molecular, physiological and pharmacological aspects. *Pflügers Arch - Eur J Physiol* 447, 469–479.
- (24) Eagle, H. (1955) Accept Terms and Conditions on JSTOR. *Science*.
- (25) D, W., N, A., N, C., and M, Y. (2002) Overexpression of the neutral amino acid transporter ASCT2 in human colorectal adenocarcinoma. *Anticancer Res* 22, 2555–2557.
- (26) Bode, B. P., Fuchs, B. C., Hurley, B. P., Conroy, J. L., Suetterlin, J. E., Tanabe, K. K., Rhoads, D. B., Abcouwer, S. F., and Souba, W. W. (2002) Molecular and functional analysis of glutamine uptake in human hepatoma and liver-derived cells. *American Journal of Physiology - Gastrointestinal and Liver Physiology* 283, G1062–G1073.
- (27) Nordberg, A. (2004) PET imaging of amyloid in Alzheimer's disease. *The Lancet Neurology* 3, 519–527.
- (28) Zhu, A., Lee, D., and Shim, H. (2011) Metabolic Positron Emission Tomography Imaging in Cancer Detection and Therapy Response. *Seminars in Oncology* 38, 55–69.
- (29) Levin, C. S. (2005) Primer on molecular imaging technology. *European Journal of Nuclear Medicine and Molecular Imaging* 32 Suppl 2, S325–45.
- (30) Townsend, D. W., and Beyer, T. (2002) A combined PET/CT scanner: the path to true image fusion. *The British Journal of Radiology* 75, S24–S30.
- (31) Ametamey, S. M., Honer, M., and Schubiger, P. A. (2008) Molecular Imaging with PET. *Chem. Rev.* 108, 1501–1516.
- (32) Böhm, H.-J., Banner, D., Bendels, S., Kansy, M., Kuhn, B., Müller, K., Obst-Sander, U., and Stahl, M. (2004) Fluorine in medicinal chemistry. *ChemBiochem* 5, 637–643.

- (33) Gillis, E. P., Eastman, K. J., Hill, M. D., Donnelly, D. J., and Meanwell, N. A. (2015) Applications of Fluorine in Medicinal Chemistry. *Journal of medicinal ...*
- (34) Vallabhajosula, S., Solnes, L., and Vallabhajosula, B. (2011) A Broad Overview of Positron Emission Tomography Radiopharmaceuticals and Clinical Applications: What Is New? *YSNUC 41*, 246–264.
- (35) Gallagher, B. M., Fowler, J. S., Gutterson, N. I., MacGregor, R. R., Wan, C. N., and Wolf, A. P. (1978) Metabolic trapping as a principle of radiopharmaceutical design: some factors responsible for the biodistribution of [18F] 2-deoxy-2-fluoro-D-glucose. *Journal of Nuclear Medicine* 19, 1154–1161.
- (36) Ido, T., Wan, C. N., Casella, V., Fowler, J. S., Wolf, A. P., Reivich, M., and Kuhl, D. E. (2006) Labeled 2-deoxy-D-glucose analogs. 18F-labeled 2-deoxy-2-fluoro-D-glucose, 2-deoxy-2-fluoro-D-mannose and 14C-2-deoxy-2-fluoro-D-glucose. *J. Label Compd. Radiopharm* 14, 175–183.
- (37) Hamacher, K., Coenen, H. H., and Stöcklin, G. (1986) Efficient stereospecific synthesis of no-carrier-added 2-[18F]-fluoro-2-deoxy-D-glucose using aminopolyether supported nucleophilic substitution. *Journal of Nuclear Medicine* 27, 235–238.
- (38) Hamacher, K. (1984) Phase-transfer catalysed synthesis of 4-S- β -d-glucopyranosyl-4-thio-d-glucopyranose (thiocellobiose) and 2-S- β -d-glucopyranosyl-2-thio-d-glucopyranose (thiosophorose). *CARBOHYDRATE RESEARCH* 128, 291–295.
- (39) Stütz, A. E. (Ed.). (2001) Glycoscience. Springer Berlin Heidelberg, Berlin, Heidelberg.
- (40) Füchtner, F., Steinbach, J., Mäding, P., and Johannsen, B. (1996) Basic hydrolysis of in the preparation of 2-[18F]fluoro-2-deoxy-d-glucose. *Applied Radiation and Isotopes* 47, 61–66.
- (41) Chen, K., and Conti, P. S. (2010) Target-specific delivery of peptide-based probes for PET imaging. *Advanced Drug Delivery Reviews* 62, 1005–1022.
- (42) Fani, M., Maecke, H. R., and Okarvi, S. M. (2012) Radiolabeled peptides: valuable tools for the detection and treatment of cancer. *Theranostics* 2, 481–501.
- (43) Rufini, V., Calcagni, M. L., and Baum, R. P. (2006) Imaging of Neuroendocrine Tumors. *Seminars in Nuclear Medicine* 36, 228–247.
- (44) Ohki-Hamazaki, H., Iwabuchi, M., and Maekawa, F. (2005) Development and function of bombesin-like peptides and their receptors. *Int. J. Dev. Biol.* 49, 293–300.
- (45) Brooks, P. C. (1996) Role of integrins in angiogenesis. *European Journal of Cancer* 32, 2423–2429.
- (46) Nieberler, M., Reuning, U., Reichart, F., Notni, J., Wester, H.-J., Schwaiger, M., Weinmüller, M., Räder, A., Steiger, K., and Kessler, H. (2017) Exploring the Role of RGD-Recognizing Integrins in Cancer. *Cancers (Basel)* 9, 116.
- (47) Haubner, R., Kuhnast, B., Mang, C., Weber, W. A., Kessler, H., Wester, H.-J., and Schwaiger, M. (2003) [18F]Galacto-RGD: Synthesis, Radiolabeling, Metabolic Stability, and Radiation Dose Estimates. *Bioconjugate Chem.* 15, 61–69.
- (48) Haubner, R., Weber, W. A., Beer, A. J., Vabulienė, E., Reim, D., Sarbia, M., Becker, K.-F., Goebel, M., Hein, R., Wester, H.-J., Kessler, H., and Schwaiger, M. (2005) Noninvasive Visualization of the Activated $\alpha\beta 3$ Integrin in Cancer Patients by Positron Emission Tomography and [18F]Galacto-RGD. *PLoS Medicine* (Ell, P., Ed.) 2, e70.

- (49) Prante, O., Hamacher, K., and Coenen, H. H. (2007) Chemoenzymatic n.c.a synthesis of the coenzyme UDP-2-deoxy-2-[18F]fluoro- α -D-glucopyranose as substrate of glycosyltransferases. *J. Label Compd. Radiopharm* 50, 55–63.
- (50) Patt, M., Sorger, D., Scheunemann, M., and Stöcklin, G. (2002) Adduct of 2-[18F]FDG and 2-nitroimidazole as a putative radiotracer for the detection of hypoxia with PET: synthesis, in vitro- and in vivo-characterization. *Applied Radiation and Isotopes* 57, 705–712.
- (51) Prante, O., Einsiedel, J., Haubner, R., Gmeiner, P., Wester, H.-J., Kuwert, T., and Maschauer, S. (2006) 3,4,6-Tri-O-acetyl-2-deoxy-2-[18F]fluoroglucopyranosyl Phenylthiosulfonate: A Thiol-Reactive Agent for the Chemoselective 18F-Glycosylation of Peptides. *Bioconjugate Chem.* 18, 254–262.
- (52) Namavari, M., Cheng, Z., Zhang, R., De, A., Levi, J., Hoerner, J. K., Yaghoubi, S. S., Syud, F. A., and Gambhir, S. S. (2009) A Novel Method for Direct Site-Specific Radiolabeling of Peptides Using [18F]FDG. *Bioconjugate Chem.* 20, 432–436.
- (53) Hultsch, C., Schottelius, M., Auernheimer, J., Alke, A., and Wester, H.-J. (2009) 18F-Fluoroglucosylation of peptides, exemplified on cyclo(RGDfK). *European Journal of Nuclear Medicine and Molecular Imaging* 36, 1469–1474.
- (54) Wuest, F., Berndt, M., Bergmann, R., van den Hoff, J., and Pietzsch, J. (2008) Synthesis and Application of [18F]FDG-Maleimidehexyloxime ([18F]FDG-MHO): A [18F]FDG-Based Prosthetic Group for the Chemoselective 18F-Labeling of Peptides and Proteins †. *Bioconjugate Chem.* 19, 1202–1210.
- (55) Kolb, H. C., Finn, M. G., and Sharpless, K. B. (2001) Click Chemistry: Diverse Chemical Function from a Few Good Reactions. *Angewandte Chemie International Edition* 40, 2004–2021.
- (56) Rostovtsev, V. V., Green, L. G., Fokin, V. V., and Sharpless, K. B. (2002) A Stepwise Huisgen Cycloaddition Process: Copper(I)-Catalyzed Regioselective “Ligation” of Azides and Terminal Alkynes. *Angew. Chem.* 114, 2708–2711.
- (57) Tornøe, C. W., Christensen, C., and Meldal, M. (2002) Peptidotriazoles on Solid Phase: [1,2,3]-Triazoles by Regiospecific Copper(I)-Catalyzed 1,3-Dipolar Cycloadditions of Terminal Alkynes to Azides. *J. Org. Chem.* 67, 3057–3064.
- (58) Thirumurugan, P., Matosiuk, D., and Jozwiak, K. (2013) Click chemistry for drug development and diverse chemical-biology applications. *Chem. Rev.* 113, 4905–4979.
- (59) Tiwari, V. K., Mishra, B. B., Mishra, K. B., Mishra, N., Singh, A. S., and Chen, X. (2016) Cu-Catalyzed Click Reaction in Carbohydrate Chemistry. *Chem. Rev.* 116, 3086–3240.
- (60) Fokin, V. V., and Matyjaszewski, K. (2012) CuAAC: The Quintessential Click Reaction, in *Organic Chemistry - Breakthroughs and Perspectives*, pp 247–277. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany.
- (61) Huisgen, R., Szeimies, G., and Möbius, L. (1967) 1.3-Dipolare Cycloadditionen, XXXII. Kinetik der Additionen organischer Azide an CC-Mehrfachbindungen. *Chemische Berichte* 100, 2494–2507.
- (62) Zhang, L., Chen, X., Xue, P., Sun, H. H. Y., Williams, I. D., Sharpless, K. B., Fokin, V. V., and Jia, G. (2005) Ruthenium-Catalyzed Cycloaddition of Alkynes and Organic Azides. *J. Am. Chem. Soc.* 127, 15998–15999.
- (63) Rasmussen, L. K., Boren, B. C., and Fokin, V. V. (2007) Ruthenium-Catalyzed Cycloaddition of Aryl Azides and Alkynes. *Org. Lett.* 9, 5337–5339.

- (64) Maschauer, S., and Prante, O. (2009) A series of 2-O-trifluoromethylsulfonyl-d-mannopyranosides as precursors for concomitant ^{18}F -labeling and glycosylation by click chemistry. *CARBOHYDRATE RESEARCH* 344, 753–761.
- (65) Maschauer, S., Haubner, R., Kuwert, T., and Prante, O. (2014) (^{18}F) -glyco-RGD peptides for PET imaging of integrin expression: efficient radiosynthesis by click chemistry and modulation of biodistribution by glycosylation. *Mol. Pharmaceutics* 11, 505–515.
- (66) Boutureira, O., D'Hooge, F., Fernández-González, M., Bernardes, G. J. L., Sánchez-Navarro, M., Koeppe, J. R., and Davis, B. G. (2009) Fluoroglycoproteins: ready chemical site-selective incorporation of fluorosugars into proteins. *Chem. Commun.* 46, 8142–8144.
- (67) Fischer, C. R., Müller, C., Reber, J., Müller, A., Krämer, S. D., Ametamey, S. M., and Schibli, R. (2012) [^{18}F]Fluoro-Deoxy-Glucose Folate: A Novel PET Radiotracer with Improved in Vivo Properties for Folate Receptor Targeting. *Bioconjugate Chem.* 23, 805–813.
- (68) Pisaneschi, F., Slade, R. L., Iddon, L., George, G. P. C., Nguyen, Q.-D., Spivey, A. C., and Aboagye, E. O. (2013) Synthesis of a new fluorine-18 glycosylated “click” cyanoquinoline for the imaging of epidermal growth factor receptor. *J. Label Compd. Radiopharm* 57, 92–96.
- (69) Maschauer, S., and Prante, O. (2014) Sweetening Pharmaceutical Radiochemistry by ^{18}F -Fluoroglycosylation: A Short Review. *BioMed Research International* 2014, 1–16.
- (70) Kim, D. H., Choe, Y. S., Jung, K.-H., Lee, K.-H., Choi, J. Y., Choi, Y., and Kim, B.-T. (2008) A ^{18}F -labeled glucose analog: Synthesis using a click labeling method and in vitro evaluation. *Arch. Pharm. Res.* 31, 587–593.
- (71) Cara, C. J., and Skropeta, D. (2015) Glycosylation and functionalization of native amino acids with azido uronic acids. *Tetrahedron* 71, 9357–9365.
- (72) “‘Clickable’ glycoconjugates – a new approach to the synthesis of bioco” by Consolato James Cara.
- (73) Dobson, C. M. (2004) Principles of protein folding, misfolding and aggregation. *Seminars in Cell & Developmental Biology* 15, 3–16.
- (74) Prince, M., Comas-Herrera, A., Knapp, M., Guerchet, M., and Karagiannidou, M. (2016) World Alzheimer report 2016: improving healthcare for people living with dementia: coverage, quality and costs now and in the future, pp 1–141.
- (75) DeToma, A. S., Salamekh, S., Ramamoorthy, A., and Lim, M. H. (2012) Misfolded proteins in Alzheimer's disease and type II diabetes. *Chem. Soc. Rev.* 41, 608–621.
- (76) Sipe, J. D. (Ed.). (2005) Amyloid Proteins. Wiley-VCH Verlag GmbH, Weinheim, Germany.
- (77) Tomlinson, J., Cullen, V., and Schlossmacher, M. G. (2010) Identifying Targets in α -Synuclein Metabolism to Treat Parkinson Disease and Related Disorders. *Protein Misfolding Diseases*, pp 817–841. John Wiley & Sons, Inc., Hoboken, NJ, USA.
- (78) Legname, G., DeArmond, S. J., Cohen, F. E., and Prusiner, S. B. (2007) Pathogenesis of Prion Diseases, in *Protein Misfolding, Aggregation, and Conformational Diseases*, pp 125–146. Springer, Boston, MA, Boston, MA.

- (79) Jurgens, C. A., Toukatly, M. N., Fligner, C. L., Udayasankar, J., Subramanian, S. L., Zraika, S., Aston-Mourney, K., Carr, D. B., Westermarck, P., Westermarck, G. T., Kahn, S. E., and Hull, R. L. (2011) β -Cell Loss and β -Cell Apoptosis in Human Type 2 Diabetes Are Related to Islet Amyloid Deposition. *The American Journal of Pathology* 178, 2632–2640.
- (80) Tvaroška, I., and Bleha, T. (1989) Anomeric and Exo-Anomeric Effects in Carbohydrate Chemistry, in *Advances in Carbohydrate Chemistry and Biochemistry Volume 47*, pp 45–123. Elsevier.
- (81) Demchenko, A. V. (2008) General Aspects of the Glycosidic Bond Formation. *Handbook of Chemical Glycosylation*, pp 1–27. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany.
- (82) Goodman, L. (1967) Neighboring-Group Participation in Sugars. *Advances in Carbohydrate Chemistry* 22, 109–175.
- (83) Crich, D., and Cai, W. (1999) Chemistry of 4,6- O-Benzylidene- d-glycopyranosyl Triflates: Contrasting Behavior between the Gluco and Manno Series. *J. Org. Chem.* 64, 4926–4930.
- (84) Crich, D., and Li, L. (2007) 4,6- O-Benzylidene-Directed β -Mannopyranosylation and α -Glucopyranosylation: The 2-Deoxy-2-fluoro and 3-Deoxy-3-fluoro Series of Donors and the Importance of the O2–C2–C3–O3 Interaction. *J. Org. Chem.* 72, 1681–1690.
- (85) Kochetkov, N. K., Khorlin, A. J., and Bochkov, A. F. (1964) New synthesis of glycosides. *Tetrahedron Letters* 5, 289–293.
- (86) Allen, J. G., and Fraser-Reid, B. (1999) n-Pentenyl Glycosyl Orthoesters as Versatile Intermediates in Oligosaccharide Synthesis. The Proteoglycan Linkage Region 1. *J. Am. Chem. Soc.* 121, 468–469.
- (87) Roberts, C., Madsen, R., and Fraser-Reid, B. (2002) Studies Related to Synthesis of Glycophosphatidylinositol Membrane-Bound Protein Anchors. 5. n-Pentenyl Ortho Esters for Mannan Components. *J. Am. Chem. Soc.* 117, 1546–1553.
- (88) Walsh, K., Sneddon, H. F., and Moody, C. J. (2014) Sustainable, mild and efficient p-methoxybenzyl ether deprotections utilizing catalytic DDQ. *Tetrahedron* 70, 7380–7387.
- (89) Jung, M. E., and Koch, P. (2011) Mild, selective deprotection of PMB ethers with triflic acid/1,3-dimethoxybenzene. *Tetrahedron Letters* 52, 6051–6054.
- (90) Wang, Z. (2010) Zemplén Deacetylation. *Comprehensive Organic Name Reactions and Reagents*. John Wiley & Sons, Inc., Hoboken, NJ, USA.
- (91) Liu, K., and DANISHEFSKY, S. J. (1994) Route from Glycals to Mannose. beta.-Glycosides. *J. Org. Chem.*
- (92) Albright, J. D., and Goldman, L. (1965) Dimethyl Sulfoxide-Acid Anhydride Mixtures. New Reagents for Oxidation of Alcohols 1. *J. Am. Chem. Soc.* 87, 4214–4216.
- (93) Lichtenthaler, F. W., and Schneider-Adams, T. (1994) 3,4,6-Tri-O-benzyl- α -d-arabino-hexopyranos-2-ulosyl Bromide: A Versatile Glycosyl Donor for the Efficient Generation of β -d-Mannopyranosidic Linkages. *Journal of Organic Chemistry* 59, 6728–6734.
- (94) Chang, C.-W. T., Hui, Y., and Elchert, B. (2001) Studies of the stereoselective reduction of ketosugar (hexosulose). *Tetrahedron Letters* 42, 7019–7023.

- (95) Gao, X., Gaddam, V., and Harmata, M. (2014) A Synthesis of 6-Deoxy-6-Fluorosucrose Suitable For PET Applications. *CARBOHYDRATE RESEARCH* 1–14.
- (96) Danishefsky, S. J., and Bilodeau, M. T. (1996) Glycals in Organic Synthesis: The Evolution of Comprehensive Strategies for the Assembly of Oligosaccharides and Glycoconjugates of Biological Consequence. *Angewandte Chemie International Edition* 35, 1380–1419.
- (97) Halcomb, R. L., and Danishefsky, S. J. (1989) On the direct epoxidation of glycals: application of a reiterative strategy for the synthesis of β -linked oligosaccharides. *J. Am. Chem. Soc.* 111, 6661–6666.
- (98) Brigl, P. (1921) Kohlenhydrate. I. Partieller Austausch von Säuregruppen in der β -Pentaacetyl-glykose. *Z. physiol. Chem* 116, 1.
- (99) Cheshev, P., Marra, A., and Dondoni, A. (2006) Direct epoxidation of d-glucal and d-galactal derivatives with in situ generated DMDO. *CARBOHYDRATE RESEARCH* 341, 2714–2716.
- (100) Maschauer, S., and Prante, O. (2009) A series of 2-O-trifluoromethylsulfonyl-d-mannopyranosides as precursors for concomitant ^{18}F -labeling and glycosylation by click chemistry. *CARBOHYDRATE RESEARCH* 344, 753–761.
- (101) Murray, R. W., and Jeyaraman, R. (1985) Dioxiranes: synthesis and reactions of methyldioxiranes. *J. Org. Chem.*
- (102) Wang, Z. (2010) Finkelstein Reaction 2nd ed. John Wiley & Sons, Inc., Hoboken, NJ, USA.
- (103) Dong, H., Pei, Z., and Ramström, O. (2006) Stereospecific Ester Activation in Nitrite-Mediated Carbohydrate Epimerization. *J. Org. Chem.* 71, 3306–3309.
- (104) Dong, H., Pei, Z., Angelin, M., Byström, S., and Ramström, O. (2007) Efficient Synthesis of β -d-Mannosides and β -d-Talosides by Double Parallel or Double Serial Inversion. *J. Org. Chem.* 72, 3694–3701.
- (105) Bock, K., and Pedersen, C. (1974) A study of ^{13}C coupling constants in hexopyranoses. *J. Chem. Soc., Perkin Trans. 2* 293–297.
- (106) Nolte, C., Ammer, J., and Mayr, H. (2012) Nucleofugality and Nucleophilicity of Fluoride in Protic Solvents. *J. Org. Chem.* 77, 3325–3335.
- (107) Card, P. J. (1983) Fluorinated carbohydrates. Use of DAST in the synthesis of fluorinated sugars. *J. Org. Chem.*
- (108) Kováč, P. (1986) A short synthesis of 2-deoxy-2-fluoro-d-glucose. *CARBOHYDRATE RESEARCH* 153, 168–170.
- (109) Furumoto, S., Shinbo, R., Iwata, R., Ishikawa, Y., Yanai, K., Yoshioka, T., and Fukuda, H. (2013) In Vitro and In Vivo Characterization of 2-Deoxy-2- ^{18}F -Fluoro-d-Mannose as a Tumor-Imaging Agent for PET. *Journal of Nuclear Medicine* 54, 1354–1361.
- (110) HARADAHIRA, T., MAEDA, M., KAI, Y., OMAE, H., and KOJIMA, M. (1985) Improved synthesis of 2-deoxy-2-fluoro-D-glucose using fluoride ion. *Chem. Pharm. Bull.* 33, 165–172.
- (111) Richardson, A. C. (1969) Nucleophilic replacement reactions of sulphonates. *CARBOHYDRATE RESEARCH* 10, 395–402.
- (112) Hale, K. J., Hough, L., Manaviazar, S., and Calabrese, A. (2014) An update of the rules for pyranoside sulfonate displacement. *Org. Lett.* 16, 4838–4841.

- (113) Aggarwal, B. B., Sundaram, C., Malani, N., and Ichikawa, H. (2007) CURCUMIN: THE INDIAN SOLID GOLD, in *The Molecular Targets and Therapeutic Uses of Curcumin in Health and Disease*, pp 1–75. Springer, Boston, MA, Boston, MA.
- (114) Strimpakos, A. S., and Sharma, R. A. (2008) Curcumin: Preventive and Therapeutic Properties in Laboratory Studies and Clinical Trials. <http://www.liebertpub.com/ars> 10, 511–546.
- (115) Gupta, S. C., Kim, J. H., Prasad, S., and Aggarwal, B. B. (2010) Regulation of survival, proliferation, invasion, angiogenesis, and metastasis of tumor cells through modulation of inflammatory pathways by nutraceuticals. *Cancer and Metastasis Reviews* 29, 405–434.
- (116) Negi, P. S., Jayaprakasha, G. K., Jagan Mohan Rao, L., and Sakariah, K. K. (1999) Antibacterial Activity of Turmeric Oil: A Byproduct from Curcumin Manufacture. *J. Agric. Food Chem.* 47, 4297–4300.
- (117) Prasad, S., Tyagi, A. K., and Aggarwal, B. B. (2014) Recent Developments in Delivery, Bioavailability, Absorption and Metabolism of Curcumin: the Golden Pigment from Golden Spice. *Cancer Res Treat* 46, 2–18.
- (118) Chandra, V., Pandav, R., Dodge, H. H., Johnston, J. M., Belle, S. H., DeKosky, S. T., and Ganguli, M. (2001) Incidence of Alzheimer's disease in a rural community in India The Indo-US Study. *Neurology* 57, 985–989.
- (119) Ng, T.-P., Chiam, P.-C., Lee, T., Chua, H.-C., Lim, L., and Kua, E.-H. (2006) Curry Consumption and Cognitive Function in the Elderly. *Am J Epidemiol* 164, 898–906.
- (120) Yang, F., Lim, G. P., Begum, A. N., Ubeda, O. J., Simmons, M. R., Ambegaokar, S. S., Chen, P. P., Kaye, R., Glabe, C. G., Frautschy, S. A., and Cole, G. M. (2005) Curcumin Inhibits Formation of Amyloid β Oligomers and Fibrils, Binds Plaques, and Reduces Amyloid in Vivo. *Journal of Biological Chemistry* 280, 5892–5901.
- (121) Narlawar, R., Pickhardt, M., Leuchtenberger, S., Baumann, K., Krause, S., Dyrks, T., Weggen, S., Mandelkow, E., and Schmidt, B. (2008) Curcumin-derived pyrazoles and isoxazoles: Swiss army knives or blunt tools for Alzheimer's disease? *ChemMedChem* 3, 165–172.
- (122) Caesar, I., Jonson, M., Nilsson, K. P. R., Thor, S., and Hammarström, P. (2012) Curcumin promotes A-beta fibrillation and reduces neurotoxicity in transgenic *Drosophila*. *PLoS ONE* 7, e31424.
- (123) Ahsan, N., Mishra, S., Jain, M. K., Surolia, A., and Gupta, S. (2015) Curcumin Pyrazole and its derivative (N-(3-Nitrophenyl)pyrazole) Curcumin inhibit aggregation, disrupt fibrils and modulate toxicity of Wild type and Mutant α -Synuclein. *Scientific Reports* 5, 9862.
- (124) Qureshi, G. A., and Parvez, S. H. (2007) Oxidative Stress and Neurodegenerative Disorders. Elsevier.
- (125) Baum, L., and Ng, A. (2004) Curcumin interaction with copper and iron suggests one possible mechanism of action in Alzheimer's disease animal models. *Journal of Alzheimer's Disease* 6, 367–377.

- (126) Mohorko, N., Repovs, G., Popović, M., Kovacs, G. G., and Bresjanac, M. (2010) Curcumin labeling of neuronal fibrillar tau inclusions in human brain samples. *J. Neuropathol. Exp. Neurol.* 69, 405–414.
- (127) Howie, A. J. (2015) Diagnosis of Amyloid Using Congo Red, in *Amyloid and Related Disorders*, pp 197–211. Humana Press, Cham, Cham.
- (128) Styren, S. D., Hamilton, R. L., Styren, G. C., and Klunk, W. E. (2000) X-34, a fluorescent derivative of Congo red: a novel histochemical stain for Alzheimer's disease pathology. *J. Histochem. Cytochem.* 48, 1223–1232.
- (129) Tu, P., Fu, H., and Cui, M. (2015) Compounds for imaging amyloid- β deposits in an Alzheimer's brain: a patent review. *Expert Opinion on Therapeutic Patents* 25, 413–423.
- (130) Pabon, H. J. J. (1964) A synthesis of curcumin and related compounds. *Recueil des Travaux Chimiques des Pays-Bas* 83, 379–386.
- (131) Eun Kyoung Ryu, Yearn Seong Choe, Kyung-Han Lee, Yong Choi, A., and Kim, B.-T. (2006) Curcumin and Dehydrozingerone Derivatives: Synthesis, Radiolabeling, and Evaluation for β -Amyloid Plaque Imaging†. *Journal of medicinal*
- (132) Rokka, J., Snellman, A., Zona, C., La Ferla, B., Nicotra, F., Salmons, M., Forloni, G., Haaparanta-Solin, M., Rinne, J. O., and Solin, O. (2014) Synthesis and evaluation of a (18)F-curcumin derivate for β -amyloid plaque imaging. *Bioorganic & Medicinal Chemistry* 22, 2753–2762.
- (133) Rokka, J., Snellman, A., Kaasalainen, M., Salonen, J., Zona, C., La Ferla, B., Nicotra, F., Re, F., Masserini, M., Forsback, S., Lopez-Picon, F., Rinne, J. O., Haaparanta-Solin, M., and Solin, O. (2016) 18 F-labeling syntheses and preclinical evaluation of functionalized nanoliposomes for Alzheimer's disease. *European Journal of Pharmaceutical Sciences* 88, 257–266.
- (134) Dolai, S., Shi, W., Corbo, C., Sun, C., Averick, S., Obeysekera, D., Farid, M., Alonso, A., Banerjee, P., and Raja, K. (2011) “Clicked” sugar-curcumin conjugate: modulator of amyloid- β and tau peptide aggregation at ultralow concentrations. *ACS Chem. Neurosci.* 2, 694–699.
- (135) Chernyak, A. Y., Sharma, G. V. M., Kononov, L. O., Krishna, P. R., Levinsky, A. B., Kochetkov, N. K., and Rama Rao, A. V. (1992) 2-Azidoethyl glycosides: glycosides potentially useful for the preparation of neoglycoconjugates. *CARBOHYDRATE RESEARCH* 223, 303–309.
- (136) Joosten, J. A. F., Tholen, N. T. H., Ait El Maate, F., Brouwer, A. J., van Esse, G. W., Rijkers, D. T. S., Liskamp, R. M. J., and Pieters, R. J. (2005) High-Yielding Microwave-Assisted Synthesis of Triazole-Linked Glycodendrimers by Copper-Catalyzed [3+2] Cycloaddition. *Eur. J. Org. Chem.* 2005, 3182–3185.
- (137) Füchtner, F., Steinbach, J., Mäding, P., and Johannsen, B. (1996) Basic hydrolysis of in the preparation of 2-[18F]fluoro-2-deoxy-d-glucose. *Applied Radiation and Isotopes* 47, 61–66.
- (138) Meyer, G. J., Matzke, K. H., Hamacher, K., Füchtner, F., Steinbach, J., Notohamiprodjo, G., and Zijlstra, S. (1999) The stability of 2-[18F]fluoro-deoxy-d-glucose towards epimerisation under alkaline conditions. *Applied Radiation and Isotopes* 51, 37–41.

- (139) Klunk, W. E., Engler, H., Nordberg, A., Wang, Y., Blomqvist, G., Holt, D. P., Bergström, M., Savitcheva, I., Huang, G.-F., Estrada, S., Ausén, B., Debnath, M. L., Barletta, J., Price, J. C., Sandell, J., Lopresti, B. J., Wall, A., Koivisto, P., Antoni, G., Mathis, C. A., and Långström, B. (2004) Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. *Annals of Neurology* 55, 306–319.
- (140) Vandenberghe, R., Van Laere, K., Ivanoiu, A., Salmon, E., Bastin, C., Triau, E., Hasselbalch, S., Law, I., Andersen, A., Korner, A., Minthon, L., Garraux, G., Nelissen, N., Bormans, G., Buckley, C., Owenius, R., Thurfjell, L., Farrar, G., and Brooks, D. J. (2010) 18F-flutemetamol amyloid imaging in Alzheimer disease and mild cognitive impairment: A phase 2 trial. *Annals of Neurology* 68, 319–329.
- (141) Rowe, C. C., Pejoska, S., Mulligan, R. S., Jones, G., Chan, J. G., Svensson, S., Cselenyi, Z., Masters, C. L., and Villemagne, V. L. (2013) Head-to-Head Comparison of 11C-PiB and 18F-AZD4694 (NAV4694) for β -Amyloid Imaging in Aging and Dementia. *Journal of Nuclear Medicine* 54, 880–886.
- (142) Ono, M., Wilson, A., Nobrega, J., Westaway, D., Verhoeff, P., Zhuang, Z.-P., Kung, M.-P., and Kung, H. F. (2003) 11C-labeled stilbene derivatives as Abeta-aggregate-specific PET imaging agents for Alzheimer's disease. *Nuclear Medicine and Biology* 30, 565–571.
- (143) Wong, D. F., Rosenberg, P. B., Zhou, Y., Kumar, A., Raymont, V., Ravert, H. T., Dannals, R. F., Nandi, A., Brasic, J. R., Ye, W., Hilton, J., Lyketsos, C., Kung, H. F., Joshi, A. D., Skovronsky, D. M., and Pontecorvo, M. J. (2010) In Vivo Imaging of Amyloid Deposition in Alzheimer Disease Using the Radioligand 18F-AV-45 (Flbetapir F 18). *Journal of Nuclear Medicine* 51, 913–920.
- (144) Kung, H. F., Choi, S. R., Qu, W., Zhang, W., and Skovronsky, D. (2009) 18F Stilbenes and Styrylpyridines for PET Imaging of A β Plaques in Alzheimer's Disease: A Miniperspective. *J. Med. Chem.* 53, 933–941.
- (145) Åslund, A., Sigurdson, C. J., Klingstedt, T., Grathwohl, S., Bolmont, T., Dickstein, D. L., Glimsdal, E., Prokop, S., Lindgren, M., Konradsson, P., Holtzman, D. M., Hof, P. R., Heppner, F. L., Gandy, S., Jucker, M., Aguzzi, A., Hammarström, P., and Nilsson, K. P. R. (2009) Novel Pentameric Thiophene Derivatives for in Vitro and in Vivo Optical Imaging of a Plethora of Protein Aggregates in Cerebral Amyloidoses. *ACS Chem. Biol.* 4, 673–684.
- (146) Klingstedt, T., Åslund, A., Simon, R. A., Johansson, L. B. G., Mason, J. J., Nyström, S., Hammarström, P., and Nilsson, K. P. R. (2011) Synthesis of a library of oligothiophenes and their utilization as fluorescent ligands for spectral assignment of protein aggregates. *Org. Biomol. Chem.* 9, 8356–15.
- (147) Klingstedt, T., Shirani, H., Mahler, J., Wegenast Braun, B. M., Nyström, S., Goedert, M., Jucker, M., and Nilsson, K. P. R. (2015) Distinct Spacing Between Anionic Groups: An Essential Chemical Determinant for Achieving Thiophene-Based Ligands to Distinguish β -Amyloid or Tau Polymorphic Aggregates. *Chem. Eur. J.* 21, 9072–9082.
- (148) Magnusson, K., Simon, R., Sjölander, D., Sigurdson, C. J., Hammarström, P., and Nilsson, K. P. R. (2014) Multimodal fluorescence microscopy of prion strain specific PrP deposits stained by thiophene-based amyloid ligands. *Prion* 8, 319–329.
- (149) Herrmann, U. S., Schütz, A. K., Shirani, H., Huang, D., Saban, D., Nuvolone, M., Li, B., Ballmer, B., Åslund, A. K. O., Mason, J. J., Rushing, E., Budka, H.,

- Nyström, S., Hammarström, P., Böckmann, A., Caflisch, A., Meier, B. H., Nilsson, K. P. R., Hornemann, S., and Aguzzi, A. (2015) Structure-based drug design identifies polythiophenes as antiprion compounds. *Science Translational Medicine* 7, 299ra123–299ra123.
- (150) Klingstedt, T., Shirani, H., Åslund, K. O. A., Cairns, N. J., Sigurdson, C. J., Goedert, M., and Nilsson, K. P. R. (2013) The Structural Basis for Optimal Performance of Oligothiophene-Based Fluorescent Amyloid Ligands: Conformational Flexibility is Essential for Spectral Assignment of a Diversity of Protein Aggregates. *Chem. Eur. J.* 19, 10179–10192.
- (151) Antoni, G., Lubberink, M., Estrada, S., Axelsson, J., Carlson, K., Lindsjö, L., Kero, T., Långström, B., Granstam, S. O., Rosengren, S., Vedin, O., Wassberg, C., Wikström, G., Westermark, P., and Sorensen, J. (2013) In Vivo Visualization of Amyloid Deposits in the Heart with 11C-PIB and PET. *Journal of Nuclear Medicine* 54, 213–220.
- (152) Nordeman, P., Johansson, L. B. G., Bäck, M., Estrada, S., Hall, H., Sjölander, D., Westermark, G. T., Westermark, P., Nilsson, L., Hammarström, P., Nilsson, K. P. R., and Antoni, G. (2016) 11C and 18F Radiolabeling of Tetra- and Pentathiophenes as PET-Ligands for Amyloid Protein Aggregates. *ACS Med. Chem. Lett.* 7, 368–373.
- (153) Allison, R. R., and Sibata, C. H. (2010) Oncologic photodynamic therapy photosensitizers: A clinical review. *Photodiagnosis and Photodynamic Therapy* 7, 61–75.
- (154) Waghorn, P. A. (2013) Radiolabelled porphyrins in nuclear medicine. *J. Label Compd. Radiopharm* (Dilworth, J. R., and Pascu, S. I., Eds.) 57, 304–309.
- (155) Pisarek, S., Maximova, K., and Gryko, D. (2014) Strategies toward the synthesis of amphiphilic porphyrins. *Tetrahedron* 70, 6685–6715.
- (156) Kushwaha, D., and Tiwari, V. K. (2013) Click Chemistry Inspired Synthesis of Glycoporphyrin Dendrimers. *J. Org. Chem.* 78, 8184–8190.
- (157) Garcia, G., Naud-Martin, D., Carrez, D., Croisy, A., and Maillard, P. (2011) Microwave-mediated 'click-chemistry' synthesis of glycoporphyrin derivatives and in vitro photocytotoxicity for application in photodynamic therapy. *Tetrahedron* 67, 4924–4932.
- (158) Mukosera, G. T., Adams, T. P., Rothbarth, R. F., Langat, H., Akanda, S., Barkley, R. G., Dolewski, R. D., Ruppel, J. V., and Snyder, N. L. (2015) Synthesis of glycosylated zinc (II) 5,15-diphenylporphyrin and zinc (II) 5,10,15,20-tetraphenylporphyrin analogs using Cu-catalyzed azide-alkyne 1,3-dipolar cycloaddition reactions. *Tetrahedron Letters* 56, 73–77.
- (159) Chen, H., Viel, S., Ziarelli, F., and Peng, L. (2013) 19F NMR: a valuable tool for studying biological events. *Chem. Soc. Rev.* 42, 7971–13.
- (160) Ruiz-Cabello, J., Barnett, B. P., Bottomley, P. A., and Bulte, J. W. M. (2010) Fluorine (19F) MRS and MRI in biomedicine. *NMR Biomed.* 24, 114–129.
- (161) Boutureira, O., D'Hooge, F., Fernández-González, M., Bernardes, G. J. L., Sánchez-Navarro, M., Koeppe, J. R., and Davis, B. G. (2009) Fluoroglycoproteins: ready chemical site-selective incorporation of fluorosugars into proteins. *Chem. Commun.* 46, 8142–4.

- (162) Salvadó, M., Amgarten, B., Castellón, S., Bernardes, G. J. L., and Boutureira, O. (2015) Synthesis of Fluorosugar Reagents for the Construction of Well-Defined Fluoroglycoproteins. *Org. Lett.* 17, 2836–2839.
- (163) Larda, S. T., Simonetti, K., Al-Abdul-Wahid, M. S., Sharpe, S., and Prosser, R. S. (2013) Dynamic Equilibria between Monomeric and Oligomeric Misfolded States of the Mammalian Prion Protein Measured by 19F NMR. *J. Am. Chem. Soc.* 135, 10533–10541.
- (164) Suzuki, Y., Brender, J. R., Soper, M. T., Krishnamoorthy, J., Zhou, Y., Ruotolo, B. T., Kotov, N. A., Ramamoorthy, A., and Marsh, E. N. G. (2013) Resolution of Oligomeric Species during the Aggregation of A β 1–40 Using 19F NMR. *Biochemistry* 52, 1903–1912.
- (165) Marsh, E. N. G., and Suzuki, Y. (2014) Using 19F NMR to Probe Biological Interactions of Proteins and Peptides. *ACS Chem. Biol.* 9, 1242–1250.
- (166) Kavali, R. R., Chul Lee, B., Seok Moon, B., Dae Yang, S., Soo Chun, K., Woon Choi, C., Lee, C.-H., and Yoon Chi, D. (2005) Efficient methods for the synthesis of 5-(4-[18F]fluorophenyl)-10,15,20-tris(3-methoxyphenyl)porphyrin as a potential imaging agent for tumor. *J. Label Compd. Radiopharm* 48, 749–758.
- (167) Entract, G. M., Bryden, F., Domarkas, J., Savoie, H., Allott, L., Archibald, S. J., Cawthorne, C., and Boyle, R. W. (2015) Development of PDT/PET Theranostics: Synthesis and Biological Evaluation of an 18F-Radiolabeled Water-Soluble Porphyrin. *Mol. Pharmaceutics* 12, 4414–4423.
- (168) Wu, X.-A., Ying, P., Liu, J.-Y., Shen, H.-S., Chen, Y., and He, L. (2009) Lithium Chloride-Assisted Selective Hydrolysis of Methyl Esters Under Microwave Irradiation. *Synthetic Communications* 39, 3459–3470.
- (169) Nakabayashi, S., Warren, C. D., and Jeanloz, R. W. (1986) A new procedure for the preparation of oligosaccharide oxazolines. *CARBOHYDRATE RESEARCH* 150, c7–c10.
- (170) Yohino, T., Sato, K.-I., Wanme, F., Takai, I., and Ishido, Y. (1992) Efficient catalysis by pyridinium sulfonate in glycosylation involving an oxazoline intermediate derived from per-O-acetyl-N-acetylglucosamine and N,N'-diacetylchitobiose. *Glycoconjugate J* 9, 287–291.
- (171) Kageshita, T., Kashio, Y., Yamauchi, A., Seki, M., Abedin, M. J., Nishi, N., Shoji, H., Nakamura, T., Ono, T., and Hirashima, M. (2002) Possible role of galectin-9 in cell aggregation and apoptosis of human melanoma cell lines and its clinical significance. *International Journal of Cancer* 99, 809–816.
- (172) Jadhav, S., Yim, C.-B., Rajander, J., Grönroos, T. J., Solin, O., and Virta, P. (2016) Solid-Supported Porphyrins Useful for the Synthesis of Conjugates with Oligomeric Biomolecules. *Bioconjugate Chem.* 27, 1023–1029.
- (173) Prapunpoj, P., and Leelawatwattana, L. (2009) Evolutionary changes to transthyretin: structure-function relationships. *FEBS J.* 276, 5330–5341.
- (174) Schwarzman, A. L., Gregori, L., Vitek, M. P., Lyubski, S., Strittmatter, W. J., Enghilde, J. J., Bhasin, R., Silverman, J., Weisgraber, K. H., and Coyle, P. K. (1994) Transthyretin sequesters amyloid beta protein and prevents amyloid formation. *Proceedings of the National Academy of Sciences* 91, 8368–8372.

- (175) Li, X., Zhang, X., Ladiwala, A. R. A., Du, D., Yadav, J. K., Tessier, P. M., Wright, P. E., Kelly, J. W., and Buxbaum, J. N. (2013) Mechanisms of Transthyretin Inhibition of β -Amyloid Aggregation In Vitro. *J. Neurosci.* 33, 19423–19433.
- (176) Quintas, A., Vaz, D. C., Cardoso, I., Saraiva, M. J., and Brito, R. M. (2001) Tetramer dissociation and monomer partial unfolding precedes protofibril formation in amyloidogenic transthyretin variants. *Journal of Biological Chemistry* 276, 27207–27213.
- (177) Choi, S., Reixach, N., Connelly, S., Johnson, S. M., Wilson, I. A., and Kelly, J. W. (2010) A Substructure Combination Strategy To Create Potent and Selective Transthyretin Kinetic Stabilizers That Prevent Amyloidogenesis and Cytotoxicity. *J. Am. Chem. Soc.* 132, 1359–1370.
- (178) Klabunde, T., Petrassi, H. M., Oza, V. B., Raman, P., Kelly, J. W., and Sacchettini, J. C. (2000) Rational design of potent human transthyretin amyloid disease inhibitors. *Nat. Struct. Biol.* 7, 312–321.
- (179) Bulawa, C. E., Connelly, S., Devit, M., Wang, L., Weigel, C., Fleming, J. A., Packman, J., Powers, E. T., Wiseman, R. L., Foss, T. R., Wilson, I. A., Kelly, J. W., and Labaudinière, R. (2012) Tafamidis, a potent and selective transthyretin kinetic stabilizer that inhibits the amyloid cascade. *Proc. Natl. Acad. Sci. U.S.A.* 109, 9629–9634.
- (180) Hammarstrom, P., Jiang, X., Hurshman, A. R., Powers, E. T., and Kelly, J. W. (2002) Sequence-dependent denaturation energetics: A major determinant in amyloid disease diversity. *Proceedings of the National Academy of Sciences* 99, 16427–16432.
- (181) Dorbala, S., Vangala, D., Semer, J., Strader, C., Bruyere, J. R., Di Carli, M. F., Moore, S. C., and Falk, R. H. (2014) Imaging cardiac amyloidosis: a pilot study using 18F-florbetapir positron emission tomography. *European Journal of Nuclear Medicine and Molecular Imaging* 41, 1652–1662.
- (182) Furukawa, K., Ikeda, S.-I., Okamura, N., Tashiro, M., Tomita, N., Furumoto, S., Iwata, R., Yanai, K., Kudo, Y., and Arai, H. (2012) Cardiac positron-emission tomography images with an amyloid-specific tracer in familial transthyretin-related systemic amyloidosis. *Circulation* 125, 556–557.
- (183) Bansal, Y., and Silakari, O. (2012) The therapeutic journey of benzimidazoles: a review. *Bioorganic & Medicinal Chemistry* 20, 6208–6236.
- (184) Wang, Z. (2010) Horner-Wadsworth-Emmons Olefination. *Comprehensive Organic Name Reactions and Reagents*. John Wiley & Sons, Inc., Hoboken, NJ, USA.
- (185) Lee, I., Choe, Y. S., Choi, J. Y., Lee, K.-H., and Kim, B.-T. (2012) Synthesis and Evaluation of 18F-Labeled Styryltrialazole and Resveratrol Derivatives for β -Amyloid Plaque Imaging. *J. Med. Chem.* 55, 883–892.
- (186) Kiddle, J. J., and Gurley, A. F. (2000) MICROWAVE IRRADIATION IN ORGANOPHOSPHORUS CHEMISTRY 1: THE MICHAELIS-ARBUZOV REACTION. *Phosphorus, Sulfur, and Silicon and the Related Elements* 160, 195–205.

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