Department of Physics, Chemistry and Biology

Bachelor's Thesis

Synthesis of azidoethyl 3,4,6-tri-O-acetyl-α–D-mannopyranoside for future bioconjugation in PET studies

Daniel Willén

2013-04-02 – 2013-06-05

LITH-IFM-x-EX--13/ 2742--SE

Linköping University
INSTITUTE OF TECHNOLOGY

Linköping University Department of Physics, Chemistry and Biology
581 83 Linköping

Department of Physics, Chemistry and Biology

Synthesis of azidoethyl 3,4,6-tri-O-acetyl-α–D-mannopyranoside for future bioconjugation in PET studies

Daniel Willén

Thesis work done at Department of Physics, Chemistry and Biology

2013-04-02 – 2013-06-05

Supervisor
Marcus Bäck
Timmy Fyrner

Examiner
Peter Konradsson

Linköping University
INSTITUTE OF TECHNOLOGY

Linköping University Department of Physics, Chemistry and Biology
581 83 Linköping

Abstract

Positron emission tomography is a powerful imaging method capable of diagnosing and studying diseases at atomic levels. The following thesis involves the synthesis of a *2*-deoxy-*2*-[^{18}F]fluoro-D-glucose derivative able to participate in click chemisty conjugation. The synthesis involves six steps with D-mannopyranose as starting material; regioselective acetylation and protection of the hydroxyl group on C-2 of the monosaccharide, glycosylation with 2-azidoethanol, removal of the selective protecting group and substitution of the hydroxyl group on C-2 with fluorine. The potential to conjugate the glycoside to other biomolecules and obtain PET data might have uses in diagnostical and medicinal chemistry.

Contents

1 Introduction

The visualization of intra- and extracellular processes *in vivo* is a very powerful and versatile way to diagnose and study diseases all the way down to the atomic level. Several techniques utilizing multiple physical phenomenons has been developed for different purposes, some examples would be magnetic resonance, ultrasound, and nuclear imaging. These methods have their own strengths and weaknesses and can be more or less suitable depending on the situation. The most sensitive method however today is the radionuclide-based positron emission tomography or PET. The principle of PET is that the molecules used as imaging agents have been labeled with a radioactive nuclide that emits a positron when it decays. Some commonly used radionuclide are ^{18}F,^{11}C,^{13}N, and^{15}O . The positron is the antiparticle to the electron, so when the positron collides with an electron they annihilate each other. This creates two γ-rays which are emitted in a relative angle of 180° from each other, which creates a line of emission. This line of emission is detected by pairs of detectors placed in the space around the object of interest, and the approximate position of annihilation can be calculated. In modern PET cameras, this data can be collected in 2D or 3D and visualized on a computer for analysis. The advantages of PET are that the technique measures the radioactivity from the tissue in absolute numbers, and therefore can detect very small amounts of concentration of biomolecules ($\approx 10^{-12}\,M$). Another advantage is that since the technique is based on positron emission, the atomic isotopes used allows the synthesis of radiopharmaceuticals that have almost the same chemical properties as the unlabeled ones.^{18}F is the most common isotope used due to its favorable sterical properties, even though its electron-withdrawing properties might change electronic, lipophilic and biological attributes.[1]

The most successful PET radiopharmaceutical to date is the 2-[^{18}F]-fluoro-deoxy-D-glucose, or [^{18}F]FDG. The molecule started out as a marker for myocardial metabolism as well as for tumor metabolism, but the uses for the molecule has expanded to other areas, neurology being the third main area apart from oncology and cardiology. The [^{18}F]FDG's main advantage is that it is very similar to the main source of energy in the body, glucose. The only difference between [^{18}F]FDG and regular glucose is the fluorinated C-2 carbon of the monosaccharide. When [^{18}F]FDG is administered to the body by intravenous injection the distribution is very similar to the regular glucose pathway. After the subsequent uptake and after the first reaction in the glucose metabolic pathway, the phosphorylation of the $-OH$ on the C-6 by the enzyme hexokinase, the [^{18}F]FDG gets metabolically trapped. Normally the next reaction involves isomerization of the glucose molecule by the enzyme glucose-6-phosphate-isomerase, which requires the $-OH$ on the C2 which the [^{18}F]FDG is missing. Hence, the [^{18}F]FDG will accumulate proportionally to the metabolism of the cell. This allows the study of tumors, since the metabolism of rapidly proliferating cancer tumors is much higher than in normal, functional cells. [2] The possibility to study the metabolism in the brain also gives the ability to diagnose diseases like Alzheimer's disease, where the metabolism of the brain is impaired in important sections.

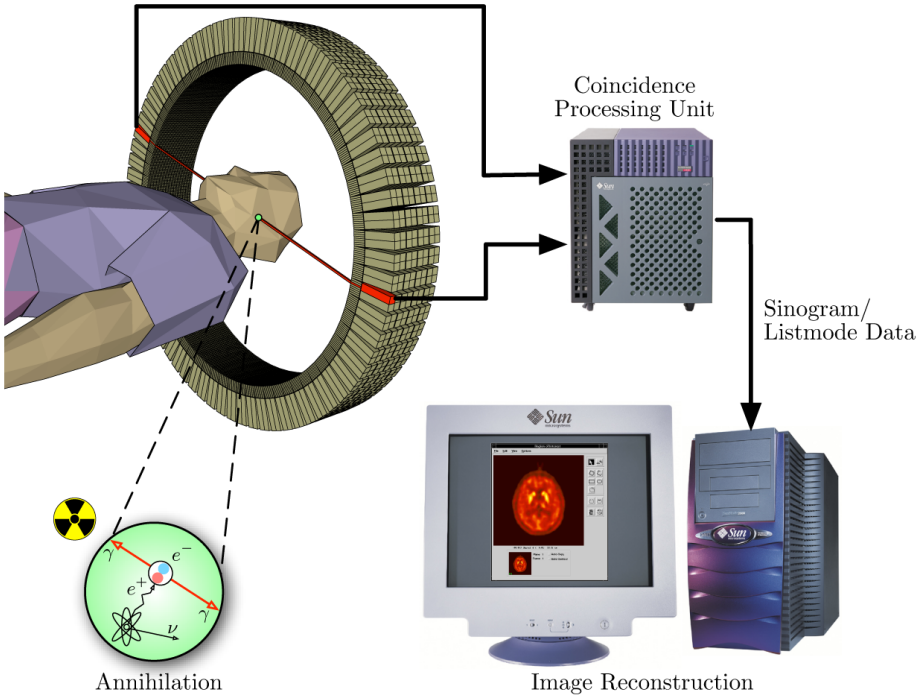
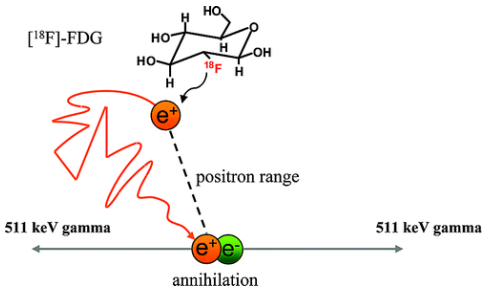
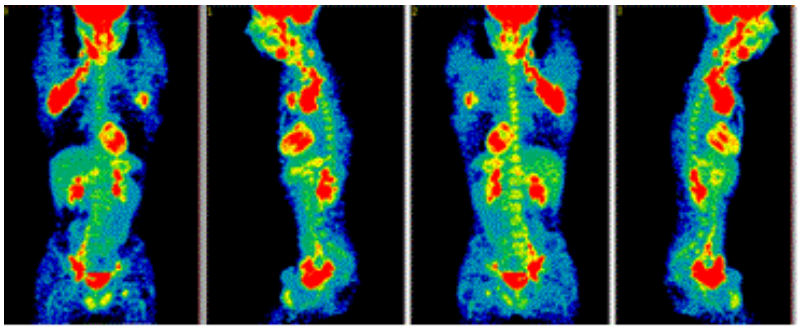


Figure 1: Acquiring of positron emssion data



(a) The mechanism of positron emission



(b) A positron emission tomography scan of the body. Image courtesy of Dr. Jorge Carrasquillo, Nuclear Medicine Department, Clinical Center, National Institutes of Health.

Many molecules are dependent on forming carbon-carbon bonds using carbonyl themed chemistry when creating new species with different structural as well as functional properties. For nature, this is not a major problem; utilizing specific enzymes and adenosine triphosphate to assemble building blocks which later is combined into the complex compounds that is required for different cellular tasks. However, it is very hard to imitate natures approach to creating these molecules with the same amount of precision using carbonyl chemistry. Without the specificity of enzyme catalysis, the reactions with functional groups might be very unspecific and therefore complicate the synthesis and make the desired compound almost impossible to reach trying to use only carbon-carbon bond forming methods. Nature on the other hand, even though the carbon-carbon bonds is not that difficult to create, prefers using carbon-hetero atom bonds creating its most important molecules: polysaccharides, polynucleotides and polypeptides. And thinking of the amount of diversity in structure and function these molecules can have, there is no wonder why large resources are invested to imitate this way of combining different modules into specific targets.

Click chemistry is an concept that was developed in the beginning of the 21th century, and it consists of several reactions which meets a strict criteria. Some of the criteria are for instance that the reaction should have simple conditions, use no solvent alternatively a solvent that is easily removed or very benign (like water) and give very high yields ($> 90\,\%$). Most common of these reactions are the carbon-hetero atom bond forming, but there are carbon-carbon bond forming reactions as well. The reactions utilizes a high thermodynamic driving force which makes them acquire these desired characteristics. One of the most common click chemistry reactions is the *$Cu(I)$-catalyzed Azide-Alkyne Cycloaddition* which fuses an azide moiety together with an terminal alkyne moiety to create 1,2,3-triazoles which is disubstituted in a 1,4 manner. Normally the reaction (which is called a *Huisgen 1,3-dipolar cycloaddition)* requires heating and proceeds slowly, but the addition of $Cu(I)$ as a catalyst rapidly speeds up the reaction and only gives the 1,4 disubstituted adduct.[3]There are many potential uses in medicinal chemistry, bio-imaging and other similar fields due to the simplicity of conjugation and the possibility of creating very specific molecules with little effort.[4]

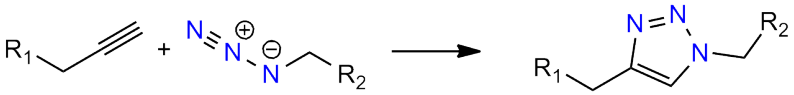


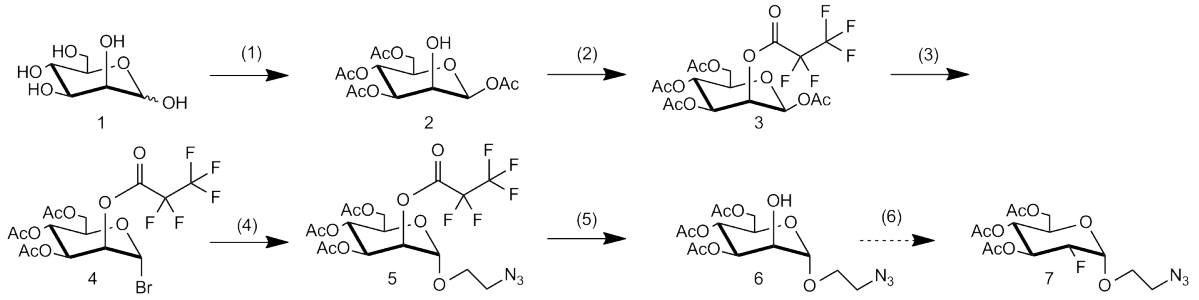
Figure 2: The *Huisgen 1,3-dipolar cycloaddition*

Ambition

The ambition of this thesis is to synthetize a molecule able to participate in click chemistry and be coupled to another biomolecule. Using this conjugated compound, targets of special interest could be analyzed using PET scanning methodology and the data obtained can be used in science related to new drugs, diagnosing diseases and analzying molecular interactions in vivo.

2 Synthesis scheme

The synthesis was divided into several parts following an outline presented by Maschauer et al [5]. with a small modification: the change from 2-bromoetanol to 2-azidoethanol, thereby removing one reaction step.



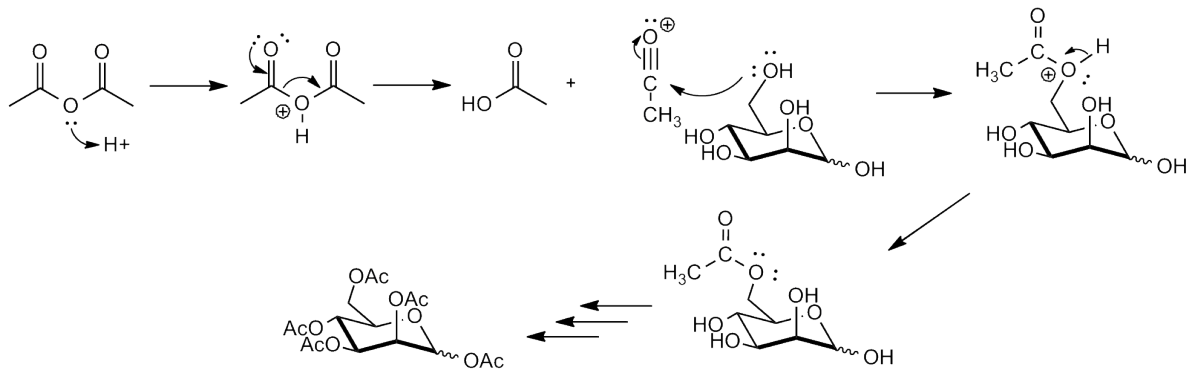
Scheme 1: (1) (i)Ac_2O, $HClO_4(60\%)$,CH_2Cl_2, $0°C$, (ii) PBr_3, CH_2Cl_2, (iii) $NaCO_3$, $0°C$; (2) $PFPA$, Pyr; (3) $HBr - AcOH\,(33\%)$, CH_2Cl_2, $0°C$ to rt;
(4) $2 - Azidoethanol$, $AgOTf$, $0°C$ to rt; (5) $EtOH$,Pyr; (6) (i)Tf_2O, Pyr, CH_2Cl_2, (ii) $TBAF$, THF

3 Reactions

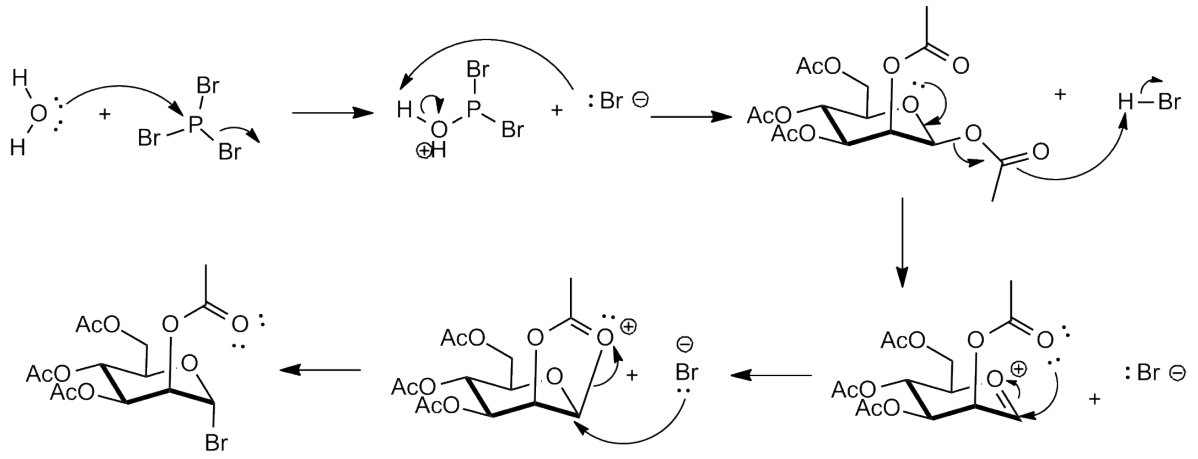
Here follows descriptions and proposed mechanisms of the different reactions involved in the synthesis, following the same order as in the scheme 1.

3.1 Regioselective installation of an anomeric acetate

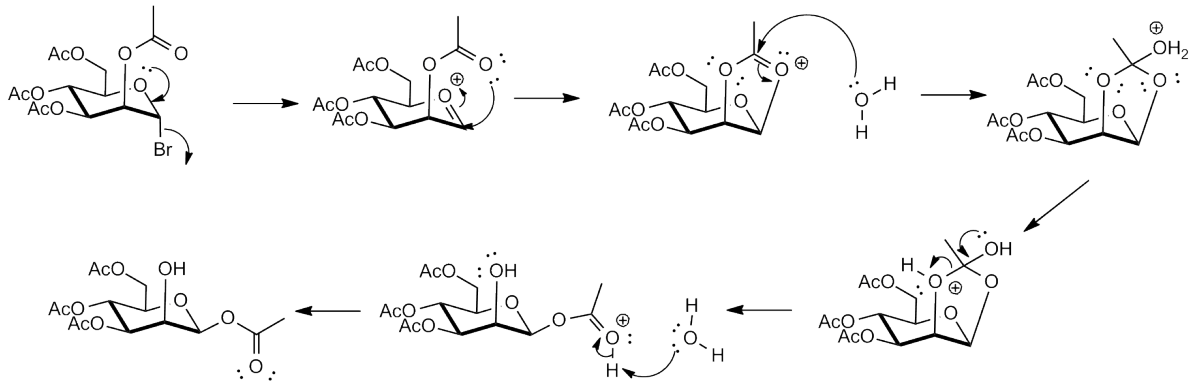
The first reaction is the acetylation of the free hydroxyl groups on the mannose sugar.
This must be performed in a regioselective manner, since the hydroxyl group on the C-2 carbon has to be free in order to proceed with the synthesis. The mechanism for the reaction is divided into three parts: acetylation, bromination and hydrolysis of the transient orthoester intermediate, all performed in a one pot manner. The perchloric acid acts as a catalyst for generating the acylium ion CH_3CO+ which then acts as an electrophile to the nucleophilic hydroxyl groups. Then the addition of the Lewis acid PBr_3 and water leads to the formation of HBr and therefore a substitution at the anomeric carbon of the acetoxy group and resulting in a glycosyl bromide. This is followed by an Sn1 reaction where the endocyclic oxygen expels the bromide leaving group. The acetoxy group at C-2 acts in a participating manner, making a nucleophilic attack on the oxocarbenium ion and creates a 1,2-acetoxonium ion. Subsequent hydrolysis of the orthoester by the presence of acetic acid and water in the reaction mixture gives the free hydroxyl group on the C-2 carbon.[6].



Scheme 2: Proposed mechanism of regiospecific acetylation: acetylation. The reaction is repeated for every hydroxyl group



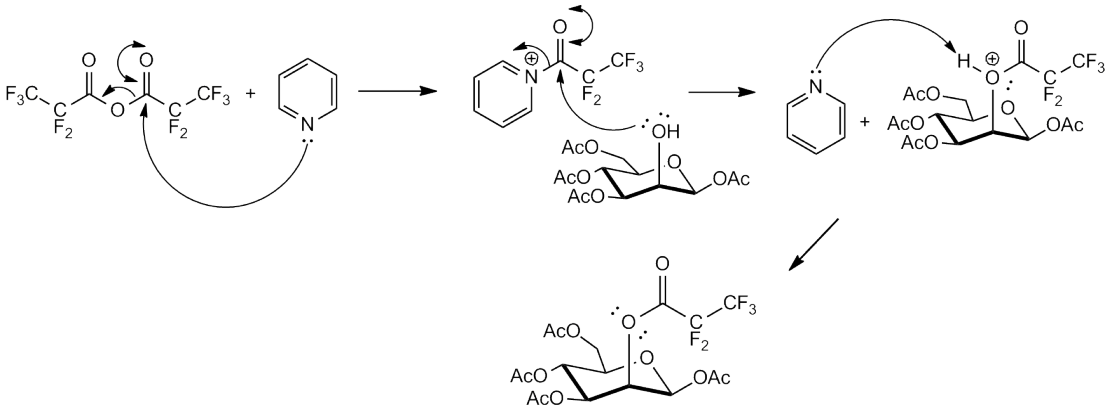
Scheme 3: Proposed mechanism of regiospecific acetylation: bromination



Scheme 4: Proposed mechanism of regiospecific acetylation: acidic opening of the intermediate orthoester

3.2 Pentafluoropropionylation

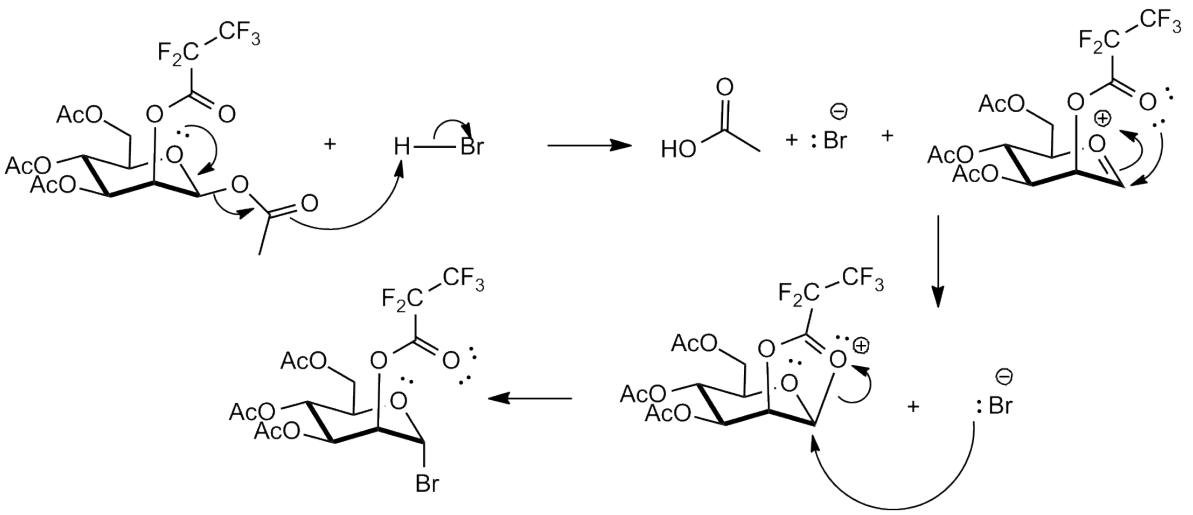
The second reaction is the introduction of a orthogonal protecting group of the C-2 hydroxyl group.
A protecting group that is stable during glycosylation but can be selectively removed in presence of the acetates is critical, hence the pentafluoropropionyl group was chosen. The reaction uses basic pyridine instead of the acidic perchloric acid as the catalyst. A nucleophilic attack by the pyridine on the pentafluoropropionic anhydride creates a tetrahedral intermediate which collapses and expels a pentafluoropropionate anion. This is followed by another nucleophilic attack by the monosaccharide which together with a deprotonization by pyridine gives the protected compound. Under these milder reaction conditions the free C-2 hydroxyl group can be protected without affecting the already protected hydroxyl groups.



Scheme 5: Proposed mechanism of pentafluoropropionylation

3.3 Bromination

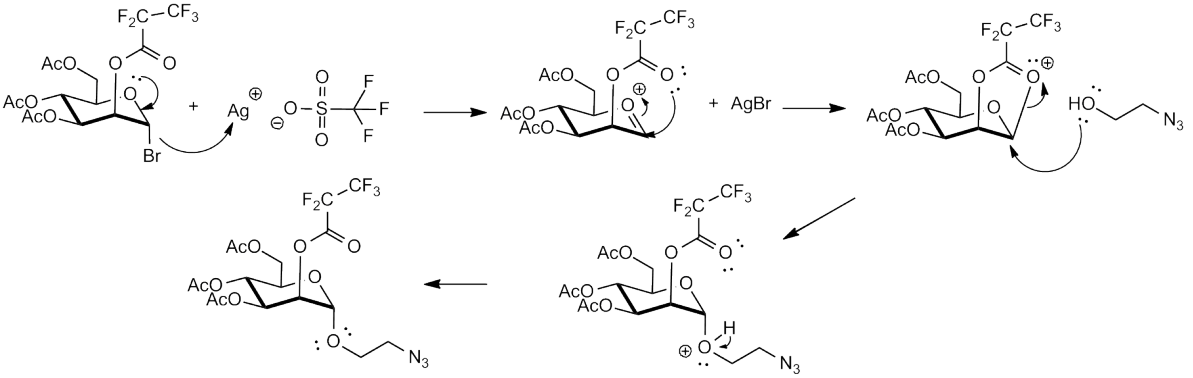
The bromination serves as a mean of creating a glycosyl donor by substituting the acetyl group at the anomeric center to a bromine atom.
The reaction takes place in a similar way as the bromination in the acetylation reaction, only this time with another final product and another way of obtaining the glycosyl bromide. This time it is accomplished by an Sn1 reaction in which HBr protonates the acetyl group, making it a better leaving group. Expulsion of the leaving group is aided by free electrons on the endocyclic oxygen, generating a oxocarbenium cation. The bromide anion then makes a nucleophilic attack on the oxocarbenium ion, completing the mechanism. Only the α anomer is obtained here due to the strong anomeric effect (the effect increases with higher electronegativity on the anomeric position) which as well as the effects of the participating protecting *Pfp* group on C-2.



Scheme 6: Proposed mechanism of bromination

3.4 Glycosylation

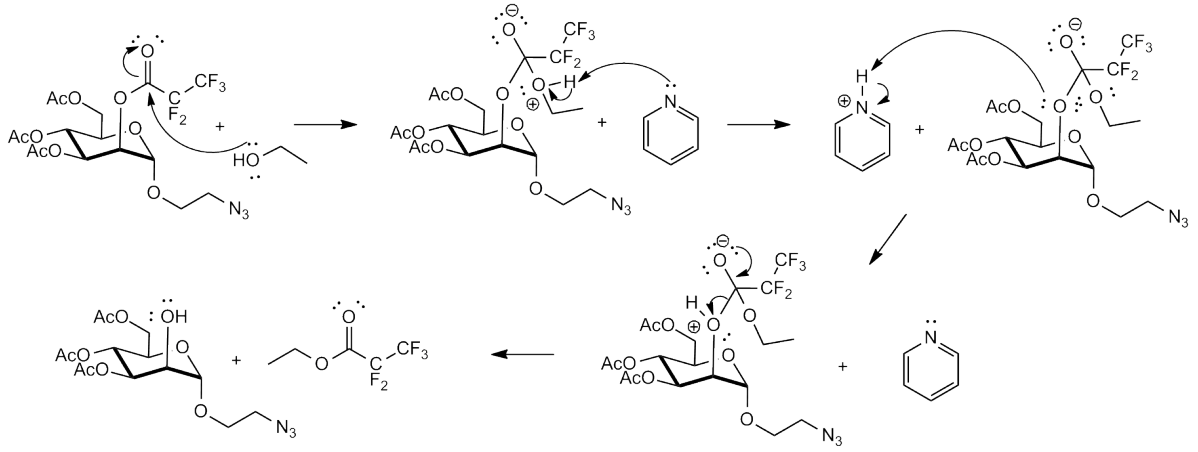
The most crucial reaction of the synthesis is the actual creation of the glycosidic bond. This method is known as the *Koenigs-Knorr* method and involves a glycosyl halide and a heavy metal salt as well as a glycosyl acceptor.[7]The glycosyl donor is activated, in this case the by the $AgOTf$ heavy metal salt. The Ag^{+}ion acts as the activator by forming the insoluble salt $AgBr$ which precipitates and removes the bromine from the saccharide and for this reason driving the formation of the oxocarbenium ion. The alcohol makes a nucleophilic attack and completing the reaction and the formation of the glycosidic bond. Another alcohol deprotonizes the glycosyl to complete the reaction. The trifluoromethanesulfonate anion (OTf) is a poor nucleophile (conjugate base to the triflic acid) and will not compete with the alcohol. The anomeric effect and the neighbouring gourp participation from the *Pfp* group is still involved, promoting only the formation of the α anomer.



Scheme 7: Proposed mechanism of the glycosylation with ethanolazide

3.5 Removal of the pentafluoropropionyl protecting group

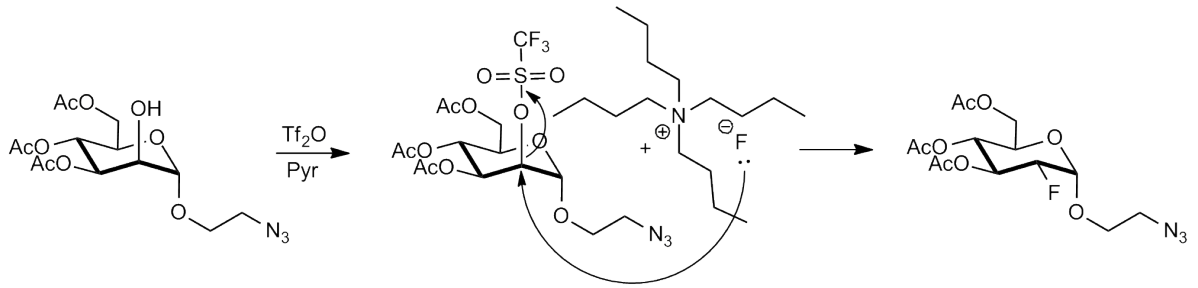
In order to epimerize mannose to glucose, an inversion of the stereochemistry at C-2 must be performed. This requires the removal of the *Pfp* group and substitution of the hydroxyl group with an efficient leaving group. This is performed using an alcohol and pyridine as a catalyst. Ethanol makes a nucleophilic attack on the carbonyl in the *Pfp* protecting group, creating a tetrahedral intermediate. Pyridine then deprotonizes the alcohol and subsequently protonizes the glycoside, making it a better leaving group. The tetrahedral intermediate then collapses and expels the glycoside, completing the reaction.



Scheme 8: Proposed mechanism of deprotection step

3.6 Interconversion of hydroxyl to *OTf* and substitution by fluorine

The conversion of the hydroxyl into the *OTf* functional group on the C-2 is intended to be performed in the same way as the pentafluoropropionylation, also catalyzed by pyridine. Since the *OTf* is a very good leaving group the nucleophilic substitution and hence the conversion from mannose to glucose is expected to proceed easily by an Sn2 reaction. The fluorine ion makes a nucleophilic attack on the C-2 carbon, expelling *OTf* while inverting the stereochemistry and epimerize mannose into glucose.



Scheme 9: Proposed mechanism of fluorine substitution

4 Results and discussion

The whole synthesis was not successfully completed during the time of which this project was performed because there was not enough time after the glycosylation to proceed with the remaining reaction (compound **7** was not synthesized). This was due to a couple of setbacks in the purification by silica column chromatography. The reaction had to be repeated several times due to the disappearance of the product on the TLC monitoring during the chromatography. It was found that the pentafluoropropionate group was deprotected for unknown reasons during the chromatography. These setbacks resulted in that the time consuming bromination reaction was repeated a few times. Moreover, synthesis of more precursors were needed. Removal of the protecting group while performing the purification led to not needing to perform the removal step in the scheme, thus reducing the amount of synthesis steps and compensating the loss of time spent repeating bromination reactions. If the cause of the removal can be identified it may be utilized to make the synthesis more effective by removing the exclusive deprotection step and proceed with the final reaction right away.

There are a few things that are worth mentioning about some of the reactions. Starting with the regioselective acetylation, this was not a very efficient reaction with only a yield of 9.4 %. This might be due to the intermediates and their formation during the one pot operation. Since it was performed as a one pot reaction there was no purification of the intermediate steps during which several undesirable byproducts could have formed and therefore affecting the yield. Especially the orthoester intermediate and its subsequent hydrolysis could be the very limiting factor in the reaction as it appears that the 1,2-acetoxonium ion in comparison with other orthoester intermediates is more labile and less likely to form. [6] Orthoesters are thermodynamically driven to end up in an axial position, so the compound of interest is a byproduct of the reaction. Although, this approach is viable since it enables larger labscale synthesis scale in a one-pot manner from which the compound of interest can be readily crystallized, other ways of performing the acetylation with higher yields might be considered or at least using another reagent for creating the orthoester intermediate.

Bromination and creation of the glycosyl donor behaved quite different from expected. Despite the observations of Maschauer et al [5] the reaction was surprisingly slow and would not be completed even with an excess 822 HBr equivalents greater than the starting material, letting the reaction continue for several days as well as applying heat to the mixture. This strange feature was observed every time the reaction was performed and the extreme inactivation of the sugar is probably due to the *Pfp* protecting group on C-2. Since the group has five fluorine molecules, it probably have a very high electronegative effect on the ring, removing electron density and therefore deactivating the ring and making the Sn1 reaction proceed extremely slow. Since this turned out to be a very ineffective reaction, an alternative route to the glycoside was tested. A method using borontrifluoride etherate ($BF_3 \cdot Ac_2O$) as an activator was tried. Unfortunately the reaction proceeded very slowly and was degraded upon heating. This is also probably is due to the protecting group deactivating the sugar. If this synthesis should be repeated another protecting group might be of interest which must be stable under acidic conditions. This require-

ment of the group is due to the conditions used in the glycosylation reaction that proceeded efficiently.

The final molecule would have really interesting properties being a glucose derivative applicable in PET imaging, but could also be a potential conjugate to molecules with other interesting biological features. This combination could be a very powerful tool in bio-imaging and the research of different molecular processes and events. One possible prospect would be to conjugate the glycoside unto a probe which may bind to amyloidal plaques, enabling monitoring via PET scanning. This might give information about the amount and location of the plaques and the data obtained could facilitate the research for new drugs or diagnoses of various mental diseases for example. This is not limited to brain research; the approach would be viable in other parts of the body as well.

5 Experimentals

5.1 General methods

Reactions were monitored with thin layer chromatography (TLC) carried out on Merck 60 F254 plates and developed with PAA [$EtOH$ (*95* %, *740* mL), H_2SO_4 (conc., *28* mL), $AcOH$ (*100* %, *8.4* mL), *4*-anisaldehyde (*20* mL)]. Flash column chromatography (FC) was carried out on silica gel Merck 60 (*40–63* μm). Proton nuclear magnetic resonance (1H NMR) were recorded on Varian 300 MHz and 500 MHz spectrometers, carbon nuclear magnetic resonance (13C NMR) was recorded on Varian 300 (*75.4* MHz) spectrometer. Chemical shift were assigned with the solvent residual peak as a reference according to Gottlieb et al. [8]

5.2 1,3,4,6-Tetra-*O*-acetyl-β-D-mannopyranose (2)

D-Mannopyranose (*25.0* g, *138.8* mmol) was dissolved in 105 mL of acetic anhydride and cooled on ice. 1 droplet of $HClO_4$ (*60* % aq.) was added to the stirred mixture while temperature was maintained at 0°C. After *40* min, PBr_3 (*20.0* mL, *210.6* mmol) was added dropwise over *10* min and stirred for *40* min. H_2O (*8.7* mL, *8.7* g, *482.9* mmol) was added dropwise followed by the addition of $NaOAc$ (*75.75* g, *923.4* mmol) in H_2O (*400* mL), in a dropwise fashion until the solution turned yellow. The mixture was extracted with CH_2Cl_2 and the organic phase was washed successively with water, $NaHCO_2$ (sat. aq.) and water. The organic phase was dried with $MgSO_4$, filtered and concentrated in vacuo. Recrystallization from 1 L anhydrous ether obtained compound 2 (*4.5* g, *9.4* %, m.p *136º* - *141º* C). ^1H-NMR ($CDCl_3, 300\,Hz$): δ 2.05 (s, 3H, OAc), 2.08 (s, 3H, OAc), 2.02 (s, 3H, OAc), 2.18 (s, 3H, OAc), 3.78 (ddd, 1H, $J_{5,6} = 2.3\,Hz$ $J_{5,6'} = 4.69\,Hz$, H-5),4.13 (dd, 1H, $J_{6,6'} = 12.3\,Hz$, H-6), 4.2 (app dt, 1H, H-2), 4.3 (dd, 1H, H-6'), 5.04 (dd, 1H, $J_{3,4} = 10.0\,Hz$, H-3), 5.4 (app t, 1H, $J_{4,5} = 10.0\,Hz$, H-4), 5.8 (d, 1H, $J_{1,2} = 1.2\,Hz$, H-1); ^{13}C-NMR ($CDCl_3, 75.4\,Hz$): δ 20.8 (CH_3), 20.9 (CH_3), 20.9 (CH_3), 21.0 (CH_3), 62.1, 65.4, 68.6, 73.0, 73.3 (C-6, C-5, C-4, C-3, C-2), 91.8 (C-1), 168.6 (CO),169.6 (CO), 170.2 (CO), 170.8 (CO)

5.3 1,3,4,6-Tetra-*O*-acetyl-2-*O*-pentafluoropropionyl-β-D-mannopyranose (3)

Compound 2 (*1* g, *2.87* mmol) was dissolved in 14 mL CH_2Cl_2. Pyridine (*420* mL, *5.17* mmol) and pentafluoropropionic anhydride (*925* mL, *4.68* mmol) was added at $0°C$ and allowed to reach room temperature. After 1.5 hours the reaction was diluted with $EtOAc$ and poured onto ice. The solution was separated and the organic phase was washed successively with glacial water, 1M HCl (aq.) and twice with brine. The organic phase was dried with $MgSO_4$, filtered and concentrated in vacuo. FC (6 : 1 toluene/EtOAc) gave compound 3 (*0.98* g, *1.98* mmol, *69 %*) as a colorless syrup. ^1H-NMR ($CDCl_3, 300\,Hz$): δ 2.01 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.08 (s, 3H, OAc), 2.10 (s, 3H, OAc), 3.86 (ddd, 1H, $J_{5,6} = 2.4$ $J_{5,6'} = 4.7\,Hz$, H-5), 4.13 (dd, 1H, $J_{6,6'} = 12.33\,Hz$, H-6),4.28 (dd, 1H, H-6'), 5.26 (unresolved dd, 1H, $J_{3,4} = 9.4\,Hz$, H-3), 5.30 (unresolved app t, 1H, $J_{4,5} = 9.4\,Hz$, H-4), 5.60 (dd, 1H, $J_{2,3}$ 2.9 Hz, H-2), 5.97 (d, 1H, $J_{1,2}$ 1.2 Hz, H-1); ^{13}C-NMR ($CDCl_3, 75.4\,Hz$): 20.3 (CH_3), 20.5 (CH_3), 20.6 (CH_3), 21.0 (CH_3) 61.6, 64.9, 70.2, 72.8 73.2 (C-6, C-5, C-4, C-3, C-2), 89.5 (C-1), 168.2 (CO),169.3 (CO), 169.7 (CO), 170.6 (CO)

5.4 Azidoethyl 3,4,6-tri-*O*-acetyl-α-mannopyranoside (6)

Compound 3 (*87* mg, *0.175* mmol) was dissolved in dry CH_2Cl_2(*1.4* mL). HBr (33%) in acetic acid (*8.32* mL) was added in a aliqouts of *0.36* mL and *0.76* mL at $0°C$, allowed to reach room temperature. The solution mixture was diluted with CH_2Cl_2 and washed with $NaHCO_2$ (sat. aq.) and water, dried with $NaSO_4$ (s), filtered and concentrated in vacuo. The crude bromosugar 4 was dissolved in dry CH_2Cl_2 (*2* mL) and cooled on ice, whereupon 2-azidoethanol (*17.3* µL, *0.030* g, *0.35* mmol) was added followed by $AgOTf$ (*47* mg, *0.184* mmol) was added at $0°C$ allowed to reach ambient temperature. After 1.5 h, the reaction was quenched with H_2O, diluted with CH_2Cl_2 and filtered through Celite®. The mixture was washed with H_2O, dried with $NaSO_4$(s) and concentrated in vacuo. FC (15 : 1 toluene/EtOAc → 9 : 1 toluene/EtOAc) afforded compound 6 (*36* mg, *0.096* mmol, *55 %*). ^1H-NMR ($CDCl_3, 500\,Hz$): δ 2.03 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.09 (s, 3H, OAc), $3.36 - 3.5$ (m, 2H, CH_2), $3.62 - 3.72$ (m, 1H,CH_2), $3.83 - 3.92$ (m, 1H, CH_2), 4.25 (ddd, 1H, $J_{5,6} = 2.4\,Hz$ $J_{5,6'} = 5.0\,Hz$, H-5), 4.09 (unresolved dd, 1H, $J_{2,3} = 3.4\,Hz$, H-2), 4.12 (unresolved dd, 1H, $J_{6,6'} = 12.2\,Hz$, H-6), 4.29 (dd, 1H, H-6'), 4.92 (d, 1H, $J_{1,2} = 1.5\,Hz$, H-1), 5.26 (dd, 1H, $J_{3,4} = 9.9\,Hz$, H-3), 5.33 (app t, 1H, $J_{4,5} = 9.9\,Hz$, H-4)

Acknowledgements

The author would like to acknowledge everyone who has participated in this project.
A special thanks to the following persons:

Timmy Fyrner and **Marcus Bäck** for excellent supervision, guidance and very pleasant company.

Peter Konradsson and **Stefan Svensson** for the opportunity to perform the project.

Mathias, Mattias, Jeff and others for help when needed and providing delightful atmosphere in the laboratory.

Ulrika Linderhed for interesting discussions, visits to my lab and for being you.

References

[1] S. M. Ametamey, M. Honer, and P. A. Schubiger. Molecular imaging with pet. *Chemical reviews*, 108(5):1501–1516, 2008. Cited By (since 1996): 190.

[2] B. Beuthien-Baumann, K. Hamacher, F. Oberdorfer, and J. Steinbach. Preparation of fluorine-18 labelled sugars and derivatives and their application as tracer for positron-emission-tomography. *Carbohydrate research*, 327(1-2):107–118, 2000. Cited By (since 1996):27.

[3] Hartmuth C. Kolb, M. G. Finn, and K. Barry Sharpless. Click chemistry: Diverse chemical function from a few good reactions. *Angewandte Chemie International Edition*, 40(11):2004–2021, 2001.

[4] K. Nwe and M. W. Brechbiel. Growing applications of "click chemistry" for bioconjugation in contemporary biomedical research. *Cancer Biotherapy and Radiopharmaceuticals*, 24(3):289–302, 2009. Cited By (since 1996):43.

[5] S. Maschauer and O. Prante. A series of 2-o-trifluoromethylsulfonyl-d-mannopyranosides as precursors for concomitant 18f-labeling and glycosylation by click chemistry, 2009. Cited By (since 1996):18.

[6] Tatsushi Toyokuni, J. S. Dileep Kumar, Paula Gunawan, Ernest S. Basarah, Jie Liu, Jorge R. Barrio, and Nagichettiar Satyamurthy. Practical and reliable synthesis of 1,3,4,6-tetra-o-acetyl-2-o-trifluoromethanesulfonyl-β-d-mannopyranose, a precursor of 2-deoxy-2-[18f]fluoro-d-glucose (fdg). *Molecular Imaging & Biology*, 6(5):324–330, 0 2004.

[7] Wilhelm Koenigs and Eduard Knorr. Ueber einige derivate des traubenzuckers und der galactose. *Berichte der deutschen chemischen Gesellschaft*, 34(1):957–981, 1901.

[8] G. R. Fulmer, A. J. M. Miller, N. H. Sherden, H. E. Gottlieb, A. Nudelman, B. M. Stoltz, J. E. Bercaw, and K. I. Goldberg. Nmr chemical shifts of trace impurities: Common laboratory solvents, organics, and gases in deuterated solvents relevant to the organometallic chemist. *Organometallics*, 29(9):2176–2179, 2010. Cited By (since 1996):165.