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# 1, 3, 4, 6-Tetra-*O*-acetyl-2-*O*-trifluoromethanesulfonyl-β-D-mannopyranose as FDG precursor

Master's work

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# ABREVIATIONS

| | |
|---|---|
| ClDG | 2-Deoxy-2-chloro-D-glucose |
| FDG | 2-Deoxy-2-fluoro-D-glucose |
| FDM | 2-Deoxy-2-fluoro-D-mannose |
| GMP | Good Manufacturing Practice |
| m.p. | Melting point |
| NMR | Nuclear Magnetic Resonance |
| PET | Positron Emission Tomography |
| Product 1 | Penta-*O*-acetyl-D-mannopyranose |
| Product 2 | Tetra-*O*-acetyl-α-D-mannopyranosyl bromide |
| Product 3 | 3,4,6-Tri-*O*-acetyl-1,2-*O*-(1-ethoxyethylidene)-β-D-mannopyranose |
| Product 4 | 1,3,4,6-Tetra-*O*-acetyl-β-D-mannopyranose |
| Product 8 | 1,3,4,6-Tetra-*O*-acetyl-2-*O*-trifluoromethanesulfonyl-β-D-mannopyranose (mannose triflate) |
| $R_f$ | Refractive index |
| TA-FDG | 2-fluoro-1,3,4,6-Tetra-*O*-acetyl-D-glucose |
| TLC | Thin-Layer Chromatography |

# 1 INTRODUCTION

FDG is a glucose analogue in which the hydroxyl group at the 2-carbon of a glucose molecule is replaced by a fluoride atom (Figure 1).

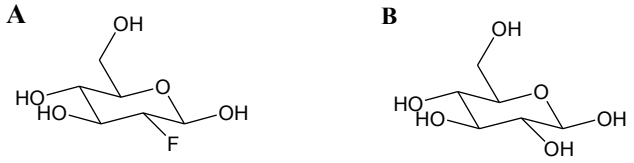



**Figure 1.** FDG (2-Deoxy-2-fluoro-D-glucose, A); glucose (B).

Due to similarity with glucose, this molecule is taken up into living cells by facilitated transport and then phosphorylated by hexokinase. FDG cannot undergo further metabolism like glucose because the hydroxyl group at the 2-carbon replaced by fluorine (Yu, S. 2006). Due to this fact, as the rate of it accumulation in the tissue is proportional to the metabolic activity of the tissue, this tracer is a good indicator of glucose uptake rates in living cells and can be used for diagnostics, treatment staging, and monitoring of cancers (Sanjiv, S. et al. 2001).

2-Deoxy-2-[$^{18}$F]fluoro-D-glucose ($^{18}$FDG - radioactive analog of FDG) is used in positron emission tomography (PET) which is the most important radio pharmaceutical diagnostic method and is called $^{18}$FDG-PET. $^{18}$FDG-PET has become a powerful scientific and clinic tool for probing biochemical processes in the human (Schlyer, D.J. 2004) and is widely used in clinical oncology.

The advanced procedures for routine preparation of [$^{18}$F]FDG are based on nucleophilic substitution of the triflate leaving group in 1,3,4,6-Tetra-*O*-acetyl-2-*O*-trifluoromethanesulphonyl-β-D-mannopyranose (mannose triflate) with [$^{18}$F]F$^{-}$ using phase-transfer conditions (Stöcklin, G. et al. 1993).

The general objectives of this work were as follows:

1. Test synthesis of mannose triflate.
2. Evaluation of this synthetic scheme for small scale production of mannose triflate as FDG precursor under GMP-like conditions.
3. Preparation of mannose triflate master drug file draft.
4. Cost estimation of synthesis this precursor.
5. Validation of the product through synthesis of “cold” FDG.

# 2 OVERVIEW

## *2.1 Synthesis of 1,3,4,6-Tetra-O-Acetyl-2-O-trifluoromethanesulfonyl-β-D-mannopyranose as FDG precursor*

The most commonly used precursor molecule in synthesis of FDG is 1,3,4,6-Tetra-*O*-Acetyl-2-*O*-trifluoromethanesulfonyl-β-D-mannopyranose (Figure 2).

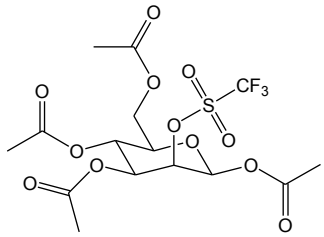



**Figure 2.** 1,3,4,6-Tetra-*O*-Acetyl-2-*O*-trifluoromethanesulfonyl-β-D-mannopyranose (mannose triflate).

Its structure is similar to that of FDG, except with a triflate group at the 2 carbon position that allowed selective fluorination in this position and acetyl groups at 1,3,4,6 position carbons via ester bonds, which can be readily broken by basic or acidic hydrolysis.

In the literature there have been two synthesis methods to get mannose triflate. The first method was described by Hamacher in 1986 where they used the synthesis of 1,3,4,6-Tetra-*O*-Acetyl-β-D-mannopyranose from D-mannose the one spot method to acetylate, brominate and hydrolysis of a transiently formed 1,2-acetoxonium ion. The second method reported by Pozsgay in 1992 (Pozsgay, V. et al. 1992) was the stepwise method to get a tetraacetyl mannose derivate via 1,2-orthoacetate intermediate hydrolysis and allowed to analyses each reaction intermediate.

The synthesis method below was described by Toyokuni et al. in 2004 and it is stepwise approach for the preparative scale synthesis of mannose triflate. Most recently Figure 3 represents steps of this synthesis.

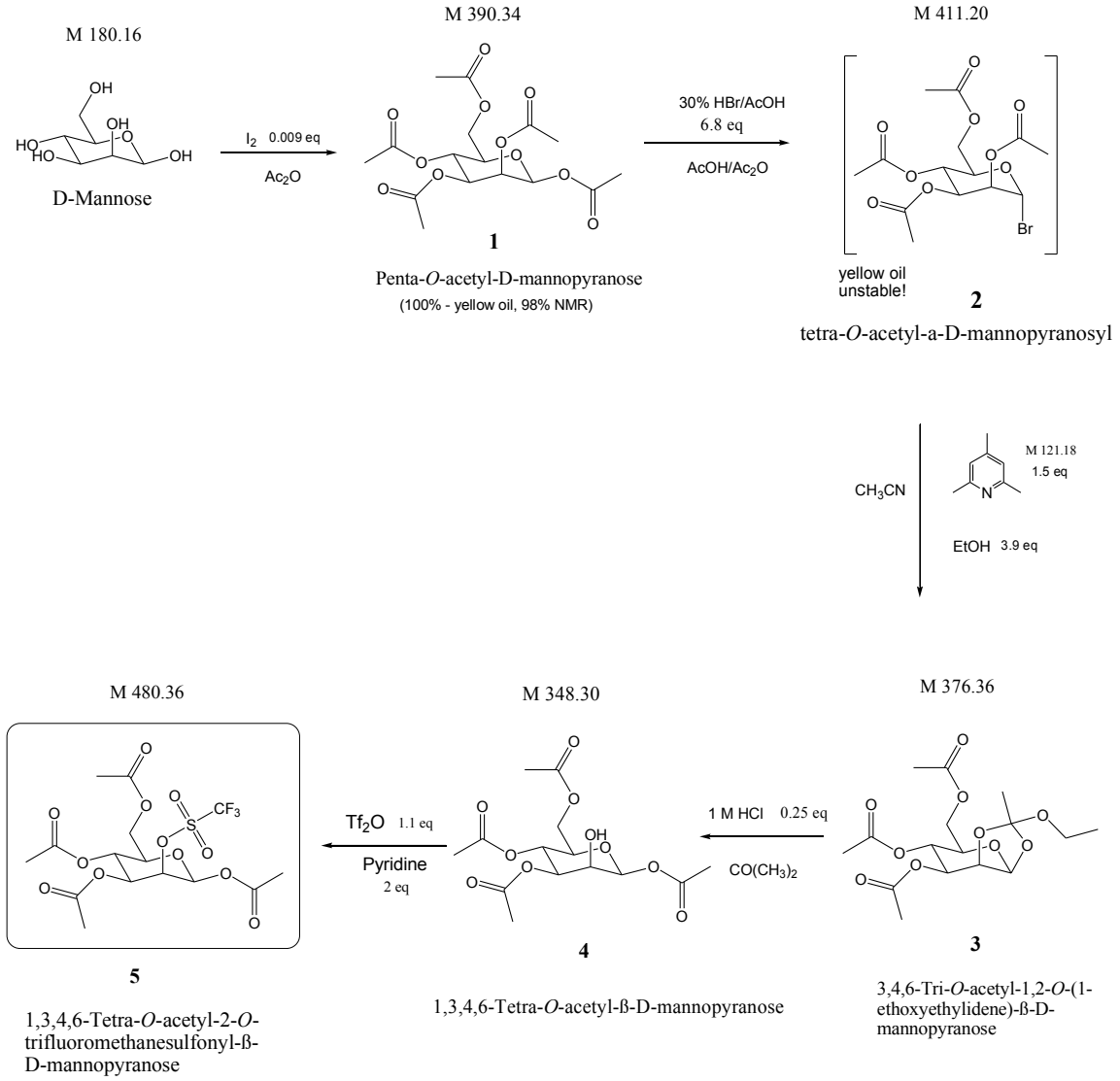



**Figure 3.** Synthesis of 1,3,4,6-Tetra-*O*-acetyl-2-*O*-trifluoromethanesulfonyl-β-D-mannopyranose (mannose triflate) (Toyokuni, T. et al. 2004).

The described procedure involves synthesis of 1,3,4,6-tetra-*O*-acetyl-beta-D-mannopyranose starting from D-mannose via the following steps:

1) Iodine-promoted acetylation with $Ac_2O$,

2) Formation of acetobromomannose with 30% HBr in AcOH,

3) 1,2-orthoester formation with EtOH in presence of 2,4,6-collidine,

4) Hydrolysis of the 1,2-orthoester with 1M aqueous HCl,

5) Triflation of the mannose tetraacetate.

### 2.1.1 Per-*O*-acetylation of carbohydrates

Per-*O*-acetylation is one of the most commonly used methods for the protection of hydroxyl groups in carbohydrates. Structural elucidations of many natural products containing carbohydrates have been confirmed by transforming them into their per-*O*-acetates. Acetylation of sugars is usually carried out using acetic anhydride in presence of basic or acid catalysts such as pyridine (Yu, B. et al. 1999), $ZnCl_2$ (Hudson C.S. et al. 1915), lithium perchlorate (Kuo-Cheng Lu. et al. 2004), $HClO_4$-$SiO_2$ (Misra A. et al. 2004), $Ce(OTf)_3$ (Bartoli G. et al. 2004), iodine (Kartha K. et al. 1997) and molecular sieves (Adinolfi M. et al. 2003).

Pyridine is the most widely used catalyst in per-*O*-acetylation of sugars. However, due to toxicity property and unpleasant odor of pyridine, handling of large volumes of it is troublesome. Therefore iodine-promoted acetylation method was chosen in this work (Figure 4).

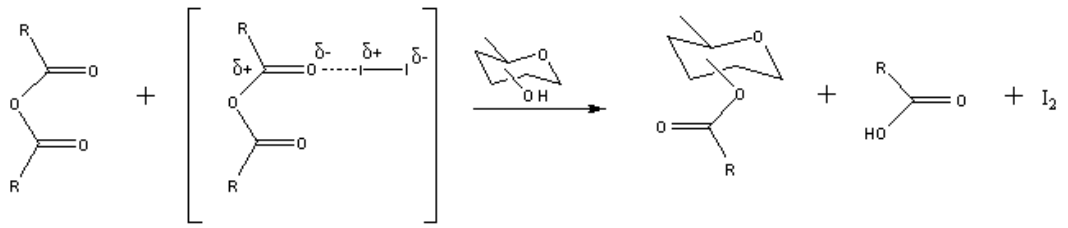



**Figure 4.** Iodine-promoted acetylation reaction (Kartha, K. et al. 1997).

Iodine is effective Lewis acid. Under controlled conditions it can bring about regioselective acetylation of carbohydrate derivatives. All of these reactions proceed in high yield (Kartha, K. et al. 1997). Iodine can be removed from the reaction mixture by simple filtration.

### 2.1.2 Substitution of acetyl hydroxyl groups with halogen in carbohydrate acetates

The substitution of acetyl hydroxyl groups with halogen can be carried out under mild conditions. This reaction is widely used in carbohydrate chemistry for getting reactionary capable acetohalogen carbohydrates. Glycoside acyl groups are easily replaced, but others acyl groups stay untouched in the same time. In many cases, more stable α-halogenide is formed, which is proceeds from both α- and β- carbohydrate (Figure 5, reaction a).

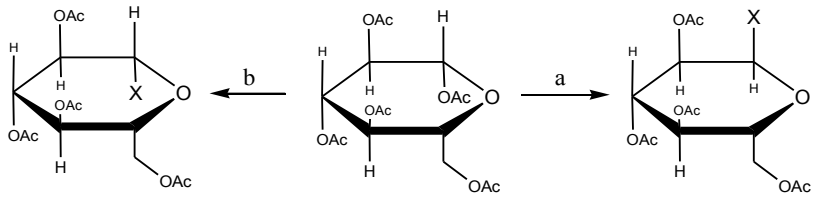



**Figure 5.** Replacement reaction of glycoside acyl group.

The reaction b shows an action of dry HCl on solvent or suspension of carbohydrate's acetate (Вейганд-Хильгетаг, 1969, p. 222).

### 2.1.3 Protection of a sugar $C_1$-$C_2$ hydroxyls via 1,2-orthoester

The ortho ester group has been used as a convenient selective protection for the $C_1$-$C_2$ hydroxyls of a sugar. Khrolin and his group (Khrolin, A.Ya. et al. 1964) showed that suitable ortho esters can be obtained in good yields by the reaction of 1,2-cis-acetohalogen carbohydrate with alcohols in ethyl acetate medium in the presence of $PbCO_3$ and $CaSO_4$ as acceptors of HBr (Figure 6).

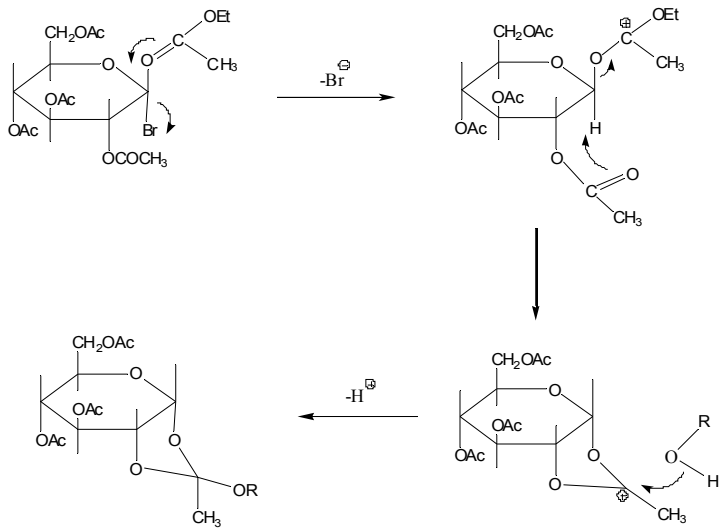



**Figure 6.** Formation of ortho ester.

The direction of reaction was explained by a double inversion of the configuration at the glycoside center.

For the preparation of 1,2-orthoester the method of Lemieux and Morgan (Lemieux, R.U. et al. 1965) was used which involved reaction of the bromo derivative with ethanol in the presence of 2,4,6-collidine (Helferich, B. et al. 1956).

## 2.1.4 Hydrolysis of ortho esters

An acyl group may migrate to a neighboring OH under acidic and neutral, but especially under basic conditions. Such migrations of acyl groups involving each carbon except $C_6$ of the hexose chain as the site of migration origin. The acyl migrations which span a large number of carbon atoms may proceed *via* a series of consecutive migrations each spanning fewer carbons (e.g., $C_2 \rightarrow C_6$ *via* $C_4 \rightarrow C_6$, $C_3 \rightarrow C_4$, and $C_2 \rightarrow C_3$) (Bonner, W. A. 1959).

Hydrolysis of ortho esters has been investigated by nuclear magnetic resonance spectroscopy (NMR) (Capon, B. et al. 1991). NMR spectroscopy is suitable for determination of unstable compounds because it allows reaction mixtures to be analyzed without extraction and chromatographic separations. NMR spectroscopy also allows a

"real-time" analysis of the reactions that proceed in the NMR tube under defined physic-chemical conditions (Akira, K. et al. 1998).

The acid-catalyzed hydrolysis of ortho esters consists of three separate reaction stages (Figure 7): generation of a dialkoxy carbonium ion (a), hydration of this ion to a hydrogen ortho ester (b), and breakdown of the latter to alcohol and carboxylic acid ester products (Ahmad, M. et al. 1979).

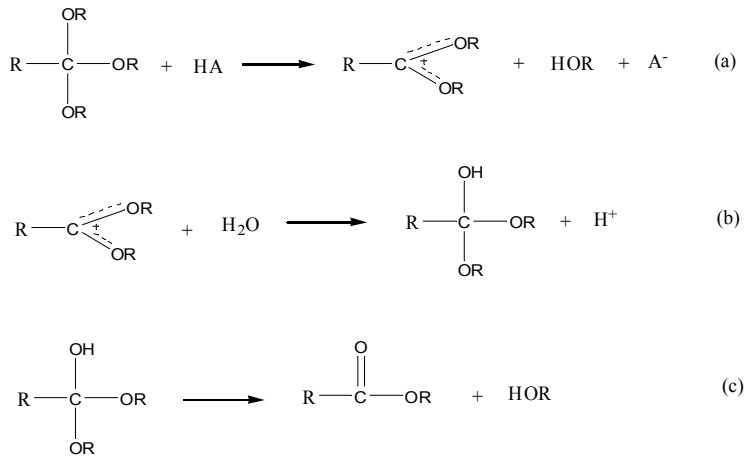



**Figure 7.** Acid-catalyzed hydrolysis of ortho esters.

In 1963 Schroeder and his group observed that the proportions of O-acetyl derivatives could be formed at higher acid concentration in acidic hydrolysis. 28 years later Capon and his group made an investigation of intermediates in the hydrolysis of ortho ester derived from D-Glucose and D-mannose (Capon, B. et al. 1991). As example, we could consider hydrolysis of 1,2-*O*-(1-exo-Ethoxyethylidene)-β-D-mannopyranose from this research (Figure 8).

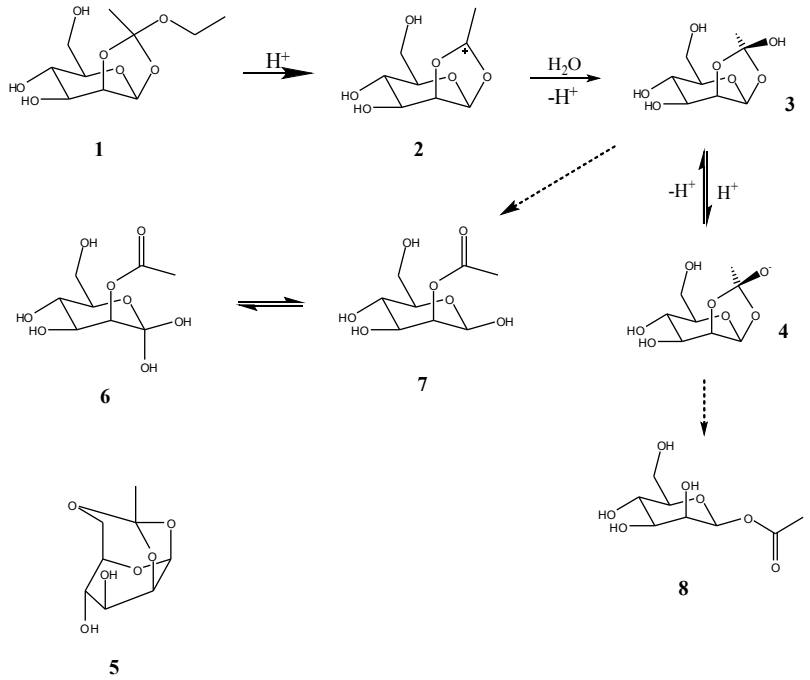



**Figure 8.** Hydrolysis of 1,2-*O*-(1-exo-Ethoxyethylidene)-β-D-mannopyranose (Capon, B. et al. 1991).

After about 5 minutes of complete conversation into the tricyclic ortho ester, 1,2,6-*O*-orthoester-β-D-mannopyranose (5), had taken place. This reaction was carried out at low acid concentration and at 25°C. After a further two hours the tricyclic ortho ester (5) had been converted into 2-*O*-acetyl-β-D-mannopyranose (7).

When hydrolysis of 1 was carried out using a higher acid concentration and lower temperature (-30°C), two additional species were observed. One of these was an intermediate between the tricyclic ortho ester (5) and 2-*O*-acetyl-β-D-mannopyranose (8) and the other was a transformation product of the latter. After about 2 h at low temperature the conversion into 2-*O*-acetyl-β-D-mannopyranose (7) was completed. On worming to room temperature, compound 7 was converted into another compound (6). It is more stable than β-anomer and the final product consists of more than 90% of compound 6.

### 2.1.5 Triflation of the mannose tetraacetate

The choice of leaving group depends on the yield, stability of precursors, ease of subsequent separation of the fluorinated product from precursors, reagents and solvents, and the formation of potential side products. Halogens are good leaving groups for aliphatic nucleophilic displacements. The sulfonic ester groups *tosylate, brosylate, nosylate,* and *mesylate* are better leaving groups than halides and are frequently used, although they tend to give lower yields (Schlyer, D.J. 2004). Other leaving groups as oxonium ions ($ROR_2^+$), fluorinated compounds *triflates* and *nonaflates* are still better, and compounds containing these groups make powerful alkylating agents (Smith, M. B. et al., 5th ed., p. 446-447).

**Table 1**

A comparision of various leaving groups listed below (Yu, S. 2006).

| Leaving group | Properties |
|---|---|
| Triflates | Very good leaving group |
| Tosylate | |
| Mesylate | |
| Iodide | Moderate leaving group |
| Bromide | |
| Chloride | Poor leaving group |

All of the leaving groups listed in the table 1 except chlorides have been used in radio-fluorination reactions, but in the synthesis of FDG ($^{18}$F-FDG) precursor triflates produces a higher and more consistent yield.

## *2.2 Synthesis of FDG*

### 2.2.1 Electrophilic fluorination

The first synthesis of FDG was carried out in 1976 by electrophilic fluorination (Fowler, J. et al. 2002). Electrophilic fluorination refers to the addition of fluorine atoms across a double bond, producing a difluoro derivative of the parent compound. As shown in Figure 9, the electrophilic fluorination reaction involves 3,4,6-tri-*O*-acetyl-D-glucal as precursor.

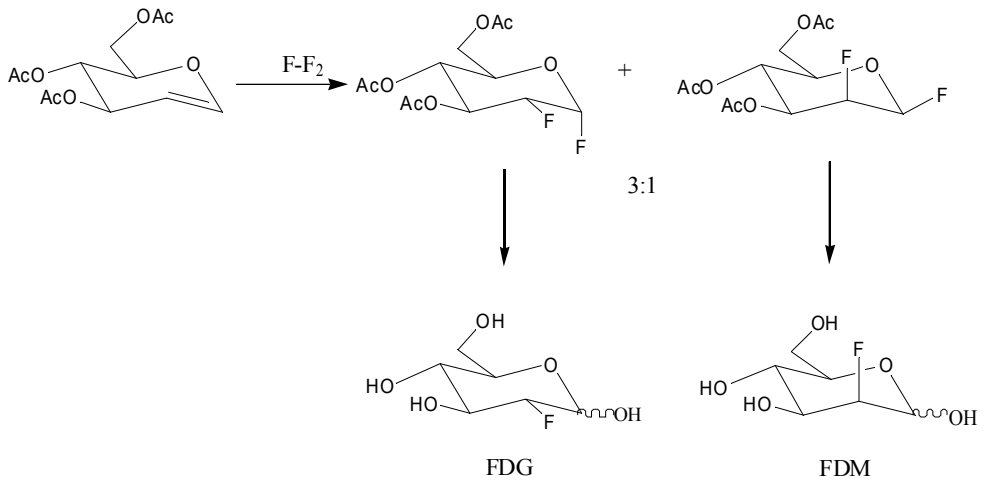



**Figure 9.** Synthesis of FDG by electrophilic fluorination.

The glucal was treated with F-$F_2$ to produce a 3:1 mixture of the F labeled 1,2-difluoroglucose isomer and the 1,2-difluoromannose isomers. The 1,2-difluoroglucose isomers was separated by preparative gas chromatography and hydrolyzed in HCl to give FDG. The yield was 8% and the synthesis time was 2 hours (Fowler, J. et al. 2002).

## 2.2.2 Nucleophilic fluorination

In 1986 were published results of investigations of Hamachr's group. They used the aminopolyether potassium complex $[K/2.2.2]^+$ $F^-$ as a phase transfer catalyst (Hamacher, K. et al. 1986). The reaction time was shorter (50 min) and yield higher (50%). On the Figure 10 is shown synthesis of FDG by nuceophilic fluorination.

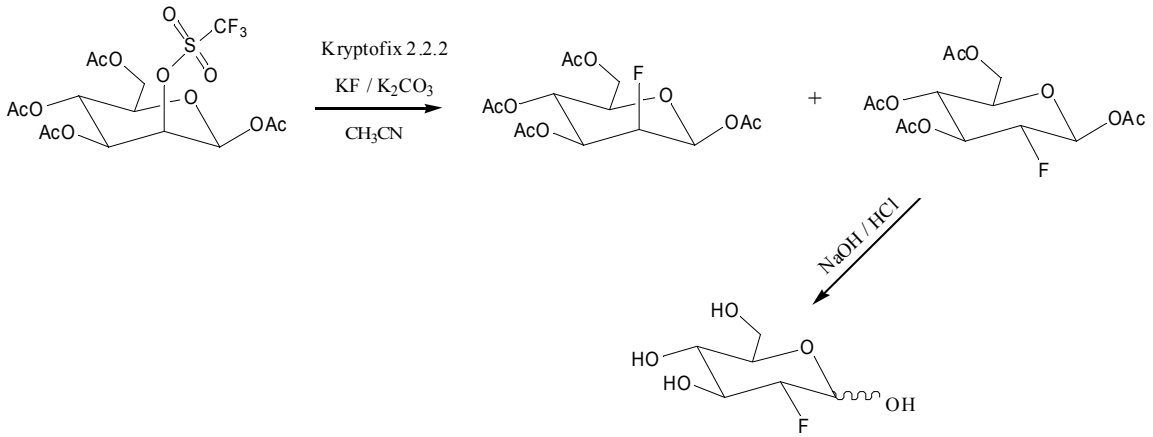



**Figure 10.** Synthesis of FDG by nuceophilic fluorination.

F ion is the nucleophile. In the presence of Kryptofix 2.2.2 as catalyst and acetonitrile as solvent, F ion approaches the mannose triflate at the 2-carbon, while the triflate group leaves the protected mannose molecule to form FDG (Yu, S. 2006).

Kryptofix 2.2.2 is used as a phase-transfer reagent to facilitate the nuceophilic reaction of fluoride in the routine preparation of 2-FDG. Kryptofix 2.2.2 is cyclic crown ether (Figure 11), which binds the potassium ion, preventing the formation of KF. Thus, potassium acts as the counter ion $F^-$ to enhance its reactivity but does not interfere with the synthesis (Yu, S. et al. 2006).

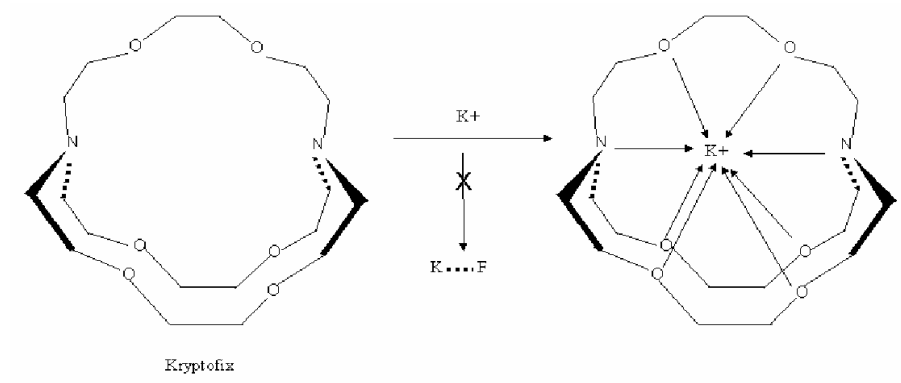



**Figure 11.** Kryptofix 2.2.2 and $K^+$ (Yu. S. 2006).

### 2.2.3 Acid and basic hydrolysis of 2-fluoro-1,3,4,6-tetra-*O*-acetyl-D-glucose

Protective acyl groups can be removed from 2-fluoro-1,3,4,6-tetra-*O*-acetyl-D-glucose (TA-FDG) by acid or basic hydrolysis (Hamache, K. et al. 1986; Füchtner, F. et al. 1995). Acid hydrolysis has been commonly used in synthesis of FDG. However, ClDG could be produced as a competing displacement reaction with chloride ion which comes from different sources including HCl in the hydrolysis step. Larger amounts of ClDG were produced when larger amounts of the triflate precursor were used. The amount of ClDG can be reduced by using sulfuric acid instead of HCl for hydrolysis (Fowler, J.S. et al. 2003).

In contrast to acid hydrolysis of TA-FDG to FDG, basic hydrolysis is shorter and produce higher yield of the final product (Füchtner, F. et al. 1995). The general course of basic hydrolysis is independent of the kind of used alkalihydroxide, ammonia, alkylammonium hydroxide or similar amines but the most suitable base for routine preparation is sodium hydroxide.

Füchtner and his co-workers observed that the rate of FDG formation increases with increasing hydroxide concentration. The maximum yield of final product was obtained at 0.3M NaOH. It was seen that all starting TA-FDG was converted into FDG under these conditions and reaction time of reaching maximum yield was only 1 min. At

higher hydroxide concentrations more $F^-$ was formed and the yield of FDG decreased (Füchtner, F. et al. 1995).

In nucleophilic substitution the yield of removing the protective groups is about 100%. In the case of electrophilic reaction the overall yield of FDG and FDM under alkaline conditions was between 75 and 80%. This is about 20% higher than using acid hydrolysis. According to TLC analysis a residue peak of a partially hydrolyzed TA-FDG was observed and this peak was up to 5% in all cases of alkaline hydrolysis (Füchtner, F. et al. 1995).

### 2.2.4 Purification of FDG

The chemical impurity test is one of the most important tests to assure safety and quality of PET radiopharmaceuticals. In the *United States Pharmacopeia (USP)* and the *European Pharmacopeia (EP)*, the permissible chemical impurity levels are defined for FDG, 2-deoxy-2chloro-D-glucose (ClDG) and Kryptofix 2.2.2 in FDG (Nakao, R. et al. 2007).

A number of analytical methods are described in literature. For example:

1. thin-layer chromatography (TLC) (Chaly, T. et al. 1989), where SilikaGel as a stationary phase and acetonitrile:water (95%:5% v/v) as eluent were used. The $R_f$ of the FDG, free $F^-$ and acetylated FDG are about 0.45, 0.0, and 0.8 to 0.95 respectively. The spots were visualized by iodine vapor method as the brown spots. The sensitivity of the iodine vapor were 2 ppm (Akhlaghi, M. et al.2006).

2. High-performance liquid chromatography (HPLC) on Lichrosorb-$NH_2$ and on ion exchange columns with ultraviolet (UV) absorbance detection (Hamacher, K. 1986).

3. HPLC methods that based on a postcolumn derivatization with 2-cyanoacetamide (2-CA) (UV detection at 276 nm, normal phase HPLC -Inertsil $NH_2$ (Nakao, R. et al. 2005) and 1-Phenyl-3-methyl-5-pyrazolone (PMP) (UV detection at 250 nm, reversed phase HPLC -Waters Xbridge $RP_{18}$ (Nakao, R. et al. 2008) were used to detect the impurities as ClDG (Cl-desoxyglycose), FDM (F-desoxymannose) and Kryptofix 2.2.2.

4. HPLC - tandem mass spectrometric method (LC/MS/MS) (Ying, Ma et al. 2000).

5. Gas chromatography (GC) with a nitrogen selective detector (Ferrieri, R. et al. 1993).

# 3 MATERIALS AND METHODS

## *3.1 Purification of solvents*

Anhydrous acetonitrile and ethanol were prepared using same method for both solvents. Excess $CaH_2$ was added portion wise and the mixture was then refluxed for some time. After reflux the solvents were distilled at constant temperature; with first 10-20 ml being discarded. Anhydrous dichloromethane was dried with $LiAlH_4$ and distilled at 45°C.

## *3.2 Analysis methods*

Pre-coated MERCK $60F_{254}$ 0.25 mm glass plates were used for TLC analyses in synthesis of mannose triflate. Eluent: EtOAc/hexanes = 1/1. Spots were visualized by 0.5% orcinol solution in $MeOH/H_2SO_4$ followed by heating (Brückner J. 1954). In the case of nucleophilic fluorination the TLC analysis was performed on plates with a stationary phase of 0.2 mm SilicaGel (DC-Alufolien, Kieselgel, Fluka). The chromatographic development was carried out in a chamber filled with ethyl acetate/ hexane = 1/1. Spots were visualized as previously. In the case of acid and alkaline hydrolysis of TA-FDG the chromotographic development was carried out with eluent: acetonitrile/0.1% TFA in $H_2O$ = 95/5 and spots were visualized by iodine vapor (Akhlaghi, M. et al. 2006). All samples were spotted on a TLC plate using glass capillars.

Bruker NMR spectrometer was used for NMR spectrum measurements. $^1H$ NMR spectrum was measured at 200 MHz and $^{13}C$ NMR spectrum at 50 Hz. $CCl_3D$ was used as a solvent. Tetramethylsilane was used as reference.

Melting point was measured by MEL-TEMP (Electrothermal).

HPLC analysis of FDG-precursor was made by using HPLC detector 430 (KONTRON Instruments) with X-Bridge C-18 P 420 column. Mobile phase was acetonitrile/water= 60/40; 1 ml/min; λ=220 nm; ρ=96.

## *3.3 Chemicals and their physical constants*

2,4,6-collidine (2,4,6-trimethylpyridine) *(Fluka; Lot & Filling code: 1332936 54207205)*, 33% HBr in AcOH *(Fluka;Lot & Filling code:1343295 54807006)*, 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane (Kryptofix 2.2.2) *(Fluka; Lot.: S36074 64107B14)*, acetic anhydride *(Fluka; Lot & Filling code: 1324226 31507218)*, acetic acid *(Fluka; Lot: 63340)*, acetone (*Fluka; Lot.: 1399413 33408240*), acetonitrile (*Rathburn; Batch number: 8B05CC*), Calcium hydride (95%) *(Sigma-Aldrich; Lot: s 36761-376*), chloroform (*Rathburn; Batch number:6J04SA*), D-Mannose *(Fluka; Lot & Filling code: 134786 14507210)*, dichloromethane (*Rathburn; Batch number: 8B64SA*), diethyl ether (*Rathburn; Batch number: 7E24DB*), hexane (*Rathburn; Batch number: 7F29EB*), hydrobromic acid (*Fluka; Lot: 1311395 20207235*), hydrochloric acid fuming 37% *(Riedel de Haën; Lot: 72220)*, iodine (*Fluka; Lot & Filling code: 1096765 23104227*), orcine monohydrate (*Fluka; Lot & Filling code: 13202821 25007046*), sodium bicarbonate (*Fluka; Lot & Filling code: 1329476 52907032*), sodium sulfate anhydrous *(Riedel-de Haën; Lot: 61380)*, sodium thiosulfate anhydrous (*Fluka; Lot & Filling code: 1146096 25004254*), sulfuric acid 95-97% *(Fluka; Lot.: 62240)***,** methanol *(Rathburn; Batch number: 8D15PA)*, potassium carbonate anhydrous *(Fluka; Lot.: 1382377 53208266)*, potassium fluoride *(Fluka; Lot.: 1331455 10308133),* pyridine (*Fluka; Lot & Filling code: 1113584 14604084*), trifluoromethanesulfonic anhydride (triflic anhydride) *(Fluka; Lot & Filling code: 1336393 53807321).*

**Table 2**

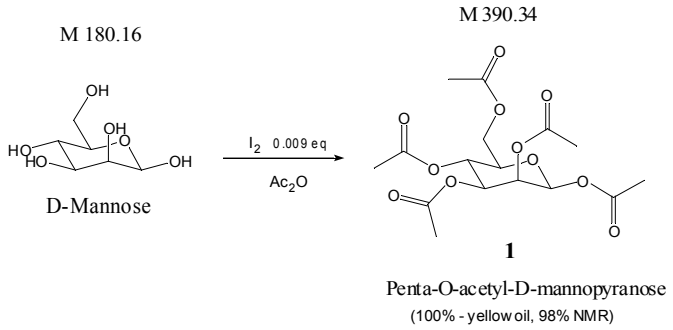
Physical properties of chemicals and solvents. Taken from www.sigmaaldrich.com (05.05.2009).

| Chemical | M, (g/mol) | m.p, (°C) | b.p, (°C/mmHg) | $d^{20}_{4}$, (g/ml) | $n_D^{20C}$ |
|---|---|---|---|---|---|
| 2,4,6-trimethylpyridine | 121.18 | -43 | 170-172 | 0.91 | 1.5 |
| 33% HBr in AcOH | 80.91 | - | - | 1.4 | - |
| Kryptofix 2.2.2 | 376.49 | 68-71 | - | - | - |
| Acetic anhydride | 102.09 | -73 | 138-140 | 1.08 | 1.39 |
| Acetic acid | 60.05 | 16.2 | 117-118 | 1.05 | 1.37 |
| Acetone | 58.08 | -94 | 56 | 0.79 | 1.36 |
| Acetonitrile | 41.05 | -48 | 81-82 | 0.79 | 1.34 |
| Calcium hydride (95%) | 42.09 | 190 | - | - | - |
| Chloroform | 119.38 | -63 | 60.5-61.5 | 1.49 | 1.45 |
| D-Mannose | 180.16 | 133-140 | - | - | - |
| Dichloromethane | 84.93 | -97 | 39.8-40 | 1.33 | 1.42 |
| Diethyl ether | 74.12 | -116 | 34.6 | 0.71 | 1.35 |
| Ethanol | 46.07 | -114 | 78 | 0.79 | 1.36 |
| Hexane | 86.18 | -95 | 68-70 | 0.67 | 1.38 |
| Hydrobromic acid | 80.91 | - | - | 1.49 | - |
| Hydrochloric acid | 36.46 | - | 100 | 1.18 | - |
| Iodine | 253.81 | 113 | 184 | - | - |
| Orcine Monohydrate | 142.16 | 56-58 | 290 | - | - |
| Sodium bicarbonate | 84.01 | - | - | - | - |
| Sodium sulfate anhydrous | 142.04 | 884 | - | 2.68 | - |
| Sodium thiosulfate anhydrous | 158.11 | - | - | - | - |
| Sulfuric acid | 98.08 | - | ~290 | 1.83 | - |
| Methanol | 32.04 | -98 | 64.7 | 0.79 | 1.33 |
| Potassium carbonate | 138.21 | 891 | - | - | - |
| Potassium fluoride | 58.1 | 858 | - | - | - |
| Pyridine | 79.1 | -42 | 115 | 0.98 | 1.51 |

| | | | | | |
|---|---|---|---|---|---|
| Trifluoromethanesulfonic anhydride | 282.14 | - | 81-83 | 1.68 | 1.32 |

## *3.4 Synthesis of 1,3,4,6-Tetra-O-acetyl-2-O-trifluoromethanesulfonyl-β-D-mannopyranose as 2-Deoxy-2-fluoro-D-glucose precursor*

### 3.4.1 Step 1: Iodine-promoted acetylation with $Ac_2O$



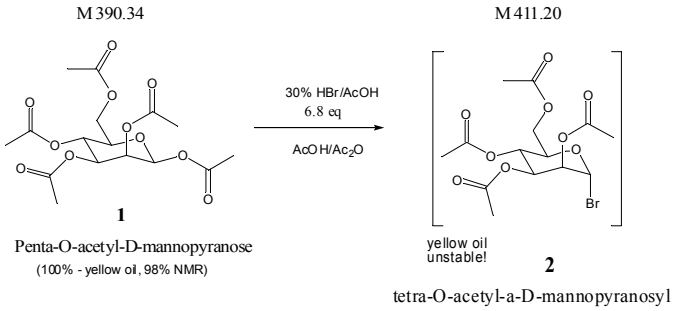

Solution of iodine (0.13 g, 0.5 mmol) in $Ac_2O$ (50 ml) was prepared in a 250 ml round flask. The reaction flask was placed into water bath (18ºC). 10.1 g (55.8 mmol) of D-mannose was added portion wise to the solution and vigorously stirred for each portion of sugar to dissolve completely after each addition. After a while the reaction mixture turned dark brown color. This reaction is somewhat exothermic, that is why it is necessary to add sugar with some care.

The reaction mixture was cooled to room temperature for about one hour. Then it was poured into 500 ml beaker with 100 ml solution of crushed ice and saturated solution of $Na_2S_2O_3$. The mixture vigorously stirred using glass stick until reaction mixture became light yellow in color. Subsequently, $NaHCO_3$ was added portion wise until the evolution of $CO_2$ ceased and oily foam started to form. The addition of $NaHCO_3$ must be done with care, because $CO_2$ evolves violently at the beginning.

Water layer removed partially by decantation and oily foam dissolved in 50 ml of $CH_2Cl_2$ and washed subsequently with pre-cooled saturated aqueous $NaHCO_3$ solution (50 ml) and water (3× 50ml). Organic layer was then dried with anhydrous $Na_2SO_4$, filtered and solvent removed at 40ºC, yielding penta-*O*-acetyl-D-mannopyranose: 23.5 g (60.2 mmol) as yellow oil (~100% from D-mannose); TLC $R_f$ 0.43.

$^{1}$H NMR (ppm) ($CCl_3D$; 500 MHz) for α: δ = 2.01 (s, 3H), 2.06 (s, 3H), 2.10 (s, 3H), 2.18 (s, 3H), 2.18 (s, 3H), 3.80-3.89 (dd, 1H), 4.061-4.11 (dd, 1H) and 4.25-4.38 (dd, 1H), 5.19 (d, J = 3.4 Hz, 1H), 5.36-5.37 (m, 2H), 6.09 (d, J = 1.8 Hz, 1H); for β: 2.006 (s, 1H), 2.11 (s, 1H), 2.22 (s, 1H), 4.12-4.17 (dd, 1H) and 4.34-4.37 (dd, 1H), 5.14 (d, J = 3 Hz, 1H), 5.25-5.28 (t, J = 5Hz, 1H), 5.48-5.50 (dd, J = 4.6; 1.2 Hz, 1H), 5.90 (d, J = 1.4 Hz, 1H) (Appendix 1). $^{13}$C NMR (50 Hz): δ = 20.46, 20.56, 20.60, 20.62, 20.67, 20.77, 62.14, 65.69, 68.37, 68.74, 70.62, 73.24, 77.36, 90.45, 90.62, 167.86, 168.16, 169.35, 169.42, 169.50, 169.56, 169.75, 169.96, 170.37 (Appendix 2).

### 3.4.2 Step 2: Formation of acetobromomannose with 33% HBr in AcOH

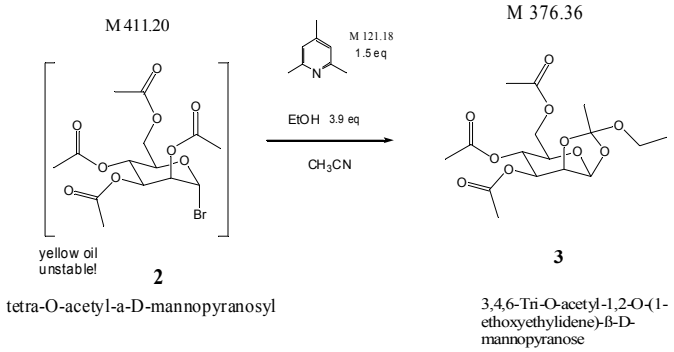



23.5 g of Product 1 (60.2 mmol) was dissolved in 25 ml glacial acetic acid containing 2 ml acetic anhydride with vigorously stirred. The solution was cooled to 0ºC on an ice bath under argon atmosphere. Then 22 ml of pre-cooled 33% HBr AcOH was added drop wise to the reagent mixture over a period of 45 minutes and mixture continuously stirred. The reaction mixture stirred at room temperature overnight. The flask was covered with aluminum foil to reduce impact of light on the decomposition of the desired product.

On the next day the mixture was diluted with 50 ml of $CHCl_3$. The organic layer was washed with pre-cooled saturated aqueous $NaHCO_3$ (3×40 ml) and water (1×40 ml). The washing with saturated aqueous $NaHCO_3$ must be done with care, because a strong back pressure of $CO_2$ initially generated. The organic layer was dried with anhydrous $Na_2SO_4$, filtered and rotated to dryness at 40ºC. Yield of crude tetra-*O*-acetyl-α-D-

mannopyranosyl bromide (Product 2) was 22.5 g (54.8 mmol) as a yellow oily product (98% from D-mannose); TLC: $R_f$ 0.57.

### 3.4.3 Step 3: 1,2-orthoester formation with EtOH in presence of 2,4,6-collidine



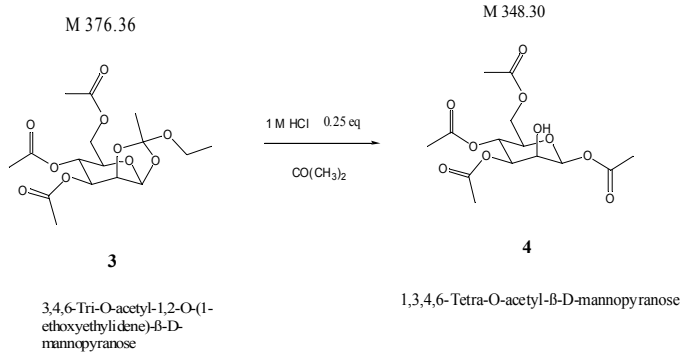

The oily residue (22.5 g) was dissolved in 8 ml (153.2 mmol) of anhydrous acetonitrile and 11 ml (82.5 mmol) of 2,4,6-collidine. 12 ml of anhydrous ethanol was added to the solution by one portion with vigorous stirring. The reaction mixture was stirred in the dark overnight at room temperature. The white solid formed on the walls of reaction flask.

The heterogeneous mixture was diluted with 60 ml of chloroform and washed with water (3×25 ml). The white solid was dissolved. The combined water layer was re-extracted with 20 ml of $CHCl_3$. The combined clear yellow organic layer was dried over anhydrous $Na_2SO_4$, filtered and rotated on rotary evaporator at 36°C. Yellow oily residue was triturated with 20 ml of hexanes to yield 3,4,6-Tri-*O*-acetyl-1,2-*O*-(1-ethoxyethylidene)-β-D-mannopyranose (Product 3). Product 3 was recrystallized with anhydrous EtOH. Yielding 10.8 g (28.7 mmol) (51% from D-mannose) as white powder, m.p 101-102°C (lit. 101-103°C (Pozsgay, V. et.al 1992)); TLC $R_f$ 0.45.

$^{1}H$ NMR ($CCl_3D$, 200 MHz): δ = 1.19 (t, J=7.2 Hz, 3H), 1.75 (s, 3H), 2.06 (s, 3H), 2.08 (s, 3H), 2.13 (s, 3H), 3.53 (br q, J=7.2 Hz, 1H) and 3.58 (br q, J=7.2 Hz, 1H), 3.50-3.72 (m, 1H), 4.09-4.17 (dd, J = 12.2; 2.8 Hz, 1H) and 4.20-4.29 (dd, J=12.2, 4.8 Hz, 1H), 4.58-4.62 (dd, J= 4.0, 2.6 Hz, 1H), 5.11-5.18 (dd, J=9.8, 3.8 Hz, 1H), 5.30 (br, J=9.6 Hz, 1H), 5.49 (d, J=2 Hz,1H) (Appendix 3). $^{13}C$ NMR (50 Hz): δ = 15.15, 20.62, 20.64, 20.69, 24.56, 58.29, 62.65, 65.97, 70.74, 71.74, 97.58, 124.28, 169.37, 170.30, 170.52 (Appendix 4).

*Note: The following reactions were carried out with fewer amounts of 1,2-orthoester: 1.0 g (2.7 mmol), 4.0 g (10.7 mmol) and 5.2 g (13.8 mmol). The using methods were the same. Therefore, synthesis of 4.0 g of Product 3 was described.*

### 3.4.4 Step 4: Hydrolysis of the 1,2-orthoester with 1M aqueous HCl




4.0 g of Product 3 dissolved in 10 ml anhydrous acetone. The reaction flask was placed into water bath (16°C) and 2.5 ml of 1.0 M HCl was added in one portion to the mixture with vigorous stirring. The reaction mixture stirred 5 min. and then was removed from water bath. The reaction mixture continued to stir at room temperature 1.5 h. The reaction was checked by TLC analysis during this period. Two major products were formed: $R_f$ 0.26 and $R_f$ 0.12. The $NaHCO_3$ solution was then added to the mixture to neutralize HCl. The reaction mixture was filtered and rotated at 20°C. The resulting white solid was dissolved in 1 ml of $CH_2Cl_2$ and mixture washed with water (3×10 ml). The

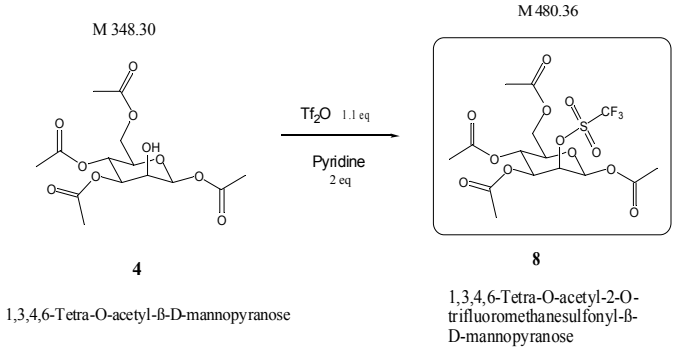
water layer was re-extracted with $CH_2Cl_2$ (2×10 ml). The organic layers were combined and dried with $Na_2SO_4$, filtrated and $CH_2Cl_2$ evaporated at 36°C, yielding products as yellow oil.

The yellow product triturated with 20 ml of diethyl ether and light yellow residue started to form. The residue was washed with diethyl ether and dried on glass filter (P4), yielding 1.1 g; 3.2 mmol (32% from product 3) of 1,3,4,6-Tetra-O-acetyl-ß-D-mannopyranose (the desired product), $R_f$ 0.12.

The filtrate was concentrated on a rotary evaporator at 36°C to give colorless oily residue. Two spots were seen on TLC plate, $R_f$ 0.17 and $R_f$ 0.35. This oily residue can be subjected to the iodine-promoted acetylation yielding the pentaacetate (STEP 1) and recycled back into the synthesis of the orthoester.

$^1$H NMR ($CCl_3D$, 200 MHz): δ = 2.05 (s, 3H), 2.09 (s, 3H), 2.11 (s, 3H), 2.18 (s, 3H), 2.45 (d, J=4Hz, 1H, lisand?), 3.48 (d, 7Hz, 1H, lisand?), 3.74-3.83 (m, 1H), 4.09-4.16 (dd, J = 14.8; 2.4 Hz, 1H) and 4.27-4.36 (dd, J=17.2, 4.8 Hz, 1H), 4.19-4.22 (t, J= 6.4, 3.4 Hz, 1H), 5.01-5.07 (dd, J=12.8, 3 Hz, 1H), 5.33-5.45 (t, J=9.8 Hz, 1H), 5.79 (s, 1H) (Appendix 5). $^{13}$C NMR (50 Hz): δ = 20.61, 20.67, 20.72, 20.81, 62.19, 65.62, 68.54, 72.98, 73.33, 91.89, 168.44, 169.50, 170.03, 170.62 (Appendix 6).

### 3.4.5 Step 5: Triflation of the mannose tetraacetate




1.1 g (3.2 mmol) of product 4 was dissolved in 8 ml of anhydrous dichloromethane containing 0.6 ml of dry pyridine. The reaction flask with mixture

placed into dry ice/EtOH bath (-15 ºC). 0.6 ml (3.24 mmol) of trifluoromethanesulfonic anhydride (triflic anhydride) was added drop wise during a period 30 min with stirring under argon atmosphere. The mixture was then allowed to reach room temperature for a period of 3 hours. The mixture was then washed with ice-cold saturated aqueous $NaHCO_3$ (2×15 ml) and water (2×15 ml). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered and concentrated on a rotary evaporator at 40ºC. The solid residue was formed and recrystallized with anhydrous ethanol. The filtrate rotated to dryness and recrystallized once more. The final product 8 is 1,3,4,6-Tetra-*O*-acetyl-2-*O*-trifluoromethanesulfonyl-β-D-mannopyranose 1.25 g, 2.6 mmol (80% from Product 4) as white needles, m.p. 118-119ºC (lit. 118-120ºC (Pozsgay, V. et.al 1992)), $R_f$ 0.54. HPLC analysis showed one peak in retention time 6.35 min and the purity was 99.7%.

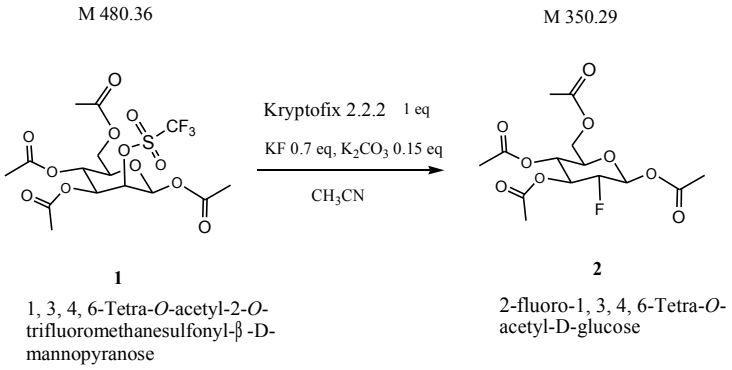
$^1$H NMR ($CCl_3D$, 200 MHz): δ = 2.08 (s, 3H), 2.11 (s, 3H), 2.12 (s, 3H), 2.17 (s, 3H), 3.79-3.92 (m, 1H), 4.11-4.35 (dd, J = 4.6; 0.6 Hz, 1H), 4.18-4.26 (m, 1H), 5.11-5.19 (m, 1H), 5.23-5.31 (t, 1H), 5.36 (s, 1H), 5.94 (s, J=1Hz, 1H) (Appendix 7). $^{13}$C NMR (50 Hz): δ = 20.35, 20.40, 20.49, 20.59, 61.89, 65.03, 69.80, 73.78, 81.35, 89.34, 115.49 (q, $J_{C,F}$ = 318.8 Hz, $CF_3$), 167.93, 169.11, 169.76, 170.48 (Appendix 8).

### 3.4.6 Recycling process of filtrate from Step 4

5.3 g of filtrate was dissolved in 27 ml of acetic anhydride, containing 0.06 g of iodine. The reaction mixture was stirred 10 min at 15°C. Then the reaction flask was removed from water bath and the mixture continued to stir 1 h at room temperature to yield the pentaacetate (Product 1). The following steps of synthesis were the same as described above, but one change was included. The product 3 was not recrystallized from anhydrous ethanol. Yield was 0.96 g (2.00 mmol) of 1,3,4,6-Tetra-*O*-acetyl-2-*O*-trifluoromethanesulfonyl-β-D-mannopyranose as white needles, m.p. 117…119°C. Combined mixture of isomers which was received from filtrate of hydrolysis of 1,2-orthoester with 1M HCl was 2.2 g.

## *3.5 Synthesis of 2-Deoxy-2-fluoro-D-glucose precursor*

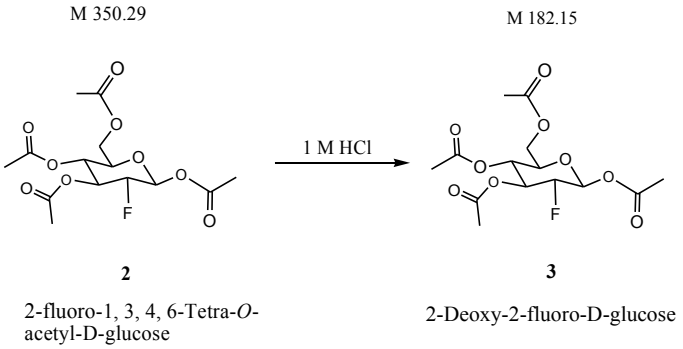
### 3.5.1 STEP 1: Nucleophilic fluorination of 1,3,4,6-Tetra-*O*-acetyl-2-*O*-trifluoromethanesulfonyl-β- D-mannopyranose




0.06 g (0.12 mmol) of mannose triflate and 0.05 g (0.13 mmol) of 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane (Kryptofix 2.2.2) were dissolved in 1.5 ml of dry acetonitrile. 5 mg (0.09 mmol) of KF and 3 mg (0.02 mmol) of $K_2CO_3$ were added to the mixture. This mixture was heated (till 80°C) with vigorous stirring under argon atmosphere for 1 h 20 min. The reaction was checked during this period with TLC analyses.

The reaction mixture was filtered and rotated at 36°C. The lightly yellow residue was dissolved in 10 ml of $CH_2Cl_2$ and washed with water (3×5 ml). Non-organic layer was re-extracted with 3 ml of $CH_2Cl_2$. Combined organic layer was dried over with anhydrous $Na_2SO_4$, filtered and rotated at 36°C. Yielding 2-fluoro-1,3,4,6-Tetra-*O*-acetyl-D-mannopyranose as yellow solid residue, Rf 0.45.

### 3.5.2 STEP 2: Acid hydrolysis of 2-fluoro-1,3,4,6-Tetra-*O*-acetyl-D-glucose




1.2 ml of 1M HCl was added to the product by one portion and heated (till 70°C) with vigorous stirring for 1 h 30 min. Then the reaction mixture was rotated at 36°C yielding dark brown heterogeneous residue.

The brown residue was neutralized with 1M NaOH (pH~7), rotated and product was separated by chromatographic column filled with silica gel (70 g, 63-200 µm; Machery-Nagel). Due to small amount of product, the column was prepared from glass pipette and eluent was acetonitrile/0.1% TFA in $H_2O$=95/5. The fraction of combined desired product was $R_f$ 0.32, yielding clear colorless product.

### 3.5.3 STEP 2: Alkaline hydrolysis of 2-fluoro-1,3,4,6-Tetra-*O*-acetyl-D-glucose

2-fluoro-1,3,4,6-Tetra-*O*-acetyl-D-mannopyranose was prepared from 0.05 g (0.1 mmol) of mannose triflate as described above (3.5.1 STEP 1). Yielding yellow solid residue, Rf 0.41. This product was dissolved in 1.5 ml of 0.3 M NaOH and heated (till 60°C) with vigorous stirring. Then the reaction mixture was rotated at 36°C. Yielding yellow oily residue was neutralized with 1M HCl (pH~7), rotated and separated as previously described by column chromatography method. Yielding clear colorless final product, which according to TLC analyses showed two spots with $R_f$ 0.32 and $R_f$ 0.7.

# 4 RESULTS AND DISCUSSION

## *4.1 Synthesis of FDG precursor*

Synthesis of 1,3,4,6-Tetra-*O*-acetyl-2-*O*-trifluoromethanesulfonyl-β-D-mannopyranose is based on previously described synthesis by Toyokuni et al. This synthesis was made in five steps. In the first step iodine-promoted acetylation was employed. D-mannose was added portion wise to a solution of iodine in acetic anhydride. Addition of the sugar must be done with care, because the reaction is exothermic. The resulting pentaacetate (Product 1) was thick yellow oil, which is comprised a mixture of α and β anomers (3:1). This product was used in the next step without further purification. The yield was ~100%, $R_f$ 0.43.

The next stage was two steps reaction. Firstly, pentaacetate (Product 1) was treated with 33% solution of bromine in acetic acid yielding acetobromomannose (Product 2), $R_f$ 0.57. Secondly, product 2 was reacted with anhydrous ethanol in the presence of 2,4,6-collidine to afford the orthoester (Product 3). It was necessary that the subsequent reaction with anhydrous ethanol was carried out on the same day due to the instability of the bromo derivative (Capon, B. 2002). The overall yield was 51% from D-mannose. Product 3 was recrystallized from anhydrous ethanol for long-term storage, $R_f$ 0.45. Determined melting point was 102-103°C which was very close to literature data (101-103°C).

The fourth step was hydrolysis of the 1,2-orthoester with 1M aqueous HCl. TLC analyses of the hydrolysis mixture showed almost equivalent amounts of two major products, the desired product (Product 4) at $R_f$ 0.12 and a byproduct at $R_f$ 0.26, together with tracer amounts of two other side products. This phenomenon is clearly described in the publication (Toyokuni, T. et al. 2004). Figure 12 shows hydrolysis products of the 1,2-orthoester.

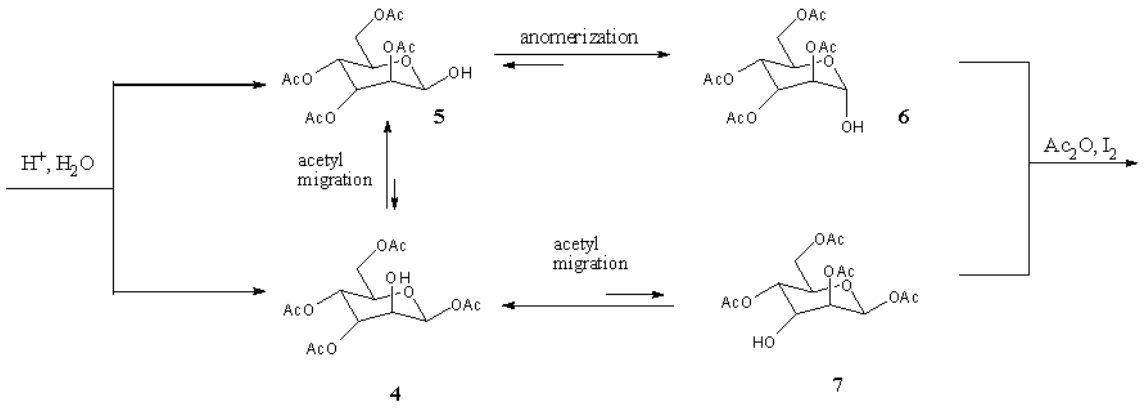



**Figure 12.** Hydrolysis products of the 1,2-orthoester (Toyokuni, T. et al. 2004).

The two fast migrating byproducts were identified as anomeric derivatives 5 and 6, which co-migrated with an authentic mixture of 5 and 6 (5>6) prepared by selective de-*O*-acetylation of the pentaacetate (Product 1) as reported. Product 5 can be anomerized to yield the more stable α-anomer 6. It was also observed that the desired product 4 was gradually converted to the anomers 5/6 with time. This is critical point of this step, because acyl migration is not favorable for this reaction. The reaction mixture can not be stored for a long time in solution. The mixture must be dried immediately after preparation. The third byproduct is tentatively identified as 1,2,4,6-tetra-*O*-acetyl-β-D-mannopyranose (7) based on O-3 to O-2 acetyl migration of 1,3,4,6-tri-*O*-acetyl-D-mannopyranose.

Formation of byproduct 5 in hydrolysis of 1,2-orthoester is more preferable than formation of desired product 4. Therefore, we tried to find optimal conditions to get higher yield of 1,3,4,6-Tetra-*O*-acetyl-β-D-mannopyranose. We investigated hydrolysis of product 3 at different acid concentrations and different solvents (acetone and acetonitrile). The kinetic of reaction was observed by TLC analyses as described above and NMR analyses. NMR analyses showed that two products were formed in one time in acid hydrolysis reaction and rate of products were 1/1(Appendix 12).

During these experiments we observed, that speed of reaction depends on concentration of acid. At the higher acid concentration it goes faster than at lower and two products were formed in the same time (desired at $R_f$ 0.12 and a byproduct at $R_f$ 0.26). We also investigated dependence between speed of reaction and amount of water.

Results of our studies showed that at lower amount of water reaction goes faster than at higher. Additionally to this, we checked influence of weaker and stronger acids such as acetic acid and hydrobromic acid. Hydrolysis was completed faster in presence of HBr than HCl, but difference was not so big. In presence of acetic acid reaction did not start at all even in acetonitrile. There was also influence of solvents. We observed that reaction was followed faster in acetonitrile than in acetone. Moreover, addition of solid $NaHCO_3$ for neutralization of acid must be excluded from synthesis, because it could increase formation of byproduct.

The final step is triflation of the mannose tetraacetate with triflic anhydride. Triflic anhydride was used in an equimolar amount (1 eq) to yield the product 8 as white needles. The product was 1,3,4,6-tetra-*O*-acetyl-2-trifluoromethanesulfonyl-β-D-mannopyranose, what was 11% from D-mannose. Melting point was 118-119°C which is very close to published value of 118-120°C.

Table 3 represents the results of three syntheses, which were started from hydrolysis of the 1,2-orthoester (Product 3) with 1M aqueous HCl. HPLC analysis showed only one peak in all probes: probe 1 with retention time 6.41 min and the purity was 99.4%; probe 2 6.35 min/99.7% and probe 3 6.32 min/95.0%. NMR spectra were identical in all probes (Appendix 7-11).

**Table 3**
Counted results of synthesis.

| Probe | Product 3 [mmol] | Product 4 [mmol] | Yield of product 4 [%] | Product 8 [mmol] | Yield of product 8 [%] | Total yield [g] | Yield from D-Mannose [%] |
|---|---|---|---|---|---|---|---|
| 1 | 2.7 | 0.6 | 22 | 0.4 | 68 | 3.0 | 11 |
| 2 | 10.7 | 3.3 | 30 | 2.6 | 80 | | |
| 3 | 13.8 | 4.9 | 35 | 3.3 | 66 | | |

In comparison the amount of final product and filtrate, which was received from hydrolysis of products 3 with 1M HCl we can see that proportion was 3:5 from the main synthesis and 1:2 from the recycling process. We can explain this moment by exclusion

of recrystallization of 1,2-orthoester in recycling synthesis, which increased the yield of the final product. However, if we want to synthesis larger amount of 1,3,4,6-Tetra-*O*-acetyl-2-trifluoromethanesulfonyl-β-D-mannopyranose (approximately 10 g), it is better to recrystallize product 3. TLC analyses showed that iodine spot had approximately placed on the same place as byproduct in the hydrolysis of 1,2-orthoester, $R_f$ 0.29. Therefore, it is harder to recognize progress of reaction. Moreover, acid-catalyzed hydrolysis of ortho ester proceeded better in recrystallized product, because presence of iodine and solvent could hamper process of hydrolysis.

We calculated approximately the price of synthesis to see economical picture. Table 4 shows amounts and prices of reagents what are needed for synthesis of 3 g of mannose triflate from 10 g of D-Mannose.

As we can see the price of reagents for synthesis of 1g of 1,3,4,6-Tetra-*O*-acetyl-2-trifluoromethanesulfonyl-β-D-mannopyranose was 331.6 EEK. In addition, it is important to take into account amount of solvents and peripherals materials what were used for washing, drying, crystallization, TLC and NMR analyses, work hours which were expended for two weeks of synthesis.

**Table 4**

Amounts and prices of reagents for synthesis of 3g of 1,3,4,6-Tetra-*O*-acetyl-2-trifluoromethanesulfonyl-β-D-mannopyranose.

| Reagent | Mass [g] | Volume [mL] | Price [EEK/g] | Price [EEK/mL] | Price [EEK] |
|---|---|---|---|---|---|
| D-Mannose | 10 | | 25.06 | | 250.60 |
| Iodine | 0.13 | | 36.56 | | 4.64 |
| Acetic anhydride | | 52 | | 0.89 | 11.24 |
| Glacial acetic acide | | 25 | | 3.21 | 15.45 |
| 30% HBr/AcOH | | 22 | | 5.36 | 117.83 |
| Acetonitrile (anhydrous) | | 8 | | 2.28 | 18.24 |
| 2, 4, 6 - collidine | | 11 | | 5.8 | 63.8 |
| EtOH (anhydrous) | | 12 | | 7.24 | 86.88 |
| Acetone | | 25 | | 0.44 | 11.00 |
| 1.0 M Hydrochloric acid | | 6.8 | | 1.65 | 11.22 |
| Dichloromethane (anhydrous) | | 7 | | 1.84 | 12.72 |
| Dry pyridine | | 1.62 | | 2.34 | 3.79 |
| Trifluoromethanesulfonic anhydride | | 1.62 | | 157.2 | 254.66 |
| Diethyl ether | | 100 | | 1.17 | 117.00 |
| Orcinol Monohydrate | 0.5 | | 31.51 | | 15.76 |
| | | | | Total | 994.83 |

## *4.2 Synthesis of "cold" FDG*

The "cold" FDG synthesis carried out as a purity control of mannose triflate. In this work nucleophilic fluorination method of FDG precursor was used and protective groups of TA-FDG were removed by acidic and alkaline hydrolysis using respectively 1M HCl and 0.3 M NaOH.

We had observed that reaction time of alkaline hydrolysis was longer than it was described in previously researches (1-2 min) (Füchtner, F. et al. 1995). Moreover, it was possible to use chromatographic column filled with silica gel for purification of FDG instead of ion exchange chromatography (Hamacher, K. et al. 1986). According to TLC analysis the clear colorless final product was determined as $R_f$ 0.32. This value was seen in both types of hydrolysis. However, in case of alkaline hydrolysis a residue peak of partially hydrolyzed TA-FDG was observed in the chromatogram ($R_f$ 0.7). This byproduct was not seen in the case of acid hydrolysis.

In our research we could not identify presents of 2-Deoxy-2-chloro-D-glucose (ClDG) in the final product, because FDG and ClDG usually move together on TLC plate ($R_f$ 0.32). The amount of ClDG can be reduced by using sulfuric acid instead of HCl (Fowler, J. S. et al. 2003).

# 5 CONCLUSIONS

In this work we studied and tested reliability of synthesis of mannose triflate (Toyokuni, T. et al. 2004). We can conclude that the suggested procedure is reliable and can be used for synthesis of bulk amounts of mannose triflate. Starting materials and most chemicals are not expensive. Moreover, no expensive special equipment is needed. The overall yield of our test synthesis was about 11% (lit. 12-16%). The synthesis has some critical steps and operations, where scaling up is not easy. A number of additional experiments of acid-catalyzed hydrolysis of 1,2-orthoester showed that increasing of the yield of the desired product (1,3,4,6-Tetra-*O*-acetyl-β-D-mannopyranose) was not possible in this step, because formation of byproduct (2,3,4,6-Tetra-*O*-acetyl-β-D-mannopyranose) was more preferable under the conditions tested. Investigation into the hydrolysis reaction of 1,2-orthoester through NMR analyses showed that the desired product and byproduct were formed at the same time and in proportion 1:1 to each other. Therefore, increasing of the yield of the final product (mannose triflate) could be increased thanking to optimization of other steps as recrystallization of intermediate products, acetylation of D-mannose, etc.

Synthesis of "cold" FDG can be used as purity control of FDG-precursor. Column chromatography can be used for separation of FDG from byproducts instead of ion exchange chromatography.

Test synthesis performed in this study seems to be a great help for planning of large-scale synthesis. Practical and reliable protocol of large scale preparation of mannose triflate was prepared and filled as an official document of PharmaSynth AS.

# 6 KOKKUVÕTE

Antud töös kontrolliti Tatsushi Toyokuni poolt kirjeldatud 1,3,4,6-Tetra-*O*-atsetüül-2-*O*-trifluorometaansulfonüül-β-D-mannopüranoosi (mannoos triflaat) sünteesi nii analüütiliste kui ka preparatiivsete reagentide hulkadega.(Toyokuni, T. et al. 2004). Leidsime, et selle sünteesi eeskirja järgi on võimalik läbi viia suurema hulga triflaadi süntees. Sünteesi lähte- ja abiained ei ole kallid. Sünteesi läbiviimine ei nõua spetsiaalset aparatuuri. Viieetapilise sünteesi produkti üldine saagis oli 11% (lit. 12-16%) lähteainest. On probleemsemaid etappe sünteesil, mis viivad üldise sünteesi saagise alla ja ei ole hästi reprodutseeritavad. 1,2-ortoestri happe-katalüütilise hüdrolüüsi lisauuringuid TMR-ga näitasid, et eelistatava produkti (1,3,4,6-Tetra-*O*-acetyl-β-D-mannopyranose) saagist oli raske suurendada, sest lisaprodukti tekkimine (2,3,4,6-Tetra-*O*-acetyl-β-D-mannopyranose) on eelistatuim protsess (produktide suhe 1:1). Järelikult, lõppprodukti saagise tõstmine on võimalik teiste etappide optimiseerimises nagu vaheproduktide kvantitatiivsem eraldamine ja puhastamine jne.

„Külma“ FDG sünteesi kasutati vaid FDG-prekursori puhtuse kontrolliks. FDG puhastamiseks kasutati edukalt kolonnkromotograafiat silikageelil ioonvahetuskromotograafia asemel.

Läbiviidud sünteesi tee annab võimaluse planeerida suuremal hulgal FDG prekursori sünteesi GMP tingimustes. Suure hulga mannoos triflaadi valmistamise usaldusväärne protokoll on esitatud PharmaSynth AS-le kui ametlik dokument.

# ACKNOWLEDGEMENTS

I would like to thank the following persons for their observant attitude and advice in the preparatory phase of the project in PharmaSynth AS: Olavi Loog, Gerda-Johanna Raidaru, Prof. Jaak Järv, Vahur Mäemets and Vladimir Stepanov. It was very interesting to work in the premises of PharmaSynth AS in the Tartu Science Park.

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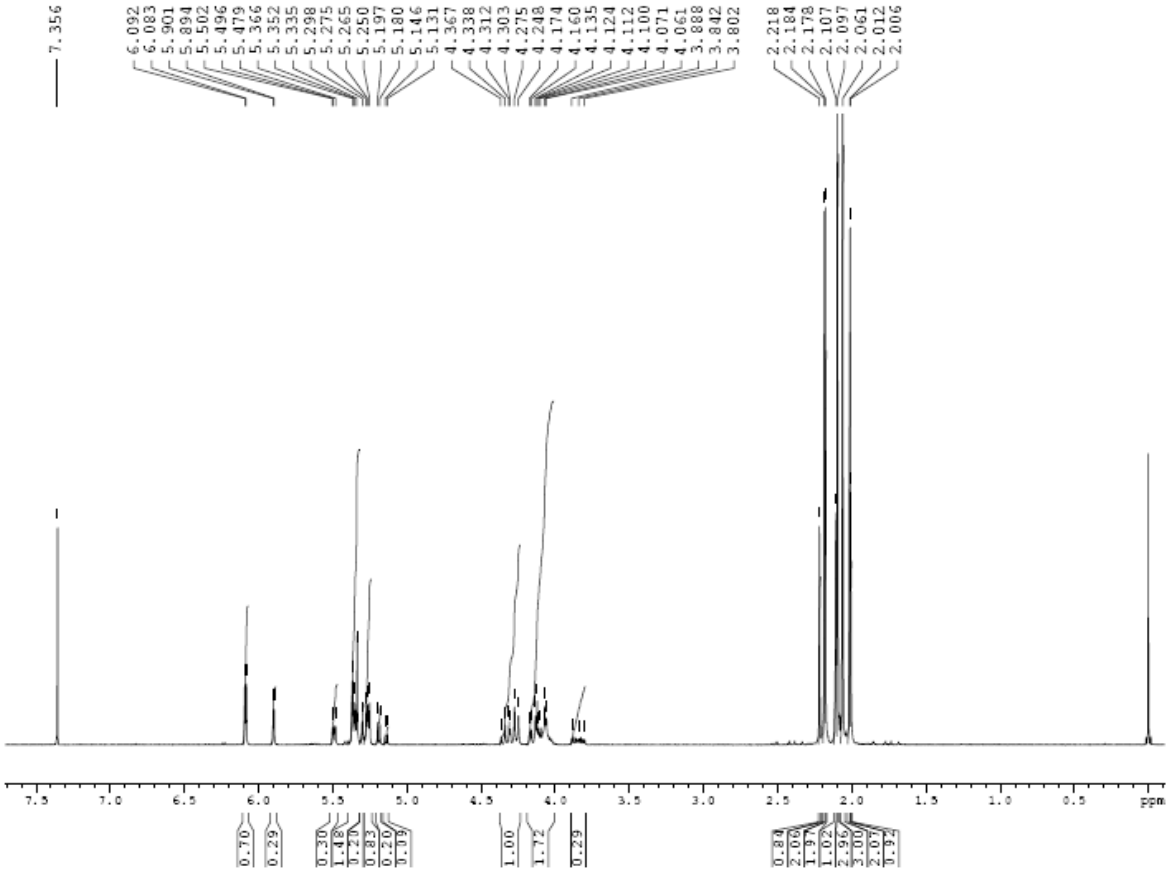
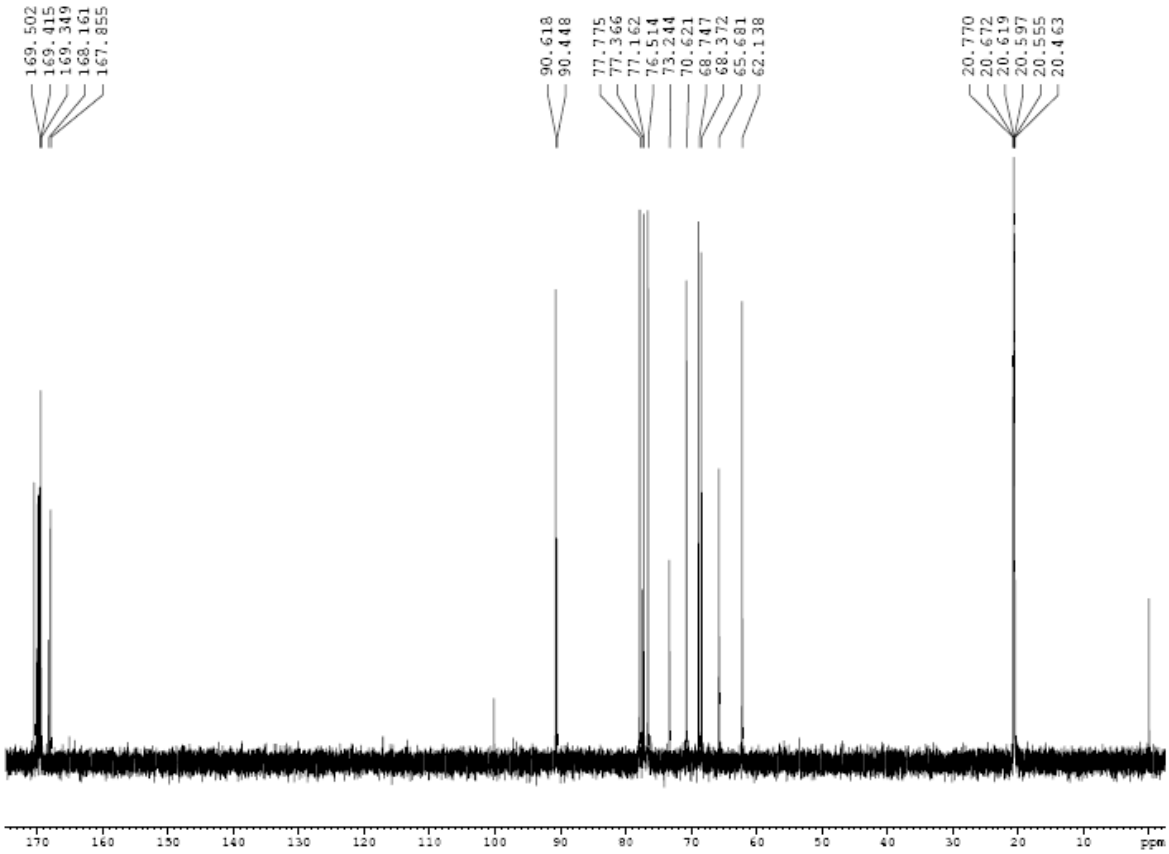
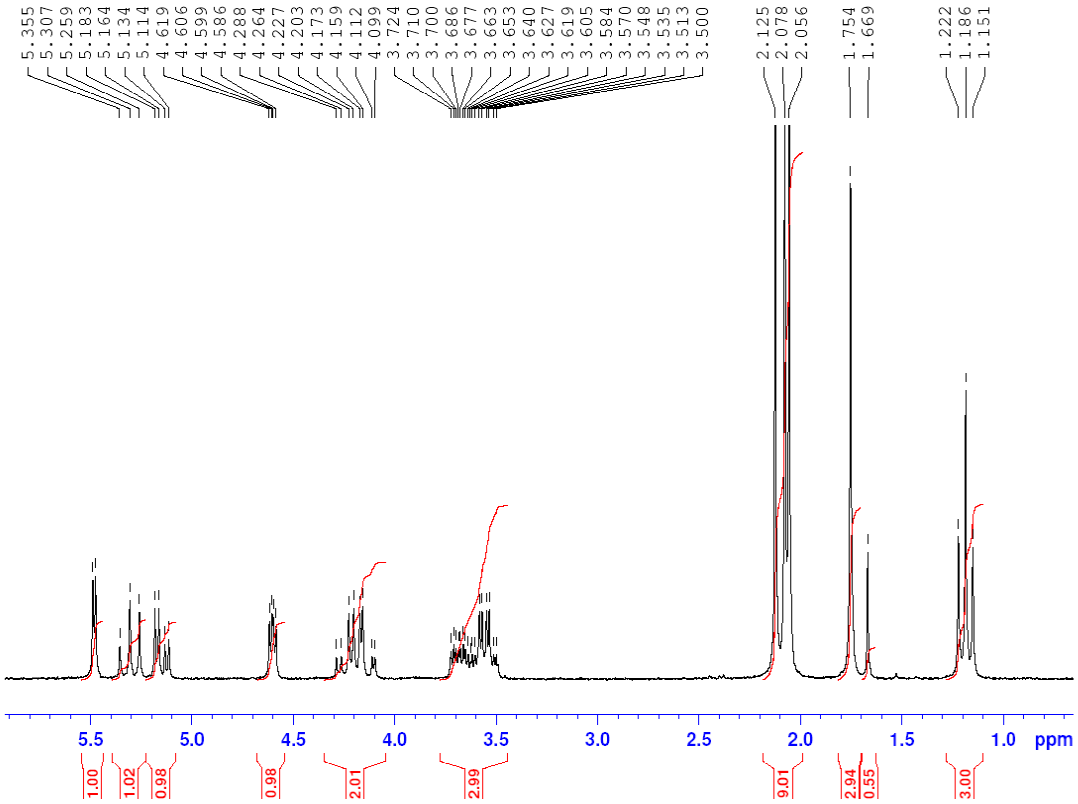
# APPENDIXES



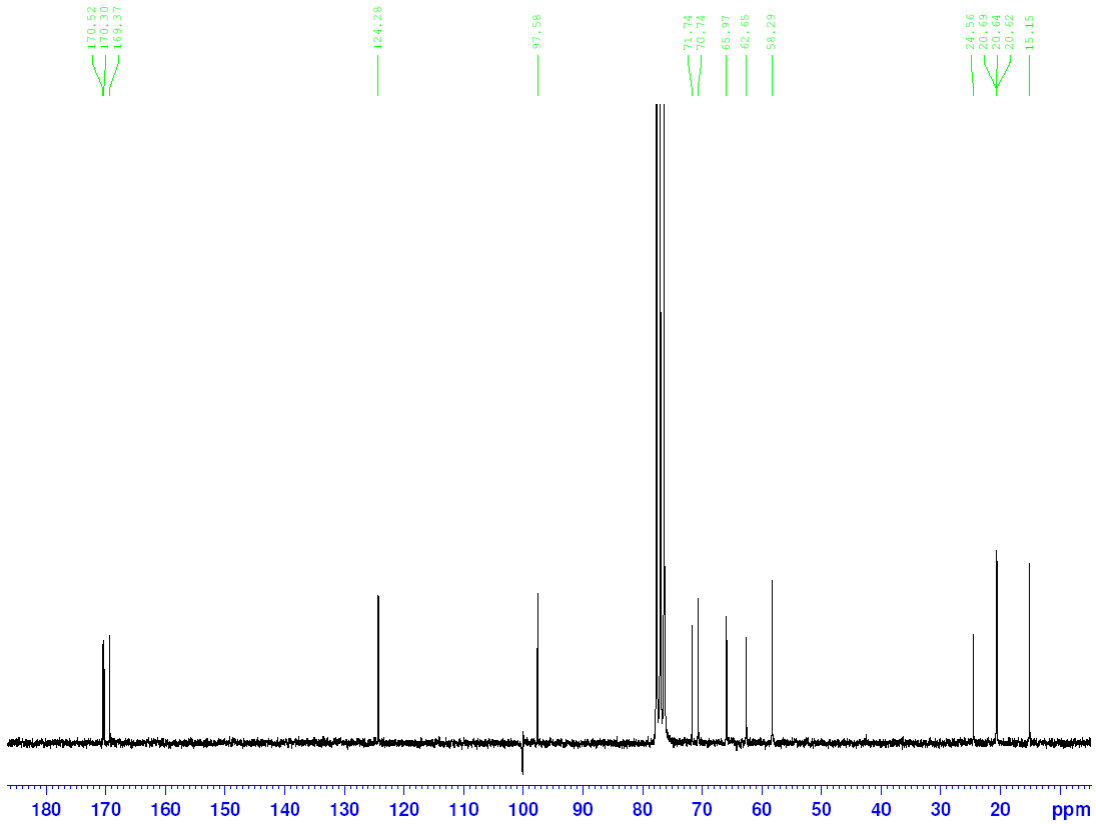

**Appendix 1.** $^{1}$H NMR (ppm) ($CCl_3D$; 200 MHz). Penta-*O*-acetyl-D-mannopyranose



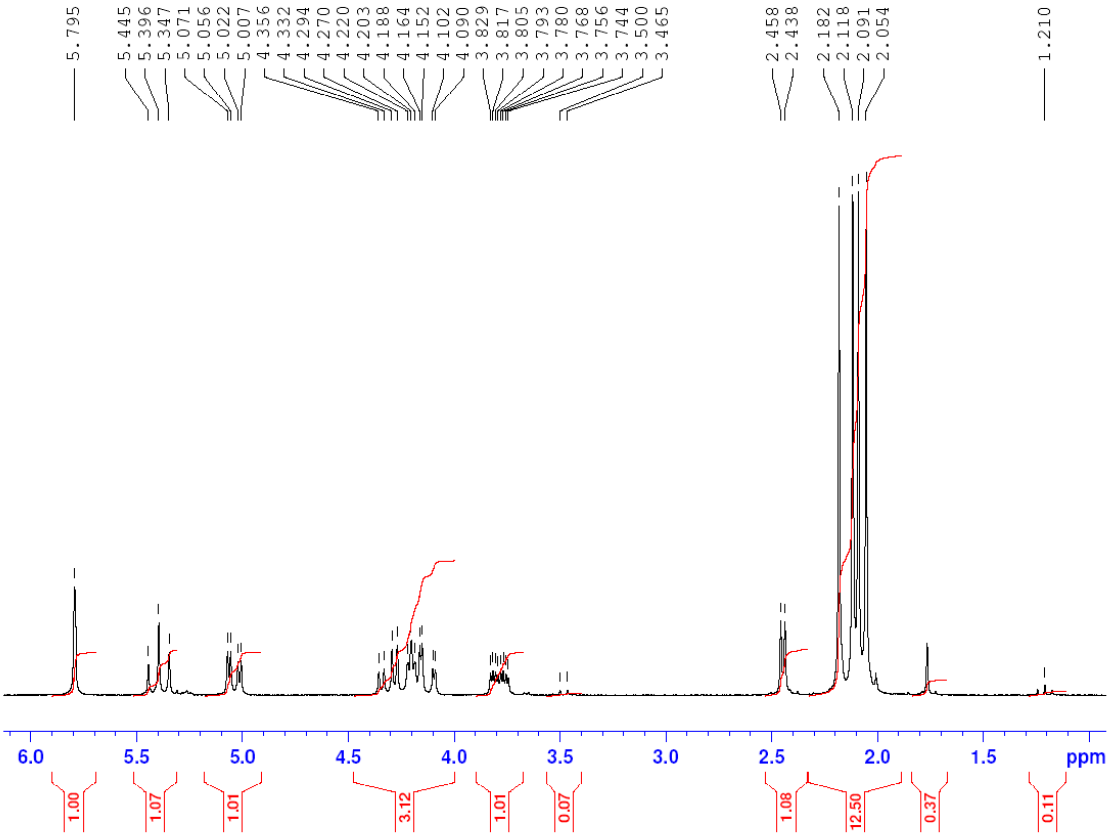

**Appendix 2.** $^{13}C$ NMR ($CCl_3D$; 50 Hz). Penta-*O*-acetyl-D-mannopyranose



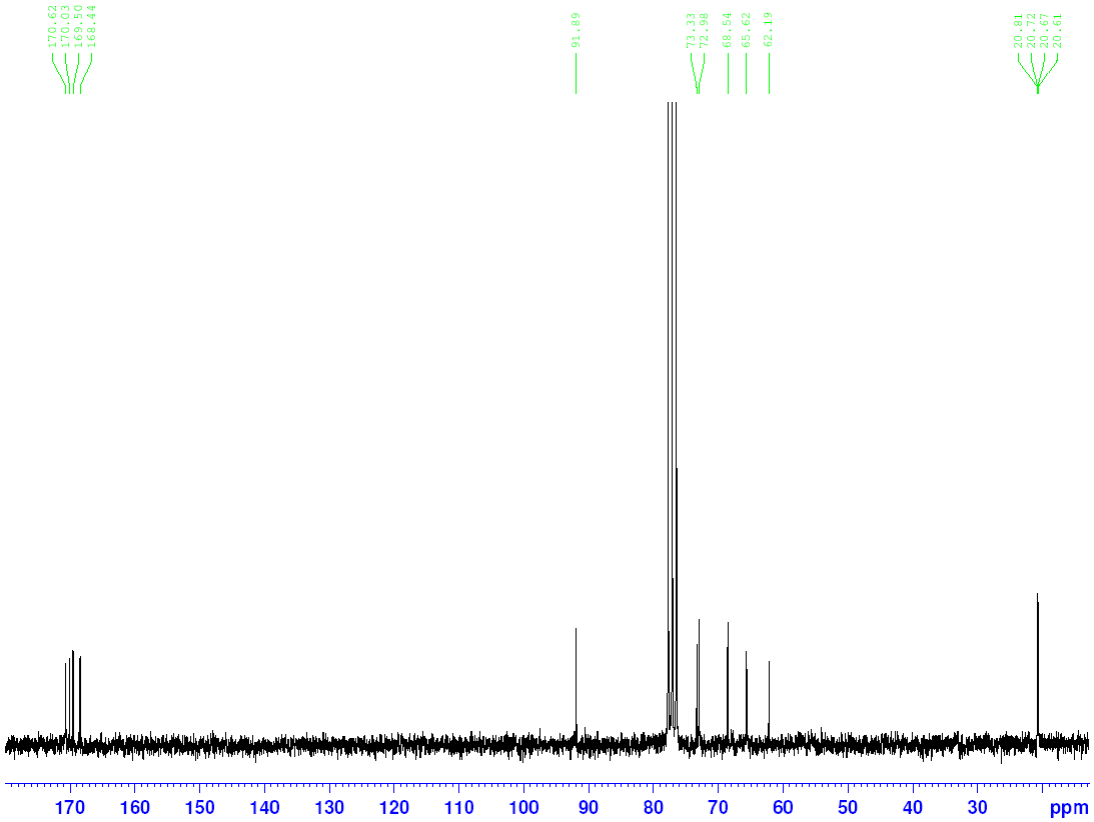

**Appendix 3.** $^{1}$H NMR (ppm) ($CCl_3D$; 200 MHz). 3,4,6-Tri-*O*-acetyl-1,2-*O*-(1-ethoxyethylidene)-β-D-mannopyranose.



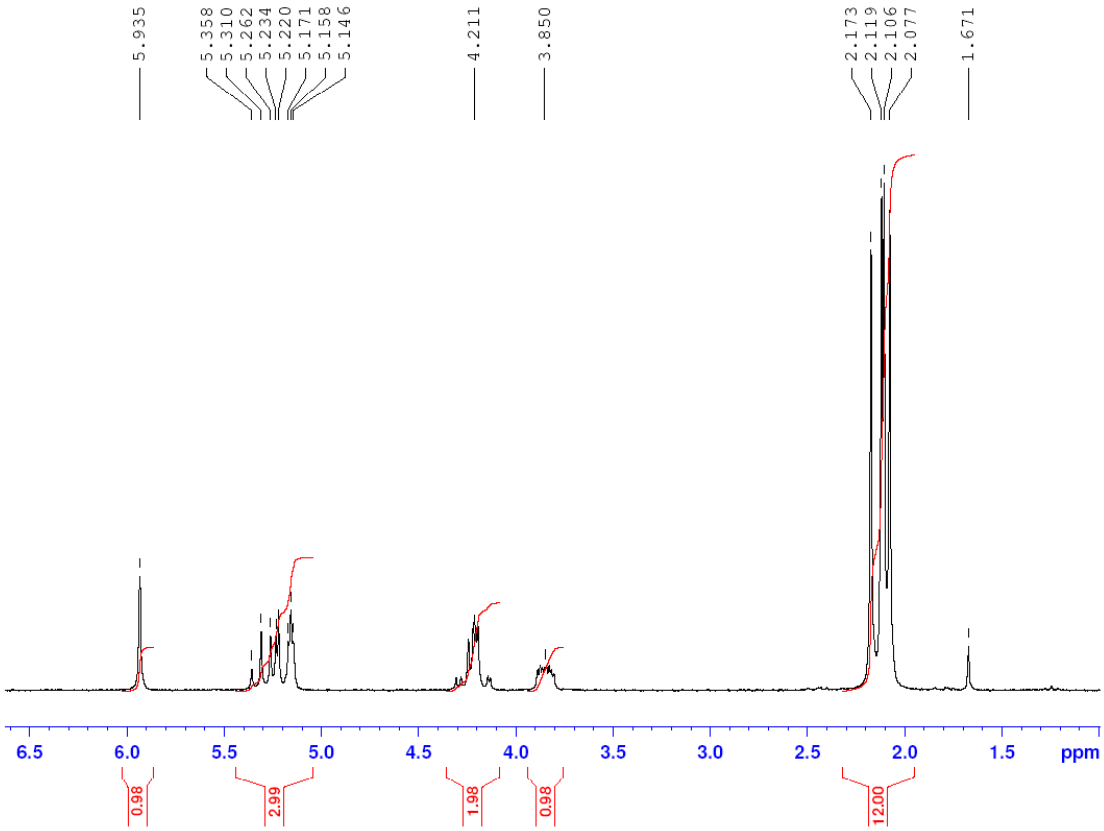

**Appendix 4.** $^{13}C$ NMR ($CCl_3D$; 50 Hz). 3,4,6-Tri-*O*-acetyl-1,2-*O*-(1-ethoxyethylidene)-β-D-mannopyranose.



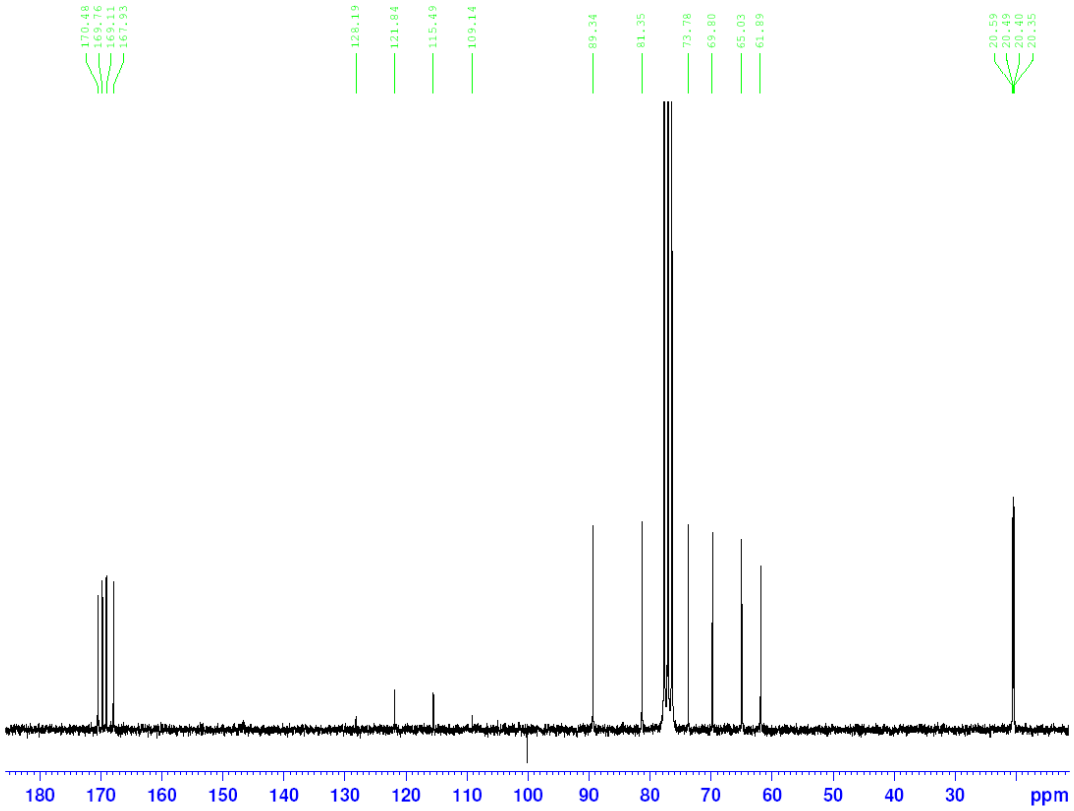

**Appendix 5.** $^{1}$H NMR (ppm) ($CCl_3D$; 200 MHz). 1,3,4,6-Tetra-*O*-acetyl-β-D-mannopyranose.



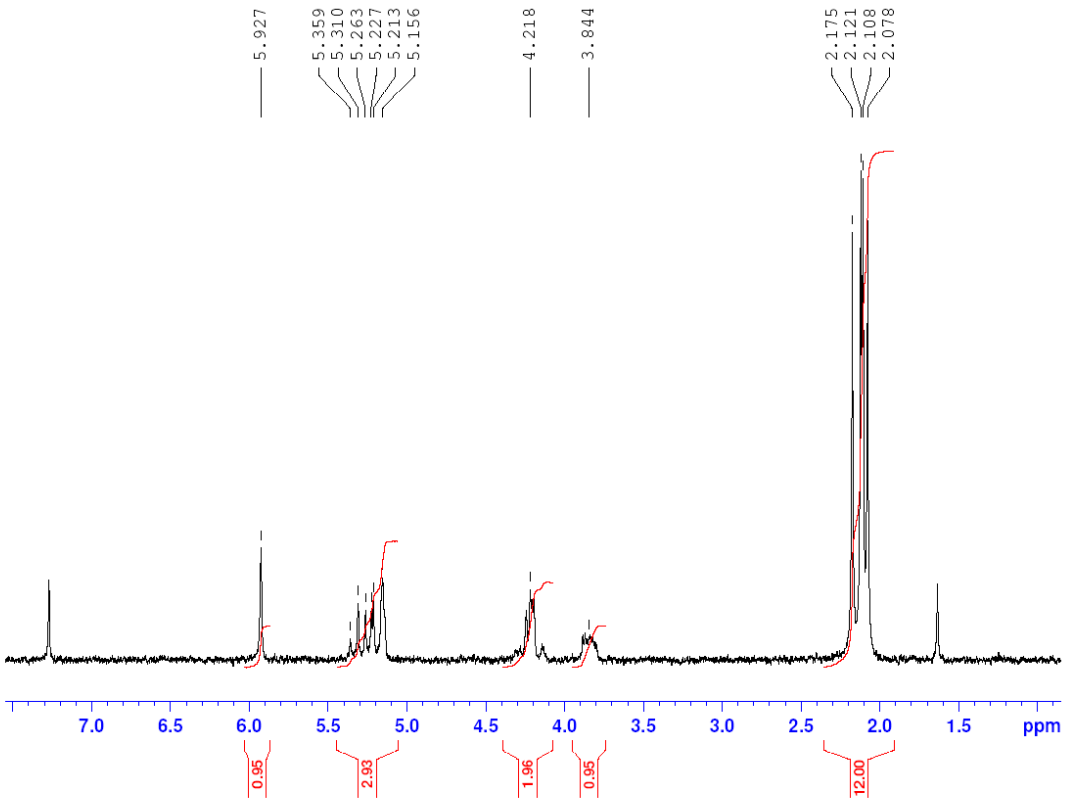

**Appendix 6.** $^{13}C$ NMR ($CCl_3D$; 50 Hz). 1,3,4,6-Tetra-*O*-acetyl-β-D-mannopyranose.



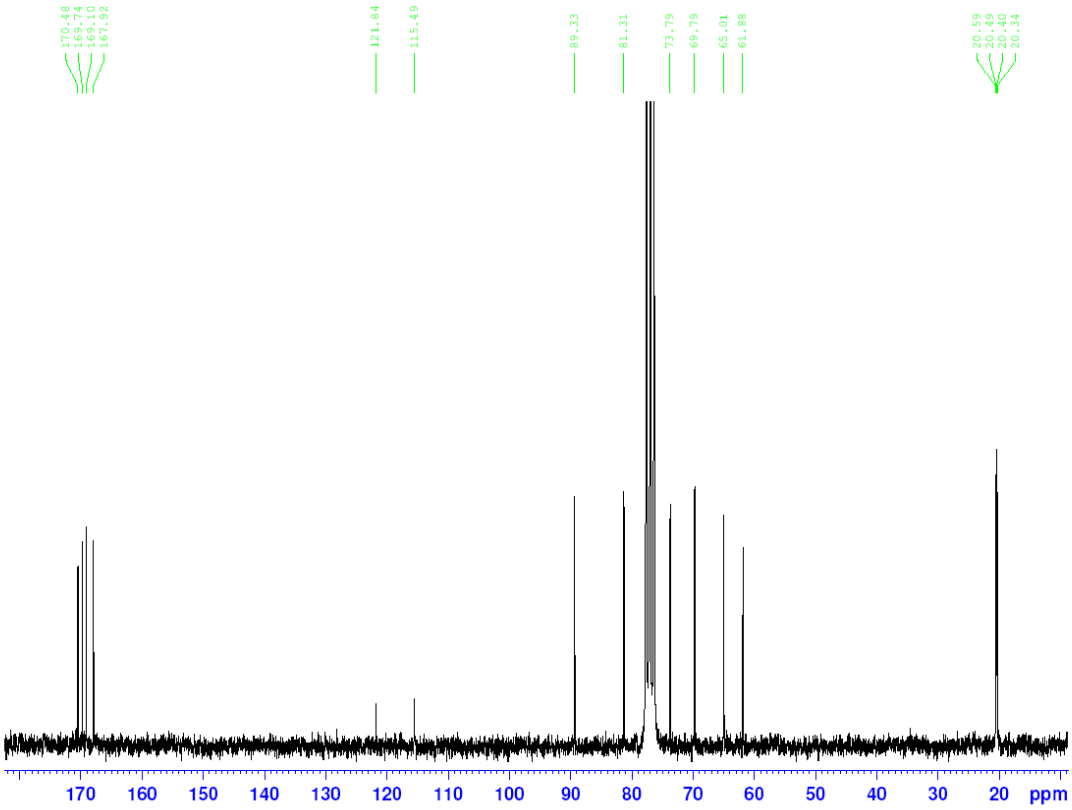

**Appendix 7.** $^{1}H$ NMR (ppm) ($CCl_3D$; 200 MHz); probe No 2: 1,3,4,6-Tetra-*O*-acetyl-2-*O*-trifluoromethanesulfonyl-β-D-mannopyranose (mannose triflate).



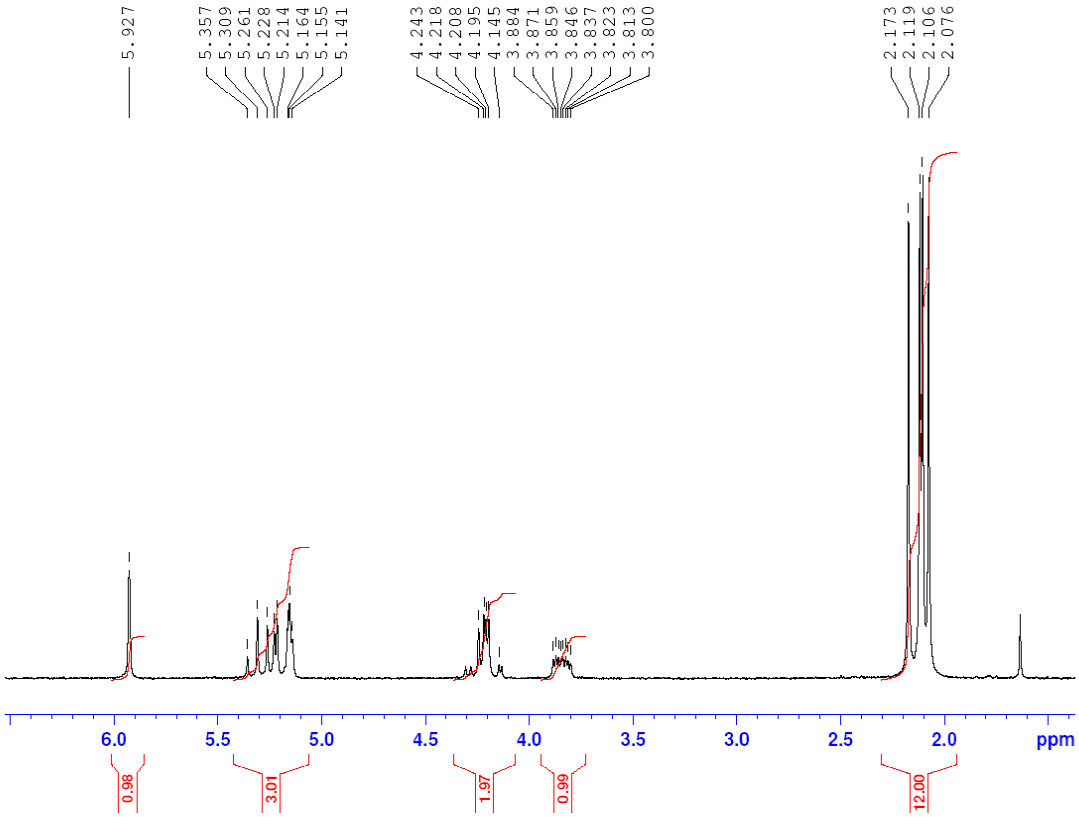

**Appendix 8.** $^{13}C$ NMR ($CCl_3D$; 50 Hz); probe No 2: 1,3,4,6-Tetra-*O*-acetyl-2-*O*-trifluoromethanesulfonyl-β-D-mannopyranose (mannose triflate).



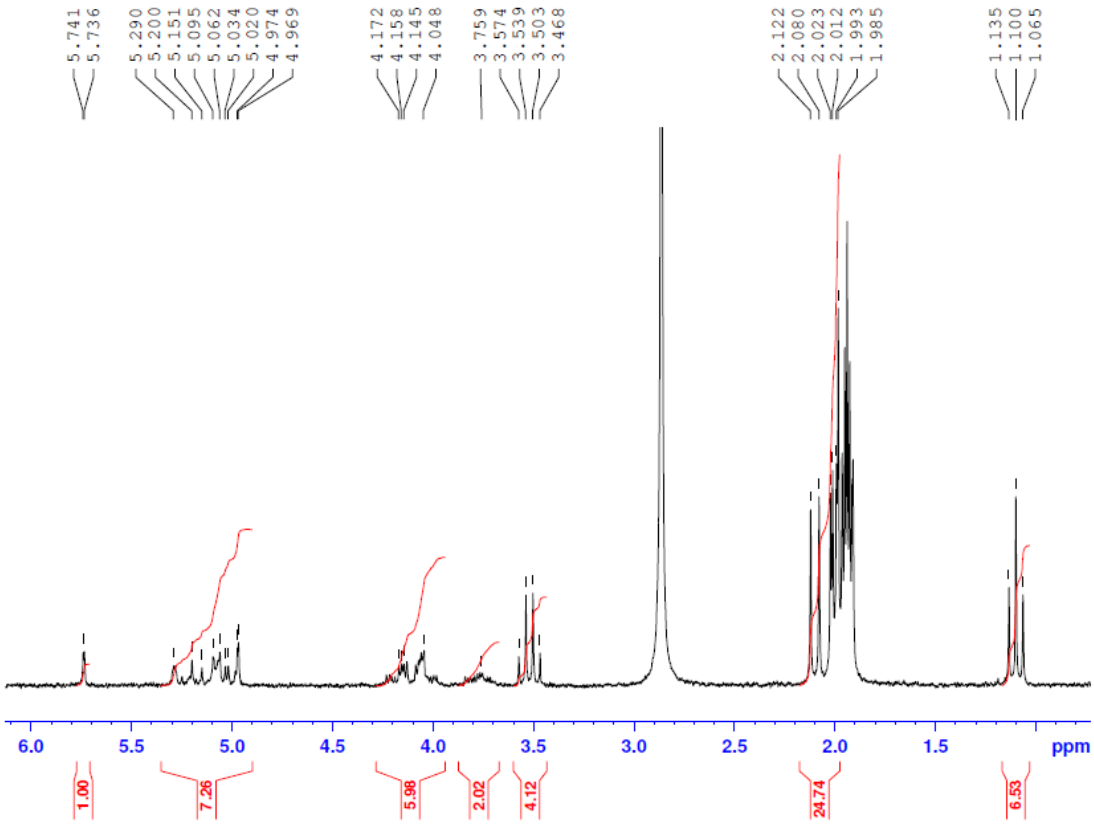

**Appendix 9.** $^{1}$H NMR (ppm) ($CCl_3D$; 200 MHz); probe No 1: 1,3,4,6-Tetra-*O*-acetyl-2-*O*-trifluoromethanesulfonyl-β-D-mannopyranose (mannose triflate).




**Appendix 10.** $^{13}C$ NMR ($CCl_3D$; 50 Hz); probe No 1: 1,3,4,6-Tetra-*O*-acetyl-2-*O*-trifluoromethanesulfonyl-β-D-mannopyranose (mannose triflate).




**Appendix 11.** $^{1}$H NMR (ppm) ($CCl_3D$; 200 MHz); probe No 3: 1,3,4,6-Tetra-*O*-acetyl-2-*O*-trifluoromethanesulfonyl-β-D-mannopyranose (mannose triflate).




**Appendix 12.** $^{1}$H NMR (ppm) ($CCl_3D$; 200 MHz). 3,4,6-Tri-*O*-acetyl-1,2-*O*-(1-ethoxyethylidene)-β-D-mannopyranose.