UNIVERSITY OF EASTERN PIEDMONT

“AMEDEO AVOGADRO”

DEPARTMENT OF PHARMACEUTICAL SCIENCES

The sustainable synthesis of

p-Tolyl-1-Thio-4,6-Benzylidene-2,3-Di-*O*-Benzyl

-β-D-Mannopyranoside

Master's Degree Course in Pharmaceutical Chemistry and Technology

Supervisor

Panza Luigi

Co-Supervisor

Imperio Daniela

Candidate

Rizwan Waleed

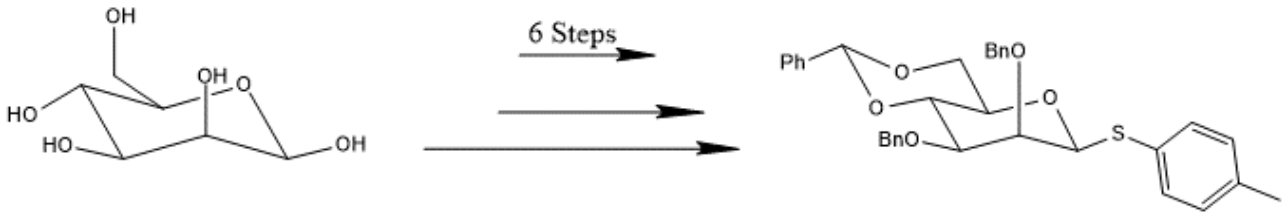
Academic year 2022/2023

Special Session

Table of Contents

1 ABSTRACT

In this thesis we aim to synthesize p-Tolyl-1-Thio-4,6-Benzylidene-2,3-Di-O-Benzyl-β-Mannopyranoside in sustainable way. Our aim was to create alternative way which would be cheaper, eco-friendly and in simplest conditions. For this molecule there has been a lot of paper published in different journals, most of them used harsh conditions.



2 ABBREVIATIONS

Py Pyridine

MeOH Methanol

DMF Dimethyl Formaldehyde

EtOAc Ethyl Acetate

PPTS Pyridinium p-toluenesulfonate

Tol Toluene

Cy Cycle Hexane

Hex n-Hexane

DMAP 4-Dimethylaminopyridine

Rt Room Temperature

EtOH Ethanol

AcOH Acetic Acid

Ac2O Acetic Anhydride

MeCN Acetonitrile

DCM Dichloromethane

CSA Camphor Sulphonic Acid

THF Tetrahydrofuran

MBTE Methyl tert-Butyl ether

MSA Methanesulfonic Acid

3 D-MANNOSE

3.1 HISTORY

D- Mannose originates from "Manna" a Biblical word (Tonnesen 1983) also known as Mannopyranose. It is a biomolecule, a food supplement and a glyconutrient that can play a significant role in human health in protein synthesis and their glycosylation process. Manna was used as an energy source during the Jews' (Israelites) journey to the Sinai Peninsula (Price 2012). The existing sources of Mannose are components of Mannan, Hemi-cellulose, or Cellulose. It is everywhere and we consume it in our daily lives. The spent Coffee ground contains 21.2%, Egg albumin holds about 2%, Mango has 0.03% and even human serum has about 0.03% mannose.(4)Hu et al., 2016)

3.2 PHYSIOCHEMICAL PROPERTIES

D-Mannose is the C- 2 epimer of D-glucose with the chemical formula $C_5H_{11}O_5CHO$. It is highly soluble in water and slightly in EtOH. Its physical appearance is a white crystalline powder. It is 0.6 times sweeter than sucrose, but it has a bitter aftertaste so that this is the main reason why it is not being used as a sweetener. It has been seen that the alpha isomer is sweet, and the beta is bitter. The melting point of Mannose is 132°C and the caloric value is 3.75 kcal/g. It is odourless and its molecular weight is 180.16 g/mol.

3.3 PRODUCTION

3.3.3 EXTRACTION

Mostly it is extracted from plants and fruits. The extraction methods are Acid hydrolysis, Thermal hydrolysis, Enzymatic hydrolysis and Microbial fermentation hydrolysis.

3.4 PRODUCTION BY CHEMICAL METHODS

It has been reported in different chemical ways, The first was using D-glucose converting into D-mannose, starting from sucrose treating with acid can produce Mannose. We can obtain it from Mannitol by oxidizing it with Chromic acid. We can make mannose starting from the depolymerization of Palm Kernel in the presence of sulphuric acid and crystallization with EtOH.

3.5 PRODUCTION BY ENZYMATIC METHOD

Three enzymes can convert Fructose to Mannose and Glucose to Mannose. Enzymes are D-mannose isomerase, D-lyxose isomerase and Cellobiose 2-epimerase.

3.6 Microbial Fermentation

It can be collected from the cell walls of certain bacteria and yeasts. The bacterium Mitsuaria chitosanitabida can manufacture D-glucose, D-mannose and D-galactose in these ratios 18:6:1.

3.7 Use Of D-Mannose

3.8 Food

It is well known to everyone the rapid growth of metabolic syndrome, so the scientists thought of mannose as an alternative to sucrose which is the main cause of metabolic syndrome. As mentioned above it is 0.6 times sweeter than sucrose and less caloric about 3.7 Kcal/g but as mentioned it has a bitter aftertaste. So, the idea was discarded and nowadays we know that polyalcohol and some other artificial sweeteners can tackle metabolic syndrome. D- Mannose is being used in the food industry such as in ice cream, processed fruits and salad dressing to improve texture because mannose has some significant physical properties as a high melting point and high solubility in water.

3.9 Animal Feed

Antibiotics have been used in animal food which increases the growth and overall health of animals. But in recent years it's been observed that bacteria have started to resist antibiotics in human beings because they are consuming meat with antibiotics. So, the alternative they found is mannose because it has antibacterial properties for example for poultry it has been used as a commercial product that has yeast cell wall fragments derived from Saccharomyces cerevisiae and the cell wall of this yeast has many mannans. D-mannose has been the most effective sugar in blocking the colonization of bacteria especially in the intestines of animals.

3.10 Cosmetics

D-Mannose and other sugars can moisturise the skin further cleansing of the skin. Aloe vera which is good for health and as well as for skin has a greater amount of mannose polysaccharide. The Beta linkage of 1-4 glycoside shows its moisturizing ability and skin toning capability. Deckner and Wivell (1997) patented a product of skin moisturizing and cleansing agents made up of sugars with these molar ratios 2.8:2.0:2.0 D-glucose, D-mannose, and D-glucuronate.

3.11 MEDICINE

D-mannose is essential for the synthesis of new secretory proteins and glycoproteins in the human body. It has been studied that mannose has an equivalent effect on probiotics like keeping an equilibrium between the good and bad bacteria in the Intestine. Mannose is used to prevent the Urinary tract infection; it blocks the adhesion of the bacteria to the epithelial cell of the urinary tract. Our body has mannose receptors so adding mannose to our daily intake can stimulate our immune system against any bacterial infection. Mannose can be used to treat the congenital disorder of glycosylation (CDG)-1b this disease is caused by the deficiency of N-glycans. The same deficiency can cause several problems in the human body like loss of proteins in the intestine, hypoglycemia and blood clotting disorders. So, the mannose was recommended to children and infants as a supplement against CDG. Further, if mannan is added to the diet it can reduce the absorption of cholesterol and glucose. Intaking mannans which can be hydrolyzed by stomach acid and converted into D-mannose can bind to macrophage to induce its activity. D-mannitol is used as a vehicle for the new drug against AIDS which is oseltamivir phosphate. Further, thanks to specific mannose transporter in the human body mannose can be used for the delivery of various drugs, drugs which have poor absorption rate and stability. For example, the nanomedicines to the mannose receptors mostly expressed cells in our immune systems, will be extremely useful in treating various cancers and other autoimmune diseases.(9)Sharma et al., 2014)

4 GLYCOSYLATION

The first glycoside was discovered by French chemists Pierre Robiquet and Antoine Boutron-Charlard in 1830. It was amygdalin glycoside which is present in most plants and some notable seeds e.g. kernel seeds, apricot seeds, apple seeds, bitter almonds, peaches, cherries and plums. So, what is glycoside? Glycoside is simply a sugar that has a functional group on its anomeric carbon linked all together with a bond called a glycosidic bond.(2)Crich, 2010)

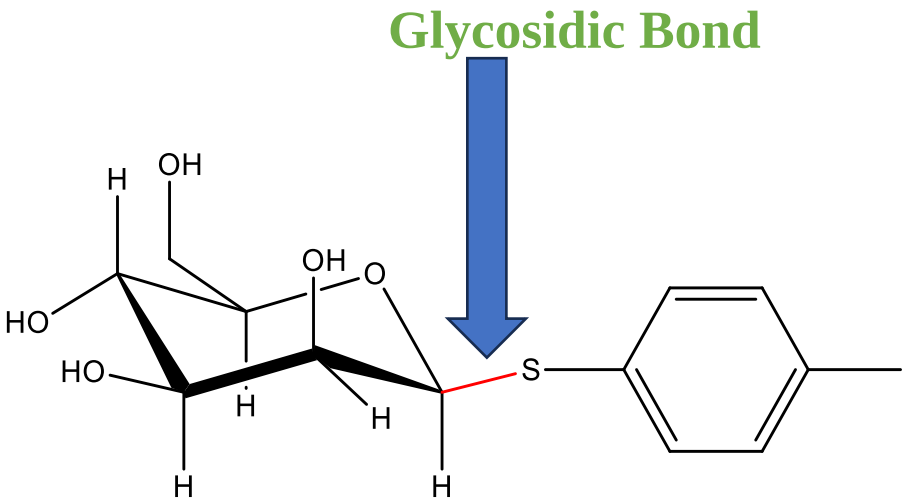
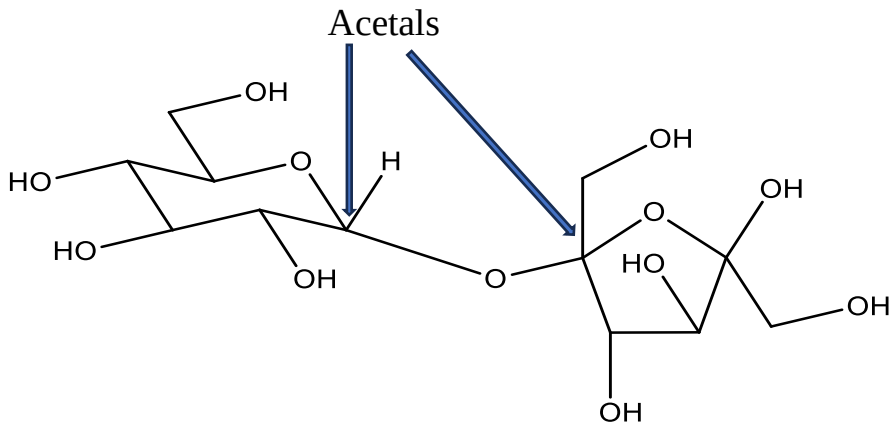


Fig. ThioMannoside

4.1 NOMENCLATURE

The nomenclature for these molecules is simple it goes back to its origin for example if the sugar that is used to form the glycoside is called glucoside. The sugar part is called glycan and the non-sugar part is called aglycone. They are also divided according to their glycosidic bond for example α glycosides and β glycosides. The glycosides are mainly derivatives of acetal and hemiacetal e.g. in the figure below you can see a great example of acetal that we commonly use in our daily life.

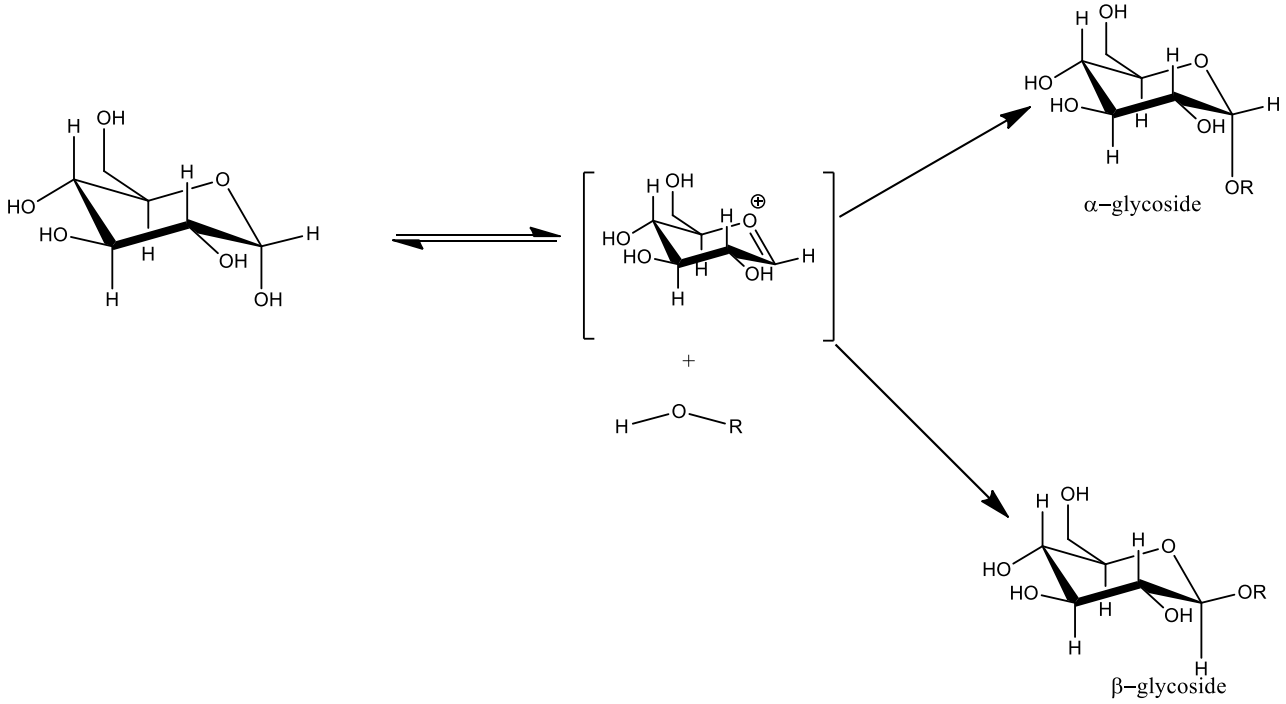


Sucrose; α-D-Glucose β-D-Fructose

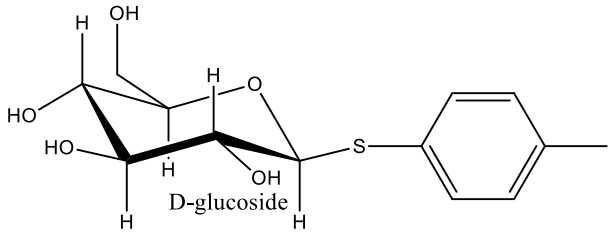
4.2 CLASSIFICATION

The glycosides are classified according to the basis of linkage between the aglycone and glycone part some examples are given below.

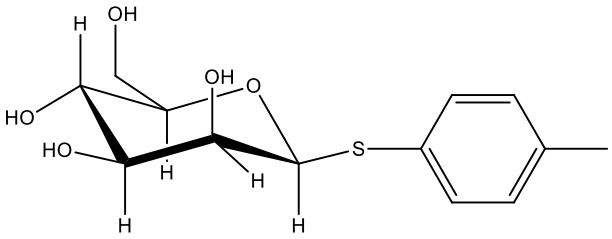
O-glycosides; sugar part linked with alcoholic or phenolic hydroxyl group. E.g. glucose acetal formation.



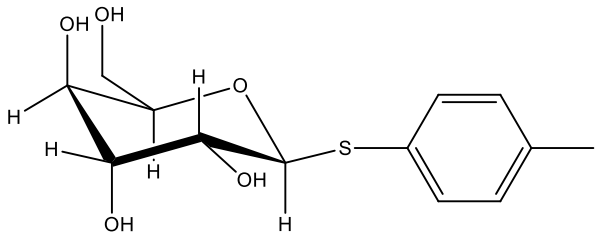
S-glycoside; The linkage of sugar to a sulphur atom of aglycone e.g. below



D-glucoside

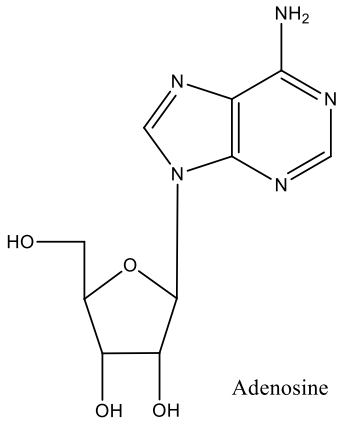


D-Mannoside



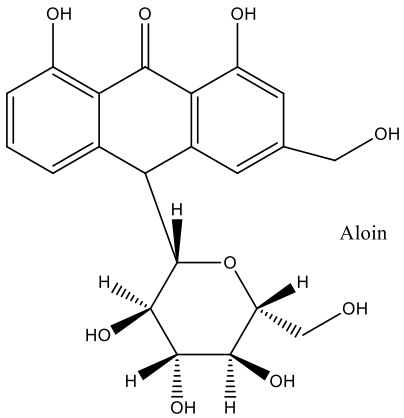
D-Galactoside

N-glycoside: The linkage of sugar to an atom of nitrogen aglycone e.g. Adenosine



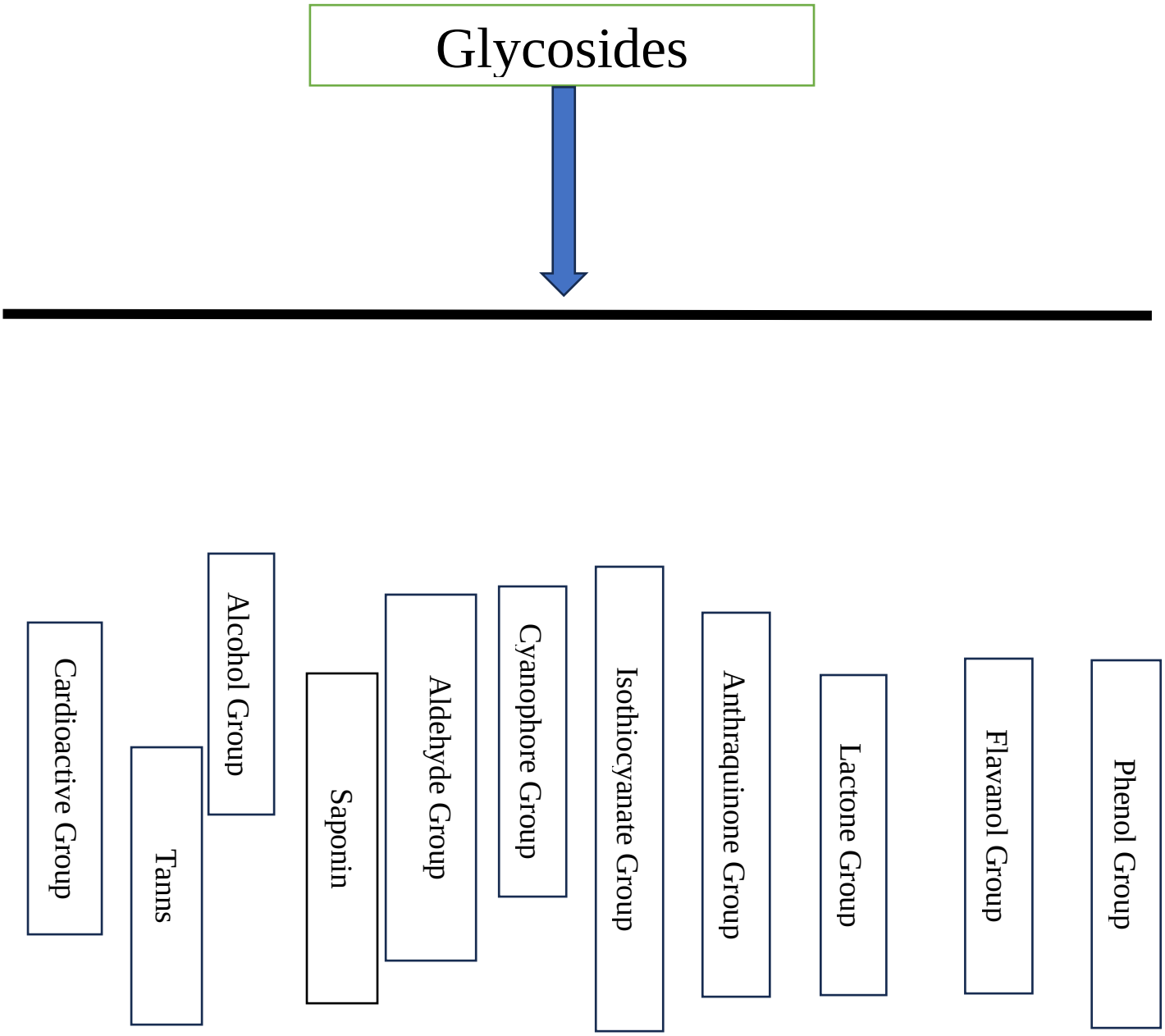
Adenosine

C-glycoside; simply just like others here we have a bond between 2 carbon atoms between glycone and aglycone. E.g. Aloin



Aloin

4.3 Classification according to Aglycone



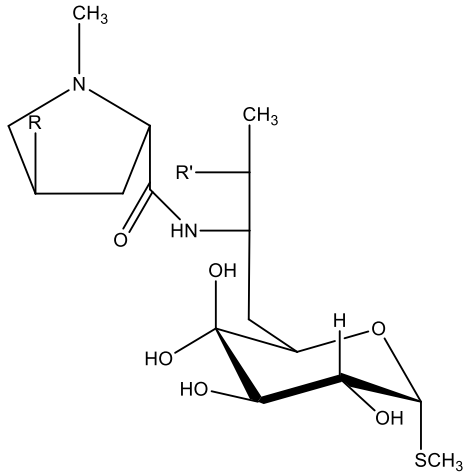
5 THIOGLYCOSIDE

Thioglycosides are hardly present in nature even though they are present in nature in the form of glucosinolates, which are O-Sulphated thiohydroximates of 1-thio-β-D-glucopyranosides. In 2011 approximately 132 thioglycosides have been studied, mostly from plants such as Barbarea vulgaris, Arabidopsis thaliana, Eruca sativa and Isatis tinctoria. (6)Lian et al., 2015)

5.1 BIOLOGICAL IMPORTANCE

Glucosinolates and their by-products have been characterized for their bactericidal, fungicidal, nematocidal and allelopathic properties. Furthermore, cruciferous vegetables such as cabbages and mustard have wound-healing properties as well as antitumor properties. For this reason, for centuries these vegetables have been used and consumed by the people. Glucosinolates and isothiocyanate have cancer chemoprotection activities attracting great interest for the development of new antitumour drugs.(1)Codée et al., 2005; 7)Nicotra et al., n.d.)

Lincomycins are another example of thioglycosides present in nature and they are antibacterial. Lincomycin A and B are antibiotics produced by Streptomyces lincolnensis. They are bacteriostatic, they inhibit proteic synthesis in bacteria, especially in Mycoplasmas, Gram-positive bacteria and cocci, but they have a lot of adverse effects because of it has been substituted by 7-chloro-7-deoxylincomycin or Clindamycin, a semi-synthetic drug. (1)Codée et al., 2005;(3)Guo et al., 2004)



Lincomycin A; R=Pr, R'=OH

Lincomycin B; R=Et, R'=OH

Clindamycin; R=Pr, R'=Cl

5.2 ADVANTAGES	5.2 DISADVANTAGES
It can be activated in very mild conditions They are very stable under a wide range of conditions for functional group. Easily available and stable under acidic and basic conditions.	They are extremely odorous and their waste is extremely toxic. They are being used in stoichiometric amount in reaction so waste management is the biggest problem. And their by-products are utterly undesirable. They can react at intramolecular level, difficulty removing these type of by-products.

6 RESEARCH AND METHODOLOGIES

6.1 AIM OF THE WORK

Our research is inserted into a more general project aiming at simplifying and make more eco-friendly the synthesis of standard building blocks typically used for the preparation of oligosaccharides through chemical glycosylation. Our target was p-tolyl 1-thio-4,6-benzylidene-2,3-di-*O*-benzyl-β-D-mannopyranoside, which is a less common intermediate with respect to the corresponding α-thioglycoside but is more challenging to obtain and, thanks to its different anomeric configuration can influence the stereochemical course of the glycosylation reaction. Finally, not many syntheses of this building block can be found in literature probably because of its not easy accessibility.

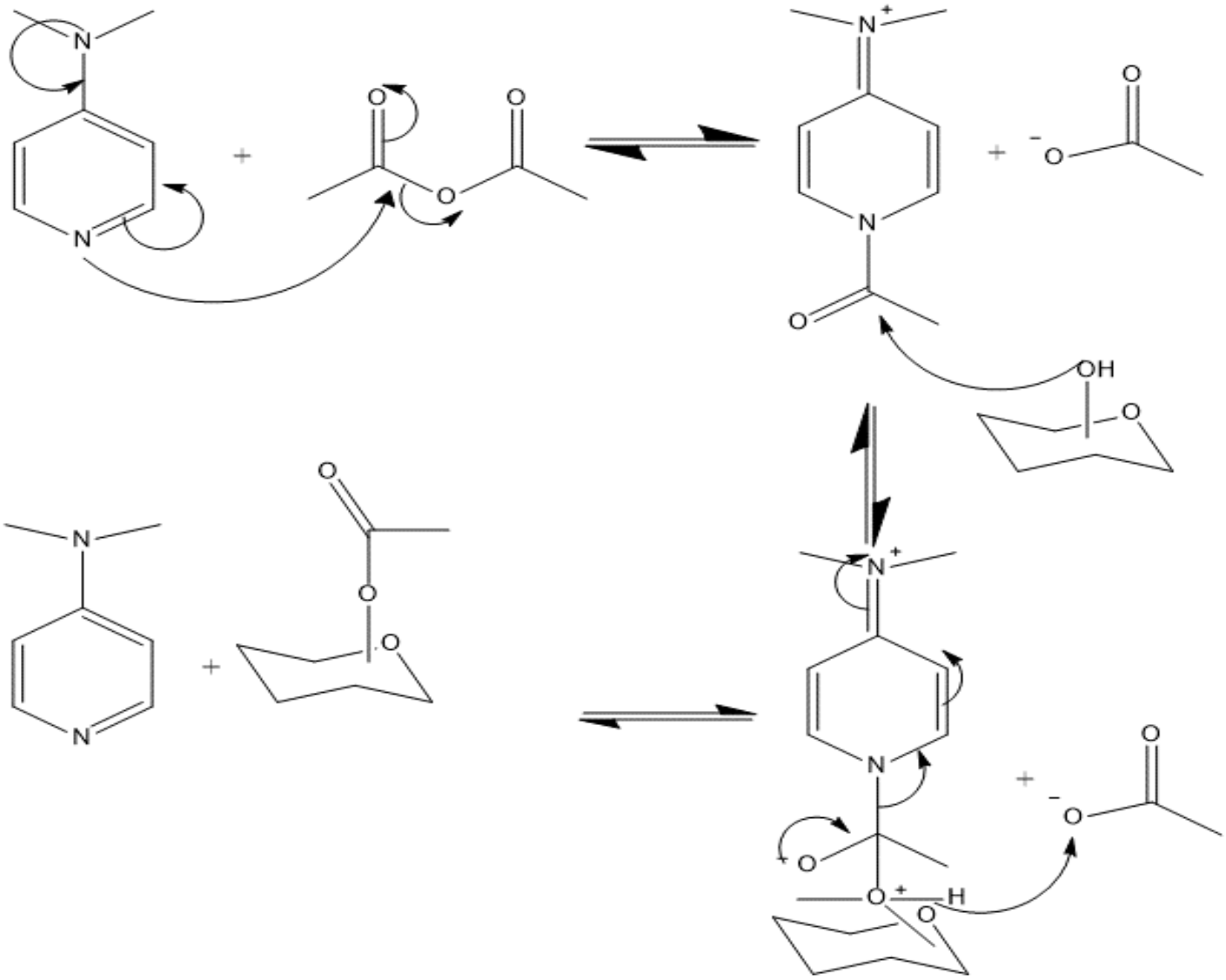
6.2 SYNTHESIS OF 1,2,3,4,6-PENTA-*O*-ACETYL-D-MANNOPYRANOSE

The monosaccharides have hydroxyl groups which are chemically and quite similar, except for the anomeric one. The first step in manipulation of monosaccharides is usually to block their reactivity by using protecting groups. It was proven during the synthesis of ascorbic acid in 1933, that there are some enzymes can be selective these enzymes are often not easily available, thus since then the organic protective groups have been used for the synthesis of complex carbohydrate molecules.

In our synthesis, we used acetyl group, easily available, and easy to install with acetic anhydride in pyridine with DMAP as a catalyst. DMAP and Py have two roles: 1° is they lower down the activation energy of O-acylation generating acylpyridinium reactive intermediates and 2° one they partly neutralize the acetic acid which was liberated during reaction.

Mechanism of reaction: -

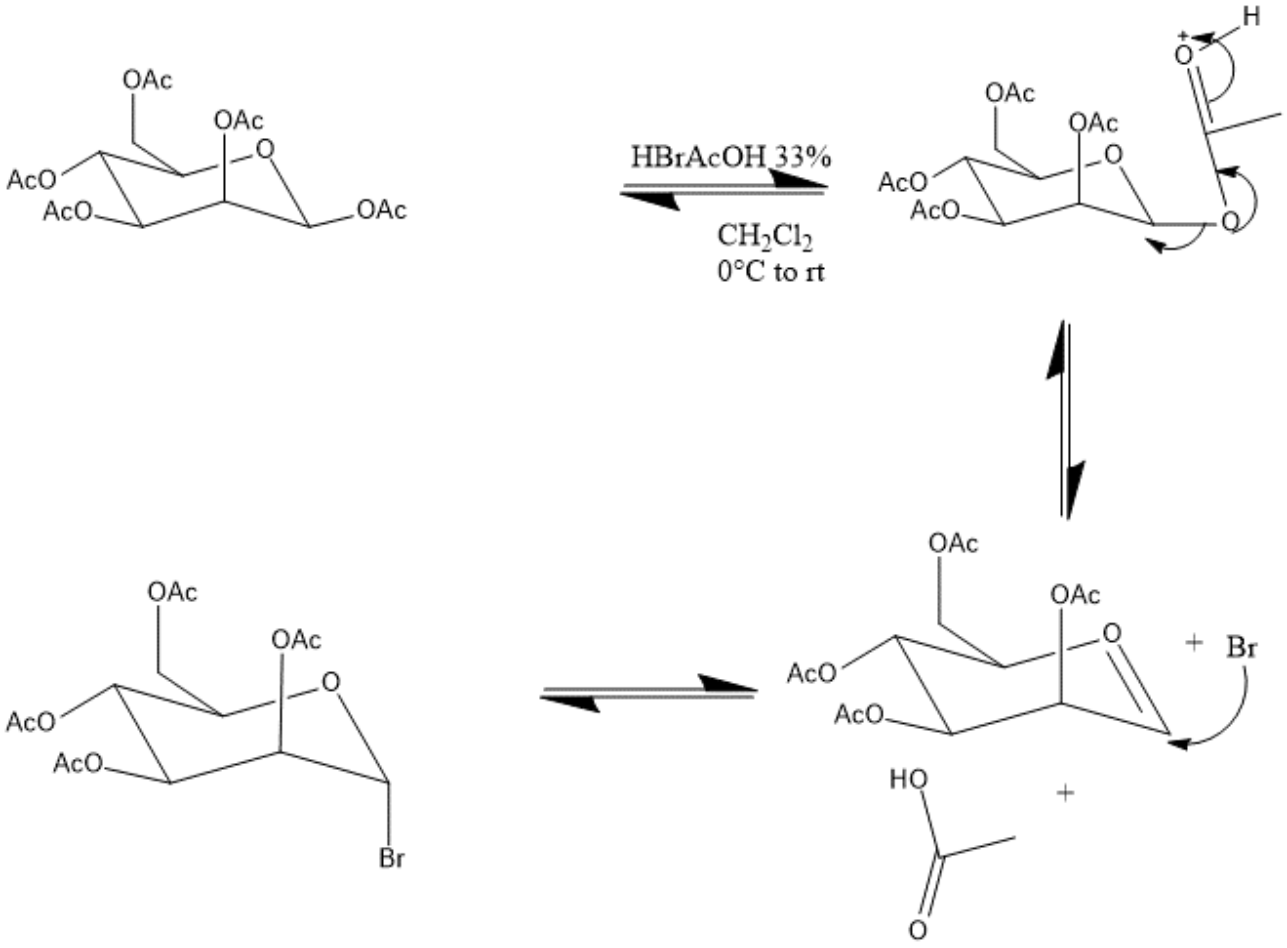
The first step is nucleophilic attack by DMAP to the Ac_2O and liberation of acetyl anion. The hydroxyl attacks the acetyl group on DMAP and the anion that was produced can grab the proton from the hydroxyl group and the further rearrangement of the molecule left us acetic acid and our protected mannose.



6.3 SYNTHESIS OF 1-BROMO-2,3,4,6-TETRA-*O*-ACETYLE- α -D-MANNOSE.

The protection has been done so the next step is to introduce a bromide onto the anomeric carbon to have a good leaving group for the next step. So, we did the reaction with HBr in acetic acid 33%. It was a simple reaction and lasted for 2 hrs: from 0°C to room temperature.

Mechanism of reaction: -



The protonation of acetate on carbonyl group of acetate on the anomeric carbon, generates a carbon cation which is attacked by the Br ion to give the expected anomeric bromide.

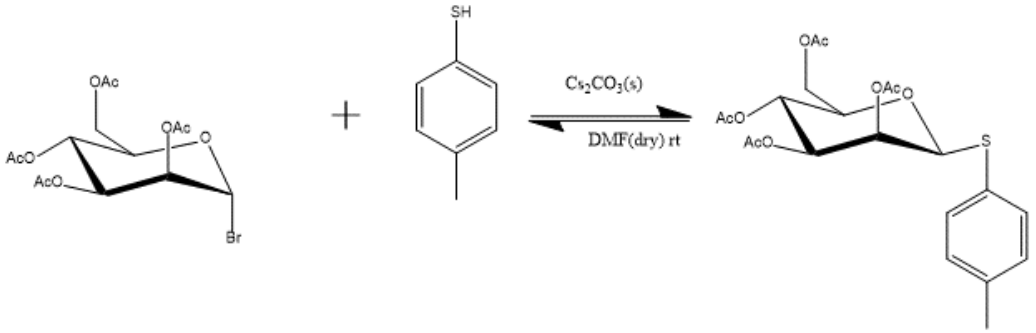
6.4 SYNTHESIS OF P-TOLYL-1-THIO-2,3,4,6-*O*-ACETYL- β -D-MANNOPYRANOSIDE

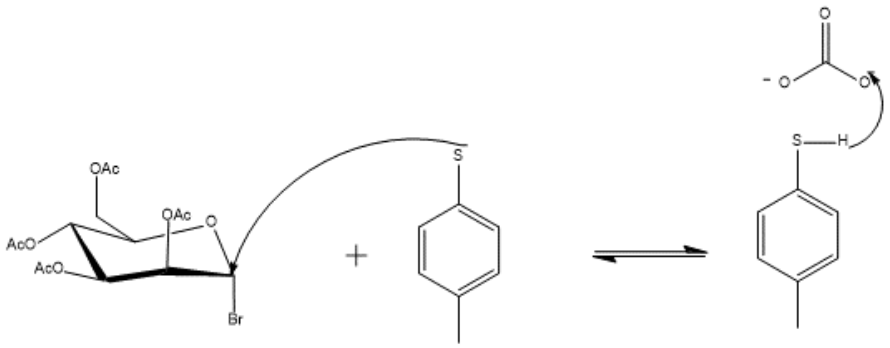
Thioglycosides are focused for the last decennial due to their stability towards acidic and basic conditions. They have been of great importance as building blocks in carbohydrates and oligosaccharides synthesis.

Different papers have been published on their preparations and the typical conditions for the reaction were usually quite harsh. We decided to try their preparation by nucleophilic substitution using a thiolate nucleophile. The conditions used for other monosaccharidic anomeric bromides did not give the expected product in good yield and purity, as either α-anomer or other byproducts were observed. Moreover, the purification of the desired product was cumbersome. After some experimentation, we found the right conditions for the reaction, and we were successful using cesium carbonate as a base in DMF as solvent at room temperature.

Mechanism of reaction: -

The carbonate ion deprotonates the SH and S- can simply attack the anomeric carbon from the top forming a beta anomer through a clean S_N2 reaction.

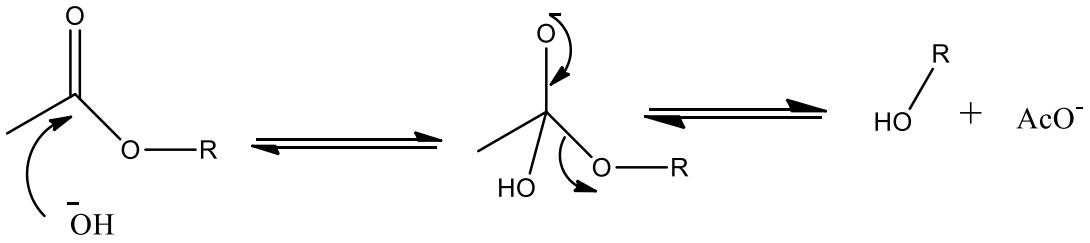




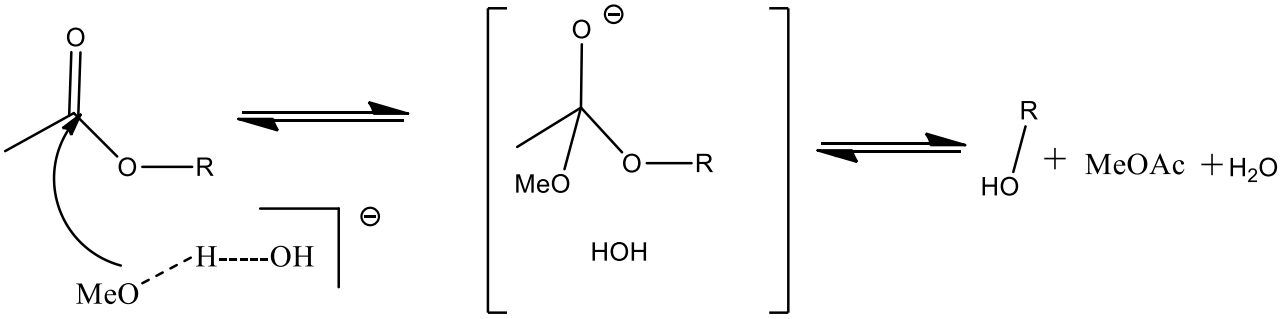
6.5 P-TOLYL-1-THIO-β-D-MANNOPYRANOSIDE

This reaction was very simple. We just followed the Zemplén reaction to remove the acetate from our product because we aimed to insert in position 4,6 a benzylidene ring followed by the benzylation on positions 2,3. Recently, the Zemplén transesterification was revisited as it was demonstrated that NaOH or KOH can remove acetate when used in catalytic amounts in MeOH.(8)Ren et al., 2015)

Mechanism of reaction: -



Expected course which would require a stoichiometric amount of base



Proposed mechanism which accounts for the use of a catalytic amount of base

6.6 SYNTHESIS OF P-TOLYL-1-THIO-4,6-BENZYLIDENE-β-D-MANNOPYRANOSIDE

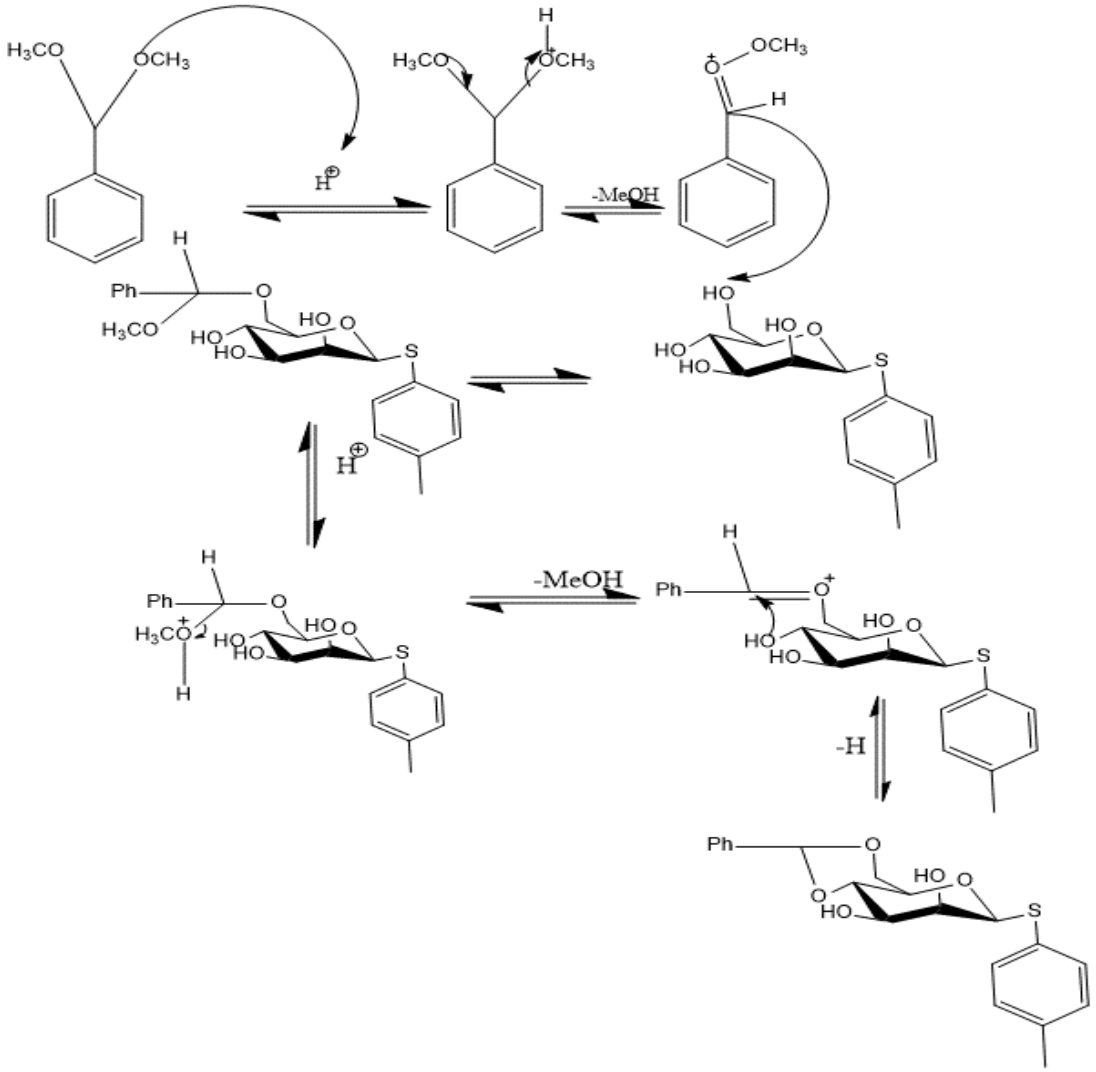
This reaction is cumbersome as the benzylidene ring can also be formed in positions 2,3 being the diol in cis relationship. It has been and has been a challenge for us. First, we tried using benzaldehyde and various solvents to obtain the desired product, but we always obtained the 2,3:4,6-di-*O*-benzylidene derivative as a by-product. The article in reference had faced the same problem, they used harsh conditions to obtain the desired product. They used protective group to get the same product even with protective groups on 4 carbon they were not able to avoid the formation of 2,3:4,6-di-*O*-benzylidene derivatives. Ultimately, we discarded the idea of using benzaldehyde which was substituted by benzaldehyde dimethyl acetal with PPTS as a catalyst in sulfolane as solvent, which is considered a green solvent. The table shown below describes the failures that we faced by using benzaldehyde in different solvents and temperatures.(5) Kasper et al., 2007; 10)Worm-Leonhard et al., 2007)

Experiments	**SOLVENT**	**CAT. ACID (Eq)**	**T°C**	**CONVERSION MONO: DI**	**TIME**	**COMPLETED**	**DATA NMR**
WR 57	TOL	CSA 0.1 Eq	145	70:30	30 min	yes	yes
WR 58	TOL	CSA 0.05 Eq	125	NA	1:45 hr	NO	No
WR 59	DCM	CSA 0.5Eq	Rt	NA	Overnight	NO	No
WR 60	EtOAc	CSA 0.05 Eq	Rt	NA	Overnight	NO	No
WR 61	MeCN	CSA 0.788	Rt to 85	NA	3 days	NO	No
WR 62	TOL	CSA 0.1 Eq	Rt to 60 to 80	NA	4 hr	NO	No
WR 63	MeCN	CSA 2.1 Eq	Rt	NA	3 days	NO	No
WR 64	MeCN	CSA 1 Eq	Rt	NA	4 days	NO	No

WR 65	THF	CSA 1.7 Eq	Rt t 65	NA	3 days	NO	No
WR 66	MeCN	CSA 0.2 Eq	84	NA	4 hr	NO	No
WR 67	MeCN	CSA 0.3 Eq	Rt	NA	overnight	NO	No
WR 68	THF & DMF	CSA 0.2 Eq	Rt to 100	NA	5 days	NO	No
WR 69	DMF	CSA 0.8 Eq	Rt to 85 to 105	0:1	4 days	NO	Yes
WR 70	DCM	CSA 0.2 Eq	Rt	NA	Overnight	NO	No
WR 71	DCM	CSA 0.2 Eq	Rt	NA	Overnight	NO	No
WR 72	NA	NA	NA	NA	NA	NA	NA
WR 73	MeCN	MSA 0.1Eq	Rt	NA	40 min	Yes	No
WR 74	DCM	MSA 0.01 Eq	Rt	NA	25 min	Yes	No
WR 75	DCM	CSA 0.1	Rt	NA	2 days	No	No
WR 76	DCM	MSA 0.01 Eq	0 to rt	NA	Overnight	No	No
WR 77	TOL	MSA 1%	0 to rt	0:1	Instant	Yes	No
WR 78	DCM	MSA	0 to rt	60:40	5 hr	Yes	Yes

Mechanism of reaction: -

It is simple protonated reaction. Benzaldehyde dimethyl acetal becomes the target of *O* of 6 carbon and further rearrangements forms the desired products.


H3CO
OCH3
H
-MeOH
Ph
HO
S
-MeOH
-H

6.7 SYNTHESIS OF P-TOLYL-1-THIO-4,6-BENZYLIDENE-2,3-DI-*O*-BENZYL-β-D-MANNOPYRANOSIDE

The final step of the project is to install benzyl in position 2,3. For this reaction, we exploited an alkylation promoted by KOH as a base in a Dean Stark apparatus, able to remove the water formed during the reaction. As a solvent we chose toluene which allows to reach at temperature high enough to bring all organic compounds in solution and to remove water by azeotropic distillation. This procedure allows to avoid the use of DMF and NaH, which are the typical conditions for this reaction, but can lead to an explosive course of the reaction, so preventing the use of expensive and potentially dangerous reagents and reaction conditions.

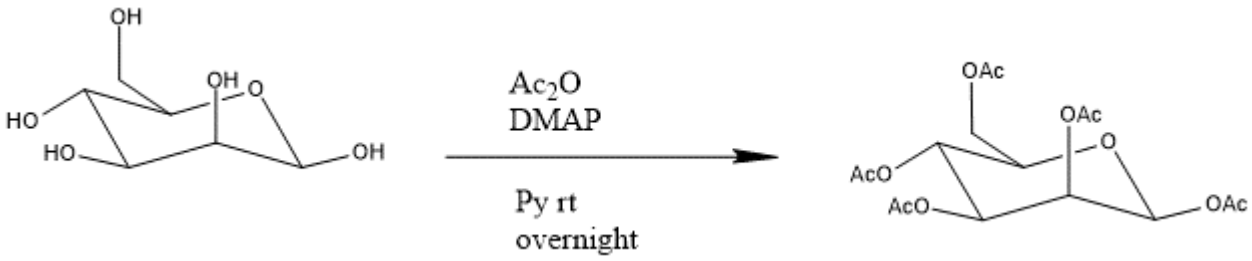
7 CONCLUSION: -

We were able to realize the synthesis of the title product in conditions which can be considered as more eco-friendly than the standard conditions described in literature and with a higher overall yield. Our product can be used as building block in oligosaccharides synthesis, and having the anomeric leaving group as b-anomer can allow to modify the stereochemical course of the glycosylation reaction.

8 EXPERIMENTAL SECTION

8.1 ACETYLATION OF D-MANNOSE

WR 1



Reagents	Wt. (g; mg)	V(ml)	D(g/ml)	MW (moles/g)	mMoles	EQ
D-Man-nose	5			180,16	27.75	1
Ac_2O		20	1.08	102.09		7.5
Py		29	0.982	79.1		
DMAP	0.35			122.17	2.86	0.1

Procedure: -

In a double-necked round bottom flask, 5 g of D-mannose were treated with Ac_2O, pyridine and DMAP and stirred under N_2 overnight at room temperature.

Work Up: -

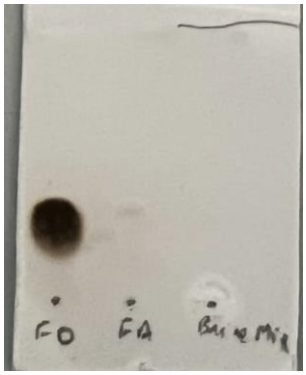
After TLC checking which showed the end of the reaction, the reaction mixture was diluted with EtOAc, washed with HCl 1M 5 times in a separatory funnel then one with NH_4Cl and one with brine. The organic phase was dried over Na_2SO_4, filtered and then evaporated under vacuum.

The final product was a yellowish syrup.

Data: -

TLC: Eluent TLC = Cy: EtOAc = 6:4; stained by H_2SO_4 5% in MeOH.

Work up



End reaction

yield = 95%

NMR report: -

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (s, 5H), 6.11 (d, *J* = 1.8 Hz, 10H), 5.44 – 5.03 (m, 34H), 4.31 (ddd, *J* = 12.3, 10.1, 5.1 Hz, 13H), 4.21 – 4.00 (m, 27H), 3.86 – 2.65 (m, 8H), 2.38 (dd, *J* = 17.7, 3.1 Hz, 4H), 2.31 – 1.83 (m, 224H), 1.27 (t, *J* = 7.1 Hz, 8H), 0.49 (d, *J* = 324.2 Hz, 6H).

8.2 ACETOBROMOMANNOSE

WR 2

Reagents	Wt (g; mg)	V(ml)	D(g/ml)	MW (moles/g)	mMoles	EQ
WR 1	4.313			390.34	11.04	1
HBr in AcOH 33%		73	1.354	80.91	1221	1.1
DCM		58	1.33	84.93		

Procedure: -

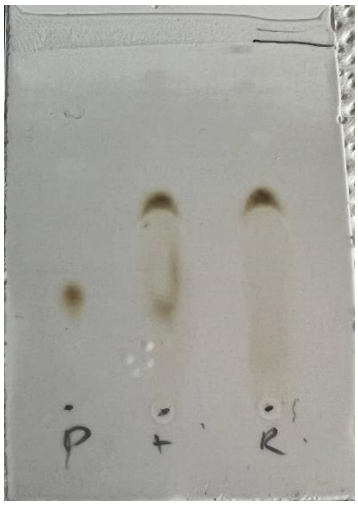
In a single-necked round bottom flask, WR1 was dissolved in DCM and HBr in AcOH was added dropwise at 0°C in 30 min. The mixture was allowed to warm to room temperature for 2 hrs.

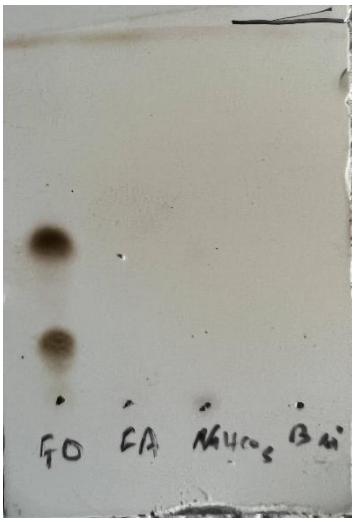
Work Up: -

The reaction was quenched with icy water to eliminate the excess of HBr with the help of a separatory funnel. The mixture was then treated with $NaHCO_3$ until its pH became neutral. Finally, the organic phase was washed with brine and dried over Na_2SO_4. Lastly, the solvent was evaporated under vacuum.

TLC: Eluent TLC = Cy: EtOAc = 6:4; stained by H_2SO_4 5% in MeOH.

End reaction work-up





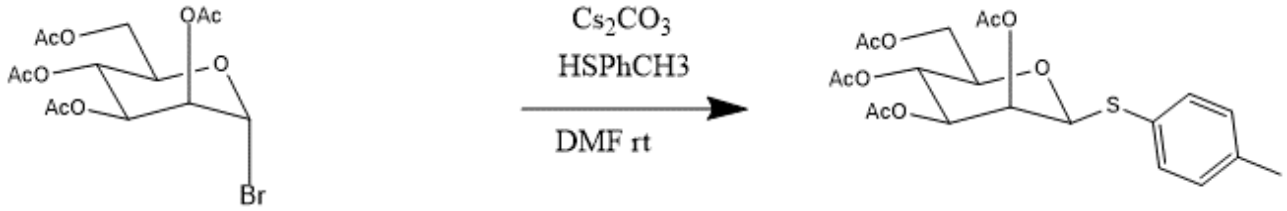
Yield= 92.78%

NMR report: -

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (d, *J* = 10.2 Hz, 31H), 7.23 – 7.13 (m, 12H), 6.32 (s, 19H), 5.74 (dd, *J* = 10.2, 3.4 Hz, 20H), 5.50 – 5.25 (m, 84H), 4.36 (dd, *J* = 12.5, 4.9 Hz, 22H), 4.30 – 4.09 (m, 44H), 3.74 (dd, *J* = 13.4, 6.5 Hz, 5H), 3.13 (d, *J* = 11.9 Hz, 4H), 2.37 (d, *J* = 6.6 Hz, 17H), 2.86 – 2.06 (m, 247H), 2.22 (d, *J* = 14.4 Hz, 79H), 2.54 – 2.06 (m, 237H), 2.16 – 1.95 (m, 194H), 1.95 – 1.81 (m, 9H), 1.95 – 1.40 (m, 15H), 1.95 – 1.22 (m, 22H).

8.3 TETRAACETATE β-MANNOTHIOGLYCOSIDE

WR 3



Reagents	Wt. (g; mg)	V(ml)	D (g/ml)	MW(g/mol)	mMoles	Eq
WR 2	4.216			411.20	10.253	1
DMF (dry)		26	0.944	73.09		
Cs2CO3	5.555			325.82	17.04	1.5
HS-Tol	1.555			124.201	12.52	1.2

Procedure: -

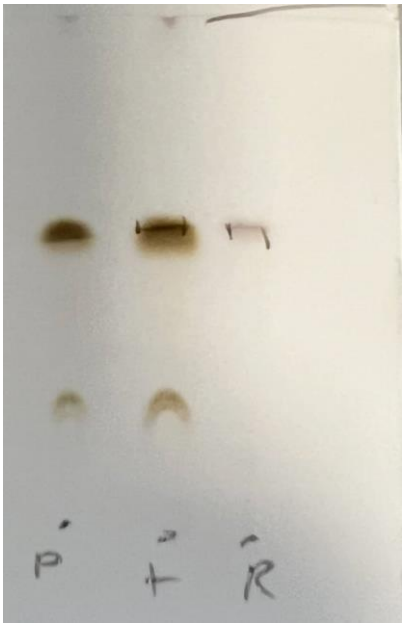
In a double-necked round bottom flask, Cs2CO3 and HS-Tol were added and DMF. After 15 minutes, WR2 was added to the flask dissolved in DMF. The reaction was left for 30 minutes.

Work Up: -

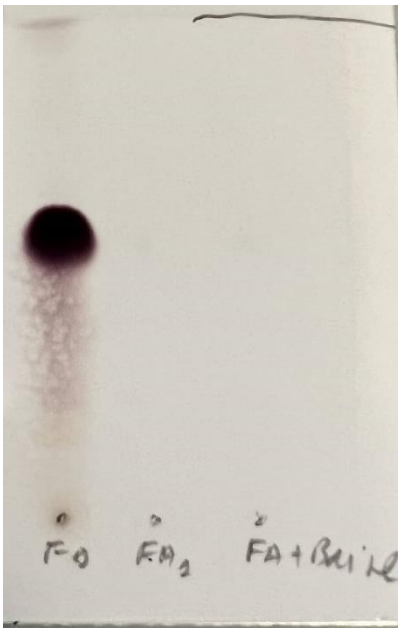
The reaction was diluted with EtOAc, and the reaction was filtered. The mixture has been washed with water 5 times, 1 time with brine and it was dried over $NaHCO_3$. It was evaporated under vacuum.

Data: - Eluent TLC= Tol: EtOAc=6.5:3.5; stained by H_2SO_4 5% in MeOH.

End reaction



Work Up



Purification: -

Further purification was done via crystallization with Et_2O: Hex 1:9. The final product was white crystals.

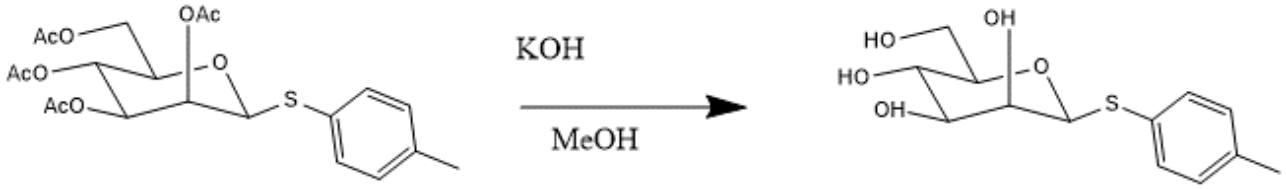
Yield=73%

NMR report: -

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 – 7.40 (m, 18H), 7.28 (s, 16H), 7.15 (d, *J* = 7.9 Hz, 18H), 5.67 (dt, *J* = 21.5, 10.7 Hz, 9H), 5.50 – 5.25 (m, 11H), 5.06 (dd, *J* = 10.1, 3.5 Hz, 9H), 4.86 (d, *J* = 1.2 Hz, 9H), 4.39 – 4.26 (m, 10H), 4.23 – 4.03 (m, 10H), 3.69 (ddd, *J* = 9.9, 6.4, 2.5 Hz, 9H), 2.39 (d, *J* = 19.7 Hz, 28H), 2.30 – 1.98 (m, 118H), 1.58 (s, 18H), 1.24 (dd, *J* = 15.9, 8.9 Hz, 2H).

8.4 DEACETYLATION OF TETRAACETATE- β - MANNOTHIOGLYCOSIDE

WR 4



Reagents	Wt. (g; mg)	V(ml)	D (g/ml)	MW(g/mol)	mMoles	Eq
WR 3	3.7			454.49	8.14	1
KOH	36mg			56.1056	0.641	0.078
MeOH		7	0.792	32.04		

Procedure: -

In a round bottom flask, WR3 and the solution of KOH in MeOH were added. The reaction was left for 30 minutes and Dowex resin was added until pH 7.

Work Up: -

The mixture was filtered to remove Dowex and evaporated under vacuum. The final product was white solid crystal powder.

Eluent TLC = Tol: EtOAc = 6:4; stained by H2SO4 5% in MeOH.

End reaction

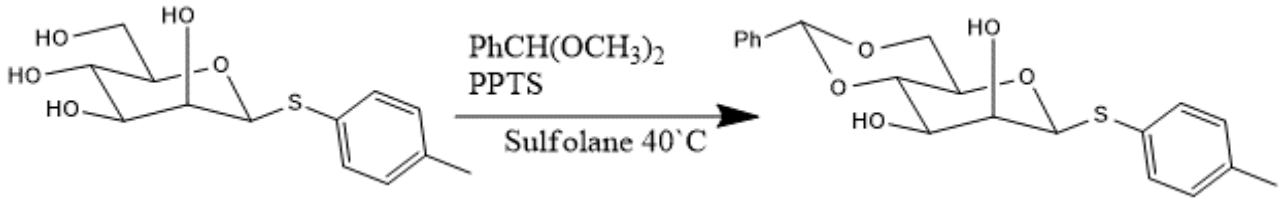
Yield=95%

NMR report: -

^{1}H NMR (400 MHz, MeOD) δ 7.46 – 7.25 (m, 85H), 7.13 (d, *J* = 7.9 Hz, 86H), 5.19 (t, *J* = 55.4 Hz, 6H), 4.92 (t, *J* = 5.4 Hz, 52H), 4.87 (s, 310H), 4.06 (dd, *J* = 3.4, 0.9 Hz, 45H), 3.97 – 3.70 (m, 99H), 3.70 – 3.47 (m, 94H), 3.47 – 3.23 (m, 130H), 2.90 – 2.70 (m, 4H), 2.57 (d, *J* = 77.0 Hz, 11H), 2.32 (s, 133H), 2.32 (s, 138H).

8.5 INSTALLATION OF 4,6-BENZYLIDENE ACETAL

WR 5



Reagents	Wt. (g; mg)	V (µl; ml)	D (g/ml)	MW(g/mol)	mMoles	Eq
WR 4	0.05			286.34	0.1747	1
PPTS	0.04			251.30	0.1591	0.1
Sulfolane		10	1.26	120.17		
PhCH $(OCH_3)_2$		207	1.014	152.19		1.5

Procedure: -

In a double-necked round bottom flask WR4 mannose, PPTS and sulfolane were added under nitrogen. The whole mixture was brought to 40°C. PhCH $(OCH_3)_2$ was added and the reaction was left for 24 hours. After 24 hours the reaction was completed.

Work Up: -

Directly into the flask 15 ml of MBTE was added and left for about 10 minutes. The whole mixture was transferred to the separatory flask and added water to the mixture caused formation of a crystal in the funnel. The crystals were dissolved in EtOAc and washed with water 5 time and 1 time with brine, dried over $NaHCO_3$ and evaporated under vacuum.

Data: -

TLCs are given; Cy:EtOAc= 5:5 were stained by H2SO4 5% in MeOH.

End Reaction

Work-up

Purification; -

The crystals were washed with MeOH to eliminate excessive MBTE.

Yield= 75%

NMR report: -

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 – 7.30 (m, 836H), 7.28 (s, 81H), 7.17 (d, *J* = 8.0 Hz, 233H), 5.59 (s, 114H), 4.89 (s, 116H), 4.48 – 4.41 (m, 9H), 4.41 – 4.26 (m, 246H), 4.07 – 3.80 (m, 380H), 3.44 (ddd, *J* = 27.7, 22.2, 17.1 Hz, 157H), 2.92 – 2.53 (m, 274H), 2.36 (d, *J* = 13.0 Hz, 367H), 2.23 (d, *J* = 11.1 Hz, 11H), 1.61 (s, 115H), 1.53 – 1.24 (m, 66H), 0.90 (d, *J* = 11.0 Hz, 16H), 0.10 (s, 26H).

8.6 INTRODUCING 2,3-DI- *O*- BENZYL

WR 6

Reagents	Wt.(g;mg)	V(ml)	D (g/ml)	MW(g/mol)	mMoles	Eq
WR 5	0.100			374.45	0.267	1
BnBr	0.110	0.0764	1.44	171.04	0.643	2.4
Toluene		4	0.867	92.14		
KOH	0.064		1.014	56.105	1.14	3.7

Procedure: -

In a round bottom flask WR5, KOH, and Tol were added. The whole mixture was brought to 130°C then BnBr was added. The reaction was checked every 30 to 40 minutes. After the first check, more 0.3 eq of BnBr was added for the complete formation of benzylation.

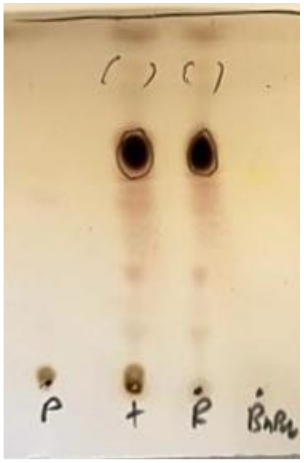
Work Up: -

The reaction was washed with water four times and one time with brine. It was dried over Na_2SO_4 and evaporated under Vacuum.

Data: -

Eluent TLC= Cy: EtOAc= 7:3

The TLC were stained by H2SO4 5% in MeOH.



Purification: -

The product was further purified with column chromatography, eluent Cy: EtOAc 95:5 and evaporated under vacuum.

Yield= 77%

NMR report: -

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 – 7.54 (m, 7H), 7.54 – 7.26 (m, 213H), 7.15 (t, *J* = 11.7 Hz, 26H), 5.17 – 4.98 (m, 15H), 5.68 – 4.53 (m, 87H), 5.43 – 4.53 (m, 73H), 4.90 (t, *J* = 11.9 Hz, 26H), 4.79 (dd, *J* = 21.1, 6.6 Hz, 26H), 4.39 – 4.27 (m, 26H), 4.20 (d, *J* = 2.2 Hz, 13H), 3.97 (t, *J* = 10.3 Hz, 14H), 3.75 (dd, *J* = 9.8, 3.1 Hz, 14H), 3.63 – 3.48 (m, 3H), 3.31 (ddd, *J* = 272.4, 138.6, 133.8 Hz, 31H), 3.30 (ddd, *J* = 306.4, 155.7, 150.8 Hz, 34H), 3.63 – 2.33 (m, 77H), 1.55 (d, *J* = 12.1 Hz, 20H), 1.28 (s, 13H), 0.91 (t, *J* = 6.8 Hz, 4H), 0.09 (d, *J* = 9.8 Hz, 3H).

9 ACKNOWLEDGEMENTS

As a Muslim it is my duty to thanks Allah almighty for giving me this success. This thesis I would dedicate to my parents especially to my father who did everything for me and give his best to give me every assist I needed for this journey.

It has been a long and adventurous journey. It has been possible thanks to Professor Luigi Panza who gave me the opportunity to work with him. I learnt a lot from him, his method of mentoring is great in all aspects. He practically pushes the students to learn and if the student has it in him, He would learn the real aspect of organic chemistry under the guidance of Professor Luigi Panza. My sincere gratitude to him.

Special thanks to Professor Daniela Imperio who guide me throughout my stay at lab. My precious thanks to my lab mates Dr Marco Brentazzoli aka Master of column chromatography I nick named him because when he was doing his project every other day, he purified its product by column chromatography so I started calling him “Maestro delle colonne”. My heartiest thanks to Dr Filippo Valloni who was in lab whenever you need something and any help that you need, He will do his best to help you He is like the Big Bro of all the undergrads.

My sincere gratitude and thanks to my friends who push me through my bad time and console me when I was down. I think If I say I am lucky when it comes to my friends and my mentors wherever I go here in Italy or in Pakistan it would not be wrong. My friends who kept me keep going through these long and frustrating journey especially Dr Riccardo Lucatorto, he helped me kept me up on my feet in my bad times He advised me, encourage me and keep me stay on the right path. I still remember when I was about to give up on my one exam, he is the one who took me with himself and I was manged to get good marks in that exam.

Another friend Dr Sara Cavicchio who made me laugh with her jokes which sometimes I couldn’t get them she would explain me the real nature of her joke. She is a great person and she also helps me with my preparation for the exam.

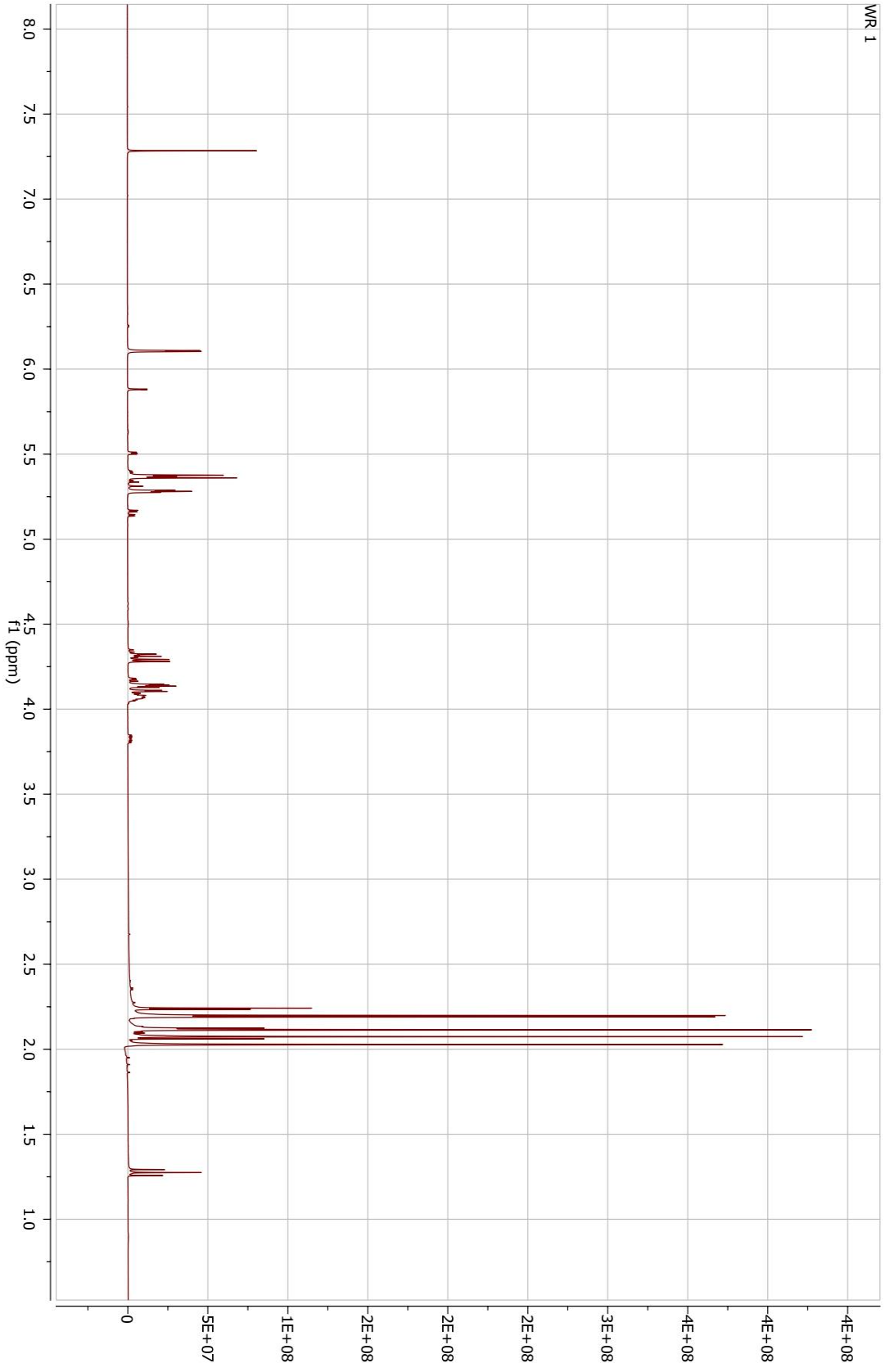
Thanks to UPO for giving me this possibility to study under great professors.

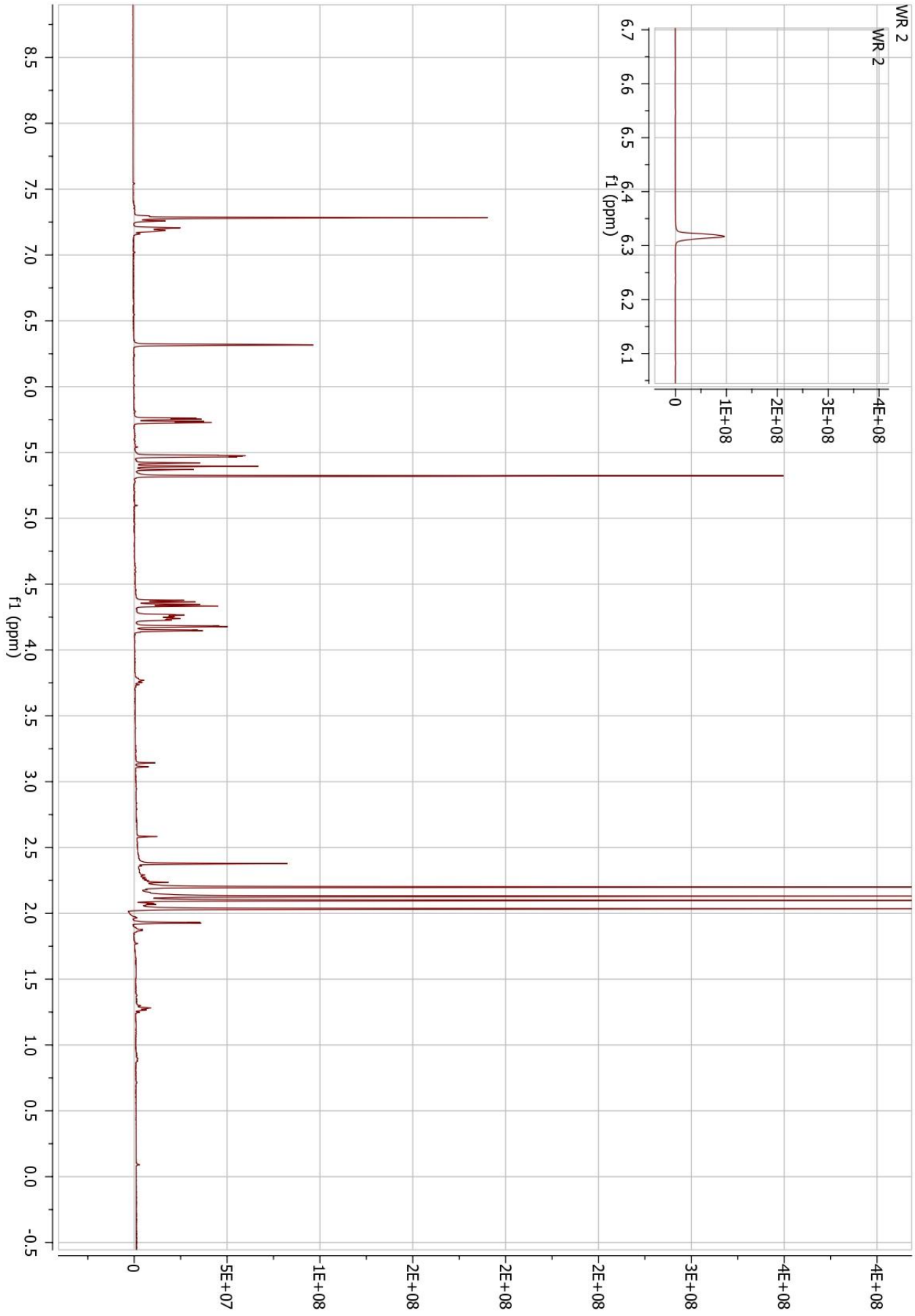
10 REFERENCES

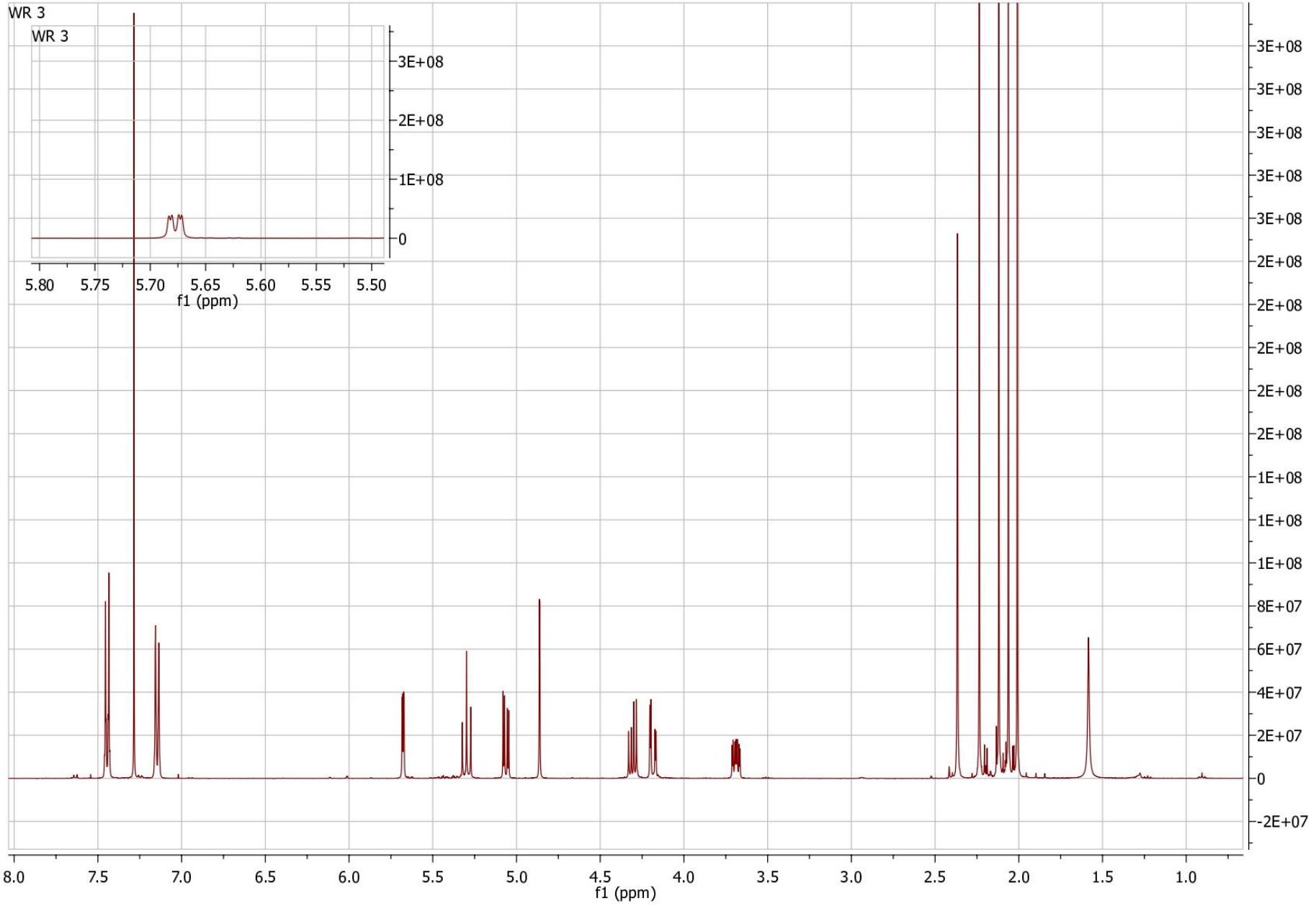
1) Codée, J. D. C., Litjens, R. E. J. N., van den Bos, L. J., Overkleeft, H. S., & van der Marel, G. A. (2005). Thioglycosides in sequential glycosylation strategies. *Chemical Society Reviews*, *34*(9), 769–782. https://doi.org/10.1039/b417138c
2) Crich, D. (2010). Mechanism of a chemical glycosylation reaction. *Accounts of Chemical Research*, *43*(8), 1144–1153. https://doi.org/10.1021/ar100035r
3) Guo, X., Cheng, C., Liu, X., Liu, X., Wang, R., Xie, D., Wang, L., & Zhao, Y. (2004). Crystallographic and theoretical study of phenyl 2,3,4,6-tetra-O-acetyl-1- thio-α-D-mannopyranoside. *Journal of Molecular Structure: THEOCHEM*, *710*(1–3), 201–205. https://doi.org/10.1016/j.theochem.2004.08.046
4) Hu, X., Shi, Y., Zhang, P., Miao, M., Zhang, T., & Jiang, B. (2016). d-Mannose: Properties, Production, and Applications: An Overview. In *Comprehensive Reviews in Food Science and Food Safety* (Vol. 15, Issue 4, pp. 773–785). Blackwell Publishing Inc. https://doi.org/10.1111/1541-4337.12211
5) Kasper, W.-L., Larsen, K., & Jensen, K. J. (2007). *4,6-O-Benzylidene Directed β-Mannosylation Without Intermediate Triflate Formation? Comparison of Trichloroacetimidate and DISAL Donors in Microwave-Promoted Glycosylations under Neutral Conditions*. *26*(7). https://doi.org/10.1080/07328300701634762?scroll=top&needAccess=true
6) Lian, G., Zhang, X., & Yu, B. (2015). Thioglycosides in Carbohydrate Research. In *Carbohydrate Research* (Vol. 403, pp. 13–22). Elsevier Ltd. https://doi.org/10.1016/j.carres.2014.06.009
7) Nicotra, F., Airoldi, C., & Cardona, F. (n.d.). .*16 Synthesis of C-and S-Glycosides*.
8) Ren, B., Wang, M., Liu, J., Ge, J., Zhang, X., & Dong, H. (2015). Zemplén transesterification: A name reaction that has misled us for 90 years. *Green Chemistry*, *17*(3), 1390–1394. https://doi.org/10.1039/c4gc02006e
9) Sharma, V., Ichikawa, M., & Freeze, H. H. (2014). Mannose metabolism: More than meets the eye. In *Biochemical and Biophysical Research Communications* (Vol. 453, Issue 2, pp. 220–228). Academic Press Inc. https://doi.org/10.1016/j.bbrc.2014.06.021
10) Worm-Leonhard, K., Larsen, K., & Jensen, K. J. (2007). 4,6-O-benzylidene directed β-mannosylation without intermediate triflate formation? Comparison of trichloroacetimidate and disal donors in microwave-

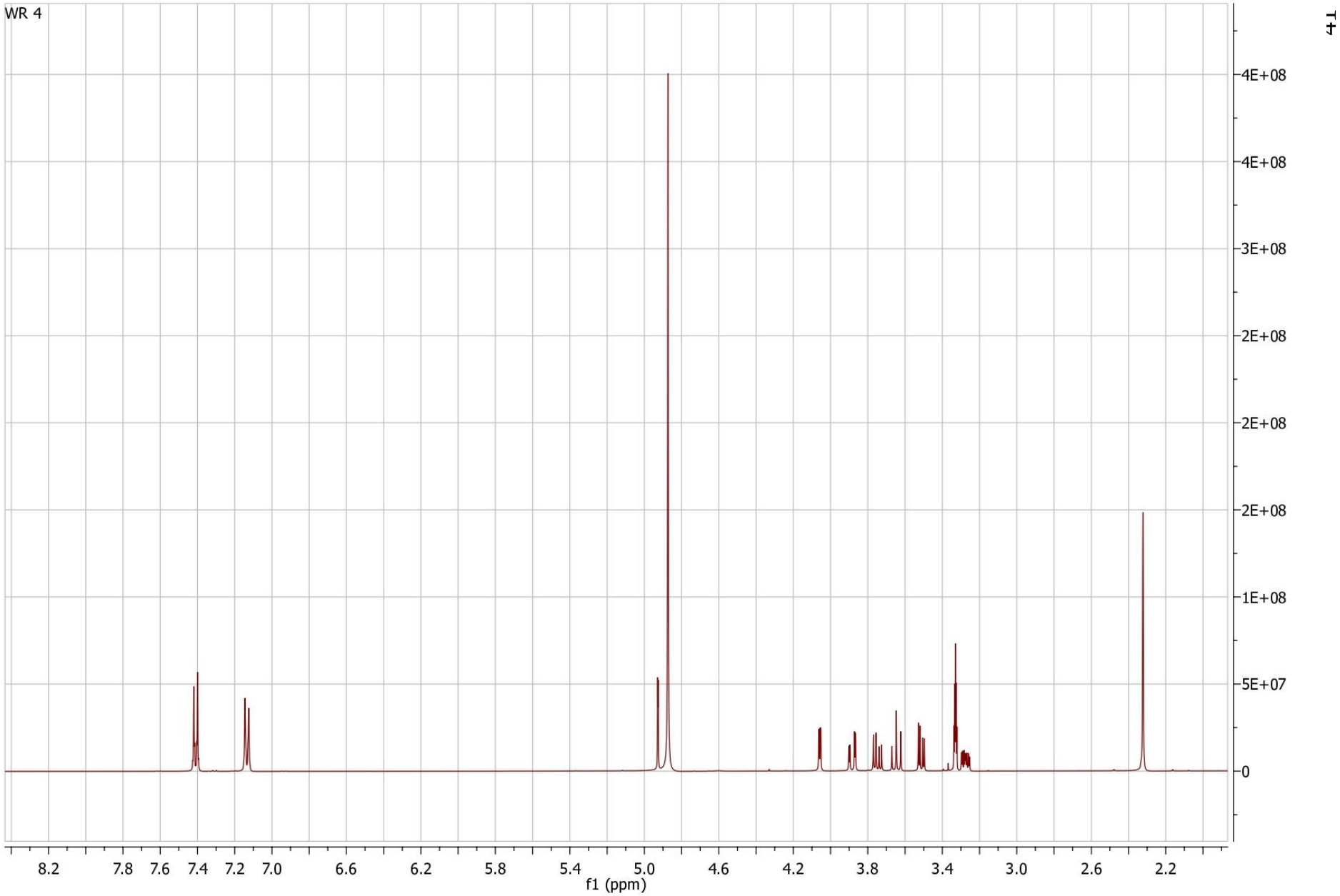
promoted glycosylations under neutral conditions. *Journal of Carbohydrate Chemistry*, *26*(7), 349–368. https://doi.org/10.1080/07328300701634762

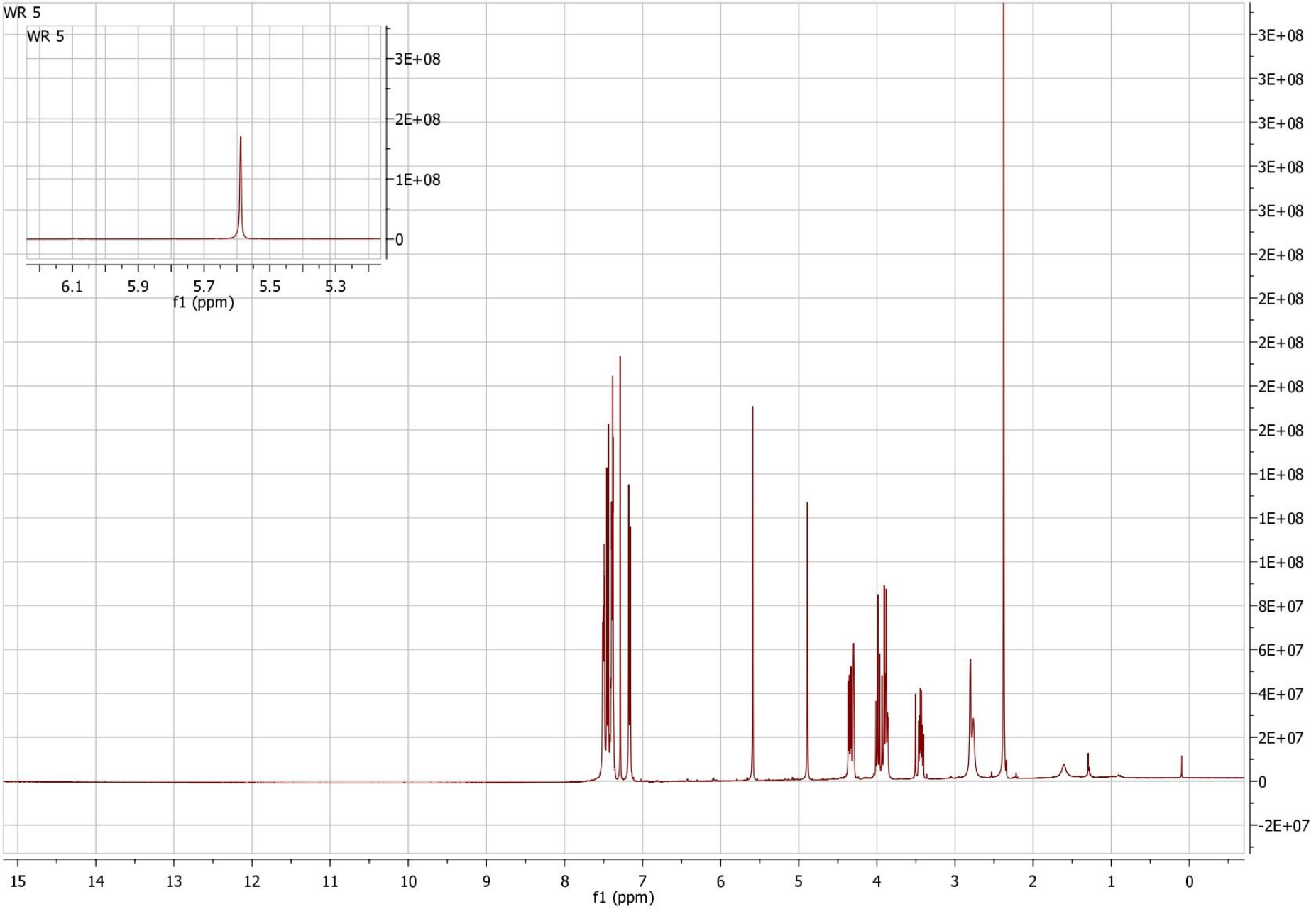
11 APPENDICES

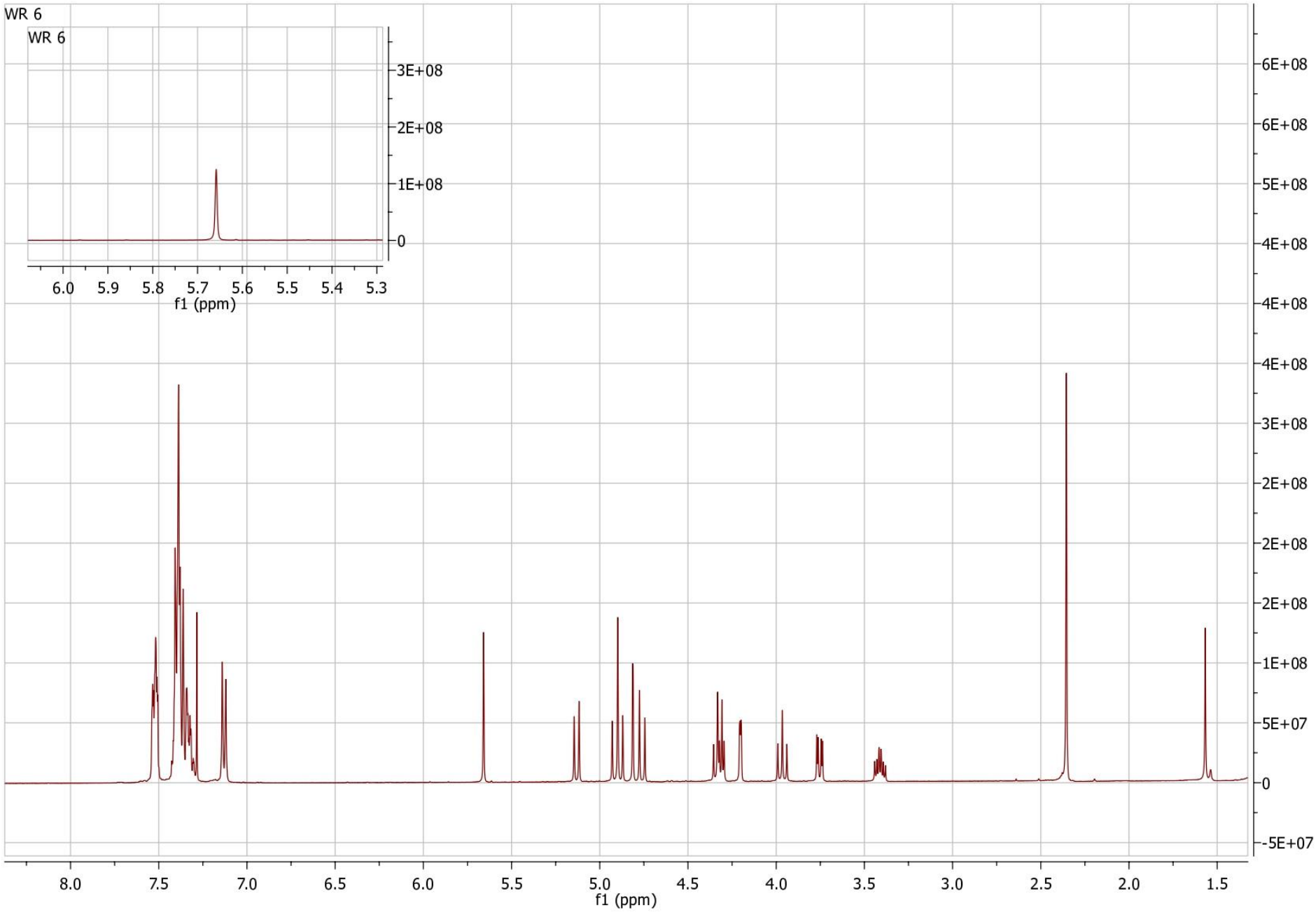



WR 2
WR 2
f1 (ppm)
f1 (ppm)


WR 3
WR 3
3E+08
2E+08
1E+08
0
5.80
5.75
5.70
5.65
5.60
5.55
5.50
f1 (ppm)
3E+08
3E+08
3E+08
3E+08
3E+08
2E+08
2E+08
2E+08
2E+08
2E+08
2E+08
1E+08
1E+08
1E+08
8E+07
6E+07
4E+07
2E+07
0
-2E+07
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)


WR 4
T4
4E+08
4E+08
3E+08
2E+08
2E+08
2E+08
1E+08
5E+07
0
8.2
7.8
7.6
7.4
7.2
7.0
6.6
6.2
5.8
5.4
5.0
4.6
4.2
3.8
3.4
3.0
2.6
2.2
f1 (ppm)


WR 5
WR 5
3E+08
2E+08
1E+08
0
6.1
5.9
5.7
5.5
5.3
f1 (ppm)
3E+08
3E+08
3E+08
3E+08
3E+08
2E+08
2E+08
2E+08
2E+08
2E+08
2E+08
1E+08
1E+08
1E+08
8E+07
6E+07
4E+07
2E+07
0
-2E+07
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)


WR 6
WR 6
3E+08
2E+08
1E+08
0
6.0 5.9 5.8 5.7 5.6 5.5 5.4 5.3
f1 (ppm)
6E+08
6E+08
5E+08
4E+08
4E+08
4E+08
3E+08
2E+08
2E+08
2E+08
1E+08
5E+07
0
-5E+07
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5
f1 (ppm)