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DIPLOMARBEIT

Synthesis of model Substances of 4-O-Methyl-Glucose and 4-O-Methyl-Glucuronic Acid in Prebiotic Polysaccharides

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1. Introduction:

1. 1.Probiotics and prebiotics

1.1.1. Probiotics

1.1.1.1. Definition

Probiotics are microorganisms, more commonly named “the good bacteria” or “the friendly bacteria”. Usually, probiotics are bacteria, most often from the groups of *Bifidobacterium* or *Lactobacillus*, although some of them are yeasts, for example *Saccharomyces boulardii*. These microorganisms are the same or similar to those commonly found in the human body. The name derives from the Latin preposition “pro” meaning for, and bios, which is a Greek word for life. The World Health Organisation and the Agriculture organization of the United Nations, define probiotics as “live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host”. They are both gram-positive and gram-negative and are settled mainly in the colon, less in the skin, saliva, oesophagus and upper intestines. There can be up to 400 different bacterial species in the colon, although 99% of the whole population normally consists of 30-40 species. Probiotics are found in fermented milk products, and have been consumed in that way for thousands of years. At the beginning of the 20th Century, Metschnikoff noted the good health and long lives of Bulgarian shepherds. He observed their daily consumption of fermented milk products, and proposed that the lactic acid bacteria provide defence against pathogenic organisms. Nowadays, probiotics are very common and can be found as foods, dietary supplements, or pharmaceutical products in many forms [1] [2] [3].

1.1.1.2. Uses of probiotics

The most important use of the probiotics is to restore and maintain a healthy balance among the bacterial flora of the intestinal tract.

- Regulation of pH values in the intestines through metabolic processes of the probiotic bacteria enables a valuable defence against the pathogenic organisms. A number of probiotic bacteria produce peptides which act as antibiotics.
- Regulation of adstringency. The usual laxatives are frequently connected with the loss of electrolytes. When probiotics are taken, no such changes are observed.
- Regulation of diarrhoea. Travelling to more exotic parts of the world may often result in diarrhoea. It usually lasts for a couple days. Taking probiotics as a prevention can help reduce the symptoms.
- Regeneration of the intestinal flora after use of antibiotics. A significant side effect of antibiotics is the loss of large numbers of intestinal bacteria which provide for general well-being. This is usually manifested in diarrhoea. Taking *Lactobacillus* products during antibiotic treatment improves the regeneration of the intestinal flora.
- Prevention and management of atopic dermatitis in children. An oral dose of *Lactobacillus rhamnosus* over a period of 5 days to 4 weeks has shown a significant reduction of symptoms and general improvement.
- Treatment of irritable bowel syndrome. Some strains of probiotic bacteria have anti-inflammatory abilities, and can reduce the irritable bowel symptoms.
- Prevention of candidiasis. The right balance of the different bacteria in the vagina is essential for the pH value. Bacteria produce lactic acid, which lowers the pH value. If the

balance is disturbed, the pH value rises, and provides a better environment for the pathogenic microorganisms to reproduce. The balance can be restored by using probiotics

- Regulation of lactose intolerance. The enzyme galactosidase which is produced by the probiotic bacteria can help reduce the symptoms of lactose intolerance. [1] [2]

1.1.2. Prebiotics

1.1.2.1. Definition

Prebiotics are non-digestible food ingredients which provide nutrition to the probiotic bacteria in the intestines. They were initially defined in 1995 as “non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improve host health.” They represent a form of nourishment for the bacteria, or influence their growth or activity. Any food ingredient which reaches the colon is a potential prebiotic. However, there are a number of criteria a prebiotic has to fulfil:

- No hydrolysis or absorption in the upper intestines
- Stimulation of a species in the colon (growth or activity)
- Ability to affect the ratio of the bacteria in the colon.

Symbiotics are products which include both probiotics and prebiotics. [3]

1.1.2.2. Uses of prebiotics

As mentioned above, the fullness of prebiotics lies in their positive effect on probiotics, so the use of both is synergistic.

1.1.2.3. Side effects of the prebiotics

Prebiotics are generally recognised as safe by a number of health organisations all over the world. However, consuming large quantities of prebiotics can lead to side effects such as flatulence, diarrhoea and intestinal pain in more sensitive patients or patients with a maladjusted intestinal flora. [4] [5]

1.1.2.4. Prebiotics today

The term prebiotic has only been used since 1995, but the market for probiotics has been growing rapidly for the past few years, probably due an increase in global health awareness. At the Technical Meeting on Prebiotics in September 2007, it was estimated that the European prebiotics market will reach 179 million \$ by 2010. A market research report in February 2010 predicts the European and US market of probiotics to reach 1.17 billion \$ by 2015 [6] [7].

1.1.2.5. Components used as prebiotics

Most of the prebiotics are polysaccharides derived from plants.

Commonly used prebiotics:

- Inulin

Inulin is a plant polysaccharide which consists of (2→1) fructosyl-fructose chains. It was first isolated from *Inula helenium* in the 19th century. It can be found in tubers of *Dahlia*, *Helianthus tuberosus*, or various types of chicory. Today, chicory is the most valuable source of inulin.

Inulin is one of the first thoroughly researched prebiotics; there are a large number of publications discussing the effects of inulin on humans. It has been legally classified as food. [8] [9]

- Fructo-oligosaccharides (FOS)

Together with inulin, FOS are the most studied and well established prebiotics in common use. They consist of short chains of fructose, and can be found in a variety of plants such as legumes, asparagus or soybeans. They are also used as sweeteners. [8] [10]

- Manno-oligosaccharides (MOS)

MOS consist of mannose short chains. They are extracted from yeast and are also used as a supplement to animal feed. It has been reported that there is a significant difference between animals fed with MOS and animals fed with feed without supplements. [11]

- Galacto-oligosaccharides (GOS)

GOS are oligosaccharides which consist of short galactose chains. GOS have a significant market share of the prebiotics market and have a long history of use. GOS are produced enzymatically from lactose. The composition of the GOS depends on the enzyme, and the lactose used for synthesis. [12]

- Xylo-oligosaccharides (XOS)

XOS favour the growth of *Bifidobacterium* species, and like FOS are used as sweeteners besides being used as prebiotics. They consist of xylose units. XOS can be found in fruits, vegetables, bamboo, honey and milk and are produced industrially from agricultural waste. [13]

- Pyrodextrins

Pyrodextrins are degradation products of roasted starch. They consist of glucose units, and their structure depends on the temperature, time of roasting, the catalyst, and the starch source. Different pyrodextrins have different roles. They are used as thickeners, glues, and prebiotics. [14]

- Lactulose

Lactulose is an artificial sugar, prepared synthetically from lactose through isomerization. It is better known for its regulation of astringency, but it also has prebiotic abilities. [15]

New prebiotics:

Pectic oligosaccharides, lactosucrose, the sugar alcohols, glucooligosaccharides, levans, resistant starch and soy oligosaccharides, have been partially studied in vitro; however there are only a few studies in humans. [7]

1.2. *Hyptis suaveolens*



1.2.1. Name and Origin

Hyptis suaveolens belongs to the family of Lamiaceae. Other latin names are: *Marrubium indicum* Blanco, or *Ballota suaveolens*. Native names are: chan, horehound, pignut, stinking Roger, wild spikenard, mumutun. It is native to tropical America, but has spread over the world as a weed, and is very common in Hawaii, Thailand, India, Australia, and New Guinea. [16]

1.2.2. Characteristics:

Hyptis suaveolens is a coarse, branched annual, which grows from 0.5 to 2m. Its leaves are ovate to obovate and are 3-5 cm long and 2-4 cm wide. Flowers are axillary and long stalked, zygomorphic and blue. Seeds are flat and mucilaginous. It is an annual, aromatic plant.

The plant favours dry open places, and is commonly found near roadsides, pastures and open forests. It forms dense thickets and favours low altitudes. [16] [17]

1.2.3. Uses of *Hyptis suaveolens*

- Wound healing

Hyptis suaveolens has been used for wound healing for centuries in folk medicine, and recent studies confirm the wound healing properties of the plant extract in animals. [18]

- Antimicrobial

The essential oil of *Hyptis suaveolens* species in northern Thailand, which prepared by steam distillation, is reported to have antibacterial and antifungal properties as well as antioxidant

abilities. The oil was tried on several strains of bacteria and fungi, with antifungal properties more noticeable than antibacterial. [19]

- Insecticidal

Leaf powder, oil and extract of *Hyptis suaveolens* are used as a natural insecticide on stored crops and oil nuts. It has been reported that the ovicide and larvicide effects are stronger than those of other plants like *Ocimum gratissimum*. [20]

- Other

Hyptis suaveolens has been used in a variety of situations in folk medicine. A decoct of the plant has been used to regulate flatulence, or externally for eczema. Crushed leaves are believed to relieve headache or boils when applied locally. Infuses are used against fever and catarrhal conditions, and juices are drunk to relieve stomach pain. In India, a paste is used to cure athlete's foot. The powdered plant is used as a sniff to stop bleeding of the nose.

In India, *Hyptis suaveolens* is used as a green manure, in Java as cattle fodder. The shoot tips are edible and are used as a spice. [17] [21]

1.2.4. The seed and mucilage of *Hyptis suaveolens*



The seeds of *Hyptis suaveolens* are the least researched part of the plant. It is known that they contain a large amount of linolic acid and have been used as a product for dry skin. The mucilage is reported to have significant absorption capacity for arsenic. The mucilage of the seeds, however, could also have prebiotic abilities, and could be used in pharmaceutical technology as a coating for probiotics. [17] [22]

1.2.5. The Polysaccharide of *Hyptis suaveolens*

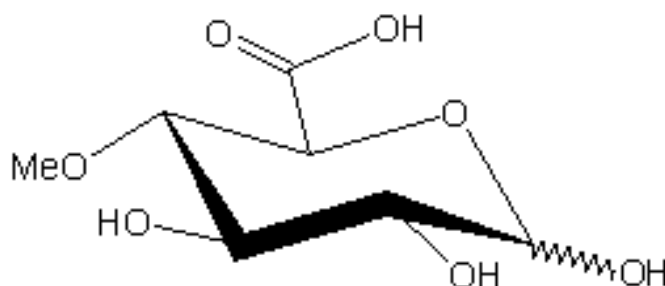
There are two polysaccharides in the seeds of *Hyptis suaveolens*: the neutral and the acid polysaccharide. Both structures have not been completely elucidated. The composition of the neutral polysaccharide is currently being investigated at the Department of Pharmaceutical Technology, University of Vienna, and research has been done on the acid polysaccharide in the past diploma thesis at this department.

The neutral polysaccharide is known to contain mannose, glucose and galactose (see Results and Discussion) and the acid polysaccharide is a heteroxylan which has a 4-linked β -D-xylose backbone and chains of 4-O-Methyl- α -D-glucuronic acid at O-2, and 2-O-L-fucopyranosyl-D-xylopyranose units at O-3.[24]

1.3. 4-O-methyl- glucuronic acid and 4-O-methyl-glucose

1.3.1. 4-O-Methyl-glucuronic acid

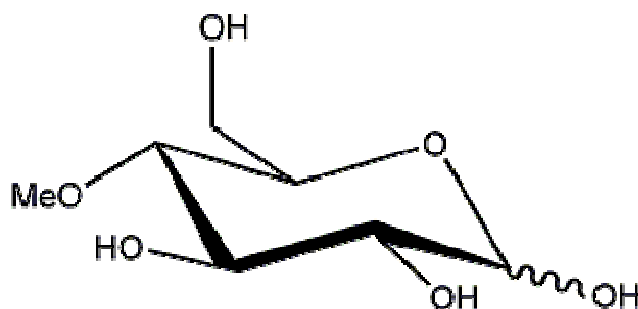
4-O-methyl glucuronic acid is a carboxylic acid derived from glucose. The constitution and configuration is that of glucose, but there is a methyl ether group at C-4, and C-6 is oxidised.



Its occurrence in nature has been observed in a variety of acid plant polysaccharides, especially hard wood xylans and polysaccharide gums. It can also be found, and has been reported in a glucosphingolipid of spermatozoa of a fresh water bivalve.[25] *Hyptis suaveolens*' acid polysaccharide contains a significant amount of this glucuronic acid.

1.3.2 4-O-methyl-glucose

4-O-methyl-glucose is a glucose derivative, O-methylated at position C-4.



It is not found in nature as often as the corresponding uronic acid, and has not been observed as a constituent of a polysaccharide. It has been found as a part of some aromatics isolated from *Bombyx batryticatus*, the larva of the Chinese silk bug, and *Cordyceps cicadae*, a mushroom, both used in traditional Chinese medicine [26]. It is not found in *Hyptis suaveolens*, but by reducing the carboxylic groups in the acid polysaccharide, 4-O-methyl-glucuronic acid is reduced to 4-O-methyl-glucose. It is the only methylated glucose which has not been crystallized. [27]

1.3.3 Synthesis and isolation of 4-O-methyl- glucuronic acid and 4-O-methyl-glucose

1.3.3.1. Isolation

Both 4-O-methyl- glucuronic acid and 4-O-methyl-glucose have been mentioned many times, but reports of isolating one of the compounds are rare. In 1957, both compounds were prepared through a series of reactions from the hemicellulose of *Populus tacamahacca*, the balsam poplar. Years later, the acid was isolated from a birch-wood xylan using xylanase (an enzyme cleaving glycosidic linkages of xylose) and glucuronidase, an enzyme which cleaves the glucuronic acids from a polysaccharide. [28] [30]

1.3.3.2. Synthesis

Since the isolation of either of the two compounds is difficult and gives low yields due to the small percentage of the compounds in polysaccharide, 4-O-methyl- glucuronic acid and 4-O-methyl-glucose have been synthesized chemically. However, most of the syntheses require special conditions, and have low yields. In 2009 a publication of the University of Agricultural sciences of Vienna showed a fairly simple procedure of the synthesis. The conditions could be reproduced, and the yields were good. The present diploma thesis relies considerably on that publication. An enzymatic synthesis of either of the compounds has not been reported, but would be an interesting field of research. [29]

2. Objectives of the present diploma thesis

The Department of Pharmaceutical Technology and Biopharmaceutics, of the University of Vienna has been investigating the contents of mucilages from different plants. Several plants have been researched, including *Ocimum basilicum*, *Hyptis suaveolens*, *Sterculia lychnophora* and *Sterculia urens*. In some of these plants, a neutral and an acidic polysaccharide occur together; these may be separated and processed further individually. In previous diploma theses, the polysaccharides of *Hyptis suaveolens* were extracted, acidic and neutral polysaccharide have been prepared, and a reduction of the carboxylic groups of the acid polysaccharide has been performed.

The usual starting analysis of any polysaccharide is its degradation to monosaccharides, and the comparison of the hydrolysate to known monosaccharides such as glucose, or xylose. Most of the standards are commercially available. However, some of them are very rarely available on the market, or not at all. 4-O-Methyl-D-glucuronic acid and 4-O-Methyl glucose belong to that group of standards. As they are frequently required for carbohydrate composition analyses, it is therefore necessary to synthesize these two compounds chemically. The present diploma thesis describes a new approach to such syntheses using a different type of protecting group for the carbohydrate intermediates.

The synthetic strategy used is partially based on a 2009 publication of Georg Sixta, Wilhelm Herok, Clemens Gruber, Hedda K. Weber, Herbert Sixta and Paul Kosma [29]. However, the pivalic ester group was used for hydroxyl protection (see the Scheme of the synthesis). Pivaloyl ester groups were found to be stable under the basic methylation conditions, while most of the other ester groups would be unstable under these conditions. Most of the intermediate products have been synthesized for the first time, and appropriate measures to characterize them have been taken, such as optical rotation, melting point and NMR spectra. While preliminary experiments were performed to oxidise intermediates to the glucuronic acid stage, it was found that such syntheses would require further development. The present diploma thesis therefore focuses on 4-O-methyl-D-glucose and the demonstration of its identity with a product formed upon hydrolysis of the carboxyl-reduced acidic polysaccharide from *Hyptis suaveolens*.

3. Materials and methods

3.1 Extraction of the polysaccharide and analysis of the monosaccharides

3.1.1 Extraction of the polysaccharide from the mucilage

- **Materials:**

- plastic beaker
- magnetic stirrer
- magnet
- sodium hydroxide 4M (NaOH 97% Sigma-Aldrich)
- distilled water
- hydrochloric acid 25% (Riedel de Haën)
- centrifuge (RC5C)
- pH meter (HANNA instruments, Microprocessor pH meter, pH 211)

- **Method:**

1g of the plant mucilage was suspended in 30 ml of 4M sodium hydroxide in a plastic beaker, covered with aluminium foil and left over night on a magnetic stirrer at 60°C. After the extraction was over, 30 ml of distilled water was added to increase the volume. The mixture was poured into a centrifugal tube, and centrifuged at 11,952 U over 5 min at 20-30°. The pellet was washed with 20 ml of 4M NaOH twice, and centrifuged again. The supernatant was collected and neutralized with 20-40 ml of 25% hydrochloric acid. [31]

3.1.2. Dialysis

- **General**

The main principle of dialysis is diffusion. Diffusion is a random movement of the molecules from one solution to another, which is separated by a semi-permeable membrane. Large molecules, in our case the polysaccharide, cannot pass the membrane and therefore stay inside the dialysis tube while the small molecules, in our case inorganic salts, diffuse out of the tube and can be removed in this manner. [32]

- **Materials**

- dialysis tube (Cellulose- Membrane, 76x49mm Sigma-Adrich)
- 5l plastic beaker
- pegs
- glass beaker
- hotplate
- distilled water
- demineralised water

- Method

A suitable piece of the dialysis tubing was cut (approx. 0.5 cm of the tube per 1 ml) and heated in a glass beaker with boiling demineralised water for 20 min. After the tube had cooled, one end of it was closed off with two pegs and the neutralized polysaccharide solution was poured into it. The filled tube was closed with two pegs at the top and was placed in a 5 l plastic beaker filled with distilled water. The beaker was left on the magnetic stirrer for 30 min, and the water was replaced with fresh 5 l of distilled water. The procedure was repeated 3 times. Finally, the beaker with the dialysis tubing was left on the magnetic stirrer overnight.

3.1.3 Lyophilization

- General

Lyophilization or freeze drying is a technique which is used to dry sensitive substances in a mild way. The main principle of lyophilization is sublimation, transition of water from the solid to the vapour state, without going to the liquid phase. First, the compound is frozen so that the water in the substance becomes ice, then the ice is evaporated under vacuum. The product of the process is a hygroscopic substance with almost no water. [33]

- Materials

- lyophiliser (HETO PowerDry LL3000)
- 500ml round-bottom flask
- dry ice
- acetone (min 99,8% Sigma- Aldrich)
- rotary evaporator (Heildolph WB 2001)
- lab jack
- Dewar bowl

- Method

The dialysed solution of the plant was poured into round-bottomed flasks and connected to a rotary evaporator. A Dewar bowl was filled with acetone and placed under the rotary evaporator. The evaporator was turned on so that only the rotary part was functional, and the rotating flask was slowly dipped into the bowl with acetone. Pieces of dry ice were then gradually put into acetone so that the temperature was constantly around -78°C. In this manner, the solution in the flasks was shell-frozen. After the whole amount was frozen, the flask was disconnected from the rotary evaporator and connected to a lyophiliser, and the water was sublimed at -54°C.

3.1.4. Ion-exchange chromatography

- General

Ion exchange chromatography is a well established technique to separate charged molecules such as proteins or polysaccharides. It is based on the interaction between a charged molecule in solution and the matrix, which is charged in the opposite direction. The starting eluant pH value is set up so that the molecules or ions in solution can be bound to the matrix. When the sample is applied, neutral molecules will pass through the column unhindered while cations will bind to a cation exchanger resin and anions will bind to an anion exchanger resin. The original counterions

of the charged species will be eluted together with the counterions originally bound to the charged groups of the respective ion exchange resin. The actual separation of the molecules is achieved by using eluants which are unfavorable to the binding of the molecule. The ionic bond between the molecule and the matrix is disrupted, and the molecule is being eluted by the buffer. Normally a gradient of a salt is used for elution, and the molecules with the weakest bond to the matrix are eluted first. When the elution is finished, the matrix is regenerated, and can be used again. [34]

- Materials

- DEAE cellulose (Express-Ion exchanger D free base- Sigma)
- distilled water
- ethanol (absolute Merck)
- TRIS 0,1M /HCl pH 7 (TRIS min 99,8% Merck, HCl 25% Riedel de Haën)
- DEAE column (height- 22cm, diameter 1,6cm, - BioRad)
- sodium chloride 0,5M (NaCl min 99,5%, Merck)
- Büchner funnel
- filter paper
- citric acid 0,01M (citric acid 99% Sigma-Aldrich, distilled water)

- Method

12.8 g of DEAE cellulose was suspended in 30 ml of distilled water, and put in a measuring cylinder. After sedimentation, the gel reached 30 ml volume. The gel was put in a Büchner funnel which was covered with filter paper, and washed in vacuo with 500ml of distilled water. Afterwards, the gel was poured into a glass beaker, and 50 ml of distilled water was added. The gel was again put in a filtration funnel, washed with 2 times 30 ml of TRIS buffer, then poured back in the glass beaker and suspended in 30 ml of TRIS buffer. It was neutralised to pH 7.6 with 0,01M citric acid, and poured into a column. *Hyptis* polysaccharide was dissolved in TRIS buffer and applied to the column. Fractions of about 10 ml were collected. First, the neutral polysaccharide was eluted with 0,1M TRIS solution. Elution was controlled by TLC (Chapter 3.1.6). After the TLC showed no more of the neutral polysaccharide being eluted, the acid polysaccharide was eluted with 0,5M sodium chloride. Both the acidic and the neutral polysaccharide were dialyzed and lyophilized.

3.1.5 Hydrolysis

- General

A polysaccharide may consist of a several monosaccharide constituents, which are bound to each other through glycosidic linkages. When a polysaccharide is treated with an acid solution over some time and at relatively high temperatures, these linkages are destroyed. A mixture of monosaccharides is the result. This method is often used for the preliminary analysis of polysaccharides. However, some of the monosaccharides are not stable in the harsh conditions necessary to accomplish hydrolysis, and may be lost.

- Materials

- Trifluoroacetic acid (TFA) (99% Sigma-Aldrich)
- distilled water
- methanol (min. 99,8%, Sigma-Aldrich)
- drying cabinet

- hot plate
- nitrogen gas (Alphagaz)
- test tubes

- Method

Approx. 5-20 mg of the polysaccharide is placed in a test tube, 2 ml of 2M trifluoroacetic acid (TFA) is added and the tube closed with a screw cap. The test tube was heated in a drying cabinet for 2h at 100°C. After two h the test tube is taken out of the cabinet, and put in a water bath previously heated to 60°C. Under a nitrogen atmosphere, the polysaccharide was diluted with methanol, then evaporated in a stream of nitrogen. This process is repeated three times. Afterwards, the evaporated compounds were dissolved in water, and again evaporated 3 times, then dissolved in water.

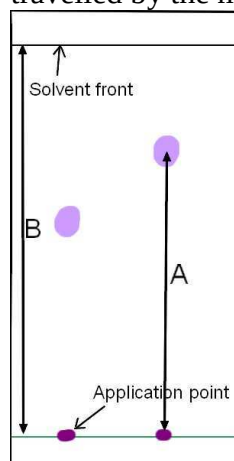
3.1.6 Thin layer chromatography (TLC)

- General

Thin layer chromatography is a form of liquid chromatography. The basic principle of a liquid chromatography is distribution of the sample between the stationary phase which is solid, and a liquid mobile phase. The sample has affinity to both phases. The mobile phase drives it forward, while the stationary phase resists, so that the molecules are “travelling”. How far a molecule “travels”, depends on its affinity toward both of the phases. If it has a greater affinity to the mobile phase, it will travel further, and vice versa.

Thin layer chromatography is a simple and inexpensive method for a preliminary identification of substances, and is one of the most important techniques of observation in chemical synthesis. The stationary phase in thin layer chromatography is on a glass, plastic or aluminium plate. The mobile phase is in a reservoir, a so called TLC trough. The sample is dissolved, and applied on a spot on the stationary phase, the application point. A line is usually drawn approx. 0.5 cm from the end of the plate. The line is known as solvent front, and it marks the front of the mobile phase. The plate is placed in a trough, and the mobile phase moves up the plate due to capillary action. After the TLC reaches the front, it is taken out of the trough, and the sample can be detected. Detection is usually achieved by spraying reagents, or measuring UV absorption. To identify the sample, one uses standards which are run simultaneously on the same plate.

R_f value, or retention factor is the distance travelled by the sample divided by the distance travelled by the mobile phase. $R_f = A/B$ [35]



- Materials

- TLC plates, 10x20cm, 5x10cm (TLC Silicagel 60 F254, Merck)
- TLC trough
- scanner
- drying cabinet
- dryer
- distilled water
- methanol (min 99,8% Sigma-Aldrich)
- acetonitrile (min. 99,9%)
- ethanol (absolute, Merck)
- 4-O- methoxy- benzaldehyde (anis aldehyde)
- thymol (min 99,5% Sigma-Aldrich)
- sulfuric acid (95-98%, Sigma-Aldrich)
- acetic acid (min 99,9% Merck)
- ethyl acetate (min99,7% Merck)
- hexane (min 95% Sigma-Aldrich)
- isopropanol (99,5% Sigma-Aldrich)
- micropipettes 10µl

* Standards:

- D-arabinose
- D-fructose
- D-fucose
- D-mannose
- D-rhamnose
- D-xylose
- D-glucose
- D-galactose
- D-glucuronic acid
- D-galacturonic acid
- maltose
- ribose

* Solvents:

- acetonitrile : water 17:1(V/V)
- hexane : ethyl acetate 1:1(V/V)
- hexane : ethyl acetate 3:1 (V/V)
- toluene : ethyl acetate 1:1(V/V)
- isopropanol : ethyl acetate 1:5 (V/V)

* Detection reagents:

- Anisaldehyde reagent: 5ml anisaldehyde, 5ml sulphuric acid, 1ml acetic acid, 95ml ethanol)
- Thymol Reagent: 0.5g thymol, 5ml sulphuric acid, 95ml ethanol [22]

- Method

Approx. 4-5µl of the sample were applied on the TLC plate together with 4-5µl of the standards. The plate was dried thoroughly, and placed in the TLC trough. After the solvent front reached the marked line, the TLC plate was taken out of the trough, dried and either sprayed with anisaldehyde reagent, or dipped into thymol reagent. The plate was then placed in the drying cabinet for 2 min at 100°C for colour development. Since the plates change colour after a while, they were scanned as soon as they were cooled down.

3.1.7 Reduction of carboxylic groups of the acidic polysaccharide

Since uronic-acid containing polysaccharides are usually more difficult to hydrolyse, the acidic polysaccharide from *Hyptis suaveolens* was subjected to carboxyl reduction. In this process, 4-O-methylglucuronic acid is converted into 4-O-methyl glucose. 4-O-Methyl glucose is found in the acid hydrolysate of the carboxyl-reduced polysaccharide and could thus be compared to the authentic standard synthesized in the present diploma thesis.

- General

Reduction of the uronic acids in the polysaccharide to the corresponding neutral sugars facilitates monosaccharide analysis. In the most frequently used application, the polysaccharide reacts with a water soluble carbodiimide to form lactones (intramolecular esters), which are subsequently reduced with sodium borohydride. [36]

- Materials

- distilled water
- pH Meter (HANNA instruments, Microprocessor pH meter, pH 211)
- 1-ethyl-3(3-dimethylaminopropyl)carbodiimide (Sigma-Aldrich)
- sodium borohydride (98% Sigma-Aldrich)
- octanol (Merck)
- 0.1M hydrochloric acid (Hydrochloric acid 37%, Sigma-Aldrich, distilled water)

- Method

50mg of the acid polysaccharide was dissolved in 10ml of distilled water, and the pH value was set to 6. 420mg of 1-ethyl-3(3-dimethylaminopropyl) carbodiimide was slowly added, and the pH value was simultaneously corrected to 4.75 with 0,1M hydrochloric acid for one hour. To avoid foaming, a drop of octanol was added. 25 ml of 4M sodium borohydride is was added dropwise, and the pH value was kept at 6 over the next two h with 0,1M hydrochloric acid. The mixture was then made acidic to destroy the remaining sodium borohydride. The solution was dialysed, then lyophilized. [36]

3.2. Chemical synthesis of 4-O-methyl glucose

General methods have been described in this chapter, materials and methods for the individual reaction steps are found in chapter 4.

The compounds were identified with the help of thin layer chromatography, optical rotation, melting point, and NMR spectroscopy. Purification was performed by recrystallisation and column chromatography.

3.2.1. Thin layer chromatography

This technique was used to follow the reactions, and to identify the products and side products. The general principle, materials and methods are described in Chapter 3.1.6.

3.2.2. Column chromatography

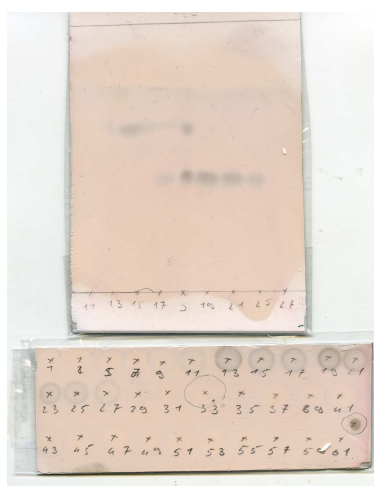
Column chromatography is a form of liquid chromatography. The general principle is the same as that of thin layer chromatography, but the stationary phase is placed in a glass column. The mobile phase is added through the top of the column and flows through the stationary phase with the help of gravity or pressure. The mobile phase was collected in fractions as it drips through the bottom of the column. Column chromatography was used to separate the desired compounds from side products.

- Materials

- glass column (different sizes)
- silica gel (Merck)
- solvents used for the mobile phase are the same as solvents used for the TLC
- dichloromethane
- methanol (min. 99,8%, Sigma-Aldrich)

- Method

The silica gel was suspended in the mobile phase and the suspension was carefully packed into the column taking care that no air bubbles form in the column. The mixture to be separated was dissolved in either dichloromethane or methanol and carefully applied to the top of the column. Fractions of different volumes were collected, depending on the size of the column, and the quantity of the target compound. Fractions were controlled by TLC. The desired fractions were gathered and evaporated.



3.2.3. Recrystallization

- General

The basic principle is dissolving the crystals in a solvent at elevated temperatures, and then cooling the solution down to room temperature or lower so that the crystals can be formed. The pure substance forms the crystals, and the impurities stay in the solvent. The most important part of recrystallization is finding the right solvent or solvent system.

- Materials

- isopropanol (99,5% Sigma-Aldrich)
- ethanol (absolute, Merck)
- Büchner funnel
- reflux cooler
- water hose
- round bottom flask
- hot plate
- water hose
- glass bowl
- tap water
- filter paper

- Method

The compound is suspended in a small amount of the solvent in a round bottomed flask. The flask is put in a glass bowl filled with water, and is connected with the reflux condenser. The mixture is heated gradually, and more solvent is added dropwise if necessary until the compound dissolves entirely. The mixture was left to cool down. When the crystals are formed, the mixture was filtrated through the Büchner funnel with filter paper and the crystals are collected.

3.2.4. Melting point

Melting points are determined on a Kofler bench. The crystals are placed on glass slides on a Kofler bench, and are observed through the microscope. The bench is heated gradually, until all of the crystals are molten. The melting point is read on the electronic thermometer.

3.2.5. Polarimetry

- General

Polarimetry is the technique of measuring the rotation of the plane of plane polarized light through optically active substances. Optically active substances, or chiral substances, have the ability to rotate the plane of polarized light either to the left (-), or to the right (+). When the plane polarised light passes through a dissolved chiral substance, the plane is rotated. How it changes depends on the concentration of the + or – enantiomers, temperature, solvent, path length and concentration of the substance. The angle by which the plane polarized light plane has changed is designated α .

$$[\alpha]_D^{20} = \frac{\alpha \cdot 100}{c \cdot l} \quad \text{specific rotation}$$

where α is the angle of the rotation in degrees $^\circ$, c is the concentration of the substance in g/100 ml and l is the path length (usually 1 dm) [37]

- Materials
 - Polarimeter "Perkin Elmer 341"
 - chloroform (Sigma-Aldrich)
 - distilled water
- Method

A specified amount of the compound was dissolved in the required amount of solvent (approx. 10 ml), and the solution placed in the glass container. The angle of rotation was measured automatically.

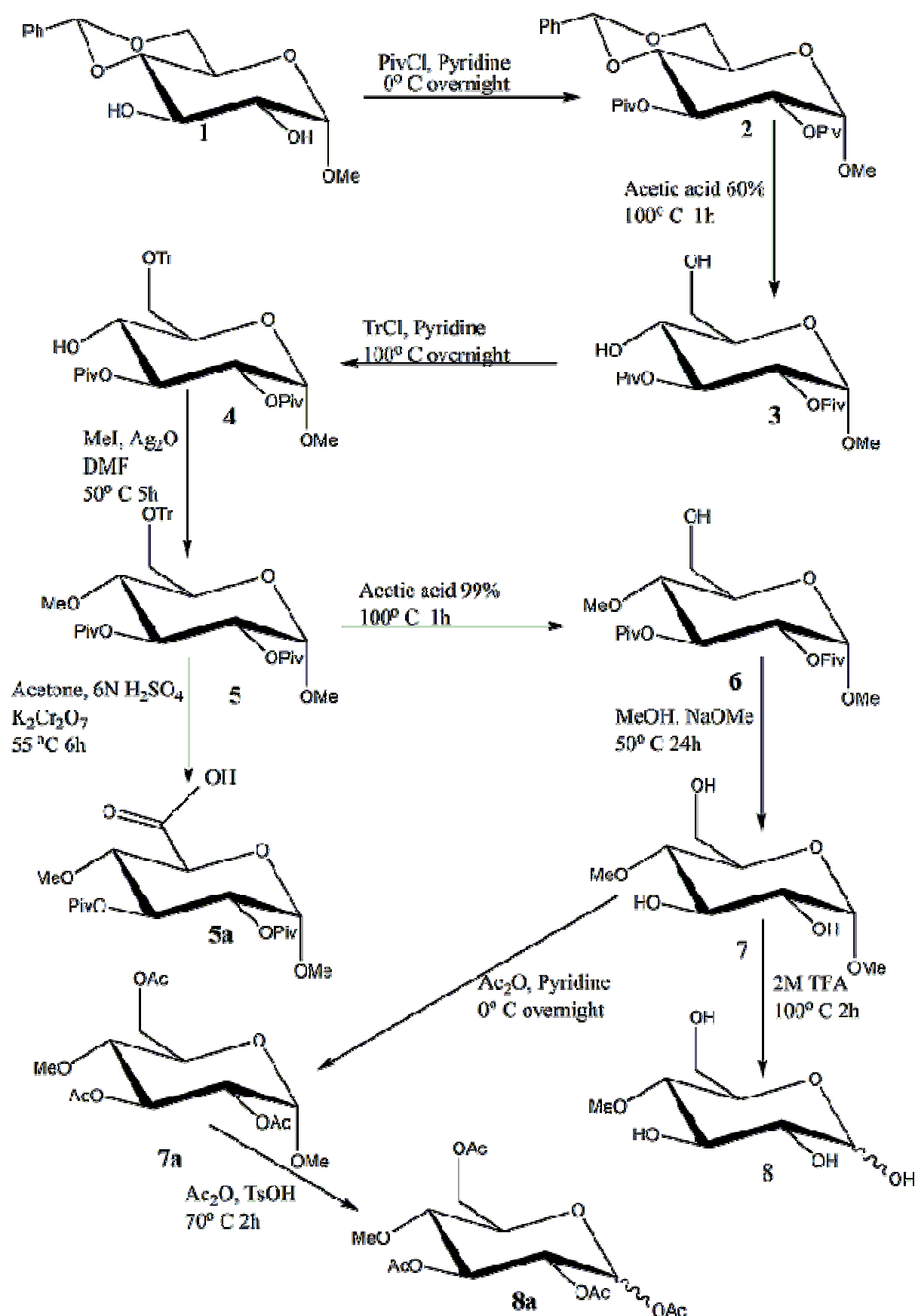
3.2.6. NMR- Spectroscopy

NMR spectroscopy is the most important technique for identification of organic compounds, and is an essential tool for following chemical syntheses. Atoms with a spin of $\frac{1}{2}$ (for example, hydrogen) can be used to record NMR spectra. If one applies an external magnetic field, the nuclei are divided into two populations, one oriented parallel to the magnetic field, the other, antiparallel to the magnetic field. The antiparallel energy state is slightly higher than the parallel one, and, by application of a characteristic radiofrequency, nuclei from the lower energy level can be transferred to the higher level. The radiofrequency required to raise nuclei to the higher energy level is referred to as the resonance frequency and depends on the chemical environment of the nucleus. Electronegativity, ring current and bond strain also affect the frequency. Proton reference frequency is the one of tetramethylsilane, and its chemical shift is set to zero in ^1H -NMR. Chemical shifts are given in parts per million of the magnetic field applied (for 500 MHz one ppm is 500 Hz). Most protons in organic molecules have chemical shifts within a range of 10 ppm (5 kHz in case of a magnetic field of 500 MHz). For ^{13}C -NMR, most carbon resonances occur within a range of 200 ppm (corresponding to 25 kHz at a magnetic field of 125 MHz). In the present diploma thesis, both proton and carbon resonance spectra have been used to characterize synthetic intermediates.

All NMR spectra were kindly recorded by Professor Wolfgang Holzer of the Department of Pharmaceutical Chemistry, Faculty of Life Sciences of the University of Vienna. [38]

- Materials
 - "Avance DRX 500" 500 and 125 MHz (Brucker)
 - Calibration:
 - ^1H -NMR: CHCl_3 in CDCl_3 7.24 ppm, H_2O in D_2O 4.79 ppm;
 - ^{13}C -NMR: CDCl_3 77.00 ppm

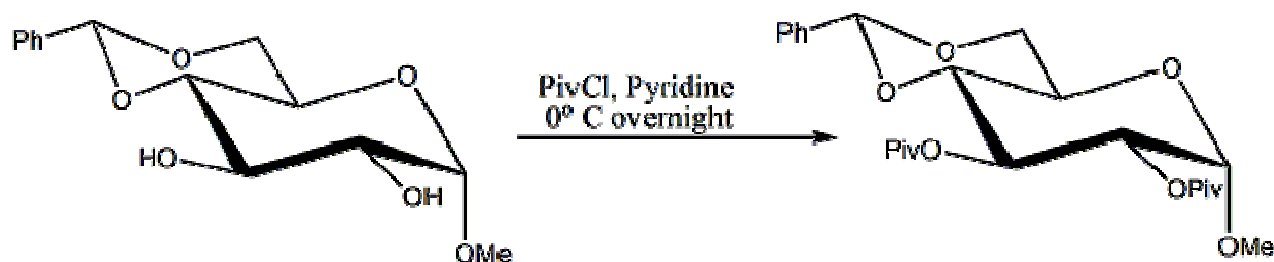
Scheme of the synthesis of 4-O-methyl glucose



4. Experimental

4.1. Synthesis of 4-O-methyl glucose

4.1.1. Methyl-2,3 –O-pivaloyl-4,6-O-benzylidene- α -glucopyranoside



Materials:

- pivaloyl chloride (99% Sigma-Aldrich)
- methyl 4,6-O-benzylidene- α -D-glucopyranoside (Glycon)
- dry Pyridine (Pyridine 99,8% Sigma_Aldrich)
- distilled Water
- ice
- dichlormethane (Merck)
- sodium hydrogencarbonate (99,5% Sigma_Aldrich)
- magnetic stirrer
- drying tube
- calcium sulfate (99%, Sigma-Aldrich)
- Drierite® 6 mesh with indicator (Sigma-Aldrich)
- magnet
- magnesium sulphate (97% Sigma-Aldrich)

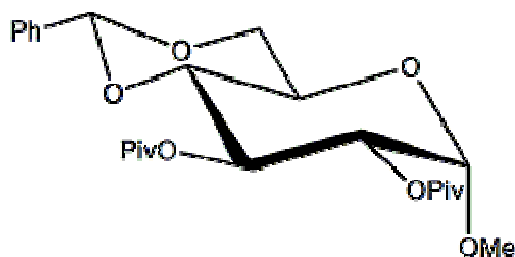
Method:

Methyl-4,6-O-benzylidene- α -glucopyranoside (5.00g, 17.7mmol) was dissolved in dry pyridine (50 ml) in an ice bath and pivaloyl chloride (10 ml) was slowly added. The ice bath was then removed, and the solution left overnight. When the TLC showed complete consumption of the starting material, the mixture was poured into iced water, extracted with dichloromethane, washed 3 times with sodium hydrogencarbonate, 3 times with water, dried over magnesium sulfate, and evaporated to give 5.52g (66.7%) of **2**. [39]

Characterisation:

$^1\text{H-NMR}$: δ 7.43-7.39 (m, 2 H, H-PhH-2,6), 7.36-7.31 (m, 3 H, PhH-3,4,5), 5.62 (dd, 1 H, $J_{3,2} = J_{3,4} = 9.9$ Hz, H-3), 5.52 (s, 1 H, PhCH), 4.94 (d, 1 H, $J_{1,2} = 3.8$ Hz, H-1), 4.86 (dd, 1 H, $J_{2,1} = 3.8$, $J_{2,3} = 9.9$ Hz, H-6), 4.32 (dd, 1 H, $J_{6,6'} = 10.3$, $J_{6,5} = 4.9$ Hz, H-6), 3.92 (ddd, 1 H, $J_{5,4} = 9.9$, $J_{5,6} = 4.9$, $J_{5,6'} = 9.9$ Hz, H-5), 3.78 (dd, 1 H, $J_{6,6'} \sim J_{6,5} \sim 10.3$), 3.67 (dd, 1 H, $J_{4,3} \sim J_{4,5} \sim 9.7$ Hz, H-4), 3.39 (s, 3 H, 1-OCH₃), 1.18, 1.16 ppm (2s, 18 H, 6xCH₃Piv).

$^{13}\text{C-NMR}$: δ 26.92, 27.07 (2x CH₃ Piv), 38.72, 38.74 (2x(CH₃)₃C Piv), 55.55 (1-O-CH₃), 62.24 (C-5), 68.43 (C-3), 68.84 (C-6), 71.40 (C-2), 79.43 (C-4), 97.68 (C-1), 101.13 (PhCH), 125.84 (PhC-2,6), 128.11 (PhC-3,5), 128.82 (PhC-4), 137.0 (PhC-1), 176.91, 177.91 ppm



C₃₅H₃₆O₈

MW: 464.55

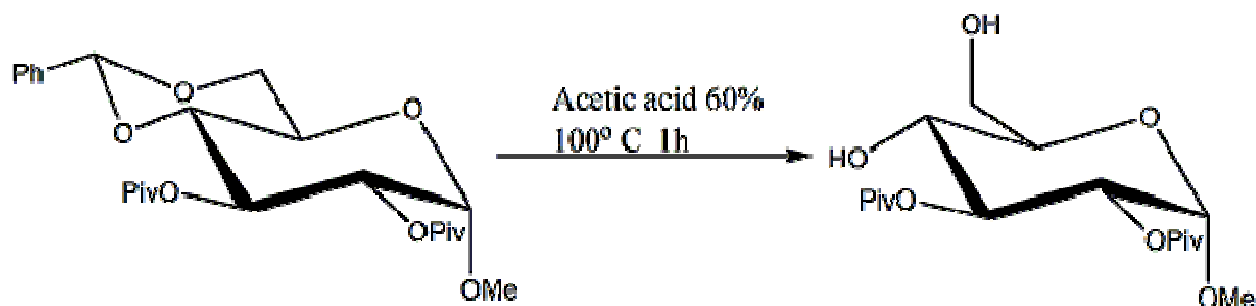
R_f (1) 0.13

R_f (2) 0.83 (ethyl acetate : toluene 1:1 V/V)

$[\alpha]_{\text{D}}^{20}$: -68.9 (20°C, 638mg/100ml water)

Melting point: 149 °C

4.1.2. Methyl-2,3 –O-pivaloyl- α -glucopyranoside



Materials:

- acetic acid (min 99,9% Merck)
- hot plate with a magnetic stirrer
- reflux condenser
- magnet
- distilled water
- dichloromethane (Merck)
- sodium hydrogencarbonate (99,5% Sigma_Aldrich)
- drying tube
- calcium sulfate (99%, Sigma-Aldrich)
- Drierite® 6 mesh with indicator (Sigma-Aldrich)
- water hose
- thermometer

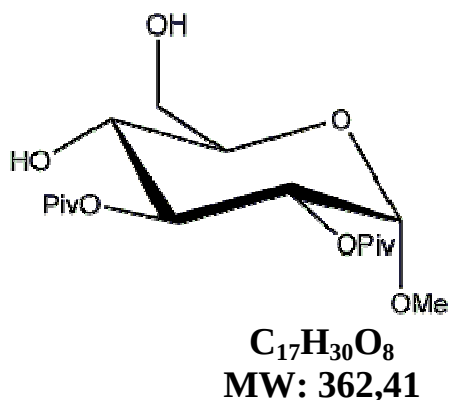
Method:

Methyl-2,3 –O-pivaloyl-4,6-O-benzylidene- α -glucopyranoside (5.21g, 11.2 mmol) was dissolved in 50% acetic acid (70 ml), and heated to 100 °. After 1 h the compound had dissolved entirely and the TLC showed complete consumption of **2**. The solution was neutralised with sodium hydrogen carbonate, extracted with dichloromethane, washed with water and evaporated to give 4.1g (100%) of **3**. [29]

Characterisation:

$^1\text{H-NMR}$: δ 5.32 (m, 1 H, $J_{3,2} \sim J_{3,4} \sim 10$ Hz, 3 H), 4.91 (d, 1H, $J_{1,2} \sim 3.5$ Hz, H-1), 4.79 (dd, 1 H, $J_{2,3} \sim 10$, $J_{2,1} \sim 3.5$ Hz, H-2), 3.90 (dd, 1 H, $J_{6,6'} = 12$, $J_{6,5} = 2.5$ Hz, H-6), 3.86 (dd, 1 H, $J_{6,6'} = 12$, $J_{6,5} = 3.5$ Hz, H-6'), 3.75-3.68(m, 2 H, distinguishable $J_{4,3} \sim J_{4,5} \sim 10$ Hz, H-4, H-5), 3.38 (s, 3 H, 1-O-CH₃), 1.19, 1.17 ppm (2s, 18H, 2x (CH₃)₃C-Piv).

$^{13}\text{C-NMR}$: δ 26.95, 27.08 (2x (CH₃)₃C-Piv), 38.73, 38.93 (2x (CH₃)₃C-Piv), 55.45 (1-O-CH₃), 61.97 (C-6), 70.04 (C-4), 70.64 (C-2), 71.16 (C-5), 73.17 (C-3), 96.75 (C-1), 177.85 (PivC=O at C-2), 179.62 ppm (PivC=O at C-3).



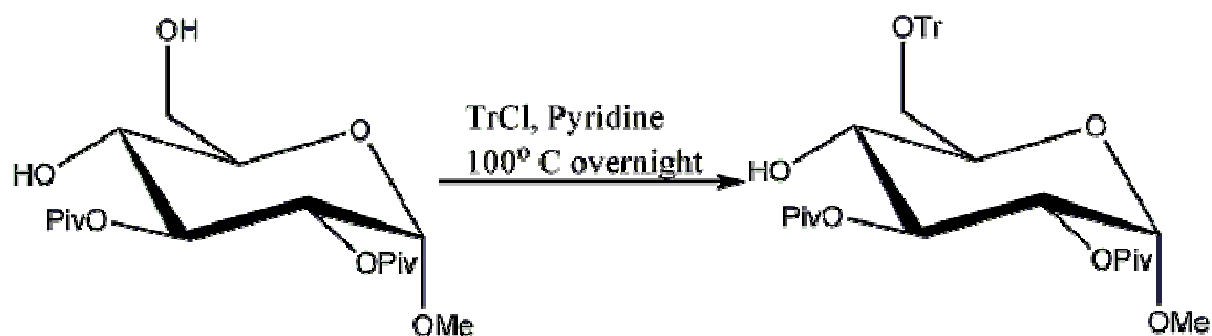
Rf (2) 0.9

Rf: (3) 0.36 (ethyl acetate: hexane 3:1V/V)

$[\alpha]_D^{20}$: +68.21 (20°C 322 mg/100ml chloroform)

Melting point: 69°C

4.1.3 Methyl-2,3-di-O-pivaloyl-6-O-trityl- α -D-glucopyranoside



Materials:

- tritylchloride (97% Sigma-Aldrich)
- dry pyridine (Pyridine 99,8% Sigma_Aldrich)
- hot plate with a magnetic stirrer
- reflux condenser
- magnet
- distilled water
- dichloromethane (Merck)
- sulfuric acid 10%(Sulfuric acid 95-98%, Sigma-Aldrich, distilled water)
- sodium hydrogencarbonate (99,5% Sigma_Aldrich)
- drying tube
- calcium sulfate (99%, Sigma-Aldrich)
- Drierite® 6 mesh with indicator (Sigma-Aldrich)
- water hose
- thermometer
- magnesium sulphate (97% Sigma-Aldrich)

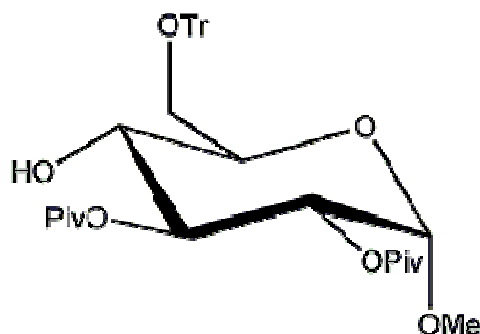
Method:

Methyl-2,3 –O-pivaloyl- α -glucopyranoside (3.91g, 10, 8mmol) was dissolved in pyridine (50 ml) and heated to 100 °C. Trityl chloride was added (3.611g, 12.9 mmol) and the mixture was left overnight. After the TLC showed complete consumption of compound 3, the mixture was poured over ice water, extracted with dichloromethane (3x50ml), washed with 10% sulphuric acid, then with sodium hydrogen carbonate until neutral, dried with magnesium sulphate and evaporated to produce 5.275g (80.8%) of white crystals. Compound **4** was recrystallized from isopropanol. [29]

Characterisation:

$^1\text{H-NMR}$: δ 7.50-7.46 (m, 6 H PhH -2,6), 7.33-7.28 (m, 6 H, Ph H -3,5), 7.27-7.22 (m, 3 H PhH-4), 5.31 (dd, 1 H, $J_{3,2} = 10.0$, $J_{3,4} = 9.4$ Hz, H 3), 4.94 (d, 1 H $J_{1,2} = 3.7$ Hz, H-1), 4.81 (dd, 1 H, $J_{2,1} = 3.8$, $J_{2,3} = 10.1$ Hz, H-2), 4.01 (heptuplett, 1 H, $^3J = 6.1$ Hz, $(\text{CH}_3)\text{CH}$ of one molecule of isopropanol of crystallization), 3.77 (ddd, 1 H, $J_{5,4} = 9.5$, $J_{5,6} = 3.6$, $J_{5,6'} = 5.6$ Hz, H-5), 3.57 (dd, $J_{4,3} \sim J_{4,5} \sim 9.5$ Hz, H-4), 3.46 (dd, $J_{6,6'} = 10.2$, $J_{6,5} = 3.6$ Hz H-6'), 3.41 (s, 3 H 1-O- CH_3), 3.37 (dd, 1 H, $J_{6,6'} = 10.2$, $J_{6',5} = 5.6$ Hz, H-6'), 1.20 (d, 6 H, $J = 6$ Hz, $(\text{CH}_3)_2$ isopropanol of crystallization), 1.19, 1.18 ppm (2s, 2x 9 H, Piv(CH_3)).

$^{13}\text{C-NMR}$: δ 25.25 ($(\text{CH}_3)_2$ isopropanol), 26.95, 27.07 (2x Piv(CH_3)), 38.69, 38.81 (2x Piv(CH_3)), 55.19 (1-O- CH_3), 63.89 (C-6), 70.23 (C-5), 70.71 (C-2), 71.05 (C-4), 72.67 (C-3), 87.00 (Ph_3C), 96.50 (C-1), 127.06 (PhC-4), 127.84 (PhC-3,5), 128.62 (PhC-2,6), 143.73 (PhC-1), 177.86, 178.89 ppm (2x PivC=O).



$\text{C}_{36}\text{H}_{44}\text{O}_7$
MW:588,73

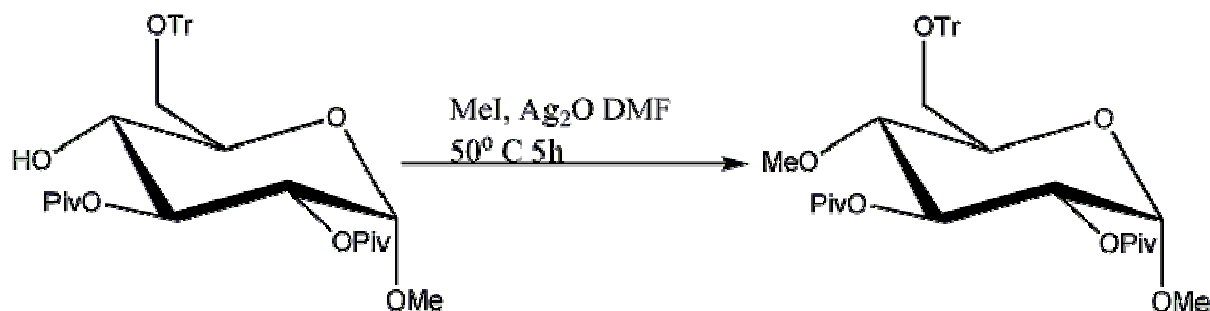
Rf (3) 0.06

Rf (4) 0.95 (ethyl acetate : hexane 3:1V/V)

$[\alpha]_{\text{D}}^{20}$: -31.87 (125mg /100ml chloroform)

Melting point: 124°C

4.1.4. Methyl-2,3-di-O-pivaloyl-4-O-methyl-6-O-trityl- α -D-glucopyranoside



Materials:

- dry DMF (99,8% Sigma_Aldrich)
- silver oxide (99% Sigma-Aldrich)
- iodomethane (99% Sigma –Aldrich)
- Celite® 545 coarse (Merck)
- hot plate with a magnetic stirrer
- reflux condenser
- magnet
- distilled water
- dichloromethane (Merck)
- drying tube
- calcium sulfate (99%, Sigma-Aldrich)
- Drierite® 6 mesh with indicator (Sigma-Aldrich)
- water hose
- thermometer
- isopropanol (99,5% Sigma-Aldrich)

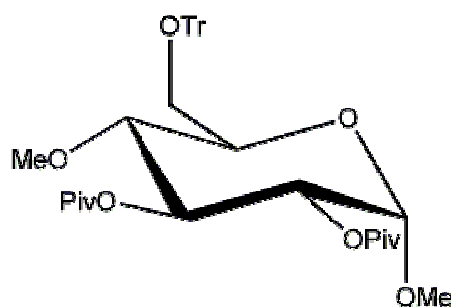
Method:

Methyl-2,3-di-O-pivaloyl-6-O-trityl- α -D-glucopyranoside (5.275g, 8.35 mmol) was dissolved in dry DMF (50 ml) at room temperature. Silver oxide (4.731g, 2 eq) was added after 15 min of stirring, together with methyl iodide (1.63 ml, 3eq). The mixture was heated to 50 °C. After 5h, another 2g of silver oxide, and 0.75 ml of methyl iodide was added, and checked with a TLC after 2h. The mixture was cooled down to room temperature, filtered over Celite, evaporated in vacuo at 55° so that DMF can be evaporated. The mixture showed white crystals which were taken from the mixture (consistency similar to honey). The crystals were recrystallized from isopropanol to receive (1.56g, 30%) of 5. [40]

Characterisation:

$^1\text{H-NMR}$: 7.50-7.49 (m, 6H, 2,6-H-arom.), 7.30 (m, 6H, H-3,5 arom.), 7.24 (3H, H-4 arom.), 5.45 (dd, 1 H, $J_{3,2} = 10.0$, $J_{3,4} = 9.5$ Hz, H-3), 7.97 (d, 1 H, $J_{1,2} = 3.7$ Hz, H-1), 4.82 (dd, 1 H, $J_{2,1} = 3.7$, $J_{2,3} = 10.2$ Hz, H-2), 3.77 (dd, 1 H, $J_{2,1} = 3.7$, $J_{2,3} = 10.2$ Hz, H-2), 3.77 (ddd, 1 H, $J_{5,4} = 10.0$, $J_{5,6} = 2.1$, $J_{5,6'} = 4.5$ Hz, H-5), 3.44 (dd, $J_{6,6'} = 10.2$, $J_{6,5} = 2.1$ Hz, H-6), 3.45 (dd, 1 H, $J_{4,5} = 10.0$, $J_{4,3} = 9.5$ Hz, H-4), 3.40 (s, 3 H, 1-OCH₃), 3.13 (dd, 1 H, $J_{6,6'} = 10.2$, $J_{6,5} = 4.5$ Hz, H-6; s, 3 H, H-4-OCH₃), 1.19, 1.18 ppm (s, 9 H, (CH₃)₃ PivO-2).

$^{13}\text{C-NMR}$: 27.0 (CH₃PivO-2), 27.2 (CH₃ PivO-3), 38.70 (C $_{\alpha}$ PivO-3), 38.74 (C $_{\alpha}$ PivO-2), 55.2 (1-O-CH₃), 60.0 (4-O-CH₃), 62.4 (C-6), 69.8 (C-5), 71.3 (C-3), 71.6 (C-2), 78.3 (C-4), 86.4 (C-Ph₃), 96.6 (C-1), 126.0 (PhC-4), 127.8 (PhC-3,5), 127.9 (PhC-2,6) 143.9 (PhC-1), 177.0, 178.1 ppm (PivC=O at C-3, C-2).



C₃₇H₄₆O₇
MW: 602,76

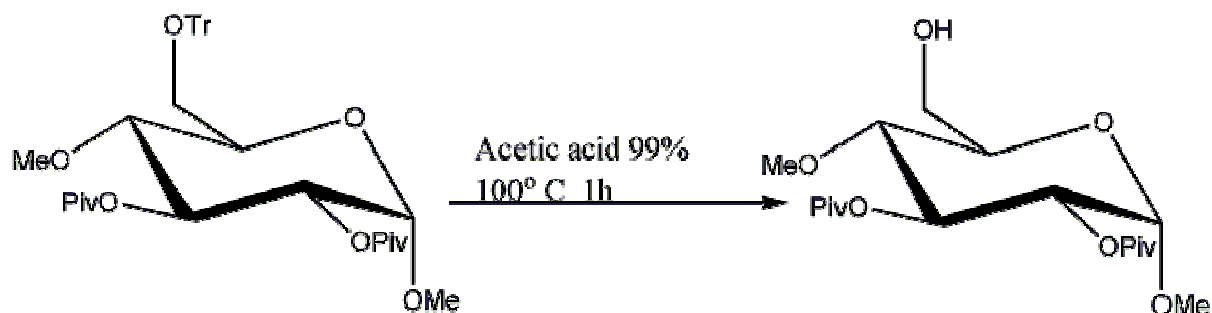
R_f (4) 0.88

R_f (5) 0.99 (ethyl acetate: hexane 1:3 V/V)

$[\alpha]_D^{20}$: +43,88 (160mg /100 ml chloroform)

Melting Point: 234°C

4.1.5. Methyl-2,3-di-O-pivaloyl-4-O-methyl- α -glucopyranoside



Materials:

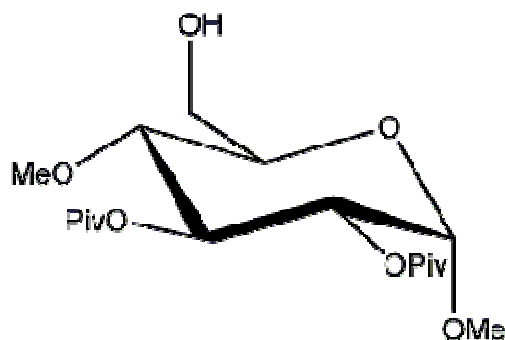
- acetic acid (min 99,9% Merck)
- hot plate with a magnetic stirrer
- reflux condenser
- magnet
- distilled water
- dichloromethane (Merck)
- sodium hydrogencarbonate (99,5% Sigma_Aldrich)
- drying tube
- calcium sulfate (99%, Sigma-Aldrich)
- Drierite® 6 mesh with indicator (Sigma-Aldrich)
- water hose
- thermometer
- magnesium sulphate (97% Sigma-Aldrich)

Method:

Methyl-2,3-di-O-pivaloyl-4-O-methyl-6-O-trityl- α -D-glucopyranoside (1.50g ,2,59 mmol) was suspended in 99% acetic acid (25ml), and water (1 ml). The mixture was heated to 100°C for one h. The compound was completely dissolved, and TLC showed complete consumption of **5**. The mixture was cooled down to room temperature, neutralized with sodium hydrogencarbonate, extracted with dichloromethane, washed with water, dried over MgSO₄, and evaporated to receive 561.1mg, 58% of **6**.^[41]

Characterisation:

$^1\text{H-NMR}$: δ 5.50 (dd, 1 H, $J_{3,2} = 10.0$, $J_{3,4} = 9.6$ Hz H-3), 5.88 (d, 1 H, $J_{1,2} = 3.8$ Hz, H-1), 4.69 (dd, 1 H, $J_{2,1} = 3.75$, $J_{2,3} = 10.2$ Hz, H-2), 3.67 (m, 1 H, $J_{6,6'} = 12.0$, $J_{6,\text{OH}} = 4.6$, $J_{6,5} = 2.7$ Hz, H-6), 3.78 (ddd, 1 H, $J_{6,6'} = 12.0$, $J_{6',\text{OH}} = 8.4$, $J_{6',5} = 3.6$ Hz, H-6'), 3.69 (ddd, 1 H, $J_{5,4} = 9.9$, $J_{5,6} = 2.7$, $J_{5,6'} = 3.6$ Hz, H-5), 3.45 (s, 3 H, 4-O- CH_3), 3.41 (dd, 1 H, $J_{4,5} \sim J_{4,3} \sim 9.6$ Hz, H-4), 3.35 (s, 3 H, 1-O- CH_3), 1.91 (dd, < 1 H, $J_{\text{OH},6} \sim 4.7$, $J_{\text{OH},6'} \sim 8.4$ Hz, 6-OH), 1.19, 1.16 ppm (2s, 2x 9H, Piv CH_3).
 $^{13}\text{C-NMR}$: δ 26.96, 27.21 (Piv- CH_3), 38.73 (Piv $(\text{CH}_3)_3\text{C}$), 55.43 (1-O- CH_3), 60.23 (4-O- CH_3), 61.52 (C-6), 70.36 (C-5), 71.12 (C-3), 71.50 (C-2), 77.58 (C-4), 96.77 (C-1), 176.95, 178.02 ppm (2xPiv $\text{C}=\text{O}$).



$\text{C}_{18}\text{H}_{32}\text{O}_8$
MW: 376,44

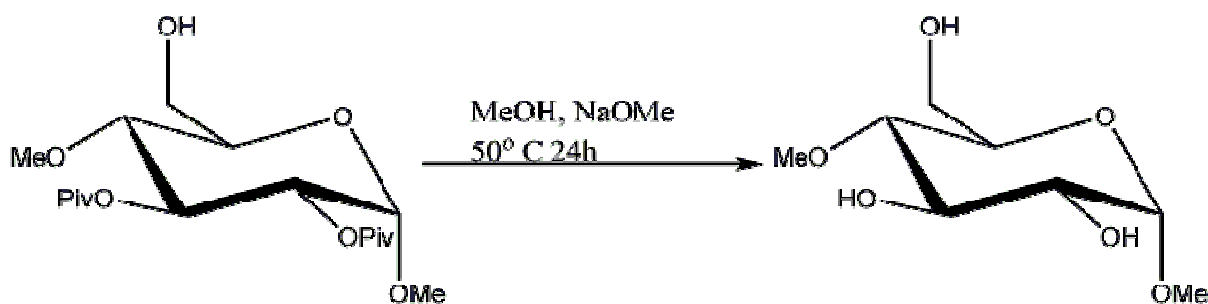
Rf (5) 0.93

Rf (6) 0.22 (ethyl acetate: hexane 3:1 V/V)

$[\alpha]_{\text{D}}^{20}$: +87.9 (136mg / 100ml chloroform)

Melting point: 85° C

4.1.6. Methyl-4-O-methyl- α -D-glucopyranoside



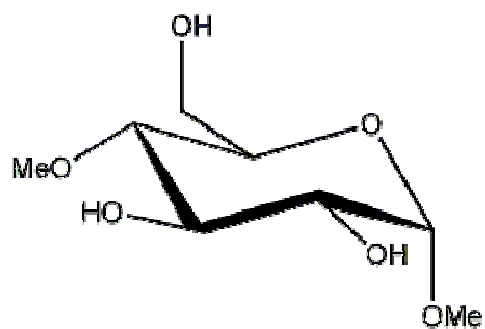
Materials:

- dry methanol (Methanol min 99,8% Sigma-Aldrich)
- sodium methoxide
- Dowex ® 50WX8-100 ion exchange resin (Sigma –Aldrich)
- hot plate with a magnetic stirrer
- reflux condenser
- magnet
- drying tube
- calcium sulfate (99%, Sigma-Aldrich)
- Drierite® 6 mesh with indicator (Sigma-Aldrich)
- water hose
- thermometer

Method:

Methyl-2,3-di-O-pivaloyl-4-O-methyl- α -glucopyranoside (395 mg, 1 mmol) was dissolved in dry methanol (5 ml), and sodium methanolate (1 ml) was added. The mixture was heated to 50°C and left for 24h. After 24 h the reaction was not over, and another 2ml of sodium methanolate was poured in, and the mixture stirred for another 1h. After TLC showed complete consumption of **5**, the mixture was left to cool to room temperature, then diluted with dry methanol (3 ml), neutralised with Dowex, filtrated and evaporated to give 152mg, 70 % of **7**. [42]

Characterisation:



C₈H₁₆O₆
MW:208,21

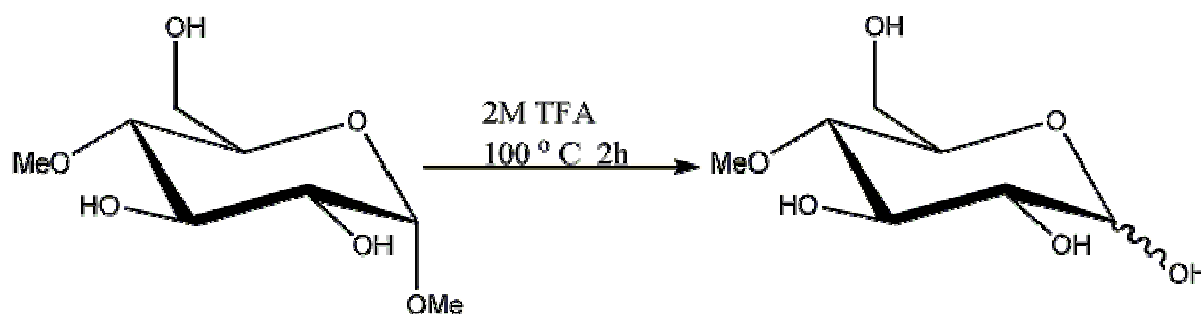
Rf (**6**) 0.93

Rf (**7**) 0.36 (isopropanol: ethyl acetate 1:5 V/V)

[α]_D²⁰: +138 (124 mg / 100 ml water)

Compound **7** is a yellow syrup.

4.1.7. 4-O-methyl-glucopyranoside



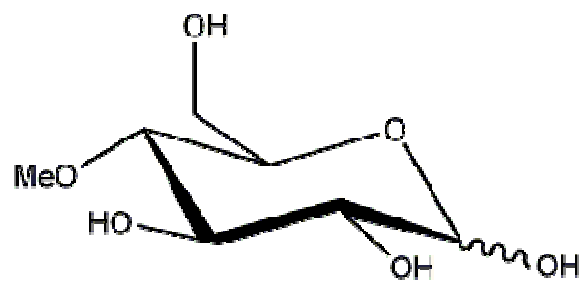
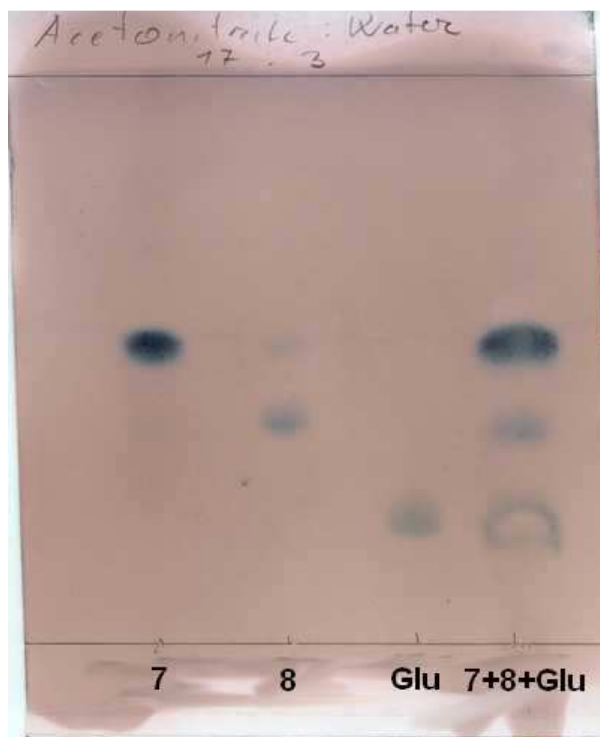
Materials:

- trifluoroacetic acid (99% Sigma-Aldrich)
- distilled water
- methanol (min. 99,8%, Sigma-Aldrich)
- drying cabinet
- hot plate
- nitrogen gas (Alphagaz)
- test tubes

Method:

Methyl 4-O-methyl- α -D-glucopyranoside (70.0 mg, 0.3mmol) was suspended in 2M trifluoroacetic acid (1ml) in a test tube. The test tube was closed with a screwcap, and placed in a drying cabinet at a temperature of 100°C for 2h. The mixture was then taken out of the drying cabinet, diluted with methanol, and evaporated in a water bath at 60 °C under nitrogen atmosphere. The residue was dissolved in methanol, and again evaporated. The process was repeated 3 times. The compound was then dissolved in water, and again evaporated to give (80mg, 124 %) of **8**. (Note that the final compound could not be purified due to very low quantities, and contains compound **7**, **8** as well as glucose. Additionally, salts of the trifluoroacetic acid are present.)

Characterisation:



$C_7H_{14}O_6$
MG 194,18

Rf (7) 0.51
Rf (8) 0.37 (acetonitrile : water 17 : 3 V/V)
Rf (glucose) 0.21

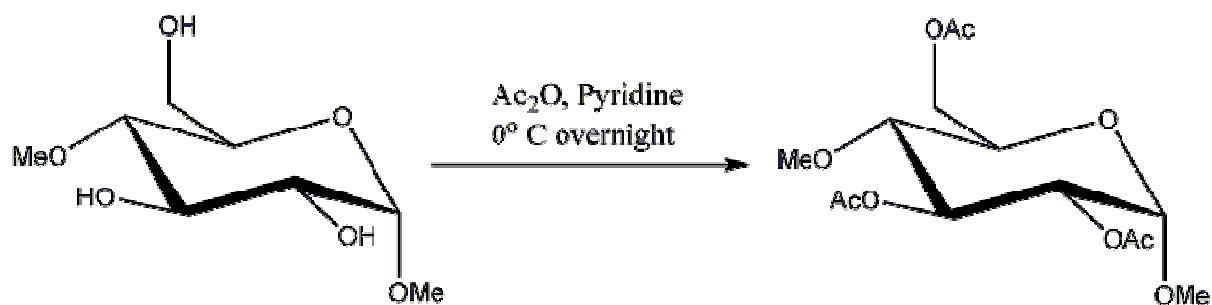
Compound **8** is yellow syrup

4.2. Attempted reactions to synthesize 4-O-methyl-glucuronic acid, and alternate route to 4-O-methyl glucopyranose

These reactions are attempted reactions, which did not bring satisfying results due to low yields, or complicated purifying methods. The quantities are very low, and the compounds are partially impure. Characterisation consists only of R_f values, as it was impossible to measure optical rotation or melting point, however, the reactions can be improved, and can provide additional information on the subject.

The oxidation of methyl-2,3-di-O-pivaloyl-4-O-methyl-6-O-trityl- α -D-glucopyranoside to methyl-2,3-di-O-pivaloyl-4-O-methyl- α -D-glucuronic acid was fairly simple and did not require special conditions such as complete absence of water, but it was difficult to control with TLC, and as the starting material was not yet consumed, an unwanted compound formed.

4.2.1. Methyl-2,3,6-tri-O-acetyl-4-O-methyl- α -D-glucopyranoside



Materials:

- acetic anhydride (99% Sigma-Aldrich)
- dry pyridine (Pyridine 99,8% Sigma_Aldrich)
- distilled water
- ice
- dichloromethane (Merck)
- sodium hydrogen carbonate (99,5% Sigma_Aldrich)
- calcium sulfate (99%, Sigma-Aldrich)
- Drierite® 6 mesh with indicator (Sigma-Aldrich)
- magnet
- magnesium sulphate (97% Sigma-Aldrich)

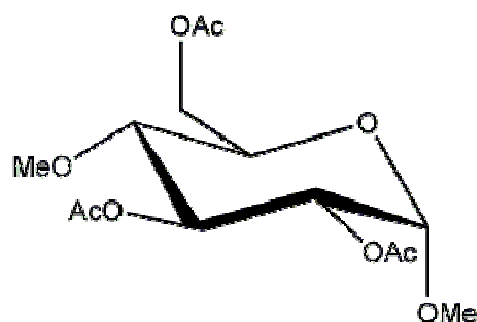
Method:

Methyl 4-O-methyl- α -D-glucopyranoside (100 mg, 0.5 mmol) was dissolved in dry pyridine (2.5 ml), and acetic anhydride (2.5 ml) was added to the flask which was closed with a drying tube. The flask was first kept in ice water, and then left overnight. The mixture was extracted with chloroform, dried over MgSO_4 , and evaporated to give (80mg 50 %) of **7a**. [43]

Characterisation:

$^1\text{H-NMR}$: δ 5.45 (dd, 1 H, $J_{3,2} = 10.1$, $J_{3,4} = 9.2$ Hz, H-3), 4.86 (d, 1 H, $J_{1,2} = 3.7$ Hz, H-1), 4.82 (dd, 1 H, $J_{2,1} = 3.7$, $J_{2,3} = 10.1$ Hz, H-2), 4.35 (dd, 1 H, $J_{6,6'} = 12.0$, $J_{6,5} = 2.3$ Hz, H-6), 4.27 (dd, 1 H, $J_{6',6} = 12.0$, $J_{6',5} = 4.7$ Hz, H-6'), 3.84 (ddd, 1 H, $J_{5,4} = 10.1$, $J_{5,6} = 2.2$, $J_{5,6'} = 4.7$ Hz, H-5), 3.42 (s, 3 H, 4-OMe), 3.38 (s, 3 H, 1-OMe), 3.33 (dd, 1 H, $J_{4,3} = 9.3$, $J_{4,5} = 10.0$ Hz, H-4) 2.12, 2.08, 2.07 ppm (3s, 9 H, OAc).

$^{13}\text{C-NMR}$: 20.77, 20.82, 20.92 (3x $\text{CH}_3\text{C=O}$), 55.25 (1-O- CH_3), 60.1 (4-O- CH_3), 62.70 (C-6), 68.19 (C-5), 71.10 (C-2), 71.90 (C-3), 77.77 (C-4), 96.80 (C-1), 169.81, 170.42, 170.72 ppm (3x $\text{CH}_3\text{C=O}$).



$\text{C}_{15}\text{H}_{22}\text{O}_{10}$

MG: 362,329

R_f (7) 0.39

R_f (7a) 0.88 (isopropanol: ethyl acetate 1:5)

5. Results and discussion

5.1. Extraction of *Hyptis suaveolens* mucilage, dialysis , and lyophilization

All three methods were optimized in previous diploma thesis, so the extraction was carried out only once. Dialysis and lyophilization techniques were described in Chapter 1. 2.00g of the mucilage was extracted, and after lyophilisation 0,783g (39 %) of the polysaccharide was prepared.

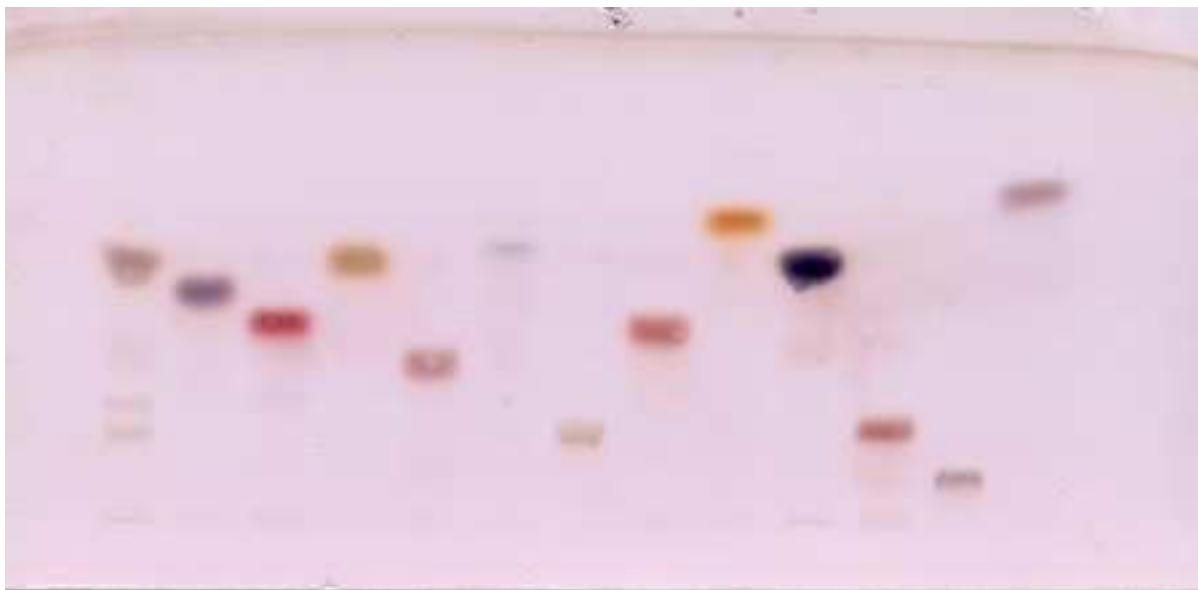
5.2. Ion exchange chromatography

Several separations of the polysaccharide were performed, and the column was always regenerated with distilled water, then with 0,1M TRIS solution. If the column had to be left unused for several days, concentrated ethanol was applied, and it was left in the cold room. It was washed with water, then with 0,1M TRIS buffer, and it could be used again. Control of the separation was carried out with TLC. A small sample was taken out of every test tube and applied as a spot on the TLC. TLC was then dipped in thymol reagent, and heated to 100°C. The neutral polysaccharide showed a reddish colour, and the acid brown. The first elution was with 0,1M TRIS buffer, until TLC showed no more of the neutral polysaccharide, then the acid polysaccharide was eluted with 0,5M sodium chloride. To reduce the volume of the fractions before dialysis, the fractions were gathered, and concentrated on rotary evaporator.

5.3. Hydrolysis and TLC of monosaccharides

Hydrolysis was carried out with 2M TFA, 1.5MTFA, and 1M TFA. The acid polysaccharide was suspended in TFA, and heated for 2h. The hydrolysis of the polysaccharide with 2M TFA has been performed in the previous diploma theses; however, we tried to see if the polysaccharide can be hydrolyzed under mild conditions. The TLC showed no difference between hydrolysis with 2M TFA and 1.5M TFA, the hydrolysis with 1M TFA showed an incomplete hydrolysis, and no new monosaccharides showed. The hydrolysis with the 2M TFA proved to be optimal.

The following TLCs were developed three times in acetonitrile/water 17:3 and dipped into thymol reagent. They were heated at 100 °C for one min. The standards concentration is approx. 15 mg in 20 ml distilled water. The first TLC which shows acid polysaccharide hydrolyzed with 2 M TFA is made using mono standards, in all of the others multiple standards per track were used.



Hydrolysis of *hyptis suaveolens* with 2M TFA (lane 1-acid polysaccharide, lane 2-arabinose, lane 3-fructose, lane 4-fucose, lane 5 - galactose, lane 6- glucose, lane 7- glucuronic acid, lane 8-mannose, lane 9- rhamnose, lane 10- xylose, lane 11- maltose, lane 12-galacturonic acid, lane 13- ribose. (TLC1)

The acidic polysaccharide contains xylose and fucose. Their Rf- Value is the same, but the colour is different. The spot with the same Rf value in the column 1 is the mixture of these colours which means that both of the monosaccharides are present. The spot with the Rf slightly higher than glucuronic acid, is 4-O-methyl-glucuronic acid.

Acidic polysaccharide hydrolyzed with 1,5M TFA (TLC2)

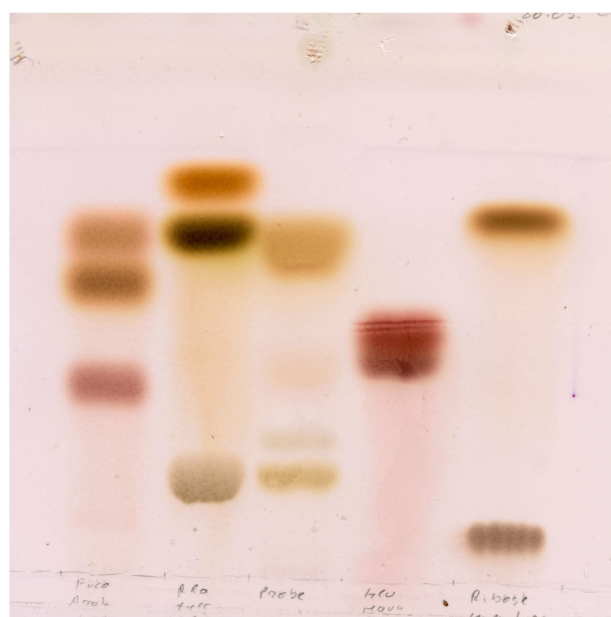
lane 1: fucose, arabinose, galactose

lane 2: rhamnose, xylose. Glucuronic acid

lane 3: acid polysaccharide

lane 4: glucose, mannose, fructose

lane 5: ribose, galacturonic acid



Acid polysaccharide hydrolyzed with 1M TFA (TLC3)

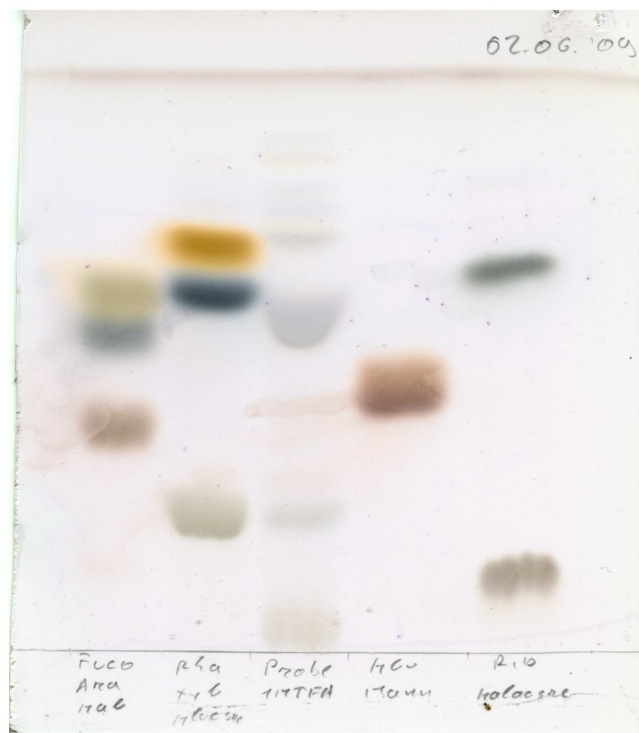
lane 1: fucose, arabinose, galactose

lane 2: rhamnose, xylose, glucuronic acid

lane 3: acid polysaccharide

lane 4: glucose, mannose

lane 5: ribose, galacturonic acid

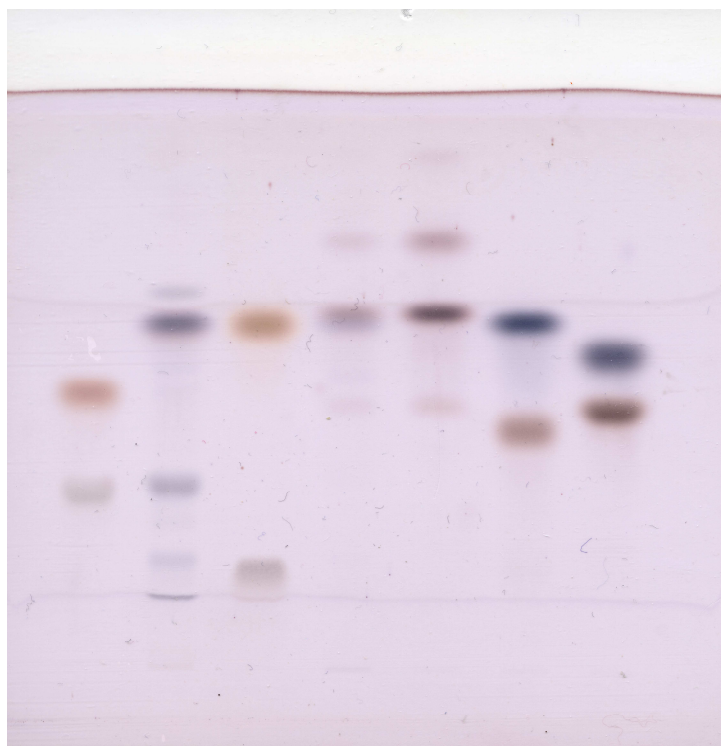


On the TLC 3 one can clearly see a brown spot at the bottom of the TLC in column 3. These are the polysaccharide remains which were not hydrolyzed. The plan of this diploma thesis involved hydrolysis with 0.5M TFA and 0.25M TFA, but since the polysaccharide was not hydrolyzed properly with 1M TFA, further hydrolysis was not necessary.

The colour on each of the TLC is different, although the identifying reagent is the same. The TLC change colour in time. TLC 2 was scanned 1 min after it was heated in the drying cabinet, TLC 3 2h later, and TLC 1 one day later. After approx. 2 days, all of the spots have similar, bluish-violet colours.

5.4. Reduction of the carboxyl groups

The method of the reduction was described in chapter 2. After the reduction and further preparation of the polysaccharide, a TLC was made to confirm the reduction. It showed two new spots, glucose and 4-O-methyl glucose. With the newly synthesized 4-O-methyl glucose, it was clear that the carboxyl groups of glucuronic acid and 4-O-methyl glucuronic acid were reduced. Glucuronic acid and 4-O-methyl glucuronic acid can however still be seen, although the spots are very weak, and the method certainly needs optimisation.



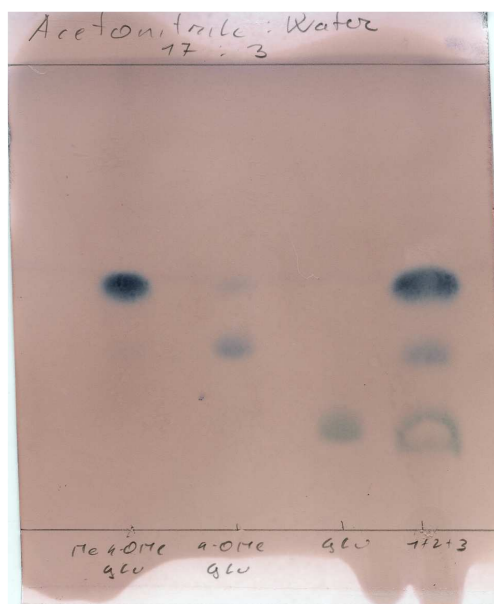
Comparison of the acid polysaccharide and the reduced acid polysaccharide with standards. Lane 1: mannose, glucuronic acid, lane 2: acid polysaccharide, lane 3: fucose, galacturonic acid, lane 4: reduced acid polysaccharide, lane 5: 4-O-methyl glucose, lane 6: xylose, galactose, lane 7: arabinose, glucose

The TLC shows the composition of each of the polysaccharide. Acid polysaccharide contains, fucose, xylose glucuronic acid and 4-O-methyl glucuronic acid, and reduced acid polysaccharide contains glucose, xylose, fucose and 4-O-methyl glucose.

5.5. Synthesis of 4-O-methyl glucose

The starting compound was Methyl-4,6-O-benzylidene- α -glucopyranoside. In order to methylate the fourth OH group, it was necessary to protect the groups 2 and 3. Since the most common protective group in carbohydrate synthesis, the acetyl group, would not be stable in drastically alkaline conditions needed for methylation, another protective group had to be used. A number of publications report the benzoyl group to be very stable against these conditions, and our first tries were introducing benzoyl group to the starting material. However, our yields were extremely small, less than 10%, despite of the attempts to optimize the reaction. We introduced the pivaloyl group, which can endure moderate alkaline conditions. The benzylidene ring was opened with acetic acid. For the protection of the OH-6, a trityl (triphenylmethyl) group was used; it has the ability of binding to OH group 6 selectively, and is also stable to alkaline conditions, but not to acidic. Methylation was constantly controlled with the TLC in order to see if the pivaloyl group holds. The reaction did not start until the mixture was heated to 50°C. It succeeded, but in 38% yield, and a number of side products were made which were then separated with column chromatography. Removing of the trityl group was carried out with concentrated acetic acid. Pivaloyl group needed a strongly alkaline environment to be separated, and heating up to 50°C.

Removing of the methyl group was done with TFA. Methyl-4-O-methyl glucose was not stable under these conditions, so a 4-O-methyl-glucose and glucose were formed. Unfortunately, we were not able to separate these compounds, due to very low quantities. TLC is made, using both of the identifying reagents, anisaldehyde reagent, and thymol reagent. All three of the compounds, glucose, 4-O-methyl-glucose, and methyl-4-O-methyl-glucose appeared red with thymol reagent, and greenish-blue with anisaldehyde.



Detection with anisaldehyde reagent

lane 1: methyl 4-O-methyl- α -D-glucopyranoside

lane 2: 4-O-methyl-glucose

lane 3: glucose

lane 4: 1+2+3



Detection with thymol reagent

lane 1: methyl 4-O-methyl- α -D-glucopyranoside

lane 2: 4-O-methyl-glucose

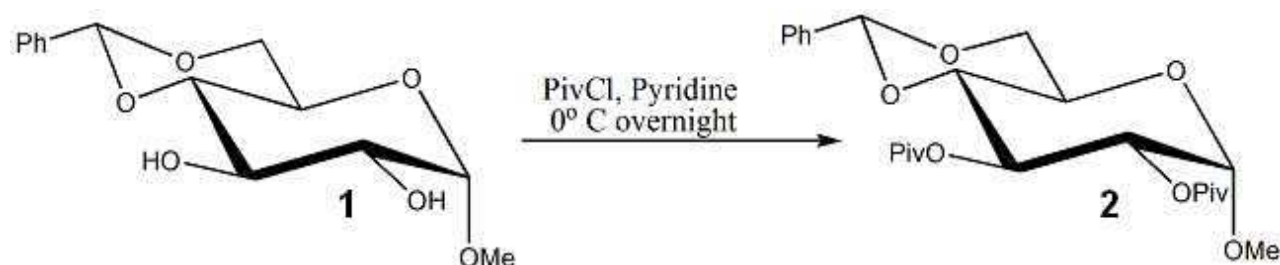
lane 3: glucose

lane 4: 1 + 2 + 3

5.5. Discussion of the synthesis using NMR spectra

Selected NMR spectra relevant to the structural identification of compounds **1** to **7a** are presented in the Appendix NMR spectra pages 56 to 73

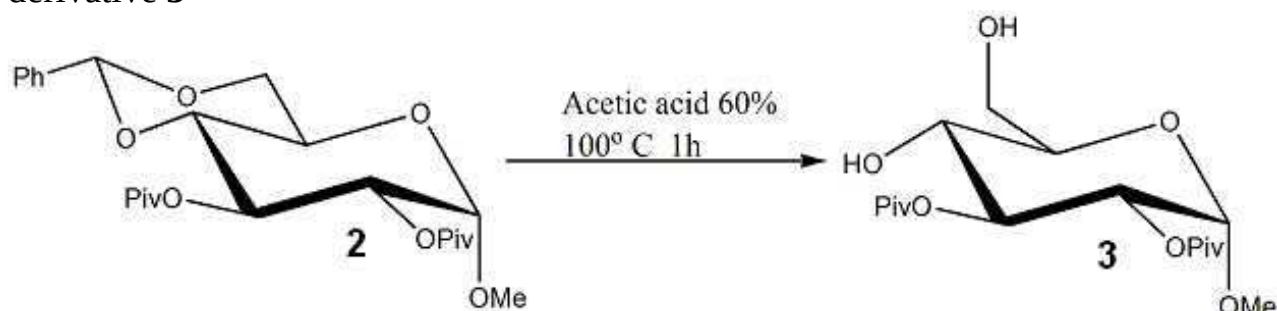
5.5.1. Conversion of compound **1** into **2**



Compound TD4 is a fully protected structure, distinguished from the starting material by the presence of two pivaloyl ester groups at C-2 and C-3. In the NMR spectrum, the six methyl groups give rise to two signals at δ 1.16 and 1.18 ppm (2 x 9 H). The signals for H-2 and H-3 expected in the range 3-4 ppm in **1**, move downfield as expected and are found at 4.85 and 5.62 ppm as expected for hydrogen atoms neighboring an ester linked oxygen atom. The H-2 signal at 4.85 ppm shows a small coupling constant due to axial-equatorial interaction with H-1 (at 4.94 ppm) and a large coupling to H-3. The signals for H-4,5,6 and 6' are not downfield shifted and are at δ 3.67, 3.91, 4.32 and 3.78 ppm, as expected for a 4,6-O-benzylidene derivative of glucose. The signal for the corresponding aldehyde hydrogen is at 5.52 ppm, and there are signals attributable to the five aromatic hydrogen atoms of the benzylidene group. The COSY spectrum is directly interpretable to prove the above assignments.

The ^{13}C – spectrum recorded in the APT (attached proton test) mode: The signals of carbon atoms with an even number of attached hydrogen atoms (0,2) point down. Those with an odd number (1,3) point up. There are two strong lines at 26.92 and 27.07 ppm representing three carbon atoms each of the pivaloyl ester groups, and two lines at 38.72 and 38.74 ppm representing the quaternary carbon atoms of the same groups. The signal for the methyl aglycone appears at 55.55 ppm. From the HSQC (two-dimensional ^{13}C - ^1H) spectrum, we assign the signals at 62.24, 68.43, 68.84, 71.40 and 78.4 ppm to C-5, C-3, C-6, C-2 and C-4 of the glucose molecule. The resonance for C-1 is at 97.65 and the aldehyde carbon signal for the benzylidene acetal is at 101.13 ppm. There are four resonances attributed to the aromatic ring carbon atoms: 125.84 (C-2, C-6), 128.11 (C-3, C-5), 128.82 (C-4) and 137.0 ppm (the quaternary C-1). Finally, the two carbonyl resonances of the pivalic acid ester groups occur at 176.91 and 177.91 ppm. Using the (two-dimensional) technique HETERONUCLEAR MULTIPLE BOND COHERENCE (HMBC) the resonance at 176.91 ppm is attributable to the pivaloyl ester group at C-3 and the one at 177.91 to the ester grouping at C-2.

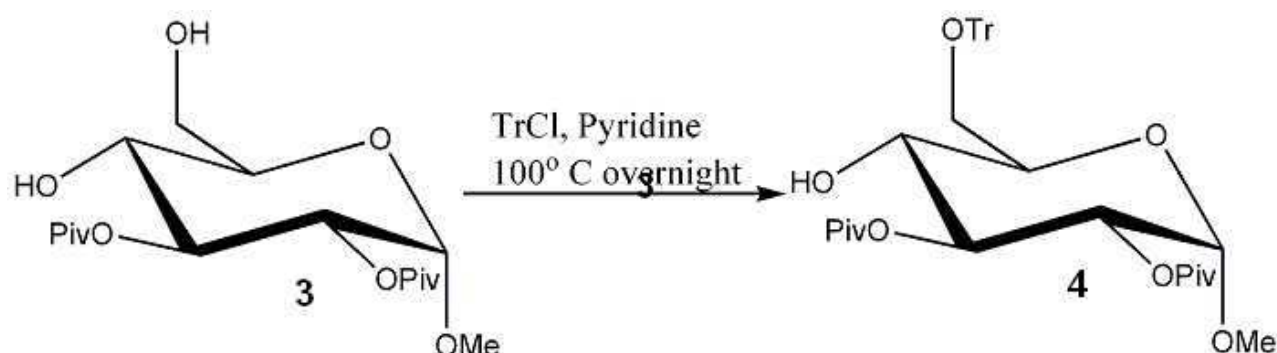
5.5.2. Treatment of the benzylidene acetal **2** with 60% acetic acid affords the diol derivative **3**



The difference between **2** and **3** lies in the absence of the benzylidene group. In the ^1H -NMR-spectrum, the signals attributed to the grouping C-1, C-2, C-3 are identical with those in the spectrum of **2**. There are no aromatic signals, and the signals for H-4, H-5 and H-6, 6' are different. The H-4 resonance is at 3.5, overlapping with the signal of H-5. The signals of H-6,6' moved upfield from those of compound **2** and are at $\sim 3\text{ppm}$. The cross peaks seen in the COSY spectrum confirm these assignments.

In the ^{13}C -NMR Spectrum of **3**, the resonance frequency of C-6 is at 61.97, the C-4 resonance is now at 70.4, the signal due to C-5 at 71.16ppm. The segment C-4-C-5-C-6 is no longer constrained in the benzylidene ring system, resulting in dramatic shifts of the corresponding carbon resonances.

5.5.3. Conversion of **3** into trityl ether **4** involves the introduction of an aromatic group $\text{C}_{19}\text{H}_{15}$

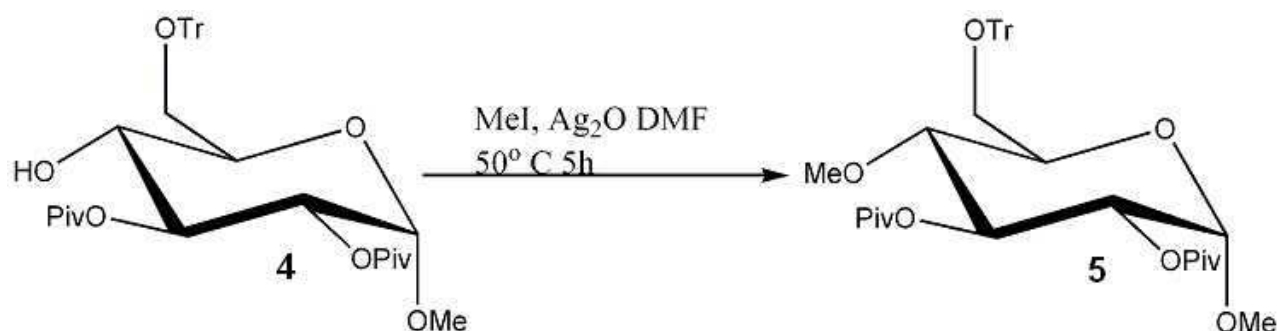


In the ^1H -NMR spectrum, the integral ratio of aromatic protons to the H-3 signal is 15:1, confirming the stoichiometric introduction of the trityl group. Chemical shift changes are observed in the range 3.3-4. ppm. The signals for H-6 and H-6' are now at 3.37 and 3.46ppm, for H-5 at 3.77 and for H-4 at 3.57ppm. The unusual septuplet at 4.01ppm is due to the CH-hydrogen atom of one molecule of 2-propanol of crystallization. The corresponding methyl resonance is a doublet (6 H) at 1.20ppm. The COSY spectrum confirms these assignments.

In the ^{13}C -NMR spectrum, there are strong additional lines due to the carbon atoms of the trityl ether group, as expected; δ 127.06, 127.17, 127.84, 127.88, 128.62, 143.73 and 146.84 ppm. The fact that the trityl ether carbon and the hydrogen atoms are not strictly exposed into four lines suggests some magnetic anisotropy in the region of the trityl ether group. The resonance of the quaternary methyl carbon atom of the trityl group is at 87.0ppm. Carbon resonances attributable to the pivaloyl ester groups and the glucose anomeric center are more or less unchanged compared to **3**. The C-6 resonance is at 63.89 and the resonances for C-5, C-2, C-4 and C-3 are at 70.23, 70.71, 71.05 and 72.67ppm.

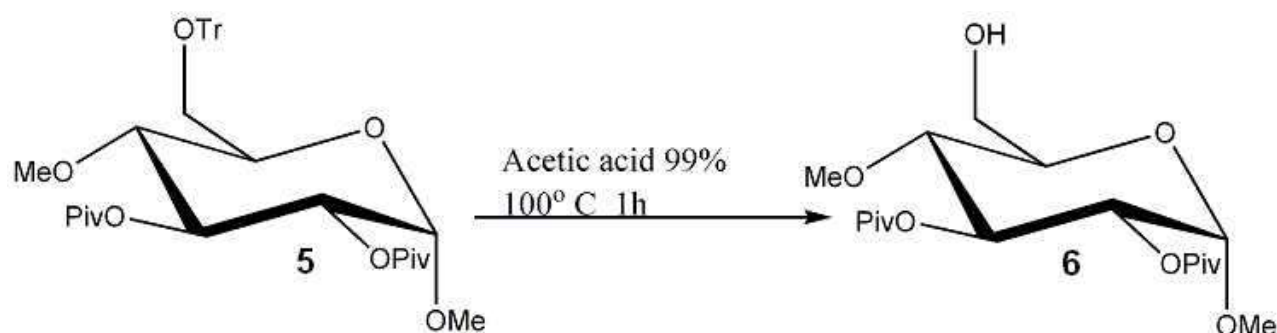
In addition, The ^{13}C -NMR spectrum shows the resonances due to the secondary carbon atom and the methyl group carbon atoms of isopropyl alcohol 64.34 and 25.25ppm.

5.5.4. Methylation of **4** to **5**



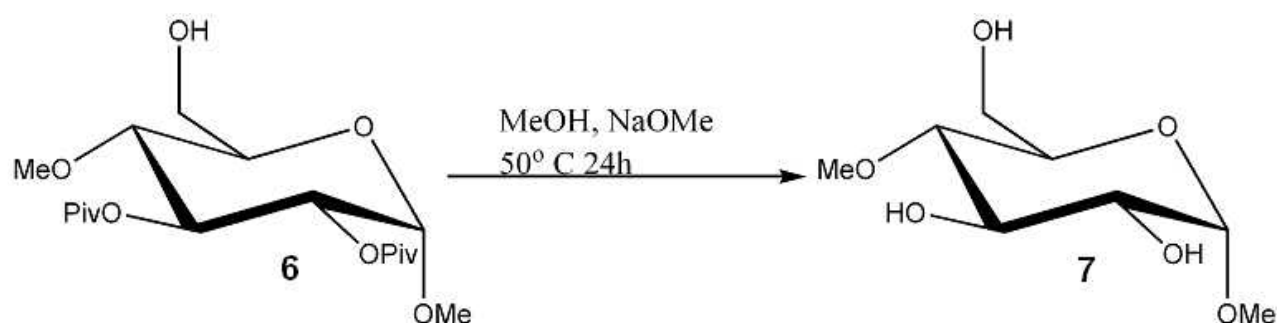
The methylation of OH group at C-4 in **4** to give **5** reflects in the ¹H-NMR-spectrum. The H-4 resonance is at ca. 3.45ppm, overlapping with the H-5 resonance. The carbon resonance attributable to the additional methoxy group is at 59.99ppm, the one for C-4 at 78.33ppm.

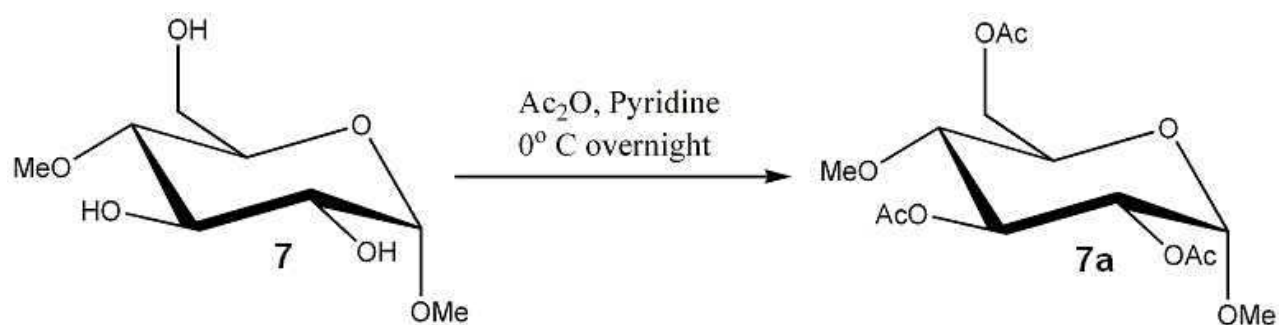
5.5.5. Removal of trityl group from **5** to give **6**



De-tritylation of the fully protected **5** is by mild acid-catalyzed hydrolysis. Therefore, it is to be expected that the features of the NMR spectrum of **6** are essentially the same as those of **5** except for the absence of the signals attributable to the trityl group and a change with respect to the signals for H-6,6'. What is observed is a downfield shift of the H-6,6' resonances from 3.13 and 3.44 to the range 3.70-3.90ppm. Furthermore, spin-spin coupling is observed between the H-6,6' and the corresponding OH whose resonance shows analogous coupling and is at δ 1.91ppm. In the ¹³C spectrum, the resonance of C-6 is at 61.52ppm. Otherwise the ¹³C-NMR spectrum of **6** is closely similar to that of **5** minus the lines attributed to the trityl group at δ 86.42, 126.97, 127.76, 127.88 and 143.92ppm.

5.5.6. Conversion of **6** to fully protected **7a**





Alcohol **6** was converted into the fully protected 4-O-methyl glucose derivative **7a** by sequential sodium methoxide catalyzed methanolysis (Zemplén saponification) and O-acetylation. The ^1H -NMR spectrum of **7a** shows the expected features. The signals for H-6,6' are shifted downfield into the range 4.20-4.40ppm (from 3.75-3.90) due to esterification. All other signals are similar to those in the ^1H -NMR spectrum of **6**, except that the signals due to the pivaloyl groups are absent and have been replaced by two acetyl signals. These are also observable in the ^{13}C -NMR spectra. Using the 2-D HSQC technique, each carbon signal can be attributed to the corresponding hydrogen resonance. (HSQC- heteronuclear single quantum coherence spectroscopy).

6. Summary

Many plant-derived polysaccharides contain the modified uronic acid, 4-O-methyl glucuronic acid (4-O-MeGlcUA). To facilitate the analysis of such polysaccharides, syntheses of 4-O-MeGlcUA as a monosaccharide standard have been reported. At the occasion of studies with a polysaccharide preparation from *Hyptis suaveolens*, we required standards for 4-O-methyl D-glucose (4-O-MeGlc) and 4-O-MeGlcUA. Since none of the published procedures are straightforward, we describe in the present diploma thesis a new synthetic approach based on the introduction of the methyl ether group into a glucose derivative with ester protection in positions 2 and 3. Preliminary results indicate that methylation of the secondary alcohol, methyl 2,3-di-O-pivaloyl -6-O-triphenylmethyl- α -D-glucopyranoside under standard conditions affords the 4-O-methyl derivative in satisfactory yield. Conventional removal of the protecting groups gives the free sugar, 4-O-MeGlc. During de-protection, intermediates with a free OH-6 have been obtained and have been found suitable for oxidation to the 4-O-MeGlcUA derivatives. The standard 4-O-MeGlc was used to demonstrate the presence of 4-O-MeGlc in monosaccharide mixtures obtained by hydrolysis of the carboxyl-reduced acidic polysaccharide from *Hyptis suaveolens*.

7. Zusammenfassung

Das Uronsäurederivat 4-O-Methylglukuronsäure (4-O-MeGlcUA) ist in zahlreichen pflanzlichen Polysacchariden enthalten. Es sind daher Synthesen von 4-O-Methylglukuronsäure als Standardsubstanz für entsprechende analytische Zwecke beschrieben worden. Im Zuge von Studien mit Polysacchariden aus *Hyptis suaveolens* benötigten wir Standards für 4-O-Methylglukose und 4-O-MeGlcUA. Da keine der veröffentlichten Synthesen einen einfachen Zugang zu diesen Substanzen bot, beschreiben wir in der vorliegenden Diplomarbeit ein neues Syntheseverfahren unter Verwendung von Substraten mit ester gebundenen Schutzgruppen in den Positionen 2 und 3. Vorläufige Ergebnisse zeigen, dass die Methylierung des sekundären Alkohols Methyl-2,3-di-O-pivaloyl-6-O-triphenylmethyl- α -D-glukopyranosid unter Standardbedingungen brauchbare Ausbeuten des 4-O-Methylderivats ergibt. Durch Entfernung der Schutzgruppen nach gebräuchlichen Verfahren erhielt man den freien Zucker 4-O-Methyl-D-glukose. Im Zuge der Entfernung der Schutzgruppen wurden auch Derivate mit freier OH-Gruppe in Position 6 erhalten, welche sich zur Oxidation zu den entsprechenden Derivaten von 4-O-MethylGlcUA eignen. In einem Anwendungsbeispiel wurde synthetisches 4-O-MeGlc als Standardsubstanz verwendet um zu zeigen, dass 4-O-MeGlc in dem Monosaccharidgemisch enthalten ist, welches durch hydrolytische Spaltung des carboxyl-reduzierten sauren Polysaccharids aus *Hyptis suaveolens* entsteht.

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Place of birth: Belgrade, Serbia
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Qualifications:

9/1990 - 6/1998	Elementary school Bora Stankovic - Belgrade, Yugoslavia
9/1998 - 6/2002	Secondary school „Fourth Belgrade Gymnasium"
6/2002	School certificate examination „4. Belgrad Gymnasium"
10/2002 – 3/2012	Studies of pharmacy – University of Vienna (Diploma thesis in the Department of Pharmaceutical Technology and Biopharmaceutics)

Professional experience:

October 2009 -running	Student employee in Team Santé Germania Apotheke, Hütteldorferstrasse 76, 1150 Vienna
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Other activities:

August 2003	Voluntary work in Malvern, England Project "The Great Outdoors"
May-August 2004	Organisation of an international theatre project "East and West in Drama" – Vienna

Languages:

English	Excellent speaking and writing
Bosnian, Croatian, Serbian	Excellent speaking and writing
German	Excellent speaking and writing

10. Appendix: NMR spectra

The Appendix NMR spectra contains selected NMR spectra relevant to structural identification of synthetic intermediates **1** through **7a** (cf. 5.5, Discussion of the synthesis using NMR spectra, p 45)

P 57: ^1H -NMR spectrum of Methyl-2,3 –O-pivaloyl-4,6-O-benzylidene- α -glucopyranoside

P 58: ^{13}C -NMR spectrum of Methyl-2,3 –O-pivaloyl-4,6-O-benzylidene- α -glucopyranoside

P 59: 2D-NMR spectrum (COSY) of Methyl-2,3 -O-pivaloyl-4,6-O-benzylidene- α -glucopyranoside

P 60: 2D-NMR spectrum (COSY) of Methyl-2,3 –O-pivaloyl- α -glucopyranoside

P 61: Partial ^1H -NMR spectrum of Methyl-2,3 –O-pivaloyl- α -glucopyranoside

P 62: ^{13}C -NMR spectrum of Methyl-2,3 –O-pivaloyl- α -glucopyranoside

P 63: ^{13}C -NMR spectrum of Methyl-2,3-di-O-pivaloyl-6-O-trityl- α -D-glucopyranoside

P 64: 2D-NMR spectrum (COSY) of Methyl-2,3-di-O-pivaloyl-6-O-trityl- α -D-glucopyranoside

P 65: Partial ^1H -NMR spectrum of Methyl-2,3-di-O-pivaloyl-6-O-trityl- α -D-glucopyranoside

P 66: 2D-NMR spectrum (COSY) of Methyl-2,3-di-O-pivaloyl-4-O-methyl-6-O-trityl- α -D-glucopyranoside

P 67: ^{13}C -NMR spectrum of Methyl-2,3-di-O-pivaloyl-4-O-methyl-6-O-trityl- α -D-glucopyranoside

P 68: Partial ^1H -NMR spectrum of Methyl-2,3-di-O-pivaloyl-4-O-methyl-6-O-trityl- α -D-glucopyranoside

P 69: ^{13}C -NMR spectrum of Methyl-2,3-di-O-pivaloyl-4-O-methyl- α -glucopyranoside

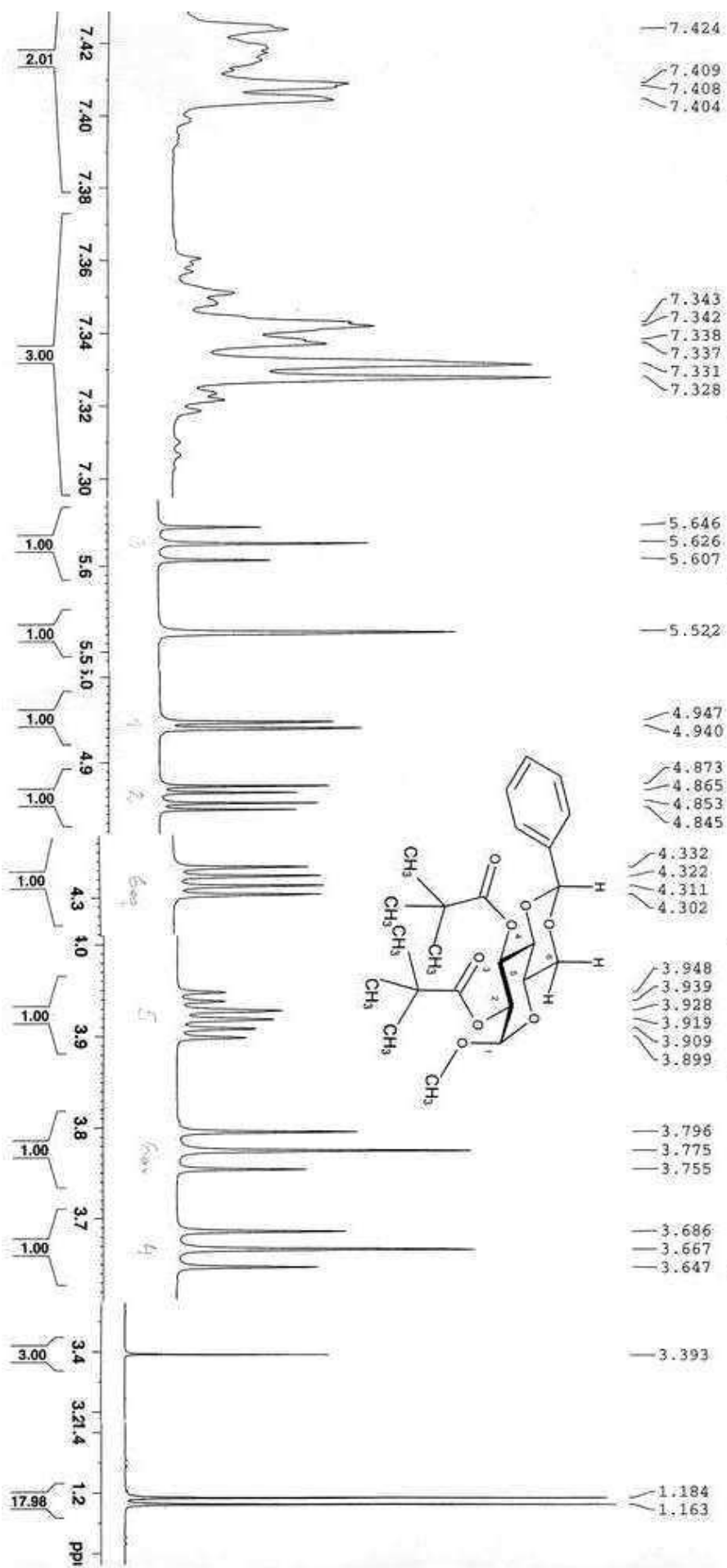
P 70: 2D-NMR spectrum (COSY) of Methyl-2,3-di-O-pivaloyl-4-O-methyl- α -glucopyranoside

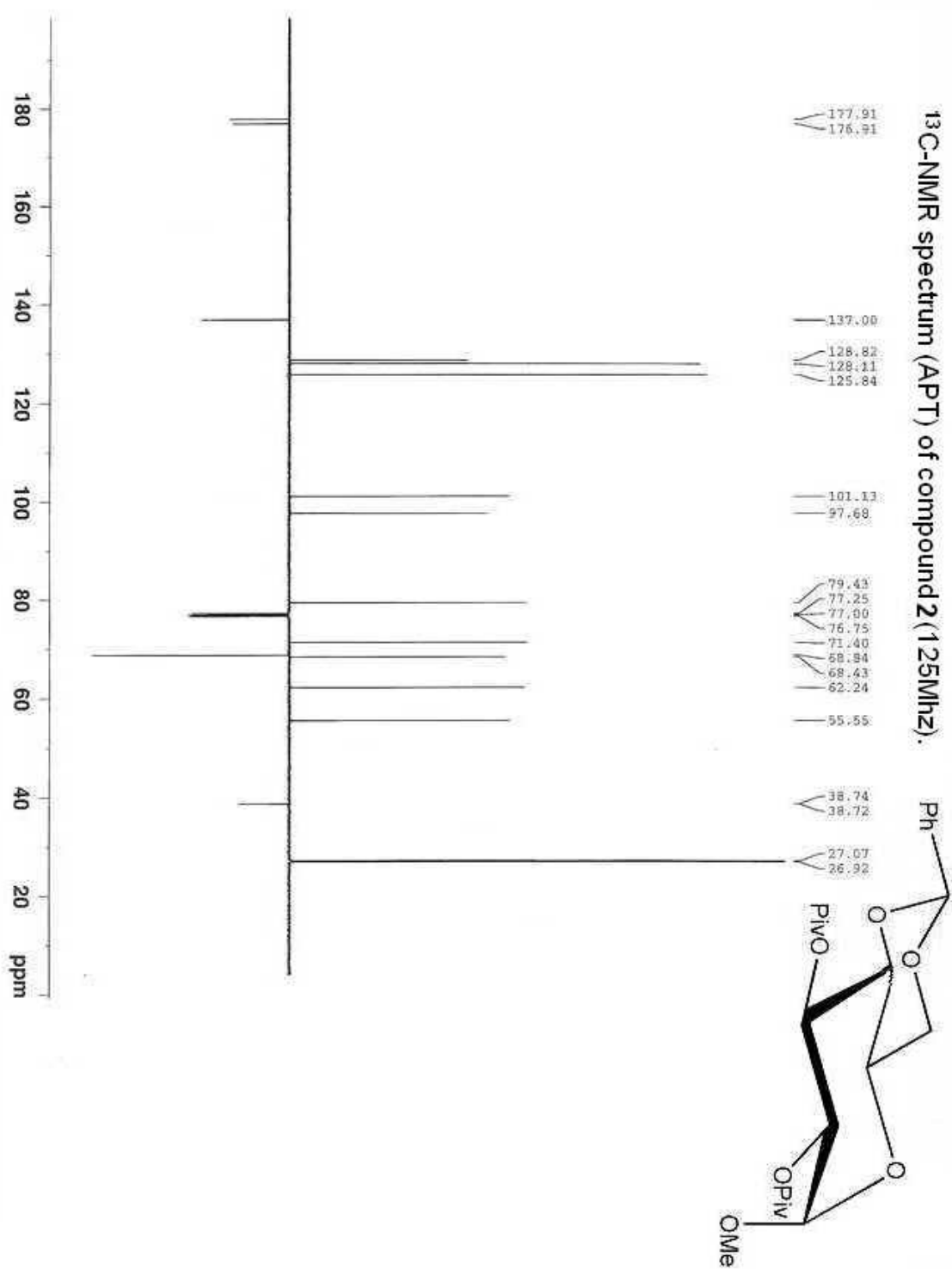
P 71: 2D-NMR spectrum (COSY) of Methyl-2,3,6-tri-O-acetyl-4-O-methyl- α -D-Glucopyranoside

P 72: ^{13}C -NMR spectrum of Methyl-2,3,6-tri-O-acetyl-4-O-methyl- α -D-Glucopyranoside

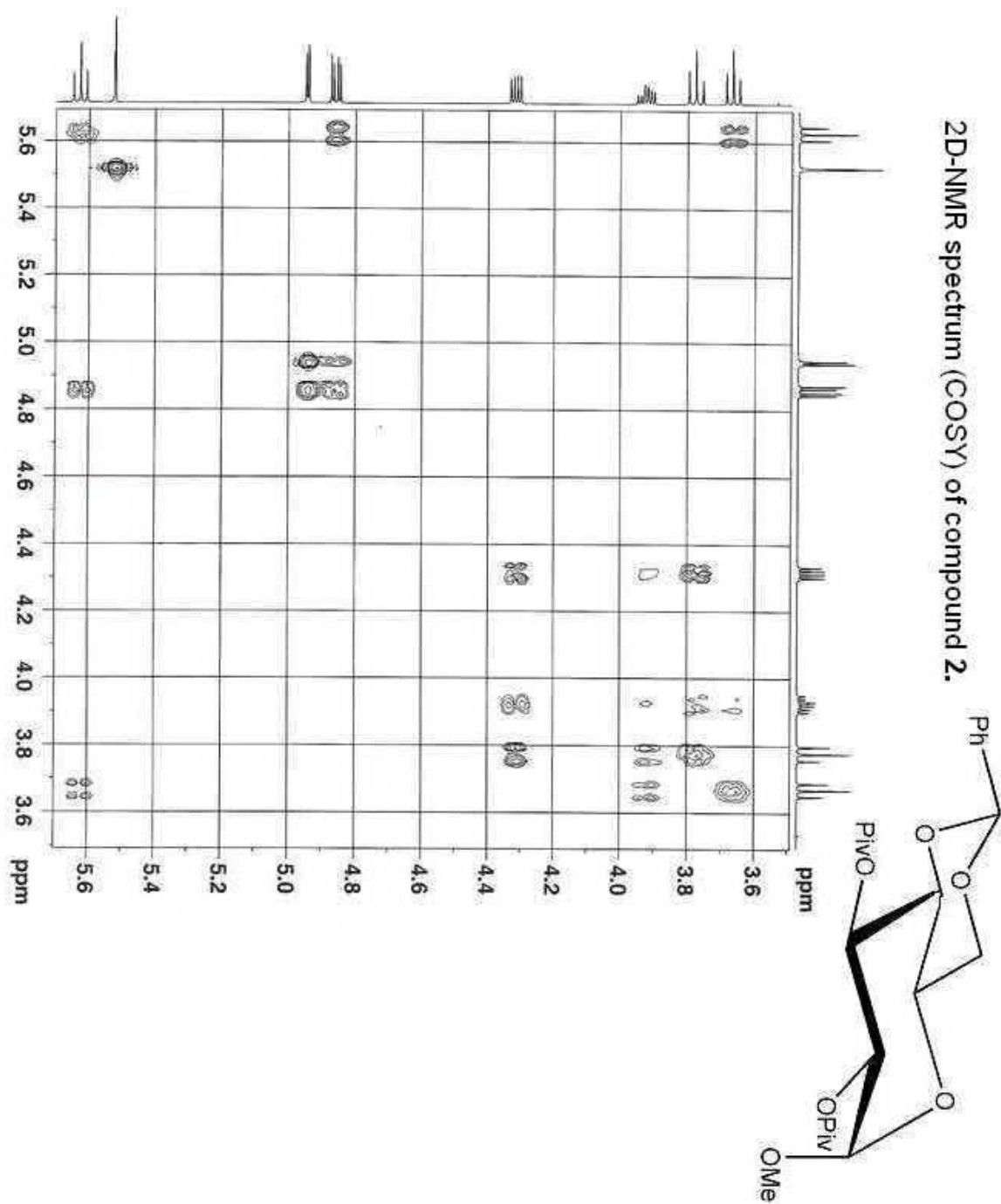
P 73: 2D-NMR spectrum (HSQC) of Methyl-2,3,6-tri-O-acetyl-4-O-methyl- α -D-Glucopyranoside

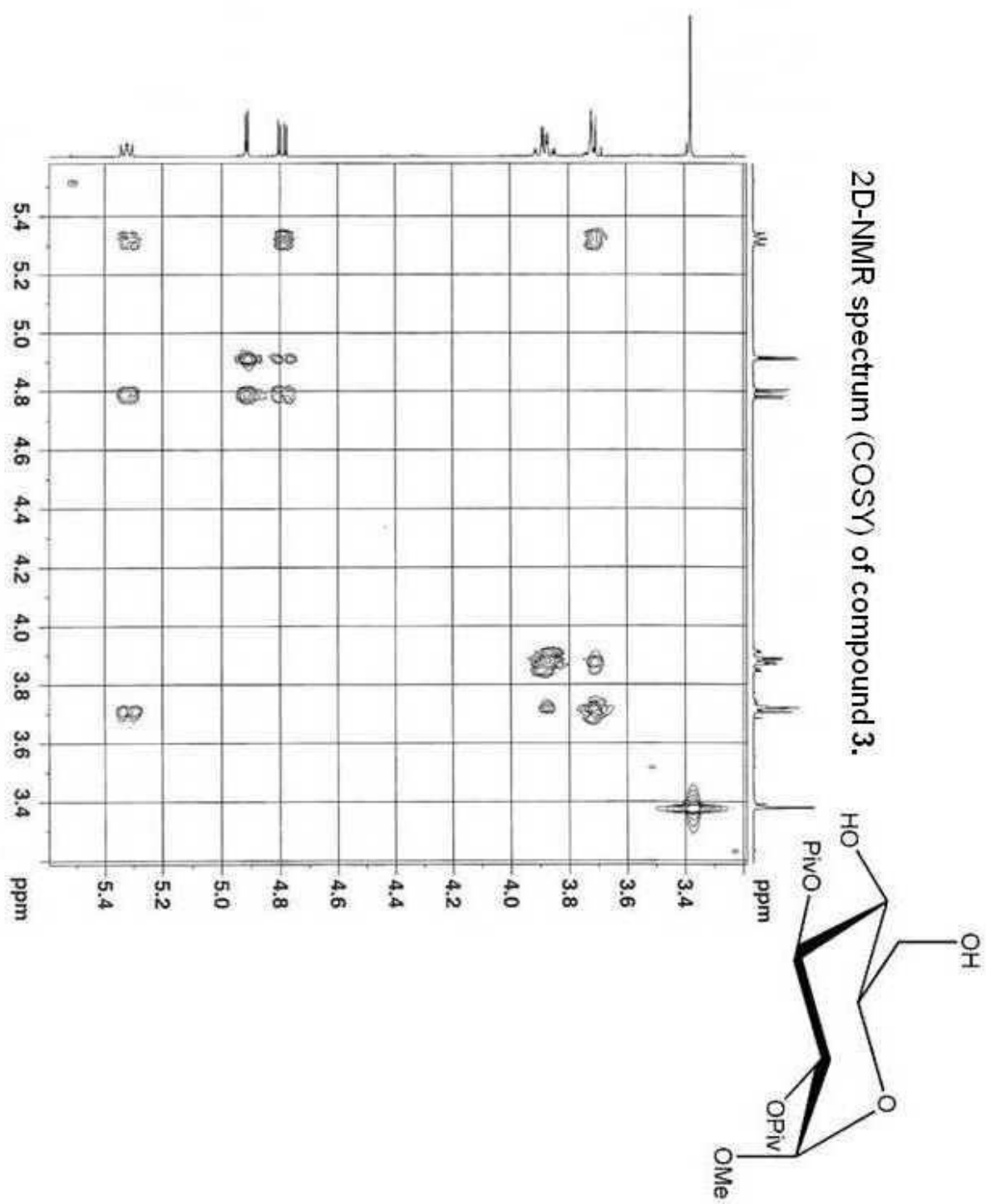
¹H-NMR spectrum (500 Mhz) of the fully protected intermediate 2. Spectral regions without signals are omitted



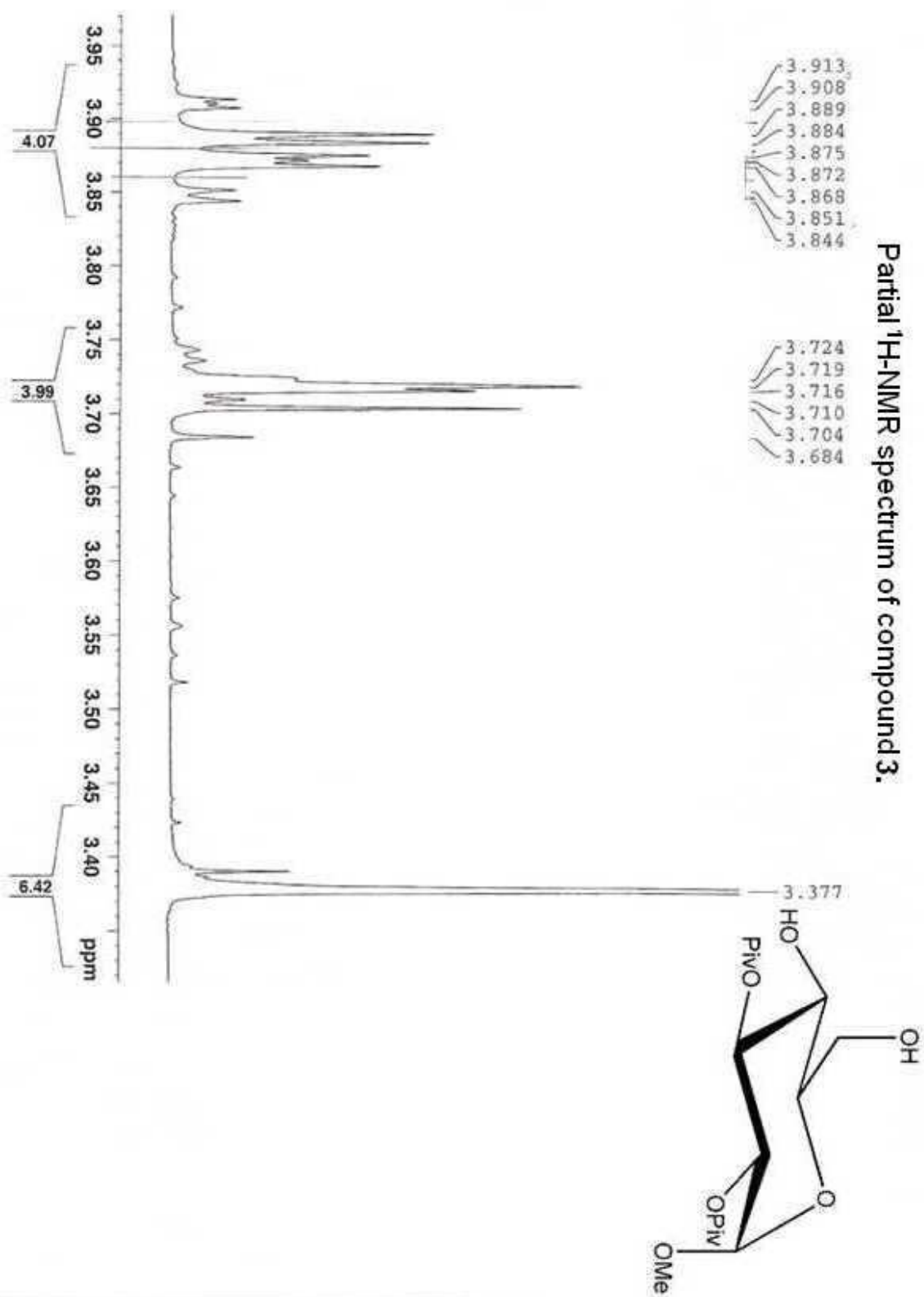


2D-NMR spectrum (COSY) of compound 2.

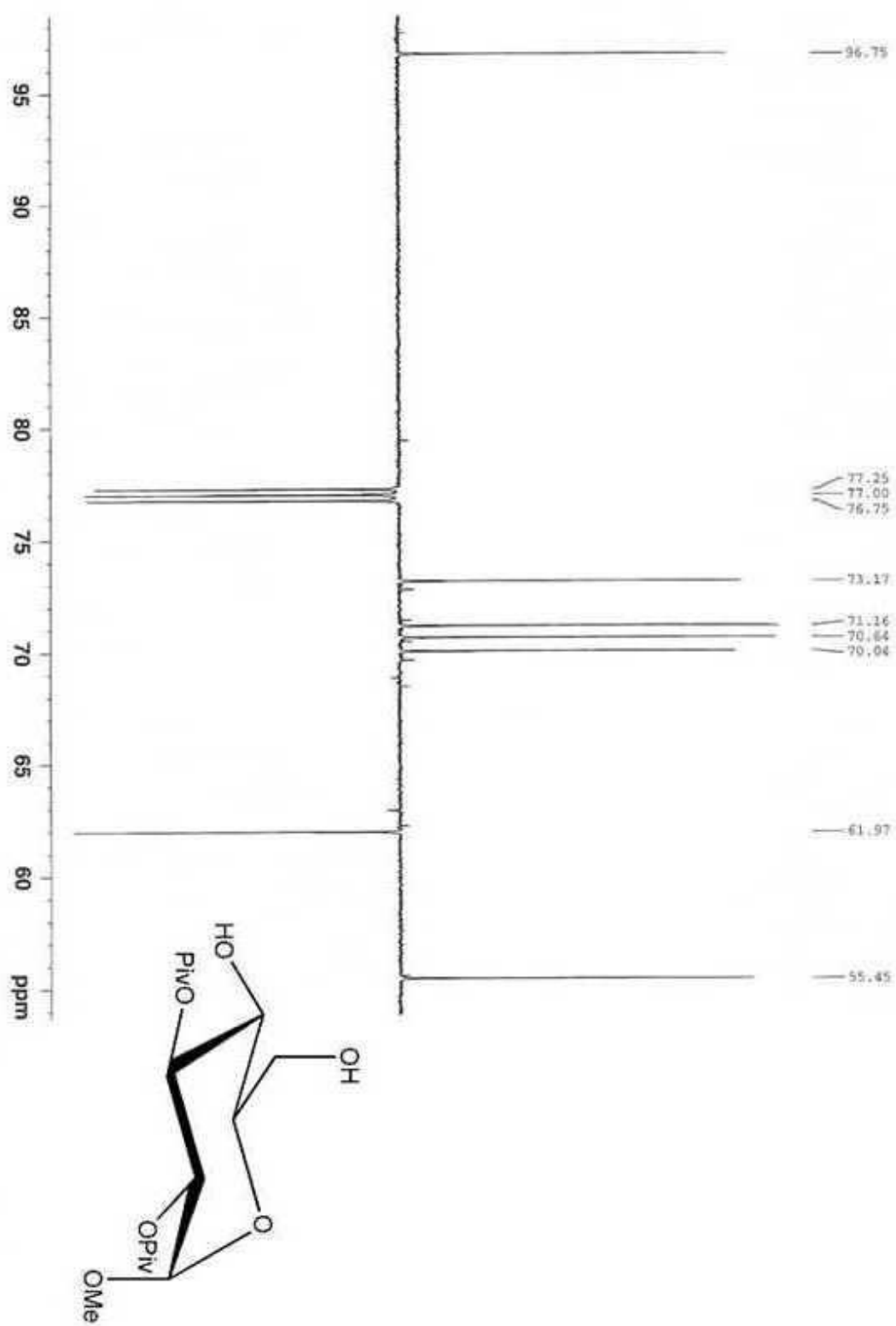




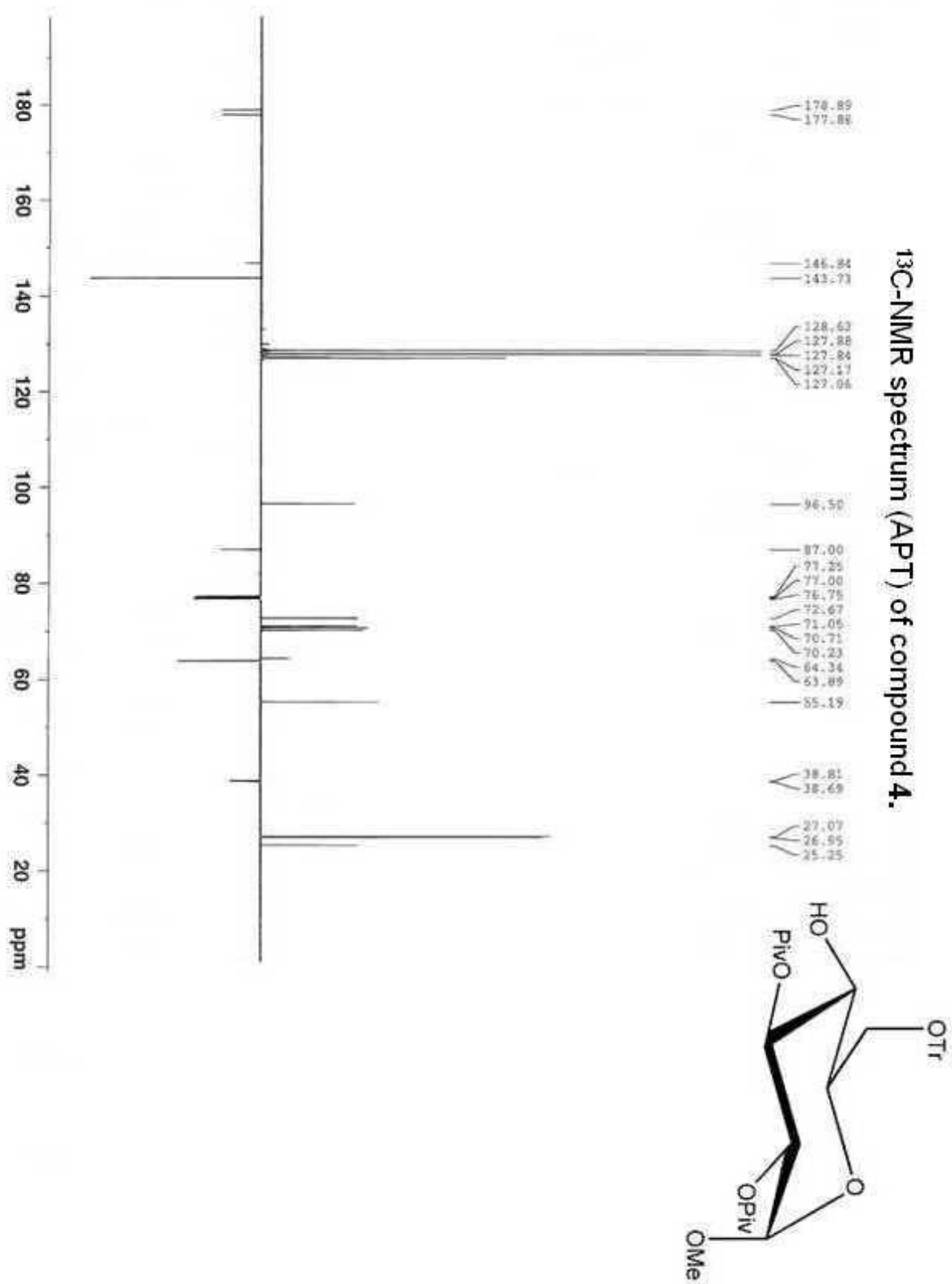
Partial ^1H -NMR spectrum of compound 3.



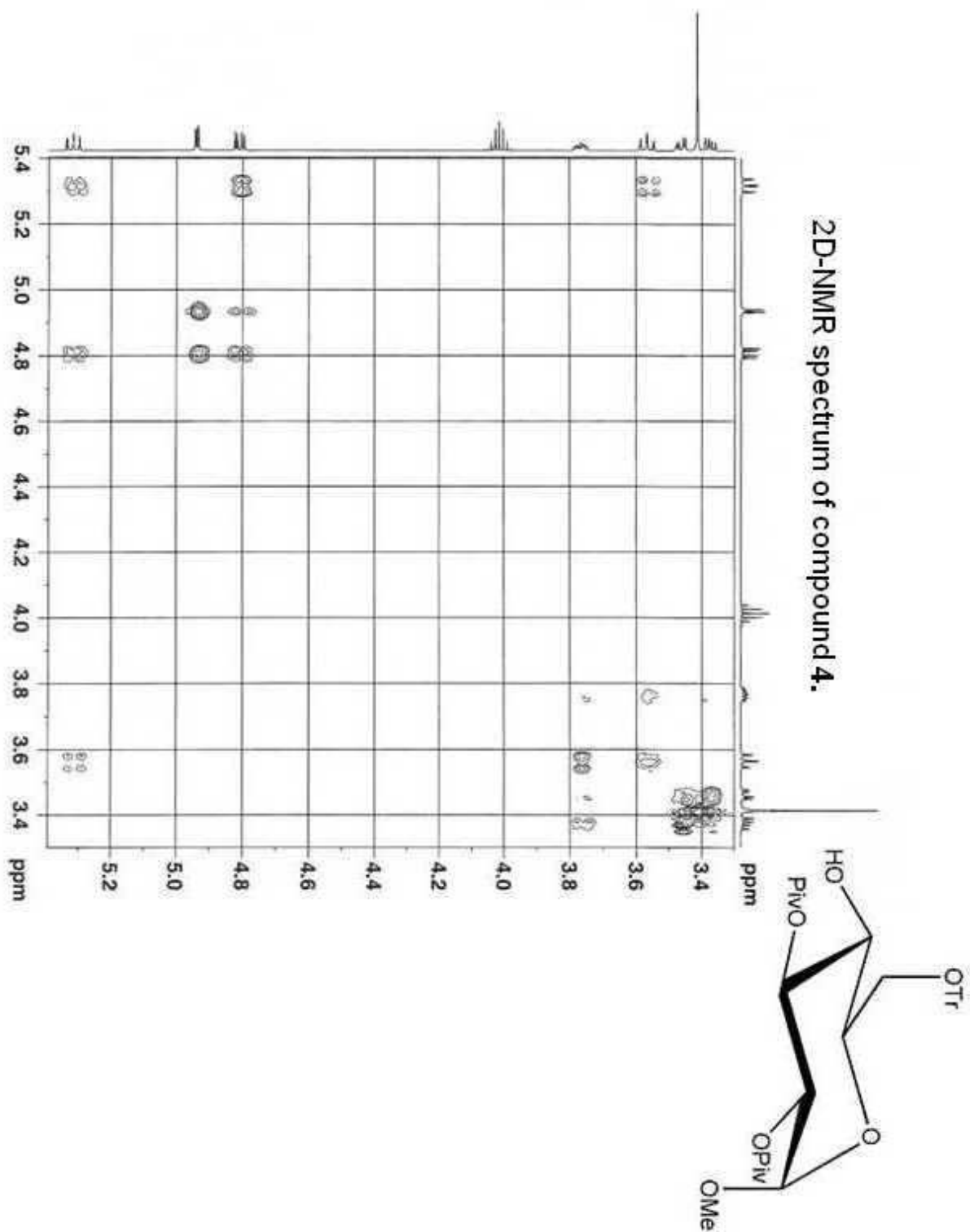
^{13}C -NMR spectrum (APT) of compound 3.



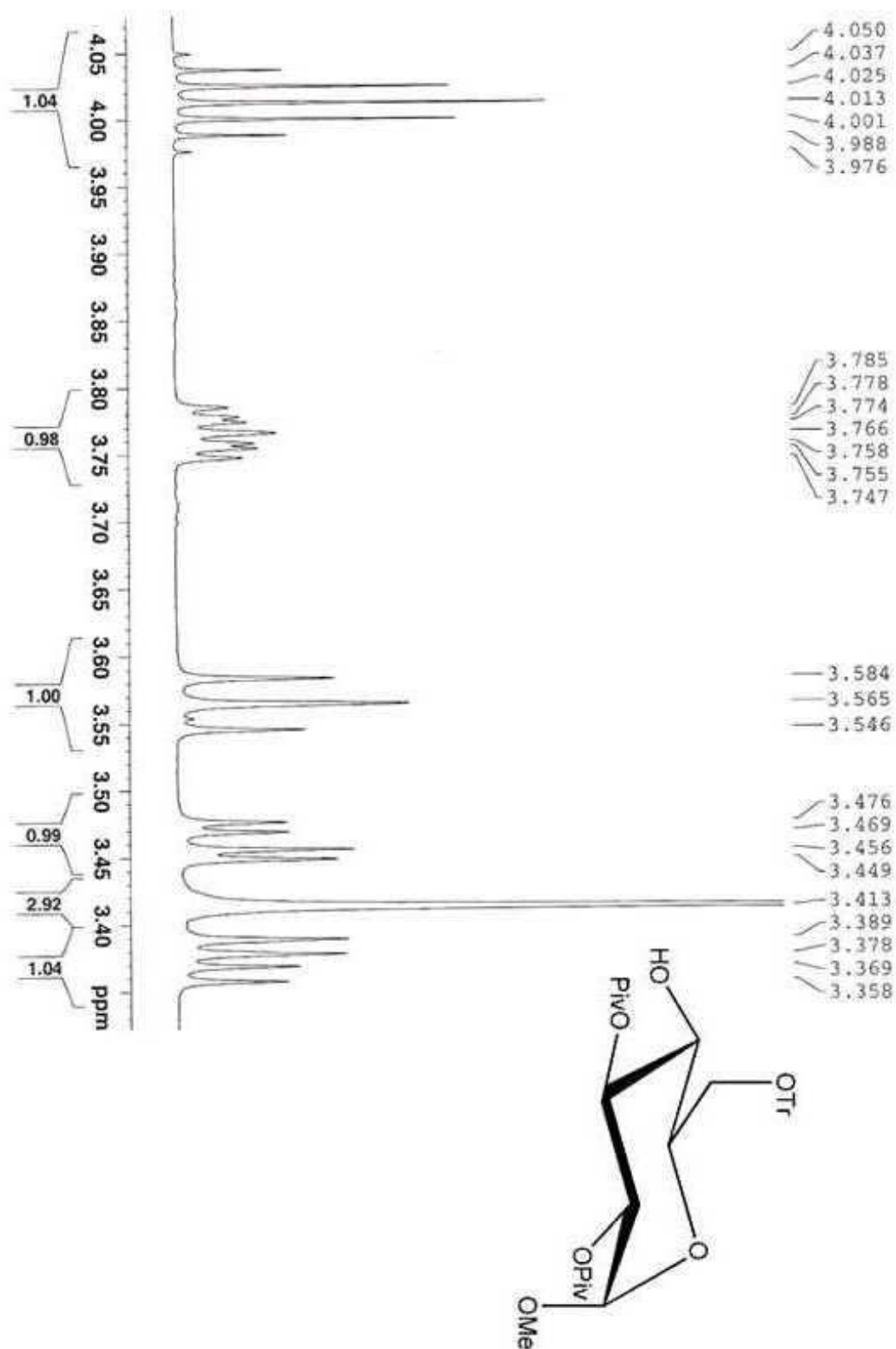
¹³C-NMR spectrum (APT) of compound 4.



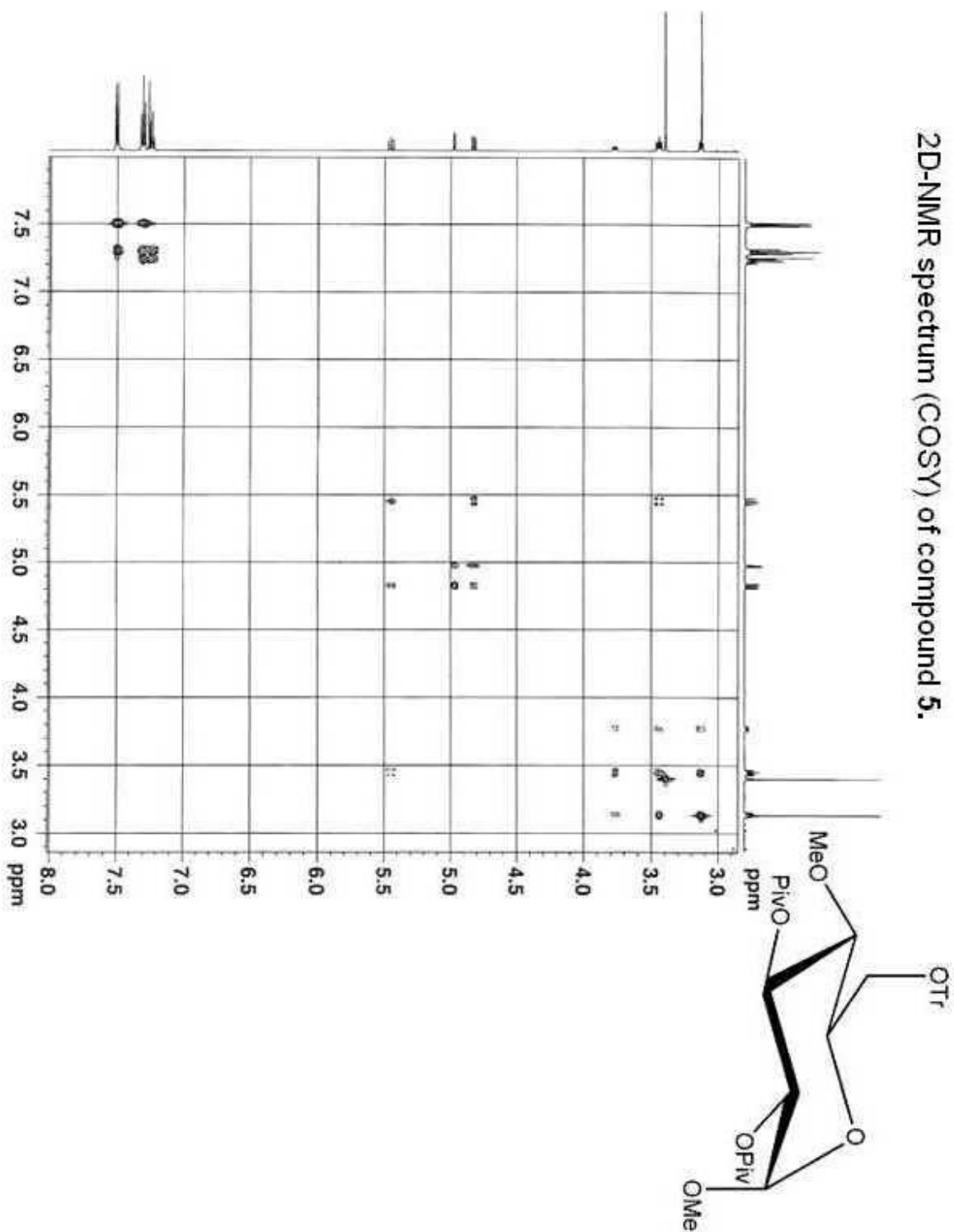
2D-NMR spectrum of compound 4.



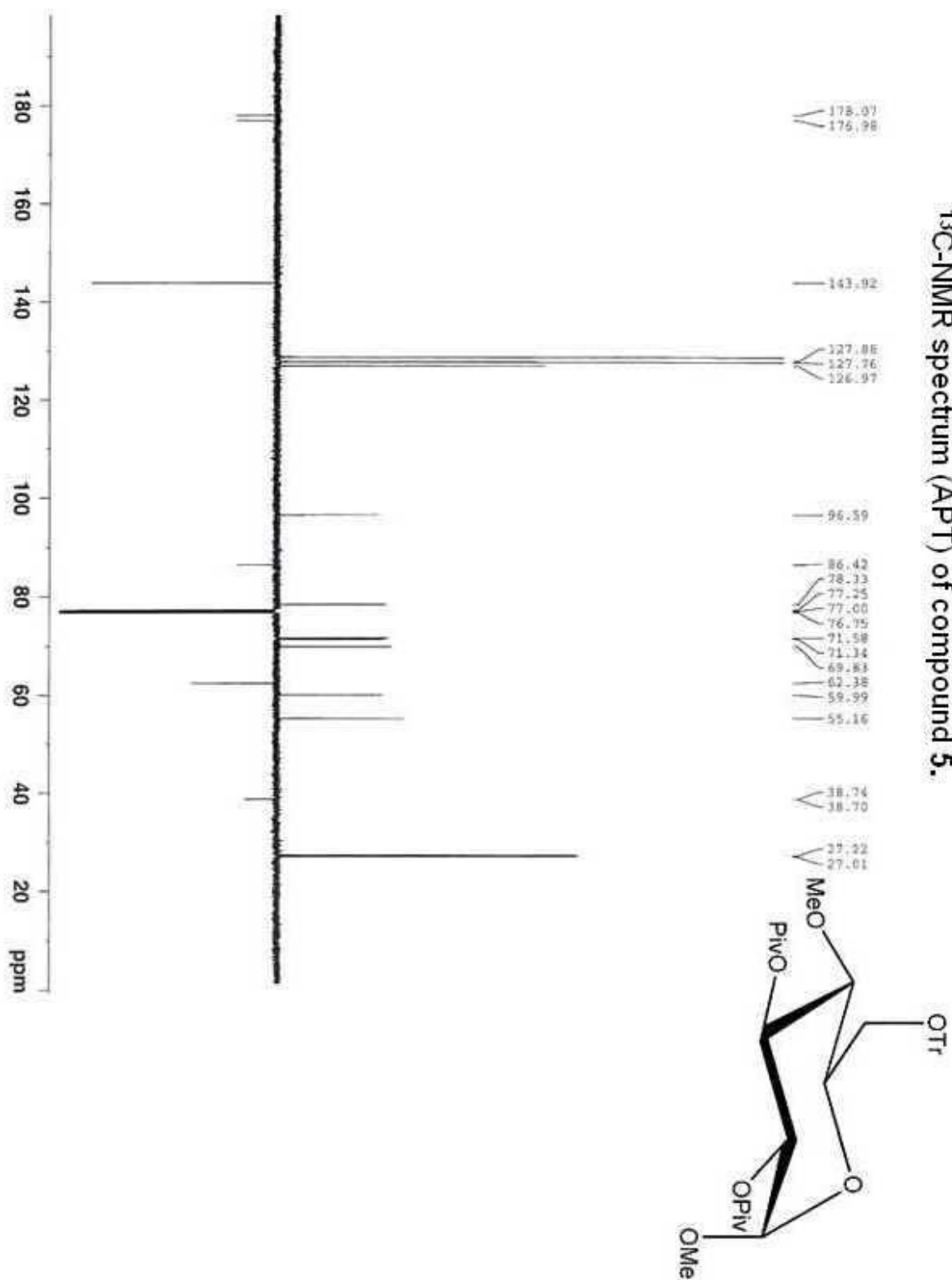
Partial ^1H -NMR spectrum of compound 4.



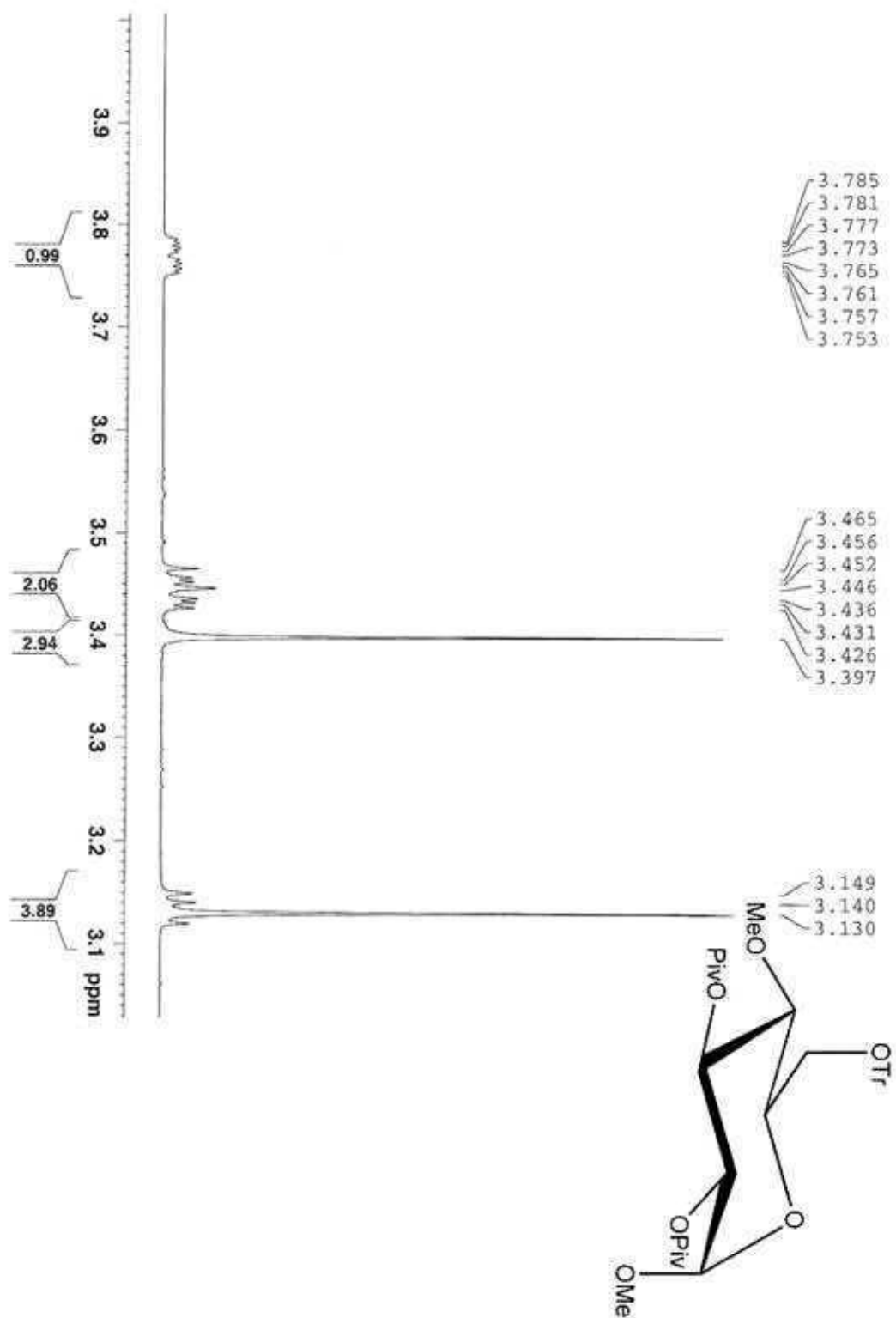
2D-NMR spectrum (COSY) of compound 5.



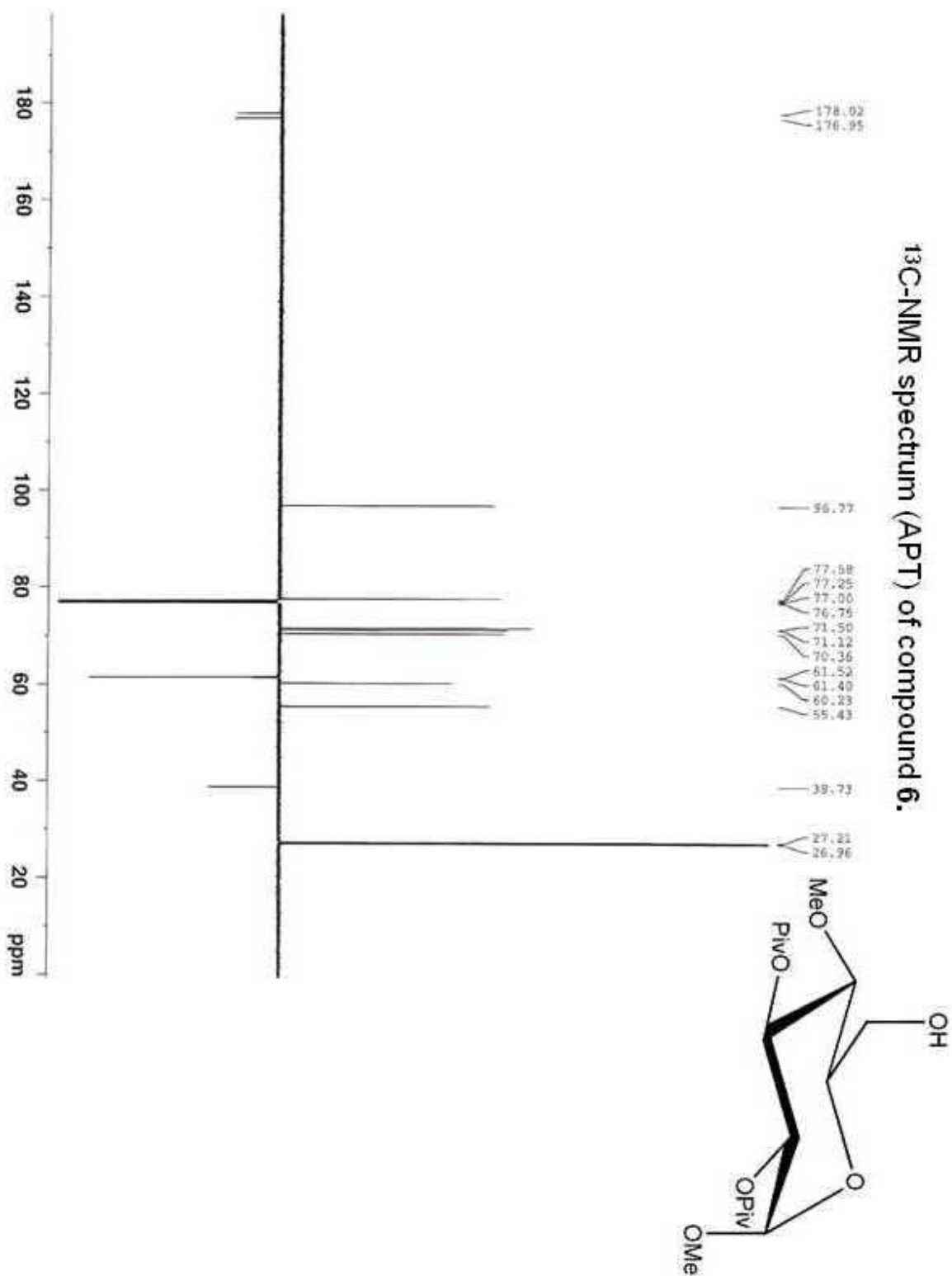
¹³C-NMR spectrum (APT) of compound 5.



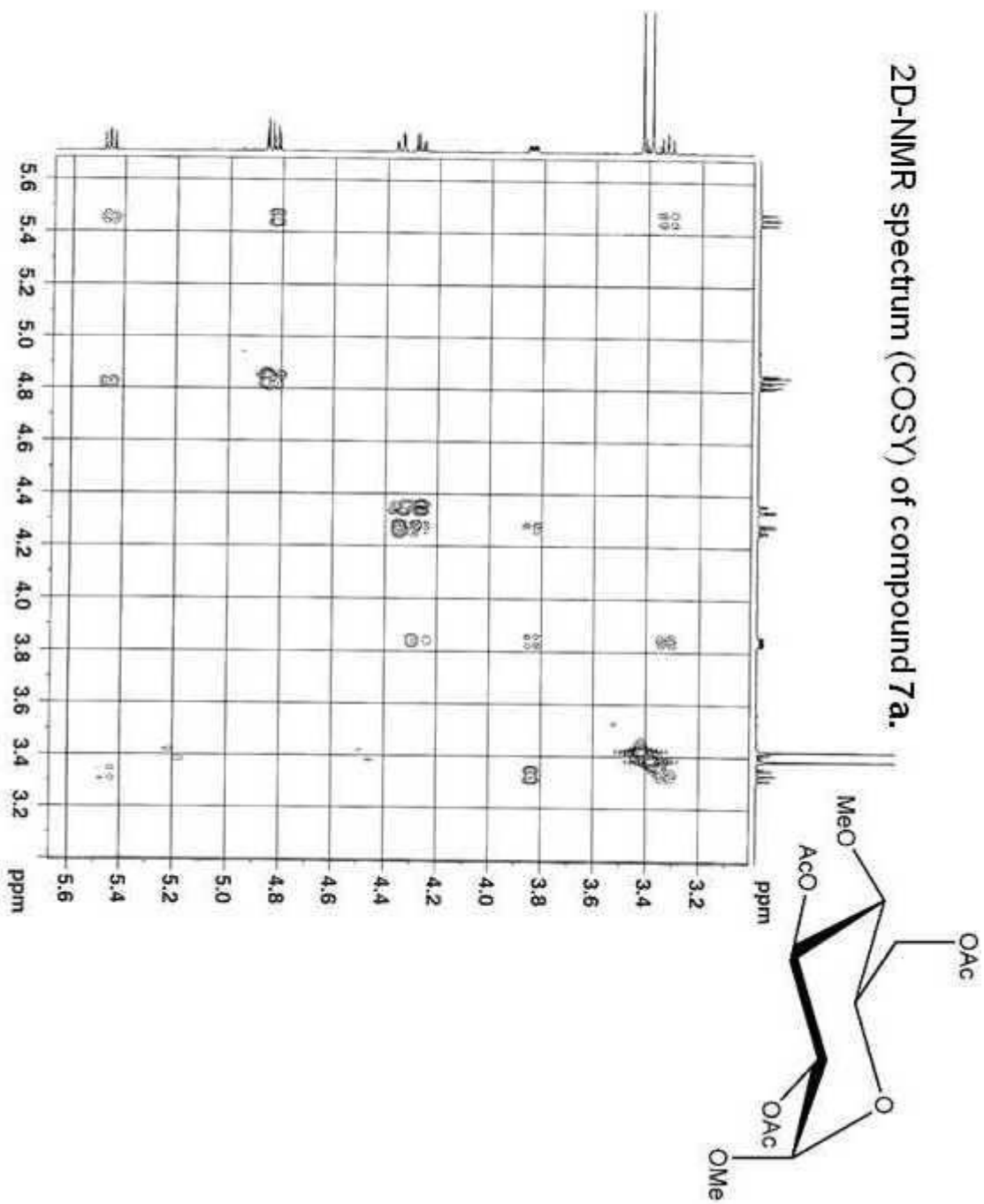
Partial ^1H -NMR spectrum of compound 5.



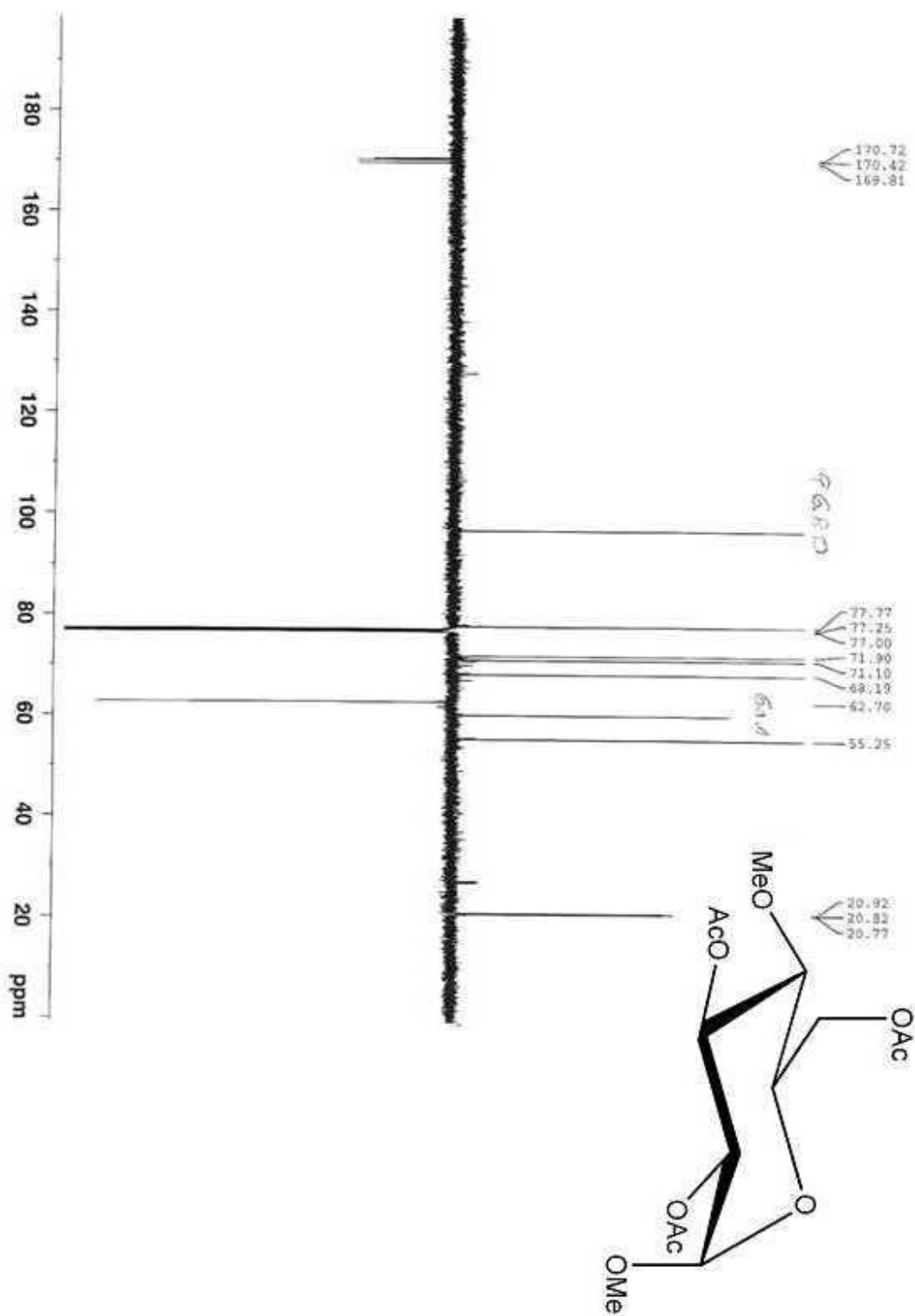
¹³C-NMR spectrum (APT) of compound 6.



2D-NMR spectrum (COSY) of compound 7a.



¹³C-NMR spectrum (APT) of compound 7a.



2D-NMR spectrum (HSQC) of compound 7a.

