Study of Stereochemical Effect of Galactoside Mixture Based On Reaction Time

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Abstract – The research project involve investigating the ratio produced by two stereoisomers of palm kernel oil derived galactosides, which are the alpha-galactosides and the beta-galactoside. The research conduct by synthesizing galactoside through three different step, which are peracytelation of galactose, glycosylation of galactoside and deacytelation of galactosdie. Three reaction time are 12 hour, 24 hour and 48 hour were done to study the α and β galactosides ratio changes. The ratio of production of both α-galactoside and β-galactoside were measured from the NMR results. Based from NMR results obtained, the ratio of more favourable to α-galactoside as time period is longer. This proven that α-galactoside takes more time to form because it is harder to stable in early codition while β-galactoside produce more in earlier stage as it is more stable, making it easier to form.

I. INTRODUCTION

The research project that had been conducted is the study of stereochemical effect of galactoside mixtures based on reaction time, referring to three different time period which are 12 hour, 24 hour and 48 hours. The reaction occur in this research is between galactose and palm kernel oil alcohol. With the present of lithium aluminium hydride, $LiAlH_4$ as catalyst, the configuration of galactoside will be formed, whether it is in α-galactoside form or β-galactoside form. The amount of production of each configuration depends on the period of time of reaction. NMR Spectroscopy is used to determine the ratio of these galactoside's configuration. In 12 hour time reaction, both α and β galactoside configuration are in an equal ratio which are 1:1. The second reaction time which is 24 hour yield a ratio of α and β galactosides configuration from ~2:1. And the final reaction time which is 48 hours had produced a ratio of ~8:1. This ratio is too high for this time reaction, which α-galactoside configuration produced is too high. There may be some error in analyzing the compound of some error during conducting the experiment. Theoretically, the ratio can be taken because the formation of α-galactoside is increase with increase of reaction time and formation of β-galactoside decrease with increase of reaction time.

II. METHODOLOGY

A. Material

All of the chemicals and materials used in this research were bought by different brands of companies. Crude Palm Kernel Oil obtained from Golden Jomaline Food Industries Sdn Bhd. The Lithium Aluminium Hydride, $LiAlH_4$ which act as catalyst was bought from Sigma Aldrich (USA). Galactose and Boron Trifluoride(BF_3) required also from Sigma Aldrich (USA). Diethyl ether and dibromomethane, ethyl acetate, acetonitrile, hexane, methanol, acetic anhydride andbutanol were purchase from Merek. Others chemical such as sodium methoxide,sodium acetate anhydrous, sodium hydrogen carbonate and amberlite were purchased fromMerek. Chlorofoam and methanol-D were purchase from Aldrich and Merek respectively.

B. Procedure

There are three types of procedure that have to run in synthesizing galactoside. The first step in synthesizing galactoside is peracytelation of galactose. At the end of peracytelation step, the product obtained is galactose pentaacetate. The second step is glycosylation of galactose and the final product in this step is glycoside. The last step in synthesizing galactosides is deacytelation of galactose. The final product in this step is galactoside, whether in α-galactosides configuration or in β-galactosides configuration.

C. Peracytelation of Galactose

Firstly, 20 g of sodium acetate is weighted in a beaker and 100 ml of acetyl anhydride is measured in a cylindrical measure. Both sodium acetate and acetyl anhydride are mixed in 1000 ml of 2 neck round bottom flask (RBF). The mixture is heated by sinking the RBF in a beaker containing oil with temperature of 120^0C and is stirred by using magnetic stirrer. The temperature of oil is maintained about 120^0C along the heating process. At the same time, 20 g of galactose is weighted in a beaker. When the mixture in RBF starting to condense, the galactose is added slowly using spatula. The purpose of adding galactose slowly is to prevent vigorous exothermic reaction to take place. The mixture of sodium acetate, acetyl anhydride and galactose left for furher heating for more than one hour until the mixture are soluble-clear mixture. Pour the hot soluble-clear mixture was poured in a container containing cold water and ice. Then stir it continuously. Left the container for about 2 minutes to make sure the product to suspend at below of container. Remove the cold water and ice, then repeat this rinse step (step 7 and step 8) for 3 times. After the rinse process, the product is filtered using suction filtration. While waiting the filtration process, 150 ml of ethanol is heated up to 60^oC to 80^oC.

The warm ethanol is poured in a conical flask containing the filtrated product. The conical flask is shake slowly until the product dissolve in warm ethanol. Then leave it to room

temperature. The conical flask is sealed using parafilm and refrigerated (refrigerator) for several days. (2 to 3 days).
After several days, the solid in conical flask is filtered using suction pump, and rinsed by using cold ethanol. The filtrated product is galactose penta-acetate.

D. Glycosylation of Galactose

6 gram of galactose penta-acetate is weighted and mixed with 3.2 gram of reduced PKO in a round bottom flask. 60 ml of dichloromethane (DCM) is poured and left to dissolve in round bottom flask. 2.3 ml of Boron Trifluoride (BF_3) is injected into round bottom flask. The time is set once this Boron Trifluoride is injected. The mixture is then left for a certain time period while being stirred using magnetic stirrer. After the certain time period, the reaction is stopped by adding aqueous sodium bicarbonate (aq. $NaHCO_3$). The mixture is stirred.
The organic layer is washed with distilled water using separating funnel for 2 times. A small amount of magnesium sulphate, $MgSO_4$ is added in purpose to remove the moisture in the mixture. It was then filtered using filter paper. The mixture is then being evaporated to remove dichloromethane at 668 mbar in the mixture. After being evaporated, 60 ml of acetonitrile is added and mixed with product in separating funnel. Then, the mixture of product and acetonitrile is washed using hexane in order to remove access alcohol (-OH) for 6 times. The first 3 times is washed by using hexane for about 60 ml and for the other 3 times for about 30 ml. Then the mixture is evaporated to remove acetonitrile at 132 mbar. The final product in glycosylation of galactose is glycoside.

E. Deacytelation of Galactose

Add 50 ml of methanol in RBF that contain product from glycosylation of galactose step. Then add sufficient amount of sodium methoxide inside mixture in RBF. The mixture is the stirred. The mixture need to be in a base condition. Litmus paper is used in order to determine whether the mixture is in base or not. It then left overnight for its further reaction. After the reaction, a few drop of amberlite is added until the mixture is neutralized. Test it by using litmus paper. Add amberlite until the mixture reaches neutral solution. It is then filtered by using filter paper, in order to separate the amberlite from the mixture. Next, the mixture is evaporated to remove methanol. The mixture is then added with n-butanol and distilled water for purifying purpose. The process is conducted by using separatin funnel. Distilled water used to wash the mixture until it separates completely. The two layer of solution will appeared in this step. Repeat this step for two times. Finally, the butanol is evaporated and the final product which is galactoside is obtained.

F. Nuclear Magnetic Resonance (NMR) Spectroscopy.

The NMR spectroscopy device is used to analyse the amount of α-galactosides and β-galactosides produced in each time period. In order to use NMR device, there are few steps must be done. Firstly, the final product, galactoside is added in NMR tube for about 8-12 mg and dissolve with methanol-D for about 2 cm high of NMR tube. Wait until it is fully mixed and then the tubes enter the NMR device for analysis purpose.

III. RESULTS AND DISCUSSION

A. Galactose Pentaacetate

The NMR results for galactose pentaacetate is as shown in Figure 1. Galactose pentaacetate is the final product in the first step which is peracytelation of galactose. This NMR results only shows the galactose pentaacetate before any reaction with PKO-OH. So there are no α-galactosides or β-galactosides in this Figure 1.

B. Gal-PKO for 12-hours

For 12 hours reaction time period, the NMR result of α-galactosides and β-galactosides can be observed. Based on Figure 2, the peak inside the purple circle represent an α-galactosides and the peak inside yellow circle represent a β-galactosides. After the result had been analyse and measured, in this 12 hour time period, the ratio between both α-galactosides and β-galactosides is ~1:1. Further information will be discussed in discussion section.

C. Gal-PKO for 24-hours

In 24 hour time period, the NMR result from Figure shows that the formation of α-galactoside is higher than formation of β-galactosides. This shows that an α-galactosides become more favourable as the time period increase. Based on Figure 3, the ratio between α-galactosides and β-galactosides is from 2:1. But after the baseline correction has been made, the ratio of α-galactosides to β-galactosides is from ~1.5:1. The baseline correction is made as the peak of α-galactosides does not reach from its baseline. So the peak is assume a little bit higher than the real peak.

D. Gal-PKO for 48-hours

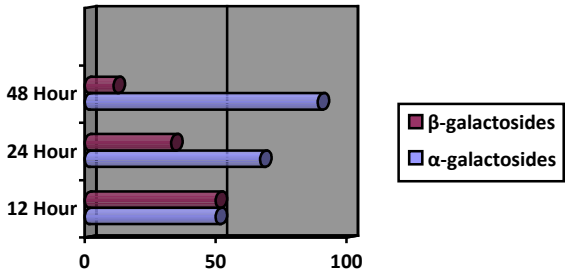
For time period of 48 hours, the α-galactosides formed is higher than β-galactosides. This can be observed from Figure 4 below. The formation of α-galactoside is far more than β-galactoside, giving a ratio from ~8:1 based on the NMR result measurement. The value is not really accurate because there are no separation between the solvent peak and the α-galactosides peak. But the value still can be taken as consideration.

E. Comparison For All Time Periods

From all three time period that had been done and observed, the different in ratio between α-galactoside and β-galactoside can be shown.

Based from the observation from all three time periods, we can see that as the time period increase, the formation of α-galactosides

will increase and formation of β-galactosides will decrease. This is because an α-galactosides is in axial configuration while β-galactoside is in equatorial configuration. Equatorial configuration stable easily compared to axial configuration. This makes β-galactoside's configuration formed higher in shorter time period, and some of this β-configuration will shift to axial configuration, which is an α-galactosides. α-galactoside is a kinetically stable while β-galactoside is thermodynamically stable. It takes more time for α-galactoside to achieve enough kinetic energy to form, while β-galactoside form through thermodynamic after the reaction takes place. The graph in Figure 5 shows the percentages of galactosides through the time periods.

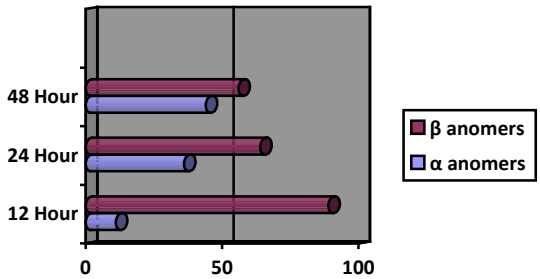


There are many other peaks in the NMR results obtained. There are a solvent peak, hydrogen peak, PKO alcohol peak and many other peaks including an impurities peak. The green circle in colour in each Figure 1,2,3 and 4 shows the hydrogen peaks and the red circle in colour represent the solvent peaks. For solvent in Figure 1, chlorofoam is used and for solvent in Figure 2, 3 and 4, solvent methanol-D is used. The black circle in Figure 2, 3 and 4 represent the PKO alcohol solvent. Table 1 shows the circle colour represent in Figures and its respective peaks.

Table 1: Circle Colour with Its Respective Peaks

Circle Colour	Peaks
Purple	α-galactoside
Yellow	β-galactoside
Green	Hydrogen
Red	Solvent
Black	PKO alcohol solvent

The result is also compared by other research partners, which she synthesizing the glucose's configuration. The results of her research is as shown in Figure 6.



IV. Conclusion

The formation of α-galactosides and β-galactosides depends on the glycosylation time. When the time period increase, the formation of α-galactosides increase and the formation of β-galactosides decrease. This is occur because the β-galactosides shift from equatorial configuration to axial configuration. Basically the amount of galactosides in the compound are the same. The value keep changing because the galactosides are shifting from β-galactoisdes to α-galactosides. The NMR device is used to analyse the ratio of formation of both α-galactosides and β-galactosides.

Acknowledgment

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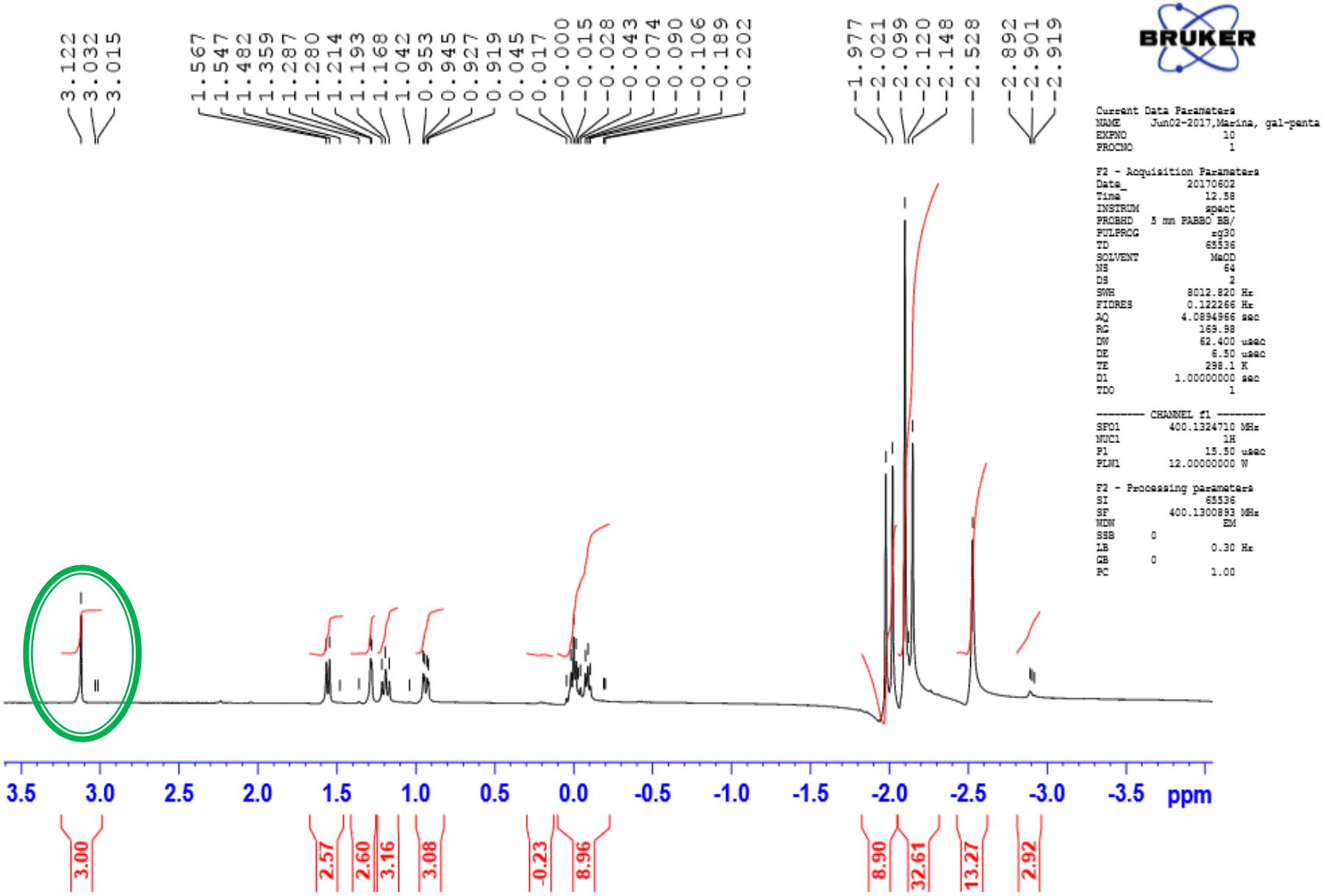

Figure 1: NMR for Galactose Pentaacetate

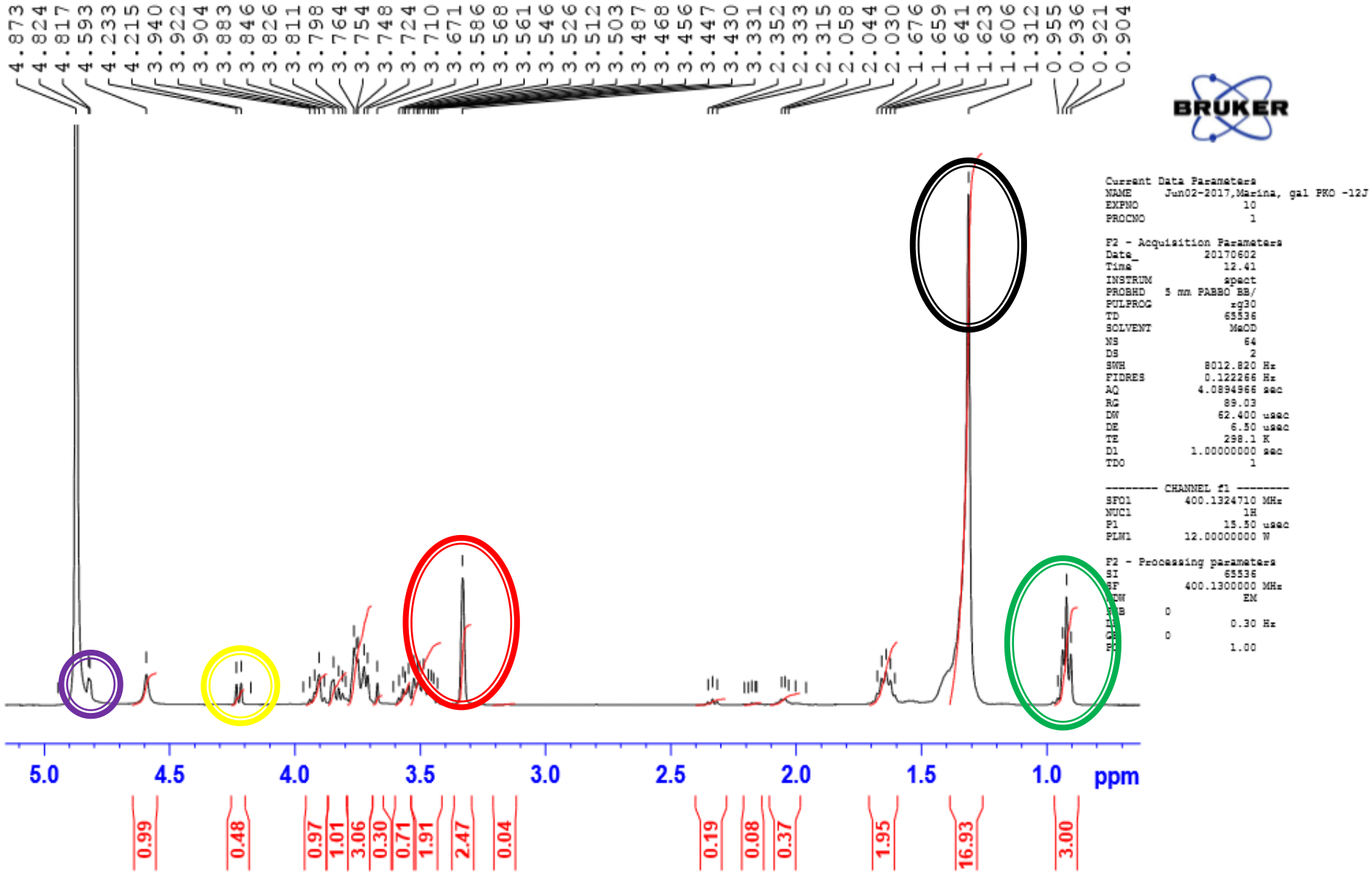

Figure 2: NMR for Gal-PKO for 12 Hour

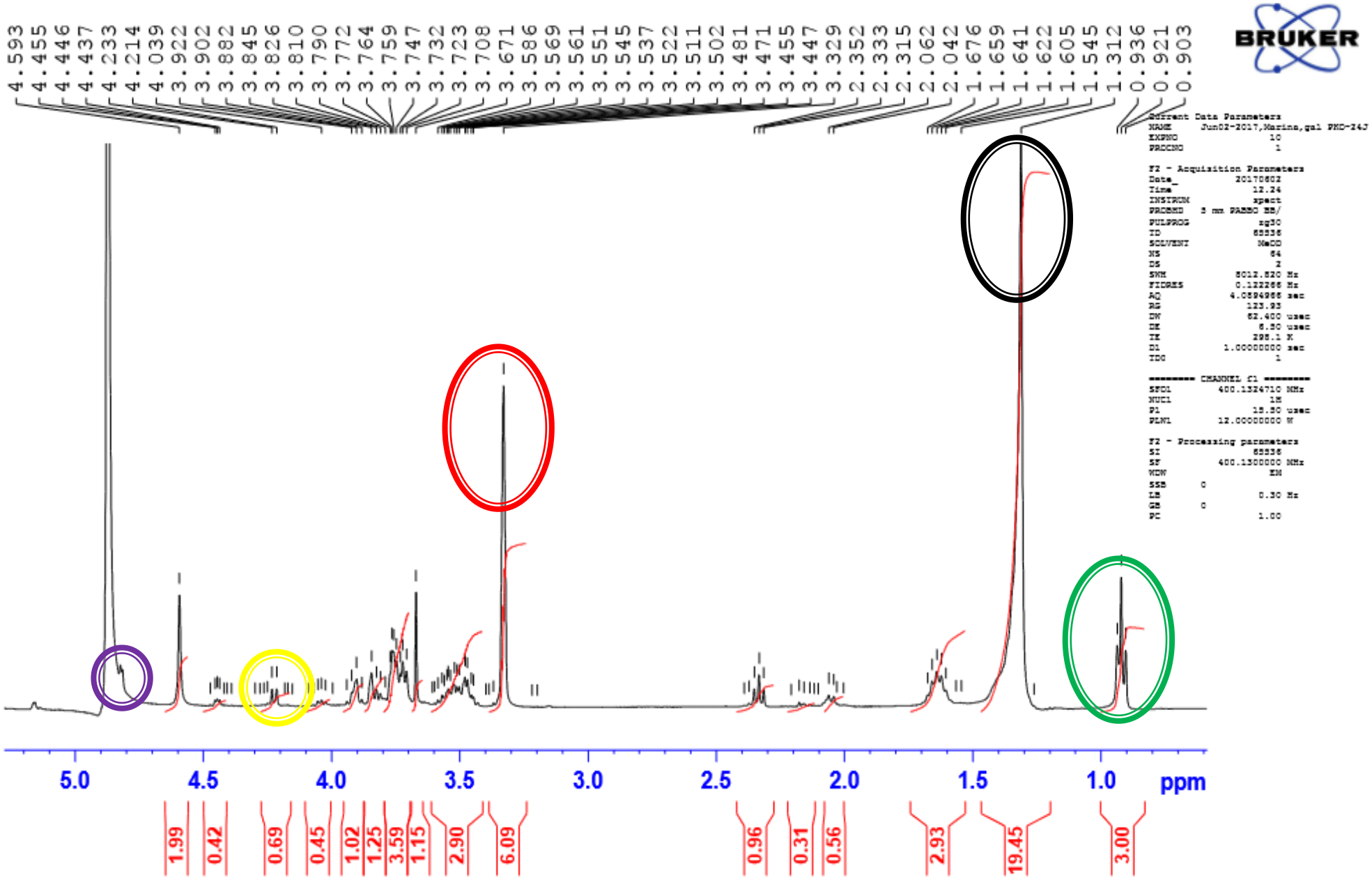

Figure 3: NMR for Gal-PKO for 24 Hour

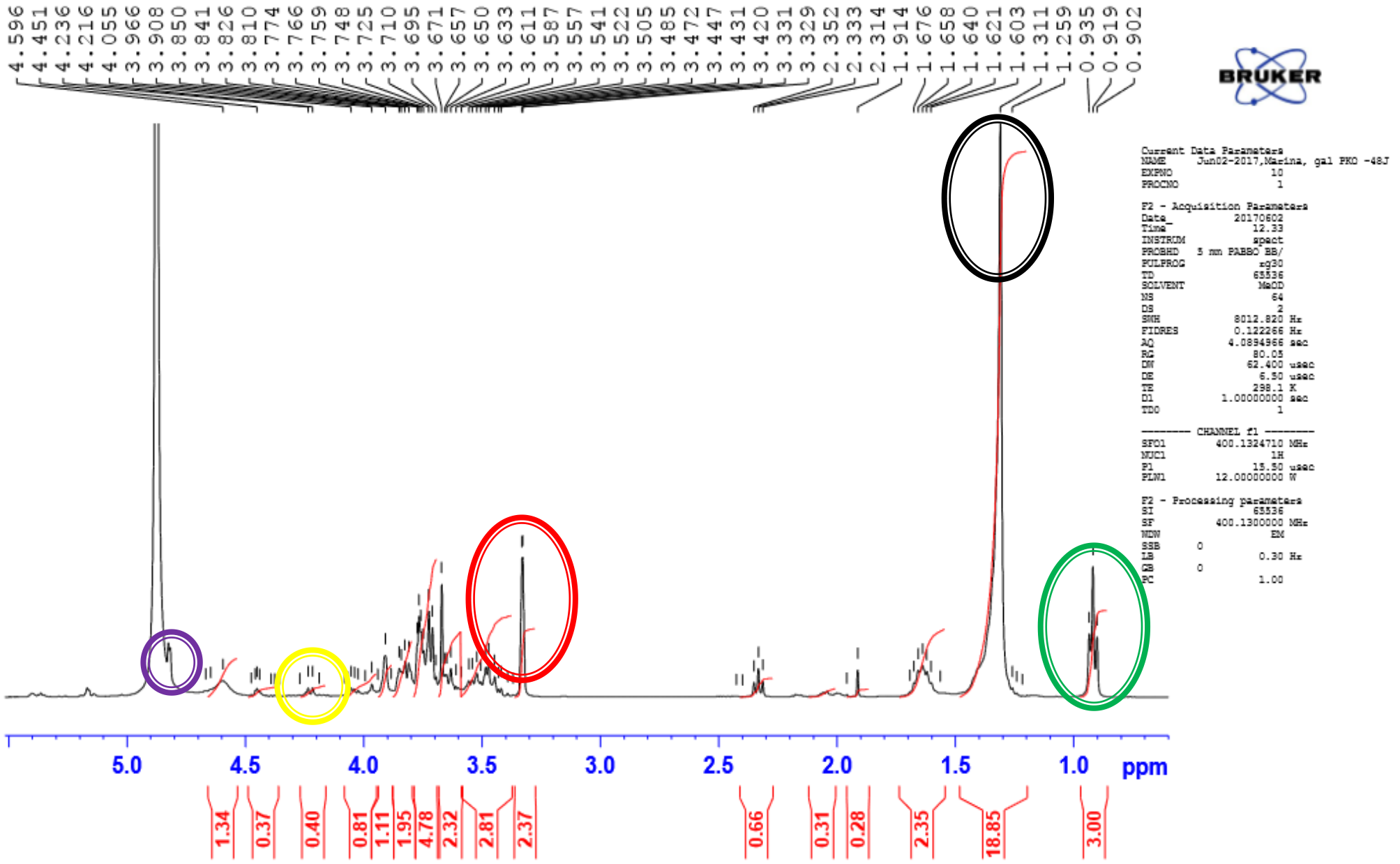

Figure 4: NMR for Gal-PKO for 48 Hour