UNIVERSITY OF MANITOBA

An investigation into the effects of L-Arabinofuranose *O*-glycosylation of hydroxyproline

By

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Abstract

The amino acid (2*S*, 4*R*)-4-hydroxyproline (Hyp) plays a critical role in animal kingdom as structural protein collagen. It is ubiquitous in plant cell walls performing various functions such as structural assembly, plant hormones, plant growth, defense against pathogens, etc. Glycosylation of Hyp is often seen in plant cell walls with L-Arabinofuranose and D-Galactopyranose and not in animal kingdom. Glycosylation is a post-translational modification, which affects characteristics of proteins and peptides.

The main objective of this thesis is to synthesize various L-arabinofuranosylated hydroxyproline model amides and investigate their thermodynamic and kinetic properties of cis/trans amide isomerization. These results are compared with the previous research of D-galactopyranosylated hydroxyproline model amides, which may provide an insight to structural implications for their stability and conformations of peptides and specificity in plants.

Both α- and β-L-arabinosylation of Hyp resulted in the stabilization of *trans* rotameric state at room temperature while the *α*-anomer leads to cis rotamer stabilization at higher temperature. Similarly, both unnatural 4S-hydroxyproline (hyp) building blocks resulted in stabilization of *trans* rotamer but α-anomer shows exo configuration instead of endo. This result shows a reverse trend when compared to galactosylated hydroxyproline building blocks as previous research results in our group. Our results may provide further insight to the role of glycosylation on protein structure and stability in plants.

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Dedication

To my parents, Nageswara Sarma and Lakshmikantham

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List of Abbreviations

[θ]	Mean residue ellipticity
[α]	specific rotation
Ac	Acetyl
Ala	L-Alanine
Asn	L-Asparagine
Aq	aqueous
Bn	Benzyl
Bz	Benzoyl
Boc	*tert*-butoxycarbonyl
c	concentration in g/100 mL
CD	Circular Dichorism
$CDCl_3$	deuterated chloroform
COSY	Correlation Spectroscopy
δ	chemical shift in parts per million
D_2O	Deuterium oxide
DCM	Dichloromethane

EtOAc	Ethyl acetate
Fmoc	9-fluorenyl methoxycarbonyl
Gal	D-Galactose
Glc	D-Glucose
GlcNAc	*N*-acetyl-D-glucosamine
Gly	Glycine
GOESY	Gradient Enhanced Nuclear Overhauser Effect Spectroscopy
HRGP	Hydroxyproline-rich Glycoprotein
HSQC	Heteronuclear single quantum coherence
Hyp	(2*S*, 4*R*)-hydroxy-L-proline
hyp	(2*S*, 4*S*)-hydroxy-L-proline
J	coupling constant in Hertz (in NMR)
k_{ct}	rate constant from cis to trans rotamers
k_{tc}	rate constant from trans to cis rotamers
$K_{trans/cis}$	Equillibrium constant of *trans:cis* amide isomers
Lys	L-Lysine
MeOH	Methanol

MS	Mass spectrometry
NHMe	N-methylamide
nOe	Nuclear Overhauser effect
NMR	Nuclear Magnetic Resonance
OMe	methoxyl group
ppm	parts per million
Pro	L-Proline
Ser	L-Serine
TFA	Trifluoroacetic acid
Thr	L-Threonine
Tyr	L-Tyrosine

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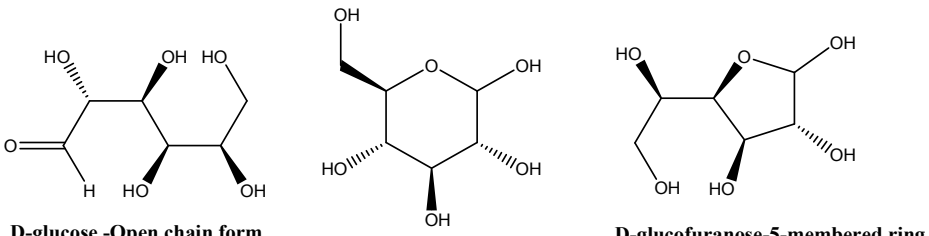
Chapter 1: Introduction and Background

1.1 Introduction to carbohydrates, glycosylation and biological roles of carbohydrates in living systems

Carbohydrates are important compounds of biological systems and generally regarded as polyhydroxy aldehydes or ketones which are hydrolysed to simpler monomers (monosaccharides) units. These exist in various chains of monosaccharides, cross linking chains of monomers in plants and animals mostly in ring forms. These compounds are classified as monosaccharides, disaccharides, trisaccharides and polysaccharides based on the linkage of one unit or multiple units of monomers. They may exist in open chain and ring forms and are interconvertible. The ring forms are mostly in 5 membered (furanose) or 6 membered (pyranose). Most of the monomers have a potential carbonyl group (ex: aldehyde in glucose and ketone in fructose) and exist in open chain or ring forms. Figure **1** shows the D-glucose in open chain and ring structure. The ring structure which contains 6 membered rings adopts the more stable chair conformation.

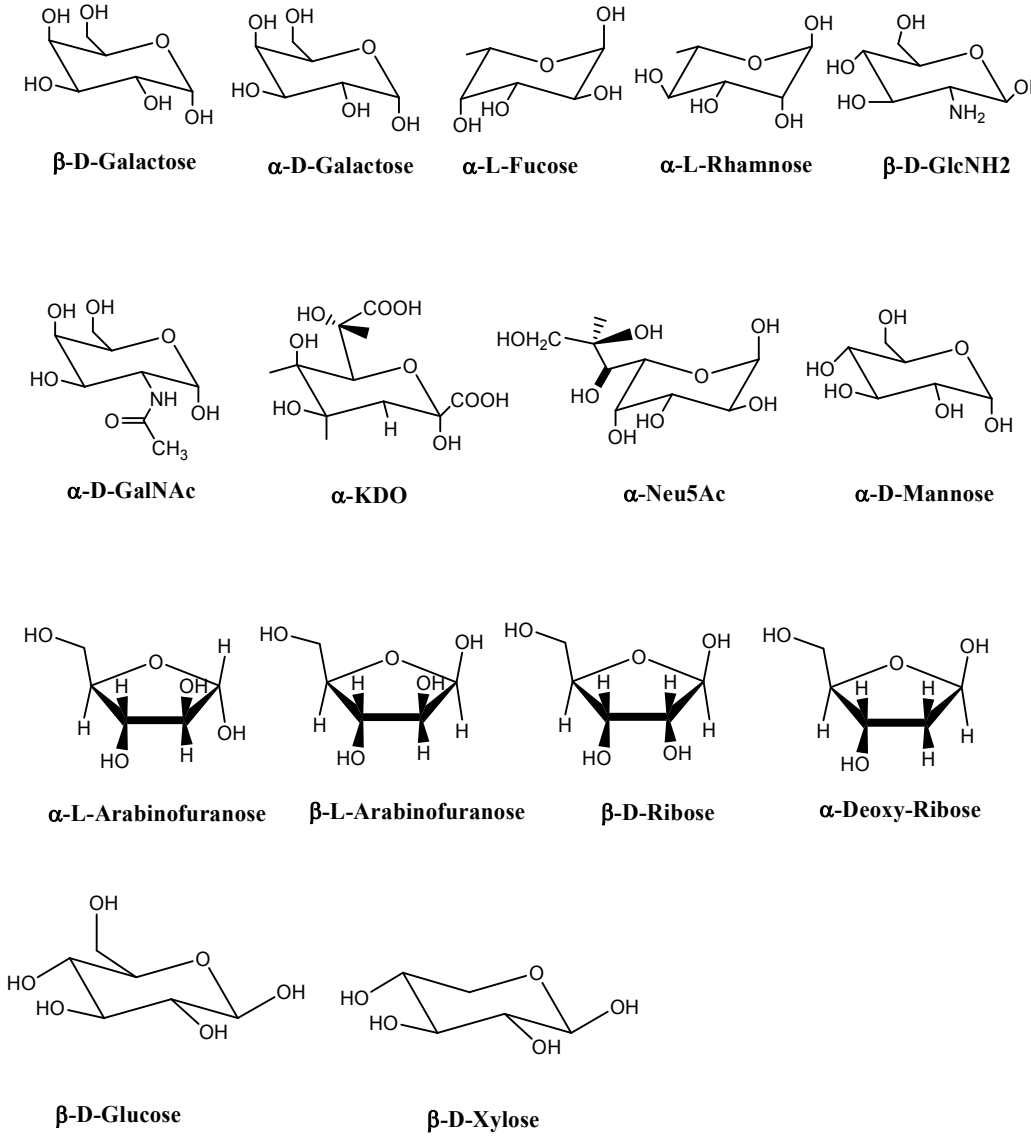
Figure 1: *D-glucose in open chain, 6-membered ring pyranose form and 5-membered ring furanose form.*



D-glucose -Open chain form **D-glucopyranose-6 membered ring** **D-glucofuranose-5-membered ring**

In most of living systems, these carbohydrates exist as chains of monomers linked to one another in various positions[1]. The most common examples of sugars in living systems are blood sugar – glucose (monomer), table sugar is sucrose (disaccharide) and polysaccharides are starch, chitin and amylase. Monosaccharides which are commonly found in Nature are given in Figure **2**.

Figure 2: *Most common monosaccharides present in biological systems in plants and animals*



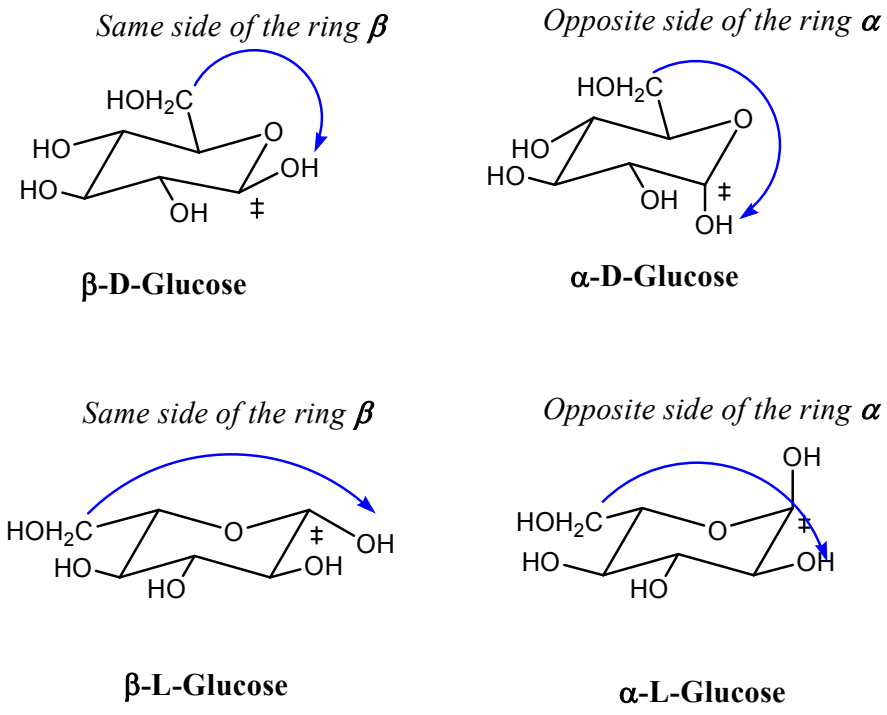
Monosaccharides are characterized by

- Placement of carbonyl group: If on first or last carbon carbonyl group if present it will be an aldehyde as in glucose a 6 carbon aldehyde.
- Number of carbon atoms: 5 membered sugars are called pentoses, 6 membered hexoses and so on.
- *The chiral handedness:* Each carbon atom has a hydroxyl group with exception of first are asymmetric and more than 2 isomers are possible for any given monosaccharide. For example glucose has 4 hydroxyl groups and the number of possible isomers are16 = 8 enantiomeric isomers.

Also, the ring forms exist in 2 anomeric forms i.e. alpha (α) and beta (β) defined by the position of hydroxyl group on the anomeric carbon which is shown for D-glucose in Figure **3**. These anomers are based on the following description and the anomeric[2] effect. Carbohydrates and some heterocyclics exhibit anomeric effect. The anomeric effect, originally defined as the preference of an electronegative substituent at the anomeric position of a carbohydrate to be axially rather than equatorially oriented, is now understood to be the result of multiple steric and stereo electronic interactions[2]. Nucleotides and proteins are linear polymers that can each have only one basic type of linkage called peptide bonds. Not many variations possible with few numbers of amino acids. In fact each monosaccharide theoretically generates an α or a β linkage to any one of several positions on another monosaccharide / amino acid in a chain or to another type of molecule (5 or 6 carbons for ring structures). For example, three nucleotide bases or

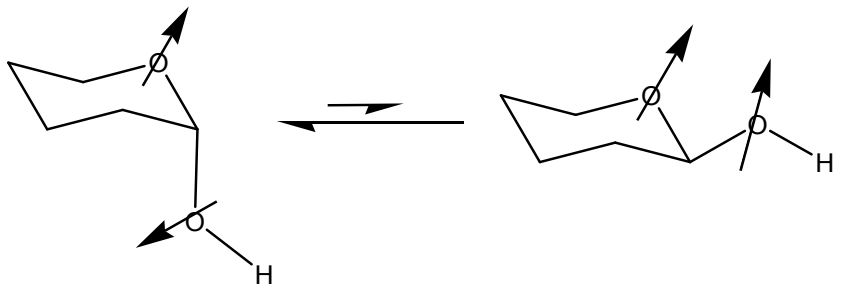
amino acids can only generate six variations, three hexoses could produce anywhere from 1,056 to 27,648 unique trisaccharides and 6 hexoses with more than a trillion unique possible combinations. As the number of carbohydrate units in the polymer increases, this difference in complexity becomes even greater. Thus, an almost unimaginable number of possible saccharide units could be theoretically present in biological systems. Thus we can conclude that carbohydrates combinations are immense and it is difficult to ascertain completely their functions in biological systems. Carbohydrates have many roles in living systems from energy storage (glycogen, starch, etc.) to structural components (celluloses in plants, chitin in some organisms) and they play important role in biosynthesis of various molecules.

Figure 3: *Assignment of **α-** and **β-** anomers of D and L-Glucose*



As it is a well-known fact that monosaccharides are polyhyroxy aldehydes or ketones with at least three carbons, one of which is a carbonyl and each remaining carbon bares a hydroxyl group. In aqueous solution, carbohydrates exist in equilibrium between their open chain and cyclic forms. Aldose sugars containing 5 or more carbons or ketoses containing 6 or more carbons cyclize to form hemiacetals in solution. Consider glucose to form a pyranose-based 6 membered ring. It will have 2 possibilities. The carbonyl carbon in the open chain becomes the anomeric carbon (*) in the cyclized structure, which is the most oxidized carbon of the ring. Cyclized aldose and ketose carbohydrates can adopt either α or β anomeric configuration depending on the orientation of the group. A simplified approach for the degree of contribution from dipole-dipole and molecular orbital interactions is depicted by Figure **4**. The alpha- anomer has bond dipole moments anti parallel (stable) but equatorial have parallel (unstable). This figure also explains the preferred stability of α-orientation of carbohydrates.

Figure 4: *Depicting the anomeric effect where α-orientation (axial) is more stable than β-orientation (equatorial) in sugar moiety due to bond dipole moments antiparallel to each other*



α orientation – axial -

bond dipole monents anti-parallel

β orientation – equatorial -

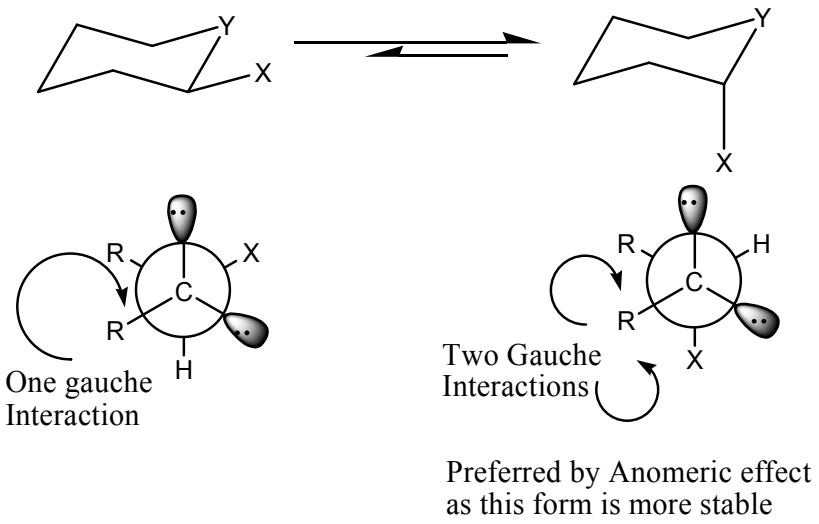
equatorial-bond dipole moments parallel

A glycosidic linkage involves the attachment of a monosaccharide to another residue of carbohydrate, peptide or lipid, typically via the hydroxyl group of this anomeric center, which can be α-linkages or β-linkages depending on the relationship of the oxygen to the anomeric carbon. Also during glycosylation reaction, the ratio of anomers α and β are unequal due to the anomeric effect.

Anomeric Effect[3]:

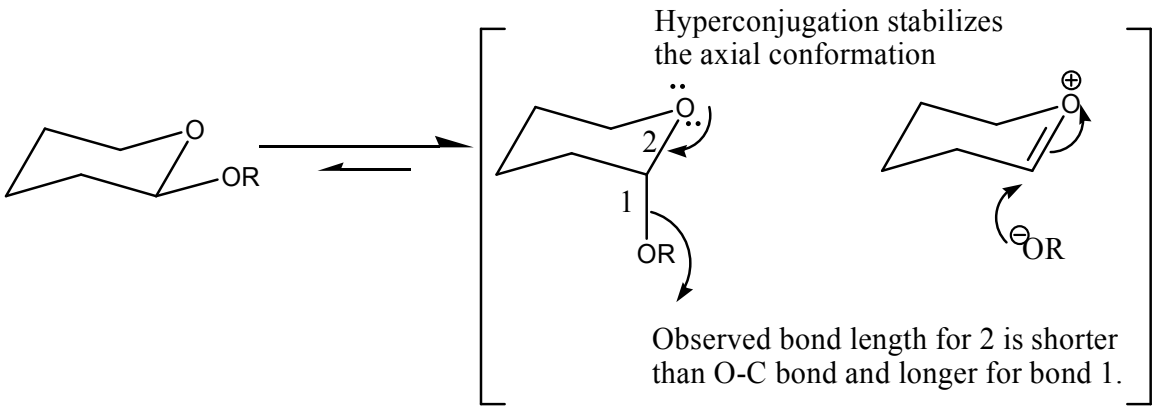
The generalized anomeric effect is a special case of general preference of gauche conformations (Figure **5**) around the bond C-Y in X-C-Y-C, where X and Y are heteroatoms with non-bonding pairs of electrons and one of them is nitrogen, oxygen or sulphur. It is affected by the solvent dipole moment as decrease of polarity increases the anomeric stabilization[4].

Figure **5**: *Anomeric effect by gauche interactions*



The most widely accepted definition of the anomeric effect is explained by hyperconjugation and antiperiplanar lone pair hypothesis (ALPH).

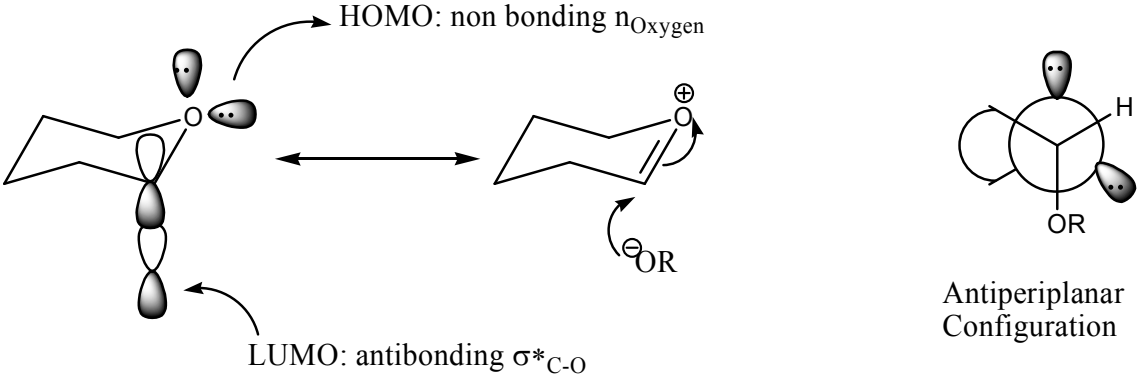
a) Hyperconjugation[5]: Figure **6**: *Anomeric effect explained by Hyperconjugation*



b) ALPH (Antiperiplanar Lone Pair Hypothesis)[1§]

There is a stabilizing 2 electron interaction by the closer examination of orbitals of HOMO (non-bonding orbital of (n_{Oxygen})) and LUMO (antibonding orbital of (σ^*_{C-O}) bond which is referred as ALPH and represented in the Figure **7**:

Figure **7**: *ALPH (Antiperiplanar Lone Pair Hypothesis)*



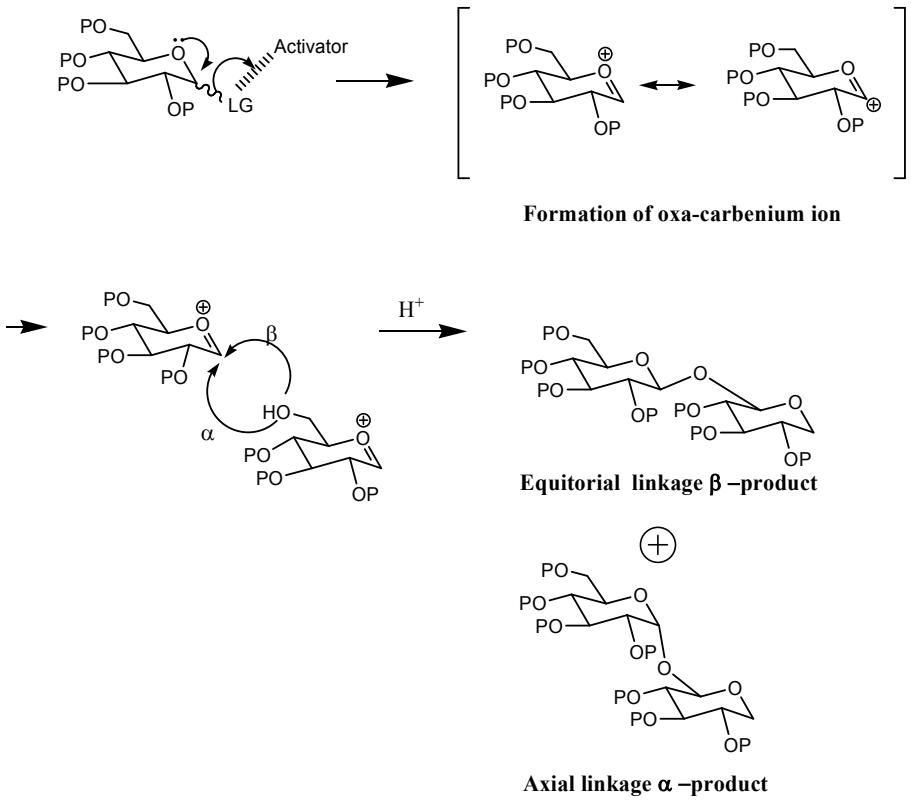
[1§] Krawczuck Paul, "Anomeric Effect – Baran group meeting" Nov 05, **2005**
http://www.scripps.edu/baran/images/grpmtgpdf/Krawczuk_Nov_05.pdf

1.2 Glycosylation of sugars in living systems: *Glycosylation reaction mechanism and their types*

Meaning of the term glycosylation:

Glycosylation is a chemical reaction between a glycosyl donor and a glycosyl acceptor which forms a bond between the anomeric oxygen and another moiety (OH of sugar, amino acid, peptide, or lipid). A sample glycosylation is shown in Figure **8**[2§].

Figure 8: *Glycosylation reaction mechanism:*

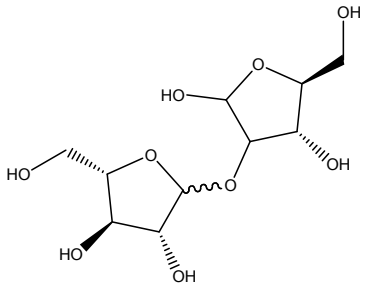


[2§] Chemical glycosylation. (2014, April 7). In *Wikipedia, The Free Encyclopedia*. Retrieved 22:53, June 18, 2014, from http://en.wikipedia.org/w/index.php?title=Chemical_glycosylation&oldid=603140535

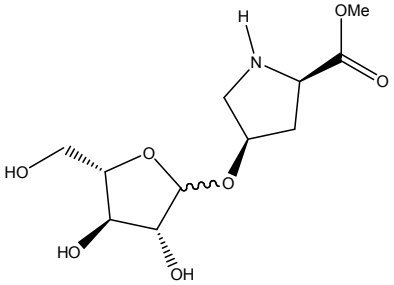
P refers to the Protecting group of OH of carbohydrate which often is benzyl, benzoyl, and Acetyl. LG refers to the leaving groups which are mostly associated with OAc or SR where R= Ph, Tol, SOPh etc.

Activators are reagents which make the leaving group leave to form an oxo-carbenium ion for resonance stabilization. Some of them are N-Iodo succinamide, AgOTf (promoter), BF_3Et_2O etc. As there are 2 orientations of attack of OH by Nucleophile to the oxo-carbenium ion, α and β, a mixture of anomers are formed. These reactions yield α- and β-anomers unequal ratios and sometimes exclusively one form either α or β governed by anomeric effect and solvent dipole moment. Most probably axial anomer is formed more than equatorial. For example, Figure **9** shows the glycosylation of L-arabinose with another *L*-arabinose moiety or hydroxyproline or a non-carbohydrate moiety like the *n*-hexyl group. If a sugar is linked to a peptide chain or protein it is referred to as a glycopeptide or glycoprotein.

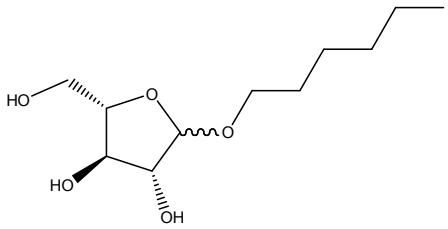
Figure 9: *Glycosylated Carbohydrates examples of L-arabinose with amino acids, non-amino acids in various combinations:*



L-Arabinose glycosylated with another L-arabinose at 1→2 glycosylation



L-Arabinose glycosylated with Hydroxy proline(amino acid)

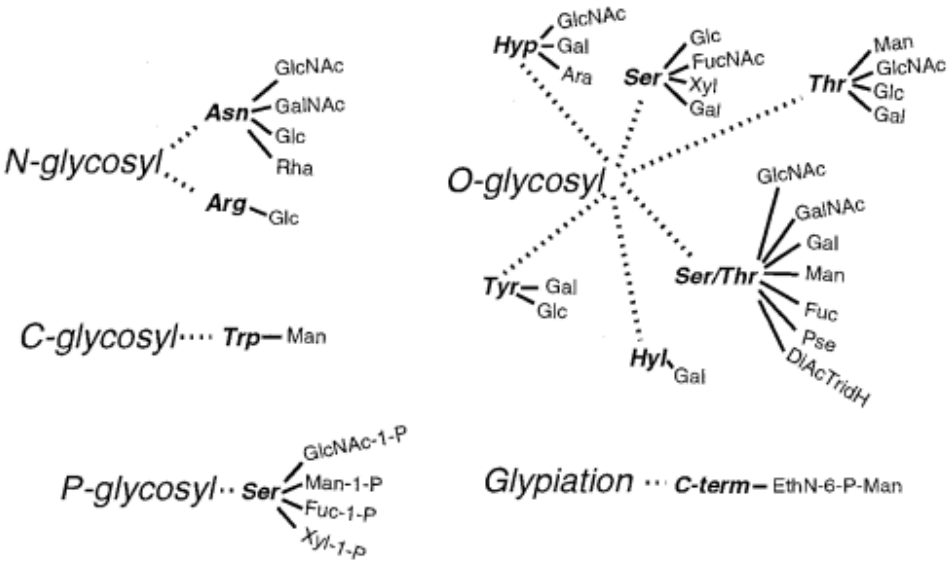


L-Arabinose glycosylated with n-Hexyl group(as in lipds)

There are major five types of glycosylation[6] found in living systems and the majority of them involve linkages to amino acids and some other carbohydrate sugars in linear and cross-linking fashion with a few peptides (in plants) observed in biological systems. They are *C*-glycosides, O-glycosides, N-glycosides, phospho serine and threonine glycosylation and GPI anchor lipid glycosylation. Some of the examples are shown in the **Figure 9**. O-glycosylation and N-

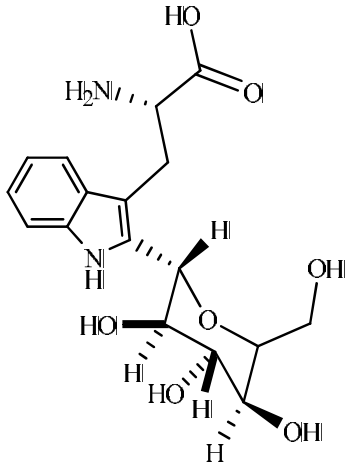
glycosylation are common and uncommon ones are C-glycosides and GPI anchor lipid glycosylation in living systems. Few examples with references of each and every type of glycosylation are presented below focussing on the type of anomeric and sugar linkages. **Figure 10** explains the types of linkages with types of sugars from an excellent review by Spiro et al[1].

Figure 10:*The occurrence of amino acid glycosylation[1] with types of sugars is obtained with permission from the journal reference*



a) <u>C-glycosides:</u> C-glycosides are common and usually involve a α-mannosidic linkage to C-2 of tryptophan[7]. These are present in RNase2, Interleukins[8], properdins[9].

Figure 11: *Tryptophan C-Glycosylation commonly seen with Mannose in living systems.*



b) N-glycosides: The β glycosyl amine (GlcNAc) linkage as well as mannose with asparagine (Asn) forms an N-glycosidic bond and they belong to class of N-glycosides. Asparagines linked glycosylation is the most common linkages found in many living systems[10]. These are mostly present along with O-glycosides too. They are present mostly in eukaryotes, plasma proteins, thryoglobulins[10], immunoglobulins, lectins etc.

c) O-glycosides: Linkages between hydroxyl group of amino acids like serine / threonine / hydroxy proline[11] with sugars form O-glycosides usually exist in both α and β-forms. In some plants and animals only one anomeric form is present. GalNAc-α-Serine linkages are predominant in mucins[12]. Variety of glycoproteins contains these linkages as in human gonadotropins, glycophorins, antifreeze glycoproteins, etc. and also show diversified linkages.

d) P-glycosides: The glycosidic bond which involves the phosphodiester linkage with the sugar and the protein are called P-glycosides. These are predominant in biological systems[13]in which sugar linkages of GlcNAc, Man, Xyl, and Fuc have been found to be

involved (Table **1**). The GlcNAc-α-1-P-Ser linkage has been found in various proteins from *Dictyostelium*[14] including proteinase-1. Man-α-1-P-Ser has been observed in several major proteins of *Leishmania* species[15], and Xyl-1-P-Ser has been found in *T. cruzi*[13]. Furthermore, evidence for the presence of Fuc-β-1-P-Ser in *Dictyostelium* has also been obtained[16].

e) Glypiated linkage: Other carbohydrate–protein connection is the GPI anchor which occurs in various biological systems[17]. In this linkage mannose is linked to phosphoethanolamine, which in turn is attached to the terminal carboxyl group of the protein. This linkage is widely distributed among biologically important cell surface glycoproteins of eukaryotes, including the Variant Surface Glycoproteins (VSGs) of trypanosomes and the Thy-1 antigen[17b].

Table 1: *The occurrence of these glycosylations in biological systems along with stereochemical descriptors are also shown in this review*[1] *but for convenience only few examples are reproduced with permission from the paper for which stereochemical anomeric descriptors are available.:*

	Linkage			Phylogenic Distribution			
Type of bond	Amino Acid	Sugar	Configuration	Eukaryotes	Archaea	Bacteria	Examples
N-glycosyl	Asn	GlcNAc	β	+	+	+	Ovalbumin, fetuin, insulin receptor
	Asn	Glc	β	+	+	-	Laminin, H. *haloblum* S-

							layer
O-glycosyl	Ser/Thr	GalNAc	α	+	-	-	Mucins, fetuin, glycophorin
	Ser/Thr	GalNAc	β	-	+	-	A. *thermoaerophilus* S-layer
	Ser/Thr	Gal	α	+	-	+	Nuclear and cytoplasmic proteins
	Ser/Thr	Man	α	+	-	-	Yeast mannoproteins
	Ser/Thr	Fuc	α	+	-	-	Coagulation and fibrinolytic factors
	Ser	FucNAc	β	-	-	+	P. *Aueruginosa pili*
	Ser	Xyl	β	+	-	-	Proteoglycans
	Thr	Man	α	+	-	-	Cell walls of plants
	Thr	GlcNAc	α	+	-	-	Dicostelium *T. Cruzi*
	Hyp	Gal	β	+	-	-	Collagen, c1q complement, core specific lectin, wheat endosperm
	Hyp	L-Ara	α	+	-	-	Plant cell walls
	Hyp	L-Ara	β	+	-	-	Potato Lectin
	Tyr	Glc	α	+	-	-	Muscle and liver glycogenin
	Tyr	Glc	β	-	-	+	C and T. *thermohydro*

							sulfuricum S-layer
C-Mannosylation	Trp	Man	α	+	-	-	RNAse 2, Interlukin 12, properdin
Phosphoglycosyl	Ser	GlcNAc	α-1-p	+	-	-	*Dicostelium* Proteinases
	Ser	Man	α-1-p	+	-	-	*L Mexicana Phosphatase*
		Fuc	β-1-p	+	-	-	*Dicostelium* proteins
Glypiation	Pr-(C-O)-EthN-6-P-Man			+	+	-	*T. Brucei VSG,* Thy-1, *Sulfolobus* Acidocaldarius

1.3 Glycosylation of sugars in living systems: *plants and animals and subtle differences*

A) Importance of glycosylation of sugars in animal kingdom:

Glycosylation in animals is a post-translational modification [18] which has diverse functions and is important in the regulation, disease management etc. Few examples for N-glycosylation effects are quoted below:

- Human acid β-glucosidase[19] enzyme is important for cleaves the glucoceramic bonds and synthetic β glucosides[20] of peptides in humans. The deficiency of this hydrolase leads to Gaucher disease which is also called lysosomal disease where the peptide is

glycosylated with sugars. Grace et al[21] have concluded that glycosylation is required for catalytic activity and non-glycosylation results in complete loss of activity.

- Haptoglobin is a protein present in humans which forms a complex with free blood-plasma protein hemoglobin, allows degradative enzymes to access hemoglobin, and prevents the loss of iron through kidneys, protecting them from damage by hemoglobin. Mutations in this gene and/or its regulatory regions cause haptoglobinemia or hypohaptoglobinemia. Haptoglobin has carbohydrate moieties of Mannose, Galactose, N-acetyl galactosamine, N-acetyl glycosamine, N-acetylneuraminic acid (sialic acid) in various cross linking chains. Vesa Kaartinen et al[22] in 1988 have conducted experiments to remove the carbohydrate portions of Haptoglobin protein using *exo*-glycosidases and studied their binding capacity for hemoglobin. Removal of Sialic acid diminished the haptoglobin-hemoglobin complex formation to 15%. Further removal of 25% galactose residues diminished i.e. 40% of carbohydrate by weight resulted in complete loss of binding of hemoglobin by haptoglobin.
- Hepatic lipase (HL) is a secretory protein present in hepatocytes and bound to liver endothelium. The N-glycosylated carbohydrates are responsible for the catalytic activity of the hydrolysis of mono, di, tri glycerides and phosphoglycerides of circulating lipoproteins, very low density and high density lipoproteins[23] and important in lipid metabolism[24]. Rat Hepatic lipase has two N-glycosylation sites to Asn-57 and Asn-376 positions (similar humans have Asn-55 and Asn-374). This rHL N-glycosylation is a post translational step for the enzyme activity. Gisala Stankhe et al altered one of the glycosylation site by oligonucleotide directed mutagenesis and incorporated into the

native HL of rat and found that there is a decrease secretion of this protein[25] and in turn lipid metabolism affected.

B) Importance of glycosylation of sugars in plant kingdom:

Glycosylation in plants is also a post-translational modification[26] which have diverse functions in plants which mainly have important role in defense mechanisms. Few examples are quoted below:

- Pearce and Ryan[27] have isolated three hydroxyproline systemin (plant hormones) from tomato plants belonging to *Lycopersicon esculentum.* These proteins have hydroxyproline and are glycosylated by secretory pathways and are important in helping young tomato plants in defense signalling in wounded conditions (i.e cut by stem). Comparision of these peptides by synthesis without glycosylation showed 1000 times less activity in defense signalling in these plants when wounded than the glycosylated counterparts.
- Pearce et al[28] also isolated tobacco systemins which is a 18 peptide amino acid and hydroxyproline is glycosylated with L-arabinose. This peptide is important for cell-cell signalling of tobacco plants. Synthetic peptide without the appended carbohydrates is 10000 times less active than normal glycosylated peptide.
- Root hairs[29] are specialized cells which help in nutrients absorption and water for plants. Their cell walls are composed of hydroxy rich glycoproteins, Extensins and Arabinogalactan proteins. These proteins are all glycosylated. Velasquez et al[30] studied the implication of prolyl 4-hydroxylases in the cell walls of Arabidopsis Thaliana for proline hydroxylation and believed to be catalyzing the arabinosylation of these proteins.

Biochemical inhibition or genetic disruption of the enzyme reduced arabinosylation and prolyl hydroxylation. This shows that *O*-glycosylation is essential for root hair growth.

1.4 Hydroxyproline-rich glycoproteins (HRGPs) in living systems and their structural aspects: plants and animals

Hydroxyproline and L-arabinose (particularly arabinofuranose) are present in plant cell walls as hydroxyproline rich glycoproteins (HRGPs), secreted peptide hormones, and extensins. These have the functions to protect the plant cells, control reproductive system, carrying out specific functions like cell-cell signalling[31] etc.

1.4.1 Occurrence of HRGPs in animals and humans:

Humans and mammals do not have HRGPs as *O*-Glycosylation of Hydroxyproline is not seen in animals and humans. Hydroxyproline is present as chains with many other amino acids in the sequence glycine-proline-X and glycine-X-hydroxyproline where X is any other amino acid other than proline and hydroxyproline in collagens of living organisms. It is believed that hydroxyproline is important for the construction of the human body's structural protein, collagen. Deficiency in collagen synthesis by human body leads to easy bruising, breakdown of ligments, increased blood vessel damage etc. Proline hydroxylases present in liver of humans requires Vitamin C as co-factor[32] for hydroxylation of proline (which can be obtained from diet) in human body. So deficiency of Vitamin C causes a disease called scurvy and poor production of hydroxyproline and in some cases it is excreted[33] in large amounts from human body due to this imbalance. Also improper production of hydroxyproline leads to defective collagen which

leads to the previous effects. Though hydroxyproline does not have any therapeutic use and is a non-essential amino acid, it has indirect effects on human body.

1.4.2 HRGPs in plants:

HRGPs are present in extracellular matrix of the plant cell wall[3] discovered by Lamport[11] in 1971. They are responsible for the strength of the plant cell wall and as a result are often referred to as proteins of plant cell wall. These glycoproteins contain long chains of repetitive hydroxyproline motifs with various amounts of *O*-glycosidic linkages to *L*-arabinofuranose and D-galactopyranose. In plant kingdom, proline is hydroxylated by an enzyme called proline-4-hydroxylase[34] and it has the capacity of hydroxylating polyproline motifs. In most of the plants 4*R* hydroxyproline is formed. But unnatural 4*S* hyp isomer is rare but found to be in free state in plant sources like Santalum Album and reported in 1970 by Radhakrishnan[35] and Chinese researchers[36] in some species of India and Middle east Asian countries. It is not much known by these researchers about 4*S* Hyp whether it is in free or bound state and still investigations are going on.

The plant cell wall is the most important for carrying out the metabolic activities. It is the source for food storage, biosynthesis of compounds required for cell growth, expansion and reproduction. HRGPs are important components of the plant cell wall and regarded as the proteins of the plant cell wall. There are more than 50 different plant cell walls but all of them fall into type I and type II cell walls. Type I cell walls are found in grasses, mosses and some algae (non flowering plants). Most of the flowering plants have type II cell walls. These two

[3] Cell wall. (2014, June 16). In *Wikipedia, The Free Encyclopedia*. Retrieved 22:51, June 18, 2014, from http://en.wikipedia.org/w/index.php?title=Cell_wall&oldid=613121739 *and* http://www.ccrc.uga.edu/~mao/intro/ouline.htm

important models of plant cell wall have been assumed and studied by Carpita[37] in 1993 for the organization of these proteins and polysaccharides in plant cell wall. HRGPs location, composition and function may be understood by below brief description of structure and organization of plant cell walls. Plant cell walls of type II consists of 3 layers:

- **Middle lamella**: This is the first layer formed during cell division. It makes up the outer wall of the cell and is shared by adjacent cells. It is composed of mainly pectic compounds and some glycoproteins. These pectic compounds are polymers of hundreds of poly-galacturonic acids (PGAs) cross linked by Ca^{+2} or Mg^{+2} salt bridges[38]. These are found as insoluble gels and are soluble in water. These PGAs can exist as weak acids and are pH dependant and play an important role in ion balance in plants. Many of the carboxyl groups are methylated to prevent hydrolysis[37, 38b].
- **Primary wall**: This is formed after the middle lamella and consists of a rigid skeleton of cellulose microfibrils embedded in a gel-like matrix composed of pectic compounds, hemicellulose, and glycoproteins. Cellulose is a polymer of glucose-typically consisting of 1000 to 10000 β-D-glucose residues. These polymers associate through hydrogen bonding in large amounts and results in the formation of micro fibers. As the hydrogen bonds are enormous between the layers of cellulose, it gives necessary tensile strength to the plant cell wall. The primary wall of cultured sycamore cells[39] for example is comprised of pectic polysaccharides (30%), cross-linking glycans (hemicellulose; ca 25%), cellulose (15-30%) and protein (20%). The actual content of the wall components varies with species and age. All plant cells have a middle lamella and primary wall. Cell enlargement[38a, 40] occurs till all the components are held together in cell walls.

Secondary wall: It is formed after cell enlargement[38a] is completed. The secondary wall is extremely rigid and provides compression strength. They also play an important role in plant defense, reproductive systems and some special functions. It is made of cellulose, hemicellulose and lignin. The secondary wall is often layered in structure.

Thus the whole cell wall network is surrounded with a variety of structural proteins and glycoproteins and is partially cross-linked by aromatic substances[41] called lignols (monolignols). These lignols are believed to be formed by biosynthesis of enzymes by radical mechanisms in plants and are the cross-linking polymers of *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol. These provide strength to cell walls, facilitate water transport, and impede the degradation of wall polysaccharides, thus acting as a major line of defense against pathogens, insects, and other herbivores. So these lignols are the basis for the woody tissue of plants and serve as fuel.

This hemicellulose is a polysaccharide comprising of a variety of sugars including *β*-D-xylose, *α*-L-arabinose, *β*-D-mannose. In most of the plants, these xyloses and arabinoses are the only sugars present. So they are called as xyloglucans or arabinoglucans etc. Hemicelluloses are often branched with cross-linking galactose / xylose with arabinoses reported by Carpita[37]. These are very hydrophilic and form gel like structure. Hemicellulose is abundant in primary cell walls but is also found in secondary cell walls.

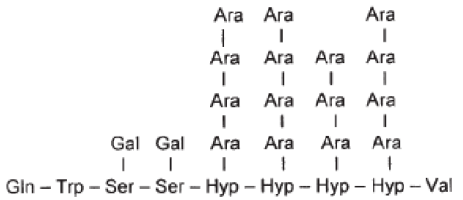
In addition to polysaccharide carbohydrates, cell walls contain a variety of glycoproteins in which various carbohydrates are linked to hydroxyproline, serine, threonine, alanine, tyrosine, tryptophan etc. These are called Hydroxyproline Rich Glycoproteins (HRGPs). In these

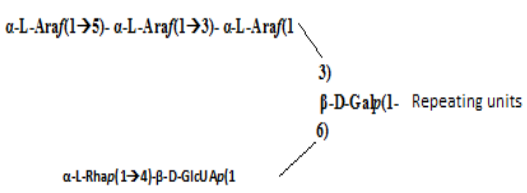
glycoproteins, the carbohydrate moiety weight is around 80% but different amounts of carbohydrates are present in different classes of HRGPs. All of these components contain many glycosylated and non-glycosylated hydroxyproline repeating motifs. In these proteins glycosylation is not well ascertained and investigations are going on. Hydroxyproline is less glycosylated in another structural cell wall protein called extensin, which is capable of forming covalent bonds with other extensin proteins through amino acid tyrosine. In these extensins, the tyrosines are evenly spaced and they wrap around other cell wall constituents like "knitting" the wall together. The amount of extension produced is dependent on mechanical wounding, infection and these responses are mediated by plant peptide hormones[42].

Enzymes needed for the biosynthesis or modification of the network (e.g peroxidase, xyloglucan, endo transglycosylase, α-fucosidase and glucanase) or for the modification of metabolites (invertase, phosphatase and ascorbic acid mutase) may be ionically or covalently bonded to the hemicelluloses network or the pectin matrix.

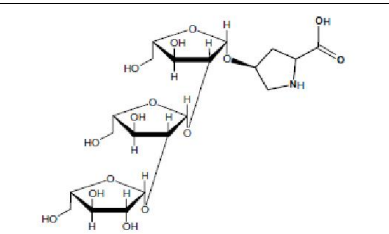
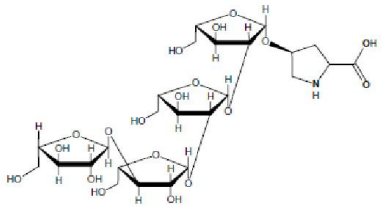
These HRGPs are broadly classified [43] into major four classes as follows and elucidated with applications in Table **2**:

Table 2: *Classes of HRGPs with their structural features, sequences in some plants and applications:*

S.No	Types of HRGPs	Structural Features	Example with peptide sequences	Uses / Applications
1	Extensins	Least glycosylated linear chains of Hyp, Serine as tetra and pentapeptide sequences as Ser-$(Hyp)_4$. Glycosylation is seen with *β*-L-Ara*f*(90%) and *β*-D-Gal*p*(10%) as Ser-D-Gal. Extensins exist as PPII helixes[44] with *α*-L-Araf glycosylation. These extensins are held together in cell walls by Iso-dityrosine linkages in plants like tomato[45], peanuts[46]	General Sequence-Hyp-(L-β-Ara*f*)$_{1\text{-}4}$[47] and Ser-Gal. Examples: a) SPPPPVKSPPPP- Maize[48] b) SPPPPYYYH- Rape[49], Soybean[50] c) SPPPPVP*SPPPPVA- Tomato[51] General sequence in Tomato Extensin  Sequence of *Chlamydomonas*[47, 52] of extensins by ^{13}C NMR is having Serine is linked to α-D-galactose and the Ser-$(Hyp)_4$ linkages are 2-*β* except the 4th residue is *α*-3 furanose linked.	a) Provide impenetrable physical barrier or immobilize the pathogens by binding to their surfaces. [43a] b) contribute to plant defense by providing protection against pathogens, elicitation and mechanical wounding[53].
2	Arabinogalactan Proteins[54]	Primarily O-linked with Hyp residues (90% to 98%) and proteins (1 to 10%). Linkages of (1-3)-*β*-D-		a) Plant growth (reproductive issues) and development [60]. By

		Gal*p* residues with substitutions of $C(O)_6$ by galactosyl side chains and terminate with α-L-Ara*f*, Rha*p* and Gal*p* residues[55]. β-Yariv reagents[67](brown-red coloured dyes with the generic structure of 1,3,5-tri-(*p*—glycosyloxyphenylazo)-2,4,5-trihydroxybenzene) used to separate protein from carbohydrate by binding β-forms of carbohydrate. This shows that AGPs contain(1-3)-β-Gal*p* and β-L-Arabinofuranose linkages[56]. These proteins have shapes of wattle blossom[57], twisted hairy rope[58], disc like ellipsoid[58] structures as in Gum Arabic isolated and as investigated in different parts of the plant. Ala-Hyp, Ser-Hyp, Thr-Hyp, Val-Pro, Gly-Pro etc. dipeptide motifs are also seen in AGPs.	General Sequence in *Nicotiana tabcuum*[59]  	RNA mutant experiments, non-glycosylated ones gave observed changes in leaf changes, stem size and underdeveloped. b) Inductive cell-cell signalling[61] interactions and controlling the fate of cells, vacuole development[62], Promoting pollen tube growth[63] c) Contributors to plant stem strength[64]. d) involved in Salt tolerance and normal root expansion[65] e) AGPs implicate the plant microbe interactions of *Agrobacterium tumefaciens.* Effects are seen by mutant changes in this plant where the plants became susceptible to attack by bacteria and decreased root growth [66]. f) AGPs are constituents of plant gums for species of Acacia Senegal which is used in candy industry

				due to low viscosity and helps in suspending flavours[67]. g) Useful in Cancer therapy too by enhancing cytotoxic activity by stimulating immune systems[68].
3	Proline Rich Proteins[43a]	Contain Proline(in major ~60%), Hydroxy proline with O-Glycosylation[69] with L-arabinose (constitutes only 3% of the mass) Tyrosine is present in these proteins which make them protected from oxidative stress responding to wounding. These proteins are mainly constituents of xylem and protoxylem tissues of cell wall.	Not much is known for these proteins as they do not get precipitated by Yariv Reagents. They don't have Ser-$(Pro)_4$ motifs. These are least glycosylated than Extensins. The glycosylation is around 3% only with L-Arabinofuranose.	a) They may act as nucleation sites for lignin deposition. b) Function as structural proteins for plants c) Prevent infection caused by mycorrhizal fungi in maize[70] and other pathogens like bacteria. d) Help in plant defense by peroxide mediative cross linking in bean and soyabean plant cells after wounding for 3 min of injury[71].
4	Glycopeptide plant hormones[31, 42, 72]	Recently some researchers found that there are also other secreted peptide components of cell wall which are important for plant growth regulation, root nodule development, cell-cell	One of the compound is isolated from tomato called Systemin in 2003 by Pearce and Ryan[27]	a) Help in defense response to attack of pathogens[31]. b) Cell proliferation and expansion c) Stem cell maintenance

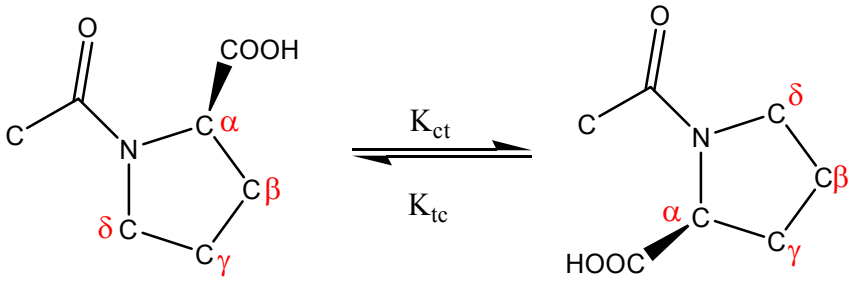
		interaction[62] etc. These are Glycopeptide hormones. These contain small chains of Hydroxy proline and L-arabinoses linkages in short chains like trioses and tetroses along with other amino acids.	  Peptide sequence in Tomato plants: a) RTOYKTOOOOTSSSOTHQ- b) GRHDSVLPOOSOKTD- c) GRHDYVASOOOOKPQ- d) DY(SO_3H)GDPSANKHDPGV[(L-Ara)$_3$]HHS e) RTVHSG[(L-Ara)$_3$]HDPLHHH *Where H and H represents Hydroxy Proline*	

Amino acid codes: *S-Ser, P-Pro, R-Arg, G-Gly, V-Val, L-Leu, O-Orn, H-Hyp, N-Asn, D-Asp, T-Thr*

1.5 Structural and conformational effects of hydroxyproline glycosylation.

Before introducing structural effects of hydroxyproline, let's examine about proline's unique properties. Proline is a unique amino acid and its side chain is cyclised onto the peptide backbone of Nitrogen. As it is a secondary amine, it has special properties when present in a polypeptide. The pyrrolidine ring does not restrict the movement of the atoms in the proline side chain. The φ angle is fixed at -75° where as in all other amino acids free rotation is possible around peptide bond. The amide bonds of the amino acids in a polypeptide chain have pseudo double bond character; through resonance stabilization, π-electrons are delocalised across the amide bond, induces a planar ω-torsional angle of the amide bond. This creates two distinct amide conformations with similar energy which are called *cis* and *trans* conformers.[73]

Figure 12: Origin of *cis* - *trans* rotamers in proline around peptide bond



Prolyl *cis* rotamer **Prolyl *trans* rotamer**

The energy difference of *cis* and *trans* rotamers of proline is less due to the similar electronic environment of α and δ carbon for N-terminal amino acid carbon. This is also due to the favourable n-π* interaction from the lone pair on the prolyl N-terminal amide carbonyl oxygen to the antibonding orbital of the prolyl C-terminyl carbonyl carbon[74] (Figure **13)**.

Thus leading to cis-trans rotamer conversion for the proline related amino acids at RT slowly and high temperature (50 - 70°C) rapidly as its rate constants can be measured by NMR experiments like Inverse Magnetization Transfer[75]. This isomerization plays an important role in folding paths of proteins in biological systems[76].

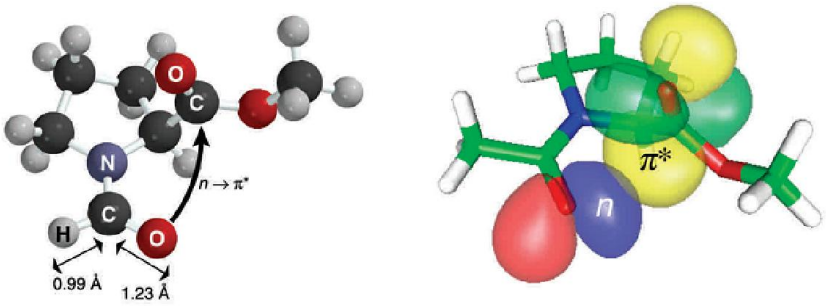


Figure A

Figure B

Figure 13: Shows the A) proposed n→π* electrostatic interaction present in the *trans* amide of N-formyl-L-proline methyl ester. B) Depiction of the n and π* molecular orbitals [Reproduced with permission, *Prot. Sci*. **2003**, *12*(6), 1188-1194. Copyright 2003 Wiley-VCH]

The N-terminal amide isomerisation of proline in peptides and proteins is slow when compared to other amino acids. Some model peptides like alanine-phenylalanine bond, the rate constants for *cis-trans* isomerisation are 0.05 sec^{-1}($k_{trans \rightarrow cis}$) and 2.3 sec^{-1}($k_{cis \rightarrow trans}$) in water at 25°C[77]. Similarly for alanine-proline amide bond 0.001 sec^{-1}($k_{trans \rightarrow cis}$) and 0.005$sec^{-1}$($k_{cis \rightarrow trans}$) in water[78] at 25°C respectively. By these results, there is a preference for *trans rotamer* amide stabilization. For proline peptide in Captropril, an ACE inhibitor useful for congenitive heart disease, the rates constants of cis trans rotamers are interconvertible at

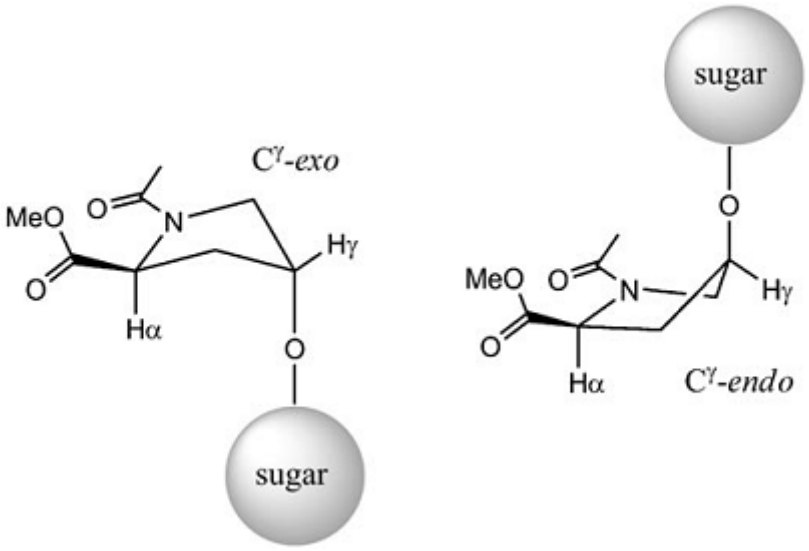
slightly above room temperature and the rate constants are measured by Inverse Magnetization Transfer NMR experiments[75]. These have an impact in protein folding in biological systems[79].

Proline *cis-trans* isomerisation around prolyl amide bond influences the gated ion channel of 5-hydroxy tryptamine receptors[80]. These peptides have proline at 8 position and which folds into two strands, which are held by hydrogen bonding. As proline has two conformers *cis* and *trans* interconverting, the *cis* form will allow the ion channels to pass through and the *trans* form does not[81]. Due to the less energy gap between *cis* and *trans* forms, this protein shuttles the ion transfer from time to time. Proline and hydroxyproline exhibit cis-trans isomerizations which have implications in biology.

1.5.1 Ring Puckers of Hydroxyproline:

Hydroxy proline a small unique modification of Proline with OH group is an important amino acid which gives the strength to collagens in mammals by stabilization of triple helix structure due to hydrogen bonding. Hydroxyproline has two geometrical isomers which are 4*R* and 4*S* forms (Hyp and hyp). These isomers adopt two conformations called exo and endo by C^{γ} bond found by some of the researchers in nature. But only Hyp form is seen in plants predominantly and not cis form. 4*S* hyp is seen only in few plants like *Santalum Album* and *Lyngbia Majuscala*. Exo form is seen in Hyp and *endo*- in hyp as found by many researchers even after glycosylation with sugars of the Hydroxyl group. Exo and endo configurations are judged by ^{3}J coupling constants and arise due to gauche configuration between peptide bonds. Structures of exo and endo forms of *O*-glycosylated Hydroxyproline is shown in Figure **14**

Figure **14**: *Exo and endo forms of Hydroxyproline from the permission of the journal*[82].

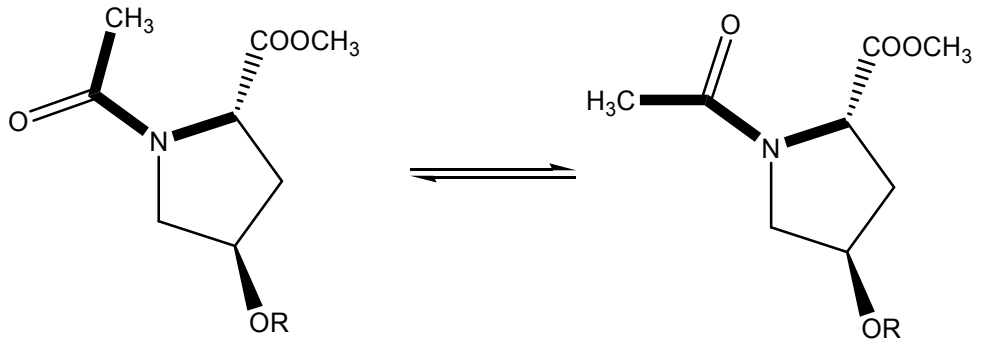


These ^{3}Jcoupling constants are calculated by NUMMRIT algorithm from the ^{1}H spectra by spinworks 3.0. As per literature, for exo configuration, the $J_{\alpha\beta1}$ and $J_{\alpha\beta2}$ of Hydroxyproline coupling constants should be from 7-11 and 9-10 Hz. For endo 7-11 and 2-4 Hz. Another method of finding the coupling constants is by decoupling methods of particular system to get the exact coupling constant of the other.

Each glycosylated Hyp or hyp have two rotamers cis and trans which are interconvertible at room temperature and faster at higher temperature i.e. 67°C. The rate of conversion of cis to trans forms the basis of this thesis. The *trans* and *cis* rotamer form of Hyp and hyp are given below based on the information in the journal[26].

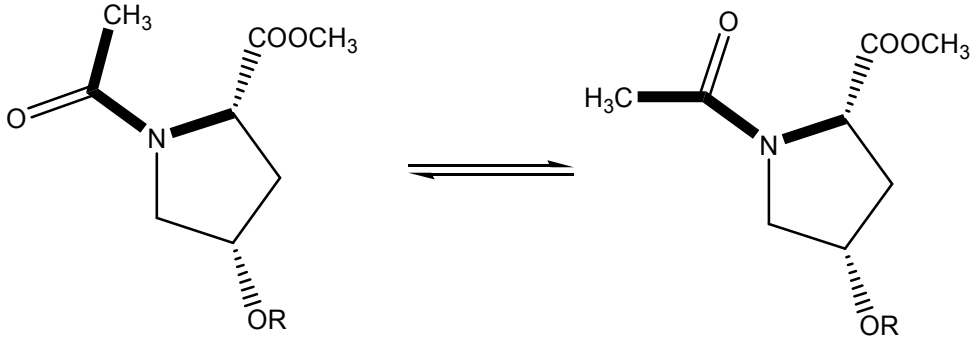
Figure **15**: *Rotamers of hydroxyproline: cis and trans*

(2S, 4R) Hyp



cis ***trans***

(2S, 4S) hyp



cis ***trans***

R=H, D-Gal*p*, L-Ara*f*

1.7 Techniques used in laboratory experiments: Introduction

The most important techniques for the study are NMR of synthesized molecules, nuclear overhauser effect (nOe) for finding out the configuration of molecules and major rotamer present in the molecule. Magnetization transfer experiments were carried out for finding out the rate constants of the *cis*, *trans* isomerisation around the peptide N-acetyl bond.

^{13}C inverse gated coupling of NMR is applied to find out the percentage of minor rotamer in the molecule. Each section will be discussed in brief with some examples.

1.7.1 Nuclear Overhauser effect technique by NMR for finding out the configurations of sugars and population of rotamers around the peptide bond:

Nuclear Overhauser effect is a common phenomenon of NMR which is a special technique employed for various purposes like finding out the stereoisomers, finding out the neighbouring groups of the molecule, its conformation[83] etc. Also in carbohydrates, it is a very useful tool for finding out the configuration of α and β anomers. For assigning the configuration of α or β anomers are judged by the 1D NOE of neighbouring protons when anomeric proton of the sugar is irradiated. In my research, for all arabinosylated hydroxyproline building blocks, irradiation of anomeric protons was performed and compared the resonances of neighbouring protons for assignment of α and β. Dr Mario Pinto and his co-workers established the monoclonal antibody strep 9 selects a local minimum conformation of Streptococcus group A trisaccharide-hapten[84] by NOE. The structure and the NOE resonances are depicted in **Figure 16**:

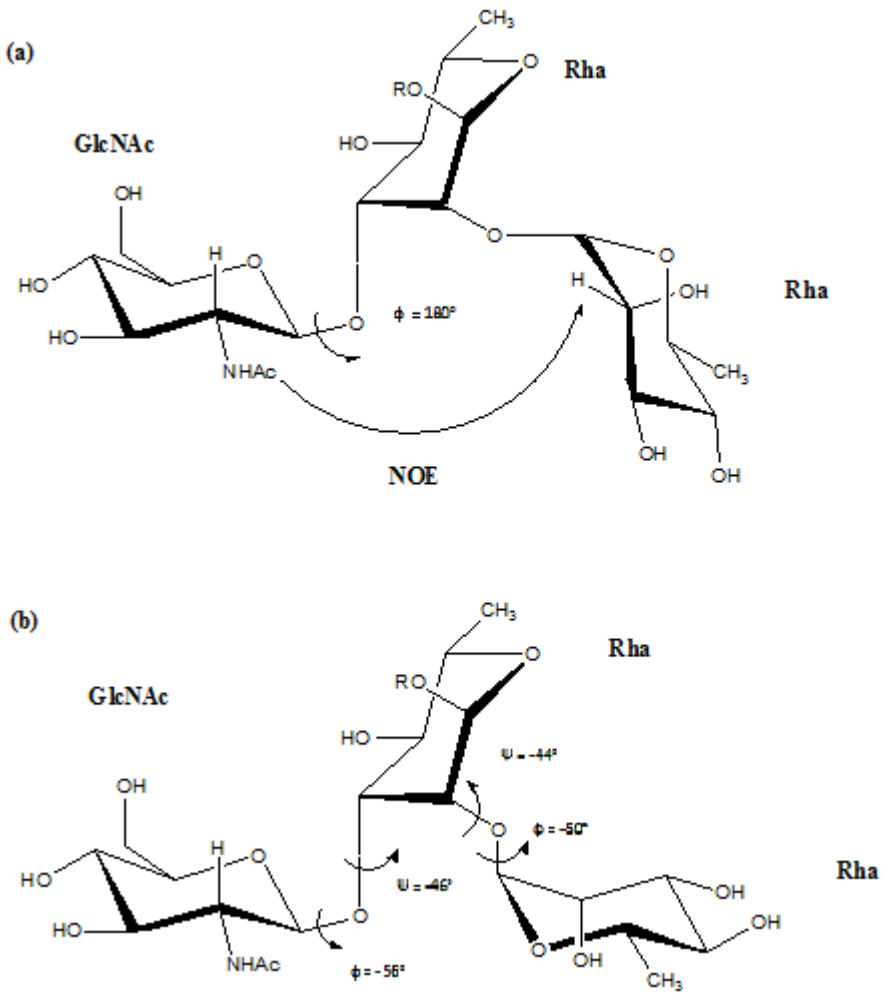


Figure 16: *Schematic diagram of Streptococcus Group A trisaccharide-hapten[84]. (a) Conformation of β-(1→3)-glycosidic linkage that accounts for the interglycosidic NOE seen. This conformation present in aqueous solution and not bound by monoclonal antibody Strep 9. (b) The conformation of the trisaccharide when bound to antibody (NOE of interglycosidic linkage not seen)*

The conformational analysis of Streptococcus Group A repeating-trisaccharide derivative of propyl 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-*O*-(*α*-L-Rhamnopyranosyl)-*α*-L-Rhamnopyranoside bound to the monoclonal antibody Strep 9 by NOE studies (long range) reveals that NOE of interglycosidic bond is not seen in bound state (Figure **16b**) and seen in aqueous solution (Figure **16a**). As a result, authors can

predict the conformation due to minimum energy conformation of the tri-saccharide to be the ψ angle changes at α-(1→2)-glycosidic linkage from its preferred + gauche orientation to – gauche orientation. The interglycosidic NOE is not seen in the bound trisaccharide complex as it is energetically disfavoured conformation. This example shows that the conformation of the carbohydrates is possible to find out for some extent by using NOE along with configuration.

1.7.2 ^{13}C Inverse gated coupling: A quantitative experiment to find out the minor rotamer percentage

Carbohydrates give complex ^{1}H NMR which most of the signals get overlapped for 5 membered furanose rings. So for finding out the rotamer population, ^{13}C quantitative experiments[85] are needed to find out the exact percentage within experimental error. So ^{13}C inverse gated coupling is one of the methods which may give accurate information about the percentage of rotamers. Some researchers applied this NMR technique for analyzing carbohydrate compositions in Honey[86] and lignin[87] samples. ^{13}C Inverse gated coupling is suitable for characterizing the lignin is due to following reasons[87]: (i) It provides the nature of all carbons of the molecule. (ii) ^{13}C spectra is not complicated by spin-spin coupling effects when decoupler is made off and gives rise to single peaks for each and every single carbon atom. (iii) ^{13}C spectra have a wider range at 500MHz. Also routine ^{13}C NMR does not lead to quantitative experiments because when proton decoupling is applied during both the relaxation delay and the acquisition period, the signal intensities do not correspond to the actual number of atoms due to nuclear Overhauser effects (nOe). To obtain a quantitative ^{13}C NMR spectrum, an inverse gated proton decoupling needs to be applied to minimize the nOe effect. In addition, the relaxation times delay must be set at least 5 times longer than the ^{13}C longitudinal

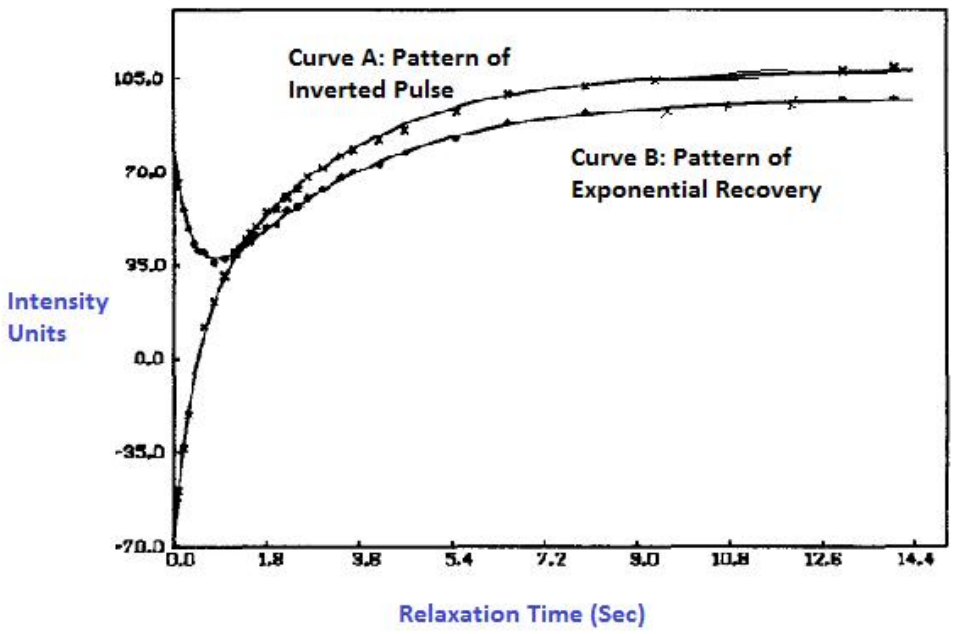
relaxation time. The outcome of this experiment is that the spectrum converts all carbons which are major to a nearly integral of same value and other minor peaks of carbons can be integrated. So these minor carbon percentages are averaged and adjusted with standard deviation to get the exact percentage of *cis* and *trans* rotamers in the compound. So this technique was employed in my Master's Research. It is a time consuming method as to one experiment takes about 13 hrs, but useful information can be obtained from this NMR experiment.

1.7.3 Inverse Magnetization experiment for finding out the rate constants of cis / trans isomerization around the peptide bond:

Inverse magnetization transfer experiment [88] of NMR is mostly used for finding out the rate constants of two isomers or rotamers exchanging within NMR time scale. Magnetization transfer experiments have a wide range of scope in peptide chemistry where the rotamer population was important, in structural biology[89], in medicine for knowing the malignant tumours of Breast Cancer[90]. This technique is referred as Soft Pulse Technique (SPT)[91] and applied to systems of any chemical systems and both populated isomers exist and capable of exchanging from one conformer to another. The experiment generally consists of a soft weak pulse of r_f of length for one of the exchanged-couple resonances to be selectively inverted. Then the response of the second conformer is studied after a variable delay (T_1) (longitudinal relaxation times) using pulse Fourier transform experiments. T_1 is characteristic of some conformers found by some researchers. Mariappan[92] has successfully demonstrated the calculation of rate constants of peptide *cis-trans* isomerization by Fourier Transform calculations. This experiment can be done using the pulse sequence to be inverted at certain temperature mostly at 67^0C on the *trans* amide singlet to see the effect of *cis* amide singlet in the isomerization. The

inverted pulse (Curve A) will be in the form of a parabola which after sometime exponentially comes back to its original position as a function of time. This affects the other rotamer recovery which is in the form of Morse curve (Curve B), initially decreases and increases till total restoration also by the function of time (Figure **17**). Thus the time required for conversion of one rotamer to another is the function of these two curves. These two curves have mathematical equations which on calculation by Mathematica program gives the values of rate constants as the function of time which is the relaxation times for conversion of one rotamer to another (*cis* to *trans*). Calculation of these parameters is depicted below in brief with references.

Figure 17[91]: *Pattern of Inverse Magnetization experiment by NMR : Curve A: Pattern of the inverted pulse. Curve B: Pattern of the exponential recovery of another conformer.*



Calculation of rate constants:

The calculation of rate constants are described in brief as per the work of J. R. Alger and J. R. Prestegard[91]:

The data can be quantitatively analyzed using a set of modified Bloch equations developed by McConnel[93]. The description for the calculation is as follows developed by Dr Joe O Neil in Mathematica program to evaluate these rate constants of cis-trans isomerization.

The time dependant magnetization transfers of the *cis* ($M_c(t)$) and *trans* ($M_t(t)$) NMR signals as a function of the inversion transfer time (t) were simultaneously fit to equations[153,109] 1 and 2 below for compounds mentioned above using Mathematica (v.7.0). In the following pulse sequence, the ^1H *trans* resonance is selectively inverted using a shaped pulse. Its recovery during t is determined by its intrinsic T_{1t}, magnetization transfer to and from the *cis* resonance, and the T_{1c} of the *cis* resonance.

π(x)sel----------t----------π/2(x,y,-x,-y)—acquire

The resonances of the *trans* and *cis* isomers show the following time dependencies by the solutions of the equations of the curves;

$$M_t(t) = c_1\tau_t(\lambda_1+1/\tau_{1c})e^{\lambda_1 t} + c_2\,\tau_c\,(\lambda_2+1/\tau_{1c})e^{\lambda_2 t} + M_{c\infty} \text{---------- 1}$$

$$M_t(t) = c_1e^{\lambda_1 t} + c_2e^{\lambda_2 t} + M_{t\infty} \text{---------- 2}$$

T_{1c} and T_{1t} are the longitudinal relaxation times of the resonances in the absence of exchange which are measured during the experiment.

τ_c and τ_t are the lifetimes of the *cis* and *trans* conformers and k_{ct} and k_{tc} are the corresponding rate constants.

τ_{1c} and τ_{1t} are the effective relaxation times of the *cis* and *trans* resonances when relaxation and exchange are both occurring and are defined below in terms of $T_{1c}$and τ_c, T_{1t} and τ_t.

λ_1 and λ_2 are related to the time constants τ_c, τ_t, τ_{1c}, and τ_{1t}, and are defined below.

c_1 and c_2 are defined below.

$M_{c\infty}$ and $M_{t\infty}$ are determined experimentally from the magnetization measured after 5 T_1 periods for the *cis* and *trans* resonances, respectively.

Mathematica program then calculates τ_t from τ_c, $M_{c\infty}$, and $M_{t\infty}$ as: $\tau_t = \tau_c * (M_{t\infty}/M_{c\infty})$

Thus,

$k_{ct} = 1/\tau_c$

$k_{tc} = 1/\tau_t$

$K_{eq} = M_{t\infty}/M_{c\infty}$

$\tau_{1c} = (T_{1c} * \tau_c)/(\tau_c + T_{1c})$

$\tau_{1t} = (T_{1t} * \tau_t)/(\tau_t + T_{1t})$

$\lambda_1 = ½\{-(1/\tau_{1c} + 1/\tau_{1t}) + [(1/\tau_{1c} + 1/\tau_{1t})^2 - 4(1/\tau_{1t}\tau_{1c} - 1/\tau_c\tau_t)^{½}\}$

$\lambda_2 = ½\{-(1/\tau_{1c} + 1/\tau_{1t}) + [(1/\tau_{1c} + 1/\tau_{1t})^2 - 4(1/\tau_{1t}\tau_{1c} - 1/\tau_c\tau_t)^{½}\}$

$c2 = 1/((\tau_c)(\lambda_1 - \lambda_2))[\tau_c(\lambda_1 + 1/\tau_{1t})(M_{0t} - M_{t\infty}) + (M_{c\infty} - M_{0c})$

$c_1 = M_{0t} - M_{t\infty} - c_2$

Thus all the values are calculated from the individual values and put into the equations by Mathematica program.

Chapter 2: Thesis Objectives

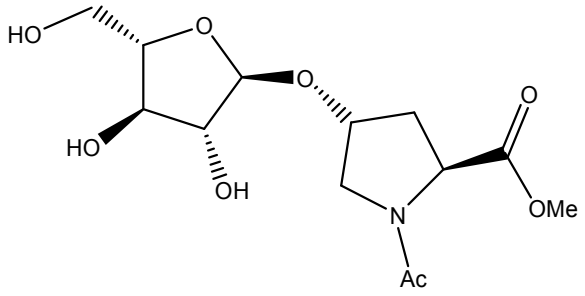
Hydroxyproline (Hyp) is found as structural proteins in plants and animals, special proteins of the cell wall of plants. In contrast, 4S-hydroxyproline (hyp) is very rare in nature but found to be present freely in nature in *Santalum Album*[35b, 36b]. The purpose of this thesis is to study the effects of L-arabinosylation of hydroxyproline in plant-derived glycopeptides. The ultimate goal of the thesis is to provide deeper insight into the roles of L-arabinosylation in plants. This requires:

- Synthesis of L-arabinosylated hydroxyproline model amides that serve as glycopeptide models.
- Exploration of the thermodynamic and kinetic parameters of hydroxyprolyl *cis*/*trans* isomerization in the glycopeptide models.

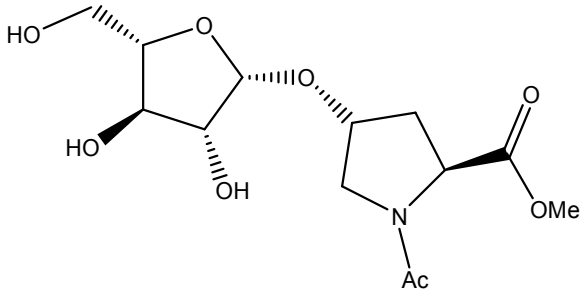
Chapter 3: Experimental Details: Effects of L-arabinofuranose glycosylation of (2*S*, 4*R*)-4-hydroxyproline and (2*S*, 4*S*)-4-hydroxyproline on the conformation, thermodynamic and kinetic of prolyl amide isomerization

3.1 Synthesis:

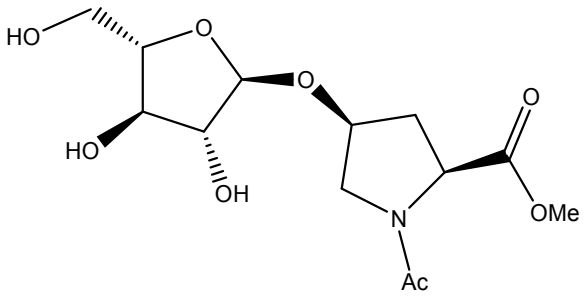
The synthesis of the target compounds **7a**, **7b**, **12a** and **12b** is shown in Figure **18**:



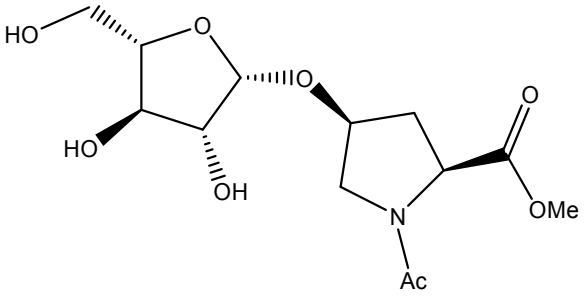
7a: 2,3,5 Triol-arab-f-NAc-Trans-alpha-Hyp-OMe



7b: 2,3,5 Triol-arab-f-NAc-Trans-beta-Hyp-OMe



12a: 2,3,5 Triol-arab-f-NAc-Cis-alpha-hyp-OMe



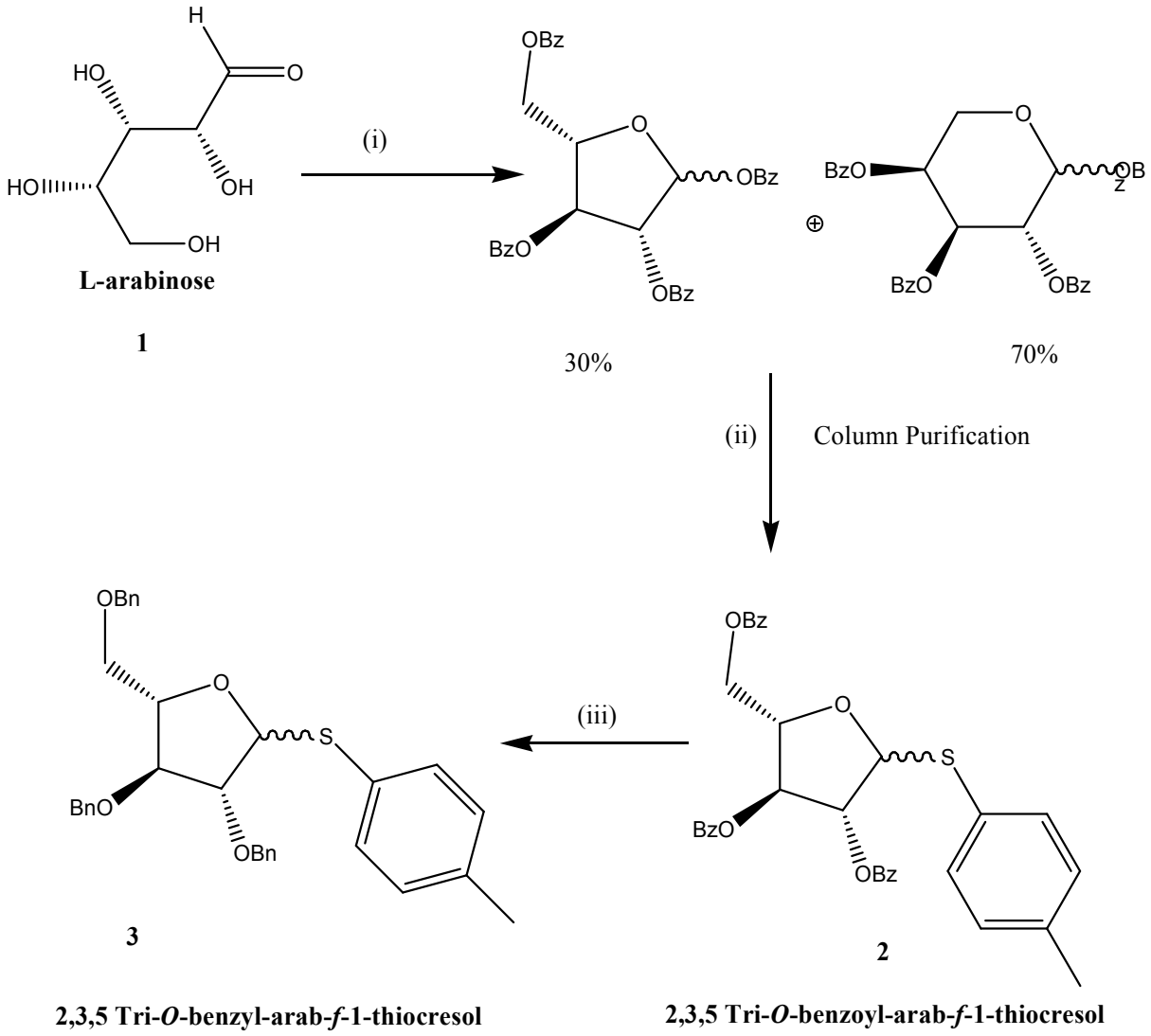
12b: 2,3,5 Triol-arab-f-NAc-Cis-beta-hyp-OMe

Figure 18: *Synthesis of mono-glycosylated L-Araf-hyp building blocks for study*

These compounds are related to D-galactopyranosylated hydroxyproline model amides in our group's previous research[82]. The synthesis of these compounds in outlined in Schemes **1-3**. The carbohydrate donor 2, 3, 5 Tri-*O*-benzyl-arab-*f*-1-thiocresol (**3**) was prepared

according to **Scheme 1**. L-arabinose (**1**) is tetra-benzoylated at 60°C using pyridine and excess of benzoylchloride. Then the crude mixture was reacted with borontrifluoride diethyl etherate ($BF_3{\cdot}Et_2O$) and *p*-thiocresol in CH_2Cl_2 at 0-5°C to afford a mixture containing protected furanose- and pyranose-based L-arabinose. Separation was achieved by careful column purification performed using 12% EtOAc in hexane to produce desired thioglycoside **3** in 29% yields. The benzoylated furanose product was debenzoylated using NaOMe / MeOH and further benzylated using benzylbromide, sodiumhydride in DMF to afford the required glycosyl donor **3** in 45% yield.

Scheme 1: *Preparation of 2, 3, 5 Tri-O-benzyl-arab-f-1-thiocresol* ***3:***

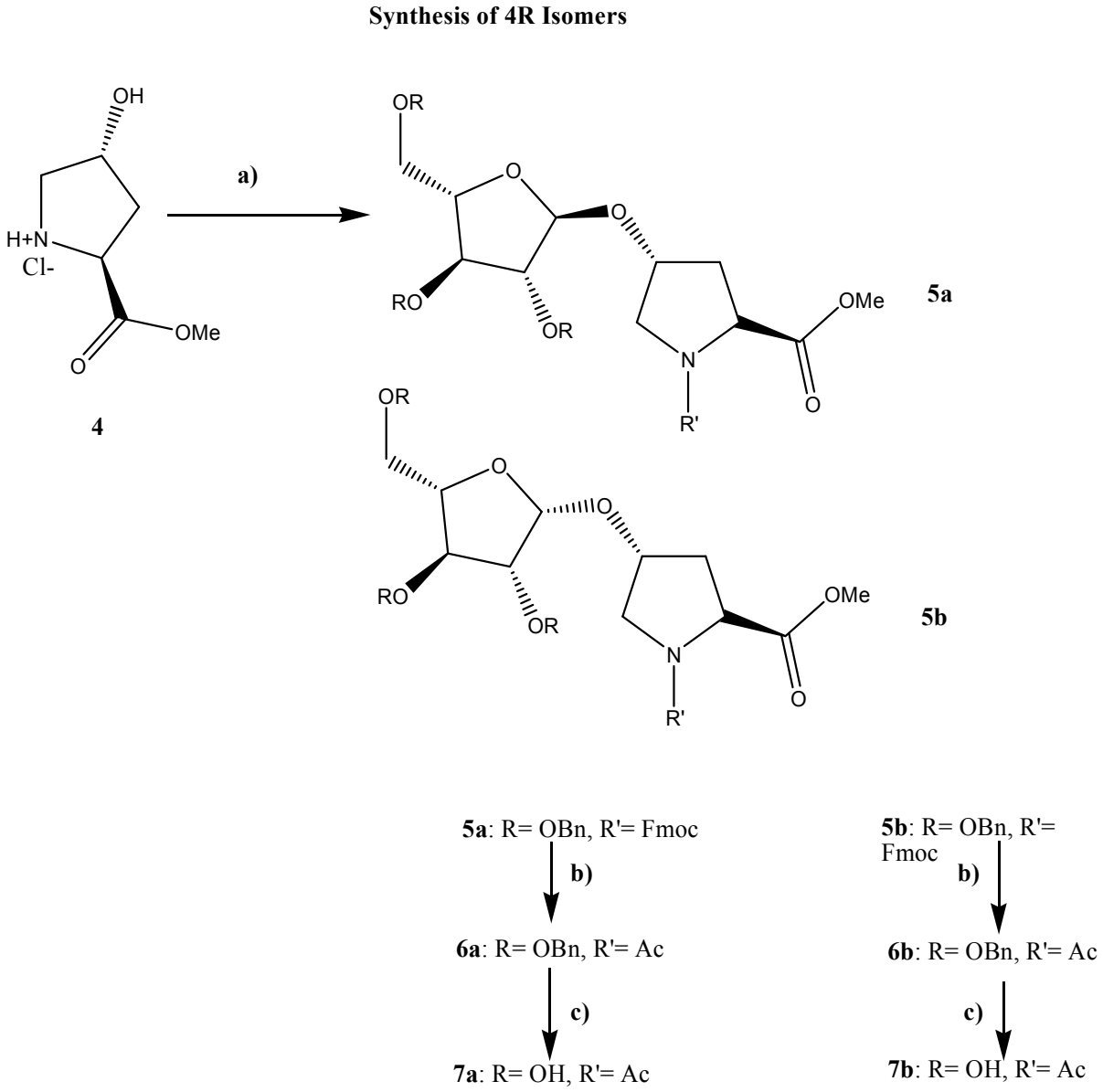


Scheme 1: *Synthesis of thio glycosyl donor* ***3****:* (i) BzCl, Py, 60°C, 3h, quantitative; (ii) $BF_3.Et_2O$, *p*-thiocresol, CH_2Cl_2, 0-5°C, 10h, quantitative; (iii) (a) NaOMe /MeOH, Amberlite strong H^+ resin, (b) BnBr, NaH, DMF, 0-5°C through RT overnight.

Model peptides of the form, N-Acetyl-Pro-OMe are well established for studying subtle effects of modification of the prolyl side chain on N-terminal amide isomerization[94]. This procedure avoids hydrogen bonding between the molecules as methyl esters do not function as hydrogen bond donors than N-Methyl amides. Hydrogen bonding in these building blocks may interfere in the equilibrium and rate constants of cis-trans isomerization while evaluation and change them.

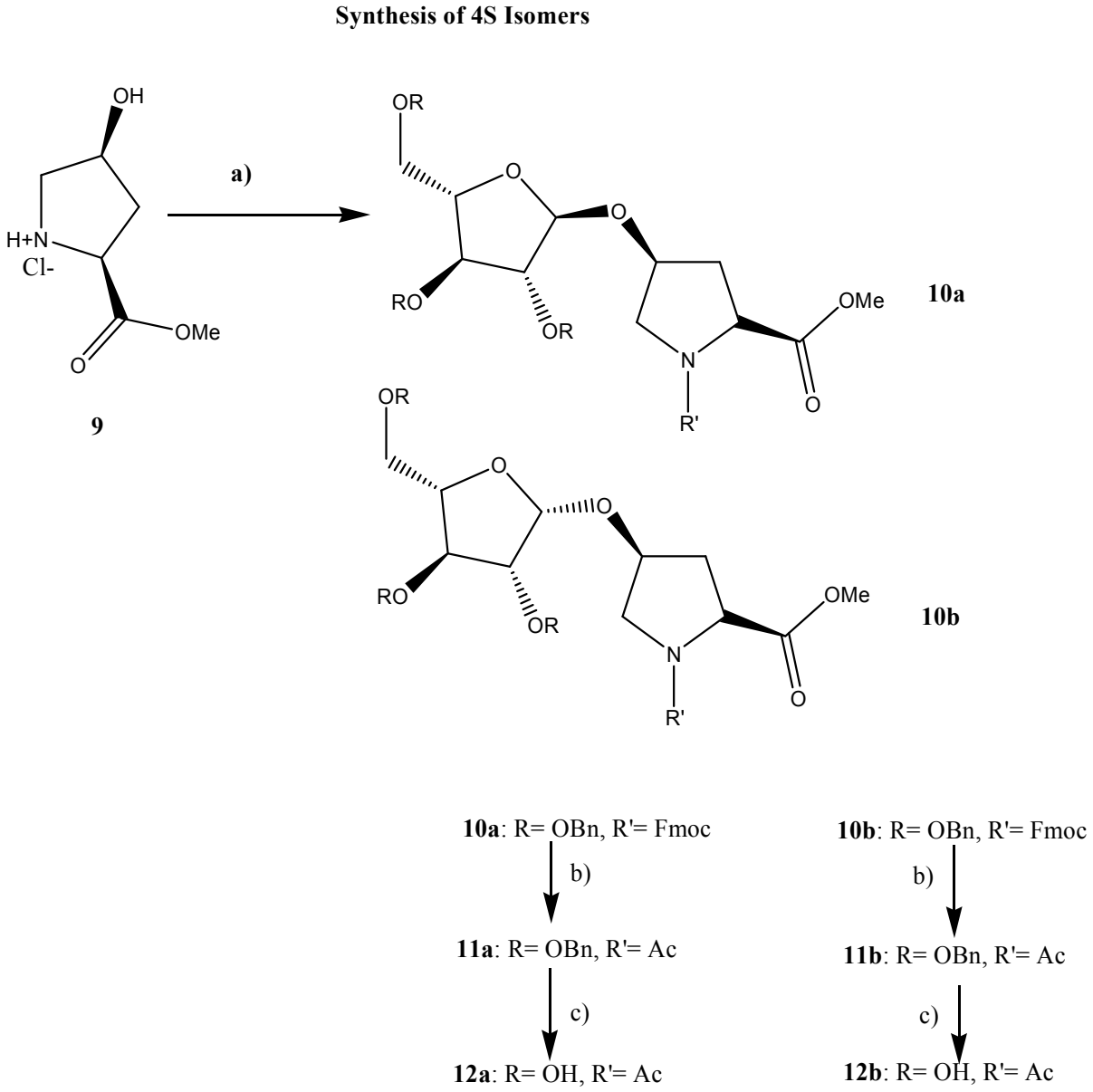
From Schemes **2** and **3** the glycosylated 4R-hydroxyproline model peptides **5a**, **5b**, **10a**, **10b** were obtained through Fmoc protection under mild basic conditions using Fmoc-Cl / $NaHCO_3$ in aqueous dioxane, followed by glycosylation using 2,3,5 tri-*O*-benzyl-arab-*f*-1-thiocresol in the presence of *N*-iodosuccinamide (NIS), silver triflate(AgOTf) in acetonitrile to yield *α*-**5a**, *β*-**5b** and *α*-**10a**, *β*-**10b** in 25% and 24% of both steps. The anomeric ratio of obtained compounds were 7:3 (α:β) in both cases. Incorporation of *N*-acetyl group was carried out by Fmoc-deprotection using piperidine in DMF followed by acylation using acetic anhydride and pyridine to give **6a**, **6b** & **11a**, **11b** in 80% yield. Removal of benzyl ether protecting groups was carried out by catalytic hydrogenation in methanol using $Pd(OH)_2$-C (10% catalyst loading) to afford model peptides **7a**, **7b** and **12a**, **12b** with 27% and 30% yields, respectively. The low overall yields of glycopeptides **12a** and **12b** required the synthesis of large amounts of starting materials as much of the material is lost in anomeric mixtures separation and isolation of pure anomers.

Scheme 2: *Synthesis of Ac-Hyp(O-α-L-Araf)-OMe, Ac-Hyp(O-β-L-Araf)-OMe and Ac-Hyp-OMe:*



Scheme 2: *Synthesis of **7a, 7b:*** a) (i)FmocCl, $NaHCO_3$, 1,4-Dioxane/H_2O(1:1), 25°C, 3h. (ii) 2,3,5 Tri-O-benzyl-L-arab-f-1-thiocresol (**3**)(prepared from Scheme **1**), MeCN, AgOTf, NIS, 0-25°C, 2h, 65% overall yield; b)(i) DMF/Piperidine (1:0.2), 25°C, 2h (ii)Ac_2O, Py, 25°C, 10h, 50% overall yield; c) H_2/$Pd(OH)_2$-C, MeOH, 25°C, 75% overall yield.

Scheme 3: *Synthesis of Ac-hyp(O-α-L-Araf)-OMe, Ac-hyp(O-β-L-Araf)-OMe and Ac-hyp-OMe:*



Scheme 3: *Synthesis of **12a** and **12b**:* a) (i)FmocCl, $NaHCO_3$, 1,4-Dioxane/H_2O(1:1), 25°C, 3h. (ii) 2,3,5 Tri-*O*-benzyl-*β*-L-arab-f-1-thiocresol(prepared from S**cheme 2**), MeCN, AgOTf, NIS, 0-25°C, 2h, 36% overall yield; b)(i) DMF/Piperidine(1:0.2), 25°C, 2h (ii)Ac_2O, Py, 25°C, 10h, 75% overall yield; (c) H_2/$Pd(OH)_2$-C, MeOH, 25°C, quantitative.

3.2 Results:

3.2.1 NMR Spectroscopic studies:

Full assignment of all the compounds was done by HSQC and COSY experiments. Assignment of the major rotamer is *trans* for all the isomers are confirmed by ^{13}C chemical shifts. As per literature, the major rotamer (trans) has the higher chemical shift of C^{δ} carbon than the minor[95] (cis rotamer). These results are tabulated below:

Table **3:** *Table showing the chemical shifts of C^{δ} carbon chemical shifts of **7a, 7b, 12a, 12b***

Compound	C^{δ} Chemical shifts (ppm)	Assignment
7a	61.17 (Minor 61.05)	Major is *trans* rotamer
7b	63.11 (Minor overlapped)	Major is *trans* rotamer
12a	61.14 (Minor 61.1)	Major is *trans* rotamer
12b	63.23 (Minor 63.21)	Major is *trans* rotamer

Final compounds **7a**, **7b**, **12a**, **12b** are studied in D_2O using DSS (4, 4-Dimethyl-4-silapentane-1-sulfonic acid 50 mM) as NMR reference standard and stabilized by phosphate buffer solution of pH 7.2.

Table 4*: NOE studies:* The NOE studies after irradiating the H_1 of the anomeric carbons of **7a**, **7b**, **12a**, **12b** and results are tabulated below indicating the rotameric state:

Compound	**nOe interactions**
(7a)	H_α-0.69%, H_γ-1.14%, H_2-0.74%, H_3-0.3%, H_4-0.09%, H_{5a}-0%, $H_{\beta1}$-0.69%, $H_{\beta2}$-0%, N-$COCH_3$-0%
(7b)	H_α-0.46%, H_γ-1.36%, H_2-2.23%, H_3-0.12%, H_4-0.13%, H_{5a}-0.22%, H_{5b}-0.53%, H_δ-0.08%, $H_{\beta1}$-0.2%, $H_{\beta2}$-0.03%, N-$COCH_3$-0.03%
(12a)	H_α-0.62%, H_γ-0.94%, H_2-0.6%, H_3-0.16%, H_4-0.24%, H_{5a}-0.34%, $H_{\beta1}$-0.84%, N-$COCH_3$-0.13%
(12b)	H_α-0.4%, H_γ-1.4%, H_2-2.52%, H_3-0.13%, H_4-0.19%, H_{5a}-0.54%, $H_{\beta1}$-0.51%, $H_{\beta2}$-1.16%, N-$COCH_3$-0.04%

The assignment of α- and β- anomer was judged by two methods. First method is by the measurement of $^3J_{H1,H2}$ coupling constant. The α-anomer shows a coupling constant of 1.6 Hz while the β-anomer shows a coupling constant of 4.6 Hz in both cases for Hyp and hyp. These results are further confirmed by similar compounds (p-Nitrophenyl β-arabinofuranosides with α-isomer with coupling constant 1.6 Hz, and β-isomer with coupling constant 4.3 Hz) synthesized by Dr Kaeothip[96] and co-workers. Second method is using ^{1}H-NMR NOEs. Irradiation of H-1 in **7a** leads to 1.14% and 0.74% NOE of H_γ and H_2, respectively while irradiation of H-1 in **7b** leads to observable NOEs of 1.36% and 2.23% for H_γ and H_2, respectively. The larger NOE effect observed for H_2 in **7b** indicates that this compound is the β-anomer in which H_1 and H_2 have a *cis* relationship. When the same

analysis is applied to compounds **12a** and **12b**, NOE resonances of H_1 and H_2 of **12b** are greater than **12a** which indicates **12b** has vicinal protons which exhibit cis relationship and confirms β-anomer (Table **5**). Our results are consistent with a previous analysis by R.A. Hoffmann. [98] Table 3 shows relevant NOE data to assign the anomeric configuration in compounds **7a**, **7b**, **12a**, and **12b** while **Table 4** contains all observable NOE effects when H_1 is irradiated in compounds **7a**, **7b**, **12a**, and **12b**.

Table 5: Showing the NOE interactions of H_γ with ^1H (anomeric carbon) from spectra

Compound	**NOE interactions of H_γ and H_2**	**Assignment**
7a	H_γ-1.14%, H_2- 0.74%	α-isomer
7b	Hγ-1.36%, H_2–2.23%	β-isomer
12a	Hγ-0.94%, H_2- 0.6%	α-isomer
12b	Hγ-1.4%, H_2–2.52%	β-isomer

3.2.2 Prolyl side chain conformation:

Alberto Lesarri et al[97] investigated the hydroxyprolines of Hyp and hyp in gas phase for determination of ring puckers. By computational calculations by the researcher revealed that the 4R isomer has low energy rotameric state which is predominantly *exo*- and 4*S endo*-form. These conformations are due to gauche interactions between the substituents on hydroxyproline[98] and stabilization due to hydrogen bonding in 4*S* isomer[97]. The exo and endo forms are judged by the coupling ^{3}J coupling constants of neighbouring carbon protons. The theoretical values of coupling constants for $J_{\alpha,\beta1}$ for typical C^{γ}-exo pucker is expected to be 7- 10 Hz and $J_{\alpha,\beta2}$, 7 – 11 Hz. For typical Cγ-endo pucker, the theoretical values are $J_{\alpha,\beta1}$ = 7-10 Hz and $J_{\alpha,\beta2}$ = 2 – 3 Hz. These prolyl puckers of **7a**, **7b**, **12b** are in compliance with theoretical values except **12a.** The selected ^{3}J coupling constants (Hz) for **7a**, **7b**, **12a**, **12b** at 25°C and (67°C) determined by NUMMRIT method by spin works program are as follows:

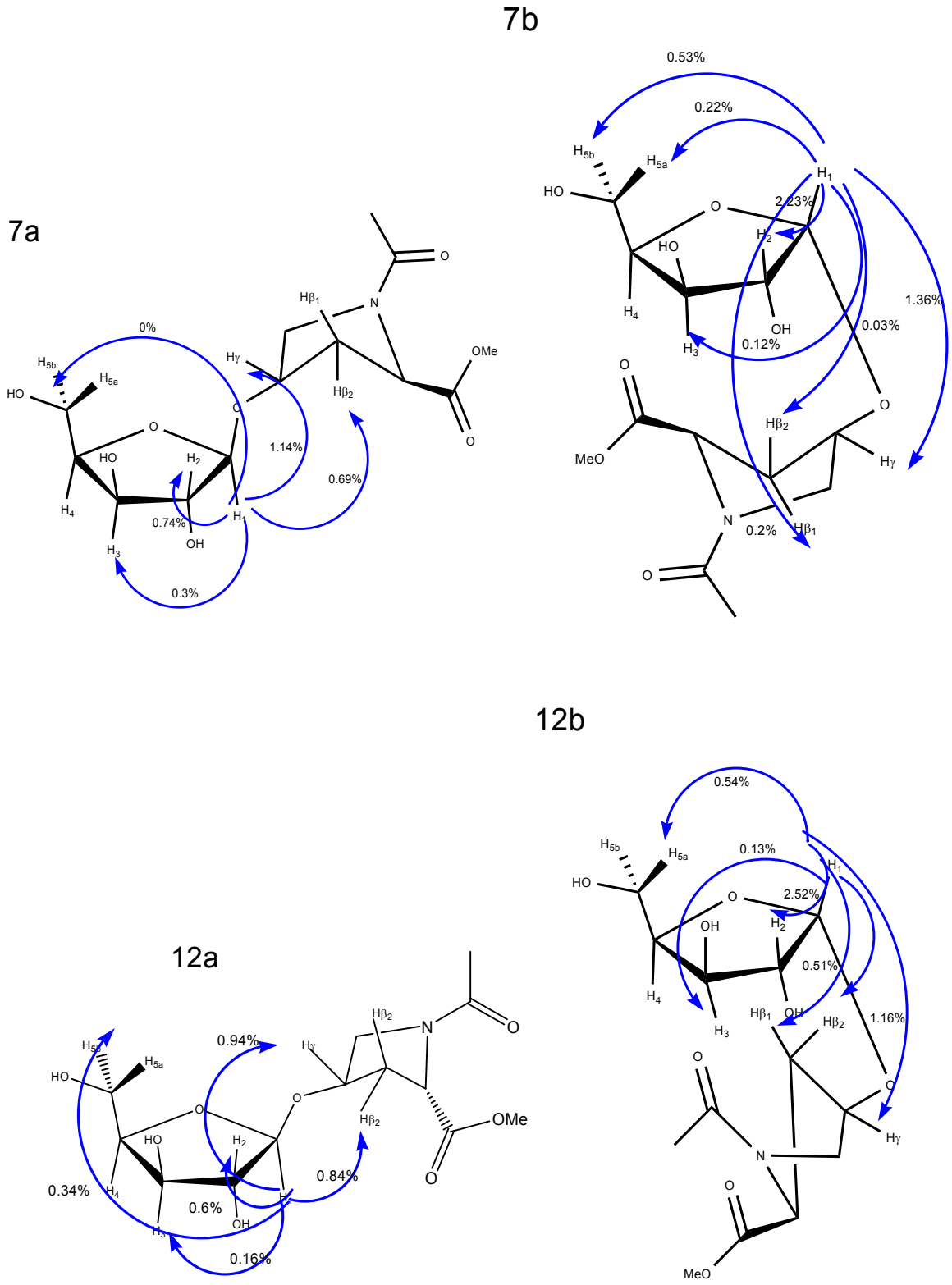
Table 6: *Prolyl side chain conformation of exo and endo configurations*

Compound	**$J_{\alpha,\beta1}$ (Hz)**	**$J_{\alpha,\beta2}$ (Hz)**	**Pucker**	**Notes**
7a	8.5 (8.4)	8.2 (8.4)	C^{γ}-exo	
7b	8.4 (8.4)	8.4(8.2)	C^{γ} -exo	
12a	8.3 (8.1)	8.4 (8.3)	C^{γ} -exo	Anomaly as it should be endo.
12b	9.7 (9.5)	2.6 (2.5)	C^{γ} -endo	

Hyp arabinofuranose building blocks (**7a** & **7b**) are having C^{γ}-exo configuration as predicted. There is anomaly regarding hyp building block **12a** which theoretically should be

an endo as **12b** (4*S* β anomer is proved to be endo by galactose building blocks[82]). But by NUMMRIT calculations, it is shown to adopt exo pucker.

Figure **19:** *Figure showing the 4 isomers of L-arabinofuranosylated Hyp(4R) and hyp(4S) with observed NOE data. (*H_α not drawn for convenience and H_β, H_{5a}, H_{5b} protons resonances are important for judging the distant contacts of prolyl ring with arabinose)



3.2.3 C^{γ} Inductive Effect:

Changes in ^{13}C chemical shifts are used to find out the electron withdrawing effects during glycosylation[99]. In reference to galactose glycosylation by our research group of the same Hyp and hyp building blocks have shown for 4*R* (Hyp) isomers about ~9ppm and 4*S* (hyp) isomers in the range of ~8ppm[100]. These values are quite higher in case with a simple acyl group of even the strongest trifluoroacetate group[101] which showed only the shift of ~3ppm. In the same manner, L-arabinosyl glycosylation produced similar effects slightly more than the galactosylation. In the case of arabinosylation the Table **7** explains the shift in ppm different for α and β isomers. For αisomer of 4R the chemical shift difference is around ~13.7 ppm and for β isomer (**7b**) the shift is around ~13.9ppm. For α isomer of 4S the shift is around ~12.2ppm and for β isomer (**12b**) it is around ~12.1ppm. These results show that glycosylation produces a local electron withdrawing effect significantly greater (~4 ppm) than galactose building blocks of Hyp and hyp.

Table **7**: *Comparison of C^{γ} L-arabinose glycosylated ones with D-Galactopyranose glycosylated compounds:*

S. No	Compound	13C Chemical Shifts of C^{γ}(ppm)	Change in Shift (~ ppm) for glycosylated ones
1	Ac-Hyp-OMe	69.6	-
2	Ac-hyp-OMe	69.9	-
3	Ac-Hyp(*O*-*α*-L-Ara*f*)OMe **7a**	83.3	~13.7
4	Ac-Hyp(*O*-*β*-L-Ara*f*)OMe **7b**	81.8	~12.2
5	Ac-hyp(*O*-*α*-L-Ara*f*)OMe **12a**	83.8	~13.9
6	Ac-hyp(*O*-*β*-L-Ara*f*)OMe **12b**	82.0	~12.1

7	Ac-Hyp(*O*-*α*-D-Gal*p*)OMe	78.9	~9
8	Ac-Hyp(*O*-*β*- D-Gal*p*)OMe	77.6	~8
9	Ac-hyp(*O*-*α*- D-Gal*p*)OMe	80.3	~10.7
10	Ac-hyp(*O*-*β*- D-Gal*p*)OMe	80.6	~11

3.2.4 ^{13}C Inverse gated Coupling[102]:

The inverse gated coupling is a quantitative experiment which gives the exact percentage of the minor rotamer present in the mixture of rotamers. This experiment was performed at 298K in D_2O with recycle time (acquisition time plus pulse delay) 5 times more than the ^{13}C-spin lattice relaxation (d1=5sec) in all the samples during analysis. This is due to avoid transient NOE and decoupler is off till the next excitation pulse. As a result each component in the mixture will have same integration of ^{13}C carbons in the spectrum. If the components are two, spectrum will show definite integration of the second compound present in it by which exact percentage of the other rotamer is found out from the average and Standard deviation of different carbons of the mixture. The trials are chosen from each of the ^{13}C spectra of different carbons of **7a**, **7b**, **12a**, **12b** with the nearly same integration and integrating the minor gave the results almost accurately in nearest percentage with less standard deviation. This experiment was attempted as there is much pronounced standard deviation in ^{1}H NMR, if we try to measure the minor integration as the signals are overlapped. The results are tabulated below:

Table 8: *^{13}C inverse gated coupling results of L-arabinosyl building blocks of hydroxy proline i.e **7a**, **7b**, **12a**, **12b** at RT i.e 24.8°C.*

S. No	Compound	Minor(Cis) Isomer %age (by ^{13}C Inverse Gated Coupling)	$K_{eq}=K_{cis/trans}$ *Average*	Standard Deviationof K_{eq}
1	Ac-Hyp(*O*-*α*-L-Ara*f*)OMe **7a**	10.52	8.60	1.09
2	Ac-Hyp(*O*-*β*-L-Ara*f*)OMe **7b**	13.98	6.15	0.22
3	Ac-hyp(*O*-*α*-L-Ara*f*)OMe **12a**	30.23	2.32	0.24
4	Ac-hyp(*O*-*β*-L-Ara*f*)OMe **12b**	30.76	2.25	0.13
5	Ac-Hyp(*O*-*α*-D-Gal*p*)OMe*	-	6	-
6	Ac-Hyp(*O*-*β*- D-Gal*p*)OMe*	-	5.9	-
7	Ac-hyp(*O*-*α*- D-Gal*p*)OMe*	-	2.9	-
8	Ac-hyp(*O*-*β*- D-Gal*p*)OMe*	-	2.9	-
9	Ac-Hyp-OMe*	-	6.2	-
10	Ac-hyp-OMe*	-	2.4	-

* *Taken from Neil's paper for comparison.*

In **Table 8** compound **7a** has the highest equilibrium constant of 8.6 which clearly shows it favours α- *trans* Hyp isomer more than the galactose building blocks.

3.2.5: Kinetics of cis-trans isomerization: *Rate constants determination*

The rate constants which are calculated by Mathematica programs using inverse magnetization transfer are summarized below in Table **9**

Table 9: *Equillibrium constants and rate constants of cis-trans isomers of the compounds researched till now:* Here "*p*" denotes pyranose sugar and "*f*" denotes furanose sugar

Compound	**k_{ct} sec^{-1}(rate constant) at 67°C**	**k_{tc} sec^{-1}(rate constant) at 67°C**	**K_{ct} at 67°C (Equal. Constant)**	**K_{ct} at 24.8°C (Equal. Constant)**
Ac-Hyp(*O*-*α*-L-Ara*f*)OMe **7a**	0.82 ± 0.2	0.14 ± 0.2	5.80 ± 0.1	8.6 ± 1.09
Ac-Hyp(*O*-*β*-L-Ara*f*)OMe **7b**	0.87 ± 0.16	0.17 ± 0.16	5.01 ± 0.2	6.15 ± 0.22
Ac-hyp(*O*-*α*-L-Ara*f*)OMe **12a**	0.96 ± 0.14	0.18 ± 0.14	5.33 ± 0.1	2.32 ± 0.24
Ac-hyp(*O*-*β*-L-Ara*f*)OMe **12b**	0.58 ± 0.12	0.25 ± 0.12	2.25 ± 0.2	2.25 ± 0.13
Ac-Hyp(*O*-*α*-D-Gal*p*)OMe*	0.85	0.19	6.0	5.37
Ac-Hyp(*O*-*β*-D-Gal*p*)OMe*	0.77	0.18	5.9	5.18
Ac-hyp(*O*-*α*-D-Gal*p*)OMe*	0.59	0.25	2.9	2.82
Ac-hyp(*O*-*β*-D-Gal*p*)OMe*	0.71	0.3	2.9	2.75
Ac-Hyp-OMe*	0.81	0.18	6.2	5.08
Ac-hyp-OMe*	0.44	0.2	2.4	2.57

Ac-Prone*	0.81	0.31	2.61	N/A
Ac-Hyphae*	0.73	0.25	2.92	N/A
Ac-Hyp(*O*-*tert*-butyl)NHMe*	0.77	0.27	2.85	N/A
Ac-Hyp(*O*-α-D-Gal*p*)NHMe*	0.83	0.27	3.07	N/A
Ac-Hyp(*O*-β-D-Gal*p*)NHMe*	0.61	0.21	2.90	N/A

* *Taken from previously published data for comparison*

From Table **8**, the rate constants of *cis*→*trans* (k_{ct}) isomerization of L-arabinofuranose glycosylated molecules i.e **7a**, **7b**, **12a**, **and 12b** give following conclusions:

- Most L-arabinosylated peptide models of Hyp and hyp do not show great variations in the isomerization rate constants. The only building block that shows a significant two-fold increase in rate is Ac-hyp (O-α-L-Araf)-OMe (**12a**).

- L-arabinosylated and D-galactosylated peptides show nearly identical rate constants at 67°C.

- Glycosylation (D-galactosylation and L-arabinosylation) of hyp results in enhanced k_{ct} constants when compared to nonglycosylated hyp while nearly identical values are obtained for Hyp.

Our results show that the nature of the sugar has an influence on the stability of the rotameric state in the model peptides. L-arabinosylation of Hyp leads to a significant

stabilization of the *trans* rotameric state when compared to D-galactose at ambient temperature. Also there is degradation observed in all the furanosylated compounds during the NMR experiment where the compounds in buffer solutions are unstable at 67 °C as exposed to that temperature for a long time. Thus, further determinations of the thermodynamic parameters were not attempted. As indicated in table 6 the rate constant values are relatively similar between furanoside-based sugars and pyranoside-based sugars.

3.2.6 Measurement of $K_{cis/trans}$:

Hydroxylation of proline modifies two properties. a) It stabilizes the prolyl side chain pucker which depends on stereochemistry of 4th position oxygen atom. 4R Hydroxy proline adopts the C^{γ} exo pucker configuration and 4S hydroxy proline C^{γ} endo pucker configuration[103]. b) Hydroxylation affects the N-terminal *cis*/*trans* equilibrium i.e. 4R isomer stabilizes the *trans* conformation and 4S isomer stabilizes *cis* conformation. The equilibrium constant for galactosylated hydroxyprolines (Table **7**) $K_{trans/cis}$ = 6.0 for 4R Hyp and 2.4 for 4S hyp[82]. This shows that 4S isomer stabilizes *cis* rotamer and 4R hydroxy proline stabilizes *trans* rotamer population. Moreover, recent studies of Mootoka et al[104] have shown that 4R hydroxyproline stabilizes the triple helix structure in collagen. These results indicate that 4*R*-hydroxyproline plays an important function to control structural properties in proteins. In order to investigate the influence of the sugar on the proline cis/trans amide isomerization we prepared L-arabinosylated hydroxyproline building blocks. By measuring the equilibrium constants for *α*- and *β*-arabinosylated Hyp we have observed a significant discrepancy between α- (K_{ct} = 8.6) and β-arabinosyalted (K_{ct} = 6.1) Hyp at room temperature. Moreover, there is a marked difference when these equilibrium constants are measured at 67.3°C and at room temperature. The results are concluded below:

- α-L-arabinosylated Hyp analog **7a** shows a strong stabilization of the *trans* amide rotamer population at room temperature when compared to their respective α- and β-galactosylated compounds. In addition, there is a much stronger temperature dependency of the equilibrium constant in the arabinosylated building blocks when compared to their galactosylated analogs.
- When temperature is increased to 67 °C, a change observed in Kct values of **12 a**. Thus *trans* Hyp amide rotamer is stabilized at higher temperature ((K_{ct}= 2.3 at 24.8°C) and at 67 °C, (K_{ct}= 5.3).
- In comparison, galactosylated derivatives display smaller differences in equilibrium constant values than L-arabinosylated building blocks.

Also the arabinofuranose glycosylated molecules are subjected to slight degradation / when subjected to Inverse Magnetization experiments at higher temperature over a prolonged time (6 hours). By NMR analysis of plants and other plant materials, it has been confirmed in majority that α and β arabinofuranoses are present in most of the flowering plants but mostly α is seen predominantly [39, 56, 59b]. β -arabinofuranoses are also seen along with α-ones in mugwort Artemesia plant as investigated by Altman et al in Ambrosia[105] and other plants like CLAVATA3 arabidopsis CLV3 peptides.

Previous research in our group has shown that galactosylation of hydroxyproline in Ac-Hyp-COOMe and Ac-Hyp-COONHMe did not have a measurable effect on the equilibrium constant. It is noteworthy that α- or β-arabinofuranosylaton of Hyp has been shown to result in a significant stabilization of the *trans* rotamer population evident by equilibrium constants.

3.3 Discussion

Previous research has shown that inductive effects in the γ-position of proline have important structural, thermodynamical and kinetic consequences of prolyl amide bond isomerization[34, 94a, b, 104, 106]. L-arabinosylation similar to D-galactosylation of Hyp induces an inductive effect which can result in stabilization of the *trans* rotameric state as in the case of L-arabinose. The combined observed ^{13}C NMR chemical shifts order (δ C^{γ}trans) is as follows: hydroxyl (**δC^{γ}=69.6**) < *tert*-butoxyl (**δC^{γ}=70.1**) < β-D-galactosyl (**δC^{γ}=77.6**) <α-D-galactosyl (**δC^{γ}=78.9**) < β-L-arabinofuranosyl (**δC^{γ}=81.8**) < α-L-Arabinofuranosyl (**δC^{γ}=83.3**)

Both α- and β- 4-*O*-galactosylation of Hyp have no apparent effect on the isomer equilibrium or the rate of isomerization[100] when compared with unglycosylated Hyp. In contrast, both α- and β- 4-*O*-L-arabinosylation induce a measurable effect. α-L-arabinosylated Hyp shows the greatest effect and leads to a significant stabilization of the *trans* rotamer population. Galactosylation of Hyp produces an inductive electron withdrawing effect on the prolyl ring and 4R-electronegative substituents stabilize the C^{γ}-exo pucker of proline. The inductive effect for L-arabinofuranose molecules are 4ppm greater in ^{13}C NMR than the galactosylated molecules (Ref: Table **6**). Nuclear Overhauser experiments indicate that the glycosylation of hyp resulted in distant contacts between proline ring and sugar linkages which is proved in both cases i.e galactopyranose and L-arabinofuranose. Due to the flexibility of arabinofuranose, nOe is judged based on the two anomers' relative resonances of neighbouring protons. This definitely induces a conformational restraint into glycopeptides in living systems. By these results, we can conclude that arabinofuranosylation of hyp too can influence cis-trans isomerization as 4*S* hydroxylation causes the substituent groups to be projected from opposite face of prolyl side chain. But by results, the α-isomer of hyp i.e **12a**

has a very good preference for *cis-trans* isomerization at higher temperature and exhibits exo configuration instead of endo. This may be due to gauche orientation of 4-hydroxyl group and prolyl nitrogen atom which may facilitate hydrogen bonding[103]. Improta et al[106c]have conducted conformational study with the aid of computational calculations, by taking 4S and 4R,hydroxyprolines, 4-Flouro proline whether inductive effect has any influence on the dipeptides for hydrogen bonding as collagen is stabilized by hydrogen bonds. Thus interaction energy of the prolines decreases of the order: proline (Pro) > hydroxyproline (Hyp) > 4-fluoroproline (Flp). The results are explained by Improta as follows:

- The hydrogen bonding capacity of the imido moiety increases with the relative stability of the substituent in which oxygen bears a formal negative charge. Thus a polar shielding group on 4-*O*-substituent not only shields the electronegative power of the substituent but also enhances the delocalization effects for dipeptides of Hyp and Flp favouring the formation of a partial N-C_{i-1}double bond. Thus in aqueous solution Hyp, Flp hydrogen bonds are stronger than Pro.

- This effect is also attributed to the gauche orientation of 4-hydroxyl group in 4S hydroxyl proline and the prolyl nitrogen atom. This orientation is further stabilized by hyperconjugative σ (C^{β}-H)→σ* (C^{γ}-O) and σ (C^{δ}-H)→σ* (C^{γ}-O) interactions. Thus by the work of Kramer et al[107], polypeptides of sequences Pro-Pro-Gly in collagen like peptides, if Hyp introduced in the sequence, it stabilizes the collagen and if hyp is introduced by synthesis, stability is decreased. This is due to the orientation of 4S substituent is on the different face of the prolyl nitrogen atom and hydrogen bonding is not much feasible.

Generally, Hyp favours *trans* amide conformation relative to proline because the C^{γ}-exo pucker forces a ψ-angle of 150°, ideal for n→π* interaction. In contrast, the C^{γ}-endo

conformation associated with hyp has been favoured to show *cis* amide conformation due to unfavourable ψ-dihedral angle for the same n→π* interaction. There may be hydrogen bonding in hyp i.e cis hydroxy proline conformation which is evidenced by R Improta et al[106c] which gives additional stability of 1.5Kcals mol^{-1} . It is between 4-hydroxyl group and C-terminal carbonyl oxygen atom in hyp, as well as electrostatic repulsion between 4-position oxygen atom and C-terminal carbonyl oxygen atom; force the prolyl ψ-angle into a poor n→π* interaction, favouring cis amide conformation relative to Pro and Hyp. The local-electronwithdrawing effect caused by the glycosylation diminishes the electrostatic repulsion between 4-Hydroxy groups of the , so that the prolyl ψ angle to relax from 180° to 150° to get favourable n→π* interaction and is very specific for C^{γ}-endo configuration for stabilization of *trans* amide rotamer stabilization[94c, 108] and this effect is not seen in C^{γ}-exo pucker. Thus we can say that Hyp favours only *trans* amide isomerization and hyp favours both trans and cis isomerization. Also glycosylation of cis hyp would eliminate the intra molecular hydrogen bonding interaction of cis hydroxyl proline isomer. By NOE studies, the galactose and arabinose moeity is not in close proximity in C^{γ}-endo pucker. This interaction is not seen in C^{γ}-exo pucker. Thus it can be predicted that there is very little or no impact of glycosylation on $K_{cis/trans}$ values. Also confirmed by Taylor et al[98], introduction of O-methylation in hyp has little effect on $K_{cis/trans}$ values. Here also in L-arabinose glycosylated ones, there is no much change in the values of $K_{cis/trans}$ values except for α-arabinosylated hyp (**12a**). In this compound, there is a very good preference for the *trans* rotamer stabilization at higher temperature i.e 67.3°C. The results of equilibrium constant values did not change much on comparison with galactosylated molecules but there is a slight preference for the *trans* conformation for **7a** more than D-galactose.

NMR inverse transfer magnetization experiments indicate that glycosylation of Hyp and hyp leads to faster rates when compared to proline. NMR magnetization inversion transfer experiments indicated that hyp model compounds of D-galactose have faster amide isomerization rates. Improta et al. have calculated that the prolyl nitrogen is more pyramidalized in the C^{γ}-exo pucker than in the C^{γ}-endo pucker[106c] which should facilitate isomerization in D-galactosylated molecules, which each have a C^{γ}-exo pucker, with respect to cis hyp isomers, each with a C^{γ}-endo pucker. In the case of L-arabinose glycosylation a similar effect is observed with an exception of **12a**. This is in contrast with the findings of Beausoleil et al[95] who found the reverse effect: hyp had a faster rate than Hyp in Ac-$(peptide)_2$-NHMe model amides in D_2O at 60°C (2.05±0.5 and 1.46±0.13 s^{-1}), which has been proved here with 4*S* α of L-arabinofuranose glycosylated molecule **12a**. This may be attributed to the intramolecular hydrogen bond in hyp reducing couloumbic repulsion between the C-terminal carbonyl oxygen atom and the prolyl nitrogen, although the values were within experimental error. Moreoever, L-arabinofuranosylated compounds are degrading over time as a result of high temperature necessary to conduct the kinetic measurements that prohibits the measurement of the thermodynamic parameters.

There is a marked difference in equillibrium constants observed in the case of L-arabinofuranose glycosylated molecules than D-galactopyranosylated building blocks. Though α- isomer of Hyp (**7a**) glycosylation showed the highest preference for *trans* stabilization at RT by equilibrium constant K_{ct}= 8.6 but its stability is reduced at increased temperature (K_{ct}=5.8, 67.3°C). Changes in *cis-trans* isomerization is generally attributed to electron withdrawing inductive effect of the prolyl γ-substituents[94b] where the γ-position group withdraws electron density from the peptide bond and there by reducing the C-N bond order[109] of peptide bond and weakens the peptide bond, makes the isomerization to occur.

But glycosylation results in local electron withdrawing effect, it does for both of the and hyp. Therefore we cannot conclude an explanation for the increase and decrease of *cis-trans* isomerization around the peptide bond due to inductive effect only.

Thus glycosylation of Hyp in compounds of D-Gal*p* and L-Arab*f* did not have much appreciable effect on the isomer equilibrium (K_{ct}) and rate of isomerization (k_{ct}, k_{tc}) in water between *cis* and *trans* isomers when compared to unglycosylated reference compounds except α-arabinofuranose glycosylated compound **7a**. But there is a clear indication of L-Arab*f* glycosylated molecules, **7a** has some cis stabilization at 67.3°C, **12a** have some *trans* stabilization which is quite unusual for the un-natural cis hyp isomer. In the case of galactosylated compounds the magnitude of change in chemical shifts is around 8 to 11 ppm. In the case of L-arabinofuranosylated compounds this magnitude change is more pronounced and is around 12 to 14 ppm [**7a** - 83.3, **7b** - 81.8, **12a** - 83.8, **12b** - 82 ppm, respectively from Table **6**]. This magnitude may be due to the sugar moeity's dipole moment (vide definition of anomers) and they are conformationally flexible. The result of the inductive effect is the lowering of pK_a of pyramidalized prolyl nitrogen and reduces the cis-trans isomerization barrier[94b] and reduces electrostatic repulsion between 4-hydroxy oxygen atom and *C*-terminyl carbon's oxygen atoms. So we can conclude that α isomer of L-arabinofuranose and β- isomer of D-galactose might be predominant in biological systems identified by some researchers [26, 110]. It is a well-known fact that electronegative substituents stabilize C^{γ}-exo pucker of proline in peptide mimics[94b] and contribute to the stability of triple helix structure of collagen by hydrogen bonds. Glycosylation was also found to stabilize the structure of collagen like poly peptides researched by our group. By Lamport hypothesis, β-glycans stabilize the PPII conformation of HRGPs in plant cell walls. But here in the case of L-arabinofuranosylated building blocks, α-isomer is the more stable one and the reason may be

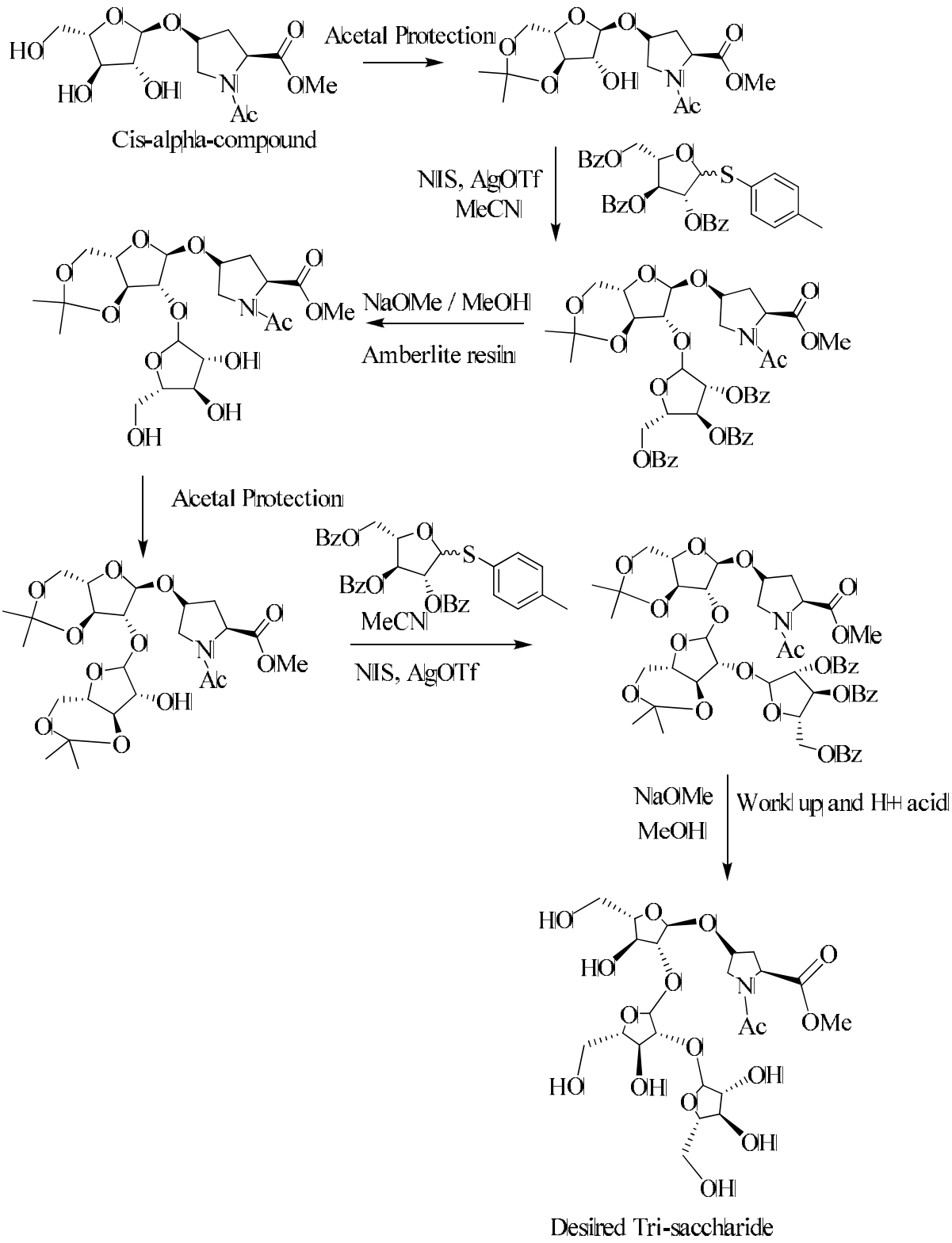
due to the stereochemistry of the sugar moiety. By computational[106c] and experimental studies[94c] stabilization of C^{γ}-exo pucker favours *trans* amide isomerization. By comparing all the bulding blocks (i.e D-galactose methyl amides, D-galactose methyl esters and L-arabinofuranose methyl esters of hydroxy proline), arabinofuranoses building blocks **7a**, **7b**, **12a**, **12b** have the trans stabilization measurable when they are comparable to un-glycosylated ones. There is a steric strain induced by C^{γ} exo pucker of Hyp by glycosylation but did not influence the *cis-trans* isomerization much but it may have more implications for the stability of collagens and plants. As tri-L-arabinofuranosides are present in plant hormones, it would be interesting to synthesize these building blocks and study their stability parameters. It may be predicted these parameters to be additive for poly peptides.

The different stereoisomers of 4-hydroxyproline provide an opportunity to understand how the prolyl ring has influence on $K_{trans/cis}$ values and the rate constants for the *cis-trans* isomerization. Hyp and hyp project the substituent or glycan spatially opposite direction; so these building blocks of D-Gal*p* and L-Arab*f* have an influence on $K_{trans/cis}$ proved experimentally but the difference is not much. But still these building blocks may be a useful tool in predicting the stability of linkages to some extent and not explaining completely the reasons behind the stability. However this research can be used to ascertain the studying carbohydrate binding interactions and the influences of glycans on peptide and protein structures in which the orientation of the glycans are important [108, 111].

Chapter 4: Conclusions and Future work:

α-linked L-arabinofuranosides linked to Hyp appear to stabilize the *trans* amide rotamer population in plants. This effect is in contrast to D-galactopyranose-linked Hyp where no measurable effect was seen. It may be hypothesized that multiple glycosylation sites on the may produce additive effects[108] and oligopeptides[112]. Synthesis of building blocks of L-arabinose tri-saccharide[59b] and tetra-saccharides[59a]of natural and un-natural analogues may have a greater impact on the results of these parameters as these tri-saccharides are ubiquitous in plants and plant hormones[59]. There are many methods of preparing tri-saccharides[113] but synthesis of arabinofuranosides so far has attracted little attention. In general the similar methods used in this thesis can be used for preparing oligosaccharides too but with some protective groups. These tri-saccharides synthesis generally involve the protection of the hydroxyl groups of the sugar and reacting with thio-donor of other sugar moiety and so on. The proposed scheme **5** is given below from one of the method[113].

Scheme 4: *Proposal for the preparation of Tri-saccharides as observed in plant hormones*



In the same way, other isomers are synthesized in the similar fashion. These tri-saccharides and their properties can be evaluated to see the effects of glycosylation i.e kinetics, equilibrium constants and thermodynamic parameters. These may provide some insight in their enhanced stability parameters.

Chapter 5: Supporting information for Chapter 3

5.1 Synthetic Procedures with NMR & Mass data - General Procedures: Reagent grade solvents were used without further purification. Thin-layer chromatography was performed on Si250F precoated plates of silica gel (250µm). Column chromatography was performed on Silica gel G60 silica gel (43-63µm). NMR spectra were analyzed on Bruker Top Spin 500 MHz spectrometer with Top Spin Works 3.0 software. The synthesized compounds are assigned based on 2D COSY and 2D HSQC experiments. For ^{1}H NMR, minor isomers are depicted in percentages of 1 proton. For ^{13}C NMR, when assigned, minor isomer carbon peaks are listed in brackets.

5.2 General Preparation of Sugar glycosyl donor: Compound 3 – 2, 3, 5 Tri-*O*-benzyl-L-arab-*f*-1-thiocresol (3): L-arabinose (5.0 gr, 0.033 mol) taken in Pyridine (65mL) and heated to 60°C under stirring at inert atmosphere. To the heated solution, benzoyl chloride (27.3mL, 0.164mol) is added dropwise for 10 min. The resulting reaction mass is stirred for 3.5 hr at 60°C and monitored upon TLC for the absence of starting material. After reaction, the reaction mass is poured onto (500mL) of ice cold water slowly under stirring. To this solution, Dichloromethane (100mL) is added for extraction. The DCM layer is washed 3 x 50mL times with Sat. $NaHCO_3$ solution, Brine and water and concentrated under vacuum. Crude NMR shows both α-pyranose and α and β of furanose forms of the tetra benzoyl deravatives. To a cooled mixture of the crude in Dichloromethane (75mL), add p-thiocresol (2.3gr, 0.0203mol) to the reaction mass and BF_3. Et_2O (10.3 mL, 0.034mol) slowly dropwise and stirred at RT for overnight for completion of reaction by TLC. Then cooled RM to 0 to 5°C, quenched with sodium bicarbonate solution and Ethyl acetate (50mL) for organic layer separation. The crude product is extracted with 2 x 50mL of Ethyl acetate and concentrated under reduced pressure. The pure furanose product **2** is obtained from flash column

purification with 12% Ethyl acetate: hexanes (careful isolation using this solvent mixture only, otherwise mixture of furanoses and pyranoses result) as first fraction. (5.3 gr, yield: 29%).

2,3,5-Tri-*O*-benzoyl-L-arab-*f*-1-thiocresol 2: **^{1}H NMR** (500MHz, $CDCl_3$, 298°K): δ = 8.14-8.08 (m, 7H), 7.63-7.65 (m, 2H), 7.53-7.44 (m, 6H), 7.41-7.39 (m, 2H), 7.3-7.28 (m, 2H), 7.75 (s, 1H), 5.70 (m, 1H), 5.64 (dd, J_1=1.4Hz, J_2=4.8Hz, H_2), 4.85 (dd, J_1=4.8Hz, J_2=8.8 Hz, H_3), 4.80 (dd, J_1=3.7Hz, J_2=11.8Hz, 1H), 4.73 (dd, J_1=5.2Hz, J_2=11.8Hz, 1H), 2.32 (s, 3H); **^{13}C NMR** (125MHz, $CDCl_3$, 298°K) 166.25, 165.68, 165.43,138.256, 133.85, 133.70, 133.63, 130.26, 130.10, 129.96, 129.93, 129.82, 129.75, 129.58, 129.38, 129.04, 128.97, 128.62, 128.59, 128.53, 128.38, 91.75(Anomeric), 82.57, 81.11, 78.18, 77.33, 77.098, 76.85, 63.63, 21.19. MS (ES): *m/z:* calcd for $C_{33}H_{28}NaO_7S$: 591.64 $[M+Na]^+$; found : 591.3 $[M+Na]^+$

Charge **2**(5.3 g, 0.009mol) in methanol (50mL), and under stirring at RT add Sodium methoxide (0.3 gr, 0.0055mol) for 3 hrs shows the absence of starting material 2. Then 3mL water was added and stirred with Amberlite 120H^+ strong acid(0.4gr, 0.06mol) resin till color of the solution disappears. Filter the resin and concentrated and subjected to short column purification and the oily product eluted at 65% ethyl acetate: Hexane. The oily 2,3,5 Triol-arab-f-1-thiocresol is suspended in 30mL dry DMF under stirring and cooled to 0 to 5°C. To the reaction mass, sodium hydride (2.8gr, 0.117mol) portion wise was added and stirred for 30 min at 0 to 5°C. Then benzyl bromide was added dropwise for 10 min. Stir RM for 2 hrs at 0 to 5°C and continued to RT and stirred overnight. After completion of reaction, charge MeOH (10mL) and stirred for 2 hrs. The solvent is evaporated and the organic layer is partitioned between ethyl acetate (30mL) and washed with sat. $NaHCO_3$ (2 X 20mL). The organic layer is washed with Brine (20mL) and water (20mL) and dried with sodium sulphate

and concentrated to give a pale yellow viscous liquid. The product **3**is purified by flash chromatography and elutes at 10% Ethyl Acetate: Hexanes. (2.0gr, yield: 45%)

2,3,5 Tri-*O*-benzyl-L-arab*f*-1-thiocresol 3: ^{1}H NMR (500MHz, $CDCl_3$, 298°K): δ = 7.44-7.22 (m, 15H), 5.18 (d, *J* = 2.8Hz, 1H), 4.72-4.52 (m, 6H), 4.5 (m, 1H), 4.16 (m, 1H), 4.02 (dd, *J*=3.3Hz, 6.8Hz, 1H), 3.68 (dd, J_1=3.9Hz, J_2=10.9Hz, 1H), 3.63 (dd, J_1=4.9Hz, J_2=10.9Hz, 1H), 2.38 (s, 3H); **^{13}C NMR** (125MHz,$CDCl_3$, 298°K) δ=138.62, 138.21, 137.96, 137.85, 137.46, 137.32, 134.52, 132.11, 131.03, 129.99-129.77, 128.70-128.25, 128.20-127.56, 124.91, 102.05, 90.66, 88.49, 84.47, 83.98, 83.61, 83.36, 80.54, 77.41, 77.16, 76.91, 73.40, 72.33, 72.17, 71.97, 69.21, 21.53, 21.19; MS (ES): *m/z:* calcd for $C_{33}H_{34}NaO_4S$: 549.69 $[M+Na]^+$; found: 549.6 $[M+Na]^+$

5.3 General Preparation of glycosylated building blocks:

5.3.1 Fmoc Stage: Compounds 5a, 5b, & 10a, 10b:

Compound **3**(1.5gr, 0.0029mol) is dissolved in dry ACN and cooled to 0-5°C under stirring. To this Fmoc-Hyp-OMe (1.13 gr, 0.0031mol) and NIS (0.78 gr, 0.0034mol), AgOT*f* (0.15 gr, 0.00058mol) were added and stirred for 3hrs at 0 – 5°C. The progress of the reaction is monitored by TLC. After completion, the solvent is removed under vacuum and partitioned between ethyl acetate and sodium bicarbonate. To this 5% aq sodium thiosulphate is added till a colourless solution obtained and stirred for 15 min. The organic layer is washed with brine (10mL), water (10mL), and dried with sodium sulphate and concentrated. The product is purified for α and β isomers by flash chromatography and the product elutes at 23% ethyl acetate and hexane(caution: the mix of solvents should be around 23 to 25% otherwise mix of anomers results and takes long time for separation). Ratio of isomers formed 8:2(α:β) and the overall yield of the products are 65% (1.3 gr mixture of anomers in both Hyp and hyp). After

careful column purification 1.5gr of Hyp isomer yielded 0.7 gr (35%) of pure α isomer and 0.15gr (6.6%) of pure β isomer after several purifications by flash column chromatography. Similarly 1.5gr of cis hyp isomer yielded 0.68gr (33%) of pure α isomer and 0.15gr (6.5%) of pure β isomer.

NMR Data:

5a) 2,3,5 Tri-O-benzyl-L-arab-*f*-α-NFmoc-Hyp-OMe:

^{1}H NMR (500MHz, $CDCl_3$, 298°K): δ=7.76 (d, 2H, *J*=7.52Hz), 7.63.-7.52 (m, 2H), 7.41-7.19 (m, 4H), 5.11 (s, 1H), 5.08 (s, 0.67H), 4.62-4.42 (m, 6H), 4.41-4.32 (m, 2H), 4.26 (t, 1H), 4.23-4.14 (m, 1H), 4.05-4.00 (m, 1H), 3.98 (dd, J_1=3.3Hz, J_2=7.1Hz, 1H), 3.86 (dd, J_1=5.3Hz, J_2=11.4 Hz, 1H), 3.77 (s, 3H), 3.73-3.68 (m, 1H), 3.68-3.63 (m, 1H), 3.62-3.57 (m, 1H), 2.36-2.26 (m, 1H), 2.1-2.02 (m, 1H); **^{13}C NMR** (125MHz, $CDCl_3$, 298°K) δ = 172.92, 172.85, 154.94, (154.36), 144.21, 144.01, 143.96, 143.78, (141.35), 141.32, (138.07), 137.98, (137.86), 137.79, 137.55, 137.46, 128.63-128.51, 128.11-127.91, 127.89-127.63, 127.20-127.05, 125.31-125.19, 125.08, 124.98, (105.32), 105.18(Anomeric), (88.77), 88.71, 83.64, (83.38), 80.95, (80.80), 77.34, 77.09, 76.84, 74.39, 73.64, 72.38, 72.34, 72.26, 69.54, (69.25), 67.70, (67.59), 57.84, (57.47), 52.46, (52.40), (47.35), 47.19, (36.26), 35.03; MS (ES): *m/z:* $C_{47}H_{47}NNaO_9$: 792.88 $[M+Na]^+$; found : 792.8 $[M+Na]^+$

5b) 2,3,5 Tri-O-benzyl-L-arab-*f*-β-NFmoc-Hyp-OMe:

^{1}H NMR (500MHz, $CDCl_3$, 298°K): δ = 7.75 (m, 2H), 7.59 (d, 1H, *J*=7.7Hz), 7.53 (dd, 2H, J_1=7.3Hz, J_2=18.9Hz), 7.42 -7.35 (m, 1H), 7.34 -7.19 (m, 2H), 4.96 (d, *J*=4.9Hz, 1H), 4.89 (d, *J*=4.9Hz, 1H)), 4.69 (dd, J_1=6.1Hz, J_2=11.9Hz, 1H), 4.61-4.48 (m, 9H), 4.48-4.39 (m, 2H), 4.33 (t, 1H), 4.30-4.25 (m, 1H), 4.24-4.15 (m, 2H), 4.13-4.03 (m, 2H), 3.73 (s, 3H), 3.65 (s, 0.6H), 3.68 (dd, J_1=5.3Hz, J_2=11.4Hz, 1H), 3.63 (m, 1H), 3.56 (dd, J_1=3.3Hz, J_2=11.4Hz,

1H), 3.53-3.48 (m, 2H), 2.39-2.32 (m, 1H), 2.09-2.02 (m, 1H); ^{13}C NMR (125MHz,$CDCl_3$, 298°K) δ = 173.01, 172.92, 154.74, 154.52, 144.12, 144.02, 143.89, 143.68, 141.34, 141.3, 138.14, 138.10, 137.92, 137.86, 137.49, 128.63-128.3, 128.21, 128.17-127.65, 127.11, 127.09, 125.15, 125.10, 125.05, 124.97, 120.02, 119.96, 100.05(Anomeric), 84.15, 83.97, 82.56, 80.12, 77.29, 77.04, 76.79, 75.64, 73.81, 73.39, 73.35, 72.69, 72.53, 72.43, 72.0, 67.72, 67.59, 58.10, 57.74, 52.37, 52.35, 51.45, 51.30, 47.26, 47.16, 37.61, 36.52; MS (ES): *m/z:* calcd for $C_{47}H_{47}NNaO_9$: 792.88 $[M+Na]^+$; found : 792.9 $[M+Na]^+$

10a) 2,3,5 Tri-O-benzyl-L-arab-*f* -α-NFmoc-hyp-OMe:

^{1}H NMR (500MHz, $CDCl_3$, 298°K): δ = 7.79 (t, 2H), 7.68 (d, *J*=7.3Hz, 2H), 7.64 (d, *J*=7.8Hz, 2H), 7.59 (t, 2H), 5.14 (s, 1H), 5.12 (s, 1H, Anomeric), 4.66-4.46 (m, 8H), 4.46-4.36 (m, 1H), 4.00 (s, 1H), 3.98-3.92 (m, 1H), 3.70 (s, 3H), 3.68-3.65 (m, 1H), 3.61 (s, 3H), 2.45-2.32 (m, 2H); **^{13}C NMR** (125MHz, $CDCl_3$, 298°K) δ = 172.14, 171.98, 154.87, 154.63, 144.23, 143.91, 141.48, 141.32, 138.15, 138.12, 137.91, 137.89, 137.61, 137.57, (129.80), (129.07), 128.02, 127.93-127.67, 127.22-127.06, 125.24, 125.12, 125.05, 125.04, 120.08, 120.05, 120.03, 105.47(anomeric), 105.43(anomeric), 88.81, 83.74, 83.62, 80.74, 80.65, 77.51, 77.25, 77.0, 74.98, 74.11, 73.49, 73.48, 72.3-72.18, 69.71, 69.66, 67.59, 67.56, 58.13, 57.85, 52.35, 52.31, 52.01, 51.39, 47.39, 38.01, 36.92; MS (ES): *m/z:* calcd for $C_{47}H_{47}NNaO_9$: 792.88 $[M+Na]^+$; found : 792.9 $[M+Na]^+$

10b) 2,3,5 Tri-O-benzyl-L-arab*f*-β-Nfmoc-hyp-OMe:

^{1}H NMR (500MHz, $CDCl_3$, 298°K): δ = 7.75 (d, , *J*=7.7Hz, 2H), 7.59 (d, *J*=7.4Hz, 2H), 7.57-7.50 (m, 2H), 7.42-7.17 (m, 2H), 4.93 (d, *J*=3.7Hz, 1H), 4.89 (d, *J*=2.3Hz, 1H), 4.68-4.49 (m, 8H), 4.49-4.42 (m, 1H), 4.38 (dd, J_1=3.5Hz, J_2=6.8Hz, 1H), 4.36-4.40 (m, 1H), 4.24 (t, 1H), 4.23-4.16 (m, 1H), 4.1-4.03 (m, 1H), 4.02-4.0 (m, 1H), 3.84-3.8 (0.3H,), 3.73-3.71

(m, 2H), 3.65 (m, 1H), 3.61 (s, 3H), 3.57-3.53 (m, 2H), 3.5 (s, 3H), 2.37-2.30 (m, 1H), 2.23-2.16 (m, 1H); ^{13}C NMR (125MHz, $CDCl_3$, 298°K) δ = 172.12, 171.96, 154.85, 154.38, 144.11, 143.83, 141.35, 141.31, 138.13, 138.09, 137.97, 137.86, 137.98, 128.55-128.24, 128.15-127.86, 127.86-127.60, 127.09, 127.05, 125.25, 125.13, 124.97, 119.99, (impurity), 99.97(anomeric), 84.97, 82.63, 82.52, 80.27, 80.13, 77.30, 77.04, 76.79, 75.34, 74.53, 73.36, 73.23, 72.53, 72.41, 72.14, 71.98, 67.60, 67.48, 57.70, 57.45, 52.74, 52.34, 52.28, 47.34, 47.22, 36.43, 35.13; MS (ES): *m/z:* calcd for $C_{47}H_{47}NNaO_9$: 792.88 $[M+Na]^+$; found : 793 $[M+Na]^+$

5.3.2 Benzyl Stage: Compounds 6a, 6b & 11a, 11b: Compounds **5a** (0.65 gr), **5b** (0.1 gr), **10a** (0.63 gr, 0.00083 mol), **and 10b** (0.1 gr, 0.000129 mol) are taken in (4 separate RB flasks) 20% piperidine in DMF (5 mL for α isomers and 2.5mL for β isomers) and stirred for 2 hrs. Progress of the reaction is monitored by TLC. After starting material disappearance, DMF is removed by high vacuum and the crudes are taken as it is for next stage. The crude products are dissolved in pyridine (10mL for α isomers and 5mL for β isomers) and Ac_2O (0.2 mL for α and 0.1 mL for β isomers) is added dropwise at 0 – 5°C and left overnight stirring at RT. The progress of the reaction is monitored by TLC. After completion, solvent is removed under vacuum and the residue is partitioned between ethyl acetate (2.5mL) and sat $NaHCO_3$ (5mL). The organic layer is washed with brine (5mL), water (5mL) and concentrated under vacuum and dried with Sodium Sulphate. The crude compounds are purified by flash chromatography and the pure products elute at 40% Ethyl Acetate: Hexanes. Yield (For Hyp α-0.12 gr (25%), β-0.07 gr (14.5%) and hyp α-0.118 gr (24%), β-0.071 gr (15%)

NMR Data:

6a) 2,3,5 Tri-*O*-benzyl-L-arab-*f* -α-NAc-Hyp-OMe:

^{1}H NMR (500MHz, $CDCl_3$, 298°K): δ = 7.41-7.17 (m, 15H), 5.07 (s, 1H), 4.59 (dd, J_1=4.6Hz, J_2=9.1Hz, 1H), 4.57-4.38 (m, 6H), 4.48-4.30 (m, 1H), 4.16-4.10 (m, 1H), 4.0 (t, 0.3H), 3.95 (dd, J_1=1.2Hz, J_2=3.4Hz, 1H), 3.92 (dd, J_1=3.4Hz, J_2= 6.8Hz, 1H), 3.90-3.85 (m, 1H), 3.65 (s, 3H), 3.62 (s, 0.6H), 3.60-3.51 (m, 3H), 3.36 (t, 0.7H), 2.4-2.33 (m, 1H), 2.32-2.26 (m, 1H), 2.08 (s, 3H), 2.02 (s, 0.7H, 3H); ^{13}C NMR (125MHz, $CDCl_3$, 298°K) δ = (171.4), 171.69, (170.15), 169.46, 168.86, 146.70, 141.04, (138.08), 138.03, (137.83), 137.80, 137.59, 137.46, 128.6-128.30, 128.12, 127.97, 127.88-127.63, (127.11), 127.04, 126.73, 125.47, 119.66, 119.78, 105.64(anomeric), (105.10-anomeric), 88.78, (88.73), (83.78), 83.49, 80.82, (80.62), 77.64, 77.19, 76.93, 75.04, (73.67), 73.43, 72.25, (69.72), 69.57, 59.14, (57.21), 54.94, 52.51, (52.23), 51.71, (47.47), (44.86), (42.52), 38.48, (36.39), (26.46), 26.22, (25.53), (24.66), 24.50, 22.36, (22.11), 21.48; MS (ES): *m/z:* calcd for $C_{34}H_{39}NNaO_8$: 612.68 $[M+Na]^+$; found : 612.6 $[M+Na]^+$

6b) 2,3,5 Tri-*O*-benzyl-L-arab-*f* -β-NAc-Hyp-OMe:

^{1}H NMR (500MHz, $CDCl_3$, 298°K): δ = 7.42-7.12 (m, 15H), 4.97 (dd, J_1=2.2Hz, J_2=4.4Hz, 0.28H), 4.89 (dd, J_1=2.1Hz, J_2=3.4Hz, 1H), 4.6-4.55 (m, 2H), 4.54-4.44 (m, 6H), 4.44 (t, 0.38H), 4.34-4.25 (m, 1H), 4.14-4.03 (m, 3H), 3.96-3.84 (m, 0.36H), 3.76-3.67 (m, 3H), 3.53-3.67 (m, 2H), 3.4 (dd, J_1=2.8Hz, J_2=11.0Hz, 1H), 2.38-2.28 (m, 1H), 2.17-2.09 (m, 1H), 2.04 (s, 3H), 1.83(s, 0.4H,); **^{13}C NMR** (125MHz, $CDCl_3$, 298°K) δ = 172.23, (172.59), (169.85), 169.30, 138.06, 137.83, 137.58, 128.81-128.8, 128.4-127.57, 100.52(Anomeric), 84.16, (83.91), 82.44, (80.10), 77.30, 77.05, 76.79, 76.34, 73.40, (73.32), (73.06), 72.76, 72.56, (72.42), (72.22), 71.92, (60.41), (58.78), 57.50, (52.79), 52.29, (50.53), 36.00, 29.72,

(29.68), 22.25, (21.07); MS (ES): *m/z:* calcd for $C_{34}H_{39}NNaO_8$: 612.68 $[M+Na]^+$; found : 612.5 $[M+Na]^+$

11a) 2,3,5 Tri-*O*-benzyl-L-arab-*f* -α-NAc-hyp-OMe:

^1H NMR (500MHz, $CDCl_3$, 298°K): δ = 7.39-7.21 (m, 15H), 5.07 (s, 0.11H), 5.06 (s, 1H), 4.58-4.44 (m, 6H), 4.4-4.32 (m, 1H), 4.16-4.10 (m, 1H), 4.05 (m, 0.31H), 3.97 (dd, J_1=1.1Hz, J_2=3.3Hz, 1H), 3.88 (dd, J_1=3.3Hz, J_2=7Hz, 1H), 3.79 (dd, J_1=5Hz, J_2=11Hz, 1H), 3.73 (s, 3H), 3.76 (s, 0.33H), 3.65-3.59 (m, 2H), 3.56 (dd, J_1=5.8Hz, J_2=11Hz, 1H), 2.38-2.32 (m, 0.19H), 2.27-2.20 (m, 1H), 2.08-2.03 (m, 1H), 2.01 (s, 3H), 1.94 (s, 0.32H); ^{13}C NMR (125MHz,$CDCl_3$, 298°K) δ = 172.74, 169.59, 137.94, 137.69, 137.38, (137.20), 128.56, 128.53-128.31, 128.08, 128, 127.93-127.67, 105.53, (105.43), (88.71), 88.62, (83.62), 80.86, (77.30), 77.05, 76.8, (75.11), 73.50, 72.41, (72.33), 69.80, 57.11, (57.04), 54.95, 52.34, (35.13), 34.82, 22.26, (21.08); MS (ES): *m/z:* calcd for $C_{34}H_{39}NNaO_8$: 612.68 $[M+Na]^+$; found : 612.5 $[M+Na]^+$

11b) 2,3,5 Tri-*O*-benzyl-L-arab-*f* -β-Nac-hyp-OMe:

^1H NMR (500MHz, $CDCl_3$, 298°K): δ = 7.37-7.21 (m, 15H), 4.93 (d, *J*=0.3H), 4.89 (d, *J*=3.7Hz, 1H), 4.68 (s, 0.37H), 4.66 (s, 0.67H), 4.61-4.48 (m, 6H), 4.38 (m, 1H), 4.26 (m, 0.34H), 4.17 (m, 1H), 4.11 (dd, J_1=7.1Hz, J_2=14.3Hz, 1H), 4.09-4.05 (m, 1H), 4.01-3.95 (m, 0.67H), 3.74-3.69 (m, 0.56H), 3.66-3.6 (m, 1H), 3.58-3.51 (m, 1H), 3.62 (s, 3H), 2.34-2.27 (m, 1H), 2.15-2.09 (m, 1H), 2.04 (s, 1H), 2.02 (s, 0.9H, 1H); ^{13}C NMR (125MHz, $CDCl_3$, 298°K) δ = (171.74), 171.70, 169.81, 169.49, 138.05, 137.97, 137.72, 128.54-128.30, 128.09, 128.02, (127.92), 127.86, 127.79, 127.76, 127.65, 100.21(Anomeric), 83.99, (83.95), 82.05, 79.94, 77.30, 77.05, 76.79, 75.72, (73.37), 73.17, 72.55, 72.47, 72.36, 72.33, 72.18, 71.82,

60.41, 56.73, 53.34, (52.77), (52.55), 52.25, 34.80, 22.19, (22.10), 21.07; MS (ES): *m/z:* calcd for $C_{34}H_{39}NNaO_8$: 612.68 $[M+Na]^+$; found : 612.5 $[M+Na]^+$

5.3.3 General Preparation of 4-*O*-arabinofuranosyl-*N*-acetyl-*N'*-methyl ester amino acids (Final compounds 7a, 7b, 12a, 12b): The protected N-Acetyl amino acid methyl esters (0.12 gr for α and 0.07gr for β isomers) was dissolved in methanol and catalyst, (20% palladium hydroxide on carbon) was added (approx. 20% w/w loading of catalyst i.e 25 mg for α isomers, 15mg for β isomers), and the flask was flushed with N_2 for 3 times. The reaction mixture was then stirred under hydrogen gas (10psi) pressure for 16 hrs, checked TLC for completion of reaction. Then the reaction mass was flushed with nitrogen and filtered using celite. The product was then concentrated under reduced pressure and co-distilled with toluene for 3 times (3 x 3mL) and subjected to C18 silica gel column to get pure compound. Sometimes if the compound is still impure by NMR, the compound is converted to tri-O-acetate by using Acetic anhydride / pyridine and purifying by column chromatography and de-acetylating by NaOMe / MeOH and solvent evaporation after that yielded thick oily compound (53mg for α and β 34mg) (yield=75%)

NMR Data:

7a) *trans*-4-*O*-(α-L-arabinofuranosyl)-*N*-acetyl-4-hydroxy-L-Proline methyl ester **(2, 3, 5 Triol-L-arab-*f* -α-NAc-Hyp-OMe):**

$[\alpha]^{D}_{23.9^{\circ}}$= -30.61 (*c*=0.3, CH_3OH); **^{1}H NMR** (500MHz, D_2O, 298°K): δ = 5.07 (d, *J*=1.6Hz, 1H, H_1), 5.05 (d, *J*=1.6Hz, 0.17H), 4.99 (d, *J*=1.4Hz, 0.13H), 4.98 (d, *J*=1.4Hz, 0.07H), 4.78-4.74 (m, 0.38H), 4.61 (dd, J_1=2.9Hz, J_2=8.8Hz, 0.2H), 4.51-4.43 (m, 2 protons, H_γ, H_4), 4.42-4.38 (m, 0.21H), 4.00 (dd, J_1=1.8Hz, J_2=3.5Hz, 1H), 3.95 (ddd, J_1=3.1Hz, J_2=5.9Hz J_3=9.1Hz, 1H), 3.87 (dd, J_1=3.6Hz, J_2=6.2Hz, 1H), 3.78 (m, 1H), 3.76 (dd, J_1=3.1Hz, J_2=12.3Hz, 1H), 3.77-3.73 (m, 2 protons, 2H), 3.70 (s, 3 H, CO-OCH_3), 3.63 (dd, J_1=5.8Hz, J_2=12.3Hz, 1H), 3.60-3.57 (m 0.12H), 3.56-3.53 (m, 0.38H), 3.350 (s, 0.03H), 3.28 (s, 0.2H), 2.49-2.43 (m, 1H), 2.37-2.30 (m, 0.57H, 1H), 2.14-2.08 (m, 1H), 2.06 (s, 3H, N-$COCH_3$), 2.01 (s, 0.15H), 1.94 (s, 0.41H); **^{13}C NMR** (125MHz, D_2O, 298°K) δ = 174.52, 173.19, 106.31, (106.60), (105.40), 83.83, 83.74, (81.82), (81.36), (76.61), 75.80, (75.39), (74.48), 61.17, (61.05), (58.73), 57.73, (57.51), 57.35, (54.43), (53.42), 53.04, (52.32), (36.67), (35.93), (35.16), 34.38, (33.92), (23.28), (21.53), 21.30, (21.15); MS (ES): *m/z:* calcd for $C_{13}H_{31}NNaO_8$: 342.31 $[M+Na]^+$; found : 342.4 $[M+Na]^+$

7b) *trans*-4-*O*-(β-L-arabinofuranosyl)-*N*-acetyl-4-hydroxy-L-proline methyl ester (**2, 3, 5 Triol –L-arab-*f* -β-NAc-Hyp-OMe):**

$[\alpha]^{D}_{23.9^{\circ}}$= 27.18 (*c*=0.3, CH_3OH); ^{1}H NMR (500MHz, D_2O, 298°K): δ = 5.06 (d, *J*=4.6Hz, 1H), 5.03 (d, J=4.9Hz, 0.15H), 4.77 (dd, J_1=7Hz, J_2=15.5Hz, 1H), 4.54-4.42 (m, 2H), 4.06 (dd, J_1=5Hz, J_2=8.5Hz, 1H), 3.94 (t, 1H), 3.84-3.77 (dd, J_1=3.3Hz, J_2=7Hz, 1H), 3.76 (s, 1H), 3.75 (s, 1H), 3.73 (d, *J*=3.9Hz, 1H), 3.70 (s, 3H, CO-OCH_3), 3.56 (dd, J_1=7.2Hz, J_2=12.8Hz, 1H), 3.49 (dd, J_1=4.9Hz, J_2=12.8Hz, 0.17H), 2.62-2.55 (m, 0.15H), 2.54-2.47 (m, 1H), 2.38-

2.31 (m, 0.19H), 2.14 (m, 2H,), 2.06 (s, 3H, N-$COCH_3$), 1.94 (s, 0.5H, N-$COCH_3$); ^{13}C NMR (125MHz, D_2O, 298°K) δ = 174.63, (174.19), 173.19, 100.28, (100.17), 81.83, 76.29, 76.14, (74.49), 74.34, 63.11, 59.07, 57.88, 53.47, (53.39), 53.03, (51.36), (37.01), 35.65, 21.29, (20.67); MS (ES): *m/z:* calcd for $C_{13}H_{31}NNaO_8$: 342.31 $[M+Na]^+$; found : 342.4 $[M+Na]^+$

12a) *cis*-4-*O*-(α-L-arabinofuranosyl)-*N*-acetyl-4-hydroxy-L-proline methyl ester (**2, 3, 5 Triol-L-arab-*f* -α-NAc-hyp-OMe):**

$[\alpha]^D_{23.9°}$= -34.11 (*c*=0.3, CH_3OH); ^1H NMR (500MHz, D_2O, 298°K): δ = 5.06(s, 1H), 5.04 (s, 0.18H), 4.75-4.73 (m, 0.2H), 4.53-4.42 (t, 2H), 3.99 (t, 1H), 3.95 (m, 1H), 3.87 (m, 1H), 3.82 (dd, J_1=4.1Hz, J_2=11.9Hz, 1H), 3.78 (s, 0.87H), 3.77-3.72 (m, 2H), 3.7 (s, 3H, CO-OCH_3), 3.63 (dd, J_1=5.9Hz, J_2=11.9Hz, 1H), 3.55 (dd, J_1=4.9Hz, J_2=12.4Hz, 0.24H), 2.57-2.50 (m, 0.2H), 2.5-2.51 (m, 1H), 2.37-2.26 (m, 0.26H), 2.15-2.07 (m, 1H), 2.06 (s, 3H, N-$COCH_3$), 1.93 (s, 0.44H), 1.91 (s, 0.08H); ^{13}C NMR (125MHz, D_2O, 298°K) δ = (173.73), 174.57, 174.52, (173.19), 106.31, (106.65), 83.84, 83.73, 76.28, (76.21), 75.81, 61.14, (61.10), 57.89, (57.52), 54.18, 53.43, (53.06), 35.70, (34.35), 21.30, (20.67);MS (ES): *m/z:* calcd for $C_{13}H_{31}NNaO_8$: 342.31 $[M+Na]^+$; found : 342.4 $[M+Na]^+$

12b) *cis*-4-*O*-(β-L-arabinofuranosyl)-*N*-acetyl-4-hydroxy L-proline methyl ester (**2, 3, 5 Triol-L-arab-*f* -β-NAc-hyp-OMe):**

$[\alpha]^D_{23.9°}$= 29.33 (*c*=0.3, CH_3OH); ^1H NMR (500MHz, D_2O, 298°K): δ=5.01 (d, *J*=4.5Hz, 1H), 4.99 (d, *J*=4.6Hz, 0.41H), 4.76 (s, 0.2H), 4.74 (s, 0.2H), 4.58 (dd, J_1=3.0Hz, J_2=9.2Hz, 1H), 4.49-4.42 (m, 1H), 4.03-3.99 (m, 1H), 3.88 (t, 1H), 3.83 (ddd, J_1=4.2Hz, J_2=7.3Hz, J_3=11.4Hz, 1H), 3.81-3.78 (m, 2H), 3.72 (s, 1.3H, $COCH_3$), 3.71 (d, *J*=3.3Hz, 0.64H), 3.62-3.58 (m, 1H), 3.56-3.53 (m, 0.7H), 2.4-2.37 (m, 0.22H), 2.37-2.34 (m, 1H), 2.31-2.29 (m, 0.22H), 2.07 (s, 3H, N-$COCH_3$), 2.01 (s, 0.46H, minor N-$COCH_3$); ^{13}C NMR (125MHz,

D_2O, 298°K) δ = (174.08), (173.79), 173.75, 173.38, 99.88 (anomeric), (99.40), 82.0, (81.97), 76.13, (76.10), 76.0, (74.52), 74.49, 63.23, (63.21), (59.38), 57.46, 54.32, (53.24), (53.16), 53.00, (35.14), 34.05, 21.27, (21.19); MS (ES): *m/z:* calcd for $C_{13}H_{31}NNaO_8$: 342.31 $[M+Na]^+$; found : 342.4 $[M+Na]^+$

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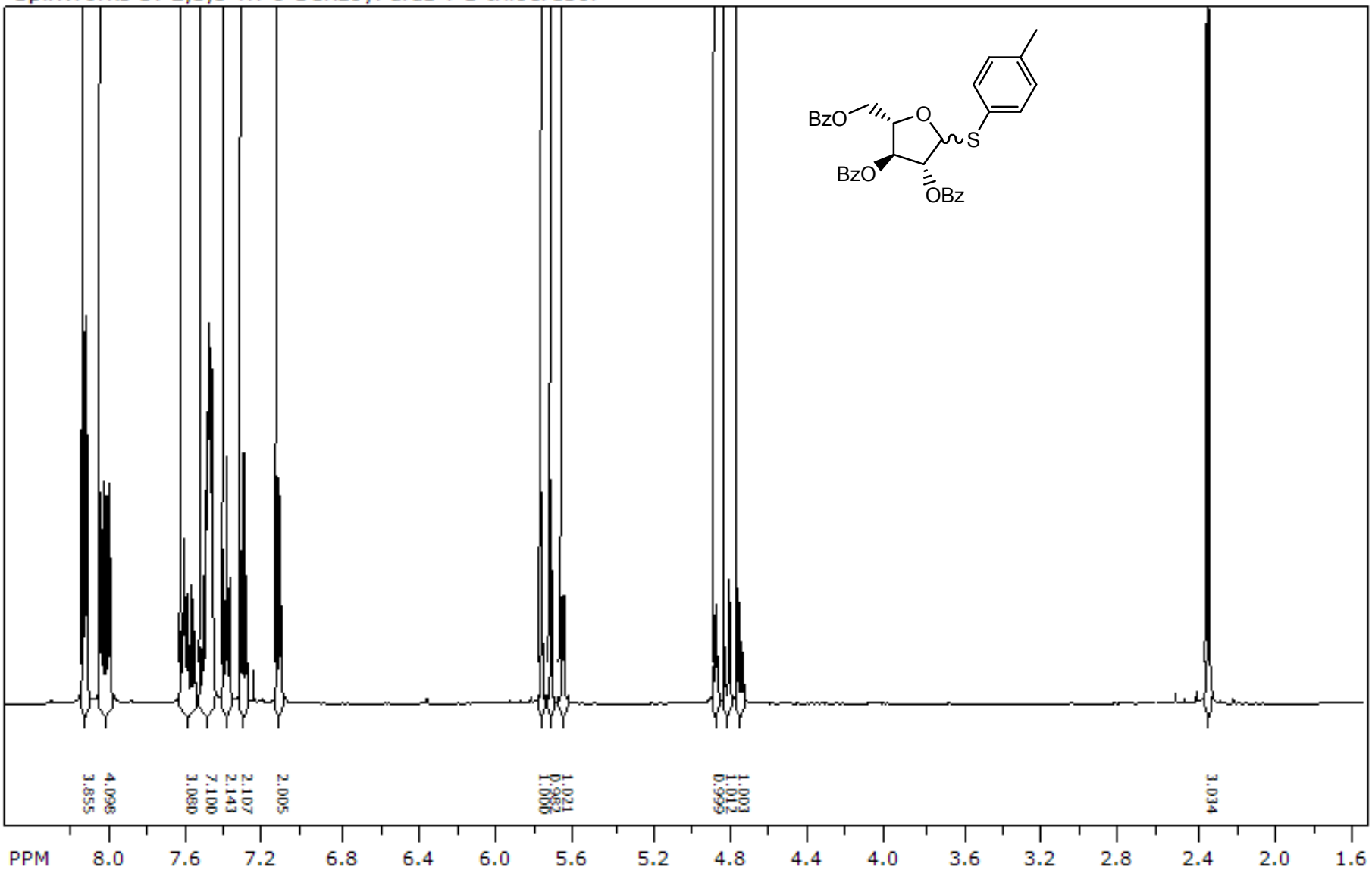
APPENDICES

NMR Data with Mass Reports

SpinWorks 3: 2,3,5 Tri-O-benzoyl-arab-f-1-thiocresol



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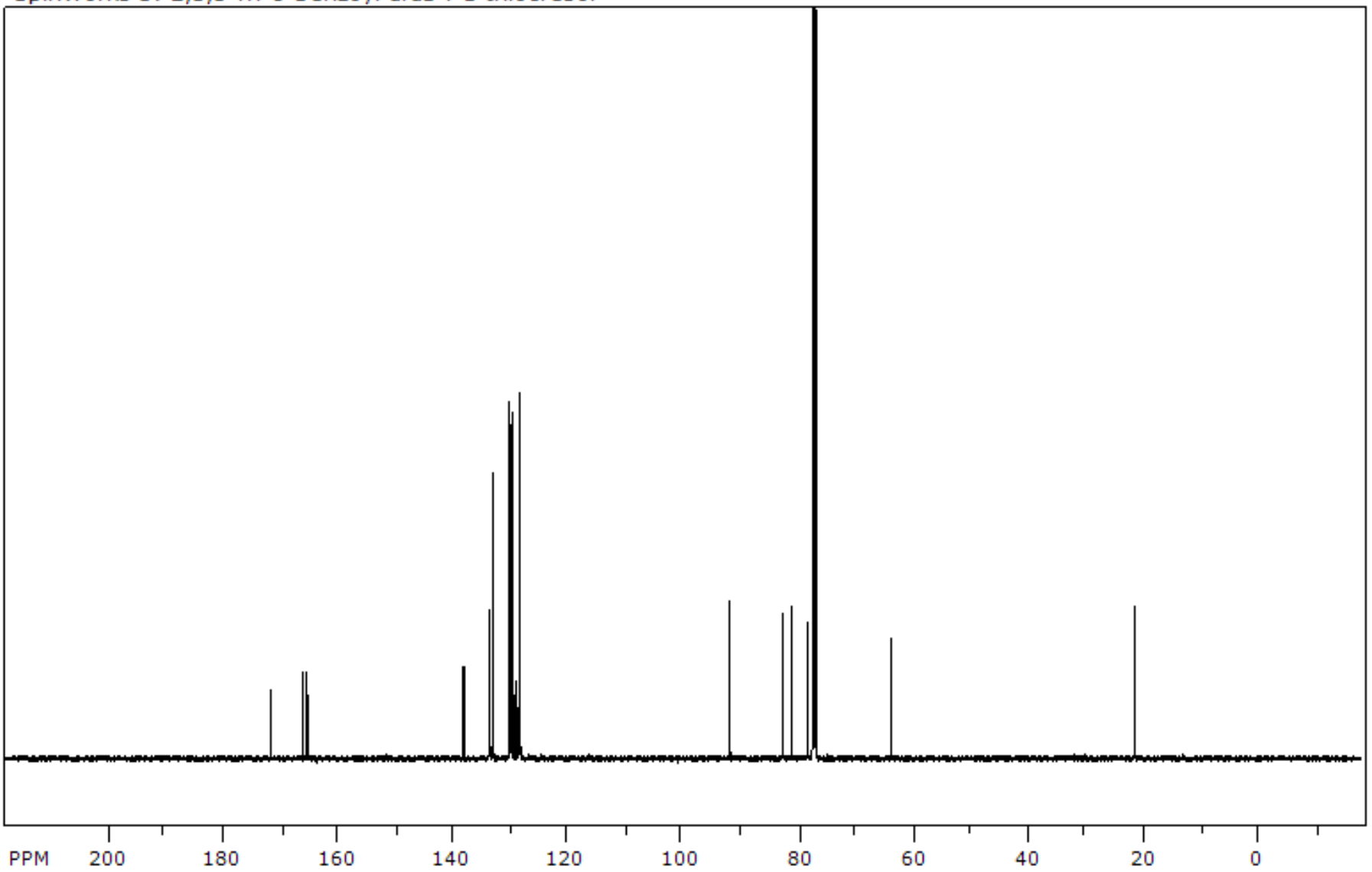
transmitter freq.: 500.133089 MHz
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 64

freq. of 0 ppm: 500.130025 MHz
processed size: 65536 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 140.253 ppm/cm: 0.28043

SpinWorks 3: 2,3,5 Tri-O-benzoyl-arab-f-1-thiocresol



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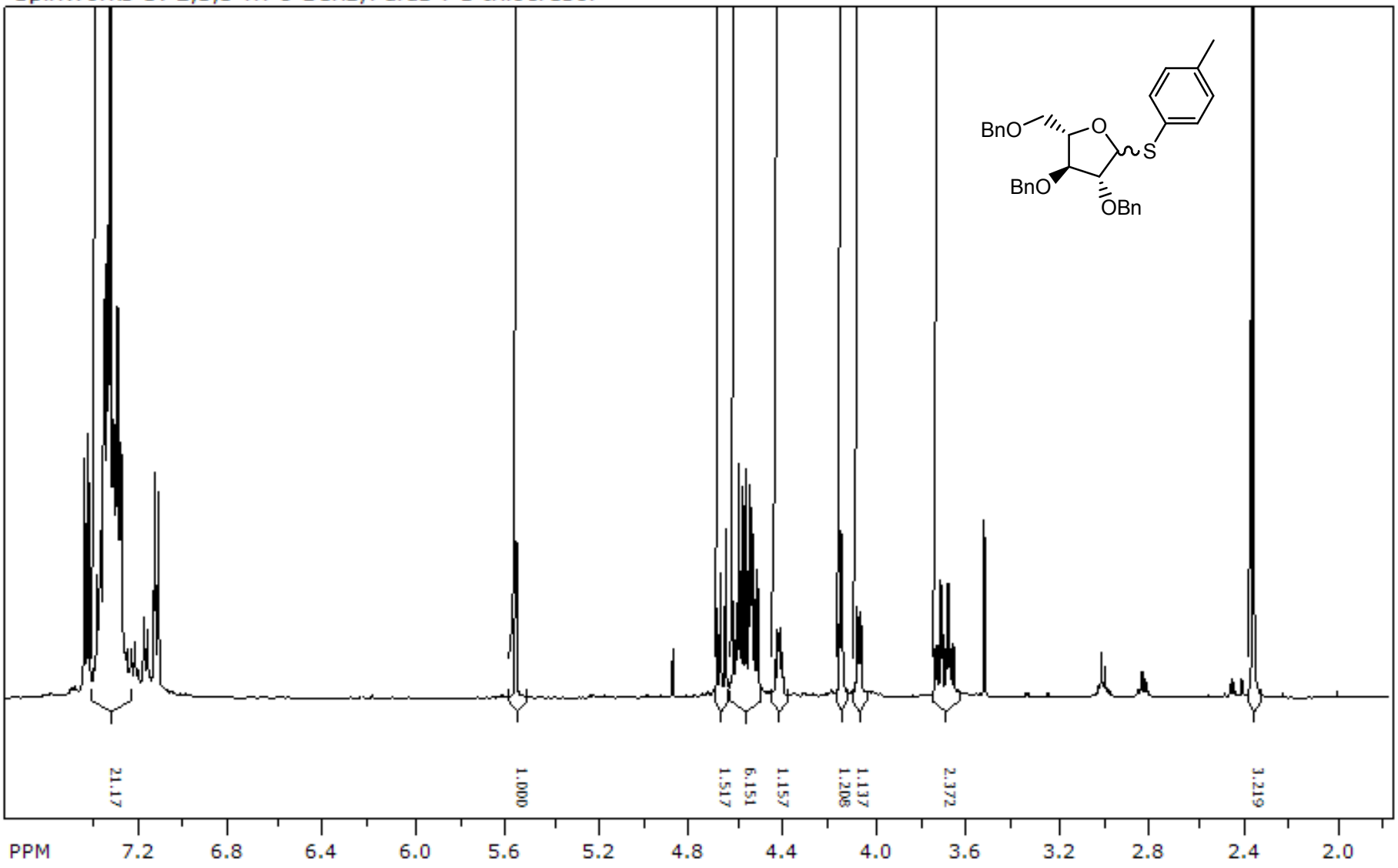
transmitter freq.: 125.770364 MHz
time domain size: 65536 points
width: 29761.90 Hz = 236.6369 ppm = 0.454131 Hz/pt
number of scans: 1024

freq. of 0 ppm: 125.757789 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000
Hz/cm: 1190.476 ppm/cm: 9.46547

Compound 3

SpinWorks 3: 2,3,5 Tri-O-Benzyl-arab-f-1-thiocresol



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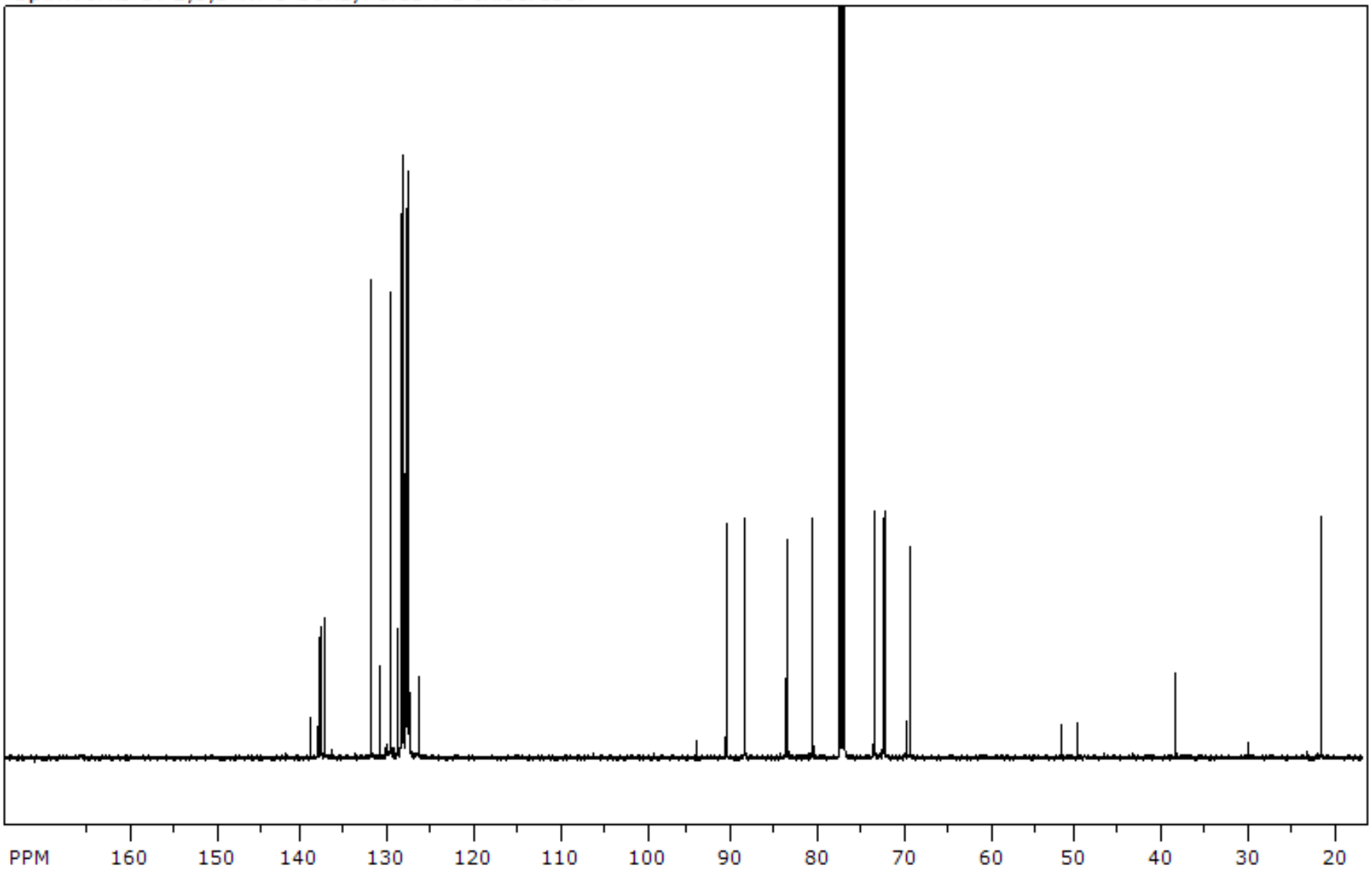
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 64

freq. of 0 ppm: 500.130025 MHz
processed size: 65536 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 120.592 ppm/cm: 0.24112

SpinWorks 3: 2,3,5 Tri-O-Benzyl-arab-f-1-thiocresol



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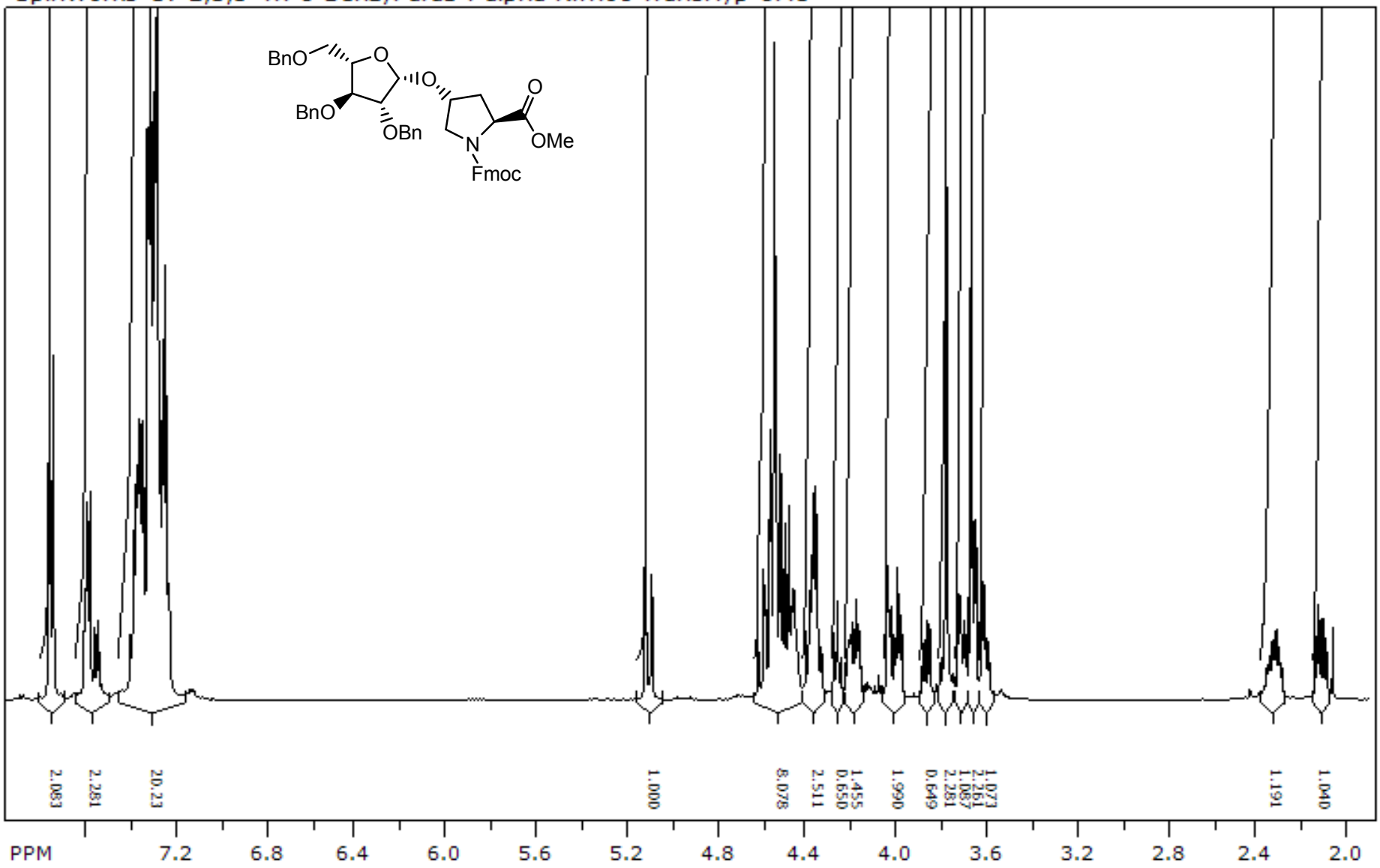
time domain size: 65536 points
width: 29761.90 Hz = 236.6369 ppm = 0.454131 Hz/pt
number of scans: 1024

freq. of 0 ppm: 125.757789 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000
Hz/cm: 796.798 ppm/cm: 6.33534

Compound 5a

SpinWorks 3: 2,3,5 Tri-O-Benzyl-arab-f-alpha-Nfmoc-TransHyp-OMe



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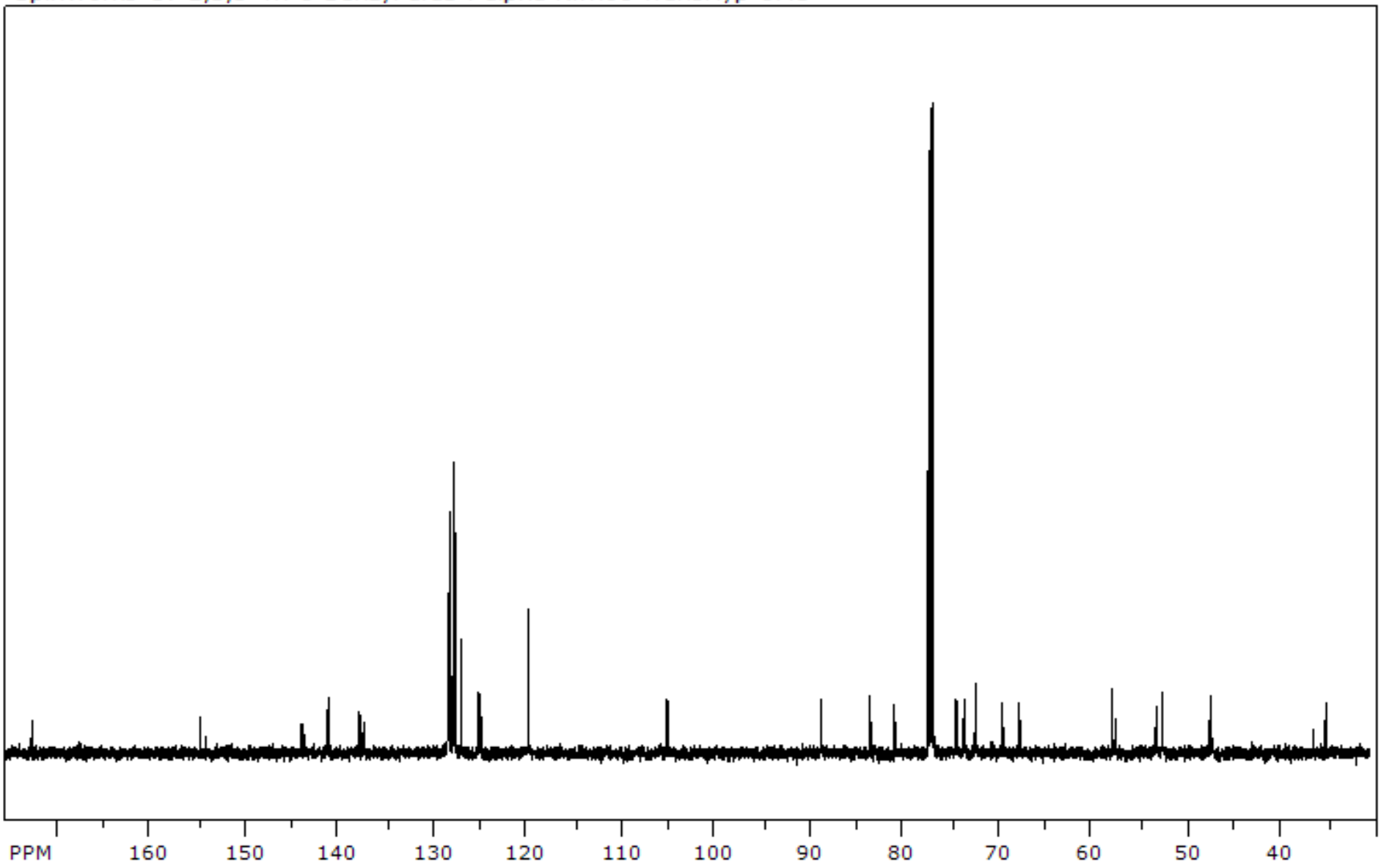
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 16

freq. of 0 ppm: 500.130025 MHz
processed size: 65536 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 126.640 ppm/cm: 0.25321

SpinWorks 3: 2,3,5 Tri-O-Benzyl-arab-f-alpha-Nfmoc-TransHyp-OMe

Compound 5a



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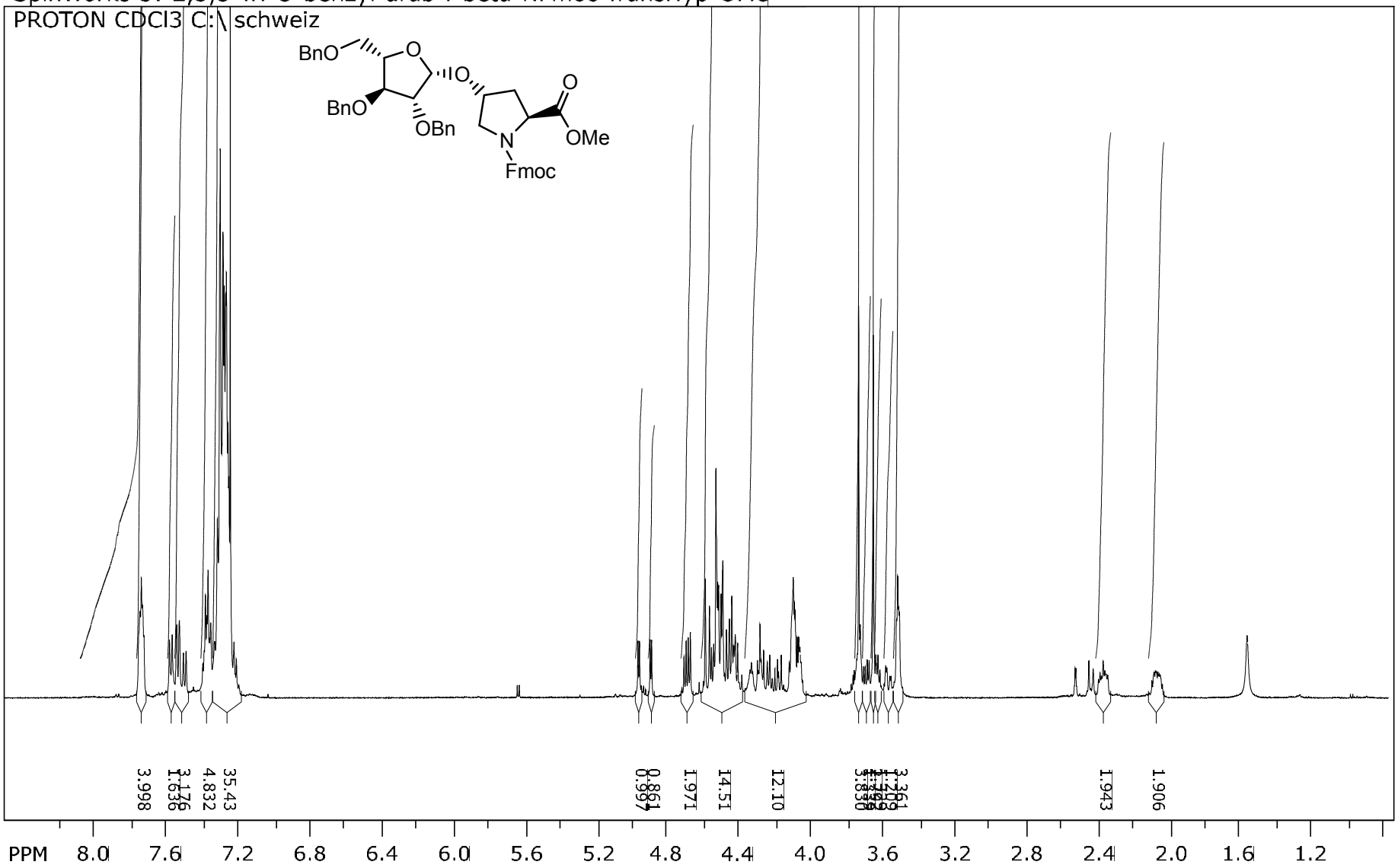
width: 29761.90 Hz = 236.6369 ppm = 0.454131 Hz/pt
number of scans: 64

freq. of 0 ppm: 125.757789 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000
Hz/cm: 762.142 ppm/cm: 6.05979

Compound 5b

SpinWorks 3: 2,3,5 Tri-O-benzyl-arab-f-beta-NFmoc-TransHyp-OMe



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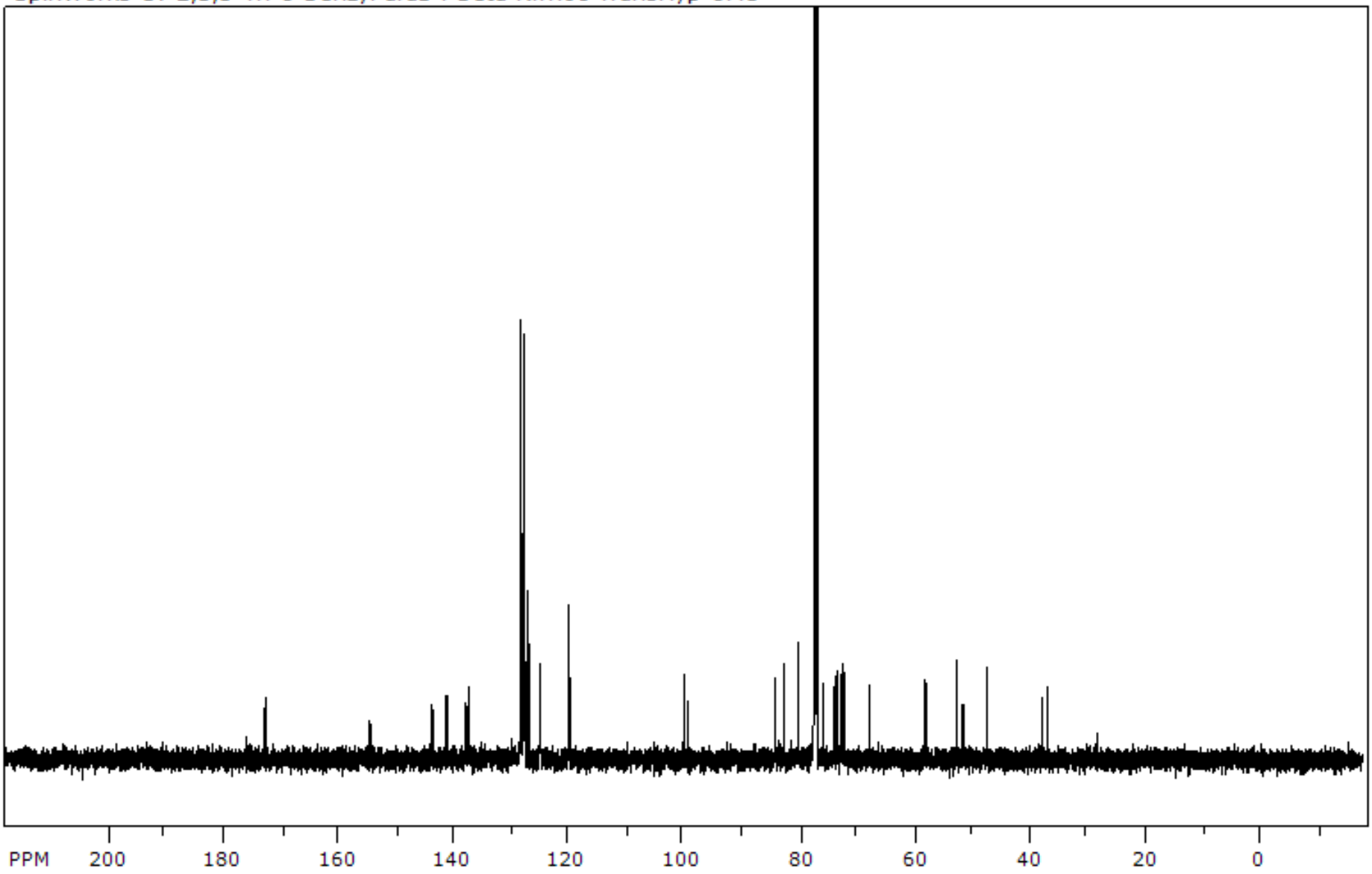
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 16

freq. of 0 ppm: 500.130025 MHz
processed size: 65536 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 155.327 ppm/cm: 0.31057

SpinWorks 3: 2,3,5 Tri-O-benzyl-arab-f-beta-Nfmoc-TransHyp-OMe

Compound 5b



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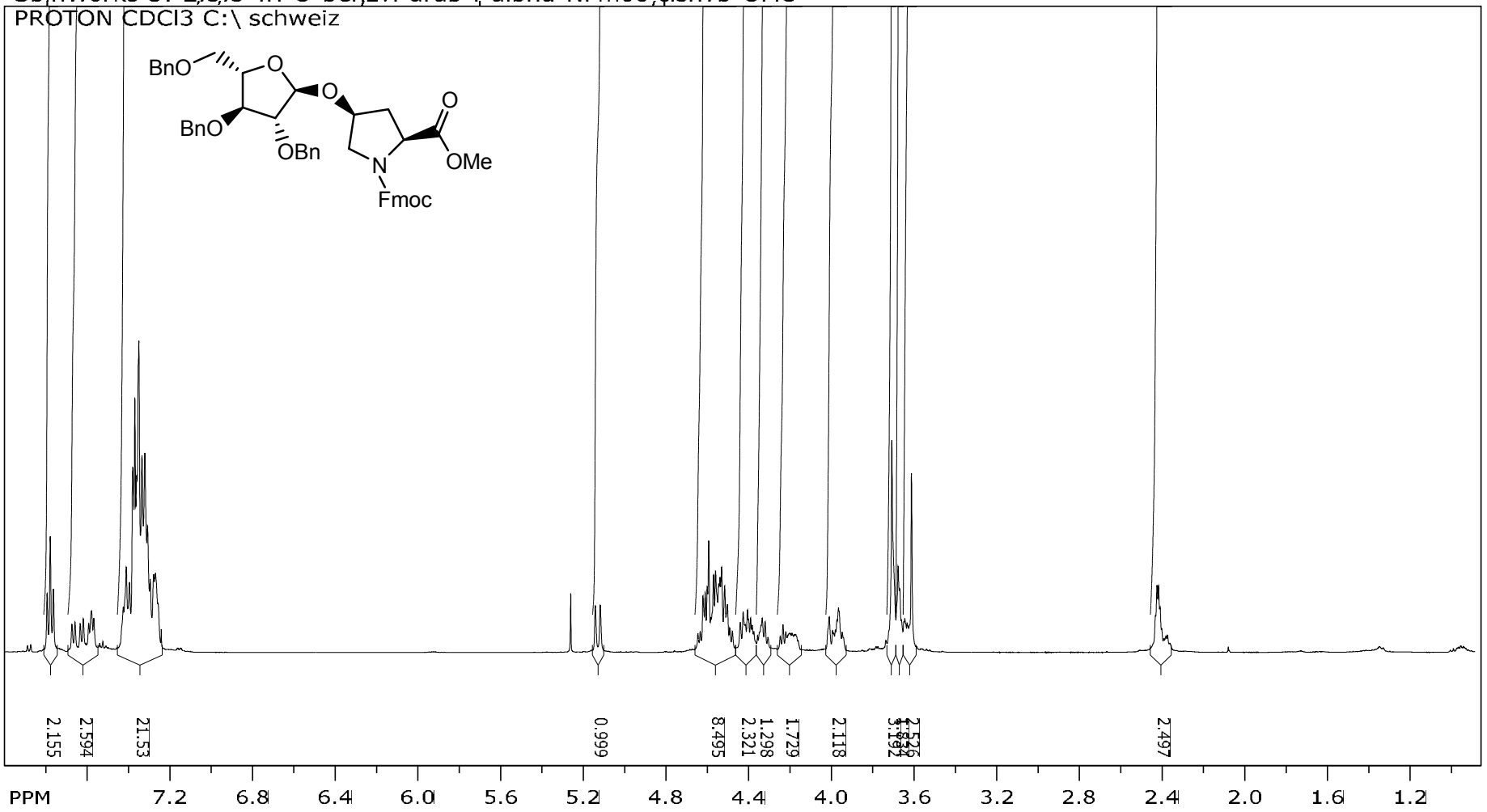
number of scans: 1258

freq. of 0 ppm: 125.757789 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000
Hz/cm: 1190.476 ppm/cm: 9.46547

SpinWorks 3: 2,3,5 Tri-O-benzyl-arab-f-alpha-NFmoc-cishyp-OMe

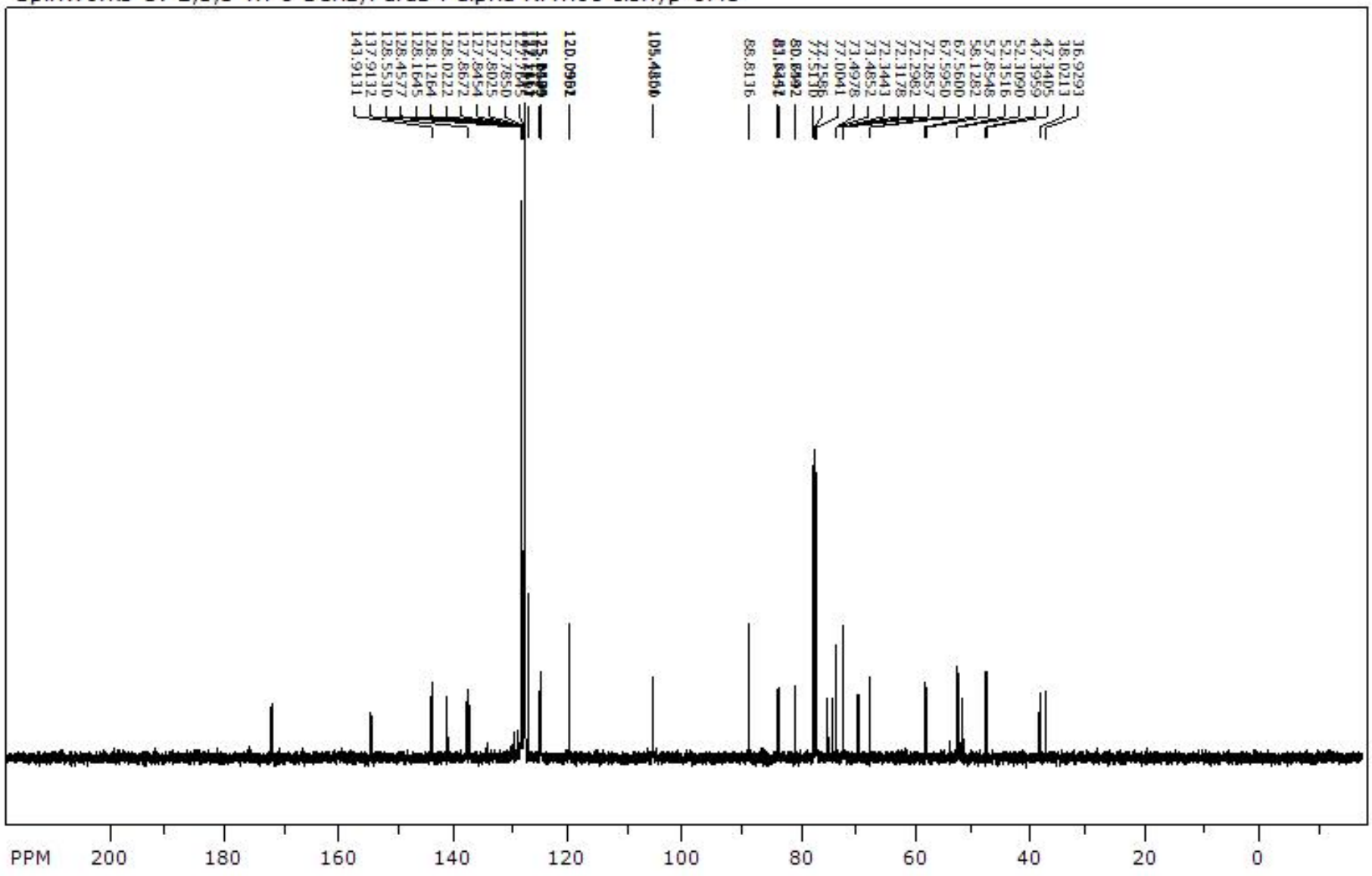


file: ...a-tobeincluded\MVR-2-101C-CA\1\fid -expt: <zg30>
transmitter freq.: 500.133089 MHz
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 16

freq. of 0 ppm: 500.130025 MHz
processed size: 65536 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 142.875 ppm/cm: 0.28567

Compound 10a

SpinWorks 3: 2,3,5 Tri-O-benzyl-arab-f-alpha-NFmoc-cishyp-OMe

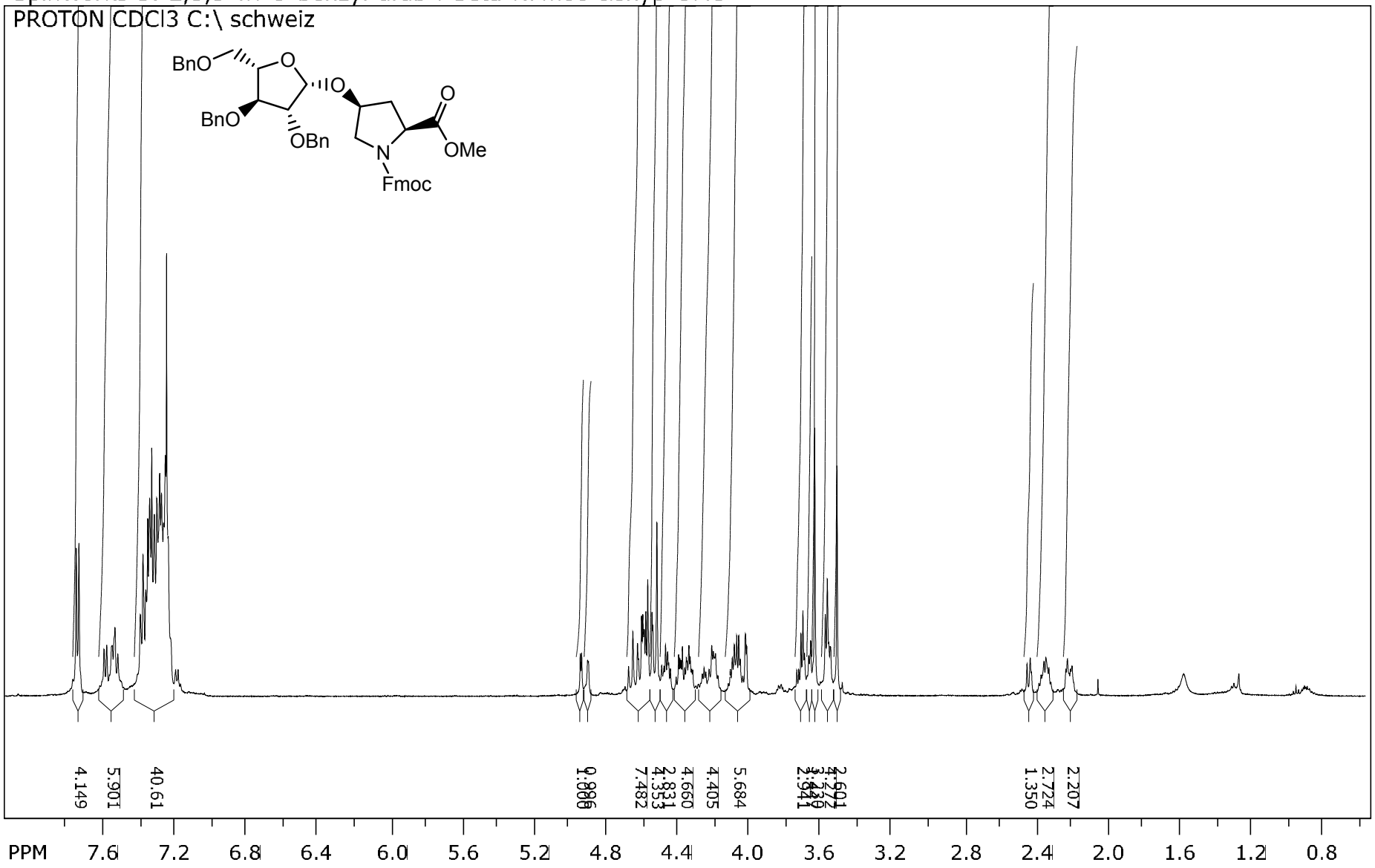


file: ...a-tobeincluded\MVR-2-101C-CA\2\fid expt: <zgpg30>
transmitter freq.: 125.770364 MHz
time domain size: 65536 points
width: 29761.90 Hz = 236.6369 ppm = 0.454131 Hz/pt
number of scans: 32

freq. of 0 ppm: 125.757789 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000
Hz/cm: 1190.476 ppm/cm: 9.46547

Compound 10b

SpinWorks 3: 2,3,5 Tri-O-benzyl-arab-f-beta-NFmoc-cishyp-OMe

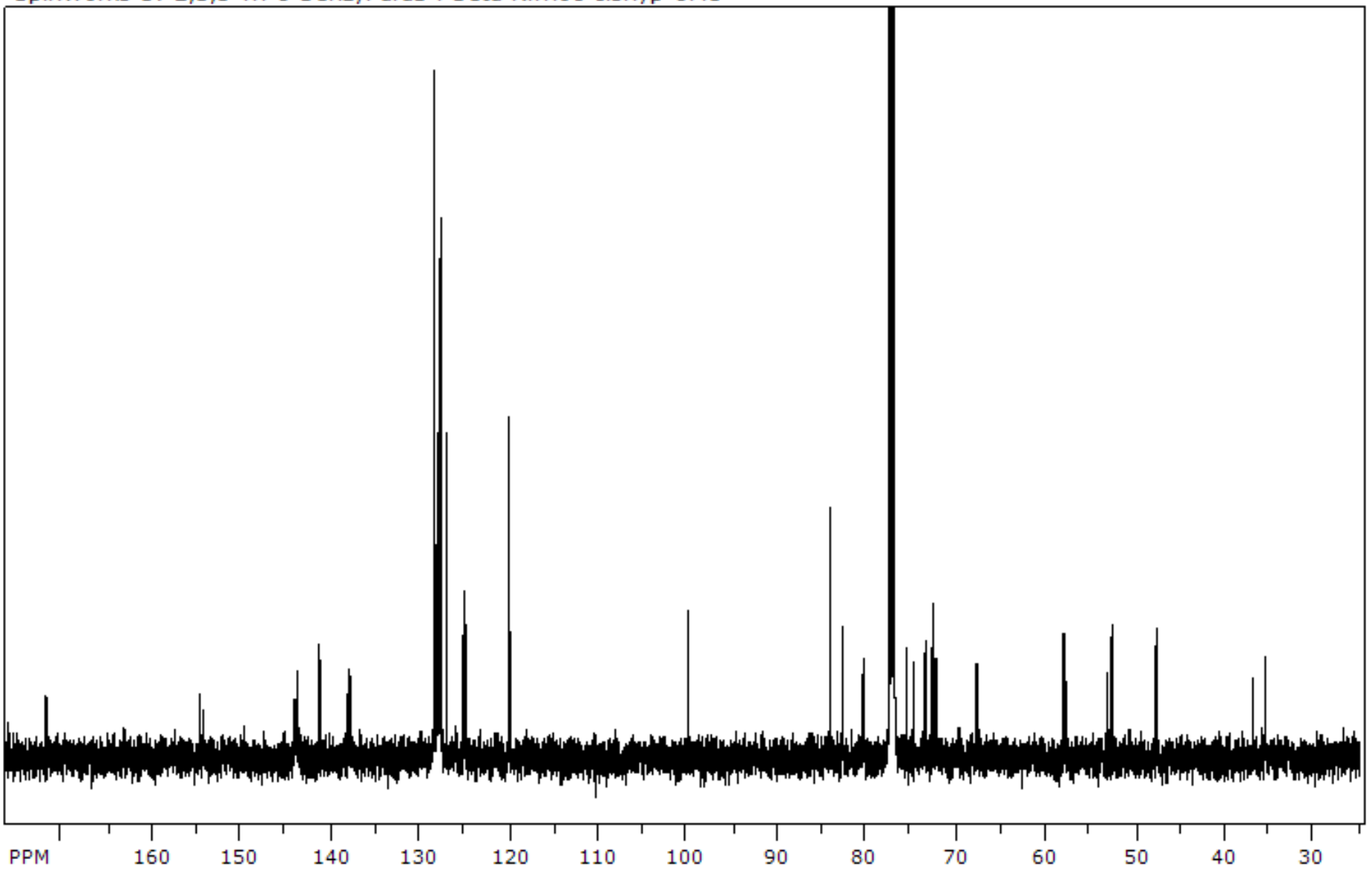


file: ...a-tobeincluded\MVR-2-101D-CB\1\fid expt: <zg30>
transmitter freq.: 500.133089 MHz
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 16

freq. of 0 ppm: 500.130025 MHz
processed size: 65536 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 152.378 ppm/cm: 0.30468

Compound 10b

SpinWorks 3: 2,3,5 Tri-O-benzyl-arab-f-beta-Nfmoc-cishyp-OMe

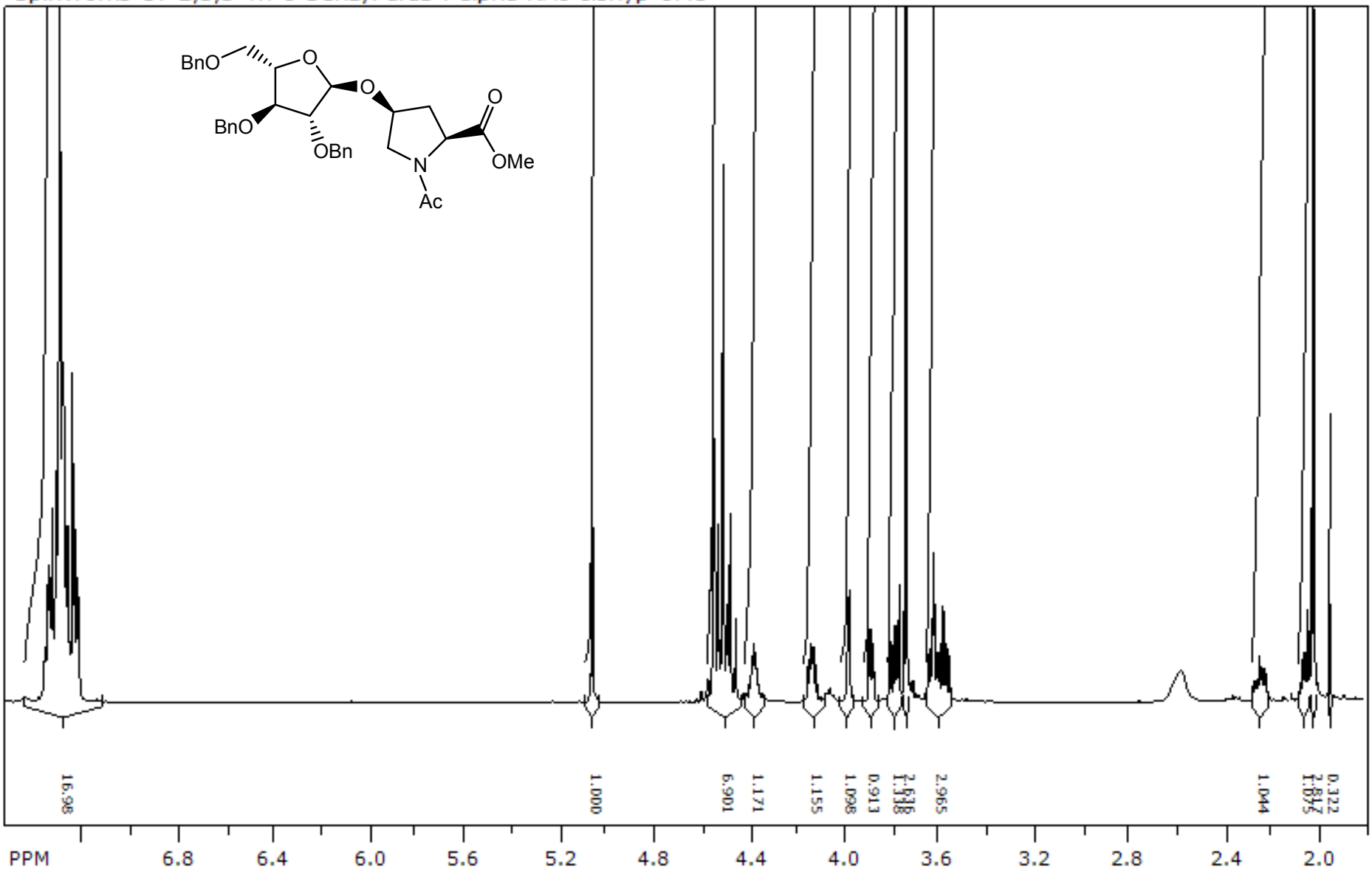


file: ...beincluded\MVR-2-101D-CB-C13\1\fid expt: <zgpg30>
transmitter freq.: 125.770364 MHz
time domain size: 65536 points
width: 29761.90 Hz = 236.6369 ppm = 0.454131 Hz/pt
number of scans: 1024

freq. of 0 ppm: 125.757789 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000
Hz/cm: 765.643 ppm/cm: 6.08763

SpinWorks 3: 2,3,5 Tri-O-benzyl-arab-f-alpha-NAc-cishyp-OMe

Compound 11a

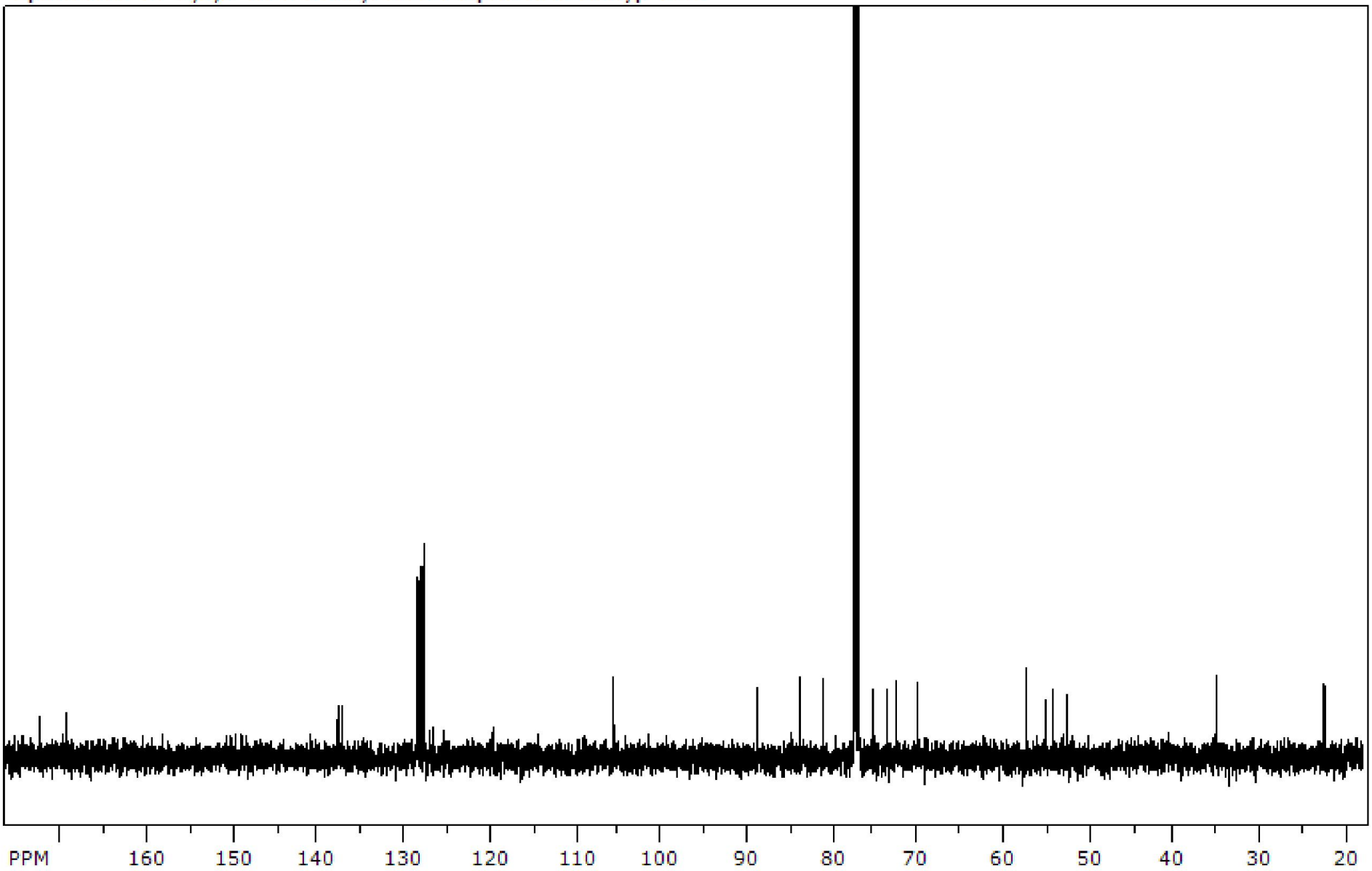


file: ...data-tobeincluded\MVR-2-100B\1\fid expt: <zg30>
transmitter freq.: 500.133089 MHz
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 16

freq. of 0 ppm: 500.130025 MHz
processed size: 65536 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 114.693 ppm/cm: 0.22933

Compound 11a

SpinWorks 3: 2,3,5 Tri-O-benzyl-arab-f-alpha-NAc-cishyp-OMe

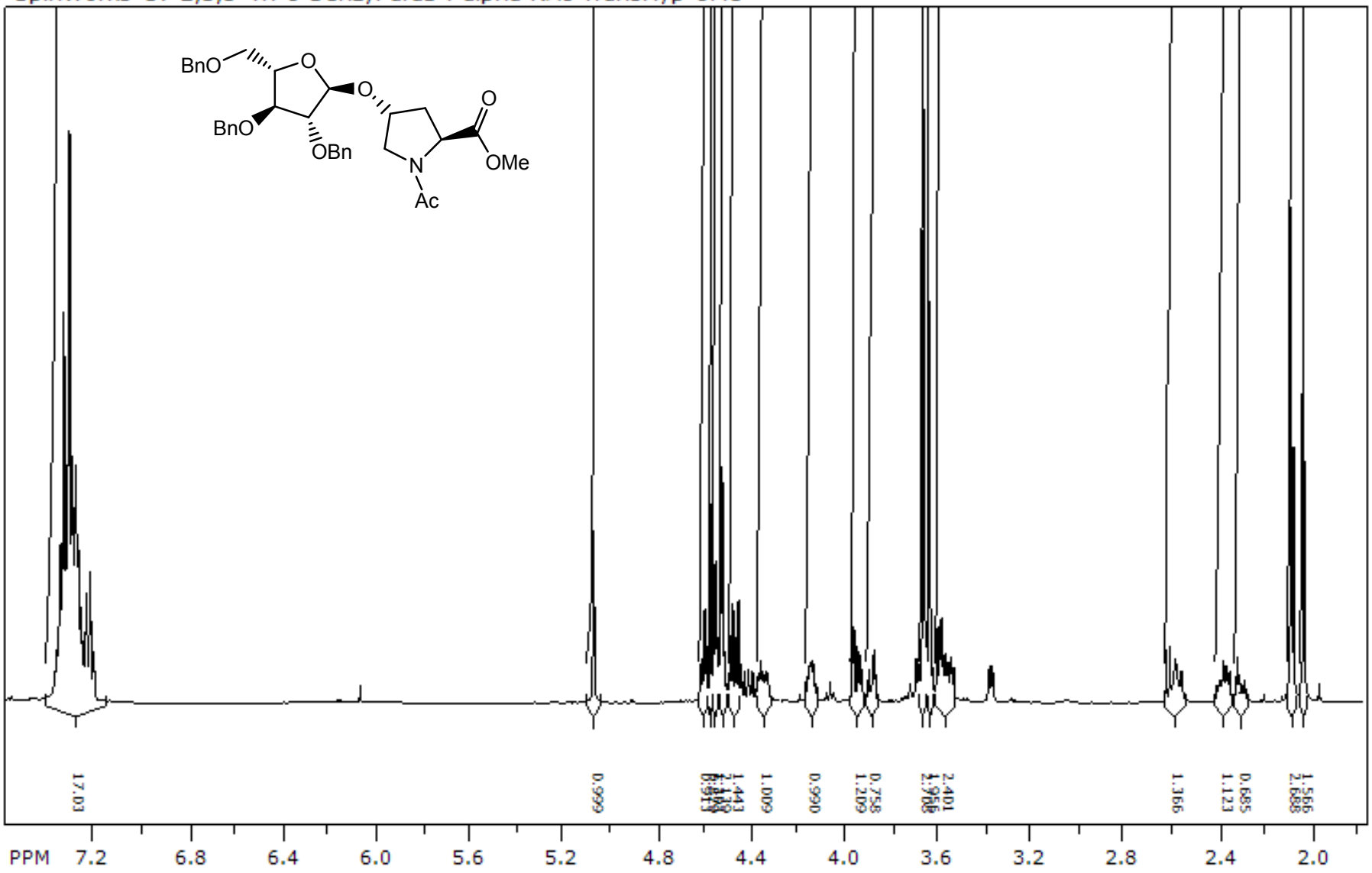


file: ...data-tobeincluded\MVR-2-100B\2\fid expt: <zgpg30>
transmitter freq.: 125.770364 MHz
time domain size: 65536 points
width: 29761.90 Hz = 236.6369 ppm = 0.454131 Hz/pt
number of scans: 64

freq. of 0 ppm: 125.757789 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000
Hz/cm: 800.574 ppm/cm: 6.36536

Compound 6a

SpinWorks 3: 2,3,5 Tri-O-benzyl-arab-f-alpha-NAc-TransHyp-OMe

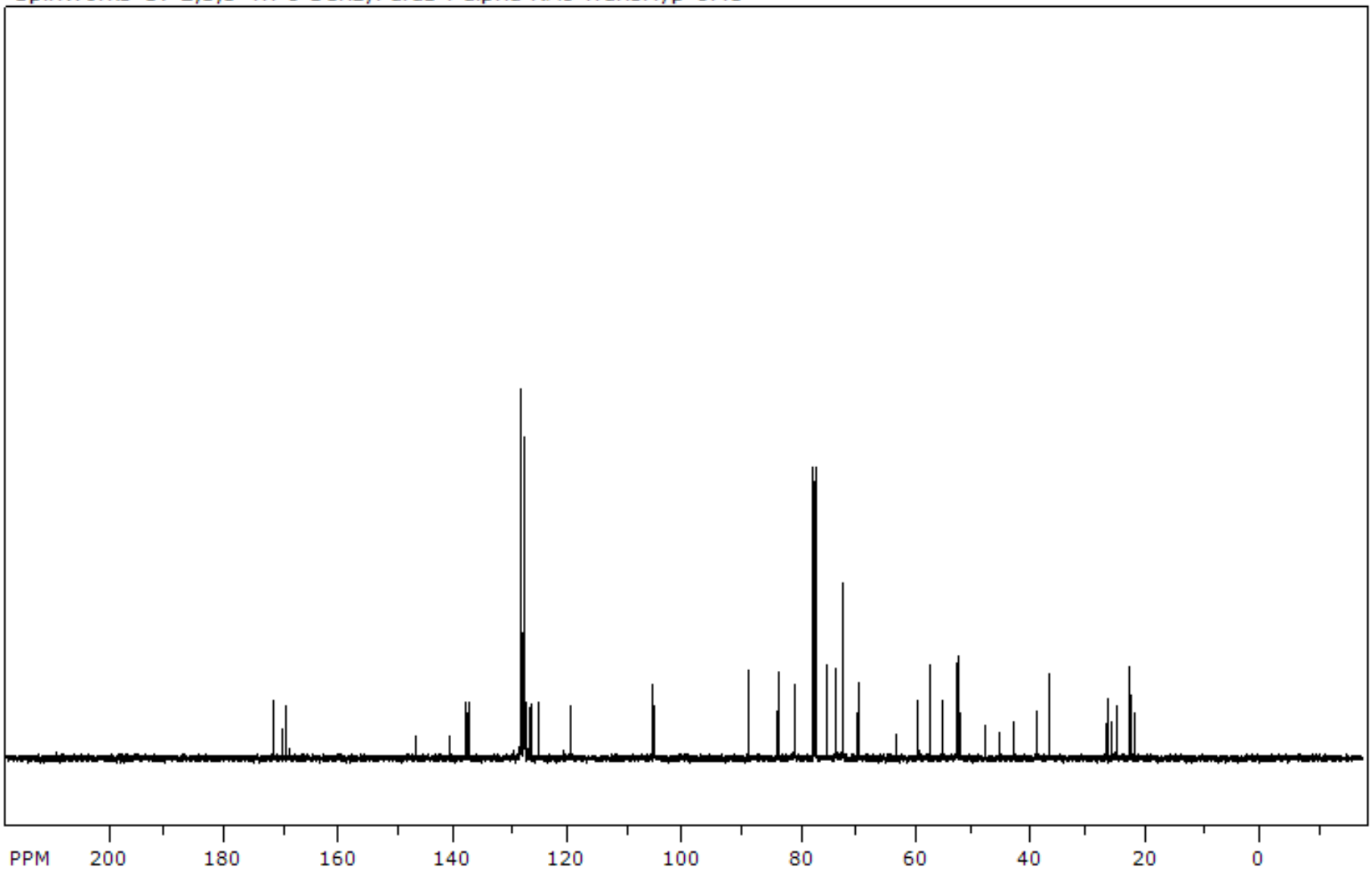


file: ...data-tobeincluded\MVR-2-100A\1\fid expt: <zg30>
transmitter freq.: 500.133089 MHz
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 16

freq. of 0 ppm: 500.130025 MHz
processed size: 65536 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 116.332 ppm/cm: 0.23260

SpinWorks 3: 2,3,5 Tri-O-benzyl-arab-f-alpha-NAc-TransHyp-OMe

Compound 6a

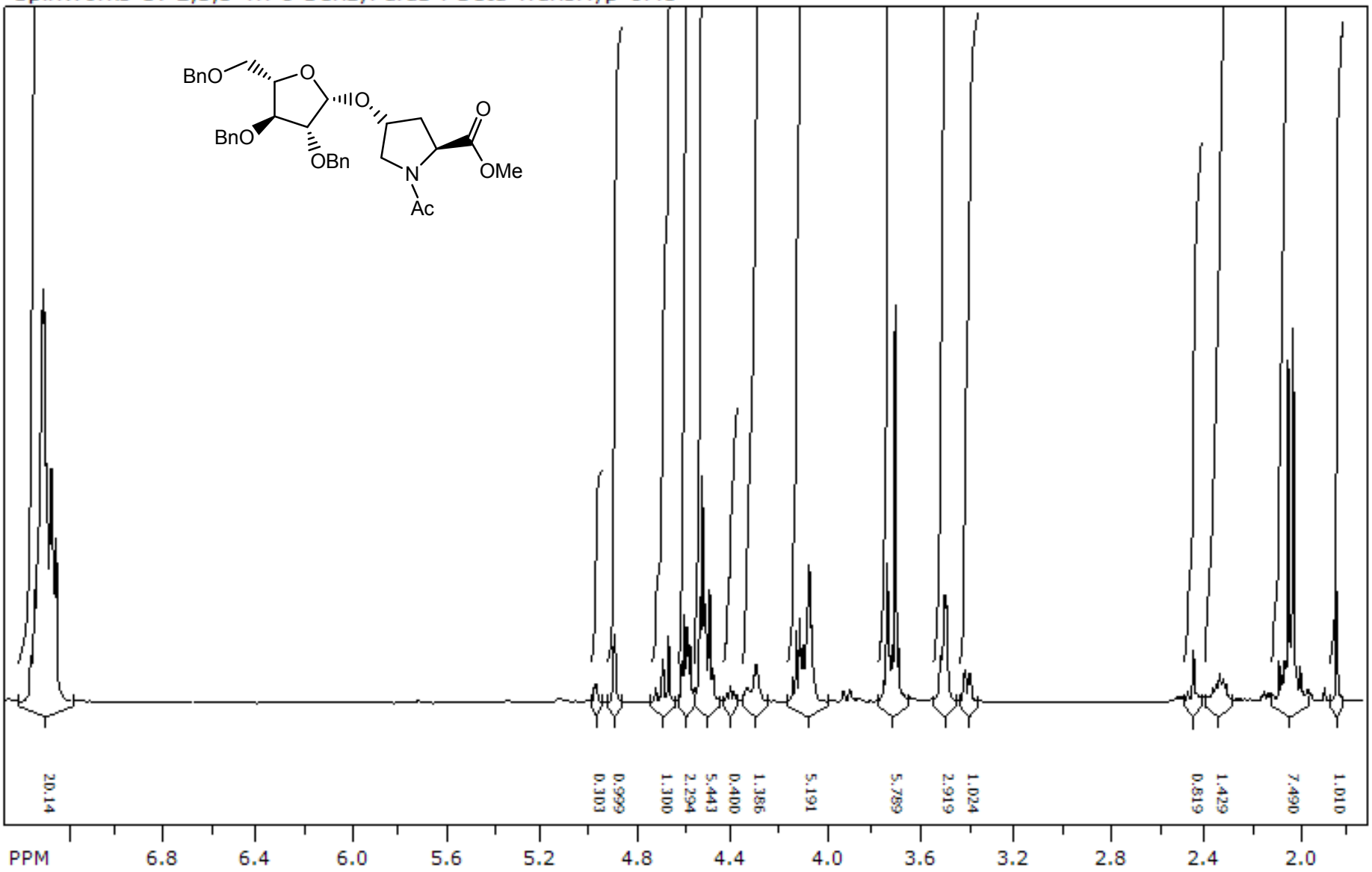


file: ...data-tobeincluded\MVR-2-100A\2\fid expt: <zgpg30>
transmitter freq.: 125.770364 MHz
time domain size: 65536 points
width: 29761.90 Hz = 236.6369 ppm = 0.454131 Hz/pt
number of scans: 64

freq. of 0 ppm: 125.757789 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000
Hz/cm: 1190.476 ppm/cm: 9.46547

Compound 6b

SpinWorks 3: 2,3,5 Tri-O-benzyl-arab-f-beta-TransHyp-OMe

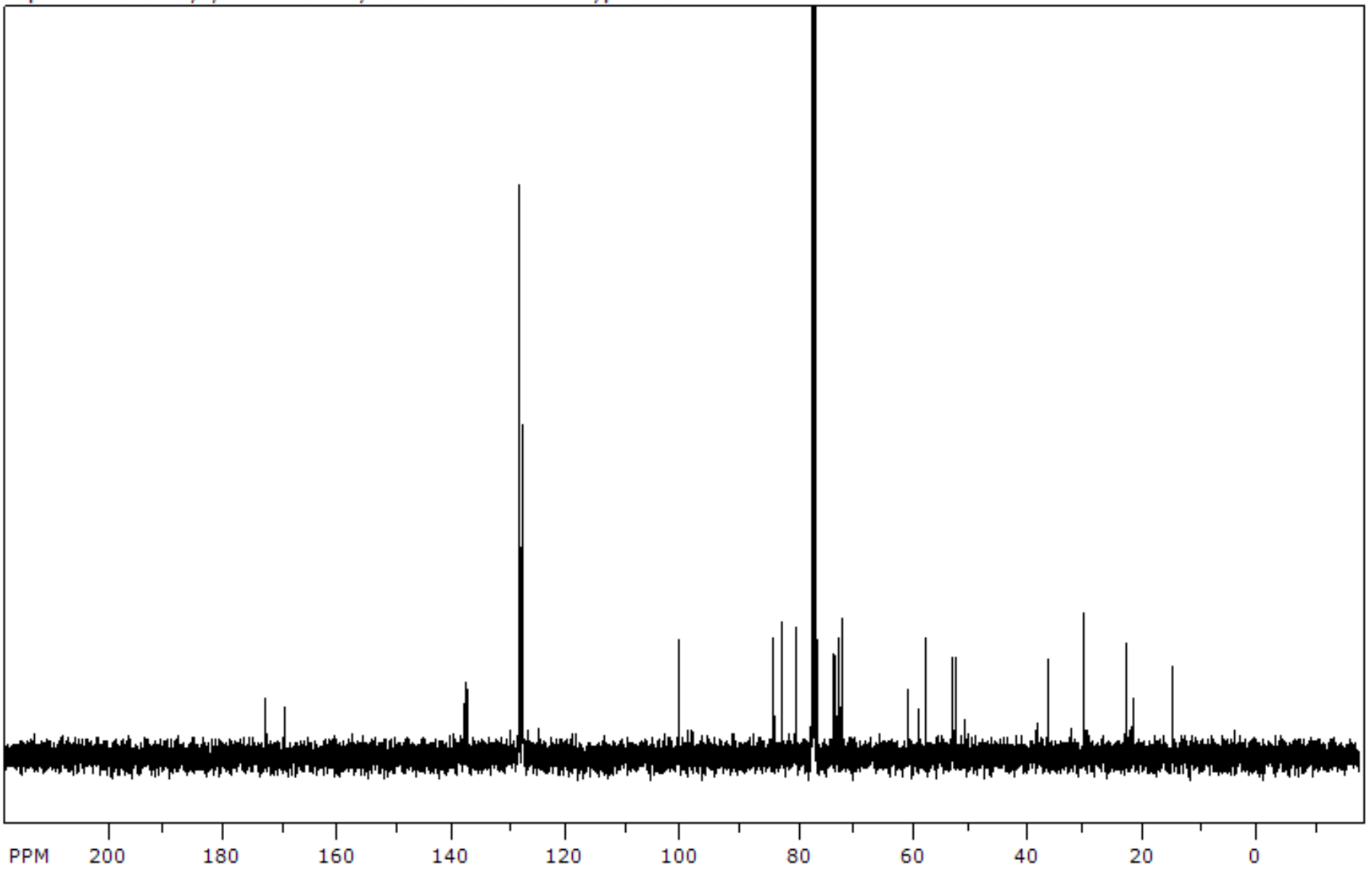


file: ... data-tobeincluded\MVR-2-99A\1\fid expt: <zg30>
transmitter freq.: 500.133089 MHz
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 16

freq. of 0 ppm: 500.130025 MHz
processed size: 65536 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 115.021 ppm/cm: 0.22998

Compound 6b

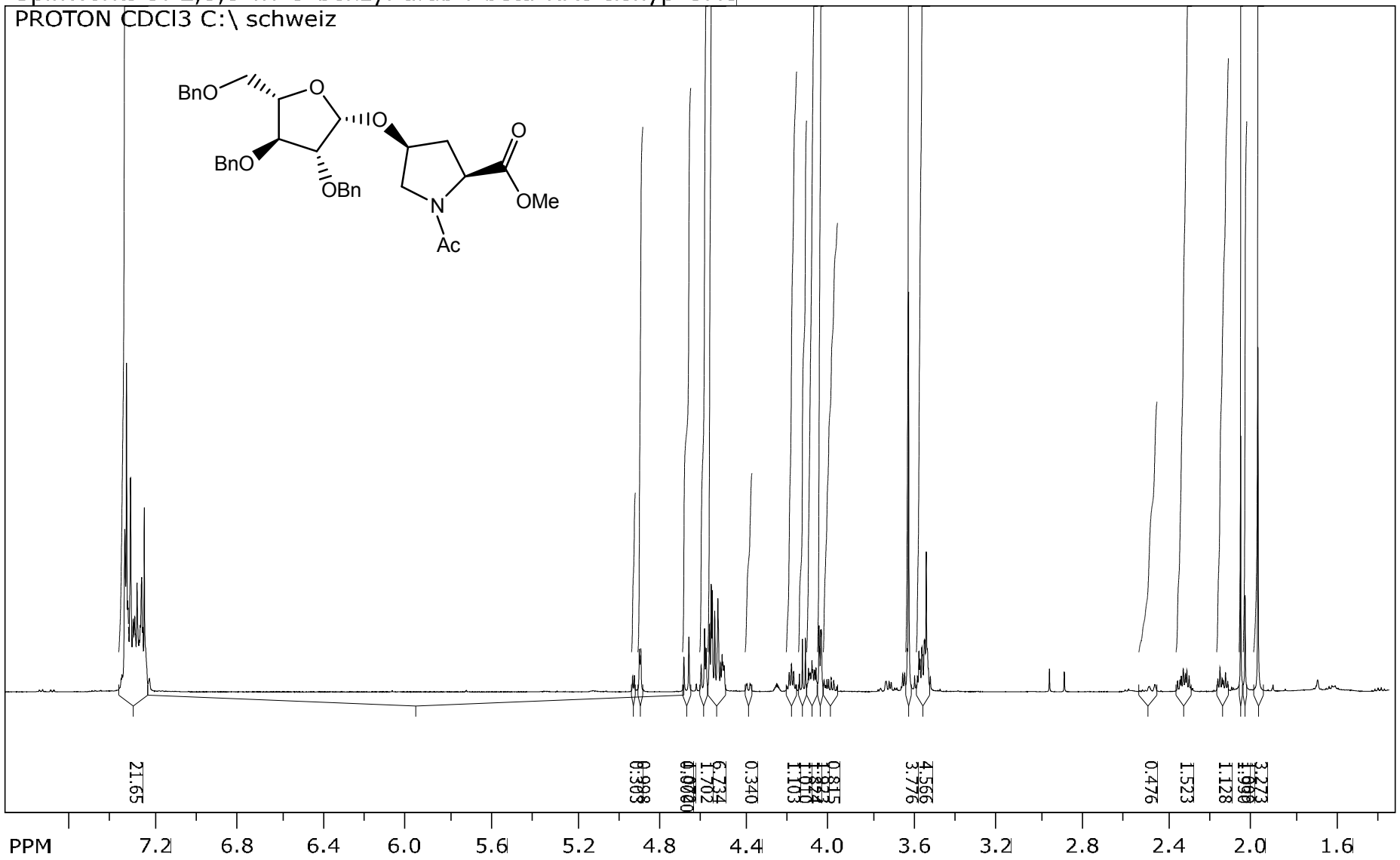
SpinWorks 3: 2,3,5 Tri-O-benzyl-arab-f-beta-TransHyp-OMe



file: ... data-tobeincluded\MVR-2-99A\2\fid expt: <zgpg30>
transmitter freq.: 125.770364 MHz
time domain size: 65536 points
width: 29761.90 Hz = 236.6369 ppm = 0.454131 Hz/pt
number of scans: 128

freq. of 0 ppm: 125.757789 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000
Hz/cm: 1190.476 ppm/cm: 9.46547

SpinWorks 3: 2,3,5 Tri-O-benzyl-arab-f-beta-NAc-cishyp-OMe

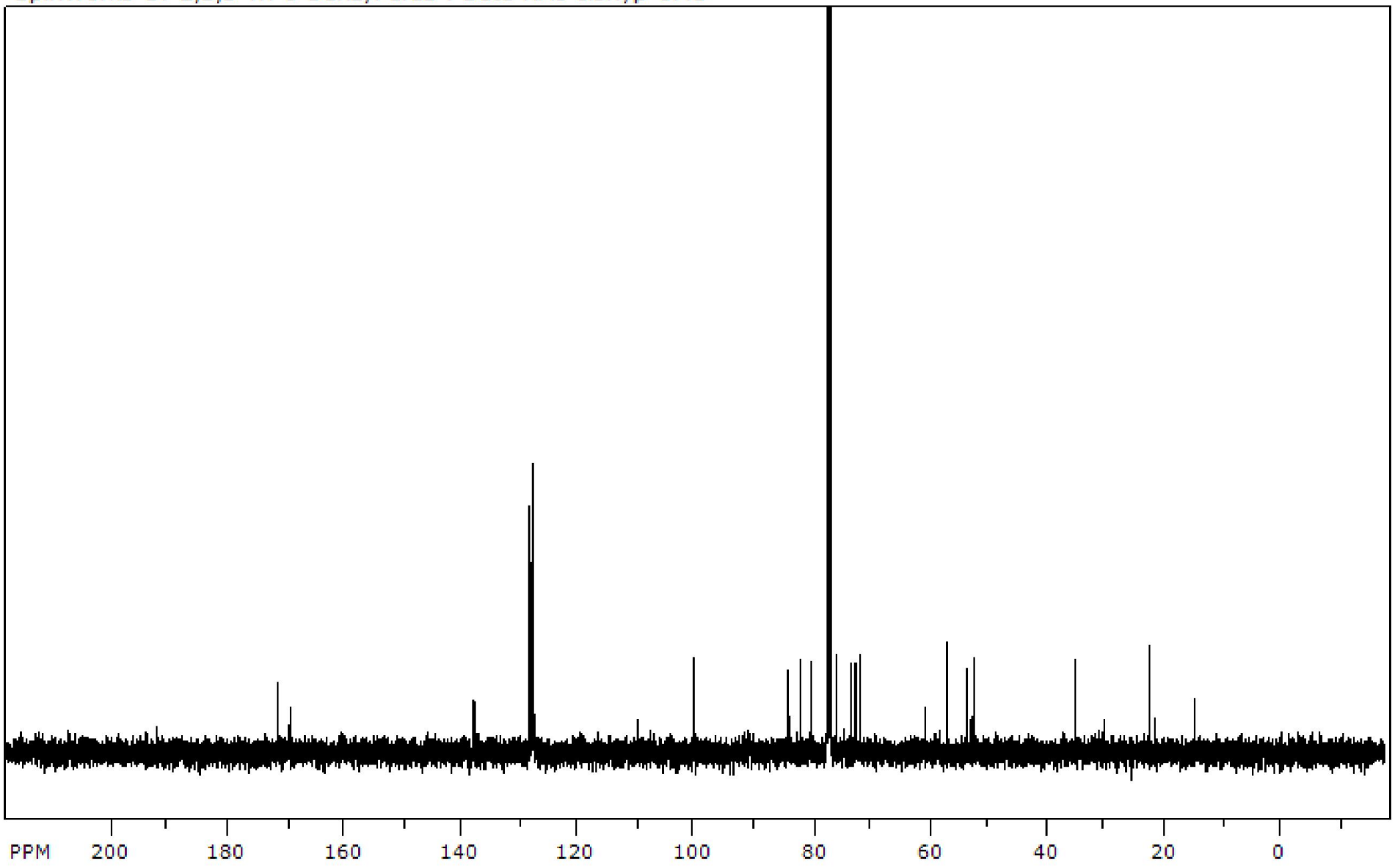


file: ... data-tobeincluded\MVR-2-99B\1\fid .expt: <zg30>
transmitter freq.: 500.133089 MHz
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 16

freq. of 0 ppm: 500.130025 MHz
processed size: 65536 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 137.222 ppm/cm: 0.27437

SpinWorks 3: 2,3,5 Tri-O-benzyl-arab-f-beta-NAc-cishyp-OMe

Compound 11b

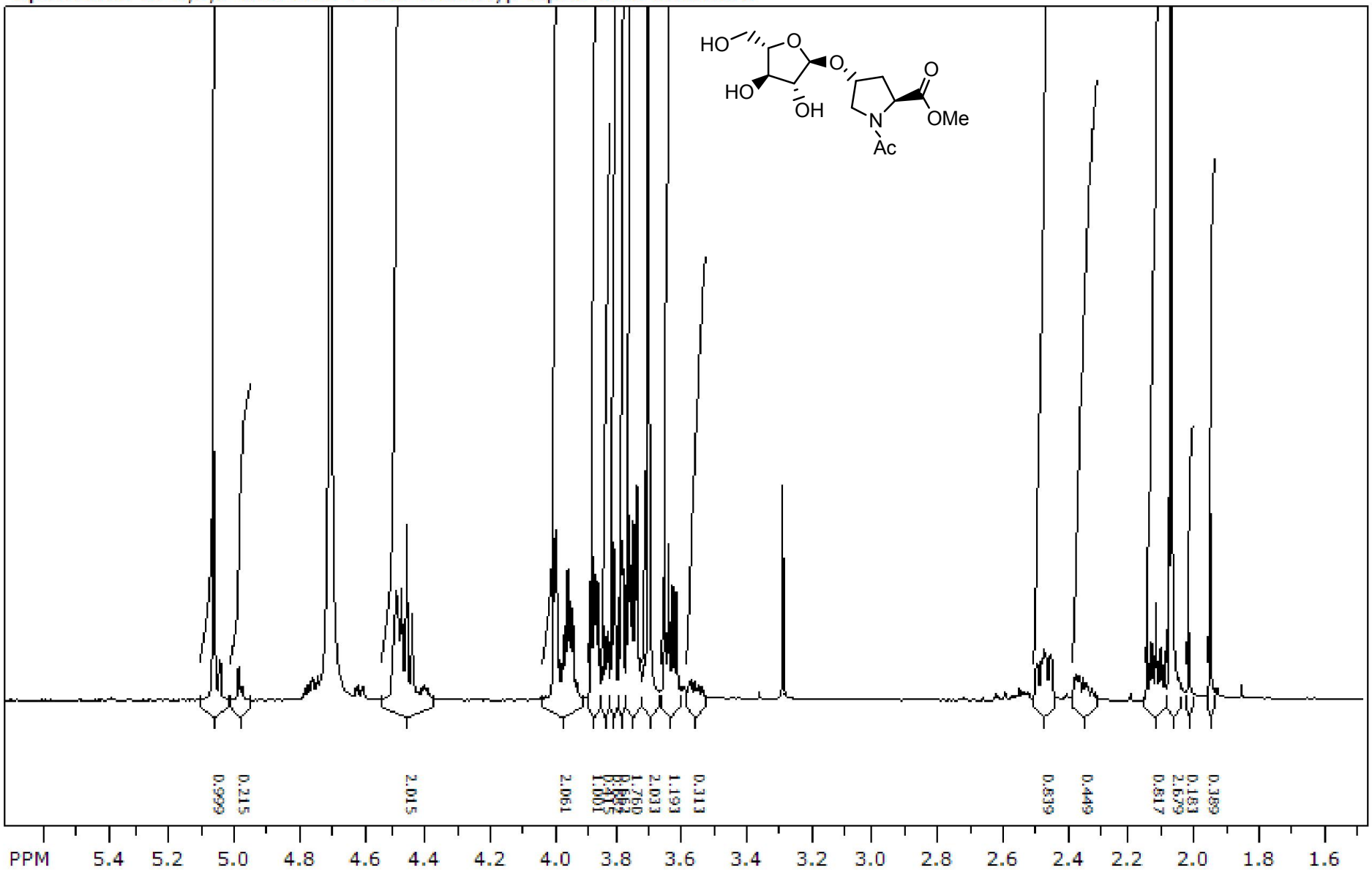


file: ... data-tobeincluded\MVR-2-99B\2\fid expt: <zgpg30>
transmitter freq.: 125.770364 MHz
time domain size: 65536 points
width: 29761.90 Hz = 236.6369 ppm = 0.454131 Hz/pt
number of scans: 128

freq. of 0 ppm: 125.757789 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000
Hz/cm: 1190.476 ppm/cm: 9.46547

Compound 7a

SpinWorks 3: 2,3,5 triol-arab-f-NAc-TransHyp-alpha-OMe anomer1

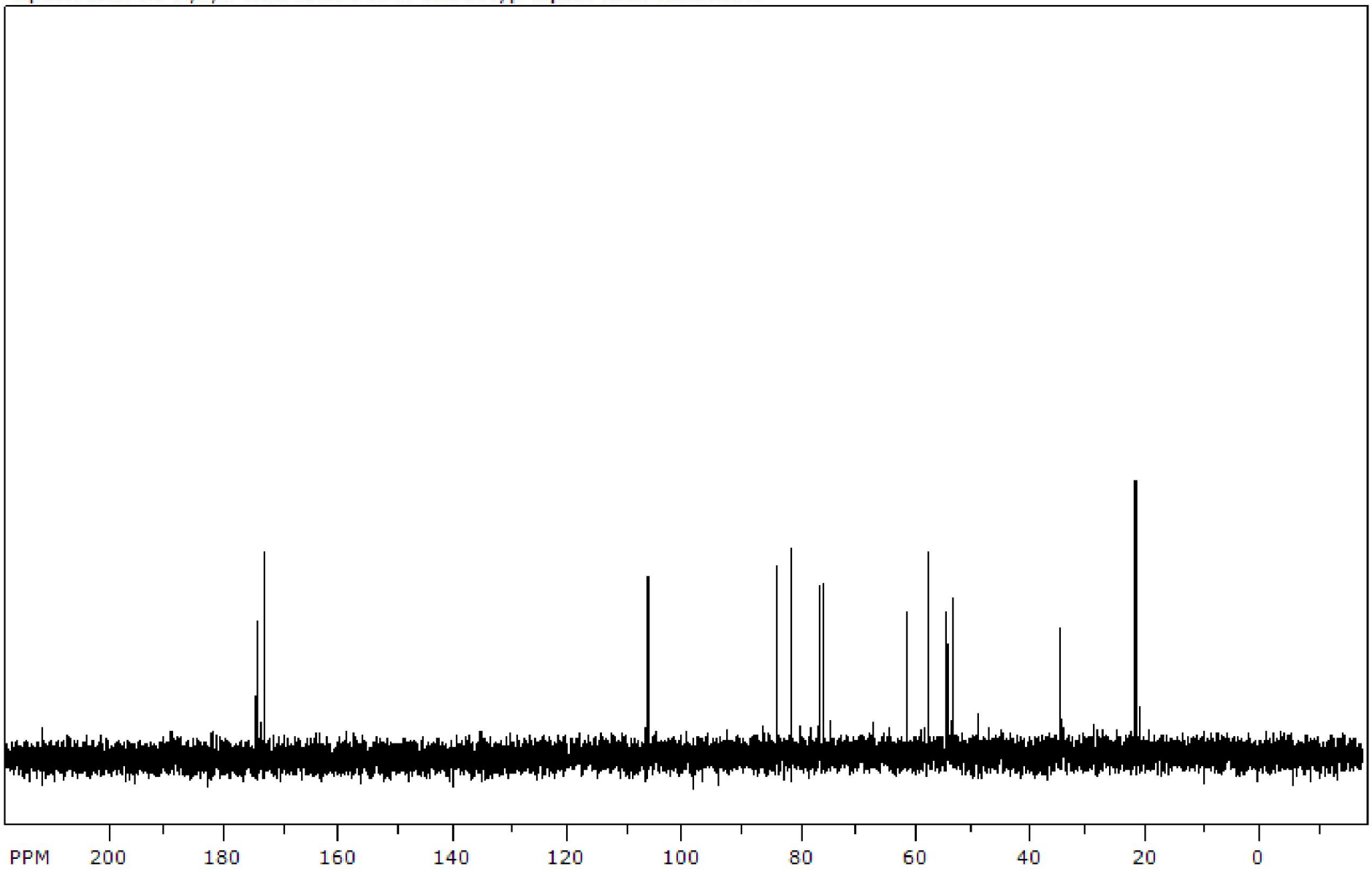


file: ...pha-arabinose-mvr-2-73A\proton\fid expt: <zg30>
transmitter freq.: 500.133089 MHz
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 32

freq. of 0 ppm: 500.130000 MHz
processed size: 65536 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 85.201 ppm/cm: 0.17036

SpinWorks 3: 2,3,5 Triol-arab-f-NAc-TransHyp-alpha-OMe anomer1

Compound 7a

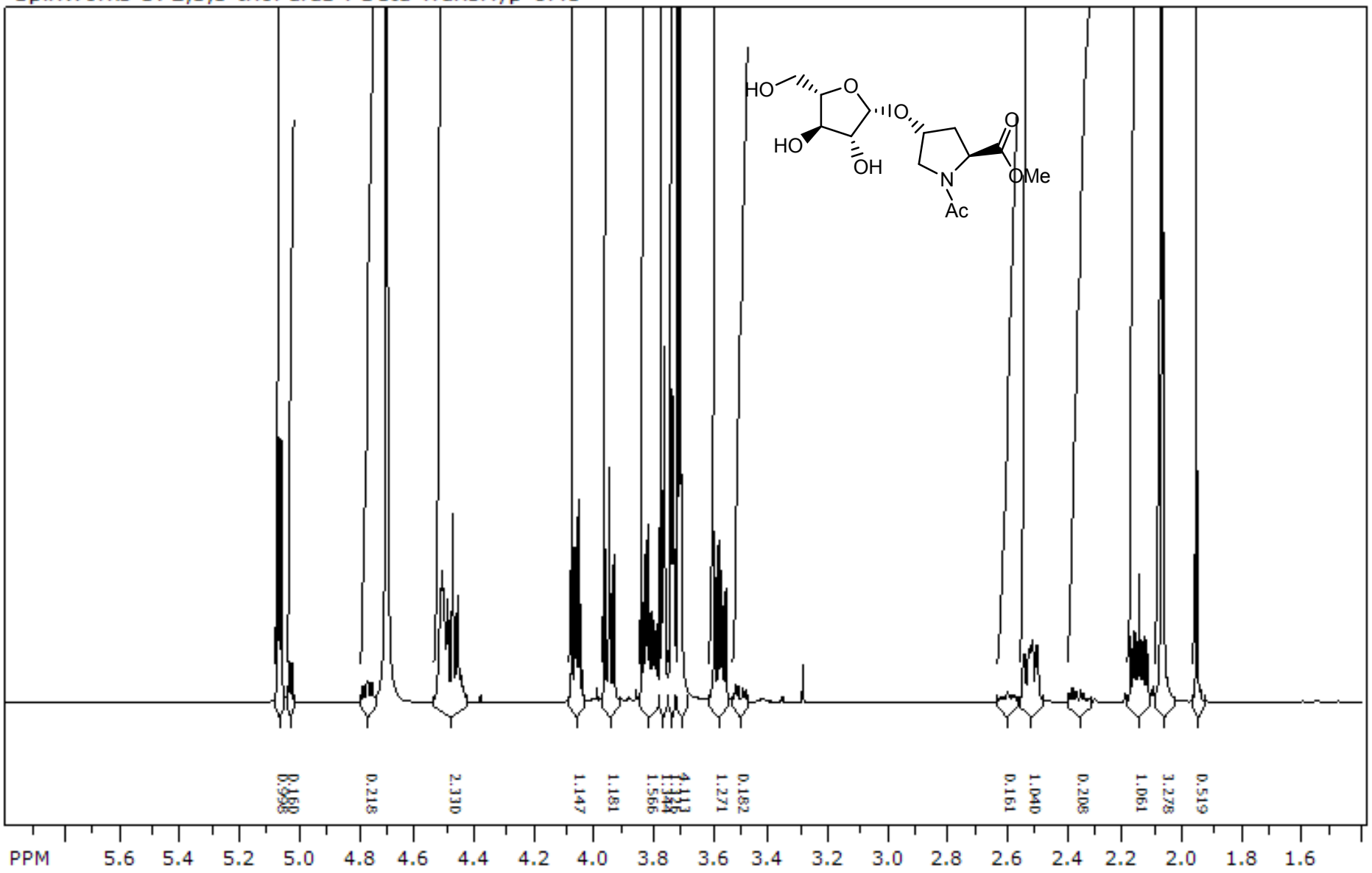


file: ...pha-arabinose-mvr-2-73A\c13ig2\fid expt: <zgig30>
transmitter freq.: 125.770364 MHz
time domain size: 65536 points
width: 29761.90 Hz = 236.6369 ppm = 0.454131 Hz/pt
number of scans: 8192

freq. of 0 ppm: 125.757789 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000
Hz/cm: 1190.476 ppm/cm: 9.46547

SpinWorks 3: 2,3,5 triol-arab-f-beta-TransHyp-OMe

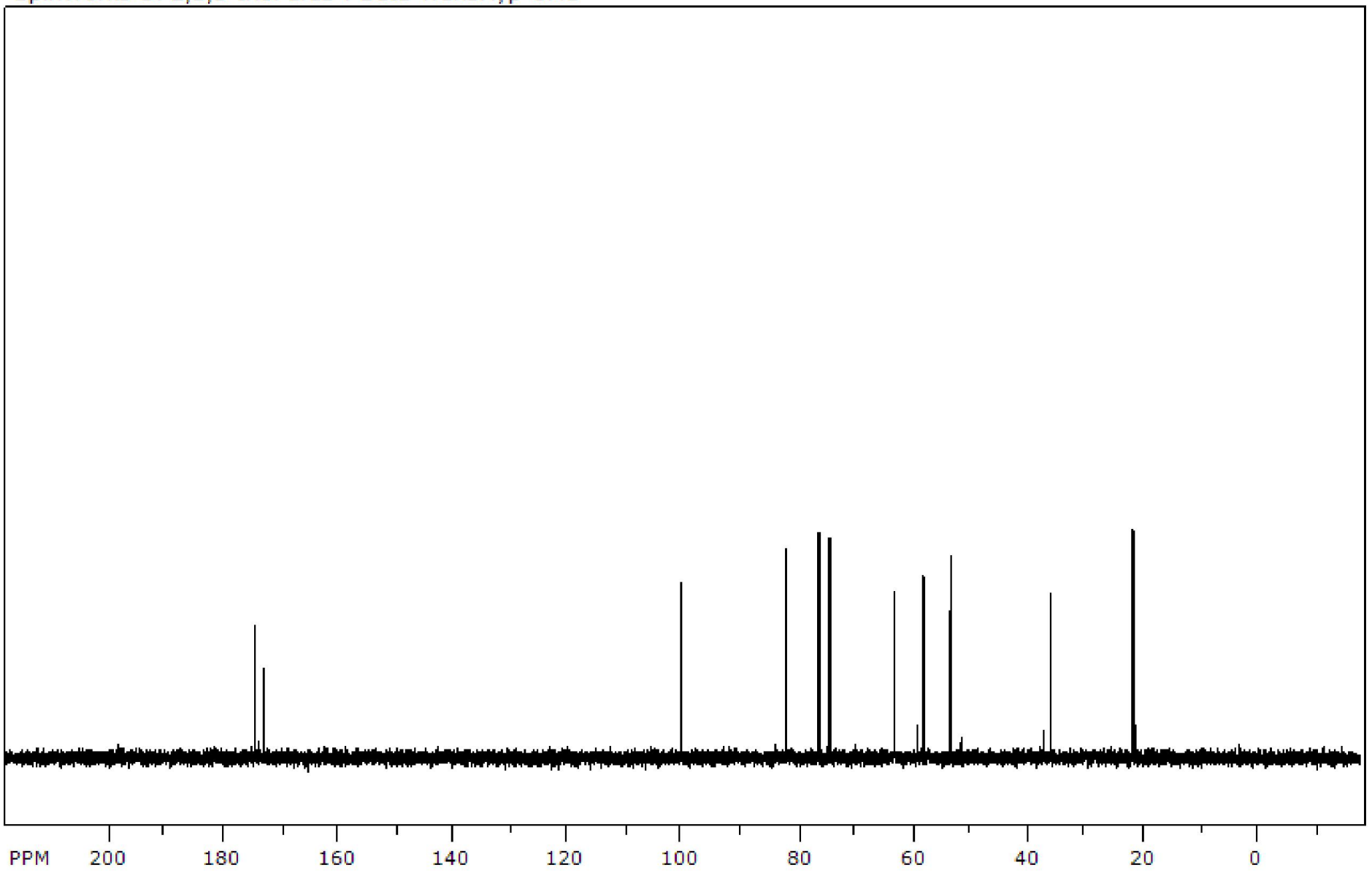
Compound 7b



file: ...arabinose-MVR-2-92A\MVR-2-92\1\fid expt: <zg30>
transmitter freq.: 500.133089 MHz
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 32

freq. of 0 ppm: 500.130000 MHz
processed size: 65536 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 92.410 ppm/cm: 0.18477

SpinWorks 3: 2,3,5 triol-arab-f-beta-TransHyp-OMe

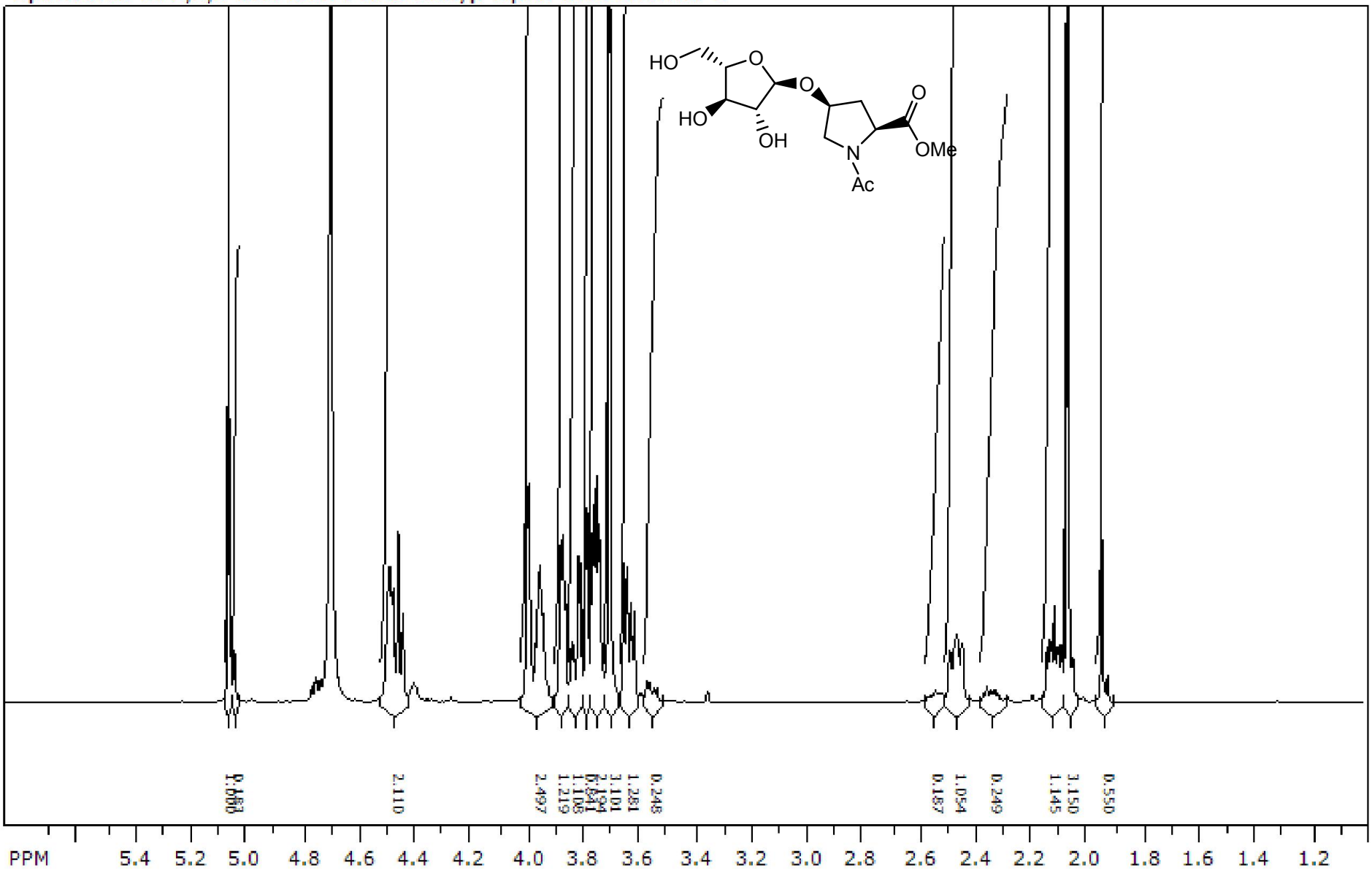


file: ...arabinose-MVR-2-92A\MVR-2-92\3\fid expt: <zgpg30>
transmitter freq.: 125.770364 MHz
time domain size: 65536 points
width: 29761.90 Hz = 236.6369 ppm = 0.454131 Hz/pt
number of scans: 128

freq. of 0 ppm: 125.757789 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000
Hz/cm: 1190.476 ppm/cm: 9.46547

Compound 12a

SpinWorks 3: 2,3,5 triol-arab-f-NAc-cishyp-alpha-OMe anomer 1

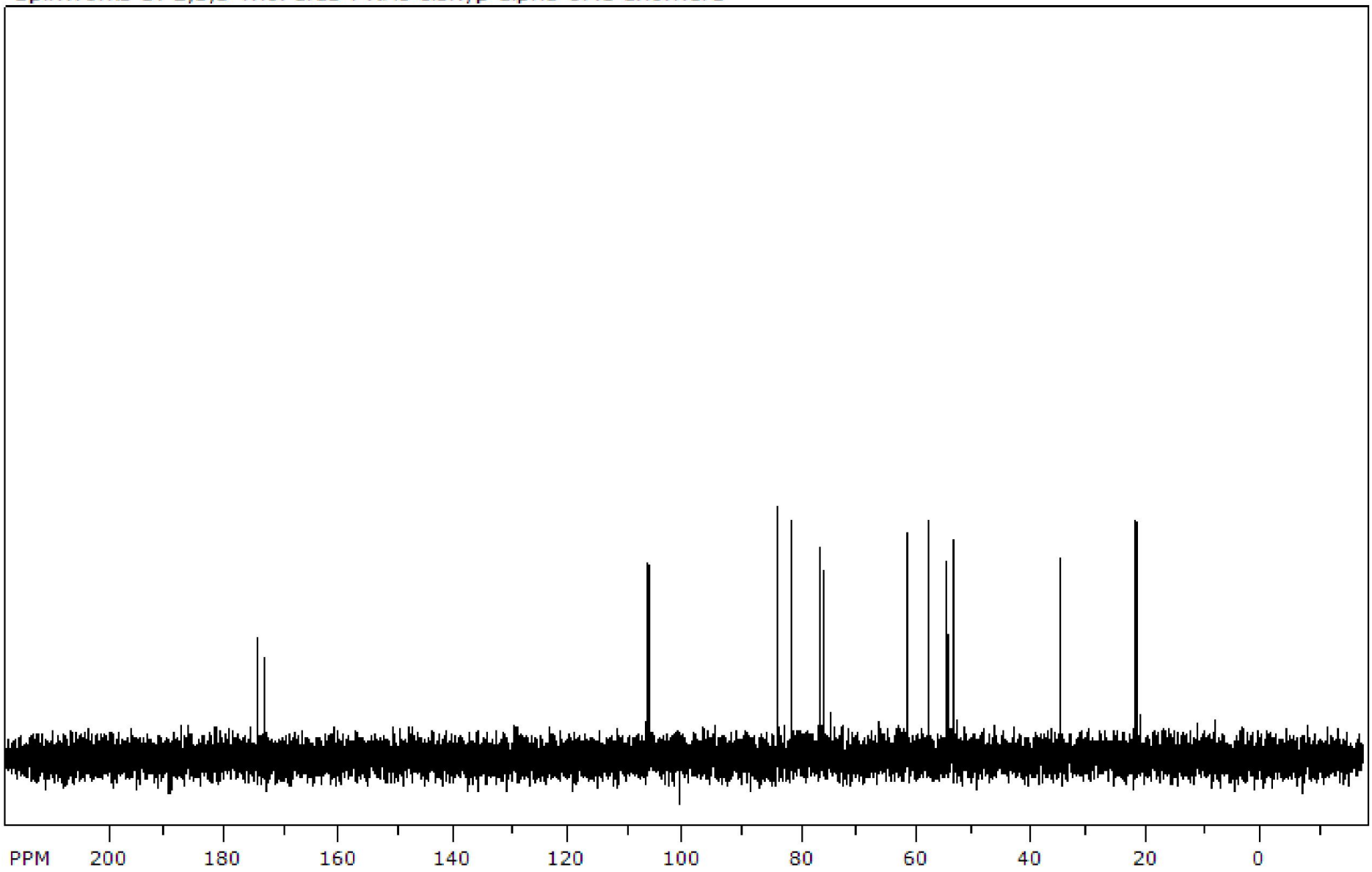


file: ...VR-2-73C-Cis-alpha-arabinose\1\fid expt: <zg30>
transmitter freq.: 500.133089 MHz
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 32

freq. of 0 ppm: 500.130000 MHz
processed size: 65536 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 97.325 ppm/cm: 0.19460

SpinWorks 3: 2,3,5 Triol-arab-f-NAc-cishyp-alpha-OMe anomer1

Compound 12a

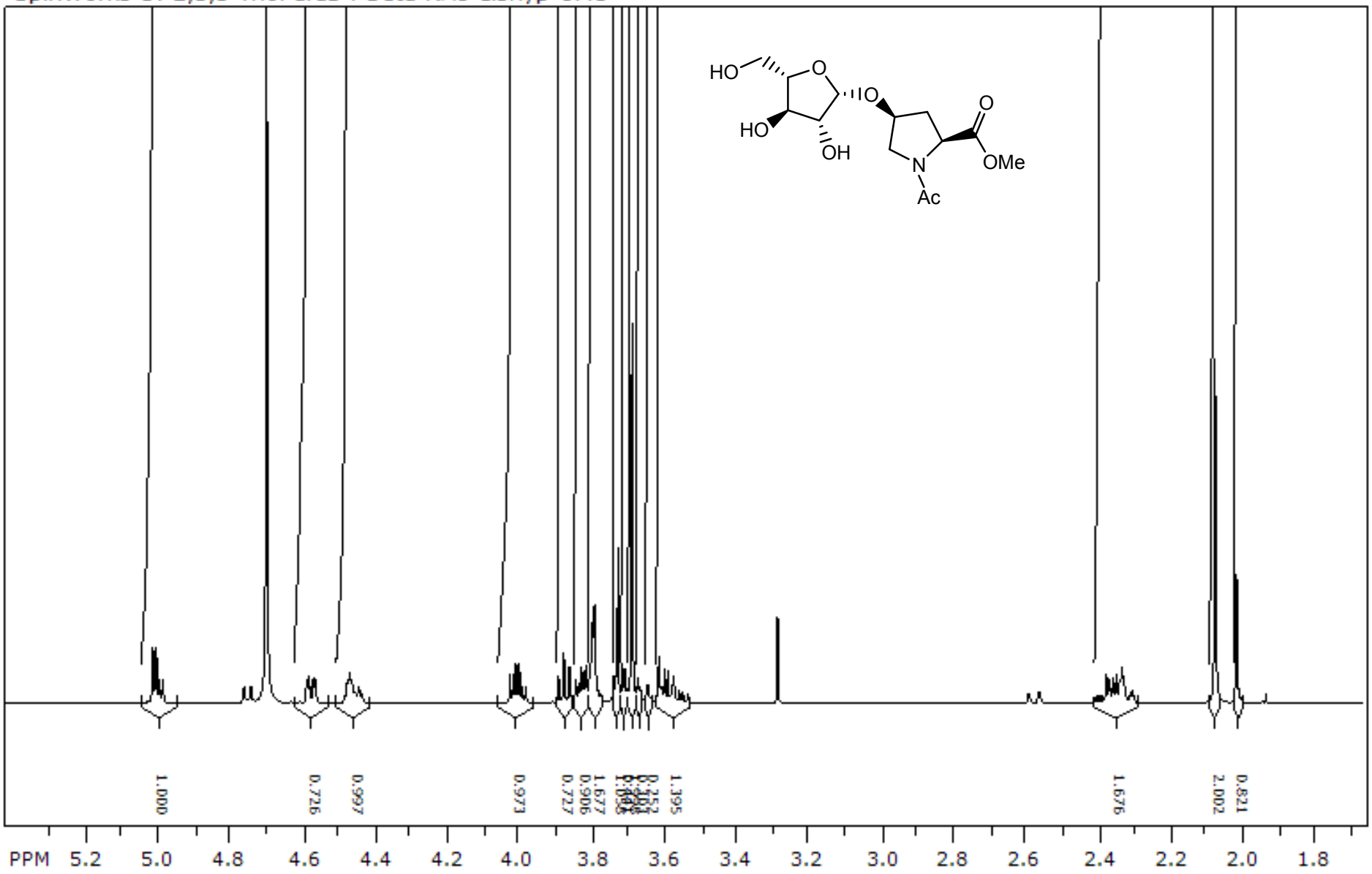


file: ...VR-2-73C-Cis-alpha-arabinose\4\fid expt: <zgpg30>
transmitter freq.: 125.770364 MHz
time domain size: 65536 points
width: 29761.90 Hz = 236.6369 ppm = 0.454131 Hz/pt
number of scans: 128

freq. of 0 ppm: 125.757789 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000
Hz/cm: 1190.476 ppm/cm: 9.46547

Compound 12b

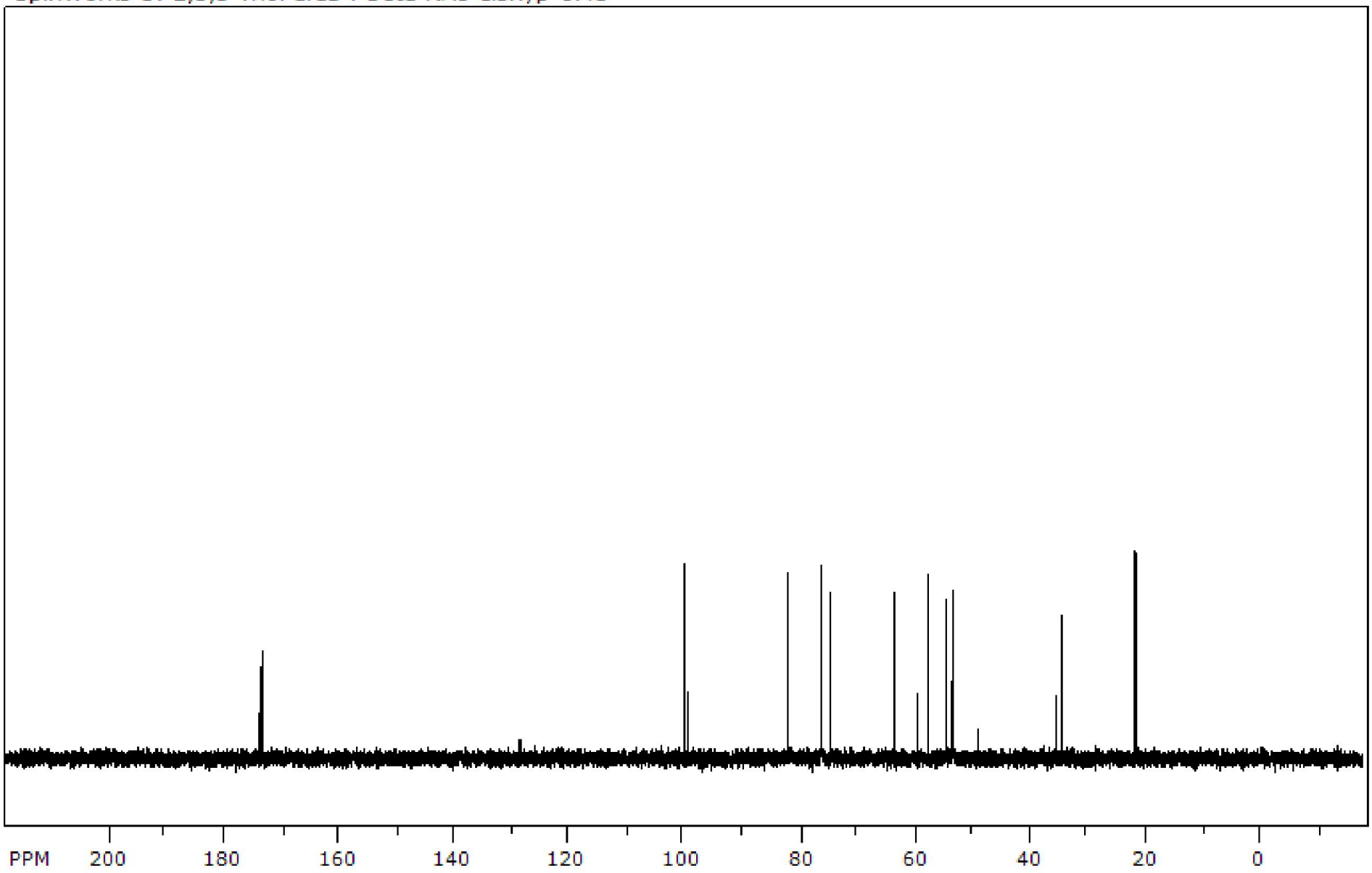
SpinWorks 3: 2,3,5 Triol-arab-f-beta-NAc-Cishyp-OMe



file: ... documents\NMR data\MVR-2-95\1\fid expt: <zg30>
transmitter freq.: 500.133089 MHz
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 64

freq. of 0 ppm: 500.130000 MHz
processed size: 65536 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 75.370 ppm/cm: 0.15070

SpinWorks 3: 2,3,5 Triol-arab-f-beta-NAc-Cishyp-OMe

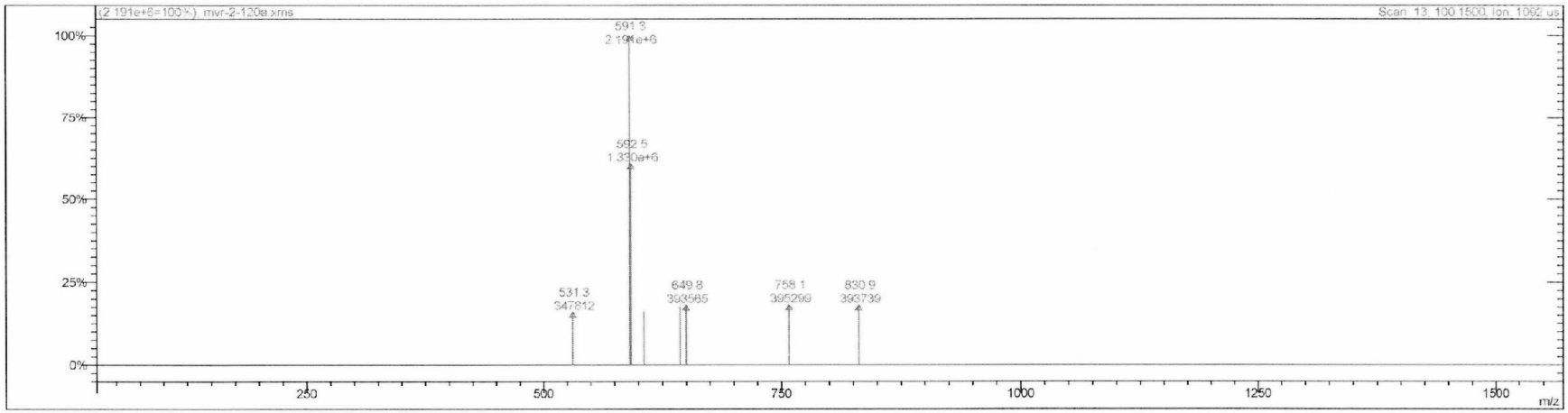


file: ... documents\NMR data\MVR-2-95\4\fid expt: <zgpg30>
transmitter freq.: 125.770364 MHz
time domain size: 65536 points
width: 29761.90 Hz = 236.6369 ppm = 0.454131 Hz/pt
number of scans: 128

freq. of 0 ppm: 125.757789 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000
Hz/cm: 1190.476 ppm/cm: 9.46547

Print Date: 18 Dec 2013 10:51:53

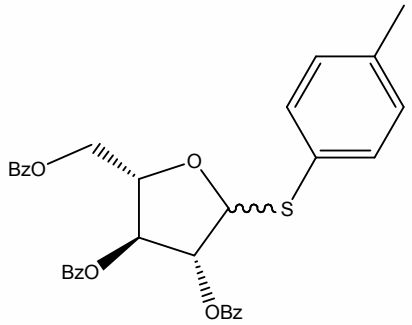
Scan 13 from c:\chemistry personal\scweizer\venkara\mvr-2-120a.xms



Spectrum from ...mistry personal\scweizer\venkara\mvr-2-120a.xms
Scan No: 13, Time: 0.179 minutes
No averaging. Not background corrected.
Comment: 0.179 min. Scan: 13 100:1500 Ion: 1092 us RIC: 4.400e+7
Pair Count: 8 MW: 0 Formula: None
CAS No: None Acquired Range: 99.5 - 1500.5 m/z

Method Time: 0.00-0.36, Centroid, Electrospray
Seg 1, Time: 0.00-0.36, Scan Functions: 1
1. 100:1500 100:1500|ESI Standard 80.0[V] Full
Product Mass Range: 99.5 - 1500.5 m/z
Scan Mass Segment 1: 99.5 - 399.5 m/z
Scan Mass Segment 2: 399.5 - 1000.5 m/z
Scan Mass Segment 3: 1000.5 - 1500.5 m/z

Ion	Int	Norm	Ion	Int	Norm	Ion	Int	Norm	Ion	Int	Norm
531.3	347812	159	592.5	1.330e+6	607	643.5	382891	175	758.1	395299	180
591.3	2.191e+6	999	605.6	352454	161	649.8	393565	179	830.9	393739	180



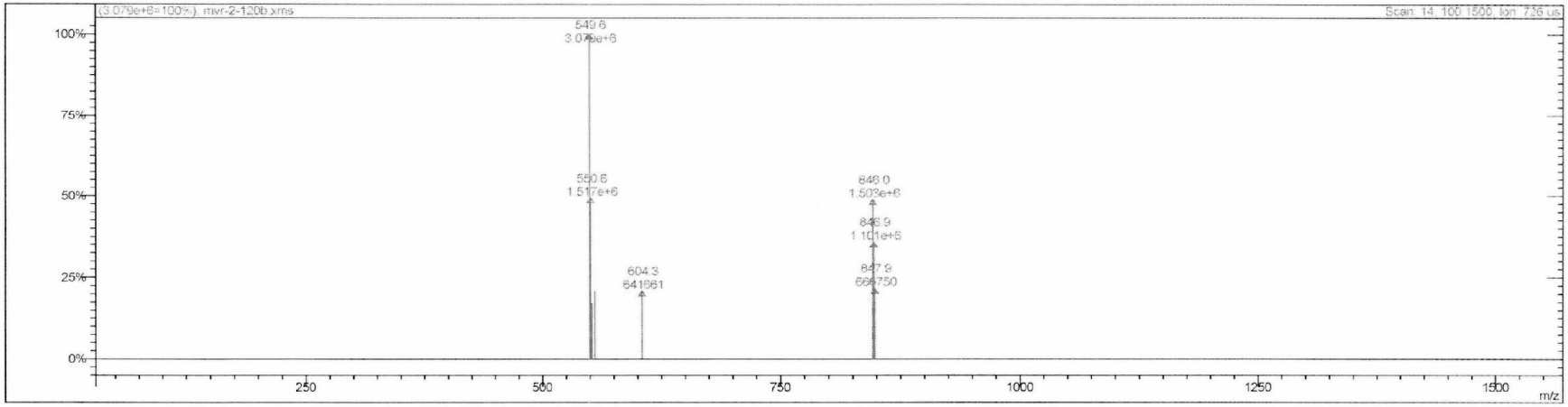
$C_{33}H_{28}O_7S$
Mol. Wt.: 568.64

2,3,5-Tri-O-benzoyl-arab-f-1-thiocresol

Print Date: 18 Dec 2013 10:52:05

Compound 3

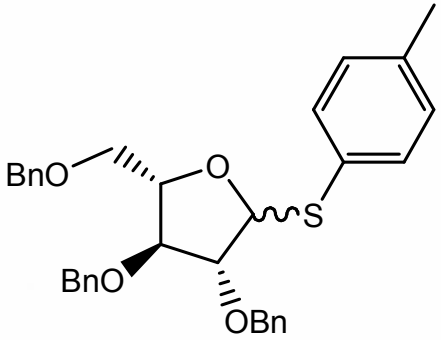
Scan 14 from c:\chemistry personal\scweizer\venkara\mvr-2-120b.xms



Spectrum from ...mistry personal\scweizer\venkara\mvr-2-120b.xms
Scan No: 14, Time: 0.193 minutes
No averaging. Not background corrected.
Comment: 0.193 min. Scan: 14 100:1500 Ion: 726 us RIC: 6.893e+7
Pair Count: 8 MW: 0 Formula: None
CAS No: None Acquired Range: 99.5 - 1500.5 m/z

Method Time: 0.00-0.42, Centroid, Electrospray
Seg 1, Time: 0.00-0.42, Scan Functions: 1
1. 100:1500 100:1500|ESI Standard 80.0[V] Full
Product Mass Range: 99.5 - 1500.5 m/z
Scan Mass Segment 1: 99.5 - 399.5 m/z
Scan Mass Segment 2: 399.5 - 1000.5 m/z
Scan Mass Segment 3: 1000.5 - 1500.5 m/z

Ion	Int Norm	Ion	Int Norm	Ion	Int Norm	Ion	Int Norm
549.6	3.079e+6 999	551.5	529206 172	604.3	641661 208	846.9	1.101e+6 357
550.6	1.517e+6 492	554.9	637414 207	846.0	1.503e+6 488	847.9	666750 216



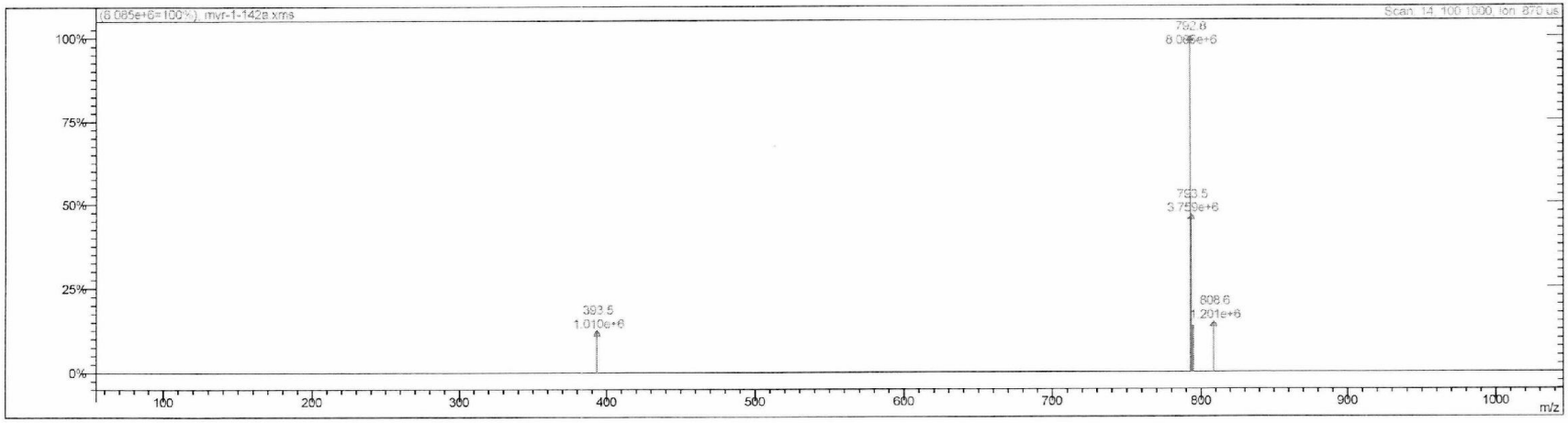
$C_{33}H_{34}O_4S$
Mol. Wt.: 526.69

2,3,5-Tri-O-benzyl-arab-f-1-thiocresol

Print Date: 21 Apr 2011 10:22:54

Compound 5a

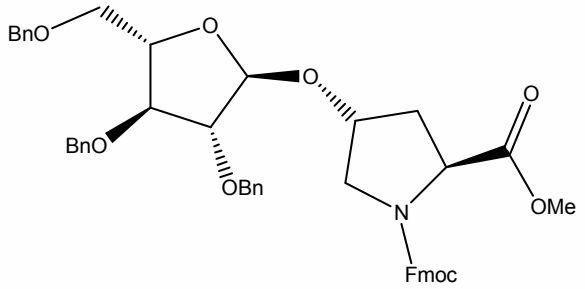
Scan 14 from c:\chemistry personal\scweizer\murthy\mvr-1-142a.xms



Spectrum from ...emistry personal\scweizer\murthy\mvr-1-142a.xms
Scan No: 14, Time: 0.129 minutes
No averaging. Not background corrected.
Comment: 0.129 min. Scan: 14 100:1000 Ion: 870 us RIC: 2.606e+7
Pair Count: 5 MW: 0 Formula: None
CAS No: None Acquired Range: 99.5 - 1000.5 m/z

Method Time: 0.00-0.48, Centroid, Electrospray
Seg 1, Time: 0.00-0.48, Scan Functions: 1
1. 100:1000 100:1000|ESI Standard 80.0[V] Full
Product Mass Range: 99.5 - 1000.5 m/z
Scan Mass Segment 1: 99.5 - 399.5 m/z
Scan Mass Segment 2: 399.5 - 1000.5 m/z

Ion	Int Norm	Ion	Int Norm	Ion	Int Norm	Ion	Int Norm
393.5	1.010e+6 125	793.5	3.759e+6 464	794.6	1.104e+6 136	808.6	1.201e+6 148
792.8	8.085e+6 999						



2,3,5 Tri-O-Benzyl-arab-f-Fmoc-Trans-alpha-Hyp-OMe

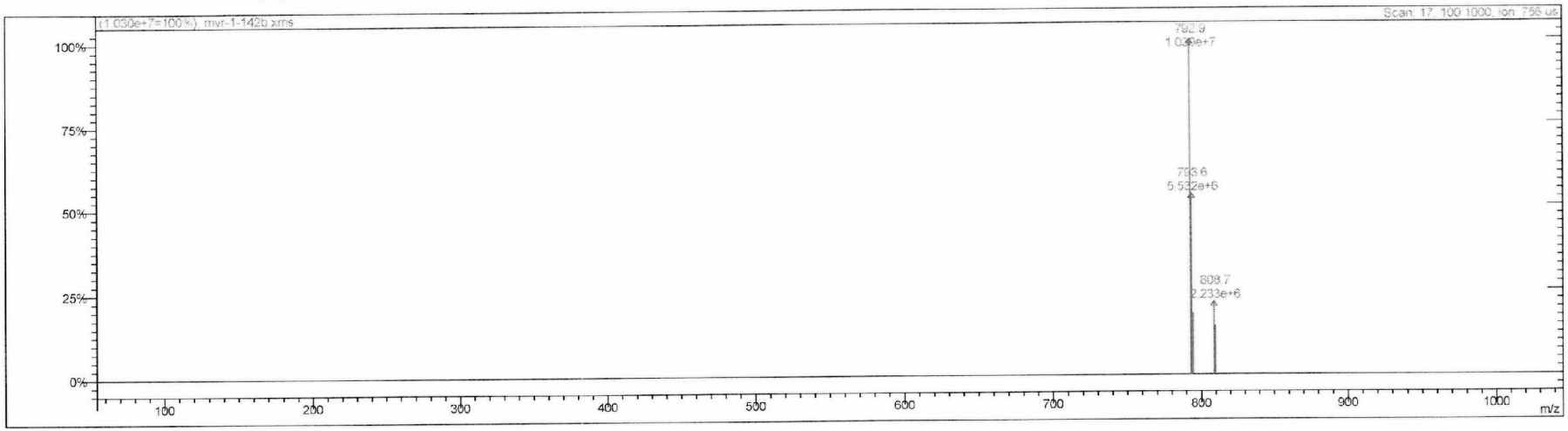
$C_{47}H_{47}NO_9$
Mol. Wt.: 769.88

NMR Code: MVR-2-101A-TA

Print Date: 21 Apr 2011 10:23:11

Compound 5b

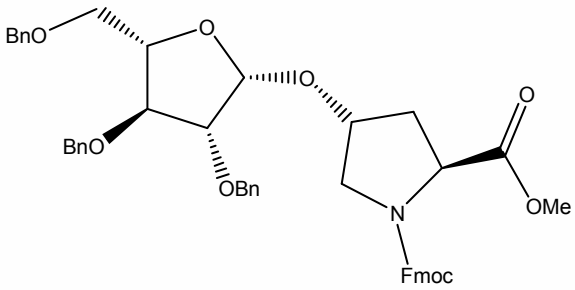
Scan 17 from c:\chemistry personal\scweizer\murthy\mvr-1-142b.xms



Spectrum from ...emistry personal\scweizer\murthy\mvr-1-142b.xms
Scan No: 17, Time: 0.159 minutes
No averaging. Not background corrected.
Comment: 0.159 min. Scan: 17 100:1000 Ion: 756 us RIC: 3.109e+7
Pair Count: 5 MW: 0 Formula: None
CAS No: None Acquired Range: 99.5 - 1000.5 m/z

Method Time: 0.00-0.26, Centroid, Electrospray
Seg 1, Time: 0.00-0.26, Scan Functions: 1
1. 100:1000 100:1000|ESI Standard 80.0[V] Full
Product Mass Range: 99.5 - 1000.5 m/z
Scan Mass Segment 1: 99.5 - 399.5 m/z
Scan Mass Segment 2: 399.5 - 1000.5 m/z

Ion	Int	Norm	Ion	Int	Norm	Ion	Int	Norm	Ion	Int	Norm
792.9	1.030e+7	999	794.6	1.879e+6	182	808.7	2.233e+6	217	809.6	1.493e+6	145
793.6	5.532e+6	536									



2,3,5 Tri-O-Benzyl-arab-f-Fmoc-Trans-beta-Hyp-OMe

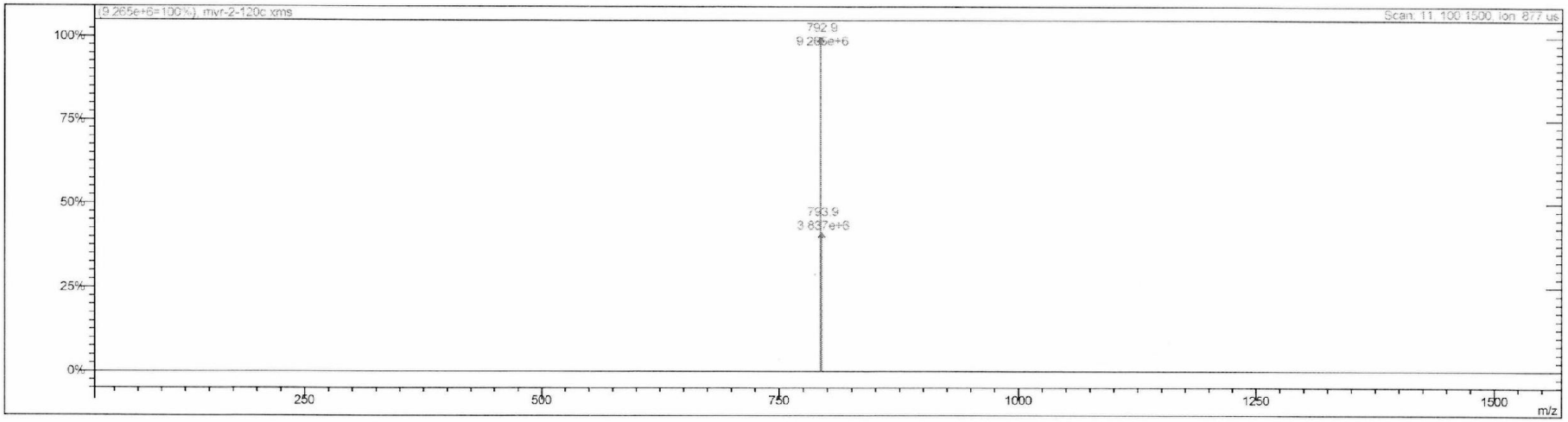
$C_{47}H_{47}NO_9$
Mol. Wt.: 769.88

NMR Code: MVR-2-101B-TB

Print Date: 18 Dec 2013 10:52:30

Compound 10a

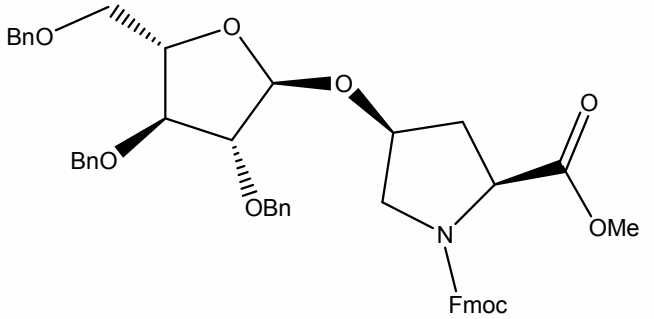
Scan 11 from c:\chemistry personal\scweizer\venkara\mvr-2-120c.xms



Spectrum from ...mistry personal\scweizer\venkara\mvr-2-120c.xms
Scan No: 11, Time: 0.149 minutes
No averaging. Not background corrected.
Comment: 0.149 min. Scan: 11 100:1500 Ion: 877 us RIC: 4.716e+7
Pair Count: 2 MW: 0 Formula: None
CAS No: None Acquired Range: 99.5 - 1500.5 m/z

Method Time: 0.00-0.18, Centroid, Electrospray
Seg 1, Time: 0.00-0.18, Scan Functions: 1
1. 100:1500 100:1500|ESI Standard 80.0[V] Full
Product Mass Range: 99.5 - 1500.5 m/z
Scan Mass Segment 1: 99.5 - 399.5 m/z
Scan Mass Segment 2: 399.5 - 1000.5 m/z
Scan Mass Segment 3: 1000.5 - 1500.5 m/z

Ion	Int Norm	Ion	Int Norm	Ion	Int Norm	Ion	Int Norm
792.9	9.265e+6 999	793.9	3.837e+6 414				



2,3,5 Tri-O-Benzyl-arab-f-Fmoc-Cis-alpha-hyp-OMe

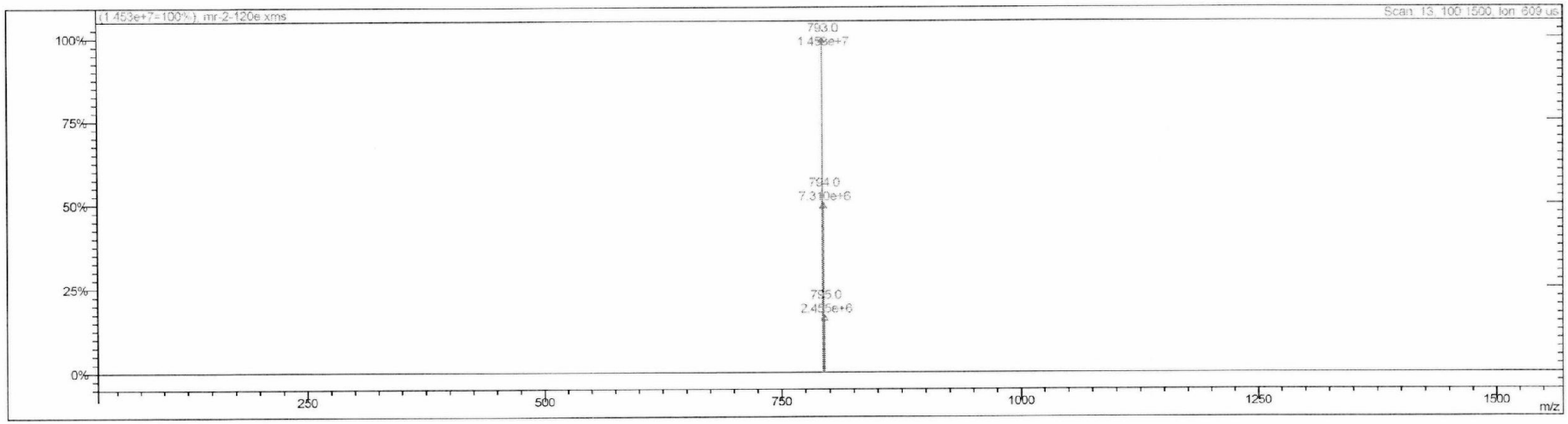
$C_{47}H_{47}NO_9$
Mol. Wt.: 769.88

NMR Code: MVR-2-101C-CA

Print Date: 18 Dec 2013 10:49:14

Compound 10b

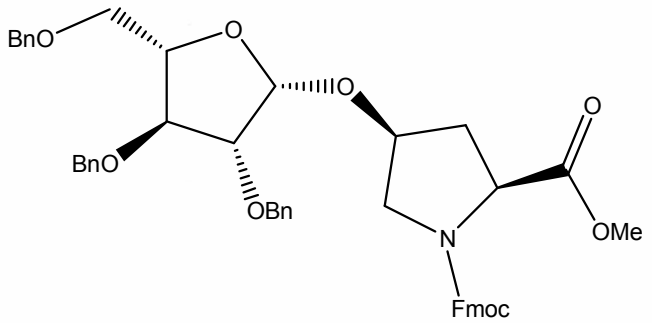
Scan 13 from c:\chemistry personal\scweizer\murthy\mr-2-120e.xms



Spectrum from ...hemistry personal\scweizer\murthy\mr-2-120e.xms
Scan No: 13, Time: 0.178 minutes
No averaging. Not background corrected.
Comment: 0.178 min. Scan: 13 100:1500 Ion: 609 us RIC: 5.994e+7
Pair Count: 3 MW: 0 Formula: None
CAS No: None Acquired Range: 99.5 - 1500.5 m/z

Method Time: 0.00-0.25, Centroid, Electrospray
Seg 1, Time: 0.00-0.25, Scan Functions: 1
1. 100:1500 100:1500|ESI Standard 80.0[V] Full
Product Mass Range: 99.5 - 1500.5 m/z
Scan Mass Segment 1: 99.5 - 399.5 m/z
Scan Mass Segment 2: 399.5 - 1000.5 m/z
Scan Mass Segment 3: 1000.5 - 1500.5 m/z

Ion	Int Norm	Ion	Int Norm	Ion	Int Norm	Ion	Int Norm
793.0	1.453e+7 999	794.0	7.310e+6 503	795.0	2.455e+6 169		



2,3,5 Tri-O-Benzyl-arab-f-Fmoc-Cis-beta-hyp-OMe

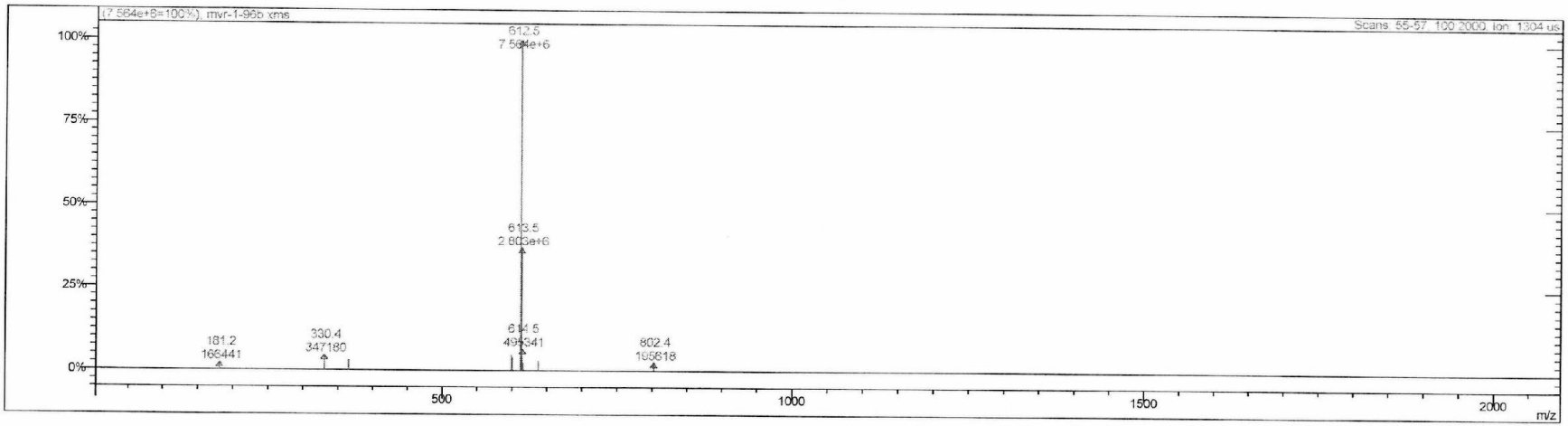
$C_{47}H_{47}NO_9$
Mol. Wt.: 769.88

NMR Code: MVR-2-101C-CB

Print Date: 13 Jan 2011 14:31:07

Compound 6b

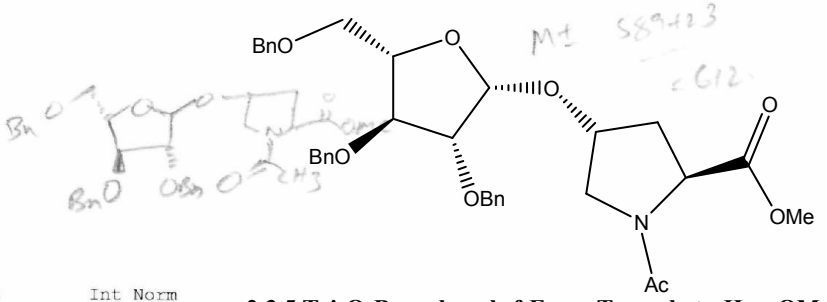
Scan 56 from c:\chemistry personal\scweizer\venkara\mvr-1-96b.xms



Spectrum from ...emistry personal\scweizer\venkara\mvr-1-96b.xms
Scan No: 56, Time: 0.942 minutes
3 points averaged. Not background corrected.
Comment: 0.942 min. Scans: 55-57 100:2000 Ion: 1303 us RIC: 3.764e+7
Pair Count: 11 MW: 0 Formula: None
CAS No: None Acquired Range: 99.5 - 2000.5 m/z

Method Time: 0.00-3.19, Centroid, Electrospray
Seg 1, Time: 0.00-3.19, Scan Functions: 1
1. 100:2000 100:2000|ESI Standard 80.0[V] Full
Product Mass Range: 99.5 - 2000.5 m/z
Scan Mass Segment 1: 99.5 - 399.5 m/z
Scan Mass Segment 2: 399.5 - 1000.5 m/z
Scan Mass Segment 3: 1000.5 - 2000.5 m/z

Ion	Int	Norm	Ion	Int	Norm	Ion	Int	Norm	Ion	Int	Norm
181.2	166441	22	599.5	349842	46	613.5	2.803e+6	370	637.4	207986	27
330.4	347180	46	600.5	293336	39	614.5	495341	65	802.4	195618	26
365.3	221927	29	612.5	7.564e+6	999	615.5	167580	22			



2,3,5 Tri-O-Benzyl-arab-f-Fmoc-Trans-beta-Hyp-OMe

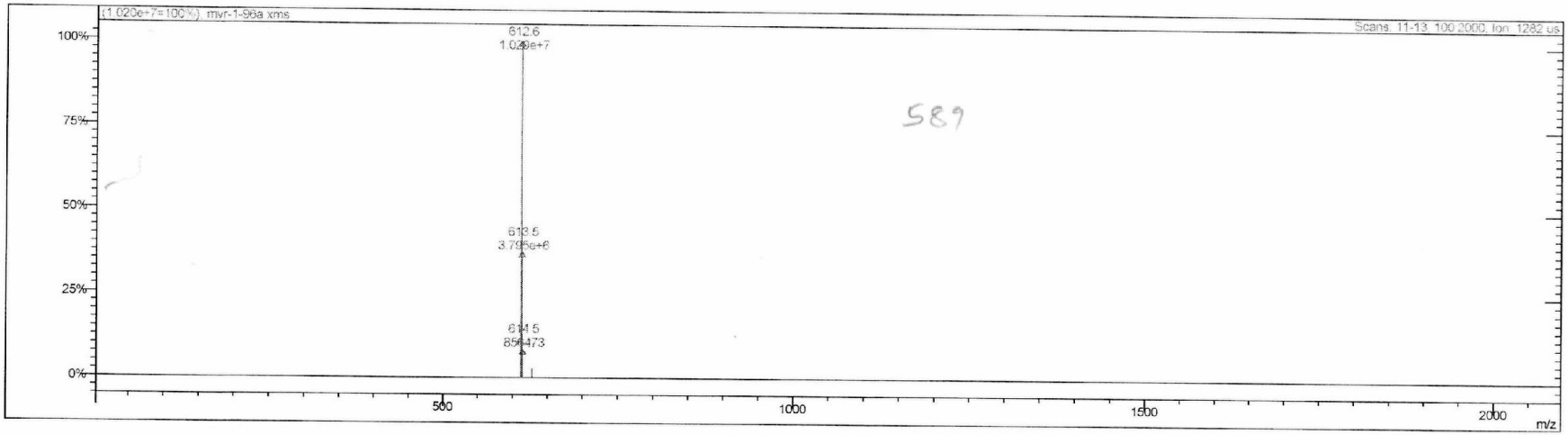
$C_{34}H_{39}NO_8$
Mol. Wt.: 589.68

NMR Code: MVR-2-99B

Print Date: 13 Jan 2011 14:07:27

Compound 6a

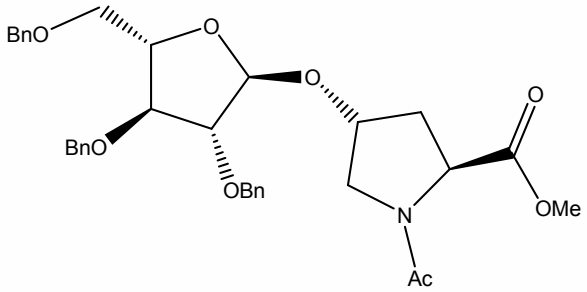
Scan 12 from c:\chemistry personal\scweizer\venkara\mvr-1-96a.xms



Spectrum from ...emistry personal\scweizer\venkara\mvr-1-96a.xms
Scan No: 12, Time: 0.189 minutes
3 points averaged. Not background corrected.
Comment: 0.189 min. Scans: 11-13 100:2000 Ion: 1282 us RIC: 3.308e+7
Pair Count: 4 MW: 0 Formula: None
CAS No: None Acquired Range: 99.5 - 2000.5 m/z

Method Time: 0.00-0.26, Centroid, Electrospray
Seg 1, Time: 0.00-0.26, Scan Functions: 1
1. 100:2000 100:2000|ESI Standard 80.0[V] Full
Product Mass Range: 99.5 - 2000.5 m/z
Scan Mass Segment 1: 99.5 - 399.5 m/z
Scan Mass Segment 2: 399.5 - 1000.5 m/z
Scan Mass Segment 3: 1000.5 - 2000.5 m/z

Ion	Int	Norm	Ion	Int	Norm	Ion	Int	Norm	Ion	Int	Norm
612.6	1.020e+7	999	613.5	3.795e+6	372	614.5	856473	84	628.4	269711	26



2,3,5 Tri-O-Benzyl-arab-f-NAc-Trans-alpha-Hyp-OMe

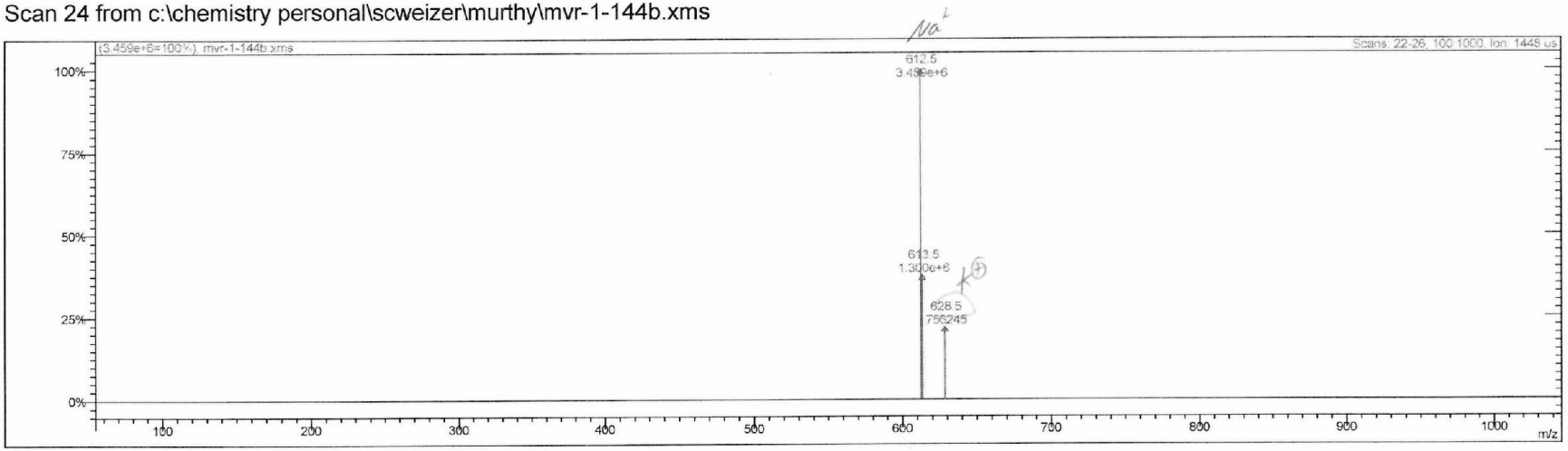
$C_{34}H_{39}NO_8$
Mol. Wt.: 589.68

NMR Code: MVR-2-99A

Print Date: 06 Apr 2011 15:33:50

Compound 11a

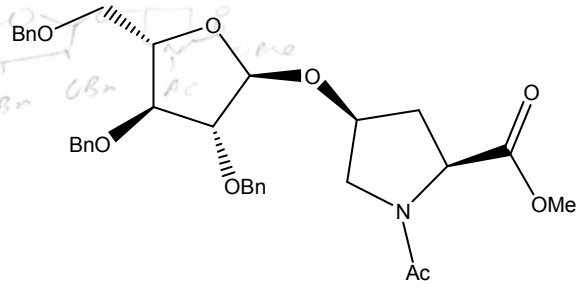
Scan 24 from c:\chemistry personal\scweizer\murthy\mvr-1-144b.xms



Spectrum from ...emistry personal\scweizer\murthy\mvr-1-144b.xms
Scan No: 24, Time: 0.349 minutes
5 points averaged. Not background corrected.
Comment: 0.349 min. Scans: 22-26 100:1000 Ion: 1447 us RIC: 1.625e+7
Pair Count: 3 MW: 0 Formula: None
CAS No: None Acquired Range: 99.5 - 1000.5 m/z

Method Time: 0.00-0.66, Centroid, Electrospray
Seg 1, Time: 0.00-0.66, Scan Functions: 1
1. 100:1000 100:1000|ESI Standard 80.0[V] Full
Product Mass Range: 99.5 - 1000.5 m/z
Scan Mass Segment 1: 99.5 - 399.5 m/z
Scan Mass Segment 2: 399.5 - 1000.5 m/z

Ion	Int Norm	Ion	Int Norm	Ion	Int Norm	Ion	Int Norm
612.5	3.459e+6 999	613.5	1.300e+6 376	628.5	756245 218		



2,3,5 Tri-O-Benzyl-arab-f-Fmoc-Cis-alpha-hyp-OMe

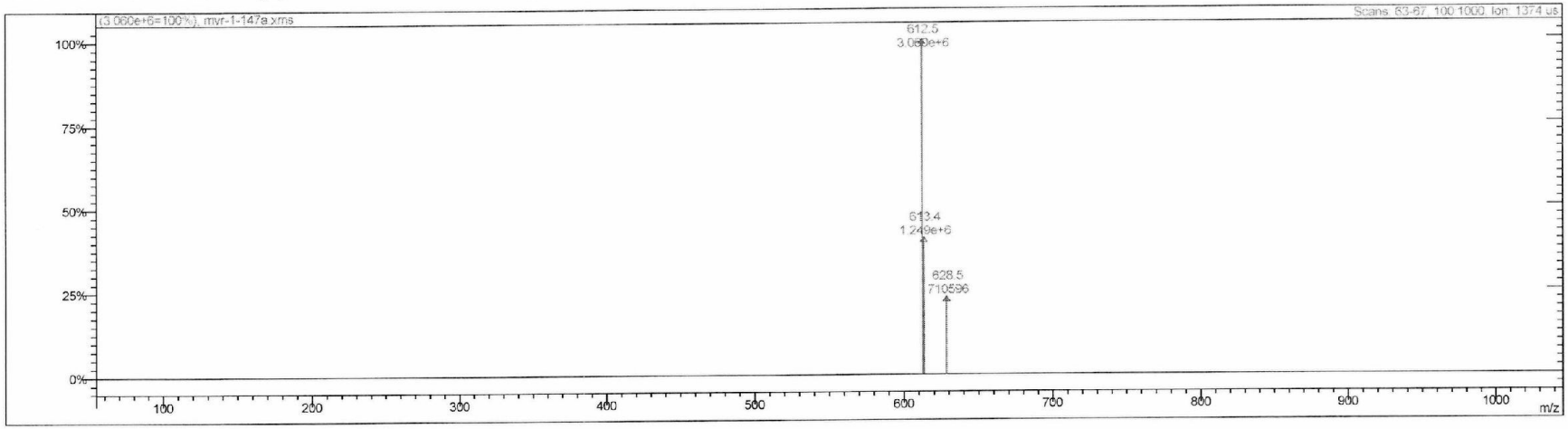
$C_{34}H_{39}NO_8$
Mol. Wt.: 589.68

NMR Code: MVR-2-100B

Print Date: 06 Apr 2011 15:40:22

Compound 11b

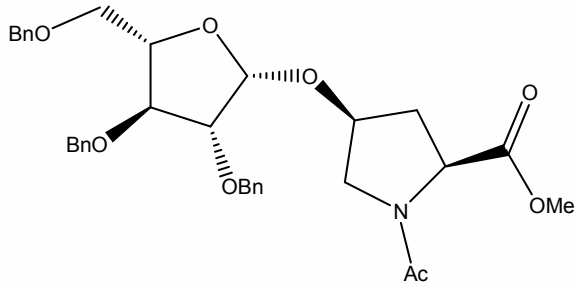
Scan 65 from c:\chemistry personal\scweizer\murthy\mvr-1-147a.xms



Spectrum from ...emistry personal\scweizer\murthy\mvr-1-147a.xms
Scan No: 65, Time: 0.677 minutes
5 points averaged. Not background corrected.
Comment: 0.677 min. Scans: 63-67 100:1000 Ion: 1373 us RIC: 1.591e+7
Pair Count: 3 MW: 0 Formula: None
CAS No: None Acquired Range: 99.5 - 1000.5 m/z

Method Time: 0.00-0.92, Centroid, Electrospray
Seg 1, Time: 0.00-0.92, Scan Functions: 1
1. 100:1000 100:1000|ESI Standard 80.0[V] Full
Product Mass Range: 99.5 - 1000.5 m/z
Scan Mass Segment 1: 99.5 - 399.5 m/z
Scan Mass Segment 2: 399.5 - 1000.5 m/z

Ion	Int Norm	Ion	Int Norm	Ion	Int Norm	Ion	Int Norm
612.5	3.060e+6 999	613.4	1.249e+6 408	628.5	710596 232		



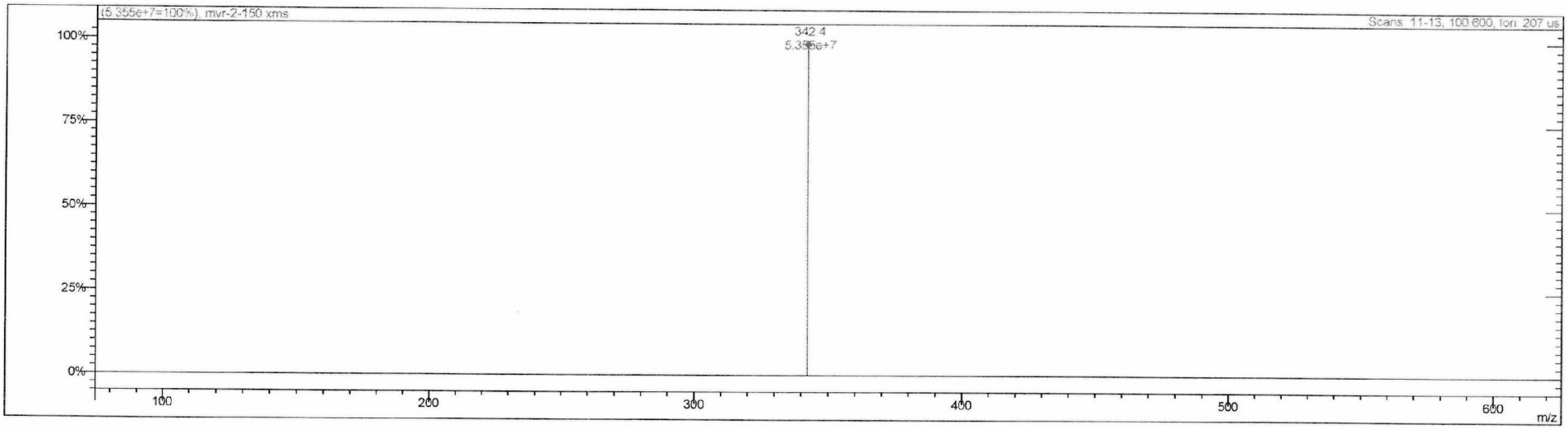
2,3,5 Tri-O-Benzyl-arab-f-Fmoc-Cis-beta-hyp-OMe

$C_{34}H_{39}NO_8$
Mol. Wt.: 589.68

NMR Code: MVR-2-100A

Scan 12 from c:\chemistry personal\scweizer\venkara\mvr-2-150.xms

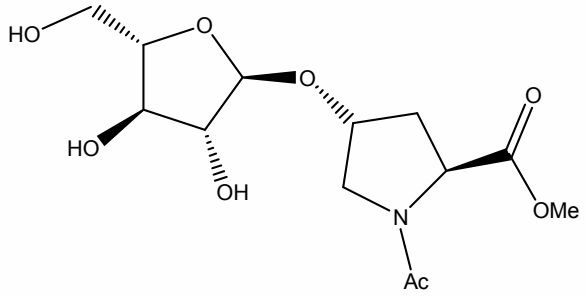
Compound 7a



Spectrum from ...emistry personal\scweizer\venkara\mvr-2-150.xms
Scan No: 12, Time: 0.093 minutes
3 points averaged. Not background corrected.
Comment: 0.093 min. Scans: 11-13 100:600 Ion: 207 us RIC: 1.069e+8
Pair Count: 1 MW: 0 Formula: None
CAS No: None Acquired Range: 99.5 - 600.5 m/z

Method Time: 0.00-0.19, Centroid, Electrospray
Seg 1, Time: 0.00-0.19, Scan Functions: 1
1. 100:600 100:600|ESI Standard 80.0[V] Full
Product Mass Range: 99.5 - 600.5 m/z
Scan Mass Segment 1: 99.5 - 399.5 m/z
Scan Mass Segment 2: 399.5 - 600.5 m/z

Ion	Int Norm	Ion	Int Norm	Ion	Int Norm	Ion	Int Norm
342.4	5.355e+7	999					



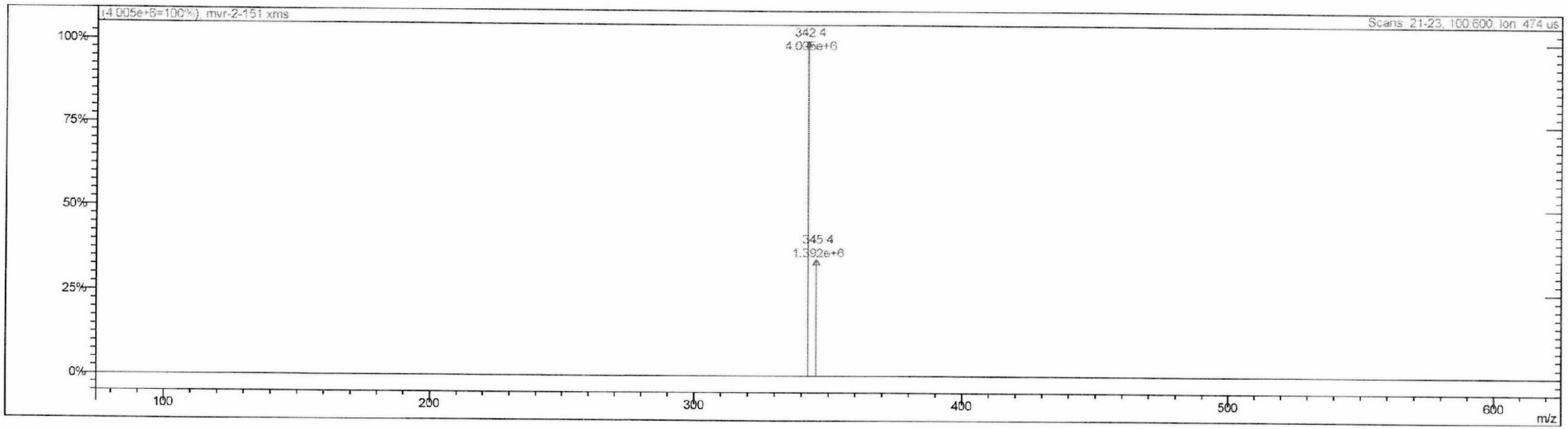
trans-4-O-(α-L-Arabinofuranosyl)-N-Acetyl-4-hydroxy L-Proline methyl ester

$C_{13}H_{21}NO_8$
Mol. Wt.: 319.31

NMR Code: MVR-2-73A

Scan 22 from c:\chemistry personal\scweizer\venkara\mvr-2-151.xms

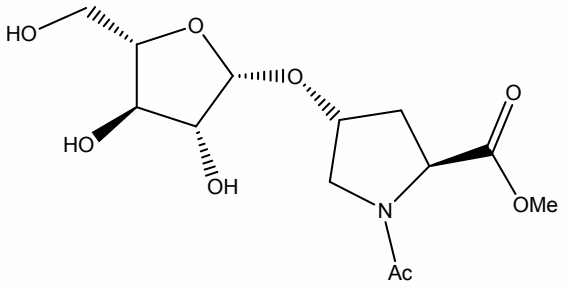
Compound 7b



Spectrum from ...emistry personal\scweizer\venkara\mvr-2-151.xms
Scan No: 22, Time: 0.214 minutes
3 points averaged. Not background corrected.
Comment: 0.214 min. Scans: 21-23 100:600 Ion: 474 us RIC: 3.264e+7
Pair Count: 2 MW: 0 Formula: None
CAS No: None Acquired Range: 99.5 - 600.5 m/z

Method Time: 0.00-0.64, Centroid, Electrospray
Seg 1, Time: 0.00-0.64, Scan Functions: 1
1. 100:600 100:600|ESI Standard 80.0[V] Full
Product Mass Range: 99.5 - 600.5 m/z
Scan Mass Segment 1: 99.5 - 399.5 m/z
Scan Mass Segment 2: 399.5 - 600.5 m/z

Ion	Int Norm	Ion	Int Norm	Ion	Int Norm	Ion	Int Norm
342.4	4.005e+6 999	345.4	1.392e+6 347				



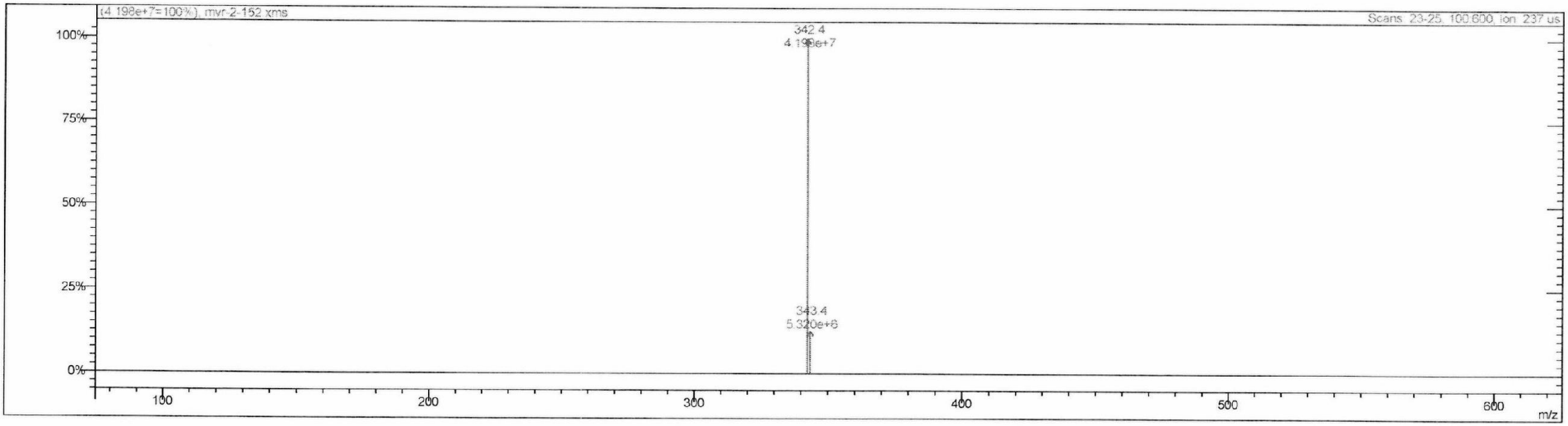
trans-4-O-(β-L-Arabinofuranosyl)-N-Acetyl-4-hydroxy L-Proline methyl ester

$C_{13}H_{21}NO_8$
Mol. Wt.: 319.31

NMR Code: MVR-2-92

Compound 12a

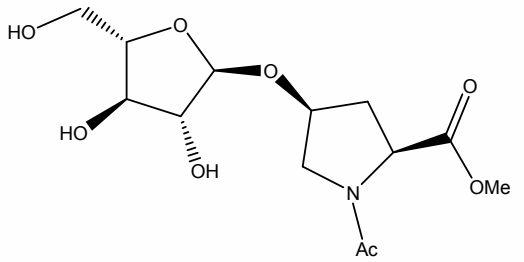
Scan 24 from c:\chemistry personal\scweizer\murthy\mvr-2-152.xms



Spectrum from ...hemistry personal\scweizer\murthy\mvr-2-152.xms
Scan No: 24, Time: 0.194 minutes
3 points averaged. Not background corrected.
Comment: 0.194 min. Scans: 23-25 100:600 Ion: 237 us RIC: 9.089e+7
Pair Count: 2 MW: 0 Formula: None
CAS No: None Acquired Range: 99.5 - 600.5 m/z

Method Time: 0.00-0.41, Centroid, Electrospray
Seg 1, Time: 0.00-0.41, Scan Functions: 1
1. 100:600 100:600|ESI Standard 80.0[V] Full
Product Mass Range: 99.5 - 600.5 m/z
Scan Mass Segment 1: 99.5 - 399.5 m/z
Scan Mass Segment 2: 399.5 - 600.5 m/z

Ion	Int Norm	Ion	Int Norm	Ion	Int Norm	Ion	Int Norm
342.4	4.198e+7 999	343.4	5.320e+6 127				



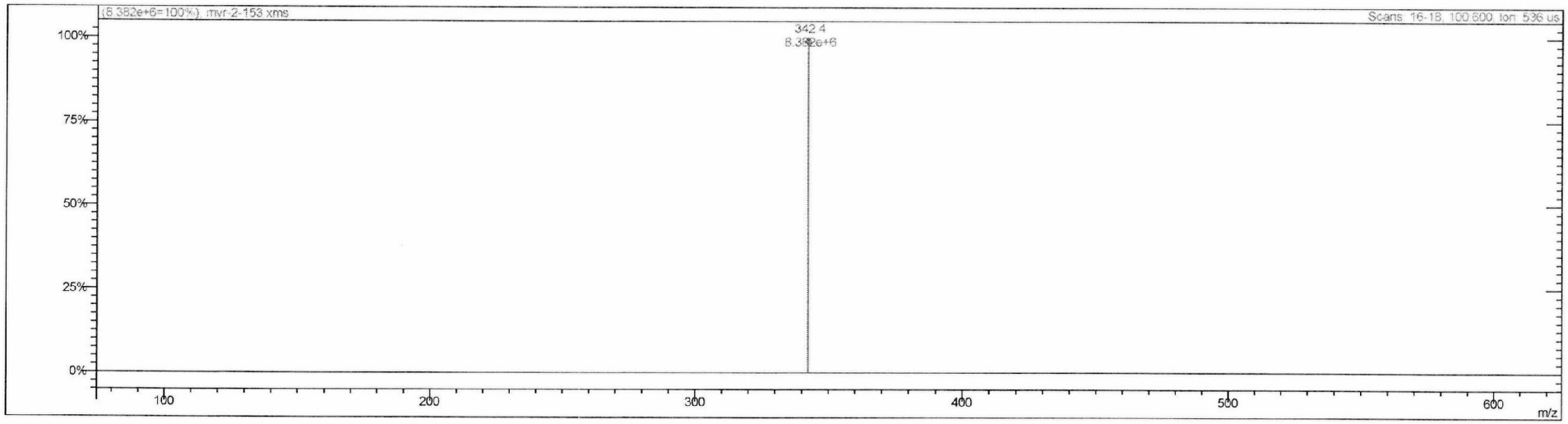
cis-4-O-(α-L-Arabinofuranosyl)-N-Acetyl-4-hydroxy L-Proline methyl ester

$C_{13}H_{21}NO_8$
Mol. Wt.: 319.31

NMR Code: MVR-2-73C

Scan 17 from c:\chemistry personal\scweizer\venkara\mvr-2-153.xms

Compound 12b

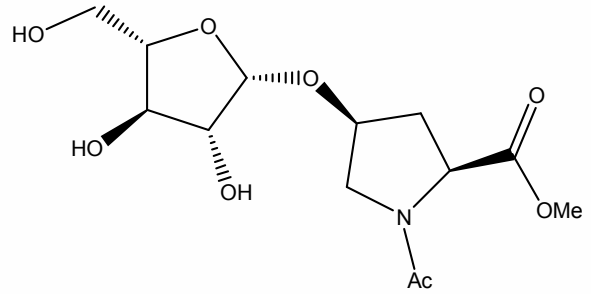


Spectrum from ...emistry personal\scweizer\venkara\mvr-2-153.xms
Scan No: 17, Time: 0.135 minutes
3 points averaged. Not background corrected.
Comment: 0.135 min. Scans: 16-18 100:600 Ion: 536 us RIC: 3.534e+7
Pair Count: 1 MW: 0 Formula: None
CAS No: None Acquired Range: 99.5 - 600.5 m/z

Method Time: 0.00-0.28, Centroid, Electrospray
Seg 1, Time: 0.00-0.28, Scan Functions: 1
1. 100:600 100:600|ESI Standard 80.0[V] Full
Product Mass Range: 99.5 - 600.5 m/z
Scan Mass Segment 1: 99.5 - 399.5 m/z
Scan Mass Segment 2: 399.5 - 600.5 m/z

Ion	Int Norm	Ion	Int Norm	Ion	Int Norm	Ion	Int Norm
342.4	8.382e+6	999					

NMR Code: MVR-2-95

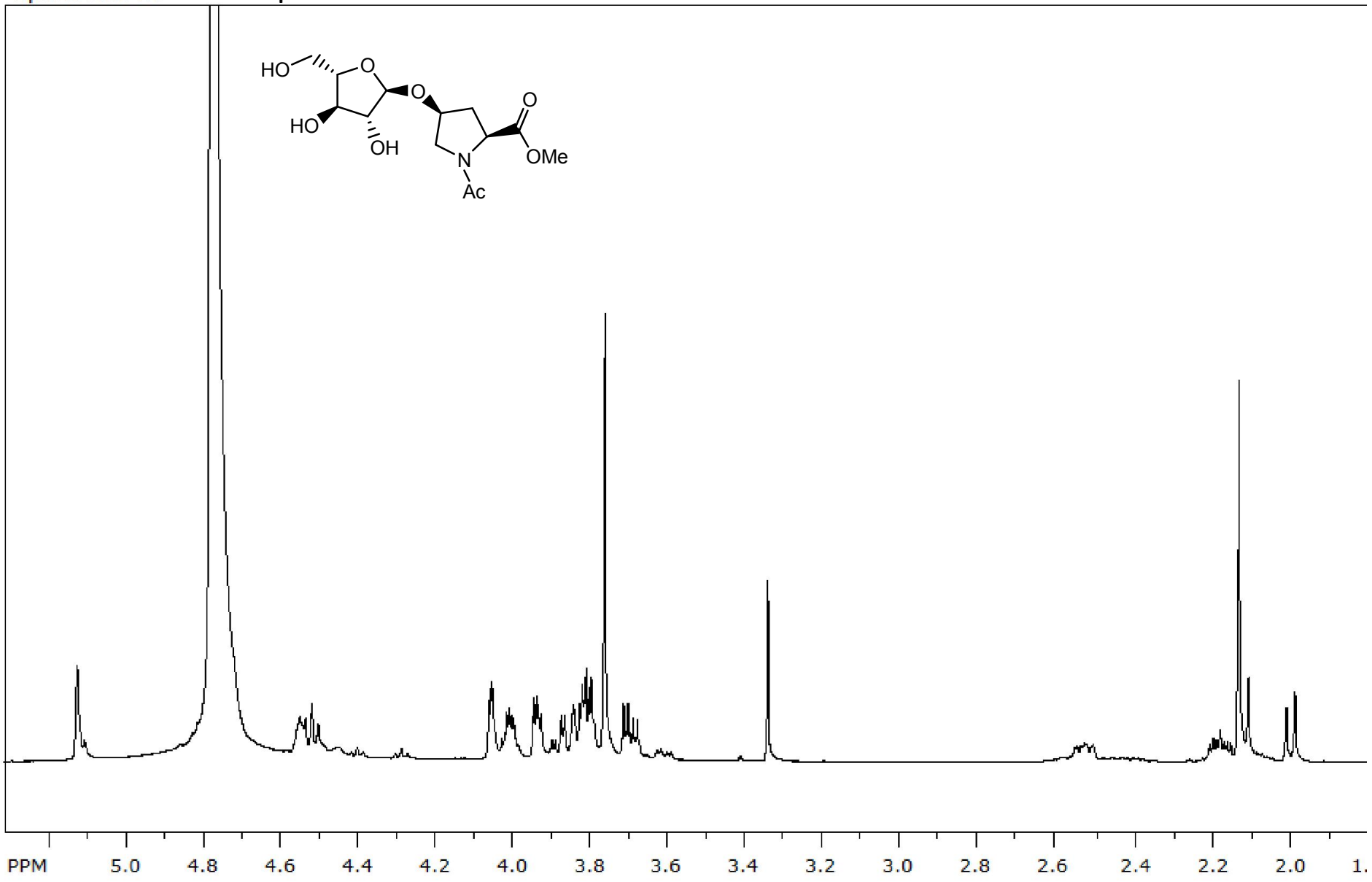


cis-4-O-(β-L-Arabinofuranosyl)-N-Acetyl-4-hydroxy L-Proline methyl ester

$C_{13}H_{21}NO_8$
Mol. Wt.: 319.31

Nuclear Overhauser Experiments (NOE) Spectra

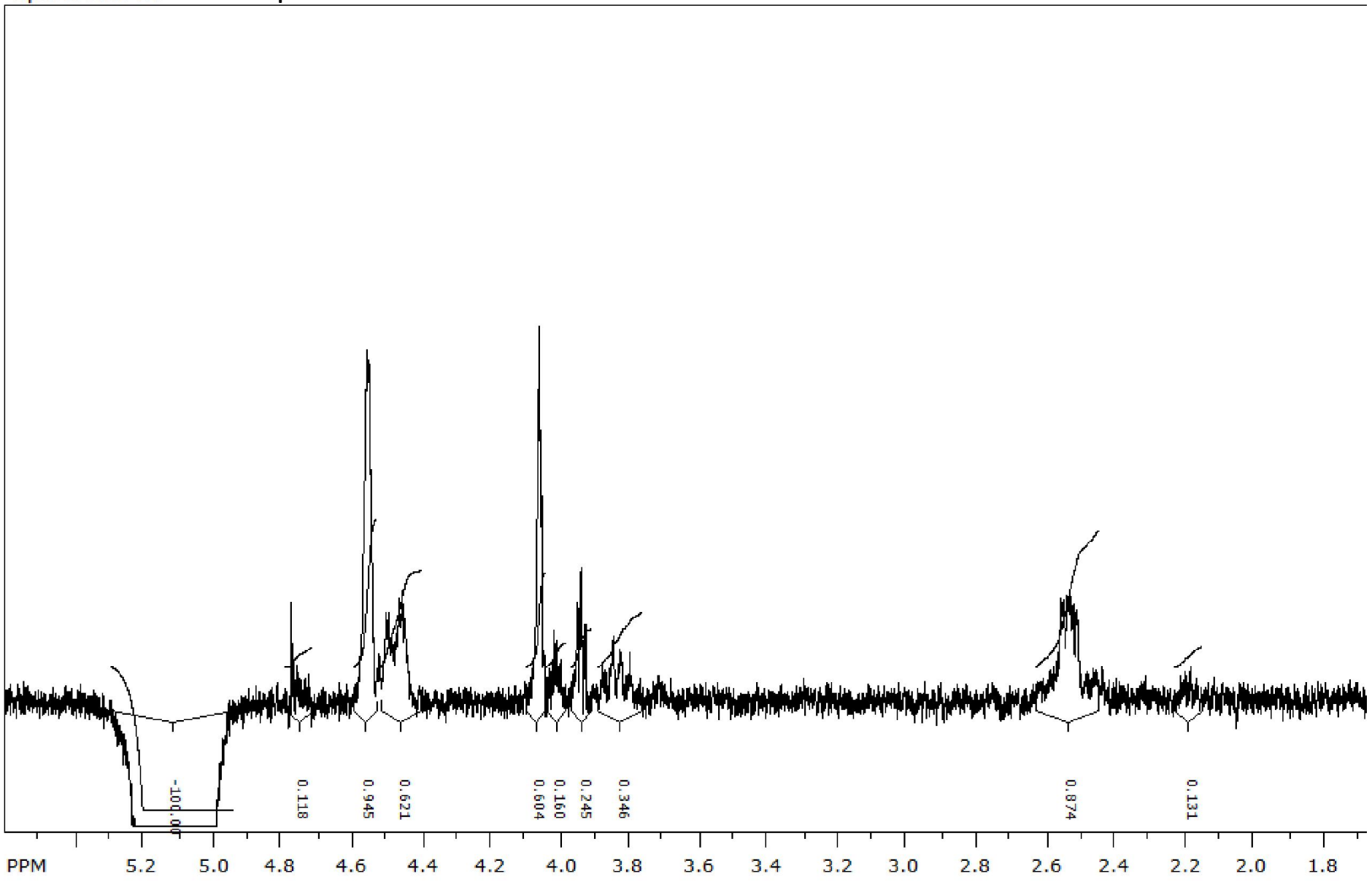
SpinWorks 3: Cis-alpha



file: ...s\Cis-alpha-arabinose-NOe-1H\1\fid expt: <zg30>
transmitter freq.: 500.133089 MHz
time domain size: 65536 points

freq. of 0 ppm: 500.130000 MHz
processed size: 65536 complex points
LB: 0.300 GF: 0.0000

SpinWorks 3: Cis-alpha

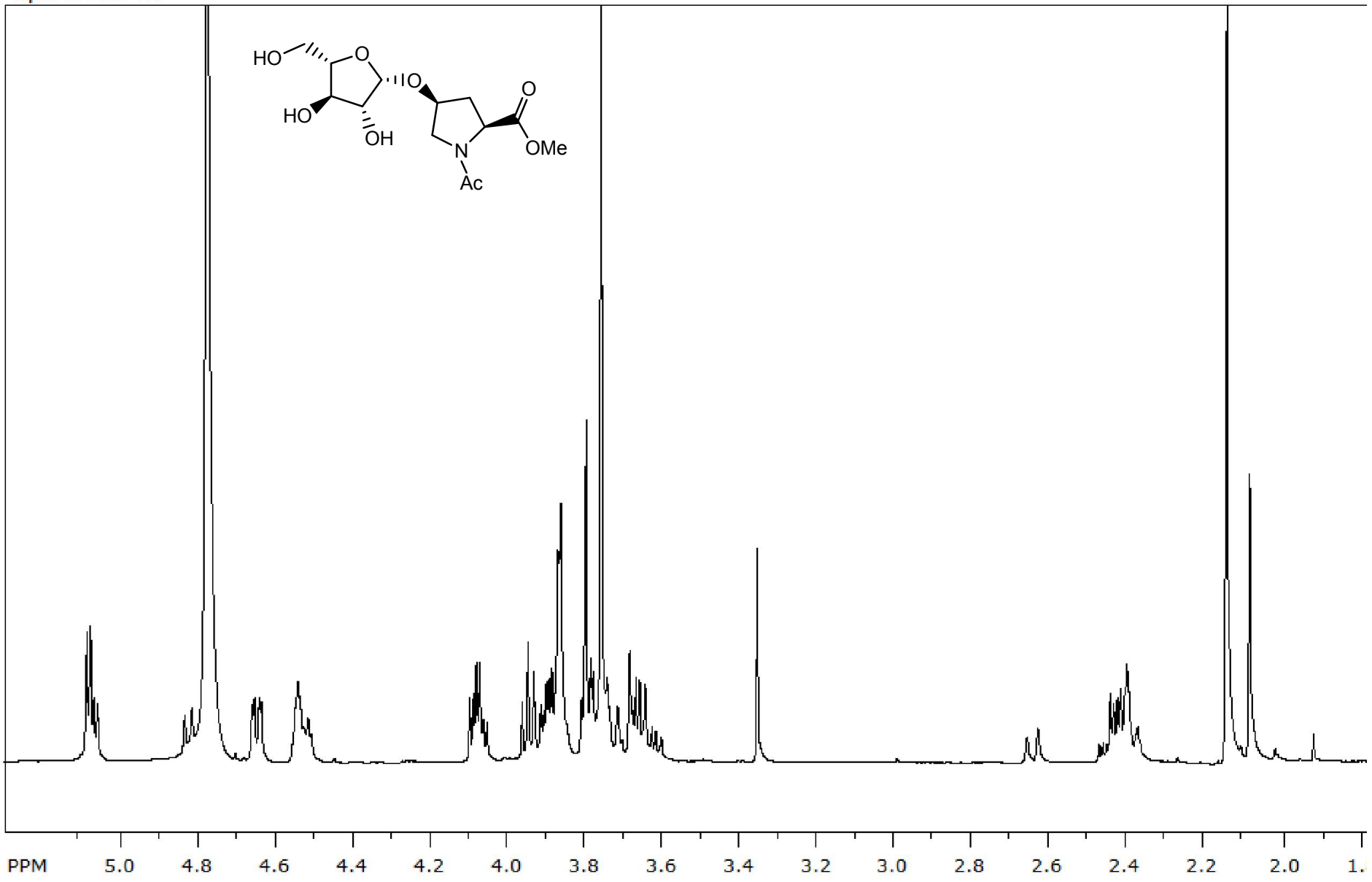


file: ...s\Cis-alpha-arabinose-NOe-1H\2\fid expt: <selnogp>
transmitter freq.: 500.133089 MHz
time domain size: 65536 points

freq. of 0 ppm: 500.130000 MHz
processed size: 65536 complex points
LB: 0.300 GF: 0.0000

SpinWorks 3: Cis-beta

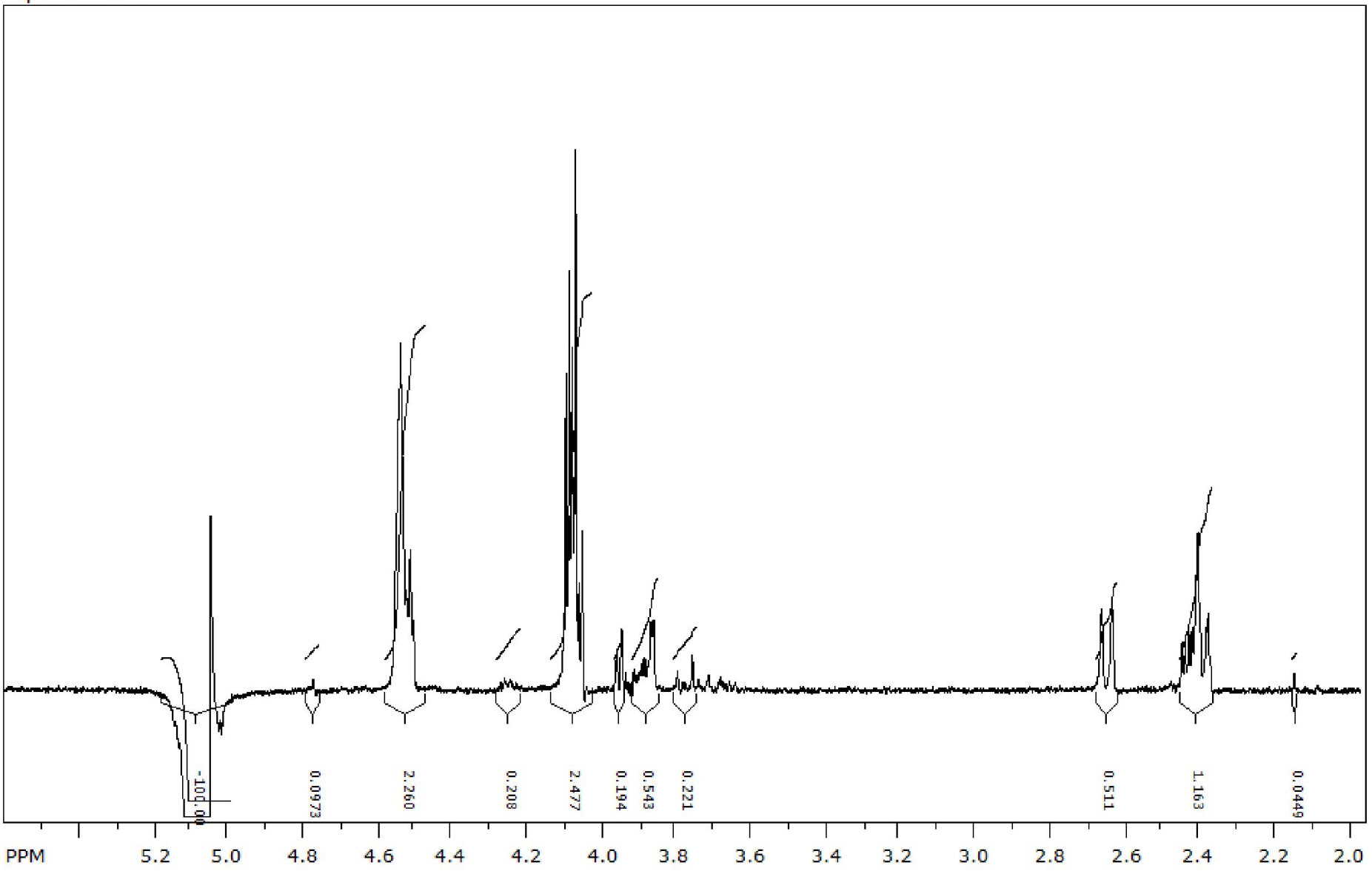
Compound 12b



file: ...es\Cis-beta-arabinose-NOe-1H\1\fid expt: <zg30>
transmitter freq.: 500.133089 MHz
time domain size: 65536 points

freq. of 0 ppm: 500.130000 MHz
processed size: 65536 complex points
LB: 0.300 GF: 0.0000

SpinWorks 3: Cis-beta

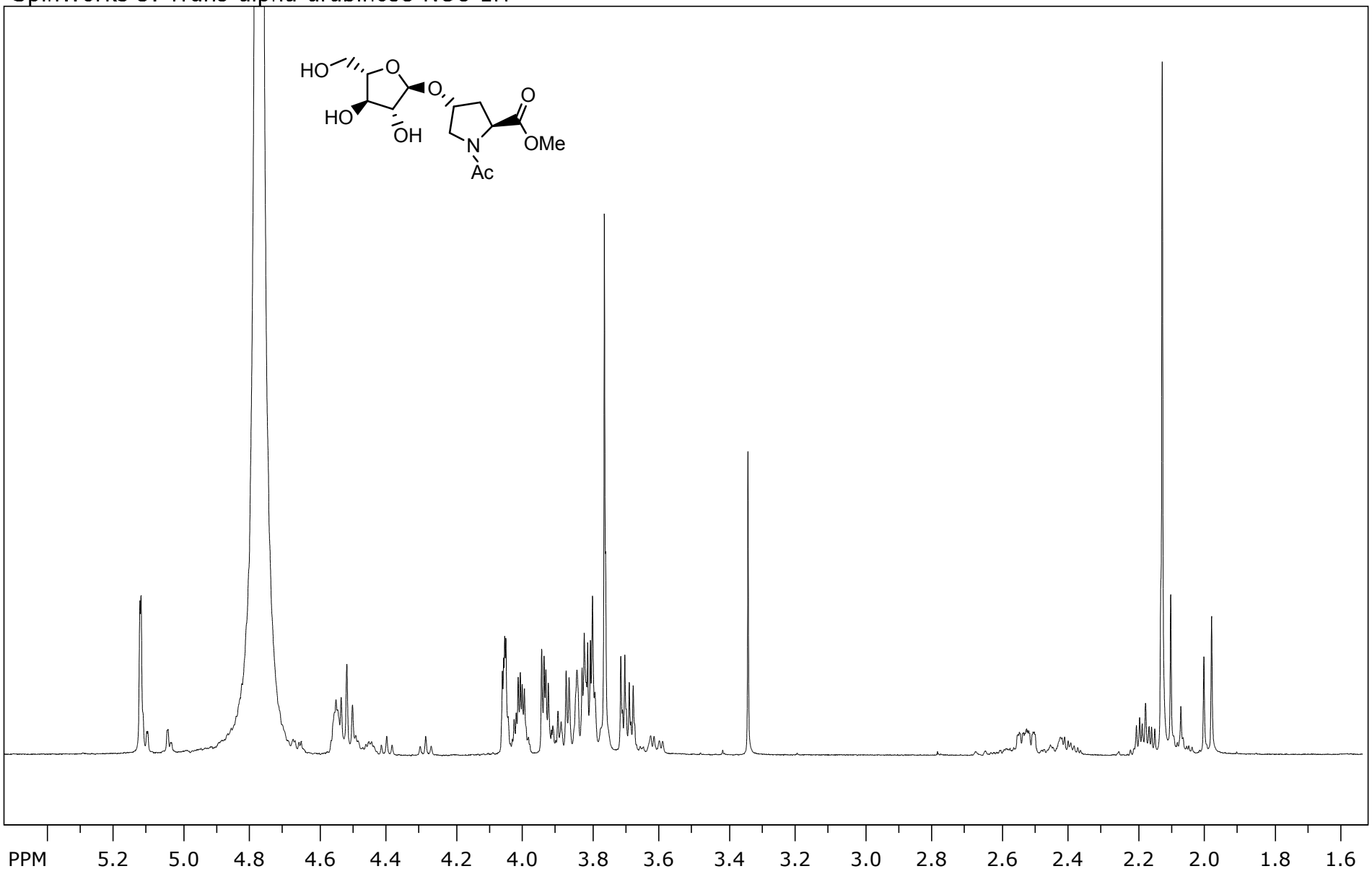


file: ...es\Cis-beta-arabinose-NOe-1H\2\fid expt: <selnogp>
transmitter freq.: 500.133089 MHz
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt

freq. of 0 ppm: 500.130000 MHz
processed size: 65536 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 73.076 ppm/cm: 0.14611

Compound 7a

SpinWorks 3: Trans-alpha-arabinose-NOe-1H

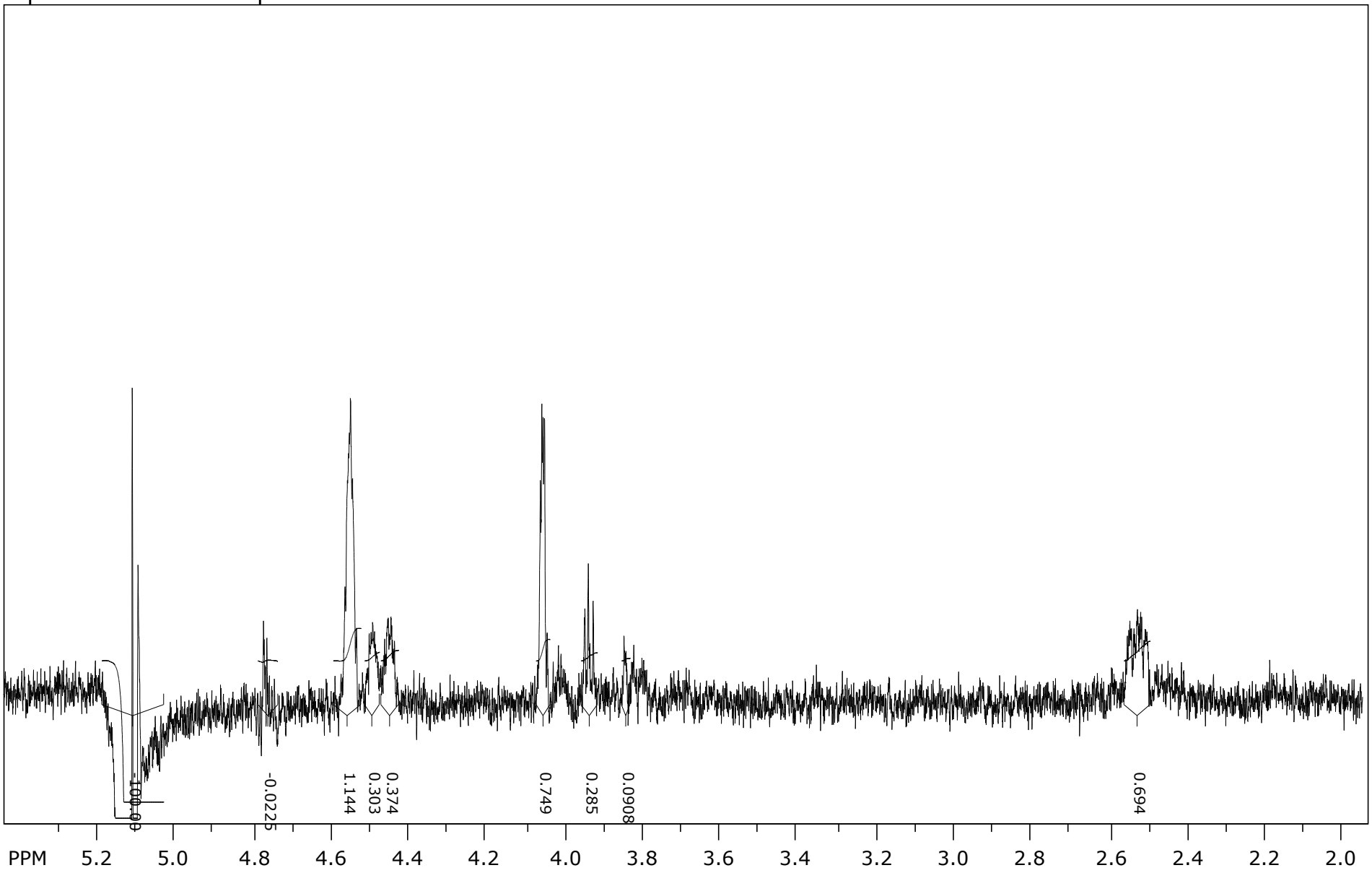


file: ...Trans-alpha-arabinose-NOe-1H\1\fid expt: <zg30>
transmitter freq.: 500.133089 MHz
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt

freq. of 0 ppm: 500.130000 MHz
processed size: 65536 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 79.958 ppm/cm: 0.15987

SpinWorks 3: Trans-alpha-arabinose-NOe-1H

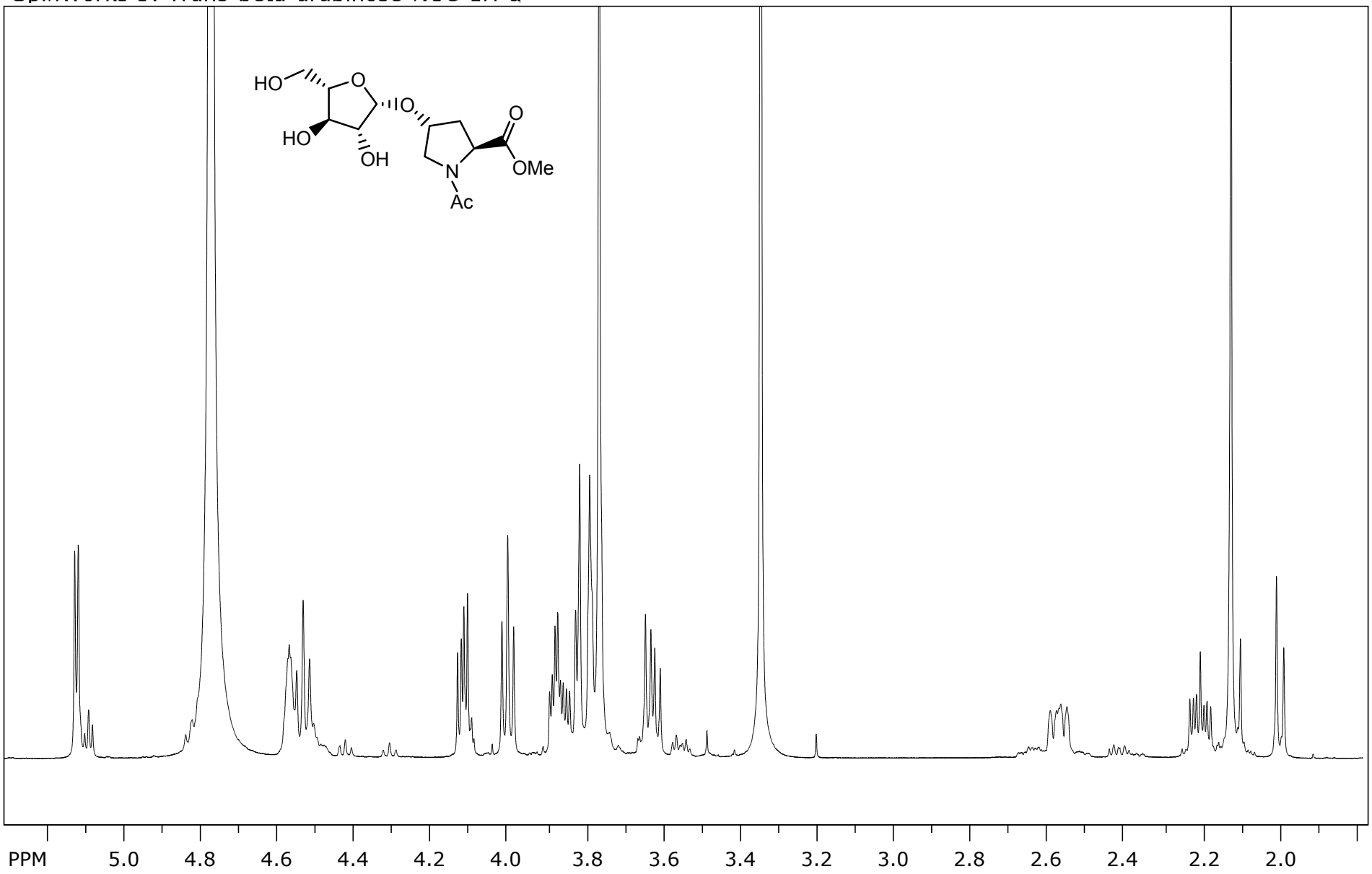
Compound 7a



file: ...Trans-alpha-arabinose-NOe-1H\2\fid expt: <selnogp>
transmitter freq.: 500.133089 MHz
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt

freq. of 0 ppm: 500.130000 MHz
processed size: 65536 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 70.127 ppm/cm: 0.14022

SpinWorks 3: Trans-beta-arabinose-NOe-1H-a

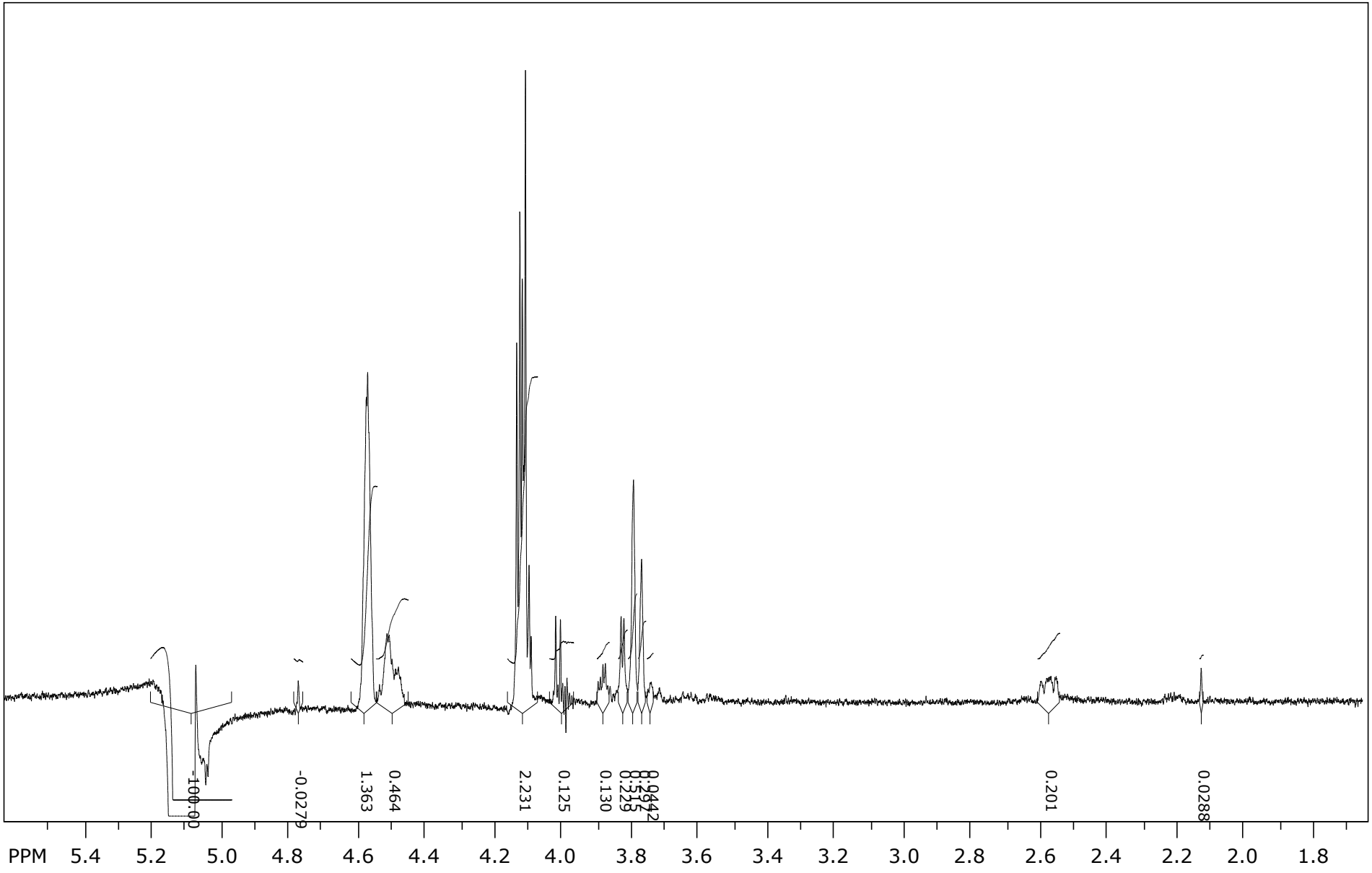


file: ...rans-beta-arabinose-NOe-1H-a\1\fid expt: <zg30>
transmitter freq.: 500.133089 MHz
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt

freq. of 0 ppm: 500.130000 MHz
processed size: 65536 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 70.782 ppm/cm: 0.14153

SpinWorks 3: Trans-beta-arabinose-NOe-1H-a

Compound 7b



file: ...rans-beta-arabinose-NOe-1H-a\2\fid expt: <selnogp>
transmitter freq.: 500.133089 MHz
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt

freq. of 0 ppm: 500.130000 MHz
processed size: 65536 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 79.958 ppm/cm: 0.15987

3J Coupling Constants by NUMMRIT Method(NMR)

Trans α Compound

sim_out.txt

*** SpinWorks simulation output, build 2008/10/11 ***

time: 14:49:44
date: 11/26/13

*** Reading spin system from:
C:\nmrdata\temp\nmr-dta-compounds\Trans-alpha-arabinose-mvr-2-73A\proton\spin_system ***

Options:

Opimize shifts and couplings? =	1
Ignore bad transitions? =	1
Auto assign observed peaks? =	1
Maximum number of iterations? =	30
RMS convergence limit =	0.020
RMS below this for autoassign =	0.250
Trans. freq. this * RMS ignored =	2.400

Groups and chemical shifts:

#	name	shift	spins	species	spin	sym
1	alpha	2232.976	1	1	1	1
2	beta1	1233.600	1	1	1	1
3	beta2	1058.149	1	1	1	1
4	gam	2249.700	1	1	1	1

Scalar coupling constants:

j[1, 2] =	j[alpha,beta1] =	8.254400
j[1, 3] =	j[alpha,beta2] =	8.410110
j[1, 4] =	j[alpha,gam] =	0.000000
j[2, 3] =	j[beta1,beta2] =	-13.672660
j[2, 4] =	j[beta1,gam] =	1.400000
j[3, 4] =	j[beta2,gam] =	4.845260

Dipolar coupling constants:

Paramaters to vary:

#	param.	range low	range hi
1	v[1]	1665.358	2806.843
2	v[3]	778.465	1347.931
3	j[1][2]	6.191	10.318
4	j[1][3]	6.308	10.513
5	j[2][3]	-10.254	-17.091
6	j[3][4]	3.634	6.057

Parameter map:

0	0	0	0
1	1	0	0
2	3	0	0
3	1	2	0
4	1	3	0
5	2	3	0
6	3	4	0

Reading assigned transitions

15 assigned transitions read
4 frequencies with no assignments read

Observed transitions read =	19
Total asigned line numbers =	15

Basis functions generated, transition information:

Species 1, up to 56 transitions, intensity 32

Total size of U3 matrix = 70 double words
Total sixe of E3 matrix = 16 double words

line	obs. freq.	calc. freq	diff.

1	1057.808	1057.653	0.155
3	2241.081	2241.340	-0.259
6	1071.298	1071.325	-0.027
9	2233.188	2232.928	0.259
11	1049.053	1049.241	-0.187
16	1052.928	1052.813	0.116
18	2233.044	2233.088	-0.044
28	2241.224	2241.340	-0.116
29	2224.720	2224.676	0.044
32	1062.974	1062.913	0.061
39	1066.562	1066.485	0.077
43	2233.044	2232.928	0.116
46	1044.317	1044.400	-0.083
51	2233.044	2233.088	-0.044
56	1057.808	1058.073	-0.265

Largest absolute difference = 0.265
Total assigned intensity = 14.986
RMS deviation of transitions = 0.19074

*** Ignored 0 transitions

*** Transitions now assigned = 15

*** Iteration # 1, new parameters are:

v[1] =	v[alpha]	2232.970	change:	-0.0065
v[2] =	v[beta1]	1233.600		
v[3] =	v[beta2]	1058.129	change:	-0.0193
v[4] =	v[gam]	2249.700		
j[1][2] =	j[alpha][beta1]	8.267	change:	0.0131
j[1][3] =	j[alpha][beta2]	8.400	change:	-0.0101
j[1][4] =	j[alpha][gam]	0.000		
j[2][3] =	j[beta1][beta2]	-13.634	change:	0.0386
j[2][4] =	j[beta1][gam]	1.400		
j[3][4] =	j[beta2][gam]	4.885	change:	0.0393

Largest absolute difference = 0.254
Total assigned intensity = 14.986
RMS deviation of transitions = 0.18779
Fractional change of RMS = -0.01545

*** Ignored 0 transitions

*** Transitions now assigned = 15

*** Iteration # 2, new parameters are:

v[1] =	v[alpha]	2232.970	change:	-0.0000
v[2] =	v[beta1]	1233.600		
v[3] =	v[beta2]	1058.128	change:	-0.0015
v[4] =	v[gam]	2249.700		
j[1][2] =	j[alpha][beta1]	8.267	change:	-0.0000
j[1][3] =	j[alpha][beta2]	8.400	change:	0.0000
j[1][4] =	j[alpha][gam]	0.000		
j[2][3] =	j[beta1][beta2]	-13.634	change:	-0.0000
j[2][4] =	j[beta1][gam]	1.400		
j[3][4] =	j[beta2][gam]	4.885	change:	-0.0000

Largest absolute difference = 0.254
Total assigned intensity = 14.986
RMS deviation of transitions = 0.18779
Fractional change of RMS = -0.00003

*** Automatic assignment has occurred ***

*** Transitions now assigned = 16

*** Iteration # 3, new parameters are:

v[1] =	v[alpha]	2232.976	change:	0.0065
v[2] =	v[beta1]	1233.600		
v[3] =	v[beta2]	1058.147	change:	0.0193
v[4] =	v[gam]	2249.700		
j[1][2] =	j[alpha][beta1]	8.254	change:	-0.0131
j[1][3] =	j[alpha][beta2]	8.410	change:	0.0101
j[1][4] =	j[alpha][gam]	0.000		
j[2][3] =	j[beta1][beta2]	-13.673	change:	-0.0386
j[2][4] =	j[beta1][gam]	1.400		
j[3][4] =	j[beta2][gam]	4.846	change:	-0.0386

Largest absolute difference = 0.186

```
   Total assigned intensity     =    16.000
   RMS deviation of transitions =   0.12792
   Fractional change of RMS     =  -0.31879


***  Ignored     0 transitions

***   Transitions now assigned =       16

***  Iteration # 4, new parameters are:
   v[1] =       v[alpha]                  2232.976 change:       0.0000
   v[2] =       v[beta1]                  1233.600
   v[3] =       v[beta2]                  1058.149 change:       0.0015
   v[4] =       v[gam]              2249.700
   j[1][2] =    j[alpha][beta1]              8.254 change:       0.0000
   j[1][3] =    j[alpha][beta2]              8.410 change:      -0.0000
   j[1][4] =    j[alpha][gam]                0.000
   j[2][3] =    j[beta1][beta2]            -13.673 change:       0.0000
   j[2][4] =    j[beta1][gam]                1.400
   j[3][4] =    j[beta2][gam]                4.846 change:       0.0000

   Largest absolute difference  =     0.188
   Total assigned intensity     =    16.000
   RMS deviation of transitions =   0.12792
   Fractional change of RMS     =  -0.00005


***  Ignored     0 transitions

***   Transitions now assigned =       16

***  Iteration # 5, new parameters are:
   v[1] =       v[alpha]                  2232.976 change:       0.0000
   v[2] =       v[beta1]                  1233.600
   v[3] =       v[beta2]                  1058.149 change:      -0.0000
   v[4] =       v[gam]              2249.700
   j[1][2] =    j[alpha][beta1]              8.254 change:      -0.0000
   j[1][3] =    j[alpha][beta2]              8.410 change:       0.0000
   j[1][4] =    j[alpha][gam]                0.000
   j[2][3] =    j[beta1][beta2]            -13.673 change:      -0.0000
   j[2][4] =    j[beta1][gam]                1.400
   j[3][4] =    j[beta2][gam]                4.846 change:      -0.0000

   Largest absolute difference  =     0.188
   Total assigned intensity     =    16.000
   RMS deviation of transitions =   0.12792
   Fractional change of RMS     =   0.00000

***   Automatic assignment has occurred  ***

***   Transitions now assigned =       16

***  Iteration # 6, new parameters are:
   v[1] =       v[alpha]                  2232.976 change:      -0.0000
   v[2] =       v[beta1]                  1233.600
   v[3] =       v[beta2]                  1058.149 change:       0.0000
   v[4] =       v[gam]              2249.700
   j[1][2] =    j[alpha][beta1]              8.254 change:      -0.0000
   j[1][3] =    j[alpha][beta2]              8.410 change:      -0.0000
   j[1][4] =    j[alpha][gam]                0.000
   j[2][3] =    j[beta1][beta2]            -13.673 change:       0.0000
   j[2][4] =    j[beta1][gam]                1.400
   j[3][4] =    j[beta2][gam]                4.846 change:       0.0000

   Largest absolute difference  =     0.188
   Total assigned intensity     =    16.000
   RMS deviation of transitions =   0.12792
   Fractional change of RMS     =   0.00000


***  Ignored     0 transitions

***   Transitions now assigned =       16

***  Iteration # 7, new parameters are:
   v[1] =       v[alpha]                  2232.976 change:      -0.0000
   v[2] =       v[beta1]                  1233.600
   v[3] =       v[beta2]                  1058.149 change:      -0.0000
   v[4] =       v[gam]              2249.700
   j[1][2] =    j[alpha][beta1]              8.254 change:       0.0000
```

```
   j[1][3] =    j[alpha][beta2]              8.410 change:       -0.0000
   j[1][4] =    j[alpha][gam]                0.000
   j[2][3] =    j[beta1][beta2]            -13.673 change:       -0.0000
   j[2][4] =    j[beta1][gam]                1.400
   j[3][4] =    j[beta2][gam]                4.846 change:       -0.0000

   Largest absolute difference  =     0.188
   Total assigned intensity     =    16.000
   RMS deviation of transitions =   0.12792
   Fractional change of RMS     =  -0.00000


***  Ignored     0 transitions

***   Transitions now assigned =       16

***  Iteration # 8, new parameters are:
   v[1] =       v[alpha]                  2232.976 change:        0.0000
   v[2] =       v[beta1]                  1233.600
   v[3] =       v[beta2]                  1058.149 change:       -0.0000
   v[4] =       v[gam]            2249.700
   j[1][2] =    j[alpha][beta1]              8.254 change:        0.0000
   j[1][3] =    j[alpha][beta2]              8.410 change:       -0.0000
   j[1][4] =    j[alpha][gam]                0.000
   j[2][3] =    j[beta1][beta2]            -13.673 change:        0.0000
   j[2][4] =    j[beta1][gam]                1.400
   j[3][4] =    j[beta2][gam]                4.846 change:       -0.0000

   Largest absolute difference  =     0.188
   Total assigned intensity     =    16.000
   RMS deviation of transitions =   0.12792
   Fractional change of RMS     =  -0.00000

*** Convergence achieved (Parameters not changing)
      This could be due to insufficient assigned transitions in a second-order spectrum

***  After final iteration:
***       current iteration count =            8
***       transitions now assigned =          16
***       current RMS =                 0.127916

***  Final parameters after 8 iterations are:
   v[1] =       v[alpha]                  2232.976 Hz. +/-       0.0452 Hz.
   v[2] =       v[beta1]                  1233.600 Hz.
   v[3] =       v[beta2]                  1058.149 Hz. +/-       0.0453 Hz.
   v[4] =       v[gam]            2249.700 Hz.
   j[1][2] =    j[alpha][beta1]              8.254 Hz. +/-       0.0906 Hz.
   j[1][3] =    j[alpha][beta2]              8.410 Hz. +/-       0.0641 Hz.
   j[1][4] =    j[alpha][gam]                0.000 Hz.
   j[2][3] =    j[beta1][beta2]            -13.673 Hz. +/-       0.0905 Hz.
   j[2][4] =    j[beta1][gam]                1.400 Hz.
   j[3][4] =    j[beta2][gam]                4.846 Hz. +/-       0.0906 Hz.

***  Parameter correlation coefficients:
   1  1.000
   2 -0.000  1.000
   3 -0.000  0.000  1.000
   4  0.000 -0.000 -0.003  1.000
   5  0.000 -0.000 -0.000 -0.000  1.000
   6 -0.000  0.000 -0.000 -0.000 -0.000  1.000


 Transitions for isotopic species 1

     46   1044.317   1044.400    -0.083    0.91257
     11   1049.053   1049.241    -0.188    0.91867
     16   1052.928   1052.812     0.116    0.92585
      1   1057.808   1057.653     0.154    0.93211
     56   1057.962   1058.072    -0.111    1.06507
     32   1062.974   1062.913     0.061    1.07504
     39   1066.562   1066.485     0.077    1.08026
      6   1071.298   1071.326    -0.028    1.09043
     52              1222.184              1.06673
     19              1223.589              1.07137
     24              1230.436              1.08398
      2              1231.841              1.08872
     55              1235.856              0.91404
     30              1237.261              0.91484
     37              1244.108              0.92973
      5              1245.513              0.93059
```

54	2224.720	2224.676	0.044	1.01442
29	2224.720	2224.676	0.044	1.01660
43	2233.044	2232.928	0.116	0.99805
9	2233.044	2232.928	0.116	0.99959
51	2233.044	2233.088	-0.044	1.00164
18	2233.044	2233.088	-0.044	1.00036
28	2241.224	2241.340	-0.116	0.98668
3	2241.224	2241.340	-0.116	0.98266
53		2246.582		1.00648
35		2246.583		1.00442
42		2247.987		1.00342
13		2247.987		1.00193
50		2251.423		0.99669
22		2251.424		0.99793
27		2252.828		0.99261
4		2252.828		0.99651

*** 32 transitions for species 1 written to plot file

RMS deviation of 11 peaks grouped within 0.050 Hz = 0.15739 Hz

*** Simulation normal exit ***
Calculation time = 0 seconds

```
***  SpinWorks simulation output, build 2008/10/11  ***

       time:  15:53:41
       date:  11/29/13


***  Reading spin system from: C:\Users\murthy\Desktop\nmr-dta-compounds
\Trans-beta-arabinose-MVR-2-92A\MVR-2-92\1\spin_system  ***

Options:

   Opimize shifts and couplings? =          1
   Ignore bad transitions? =                1
   Auto assign observed peaks? =            1
   Maximum number of iterations? =         30
   RMS convergence limit =              0.020
   RMS below this for autoassign =      0.250
   Trans. freq. this * RMS ignored =    2.400


Groups and chemical shifts:

  #      name     shift           spins    species spin     sym

  1       alpha   2240.618          1        1        1       1
  2       beta1   1257.000          1        1        1       1
  3       beta2   1072.369          1        1        1       1
  4         gam   2258.000          1        1        1       1


Scalar coupling constants:

   j[ 1, 2] =   j[alpha,beta1] =         8.490230
   j[ 1, 3] =   j[alpha,beta2] =         8.368170
   j[ 1, 4] =   j[alpha,gam] =   0.000000
   j[ 2, 3] =   j[beta1,beta2] =        -13.483640
   j[ 2, 4] =   j[beta1,gam] =   1.500000
   j[ 3, 4] =   j[beta2,gam] =   4.753340


Dipolar coupling constants:


Paramaters to vary:

  #       param.          range low        range hi
```

1	v[1]	1670.981	2816.577
2	v[3]	789.311	1365.403
3	j[1][2]	6.368	10.613
4	j[1][3]	6.276	10.460
5	j[2][3]	-10.113	-16.855
6	j[3][4]	3.565	5.942

Parameter map:

0	0	0	0
1	1	0	0
2	3	0	0
3	1	2	0
4	1	3	0
5	2	3	0
6	3	4	0

Reading assigned transitions

11 assigned transitions read

Observed transitions read = 11
Total asigned line numbers = 11

Basis functions generated, transition information:

Species 1, up to 56 transitions, intensity 32

Total size of U3 matrix = 70 double words
Total sixe of E3 matrix = 16 double words

line	obs. freq.	calc. freq	diff.
1	1071.970	1071.921	0.048
6	1085.752	1085.405	0.347
9	2241.049	2240.710	0.339
11	1063.156	1063.551	-0.395
28	2248.741	2249.080	-0.339
29	2232.235	2232.222	0.012
39	1080.624	1080.656	-0.032
46	1059.149	1058.802	0.347
51	2240.568	2240.593	-0.025
54	2232.235	2232.222	0.012
56	1071.970	1072.285	-0.315

Largest absolute difference = 0.395
Total assigned intensity = 11.018
RMS deviation of transitions = 0.38237

```
***  Ignored     0 transitions

***   Transitions now assigned =       11

***  Iteration # 1, new parameters are:
   v[1] =       v[alpha]                  2240.618 change:       0.0000
   v[2] =       v[beta1]                  1257.000
   v[3] =       v[beta2]                  1072.369 change:       0.0000
   v[4] =       v[gam]            2258.000
   j[1][2] =    j[alpha][beta1]              8.490 change:      -0.0000
   j[1][3] =    j[alpha][beta2]              8.368 change:      -0.0000
   j[1][4] =    j[alpha][gam]                0.000
   j[2][3] =    j[beta1][beta2]            -13.484 change:      -0.0000
   j[2][4] =    j[beta1][gam]                1.500
   j[3][4] =    j[beta2][gam]                4.753 change:      -0.0000

   Largest absolute difference  =     0.395
   Total assigned intensity     =    11.018
   RMS deviation of transitions =   0.38237
   Fractional change of RMS     =  -0.00000


***  Ignored     0 transitions

***   Transitions now assigned =       11

***  Iteration # 2, new parameters are:
   v[1] =       v[alpha]                  2240.618 change:       0.0000
   v[2] =       v[beta1]                  1257.000
   v[3] =       v[beta2]                  1072.369 change:       0.0000
   v[4] =       v[gam]            2258.000
   j[1][2] =    j[alpha][beta1]              8.490 change:      -0.0000
   j[1][3] =    j[alpha][beta2]              8.368 change:       0.0000
   j[1][4] =    j[alpha][gam]                0.000
   j[2][3] =    j[beta1][beta2]            -13.484 change:       0.0000
   j[2][4] =    j[beta1][gam]                1.500
   j[3][4] =    j[beta2][gam]                4.753 change:       0.0000

   Largest absolute difference  =     0.395
   Total assigned intensity     =    11.018
   RMS deviation of transitions =   0.38237
   Fractional change of RMS     =   0.00000


***  Ignored     0 transitions
```

```
***  Ignored     0 transitions

***   Transitions now assigned =       11

***  Iteration # 4, new parameters are:
   v[1] =       v[alpha]                  2240.618 change:       0.0000
   v[2] =       v[beta1]                  1257.000
   v[3] =       v[beta2]                  1072.369 change:       0.0000
   v[4] =       v[gam]            2258.000
   j[1][2] =    j[alpha][beta1]              8.490 change:       0.0000
   j[1][3] =    j[alpha][beta2]              8.368 change:      -0.0000
   j[1][4] =    j[alpha][gam]                0.000
   j[2][3] =    j[beta1][beta2]            -13.484 change:      -0.0000
   j[2][4] =    j[beta1][gam]                1.500
   j[3][4] =    j[beta2][gam]                4.753 change:       0.0000

   Largest absolute difference  =     0.395
   Total assigned intensity     =    11.018
   RMS deviation of transitions =   0.38237
   Fractional change of RMS     =  -0.00000


***  Ignored     0 transitions

***   Transitions now assigned =       11

***  Iteration # 5, new parameters are:
```

```
***   Transitions now assigned =       11

***  Iteration # 3, new parameters are:
   v[1] =       v[alpha]                  2240.618 change:       0.0000
   v[2] =       v[beta1]                  1257.000
   v[3] =       v[beta2]                  1072.369 change:      -0.0000
   v[4] =       v[gam]            2258.000
   j[1][2] =    j[alpha][beta1]              8.490 change:      -0.0000
   j[1][3] =    j[alpha][beta2]              8.368 change:       0.0000
   j[1][4] =    j[alpha][gam]                0.000
   j[2][3] =    j[beta1][beta2]            -13.484 change:       0.0000
   j[2][4] =    j[beta1][gam]                1.500
   j[3][4] =    j[beta2][gam]                4.753 change:      -0.0000

   Largest absolute difference  =     0.395
   Total assigned intensity     =    11.018
   RMS deviation of transitions =   0.38237
   Fractional change of RMS     =  -0.00000
```

```
   v[1] =       v[alpha]                2240.618 change:      -0.0000
   v[2] =       v[beta1]                1257.000
   v[3] =       v[beta2]                1072.369 change:      -0.0000
   v[4] =       v[gam]           2258.000
   j[1][2] =    j[alpha][beta1]            8.490 change:      -0.0000
   j[1][3] =    j[alpha][beta2]            8.368 change:       0.0000
   j[1][4] =    j[alpha][gam]              0.000
   j[2][3] =    j[beta1][beta2]          -13.484 change:       0.0000
   j[2][4] =    j[beta1][gam]              1.500
   j[3][4] =    j[beta2][gam]              4.753 change:       0.0000

   Largest absolute difference  =     0.395
   Total assigned intensity     =    11.018
   RMS deviation of transitions =   0.38237
   Fractional change of RMS     =   0.00000


***  Ignored     0 transitions

***   Transitions now assigned =       11

***  Iteration # 6, new parameters are:
   v[1] =       v[alpha]                2240.618 change:       0.0000
   v[2] =       v[beta1]                1257.000
   v[3] =       v[beta2]                1072.369 change:       0.0000
   v[4] =       v[gam]           2258.000
   j[1][2] =    j[alpha][beta1]            8.490 change:       0.0000
   j[1][3] =    j[alpha][beta2]            8.368 change:       0.0000
   j[1][4] =    j[alpha][gam]              0.000
   j[2][3] =    j[beta1][beta2]          -13.484 change:      -0.0000
   j[2][4] =    j[beta1][gam]              1.500
   j[3][4] =    j[beta2][gam]              4.753 change:      -0.0000

   Largest absolute difference  =     0.395
   Total assigned intensity     =    11.018
   RMS deviation of transitions =   0.38237
   Fractional change of RMS     =   0.00000


***  Ignored     0 transitions

***   Transitions now assigned =       11

***  Iteration # 7, new parameters are:
   v[1] =       v[alpha]                2240.618 change:      -0.0000
   v[2] =       v[beta1]                1257.000
   v[3] =       v[beta2]                1072.369 change:       0.0000
```

```
   v[4] =       v[gam]            2258.000
   j[1][2] =    j[alpha][beta1]              8.490 change:      -0.0000
   j[1][3] =    j[alpha][beta2]              8.368 change:       0.0000
   j[1][4] =    j[alpha][gam]                0.000
   j[2][3] =    j[beta1][beta2]            -13.484 change:       0.0000
   j[2][4] =    j[beta1][gam]                1.500
   j[3][4] =    j[beta2][gam]                4.753 change:      -0.0000

   Largest absolute difference  =     0.395
   Total assigned intensity     =    11.018
   RMS deviation of transitions =   0.38237
   Fractional change of RMS     =  -0.00000


***  Ignored     0 transitions

***   Transitions now assigned =       11

***  Iteration # 8, new parameters are:
   v[1] =       v[alpha]                  2240.618 change:      -0.0000
   v[2] =       v[beta1]                  1257.000
   v[3] =       v[beta2]                  1072.369 change:      -0.0000
   v[4] =       v[gam]            2258.000
   j[1][2] =    j[alpha][beta1]              8.490 change:      -0.0000
   j[1][3] =    j[alpha][beta2]              8.368 change:       0.0000
   j[1][4] =    j[alpha][gam]                0.000
   j[2][3] =    j[beta1][beta2]            -13.484 change:       0.0000
   j[2][4] =    j[beta1][gam]                1.500
   j[3][4] =    j[beta2][gam]                4.753 change:       0.0000

   Largest absolute difference  =     0.395
   Total assigned intensity     =    11.018
   RMS deviation of transitions =   0.38237
   Fractional change of RMS     =   0.00000

*** Convergence achieved (Parameters not changing)
      This could be due to insufficient assigned transitions in a second-order
spectrum

***  After final iteration:
***      current iteration count =            8
***      transitions now assigned =          11
***      current RMS =                 0.382374

***  Final parameters after 8 iterations are:
   v[1] =       v[alpha]                  2240.618 Hz. +/-       0.1759 Hz.
   v[2] =       v[beta1]                  1257.000 Hz.
```

```
   v[3] =       v[beta2]                 1072.369 Hz. +/-       0.1563 Hz.
   v[4] =       v[gam]             2258.000 Hz.
   j[1][2] =    j[alpha][beta1]            8.490 Hz. +/-       0.3523 Hz.
   j[1][3] =    j[alpha][beta2]            8.368 Hz. +/-       0.2602 Hz.
   j[1][4] =    j[alpha][gam]              0.000 Hz.
   j[2][3] =    j[beta1][beta2]          -13.484 Hz. +/-       0.3557 Hz.
   j[2][4] =    j[beta1][gam]              1.500 Hz.
   j[3][4] =    j[beta2][gam]              4.753 Hz. +/-       0.3562 Hz.

***  Parameter correlation coefficients:
   1  1.000
   2 -0.000  1.000
   3  0.182  0.000  1.000
   4  0.123 -0.000 -0.126  1.000
   5  0.045 -0.000 -0.045  0.365  1.000
   6 -0.045  0.000  0.045 -0.365 -0.422  1.000


 Transitions for isotopic species 1

     46   1059.149   1058.802     0.347    0.91727
     11   1063.156   1063.551    -0.395    0.92352
     16              1067.172              0.93071
      1   1071.970   1071.922     0.048    0.93713
     56   1071.970   1072.285    -0.315    1.06047
     32              1077.034              1.07007
     39   1080.624   1080.656    -0.032    1.07552
      6   1085.752   1085.405     0.347    1.08531
     52              1245.490              1.06159
     19              1246.994              1.06622
     24              1253.977              1.07942
      2              1255.481              1.08414
     55              1258.973              0.91819
     30              1260.477              0.91950
     37              1267.460              0.93479
      5              1268.964              0.93616
     54   2232.235   2232.222     0.012    1.01482
     29   2232.235   2232.222     0.012    1.01696
     51   2240.568   2240.593    -0.025    1.00193
     18              2240.593              1.00080
     43              2240.710              0.99777
      9   2241.049   2240.710     0.339    0.99912
     28   2248.741   2249.080    -0.339    0.98625
      3              2249.081              0.98235
     53              2254.879              1.00652
     35              2254.879              1.00451
     42              2256.383              1.00315
```

```
     13              2256.383              1.00185
     50              2259.628              0.99695
     22              2259.628              0.99803
     27              2261.132              0.99261
      4              2261.132              0.99638

***  32 transitions for species 1 written to plot file

  RMS deviation of      9 peaks grouped within      0.050 Hz =    0.46981 Hz


***  Simulation normal exit  ***
     Calculation time = 0 seconds
```

sim_out.txt

*** SpinWorks simulation output, build 2008/10/11 ***

time: 15:19:17
date: 11/26/13

*** Reading spin system from:
C:\nmrdata\temp\nmr-dta-compounds\MVR-2-73C-Cis-alpha-arabinose\1\spin_system ***

Options:

Opimize shifts and couplings? =	1
Ignore bad transitions? =	1
Auto assign observed peaks? =	1
Maximum number of iterations? =	30
RMS convergence limit =	0.020
RMS below this for autoassign =	0.250
Trans. freq. this * RMS ignored =	2.400

Groups and chemical shifts:

#	name	shift	spins	species	spin	sym
1	alpha	2232.000	1	1	1	1
2	beta1	1231.600	1	1	1	1
3	beta2	1056.700	1	1	1	1
4	gam	2248.300	1	1	1	1

Scalar coupling constants:

j[1, 2] =	j[alpha,beta1] =	8.200000	
j[1, 3] =	j[alpha,beta2] =	8.400000	
j[1, 4] =	j[alpha,gam] =	0.000000	
j[2, 3] =	j[beta1,beta2] =	-13.700000	
j[2, 4] =	j[beta1,gam] =	1.400000	
j[3, 4] =	j[beta2,gam] =	4.800000	

Dipolar coupling constants:

Paramaters to vary:

#	param.	range low	range hi
1	v[1]	1664.663	2805.563
2	v[3]	777.394	1346.094
3	j[1][2]	6.150	10.250
4	j[1][3]	6.300	10.500
5	j[2][3]	-10.275	-17.125
6	j[3][4]	3.600	6.000

Parameter map:

0	0	0	0
1	1	0	0
2	3	0	0
3	1	2	0
4	1	3	0
5	2	3	0
6	3	4	0

Reading assigned transitions

16 assigned transitions read

Observed transitions read =	16
Total asigned line numbers =	16

Basis functions generated, transition information:

Species 1, up to 56 transitions, intensity 32

Total size of U3 matrix = 70 double words
Total sixe of E3 matrix = 16 double words

line	obs. freq.	calc. freq	diff.
1	1056.415	1056.161	0.255

```
        3         2240.208        2240.332          -0.123
        6         1069.807        1069.861          -0.053
        9         2231.943        2231.930           0.013
       11         1047.581        1047.759          -0.177
       16         1051.126        1051.366          -0.240
       18         2231.943        2232.134          -0.191
       28         2240.258        2240.332          -0.074
       29         2223.677        2223.732          -0.055
       32         1061.480        1061.459           0.021
       39         1065.137        1065.066           0.071
       43         2232.190        2231.930           0.260
       46         1042.799        1042.964          -0.165
       51         2231.943        2232.134          -0.191
       54         2223.677        2223.732          -0.055
       56         1056.415        1056.664          -0.248
   Largest absolute difference  =     0.260
   Total assigned intensity     =    16.000
   RMS deviation of transitions =   0.20466


***  Ignored     0 transitions

***   Transitions now assigned =       16

***  Iteration # 1, new parameters are:
   v[1] =       v[alpha]                  2231.948 change:       -0.0521
   v[2] =       v[beta1]                  1231.600
   v[3] =       v[beta2]                  1056.633 change:       -0.0672
   v[4] =       v[gam]              2248.300
   j[1][2] =    j[alpha][beta1]              8.342 change:        0.1424
   j[1][3] =    j[alpha][beta2]              8.382 change:       -0.0178
   j[1][4] =    j[alpha][gam]                0.000
   j[2][3] =    j[beta1][beta2]            -13.730 change:       -0.0295
   j[2][4] =    j[beta1][gam]                1.400
   j[3][4] =    j[beta2][gam]                4.957 change:        0.1568

   Largest absolute difference  =     0.268
   Total assigned intensity     =    16.000
   RMS deviation of transitions =   0.16393
   Fractional change of RMS     =  -0.19900


***  Ignored     0 transitions

***   Transitions now assigned =       16

***  Iteration # 2, new parameters are:
   v[1] =       v[alpha]                  2231.947 change:       -0.0005
   v[2] =       v[beta1]                  1231.600
   v[3] =       v[beta2]                  1056.634 change:        0.0014
   v[4] =       v[gam]              2248.300
   j[1][2] =    j[alpha][beta1]              8.342 change:        0.0000
   j[1][3] =    j[alpha][beta2]              8.382 change:       -0.0000
   j[1][4] =    j[alpha][gam]                0.000
   j[2][3] =    j[beta1][beta2]            -13.730 change:       -0.0000
   j[2][4] =    j[beta1][gam]                1.400
   j[3][4] =    j[beta2][gam]                4.957 change:        0.0000

   Largest absolute difference  =     0.267
   Total assigned intensity     =    16.000
   RMS deviation of transitions =   0.16393
   Fractional change of RMS     =  -0.00003

***   Automatic assignment has occurred  ***

***   Transitions now assigned =       16

***  Iteration # 3, new parameters are:
   v[1] =       v[alpha]                  2231.923 change:       -0.0247
   v[2] =       v[beta1]                  1231.600
   v[3] =       v[beta2]                  1056.634 change:        0.0000
   v[4] =       v[gam]              2248.300
   j[1][2] =    j[alpha][beta1]              8.293 change:       -0.0496
   j[1][3] =    j[alpha][beta2]              8.419 change:        0.0373
   j[1][4] =    j[alpha][gam]                0.000
   j[2][3] =    j[beta1][beta2]            -13.730 change:       -0.0000
   j[2][4] =    j[beta1][gam]                1.400
   j[3][4] =    j[beta2][gam]                4.957 change:        0.0000

   Largest absolute difference  =     0.249
```

```
   Total assigned intensity     =    16.000
   RMS deviation of transitions =   0.13738
   Fractional change of RMS     =  -0.16196


***  Ignored     0 transitions

***   Transitions now assigned =       16

***  Iteration # 4, new parameters are:
   v[1] =       v[alpha]                    2231.923 change:       0.0001
   v[2] =       v[beta1]                    1231.600
   v[3] =       v[beta2]                    1056.634 change:       0.0001
   v[4] =       v[gam]              2248.300
   j[1][2] =    j[alpha][beta1]                8.293 change:      -0.0000
   j[1][3] =    j[alpha][beta2]                8.419 change:       0.0000
   j[1][4] =    j[alpha][gam]                  0.000
   j[2][3] =    j[beta1][beta2]              -13.730 change:       0.0000
   j[2][4] =    j[beta1][gam]                  1.400
   j[3][4] =    j[beta2][gam]                  4.957 change:       0.0000

   Largest absolute difference  =     0.248
   Total assigned intensity     =    16.000
   RMS deviation of transitions =   0.13738
   Fractional change of RMS     =  -0.00000


***  Ignored     0 transitions

***   Transitions now assigned =       16

***  Iteration # 5, new parameters are:
   v[1] =       v[alpha]                    2231.923 change:       0.0000
   v[2] =       v[beta1]                    1231.600
   v[3] =       v[beta2]                    1056.634 change:      -0.0000
   v[4] =       v[gam]              2248.300
   j[1][2] =    j[alpha][beta1]                8.293 change:      -0.0000
   j[1][3] =    j[alpha][beta2]                8.419 change:       0.0000
   j[1][4] =    j[alpha][gam]                  0.000
   j[2][3] =    j[beta1][beta2]              -13.730 change:       0.0000
   j[2][4] =    j[beta1][gam]                  1.400
   j[3][4] =    j[beta2][gam]                  4.957 change:      -0.0000

   Largest absolute difference  =     0.248
   Total assigned intensity     =    16.000
   RMS deviation of transitions =   0.13738
   Fractional change of RMS     =  -0.00000

***   Automatic assignment has occurred  ***

***   Transitions now assigned =       16

***  Iteration # 6, new parameters are:
   v[1] =       v[alpha]                    2231.923 change:       0.0000
   v[2] =       v[beta1]                    1231.600
   v[3] =       v[beta2]                    1056.634 change:      -0.0000
   v[4] =       v[gam]              2248.300
   j[1][2] =    j[alpha][beta1]                8.293 change:       0.0000
   j[1][3] =    j[alpha][beta2]                8.419 change:      -0.0000
   j[1][4] =    j[alpha][gam]                  0.000
   j[2][3] =    j[beta1][beta2]              -13.730 change:      -0.0000
   j[2][4] =    j[beta1][gam]                  1.400
   j[3][4] =    j[beta2][gam]                  4.957 change:      -0.0000

   Largest absolute difference  =     0.248
   Total assigned intensity     =    16.000
   RMS deviation of transitions =   0.13738
   Fractional change of RMS     =   0.00000


***  Ignored     0 transitions

***   Transitions now assigned =       16

***  Iteration # 7, new parameters are:
   v[1] =       v[alpha]                    2231.923 change:      -0.0000
   v[2] =       v[beta1]                    1231.600
   v[3] =       v[beta2]                    1056.634 change:       0.0000
   v[4] =       v[gam]              2248.300
   j[1][2] =    j[alpha][beta1]                8.293 change:      -0.0000
```

```
   j[1][3] =     j[alpha][beta2]              8.419 change:        0.0000
   j[1][4] =     j[alpha][gam]                0.000
   j[2][3] =     j[beta1][beta2]            -13.730 change:        0.0000
   j[2][4] =     j[beta1][gam]                1.400
   j[3][4] =     j[beta2][gam]                4.957 change:        0.0000

   Largest absolute difference  =     0.248
   Total assigned intensity     =    16.000
   RMS deviation of transitions =   0.13738
   Fractional change of RMS     =   0.00000


***  Ignored     0 transitions

***   Transitions now assigned =       16

***  Iteration # 8, new parameters are:
   v[1] =        v[alpha]                  2231.923 change:        0.0000
   v[2] =        v[beta1]                  1231.600
   v[3] =        v[beta2]                  1056.634 change:        0.0000
   v[4] =        v[gam]              2248.300
   j[1][2] =     j[alpha][beta1]              8.293 change:       -0.0000
   j[1][3] =     j[alpha][beta2]              8.419 change:        0.0000
   j[1][4] =     j[alpha][gam]                0.000
   j[2][3] =     j[beta1][beta2]            -13.730 change:        0.0000
   j[2][4] =     j[beta1][gam]                1.400
   j[3][4] =     j[beta2][gam]                4.957 change:        0.0000

   Largest absolute difference  =     0.248
   Total assigned intensity     =    16.000
   RMS deviation of transitions =   0.13738
   Fractional change of RMS     =  -0.00000

*** Convergence achieved (Parameters not changing)
      This could be due to insufficient assigned transitions in a second-order spectrum

***  After final iteration:
***       current iteration count =            8
***       transitions now assigned =          16
***       current RMS =                 0.137377

***  Final parameters after 8 iterations are:
   v[1] =        v[alpha]                  2231.923 Hz. +/-       0.0486 Hz.
   v[2] =        v[beta1]                  1231.600 Hz.
   v[3] =        v[beta2]                  1056.634 Hz. +/-       0.0486 Hz.
   v[4] =        v[gam]              2248.300 Hz.
   j[1][2] =     j[alpha][beta1]              8.293 Hz. +/-       0.0973 Hz.
   j[1][3] =     j[alpha][beta2]              8.419 Hz. +/-       0.0688 Hz.
   j[1][4] =     j[alpha][gam]                0.000 Hz.
   j[2][3] =     j[beta1][beta2]            -13.730 Hz. +/-       0.0971 Hz.
   j[2][4] =     j[beta1][gam]                1.400 Hz.
   j[3][4] =     j[beta2][gam]                4.957 Hz. +/-       0.0973 Hz.

***  Parameter correlation coefficients:
   1  1.000
   2 -0.000  1.000
   3 -0.000  0.000  1.000
   4  0.000 -0.000 -0.003  1.000
   5  0.000 -0.000 -0.000 -0.000  1.000
   6 -0.000  0.000 -0.000 -0.000 -0.000  1.000


 Transitions for isotopic species 1

     46   1042.799   1042.794     0.005    0.91197
     11   1047.581   1047.746    -0.164    0.91818
     16   1051.126   1051.215    -0.089    0.92526
      1   1056.415   1056.167     0.248    0.93163
     56   1056.415   1056.523    -0.108    1.06547
     32   1061.480   1061.475     0.005    1.07570
     39   1065.137   1064.945     0.192    1.08067
      6   1069.807   1069.896    -0.089    1.09111
     52              1220.139              1.06720
     19              1221.544              1.07192
     24              1228.429              1.08451
      2              1229.834              1.08932
     55              1233.868              0.91350
     30              1235.273              0.91424
     37              1242.159              0.92927
      5              1243.564              0.93004
```

54	2223.677	2223.599	0.078	1.01442
29	2223.677	2223.599	0.078	1.01667
43	2231.943	2231.889	0.053	0.99798
9	2231.943	2231.889	0.053	0.99961
51	2231.943	2232.020	-0.078	1.00170
18	2231.943	2232.021	-0.078	1.00035
28	2240.258	2240.311	-0.053	0.98674
3	2240.258	2240.311	-0.053	0.98253
53		2245.127		1.00661
35		2245.127		1.00448
42		2246.532		1.00356
13		2246.532		1.00198
50		2250.079		0.99657
22		2250.079		0.99787
27		2251.484		0.99243
4		2251.484		0.99651

*** 32 transitions for species 1 written to plot file

RMS deviation of 10 peaks grouped within 0.050 Hz = 0.17657 Hz

*** Simulation normal exit ***
Calculation time = 0 seconds

sim_out.txt

*** SpinWorks simulation output, build 2008/10/11 ***

time: 10:43:55
date: 12/06/13

*** Reading spin system from:
C:\nmrdata\temp\nmr-dta-compounds\Cis-beta-arabinose-MVR-2-95\1\spin_system ***

Options:

Opimize shifts and couplings? =	1
Ignore bad transitions? =	1
Auto assign observed peaks? =	1
Maximum number of iterations? =	30
RMS convergence limit =	0.020
RMS below this for autoassign =	0.250
Trans. freq. this * RMS ignored =	2.400

Groups and chemical shifts:

#	name	shift	spins	species	spin	sym
1	H-a	2291.000	1	1	1	1
2	H-b1	1185.000	1	1	1	1
3	H-b2	1161.000	1	1	1	1
4	H-g	2238.000	1	1	1	1

Scalar coupling constants:

j[1, 2] =	j[H-a,H-b1] =	9.200000
j[1, 3] =	j[H-a,H-b2] =	3.100000
j[1, 4] =	j[H-a,H-g] =	0.000000
j[2, 3] =	j[H-b1,H-b2] =	-13.500000
j[2, 4] =	j[H-b1,H-g] =	4.500000
j[3, 4] =	j[H-b2,H-g] =	3.000000

Dipolar coupling constants:

Paramaters to vary:

#	param.	range low	range hi
1	v[1]	1711.331	2875.281
2	v[2]	873.450	1506.750
3	v[3]	859.725	1469.625
4	j[1][2]	6.900	11.500
5	j[1][3]	2.325	3.875
6	j[2][3]	-10.125	-16.875
7	j[2][4]	3.375	5.625
8	j[3][4]	2.250	3.750

Parameter map:

0	0	0	0
1	1	0	0
2	2	0	0
3	3	0	0
4	1	2	0
5	1	3	0
6	2	3	0
7	2	4	0
8	3	4	0

Reading assigned transitions

24 assigned transitions read

Observed transitions read =	24
Total asigned line numbers =	24

Basis functions generated, transition information:

Species 1, up to 56 transitions, intensity 32

Total size of U3 matrix = 70 double words
Total sixe of E3 matrix = 16 double words

line	obs. freq.	calc. freq	diff.
1	1154.009	1155.747	-1.737
2	1186.811	1186.625	0.186
4	2297.189	2297.171	0.018
5	1200.925	1200.125	0.800
6	1168.518	1169.246	-0.729
11	1151.329	1152.665	-1.336
15	1182.238	1182.207	0.031
17	2294.035	2293.689	0.346
20	1151.486	1152.264	-0.778
22	2287.963	2288.354	-0.390
24	1177.507	1177.808	-0.301
27	2297.189	2297.171	0.018
30	1196.352	1195.707	0.645
31	1165.364	1166.165	-0.801
35	2284.888	2284.871	0.017
38	1191.542	1191.307	0.234
39	1165.285	1165.764	-0.479
42	2294.114	2293.647	0.466
46	1148.490	1149.141	-0.651
49	2287.963	2288.395	-0.432
52	1173.091	1173.431	-0.340
53	2284.888	2284.871	0.017
55	1186.811	1186.931	-0.120
56	1162.446	1162.641	-0.195

Largest absolute difference = 1.737
Total assigned intensity = 23.988
RMS deviation of transitions = 0.76314

*** Ignored 0 transitions

*** Transitions now assigned = 24

*** Iteration # 1, new parameters are:

v[1] =	v[H-a]	2291.007	change:	0.0074
v[2] =	v[H-b1]	1185.208	change:	0.2077
v[3] =	v[H-b2]	1160.096	change:	-0.9042
v[4] =	v[H-g]	2238.000		
j[1][2] =	j[H-a][H-b1]	9.719	change:	0.5190
j[1][3] =	j[H-a][H-b2]	2.542	change:	-0.5577
j[1][4] =	j[H-a][H-g]	0.000		
j[2][3] =	j[H-b1][H-b2]	-14.035	change:	-0.5355
j[2][4] =	j[H-b1][H-g]	4.693	change:	0.1931
j[3][4] =	j[H-b2][H-g]	2.798	change:	-0.2022

Largest absolute difference = 0.354
Total assigned intensity = 23.985
RMS deviation of transitions = 0.18738
Fractional change of RMS = -0.75447

*** Ignored 0 transitions

*** Transitions now assigned = 24

*** Iteration # 2, new parameters are:

v[1] =	v[H-a]	2291.006	change:	-0.0015
v[2] =	v[H-b1]	1185.057	change:	-0.1511
v[3] =	v[H-b2]	1160.249	change:	0.1528
v[4] =	v[H-g]	2238.000		
j[1][2] =	j[H-a][H-b1]	9.757	change:	0.0381
j[1][3] =	j[H-a][H-b2]	2.504	change:	-0.0381
j[1][4] =	j[H-a][H-g]	0.000		
j[2][3] =	j[H-b1][H-b2]	-14.036	change:	-0.0000
j[2][4] =	j[H-b1][H-g]	4.703	change:	0.0097
j[3][4] =	j[H-b2][H-g]	2.788	change:	-0.0097

Largest absolute difference = 0.277
Total assigned intensity = 23.984
RMS deviation of transitions = 0.13262
Fractional change of RMS = -0.29222

*** Automatic assignment has occurred ***

*** Transitions now assigned = 23

*** Iteration # 3, new parameters are:

```
   v[1] =       v[H-a]                    2291.016 change:       0.0097
   v[2] =       v[H-b1]                   1185.054 change:      -0.0025
   v[3] =       v[H-b2]                   1160.290 change:       0.0415
   v[4] =       v[H-g]              2238.000
   j[1][2] =    j[H-a][H-b1]                 9.767 change:       0.0094
   j[1][3] =    j[H-a][H-b2]                 2.553 change:       0.0492
   j[1][4] =    j[H-a][H-g]                  0.000
   j[2][3] =    j[H-b1][H-b2]              -14.016 change:       0.0191
   j[2][4] =    j[H-b1][H-g]                 4.696 change:      -0.0064
   j[3][4] =    j[H-b2][H-g]                 2.872 change:       0.0840

   Largest absolute difference  =     0.189
   Total assigned intensity     =    23.414
   RMS deviation of transitions =   0.09304
   Fractional change of RMS     =  -0.29847


***  Ignored     0 transitions

***   Transitions now assigned =       23

***  Iteration # 4, new parameters are:
   v[1] =       v[H-a]                    2291.016 change:      -0.0001
   v[2] =       v[H-b1]                   1185.060 change:       0.0056
   v[3] =       v[H-b2]                   1160.285 change:      -0.0053
   v[4] =       v[H-g]              2238.000
   j[1][2] =    j[H-a][H-b1]                 9.765 change:      -0.0013
   j[1][3] =    j[H-a][H-b2]                 2.555 change:       0.0013
   j[1][4] =    j[H-a][H-g]                  0.000
   j[2][3] =    j[H-b1][H-b2]              -14.016 change:       0.0000
   j[2][4] =    j[H-b1][H-g]                 4.696 change:      -0.0004
   j[3][4] =    j[H-b2][H-g]                 2.873 change:       0.0004

   Largest absolute difference  =     0.195
   Total assigned intensity     =    23.414
   RMS deviation of transitions =   0.09291
   Fractional change of RMS     =  -0.00137


***  Ignored     0 transitions

***   Transitions now assigned =       23

***  Iteration # 5, new parameters are:
   v[1] =       v[H-a]                    2291.016 change:       0.0000
   v[2] =       v[H-b1]                   1185.060 change:       0.0000
   v[3] =       v[H-b2]                   1160.285 change:      -0.0000
   v[4] =       v[H-g]              2238.000
   j[1][2] =    j[H-a][H-b1]                 9.765 change:      -0.0001
   j[1][3] =    j[H-a][H-b2]                 2.555 change:       0.0001
   j[1][4] =    j[H-a][H-g]                  0.000
   j[2][3] =    j[H-b1][H-b2]              -14.016 change:       0.0000
   j[2][4] =    j[H-b1][H-g]                 4.696 change:      -0.0000
   j[3][4] =    j[H-b2][H-g]                 2.873 change:       0.0000

   Largest absolute difference  =     0.195
   Total assigned intensity     =    23.414
   RMS deviation of transitions =   0.09291
   Fractional change of RMS     =  -0.00000

***   Automatic assignment has occurred  ***

***   Transitions now assigned =       22

***  Iteration # 6, new parameters are:
   v[1] =       v[H-a]                    2291.016 change:      -0.0000
   v[2] =       v[H-b1]                   1185.102 change:       0.0427
   v[3] =       v[H-b2]                   1160.277 change:      -0.0076
   v[4] =       v[H-g]              2238.000
   j[1][2] =    j[H-a][H-b1]                 9.722 change:      -0.0431
   j[1][3] =    j[H-a][H-b2]                 2.555 change:       0.0002
   j[1][4] =    j[H-a][H-g]                  0.000
   j[2][3] =    j[H-b1][H-b2]              -14.059 change:      -0.0429
   j[2][4] =    j[H-b1][H-g]                 4.614 change:      -0.0822
   j[3][4] =    j[H-b2][H-g]                 2.869 change:      -0.0037

   Largest absolute difference  =     0.155
   Total assigned intensity     =    22.992
   RMS deviation of transitions =   0.07092
   Fractional change of RMS     =  -0.23672
```

```
 ***  Ignored      0 transitions

 ***   Transitions now assigned =       22

 ***  Iteration # 7, new parameters are:
    v[1] =       v[H-a]                    2291.016 change:       0.0002
    v[2] =       v[H-b1]                   1185.090 change:      -0.0126
    v[3] =       v[H-b2]                   1160.289 change:       0.0122
    v[4] =       v[H-g]          2238.000
    j[1][2] =    j[H-a][H-b1]                 9.725 change:       0.0031
    j[1][3] =    j[H-a][H-b2]                 2.552 change:      -0.0031
    j[1][4] =    j[H-a][H-g]                  0.000
    j[2][3] =    j[H-b1][H-b2]              -14.059 change:       0.0000
    j[2][4] =    j[H-b1][H-g]                 4.615 change:       0.0006
    j[3][4] =    j[H-b2][H-g]                 2.868 change:      -0.0006

    Largest absolute difference  =     0.143
    Total assigned intensity     =    22.992
    RMS deviation of transitions =   0.07009
    Fractional change of RMS     =  -0.01162


 ***  Ignored      0 transitions

 ***   Transitions now assigned =       22

 ***  Iteration # 8, new parameters are:
    v[1] =       v[H-a]                    2291.016 change:      -0.0000
    v[2] =       v[H-b1]                   1185.090 change:      -0.0000
    v[3] =       v[H-b2]                   1160.289 change:       0.0000
    v[4] =       v[H-g]          2238.000
    j[1][2] =    j[H-a][H-b1]                 9.725 change:       0.0001
    j[1][3] =    j[H-a][H-b2]                 2.552 change:      -0.0001
    j[1][4] =    j[H-a][H-g]                  0.000
    j[2][3] =    j[H-b1][H-b2]              -14.059 change:      -0.0000
    j[2][4] =    j[H-b1][H-g]                 4.615 change:       0.0000
    j[3][4] =    j[H-b2][H-g]                 2.868 change:      -0.0000

    Largest absolute difference  =     0.143
    Total assigned intensity     =    22.992
    RMS deviation of transitions =   0.07009
    Fractional change of RMS     =   0.00000

 ***   Automatic assignment has occurred  ***

 ***   Transitions now assigned =       21

 ***  Iteration # 9, new parameters are:
    v[1] =       v[H-a]                    2291.016 change:      -0.0000
    v[2] =       v[H-b1]                   1185.095 change:       0.0054
    v[3] =       v[H-b2]                   1160.258 change:      -0.0317
    v[4] =       v[H-g]          2238.000
    j[1][2] =    j[H-a][H-b1]                 9.719 change:      -0.0061
    j[1][3] =    j[H-a][H-b2]                 2.584 change:       0.0319
    j[1][4] =    j[H-a][H-g]                  0.000
    j[2][3] =    j[H-b1][H-b2]              -14.091 change:      -0.0316
    j[2][4] =    j[H-b1][H-g]                 4.605 change:      -0.0092
    j[3][4] =    j[H-b2][H-g]                 2.929 change:       0.0607

    Largest absolute difference  =     0.104
    Total assigned intensity     =    22.562
    RMS deviation of transitions =   0.05315
    Fractional change of RMS     =  -0.24165


 ***  Ignored      0 transitions

 ***   Transitions now assigned =       21

 ***  Iteration # 10, new parameters are:
    v[1] =       v[H-a]                    2291.016 change:      -0.0000
    v[2] =       v[H-b1]                   1185.086 change:      -0.0091
    v[3] =       v[H-b2]                   1160.267 change:       0.0091
    v[4] =       v[H-g]          2238.000
    j[1][2] =    j[H-a][H-b1]                 9.721 change:       0.0024
    j[1][3] =    j[H-a][H-b2]                 2.581 change:      -0.0024
    j[1][4] =    j[H-a][H-g]                  0.000
    j[2][3] =    j[H-b1][H-b2]              -14.091 change:       0.0000
```

```
   j[2][4] =    j[H-b1][H-g]                    4.606 change:       0.0005
   j[3][4] =    j[H-b2][H-g]                    2.928 change:      -0.0005

   Largest absolute difference  =     0.095
   Total assigned intensity     =    22.563
   RMS deviation of transitions =   0.05257
   Fractional change of RMS     =  -0.01094


***  Ignored     0 transitions

***   Transitions now assigned =       21

***  Iteration # 11, new parameters are:
   v[1] =       v[H-a]                       2291.016 change:      -0.0000
   v[2] =       v[H-b1]                      1185.086 change:      -0.0000
   v[3] =       v[H-b2]                      1160.267 change:       0.0000
   v[4] =       v[H-g]               2238.000
   j[1][2] =    j[H-a][H-b1]                    9.722 change:       0.0001
   j[1][3] =    j[H-a][H-b2]                    2.581 change:      -0.0001
   j[1][4] =    j[H-a][H-g]                     0.000
   j[2][3] =    j[H-b1][H-b2]                 -14.091 change:      -0.0000
   j[2][4] =    j[H-b1][H-g]                    4.606 change:       0.0000
   j[3][4] =    j[H-b2][H-g]                    2.928 change:      -0.0000

   Largest absolute difference  =     0.095
   Total assigned intensity     =    22.563
   RMS deviation of transitions =   0.05257
   Fractional change of RMS     =   0.00000

***   Automatic assignment has occurred  ***

***   Transitions now assigned =       21

***  Iteration # 12, new parameters are:
   v[1] =       v[H-a]                       2291.006 change:      -0.0099
   v[2] =       v[H-b1]                      1185.087 change:       0.0012
   v[3] =       v[H-b2]                      1160.266 change:      -0.0004
   v[4] =       v[H-g]               2238.000
   j[1][2] =    j[H-a][H-b1]                    9.709 change:      -0.0124
   j[1][3] =    j[H-a][H-b2]                    2.595 change:       0.0133
   j[1][4] =    j[H-a][H-g]                     0.000
   j[2][3] =    j[H-b1][H-b2]                 -14.092 change:      -0.0010
   j[2][4] =    j[H-b1][H-g]                    4.604 change:      -0.0015
   j[3][4] =    j[H-b2][H-g]                    2.932 change:       0.0033

   Largest absolute difference  =     0.102
   Total assigned intensity     =    22.562
   RMS deviation of transitions =   0.05467
   Fractional change of RMS     =   0.03997


***  Ignored     0 transitions

***   Transitions now assigned =       21

***  Iteration # 13, new parameters are:
   v[1] =       v[H-a]                       2291.006 change:       0.0000
   v[2] =       v[H-b1]                      1185.087 change:      -0.0003
   v[3] =       v[H-b2]                      1160.267 change:       0.0003
   v[4] =       v[H-g]               2238.000
   j[1][2] =    j[H-a][H-b1]                    9.709 change:       0.0001
   j[1][3] =    j[H-a][H-b2]                    2.595 change:      -0.0001
   j[1][4] =    j[H-a][H-g]                     0.000
   j[2][3] =    j[H-b1][H-b2]                 -14.092 change:       0.0000
   j[2][4] =    j[H-b1][H-g]                    4.604 change:       0.0000
   j[3][4] =    j[H-b2][H-g]                    2.932 change:      -0.0000

   Largest absolute difference  =     0.102
   Total assigned intensity     =    22.562
   RMS deviation of transitions =   0.05467
   Fractional change of RMS     =  -0.00001


***  Ignored     0 transitions

***   Transitions now assigned =       21

***  Iteration # 14, new parameters are:
   v[1] =       v[H-a]                       2291.006 change:      -0.0000
```

```
   v[2] =       v[H-b1]                    1185.087 change:       0.0000
   v[3] =       v[H-b2]                    1160.267 change:      -0.0000
   v[4] =       v[H-g]           2238.000
   j[1][2] =    j[H-a][H-b1]                  9.709 change:       0.0000
   j[1][3] =    j[H-a][H-b2]                  2.595 change:      -0.0000
   j[1][4] =    j[H-a][H-g]                   0.000
   j[2][3] =    j[H-b1][H-b2]               -14.092 change:      -0.0000
   j[2][4] =    j[H-b1][H-g]                  4.604 change:       0.0000
   j[3][4] =    j[H-b2][H-g]                  2.932 change:      -0.0000

   Largest absolute difference  =     0.102
   Total assigned intensity     =    22.562
   RMS deviation of transitions =   0.05467
   Fractional change of RMS     =   0.00000

***   Automatic assignment has occurred  ***

***   Transitions now assigned =       20

***  Iteration # 15, new parameters are:
   v[1] =       v[H-a]                     2291.006 change:       0.0000
   v[2] =       v[H-b1]                    1185.087 change:       0.0002
   v[3] =       v[H-b2]                    1160.235 change:      -0.0321
   v[4] =       v[H-g]           2238.000
   j[1][2] =    j[H-a][H-b1]                  9.708 change:      -0.0015
   j[1][3] =    j[H-a][H-b2]                  2.639 change:       0.0441
   j[1][4] =    j[H-a][H-g]                   0.000
   j[2][3] =    j[H-b1][H-b2]               -14.069 change:       0.0233
   j[2][4] =    j[H-b1][H-g]                  4.604 change:      -0.0003
   j[3][4] =    j[H-b2][H-g]                  3.017 change:       0.0855

   Largest absolute difference  =     0.070
   Total assigned intensity     =    21.002
   RMS deviation of transitions =   0.03946
   Fractional change of RMS     =  -0.27826

***  Ignored     0 transitions

***   Transitions now assigned =       20

***  Iteration # 16, new parameters are:
   v[1] =       v[H-a]                     2291.006 change:      -0.0000
   v[2] =       v[H-b1]                    1185.094 change:       0.0068
   v[3] =       v[H-b2]                    1160.228 change:      -0.0066
   v[4] =       v[H-g]           2238.000
   j[1][2] =    j[H-a][H-b1]                  9.706 change:      -0.0020
   j[1][3] =    j[H-a][H-b2]                  2.641 change:       0.0019
   j[1][4] =    j[H-a][H-g]                   0.000
   j[2][3] =    j[H-b1][H-b2]               -14.068 change:       0.0001
   j[2][4] =    j[H-b1][H-g]                  4.604 change:      -0.0004
   j[3][4] =    j[H-b2][H-g]                  3.017 change:       0.0003

   Largest absolute difference  =     0.065
   Total assigned intensity     =    21.002
   RMS deviation of transitions =   0.03904
   Fractional change of RMS     =  -0.01067

*** Convergence achieved (Fractional decrease)

***  After final iteration:
***       current iteration count =          16
***       transitions now assigned =         20
***       current RMS =                0.039039

***  Final parameters after 16 iterations are:
   v[1] =       v[H-a]                     2291.006 Hz. +/-       0.0138 Hz.
   v[2] =       v[H-b1]                    1185.094 Hz. +/-       0.0164 Hz.
   v[3] =       v[H-b2]                    1160.228 Hz. +/-       0.0197 Hz.
   v[4] =       v[H-g]           2238.000 Hz.
   j[1][2] =    j[H-a][H-b1]                  9.706 Hz. +/-       0.0222 Hz.
   j[1][3] =    j[H-a][H-b2]                  2.641 Hz. +/-       0.0260 Hz.
   j[1][4] =    j[H-a][H-g]                   0.000 Hz.
   j[2][3] =    j[H-b1][H-b2]               -14.068 Hz. +/-       0.0235 Hz.
   j[2][4] =    j[H-b1][H-g]                  4.604 Hz. +/-       0.0326 Hz.
   j[3][4] =    j[H-b2][H-g]                  3.017 Hz. +/-       0.0422 Hz.

***  Parameter correlation coefficients:
   1  1.000
   2 -0.000  1.000
```

```
  3 -0.000 -0.157  1.000
  4 -0.000 -0.164  0.071  1.000
  5  0.000  0.043 -0.236 -0.138  1.000
  6  0.000 -0.145  0.086  0.098  0.113  1.000
  7  0.000 -0.176  0.042  0.130 -0.028  0.123  1.000
  8  0.000  0.017 -0.249 -0.031  0.417  0.147 -0.116  1.000

Transitions for isotopic species 1

    46                1148.176                0.43330
    11    1151.329    1151.313      0.016     0.54470
    20    1151.329    1151.329     -0.000     0.46405
     1                1154.415                0.56763
    56                1162.245                1.55502
    31    1165.364    1165.381     -0.017     1.45488
    39    1165.364    1165.397     -0.033     1.53609
     6    1168.518    1168.484      0.034     1.44348
    52    1173.091    1173.064      0.027     1.54853
    24    1177.507    1177.532     -0.026     1.53196
    15    1182.238    1182.274     -0.036     1.45916
     2    1186.811    1186.792      0.019     1.45067
    55                1187.132                0.42671
    38    1191.542    1191.601     -0.059     0.45989
    30    1196.352    1196.342      0.010     0.54900
     5    1200.925    1200.860      0.065     0.57492
    54                2234.197                1.00667
    29                2234.197                1.00771
    14                2237.299                1.00159
    51                2237.349                1.00068
    44                2238.665                0.99864
     9                2238.715                0.99815
    28                2241.818                0.99332
     3                2241.818                0.99238
    53    2284.888    2284.856      0.033     1.01159
    35    2284.888    2284.856      0.033     1.01069
    22    2287.963    2287.942      0.021     1.00181
    49    2287.963    2287.992     -0.028     1.00151
    42    2294.035    2294.065     -0.031     0.98998
    17    2294.114    2294.115     -0.002     0.99113
    27    2297.189    2297.202     -0.013     0.98852
     4    2297.189    2297.202     -0.013     0.98932

***  32 transitions for species 1 written to plot file

  RMS deviation of    15 peaks grouped within     0.050 Hz =    0.04701 Hz


***  Simulation normal exit  ***
     Calculation time = 0 seconds
```

Inverse Magnetization Transfer Experiment data

Compound 7a Major Amide singlet irradiation

Major - Tα

Tue Nov 15 09:22:16 CST 2011

Dataset :
E:/data/schweiz/nmr/2,3,5 Triol-arab-f-NAC_Trans-alpha-Hyp-OMe_67.3C/2/pdata/
INTENSITY fit :
I[t]=I[0]+P*exp(-t/T1)
32 points for Peak 1, Peak Point at 2.455 ppm
Results Comp. 1
I[0] = 5.836e-001
P = -1.472e+000
T1 = 1.402s
SD = 6.695e-002

tau	ppm	integral	intensity
1.000m	2.455	-4.3038e+007	-2.6439e+006
10.000m	2.455	-4.2735e+007	-2.9216e+006
150.000m	2.455	-3.5797e+007	-2.421e+006
20.000m	2.454	-4.1885e+007	-2.8163e+006
30.000m	2.454	-4.1244e+007	-2.8106e+006
40.000m	2.454	-4.048e+007	-2.6397e+006
50.000m	2.454	-3.9795e+007	-2.4496e+006
80.000m	2.454	-3.8166e+007	-2.2384e+006
100.000m	2.454	-3.7052e+007	-2.0973e+006
150.000m	2.454	-3.4598e+007	-1.9093e+006
200.000m	2.454	-3.2223e+007	-1.7328e+006
300.000m	2.454	-2.794e+007	-1.4836e+006
400.000m	2.454	-2.3973e+007	-1.2663e+006
500.000m	2.454	-2.0343e+007	-1.0808e+006
600.000m	2.454	-1.6962e+007	-9.0712e+005
800.000m	2.454	-1.0991e+007	-6.246e+005
1.000s	2.454	-5.7766e+006	-3.8432e+005
1.200s	2.454	-1.2082e+006	-1.7686e+005
1.500s	2.457	4.653e+006	89826
2.000s	2.454	1.2487e+007	4.348e+005
3.000s	2.454	2.3161e+007	9.1272e+005
4.000s	2.454	2.9855e+007	1.208e+006
5.000s	2.454	3.4238e+007	1.3913e+006
6.000s	2.454	3.7112e+007	1.5126e+006
8.000s	2.454	4.0618e+007	1.669e+006
10.000s	2.454	4.2423e+007	1.7424e+006
12.000s	2.454	4.3458e+007	1.7706e+006

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15.000s	2.454	4.4412e+007	1.8073e+006
18.000s	2.454	4.4981e+007	1.8252e+006
20.000s	2.454	4.5201e+007	1.8322e+006
25.000s	2.454	4.5744e+007	1.8634e+006
30.000s	2.454	4.5975e+007	1.8694e+006

Minor - T1

Tue Nov 15 09:23:03 CST 2011

Compound 7a Minor amide exponential recovery

Dataset :
E:/data/schweiz/nmr/2,3,5 Triol-arab-f-NAC_Trans-alpha-Hyp-OMe_67.3C/2/pdata/
INTENSITY fit :
I[t]=I[0]+P*exp(-t/T1)
32 points for Peak 2, Peak Point at 2.336 ppm
Results Comp. 1
I[0] = 4.512e-001
P = 5.750e-001
T1 = 144.137m
SD = 1.308e-001

tau	ppm	integral	intensity
1.000m	2.336	8.4499e+006	4.7061e+005
10.000m	2.336	8.2749e+006	5.0378e+005
150.000m	2.336	6.6795e+006	4.035e+005
20.000m	2.336	8.079e+006	4.8913e+005
30.000m	2.335	7.9414e+006	4.8563e+005
40.000m	2.335	7.8249e+006	4.5934e+005
50.000m	2.335	7.6551e+006	4.2678e+005
80.000m	2.335	7.3169e+006	3.8676e+005
100.000m	2.335	7.0943e+006	3.6064e+005
150.000m	2.335	6.5917e+006	3.2392e+005
200.000m	2.335	6.1465e+006	2.9139e+005
300.000m	2.335	5.4078e+006	2.4752e+005
400.000m	2.335	4.776e+006	2.1305e+005
500.000m	2.335	4.2979e+006	1.8652e+005
600.000m	2.335	3.8787e+006	1.6287e+005
800.000m	2.335	3.3051e+006	1.3492e+005
1.000s	2.335	2.9963e+006	1.188e+005
1.200s	2.335	2.8418e+006	1.1037e+005
1.500s	2.335	2.8493e+006	1.1108e+005
2.000s	2.335	3.1865e+006	1.2447e+005
3.000s	2.335	4.2527e+006	1.7262e+005
4.000s	2.335	5.189e+006	2.1299e+005
5.000s	2.335	5.8819e+006	2.4202e+005
6.000s	2.335	6.3836e+006	2.6207e+005
8.000s	2.335	6.9902e+006	2.8975e+005
10.000s	2.335	7.3388e+006	3.0312e+005
12.000s	2.335	7.5036e+006	3.0752e+005

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15.000s	2.335	7.691e+006	3.1443e+005
18.000s	2.335	7.765e+006	3.1716e+005
20.000s	2.335	7.8072e+006	3.1846e+005
25.000s	2.335	7.9144e+006	3.2465e+005
30.000s	2.335	7.9199e+006	3.2453e+005

Major Tβ

Compound 7b Major amide singlet irradiation

Fri Dec 16 09:20:49 CST 2011

Dataset :
E:/data/schweiz/nmr/2,3,5 Triol-arab-f-NAC_Trans-beta-hyp-OMe_67.3C/2/pdata/1
AREA fit :
I[t]=I[0]+P*exp(-t/T1)
32 points for Integral 1, Integral Region from 2.486 to 2.416 ppm
Results Comp. 1
I[0] = 9.369e-001
P = -1.681e+000
T1 = 2.137s
SD = 3.262e-002

tau	ppm	integral	intensity
1.000m	2.458	-2.4574e+008	-1.8202e+007
10.000m	2.458	-2.4436e+008	-1.7829e+007
15.000m	2.458	-2.4355e+008	-1.7587e+007
20.000m	2.458	-2.4099e+008	-1.735e+007
30.000m	2.458	-2.3692e+008	-1.6873e+007
40.000m	2.458	-2.3283e+008	-1.6346e+007
50.000m	2.458	-2.2885e+008	-1.578e+007
80.000m	2.458	-2.1926e+008	-1.4902e+007
100.000m	2.458	-2.1269e+008	-1.4239e+007
150.000m	2.458	-1.9838e+008	-1.3155e+007
200.000m	2.458	-1.8479e+008	-1.2114e+007
300.000m	2.458	-1.5938e+008	-1.049e+007
400.000m	2.458	-1.3596e+008	-9.0115e+006
500.000m	2.458	-1.1423e+008	-7.6809e+006
600.000m	2.458	-9.3995e+007	-6.4589e+006
800.000m	2.458	-5.7852e+007	-4.5067e+006
1.000s	2.458	-2.6171e+007	-2.8365e+006
1.200s	2.458	1.7959e+006	-1.3931e+006
1.500s	2.458	3.7928e+007	4.6812e+005
2.000s	2.459	8.7105e+007	2.9603e+006
3.000s	2.459	1.5656e+008	6.4694e+006
4.000s	2.459	2.0028e+008	8.62e+006
5.000s	2.459	2.2854e+008	9.9742e+006
6.000s	2.459	2.4868e+008	1.0928e+007
8.000s	2.459	2.7268e+008	1.208e+007
10.000s	2.459	2.8586e+008	1.2673e+007
12.000s	2.459	2.9392e+008	1.3021e+007

15.000s	2.459	3.019e+008	1.3427e+007
18.000s	2.459	3.0728e+008	1.3735e+007
20.000s	2.459	3.1042e+008	1.3989e+007
25.000s	2.459	3.1575e+008	1.4262e+007
30.000s	2.459	3.1926e+008	1.4465e+007

Minor Tβ

Compound 7b Minor amide exponential recovery

Fri Dec 16 09:21:59 CST 2011

Dataset :
E:/data/schweiz/nmr/2,3,5 Triol-arab-f-NAC_Trans-beta-hyp-OMe_67.3C/2/pdata/1
AREA fit :
I[t]=I[0]+P*exp(-t/T1)
32 points for Integral 2, Integral Region from 2.365 to 2.331 ppm
Results Comp. 1
I[0] = 6.813e-001
P = 2.951e-001
T1 = 108.364m
SD = 1.900e-001

tau	ppm	integral	intensity
1.000m	2.343	5.9974e+007	3.3127e+006
10.000m	2.343	5.9805e+007	3.2449e+006
15.000m	2.343	5.9383e+007	3.2081e+006
20.000m	2.343	5.8815e+007	3.1629e+006
30.000m	2.343	5.7997e+007	3.0723e+006
40.000m	2.343	5.7197e+007	2.9799e+006
50.000m	2.343	5.6334e+007	2.8794e+006
80.000m	2.343	5.4028e+007	2.7013e+006
100.000m	2.343	5.257e+007	2.5821e+006
150.000m	2.343	4.9239e+007	2.3622e+006
200.000m	2.343	4.6209e+007	2.1646e+006
300.000m	2.343	4.0943e+007	1.8596e+006
400.000m	2.343	3.6509e+007	1.6081e+006
500.000m	2.343	3.2933e+007	1.4033e+006
600.000m	2.343	3.0074e+007	1.241e+006
800.000m	2.343	2.5941e+007	1.0323e+006
1.000s	2.343	2.3529e+007	9.1239e+005
1.200s	2.343	2.2431e+007	8.5813e+005
1.500s	2.343	2.2497e+007	8.5659e+005
2.000s	2.343	2.5307e+007	9.7691e+005
3.000s	2.344	3.3371e+007	1.3561e+006
4.000s	2.344	4.0453e+007	1.6784e+006
5.000s	2.344	4.5557e+007	1.9119e+006
6.000s	2.344	4.9353e+007	2.0832e+006
8.000s	2.344	5.4171e+007	2.2987e+006
10.000s	2.344	5.6903e+007	2.4097e+006
12.000s	2.344	5.8522e+007	2.4778e+006

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15.000s	2.344	6.001e+007	2.55e+006
18.000s	2.344	6.124e+007	2.609e+006
20.000s	2.343	6.1822e+007	2.6537e+006
25.000s	2.343	6.2821e+007	2.7012e+006
30.000s	2.343	6.361e+007	2.7409e+006

Major Cα

Compound 12a Major amide singlet irradiation

Thu Nov 17 09:28:19 CST 2011

Dataset :
E:/data/schweiz/nmr/2,3,5 Triol-arab-f-NAC_Cis-alpha-hyp-OMe_67.3C/2/pdata/1
AREA fit :
I[t]=I[0]+P*exp(-t/T1)
32 points for Integral 1, Integral Region from 2.482 to 2.439 ppm
Results Comp. 1
I[0] = 9.405e-001
P = -1.837e+000
T1 = 2.083s
SD = 3.477e-002

tau	ppm	integral	intensity
1.000m	2.460	-9.3855e+007	-6.6201e+006
10.000m	2.460	-9.2803e+007	-6.9244e+006
150.000m	2.460	-7.8596e+007	-6.185e+006
20.000m	2.460	-9.1397e+007	-6.9785e+006
30.000m	2.460	-9.0124e+007	-6.682e+006
40.000m	2.460	-8.8767e+007	-6.3567e+006
50.000m	2.460	-8.7475e+007	-6.0359e+006
80.000m	2.460	-8.4115e+007	-5.6631e+006
100.000m	2.460	-8.1859e+007	-5.3809e+006
150.000m	2.460	-7.6686e+007	-4.9615e+006
200.000m	2.460	-7.183e+007	-4.588e+006
300.000m	2.460	-6.2832e+007	-3.9692e+006
400.000m	2.460	-5.4572e+007	-3.3751e+006
500.000m	2.460	-4.6872e+007	-2.8782e+006
600.000m	2.460	-3.9734e+007	-2.4369e+006
800.000m	2.460	-2.7053e+007	-1.7182e+006
1.000s	2.460	-1.5947e+007	-1.1074e+006
1.200s	2.460	-6.2716e+006	-5.7445e+005
1.500s	2.462	6.4065e+006	1.1135e+005
2.000s	2.460	2.3158e+007	9.9789e+005
3.000s	2.460	4.6543e+007	2.2358e+006
4.000s	2.460	6.1605e+007	3.0175e+006
5.000s	2.460	7.154e+007	3.5117e+006
6.000s	2.460	7.8307e+007	3.8329e+006
8.000s	2.460	8.6513e+007	4.221e+006
10.000s	2.461	9.088e+007	4.4179e+006
12.000s	2.461	9.3569e+007	4.5127e+006

15.000s	2.460	9.6091e+007	4.6315e+006
18.000s	2.460	9.7741e+007	4.6852e+006
20.000s	2.460	9.847e+007	4.7083e+006
25.000s	2.460	9.9927e+007	4.7775e+006
30.000s	2.460	1.007e+008	4.7991e+006

Minor Cα

Thu Nov 17 09:30:39 CST 2011

Dataset :
E:/data/schweiz/nmr/2,3,5 Triol-arab-f-NAC_Cis-alpha-hyp-OMe_67.3C/2/pdata/1
AREA fit :
I[t]=I[0]+P*exp(-t/T1)
32 points for Integral 2, Integral Region from 2.356 to 2.330 ppm

Results	Comp. 1
I[0] =	6.287e-001
P =	4.051e-001
T1 =	128.527m
SD =	1.941e-001

tau	ppm	integral	intensity
1.000m	2.342	2.0109e+007	1.1778e+006
10.000m	2.342	1.9861e+007	1.2108e+006
150.000m	2.341	1.5873e+007	9.9929e+005
20.000m	2.341	1.9427e+007	1.2024e+006
30.000m	2.341	1.909e+007	1.154e+006
40.000m	2.341	1.8807e+007	1.105e+006
50.000m	2.341	1.8429e+007	1.0526e+006
80.000m	2.341	1.7583e+007	9.7987e+005
100.000m	2.341	1.702e+007	9.2617e+005
150.000m	2.342	1.5765e+007	8.4155e+005
200.000m	2.341	1.459e+007	7.6621e+005
300.000m	2.342	1.2576e+007	6.444e+005
400.000m	2.342	1.0918e+007	5.4064e+005
500.000m	2.342	9.6205e+006	4.6367e+005
600.000m	2.342	8.5149e+006	4.0254e+005
800.000m	2.342	6.9664e+006	3.2263e+005
1.000s	2.342	6.1278e+006	2.8076e+005
1.200s	2.342	5.7561e+006	2.5855e+005
1.500s	2.342	5.7234e+006	2.5692e+005
2.000s	2.341	6.5826e+006	3.0162e+005
3.000s	2.341	9.2214e+006	4.3296e+005
4.000s	2.341	1.1597e+007	5.4806e+005
5.000s	2.341	1.336e+007	6.3264e+005
6.000s	2.341	1.4598e+007	6.8739e+005
8.000s	2.342	1.6198e+007	7.5744e+005
10.000s	2.342	1.7065e+007	7.9376e+005
12.000s	2.342	1.7568e+007	8.1347e+005

$k_{ct} = 0.659153$

$k_{tc} = 0.123504$

$K_{eq} = 5.337$

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15.000s	2.341	1.7998e+007	8.3536e+005
18.000s	2.342	1.8326e+007	8.4386e+005
20.000s	2.341	1.8492e+007	8.477e+005
25.000s	2.342	1.8787e+007	8.6276e+005
30.000s	2.341	1.8868e+007	8.6461e+005

Major cβ

Compound 12b Major amide singlet irradiation

Fri Dec 16 09:25:56 CST 2011

Dataset :
 E:/data/schweiz/nmr/2,3,5 Triol-arab-f-NAC_Cis-beta-hyp-OMe_67.3C/2/pdata/1
AREA fit :
 I[t]=I[0]+P*exp(-t/T1)
32 points for Integral 1, Integral Region from 2.502 to 2.436 ppm
Results   Comp. 1
I[0]   =   9.383e-001
P   =   -1.685e+000
T1   =   1.731s
SD   =   3.467e-002

tau	ppm	integral	intensity
1.000m	2.473	-8.6252e+008	-5.0091e+007
10.000m	2.473	-8.4948e+008	-4.9497e+007
15.000m	2.473	-8.4302e+008	-4.8955e+007
20.000m	2.473	-8.365e+008	-4.8656e+007
30.000m	2.473	-8.2242e+008	-4.787e+007
40.000m	2.473	-8.0807e+008	-4.7227e+007
50.000m	2.473	-7.9521e+008	-4.6469e+007
80.000m	2.473	-7.5749e+008	-4.4469e+007
100.000m	2.473	-7.3176e+008	-4.2924e+007
150.000m	2.473	-6.7174e+008	-3.9749e+007
200.000m	2.473	-6.1352e+008	-3.6652e+007
300.000m	2.473	-5.0672e+008	-3.0939e+007
400.000m	2.473	-4.0985e+008	-2.5695e+007
500.000m	2.473	-3.2113e+008	-2.1094e+007
600.000m	2.473	-2.3912e+008	-1.6742e+007
800.000m	2.473	-9.456e+007	-9.1801e+006
1.000s	2.473	2.7486e+007	-2.745e+006
1.200s	2.473	1.3305e+008	2.8659e+006
1.500s	2.473	2.6469e+008	9.8113e+006
2.000s	2.473	4.3348e+008	1.87e+007
3.000s	2.473	6.5343e+008	3.0364e+007
4.000s	2.473	7.8574e+008	3.735e+007
5.000s	2.473	8.6957e+008	4.1893e+007
6.000s	2.473	9.251e+008	4.4937e+007
8.000s	2.473	9.9055e+008	4.8433e+007
10.000s	2.473	1.0259e+009	5.037e+007
12.000s	2.473	1.0486e+009	5.1271e+007

K_{ct} = 0.604159

K_{tc} = 0.179291

K_{eq} = 5.5581 / 2.4[illegible]

= 2.2541

Fri Dec 16 09:25:56 CST 2011

15.000s	2.473	1.0717e+009	5.1997e+007
18.000s	2.472	1.0874e+009	5.271e+007
20.000s	2.473	1.0965e+009	5.306e+007
25.000s	2.473	1.1096e+009	5.4367e+007
30.000s	2.473	1.1177e+009	5.6022e+007

Minor Cβ

Compound 12b Minor amide exponential recovery

Fri Dec 16 09:27:15 CST 2011

Dataset :
E:/data/schweiz/nmr/2,3,5 Triol-arab-f-NAC_Cis-beta-hyp-OMe_67.3C/2/pdata/1
AREA fit :
I[t]=I[0]+P*exp(-t/T1)
32 points for Integral 2, Integral Region from 2.434 to 2.378 ppm
Results Comp. 1
I[0] = 3.654e+000
P = -2.918e+000
T1 = 258.602s
SD = 1.441e-001

tau	ppm	integral	intensity
1.000m	2.405	4.6494e+008	2.0468e+007
10.000m	2.405	4.6173e+008	2.0308e+007
15.000m	2.405	4.5869e+008	2.0106e+007
20.000m	2.405	4.5602e+008	2.0032e+007
30.000m	2.405	4.5048e+008	1.9779e+007
40.000m	2.405	4.4657e+008	1.9531e+007
50.000m	2.405	4.417e+008	1.9284e+007
80.000m	2.405	4.2768e+008	1.8626e+007
100.000m	2.405	4.1933e+008	1.8119e+007
150.000m	2.405	3.9852e+008	1.715e+007
200.000m	2.405	3.8011e+008	1.6243e+007
300.000m	2.405	3.4822e+008	1.4675e+007
400.000m	2.405	3.211e+008	1.3355e+007
500.000m	2.405	2.9993e+008	1.2322e+007
600.000m	2.405	2.832e+008	1.149e+007
800.000m	2.405	2.597e+008	1.0366e+007
1.000s	2.405	2.4681e+008	9.7393e+006
1.200s	2.405	2.4185e+008	9.4837e+006
1.500s	2.405	2.4431e+008	9.5729e+006
2.000s	2.405	2.6281e+008	1.0405e+007
3.000s	2.405	3.137e+008	1.2784e+007
4.000s	2.405	3.5783e+008	1.4859e+007
5.000s	2.405	3.8993e+008	1.6406e+007
6.000s	2.405	4.1253e+008	1.7507e+007
8.000s	2.405	4.401e+008	1.8827e+007
10.000s	2.405	4.5481e+008	1.9579e+007
12.000s	2.405	4.6447e+008	1.9918e+007

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15.000s	2.405	4.7494e+008	2.0207e+007
18.000s	2.405	4.8352e+008	2.0473e+007
20.000s	2.405	4.8792e+008	2.0624e+007
25.000s	2.405	4.933e+008	2.1091e+007
30.000s	2.405	4.9584e+008	2.1668e+007

Inverse Magnetization Transfer – Mathematica Programs with Graphs

Fitting Inversion Magnetization Transfer NMR Experiments

DESCRIPTION

Magnetization transfer is used to measure the following Proline isomerization:

kct
cis <-------> trans
ktc

where Keq = kct/ktc = taut/tauc = Mtinf/Mcinf.

This notebook will simultaneously fit the time-dependent magnetization transfers of Pro 1H or 13C *cis* (Mct) and *trans* (Mtt) NMR signals as a function of the inversion transfer time (t) in Inversion Magnetization Transfer experiments. In the following pulse sequence, the 13C or 1H *cis* resonance is selectively inverted using a shaped pulse. Its recovery during t is determined by its intrinsic T1c, magnetization transfer to and from the *trans* resonance, and the T1t of the *trans* resonance. Similarly, the steady-state magnetization of the *trans* resonance is modulated by exchange with the *cis* resonance:

p(x)sel ----------t---------- p/2(x,y.-x.-y)---acquire

The resonance of the *trans* isomer of Pro (Mtt) shows the following time dependence:

Mtt = (c1)(t t)(l 1+1/t 1c)exp(l 1*t) + (c2)(t 2)(l 2+1/t 1c)exp(l 2t) + Mtinf

The resonance of the *cis* isomer of Pro (Mtc) shows the following time dependence:

$$Mtc = (c1)\exp(\lambda 1 * t) + (c2)\exp(\lambda 2 t) + Mcinf$$

T1c and T1t are the longitudinal relaxation times the resonances would have in the absence of exchange.

τc and τt are the lifetimes of the *cis* and *trans* conformers and kct and ktc are the corresponding rate constants.

τ1c and τ1t are the effective relaxation times of the *cis* and *trans* resonances when relaxation and exchange are both occuring and are defined below in terms of T1c and τc, T1t and τt.

λ1 and λ2 are related to the time constants τc, τt, τ1c, and τ1t, and are defined below.

c1 and c2 are defined below.

The user must enter Mcinf and Mtinf, the magnetization measured after 5 T1 periods for the *cis* and *trans* resonances.

The program calculates τt from τc, Mcinf, and Mtinf as: τt = τc * Mtinf/Mcinf.

The notebook will also generate graphs of the data and the fitted curve and statistical information on the goodness of the fit. A table of points can be produced to permit export of the theoretical curve.

References:

Alger and Prestegard (1977) J. Magn. Reson. 27, 137-41.
Mariappan and Rabenstein (1992) J. Magn. Reson. 100, 183-8.

Thanks to *Maxim A. Dubinnyi* for advice on how to fit multiple data sets.

INSTRUCTIONS

Hit the down arrow to move from cell to cell and press "enter" (not return) to execute each calculation. Follow the example below to see the fitting results for one set of data for hydroxy-Pro.

The user may enter data manually, in the form of {Transfer Time, Intensity} pairs, or read in a data files. For the latter, set the directory containing the data in the first line below. If you are not reading in files, move the cursor to the 4th line ("list2") and replace the pairs of points in "list2" with your own data. Do the same with "list3". Alternatively, press enter on the line "list2" and follow the fitting of those data.

In the first 3 lines below we set a directory, read in a data file, and check the number of points in the file.

```
SetDirectory["~/Documents/JOE/Mathematica/Research/FrankSchweizer/Venkata"];
```

```
translist2 = Import["NaxTransHypOmeUnGlyT.xls"];
cislist3 = Import["NaxTransHypOmeUnGlyC.xls"];
```

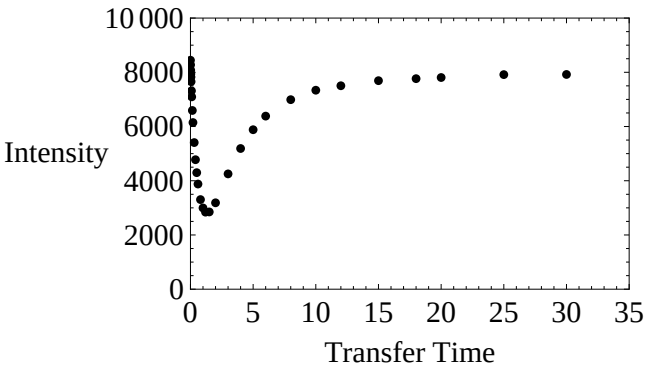
```
cislist := {{0.001, 8444.9}, {0.01, 8274.9}, {0.02, 8079}, {0.03, 7941.4}, {0.04, 7824.9},
  {0.05, 7655.1}, {0.08, 7316.9}, {0.1, 7094.3}, {0.15, 6591.7}, {0.2, 6146.5}, {0.3, 5407.8},
  {0.4, 4776}, {0.5, 4297.9}, {0.6, 3878.7}, {0.8, 3305.1}, {1, 2996.3}, {1.2, 2841.8},
  {1.5, 2849.3}, {2, 3186.5}, {3, 4252.7}, {4, 5189}, {5, 5881.9}, {6, 6383.6}, {8, 6990.2},
  {10, 7338.8}, {12, 7503.6}, {15, 7691}, {18, 7765}, {20, 7807.2}, {25, 7914.4}, {30, 7919.9}}
```

```
translist := {{0.001, -43048}, {0.01, -42375}, {0.02, -41885}, {0.03, -41244},
  {0.04, -40480}, {0.05, -39795}, {0.08, -38166}, {0.1, -37052}, {0.15, -34598},
  {0.2, -32223}, {0.3, -27940}, {0.4, -23973}, {0.5, -20343}, {0.6, -16962},
  {0.8, -10991}, {1, -5776.6}, {1.2, -1208.2}, {1.5, 4653}, {2, 12487},
  {3, 23161}, {4, 29855}, {5, 34238}, {6, 37112}, {8, 40618}, {10, 42423},
  {12, 43458}, {15, 44412}, {18, 44981}, {20, 45201}, {25, 45744}, {30, 45975}}
```

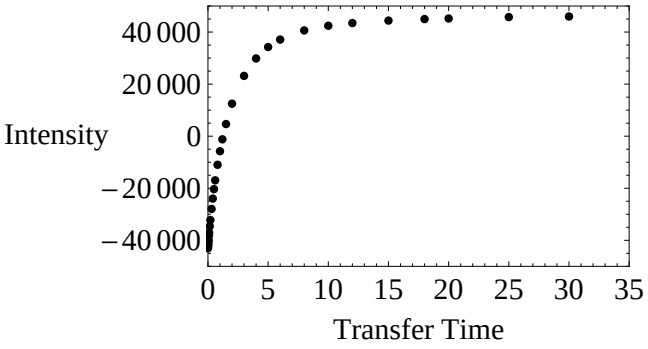
```
totalres4 = Length[translist];
totalres6 = Length[cislist]
```

```
31
```

```
lp2 = ListPlot[cislist, PlotStyle → {PointSize[0.02], Black},
  PlotRange → {{0, 35}, {0, 10 000}}, Frame → True, RotateLabel → False,
  BaseStyle → {14, FontFamily → "Times"}, FrameLabel → {"Transfer Time", "Intensity"},
  Axes → False, BaseStyle → {14, FontFamily → "Times"}]
```




```
lp3 = ListPlot[translist, PlotStyle → {PointSize[0.02], Black},
  PlotRange → {{0, 35}, {-50 000, 50 000}}, Frame → True, RotateLabel → False,
  BaseStyle → {14, FontFamily → "Times"}, FrameLabel → {"Transfer Time", "Intensity"},
  Axes → False, BaseStyle → {14, FontFamily → "Times"}]
```




```
CombinedData = Join[Insert[#, 1, 2] & /@ translist, Insert[#, 2, 2] & /@ cislist];
CombinedData // TableForm
```

```
0.001  1  -43 048
0.01   1  -42 375
```

```
0.02   1  -41 885
0.03   1  -41 244
0.04   1  -40 480
0.05   1  -39 795
0.08   1  -38 166
0.1    1  -37 052
0.15   1  -34 598
0.2    1  -32 223
0.3    1  -27 940
0.4    1  -23 973
0.5    1  -20 343
0.6    1  -16 962
0.8    1  -10 991
1      1  -5776.6
1.2    1  -1208.2
1.5    1  4653
2      1  12 487
3      1  23 161
4      1  29 855
5      1  34 238
6      1  37 112
8      1  40 618
10     1  42 423
12     1  43 458
15     1  44 412
18     1  44 981
20     1  45 201
25     1  45 744
30     1  45 975
0.001  2  8444.9
0.01   2  8274.9
0.02   2  8079
0.03   2  7941.4
0.04   2  7824.9
0.05   2  7655.1
0.08   2  7316.9
0.1    2  7094.3
0.15   2  6591.7
0.2    2  6146.5
0.3    2  5407.8
0.4    2  4776
0.5    2  4297.9
0.6    2  3878.7
0.8    2  3305.1
1      2  2996.3
```

```
1.2    2 2841.8
1.5    2 2849.3
2      2 3186.5
3      2 4252.7
4      2 5189
5      2 5881.9
6      2 6383.6
8      2 6990.2
10     2 7338.8
12     2 7503.6
15     2 7691
18     2 7765
20     2 7807.2
25     2 7914.4
30     2 7919.9
```

```
Clear[T1t, T1c, taut, tauc, M0t, M0c, Mtinf, Mcinf, tau1t,
 tau1c, lam1, lam2, c1, c2, Mct, Mtt, Keq, kct, ktc, param1, NLM1]
```

Users : Enter your values for Mcinf and Mtinf below :

```
Mcinf := 7919.9
```

```
Mtinf := 45 975
```

```
taut := ((tauc) * (Mtinf)) / (Mcinf)

kct := 1 / tauc

ktc := 1 / taut

Keq := (Mtinf) / (Mcinf)

tau1c := ((T1c) * (tauc)) / ((tauc) + (T1c))

tau1t := ((T1t) * (taut)) / ((taut) + (T1t))

lam1 :=
 (0.5) * (- (((1) / (tau1c)) + ((1) / (tau1t))) + ((((((1) / (tau1c)) + ((1) / (tau1t)))^(2)) - (4) *
          ((((1) / (tau1c)) * ((1) / (tau1t))) - (((1) / (tauc)) * ((1) / (taut))))))^(0.5))

lam2 :=
 (0.5) * (- (((1) / (tau1c)) + ((1) / (tau1t))) - ((((((1) / (tau1c)) + ((1) / (tau1t)))^(2)) - (4) *
          (((((1) / (tau1c))) * (((1) / (tau1t)))) - (((1) / (tauc)) * ((1) / (taut))))))^(0.5))

c2 := ((1) / ((taut) * ((lam1) - (lam2)))) *
  (((taut) * ((lam1) + (((1) / (tau1c))))) * ((M0c) - (Mcinf))) + ((Mtinf) - (M0t)))

c1 := M0c - Mcinf - c2

Mtt := ((c1) * (taut) * ((lam1) + (((1) / (tau1c))))) * (Exp[(lam1) * (x)])) +
  ((c2) * (taut) * ((lam2) + (((1) / (tau1c))))) * (Exp[(lam2) * (x)])) + Mtinf

Mct := ((c1) * (Exp[(lam1) * (x)])) + ((c2) * (Exp[(lam2) * (x)])) + (Mcinf)

CombinedMctMtt :=
 Which[MDL == 1, Evaluate@Mtt,
    MDL == 2, Evaluate@Mct,
    True,   0];

taut

tau1c

tau1t

1 / tau1c

1 / tau1t
```

$$5.805\ \text{tauc}$$

$$\frac{\text{T1c tauc}}{\text{T1c} + \text{tauc}}$$

$$\frac{5.805\ \text{T1t tauc}}{\text{T1t} + 5.805\ \text{tauc}}$$

$$\frac{T1c + tauc}{T1c\ tauc}$$

$$\frac{0.172265\ (T1t + 5.805\ tauc)}{T1t\ tauc}$$

Users : You may want to adjust the best guesses for the parameters, the upper and lower limits, and the number of interations below :

```
NLM1 = NonlinearModelFit[CombinedData, CombinedMctMtt,
  {{T1c, 1.7, 0.1, 100}, {T1t, 1.7, 0.1, 100}, {tauc, 1, 0.1, 100}, {M0c, 8500, 5000, 10 000},
   {M0t, -43 000, -60 000, -30 000}}, {x, MDL}, MaxIterations → 1 000 000]
```

NonlinearModelFit::lstol :

The line search decreased the step size to within tolerance specified by AccuracyGoal and PrecisionGoal but was unable to find a sufficient decrease in the norm of the residual. You may need more than MachinePrecision digits of working precision to meet these tolerances. ≫

FittedModel[Which[MDL == 1, ≪4≫, 0]]

```
NLM1[{"ParameterTable", "RSquared"}]
```

FittedModel::constr :

The property values {ParameterTable} assume an unconstrained model. The results for these properties may not be valid, particularly if the fitted parameters are near a constraint boundary. ≫

	Estimate	Standard Error	t Statistic	P-Value
T1c	15.4307	12.4031	1.24411	0.218552
T1t	2.34505	0.0410966	57.062	5.5155×10^{-52}
tauc	1.21067	0.0982919	12.3171	1.05702×10^{-17}
M0c	8699.18	258.843	33.6079	2.87267×10^{-39}
M0t	−42 090.7	253.905	−165.774	3.36472×10^{-78}

, 0.99919}

```
param1 = NLM1["BestFitParameters"]
```

{T1c → 15.4307, T1t → 2.34505, tauc → 1.21067, M0c → 8699.18, M0t → −42 090.7}

```
T1c = T1c /. param1
T1t = T1t /. param1
tauc = tauc /. param1
M0t = M0t /. param1
M0c = M0c /. param1
```

15.4307

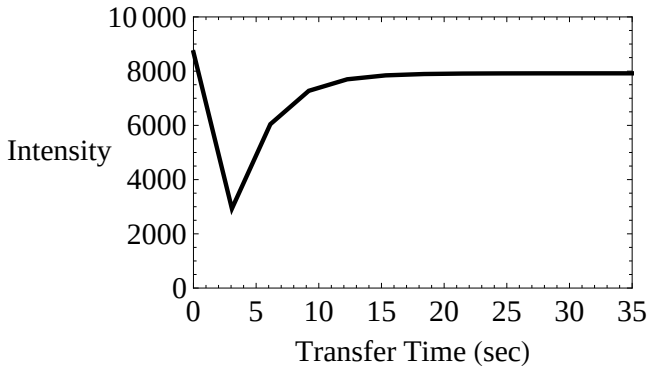
2.34505

1.21067

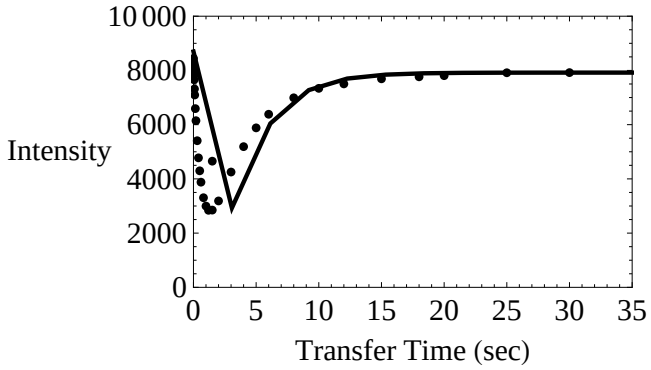
−42 090.7

8699.18

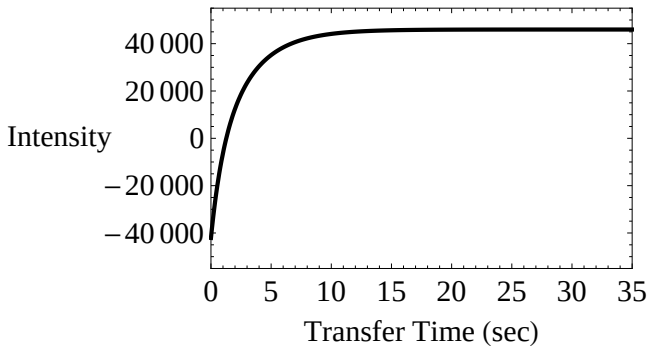
```
Plot[Mct, {x, 0, 10 000}, PlotStyle → {Thickness[0.01], Black},
 PlotRange → {{0, 35}, {0, 10 000}}, Frame → True,
 RotateLabel → False, BaseStyle → {14, FontFamily → "Times"},
 FrameLabel → {"Transfer Time (sec)", "Intensity"}, Axes → False]
```



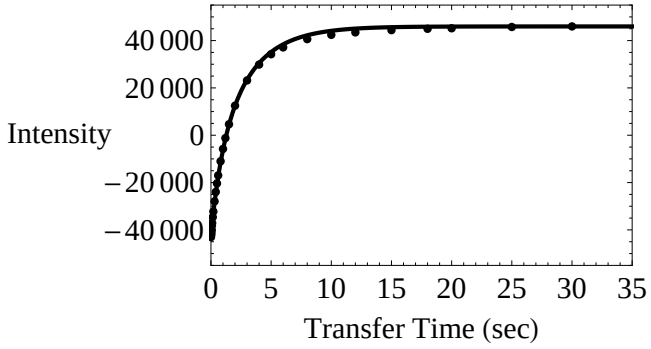

```
Show[%, lp2]
```




```
Plot[Mtt, {x, 0, 35}, PlotStyle → {Thickness[0.01], Black},
 PlotRange → {{0, 35}, {-55 000, 55 000}}, Frame → True,
 RotateLabel → False, BaseStyle → {14, FontFamily → "Times"},
 FrameLabel → {"Transfer Time (sec)", "Intensity"}, Axes → False]
```



```
Show[%, lp3]
```



Following are the rate constants in sec-1 and the equilibrium constant:

```
kct
```

```
0.82599
```

```
ktc
```

```
0.14229
```

```
Keq
```

```
5.805
```

The next line calculates points from the theoretical curve in case you want to graph the curve in another application:

```
theorMtt = Table[{x, Mtt}, {x, 0, 15, 0.01}]
```

```
theorMct = Table[{x, Mct}, {x, 0, 15, 0.01}]
```

The next line exports a file containing the theoretical points just calculated.

```
Export["OHPro1.txt", theorMtt, "Table"]
```

```
Export["OHPro2.txt", theorMct, "Table"]
```

Just to confirm, here are graphs of the theoretical points and the data.

```
ListPlot[theorMtt, PlotStyle → {PointSize[0.01], Black}, PlotRange → {{0, 15}, {70, +120}},
 Frame → True, RotateLabel → False, BaseStyle → {14, FontFamily → "Times"},
 FrameLabel → {"Transfer Time (sec)", "Intensity"}, Axes → False]
```

```
Show[%, lp2]
```

```
ListPlot[theorMct, PlotStyle → {PointSize[0.01], Black}, PlotRange → {{0, 15}, {-75, +50}},
 Frame → True, RotateLabel → False, BaseStyle → {14, FontFamily → "Times"},
 FrameLabel → {"Transfer Time (sec)", "Intensity"}, Axes → False]
```

```
Show[%, lp3]
```

```
Clear[T1t, T1c, taut, tauc, M0t, M0c, Mtinf, Mcinf, tau1t,
 tau1c, lam1, lam2, c1, c2, Mct, Mtt, Keq, kct, ktc, param1, NLM1]
```

END

Conditions of Use:

This software is in the public domain and may be distrubuted and modified freely by any user. It is provided "as is", with absolutely no warranty expressed or implied. Any use is at your own risk.

Errors, suggestions for improvement, or other comments may be sent to:

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Last updated: June 20 2007

Fitting Inversion Magnetization Transfer NMR Experiments

DESCRIPTION

Magnetization transfer is used to measure the following Proline isomerization:

kct
cis <-------> trans
ktc

where Keq = kct/ktc = taut/tauc = Mtinf/Mcinf.

This notebook will simultaneously fit the time-dependent magnetization transfers of Pro 1H or 13C *cis* (Mct) and *trans* (Mtt) NMR signals as a function of the inversion transfer time (t) in Inversion Magnetization Transfer experiments. In the following pulse sequence, the 13C or 1H *cis* resonance is selectively inverted using a shaped pulse. Its recovery during t is determined by its intrinsic T1c, magnetization transfer to and from the *trans* resonance, and the T1t of the *trans* resonance. Similarly, the steady-state magnetization of the *trans* resonance is modulated by exchange with the *cis* resonance:

p(x)sel ----------t---------- p/2(x,y.-x.-y)---acquire

The resonance of the *trans* isomer of Pro (Mtt) shows the following time dependence:

Mtt = (c1)(t t)(l 1+1/t 1c)exp(l 1*t) + (c2)(t 2)(l 2+1/t 1c)exp(l 2t) + Mtinf

The resonance of the *cis* isomer of Pro (Mtc) shows the following time dependence:

$$Mtc = (c1)\exp(\lambda 1 * t) + (c2)\exp(\lambda 2 t) + Mcinf$$

T1c and T1t are the longitudinal relaxation times the resonances would have in the absence of exchange.

τc **and** τt **are the lifetimes of the *cis* and *trans* conformers and kct and ktc are the corresponding rate constants.**

$\tau 1c$ **and** $\tau 1t$ **are the effective relaxation times of the *cis* and *trans* resonances when relaxation and exchange are both occuring and are defined below in terms of T1c and** τc**, T1t and** τt**.**

$\lambda 1$ **and** $\lambda 2$ **are related to the time constants** τc**,** τt**,** $\tau 1c$**, and** $\tau 1t$**, and are defined below.**

c1 and c2 are defined below.

The user must enter Mcinf and Mtinf, the magnetization measured after 5 T1 periods for the *cis* and *trans* resonances.

The program calculates τt **from** τc**, Mcinf, and Mtinf as:** $\tau t = \tau c$ *** Mtinf/Mcinf.**

The notebook will also generate graphs of the data and the fitted curve and statistical information on the goodness of the fit. A table of points can be produced to permit export of the theoretical curve.

References:

Alger and Prestegard (1977) J. Magn. Reson. 27, 137-41.
Mariappan and Rabenstein (1992) J. Magn. Reson. 100, 183-8.

Thanks to *Maxim A. Dubinnyi* for advice on how to fit multiple data sets.

INSTRUCTIONS

Hit the down arrow to move from cell to cell and press "enter" (not return) to execute each calculation. Follow the example below to see the fitting results for one set of data for hydroxy-Pro.

The user may enter data manually, in the form of {Transfer Time, Intensity} pairs, or read in a data files. For the latter, set the directory containing the data in the first line below. If you are not reading in files, move the cursor to the 4th line ("list2") and replace the pairs of points in "list2" with your own data. Do the same with "list3". Alternatively, press enter on the line "list2" and follow the fitting of those data.

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```
SetDirectory["~/Documents/JOE/Mathematica/Research/FrankSchweizer/Venkata"];
```

```
translist2 = Import["NaxTransHypOmeUnGlyT.xls"];
cislist3 = Import["NaxTransHypOmeUnGlyC.xls"];
```

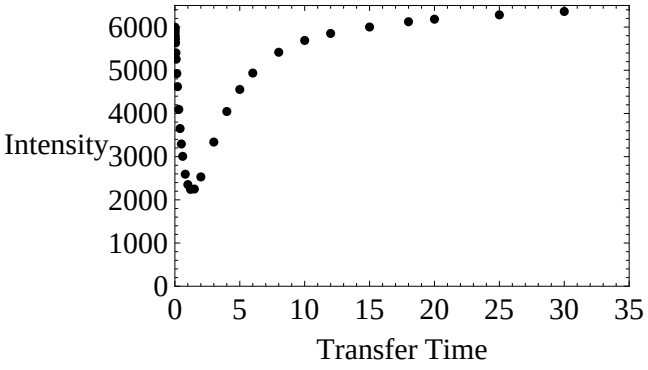
```
cislist := {{0.001, 5997.4}, {0.01, 5980.5}, {0.015, 5938.3}, {0.02, 5881.5},
  {0.03, 5799.7}, {0.04, 5719.7}, {0.05, 5633.4}, {0.08, 5402.8}, {0.1, 5257},
  {0.15, 4923.9}, {0.2, 4620.9}, {0.3, 4094.3}, {0.4, 3650.9}, {0.5, 3293.3},
  {0.6, 3007.4}, {0.8, 2594.1}, {1, 2352.9}, {1.2, 2243.1}, {1.5, 2249.7}, {2, 2530.7},
  {3, 3337.1}, {4, 4045.3}, {5, 4555.7}, {6, 4935.3}, {8, 5417.1}, {10, 5690.3},
  {12, 5852.2}, {15, 6001}, {18, 6124}, {20, 6182.2}, {25, 6282.1}, {30, 6361}}

translist := {{0.001, -24574}, {0.01, -24436}, {0.015, -24355}, {0.02, -24099},
  {0.03, -23692}, {0.04, -23283}, {0.05, -22885}, {0.08, -21926}, {0.1, -21269},
  {0.15, -19838}, {0.2, -18479}, {0.3, -15938}, {0.4, -13596}, {0.5, -11423},
  {0.6, -9399.5}, {0.8, -5785.2}, {1, -2617.1}, {1.2, 179.59}, {1.5, 3792.8},
  {2, 8710.5}, {3, 15656}, {4, 20028}, {5, 22854}, {6, 24868}, {8, 27268}, {10, 28586},
  {12, 29392}, {15, 30190}, {18, 30728}, {20, 31042}, {25, 31575}, {30, 31926}}
```

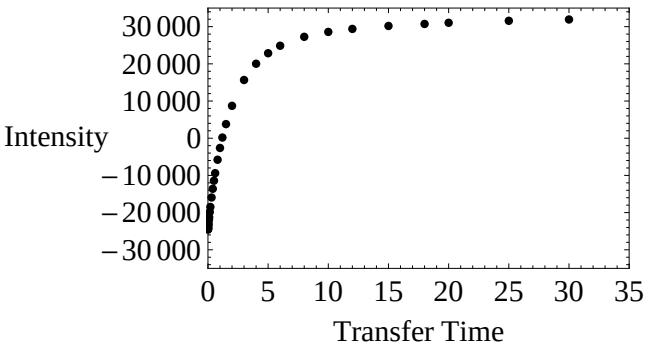
```
totalres4 = Length[translist];
totalres6 = Length[cislist]
```

```
32
```

```
lp2 = ListPlot[cislist, PlotStyle → {PointSize[0.02], Black},
  PlotRange → {{0, 35}, {0, 6500}}, Frame → True, RotateLabel → False,
  BaseStyle → {14, FontFamily → "Times"}, FrameLabel → {"Transfer Time", "Intensity"},
  Axes → False, BaseStyle → {14, FontFamily → "Times"}]
```




```
lp3 = ListPlot[translist, PlotStyle → {PointSize[0.02], Black},
  PlotRange → {{0, 35}, {-35 000, 35 000}}, Frame → True, RotateLabel → False,
  BaseStyle → {14, FontFamily → "Times"}, FrameLabel → {"Transfer Time", "Intensity"},
  Axes → False, BaseStyle → {14, FontFamily → "Times"}]
```




```
CombinedData = Join[Insert[#, 1, 2] & /@ translist, Insert[#, 2, 2] & /@ cislist];
CombinedData // TableForm
```

```
0.001  1  −24 574
0.01   1  −24 436
0.015  1  −24 355
```

```
0.02   1  -24 099
0.03   1  -23 692
0.04   1  -23 283
0.05   1  -22 885
0.08   1  -21 926
0.1    1  -21 269
0.15   1  -19 838
0.2    1  -18 479
0.3    1  -15 938
0.4    1  -13 596
0.5    1  -11 423
0.6    1  -9399.5
0.8    1  -5785.2
1      1  -2617.1
1.2    1  179.59
1.5    1  3792.8
2      1  8710.5
3      1  15 656
4      1  20 028
5      1  22 854
6      1  24 868
8      1  27 268
10     1  28 586
12     1  29 392
15     1  30 190
18     1  30 728
20     1  31 042
25     1  31 575
30     1  31 926
0.001  2  5997.4
0.01   2  5980.5
0.015  2  5938.3
0.02   2  5881.5
0.03   2  5799.7
0.04   2  5719.7
0.05   2  5633.4
0.08   2  5402.8
0.1    2  5257
0.15   2  4923.9
0.2    2  4620.9
0.3    2  4094.3
0.4    2  3650.9
0.5    2  3293.3
0.6    2  3007.4
0.8    2  2594.1
```

```
1      2 2352.9
1.2    2 2243.1
1.5    2 2249.7
2      2 2530.7
3      2 3337.1
4      2 4045.3
5      2 4555.7
6      2 4935.3
8      2 5417.1
10     2 5690.3
12     2 5852.2
15     2 6001
18     2 6124
20     2 6182.2
25     2 6282.1
30     2 6361
```

```
Clear[T1t, T1c, taut, tauc, M0t, M0c, Mtinf, Mcinf, tau1t,
 tau1c, lam1, lam2, c1, c2, Mct, Mtt, Keq, kct, ktc, param1, NLM1]
```

Users : Enter your values for Mcinf and Mtinf below :

```
Mcinf := 6361
```

```
Mtinf := 31 926
```

```
taut := ((tauc) * (Mtinf)) / (Mcinf)

kct := 1 / tauc

ktc := 1 / taut

Keq := (Mtinf) / (Mcinf)

tau1c := ((T1c) * (tauc)) / ((tauc) + (T1c))

tau1t := ((T1t) * (taut)) / ((taut) + (T1t))

lam1 :=
 (0.5) * (- (((1) / (tau1c)) + ((1) / (tau1t))) + ((((((1) / (tau1c)) + ((1) / (tau1t)))^(2)) - (4) *
          ((((1) / (tau1c)) * ((1) / (tau1t))) - (((1) / (tauc)) * ((1) / (taut))))))^(0.5))

lam2 :=
 (0.5) * (- (((1) / (tau1c)) + ((1) / (tau1t))) - ((((((1) / (tau1c)) + ((1) / (tau1t)))^(2)) - (4) *
          (((((1) / (tau1c))) * (((1) / (tau1t)))) - (((1) / (tauc)) * ((1) / (taut))))))^(0.5))

c2 := ((1) / ((taut) * ((lam1) - (lam2)))) *
  (((taut) * ((lam1) + (((1) / (tau1c))))) * ((M0c) - (Mcinf))) + ((Mtinf) - (M0t)))

c1 := M0c - Mcinf - c2

Mtt := ((c1) * (taut) * ((lam1) + (((1) / (tau1c))))) * (Exp[(lam1) * (x)])) +
  ((c2) * (taut) * ((lam2) + (((1) / (tau1c))))) * (Exp[(lam2) * (x)])) + Mtinf

Mct := ((c1) * (Exp[(lam1) * (x)])) + ((c2) * (Exp[(lam2) * (x)])) + (Mcinf)

CombinedMctMtt :=
 Which[MDL == 1, Evaluate@Mtt,
    MDL == 2, Evaluate@Mct,
    True,   0];

taut

tau1c

tau1t

1 / tau1c

1 / tau1t
```

$$\frac{31\,926\ \text{tauc}}{6361}$$

$$\frac{\text{T1c tauc}}{\text{T1c} + \text{tauc}}$$

$$\frac{31\,926\ \text{T1t tauc}}{6361\left(\text{T1t}+\frac{31\,926\ \text{tauc}}{6361}\right)}$$

$$\frac{\text{T1c}+\text{tauc}}{\text{T1c tauc}}$$

$$\frac{6361\left(\text{T1t}+\frac{31\,926\ \text{tauc}}{6361}\right)}{31\,926\ \text{T1t tauc}}$$

Users : You may want to adjust the best guesses for the parameters, the upper and lower limits, and the number of interations below :

```
NLM1 = NonlinearModelFit[CombinedData, CombinedMctMtt,
  {{T1c, 1.8, 0.5, 5}, {T1t, 1.8, 0.5, 5}, {tauc, 1, 0.1, 100}, {M0c, 6000, 3000, 12 000},
   {M0t, -25 000, -35 000, -15 000}}, {x, MDL}, MaxIterations → 1 000 000]
```

FindMinimum::reged :

The point {5., 2.63505, 1.14774, 6000.01, −25000.} is at the edge of the search region $\left\{\frac{1}{2}, 5\right\}$ in coordinate 1 and the computed search direction points outside the region. ≫

FittedModel[≪1≫]

```
NLM1[{"ParameterTable", "RSquared"}]
```

FittedModel::constr :

The property values {ParameterTable} assume an unconstrained model. The results for these properties may not be valid, particularly if the fitted parameters are near a constraint boundary. ≫

	Estimate	Standard Error	t Statistic	P-Value
T1c	5.	2.16016	2.31464	0.0241315
T1t	2.63505	0.0841792	31.3029	1.89899×10^{-38}
tauc	1.14774	0.143342	8.00703	5.32396×10^{-11}
M0c	6000.01	235.089	25.5223	1.42514×10^{-33}
M0t	−25 000.	224.381	−111.417	2.6596×10^{-70}

, 0.998259

```
param1 = NLM1["BestFitParameters"]
```

{T1c → 5., T1t → 2.63505, tauc → 1.14774, M0c → 6000.01, M0t → −25 000.}

```
T1c = T1c /. param1
T1t = T1t /. param1
tauc = tauc /. param1
M0t = M0t /. param1
M0c = M0c /. param1
```

```
5.
```

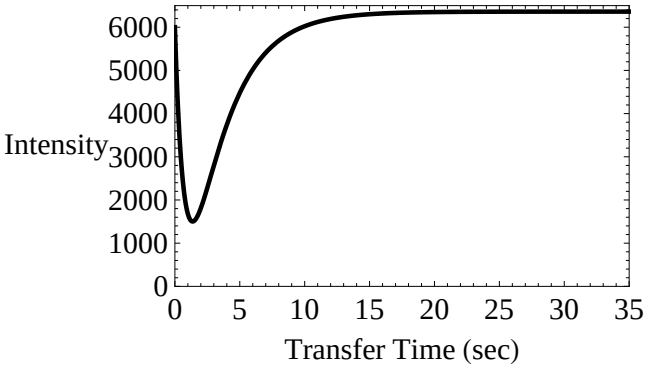
```
2.63505
```

```
1.14774
```

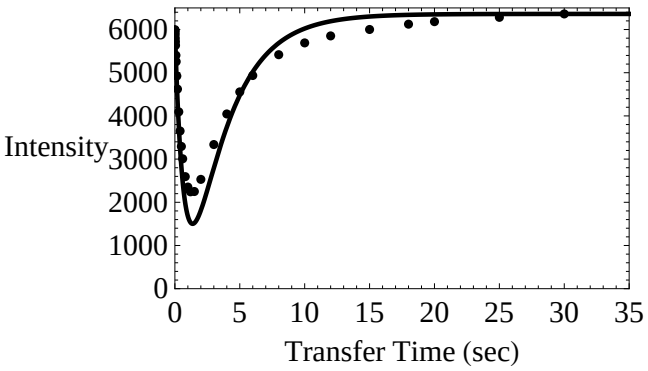
```
-25000.
```

```
6000.01
```

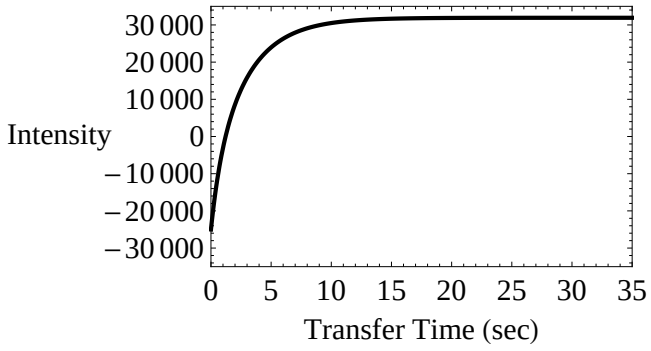
```
Plot[Mct, {x, 0, 35}, PlotStyle → {Thickness[0.01], Black}, PlotRange → {{0, 35}, {0, 6500}},
 Frame → True, RotateLabel → False, BaseStyle → {14, FontFamily → "Times"},
 FrameLabel → {"Transfer Time (sec)", "Intensity"}, Axes → False]
```



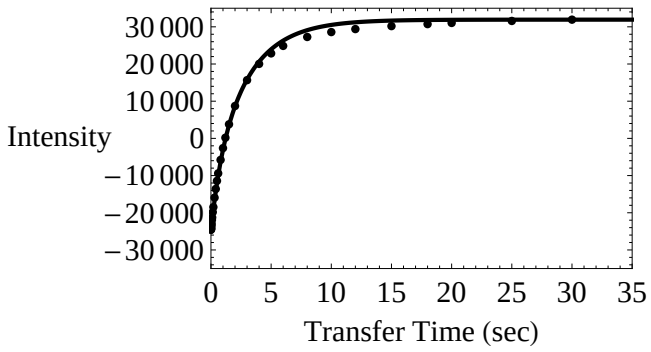

```
Show[%, lp2]
```



```
Plot[Mtt, {x, 0, 35}, PlotStyle → {Thickness[0.01], Black},
 PlotRange → {{0, 35}, {-35 000, 35 000}}, Frame → True,
 RotateLabel → False, BaseStyle → {14, FontFamily → "Times"},
 FrameLabel → {"Transfer Time (sec)", "Intensity"}, Axes → False]
```




```
Show[%, lp3]
```



Following are the rate constants in sec-1 and the equilibrium constant:

```
kct
```

```
0.871275
```

```
ktc
```

```
0.173595
```

```
Keq
```

$$\frac{31\,926}{6361}$$

The next line calculates points from the theoretical curve in case you want to graph the curve in another application:

```
theorMtt = Table[{x, Mtt}, {x, 0, 15, 0.01}]
```

```
theorMct = Table[{x, Mct}, {x, 0, 15, 0.01}]
```

The next line exports a file containing the theoretical points just calculated.

```
Export["OHPro1.txt", theorMtt, "Table"]

Export["OHPro2.txt", theorMct, "Table"]
```

Just to confirm, here are graphs of the theoretical points and the data.

```
ListPlot[theorMtt, PlotStyle → {PointSize[0.01], Black}, PlotRange → {{0, 15}, {70, +120}},
 Frame → True, RotateLabel → False, BaseStyle → {14, FontFamily → "Times"},
 FrameLabel → {"Transfer Time (sec)", "Intensity"}, Axes → False]

Show[%, lp2]

ListPlot[theorMct, PlotStyle → {PointSize[0.01], Black}, PlotRange → {{0, 15}, {-75, +50}},
 Frame → True, RotateLabel → False, BaseStyle → {14, FontFamily → "Times"},
 FrameLabel → {"Transfer Time (sec)", "Intensity"}, Axes → False]

Show[%, lp3]

Clear[T1t, T1c, taut, tauc, M0t, M0c, Mtinf, Mcinf, tau1t,
 tau1c, lam1, lam2, c1, c2, Mct, Mtt, Keq, kct, ktc, param1, NLM1]
```

END

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Last updated: June 20 2007

Fitting Inversion Magnetization Transfer NMR Experiments

DESCRIPTION

Magnetization transfer is used to measure the following Proline isomerization:

kct
cis <-------> trans
ktc

where Keq = kct/ktc = taut/tauc = Mtinf/Mcinf.

This notebook will simultaneously fit the time-dependent magnetization transfers of Pro 1H or 13C *cis* (Mct) and *trans* (Mtt) NMR signals as a function of the inversion transfer time (t) in Inversion Magnetization Transfer experiments. In the following pulse sequence, the 13C or 1H *cis* resonance is selectively inverted using a shaped pulse. Its recovery during t is determined by its intrinsic T1c, magnetization transfer to and from the *trans* resonance, and the T1t of the *trans* resonance. Similarly, the steady-state magnetization of the *trans* resonance is modulated by exchange with the *cis* resonance:

p(x)sel ----------t---------- p/2(x,y.-x.-y)---acquire

The resonance of the *trans* isomer of Pro (Mtt) shows the following time dependence:

Mtt = (c1)(t t)(l 1+1/t 1c)exp(l 1*t) + (c2)(t 2)(l 2+1/t 1c)exp(l 2t) + Mtinf

The resonance of the *cis* isomer of Pro (Mtc) shows the following time dependence:

$$Mtc = (c1)\exp(\lambda 1 * t) + (c2)\exp(\lambda 2 t) + Mcinf$$

T1c and T1t are the longitudinal relaxation times the resonances would have in the absence of exchange.

τc **and** τt **are the lifetimes of the *cis* and *trans* conformers and kct and ktc are the corresponding rate constants.**

$\tau 1c$ **and** $\tau 1t$ **are the effective relaxation times of the *cis* and *trans* resonances when relaxation and exchange are both occuring and are defined below in terms of T1c and** τc**, T1t and** τt**.**

$\lambda 1$ **and** $\lambda 2$ **are related to the time constants** τc**,** τt**,** $\tau 1c$**, and** $\tau 1t$**, and are defined below.**

c1 and c2 are defined below.

The user must enter Mcinf and Mtinf, the magnetization measured after 5 T1 periods for the *cis* and *trans* resonances.

The program calculates τt **from** τc**, Mcinf, and Mtinf as:** $\tau t = \tau c$ ***** **Mtinf/Mcinf.**

The notebook will also generate graphs of the data and the fitted curve and statistical information on the goodness of the fit. A table of points can be produced to permit export of the theoretical curve.

References:

Alger and Prestegard (1977) J. Magn. Reson. 27, 137-41.
Mariappan and Rabenstein (1992) J. Magn. Reson. 100, 183-8.

Thanks to *Maxim A. Dubinnyi* for advice on how to fit multiple data sets.

INSTRUCTIONS

Hit the down arrow to move from cell to cell and press "enter" (not return) to execute each calculation. Follow the example below to see the fitting results for one set of data for hydroxy-Pro.

The user may enter data manually, in the form of {Transfer Time, Intensity} pairs, or read in a data files. For the latter, set the directory containing the data in the first line below. If you are not reading in files, move the cursor to the 4th line ("list2") and replace the pairs of points in "list2" with your own data. Do the same with "list3". Alternatively, press enter on the line "list2" and follow the fitting of those data.

In the first 3 lines below we set a directory, read in a data file, and check the number of points in the file.

```
SetDirectory["~/Documents/JOE/Mathematica/Research/FrankSchweizer/Venkata"];
```

```
translist2 = Import["NaxTransHypOmeUnGlyT.xls"];
cislist3 = Import["NaxTransHypOmeUnGlyC.xls"];
```

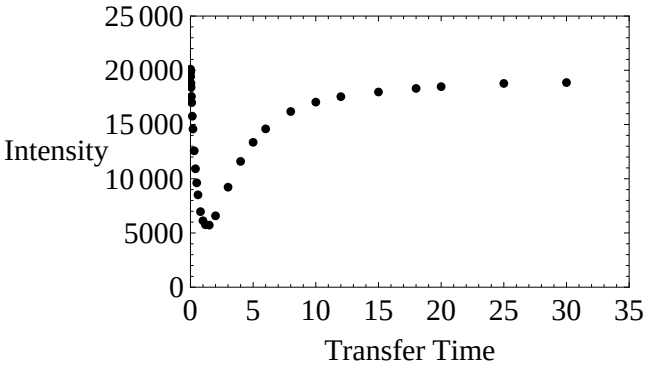
```
cislist := {{0.001, 20 109}, {0.01, 19 861}, {0.02, 19 427}, {0.03, 19 909}, {0.04, 18 807},
  {0.05, 18 429}, {0.08, 17 583}, {0.1, 17 020}, {0.15, 15 765}, {0.2, 14 590}, {0.3, 12 576},
  {0.4, 10 918}, {0.5, 9620.5}, {0.6, 8514.9}, {0.8, 6966.4}, {1, 6127.8}, {1.2, 5756.1},
  {1.5, 5723.4}, {2, 6582.6}, {3, 9221.4}, {4, 11 597}, {5, 13 360}, {6, 14 598}, {8, 16 198},
  {10, 17 065}, {12, 17 568}, {15, 17 998}, {18, 18 326}, {20, 18 492}, {25, 18 787}, {30, 18 868}}

translist := {{0.001, -93 855}, {0.01, -92 803}, {0.02, -91 397}, {0.03, -90 124},
  {0.04, -88 767}, {0.05, -87 475}, {0.08, -84 115}, {0.1, -81 859}, {0.15, -76 686},
  {0.2, -71 830}, {0.3, -62 832}, {0.4, -54 572}, {0.5, -46 782}, {0.6, -39 734},
  {0.8, -27 053}, {1, -15 947}, {1.2, -6271.6}, {1.5, 6406.5}, {2, 23 158},
  {3, 46 543}, {4, 61 605}, {5, 71 540}, {6, 78 307}, {8, 86 513}, {10, 90 880},
  {12, 93 569}, {15, 96 091}, {18, 97 741}, {20, 98 470}, {25, 99 927}, {30, 100 700}}
```

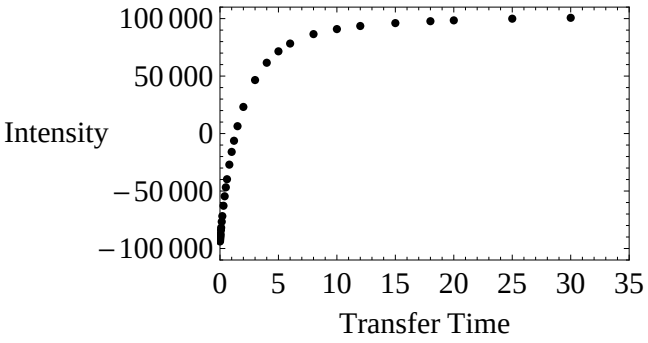
```
totalres4 = Length[translist];
totalres6 = Length[cislist]
```

```
31
```

```
lp2 = ListPlot[cislist, PlotStyle → {PointSize[0.02], Black},
  PlotRange → {{0, 35}, {0, 25 000}}, Frame → True, RotateLabel → False,
  BaseStyle → {14, FontFamily → "Times"}, FrameLabel → {"Transfer Time", "Intensity"},
  Axes → False, BaseStyle → {14, FontFamily → "Times"}]
```




```
lp3 = ListPlot[translist, PlotStyle → {PointSize[0.02], Black},
  PlotRange → {{0, 35}, {-110 000, 110 000}}, Frame → True, RotateLabel → False,
  BaseStyle → {14, FontFamily → "Times"}, FrameLabel → {"Transfer Time", "Intensity"},
  Axes → False, BaseStyle → {14, FontFamily → "Times"}]
```




```
CombinedData = Join[Insert[#, 1, 2] & /@ translist, Insert[#, 2, 2] & /@ cislist];
CombinedData // TableForm
```

```
0.001  1  -93 855
0.01   1  -92 803
```

```
0.02   1  -91 397
0.03   1  -90 124
0.04   1  -88 767
0.05   1  -87 475
0.08   1  -84 115
0.1    1  -81 859
0.15   1  -76 686
0.2    1  -71 830
0.3    1  -62 832
0.4    1  -54 572
0.5    1  -46 782
0.6    1  -39 734
0.8    1  -27 053
1      1  -15 947
1.2    1  -6271.6
1.5    1  6406.5
2      1  23 158
3      1  46 543
4      1  61 605
5      1  71 540
6      1  78 307
8      1  86 513
10     1  90 880
12     1  93 569
15     1  96 091
18     1  97 741
20     1  98 470
25     1  99 927
30     1  100 700
0.001  2  20 109
0.01   2  19 861
0.02   2  19 427
0.03   2  19 909
0.04   2  18 807
0.05   2  18 429
0.08   2  17 583
0.1    2  17 020
0.15   2  15 765
0.2    2  14 590
0.3    2  12 576
0.4    2  10 918
0.5    2  9620.5
0.6    2  8514.9
0.8    2  6966.4
1      2  6127.8
```

```
1.2    2  5756.1
1.5    2  5723.4
2      2  6582.6
3      2  9221.4
4      2  11 597
5      2  13 360
6      2  14 598
8      2  16 198
10     2  17 065
12     2  17 568
15     2  17 998
18     2  18 326
20     2  18 492
25     2  18 787
30     2  18 868
```

```
Clear[T1t, T1c, taut, tauc, M0t, M0c, Mtinf, Mcinf, tau1t,
 tau1c, lam1, lam2, c1, c2, Mct, Mtt, Keq, kct, ktc, param1, NLM1]
```

Users : Enter your values for Mcinf and Mtinf below :

```
Mcinf := 18 868
```

```
Mtinf := 100 700
```

```
taut := ((tauc) * (Mtinf)) / (Mcinf)

kct := 1 / tauc

ktc := 1 / taut

Keq := (Mtinf) / (Mcinf)

tau1c := ((T1c) * (tauc)) / ((tauc) + (T1c))

tau1t := ((T1t) * (taut)) / ((taut) + (T1t))

lam1 :=
 (0.5) * (- (((1) / (tau1c)) + ((1) / (tau1t))) + ((((((1) / (tau1c)) + ((1) / (tau1t)))^(2)) - (4) *
          ((((1) / (tau1c)) * ((1) / (tau1t))) - (((1) / (tauc)) * ((1) / (taut))))))^(0.5))

lam2 :=
 (0.5) * (- (((1) / (tau1c)) + ((1) / (tau1t))) - ((((((1) / (tau1c)) + ((1) / (tau1t)))^(2)) - (4) *
          (((((1) / (tau1c))) * (((1) / (tau1t)))) - (((1) / (tauc)) * ((1) / (taut))))))^(0.5))

c2 := ((1) / ((taut) * ((lam1) - (lam2)))) *
  (((taut) * ((lam1) + (((1) / (tau1c))))) * ((M0c) - (Mcinf))) + ((Mtinf) - (M0t)))

c1 := M0c - Mcinf - c2

Mtt := ((c1) * (taut) * ((lam1) + (((1) / (tau1c))))) * (Exp[(lam1) * (x)])) +
  ((c2) * (taut) * ((lam2) + (((1) / (tau1c))))) * (Exp[(lam2) * (x)])) + Mtinf

Mct := ((c1) * (Exp[(lam1) * (x)])) + ((c2) * (Exp[(lam2) * (x)])) + (Mcinf)

CombinedMctMtt :=
 Which[MDL == 1, Evaluate@Mtt,
    MDL == 2, Evaluate@Mct,
    True,   0];

taut

tau1c

tau1t

1 / tau1c

1 / tau1t
```

$$\frac{475\ \text{tauc}}{89}$$

$$\frac{\text{T1c tauc}}{\text{T1c} + \text{tauc}}$$

$$\frac{475\ \text{T1t tauc}}{89\left(\text{T1t}+\frac{475\ \text{tauc}}{89}\right)}$$

$$\frac{\text{T1c}+\text{tauc}}{\text{T1c tauc}}$$

$$\frac{89\left(\text{T1t}+\frac{475\ \text{tauc}}{89}\right)}{475\ \text{T1t tauc}}$$

Users : You may want to adjust the best guesses for the parameters, the upper and lower limits, and the number of interations below :

```
NLM1 = NonlinearModelFit[CombinedData, CombinedMctMtt,
  {{T1c, 2, 0.5, 5}, {T1t, 2, 0.5, 5}, {tauc, 1, 0.1, 100}, {M0c, 20 000, 10 000, 30 000},
   {M0t, -95 000, -150 000, -30 000}}, {x, MDL}, MaxIterations → 1 000 000]
```

FindMinimum::reged :

The point {5., 2.54472, 1.04081, 20000., −95000.} is at the edge of the search region $\left\{\frac{1}{2}, 5\right\}$ in coordinate 1 and the computed search direction points outside the region. ≫

FittedModel[Which[MDL == 1, ≪1≫, MDL == 2, $18\,868+\frac{\ll 1\gg}{\ll 1\gg}+e^{0.5\,(\ll 1\gg)\,x}\left(-18\,868+\ll 19\gg-\frac{89\ \ll 1\gg\ (\ll 1\gg\ \ll 1\gg)}{\ll 1\gg}\right)$, True, 0]]

```
NLM1[{"ParameterTable", "RSquared"}]
```

FittedModel::constr :

The property values {ParameterTable} assume an unconstrained model. The results for these properties may not be valid, particularly if the fitted parameters are near a constraint boundary. ≫

{

	Estimate	Standard Error	t Statistic	P-Value
T1c	5.	2.11033	2.3693	0.0212319
T1t	2.54472	0.0719304	35.3776	1.75839×10^{-40}
tauc	1.04081	0.110532	9.41641	3.24919×10^{-13}
M0c	20 000.	766.051	26.1079	2.14428×10^{-33}
M0t	−95 000.	724.769	−131.076	2.1152×10^{-72}

, 0.998634}

```
param1 = NLM1["BestFitParameters"]
```

{T1c → 5., T1t → 2.54472, tauc → 1.04081, M0c → 20 000., M0t → −95 000.}

```
T1c = T1c /. param1
T1t = T1t /. param1
tauc = tauc /. param1
M0t = M0t /. param1
M0c = M0c /. param1
```

```
5.
```

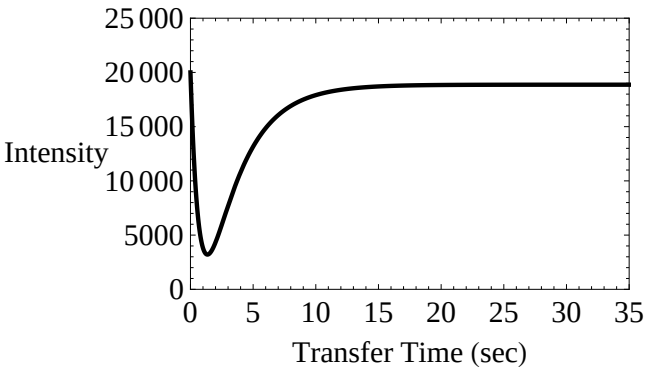
```
2.54472
```

```
1.04081
```

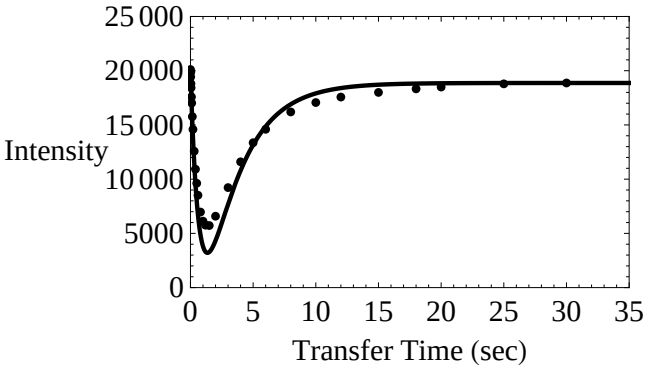
```
-95 000.
```

```
20 000.
```

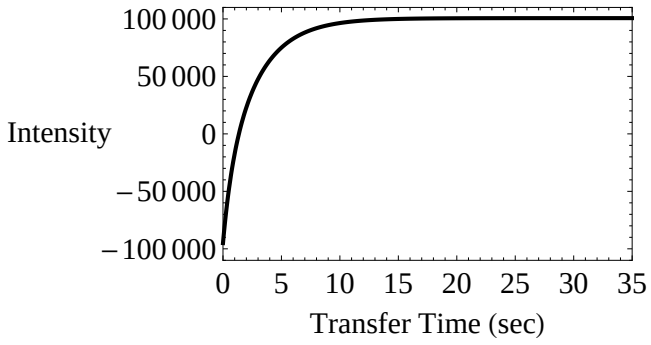
```
Plot[Mct, {x, 0, 35}, PlotStyle → {Thickness[0.01], Black},
 PlotRange → {{0, 35}, {0, 25 000}}, Frame → True,
 RotateLabel → False, BaseStyle → {14, FontFamily → "Times"},
 FrameLabel → {"Transfer Time (sec)", "Intensity"}, Axes → False]
```



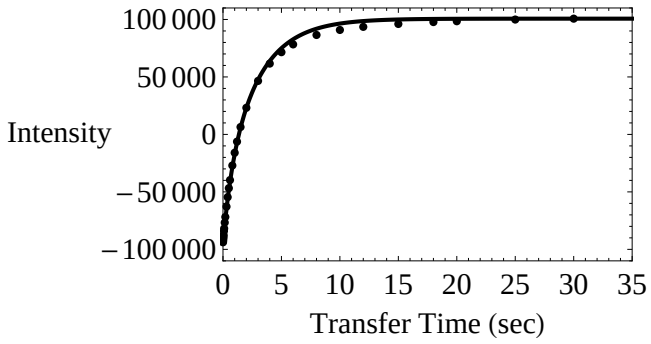

```
Show[%, lp2]
```



```
Plot[Mtt, {x, 0, 35}, PlotStyle → {Thickness[0.01], Black},
 PlotRange → {{0, 35}, {-110 000, 110 000}}, Frame → True,
 RotateLabel → False, BaseStyle → {14, FontFamily → "Times"},
 FrameLabel → {"Transfer Time (sec)", "Intensity"}, Axes → False]
```




```
Show[%, lp3]
```



Following are the rate constants in sec-1 and the equilibrium constant:

```
kct
```

```
0.960788
```

```
ktc
```

```
0.180021
```

```
Keq
```

$$\frac{475}{89}$$

The next line calculates points from the theoretical curve in case you want to graph the curve in another application:

```
theorMtt = Table[{x, Mtt}, {x, 0, 15, 0.01}]
```

```
theorMct = Table[{x, Mct}, {x, 0, 15, 0.01}]
```

The next line exports a file containing the theoretical points just calculated.

```
Export["OHPro1.txt", theorMtt, "Table"]

Export["OHPro2.txt", theorMct, "Table"]
```

Just to confirm, here are graphs of the theoretical points and the data.

```
ListPlot[theorMtt, PlotStyle → {PointSize[0.01], Black}, PlotRange → {{0, 15}, {70, +120}},
 Frame → True, RotateLabel → False, BaseStyle → {14, FontFamily → "Times"},
 FrameLabel → {"Transfer Time (sec)", "Intensity"}, Axes → False]

Show[%, lp2]

ListPlot[theorMct, PlotStyle → {PointSize[0.01], Black}, PlotRange → {{0, 15}, {-75, +50}},
 Frame → True, RotateLabel → False, BaseStyle → {14, FontFamily → "Times"},
 FrameLabel → {"Transfer Time (sec)", "Intensity"}, Axes → False]

Show[%, lp3]

Clear[T1t, T1c, taut, tauc, M0t, M0c, Mtinf, Mcinf, tau1t,
 tau1c, lam1, lam2, c1, c2, Mct, Mtt, Keq, kct, ktc, param1, NLM1]
```

END

Conditions of Use:

Errors, suggestions for improvement, or other comments may be sent to:

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University of Manitoba
390 Parker Building
144 Dysart Rd.
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R3T 2N2
TELE: (204) 474-6697
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Last updated: June 20 2007

Fitting Inversion Magnetization Transfer NMR Experiments

DESCRIPTION

Magnetization transfer is used to measure the following Proline isomerization:

kct
cis <-------> trans
ktc

where Keq = kct/ktc = taut/tauc = Mtinf/Mcinf.

This notebook will simultaneously fit the time-dependent magnetization transfers of Pro 1H or 13C *cis* (Mct) and *trans* (Mtt) NMR signals as a function of the inversion transfer time (t) in Inversion Magnetization Transfer experiments. In the following pulse sequence, the 13C or 1H *cis* resonance is selectively inverted using a shaped pulse. Its recovery during t is determined by its intrinsic T1c, magnetization transfer to and from the *trans* resonance, and the T1t of the *trans* resonance. Similarly, the steady-state magnetization of the *trans* resonance is modulated by exchange with the *cis* resonance:

p(x)sel ----------t---------- p/2(x,y.-x.-y)---acquire

The resonance of the *trans* isomer of Pro (Mtt) shows the following time dependence:

Mtt = (c1)(t t)(l 1+1/t 1c)exp(l 1*t) + (c2)(t 2)(l 2+1/t 1c)exp(l 2t) + Mtinf

The resonance of the *cis* isomer of Pro (Mtc) shows the following time dependence:

$$Mtc = (c1)\exp(\lambda 1 * t) + (c2)\exp(\lambda 2 t) + Mcinf$$

T1c and T1t are the longitudinal relaxation times the resonances would have in the absence of exchange.

τc and τt are the lifetimes of the *cis* and *trans* conformers and kct and ktc are the corresponding rate constants.

τ1c and τ1t are the effective relaxation times of the *cis* and *trans* resonances when relaxation and exchange are both occuring and are defined below in terms of T1c and τc, T1t and τt.

λ1 and λ2 are related to the time constants τc, τt, τ1c, and τ1t, and are defined below.

c1 and c2 are defined below.

The user must enter Mcinf and Mtinf, the magnetization measured after 5 T1 periods for the *cis* and *trans* resonances.

The program calculates τt from τc, Mcinf, and Mtinf as: τt = τc * Mtinf/Mcinf.

The notebook will also generate graphs of the data and the fitted curve and statistical information on the goodness of the fit. A table of points can be produced to permit export of the theoretical curve.

References:

Alger and Prestegard (1977) J. Magn. Reson. 27, 137-41.
Mariappan and Rabenstein (1992) J. Magn. Reson. 100, 183-8.

Thanks to *Maxim A. Dubinnyi* for advice on how to fit multiple data sets.

INSTRUCTIONS

Hit the down arrow to move from cell to cell and press "enter" (not return) to execute each calculation. Follow the example below to see the fitting results for one set of data for hydroxy-Pro.

The user may enter data manually, in the form of {Transfer Time, Intensity} pairs, or read in a data files. For the latter, set the directory containing the data in the first line below. If you are not reading in files, move the cursor to the 4th line ("list2") and replace the pairs of points in "list2" with your own data. Do the same with "list3". Alternatively, press enter on the line "list2" and follow the fitting of those data.

In the first 3 lines below we set a directory, read in a data file, and check the number of points in the file.

```
SetDirectory["~/Documents/JOE/Mathematica/Research/FrankSchweizer/Venkata"];
```

```
translist2 = Import["NaxTransHypOmeUnGlyT.xls"];
cislist3 = Import["NaxTransHypOmeUnGlyC.xls"];
```

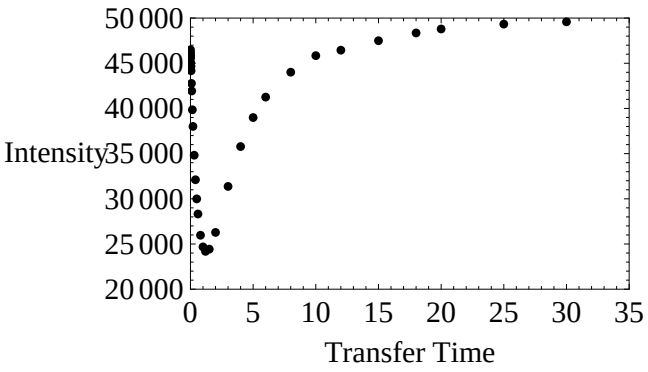
```
cislist := {{0.001, 46 494}, {0.01, 46 173}, {0.015, 45 869}, {0.02, 45 602},
  {0.03, 45 048}, {0.04, 44 657}, {0.05, 44 170}, {0.08, 42 768}, {0.1, 41 933},
  {0.15, 39 852}, {0.2, 38 011}, {0.3, 34 822}, {0.4, 32 110}, {0.5, 29 993},
  {0.6, 28 320}, {0.8, 25 970}, {1, 24 681}, {1.2, 24 185}, {1.5, 24 431}, {2, 26 281},
  {3, 31 370}, {4, 35 783}, {5, 38 993}, {6, 41 253}, {8, 44 010}, {10, 45 841},
  {12, 46 447}, {15, 47 494}, {18, 48 352}, {20, 48 792}, {25, 49 330}, {30, 49 584}}

translist := {{0.001, -86 252}, {0.01, -84 948}, {0.015, -84 032}, {0.02, -83 650},
  {0.03, -82 242}, {0.04, -80 807}, {0.05, -79 521}, {0.08, -75 749}, {0.1, -73 176},
  {0.15, -67 174}, {0.2, -61 352}, {0.3, -50 672}, {0.4, -40 985}, {0.5, -32 113},
  {0.6, -23 912}, {0.8, -9456}, {1, 2748.6}, {1.2, 13 305}, {1.5, 26 469}, {2, 43 348},
  {3, 65 343}, {4, 78 574}, {5, 86 957}, {6, 92 510}, {8, 99 055}, {10, 102 590},
  {12, 104 860}, {15, 107 170}, {18, 108 740}, {20, 109 650}, {25, 110 960}, {30, 111 770}}
```

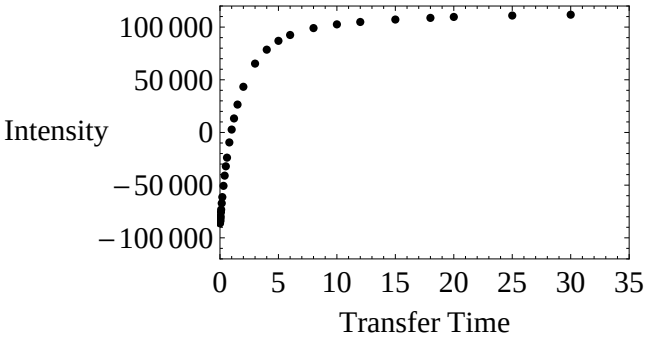
```
totalres4 = Length[translist];
totalres6 = Length[cislist]
```

```
32
```

```
lp2 = ListPlot[cislist, PlotStyle → {PointSize[0.02], Black},
  PlotRange → {{0, 35}, {20 000, 50 000}}, Frame → True, RotateLabel → False,
  BaseStyle → {14, FontFamily → "Times"}, FrameLabel → {"Transfer Time", "Intensity"},
  Axes → False, BaseStyle → {14, FontFamily → "Times"}]
```




```
lp3 = ListPlot[translist, PlotStyle → {PointSize[0.02], Black},
  PlotRange → {{0, 35}, {-120 000, 120 000}}, Frame → True, RotateLabel → False,
  BaseStyle → {14, FontFamily → "Times"}, FrameLabel → {"Transfer Time", "Intensity"},
  Axes → False, BaseStyle → {14, FontFamily → "Times"}]
```




```
CombinedData = Join[Insert[#, 1, 2] & /@ translist, Insert[#, 2, 2] & /@ cislist];
CombinedData // TableForm
```

```
0.001  1  -86 252
0.01   1  -84 948
0.015  1  -84 032
```

0.02	1	−83 650
0.03	1	−82 242
0.04	1	−80 807
0.05	1	−79 521
0.08	1	−75 749
0.1	1	−73 176
0.15	1	−67 174
0.2	1	−61 352
0.3	1	−50 672
0.4	1	−40 985
0.5	1	−32 113
0.6	1	−23 912
0.8	1	−9456
1	1	2748.6
1.2	1	13 305
1.5	1	26 469
2	1	43 348
3	1	65 343
4	1	78 574
5	1	86 957
6	1	92 510
8	1	99 055
10	1	102 590
12	1	104 860
15	1	107 170
18	1	108 740
20	1	109 650
25	1	110 960
30	1	111 770
0.001	2	46 494
0.01	2	46 173
0.015	2	45 869
0.02	2	45 602
0.03	2	45 048
0.04	2	44 657
0.05	2	44 170
0.08	2	42 768
0.1	2	41 933
0.15	2	39 852
0.2	2	38 011
0.3	2	34 822
0.4	2	32 110
0.5	2	29 993
0.6	2	28 320
0.8	2	25 970

```
1      2  24 681
1.2    2  24 185
1.5    2  24 431
2      2  26 281
3      2  31 370
4      2  35 783
5      2  38 993
6      2  41 253
8      2  44 010
10     2  45 841
12     2  46 447
15     2  47 494
18     2  48 352
20     2  48 792
25     2  49 330
30     2  49 584
```

```
Clear[T1t, T1c, taut, tauc, M0t, M0c, Mtinf, Mcinf, tau1t,
 tau1c, lam1, lam2, c1, c2, Mct, Mtt, Keq, kct, ktc, param1, NLM1]
```

Users : Enter your values for Mcinf and Mtinf below :

```
Mcinf := 49 584
```

```
Mtinf := 111 770
```

```
taut := ((tauc) * (Mtinf)) / (Mcinf)

kct := 1 / tauc

ktc := 1 / taut

Keq := (Mtinf) / (Mcinf)

tau1c := ((T1c) * (tauc)) / ((tauc) + (T1c))

tau1t := ((T1t) * (taut)) / ((taut) + (T1t))

lam1 :=
 (0.5) * (- (((1) / (tau1c)) + ((1) / (tau1t))) + ((((((1) / (tau1c)) + ((1) / (tau1t)))^(2)) - (4) *
          ((((1) / (tau1c)) * ((1) / (tau1t))) - (((1) / (tauc)) * ((1) / (taut))))))^(0.5))

lam2 :=
 (0.5) * (- (((1) / (tau1c)) + ((1) / (tau1t))) - ((((((1) / (tau1c)) + ((1) / (tau1t)))^(2)) - (4) *
          (((((1) / (tau1c))) * (((1) / (tau1t)))) - (((1) / (tauc)) * ((1) / (taut))))))^(0.5))

c2 := ((1) / ((taut) * ((lam1) - (lam2)))) *
  (((taut) * ((lam1) + (((1) / (tau1c))))) * ((M0c) - (Mcinf))) + ((Mtinf) - (M0t)))

c1 := M0c - Mcinf - c2

Mtt := ((c1) * (taut) * ((lam1) + (((1) / (tau1c))))) * (Exp[(lam1) * (x)])) +
  ((c2) * (taut) * ((lam2) + (((1) / (tau1c))))) * (Exp[(lam2) * (x)])) + Mtinf

Mct := ((c1) * (Exp[(lam1) * (x)])) + ((c2) * (Exp[(lam2) * (x)])) + (Mcinf)

CombinedMctMtt :=
 Which[MDL == 1, Evaluate@Mtt,
    MDL == 2, Evaluate@Mct,
    True,   0];

taut

tau1c

tau1t

1 / tau1c

1 / tau1t
```

$$\frac{55\,885\ \text{tauc}}{24\,792}$$

$$\frac{\text{T1c tauc}}{\text{T1c} + \text{tauc}}$$

$$\frac{55\,885\ \text{T1t}\ \text{tauc}}{24\,792\ \left(\text{T1t} + \frac{55\,885\ \text{tauc}}{24\,792}\right)}$$

$$\frac{\text{T1c} + \text{tauc}}{\text{T1c}\ \text{tauc}}$$

$$\frac{24\,792\ \left(\text{T1t} + \frac{55\,885\ \text{tauc}}{24\,792}\right)}{55\,885\ \text{T1t}\ \text{tauc}}$$

Users : You may want to adjust the best guesses for the parameters, the upper and lower limits, and the number of interations below :

```
NLM1 = NonlinearModelFit[CombinedData, CombinedMctMtt,
  {{T1c, 1.4, 0.5, 5}, {T1t, 2, 0.1, 10}, {tauc, 1, 0.1, 100}, {M0c, 50 000, 10 000, 80 000},
   {M0t, -90 000, -200 000, -10 000}}, {x, MDL}, MaxIterations → 1 000 000]
```

NonlinearModelFit::lstol :

The line search decreased the step size to within tolerance specified by AccuracyGoal and PrecisionGoal but was unable to find a sufficient decrease in the norm of the residual. You may need more than MachinePrecision digits of working precision to meet these tolerances. ≫

```
FittedModel[ Which[MDL == 1, ≪4≫, 0] ]
```

```
NLM1[{"ParameterTable", "RSquared"}]
```

FittedModel::constr :

The property values {ParameterTable} assume an unconstrained model. The results for these properties may not be valid, particularly if the fitted parameters are near a constraint boundary. ≫

{

	Estimate	Standard Error	t Statistic	P-Value
T1c	4.43998	0.684587	6.48563	1.99594×10^{-8}
T1t	2.50305	0.0625134	40.0402	1.78701×10^{-44}
tauc	1.72364	0.0969296	17.7824	2.23594×10^{-25}
M0c	46 957.8	611.354	76.8095	7.76098×10^{-61}
M0t	−85 207.4	581.78	−146.46	2.772×10^{-77}

, 0.99921}

```
param1 = NLM1["BestFitParameters"]
```

{T1c → 4.43998, T1t → 2.50305, tauc → 1.72364, M0c → 46 957.8, M0t → −85 207.4}

```
T1c = T1c /. param1
T1t = T1t /. param1
tauc = tauc /. param1
M0t = M0t /. param1
M0c = M0c /. param1
```

```
4.43998
```

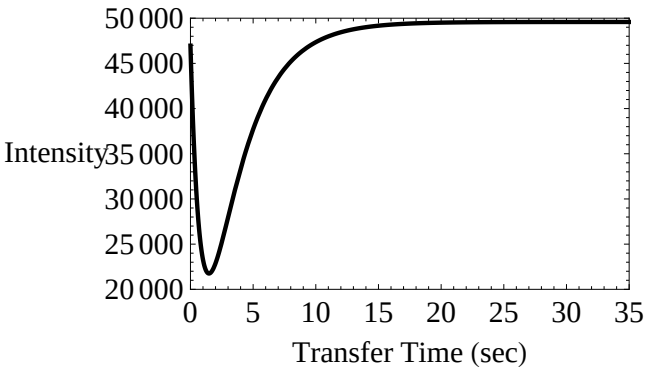
```
2.50305
```

```
1.72364
```

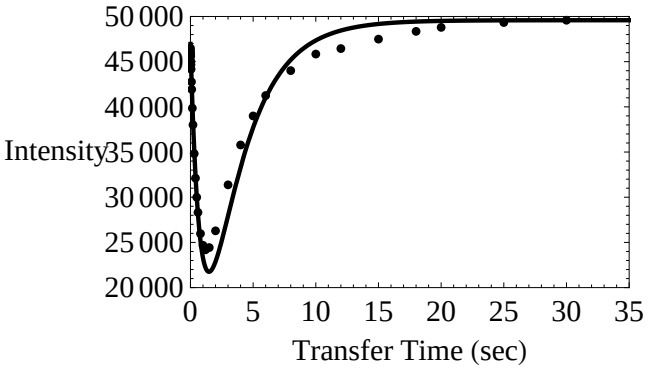
```
-85 207.4
```

```
46 957.8
```

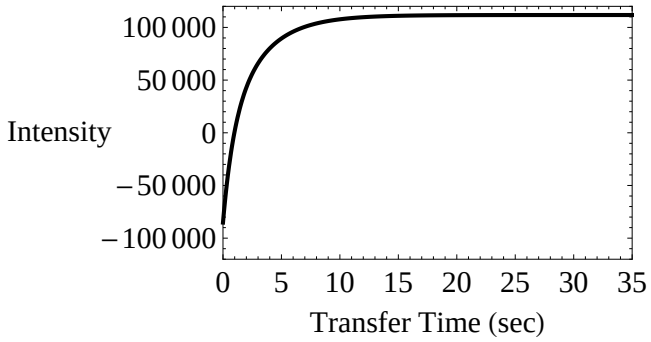
```
Plot[Mct, {x, 0, 35}, PlotStyle → {Thickness[0.01], Black},
 PlotRange → {{0, 35}, {20 000, 50 000}}, Frame → True,
 RotateLabel → False, BaseStyle → {14, FontFamily → "Times"},
 FrameLabel → {"Transfer Time (sec)", "Intensity"}, Axes → False]
```



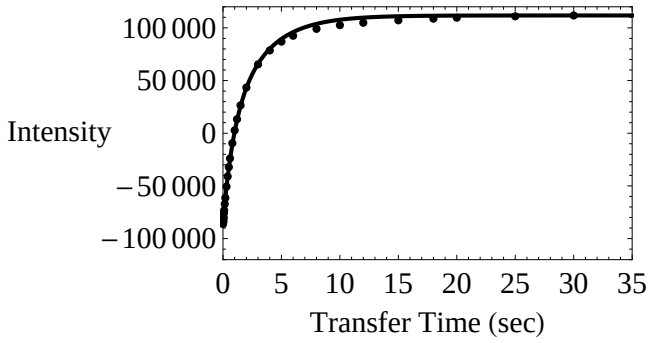

```
Show[%, lp2]
```



```
Plot[Mtt, {x, 0, 35}, PlotStyle → {Thickness[0.01], Black},
 PlotRange → {{0, 35}, {-120 000, 120 000}}, Frame → True,
 RotateLabel → False, BaseStyle → {14, FontFamily → "Times"},
 FrameLabel → {"Transfer Time (sec)", "Intensity"}, Axes → False]
```




```
Show[%, lp3]
```



Following are the rate constants in sec-1 and the equilibrium constant:

```
kct
```

```
0.580168
```

```
ktc
```

```
0.257377
```

```
Keq
```

$$\frac{55\,885}{24\,792}$$

The next line calculates points from the theoretical curve in case you want to graph the curve in another application:

```
theorMtt = Table[{x, Mtt}, {x, 0, 15, 0.01}]
```

{{0., −85 207.4}, {0.01, −83 934.3}, {0.02, −82 672.3}, {0.03, −81 421.3}, {0.04, −80 181.1},
{0.05, −78 951.8}, {0.06, −77 733.1}, {0.07, −76 524.9}, {0.08, −75 327.2}, {0.09, −74 139.8},
{0.1, −72 962.7}, {0.11, −71 795.6}, {0.12, −70 638.6}, {0.13, −69 491.5}, {0.14, −68 354.3},
{0.15, −67 226.7}, {0.16, −66 108.8}, {0.17, −65 000.4}, {0.18, −63 901.4}, {0.19, −62 811.7},
{0.2, −61 731.3}, {0.21, −60 660.}, {0.22, −59 597.8}, {0.23, −58 544.6}, {0.24, −57 500.2},
{0.25, −56 464.5}, {0.26, −55 437.6}, {0.27, −54 419.3}, {0.28, −53 409.5},
{0.29, −52 408.1}, {0.3, −51 415.1}, {0.31, −50 430.4}, {0.32, −49 453.8}, {0.33, −48 485.3},
{0.34, −47 524.9}, {0.35, −46 572.3}, {0.36, −45 627.7}, {0.37, −44 690.8},
{0.38, −43 761.6}, {0.39, −42 840.}, {0.4, −41 926.}, {0.41, −41 019.5}, {0.42, −40 120.4},
{0.43, −39 228.6}, {0.44, −38 344.}, {0.45, −37 466.6}, {0.46, −36 596.4}, {0.47, −35 733.2},
{0.48, −34 876.9}, {0.49, −34 027.6}, {0.5, −33 185.}, {0.51, −32 349.3}, {0.52, −31 520.2},
{0.53, −30 697.8}, {0.54, −29 882.}, {0.55, −29 072.6}, {0.56, −28 269.7}, {0.57, −27 473.2},
{0.58, −26 683.}, {0.59, −25 899.}, {0.6, −25 121.2}, {0.61, −24 349.6}, {0.62, −23 584.},
{0.63, −22 824.5}, {0.64, −22 070.9}, {0.65, −21 323.1}, {0.66, −20 581.3},
{0.67, −19 845.2}, {0.68, −19 114.8}, {0.69, −18 390.1}, {0.7, −17 671.}, {0.71, −16 957.5},
{0.72, −16 249.4}, {0.73, −15 546.9}, {0.74, −14 849.7}, {0.75, −14 157.9},
{0.76, −13 471.3}, {0.77, −12 790.}, {0.78, −12 113.9}, {0.79, −11 443.}, {0.8, −10 777.1},
{0.81, −10 116.3}, {0.82, −9460.52}, {0.83, −8809.66}, {0.84, −8163.71}, {0.85, −7522.61},
{0.86, −6886.31}, {0.87, −6254.79}, {0.88, −5627.98}, {0.89, −5005.84}, {0.9, −4388.33},
{0.91, −3775.41}, {0.92, −3167.03}, {0.93, −2563.15}, {0.94, −1963.72}, {0.95, −1368.71},
{0.96, −778.073}, {0.97, −191.766}, {0.98, 390.251}, {0.99, 968.018}, {1., 1541.57},
{1.01, 2110.96}, {1.02, 2676.21}, {1.03, 3237.38}, {1.04, 3794.48}, {1.05, 4347.57},
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The next line exports a file containing the theoretical points just calculated.

```
Export["OHPro1.txt", theorMtt, "Table"]
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OHPro1.txt

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Export["OHPro2.txt", theorMct, "Table"]
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OHPro2.txt

Just to confirm, here are graphs of the theoretical points and the data.

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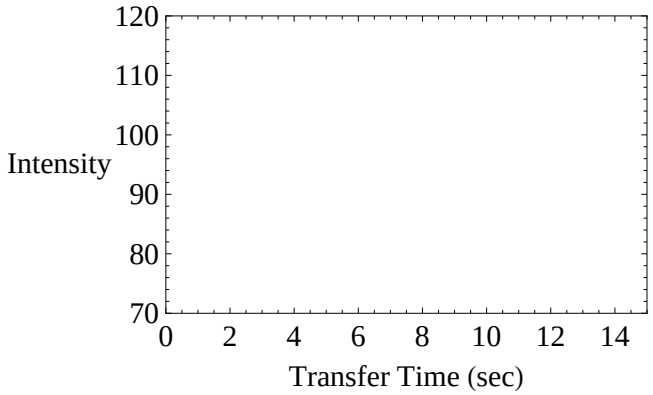

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Show[%, lp2]
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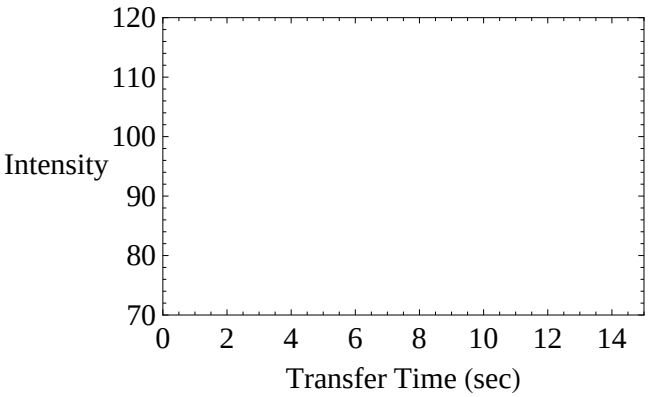


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Show[%, lp3]
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END

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