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Thioglycoside activation using bismuth(V) chemistry

by

Manibarsha Goswami

A dissertation submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Chemistry (Organic Chemistry)

Program of Study Committee:
Nicola L. B. Pohl, Co-major Professor
Jason S. Chen, Co-major Professor
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Iowa State University
Ames, Iowa
2014

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"*The true sign of intelligence is not knowledge but imagination."*– Albert Einstein

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LIST OF ABBREVIATIONS

Ac	Acetyl
AcOH	Acetic acid
All	Allyl
BAIB	Bisacetoxyiodobenzene
$BF_3 \bullet OEt_2$	Boron trifluoride diethyletherate
Bn	Benzyl
Bu_4N	*N,N,N,N,*-Tetrabutylammonium
Bz	Benzoyl
C_6F_5	Pentafluorobenzene
CH_3	Methyl
CH_2Cl_2	Dichloromethane
ClO_4	Perchlorate
COSY	Correlation spectroscopy
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM	Dichloromethane
DCE	Dichloroethane
Dec	Decomposed
DNA	Deoxyribonucleic acid
DOSY	Diffusion-ordered spectroscopy
DMF	*N, N,*-Dimethylformamide
EIC	Extracted Ion Chromatogram
ESI	Electrospray ionization
Et-O-Et	Diethylether
Et-O-Ac	Ethyl acetate
Fuc	Fucose
FSPE	Fluorous solid-phase extraction
F-tag	Fluorous tag
Gal	Galactose
GCMS	Gas chromatography mass spectrometry
Glc	Glucose
HMBC	Hetero multi-bond correlation spectroscopy
HPLC	High-performance liquid chromatography
HRMS	High resolution mass spectrometry
HSQC	Heteronuclear single-quantum correlation spectroscopy
MALDI-TOF	Matrix-assisted laser desorption ionization-Time of flight
Man	Mannose
Me	Methyl
MeCN	Acetonitrile
MeOH	Methanol
MeOTf	Methyl trifluoromethanesulfonate
m/z	Mass/charge ratio
N_3^-	Azide

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NaH	Sodium hydride
NBS	*N*-bromosuccinimide
NIS	*N*-iodosuccinimide
NMR	Nuclear magnetic resonance
NOE	Nuclear Overhauser effect
NOESY	Nuclear Overhauser enhancement spectroscopy
PF_6^-	Hexafluorophosphate
Ph	Phenyl
Phth	Phthalimido
Pr	Propyl
PrSH	Propanethiol
QTOF	Quadrupole time-of-flight mass spectrometer
ROESY	Rotating frame Overhauser effect spectroscopy
SPE	Solid phase extraction
TEA	Triethylamine
TEMPO	2,2,6,6-Tetramethylpiperidinyloxy
TFA	Trifluoroacetic acid
TfO^-	Trifluoromethanesulfonate
TfOH	Trifluoromethanesulfonic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMG	1,1,3,3-Tetramethylguanidine
TMS	Trimethylsilyl
Tol	Toluene
TOCSY	Total correlation spectroscopy
Tr	Trimethylphenyl

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ABSTRACT

Carbohydrates are considered important biomolecules and are the most efficient sources of energy, which is the reason they are known as the "fuel of life." Apart from nutritional functions, they play significant roles in various cell-cell recognition and differentiation, inflammatory responses, pathogen invasion, etc. To understand their functions better, chemical syntheses of these crucial molecules have been attempted but scientists over the years have found this task to be challenging. Various worthwhile iterative methods/protocols have been developed and a range of glycosyl donors have been utilized to build oligosaccharides. Chapter 1 is a review on thioglycosides as important glycosyl donors in these processes. The chapter enlists common methods for their synthesis as well as accounts the available methods for their activation along with advantages/limitations.

In lieu of the existing limitations in thioglycoside activation, a simple and efficient methodology was developed for activating thiopropylglycosides utilizing a unique Bi(V) containing promoter. Chapter 2 discusses the successes and failures in synthetic trials towards the discovery of this promoter. A variety of glycosyl donors containing different protecting groups could be coupled to simple and complex glycosyl acceptors in high to excellent yields. The method does not require low temperatures, or additional additives/co-promoters and tolerates different functional groups including alkenes.

After the success of the developed method with alkene-containing compounds, it was applied to the acceptors containing alkynes. Chapter 3 describes how this strategy

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was utilized towards the synthesis of a particular alkynyl D-mannose analogue that was then coupled to a class of pentablock copolymers via Huisgen cycloaddition and quantified by various methods. The Mallapragada group has developed these copolymers as potential vaccine adjuvants candidates. Addition of carbohydrates like mannose to these adjuvants has proved helpful towards building protective immunity against viruses.

The bismuth-mediated thioglycoside activation protocol is one of the first demonstrations of Bi(V) in carbohydrate chemistry and to our knowledge, is also one of the first reports of using less than stoichiometric amounts of promoter in thioglycoside activation. These interesting features led to us to study this reaction in depth, with a vision to find possible ways to further improve the activation (make it catalytic), fasten reaction times, and also induce stereoselectivity in products. Chapter 4 constitutes a detailed mechanistic investigation of the activation with a variety of benchtop and analytical tools like 1D & 2D-NMR, GCMS, kinetics modeling etc. Role of various reactants on the rate of the reaction were studied as well as different by-product analyses were done. While studying the kinetics, an unprecedented isomerization was also discovered. This observation was utilized to improve reaction times and implement better diastereoselectivity in products. The hypothesis behind developing the activation protocol was based on the thiophilicity of bismuth and this was successfully probed as an in situ bismuth-sulfonium species could be detected by various NMR methods.

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CHAPTER 1. A REVIEW ON THIOGLYCOSIDES AS IMPORTANT GLYCOSYL DONORS

1.1. INTRODUCTION

1.1.1. Oligosaccharides

Carbohydrates are the most abundant group of natural products found in nature, comprising both plant and animal sources. In the past two decades, oligosaccharides, a class of carbohydrates has gained considerable interest in the field of glycoscience and its applications.[1] Alongside, the synthetic difficulties associated with the building of the oligosaccharides from monosaccharides have also come to the forefront.[2] The synthesis of oligosaccharides involves the joining of two saccharide units: a glycosyl donor and a glycosyl acceptor, by a process known as chemical glycosylation[3] (**Scheme 1**). This step is generally done in the presence of a promoter/activator or a catalyst and is considered to be the most crucial step in any carbohydrate synthesis. However, building these complex molecules can be very difficult and time-consuming. An ideal strategy would then be to automate iterative oligosaccharide synthesis,[4] as done in peptide or nucleic-acid building block synthesis. So far, excellent automated methods comprising; solid-phase,[5] solution-phase,[6] HPLC-assisted,[7] etc. have been developed for making a number of oligosaccharide libraries. The Pohl group has developed their own unique automated solution-phase synthesis technique, which utilizes fluorous tag chemistry.[6b, 8] Among the various advantages of solution-phase over solid-phase strategies, the most significant ones in carbohydrate synthesis are: no requirement of excess reagents, or sugar building-blocks, ability to monitor progress of reaction and product identity as well as

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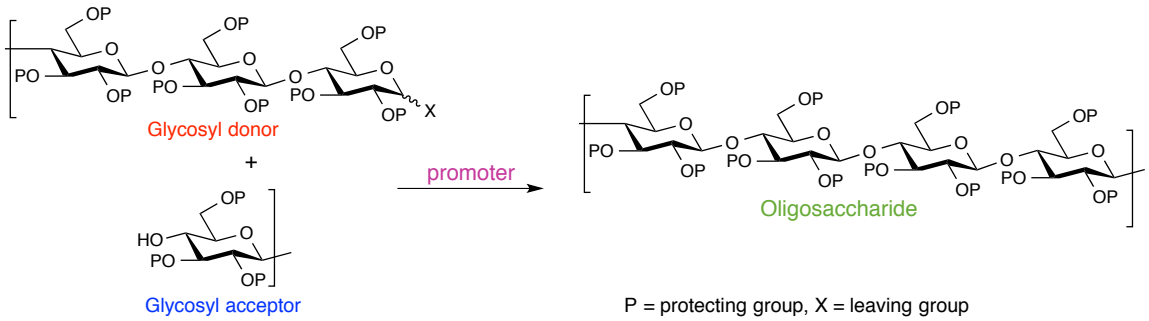
purity by various chromatographic/spectroscopic methods during a multi-step synthesis. Further, the use of fluorous-tags makes the Pohl method distinctive as it helps in efficient purification as well as to immobilize carbohydrates on a fluorous microarray[9] platforms.

In all of the above-mentioned methods, different classes of glycosyl donors have been used to build the desired oligosaccharides. Traditionally, the halide donors were used for the preparation of oligosaccharides, but they suffered from distinct disadvantages: strong acidic conditions were required to prepare these donors and they were typically activated by stoichiometric amounts of heavy metal salts like Ag, Hg, etc., which can be expensive and/or toxic. Since their discovery by Schmidt and co-workers, trichloroacetimidate donors have become very popular.[3c] Although these donors have proved to be better than the halide donors, they also suffer limitations. They are extremely labile and have to be prepared more or less just prior to a glycosylation. Hence, they cannot be stored for a long time and also are not suitable for orthogonal activation in presence of other donors. As such, their use in iterative automated solution- or solid- phase protocols become very tricky and hence less useful at times.



Scheme 1. *General scheme of chemical glycosylation*

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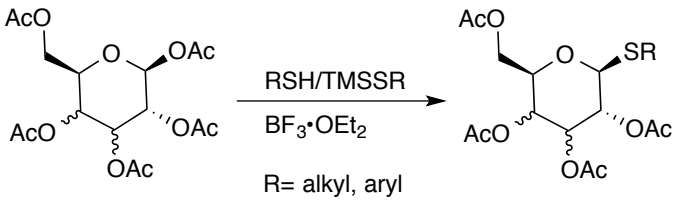
1.1.2. Thioglycosides: synthesis, advantages

Because of the aforementioned disadvantages with other glycosyl donors,[3c] thioglycosides[10] have become more widely used donors. Thioglycosides were discovered around a century ago in 1910 by Fischer and Delbrück.[11] Since then, these donors have been prepared by a variety of methods, which are mostly based on nucleophilic substitutions at the anomeric center. Thioglycosides can be obtained from mercaptolysis of different glycosyl halides,[12] or trichloroacetimidates, etc., from ring-opening of 1,2-anhydrosugars, or by alkylations of 1-thiosugars. Another common procedure to make thioglycosides is the Lewis acid-catalyzed reaction of thiols[13] or trimethylsilyl (TMS) ethers of thiols[14] with glycosyl acetates (**Scheme 2**), especially to prepare a 1,2-*trans* relationship.



***Scheme 2**: Example of Lewis-acid promoted thioglycoside formation*

Apart from their easy preparation, thioglycosyl donors offer distinct advantages.[3b, 10c] They have remarkable stability as they have long shelf lives and remain intact in various functional group manipulations. Additionally, they are stable in several glycosylation conditions, thereby offering orthogonality in their activation in the presence of other glycosyl donors.[10c] This feature also facilitates their use as potential glycosyl acceptors amongst different sugar building blocks, which later can be used as

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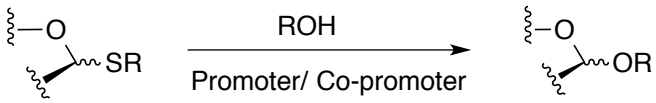
donors. Moreover, thioglycosides can be easily converted to other glycosyl donors. Overall, these attributes make thioglycoside donors to be the most versatile and popular donor in oligosaccharide synthesis.

1.1.3. Thioglycoside activation: promoters, applications, and limitations

Although thioglycosides were discovered a long time ago, their use in glycosylation as glycosyl donors was done only a few decades ago. Since then, a wide variety of promoters have been developed for their activation (**Scheme 3**) and are listed as follows:



Scheme 3: *General scheme of thioglycoside activation*

1.1.3.1. Metal-based promoters:

Ferrier *et al.* explored the well known high-affinity of mercury(II) for sulfide and became the first research group to activate thioglycosides using heavy metal salts like mercuric sulfate ($HgSO_4$) and mercuric acetate [$Hg(OAc)_2$].[15] In later years, various other mercury salts like $HgCl_2$, PhHgOTf,[16] $Hg(OBz)_2$,[17] etc. were used as promoters, however all of these failed to give high yielding glycosylations. Another way of activating thiopyridinyl donors was to convert them to in situ glycosyl halides by the use of reagents like $(Bu_4N)_2CuBr_4$. Addition of glycosyl acceptor and AgOTf then produces the desired glycoside,[18] although this method was not useful with the common thioaryl or alkylglycosides.

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Apart from low yielding activations, few other disadvantages exist with this class of promoters. Most of these metals like Hg, Pb, etc. are extremely toxic in nature and are not preferred for use with potential biologically-active substrates. Furthermore, using these in presence of thioglycosides and glycosyl halide donors can activate both to form undesired products.

1.1.3.2. Alkylating promoters:

Soon after the discovery of metal containing promoters, Lönn used MeOTf[19] to successfully activate alkyl and aryl thioglycosyl donors. Although fast and effective, this method has several disadvantages. MeOTf is toxic, difficult to handle and in the presence of unreactive donors, it can alkylate free hydroxyl groups in acceptors instead of forming the desired glycosides. A much milder promoter, methyl iodide (MeI),[20] was reported with activation of 2-pyridyl thioglycosides during the same time. But this reagent was found to be very slow in activating any other alkyl or aryl thioglycosides.

1.1.3.3. Halonium-based promoters:

Currently, methods employing halonium-based reagents in thioglycoside glycosylation are perhaps the most popular ones. The earliest example of such a promoter was *N*-bromosuccinamide (NBS)[21] used by Nicolaou and group to activate phenyl thioglycosides. This mild bromonium source was used effectively to couple various simple to complex acceptors. During the years of 1990-1991, van Boom and colleagues discovered some of the more efficient halonium promoters. These were

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based on "soft" iodonium species, and iodonium dicollidine perchlorate (IDCP)[22] was the first of its kind to be used to couple different alkyl thio-rhamnosyl and fucosyl donors. This was followed shortly by iodonium dicollidine triflate (IDCTf),[23] which was less toxic than IDCP. In the same year, another important contribution by Veeneman & Van Boom was the use of stoichiometric amounts of *N*-iodosuccinamide (NIS) in presence of catalytic triflic acid (TfOH)[24] to give very fast thioglycoside activations. This combination of reagents was also discovered by the Fraser-Reid group independently in the same year[25] to activate *n*-pentenyl donors. Since then, many other methods utilizing excess or stoichiometric amounts of NBS or NIS with catalytic amounts of co-promoters for e.g. NBS/TfOH[26] or Ph_2IOTf, Bu_4NOTf, or Bu_4NClO_4[27] or $Bi(OTf)_3$,[28] NIS/TMSOTf or AgOTf or $TrB(C_6F_5)_4$[29] or $Yb(OTf)_3$[30] etc. have been developed. To resolve some of the undesired products issues, another reagent *N*-iodosaccharin (NISac)[31] was discovered. Other halonium sources like, I_2 or ICl or IBr in presence of co-promoters like AgOTf[32] and PhIO in presence of $Sn(OTf)_2$ or $SnCl_2$-$AgClO_4$,[33] etc. have also been successfully explored to activate ethyl, phenyl thioglycosides as well as ethyl *S*-glycosyl xanthates. Bennett *et al*. have also recently developed a new air- and water-stable iodonium salt phenyl(trifluoroethyl)iodonium triflimide (IPTIT)[34] which activates thioglycosides at room temperature.

Although these methods are efficient, several disadvantages are still encountered while performing glycosylations. Most of them need excess reagents or additives/co-promoters to form reactive intermediates and as such reagent handling as well as storage of these intermediates pose a problem. Some of the popular activators

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like NIS/TfOH, TMSOTf, etc. are mostly used at very low temperatures (<–20 °C), so as to control reactivity or formation of unwanted side-products. Another limitation of NIS or NBS kind of reagents is that the high nucleophilicity of the counterion can lead to various *N*-succinimide products,[35] especially with unreactive acceptors. In addition, the presence of "soft" nucleophilic centers like alkenes in donors or acceptors has been a concern for these classes of promoters. Even though controlled activations[36] have been performed, they still form various addition products or undesired cyclic intermediates as seen with some *O*-allyl containing glycosyl acceptors.[37] This further restricts their use in presence of the *n*-pentenyl glycosyl donors,[38] that are another important class of donors frequently encountered in oligosaccharide synthesis.

1.1.3.4. Sulfonium, selenium-based promoters:

Organosulfur-based reagents are also commonly used with thioglycosides. Fügedi and group reported one of the first sulfonium promoter; Dimethyl(thiomethyl)sulfonium triflate (DMTST)[39] to activate alkyl and phenyl thioglycosides to form various disaccharides. Other similar alkylsulfenylating agents such as methylsulfenyl triflate (MeSOTf),[40] methylsulfenyl bromide (MeSBr),[41] phenylsulfenyl triflate (PhSOTf)[42] and *N*-ethylsulfenyl phthalimide (PhthNSEt)[43] in combination with Lewis acid $TrB(C_6F_5)_4$ have been also used. More recently, powerful sulfur reagents in the presence of Tf_2O like, *N*-(phenylthio)-ε-caprolactam/Tf_2O,[44] benzenesulfinylpiperidine-triflic anhydride (BSP)/ Tf_2O,[45] diphenyl sulfoxide (Ph_2SO/Tf_2O),[46] S-(4-methoxyphenyl)benzenethiosulfinate (MPBT)/Tf_2O[47] have replaced

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the previous generation of promoters used to couple complex sugars like sialic acids and hyaluronic acid oligomers. Another cheap and efficient promoter; dimethyl disulfide-triflic anhydride (Me_2S/Tf_2O)[48] was recently developed for alkylthioglycosides.

Similar to the sulfenyl reagents, arylselenylating reagents have also become quite prevalent, for e.g. 1-benzeneselenyl triflate,[49] *N*-phenylselenylphthalimide (PhSeNPhth)-TMSOTf[50] or $MgClO_4$[51] have been used too. Recently, Zhu et al. used thioperoxide in combination with TMSOTf[52] as a powerful thiophilic promoter system, capable of activating different thioglycosides.

1.1.3.5. Single electron transfer promoters:

Electron transfer reagents like tris(4-bromophenyl)ammoniumyl hexachloroantimonate (TBPA)[53] and nitrosyl tetrafluoroborate ($NOBF_4$)[54] were discovered in early 1990s to activate thioglycosyl donors. Another example is the $TrB(C_6F_5)$-$NaIO_4$[55] promoter which has also been studied. However, single electron activation of thioglycosides still is relatively unexplored, probably since these reagents are generally not very selective and can give low yielding glycosylations. A most recent method applies single electron transfer using visible light and ruthenium or iridium-containing catalysts[56] to activate thioglycosides.

1.1.3.6. Other methods:

$AgPF_6$[57] and electrochemical oxidation[58] has been reported separately to activate thioglycosides too.

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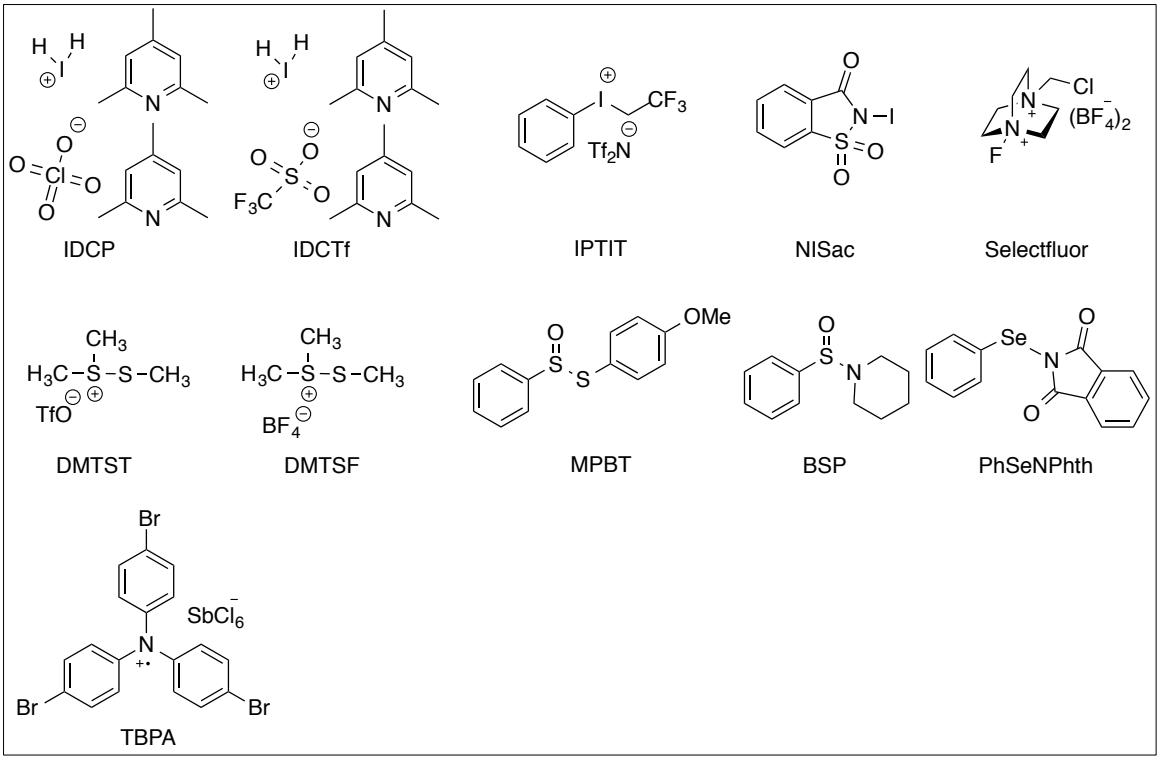

***Scheme 4**: Structures of some thioglycoside promoters*

1.1.4. Other S-containing glycosyl donors:

Apart from alkyl or aryl thioether-type glycosides, many other thio-containing glycosyl donors have been popular, for e.g. glycosyl sulfoxides, sulfones, thioimidates, etc.[3, 10b] Since their discovery, various methods for their activation have been developed. Moreover, orthogonal glycosylations of these donors in presence of thioglycosides have also been well studied and utilized in carbohydrate synthesis.

1.2. CONCLUSION

In summary, in the past decade, numerous new methods for the preparation and activation of thioglycosides have been reported. As thioglycosides are among the most versatile donors, they will continue to play an important role in oligosaccharide

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synthesis. Although the field of thioglycoside activation has progressed leaps and bounds in the last 25-30 years, there are still limitations that interfere in efficient glycosylation with these donors. These issues with solubility, undesired by-products, stability or reagent handling, co-activation of other glycosyl donors are particularly problematic in the context of the development of robust automated protocols for oligosaccharide synthesis and hence need to be dealt with.

1.3. REFERENCES

1. (a) DeMarco, M. L.; Woods, R. J., Structural glycobiology: A game of snakes and ladders. *Glycobiology* **2008,** *18* (6), 426-440; (b) Koeller, K. M.; Wong, C.-H., Emerging themes in medicinal glycoscience. *Nat. Biotechnol.* **2000,** *18* (8), 835; (c) Merry, A. H.; Merry, C. L. R., *Glycoscience finally comes of age*. 2005; Vol. 6, p 900-903; (d) Woods, R. J.; Tessier, M. B., Computational glycoscience: characterizing the spatial and temporal properties of glycans and glycan–protein complexes. *Curr. Opin. Struct. Biol.* **2010,** *20* (5), 575-583.

2. (a) Boons, G.-J., Strategies in Oligosaccharide Synthesis. *Tetrahedron* **1996,** *52* (4), 1095-1121; (b) Davis, B. G., Recent developments in oligosaccharide synthesis. *J. Chem. Soc., Perkin Trans. 1* **2000,** (14), 2137-2160; (c) Garegg, P. J., Synthesis and reactions of glycosides. In *Adv. Carbohydr. Chem. Biochem.*, Academic Press: 2004; Vol. Volume 59, pp 69-134; (d) Paulsen, H., Haworth Memorial Lecture. Synthesis of complex oligosaccharide chains of glycoproteins. *Chem. Soc. Rev.* **1984,** *13* (1), 15-45.

3. (a) Demchenko, A., *Handbook of Chemical Glycosylation*. Wiley- VCH: Weinhem, 2008; (b) Fügedi, P., *The Organic Chemistry of Sugars*. CRC: Boca Raton, FL, 2005; (c) Zhu, X.; Schmidt, R. R., New Principles for Glycoside-Bond Formation. *Angew. Chem., Int. Ed.* **2009,** *48* (11), 1900-1934.

4. (a) Zhang, Z.; Ollmann, I. R.; Ye, X.-S.; Wischnat, R.; Baasov, T.; Wong, C.-H., Programmable One-Pot Oligosaccharide Synthesis. *J. Am. Chem. Soc.* **1999,** *121* (4), 734-753; (b) Hsu, C.-H.; Hung, S.-C.; Wu, C.-Y.; Wong, C.-H., Toward Automated Oligosaccharide Synthesis. *Angew. Chem., Int. Ed.* **2011,** *50* (50), 11872-11923.

5. Seeberger, P. H., Automated oligosaccharide synthesis. *Chem. Soc. Rev.* **2008,** *37* (1), 19-28.

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6. (a) Nicola, L. P., Automated Solution-Phase Oligosaccharide Synthesis and Carbohydrate Microarrays: Development of Fluorous-Based Tools for Glycomics. In *Chemical Glycobiology*, American Chemical Society: 2008; Vol. 990, pp 272-287; (b) Jaipuri, F. A.; Pohl, N. L., Toward solution-phase automated iterative synthesis: fluorous-tag assisted solution-phase synthesis of linear and branched mannose oligomers. *Org. Biomol. Chem.* **2008,** *6* (15), 2686-2691; (c) Weinbrenner, S.; Tzschucke, C. C., Purification Principles in High-Speed Solution-Phase Synthesis. In *Combinatorial Chemistry*, Wiley-VCH Verlag GmbH & Co. KGaA: 2006; pp 1-31.

7. Ganesh, N. V.; Fujikawa, K.; Tan, Y. H.; Stine, K. J.; Demchenko, A. V., HPLC-Assisted Automated Oligosaccharide Synthesis. *Org. Lett.* **2012,** *14* (12), 3036-3039.

8. (a) Park, G.; Ko, K.-S.; Zakharova, A.; Pohl, N. L., Mono- vs. di-fluorous-tagged glucosamines for iterative oligosaccharide synthesis. *J. Fluorine Chem.* **2008,** *129* (10), 978-982; (b) Studer, A.; Hadida, S., Fluorous synthesis: A fluorous-phase strategy for improving separation efficiency in organic synthes. *Science* **1997,** *275* (5301), 823-826; (c) Zhang, W., Fluorous Linker-Facilitated Chemical Synthesis. *Chem. Rev. (Washington, DC, U. S.)* **2009,** *109* (2), 749-795.

9. (a) Collet, B. Y. M.; Nagashima, T.; Yu, M. S.; Pohl, N. L. B., Fluorous-based peptide microarrays for protease screening. *J. Fluorine Chem.* **2009,** *130* (11), 1042-1048; (b) Mamidyala, S. K.; Ko, K.-S.; Jaipuri, F. A.; Park, G.; Pohl, N. L., Noncovalent fluorous interactions for the synthesis of carbohydrate microarrays. *J. Fluorine Chem.* **2006,** *127* (4–5), 571-579.

10. (a) Codee, J. D. C.; Litjens, R. E. J. N.; van den Bos, L. J.; Overkleeft, H. S.; van der Marel, G. A., Thioglycosides in sequential glycosylation strategies. *Chem. Soc. Rev.* **2005,** *34* (9), 769-782; (b) Garegg, P. J., Thioglycosides as Glycosyl Donors in Oligosaccharide Synthesis. In *Adv. Carbohydr. Chem. Biochem.*, Derek, H., Ed. Academic Press: 1997; Vol. Volume 52, pp 179-205; (c) Oscarson, S.; Ernst, B.; Hart, G. W.; Sinaý, P., Thioglycosides. In *Carbohydrates in Chemistry and Biology*, Wiley-VCH Verlag GmbH: 2008; pp 93-116.

11. Fischer, E.; Delbrück, K., Über Thiophenol-glucoside. *Berichte der deutschen chemischen Gesellschaft* **1909,** *42* (2), 1476-1482.

12. Blanc-Muesser, M.; Defaye, J.; Driguez, H., Syntheses stereoselectives de 1-thioglycosides. *Carbohydr. Res.* **1978,** *67* (2), 305-328.

13. Ferrier, R. J.; Furneaux, R. H., Synthesis of 1,2-trans-related 1-thioglycoside esters. *Carbohydr. Res.* **1976,** *52* (1), 63-68.

www.manaraa.com

14. Pozsgay, V.; Jennings, H. J., A new, stereoselective synthesis of methyl 1,2-trans-1-thioglycosides. *Tetrahedron Lett.* **1987,** *28* (13), 1375-1376.

15. Ferrier, R. J.; Hay, R. W.; Vethaviyasar, N.]A potentially versatile synthesis of glycosides. *Carbohydr. Res.* **1973,** *27* (1), 55-61.

16. Garegg, P. J.; Henrichson, C.; Norberg, T., A reinvestigation of glycosidation reactions using 1-thioglycosides as glycosyl donors and thiophilic cations as promoters. *Carbohydr. Res.* **1983,** *116* (1), 162-165.

17. Van Cleve, J. W., Reinvestigation of the preparation of cholesteryl 2,3,4,6-tetra-O-benzyl-α-D-glucopyranoside. *Carbohydr. Res.* **1979,** *70* (1), 161-164.

18. Sato, S.; Mori, M.; Ito, Y.; Ogawa, t., An efficient approach to O-glycosides through CuBr2-Bu4NBr mediated activation of glycosides. *Carbohydr. Res.* **1986,** *155* (0), C6-C10.

19. Lönn, H., Synthesis of a tri- and a hepta-saccharide which contain α-l-fucopyranosyl groups and are part of the complex type of carbohydrate moiety of glycoproteins. *Carbohydr. Res.* **1985,** *139* (0), 105-113.

20. Reddy, G. V.; Kulkarni, V. R.; Mereyala, H. B., A mild general method for the synthesis of ∝-linked disaccharides. *Tetrahedron Lett.* **1989,** *30* (32), 4283-4286.

21. Nicolaou, K. C.; Seitz, S. P.; Papahatjis, D. P., A mild and general method for the synthesis of 0-glycosides. *J. Am. Chem. Soc.* **1983,** *105* (8), 2430-2434.

22. Veeneman, G. H.; van Boom, J. H., An efficient thioglycoside-mediated formation of α-glycosidic linkages promoted by iodonium dicollidine perchlorate. *Tetrahedron Lett.* **1990,** *31* (2), 275-278.

23. Veeneman, G. H.; Van Leeuwen, S. H.; Zuurmond, H.; Van Boom, J. H., Synthesis of Carbohydrate-Antigenic Structures of Mycobacterium Tuberculosis using an Iodonium Ion Promoted Glycosidation Approach. *J. Carbohydr. Chem.* **1990,** *9* (6), 783-796.

24. Veeneman, G. H.; van Leeuwen, S. H.; van Boom, J. H., Iodonium ion promoted reactions at the anomeric centre. II An efficient thioglycoside mediated approach toward the formation of 1,2-trans linked glycosides and glycosidic esters. *Tetrahedron Lett.* **1990,** *31* (9), 1331-1334.

25. Konradsson, P.; Mootoo, D. R.; McDevitt, R. E.; Fraser-Reid, B., Iodonium ion generated in situ from N-iodosuccinimide and trifluoromethanesulphonic acid promotes direct linkage of 'disarmed' pent-4-enyl glycosides. *J. Chem. Soc., Chem. Commun.* **1990,** (3), 270-272.

www.manaraa.com

26. Sasaki, M.; Tachibana, K.; Nakanishi, H., An efficient and stereocontrolled synthesis of the nephritogenoside core structure. *Tetrahedron Lett.* **1991,** *32* (47), 6873-6876.

27. Fukase, K.; Hasuoka, A.; Kinoshita, I.; Aoki, Y.; Kusumoto, S., A stereoselective glycosidation using thioglycosides, activation by combination of N-bromosuccinimide and strong acid salts. *Tetrahedron* **1995,** *51* (17), 4923-4932.

28. Valerio, S.; Iadonisi, A.; Adinolfi, M.; Ravidà, A., Novel Approaches for the Synthesis and Activation of Thio- and Selenoglycoside Donors. *J. Org. Chem* **2007,** *72* (16), 6097-6106.

29. Takeuchi, K.; Tamura, T.; Mukaiyama, T., Stereoselective Glycosylation of Thioglycosides Promoted by Respective Combinations of N-Iodo- or N-Bromosuccinimide and Trityl Tetrakis(pentafluorophenyl)borate. Application to One-Pot Sequential Synthesis of Trisaccharide. *Chem. Lett.* **2000,** *29* (2), 124-125.

30. Chung, S.-K.; Park, K.-H., A novel approach to the stereoselective synthesis of β-d-mannopyranosides. *Tetrahedron Lett.* **2001,** *42* (24), 4005-4007.

31. Aloui, M.; Fairbanks, A. J., N-Iodosaccharin: A Potent New Activator of Thiophenylglycosides. *Synlett* **2001,** *2001* (06), 0797-0799.

32. Ercegovic, T.; Meijer, A.; Magnusson, G.; Ellervik, U., Iodine Monochloride/Silver Trifluoromethanesulfonate (ICl/AgOTf) as a Convenient Promoter System for O-Glycoside Synthesis. *Org. Lett.* **2001,** *3* (6), 913-915.

33. Fukase, K.; Kinoshita, I.; Kanoh, T.; Nakai, Y.; Hasuoka, A.; Kusumoto, S., A novel method for stereoselective glycosidation with thioglycosides: Promotion by hypervalent iodine reagents prepared from PhIO and various acids. *Tetrahedron* **1996,** *52* (11), 3897-3904.

34. Chu, A.-H. A.; Minciunescu, A.; Montanari, V.; Kumar, K.; Bennett, C. S., An Air- and Water-Stable Iodonium Salt Promoter for Facile Thioglycoside Activation. *Org. Lett.* **2014,** *16* (6), 1780-1782.

35. (a) Krog-Jensen, C.; Oscarson, S., Synthesis of d-Fructofuranosides Using Thioglycosides as Glycosyl Donors. *J. Org. Chem* **1996,** *61* (4), 1234-1238; (b) Oscarson, S.; Tedebark, U.; Turek, D., Synthesis of colitose-containing oligosaccharide structures found in polysaccharides from Vibrio cholerae O139 synonym Bengal using thioglycoside donors. *Carbohydr. Res.* **1997,** *299* (3), 159-164.

36. Zuurmond, H. M.; van der Laan, S. C.; van der Marel, G. A.; van Boom, J. H., Iodonium ion-assisted glycosylation of alkyl (aryl) 1-thio-glycosides: regulation of stereoselectivity and reactivity. *Carbohydr. Res.* **1991,** *215* (2), C1-C3.

www.manaraa.com

37. (a) Demchenko, A. V.; De Meo, C., Semi-orthogonality of O-pentenyl and S-ethyl glycosides: application for the oligosaccharide synthesis. *Tetrahedron Lett.* **2002,** *43* (49), 8819-8822; (b) López, J. C.; Gómez, A. M.; Uriel, C.; Fraser-Reid, B., Thioglycoside and trichloroacetimidate donors in regioselective glycosidations. Comparison with n-pentenyl glycosides. *Tetrahedron Lett.* **2003,** *44* (7), 1417-1420.

38. (a) Mootoo, D. R.; Date, V.; Fraser-Reid, B., n-Pentenyl glycosides permit the chemospecific liberation of the anomeric center. *J. Am. Chem. Soc.* **1988,** *110* (8), 2662-2663; (b) Gigg, J.; Gigg, R.; Payne, S.; Conant, R., The allyl group for protection in carbohydrate chemistry. Part 20. Synthesis of 1L-1-O-methyl-myo-inositol [(+)-bornesitol] by resolution of (+/-)-1,2,4-tri-O-benzyl-myo-inositol. *J. Chem. Soc., Perkin Trans. 1* **1987,** (0), 1757-1762.

39. Fügedi, P.; Garegg, P. J., A novel promoter for the efficient construction of 1,2-trans linkages in glycoside synthesis, using thioglycosides as glycosyl donors. *Carbohydr. Res.* **1986,** *149* (1), C9-C12.

40. Dasgupta, F.; Garegg, P. J., Alkyl sulfenyl triflate as activator in the thioglycoside-mediated formation of β-glycosidic linkages during oligosaccharide synthesis. *Carbohydr. Res.* **1988,** *177* (0), c13-c17.

41. Dasgupta, F.; Garegg, P. J., Use of the methylsulfenyl cation as an activator for glycosylation reactions with alkyl (aryl) l-thioglycopyranosides: synthesis of methyl O-(2-acetamido-2-deoxy- β -d-glucopyranosyl)-(1$\rightarrow$ 2)- α -d-glucopyranoside, a derivative of the core trisaccharide of E. coli K12. *Carbohydr. Res.* **1990,** *202* (0), 225-238.

42. Martichonok, V.; Whitesides, G. M., Stereoselective α-Sialylation with Sialyl Xanthate and Phenylsulfenyl Triflate as a Promotor. *J. Org. Chem* **1996,** *61* (5), 1702-1706.

43. Jona, H.; Takeuchi, K.; Saitoh, T.; Mukaiyama, T., Effective Activation of ‘Armed’ Thioglycoside with a New Combination of Trityl Tetrakis(pentafluorophenyl)borate [TrB(C6F5)4](Ethylthio)phthalimide (PhthNSEt). *Chem. Lett.* **2000,** *29* (10), 1178-1179.

44. Durón, S. G.; Polat, T.; Wong, C.-H., N-(Phenylthio)-ε-caprolactam: A New Promoter for the Activation of Thioglycosides. *Org. Lett.* **2004,** *6* (5), 839-841.

45. Crich, D.; Smith, M., 1-Benzenesulfinyl Piperidine/Trifluoromethanesulfonic Anhydride: A Potent Combination of Shelf-Stable Reagents for the Low-Temperature Conversion of Thioglycosides to Glycosyl Triflates and for the Formation of Diverse Glycosidic Linkages. *J. Am. Chem. Soc.* **2001,** *123* (37), 9015-9020.

www.manaraa.com

46. Codée, J. D. C.; Litjens, R. E. J. N.; den Heeten, R.; Overkleeft, H. S.; van Boom, J. H.; van der Marel, G. A., Ph2SO/Tf2O: a Powerful Promotor System in Chemoselective Glycosylations Using Thioglycosides. *Org. Lett.* **2003,** *5* (9), 1519-1522.

47. Crich, D.; Smith, M., S-(4-Methoxyphenyl) Benzenethiosulfinate (MPBT)/Trifluoromethanesulfonic Anhydride: A Convenient System for the Generation of Glycosyl Triflates from Thioglycosides. *Org. Lett.* **2000,** *2* (25), 4067-4069.

48. Tatai, J.; Fügedi, P., A New, Powerful Glycosylation Method: Activation of Thioglycosides with Dimethyl Disulfide–Triflic Anhydride. *Org. Lett.* **2007,** *9* (22), 4647-4650.

49. Ito, Y.; Ogawa, T., Benzeneselenenyl triflate as a promoter of thioglycosides : A new method for O-glycosylation using thioglycosides. *Tetrahedron Lett.* **1988,** *29* (9), 1061-1064.

50. Shimizu, H.; Ito, Y.; Ogawa, T., PhSeNPhth-TMSOTf as a Promotor of Thioglycoside. *Synlett* **1994,** *1994* (07), 535-536.

51. Fukase, K.; Nakai, Y.; Kanoh, T.; Kusumoto, S., Mild but Efficient Methods for Stereoselective Glycosylation with Thioglycosides: Activation by [N-Phenylselenophthalimide-Mg(ClO4)2] and [PhIO-Mg(ClO4)2]. *Synlett* **1998,** *1998* (01), 84-86.

52. He, H.; Zhu, X., Thioperoxide-Mediated Activation of Thioglycoside Donors. *Org. Lett.* **2014,** *16* (11), 3102-3105.

53. Marra, A.; Mallet, J.-M.; Amatore, C.; Sinaÿ, P., Glycosylation Using a One-Electron-Transfer Homogeneous Reagent: A Novel and Efficient Synthesis of β-Linked Disaccharides. *Synlett* **1990,** *1990* (10), 572-574.

54. Pozsgay, V.; Jennings, H. J., A new method for the synthesis of O-glycosides from S-glycosides. *J. Org. Chem* **1987,** *52* (20), 4635-4637.

55. Uchiro, H.; Mukaiyama, T., An Efficient Method for Catalytic and Stereoselective Glycosylation with Thioglycosides Promoted by Trityl Tetrakis(pentafluorophenyl)borate and Sodium Periodate. *Chem. Lett.* **1997,** *26* (2), 121-122.

56. Wever, W. J.; Cinelli, M. A.; Bowers, A. A., Visible Light Mediated Activation and O-Glycosylation of Thioglycosides. *Org. Lett.* **2012,** *15* (1), 30-33.

57. Lear, M. J.; Yoshimura, F.; Hirama, M., A Direct and Efficient α-Selective Glycosylation Protocol for the Kedarcidin Sugar, L-Mycarose: AgPF6 as a

www.manaraa.com

Remarkable Activator of 2-Deoxythioglycosides. *Angew. Chem., Int. Ed.* **2001,** *40* (5), 946-949.

58. Suzuki, S.; Matsumoto, K.; Kawamura, K.; Suga, S.; Yoshida, J.-i., Generation of Alkoxycarbenium Ion Pools from Thioacetals and Applications to Glycosylation Chemistry. *Org. Lett.* **2004,** *6* (21), 3755-3758.

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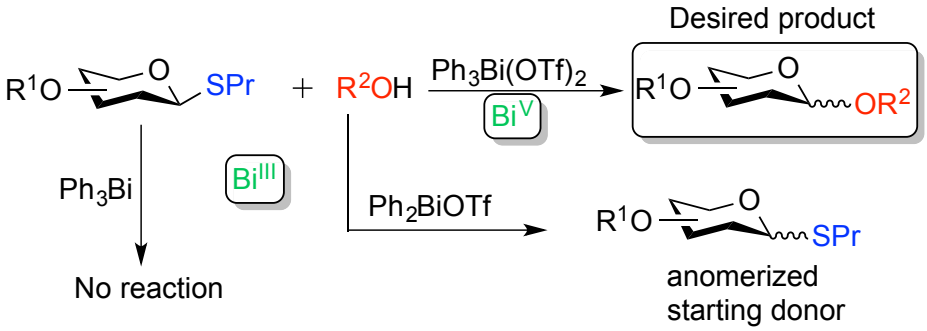
CHAPTER 2. BISMUTH(V)-MEDIATED THIOGLYCOSIDE ACTIVATION

Paper published in part in *Angewandte Chemie International Edition*[1]



Abstract

A straightforward method utilizing a bismuth(V) compound was developed for the activation of thiopropylglycosides for coupling to various acceptors; good to excellent yields were obtained without applying additional additives/co-promoters. The method does not require low temperatures, is applicable to a wide variety of carbohydrates, and tolerates different functional groups including alkenes.

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2.1. INTRODUCTION

In the past decade, main group metal and transition metal catalysis have become immensely popular in synthetic organic chemistry. Chemical glycosylation is also one of these synthetic areas, which has been actively explored too. A range of transition metals like Pd, Ni, Zn, Ti, Au etc. have been effectively used to activate various glycosyl donors like glycosyl trichloroacetimidate, orthoalkynyl benzoate, halide donors etc.[1] Unlike with the above donors, fewer examples of transition metals like Hg, Cu, etc. have been utilized in the area of thioglycosyl donors, although heavy-metal cation based promoters were among the first thioglycoside activation methods to be discovered.[2] When it comes to main-group metals, even fewer examples can be found with thioglycosides. In lieu of the available heavy metal cation-based promoters, bismuth presents interesting possibilities. Bismuth is a post transition metal and like its neighboring metals such as Hg and Pb—is considered thiophilic[3] as well as soft Lewis acidic.[4] In nature, it occurs in various forms which suggests its thiophilicity; from simple ores like bismuthinite (Bi_2S_3) to complex minerals like matildite ($AgBiS_2$), tetradymite (Bi_2TeS), galenobismutite ($PbBi_2S_4$), etc.[5]

In comparison to other transition and post transition metals, bismuth is relatively inexpensive and is frequently used in metallurgy, electronics, and ceramic industries.[3b, 5-6] Also considering its heavy metal status, bismuth is one of the rare elements treated to be safe as it is nontoxic and non-carcinogenic[7] unlike Hg and Pb. In fact, bismuth compounds have been used in the pharmaceutical and cosmetic industries for more than a century.[8]

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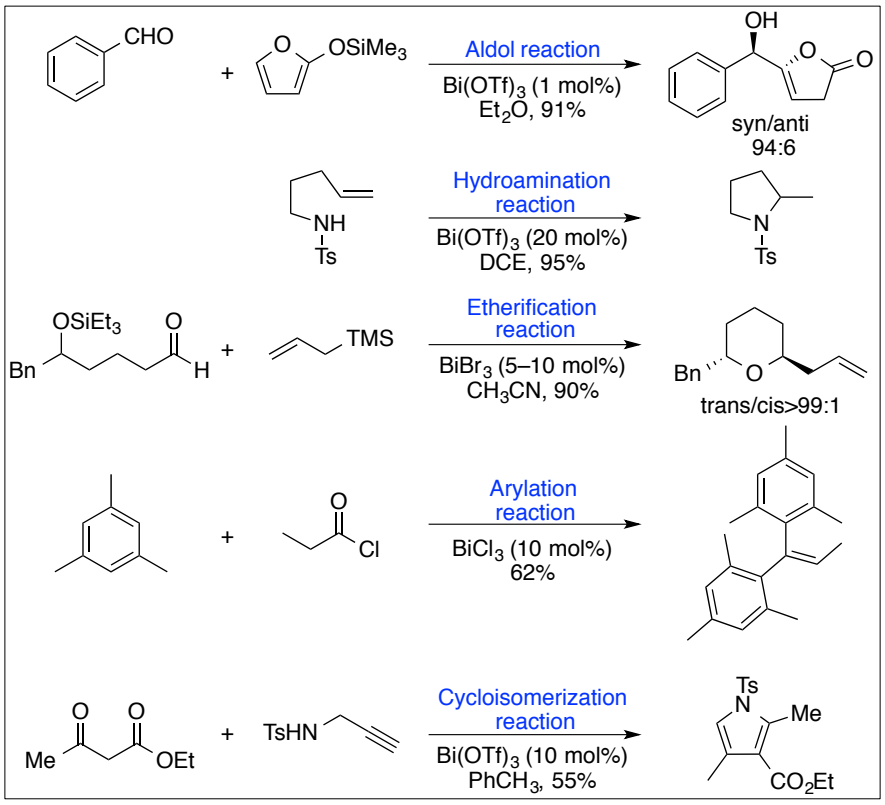
2.1.1. *Applications of Bi^{III} in functional group transformations & in carbohydrate chemistry*

Unfortunately, despite its popularity as a treatment for digestive problems, the synthetic utility of bismuth compounds remain relatively unexplored. However, the chemistry of this element is gaining considerable interest over the past decade. Various bismuth(III) compounds have been discovered which play crucial roles in different functional groups transformations (**Scheme 1**).[9] Bi^{III} reagents like bismuth triflate [$Bi(OTf)_3$], bismuth chloride or bromide ($BiCl_3$ or BiI_3), etc. have been used in a variety of reactions in stoichiometric or catalytic amounts, for e.g.; aldol reactions, epoxide opening reactions, alkylation or arylation, cycloisomerization, oxycarbonylations and many more.



***Scheme 1**: Examples of Bi(III)-catalyzed reactions*

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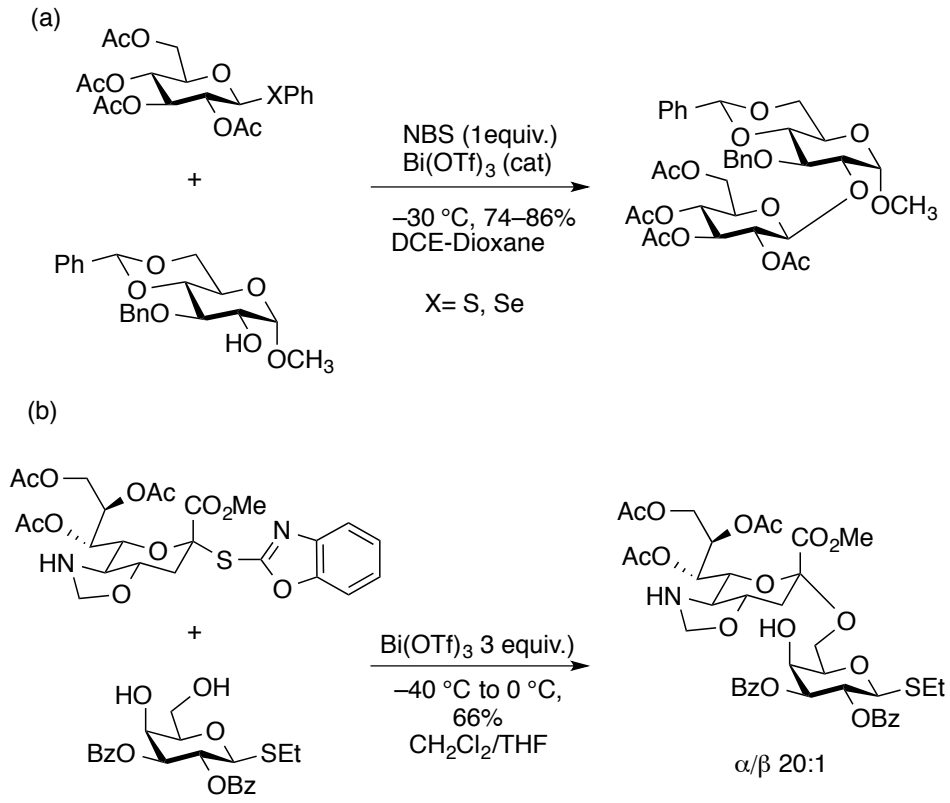
In carbohydrate chemistry, bismuth (III) triflate [$Bi(OTf)_3$] combined with NBS has been reported as a promoter for the activation of thio- and seleno-glycosides [**Scheme 2**(a)].[10] Moreover, $Bi(OTf)_3$ by itself can also be used for selective activation of an *S*-benzoxazolyl (SBox) sialyl donor over a galactosyl acceptor equipped with a thioethyl anomeric moiety [**Scheme 2**(b)].[11] However, in both these cases, it was observed that $Bi(OTf)_3$ was not only used in excess amounts, but due to its insolubility in the organic solvents often used for glycosylations, it has to be used in the presence of co-solvents like 1,4-dioxane and tetrahydrofuran that play a significant role in the diastereoselectivity[12] of the glycosylation products.



***Scheme 2**: Glycosylation schemes using $Bi(OTf)_3$ with (a) Thio, seleno-glycosides, (b) SBox donors*

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2.2. RESULTS AND DISCUSSION

2.2.1. Preparation of thiopropylglycosides

A number of alkyl and aryl-containing thiols were examined for the preparation of thioglycoside donors. Thiols in general are difficult to handle owing to their malodorous property. We therefore eschewed the more common volatile methane/ethanethiols and the highly pungent, more toxic aryl thiols and settled on *n*-propylthiol, a compound safe enough to be approved as a food additive for its savory onion-like smell.[13]

A range of thiopropyl analogues of galactosyl (**1a-b)**, glucosyl (**2a-b)**, fucosyl **(3a)** donors were prepared (**Scheme 3**). The acetylated sugars (**1**, **2**, **3**) were converted to the corresponding thiopropylglycosides (**1a, 2a, 3a**) by reacting with *n*-propylthiol in the presence of a Lewis acid ($BF_3 \bullet OEt_2$).[14] Further, the perbenzylated donors (**1b, 2b**) were then obtained by deacetylation of the 2,3,4,6- hydroxyls and then re-protection with a benzylating reagent (BnBr).

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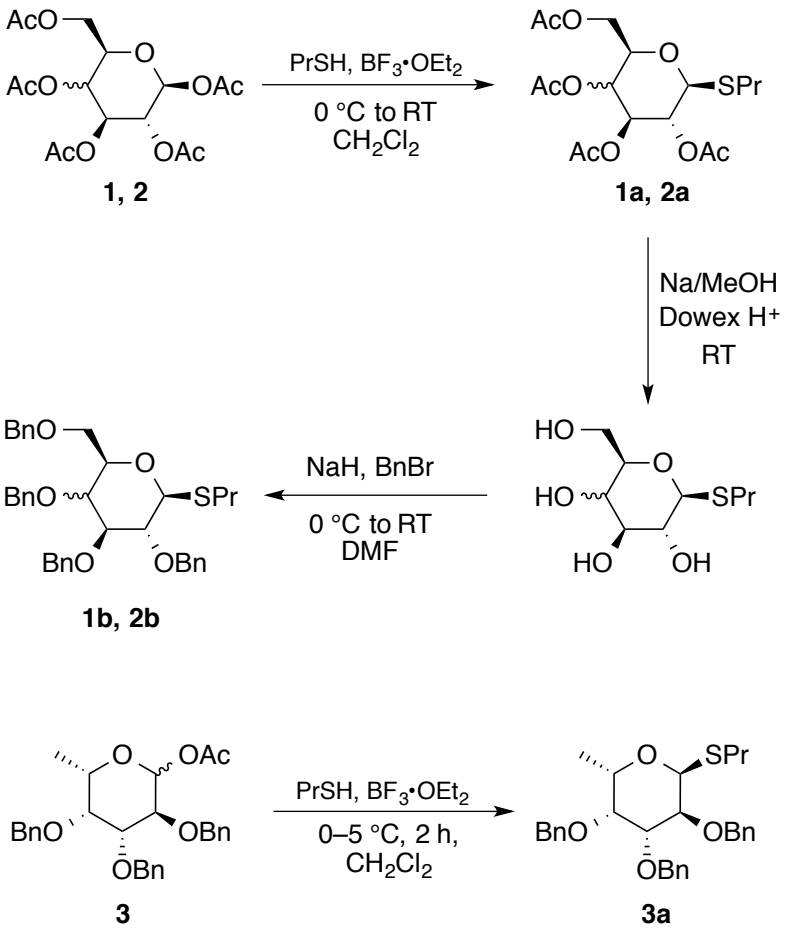

***Scheme 3**: Preparation of thioglycosyl donors*

2.2.2. Overview of synthesis & characterization of Bi^{III} compounds

As noted earlier, various Bi^{III} compounds like $Bi(OTf)_3$, BiX_3 (X= Cl, Br, I) have been applied to chemical glycosylation of various donors like glycosyl phosphites, glycosyl thioimidates, thioalkyl-, aryl-glycosides etc., however they still suffer from limitations. Ideally, a method for thioglycoside activation using bismuth chemistry could be developed that avoided the use of additives, co-solvents, low temperatures and even the requirement for excess promoter.

Among different existing oxidation states for bismuth, Bi(III) is the most stable

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and prevalent oxidation state. Since most of the Bi^{III} promoters that have been used in glycosylations are Lewis acidic, our initial aim was to discover a superior Bi^{III} Lewis acid, which will selectively activate thiopropylglycosides and evade some of the aforementioned limitations. At first, we tried to use some of the commercially available Bi^{III} compounds to activate the prepared thioglycosides. In spite of many trials and varying reaction conditions, we failed to get any success. Among the different Bi^{III} compounds, $Bi(OTf)_3$ has been used previously to activate SBox donors in presence of thioglycosides, although it suffers from low solubility issues. So, we imagined long-chain ligands might increase solubility of Bi^{III} compounds. Also, stronger Lewis acidity might activate thioglycosides as they are usually harder to activate than thioimidates.[2b] Bearing these two ideas in mind, we proposed some Bi^{III} compounds analogous to the reported bismuth(III) carboxylates and sulfonates: $Bi(OCOC_nF_{2n+1})_3$, $Bi(OSO_2C_nF_{2n+1})_3$ (n=7,8,9). The long fluorous chains were chosen since these tend to increase solubility. Moreover, these fluorous tails help in selective solid-phase purification, a feature that might be fruitful for promoter recovery at the end of an activation. Since none of the reported thioglycoside promoters are recyclable, this strategy can make our methodology more efficient than others.

To prepare some of the proposed Bi^{III} compounds, several methods were investigated using a variety of bismuth starting precursors (**Scheme 4**). Although Bi(III) carboxylates have been reported earlier, it was surprising to us that chemistry of bismuth sulfonates is limited to $Bi(OTf)_3$.[9b]

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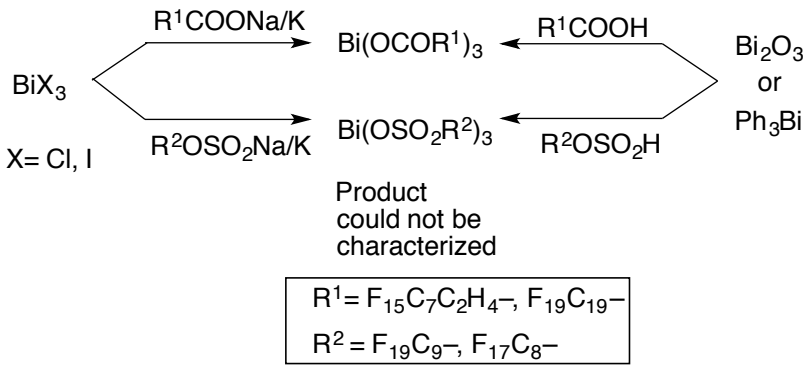

***Scheme 4**: Synthetic trials for preparation of Bi^{III} compounds*

The first reagent of choice was bismuth oxide (Bi_2O_3), since it is commonly used in the synthesis of $Bi(OTf)_3$. It was reacted with various fluorous sulfonic and carboxylic acids in presence of different solvents like THF, toluene, ethanol, etc. Unluckily, several difficulties were faced with these trials: like monitoring reaction progress and separation of excess reactants from products. A similar situation was faced when synthetic trials were done with bismuth halides like BiX_3 (X= Cl, I) as starting materials. Several solvents at various temperatures were screened, but extraction of desired product posed as the major obstacle.

Another common Bi-precursor: triphenyl bismuth (Ph_3Bi) was then chosen. This compound is UV-active; hence it can be monitored by TLC during an ongoing reaction. With this thought, different sulfonic and carboxylic acids were then reacted to form the desired Bi^{III} sulfonates and carboxylates respectively. As expected, the disappearance of the starting compound could be noticed. But the separation of the products by various techniques, like fluorous solid-phase extraction (FSPE), recrystallization etc. still remained unsuccessful. One of our aims with these proposed Bi(III) compounds was to

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increase solubility but in spite of using the long fluorous tails, we still encountered low solubility issues with all of our product mixtures.

Nevertheless, we decided to analyze our product mixtures to have a deeper understanding of our failed synthetic schemes and possibly troubleshoot them. Looking into literature, we found that the techniques mostly used to characterize Bi-containing compounds are X-ray crystallography, NMR spectroscopy and to some extent IR spectroscopy. When it comes to variety of bismuth compounds, this metal exist mainly in +3 state, but other oxidation states of +4, +5, etc. are also commonly seen. Another possibility in bismuth chemistry, is the element's ability to form chelation compounds with various heteroatoms like O, S, N, etc.[15]

Initially, various 1-D NMR techniques were utilized to analyze the mixtures, but these did not prove to be informative, as the spectra of starting reactants and products were almost identical. Sine recrystallization attempts failed, X-ray crystallography could not be used either. On the other hand, IR spectroscopy did show us bands characteristic of Bi-O bonds but did not help in identifying the structure of the product. Next, we turned to one of the most sensitive and useful analytical technique: high-resolution mass spectrometry (HRMS). Though we did not aim for product separation, it was anticipated that this method would inform if the desired high-molecular weight products were being formed at all. Unfortunately, this effort also failed to give any insight since inconsistent data was obtained with different trials of the same analyte. Going back to literature, it was surprising to us that there are only a few reports on

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structural analysis of synthetic Bi-compounds by MS,[9b] although this technique is frequently used with bismuth-protein complexes. These studies emphasized the difficulty of obtaining accurate molecular ion peaks for bismuth compounds or a reliable MS spectrum, as these do not follow regular fragmentation patterns.[9b] So, although high mass molecular ion peaks were observed, verification of the structure of the products failed. Recently, solid state Bismuth-NMR has been utilized by Schurko group[16] for structural identification. However, ^{209}Bi NMR spectroscopy is very limited because of its large nuclear quadrupole moment and complex nature. To date, only a handful research groups in the world have attempted this technique.

2.2.3. Synthesis of Bi^{V} compounds: $Ph_3Bi(OTf)_2$ & X-ray structure

Soon after discovering the challenges of trying to synthesize and characterize new bismuth(III)-containing compounds, we decided to look into other aspects of bismuth chemistry. The next most stable oxidation state for bismuth compounds after +3 is +5. Surprisingly, a variety of bismuth(III) reagents have been used in arylation, hydroamination and glycosylation reactions, but applications of bismuth(V) compounds remain relatively unexplored. Recent reports[15, 17] demonstrate the use of Bi^{V} salts and ylides in carbon-carbon, carbon-heteroatom bond-forming and oxidation reactions (**Scheme 5**).

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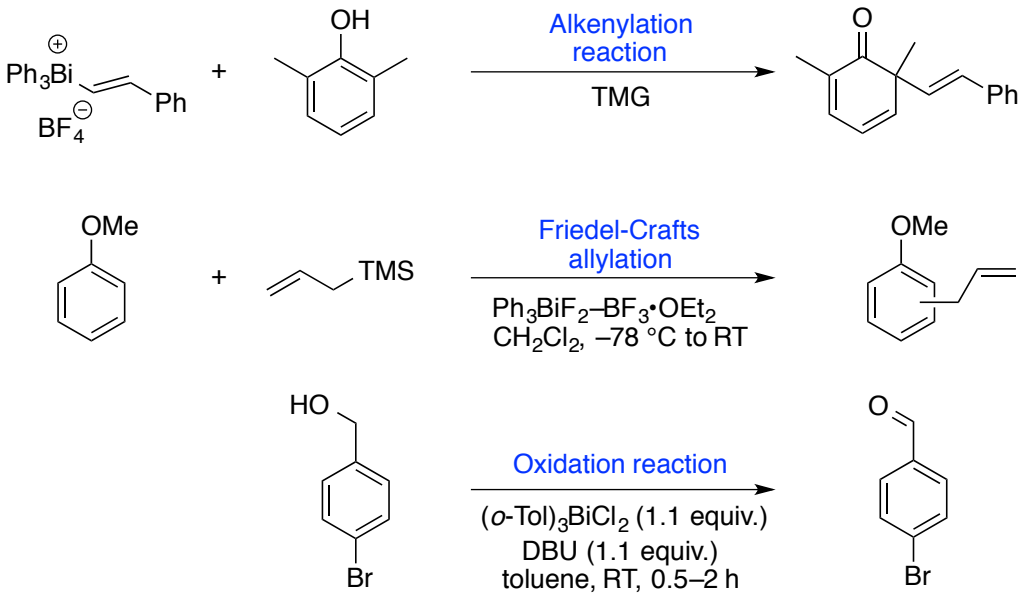

***Scheme 5**: Examples of Bi(V)-assisted reactions*

Observing the reported bismuth(V) compounds, we found that the common feature in all of these was the presence of phenyl or substituted-phenyl ring ligands. To our delight, most of these Bi^{V} compounds were soluble in most organic solvents like CH_2Cl_2, $CHCl_3$, toluene, etc. probably due to presence of the aryl rings. Hence, we were curious with the possibility of the addition of solubilizing ligands on Bi^{III} and the thiophilicity of Bi^{V} complexes. Given the current limitations in the definitive characterization of new bismuth compounds in solution,[3b] we next sought a complex that was amenable to crystallization and thereby went to a pentavalent bismuth compound containing three phenyl (Ph) and two triflate (OTf) groups, namely triphenyl bismuth ditriflate [$Ph_3Bi(OTf)_2$] **5**. $Ph_3Bi(OTf)_2$ was synthesized in two steps (**Scheme 6**) starting from relatively inexpensive triphenyl bismuth, which was first oxidized to triphenyl bismuth diacetate **4** and then later converted to the desired compound **5** in an

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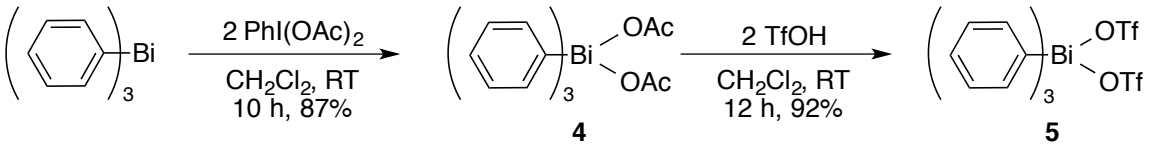
80% overall yield. Compound **5** is a colorless white solid that crystallizes in a highly disordered orthorhombic space group with trigonal bipyramidal coordination geometry (crystallographic data collected and solved by Dr. Arkady Ellern) around the bismuth metal center, which is similar to previously reported Bi^{V}-containing compounds[15] (**Figure 1**). More importantly, the compound surprisingly proved to have activity in an initial glycosylation reaction screen.



Scheme 6. *Preparation of bismuth(V) promoter*

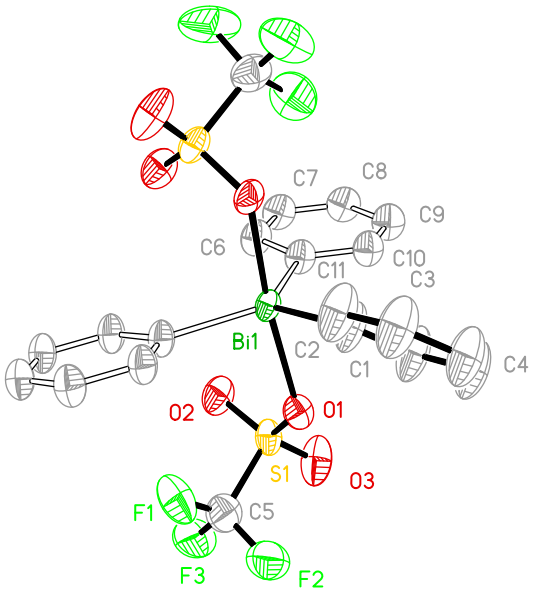


Figure 1: *X-ray structure of compound* ***5*** [$Ph_3Bi(OTf)_2$]. (Data by Dr. Arkady Ellern)

Crystal data: $C_{20}H_{19}BiF_6O_6S_2$, FW 780.45; orthorhombic, *Cmcm*; *a* = 10.7232(9), *b* = 18.469(2), *c* = 14.1481(11) Å; *V* = 2802.0(4) $Å^3$; *Z* = 4; D_{calcd} = 1.850 g/cm^{-3}; *F*(000) = 1496; *T* = 173 K; *R* = 0.0388; *R*w = 0.1184 for 2315 observed data. Intensity data were collected on a Bruker APEX 2 diffractometer with Mo K_a-radiation (l = 0.71073 Å). Molecule occupies a special crystallographic position *m2m* and it is disordered by symmetry. SIMU and DELU similarity restrains were used for all carbon atoms. Hydrogen atoms were placed at idealized positions and were allowed to ride on the neighboring atoms with relative isotropic displacement coefficients. Two isolated water molecules per cluster were observed. CCDC 937346

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contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif

Further exploration of this Bi^{V} reagent revealed several advantages. Solubility has been a major drawback with most reported promoters, thereby complicating their employment in automation platforms (solution or solid-phase) that carry out iterative oligosaccharide synthesis. As predicted, compound **5** was found to be readily soluble in most organic solvents, particularly dichloromethane and toluene that are desirable non-participating solvents in glycosylation. In addition, the promoter was also found to be oxygen and light stable. No degradation or decomposition was seen when kept under anhydrous conditions for months. So, unlike many thioglycoside promoters, complex **5** does not need to be synthesized just prior to the activation reaction, but can be made in batches and stored. In addition, the promoter **5** does not require a co-promoter like NBS/NIS to first make a soft electrophilic halonium species to attach to the soft nucleophilic sulfur. Finally, the activation does not require extreme low temperatures (−20 to −78 °C) primarily to control side reactions or unwanted by-products.

2.2.4. Coupling of various glycosyl donors with different acceptors

To test the scope of the developed methodology, a range of thiopropyl analogues of galactosyl (**1a-b**), glucosyl (**2a-b**), fucosyl (**3a**) donors were prepared. Next, the solvent chosen for the activation was dichloromethane as it is relatively inert, easy to handle, has negligible solvent effects[12] and completely solubilizes the donor/acceptor/promoter. Examining various temperature conditions (0 °C–reflux), the glycosylations were found to be best (without degradation of starting material) carried

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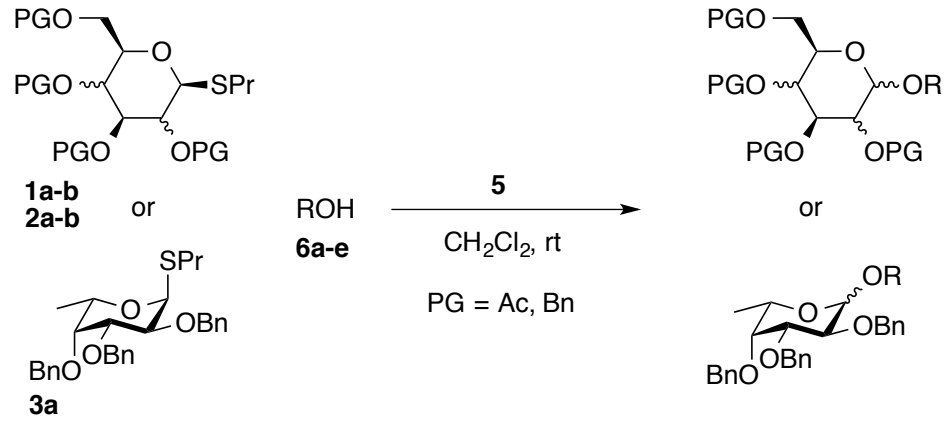
out at ambient temperature. These propanethiol-modified sugars were then subjected to these optimized reaction conditions with a range of glycosyl acceptors in presence of **5** (**Scheme 7** and **Table 1**).



Scheme 7. *Activation of thiopropylglycosides with* ***5***

As listed in **Table 1**, the study of thioglycoside activation with our model promoter **5** was started with a simple acceptor: allyl alcohol **6a**. The evident feature here is the presence of double bonds, which is generally avoided in donor/acceptor, as they tend to compete as a potential soft nucleophilic center to sulfur. Though some controlled activation[18] protocols can avoid these issues, alkenyl protecting groups always have the potential to be cleaved.[19] Fortunately, the "armed"[20] perbenzylated galactosyl donor **1b** and the "disarmed"[20] peracetylated galactosyl donor **1a** gave the desired *O*-allyl galactosides in high yields (entries 1-2). The "armed" glucosyl donor **2b** could also be activated (entry 3) to give the *O*-allyl glucoside in good yield. The alkenyl system remained intact throughout these reactions and formation of addition side product was not observed.

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With our initial success with acceptor **6a**, the method was applied to various glycosyl acceptors containing a wide range of functional groups. The glucosyl acceptor **6b** was selected to test the method for the formation of 1→6 linked disaccharides as well as glycosylation with a primary hydroxyl acceptor. Both the galactosyl donors' **1a** and **1b** (entries 4-5) gave the disaccharides in excellent yields. As predicted, the 1,2-*trans*-glycosides were favored for the "disarmed" thioglycosides and a low stereoselectivity was observed in the case of "armed" thioglycosides. Similarly, the armed glucosyl donor **2b** (entry 6) was also activated to give the 1→6 disaccharide in very high yield with a slight preference for the α-anomer.

Next, from common sugars like D-glucose and D-galactose, we moved forward to less common sugars. An "armed" L-fucosyl donor **3a** was chosen for this purpose and was synthesized from its acetate analogue (see Supporting Information). The activations were investigated with α-thiopropyl L-fucoside donors; these are the more stable anomers for L-fucose. Interestingly, the initial glycosylation with the glucosyl acceptor **6b** (entry 7) was extremely fast as the donor was consumed in 12 min to give the fucose-1→6-linked glycoside in good yield. The anomeric selectivity of the disaccharide was seen to be more α-favoring, as is generally seen with fucose analogues.[21]

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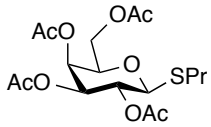
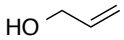
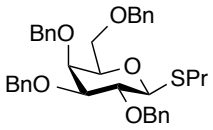
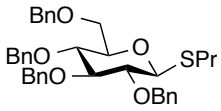
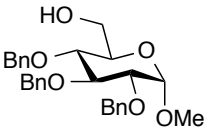
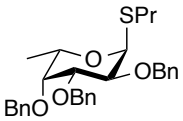
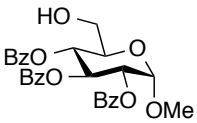
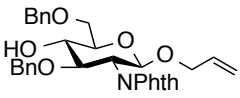
Table 1. *Reaction of glycosyl donors and acceptors.*

Entry	Donor	Acceptor	*t* [h]	Isolated Yield [%][b]	α:β[c]
1	**1a** 	**6a** 	4.5	78	1:10
2	**1b** 	**6a**	1	86	2:1
3	**2b** 	**6a**	3	69	1.2:1
4	**1a**	**6b** 	6	86	1:9
5	**1b**	**6b**	0.5	91	1.5:1
6	**2b**	**6b**	3.5	87	1.2:1
7	**3a** 	**6b**	0.2	80	2.3:1
8	**1a**	**6c** 	8	71	1:12
9	**1b**	**6c**	1	81	1:1
10	**2b**	**6c**	4	64	2:1
11	**2b**	**6d** 	0.5	72	2:1
12	**3a**	**6d**	0.33	85	7:1

[a] Reaction conditions: donor (1 equiv.), acceptor (0.9 equiv.), **5** (1 equiv.), CH_2Cl_2, RT, 0.5 M
[b] Yield after silica gel chromatography [c] calculated by NMR spectroscopy

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To explore another acceptor containing a commonly used protecting group, acceptor **6c** was selected. Unlike the benzyls (OBn) on **6b**, the benzoates (OBz) on **6c** made it very deactivated**.** This particular acceptor was selected as previously[22] it has been observed that coupling similarly deactivated acceptors with reactive donors in the presence of NIS resulted in the formation of irreversible *N*-succinimide glycosides of donor as major products. However, using promoter **5**, the required disaccharides were obtained in high to good yields with both reactive donors (entry 9-10) and a deactivated donor (entry 8). Interestingly, the rate of glycosylation did not differ much with alterations in the electronics of the acceptor (entries 5 and 9, 6 and 8), but a change from disarmed to armed donors (entries 8-9) had a significant impact on reaction times.

To extend the method to amino-sugars, a glucosamine acceptor **6d** containing a variety of functional groups including benzyl and allyl as alcohol protecting groups and phthalimido (Phth) as amine protecting group was chosen. This acceptor with a free 4-hydroxyl group would also validate our promoter for making 1→4-linked disaccharides, which in general are difficult to construct due to the low reactivity of the C-4 hydroxyl.[23] On reaction of acceptor **6c** with the perbenzylated galactosyl donor **1b** (entry 11) using promoter **5**, the 1→4-linked galactose-glucosamine disaccharide was obtained in 72% yield without any interference with the other functional groups. Coupling of acceptor **6d** to fucosyl donor **3a** (entry 12) was met with similar success. The glycosylation was observed to be very fast, resulting in the formation of the 1→4-linked fucose-glucosamine disaccharide in very high yield favoring the α-anomer. Comparing all the

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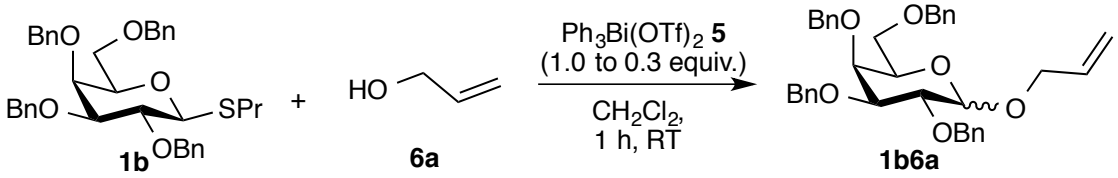
entries in **Table 1**, we can conclude that the glycosylation time depends on the nature of the donor (armed or disarmed) more so than on the incoming acceptor.

2.2.5. Stoichiometry studies

Another noteworthy limitation with available thioglycoside activators has been the amounts of promoter/co-promoter needed for thioglycoside activation. To the best of our knowledge, none of the available methods to date require less than stoichiometric amounts of promoter. Considering this and the above successful glycosylations, experiments were then designed to probe the amounts needed for full consumption of the glycosyl donor (**Table 2**) and isolation of the desired glycosides. Here, the coupling of benzylated galactoside donor **1b** to allyl acceptor **6a** in the presence of promoter **5** was considered as our model thioglycoside activation reaction. Moreover, to make accurate and consistent comparison of the differential loading, we quenched the reactions (**Table 2**) after a 1 h run, as the total reaction time was already determined for the same reaction in **Table 1**, entry 2.

Table 2. *Promoter equivalence studies with model glycosylation of donor **1b** and acceptor **6a***



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Entry	Promoter Equivalence	Isolated Yield [%][a]
1	1	86
2	0.7	82
3	0.5	76
4	0.3	68

Reaction conditions: donor, acceptor (1 equiv.), RT, CH_2Cl_2, 1 h
[a] Yield after silica gel chromatography

Fortunately, very high to good conversion rates were still seen as the amount of promoter was steadily decreased (**Table 2**). Only a modest decrease in isolated yields (86% to 68%) was observed as the loading was decreased from 100% to 30%. Nevertheless, a very high isolated yield of 76% was obtained even when cutting in half the amount of promoter previously used. This result turned out to be particularly remarkable as none of the previously reported thioglycoside promoters have been able to effect such activations with less than stoichiometric amounts of promoter without other additives or co-promoters. To further authenticate as well as to confirm our finding with another thioglycoside activation, we chose the glycosylation of fucosyl donor **3a** and glucosamine acceptor **6d** (**Scheme 8**) with only half the amount of promoter previously used**.** The activation was achieved in similar times (**Table 1** entry 12). The donor was completely consumed and the yield was also comparable to the earlier trial. These trials show that a less than stoichiometric amount of promoter **5** (≤0.7 equiv.) is sufficient for complete activation.

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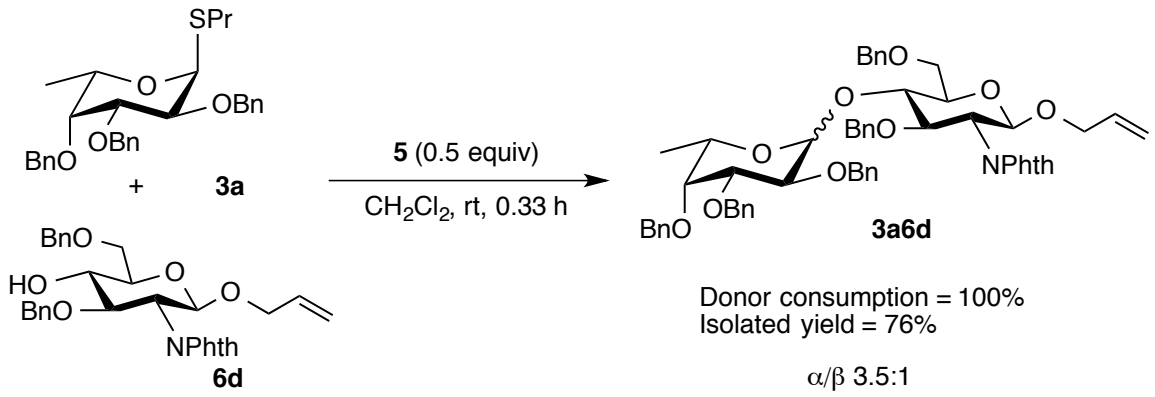

Scheme 8. *Glycosylation with donor **1a** and acceptor **6d***

2.2.6. Control experiments

The two common oxidation states for Bi are Bi^{III} and Bi^{V}; the +3 state is to-date better known. For a preliminary investigation into the activity of the Bi species, a set of control experiments (**Scheme 8**) was designed. For these studies, we went back to the model glycosylation of donor **1b** and acceptor **6a**, which was also selected for the loading experiments. To have a consistent comparison of the reactivity with the previous study (**Table 2**), the monitoring of the reaction was also done after 1 h of reaction time. At first, a trial glycosylation using only Ph_3Bi [**Scheme 8**, (a)] as a promoter was performed. However, no change in the reaction mixture or formation of product at 1 h or even after an overnight reaction time was observed. The next trial was a 1:2 mixture of Ph_3Bi and triflic acid (TfOH) as an activator [**Scheme 8**, (b)], which resembles the composition of the promoter [$Ph_3Bi(OTf)_2$]. Product formation again was not observed, although a slow anomerization of the β-galactosyl donor was seen, likely owing to the presence of the strong acid, TfOH. Examining the reaction over time, the

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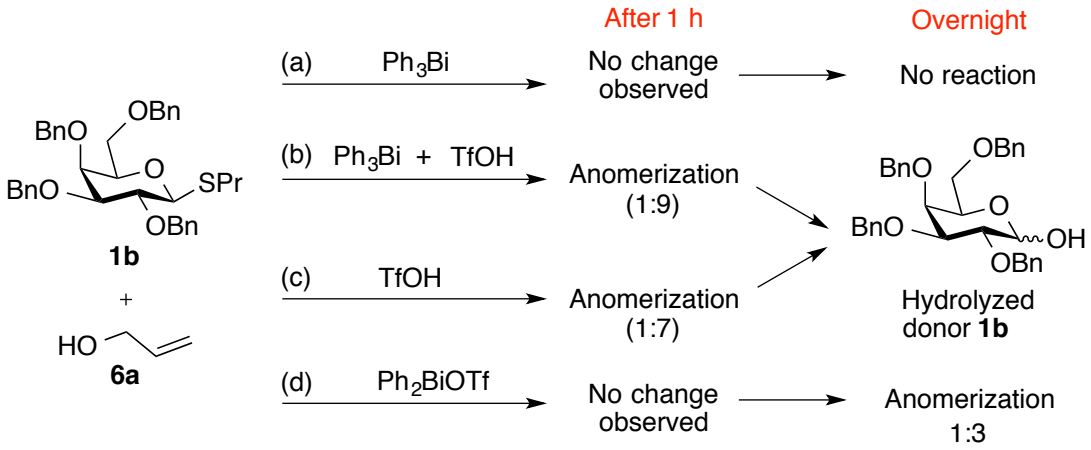
donor was hydrolyzed completely without any formation of desired product. This result led to the inference that Bi in the Bi^{V} state is necessary; it is not a mixture of Ph_3Bi and TfOH performing the activation.



***Scheme 9**. Control studies with model glycosylation of donor **1b** and acceptor **6a**. Reaction conditions: donor, acceptor, promoter (1 equiv.) (a)–(c) CH_2Cl_2, RT; (d) CH_3CN, RT*

Next, the activity of TfOH [**Scheme 9**, (c)] as a promoter in thioglycoside activation was tested. The donor anomerized slightly faster, indicating that previously the reactivity of TfOH was slowed perhaps in association with Ph_3Bi. The hydrolyzed donor was also found to be the major product after an overnight reaction. Finally, another Bi^{III} compound (Ph_2BiOTf)[24] was checked for its reactivity, as it resembles the promoter [$Ph_3Bi(OTf)_2$]. The mechanistic pathway of the activation is still unclear and is currently under study, yet the soft Lewis acidity of the Bi compound can be imagined to play a pivotal role. The glycosylation [**Scheme 9**, (d)] was closely monitored but no formation of our desired product was seen in 1 h. After an overnight observation, the β-galactosyl donor was noticed to be anomerizing to the α-anomer, yet still no product formation

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was seen. However, the donor was not seen hydrolyzing in this case. Considering all of the results above, we can assume that bismuth(V) is responsible for the activation of thioglycosides rather than bismuth(III). Previously Bi^{V} has seen used in oxidation and some addition reactions, particularly phenylation. David and Thieffry et al also tried to selectively oxidize carbohydrate alcohols with $Ph_3Bi(OAc)_2$, which incidentally is the first example of Bi^{V} in carbohydrate chemistry.[25] Nonetheless, formation of such addition products was not observed with the thioglycosyl donors or glycosyl acceptors under consideration.

2.3. CONCLUSION

In summary, the first demonstration of the catalytic utility of a pentavalent bismuth complex, specifically $Ph_3Bi(OTf)_2$, has been shown in the context of a thioglycoside activation reaction. This new promoter has shown distinct advantages over most current thioglycoside activators, namely high solubility, air/light stability and a long shelf life. Most importantly, this promoter can activate thioglycosides with as little as 0.5 equiv. and at room temperatures. The scope of reactivity was studied with a wide variety of sugar donors carrying diverse protecting groups and products were seen to form in good to excellent yields. The diastereoselectivity of the products seem to follow trends reported in literature. Unexpectedly, the activation was found to be uniquely related to pentavalent and not trivalent bismuth, a fact that should spur additional work in developing the chemistry of this relatively nontoxic metal.

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2.4. EXPERIMENTAL SECTION

Air- and moisture-sensitive reactions were carried out in oven-dried or flame-dried glassware, septum-capped under atmospheric pressure of argon. Commercially available compounds were used without further purification unless otherwise stated. Air- and moisture-sensitive liquids and solutions were transferred via syringe or stainless steel cannula. All saccharides and promoters were pre-treated by azeotropic removal of water using toluene. Flash silica gel column chromatography was performed employing Silica Gel 60 Sorbent (40-63 µm, 230-400 mesh). Thin–layer chromatography (analytical) was performed using glass plates pre-coated to a depth of 0.25 mm detected under UV light and by spraying the plates with a 0.02 M solution of resorcinol in 20% ethanolic H_2SO_4 solution followed by heating.

2.4.1. Instrumentation

Proton (^{1}H) NMR, carbon (^{13}C) NMR and fluorine (^{19}F) spectra were recorded on a 600 MHz, 125 MHz and 565 MHz instrument respectively using the residual signals from chloroform ($CDCl_3$), 7.26 ppm and 77.0 ppm, and acetonitrile (CD_3CN), 1.94 ppm and 118.69 ppm, 1.39 ppm, as internal references for ^{1}H and ^{13}C chemical shifts (δ) respectively. Trifluoroacetic acid (CF_3COOH) with δ −76.55 ppm was used as an external reference for ^{19}F NMR. ESI-HRMS mass spectrometry was carried out on an Agilent 6540 QTOF. X-ray crystallography was done on a Bruker APEX2 CCD System. Optical rotations were measured at 20 °C using an automatic polarimeter AP300. Melting points were recorded in capillary tubes on a Digimelt SRS.

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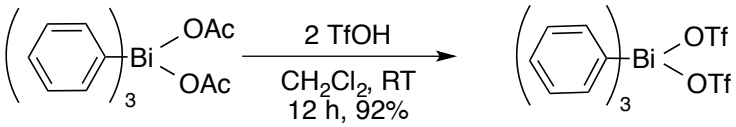
2.4.2. Synthetic protocols and characterization of new compounds

Preparation of triphenyl bismuth ditriflate (5):



4 **5**

$C_{20}H_{15}BiF_6O_6S_2$
(738.43)

To a solution of **4**[26] (2.3 g, 4.1 mmol) in anhydrous dichloromethane (20 mL), trifluoromethane sulfonic acid (TfOH, 1.2 g, 8.2 mmol) was added dropwise at −78 °C. The reaction was stirred at ambient temperature for 12 h. The solvent was removed under reduced pressure to obtain a pale white crude solid. Upon recrystallization with a 1:1 mixture of dichloromethane and hexanes, colorless transparent crystals of the desired Bi^V compound **5** (2.8 g, 3.7 mmol, 92%) were obtained; m.p. 165-166 °C (dec); ^{1}H NMR (600 MHz, [D-1] $CDCl_3$, 25 °C, TMS) δ 8.14 (d, *J* = 7.9 Hz, 6H; H-1 Ph), 7.91 (t, *J* = 7.8 Hz, 6H; H-2 Ph), 7.73 (t, *J* = 7.5 Hz, 3H; H-3 Ph); ^{13}C NMR (151 MHz, [D-1] $CDCl_3$, 25 °C, TMS) δ 155.56 (3C; Bi-*C*, C-1 C_{Ph}) 134.78, 134.02, 133.53 (9C; C-2,3,4 C_{Ph}), 119.76, 117.65 (2C; *C*F_3); ^{19}F NMR (565 MHz, $CDCl_3$, ext. std. CF_3COOH) δ -77.53 (6F, OSO_2C*F_3*).

X-ray structure and data

CCDC 937346 contains the supplementary crystallographic data for $Ph_3Bi(OTf)_2$. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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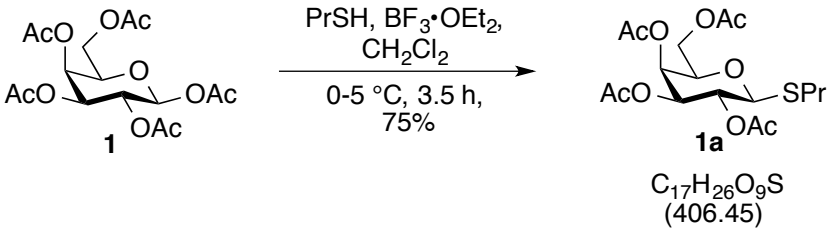
Standard procedure for preparation of thiopropyl glycosides

A 0.50 M solution of glycosyl acetate donor (1.0 equiv.) and propanethiol (PrSH, 1.3 equiv.) were stirred for 0.5 h in anhydrous dichloromethane at 0 °C. Then, boron trifluoride dietherate ($BF_3{\cdot}OEt_2$, 1.5 equiv.) was added dropwise to the reaction mixture and it was stirred over an ice bath (0–5 °C) until consumption of the starting donor was seen by TLC. The reaction was quenched with excess triethylamine, diluted with CH_2Cl_2, filtered, and washed sequentially with 2 M aqueous HCl, saturated aqueous $NaHCO_3$, and water. The organic layer was dried over $MgSO_4$, concentrated under reduced pressure at 40 °C, and the resulting residue was purified by silica gel column chromatography.

***n*-Propyl-2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (1a)**



$C_{17}H_{26}O_9S$
(406.45)

The donor **1**[27] (2.5 g, 6.4 mmol) with PrSH (0.63 g, 8.3 mmol) were reacted and the reaction mixture was purified by flash silica gel column chromatography (Ethyl acetate:hexanes, 1:3) to give **1a**[28] (2.0 g, 4.8 mmol) as a white solid; m.p. 80.2–81.5 °C; R_f 0.48 (Ethyl acetate:hexanes 1:3); $[\alpha]_D$ + 83.3 $cm^3\ g^{-1}dm^{-1}$ (c 0.12 g cm^{-3}, $CHCl_3$; ^1H NMR (600 MHz, [D-1] $CDCl_3$, 25 ^oC, TMS) δ = 5.41 (d, *J*=7.0, 1H; H-1), 5.21 (t, *J*=10.0, 1H; H-4), 5.02 (dd, *J*=10.0, 3.4, 1H; H-2), 4.46 (d, *J*=10.0, 1H; H-3), 4.11 (ddd, *J*=11.3, 6.7, 2H; H-

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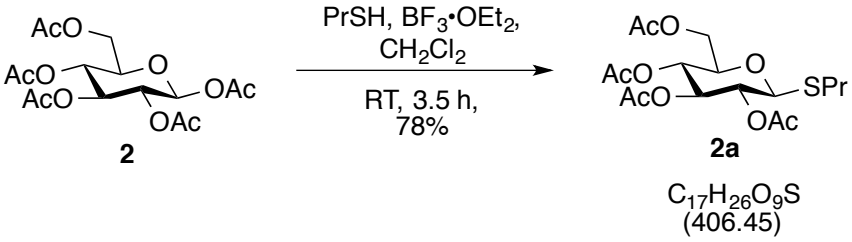
6a,6b), 3.91 (td, *J*=6.9, 1.4, 1H; H-5), 2.72 – 2.57 (m, 2H; SC*H*$_2$CH$_2$CH$_3$), 2.13, 2.04, 2.03, 1.96 (4s, 12H; 24×C*H*$_3$C=O) 1.64 (dt, *J*=14.6, 7.4, 2H; SCH$_2$C*H*$_2$CH$_3$), 0.97 (t, *J*=7.4, 3H; SCH$_2$CH$_2$C*H*$_3$); ^{13}C NMR (151 MHz, [D-1] CDCl$_3$, 25 ^{o}C, TMS) δ 170.46, 170.32, 170.16, 169.66 (4C; *C*=O), 84.38 (1C; C-1), 74.47 (1C; C-2), 72.03 (2C; C-3), 67.41 (1C; C-4), 67.38 (1C; C-5), 61.57 (1C; C-6ab), 32.45 (1C; S*C*H$_2$CH$_2$CH$_3$), 23.21 (1C; SCH$_2$*C*H$_2$CH$_3$), 20.92, 20.78, 20.77, 20.70 (4C; *C*H$_3$C=O), 13.50 (1C; SCH$_2$CH$_2$*C*H$_3$); HRMS (ESI-QTOF) *m/z* calc. for $C_{17}H_{26}O_9$ SNa$^+$ 429.1195 found 420.1200.

_n_-Propyl-2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranoside (2a)



The donor **2**[29] (2.3 g, 5.9 mmol) with PrSH (0.58 g, 7.6 mmol) were reacted and the reaction mixture was purified by silica gel column chromatography (Ethyl acetate:hexanes, 1:3) to give **2a**[30] (1.9 g, 4.6 mmol) as a white solid; m.p. 83.2–84.1 °C; R$_f$ 0.39 (Ethyl acetate:hexanes 1:3); [α]$_D$ +58.8 cm^3 g^{-1}dm^{-1} (c 0.17 g cm^{-3}, CHCl$_3$); ^{1}H NMR (600 MHz, [D-1] CDCl$_3$, 25 ^{o}C, TMS) δ = 5.19 (t, *J*=9.4, 1H; H-2), 5.05 (t, *J*=9.8, 1H; H-3), 5.00 (t, *J*=9.7, 1H; H-4), 4.46 (d, *J*=10.1, 1H; H-1), 4.21 (dd, *J*=12.3, 5.0, 1H; H-6a), 4.11 (dd, *J*=12.3, 2.4, 1H; H-6b), 3.70 – 3.66 (m, 1H; H-5), 2.69 – 2.55 (m, 2H; SC*H*$_2$CH$_2$CH$_3$), 2.05, 2.03, 2.00, 1.98 (s, 12H; 4×C*H*$_3$C=O), 1.60 (dt, *J*=15.3, 7.7, 2H; SCH$_2$C*H*$_2$CH$_3$), 0.96 (t, *J*=7.3, 3H; SCH$_2$CH$_2$C*H*$_3$); ^{13}C NMR (151 MHz, [D-1] CDCl$_3$, 25 ^{o}C, TMS) δ 170.70, 170.27,

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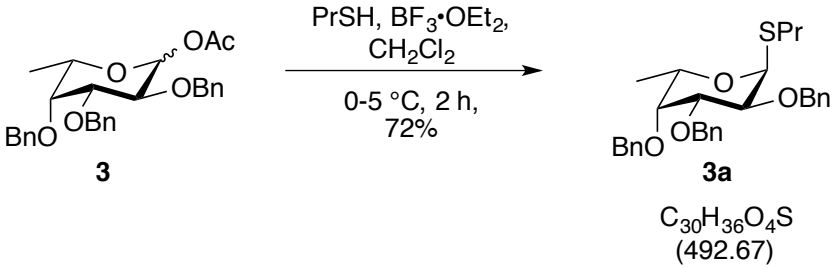
169.49, 169.46 (4C; <u>C</u>=O), 83.78 (1C; C-1), 75.93 (1C; C-2), 74.00 (1C; C-3), 70.02 (1C; C-4), 68.46 (1C; C-5), 62.28 (1C; C-6ab), 32.21 (1C; S<u>C</u>$H_2CH_2CH_3$), 23.17 (1C; SCH_2<u>C</u>H_2CH_3), 20.81, 20.71, 20.68 (4C; <u>C</u>H_3C=O), 13.48 (1C; SCH_2CH_2<u>C</u>H_3); HRMS (ESI-QTOF) *m/z* calc. for $C_{17}H_{26}O_9$ SNa^+ 429.1195 found 420.1196.

_n_-Propyl-2,3,4-tri-_O_-benzyl-1-thio-α-L-fucopyranoside (3a)



The donor **3**[31] (1.2 g, 2.5 mmol) with PrSH (0.25 g, 3.3 mmol) were reacted and the reaction mixture was purified by silica gel column chromatography (Ethyl acetate:hexanes, 1:4) to give **3a** (0.88 g, 4.6 mmol) as a yellow oil; R_f 0.86 (Ethyl acetate:hexanes 1:3), $[\alpha]_D$ -105.2 cm^3 $g^{-1}dm^{-1}$ (c 0.13 g cm^{-3}, $CHCl_3$); ^{1}H NMR (600 MHz, [D-3] CD_3CN, 25 ^oC, TMS) δ = 7.39 – 7.28 (m, 15H; <u>*Ph*</u>CH_2O), 5.53 (d, *J*=3.9, 1H; H-1), 4.87 (d, *J*=11.6, 1H; PhC<u>*H*$_2$</u>O), 4.74 (s, 2H; PhC<u>*H*$_2$</u>O), 4.71 (d, *J*=11.6, 1H; PhC<u>*H*$_2$</u>O), 4.59 (d, *J*=11.3, 2H; PhC<u>*H*$_2$</u>O), 4.21 – 4.15 (m, 1H; H-5), 4.10 (dd, *J*=10.0, 5.5, 1H; H-2), 3.80 (dd, *J*=3.1, 1.2, 1H; H-3), 3.74 (dd, *J*=10.0, 3.0, 1H; H-4), 2.49 (ddd, *J*=12.9, 7.8, 6.7, 2H; SC<u>*H*$_2$</u>CH_2CH_3), 1.61 (tq, *J*=14.0, 7.2, 2H; SCH_2C<u>*H*$_2$</u>CH_3), 1.16 (d, *J*=6.5, 3H; C<u>*H*$_3$</u>), 0.98 (t, *J*=7.3, 3H; SCH_2CH_2C<u>*H*$_3$</u>); ^{13}C NMR (151 MHz, [D-3] CD_3CN, 25 ^oC, TMS) δ 129.10, 129.08, 129.02, 128.88, 128.68, 128.40, 128.37, 128.32, 128.24 (15C; C_{Ph}), 84.12 (1C; C-1), 80.10 (1C; C-4), 78.75 (1C; C-3), 76.51 (1C; C-2), 75.79, 73.08, 72.31 (3C; Ph<u>C</u>H_2O), 67.40 (1C; C-

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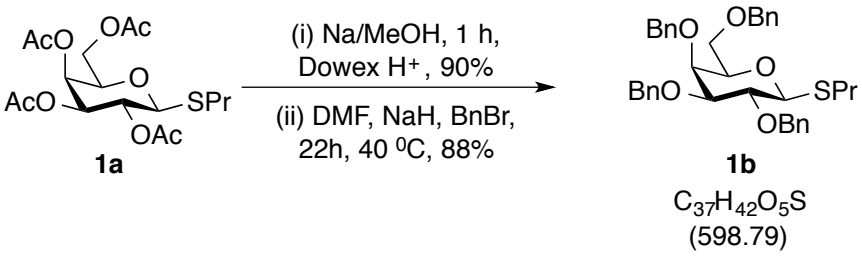
5), 32.02 (1C; S<u>C</u>H$_2$CH$_2$CH$_3$), 23.61 (1C; SCH$_2$<u>C</u>H$_2$CH$_3$), 16.78 (1C; <u>C</u>H$_3$), 13.66 (1C; SCH$_2$CH$_2$<u>C</u>H$_3$); HRMS (ESI-QTOF) *m/z* calc. for $C_{30}H_{36}O_4$ SNa^+ 515.2227 found 515.2218.

_n_-Propyl-2,3,4,6-tetra-_O_-benzyl-1-thio-β-D-galactopyranoside (1b)



To a solution of **1a** (1.2 g, 2.9 mmol) in 30 mL anhydrous (MeOH), 0.50 mL of 0.5 N sodium methoxide (freshly prepared by dissolving sodium in anhydrous methanol) was added. The reaction mixture was stirred at room temperature for 1 h, neutralized with Dowex 50WX8 (H^+) cation exchange resin and filtered. The methanol was removed *in vacuo* and trace methanol or water was removed by co-evaporation with toluene (3 x 5 mL). The crude product (0.63 g, 2.6 mmol) was used directly in the next step and mixed in 10 mL anhydrous DMF. To the solution, NaH was added (0.63 g, 26 mmol) at 0 °C and left to stir for 20 min. Then, BnBr (2.7 g, 16 mmol) was added to the reaction mixture and was stirred for 22 h, until consumption of starting material. The reaction was quenched with MeOH, diluted with CH_2Cl_2, filtered, and washed sequentially with 2 M aqueous HCl, saturated aqueous $NaHCO_3$, and water. The organic layer was dried over $MgSO_4$, concentrated under reduced pressure at 40 °C, and the residue was purified by silica gel column chromatography (Ethyl acetate:hexanes, 1:6) to obtain a pale white solid **1b** (1.4 g, 2.3 mmol); m.p. 79.0-79.4 °C; R_f 0.84 (Ethyl acetate:hexanes 1:3), $[\alpha]_D$

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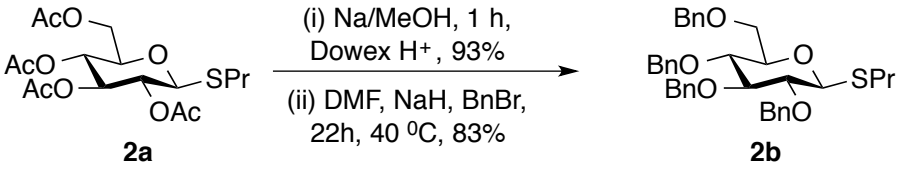
+71.4 $cm^3\ g^{-1}dm^{-1}$ (c 0.14 g cm^{-3}, $CHCl_3$); ^{1}H NMR (600 MHz, [D-1] $CDCl_3$, 25 ^oC, TMS) δ = 7.36 – 7.26, 7.25 – 7.18 (2m, 20H; *Ph*CH_2O), 4.90 (d, *J*=11.7, 1H; PhC*H*$_2$O), 4.83 (d, *J*=10.2, 1H; PhC*H*$_2$O), 4.74 (d, *J*=10.2, 1H; PhC*H*$_2$O), 4.67 (s, 2H; PhC*H*$_2$O), 4.56 (d, *J*=11.7, 1H; PhC*H*$_2$O), 4.40 (d, *J*=11.7, 1H; PhC*H*$_2$O), 4.36 (d, *J*=8.9, 1H; H-1), 4.34 (s, 1H; PhC*H*$_2$O), 3.90 (d, *J*=2.8, 1H; H-2), 3.77 (t, *J*=9.5, 1H; H-3), 3.54 (dd, *J*=6.3, 2.9, 2H; H-4,5), 3.53 – 3.48 (m, 2H; H-6ab), 2.77 – 2.43 (m, 2H; SC*H*$_2$$CH_2CH_3$), 1.68 – 1.54 (m, 2H; SCH_2C*H*$_2$$CH_3$), 0.93 (t, *J*=7.3, 3H; SCH_2CH_2C*H*$_3$); ^{13}C NMR (151 MHz, [D-1] $CDCl_3$, 25 ^oC, TMS) δ 138.91, 138.53, 138.46, 138.03 (4C; 4×C-1' C_{Ph}), 128.59, 128.55, 128.44, 128.30, 128.13, 128.04, 127.93, 127.85, 127.76, 127.67 (20C; C_{Ph}), 85.68 (1C; C-1), 84.26, 78.65, 75.93, 74.54 (4C; Ph*C*H_2O), 73.73 (1C; C-2), 73.70 (1C; C-3), 72.88 (1C; C-3), 68.98 (1C; C-4), 32.89 (1C; S*C*$H_2CH_2CH_3$), 23.44 (1C; SCH_2*C*H_2CH_3), 13.72 (1C; SCH_2CH_2*C*H_3); HRMS (ESI-QTOF) *m/z* calc. for $C_{37}H_{42}O_5\ SEt_3NH^+$ 700.4035 found 700.4022, *m/z* calc. for $C_{37}H_{42}O_5Na^+$ 621.2645 found 621.2638.

***n*-Propyl-2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (2b)**



2a **2b**

$C_{37}H_{42}O_5S$
(598.79)

To a solution of **2a** (0.90 g, 2.2 mmol) in 30 mL anhydrous (MeOH), 0.5 mL of 0.5 N sodium methoxide (freshly prepared by dissolving sodium in anhydrous methanol) was added. The reaction mixture was stirred at room temperature for 1 h, neutralized with

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Dowex 50WX8 (H^+) cation exchange resin and filtered. The methanol was removed *in vacuo* and trace methanol or water was removed by co-evaporation with toluene (3 x 5 mL). The crude product (0.49 g, 2.1 mmol) was used directly in the next step and mixed in 10 mL anhydrous DMF. To the solution, NaH was added (0.49 g, 21 mmol) at 0 °C and left to stir for 20 min. Then, BnBr (2.1 g, 12 mmol) was added to the reaction mixture and was stirred for 22 h, until consumption of starting material. The reaction was quenched with MeOH, diluted with CH_2Cl_2, filtered, and washed sequentially with 2 M aqueous HCl, saturated aqueous $NaHCO_3$, and water. The organic layer was dried over $MgSO_4$, concentrated under reduced pressure at 40 °C, and the residue was purified by silica gel column chromatography (Ethyl acetate:hexanes, 1:6) to obtain a pale white solid **2b**[32] (1.0 g, 2.3 mmol); m.p. 81.0–82.2 °C; R_f 0.81 (Ethyl acetate:hexanes 1:3), $[\alpha]_D$ +71.4 $cm^3\ g^{-1}dm^{-1}$ (c 0.09 g cm^{-3}, $CHCl_3$); ^{1}H NMR (600 MHz, [D-3] CD_3CN, 25 ^{o}C, TMS) δ = 7.43 – 7.17 (m, 20H; *Ph*CH_2O), 4.86 (dd, *J*=10.9, 1.7, 2H; PhC*H*$_2$O), 4.82 (d, *J*=11.1, 1H; PhC*H*$_2$O), 4.78 (d, *J*=11.0, 1H; PhC*H*$_2$O), 4.73 (d, *J*=10.6, 1H; PhC*H*$_2$O), 4.57 (d, *J*=7.4, 1H; H-1), 4.57 (d, *J*=17.0, 1H; PhC*H*$_2$O), 4.52 (d, *J*=12.0, 1H; PhC*H*$_2$O), 4.48 (d, *J*=9.8, 1H; PhC*H*$_2$O), 3.72 (dd, *J*=11.0, 1.9, 1H; H-2), 3.65 (m, 2H; H-6ab), 3.53 (dd, *J*=9.8, 8.8, 1H; H-3), 3.47 (ddd, *J*=9.9, 4.7, 1.9, 1H; H-5), 3.37 (dd, *J*=9.8, 8.7, 1H; H-4), 2.69 (ddd, *J*=12.7, 7.8, 6.7, 2H; SC*H*$_2$$CH_2CH_3$), 1.74 – 1.59 (m, 2H; SCH_2C*H*$_2$$CH_3$), 0.98 (t, *J*=7.3, 3H; SCH_2CH_2C*H*$_3$); ^{13}C NMR (151 MHz, [D-3] CD_3CN) δ 139.89, 139.57, 139.55, 139.54 (4C; 4×C-1' C_{Ph}), 129.30, 129.25, 129.19, 128.96, 128.88, 128.78, 128.73, 128.57, 128.52, 128.46 (20C; C_{Ph}), 87.20 (1C; C-1), 85.68, 82.65, 79.46, 79.01(4C; Ph*C*H_2O), 76.10(1C; C-2), 75.65(1C; C-3), 75.48 (1C; C-3), 73.78 (1C; C-4), 70.10 (1C; C-5), 33.31 (1C;

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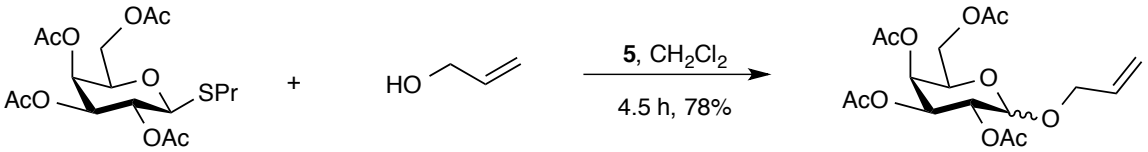
S<u>C</u>H$_2$CH$_2$CH$_3$), 24.12 (1C; SCH$_2$<u>C</u>H$_2$CH$_3$), 13.74 (1C; SCH$_2$CH$_2$<u>C</u>H$_3$); HRMS (ESI-QTOF) *m/z* calc. for $C_{37}H_{42}O_5SEt_3NH^+$ 700.4035 found 700.4013, *m/z* calc. for $C_{37}H_{42}O_5Na^+$ 621.2645 found 621.2637.

General procedure for thioglycoside activation

A 0.50 M solution of thioglycoside donor (1.0 equiv.) and acceptor (0.90 equiv.) were stirred together in anhydrous dichloromethane (CH_2Cl_2) for 0.5 h in a flask filled with Ar at room temperature. To it a solution of the promoter (1.0 equiv.) in anhydrous CH_2Cl_2 was added and the reaction was stirred at room temperature till the consumption of donor was seen by TLC. The reaction was then quenched with triethylamine, filtered through a Celite pad, concentrated under vacuum and purified by silica gel column chromatography.

Entry 1:

Allyl-2,3,4,6-tetra-*O*-acetyl-1-*O*-D-galactopyranoside (1a6a)



1a **6a** **1a6a**

1a (0.030 g, 0.070 mmol) and **6a** (0.0040 g, 0.070 mmol) were reacted in presence of **5** (0.054 g, 0.074 mmol) to yield **1a6a**[33] (0.022 g, 0.058 mmol) as a white gel after purification by silica gel column chromatography (Ethyl acetate:hexanes, 1:3). The ^{1}H NMR and ^{13}C NMR data matched those previously reported.[33]

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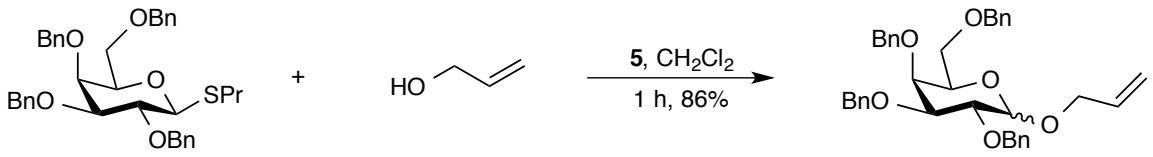
Entry 2:

Allyl-2,3,4,6-tetra-*O*-benzyl-1-*O*-D-galactopyranoside (1b6a)

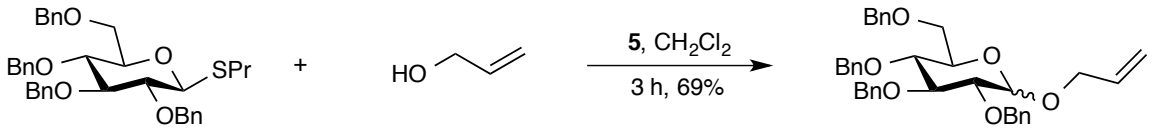


1b **6a** **1b6a**

1b (0.030 g, 0.050 mmol) and **6a** (0.0030 g, 0.050 mmol) were reacted in presence of **5** (0.037 g, 0.050 mmol) to yield **1b6a**[34] (0.025 g, 0.043 mmol) as a white gel after purification by silica flash column chromatography (Ethyl acetate:hexanes, 1:5). The ^{1}H NMR and ^{13}C NMR data matched those previously reported.[34]

Entry 3

Allyl-2,3,4,6-tetra-*O*-benzyl-1-*O*-β-D-glucopyranoside (2b6a)



2b **6a** **2b6a**

2b (0.030 g, 0.050 mmol) and **6a** (0.0030 g, 0.050 mmol) were reacted in presence of **5** (0.037 g, 0.050 mmol) to yield **2b6a**[34] (0.020 g, 0.035 mmol) as a pale yellow gel after purification by silica gel column chromatography (Ethyl acetate:hexanes, 1:5). The ^{1}H NMR and ^{13}C NMR data matched those previously reported.[34]

Entry 4:

Methyl-(2',3',4',6'-tetra-*O*-acetyl-D-galactopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (1a6b)

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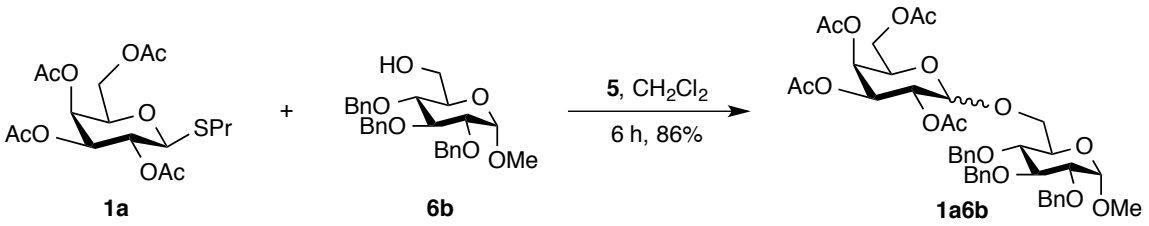
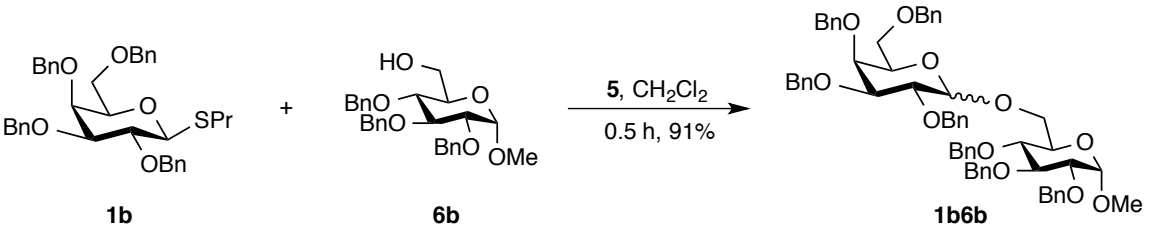


1a (0.030 g, 0.074 mmol) and **6b**[35] (0.031 g, 0.066 mmol) were reacted in presence of **5** (0.054 g, 0.074 mmol) to yield **1a6b**[36] (0.031 g, 0.039 mmol) as a white foam after purification by silica gel column chromatography (Ethyl acetate:hexanes, 1:3). The ^{1}H NMR, ^{13}C NMR, HRMS data matched those previously reported.[36]

Entry 5:

Methyl-(2',3',4',6'-tetra-*O*-benzyl-D-galactopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (1b6b)



1b (0.035 g, 0.058 mmol) and **6b** (0.024 g, 0.052 mmol) were reacted in presence of **5** (0.043 g, 0.058 mmol) to yield **1b6b**[37] (0.047 g, 0.047 mmol) as a pale yellow gel after purification by silica gel column chromatography (Ethyl acetate:hexanes, 1:4). The ^{1}H NMR, ^{13}C NMR, HRMS data matched those previously reported.[37]

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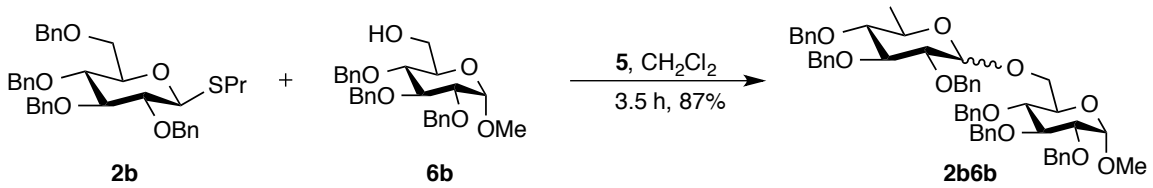
Entry 6:

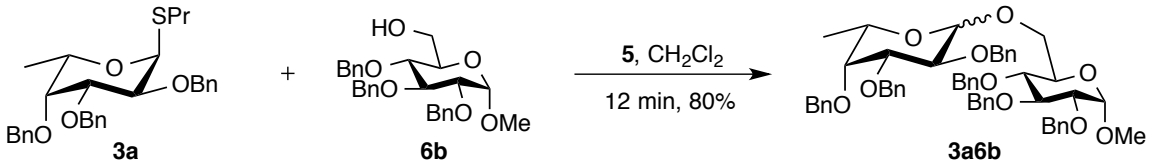
Methyl-(2',3',4',6'-tetra-*O*-benzyl-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (2b6b)



2b (0.035 g, 0.058 mmol) and **6b** (0.024 g, 0.052 mmol) were reacted in presence of **5** (0.043 g, 0.058 mmol) to yield **2b6b**[36] **(**0.045 g, 0.045 mmol) as a pale yellow foam after purification by silica gel column chromatography (Ethyl acetate:hexanes, 1:4). The ^{1}H NMR and ^{13}C NMR data matched those previously reported.[36]

Entry 7:

Methyl-(2',3',4'-tri-*O*-benzyl-L-fucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (3a6b)



3a (0.035 g, 0.071 mmol) and **6b** (0.030 g, 0.064 mmol) were reacted in presence of **5** (0.052 g, 0.071 mmol) to yield **3a6b**[38] (0.045 g, 0.051 mmol) as a white solid after purification by silica gel column chromatography (Ethyl acetate:hexanes, 1:6). The ^{1}H NMR and ^{13}C NMR data matched those previously reported.[38]

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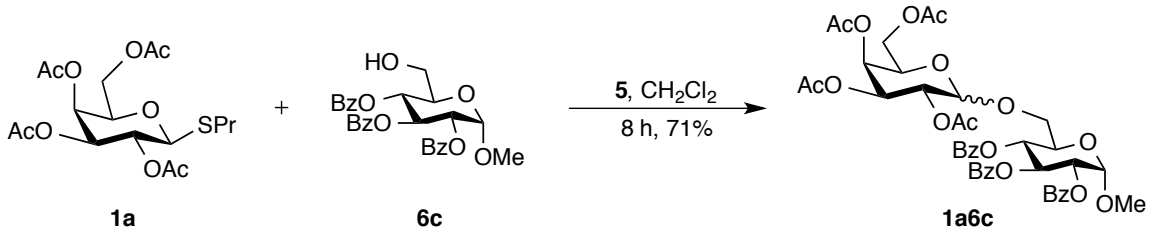
Entry 8:

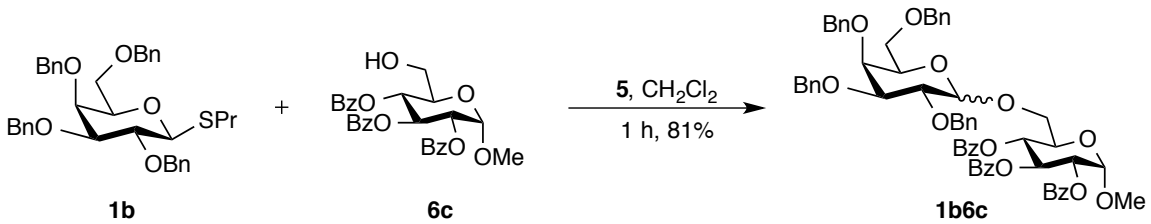
Methyl-(2',3',4',6'-tetra-*O*-acetyl-D-galactopyranosyl)-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-glucopyranoside (1a6c)



1a (0.038 g, 0.093 mmol) and **6c**[39] (0.042 g, 0.084 mmol) were reacted in presence of **5** (0.069 g, 0.093 mmol) to yield **1a6c**[40] (0.049 g, 0.059 mmol) as a pale white foam after purification by silica gel column chromatography (Ethyl acetate:hexanes, 1:3). The ^{1}H NMR, ^{13}C NMR, HRMS data matched those previously reported.[40]

Entry 9:

Methyl-(2',3',4',6'-tetra-*O*-benzyl-D-galactopyranosyl)-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-glucopyranoside (1b6c)



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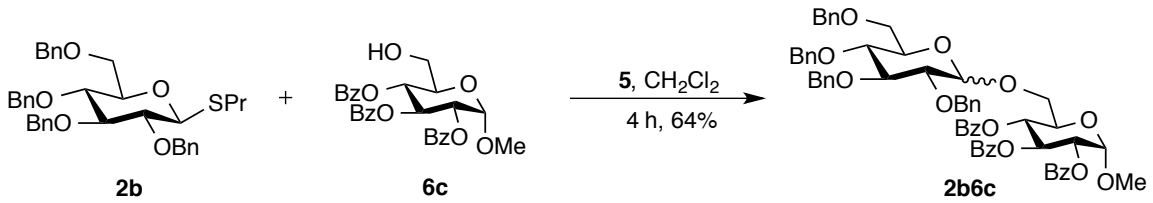
1b (0.030 g, 0.050 mmol) and **6c** (0.023 g, 0.045 mmol) were reacted in presence of **5** (0.037 g, 0.050 mmol) to yield **1b6c**[38] (0.037 g, 0.036 mmol) as a pale white gel after purification by silica gel column chromatography (Ethyl acetate:hexanes, 1:3). The ^{1}H NMR and ^{13}C NMR data matched those previously reported.[38]

Entry 10:

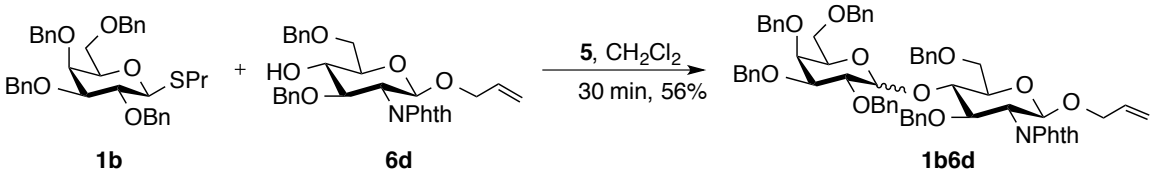
Methyl-(2',3',4',6'-tetra-*O*-benzyl-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-glucopyranoside (2b6c)



1b (0.030 g, 0.050 mmol) and **6c** (0.023 g, 0.045 mmol) were reacted in presence of **5** (0.037 g, 0.050 mmol) to yield **2b6c**[41] (0.029 g, 0.028 mmol) as a pale white gel after purification by silica gel column chromatography (Ethyl acetate:hexanes, 1:3). The ^{1}H NMR and ^{13}C NMR data matched those previously reported.[41]

Entry 11:

Allyl-(2',3',4',6'-tetra-*O*-benzyl-D-galactopyranosyl)(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-*N*-phthalimido-D-glucopyranoside (1b6d)



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1b (0.035 g, 0.058 mmol) and **6d**[42] (0.028 g, 0.052 mmol) were reacted in presence of **5** (0.043 g, 0.058 mmol) to yield **1b6d** (0.039 g, 0.037 mmol) as a pale white gel after purification by silica gel column chromatography (Ethyl acetate:hexanes; 1:5). R_f 0.64 (Ethyl acetate:hexanes 1:3); $[\alpha]_D$ +71.2 $cm^3\ g^{-1}dm^{-1}$ (c 0.13 g cm^{-3}, $CHCl_3$); ^{1}H NMR (600 MHz, [D-3] CD_3CN, 25 ^oC, TMS) δ = 7.83 – 7.69 (m, 4H; N*Phth*), 7.43 – 7.21 (m, 26H; 5×*Ph*CH_2O, *Ph(H-4)*CH_2OC-3), 6.90 – 6.81 (m, 4H; *Ph(H-2,3)*CH_2OC-3), 5.71 (ddd, *J*=16.5, 11.0, 5.5, 1H; OCH_2CH=CH_2), 5.13 (d, *J*=8.5, 1H; H-1), 5.11 – 4.94 (m, 2H; OCH_2CH=CH_2), [4.84 (d, *J*=11.4), 4.80 – 4.75 (m), 4.74-4.68 (m), 4.3 – 4.51 (m), 9H; PhCH_2O], 4.50 (d, *J*=3.8, 1H; H-1'), [4.47 – 4.45 (2s), 4.41 (d, *J*=11.8), 4.36 (d, *J*=11.8), 4.31 (d, *J*=7.1), 3H; PhCH_2O], 4.26 – 4.16 (m, 2H; 1× OCH_2CH=CH_2, H-4'), 4.06 – 4.01 (m, 1H; H-2'), [4.00 – 3.93 (m) , 3.93 – 3.89 (m), 3.87 (dd, *J*=10.9, 3.8), 4H; H6a'6b', H-2,4], 3.72 (dd, *J*=10.9, 1.8, 1H; 1× OCH_2CH=CH_2), 3.66 – 3.56 (m, 3H; H-3,3', H6a), 3.54 – 3.46 (m, 3H; H-5,5', H6b); ^{13}C NMR (151 MHz, [D-3] CD_3CN, 25 ^oC, TMS) 138.25, 138.22, 135.24, 135.15, 135.00, 134.97, 132.71, 132.51 (10C; C_{Phth}, C_{Ph}-C-1), 131.26 (1C; OCH_2CH=CH_2), 129.21, 129.16, 129.06, 129.00, 128.95, 128.87, 128.85, 128.77, 128.66, 128.60, 128.55, 128.48, 128.41, 128.38, 128.36, 128.31, 128.24, 128.13, 127.97 (30C; C_{Ph}-C 2,3,4), 124.01 (2C; C_{Phth}), 117.29 (1C; OCH_2CH=CH_2), 103.44 (1C; C-1), 98.36 (1C; C-1'), 82.84, 80.64, 78.07, 77.83 (4C; C-2',3',4',4), 75.66, 75.30, 75.05, 74.90, 74.21, 74.03, 73.74, 73.54, 73.10, 72.92 (9C; 6×PhCH_2O, C-3,5,5'), 70.34, 69.47, 68.83 (3C; C6a'b',C6ab, OCH_2CH=CH_2), 56.53(1C; C-2); HRMS (ESI-QTOF) *m/z* calc. for $C_{65}H_{65}NO_{12}Et_3NH^+$ 1153.5790 found 1153.5721, *m/z* calc. for $C_{65}H_{65}NO_{12}Na^+$ 1074.4404 found 1074.4343.

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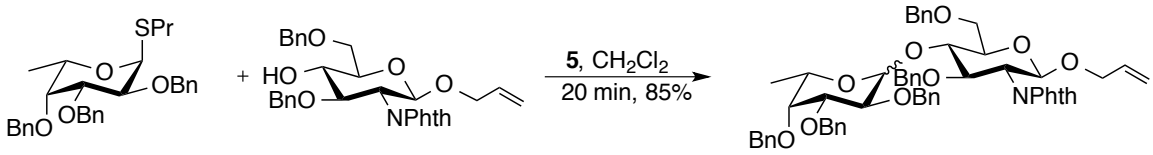
Entry 12:

Allyl-(2',3',4'-tri-*O*-benzyl-L-fucopyranosyl)(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-*N*-phthalimido-D-glucopyranoside (3a6d)



3a (0.030 g, 0.060 mmol) and **6d** (0.028 g, 0.054 mmol) were reacted in presence of **5** (0.044 g, 0.060 mmol) to yield **3a6d** (0.043 g, 0.046 mmol) as a white foam after purification by silica flash silica gel column chromatography (Ethyl acetate:hexanes, 1:5); R_f 0.54 (Ethyl acetate:hexanes 3:1); $[\alpha]_D$ - 54.2 $cm^3\ g^{-1}dm^{-1}$ (c 0.13 g cm^{-3}, $CHCl_3$); ^1H NMR (600 MHz, [D-3] CD_3CN, 25 ^oC, TMS) δ = 7.79 – 7.74 (m, 4H; N*Phth*), 7.41 – 7.23 (m, 20H; 4×*Ph*CH_2O), 6.97 (dd, *J*=41.6, 7.1, 2H; *Ph(H-2)*CH_2OC-3), 6.88 (d, *J*=7.0, 2H; *Ph(H-1)*CH_2OC-3), 6.74 (d, *J*=4.2, 1H; *Ph(H-3)*CH_2OC-3), 5.70 (m, 1H; $OCH_2CH{=}CH_2$), 5.15 (d, *J*=8.5, 1H; H-1β), 5.11 (d, *J*=8.48, 1H; H-1'β), 5.09 (d, *J*=3.41, H-1α), 5.07–4.91 (m, 2H; $OCH_2CH{=}CH_2$), 4.84 (d, *J*=11.3, 1H; H-1'α), 4.80 – 4.69, 4.65 – 4.58, 4.55, 4.53, 4.5, (m, 8H; $PhCH_2O$), 4.36 (dd, *J*=10.8, 8.5, 1H; H-2'), 4.32 – 4.26 (m, 1H; $PhCH_2O$) 4.17 (dtt, *J*=13.3, 5.2, 1.6, 1H; $OCH_2CH{=}CH_2$), 4.17 (d, *J*=18.2, 1H; $PhCH_2O$), 4.01 (dd, *J*= 11.29,1.88, 1H; H-3'), 3.97 (ddt, *J*=11.9, 6.1, 1.5, 1H; $OCH_2CH{=}CH_2$), 3.92 (m, 1H; H-2) 3.87 (dd, *J*=10.3, 3.5, 1H; H-4'), 3.82 (m, 1H; H6a6b), 3.74 (m, 1H; H-5'), 3.67 (m, 1H; H6a6b), 3.56 (m, 2H; H-4,5) 3.50 (dd, *J*=6.5, 1.2, 1H; H-3) 1.18 [(d, *J*=6.4, 1H)-0.84 (d, *J*=6.5, 2H), 3H; CH_3]; ^{13}C NMR (151 MHz, [D-3] CD_3CN, 25 ^oC, TMS) δ 140.22, 139.99, 139.96, 139.87,

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139.11, 139.07 (5C; C_{Ph}-C1) 135.40, 135.33, 135.07 (4C; C_{Phth}), 132.32 (1C; $OCH_2\underline{C}H=CH_2$),129.31, 129.28, 129.26, 129.20, 129.18, 129.13, 129.05, 129.02, 129.00, 128.94, 128.86, 128.69, 128.67, 128.61, 128.56, 128.54, 128.44, 128.33, 128.31, 128.27, 128.18 (20C; C_{Ph}-C 2,3,4), 124.19, 124.14 (2C, C_{Phth}), 117.37 (1C, $OCH_2CH=\underline{C}H_2$), 103.45 (1C; C-1), 98.40, 98.19 (1C; C-1'), 83.23, 81.11, 80.36, 80.21 (4C; C-2',3',4',4), 79.71, 78.76, 78.14, 76.11, 75.71, 75.59, 75.57, 74.56, 73.76, 72.90 (5C; $Ph\underline{C}H_2O$), 71.32, 70.77, 70.48, 70.46, 69.84, 67.78 (4C; C-3,5,6, $O\underline{C}H_2CH=CH_2$), 57.04 (1C; C-2') 16.92, 16.76 (1C; $\underline{C}H_3$); HRMS (ESI-QTOF) *m/z* calc. for $C_{58}H_{59}N_2O_{11}Et_3NH^+$ 1047.5370 found 1047.5400, *m/z* calc. for $C_{58}H_{59}N_2O_{11}Na^+$ 968.3985 found 968.3994.

Promoter equivalence studies (Table 2)

A 0.5 M solution of thioglycoside donor **1b** (0.030 g, 0.050 mmol) and acceptor **6a** (0.0029 g, 0.050 mmol) was stirred together in anhydrous CH_2Cl_2 for 0.5 h in a flask filled with Ar at room temperature. To it a solution of the promoter **5** (see below for amounts) in anhydrous CH_2Cl_2 was added and the reaction was stirred at room temperature for 1 h. The progress was monitored by TLC and then the reaction was quenched by triethylamine, filtered through a Celite pad, concentrated under vacuum and the product purified by silica gel column chromatography.

Entry 1: same as table 1 entry 2.

2: promoter **5** (0.026 g, 0.035 mmol),

product **1b6a** obtained (0.024 g, 0.041 mmol, 82%)

3: promoter **5** (0.018 g, 0.025 mmol),

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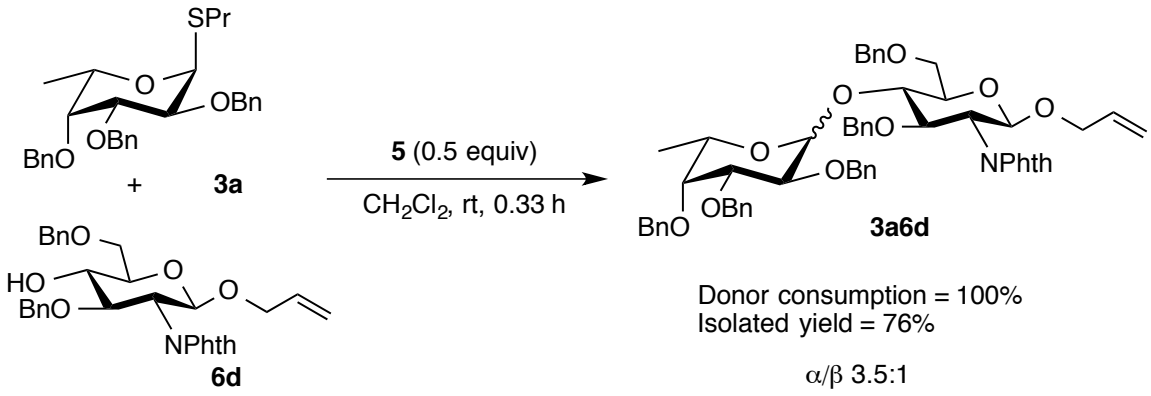
product **1b6a** obtained (0.022 g, 0.038 mmol, 76%)

4: promoter **5** (0.011 g, 0.015 mmol),

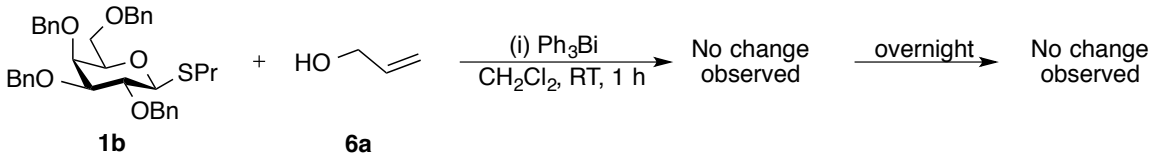
product **1b6a** obtained (0.020 g, 0.034 mmol, 68%)



3a (0.030 g, 0.060 mmol) and **6d** (0.028 g, 0.054 mmol) were reacted in presence of **2** (0.022 g, 0.030 mmol) to yield **3a6d** (0.038 g, 0.041 mmol) as a white foam after purification by silica flash silica gel column chromatography (Ethyl acetate:hexanes, 1:5); R_f 0.54 (Ethyl acetate:hexanes 3:1). The ^{1}H NMR and ^{13}C NMR matched with table 1 entry 12.

Control experiments

(i)



A 0.5 M solution of thioglycoside donor (0.030 g, 0.050 mmol) and acceptor (0.0029 g, 0.05 mmol) were stirred together in anhydrous dichloromethane (CH_2Cl_2) for 0.5 h in a

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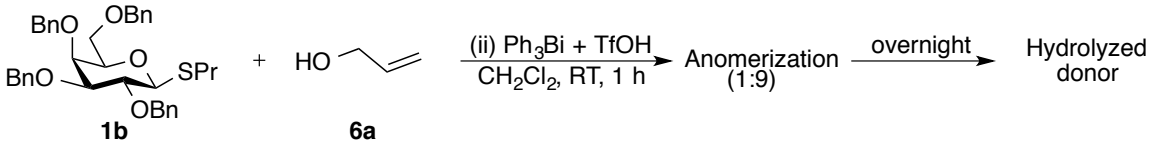
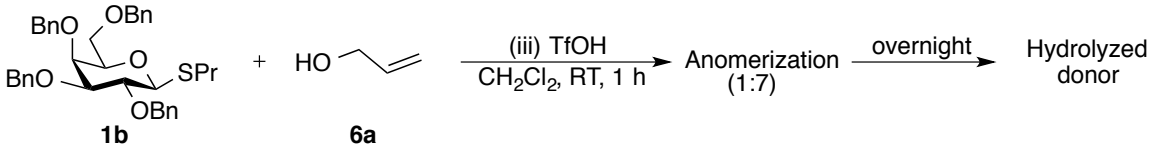
flask filled with Ar at room temperature. To it a solution of the triphenyl bismuth (Ph_3Bi, 0.022 g, 0.050 mmol, 1 equiv.) in anhydrous CH_2Cl_2 was added and the reaction was monitored by TLC. There was no change seen in the donor consumption and no product formation was observed after an overnight reaction time.

(ii)



A 0.5 M solution of thioglycoside donor (0.030 g, 0.050 mmol) and acceptor (0.0029 g, 0.050 mmol) were stirred together in anhydrous dichloromethane (CH_2Cl_2) for 0.5 h in a flask filled with Ar at room temperature. To it a solution of Ph_3Bi (0.022 g, 0.050 mmol) and TfOH (0.0075 g, 0.050 mmol) in anhydrous CH_2Cl_2 was added and the reaction was monitored by TLC. After 1 h, a slight anomerization of the β anomer to α anomer was observed, which increased after an overnight reaction time. No product formation was observed by TLC. On the other hand, the hydrolyzed donor was found to be the only product in the end.

(iii)



A 0.5 M solution of thioglycoside donor (0.030 g, 0.050 mmol) and acceptor (0.0029 g, 0.050 mmol) were stirred together in anhydrous dichloromethane (CH_2Cl_2) for 0.5 h in a flask filled with Ar at room temperature. To the reaction mixture, TfOH (0.0070 g, 0.050

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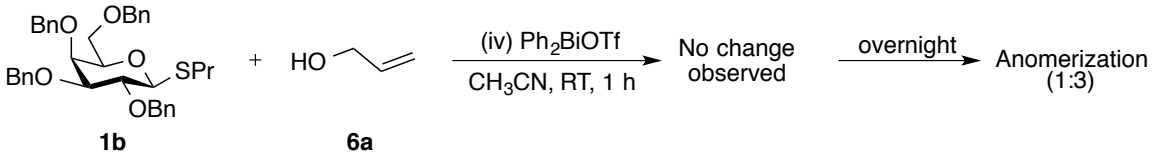
mmol) was added and reaction was monitored by TLC. There was no product formation after an overnight reaction. In this case as well, the hydrolyzed donor was found to the only product.

(iv)



A 0.5 M solution of thioglycoside donor (0.030 g, 0.050 mmol) and acceptor (0.0029 g, 0.050 mmol) were stirred together in anhydrous acetonitrile (CH_3CN) for 0.5 h in a flask filled with Ar at room temperature. To the reaction mixture, Ph_2BiOTf (0.026 g, 0.050 mmol) was added and the reaction was monitored by TLC. There was no change observed after 1 h, although anomerization of the β anomer to α anomer was observed after an overnight reaction time. No product formation was observed in this case as well.

2.5. REFERENCES

1. (a) McKay, M. J.; Nguyen, H. M., Recent Advances in Transition Metal-Catalyzed Glycosylation. *ACS Catal.* **2012,** *2* (8), 1563-1595; (b) Li, X.; Zhu, J., Recent Advances in Transition Metal–Catalyzed O-Glycosylations. *J. Carbohydr. Chem.* **2012,** *31* (4-6), 284-324.

2. (a) Fügedi, P., *The Organic Chemistry of Sugars*. CRC: Boca Raton, FL, 2005; (b) Oscarson, S.; Ernst, B.; Hart, G. W.; Sinaý, P., Thioglycosides. In *Carbohydrates in Chemistry and Biology*, Wiley-VCH Verlag GmbH: 2008; pp 93-116.

3. (a) Agocs, L.; Burford, N.; Cameron, T. S.; Curtis, J. M.; Richardson, J. F.; Robertson, K. N.; Yhard, G. B., Spectroscopic, Structural, and Mass Spectrometric Studies on Two Systematic Series of Dithiabismuth(III) Heterocycles: Identification of Bismuthenium Cations and Their Solvent Complexes. *J. Am. Chem. Soc.* **1996,** *118* (13), 3225-3232; (b) Burford, N.; Eelman, M. D.; Mahony,

www.manaraa.com

D. E.; Morash, M., Definitive identification of cysteine and glutathione complexes of bismuth by mass spectrometry: assessing the biochemical fate of bismuth pharmaceutical agents. *Chem Commun (Camb)* **2003,** *81* (1), 146-7; (c) Ge, R.; Sun, H., Bioinorganic Chemistry of Bismuth and Antimony: Target Sites of Metallodrugs. *Acc. Chem. Res.* **2007,** *40* (4), 267-274.

4. (a) Pearson, R. G., Hard and Soft Acids and Bases. *J. Am. Chem. Soc.* **1963,** *85* (22), 3533-3539; (b) Rueping, M.; Nachtsheim, B., Bismuth Salts in Catalytic Alkylation Reactions. In *Bismuth-Mediated Organic Reactions*, Ollevier, T., Ed. Springer Berlin Heidelberg: 2012; Vol. 311, pp 115-141; (c) Sanderson, J.; Bayse, C. A., The Lewis acidity of bismuth(III) halides: a DFT analysis. *Tetrahedron* **2008,** *64* (33), 7685-7689.

5. Suzuki, H., Chapter 1 - Introduction. In *Organobismuth Chemistry*, Suzuki, H.; Matano, Y., Eds. Elsevier Science: Amsterdam, 2001; pp 1-20.

6. Salvador, J. A. R.; Silvestre, S. M.; Pinto, R. M. A.; Santos, R. C.; LeRoux, C., New Applications for Bismuth(III) Salts in Organic Synthesis: From Bulk Chemicals to Steroid and Terpene Chemistry. In *Bismuth-Mediated Organic Reactions*, Ollevier, T., Ed. Springer Berlin Heidelberg: 2012; Vol. 311, pp 143-177.

7. Mohan, R., Green bismuth. *Nat. Chem.* **2010,** *2* (4), 336-336.

8. (a) Briand, G. G.; Burford, N., Bismuth Compounds and Preparations with Biological or Medicinal Relevance. *Chem. Rev. (Washington, DC, U. S.)* **1999,** *99* (9), 2601-2658; (b) Kotani, T.; Nagai, D.; Asahi, K.; Suzuki, H.; Yamao, F.; Kataoka, N.; Yagura, T., Antibacterial Properties of Some Cyclic Organobismuth(III) Compounds. *Antimicrob. Agents Chemother.* **2005,** *49* (7), 2729-2734; (c) Mahony, D. E.; Lim-Morrison, S.; Bryden, L.; Faulkner, G.; Hoffman, P. S.; Agocs, L.; Briand, G. G.; Burford, N.; Maguire, H., Antimicrobial Activities of Synthetic Bismuth Compounds against Clostridium difficile. *Antimicrob. Agents Chemother.* **1999,** *43* (3), 582-588.

9. (a) Bothwell, J. M.; Krabbe, S. W.; Mohan, R. S., Applications of bismuth(III) compounds in organic synthesis. *Chem. Soc. Rev.* **2011,** *40* (9), 4649-4707; (b) Matano, Y.; Ikegami, T., Chapter 2 - Organobismuth(III) Compounds. In *Organobismuth Chemistry*, Suzuki, H.; Matano, Y., Eds. Elsevier Science: Amsterdam, 2001; pp 21-245; (c) Ollevier, T., New trends in bismuth-catalyzed synthetic transformations. *Org. Biomol. Chem.* **2013,** *11* (17), 2740-2755.

10. Valerio, S.; Iadonisi, A.; Adinolfi, M.; Ravidà, A., Novel Approaches for the Synthesis and Activation of Thio- and Selenoglycoside Donors. *J. Org. Chem* **2007,** *72* (16), 6097-6106.

www.manaraa.com

11. (a) Harris, B. N.; Patel, P. P.; Gobble, C. P.; Stark, M. J.; De Meo, C., C-5 Modified S-Benzoxazolyl Sialyl Donors: Towards More Efficient Selective Sialylations. *Eur. J. Org. Chem.* **2011,** *2011* (20-21), 4023-4027; (b) Kaeothip, S.; Pornsuriyasak, P.; Rath, N. P.; Demchenko, A. V., Unexpected Orthogonality of S-Benzoxazolyl and S-Thiazolinyl Glycosides: Application to Expeditious Oligosaccharide Assembly. *Org. Lett.* **2009,** *11* (4), 799-802.

12. Satoh, H.; Hansen, H. S.; Manabe, S.; van Gunsteren, W. F.; Hünenberger, P. H., Theoretical Investigation of Solvent Effects on Glycosylation Reactions: Stereoselectivity Controlled by Preferential Conformations of the Intermediate Oxacarbenium-Counterion Complex. *J. Chem. Theory Comput.* **2010,** *6* (6), 1783-1797.

13. Gürbüz, O.; Rouseff, J.; Talcott, S. T.; Rouseff, R., Identification of Muscadine Wine Sulfur Volatiles: Pectinase versus Skin-Contact Maceration. *J. Agric. Food Chem.* **2013,** *61* (3), 532-539.

14. Ferrier, R. J.; Furneaux, R. H., Synthesis of 1,2-trans-related 1-thioglycoside esters. *Carbohydr. Res.* **1976,** *52* (1), 63-68.

15. Silvestru, C.; Breunig, H. J.; Althaus, H., Structural Chemistry of Bismuth Compounds. I. Organobismuth Derivatives. *Chem. Rev.* **1999,** *99* (11), 3277-3328.

16. Hamaed, H.; Laschuk, M. W.; Terskikh, V. V.; Schurko, R. W., Application of Solid-State 209Bi NMR to the Structural Characterization of Bismuth-Containing Materials. *J. Am. Chem. Soc.* **2009,** *131* (23), 8271-8279.

17. (a) Luan, J.; Zhang, L.; Hu, Z., Synthesis, Properties Characterization and Applications of Various Organobismuth Compounds. *Molecules* **2011,** *16* (5), 4191-4230; (b) Matano, Y., Pentavalent Organobismuth Reagents in Organic Synthesis: Alkylation, Alcohol Oxidation and Cationic Photopolymerization. In *Bismuth-Mediated Organic Reactions*, Ollevier, T., Ed. Springer Berlin Heidelberg: 2012; Vol. 311, pp 19-44; (c) Matano, Y.; Ikegami, T., Chapter 3 - Organobismuth(V) Compounds. In *Organobismuth Chemistry*, Suzuki, H.; Matano, Y., Eds. Elsevier Science: Amsterdam, 2001; pp 247-327.

18. Zuurmond, H. M.; van der Laan, S. C.; van der Marel, G. A.; van Boom, J. H., Iodonium ion-assisted glycosylation of alkyl (aryl) 1-thio-glycosides: regulation of stereoselectivity and reactivity. *Carbohydr. Res.* **1991,** *215* (2), C1-C3.

19. (a) Gigg, J.; Gigg, R.; Payne, S.; Conant, R., The allyl group for protection in carbohydrate chemistry. Part 20. Synthesis of 1L-1-O-methyl-myo-inositol [(+)-bornesitol] by resolution of (+/-)-1,2,4-tri-O-benzyl-myo-inositol. *Journal of the Chemical Society, Perkin Transactions 1* **1987,** (0), 1757-1762; (b) Mootoo, D. R.;

www.manaraa.com

Date, V.; Fraser-Reid, B., n-Pentenyl glycosides permit the chemospecific liberation of the anomeric center. *J. Am. Chem. Soc.* **1988,** *110* (8), 2662-2663.

20. (a) Fraser-Reid, B.; Wu, Z.; Andrews, C. W.; Skowronski, E.; Bowen, J. P., Torsional effects in glycoside reactivity: saccharide couplings mediated by acetal protecting groups. *J. Am. Chem. Soc.* **1991,** *113* (4), 1434-1435; (b) Fraser-Reid, B.; Wu, Z.; Udodong, U. E.; Ottosson, H., Armed/disarmed effects in glycosyl donors: rationalization and sidetracking. *J. Org. Chem* **1990,** *55* (25), 6068-6070; (c) Mootoo, D. R.; Konradsson, P.; Udodong, U.; Fraser-Reid, B., Armed and disarmed n-pentenyl glycosides in saccharide couplings leading to oligosaccharides. *J. Am. Chem. Soc.* **1988,** *110* (16), 5583-5584.

21. (a) Flowers, H. M., Effect of a nucleophilic substituent on the stero-chemistry of the koenigs-knorr reaction. stereospecific, synthesis of some a and β -linked disaccharides of l-fucose. *Carbohydr. Res.* **1979,** *74* (1), 177-185; (b) Staudacher, E.; 西原, 祥., α 1,3-フコース転移酵素群. *Trends in Glycoscience and Glycotechnology* **1996,** *8* (44), 391-408.

22. (a) Krog-Jensen, C.; Oscarson, S., Synthesis of d-Fructofuranosides Using Thioglycosides as Glycosyl Donors. *J. Org. Chem* **1996,** *61* (4), 1234-1238; (b) Oscarson, S.; Tedebark, U.; Turek, D., Synthesis of colitose-containing oligosaccharide structures found in polysaccharides from Vibrio cholerae O139 synonym Bengal using thioglycoside donors. *Carbohydr. Res.* **1997,** *299* (3), 159-164.

23. Miljković, M., Relative Reactivity of Hydroxyl Groups in Monosaccharides. In *Carbohydrates*, Springer New York: 2009; pp 113-142.

24. Beckmann, J.; Bolsinger, J.; Duthie, A.; Finke, P.; Lork, E.; Lüdtke, C.; Mallow, O.; Mebs, S., Mesityltellurenyl Cations Stabilized by Triphenylpnictogens [MesTe(EPh3)]+ (E = P, As, Sb). *Inorg. Chem.* **2012,** *51* (22), 12395-12406.

25. David, S.; Thieffry, A., Conversion of one hydroxy group in a diol to a phenyl ether with triphenylbismuth diacetate, a new glycol reaction showing strong axial preference in six-membered rings. *J. Org. Chem.* **1983,** *48* (4), 441-447.

26. Combes, S.; Finet, J.-P., Triarylbismuthane - iodobenzene diacetate: One-pot system for copper-catalysed N-arylation under neutral conditions. *Tetrahedron* **1998,** *54* (17), 4313-4318.

27. Mukthavaram, R.; Marepally, S.; Venkata, M. Y.; Vegi, G. N.; Sistla, R.; Chaudhuri, A., Cationic glycolipids with cyclic and open galactose head groups for the selective targeting of genes to mouse liver. *Biomaterials* **2009,** *30* (12), 2369-2384.

www.manaraa.com

28. Bogusiak, J.; Szeja, W., Synthesis of 1-thioglycosides in a catalytic two-phase system. *Pol. J. Chem.* **1985,** *59* (3), 293–298.

29. Wolfrom, M. L.; Thompson, A., Reaction of carbohydrates, Methods in Carbohydrate Chemistry Academic Press: New York, 1963; Vol. II, pp 211–215.

30. Fujihira, T.; Takido, T.; Seno, M., Synthesis of alkyl and arylthioglycosides and thiodisaccharides via thioiminium salts in a two-phase system. *J. Mol. Catal. A: Chem.* **1999,** *137* (1–3), 65-75.

31. Nashed, M. A.; Anderson, L., Oligosaccharides from "standardized intermediates." Synthesis of a branched tetrasaccharide glycoside related to the blood group B determinant. *J. Am. Chem. Soc.* **1982,** *104* (25), 7282-7286.

32. Griffin, Frank K.; Paterson, Duncan E.; Murphy, Paul V.; Taylor, Richard J. K., A New Route to exo-Glycals Using the Ramberg−Bäcklund Rearrangement. *Eur. J. Org. Chem.* **2002,** *2002* (7), 1305-1322.

33. Khamsi, J.; Ashmus, R. A.; Schocker, N. S.; Michael, K., A high-yielding synthesis of allyl glycosides from peracetylated glycosyl donors. *Carbohydr. Res.* **2012,** *357* (0), 147-150.

34. Zeng, J.; Vedachalam, S.; Xiang, S.; Liu, X.-W., Direct C-Glycosylation of Organotrifluoroborates with Glycosyl Fluorides and Its Application to the Total Synthesis of (+)-Varitriol. *Org. Lett.* **2010,** *13* (1), 42-45.

35. Alper, P. B.; Hendrix, M.; Sears, P.; Wong, C.-H., Probing the Specificity of Aminoglycoside−Ribosomal RNA Interactions with Designed Synthetic Analogs. *J. Am. Chem. Soc.* **1998,** *120* (9), 1965-1978.

36. Mukhopadhyay, B.; Maurer, S. V.; Rudolph, N.; van Well, R. M.; Russell, D. A.; Field, R. A., From Solution Phase to “On-Column” Chemistry: Trichloroacetimidate-Based Glycosylation Promoted by Perchloric Acid−Silica. *J. Org. Chem* **2005,** *70* (22), 9059-9062.

37. Nguyen, H. M.; Chen, Y.; Duron, S. G.; Gin, D. Y., Sulfide-Mediated Dehydrative Glycosylation. *J. Am. Chem. Soc.* **2001,** *123* (36), 8766-8772.

38. Lu, S.-R.; Lai, Y.-H.; Chen, J.-H.; Liu, C.-Y.; Mong, K.-K. T., Dimethylformamide: An Unusual Glycosylation Modulator. *Angewandte Chemie International Edition* **2011,** *50* (32), 7315-7320.

39. Zhang, F.; Zhang, W.; Zhang, Y.; Curran, D. P.; Liu, G., Synthesis and Applications of a Light-Fluorous Glycosyl Donor. *J. Org. Chem* **2009,** *74* (6), 2594-2597.

www.manaraa.com

40. Lucas-Lopez, C.; Murphy, N.; Zhu, X., Catalytic Glycosylation with Glycosyl Thioimidate Donors. *Eur. J. Org. Chem.* **2008,** *2008* (26), 4401-4404.

41. Hashimoto, S.-i.; Umeo, K.; Sano, A.; Watanabe, N.; Nakajima, M.; Ikegami, S., An extremely mild and stereocontrolled construction of 1,2-trans-β-glycosidic linkages capitalizing on benzyl-protected glycopyranosyl diethyl phosphites as glycosyl donors. *Tetrahedron Lett.* **1995,** *36* (13), 2251-2254.

42. Wang, L.-X.; Li, C.; Wang, Q.-W.; Hui, Y.-Z., Chemical synthesis of NodRm-1: the nodulation factor involved in Rhizobium meliloti-legume symbiosis. *Journal of the Chemical Society, Perkin Transactions 1* **1994,** (6), 621-628.

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CHAPTER 3. BISMUTH(V) ACTIVATION OF ALKYNES: APPLICATIONS TO CARBOHYDRATE FUNCTIONALIZATION OF PENTABLOCK COPOLYMERS AS POTENTIAL VACCINE ADJUVANTS

Collaboration with Justin R. Adams, Surya K. Mallapragada, Department of Chemical Engineering, Iowa State University, Ames IA 50011

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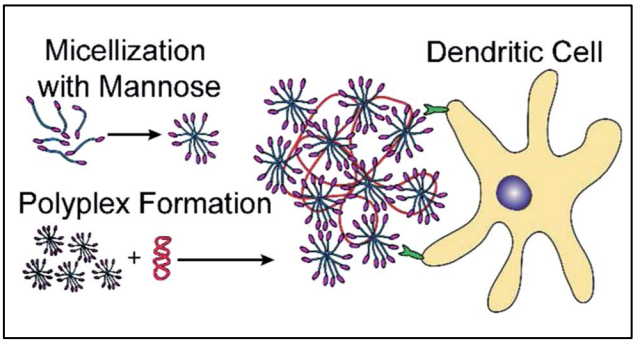

Abstract

A family of amphiphilic pentablock polymers with different cationic blocks and with controlled architectures as potential vaccine carriers for subunit vaccines has been developed and their properties as a gene and protein delivery vehicle has been studied by Mallapragada *et al*. Modification of other polymer systems with carbohydrates like mannose has been shown to enhance immunogenicity by activating pattern recognition receptors on antigen presenting cells and increasing uptake in these cells. Here, we report the synthesis of a virus-mimicking pentablock copolymer vaccine platform by successful functionalization of these polymers with mannose through an azide-alkyne Huisgen cycloaddition. The synthesis of a mannoside with the alkyne linker was achieved by a recently reported bismuth(V)-mediated activation of a thioglycoside that left the alkyne intact. The carbohydrate modification was shown not to interfere with the ability of these virus-mimicking block copolymers to complex DNA, thereby making this family of modified materials promising candidates for DNA-based vaccine delivery.

1. *RSC Adv*., 2014, *4*, 15655–15663; Reproduced by permission of The Royal Society of Chemistry http://pubs.rsc.org/en/content/articlelanding/2014/ra/c3ra47687a - !divAbstract

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3.1. INTRODUCTION

3.1.1. Importance of alkenes and alkynes in organic synthesis

Among different classes of functional groups seen in synthetic organic chemistry, alkenes constitute one of the most significant categories. In carbohydrate chemistry, alkene functionality is regularly used as protecting groups for: alcohols like allyl ethers, prenyl ethers, alkyl allyl carbonates (Alloc–OR);[1] acids like allyl esters;[2] amines like *N*-prenyl amines, allyl carbamates, etc.[3] Unsaturated carbohydrates like glycals are used commonly in oligosaccharide synthesis to build *O*- or *C*-glycosides.[4] In chemical glycosylation, Fraser-Reid *et al.* developed the multifaceted *n*-pentenyl glycosyl donors,[5] while Boons and co-workers introduced alkenyl glycosides for latent-active glycosylations.[6] However, as mentioned previously, alkenyl groups have posed major challenges with thioglycosyl donors and their activation. Fortunately, we developed a unique bismuth(V) promoted thioglycoside activation protocol,[7] which could be applied to couple a variety of donors with alkene-containing acceptors. Unlike many thioglycoside activation methods that rely on promoter interactions with the soft sulfur that preclude the facile use of alkenes anywhere in the glycosyl donor or acceptor, this bismuth-mediated method was shown to work effectively in the presence of alkenyl groups.

Another essential functional group in chemical synthesis similar in reactivity to alkenes is the alkyne functionality. Like alkenes, they are found in many protecting groups for alcohols, acids and amines:[1-3] for e.g. propargyl ethers, prop-2-ynyl esters

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and propargyl carbamates respectively. More importantly, alkynes have become hugely popular functional groups in chemical synthesis as they can undergo Huisgen cycloaddition[8] with azides to form extremely stable cyclized products. This efficient click reaction has become quintessential in pharmaceutical, material and other industries as it can generate large libraries of compounds for screening in discovery research. Although alkynes are thermodynamically less stable than alkenes and can easily undergo hydrogenation to alkenes, they are less reactive towards electrophilic substitution reactions. Nevertheless, they can easily form various addition and rearranged products with electrophilic reagents like X_2 (Br_2, I_2), HX (HCl, HI), etc., which are commonly used promoters in glycosylation. Additionally, they can also undergo hydration in presence of acids to give keto-enol tautomers. Because of these reasons, they are difficult to handle during a glycosylation and general avoided in glycosyl donors or acceptors. They are also prone to rearrangement and eventual cleavage in presence of transition metals like Pd, Au, Sn etc.,[9] a feature which was recently used by Zhu *et al*. to activate S-prop-2-ynyl thioglycosides to prepare 2-deoxy glycosides.[10] Moreover, Sen and co-workers used I_2 as an efficient and chemoselective activator with glycosyl ortho-alkynylbenzoates donor in presence of thioglycosides,[11] which suggests the higher reactivity of alkynes towards electrophilic reagents.

After the initial success with alkenes, we wanted to further test the scope of our thioglycoside activation methodology in presence of alkynes. Fortunately, the Bi(V)-mediated method could be used successfully with alkynes and assisted in

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functionalization of virus-mimicking pentablock co-polymers with pathogen-associated carbohydrates via click chemistry.

3.1.2. Pentablock copolymers as potential vaccine adjuvants

Block copolymers based on Pluronics have been used in a variety of applications, including sustained drug delivery, therapeutic cellular transfection and as novel vaccine adjuvants.[12] Pluronic F127 is an FDA-approved surfactant that exhibits temperature and pH-dependent gelation properties that make it an ideal carrier for hydrophobic drugs such as paclitaxel, aspirin and antibiotics.[13] Pluronic F127 has a central hydrophobic chain of polyoxypropylene and two outer hydrophilic chains of polyoxyethylene.[14] The hydrophobic interactions between the collapsed polyoxypropylene blocks result in the formation of micelles.[15] There are several advantageous properties of Pluronic that make it suitable for antigen delivery including cellular uptake through endocytosis and high gene expression.[12c, 13d, 14, 16] There is also evidence that hydrophobic portions of synthetic polymers can initiate immune responses.[17]

The Mallapragada group has previously synthesized a family of cationic pentablock copolymers based on Pluronics for drug delivery.[18] The pentablock copolymers, which are synthesized using Pluronic macroinitiators and atom transfer radical polymerization (ATRP) of the outer cationic blocks, offer several critical advantages for DNA-based vaccine delivery. The amine groups on the pentablock copolymer outerblocks can form nanoscale complexes with DNA spontaneously as a result of electrostatic interactions.[19] Detailed studies with one of these pentablock

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copolymers with poly(2-diethylaminoethyl methacrylate) (PDEAEM) cationic blocks, have shown that the polymer micelles aggregate to form physical gels at high polymer concentrations. This enables the pentablock copolymers to serve as a unique multi-purpose platform to condense DNA, enable sustained gene delivery from the pentablock copolymer gels, and also provide combinational therapy of gene and drug co-delivery from the same platform.[15, 20] However, the effect of different cationic blocks on DNA complexation ability as well as cytotoxicity of the copolymers has not been investigated. To have a deeper understanding of the functions of various chain lengths on gene or drug-delivery, they synthesized four other pentablock copolymers based on Pluronic with end blocks of PDEAEM, poly(2-dimethylaminoethyl methacrylate), poly(2-diisopropylaminoethyl methacrylate) or poly-(tert-butylaminoethyl methacrylate) abbreviated PDMAEM, PDiPAEM and PtBAEM, respectively [work done by Justin Adams]. The PDEAEM and PDMAEM pentablock copolymers appeared to be the optimal subunit vaccine candidates due to their low cytotoxicity and relatively small Nitrogen/Phosphorous (N/P) ratios required for DNA condensation. The PDiPAEM pentablock copolymer was insoluble in water, requiring the use of slightly cytotoxic DMSO solvent or use of sonication, and did not complex with the DNA plasmid at relatively low N/P ratios. Although the P*t*BAEM pentablock copolymer complexed with DNA at a low N/P ratios, it proved to be highly cytotoxic.

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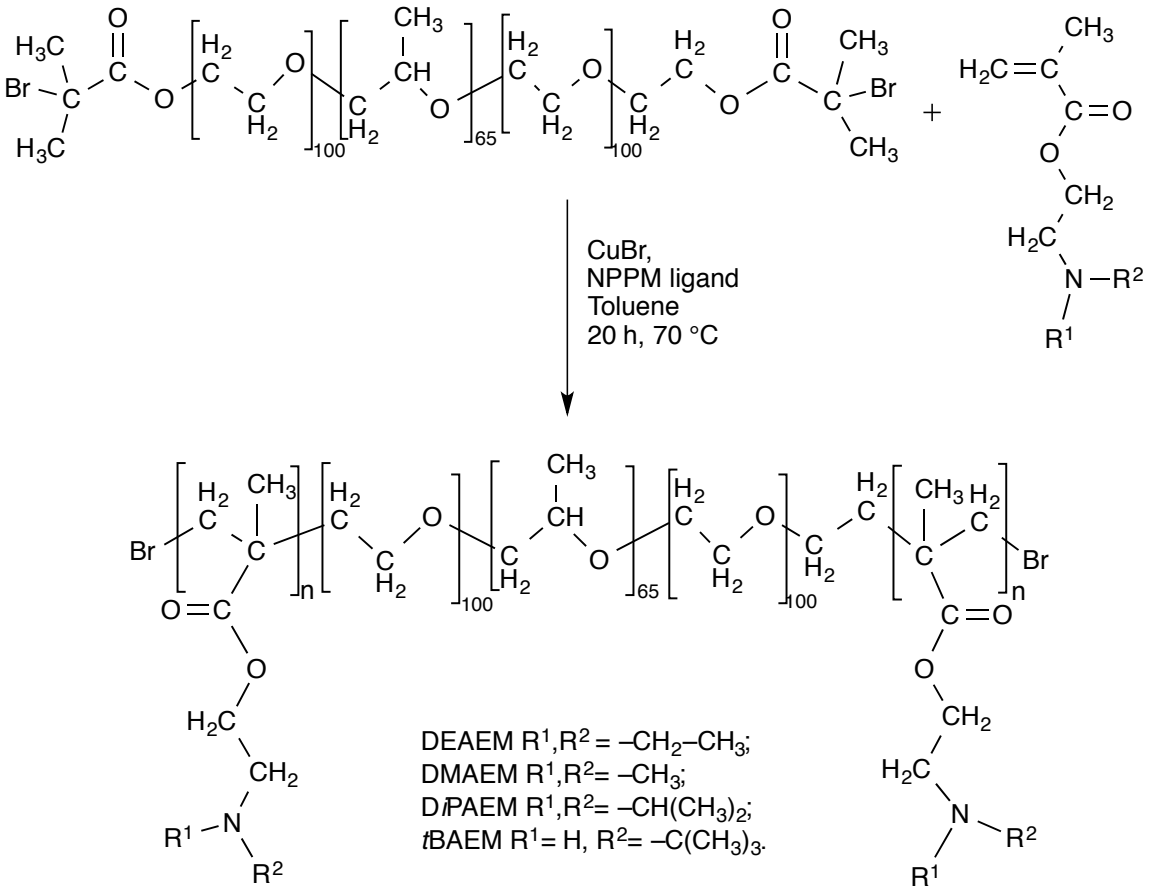

Scheme 1. *ATRP synthesis of block copolymer family (work done by Justin Adams)*

3.1.3. Modification with carbohydrates: advantages

To establish protective immunity, vaccines can enhance an innate immune response by activating antigen presenting cells (APCs).[21] Dendritic cells are a critical type of APCs that are present in all body tissues.[22] Immature dendritic cells can activate directly from pattern recognition receptors (PRRs) that recognize pathogen-associated molecular patterns (PAMPs).[22] Carbohydrates such as mannose have been shown to act as PAMPs, which can target carbohydrate-recognition recognition receptors, specifically C-type lectin receptors abundantly expressed on dendritic cells.[23] Ligation of C-type

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lectin receptors with mannose structures displayed by pathogen glycoproteins leads to internalization of pathogens for antigen loading of major histocompatibility molecules.[23b, 24] Therefore, nano-scale adjuvants with attached carbohydrates can act as virus-mimicking particles such as mannose containing HIV-1 and measles virus, and increase dendritic cell transfection efficiencies through receptor mediated endocytosis and presentation of processed antigen to T cells.[23c] **Figure 1** is a representation of the proposed interaction of mannose functionalized pentablock copolymer DNA polyplexes interacting with PRRs on a dendritic cell.

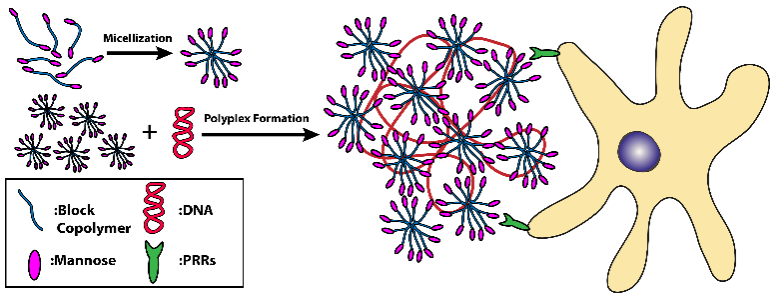

Figure 1. *Schematic diagram showing polyplex formation and interaction with dendritic cells*

3.2. RESULTS AND DISCUSSION

We utilized the simple and effective azide-alkyne Huisgen cycloaddition to couple the pathogen-associated carbohydrates, specifically D-mannose to the pentablock co-polymers. This was done by functionalizing the polymer with an azide group and the mannose with an alkyne linker. As previously reported,[25] bromo-terminated polyacrylates can be easily substituted by the nucleophilic azide group hence the halogen end groups of the pentablock copolymers were replaced by azide (**Scheme**

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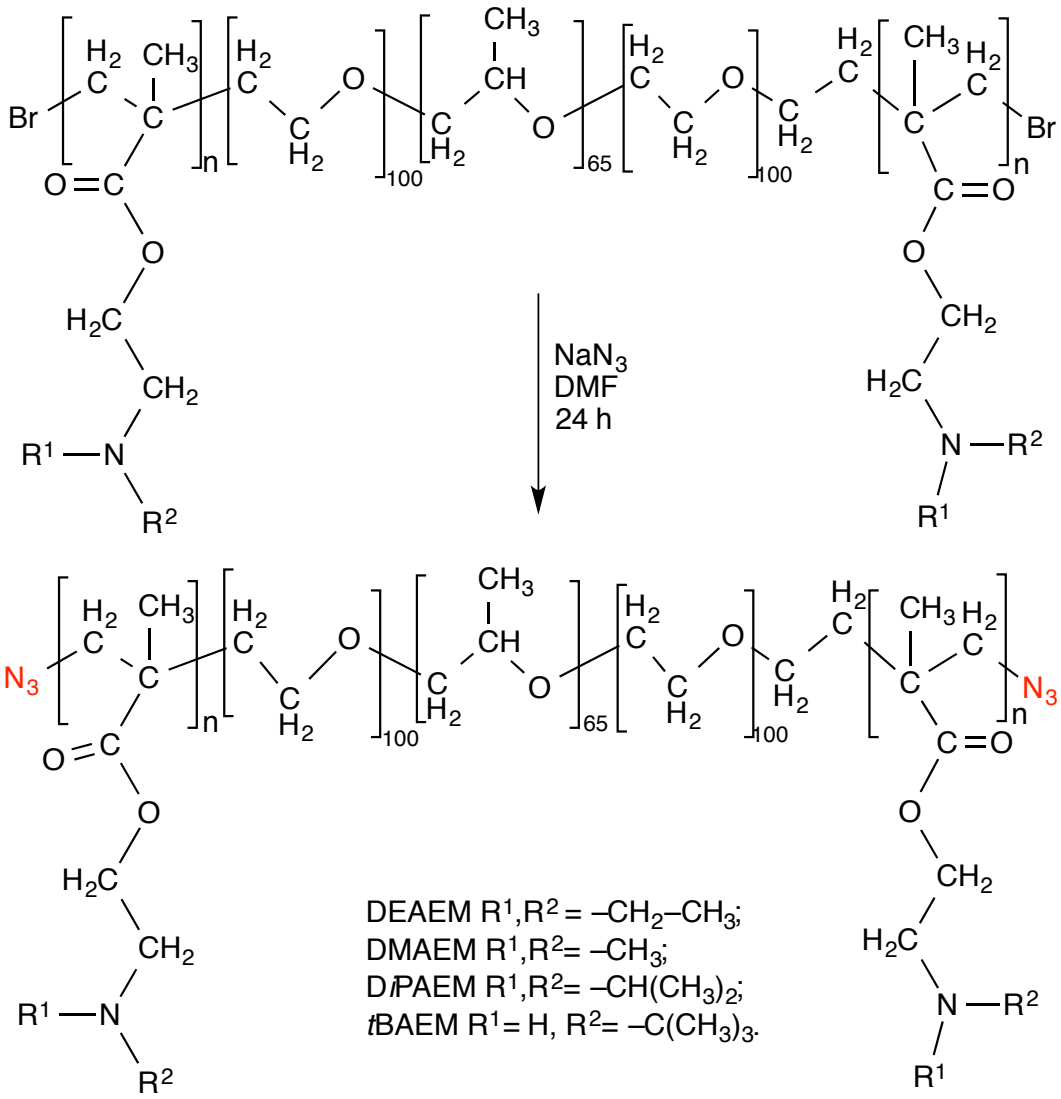
2). A distinct IR stretch band present between 2100 and 2300 cm^{-1} represents the azide functionalization of the pentablock copolymers (**Figure 2**) that was previously absent in the non-azide functionalized polymers [work done along with Justin Adams].



***Scheme 2**: Azide functionalization of block copolymer family*

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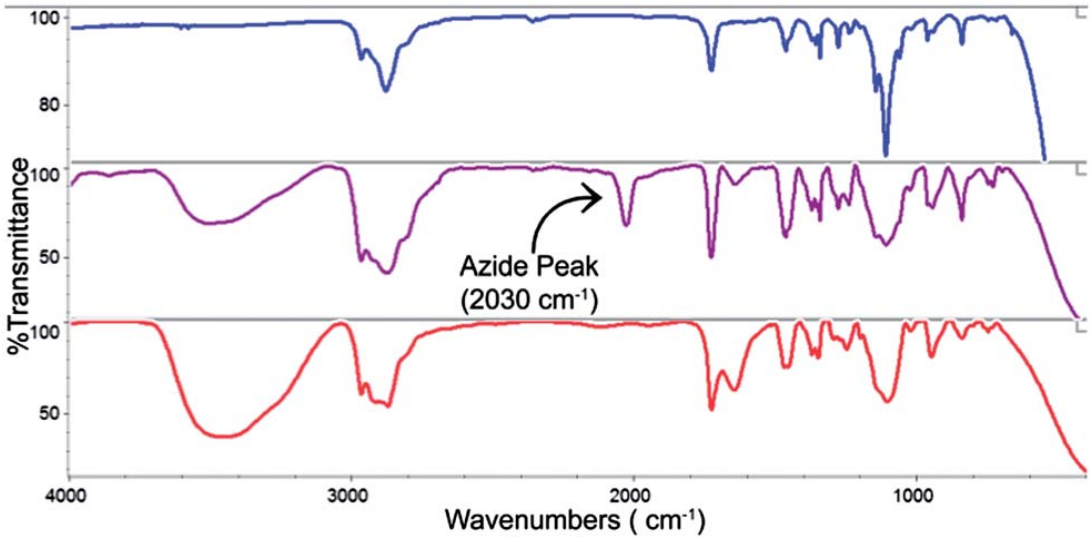

***Figure 2**: IR spectrum comparison of PDEAEM before azide functionalization (top), after azide functionalization (middle) and after azide-alkyne Huisgen reaction with mannose analogue **4** (bottom).*

3.2.1. Synthesis of thiopropyl mannosyl donors

The synthesis of the desired mannose analogue (**Scheme 3**) containing the alkyne moiety started with peracetylation of commercially available D-mannose, which was then converted to a new thiopropyl mannoside donor **2**. The donor **2** was then activated with propargyl alcohol using our developed promoter, $Ph_3Bi(OTf)_2$[7] to give the alkyne linked mannose **3** in 79% yield, without any interference with the alkyne moiety. Finally, **3** was deacetylated to give the final alkynyl mannose **4** in overall 50% yield in 5 steps.

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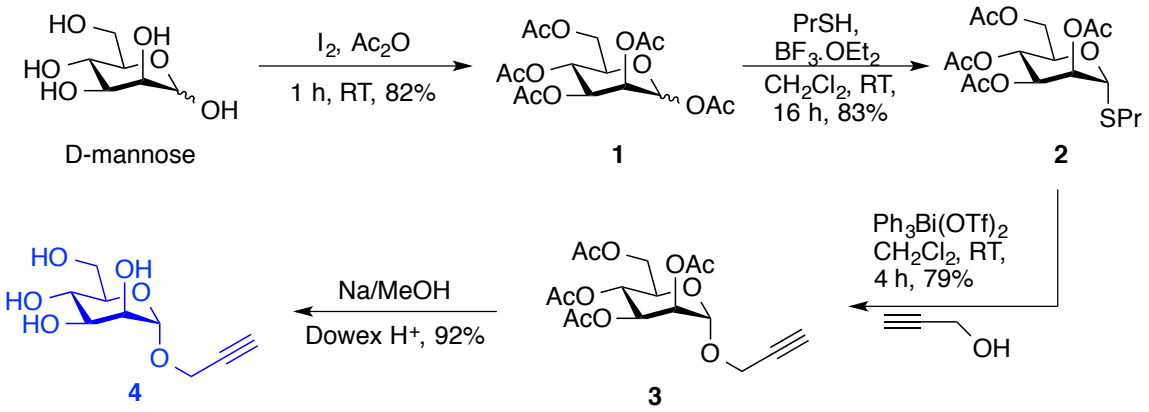

Scheme 4: *Preparation of D-mannose with the alkyne linker*

3.2.2. Attachment of D-mannose to copolymers

After the azide and alkyne functionalization of the polymer and mannose respectively, they were coupled together via a Huisgen azide-alkyne click reaction catalyzed by a mixture of $CuSO_4 \bullet 5H_2O$ and L-ascorbic acid (**Scheme 5**).

3.2.3. Purification and Analysis of the Attachment of D -mannose to Pentablock Copolymers: IR Spectroscopy, and Phenol Sulfuric Acid Assay

The next crucial part was to characterize the attachment as well as quantify the amount of sugar attached to the polymer. Unfortunately, the initial studies using mass spectroscopy (specifically MALDI-TOF) and ^{15}N labelled 1-D & 2-D NMR spectroscopy were not definitive. IR spectroscopy results showed the presence and absence of peaks, particularly a distinct azide peak (~2050–2300 cm^{-1}) (**Figure 2**) before/after reaction, but did not quantify the coupling. The ^{1}H NMR spectra of the pentablock copolymers before and after attachment (Supporting Information) also showed new peaks in the region of 5-7 ppm, which are probably from the triazole ring protons and the mannose protons after the click reaction. However, since these protons were too small in number

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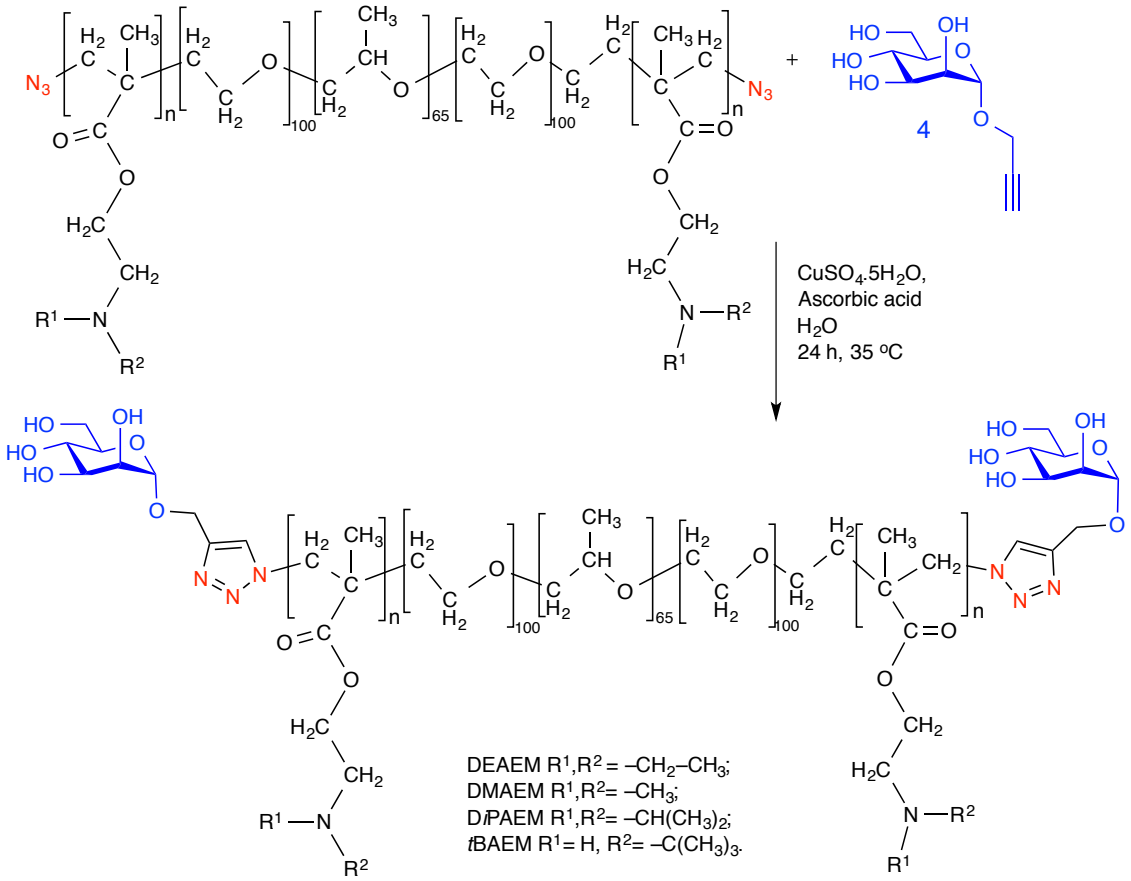
compared to the copolymer protons, they were difficult to accurately integrate to quantify the attachment.



Scheme 5: *Azide-alkyne Huisgen cycloaddition between D-mannose and the various pentablock copolymers*

In order to accurately quantify the amount of mannose attached to the polymer, a phenol sulfuric acid assay was carried out by dissolving the pentablock copolymers in water and reacting the carbohydrates with phenol in the presence of sulfuric acid (**Figure 3**). Prior to these assays, the mannose functionalized pentablock copolymers were purified from the unreacted sugar/excess reagents by utilizing dialysis membrane cassettes to rule out detection of unattached sugar. The separation of the excess sugar

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as well as excellent coupling of the two were clearly evident in the phenol sulfuric acid assay values from before and after dialysis samples. The PDiPAEM pentablock copolymer was slightly soluble in water with vortexing but continuously precipitated out of solution at relatively low concentrations. For this reason, sonication was used for the mannose functionalized PDiPAEM pentablock copolymer for all characterization assays. Overall, it was determined that the dialyzed pentablock copolymers had approximately two moles of mannose present per mole polymer indicating that the azide-alkyne click reaction produced near-complete conversion.

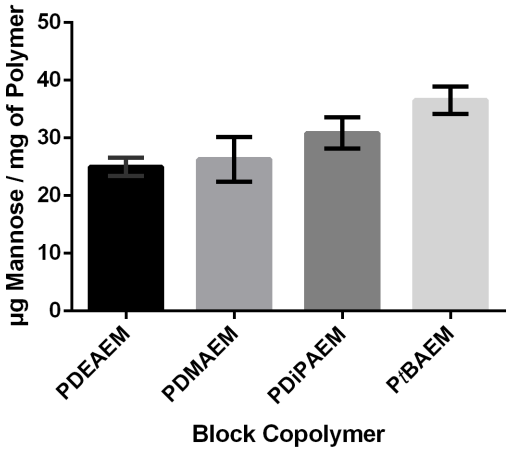

Figure 3. *Quantification of mannose functionalized pentablock copolymers. The data presented are 95% confidence intervals for the mean for four independent experiments*

3.2.4. DNA Condensation of Mannose Functionalized Block Copolymers
(Work done by Justin Adams)

We envisioned that the addition of the mannose to the pentablock copolymers should not hamper their ability to condense DNA since this ability comes from the tertiary amine groups present in the outerblocks. Nevertheless, we performed gel

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electrophoresis on the mannose attached copolymers to test this hypothesis. The minimum N/P ratios for DNA complexation did not change for any of the four pentablock copolymers after the azide-alkyne Huisgen reaction indicating that the attached mannose did not impact the capability of the block copolymers to condense DNA (**Figure 4**).

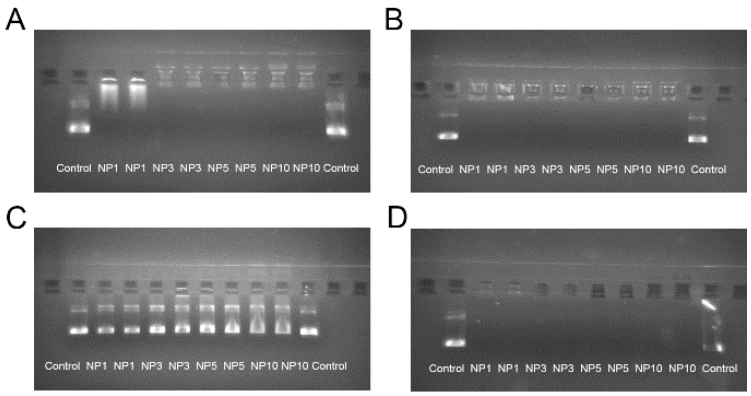

***Figure 4**. Agarose gel electrophoresis of mannose functionalized pentablock copolymer polyplexes at N/P ratios of 1, 3, 5 and 10 with a DNA only control (A) PDEAEM (B) PDMAEM (C) PDiPAEM (D) PtBAEM*

3.2.5. Further Discussion

C-type lectin receptors expressed by dendritic cells and macrophages internalize pathogens after binding carbohydrate structures on their surface.[26] Recently, a carbohydrate-functionalized polymer synthesized by Charville *et al*. demonstrated that copolymers containing a higher degree of mannose functionalization were more prone to binding lectin receptors.[27] Our method that includes the functionalization with mannose alkyne linker synthesized by bismuth(V)-mediated activation of a thioglycoside has yielded pentablock copolymers with hydrophobic polyoxyproplyene blocks that self-

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assemble into micelles. These pentablock copolymers are capable of condensing DNA for gene delivery through tertiary amine groups in the outer blocks.

Carbohydrate functionalized particles have been shown to enhance the expression of MHC II, costimulatory molecules and c-type lectin receptors by dendritic cells.[22] The high carbohydrate- functionalization of the pentablock copolymers may provide benefits when compared to other reported functionalized vaccine platforms, which typically focus on the decoration of particle surfaces.[28] The continued presence of sugars attached to polymer may provide increased and longer-lasting activation of immune cells as compared to a sugar-decorated particle, which may erode and slough off the sugars quickly.

3.3. CONCLUSION

The developed Bi(V)-mediated thioglycoside activation strategy proved to be applicable in presence of alkynes. The activation of thioproyl mannoside donor could be done to prepare alkynyl mannose in high yield. All four of the block copolymers, namely PDEAEM, PDMAEM, PDiPAEM and PtBAEM were then successfully functionalized with mannose by a high-yielding azide-alkyne Huisgen reaction. The addition of mannose to the polymer did not hamper the ability of the polymers to complex DNA and therefore these new sugar-modified materials have the potential to activate APCs. These promising virus-mimicking nanoparticles for DNA-based vaccine delivery will be evaluated in future studies for APC activation and immune response generation.

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3.4. EXPERIMENTAL SECTION

3.4.1. General methods

Air- and moisture-sensitive reactions were carried out in oven-dried or flame-dried glassware, septum-capped under atmospheric pressure of argon. Commercially available compounds were used without further purification unless otherwise stated. The exact reaction conditions are given in the respective procedures. Air- and moisture-sensitive liquids and solutions were transferred via syringe or stainless steel cannula. All saccharides and the Bi(V) reagent were pre-dried by azeotropic removal of water using anhydrous toluene. Flash silica gel column chromatography was performed employing Silica Gel 60 Sorbent (40-63 µm, 230-400 mesh). Thin-layer chromatography (analytical) was performed using glass plates pre-coated to a depth of 0.25 mm detected under UV light and by spraying the plates with a 0.02 M solution of resorcinol in 20% ethanolic H_2SO_4 solution followed by heating.

Proton (^{1}H) NMR, carbon (^{13}C) NMR, ^{1}H-^{13}C HSQC were recorded on a Bruker Avance III 600 MHz, 151 MHz instrument respectively using the residual signals from chloroform ($CDCl_3$), 7.26 ppm and 77.0 ppm as internal references for ^{1}H and ^{13}C chemical shifts (δ) respectively. Electrospray ionization high-resolution mass spectrometry (ESI-HRMS) was carried out on an Agilent 6540 QTOF. Optical rotations were measured at 20 °C using an automatic polarimeter AP300. Melting points were recorded in capillary tubes on a Digimelt SRS. IR spectroscopy was recorded by putting a thin film of the analyte on a salt plate on a Perkin-Elmer instrument.

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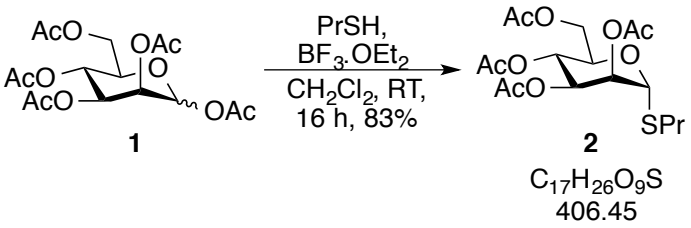
3.4.2. Synthesis of Alkynyl Mannose Analogue

_n_-Propyl-2,3,4,6-tetra-_O_-acetyl-1-thio-α- D-mannopyranoside (2)



A 0.500 M solution of peracetylated donor **1**[29] (2.00 g, 5.77 mmol, 1.0 equiv.) and propanethiol (PrSH, 0.571 g, 7.50 mmol, 1.3 equiv.) was stirred for 0.5 h in anhydrous dichloromethane at 0 °C. Then, boron trifluoride dietherate ($BF_3{\cdot}OEt_2$, 1.23 g, 8.65 mmol, 1.5 equiv.) was added drop wise at 0 °C to the reaction mixture and then the reaction was left to stir at ambient temperature for 16 h. The reaction was quenched with excess triethylamine, diluted with CH_2Cl_2, filtered, and washed sequentially with 2 M aqueous HCl, saturated aqueous $NaHCO_3$, and water. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure at 40 °C; the resulting residue was purified by silica gel column chromatography with hexane:ethyl acetate (4:1) to yield **2** as a white solid (1.95 g, 4.78 mmol, 83%); R_f 0.48 (hexanes:ethyl acetate 3:1); $[\alpha]^D$ + 73.3 cm^3 g-1dm-1 (c 0.012 g cm^{-3}, $CHCl_3$; ^{1}H NMR (600 MHz, [D-1] $CDCl_3$, 25 °C, TMS) δ = 5.32 (dd, *J*=3.1, 1.6, 1H; H-3), 5.28 (m, 1H; H-2), 5.25 (d, *J*=3.1, 1H; H-1α), 5.23 (m, 1H; H-4), 4.37 (ddd, *J*=9.3, 5.4, 2.3, 1H; H-5), 4.28 (dd, *J*=12.2, 5.5, 1H; H-6a), 4.07 (dd, *J*=12.3, 2.4, 1H; H-6b), 2.66 – 2.47 (m, 2H; $SCH_2CH_2CH_3$), 2.15 – 1.94 (m, 12H; 4×CH_3C=O), 1.69 – 1.57 (m, 2H; $SCH_2CH_2CH_3$), 0.97 (t, *J*=7.3, 3H; $SCH_2CH_2CH_3$). ^{13}C NMR (151 MHz, [D-1] $CDCl_3$, 25 °C, TMS) δ = 170.56, 169.96, 169.75, 169.72 (4C; C=O), 82.64

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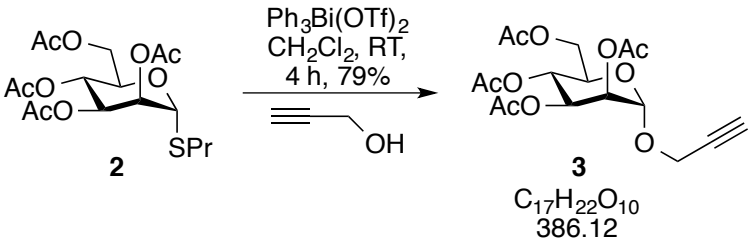
(1C; C-1), 71.21, 69.46, 68.91, 66.37 (4C; C-2,3,4,5) 62.45 (1C; C-6), 33.48 (1C; S*C*$H_2CH_2CH_3$), 22.80 (1C; SCH_2*C*H_2CH_3), 20.92, 20.70, 20.69, 20.62 (4C; 4×*C*H_3C=O), 13.35 (1C; SCH_2CH_2*C*H_3), HRMS (ESI-QTOF) m/z calc. for $C_{17}H_{26}O_9SNa^+$ 429.119 found 429.126.

2-Propynyl-2,3,4,6-tetra-*O*-acetyl-1-*O*-α- D-mannopyranoside (3)



A 0.50 M solution of thioglycoside donor **2** (0.250 g, 0.615 mmol, 1.0 equiv.) and propargyl alcohol (0.035 g, 0.615 mmol, 1.0 equiv.) were stirred together in anhydrous dichloromethane (CH_2Cl_2) for 0.5 h in a flask filled with Ar at ambient temperature. A solution of $Ph_3Bi(OTf)_2$[27] (0.317 g, 0.430 mmol, 0.700 equiv.) in anhydrous CH_2Cl_2 was added and the reaction was stirred at ambient temperature for 4 h (until the consumption of donor was seen by TLC). The reaction was then quenched by triethylamine, filtered through a Celite pad, and concentrated under reduced pressure; the resulting residue was purified by silica gel column chromatography with hexane:ethyl acetate (3:1)to give a pale white solid **3** (0.188 g, 0.486 mmol, 79%). The ^{1}H NMR, ^{13}C NMR, HRMS matched with data in the literature.[30]

^{1}H NMR (600 MHz, [D-1] $CDCl_3$, 25 °C, TMS) δ [5.35 (dd, J = 10.0, 3.4 Hz), 5.31 (d, J = 9.8 Hz), 5.29 – 5.27 (m) (3H; H-2,3,4)], 5.03 (d, J = 1.8 Hz, 1H; H-1), 4.31 – 4.28 (m, 1H, H-6a),

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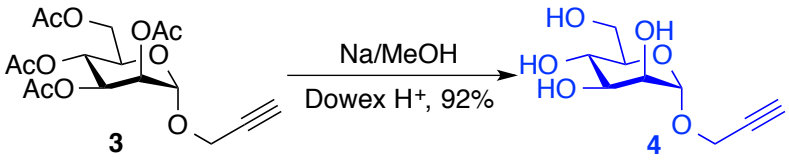
4.28 (d, J = 2.3 Hz, 2H, C*H*$_2$CCH), 4.11 (dd, J = 12.3, 2.5 Hz, 1H, H-6b), 4.02 (ddd, J = 9.7, 5.2, 2.4 Hz, 1H, H-5), 2.47 (t, *J* = 2.4 Hz, 1H, CH$_2$CC*H*), 2.17 (s, 3H), 2.11 (s, 3H), 2.04 (s, 3H), 1.99 (s, 3H) (4×CH$_3$C=O).

^{13}C NMR (151 MHz, [D-1] CDCl$_3$, 25 °C, TMS) δ 96.23 (1C; C-1), 77.92, 75.62 (2C, CH$_2$*CC*H*)*, 69.33, 68.97, 68.91, 66.00 (4C; C-2,3,4,5), 62.30 (1C; C-6), 54.94 (1C; *C*H$_2$CCH), 20.85, 20.73, 20.68, 20.64 (4C; 4×CH$_3$C=O).

HRMS (ESI-QTOF) m/z calc. for $C_{17}H_{22}O_{10}Na^+$ 409.110 found 409.111.

2-Propynyl-1-*O*-α- D-mannose (4)



To a solution of **3a** (0.188 g, 0.486 mmol) in 10.0 mL anhydrous methanol (MeOH), 0.2 mL of 0.5 N sodium methoxide (freshly prepared by dissolving sodium in anhydrous MeOH) was added. The reaction mixture was stirred at ambient temperature for 1 h, neutralized with Dowex 50WX8 (H^+) cation exchange resin and filtered. The methanol was removed under reduced pressure and trace methanol or water was removed by co-evaporation with toluene (3 x 5 mL) to give the desired product **4** as pale yellow solid (0.098 g, 0.447 mmol, 92%), which was directly used in the click reaction.

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General Procedure for Azide-Alkyne Huisgen Reaction

To a solution of the pentablock copolymer (1.00 equiv.) and **4** (10 equiv.) in H_2O, aqueous solutions of $CuSO_4 \bullet 5H_2O$ (0.20 equiv.) and L-ascorbic acid (0.40 equiv.) were sequentially added. The mixture was stirred at 35 °C for 24 h. The solvent was removed under reduced pressure and residual mixture was dried under vacuum overnight.

3.4.3. Purification of Mannose Functionalized Pentablock Copolymers/Dialysis

The residue after the click reaction was dissolved in minimum amount (<3.0 mL) of solvent (33% ethanol in water, except for PDiPAEM in which case 66% ethanol/water was used). The solution was then injected into a Slide-A-Lyzer® dialysis cassette (10KDa MW cutoff), and left for stirring overnight for 20 h in nanopure water. The leftover solvent inside the cassette was then evaporated under reduced pressure and the dialyzed sample was kept under vacuum overnight for complete dryness.

3.4.4. Phenol Sulfuric Acid Assay

The sugar attached pentablock copolymers were dissolved in nanopure water at a concentration of 1 mg/mL and 100 µL volumes per well was added to a 96 well plate. A 150 µL volume of stock sulfuric acid was added to each well following a 30 µL volume of 5% phenol. The 96 well plate was heated for 30 min at 90 °C and the absorbance were recorded in a microplate reader at the 490 nm wavelength.

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3.4.6. Statistical Analysis

All error bars represent 95% confidence intervals for the mean. Differences between groups were analysed with one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. The denoted statistical significance indicates a p-value ≤ 0.05.

3.5. REFERENCES

1. Wuts, P. G. M.; Greene, T. W., Protection for the Hydroxyl Group, Including 1,2- and 1,3-Diols. In *Greene's Protective Groups in Organic Synthesis*, John Wiley & Sons, Inc.: 2006; pp 16-366.

2. Wuts, P. G. M.; Greene, T. W., Protection for the Carboxyl Group. In *Greene's Protective Groups in Organic Synthesis*, John Wiley & Sons, Inc.: 2006; pp 533-646.

3. Wuts, P. G. M.; Greene, T. W., Protection for the Amino Group. In *Greene's Protective Groups in Organic Synthesis*, John Wiley & Sons, Inc.: 2006; pp 696-926.

4. Hecht, S. M., *Bioorganic Chemistry: Carbohydrates*. Oxford University Press: 1999.

5. (a) Mootoo, D. R.; Date, V.; Fraser-Reid, B., n-Pentenyl glycosides permit the chemospecific liberation of the anomeric center. *J. Am. Chem. Soc.* **1988,** *110* (8), 2662-2663; (b) Mootoo, D. R.; Konradsson, P.; Udodong, U.; Fraser-Reid, B., Armed and disarmed n-pentenyl glycosides in saccharide couplings leading to oligosaccharides. *J. Am. Chem. Soc.* **1988,** *110* (16), 5583-5584.

6. Boons, G.-J.; Heskamp, B.; Hout, F., Vinyl Glycosides in Oligosaccharide Synthesis: A Strategy for the Preparation of Trisaccharide Libraries Based on Latent-Active Glycosylation. *Angew. Chem., Int. Ed.* **1996,** *35* (23-24), 2845-2847.

7. Goswami, M.; Ellern, A.; Pohl, N. L. B., Bismuth(V)-Mediated Thioglycoside Activation. *Angew. Chem., Int. Ed.* **2013,** *52* (32), 8441-8445.

8. (a) Kolb, H. C.; Finn, M. G.; Sharpless, K. B., Click Chemistry: Diverse Chemical Function from a Few Good Reactions. *Angewandte Chemie (International ed. in English)* **2001,** *40* (11), 2004-2021; (b) Evans, R. A., The Rise of Azide–Alkyne 1,3-Dipolar 'Click' Cycloaddition and its Application to Polymer Science and Surface Modification. *Aust. J. Chem.* **2007,** *60* (6), 384-395.

www.manaraa.com

9. McKay, M. J.; Nguyen, H. M., Recent Advances in Transition Metal-Catalyzed Glycosylation. *ACS Catalysis* **2012,** *2* (8), 1563-1595.

10. Adhikari, S.; Baryal, K. N.; Zhu, D.; Li, X.; Zhu, J., Gold-Catalyzed Synthesis of 2-Deoxy Glycosides Using S-But-3-ynyl Thioglycoside Donors. *ACS Catalysis* **2012,** *3* (1), 57-60.

11. Dutta, S.; Sarkar, S.; Gupta, S. J.; Sen, A. K., Use of iodine for efficient and chemoselective glycosylation with glycosyl ortho-alkynylbenzoates as donor in presence of thioglycosides. *Tetrahedron Lett.* **2013,** *54* (8), 865-870.

12. (a) Batrakova, E. V.; Kabanov, A. V., Pluronic block copolymers: Evolution of drug delivery concept from inert nanocarriers to biological response modifiers. *J. Controlled Release* **2008,** *130* (2), 98-106; (b) Determan, M. D.; Cox, J. P.; Mallapragada, S. K., Drug release from pH-responsive thermogelling pentablock copolymers. *J. Biomed. Mater. Res., Part A* **2007,** *81A* (2), 326-333; (c) Hunter, R.; Strickland, F.; Kézdy, F., The adjuvant activity of nonionic block polymer surfactants. I. The role of hydrophile-lipophile balance. *J. Immunol.* **1981,** *127* (3), 1244-1250.

13. (a) Adams, J. R.; Mallapragada, S. K., Novel Atom Transfer Radical Polymerization Method to Yield Copper-Free Block Copolymeric Biomaterials. *Macromol. Chem. Phys.* **2013,** *214* (12), 1321-1325; (b) Escobar-Chavez, J. J.; Lopez-Cervantes, M.; Naik, A.; Kalia, Y. N.; Quintanar-Guerrero, D.; Ganem-Quintanar, A., Applications of thermo-reversible pluronic F-127 gels in pharmaceutical formulations. *J. Pharm. Pharm. Sci.* **2006,** *9* (3), 339-358; (c) Wenzel, J. G.; Balaji, K. S.; Koushik, K.; Navarre, C.; Duran, S. H.; Rahe, C. H.; Kompella, U. B., Pluronic F127 gel formulations of deslorelin and GnRH reduce drug degradation and sustain drug release and effect in cattle. *J. Controlled Release* **2002,** *85* (1-3), 51-59; (d) Zhang, W.; Shi, Y.; Chen, Y.; Ye, J.; Sha, X.; Fang, X., Multifunctional Pluronic P123/F127 mixed polymeric micelles loaded with paclitaxel for the treatment of multidrug resistant tumors. *Biomaterials* **2011,** *32* (11), 2894-2906.

14. Kabanov, A. V.; Lemieux, P.; Vinogradov, S.; Alakhov, V., Pluronic® block copolymers: novel functional molecules for gene therapy. *Adv. Drug Delivery Rev.* **2002,** *54* (2), 223-233.

15. Agarwal, A.; Unfer, R. C.; Mallapragada, S. K., Dual-role self-assembling nanoplexes for efficient gene transfection and sustained gene delivery. *Biomaterials* **2008,** *29* (5), 607-617.

16. Hunter, R. L.; McNicholl, J.; Lal, A. A., Mechanisms of action of nonionic block copolymer adjuvants. *AIDS Res Hum Retroviruses* **1994,** *10 Suppl 2*, S95-S98.

www.manaraa.com

17. (a) Reddy, S. T.; Swartz, M. A.; Hubbell, J. A., Targeting dendritic cells with biomaterials: developing the next generation of vaccines. *Trends Immunol.* **2006,** *27* (12), 573-579; (b) Seong, S. Y.; Matzinger, P., Hydrophobicity: an ancient damage-associated molecular pattern that initiates innate immune responses. *Nat. Rev. Immunol.* **2004,** *4* (6), 469-478.

18. Determan, M. D.; Cox, J. P.; Seifert, S.; Thiyagarajan, P.; Mallapragada, S. K., Synthesis and characterization of temperature and pH-responsive pentablock copolymers. *Polymer* **2005,** *46* (18), 6933-6946.

19. Agarwal, A.; Mallapragada, S. K., Synthetic sustained gene delivery systems. *Current topics in medicinal chemistry* **2008,** *8* (4), 311-320.

20. (a) Zhang, B.; Jia, F.; Fleming, M. Q.; Mallapragada, S. K., Injectable self-assembled block copolymers for sustained gene and drug co-delivery: an in vitro study. *Int. J. Pharm.* **2012,** *427* (1), 88-96; (b) Zhang, B.; Kanapathipillai, M.; Bisso, P.; Mallapragada, S., Novel pentablock copolymers for selective gene delivery to cancer cells. *Pharm. Res.* **2009,** *26* (3), 700-713.

21. Pashine, A.; Valiante, N. M.; Ulmer, J. B., Targeting the innate immune response with improved vaccine adjuvants. *Nat Med* **2005,** *11* (4 Suppl), S63-S68.

22. Wilson-Welder, J. H.; Torres, M. P.; Kipper, M. J.; Mallapragada, S. K.; Wannemuehler, M. J.; Narasimhan, B., Vaccine adjuvants: current challenges and future approaches. *J. Pharm. Sci.* **2009,** *98* (4), 1278-1316.

23. (a) Carrillo-Conde, B.; Song, E. H.; Chavez-Santoscoy, A.; Phanse, Y.; Ramer-Tait, A. E.; Pohl, N. L.; Wannemuehler, M. J.; Bellaire, B. H.; Narasimhan, B., Mannose-functionalized "pathogen-like" polyanhydride nanoparticles target C-type lectin receptors on dendritic cells. *Mol. Pharm.* **2011,** *8* (5), 1877-1886; (b) Figdor, C. G.; van Kooyk, Y.; Adema, G. J., C-type lectin receptors on dendritic cells and Langerhans cells. *Nat. Rev. Immunol.* **2002,** *2* (2), 77-84; (c) Geijtenbeek, T. B.; Gringhuis, S. I., Signalling through C-type lectin receptors: shaping immune responses. *Nat. Rev. Immunol.* **2009,** *9* (7), 465-479.

24. Tabarani, G.; Reina, J. J.; Ebel, C.; Vives, C.; Lortat-Jacob, H.; Rojo, J.; Fieschi, F., Mannose hyperbranched dendritic polymers interact with clustered organization of DC-SIGN and inhibit gp120 binding. *FEBS Lett.* **2006,** *580* (10), 2402-2408.

25. Coessens, V.; Matyjaszewski, K., End group transformation of polymers prepared by ATRP, substitution to azides. *J. Macromol. Sci. Part A* **1999,** *36* (5-6), 667-679.

26. Koppel, E. A.; Ludwig, I. S.; Hernandez, M. S.; Lowary, T. L.; Gadikota, R. R.; Tuzikov, A. B.; Vandenbroucke-Grauls, C. M.; van Kooyk, Y.; Appelmelk, B. J.; Geijtenbeek, T. B., Identification of the mycobacterial carbohydrate structure

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that binds the C-type lectins DC-SIGN, L-SIGN and SIGNR1. *Immunobiology* **2004,** *209* (1-2), 117-127.

27. Charville, H.; Jin, J.; Evans, C. W.; Brimble, M. A.; Williams, D. E., The synthesis and lectin-binding properties of novel mannose-functionalised polymers. *RSC Adv.* **2013,** *3* (35), 15435-15441.

28. (a) Garinot, M.; Fievez, V.; Pourcelle, V.; Stoffelbach, F.; des Rieux, A.; Plapied, L.; Theate, I.; Freichels, H.; Jerome, C.; Marchand-Brynaert, J.; Schneider, Y. J.; Preat, V., PEGylated PLGA-based nanoparticles targeting M cells for oral vaccination. *Journal of controlled release : official journal of the Controlled Release Society* **2007,** *120* (3), 195-204; (b) Martin, A. L.; Li, B.; Gillies, E. R., Surface functionalization of nanomaterials with dendritic groups: toward enhanced binding to biological targets. *J. Am. Chem. Soc.* **2009,** *131* (2), 734-741; (c) Fischer, S.; Foerg, C.; Ellenberger, S.; Merkle, H. P.; Gander, B., One-step preparation of polyelectrolyte-coated PLGA microparticles and their functionalization with model ligands. *Journal of controlled release : official journal of the Controlled Release Society* **2006,** *111* (1-2), 135-144; (d) Rieger, J.; Freichels, H.; Imberty, A.; Putaux, J. L.; Delair, T.; Jerome, C.; Auzely-Velty, R., Polyester nanoparticles presenting mannose residues: toward the development of new vaccine delivery systems combining biodegradability and targeting properties. *Biomacromolecules* **2009,** *10* (3), 651-657; (e) Munier, S.; Messai, I.; Delair, T.; Verrier, B.; Ataman-Onal, Y., Cationic PLA nanoparticles for DNA delivery: comparison of three surface polycations for DNA binding, protection and transfection properties. *Colloids Surf., B* **2005,** *43* (3-4), 163-173.

29. Kaufman, R. J.; Sidhu, R. S., Synthesis of aryl cluster glycosides by cyclotrimerization of 2-propynyl carbohydrate derivatives. *J. Org. Chem* **1982,** *47* (25), 4941-4947.

30. Roy, R.; Das, S. K.; Santoyo-González, F.; Hernández-Mateo, F.; Dam, T. K.; Brewer, C. F., Synthesis of “Sugar-Rods” with Phytohemagglutinin Cross-Linking Properties by Using the Palladium-Catalyzed Sonogashira Reaction. *Chem. – Eur. J.* **2000,** *6* (10), 1757-1762.

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CHAPTER 4. MECHANISTIC INSIGHTS OF Bi^{V} MEDIATED THIOGLYCOSIDE ACTIVATION

Abstract

The developed thioglycoside activation strategy is unique in various ways for e.g., less than stoichiometric amount of promoter was needed for activation, no additive/co-promoter or low temperature is required and most importantly, it is one of the first examples of Bi(V) chemistry in chemical glycosylation, or indeed in all of carbohydrate chemistry. These interesting features led our curiosity to conduct a detailed mechanistic investigation of the glycosylation. A variety of benchtop and analytical tools like: 1D & 2D-NMR, GCMS, kinetics modeling etc. were used to gain a deeper understanding. This chapter is one of the first in-depth kinetics analyses in thioglycoside activation, and in glycosylation. The roles of various reactants on the rate of the reaction were explained and different by-product analyses were done. While studying the kinetics, an unprecedented anomerization was also discovered, which was utilized to reduce reaction times and enhance stereoselectivity. Interestingly, a probable bismuth-sulfonium reactive intermediate was also detected and studied using different NOE experiments. Also, other possible mechanistic pathways were also probed and some possible by-products as well as effect of ligands were also studied.

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4.1 INTRODUCTION

4.1.1. General introduction to mechanistic studies in chemical glycosylation

Carbohydrate or oligosaccharide synthesis has always been a complex area of research in organic synthesis, owing to the high number of stereocenters as well as multiple functional groups present in these molecules. To build these important biomolecules, chemical glycosylation is perhaps the most pivotal reaction and has been extensively studied. A variety of glycosyl donors can be coupled to simple and complex acceptors in the presence of promoters/activators to form unique oligosaccharides via this reaction. However, the complexities associated with the prediction and control of stereoselectivity of products has been some of the main hurdles of this important reaction. Carbohydrate chemists have made continuous efforts to optimize this reaction in the past century.

"*Deciphering a reaction mechanism is the most enabling knowledge that a chemist has to control the outcome of a reaction*."[1] To have a deeper understanding of the reaction pathways, a variety of mechanistic studies employing various analytical techniques have been done. Pioneering work has been done by Crich,[2] Gin,[3] Bol,[4] Woerpel,[5] etc. which has revolutionized mechanistic studies in carbohydrate chemistry in the past 15 years. Apart from experimental and analytical methods, quantum mechanical studies and computational modeling studies have been explored by Whitfield *et al*[6] and other research groups. Considering all of these studies, general mechanistic pathways for glycosylation have been postulated,[2a, 7] for both non-

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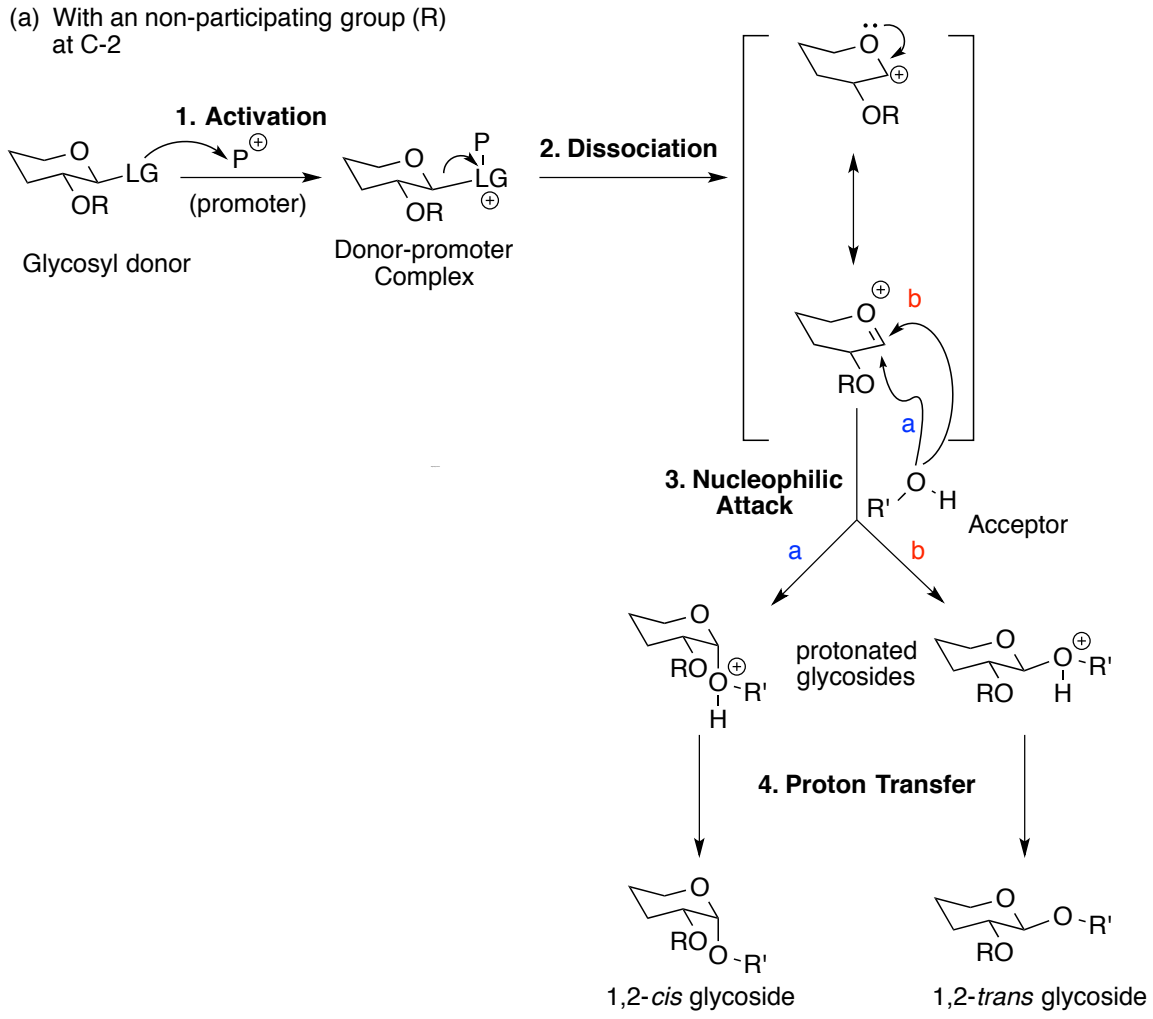
participating (**Scheme 1**) and participating groups (**Scheme 2**) present on C-2 carbon of the glycosyl donor. This schemes have been adapted from a review[7b] on chemical glycosylation mechanisms.



***Scheme 1**: General glycosylation mechanism with a C-2 non-participating group*

Glycosylation is perceived to consist of primarily four steps; firstly, the donor reacts with the promoter to form a complex, which can be reversible or irreversible depending on the system involved. Then, the anomeric group leaves to form a glycosyl

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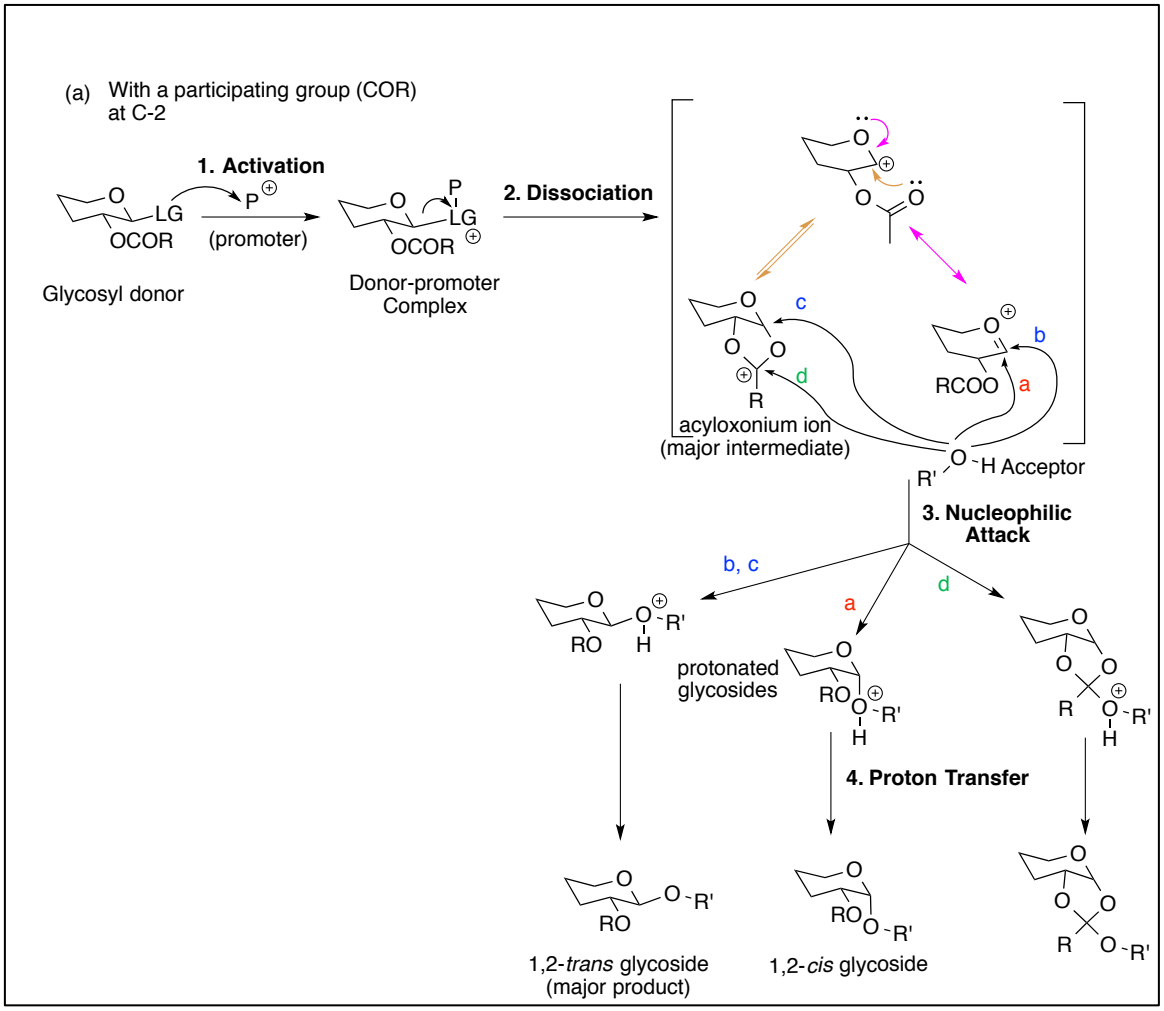
oxacarbenium ion, which is typically an irreversible act. This formation is sometimes speculated to be the slowest step or the rate-determining step. Next is a nucleophilic attack by an acceptor on the reactive species, after which proton transfer gives the 1,2 *cis*- or *trans*-glycoside product (**Scheme 1**). In presence of a participating group like an ester, the nucleophilic carboxyl oxygen can form a reactive acyloxonium intermediate, which typically blocks the α-face attack by a nucleophile. This kind of anchimeric assistance results in a 1,2 *trans*-glycoside as the major product (**Scheme 2**).



Scheme 2: *General glycosylation mechanism with a C-2 participating group (anchimeric assistance)*

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4.1.2 Previous mechanistic studies on thioglycosides and limitations

In the case of thioglycosyl donors, some initial mechanistic studies have been done mainly focusing on the initial activation step. The underlying theme of most of the thioglycoside activations is the use of an electrophilic reagent which complexes or activates the anomeric sulfur group and then assists in its departure as a leaving group. For e.g., soft Lewis acid metals like Hg, Ag, Pb, etc. usually complex with the soft sulfur atom, or halonium based promoters are perceived to do a electrophilic halogen addition to the sulfur group, and sulfonium or selenium based promoters are argued to form disulfides or stable selenyl–sulfides respectively with the thio-alkyl or aryl moiety [**Scheme 3** (adapted from review[7a])]. All these processes then help in the removal of the reactive anomeric group to form an oxacarbenium intermediate.

Schuerch *et al.* were among the first groups to report in situ generation of glycosyl sulfoniums while investigating generation of α-glycosides.[8] Since then, various research groups have proposed sulfonium intermediates as a route for stereocontrolled glycosylations.[9] Recently Mydock *et al.* reported the formation and isolation of stable glycosyl sulfonium salts while investigating thioethylglycoside activation using MeOTf[10] and also found ethylmethyl sulfide (EtSMe) as one of the major by-products. Surprisingly, disulfide compounds have been observed as a common by-product with different classes of promoters. Crich and co-workers reported disulfides with several promoters like PhSOTf[11] and *p*-Nitrobenzenesulfenyl chloride (*p*-NO_2PhCl)/TMSOTf,[12] while Huang found the presence of *p*-tolyl disulfide with p-ToSCl/AgOTf as a promoter.[13]

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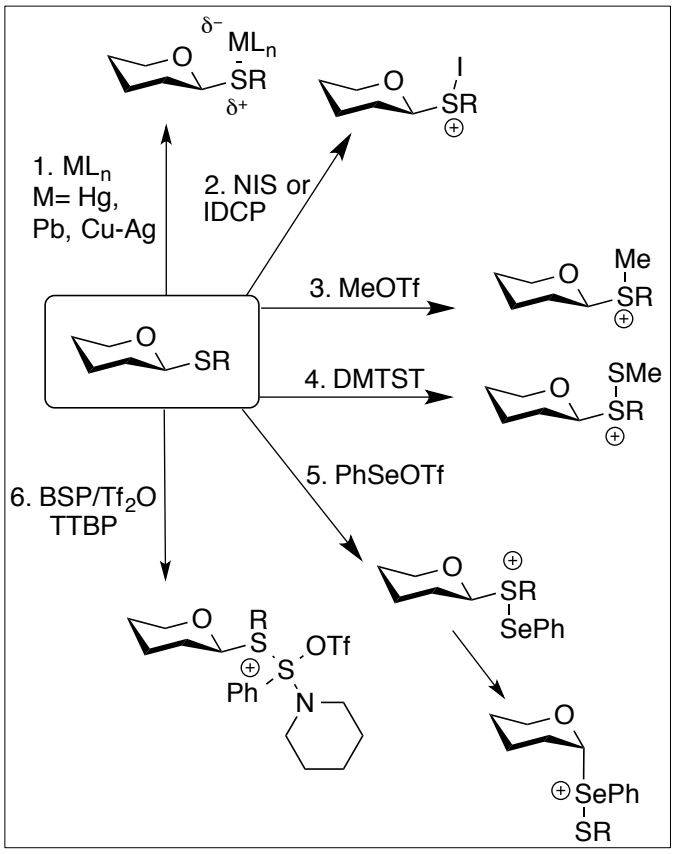
Presence of PhSSPh was also observed by mass spectrometry and HPLC by the Sinay group while using electrochemical oxidation to activate phenylthioglycosides.[14]



***Scheme 3**: Common thioglycoside activation intermediates*

Although detailed by-product studies and a few intermediate studies have been performed, yet a lack of comprehensive and detailed investigation of thioglycoside activation pathways, or in fact chemical glycosylation can still be seen. For e.g. kinetics-rate order studies are extremely vital in any reaction mechanism study, but they are very limited in chemical glycosylations. Consequently, topics like rate-determining step in a glycosylation or rate dependence of the reactants are still debatable.

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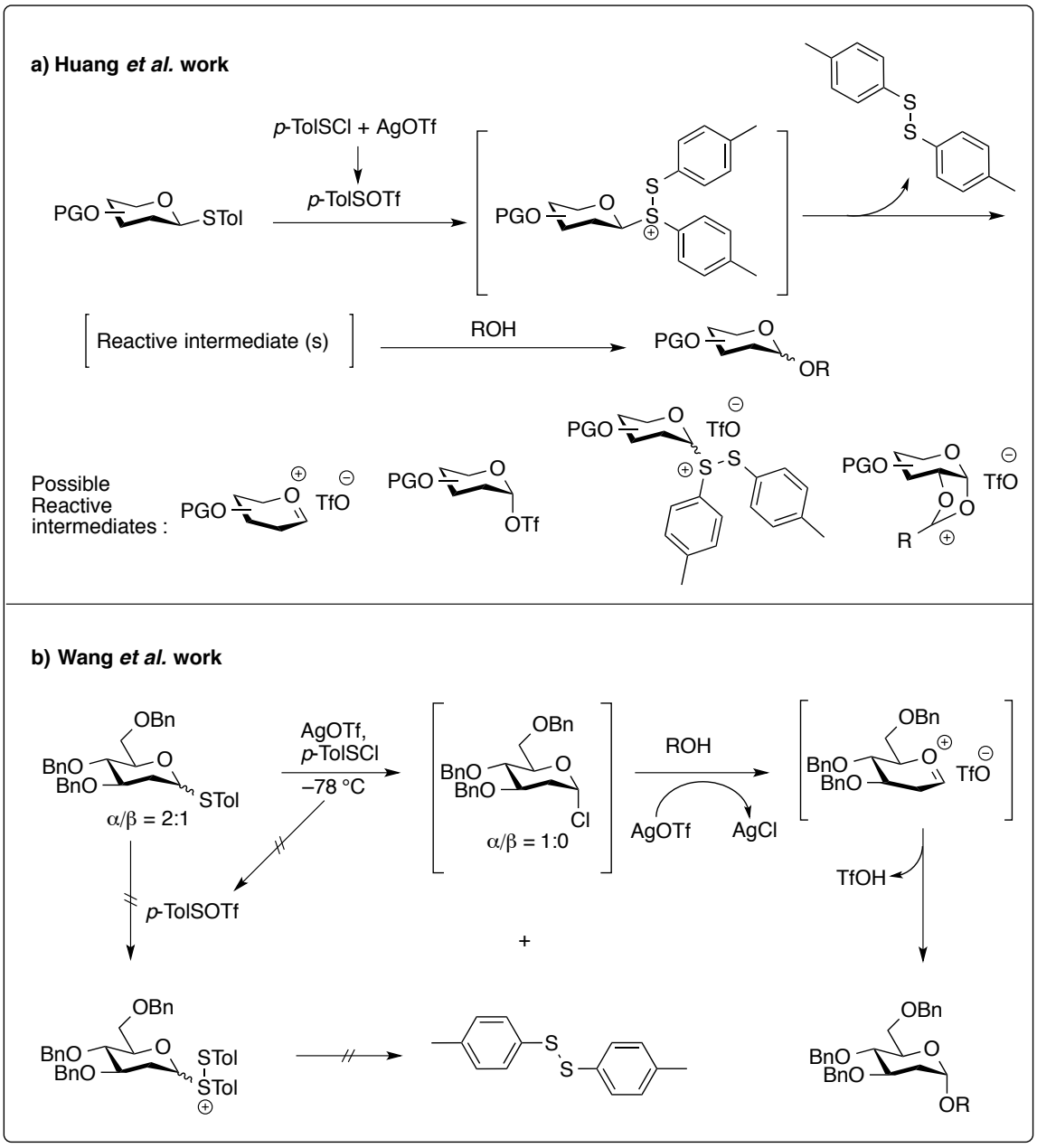

***Scheme 4**: Proposed thioglycoside activation*

The breakdown of the glycosyl donor-promoter complex has been regarded as the rate-determining step,[7b] but Huang *et al.* reported the attack of the glycosyl acceptor to the reactive intermediate as the slow step[15] while calculating relative reactivity rates of various thioglycoside donors. Similarly, halonium or sulfonium type promoters like

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NIS/NBS, *p*-TolSCl/AgOTf, etc. have always been presumed to form a transient sulfur-halide or sulfur-sulfur intermediate species. Interestingly, Verma and Wang recently reported[16] mechanistic studies with deoxy thio-donors, which imply that the thioglycoside activation does not proceed via these sulfonium intermediates but via a glycosyl halide donor (**Scheme 4b**). Using the same promoter in more common sugars, Huang group observed the glycosyl triflate and bridging dioxalenium ions to be the major reactive intermediates in a thioglycoside pre-activation strategy (**Scheme 4a**).[17] Therefore, it is evident that more detailed mechanistic studies are required to decipher the complicated glycosylation pathways and which will possibly provide insights to improve current methodologies.

4.1.3 Approach of our work

We recently developed a unique method for activation of thiopropylglycosyl donors for coupling to various acceptors utilizing bismuth chemistry.[18] Very good to excellent yields were obtained without using additional co-promoters/additives and the strategy was applied to a wide variety of carbohydrates tolerating different functional groups, even alkenes and alkynes. Surprisingly, the methodology could be carried out uniquely with a bismuth(V) [and not with the more common Bi(III)] containing promoter and is one of the first demonstrations of its kind in carbohydrate chemistry. Additionally, unlike most current methods in thioglycoside activation, we observed that our technique could be not only carried out with less than stoichiometric amounts of the promoter but also be performed at room temperatures. These exciting observations led

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us to study this interesting activation and possibly shed light into its mechanistic pathways. One of the primary goals of our work was to perform a comprehensive analysis of this reaction utilizing various spectroscopic techniques and sophisticated computation modeling in parallel with benchtop experiments. However, it needs to be mentioned here that these types of extensive studies are a rare sight in the field of bismuth catalysis/reactivity. Specifically, computational analysis on bismuth chemistry[19] is not as prevalent as studies on other transition metals or lighter main group metals. With more insight into the mechanism, we envision to further improve our existing Bi(V) promoter or discover superior alternatives, which in turn could increase the glycosylation rate and possibly induce higher stereoselectivity in products. We imagine that these types of investigations will open doors for exploration of new features of this non-toxic, cheap metal and its continued use in organic synthesis.

4.2 RESULTS AND DISCUSSION

4.2.1 GCMS studies

One of the most useful insights for studying a reaction mechanism is a possible balanced equation for the reaction. In our glycosylation containing a thioether activation, some probable by-products containing sulfur can be anticipated, for e.g. the obvious free thiol (PrSH), oxidation of thiol to a disulfide (PrSSPr), or a mixed thioether (PhSPr) formed most likely by a phenyl group transfer from the Bi(V) promoter. We chose the sensitive gas chromatography-mass spectrometry (GCMS) technique to monitor the presence of these compounds during and after a thioglycoside activation

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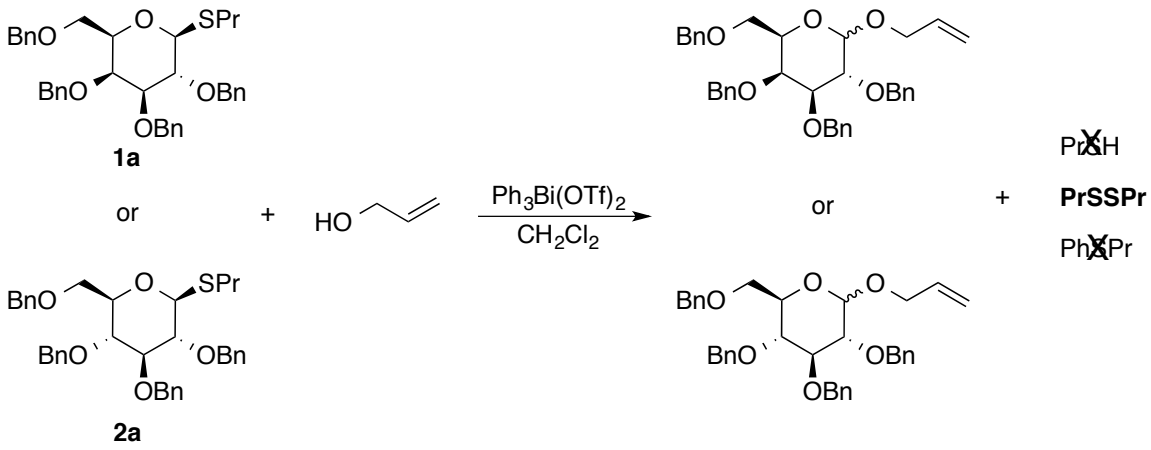
promoted by $Ph_3Bi(OTf)_2$ (**Scheme 5**). We selected two glycosyl donors: perbenzylated thiopropylgalactoside (**1a**) and perbenzylated thiopropylglucoside (**2a**) for these studies and reacted them with acceptor allyl alcohol in presence of our promoter: $Ph_3Bi(OTf)_2$. These reactions were then monitored by GCMS at different stages of reaction progress namely in the beginning (t=0), around halfway~t=1/2, at completion (t=1).



***Scheme 5**: Model glycosylation monitored by GCMS*

It was observed that out of proposed by-products, PrSSPr was the only compound found in the reaction mixture and it was detected only towards the end of the reaction (See supporting information). In addition, the free thiol was never detected during the course of the reaction. This led to our conclusion that the formation of dipropyl disulfide was perhaps not by the oxidation of two free thiols but by the possible interaction of bismuth(V) with the thiopropyl moiety, which then promotes its oxidation. This experiment supported our initial hypothesis that the first step of activation might be formation of a sulfur-bismuth complex as bismuth metal is highly thiophillic. However, it was observed that the amounts of PrSSPr detected at the end of

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the activation was quite low (~20%) compared to the expected amounts, if all of the SPr moiety was considered to be converting to PrSSPr.

4.2.2 Reaction monitoring kinetic studies

Moving forward, we decided to investigate the rate law equation governing the activation. As mentioned before, kinetic rate order studies are limited not only in the area of thioglycoside activation but generally in chemical glycosylation. Wallace and Schroeder were among the first to perform kinetic rate studies with Koenigs-Knorr type glycosylation by using polarimetry and quantitative Gas Liquid Chromatography.[20] Huang *et al* also tried to calculate relative rate orders in thioglycosides along with various substituents effects by using HPLC experiments.[15] Though low-temperature NMR spectroscopy has been extensively used to study reactive intermediates in glycosylation, NMR in general, is not the preferred method for kinetic rate order studies, owing to the complexity of overlapping signals in a carbohydrate mixture NMR spectrum. Nevertheless, we decided to use this powerful technique to investigate the rate order equation of our activation.

The armed benzylated β-thiopropyl galactoside was selected to be donor (**β-D**) and for sake of simplicity, methanol (MeOH) was chosen as the acceptor (**A**) with the promoter (**P**) being $Ph_3Bi(OTf)_2$ (**Scheme 6**). Although the activations are usually carried out in CH_2Cl_2, the kinetics studies were carried in solvent $CDCl_3$, as it does not have overlapping signals with the sugar-ring protons, thiopropyl, or the Bi(V) compound. A number of kinetics experiments were performed at various temperatures and 313 K (40

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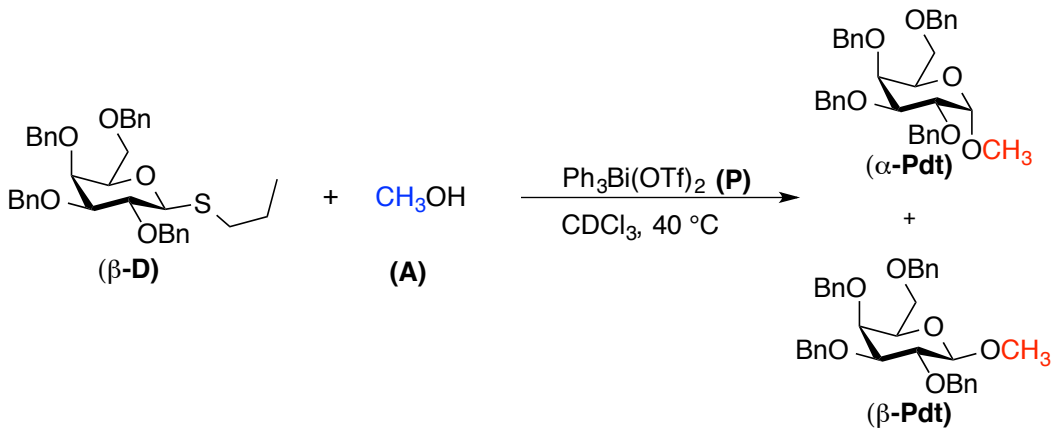
°C) was found to be the optimum temperature to carry the remaining studies, considering the required reaction time of a single experiment on the NMR machine.



***Scheme 6**: Model glycosylation with β-donor for kinetic rate-order studies*

Figure **1** shows a full ^{1}HNMR kinetics spectrum (FIDs shown after every 10 min) of the model reaction (**Scheme 6**), where equimolar amounts of all the reactants were taken. Within an hour reaction time, the activation was complete and several peaks appeared/disappeared, grew/shrunk and even shifted during the progress of the reaction.

To monitor the progress of the reaction, the disappearance and appearance of the methoxy peak (OMe) in the reactant (**A**) and β-product (**β-Pdt**) were quantified. These peaks were selected as these did not overlap with the sugar ring, benzyl or the phenyl protons on the promoter. Hence, these could be integrated separately as shown in **figure 2**. On plotting these peak concentrations against time of the reaction, a gradual decrease of the OMe peak of **A** correlating with increase of the OMe peak of the β-**Pdt**

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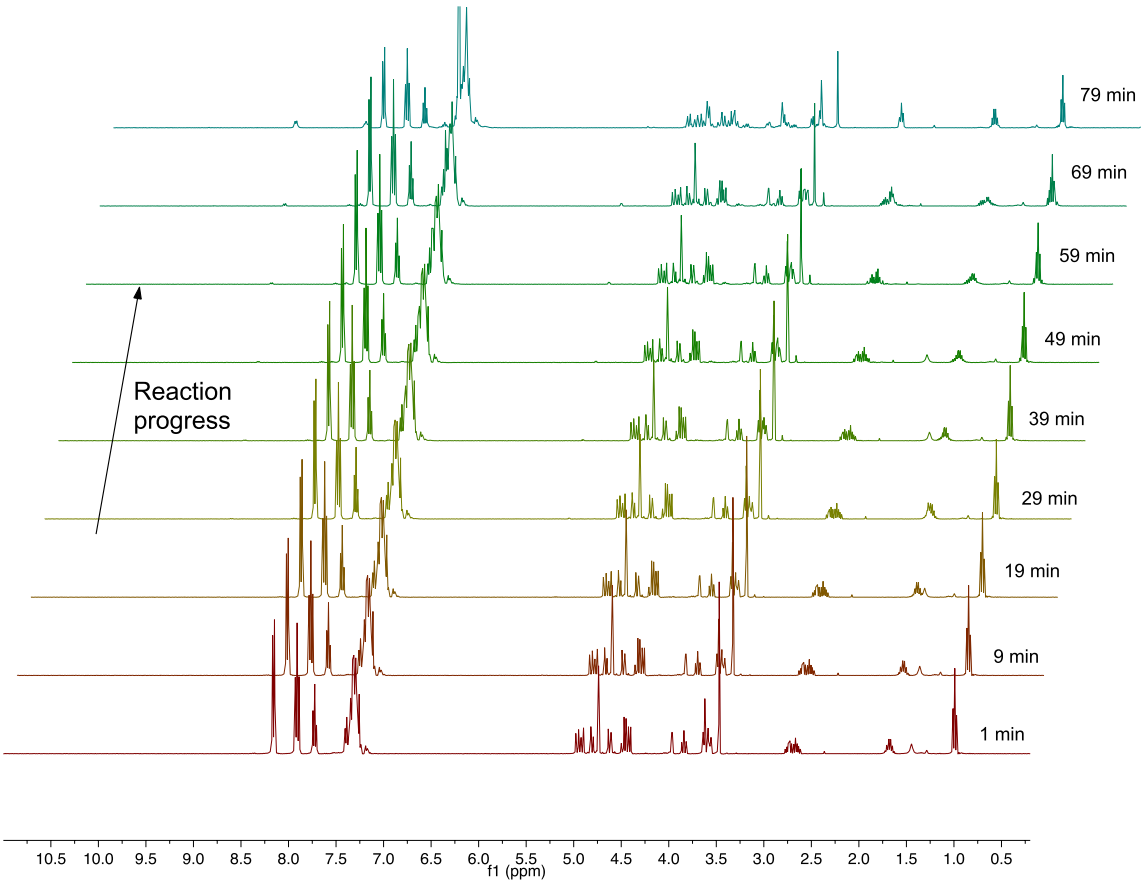

***Figure 1**: Full kinetics spectrum of model glycosylation of β-donor (Scheme 2)*

could be noticed. Interestingly, it could be noticed that after an initial decay of **A** (or vice versa increase of **Pdt**), there was a sudden drop (or rise in **Pdt)** in the peak concentration. This non-linear behavior of the kinetics curve is a clear indication of the complicated nature of the reaction. The initial lag phase is suggestive of existence of an induction period for the Bi(V) promoter to form sort of an "active species". This kind of sigmoidal curve is fairly uncommon in synthetic organic chemistry and additional experiments were required to understand the complexity of the activation.

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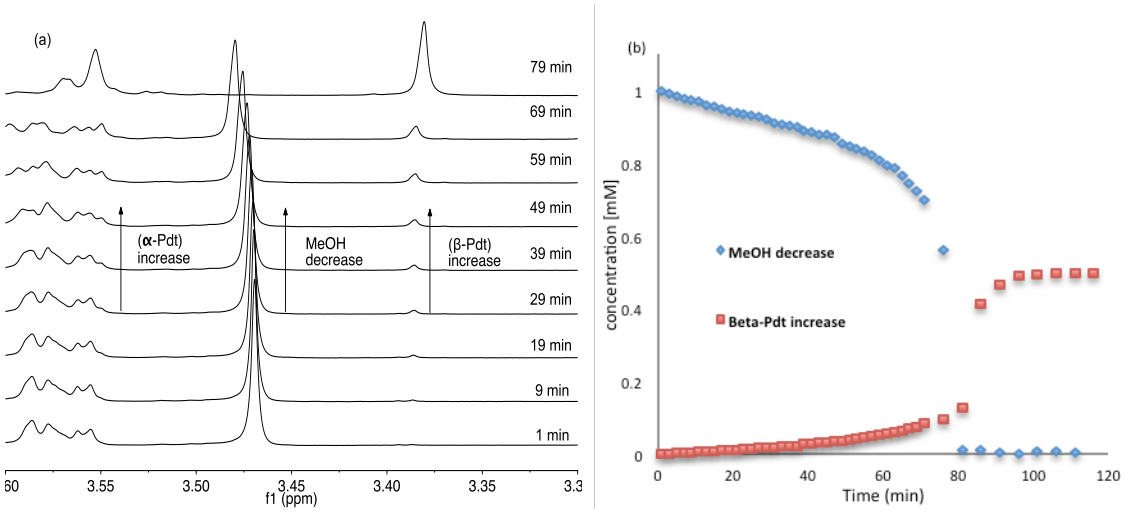

***Figure 2**: (a) Expanded kinetics spectrum of model glycosylation (**Scheme 6**) showing the peaks of interest (b) Product and acceptor concentration vs. time model glycosylation. Reaction conditions: Donor:Promoter:Acceptor::1:1:1 equiv., 40 °C, $CDCl_3$.*

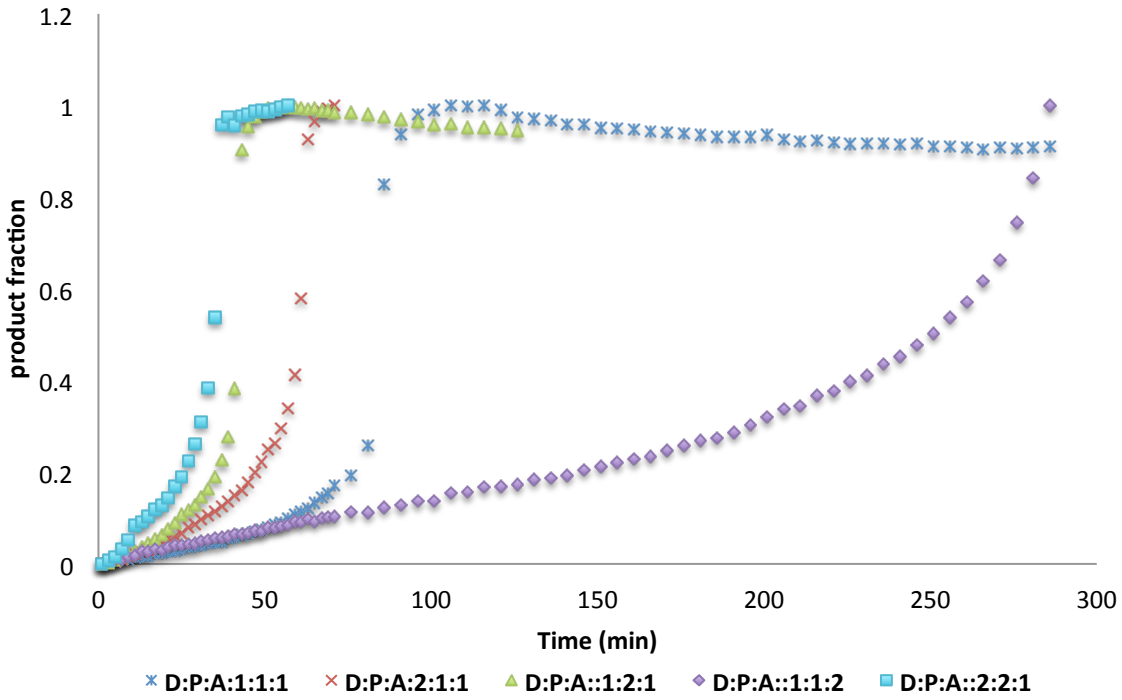

***Figure 3**: Comparison of various kinetics experiments with varying concentration of reactants. Legend entry: D:P:A::x:y:z x:y:z= ratio of concentrations of reactants (mM)*

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4.2.2.1 Rate-order studies of reactants: Pseudo-first order kinetics & Initial rate studies

To investigate the dependence of reactants on the rate of the reaction, we designed a number of kinetics experiments varying the concentrations of the reactants and compared them to each other (**Figure 3**).

Investigating the effect of different stoichiometry of reactants on the reaction kinetics, we observe that changes in concentrations of all the reactants, i.e., donor (D), promoter (P) as well as acceptor (A) have an impact on reaction kinetics. To understand these trends better, some pseudo-first order kinetics experiments were designed. Pseudo-first order rate studies are fairly commonly done to analyze second– or higher– order reactions, catalytic reactions, etc., where simultaneous monitoring of all the reactants is not feasible. In addition, to estimate rate order of each reactant, initial rate kinetics method was used, which has several advantages. Because the initial rate is measured under nearly pseudo-zero-order conditions, the determination of slope is easier as the change in concentration with time is effectively linear. Finally, as the reaction of interest progresses competing reactions may develop, complicating the kinetics—using the initial rate eliminates these complications. The sigmoid nature of the kinetics curve of the activation makes the calculation of slope quite tricky. However, this method sometimes suffers from the limitation that there may be insufficient time for completely mixing of the reactants. Also, since we have a sigmoidal curve here and a possible

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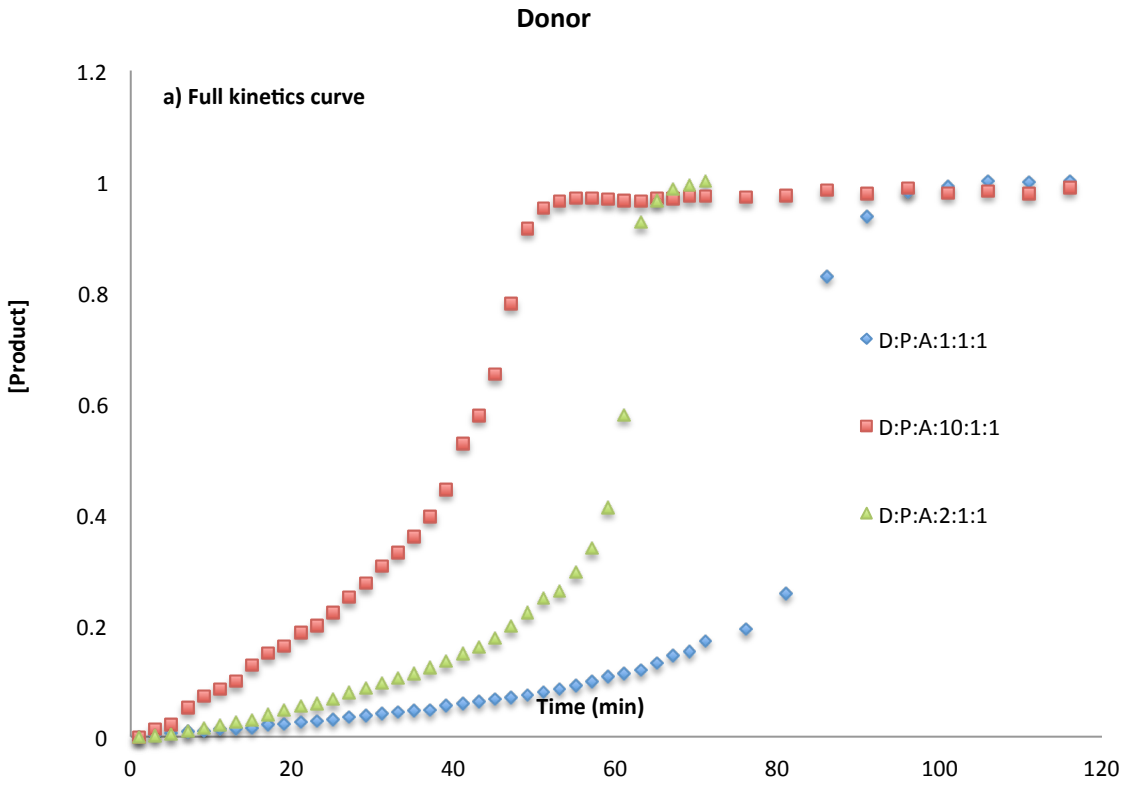
induction period, it needs to stated here that the initial rate method was utilized to study the rates of the induction phase and not the entire reaction.

4.2.2.1.1 Role of donor (D)

The donor concentration was varied in the model glycosylation and compared with each other [**Figure 3 (a)**]. It was observed that the reaction accelerated as donor amounts increased. Applying the initial-rate kinetics method [**Figure 3 (b)**], it can be seen that doubling the donor concentration, the induction period rate halves and with excess donor, the reaction becomes extremely fast. This kind of response is suggestive of first-order kinetics and a possible role in the rate-limiting step.



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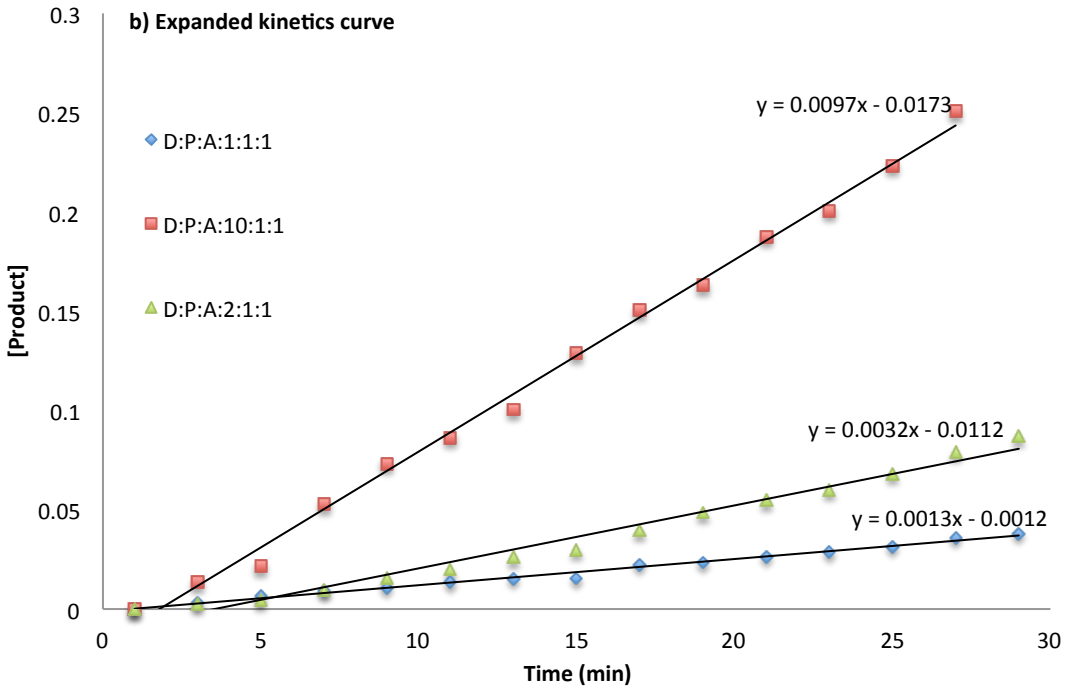

***Figure 4**: Kinetics data for different donor concentrations*

4.2.2.1.2. *Role of promoter (P)*

Figure 5 (a) demonstrates the comparison of kinetics runs for different concentrations of promoter (P) in the model thioglycosidation. From this data, it was observed that the reaction rate increased on doubling the promoter concentration (D:P:A::1:2:1). Unlike the case of donor kinetics, taking the promoter in excess amounts (D:P:A::1:10:1) initially accelerates reaction rate, but surprisingly the total reaction time is longer than the entry D:P:A::1:2:1. **Figure 5 (b)** shows the induction rate comparison of the above entries and as discussed above, the initial rate increases by a factor of 3 when donor concentration was doubled and by ~7 when used in excess. All the

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observations clearly indicate that the rate order of the promoter is probably different from the donor, and is more complicated.

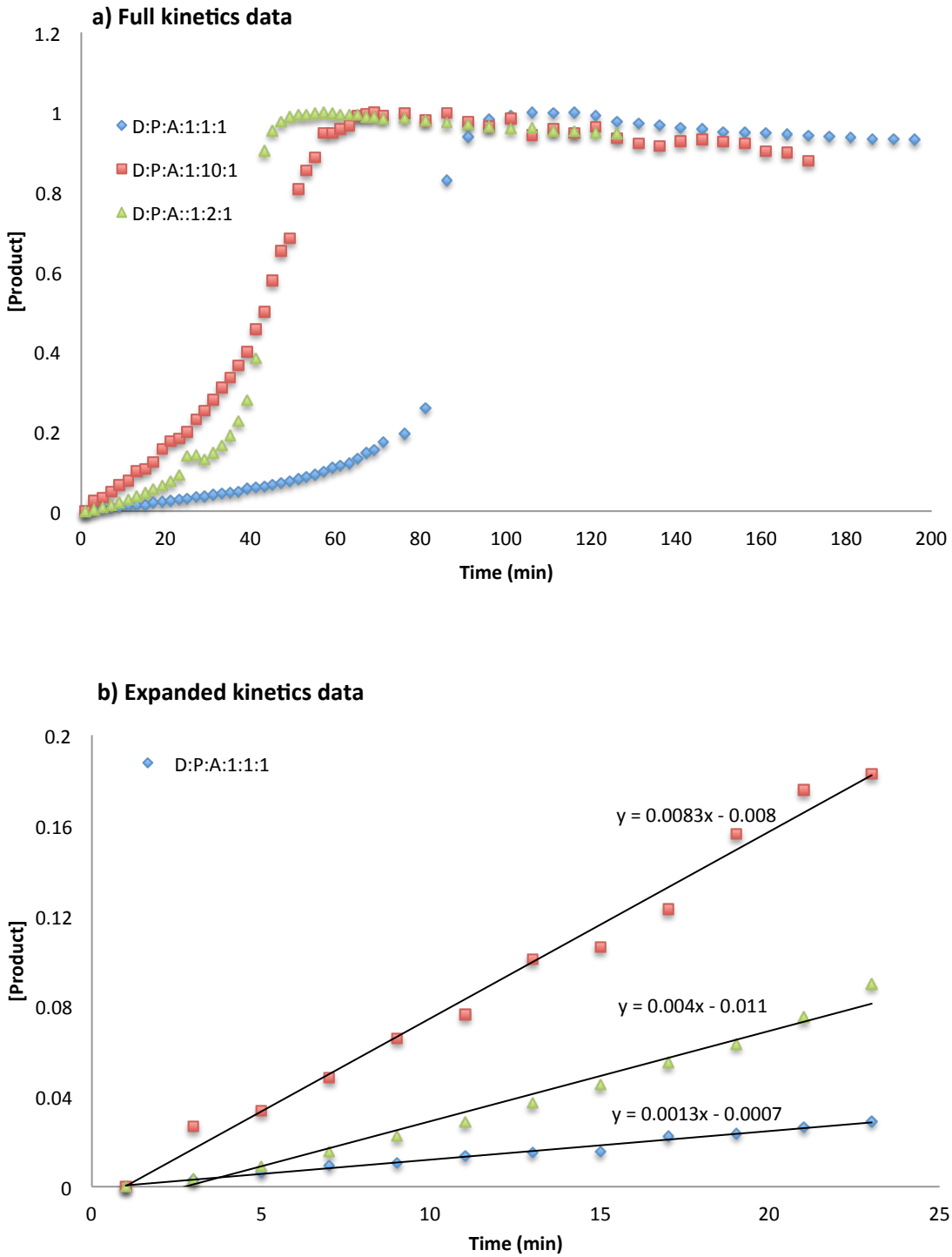

***Figure 5**: Kinetics data for different promoter concentrations*

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4.2.2.1.3. Role of acceptor

Next, we looked at the effects of changing concentrations of acceptor (A) in the model glycosylation. Unexpectedly, as the amounts of the acceptor were increased, the activation progressively slowed down. Interestingly, the initial rate measurements showed that when doubling the acceptor concentration, the induction period rate decreased by a factor of 0.7, but the total reaction time increased from 80 min (1.3 h, D:P:A::1:1:1) to 286 min (~5 h, D:P:A::1:1:2). In fact, using excess acceptor (D:P:A::1:1:10), the initial rate as well as the total reaction time is comparable to the D:P:A::1:1:2.

Previously, Huang *et al*. in their mechanistic studies of thioglycoside activation with *N*-iodosuccinamide (NIS)/triflic acid as promoter, suggested that the first step of electrophilic addition of the promoter to the donor is the fast step, and the next step of addition of the acceptor to the reactive intermediate is the rate limiting step. In our case, increase in both donor and promoter accelerated the reaction kinetics and excess acceptor unusually retarded the reaction rate.

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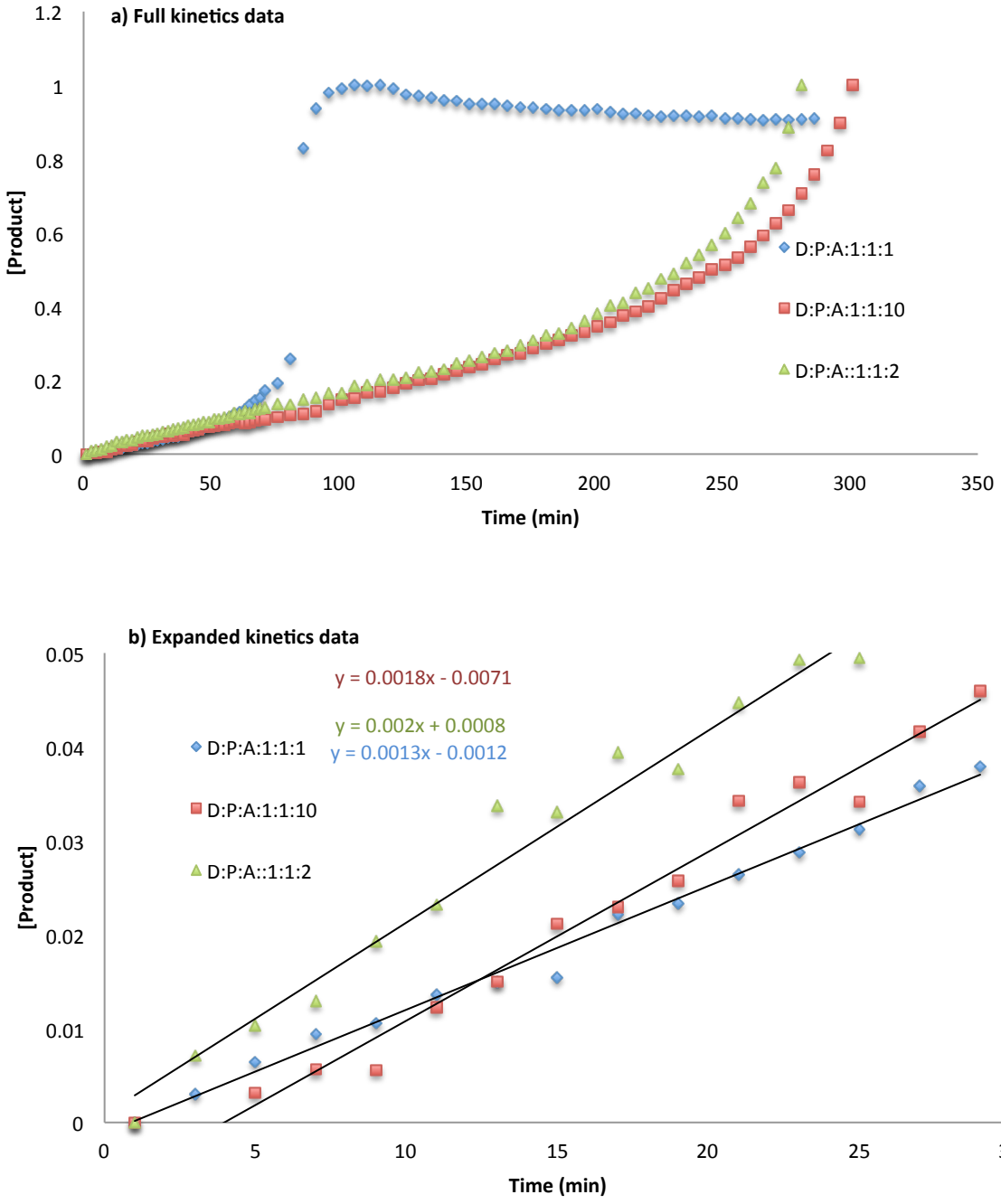

***Figure 6**: Kinetics data for different acceptor concentrations*

4.2.2.2. Comparison of pseudo-first order kinetics studies

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As referred before, pseudo-first order rate studies can be crucial in solving rate kinetics of complex reaction mechanisms. We decided to make use of this technique to

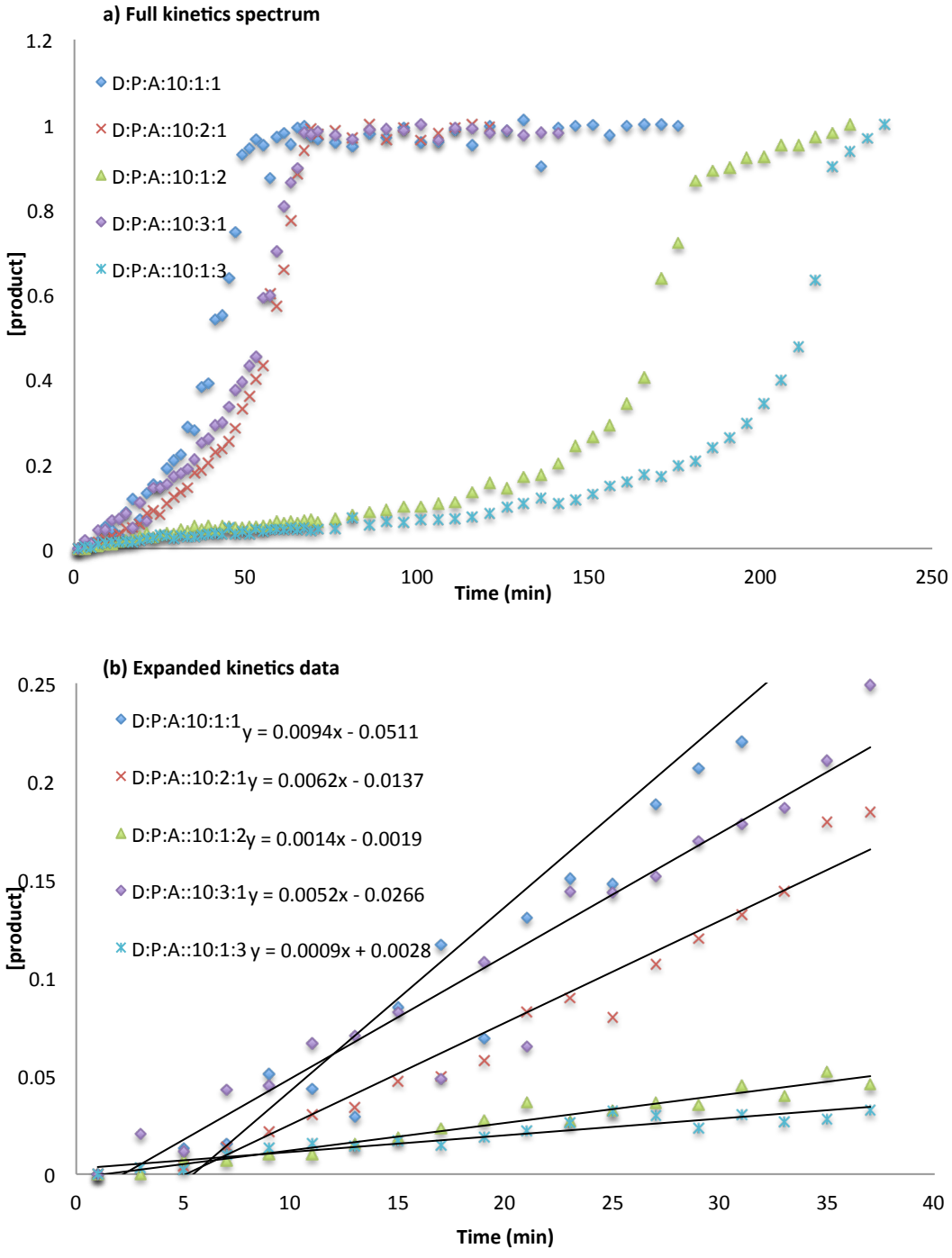

***Figure 7**: Comparison of kinetics data with pseudo-first order kinetics in donor*

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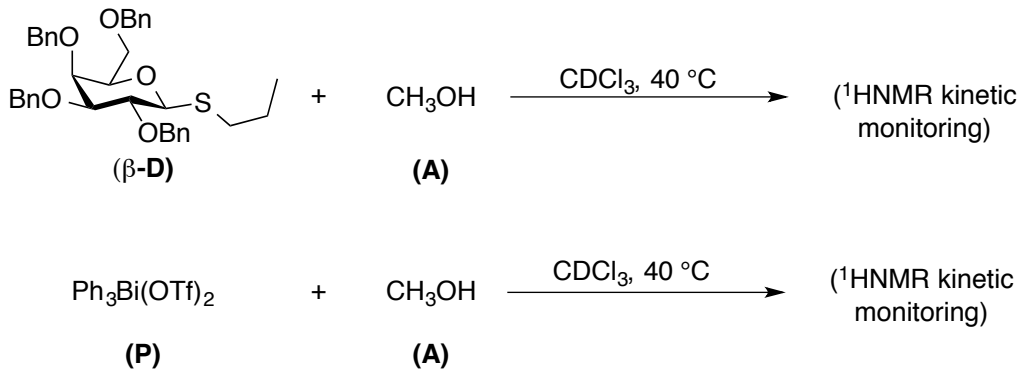
look into the complicated roles played by the acceptor and promoter. Several experiments comprising different reactant concentrations were done by taking excess amounts of donor (pseudo-first order) compared to promoter and acceptor amounts (**Figure 7**). Surprisingly, increasing promoter amounts in these experiments did not accelerate the reaction rate, in fact the initial rate slowed down slightly. However, increasing acceptor amounts significantly retarded the overall reaction rate, although not much change was observed in the initial rate. Additional experiments were hence required to understand the unorthodox behavior of the reactants.

4.2.2.3. Probing the function of acceptor: control experiments & glycosyl acceptor kinetics studies



***Scheme 7**: Control experiments with donor, promoter and acceptor*

The control experiment (**Scheme 7**) showed that the donor and acceptor did not react with each other in absence of the promoter as no changes were observed in the ^{1}HNMR kinetics studies. However, when the acceptor with the promoter alone was reacted, some changes though minimal could still be seen. It appeared that these two

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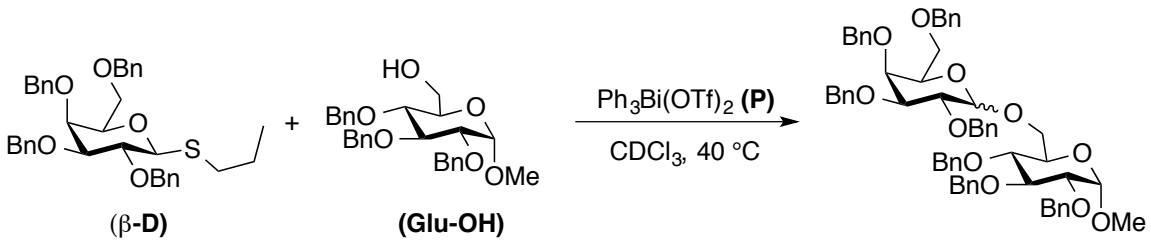
reactants together formed some complexes/side-products after a certain time (>4h, longer than usual reaction time). Bi^{V} complexes have been tested to facilitate oxidation of secondary alcohol to ketones. Consequently, it was observed that if the promoter was added to the acceptor first and then reacted with the donor, the reaction rate slightly slowed down compared to when the promoter was first mixed with acceptor and then added to the donor. However, since bismuth is perhaps more thiophilic[21] than oxophilic, the reaction of promoter with the donor is more favorable than with the acceptor, because of which lowering of reaction yields was not observed but only increase in total reaction time was seen.

To study if the effects of the acceptor, MeOH are general to other alcohols as well, a glycosyl acceptor (**Glu-OH**, **Scheme 8**) was selected and few kinetics studies (**Figure 8**) were done.



***Scheme 8**: [1]HNMR kinetics experiment of a glycosylation with a glycosyl acceptor*

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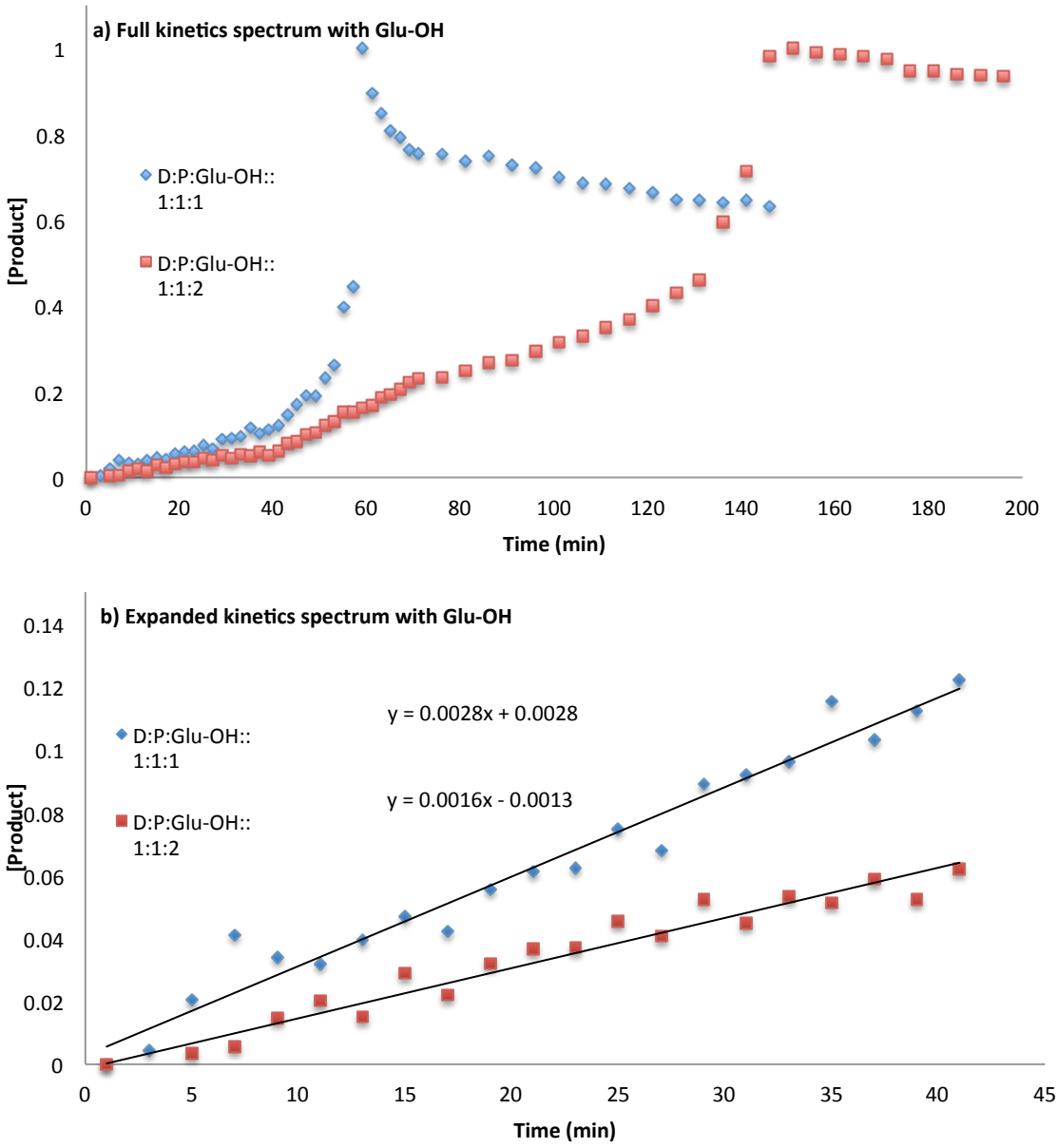

***Figure 8**: Comparative kinetics data for glycosyl acceptor concentrations*

Plotting the product concentration against the time of reaction, similar trends were observed in the ^{1}HNMR kinetics studies conducted with the glycosylation of **β-D** with **Glu-OH**. After an initial slow increase, there was a sudden increase in product formation, as seen with MeOH. When the amount of the **Glu-OH** was doubled, the

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reaction rate decreased, i.e., the reaction time lengthened from 59 min to 151 min. The initial rate measurements also showed decrease in reaction rate by a factor of ~0.5 as we went from D:P:Glu-OH::1:1:1 to D:P:Glu-OH::1:1:2. This decrease however was less pronounced than the case of using MeOH as an acceptor. Taking these observations into account, we concluded that excess acceptor does retard the reaction rate, possibly interacting with the Bi(V) reagent. Fortunately, the overall reaction yield remains same in both cases, which suggests that the interaction probably is a reversible equilibrium step, hence the promoter is not blocked from interacting with the donor. Interestingly, the product concentration starts going down once it has reached its maximum indicating that the glycosidic bond between the two saccharides is prone to anomerization or hydrolysis when the reaction is left running for long.

4.2.2.4. Further discussion and summary of kinetics studies

In light of all of the above ^{1}HNMR kinetics studies, it can be concluded that the Bi^{V}-mediated thioglycoside activation does not follow a straightforward kinetics pathway and the kinetics modeling has proved to be quite tricky. More importantly, these studies have helped us to better understand the impact of the various reactants in the initial induction phase. In addition, it was noticed that the order of addition of reactants into the reaction mixture plays a crucial role in the rate of the reaction. Although we have progressed in our understanding, the unique sigmoidal behavior of the kinetics curve showcased by the Bi(V) glycosylation needs more investigation. Interestingly, comparable nonlinear behavior of the curve has been observed earlier

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with autocatalytic systems.[22] The initial slow increase of the product is considered to be the induction or resting period for the catalyst, followed by the sharp increase as the autocatalysis starts playing a part. In some of the preliminary investigation to study autocatalysis, we have observed a consistent rate shift at around 20% conversion. (We thank Prof. Jason Hein, UC Merced for his valuable help here). In future, detailed kinetics experiments need to be investigated to confirm these initial observations.

4.2.3. 1D & 2D NMR Studies: new direction to the mechanistic studies

So far, with the GC-MS and various kinetics experiments, we have been able to make quite a few discoveries regarding our reaction mechanism: specifically with the interaction of reactants with each other, rate order calculations and rate law equations. NMR spectroscopy had proved to be a very effective tool in analyzing these observations. So, going back to the initial ^{1}HNMR-kinetics spectrum, we observed a few other interesting features, apart from the disappearance and appearance of the methoxy peak (**Figure 1**). Particularly, a peak in the chemical shift range of δ 5.5–5.6 ppm (d, *J* = 4.8–5.2 Hz, 1H) was seen to grow (**Figure 9**) during the course of reaction and disappear as soon as all of the reactant got consumed (OMe of MeOH). Also, multiplets at δ 2.7 and 1.6 ppm seemed to be split into two sets (**Figure 9**) as the reaction proceeded and similarly this trend disappeared at the end of the reaction. These interesting observations led us to believe that perhaps, the formation and disappearance of a reactive intermediate was being detected in the NMR instrument. This was a very exciting discovery since observing a reactive intermediate in an ongoing

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reaction on the NMR time-scale (especially without low temperature) is rare, not to mention that these kinds of thioglycoside based intermediates are uncommon in glycosylation literature.

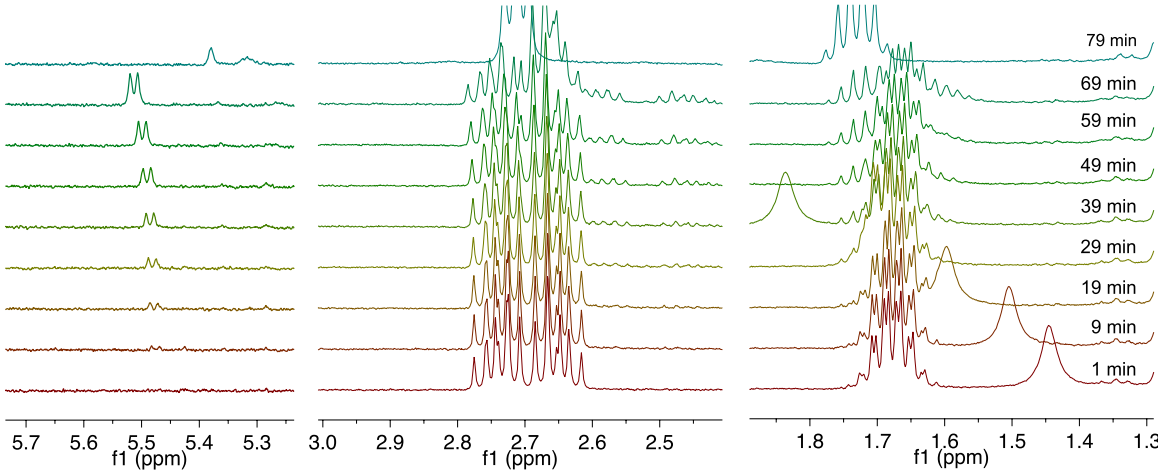

***Figure 9**: Expanded regions of the NMR spectrum focusing on the transient peaks*

Another unique feature we noticed with the growing doublet peak around δ 5.5 ppm, which was assumed to be a beta (β)-anomeric H-1 peak (usually δ 4.5-6.5 ppm), was that it shifted around 0.2 ppm during the course of the reaction. Moreover, the peak initially seemed to have a coupling constant of 4.8 Hz, which later slightly increased to around 5.5 Hz. This was very intriguing as well as baffling to us as we suspected a β-anomeric H-1 peak of a glycosyl sulfonium intermediate to have a more deshielded chemical shift than δ 5.5 ppm as well as the coupling constant to be in the range of 9-11 Hz. In fact, the observed shifts/coupling constants were indicative of an alpha (α)-anomeric H-1 peak of a glycosyl compound. Hence, a number of 1D & 2D correlation NMR studies were done to investigate this mysterious intermediate. At first,

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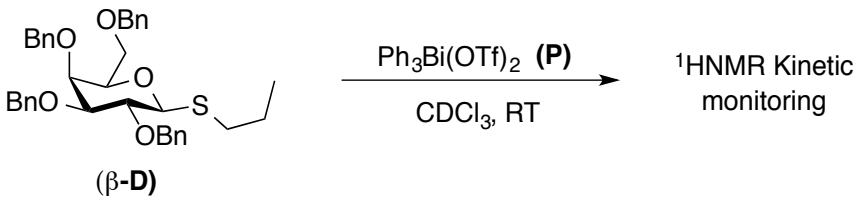
a kinetics experiment was monitored reacting only the **β-D** and the $Ph_3Bi(OTf)_2$ (**P**) at various temperatures [0 °C, RT(~25 °C), 40 °C]. Similar trends were observed with the proton peaks (as mentioned before) and the unknown intermediate still could be seen to be forming/disappearing in the absence of the acceptor [See experimental section]. This result confirmed our initial hypothesis that the detected intermediate is a glycoside-based compound. To investigate more, another experiment was set up by reacting only donor (β-**D**) and promoter (**P**) at room temperature (~25 °C) in absence of an acceptor (**A**) to analyze the intermediate (**Scheme 9**).



***Scheme 9**: Activation of β-donor with promoter in absence of acceptor*

As soon as the reactive intermediate was building up, the kinetics experiment was halted and 1D & 2D NMRs were taken. The benzylated α-thiopropyl galactoside donor was synthesized following literature methods and its NMRs were then compared to the intermediate's NMRs. Correlating ^{1}H (**Figure 10**), ^{13}C as well as phase-edit HSQC spectra (**Figure 11**), we noticed that this intermediate was in fact the α-donor! In other words, the β-thioglycosyl donor underwent anomerization to the α-donor before getting activated to the desired product. This finding was a revelation in our mechanism as this kind of β to α anomerization of a glycosyl donor is a rare sight in carbohydrate literature.

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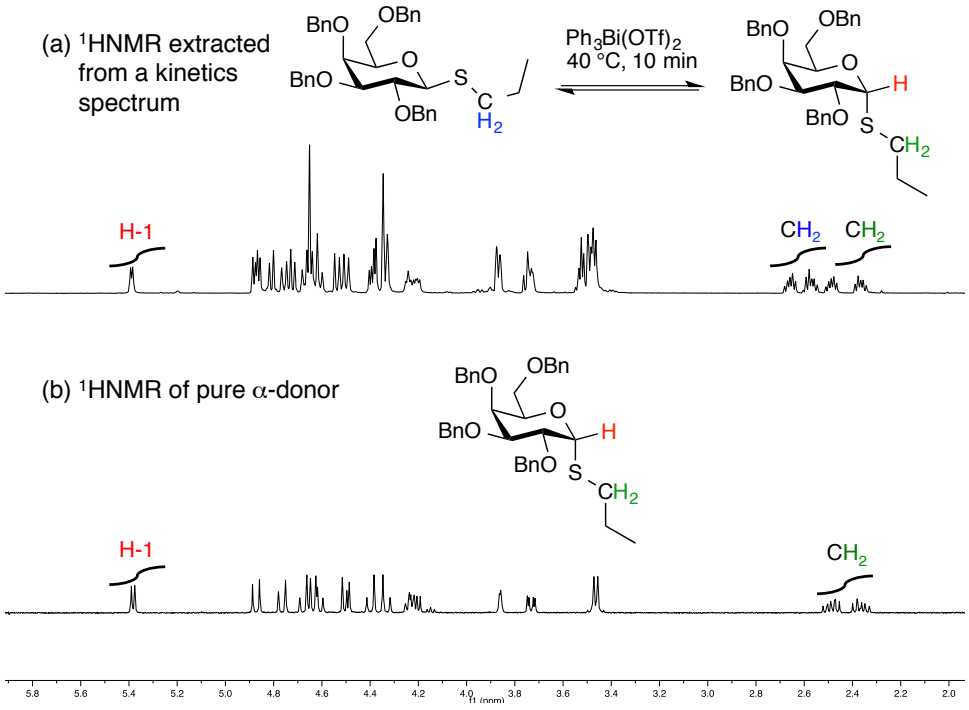

***Figure 10**: Expanded ¹HNMR comparison of the reaction mixture and the α-donor*

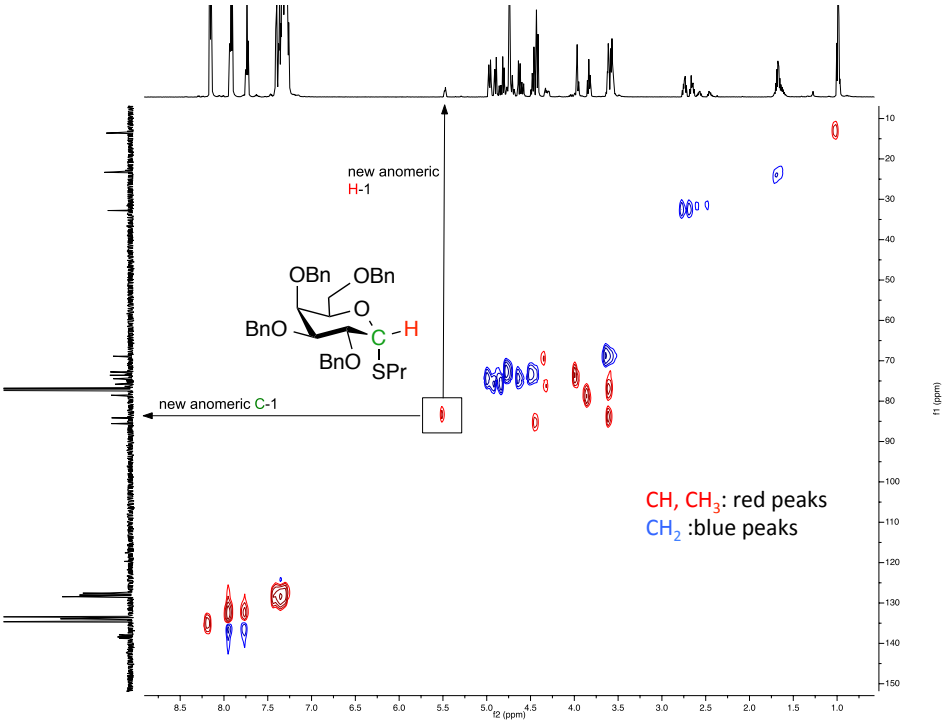

***Figure 11**: HSQC (Phase-edited) of the reaction mixture and compared to the α-donor*

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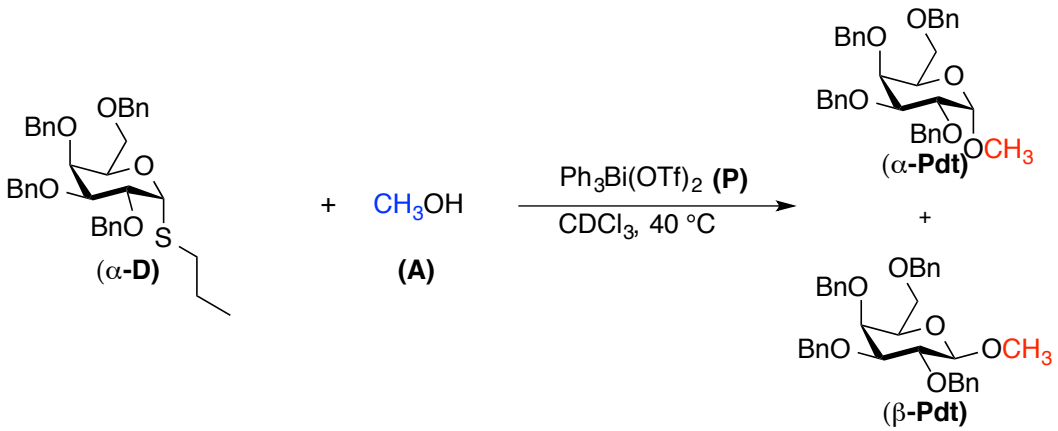
4.2.3.1. Alpha (α)- donor kinetics

To further authenticate our results, another ^{1}HNMR kinetics experiment of the same model glycosylation was done using the alpha-donor instead (**Scheme 10**). If our assumption was correct, we suspected to observe no isomerization, i.e., decrease of the H-1 of the alpha-donor and overall faster kinetics.



***Scheme 10**: Model glycosylation with α-donor for kinetics rate-order study*

Confirming our predictions, the kinetics spectrum of alpha-donor (**Figure 12**) shows no increase but rather decrease of the H-1 peak at δ 5.5 ppm, supporting our hypothesis that the reactive intermediate, which formed in Figure 1, was in fact the α-isomer. Moreover, the mulitplets at δ 2.7 & 1.6 ppm can no longer be seen to be splitting with reaction progress. Instead, the cleaved off methylene protons of the thiopropyl containing by-product can be seen growing. As expected, the α-donor activation was indeed faster than the β-donor. **Figure 13** shows the comparative kinetics

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spectrum of the activation done with both the beta and the alpha donor. By initial-rate kinetics method, the increase in rate is evident between the two trials.

Apart from the improved reaction time, it was noticed that the diastereoselectivity of the obtained products also enhanced from a lower 1:1 to higher 4:7 ratio. This discovery can be counted as an advantage to our developed methodology as our current protocol has not yet induced high stereoselectivity in products and followed general trends. We envision that this strategy will be explored more in the future, to set tougher stereoselectivity and difficult linkages with different thioglycosyl donors.

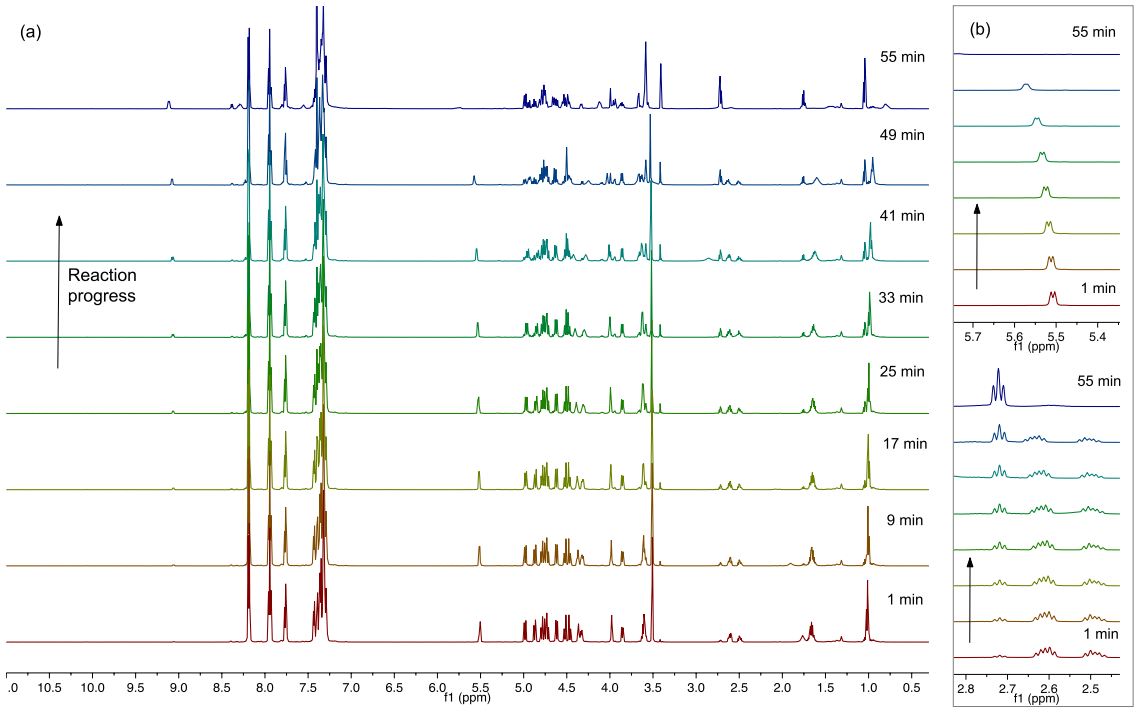

***Figure 12**: (a) Full kinetics spectrum of model glycosylation of α-donor (b) Expanded kinetics spectrum showing regions of δ 2.4-2.8 ppm and δ 5.3-5.8 ppm*

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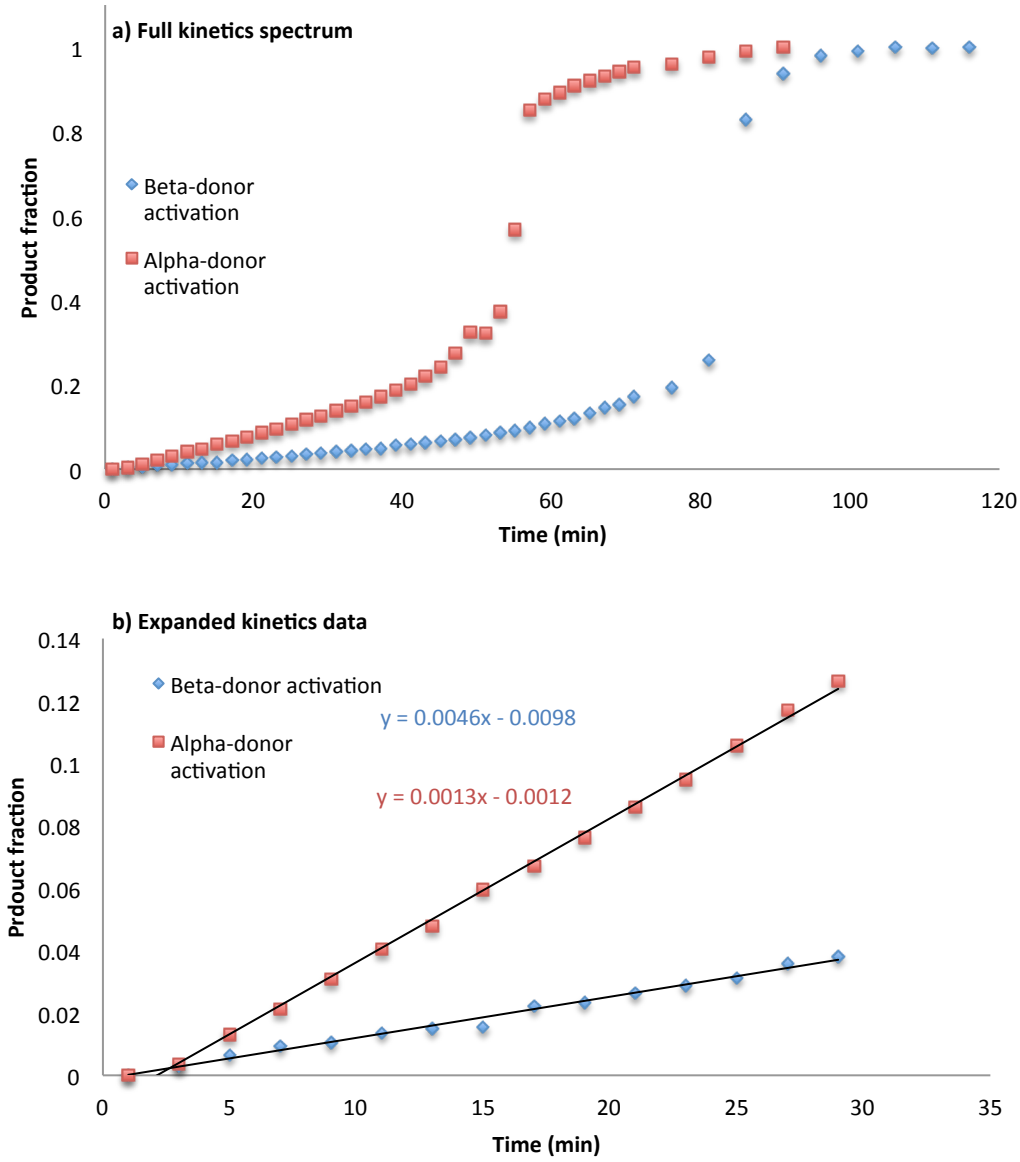

***Figure 13**: Comparison between kinetics data of α vs. β-donor*

4.2.4. *Probing reactive intermediates 1D & 2D correlation studies: trials and successes*

This interesting discovery of unusual $\beta \rightarrow \alpha$ anomerization led us to speculate the reason behind this event and in what ways it contributes towards the thioglycoside activation. As mentioned earlier, our methodology was based on the hypothesis that

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bismuth is inherently thiophilic and can possibly attach to the sulfur atom on the glycoside to form reactive sulfonium species, which then help in its cleavage. The aforementioned GC-MS studies also suggested that the formation of by-product, PrSSPr was probably due to this bismuth-sulfonium species. To investigate further, we chose the informative NMR spectroscopy. Although, correlations of protons in a reactive intermediate are difficult to detect on the NMR machine as the dynamic motion of an intermediate is generally faster than the NMR time-scale, especially at room temperature.

4.2.4.1. Through–bond NMR & other methods

A number of NMR experiments were explored to probe the reactive intermediate formed in situ during the thioglycoside activation. At first, diffusion based spectroscopy (DOSY) was utilized with the intention of separating the constituents in the NMR mixture relative to their diffusion coefficient. But this technique failed to distinguish the mixture and overlapping peaks were observed. The rationale behind this observation is probably the similarity in size and shape of the reactants/intermediates, which is not uncommon in DOSY spectrum of high-molecular weight species.

Next, a variety of 1D-selective & 2D through-bond based (COSY, HMBC, HSQC, TOCSY, etc.) methods were employed to chase the reactive intermediate. A multi ^{1}H-^{13}C HMBC experiment was done where spectra was recorded for the β-donor activation (**Scheme 9**) at various stages of the reaction (**Figure 14**). From the figure, correlations of the reactant donor protons with sugar ring carbons and the promoter protons with

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phenyl ring carbon could be seen throughout the reaction, but correlations between the donor and promoter were not detected. The HMBC at the end of the reaction also failed to show any promising peaks of the by-products formed.

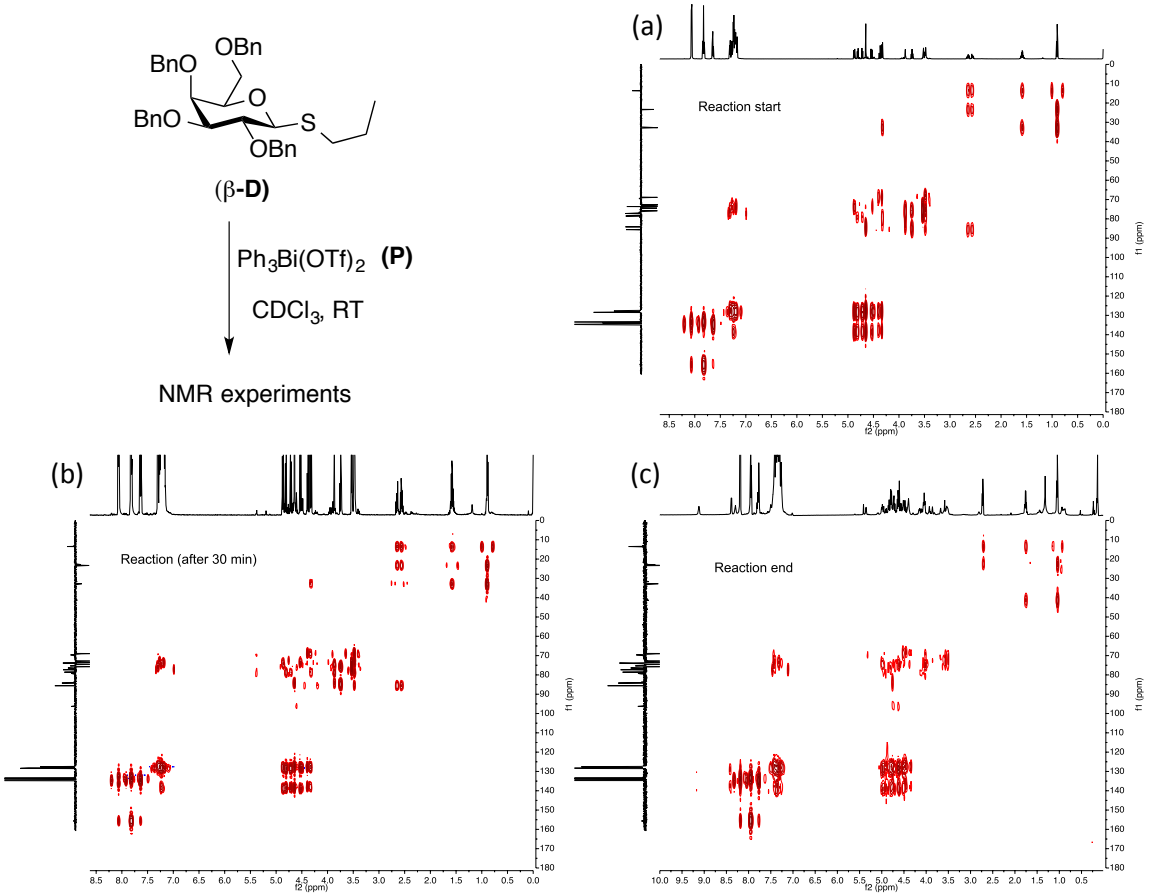

***Figure 14**: ^{1}H-^{13}C HMBC of β-donor activation at various reaction times: a) start; b) after 30 min; c) end*

Some similar experiments comprising ^{1}H-^{13}C HMBC and 1D-selective TOCSYs were done starting with the α-donor (**Figure 15**). Of these experiments, particularly an HMBC taken towards the end of the activation was particularly interesting since it showed correlations of the cleaved off SCH_2 of the thiopropyl group to the phenyl ring protons on the Bi-promoter. This suggested that other than PrSSPr, some other PhBi-S

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containing by-products forms during the course of the reaction. This also explained the detection of lower than expected amounts of disulfide at the reaction end.

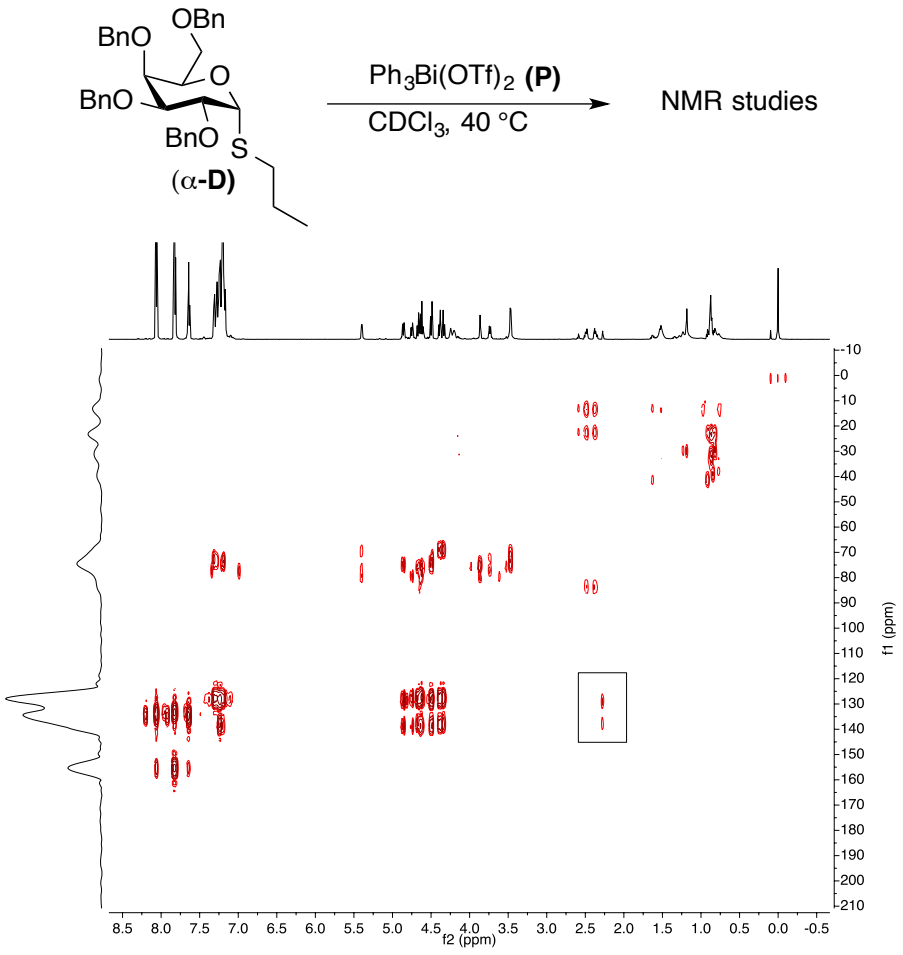

***Figure 15**: ^{1}H-^{13}C HMBC of α-donor activation after 30 min*

Other 1D and 2D through-bond based NMR techniques like COSY, HSQC, etc. were also done for both α and β-donor activation. Unfortunately, these also failed to give much structural insights of the reactive complex. The presence of various spin systems on the same molecule along with the distance between the "interested"

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protons of the large bismuth-sulfonium species possibly posed as the main limitations for the through bond approaches.

4.2.4.2. Through-space NMR methods

Luckily, the selective through space approach; nuclear Overhauser effect (NOE)[23] experiments (NOESY, ROESY, etc.) helped us to identify the possible intermediate. At first, we tested various 1D and 2D NOESY and ROESY experiments with the β-donor activation (**Scheme 9**). The comparative stacked spectra [**Figure 16 (b), (c), (d), (e)**] shows the 1-D selective NOESY's of the different pulsed peaks selected from the ^{1}HNMR of the in-situ reactive intermediate [**Figure 16 (a)**].

These studies in figure **16** showed: (b) when phenyl protons present on the bismuth promoter (9.2 ppm) were selectively pulsed, they correlated with peaks on the glycoside, specifically the methyl (CH_3) on thiopropyl group; (c) when transient H-1 peak on the thioglycoside was pulsed (5.5 ppm), it correlated with the phenyl protons on the promoter as well as other sugar ring protons; (d) CH_2 on the thiopropyl group (2.6 ppm) on pulsing surprisingly showed strong correlation signals with the phenyl protons, whereas showed weak signals with the rest of the thiopropyl protons; (e) when CH_3 (1.0 ppm) of the thiopropyl group was pulsed, it again correlated with the phenyl protons on the bismuth promoter. To explain these correlations, we came up with a possible intermediate structure (**Figure 16**). The structure shows that the phenyl protons on the bismuth core are probably closer to CH_3 of the thiopropyl group (as this part can rotate freely and can be in the proximity of the phenyl ring), which may also be the reason of

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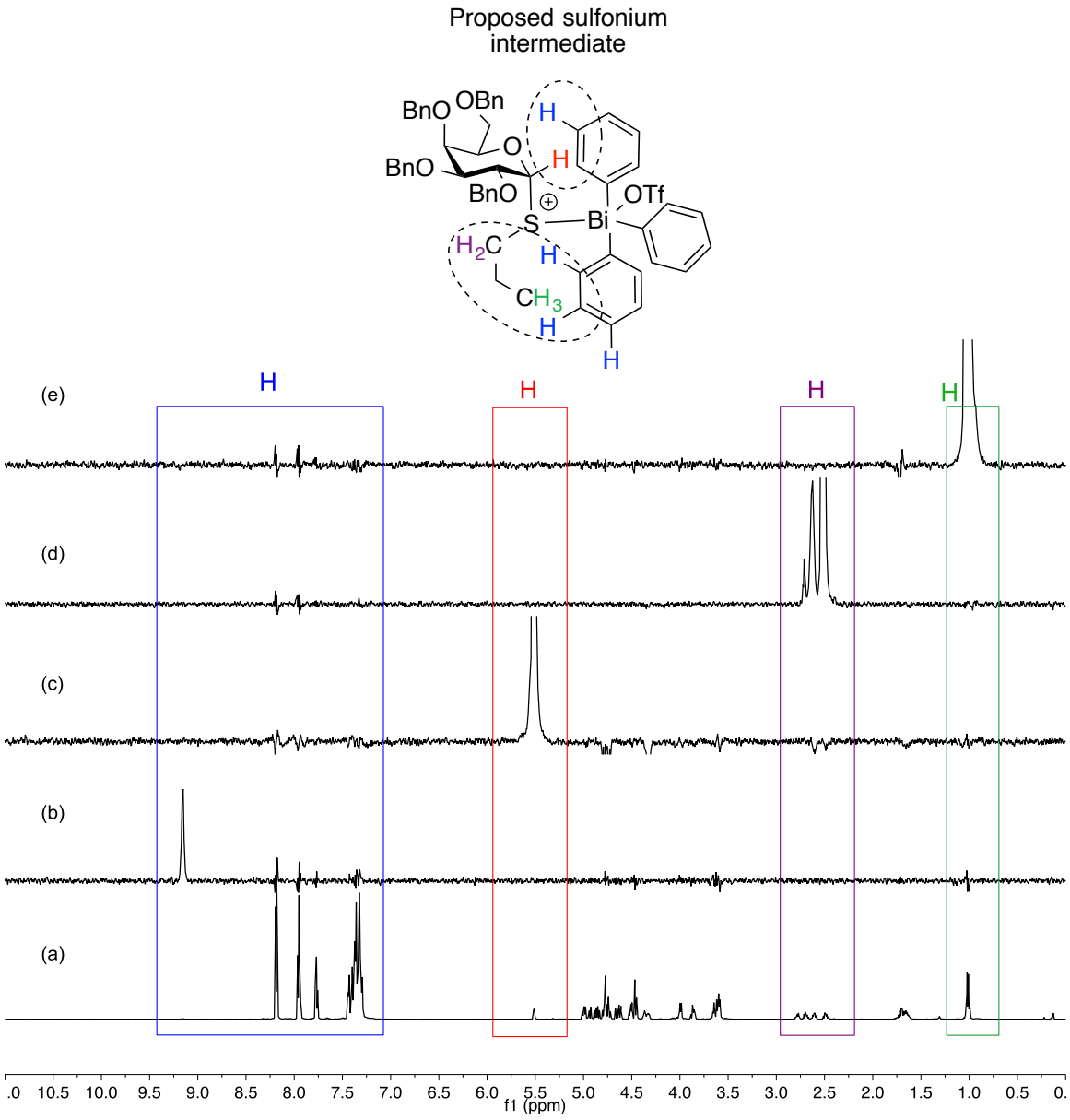

***Figure 16**: a) [1]HNMR of the reactive intermediate; 1D selective NOESY of peaks at chemical shifts δ (b) 9.2 ppm (c) 5.5 ppm (d) 2.6 ppm (e) 1.0 ppm showing correlation with other peaks.*

seeing correlations through space and not through bonds. Most importantly, the correlations of the anomeric proton present on the glycosyl ring to the rest of the ring protons and especially to the phenyl protons on the bismuth core provide strong evidence for existence of the bismuth-sulfonium species. To support the existence of

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this sulfonium intermediate, the behavior of the H-1 of the alpha-donor during the kinetics experiment can be considered. Close examination of **Figure 12 (b)** shows that the particular proton peak shifts by 0.05 ppm on the NMR scale during glycosylation, while the coupling constant changes from 4.8–5.5 Hz. These features along with the peak shape change from a distinct doublet to a broad singlet before getting consumed are definite indications of a complex formation.

4.2.5. Superarmed donor kinetics rate studies

Anchimeric assistance or neighboring group participation (NGP) is one of most crucial strategy that is utilized in oligosaccharide synthesis to construct 1,2-*trans* linkages. Usually, the 2-OH of the glycosyl donor is protected with a participating ester group like acetates (OAc) or benzoates (OBz), which in general blocks any attack from one of the faces, giving typically one product diastereoisomer.

Since the discovery of the atypical $\beta \rightarrow \alpha$ anomerization in our thioglycoside activation, we wanted to investigate if this phenomenon will still occur with an acetate group present on the 2-OH of the thioglycosyl donor. For this purpose, we synthesized a thiopropyl galactoside donor **(β-SD**) and performed NMR reaction kinetics (**Scheme 11**). This particular class of donors which has benzyl ethers on 3, 4, 6-OH and a participating group like OAc or OBz on a 2-OH are called superarmed donors,[24] as glycosylation with these donors are extremely fast. As expected, we found a similar trend with the kinetics experiment, wherein the rate of the β-superarmed donor (**β-SD**) activation was way faster than the previous β-donor (**1a**, **β-D**).

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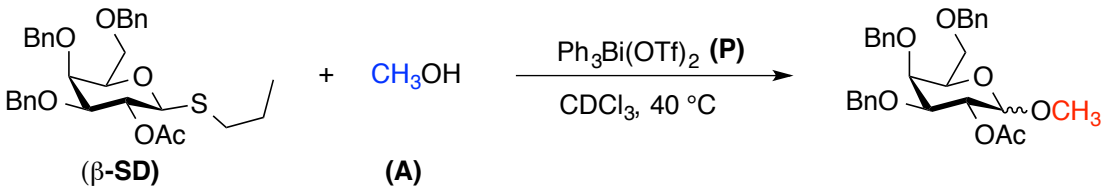

***Scheme 11**: Glycosylation of the superarmed donor for kinetics studies*

Because the activation was fast, the appearance/growing of peaks were not as distinct as with the other β-donor. However, on closer look at the ^{1}HNMR kinetics spectrum, we noticed that a mixture of two isomers was formed as seen in previous studies (**Figure 9**). For accurate analysis, we synthesized the α-superarmed donor (**α-SD**) and compared the kinetics spectrum (**Figure 17**).

As evident from the **Figure 18**, the ^{1}HNMR taken during the ongoing activation (**Figure 17**) contains the peaks for the α-donor, in addition to the β-donor and other products. This implies that the β-donor interconverts to the α-donor during the activation! This finding is very surprising as the presence of NGP on 2-OH of a galactosyl donor blocks off the α- face and so, formation of the α-donor was unprecedented. Hence, it can be concluded that the β→α anomerization is quite favorable with the Bi^{V} activation even in the presence of anchimeric assistance.

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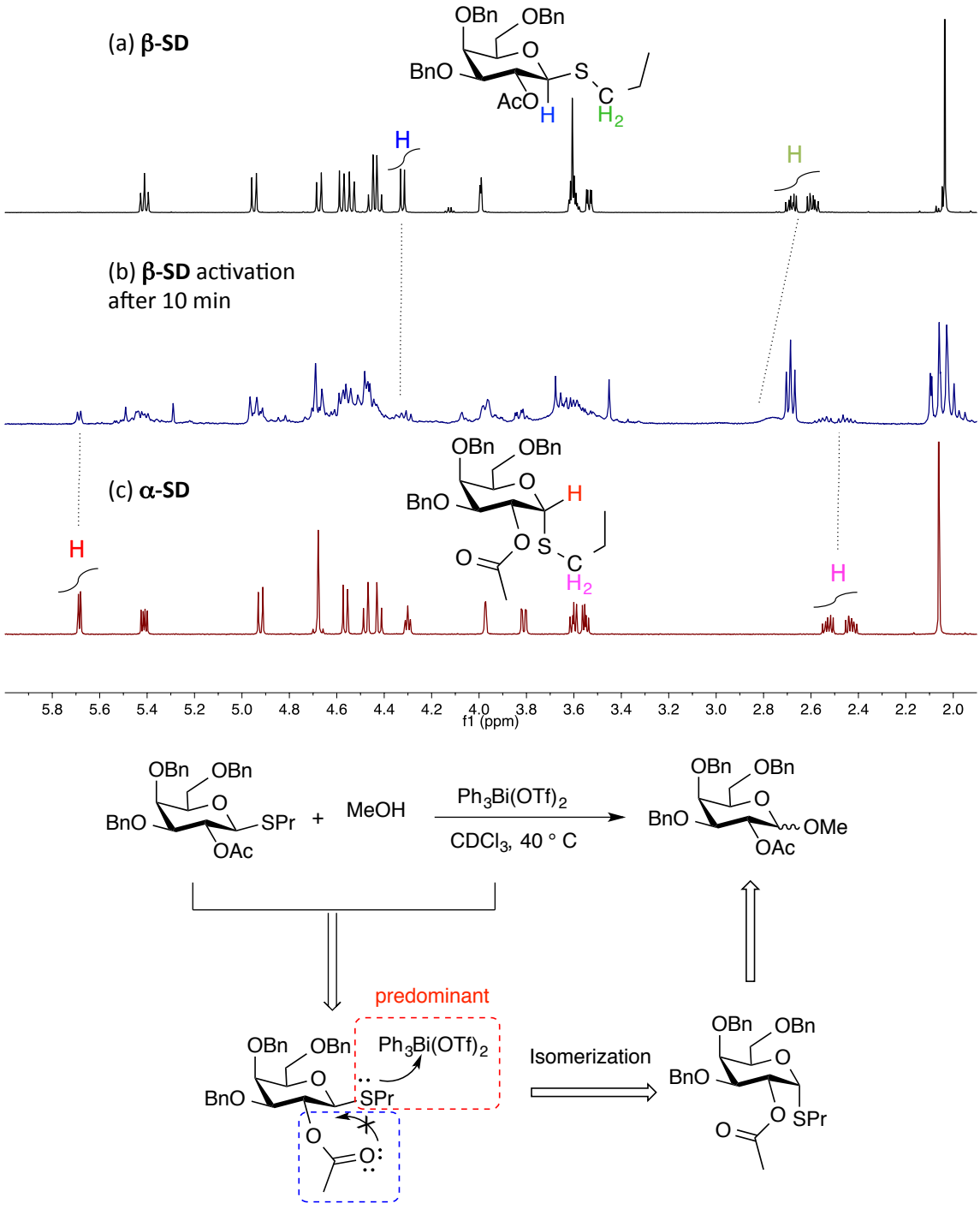

Figure 17: *[1]HNMR comparison of the activation; a) pure β-superarmed donor (**β-SD**); b) reaction mixture of activation of β-donor (scheme shown in figure) after 10 min; c) pure α-superarmed donor (**α-SD**)*

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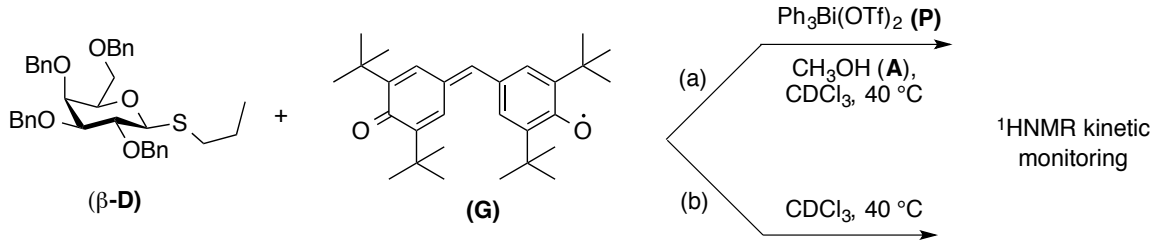
4.2.6. Probing single electron transfer (SET) pathway

The activation of thioglycosides is commonly done via the formation of a reactive sulfonium intermediate through a two-electron process. Another common way to generate a carbenium center is through an electron transfer activation of the sulfide group to give a reactive radical cation, which was exploited with Amatore and Sinay[14] and recently by Bowers and co-workers.[25] To probe if our developed methodology also 8follows a radical pathway, we designed a few kinetics experiments with a radical scavenger, galvinoxyl. The approach was that if the mechanism is undergoing a single-electron radical pathway, including a radical scavenger in the reaction mixture should quench or inhibit the activation. Galvinoxyl is a long-lived free radical and is highly efficient in scavenging short-lived free radicals. It has been previously used as a mechanistic probe for radical chain processes and also as an inhibitor to prevent undesired competitive radical reactions.[26] A few ^{1}HNMR-kinetics experiments were done with the β-galactosyl donor in presence of galvinoxyl (**Scheme 12**) to gain mechanistic insights.



***Scheme 12**: Kinetics studies of model glycosylation with galvinoxyl*

From the kinetics spectrum of the activation [**Scheme 12 (a)**], it was observed that the donor was activated in spite of the presence of the scavenger [see experimental

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section]. A control kinetics experiment was also done with just the donor and the galvinoxyl radical, so as to rule out any activation by the radical itself. This study showed that galvinoxyl, by itself has no affect on the thioglycoside donor. Hence, with these studies, it can be concluded that $Ph_3Bi(OTf)_2$-mediated activation of thiopropylglycosides do not follow a single-electron radical mechanistic pathway.

4.2.7. Miscellaneous studies

4.2.7.1. By-product analysis

Although PrSSPr was found to be one of the sulfur by-products in the reaction of study, we still did not have much knowledge of the other bismuth- or sulfur-containing by-products. By-product studies are very important in understanding of a reaction and sometimes prove to critical, especially in catalytic systems. Previously, it had been reported that in reactions involving phenyl-containing Bi(V) reagents, the major by-product is Ph_3Bi. To test this hypothesis, we went back to our activation analysis by NMR spectroscopy. Since we already had Ph_3Bi in hand, we thought that adding this reagent at the end of an activation would either increase or create newer peaks in the NMR spectrum, depending on whether it is a by-product or not (**Scheme 13**).

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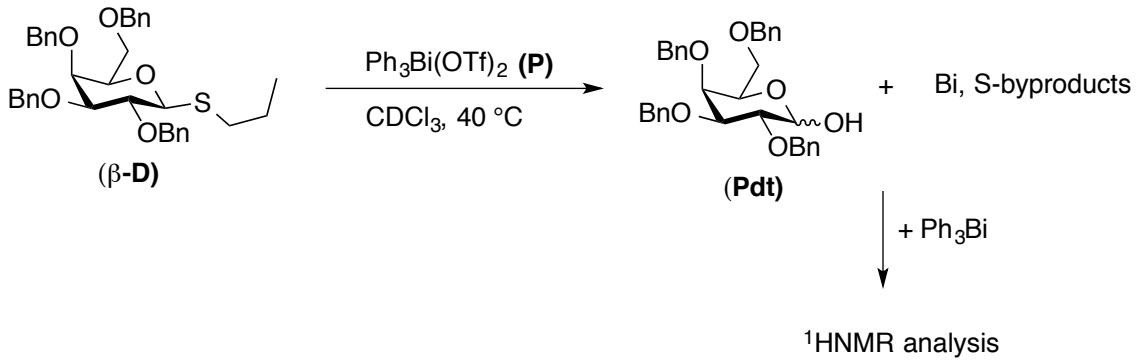

***Scheme 13**: By-product analysis*

By NMR analysis, we found that there was no increase in intensity of the residual peaks of the phenyl protons of bismuth promoter present at the end of the reaction (see experimental section). In fact, new peaks emerged in the spectrum, which confirmed that the Ph_3Bi is not a by-product of the activation. These studies along with the earlier HMBC experiment (**Figure 16**) suggest the formation of a phenylbismuth-sulfur containing by-product.

4.2.7.2. Chloride vs. triflate promoter study

In our earlier work,[18] we found that the activation protocol is uniquely related to pentavalent bismuth chemistry. To further investigate if this observation was more general, we tried a bench-top and a kinetics experiment of the model glycosylation of the β-donor with a new promoter triphenylbismuth dichloride (Ph_3BiCl_2)[27] [**Scheme 14**]. Surprisingly, no change was observed by TLC analysis or even by ^{1}HNMR kinetics study over a span of 3 h.

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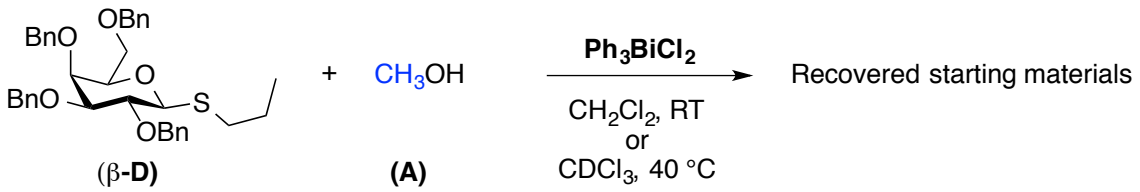

***Scheme 14**: Activation trials with Ph_3BiCl_2*

4.2.8. Plausible mechanistic pathway

Putting all our observations/estimations together, a plausible mechanistic pathway can be proposed (**Scheme 15**). The β-donor in presence of $Ph_3Bi(OTf)_2$ anomerizes to the α-donor. This α-donor then possibly forms a bismuth-sulfonium species, which in all probability is in equilibrium with the β-sulfonium species. Once this reactive intermediate forms, it can disintegrate to form an oxacarbenium species, which has been suggested by earlier mechanistic studies with different glycosyl donors. In this process, the Bi-S portion breaks off to form some by-products which are yet to be determined. Some of the PrS- fragments can possibly come together to form a disulfide (PrSSPr) by a possible aerobic oxidation (or some other process) that has been detected in our studies. The reactive intermediate can readily be then attacked by the incoming nucleophile via an S_N1-like mechanism to give the desired products (Path A) in 1:1 ratio.

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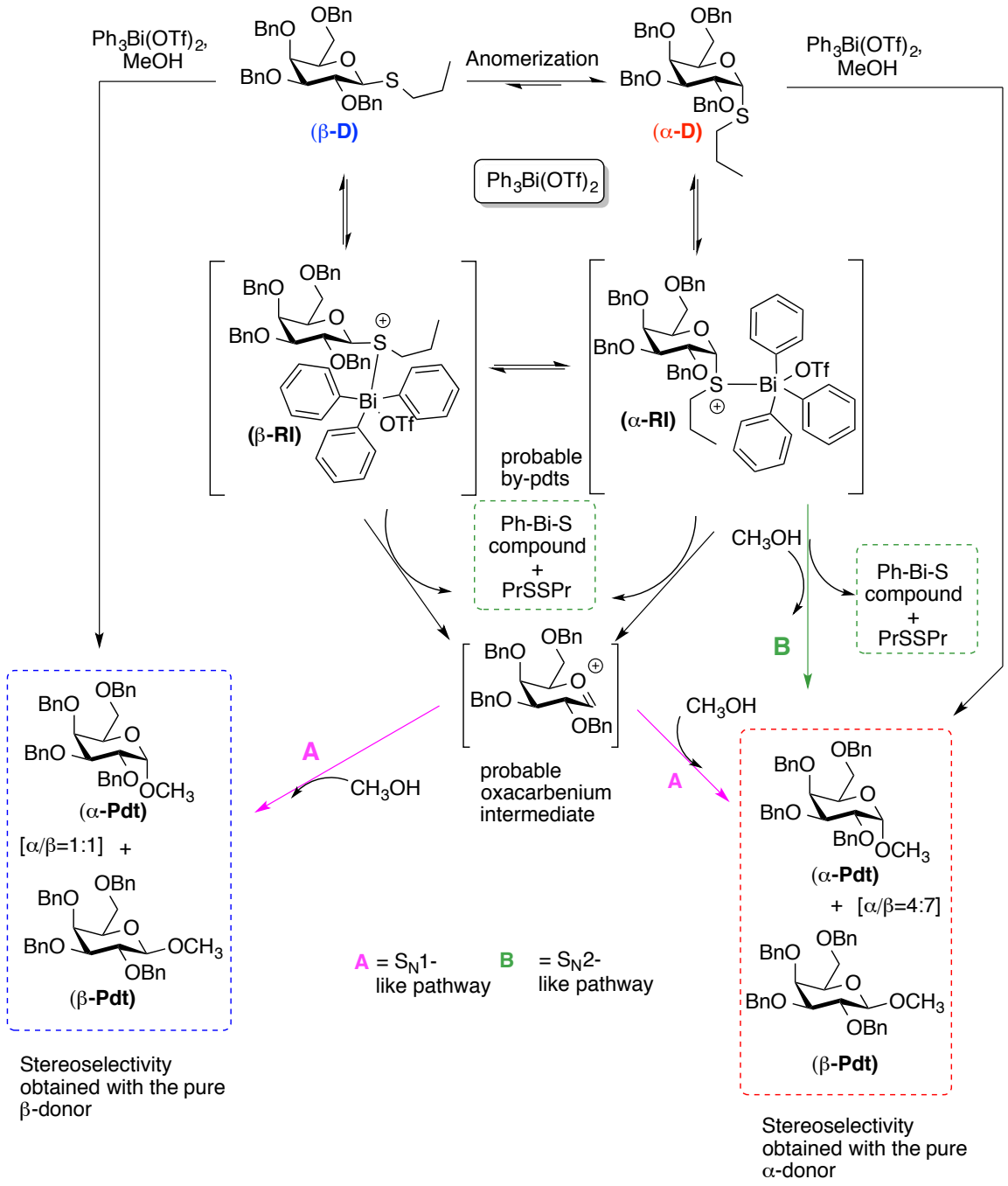

***Scheme 15**: Proposed mechanistic pathway*

However, another pathway is also plausible, especially when the activation is done with the α-donor. Since the α-donor does not undergo anomerization to the β-

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donor, the in situ formed bismuth-sulfonium species is probably enriched in more α-form than the β-form. Experimental evidence also shows that the diastereoselectivity of product obtained with α-donor activation is slightly enhanced. Hence, we can see envision an S_N2-like pathway to give beta product preferentially over alpha product. However, mention need to made that the product selectivity might also result from other effects as a S_N2 attack on the sulfonium with the bulky bismuth and phenyl ligands present on the molecule seems a bit far-fetched. Nevertheless, with our studies we have managed to come up with a possible mechanistic pathway for this extraordinary activation.

4.3. CONCLUSION

In summary, we investigated a bismuth(V) mediated thiopropylglycosides activation with various bench-top experiments and analytical methods. The kinetics rate order studies gave insight into the effect of each reactant on the rate of the concerned glycosylation. Uniquely, these NMR kinetics studies showed that the beta anomer preferred to isomerize to the alpha anomer during the course of the reaction. On closer observation with a variety of 1D and 2D NMR studies, formation of an in-situ bismuth-sulfonium reactive intermediate could be detected. Once this reactive species formed, it reacted quickly with the incoming nucleophile to form the desired *O*-glycoside. Other mechanistic pathways like: oxidation of the *S*-glycoside to form sulfoxides, single-electron thioglycoside radical formation, phenyl group transfer on the *S*-glycoside, etc., were also examined but were disregarded as they failed to give any evidence of

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occurrence. These in-depth mechanistic studies, notably rate-order kinetics, are one of the first of its kind in thioglycoside activation as well as in chemical glycosylation. This kind of detained mechanistic examination is also extremely rare in bismuth-mediated functional group transformations. In future, we hope this report will spur additional work on the unique bismuth metal chemistry and the quintessential chemical glycosylation.

4.4. EXPERIMENTAL SECTION

4.4.1. General methods

All moisture-sensitive manipulations were carried out with standard Schlenk techniques under argon. Commercially available compounds were used without further purification unless otherwise stated. Commercial solvent/reagents used in GC-MS, kinetics measurements, NMR studies specifically MeOH, CH_2Cl_2, $CDCl_3$, AllOH were dried and distilled before use. All saccharides were pre-treated by azeotropic removal of water using toluene. These saccharides along with the promoter [$PhBi_3(OTf)_2$] were kept in vacuum desiccator containing P_2O_5 overnight before use. Flash silica gel column chromatography was performed employing Silica Gel 60 Sorbent (40-63 µm, 230-400 mesh). Thin–layer chromatography (analytical) was performed using glass plates pre-coated to a depth of 0.25 mm detected under UV light and by spraying the plates with a 0.02 M solution of resorcinol in 20% ethanolic H_2SO_4 solution followed by heating.

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4.4.2. Instrumentation

GC-MS analyses were done on a GCT GCMS is an orthogonal time-of-flight mass spectrometer from Waters Inc., Milford, MA. The system also includes a model 6890 GC from Agilent, Santa Clara, CA, which is equipped with a model 7683 Autoinjector from Agilent. ^{1}HNMR kinetics measurements were done on a Bruker AVII 600 MHz and Bruker DRX 400 MHz spectrometer. Proton (^{1}H) NMR, carbon (^{13}C) NMR, etc. were recorded on a 600 MHz and 125 MHz instrument respectively using the residual signals from chloroform ($CDCl_3$), 7.26 ppm and 77.0 ppm, as internal references for ^{1}H and ^{13}C chemical shifts (δ) respectively. All 1D-selective and 2D NMR experiment was done on a Bruker AVII 600 spectrometer. The DOSY experiment was done on a Bruker DRX 400 MHz spectrometer. ESI-HRMS mass spectrometry was carried out on an Agilent 6540-QTOF.

4.4.3. General procedures

4.4.3.1. GC-MS analyses

Materials: PrSH was commercially bought from Sigma Aldrich and distilled before use. PrSSPr and PhSPr were synthesized following literature methods and distilled before use.

Separation of analytes procedure: To find the retention times of the analytes, a stock solution was prepared by mixing 1 μL of each of the analyte, namely PrSH, PrSSPr, PhSPr and an internal standard (dodecane) in 100 mL CH_2Cl_2 and analyzed on the GC-MS

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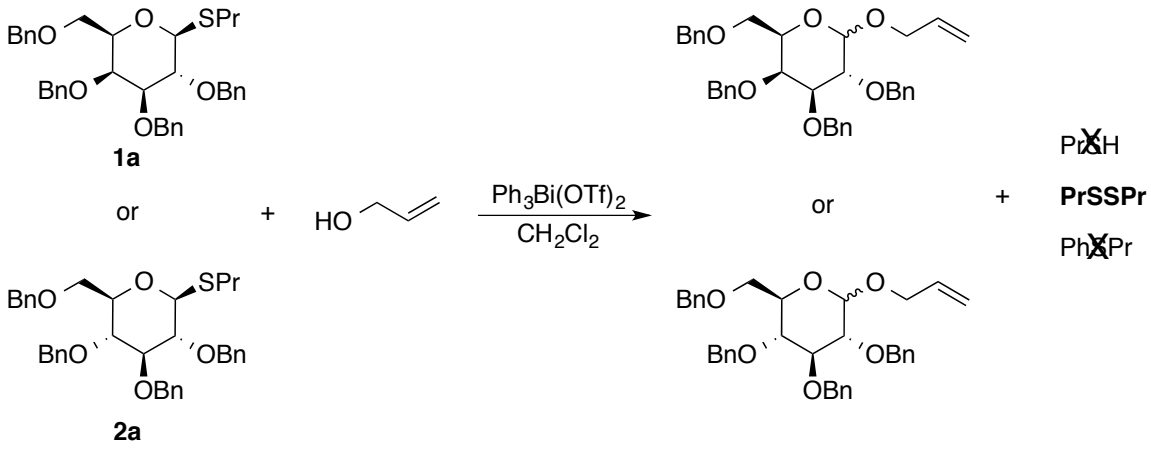
instrument. The retention times were recorded and the relative response factors (RRF) were then calculated. All the analytes were separated on the chromatogram and could be monitored.

Analyte	Formula	MW	RT (min)	Peak height	Peak Area	Density	Stock Conc.	RRf
PrSH	C3H8S	76	2.124	870	21.54	0.84	42	3.375
PrSSPr	C6H14S2	150	8.166	1680	34.37	0.96	48	4.471
Dodecane	C12H26	170	9.092	271	5.69	0.75	37.45	1.000
PhSPr	C9H12S	152	9.575	2449	54.06	0.99	50	7.116

General procedure of reaction monitoring by GC-MS:



The glycosyl donor (1 equiv., 0.033 mmol, 0.020 g) and allyl alcohol (1 equiv., 0.033 mmol, 2.46 μL) were taken a in a tightly sealed oven dried flask under argon. To it a solution of promoter (1 equiv., 0.033 mmol, 0.025 g) in CH_2Cl_2 was added and reaction

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left to stir under argon. Aliquots (1 μL) from the reaction mixture at various times were then taken out, mixed with dodecane to make 50 mL stock solutions in CH_2Cl_2 and then immediately analyzed by GC-MS. The donor consumption was also monitored by TLC.

4.4.3.2 NMR kinetics studies

All these experiments were done on the Bruker DRX 400 spectrometer. Various temperatures were tried for monitoring the kinetics and out of these 40 °C (313 K) was found to be the optimum temperature, considering the total reaction time. NMR tubes were oven-dried before use.

Typical procedure: The glycosyl donor and promoter were weighed in a septum-capped oven dried 1-dram vial, purged with argon, and then taken to the NMR instrument. The thermostat of the NMR instrument was allowed to stabilize at 313 K. Then, the instrument was locked and shimmed using an NMR tube containing only $CDCl_3$. A stock solution of the acceptor in deuterated $CDCl_3$ was prepared and then the required amount was added to the vial via a syringe. The vial was shaken until homogenous (generally ~5-7 sec), transferred to another NMR tube and immediately taken into the NMR machine for data acquisition. This was taken as time zero and automatic spectra, of 4 scans with a 24 s acquisition delay (D1) between scans, were registered automatically every 120 seconds for the first 69 FIDs, after which scans were taken at the intervals of 300 seconds.

The spectra obtained were processed manually in mestrenova software to obtain the concentration of reactant and product species during the reaction.

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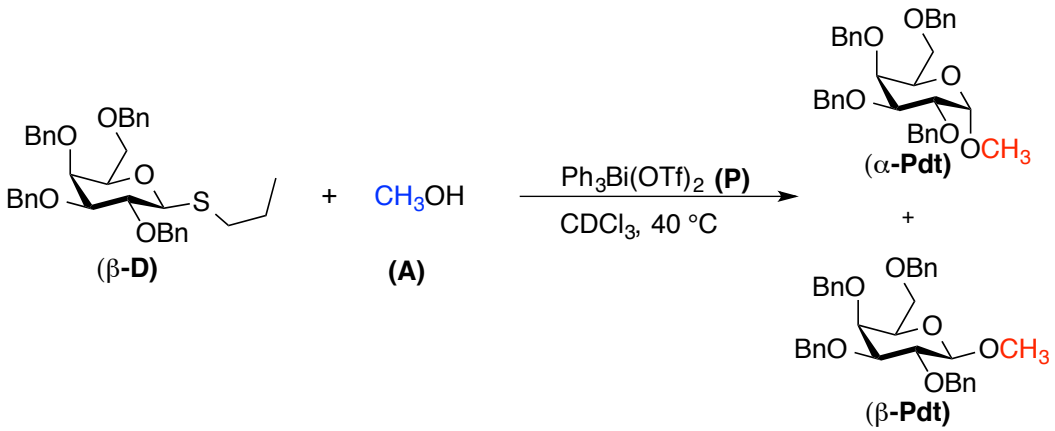
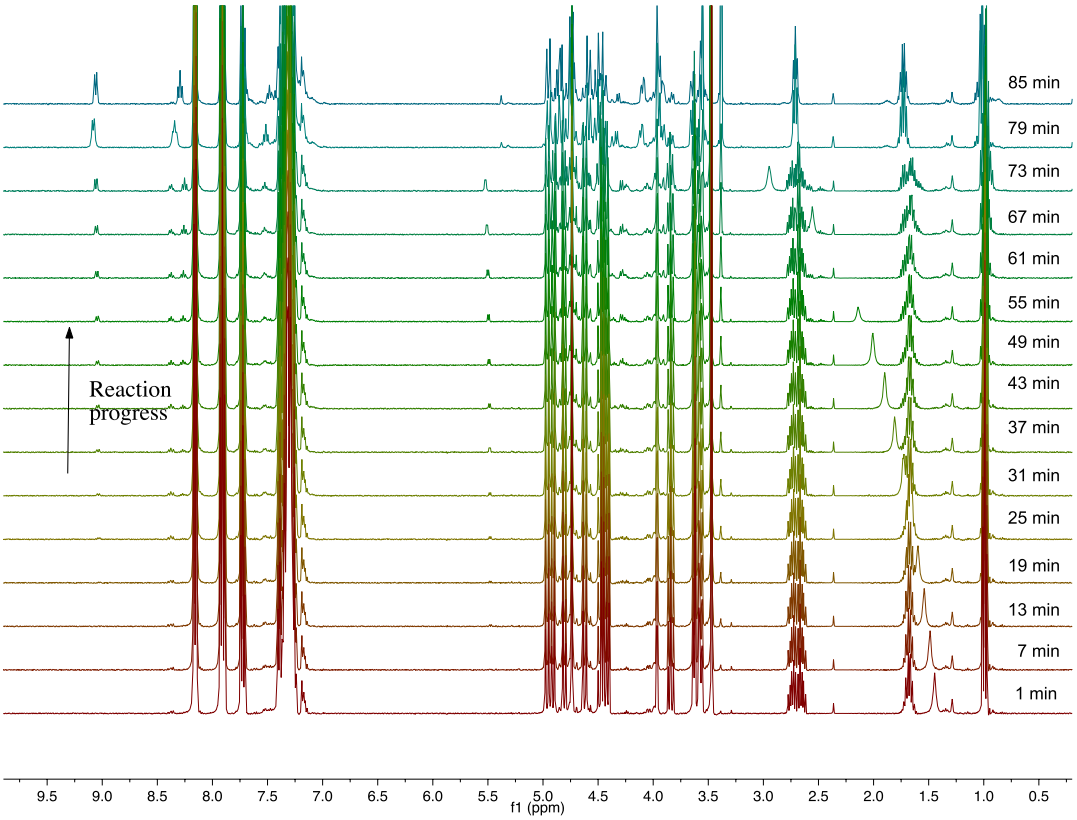
(i) A typical kinetics spectrum of the model glycosylation (D:P:A::1:1:1) looked as follows:





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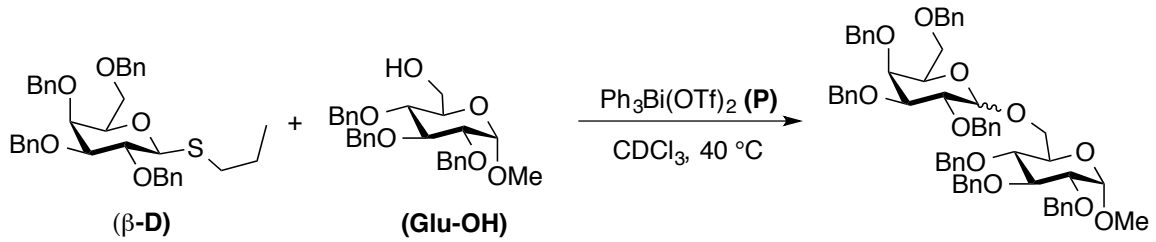
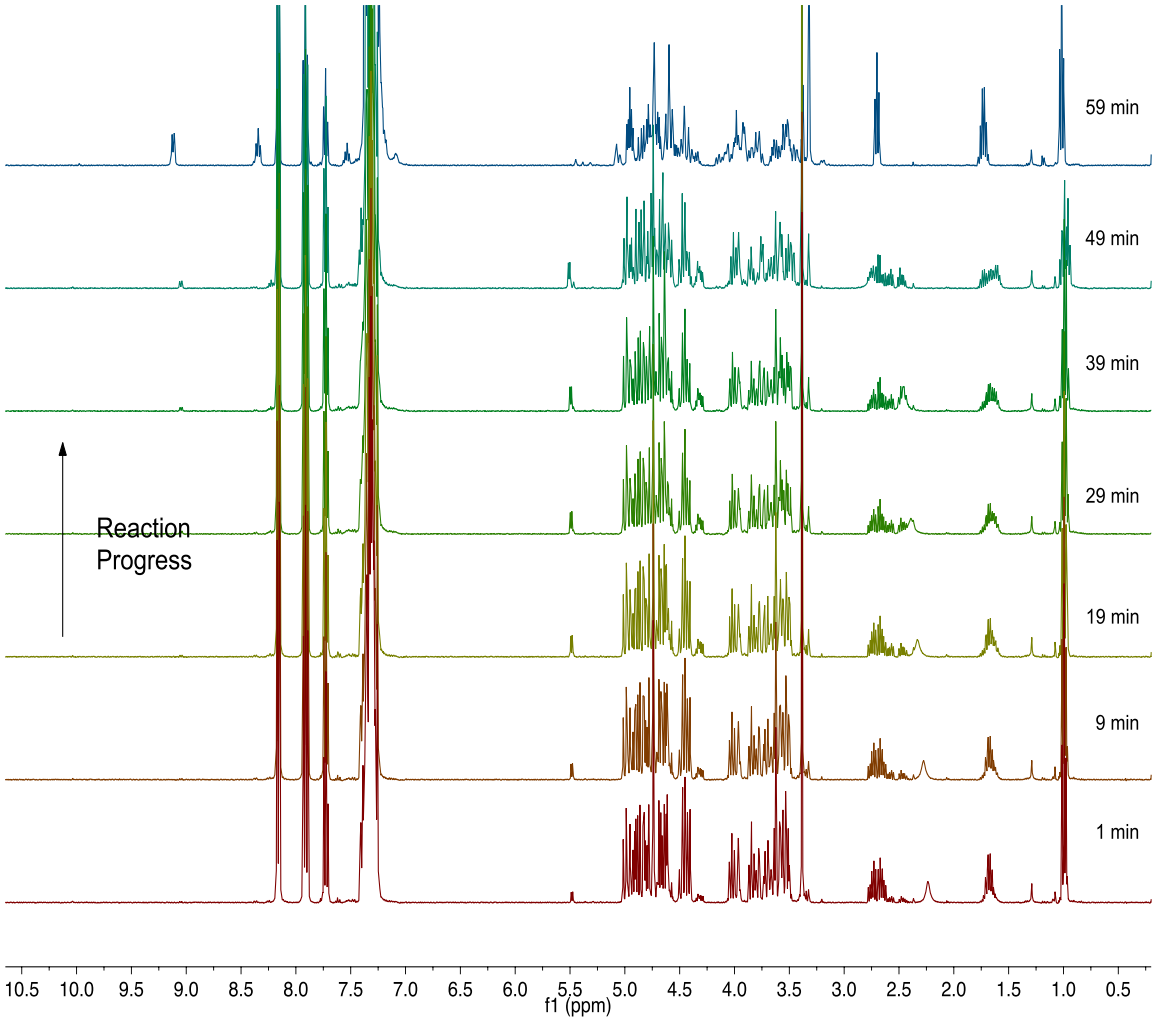
(ii) A typical kinetics spectrum of glycosylation with a glycosyl acceptor (D:P:Glu-OH:1:1:1)





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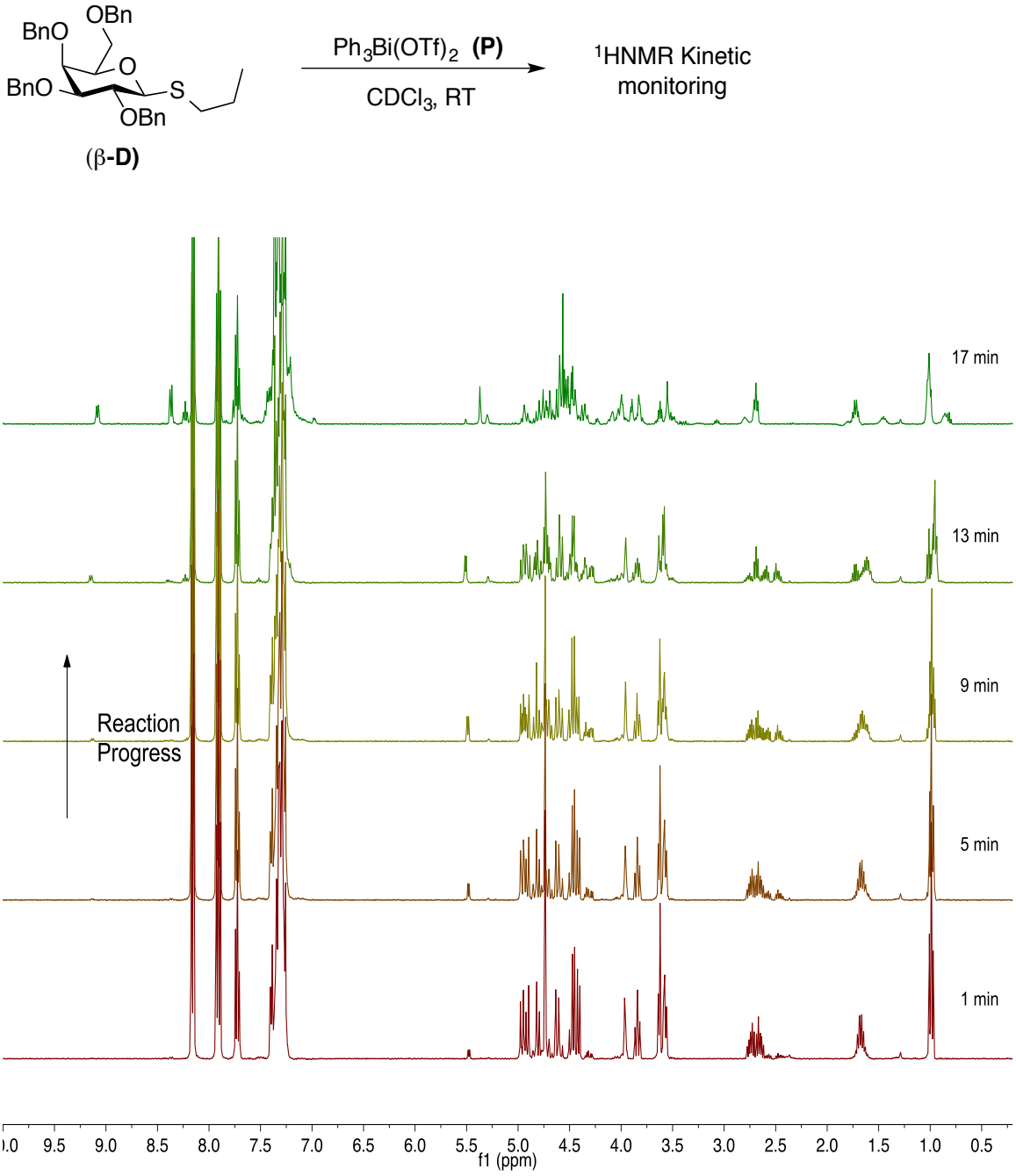
(iii) Kinetics spectrum of reaction between donor and promoter:



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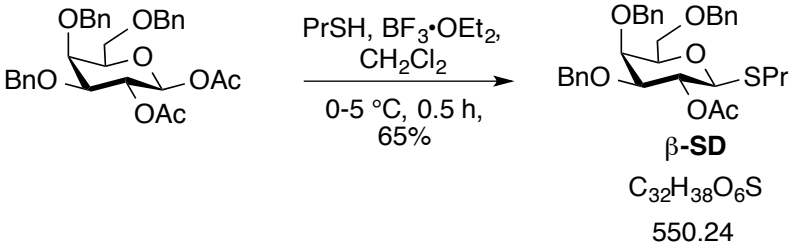
Synthesis of donor:

_n_-Propyl-2-_O_-acetyl-3,4,6-tetra-_O_-benzyl-1-thio-β-D-galactopyranoside (β-SD)



β-SD

$C_{32}H_{38}O_6S$

550.24

A 0.50 M solution of glycosyl acetate donor[28] (1.50 g, 2.81 mmol) and propanethiol (PrSH, 0.256 g, 3.37 mmol) was stirred for 0.5 h in anhydrous dichloromethane at 0 °C. Then, boron trifluoride dietherate ($BF_3{\cdot}OEt_2$, 1.19 g, 8.42 mmol) was added dropwise to the reaction mixture and it was stirred over an ice bath (0–5 °C) until consumption of the starting donor was seen by TLC. The reaction was quenched with excess triethylamine, diluted with CH_2Cl_2, filtered, and washed sequentially with 2 M aqueous HCl, saturated aqueous $NaHCO_3$, and water. The organic layer was dried over $MgSO_4$, concentrated under reduced pressure at 40 °C, and the resulting residue was purified by silica gel column chromatography by a solvent system (Ethyl acetate:hexanes, 1:7), which yielded **β-SD** as a white solid (0.99 g, 65%); R_f 0.62 (Ethyl acetate:hexanes 1:5); $[\alpha]_D$ + 67.3 $cm^3\ g^{-1}dm^{-1}$ (c 0.12 g cm^{-3}, $CHCl_3$; ^{1}H NMR (600 MHz, [D-1] $CDCl_3$, 25 ^oC, TMS) ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.31 (m, 15 H) 5.41 (t, *J* = 9.7 Hz, 1H), 4.95 (d, *J* = 11.7 Hz, 1H), 4.68 (d, *J* = 12.2 Hz, 1H), 4.58-4.55 (2 d, *J* = 11.9 Hz, 2H), 4.48 – 4.39 (m, 2H), 4.32 (d, *J* = 9.9 Hz, 1H), 3.99 (dd, *J* = 2.8, 0.9 Hz, 1H), 3.62 – 3.57 (m, 3H), 3.54 (dd, *J* = 9.7, 2.8 Hz, 1H), 2.64 (ddd, *J* = 12.4, 8.3, 6.5 Hz, 2H), 1.64 – 1.56 (m, 2H), 0.94 (t, *J* = 7.4 Hz, 3H); ^{13}C

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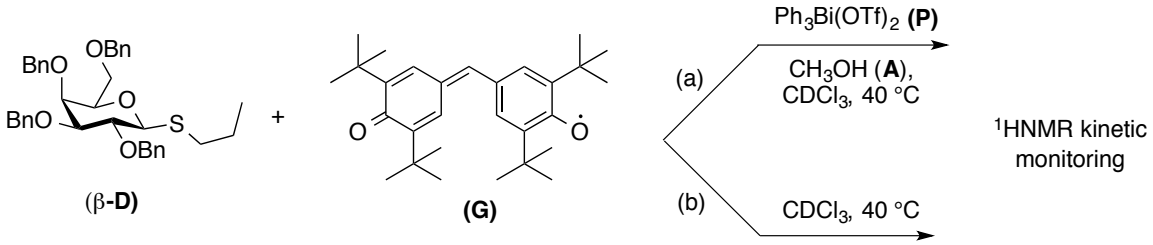
NMR (151 MHz, $CDCl_3$) δ 169.76, 138.72, 138.13, 137.95, 128.55, 128.53, 128.29, 128.06, 128.04, 127.95, 127.85, 127.57, 127.55, 83.94, 81.61, 77.59, 77.37, 77.16, 76.95, 74.50, 73.68, 73.03, 72.07, 69.85, 68.68, 31.66, 23.24, 21.19, 13.63; HRMS (ESI-QTOF) *m/z* calc. for $C_{32}H_{38}O_6SNa^+$ 573.2286 found 573.2291.

(iii) Galvinoxyl kinetics



The general kinetics experiment procedure was followed here too, except the galvinoxyl was also weighed in the dark in the vial along with the donor and promoter (all are 1:1:1). The vial was wrapped in aluminum foil to avoid light exposure when taken to the NMR instrument.

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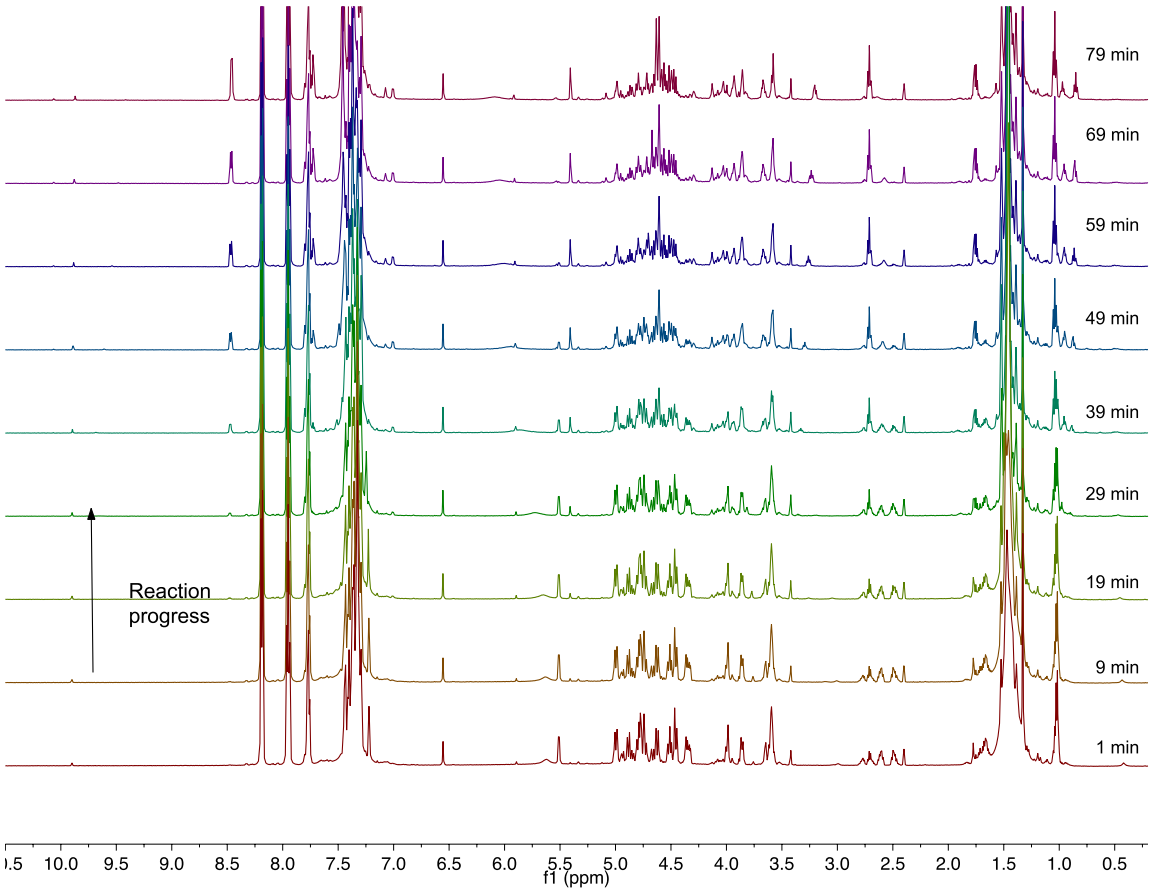
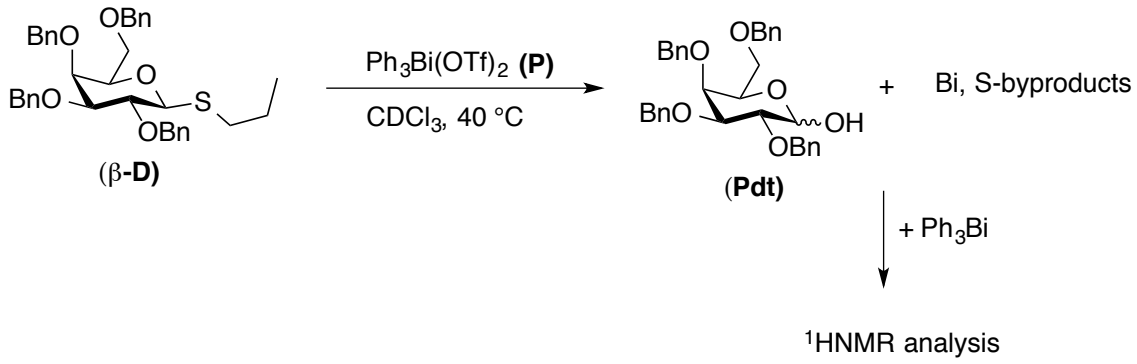


(iv) By-product studies and the ^{1}HNMR comparison



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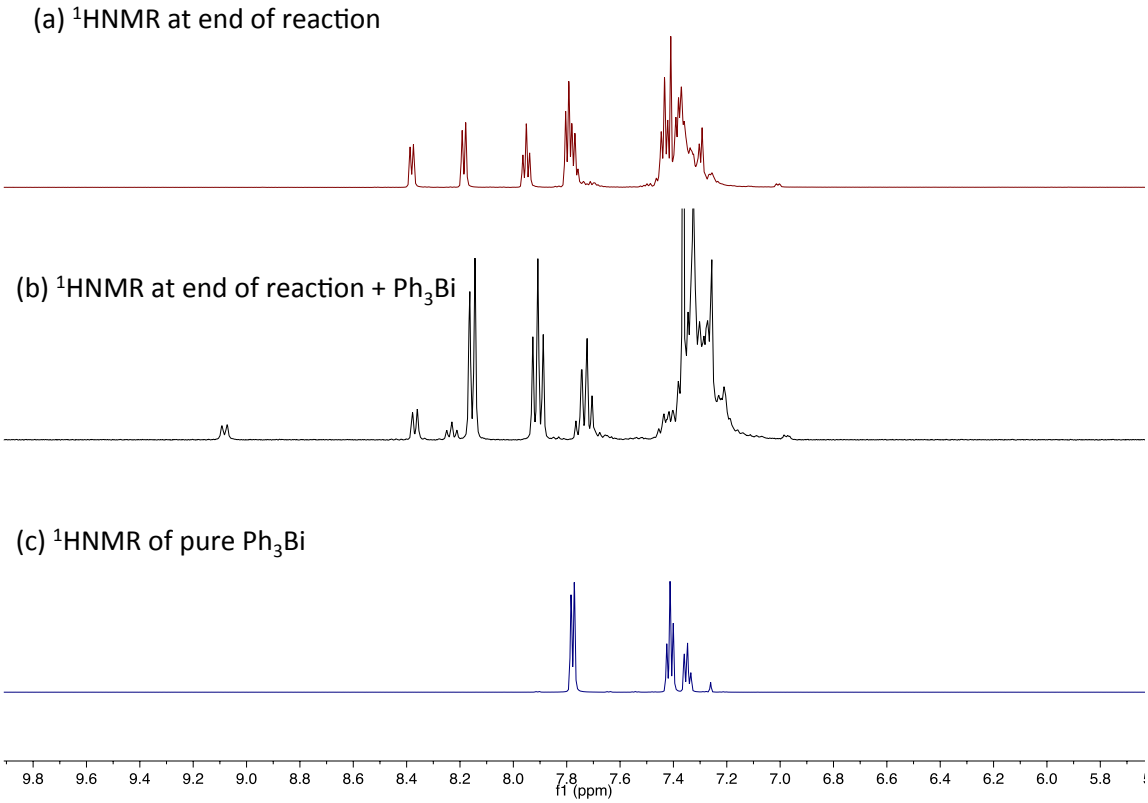

4.5. REFERENCES

1. Anslyn, E. V.; Dougherty, D. A., *Modern Physical Organic Chemistry*. University Science: 2006; p 355-417.

2. (a) Crich, D., Mechanism of a Chemical Glycosylation Reaction. *Acc. Chem. Res.* **2010,** *43* (8), 1144-1153; (b) Huang, M.; Retailleau, P.; Bohé, L.; Crich, D., Cation Clock Permits Distinction Between the Mechanisms of α- and β-O- and β-C-Glycosylation in the Mannopyranose Series: Evidence for the Existence of a Mannopyranosyl Oxocarbenium Ion. *J. Am. Chem. Soc.* **2012,** *134* (36), 14746-14749; (c) Crich, D.; Sun, S., Direct Formation of β-Mannopyranosides and Other Hindered Glycosides from Thioglycosides. *J. Am. Chem. Soc.* **1998,** *120* (2), 435-436; (d) Crich, D.; Dudkin, V., Why Are the Hydroxy Groups of Partially Protected N-Acetylglucosamine Derivatives Such Poor Glycosyl Acceptors, and What Can Be Done about It? A Comparative Study of the Reactivity of N-Acetyl-, N-Phthalimido-, and 2-Azido-2-deoxy-glucosamine Derivatives in Glycosylation. 2-Picolinyl Ethers as Reactivity-Enhancing Replacements for Benzyl Ethers. *J. Am. Chem. Soc.* **2001,** *123* (28), 6819-6825; (e) Crich, D.; Vinogradova, O., On the

www.manaraa.com

Influence of the C2−O2 and C3−O3 Bonds in 4,6-O-Benzylidene-Directed β-Mannopyranosylation and α-Glucopyranosylation. *J. Org. Chem* **2006,** *71* (22), 8473-8480.

3. (a) Garcia, B. A.; Gin, D. Y., Dehydrative Glycosylation with Activated Diphenyl Sulfonium Reagents. Scope, Mode of C(1)-Hemiacetal Activation, and Detection of Reactive Glycosyl Intermediates. *J. Am. Chem. Soc.* **2000,** *122* (18), 4269-4279; (b) Garcia, B. A.; Poole, J. L.; Gin, D. Y., Direct Glycosylations with 1-Hydroxy Glycosyl Donors using Trifluoromethanesulfonic Anhydride and Diphenyl Sulfoxide. *J. Am. Chem. Soc.* **1997,** *119* (32), 7597-7598; (c) Nguyen, H. M.; Chen, Y.; Duron, S. G.; Gin, D. Y., Sulfide-Mediated Dehydrative Glycosylation. *J. Am. Chem. Soc.* **2001,** *123* (36), 8766-8772; (d) Ryan, D.; Demchenko, D.; Ryan, X.; Gin, R.; Zhu; Schmidt; Demchenko, A. V., *Glycoside Synthesis from 1-Oxygen Substituted Glycosyl Donors: Sections 3.1 and 3.2 Handbook of Chemical Glycosylation - Advances in Stereoselectivity and Therapeutic Relevance*. 2008; p 95-185.

4. (a) Jensen, H. H.; Nordstrøm, L. U.; Bols, M., The Disarming Effect of the 4,6-Acetal Group on Glycoside Reactivity: Torsional or Electronic? *J. Am. Chem. Soc.* **2004,** *126* (30), 9205-9213; (b) McDonnell, C.; López, O.; Murphy, P.; Fernández Bolaños, J. G.; Hazell, R.; Bols, M., Conformational Effects on Glycoside Reactivity: Study of the High Reactive Conformer of Glucose. *J. Am. Chem. Soc.* **2004,** *126* (39), 12374-12385; (c) Pedersen, C. M.; Marinescu, L. G.; Bols, M., Glycosyl donors in "unusual" conformations – influence on reactivity and selectivity. *C. R. Chim.* **2011,** *14* (1), 17-43.

5. (a) Romero, J. A. C.; Romero, S.; Tabacco, K. A.; Woerpel, Stereochemical Reversal of Nucleophilic Substitution Reactions Depending upon Substituent: Reactions of Heteroatom-Substituted Six-Membered-Ring Oxocarbenium Ions through Pseudoaxial Conformers. *J. Am. Chem. Soc.* **2000,** *122* (1), 168-169; (b) Ayala, L.; Ayala, C.; Lucero, J. A. C.; Romero, S.; Tabacco, K. A.; Woerpel, Stereochemistry of Nucleophilic Substitution Reactions Depending upon Substituent: Evidence for Electrostatic Stabilization of Pseudoaxial Conformers of Oxocarbenium Ions by Heteroatom Substituents. *J. Am. Chem. Soc.* **2003,** *125* (50), 15521-15528; (c) Shenoy, S.; Shenoy, K. A.; Woerpel, Investigations into the Role of Ion Pairing in Reactions of Heteroatom-Substituted Cyclic Oxocarbenium Ions. *Org. Lett.* **2005,** *7* (6), 1157-1160; (d) Billings, S.; Billings, K. A.; Woerpel, Nucleophilic Substitution Reactions of Sulfur-Substituted Cyclohexanone Acetals: An Analysis of the Factors Controlling Stereoselectivity. *J. Org. Chem.* **2006,** *71* (14), 5171-5178.

6. (a) Nukada, T.; Nukada, A.; Berces, M.; Zgierski, D.; Whitfield, Exploring the Mechanism of Neighboring Group Assisted Glycosylation Reactions. *J. Am. Chem. Soc.* **1998,** *120* (51), 13291-13295; (b) Bérces, A.; Bérces, G.; Enright, T.; Nukada,

www.manaraa.com

D.; Whitfield, The Conformational Origin of the Barrier to the Formation of Neighboring Group Assistance in Glycosylation Reactions: A Dynamical Density Functional Theory Study. *J. Am. Chem. Soc.* **2001,** *123* (23), 5460-5464; (c) Whitfield, D.; Whitfield, Chapter 4 Computational Studies of the Role of Glycopyranosyl Oxacarbenium Ions in Glycobiology and Glycochemistry. Advances in Carbohydrate Chemistry and Biochemistry Volume 62. *Adv. Carbohydr. Chem. Biochem.* **2009,** *62*, 83-159.

7. (a) Ranade, S. C.; Demchenko, A. V., Mechanism of Chemical Glycosylation: Focus on the Mode of Activation and Departure of Anomeric Leaving Groups. *J. Carbohydr. Chem.* **2013,** *32* (1), 1-43; (b) Mydock, L. K.; Demchenko, A. V., Mechanism of chemical O-glycosylation: from early studies to recent discoveries. *Org. Biomol. Chem.* **2010,** *8* (3), 497-510.

8. West, A. C.; Schuerch, C., Reverse anomeric effect and the synthesis of a-glycosides. *J. Am. Chem. Soc.* **1973,** *95* (4), 1333-1335.

9. (a) Park, J.; Kawatkar, S.; Kim, J.-H.; Boons, G.-J., Stereoselective Glycosylations of 2-Azido-2-deoxy-glucosides Using Intermediate Sulfonium Ions. *Org. Lett.* **2007,** *9* (10), 1959-1962; (b) Nokami, T.; Shibuya, A.; Manabe, S.; Ito, Y.; Yoshida, J.-i., α- and β-Glycosyl Sulfonium Ions: Generation and Reactivity. *Chem. – Eur. J.* **2009,** *15* (10), 2252-2255; (c) Stalford, S. A.; Kilner, C. A.; Leach, A. G.; Turnbull, W. B., Neighbouring group participation vs. addition to oxacarbenium ions: studies on the synthesis of mycobacterial oligosaccharides. *Org. Biomol. Chem.* **2009,** *7* (23), 4842-4852.

10. Mydock, L. K.; Kamat, M. N.; Demchenko, A. V., Direct Synthesis of Diastereomerically Pure Glycosyl Sulfonium Salts. *Org. Lett.* **2011,** *13* (11), 2928-2931.

11. Crich, D.; Sun, S., Direct chemical synthesis of β-mannopyranosides and other glycosides via glycosyl triflates. *Tetrahedron* **1998,** *54* (29), 8321-8348.

12. Crich, D.; Cai, F.; Yang, F., A stable, commercially available sulfenyl chloride for the activation of thioglycosides in conjunction with silver trifluoromethanesulfonate. *Carbohydr. Res.* **2008,** *343* (10–11), 1858-1862.

13. Huang, X.; Huang, L.; Wang, H.; Ye, X.-S., Iterative One-Pot Synthesis of Oligosaccharides. *Angewandte Chemie International Edition* **2004,** *43* (39), 5221-5224.

14. Amatore, C.; Jutand, A.; Mallet, J.-M.; Meyer, G.; Sinay, P., Electrochemical glycosylation using phenyl S-glycosides. *J. Chem. Soc., Chem. Commun.* **1990,** (9), 718-719.

www.manaraa.com

15. Li, X.; Huang, L.; Hu, X.; Huang, X., Thio-arylglycosides with various aglyconpara-substituents: a probe for studying chemical glycosylation reactions. *Org. Biomol. Chem.* **2009,** *7* (1), 117-127.

16. Verma, V. P.; Wang, C.-C., Highly Stereoselective Glycosyl-Chloride-Mediated Synthesis of 2-Deoxyglucosides. *Chem. – Eur. J.* **2013,** *19* (3), 846-851.

17. Zeng, Y.; Wang, Z.; Whitfield, D.; Huang, X., Installation of Electron-Donating Protective Groups, a Strategy for Glycosylating Unreactive Thioglycosyl Acceptors using the Preactivation-Based Glycosylation Method(1). *J. Org. Chem* **2008,** *73* (20), 7952-7962.

18. Goswami, M.; Ellern, A.; Pohl, N. L. B., Bismuth(V)-Mediated Thioglycoside Activation. *Angew. Chem., Int. Ed.* **2013,** *52* (32), 8441-8445.

19. (a) Reith, L. M.; Stiftinger, M.; Monkowius, U.; Knör, G. n.; Schoefberger, W., Synthesis and Characterization of a Stable Bismuth(III) A3–Corrole. *Inorg. Chem.* **2011,** *50* (14), 6788-6797; (b) Monakhov, K. Y.; Linti, G., Theoretical Study of Structure, Bonding, and Electronic Behavior of Low-Valent Bismuth Cyclopentadienyl and Pentamethylcyclopentadienyl Half-Sandwich Compounds. *Inorg. Chem.* **2009,** *48* (14), 6986-6996; (c) Auer, A. A.; Mansfeld, D.; Nolde, C.; Schneider, W.; Schürmann, M.; Mehring, M., Bismuth−Arene π-Interaction: A Combined Experimental and Theoretical Approach. *Organometallics* **2009,** *28* (18), 5405-5411.

20. Wallace, J. E.; Schroeder, L. R., Koenigs-Knorr reactions. Part II. A mechanistic study of mercury(II) cyanide-promoted reactions of 2,3,4,6-tetra-O-methyl-[small alpha]-D-glucopyranosyl bromide with cyclohexanol in benzene-nitromethane. *Journal of the Chemical Society, Perkin Transactions 2* **1976,** (14), 1632-1636.

21. (a) Agocs, L.; Burford, N.; Cameron, T. S.; Curtis, J. M.; Richardson, J. F.; Robertson, K. N.; Yhard, G. B., Spectroscopic, Structural, and Mass Spectrometric Studies on Two Systematic Series of Dithiabismuth(III) Heterocycles: Identification of Bismuthenium Cations and Their Solvent Complexes. *J. Am. Chem. Soc.* **1996,** *118* (13), 3225-3232; (b) Burford, N.; Eelman, M. D.; Mahony, D. E.; Morash, M., Definitive identification of cysteine and glutathione complexes of bismuth by mass spectrometry: assessing the biochemical fate of bismuth pharmaceutical agents. *Chem Commun (Camb)* **2003,** *81* (1), 146-7.

22. (a) Burés, J.; Armstrong, A.; Blackmond, D. G., Mechanistic Rationalization of Organocatalyzed Conjugate Addition of Linear Aldehydes to Nitro-olefins. *J. Am. Chem. Soc.* **2011,** *133* (23), 8822-8825; (b) Blackmond, D. G., Kinetics aspects of non-linear effects in asymmetric synthesis, catalysis, and autocatalysis. *Tetrahedron: Asymmetry* **2010,** *21* (11–12), 1630-1634.

www.manaraa.com

23. Anet, F. A. L.; Bourn, A. J. R., Nuclear Magnetic Resonance Spectral Assignments from Nuclear Overhauser Effects 1. *J. Am. Chem. Soc.* **1965,** *87* (22), 5250-5251.

24. Pedersen, C. M.; Nordstrøm, L. U.; Bols, M., "Super Armed" Glycosyl Donors: Conformational Arming of Thioglycosides by Silylation. *J. Am. Chem. Soc.* **2007,** *129* (29), 9222-9235.

25. Wever, W. J.; Cinelli, M. A.; Bowers, A. A., Visible Light Mediated Activation and O-Glycosylation of Thioglycosides. *Org. Lett.* **2012,** *15* (1), 30-33.

26. Rajan Babu, T. V., *Handbook of Reagents for Organic Synthesis, Reagents for Radical and Radical Ion Chemistry*. Wiley: 2009; p 303.

27. Barton, D.; Barton, D. H. R.; Barton, N.; Bhatnagar, J.-P.; Finet, W.; Motherwell, Pentavalent organobismuth reagents. Part vi. Comparative migratory aptitudes of aryl groups in the arylation of phenols and enols by pentavalent bismuth reagents. *Tetrahedron* **1986,** *42* (12), 3111-3122.

28. Trumtel, M.; Trumtel, P.; Tavecchia, A.; Veyrières, P.; Sinaÿ, The synthesis of 2′ -deoxy-β-disaccharides: Novel approaches. *Carbohydr. Res.* **1989,** *191* (1), 29-52.

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CHAPTER 5: CONCLUSIONS AND FUTURE DIRECTIONS

Oligosaccharide synthesis has been a challenging task for carbohydrate chemists and they have made continuous efforts to improve current synthetic strategies. This dissertation describes the development of an efficient and straightforward protocol that can be used to couple thioglycosyl donors to a wide variety of simple and complex acceptors to form highly functionalized monosaccharide as well as disaccharides. The activation methodology utilizes bismuth(V) chemistry, and is one of the first reports of its type in carbohydrate chemistry. The method offers distinct advantages: no requirement of excess promoter, low temperatures or additional additives/co-promoters and can be used with various functional groups, even alkenes.

This strategy was further extended to alkynes and was successfully used to synthesize an alkynyl mannose analogue. This mannose alkyne was then attached to a series of pentablock copolymers. These copolymers, developed by the Mallapragada group, are thermo-sensitive in nature and have the ability to polyplex with RNA. Cell-based studies are underway in Mallapragada lab, in association with the Carpenter group, on these decorated particles as potential vaccine adjuvants against the influenza virus.

In our studies, it was observed that the bismuth-mediated activation protocol was uniquely related to less common Bi(V), rather than the more explored Bi(III), chemistry. To gain mechanistic insights into this extraordinary activation, a detailed analytical investigation of the reaction was carried out. Interestingly, kinetics studies showed an uncommon non-linear growth curve in concentration vs. time plots. This kind of

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behavior is suggestive of a resting “induction” phase of the promoter/catalyst. Once this phase is passed, the rate suddenly escalates to reach reaction completion and this trend is generally seen with autocatalytic systems. In the future, theoretical modeling of the kinetics data could perhaps shed more light on this mysterious mechanistic pathway. These studies can also suggest ways to make the activation catalytic, which would be desirable in iterative automated oligosaccharide synthesis.

Another aspect, which was discovered with this work, was an unexpected beta to alpha isomerization during the activation of the β-donor. This finding was further utilized to improve reaction times and enhance stereoselectivity. Also, an in situ sulfonium intermediate could be probed with various NMR techniques. To have deeper understanding of these exciting mechanistic pathways, a collaboration was initiated with the Baik Lab (with graduate student Daniel Ashley, Indiana University), who are experts in the field of computational modeling. They are in the process of attempting numerous methods to accurately compute the energetics of the thioglycosides, the bismuth promoter and their interactions. Initial results have shown that the alpha and beta perbenzylated thiopropylglycosides are in fact quite close in energies and an isomerization is indeed feasible. These theoretical studies promise to help in deciphering the role of the bismuth promoter in the glycosyl activation and will open up possibilities to improve its efficiency.

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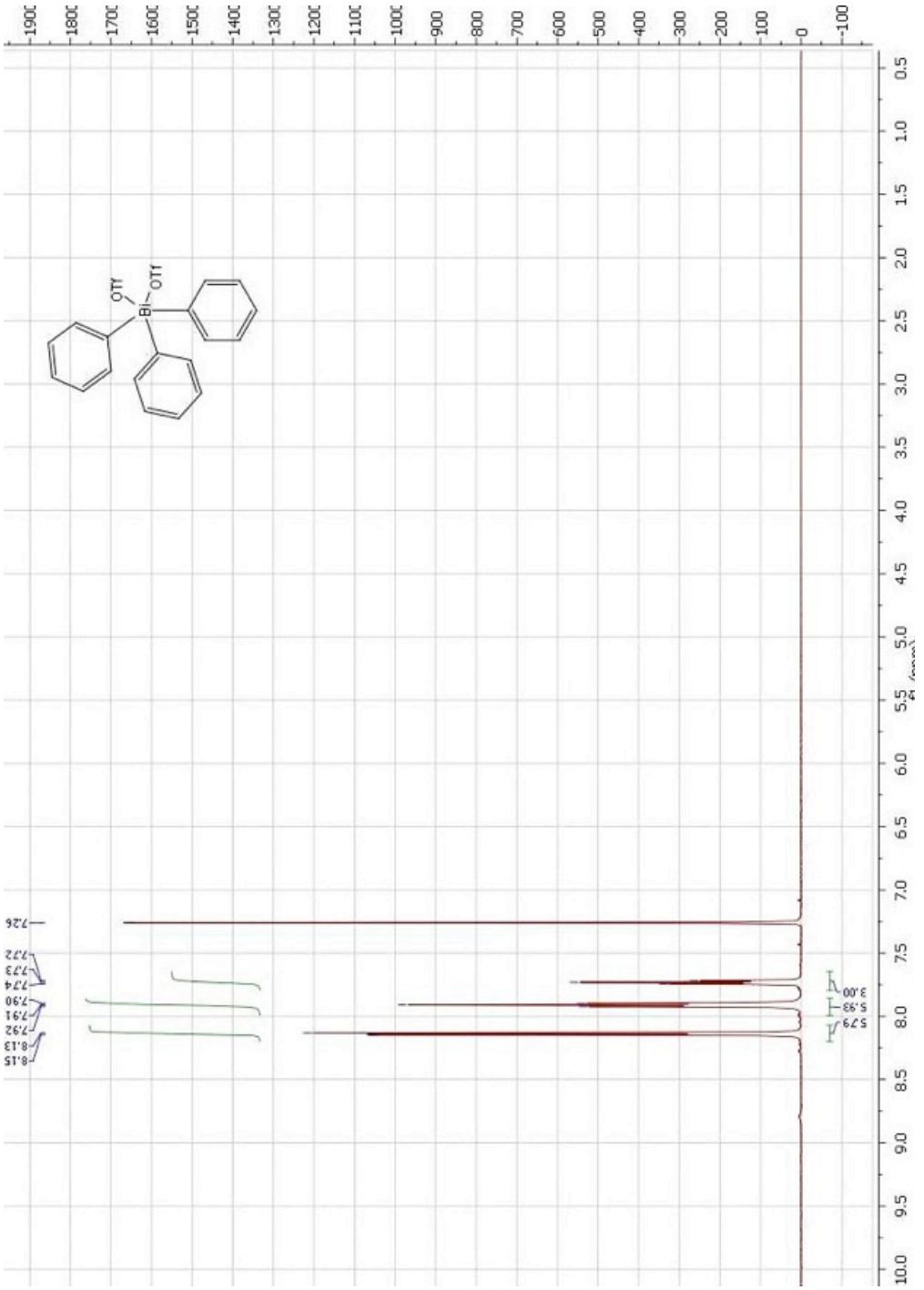
APPENDIX A. CHAPTER 2 1D & 2D NMR SPECTRA, XRD SPECTRA

Triphenyl bismuth ditriflate (5)



^{1}H NMR (600 MHz, $CDCl_3$)

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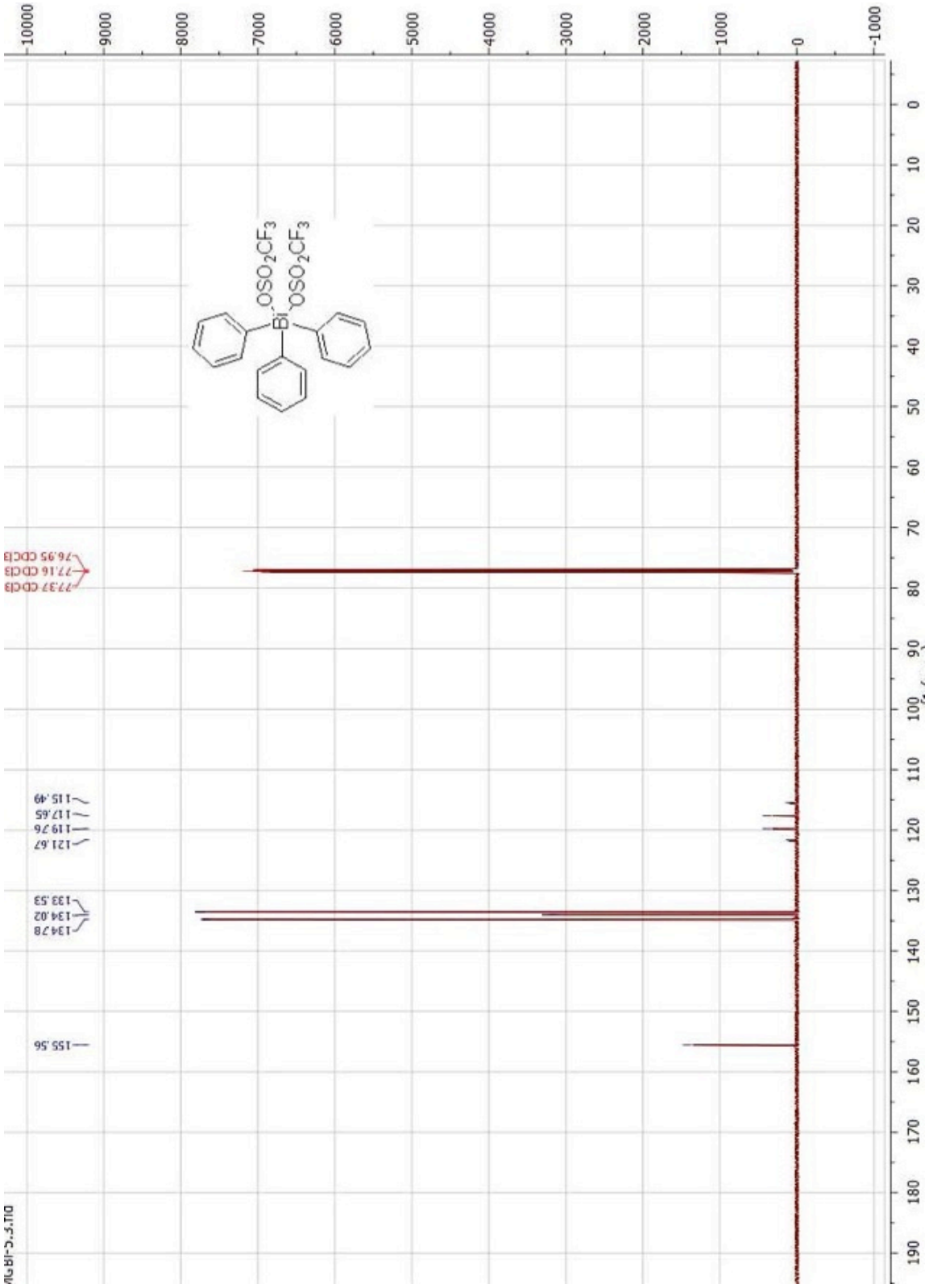

^{13}C NMR (151 MHz, $CDCl_3$)

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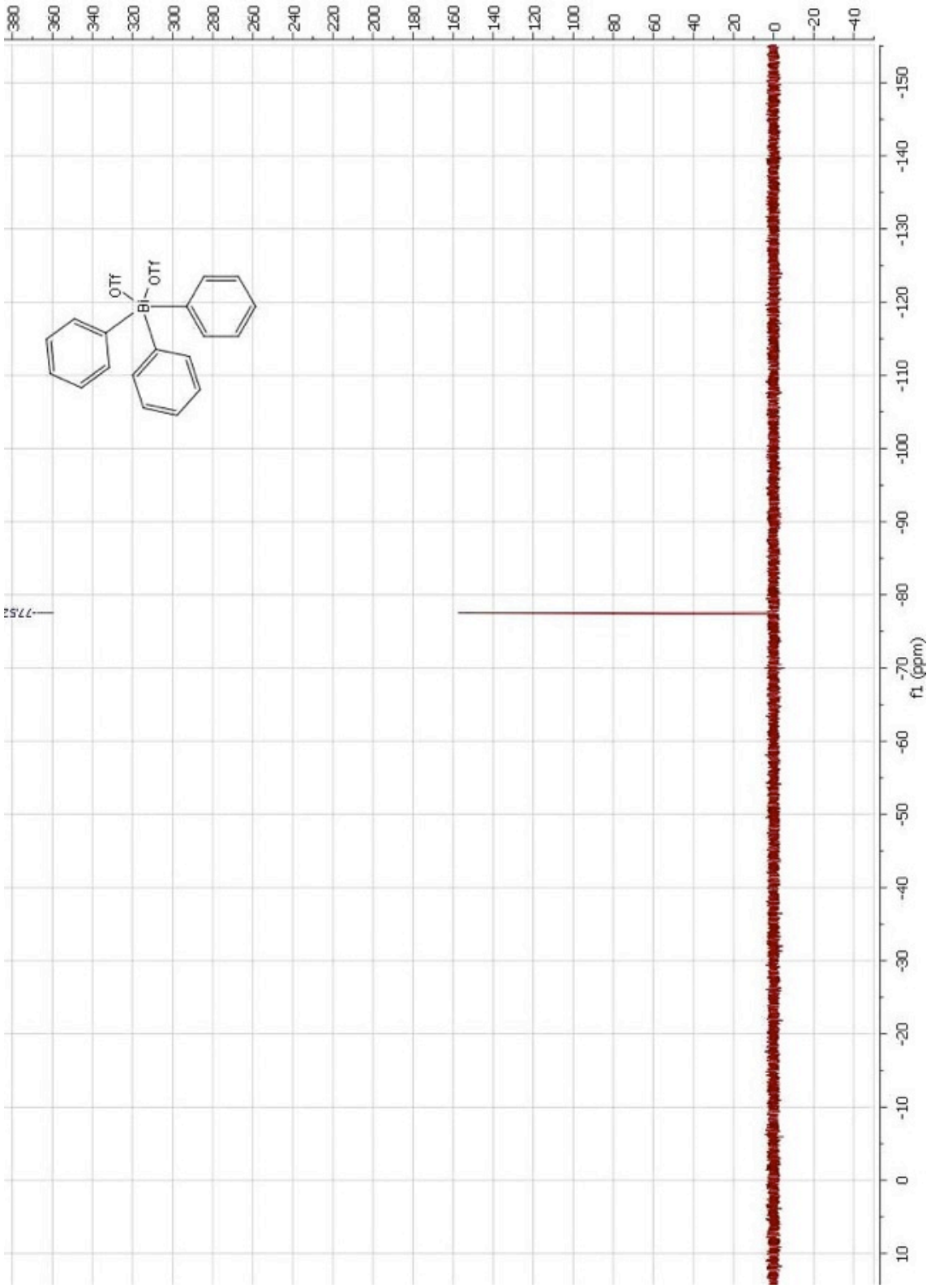

^{19}F NMR (565 MHz, $CDCl_3$)

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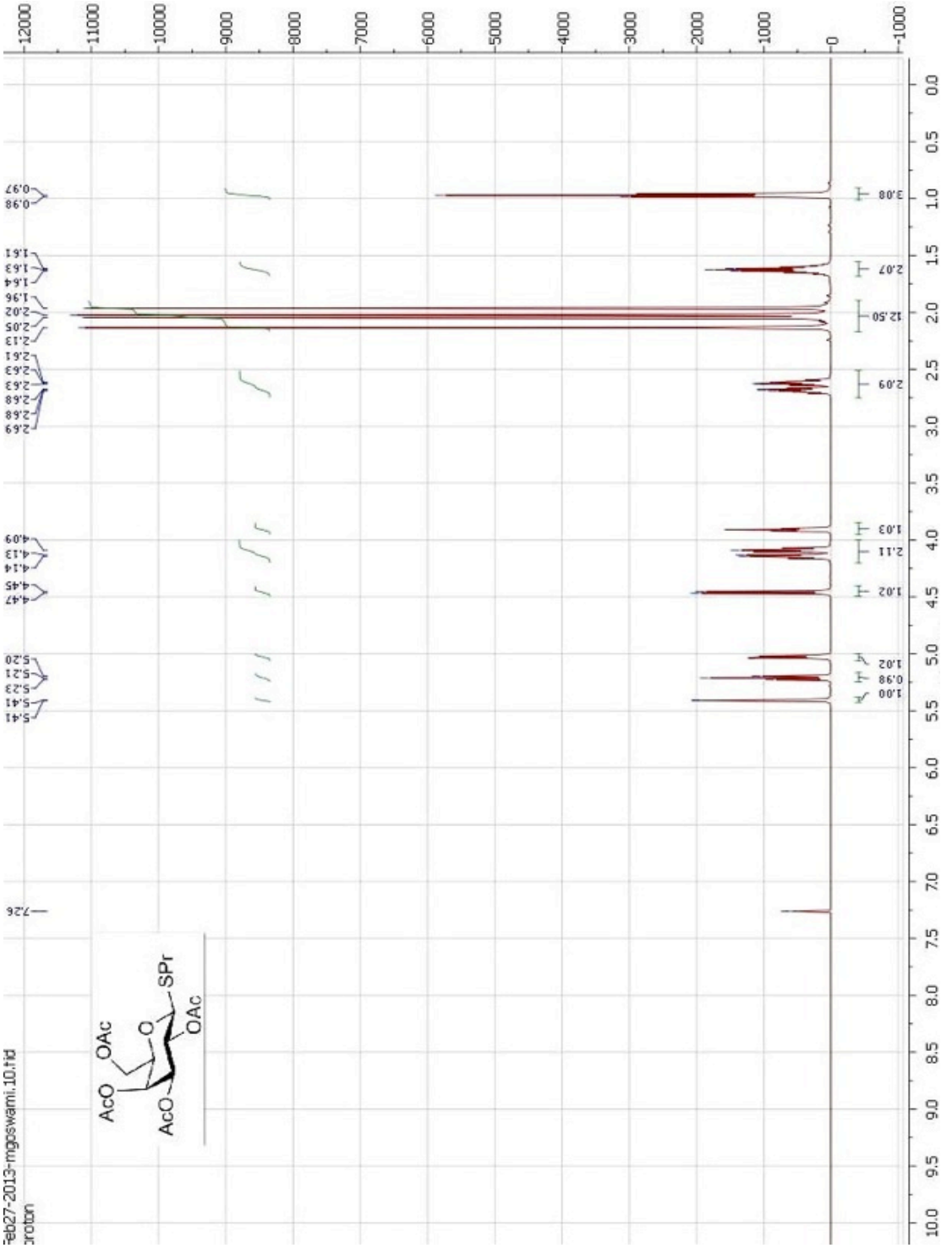
***n*-Propyl-2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (1a)**



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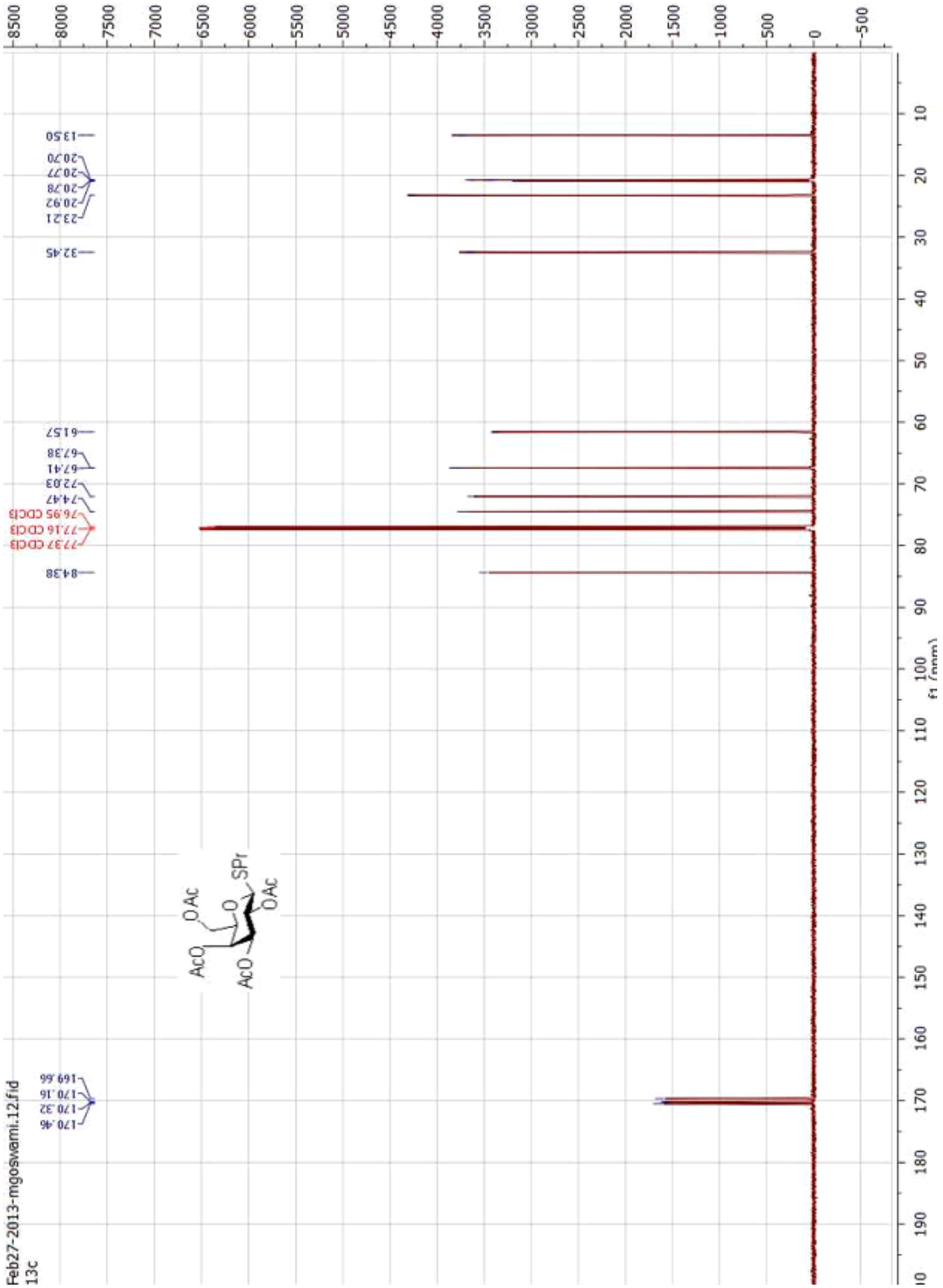

^{13}C NMR (151 MHz, $CDCl_3$)

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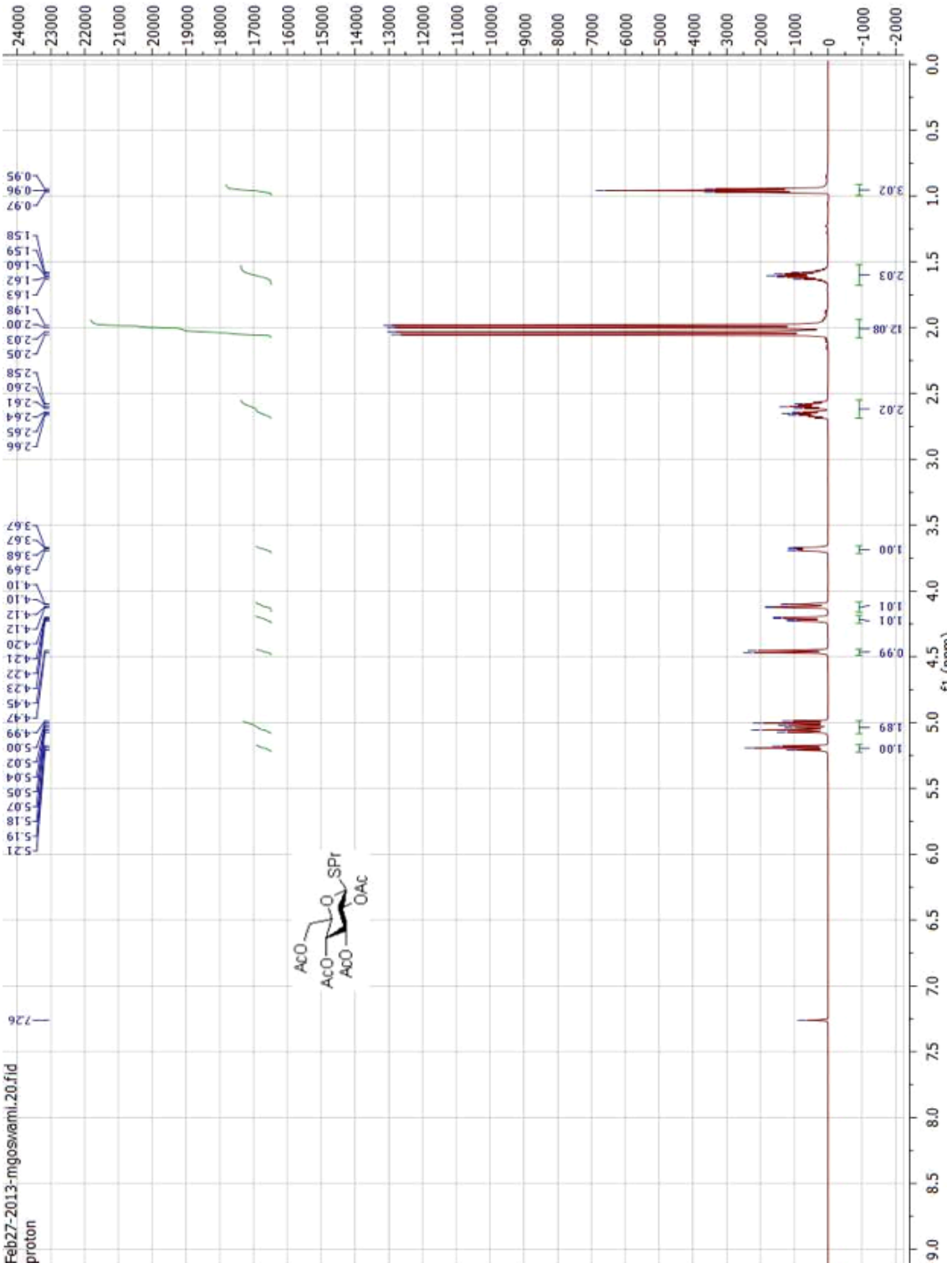

^{1}H NMR (600 MHz, $CDCl_3$)

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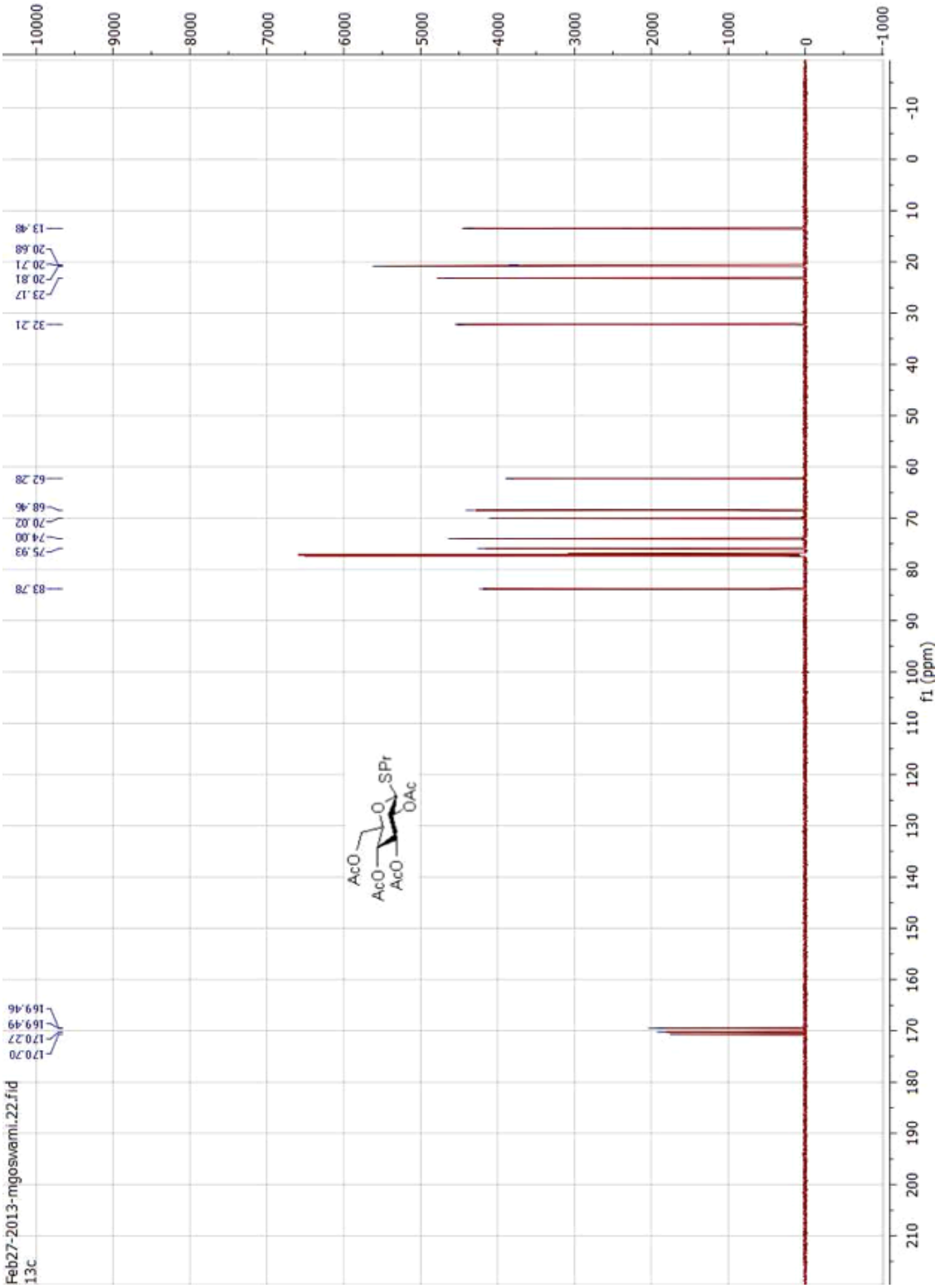

^{13}C NMR (151 MHz, $CDCl_3$)

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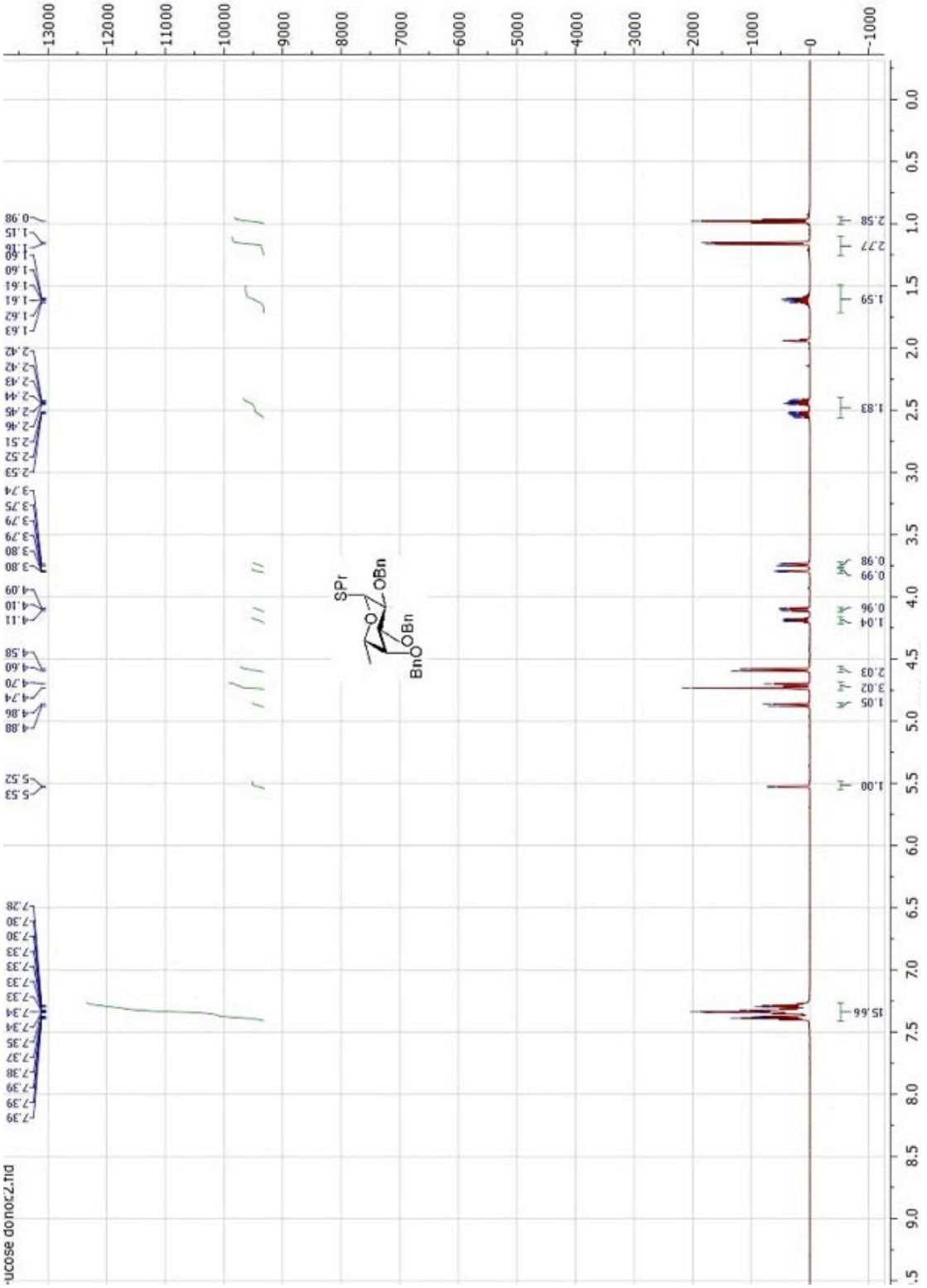

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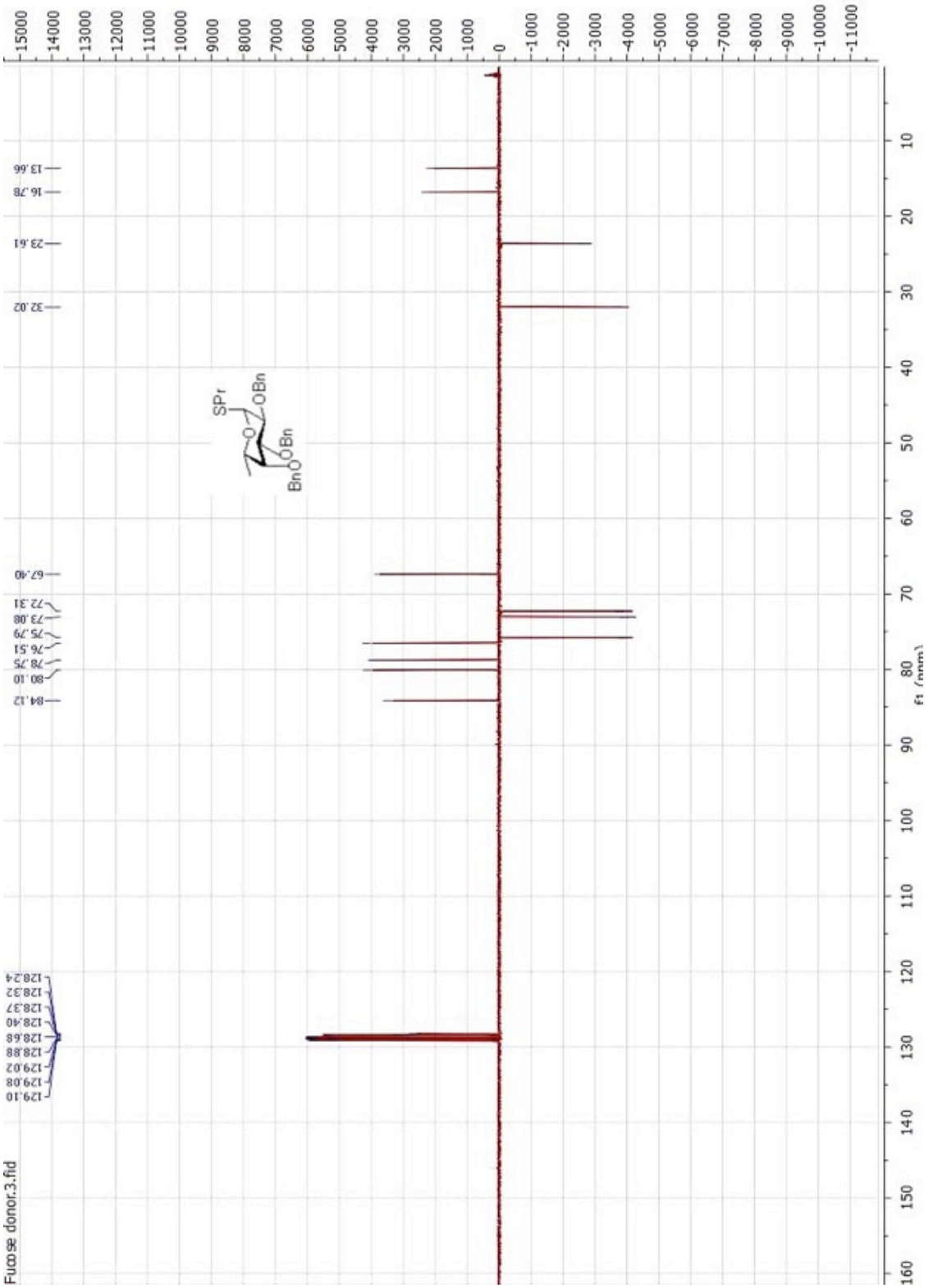

^{13}C NMR (151 MHz, CD_3CN)

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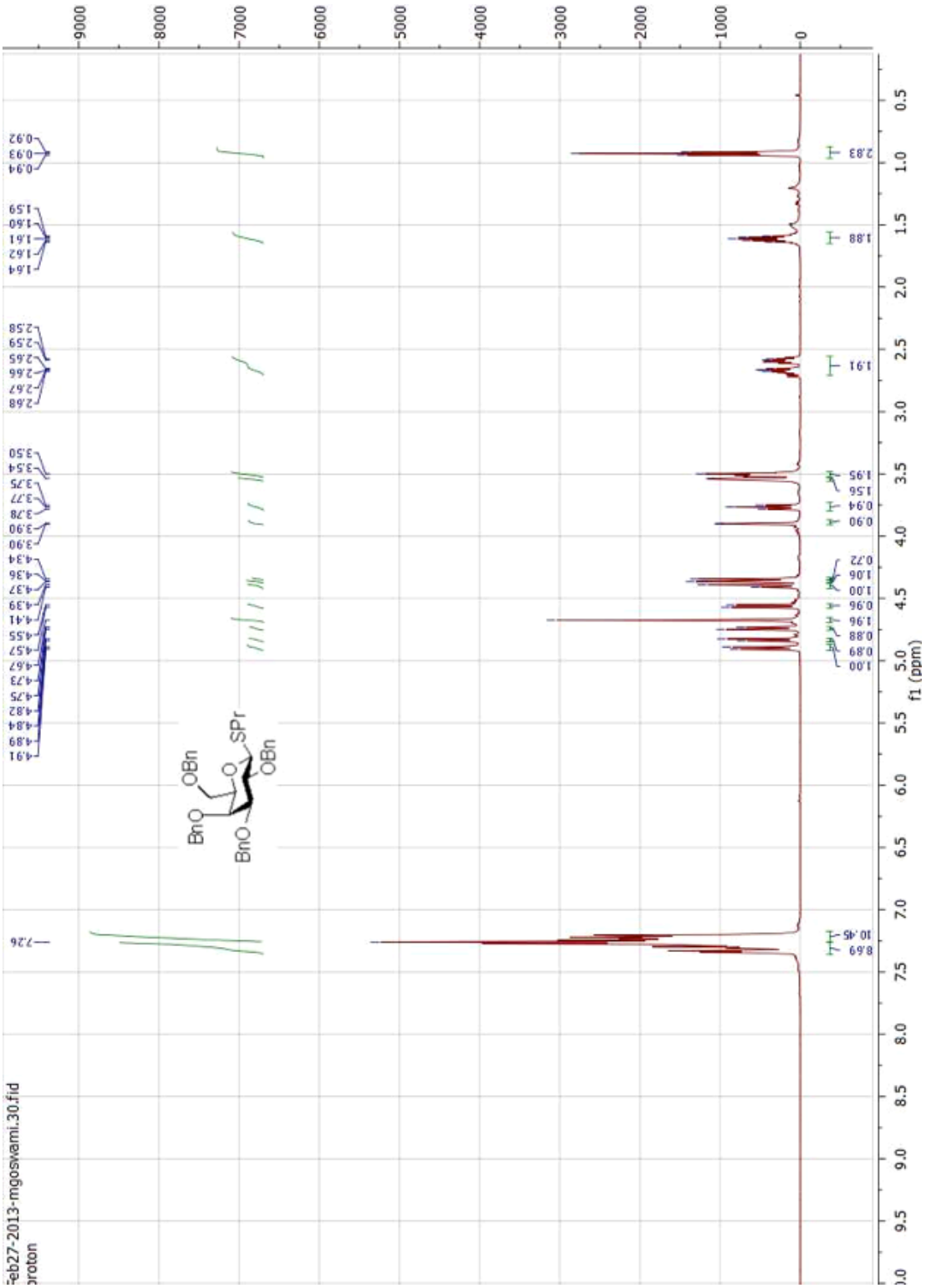
***n*-Propyl-2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (1b)**



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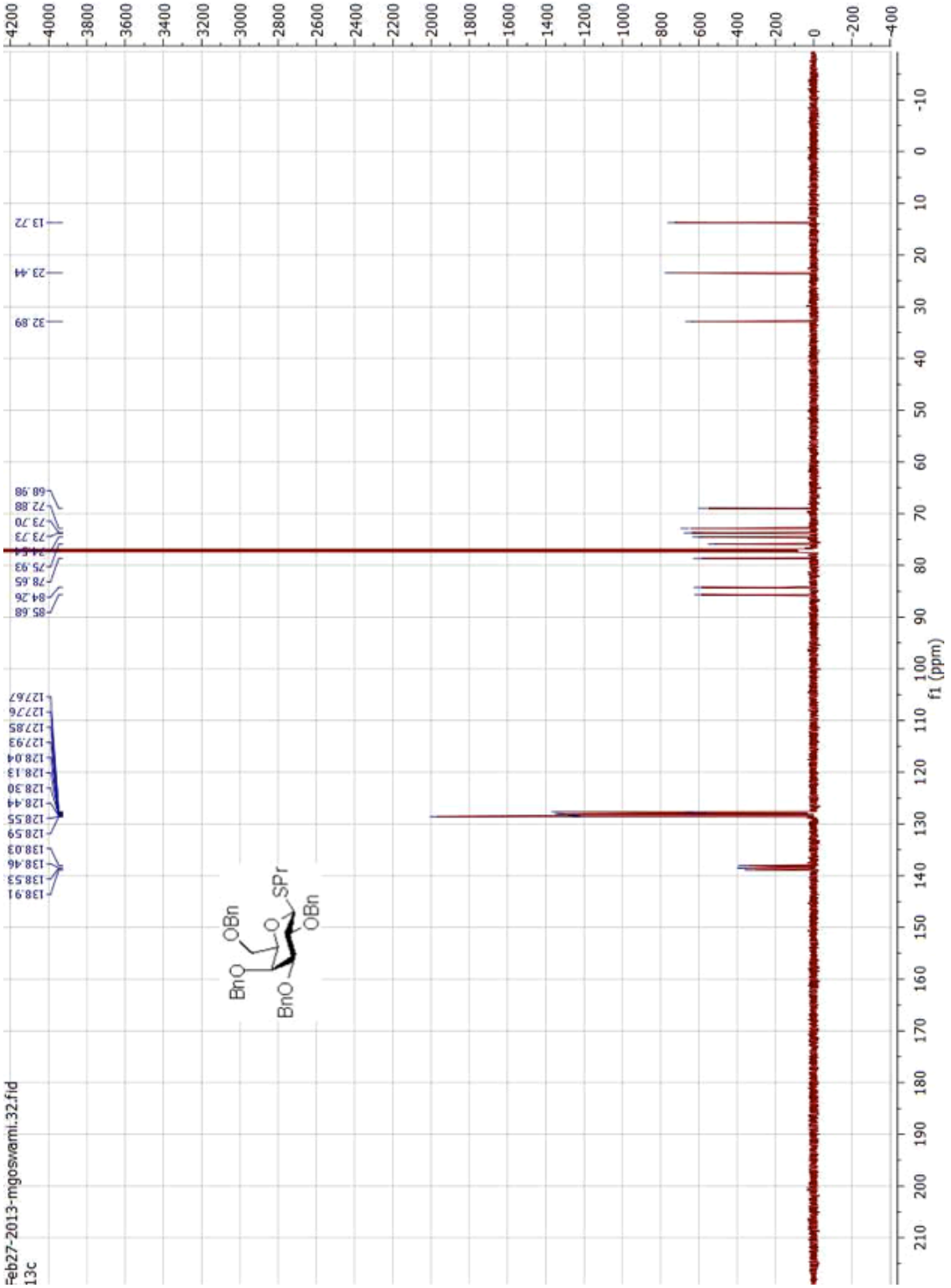

^{13}C NMR (151 MHz, $CDCl_3$)

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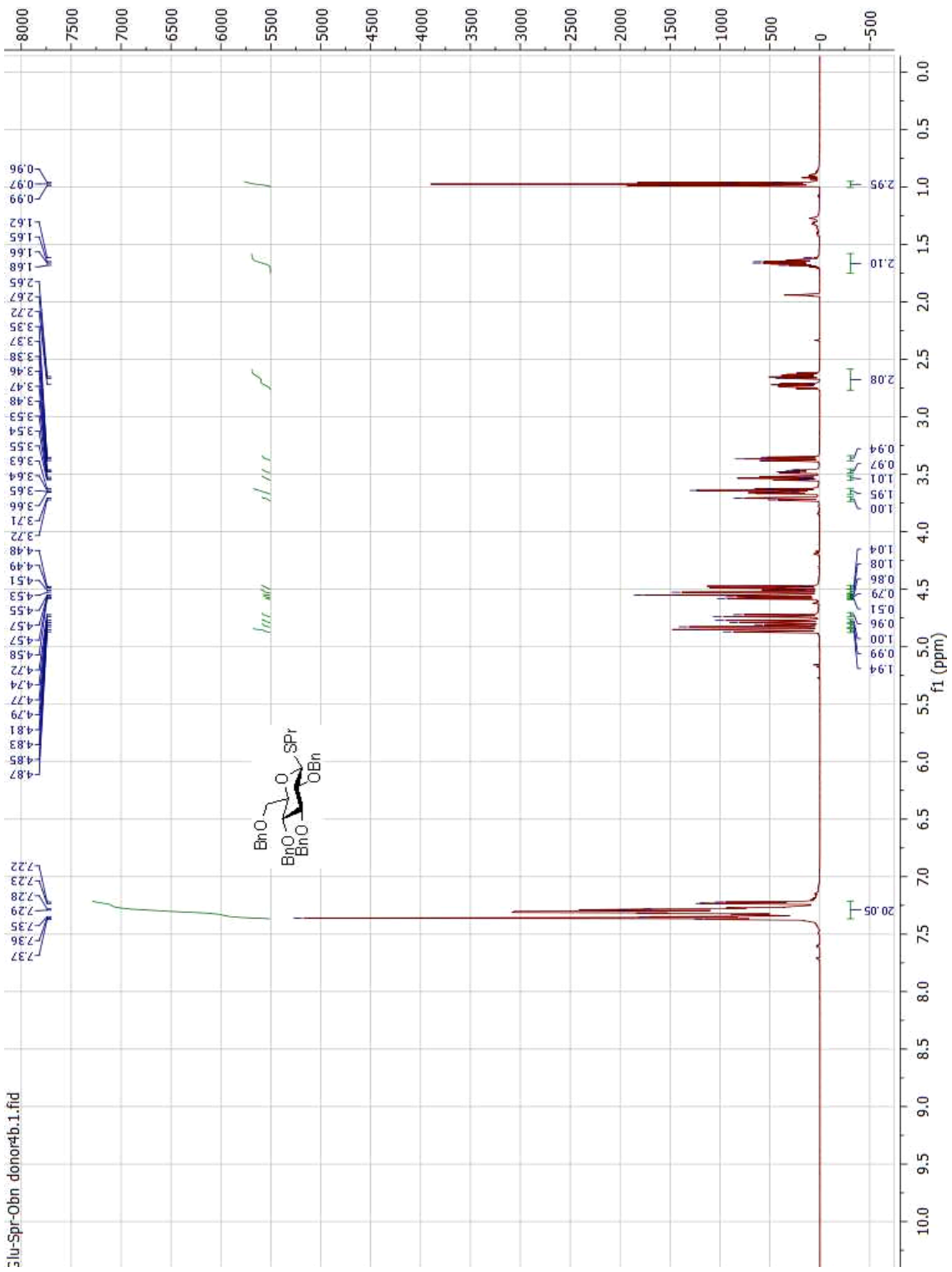
***n*-Propyl-2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (2b)**



^{1}H NMR (600 MHz, CD_3CN)

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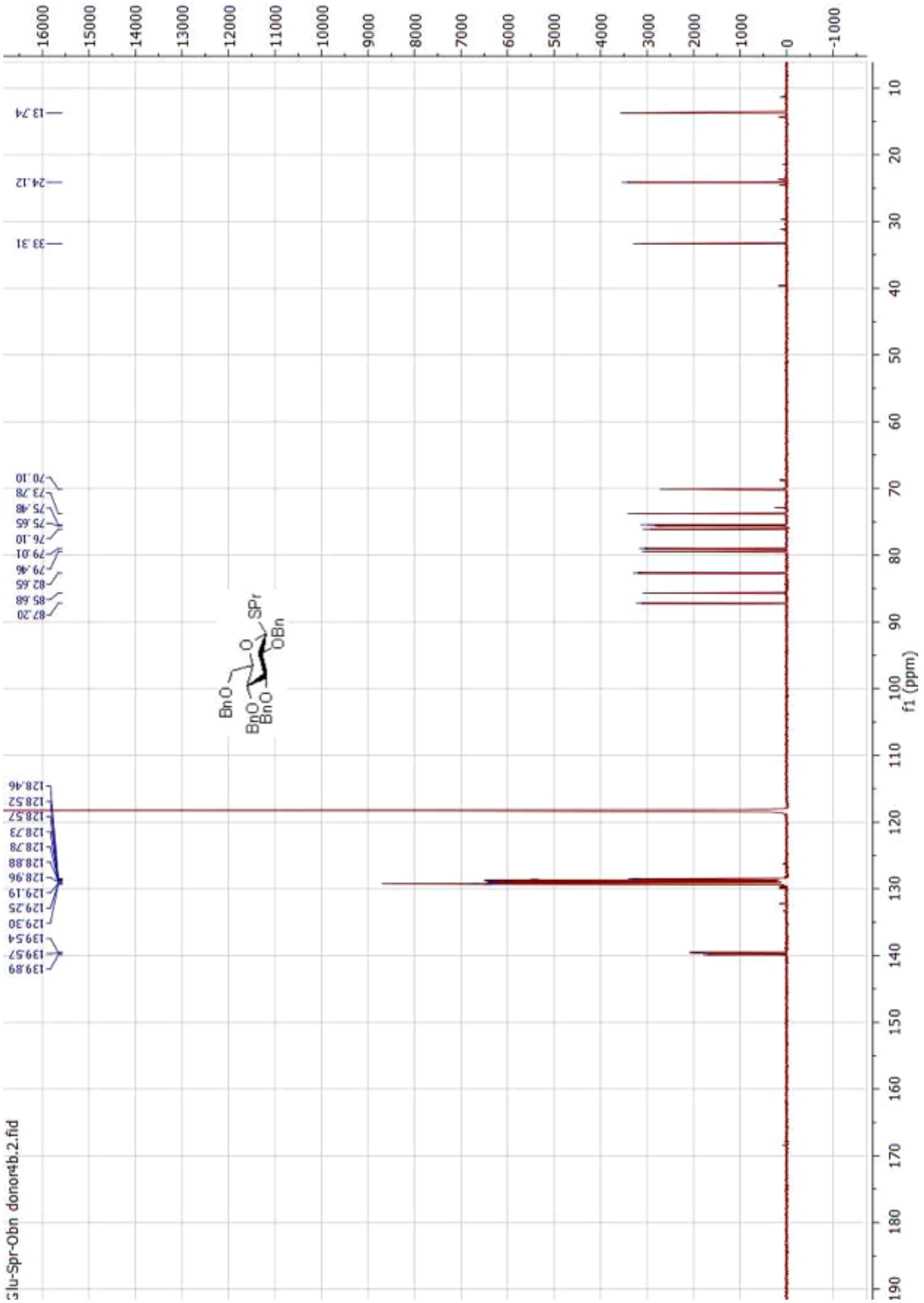

^{13}C NMR (151 MHz, CD_3CN)

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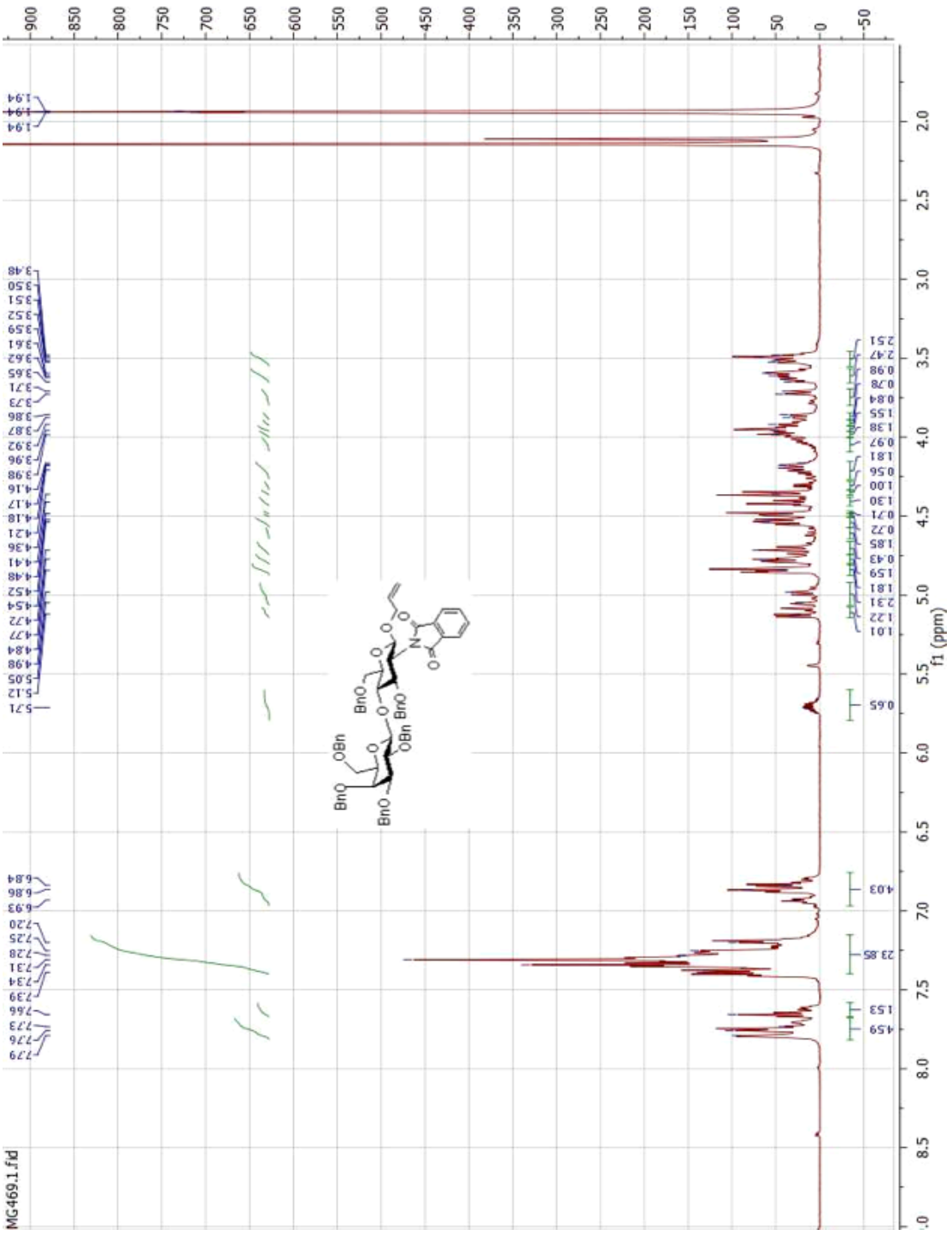
Allyl-(2',3',4',6'-tetra-*O*-benzyl-D-galactopyranosyl)(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-*N*-phthalimido-D-glucopyranoside (1b6d)



^{1}H NMR (600 MHz, CD_3CN)

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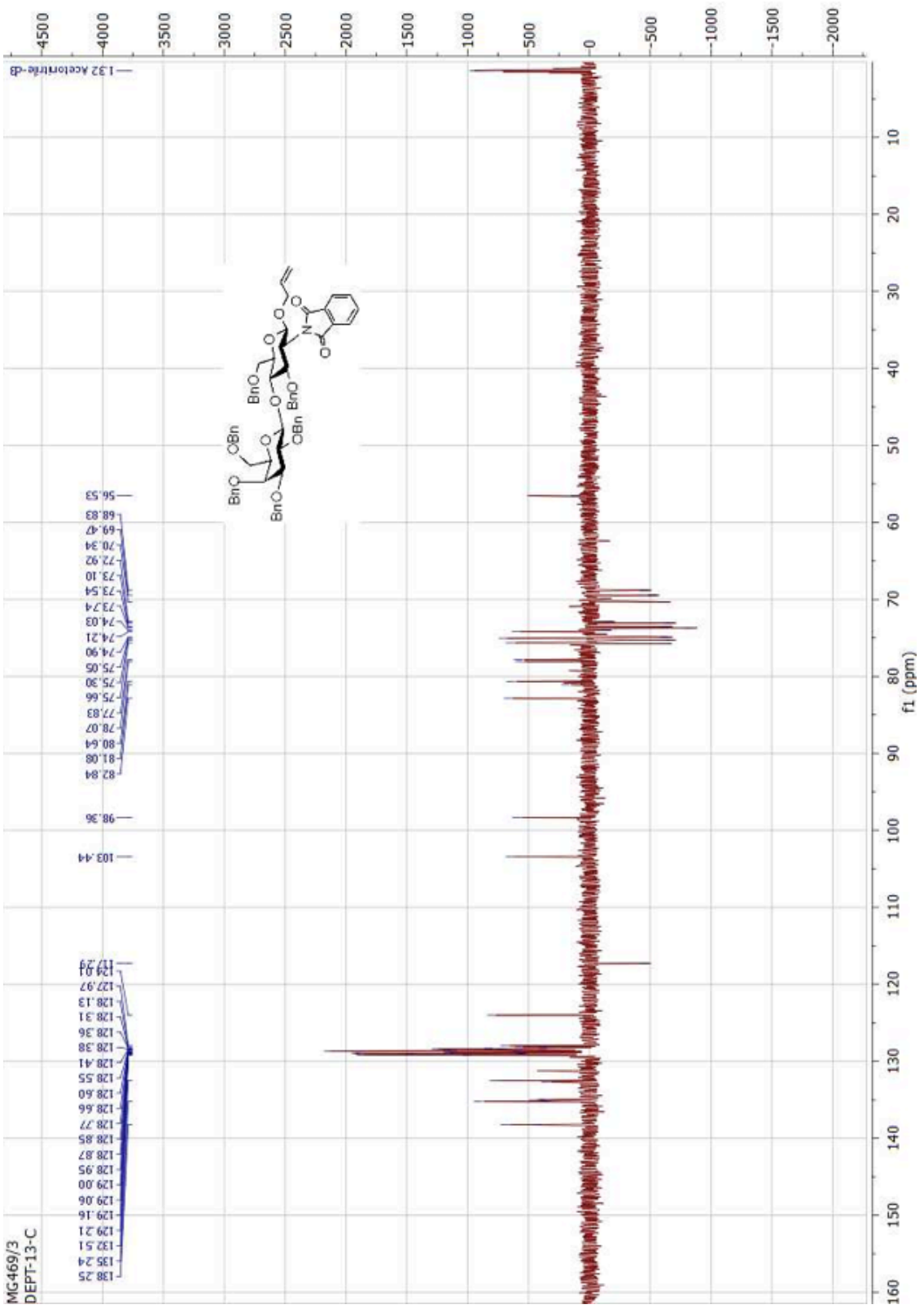

^{13}C NMR (DEPT) (151 MHz, CD_3CN)

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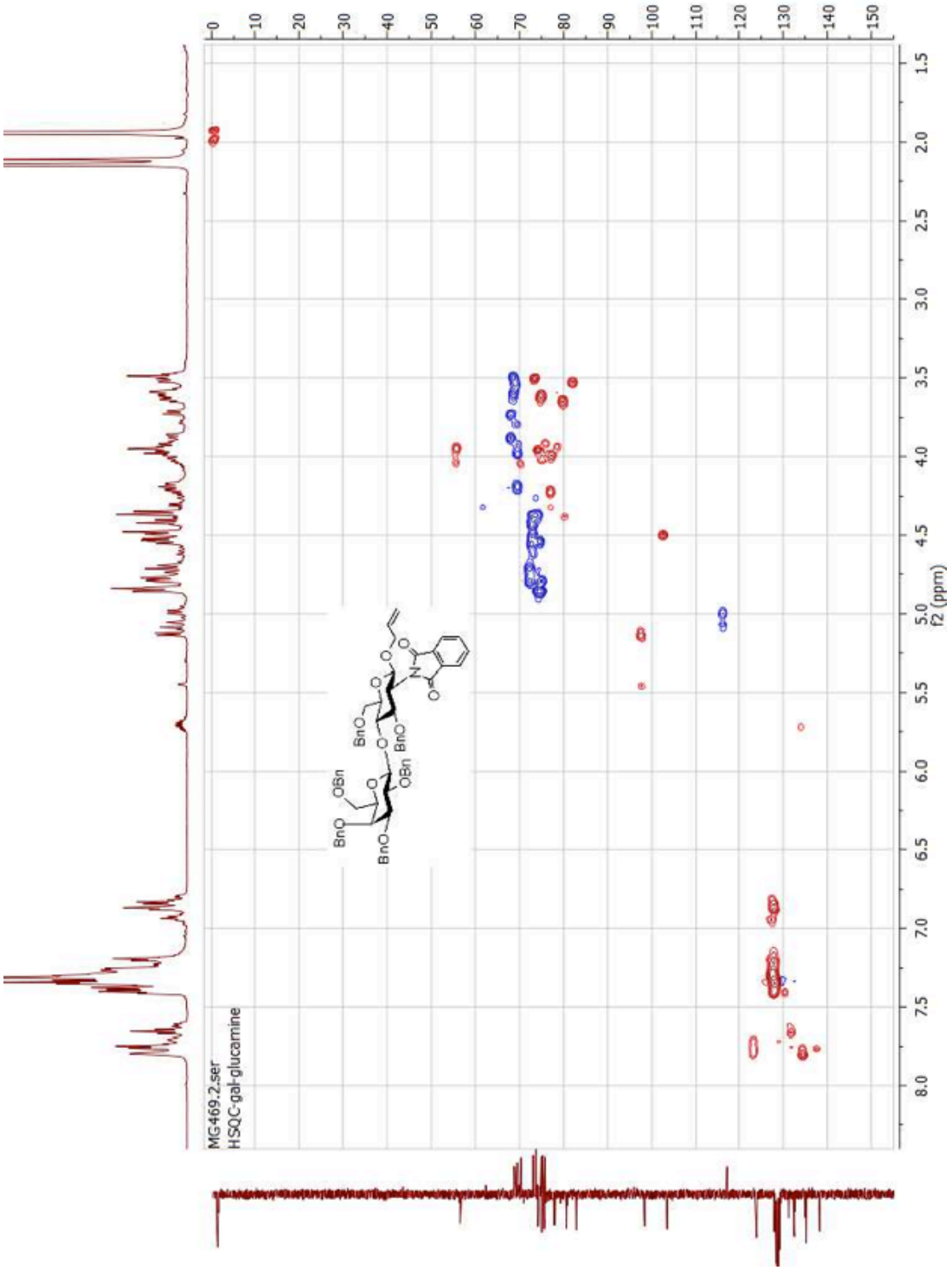

HSQC (151 MHz, CD_3CN)

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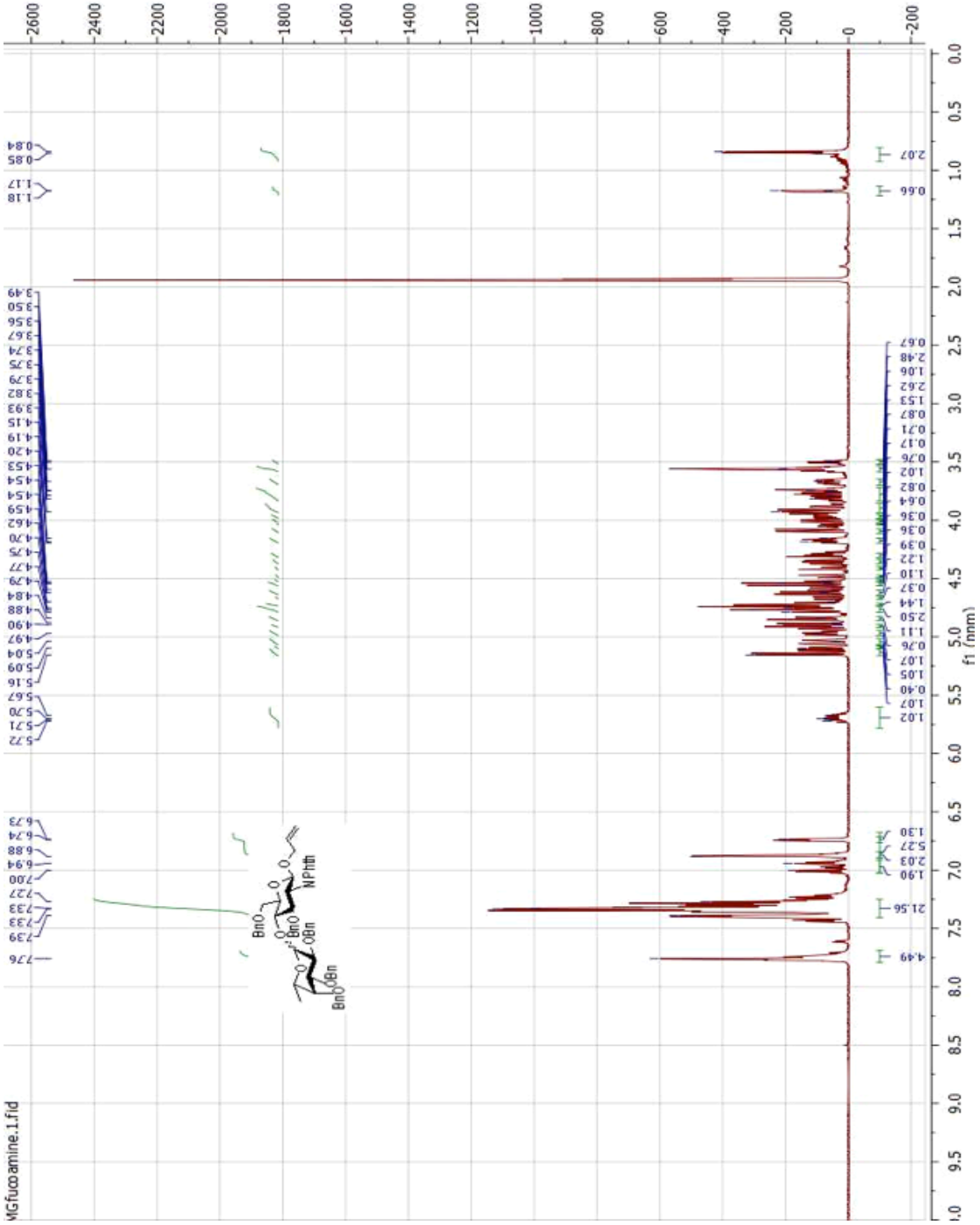
Allyl-(2',3',4'-tri-*O*-benzyl-L-fucopyranosyl)(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-*N*-phthalimido-D-glucopyranoside (3a6d)



^{1}H NMR (600 MHz, CD_3CN)

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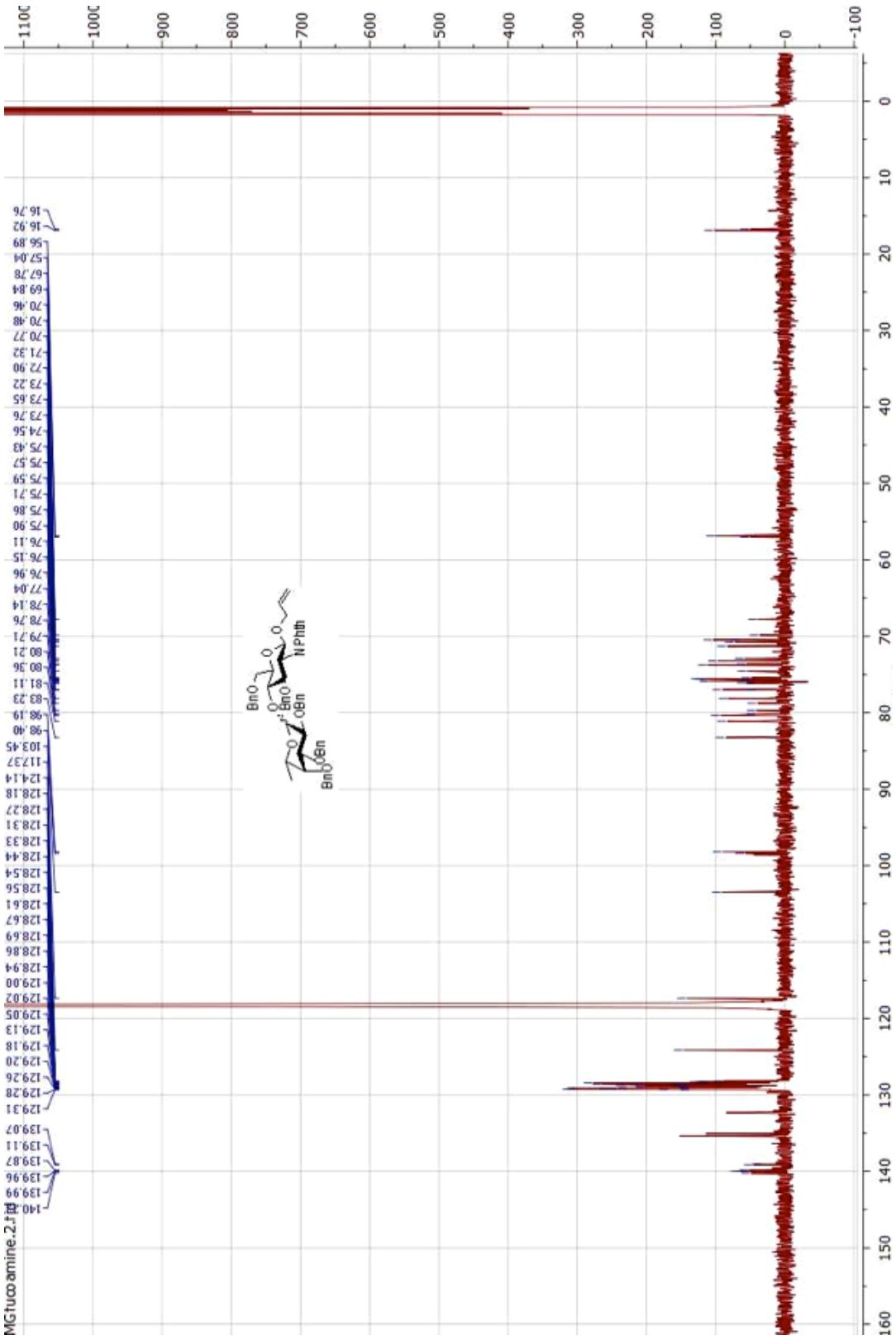

^{13}C NMR (150 MHz, CD_3CN)

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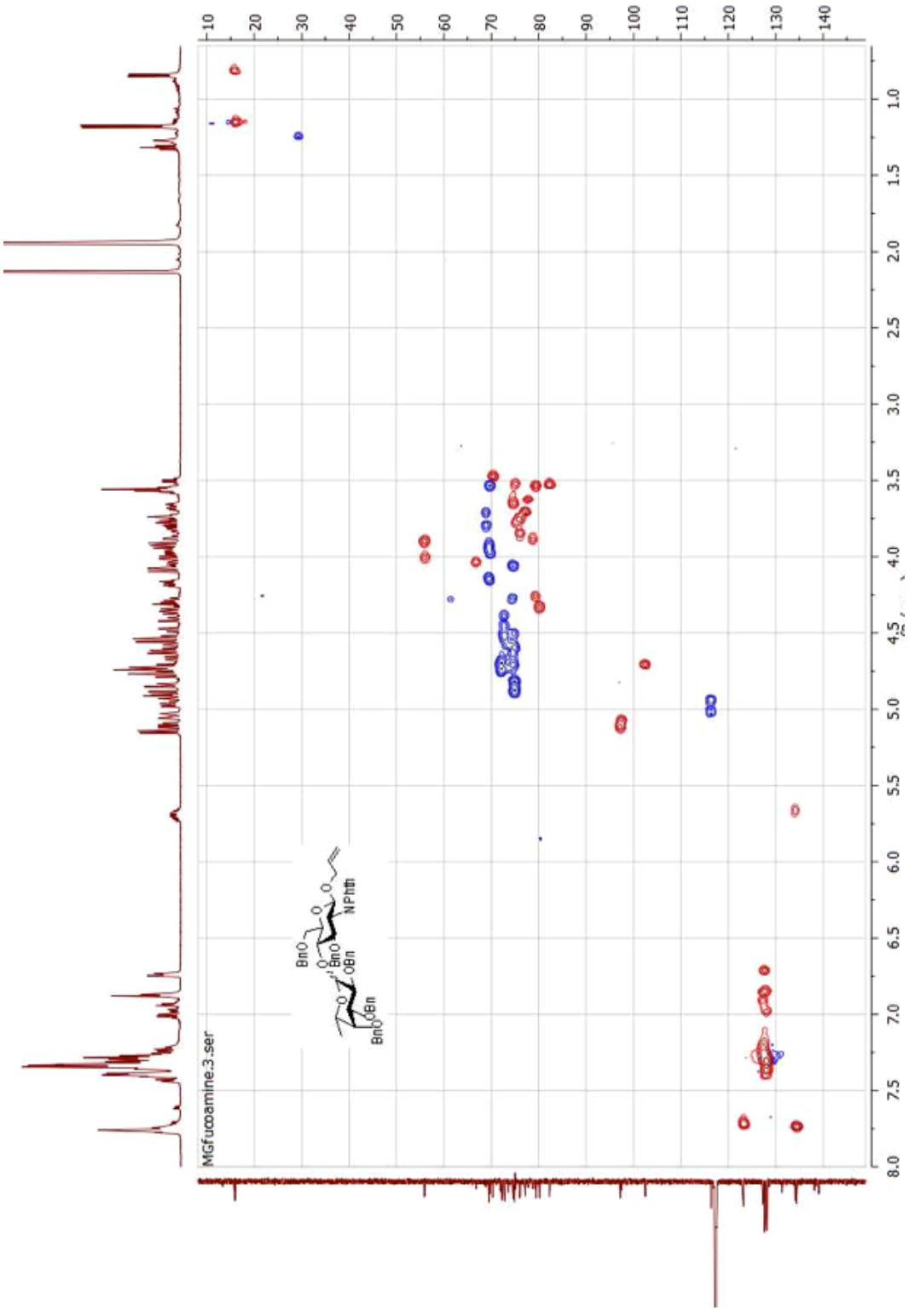

HSQC (151 MHz, CD_3CN)

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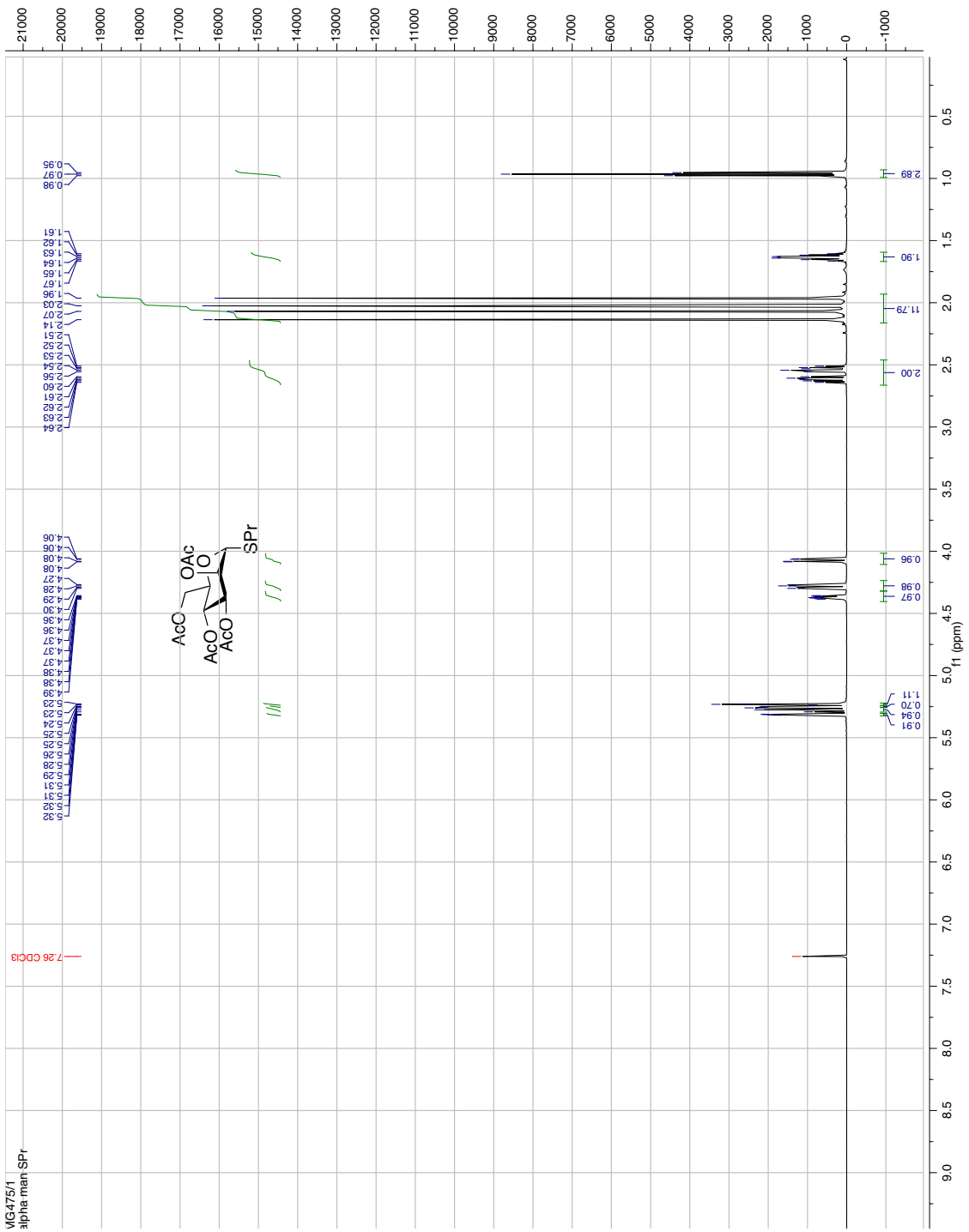
APPENDIX B. CHAPTER 3 1D & 2D NMR SPECTRA

***n*-Propyl-2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranoside (2)**



(^{1}HNMR, 600MHz, $CDCl_3$)

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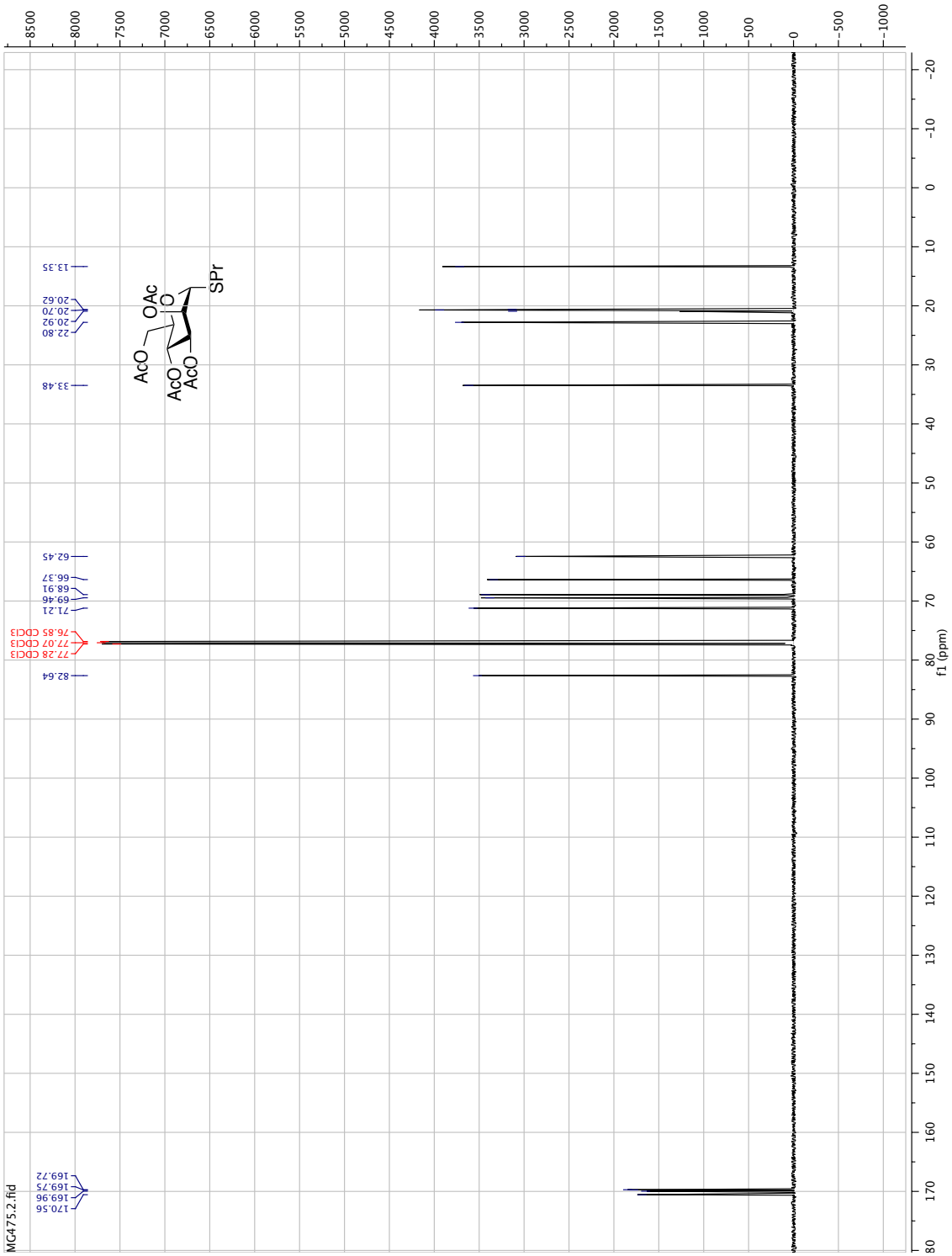

(^{13}CNMR, 151 MHz, $CDCl_3$)

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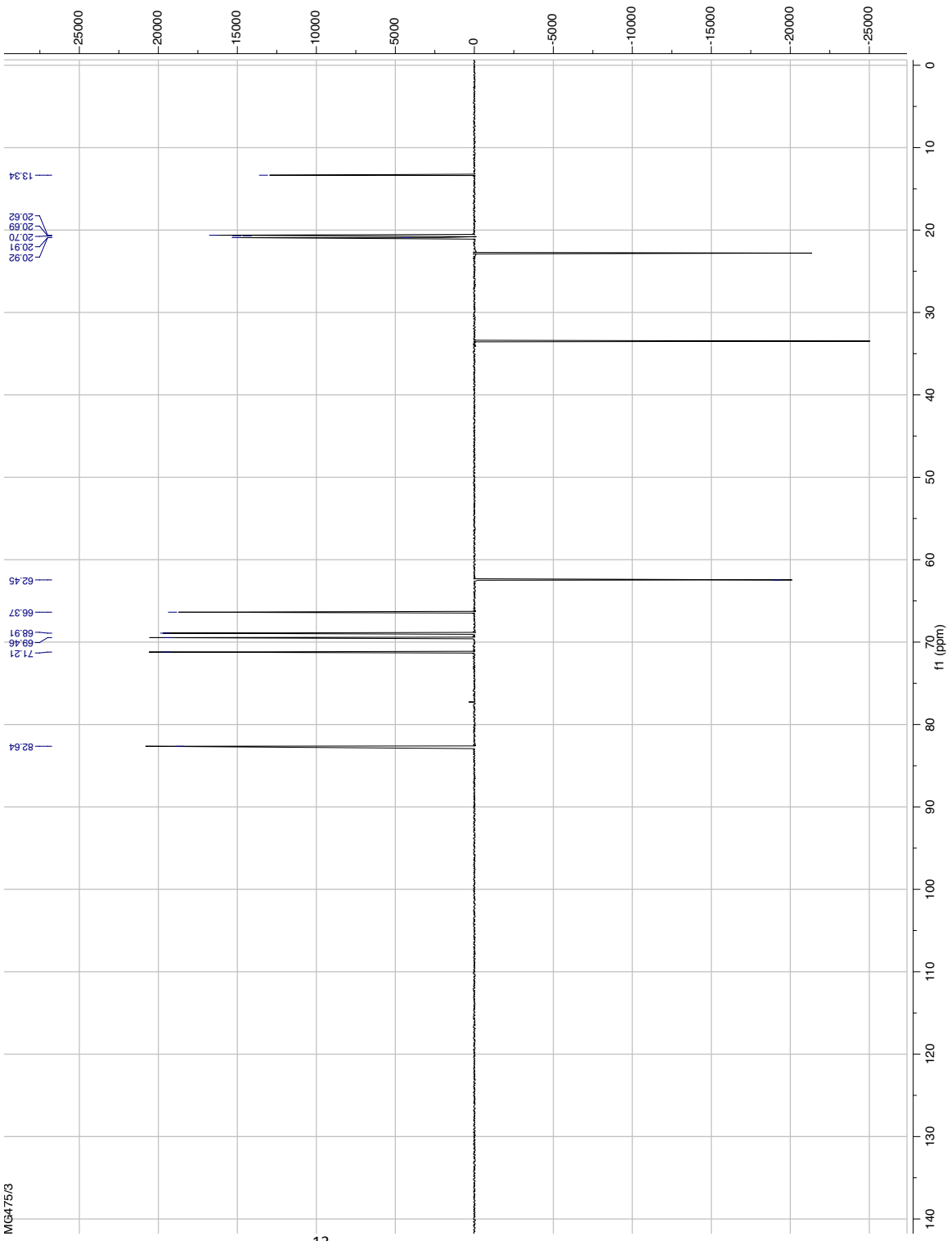

(^{13}CNMR-DEPT, 151 MHz, $CDCl_3$)

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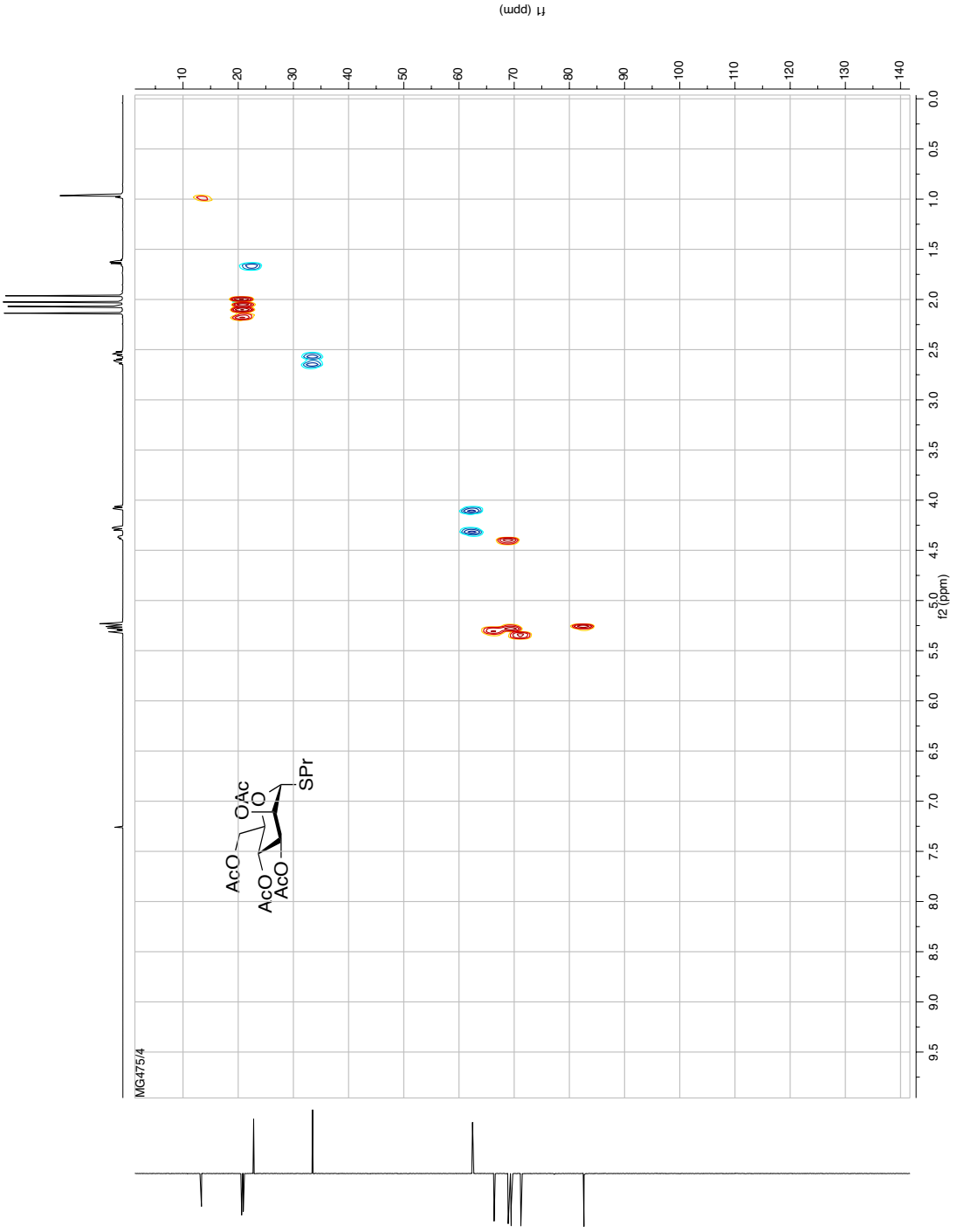

(^{1}H-^{13}C HSQC-Phase edit, 600 MHz, $CDCl_3$)

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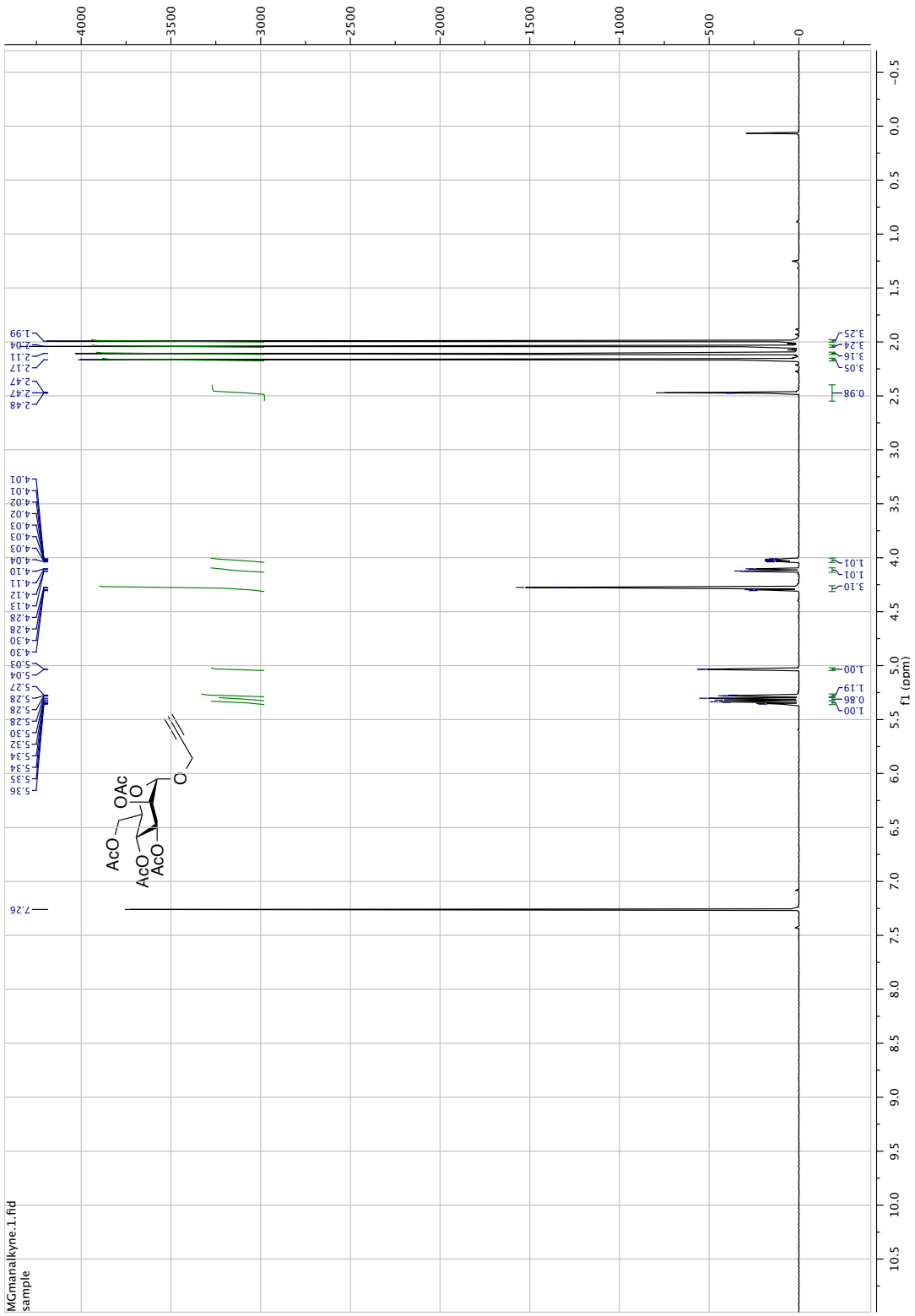
2-Propynyl-2,3,4,6-tetra-*O*-acetyl-1-*O*-α-D-mannopyranoside (3)



(^{1}HNMR, 600 MHz, $CDCl_3$)

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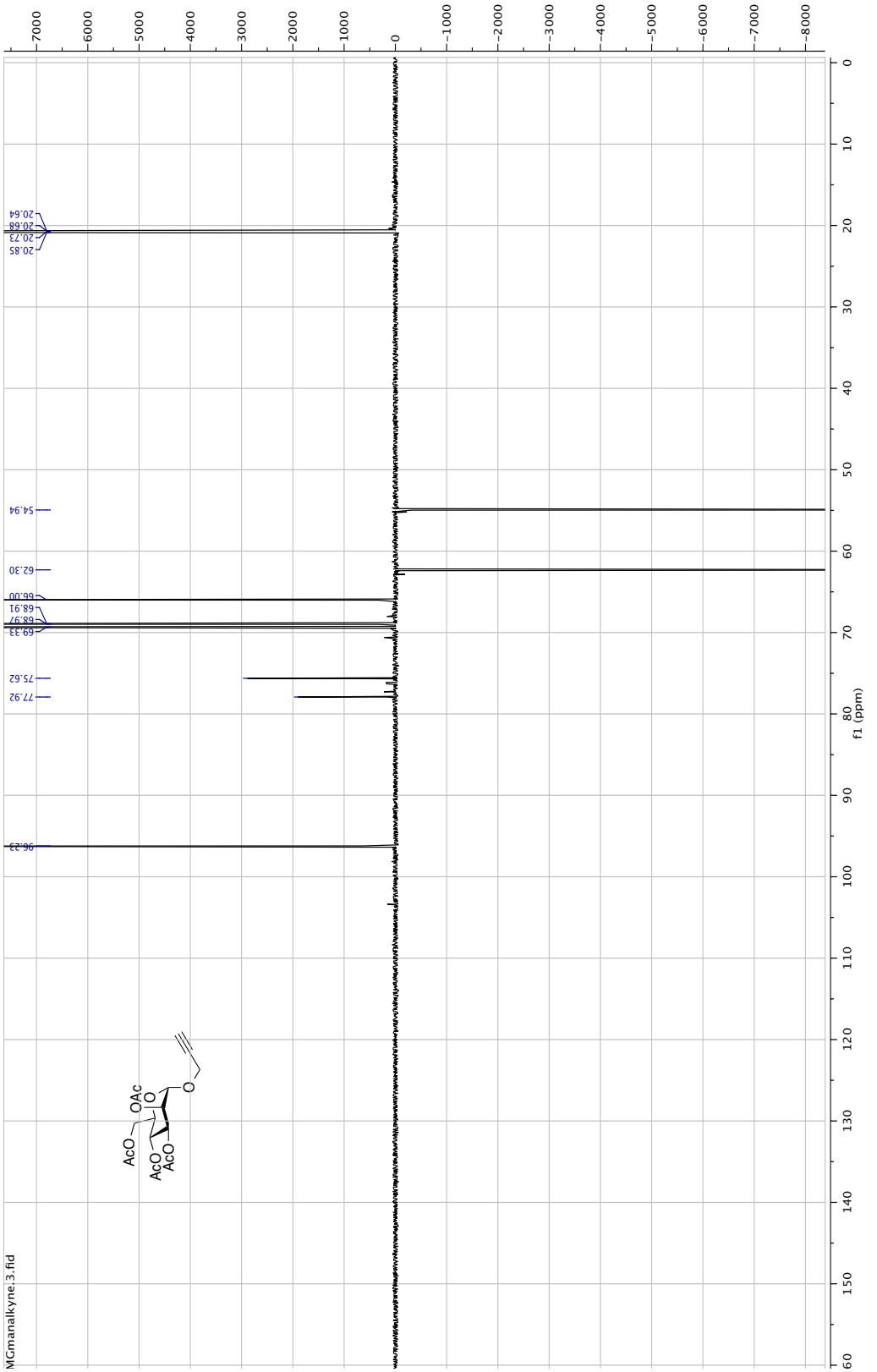

(^{13}CNMR-DEPT, 151 MHz, $CDCl_3$)

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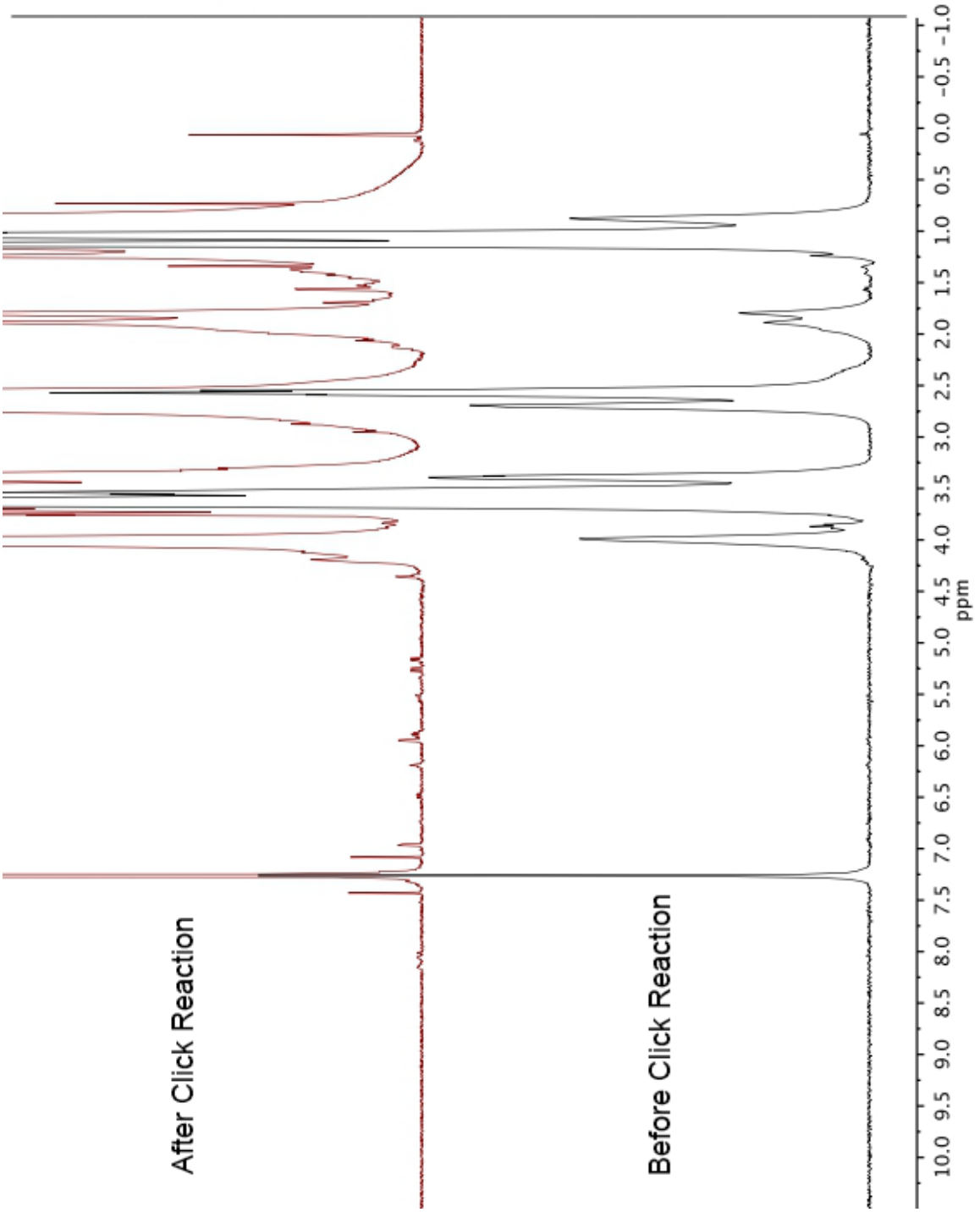
^{1}H NMR comparison of the PDEAEM pentablock copolymer before/after coupling to mannose



(^{1}HNMR, 600 MHz, $CDCl_3$)

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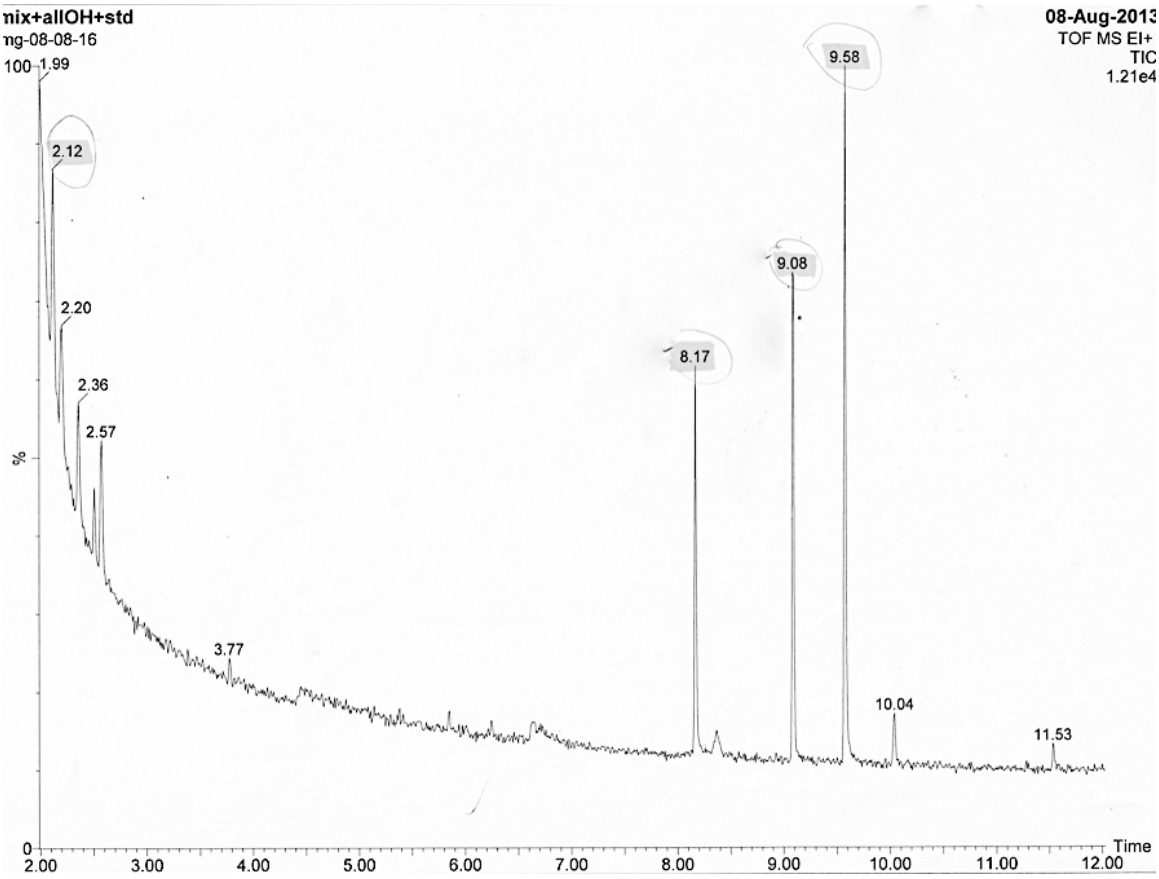
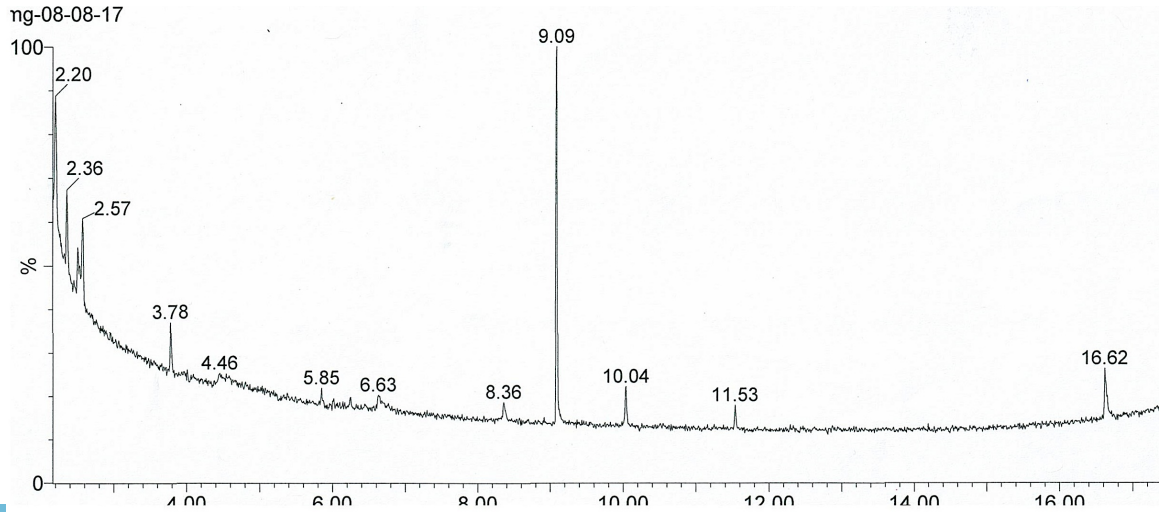
APPENDIX C. CHAPTER 4 1D & 2D NMR SPECTRA, KINETICS RATE GRAPHS, GC-MS SPECTRA

GC-MS of mixture of analyte



Reaction monitoring

At T=0



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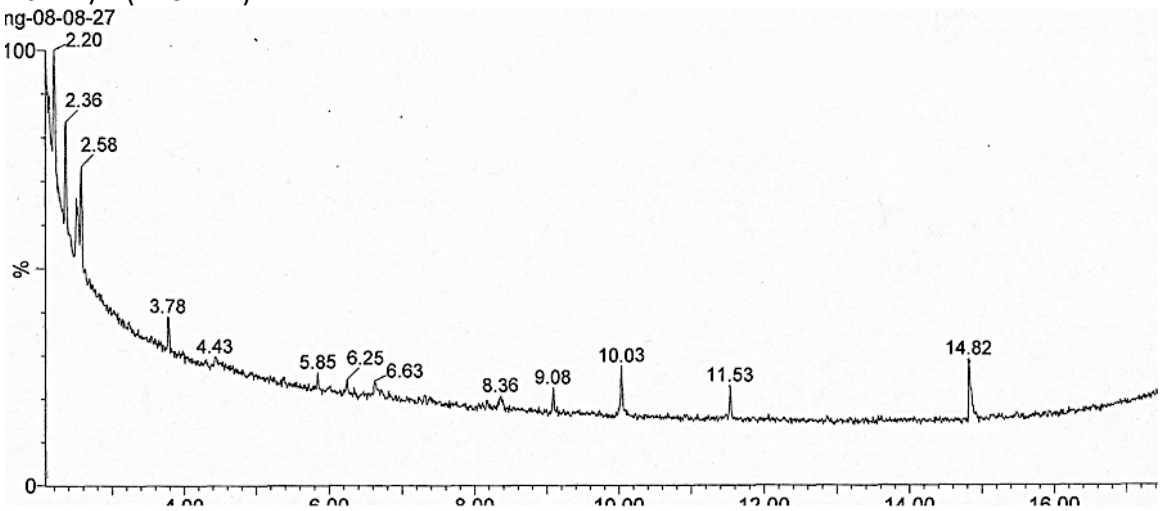
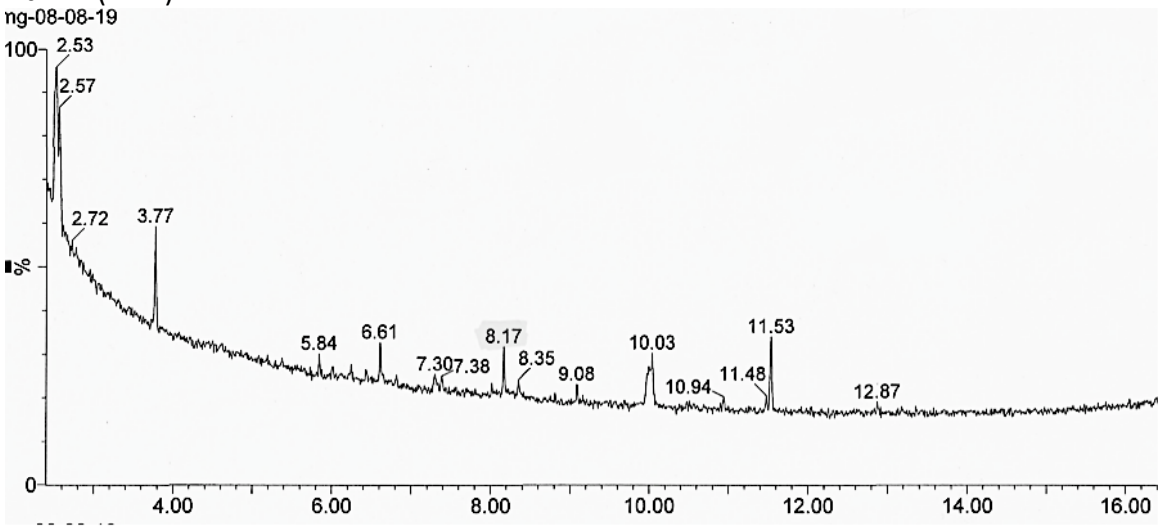
At T=1/2 (~40 min)



At T=1 (~1 h)



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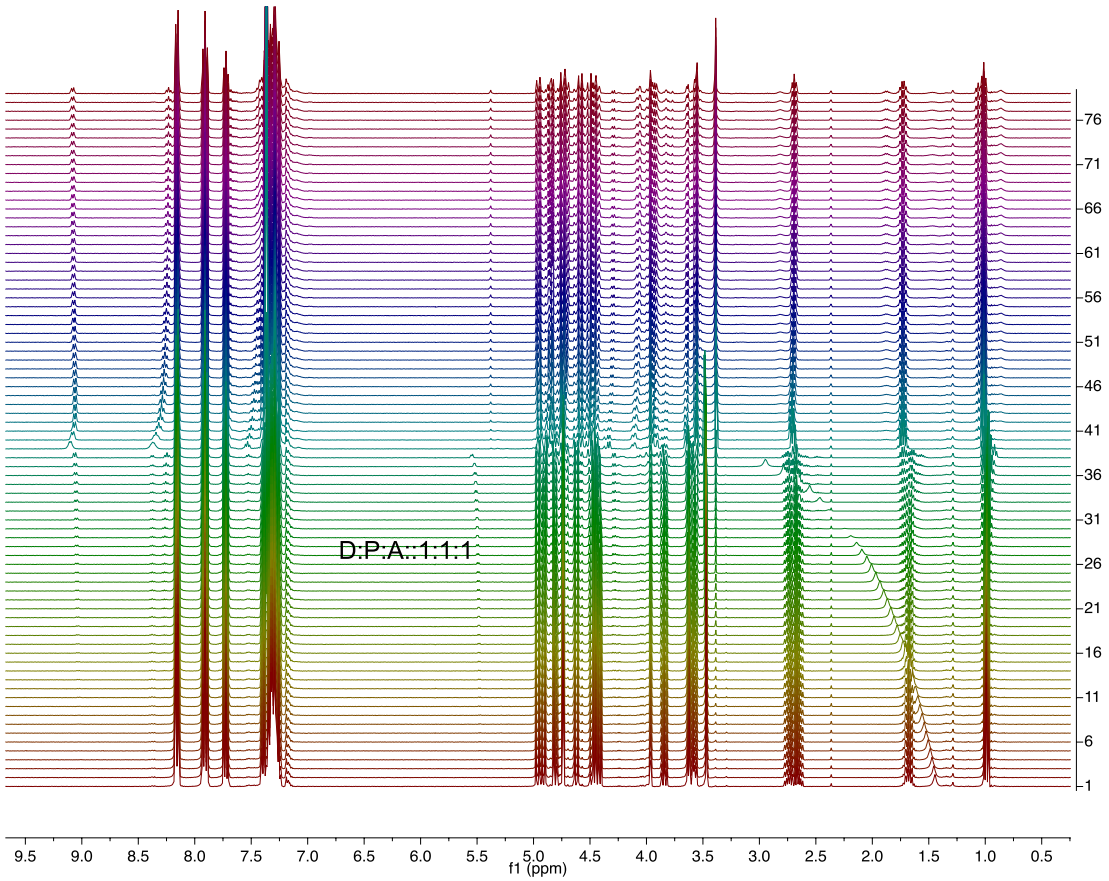
Full kinetics spectra

FID #	Time (min)	FID #	Time (min)	FID #	Time (min)	FID #	Time (min)	FID #	Time (min)
1	1	17	33	33	65	49	136	65	216
2	3	18	35	34	67	50	141	66	221
3	5	19	37	35	69	51	146	67	226
4	7	20	39	36	71	52	151	68	231
5	9	21	41	37	76	53	156	69	236
6	11	22	43	38	81	54	161	70	241
7	13	23	45	39	86	55	166	71	246
8	15	24	47	40	91	56	171	72	251
9	17	25	49	41	96	57	176	73	256
10	19	26	51	42	101	58	181	74	261
11	21	27	53	43	106	59	186	75	266
12	23	28	55	44	111	60	191	76	271
13	25	29	57	45	116	61	196	77	276
14	27	30	59	46	121	62	201	78	281
15	29	31	61	47	126	63	206	79	286
16	31	32	63	48	131	64	211	80	290



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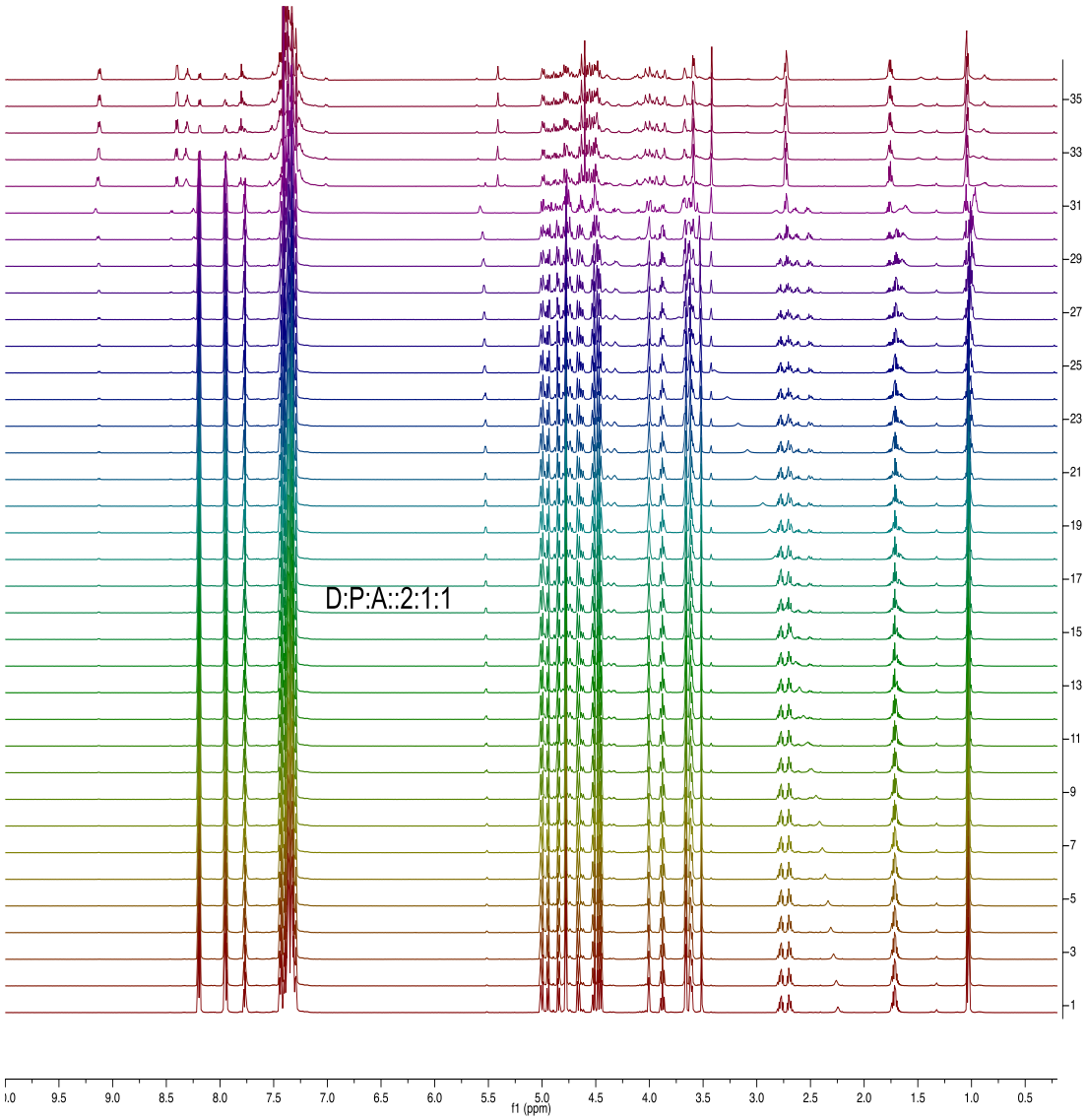

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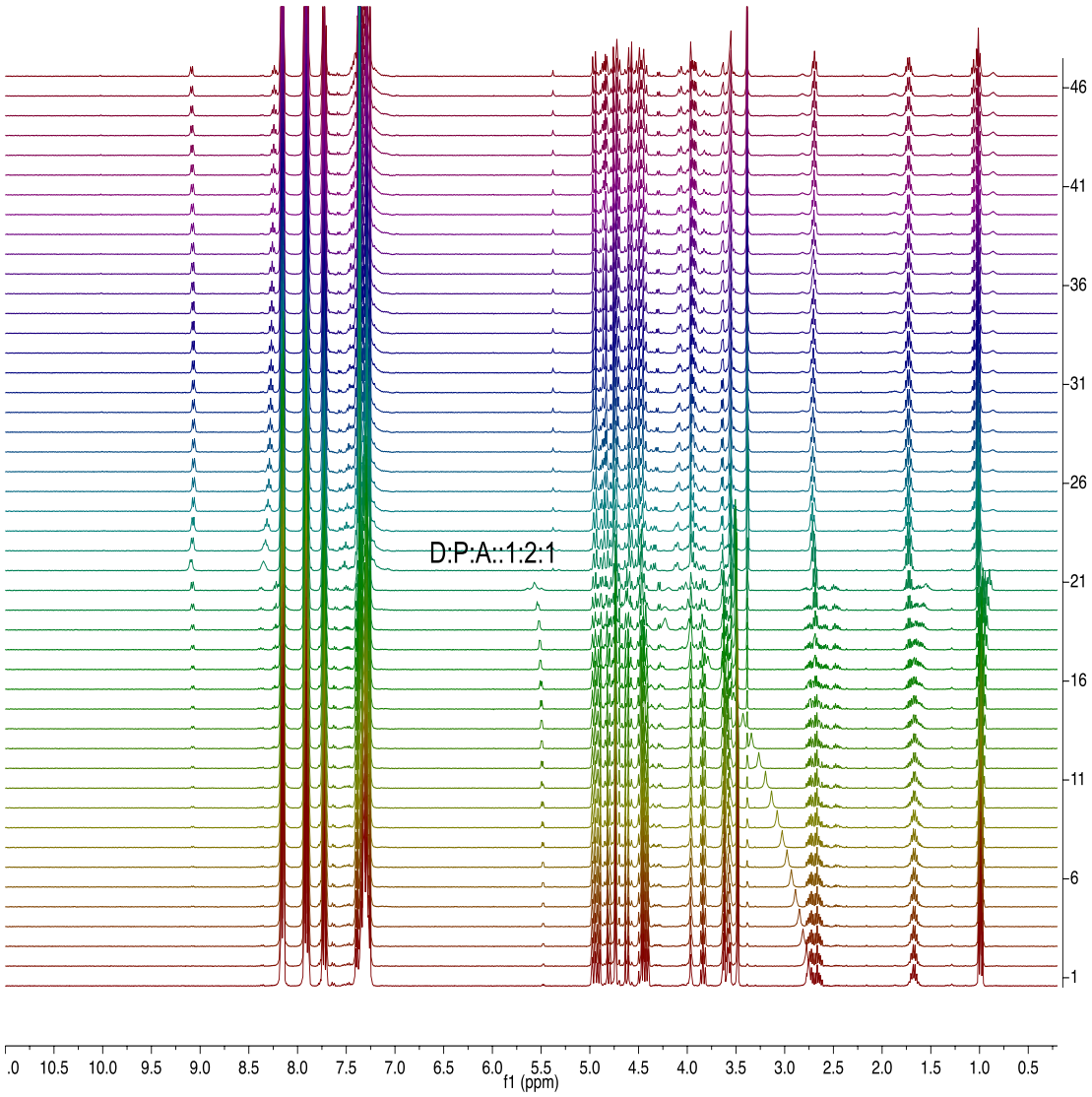

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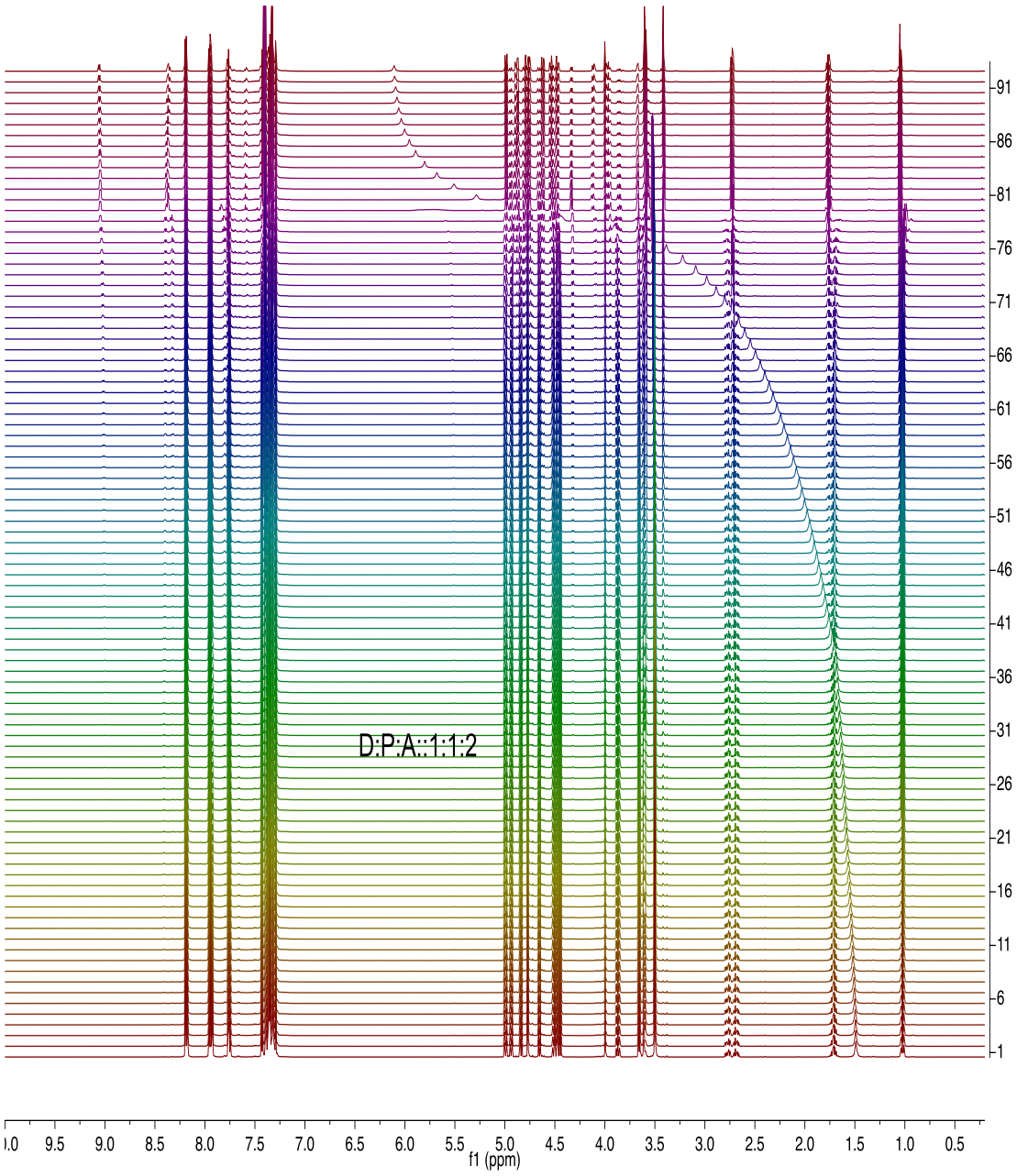

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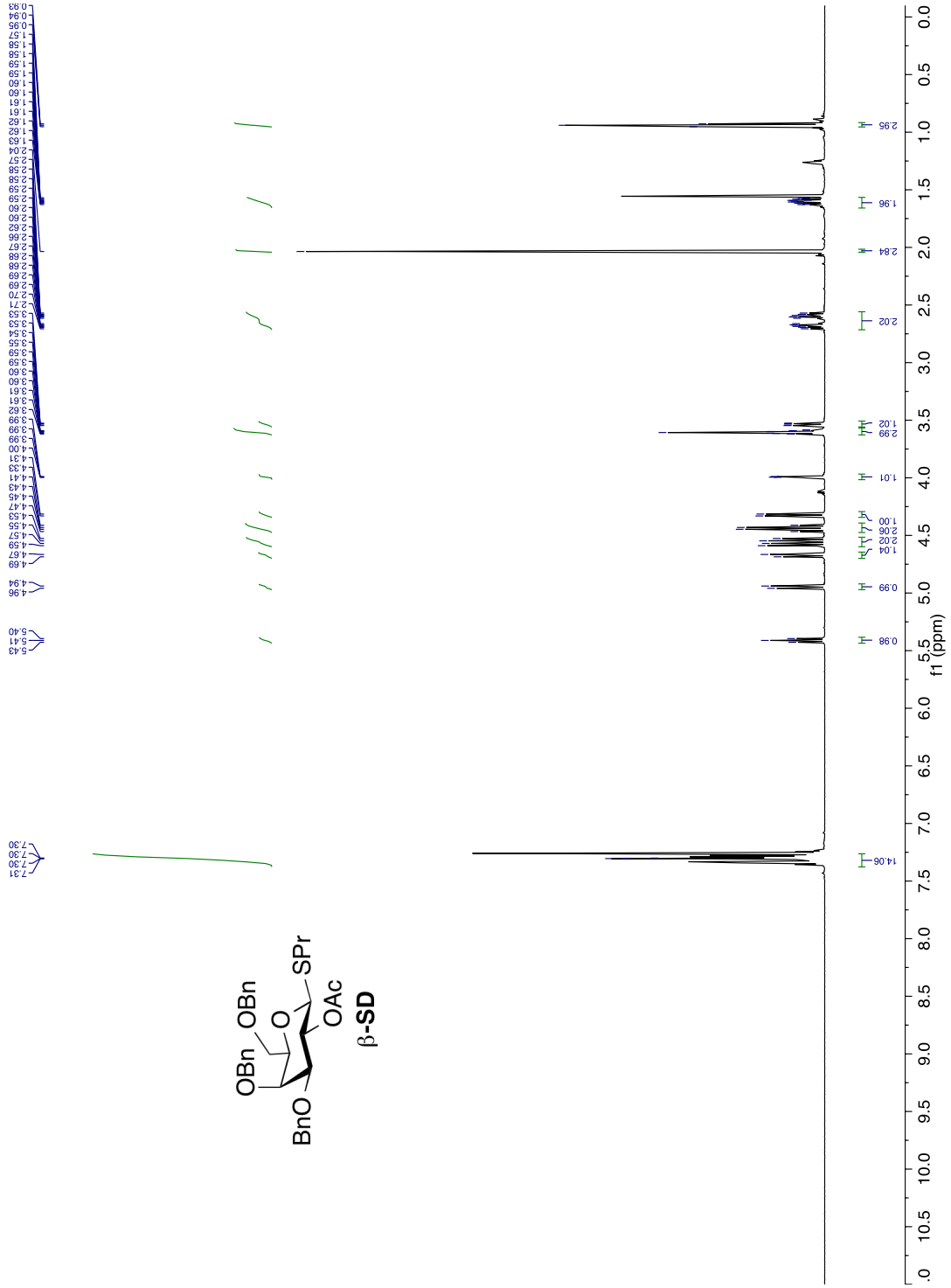
***n*-Propyl-2-*O*-acetyl-3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (β-SD)**



^{1}HNMR ($CDCl_3$, 600 MHz)

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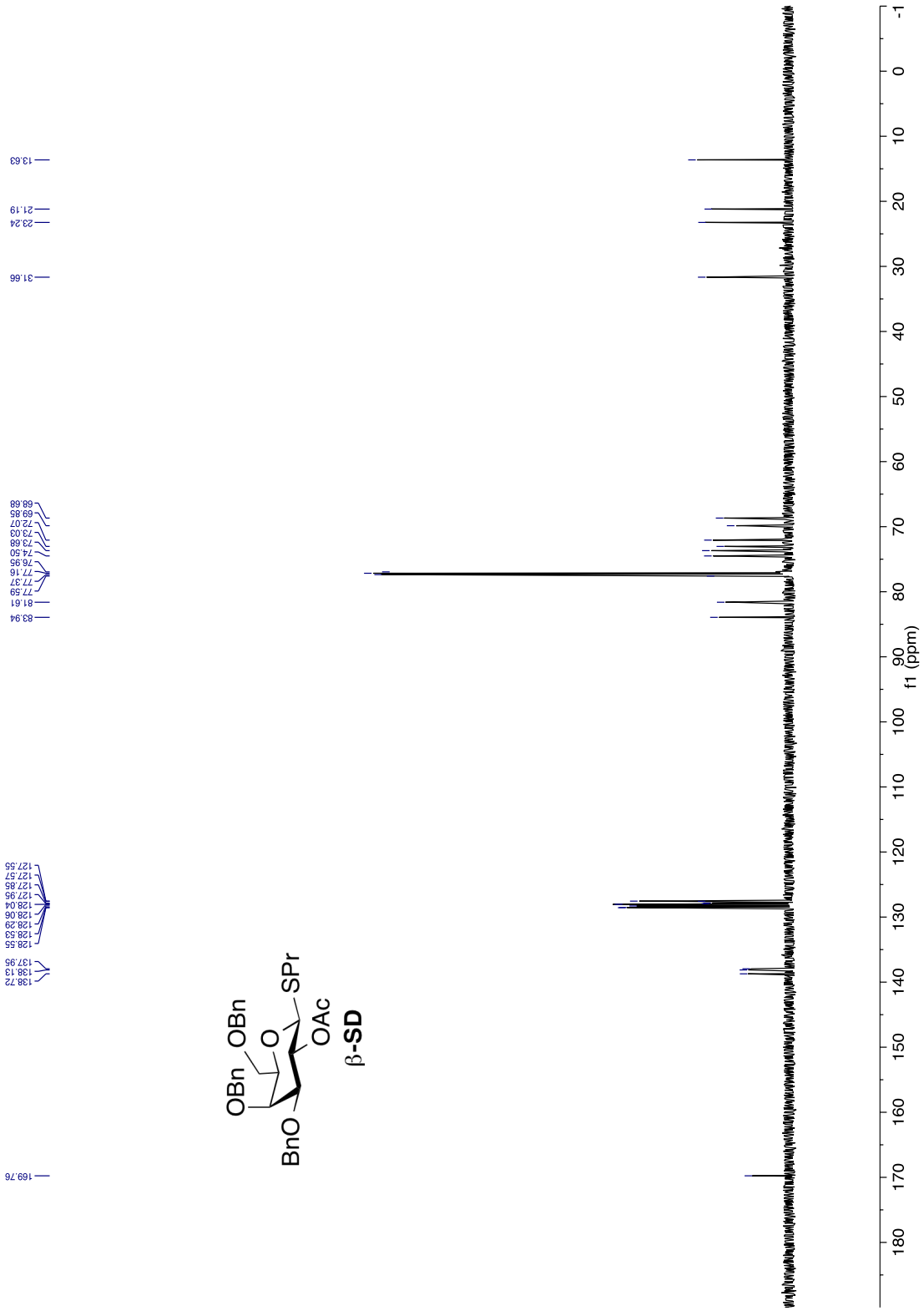

^{13}CNMR ($CDCl_3$, 151 MHz)

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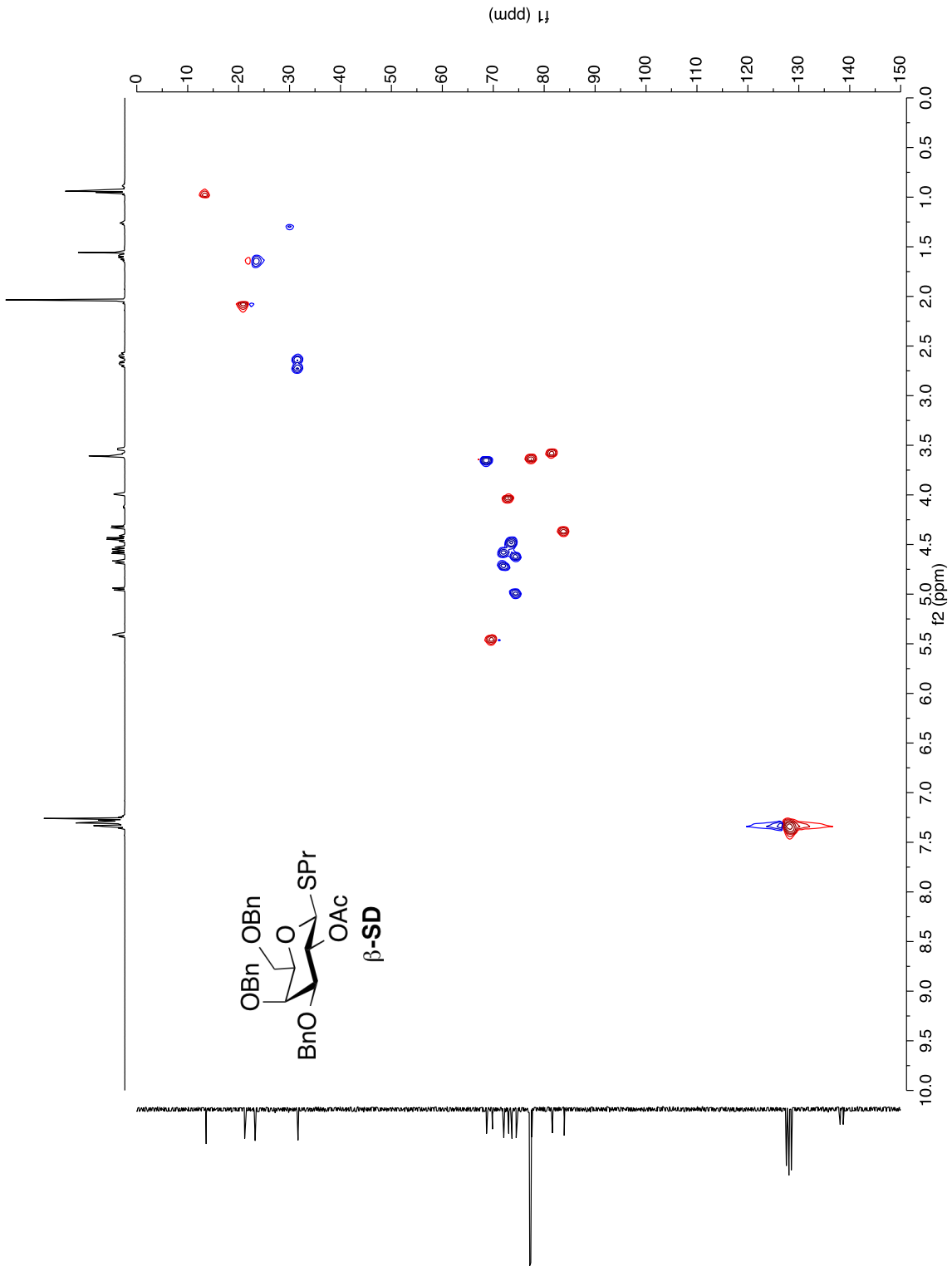

HSQC ($CDCl_3$, 600 MHz)

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