國立交通大學

應用化學研究所

博士論文

(1) Synthesis of Analogues of *N*-acetyl Neuraminic Acid Using Pd(0)-catalyzed Approach

(2) Preparation of α-Glycosyl Chloride via TCT/DMF

(3) DMF-mediated α-Stereoselective Glycosylation

研究生：張智為 撰

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ABSTRACTS

This dissertation is divided into four parts:

Part I. A highly effective regio- and stereoselective palladium-catalyzed allylic substitution of 2,3-unsaturated derivatives of *N*-acetyl neuraminic acid (Neu5Ac2en) has been developed. We show that the efficiency of the allylation reaction depends on suitable protecting groups on the starting material and that, with sodium malonate anion as a nucleophile, regioselectivity can be fine tuned by the nature of the ligands associated with the palladium complex. The reaction was also applied to other nucleophiles for constructing C-C, C-N and C-O bonds and led to the major formation of the C-4 regioisomers. The selective transformation of some of the substitution products provided easy entry to a variety of modified sialic acid derivatives.

Part II. Preparation of α-glycosyl chloride derived from glycosyl hemiacetals (1-hydroxy sugars) can be performed by treatment of Vilsmeier-Haack complex generated from stoichimetric 2,4,6-trichloro-1,3,5-triazine (TCT) and *N*,*N*-dimethylformamide (DMF) to afford the corresponding chloride in excellent yields. This mild condition has been approved being compatible with acid-labile functionalities. In addition, this protocol can be applied to the synthesis of *N*-acetylneuraminic acid glycal in one-pot manner.

Part III. DMF-mediated α−selective glycosylation has been systematically investigated in aspects of mechanistic understanding, the applicable scope and experimental manipulation. We found the several distinguished features:**1.** Only stoichiometric DMF is required; **2.** Better α−selectivity is usually obtained at the low temperature; **3.** Pre-activation of glycosyl chlorides with DMF is for the first time proved feasible. **4.** Plausible IAD mechanism may be

contributed to stereoselectivity. This Applications by using DMF as an additive could be practical in glycol-assembly.

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Part IV. A tetrasaccharide containing the core structure of a glycoglycerolipid isolated from Meiothermus taiwanensis ATCC BAA-400 has been efficiently synthesized by using the convergent [2+2] glycoyslation strategy. DMF-mediated α–selective and low-concentration 1,2-*trans* β–selective glycosylation strategy are for the first time applied to construct the specific α– or β–linkage in oligosaccharide synthesis.

RÉSUMÉ

La première partie: Nous avons mis au point une méthodologie sur l'acide sialique en utilisant la réaction de Tsuji-Trost pour obtenir des produits de couplage avec de bons rendements et de bonnes sélectivités. De plus, selon la nature de la phosphine utilisée, nous avons pu constater différentes régiosélectivité en position 2 ou 4 avec diméthylmalonate. Nous avons aussi étendu la réaction à d'autres nucléophiles et nous avons pu construire diverses liaisons. Certaines modifications des dérivés en C-2 et C-4 ont été effectuées.

La deuxième partie: Après je suis revenu à Taïwan et nous avons développer une méthode par le réactif TCT/DMF pour la préparation de α-chlorure de glycosides. Cette condition est compatibile avec diverses fonctions sensibles aux acides. L'application en chimie des sucres par une stratégie 'one-pot' a été examiné.

La troisième partie: Dans le projet TCT/DMF, nous avons constaté que le DMF est très important pour la sélectivité α de la glycosylation. Des études mécanistiques ont montré la formation intermédiaire d'anion glycosyl iminium.

La dernière partie: Nous avons aussi appliqué cette méthode de glycosylation α -sélective à la synthèse d'oligosaccharides.

謝誌

此論文得以順利完成，絕非僅靠個人的努力達成目標，在其背後，有太多需要感謝的人，這些人除了在實務上與精神上的支持，更是幫助我闖過層層困難良師益友，因為有這一群人，此論文才得以產生。

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TABLE OF CONTENTS

LIST OF FIGURES

Chapter 1

Chapter 2

Chapter 3

LIST OF TABLES

Chapter 1

Chapter 2

Chapter 3

LIST OF SCHEMES

Chapter 1

Chapter 2

Chapter 3

Chapter 4

LIST OF ABBREVIATIONS

Ac	acetyl
Ac_2O	acetic anhydride
AgOTf	silve trifluoromethanesulfonate
All	allyl
Boc	*tert*-butyloxycarbonyl
Bn	benzyl
bs	broad singlet
Bz	benzoyl
Bu	butyl
CH_3CN	acetonitrile
c	concentration
cat.	catalytic
CIP	contact ion-pair
Cq	quarternary carbon atom
CSA	camphor sulfonic acid
δ	chemical shift
d	doublet
dba	dibenzylideneacetone
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCE	dichloroethane
DCM	dichloromethane

DCMME	α-dichloromethyl methyl ether
dd	doublet of doublets
DMA	*N*,*N*-dimethyl acetamide
DMAP	4-dimethylaminopyridine
DMF	*N*,*N*-dimethylformamide
DAMA	*N*-acetyl acetamide
DMSO	dimethylsulfoxide
DN	donacity
dppb	bis(diphenylphosphino)butane
dppe	bis(diphenylphosphino)ethane
DPS	diphenylsulfoxide
DTBMP	2,6-di-*tert*-butyl-4-methylpyridine
equiv.	equivalent
EDCI	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide
ESI	electrospray ionization
Et	ethyl
EtCN	propionitrile
EtOAc	ethyl acetate
Et_3N	triethylamine
Fmoc	9H-fluoren-9-ylmethoxycarbonyl
h	hour
Hex	hexane
HMPA	hexamethylphosphoramide
HMPT	hexamethylphosphoric triamide

HOBT	hydroxybenzotriazole
HRMS	high resolution mass spectrometry
Hz	Hertz
IAD	intramolecular aglycon delivery
IDCP	iodonium di-collidine perchlorate
IR	infrared spectroscopy
J	coupling constant
KHMDS	potassium hexamethyldisilazide
LG	leaving Group
m	multiplet
M	molar
min	minute
mp	melting point
Me	methyl
MS	molecular sieves
n	normal
NANA	*N*-acetylneuraminic acid
NBS	*N*-bromosuccinimide
Neu5Ac2en	5-acetamido-2,6-anhydro-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-enoic acid
NIS	*N*-iodosuccinimide
NMM	*N*-methylmorpholine
NMR	nuclear magnetic resonance
NuH	nucleophile

PG	protecting group
Ph	phenyl
PMB	*p*-methoxybenzyl
ppm	parts per million
PTSA	*p*-toluenesulfonic acid
Py	pyridine
R_f	retardation factor
RT	room temperature
s	singlet
SSIP	solvent-separated ion pair
t	tertiary
t	triplet
TBAB	tetra-n-butylammonium bromide
TBAF	tetra-n-butylammonium fluoride
TBS	tert-butyldimethylsilyl
TBSOTf	tert-butyldimethylsilyl methanesulfonate
TCT	2,4,6-trichloro-1,3,5-triazine
tert	tertiary
TFA	trifluoroacetic acid
Tf_2O	trifluoromethanesulfonic anhydride
TfOH	trifluoromethanesulfonic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl

TMSOTf	trimethylsilyl methanesulfonate
TMU	1,1,3,3 tetramethylurea
t.o.f	turn over frequency
VH	Vilsmeier-Haack

Chapter 1

Regio- and stereoselective Pd(0)-catalyzed allylic substitution of sialic acid derivatives

1. Introduction

1.1. General information of influenza virus and the related therapies

Very recently, the new influenza A (H1N1) reassorted from the swine viruses first emerged in North America in February 2009. The virus has readily spread in other regions and become the worldwide pandemic. Generally, its higher mortality than seasonal influenza may be explained because this new strain virus is capable of affecting the lower respiratory tract and cause potential severe syndromes in some serious cases.[1] In the human history, three influenza pandemics occurred in the 20th century and killed tens of millions of people, with each of these pandemics being caused by the appearance of a new strain of the virus in humans. Often, these new strains result from the spread of an existing flu virus to humans from other animal species. Moreover, a deadly avian strain of H5N1 has posed the greatest risk for a new influenza pandemic since it first killed humans in Asia in the 1990s.[2]

Although vaccination is the primary strategy for the prevention of influenza, an antigenic drift in the virus may occur after the seasonal formulation of vaccine has been used, rendering the vaccine less protective. Furthermore, vaccine production by current methods cannot be produced with the speed required to halt the progress of a new strain of influenza virus; therefore, it is likely that vaccine would not be available for the first wave of spread of virus.

According to the infection and replication cycle of influenza viruses, several anti-viral

agents were developed against spreading of viruses in different stages (Figure 1). These antiviral drugs are able to affect the virus itself and may be used for either prevention or treatment.

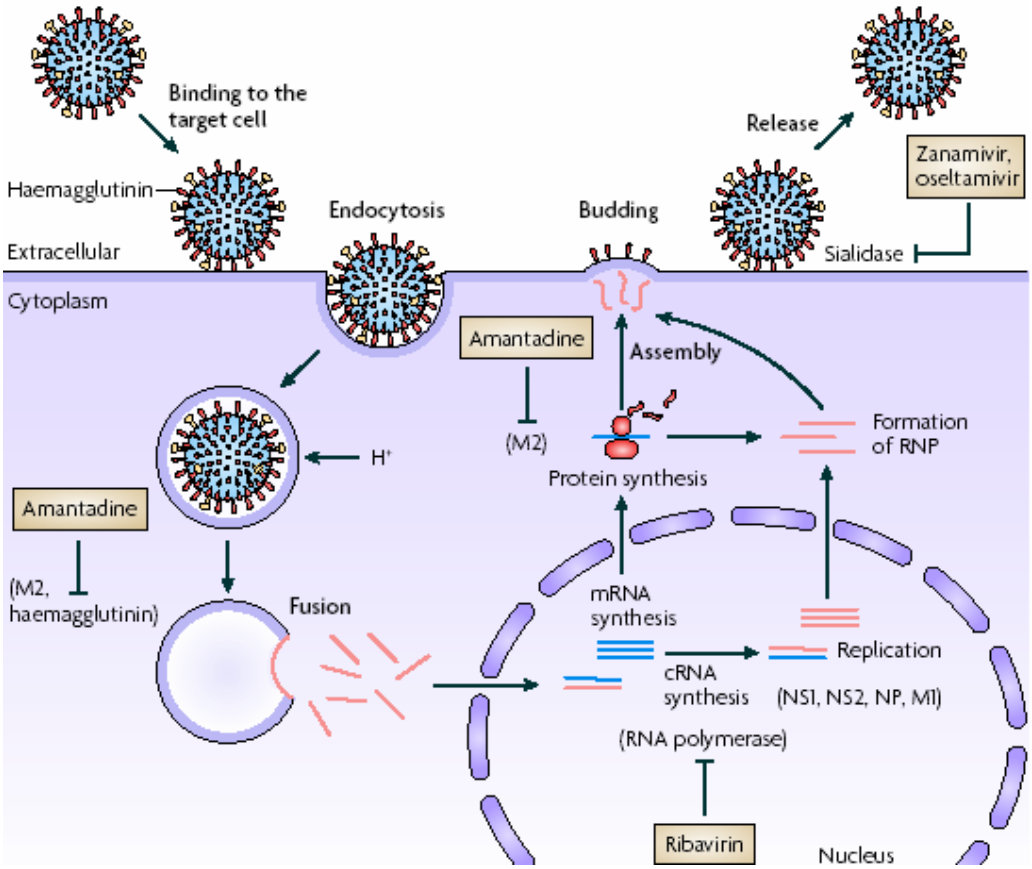
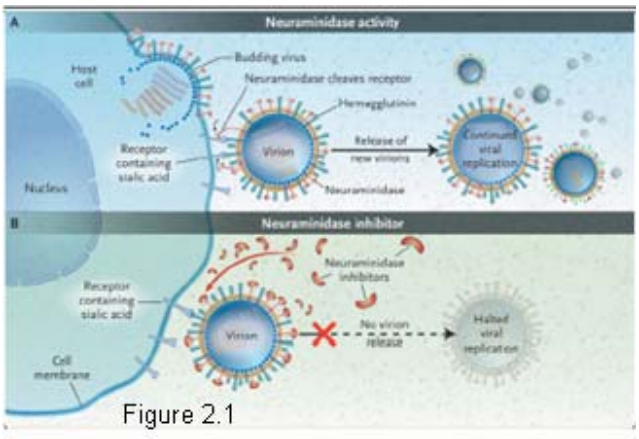


Figure 1 - Possible pathways for preventing the infection of influenza viruses

Neuraminidase (NA) is a glycohydrolase enzyme (EC 3.2.1.18) which catalyzes the cleavage of terminal sialic acid α-ketosidically linked to antigenic glycoproteins on the surface of the influenza virus. This action can prevent aggregation of viruses and promote the release of progeny viruses from infected cells. Administration of chemical inhibitors of neuraminidase is a treatment that limits the severity and spread of viral infections (Figiure 2.1).[3]

Hemagglutinin (HA) is a homotrimeric glycoprotein found on the surface of the influenza viruses which possesses the three binding sites. It is responsible for binding the virus to the

terminal sialylated glycoprotein on the membrane surface of the host cell followed by embedding into the cell (Figure 2.2).[4]



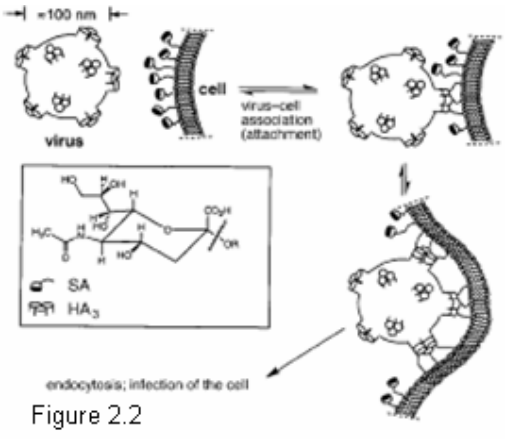


Figure 2 - Intervention of neuraminidase inhibitors and process of anchoring to host cell through HA

The subtypes of influenzas are characterized and named according to the type of nuclear materials, the origin of area, and different groups of hemagglutinin (HA) and neuraminidase (NA), such as the stereotype of influenza A viruses, found in Fujian, China in 2002, hence named as A/Fujian/411/2002/H3N2 (Figure 3).

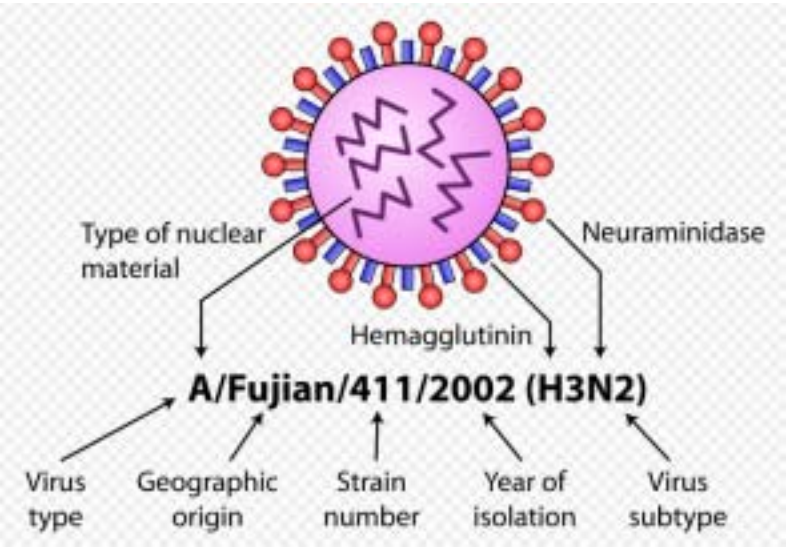


Figure 3 – Nomenclatures of different subtypes of influenza virus

Among these existing therapeutic treatments, the neuraminidase inhibitors are

particularly effective and convenient. As a mater of fact, 2,3-unsaturated *N*-acetylneuraminic acid **2** (5-acetamido-2,6-anhydro-3,5-dideoxy-D-glycero-D-galacto-non-2-enoic acid, Neu5Ac -2en) was first discovered by Meindl and Tuppy in 1969 as a potent neuraminidase inhibitor (Figure 4).[5-7] According to the enzyme-based rational design mimicking the cleaved intermediate **3**, inhibitors towards neuraminidase of viruses were discovered and developed as the two anti-influenza drugs currently used to treat infected patients, Zanamivir **1** (Relenza®) and Oseltamivir phosphate **4** (Tamiflu®).[8-10]

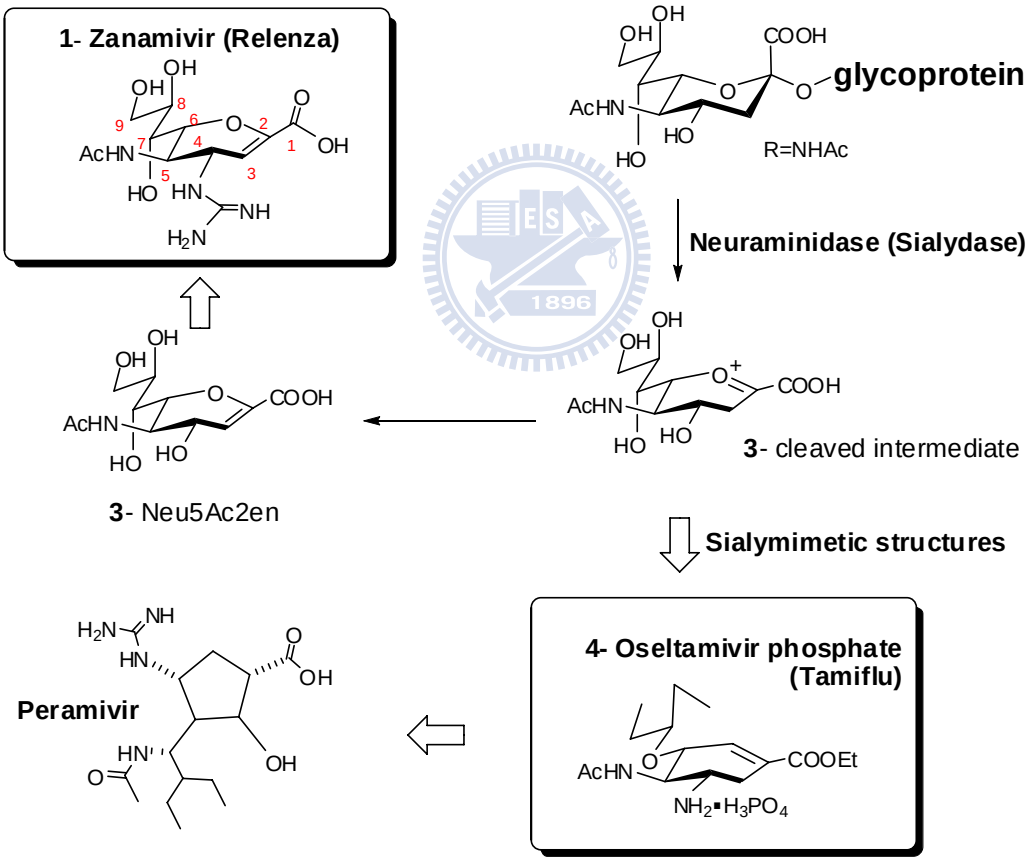


Figure 4 - Design of neuraminidase inhibitors mimicking the cleaved intermediate **3**

Even though the anti-influenza drugs are available for the treatment, the emergence of drug resistance to oseltamivir[11,12] still occurred recently and caused the humans health risk.

Crystal structures of oseltamivir-resistant influenza virus neuraminidase mutants have been reported by Collins *et al.* in UK.[13] They demonstrated that the hydrophobic pentyloxy group of oseltamivir is an influential group. To bind with pentyloxy group of oseltamivir, the carboxyl group of Glu 276 needs a conformational change (Figure 5-c). In contrast, the polar glycerol group of Zanamivir has the tight affinity with Glu276 through hydrogen bonding (Figure 5-d). However, the mutated NA bearing His294Try residue affects the binding between the side chain of oseltamivir and Glu276 (Figure 5-f). That is a key factor which resulted in the drug resistance of oseltamivir. Therefore, the development of next-generation chemotherapeutic agents derived from Zanamivir would be advantageous instead of oseltamivir analogues.

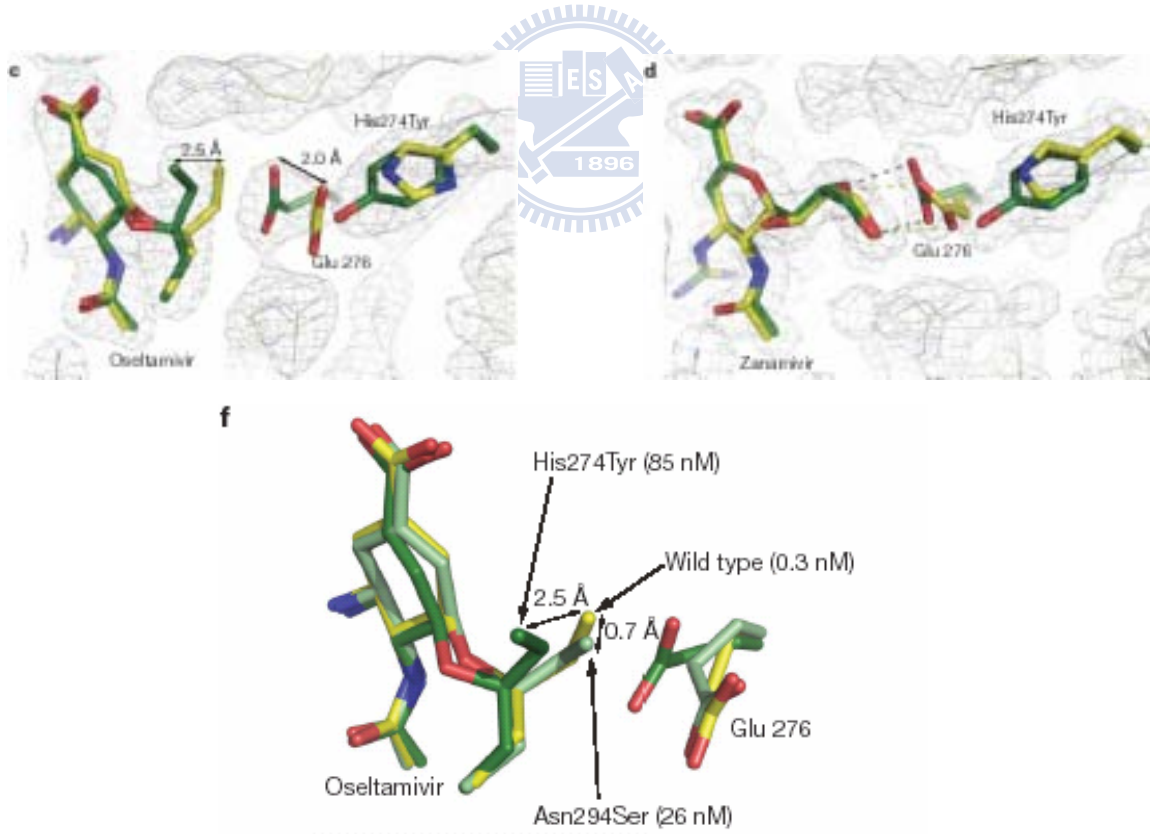


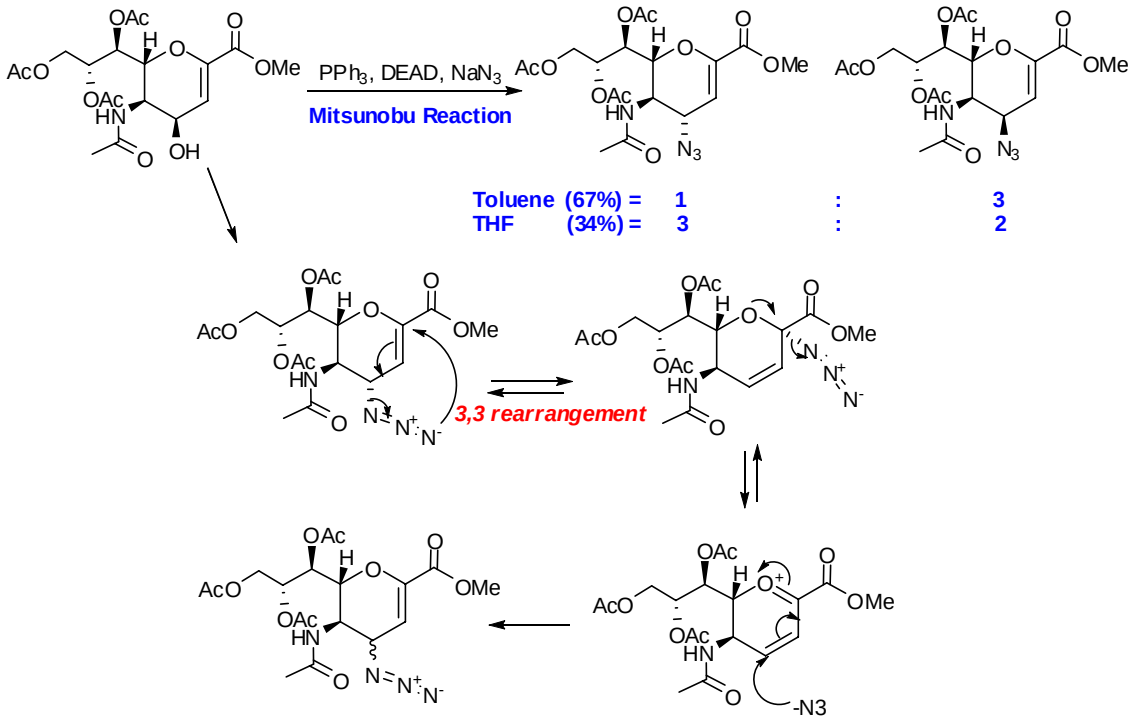
Figure 5 – The binding of inhibitors with oseltamivir-resistant influenza virus neuraminidase

1.2. Chemical synthesis of neuraminidase inhibitors from Neu5Ac2en

In 1993, von Itzstein *et al.* developed the anti-influenza drug, Zanamivir **1** (Relenza®) based on the structure-basis rationale involving the probable sialosyl cation transition-state intermediate.[14] Afterwards, a large number of sialymimetic analogues were synthesized and evaluated for probing the biological binding activity and indeed encouraged the current drug design.[15,16] In this dissertation, we will focus on the synthetic strategies derived from Neu5Ac2en.

1.2.1. The first synthesis of 4-azido derivatives using Mitsunobu Reaction

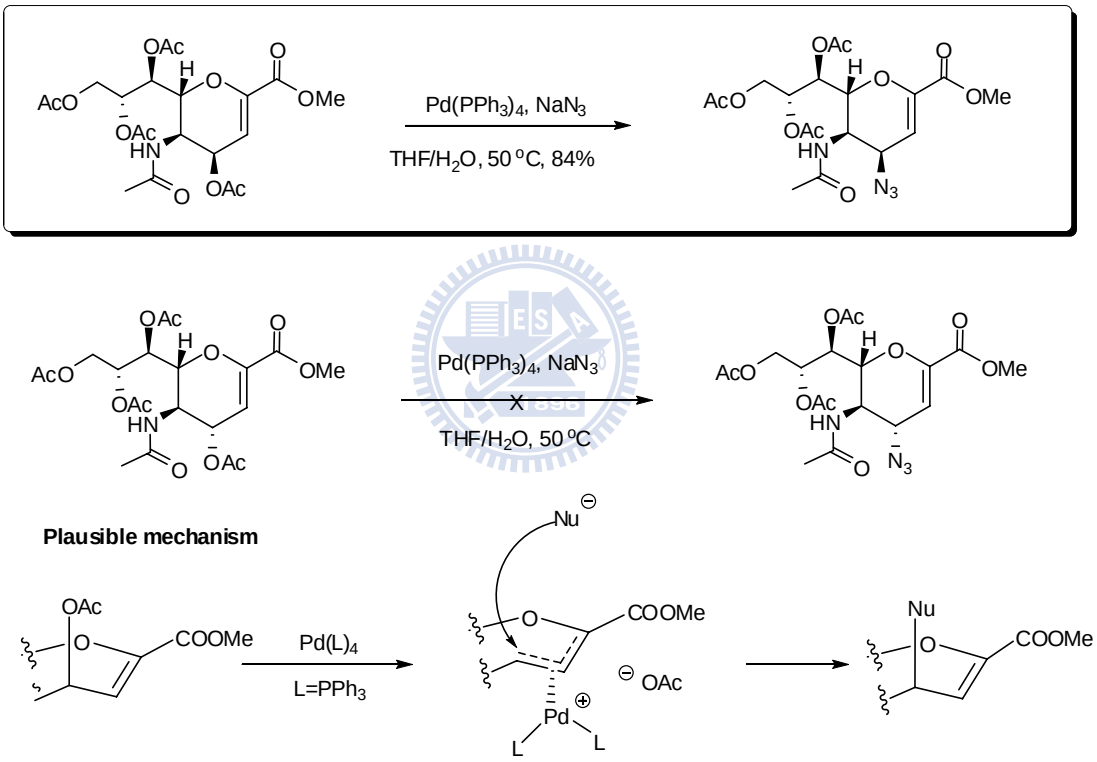
The first chemical synthesis for the azido derivatives of Neu5Ac2en has been reported by Schauer *et al.* in 1991.[17] The azido product suffered from 3,3 sigmatropic rearrangement and lost its chirality at C-4 (Scheme 1).



Scheme 1 - The first chemical synthesis for the azido derivatives of Neu5Ac2en

1.2.2. The first synthesis for 4-epi-4-azido-Neu5Ac2en using Pd(0)

The pioneering attempt to use the organometallic catalyst in synthesizing the analogue of Neu5Ac2en was conducted by von Itzstein *et al.* in 1997. However, only epi-Neu5Ac2en allowed to be accessed by this method.[18] They demonstrated that a pseudo-axially disposed acetate better facilitate the initial oxidation addition of the allyl acetate to Pd(0) (Scheme 2).

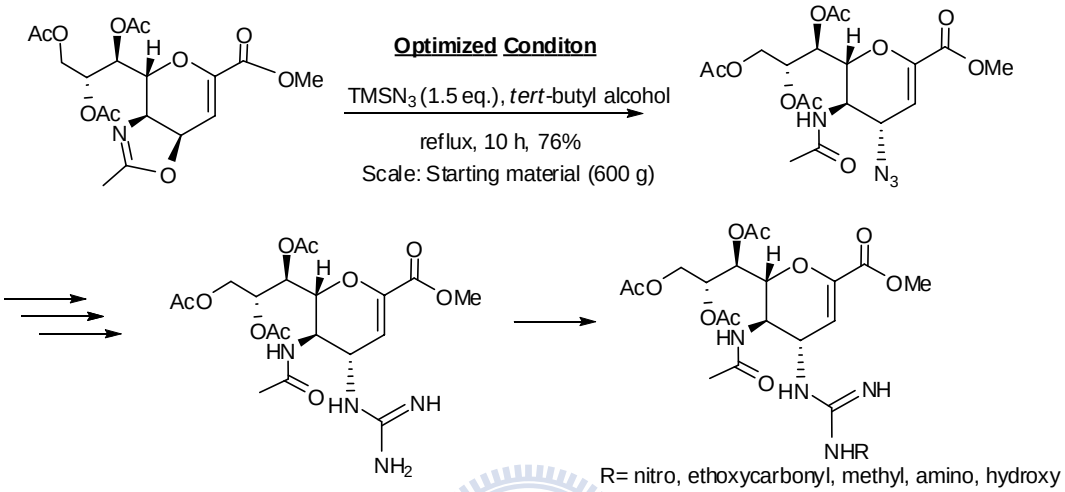


Scheme 2 - The first synthesis of 4-epi-4-azido-Neu5Ac2en using Pd(0)

1.2.3. Optimized condition for 4-azidation of Neu5Ac2en and activity evaluation of the 4-guanidino Neu5Ac2en derivatives

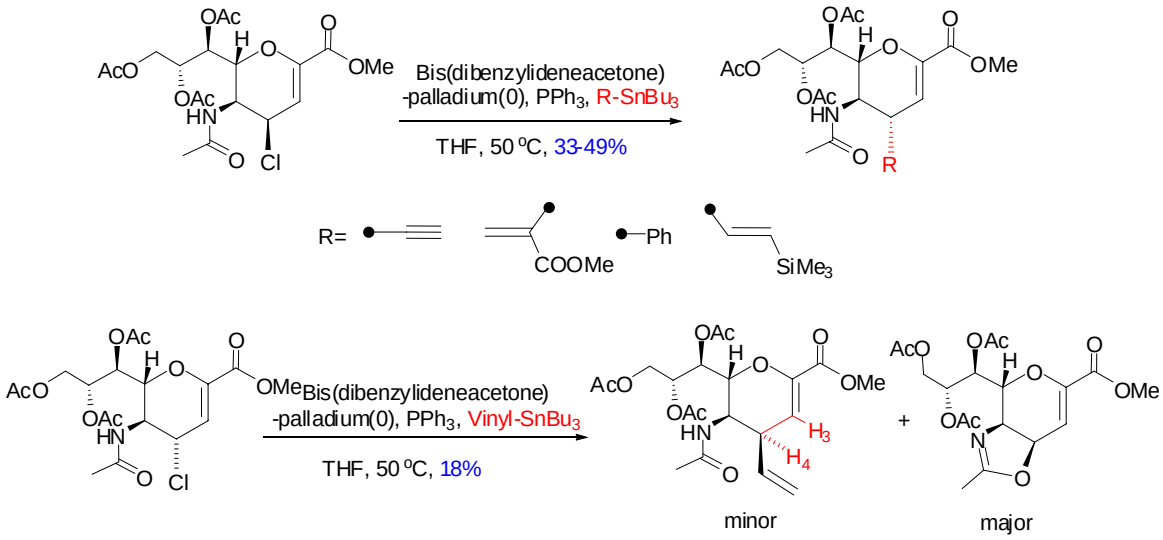
A number of derivatives of 4-guanidino Neu5Ac2en have been tested for enzyme

inhibition. None of them showed a better inhibitory activity than Zanamivir. It is worth of note that the optimized procedure for synthesizing the 4-azido Neu5Ac2en has been scaled up to a large quantity (600 g) (Scheme 3).[19]



Scheme 3 - Optimized condition for azidation of Neu5Ac2en

1.2.4. C4 – Analogues synthesis from 4-chloro Neu5Ac2en at C-4 by Stille coupling

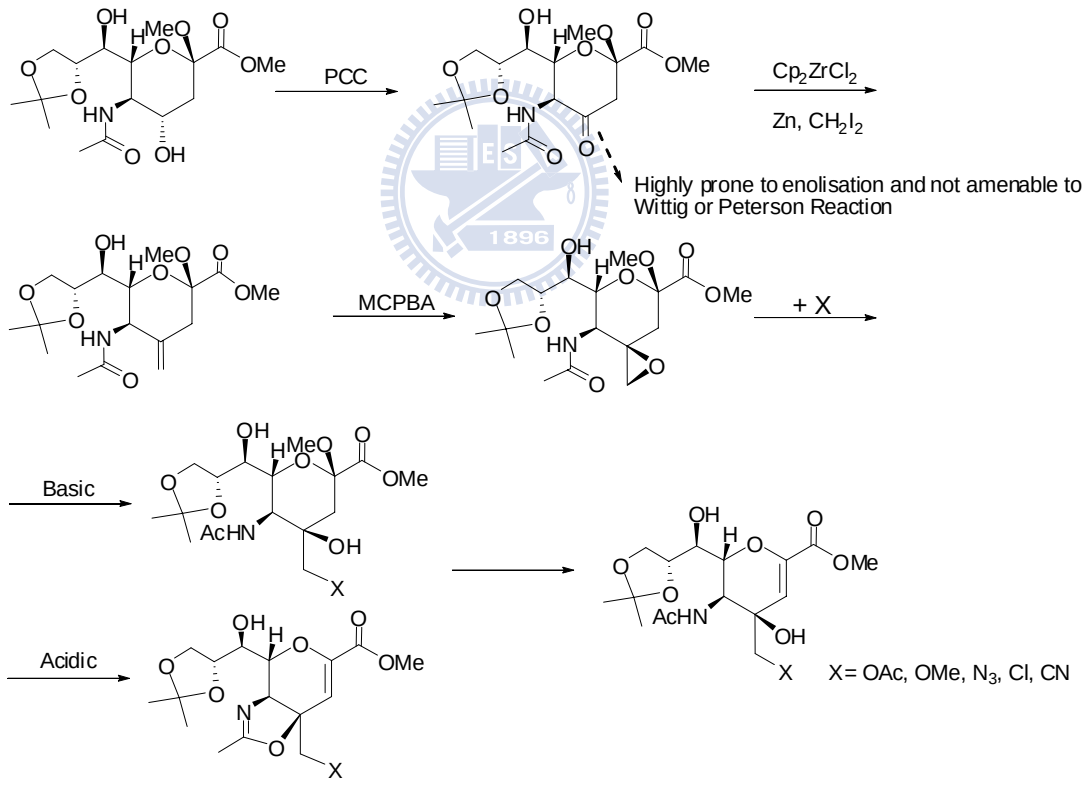


Scheme 4 - Analogues synthesis from 4-chloro Neu5Ac2en

Kok and von Itzstein developed an organometallic-mediated approach (Stille coupling) for the stereoselective C-C bond formation at C-4. However, they emphasized that the instability of the chlorosubstrate would be a problematic issue. Moreover, the excessive use of toxic stannyl reagents limits the scope of this method (Scheme 4).[20, 21]

1.2.5. Methylenation of ketone to the disubstituted analogues at C-4

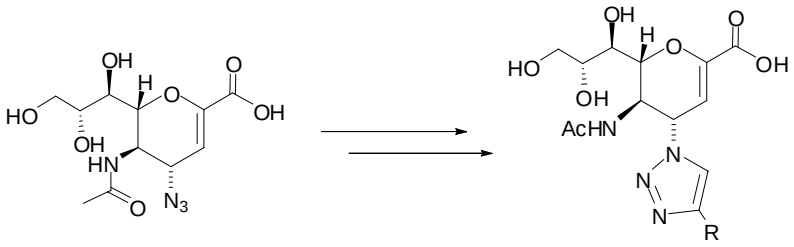
The serial studies for synthesizing C-4 disubstituted derivatives of Neu5Ac2en from the olefinated moiety have been also reported by von Itzstein's group (Scheme 5).[22, 23]



Scheme 5 - Methylenation of ketone to the disubstituted analogues at C-4

1.2.6. Derivatives synthesis of 4-azido Neu5Ac2en derivatives– using Click chemistry

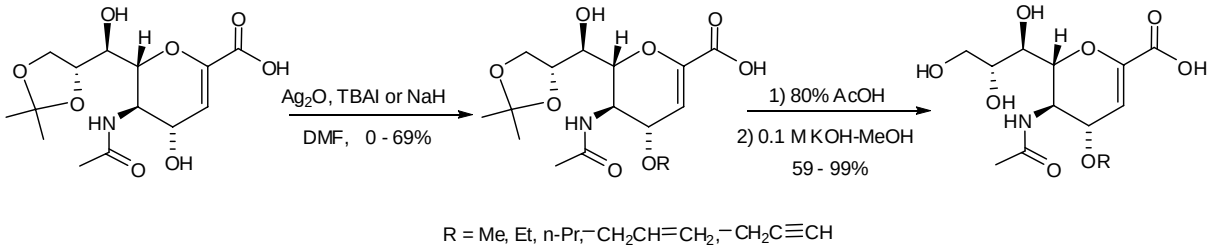
Several Zanamivir analogues with different substituted triazoles derived from 4-azido NeuAc2en using click chemistry has been described by Zuo *et al.* and also evaluated in anti-avian influenza virus (H5N1) activities, which showed the comparable inhibition with Zanamivir **1** (Scheme 6).[24]



Scheme 6 - The derivatives synthesis using Click chemistry

1.2.7. Modification of Neu5Ac2en at C-4 for the inhibition evaluation of hPIV-1

Human parainfluenza virus type 1 (hPIV-1) is an important pathogen causing upper and lower respiratory disease in infants and young children. Referred to the reported information of enzyme interaction, a serial of C-4 modified inhibitors of Neu5Ac2en showed the superior inhibitory activity than Zanamivir (Scheme 7). This finding implied that these analogues showed the different binding affinity with neuraminidases from the different strain of virus.[25]



Scheme 7 - C4 – Modification of Neu5Ac2en at C4

1.2.8. Analogues derived from other positions

Modifications of Neu5Ac2en at C-4, 5, 6, 7, 8, 9 - The truncated 6-glycerol substituted series related to Zanamivir also contributed the significant binding information with neuraminidase.[13] Variations at other positions, such as C-5, C-7, C-8, C-9 were also investigated for the inhibitory activity against neuraminidase (Figure 6).

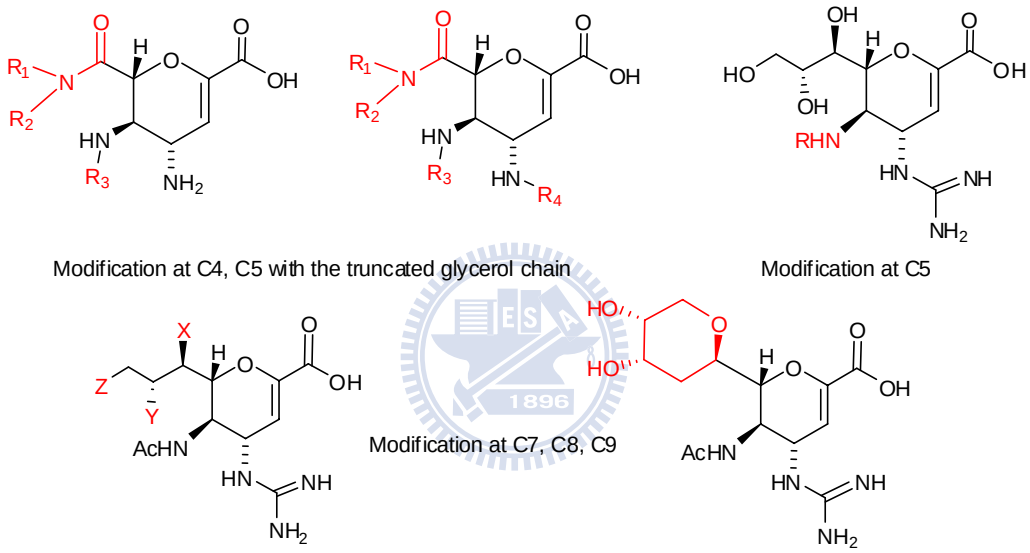


Figure 6 - Modifications of Neu5Ac2en at C4, 5, 6, 7, 8, 9

1.2.9. Polymeric sialosides

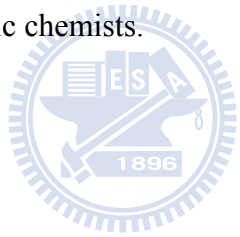
Multivalent compounds derived from C-4 - Polymeric 4-*N*-linked sialoside - Polymeric sialosides have been synthesized and acted as multivalent inhibitors of hemagglutinin.[26]

Multivalent compounds derived from C-7 - Polymeric 7-*O*-linked sialoside - All polymeric sialosides displayed less potent activity for the inhibition of influenza A sialidase. However, a much greater *in vivo* efficacy against influenza A in the animal mice model by intranasal

administration than Zanamivir was reported.[27, 28]

Multivalent compounds derived from C-7 - Polymeric , tri- or tetrameric 7-*O*-linked sialoside - The X-ray crystal structure of Zanamivir bound in the influenza NA active sites shows that 7-OH group is not involved in any significant interactions. Some polymeric sialosides designed by Krippner *et al.* and derived from C-7 showed the prolonged efficacy due to the slow lung clearance of high molecular weight (500 kD) compounds.[29, 30]

After a survey of the recent reports regarding the synthesis of neuraminidase inhibitors, many versatile molecules were built up to address either *in-vivo* or *in-vitro* potency against the influenza viruses. However, the new synthetic routes to prepare the novel constructs and more economic direct-access approach derived from each position of sialic acid are still challenging and of the great interest for the organic chemists.



1.3. Our initial approach

Pd(0)-catalyzed allylic substitution is a powerful tool for the different types of bond formation, such as C-C, C-N, C-S, C-O, C-P, and widely applied in asymmetric synthesis in the past decades.[31-33] Although certain synthetic methodologies using organo- metallic catalysts, such as the Stille coupling mentioned above, have been reported on Neu5Ac2en, we envisaged that the starting substrate with the different protecting or functional groups probably give us certain advantages in terms of reactivity and selectivity. Our initial idea was to synthesize the protected substrate and test its feasibility in Pd(0)-catalyzed substitution, also named "Tsuji-Trost" reaction. The successive formation of Pd π-allyl complex would be a key intermediate for the later nucleophilic substitution (Figure 7).

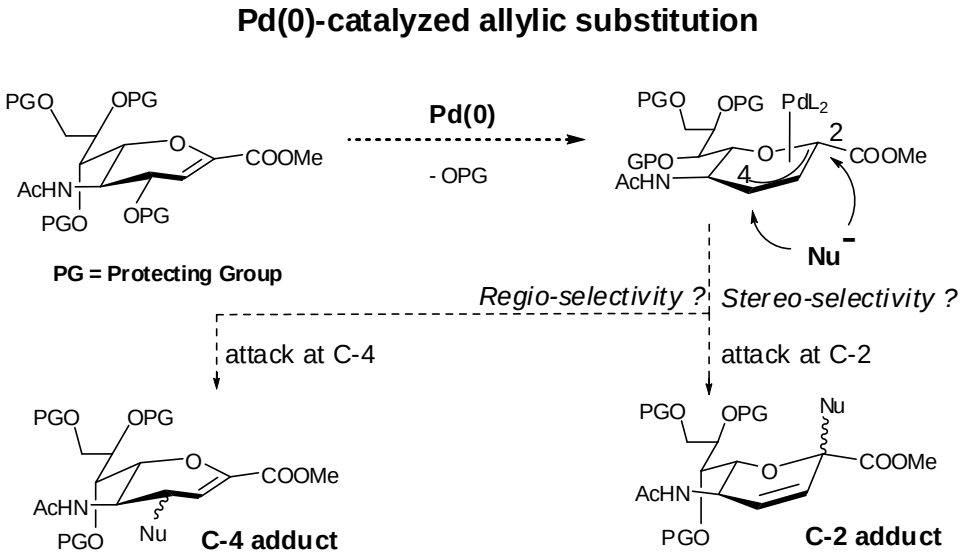


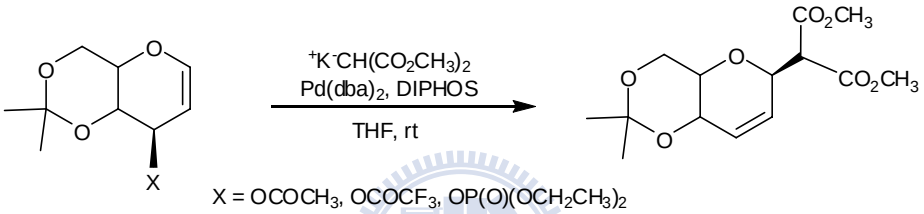
Figure 7 - Pd(0)-catalyzed allylic substitution on Neu5Ac2en

Before proving this concept, the outcome in reactivity, regioselectivity and stereoselectivity is almost unpredictable due to the functional complexity for this type of sugar substrate. However, similar strategies using palladium catalyst on the pyranose systems have been developed for *C*- and *O*-glycosylation on the relatively simple structures. Those results will be summarized in the later section and expected as an useful information to guide us for the following investigation.

1.3.1. Earlier methods: Pd(0)-catalyzed *C*- and *O*-glycosylation on sugars

Pd(0)-catalyzed *C*-glycosylation

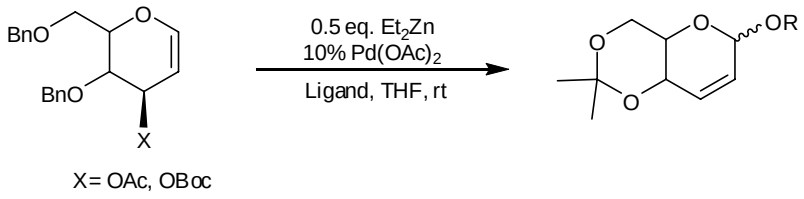
RajanBabu *et al.* reported a general method for Pd(0)-catalyzed *C*-glycosylation on glycols.[23] Several *C*-glycosides derived from C-1 were easily accessed in excellent regio- and stereoselectivity due to the anomeric stabilization in the pyranose ring (regioselectivity) and the preferential substitution from the opposite face of π-allyl Pd complex (stereoselectivity) (Scheme 8).



Scheme 8 - Pd(0)-catalyzed *C*-glycosylation at anomeric carbon

Pd(0)-catalyzed *O*-glycosylation

Lee *et al.* developed a new method for *O*-glycosylation of glycal with the aid of zinc(II) alkoxide under Pd(0)-mediation.[34] The variation of α/β stereoselectivity is dependant of the different ligand used (Scheme 9). The mechanistic explanations for the excellent selectivity have not been mentioned in this context.



Scheme 9 - Pd(0)-catalyzed *O*-glycosylation

Pd(0)-catalyzed *O*-glycosylation and it's application in oligosaccharide synthesis

Feringa *et al.* also described a Pd(0)-catalyzed approach for *O*-glycosylation in enone-pyranose system. The property of enone enables the following *O*-glycosylations.[35] Interestingly, at almost the same time, O'Doherty *et al.* published similar results using a lower Pd catalyst loading and further applied this method for the total synthesis of anthrax tetrasaccharide (Figure 8).[36]

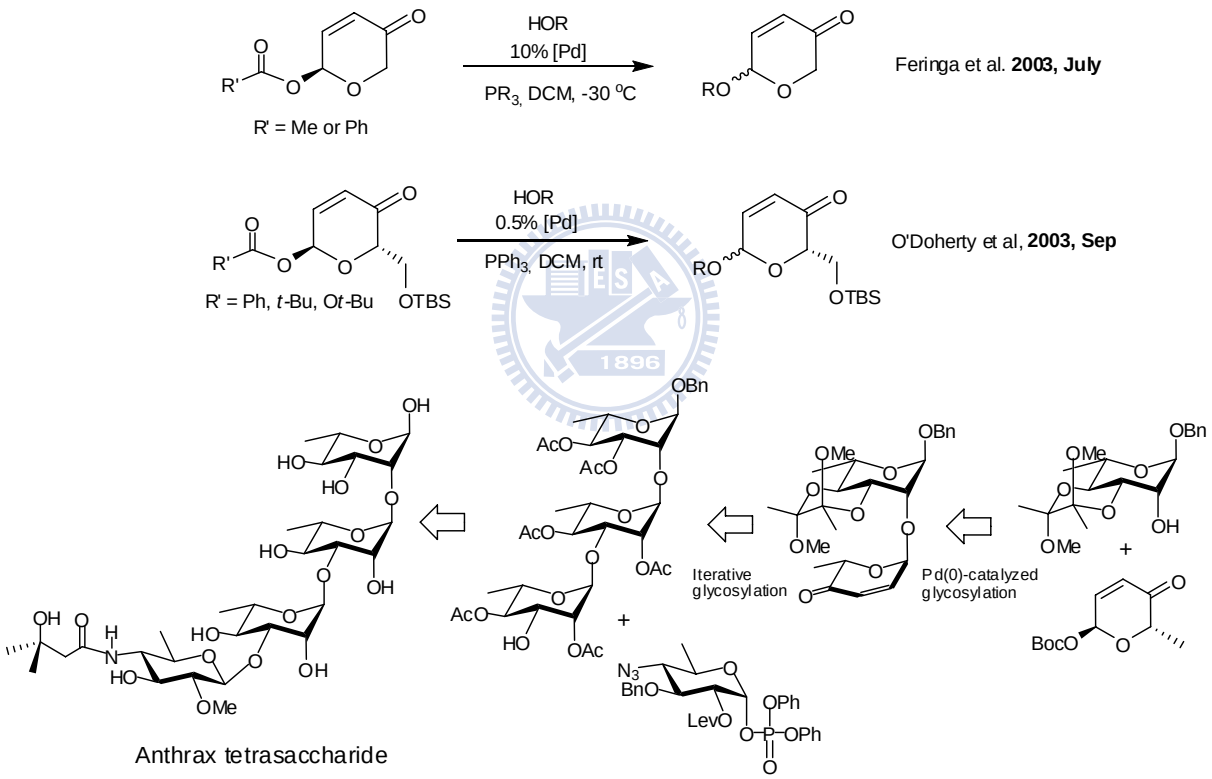


Figure 8 - Pd(0)-catalyzed *O*-glycosylation and it's application in oligosaccharide synthesis

Pd(0)-catalyzed regioselective allylic substitution induced by a carbonyl group

In the enone system, the excellent regioselectivity at the γ carbon with *C*-, *N*-, and *O*-nucleophile using Pd(0)-catalyzed allylic substitution approach is attributed to the electron-withdrawing property of carbonyl group. The other stereoisomer run through

π–α–π rearrangement can be converted to the same reactive species in line with the geometry of intermediate complex, thus leading to the same product (Figure 9).[37]

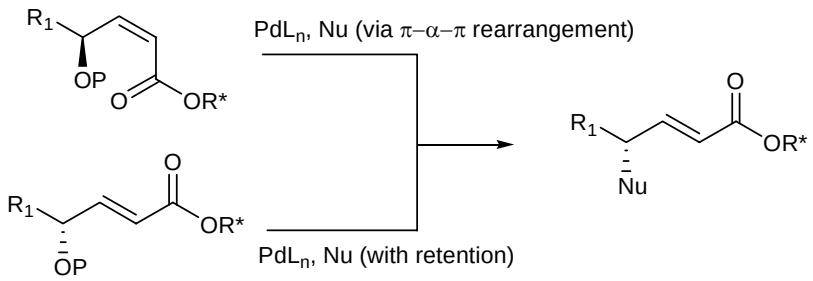


Figure 9 - Pd(0)-catalyzed regioselective allylic substitution

1.3.2. Paradox in regioselectivity - Pd(0)-catalyzed substitution in Neu5Ac2en

The unsaturated Neu5Ac2en bearing carboxylate moiety at C-1, an acetamido group at C-5, and a glycerol chain at C-6 provides a unique electronic environment, usually make synthetic endeavors less productive, such as the low yield in *O*-sialylation.[38] In general, the electron-withdrawing property of carbonyl at carboxylate group reduces electron density at C-4 and further induce the regioselectivity at C-4 position. In contrast, more electronegative oxygen in pyranose ring could stabilize the anomeric cation to facilitate the attack of the incoming nucleophile (Figure 10).[39] Apparently, two types of forces from oxygen and carboxylate seem opposite to each other and possibly cause regio-discrimination and even diminish the reactivity as well. Besides, unexpected factors may be arising from other functionalities, such as the acetamide at C-5 and substitutents at the glycerol chain, could make the entire process more complicated.

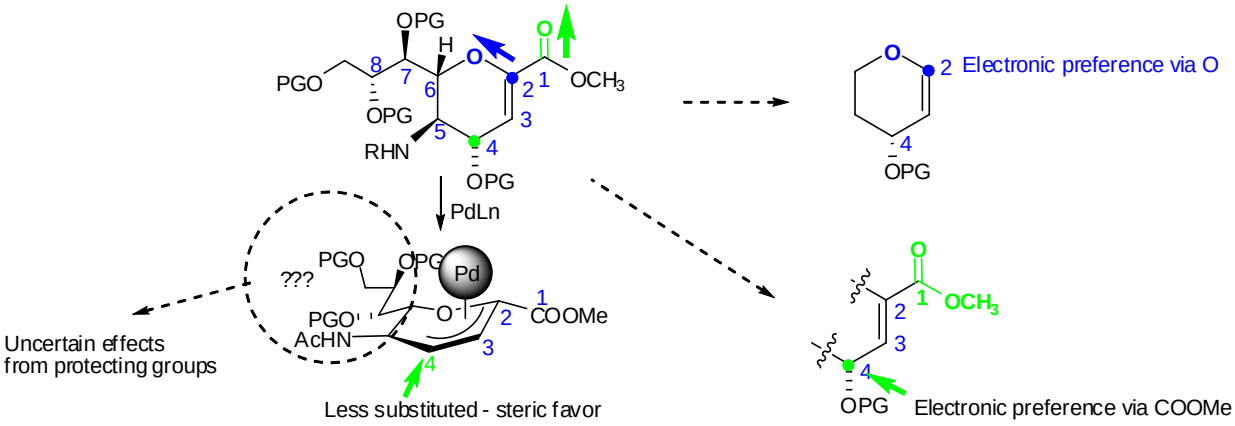


Figure 10 - Paradox in regioselectivity - Pd(0)-catalyzed substitution in Neu5Ac2en

1.3.3. General catalytic cycle in Pd(0)-catalyzed allylic substitution (Tsuji-Trost reaction)

Palladium-catalyzed allylic substitution is a versatile process. η3-allylpalladium complexes were first isolated and identified over 30 years. In 1965, Tsuji first disclosed the bond forming involved π-allylpalladium complex with certain nucleophiles.[40,41] Afterwards, numerous mechanistic studies and applications regarding to this approach were profoundly investigated in regioselectivity, enantioselectivity and a wide range of substrates and their nucleophilic partners. From Pd(0) catalylic cycle (Figure 11), substrates bearing the different leaving groups in combination with the diverse Pd(0) sources, ligand (mono/bidentate/chiral/ hetero), types of nucleophiles (measured by pKa or steric dimension) and counter ions under different concentrations, solvent and temperature usually influence the distinct scenario in regioselectivity and enantioselectivity.[42-45] Among these parameters, the derivatives of highly-functionalized Neu5Ac2en possess an unique electronic and steric environment probably inducing more difficulties in organometallic-mediated reaction. For examples, interconversion of intermediate complex, uncertain interactions between substrates, metals

and ligands may cause problems during the reaction course.

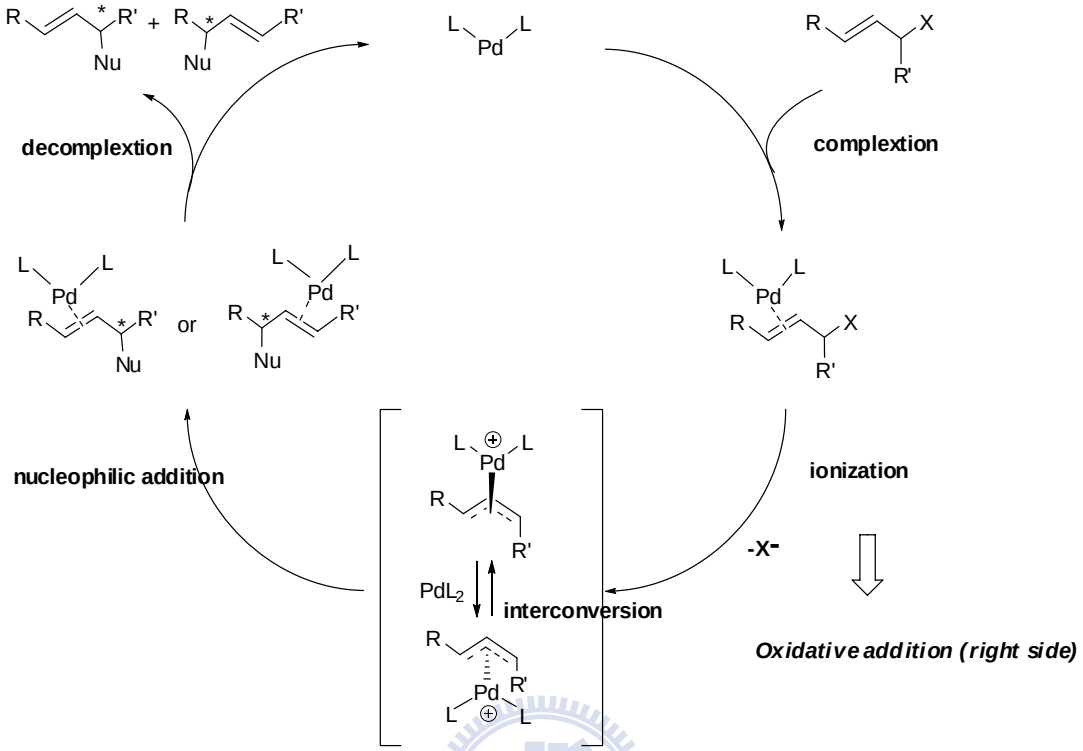
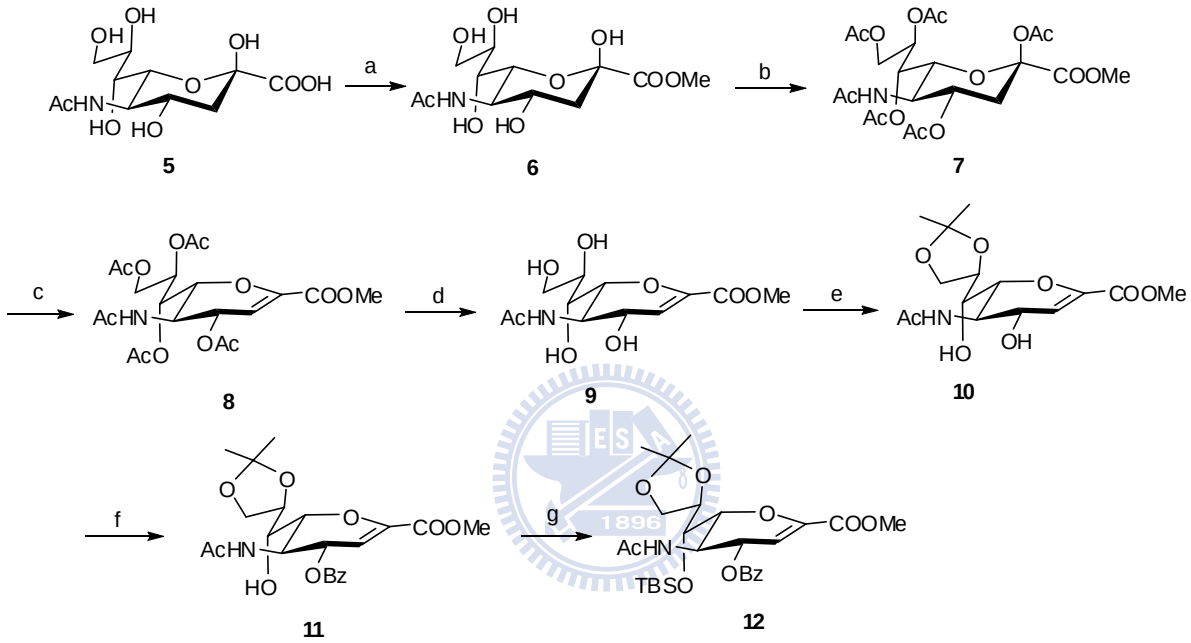


Figure 11 - General catalytic cycle in Pd(0)-catalyzed allylic substitution

2. Results and discussion

2.1. Substrate synthesis



Reagent and conditions : a) Dowex 50 H^+, MeOH, rt, 24 h, 99%; b) Ac_2O, 0 °C to rt, 24 h, 99%; c) TMSOTf (2.5 equiv.) AcOEt, 0 °C to rt, 4 h, 89%; d) NaOMe, MeOH, rt, 30 min; e) CSA, 2,2-dimethoxypropane, acetone, reflux, 1-2 h; f) BzCl, pyridine, DCM, 0 °C, 2 h , 72% for the 3 steps; g) TBSOTf, DCM, lutidine, rt, 18 h, 88%.

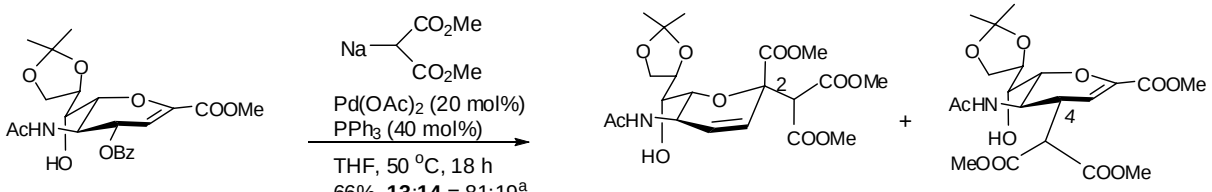
Scheme 10 - Synthetic route of substrates

For the purpose of this study, the substrate synthesis started from sialic acid **5** to proceed in esterification with methanol followed by acetylation in the presence of pyridine and acetic anhydride.[19] The resulting per-acetylated methyl ester **7** was treated carefully with trimethylsilyl triflate (TMSOTf) in ethyl acetate to induce β-elimination to obtain the unsaturated derivative **8** in an excellent 89% yield. Zemplén de-*O*-acetylation followed by 8,9-isopropylidenation furnished a diol **10**,[46] which was subsequently selectively protected with benzoyl group to furnish the benzoate **11** in 72% yield over 3 steps.[47] In order to prevent

the influence of the 7-OH group, **11** was further silylated with *tert*-butyldimethylsilyl triflate (TBSOTf) to give the fully-protected derivative **12**.[48]

2.2. Preliminary test using sodium dimethylmalonate as a nucleophile

In a first trial, the treatment with per-acetylated Neu5Ac2en **8** under Pd(0)-catalyzed allylic substitution conditions using freshly-prepared sodium dimethylmalonate as a nucleophile failed to give any desired product, and only starting materials were recovered quantitatively. This result agrees with the previous works reported by von Itzstein,[18] who undertook azidation under Pd(0)-mediation in use of Neu5Ac2en **7** (Section 1.2.2). Thus, we turned our attention to other substrates. In contrast, allylic benzoate **11** readily reacted with sodium dimethylmalonate in the presence of $Pd(OAc)_2$ (20 mol%) in combination with PPh_3 (40 mol%). The reaction, carried out at 50 ^oC for 18 h, provided regioselectively alkylated product **13** at C-2 on the α face of the sugar (C-2/C-4 ratio of 81:19, 66% yield; Scheme 11). Different palladium sources {$[Pd_2(dba)_3]$•$CHCl_3$, $[(allylPdCl)_2]$, etc.} associated with different ligands (PPh_3, PBu_3, dppb, etc.) did not significantly improve this transformation.

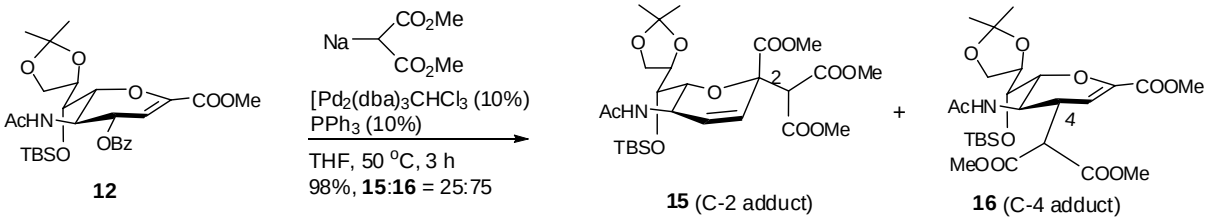


11 **13** (C-2 adduct) + **14** (C-4 adduct)

[a] The ratio of C-2/C-4 was determined by ^{1}H-NMR.

Scheme 11 - Allylic substitution of Neu5Ac2en derivative **11**

To our surprise, silylated allylic benzoate **12** provided the major alkylated product **16** at C-4 in an excellent 98% yield (C-2/C-4 ratio of 25:75, Scheme 12) under similar conditions. Before investigating these inverse regioselectivity between C-2 and C-4, exact configurations and conformations of these isomers **13**, **14**, **15** and **16** need to be characterized.



[a] The ratio of C-2/C-4 was determined by ^{1}H-NMR.

Scheme 12 - Allylic substitution of Neu5Ac2en derivative **12**

2.3 Characterization of isomers

2.3.1. Possible isomers

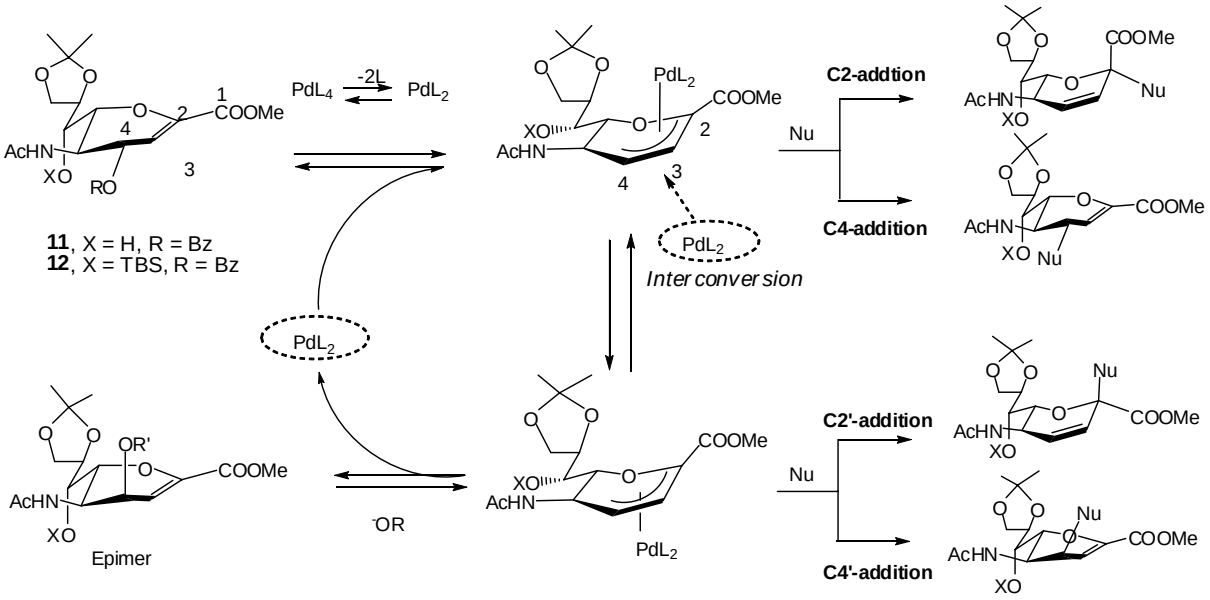


Figure 12 - Possible routes for the formation of isomers

Stereoselectivity of allylic substitution in use of metal-olefin complex is commonly modulated by the conformation of intermediate to form the "stereo-retention" product. In reality, the stability of the complex varies widely in the reversible catalytic cycle and finally leads the reaction to the state in the compromised energy level. As illustrated in Figure 11, the substrate is catalyzed by Pd(0) to form the intermediate complex which was called "complexation and ionization", followed by either undergoing interconversion to the "stereo-inverse" complex or directly coupling with the nucleophiles to achieve the "stereo-retention" product in line with the regio-dominance regulated by the electronic and steric factors of active species.

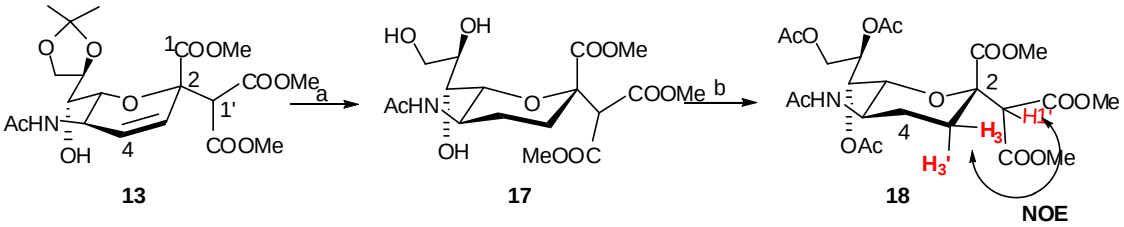
2.3.2 Characterization of 13 and 16

Configuration of two regioisomers **13** and **16** were determined using the relevant 2D-NMR (COSY, HMQC) in combination with DEPT and ^{1}H-NMR to assign carbons and protons.

Characterization of regioisomers (C-2 or C-4)

In terms of regioselectivity, the chemical shifts of the particular protons (**13**, H-3 = 6.2 ppm, H-4 = 6.0 ppm, H-5 = 4.6 ppm; **16**, H-3 = 6.0 ppm, H-4 = 3.5 ppm, H-5 = 3.7 ppm) provide the significant evidence to explain the alkylated position. It would be attributed to the allylic downshifting effect and further approved by hydrogenation of **13** with two additional methylene group (CH_2). Thereupon peracetylation of **17** was made to facilitate the observation of NOE. Unfortunately, the overlapped signals between H-3(axial) and H-3(equatorial) makes

the judgment more difficult in stereoselectivty of the nucleophilic attack (Scheme 13).

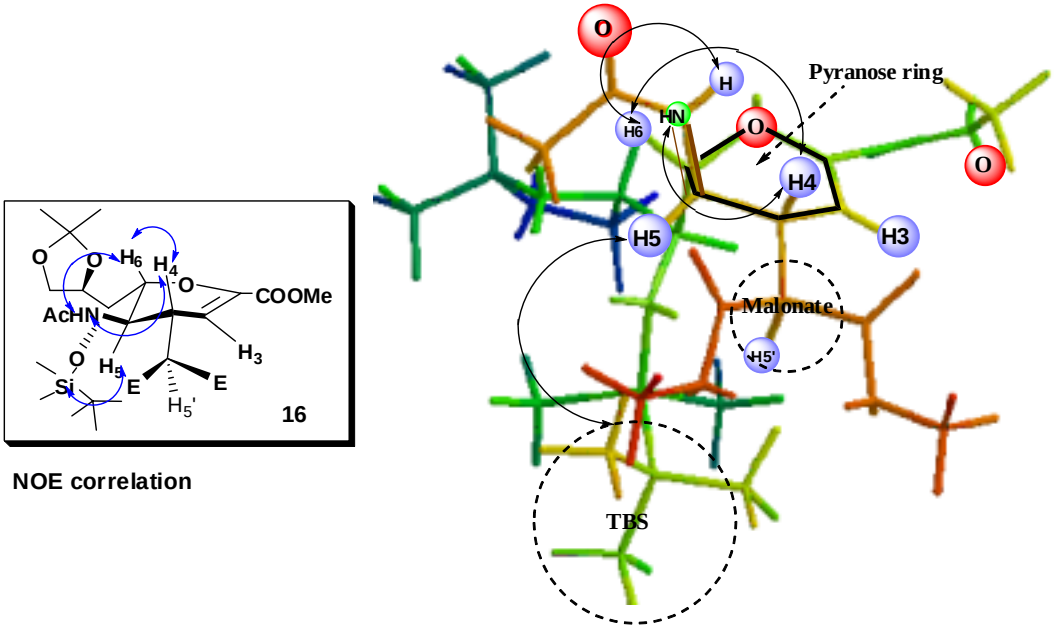


Reagent and conditions : (a) 5% Pd/C, H_2, MeOH, rt, 24h,; (b) Ac_2O, Py, rt, 80% (over 2 steps).

Scheme 13 - Characterization of regioisomers

Configuration characterization of 16 via coupling constant ($J_{H\text{-}H}$) and NOESY

To solve problem, based on the ^{1}H-NMR analysis of **16**, the coupling constant calculation of H-4, H-5 and H-6 can provide the crucial information to determine the absolute configuration in a direct manner. Therefore, the large coupling constants ($J_{H5,H4}$ = 10.0 Hz, $J_{H6,H5}$ = 10.0 Hz, $J_{H4,H5}$ = 10.1 Hz) which were respectively found in ^{1}H-NMR succinctly revealed the *trans* relationship between H-4, H-5 and H-6 (Figure 13).



The 3D energy-minimized drawing is created by Chem3D software

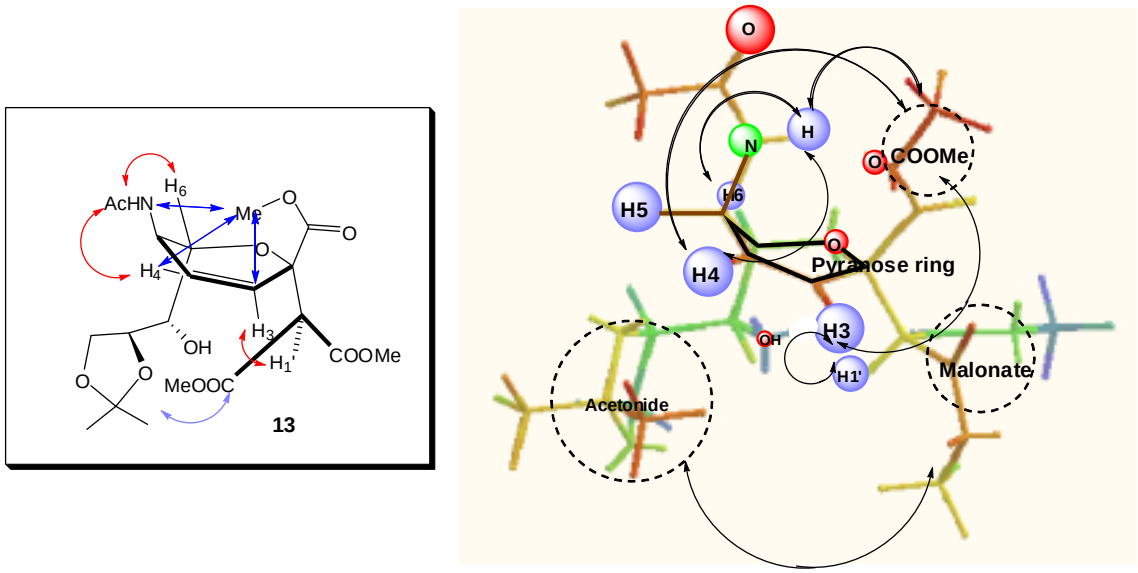
Figure 13 - NOE correlation of **16** and 3D diagram

In principle, two neighboring protons eclipsed with the larger dihedral angle correspond to a larger coupling constant. This relationship has been established by Karplus. Additionally, to reconfirm the assignment of configuration, conducting NOESY experiments to realize the spatial relationships of each proton can be a complementary route.

As illustrated in Figure 12, H-6 of compound **16** showed the evident NOE signals with either H-4 or NH, which further indicate the relatively closer relationship and ensure the chirality of C-4. Meanwhile, we also observed the proximity between TBS group and H-5. This evidence supports that malonate group might be away from TBS group due to the steric repulsion.

Characterization of 13 via NOESY

Similarly, compound **13** could be examined with the same procedure and certain unexpected messages from NOESY confirmed our initial assumption. Firstly, we found the long-range and larger coupling constant of $J_{H3\text{-}H5}$ (~2.6 Hz) than $J_{H3\text{-}H4}$ (~1.7 Hz). The preliminary assignment of H-3 and H-4 in conflict with the later NOESY correlation pattern suggested us to examine their correspondence in the related spectrums. From the singlet peak of H-1', the corresponding NOE signal with H-3 was observed. The methyl ester of C-1 seems bent inward and contributes NOE signals with either H-3 or H-4. In addition, NH of amido group also correlated to H-6 and H-4. All of evidences pointed out that dimethylmalonate and protected glycerol side chain are prone to situate on the same face with the boat-like pyranose ring (Figure 14).



The 3D energy-minimized drawing is created by Chem3D software

Figure 14 - NOE correlation of **13** and 3D diagram

X-ray of 15

To prove our assignment, **13** was protected with TBS group at 7-OH leading to the same product with **15** (complete matching of ^{1}H-NMR). Furthermore, the configuration of **15** can be reconfirmed by X-ray analysis (Figure 15).

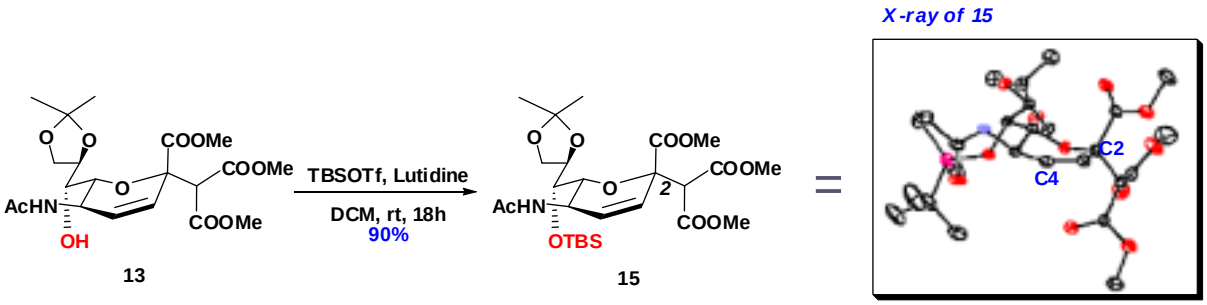
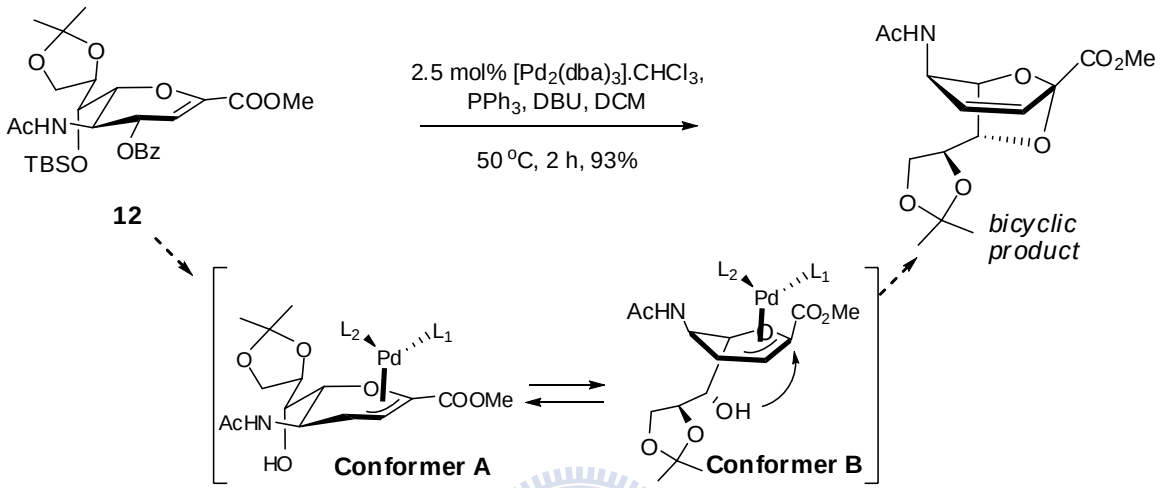


Figure 15 - TBS protection of **13** and X-ray of **15**

On the other hand, bicyclic acetal derivative was formed in high yield by treating **12** with the palladium catalyst without an external nucleophile (Scheme 14). This revealed a probable

transitory π-allyl palladium complex close in geometry to the bicyclic product and therefore to conformer B (Scheme 14). This explains logically the selective formation of the C-2 adduct based on steric grounds, with the bulky side chain at C-6 screening the C-4 attack of the incoming nucleophile.



Scheme 14 - Formation of bicyclic product

2.3.3. Reaction procedure

During the catalytic cycle of Pd(0)-catalyzed allylic substitution, the first step is to form active Pd(0) species stabilized by the ligands, followed by adding the substrate and nucleophile successively. We intended to examine the different adding sequences in order to streamline the operational steps.

Procedure A - To the starting material was added the solution of Pd(0) followed by addition of ligand, and stirred at rt until the solution turned to be the corresponding color which implied the formation of the pre-catalyst. After addition of freshly-prepared nucleophile, the reaction mixture was stirred at rt for 10 mins, and then heated at the indicated temperature (usually adopt THF as a solvent).

Procedure B - To the starting material was added the solution of Pd(0) followed by addition of ligand and freshly-prepared nucleophile. The reaction mixture was heated at the indicated temperature.

Procedure C – To begin mixing all of the solid reactants and additives was followed by adding the liquid reactants and solvent. The reaction mixture was directly heated at the indicated temperature.

At beginning, we used **12** as a model substrate to carry out the Pd(0)-catalyzed allylic substitution and added sodium dimethyl malonate (either solution or solid form) as a nucleophile. After examining these different procedures **A, B** and **C**, the similar reaction time and product distribution were found respectively. The explanation towards the results is given that the following formation of π-allylpalladium complex derived from **12** or the rate-determining step of decomplexation might occur at the higher temperature (50 ^{o}C). Generally, we adopted the procedure **C** as our standard protocol unless specified in some

unusual conditions.

2.4. Allylic substitution of 12 using sodium dimethylmalonate as a nucleophile

2.4.1. Ligand effect

As described in the first trial of **12** (4-OBz-7OTBS), the isomer from C-4 addition coupling with dimethyl malonate was first obtained. To realize the role of ligand in the reaction, a serial of conditions have been examined by a panel of ligands, such as dppe, dppb, dppf, $P(OPh)_3$. Surprisingly, the inverse regioselectivity on the substrate **12** using the bidendate ligands was achieved (entry 2-8 of Table 1). The preliminary results are summarized in Table 1, categorized by each individual ligand, the ratio of Pd/ligand. Using 10% of $Pd_2(dba)_3 .CHCl_3$ as Pd source in the absence of the external ligands as a control reaction proceed the poor regioselectivity with ratio of 47/53 (C-2/C-4) in a moderate yield (66%, entry 1 of Table 1). While adding the bidentate ligands (dppe, dppb and dppf), C-2 adducts have been preferably obtained within 2 h with excellent yields (84-96%) (entry 2-8). On the other hand, the similar regioselectivity was performed with more basic triphenylphophite, which possess the stronger π-acceptor property and caused the larger electronic difference between C-2 and C-4 (entry 9, Table 1). An elegant series of ^{13}C NMR experiments support this assumption (Figure 16).[49, 50] In general, the more stable position for the positive charge character is the more substituted terminus, and therefore this centre becomes more reactive to an incoming nucleophile. To realize the inverse regioselectivity mediated by bidentate ligands, we set out the more detailed investigation in aspects of functions of ligands.

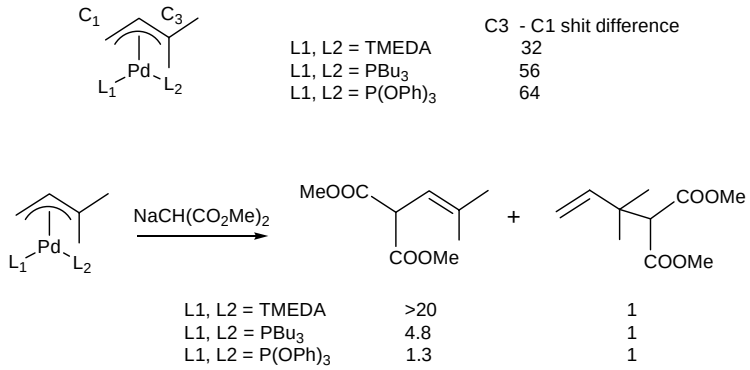


Figure 16 - Regioselectivity predicted by chemical shift of ^{13}C-NMR

Table 1 - Conditions for the regioselective allylic substitution of **12**

Entry	Pd source (mol%)	Ligand (mol%)	Pd/P [e]	Time	Yield[f]	**15:16** (C-2/C-4)[f]
1	$Pd_2(dba)_3.CHCl_3$ - 10%	None		3 h	66%	47:53
2	Allylpalladium(II) chloride dimmer [a] - 10%	dppe[b] (40)	1/4	3 h	96%	93:7
3	Bis(benzonitrile)-palladium (II) Chloride[a] -10%	dppe[b] (40)	1/4	3 h	86%	>95:5
4	$Pd_2(dba)_3.CHCl_3$ - 10%	dppe[b] (40)	1/4	3 h	91%	>95:5
5	$Pd_2(dba)_3.CHCl_3$ - 10%	dppb[c] (40)	1/4	2 h	91%	>95:5
6	$Pd_2(dba)_3.CHCl_3$ - 5%	dppb[c] (20)	1/4	2 h	84%	89:11
7	$Pd_2(dba)_2.CHCl_3$ - 2,5%	dppb[c] (20)	1/8	3 h	87%	93:7
8	$Pd_2(dba)_3.CHCl_3$ - 10%	dppf[d] (40)	1/4	19 h	94%	>95:5
9	$Pd_2(dba)_3.CHCl_3$ - 10%	$P(OPh)_3$ (40)	1/2	19 h	87%	87:13
10	$Pd_2(dba)_3.CHCl_3$ - 10%	PBu_3 (40)	1/2	2,5 h	95%	5:95
11	$Pd_2(dba)_3.CHCl_3$ - 10%	PCy_3 (40)	1/2	2,5 h	91%	9:91
12	$Pd_2(dba)_3.CHCl_3$ - 10%	PPh_3 (20)	1/1	20 h	98%	5:95

[a] Allylpalladium(II) chloride dimer contains two Pds; Bis(benzonitrile)-palladium(II) Chloride contains one Pd,

[b] dppe = 1,2-Bis(diphenylphosphino)ethane ; [c] dppf = 1,1'-bis(diphenylphosphino)ferrocene; [d] dppb = 1,4-bis(diphenylphosphino)butane

[e] bidentate ligand contains two phosphors

[f] the reaction mixture was purified via column chromatography and product ratio was determined by ^{1}H-NMR.

In terms of the property of mono ligand, “cone angle” was introduced by Tolman to describe the size of ligand which further influences the reactivity in the catalytic process (Figure 17).[51] However; the differences in cone angle can not address the question concering about the dramatic change in regioselectivity so far.

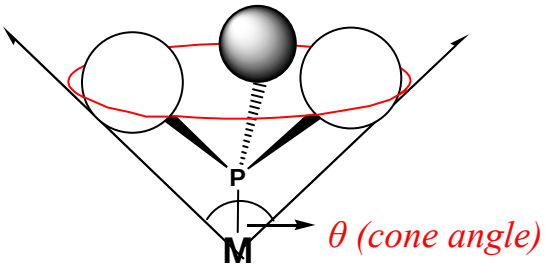


Figure 17 - Tolman's cone angle

To answer it, the resulting bite angle for each intermediate complex with bidentate ligand needs to be taken into account. Thus, in 2001, van Leeuwen *et al.* demonstrated that the distorted coordination of a substituted allyl moiety to palladium can induce more electronic preference on more substituted electrophilic carbon which simply means “*geometry induce electronic effect*”.[52] This relevant studies on the simple substrate possibly render a plausible explanation.

In principle, coordination geometry of π-allylpalladium is symmetrically square planar. The bidentate ligand bonding to palladium will disturb the symmetry due to the nature of the fixed linker. Thus, this distortion of an η^3 toward an η^1,η^2 bonding mode will result in a lower value of the overlap integral on the η^2 site and a higher value of this integral on the η^1 site. On the η^2 site, both the donating interaction (ligand to metal) and the back-donating interaction (metal to ligand) will be decreased. At the same time, the Pd-C1 distance is decreased and the C1-C2 distance is increased so that the unsymmetrically π-bonded allyl moiety is formed accordingly. This assumption has been approved by the crystal structure of π-allylpalladium

complex with the bidentate ligand. It implies that an increase in the bite angles results in a decrease of the back-donation. As a consequence, the allylic LUMO orbital, which are the sites of reaction in the nucleophilic attack, remain less occupied. Therefore, a larger bite angle results in an increase of the reactivity of the allyl moiety toward nucleophilic attack. This has indeed been observed in this case of *trans*-but-2-enyl acetate (Figure 18 and Table 2).[53, 54]

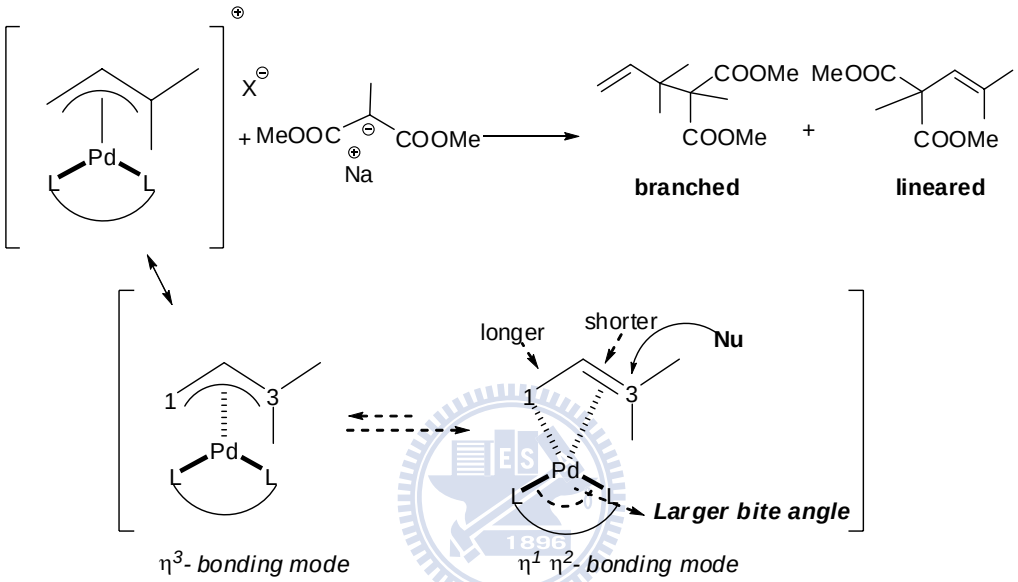


Figure 18 - Conversion between η3 and η1 η2 bonding mode

Table 2 - Regioselectivity in relation to bite angle

Ligand	bite angle (deg)	% branched	% linear
dppe	85.77	8	92
dppp	95	5	95
dppb	99	11	89
dppf	101.2	13	87
DPEphos	103.93	41	59
Xantphos	108.11	61	39

In earlier time, the steric interference in regioselectity has been also investigated by van Leeuwen and co-workers. They conducted the mechanistic study in the relatively simple

substrate (2-hexenylacetate) through molecular modeling approach.[55] It is believed that the larger bite angle of ligand will force the substituents of phosphorus to the increasing embracement of the allyl fragment followed by creating the steric repellence. The involved nucleophile will encounter more steric resistance on more substituted electrophilic center and prefer to attack less substituted position, which explains the higher bite angle ligand leading to the linear substitution product (Figure 19). Furthermore, the modest steric demands of the π-allyl ligand, such as dppb, possess the superior reactivity (higher t.o.f) due to strain release. However, when the bite angle of the disphophine is over 100^{o} or larger, the stronger π-acceptor property will hamper the ligand interaction with palladium and decrease the initial turnover frequencies (Table 3).

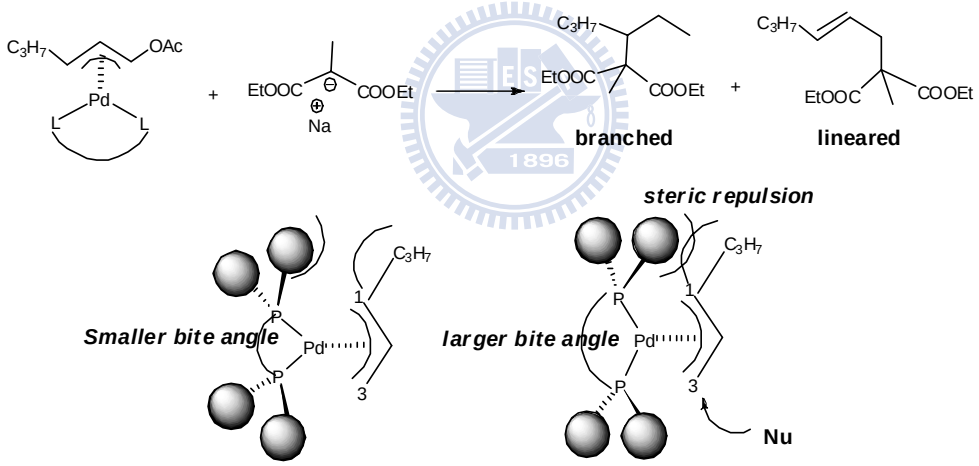


Figure 19 - Steric repulsion between ligand and substrate

Table 3 - Regioselectivity in relation to bite angle

Ligand	bite angle (deg)	t.o.f (mol/mol Pd/hr)	% branched	% linear
dppe	78.1	82	3.8	96.2
dppp	86.2	111	3.4	96.6
dppb	98.6	393	2.3	97.7
dppf	99.07	118	1	99
DPEphos	102.7	114	0.3	99.7
Xantphos	110	22	0	100

These two explanations mentioned above were proposed by the same research group and applied to the different substrates and nucleophiles (methyl dimethyl malonate and methyl diethyl malonate). However, the trend of regioselectivity in these two substrates (*trans*-but-2-enyl acetate and 2-hexenylacetate) is totally reversed. The paradox from their statement implied not only the single effect involved, but also some uncertain factors may contribute. In the case of 2-hexenylacetate, the ratio of linear/branched product showed a trivial difference for each ligand (Table 3). Their conclusion is hard to explain our findings on the cyclic pyranose system. Therefore, the bonding distortion between η^3 and η^1,η^2 mode probably provide insight into the mechanism for the elucidation of our preliminary results.

In contrast, the completely-inversed regioselectivity of allylic substitution at C-4 was achieved by using trialkyl phosphine, such as PCy_3 and PBu_3, which possess the better σ-donor property to tightly coordinate with palladium (entry 9-12, Table 1). Furthermore, these mono ligands also possess poor π-acceptor property, which may cause the later decomplexation step more slowly. Therefore, the substituent of phosphorus will be closer to the reaction center and create the more-hindered environment, which allow the nucleophile to approach from the less-substituted side (Figure 20).

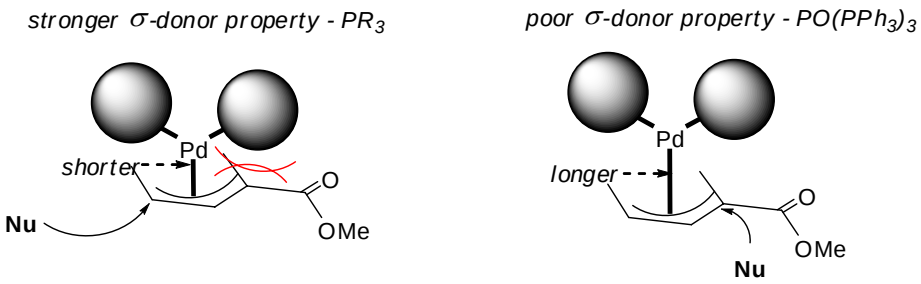


Figure 20 - Plausible conformation due to ligand property

The importance of the nature of the ligands and of the Pd/ ligand ratio in obtaining high regioselectivities in the alkylation of the 7-OTBS derivative **12** suggests that different π-allyl

palladium species could be involved. The further studies regarding the regioselectivty with the different ratios of Pd/ligand will be disclosed in the next section.

2.4.2. Effect of the ratio of Pd/Ligand on the substrate 12

Table 4 - Dppb as a ligand and examination of Pd/P ratio in regioselectivity of **12**[a]

Entry	Pd source (mol%)	Ligand (mol%)	Pd/P	Time	yield[b]	SM[b]	**15:16** (C-2/C-4)[c]
1	$Pd_2(dba)_3.CHCl_3$ - 10%	dppb (20)	1/2	45 min	84%		60:40
2	$Pd_2(dba)_3.CHCl_3$ - 10%	dppb (30)	1/3	2 h	96%		52:48
3	$Pd_2(dba)_3.CHCl_3$ - 10%	dppb (40)	1/4	2 h	91%		>95:5
4	$Pd_2(dba)_2.CHCl_3$ - 10%	dppb (60)	1/6	2 h	95%		>95:5
5	$Pd(OAc)_2$ - 10%	dppb (40)	1/8	18 h	64%	12%	82:18
6	$Pd_2(dba)_3.CHCl_3$ - 5%	dppb (40)	1/8	18 h	92%		>95:5
7	$Pd_2(dba)_3.CHCl_3$ - 2.5%	dppb (20)	1/8	3 h	87%	10%	93:7
8	$Pd_2(dba)_3.CHCl_3$ - 2.5%	dppb (40)	1/16	3 h	86%	9%	95:5

[a]The reactions with substrate **12** were carried out in a degassed solvent at a concentration of 0.08 M with 2 equiv of sodium dimethylmalonate as a nucleophile at 50 °C.

[b] SM = starting material; isolated yield following chromatography.

[c]The ratio of C-2/C-4 was determined by ^{1}H-NMR spectroscopy of the crude reaction mixture.

In the case of dppb as a ligand, a correlation of regioselectivity with the ratios of Pd/P (dppb) is shown that 1 equiv of Pd in company with at least 2 equiv of dppb begins to show the superior selectivity at C-2 (entry 1-3 of Table 4) but the excessive quantity of dppb brings no significant loss in either selectivity or reaction time (entry 4-8). More usefully, the less loading of palladium has been proved possible (entry 6-8).

The similar trend using dppe as a ligand is shown in Table 5. On the other hand, it should be noted that the relatively less amount of dppe than dppb starts to exhibit the regioselectivity

at C-2 (entry 2, Table 5). The distinction between dppb and dppe may imply that the formation of the didendate complex proceed in either the slightly different reaction rate or conformers.

Table 5 - Dppe as a ligand and study of Pd/P ratio in regioselectivity of **12**

Entry	Pd source (mol%)	Ligand (mol%)	Pd/P	Time	yield	**15:16** (C-2/C-4)
1	$Pd_2(dba)_3.CHCl_3$ - 10%	dppe (20)	1/2	2 h	81%	76:24
2	$Pd_2(dba)_3.CHCl_3$ - 10%	dppe (30)	1/3	2 h	97%	95:5
3	Allylpalladium(II) chloride dimer - 10%	dppe (40)	1/4	3 h	96%	93:7
4	Bis(benzonitrile)-palladium(II) Chloride -10%	dppe (40)	1/4	3 h	86%	>95:5
5	$Pd_2(dba)_3.CHCl_3$ - 10%	dppe (40)	1/4	3 h	88%	>95:5

Table 6 – PPh_3 as ligand and study of Pd/P ratio in regioselectivity of **12**

Entry	Pd source (mol%)	Ligand (mol%)	Pd/P	Time	yield	SM	**15:16** (C-2/C-4)
1	$Pd_2(dba)_3.CHCl_3$ - 10%	PPh_3 (20)	1/1	20 h	98%		5:95
2	$Pd_2(dba)_3.CHCl_3$ - 5%	PPh_3 (10)	1/1	18 h	87%		24:76
3	$Pd_2(dba)_3.CHCl_3$ - 10%	PPh_3 (40)	1/2	20 h	80%		15:85
4	$Pd_2(dba)_3.CHCl_3$ - 10%	PPh_3 (80)	1/4	3 h	No reaction		
5	Allylpalladium(II) chloride dimer - 10%	PPh_3 (40)	1/2	3 h	88%		19:81
6	Bis(benzonitrile)-palladium(II) Chloride -10%	PPh_3 (40)	1/2	19 h	37%	41%	34:66
7	$Pd(OAc)_2$ - 20%	PPh_3 (40)	1/2	18 h	64%	8%	31:69
8	$Pd(OAc)_2$ - 10%	PPh_3 (20)	1/2	18 h	55%	42%	24:76

The regioselectivity at C-4 using PPh_3 instead of bidentate ligand was improved by the decreasing amount of phosphine (entry 1-3, Table 6) but the more excessive ligand shut down

the reaction (entry 4, Table 6). However, the selection of other Pd catalyst associated with PPh_3 showed the similar trend in regioselectivity (entry 5 and 6, Table 6).

In Table 7, a similar tendency was found while PBu_3 served as a ligand, which showed the more tolerance for the ratio change of Pd/P.

Table 7 - PBu_3 as a ligand and study of Pd/P ratio in regioselectivity of **12**

Entry	Pd source (mol%)	Ligand (mol%)	Pd/P	Time	yield	SM	**15:16** (C-2/C-4)
1	$Pd_2(dba)_3.CHCl_3$ - 10%	PBu_3(20)	1/1	30 min	93%		13:87
2	Bis(benzonitrile)-palladium(II) Chloride -10%	PBu_3 (40)	1/2	2,5 h	95%		5:95
3	$Pd_2(dba)_3.CHCl_3$ - 5%	PBu_3 (20)	1/2	3 h	87%	6%	13:87
4	$Pd_2(dba)_3.CHCl_3$ - 10%	PBu3 (50)	1/2.5	3 h	91%		21:79

In general, the symmetric active PdL_2 species is considered as the reactive catalyst in a catalytic process. Nevertheless, the question is: why only 1 equiv of ligand to Pd renders a higher regioselectivity and reactivity? The sufficient quantity is supposed to be 2 equiv of monoligands corresponding to 1 equiv of Pd. From the control experiment of $Pd_2(dba)_3.CHCl_3$ without added ligands, it indicates that dba is able to serve a ligand as well to participate in the reaction but obtain the mediocre selectivity.[56, 57] In terms of mechanism of ligand dissociation and association, 1 equiv of monoligands is impossible to generate the dominant species – PdL_2 because $Pd(dba)_2$ could be involved in the catalytic course and destroyed the regioselectivity.

Therefore, we assumed that Pd(dba)L is a dominant species in favor of C-4 addition through the *trans*-influence via phosphorus. In general, trialkyl phosphine ligand with *d*-orbital possesses the better *trans*-effect property than the ligand "dba" and introduces the incoming

nucleophile in the position trans to phosphine. Moreover, a possible explanation for the inert condition using excessive PPh_3 could be given that the abundant ligands will force the active PdL_2 back to the more stable and inactive PdL_4 (Figure 21). These results also led us to consider the involvement of a neutral complex with monophosphine ligands.

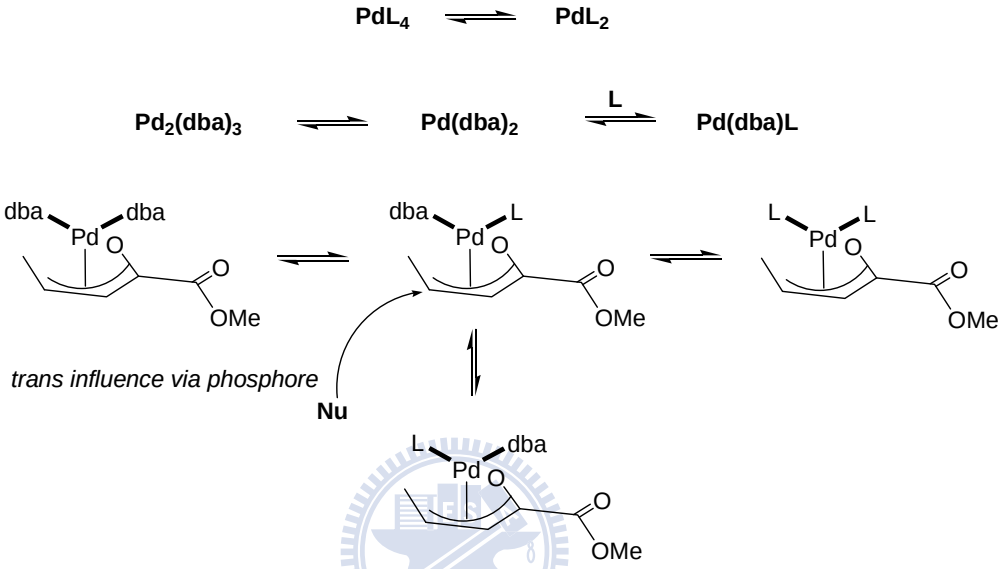
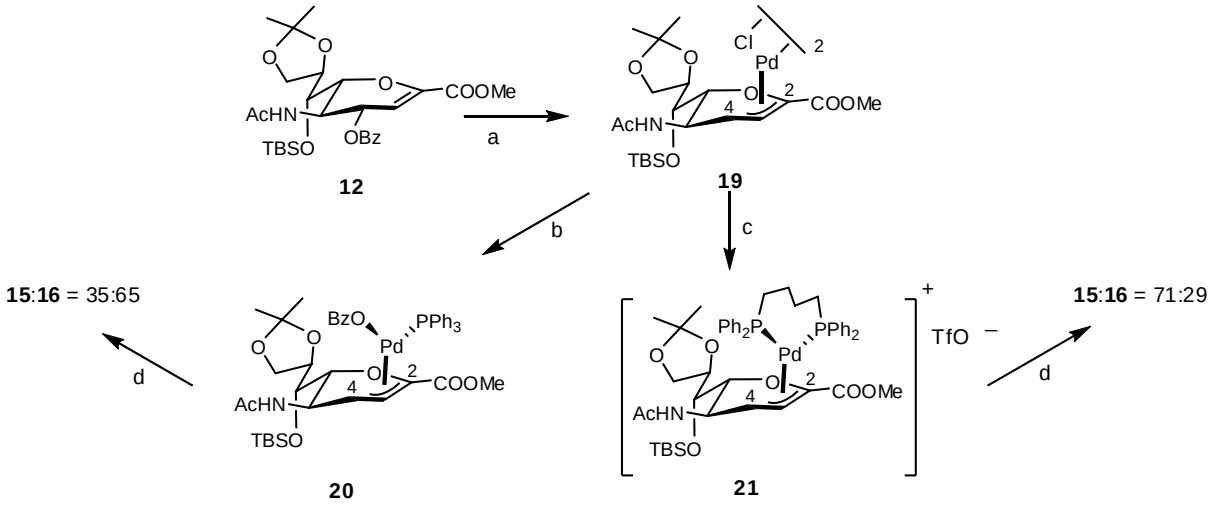


Figure 21 - Trans effect induces regioselective substitution

To better rationalize these results, the neutral complex **20** and the cationic complex **21** were prepared from the chloro-bridged dimer **19** (Scheme 15). For the neutral complex **19**, detailed NMR analysis readily showed that C-4 is trans to the phosphine ligand, because of a shift of this carbon to higher frequency and the large value of the coupling constant of 28 Hz.[49] The two complexes **20** and **21** were then engaged in the stoichiometric reaction with sodium dimethylmalonate in THF at 50 °C. The neutral complex **20** led to a 35:65 mixture of **15** and **16**, a selectivity in agreement with the catalytic reaction, with the favored attack of the nucleophile at C-4, trans to the best acceptor ligand.[58] The cationic complex **21** also gave a selectivity (a 71:29 mixture of **15** and **16**) consistent with the catalytic reaction, with the preferential formation of the 2-C alkylated compound. This is in line with a longer Pd—C-2

bond as detected by ^{13}C NMR spectroscopy in complex **20**.[59, 60]



Reagents and conditions: a) $Pd(dba)_2$, LiCl, THF, rt, 72%; b) PPh_3 (2 equiv), $CDCl_3$ then AgOBz (2 equiv); c) dppb (3 equiv), $CDCl_3$ then AgOTf (2.2 equiv); d) sodium malonate anion, THF, 50 ^oC. dppb=1,4-bis(diphenylphosphino)butane.

Scheme 15 - Preparation of the palladium complexes and their reaction

Upon the detailed screening of the conditions, the different combination with the ligand, catalyst and corresponding ratio on the substrate **12** resulted in the certain change in regioselectivity. The optimal condition could be summarized and listed as below:

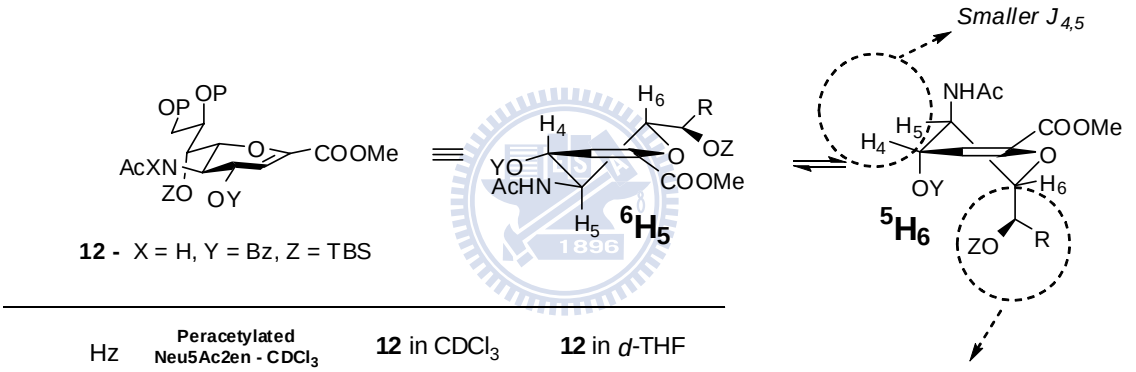
While Pd/dppb → **Pd/P≧1/4** and Pd/dppe → **Pd/P≧1/3**; regioselectivity increases.

While Pd/ PPh_3 → **Pd/P≦1/2** and Pd/ PBu_3 → **Pd/P≦1/2.5**; regioselectivity increases.

Based on these observations, we usually added dppb (40~80 mol% of Pd) as a ligand partner with $Pd_2(dba)_3$.$CHCl_3$ (10~20 mol% of substrate) for the expected C-2 adduct. In contrast, Pd/ PBu_3 with an appropriate ratio were employed to obtain the C-4 adduct. Prior to explore its scope for this methodology with the different nucleophiles, informations regarding to the substrate conformation evaluated from NMR analysis and the effect of solvents would be helpful for the following studies.

2.5. Conformation highlight

The conformational studies in glycals have been described by Hall *et al.* since 1974.[61-64] They demonstrated that the proton coupling constant (*J*) in glycals is reflected in a rapid interconversion equilibrium between the two possible half-chair conformation ($^{6}H_5 \leftrightarrow {}^{5}H_6$) (Figure 22). It probably can explain that the higher proportion (determined from the average *J* value measured in *d*-solution) of an adequate leaving group at pseudo-axial allylic position capable facilitating the initial oxidative addition in conformers $^{5}H_6$.



12 - X = H, Y = Bz, Z = TBS

Hz	Peracetylated Neu5Ac2en - $CDCl_3$	**12** in $CDCl_3$	**12** in *d*-THF
$J_{3,4}$	**3.4**	**4.0**	3.4
$J_{4,5}$	**7.0**	**5.4**	6.7
$J_{5,6}$	**8.9**	**7.3**	ND

Figure 22 - *J* value between peracetylated Neu5Ac2en and **12**

To address this issue from this point, we compared the coupling constants between per-acetylated Neu5Ac2en (failed to work on allylic substitution with sodium dimethylmalonate) and the standard substrate **12** (7-OTBS). The significant difference of $J_{4,5}$ implied that the conformation and electronic effects in glycals could be modulated by a simple change of the protecting groups (P). Further, it could be anticipated that designing a more

efficient substrate should be possible.

2.6. Solvent effect

Table 8 - Solvent effect

Entry[a]	Solvent	Ligand (mol%)	Pd/P	Time	yield[b]	SM[b]	**15:16** (C-2/C-4)[c]
1	DCM	dppb (40)	1/4	1 h	95%		>95:5
2	DCM	PPh_3 (20)	1/1	1 h	92%		39:61
3	DCM	PBu_3 (40)	1/2	1 h	94%		20:80
4	DCE	dppb (40) less soluble	1/4	18 h	41%	55%	20:80
5	DCE	PBu_3 (20)	1/1	18 h	26%	72%	>95:5
6	CH_3CN	dppb (40) less soluble	1/4	2 h	84%		63:37
7	CH_3CN	PPh_3 (20)	1/1	2 h	89%		62:38
8	CH_3CN	PPh_3 (40)	1/2	2 h	91%		84:16
9	DMF	dppb (40) less soluble	1/4	60 h	64%	21%	43:57
10	DMF	PPh_3 (40)	1/2	60 h	30%	56%	40:60
11	toluene	dppb (40)	1/4	2 h	77%		78:22
12	toluene	PPh_3 (40)	1/2	60 h	trace		ND

[a]The reactions with substrate **12** were carried out in a degassed solvent at a concentration of 0.08 M with 2 equiv of sodium dimethylmalonate as a nucleophile by using 10 mmol% of $[Pd_2(dba)_3.CHCl_3]$ at 50 ℃

[b] SM = starting material

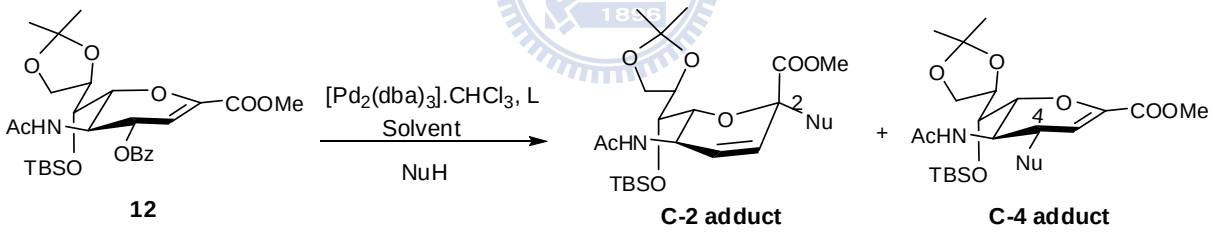
[c]The ratio of C-2/C-4 was determined by ^{1}H-NMR spectroscopy of the crude reaction mixture.

Several solvents have been surveyed to compare their reactivity and selectivity. In the case of dppb as a ligand, DCM, CH_3CN and toluene (entry 1, 6 and 11, Table 8) showed the competitive reactivity with THF but mostly decreased the regioselectivity, except for DCM showing the excellent regioseletivity at C-2 (entry 1). As for monoligands, the efficient conversion can be observed in DCM, however, it showed mediocre regioselectity at C-4 (entry 2 and 3). More importantly, using monoligands (PPh_3 or PBu_3) instead of bidentate

ligand (dppb) can not improve the ratio of C-4 adduct (entry 6, 7 and 8, Table 8), even lead to the formation of C-2 adduct (entry 8). For other solvents, the reactivity sometimes dramatically dropped probably due to the lower solubility of each ligand in the different solvents, which further resulted in an inappropriate ratio of Pd/P (entry 4, 6 and 9). There is no doubt that the solvent property definitely plays a significant role in control of reactivity and stereochemistry during the reaction. Although the complete conversion can be achieved in many cases, the solvent effect severely detracts from the overall regioselectivity and broad utility of this strategy. Nevertheless, DCM has been proved as an alternative solvent with THF, which indeed accelerate the reaction progress.

2.7. Allylic substitution of 12 with various nucleophiles

To access the true extent of the current method, other types of nucleophiles should be surveyed for synthesizing various derivatives of neuraminic acids (Scheme 16). Therefore, the substrate 7-OTBS **12** was employed as a model substrate and herein we classified them in four types: **I.** Symmetric malonate type; **II.** Asymmetric malonate type; **III**. Amine; **IV.** Alcohol. In the case of type **I** and **II**, procedure A is usually adopted in THF (see section 2.3.3). In type **III** and **IV**, the procedure B with a higher loading of catalyst (20%) and the corresponding ratio of dppb as a ligand in DCM is usually exploited. Referring to some related works in the literatures, some additives, such as base, phase transfer reagent, or metal alkoxide, would be introduced to the reaction for improving their efficiency.



Scheme 16 - Standard reaction

2.7.1. Type I: Symmetric malonate

Apart from dimethyl malonate, very little has been reported on the use of bis(phenylsul -fonyl)methane as a nucleophile, and prior to this study, little was known of their reactivity. We found that the higher temperature (80 ^{o}C) is required to speed up the reaction rate (Figure 22).[65] Upon consumption of the starting materials, the desired product **22** was obtained in a moderate 46% yield and a certain unknown byproduct was separated by column

chromatography being identified as the epimerized product (Figure 23).

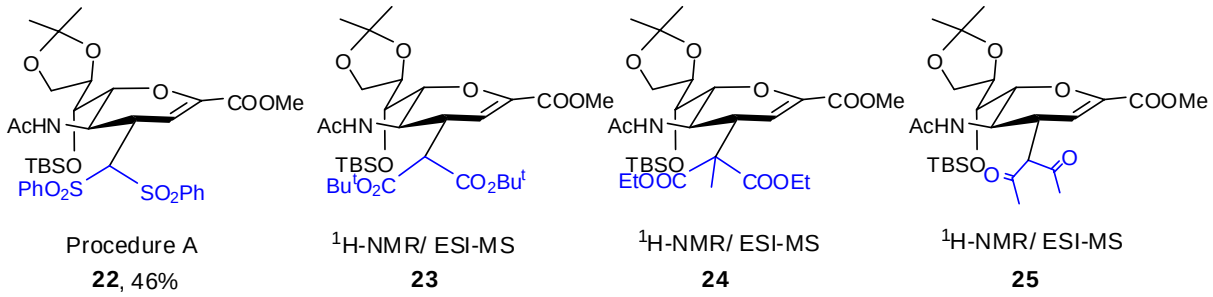


Figure 23 - Symmetric malonate type

It indicates that the reaction with the more bulky nucleophile should be conducted under the harsh condition to overcome the energy barrier. In addition, the OTBS group may locate on the bottom face and facilitate interconversion process by the other palladium atom. However, the formation of epimer also implied that it is not easy to introduce the bulky nucleophile on the face close to acetamido group, except for the smaller and accessible benzoyl anion (Figure 24)

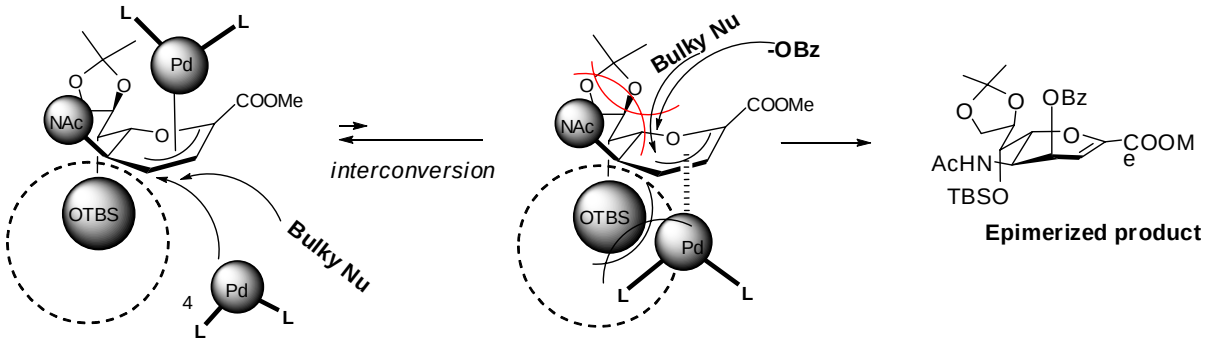


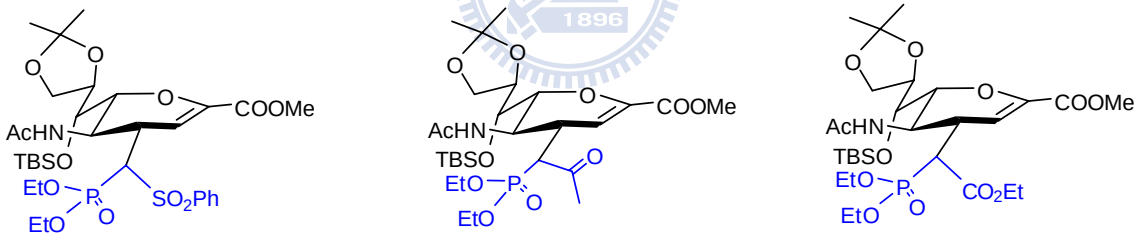
Figure 24 - Plausible epimerization

For the other similar nucleophiles, upon completion of the reaction, the existence of the

C-4 adducts (**23**, **24**, **25**) were approved via ^{1}H-NMR and ESI-MS analysis. Unfortunately, the purified products can not be identified as a single isomer, which cause the difficulty in characterization. In the case of **23**, the decomposition of the reaction mixture during purification via silica gel chromatography was also observed and probably attributed to the occurrence of de-carboxylation due to the instability of the acid-labile *tert*-butyl group. Moreover, the conversion rate of **25** is less than 30%, measured by ^{1}H-NMR, most of starting material still remained.

2.7.2. Type II: Unymmetric malonate

2.7.2.1. Phosphonate derivatives



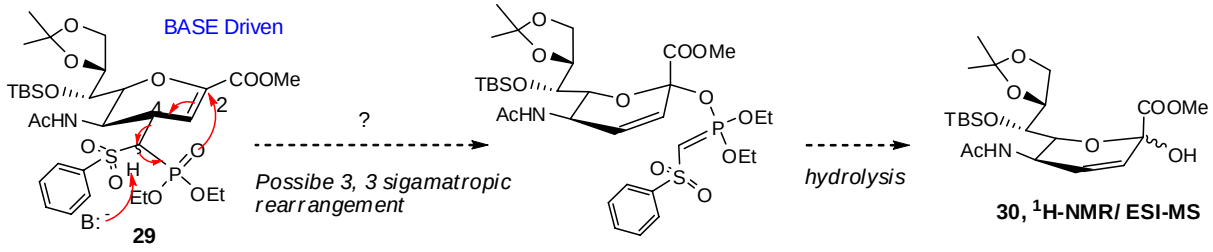
^{1}H-NMR/ ESI-MS **^{1}H-NMR/ ESI-MS** **^{1}H-NMR/ ESI-MS**

26 **27** **28**

Figure 25 - Phosphonate derivatives

We are also interested in preparation of phosphonate derivatives **26**, **27** and **28** using PBu_3 as a ligand (Figure 25). The crude mixtures indicated the conversion rate is around 20 to 60% estimated by ^{1}H-NMR. The common hydrolyzed product **30** in three examples was probably derived from the plausible 3, 3-sigmatropic rearrangement followed by the hydrolysis of the phosphonate group (Scheme 17). We made much effort in characterizing each obtained product and tried to prove the proposed pathway. However, the instability of products and possible

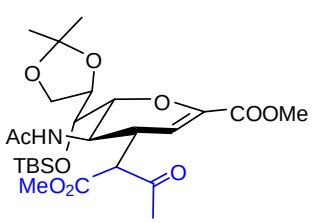
conversion in an acidic media of silica gel rendered the further purification more difficult. Furthermore, the scarce amount of each isolated compound can not afford the further analysis, such as C^{13}-NMR, HMQC, NOESY, etc.



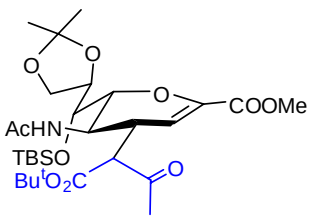
Scheme 17 - The formation of byproduct via 3,3-sigmatropic rearrangement

2.7.2.2. Acetoacetate derivatives

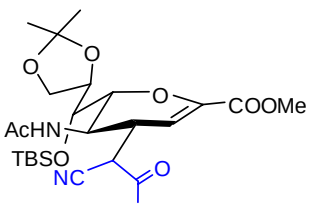
The first trial reaction in a 20 μmole scale of the substrate **12** led to the clean conversion of a diastereomeric mixture (1:1) **31** at C-4 using PBu_3 as a ligand in presence of allylpalladium(II) chloride dimer complex. Unfortunately, the larger scale of this reaction can not work perfectly as the preliminary result (Figure 26). The derivative **32** has the similar problem with **23** in purification and characterization. As for the adduct **33**, we intended to apply the general condition [$Pd_2(dba)_3$.$CHCl_3$] for the first trial, but no any desired product was found. However, this reaction performed better under the catalysis of π-allylpalladium(II) chloride dimer complex, but several stereoisomers at either C-4 or C-2 can not be purified via a routine column chromatography.



^{1}H-NMR/ ESI-MS
31



^{1}H-NMR/ ESI-MS
32



^{1}H-NMR/ ESI-MS
33

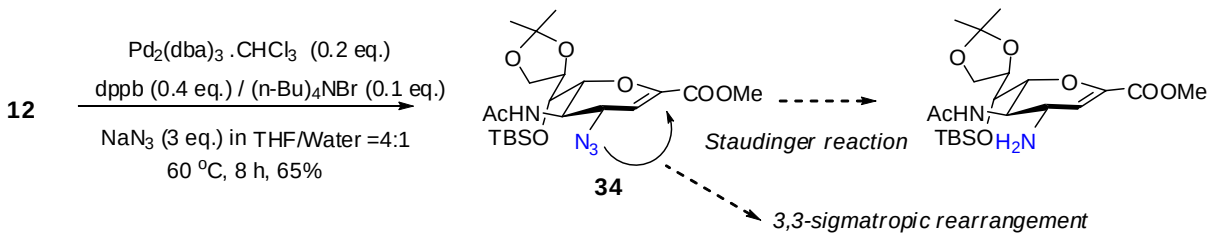
Figure 26 - Acetoacetate derivatives

Therefore, based on these preliminary results, π-allylpalladium(II) chloride dimer complex could be a more efficient catalyst in allylic alkylation reaction, especially for some specific nucleophiles. Besides that, some potential problems need to be considered, such as the inseparable stereoisomers, the instability of products or variable results while an attempt to repeat the reaction in a larger scale was performed.

2.7.3. Type III: Amine derivative as nucleophile

2.7.3.1. Allylic Azidation

Amine group is always a moiety as the key fragment in biological activity. Allylic amination using our current methodology would be an intriguing topic.[65,66] In general, azidation in allylic moiety using Tsuji-Trost conditions has been reported.[67,68] This type of reaction is suitable to examine the feasibility of our method. An attempt to use sodium azide as a nucleophile in biphasic solution (THF/water = 4/1) with triphenyl phosphine (PPh_3) failed to obtain the desired product and only starting material was recovered from the reaction mixture.

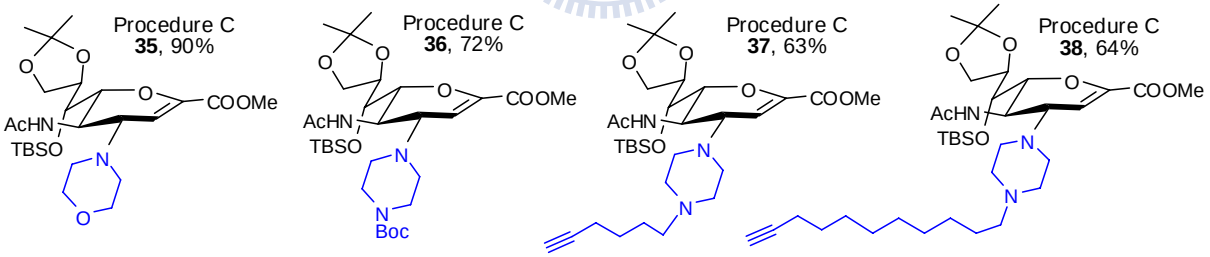


Scheme 18 - Allylic azidation of **12**

In point of reactivity, the bidentate ligand with the larger bite angle usually serves a better

partner to palladium in allylic substitution. Thus we repeated the reactions using dppb as a ligand in the presence of phase transfer reagent TBAB [$(n\text{-}Bu)_4NBr$] as an additive and the desired azidation product **34** has been obtained and isolated in 65% yield. It is worthwhile noted that longer reactions time resulted in the poor 26% yield probably due to the formation of the byproduct derived from either 3,3-sigmatropic rearrangement[17] or Staudinger reaction[69] (which usually occurs in the presence of phosphorus ligand, THF, and water). Thus the reaction should be monitored carefully and halt heating in time. Otherwise, the reaction condition in the different scale is hard to be generalized and the reaction time is not predictable (Scheme 18).

2.7.3.2. Secondary amine: morpholine derivatives



Condition: The reactions were carried out in a degassed solvent at a concentration of 0.08 M by treatment of 20 mol% of $[Pd_2(dba)_3]\cdot CHCl_3$, 3 equiv of the amine with 10 equiv of Et_3N at 60–80 ℃ for 8–15 h.

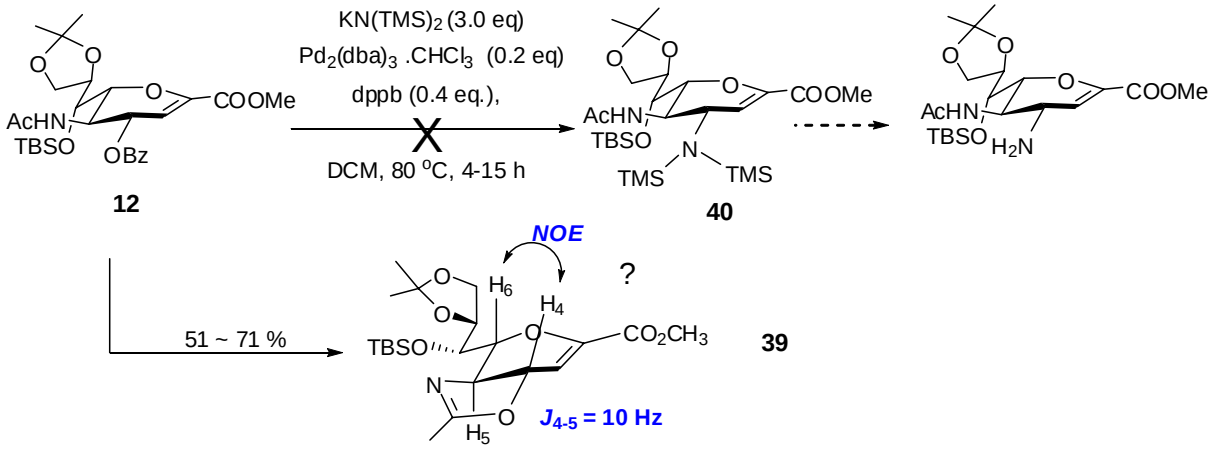
Figure 27 - morpholine derivatives

For the secondary amine, we first started the reaction with the simplest morpholine as a nucleophile (Figure 27). For most of morpholine derivatives, the desired C-4 adducts (**35**, **36**, **37** and **38**) can be obtained in moderate to high yield (63-90%) with excellent C-4. regioselectivity. In addition, this condition has proved to be harmless for certain functional

groups, such as Boc group and the terminal alkynes, rendering the following derivation feasible (**36**, **37** and **38**).

2.7.3.3. Secondary amine: hexamethyldisilazide (KHMDS) as a nucleophile

The good efficiency of morpholine-like nucleophiles inspired us to think that the secondary amines may serve as a good nucleophile to lead to a clean conversion with a high yield and regioselectivity at C-4. One of the objectives for the green chemistry is to prepare the Zanamivir **1** without using the hazardous sodium azide. That is why we selected potassium hexamethyldisilazide (KHMDS) as nitrogen source and applied our method to attain this aim. Unexpectedly, a possible *trans*-fused oxazoline derivative **39** may be formed as a major product, probably due to intramolecular cyclization mediated by π-allylpalladium complex intermediates. The formation of the putative oxazoline **39** can be reproduced in 51-71 yields (Scheme 19). Of the later trials by using the different types of nucleophiles, the putative structure **39** sometimes can be isolated and identified as the same product, based on the complete matching of ^{1}H-NMR and ESI-MS data. To characterize this unusual *trans*-bicyclic structure which is rarely reported in the literatures, the related 2D-NMR spectra and HRMS are recorded for this purpose.

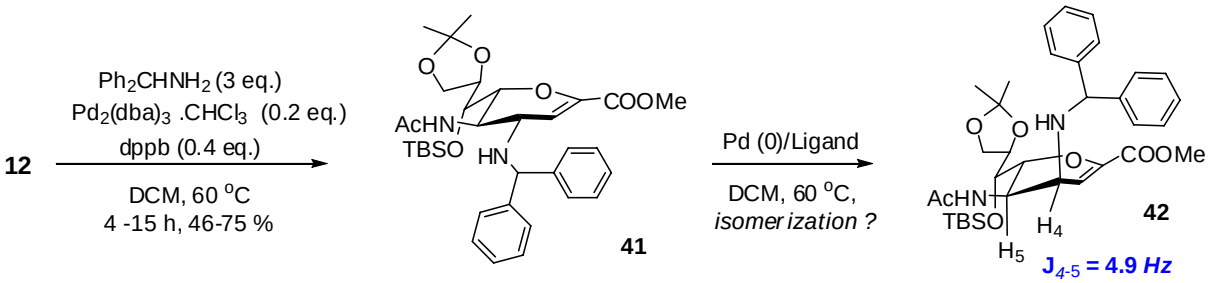


Scheme 19 - KHMDS as a nucleophile

From NOESY, an obvious NOE signal between H-4 and H-6 was observed to prove their spatial proximity, likewise a larger coupling constant J = 10 Hz between H-4 and H-5 shows the large dihedral angle which implied the *trans*-fused relationship. A point deserved to mention is that this putative product **39** is quite unstable in a solution of $CDCl_3$ unless added a few Et_3N to neutralize the media. Thus, it may be able to serve as the other type of substrate with an additional benefit.

2.7.3.4. Diphenyl methanamine as a nucleophile

To achieve the installation of nitrogen at C-4, another amino source (diphenyl methanamine as a nucleophile) was also investigated. We envisaged that diphenyl methananime group is more easily transformed to the free amine without damage of the internal double bond between C-2 and C-3. In the beginning, the result in a larger scale (20 up to 100 mmole) was not reproducible. To overcome the problem, a number of conditions have been tested. We observed that: **I**. At least two by-products were involved in this reaction. One is inseparable and the other one can be isolated and temporarily identified as the epimer of **42** at C-4 (possessed a smaller value of $J_{4\text{-}5}$). **II.** In addition, the appropriate concentration range (c = 0.1-0.15 M) is recommended. **III.** The increasing proportion of **42** with the prolonged reaction time implied that isomerization of **41** probably occurred under Pd(0)-catalyzed condition (Scheme 20). To remove the diphenyl group, several general conditions have been surveyed. Unfortunately, this group is quite stable even under some extremely acidic conditions.



Scheme 20 - Diphenyl methanamine as a nucleophile

2.7.3.5. Aniline and benzylamine derivative as nucleophiles

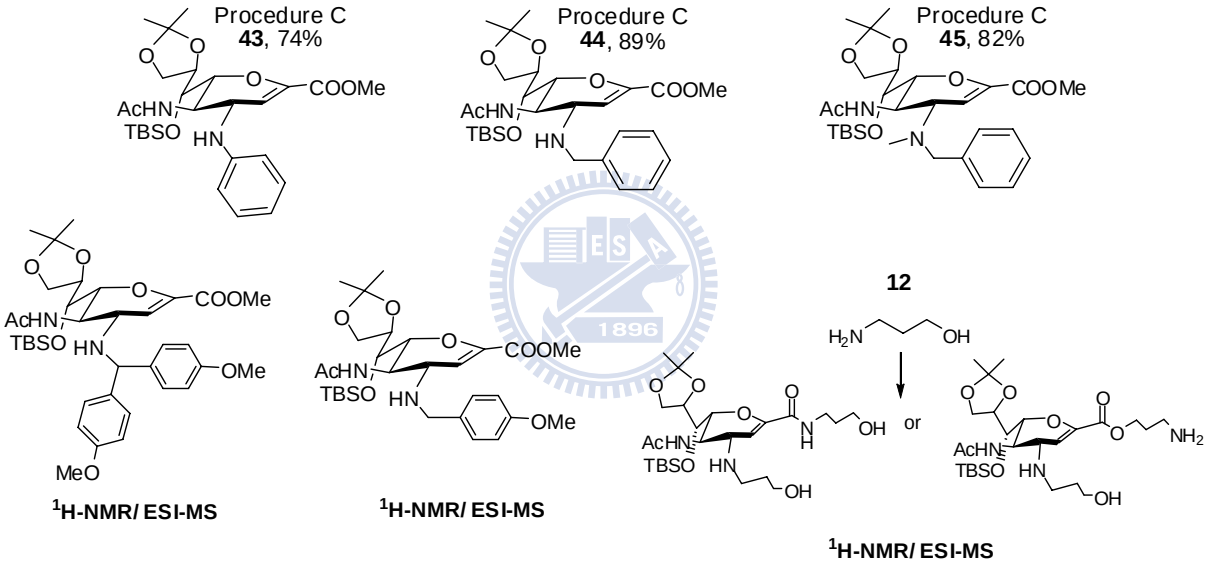


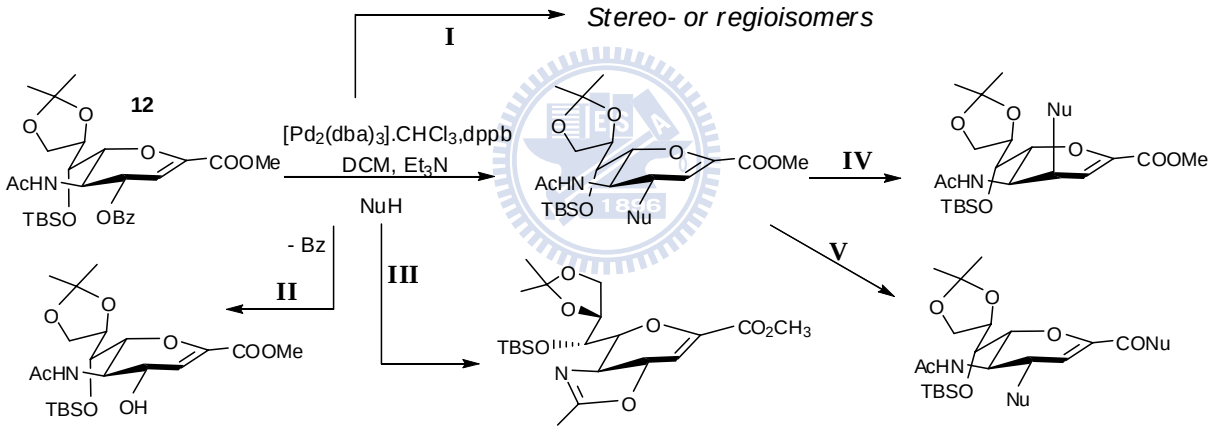
Figure 28 - Aniline and benzylamine derivative

To extend the scope to other amine derivatives, a panel of amine nucleophiles was screened. For the aniline, benzylamine, *N*-methyl benzylamine, the corresponding products **43**, **44**, and **45** were prepared in excellent yields (74-89%) (Figure 28). While using *p*-methoxy diphenyl methanamine or *p*-methoxy benzyl amine as nucleophiles, the half conversion was observed but several unknown products were also detected on ^{1}H-NMR.

In this case of 3-amino 1-propanol as a nucleophile, it is anticipated that *N*-alkylation dominates in this reaction. The result was shown that the chemoselectivity between O and N

can not be distinguished from ^{1}H-NMR. From the ESI-MS analysis of the purified mixtures, the possible transesterification via *N*- or *O*-attack at C-1 carboxylate moiety brings about the complexity of this reaction (Figure 28).

In amination of the substrate **12**, several plausible side reactions are summarized as below (Scheme 21). **I.** Among the poor examples, most of stereo- or regioisomers are inseperable; **II.** For some trials, the nucleophile with stronger basicity induces de-benzoylation of **12**. The deprotected substrate is usually unreactive and mixed with the product. **III.** Intramolecular cyclization via Pd(0)-mediation probably occurs under some specific conditions. **IV.** Isomerization with the prolonged reaction is observed in certain cases.



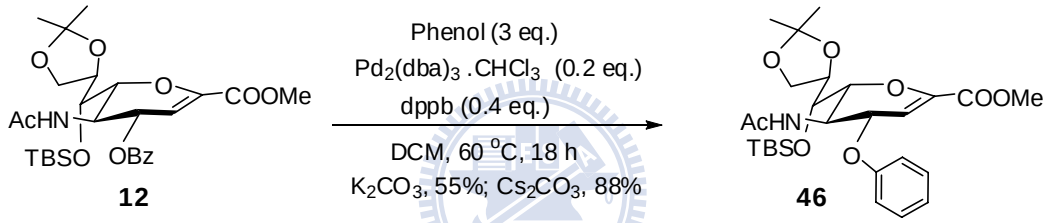
Scheme 21 - Plausible side reactions

V. The carboxylate function could be another electrophilic center leading to certain transformations, such as transesterification. To address these problems, a more reactive substrate or appropriate combination between ligands, catalysts and the additives might be taken into consideration to prevent the successive side-reactions.

2.7.4. Alcohol as a nucleophile

2.7.4.1. Phenol as a nucleophile

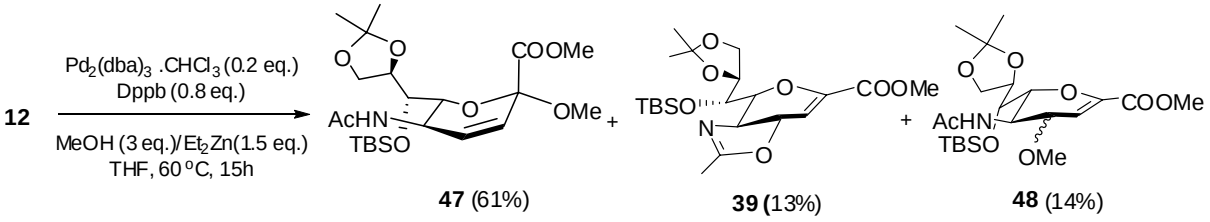
With the aim of extending the scope of our method for versatile nucleophiles, phenol as a standard moiety was tested first. Following the standard procedure C in the presence of K_2CO_3, the *O*-substituted derivative **46** was obtained in moderate 55% yield. Replacement of base instead of Cs_2CO_3 dramatically improves the yield up to 88% (Scheme 22).



Scheme 22 - Phenol as a nucleophile

2.7.4.2. Zinc alkoxide as a nucleophile

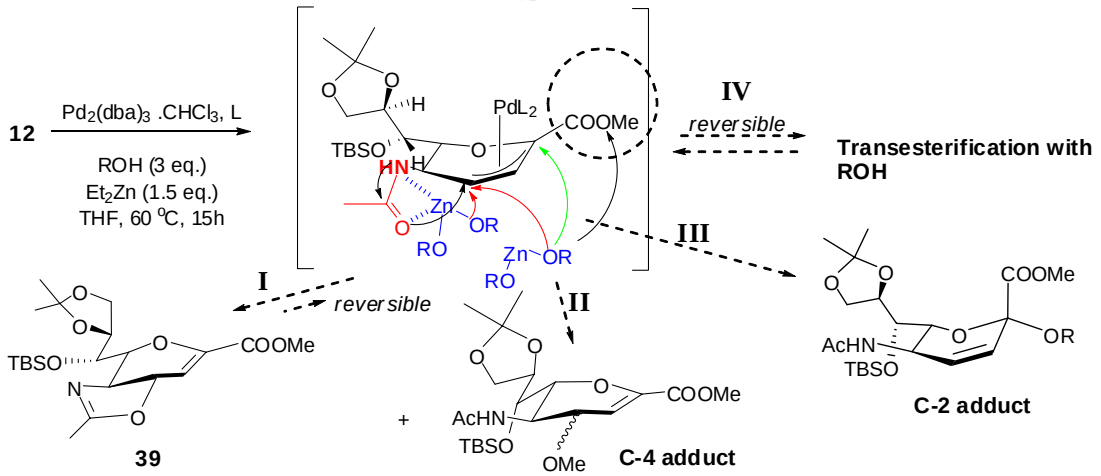
To improve the nucleophilicity of alcohol, the preparation of zinc alkoxide (2 equiv of alcohol and 1 equiv of Et_2Zn) was followed as the reported procedure.[34] Upon treatment of zinc(II) methoxide, the reaction proceed very slowly to furnish the C-2 adduct **47** with an isolated 61% yield (Scheme 23).



Scheme 23 - Zinc methoxide as a nucleophile

In addition, we also found that two less polar products which were identified as an oxazoline derivative **39** and certain regio-isomers **48** at C-4, respectively (Scheme 23). This result inspired us to investigate other available alcohols and thereby the benzyl alcohol employed as a nucleophile also gave us a clean conversion. However, the transesterification between the adding alcohol and carboxylate methyl ester renders the resulting reaction mixture unfit for the following purification and characterization (Scheme 24).

In perspective of mechanism, we monitored the reaction by ^{1}H-NMR, which indicated that the oxazoline derivative was first produced and the subsequent emergence of the desired *O*-alkylated product was detected during the prolonged reaction time. The plausible pathways may include several stages: **I.** Zinc metal activates the carbonyl of acetamido group. In the meantime, π-allypalladium complex serves as an electrophilic center susceptible to the intramolecular oxazoline formation. Thi result also suggests that palladium and zinc can be compatible under this condition (Scheme 24).



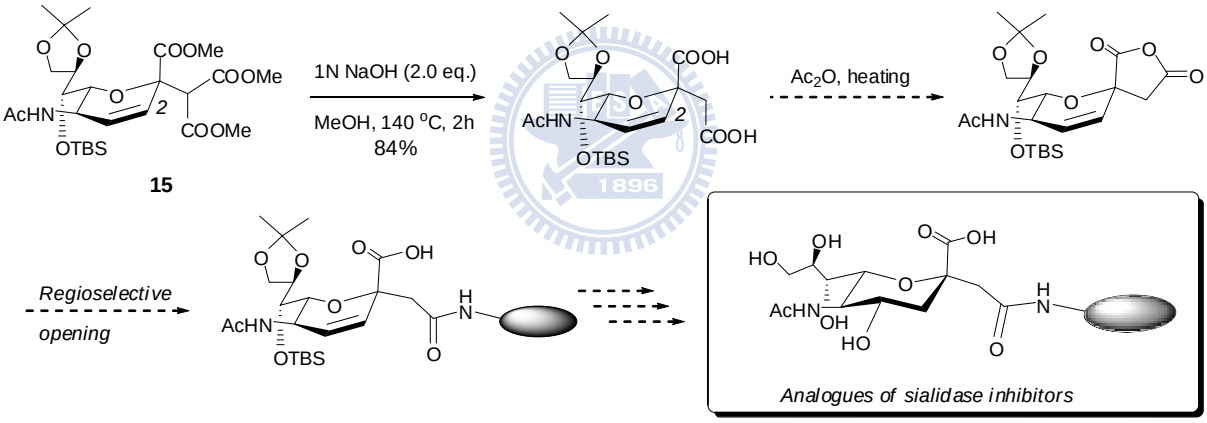
Scheme 24 - Plausible mechanism via zinc-alkoxide mediation

II. III. Due to the steric blockage of π-allypalladium complex, the alkoxide is delivered from the opposite side of Pd leading to C-2 and C-4 adduct. Interestingly, the favorable regioselectivity at C-2 is in accord with the case of sodium dimethylmalonate while

employing dppb as a ligand. **IV.** A reversible transesterification between zinc alkoxide and methyl ester of neuraminic acid emerges simultaneously. The unexpected transformation indeed hampers the following development for the synthesis of *O*-alkylated derivatives using this method.

2.8. Fuctionalization of synthesized derivatives

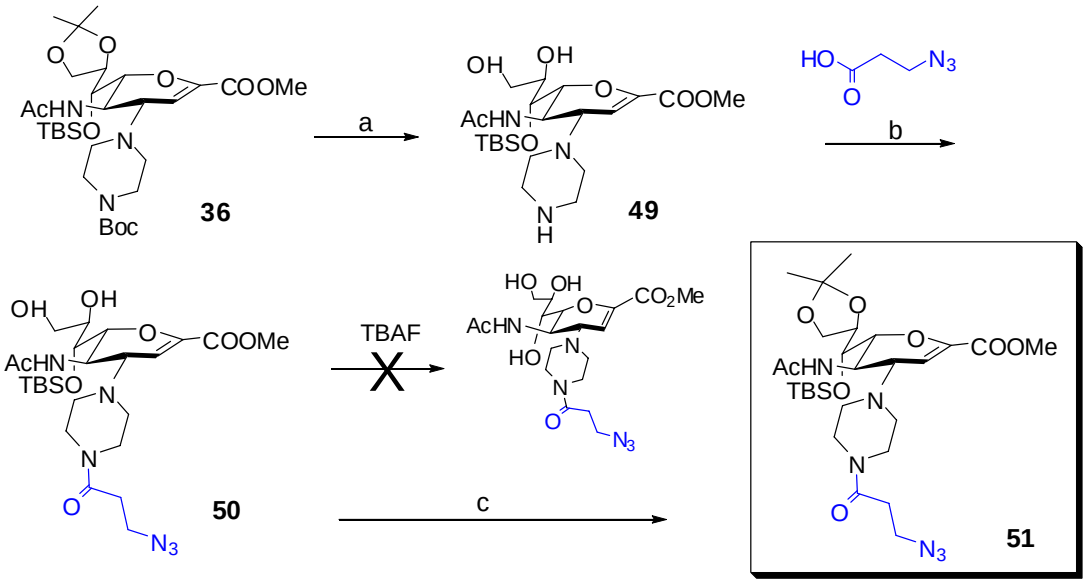
2.8.1. Dimethylmalonate derivative at C-2



Scheme 25 - Functionalization of C-2 dimethylmalonate derivative

The C-2 dimethylmalonate derivative **15** can be readily accessed with the less loading of catalyst $Pd_2(dba)_3.CH_3Cl$ (5%) and dppb (20%). The functionalized **15** is envisaged as a potential sialidase inhibitor (Scheme 25). At the beginning, a hydrolysis followed by the tandem decarboxylation was carried out at 140 ^oC furnishing the diacid in a 74% yield. The subsequent intramolecular lactonization, regioselective opening by amines and several synthetic steps to prepare the target molecule should be possible.

2.8.2. Piperazine derivative at C-4



Reagent and conditions : (a) TFA, DCM, rt, 2 h; (b) EDCI, HOBT, 0 °C, 1 h; (c) CSA, 2,2 Dimethoxypropane, $CHCN_3$, rt, 15 h, (3 steps, 58%)

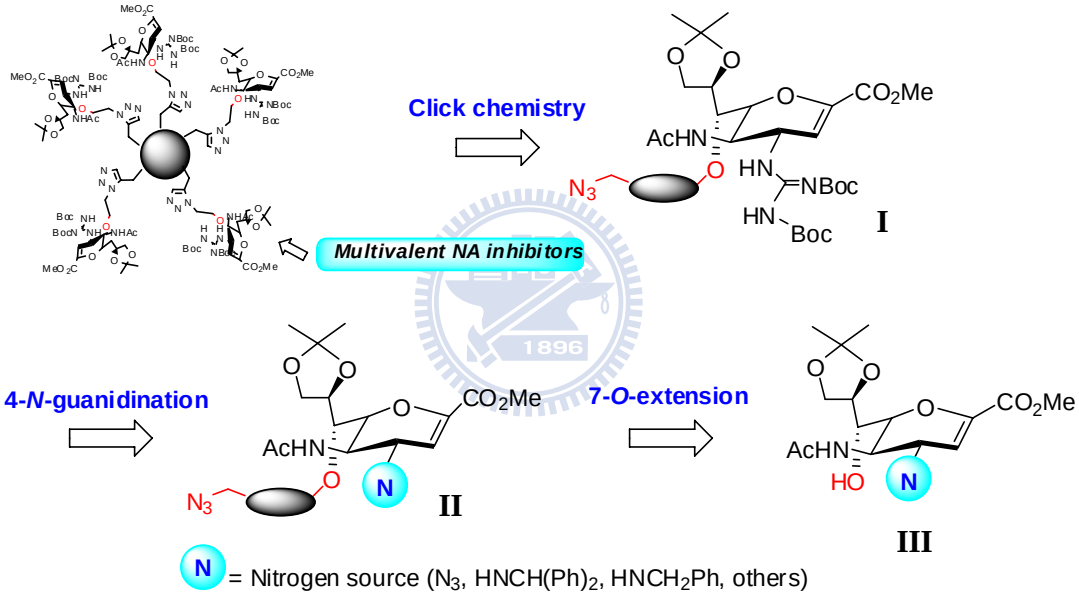
Scheme 26 - Functionalization of piperazine derivative at C-4

Based on our method, the preparation of piperazine derivative at C-4 can be reproduced in gram scale with excellent yields (78-89%). To extend its deviation, the removal of Boc by treatment of TFA concurrently deprotected isopropylidene at C-8 and C-9 (Scheme 26). The crude residue was basified by the resin AG 1-X8, followed by evaporation of solvent. The crude product was treated by the reagent (EDCI and HOBT), further coupling with 3-azidopropanoic acid to obtain the derivative **50**. The remaining polar EDCI and HOBT can be simply removed by Sephadex H-20^{+}. Protection of isopropylene to obtain **51** made the purification much easier**.**

2.8.3. An attempt to synthesize the multivalent derivatives

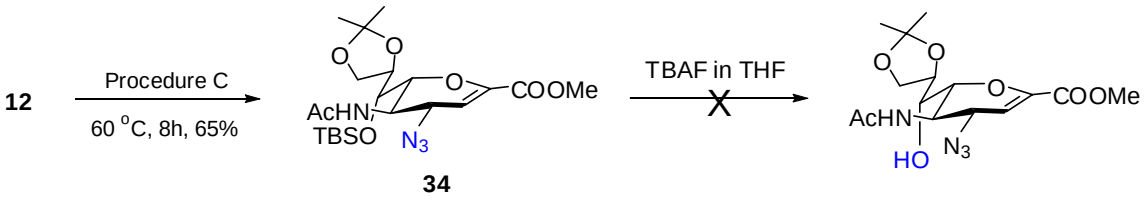
Based on recent reports addressing the interaction between neuraminidase and its

potential inhibitors, they demonstrated that the polymeric alkyl ether analogues derived from the C-7 position of 4-guanidino-Neu5Ac2en shows a better bioavailability in comparison with Zanamivir due to the multivalent effect and less influential actions with the target enzyme. For this purpose, a retrosynthetic route for the synthesis of multivalent derivatives may come from a modification of **III** (Scheme 27). The subsequent installation of alkyl chain with the function of terminal azide at 7-OH, followed by guanidination at 4-NH_2 could be a potential precursor for the preparation of multivalent Zanamivir.



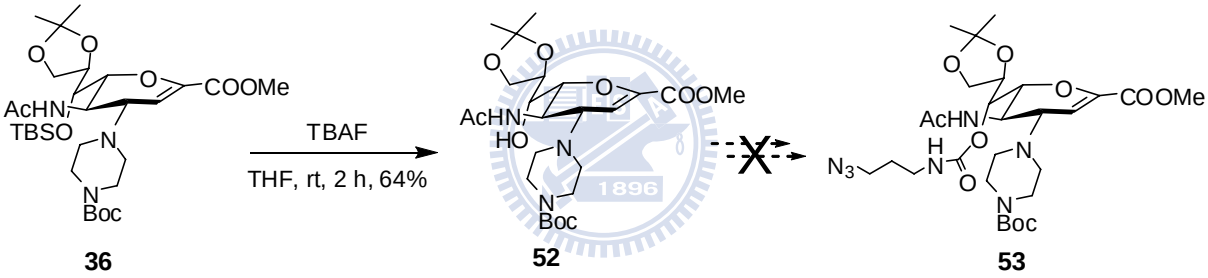
Scheme 27 - Retrosynthesis of multivalent NA inhibitors

In fact, the azido adduct **34** at C-4 has been prepared by using our method. However, the fluctuate results in yields make the large-scale preparation more difficult (Section 2.7.3.1). Besides, a deprotection of TBS at C-7 by treatment of TBAF failed to give any desired product probably due to the instability of azido group in the presence of basic TBAF (Scheme 28). The similar result was also observed in the transformation of **50** (Section 2.8.2).



Scheme 28 - TBS deprotection of **34**

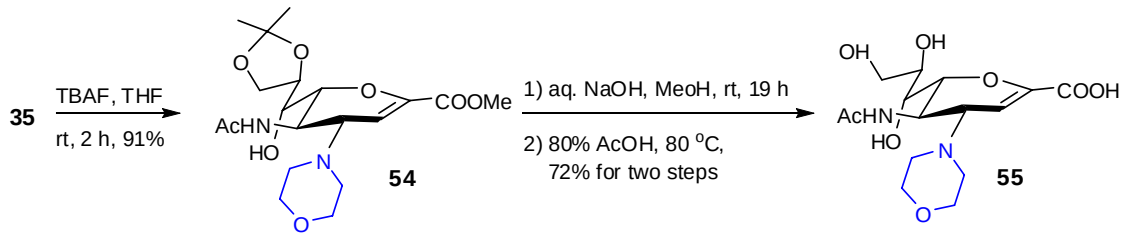
After a survey of literature, the derivation from the hindered C-7 is found problematic because the certain harsh and basic condition is not compatible with the highly-functionalized sialic acid. As following the reported procedure (4-nitrophenyl chloroformate as a junction of carbamate), an attempt to extend the azido chain from C-7 failed to give the desired product **53** (Scheme 29)



Scheme 29 - Functionalization of piperazine derivative **36**

2.8.4. Global deprotection of these synthesized derivatives

The general procedure for deprotection of the morpholine derivative **35** led to the affording triol **55** in a high overall yield (Scheme 30). It would be anticipated that other deprotected derivatives could be prepared in a similar manner.



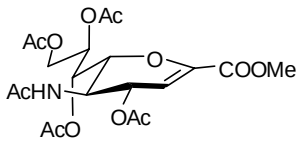
Scheme 30 - Global deprotection of the morpholine derivative **35**

3. Conclusion

In summery, we have developed a stereo- and regioselective allylic substitution on simple Neu5Ac2en derivatives that ensures the control of the regio- and stereoselectivity and affords the C-2 or C-4 products with high efficiency. **I.** The simple replacement of the protecting group on the glycerol chain might induce certain conformational changes which further promote the reactivity. **II.** The more reactive catalyst cooperated with the appropriate ligands and even the ratio between ligand and catalyst can be finely tuned to investigate the optimal conditions, especially for the retest of the unsuccessful cases. **III.** The selected additives for the improvement of efficiency, such as phase transfer reagent, base, metal, etc., could be possible. **IV.** The functionalized products provide an easy entry to a variety of modified sialic acid derivatives, which can serve as useful sialyl building blocks for biological research.

4. Experimental

General. Unless otherwise stated, all reactions were carried out under argon. THF and DCM were purified using a PureSolv solvent purification system (Innovative Technology Inc.). Reactions were monitored with analytical thin-layer chromatography (TLC) on silica gel 60 F_{254} plates and visualized under UV (254 nm) and/or by staining with $KMnO_4$. Silica gel SDS 60 ACC 35-70 mm was used for column chromatography. NMR spectra were recorded with AM 300, AVANCE 300 and AVANCE 500 Brüker spectrometers. Chemical shifts are given in parts per million, referenced to the solvent peak of $CDCl_3$, defined at 77.0 ppm (^{13}C NMR) and 7.26 ppm (^{1}H NMR). Melting points (uncorrected) were determined with the aid of a Büchi B-540 apparatus. IR spectra were recorded on a Perkin-Elmer Spectrum BX instrument with an FT-IR system. Optical Rotations were measured on a JASCO-810 polarimeter using a cell of 1 dm-length path.

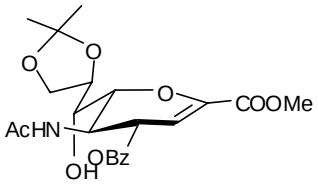


8

Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-enonate (8)

Preparation of (8): To a solution of Neu5Ac (3.5 g, 6.67 mmol) in anhydrous ethyl acetate (30 mL) was added dropwise TMSOTf (2.68 mL, 14.82 mmol) at 0 ^{o}C over a period of 5 mins. The mixture solution was warmed to room temperature and stirred for another 4 hrs. The reaction was monitored by TLC, quenched by Et_3N (5 mL) and water (50 mL) and then extracted by ethyl acetate (2 × 30 mL). The combined organic layer was washed with brine (30 mL), dried (Na_2SO_4) and concentrated to obtain the crude product which was purified by silica gel

chromatography (DCM/MeOH : 97/3) to afford Neu5Ac2en (2.73 g, 5.84 mmol, 89 %) as a white solid.[70]

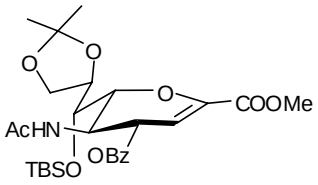


11

Methyl 5-acetamido-2,6-anhydro-4-*O*-benzoyl-3,5-dideoxy-8,9-*O*-isopropylidene-D-*glycero*-D-galacto-non-2-enonate (11)

Preparation of (11): Neu5Ac2en (5.9 g, 12.63 mmol) was dissolved in freshly-distilled methanol (100 mL) and sodium (38 mg, 0.61 mmol) was added at room temperature. While the clear solution turned to be cloudy (1 h), the reaction was completed. Dowex 50 (H^+) resin was added and the reaction mixture was filtered and washed with hot methanol. The filtrates were evaporated in *vacuo* to give a pale-yellow foam (3.85 g), which was used directly for the next step. To the crude mixture of de-acetylated product (3.85 g) and CSA (0.15 g, 0.73 mmol) in anhydrous acetone (200 mL) was added 2,2-dimethoxypropane (12 mL). The reaction mixture was heated to reflux for 2 hrs. After being cooled, the solvent was evaporated. To a solution of the crude product (4.50 g) in DCM (100 mL) was added pyridine (10 mL) followed by dropwise addition of BzCl (2.14 ml, 18.50 mmol) at 0°C. The reaction was completed (1~2 hrs) monitoring by TLC and co-evaporated with toluene (3 × 50 mL). The residue was diluted with DCM (200 mL), washed with HCl (0.5% in water, 3 × 10 mL), water (10 mL), and saturated $NaHCO_3$ aqueous solution (2 × 10 mL). After evaporation of the solvents, the crude residue was purified by silica gel chromatography (DCM/MeOH : 100/0 to 98/2) gave the expected product **8** (4.09 g, three steps 72%) as a white solid. m.p. 230-232ºC; $[\alpha]_D^{25} = +69.8$ ($c = 1.05$ in $CHCl_3$); **^{1}H NMR** (300 MHz, $CDCl_3$, 25°C): $\delta = 8.02$ (d, $^3J_{H,H} = 7.0$ Hz, 2H, H-Ph), 7.60 (dd,

$^3J_{H,H}$ = 7.4 Hz, $^3J_{H,H}$ = 8.4 Hz, 1H, H-Ph), 7.45 (dd, $^3J_{H,H}$ = 7.3 Hz, $^3J_{H,H}$ =8.4 Hz, 2H, H-Ph), 6.07 (d, $^3J_{NH,H-5}$ = 7.0 Hz, 1H, NH), 5.96 (d, $^3J_{H-3,H-4}$ = 2.6 Hz, 1H, H-3), 5.95 (dd, $^3J_{H-4,H-3}$ = 2.6 Hz, $^3J_{H-4,H-5}$ = 11.5 Hz, 1H, H-4), 4.78 (d, $^3J_{OH,H-7}$ = 4.5 Hz, 1H, OH), 4.44-4.31 (m, 2H, H-5, H-8), 4.19- 4.08 (m, 3H, H-6, H-9, H-9'), 3.79 (s, 3H, OCH_3), 3.50 (dd, $^3J_{OH,H-7}$ = 4.5 Hz, $^3J_{H,H}$ = 8.3 Hz, 1H, H-7), 1.99 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.36 (s, 3H, CH_3) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$, 25°C): δ = 173.2 (*C*O), 167.5 (*C*O), 161.9 (*C*O), 146.6 (C-2), 133.9 (Ph), 129.9 (Ph), 128.7 (Cq-Ph), 128.6 (Ph), 109.2 (Cq-acetonide), 106.7 (C-3), 77.9 (C-6), 74.0 (C-8), 69.7 (C-7), 69.1 (C-4), 67.3 (C-9), 52.4 (O*C*H_3), 49.4 (C-5), 27.1 (*C*H_3), 25.2 (*C*H_3), 23.1 ppm (NHCO*C*H_3) ppm; IR (neat): ν bar = 3280, 3093, 2924, 1727, 1667, 1638, 1562, 1556, 1454, 1439, 1390, 1250, 1127, 1067, 1043, 975, 860, 801 cm^{-1} ; ESI HRMS for $C_{22}H_{27}O_9NNa$ [M + Na]$^+$: found 472.1589, calcd 472.1578; elemental analysis calcd (%) for $C_{22}H_{27}O_9N$: C 58.79, H 6.06, N 3.12, O 32.04; found: C 59.01, H 6.08, N 3.05, O 31.99.



12

Methyl 5-acetamido-2,6-anhydro-4-*O*-benzoyl-7-*O*-tertbutyldimethylsilyl-3,5-dideoxy -8,9-*O*-isopropylidene-D-*glycero*-D-*galacto*-non-2-enonate (12)

Preparation of (12): To a solution of **7** (168 mg, 0.37 mmol) in anhydrous DCM (1.5 mL) was added 2,6-Lutidine (0.22 mL, 1.89 mmol) followed by slow addition of TBSOTf (0.30 mL, 1.31 mmol) at room temperature. After being stirred overnight, the reaction mixture was quenched by Et_3N and poured into water, washed by HCl (0.5% in water, 3 × 3 mL), water (3 mL), saturated $NaHCO_3$ aqueous solution (3 mL), brine (3 mL) and dried ($MgSO_4$). After

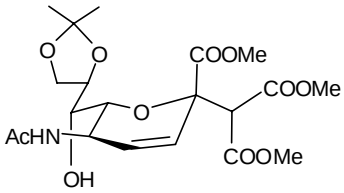
concentration under reduced pressure, the residue was purified by silica gel chromatography (DCM/MeOH : 100/0 to 97.5/1.5) to furnish **12** (185 mg, 88%) as a white solid. m.p. 152-154ºC; $[\alpha]_D^{25}$ = + 103.5 (c = 0.8 in $CHCl_3$); **^{1}H NMR** (300 MHz, $CDCl_3$, 25°C): δ = 8.02 (d, $^3J_{H,H}$ = 7.9 Hz, 1H, H-Ph), 7.57 (dd, $^3J_{H,H}$ = 7.6 Hz, $^3J_{H,H}$ = 8.0 Hz, 1H, H-Ar), 7.44 (dd, $^3J_{H,H}$ = 7.6 Hz, $^3J_{H,H}$ = 7.9 Hz, 2H, H-Ph), 6.13 (d, $J_{H\text{-}3,H\text{-}4}$ = 4.0 Hz, 1H, H-3), 5.70 (dd, $^3J_{H\text{-}4,H\text{-}3}$ = 4.0 Hz, $^3J_{H\text{-}4,H\text{-}5}$ = 5.4 Hz, 1H, H-4), 5.55 (d, 1H, NH, $J_{NH,5}$ = 7.7 Hz), 4.49-4.32 (m, 4H, H-5, H-6, H-7, H-8), 4.06 (dd, $^3J_{H\text{-}9,H\text{-}8}$ = 6.4 Hz, $^2J_{H\text{-}9,H\text{-}9'}$ = 7.7 Hz, 1H, H-9), 3.93 (t, $^3J_{H\text{-}9',H\text{-}9}$ = $^3J_{H\text{-}9',H\text{-}8}$ = 7.6 Hz, 1H, H-9'), 3.79 (s, 3H, OCH_3), 1.93 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 1.26 (s, 3H, CH_3), 0.90 (s, 9H, CH_3), 0.11 (s, 3H, CH_3), 0.06 (s, 3H, CH_3) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$, 25°C): δ =169.7 (*C*O), 165.9 (*C*O), 162.1 (*C*O), 145.2 (C-2), 133.4 (Ph), 129.9 (Ph), 129.3 (Cq-Ph), 128.5 (Ph), 108.6 (Cq-acetonide), 106.7 (C-3), 79.0 (C-6), 75.6 (C-7), 70.0 (C-8),), 67.7 (C-4), 64.9 (C-9), 52.4 (OCH_3), 47.5 (C-5), 26.6 (*C*H_3), 25.9 (SiC(*C*H_3)$_3$), 25.2 (*C*H_3), 23.3 (NHCO*C*H_3), 18.3 (Si*C*), -3.6 (Si*C*H_3), -4.5 (Si*C*H_3) ppm; IR (neat): ν bar = 2929, 2854, 2870, 1746, 1734, 1725, 1643, 1530, 1450, 1370, 1249, 1225, 1058, 1093, 1024, 918, 851, 834, 772, 711 cm^{-1} ; ESI HRMS for $C_{28}H_{41}O_9NNaSi$ $[M + Na]^+$: found 586.2455, calcd 586.2443; elemental analysis calcd (%) for $C_{28}H_{41}NO_9Si$: C 59.66, H 7.33, N 2.48; found: C 59.69, H 7.35, N 2.44.

General procedures for palladium-mediated allylic substitution

A- with sodium malonate as nucleophile: In a Schlenk tube, a solution of the palladium source and the appropriate ligand in degassed THF (0.25 M) was added to **11** or **12**. After stirring for 10 min, a solution of freshly-prepared sodium malonate in THF (0.33 M) was added and the resulting mixture was heated at 50°C for 0.5 - 18h. After cooling, the solvents were removed under reduced pressure and the residue was purified by flash column chromatography

on silica gel.

B-with other nucleophiles: In a Schlenk tube, a mixture of **12**, $Pd_2(dba)_3.CHCl_3$, the appropriate ligand, the nucleophile and the base or the additive in degassed solvent (0.07 M) were heated at the indicated temperature. After cooling, the solvents were removed under reduced pressure and the residue was purified by flash column chromatography on silica gel.

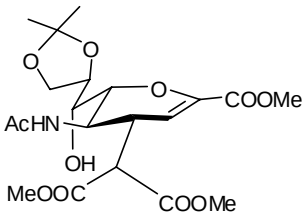


13

Methyl 5-acetamido-2,6-anhydro-2-*C*-(1-di-methoxycarbonyl-methyl)-8,9-*O*-isopropy -lidene-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-3-enonate (13)

(13) : m.p. 158-160 ºC; $[\alpha]_D^{25}$ = -1.7 (c = 0.4 in $CHCl_3$); **^{1}H NMR** (500 MHz, $CDCl_3$, 25°C): δ = 6.04 (dd, $^3J_{H\text{-}3,H\text{-}4}$ = 9.5 Hz, $^4J_{H\text{-}3,H\text{-}5}$ = 2.0 Hz, 1H, H-3), 5.92 (dd, $^3J_{H\text{-}4,H\text{-}5}$ = 2.0 Hz, $^3J_{H\text{-}4,H\text{-}3}$ = 9.5 Hz, 1H, H-4), 5.56 (d, $^3J_{NH,H\text{-}5}$ = 9.5 Hz, 1H, NH), 4.66 (tt, $^3J_{H\text{-}5,H\text{-}4}$ = $^4J_{H\text{-}5,H\text{-}3}$ = 2.0 Hz, $^3J_{H\text{-}5,NH}$ = $^3J_{H\text{-}5,H\text{-}6}$ = 9.5 Hz, 1H, H-5), 4.25 (q, $^3J_{H\text{-}8,H\text{-}9}$ = $J_{H\text{-}8,H\text{-}9'}$ = 6.5 Hz, 1H, H-8), 4.09 (s, 1H, H-malonyl), 4.05 (dd, $^3J_{H\text{-}9,H\text{-}8}$ = 6.5 Hz, $^2J_{H\text{-}9,H\text{-}9'}$ = 8.5 Hz, 1H, H-9), 3.99 (dd, $^3J_{H\text{-}9',H\text{-}8}$ = 6.5 Hz, $^2J_{H\text{-}9',H\text{-}9}$ = 8.5 Hz, 1H, H-9'), 3.75-3.77 (m, 1H, H-6), 3.74 (s, 3H, OCH_3), 3.72 (s, 6H, OCH_3), 3.65-3.67 (m, 1H, H-7), 3.54-3.58 (br, 1H, OH), 1.99 (s, 3H, NHCOCH_3), 1.37 (s, 3H, CH_3), 1.33 (s, 3H, CH_3) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 171.4 (*C*O), 170.1 (*C*O), 166.6 (*C*O), 166.4 (*C*O), 131.7 (C-4), 127.0 (C-3), 108.5 (Cq), 78.7 (C-2), 76.4 (C-8), 75.3 (C-6), 69.5 (C-7), 66.0 (C-9), 58.6 (C-malonyl), 53.0 (O*C*H_3), 52.9 (O*C*H_3), 41.3 (C-5), 26.8 (*C*H_3), 25.7 (*C*H_3), 23.3 (NHCO*C*H_3); IR (neat): ν bar = 3476 (br), 3267 (br), 2957, 2358, 1735, 1643, 1550, 1433, 1370, 1331, 1246, 1155, 1111, 1058, 1012, 890, 853, 757, 710 cm^{-1} ; ESI HRMS for

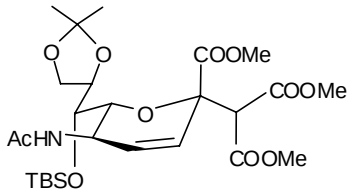
$C_{20}H_{29}NO_{11}Na$ $[M + Na]^+$: found 482.1642, calcd 482.1638; elemental analysis calcd (%) for $C_{20}H_{29}NO_{11}$: C 52.28, H 6.36, N 3.05, O 38.31; found: C 51.99, H 6.32, N 3.01, O 38.05.



14

Methyl 5-acetamido-2,6-anhydro-4-*C*-(1-di-methoxycarbonyl-methyl)-8,9-*O*-isopro -pylidene-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (14)

(14) : mp 191-193 ºC; $[\alpha]_D^{25}$ = + 20.2 (*c* = 0.43 in $CHCl_3$) ; **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 5.97 (d, $^3J_{NH,H-5}$ = 8.0 Hz, 1H, NH), 5.88 (d, $^3J_{H-3,H-4}$ = 2.5 Hz, 1H, H-3), 4.67-4.58 (bs, 1H, O*H*), 4.39 (m, 1H, H-8), 4.18 (dd, $^2J_{H-9,H-9'}$ = 8.5 Hz, $^3J_{H-9,H-8}$ = 6.0 Hz, 1H, H-9), 4.12 (dd, $^3J_{H-9',H-8}$ = 5.0 Hz, $^2J_{H-9',H-9}$ = 8.5 Hz, 1H, H-9'), 4.05 (td, $^3J_{H-5,NH}$ = $^3J_{H-5,H-4}$ = 8.0 Hz, $^3J_{H-5,H-6}$ = 10.5 Hz, 1H, H-5), 3.85 (d, $^3J_{H-6,H-5}$ = 10.5 Hz, 1H, H-6), 3.81 (s, 3H, OC*H*$_3$), 3.79 (s, 3H, OC*H*$_3$), 3.76 (s, 3H, OC*H*$_3$), 3.59 (d, $^3J_{H-malonyl,H-4}$ = 5.5 Hz, 1H, H-malonyl), 3.55 (d, $J_{H-7,H-8}$ = 8.0 Hz, 1H, H-7), 3.30 (ddd, $^3J_{H-4,H-3}$ = 2.5 Hz, $^3J_{H-malonyl,H-4}$ = 5.5 Hz, $^3J_{H-4,H-5}$ = 8.0 Hz, 1H, H-4), 2.06 (s, 3H, C*H*$_3$), 1.42 (s, 3H, C*H*$_3$), 1.38 (s, 3H, C*H*$_3$) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 172.8 (*C*O), 168.7 (*C*O), 168.3 (*C*O), 162.3 (*C*O), 145.3 (C-2), 109.1 (Cq), 108.9 (C-3), 77.9 (C-6), 74.4 (C-8), 69.5 (C-7), 67.2 (C-9), 53.9 (O*C*H3), 53.1 (C-malonyl, O*C*H$_3$), 52.3 (O*C*H$_3$), 46.7 (C-5), 37.9 (C-4), 27.0 (*C*H$_3$), 25.3 (CH$_3$), 23.0 (NHCO*C*H$_3$) ppm; IR (neat): ν bar = 3271 (br), 2955, 2359, 1754, 1726, 1641, 1556, 1435, 1370, 1311, 1256, 1227, 1158, 1043, 920, 862, 777, 731 cm^{-1}; ESI HRMS for $C_{20}H_{29}NO_{11}Na$ $[M + Na]^+$: found 482.1634, calcd 482.1638; elemental analysis calcd (%) for $C_{20}H_{29}NO_{11}$: C 52.28, H 6.36, N 3.05, O 38.31 ; found: C 52.17, H 6.36, N 3.11, O 38.05.

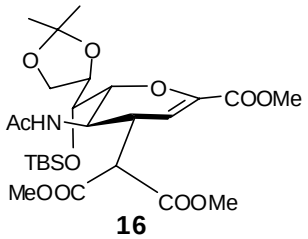


15

Methyl 5-acetamido-2,6-anhydro-7-*O*-tertbutyldimethylsilyl-2-*C*-(1-di-methoxycarbonyl -methyl)-8,9-*O*-isopropylidene-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-3-enonate (15)

Preparation of (15): In a Schlenk tube, a solution of $[Pd_2(dba)_3]$•$CHCl_3$ (5.1 mg, 5 mmol) and dppb (8 mg, 20 mmol) in degassed THF (0.2 mL) was added to **8** (28.1 mg, 50 mmol). After stirring for 10 min, a solution of freshly-prepared sodium dimethyl malonate in THF (0.3 mL, 0.1 mmol) was added and the resulting mixture was heated at 50 ^{o}C for 3 h. After cooling, the solvents were removed under reduced pressure and the residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 1:0 to 49:1) to give **15** as a colorless solid (24 mg, 84%). m.p. 143 ^{o}C; $[\alpha]_D^{25}$ = -3.3 (c=1.3 in $CHCl_3$); **^{1}H NMR** (300 MHz, $CDCl_3$): δ= 6.02 (dd, $^3J_{H\text{-}3,H\text{-}5}$ =1.5 Hz, $^3J_{H\text{-}3,H\text{-}4}$ =10.4 Hz, 1H; H-3), 5.99 (dd, $^3J_{H\text{-}4,H\text{-}5}$ =1.5 Hz, $^3J_{H\text{-}4,H\text{-}3}$ = 10.4 Hz, 1H; H-4), 5.48 (d, $^3J_{NH,H\text{-}5}$ =7.5 Hz, 1H; NH), 4.36 (ddt, $^3J_{H\text{-}5,H\text{-}3}$ = $^3J_{H\text{-}5,H\text{-}4}$ =1.5 Hz, $^3J_{H\text{-}5,NH}$ = 7.5 Hz, $^3J_{H\text{-}5,H\text{-}6}$ = 9.3 Hz, 1H; H-5), 4.25 (td, $^3J_{H\text{-}8,H\text{-}7}$ =3.0 Hz , $^3J_{H\text{-}8,H\text{-}9}$ = $^3J_{H\text{-}8,H\text{-}9'}$ = 7.5 Hz, 1H; H-8), 4.15 (dd, $^3J_{H\text{-}7,H\text{-}6}$ =1.5 Hz, $^3J_{H\text{-}7,H\text{-}8}$ =3.0 Hz, 1H; H-7), 3.96 (t, $^3J_{H\text{-}9,H\text{-}9'}$ = $^3J_{H\text{-}8,H\text{-}9'}$ = 7.5 Hz, 1H; H-9), 3.95 (s, 1H; H-malonyl), 3.88 (dd, $^3J_{H\text{-}6,H\text{-}7}$ =1.5, $^3J_{H\text{-}6,H\text{-}5}$ = 9.3 Hz, 1H; H-6), 3.82 (t, $^3J_{H\text{-}9',H\text{-}8}$= $^3J_{H\text{-}9,H\text{-}9'}$ = 7.5 Hz, 1H; H-9'), 3.74 (s, 3H; OCH_3), 3.71 (s, 3H; OCH_3), 3.70 (s, 3H; OCH_3), 1.94 (s, 3H; $NHCOCH_3$), 1.40 (s, 3H; CH_3), 1.31 (s, 3H; CH_3), 0.89 (s, 9H; CH_3), 0.12 (s, 3H; CH_3), 0.11 ppm (s, 3H; CH_3); **^{13}C NMR** (75 MHz, $CDCl_3$): δ=170.1 (CO), 169.8 (CO), 166.2 (CO), 166.0 (CO), 132.3 (C-4), 125.1 (C-3), 107.7 (Cq), 78.9 (C-2), 77.4 (C-8), 76.6 (C-6), 70.8 (C-7), 64.7(C-9), 59.0 (C-malonyl), 52.7 (OCH_3), 52.5 (OCH_3), 44.3

(C-5), 26.3 (CH_3), 26.0 ($SiC(CH_3)_3$), 24.8 (CH_3), 23.3 (CH_3 $NHCOCH_3$), 18.2 (SiC), -4.9 ($SiCH_3$), -3.9 ppm (q, $SiCH_3$); IR (neat): ν bar = 2951, 1740, 1646, 1542, 1433, 1243, 1156, 1015, 835, 776, 714 cm^{-1}; ESI HRMS: m/z: calcd for $C_{26}H_{43}NO_{11}SiNa$ $[M+Na]^+$: 596.2503; found 596.2500; elemental analysis calcd (%) for $C_{26}H_{43}NO_{11}Si$: C 54.43, H 7.55, N 2.44; found: C 54.51, H 7.53, N 2.36.

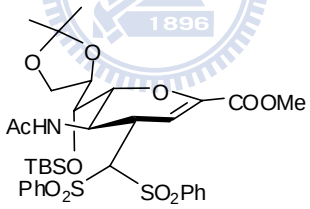


16

Methyl 5-acetamido-2,6-anhydro-4-*C*-(1-di-methoxycarbonyl-methyl)-8,9-*O*-isopropyli -dene-7-*O*-tertbutyldimethylsilyl-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (16)

Preparation of (16): In a Schlenk tube, a solution of $[Pd_2(dba)_3]$•$CHCl_3$ (51 mg, 0.05 mmol) and PBu_3 (50 mL, 0.2 mmol) in degassed THF (2 mL) was added to **12** (281 mg, 0.5 mmol). After stirring for 10 min, a solution of freshly-prepared sodium dimethyl malonate in THF (3 mL, 1 mmol) was added and the resulting mixture was heated at 50 ^oC for 15 h. After cooling, the solvents were removed under reduced pressure and the residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 1:0 to 99:1) to give **16** as a colorless solid (261 mg, 92%). m.p.78 ^oC; $[\alpha]_D^{25}$ = +30.2 (c=1 in $CHCl_3$); **^{1}H NMR** (300 MHz, $CDCl_3$, 25 ^oC): δ = 6.02 (d, $^3J_{H\text{-}3,H\text{-}4}$ = 2.5 Hz, 1H; H-3), 5.62 (d, $^3J_{NH,H\text{-}5}$ = 8.0 Hz, 1H; NHAc), 4.38 (dd, $^3J_{H\text{-}6,H\text{-}7}$ = 1.2, $^3J_{H\text{-}6,H\text{-}5}$ = 10.0 Hz, 1H; H-6), 4.29 (td, $^3J_{H\text{-}8,H\text{-}7}$ = 5.0 Hz, $^3J_{H\text{-}8,H\text{-}9}$ = $^3J_{H\text{-}8,H\text{-}9'}$ = 6.6 Hz, 1H; H-8), 4.14 (dd, $^3J_{H\text{-}7,H\text{-}6}$ =1.2 Hz, $^3J_{H\text{-}7,H\text{-}8}$ = 5.0 Hz, 1H; H-7), 4.09(dd, $^3J_{H\text{-}9,H\text{-}8}$ = 6.6, $^3J_{H\text{-}9,H\text{-}9'}$ = 8.1 Hz, 1H; H-9), 3.96 (dd, $^3J_{H\text{-}9',H\text{-}8}$ = 6.6 Hz, $^3J_{H\text{-}9',H\text{-}9}$ = 8.1 Hz, 1H; H-9'), 3.83 (td, $^3J_{H\text{-}5,NH}$ = 8.0 Hz, $^3J_{H\text{-}5,H\text{-}6}$ = $^3J_{H\text{-}5,H\text{-}4}$ =10.0 Hz, 1H; H-5), 3.78 (s, 3H; OCH_3),

3.77 (s, 3H; OCH_3), 3.75 (s, 3H; OCH_3), 3.72 (d, $^3J_{H\text{-malonyl},H\text{-}4}$ = 4.5 Hz, 1H; H-malonyl), 3.54 (ddd, $^3J_{H\text{-}4,H\text{-}3}$ = 2.5 Hz, $^3J_{H\text{-}4,\ H\text{-malonyl}}$ = 4.5, $^3J_{H\text{-}4,H\text{-}5}$ =10.0 Hz, 1H; H-4), 1.96 (s, 3H; CH_3), 1.40 (s,3H; CH_3), 1.30 (s, 3H, CH_3), 0.87 (s, 9H; 3 x CH_3), 0.12 (s, 3H; CH_3), 0.11 ppm (s, 3H; CH_3); **^{13}C NMR** (75 MHz, $CDCl_3$, 25 °C): δ = 170.2 (CO), 168.7 (CO), 167.9 (CO), 162.3 (CO), 144.1 (C-2), 109.9.1 (C-3), 108.3 (Cq), 77.5 (C-6), 76.3 (C-8), 71.5 (C-7), 65.6 (C-9), 52.8 (OCH_3), 52.6 (OCH_3), 52.1 (C-malonyl, OCH_3), 47.7 (C-5), 38.2 (C-4), 26.6 (CH_3), 26.0 ($SiC(CH_3)_3$), 24.9 (CH_3), 23.6 (CH_3), 18.4 (SiC), -3.3 ($SiCH_3$), -4.3 ppm ($SiCH_3$); IR (neat): νbar = 3476 (br), 3267 (br), 2957, 2358, 1735, 1643, 1550, 1433, 1370, 1331, 1246, 1155, 1111, 1058, 1012, 890, 853, 757, 710 cm^{-1}; ESI HRMS: m/z: calcd for $C_{26}H_{43}NO_{11}SiNa$ $[M+Na]^+$: 596.2503; found 596.2503; elemental analysis calcd (%) for $C_{26}H_{43}NO_{11}Si$: C 54.43, H 7.55, N 2.44; found: C 54.31, H 7.54, N 2.38.

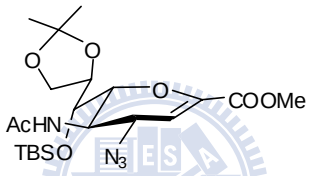


22

Methyl 5-acetamido-2,6-anhydro-7-*O*-tertbutyldimethylsilyl-3,4,5-trideoxy-4-*C*-(1-diben -zenesulfonyl-methyl)-8,9-*O*-isopropylidene-D-*glycero*-D-*galacto*-non-2-enonate (22)

(22): m.p. 177-179 ºC; $[\alpha]_D^{25}$ = + 2.3 (c = 0.3 in $CHCl_3$); **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.05 (d, 2H, *J* = 7.8 Hz, Ph), 7.80 (d, 2H, *J* = 7.7 Hz, Ph), 7.67-7.56 (m, 2H, Ph), 7.45-7.54 (m, 4H, Ph), 6.18 (d, 1H, $J_{3,4}$ = 2.1 Hz, H-3), 5.68 (d, 1H, $J_{NH,5}$ = 7.8 Hz, NH), 5.65 (s, 1H, $HC(SO_2Ph)_2$), 4.71 (td, 1H, $J_{5,NH}$ = 7.8 Hz, $J_{5,4}$ = $J_{5,6}$ = 9.5 Hz, H-5), 4.28-4.18 (m, 2H, H-7, H-8), 4.05 (dd, 1H, $J_{9,8}$ = 5.8 Hz, $J_{9,9'}$ = 7.5 Hz, H-9), 3.94 (d, 1H, $J_{6,5}$ = 9.5 Hz, H-6), 3.88 (t, 1H, $J_{9',8}$ = $J_{9',9}$ = 7.5 Hz, H-9'), 3.77 (s, 3H, CH_3), 3.58 (dd, 1H, $J_{4,3}$ = 2.1 Hz, $J_{4,5}$ = 9.5 Hz,

H-4), 1.86 (s, 3H, CH_3), 1.31 (s, 3H, CH_3), 1.23 (s, 3H, CH_3), 0.89 (s, 9H, 3 x CH_3),0.14 (s, 6H, 2 x CH_3); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 171.3 (CO), 161.8 (CO), 144.9 (C-2), 140.0 (Cq Ph), 137.2 (Cq Ph), 134.7 (Ph), 134.1 (Ph), 129.7 (Ph), 129.4 (Ph), 129.3 (Ph), 108.3 (Cq), 107.6 (C-3), 80.7 ($C(SO_2Ph)_2$), 78.1 (C-7), 75.9 (C-8), 71.9 (C-6), 65.2 (C-9), 52.3 (OCH_3), 47.5 (C-5), 41.4 (C-5), 26.5 (CH_3), 26.0 ($SiC(CH_3)_3$), 25.0 (CH_3), 23.5 ($NHCOCH_3$), 18.3 (SiC), -3.8 ($SiCH_3$), -4.5 ($SiCH_3$); IR (neat): ν bar = 2927, 1730, 1666, 1650, 1536, 1309, 1277, 1253, 1145, 1078, 832, 745, 727, 625 cm^{-1}; ESI HRMS for $C_{34}H_{47}NO_{11}S_2SiNa$: found 760.2247, calcd 760.2258.

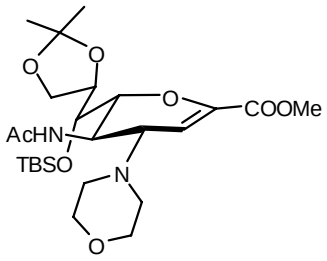


34

Methyl 5-acetamido-2,6-anhydro-4-azido-7-*O*-tertbutyldimethylsilyl-3,4,5-trideoxy-8,9 -*O*-isopropylidene-D-*glycero*-D-*galacto*-non-2-enonate (34)

(34): $[\alpha]_D^{25}$ = +65.6 (c = 1.2 in $CHCl_3$); 1H NMR (500 MHz, $CDCl_3$): δ 5.97 (d, 1H, $J_{3,4}$ = 2.5 Hz, H-3), 5.69 (d, 1H, $J_{NH,5}$ = 8.5 Hz, NH), 4.68 (dd, 1H, $J_{6,7}$ = 3 Hz, $J_{6,5}$ = 8.5 Hz, H-6), 4.65 (dd, 1H, $J_{4,3}$ = 2.5 Hz, $J_{4,5}$ = 8.5 Hz, H-4), 4.29 (q, 1H, $J_{8,9}$ = $J_{8,9'}$ = $J_{8,7}$ = 7.0 Hz, H-8), 4.18-4.14 (m, 1H, H-7), 4.11 (t, 1H, $J_{9,8}$ = $J_{9,9'}$ = 7.0 Hz, H-9), 3.94 (t, 1H, $J_{9',8}$ = $J_{9',9}$ = 7.0 Hz, H-9'), 3.82 (s, 3H, OCH_3), 3.61 (q, 1H, $J_{5,4}$ = $J_{5,NH}$ = $J_{5,6}$ = 8.5 Hz, H-5), 2.04 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 1.35 (s, 3H, CH_3), 0.91 (s, 9H, 3 x CH_3), 0.16 (s, 3H, CH_3), 0.14 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.4 (CO), 161.8 (CO), 145.3 (C-2), 108.5 (Cq), 106.7 (C-3), 77.5 (C-6), 76.3 (C-8), 70.7 (C-7), 65.5 (C-9), 56.6 (C-4), 52.4 (OCH_3), 50.5 (C-5), 26.5 (CH_3), 26.0 ($SiC(CH_3)3$), 24.9 (CH_3), 23.5 ($NHCOCH_3$), 18.5 (SiC), -3.2 ($SiCH_3$), -4.3

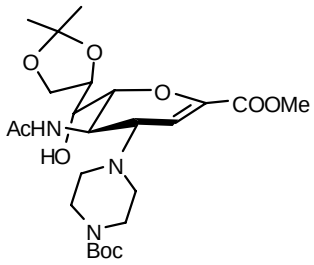
($SiCH_3$); IR (neat): 2927, 2854, 2357, 2096, 1731, 1650, 1659, 1537, 1438, 1370, 1249, 1129, 1070, 832, 776, 712 cm^{-1}; ESI HRMS for $C_{21}H_{36}N_4O_7Si$ Na: found 507.2271, calcd 507.2251;



35

Methyl 5-acetamido-2,6-anhydro-7-*O*-tertbutyldimethylsilyl-3,4,5-trideoxy-4-*N*-morpho -lino-8,9-*O*-isopropylidene-D-*glycero*-D-*galacto*-non-2-enonate (35)

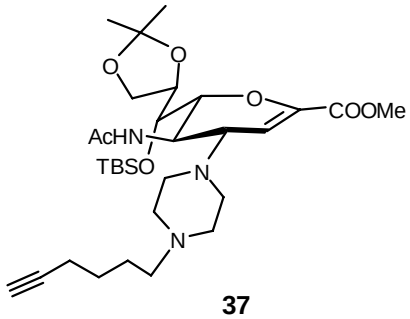
(35): m.p. 61-63 ºC; $[\alpha]_D^{25}$ = +45.8 (c = 1.5 in $CHCl_3$); **^{1}H NMR** (300 MHz, $CDCl_3$): δ 6.07 (d, 1H, $J_{3,4}$ = 3.6 Hz, H-3), 5.46 (d, 1H, $J_{NH,5}$ = 7.2 Hz, NH), 4.38-4.24 (m, 3H, H-6, H-7, H-8), 4.07 (q, 1H, $J_{5,6} = J_{5,NH} = J_{5,4}$ = 7.2 Hz, H-5), 4.06 (t, 1H, $J_{9,8} = J_{9,9'}$ = 7.3 Hz, H-9), 3.95 (t, 1H, $J_{9',8} = J_{9',9}$ = 7.3 Hz, H-9'), 3.81 (s, 3H, OCH_3), 3.72-3.68 (m, 4H, 2 x CH_2), 3.34 (dd, 1H, $J_{4,3}$ = 3.6 Hz, $J_{4,5}$ = 7.2 Hz, H-4), 2.76-2.58 (m, 4H, 2 x CH_2), 1.96 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.31 (s, 3H, CH_3), 0.87 (s, 9H, 3 x CH_3), 0.08 (s, 3H, CH_3), 0.04 (s, 3H, CH_3); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 170.1 (CO), 162.5 (CO), 144.9 (C-2), 108.6 (Cq), 108.1 (C-3), 79.2 (C-8), 76.2 (C-6), 70.5 (C-7), 67.5 (OCH_2), 65.2 (C-9), 61.3 (C-4), 52.4 (OCH_3), 50.2 (CH_2N), 45.6 (C-5), 26.8 (CH_3), 26.2 ($SiC(CH_3)_3$), 25.4 (CH_3), 23.7 ($NHCOCH_3$), 18.6 (SiC), -3.1 ($SiCH_3$), -4.2 ($SiCH_3$); IR (neat): ν bar = 2929, 2854, 1731, 1650, 1537, 1437, 1369, 1250, 1152, 1114, 1004, 832, 776, 692 cm^{-1}; ESI HRMS for $C_{25}H_{44}N_2O_8SiNa$: found 551.2767, calcd 551.2765; Anal. Calcd for $C_{25}H_{44}N_2O_8Si$: C, 56.79; H, 8.39; N, 5.30; Found: C, 56.67; H, 8.51; N, 5.07.



36 (-TBS)

Methyl 5-acetamido-2,6-anhydro-3,4,5-trideoxy-4-(4-tertbutoxycarbonyl-piperazin-1-yl) -8,9-*O*-isopropylidene-D-*glycero*-D-*galacto*-non-2-enonate

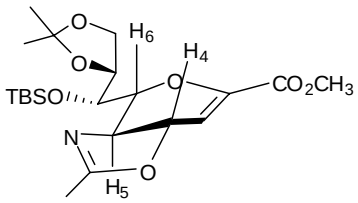
(36 - TBS): mp: 183 ºC; $[\alpha]_D^{25}$ = +40.6 (c 0.81, $CHCl_3$) ; **^{1}H NMR** (300 MHz, $CDCl_3$): δ 5.93 (d, 1H, H-3, J3,4 = 3.9 Hz); 5.87 (d, 1H, NH, $J_{NH,5}$ = 7.7 Hz), 4.24-4.32 (m, 2H, H-5, H-7), 4.09-4.17 (m, 2H, H-9', H-8), 4.01 (dd, 1H, H-9, J = 5.0, 8.7 Hz), 3.75 (s, 3H, OCH_3), 3.52 (d, 1H, H-6, J = 8.3 Hz), 3.38 (br, 4H, piperazine), 3.17 (dd, 1H, H-4, J = 5.0, 5.0 Hz), 2.59 (br, 4H, piperazine), 2.00 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 1.36 (s, 3H, CH_3),1.32 (s, 3H, CH_3); **^{13}C NMR** (75 MHz, $CDCl_3$): 171.5 (CO); 162.2 (CO), 154.5 (CO), 146.0 (C-2), 109.2 (Cq), 105.2 (C-3), 77.5 (C-8), 74.4 (C-5), 72.1 (C-6), 67.4 (C-9), 60.7 (C-4), 52.4 (OCH_3), 49.4 (CH_2 of piperazine), 45.3 (C-7), 43.4 (CH_2-piperazine), 28.4 (CH_3-Boc), 27.0 (CH_3-acetonide), 25.3 (CH_3-acetonide), 23.7 (NHCO*C*H_3); IR (neat): ν bar = 3282(br), 2980, 2931, 1728, 1690, 1646, 1552, 1409, 1367, 1246, 1153, 1120, 1062, 1004, 914, 847, 764, 719 cm^{-1}; ESI HRMS for $C_{24}H_{39}N_3O_9Na$:: found: 536.2572 calcd 536.2584;; Anal. Calcd for $C_{24}H_{39}N_3O_9$: C, 56.13; H, 7.65; N, 8.18; O, 28.04; Found: C, 55.43; H, 7.18; N, 7.57; O, 27.68.



37

Methyl 5-acetamido-2,6-anhydro-7-*O*-tertbutyldimethylsilyl-3,4,5-trideoxy-4-(4-(hex-5-ynyl)-piperazin-1-yl)-8,9-*O*-isopropylidene-D-*glycero*-D-*galacto*-non-2-enonate (37)

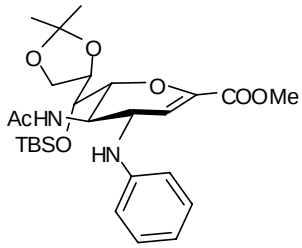
(37): m.p. 55-56 ºC; $[\alpha]_D^{25}$ = +53.2 (c = 0.9 in $CHCl_3$); **^{1}H NMR** (500 MHz, $CDCl_3$): δ 6.08 (d, 1H, H-3, $J_{3,4}$ = 3.0 Hz), 5.52 (d, 1H, $J_{NH,5}$ = 7.0 Hz, NH), 4.36-4.31 (m, 1H, H-8), 4.31-4.26 (m, 1H, H-7), 4.24-4.18 (m, 1H, H-6), 4.01 (t, 1H, $J_{9,8} = J_{9,9'}$ = 7.0 Hz, H-9), 3.98 -3.92 (m, 1H, H-5), 3.91 (t, 1H, $J_{9',8} = J_{9',9}$ = 7.0 Hz, H-9'), 3.76 (s, 3H, OCH_3), 3.44-3.40 (m, 1H, H-4), 2.75-2.62 (m, 4H, NCH_2), 2.52-2.39 (m, 4H, NCH_2), 2.38-2.32 (m, 2H, CH_2), 2.24-2.20 (m, 2H, CH_2), 1.95 (s, 3H, CH_3), 1.91 (s, 1H, CH-alkyne), 1.72-1.48 (m, 6H, CH_2), 1.40 (s, 3H, CH_3), 1.31 (s, 3H, CH_3), 0.88 (s, 9H, CH_3), 0.09 (s, 3H, CH_3), 0.06 (s, 3H, CH_3); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 170.0 (CO), 162.4 (CO), 144.3 (C-2), 108.7 (Cq), 108.3 (C-3), 84.3 (Calkyne), 79.1 (C-6), 76.1 (C-8), 70.0 (C-7), 68.5 (CH-alkyne), 64.8 (C-9), 60.2 (C-4), 58.0 (CH_2), 53.5 (CH_2), 52.2 (OCH_3), 49.4 (CH_2), 45.9 (C-5), 26.5 (CH_3), 26.4 (CH_2), 26.0 (CH_3), 25.9 (CH_2), 25.4 (CH_3), 23.5 ($NHCOCH_3$), 18.3 (SiC, CH_2), -3.0 ($SiCH_3$), -4.5 ($SiCH_3$); IR (neat): νbar = 3268, 2930, 1731, 1650, 1436, 1369, 1251, 1119, 1070, 1006, 833, 776, 695 cm^{-1}; ESI HRMS for $C_{31}H_{54}N_3O_7Si$: found 608.3730, calcd 608.3731;



39

Methyl 2,6-anhydro-7-*O*-tertbutyldimethylsilyl-8,9-*O*-isopropylidene-4',5'-dihydro-2'-methyloxazolo<5.4-d>-2,3,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (39)

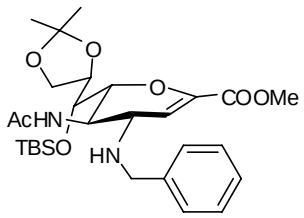
Preparation of (39): In a Schlenk tube, the mixture of **12** (11.2 mg, 20 μmol), [$Pd_2(dba)_3.CHCl_3$] (4 mg, 4μmol), dppb (6.8 mg, 16 μmol) and potassium hexamethyl -disilazide (KHMDS, 20 mg, 100 μmol) in degassed DCM (1 mL) was heated at 80 ^oC for 15 h. After purification via column chromatography over silica gel (DCM/MeOH = 99:1 to 98:2), the bicyclic oxazoline **39** was obtained as a colorless syrup (12.5 mg, 71%). For **39**, $[\alpha]_D^{25}$ = -7.4 (c 1.48, $CHCl_3$) ; **^{1}H NMR** (300 MHz, $CDCl_3$): δ 6.32 (d, 1H, H-3, $J_{3,4}$ = 4.0 Hz), 4.79 (dd, 1H, H-4, $J_{4,3}$ = 3.7 Hz, $J_{4,5}$ = 8.0 Hz), 4.23-4.28 (m, 2H, H-7, H-8), 4.11-4.15 (m, 2H, H-5, H-9'), 3.98 (dd, 1H, H-9, $J_{9,8}$ = 7.6, 8.9 Hz), 3.79 (s, 3H, OCH_3), 3.22 (d, 1H, H-6, $J_{6,5}$ = 10.4 Hz), 1.97 (s, 3H, NAc), 1.40 (s, 3H, CH_3), 1.31 (s, 3H, CH_3), 0.89 (s, 9H, CH_3), 0.19 (s, 3H, CH_3), 0.13 (s, 3H, CH_3); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 166.4 (CO), 162.4 (CO), 147.8 (C-2), 108.1 (Cq), 106.7 (C-3), 79.6 (C-6), 77.1 (C-8), 72.6 (C-4), 70.2 (C-7), 65.7 (C-9), 61.2 (C-5), 52.4 (OCH_3), 26.5 (CH_3), 26.0 ($SiC(CH_3)_3$), 25.2 (CH_3), 18.4 (SiC), 14.1 ($NHCOCH_3$), -3.7 ($SiCH_3$), -4.0 ($SiCH_3$). ESI HRMS for $C_{21}H_{35}NO_7SiNa$: found 464.2079, calcd 464.2080;



43

Methyl 5-acetamido-2,6-anhydro-7-*O*-tertbutyldimethylsilyl-3,4,5-trideoxy-4-phenyl -amino-8,9-*O*-isopropylidene-D-*glycero*-D-*galacto*-non-2-enonate (43)

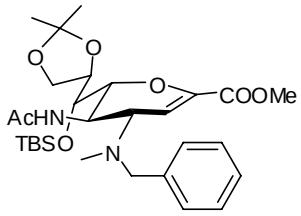
(43): $[\alpha]_D^{25}$ = +31.6 (c = 0.6 in $CHCl_3$) ; **^{1}H NMR** (500 MHz, $CDCl_3$): δ 7.21 (dd, 2H, *J* = 8.0, 8.5 Hz, Ph), 6.76 (t, 1H, *J* = 8.0 Hz, Ph), 6.66 (d, 2H, *J* = 8.0 Hz, Ph), 6.10 (d, 1H, $J_{3,4}$ = 2.5 Hz, H-3), 5.89 (d, 1H, J_{NH}, 5 = 8.5 Hz, NH), 4.61 (d, 1H, $J_{NH,4}$ = 6.5 Hz, NH), 4.30 (dd, 1H, $J_{6,7}$ = 2.0 Hz, $J_{6,5}$ = 8.5 Hz, H-6), 4.30-4.23 (m, 3H, H-4, H-7, H-8), 4.10 (q, 1H, $J_{5,NH}$ = $J_{5,4}$ = $J_{5,6}$ = 8.5 Hz, H-5), 4.06-4.02 (m, 1H, H-9), 3.92-3.88 (m, 1H, H-9'), 3.79 (s, 3H, OCH_3), 1.97 (s, 3H, CH_3), 1.44 (s, 3H, CH_3), 1.30 (s, 3H, CH_3), 0.93 (s, 9H, 3 x CH_3), 0.18 (s, 3H, CH_3), 0.17 (s, 3H, CH_3); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 171.2 (CO), 162.4 (CO), 146.7 (Cq), 143.5 (C-2), 129.5 (Ph), 118.1 (Ph), 113.2 (Ph), 110.7 (C-3), 108.5 (Cq), 78.4 (C-8), 75.6 (C-6), 71.8 (C-7), 65.3 (C-9), 53.5 (C-4), 52.2 (OCH_3), 49.4 (C-5), 26.5 (CH_3), 25.7 (SiC(CH_3)$_3$), 25.1 (CH_3), 23.4 (NHCOCH_3), 18.3 (SiC), -3.7 ($SiCH_3$), -4.5 ($SiCH_3$); IR (neat): 3284, 2929, 2855, 1731, 1650, 1601, 1504, 1435, 1370, 1307, 1249, 1143, 1069, 835, 748, 730 cm^{-1} ; ESI HRMS for $C_{27}H_{42}N_2O_7SiNa$: found 557.2695, calcd 557.2659;



44

Methyl 5-acetamido-2,6-anhydro-4-benzylamino-7-*O*-tertbutyldimethylsilyl-3,4,5-tride -oxy-8,9-*O*-isopropylidene-D-*glycero*-D-*galacto*-non-2-enonate (44)

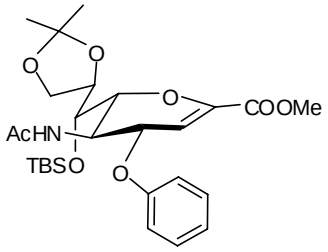
(44): m.p. 90-92 ºC; $[\alpha]_D^{25}$ = +41.1 (c = 0.5 in $CHCl_3$); **^{1}H NMR** (500 MHz, $CDCl_3$): δ 7.37-7.27 (m, 5H, Ph), 6.11 (d, 1H, $J_{3,4}$ = 3.0 Hz, H-3), 5.57 (d, 1H, $J_{NH,5}$ = 6.0 Hz, NH), 4.29 (td, 1H, $J_{8,7}$ = 3.5 Hz, $J_{8,9}$ = $J_{8,9'}$ = 6.5 Hz, H-8), 4.25 (t, 1H, $J_{7,6}$ = $J_{7,8}$ = 3.5 Hz, H-7), 4.19 (dd, 1H, $J_{6,7}$ = 3.5 Hz, $J_{6,5}$ = 8.5 Hz, H-6), 4.03 (dd, 1H, $J_{9,8}$ = 6.5 Hz, $J_{9,9'}$ = 8.0 Hz, H-9), 3.95-3.86 (m, 3H, H-5, H-9', NC*H*$_2$Ph), 3.80-3.74 (m, 5H, NH, NC*H*$_2$Ph, OCH_3), 3.48 (dd, 1H, $J_{4,3}$ = 3.0 Hz, $J_{4,5}$ = 7.0 Hz, H-4), 1.93 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.29 (s, 3H, CH_3), 0.88 (s, 9H, 3 x CH_3), 0.10 (s, 3H, CH_3), 0.07 (s, 3H, CH_3); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 170.2 (CO), 162.7 (CO), 143.6 (C-2), 140.0 (Cq), 128.6 (Ph), 128.5 (Ph), 128.3 (Ph), 127.1 (Ph), 126.8 (Ph), 110.9 (C-3), 108.4 (Cq), 78.8 (C-6), 76.0 (C-8), 70.8 (C-7), 65.3 (C-9), 54.6 (C-4), 52.2 (OCH_3), 50.4 (N*C*H$_2$Ph), 48.8 (C-5), 26.6 (CH_3), 26.0 (SiC(*C*H$_3$)$_3$), 25.2 (CH_3), 23.5 ($NHCOCH_3$), 18.3 (SiC), -3.4 ($SiCH_3$), -4.4 ($SiCH_3$); IR (neat): 3320 (br), 2930, 2854, 1713, 1650, 1537, 1436, 1370, 1252, 1138, 1027, 832, 776, 742, 695 cm^{-1}; ESI HRMS for $C_{28}H_{44}N_2O_7SiNa$: found 549.2997; calcd 549.2996.



45

Methyl 5-acetamido-2,6-anhydro-4-(*N*-benzyl-*N*-methyl)amino-8,9-*O*-isopropylidene -7-*O*-tertbutyldimethylsilyl-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (45)

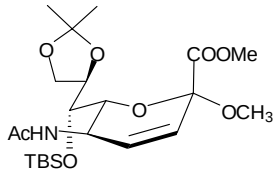
(45): m.p. 95-97 ºC; $[\alpha]_D^{25}$ = +59.8 (c = 0.65 in THF); **^{1}H NMR** (500 MHz, $CDCl_3$): δ 7.33-7.27 (m, 5H, Ph), 6.14 (s, 1H, H-3), 5.31 (bs, 1H, NH), 4.36 (d, 1H, $J_{6,5}$ = 7.0 Hz, H-6), 4.29 (q, 1H, $J_{8,7} = J_{8,9} = J_{8,9'}$ = 6.5 Hz, H-8), 4.14 (d, 1H, $J_{7,8}$ = 6.5 Hz, H-7), 4.12 (dd, 1H, $J_{9,8}$ = 6.5 Hz, $J_{9,9'}$ = 8.0 Hz, H-9), 3.99 (dd, 1H, $J_{9,8}$ = 6.5 Hz, $J_{9,9'}$ = 8.0 Hz, H-9), 3.97-3.91 (m, 1H, H-5), 3.84-3.74 (m, 4H, NCH_2Ph, OCH_3), 3.72-3.66 (m, 1H, H-4), 3.59 (d, 1H, *J* = 13.0 Hz, NCH_2Ph), 2.25 (s, 3H, CH_3), 1.94 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 1.29 (s, 3H, CH_3), 0.87 (s, 9H, 3 x CH_3), 0.11 (s, 3H, CH_3), 0.10 (s, 3H, CH_3); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 170.0 (CO), 162.4 (CO), 145.5 (C-2), 139.2 (Cq), 128.7 (Ph), 128.4 (Ph), 127.2 (Ph), 109.0 (Cq), 108.3 (C-3), 78.2 (C-8), 76.3 (C-6), 71.1 (C-7), 65.8 (C-9), 59.3 (C-4), 58.6 (NCH_2Ph), 52.1(OCH_3), 46.6 (C-5), 37.7 (NCH_3), 26.6 (CH_3), 26.0 ($SiC(CH_3)_3$), 25.0 (CH_3), 23.7 ($NCOCH_3$), -3.1 ($SiCH_3$), 18.5 (SiC), -4.1 ($SiCH_3$); IR (neat): 2927, 2854, 1736, 1650, 1547, 1454, 1369, 1250, 1133, 1071, 832, 776, 741, 697 cm^{-1}; ESI HRMS for $C_{29}H_{47}N_2O_7Si$: found: 563.3156, calcd 563.3152; Anal. Calcd for $C_{29}H_{47}N_2O_7Si$: C, 61.89; H, 8.24; N, 4.98; Found: C, 62.19; H, 8.45; N, 4.31.



46

Methyl 5-acetamido-7-*O*-tertbutyldimethylsilyl-3,5-trideoxy-2,6-anhydro-4-*O*-phenyl -8,9-*O*-isopropylidene-D-*glycero*-D-*galacto*-non-2-enonate (46)

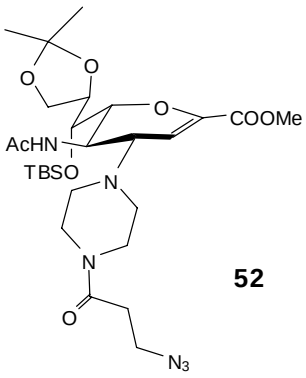
(46): m.p. 87-89 ºC; $[\alpha]_D^{25}$ = +62.0 (c 0.9 in $CHCl_3$); **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.28 (dd, 2H, *J* = 7.2 Hz, *J* = 7.2 Hz, 8.7 Hz, Ph), 7.02-6.95 (m, 3H, Ph), 6.16 (d, 1H, $J_{3,4}$ = 3.9 Hz, H-3), 5.72 (d, 1H, $J_{NH,5}$ = 6.0 Hz, NH), 5.19 (dd, 1H, $J_{4,3}$ = 3.9 Hz, $J_{4,5}$ = 6.0 Hz, H-4), 4.55 (t, 1H, $J_{6,5}$ = $J_{6,7}$ = 6.0 Hz, H-6), 4.38 (dd, 1H, $J_{7,8}$ = 3.9 Hz, $J_{7,6}$ = 6.0 Hz, H-7), 4.21 (ddd, 1H, $J_{8,7}$ = 3.9 Hz, $J_{8,9}$ = 6.6, $J_{8,9'}$ = 7.8 Hz, H-8), 4.15 (q, 1H, $J_{5,6}$ = $J_{5,NH}$ = $J_{5,4}$ = 6.0 Hz, H-5), 3.96 (dd, 1H, $J_{9,8}$ = 6.6 Hz, $J_{9,9'}$ = 7.8 Hz, H-9), 3.93 (t, 1H, $J_{9',9}$ = $J_{9',8}$ = 7.8 Hz, H-9'), 3.82 (s, 3H, OCH_3), 1.93 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.18 (s, 3H, CH_3), 0.90 (s, 9H, 3 x CH_3), 0.11 (s, 3H, CH_3), 0.06 (s, 3H, CH_3); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 170.2 (CO), 162.4 (CO), 156.9 (Ph), 144.5 (C-2), 129.8 (Ph), 121.8 (Ph), 115.7 (Ph), 108.4 (Cq), 106.9 (C-3), 79.1 (C-6), 76.1 (C-8), 69.3 (C-7), 68.9 (C-4), 64.4 (C-9), 52.4 (OCH_3), 48.8 (C-5), 26.4 (CH_3), 26.0 (SiC(CH_3)3), 25.0 (CH_3), 23.4 (NHCOCH_3), 18.4 (SiC), -3.4 ($SiCH_3$), -4.6 ($SiCH_3$); IR (neat): 2928, 2854, 1731, 1705, 1650, 1537, 1370, 1251, 1219, 1114, 1069, 1004, 834, 776, 691 cm^{-1}; ESI HRMS for $C_{27}H_{41}NO_8SiNa$: found 558.2491, calcd 558.2499; Anal. Calcd for $C_{27}H_{41}NO_8Si$: C, 60.54; H, 7.71; N, 2.61; Found: C, 60.35; H, 7.82; N, 2.51.



47

Methyl 5-acetamido-2,6-anhydro-7-*O*-tertbutyldimethylsilyl-8,9-*O*-isopropylidene-2-*O*-methyl-3,4,5-trideoxy -D-*glycero*-D-*galacto*-non-2-enonate (47)

Preparation of (47): In a Schlenk tube, to the mixture of **12** (56.3 mg, 100 μmol), [$Pd_2(dba)_3.CHCl_3$] (20 mg, 20 μmol), dppb (34 mg, 80 μmol) in degassed THF (0.56 mL) was added a suspension of $Zn(OMe)_2$ (500 μmol) in cosolvent of THF and hexane [$Zn(OMe)_2$ was freshly prepared from MeOH (48 μL), Et_2Zn (1 M in hexane, 0.6 mL) in degassed THF (0.48 mL)]. The reaction mixture was heated at 60 ^oC for 15 h. The cooled residue was directly subjected to column chromatography over silica gel and the product **47** was obtained as a colorless syrup (29.2 mg, 62%). For **47**, **^{1}H NMR** (300 MHz, $CDCl_3$): δ 5.98 (d, 1H, H-4, $J_{4,5}$ = 9.8 Hz), 5.87 (dd, 1H, H-3, $J_{3,5}$ = 2.4 Hz, $J_{3,4}$ = 10.1 Hz), 5.38 (d, 1H, NH, $J_{NH,5}$ = 7.6 Hz), 4.63 (dt, 1H, H-5, $J_{5,3}$ = 1.8 Hz, $J_{5,6}$ = $J_{5,NH}$ = 8.5 Hz), 4.29 (ddd, 1H, H-8, $J_{8,7}$ = 6.1, 6.7, 6.7 Hz), 3.87 (dd, 1H, H-9, $J_{9,8}$ = 7.0, 8.1 Hz), 4.05 (dd, 1H, H-9', $J_{9',8}$ = 6.7, 7.9 Hz), 3.92 (d, 1H, H-7, $J_{7,8}$ = 6.7 Hz), 3.84 (d, 1H, H-6, $J_{6,5}$ = 10.5 Hz), 3.76 (s, 3H, OCH_3), 3.33 (s, 3H, OCH_3), 1.97 (s, 3H, NAc), 1.38 (s, 3H, CH_3), 1.30 (s, 3H, CH_3), 0,89 (s, 9H, CH_3), 0.15 (s, 3H, CH_3), 0.11 (s, 3H, CH_3); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 169.8 (CO), 169.1 (CO), 133.2 (C-4), 126.0 (C-3), 108.7 (Cq), 96.3 (C-2), 74.9 (C-8), 73.0 (C-7), 71.2 (C-6), 66.8 (C-9), 52.6 (OCH_3), 51.6 (OCH_3), 44.2 (C-5), 26.7 (CH_3), 25.9 (SiC(CH_3)$_3$), 25.1 (CH_3), 23.3 (NHCOCH_3), 18.4 (SiC), -4.3 ($SiCH_3$), -3.8 ($SiCH_3$); ESI HRMS for $C_{22}H_{39}NO_8SiNa$: found 496.2338, calcd 496.2343.



52

Methyl 5-acetamido-2,6-anhydro-7-*O*-tertbutyldimethylsilyl-3,4,5-trideoxy-4-*N*-[4-(3-azido-propionylamino)-piperazin-1-yl]-8,9-*O*-isopropylidene -D-*glycero*-D-*galacto*-non-2-enopyranosonate (52)

(52): m.p. 77 ºC; $[\alpha]_D^{25}$ = +73.9 (c 0.89 in $CHCl_3$); **^{1}H NMR** (300 MHz, MeOD) δ 6.07 (d, $J_{3,4}$ = 2.7 Hz, 1H, H-3), 4.42-4.30 (m, 2H, H-5, H-8), 4.18 (dd, $J_{6,7}$ = 2.1 Hz, $J_{6,5}$ = 10.2 Hz, H-6), 4.14 (dd, $J_{9,8}$ = 6.3 Hz, $J_{9,9}$= 8.1 Hz, 1H, H-9), 4.06 (dd, $J_{7,6}$ = 1.5 Hz, $J_{7,8}$ = 6.9 Hz, 1H, H-7), 3.99 (dd, $J_{9',8}$ = 6.0 Hz, $J_{9',9}$ = 8.4 Hz, 1H, H-9'), 3.81 (s, 3H), 3.64-3.52 (m, 7H, CH_2-piperazine, C$H_2$$CH_2CH_2N_3$, H-4), 2.76 (br, 2H, CH_2-piperazine), 2.68 (t, J = 6.3 = 6.3 Hz, 2H, $CH_2CH_2CH_2N_3$), 2.55 (br, 2H, CH_2-piperazine), 2.00 (s, 3H, NHCOCH_3), 1.41 (s, 3H, acetonide), 1.34 (s, 3H, CH_3), 1.21 (t, J = 7.0 Hz, J = 7.0 Hz, 2H, $CH_2CH_2CH_2N_3$), 0.92 (s, 9H, CH_3), 0.18 (s, 3H, CH_3), 0.14 (s, 3H, CH_3); **^{13}C NMR** (75 MHz, MeOD): δ 173.97 (CO), 171.9 (CO), 164.5 (CO), 147.2 (C-2), 111.0 (C-3), 110.8 (Cq), 80.4 (C-6), 77.7 (C-8), 74.1 (C-7) , 68.6 (C-9), 64.8 (C-4), 53.5 (O CH_3), 51.0 (CH_2) , 49.1 (CH_2), 48.1 (CH_2), 45.6 (H-5), 44.4 (CH_2), 34.0 (CH_2), 27.9 (CH_3), 27.5 ($SiC(CH_3)_3$), 26.2(CH_3), 23.7 ($NHCOCH_3$), 20.3 (SiC), -1.7 ($SiCH_3$), -2.7 ($SiCH_3$).

5. References

(1) Feizi, T.; Childs, R.; Palma, A.; Wharton, S.; Matrosovich, T.; Liu, Y.; Chai, W. G.; Campanero-Rhodes, M.; Zhang, Y. B.; Eickmann, M.; Kiso, M.; Matrosovich, M.; Feizi, T. *Nature Biotechnol.* **2009**, *27*, 797-799.

(2) De Clercq, E. *Nature Reviews Drug Discovery* **2006**, *5*, 1015-1025.

(3) Moscona, A. *New Eng. J. Med* **2005**, *353*, 1363-1373.

(4) Mammen, M.; Choi, S. K.; Whitesides, G. M. *Angew. Chem. Int. Ed.* **1998**, *37*, 2755-2794.

(5) Meindl, P.; Tuppy, H. *Physiol. Chem.* **1969**, *350*, 1088-1092.

(6) Beau, J. M.; Schauer, R. *Glycoconjugates* **1971**, *26*, 792-797.

(7) Nohle, U.; Beau, J. M.; Schauer, R. *Eur. J. Biochem.* **1982**, *126*, 543-548.

(8) von Itzstein, M. *Nature Reviews Drug Discovery* **2007**, *6*, 967-974.

(9) Farina, V.; Brown, J. D. *Angew. Chem. Int. Ed.* **2006**, *45*, 7330-7334.

(10) Shibasaki, M.; Kanai, M. *Eur. J. Org. Chem.* **2008**, 1839-1850.

(11) Ward, P.; Small, I.; Smith, J.; Suter, P.; Dutkowski, R. *J. Antimicrob. Chemother.* **2005**, *55*, i5-i21.

(12) Ferraris, O.; Lina, B. *J. Clin. Virol.* **2008**, *41*, 13-19.

(13) Collins, P. J.; Haire, L. F.; Lin, Y. P.; Liu, J. F.; Russell, R. J.; Walker, P. A.; Skehel, J. J.; Martin, S. R.; Hay, A. J.; Gamblin, S. J. *Nature* **2008**, *453*, 1258-1262.

(14) von itzstein, M.; Wu, W. Y.; Kok, G. B.; Pegg, M. S.; Dyason, J. C.; Jin, B.; Phan, T. V.; Smythe, M. L.; White, H. F.; Oliver, S. W.; Colman, P. M.; Varghese, J. N.; Ryan, D. M.; Woods, J. M.; Bethell, R. C.; Hotham, V. J.; Cameron, J. M.; Penn, C. R. *Nature* **1993**, *363*, 418-423.

(15) Klefel, M. J.; von Itzstein, M. *Chem. Rev.* **2002**, *102*, 471-490.

(16) Hemeon, I.; Bennet, A. J. *Synthesis-Stuttgart* **2007**, 1899-1926.

(17) Schreiner, E. Z., E; Kleineidam, RG; Schauer, R. *Liebigs Ann. Chem.* **1991**, *2*, 129-134.

(18) Kok, G. B.; von Itzstein, M. *Carbohydr. Res.* **1997**, *302*, 237-240.

(19) Chandler, M.; Bamford, M. J.; Conroy, R.; Lamont, B.; Patel, B.; Patel, V. K.; Steeples, I. P.; Storer, R.; Weir, N. G.; Wright, M.; Williamson, C. *J. Chem. Soc. Perkin Trans. 1* **1995**, 1173-1180.

(20) Kok, G. B.; von Itzstein, M. *J. Chem. Soc. Perkin Trans. 1* **1998**, 905-908.

(21) Norton, A. K.; Kok, G. B.; von Itzstein, M. *J. Carbohyr. Chem.* **2001**, *20*, 507-517.

(22) Kok, G. B.; Groves, D.; von Itzstein, M. *J. Chem. Soc. Perkin Trans. 1* **1999**, 2109-2115.

(23) Rajanbabu, T. V. *J.Org. Chem.* **1985**, *50*, 3642-3644.

(24) Li, J.; Zheng, M. Y.; Tang, W.; He, P. L.; Zhu, W. L.; Li, T. X.; Zuo, J. P.; Liu, H.; Jiang, H. L. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5009-5013.

(25) Ikeda, K.; Sato, K.; Kitani, S.; Suzuki, T.; Maki, N.; Suzuki, Y.; Sato, M. *Bioorg. Med. Chem.* **2006**, *14*, 7893-7897.

(26) Wu, W.-Y.; Jin, B.; Krippner, G. Y.; Watson, K. G. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 341-343.

(27) Wen, W.-H.; Lin, M.-G.; Su, C.-Y.; Wang, S.-Y.; Cheng, Y.-S.; Fang, J.-M.; Wong, C.-H. *J. Med. Chem.* **2009**, *52*, 4903-4910.

(28) Honda, T.; Yoshida, S.; Arai, M.; Masuda, T.; Yamashita, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1929-1932.

(29) Watson, K. G.; Cameron, R.; Fenton, R. J.; Gower, D.; Hamilton, S.; Jin, B.; Krippner, G. Y.; Luttick, A.; McConnell, D.; MacDonald, S. J. F.; Mason, A. M.; Nguyen, V.; Tucker, S. P.; Wu, W.-Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1589-1592.

(30) Macdonald, S. J. F.; Cameron, R.; Demaine, D. A.; Fenton, R. J.; Foster, G.; Gower, D.; Hamblin, J. N.; Hamilton, S.; Hart, G. J.; Hill, A. P.; Inglis, G. G. A.; Jin, B.; Jones, H. T.; McConnell, D. B.; McKimm-Breschkin, J.; Mills, G.; Van, N.; Owens, I. J.; Parry, N.; Shanahan, S. E.; Smith, D.; Watson, K. G.; Wu, W. Y.; Tucker, S. P. *J. Med. Chem.* **2005**, *48*, 2964-2971.

(31) Frost, C. G.; Howarth, J.; Williams, J. M. J. *Tetrahedron: Asymmetry* **1992**, *3*, 1089-1122.

(32) Trost, B. M.; Van Vranken, D. L. In *Chem. Rev.* 1996; Vol. 96, p 395-422.

(33) Trost, B. M.; Crawley, M. L. *Chem. Rev.* 2003; Vol. 103, p 2921-2944.

(34) Kim, H.; Men, H.; Lee, C. *J. Am. Chem. Soc.* **2004**, *126*, 1336-1337.

(35) Babu, R. S.; O'Doherty, G. A. *J. Am. Chem. Soc.* **2003**, *125*, 12406-12407.

(36) Guo, H.-B.; O'Doherty, G. A. *Angew. Chem. Int. Ed.* **2007**, *46*, 5206-5208.

(37) Pedersen, T. M.; Hansen, L.; Kane, J.; Rein, T.; Helquist. *J. Am. Chem. Soc.* **2001**, *123*, 9738-9742.

(38) Boons, G. J.; Demchenko, A. V. *Chem. Rev.* **2000**, *100*, 4539-4565.

(39) Fiaud, J. C.; Aribizouioueche, L. *Chem. Commun.* **1986**, 390-392.

(40) Tsuji, J.; Takahashi, H.; Morikawa, M. *Tetrahedron Lett.* **1965**, *6*, 4387-4388.

(41) Tsuji, J.; Shimizu, I.; Minami, I.; Ohashi, Y.; Sugiura, T.; Takahashi, K. *J. Org. Chem.* **1985**, *50*, 1523-1529.

(42) Amotore, C.; Jutand, A.; Meyer, G.; Mottier, L. *Chem. Eur. J.* **1999**, *5*, 466-473.

(43) Trost, B. M.; Verhoeven, T. R. *J. Am. Chem. Soc.* **1980**, *102*, 4130-4743.

(44) van Leeuwen, P.; Kamer, P. C. J.; Reek, J. N. H.; Dierkes, P. *Chem. Rev.* **2000**, *100*, 2741-2769.

(45) Fristrup, P.; Jensen, T.; Hoppe, J.; Norrby, P. O. *Chem. Eur. J.* **2006**, *12*, 5352-60.

(46) Okamoto, K.; Kondo, T.; Coto, T. *Tetrahedron* **1987**, *43*, 5905-5918.

(47) Tsvetkov, Y. E.; Nifantiev, N. E. *Synlett* **2005**, *9*, 1375-1380.

(48) Crimmins, M. T.; Christie, H. S.; Chaudhary, K.; Long, A. *J. Am. Chem. Soc.* **2005**, *127*, 13810-13812.

(49) Åkermark, B.; Krakenberger, B.; Hansson, S.; Vitagliano, A. *Organometallics* **1987**, *6*, 620-628.

(50) Åakermark, B.; Aakermark, G.; Hegedus, L. S.; Zetterberg, K. *J. Am. Chem. Soc.* **1981**, *103*, 3037-3040.

(51) Tolman, C. A. *Chem. Rev.* **1977**, *77*, 313-348.

(52) van Haaren, R. J.; Goubitz, K.; Fraanje, J.; van Strijdonck, G. P. F.; Oevering, H.; Coussens, B.; Reek, J. N. H.; Kamer, P. C. J.; van Leeuwen, P. *Inorganic Chemistry* **2001**, *40*, 3363-3372.

(53) Gogoll, A.; Ornebro, J.; Grennberg, H.; Backvall, J. E. *J. Am. Chem. Soc.* **1994**, *116*, 3631-3632.

(54) Hansson, S.; Norrby, P. O.; Sjogren, M. P. T.; Akermark, B.; Cucciolito, M. E.; Giordano, F.; Vitagliano, A. *Organometallics* **1993**, *12*, 4940-4948.

(55) van Leeuwen, P.; Kamer, P. C. J.; Reek, J. N. H. *Pure Appl. Chem.* **1999**, *71*, 1443-1452.

(56) Pierpont, C. G.; Mazza, M. C. *Inorg. Chem.* **1974**, *13*, 1891-1895.

(57) Mazza, M. C.; Pierpont, C. G. *Inorg. Chem.* **1973**, *12*, 2955-2959.

(58) Mann, B. E.; Pietropaolo, R.; Shaw, B. L. *J. Chem. Soc. Chem. Commun.* **1971**, 790-791.

(59) von Matt, P.; Lloyd-Jones, G. C.; Minidis, A. B. E.; Pfaltz, A.; Macko, L.; Neuburger, M.; Zehnder, M.; Rüegger, H.; Pregosin, P. S. *Hel. Chim. Acta* **1995**, *78*, 265-284.

(60) van Haaren, R. J.; Oevering, H.; Coussens, B. B.; cvan Strijdonck, G. P. F.; Reek, J. N. H.; Kamer, P. C. J.; van Leeuwen, P. *Eur. J. Inorg. Chem.* **1999**, 1237-1241.

(61) Hall, R. H.; A., C. A. *J. Chem. Soc. Perkin Trans. 2* **1974**, 728-732.

(62) Rico, m.; Santoro, J. *Org. Magn. Res.* **1976**, *8*, 49-55.

(63) Thiem, J.; Ossowski, P. *J. Carbohyr. Chem.* **1984**, *3*, 287-313.

(64) Horton, D.; Priebe, W.; Sznaidman, M. *Carbohydr. Res.* **1990**, *205*, 71-86.

(65) Watson, I. D. G.; Yudin, A. K. *J. Am. Chem. Soc.* **2005**, *127*, 17516-17529.

(66) Bolitt, V.; Chaguir, B.; Sinou, D. *Tetrahedron Lett.* **1992**, *33*, 2481-2484.

(67) de Oliveira, R. N.; Cottier, L.; Sinou, D.; Srivastava, R. A. *Tetrahedron* **2005**, *61*, 8271-8281.

(68) Murahashi, S.; Taniguchi, Y.; Imada, Y.; Tanigawa, Y. *J. Org. Chem.* **1989**, *54*, 3292-3303.

(69) Staudinger, H.; Meyer, J. *Helv. Chim. Acta.* **1919**, *2*, 635-646.

(70) Chandler, M.; Bamford, M. J.; Conroy, R.; Lanmont, B.; Patel, B.; Patel, V. K.; Steeples, L. P.; Storer, R.; Weir, N. G.; M., W.; Williamson, C. *J. Chem. Soc. Perkin Trans. 1* **2001**, 1098-1102.

Chapter 2

An Easy Access to α-Glycosyl Chloride via TCT/DMF

1. Introduction

1.1. Previous methods in preparations of α-glycosyl chlorides

In the past decades, glycosyl halides are widely employed as donors in constructions of *O*-, *C*-, *N*-glycosides.[1-3] A vast number of methods have been established. Among them, glycosyl chloride received much less attention and elaborated studies towards its advantage are rarely described.[4] Usually, per-*O*-acetylated sugars are straightforwardly converted to the corresponding glycosyl chlorides by treatment of anhydrous HCl in various sovlents, AcCl, $SOCl_2$/AcOH, $SOCl_2$/$SnCl_4$, PCl_5, $AlCl_3$, $ZnCl_2$, $TiCl_4$, $BiCl_3$/$MeSiCl_3$, triphosgene or α-dichloromethyl methyl ether (DCMME) (Figure 1).[5-9] Unfortunately, the acid-labile groups can not survive under those harsh conditions, and the generally-adopted reagent (DCMME) is a lachrymatory, quite expensive and toxic, which impedes its large-scale preparation.[10-11]

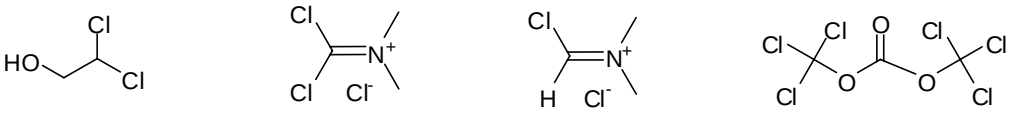
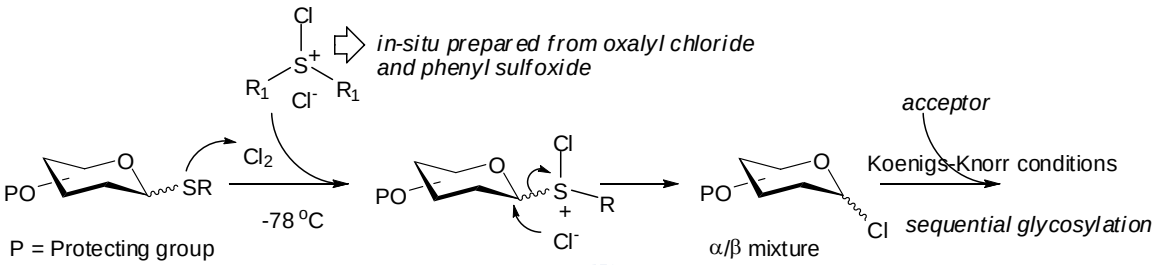


Figure 1 - Common chlorinating reagent for preparation of glycosyl chlorides

Activation of thioglycoside via ICl to prepare glycosyl chlorides also has been reported, however, the instant realease of HCl may impair the acid-sensitive groups, which limit its scope.[12] Recently, a mild protocol through chlorosulfonium chloride reagent converting thioglycoside to α/β. mixed glycosyl chloride at –78 °C has been disclosed and the crude chloride was directly subjected to the following Koenigs-Knorr glycosylation with the several acceptors (Scheme 1).[13]

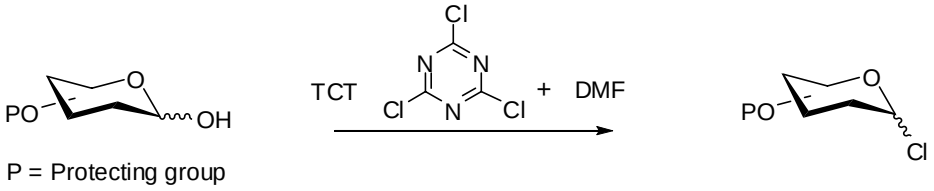


Scheme 1 - Conversion from thioglycoside to glycosyl chloride via chlorosulfonium chloride

The other basic or neutral methods derived from sugar hemiacetals, such as PPh_3/CCl_4, $(Me_2N)_3P/CCl_4$,[14] n-BuLi/$ClPO(OPh)_2$,[15] chloroenamines (Viehe's salt or Vilsmeier-Haack reagent),[16] TsCl/DMAP/Et_3N,[17] have been mentioned (Figure 1). In those aforementioned procedures, handling hazardous, costly, and excess reagents, even sometimes at lower temperature might arouse several potential drawbacks. Therefore, developing a milder, bench-top and cheap reagent to transform hemiacetals to the corresponding glycosyl chlorides is highly desirable.

Therefore, we envisaged that a new method to prepare glycosyl chloride from hemiacetal using Vilsmeier-Haack (VH) reagent in-situ generated from 2,4,6-trichloro-1,3,5-triazine (TCT) and *N,N*-dimethylformamide (DMF) (Scheme 2). Using the complex TCT/DMF converting amide to nitrile was firstly reported by Rodriguez *et al.* in 1997,[18]and many applications using TCT appeared in the past decade.[19-25] A mild chlorination and

chemoselective formylation of alcohol via TCT/DMF complex have been reported by Giacomelli *et al*.[26, 27] Their findings prompted us to consider its use in synthetic carbohydrate chemistry (Scheme 2).



Scheme 2 - Preparation of glycosyl chloride via TCT/DMF

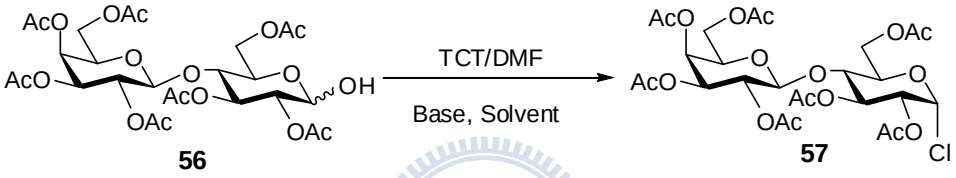
2. Results and discussion

2.1. Preliminary results and condition optimizations

In the first trial, according to the reported procedure, in-situ generation of VH-reagent via TCT (1.2 equiv.) and DMF (4.0 equiv.) at ambient temperature followed by addition of per-*O*-acetylated lactosyl hemiacetal **56** as a model substrate in dichloromethane (DCM) showed a sluggish result with occurrence of several products on TLC tracking (Table 1). After 48 h, the desired α-lactosyl chloride was isolated in moderate yield (58%) (entry 1). Repeating this reaction at 60 ^{o}C in 1,2-dichloroethane (DCE) provided a clean conversion from **56** to **57** in 4 h in good yield (85%) (entry 2). In addition, 0.9 equivalent of 1, 8-diazabicyclo-[5.4.0]undec-7-ene (DBU) as an acid scavenger was able to shorten the reaction time (entry 3), whereas an excess of homogenous base retarded the conversion (entry 5). A longer reaction time in line with a lower temperature was also observed (entry 4). On

the other hand, *N*-methylmorpholine (NMM), Et_3N, or inorganic base (K_2CO_3) performed similarly as DBU (entry 6-8). Other solvents, such as CH_3CN and toluene, also worked efficiently as DCE (entry 9 and 10). During the reaction course, we observed that the final mixture became suspended upon completion of the conversion. To exploit the advantage of this physical property, pouring ether into resulting mixture can precipitate most of used reagent, which was subsequently filtered off. The collected filtrate was concentrated, providing crude chloride for use in the next step without tedious work-up procedures.

Table 1 - Lactosyl hemiacetal **56** as a model substrate for condition optimizations

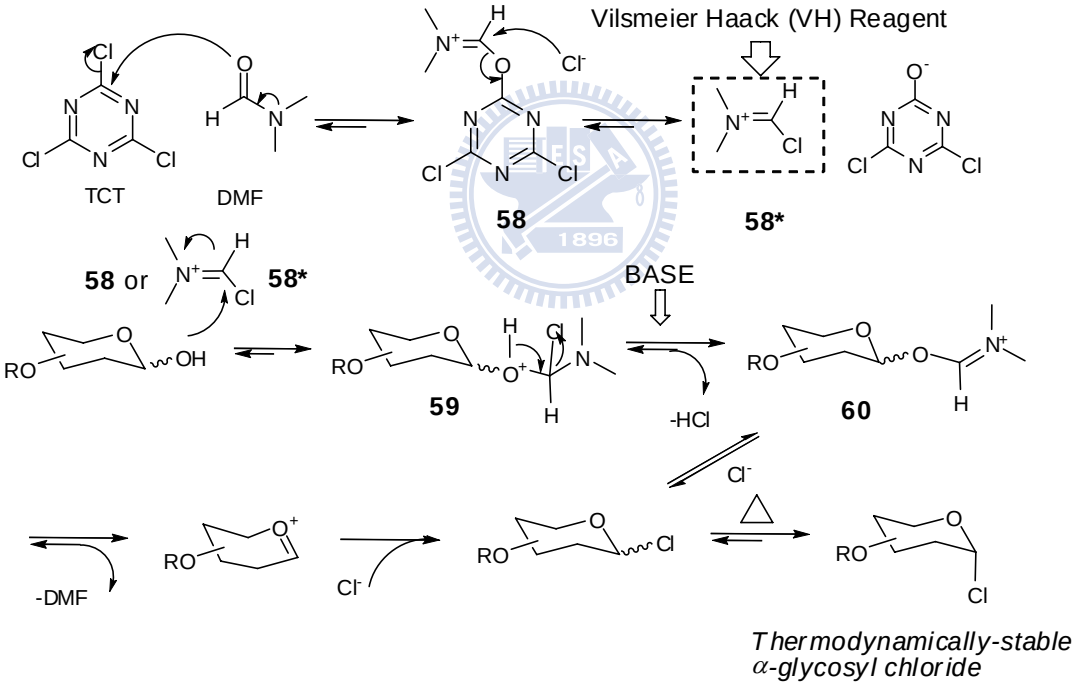


Entry	Base	Solvent	*T* ℃/t (h)	Yield of **57** (%)[a]
1	none	DCM	25/48h	58
2	none	DCE	60/4h	85
3	DBU	DCE	60/1h	87
4	DBU	DCE	50/2.5h	83
5	DBU	DCE	60/48h	25[b]
6	Et_3N	DCE	50/4h	81
7	NMM	DCE	50/4h	79
8	K_2CO_3	DCE	50/4h	75[c]
9	DBU	CH_3CN	60/3h	82
10	DBU	Toluene	60/3h	81

[a] Isolated yield via SiO_2 column chromatography; [b] 5.0 equiv. of DBU was used; [c] 5.0 equiv. of $K_2CO_3$was used.

2.2. Mechanistic aspect

In terms of mechanism, the preliminary results suggested that the formation of exclusive α-glycosyl chloride should be a thermodynamically-driven process involving several equilibrated intermediates. To clarify the role of reagent derived from TCT/DMF during the course of the reaction, treatment of TCT alone with hemiacetal **56** in the presence of DBU gave us a coupled product between **56** and TCT, in accordance with the results reported by Schimdt *et al.*[28]



Scheme 3 - Plausible mechanism for TCT/DMF chlorination at C-1

It showed that DMF participates in the formation of VH reagent resulted and the cationic species (either **58** or chloroiminum salt **58***-VH reagent) associate with the anomeric hydroxy groups as an intact ion pair **59**. Elimination of HCl under the assistance of base is able to generate the more reactive intermediate **60**, which allows the dissociation of DMF to form the

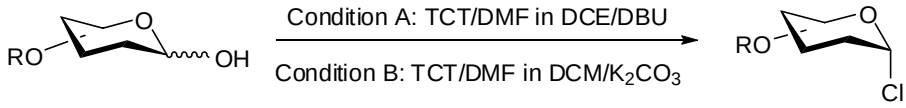
oxocarbenium followed by attachment of chloride. The α/β mixed anomers likely proceed by anomerization to the more stable α-chloride (Scheme 3). The direct nucleophilic substitution via chloride from **60** to α/β mixed chlorides is also considered as a possible route.

2.3. Applications in carbohydrate chemistry

2.3.1. Preparation of α-glycosyl chlorides from glycosyl hemiacetals

The optimized conditions can be generally performed with a variety of hemiacetals to yield the corresponding α-glycosyl chlorides (65-92%) (Table 2). It should be mentioned that ester-type of substrates usually require a higher temperature (60 ^{o}C) to complete the reaction. (**61b**-**68b** and **72b**). This protocol also can be applied to prepare **64b** (1-chloro-3,4,6-*O*-acetyl-2-azido -D-glucopyranoside) in gram-scale with a satisfying 76% yield. Noticeably, this protocol shows the comparable result with the current methods by use of azidonitration[29] or DCMME/$ZnCl_2$.[30] Moreover, substrates (**69b**, **70b, 71b**, **73b**) bearing electron-donating groups usually adopt DCM as a solvent in the presence of K_2CO_3 at 45 ^{o}C. Upon simple filtration, further purification was roughly accomplished by short column chromatography. The success of preparations of the corresponding chlorides possessing 4,6-*O*-benzylidene, 2,3-*O*-isopropylidene, 5,6-*O*-isopropylidene (**70b**, **71b**, **73b**) manifested the considerable functional group tolerance. Besides that, it should be noticed that VH-reagent generated via $POCl_3$/DMF converts 6-*O*-TBS ether groups of glucals into 6-*O*-formylated derivatives, which implied incompatibility between VH-reagent and silyl groups.[31] Apparently, the survival of **72b** under the current conditions demonstrated that TCT/DMF is more benign than other types of VH reagents.

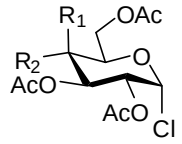
Table 2 - Preparation of α-glycosyl chlorides via TCT/DMF



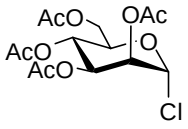
61a–73a
hemiacetals of sugars

61b–73b
α-glycosyl chlorides

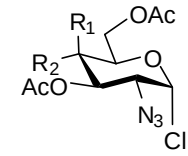
substrate	*T* ℃/t (h)	Time	condition	product	isolated yield (%)
61a	60	5 h	A	**61b**	89
62a	60	3 h	A	**62b**	90
63a	60	2.5 h	A	**63b**	92
64a	60	4 h	A	**64b**	76
65a	60	3 h	A	**65b**	88
66a	60	5 h	A	**66b**	75
67a	60	1 h	A	**67b**	79
68a	60	1 h	A	**68b**	80
69a	60	5 h	B	**69b**	82
70a	45	1 h	B	**70b**	85
71a	45	3 h	B	**71b**	82
72a	60	2 h	A	**72b**	65
73a	45	1 h	B	**73b**	89



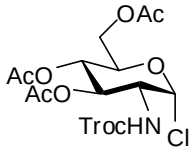
61b, R_1=H, R_2=OAc
62b, R_1=OAc, R_2=H



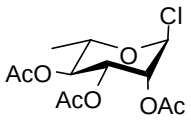
63b



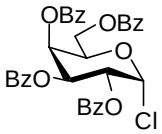
64b, R_1=H, R_2=OAc
65b, R_1=OAc, R_2=H



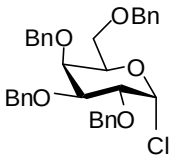
66b



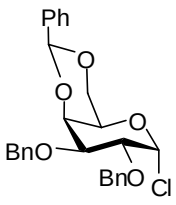
67b



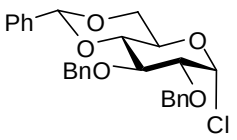
68b



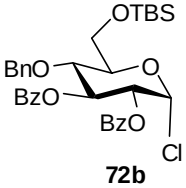
69b



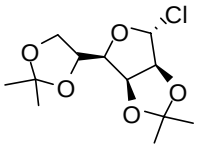
70b



71b



72b

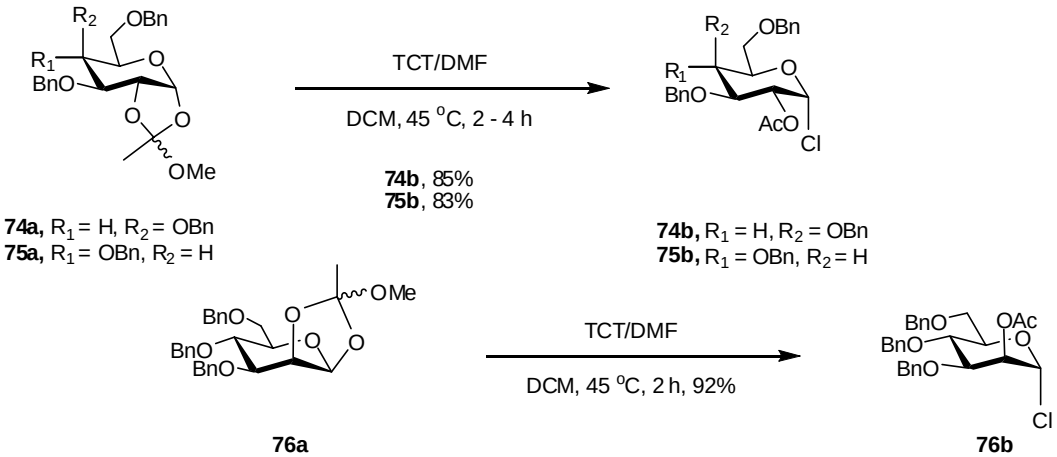


73b

In addition, the known bisacetonide **73a** was converted to the corresponding chloride **73b** using TCT/DMF with an improved yield of 89%, which is superior to the reported procedures.[32,33] Moreover, **73b** can be served as a useful chiral intermediate for the synthesis of natural products.[34]

2.3.2. Preparation of α-glycosyl chlorides from glycosyl orthoesters

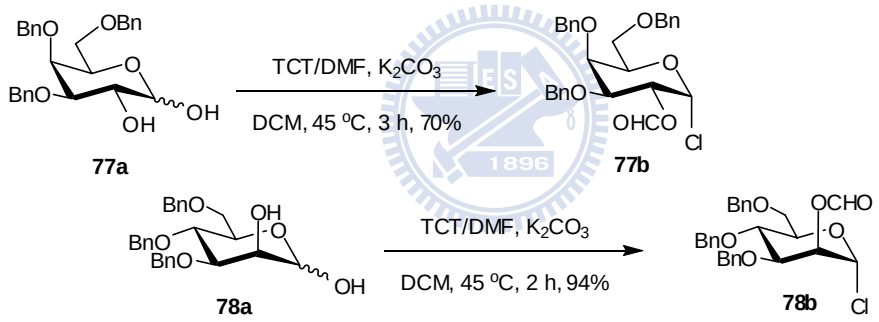
In addition, we envisaged that omitting the base in TCT–DMF chlorination can effect a one-pot conversion of glycosyl orthoester to glycosyl chloride. Though TMSCl was known to carry out this type of transformation,[35] TCT/DMF reagent renders a milder route to prepare the related building blocks. It should be mentioned that similar conversion explained in previous study requires four reaction steps.[36] Thus treatment of glycosyl orthoesters **74a**, **75a** , **76a** with the modified procedure of condition B resulted in the formation of glycosyl chlorides **74b**, **75b** , **76b** in high yields.



Scheme 4 - Preparation of α-glycosyl chlorides from glycosyl orthoesters

2.3.3. Chemoselective chlorination and formylation

As TCT/DMF is also effective for formylation of aliphatic hydroxyl function, it may enable chemoselective hydroxyl formylation at C-2 and anomeric chlorination in one-pot manner. Thus 3,4,6-tri-*O*-benzyl-D-galactopyranose **77a** and 3,4,6-tri-*O*-benzyl-D-mannopy -ranose **78a** were treated with the chlorination protocol. Gratefully, the expected glycosyl chlorides **77b** and **78b** were obtained cleanly in high yields (Scheme 5). This result also clearly represents the intrinsic differences in reactivity between the anomeric hydroxyl group and the other ones on pyranose ring.

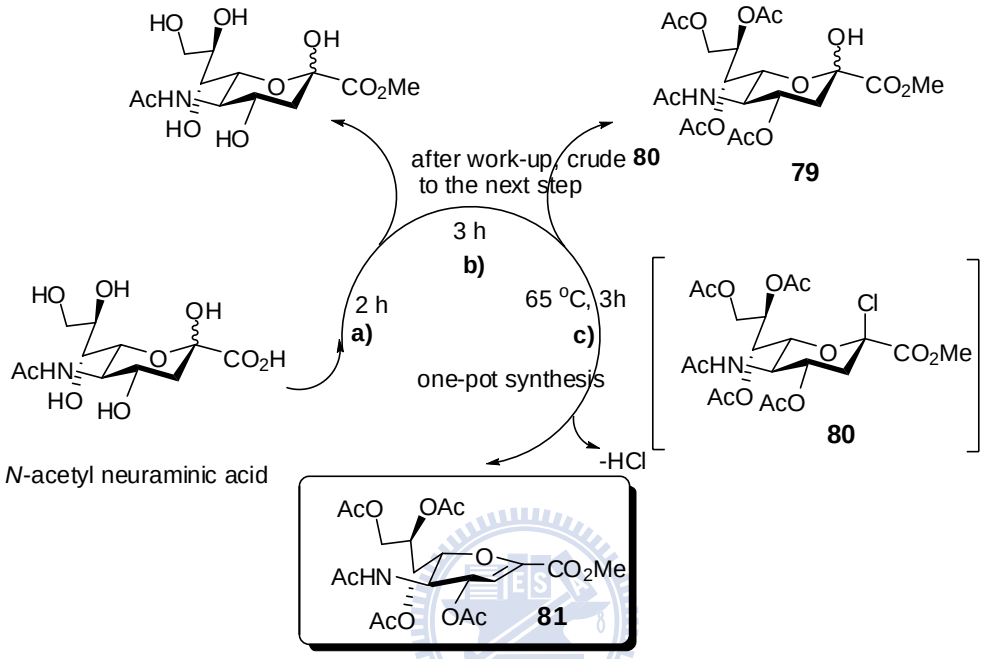


Scheme 5 - Chemoselective chlorination and formylation of **77a** and **78a**

2.3.4. Synthesis of *N*-acetylneuraminic acid glycal

The importance of the glycal **81** for approaching to biologically-relevant targets has been stated recurrently.[37,38] The facile preparation of **79** from free *N*-acetylneuraminic acid (NANA) under catalysis of TsOH has been introduced by our group.[39] Accordingly, treatment of TCT/DMF reagent with **79** in the presence of DBU led to *N*-acetylneuraminic acid glycal **81** in a 72% yield via the transitional Neu5Ac glycosyl chloride **80** using the one-pot strategy

(Scheme 6), which favorably compared to the current methods.[40,41] Thus this modified procedure based on TCT/DMF can be an efficient alternative to spare the time and expense for its synthesis.



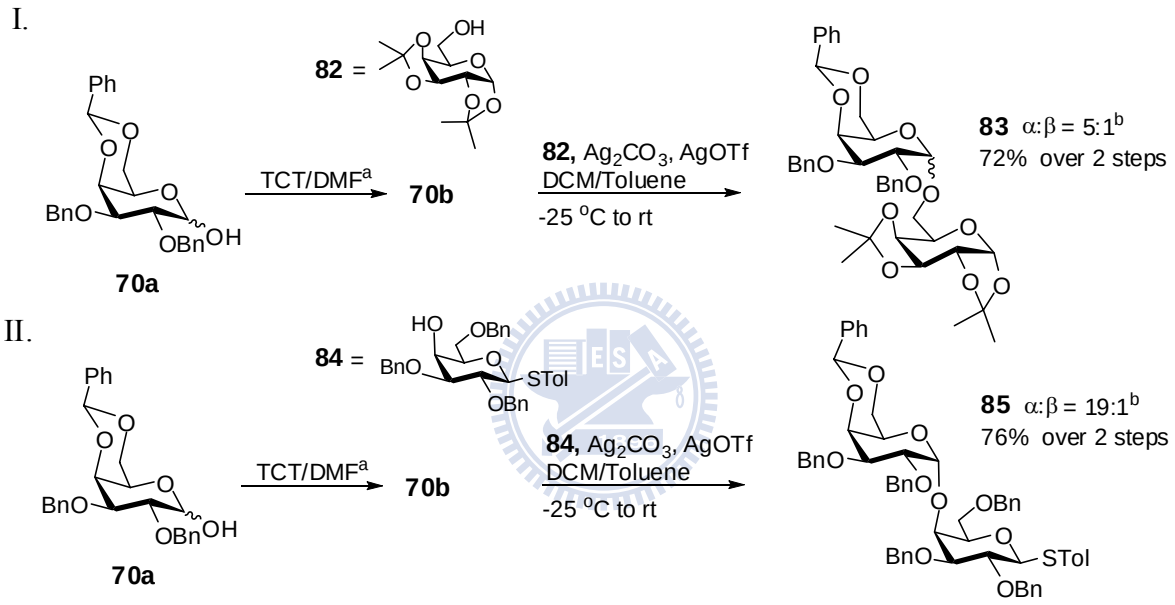
Condition: a) MeOH, PTSA (cat.), rt, 2 h; b) Ac_2O, CH_3CN, rt, 3 h; c) TCT (1.5 eq.), DMF (4 eq.), DBU (1.5 eq.), DCE, 65 °C, 3 h, 72% over 4 steps from *N*-acetyl neuraminic acid

Scheme 6 - One-pot synthesis of NANA glycal **81**

2.3.5. Sequential chlorination-thioglycosidation

As glycosyl chlorides are versatile donors for Koenigs–Knorr glycosylation, it is reasonable to streamline TCT–DMF chlorination and Koenigs–Knorr glycosylation to a sequential process. For this attempt, D-galactopyranosyl hemiacetal **70a** was first treated with TCT–DMF chlorination protocol giving galactopyranosyl chloride **70b** (Scheme 7). Crude galactopy- ranosyl chloride **70b** obtained after simple filtration and solvent removal was used directly as a donor for glycosylation of acceptor **82** without tedious chromatography isolation

of glycosyl chloride. Desired disaccharide **83** was obtained in 72% overall yield as a 5:1 α:β-anomeric mixture. Particularly, the construction of 1,2-*cis*-*O*-linkage for the primary hydroxyl acceptor is always challenging.[42] Such sequential chlorination – glycosylation also works well for thioglycoside acceptor rendering an orthogonal glycosylation possible.[43] Thus **70a** was chlorinated and thereof glycosylated with thiogalactopyranoside **84** furnishing thioglycoside **85** in 76% overall yield and excellent α-selectivity.



[a]TCT (1.1 eq.), DMF (4 eq.), K_2CO_3 (5 eq.) in DCM at 45 °C; [b]ratio was determined via ^{1}H-NMR analysis.

Scheme 7 - Sequential chlorination–glycosylation

3. Conclusion

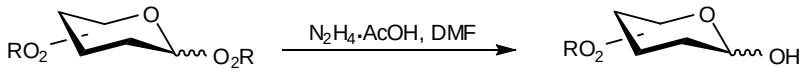
In summary, we developed a mild and efficient TCT-DMF chlorination method for different carbohydrate substrates including glycosyl hemiacetals and glycosyl orthoesters. A new protocol in utilization of stoichimetric TCT/DMF for preparation of an array of α-glycosyl chlorides and *N*-acetyl neuraminic acid glycal with ease of manipulation was also

described accordingly. Readily-accessible glycosyl chlorides allow a new entry to explore its practical use in synthetic carbohydrate chemistry. Furthermore, based on this new chlorination method, a simple sequential chlorination-glycosylation strategy is developed, which should find useful for oligosaccharide synthesis.

4. Experimental

General: Chemicals used in this study were purchased as reagent grade from commercial venders and used without purification. All solvents used in the experiments were dried and distilled by standard techniques including: (1) distillation over CaH_2 for CH_2Cl_2, DMF, toluene, MeOH and (2) drying over molecular sieve for $C_2H_4Cl_2$. Optical rotations were measured with a JASCO DIP-1000 polarimeter at 27 ˚C. Flash column chromatography was performed on silica gel 60 (70–230 mesh, E. Merck). ^1H and ^{13}C NMR spectra were recorded with 300 MHz and 75 MHz spectrometers by either the Brüker console or Varian Unity-300. Chemical shift (δ ppm) was calibrated against the residual proton and ^{13}C signal of deuterated chloroform ($CDCl_3$). Coupling constant(s) in hertz (Hz) were measured from ^1H NMR spectra. Molecular weights of disaccharides $[M + Na]^+$ were determined by BioTOF Ultraflex II (Bruker Daltonics, Billeriaca, MA 01821, USA).

General procedure for preparation of glycosyl hemiacetals: 56, 61a–68a

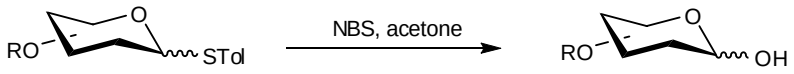


s56, s61-s68 **56, 61a-68a**

Per-*O*-acyl hexopyranosyl substrate (1 equiv of **s56**, **s61-s68**) in DMF solution (4 mL DMF per 1 mmol of sugar substrate) was stirred with hydrazine acetate (1.5 equiv) at rt.[44] Upon completion of the anomeric deacetylation (*ca.* 1–3 h), 1 mL of water was added and DMF was removed under reduced pressure. The residue is dissolved in EtOAc followed by washing with 1% HCl, saturated $NaHCO_3$, brine, dried over $MgSO_4$, filtered and concentrated to give the

crude glycosyl hemiacetal (**56**, **61a-68a**) for TCT/DMF chlorination. For per-*O*-benzoyl galactopyranosyl benzoate **s68**, the procedure is the same as above, but a higher temperature (45 °C) was required and the complete removal of anomeric benzoate needed 15 h.

General procedure for preparation of glycosyl hemiacetals: 11a–14a:



s69–s72 **69a–72a**

To a 9:1 acetone/H_2O solution of thioglycoside (1.3 mmol of **s69-s72**) (30 mL) was added NBS (0.79 g, 4.4 mmol) and the mixture was stirred at 0 °C for 30 min.[45] After then saturated $NaHCO_3$ (40 mL) was added to quench the reaction, followed by the removal of acetone under reduced pressure. The resulting residue was diluted with CH_2Cl_2, and washed with saturated $Na_2S_2O_3$, brine , dried over $MgSO_4$ and concentrated for standard column chromatography to furnish the desired glycosyl hemiacetal (**69a-72a**).

Preparation of D-mannofuranosyl hemiacetal **73a**,[32] glycosyl orthoesters **74a**,[46] **76a**[47] and hexopyranosyl diols **77a**,[46] **78a**[48] were based on literature procedures.

TCT-DMF chlorination protocol A: DMF (1.55 mL, 20.0 mmol) was added to 2,4,6-trichloro-[1,3,5]-triazine (TCT) (1.0 g, 5.5 mmol) and the resulting suspension was stirred at rt for 15 min under N_2. Glycosyl hemiacetal (5.0 mmol) (**56**, **61a-68a** or **72a**) in dichloroethane solution (DCE) was added to the TCT-DMF suspension, and followed by addition of DBU (0.8 mL, 5.5 mmol). The reaction mixture was stirred at 60˚C and progress of

reaction was monitored by TLC (ca. 1–4 h). Upon completion of chlorination, the temperature was brought to rt and Et_2O was added to the mixture for the precipitation of cyanuric salt. After removal of cyanuric salt by filtration, the combined filtrate was concentrated to yield the crude glycosyl chloride. Further purification was performed by fast chromatography elution over a short pad of silica gel to furnish the respective α-glycosyl chloride **57**, **61b-68b** or **72b** TCT-DMF chlorination protocol B for substrates **69a**, **70a**, **71a**, **73a**, **74a**, **76a**, **77a**, **78a**: Similar to protocol A except that CH_2Cl_2 and excess K_2CO_3 (5 mol equiv) were used as solvent and proton scavenger respectively to replace DCE and DBU in protocol A. The reaction was stirred at 45 ˚C and reaction was monitored by TLC examination. For glycosyl orthoesters **74a** and **76a**, K_2CO_3 was omitted to achieve cleavage of orthoester function. Subsequent workup followed the same procedure as described above (protocol A) to obtain the respective α-glycosyl chloride **69b**, **70b**, **71b**, **73b**, **74b**, **76b**, **77b**, **78b**..

2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyranosyl chloride (**57**). Protocol A; eluent for column chromatographic purification, EtOAc/Hexane = 1/1, white solid (87% yield); R_f = 0.45 (TLC developing solution: EtOAc/Hexane = 1/1); **^{1}H NMR** (300 MHz, $CDCl_3$) : δ 6.14 (d, J = 3.0 Hz, 1H, H-1'), 5.49 (t, J = 9.0 Hz, 1H), 5.29 (dd, J = 3.3, 1.0 Hz, 1H), 5.07 (dd, J = 10.7, 7.8 Hz, 1H), 4.95 (dd, J = 10.5, 3.4 Hz, 1H), 4.92 (dd, J = 10.0, 3.9 Hz, 1H), 4.46 (d, J = 7.8 Hz, 1H), 4.01–4.23 (m, 5H), 3.75–3.89 (m, 2H), 2.10 (s, 3H), 2.07 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.90 (s, 3H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 170.2, 170.1, 170.0, 169.9, 169.2, 168.8, 100.7 (C-1'), 89.9 (C-1), 75.0, 71.2, 70.8, 70.9, 70.7, 68.9, 68.8, 66.5, 61.1, 60.8, 60.3, 20.6, 20.5, 20.3. The spectroscopic data agrees with the literature values.[49]

2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl chloride (**61b**): Protocol A; eluent for column chromatographic purification, EtOAc/Hexane = 1/2, white solid (89% yield); R_f = 0.42 (TLC developing solution: EtOAc/Hexane = 1/2); **^{1}H NMR** (300 MHz, $CDCl_3$) : δ 6.24 (d, J = 4.0 Hz, 1H, H-1), 5.50 (t, J = 9.8 Hz, 1H), 5.08 (t, J = 9.8 Hz, 1H), 4.96 (dd, J = 9.8, 4.0 Hz, 1H), 4.34–4.19 (m, 2H), 4.07 (dd, J = 14.1, 3.7 Hz, 1H), 2.04 (s, 3H), 2.03 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) : δ 170.4, 169.78, 169.7, 169.3, 90.0 (C-1), 70.6, 70.3, 69.3, 67.3, 61.0, 20.6, 20.5, 20.5, 20.4. The spectroscopic data agrees with the literature values.[49]

2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl chloride (**62b**): Protocol A; eluent for column chromatographic purification, EtOAc/Hexane = 1/2, white solid (90 % yield); R_f = 0.42 (TLC developing solution: EtOAc/Hexane = 1/2); **^{1}H NMR** (300 MHz, $CDCl_3$) : δ 6.34 (d, J = 3.9 Hz, 1H, H-1), 5.49 (dd, J = 3.2, 1.2 Hz, 1H), 5.39 (dd, J = 10.7, 3.3 Hz, 1H), 5.22 (dd, J = 10.7, 3.9 Hz, 1H), 4.49 (t, J = 6.3 Hz, 1H), 4.28–3.95 (m, 2H), 2.12 (s, 3H), 2.08 (s, 3H), 2.03 (s, 3H), 1.98 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) : δ 170.3, 170.1, 169.9, 169.7, 91.1 (C-1), 69.3, 67.8, 67.2, 67.1, 61.0, 20.7, 20.6, 20.6, 20.5. The spectroscopic data agrees with the literature values.[5]

2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl chloride (**63b**): Protocol A; eluent for column chromatographic purification, EtOAc/Hexane = 1/2, white solid (92 % yield); R_f = 0.40 (TLC developing solution: EtOAc/Hexane = 1/2); **^{1}H NMR** (300 MHz, $CDCl_3$) : δ 5.96 (d, J = 0.9 Hz, 1H, H-1), 5.59 (dd, J = 10.1, 3.3 Hz, 1H), 5.42–5.25 (m, 2H), 4.37–4.04 (m, 3H), 2.16 (s, 3H), 2.09 (s, 3H), 2.05 (s, 3H), 1.99 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) : δ 170.8, 170.0, 169.9, 169.8, 89.0 (C-1), 71.8, 71.5, 68.0, 65.5, 61.9, 21.0, 21.0, 20.9, 20.8. The spectroscopic data agrees with the literature values.[5]

3,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl chloride (**64b**): Protocol A; eluent for column chromatographic purification, EtOAc/Hexane = 1/2, white solid (76% yield); R_f = 0.40 (TLC developing solution: EtOAc/Hexane = 1/2); **^{1}H NMR** (300 MHz, $CDCl_3$) : δ 6.09 (d, J = 3.8 Hz, 1H, H-1), 5.52 (t, J = 9.0 Hz, 1H), 5.08 (t, J = 9.6 Hz, 1H), 4.35–4.26 (m, 2H), 4.10 (d, J = 4.0 Hz, 1H), 3.84 (dd, J = 10.3, 3.8 Hz, 1H), 2.07 (s, 3H), 2.06 (s, 3H), 2.02 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) : δ 170.8, 170.1, 170.0, 92.0 (C-1), 71.0, 67.9, 62.5, 61.4, 21.0, 20.9, 20.8. The spectroscopic data agrees with the literature values.[54]

3,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl chloride (**65b**): Protocol A; eluent for column chromatographic purification, EtOAc/Hexane = 1/2, white solid (88% yield); R_f = 0.40 (TLC developing solution: EtOAc/Hexane = 1/2); **^{1}H NMR** (300 MHz, $CDCl_3$) : δ 6.13 (d, J = 3.8 Hz, 1H, H-1), 5.45 (dd, J = 3.1, 1.1 Hz, 1H), 5.31 (dd, J = 10.9, 3.2 Hz, 1H), 4.45 (t, J = 6.5 Hz, 1H), 4.11–4.02 (m, 3H), 2.10 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) : δ 170.2, 169.7, 169.4, 92.5 (C-1), 69.6, 68.6, 66.6, 60.8, 58.4, 20.5, 20.4, 20.3. The spectroscopic data agrees with the literature values.[29]

3,4,6-Tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbamyl)-α-D-glucopyranosyl chloride (**66b**): Protocol A; eluent for column chromatographic purification, EtOAc/Hexane = 1/2, white solid (75% yield); R_f = 0.40 (TLC developing solution: EtOAc/Hexane = 1/2); **^{1}H NMR** (300 MHz, $CDCl_3$) : δ 6.17 (d, J = 3.6 Hz, 1H), 5.44 (d, J = 9.2 Hz, 1H), 5.33 (t, J = 10.0 Hz, 1H), 5.17 (t, J = 9.8 Hz, 1H), 4.78 (d, J = 12.0 Hz, 1H), 4.62 (d, J = 12.0 Hz, 1H), 4.36–4.17 (m, 3H), 4.10 (d, J = 11.2 Hz, 1H), 2.07 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H); **^{13}C NMR** (75 MHz, $CDCl_3$) : δ 170.9, 170.5, 169.2, 154.0, 95.1, 93.3, 74.7, 70.9, 69.8, 67.0, 61.1, 55.4, 20.6, 20.6, 20.5. The spectroscopic data agrees with the literature values.[49]

2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl chloride (**67b**): Protocol A; eluent for column chromatographic purification, EtOAc/Hexane = 1/5, a colorless syrup (79% yield); R_f = 0.45 (TLC developing solution: EtOAc/Hexane = 1/4); **^{1}H NMR** (300 MHz, $CDCl_3$) : δ 5.89 (d, J =1.8 Hz, 1H, H-1), 5.52 (dd, J = 10.0, 3.4 Hz, 1H), 5.33 (dd, J = 3.3, 1.6 Hz, 1H), 5.09 (t, J = 10.1 Hz, 1H), 4.14-4.10 (m, 1H), 2.13 (s, 3H), 2.03 (s, 3H), 1.96 (s, 3H), 1.23 (d, J = 6.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) : δ 169.8, 169.7, 169.6, 89.0 (C-1), 71.8, 70.3, 69.4, 67.7, 20.8, 20.7, 20.6, 17.1. The spectroscopic data agrees with the literature values.[50]

2,3,4,6-Tetra-*O*-benzoyl-α-D-galactopyranosyl chloride (**68b**): Protocol A; eluent for column chromatographic purification, EtOAc/Hexane = 1/4, white solid (80% yield); R_f = 0.48 (TLC developing solution: EtOAc/Hexane = 1/3); $[\alpha]^{27}_D$ = +58.5 (c = 1.21, $CHCl_3$); **^{1}H NMR** (300 MHz, $CDCl_3$) : δ 8.14–7.93 (m, 6H), 7.87–7.77 (m, 2H), 7.59–7.48 (m, 10H), 7.24 (dd, J = 8.2, 7.2 Hz, 3H), 6.66 (d, J = 3.9 Hz, 1H, H-1), 6.17–6.00 (m, 2H), 5.85 (dd, J = 10.4, 3.9 Hz, 1H), 4.94 (t, J = 6.4 Hz, 1H), 4.63 (dd, J = 11.5, 6.7 Hz, 1H), 4.44 (dd, J = 11.5, 6.1 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) : δ 165.9, 165.6, 165.4, 165.3, 133.7, 133.3, 133.3, 129.9, 129.9, 129.8, 129.7, 129.2, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 91.5 (C-1), 70.0, 68.7, 68.3, 67.9, 61.7.

2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl chloride (**69b**): Protocol B; eluent for column chromatographic purification, EtOAc/Hexane = 1/9, a colorless syrup (85% yield); R_f = 0.35 (TLC developing solution: EtOAc/Hexane = 1/9); **^{1}H NMR** (300 MHz, $CDCl_3$) : δ 7.48–7.25 (m, 20H), 6.17 (d, J = 3.8 Hz, 1H, H-1), 4.97 (d, J = 11.3 Hz, 1H), 4.88 (d, J = 11.7 Hz, 1H), 4.81–4.70 (m, 3H), 4.58 (d, J = 11.3 Hz, 1H), 4.50 (d, J = 11.8 Hz, 1H), 4.50 (d, J = 11.8 Hz, 1H), 4.27–4.20 (m, 2H), , 4.04– 3.96 (m, 2H), 3.56 (d, J = 6.7 Hz, 2H); **^{13}C NMR** (75 MHz, $CDCl_3$) : δ 138.4, 138.2, 137.8, 137.6, 128.4, 128.3, 128.2, 127.9, 127.8, 127.8, 127.7, 127.6,

127.5, 94.9 (C-1), 78.3, 76.2, 75.0, 74.3, 73.4, 73.3, 73.0, 72.3, 67.9. The spectroscopic data agrees with the literature values.[13]

2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl chloride (**70b**): Protocol B; eluent for column chromatographic purification, EtOAc/Hexane/CH_2Cl_2 = 1/4/1, a colorless syrup (85% yield); R_f = 0.32 (TLC developing solution: EtOAc/Hexane = 1/4); $[\alpha]^{27}_D$ = +88.1 (c = 0.81, $CHCl_3$); **^{1}H NMR** (300 MHz, $CDCl_3$) : δ 7.53–7.24 (m, 15H), 6.23 (d, J = 3.6 Hz, 1H, H-1), 5.51 (s, 1H), 4.92–4.70 (m, 4H), 4.29–4.11 (m, 3H), 4.09–4.01 (m, 2H), 3.93 (bs, 1H); **^{13}C NMR** (75 MHz, $CDCl_3$) : δ 138.3, 137.7, 137.4, 128.9, 128.3, 128.2, 128.1, 127.8, 127.6, 127.6, 126.1, 100.8, 95.2 (C-1), 75.3, 75.1, 73.9, 73.2, 72.3, 68.7, 65.5. The spectroscopic data agrees with the literature values.[13]

2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl chloride (**71b**): Protocol B; eluent for column chromatographic purification, EtOAc/Hexane/CH_2Cl_2 = 1/4/1, a colorless syrup (82% yield); R_f = 0.30 (TLC developing solution: EtOAc/Hexane = 1/4); $[\alpha]^{27}_D$ = +26.1 (c = 1.16, $CHCl_3$); **^{1}H NMR** (300 MHz, $CDCl_3$) : δ 7.65–7.23 (m, 15H), 6.04 (d, J = 4.0 Hz, 1H, H-1), 5.59 (s, 1H), 4.91–4.74 (m, 4H), 4.34 (dd, J = 10.1, 4.9 Hz, 1H), 4.33–4.10 (m, 2H), 3.85–3.60 (m, 3H); **^{13}C NMR** (75 MHz, $CDCl_3$) : δ 138.3, 137.3, 136.9, 129.0, 128.5, 128.3, 128.3, 128.2, 128.1, 128.0, 127.9, 127.6, 126.0, 101.3, 93.3 (C-1), 80.9, 79.1, 77.7, 75.3, 73.2, 68.2, 65.2.

2,3-Di-*O*-benzoyl-3-*O*-benzyl-6-*O*-*t*-butyldimethylsilyl-α-D-glucopyranosyl chloride (**72b**): **72b** was obtained as a colorless syrup (70% yield) upon column chromatography purification over silica gel with 1/9 EtOAc/Hexane elution. For **72b**, R_f = 0.35 (TLC developing solution:

EtOAc/Hexane = 1/9); $[\alpha]^{27}_{D}$ = +108.8 (*c* = 0.45, $CHCl_3$); **^{1}H NMR** (300 MHz, $CDCl_3$) : δ 8.06-8.03 (m, 4H), 7.61 (t, *J* = 7.4 Hz, 2H), 7.47 (dd, *J* = 10.5, 4.7 Hz, 4H), 7.24 (s, 5H), 6.55 (d, *J* = 3.9 Hz, 1H), 6.18 (t, *J* = 9.7 Hz, 1H), 5.35 (dd, *J* = 10.1, 4.0 Hz, 1H), 4.74 (q, *J* = 10.9 Hz, 2H), 4.33– 4.09 (m, 4H), 4.02 (dd, *J* = 12.0, 1.5 Hz, 1H), 1.06 (s, 9H), 0.24 (s, 3H), 0.22 (s, 3H); **^{13}C NMR** (75 MHz, $CDCl_3$) : δ 165.6, 165.5, 137.3, 133.6, 133.2, 130.0, 129.7, 129.4, 128.6, 128.5, 128.3, 128.3, 128.0, 127.8, 91.3, 75.0, 74.6, 74.6, 72.0, 71.8, 61.1, 25.9, 18.4.

2,3:5,6-Di-*O*-isopropylidene-α-D-mannofuranosyl chloride (**73b**): **73b** was obtained as a colorless syrup (89% yield) upon column chromatography purification over silica gel with 1/5 EtOAc/Hexane elution. For **73b**, R_f = 0.30 (TLC developing solution: EtOAc/Hexane = 1/9); **^{1}H NMR** (300 MHz, $CDCl_3$) : δ 6.02 (s, 1H, H-1), 4.91 (d, *J* = 5.8, 1H), 4.84 (dd, *J* = 5.8, 3.6 Hz, 1H), 4.40–4.38 (m, 1H), 4.16 (dd, *J* = 11.0, 3.6 Hz, 1H), 4.05 (dd, *J* = 8.9 Hz, 6.1 Hz, 1H), 3.97 (dd, *J* = 8.8, 4.4 Hz, 1H), 1.42 (s, 6H), 1.33 (s, 3H), 1.28 (s, 3H); **^{13}C NMR** (75 MHz, $CDCl_3$) : δ 113.2, 109.4, 97.5, 89.1, 82.3, 78.4, 72.2, 66.6, 26.8, 25.7, 25.0, 24.5. The spectroscopic data agrees with the literature values.[32]

2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl chloride (**74b**): Galactopyranosyl hemiacetal **74a** was prepared according to the reported procedure.[8] Preparation of **74b** from **74a** employed protocol A as described in the general procedure. Chromatography purification of **74b** was achieved by 1/4 EtOAc/Hexane elution and **74b** was obtained a colorless syrup (85% yield). For **74b**, R_f = 0.48 (TLC developing solution: EtOAc/Hexane = 1/4); **^{1}H NMR** (300 MHz, $CDCl_3$) : δ 7.51–7.24 (m, 15H), 6.46 (d, *J* = 3.9 Hz, 1H, H-1), 5.48 (dd, *J* = 9.9, 3.9 Hz, 1H), 4.99 (d, *J* = 11.3 Hz, 1H), 4.75 (s, 2H), 4.63 (d, *J* = 11.3 Hz, 1H), 4.52 (d, *J* = 11.0 Hz, 1H), 4.48 (d, *J* = 11.0 Hz, 1H), 4.30 (t, *J* = 6.5 Hz, 1H), 4.14–4.01 (m, 2H), 3.76–3.54 (m, 2H),

2.12 (s, 3H); **^{13}C NMR** (75 MHz, $CDCl_3$) : δ 170.0, 138.0, 137.8, 137.5, 128.7, 128.3, 128.29, 128.1, 128.1, 128.0, 127.8, 127.7, 127.6, 127.5, 127.3, 127.0, 93.0 (C-1), 76.1, 74.9, 73.8, 73.3, 72.8, 72.3, 70.8, 67.7, 20.7. The spectroscopic data agrees with the literature values.[36]

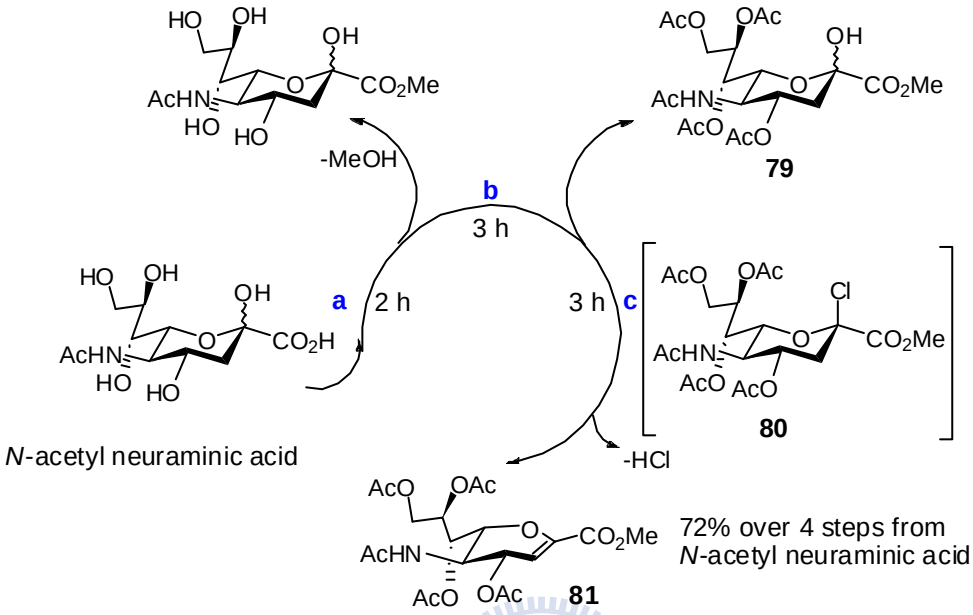
2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl chloride (**76b**): Mannopyranosyl hemiacetal **76a** was prepared according to the reported procedure.[12] Preparation of **76b** from **76a** employed protocol A as described in the general procedure. Chromatography purification of **76b** was achieved by 1/5 EtOAc/Hexane elution and **76b** was obtained a colorless syrup (92% yield). For **76b**, R_f = 0.45 (TLC developing solution: EtOAc/Hexane = 1/5); **^{1}H NMR** (300 MHz, $CDCl_3$) : δ 7.42– 7.12 (m, 15H), 6.07 (d, J = 1.6 Hz, 1H, H-1), 5.47 (dd, J = 3.3, 1.8 Hz, 1H), 4.87, 4.52 (2d, J = 10.7 Hz, 2H), 4.70, 4.56 (2d, J = 11.7 Hz, 2H), 4.63, 4.46 (2d, J = 13.0 Hz, 2H), 4.26 (dd, J = 9.1, 3.3 Hz, 1H), 4.05–4.00 (m, 1H), 3.96 (t, J = 9.6 Hz, 1H), 3.84 (dd, J = 11.1, 3.7 Hz, 1H), 3.69 (dd, J = 11.1, 1.8 Hz, 1H), 2.16 (s, 3H); **^{13}C NMR** (75 MHz, $CDCl_3$) : δ 170.0, 138.0, 137.8, 137.4, 128.4, 128.3, 128.1, 127.9, 127.8, 127.8, 127.7, 127.7, 90.3 (C-1), 76.6, 75.3, 74.2, 73.5, 73.4, 72.1, 70.9, 67.9, 21.0. The spectroscopic data agrees with the literature values.[51]

3,4,6-Tri-*O*-benzyl-2-*O*-formyl-α-D-galactopyranosyl chloride (**77b**): Galactopyrano- syl hemiacetal **77a** was prepared according to the reported procedure.[14] Preparation of **77b** from **77a** employed protocol A as described in the general procedure. Chromatography purification of **77b** was achieved by 1/5 EtOAc/Hexane elution and **77b** was obtained as a colorless syrup (70% yield). For **77b**, R_f = 0.42 (EtOAc/Hexane = 1/5); $[\alpha]^{27}_D$ = +50.6 (c = 0.91, $CHCl_3$); **^{1}H NMR** (300 MHz, $CDCl_3$) : δ 8.10 (d, J = 0.9 Hz, 1H), 7.42–7.20 (m, 15H), 6.37 (d, J = 3.7 Hz, 1H, H-1), 5.58–5.47 (m, 1H), 4.93 (d, J = 11.3 Hz, 1H), 4.70 (s, 2H), 4.55 (d, J = 11.3 Hz, 1H),

4.49 (d, J = 11.8 Hz, 1H), 4.42 (d, J = 11.8, 1H), 4.27–4.23 (m, 1H), 4.08–3.99 (m, 2H), 3.66–3.51 (m, 2H); **^{13}C NMR** (75 MHz, $CDCl_3$) : δ 160.2, 138.2, 138.0, 137.8, 128.8, 128.7, 128.6, 128.4, 128.2, 128.1, 128.0, 127.7, 92.7 (C-1), 76.3, 75.3, 74.1, 73.8, 73.2, 72.7, 70.8, 68.0.

3,4,6-Tri-*O*-benzyl-2-*O*-formyl-α-D-mannopyranosyl chloride (**78b**): Hemiacetal **78a** was prepared according to the reported procedure.[50] Preparation of **78b** from **78a** employed the protocol A as described in the general procedure. Column chromatography purification of **78b** was achieved by 1/5 EtOAc/Hexane elution and **78b** was obtained as a colorless syrup (94% yield). For **78b**, R_f = 0.41 (TLC developing solution: EtOAc/Hexane = 1/5); $[\alpha]^{27}_D$ = +48.5 (c = 1.56, $CHCl_3$); **^{1}H NMR** (300 MHz, $CDCl_3$) : δ 8.17 (s, 1H), 7.44–7.08 (m, 15H), 6.10 (d, J = 1.4 Hz, 1H, H-1), 5.59 (d, J = 1.3 Hz, 1H), 4.87 (d, J = 10.7 Hz, 1H), 4.75–4.60 (m, 3H), 4.56–4.46 (m, 2H), 4.30 (dd, J = 8.6, 3.0 Hz, 1H), 4.10–3.96 (m, 2H), 3.84 (dd, J = 11.0, 3.2 Hz, 1H), 3.69 (dd, J = 11.0, 1.5 Hz, 1H); **^{13}C NMR** (75 MHz, $CDCl_3$) : δ 159.6, 137.8, 137.7, 137.1, 128.5, 128.3, 128.2, 128.0, 127.9, 127.9, 127.8, 127.7, 127.7, 89.8 (C-1), 76.4, 75.3, 74.2, 73.4, 73.2, 72.3, 70.4, 67.6.

Preparation of methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2,6-anhydro-D-glycero-D-galacto-non-2-enonate, Neu5Ac glycal (81):

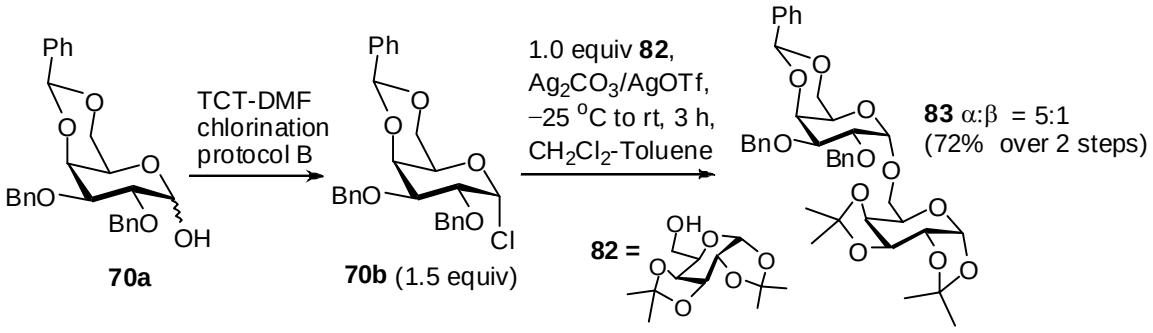


Conditions and reagents: (a) (b) One-pot Fischer glycosidation acetylation, 6–7 h;[39] (c) TCT (1.1 equiv), DMF (4 equiv), DBU (1.1 equiv) in DCE at 65˚C, 3 h. (72% over 4 steps)

To a suspension of *N*-acetyl neuraminic acid (0.62 g, 2.0 mmol) in dry MeOH (20 mL) was added pre-dried *p*-toluenesulfonic acid monohydrate (37 mg, 0.2 mmol), and the suspension was stirred at rt for 2 h. The turbid mixture gradually became clear and upon complete acetylation, the mixture was concentrated to give crude NANA methyl ester. To NANA ester was added acetic anhydride (1.5 mL, 16 mmol) and CH_3CN (1.5 mL) and the resulting suspension was stirred at rt for 3 h until becoming a clear solution. After then, saturated $NaHCO_3$ solution was added to quench the reaction, followed by addition with CH_2Cl_2. The resulting CH_2Cl_2 was washed by saturated $NaHCO_3$ (× 3), brine, dried over $MgSO_4$, filtered and concentrated to furnish the crude product **79** directly used for the next step. To the mixture of TCT (0.43 g, 2.4 mmol) and DMF (0.61 mL, 8 mmol) was added a solution of

79 in DCE (10 mL) followed by addition of DBU (0.60 mL, 4 mmol). The reaction mixture was then stirred at 65 ˚C for 3 h. After cooling to rt, the resulting mixture was diluted with CH_2Cl_2 (30 mL), which was then washed with 1% HCl, saturated $NaHCO_3$, brine, dried over $MgSO_4$, filtered and concentrated for column chromatography purification over silica gel (elution by CH_2Cl_2/MeOH mixture gradient from 1/0 to 49/1) to give peracetyl Neu5Ac glycal **81** as a white glassy solid (0.66 g, 72% over 4 steps). For **Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-enonate** (**81**). **^{1}H NMR** (300 MHz, $CDCl_3$): δ 6.08 (d, J = 8.9 Hz, 1H), 5.92 (d, J = 3.1 Hz, 1H), 5.48–5.45 (m, 2H), 5.30–5.24 (m, 1H), 4.59 (dd, J = 12.3, 3.1 Hz, 1H), 4.34–4.25 (m, 1H), 4.13 (dd, J = 12.3, 7.3 Hz, 1H), 3.74 (s, 3H), 2.06 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.86 (s, 3H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 170.7, 170.5, 170.1, 170.1, 170.1, 161.5, 145.0, 107.9, 76.6, 70.8, 68.0, 67.6, 61.9, 52.5, 46.3, 23.0, 20.7, 20.6. The spectroscopic data agrees with the literature values.[52]

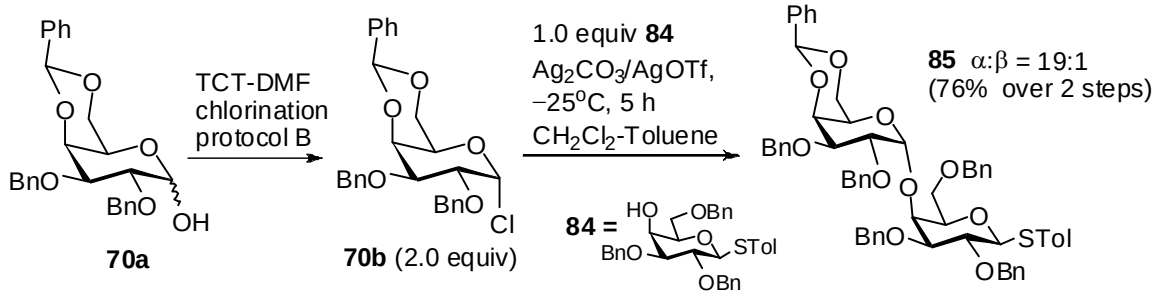
Sequential tandem chlorination–glycosylation



A mixture of commercially available glycosyl acceptor **82** (0.26 g, 1.0 mmol) and AW300 MS (1.0 g) in 4/1 CH_2Cl_2/toluene (5 mL) was stirred at rt under N_2 for 30 min. The mixture was then cooled at –25 ˚C cooling bath followed by addition of $AgCO_3$ (1.1 g, 4.0 mmol) and

AgOTf (0.13 g, 0.5 mmol). Freshly prepared **70b** (0.67 g, 1.5 mmol of **70a**) in CH_2Cl_2 (5 mL) was gradually delivered to the reaction mixture over 1 h by the syringe pump. The resulting mixture was subsequently stirred from –25 ˚C to rt. Upon the complete consumption of **82** as shown by TLC, the resulting mixture was filtered through Celite to remove the silver salts. The filtrate was concentrated for column chromatography purification over silica gel (EtOAc/Hexane elution: gradient from 0/1 to 3/7) to give the disaccharide **83** as a 5:1 α/β-anomeric mixture. **(2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl)-(1→6)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose** (**83α**): white solid, mp = 108 ˚C; R_f = 0.55 (TLC developing solution: EtOAc/Hexane = 2/3); $[\alpha]^{27}_D$ = +29.1 (*c* = 1.20, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) : δ 7.50 (dd, *J* = 7.3, 2.2 Hz, 2H), 7.44–7.25 (m, 13H), 5.57–5.39 (m, 2H), 5.03 (d, *J* = 3.3 Hz, 1H, H-1), 4.85–4.69 (m, 5H), 4.56 (dd, *J* = 7.7, 2.3 Hz, 1H), 4.34–4.11 (m, 5H), 4.07–3.87 (m, 4H), 3.78–3.69 (m, 3H), 1.48 (s, 3H), 1.42 (s, 3H), 1.30 (s, 3H), 1.29 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) : δ 138.8, 138.7, 137.9, 128.8, 128.2, 128.0, 127.7, 127.6, 127.5, 127.4, 126.3, 109.3, 108.5, 101.03, 98.2 ($J_{C\text{-}H}$ = 169 Hz, anomeric CH), 96.3 ($J_{C\text{-}H}$ = 179 Hz, anomeric CH), 75.9, 75.5, 74.7, 73.2, 72.0, 71.0, 70.6, 70.5, 69.4, 67.0, 66.6, 62.5, 26.1, 26.0, 24.9, 24.5; HRMS (Bio-ToFII): calcd for $C_{39}H_{46}O_{11}Na$ requires 713.2938; found: *m*/*z* = 713.2932 $[M + Na]^+$.

Sequential tandem chlorination–orthogonal glycosylation



A mixture of thioglycosyl acceptor **84** (0.56 g, 1.0 mmol) and AW300 MS (1.0 g) in 4/1 CH_2Cl_2/toluene (5 mL) was stirred at rt under N_2 for 30 min. The mixture was then cooled at –25 ˚C cooling bath followed by addition of $AgCO_3$ (1.1 g, 4.0 mmol) and AgOTf (0.13 g, 0.5 mmol). Freshly prepared **70b** (0.90 g, 2.0 mmol of **70a**) in CH_2Cl_2 (5 mL) was gradually delivered to the reaction mixture over 4 h. The mixture was subsequently stirred at –25 ˚C until the complete consumption of **84** as shown by TLC, the resulting mixture was filtered through Celite to remove the silver salts. The filtrate was concentrated for column chromatography purification over silica gel (EtOAc/Hexane/CH_2Cl_2 elution: gradient from 0/8/2 to 2/6/2) to give the disaccharide **85** as the single α anomer (0.75 g, 76%). The stereochemistry of α anomer was determined via the method described above.[53] For **Tolyl 4-*O*-(2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosy-l)-(1→4)-*O*-2,3,6-tri-benzyl–thio-β-D-galactopyranoside (85)**: white solid (recrystallized from Et_2O and hexane), melting point can not be measured due to decomposition over 180 ˚C; R_f = 0.46 (TLC developing solution: EtOAc/Hexane = 2/3); $[\alpha]^{27}_D$ = +50.7 (*c* = 1.32, $CHCl_3$); **^{1}H NMR** (300 MHz, $CDCl_3$) : δ 7.68 (d, *J* = 8.1 Hz, 2H), 7.59–7.31 (m, 30H), 7.12 (d, *J* = 8.1 Hz, 2H), 5.40 (s, 1H), 5.25 (d, *J* = 3.3 Hz, 1H, H-1'), 5.12 (d, *J* = 11.7 Hz, 1H), 4.87–4.75 (m, 6H), 4.68–4.59 (m, 2H), 4.44–4.01 (m, 9H), 3.87 (t, *J* = 9.4 Hz, 1H), 3.75–3.54 (m, 3H), 3.46 (d, *J* = 12.5 Hz, 1H), 2.22 (s, 3H); **^{13}C NMR** (75 MHz, $CDCl_3$) : δ 138.8, 138.7, 138.1, 138.0, 137.9, 137.7, 136.8, 131.9, 129.92, 129.5, 128.7, 128.3, 128.2, 128.2, 128.0, 127.8, 127.8, 127.7, 127.5, 127.4, 127.3, 127.24, 126.8, 126.2, 100.6, 99.9 ($J_{C\text{-}H}$ = 169 Hz, anomeric CH), 86.9, 82.7, 77.1, 76.5, 75.8, 75.5, 75.1, 74.3, 73.9, 73.1, 72.7, 71.9, 70.7, 69.2, 67.0, 62.4, 20.9. HRMS (Bio-ToFII): calcd for $C_{61}H_{62}O_{10}SNa$ requires 1009.3961; found: *m*/*z* = 1009.3956 $[M + Na]^+$.

5. References

(1) Toshima, K. *Glycosyl Halides. In Glycoscience; Fraser-Reid, B., Tatsuta, K. Thiem, J., Eds,; Springer: Berlin, Heidelberg* **2008**, pp 429-449.

(2) Demchenko, A. V. *Handbook of Chemical Glycosylation; Wiley-VCH;* **2008**, pp 29-85.

(3) Brito-Arias, M. *Synthesis and Characterization of Glycosides;* Springer*;* **2006**, pp 151-161.

(4) Köenigs, W.; Knorr, E. *Chem. Ber.* **1901**, *34*, 957.

(5) Montero, J.-L.; Winum, J.-Y., et al. *Carbohydr. Res.* **1997**, *297*, 175-180.

(6) Chosh, R.; Chakraborty, A.; Maiti, S. *Tetrahedron* **2001**, *57*, 9631-9634.

(7) Wang, Q.-B.; Fu, J.; Zhang, J.-B. *Cabohydr. Res.* **2008**, *343*, 2989-2991.

(8) Cicchillo, R. M.; Norris, P. *Cabohydr. Res.* **2000**, *328*, 431-434.

(9) Pozsgay, V.; Dubois, E. P.; Pannell, L. *J. Org. Chem.* **1997**, *62*, 2832-2846.

(10) Khan, S. H.; O'Neill, R. A. *Modern Methods in Carbohydrate Synthesis;* Kováč, P.*;* CRC Press*,* **1996**, pp 55-80.

(11) Edgar, K. J.; J. E. K. *J. Org. Chem.* **1992**, *57*, 2455-2467.

(12) Kartha, K. P. R.; Cura, P.; Aloui, M.; Readman, S. K.; Rutherford, T. J.; Field, R. A. *Tetrahedron:Asymmetry* **2000**, *11*, 581-593.

(13) Sugiyama, S.; Diakur, J. M. *Org. Lett.* **2000**, *2*, 2713-2715.

(14) Chretien, F.; Chapleur, Y.; Castro, B.; Gross, B. *J. Chem. Soc. Perkin Trans. 1* **1980**, 381-284.

(15) Hung, S.-C.; Wong, C.-H. *Tetrahedron Lett.* **1996**, *37*, 4903-4906.

(16) Copeland, C.; McAdam, D. P.; Stick, R. V. *Aust. J. Chem.* **1983**, *36*, 1239-1247.

(17) Hwang, C.-K.; Li, W.-S.; Nicolaou, K. C. *Tetrahedron Lett.* **1984**, *25*, 2295-2296.

(18) Maetz, P.; Rodriguez, M. *Tetrahedron Lett.* **1997**, *38*, 4221-4222.

(19) Blotny, G. *Tetrahedron* **2006**, *62*, 9507-9522.

(20) Giacomelli, G.; Porcheddu, A.; De Luca, L. *Curr. Org.Chem.* **2004**, *8*, 1497-1519.

(21) Bandgar, B. P.; Joshi, N. S.; Kamble, V. T.; Sawant, S. S. *Aust. J. Chem.* **2008**, *61*, 231-234.

(22) De Luca, L.; Giacomelli, G.; Nieddu, G. *J. Org. Chem.* **2007**, *72*, 3955-3957.

(23) Kolmakov, K. A. *Can. J. Chem.* **2007**, *85*, 1070-1074.

(24) Sun, L.; Guo, Y.; Peng, G.-S.; Li, C.-B. *Synthesis-Stuttgart* **2008**, *21*, 3487-3491.

(25) Zhang, Z.-H.; Tao, X. Y. *Aust. J. Chem.* **2008**, *61*, 77-79.

(26) De Luca, L.; Giacomelli, G.; Porcheddu, A. *J. Org. Chem.* **2002**, *67*, 5152-5155.

(27) De Luca, L.; Giacomelli, G.; Porcheddu, A. *Org. Lett.* **2002**, *4*, 553-555.

(28) Huchel, U.; Schimdt, C.; Schimdt, R. R. *Eur. J. Org. Chem.* **1998**, 1353-1360.

(29) Lemieux, R. U.; Ractliffe, R. M. *Can. J. Chem.* **1979**, *57*, 1244-1251.

(30) Pavliak, V.; Kováč, P.; Glaudeman, C. P. J. *Carbohydr. Res.* **1992**, *229*, 103-116.

(31) Lellouche, J. P.; Kotlyar, V. *Synlett* **2004**, 564-571.

(32) Kim, C.; Hoang, R.; Theodorakis, E. A. *Org. Lett.* **1999**, *1*, 1295-1297.

(33) Oishi, T.; Inomiya, K.; Sato, H.; Lida, M.; Chida, N. *Bull. Chem. Soc. Jpn.* **2002**, *75*, 1927-1948.

(34) Brady, T. P.; Kim, S.-H.; Wen, K.; Kim, C.; Theodorakis, E. A. *Chem. Eur. J.* **2005**, *11*, 7175-7190.

(35) Newman, M. S.; Olson, D. R. *J. Org. Chem.* **1973**, *38*, 4203-4204.

(36) Wotovic, A.; Jacquinet, J. C. *Carbohydr. Res.* **1990**, *205*, 235-245.

(37) Boons, G. J.; Demchenko, A. V. *Chem. Rev.* **2000**, *100*, 4539-4565.

(38) Burkart, M. D.; Vincent, S. P.; Wong, C.-H. *J. Chem. Soc. Chem. Commun.* **1999**,

1525-1526.

(39) Chao, C.-S.; Chen, C.-C.; Lin, S.-C.; Mong, K.-K. T. *Cabohydr. Res.* **2008**, *343*, 957-964.

(40) Kulikova, N. Y.; Shpirt, A. M.; Kononov, L. O. *Synthesis* **2006**, *24*, 4113-4114.

(41) Okamoto, K.; Kondo, T. ; Goto, T. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 631-636.

(42) Demchenko, A. V. *Curr. Org. Chem.* **2003**, *7*, 35-79.

(43) Ekelf, K.; Oscarson, S. *J. Org. Chem.* **1996**, *61*, 7711-7718.

(44) Mori, M.; Ito, Y.; Ogawa, T. *Carbohydr. Res.* **1990**, *195*, 199-224.

(45) Oshitar, T.; Shibasaki, M.; Yoshizawa, T.; Tomita, M.; Takao, K.-I.; Kobayashi, S. *Tetrahedron* **1997**, *53*, 10993-11006.

(46) Asai, N.; Fusetani, N.; Matsunaga, S. *J. Nat. Prod.* **2001**, *64*, 1210-1215.

(47) Garegg, P. J.; Maron, L. *Acta Chem. Scand., Ser. B.* **1979**, *B33*, 39-41.

(48) Jonke, S.; Liu, K.-G.; Schimdt, R. R. *Chem. Eur. J.* **2006**, *12*, 1274-1290.

(49) Zhang, Z. Y.; Magnusson, G. *Carbohydr. Res.* **1996**, *295*, 41-55.

(50) Donahur, M. G.; Johnston, J. N. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5602-5604.

(51) Du, Y.; Kong, F. Z. *J. Carbohydr. Chem.* **1995**, *14*, 341-352.

(52) Bhaskar, K. V.; Duggan, P. J.; et al. *J. Chem. Soc. Perkin Trans. 1* **2001**, 1098-1102.

(53) Bock, K.; Pedersen, C. *J. Chem. Soc. Perkin Trans. 2* **1974**, 293-297.

(54) Paulsen, H.; Richter, A.; Sinnwell, V.; Stenzel, W. *Carbohydr. Res.* **1978**, *64*, 339-364.

Chapter 3

DMF functions as a “brake” molecule in highly α-stereoselective glycosylation

1. Introduction of α-stereoselective *O*-glycosylation

In recent decades, the biological functions of the natural oligosaccharides have been intensively explored. They are found to play important roles in cellular trafficking, viral infections, cell proliferation, differentiation, apoptosis, immune response, even enzyme folding etc. Most of these activities are mediated through the carbohydrate-protein recognitions (Figure 1).[1-4]

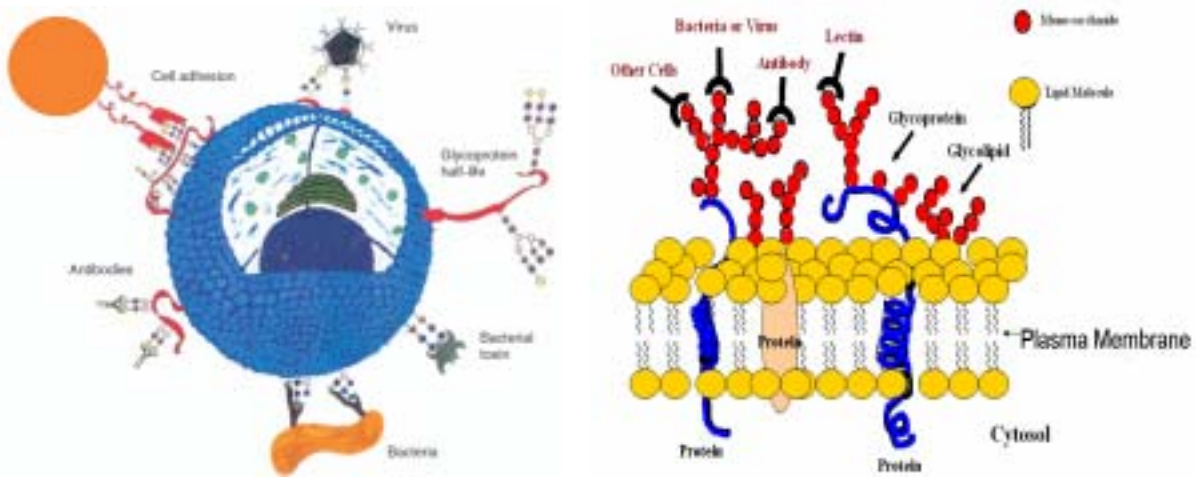


Figure 1 - Biological functions of glycoconjugates

Extraction of pure glycans from nature is inefficient and tedious. Synthesis of oligosaccharide usually relied on chemical methods. Even though significant progresses on

stereoselective glycosylations have been achieved, none of these methods can work perfectly as an enzyme, which implies that the mixed isomers are always obtained. However, the higher homogeneity of synthetic oligosaccharides could be employed as a target for the biological evaluation, which may give us the more credible results. For this purpose, the new method for the development in highly sereoselective glycosylation still draws a great attention for the synthetic chemists.

1,2-*cis*-glycosidic linkages are widely occurred in various natural glycoconjugates. For example, α-D-gluco-, α-L-fuco-, α-D-galacto-, β-D-mannopyranosides are the common blocks of numerous glycans, such as polysulfated glycosaminoglycans, α-Gal Ceramide (KRN7000),[5] globotriaosylceramide (Gb_3),[6] Lewis (Le) antigens,[7] *O*-linked glycolproteins, *N*-linked glycans (Figure 2).

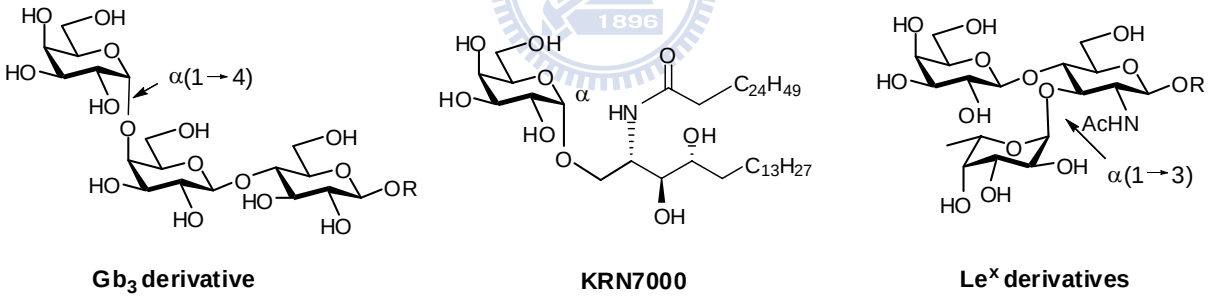


Figure 2 - Examples of bioactive α-glycosides

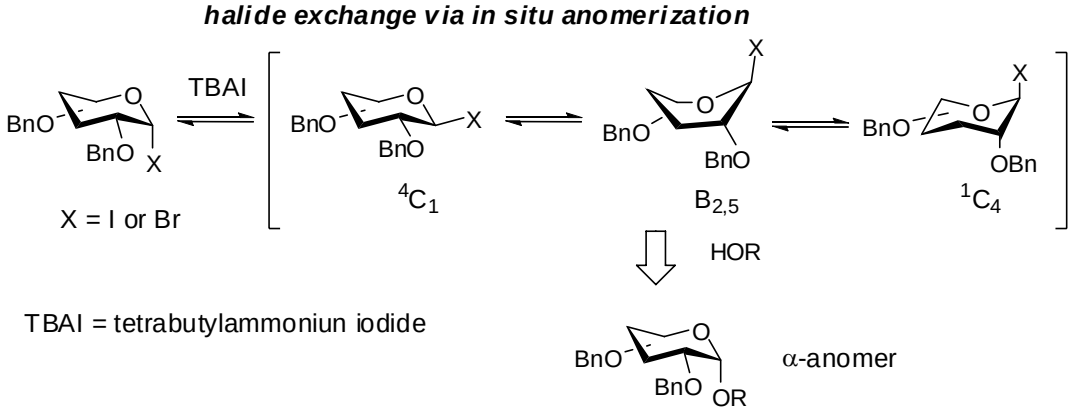
The construction of 1,2-*cis*-*O*-linkage in mannosides is a particular topic in which the different strategies were usually adopted, therefore the details regarding to the recent advances for β-mannosylation will be excluded in this context. In addition, 1,2-*cis*-gluco- and galactopyranosides are defined as α-anomers, which represent axially *O*-linked saccharides. In this chapter, the reported approaches and our findings for α-selereoselective glycosylations in a series of gluco- and galactosides will be described in the later section. Generally, the

formation of α-anomers is favorable due to the stronger anomeric effect. In reality, many factors still render the stereochemistry outcomes fluctuate, which prompts the synthetic chemist to work out this challenge.

1.1. Earlier strategies for α-selective *O*-glycosylation

1.1.1. Lemieux's in situ anomerization strategy

In 1968, Lemieux first announced in situ anomerization α-glycosylation.[8] This elegant work is so called "halide-catalyzed glycosylation" for preparation of α-glycosides.[9] It triggers a series of elaborative studies in relation to mechanisms and applications to oligosaccharide syntheses. Subsequent developments of α–glycosylation methods are largely based on this in situ anomerization concept. A brief mechanistic elaboration is given as follows (Scheme 1). The other approaches to assemble α-linkages are overwhelmed by in situ anomerization strategy for several decades until some new methods were described very recently.

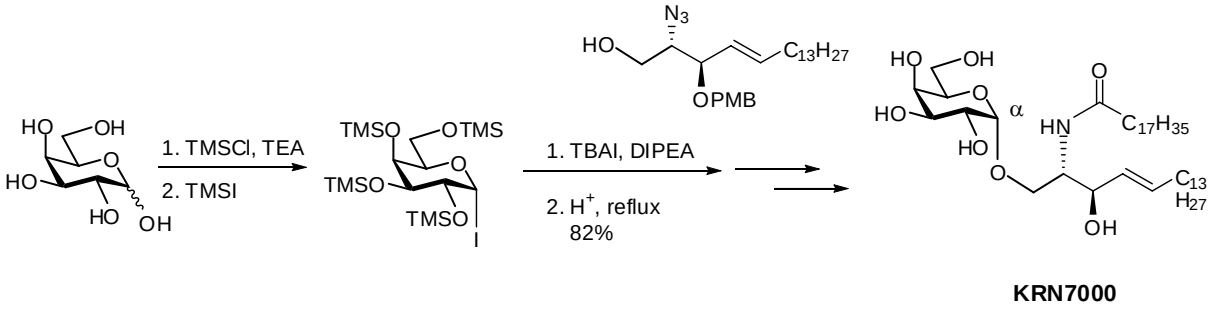


Scheme 1 - α-selective glycosylation via in situ anomerization

Upon activation by free halides, they observed that the tetra-*O*-benzyl-α-D-glycopyranosyl halides undergoes in situ anomerization to form β-glycosyl halide via the simple S_N2-like

substitution by iodide nucleophile from tetrabutylammoniun iodide (TBAI). Such a β-glycosyl halide is highly reactive species, which is attacked by nucleophlic acceptors from α-face to provide α-glycosides exclusively. The conformational change of the oxocarbenium associated with the solvents or free halides may temporarily form contact-ion pair in order to facilitate the glycosylation in a favorable energy state.[9] Although good α-selectivity can be obtained in some cases, the prolonged reaction time and the restricted application to armed per-*O*-benzylated glycosyl donor severely limits the synthetic utility of this strategy.

Recently, Gervay *et al.* reported a glycosylation protocol using trimethylsilyl iodide (TMSI) as reagent for in situ preparation of glycosyl iodide, which was used as a glycosyl donor for α–glycosylation.[10] The application of this method was demonstrated in synthesis of carbohydrate antigen KRN7000 as killer T-cell activators (Scheme 2).[11] It shoud be noted that this approach is still a modification of in situ anomerization concept initiated by Lemieux.



Scheme 2 - Gervay's synthesis of KRN7000

1.1.2. Solvent influence in α-selective glycosylations

In 1974, Schuerch *et al.* for the first time reported the use of l-*O*-tosyl-D-glucopyranose derivative with nonparticipating group at C-2 position to react with alcohol acceptor furnishing α–glycosylation product in ether solvent.[12,13] It was later reasoned that ether-type

solvents can solvate oxocarbenium ion (Figure 3). The dipole-dipole moment changes direction opposite to the dipole induced by oxygen atom in pyranose ring. This resulting force is named as "reverse anomeric effect",[14] which directs α-species to β-solvated intermediate. Therefore, the acceptor attacks from α-face favorably to form α-glycosylated product. In addition, cyclopentyl methyl ether (CPME), 1,4-dioxane, and 1,2-dimethoxyethane (DME) have been also explored as solvents to enhance selectivity in α-glycosylations. Further investigation of mixed solvent systems (combined use with the halogenated solvent, such as $CHCl_3$, DCE) indicated that α-selectivity is affected by solvents.[15] However, the solvent participation in α-selectivity has never been a single force. Other experimental factors, such as promoter system, leaving group or substrate structures are believed to exert a synergistic effect in glycosylation processes.

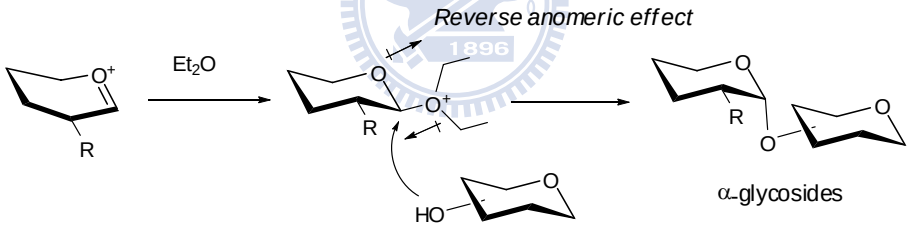
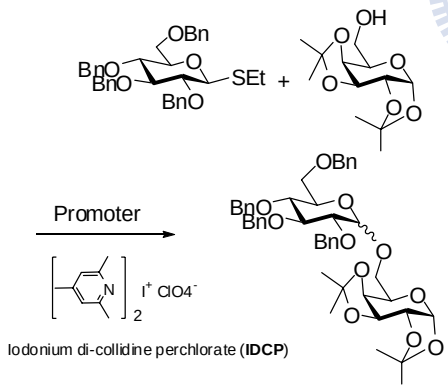


Figure 3 - Ethereal solvents induce α-selective glycosylation

1.1.3. α-Selectivitive glycosylations enhanced by promoters

Regarding the effect of promoters in α-selective glycosylation, Boons *et al*. have reported a systematic investigation.[16] The use of iodonium di-collidine perchlorate (IDCP) as a promoter for activation of thioglycoside in the dioxane-mixed cosolvent resulted in an improvement of α-selectivity. The study indicated that counter ions of Lewis acid promoter affect the stereoselectivity (Table 1). However, IDCP is not commercially available; its use requires additional preparations. In some cases, glycosylations promoted by IDCP do not complete regardless of the amount of promoter added. Taking these together limits its wide applications.

Table 1 - The effect of promoter for activation of thioglycosides



Entry	Promoter system	α/β ratio*
1	IDCP	18.0:1
2	MeOTf	6.6:1
3	NIS/TMSOTf	8.6:1
4	NIS/$TrClO_4$	8.1:1
5	NIS/Tf_2O	6.3:1
6	NIS/TfOH	5.4:1
7	NIS/TBDMSOTf	4.3:1

*All reactions were performed in the presence of MS 4A in toluene-dioxane (1:3 v/v) at RT

Besides IDCP, the $ClO4^-$ counter ion has been shown to contribute to α–selectivity.[17] It is proposed that activation of glycosyl diphenyl phosphate produces an equilibrated oxocarbenium-perchlorate contact ion-pair (CIP) and α–/β– glycosyl perchlorates (Figure 4). The counter ion may occupy either β- or α-face of the oxocarbenium ion. Furthermore, ethereal solvent can solvate CIP to give the solvent-separated ion pair (SSIP). The catalytic

amount of $ClO4^-$ counter ion plays a key role to enhance α-selectivity. However, the mechanistic study for this above reaction has not been elucidated.

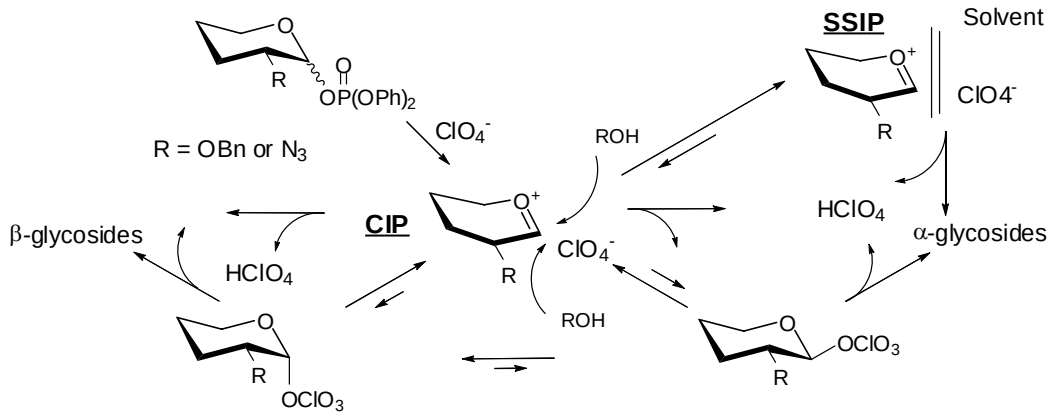
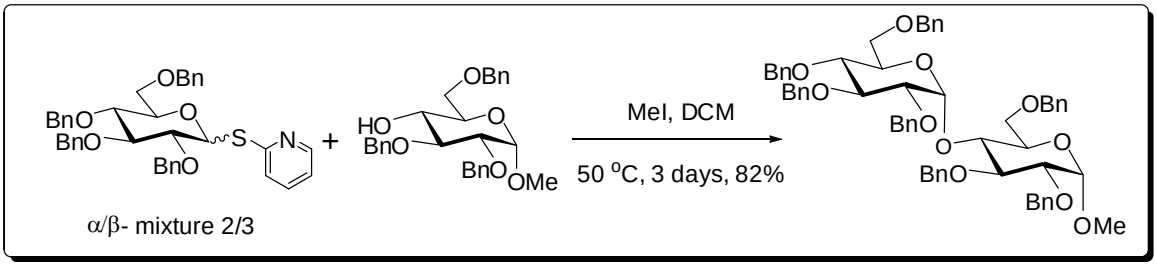


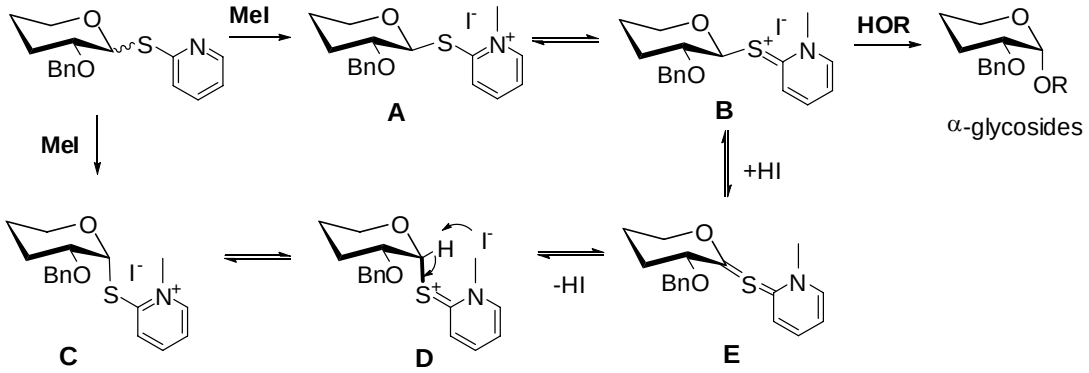
Figure 4 - Proposed mechanism of $ClO4^-$ playing a role in α-selective glycosylation

1.1.4. α-Selective glycosylation with special anomeric leaving groups

In the current glycosylation methods, the anomeric leaving groups have been explored in α–selective glycosylations. The stereochemical preference of using the different leaving groups is less consistent. For example, glycosyl phosphate has been examined as a glycosyl donor to impart α–glycosylaton. The experimental conditions invoke the use of ethereal solvent, higher temperature (RT or above). Apparently more than one factor is involved in the studies. Mereyala *et al.* also reported that the use of per-*O*-benzylated thiopyridyl glycosides to achieve α(1→4)-linked disaccharides in an excellent 82% yield.[18] The mechanism of α–selective glycosylation is depicted in Scheme 3. *N*-Methylation of nitrogen of pyridine triggers the formation of **B** via an equilibrium network. The resonance **B** intermediate is stabilized by the reverse anomeric effect that directs the attack of alcohol acceptor from α-face.



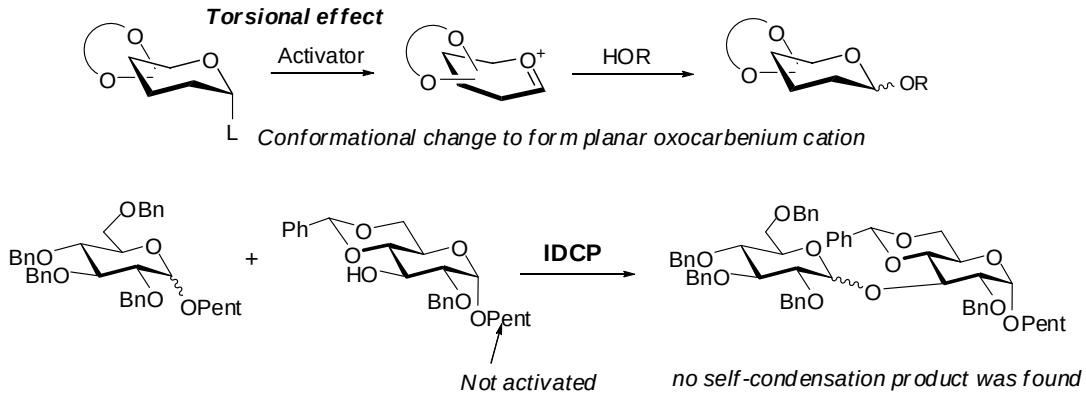
Plausible mechanism:



Scheme 3 - Thiopyridyl glycosides activated by MeI and the plausible mechanism

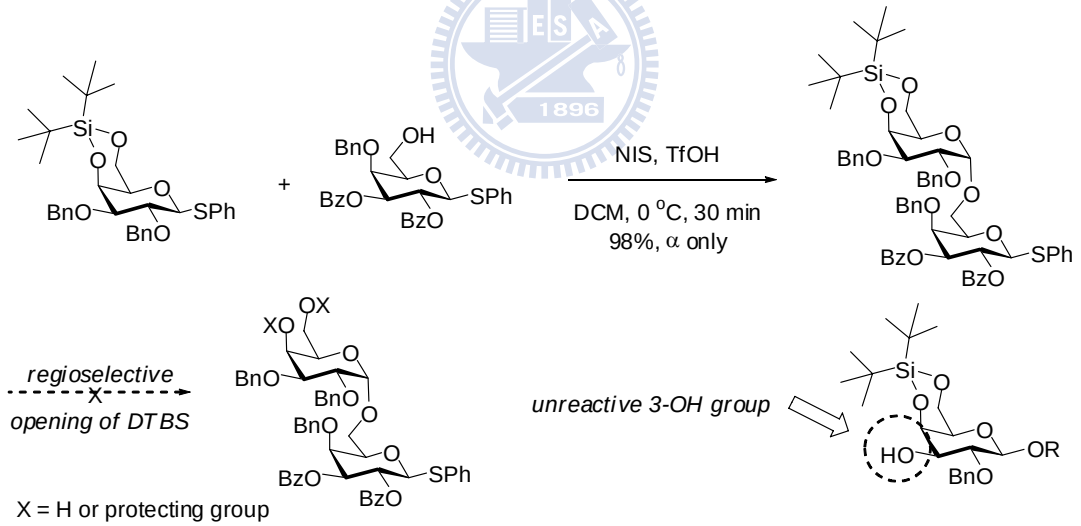
1.1.5. α-Selective glycosylation by special protecting group

There is no doubt that the nature of protecting groups in glycosides strongly affects the reactive property of either donors or acceptors. In line with this idea, the diol protecting groups, such as the benzylidene, anisylidene, cyclic carbonate, cyclic silyl ethers, oxazolidinones and isopropylidene acetals have been developed in stereoseletive glycosylations.[19,20] Fraser-Reid *et al.* observed that cyclic acetals protecting group on glycosyl donors affect either reactivity or stereoselectivity via a torsional effect.[21] To maintain the rigidity of the fixed bicyclic rings, the formation of oxocarbenium ion through a conformational change from chair to boat-like form renders the subsequent glycosylation more difficult (Scheme 4), which further reduce the reactivity of the corresponding glycosyl donor. Furthermore, this feature was successfully applied in orthogonal glycosylations by the use of glycosyl pairs with the same pentenyl leaving group.[21]



Scheme 4 - Torsional effects in donor activation and examples.

The bicyclic protecting group on sugars not only influences the reactivity but also the stereoselectivity. Among the recent advanced developments, Kiso *et al.* has reported one method so called "di-*tert*-butylsilylene-(DTBS) directed α-selective glycosylation strategy".[22]



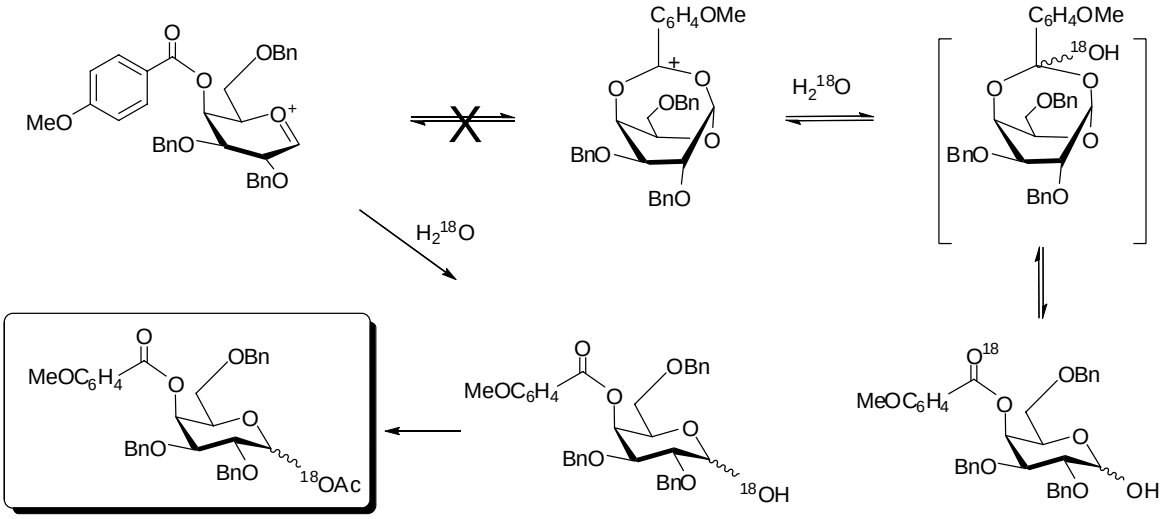
Scheme 5 - DTBS directed α-selective galactosylation

They demonstrated that the approach of the acceptor from β-face is blocked by the bulky bicyclic silyl group directing the nucleophilic attack more easily from α-face. However, there are some drawbacks for this method. **I.** The installation of DTBS residue by the expensive silylation reagent makes this process impractical for the route use. **II.** Methods for

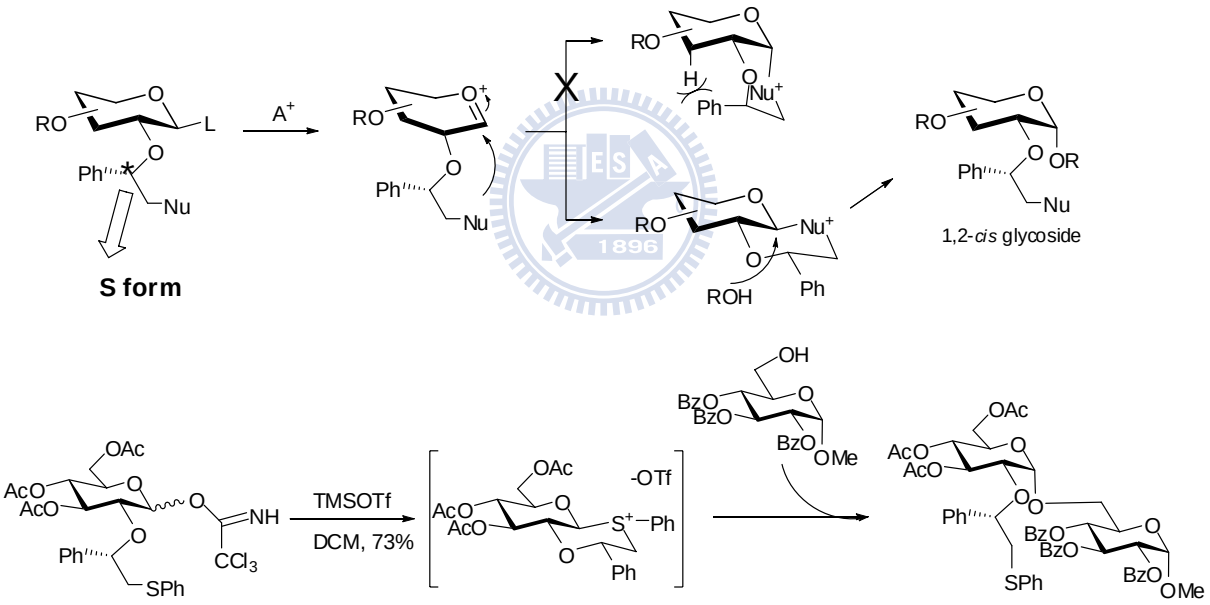
regioselective-opening of silylidene acetal for the following conjugation have not been discovered. **III.** Due to the bulkiness of the silyl group, glycosylation at 3-hydroxyl position is prevented (Scheme 5).

1.1.6. α-Selective glycosylations by neighboring group participation

Neighboring group participation has long been exploited to control the stereochemistry of glycosylation. In principle, the oxacarbenium ion could react with the participating group to form the more stable intermediate, which enable the nucleophilic attack from the opposite face. The earlier example inspired by this concept was described by Schuerch *et al.*[23] They replaced the 6-*O*-benzyl group with the ester or carbamate group bringing about the higher α-seletivity in glycosylations. However, they speculated that a substituent with a stronger donating function at C-6 might influence the looseness or tightness of the ion pair. Such an effect can induce α-selectivity but they also mentioned that the tight covalent bonding between the participating group and oxocabenium cation can not be conclusive. The role of neighboring group participation remains controversial until Crich *et al.* reported a mechanistic study using an isotopic labeling probe towards the influence of esters at the 3-*O*-axial and -equorial, 4-*O*-axial and -equatorial, and 6-*O*-sites (Scheme 6).[24] However, they found that no evidence can convince them of the occurrence of the neighboring group participation induced by carbonate esters at these positions, which implied that not only one single factor can induce a certain anomeric selectivity. However, the exemplified cases at other positions are so limited that the current debate over this issue is still going on. In the recent years, the utilization of participating group helping the construction of 1,2-*cis*-*O*-glycocidic bond seems prevalent in this field. Thus those advances regarding this strategy will be briefly introduced in the later context.



Scheme 6 - An isotopic labeling probe to investigate the effect of ester participating groups

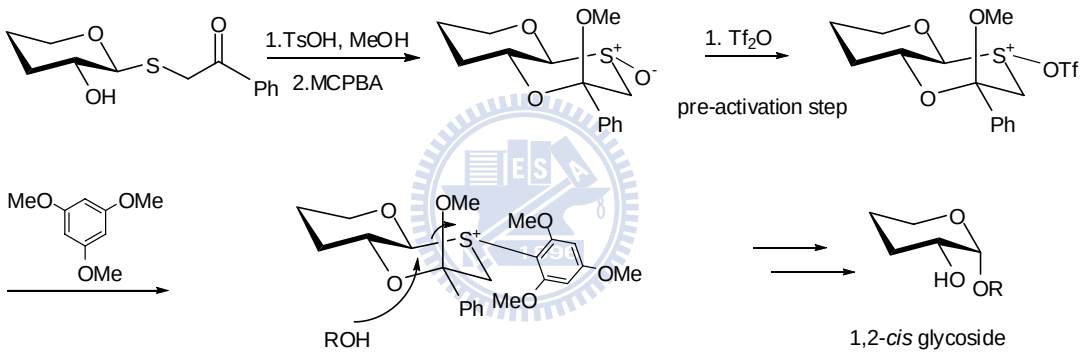


Scheme 7 - Neighboring group participation by a *S* auxiliary at C-2 leading to 1,2-*cis*-glycosides

A novel strategy using a chiral auxiliary at the C-2 of a glycosyl donor was reported by Boons *et al* (Scheme 7).[25] They demonstrated that an activated oxocarbenium ion could be associated with the nucleophilic atom of the auxiliary moiety followed by the formation of either a *trans*- or a *cis*-decalin system. The *trans*-decalin conformation dominates due to the favorable steric effect with *S*-form phenyl substituent. The subsequent attack by a glycosyl

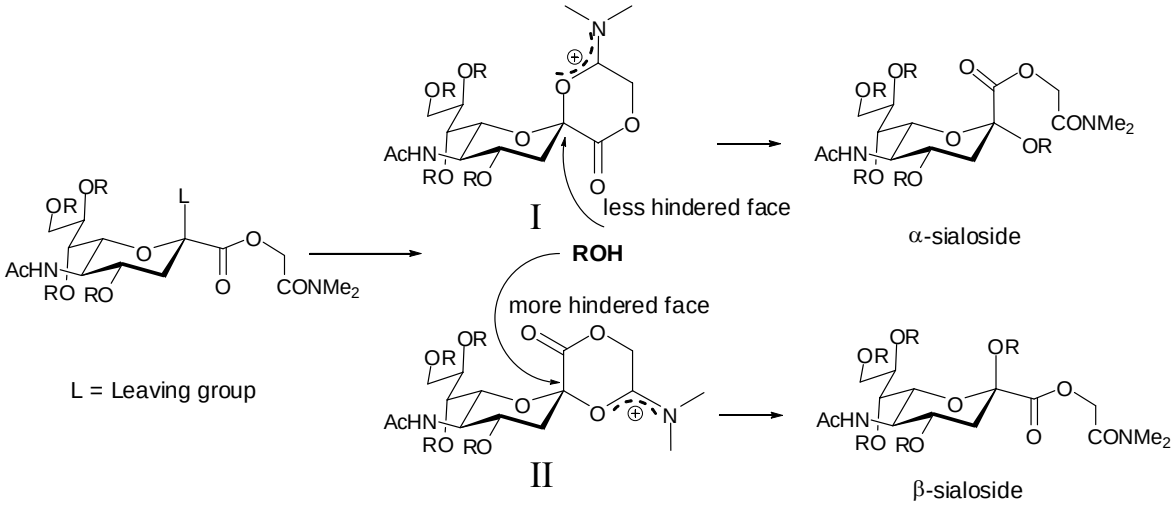
acceptor facilitates the formation of 1,2-*cis*-α-glycoside, whereas the use of the opposite chiral auxiliary (*R* form) would lead to 1,2-*trans*-β-glycoside (Scheme 7).

Lately, based on the "locked-decalin" concept, Turnbull *et al.* presented that a new oxathiane glycosyl donor involving cyclization of auxiliary chain at C-1 could be formed as a more stable bicyclic thioglycoside as well.[26] Upon pre-activation of glycosyl sulfoxide via triflic anhydride (Tf_2O) and 1,3,5-trimethoxybenzene, the resulting sulfonium ion intermediate is capable of glycosylation with a variety of acceptors in excellent α-stereoselectivity (Scheme 8).



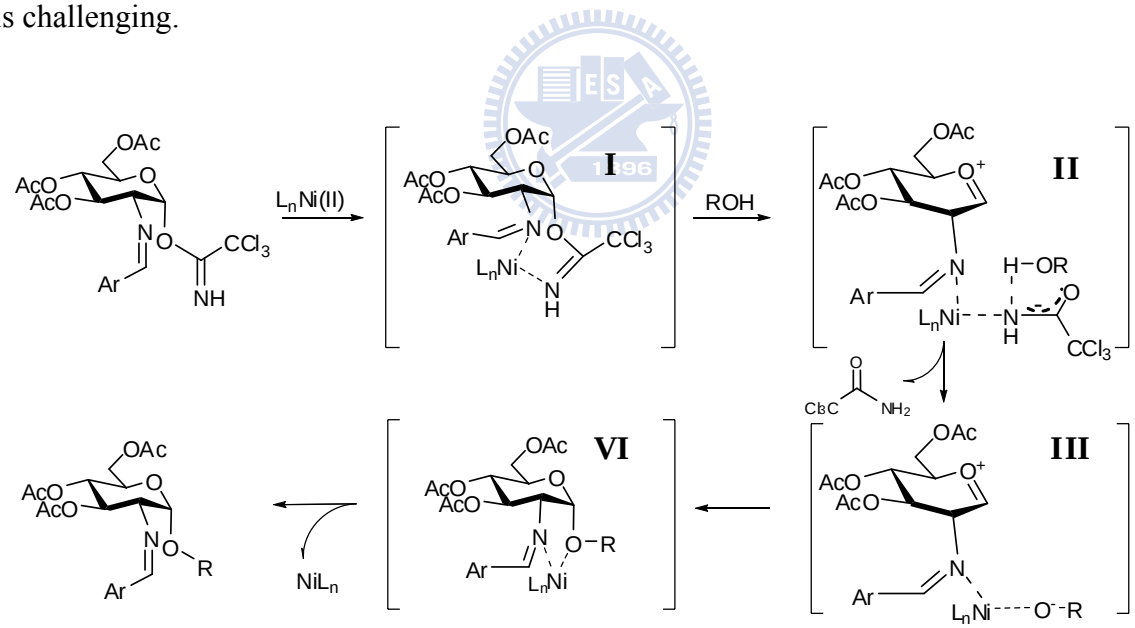
Scheme 8 - Oxathiane glycosyl donors direct α-selective glycosylation

Glycoconjugates incorporating α-sialosides are widely occurred in nature. α-sialylation is usually a challenging task. Gin *et al.* presented a strategy by installation of *N,N*-dimethylglycolamide auxiliary (-OCH_2-$CONMe_2$) at C-1.[27] It was anticipated that the nature of amide can stabilize the oxocarbenium ion to form the two putative intermediates **I** and **II**. The predominant intermediate **I** (due to anomeric effect via oxygen in pyranose ring) is likely more susceptible to the attack of acceptor from α-face, which is believed as a less hindered direction (Scheme 9).



Scheme 9 - Gin's proposed mechanism of α-sialylation via the amide auxiliary

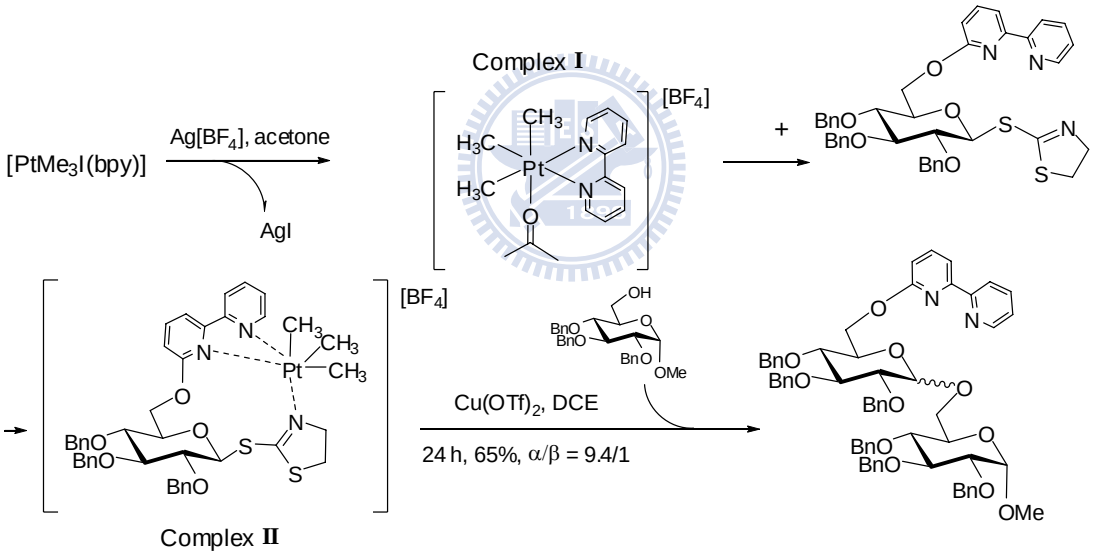
The 2-deoxy-2-amino α-gluco- and α-galactosides are prominent components of various biofunctional glycoconjugates. Assembly of 1,2-*cis*-*O*-glycosidic linkage for these substrates is challenging.



Scheme 10 - Nguyen's proposed mechanism of α-selective glycosylation via nickel-mediation

Recently, Nguyen *et al.* developed a nickel-catalyzed glycosylation to achieve α-selective glycosylations with 2-deoxy-2-amino glycosyl substrates.[28] A plausible mechanism is given that nickel metal coordinates the nitrogen atom of the imidate at C-1 and imine at C-2 forming a putative seven-membered ring complex **I**. They assumed that the

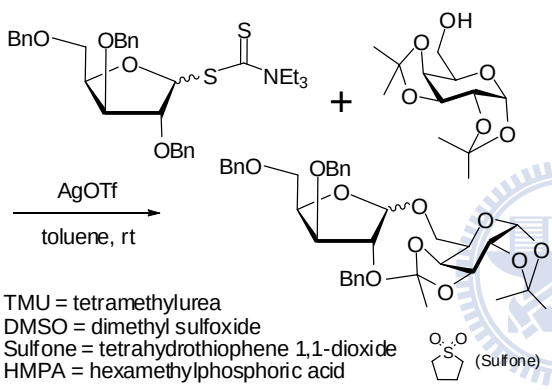
alcohol acceptor leads to formation of oxocarbenium complex **II**. Followed by departure of imidate gives the oxocarbenium intermediate **III**, which guides the attack of alcohol from α-face (Scheme 10). In the meantime, Demchenko *et al.* utilized a coordination chemistry to address the α-selectivity issue.[29] Their investigation showed that a suitable multi-dentate metal ligand could strongly coordinate to both of the leaving group and specific atoms of substituent in the C-6 hydroxyl protecting function forming a coordinating complex **II** (Scheme 11). Subsequent activation via $Cu(OTf)_2$ followed by reacting with nucleophilic acceptors provides α-selective glycosylations. This α-selectivity is ascribed to the more hindered β–face caused by the temporary coordination of metal.



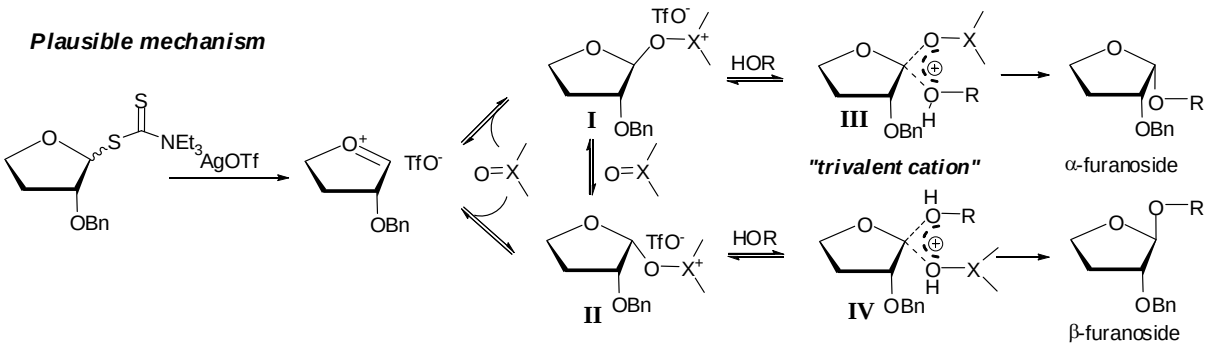
Scheme 11 - Demchenko's α-selective glycosylation via the metal-coordinated glycosyl donor

1.1.7. α-Selective glycosylation by additives.

Though the use of chiral auxiliary participating group in α-glycosylation is an elegant concept, installation of auxiliary functions is non-trivial that limits its wider application. Therefore, it is reasonable for developing a more convenient strategy. In this regard, the addition of certain additives to interfere in the glycosylation process may provide an alternative to obtain the desired α-stereochemical outcome.



Entry	Additive	Yield	α/β ratio*
1	none	78%	2:3
2	TMU	78%	3:1
3	DMSO	75%	3:1
4	HMPA	87%	10:1
5	Sulfone	83%	1:1



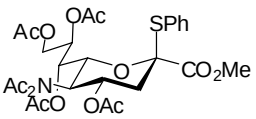
Scheme 12 - Bogusiak's α-selective glycosylation enhanced by the polar additives

For this purpose, Bogusiak *et al.* reported that the addition of a stoichiometric amount of hexamethylphosphoramide (HMPA) additive would effect 1,2-*cis*-α-furanoside formation

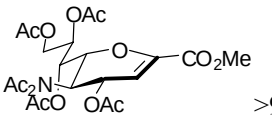
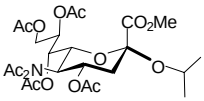
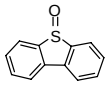
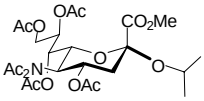
when glycosyl xanthates were used as glycosyl donors.[30,31] For other additives (DMSO, TMU, sulfone, HMPA), the donicity (DN) of these molecules may provide a rationale towards the higher α-selectivity achieved via addition of additives with a higher DN (HMPA = 38.8). The additive molecules can presumably trap oxocarbenium ion to form intermediate **I** and **II** followed by reactions with the alcohol acceptor to form trivalent cations **III** and **IV** (Scheme 12). They also conducted quantum chemical calculation, which suggests that the more stable intermediate **IV** unfavorably proceed via the dissociation of additive leading to the β-furanoside, whereas the α-furanoside is more easily accessed via the intermediate **III**.

Few years later, Crich *et al.* adopted the combined reagents of a diaryl sulfoxide (Ar_2SO) and triflic anhydride (Tf_2O) as a promoting system which was developed by Gin's group for the activation of thiosialoside.[32,33] Interestingly, excessive amount of diaryl sulfoxide was found to play a critical role for α-sialylation. When stoichiometric amount of sulfoxide was used, only the elimination product was found (entry1, Table 2).

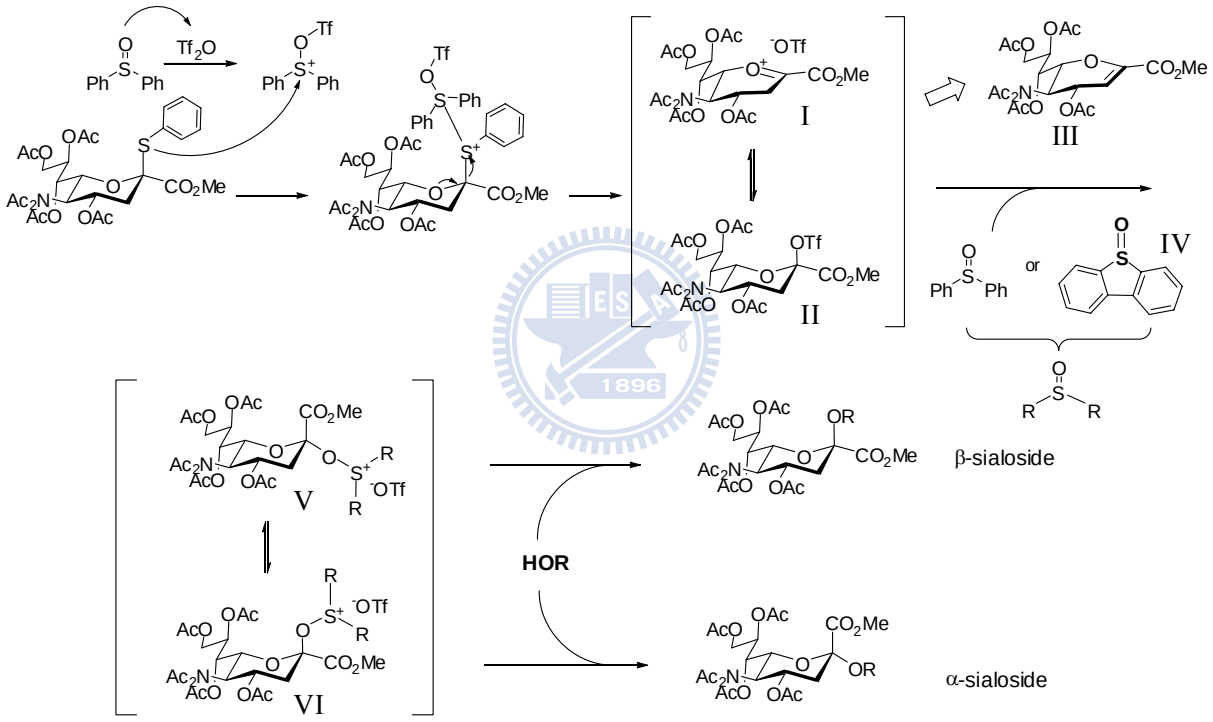
Table 2 - Crich's study: The effect of diaryl sulfoxides for α-sialylation using 2-propanol as an acceptor



TTBP = 2,4,6-Tri-tert-butylpyrimidine
Ph_2SO = Diphenyl sulfoxide
*All of the reactions were performed at -78 °C.

Entry	Ph_2SO (equiv)	Tf_2O (equiv)	TTBP (equiv)	Additive (equiv)	Product, yield (α/β ratio)
1	1.0	1.0	2.0	none	 >90%
2	**3.0**	1.0	2.0	none	 97% (2.3/1)
3	3.0	1.1	2.0	**3.0** 	 95% (6/1)

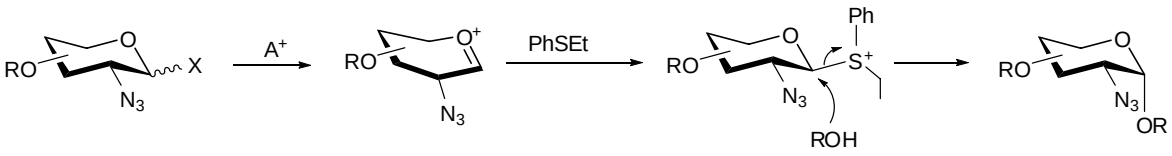
In contrast, the addition of two more equivalents of diaryl sulfoxide dramatically increased the glycosylation yield but the moderate stereoselectivity obtained (entry 2, Table 2). They concluded that diary sulfoxide not only functions as a promoter but also it would couple with the unstable oxocarbenium ion to form the intermediates **V** and **VI** (Scheme 13)**.** For validation of their assumption, they also screened a panel of sulfoxides and found that dibenzothiophene **IV** was a better molecule to engage in α-sialylations (entry 3, Table 2).



Scheme 13 - Crich's proposed mechanism: Sulfoxide derivatives involving in α-selective sialylation

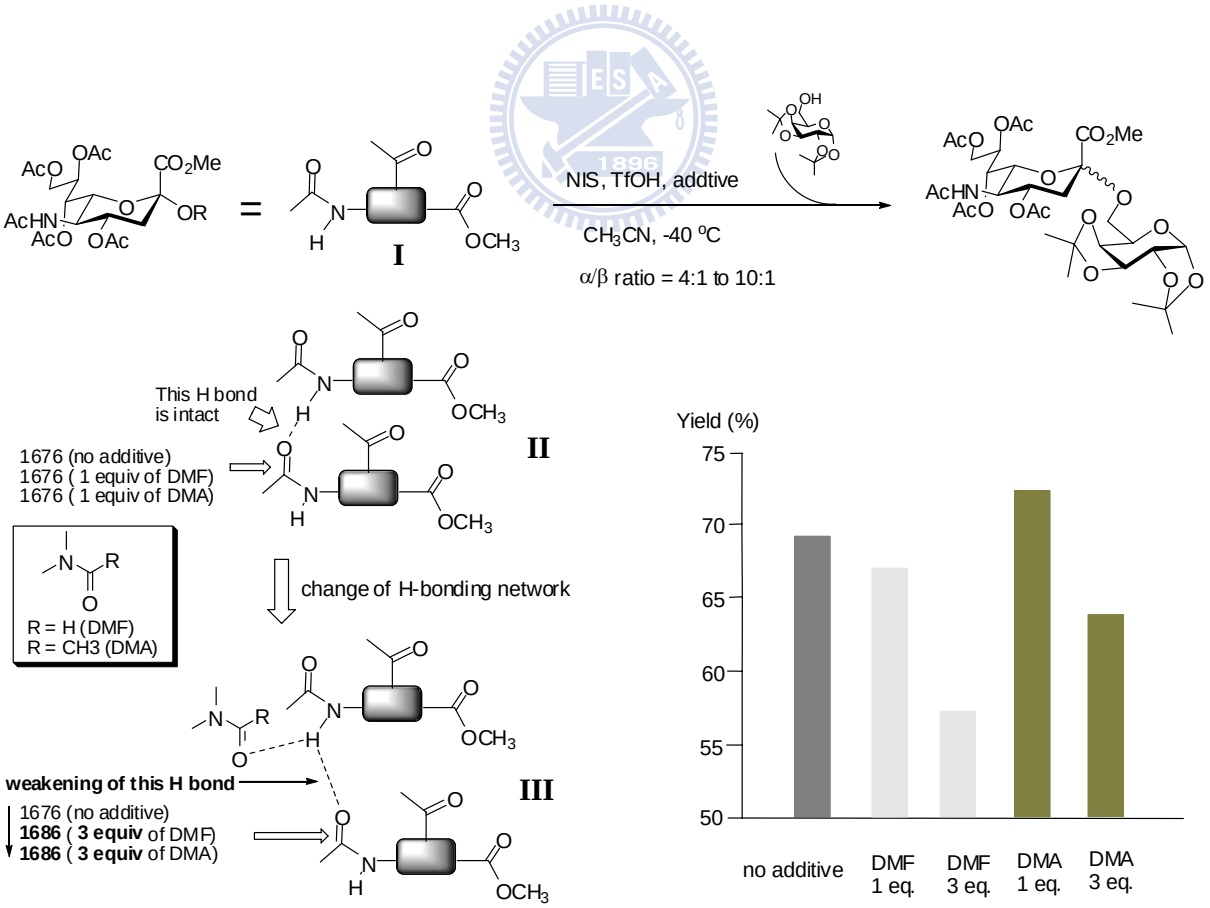
Recently, Boons *et al.* utilized a similar approach and demonstrated that the addition of excessive amount of PhSEt or thiophene could induce α-selectivity while 2-azido-2-deoxy glucosyl trichloroacetimidate was used as a donor (Scheme 14).[34] With NMR and computational studies, the formation of β-anomeric sulfonium intermediate is favored due to the reverse anomeric effect and steric factor. Thus, the predominant β-species could direct the

incoming acceptor from α−face. However, selectivity of per-*O*-acetylated 2-azido-2-deoxy glucosyl donor is found higher than that of per-*O*-benzylated donor. There may be other structural factors affecting the stereochemical outcome.



Scheme 14 - Boon's method: PhSEt as an additives involved in α-selective glycosylation

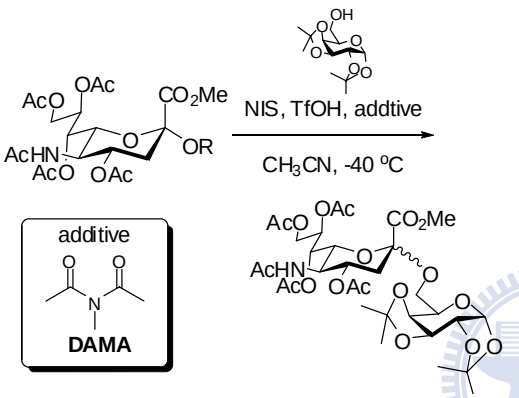
Other than Crich and Boons, Kononov *et al.* reported the influence of the additives on the physical properties of glycosyl donors.[35] IR spectroscopy was employed to record the absorption shift of carbonyl group at 5-NAc of sialic acid (Scheme 15).



Scheme 15 - The influence of additives for supramer structures via IR spectroscopy

This result indicated that the H-bonding properties would be altered by the addition of the excessive additives molecules, such as DMF, DMA. The resulting phenomenon affects reaction yields and even the stereoselectivity. However, the trivial difference in yields and α/β−selectivities is hard to draw a conclusion that weakening the hydrogen bonding by additives is a single reason to contribute in α-selective sialylation (Table 3).[36]

Table 3 - Comparative study of DAMA as an additive in α-*O*-sialylation

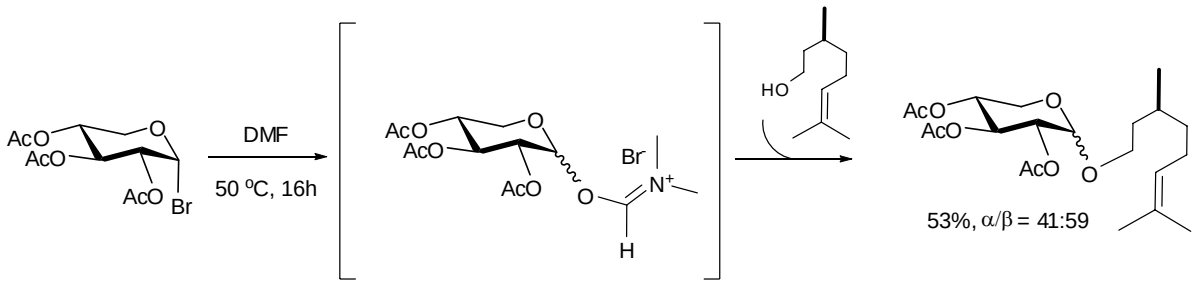


Entry	Additives (equiv.)	Time	Yield (%)[a]	α/β ratio
1	none	15 min	62	13:1
2	DAMA (1.0)	15 min	64	27:1
3	DAMA (3.0)	15 min	57	18:1
4	none	3 h	69	7:1
5	DAMA (1.0)	3 h	80	8:1
6	DAMA (3.0)	3 h	72	7:1

1.1.8. α-Selective *O*-glycosylation mediated by DMF-type molecules

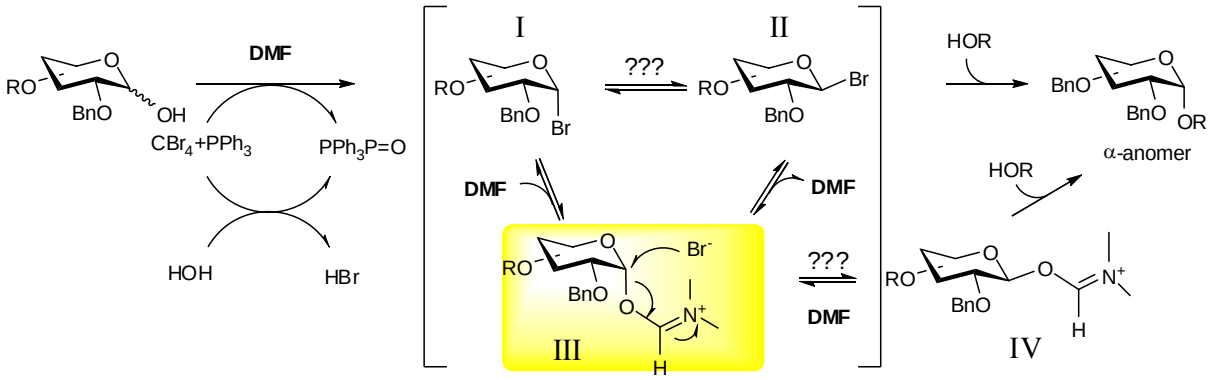
In June 2009, Muzart published an interesting review article whose title is "*N,N*-Dimethylformamide (DMF): much more than a solvent". He wrote that "*The O-atom of DMF can act as a donating moiety. Besides, DMF can react as either an electrophilic or a nucleophilic agent, and, in addition, can be the source of various key intermediates mediating reactions.*"[37] In his previous work, he found that the 2,3,4-triacetyl-1-bromo-α-D-xylo -pyranose could glycosylate with a serials of terpenols in DMF instead of applying the conventional Koenigs–Knorr conditions (Scheme 16).[38] He also referred to ^{1}H-NMR studies conducted by Nishida and co-workers who suggest that the formation of Vilsmeier–Haack

intermediate between glycosyl bromides and DMF make the glycosylation possible even in the absence of any additional promoting reagent, such as Ag, Hg metal.



Scheme 16 - Xylosylation of terpenols in DMF

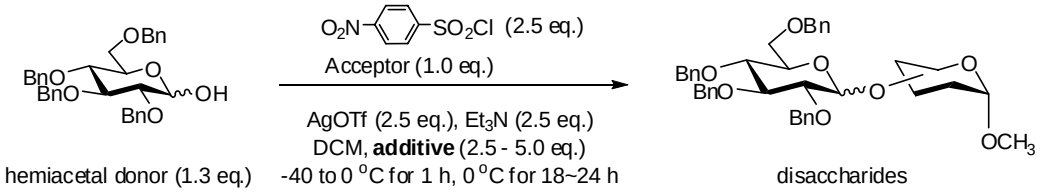
More interestingly, 35 years ago Lemieux has ever reminded us that the addition of the small amount of DMF enable the more efficient halide-catalyzed glycosylations (in situ anomerization process).[9] However, no clear explanations were given in his following works until Nishida *et al.* developed a new dehydrated glycosylation protocol using Appel agents in DMF and further demonstrated the role of DMF in glycosylations.[39] According to the evident NMR spectra for these possible intermediates, these signals indicated that the in situ prepared α-glycosyl bromide **I** by using Appel agent could be transformed to α-glycosyl iminium bromide salt **III** while DMF is used as a solvent (Scheme 17).



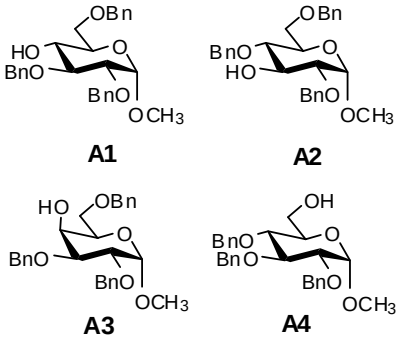
Scheme 17 - Nishida's dehydrative glycosylation method using Appel agents in DMF

Based on Lemieux's in situ anomerization mechanism, it could be rationalized that the β-glycosyl bromide **II** derived from α-glycosyl iminium intermdiate would be a real reactive donor for α-selective glycosylation. Furthermore, 2 years later, they made an exhaustive effort to verify the existence of β-substituted donors.[40] Unfortunately, they failed to find out the strong evidence for supporting their speculation. Nevertheless, they still suspect that not only β-glycosyl bromide **II** but also β-glycosyl imidate **IV** could be the key species to bring about the stereochemical outcome. In contrast to thermodynamically stable α-anomer, they emphasized that the relatively small proportion (*ca.* 5%) of β-substituted intermediates should be a kinetic moiety and its NMR signal may not be accessible at the ambient temperature due to the rapid equilibration. Moreover, the frozen property of d_7-DMF would not allow the further NMR measurement at the low temperature. To date, in terms of mechanistic investigation, the real entity for this reaction is still not successfully identified by any means.

Table 4 - DMA as an additive in α-*O*-glucosylation



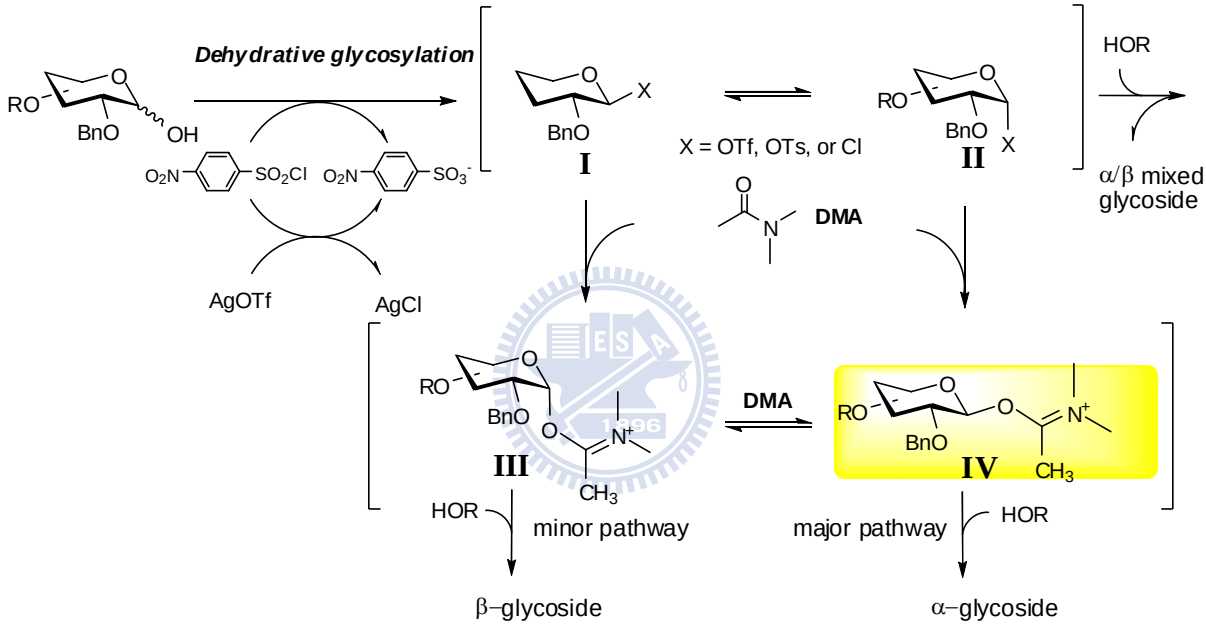
Acceptors



[a]DMF is used a solvent

Entry	Acceptor	Additive (equiv.)	Yield	α/β ratio
1	**A1**	DMF[a]	58%	77:23
2	**A1**	DMF (2.5)	92%	88:12
3	**A1**	DMA (2.5)	86%	93: 7
4	**A1**	DMA (5.0)	73%	86:14
5	**A2**	DMA (2.5)	85%	89:11
6	**A3**	DMA (2.5)	87%	90:10
7	**A4**	DMA (2.5)	91%	47:53
8	**A4**	DMA (5.0)	88%	73:27

Based on the literature reviews, Koto first reported the use of small amide molecules to achieve α–selective glycosylation (Table 4).[41] They proposed a mechanism to account for their observatioin. Upon the dehydrative activation of glycosyl hemiacetal donors, the reactive intermediates **I** and **II** readily associate with *N,N*-dimethylacetamide (DMA) forming the glycosyl iminiums **III** and **IV**. Coupling with **III** and **IV** with the alcohol acceptor gives the glycosylation products (Scheme 18).



Scheme 18 - Koto's plausible mechanism using DMA as an additive in α-*O*-glucosylation

α-Configuration **III** is thermodynamically more stable than its β-counterpart. In contrast, β-configuration **IV** is highly reactive and leading to α-selective glucosylations. Though the operation is simple, no elaborative studies were followed.

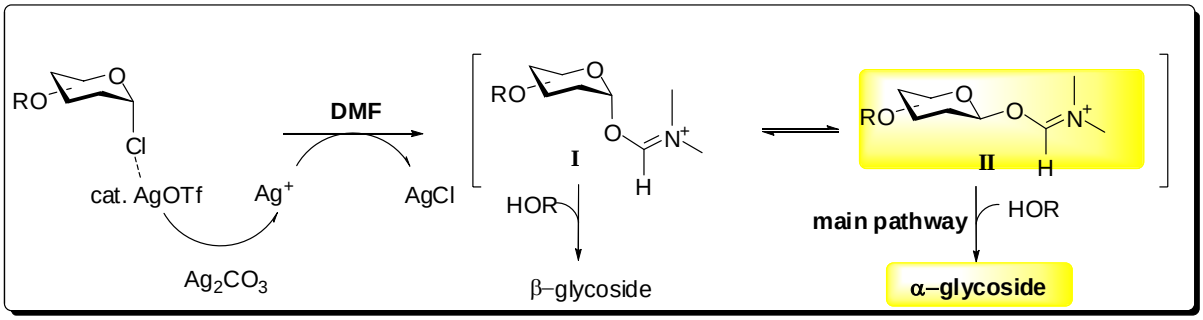
However, in our previous works, a good α-selectivity of glycosylation in the aforementioned Chapter 2 (the sequential chlorination-glycosylation protocol) was obtained. Upon closed examination, it was found that DMF residue in crude glycosyl chloride is the key for α-selectivity observed. This finding sparks our interests to investigate the role, mechanism

and scope of application for DMF additive in α-glycosylation. Some parts of our findings could be very different from others. The more detailed studies would be described in the later sections.

2. Our strategy using DMF as an additive in α-selective glycosylation

As mentioned in the above context, this is certainly not the first time that DMF was used for the stereoselective glycosylation. However, the debates concerning the role of DMF still lie ahead. To clear it up further, a couple of the essential questions should be proposed in advance. **I.** Does the reaction go through the common β-substituted intermediate or multiple pathways simultaneously? **II.** Is the excess amount of DMF (even used as a solvent) required for the optimal result? **III.** Does the reaction need to be carried out at a relatively higher temperature? What effect does the temperature bring out? **IV.** To date, the reported examples are only limited to the use of glycosyl halides as the reactive donors. We don't know whether DMF can be applied to other glycosyl donor systems, such as a common-used thioglycoside. **V.** The more challenging task is to realize the interactions between DMF, promoter, base and the related counter ions during the reaction course.

Therefore, according to the literature survey and our initial findings, we envisaged that DMF may function as a "brake" molecule to allow the reactions bypass the more reactive pathways via the α/β mixed glycosyl iminium intermediate, which can further result in the improved α-selectivity (Scheme 19).



Scheme 19 - DMF-mediated α-selective glycosylation via glycosyl iminium intertemediates

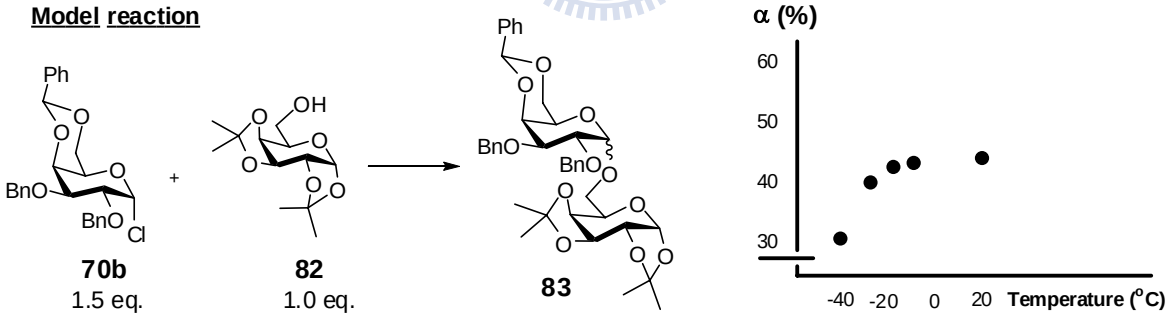
3. Results and discussion

To study the DMF-mediated stereoselective glycosylations, we decided to choose α-glycosyl chlorides as the standard glycosyl donor. In our lab, TCT/DMF chlorination method has been proven being a practical and reliable procedure for the preparation of a wide range of α-glycosyl chlorides. In spite of this advantage, a numbers of other reasons are given that: **I.** Activation of glycosyl chloride by using the relatively simple promoting system (usually the combinations of AgOTf/Ag_2CO_3, AgOTf/TMU, or $AgClO_4$/TMU) probably can avoid the potential problem derived from the complex reaction mixtures. **II.** The configuration of leaving group has been determined as an influential factor in glycosylation processes. The specific α-oriented glycosyl chloride has its merit. **III.** The compatibility of glycosyl chlorides with other latent leaving groups has been well known with the applications of orthogonal glycosylation strategies.[42]

Prior to the investigation, we surveyed the typical Koenigs-Knorr glycosylation conditions reported in the literatures and intended to find out the general operations.[43-46] However, the versatile procedures can not provide the useful message and rationales for their protocols. For examples, the excess amount of reagents was generally used and the reaction temperature usually varied in a broad range. Thus its paucity of information about the experimental details prompted us to work out this problem. In the absence of the additives, therefore, α-glycosyl chloride **70b** was exploited as a testing donor with the more reactive and commercial available diacetone galactosyl acceptor **82** in the preliminary trials. A panel of Koenigs-Knorr conditions was examined to realize which factors significantly contribute to the reactivity and stereoselectivity. Several characteristics for this reaction could be

summarized as below: **I.** The solid Ag_2CO_3 provides the source of silver cation for promoting the glycosyl halide and it is also an acid scavenger to neutralize the reaction media. The slightly excessive use of Ag_2CO_3 is sufficient for the completion of the reactions. Ag_2CO_3 can be replaced by TMU which usually serves as a base. **II.** We found that the conversion proceeded very slowly without the treatment of AgOTf. At lower temperature, the increasing amount of AgOTf was required to accelerate the reaction. At ambient temperature the minimum usage of Ag_2CO_3 (1.6 equiv) and AgOTf (0.05 equiv) as a combined promoting system render this glycosylation protocol inexpensive. **III.** α-glycosyl chloride **70b** can be easily activated under this condition, even at -70 °C. The α/β ratio is highly correlated to the temperature (β-anomer was favorably formed at lower temperature) probably due to the intrinsic stereochemistry of α-halide which may prefer S_N2-like glycosylation pathway (entry1-6, Table 5).

Table 5 - Optimization for the conventional Koenigs-Knorr conditions



Entry	Ag_2CO_3 (equiv.)	AgOTf (equiv.)	Additive (equiv.)	Time	Temp (°C)	Yield	α/β- via ^{1}H-NMR
1	4.0	0.5	none	6 h	-40	65%	29:71
2	1.6	0.5	none	6 h	-40	68%	29:71
3	1.6	0.5	none	6 h	-25	70%	30:70
4	1.6	0.2	none	15 h	-25	75%	34:66
5	1.6	0.2	none	3 h	-10	82%	41:59
6	1.6	0.05	none	30 min	RT	84%	43:57
7	1.6	0.05	DCM:Ether = 1:1	1 h	RT	79%	37:63

IV. Improving α-stereoselectivity by using the ethereal solvent seems ineffective (entry 7, Table 5). **V.** The general procedure is conveniently performed by such the adding sequence: To a mixture of Ag_2CO_3 (1.6 equiv.), molecular sieve (AW300), the glycosyl acceptor (1.0 equiv.) and glycosyl donor (1.5 equiv.) in DCM was added a catalytic amount of AgOTf (0.05–0.5 equiv) at the given temperature. Upon completion of the reaction, the subsequent treatment of Et_3N allows the reaction mixture directly subjected to SiO_2 column chromatography. We observed that a modification of the procedure, such as the reverse addition and the slow addition of glycosyl chlorides, could not provide the significant improvement in stereoselectivity. All in all, this protocol would be taken as a standard manipulation unless any change for the certain purpose will be notified in the following context.

3.1. DMF as an additive: Effect in stereoselectivity of glycosylations

Though DMF can improve the α-stereoselectivity under Koenigs-Knorr conditions, the exact function of DMF is not clearly elucidated. To clarify the role of DMF, glycosyl donor **70b** and acceptor **82** were employed as a model for glycosylation study (Table 6). Without addition of DMF, no selectivity (α/β ratio = 43:57) was obtained (entry 1, Table 6), whereas with addition of a stoichiometric amount of DMF to the reaction mixture α-selectivity was dramatically boosted to 79:21 (α/β, entry 2, Table 6). Further increase of DMF up to 6 equivalents slightly improved α-selectivity (α/β ratio = 82:18) (entry 4, Table 6). However, we are still wondering why the excessive additive (at least 2.5 equivalents of DMF) needs to be used. If Koto's proposed mechanism is correct, a stoichiometric amount of DMF in comparison to donors should be sufficient. To attest their speculation, the addition of the

minimum 1.5 equiv of DMF and the reduced AgOTf (from 1.0 to 0.05 equiv) provided a considerably better α-selectivity (α/β ratio = 87:13, entry 5, Table 6) without compromising the efficiency. In contrast, Ag_2CO_3 can slowly promote the reaction even in the absence of AgOTf (entry 3, Table 6).

Table 6 - DMF as an additive: Optimization of conditions in α-selective glycosylations using **70b** and **82**

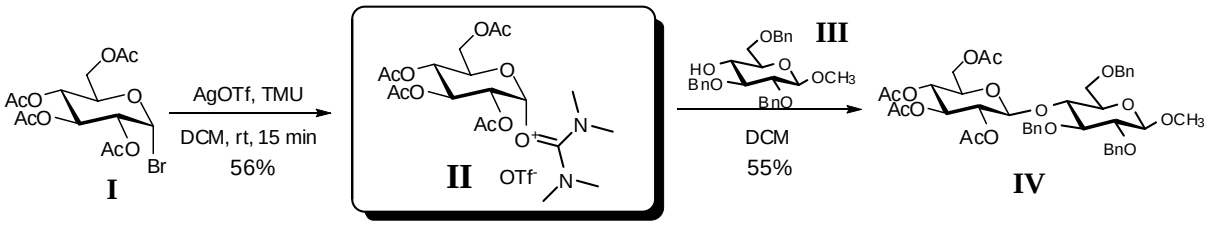
Entry	Ag_2CO_3 (equiv.)	AgOTf (equiv.)	DMF (equiv.)	Time	Temp (^oC)	Yield	α/β- via ^{1}H-NMR
1	1.6	0.05	none	30 min	rt	84%	43:57
2	**1.6**	**1.0**	**1.5**	**30 min**	**rt**	**85%**	**79:21**
3	1.6	none	1.5	18 h	rt	62%	74:26
4	1.6	1.0	6.0	30 min	rt	80%	82:18
5	1.6	0.05	1.5	30 min	rt	87%	87:13
6	**1.6**	**0.3**	**1.5**	**24 h**	**-25**	**88%**	**94:6**
7	1.6	0.5	1.5	48 h	-40	59%[a]	>95:5

[a] Some unidentified byproduct is inseparable with the product.

For the effect of temperature in the present glycosylation, an excellent α-selectivity (α/β ratio = 94:6) with a good yield (88%) was obtained at -25 ^oC (entry 6, Table 6). Further lowering the temperature (-40 ^oC) halted the glycosylation coupling but instead an unknown compound was observed as an inseparable mixture with the desired product (entry 7, Table 6). By tracking the reactions, we observed that the reaction proceeded very slowly with treatment of 0.05 equiv of AgOTf. Increasing amount of AgOTf led to a faster conversion. DMF was proved to be an effective additive for tuning the stereochemical outcome in glycosylation reactions. With reviewing the earlier reports, the other additives should be revisited and compared with DMF in terms of reactivity and selectivity.

3.2. Screening of additives

It has been long described that 1,1,3,3 tetramethylurea (TMU) in glycosylaton reactions serves not only as a base but also reacts with the glycosyl halide to form an ionic compound.[47]

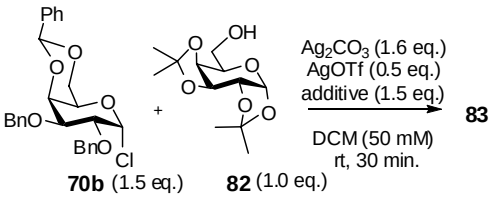


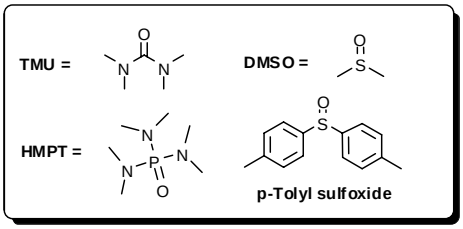
Scheme 20 - The formation of per-*O*-acetylated glucopyranosyl uronium triflate

Thus, treatment of 2,3,4,6-tetra-*O*-acetyla-α-D-glucopyranosyl bromide **I** in DCM with AgOTf and TMU at room temperature for 15 min gave 1,1,3,3-tetramethyl-2-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl) uronium triflate **II** in 56% yield (Scheme 20). Glycosylation of **II** with **III** in DCM gave 62% yield of β-linked dissacharide **IV**. The aforementioned examples employing TMU for the construction of 1,2-*cis*-*O*-linkage was briefly introduced in section 1.1.7 of this chapter.

However, addition of TMU in our case failed to improve α–selectivity (entry 2, Table 7). Triphenyphosphine oxide and hexamethylphosphoric triamide (HMPT) were shown as similar outcomes (entry 4 and 5, Table 7). Interestingly, sulfoxide-type additives provided the considerable enhancement as DMF, which suggests that the donating ability of oxygen atom in sulfoxide molecule could bring about the possible interaction with the reacting intermediates (entry 3 and 7, Table 7).

Table 7 - Screening of the additive in α-selective glycosylations using **70b** and **82**



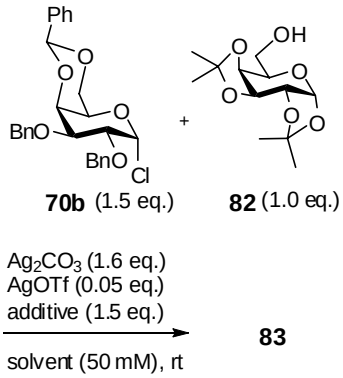


Entry	Additive (equiv.)	Yield	α/β ratio- via ^{1}H-NMR
1	DMF	80%	79:21
2*	TMU	75%	57:43
3*	DMSO	82%	75:25
4	$O=P(Ph)_3$	85%	67:32
5*	HMPT	89%	57:43
6	$P(OMe)_3$	90%	39:61
7	p-Tolyl sulfoxide	84%	74:26

3.3. Examination of solvent effect

To access the influence of solvents in DMF-mediated α-selective glycosylations, further investigation also exploited the same glycosyl substrates and the systematic screening with solvents was conducted in a parallel setting. The results was shown in Table 8 that 1,2 dichloroethane (DCE), ether, and toluene could be used alternatives for the clean conversion with the similar selectivity (entry 2, 4 and 6, Table 8). Among these, it is generally believed that ethereal solvents are capable directing α-selectivity.

Table 8 - Examination of solvent effect using **70b** and **82**



Entry	Solvent	Time	Yield	α/β- via ^{1}H-NMR
1	DCM	30 min	87%	87:13
2	DCE	1 h	78%	85:15
3	CH_3CN	1 h	61%	47:53
4	Ether	1 h	75%	89:11
5	1,4-Dioxane	1 h	trace	ND
6	Toluene	1 h	82%	86:14

For our result, this represented a synergistic effect in a certain level. However, the reaction was sluggish in 1,4-dioxane (entry 5, Table 8). Furthermore, β-directed nitrile solvent (CH_3CN) could act as a competitive role with DMF and its α-selectivity was not obvious in CH_3CN (entry 3, Table 8).

In comparison with the similar reports by using DMF-type molecules to enhance α-selectivity, our systematic investigations and results pose the several features distinguished from others. For examples: **I.** Good α-selectivity can be achieved at lower temperature. In most of existing approaches, glycosylations are usually performed at higher temperature, which favors the formation of thermodynamically-stable α-glycosides; **II.** The reaction rate and selectivity can be tuned by changing the amount of promoter; **III.** The minimum amount of reactants would be a great benefit for the large-scale preparation in oligosaccharide synthesis; **IV.** The simple procedure would be helpful for a practical use.

Despite of these advantages mentioned above, the reasons for the resulting α-selectivity at the low temperature are still not clear. Therefore, the further mechanistic investigation to recognize the behavior of DMF is definitely an intriguing topic for the following study.

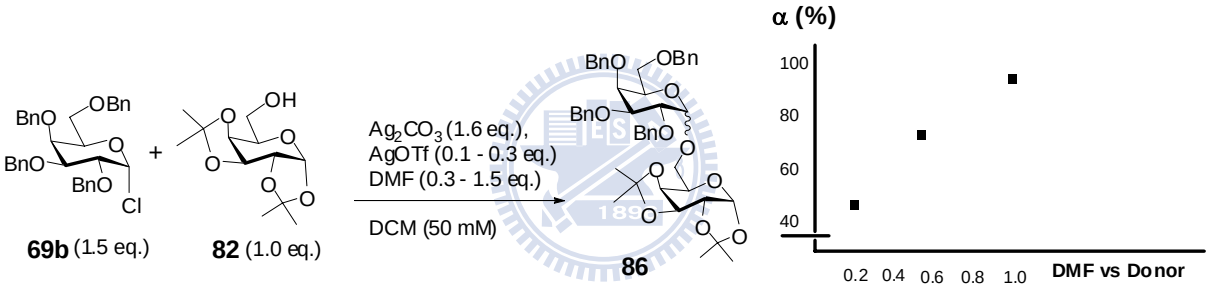
3.4. Mechanistic investigation

3.4.1. The correlation of α/β ratio and amount of DMF

Although Koto and co-workers have emphasized that increasing amount of DMF-type molecule is advantageous for pursuing a better α-stereoselectivity. The study of the reducing amount of DMF for the effect of selectivity is rarely mentioned. However, according to our findings, a stoichiometric amount of DMF is sufficient for obtaining a good selectivity, which

suggests the possible formation of common intermediate. To clarify this argument, we set out a panel of conditions and used the more reactive glycosyl donor **69b** as our model reaction. In the presence of catalytic amount of AgOTf (0.1 equiv.) and the stoichiometric amount of DMF the glycosylation proceeded very slowly unless the reaction was warmed to 0 ^{o}C (entry 1 and 2, Table 9). This phenomenon implied that the glycosylation is temperature-dependent, which is consistent with previous observations. Therefore, the fixed amount of AgOTf (0.3 equiv.) was exploited to allow the faster conversion, followed by examining the stereochemical outcome in the presence of the varying amount of DMF (entry 3-5, Table 9).

Table 9 -The correlation diagram regarding to α/β ratio and amount of DMF



Entry	Promoter (equiv.)	Additive (equiv.)	Temp (℃)	Time	Yield	α/β ratio - via NMR
1	AgOTf (0.1)	DMF (1.5)	-25 to 0	2 h	69%	83:17
2	AgOTf (0.2)	DMF (1.5)	-25 to -5	1.5 h	77%	83:17
3	AgOTf (0.3)	DMF (1.5)	-25 to -15	1 h	74%	85:15
4	AgOTf (0.3)	DMF (0.8)	-25 to -15	1 h	81%	66:34
5	AgOTf (0.3)	DMF (0.3)	-25 to -15	1 h	92%	42:58

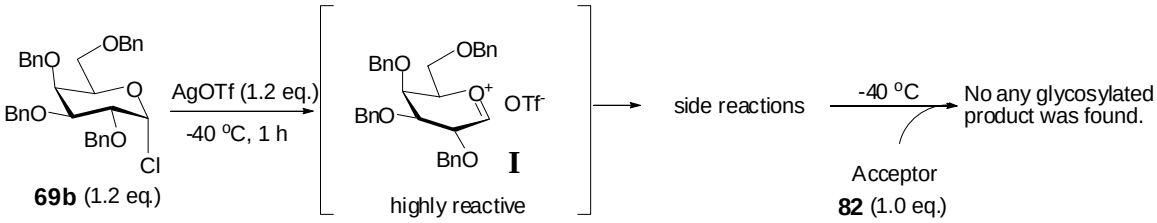
It was found the α-selectivity obtained was correlated with the amount of DMF. This result suggested that DMF is likely to react faster with the oxocarbenium ion than alcohol acceptor and direct the subsequent α-glycosylation. The stronger affinity of DMF to oxocarbenium ion is generally ascribed to the contribution from the resonances forms between the oxygen and the lone pair electron of nitrogen atom. If it is true, DMF could

function as a “brake” molecule to halt the reaction, which further bypasses the general glycosylaton pathways.

In addition, the reaction rate becomes slower in the presence of DMF. Upon the formation of the putative intermediates, the resulting α/β glycosyl iminium mixtures would be the newly formed intermediates with higher stability.

3.4.2. Pre-activation strategy for the mechanistic investigations

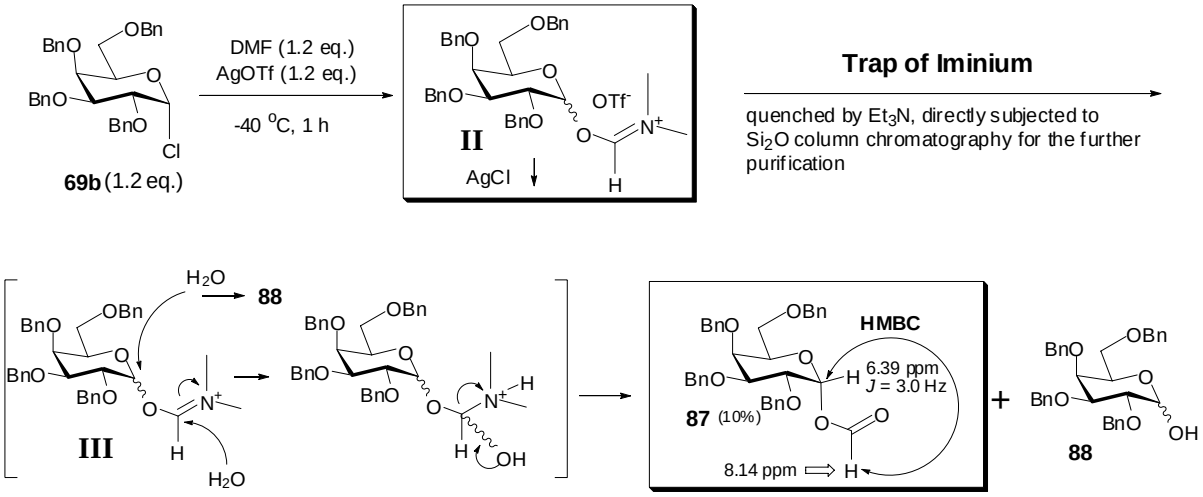
For confirmation of above speculation, this study laid out a model study. Thus, mixture of DMF (1.5 equiv.) and glycosyl chloride (1.5 equiv.) were simply activated by AgOTf (1.5 equiv.) (so called “pre-activation” approach) at -40 ^{o}C to generate the glycosyl iminum species. Subsquent addition of alcohol acceptor **82** should lead to the glycosylation product. In past decades, similar “pre-activation” strategies have emerged,[48,49] which are found useful for sequential glycosylations and provide a convenient route for oligosaccharide synthesis.



Scheme 21 - Control experiment without addition of DMF

In the begining, the control experiment was carried out without addition of DMF. After activation of glycosyl chloride **69b** by AgOTf at -40 ^{o}C (Scheme 21), the reaction mixture was standing by at the same temperature for 1 h and followed by the addition of acceptor. By

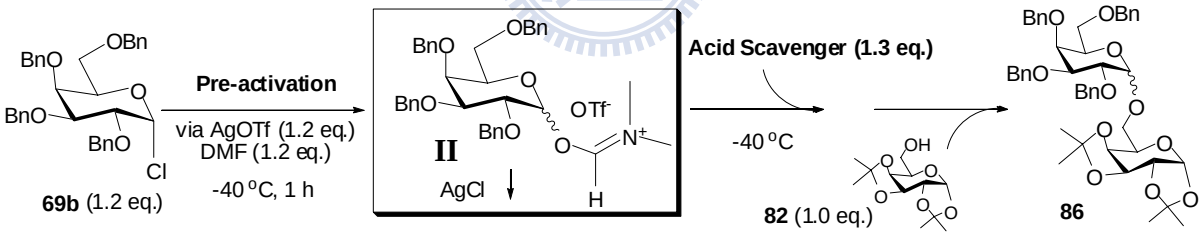
TLC examination, no traces of glycosylation product were observed. It is clear that the highly reactive glycosyl oxocarbenium species is readily decomposed and undergoes other side reactions in the absence of DMF. In the presence of DMF, the formation of new glycosyl intermediates could be detected by TLC analysis, which was a benchmark of activation. A less polar product (*ca.* 50%) was observed from TLC analysis with neutralized eluent in the presence of Et_3N. The reaction mixture was quenched by Et_3N following by a direct purification over silica gel chromatography. The less polar product could be obtained in 10% yield and determined by 1D- and 2D-NMR spectroscopy to be 2,3,4,6-tetra-*O*-benzyl-l-*O*-formyl-D-galactopyranose **87** (Scheme 22). In addition, the other polar product is identified as a hydrolyzed donor **88**. This result suggests that the oxocarbenium ion can be trapped by DMF to form the glycosyl iminium salts **III**. However, the interception of oxocarbenium by DMF may not be 100% completed but should be a predominent pathway. The occurrence of 1-*O*-formyl derivative **87** was an indirect proof for the presence of glycosyl iminium salt **III.** **III** was converted to either the product **88** or 1-*O*-formyl derivative **87** through the hydrolysis.



Scheme 22 - Trap of oxocarbenium using DMF as a "brake" molecule

Being encouraged by the experimental results, we were prompted to develop a pre-activation strategy for glycosyl chloride donors. Gratifyingly, subsequent addition of glycosyl acceptor **82** to the mixture led to a clean conversion with the mediocre α-selectivity (α/β ratio = 61:39) without treatment of Ag_2CO_3 (entry 1, Table 10). With this procedure, the amount of glycosyl chloride could be reduced to a nearly stoichiometric use. Thus, this stirred our interest to investigate the specific function played by Ag_2CO_3. To this end, 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) used to replace Ag_2CO_3, and yet similar glycosylation results were obtained (entry 2 and 3, Table 10). At -40 °C, α-selectivity (α/β ratio = 88:12) can be improved further, but at the expense of glycosylation yield (entry 4, Table 10).

Table 10 - Glycosylation with the putative glycosyl iminium **II**



Entry	Base (equiv.)	Temp (°C)	Time	Yield	α/β ratio - via ^{1}H-NMR
1	none	-40 to -5	3 h	89%	61:39
2	Ag_2CO_3 (2.4)	-40 to -5	3 h	81%	79:21
3	DTBMP (2.4)	-40 to -5	3 h	85%	78:22
4	DTBMP (2.4)	-40	10 h	55%	88:12

For detection of the proposed glycosyl iminium, ^{1}H-NMR spectroscopy was performed. A pre-mixed solution of glycosyl donor (1.0 equiv.) and DMF (1.0 equiv.) in $CDCl_3$ (25 mM) was treated with AgOTf (1.0 equiv.) at -50 °C. Unfortunately, the high viscosity of the resultant mixture at the low temperature made the solvent inhomogeneous and further

disturbed the signal detection. Upon warming the mixture to RT, except for the unreacted glycosyl chloride **69b**, a set of broad peaks (6.44 ppm and 8.95 ppm) were observed at the frist 5 min of activation (spectrum C, Figure 5). These signals should be contributed from glycosyl intermediates, which are proven distinguished from the earlier 1-*O*-formyl derivative **87** (spectrum B, Figure 5).

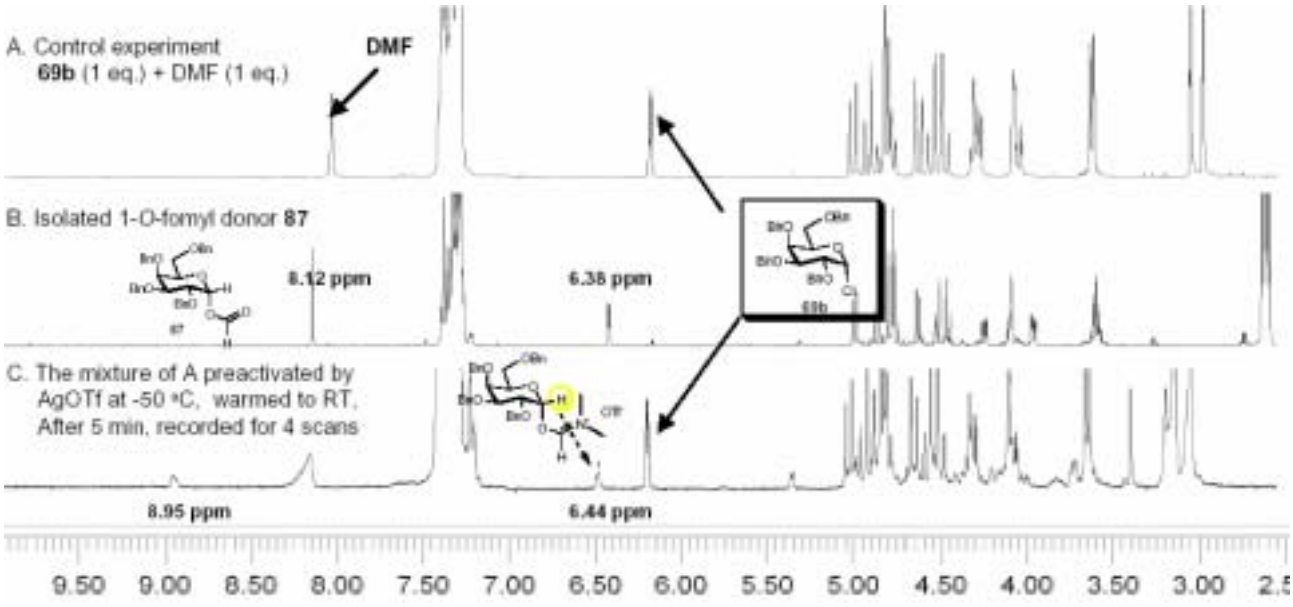
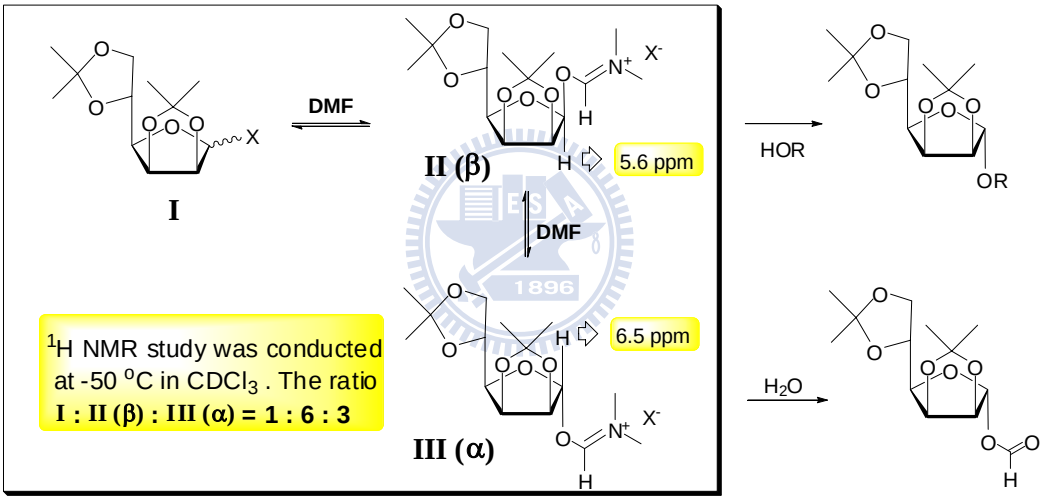


Figure 5 -Mechanistic investigation via ^{1}H-NMR

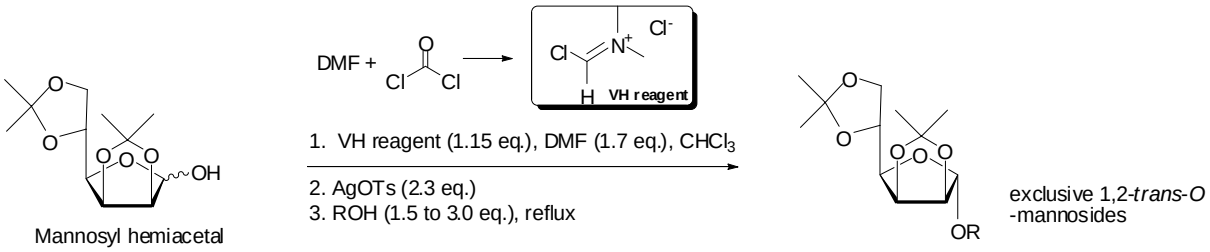
Then, after 15 min, the proton signal of the well-mixed solution with the precipitation of silver salt showed the similar pattern as the control experiment (Scheme 21). The rationale is the fact that the possible self-condensation and decomposition of the reactive cationic species may occur in the absence of the trapping molecules, such as DMF or alcohol acceptor. Moreover, we assumed that the kinetically-stable β-glycosyl iminium specie is not allowed to be observed at the higher temperature probably due to either the limited time-scale of NMR or the relatively small proportion or a short lifetime. Nevertheless, we believed that the little quantity of the more reactive β-iminum species is sufficient enough to drive the reaction to completion. However, we indeed require the more solid evidences to prove that. While we

tried to tackle this problem, we were pleased to find out some interesting papers published 30 years ago. Prof. Gross in France has done a pioneering work to identify the existence of glycosyl iminium species at -50 °C via NMR spectroscopy (Scheme 23).[50] More interestingly, they told us that "*We know that condensation of an alcohol with mannosyl donors usually gives a mixture of α- and β-mannosides. So we believe that DMF plays a specific role to give only α-mannosides. It can exert a very strong solvating effect at C-l on the reactive species or, possibly, form a small amount of a very reactive β-iminium salt at C-l,* ***II*** *or* ***III****, through the equilibrium.*"



Scheme 23 - ^{1}H-NMR study to investigate the existence of glycosyl iminium salts

Based on this assumption, they successfully applied the glycosyl imium salts as the donor precursor to construct a variety of α-mannosides under heating conditions (Scheme 24),



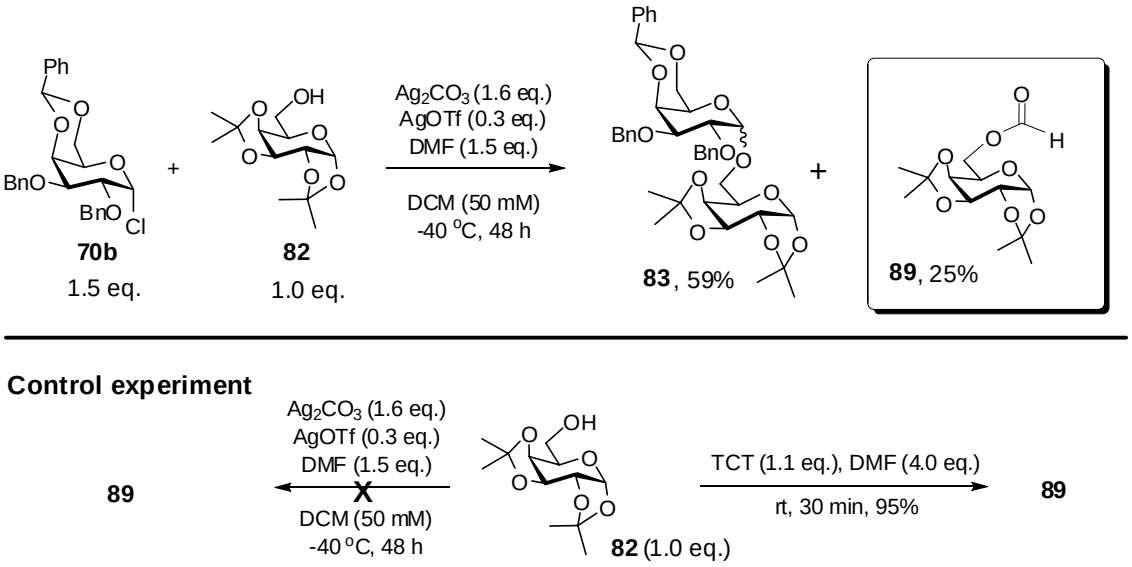
Scheme 24 - Dehydrative α-mannosylation mediated by DMF

which suggest us that such a type of glycosylation should be thermodynamically-driven in favor of 1,2-*trans*-*O*-linkage assembly in the case of mannosides.[51]

Apparently, their way of thinking is opposite to our present work. On the other hand, they first reported the observed ratio between α-mannosyl chloride, β-iminum salt, and α-iminum salt [**I**: **II**(β): **III**(α)] = 1: 6: 3 which identified the typical peaks of proton at C-1 respectively (Scheme 23). Our measured peaks (6.44 ppm and 8.95 ppm) for α-glycosyl iminium salt are in accordance to their reported value (spectrum C, Figure 1). In addition, the existence of the dominant β-iminium salt at -50 ^{o}C prompted us to consider that the greater proportion of β-iminium salt could be contributed to the improvement of α-selectivity in our cases, especially for the superior selectivity obtained at low temperature.

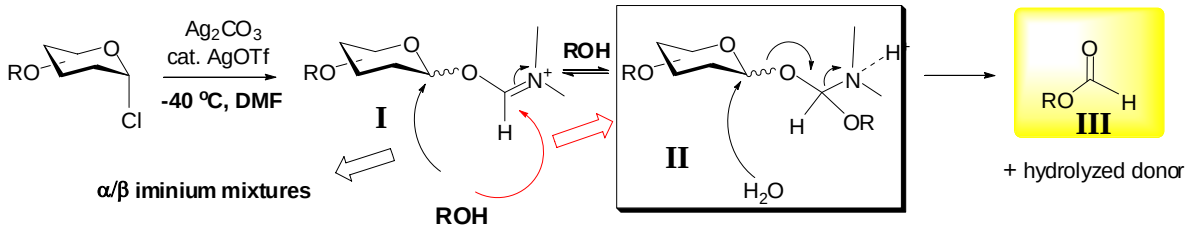
3.4.3. Identification of the 6-*O*-formyl derivative from glycosylation results

In entry 7 of Table 6, it was noted that the unidentified byproducts were formed along with the desired product. During the course of investigation, this byproduct frequently occurred at low reaction temperature and reduced the reaction yield. Moreover, its presence made purification tedious. To isolate this byproduct, a considerable effort was taken and it was proved to be 6-*O*-formyl derivative **89** (Scheme 25). For further confirmation, the glycosyl acceptor **82** was treated with TCT/DMF as a well-known formylating reagent to formylate the hydroxyl group at C-6 to afford the same compound **89**. In the meantime, a control experiment using **82** as a starting material was treated with promoting reagent alone, but no traces of the desired product **89** were obtained. As a result, the formylation should be triggered by a reactive intermediate arising from activation of glycosyl chloride.



Scheme 25 - Identification of 6-*O*-formyl derivative **89**

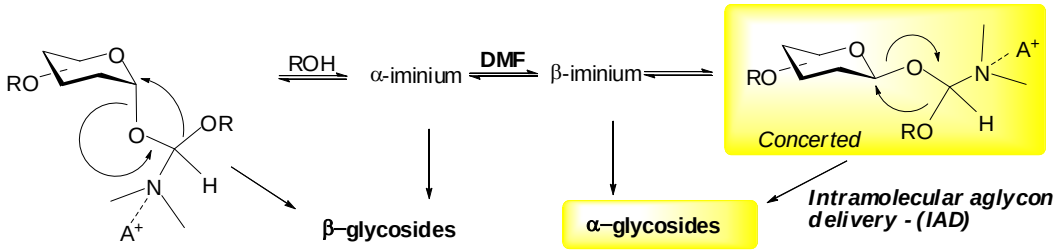
These interesting findings guided us to assume that the intermediates derived from the glycosyl donor provided a source of formylating agent. In glycosylation coupling, glycosyl acceptor is presumably attacked at C-1. The possible mechanism of formylation is that there is other electrophilic carbon in glycosyl iminum **I** susceptible to the nucleophilic attack of alcohol acceptor (Scheme 26). The resulting orthoester **II** is reversibly converted to the imium form **I** in an equilibrium state. Upon hydrolysis of orthoester **II**, the formyl derivative **III** was produced.



Scheme 26 - Plausible mechanism for the formation of 6-*O*-formyl derivative **89**

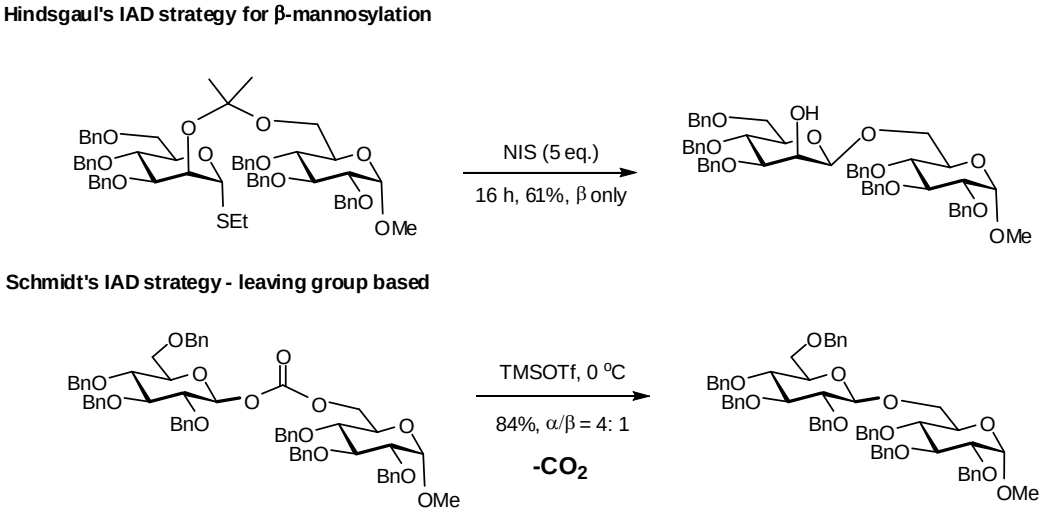
Based on the formation of orthoester **II**, it is reasonable to consider **II** as a potential intermediate for α-selective glycosylations. We hypothesized that an aglycon moiety could be

easily transferred to the anomeric center through an intramolecular delivery (IAD) (Scheme 27).



Scheme 27 - Plausible mechanism for α-selective glycosylation through IAD

Intramolecular aglycon delivery (IAD) to construct 1,2-cis-b-mannosidic bond was first introduced by Barresi and Hindsgaul,[52,53] and later elaborated by Stork and co-workers (Scheme 28).[54,55] However, the leaving group based IAD strategy was subsequently disclosed by Schmidt in 1,2-*cis*-*O*-glucosylation.[56,57] To our knowledge, the proposed IAD mechanism shown in Scheme 27 has never been reported in literatures.



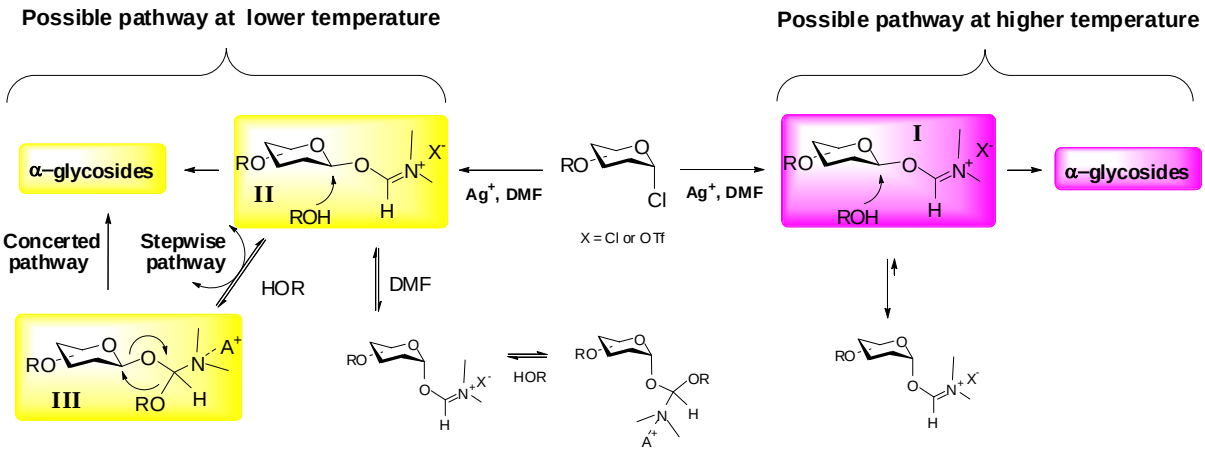
Scheme 28 - IAD approaches in the literature

This study proposes that such an IAD mechanism should be one part of possible pathways for α-glycosylations. Some facts were found as below: **I.** The reaction rate at low temperature is in proportion to the increasing amount of promoter. This phenomenon is

occasionally mentioned in IAD-related glycosylation methods.[58] **II.** The formyl-tansferred product has been observed in other cases, which implied that the formation of *N*,*O*-orthoester intermediate is likely to be universal. **III.** With tracking the progress of pre-activation procedure, upon the addition of the acceptor, the several sugar moieties appeared at the initial stage and finally converted to the desired product with the prolonged time. This observation suggests that the reactive intermediates are unlikely to be a single species.

3.4.4. Summarize the plausible reaction pathways

Indeed, our data have provided the experimental evidences to support the proposed mechanism. The possible reaction routes are outlined in Scheme 29. **(1).** At higher reaction temperature, the reactive β-glycosyl iminium **I** participates in production of α-glycoside. This putative β-glycosyl iminium **I** is similar to β-halide proposed earlier by Lemieux.



Scheme 29 - The plausible reaction pathways

However, detection of β-iminium at high temperature is difficult for the technical problems. **(2)**. At lower temperature, the equilibrium between α- and β-iminium species is considerably shifted to the kinetically-stable β-glycosyl iminium intermediate **II**, which is believed as an

advantage in favor of α-selectivity. **(3).** Although the existence of orthoester intermediate **III** remains speculative at this stage, isolation of 6-*O*-fomyl derivatives should provide indirect support for IAD mechanism.

To prove further these hypotheses, more questions need to be addressed, such as the possibility to confirm the generation of β-glycosyl iminium via low-temperature NMR analysis. Moreover, the other possibility should be considered that the orthoester intermediates may not only proceed into the concerted pathway to form α-glycosides, but also reversibly converted to glycosyl iminiums to which the nearby acceptor is more readily accessed (Scheme 29).

3.5. Standardizing the reaction procedure

To aim at developing a general DMF-mediated α-selective glycosylation, an extentive set of experiments under the different conditions were conducted. By some efforts, these glycosylation protocols were devised and found suitable for different glycosyl substrates.

Procedure A at the room temperature – Following the adding sequence - Ag_2CO_3 (1.3 equiv.), molecular sieve (AW300, 50 mg/per 1mL of solvent), the alcohol acceptor (1.0 equiv.), DMF (1.2 equiv.), the glycosyl donor (1.2 equiv.) – The final concentration was adjusted to 50 mM by DCM. To the reaction mixture a solution of AgOTf (0.05 equiv, con. = 0.2 M of AgOTf in toluene) was added at room temperature. Upon completion of reaction via TLC monitoring, the reaction was quenched by Et_3N (10 equiv.) and directly subjected to SiO_2 column chromatography. In a small scale (less than 0.1 mmol of the alcohol acceptor), 1.5 equiv of α-glycosyl chloride is recommended. Furthermore, the used amount of other reactants (Ag_2CO_3 and DMF) should be modified according to the principle mentioned earlier (Ag_2CO_3

is 1.1 fold equivalent vs. glycosyl chloride; equivalent of DMF is identical to glycosyl chloride).

Procedure B at -25 ^{o}C - The adding and work-up sequence follow the procedure A. The use of more α-glycosyl chloride (1.5 - 2.0 equiv.) is a compromise between the yield, efficiency and possible decomposition of the reactive intermediates during the reaction course.

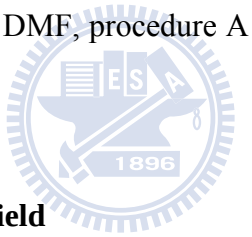
Procedure C – **Pre-activation glycosylation** – Mixture of DMF (1.2 equiv.), glycosyl chloride (1.2 equiv.) and molecular sieve (AW300, 50 mg/per 1mL solvent) in DCM was cooled to -40 ^{o}C to which a solution of AgOTf (1.2 equiv, con. = 0.2 M of AgOTf in toluene) was added. The resulting mixture was stirred at -40 ^{o}C for 1 h, which was added either Ag_2CO_3 (1.3 equiv.) or [DTBMP (1.3 equiv.)] and the alcohol acceptor (1.0 equiv.) at -40 ^{o}C. Upon completion of glycosylation, the reaction was quenched by excess Et_3N at -40 ^{o}C, then gradually warmed to the room temperature and filtered by Celite. The filtrate was diluted with DCM, washed with saturated sodium bicarbonate solution, brine, dried with $MgSO_4$. The crude product was purified by column chromatography over silica gel.

Procedure D – **Inverse addition protocol** (especially for the less reactive secondary glycosyl acceptor) – Mixture of molecular sieve (AW300, 50 mg/per 1mL solvent) and the alcohol acceptor (1.0 equiv.) in DCM was cooled to -40 ^{o}C and treated with TMSOTf (2.0 equiv.) The resulting mixture was stirred at -40 ^{o}C for 10 min ensuring the complete formation of TMS-ether. To the mixture is dropwise added DMF (1.5 equiv.) and a solution of AgOTf (3.0 equiv, con. = 0.2 M of AgOTf in toluene) followed by the addition of glycosyl donor (3.0 equiv, 100 mg donor in 1 mL of DCM) in 5 min at -40 ^{o}C. Upon completion of glycosylation, the reaction was quenched by excess Et_3N at -40 ^{o}C, then gradually warmed to the room temperature and filtered by Celite. The filtrate was diluted with DCM, washed with saturated sodium bicarbonate solution, brine, dried with $MgSO_4$. The crude product was subsequently

purified by column chromatography over silica gel. For this inverse addition protocol, the reasons why we applied it to the secondary glycosyl acceptor will be discussed in the later section.

3.6. Extend the scope to other donors and acceptors

With DMF-mediated α-selective glycosylation and differing procedures in hand, we attempted to apply our method to prepare 1,2-*cis*-*O*-linkage with a range of acceptors by the use of the common promoting system ($AgOTf/Ag_2CO_3$) under Koenigs-Knorr conditions. In addition, we decided to choose a serial of galacto- and glucopyranosyl chlorides as model donors to evaluate the performance of DMF in stereoseletivity and yield. For comparison, three protocols (procedure A without DMF, procedure A at room temperature, procedure B at -25 ^{o}C) were carried out in parallel.

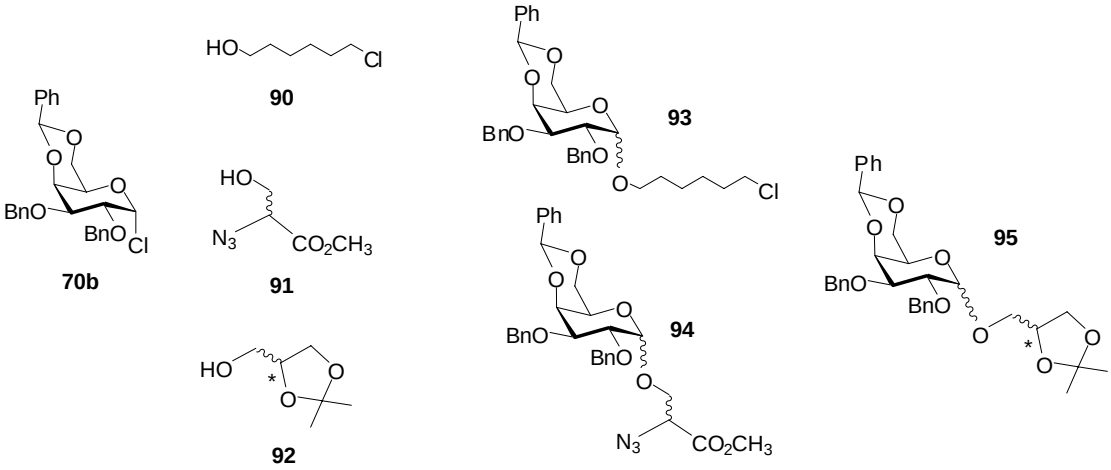


Characterization of α/β ratio and yield

Upon the characterization via ^{1}H, ^{13}C, COSY, HMQC, α/β ratio of the coupled saccharides from the crude product are determined by the integration of the typical peaks for α- and β-anomers through ^{1}H NMR. For the difficult cases, the intensities of ^{13}C peaks between 90 to 110 ppm through ^{13}C NMR were recruited as assessment of α/β ratio. For instance, the range between 90 and 100 usually indicate α-*O*-linkage, whereas the range between 100 and 110 usually indicate β–*O*-linkage. This can be further confirmed by $^{1}J_{C1,H1}$ (~170 Hz) for α linkages and $^{1}J_{C1,H1}$ (~160 Hz) for β linkages via the non-decoupling experiment. After purification, the collected α/β mixed product was recorded in an isolated yield.

3.6.1. Glycosylation of α-Galactosyl chlorides with primary alcohol acceptors

Table 11 - Glycosylation of **70b** with the simple primary alcohol acceptors

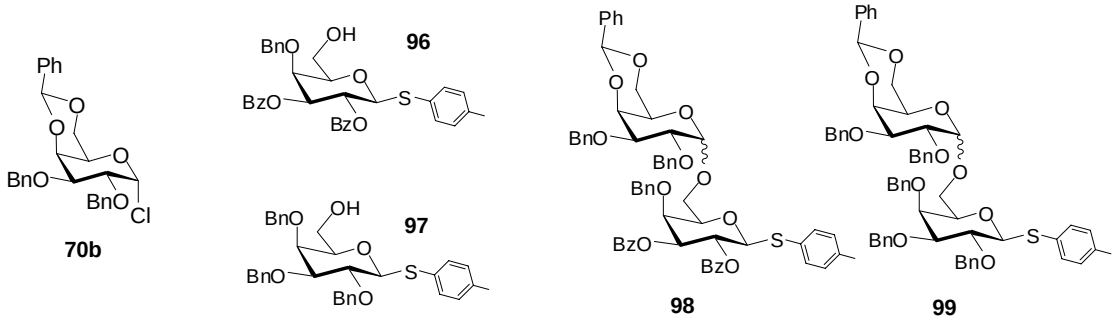


Entry	Donor	Acceptor	Product	Procedure	Time	Yield	α/β ratio
1	**70b**	**90**	**93**	No DMF	30 min	88%	8:92
2	**70b**	**90**	**93**	A	30 min	89%	84:16
3	**70b**	**90**	**93**	B	5 h	84%	94:6
4	**70b**	**91**	**94**	A	30 min	85%	>95:5
5	**70b**	**92**	**95**	No DMF	30 min	72%	56:44
6	**70b**	**92**	**95**	B	5 h	75%	>95:5

The scope of DMF-mediated α-selective glycosylation was examined with a number of primary alcohol acceptors. For glycosylation of 6-chloro hexanol **90**, β-anomer **93β** was obtained as a major product with α/β ratio (8:92) in the absence of DMF (entry 1, Table 11). While addition of just stoichoimetric DMF dramatically reversed the selectivity to the formation of α-anomer **93α** (entry 2, Table 11). Furthermore, the greater α-selectivity (α/β ratio = 94:6) at -25 ^{o}C was obtained in a good 84% yield (entry 3, Table 11). These results indeed support our assumption: α-selectivity could be better at lower temperature. In

line with the result of acceptor **90**, both of acceptors **91** and **92** successfully coupled to **70b** with good α-selectivities (entry 4-6, Table 11). However, the racemic acceptors as starting materials resulted in the diastereomeric mixtures of products clearly shown in ^{13}C NMR analysis.

Table 12 - Orthogonal glycosylation of **70b** with the primary glycosyl acceptors

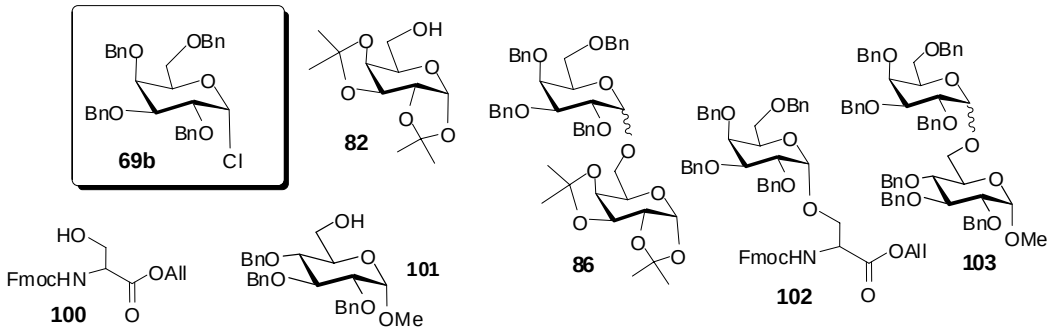


Entry	Donor	Acceptor	Product	Procedure	Time	Yield	α/β ratio
1	**70b**	**96**	**98**	No DMF	30 min	61%	75:25
2	**70b**	**96**	**98**	A	3 h	84%	94:6
3	**70b**	**96**	**98**	B	3 h	40%	>95:5
4	**70b**	**97**	**99**	No DMF	30 min	85%	35:65
5	**70b**	**97**	**99**	A	30 min	89%	93:7

The orthogonal glycosylation steps are based on the selective activation of a leaving group, which can considerably provide an efficient route to construct the oligosaccahrides. The compatibility of glycosyl halides and thioglycosides has been long well known. Gratifyingly, both of thioglycosides **96** and **97** enable coupling with the galactosyl chloride **70b** in excellent yields (84–89%) under these conditions (entry 2 and 5, Table 12). In comparison to the results without mediation of DMF (entry 1, 2, 4 and 5), the significant

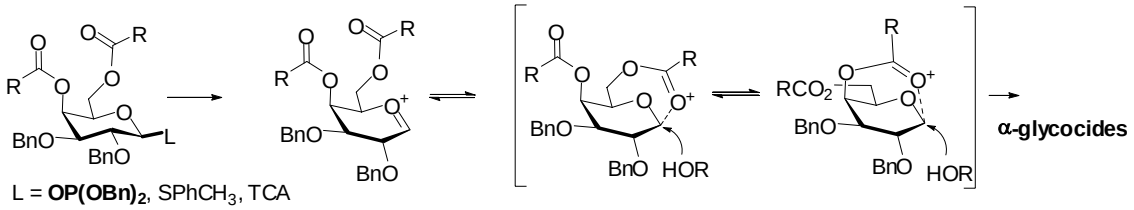
improvement (α/β ratio from 35:65 to 93:7 and 75:25 to 94:6) has been achieved no matter by which the arming or disarming acceptor was exploited.

Table 13 - Glycosylation of **69b** with the primary alcohol acceptors



Entry	Donor	Acceptor	Product	Procedure	Time	Yield	α/β ratio
1	**69b**	**82**	**86**	No DMF	30 min	85%	55:45
2	**69b**	**82**	**86**	A	30 min	94%	85:15
3	**69b**	**82**	**86**	B	18 h	90%	88:12
4	**69b**	**100**	**102**	No DMF	30 min	92%	90:10
5	**69b**	**100**	**102**	B	18 h	90%	>95:5
6	**69b**	**101**	**103**	B	15 h	72%	75:25

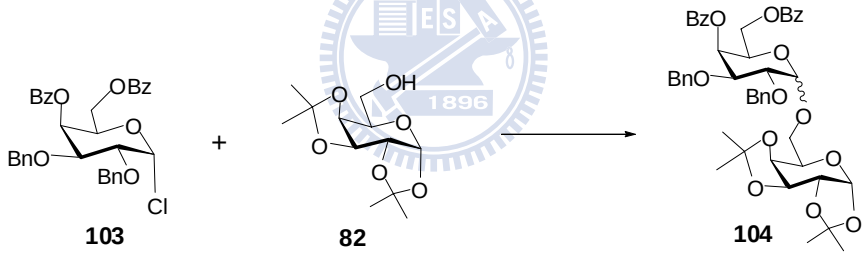
When per-*O*-benzylated galactosyl chloride **69b** was used as a model donor,[3] similar α-stereochemical preference was observed (entry 1-3, Table 13). Interestingly, serinyl acceptor **100** presented an innate property for α–selectivity (α/β ratio = 90:10, entry 4, Table 13). However, the application of DMF-mediated glycosylation to **100** further boosted α–selectivity (α/β ratio > 95:5, entry 5, Table 13). Notably, α–selectivity by coupling with **101** slightly decreased (α/β ratio = 75:25) and the reason is not clear at present.



Scheme 30 - Lin's proposed mechanism for α-selective galactosylation via 4- and 6-acyl remote participation.

In 2002, Lin *et al.* found that the 2,3-*O*-dibenzyl-4,6-*O*-dibenzoyl galactosyl phosphite donor was shown to be an excellent donor for α–selective galactosylation.[59] The remote participation of the 4- and 6-acyl group is proposed to account for the results (Scheme 30). More interestingly, they also emphasized that α–selectivity is temperature-dependant and a higher α–selectivity was obtained at higher glycosylation temperature.

Table 14 - Glycosylation of **103** with the primary alcohol acceptor **82**



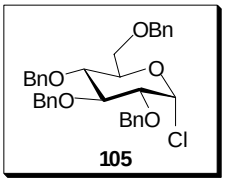
Entry	Donor	Acceptor	Product	Procedure	Time	Yield	α/β ratio
1	**103**	**82**	**104**	No DMF	30 min	86%	77:23
2	**103**	**82**	**104**	A	30 min	94%	91:9
3	**103**	**82**	**104**	B	15 h	59%	>95:5

Their report prompted us to exploit 2,3-*O*-dibenzyl-4,6-*O*-dibenzoyl galactosyl chloride **103** as a glycosyl donor for coupling with the diacetone galactoside **82**. Indeed, a higher α ratio (α/β ratio = 77:23) was shown in a control experiment. However, by following the procedure A, α/β ratio can be significantly improved to 91:9 (entry 2, Table 14). At the lower

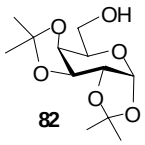
temperature, the reaction was not completed within 15 h. The moderate 59% yield was obtained with better α–selectivity (α/β ratio >95:5, entry 3, Table 14).

3.6.2. Glycosylation of α-Glucosyl chlorides with primary alcohol acceptors

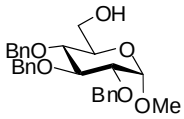
Table 15 - Glycosylation of **105** with the primary glycosyl acceptors



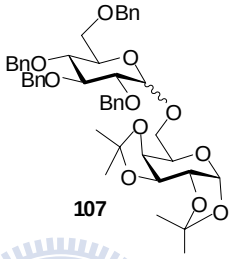
105



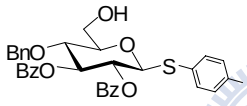
82



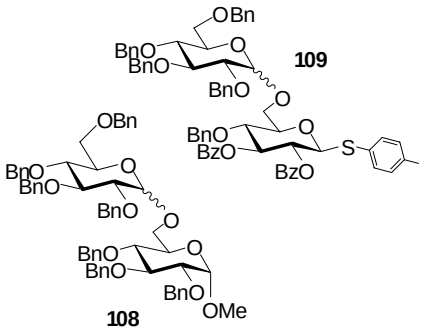
101



107



106



108

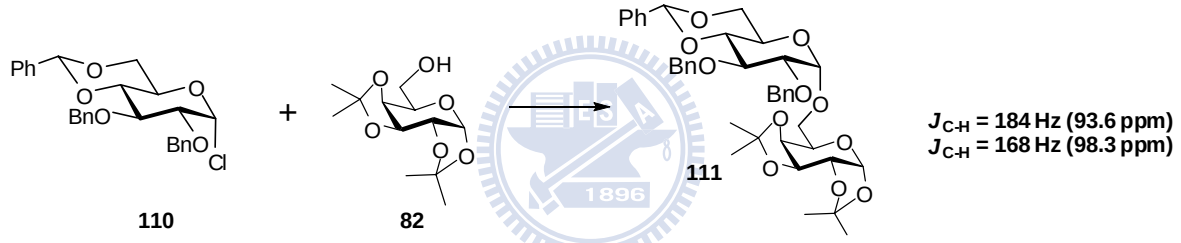
Entry	Donor	Acceptor	Product	Procedure	Time	Yield	α/β ratio
1	**105**	**82**	**107**	No DMF	30 min	65%	44:56
2	**105**	**82**	**107**	A	1 h	72%	63:37
3	**105**	**82**	**107**	B	18 h	84%	71:29
4	**105**	**101**	**108**	No DMF	30 min	71%	49:51
5	**105**	**101**	**108**	A	1 h	83%	64:36
6	**105**	**101**	**108**	B	18 h	84%	77:23
7	**105**	**106**	**109**	No DMF	30 min	87%	56:44
8	**105**	**106**	**109**	A	1 h	72%	79:17
9	**105**	**106**	**109**	B	18 h	38%*	83:17

***** Glycosylation didn't emerge at -25 °C. Until warming to 0 °C, the reaction proceeded very slowly.

Encouraged by the positive results in α–selective galactosylations, this study turned to investigate the gluco- series. Therefore, per-*O*-benzylated glucosyl chloride **105** was readily prepared from the glycosyl hemiacetal by following TCT/DMF chlorination protocol. Parallel

glycosylations of **105** with the three different glycosyl acceptor (**82**, **101** and **106**) were performed by three procedures (no DMF, A and B).[3] Most of the resultant disaccharides were obtained in good yield (65-87%), but only moderate α–selectivities (at least, poorer than galactose) was obtained (entry 1-9, Table 15). Nevertheless, the temperature-dependent feature remains consistent with out previous examples. It is noteworthy that glycosylation of glycosyl acceptor **109** could not be initiated at -25 °C until warming to 0 °C (entry 9, Table 15). Despite of this problem, this reaction was still sluggish, giving the poorer coupling yield (38%).

Table 16 - Glycosylation of **110** with the primary glycosyl acceptor **82**



110 **82** **111**

Entry	Donor	Acceptor	Product	Procedure	Time	Yield	α/β ratio
1	**110**	**82**	**111**	No DMF	30 min	88%	85:15
2	**110**	**82**	**111**	A	18 h	32%[a]	>95:5
3	**110**	**82**	**111**	B	18 h	10%[b]	>95:5
4	**110**	**82**	**111**	C	5 h	76%[c]	>95:5

[a] Some of glycosyl chloride was recovered after purification.

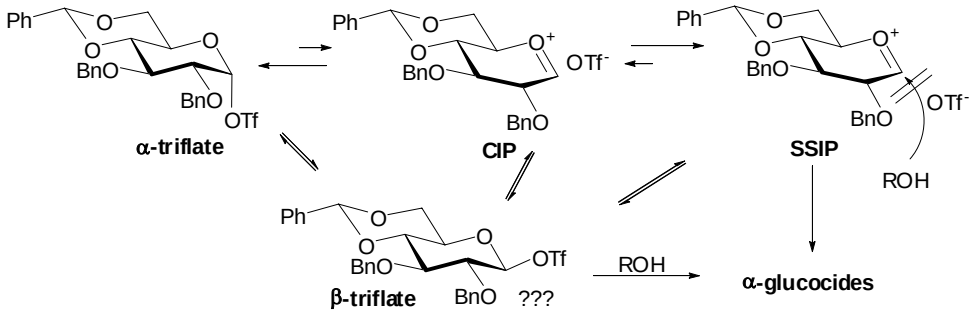
[b] Run at -25 ℃ for 18 h. Most of glycosyl chloride was recovered.

[c] Use the pre-activation procedure at -25 ℃. Upon the addition of acceptor, the reaction was gradually warmed to the room temperature. The crude product contains some 'Formyl-transfer' product.

In order to achieve a better α–selectivity in glucosylations, an attempt to prepare 4,6-*O*-benzylidene-D-glucopyranosyl chloride **110** was made to investigate its performance in α–selective glycosylations. Unexpectedly, glycosylation of **110** with acceptor **82** led to the

desired disaccharide **111** in 88% yield with a good α–selectivity (α/β ratio = 85:15) even in the absence of DMF (entry 1, Table 16). For the other comparative experiment by the use of DMF, most of glycosyl chloride still remained and the poor 32% yield of the disaccharides **111** was obtained (entry 2, Table 16). In addition, only traces of glycosylation product were observed when the reaction was carried out at -25 ^{o}C (entry 3, Table 16).

Based on the literature search, 4,6-*O*-benzylidene-D-glycopyranosyl chloride **110** has not been employed as a glycosyl donor for α-selective glycosyltions. Though corresponding donors with different leaving groups were explored, previous stereoselective outcomes observed were variable in some cases.[60-65] The related studies regarding to benzylidene protected glycosyl halides is rarely mentioned.[63] In an effort to study the role of DMF, the pre-activation protocol was adopted to elucidate the factor resulting in slow conversions. Upon the pre-activation of chloride **110** followed by adding acceptor **82**, the glycosylation was completed within 5 h and the dissachride **111** was produced in 76% yield with an exclusive α–selectivity (entry 4, Table 16). In general, one equivalent of AgOTf was used as the promoter under pre-activation conditions. The success of pre-activation strategy suggested that the increasing quantity of promoter could accelerate the reaction. This finding could be useful in the following investigation. Although the reason for the superior α–selectivity in coupling of **110** with the primary glycosyl acceptor **82** is still unclear, we assumed that α–selectivity might result from the innate property of glycosyl chloride with the benzylidene protecting group and even the promoters.



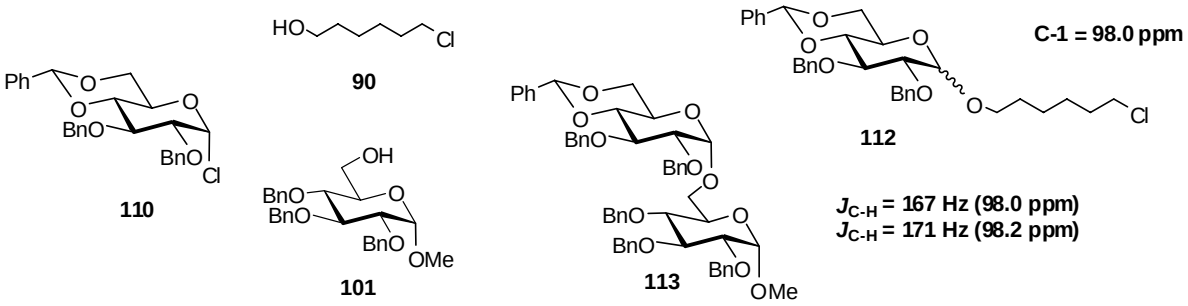
Scheme 31 - Proposed mechanism for α-selective glucosylation effected by a bicyclic protecting group

It is well established that *trans*-fused bicyclic protecting group in glycosides not only affects the stereochemical course in glycosylation but also the reactivity of donors or acceptors. Among these protecting functions, 4,6-*O*-benzylidene protected thioglycosides has been intensively investigated to address its unique property in α–selective glycosylations. The reason is generally given that the benzylidene acetal exerts a torsional effect disfavoring the formation of oxocarbenium ion. It is believed that α–triflate is not the true reaction species even though a greater proportion of α–triflate may exist in the reaction media. However, in order to maintaining C5-C6 bond in the most electron-withdrawing tg conformer, the minor contact ion pair (CIP) is not a favorable intermediate in an equilibrium state (Scheme 31).[66] Accordingly, the solvent separated ion pair (SSIP) would be most likely the true species participating in formation of α–glycosides due to anomeric effect. Based on the notion of the reactive β–halide described by Lemieux, β–triflate could be a potential reacting species even though β–triflate has never been detected.

As part of our continuing interest, we anticipated that this phenomenon could be observed while other simple alcohols serve as glycosyl acceptors. In the case of 6-chlorohexanol **90**, the similar results with the glycosyl pairs of **110** and **82** was presented in

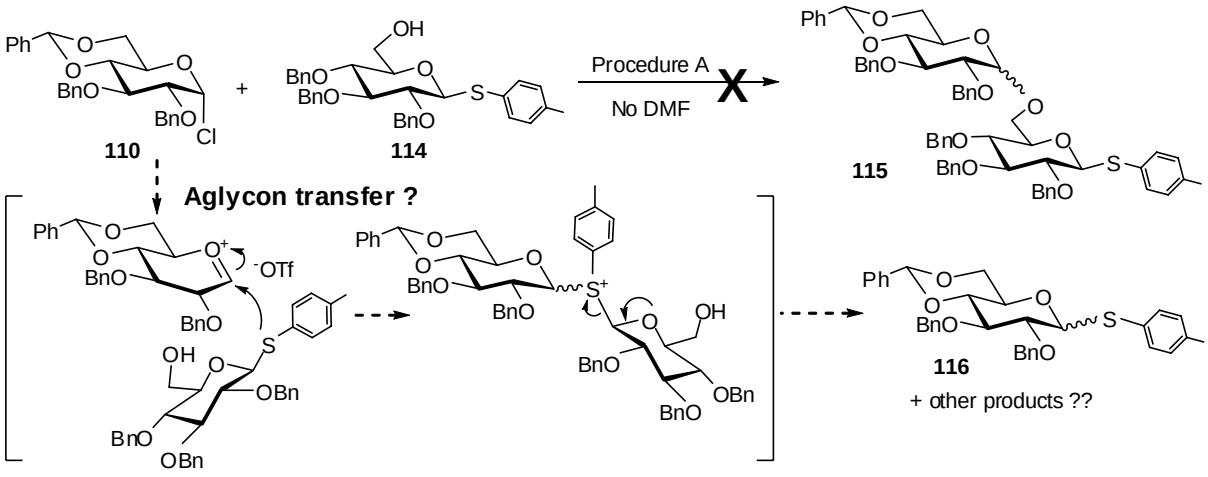
entry 1-3 of Table 17, except for the slightly lower α–selectivity obtained without the presence of DMF (entry 1, Table 17).

Table 17 - Glycosylation of **110** with the primary alcohol acceptors



Entry	Donor	Acceptor	Product	Procedure	Time	Yield	α/β ratio
1	**110**	**90**	**112**	No DMF	12 h	90%	87:13
2	**110**	**90**	**112**	A	24 h	31%	>95:5
3	**110**	**90**	**112**	B	24 h	21%	>95:5
4	**110**	**101**	**113**	No DMF	5 h	72%	90:10

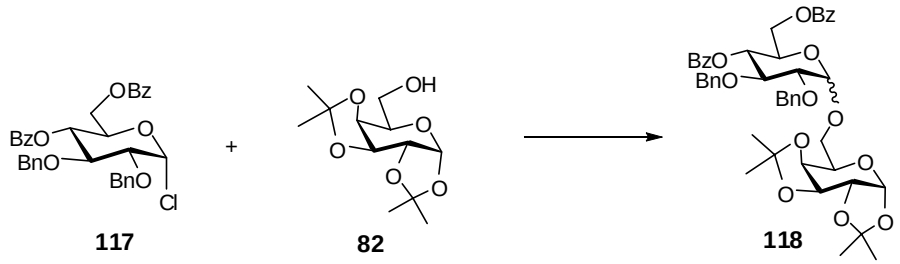
In our observations, it should be noted that α–chloride **110** with a greater stability can be tolerate under silica gel purification conditions without the significant loss of materials. It could be reasonably ascribed to the disarming property resulted from the benzylidene functions.



Scheme 32 - Orthogonal glycosylation of **110** with the primary glycosyl acceptor **114**

Furthermore, it is not surprising that coupling of **110** with the more reactive thioglycosyl acceptor **114** failed to give any desired disaccharide **115** probably owing to the potential aglycon transfer which usually emerges in the glycosyl pairs of the disarming donor and arming acceptor with a latent leaving group (Scheme 32).[69]

Table 18 - Glycosylation of **117** with the primary alcohol acceptor **82**



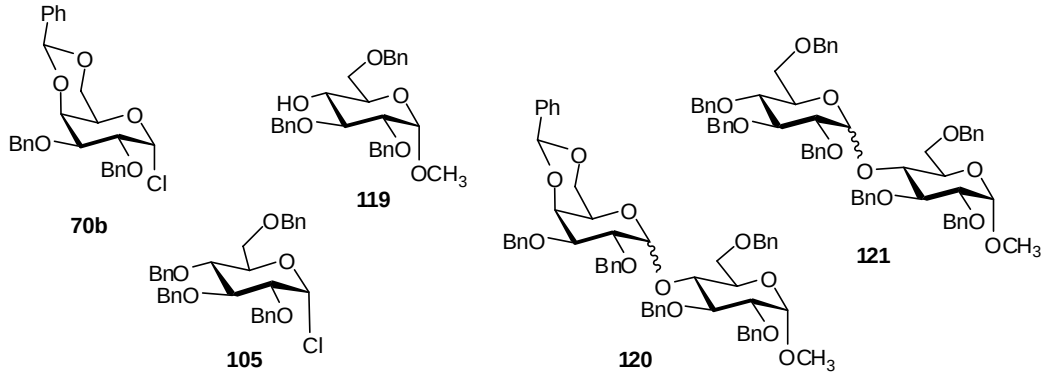
117 **82** **118**

Entry	Donor	Acceptor	Product	Procedure	Time	Yield	α/β ratio
1	**117**	**82**	**118**	No DMF	2 h	74%	72:28
2	**117**	**82**	**118**	A	3 h	62%	87:13
3	**117**	**82**	**118**	B	48 h	79%	93:7

With the same procedures, glycosylation of acceptor **82** with 2,3-*O*-dibenzyl-4,6-*O*-dibenzoyl α–glucosyl chloride **117** at -25 ^{o}C provided the expected disaccharide **118** in 79% yield with an improved α–selectivity (α/β ratio = 93:7) (entry 3, Table 18).

3.6.3. The secondary alcohol acceptor

Table 19 - Glycosylation with the secondary alcohol acceptor **119**



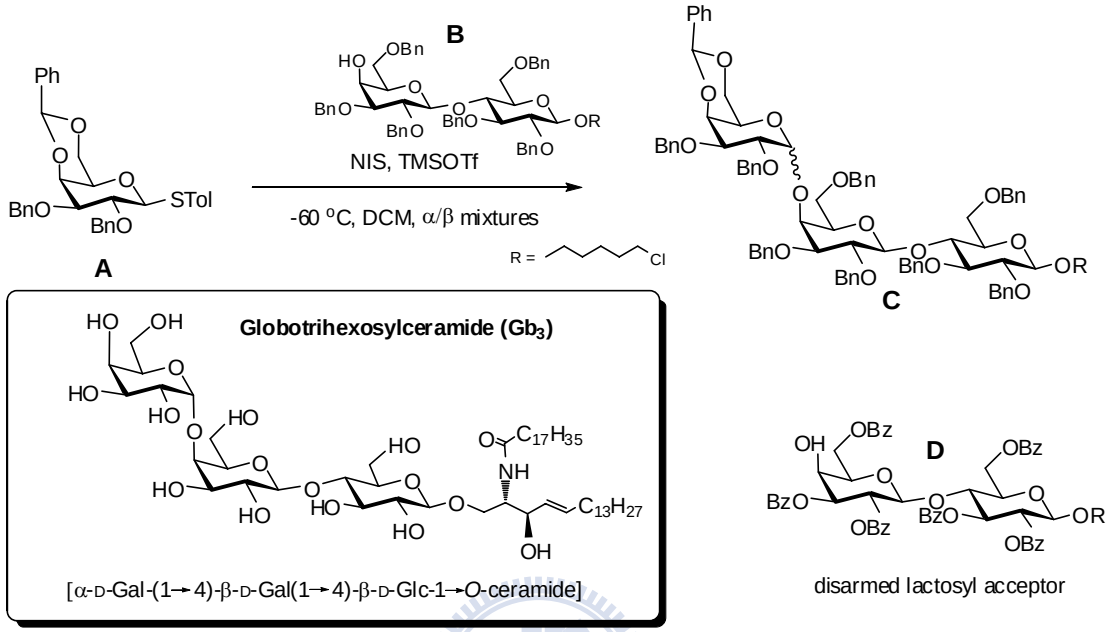
Entry	Donor (equiv.)	Ag_2CO_3 (equiv.)	TMSOTf (equiv.)	AgOTf (equiv.)	Procedure	Product	Time	Yield	α/β ratio
1	**70b** (2.0)	2.2	none	2.0	No DMF	**120**	12 h	0%	
2	**70b** (2.0)	2.2	none	2.0	A	**120**	18 h	58%	>95:5
3	**70b** (2.0)	2.2	none	2.0	B	**120**	18 h	18%	>95:5
4	**70b** (3.0)	none	2.0	3.0	D	**120**	1.5 h	85%	>95:5
5	**70b** (2.0)	none	none	2.0	D	**120**	2 h	62%	>95:5
6	**70b** (2.0)	none	2.0	2.0	D	**120**	2 h	82%	>95:5
7	**70b** (2.0)	none	1.0	2.0	D	**120**	2 h	75%	>95:5
8	**105** (2.0)	2.2	none	2.0	D	**121**	48 h	53% [a]	85:15

After screening of the primary acceptors, we set out to examine the feasibility of this method for coupling with secondary alcohol acceptor. Unfortunately, glycosylations of **70b** with acceptor **119** for the assembly of α–(1→4) linkage were inefficient regardless of the procedure used (entry 2 and 3, Table 19). Even though the moderate yield (58%) was obtained in a high α–selectivity (α/β ratio > 95:5), the overall efficiency is not acceptable. It is noteworthy that no traces of glycosylated products were found in the reaction mixture while DMF was not used an additive (entry 1, Table 19). It seemed that the more hindered

secondary alcohol is difficult to react with the activated intermediate. This negative result could imply that the highly unstable oxocarbenium ion likely needs to be stabilized by some species, such as DMF, to prolong its survival time, which allows the acceptor for the further coupling. However, optimizing the conditions for the secondary alcohol acceptor is imperative for our present program. Thus, several approaches could be considered. **I.** In the previous works, increasing amount of promoters may accelerate the reaction. **II**. By referring to the earlier reports in the literatures,[68-71] the alcohol acceptor protected by the silyl group can increase the nucleophilicity of hydroxyl functions. TMSOTf is well known as a silylating reagent and mixing TMSOTf with acceptors may lead to the formation of TMS-ether acceptor. **III.** To this aim, the inverse addition procedure was undertaken, such that the acceptor is first added, followed by mixing with TMSOTf to prepare TMS-ether. The glycosyl donor is the last reagent adding to the mixture. Based on these assumptions mentioned above, the inverse addition procedure D was adopted, particularly for glycosylations with the secondary alcohol acceptor (the detailed manipulation is described in the earlier section). Gratifyingly, applying the procedure D led to the formation of the disaccharide **120** in an excellent 82% yield with an exclusive α–selectivity (entry 4-7, Table 19). Reducing the used amount of donor and TMSOTf makes this protocol more practical (entry 6, Table 19).

Although the coupling efficiency with the secondary acceptor is not high as the primary acceptor, the more detailed studies are needed to breakthrough the present limitation. Therefore, the synthesis of Gb_3 derivative was selected as our next target molecule. Gb_3 ceramide is a member of the glycosphingolipids (Scheme 31).[71] It was found highly expressed in several types of tumor cells, such as teratocarcinoma, embryonal carcinoma. Recently, the tumor-associated carbohydrate antigens based on the core trisaccharides of Gb_3 ceramides were synthesized for the development of vaccine against the related cancers. Its biological

significance attracted a lot of attention in pursuing its efficient synthesis. In the past decades, Gb_3, also known as the P_k antigen, has been prepared by the different groups (Scheme 33).[73-75]

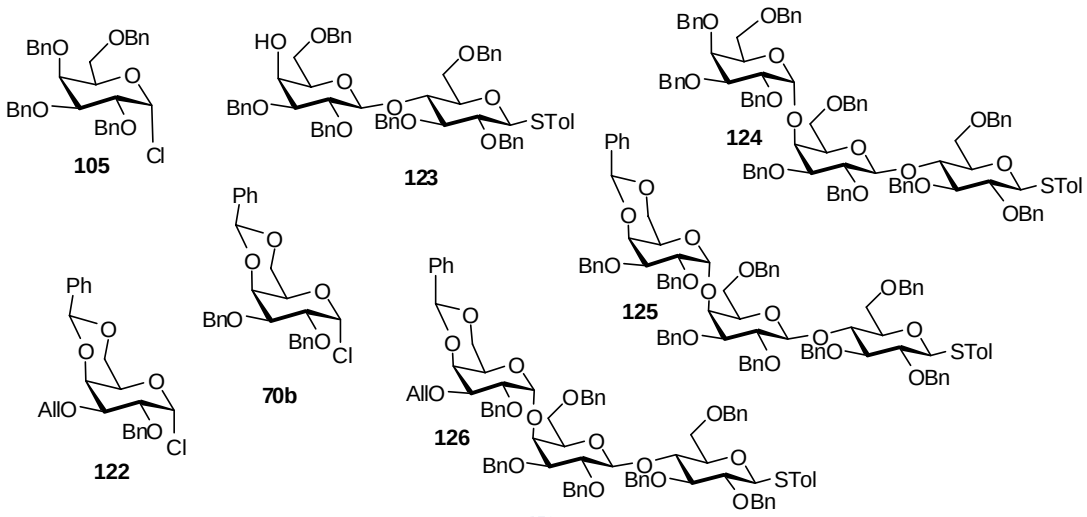


Scheme 33 - Previous synthesis of Gb_3 derivative in our lab

However, the construction of Gal-α-(1-4)-Gal linkage is the main challenge in the synthesis of Gb_3 derivatives.[49] Indeed, an attempt to assemble the thioglycoside donor **A** with lactosyl acceptor **B** failed to give us a good α–selectivity in our lab. More efforts have been made to work on this problem, such as the replacement of solvents, temperature, promoters, which have proved ineffective for a better α–selectivity. In view of the previous approaches reported by other groups, the disarmed lactosyl acceptor **D** was generally adopted as a suitable glycosyl partner to achieve α–selective glycosylaton.[74] To test whether DMF-mediated glycosylation can be performed as an alternative strategy to solve the problem, we begun with the glycosyl donor-acceptor pairs of per-*O*-benzylated glactosyl chloride **105** and per-*O*-benzylated lactoside **123** in probing the influence of DMF. Without any optimization, the desired trisaccharide was obtained in 45% yield with an exclusive

α−selectivity (entry 2, Table 20), whereas the random α/β selectivity (53:47) was given in the absence of DMF (entry 1, Table 20).

Table 20 - Glycosylation with the lactosyl acceptor **123**



Entry	Donor (equiv.)	Ag_2CO_3 (equiv.)	TMSOTf (equiv.)	AgOTf (equiv.)	Procedure	Product	Time	Yield	α/β ratio
1	**105** (2.0)	2.2	none	2.0	No DMF	**124**	4 h	32%	53:47
2	**105** (2.0)	2.2	none	2.0	D	**124**	4 h	45%	>95:5
3	**70b** (2.0)	2.2	none	2.0	D	**125**	15 h	20%	>95:5
4	**70b** (3.0)	3.3	none	3.0	D	**125**	15 h	35%	>95:5
5	**70b** (3.0)	none	2.0	3.0	D	**125**	4 h	75%	>95:5
6	**70b** (2.0)	none	2.0	2.0	D	**125**	8 h	60%	>95:5
7	**122** (3.0)	none	2.0	3.0	D	**126**	4 h	78%	>95:5

As mentioned earlier for optimal conditions towards the secondary alcohol acceptor, coupling glycosyl donor **70b** with lactosyl acceptor **123** served as a model reaction to examine the reproducibility of the established inverse-addition protocol. The results were depicted in entry 3-6 of Table 20, which shows a consistency with our previous findings. Again, TMSOTf has been proved effective in completion of the reactions. This fact could be

rationalized that TMSOTf not only increases the nucleophilicity of acceptor via the temporary TMS-ether but also provides more of the free triflate anion in the reaction mixture. As a result, the triflate anion might provoke the reactions through association between itself and any possible reactive intermediates, such as iminium species. To prevent the potential decomposition or deprotection of reactants under the presence of excess triflate anion, the inverse-addition protocol needs to be executed at lower temperature (from -40 to -25 ^{o}C). On the other hand, it should be noted that an attempt to reduce the amount of glycosyl donor **70b** from 3.0 to 2.0 equivalents led to a slightly lower yield (60%) of trisaccharide (entry 6, Table 20). By following up this standard protocol, the trisaccharide **126** from the glycosyl pairs of **122** and **123** was readily accessed with a satisfying 78% yield and an exclusive α–selectivity (entry 7, Table 20). This reaction has been successfully repeated for at least 3 times in the different scales, and the stereochemistry of the resulting trisaccharide **126** was fully characterized via NMR, HMRS, non-decoupling experiment.

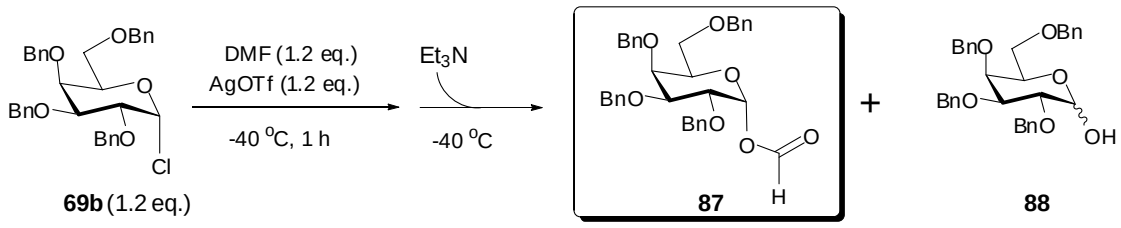
4. Conclusion

In conclusion, DMF-mediated α–selective glycosylation has been systematically investigated in aspects of mechanistic understanding, the applicable scope and experimental manipulation. This method provides the several distinct features: **I.** Only stoichiometric DMF is required; **II.** The increasing selectivity is shown to be temperature-dependant in a reverse manner. Better α–selectivity is usually obtained at the low temperature; **III.** Pre-activation of glycosyl chlorides with DMF is for the first time proved feasible under Koenigs-Knorr conditions. **IV**. TMSOTf increases the conversion rate; **V.** The inverse addition is adopted,

particularly for the secondary alcohol acceptor. Based on these advantages, this convenient method could be found useful in glycol-assembly. .

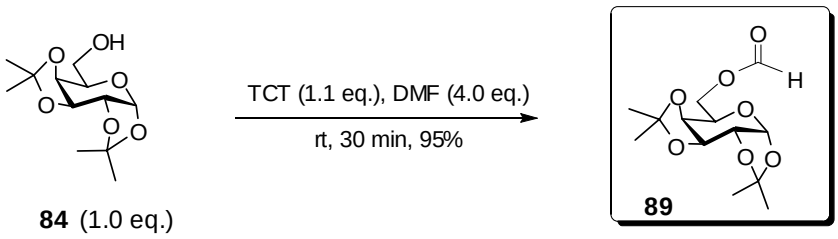
5. Experimental

General: Chemicals used this study were purchased as reagent grade from commercial venders and used without purification. All solvents used in the experiments were dried and distilled by standard techniques including: (1) distillation over CaH_2 for CH_2Cl_2, $C_2H_4Cl_2$, MeOH and (2) *N*,*N*-dimethylformamide (DMF) was distilled over calcium hydride and stocked with flame-dried molecular sieves(MS) under N_2. Optical rotations were measured with a JASCO DIP-1000 polarimeter at 27 ˚C. Flash column chromatography was performed on silica gel 60 (70–230 mesh, E. Merck). ^1H and ^{13}C NMR spectra were recorded with 300 MHz and 75 MHz spectrometers by Varian Unity-300 or with 500 MHz and 125 MHz by a Varian console as specified. Chemical shift (δ ppm) was calibrated against the residual proton of TMS and ^{13}C signal of deuterated chloroform ($CDCl_3$). Coupling constant(s) in hertz (Hz) were measured from ^1H NMR spectra. Molecular weights of disaccharides $[M + Na]^+$ were determined by BioTOF Ultraflex II (Bruker Daltonics, Billeriaca, MA 01821, USA).



Formyl 2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranoside (87): A mixture of per-*O*-benzyl galactosyl chloride **69b** (56 mg, 0.1 mmol) , DMF (8 μL, 0.1 mmol) and AW300 MS (100 mg) in CH_2Cl_2 (1.5 mL) was stirred at rt under N_2 for 30 min. The mixture was then cooled at –40 ˚C followed by addition of a solution of AgOTf (25 mg, 0.1 mmol, 1.0 equiv, con. = 0.2 M of

AgOTf in toluene) and stirred at –40 ˚C for 1 hr. The reaction was quenched by Et_3N (0.2 mL) and directly subjected to column chromatography over silica gel (EtOAc/Hexane elution: gradient from 1/9 to 3/7) to give the 1-*O*-Formyl derivative **87** as the single α anomer (5 mg, 10%) and the hydrolyzed product **88** (42 mg, 75%). The structure of **87** was fully characterized by 1D, 2D-NMR and HMBC. **87:** R_f = 0.42 (TLC developing solution: EtOAc/Hexane = 1/4); **^{1}H NMR** (300 MHz, $CDCl_3$) : δ 8.14 (s, 1H), 7.48-7.20 (m, 20H), 6.40 (d, J = 3.4 Hz, 1H, H-1), 4.95 (d, J = 11.4 Hz, 1H), 4.82 (d, J = 11.7 Hz, 1H), 4.77-4.69 (m, 3H), 4.58 (d, J = 11.4 Hz, 1H), 4.46 (d, J = 11.8 Hz, 1H), 4.39 (d, J = 11.8 Hz, 1H), 4.19 (dd, J = 10.1, 3.5 Hz, 1H), 4.07-3.98 (m, 2H), 3.90 (dd, J = 10.0, 2.6 Hz, 1H), 3.52 (dd, J = 6.3, 2.1 Hz, 2H); **^{13}C NMR** (75 MHz, $CDCl_3$) : δ 159.41, 138.31, 138.23, 137.64, 137.59, 128.31, 128.30, 128.26, 128.21, 128.17, 128.15, 128.09, 128.06, 128.04, 127.99, 127.88, 127.83, 127.79, 127.76, 127.73, 127.71, 127.58, 127.51, 127.47, 127.45, 127.44, 127.39, 127.32, 127.22, 91.08 (C-1), 78.64, 75.16, 74.91, 74.48, 73.73, 73.55, 73.16, 72.20, 68.39.



84 (1.0 eq.)

6-*O*-Formyl-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (89): The procedure for preparation of 6-*O*-formyl derivative **89** followed the TCT/DMF protocol described in Chapter 2. The final mixture was precipitated by the addition of ether and filtered by Celite. The filtrate was concentrated and purified by column chromatography over silica gel (EtOAc/Hexane elution: 2/8). For **89:** R_f = 0.38 (TLC developing solution: EtOAc/Hexane =

1/4); **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.10 (s, 1H, CHO), 5.55 (d, *J* = 5.0 Hz, 1H. H-1), 4.64 (dd, *J* = 7.9, 2.5 Hz, 1H), 4.40-4.28 (m, 4H), 4.25 (dd, *J* = 7.9, 1.9 Hz, 1H), 4.05 (ddd, *J* = 7.1, 4.9, 1.9 Hz, 1H), 1.52 (s, 3H), 1.46 (s, 3H), 1.35 (s, 3H), 1.34 (s, 3H); **^{13}C NMR** (75 MHz, CDCl3): δ 160.76, 109.66, 108.75, 96.20 (C-1), 70.88, 70.62, 70.28, 65.80, 62.91, 25.93, 25.87, 24.84, 24.40. The spectroscopic data agrees with the literature values.[76]

General procedure for DMF-mediated α-stereoselective glycosylation

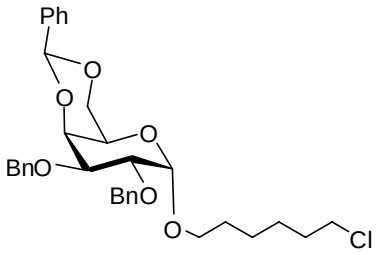
Procedure A at the room temperature – Following the adding sequence - Ag_2CO_3 (1.3 equiv.), molecular sieve (AW300, 50 mg/per 1mL of solvent), the alcohol acceptor (1.0 equiv.), DMF (1.2 equiv.), the glycosyl donor (1.2 equiv.) – The final concentration was adjusted to 50 mM by DCM. To the reaction mixture a solution of AgOTf (0.05 equiv, con. = 0.2 M of AgOTf in toluene) was added at room temperature. Upon completion of reaction via TLC monitoring, the reaction was quenched by Et_3N (10 equiv.) and directly subjected to SiO_2 column chromatography. In a small scale (less than 0.1 mmol of the alcohol acceptor), 1.5 equiv of α-glycosyl chloride is recommended. Furthermore, the used amount of other reactants (Ag_2CO_3 and DMF) should be modified according to the principle mentioned earlier (Ag_2CO_3 is 1.1 fold equivalent vs. glycosyl chloride; equivalent of DMF is identical to glycosyl chloride).

Procedure B at -25 ^{o}C - The adding and work-up sequence follow the procedure A. The use of more α-glycosyl chloride (1.5 - 2.0 equiv.) is a compromise between the yield, efficiency and possible decomposition of the reactive intermediates during the reaction course.

Procedure C – **Pre-activation glycosylation** – Mixture of DMF (1.2 equiv.), glycosyl chloride (1.2 equiv.) and molecular sieve (AW300, 50 mg/per 1mL solvent) in DCM was cooled to

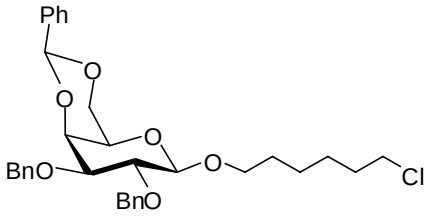
-40 ^{o}C to which a solution of AgOTf (1.2 equiv, con. = 0.2 M of AgOTf in toluene) was added. The resulting mixture was stirred at -40 ^{o}C for 1 h, which was added either Ag_2CO_3 (1.3 equiv.) or [DTBMP (1.3 equiv.)] and the alcohol acceptor (1.0 equiv.) at -40 ^{o}C. Upon completion of glycosylation, the reaction was quenched by excess Et_3N at -40 ^{o}C, then gradually warmed to the room temperature and filtered by Celite. The filtrate was diluted with DCM, washed with saturated sodium bicarbonate solution, brine, dried with $MgSO_4$. The crude product was purified by column chromatography over silica gel.

Procedure D – Inverse addition protocol (especially for the less reactive secondary glycosyl acceptor) – Mixture of molecular sieve (AW300, 50 mg/per 1mL solvent) and the alcohol acceptor (1.0 equiv.) in DCM was cooled to -40 ^{o}C and treated with TMSOTf (2.0 equiv.) The resulting mixture was stirred at -40 ^{o}C for 10 min ensuring the complete formation of TMS-ether. To the mixture is dropwise added DMF (1.5 equiv.) and a solution of AgOTf (3.0 equiv, con. = 0.2 M of AgOTf in toluene) followed by the addition of glycosyl donor (3.0 equiv, 100 mg donor in 1 mL of DCM) in 10 min at -40 ^{o}C. Upon completion of glycosylation, the reaction was quenched by excess Et_3N at -40 ^{o}C, then gradually warmed to the room temperature and filtered by Celite. The filtrate was diluted with DCM, washed with saturated sodium bicarbonate solution, brine, dried with $MgSO_4$. The crude product was subsequently purified by column chromatography over silica gel.



93α

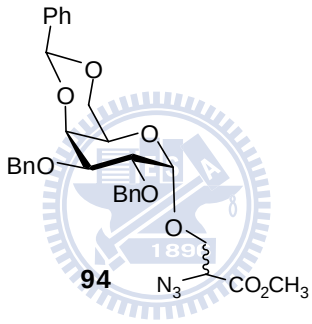
6-Chloro-n-hexyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranoside (93α): Follow the procedure B. **93α** (48 mg, 84%); R_f = 0.52 (TLC developing solution: EtOAc/ Hexane = 1/4); $[\alpha]^{27}_D$ = +87.6 (*c* = 0.35, $CHCl_3$); **^{1}H NMR** (300 MHz, $CDCl_3$):δ 7.52 (dd, *J* = 7.5, 2.1 Hz, 2H), 7.42-7.23 (m, 13H), 5.49 (s, 1H), 4.90 (d, *J* = 3.6 Hz, 1H, H-1), 4.86 (d, *J* = 11.7 Hz, 1H), 4.82 (d, *J* = 12.0 Hz, 1H), 4.74 (d, *J* = 12.0 Hz, 1H), 4.65 (d, *J* = 12.0 Hz, 1H), 4.22-4.17 (m, 2H), 4.08-3.96(m, 3H), 3.67-3.59 (m, 2H), 3.51 (t, *J* = 6.6 Hz, 2H), 3.47 -3.41 (m, 1H), 1.79-1.71 (m, 2H), 1.67-1.56 (m, 2H), 1.49-1.40 (m, 2H), 1.40-1.32 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.86, 138.77, 137.84, 128.80, 128.24, 128.23, 128.05, 127.80, 127.57, 127.55, 127.45, 126.31, 101.04, 98.09 (C-1, $J_{C\text{-}H}$ = 168 Hz), 76.07, 75.63, 74.73, 73.48, 72.04, 69.46, 68.13, 62.61, 44.98, 32.46, 29.19, 26.59, 25.44; HRMS (Bio-ToFII): calcd for $C_{33}H_{39}ClO_6Na$ requires 589.2333; found: *m*/*z* = 589.2358 $[M + Na]^+$.



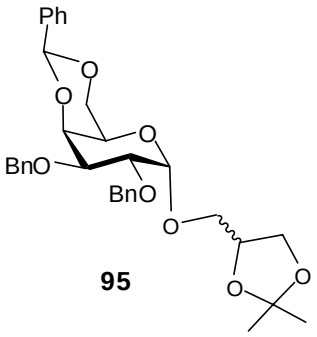
93β

6-Chloro-n-hexyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside (93β): Follow the procedure A without addition of DMF. **93β** (50 mg, 88%) R_f = 0.36 (TLC developing solution: EtOAc/Hexane = 1/4); $[\alpha]^{27}_D$ = +26.1 (*c* = 0.28, $CHCl_3$); **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.55 (dd, *J* = 7.5, 2.1 Hz, 2H), 7.44-7.21 (m, 13H), 5.50 (s, 1H), 4.92 (d, *J* =

10.9 Hz, 1H), 4.79 (d, J = 11.7 Hz, 1H), 4.76 (s, 2H), 4.38 (d, J = 7.7 Hz, 1H, H-1), 4.30 (d, J = 12.4 Hz, 1H), 4.11 (d, J = 3.5 Hz, 1H), 4.01 (dd, J = 1.5 Hz, J = 11.7 Hz, 1H), 4.00-3.94 (m, 1H), 3.83 (dd, J = 9.6, 7.8 Hz, 1H), 3.55 (dd, J = 9.6, 3.6 Hz 1H), 3.53-3.45 (m, 3H), 3.31 (s, 1H), 1.79-1.60 (m, 4H), 1.49-1.37 (m, 4H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 138.90, 138.44, 137.85, 128.87, 128.31, 128.23, 128.08, 127.88, 127.71, 127.62, 127.48, 126.47, 103.64 (C-1, $J_{C\text{-}H}$ = 155 Hz), 101.28, 79.22, 78.43, 75.19, 74.00, 71.97, 69.66, 69.25, 66.38, 45.02, 32.50, 29.54, 26.67, 25.45. HRMS (Bio-ToFII): calcd for $C_{33}H_{39}ClO_6Na$ requires 589.2333; found: m/z = 589.2344 $[M + Na]^+$.

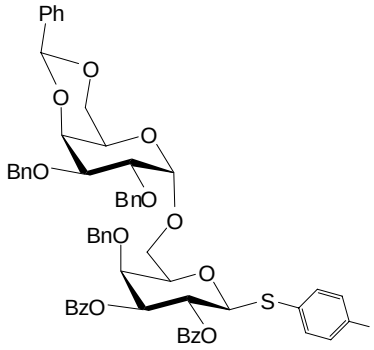


1-*O*-(2-Azido-2-methoxycarbonyl-ethyl)-2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranoside (94): Follow the procedure B. R_f = 0.32 (TLC developing solution: EtOAc/Hexane = 3/7). For 1:1 mixture of **94** diastereoisomers (49 mg, 85%): **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.55-7.44 (m, 6H), 7.42-7.22 (m, 24H), 5.47 (s, 2H), 4.94 (d, J = 3.6 Hz, 1H, H-1$_a$), 4.89 (d, J = 3.6 Hz, H-1$_b$), 4.88-4.65 (m, 7H), 4.64 (d, J = 3.3 Hz, 1H), 4.23 (d, J = 5.1 Hz, 1H), 4.24-4.20 (m, 2H), 4.20-4.12 (m, 5H), 4.12-3.77 (m, 4H), 3.75 (s, 1H), 3.70 (s, 3H), 3.69 (s, 3H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 168.62, 168.49, 138.74, 138.64, 138.62, 137.72, 128.83, 128.30, 128.25, 128.23, 128.13, 128.07, 127.82, 127.70, 127.66, 127.63, 127.58, 127.51, 127.49, 126.26, 100.99, 100.97, 99.24 (C-1$_a$), 98.93 (C-1$_b$), 75.54, 75.51, 75.31, 74.59, 73.67, 73.29, 72.16, 69.30, 68.78, 68.26, 63.20, 63.14, 61.60, 61.46, 52.79, 52.77; HRMS (Bio-ToFII): calcd for $C_{31}H_{33}N_3O_8Na$ requires 598.2165; found: m/z = 598.2184 $[M + Na]^+$.



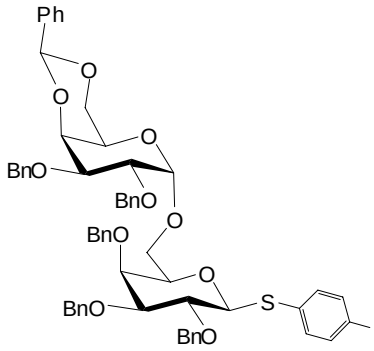
95

1,2-Isopropylidene-3-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl)-*rac*-glyc-erol (95): Follow the procedure B. R_f = 0.31 (TLC developing solution: EtOAc/Hexane = 3/7); For 1:1 mixture of **95** diastereoisomers (42 mg, 75%): **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.55-7.47 (m, 4H), 7.43-7.23 (m, 26H), 5.48 (s, 2H), 4.98 (d, *J* = 3.3 Hz, 1H, H-1), 4.89 (d, *J* = 3.6 Hz, H, H-1'), 4.86 (d, *J* = 12.6 Hz, 2H), 4.81 (d, *J* = 13.2 Hz, 3H), 4.72 (d, *J* = 12.3 Hz, 3H), 4.67 (d, *J* = 2.9 Hz, 1H), 4.63 (d, *J* = 2.8 Hz, 1H), 4.36-4.27 (m, 2H), 4.24-4.13 (m, 4H), 4.12-3.93 (m, 7H), 3.81-3.60 (m, 5H), 3.59-3.51(m, 1H), 3.45 (dd, *J* = 5.4 Hz, *J* = 10.2 Hz, 1H), 1.39 (s, 3H), 1.38 (s, 3H), 1.35 (s, 3H), 1.35 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.78, 138.70, 138.69, 138.63, 137.76, 137.75, 128.89, 128.82, 128.27, 128.26, 128.24, 128.14, 128.06, 127.96, 127.83, 127.77, 127.70, 127.68, 127.60, 127.50, 127.47, 126.41, 126.40, 126.28, 109.43, 109.33, 101.00, 98.64 (C-1), 98.53 (C-1'), 75.93, 75.66, 75.54, 75.50, 74.65, 74.57, 74.40, 73.59, 73.41, 72.06, 71.98, 69.61, 69.37, 69.31, 68.68, 66.78, 66.55, 62.71, 62.61, 26.87, 26.68, 25.47, 25.38; HRMS (Bio-ToFII): calcd for $C_{33}H_{38}O_8Na$ requires 585.2464; found: *m*/*z* = 585.2425 $[M + Na]^+$.



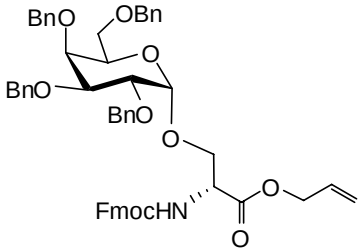
98

p-Tolyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl-(1→6)-2,3-di-*O*-benzo -yl-4-*O*-benzyl-1-thio-β-D-galactopyranoside (98): Follow the procedure A. (85 mg, 84%); R_f = 0.38 (TLC developing solution: EtOAc/Hexane = 3/7); $[\alpha]^{27}_D$ = +67.3 (*c* = 0.49, $CHCl_3$); **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.96 (d, *J* = 7.2 Hz, 2H), 7.95 (d, *J* = 7.2 Hz, 2H), 7.79 (d, *J* = 7.2 Hz, 2H), 7.78 (d, *J* = 7.2 Hz, 2H), 7.62-7.43 (m, 4H), 7.42-7.16 (m, 16H), 7.15-6.98 (m, 6H), 5.69 (t, *J* = 9.3 Hz, 1H), 5.49 (s, 1H), 5.32 (t, *J* = 9.3 Hz, 1H), 5.16 (d, *J* = 3.4 Hz, 1H, H-1'), 4.85 (d, *J* = 9.9 Hz, 1H, H-1), 4.82-4.71 (m, 3H), 4.65 (d, *J* = 11.7 Hz, 2H), 4.49 (s, 2H), 4.22 (d, *J* = 12.6 Hz, 1H), 4.13-4.05 (m, 2H), 4.03-3.72 (m, 5H), 3.65 (s, 1H), 2.24 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.56, 165.17, 138.67, 138.52, 138.18, 137.87, 137.35, 133.10, 133.01, 129.80, 129.73, 129.71, 129.34, 129.27, 128.82, 128.41, 128.27, 128.25, 128.20, 128.18, 128.07, 127.99, 127.93, 127.86, 127.71, 127.54, 127.52, 127.48, 126.30, 100.99, 98.01 (C-1'), 85.73 (C-1), 79.47, 76.31, 75.98, 75.86, 75.50, 74.57, 73.44, 71.85, 70.80, 69.38, 65.60, 62.60, 21.06; HRMS (Bio-ToFII): calcd for $C_{61}H_{58}O_{12}SNa$ requires 1037.3547; found: *m*/*z* = 1037.3598 $[M + Na]^+$.



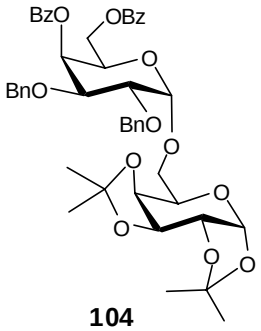
99α

p-Tolyl 2,3-di-*O*-benzly-4,6-*O*-benzylidene-α-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benz -yl-1-thio-β-D-galatopyranoside (99α): Follow the procedure A. (0.88 g, 89%); R_f = 0.45 (TLC developing solution: EtOAc/Hexane = 3/7); $[\alpha]^{27}_D$ = +42.6 (*c* = 1.04, $CHCl_3$); **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.56-7.43 (m, 2H), 7.42-7.15 (m, 31H), 6.97 (d, *J* = 8.4 Hz, 2H), 5.4 (s, 1H), 4.92 (d, *J* = 10.8 Hz, 1H), 4.83 (d, *J* = 11.7 Hz, 1H), 4.80-4.69 (m, 8H), 4.66 (d, *J* = 9.6 Hz, 1H, H-1), 4.60 (d, *J* = 12.3 Hz, 1H), 4.58 (d, *J* = 11.7 Hz, 1H), 4.15 (d, *J* = 11.1 Hz, 1H), 4.08-4.03 (m, 2H), 4.02 (d, *J* = 3.9 Hz, 1H), 3.98-3.85 (m, 2H), 3.84-3.63 (m, 3H), 3.60 (dd, *J* = 9.6, 2.4 Hz, 1H), 3.32 (dd, *J* = 9.9, 3.9 Hz, 1H), 2.23 (s, 3H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 138.49, 138.28, 138.11, 137.92, 137.85, 137.62, 136.42, 130.55, 130.32, 129.33, 128.55, 128.23, 128.11, 128.08, 128.05, 128.01, 127.81, 127.76, 127.75, 127.56, 127.44, 127.39, 127.28, 127.17, 126.03, 100.67, 97.76 (C-1', $J_{C'\text{-}H'}$ = 171 Hz), 86.67 (C-1, $J_{C\text{-}H}$ = 152 Hz), 83.87, 77.05, 76.89, 76.06, 75.44, 75.07, 74.06, 73.93, 73.65, 72.77, 71.48, 69.22, 67.47, 62.17, 20.84; HRMS (Bio-ToFII): calcd for $C_{61}H_{64}O_{10}SNa$ requires 1009.3961; found: *m*/*z* = 1009.3928 $[M + Na]^+$.



102

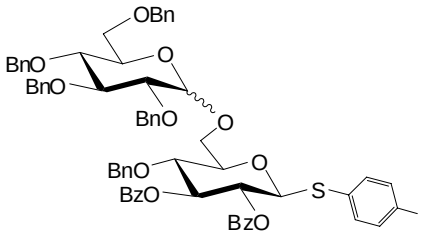
***N*-(9-Fluorenylmethoxycarbonyl)-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl)-L-serine allyl ester (102)**: Follow the procedure B. (90 mg, 90%);R_f = 0.55 (TLC developing solution: EtOAc/Hexane = 1/4); $[\alpha]^{27}_D$ = +24.8 (*c* = 0.36, $CHCl_3$); **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.73 (d, *J* = 7.5 Hz, 2H), 7.55 (d, *J* = 9.1 Hz, 2H), 7.40-7.19 (m, 24H), 6.33 (d, *J* = 8.6 Hz, 1H), 5.96-5.78 (m, 1H), 5.33-5.24 (m, 1H), 5.17 (d, *J* = 10.5 Hz, 1H), 4.93 (d, *J* = 11.4 Hz, 1H), 4.83-4.47 (m, 7H), 4.45-4.13 (m, 6H), 4.04 (dd, *J* = 9.6, 3.6 Hz, 1H), 3.99-3.78 (m, 4H), 3.60-3.50 (m, 3H), 3.46 (dd, *J* = 9.0, 6.3 Hz, 1H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 169.77, 156.12, 143.87, 143.81, 141.24, 141.22, 138.63, 138.46, 137.84, 131.57, 128.42, 128.35, 128.31, 128.22, 128.10, 128.03, 127.83, 127.74, 127.69, 127.61, 127.51, 127.43, 127.33, 127.28, 127.06, 127.02, 125.17, 125.13, 119.90, 118.63, 99.72 (C-1, $J_{C\text{-}H}$ = 168 Hz), 78.65, 76.39, 74.80, 74.72, 73.35, 73.06, 70.01, 68.94, 67.04, 66.11, 54.77, 47.07; HRMS (Bio-ToFII): calcd for $C_{55}H_{55}NO_{10}Na$ requires 912.3724; found: *m*/*z* = 912.3730 [M + Na]$^+$.



104

6-*O*-(4,6-Di-*O*-benzoyl-2,3-di-*O*-benzyl-α-D-galactopyranosyl)-(1→6)-*O*-1,2:3,4-di-*O*-isop

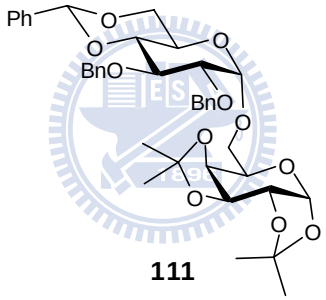
ropylidene-α-D-galactopyranose (104): Follow the procedure B. (38 mg, 94%); R_f = 0.56 (TLC developing solution: EtOAc/Hexane = 3/7); $[\alpha]^{27}_D$ = +10.1 (*c* = 0.45, $CHCl_3$); **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.03 (d, *J* = 7.2 Hz, 4H), 7.61-7.50 (m, 1H), 7.49-7.36 (m, 5H), 7.36-7.17 (m, 10H), 5.92 (d, *J* = 3.3 Hz, 1H), 5.51 (d, *J* = 4.5 Hz, 1H, H-1), 5.07 (d, *J* = 3.3 Hz, 1H, H-1'), 4.85 (d, *J* = 12.0 Hz, 1H), 4.81 (d, *J* = 12.0 Hz, 1H), 4.72 (d, *J* = 12.0 Hz, 1H), 4.64-4.45 (m, 4H), 4.34-4.25 (m, 3H), 4.14 (dd, *J* = 10.0, 3.3 Hz , 1H), 4.05 (td, *J* = 6.0, 1.5 Hz, 1H), 3.96 (dd, *J* = 10.5, 3.6 Hz, 1H), 3.87-3.81 (m, 2H), 1.50 (s, 3H), 1.39 (s, 3H), 1.31 (s, 3H), 1.28 (s, 3H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 166.05, 165.74, 138.37, 138.10, 133.06, 132.93, 129.87, 129.83, 129.75, 129.69, 128.48, 128.46, 128.41, 128.38, 128.37, 128.34, 128.31, 128.28, 128.24, 128.14, 128.09, 128.07, 127.88, 127.85, 127.83, 127.78, 127.69, 127.59, 127.38, 109.23, 108.49, 97.93 (C-1', $J_{C'\text{-}H}$ = 169 Hz), 96.27 (C-1, $J_{C\text{-}H}$ = 180 Hz), 76.11, 75.10, 73.15, 71.98, 71.02, 70.68, 70.46, 68.71, 67.24, 67.06, 66.44, 63.06, 26.05, 25.97, 24.84, 24.53; HRMS (Bio-ToFII): calcd for $C_{46}H_{50}O_{13}Na$ requires 833.3144; found: *m*/*z* = 833.3341 $[M + Na]^+$.



109

p-Tolyl 6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→6)-*O*-2,3-di-*O*-benzoyl-4-*O*-benzyl-1-thio-β-D-glucopyranoside (109): Follow the procedure B. (42 mg, 38% yield, α/β ratio = 83:17); R_f = 0.54 (TLC developing solution: EtOAc/Hexane = 3/7); **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.95 (d, *J* = 8.2 Hz, 2H), 7.77 (d, *J* = 8.2 Hz, 2H), 7.49 (d, *J* = 7.9 Hz, 2H), 7.43-6.97 (m, 33H), 5.66 (t, *J* = 9.4 Hz, 1H), 5.29 (t, *J* = 9.6 Hz, 1H), 5.12 (d, *J* = 3.4 Hz, 1H,

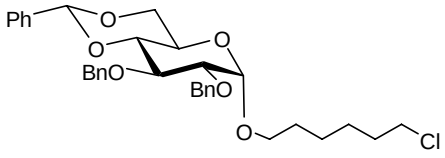
H-1), 5.02 (d, J = 10.9 Hz, 1H), 4.94-4.75 (m, 3H), 4.74-4.59 (m, 3H), 4.48-4.39 (m, 4H), 4.02 (t, J = 9.3 Hz, 1H), 3.95-3.84 (m, 3H), 3.81-3.39 (m, 6H), 2.20 (s, 4H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 165.58, 165.17, 138.77, 138.47, 138.30, 138.12, 137.98, 137.25, 134.02, 133.38, 133.06, 132.95, 129.79, 129.74, 129.71, 129.67, 129.58, 129.40, 129.31, 128.61, 128.50, 128.35, 128.33, 128.26, 128.23, 128.16, 128.14, 128.09, 128.07, 127.90, 127.88, 127.87, 127.84, 127.81, 127.76, 127.73, 127.68, 127.60, 127.52, 127.48, 127.34, 97.02 (C-1, $J_{C\text{-}H}$ = 169 Hz), 86.56 (C-1', $J_{C\text{-}H}$ = 159 Hz), 81.83, 80.24, 79.55, 77.68, 76.24, 75.62, 75.54, 75.02, 74.52, 73.36, 72.83, 70.96, 70.29, 68.52, 65.14, 21.04; HRMS (Bio-ToFII): calcd for $C_{68}H_{66}O_{12}SNa$ requires 1129.4173; found: m/z = 1129.4251 $[M + Na]^+$.



111

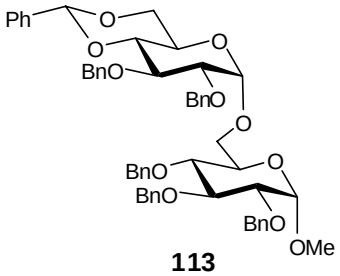
6-*O*-(2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-(1→6)-*O*-1,2:3,4-di-*O*-isop-ropylidene-α-D-galactopyranose (111): Follow the procedure C. (105 mg, 76% yield, α/β ratio > 95:5); R_f = 0.49 (TLC developing solution: EtOAc/Hexane = 3/7); **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.54-7.44 (m, 1H), 7.43-7.21 (m, 14H), 5.55 (s, 1H), 5.53 (d, J = 4.5 Hz, 1H, H-1), 4.93 (d, J = 3.6 Hz, 1H, H-1'), 4.92 (d, J = 11.4 Hz, 1H), 4.83 (d, J = 11.4 Hz, 1H), 4.78-4.74 (m, 2H), 4.63-4.53 (m, 2H), 4.35 (dd, J = 7.8, 2.1 Hz, 1H), 4.33-4.25 (m, 2H), 4.10-4.00 (m, 2H), 3.98-3.84 (m, 1H), 3.83-3.67 (m, 2H), 3.66-3.52 (m, 2H), 1.55 (s, 3H), 1.46 (s, 3H), 1.33 (s, 3H), 1.32 (s, 3H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 138.82, 138.25, 137.45, 128.82, 128.47, 128.39, 128.32, 128.22, 128.15, 127.99, 127.88, 127.83, 127.74, 127.69, 127.65, 127.54, 127.52, 127.47, 126.00, 125.96, 109.18, 108.62, 101.16, 98.31(C-1', $J_{C\text{-}H}$ = 168 Hz), 96.28

(C-1, , $J_{C\text{-}H}$ = 182 Hz), 82.06, 79.22, 78.50, 75.21, 72.86, 70.79, 70.60, 68.98, 66.85, 65.89, 62.42, 26.12, 26.03, 24.89, 24.58; HRMS (Bio-ToFII): calcd for $C_{39}H_{48}O_{11}Na$ requires 713.2938; found: m/z = 713.2910 $[M + Na]^+$.



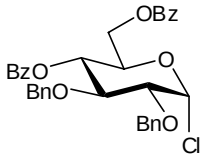
112

6-Chloro-n-hexyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (112): Follow the procedure A without addition of DMF. (51 mg, 88% yield, α/β ratio = 87:13); R_f = 0.65 (TLC developing solution: EtOAc/Hexane = 1/4); $[\alpha]^{27}_D$ = +6.2 (c = 0.42, $CHCl_3$); **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.53-7.44 (m, 2H), 7.44-7.23 (m, 13H), 5.56 (s, 1H), 4.93 (d, J = 11.2 Hz, 1H), 4.84 (d, J = 11.2 Hz, 2H), 4.73 (d, J = 3.3 Hz, 1H, H-1), 4.67 (d, J = 12.1 Hz, 1H), 4.62-4.48 (m, 2H), 4.25 (dd, J = 10.0, 4.6 Hz, 1H), 4.05 (t, J = 9.2 Hz, 1H), 3.83 (dd, J = 9.9, 4.6Hz, 1H), 3.77-3.38 (m, 5H), 1.82-1.71 (m, 2H), 1.70-1.60 (m, 2H), 1.51-1.34(m, 4H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 138.79, 138.30, 137.37, 128.87, 128.39, 128.34, 128.32, 128.27, 128.20, 128.02, 127.99, 127.93, 127.92, 127.86, 127.82, 127.74, 127.68, 127.65, 127.57, 127.53, 125.97, 101.17, 98.01 (C-1), 82.24, 79.40, 78.58, 75.29, 73.51, 69.07, 68.24, 62.42, 45.02, 32.47, 29.24, 26.64, 25.47; HRMS (Bio-ToFII): calcd for $C_{33}H_{41}ClO_6Na$ requires 589.2333; found: m/z = 589.2356 $[M + Na]^+$.



113

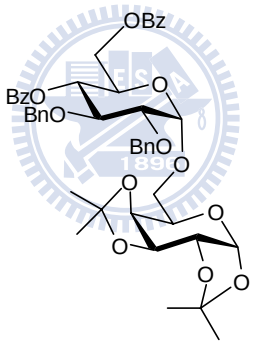
Methyl 6-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-(1 → 6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (113): Follow the procedure A without addition of DMF. (64 mg, 76% yield, α/β ratio = 90:10); R_f = 0.62 (TLC developing solution: EtOAc/Hexane = 1/4); **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.51-7.41 (m, 2H), 7.41-7.16 (m, 28H), 5.53 (s, 1H), 4.99-4.85 (m, 4H), 4.84-4.75 (m, 3H), 4.73-4.63 (m, 3H), 4.62-4.53 (m, 3H), 4.20 (dd, *J* = 10.1, 4.7 Hz, 1H), 4.05-3.92 (m, 3H), 3.87 (dd, *J* = 9.9, 4.7 Hz, 1H), 3.83-3.72 (m, 2H), 3.71-3.57 (m, 2H), 3.53 (dd, *J* = 9.2, 3.6 Hz, 1H), 3.43 (dd, *J* = 9.6, 3.5 Hz, 1H), 3.34 (s, 3H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 138.79, 138.66, 138.33, 138.29, 138.12, 137.47, 128.85, 128.38, 128.33, 128.32, 128.23, 128.19, 128.16, 128.11, 127.98, 127.97, 127.93, 127.84, 127.81, 127.73, 127.70, 127.58, 127.56, 127.52, 127.47, 126.02, 101.27, 98.18 (C-1), 97.96 (C-1'), 80.06, 79.31, 77.89, 77.70, 75.67, 75.00, 73.32, 72.83, 70.33, 69.05, 66.31, 62.51, 55.17. Ref: JACS_2008_8537; LiebigsAnnalen_1997_2573.



117

4,6-Di-*O*-benzoyl-2,3-di-*O*-benzyl-α-D-glucopyranosyl chloride (117): Follow the TCT/DMF chlorination procedure B in Chapter 2 (Replace DCM by DCE for shortening the reaction time). The reaction mixture was heated at 60 °C for 4 hrs. Upon work-up and purification via short column, α-glycosyl chloride **122** was obtained as the colorless syrup

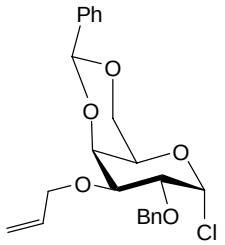
(0.22 g, 83%); R_f = 0.62 (TLC developing solution: EtOAc/Hexane = 1/4); $[\alpha]^{27}_D$ = +53.9 (*c* = 0.95, $CHCl_3$); **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.10-7.92 (m, 4H), 7.63-7.44 (m, 3H), 7.43-7.19 (m, 13H), 6.08 (d, *J* = 3.8 Hz, 1H, H-1), 5.51 (t, *J* = 11.2 Hz, 1H), 4.84 (d, *J* = 11.1 Hz, 1H), 4.78 (d, *J* = 11.0 Hz, 1H), 4.74 (d, *J* = 11.0 Hz, 1H), 4.66 (d, *J* = 11.1 Hz, 1H), 4.56 (dd, *J* = 12.2, 2.5 Hz, 1H), 4.53-4.45 (m, 1H), 4.34 (dd, *J* = 12.2, 4.5 Hz, 1H), 4.17 (t, *J* = 8.7 Hz, 1H), 3.87 (dd, *J* = 9.3, 3.8 Hz, 1H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 165.93, 164.97, 137.52, 137.14, 133.30, 132.97, 129.76, 129.66, 129.47, 129.15, 128.49, 128.42, 128.35, 128.28, 128.22, 128.11, 128.08, 128.00, 127.88, 127.78, 127.57, 92.69 (C-1), 79.62, 78.07, 75.48, 73.03, 70.78, 69.43, 62.26.



118

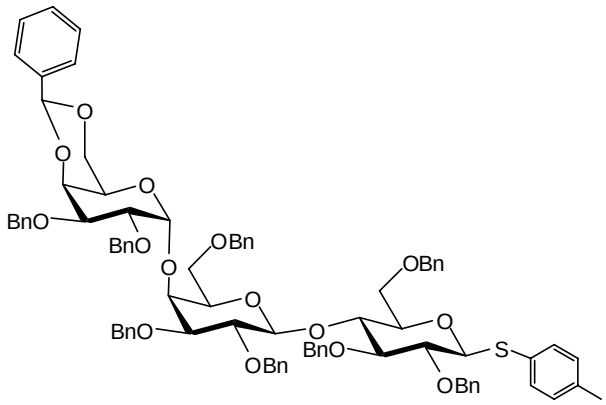
6-*O*-(4,6-Di-*O*-benzoyl-2,3-di-*O*-benzyl-α-D-glucopyranosyl)-(1→6)-*O*-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (118): Follow the procedure B. (32 mg, 79% yield, α/β ratio = 93:7); $[\alpha]^{27}_D$ = +10.1 (*c* = 0.45, $CHCl_3$); R_f = 0.55 (TLC developing solution: EtOAc/Hexane = 3/7); **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.06-7.94 (m, 4H), 7.58-7.46 (m, 2H), 7.45-7.26 (m, 9H), 7.13-7.08 (m, 5H), 5.54 (d, *J* = 3.6 Hz, 1H, H-1), 5.43 (t, *J* = 9.6 Hz, 1H), 5.00 (d, *J* = 3.6 Hz, 1H, H-1'), 4.83 (d, *J* = 11.1 Hz, 1H), 4.78-4.71 (m, 2H), 4.68-4.57 (m, 2H), 4.50 (d, *J* = 9.9, 4.5 Hz, 1H), 4.40-4.25 (m, 3H), 4.17-4.03 (m, 3H), 3.89-3.81 (m, 2H), 3.70 (dd, *J* = 9.5, 3.5 Hz, 1H), 1.57 (s, 3H), 1.43 (s, 3H), 1.34 (s, 3H), 1.31 (s, 3H); **^{13}C NMR**

(75 MHz, $CDCl_3$) δ 166.22, 165.12, 138.00, 137.99, 133.08, 132.79, 129.78, 129.73, 129.68, 129.62, 128.52, 128.36, 128.27, 128.20, 128.12, 128.06, 127.96, 127.93, 127.84, 127.76, 127.69, 127.39, 109.31, 108.52, 96.89 (C-1'), 96.28 (C-1), 79.57, 78.97, 75.37, 72.75, 71.00, 70.71, 70.65, 70.46, 67.61, 67.15, 66.29, 63.14, 26.07, 25.95, 24.90, 24.52; HRMS (Bio-ToFII): calcd for $C_{46}H_{52}O_{13}Na$ requires 833.3144; found: m/z = 833.3317 $[M + Na]^+$.



122

3-*O*-allyl-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl chloride (122): Follow the TCT/DMF chlorination procedure B in Chapter 2 (Replace DCM by DCE for shortening the reaction time). The reaction mixture was heated at 60 °C for 4 hrs. Upon work-up and purification via short column, α-glycosyl chloride **122** was obtained as the colorless syrup (0.54 g, 86%); R_f = 0.58 (TLC developing solution: EtOAc/Hexane = 1/4); **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.54-7.44 (m, 3H), 7.41-7.23 (m, 7H), 6.20 (d, J = 3.6 Hz, 1H, H-1), 6.05-5.86 (m, 1H), 5.53 (s, 1H), 5.33 (dd, J = 17.2, 1.6 Hz,1H), 5.25-5.13 (m, 1H), 4.78 (d, J = 11.8 Hz, 1H), 4.66 (d, J = 11.8 Hz, 1H), 4.33-4.12 (m, 4H), 4.09-3.87 (m, 4H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 137.71, 137.35, 134.73, 128.85, 128.26, 128.00, 127.69, 126.07, 117.11, 100.76, 95.20 (C-1), 75.01, 74.81, 73.88, 73.09, 71.38, 68.64, 65.54.

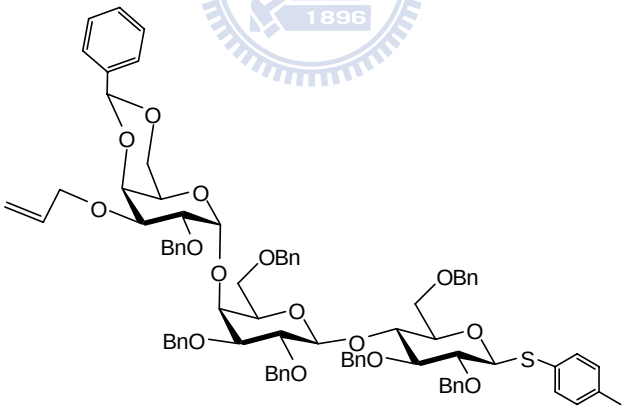


125

p-Tolyl 6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (125):

Follow the procedure D (Inverse addition protocol): Mixture of molecular sieve (AW300, 50 mg/per 1mL solvent) and the alcohol acceptor **123** (250 mg, 0.25 mmol, 1.0 equiv.) in DCM (2.8 mL) was cooled to -40 ^{o}C and treated with TMSOTf (91 μL, 0.50 mmol, 2.0 equiv.). The resulting mixture was stirred at -40 ^{o}C for 10 min ensuring the complete formation of TMS-ether. To the mixture is dropwise added DMF (30 μL, 0.375 mmol, 1.5 equiv.) and a solution of AgOTf [192 mg, 0.75 mmol, 3.0 equiv, 3.7 mL (con. = 0.2 M of AgOTf in toluene)] followed by the addition of glycosyl donor **70b** [350 mg, 3.0 equiv., 3.5 mL (100 mg donor in 1 mL of DCM)] in 10 min at -40 ^{o}C. Upon completion of glycosylation, the reaction was quenched by excess Et_3N at -40 ^{o}C, then gradually warmed to the room temperature and filtered by Celite. The filtrate was diluted with DCM, washed with saturated sodium bicarbonate solution, brine, dried with $MgSO_4$. The crude product was subsequently purified by column chromatography over silica gel (EtOAc/Hexane/DCM elution: 0.5/7/2 to 1/7/2) to obtain the single α-anomer **125** as the amorphous solid (266 mg, 75%). **(125)**: R_f = 0.54 (TLC developing solution: EtOAc/Hexane/DCM = 1/7/2); $[\alpha]^{27}_D$ = +48.5 (*c* = 1.13, $CHCl_3$); **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.50-7.05 (m, 47H), 7.01 (d, *J* = 8.2 Hz, 2H), 5.16 (s, 1H), 5.13 (d, *J* = 3.0 Hz, 1H, H-1''), 5.12 (s, 1H), 4.86-4.66 (m, 9H), 4.60-4.52 (m, 3H), 4.51 (d, *J* =

11.7 Hz, 1H), 4.50-4.44 (m, 2H), 4.40 (d, J = 11.7 Hz, 1H), 4.32 (d, J = 11.7 Hz, 1H), 4.23 (d, J = 12.0 Hz, 1H), 4.23-4.06 (m, 3H), 4.05-3.94 (m, 4H), 3.92-3.72 (m, 3H), 3.64 (d , J = 8.7 Hz, 1H), 3.61-3.49 (m, 2H), 3.48-3.27 (m, 7H); **^{13}C NMR** (125 MHz, $CDCl_3$): δ 138.91, 138.77, 138.55, 138.43, 138.33, 138.28, 138.22, 138.16, 137.96, 137.58, 132.74, 129.55, 128.66, 128.56, 128.36, 128.31, 128.24, 128.22, 128.19, 128.14, 128.12, 128.09, 128.06, 127.93, 127.88, 127.80, 127.75, 127.61, 127.56, 127.52, 127.48, 127.45, 127.43, 127.41, 127.36, 127.27, 127.03, 126.26, 103.03 (C-1', $J_{C'\text{-}H'}$ = 160 Hz), 100.95 (C-1'', $J_{C''\text{-}H''}$ = 169 Hz), 100.64, 87.54 (C-1, $J_{C\text{-}H}$ = 158 Hz), 84.53, 81.23, 79.94, 79.29, 78.62, 76.31, 75.40, 75.31, 74.87, 74.70, 74.06, 73.89, 73.09, 73.03, 73.00, 71.99, 71.57, 69.18, 68.36, 67.21, 62.93, 21.05; HRMS (Bio-ToFII): calcd for $C_{88}H_{90}O_{15}SNa$ requires 1441.5893; found: m/z = 1441.5899 $[M + Na]^+$.



126

p-Tolyl 6-*O*-(4-*O*-allyl-2-*O*-benzyl-4,6-*O*-di-benzylidene-α-D-galactopyranosyl)-(1 → 4)-4-*O*-(2,3,6-tri-O-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-1-thio-β-D-gluc-opyranoside (126): Follow the procedure D (Inverse addition protocol): Mixture of molecular sieve (AW300, 50 mg/per 1mL solvent), and the alcohol acceptor **123** (593mg, 0.60 mmol, 1.0 equiv.) in DCM (7.7 mL) was cooled to -40 ^{o}C and treated with TMSOTf (216 μL, 1.20 mmol, 2.0 equiv.). The resulting mixture was stirred at -40 ^{o}C for 10 min ensuring the

complete formation of TMS-ether. To the mixture is dropwise added DMF (70 μL, 0.90 mmol, 1.5 equiv.) and a solution of AgOTf [460 mg, 1.8 mmol, 3.0 equiv, 8.8 mL (con. = 0.2 M of AgOTf in toluene)] followed by the addition of glycosyl donor **122** [750 mg, 3.0 equiv., 7.5 mL (100 mg donor in 1 mL of DCM)] in 10 min at -40 ^{o}C. Upon completion of glycosylation, the reaction was quenched by excess Et_3N at -40 ^{o}C, then gradually warmed to the room temperature and filtered by Celite. The filtrate was diluted with DCM, washed with saturated sodium bicarbonate solution, brine, dried with $MgSO_4$. The crude product was subsequently purified by column chromatography over silica gel (EtOAc/Hexane/DCM elution: 0.5/7/2 to 1/7/2) to obtain the single α-anomer **126** as the amorphous solid (646 mg, 78%). **(126)**: R_f = 0.54 (TLC developing solution: EtOAc/Hexane/DCM = 1/7/2); $[\alpha]^{27}_D$ = +43.9 (*c* = 0.84, $CHCl_3$); **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.47 (dd, *J* = 8.7, 5.9 Hz, 2H), 7.44-7.14 (m, 40H), 7.03 (d, *J* = 8.0 Hz, 2H), 5.86-5.67 (m, 1H), 5.39 (s, 1H), 5.22-5.10 (m, 3H, H-1'), 5.05 (dd, *J* = 9.8, 1.7 Hz, 1H), 4.91-4.80 (m, 4H), 4.79-4.66 (m, 5H), 4.64-4.56 (m, 4H), 4.54 (d, *J* = 8.7 Hz, 1H, H-1), 4.43 (d, *J* = 11.9 Hz, 1H), 4.34 (d, *J* = 11.7 Hz, 1H), 4.28-4.14 (m, 3H), 4.16-4.04 (m, 4H), 4.04-3.85 (m, 5H), 3.82 (d, *J* = 9.8 Hz, 1H), 3.69-3.48 (m, 5H), 3.47-3.30 (m, 3H), 2.31 (s, 3H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 141.46, 138.82, 138.51, 138.36, 138.27, 138.20, 138.14, 138.13, 137.85, 137.55, 135.12, 132.70, 129.52, 129.49, 128.66, 128.51, 128.33, 128.29, 128.18, 128.16, 128.12, 128.10, 128.03, 128.02, 127.91, 127.79, 127.77, 127.70, 127.65, 127.59, 127.53, 127.48, 127.45, 127.42, 127.37, 126.96, 126.27, 116.24, 102.91(C-1', $J_{C'-H'}$ = 159 Hz), 100.94 (C-1'', $J_{C''-H''}$ = 168 Hz), 100.68, 87.46 (C-1, J_{C-H} = 155 Hz), 84.47, 81.18, 79.80, 79.19, 78.45, 77.20, 76.77, 75.98, 75.27, 75.22, 74.77, 74.72, 73.95, 73.80, 72.95, 72.93, 71.90, 70.75, 69.16, 68.27, 67.08, 62.91, 21.03; HRMS (Bio-ToFII): calcd for $C_{84}H_{88}O_{15}SNa$ requires 1391.5742; found: *m*/*z* = 1391.5719 $[M + Na]^+$.

6. References

(1) Lindahl, L. K. a. U. *Annu. Rev. Biochem.* **1991**, *60*, 443-475.

(2) Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683-720.

(3) Hecht, M. L.; Stallforth, P.; Silva, D. V.; Adibekian, A.; Seeberger, P. H. *Curr. Opin. Chem. Biol.* **2009**, *13*, 354-359.

(4) Bertozzi, C. R.; Kiessling, L. L. *Science* **2001**, *291*, 2357-2364.

(5) Kawano, T.; Cui, J. Q.; Koezuka, Y.; Toura, I.; Kaneko, Y.; Motoki, K.; Ueno, H.; Nakagawa, R.; Sato, H.; Kondo, E.; Koseki, H.; Taniguchi, M. *Science* **1997**, *278*, 1626-1629.

(6) Siddiqui, B.; Hakomori, S. *J. Biol. Chem* **1971**, *246*, 2271-2277.

(7) Lemieux, R. U.; Driguez, H. *J. Am. Chem. Soc.* **1975**, *97*, 4063-4069.

(8) Lemieux, R. U. Gordon Research Conference, The Chemistry of Carbohydrates, Tilton, N. H., June 10-14. **1968**.

(9) Lemieux, R. U.; Hendriks, R. V.; Stick, R. V.; James, K. *J. Am. Chem. Soc.* **1975**, *97*, 4056-4062.

(10) Hadd, M. J.; Gervay, J. *Carbohydr. Res.* **1999**, *320*, 61-69.

(11) Du, W.-J.; Gervay-Hague, J. *Org. Lett.* **2005**, *7*, 2063-2065.

(12) Lucas, T. J.; Schuerch, C. *Carbohydr. Res.* **1975**, *39*, 39-45.

(13) Marousek, V.; Lucas, T. J.; Wheat, P. E.; Schuerch, C. *Carbohydr. Res.* **1978**, *60*, 85-96.

(14) Perrin, C. L. *Tetrahedron* **1995**, *51*, 11901-11935.

(15) Ishiwata, A.; Munemura, Y.; Ito, Y. *Tetrahedron* **2008**, *64*, 92-102.

(16) Demchenko, A. V.; Stauch, T.; Boons, G. J. *Synlett* **1997**, 818-819.

(17) Koshiba, M.; Suzuki, N.; Arihara, R.; Tsuda, T.; Nambu, H.; Nakamura, S.; Hashimoto, S. *Chem. Asian. J.* **2008**, *3*, 1664-1677.

(18) Reddy, G. V.; Kulkarni, V. R.; Mereyala, H. B. *Tetrahedron Lett.* **1989**, *30*, 4283-4286.

(19) Litjens, R.; van den Bos, L. J.; Codee, J. D. C.; Overkleeft, H. S.; van der Marel, G. A. *Carbohydr. Res.* **2007**, *342*, 419-429.

(20) Ley, S. V.; Baeschlin, D. K.; Dixon, D. J.; Foster, A. C.; Ince, S. J.; Priepke, H. W. M.; Reynolds, D. J. *Chem. Rev.* **2001**, *101*, 53-80.

(21) Fraser-Reid, B.; Wu, Z. F.; Andrews, C. W.; Skowronski, E.; Bowen, J. P. *J. Am. Chem. Soc.* **1991**, *113*, 1434-1435.

(22) Imamura, A.; Kimura, A.; Ando, H.; Ishida, H.; Kiso, M. *Chem. Eur. J.* **2006**, *12*, 8862-8870.

(23) Schuerch, C.; Eby, R. *Carbohydr. Res.* **1974**, *34*, 79-90.

(24) Crich, D.; Hu, T. S.; Cai, F. *J. Org. Chem.* **2008**, *73*, 8942-8953.

(25) Kim, J.-H.; Yang, H.; Boons, G. J. *Angew. Chem. Int. Ed.* **2005**, *44*, 947-949.

(26) Fascione, M. A.; Adshead, S. J.; Stalford, S. A.; Kilner, C. A.; Leachb, A. G.; Turnbull, W. B. *Chem. Commun.* **2009**, 5841-5843.

(27) Gin, D.; Haberman, J. M. *Org. Lett.* **2001**, *3*, 1665-1668.

(28) Nguyen, H. M.; Mensah, E. A. *J. Am. Chem. Soc.* **2009**, *131*, 8778-8780.

(29) Pornsriyasak, P.; Vetter, C.; Kaeothip, S.; Kovermann, M.; Balbach, J.; Steinborn, D.; Demchenko, A. V. *Chem. Commun.* **2009**, 6379-6381.

(30) Bogusiak, J.; Szeja, W. *Synlett* **1997**, 661-662.

(31) Bogusiak, J.; Szejab, W. *Carbohydr. Res.* **2001**, *330*, 141-144.

(32) Crich, D.; Li, W. *Org. Lett.* **2006**, *8*, 959-962.

(33) Gin, D.; Haberman, J. M. *Org. Lett.* **2003**, *5*, 2539-2541.

(34) Park, J.; Kawatkar, S.; Kim, J.-H.; Boons, G. J. *Org. Lett.* **2007**, *9*, 1959-1962.

(35) Kononov, L. O.; Malysheva, N. N.; Orlova, A. V. *Eur. J. Org. Chem.* **2009**, 611-616.

(36) Kononov, L. O.; Malysheva, N. N.; Kononova, E. G.; Orlova, A. V. *Eur. J. Org. Chem.* **2008**, 3251-3255.

(37) Muzart, J. *Tetrahedron* **2009**, *65*, 8313-8323.

(38) Satge, C.; Le Bras, J.; Henin, F.; Muzart, J. *Tetrahedron* **2005**, *61*, 8405-8409.

(39) Nishida, Y.; Shingu, Y.; Dohi, H.; Kobayashi, K. *Org. Lett.* **2003**, *5*, 2377-2380.

(40) Shingu, Y.; Miyachi, A.; Miura, Y.; Kobayashi, K.; Nishida, Y. *Carbohydr. Res.* **2005**, *340*, 2236-2244.

(41) Koto, S.; Morishima, N.; Owa, M.; Zen, S. *Carbohydr. Res.* **1984**, *130*, 73-83.

(42) Norberg, T.; Walding, M.; Westman, E. *J. Carbohydr. Chem.* **1988**, *7*, 283-292.

(43) Ruttens, B.; Saksena, R.; Kovác, P. *Eur. J. Org. Chem.* **2007**, 4366-4375.

(44) Kovác, P.; Edgar, K. J. *J. Org. Chem.* **1992**, *57*, 2455-2467.

(45) Ogawa, T.; Sahajima, K. *Carbohydr. Res.* **1981**, *93*, 67-81.

(46) Ogawa, T.; Nukada, T. *Carbohydr. Res.* **1985**, *136*, 135-152.

(47) Refin, S.; Guzman, J. F. B.; Bock, K. *Carbohydr. Res.* **1992**, *232*, 353-357.

(48) Huang, L.; Huang, X. *Chem. Eur. J.* **2007**, *13*, 529-540.

(49) Wang, Z.; Zhou, Luyuan. Y.; El-Boubbou, K.; Ye, X.-S.; Huang, X.-F. *J. Org. Chem.* **2007**, *72*, 6409-6420.

(50) Gross, B.; Cesare, P. D. *Carbohydr. Res.* **1977**, *58*, C1-C3.

(51) Dourtoglou, V.; Gross, B. *J. Carbohydr. Chem.* **1983**, *2*, 57-73.

(52) Barresi, F.; Hindsgaul, O. *J. Am. Chem. Soc.* **1991**, *113*, 9376-9377.

(53) Barresi, F.; Hindsgaul, O. *Synlett* **1992**, 759-761.

(54) Stork, G.; Kim, G. *J. Am. Chem. Soc.* **1992**, *114*, 1087-1088.

(55) Bols, M. *J. Chem. Soc. Chem. Commum.* **1993**, 791-792.

(56) Scheffler, G.; Schimdt, R. R. *J. Org. Chem.* **1999**, *64*, 1319-1325.

(57) Scheffler, G.; Schimdt, R. R. *Tetrahedron Lett.* **1997**, *38*, 2843-2846.

(58) Yang, Z.-Y.; Lin, W.-B.; Yu, B. *Carbohydr. Res.* **2000**, *329*, 879-884.

(59) Cheng, Y.-P.; Chen, H.-T.; Lin, C.-C. *Tetrahedron Lett.* **2002**, *43*, 7721-7723.

(60) Kim, K.-S.; Kang, S.-S.; Seo, Y.-S.; Kim, H.-J.; Lee, Y. -J.; Jeong, K.-S. *Synlett* **2003**, *2003*, 1311-1314.

(61) Kim, K.-S.; Fulse, D.-B.; Baek, J.-Y.; Lee, B.-Y.; Jeon, H.-B. *J. Am. Chem. Soc.* **2008**, *130*, 8537-8547.

(62) Crich, D.; Cai, W. L. *J. Org. Chem.* **1999**, *64*, 4926-4930.

(63) Schimd, U.; Waldmann, H. *Liebigs Ann.* **1997**, 2573-2517.

(64) Crich, D.; Smith, M. *J. Am. Chem. Soc.* **2001**, *123*, 9015-9020.

(65) Crich, D.; Vinod, A. U. *Org. Lett.* **2003**, *5*, 1297-1300.

(66) Crich, D.; Vinogradova, O. *J. Org. Chem.* **2006**, *71*, 8473-8480.

(67) Li, Z.; Gildersleeve, J. C. *J. Am. Chem. Soc.* **2006**, *128*, 11612-11619.

(68) Hashimoto, S.; Hayashi, M.; Noyori, R. *Tetrahedron Lett.* **1984**, *25*, 1379-1382.

(69) Mukaiyama, T.; Shimpuku, T.; Takashima, T.; Kobayashi, K. *Chem. Lett.* **1989**, 145-148.

(70) Charette, A. B.; Marcoux, J. F.; Cdte, B. *Tetrahedron Lett.* **1991**, *32*, 7215-7218.

(71) Plante, O. J.; Palmacci, E. R.; Andrade, R. B.; Seeberger, P. H. *J. Am. Chem. Soc.* **2001**, *123*, 9545-9554.

(72) Nicolaou, K. C.; Caulfield, T.; Kataoka, H.; Kumazawa, T. *J. Am. Com. Soc* **1988**, *110*, 7910-7912.

(73) Wang, C.-N.; Wang, H.-S.; Zhang, L.-H.; Ye, X.-S. *Tetrahedron* **2006**, *62*, 11657-11662.

(74) Hsieh, S.-Y.; Jan, M.-D.; Patkar, L.-N.; Chen, C.-T.; Lin, C.-C. *Carbohydr. Res.* **2005**, *340*,

49-57.

(75) Ekelf, K.; Oscarson, S. *J. Org. Chem.* **1996**, *61*, 7711-7718.

(76) Hodosi, G.; Podanyi, B.; Kuszmann, J. *Carbohydr. Res.* **1992**, *230*, 327-342.

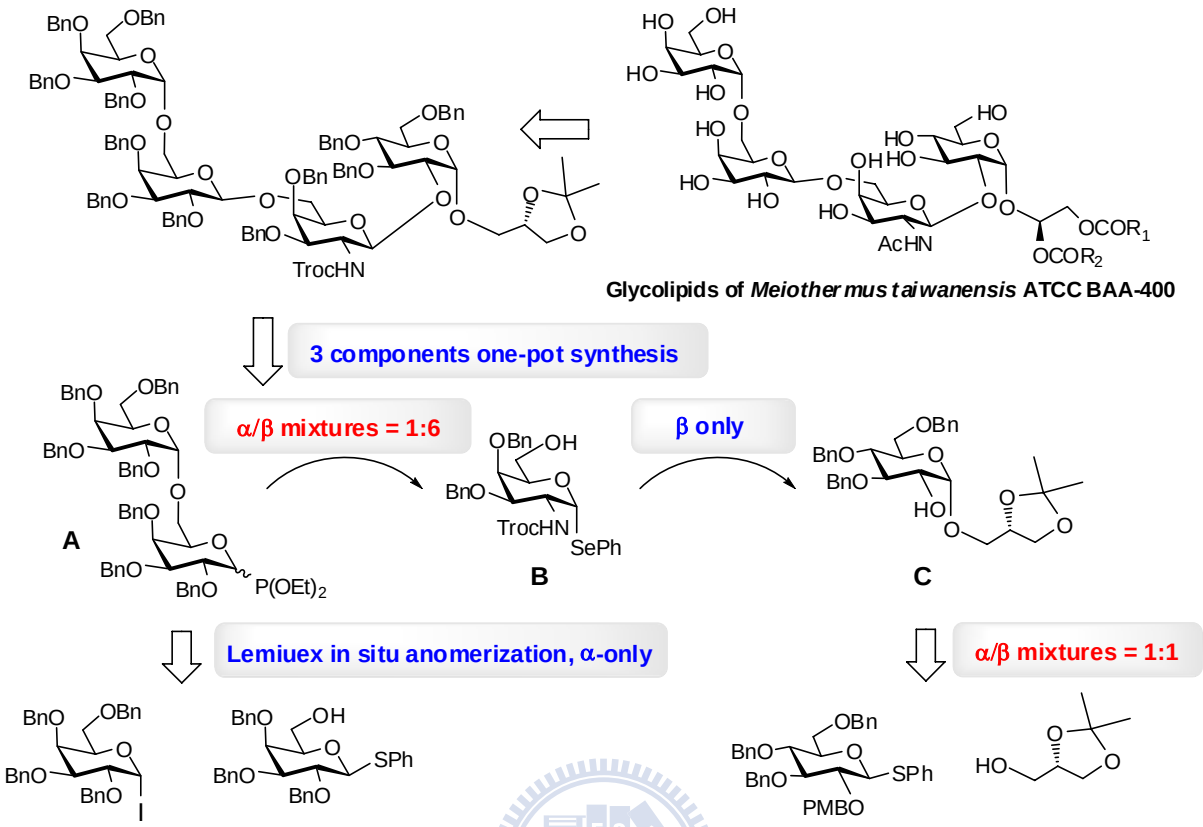
Chapter 4

Synthesis of oligosaccharides -

Glycolipids derivative of Meiothermus taiwanensis ATCC BAA-400 using DMF-mediated α-selective glycosylation strategy

1. Introduction

DMF-mediated α-stereoselective glycosylation method has been established in the previous chapter. To apply this method to oligosaccharide synthesis, we selected a glycolipid derivative as our synthetic target. This glycolipid was isolated from the thermophilic bacteria Meiothermus taiwanensis ATCC BAA-400.[1] It was found in the hot spring in Taiwan and subsequently characterized by Wu *et al.* in 2004. Among the glycoconjucates, the glycolipids also play significant biological functions in many species.[2-6] In Wu's lab, their preliminary biological studies suggest that this glycolipid may be a potential immunomodulator. They identified these glycolipids comprising a tetrasaccharide core with the tethered lipid. Interestingly, they indicate that the tetrasaccharide shows the high homogeneity, whereas the tether lipids are composed of the various types of moieties. To realize its potential use, the first synthesis of this glycolipid precursor has been reported by the same group in 2007.[7]

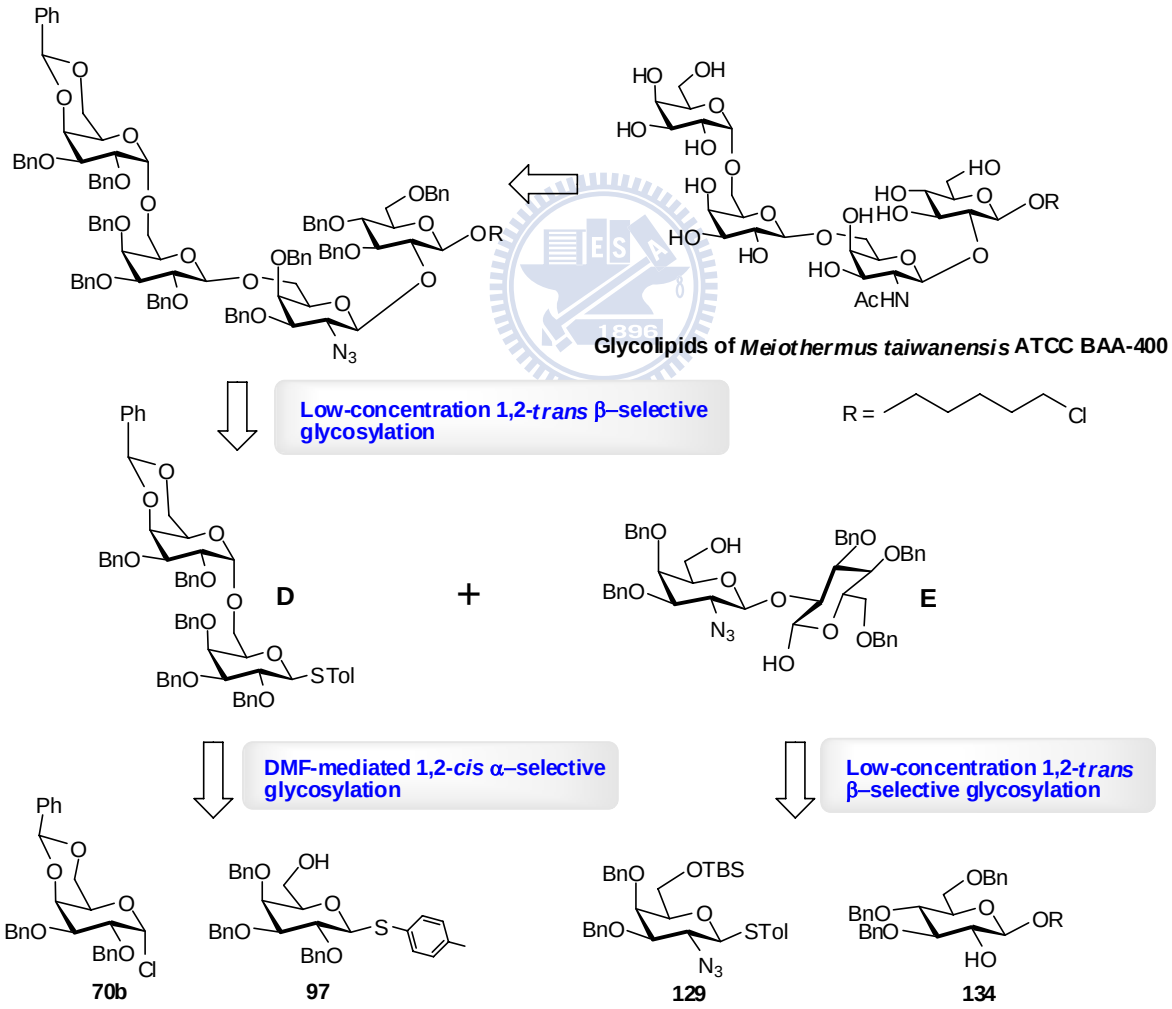


Scheme 1 - The first synthesis of the precursor of glycolipid isolated from Meiothermus taiwanensis ATCC BAA-400

In the first synthesis of the precursor of the tetrasaccharide lipids, the core structure could be divided into four individual building blocks with these linkages (α–D-Gal-(1→6)-β–D-Gal(1→6)-β–D-GalNAc(1→2)-α–D-Glc-1-*O*-Glycerol). The precursor of disaccharides **A** was prepared by coupling of per-*O*-benzylated galactosyl iodide with 2,3,4-tri-*O*-benzyl -1-β-D-thioglucopyranoside with an exclusive α–linkage via Lemieux's in situ anomerization strategy (Scheme 1). To facilitate the following orthogonal glycosylation, the phosphite group was introduced to the disaccaharide **A**, and the selenyl group was assembled to the building block **B** respectively. Coupling of glycerol chain with glucosyl building block resulted in inseparable α/β mixtures (1:1) subsequently treated with DDQ to remove PMB group. Then, this α/β mixture product was allowed for silica gel purification to obtain the pure α anomer of

glucose building block **C**. The one-pot glycosylation with three components **A**, **B** and **C** was performed as a key step. However, in the first coupling step to glycosylate **A** with **B**, α/β mixtures (*ca.* 1:6) of trisaccharide was obtained, followed by the addition of the third component to form β–linkage through the neighboring group participation of NTroc and the overall yield of the tetrasaccharides for one-pot glycosylation is around 46%.

2. Retrosynthesis of glycolipid derivatives – convergent (2+2) synthesis



Scheme 2 - Retrosynthesis of tetrasaccharide derivatives

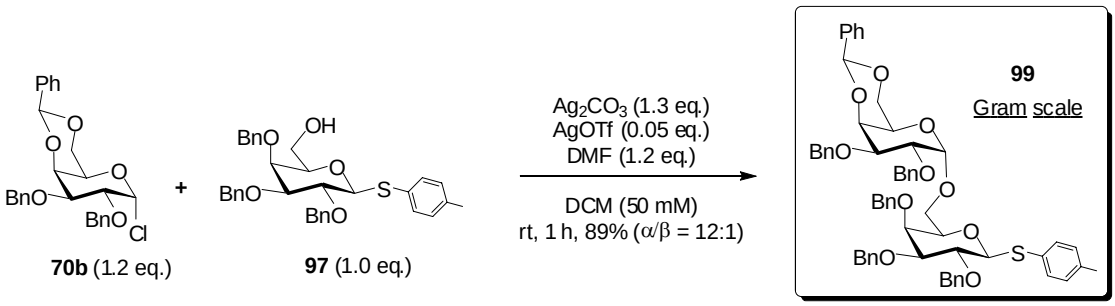
Retrosynthetically, we decided to apply the convergent [2+2] synthesis instead of one-pot glycosylation in order to obtain the better synthetic yield. In the first step, DMF-mediated α–selective glycosylation could be employed for preparation of the disaccharide **D** with a favorable α-Gal-(1→6)-Gal linkage. In addition, without the assistance of the neighboring group participation to construct β–glycosides, low-concentration 1,2-*trans* β–selective glycosylation strategy has been well established in our lab.[8] We envisaged that the β–linkage of disaccharide **D** and the final [2+2] coupling of disaccharides **D** and **E** can be precisely installed by the first use of this strategy in tetrasaccharide synthesis.

3. Results and discussion

3.1. Synthesis of building blocks

3.1.1. Preparation of disaccharide 99

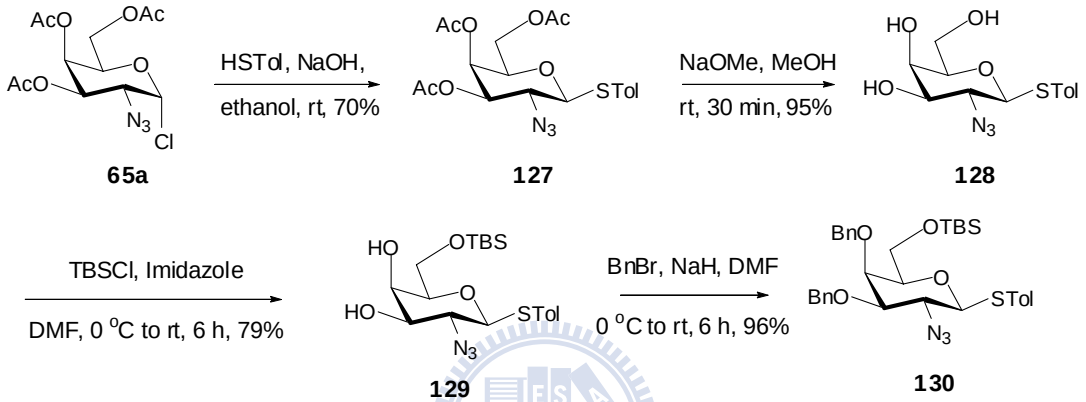
Preparation of the disaccharide **99** at room temperature by following procedure A (see Chapter 3) has been reproduced in the gram scale with a good α–selectivity (α/β ratio = 93:7) (Scheme 3).



Scheme 3 - Preparation of disaccharide **99**

Moreover, the better α–selectivity should be obtained at -25 °C under the similar conditions. The further optimization in selectivity would be taken for granted in the future.

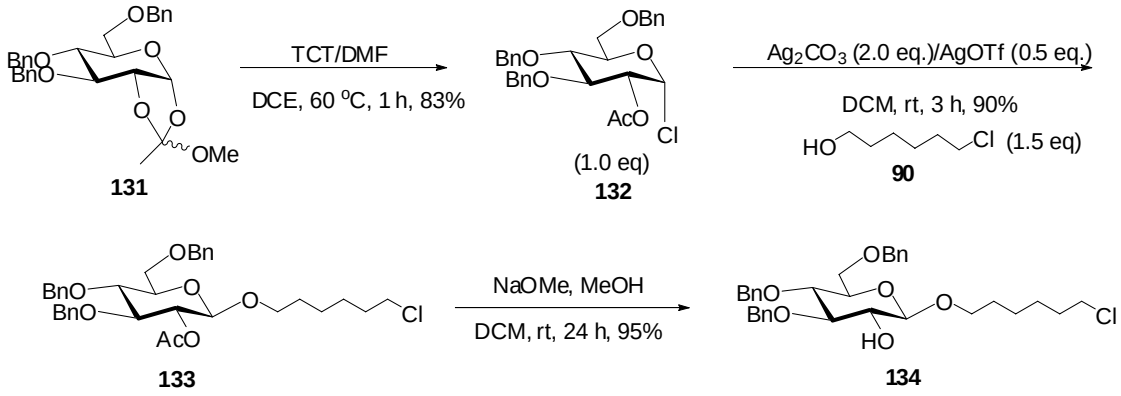
3.1.2. Preparation of Gal-N_3 donor 130



Scheme 4 - Synthetic route for preparation of Gal-N_3 donor **130**

3,4,6-Tri-O-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl chloride **65a** was prepared by TCT/DMF chlorination protocol, followed by the S_N2 replacement of thio group to afford the β–thioglycoside **127** in 70% yield under basic conditions (Scheme 4).[9] Upon Zemplén de-*O*-acetylation of **127**, treatment of TBSCl led to regioselective protection at 6-OH group of **127**. Subsequent benzylation of 3- and 4-OH group gave the GalNAc donor **130** in an isolated 75% yield over three steps.

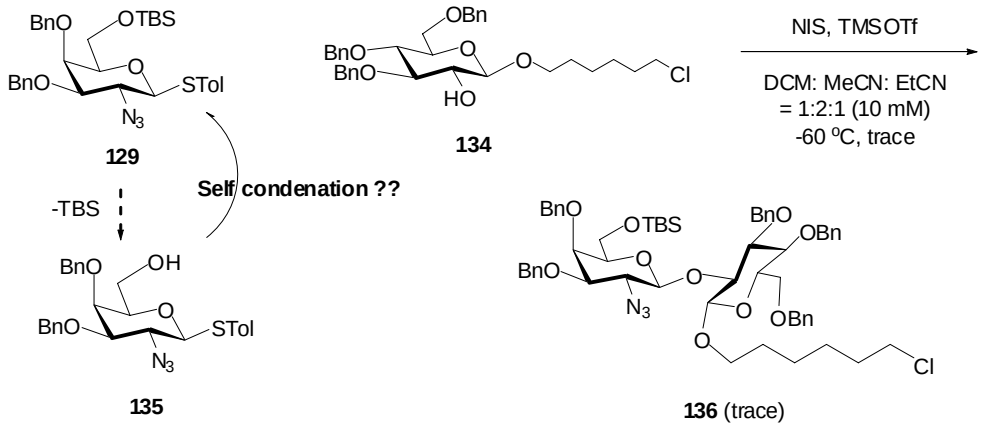
3.1.3. Preparation of glucosyl acceptor 134



Scheme 5 - Preparation of glucosyl acceptor **134**

The synthesis of glycosyl orthoester **131** followed the reported procedures. Upon opening of orthoester **131** and chlorination via one-pot procedure, α–glycosyl chloride **132** was obtained in an excellent 83% yield. α–chloride **132** served as a glycosyl donor to couple with 6-chloro hexanol under Koenigs-Knorr glycosylation conditions. Deacetylation of **133** led to glucosyl acceptor **134** quantitatively (Scheme 5).

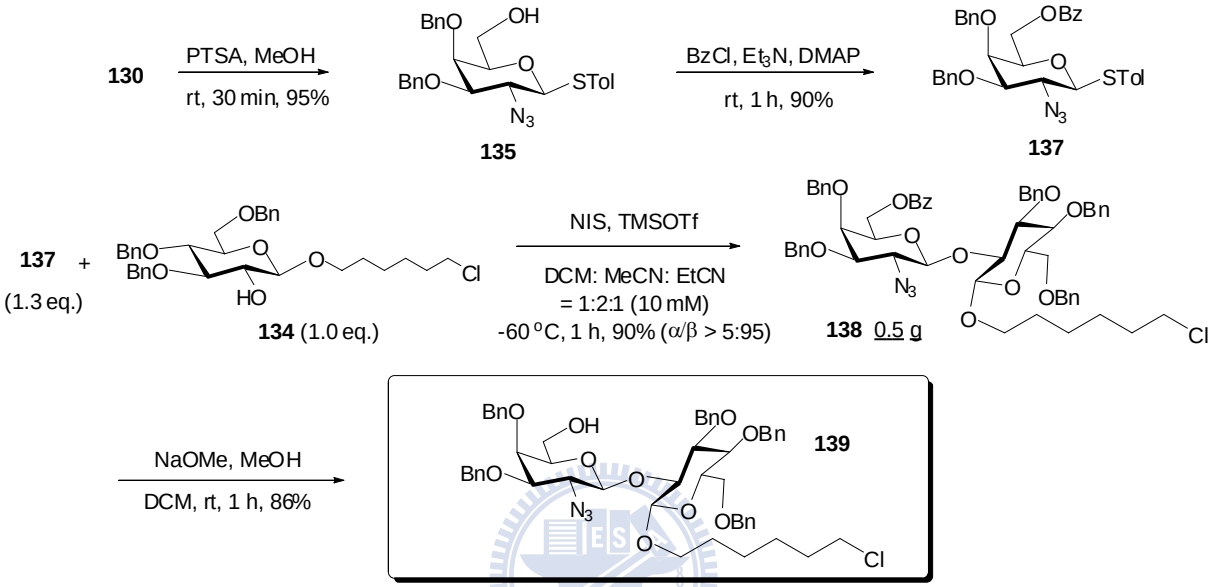
3.1.4. Preparation of disaccharide E - 136



Scheme 6 - Preparation of disaccharide **136**

With the available GalNAc donor **130** and glucosyl acceptor **134** in hand, we set out to apply the low-concentration 1,2-*trans* β–selective glycosylation to prepare the disaccharide

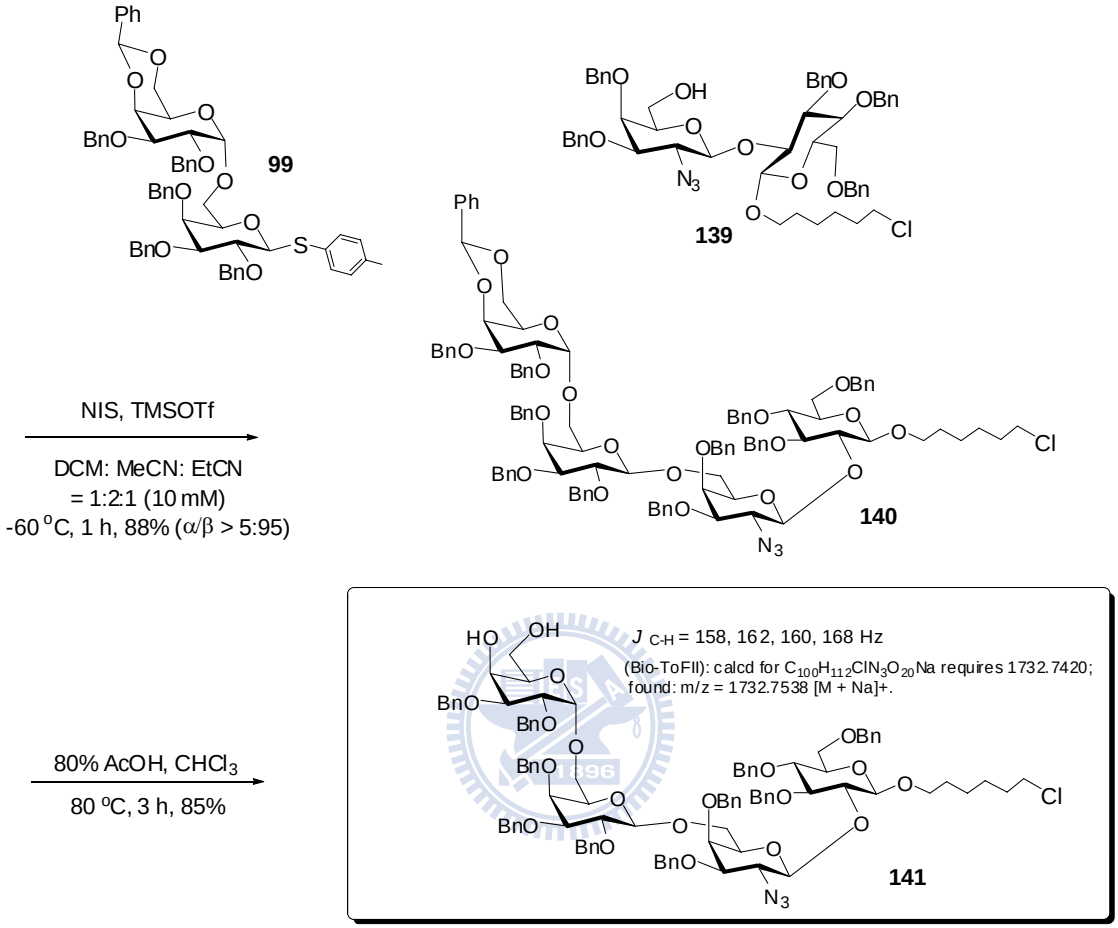
136. Unfortunately, only traces of the desired product were obtained in the product mixture (Scheme 6). This failure was attributed to acid-labile 6-*O*-TBS protecting group which did not survive NIS/TMSOTf activation conditions.[10]



Scheme 7 - Preparation of disaccharide **139**

To address this problem, a more robust benzoyl group was introduced to protect 6-OH of **135** furnishing Gal-N_3 donor **137** (Scheme 7). By following the same procedure for the construction of β–linkage, the discaccharide **138** was given with an exclusive β–selectivity. Subsequent deacetylation of **138** led to the desired disaccharide precursor **139** in an excellent 88% yield over 2 steps.

3.1.5. Convergent [2+2] approach for synthesis of tetrasaccharide 140



Scheme 8 - Convergent [2+2] synthesis of tetrasaccharide **140**

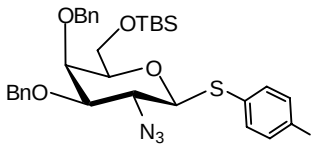
To this end, following the same procedure in the convergent [2+2] glycosylations with the two disaccharides **99** and **139** successfully led to the high yield of tetrasaccharide **140** with an excellent β–selectivity (Scheme 8). The stereochemistry of each linkage was confirmed by NMR analyses including non-decoupling experiment. ($J_{C\text{-}H}$ = 151, 155, 158, 167 Hz). However, the further MALDI-TOF-HRMS analysis failed to give the expected value probably due to the highly hydrophobic property. Therefore, subsequent removal of benzylidene ring gave the tetrasaccharide diol **141** suitable for the HRMS measurement. The data was shown in Scheme 8.

4. Conclusion

In summary, a protected tetrasaccharide **141** containing the core structure of a glycoglycerolipid isolated from Meiothermus taiwanensis ATCC BAA-400 has been efficiently synthesized by using the convergent [2+2] glycoyslation strategy. Either DMF-mediated α–selective or low-concentration 1,2-*trans* β–selective glycosylation strategy were for the first time recognized as a promising method for the highly stereoselective assembly of *O*-linkage in oligosaccharide synthesis.

5. Experimental

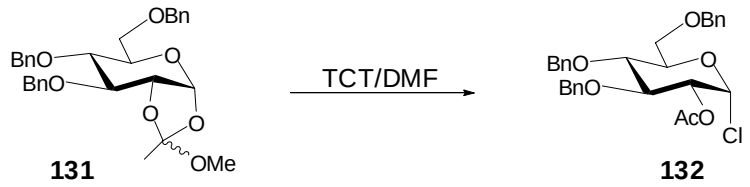
General: Followed the standard operations as described in the previous chapters.



130

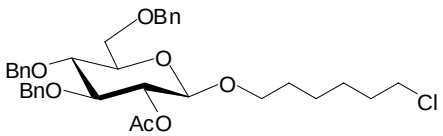
p-Tolyl 2-azido-3,4-di-*O*-benzyl-6-*O*-tert-butyldimethylsilyl-2-deoxy-1-thio-β-D-galacto -pyranoside (130): *p*-Tolyl 2-azido-6-*O*-tert-butyldimethylsilyl-2-deoxy-1-thio-β-D-galacto -pyranoside **129** was prepared according to the reported procedure. To a mixture of **129** (0.78 g, 1.83 mmol) and BnBr (0.55 mL, 4.6 mmol) in THF (10 mL) and DMF (3 mL) was portionwise added 60% NaH (0.20 g, 5.0 mmol) at 0 ^{o}C. The resulting mixture was stirred at 0 ^{o}C for 30 min, then quenched by saturated NH_4Cl solution in an ice bath followed by removal of THF. The crude residue was washed by 0.5% HCl aqueous solution and saturated sodium bicarbonate solution, brine, dried with $MgSO_4$. The concentrated mixture was subjected to column chromatography over silica gel (EtOAc/Hexane = 1/9 to 3/7) to furnish the β-thio -glycoside **129** as a syrup (1.06 g, 96%). For **(126)**: R_f = 0.60 (TLC developing solution: EtOAc/ Hexane = 1/9) ; $[\alpha]^{27}_D$ = -3.3 (c = 0.65, $CHCl_3$); **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.46 (d, J = 8.1 Hz, 2H), 7.37-7.24 (m, 10H), 7.01 (d, J = 7.9 Hz, 2H), 4.87 (d, J = 11.3 Hz, 1H), 4.70 (s, 2H), 4.55 (d, J = 11.3 Hz, 1H), 4.33 (d, J = 10.0 Hz, 1H, H-1), 3.89 (d, J = 2.2 Hz, 1H), 3.80 (t, J = 9.9 Hz, 1H), 3.76-3.69 (m, 2H), 3.44-3.35 (m, 2H), 2.30 (s, 3H), 0.88 (s, 9H), 0.02 (s, 6H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 138.64, 137.97, 137.49, 133.31, 129.58, 128.48, 128.06, 127.96, 127.87, 127.81, 127.40, 127.30, 82.45 (C-1), 78.88, 74.34, 72.45, 71.91, 61.42, 61.13, 25.85, 21.14, 18.15.

HRMS (Bio-ToFII): calcd for $C_{33}H_{43}N_3O_4SSiNa$ requires 628.2641; found: *m*/*z* = 628.2652 [M + Na]$^+$.



131 **132**

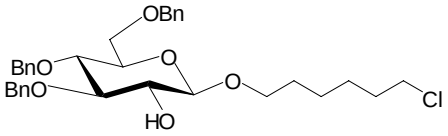
2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl chloride (132) : Glycosyl orthoester **131** was prepared according to the reported procedure.[11] Preparation of α-glucosyl chloride employed protocol B as described in Chapter 2. Short-column chromatographic purification of glucosyl chloride was achieved by 1/4 EtOAc/Hexane elution and glycosyl chloride **132** was obtained a colorless syrup (92% yield). For **(131)**, R_f = 0.42 (TLC developing solution: EtOAc/Hexane = 1/4); **^{1}H NMR** (300 MHz, $CDCl_3$) :δ 7.38–7.22 (m, 13H), 7.21–7.09 (m, 2H), 6.31(d, *J* = 3.9 Hz, 1H), 4.97 (dd, J = 9.8, 3.9 Hz, 1H), 4.87–4.69 (m, 3H), 4.61–4.40 (m, 3H), 4.09 (m, 2H), 3.90–3.72 (m, 2H), 3.66 (dd, J = 11.1, 1.8, 1H), 2.02 (s, 3H).; **^{13}C NMR** (75 MHz, $CDCl_3$) :δ 69.9, 138.1, 137.6, 137.5, 128.3, 128.1, 127.8, 127.8, 127.7, 127.6, 127.5, 91.7 (C-1), 79.4, 76.4, 75.5, 75.2, 73.5, 73.3, 73.3, 67.5, 20.6. The spectroscopic data agrees with the literature values.[12]



133

6-Chloro-n-hexyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranoside (133): To a mixture of Ag_2CO_3 (2.80 g, 10.20 mmol), molecular sieve (AW300, 50 mg/per 1mL solvent), α-glucos -yl chloride **132** (2.60 g, 5.09 mmol) and 6-chloro hexanol **90** (0.79 mL, 6.1 mmol) in DCM

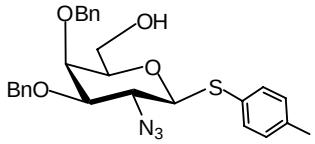
(20 mL) was added a solution of AgOTf [0.13 g, 0.5 mmol (con. = 0.2 M of AgOTf in toluene)] at rt. The resulting mixture was stirred at rt for another 3 hrs, quenched by excess Et_3N, then filtered by Celite and concentrated to give the crude product which was directly purified by column chromatography over silica gel (EtOAc/Hexane = 1/9 to 1/4) to furnish the single β-anomer **133** as a colorless syrup (2.80 g, 90%). For **133**: R_f = 0.46 (TLC developing solution: EtOAc/ Hexane = 1/4); $[\alpha]^{27}_D$ = -32.2 (*c* = 0.25, $CHCl_3$); **^{1}H NMR** (300 MHz, $CDCl_3$):δ 7.41-7.09 (m, 15H), 4.99 (d, *J* = 8.3 Hz, 1H), 4.79 (d, *J* = 10.2 Hz, 1H), 4.78 (d, *J* =10.0 Hz, 1H), 4.66 (d, *J* = 10.0 Hz, 1H), 4.58 (d, *J* = 9.6 Hz, 2H), 4.54 (d, *J* = 10.8 Hz, 1H), 4.34 (d, *J* = 8.4 Hz, 1H, H-1), 3.91-3.69 (m, 4H), 3.67 (t, *J* = 5.4 Hz, 1H), 3.55-3.40 (m, 4H), 1.95 (s, 3H), 1.80-1.50 (m, 4H), 1.49-1.19 (m, 4H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 169.41, 138.09, 138.01, 137.76, 128.37, 128.30, 127.99, 127.87, 127.81, 127.79, 127.73, 127.68, 127.56, 100.90 (C-1), 82.86, 77.97, 75.06, 74.99, 74.96, 73.42, 73.07, 69.34, 68.68, 44.97, 32.44, 29.24, 26.48, 25.13, 20.87; HRMS (Bio-ToFII): calcd for $C_{35}H_{43}ClO_7Na$ requires 633.2590; found: *m*/*z* = 633.2578 $[M + Na]^+$.



134

6-Chloro-n-hexyl 3,4,6-tri-*O*-benzyl-β-D-galactopyranoside (134): To a solution of **133** (0.16 g, 0.26 mmol) in MeOH (2 mL) and DCM (2 mL) was added sodium (5 mg, 0.21 mmol) at RT. The resulting solution was stirred for 24 hrs and concentrated to obtain the crude residue followed by purification via chromatography over silica gel (EtOAc/Hexane = 1/4 to 3/7) to give **134** as a colorless syrup (0.14 g, 95%). For **134**: R_f = 0.46 (TLC developing solution: EtOAc/ Hexane = 1/4); $[\alpha]^{27}_D$ = +0.6 (*c* = 0.56, $CHCl_3$); **^{1}H NMR** (300 MHz,

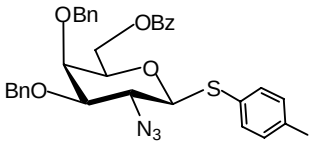
$CDCl_3$): δ 7.56-7.05 (m, 15H), 4.90 (d, *J* = 11.3 Hz, 1H), 4.80 (d, *J* = 12.5 Hz, 2H), 4.58 (d, *J* = 12.2 Hz, 1H), 4.54-4.45 (m, 3H), 4.21 (d, *J* = 7.2 Hz, 1H, H-1), 3.93-3.83 (m, 1H), 3.70-3.61 (m, 3H), 3.60-3.40 (m, 5H), 1.78-1.68 (m, 2H), 1.60 (m, 2H), 1.52-1.27 (m, 4H); **^{13}C NMR** (75 MHz, CDCl3): δ 138.52, 137.99, 137.93, 128.37, 128.31, 128.27, 127.89, 127.84, 127.78, 127.71, 127.63, 127.55, 102.62 (C-1), 84.41, 77.49, 75.02, 74.92, 74.58, 73.38, 69.74, 68.78, 44.95, 32.41, 32.38, 29.36, 26.56, 26.53, 25.21; HRMS (Bio-ToFII): calcd for $C_{33}H_{41}ClO_6Na$ requires 591.2484; found: *m*/*z* = 591.2455 $[M + Na]^+$.



135

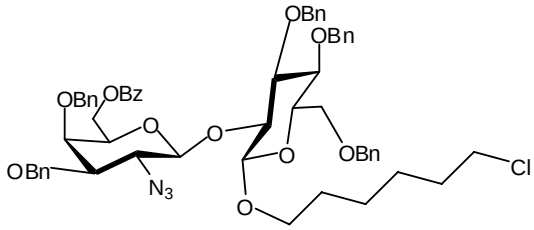
p-Tolyl 2-azido-3,4-di-*O*-benzyl-2-deoxy-1-thio-β-D-galactopyranoside (135): To a soluti -on of **130** (0.50 g, 0.82 mmol) in MeOH (5 mL) and DCM (5 mL) was added PTSA (0.10 g, 0.58 mmol) at RT. The resulting solution was stirred for 30 min and concentrated to obtain the crude residue followed by purification via chromatography over silica gel (EtOAc/Hexane = 1/4 to 2/3) to give **135** as a colorless syrup (0.38 g, 95%). For **135**: R_f = 0.48 (TLC developing solution: EtOAc/ Hexane = 2/3); $[\alpha]^{27}_D$ = -19.6 (*c* = 0.95, $CHCl_3$); **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.46 (d, *J* = 8.1 Hz, 2H), 7.41-7.29 (m, 10H), 7.05 (d, *J* = 7.9 Hz, 2H), 4.89 (d, *J* = 11.6 Hz, 1H), 4.73 (s, 2H), 4.54 (d, *J* = 11.6 Hz, 1H), 4.34 (d, *J* = 10.1 Hz, 1H, H-1), 3.87-3.76 (m, 4H), 3.55 (s, 1H, OH), 3.41 (dd, *J* = 9.6, 3.3 Hz, 1H), 3.41 (td, *J* = 6.6, 1.2 Hz, 1H), 2.31 (s, 3H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 138.05, 137.97, 137.15, 133.13, 129.59, 128.42, 128.16, 127.92, 127.73, 127.72, 127.59, 127.50, 86.21 (C-1), 82.38, 78.84, 74.02, 72.39, 71.85, 61.90, 61.25, 21.02; HRMS (Bio-ToFII): calcd for $C_{27}H_{29}N_3O_4SNa$ requires

514.1771; found: m/z = 514.1788 $[M + Na]^+$.



137

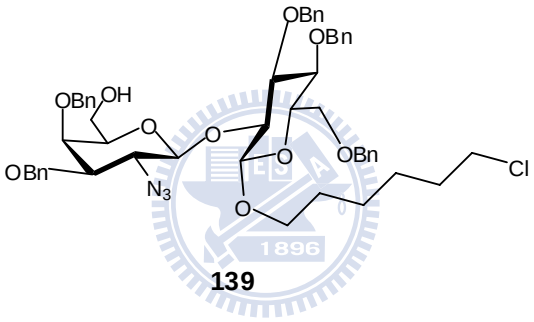
p-Tolyl 2-azido-6-*O*-benzoyl-3,4-di-*O*-benzyl-2-deoxy-1-thio-β-D-galactopyranoside (137): To a solution of **135** (0.36 g, 0.71 mmol) and BzCl (86 μL, 0.74 mmol) in DCM (6 mL) was added Et_3N (400 μL, 2.89 mmol) at 0 °C. The resulting solution was stirred for 30 min at RT. Upon completion of reaction, the reaction mixture was diluted with DCM (10 mL) and washed by 1% HCl aqueous solution and saturated sodium bicarbonate solution, brine, dried with $MgSO_4$. The collected DCM layer was concentrated to obtain the crude residue which was purified by chromatography over silica gel (EtOAc/Hexane = 1/9 to 3/7) to give **137** as a colorless syrup (0.38 g, 90%). For **137**: R_f = 0.62 (TLC developing solution: EtOAc/ Hexane = 3/7); $[\alpha]^{27}_D$ = -30.1 (c = 0.62, $CHCl_3$); **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.96 (d, J = 10.5 Hz, 2H), 7.60 (t, J = 7.4 Hz, 1H), 7.54-7.16 (m, 14H), 6.95 (d, J = 8.1 Hz, 2H), 4.93 (d, J = 11.4 Hz, 1H), 4.75 (s, 2H), 4.59 (d, J = 11.5 Hz, 1H), 4.51 (dd, J = 11.3, 7.0 Hz, 1H, H-6a), 4.36 (d, J = 10.2 Hz, 1H, H-1), 4.35-4.31 (m, 1H, H-6b), 3.83-3.87 (m, 2H, H-2, H-4), 3.70 (t, J = 6.1 Hz, 1H, H-5), 3.44 (dd, J = 9.7, 2.6 Hz, 1H, H-3), 2.27 (s, 3H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 166.13, 138.05, 137.91, 137.26, 133.20, 129.69, 129.59, 128.57, 128.46, 128.37, 128.29, 128.18, 128.10, 127.95, 127.73, 86.86 (C-1), 82.66, 76.15, 74.35, 72.91, 72.14, 63.68, 61.54, 21.13; HRMS (Bio-ToFII): calcd for $C_{34}H_{33}N_3O_5SNa$ requires 618.2039; found: m/z = 618.2038 $[M + Na]^+$.



138

6-Chloro-n-hexyl 2-*O*-(2-azido-6-*O*-benzoyl-3,4-di-*O*-benzyl-2-deoxy-β-D-galactopyrano -syl)-(1→2)-3,4,6-tri-*O*-benzyl-β-D-glucopyranoside (138): General procedure for low-con -centration 1,2-*trans* β-selective glycosylation was depicted in the supporting information of our reported work.(ref). For preparation of the dissacharide **138**: A mixture of thioglycoside **137** (0.35 g, 0.74 mmol), acceptor **134** (0.32 g, 0.57 mmol), and flame-dried molecular sieves (AW300) were suspended in 1:2:1 v/v DCM–CH_3CN–EtCN solvent mixture such that the final concentrations of thioglycoside and acceptor were 10 mM. The resulting mixture was stirred at room temperature for 10 min and at -60 ^oC for an additional 20 min under N_2, and followed by the addition of NIS (0.20 g, 0.89 mmol) and TMSOTf (50 μL, 0.28 mmol). The reaction mixture was stirred at -60 ^oC for 40 mins. Upon completion of glycosylation monitored by TLC analysis, excess of Et_3N, a small volume of saturated $NaHCO_3$ and small lumps of $Na_2S_2O_3$(s) were added to the mixture, followed by vigorous stirring until the deep-red color of the reaction mixture turned to pale yellow. Then the molecular sieves were removed by filtration over celite. The filtrate was dried over $MgSO_4$, filtered, and concentrated for flash-chromatography purification over silica gel (EtOAc/Hexane = 1/9 to 1/4) to give the disaccharide **138** as a colorless syrup (0.53 g, 90%). For **138**: R_f = 0.45 (TLC developing solution: EtOAc/ Hexane = 1/4); $[\alpha]^{27}_D$ = -22.2 (c = 0.70, $CHCl_3$); **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.91 (d, J = 7.2 Hz, 2H), 7.55 (d, J = 7.5 Hz, 1H), 7.45-7.07 (m, 27H), 5.01 (d, J = 10.8 Hz, 1H), 4.94 (d, J =11.7 Hz, 1H), 4.85 (d, J = 10.8 Hz, 1H), 4.78 (d, J = 10.8 Hz, 1H), 4.75-4.71 (m, 2H), 4.64 (d, J = 8.1 Hz, 1H, H-1), 4.63-4.49 (m, 4H), 4.45-4.36 (m, 2H,

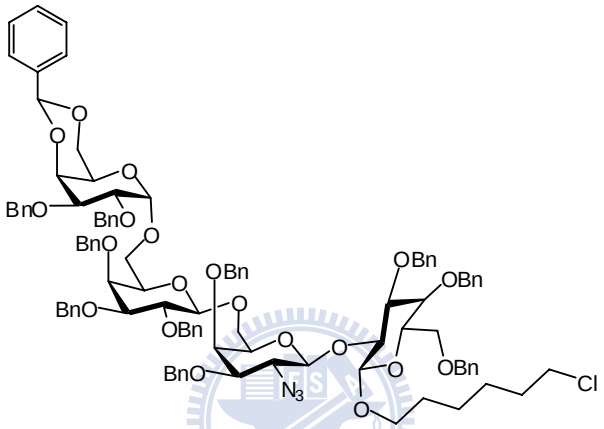
H-1'), 3.90-3.79 (m, 3H), 3.78-3.65 (m, 4H), 3.64-3.52 (m, 3H), 3.51-3.34 (m, 5H), 1.70-1.63 (m, 2H), 1.60-1.47 (m, 2H), 1.37-1.32 (m, 4H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 165.83, 138.39, 138.01, 137.83, 137.78, 137.21, 133.09, 129.51, 129.46, 128.43, 128.28, 128.25, 128.23, 128.20, 128.09, 127.91, 127.89, 127.85, 127.76, 127.67, 127.61, 127.57, 127.50, 127.44, 101.79 (C-1, $J_{C\text{-}H}$ = 157 Hz), 101.18 (C-1', $J_{C'\text{-}H'}$ = 161 Hz), 84.65, 81.37, 80.13, 77.92, 75.43, 74.76, 74.57, 74.42, 73.29, 72.67, 72.08, 71.62, 69.15, 68.74, 63.49, 62.73, 44.99, 32.28, 29.41, 26.41, 24.94; HRMS (Bio-ToFII): calcd for $C_{60}H_{66}ClN_3O_{11}Na$ requires 1062.4278; found: *m*/*z* = 1062.4230 $[M + Na]^+$.



139

6-Chloro-n-hexyl 2-*O*-(2-azido-3,4-di-*O*-benzyl-2-deoxy-β-D-galactopyranosyl)-(1→2)-3, 4,6-tri-*O*-benzyl-β-D-glucopyranoside (139): To a solution of **138** (0.30 g, 0.29 mmol) in MeOH (1 mL) and DCM (5 mL) was added sodium (20 mg, 0.87 mmol) at RT. The resulting solution was stirred for 1 h and concentrated to obtain the crude residue followed by purification via chromatography over silica gel (EtOAc/Hexane = 3/7 to 2/3) to give **139** as a colorless syrup (0.24 g, 86%). For **139**: R_f = 0.28 (TLC developing solution: EtOAc/ Hexane = 3/7); $[\alpha]^{27}_D$ = -22.2 (*c* = 0.70, $CHCl_3$); **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.43-7.21 (m, 23H), 7.18-7.10 (m, 2H), 5.00 (d, *J* = 10.6 Hz, 1H), 4.87 (d, *J* = 11.1 Hz, 2H), 4.78 (d, *J* = 10.9 Hz, 1H), 4.69 (s, 2H), 4.58 (d, *J* = 7.8 Hz, 1H, H-1), 4.57- 4.48 (m, 5H), 4.37 (d, *J* = 6.9 Hz, 1H, H-1'), 3.91-3.78 (m, 3H), 3.77-3.54 (m, 8H), 3.34-3.23 (m, 2H), 1.80-1.48 (m, 5H), 1.45-1.31 (m, 5H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 138.41, 137.96, 137.81, 137.29, 128.47,

128.31, 128.27, 128.25, 128.21, 127.94, 127.90, 127.84, 127.81, 127.77, 127.75, 127.66, 127.52, 101.77 (C-1'), 101.50 (C-1), 84.84, 81.14, 79.84, 78.09, 75.39, 74.77, 74.75, 74.57, 74.23, 73.35, 72.47, 71.65, 69.60, 68.68, 63.50, 61.61, 45.04, 32.30, 29.25, 26.37, 24.89; HRMS (Bio-ToFII): calcd for $C_{53}H_{62}ClN_3O_{10}Na$ requires 958.4021; found: *m*/*z* = 958.4033 [M + Na]$^+$.

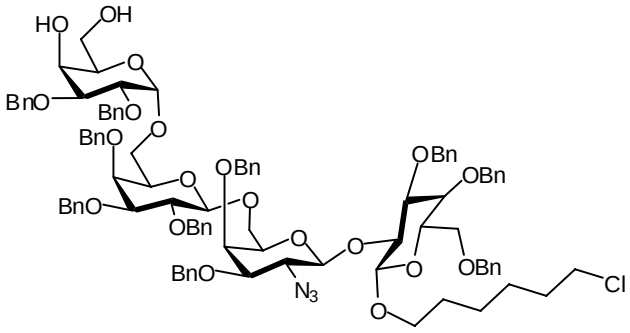


140

6-Chloro-n-hexyl 2,3-di-*O*-benzly-4,6-*O*-benzylidene-α-D-galactopyranosyl-(1 → 6)-2,3, 4-*O*-tri-*O*-benzyl-β-D-galatopyranosyl-(1 → 6)-2-azido-3,4-di-*O*-benzyl-2-deoxy-β-D-gala ctopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-β-D-galactopyranoside (140):

For preparation of the tetrasacharide **140**: A mixture of thioglycoside **99** (0.34 g, 0.35 mmol), acceptor **139** (0.27 g, 0.29 mmol), and flame-dried molecular sieves (AW300) were suspended in 1:2:1 v/v DCM–CH_3CN–EtCN solvent mixture such that the final concentrations of thioglycoside and acceptor were 10 mM. The resulting mixture was stirred at room temperature for 10 min and at -70 ^oC for an additional 20 min under N_2, and followed by the addition of NIS (0.09 g, 0.41 mmol) and TMSOTf (27 μL, 0.15 mmol). The reaction mixture was stirred at -70 ^oC for 15 mins. Upon completion of glycosylation monitored by TLC analysis, excess of Et_3N, a small volume of saturated $NaHCO_3$ and small lumps of

$Na_2S_2O_3$(s) were added to the mixture, followed by vigorous stirring until the deep-red color of the reaction mixture turned to pale yellow. Then the molecular sieves were removed by filtration over celite. The filtrate was dried over $MgSO_4$, filtered, and concentrated for flash-chromatography purification over silica gel (EtOAc/Hexane = 1/9 to 3/7) to give the tetrasaccharide **140** as the amophorous solid (0.46 g, 88%). For **140**: R_f = 0.51 (TLC developing solution: EtOAc/ Hexane = 3/7); $[\alpha]^{27}_D$ = +25.9 (*c* = 1.60, $CHCl_3$); **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.59-7.45 (m, 3H), 7.44-7.06 (m, 52H), 5.45 (s, 1H), 5.00-4.74 (m, 10H), 4.73 (d, *J* =2.1 Hz, 1H, H-1'''), 4.72-4.45 (m, 12 H), 4.42 (d, *J* = 7.5 Hz, 1H, H-1), 4.32 (d, *J* = 7.5 Hz, 1H, H-1'), 4.19 (d, *J* = 2.1 Hz, 1H), 4.14-3.94 (m, 6H), 3.93-3.67 (m, 6H), 3.66-3.40 (m, 8H), 3.39-3.28 (m, 4H), 3.19 (dd, *J* = 2.7 Hz, J = 10.5 Hz, 2H), 1.64-1.47 (m, 4H), 1.37-1.24 (m, 4H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 138.75, 138.59, 138.44, 138.39, 138.37, 138.30, 138.03, 137.87, 137.73, 137.42, 128.80, 128.36, 128.33, 128.26, 128.21, 128.13, 128.11, 128.09, 128.04, 127.96, 127.84, 127.78, 127.72, 127.65, 127.61, 127.58, 127.48, 127.41, 127.19, 126.38, 126.23, 103.40 (C-1, $J_{C\text{-}H}$ = 157 Hz), 101.83 (C-1', $J_{C'\text{-}H'}$ = 155 Hz), 101.17 (C-1'', $J_{C''\text{-}H''}$ = 162 Hz), 100.87, 98.85 (C-1''', $J_{C'''\text{-}H'''}$ = 167 Hz), 85.05, 82.04, 80.88, 79.41, 78.96, 78.24, 77.20, 76.22, 75.43, 75.11, 74.94, 74.79, 74.65, 74.54, 74.41, 74.09, 73.84, 73.49, 73.30, 73.02, 72.88, 72.76, 71.97, 71.49, 69.47, 69.28, 68.82, 66.61, 63.45, 62.66, 45.10, 32.29, 29.31, 26.33, 24.86; HRMS (Bio-ToFII): calcd for $C_{107}H_{116}ClN_3O_{20}Na$ requires 1820.7738; found: *m*/*z* = 1820.7936 $[M + Na]^+$.



141

6-Chloro-n-hexyl 2,3-di-*O*-benzly-α-D-galactopyranosyl-(1→6)-2,3,4-*O*-tri-*O*-benzyl-β-D-galatopyranosyl-(1→6)-2-azido-3,4-di-*O*-benzyl-2-deoxy-β-D-galactopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-β-D-galactopyranoside (141): A solution of the tetrasaccharide **140** (0.19 g, 0.10 mmol), AcOH (2.6 mL), H_2O (0.4 mL) in CH_3Cl (3 mL) was heated at 90 ^oC for 15 hrs. The remaining solvents were removed via co-evaporation with toluene for three times. The concentrated residue was directly subjected to column chromatography over silica gel (EtOAc/Hexane/DCM = 2/8/2 to 5/5/2) to furnish the partially deprotected tetrasaccharide **141** as the amophorous solid (62 mg, 53%). For **141**: R_f = 0.35 (TLC developing solution: EtOAc/ Hexane = 3/7); $[\alpha]^{27}_D$ = +12.9 (*c* = 0.43, $CHCl_3$); **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.41-7.14 (m, 48H), 7.13-7.06 (m, 2H), 5.00-4.85 (m, 9H), 4.84 (d, *J* = 2.7 Hz, 1H, H-1'''), 4.81-4.65 (m, 5H), 4.64 (d, *J* = 7.5 Hz, 1H, H-1'), 4.63-4.54 (m, 8H), 4.53-4.49 (m, 4H), 4.48 (d, *J* = 6.9 Hz, 1H, H-1''), 4.33 (d, *J* = 7.5 Hz, 1H, H-1), 4.10 (s, 1H), 3.91-3.61 (m, 13H), 3.60-3.39 (m, 5H), 3.38-3.28 (m, 4H), 3.15 (dd, *J* = 7.2, 2.4 Hz, 1H), 2.87 (s, 1H), 2.63 (br, 1H), 1.65-1.57 (m, 2H), 1.56-1.49 (m, 2H), 1.37-1.21 (m, 2H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 138.93, 138.66, 138.57, 138.49, 138.25, 138.08, 137.98, 137.90, 137.46, 128.47, 128.42, 128.36, 128.29, 128.26, 128.24, 128.19, 128.14, 128.06, 127.88, 127.83, 127.69, 127.59, 127.56, 127.52, 127.47, 127.40, 127.33, 103.30 (C-1, $J_{C\text{-}H}$ = 160 Hz), 101.87 (C-1', $J_{C'\text{-}H'}$ = 158 Hz), 101.23 (C-1'', $J_{C''\text{-}H''}$ = 162 Hz), 98.57 (C-1''', $J_{C'''\text{-}H'''}$ = 168 Hz), 85.26, 82.11,

80.89, 79.69, 78.70, 78.32, 77.55, 77.20, 75.44, 75.38, 74.92, 74.79, 74.62, 74.39, 74.28, 73.97, 73.79, 73.47, 73.30, 73.16, 73.06, 72.31, 72.19, 71.49, 69.62, 69.42, 68.89, 68.72, 67.92, 66.76, 63.41, 62.87, 45.13, 32.33, 29.41, 26.42, 24.91; HRMS (Bio-ToFII): calcd for $C_{100}H_{112}ClN_3O_{20}Na$ requires 1732.7420; found: m/z = 1732.7538 $[M + Na]^+$.

6. References

(1) Yang, F.-L.; Lu, C.-P.; Chen, C.-S.; Chen, M.-Y.; Hsiao, H.-L.; Su, Y.; Tsay, S.-S.; Zou, W.; Wu, S.-H. *Euro. J. Biochem.* **2004**, *271*, 4545-4551.

(2) Burdin, N.; Brossay, L.; Koezuka, Y.; Smiley, S. T.; Grusby, M. J.; Gui, M.; Taniguchi, M.; Hayakawa, K.; Kronenberg, M. *J. Immunol.* **1998**, *161*, 3271-3281.

(3) Sharif, S.; Arreaza, G. A.; Zucker, P.; Mi, Q. S.; Sondhi, J.; Naidenko, O. V.; Kronenberg, M.; Koezuka, Y.; Delovitch, T. L.; Gombert, J. M.; Leite-De-Moraes, M.; Gouarin, C.; Zhu, R.; Hameg, A.; Nakayama, T.; Taniguchi, M.; Lepault, F.; Lehuen, A.; Bach, J. F.; Herbelin, A. *Nature Medicine* **2001**, *7*, 1057-1062.

(4) Cross, G. A. M. *Annual Review of Cell Biology* **1990**, *6*, 1-39.

(5) Kronenberg, M. *Annual Review of Immunology* **2005**, *23*, 877-900.

(6) Leone, S.; Molinaro, A.; Lindner, B.; Romano, I.; Nicolaus, B.; Parrilli, M.; Lanzetta, R.; Holst, O. *Glycobiology* **2006**, *16*, 766-775.

(7) Ren, C.-T.; Tsai, Y.-H.; Yang, Y.-L.; Zou, W.; Wu, S.-H. *J. Org. Chem.* **2007**, *72*, 5427-5430.

(8) Chao, C.-S.; Li, C.-W.; Chen, M.-C.; Chang, S.-S.; Mong, K. K. T. *Chem. Eur. J.* **2009**, 10972-10982.

(9) Lüning, B.; Norberg, T.; Tejbrant, J. *Glycoconjugate* **1989**, *6*, 5-19.

(10) Zegelaar-Jaarsveld, K.; Smits, S. A. W.; Marel, V. D.; Boom, J. H. V. *Bioorg. Med. Chem.* **1996**, *4*, 1819-1832.

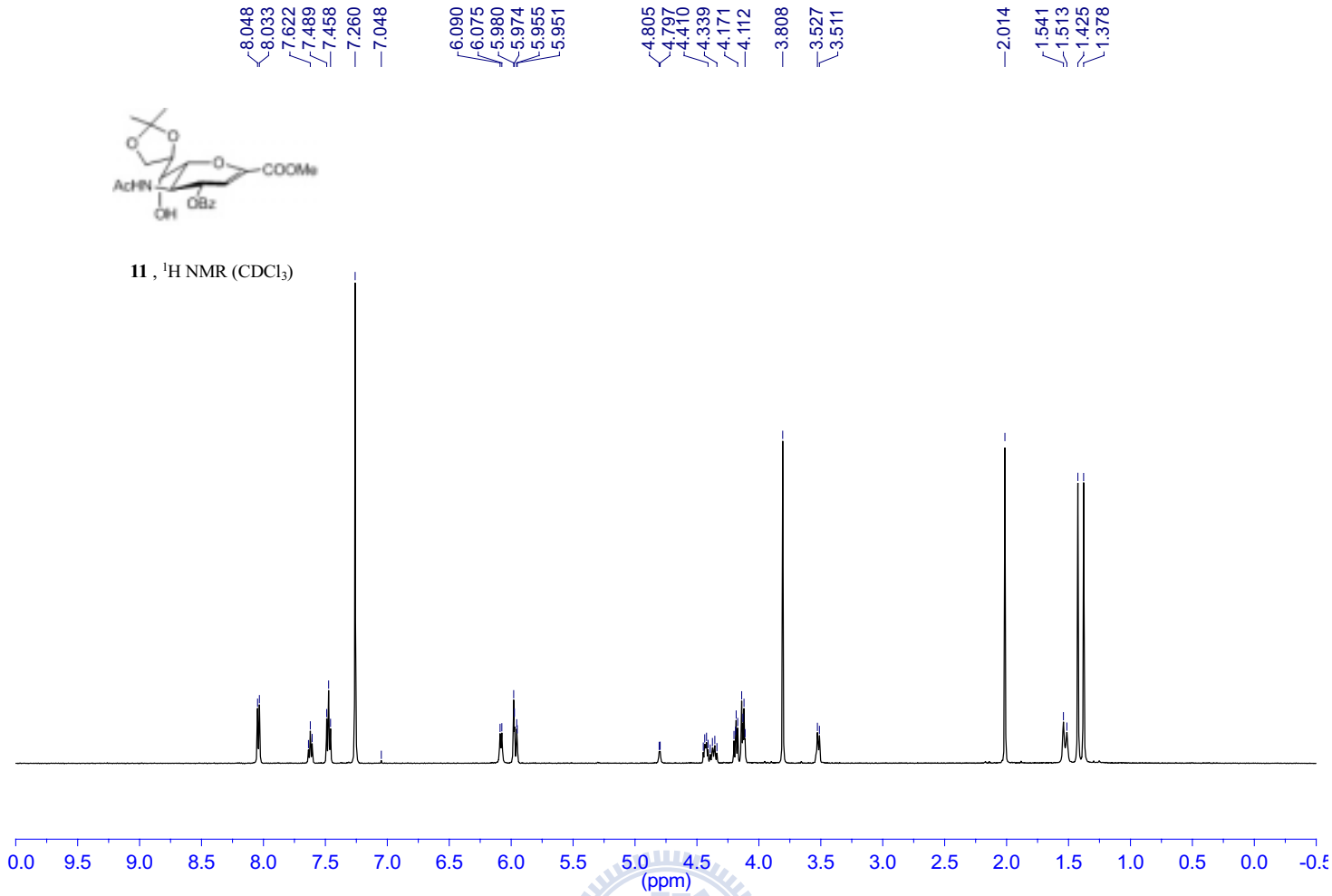
(11) Garegg, P. J.; Maron, L. *Acta Chem. Scand., Ser. B* **1979**, *B33*, 39-41.

(12) Mouton, C.; Tillequin, F.; Seguin, E.; Monneret, C. *J. Chem. Soc., Perkin Trans. 1* **1998**,

2055-2060.

APPENDIX

Spectra list of the synthesized compounds



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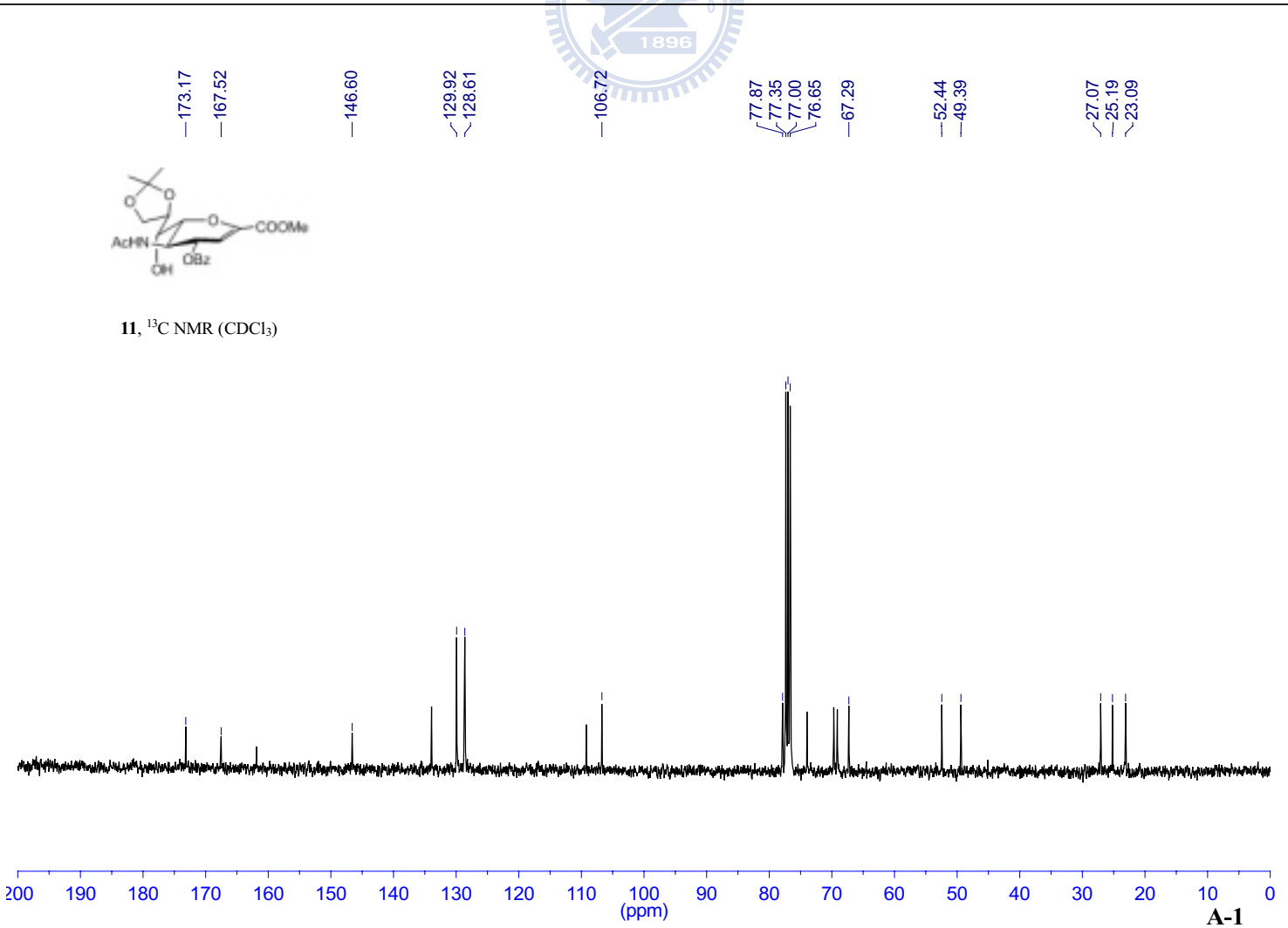

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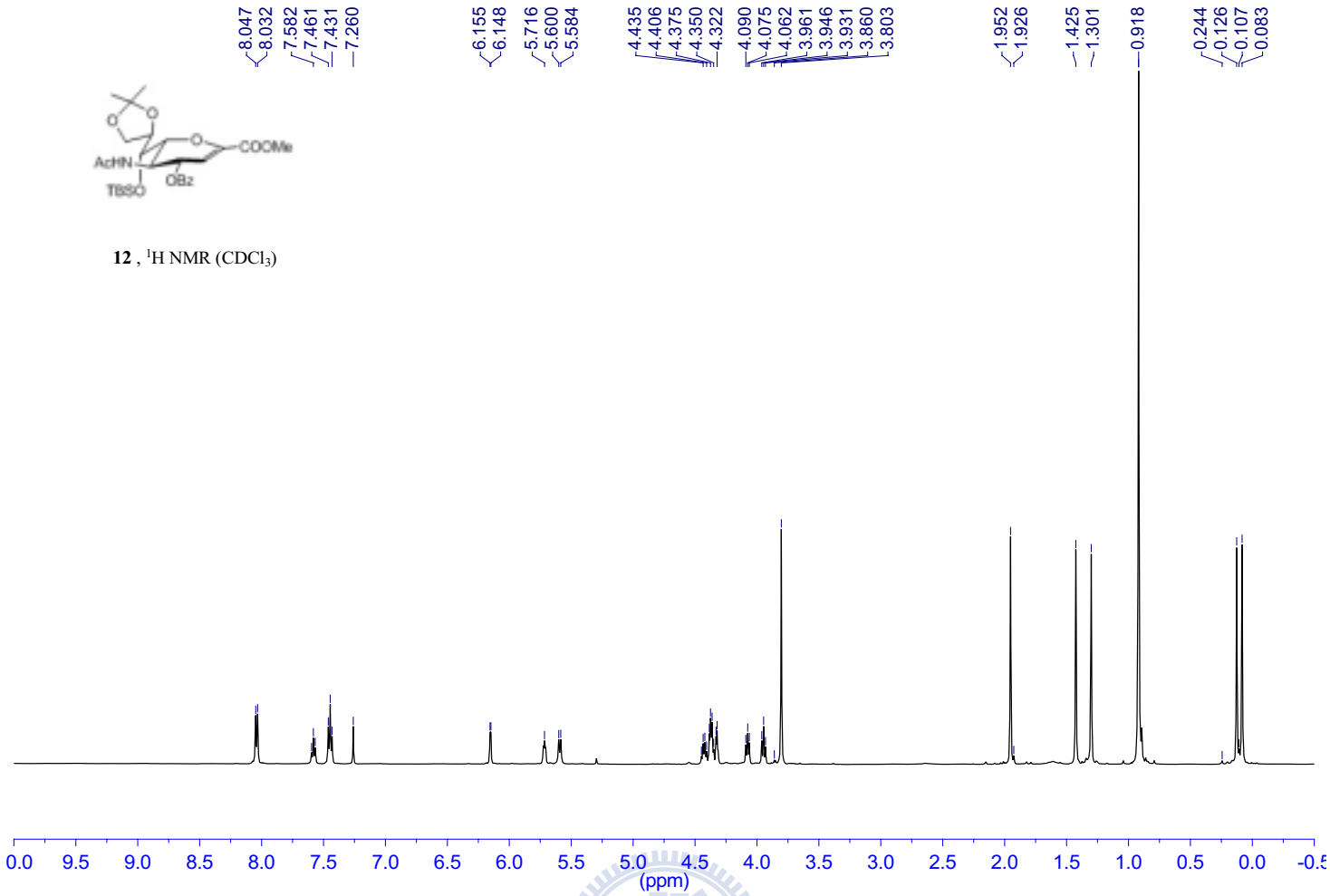

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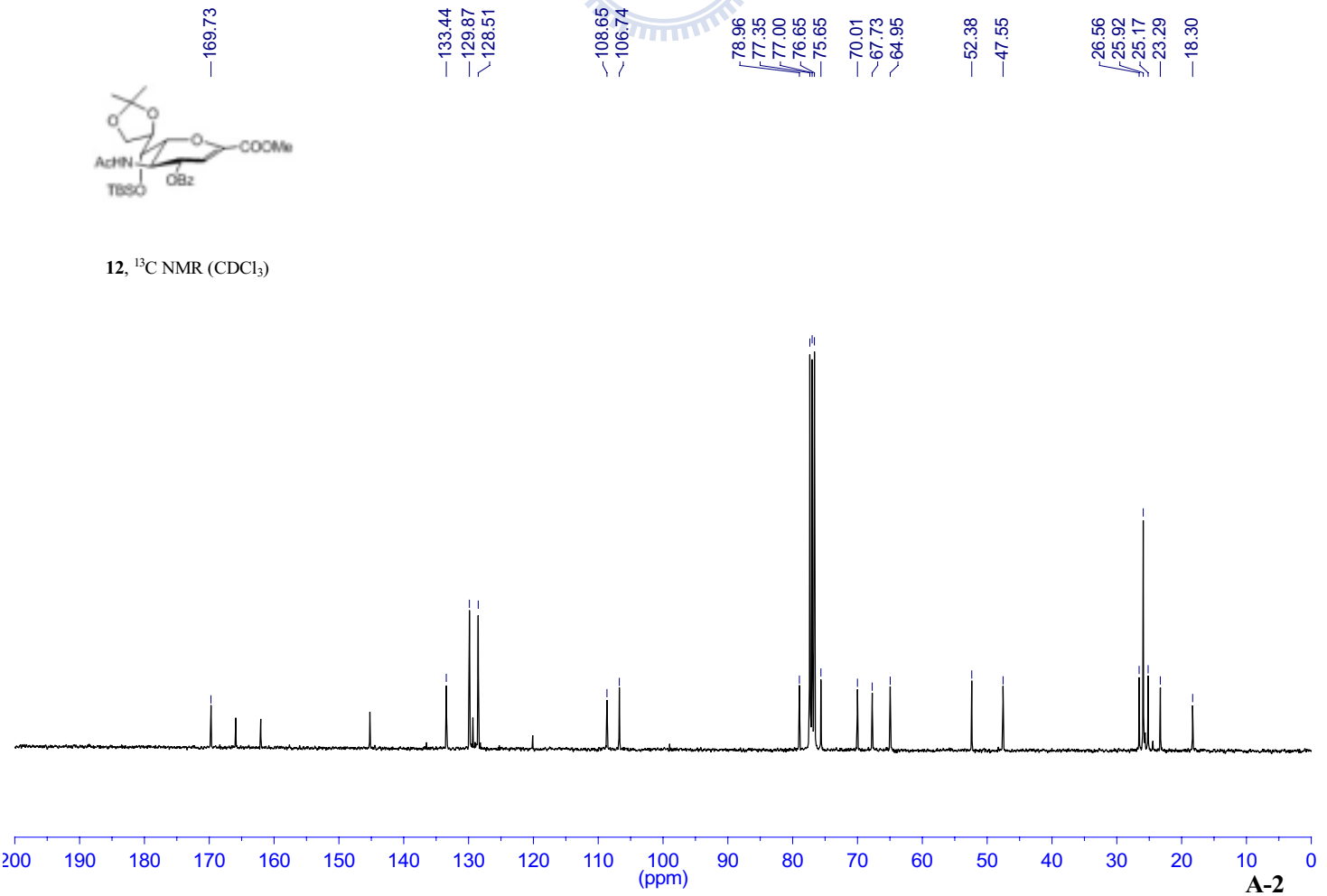

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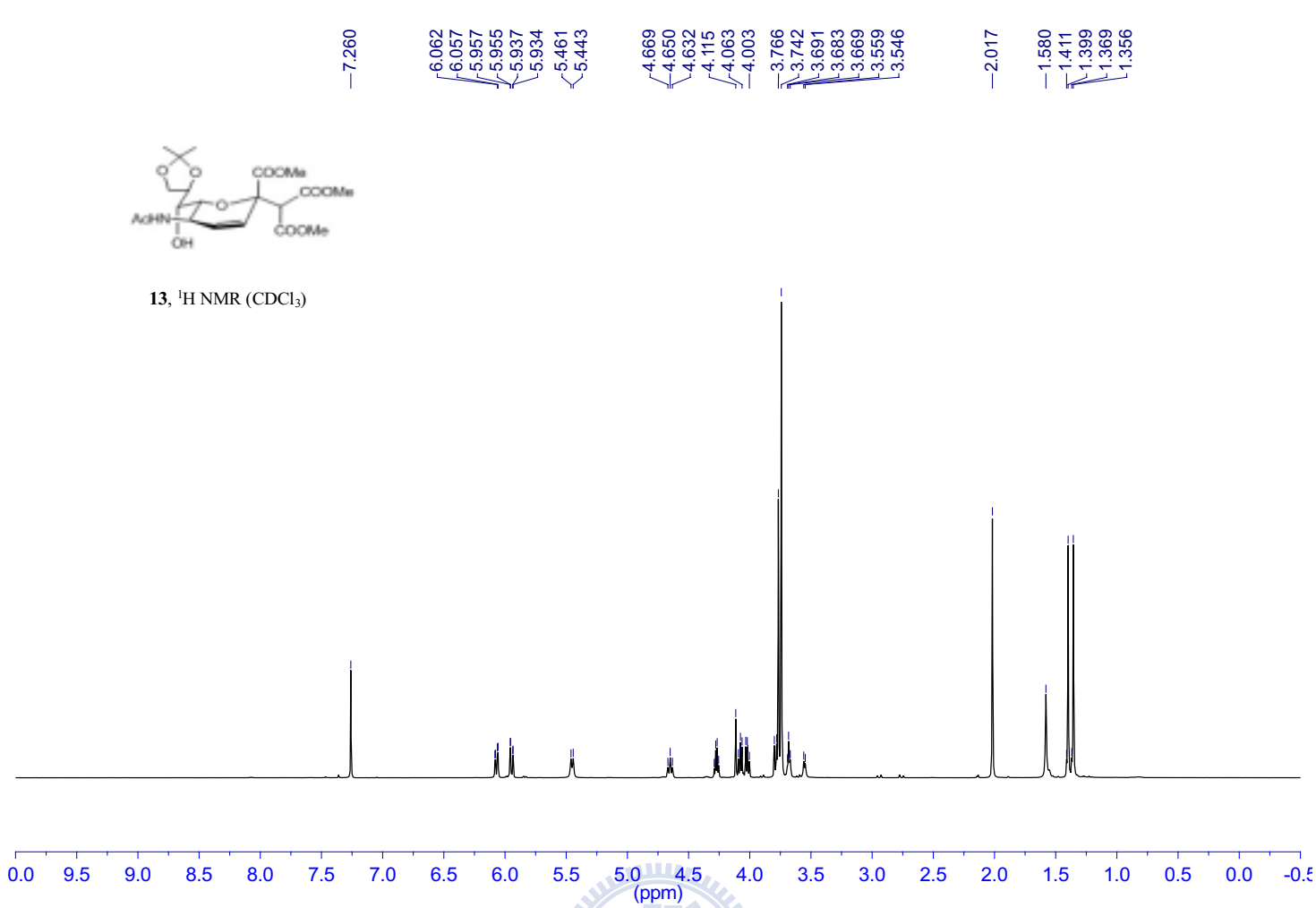

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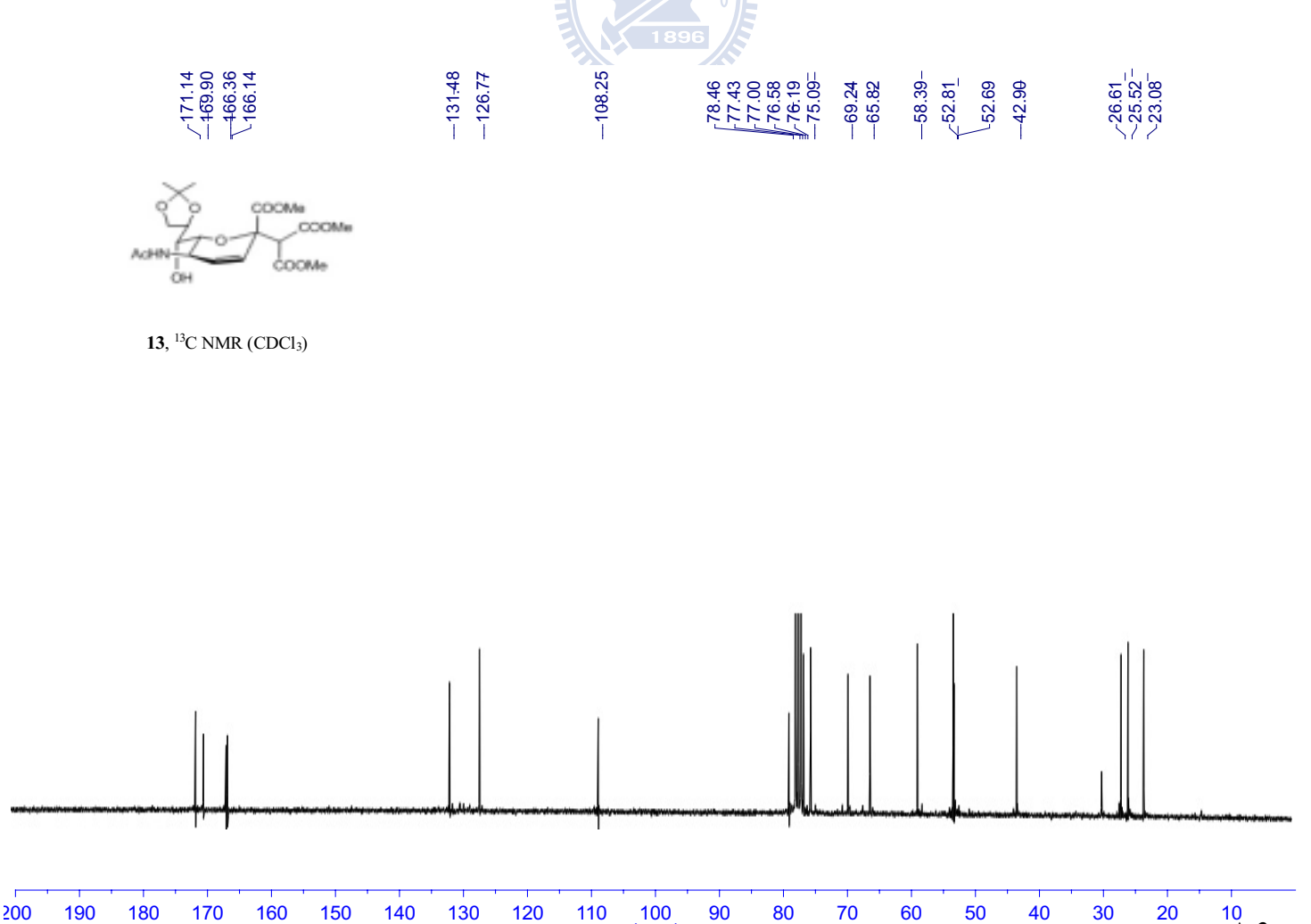

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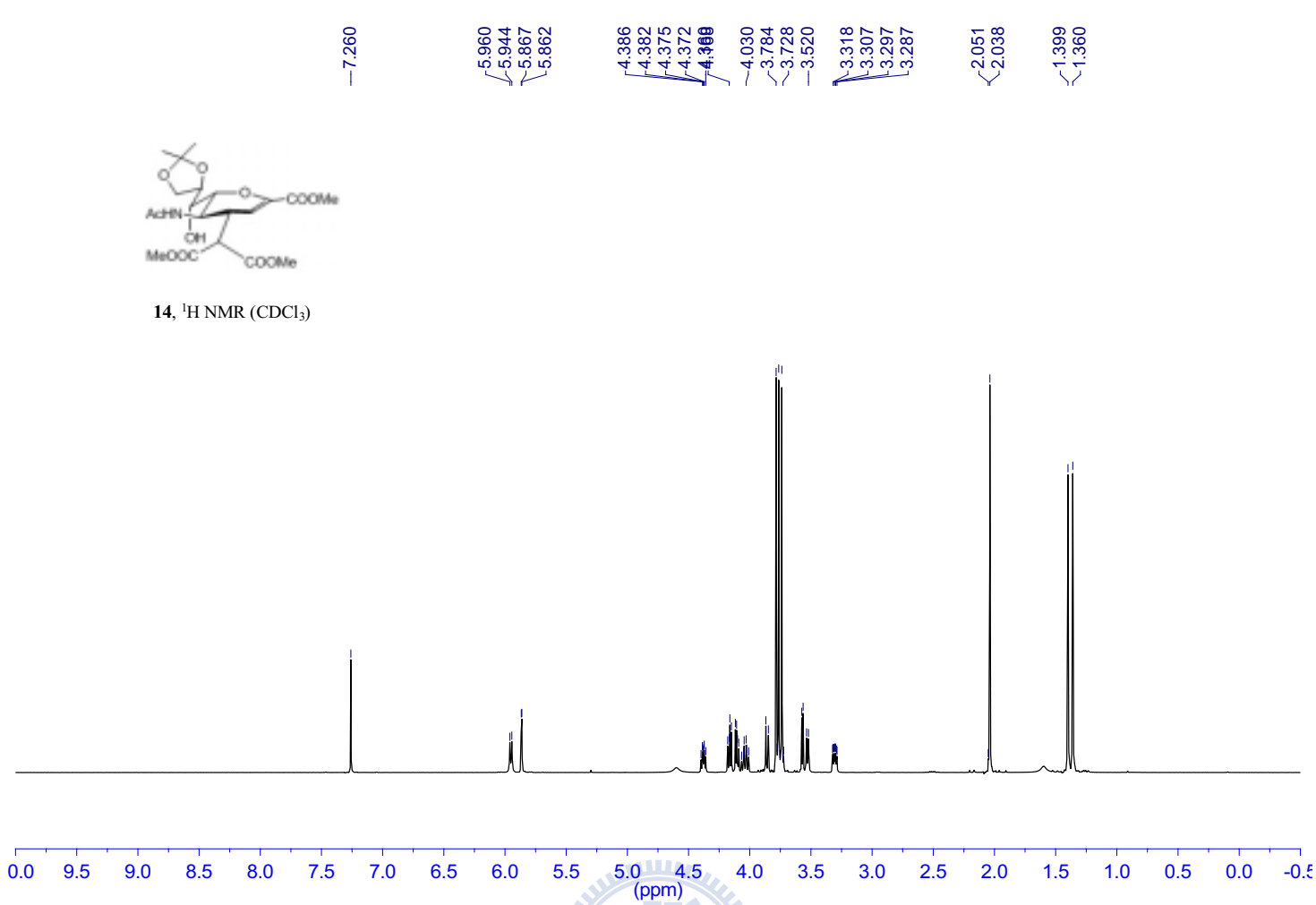

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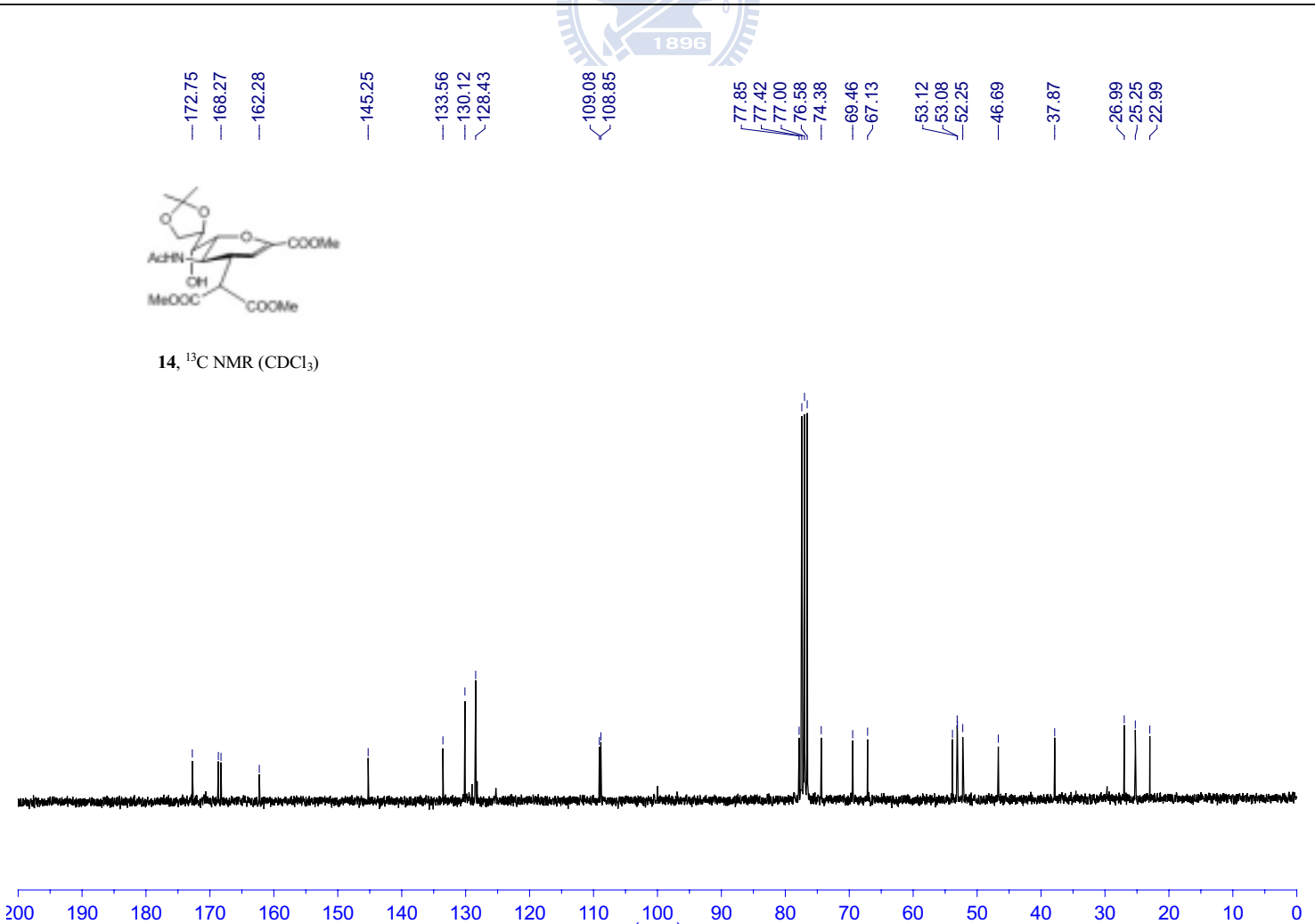

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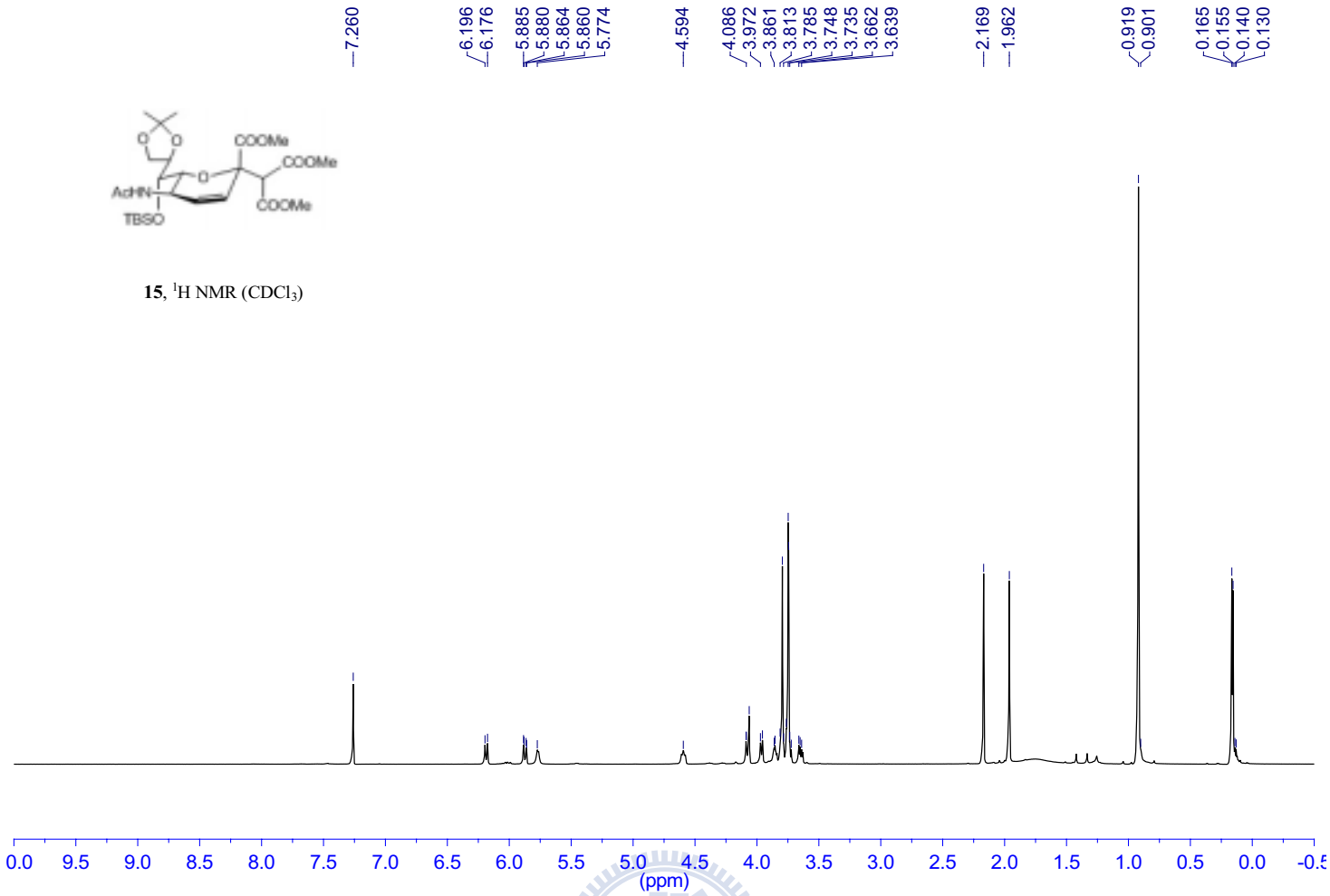

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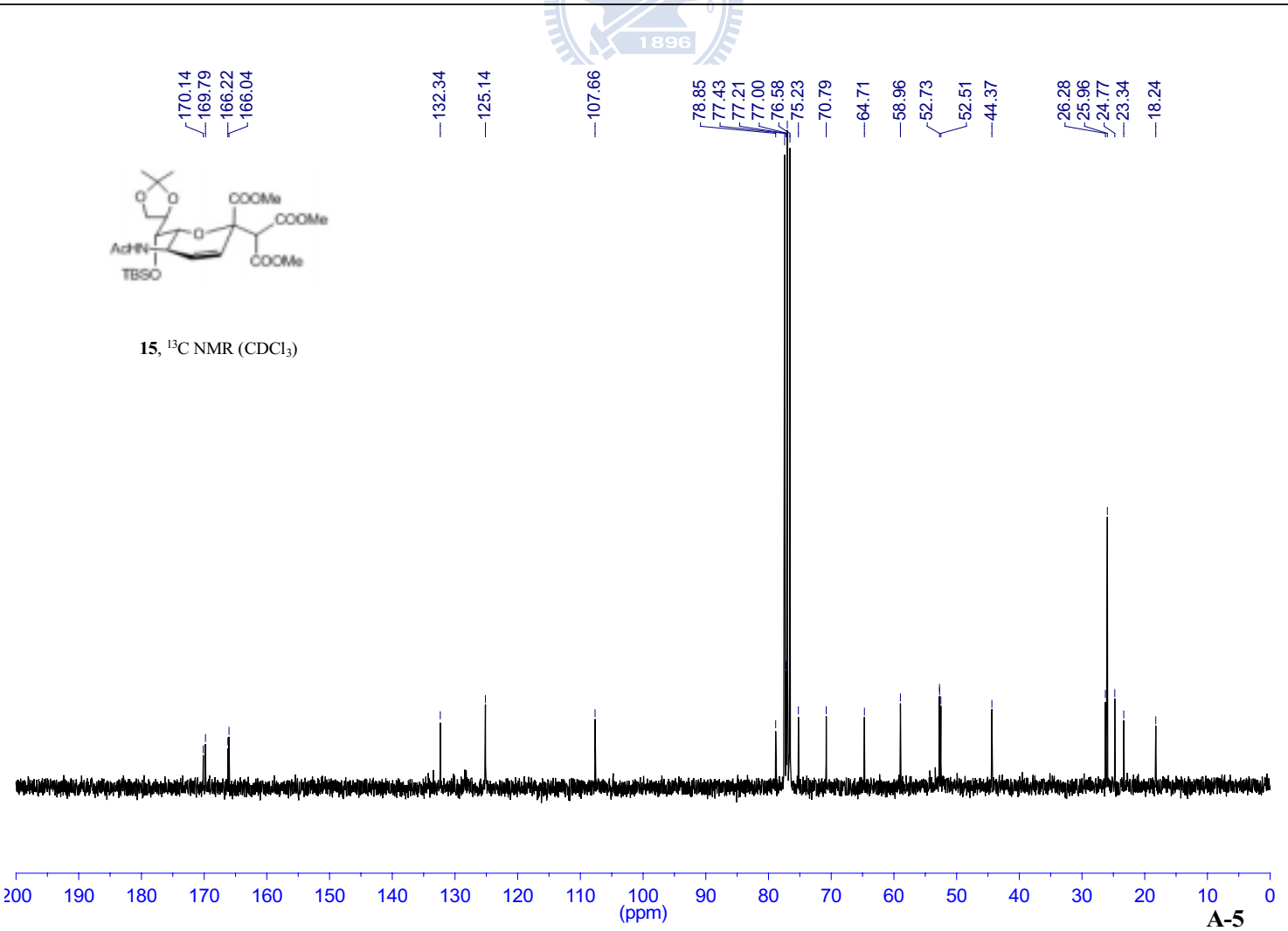

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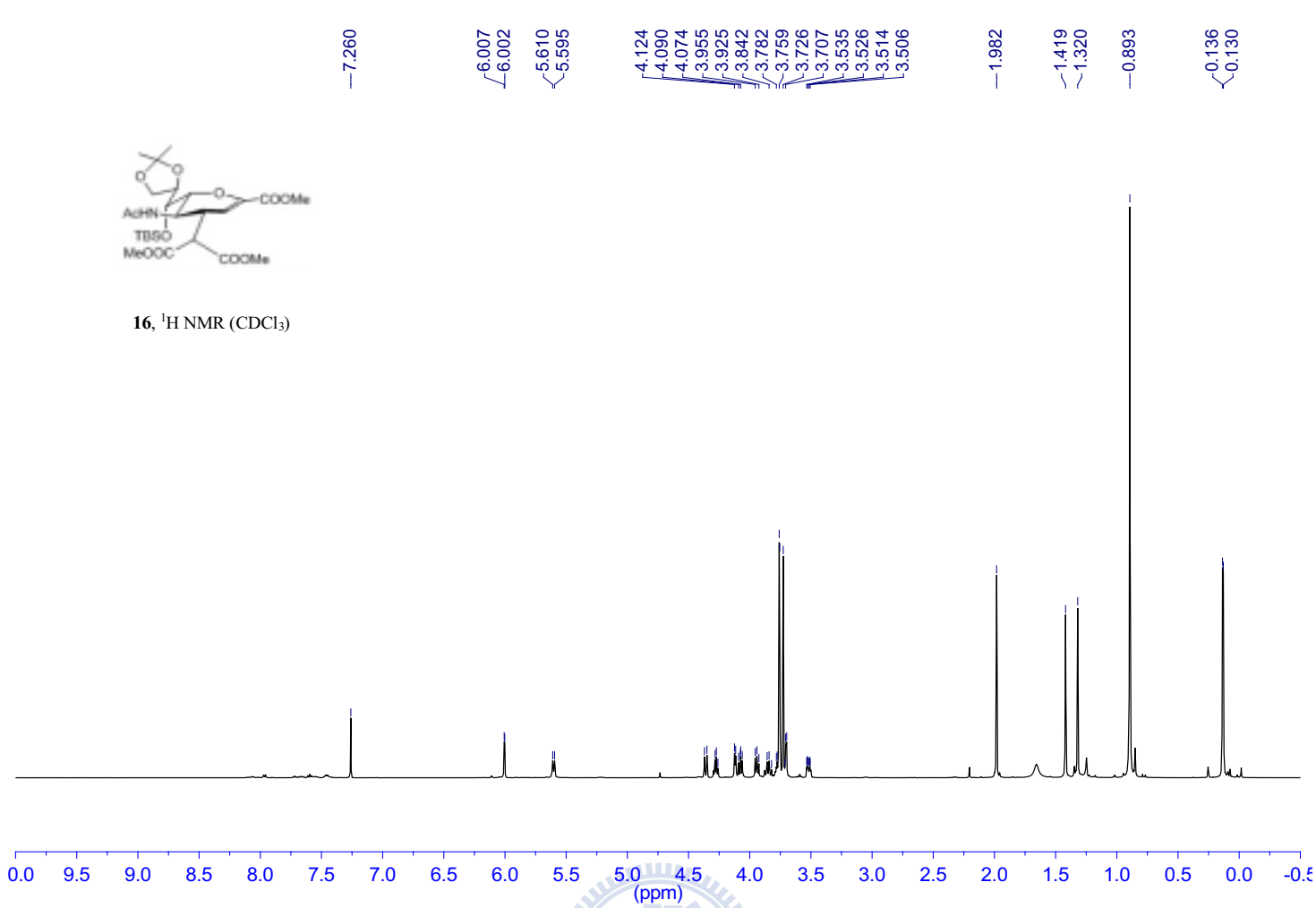

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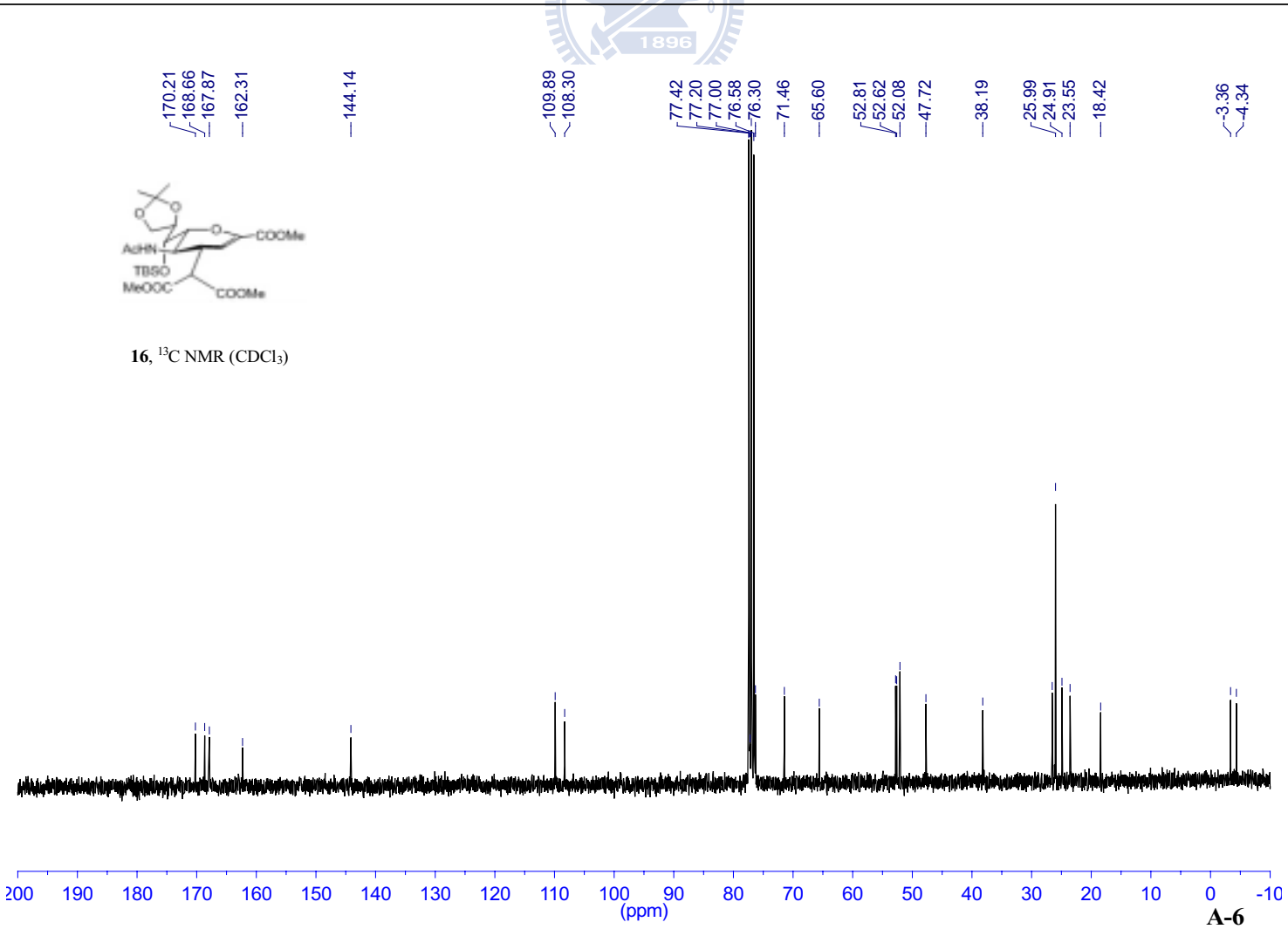

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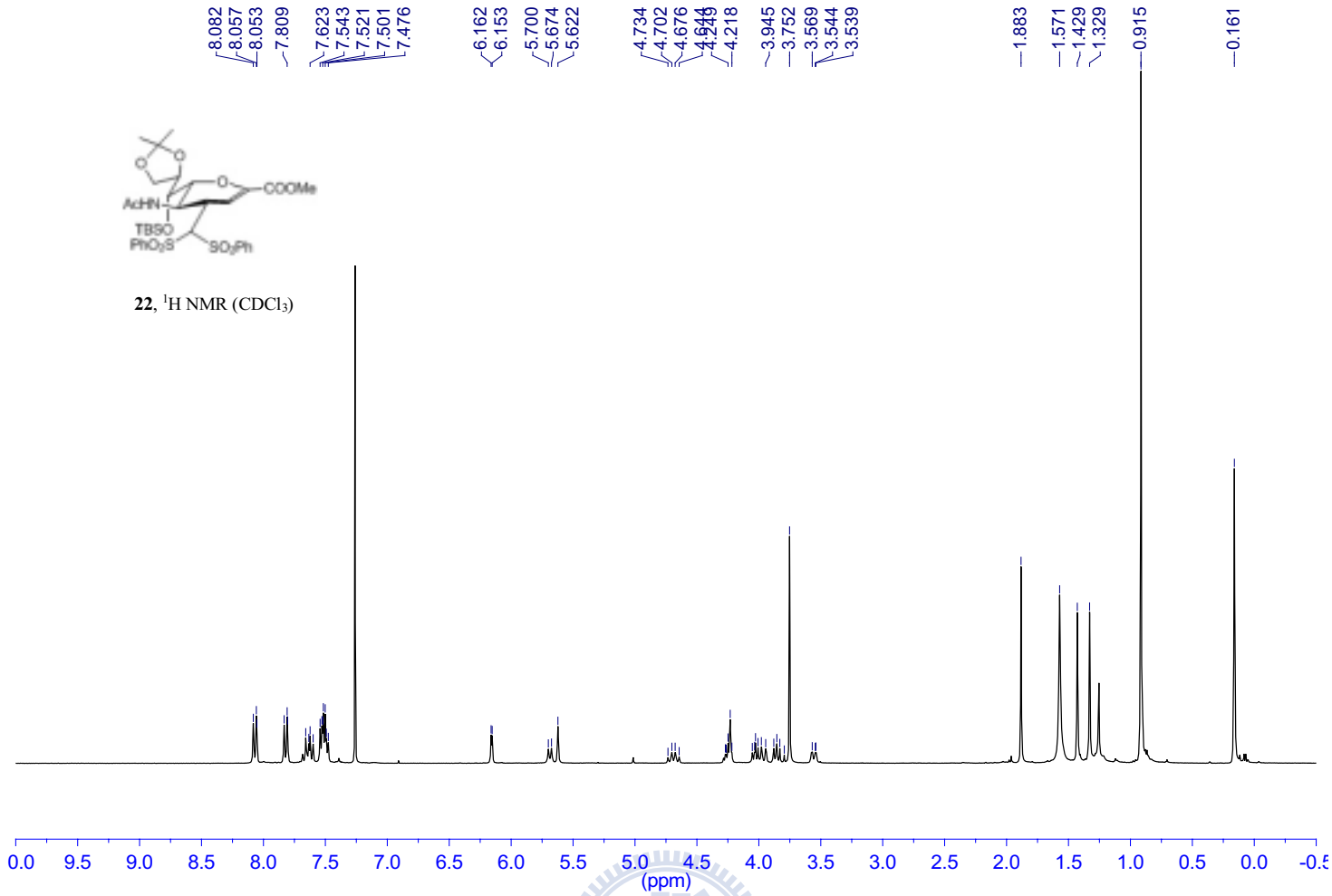

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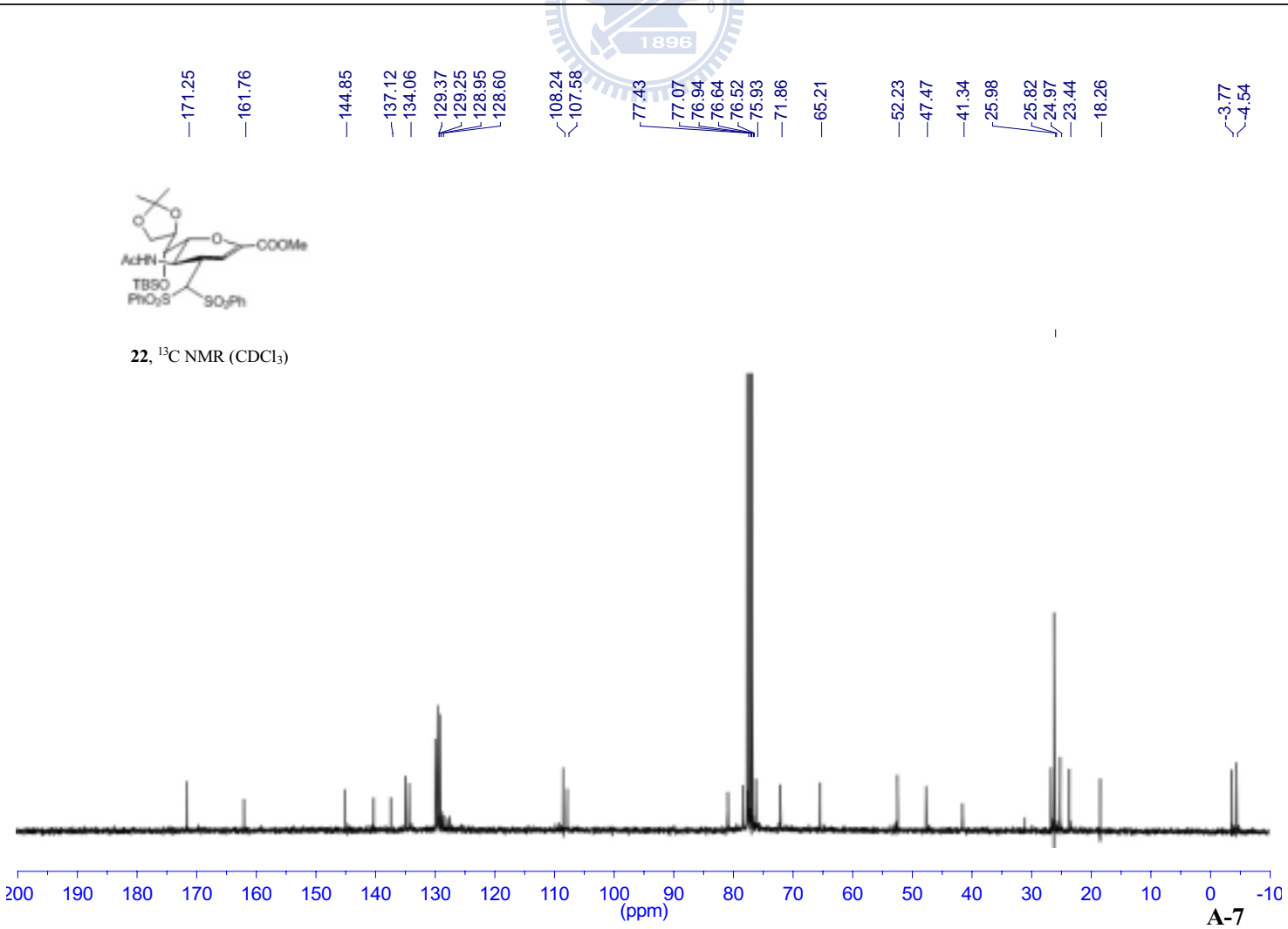

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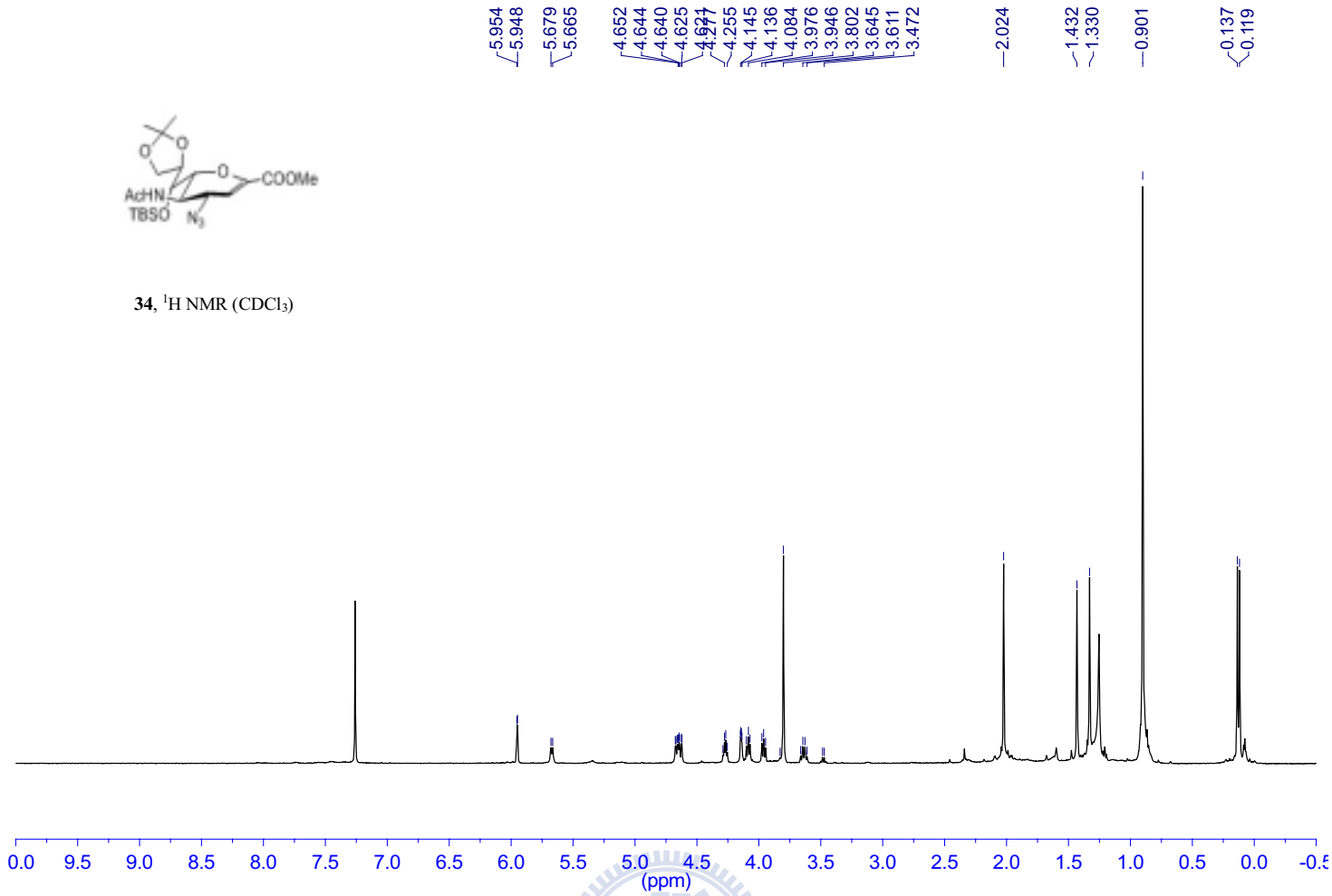

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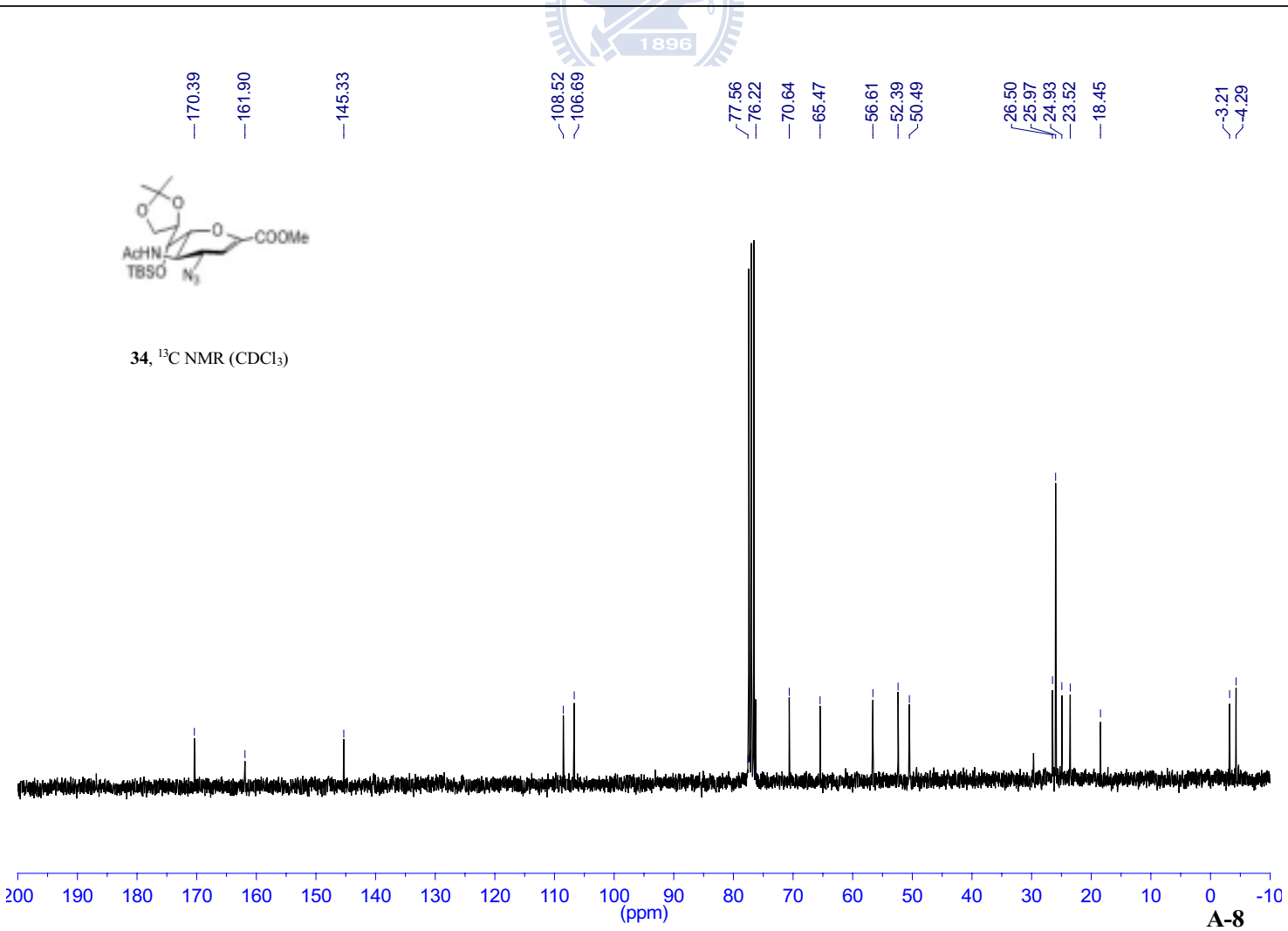

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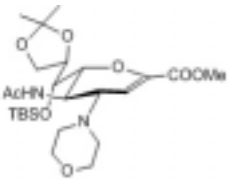

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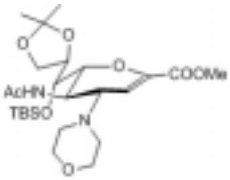

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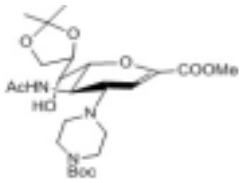

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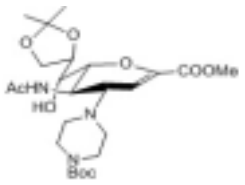
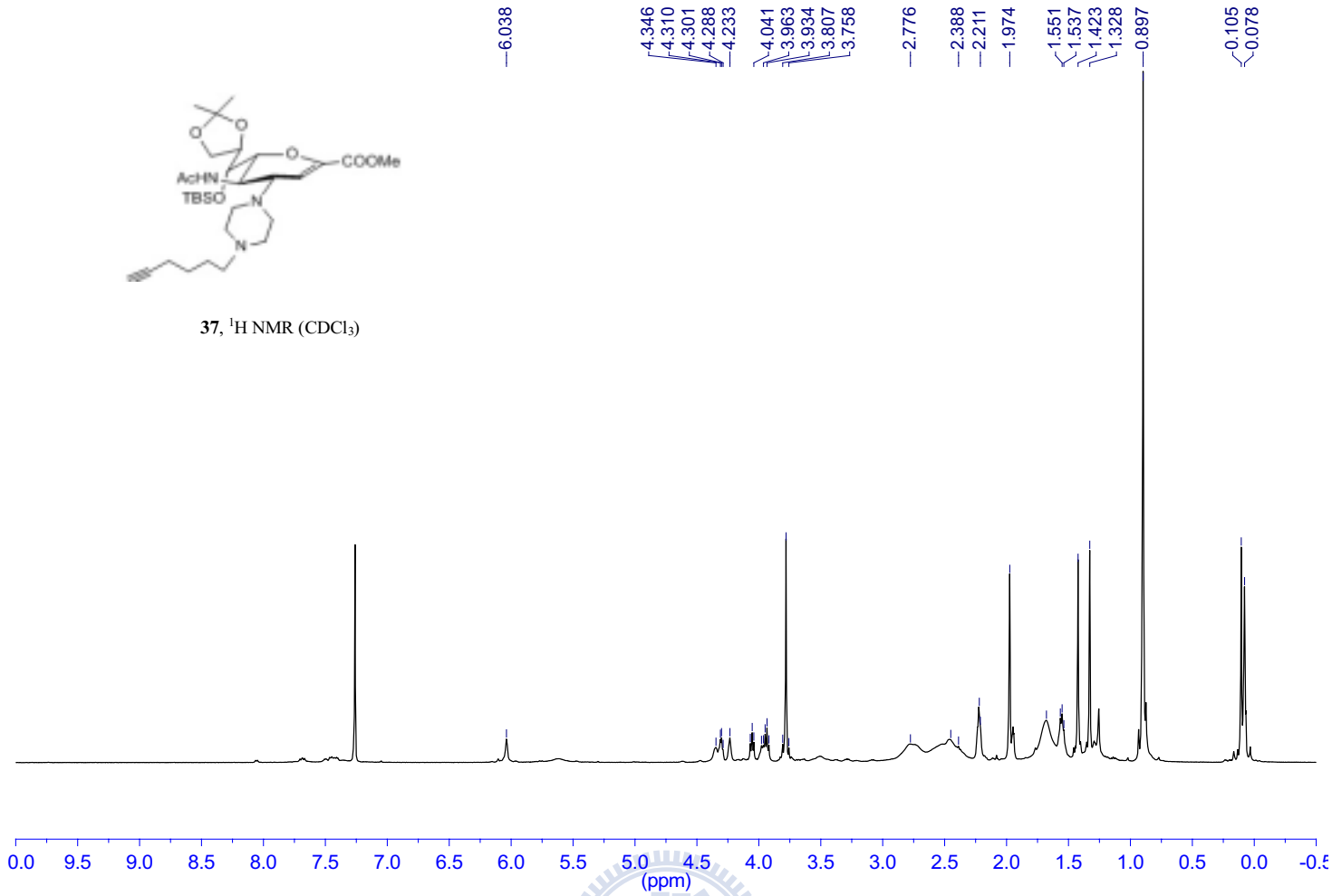


36-TBS, ^{13}C NMR ($CDCl_3$)



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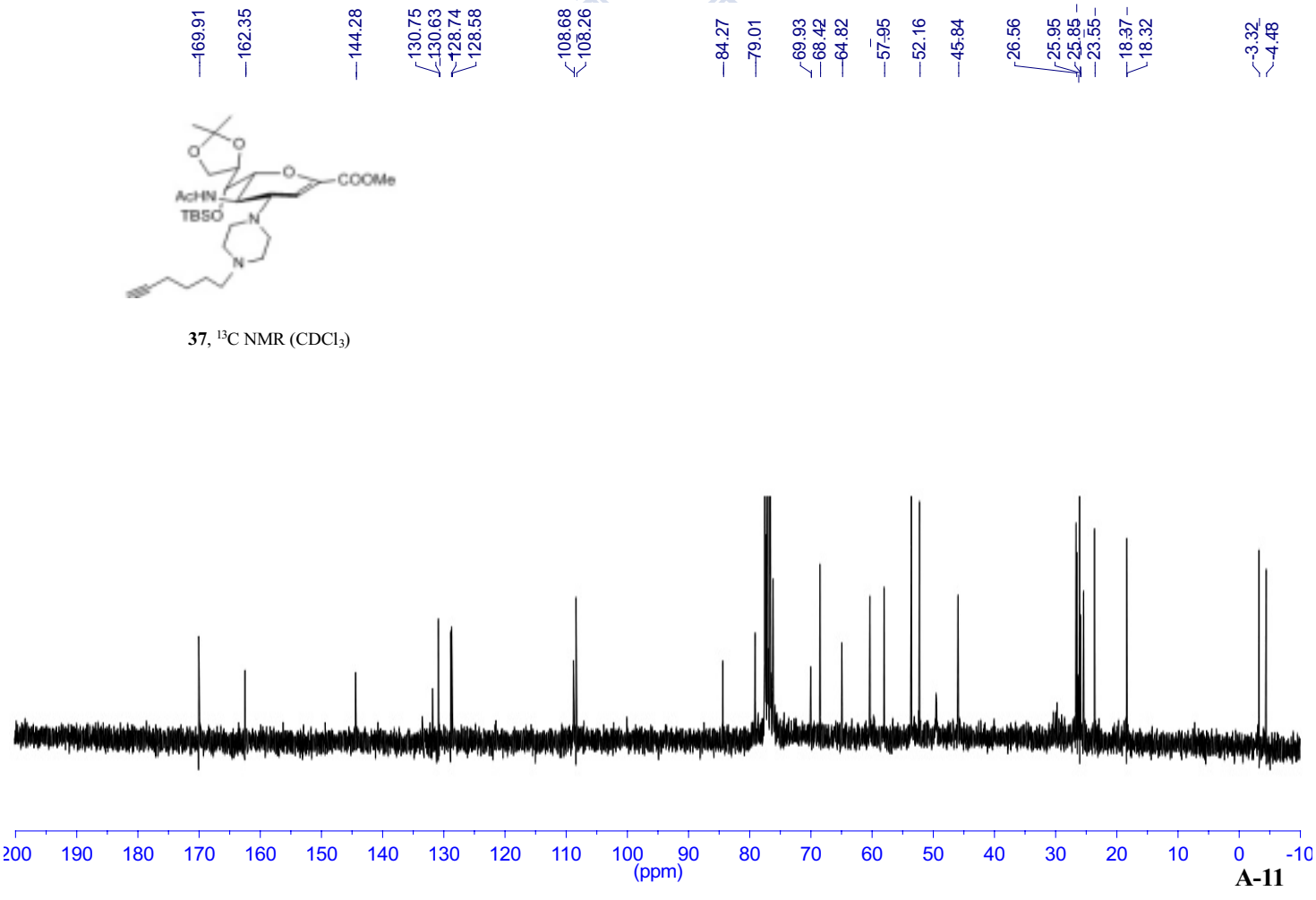

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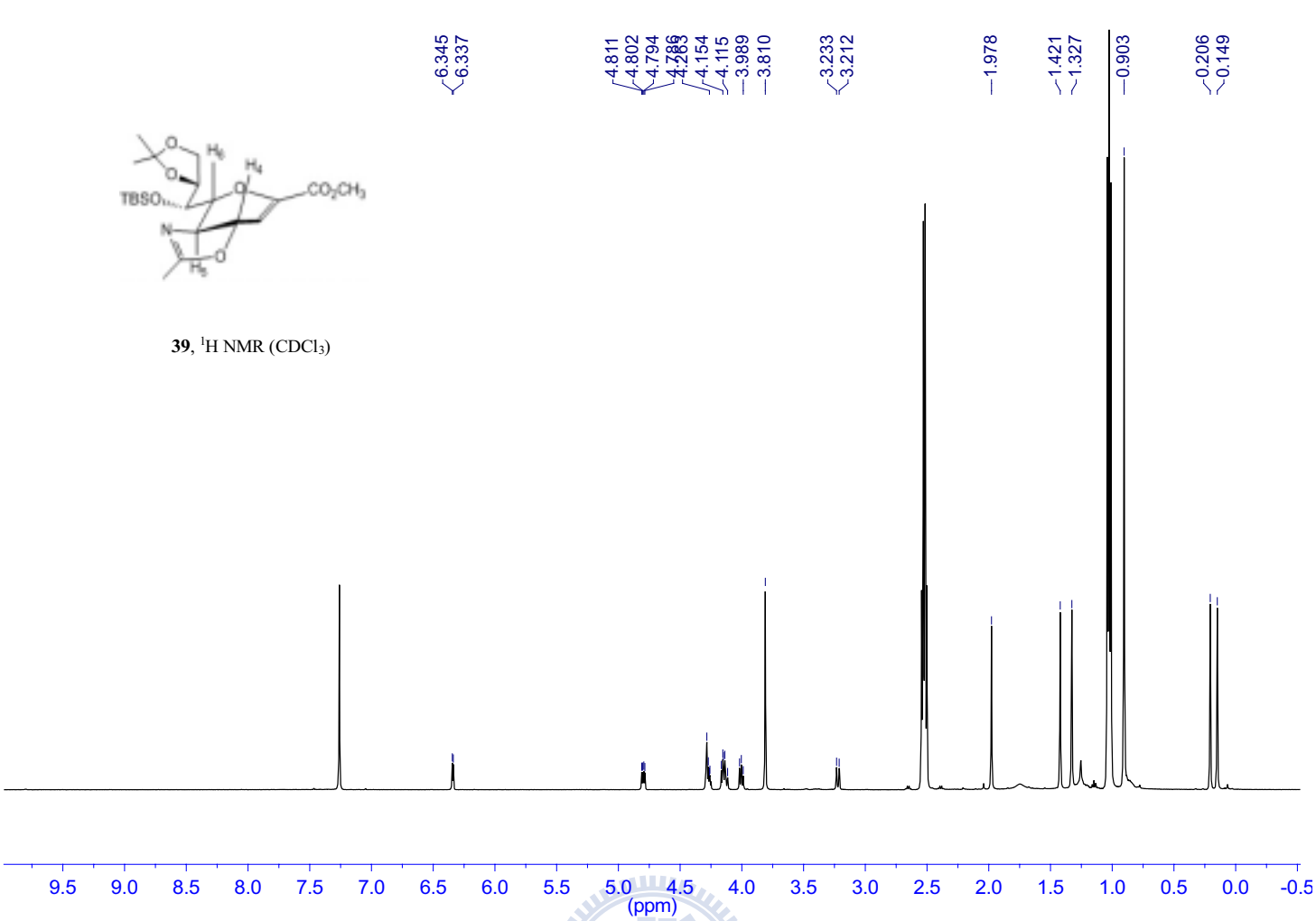

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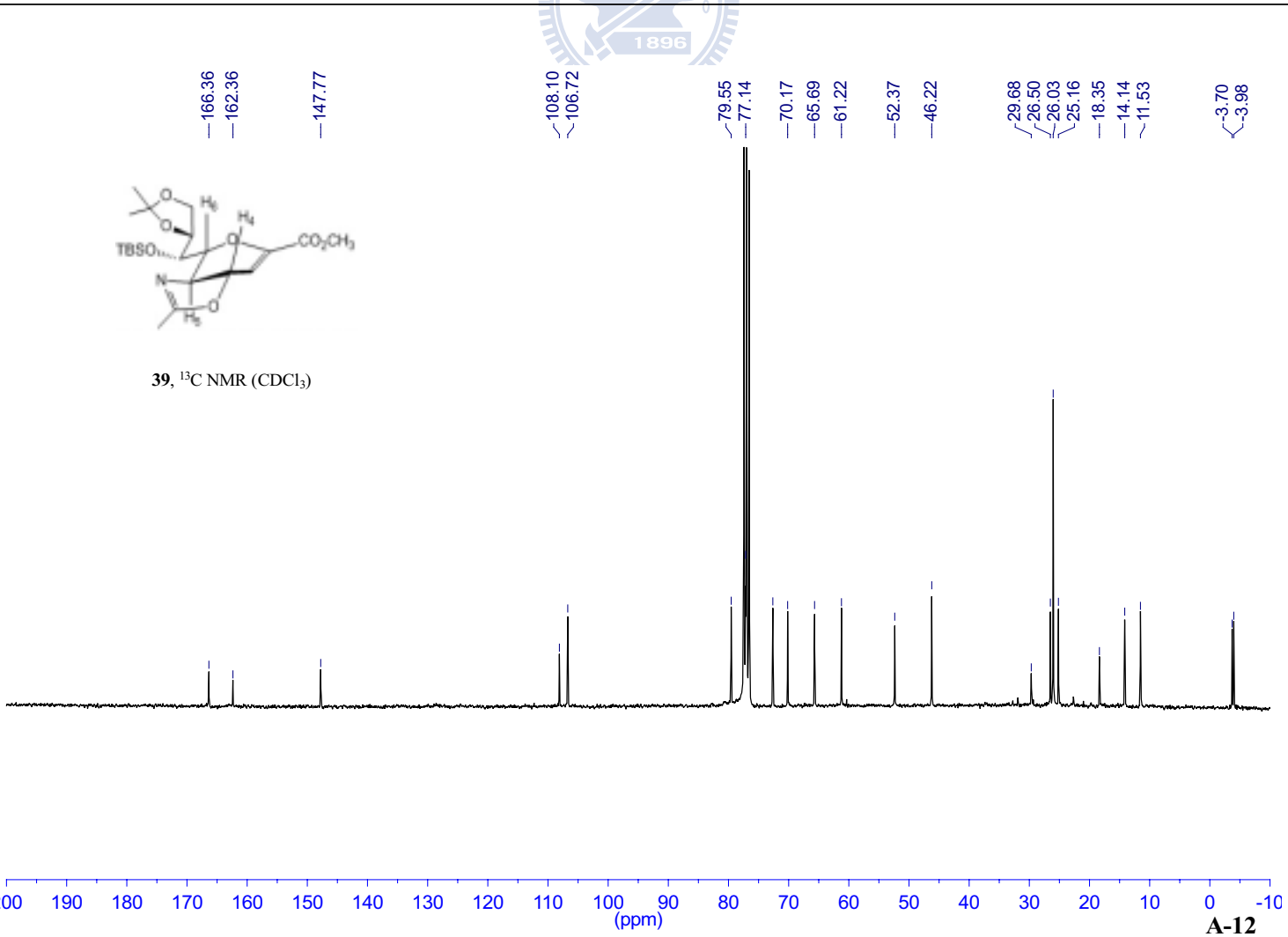

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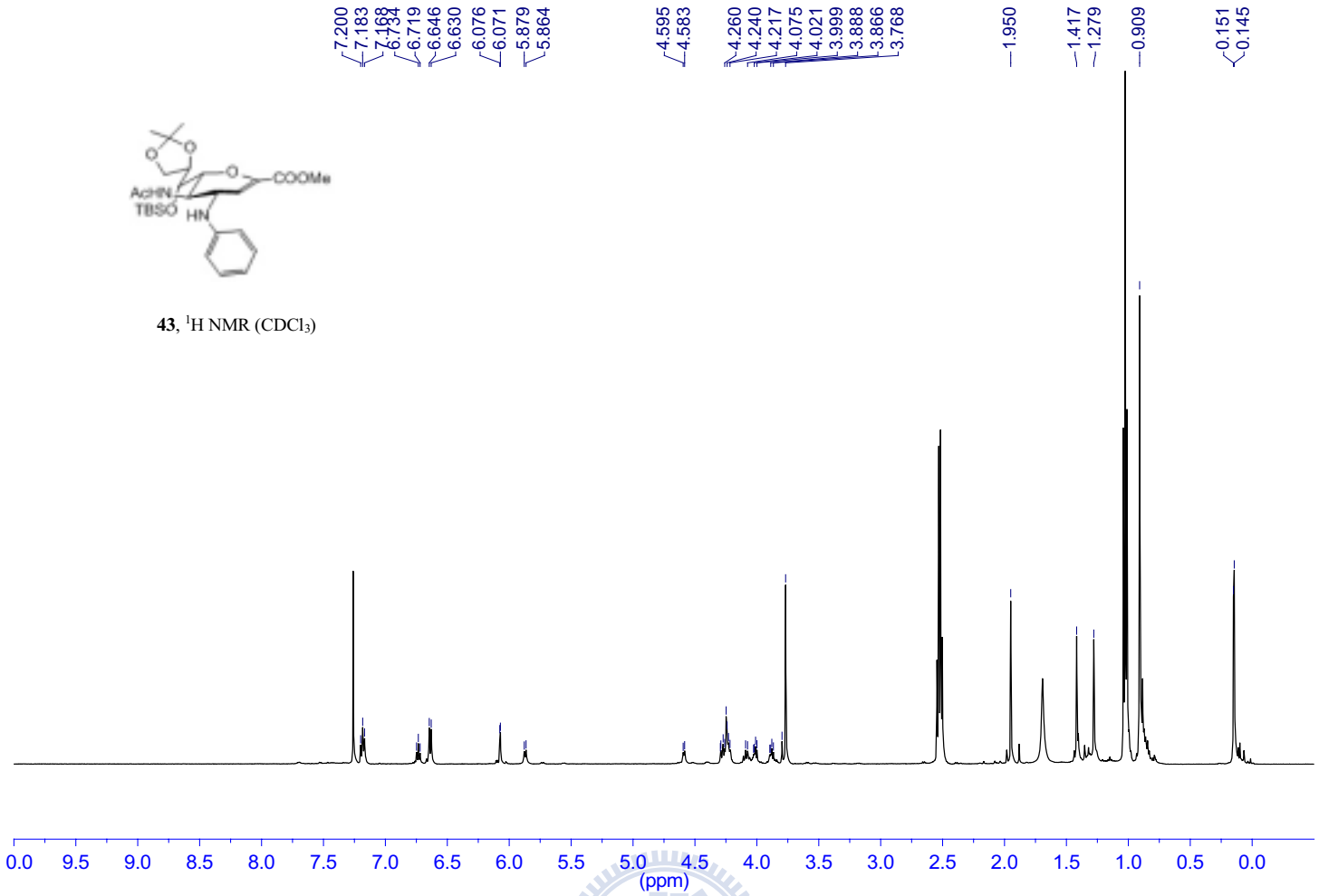

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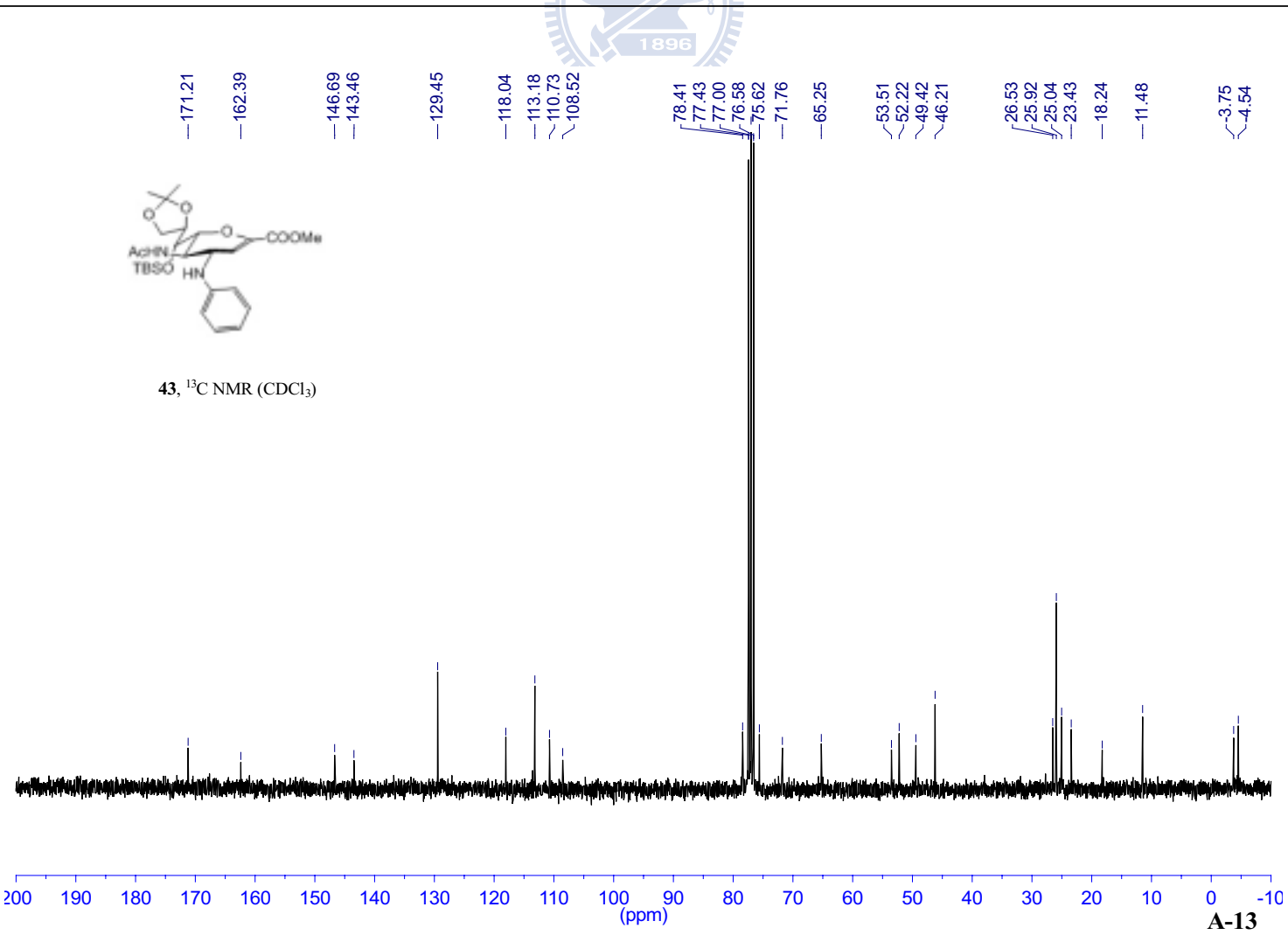

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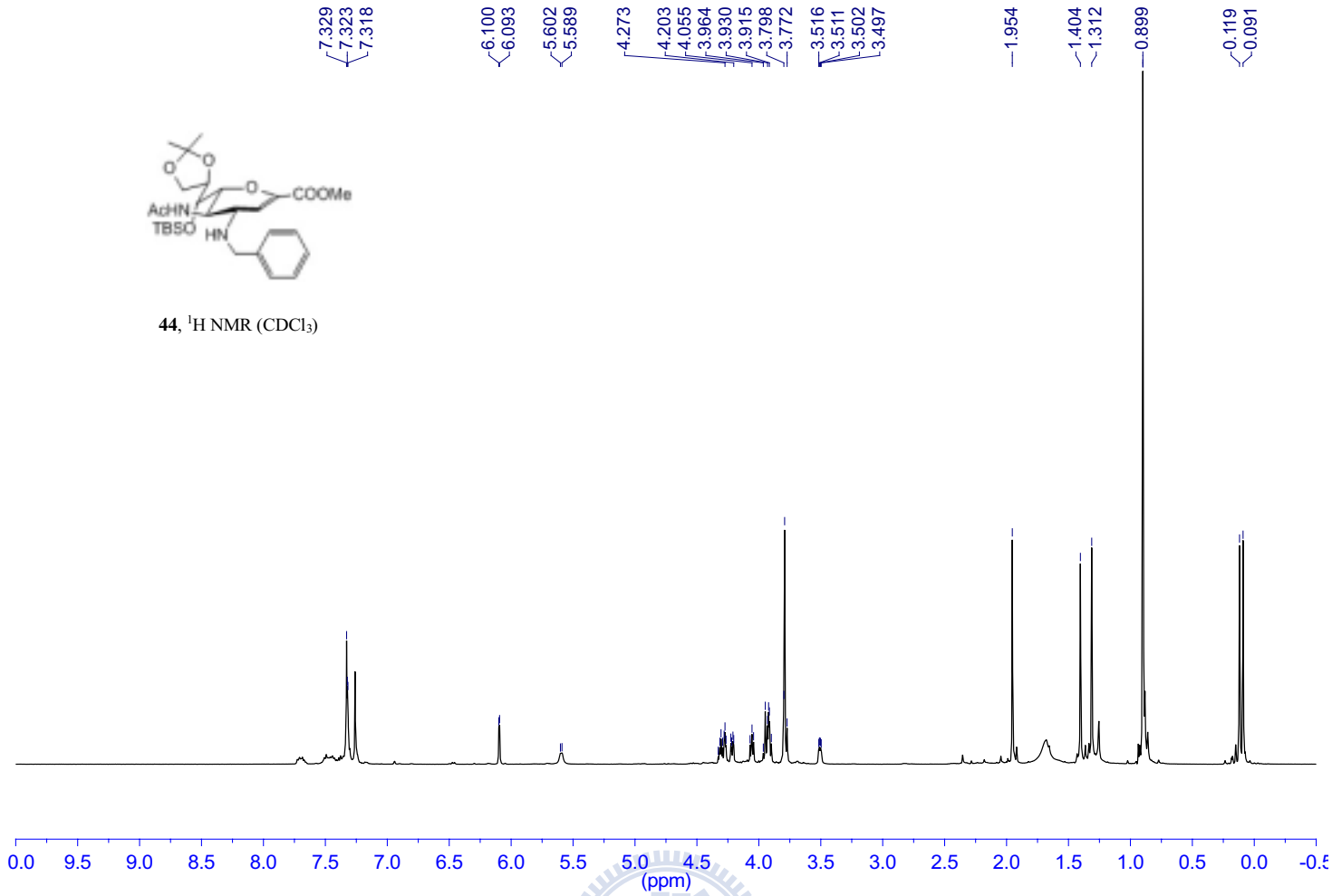

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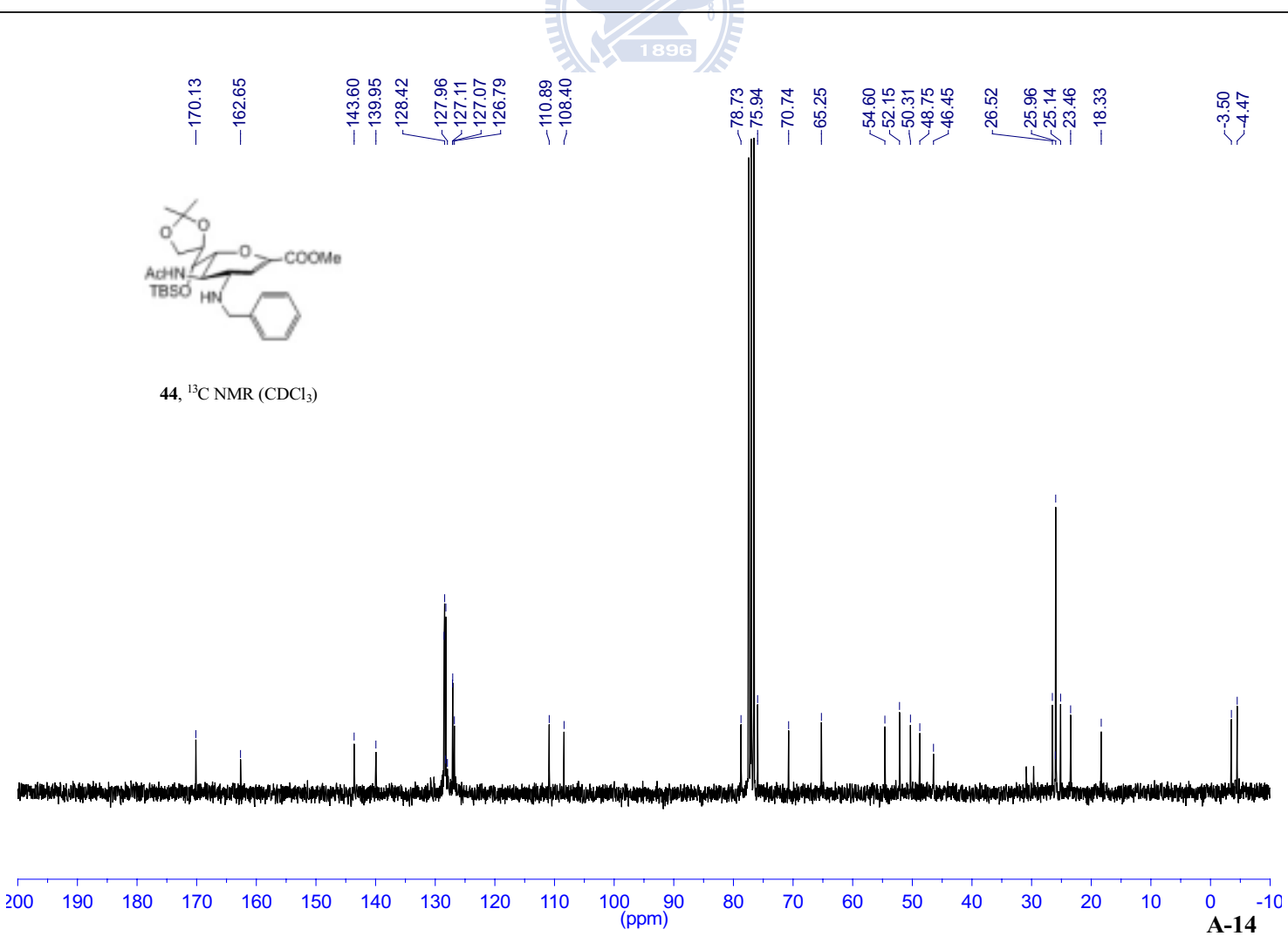

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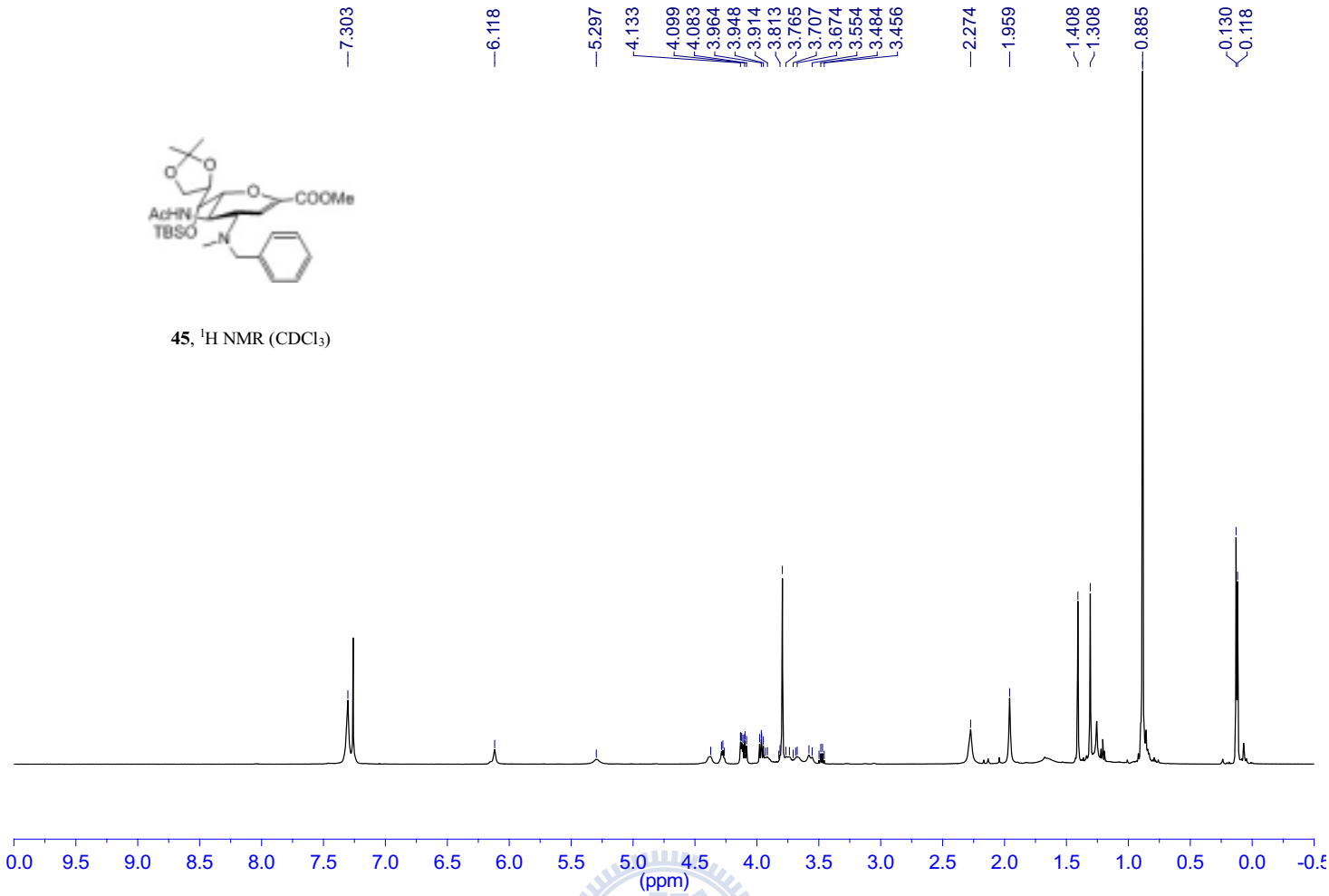
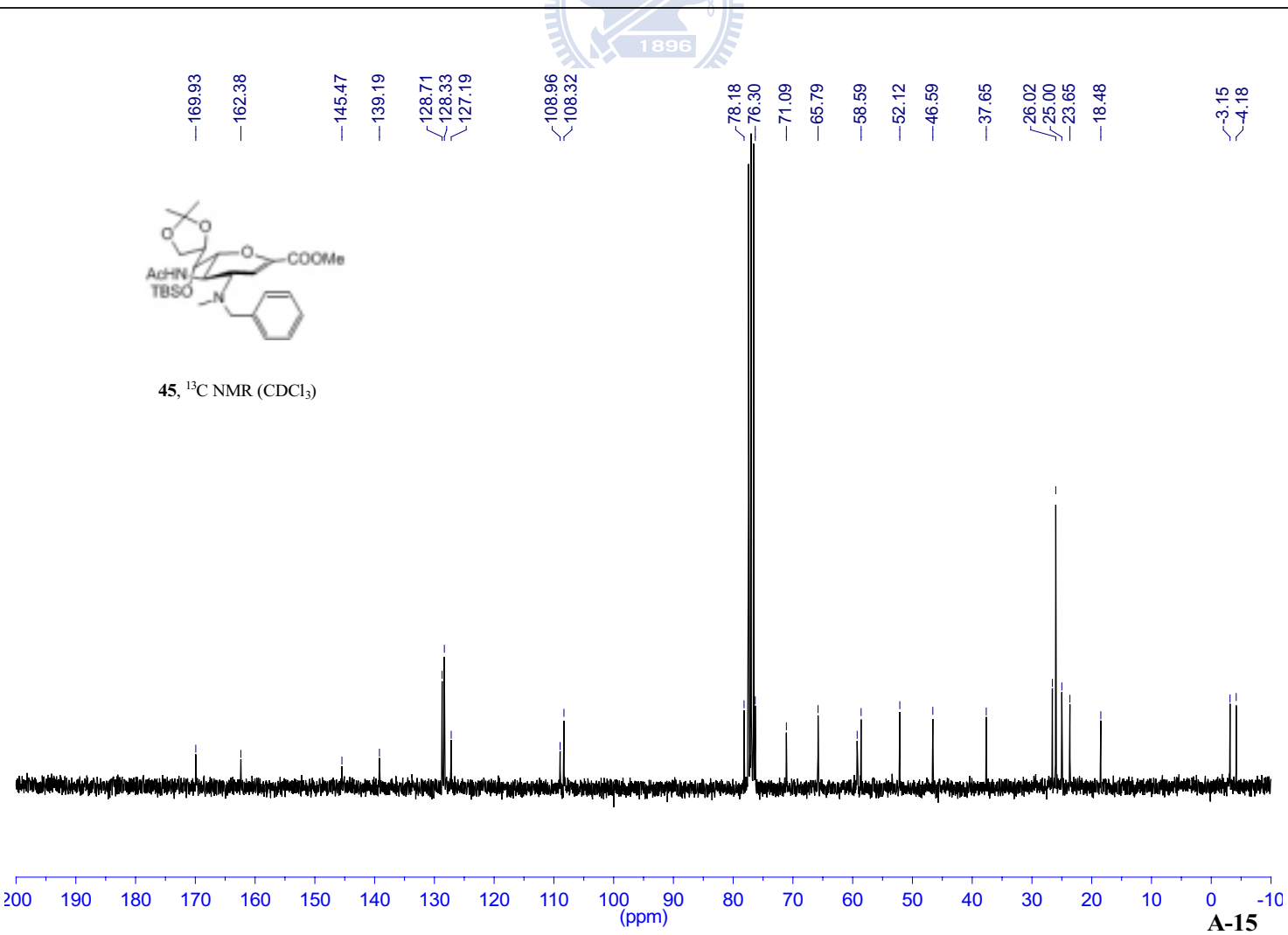
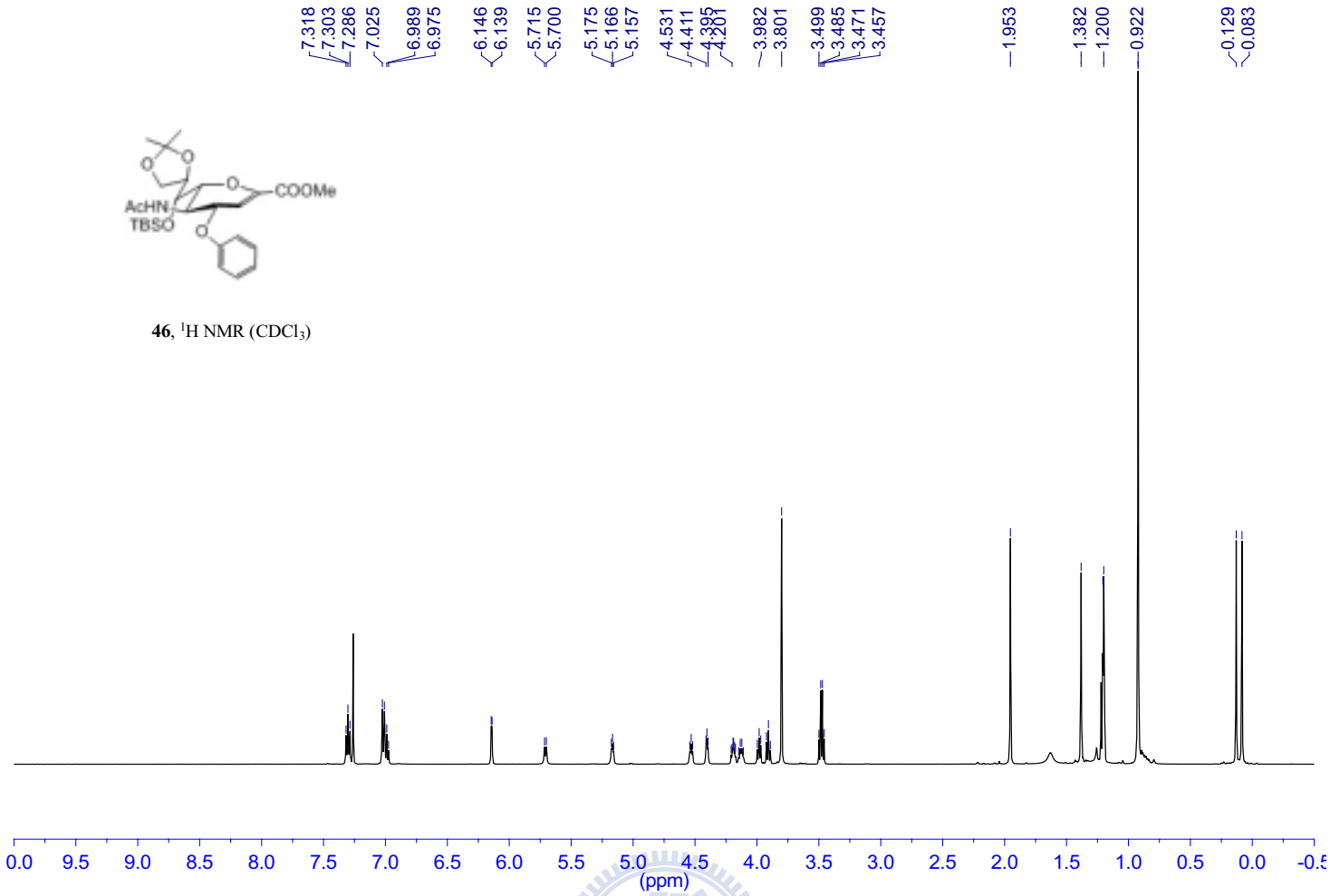


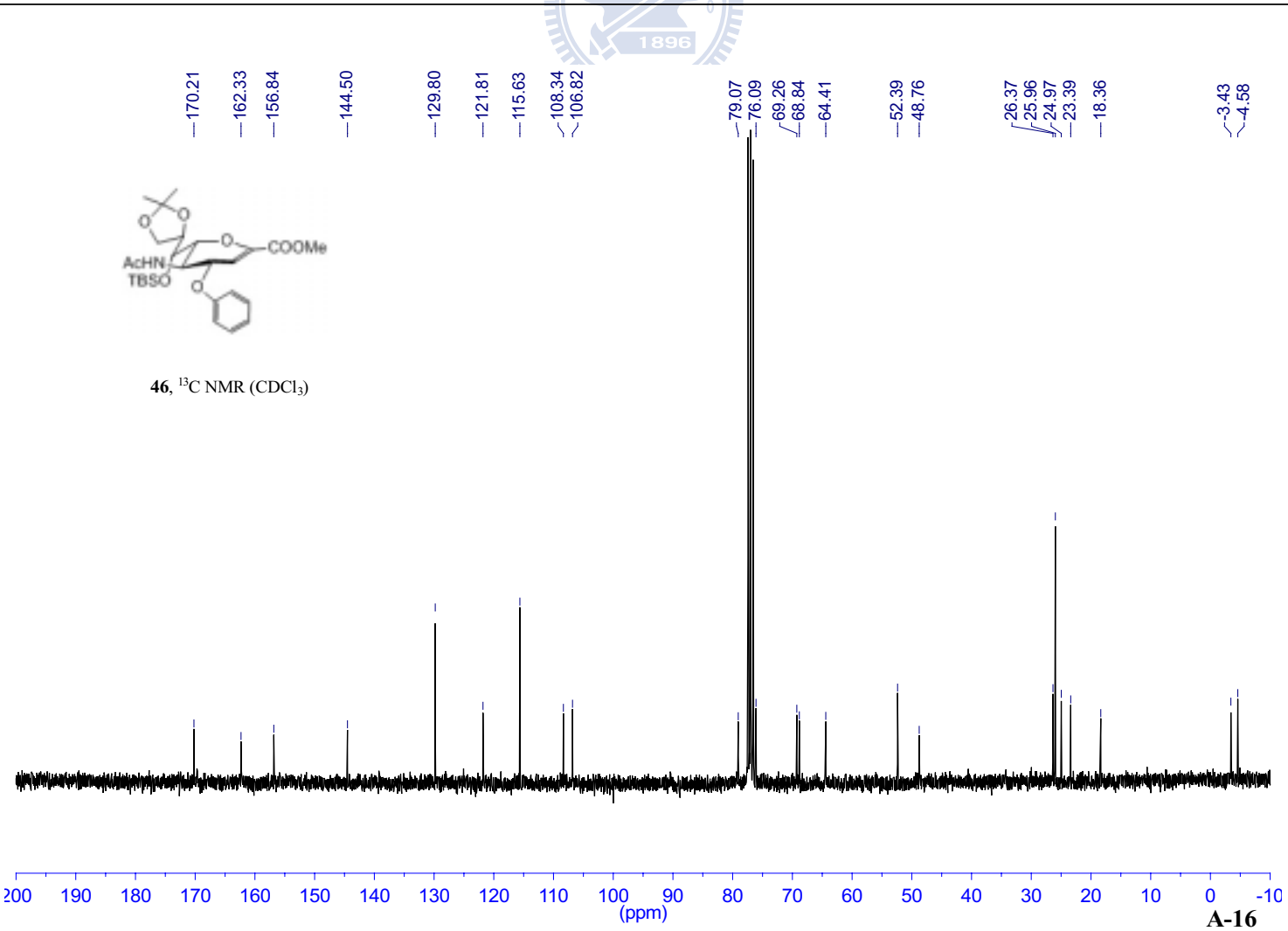
45, ^{1}H NMR ($CDCl_3$)



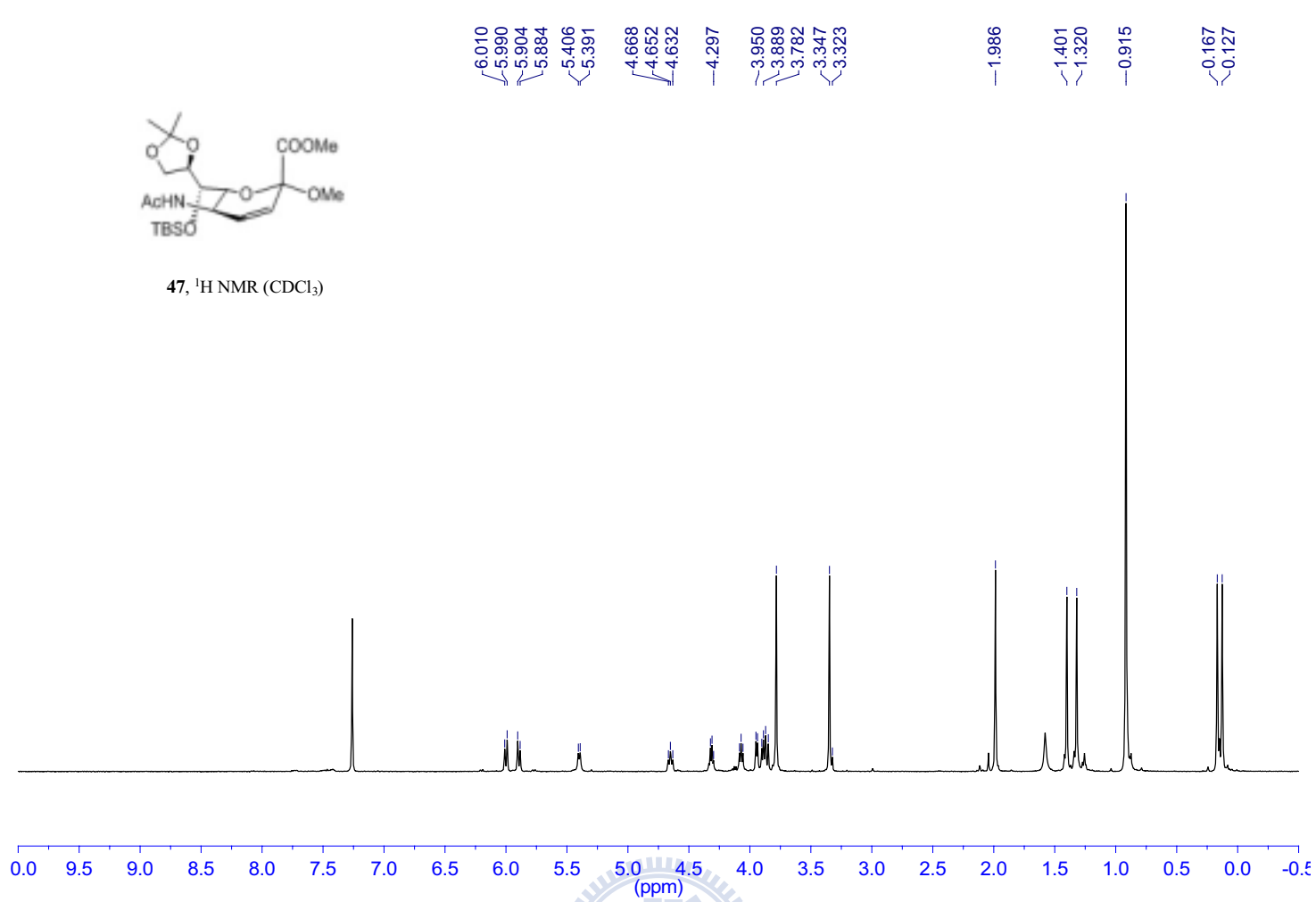
45, ^{13}C NMR ($CDCl_3$)



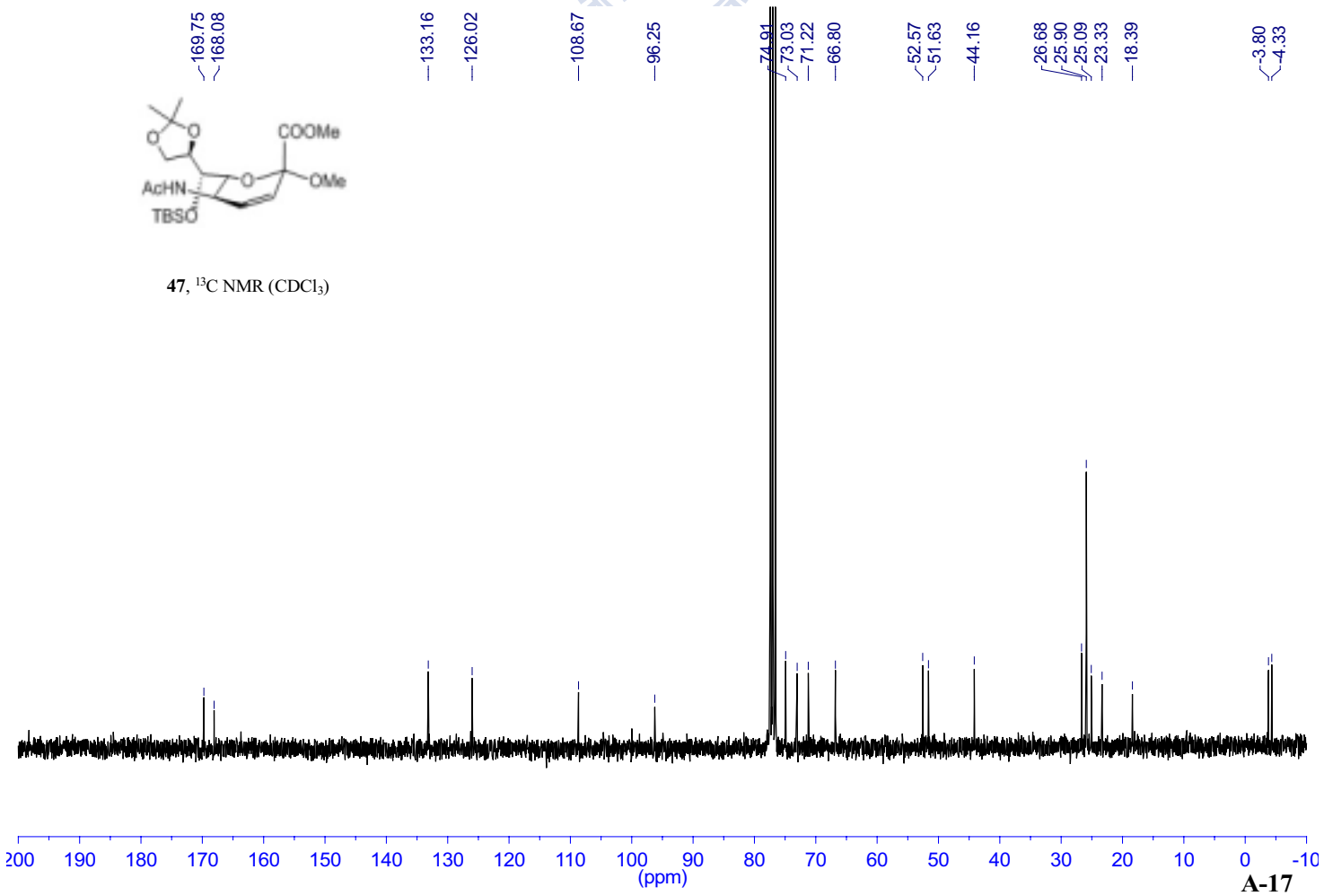
46, ^{1}H NMR ($CDCl_3$)



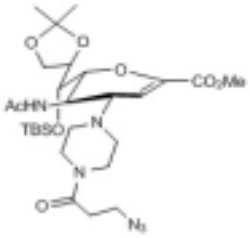
46, ^{13}C NMR ($CDCl_3$)



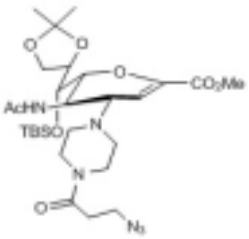
47, ^{1}H NMR ($CDCl_3$)



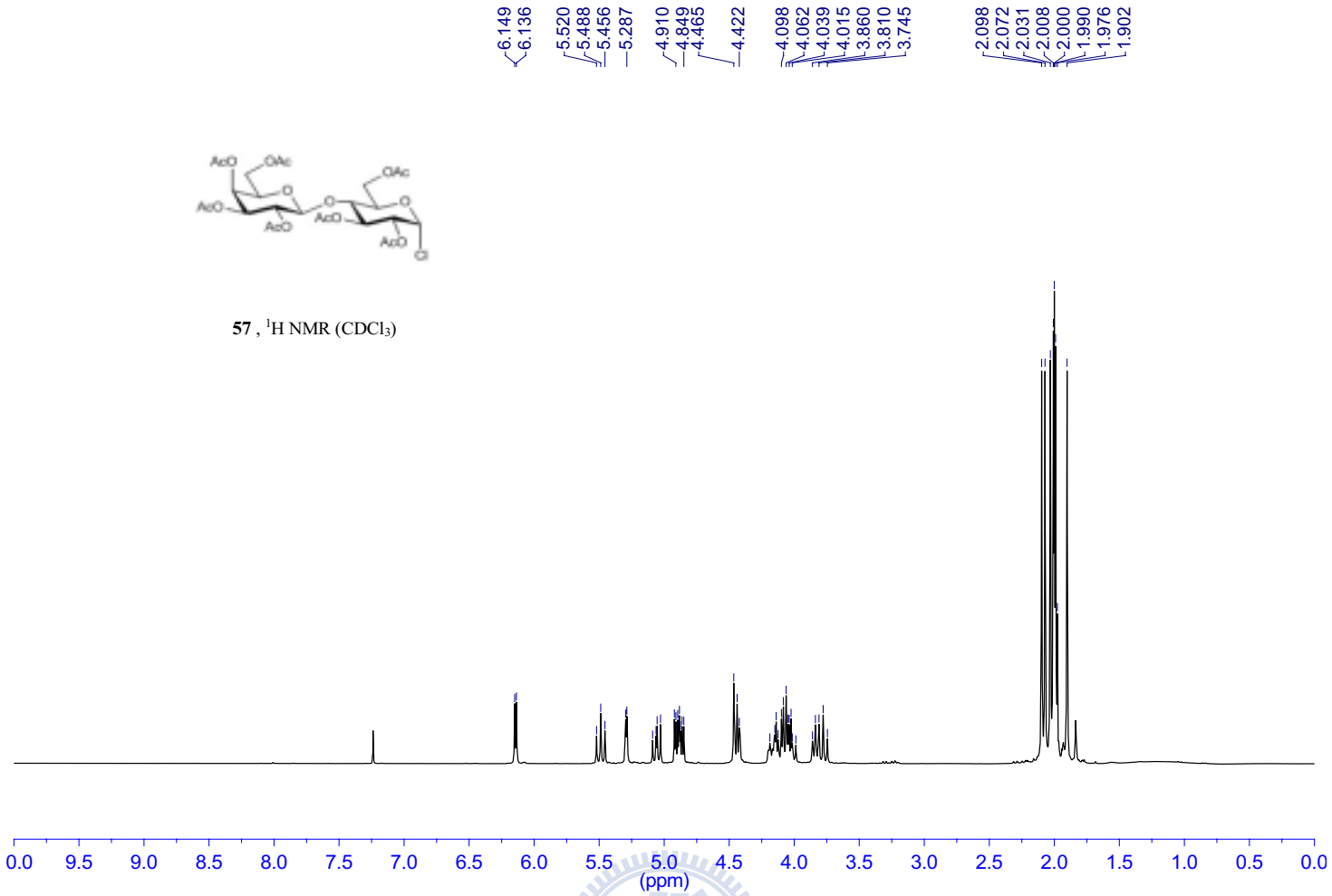
47, ^{13}C NMR ($CDCl_3$)



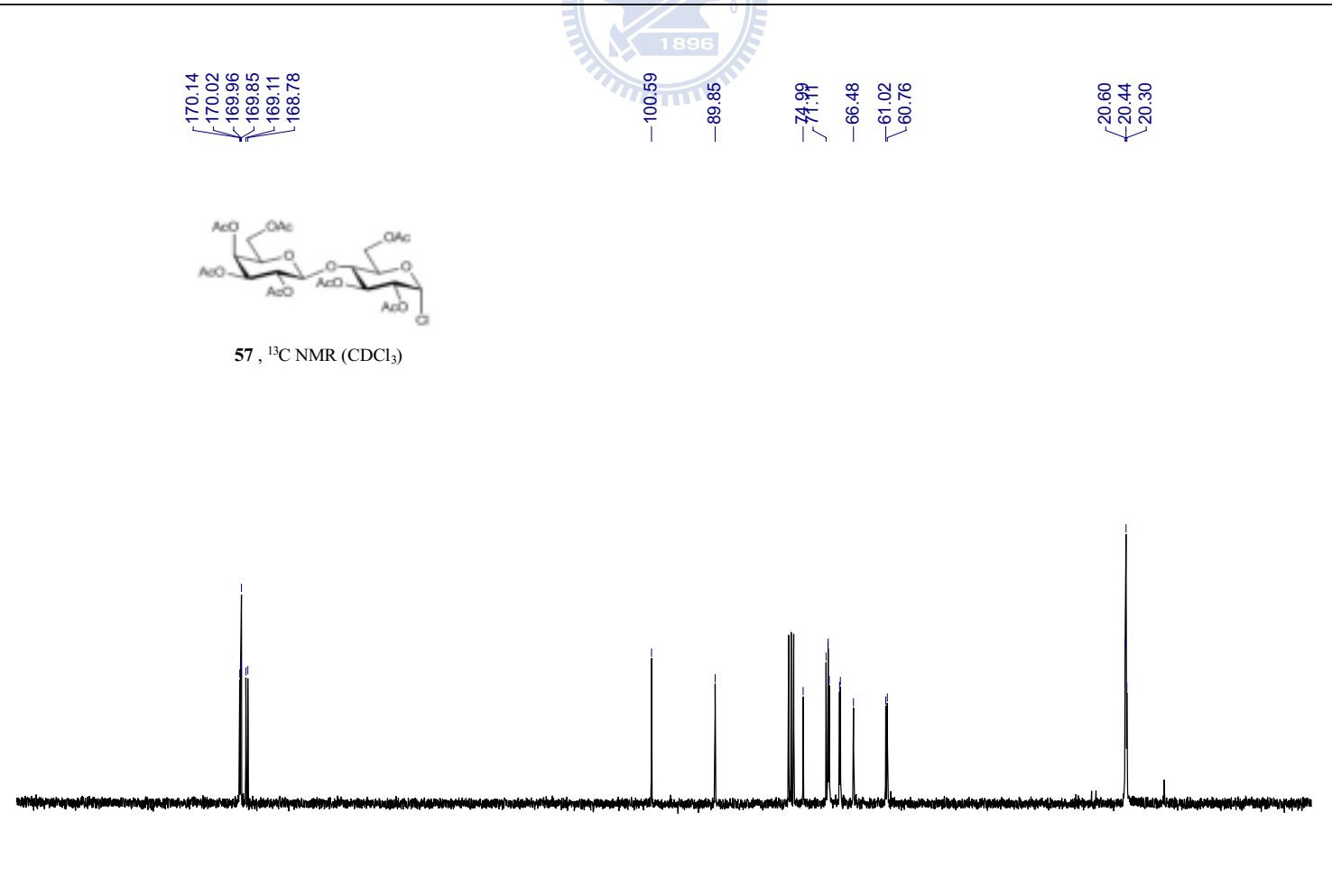
52, ^{1}H NMR (CD_3OD)



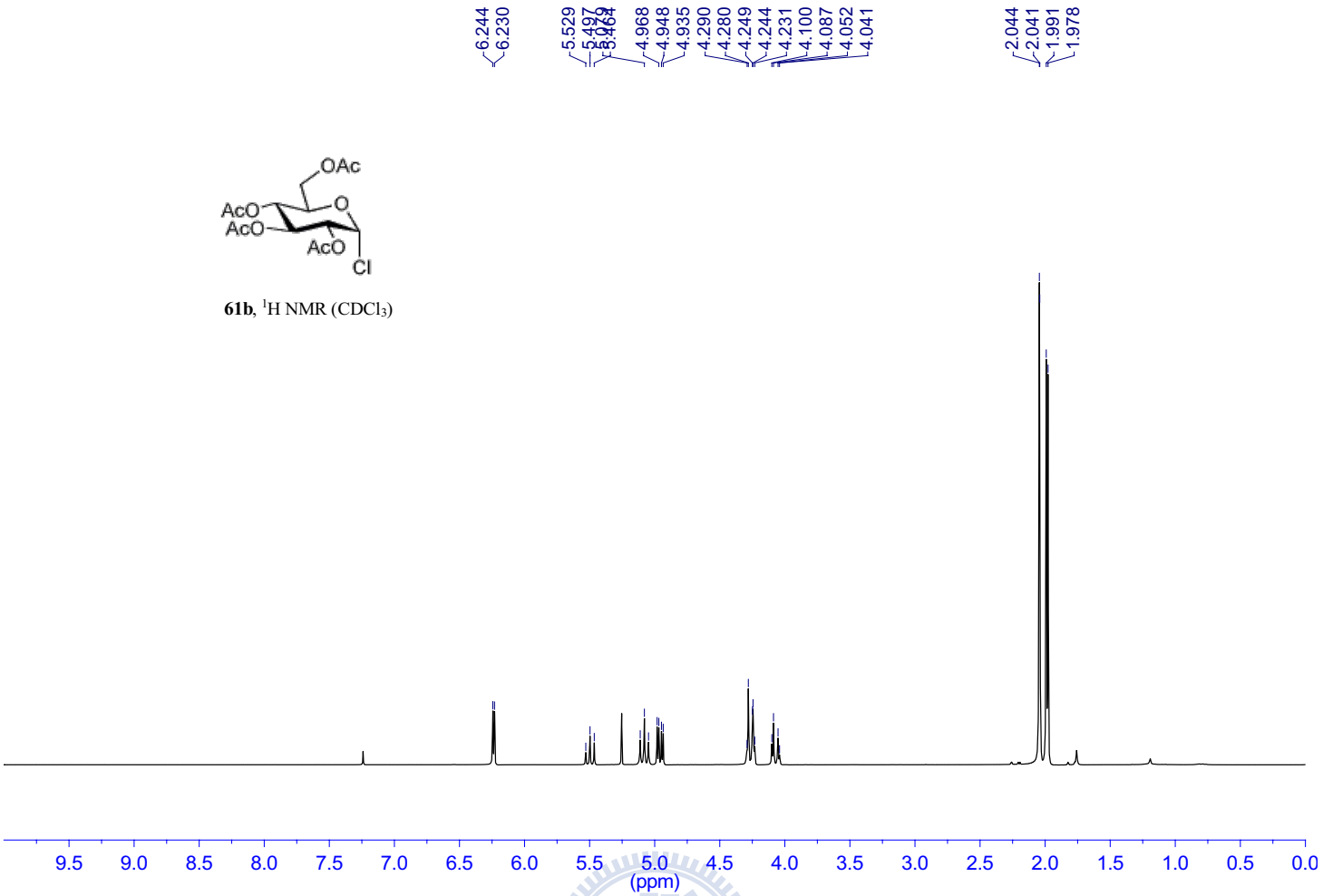
52, ^{13}C NMR (CD_3OD)



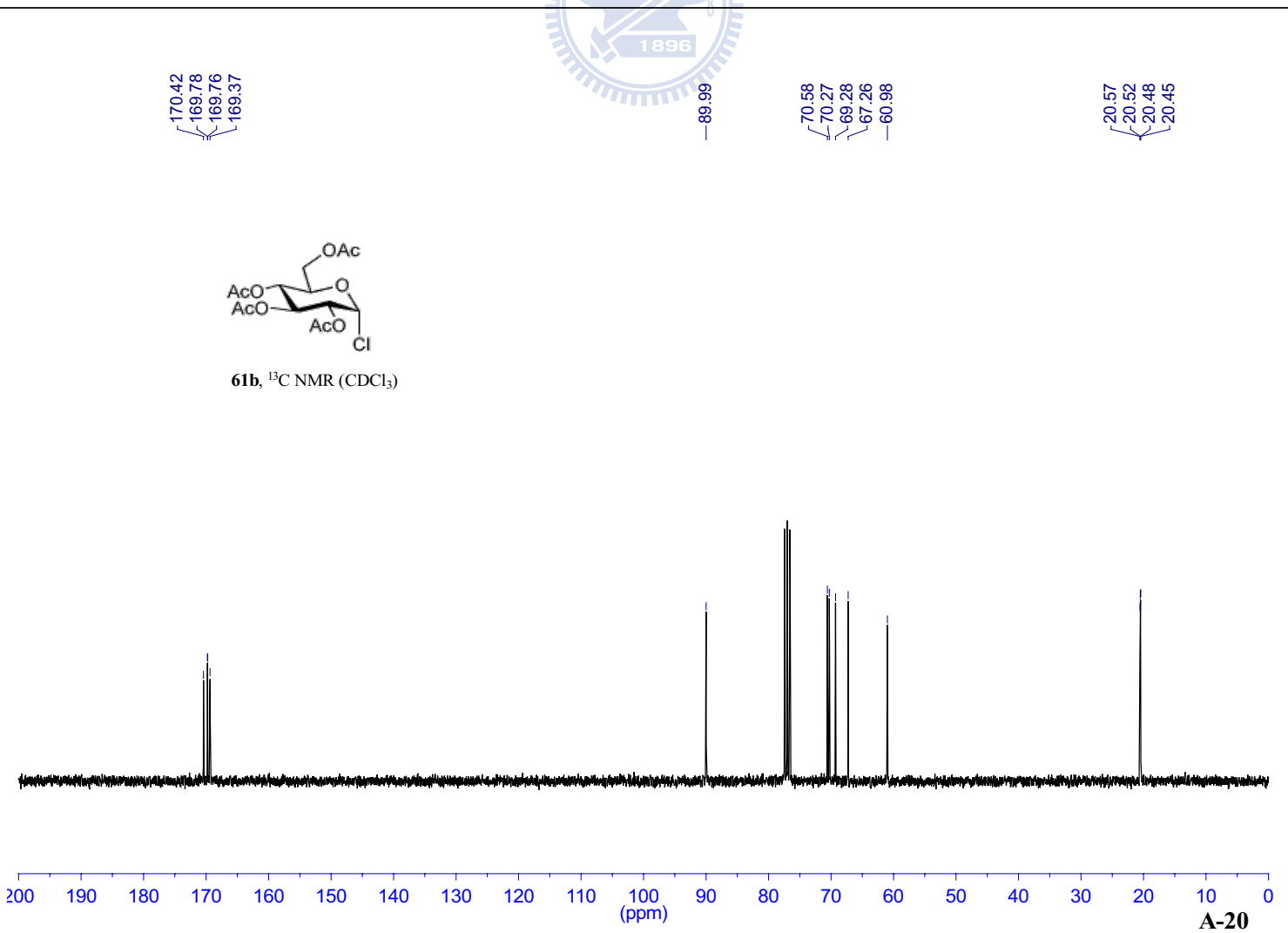
57 , ^{1}H NMR ($CDCl_3$)



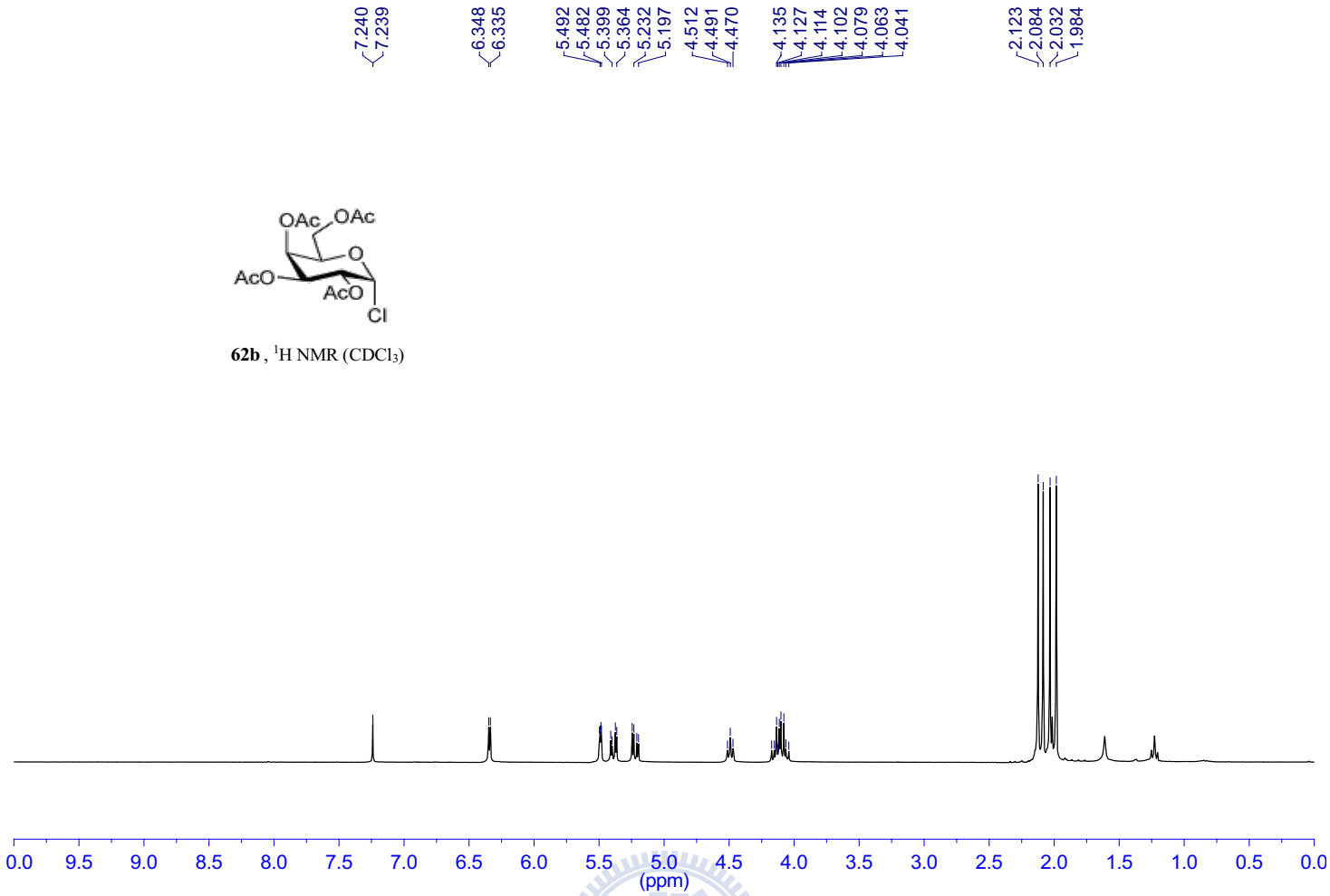
57 , ^{13}C NMR ($CDCl_3$)



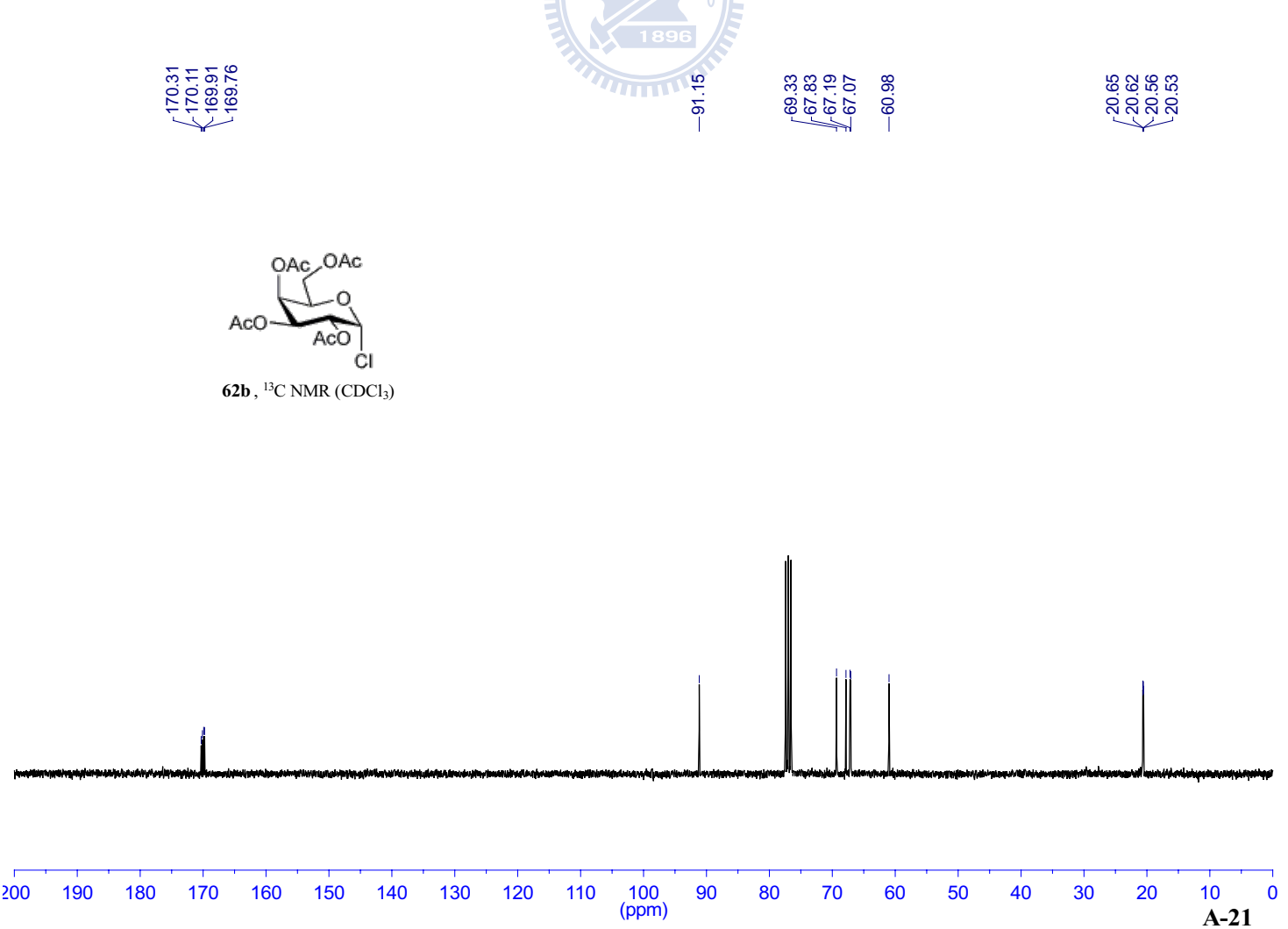
61b, ^{1}H NMR ($CDCl_3$)



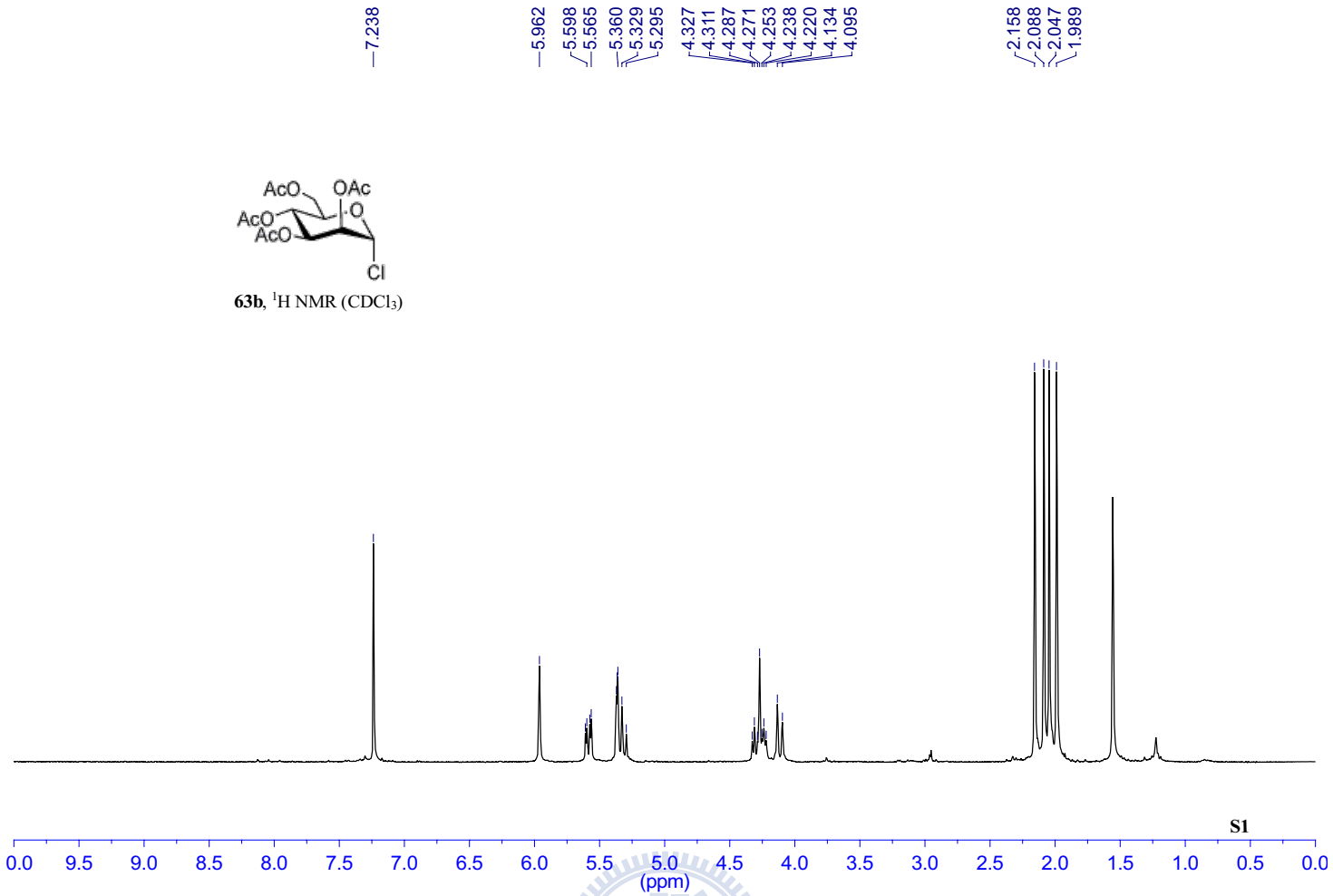
61b, ^{13}C NMR ($CDCl_3$)



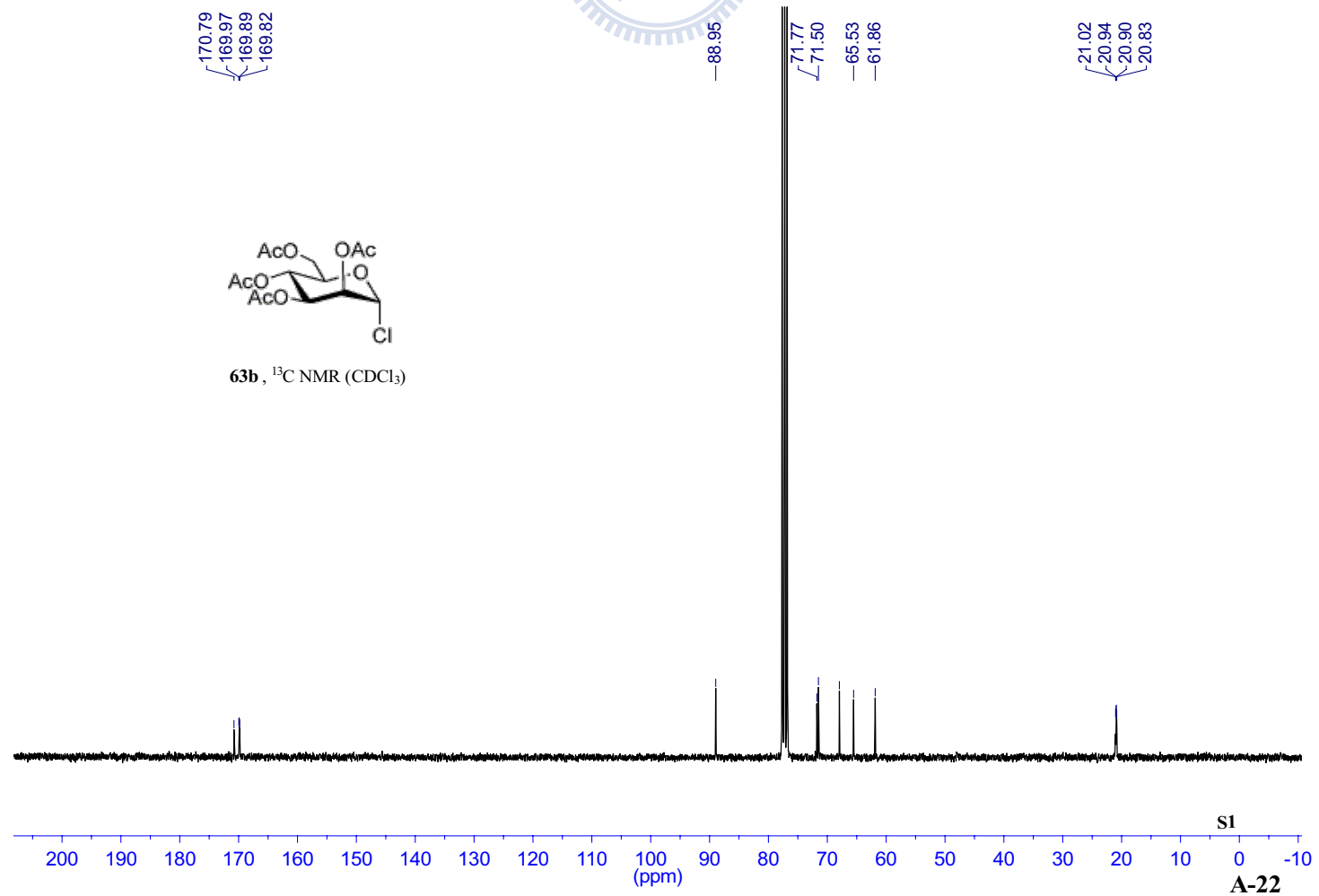
62b , ^{1}H NMR ($CDCl_3$)



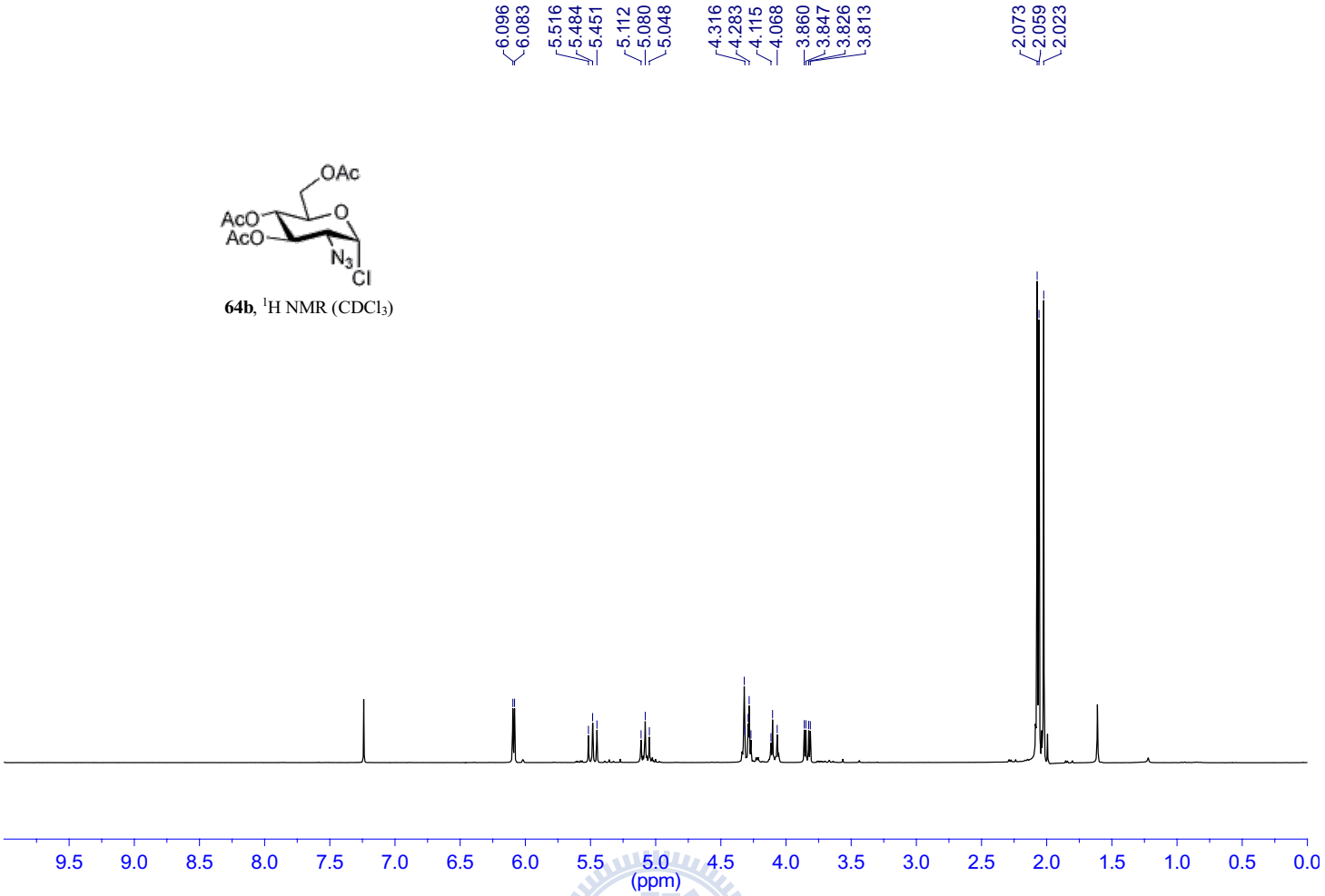
62b , ^{13}C NMR ($CDCl_3$)



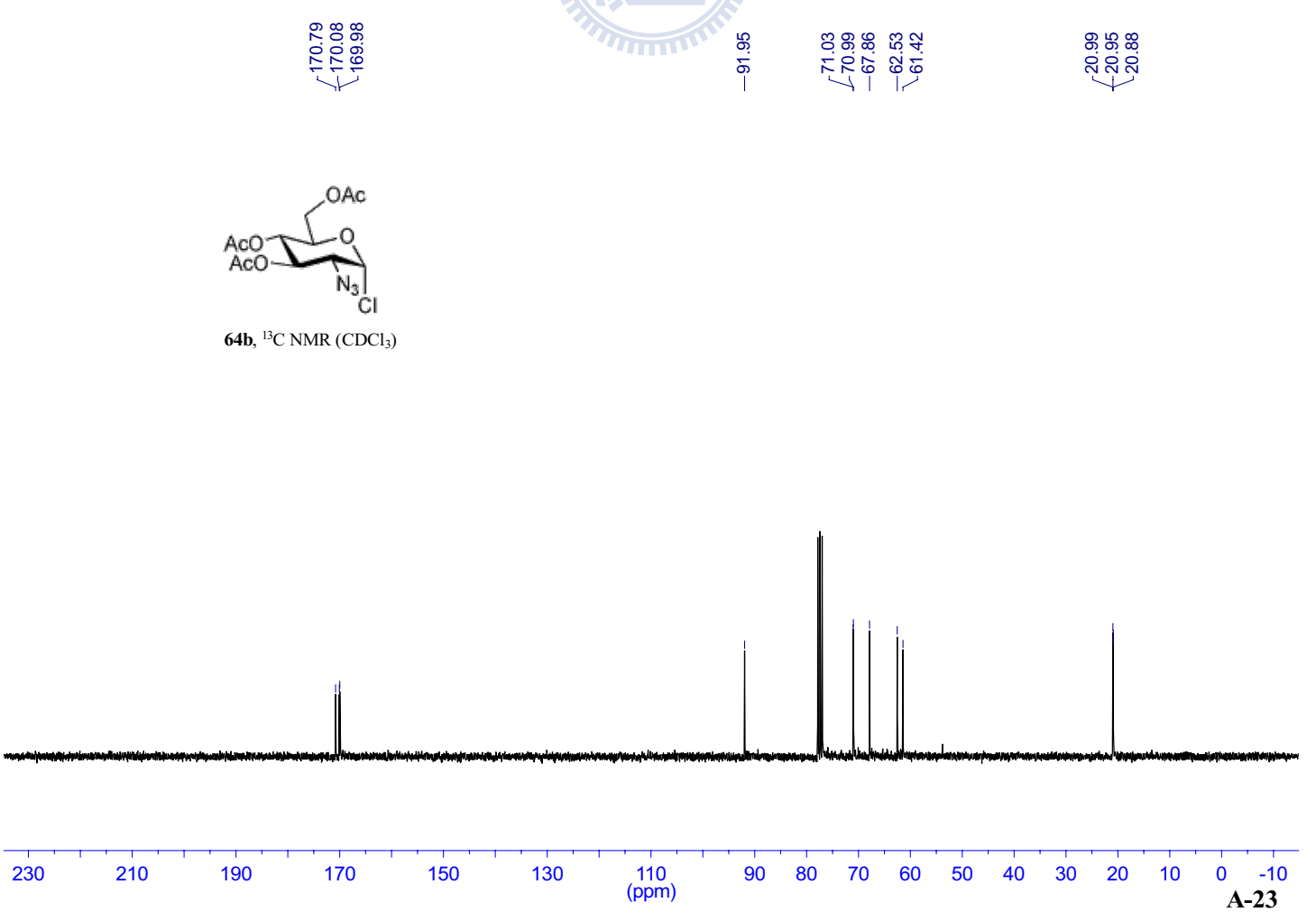
63b, ^{1}H NMR ($CDCl_3$)



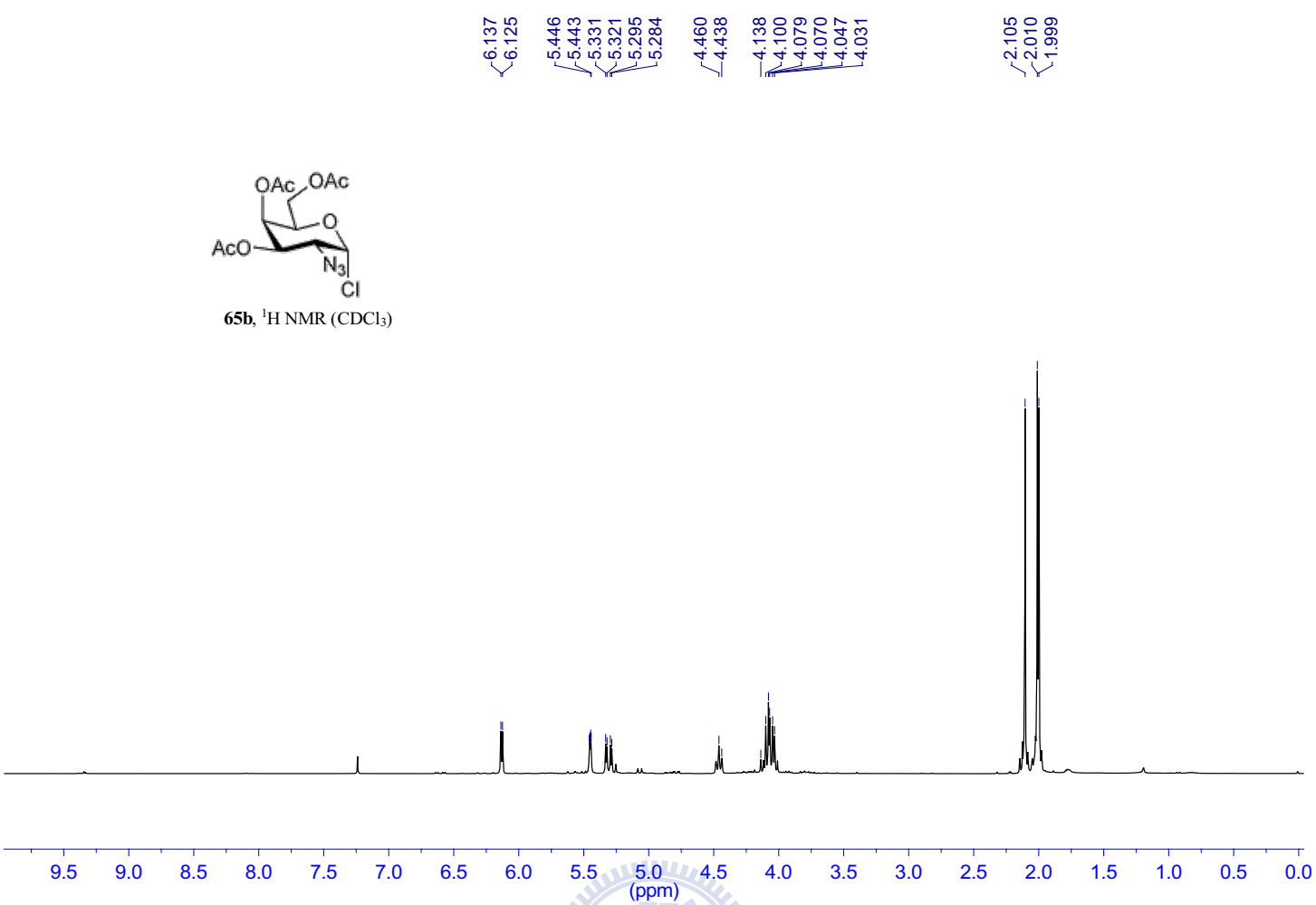
63b , ^{13}C NMR ($CDCl_3$)



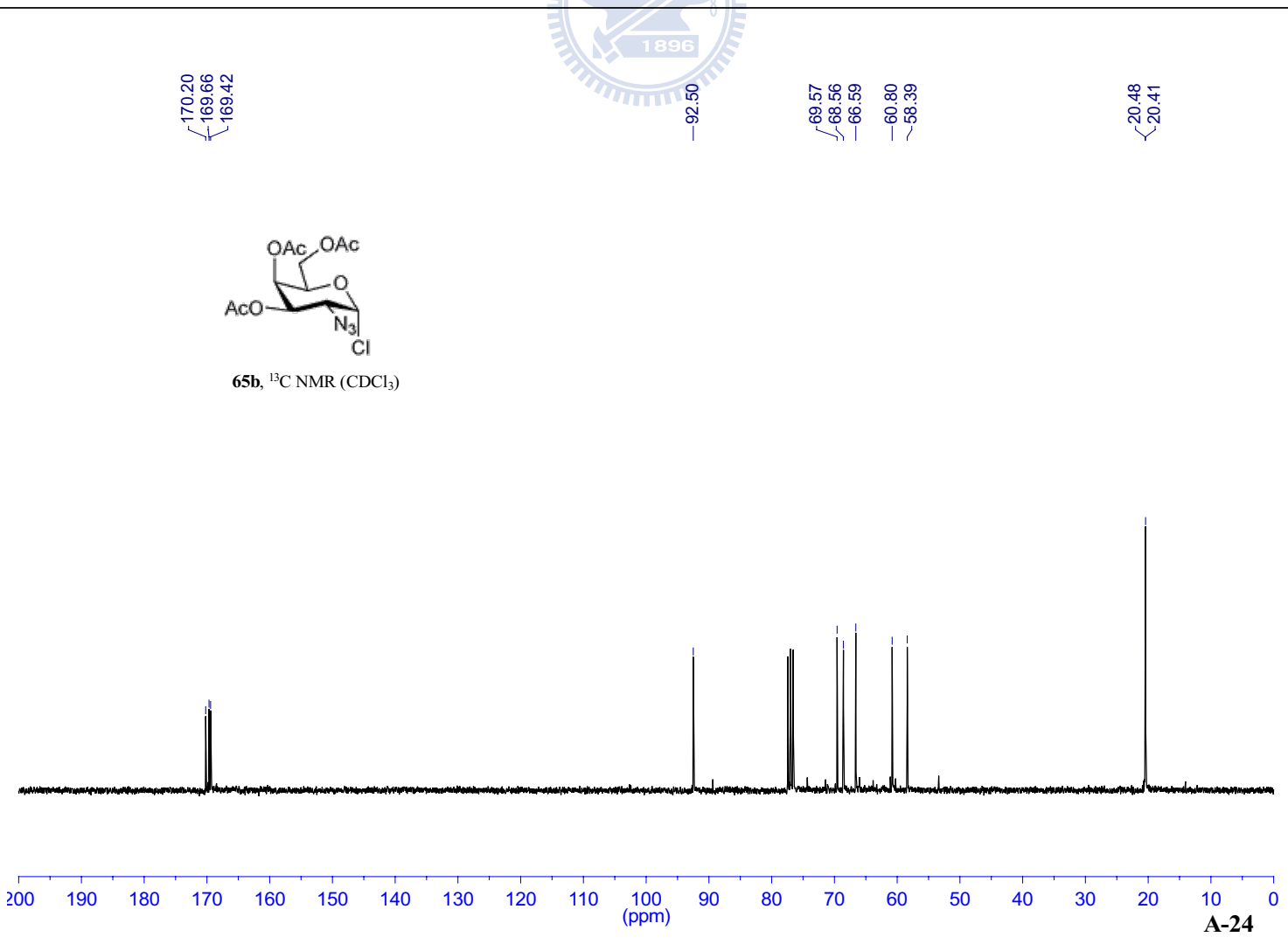
64b, ^{1}H NMR ($CDCl_3$)



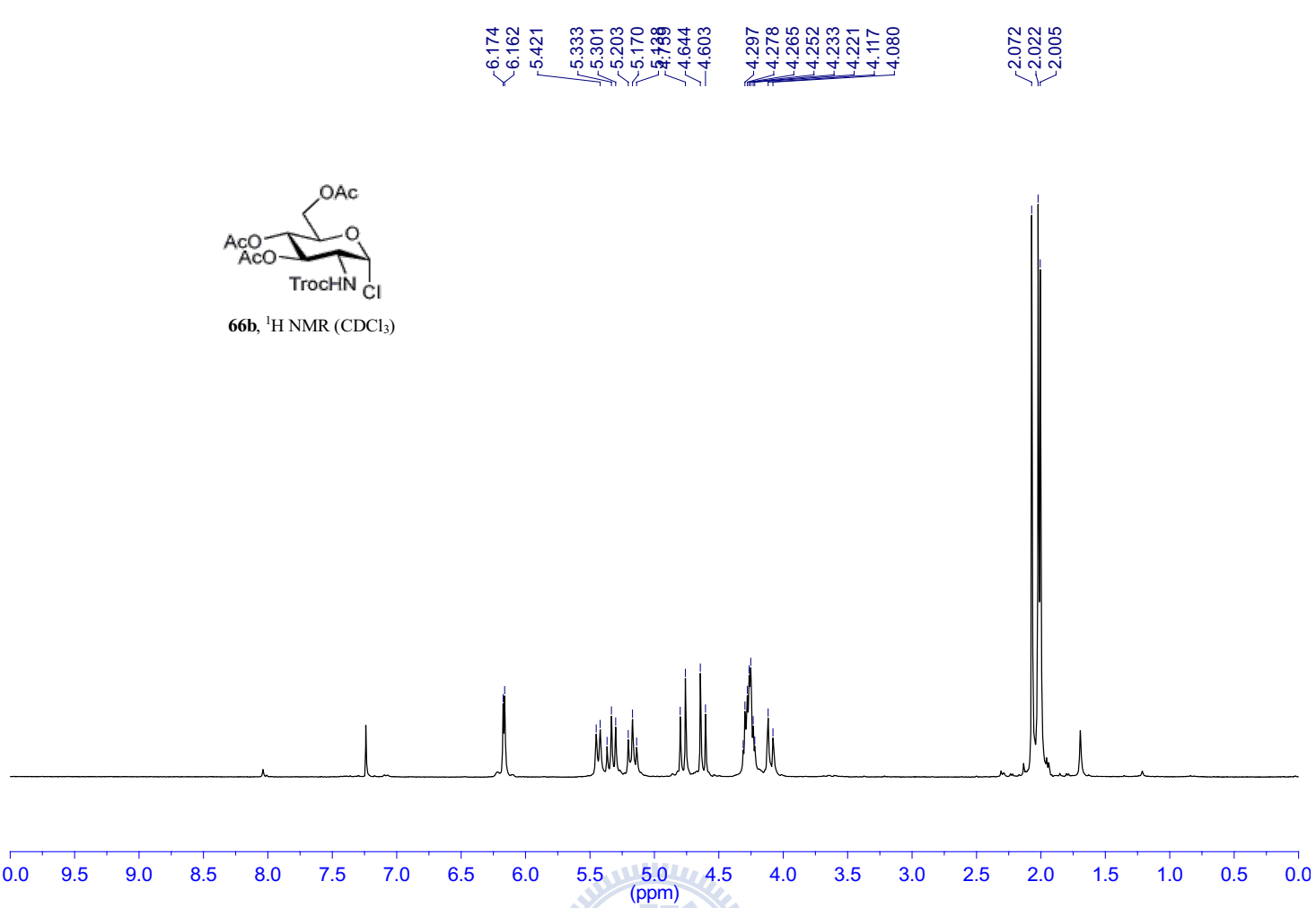
64b, ^{13}C NMR ($CDCl_3$)



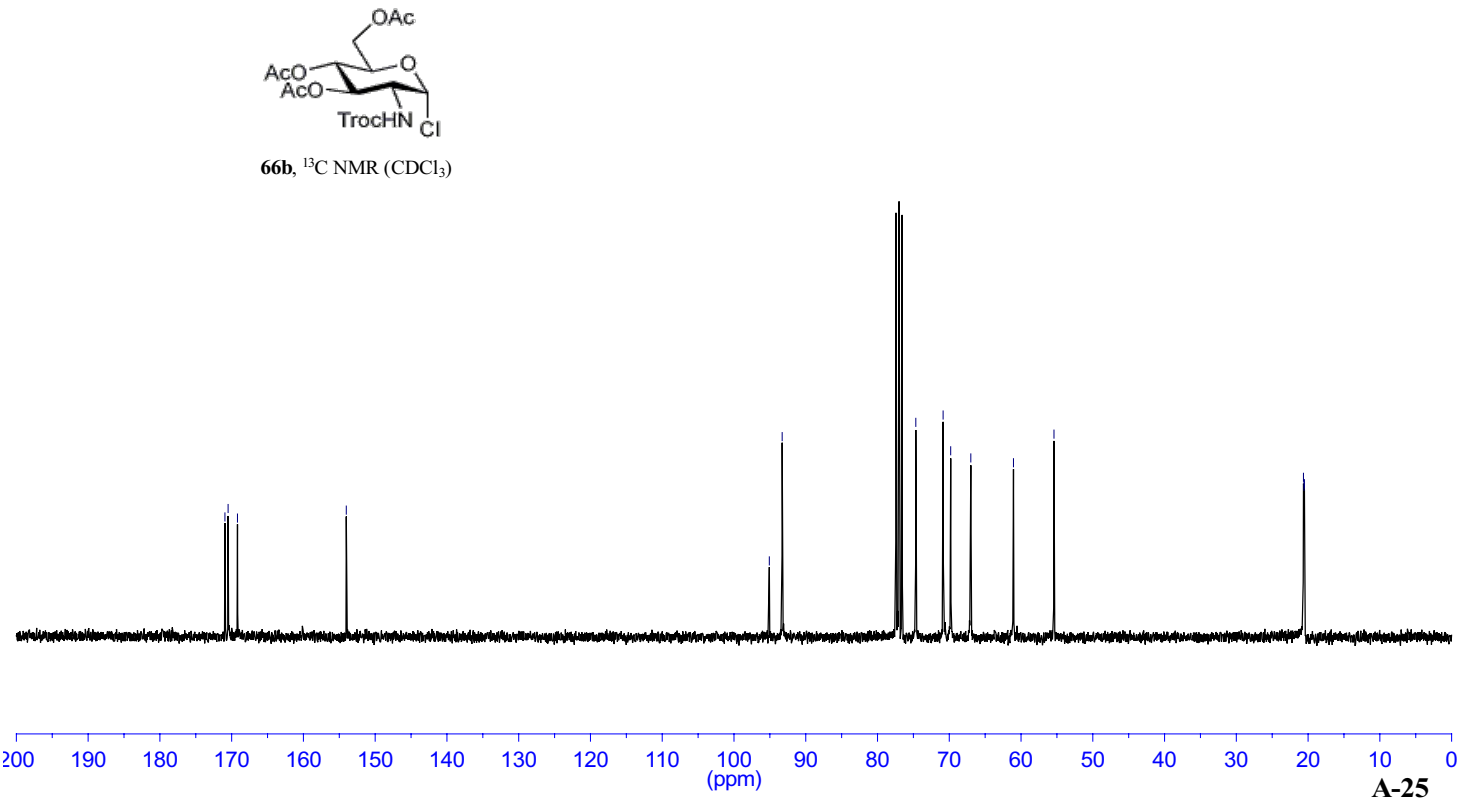
65b, ^{1}H NMR ($CDCl_3$)



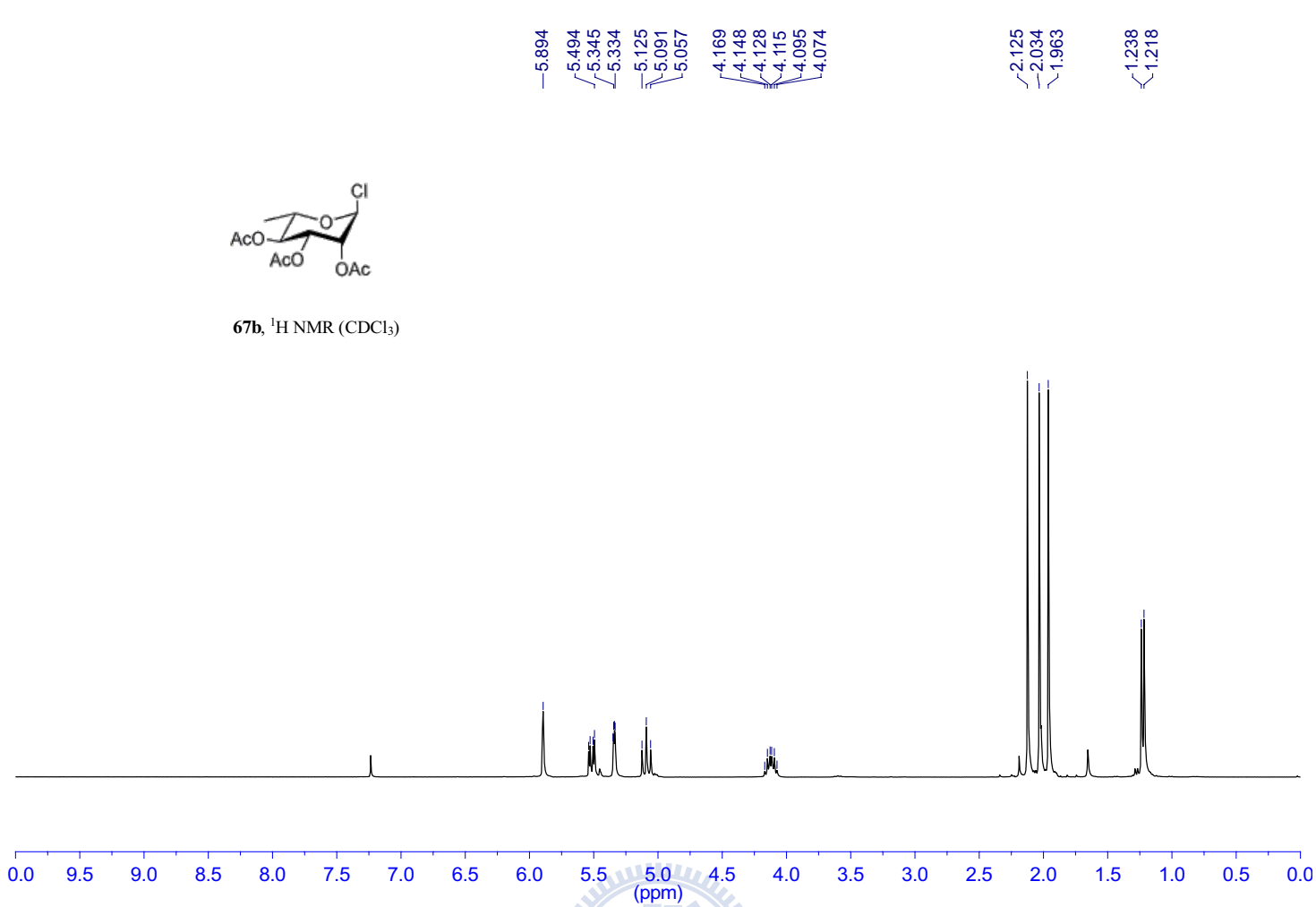
65b, ^{13}C NMR ($CDCl_3$)



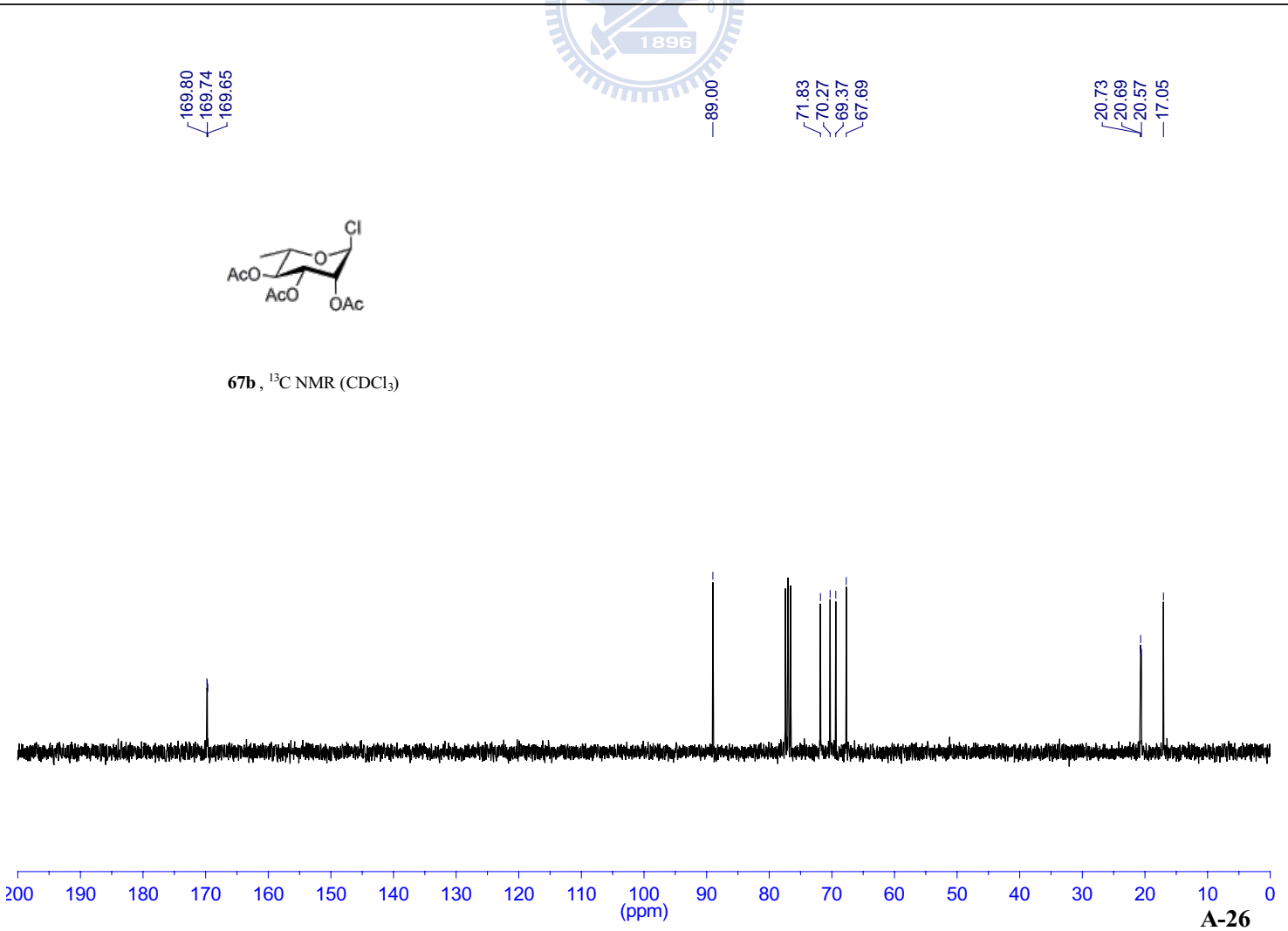
66b, ^{1}H NMR ($CDCl_3$)



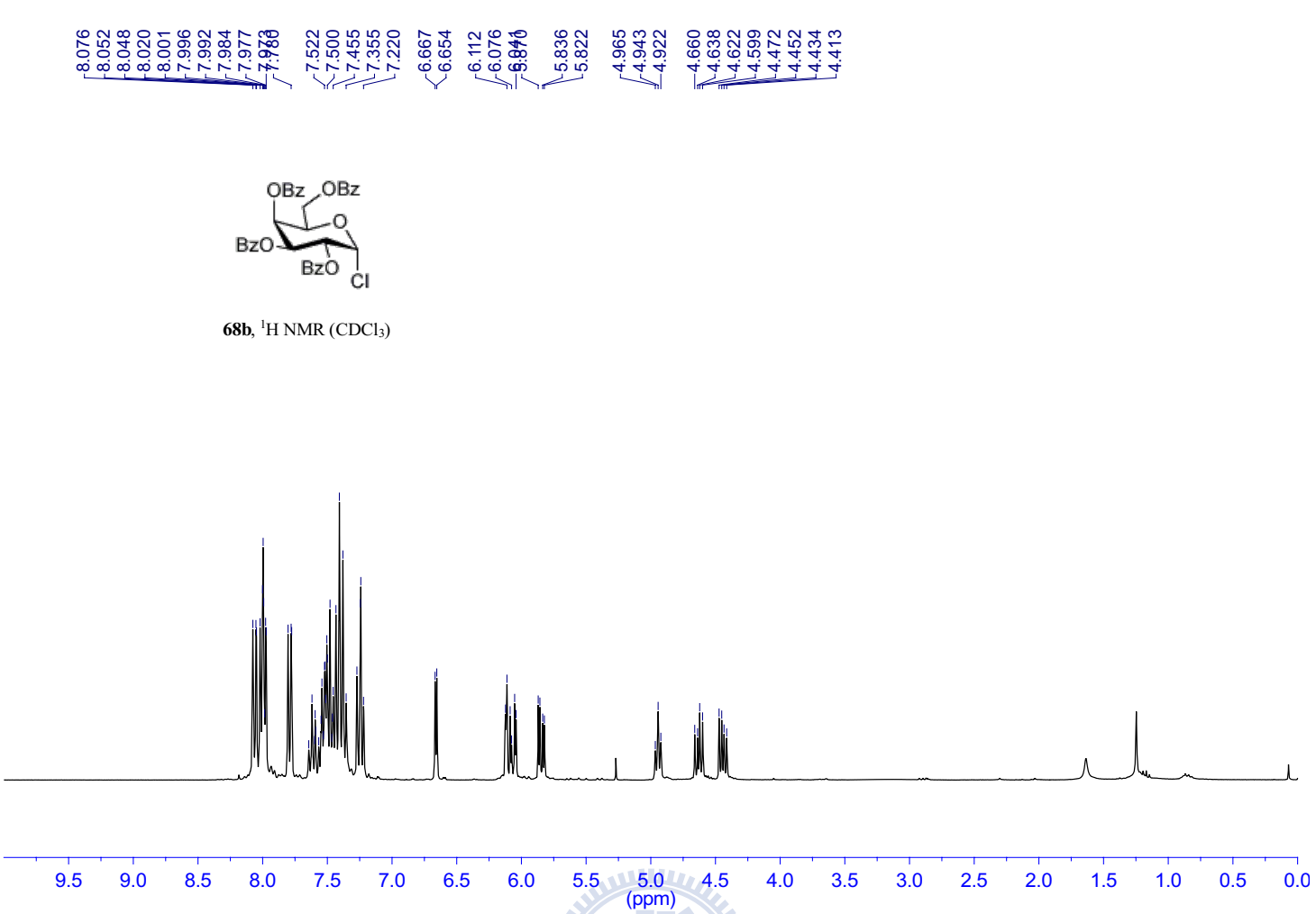
66b, ^{13}C NMR ($CDCl_3$)



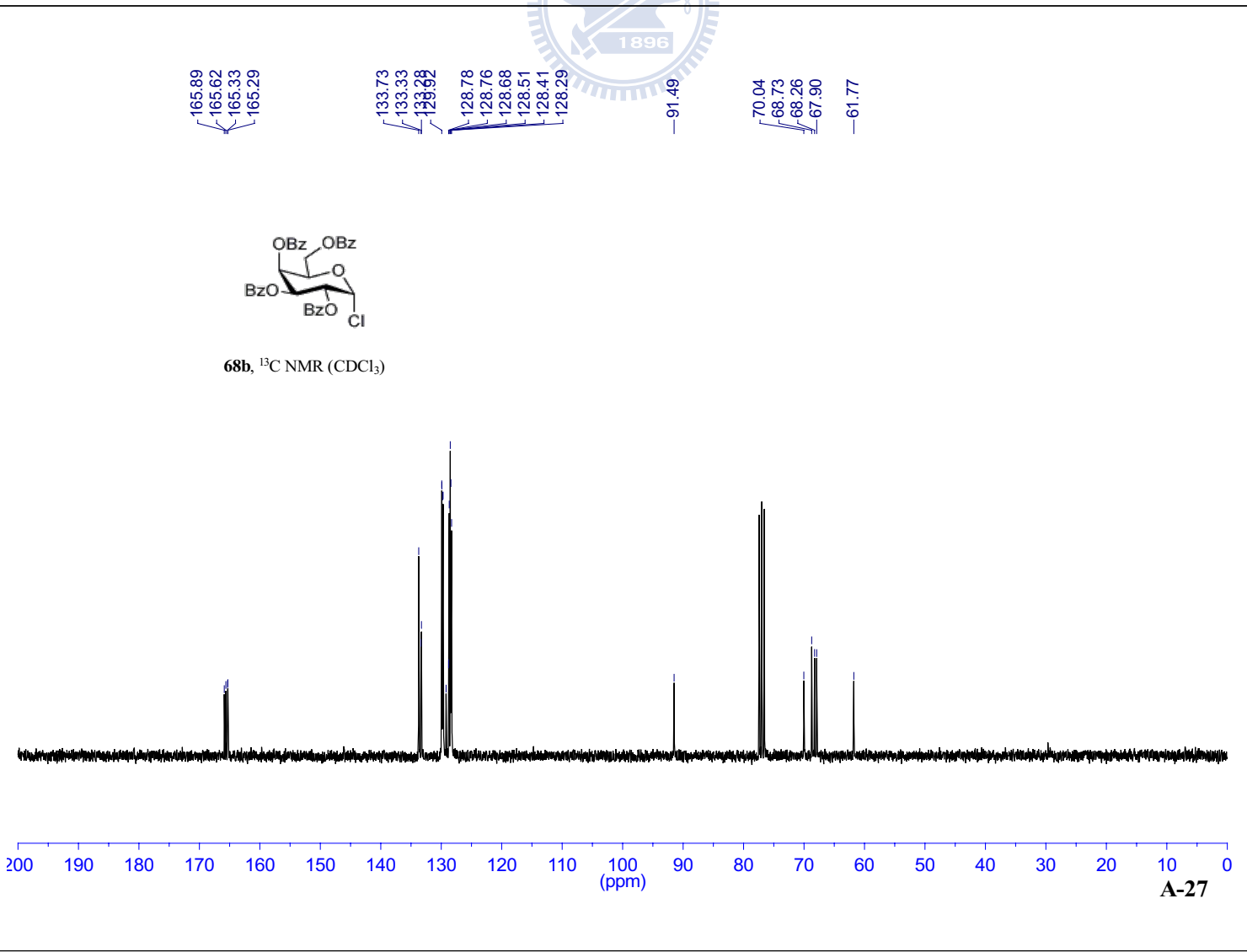
67b, ^{1}H NMR ($CDCl_3$)



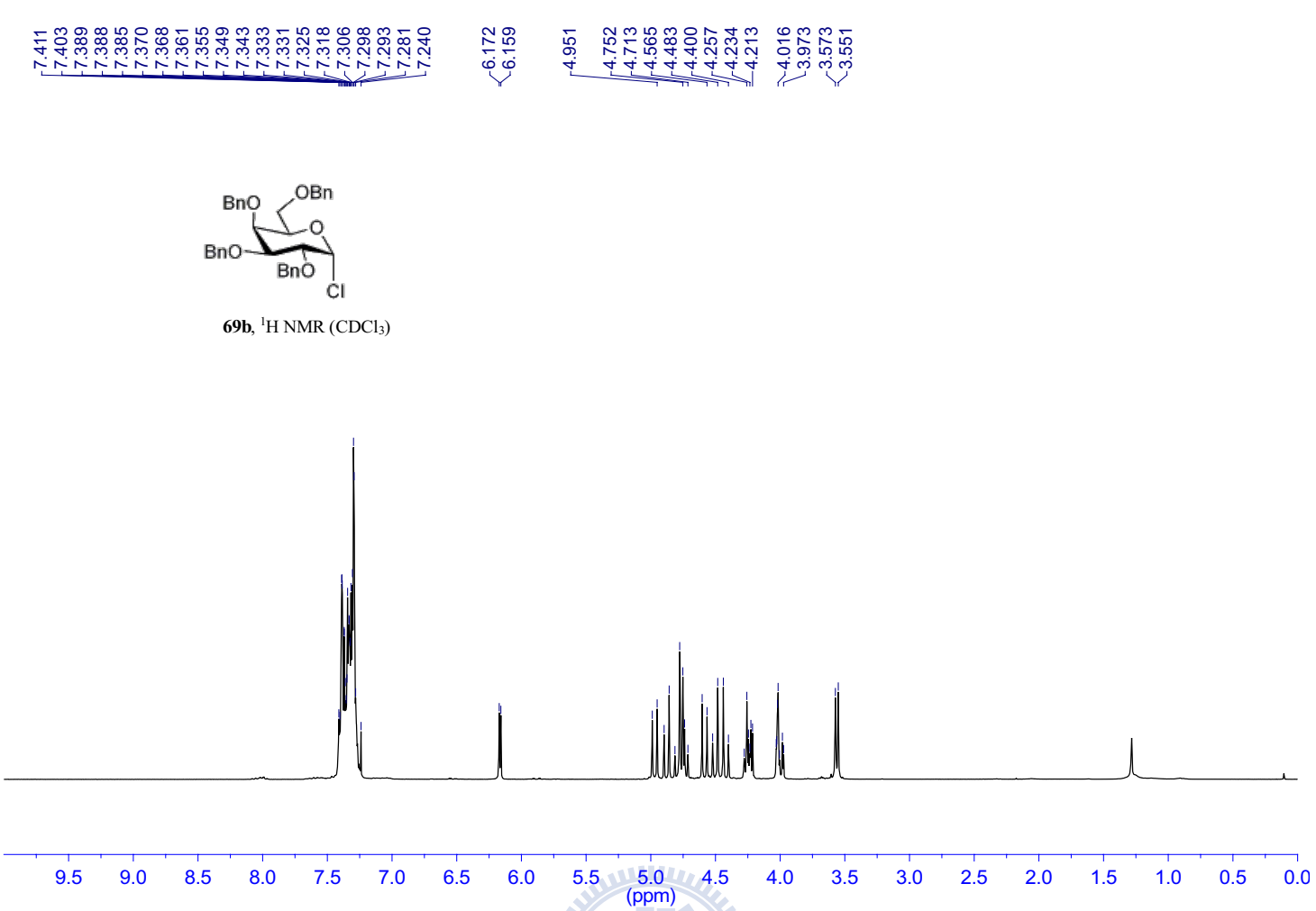
67b , ^{13}C NMR ($CDCl_3$)



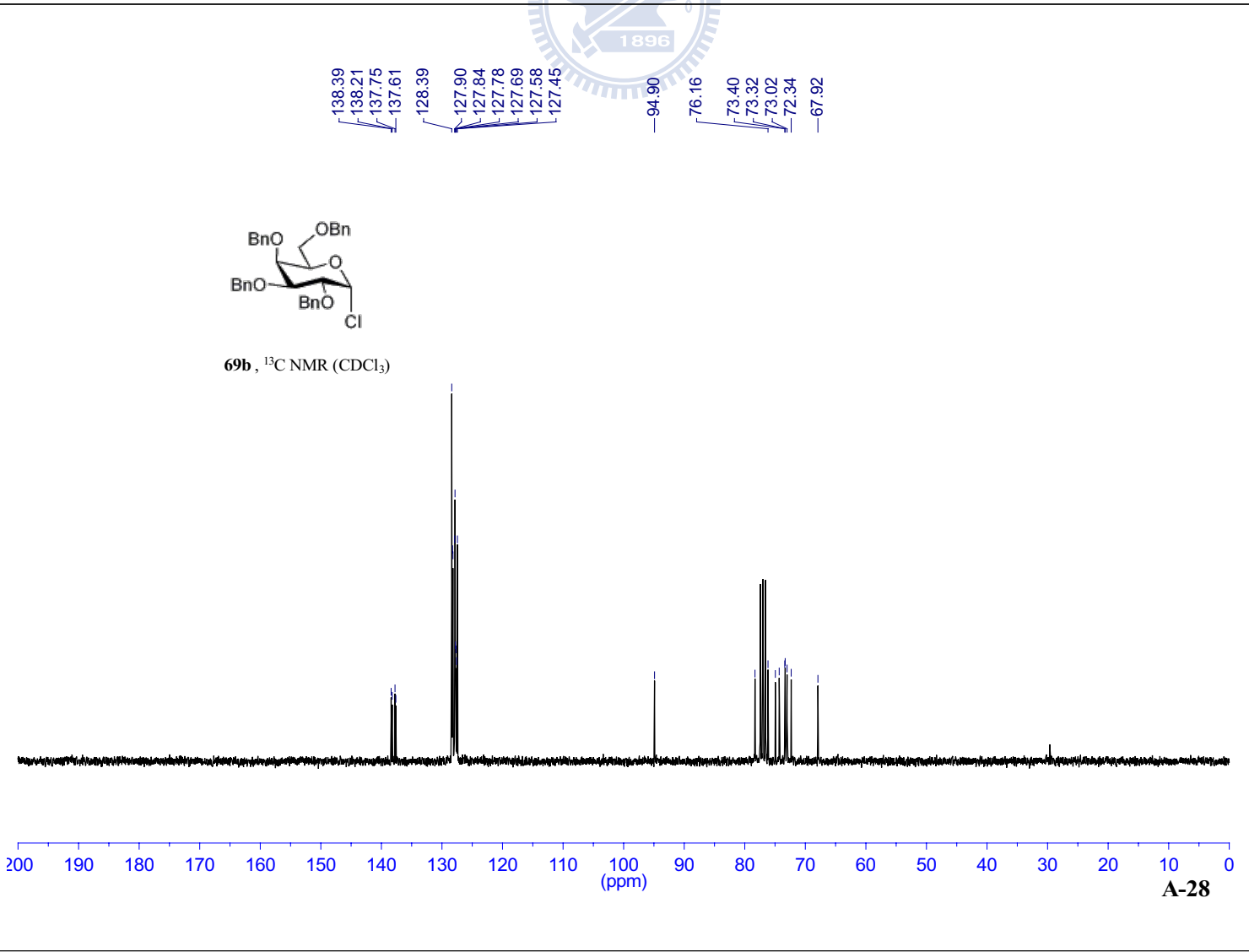
68b, ^{1}H NMR ($CDCl_3$)



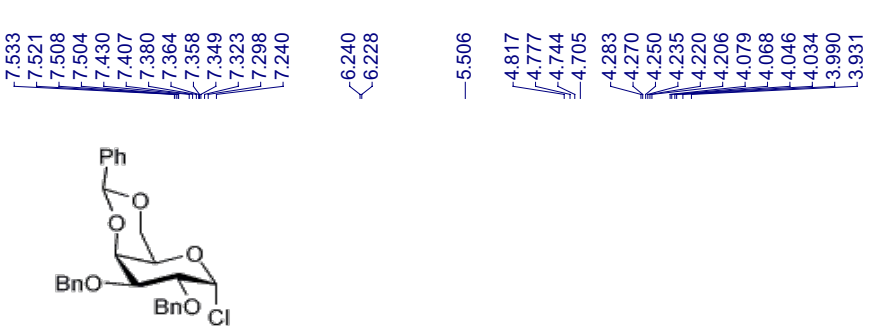
68b, ^{13}C NMR ($CDCl_3$)



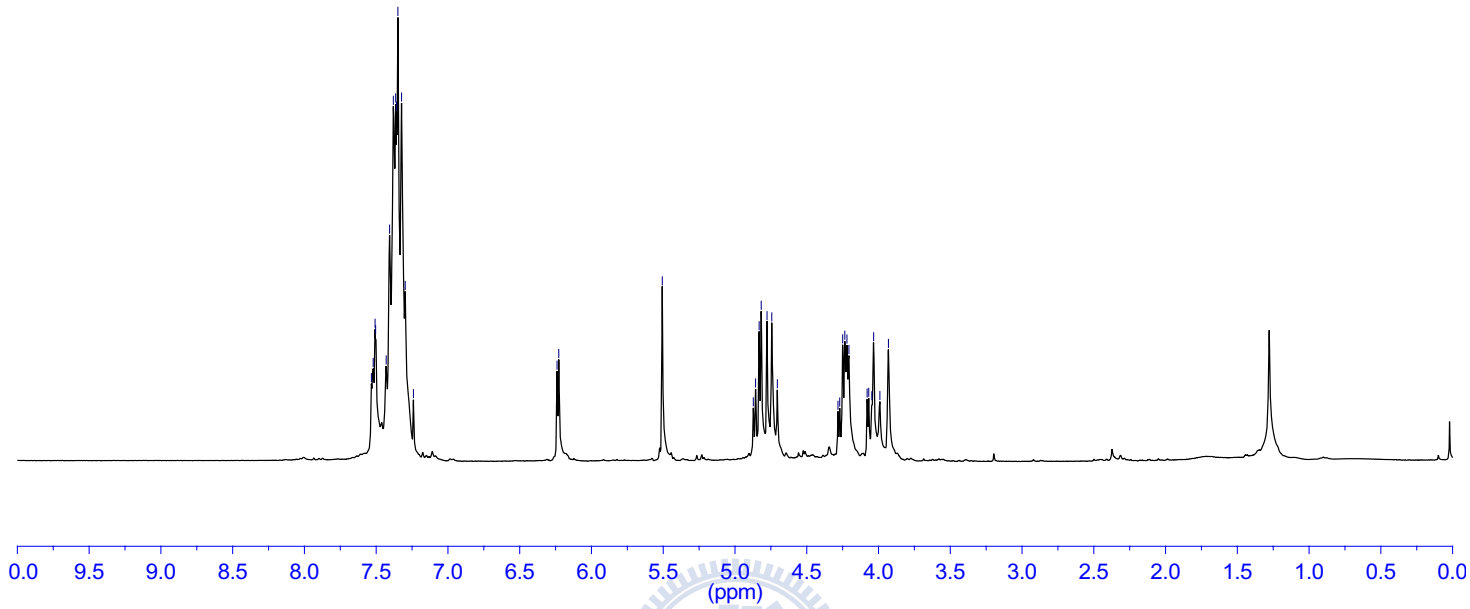
69b, ^{1}H NMR ($CDCl_3$)

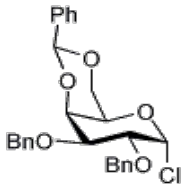


69b , ^{13}C NMR ($CDCl_3$)

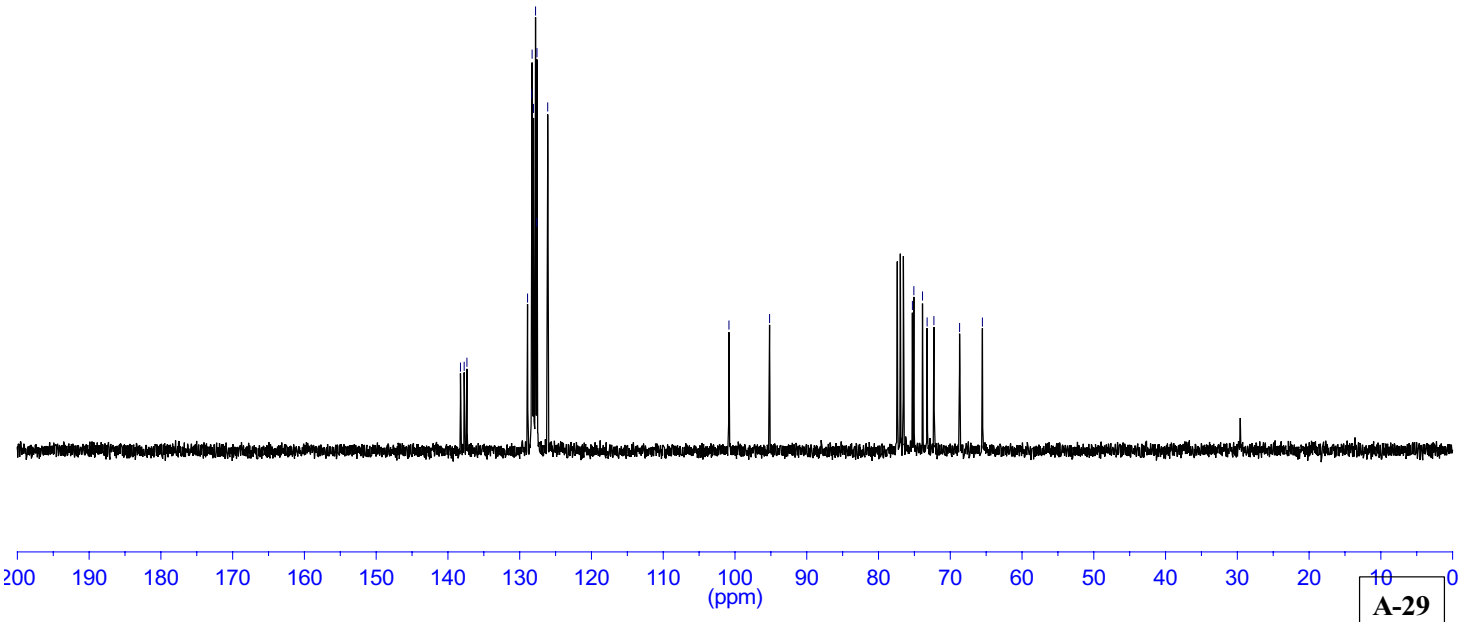


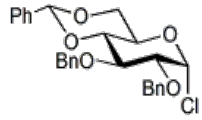
70b, ^{1}H NMR ($CDCl_3$)



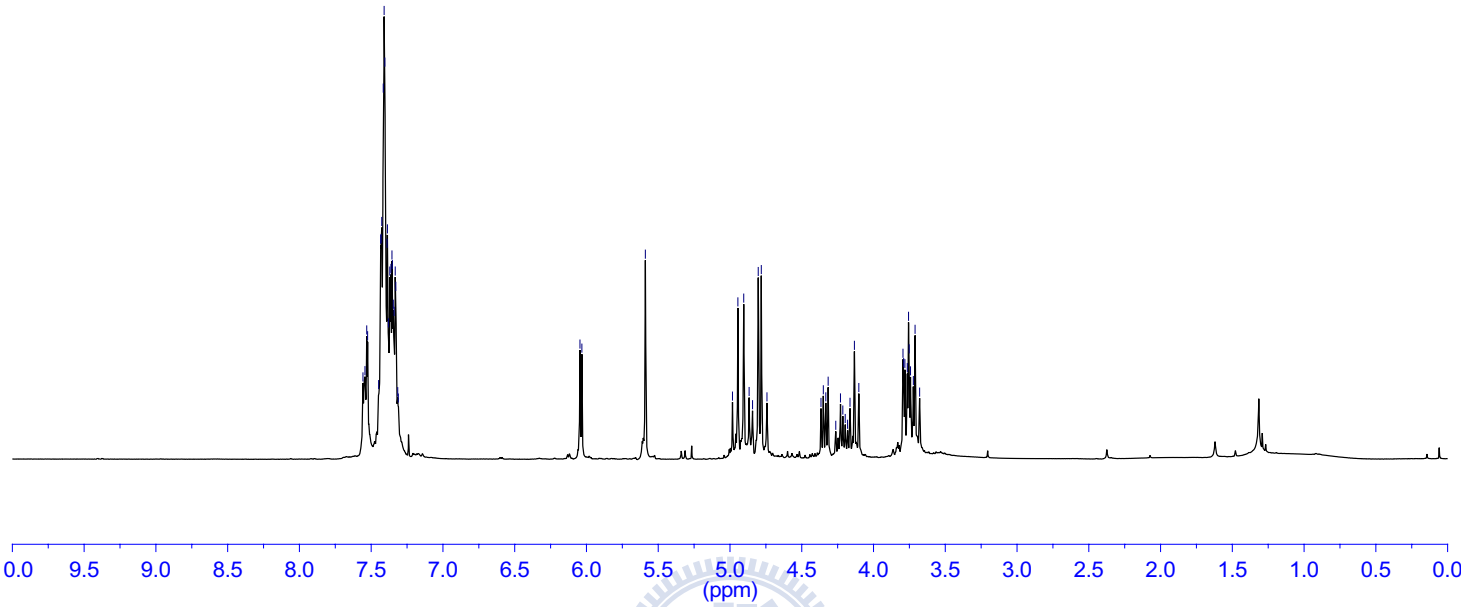


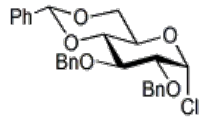
70b , ^{13}C NMR ($CDCl_3$)



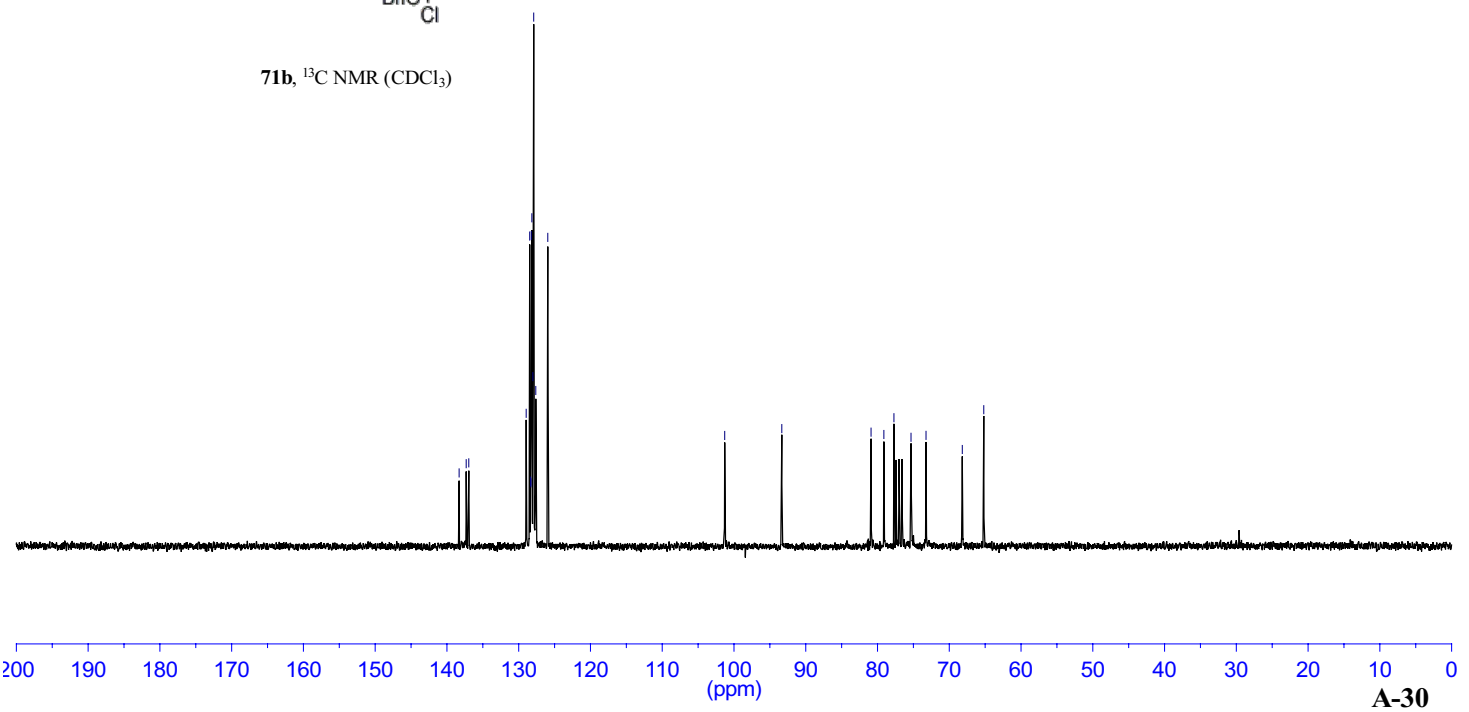


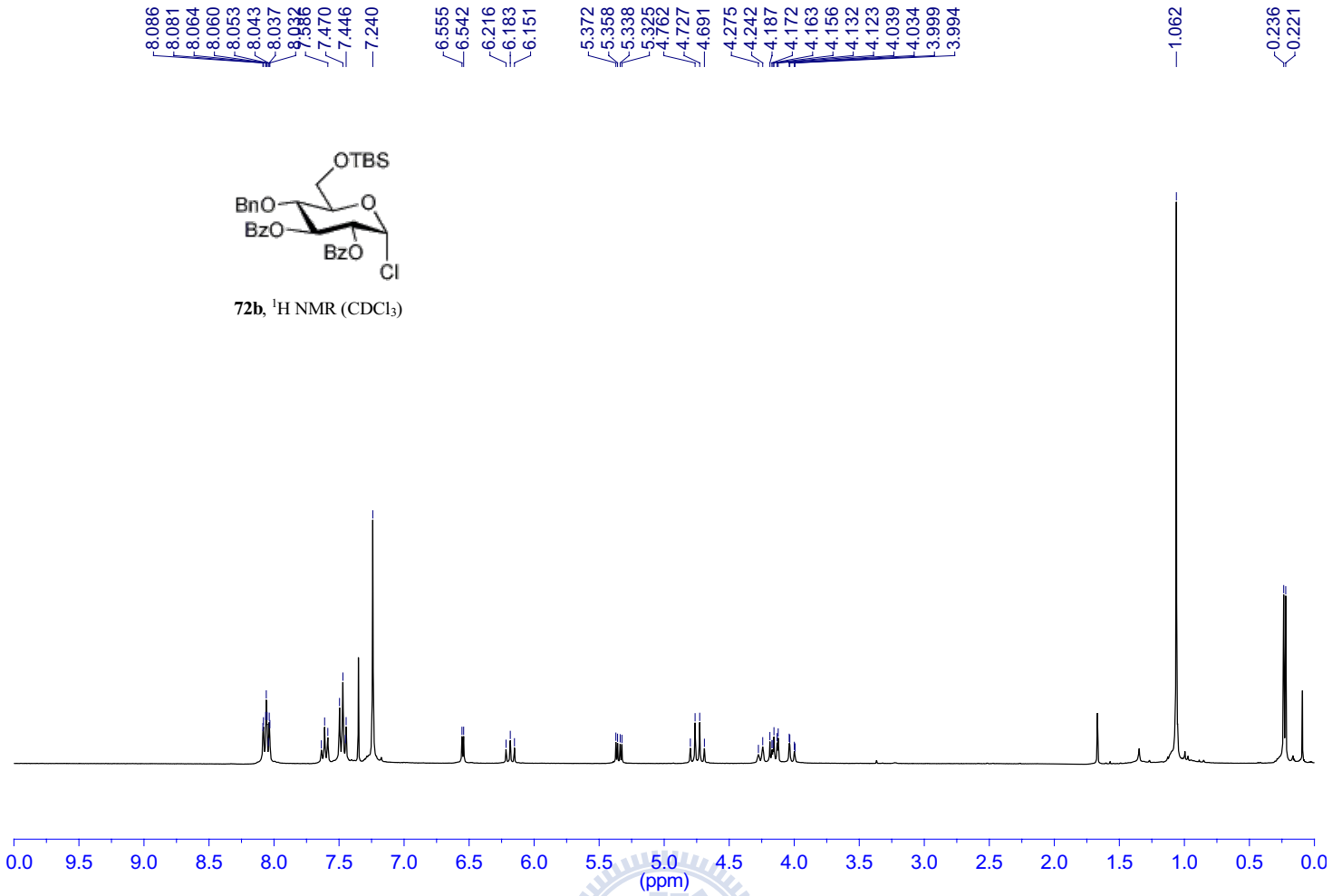
71b, ^{1}H NMR ($CDCl_3$)



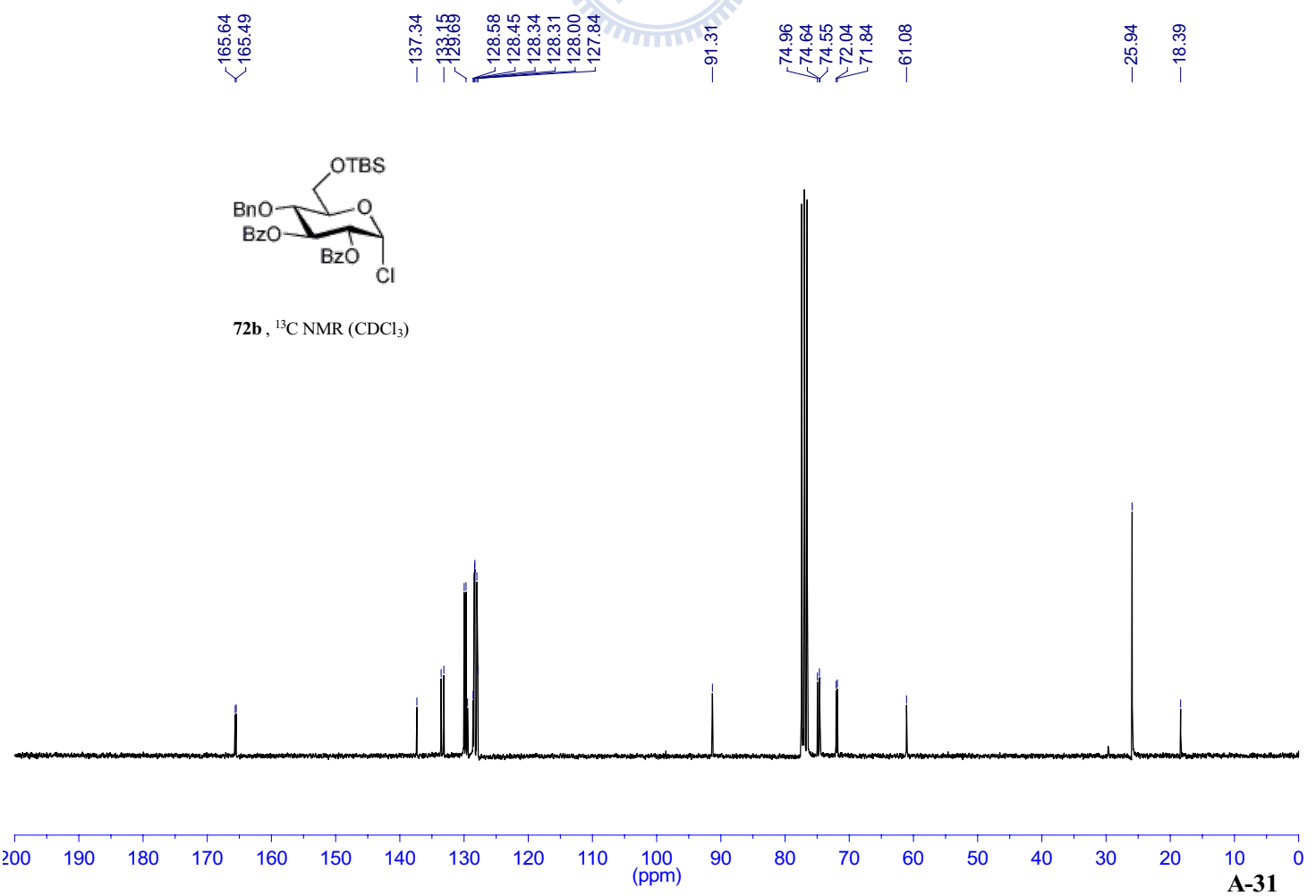


71b, ^{13}C NMR ($CDCl_3$)

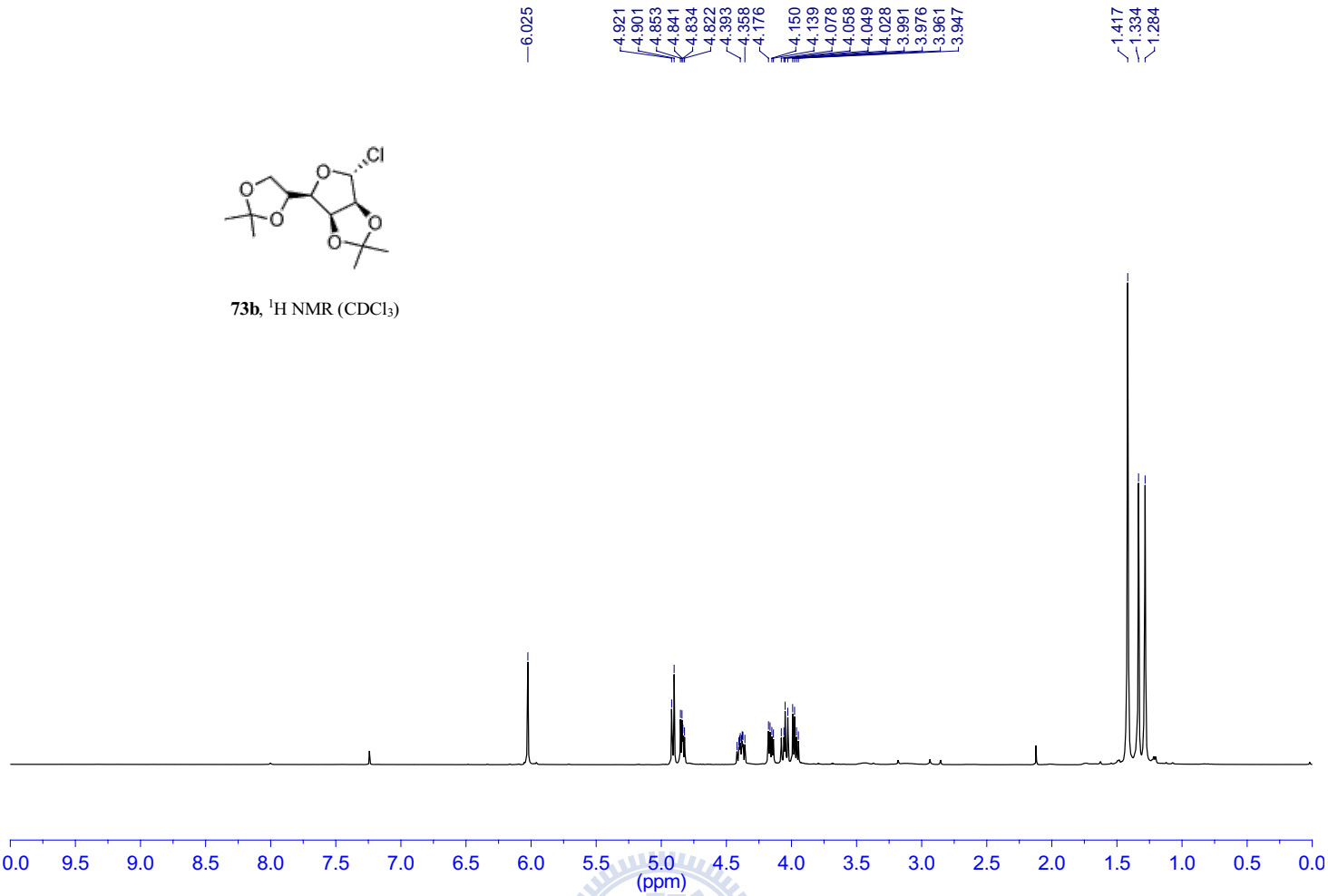




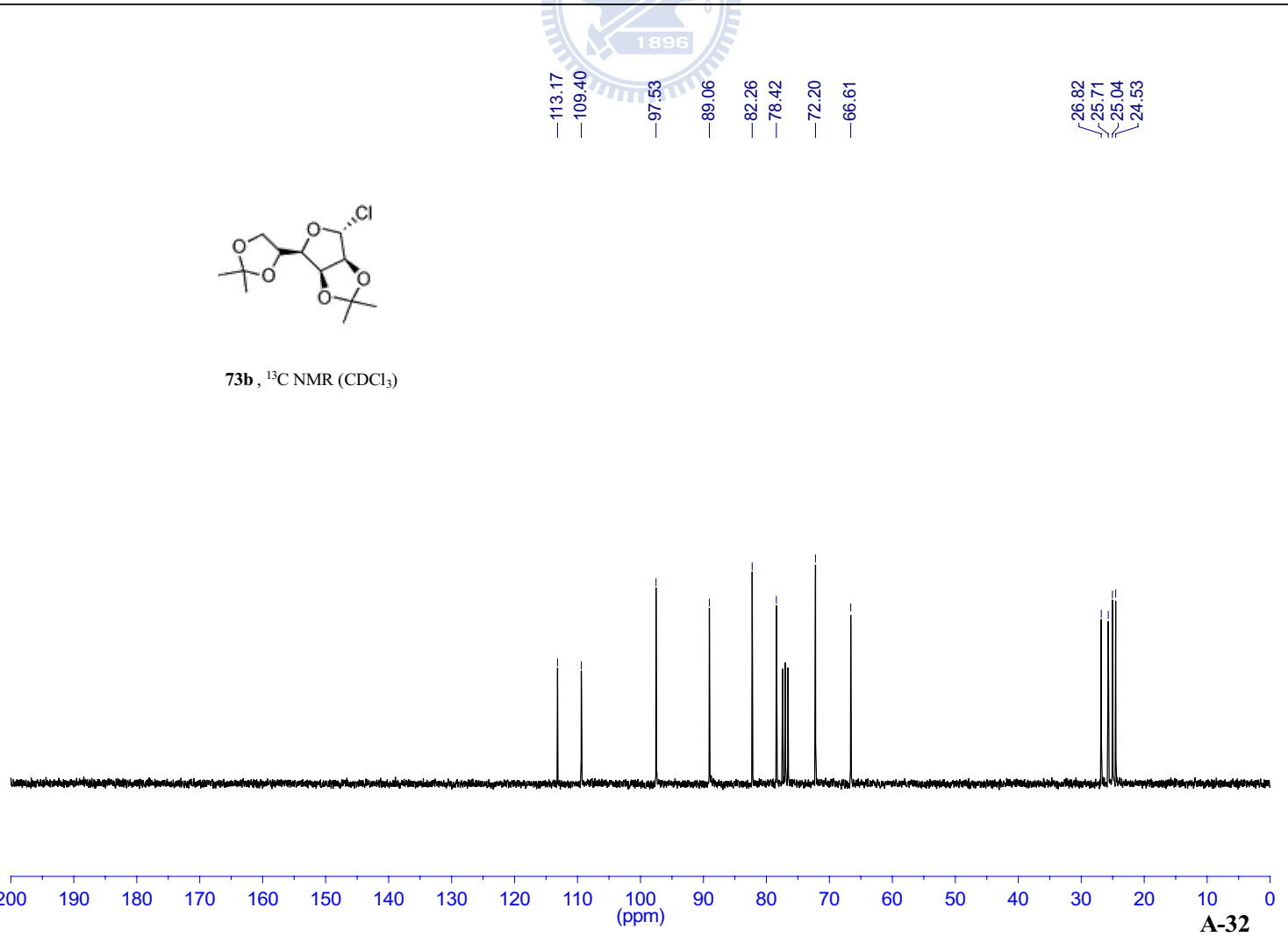
72b, ^{1}H NMR ($CDCl_3$)



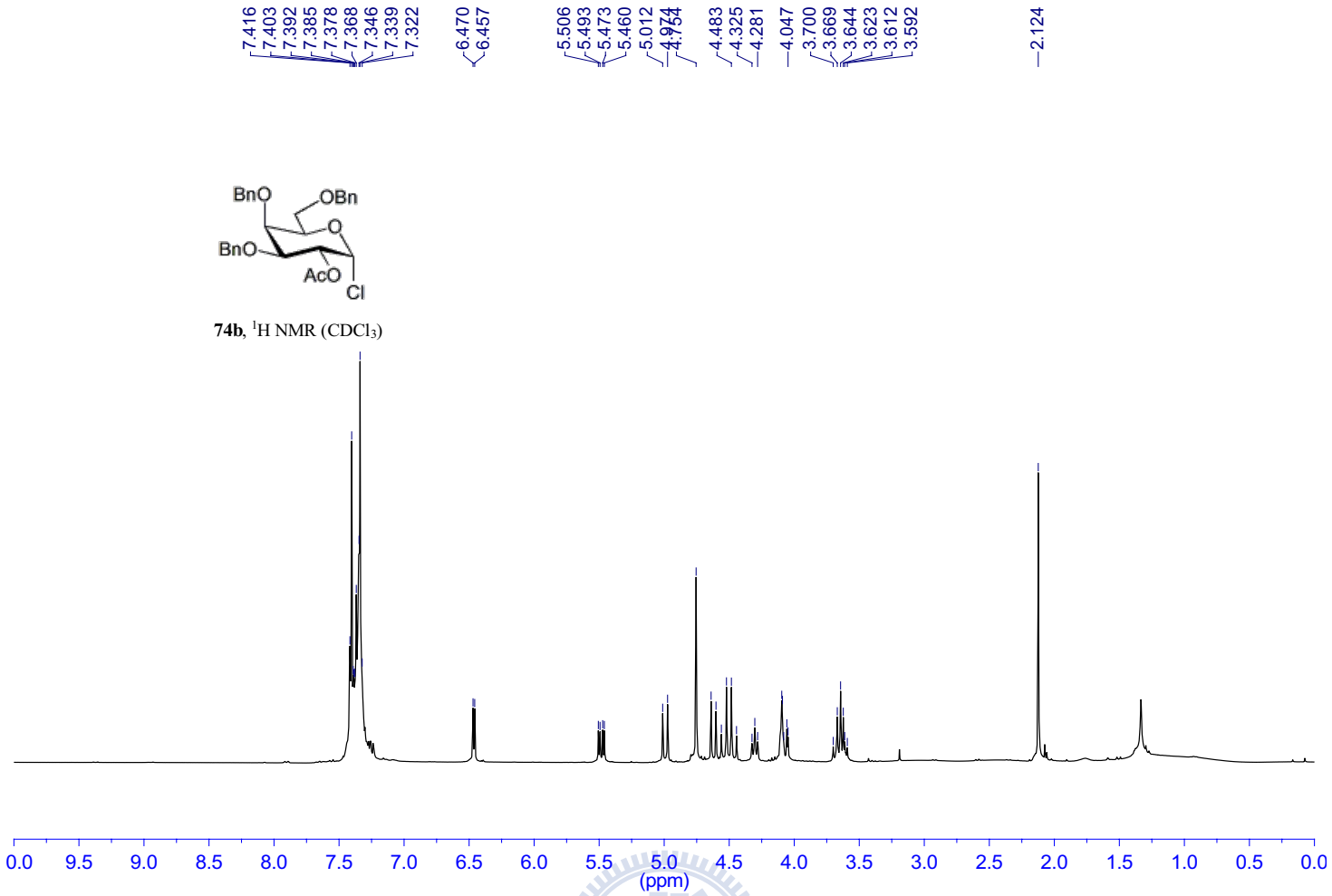
72b , ^{13}C NMR ($CDCl_3$)



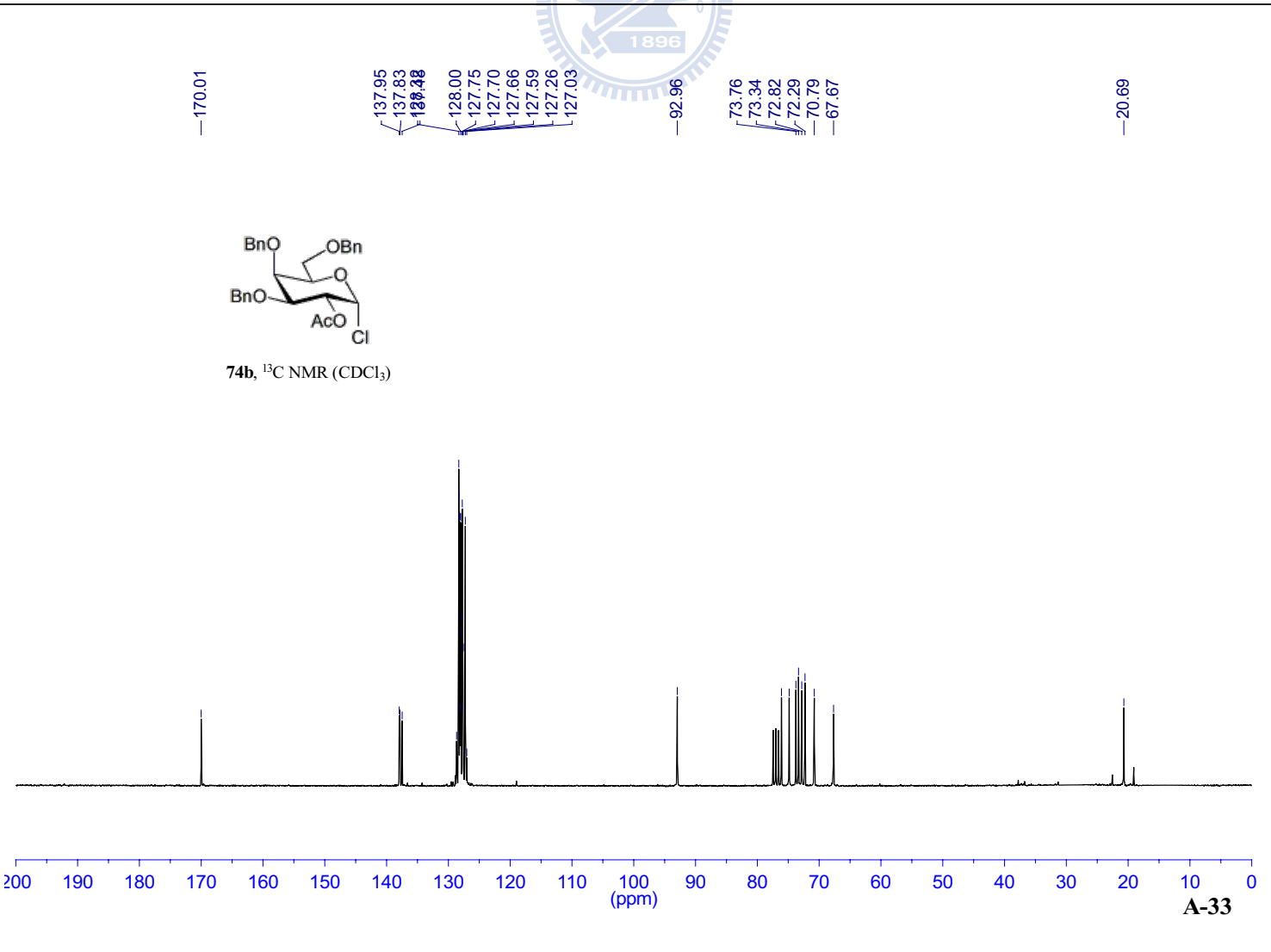
73b, ^{1}H NMR ($CDCl_3$)



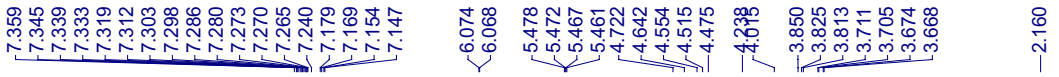
73b , ^{13}C NMR ($CDCl_3$)

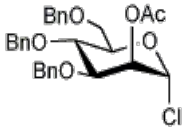


74b, ^{1}H NMR ($CDCl_3$)

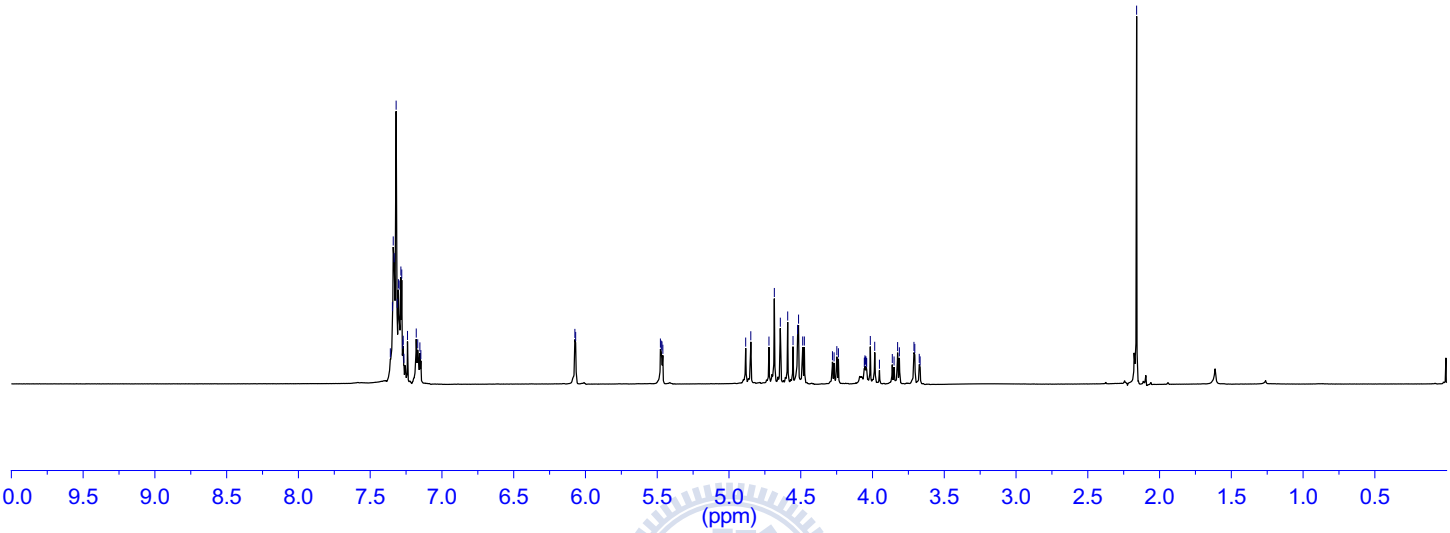


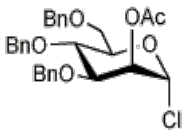
74b, ^{13}C NMR ($CDCl_3$)



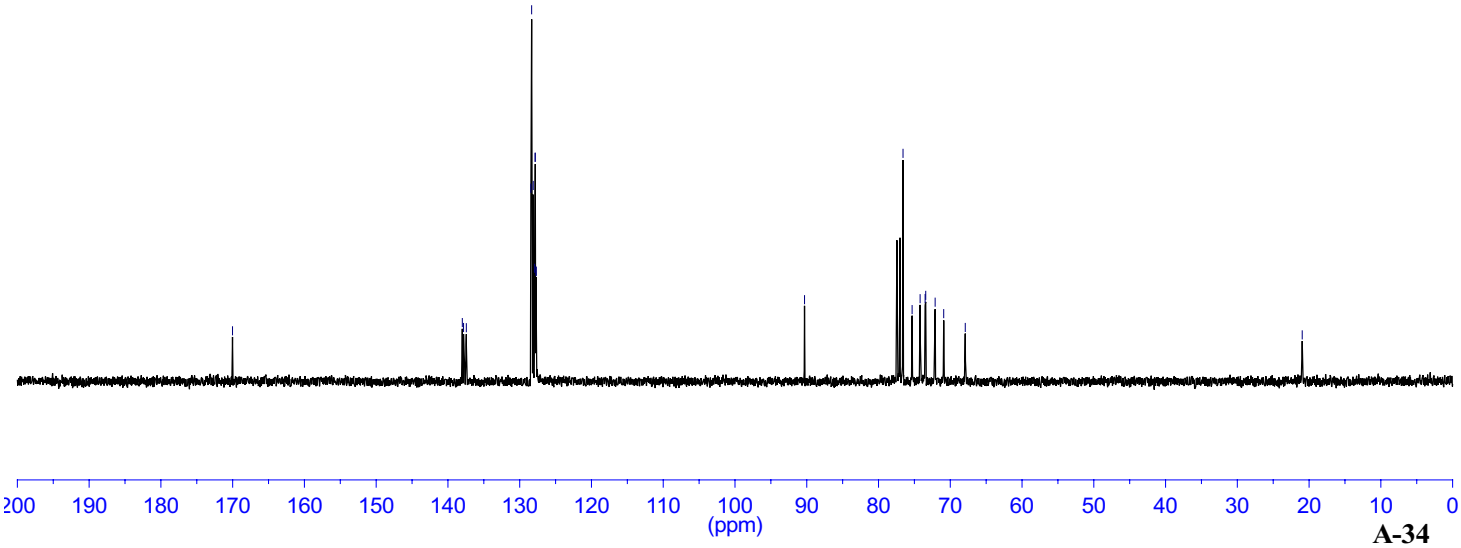


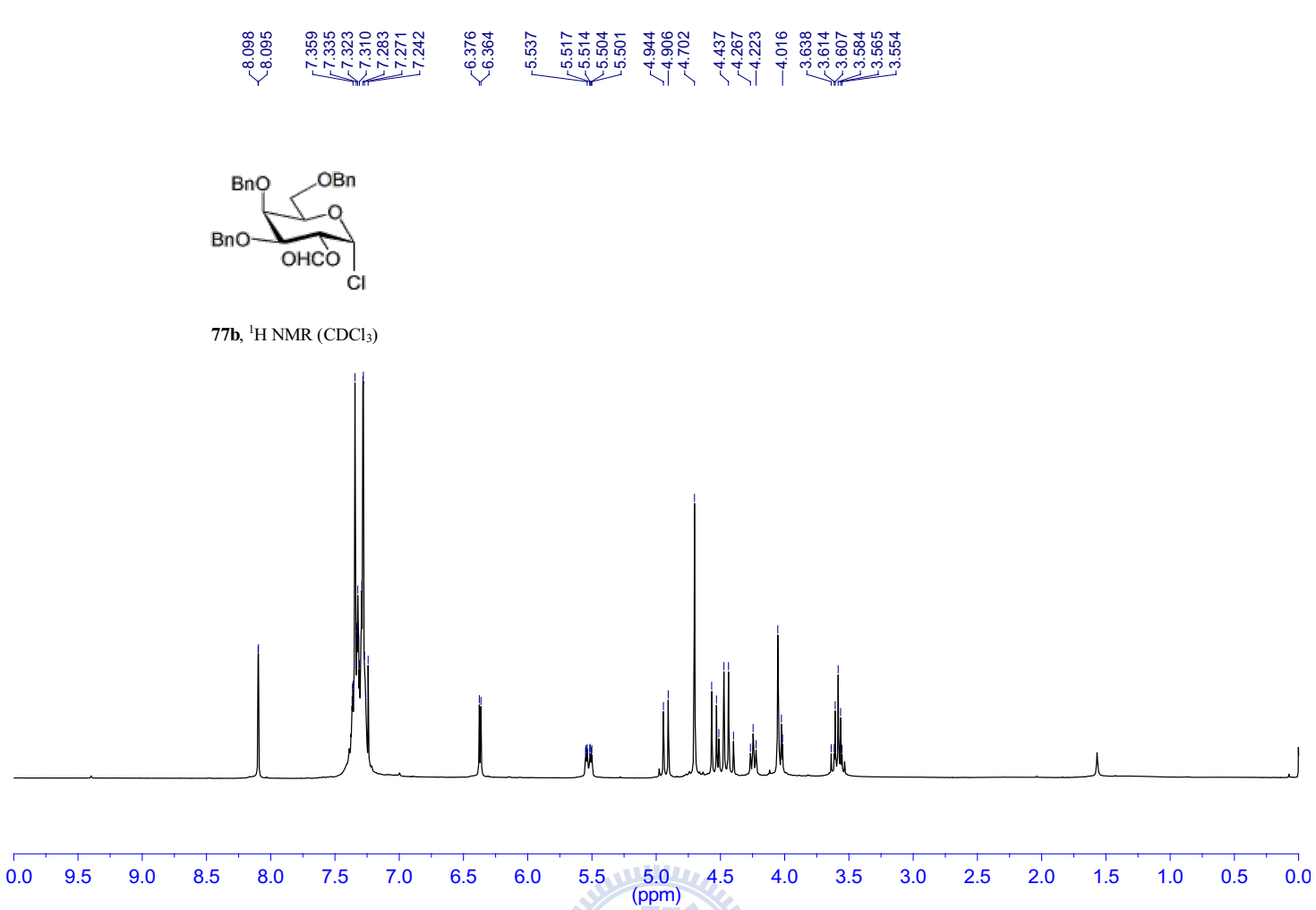
76b, ^{1}H NMR ($CDCl_3$)



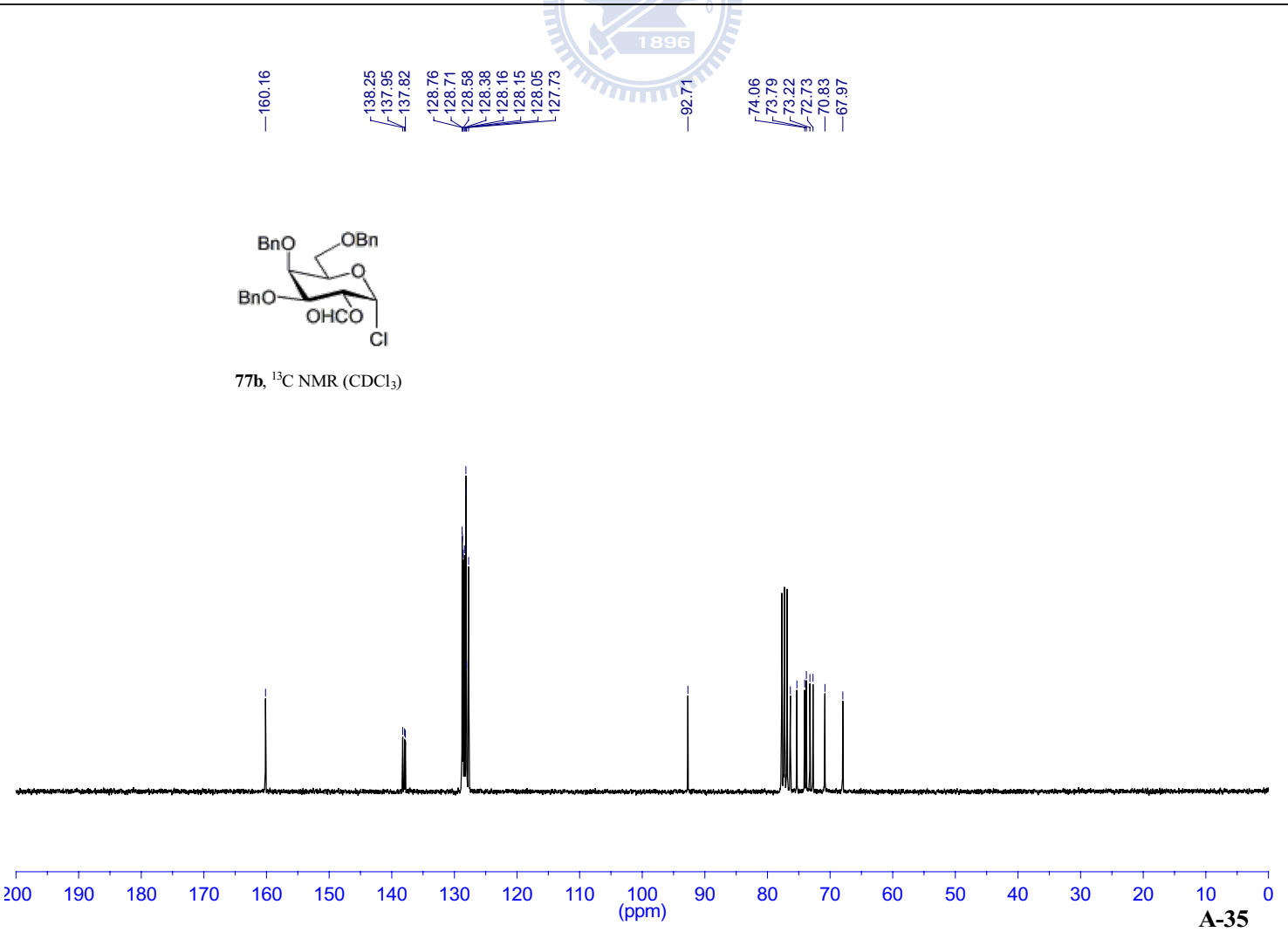


76b, ^{13}C NMR ($CDCl_3$)

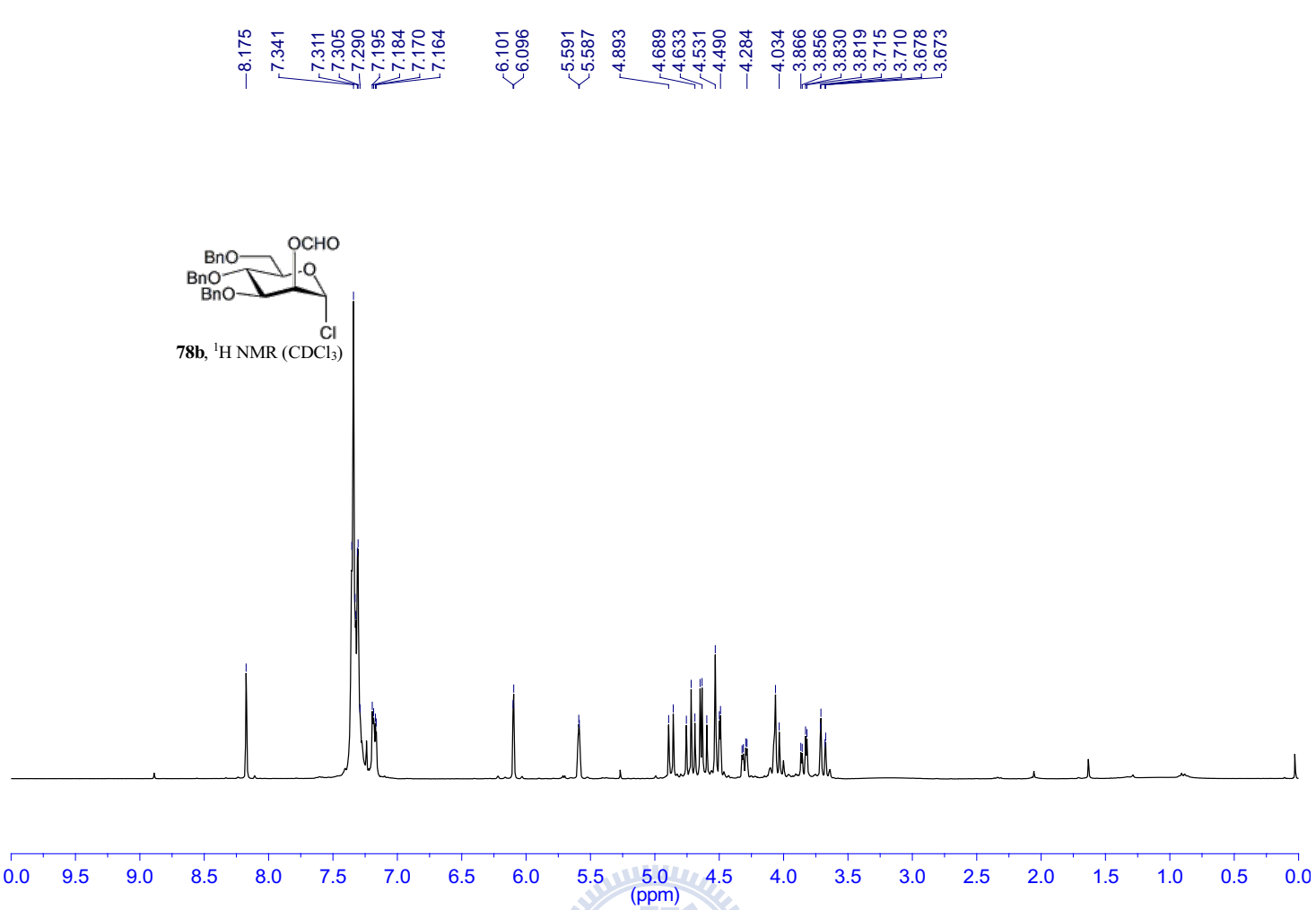




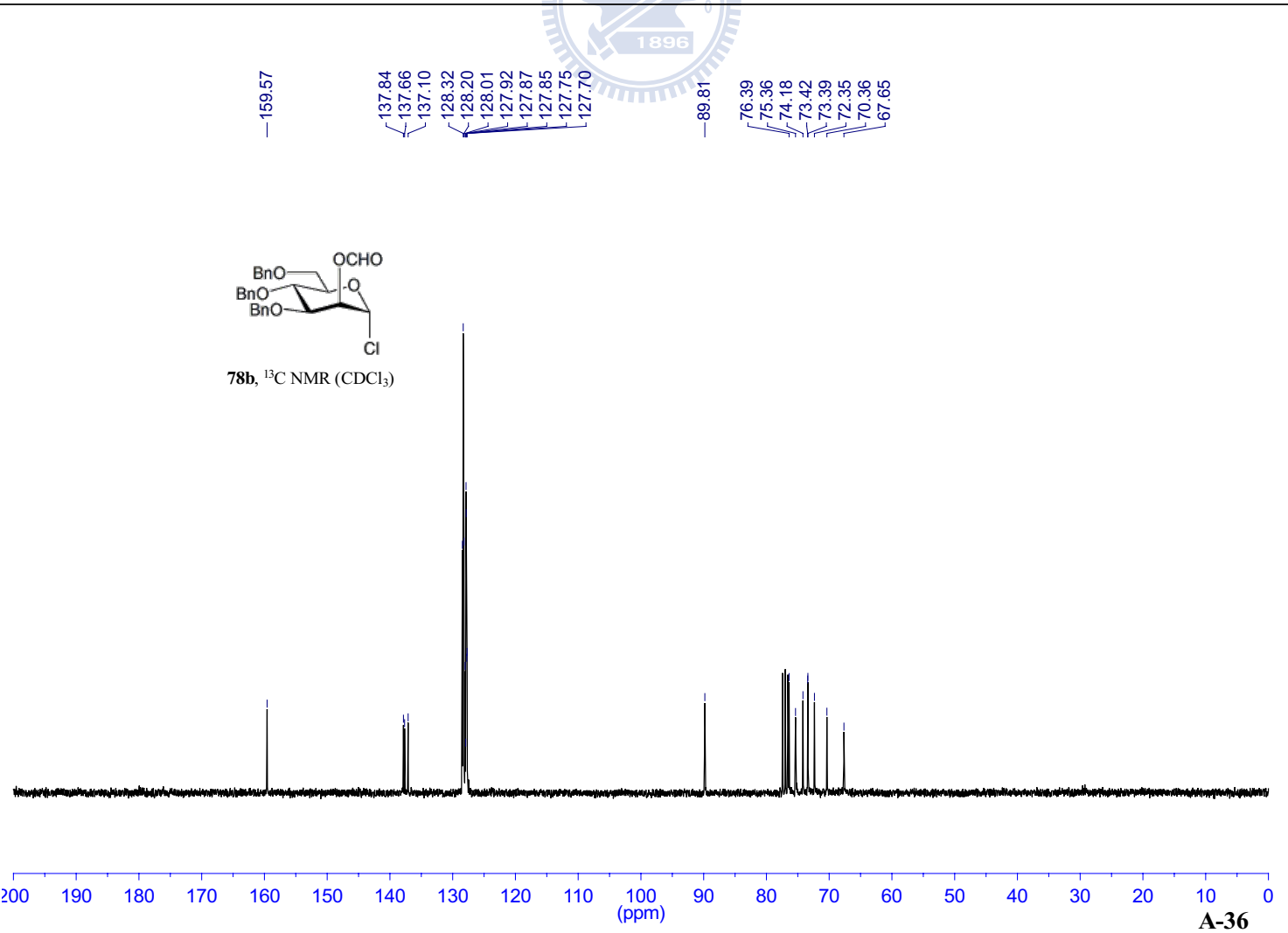
77b, ^{1}H NMR ($CDCl_3$)



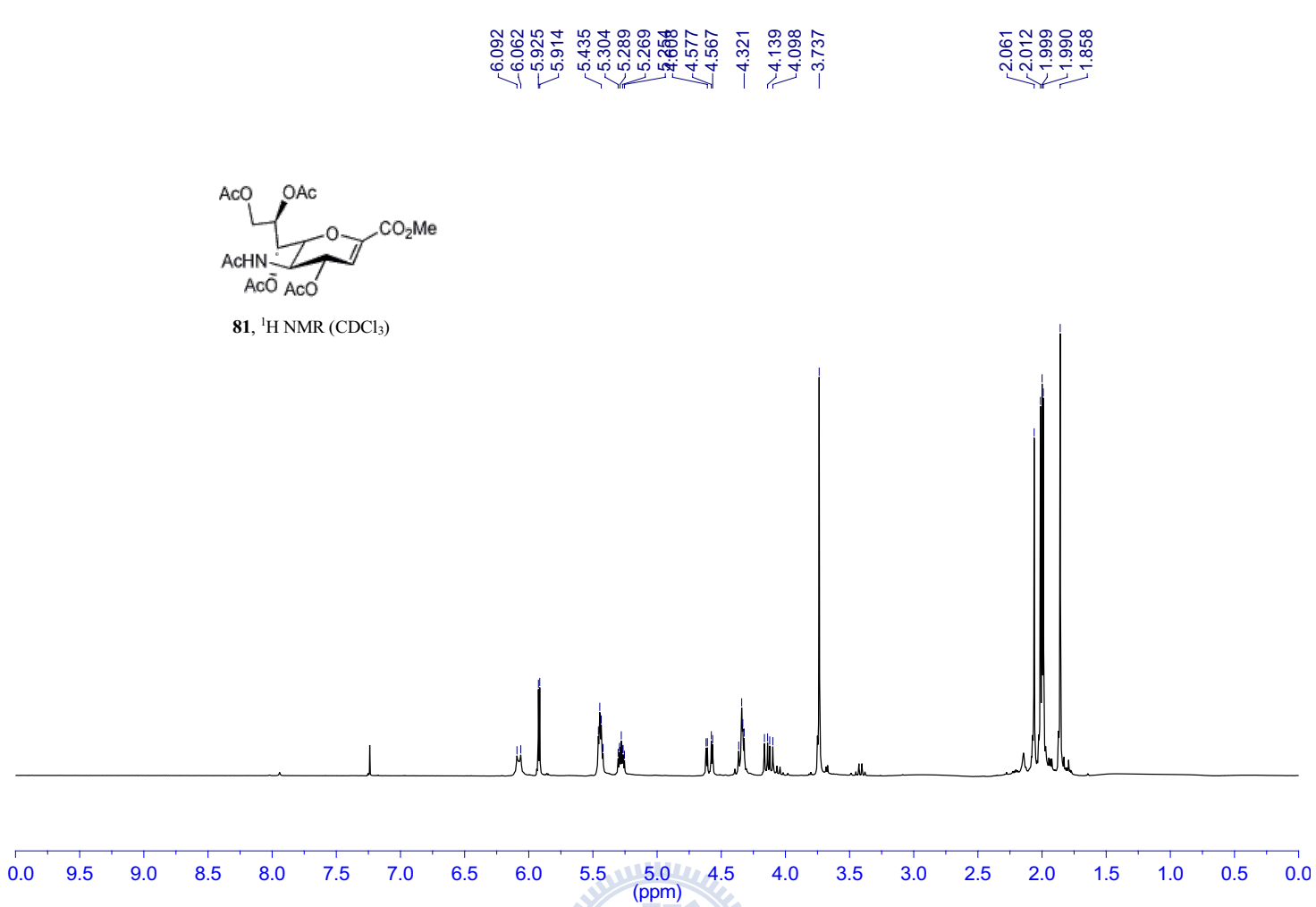
77b, ^{13}C NMR ($CDCl_3$)



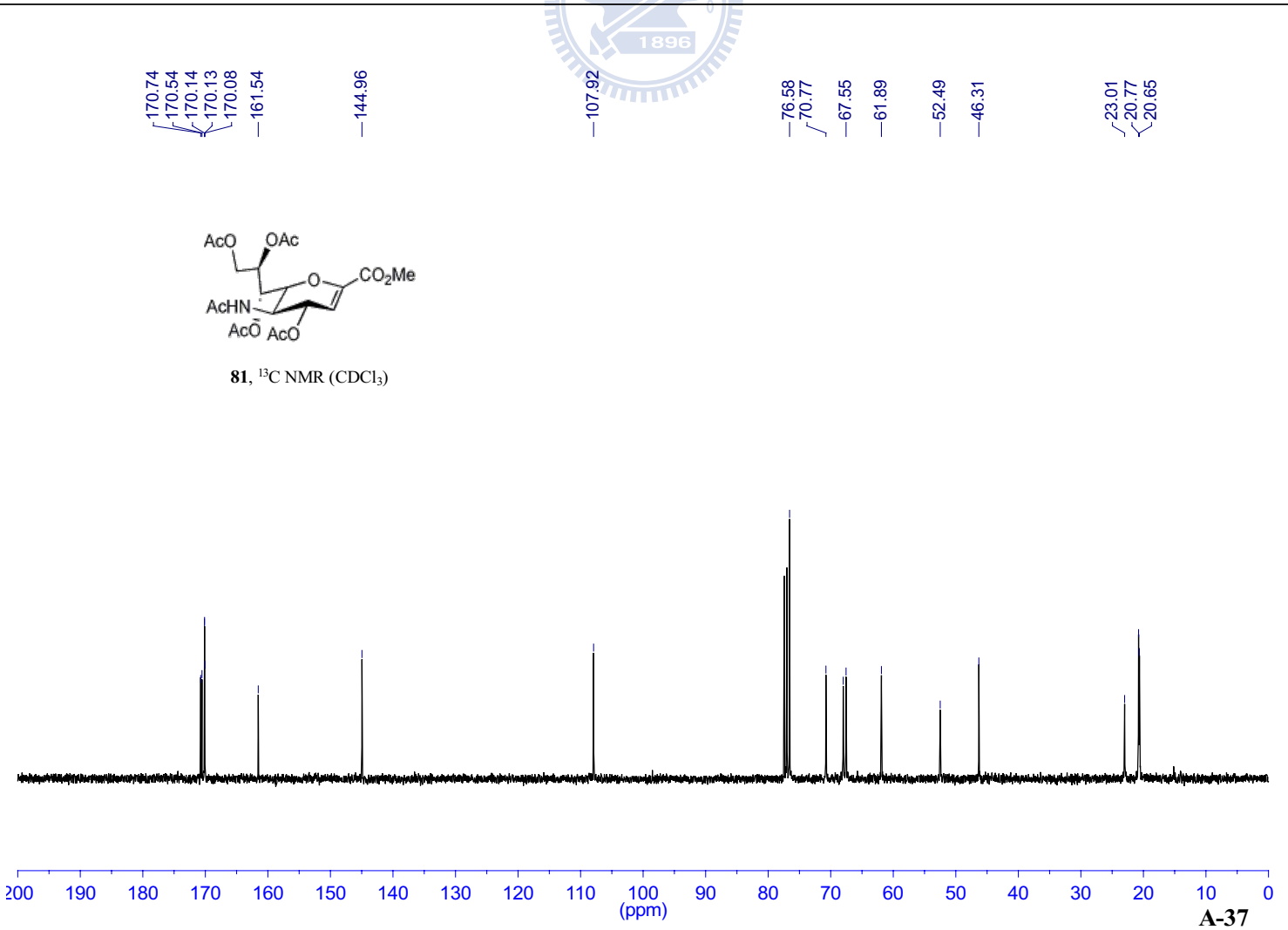
78b, ^{1}H NMR ($CDCl_3$)



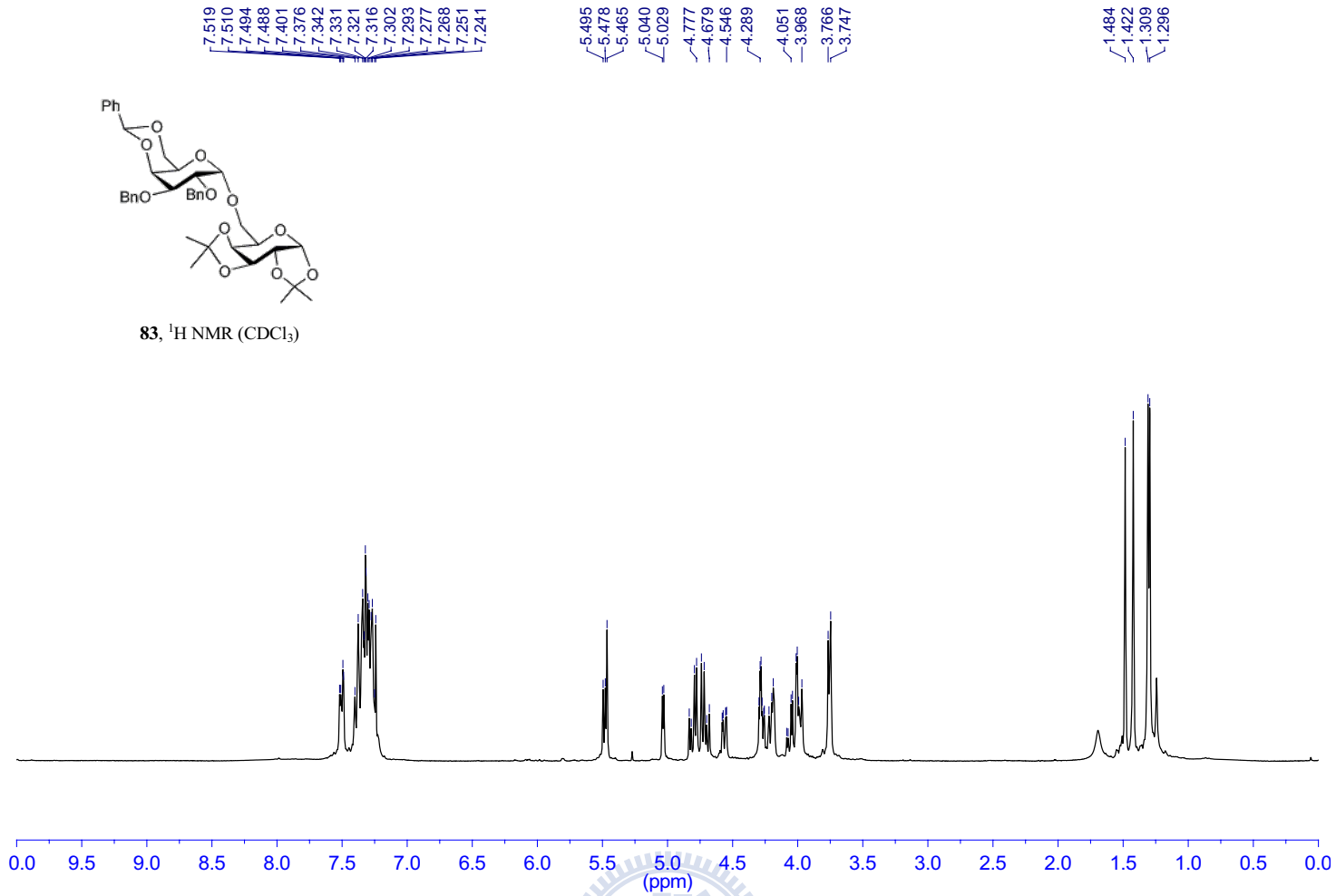
78b, ^{13}C NMR ($CDCl_3$)



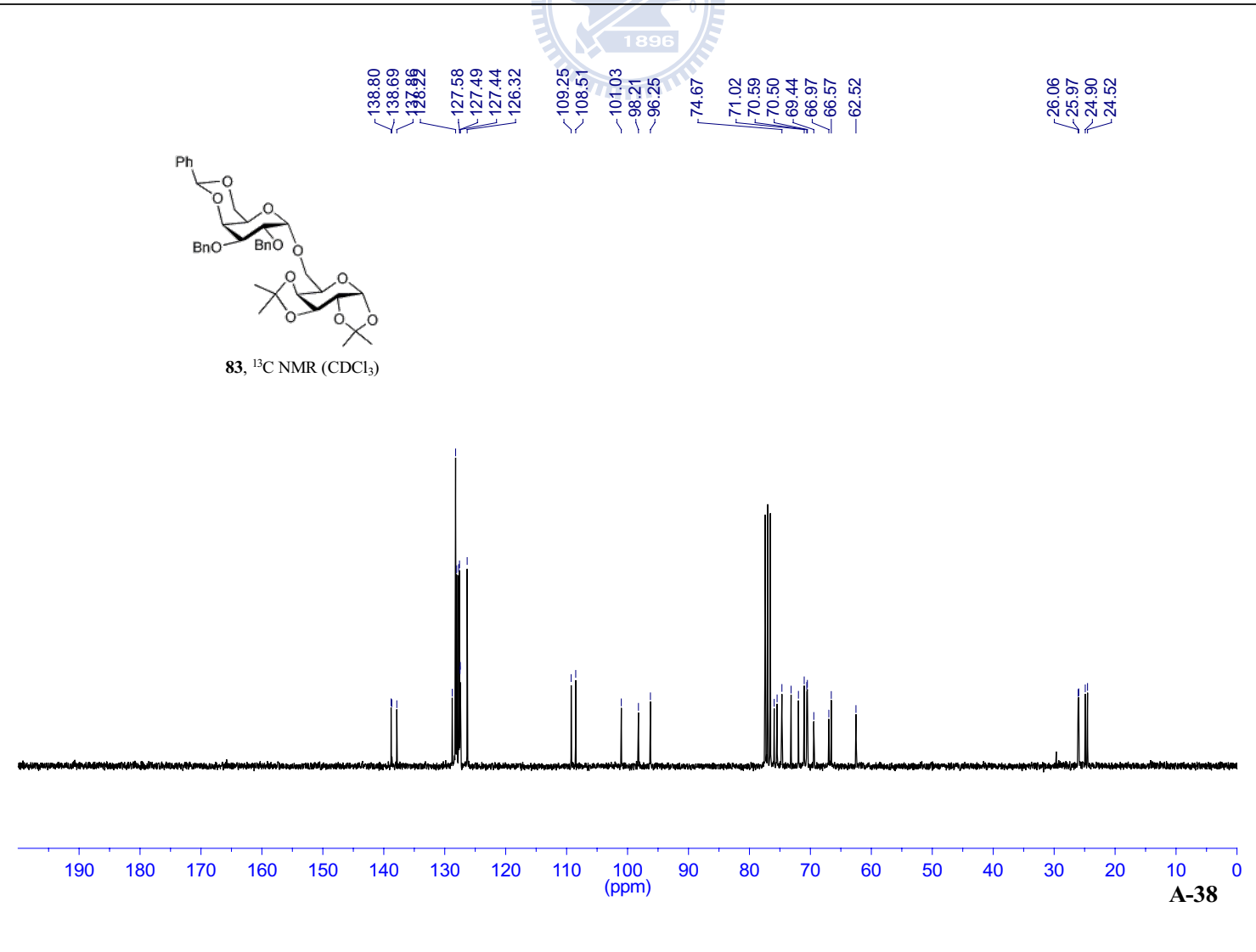
81, ^{1}H NMR ($CDCl_3$)



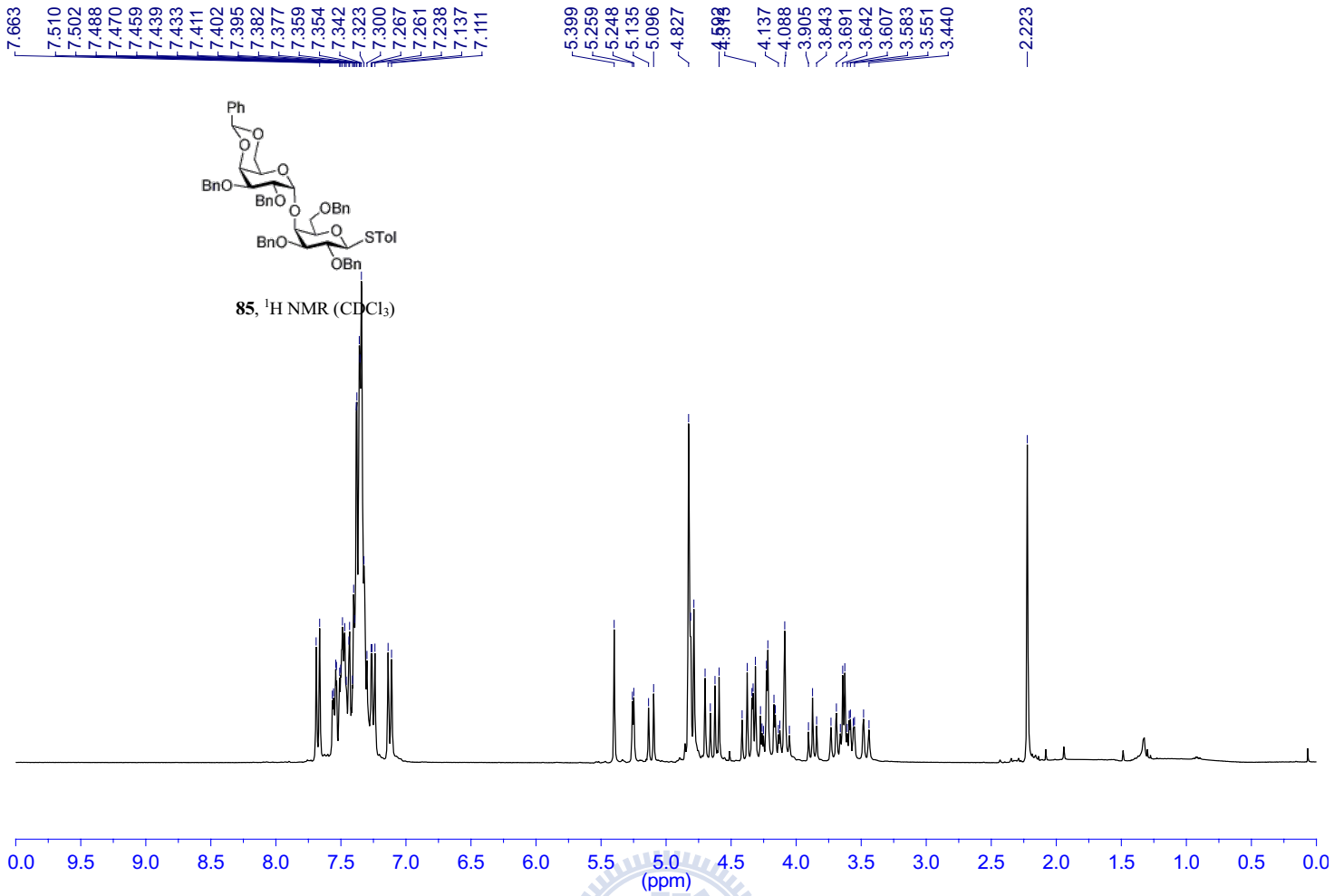
81, ^{13}C NMR ($CDCl_3$)



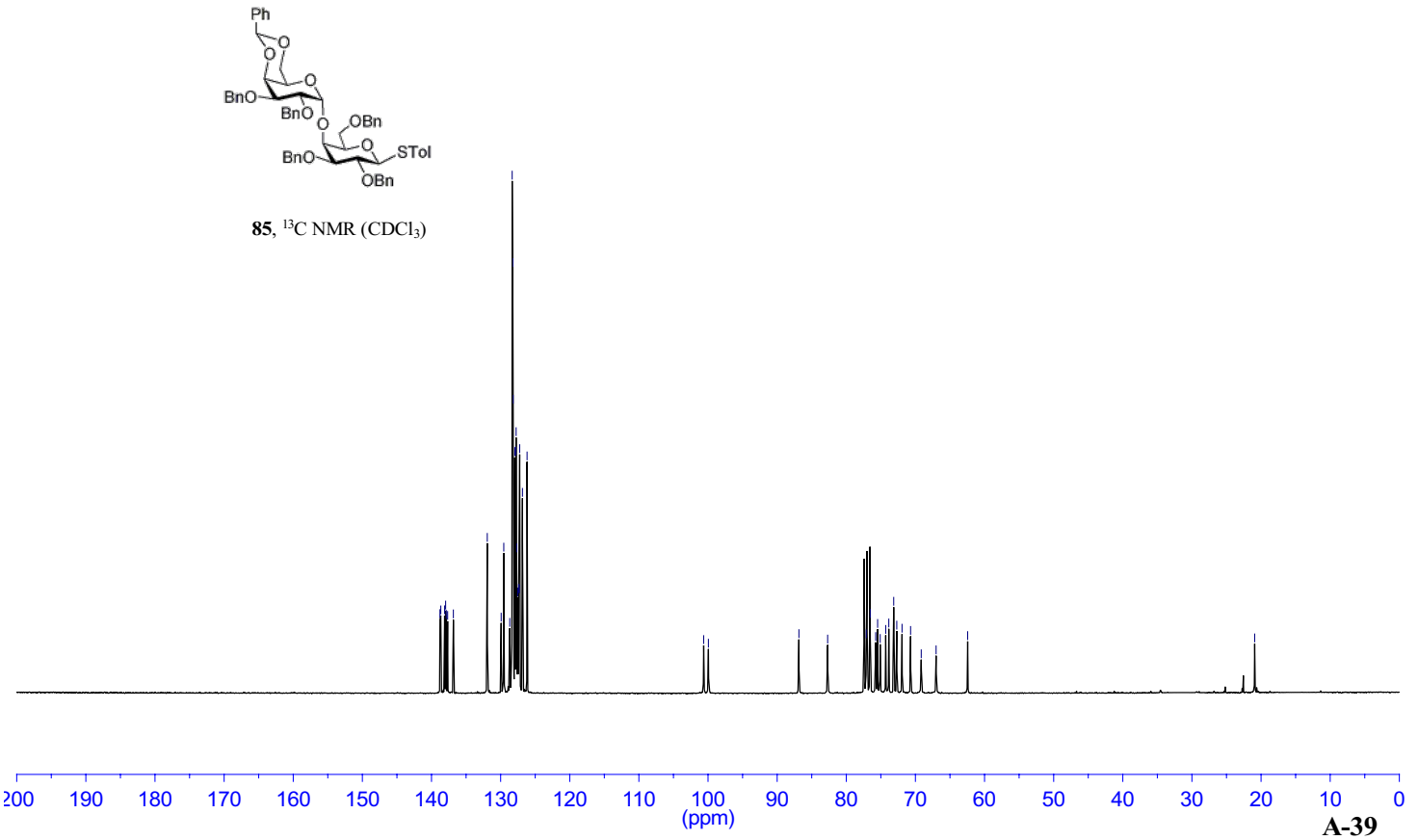
83, ^{1}H NMR ($CDCl_3$)



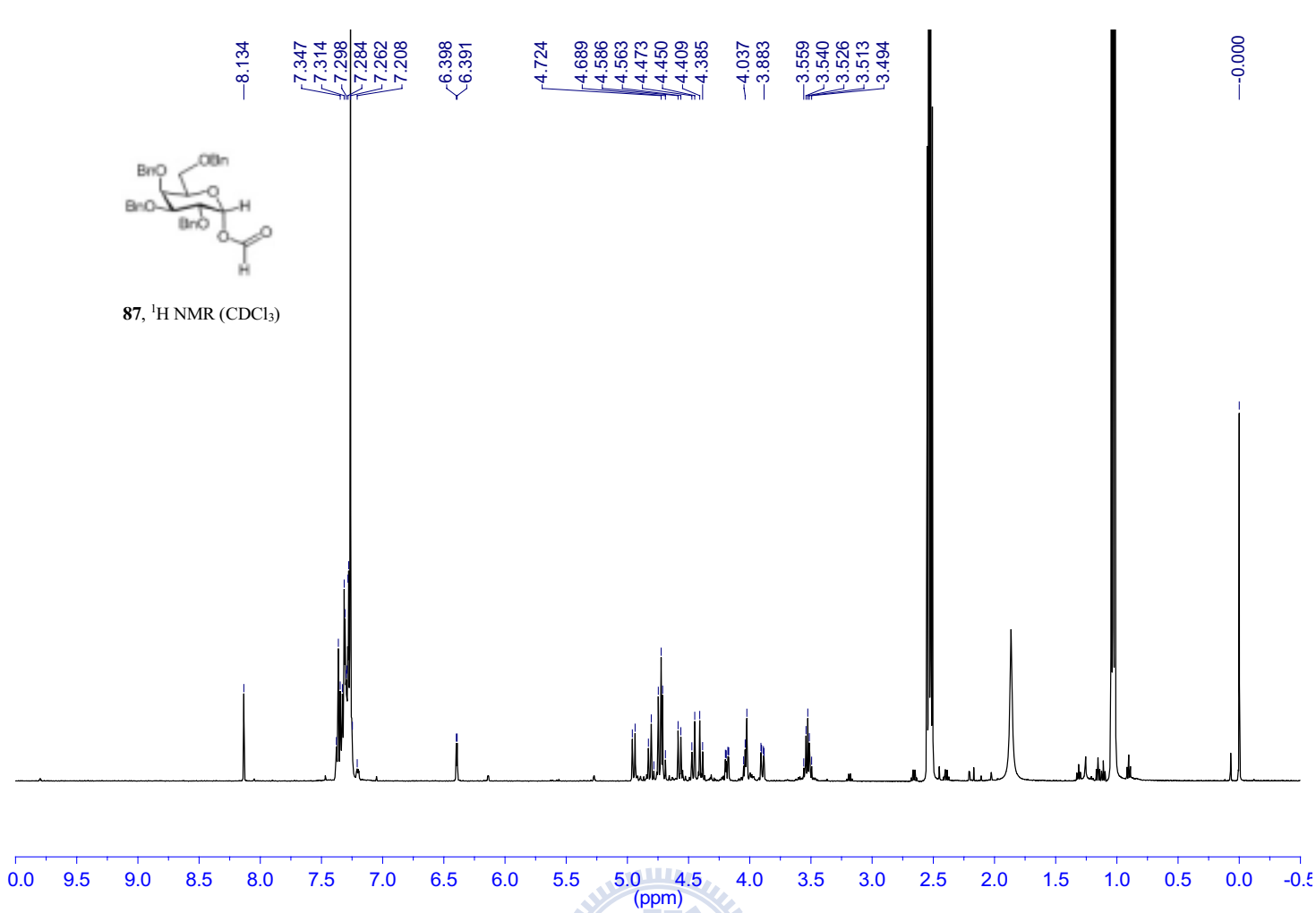
83, ^{13}C NMR ($CDCl_3$)



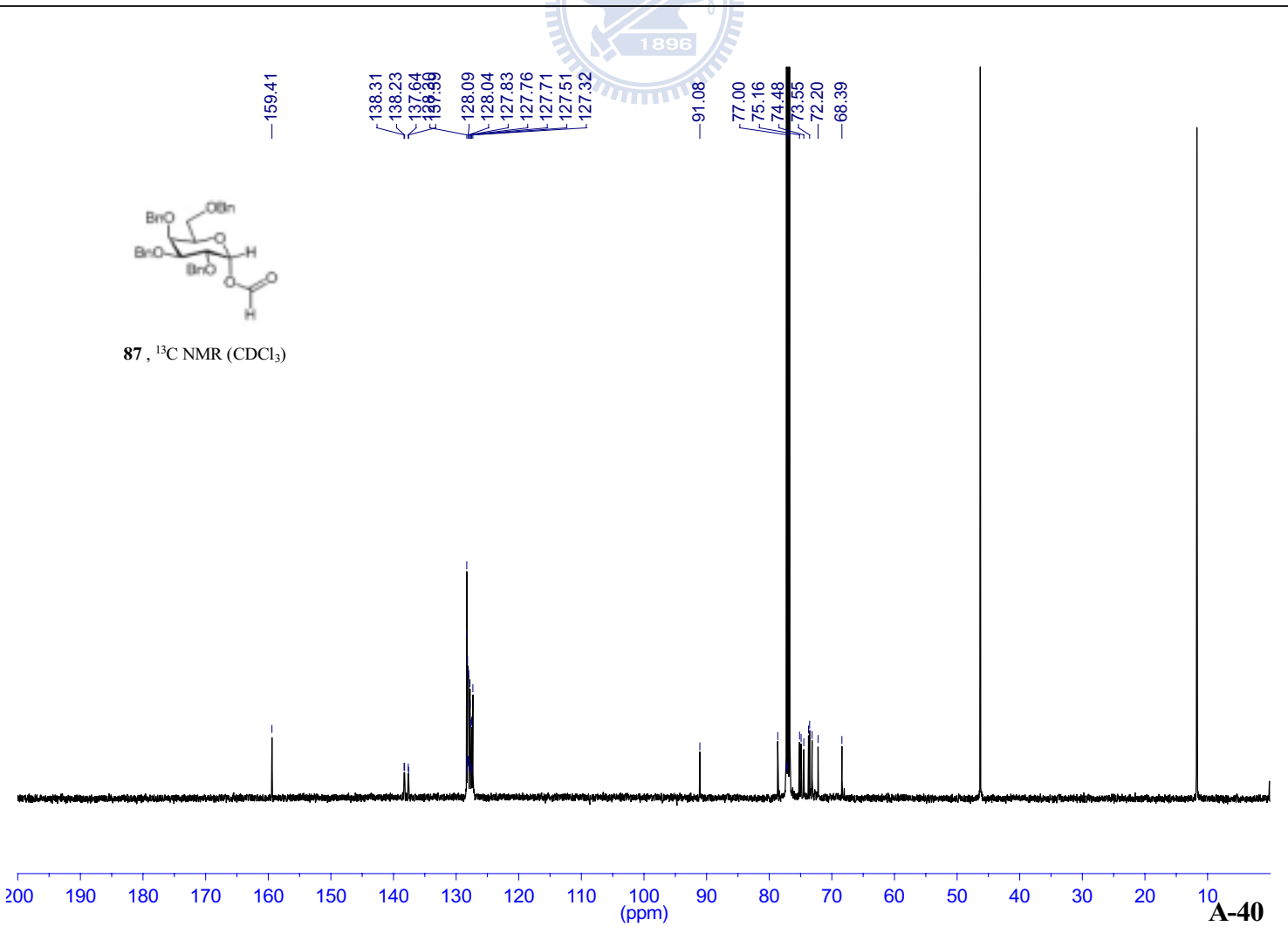
85, ^{1}H NMR ($CDCl_3$)



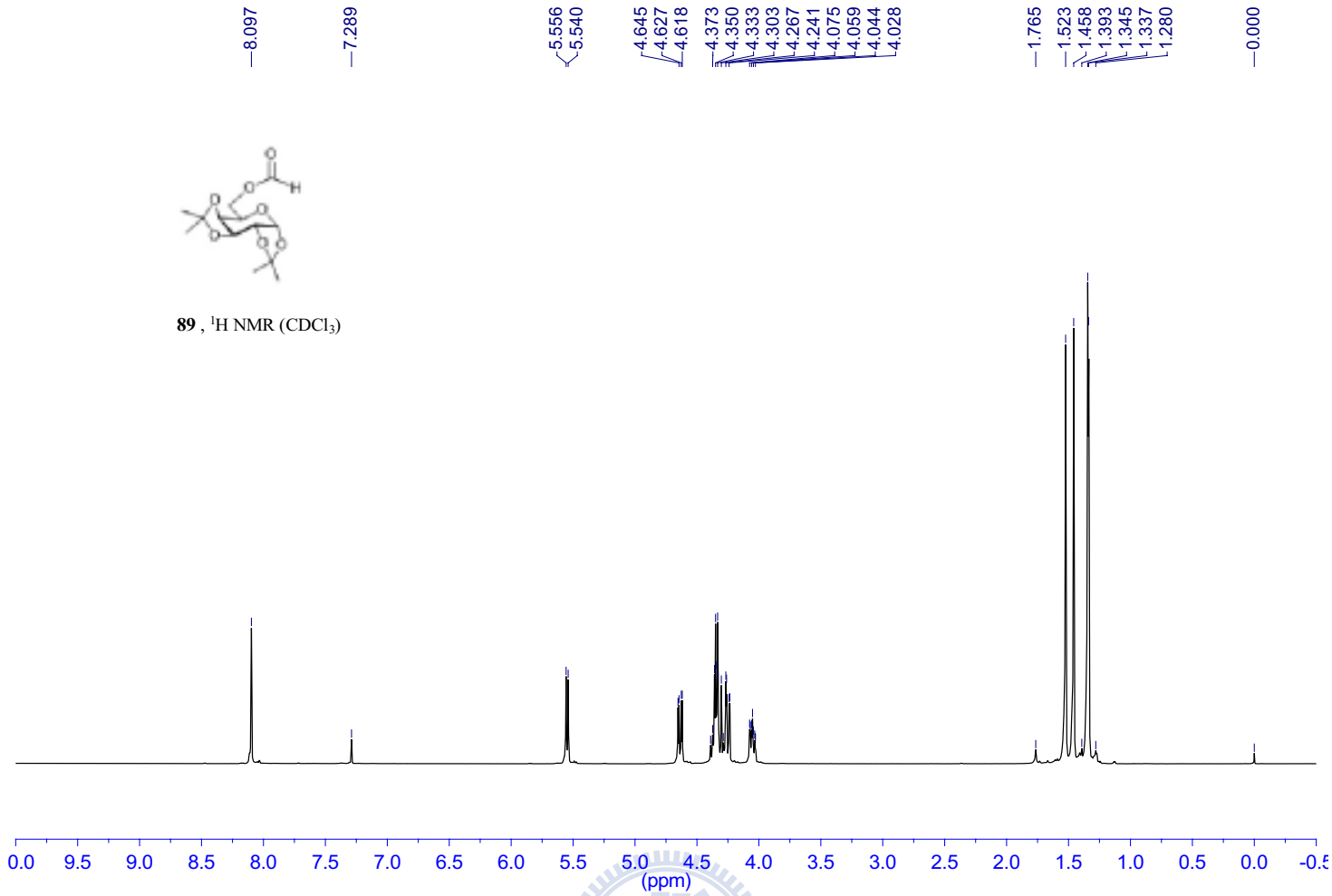
85, ^{13}C NMR ($CDCl_3$)



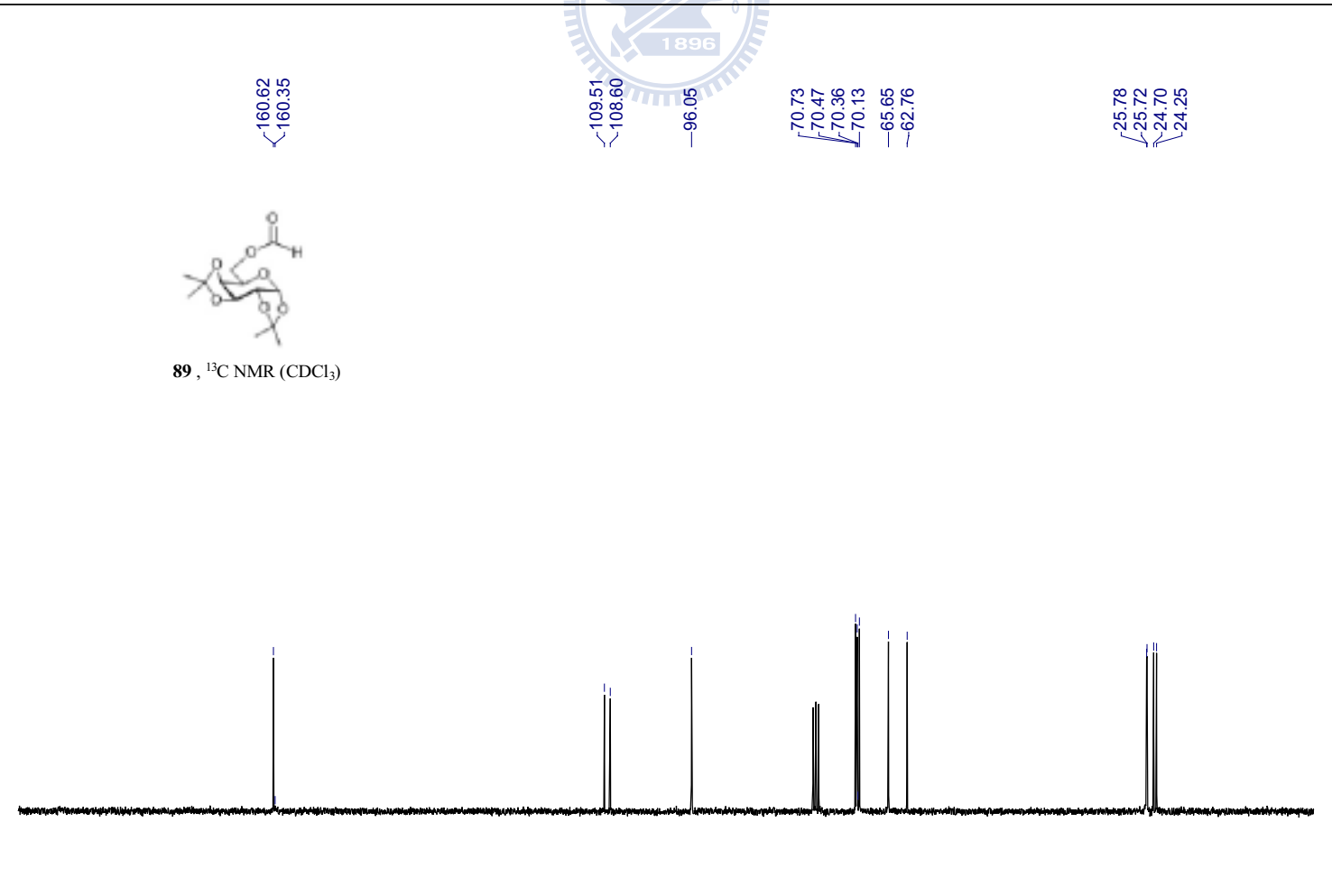
87, ^{1}H NMR ($CDCl_3$)



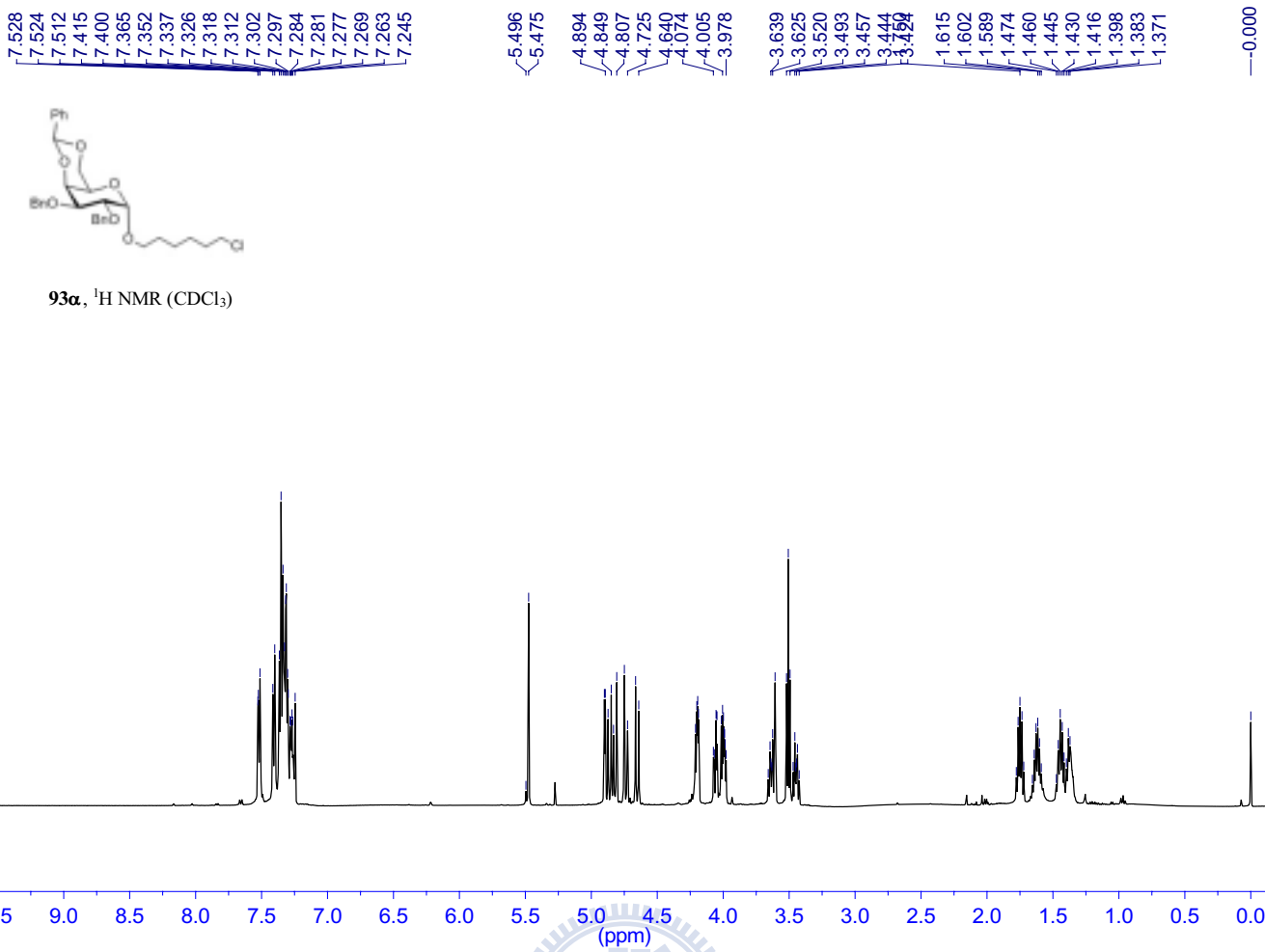
87 , ^{13}C NMR ($CDCl_3$)



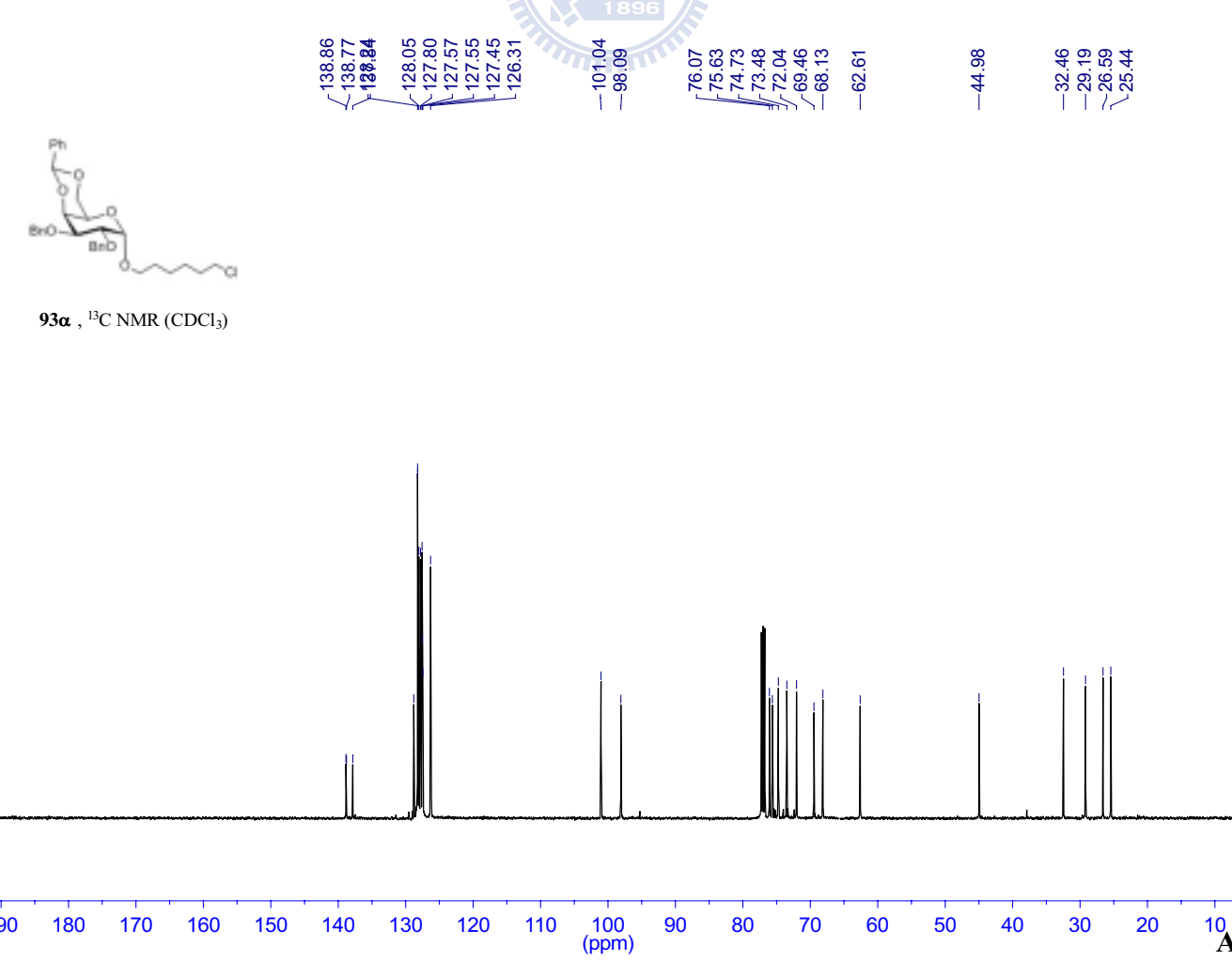
89 , ^{1}H NMR ($CDCl_3$)



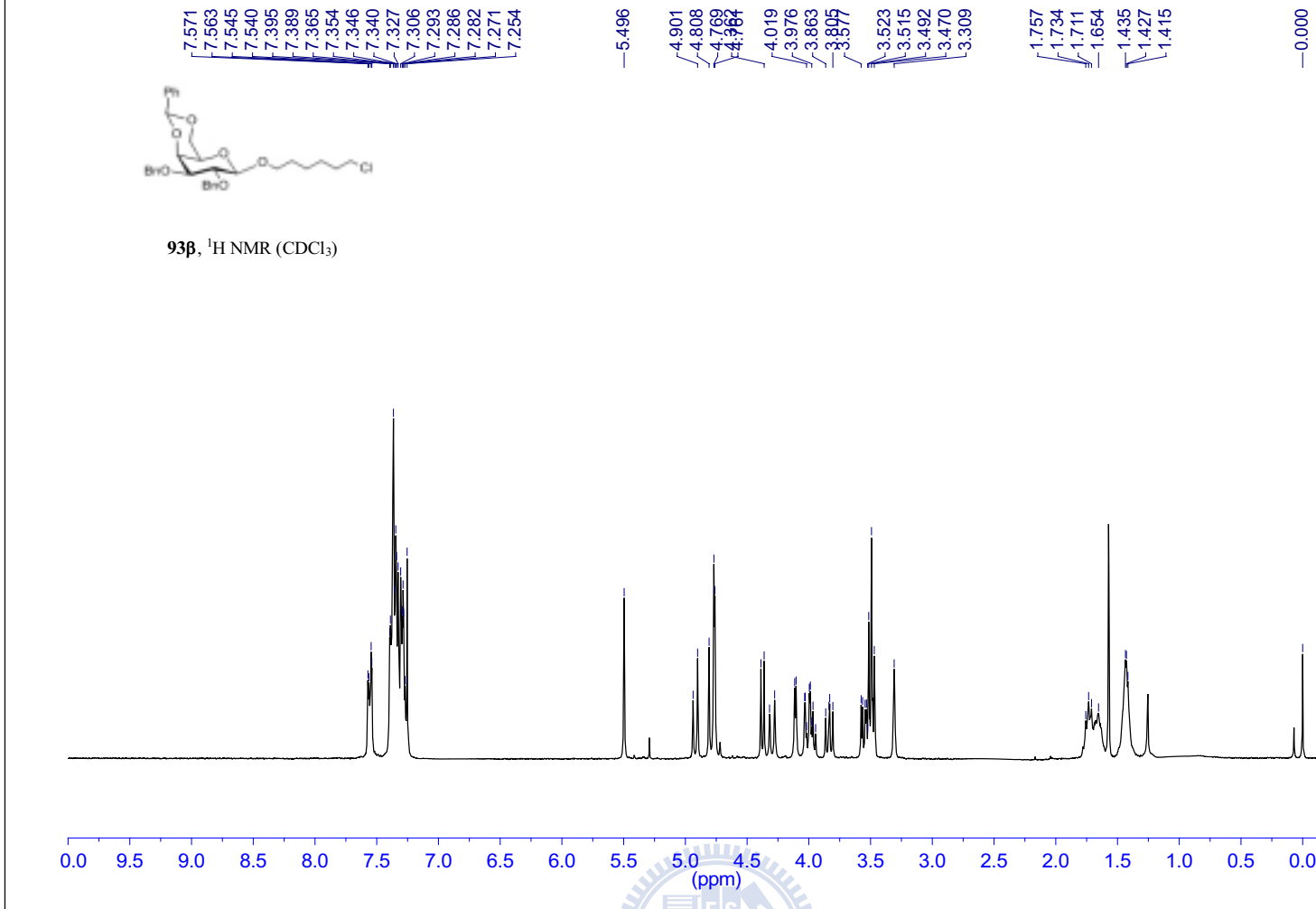
89 , ^{13}C NMR ($CDCl_3$)



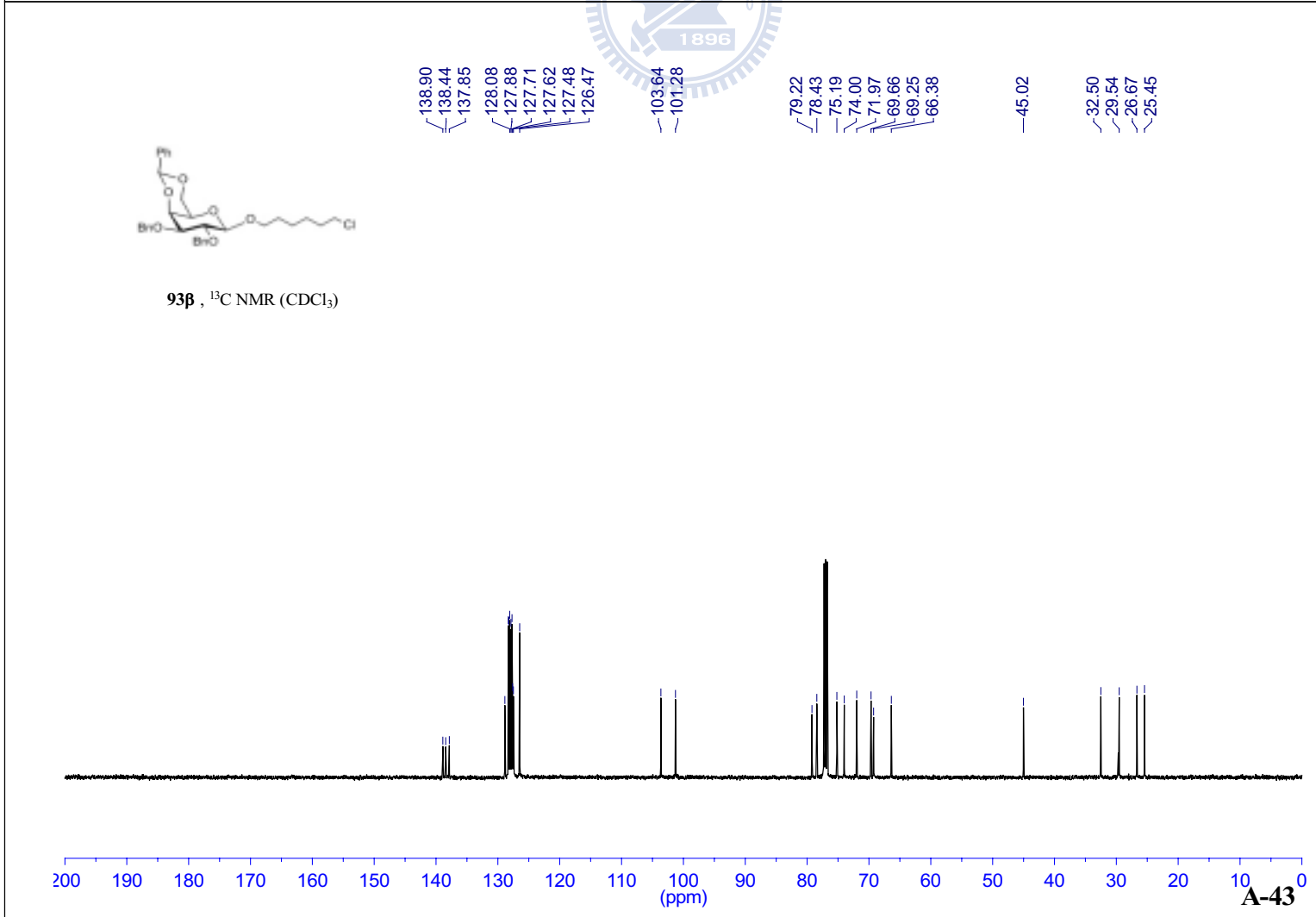
93α, ^{1}H NMR ($CDCl_3$)



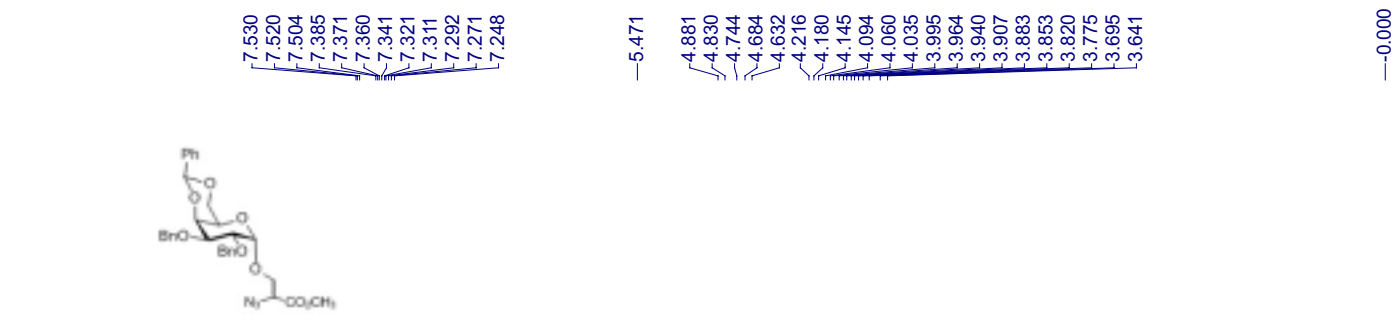
93α , ^{13}C NMR ($CDCl_3$)



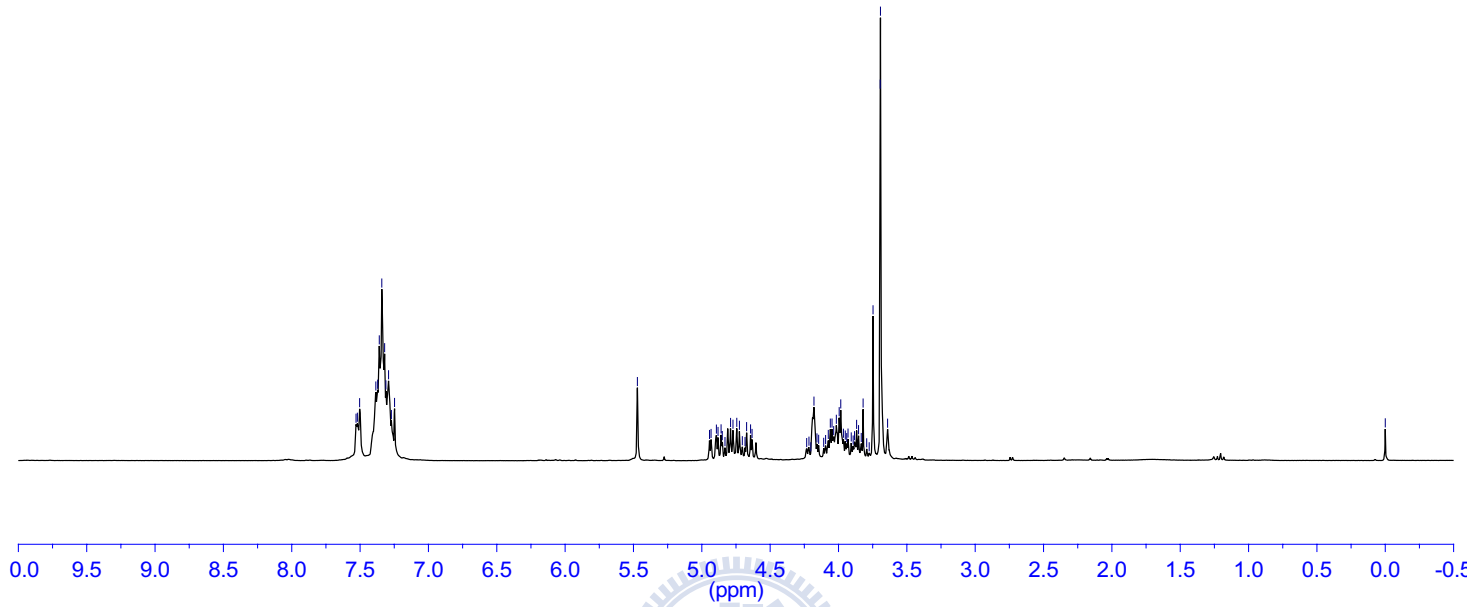
93β, ^{1}H NMR ($CDCl_3$)

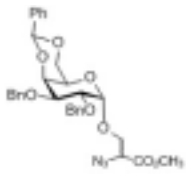


93β , ^{13}C NMR ($CDCl_3$)

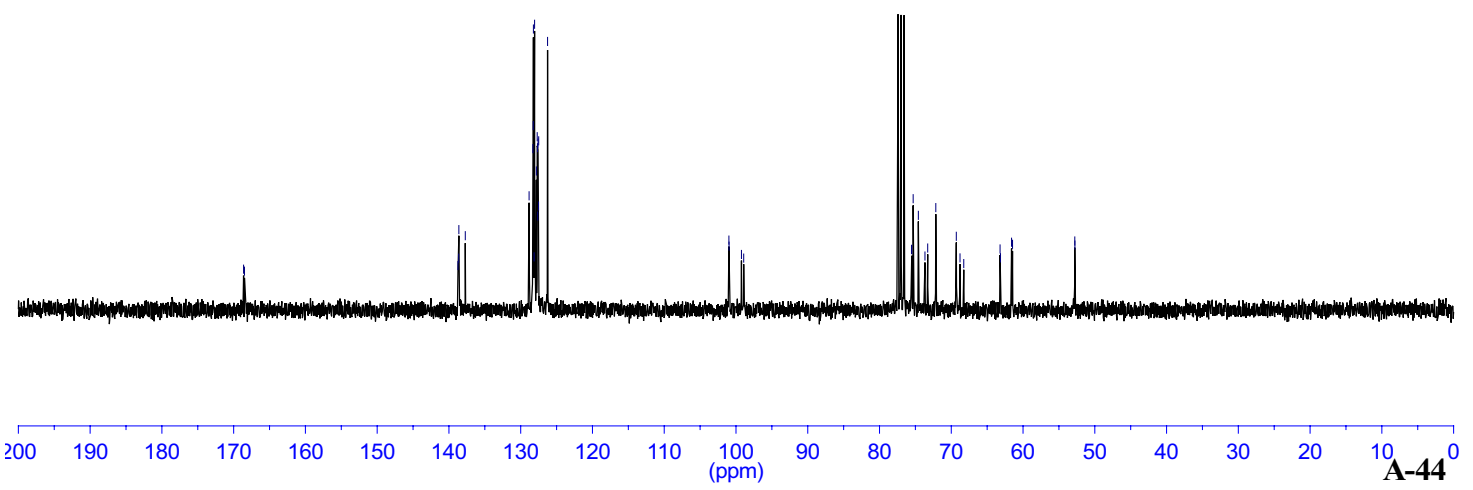


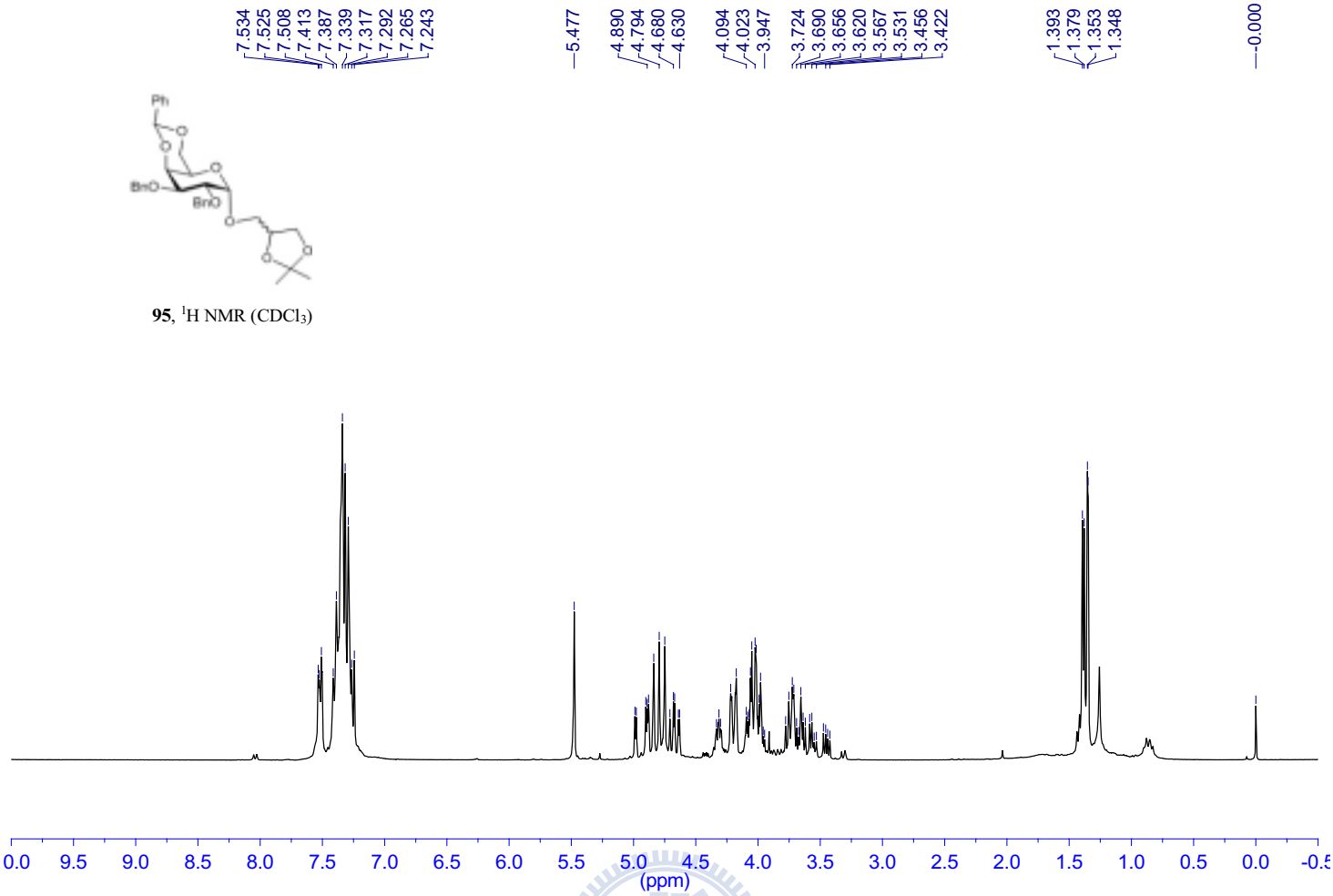
94, ^{1}H NMR ($CDCl_3$)



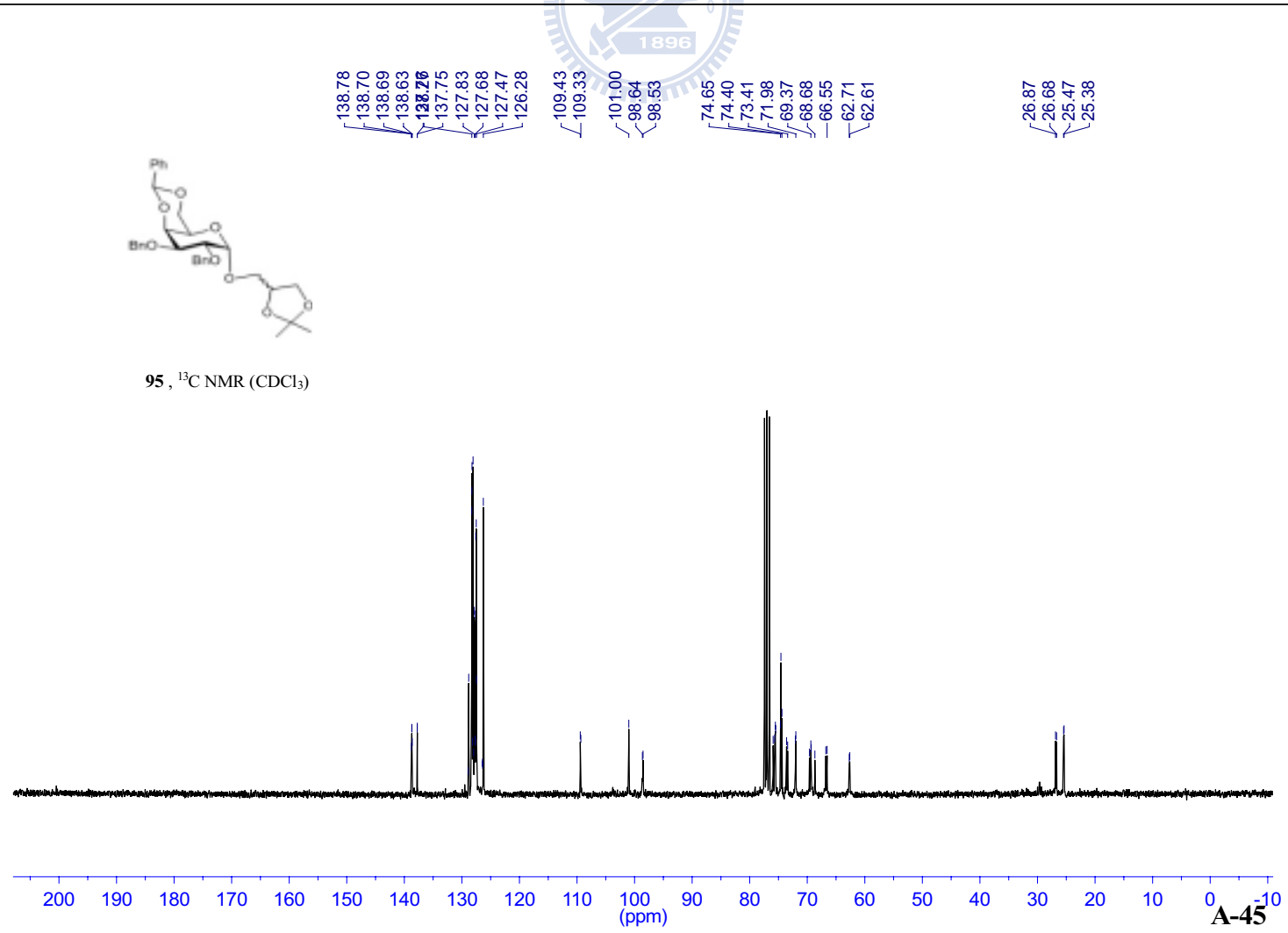


94 , ^{13}C NMR ($CDCl_3$)

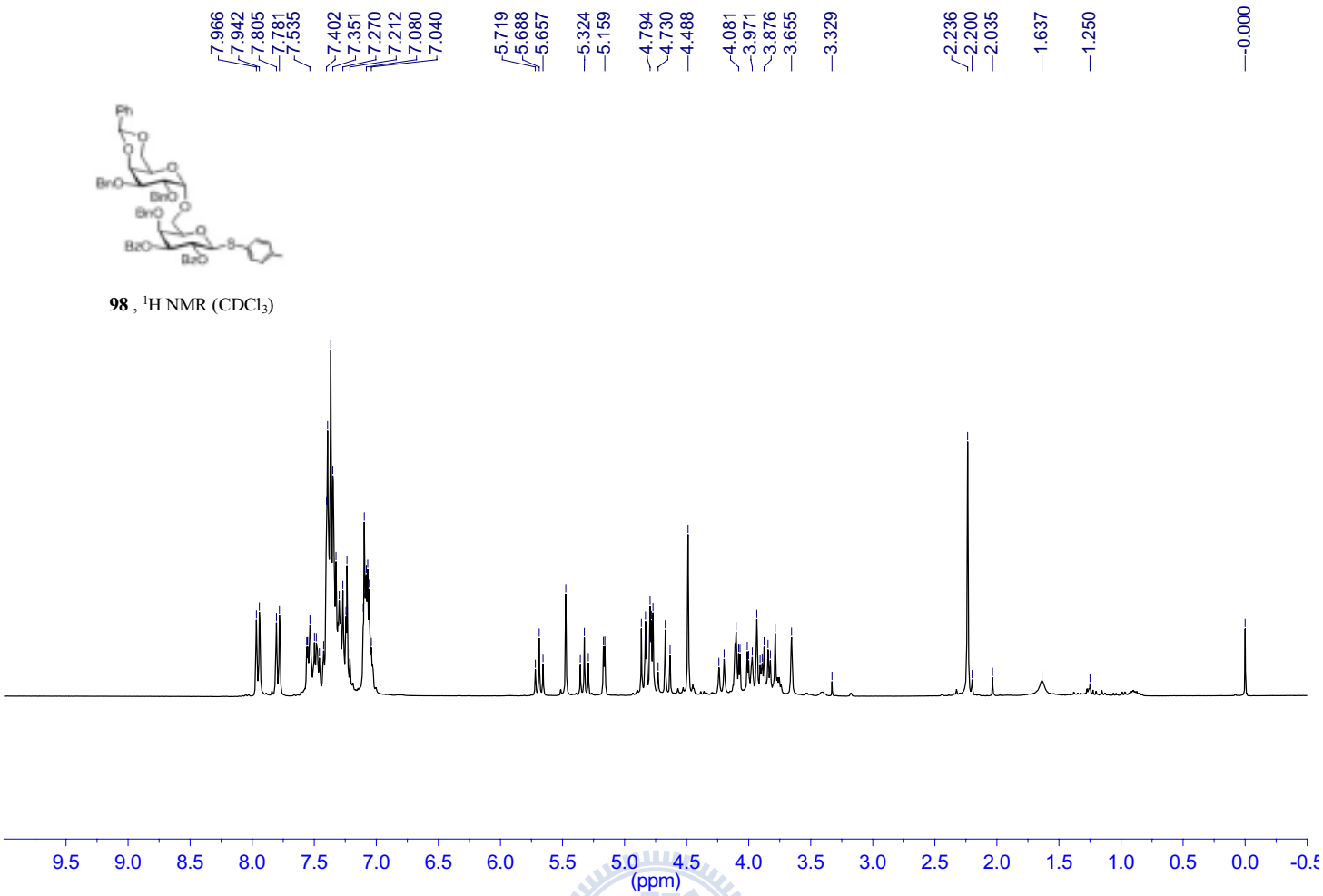




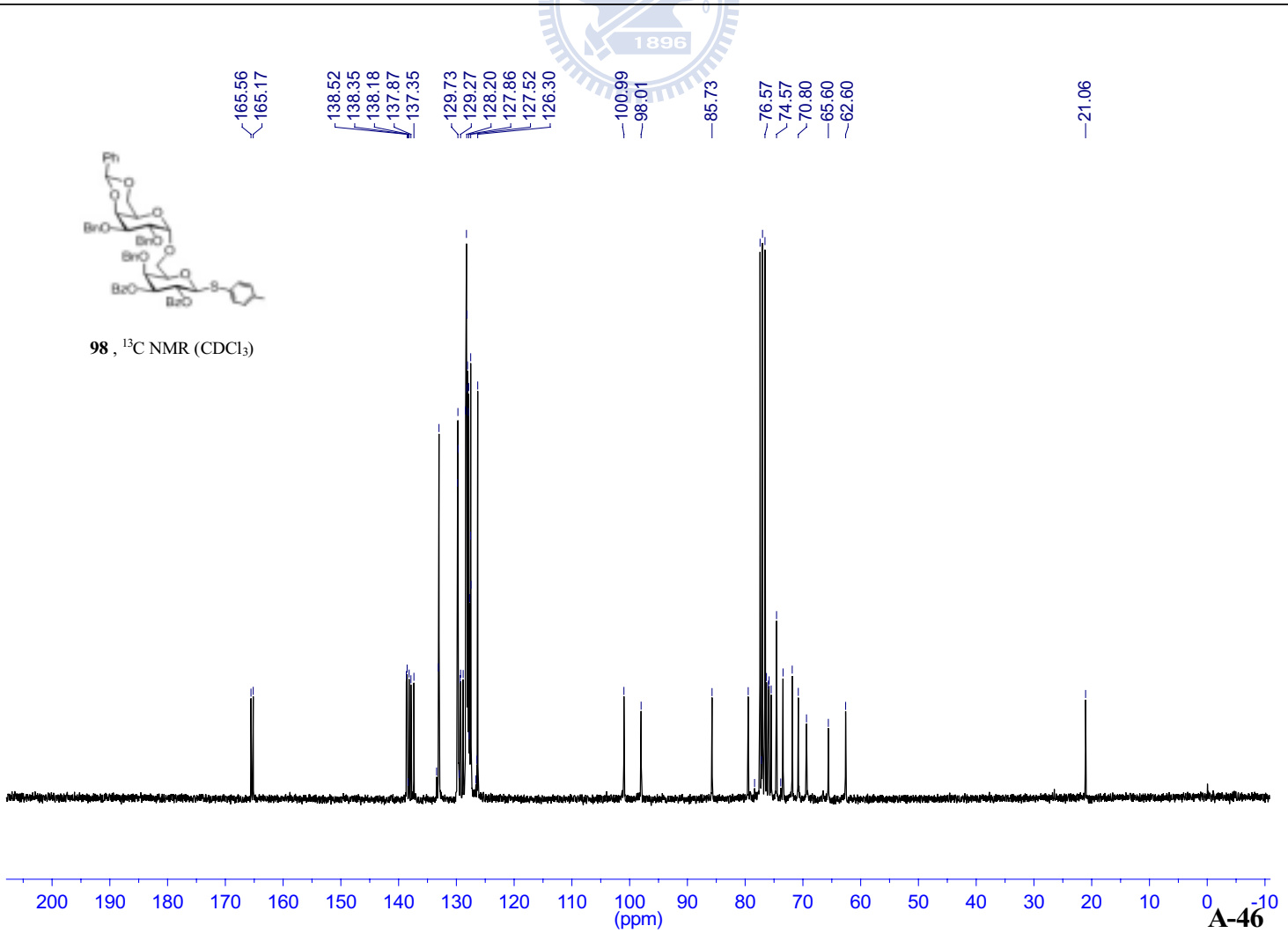
95, ^{1}H NMR ($CDCl_3$)



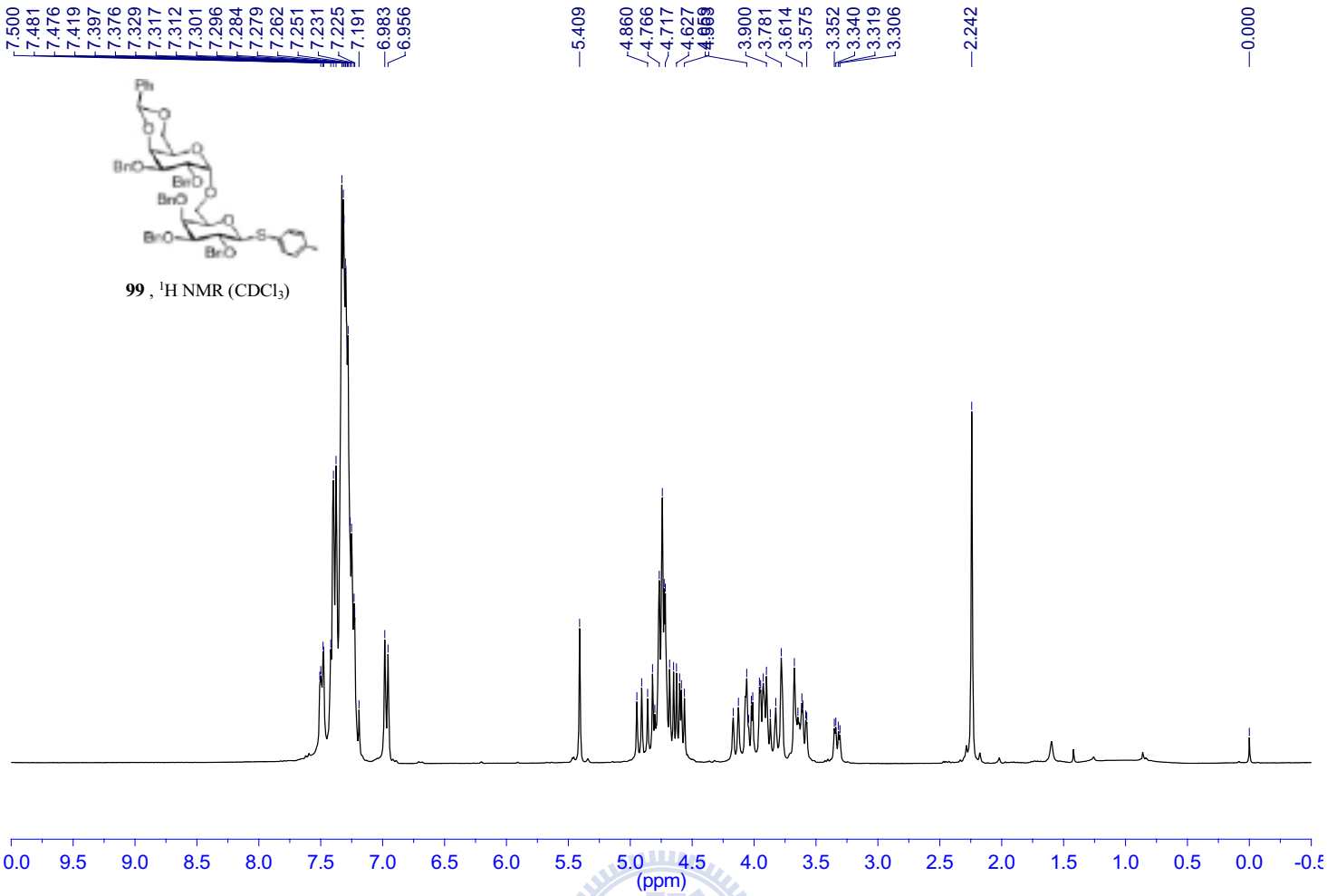
95 , ^{13}C NMR ($CDCl_3$)



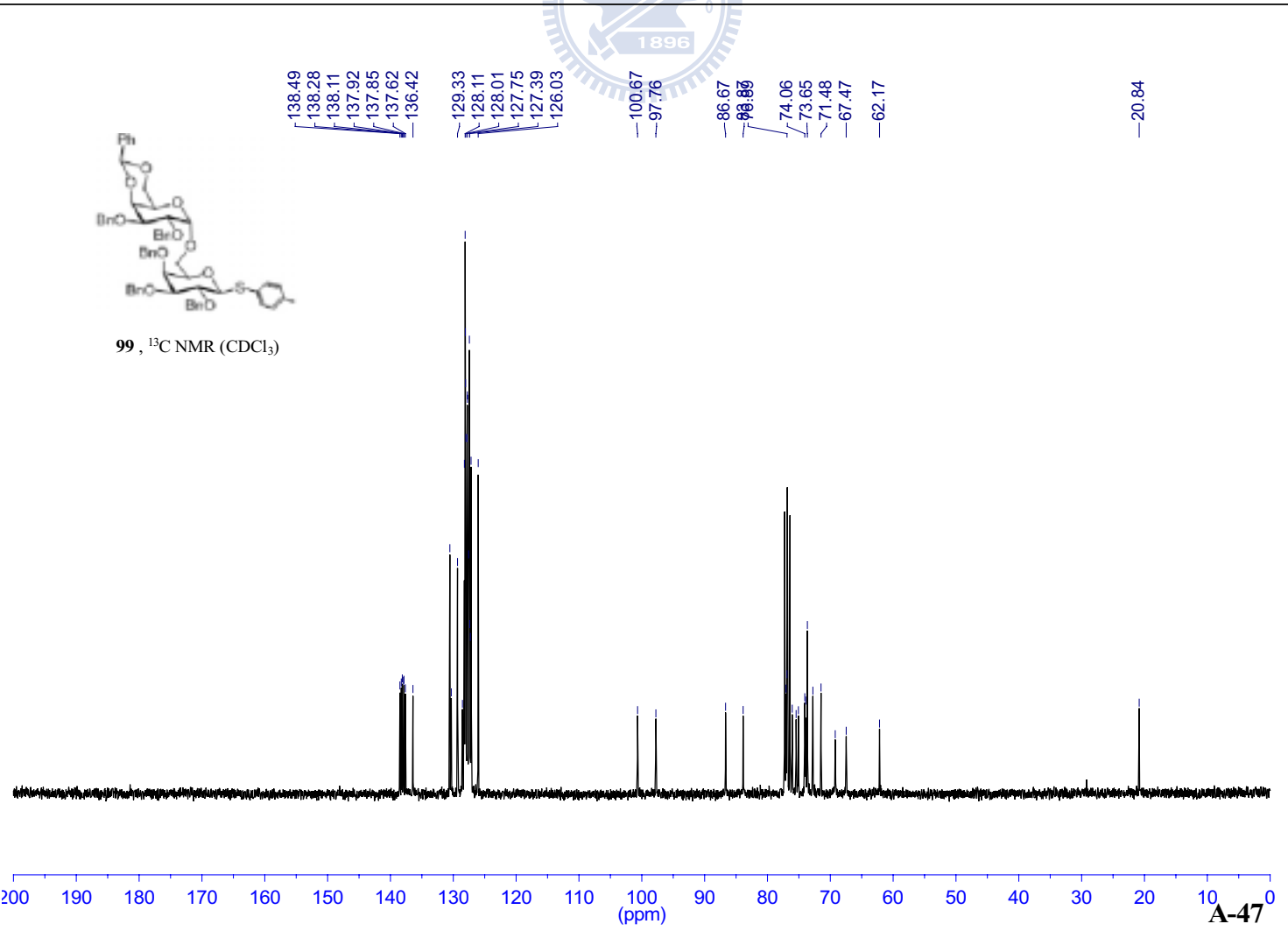
98 , ^{1}H NMR ($CDCl_3$)



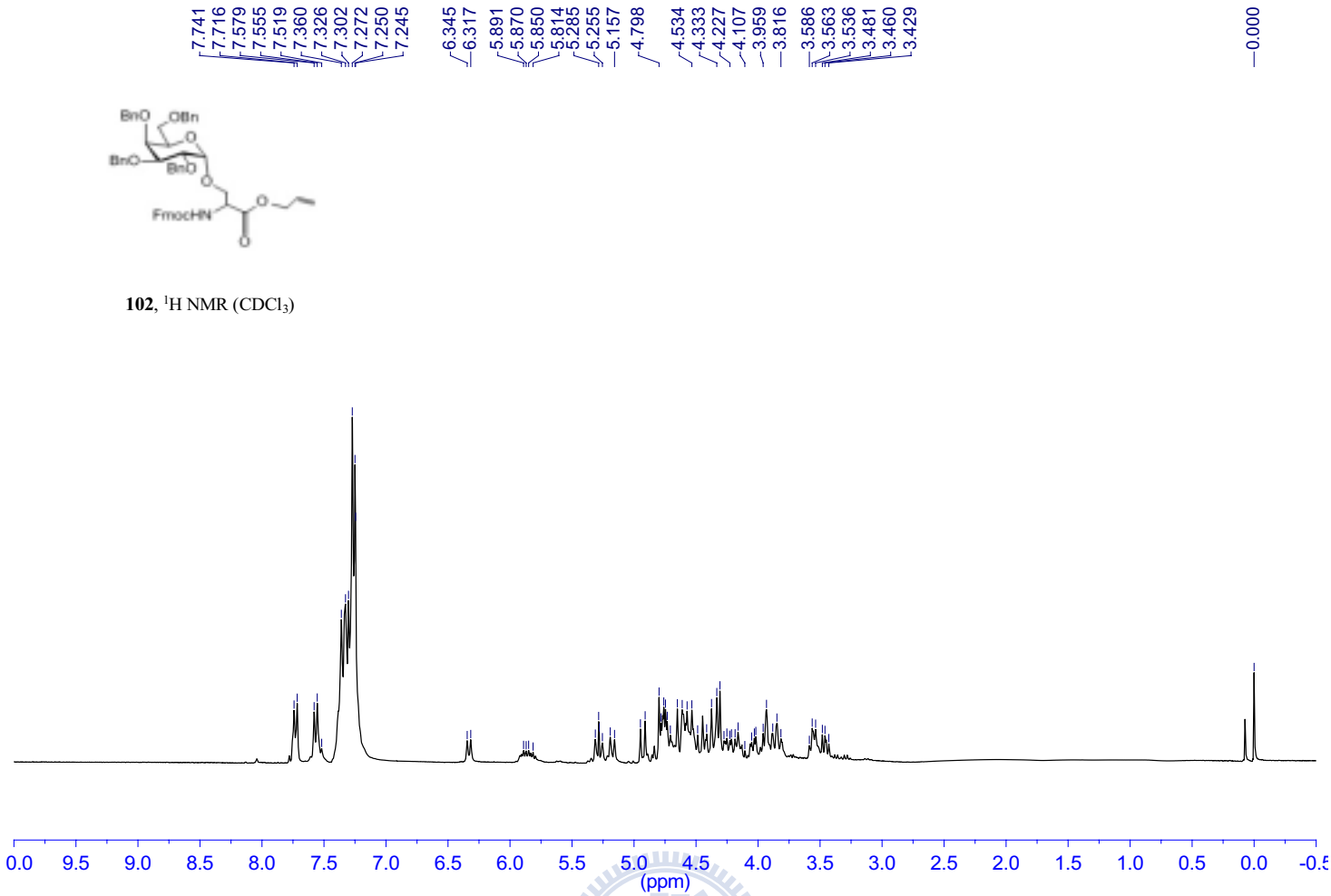
98 , ^{13}C NMR ($CDCl_3$)



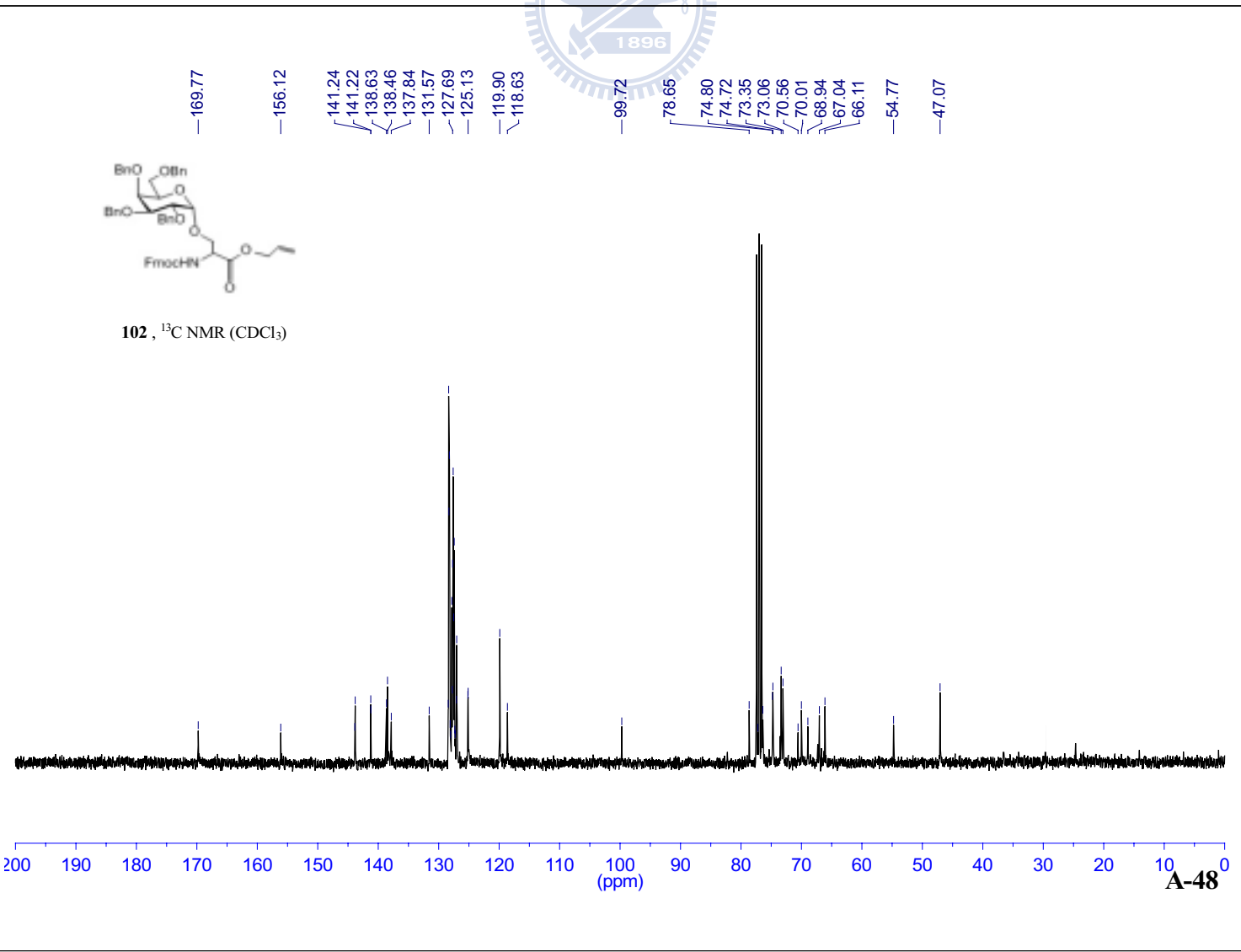
99 , ^{1}H NMR ($CDCl_3$)



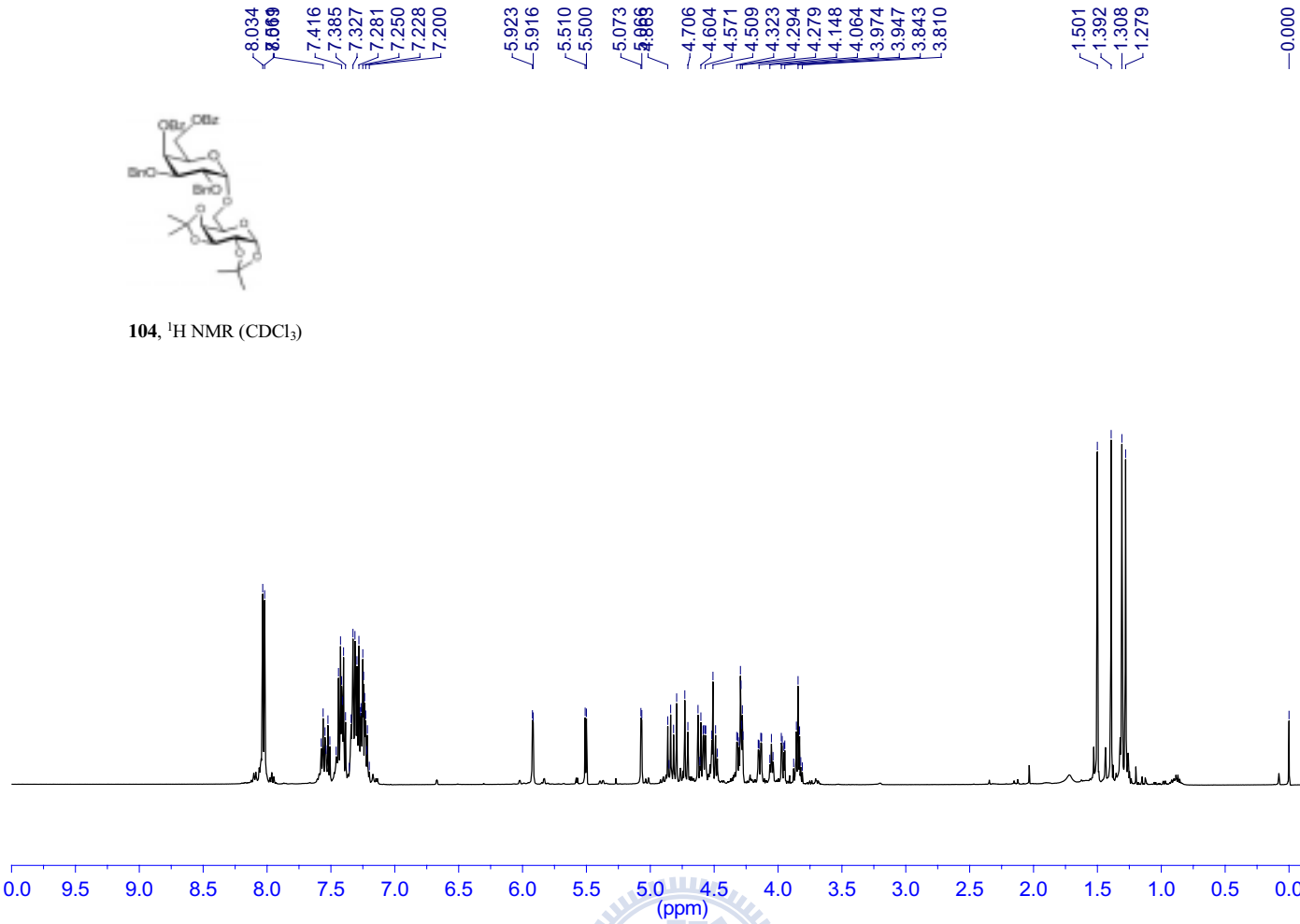
99 , ^{13}C NMR ($CDCl_3$)



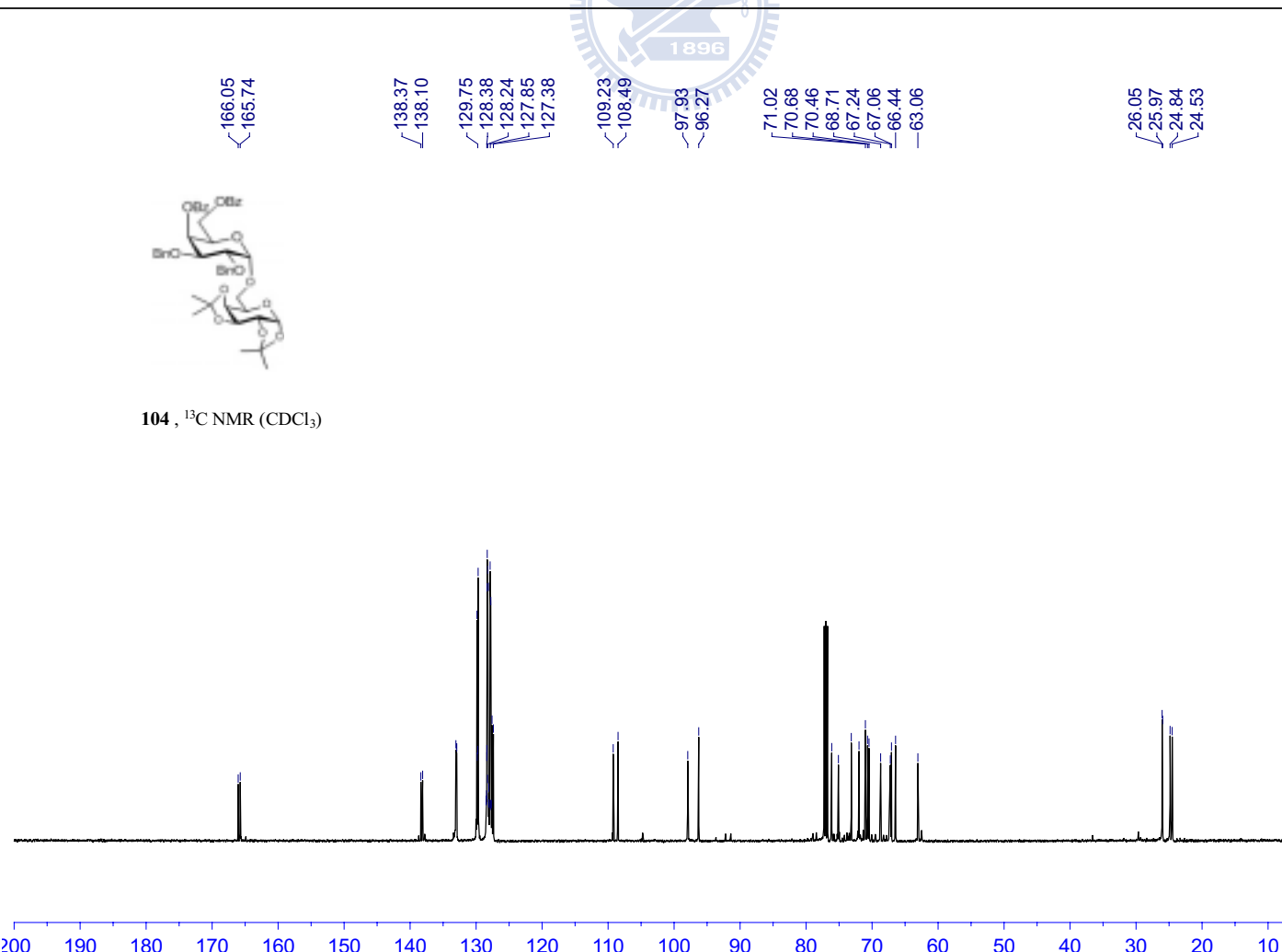
102, ^{1}H NMR ($CDCl_3$)



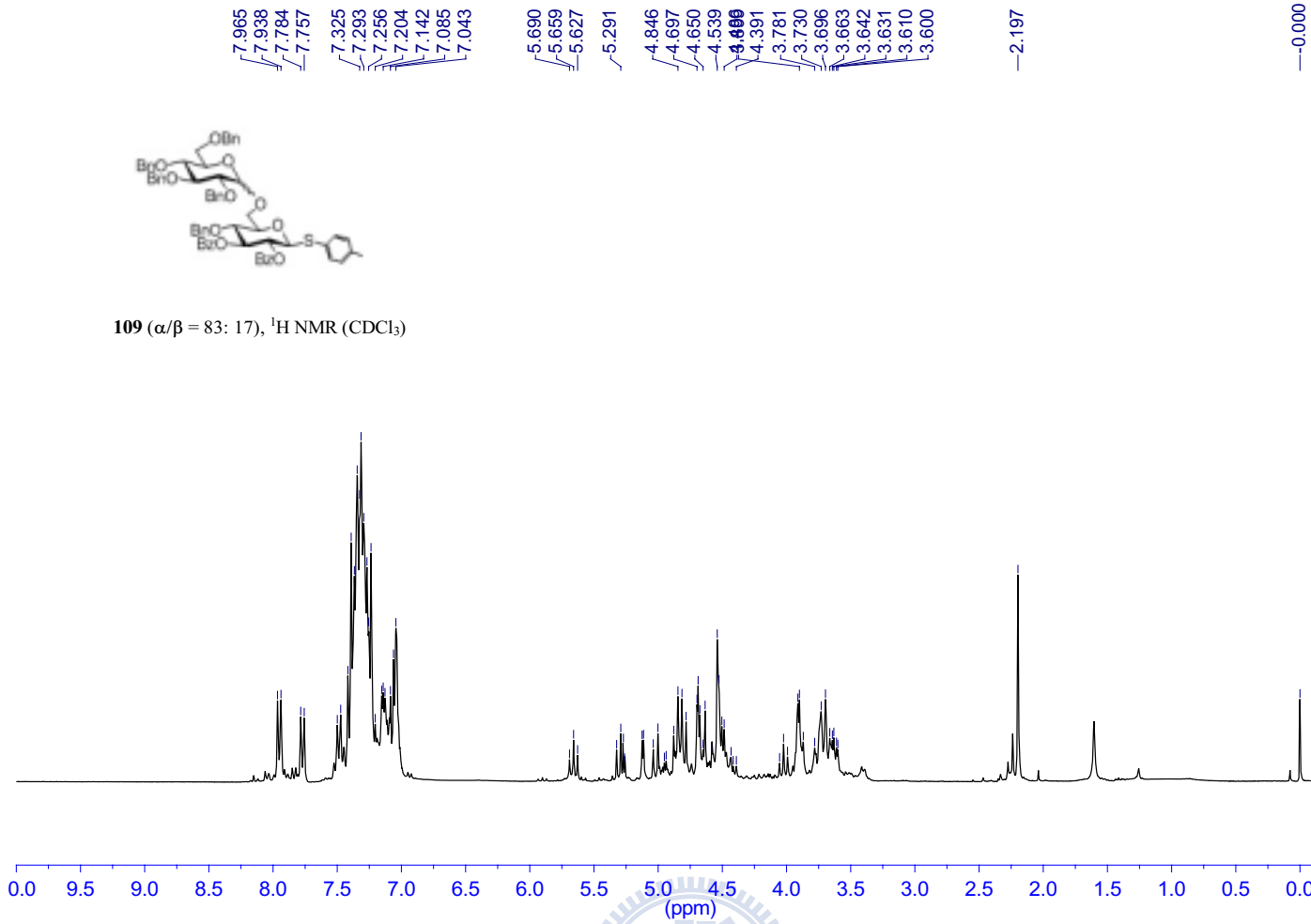
102 , ^{13}C NMR ($CDCl_3$)



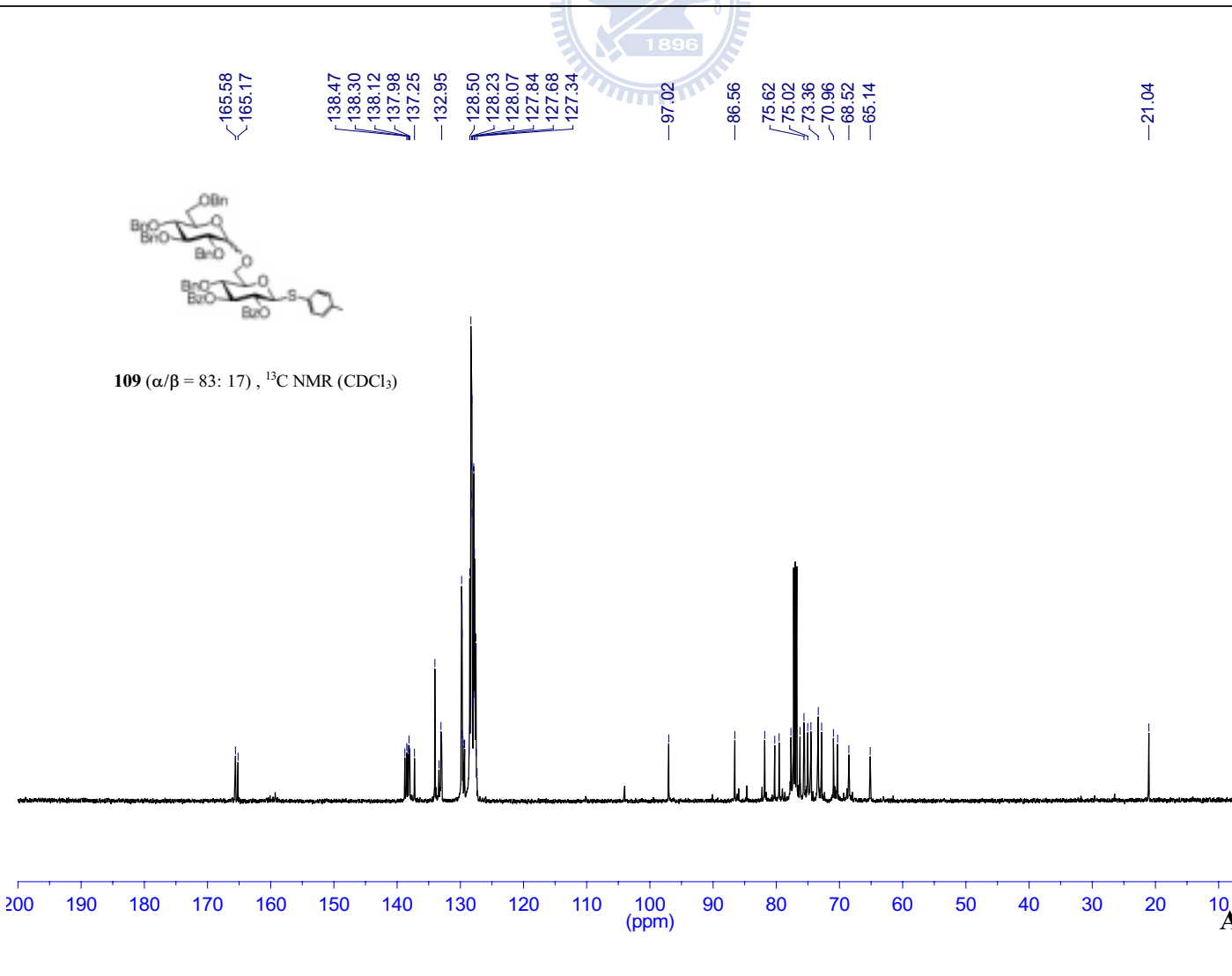
104, ^{1}H NMR ($CDCl_3$)



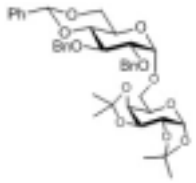
104 , ^{13}C NMR ($CDCl_3$)



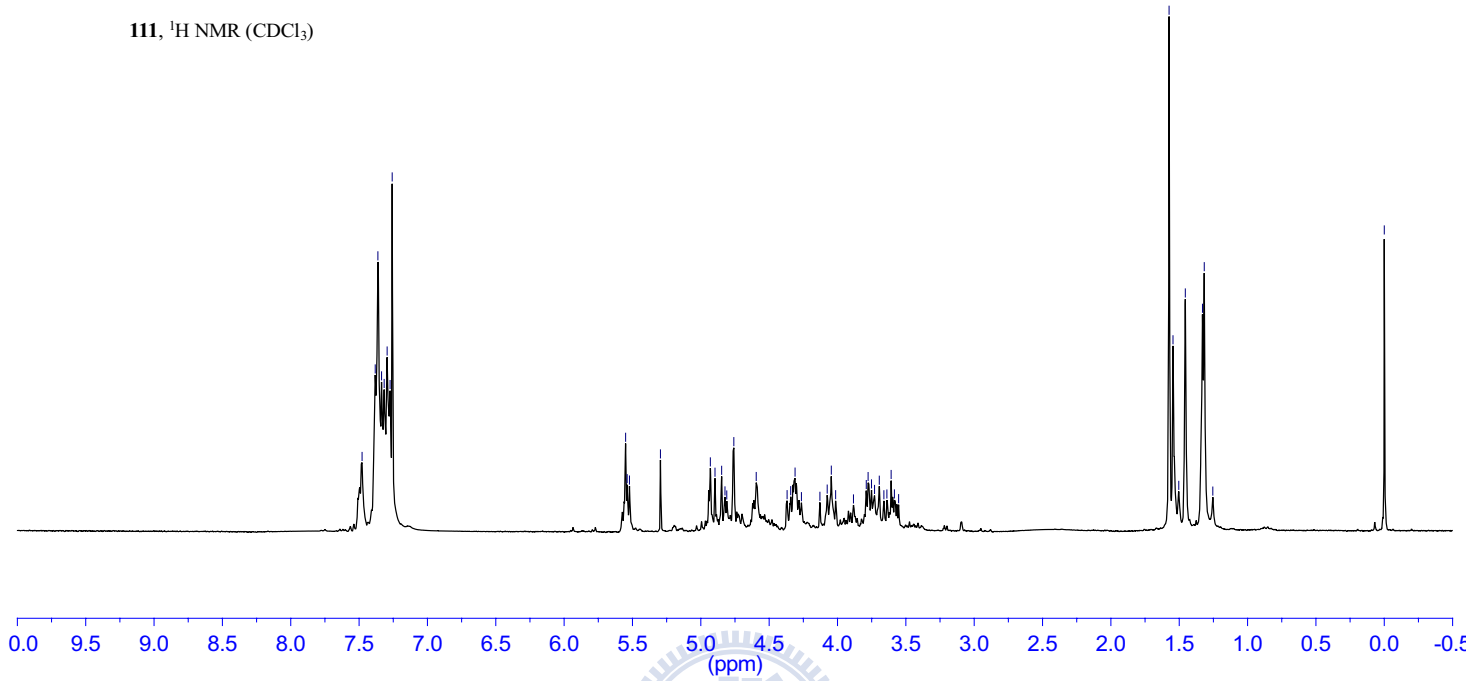
109 (α/β = 83: 17), ^{1}H NMR ($CDCl_3$)

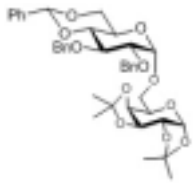


109 (α/β = 83: 17) , ^{13}C NMR ($CDCl_3$)

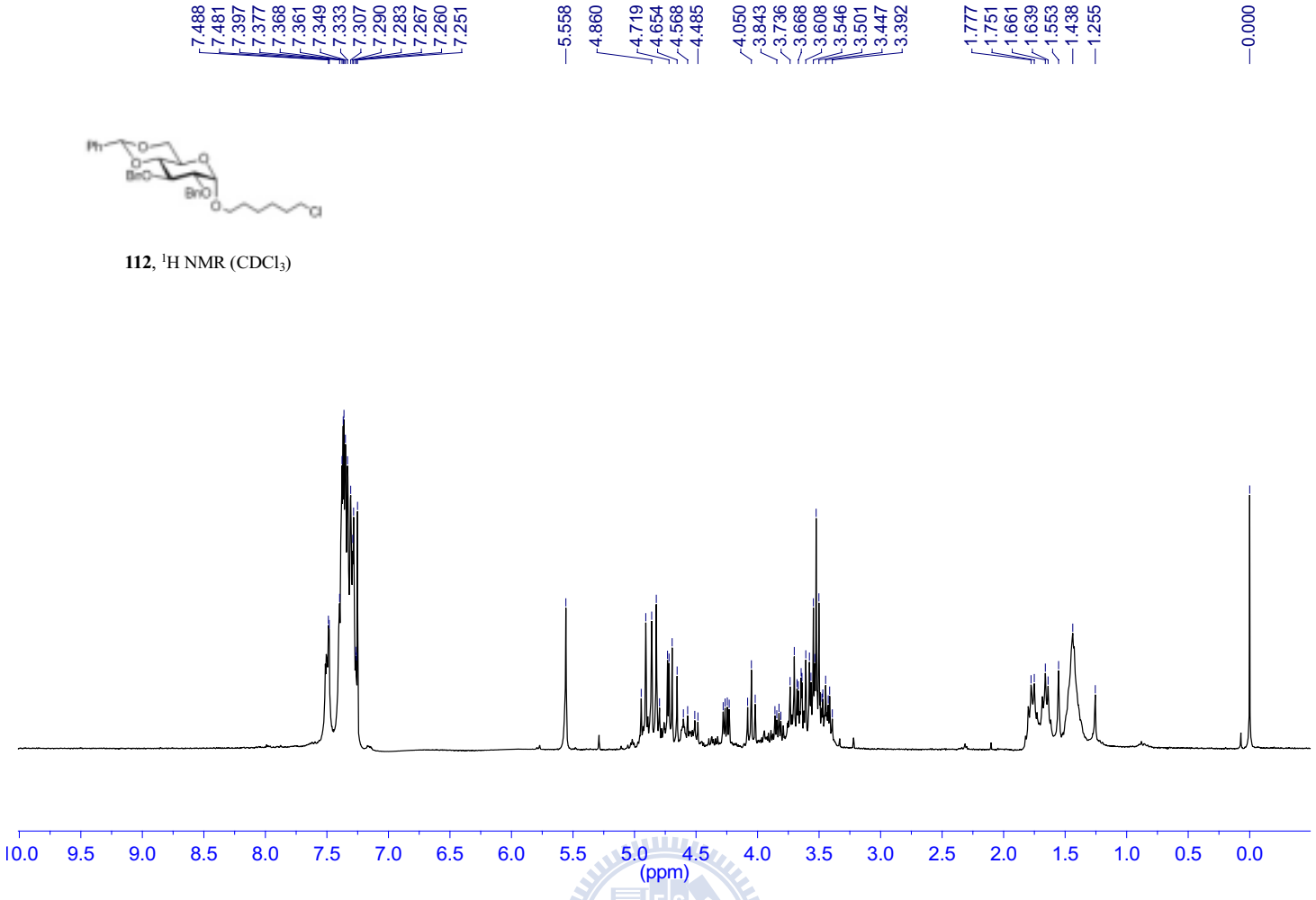


111, ^{1}H NMR ($CDCl_3$)

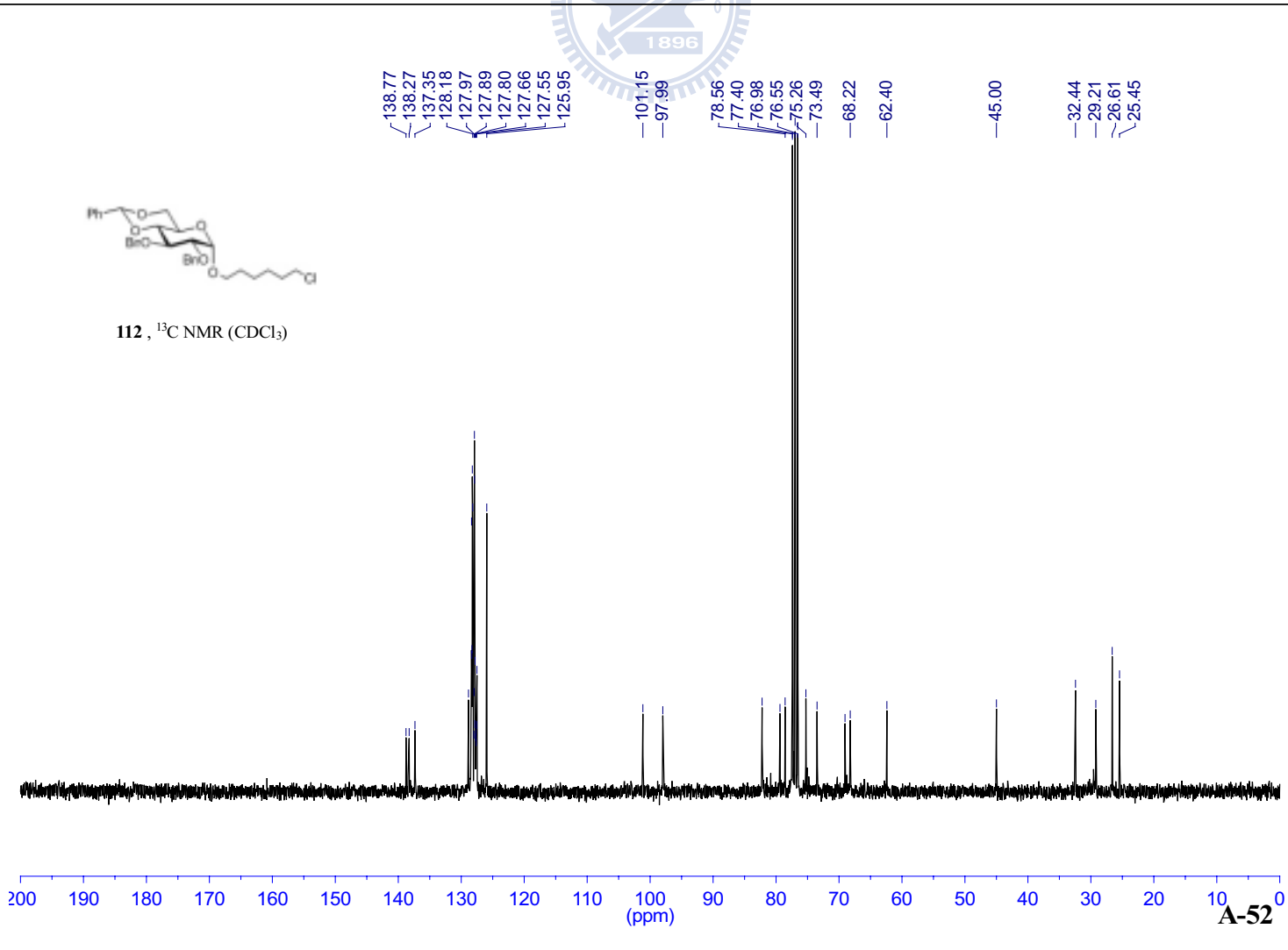




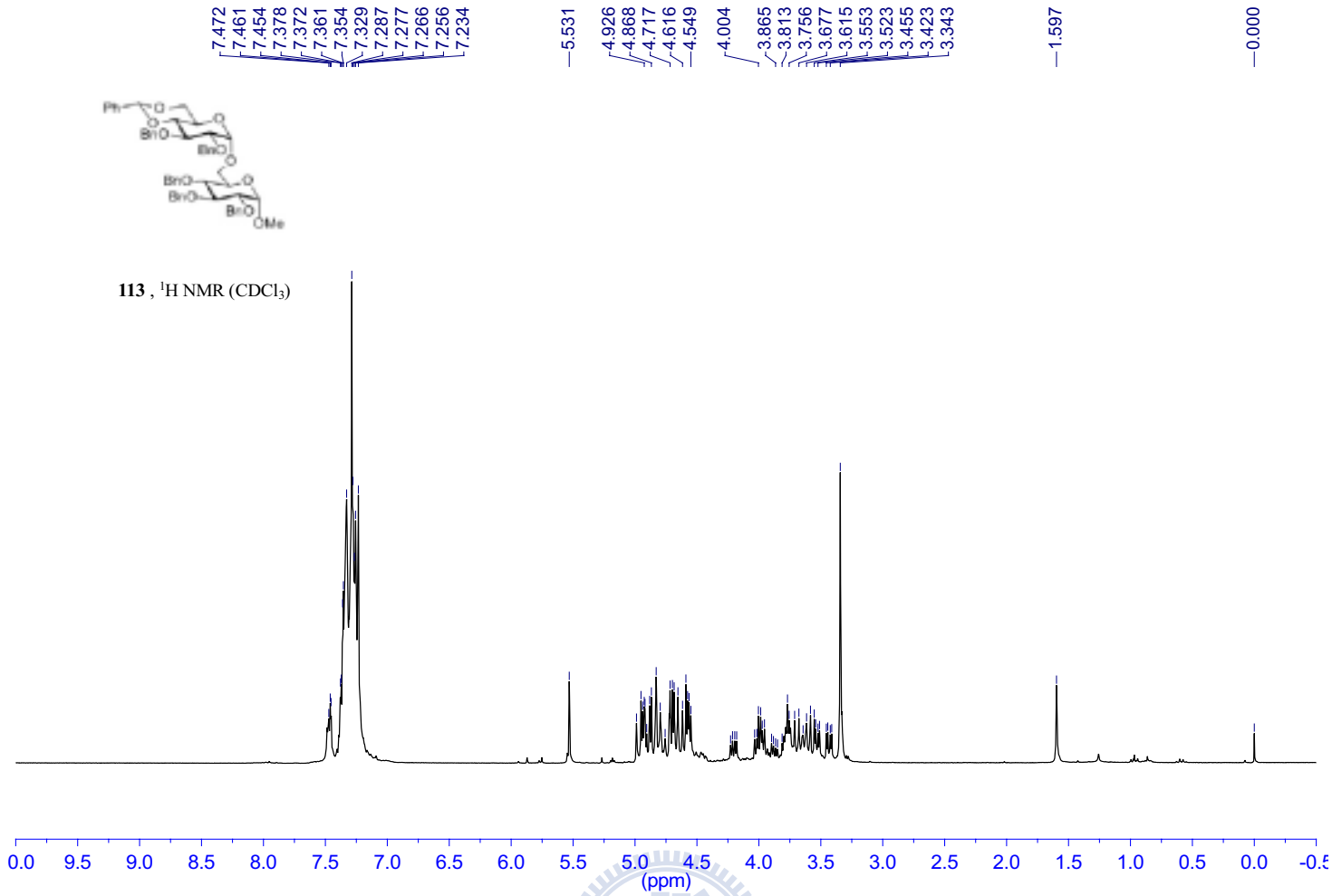
111 , ^{13}C NMR ($CDCl_3$)



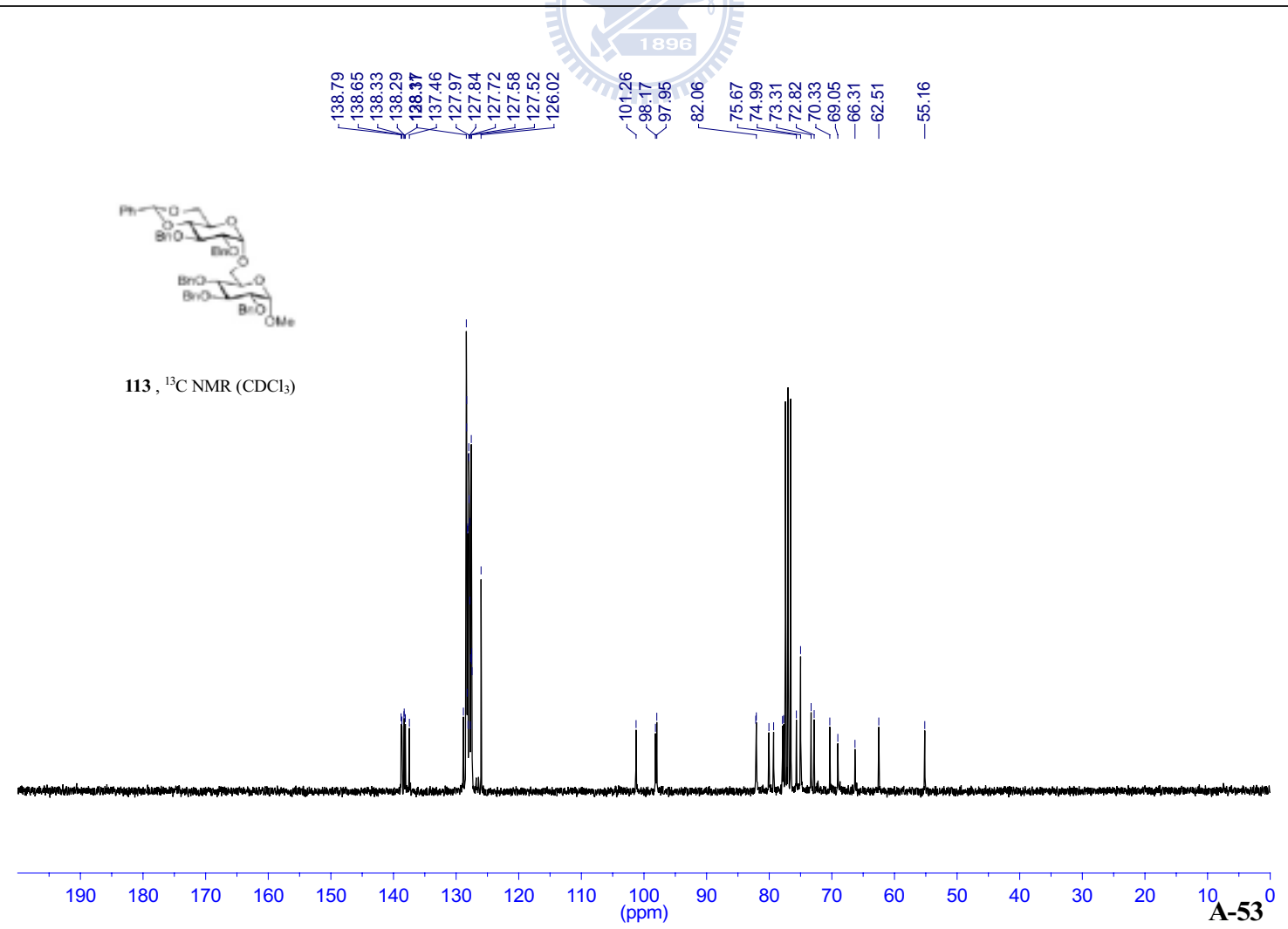
112, ^{1}H NMR ($CDCl_3$)



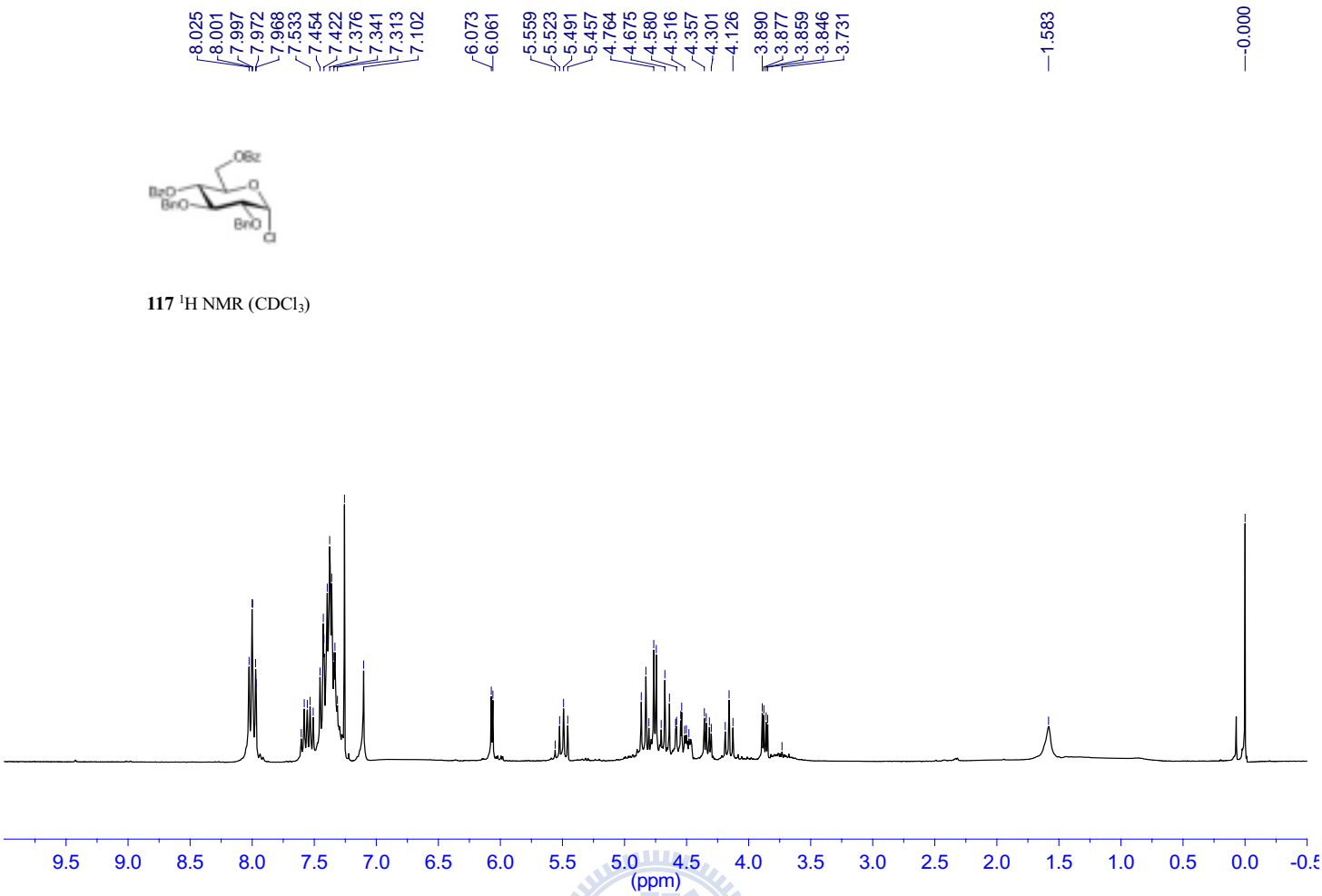
112 , ^{13}C NMR ($CDCl_3$)



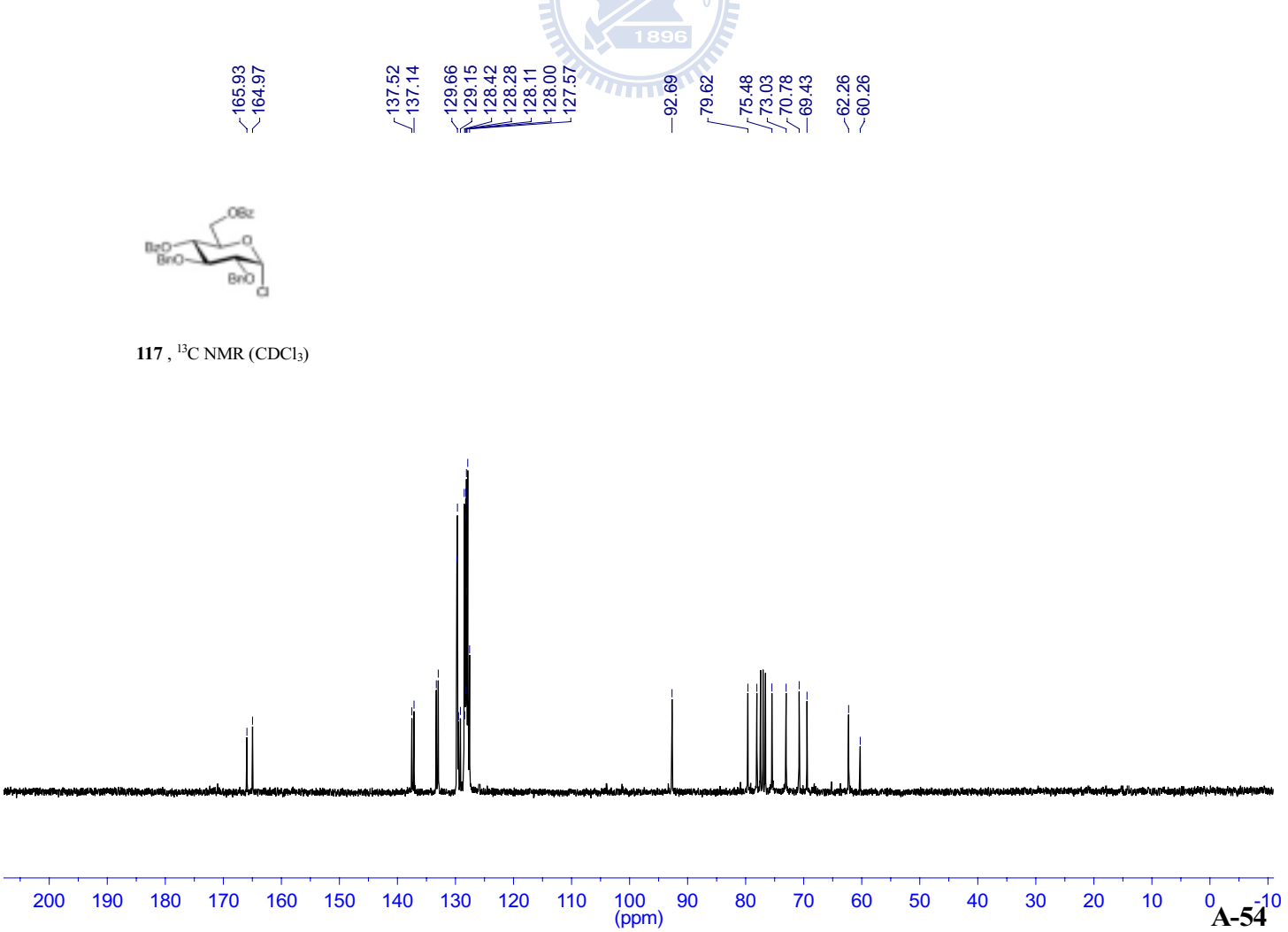
113 , ^{1}H NMR ($CDCl_3$)



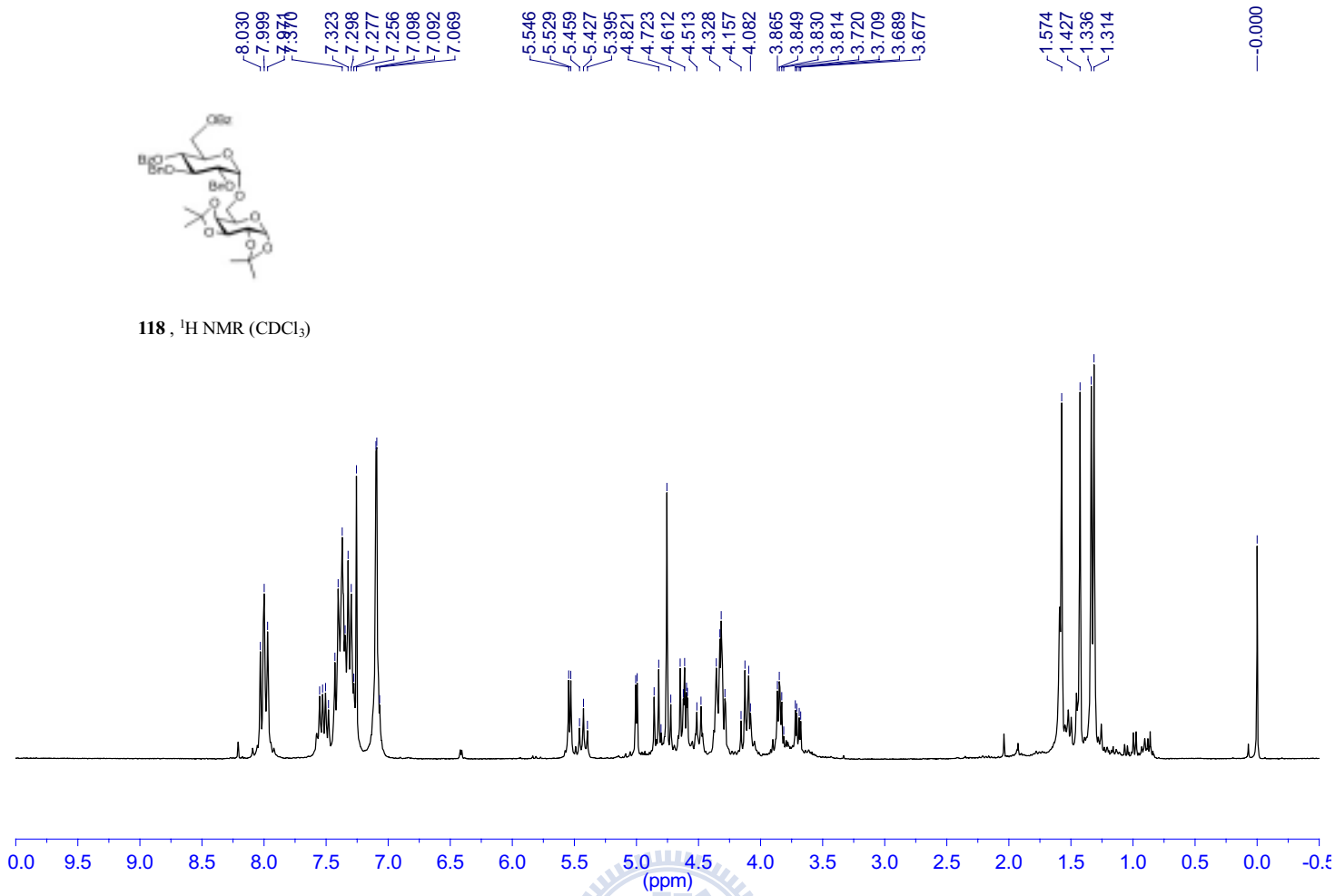
113 , ^{13}C NMR ($CDCl_3$)



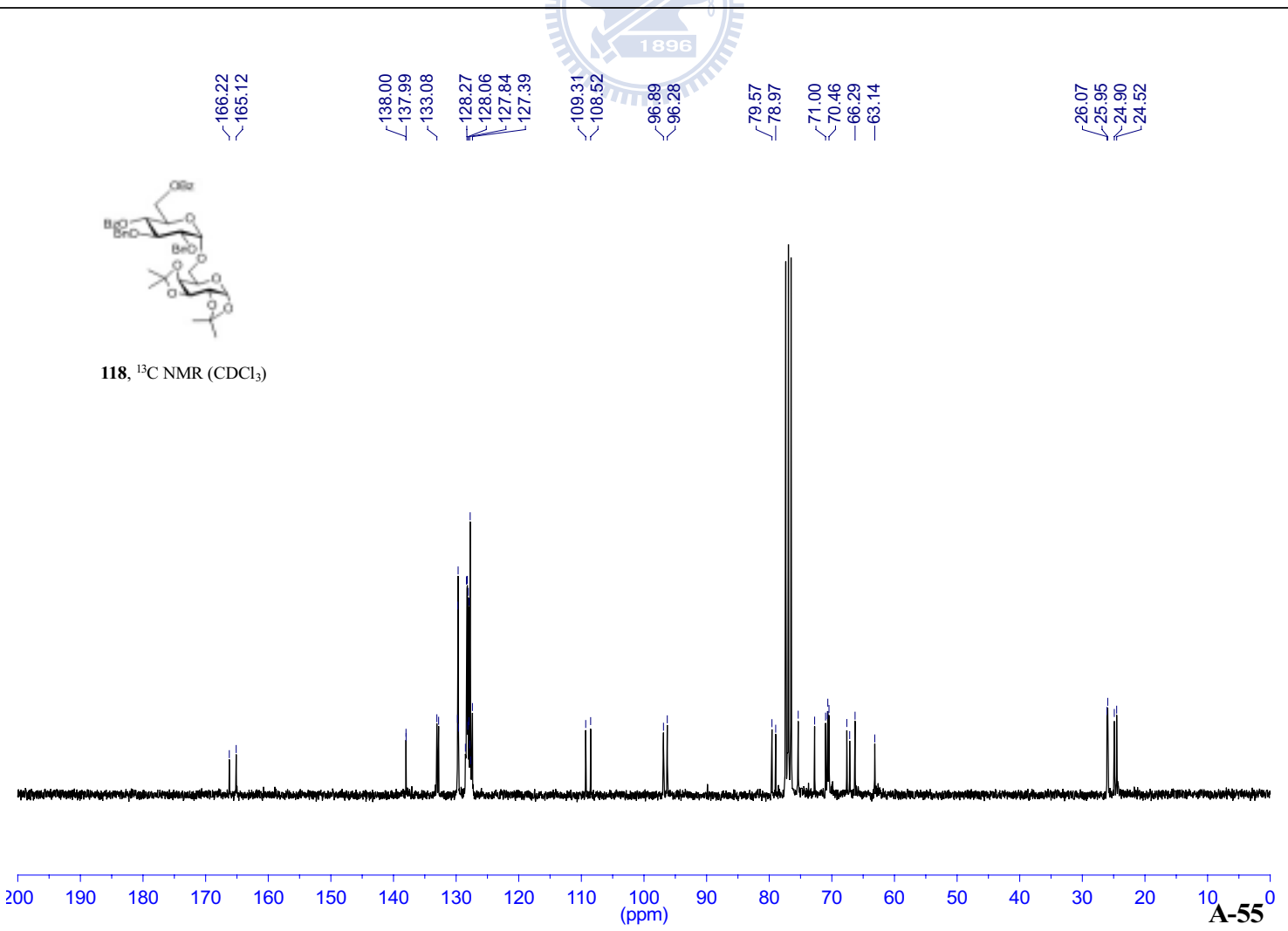
117 ^{1}H NMR ($CDCl_3$)



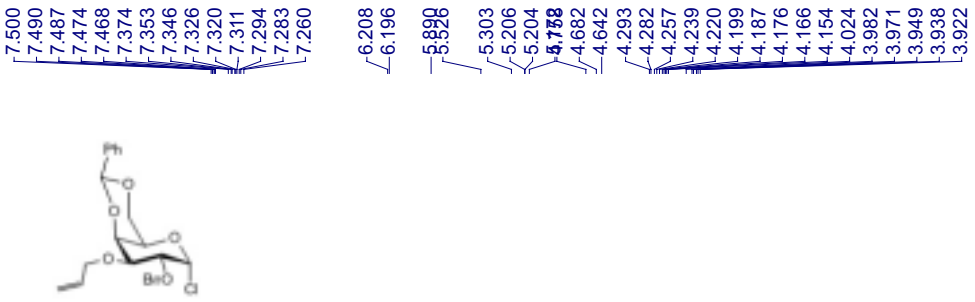
117 , ^{13}C NMR ($CDCl_3$)



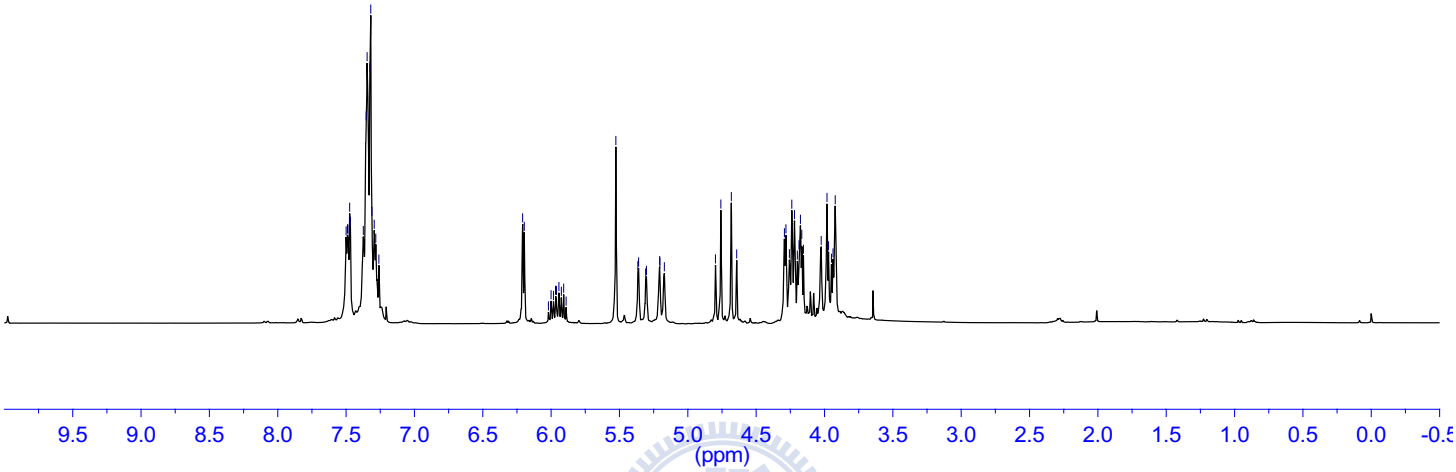
118 , ^{1}H NMR ($CDCl_3$)



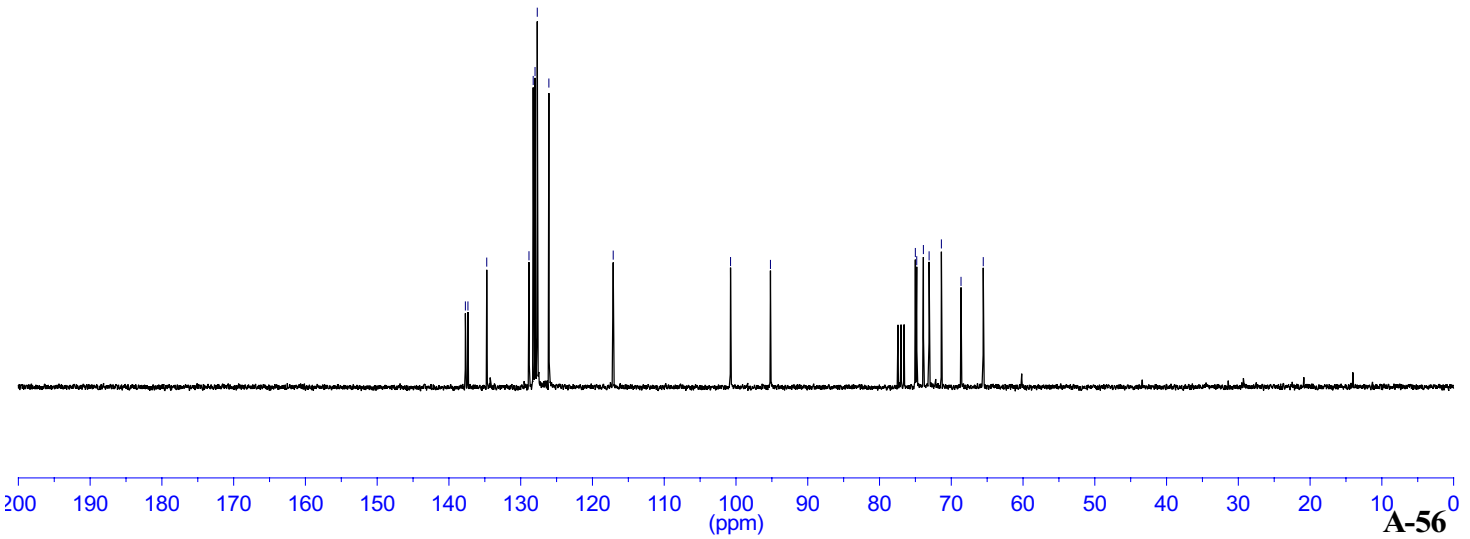
118, ^{13}C NMR ($CDCl_3$)

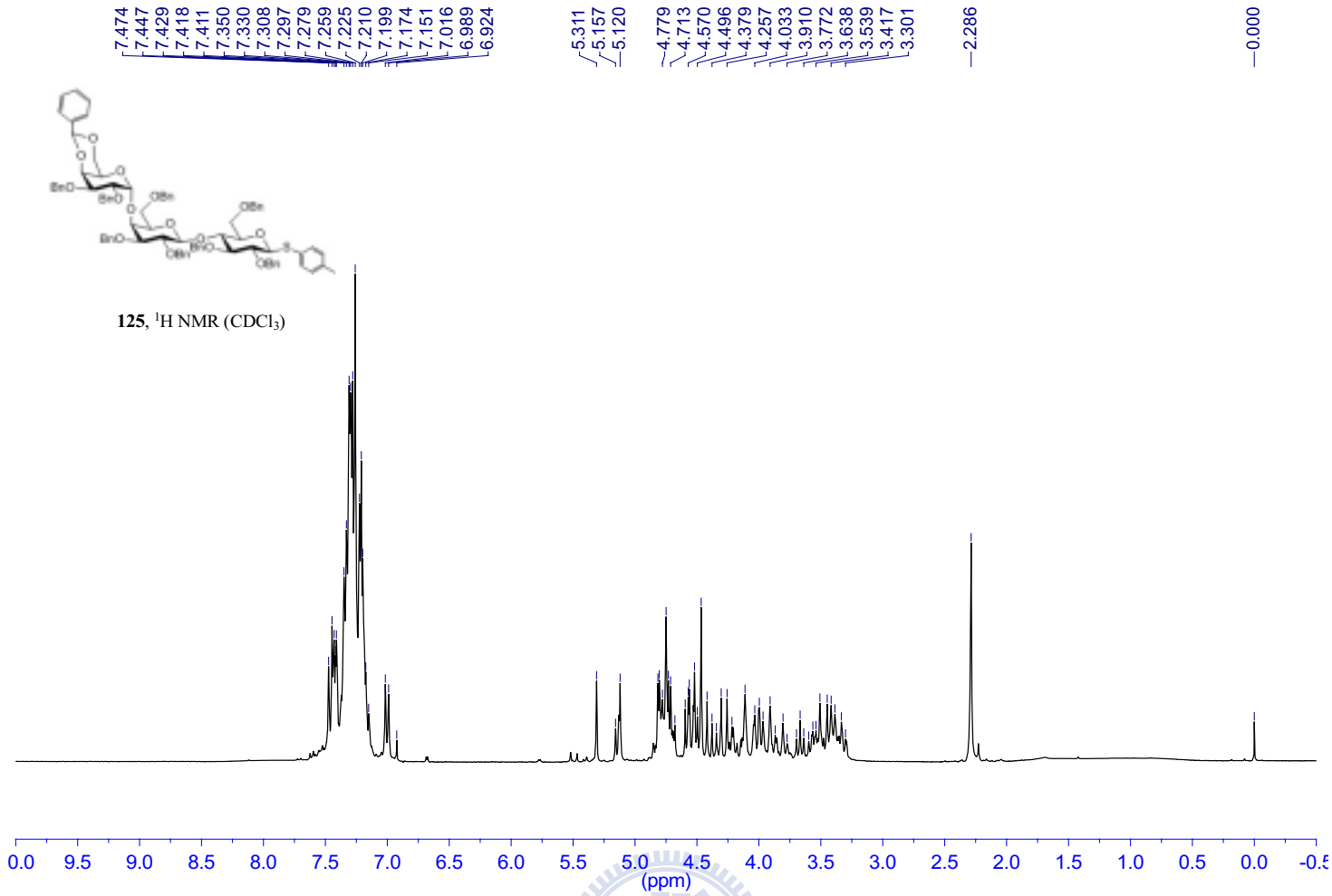


122, ^{1}H NMR ($CDCl_3$)

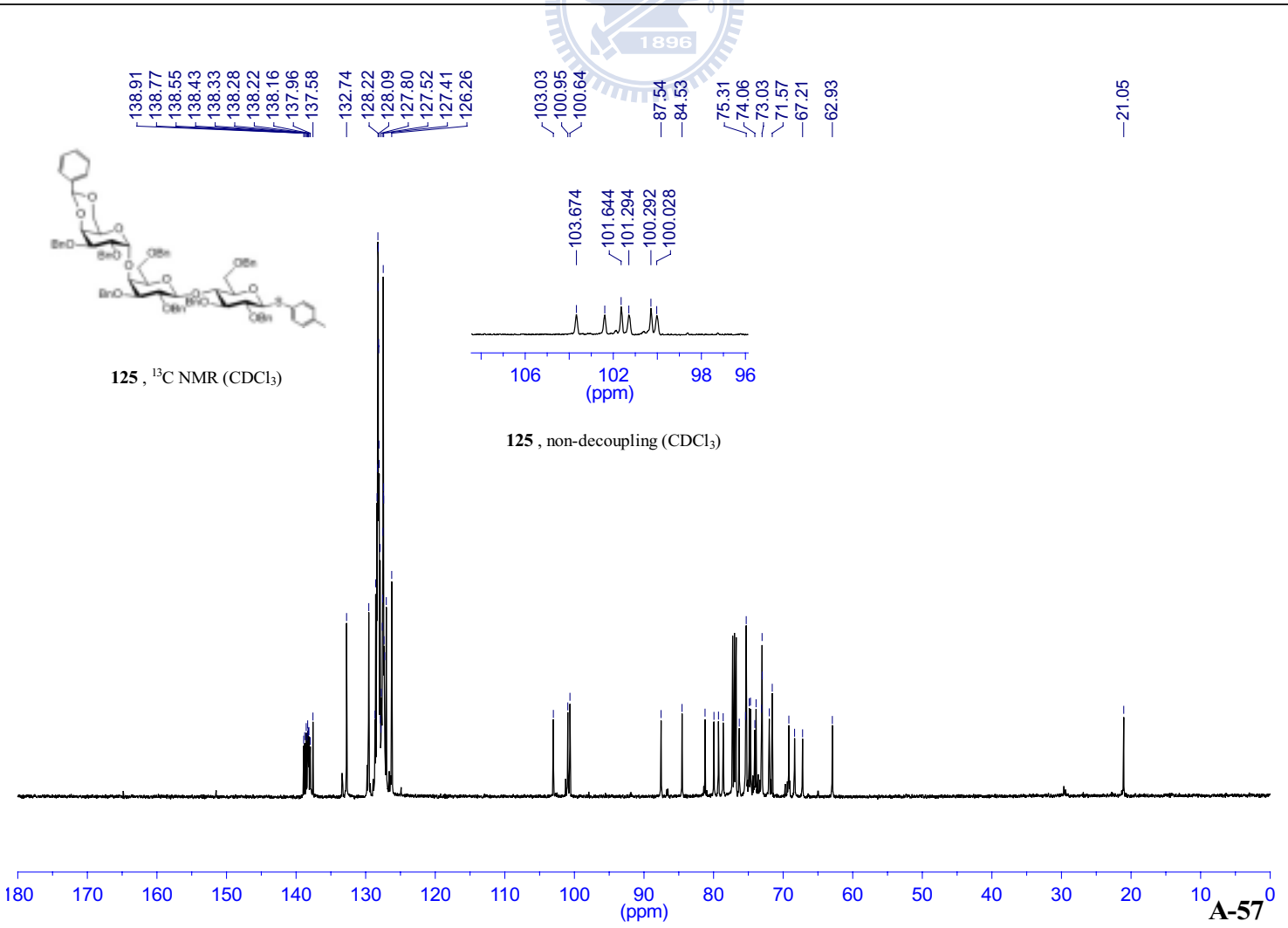


122 , ^{13}C NMR ($CDCl_3$)



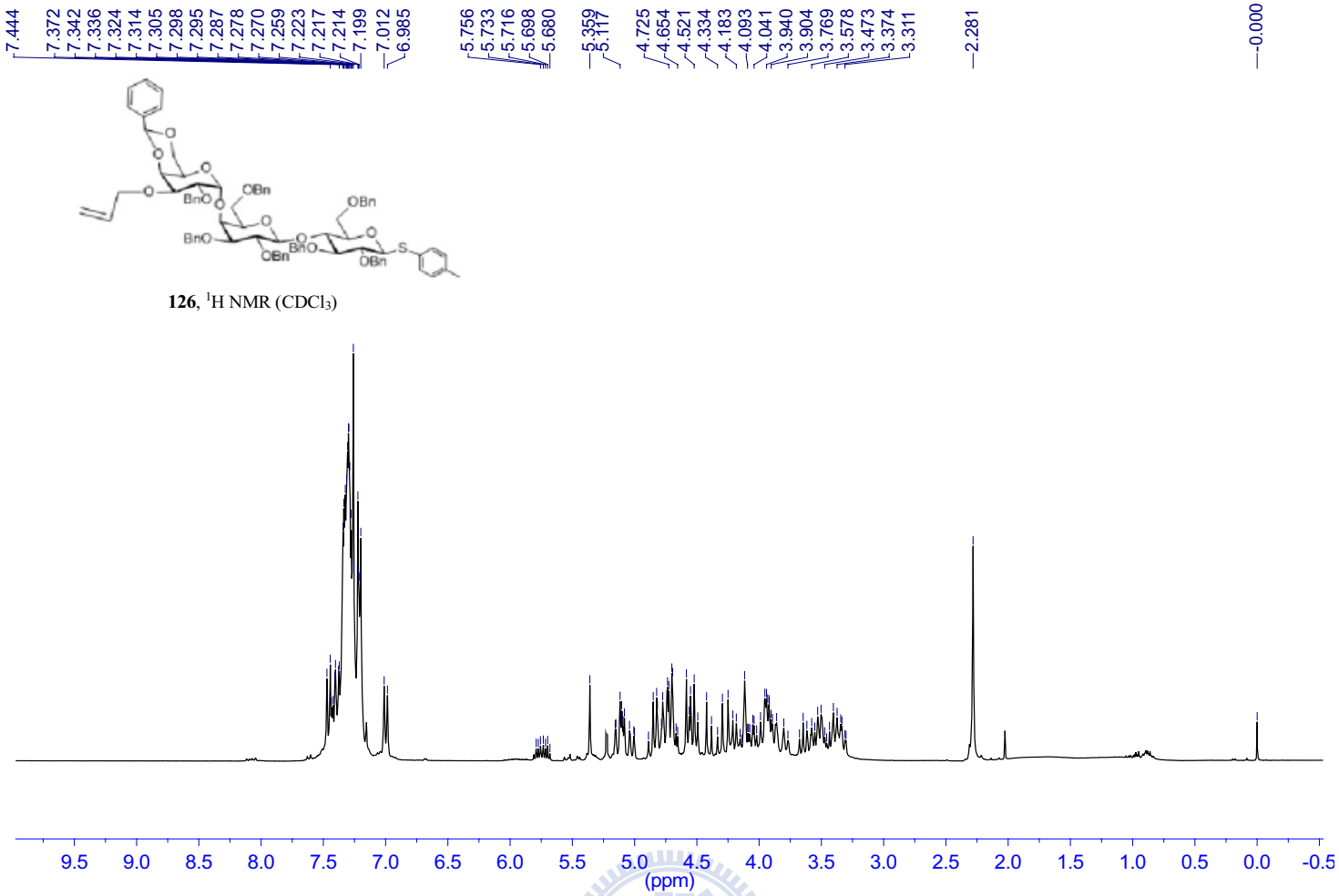


125, ^{1}H NMR ($CDCl_3$)

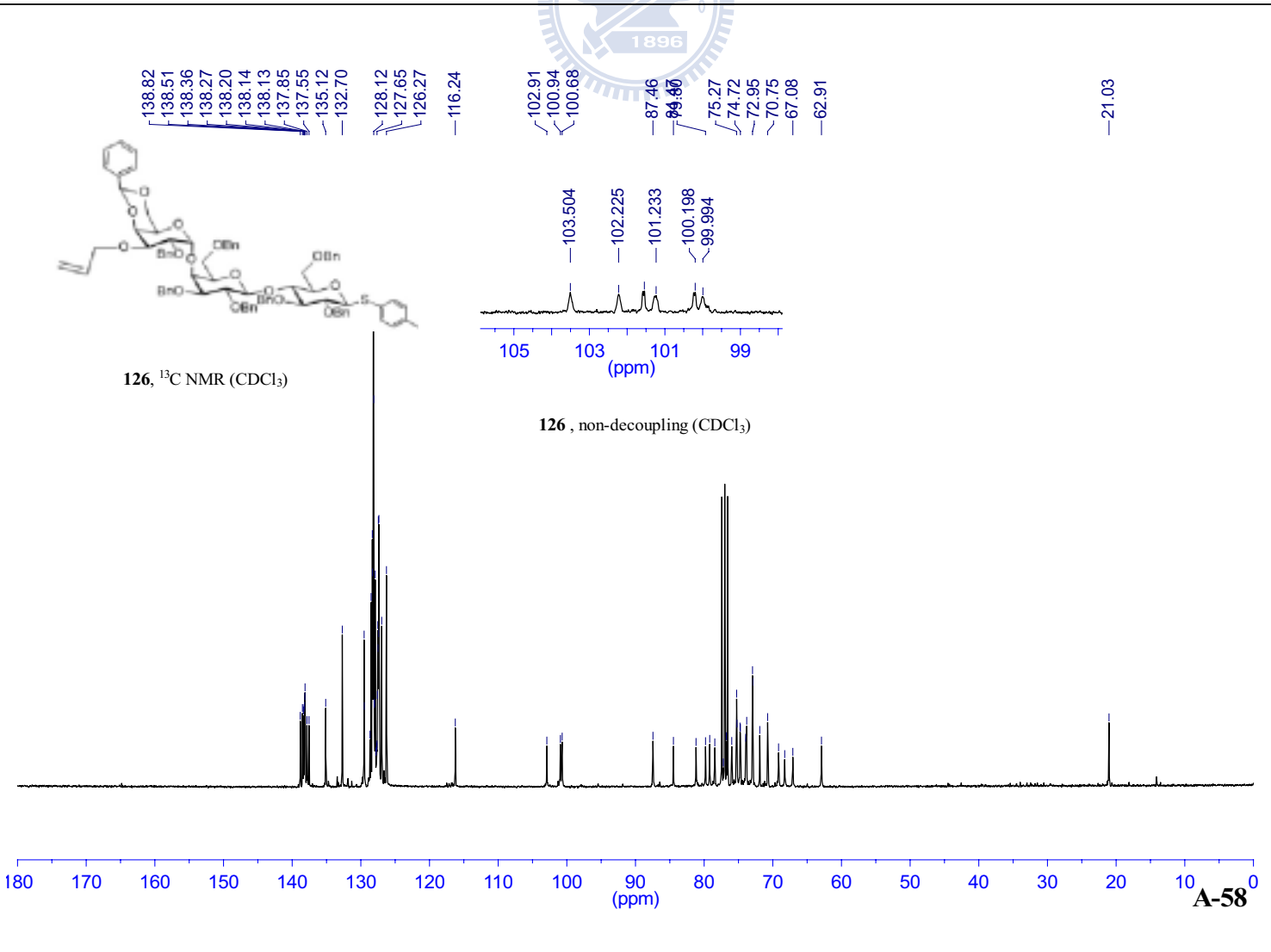


125 , ^{13}C NMR ($CDCl_3$)

125 , non-decoupling ($CDCl_3$)

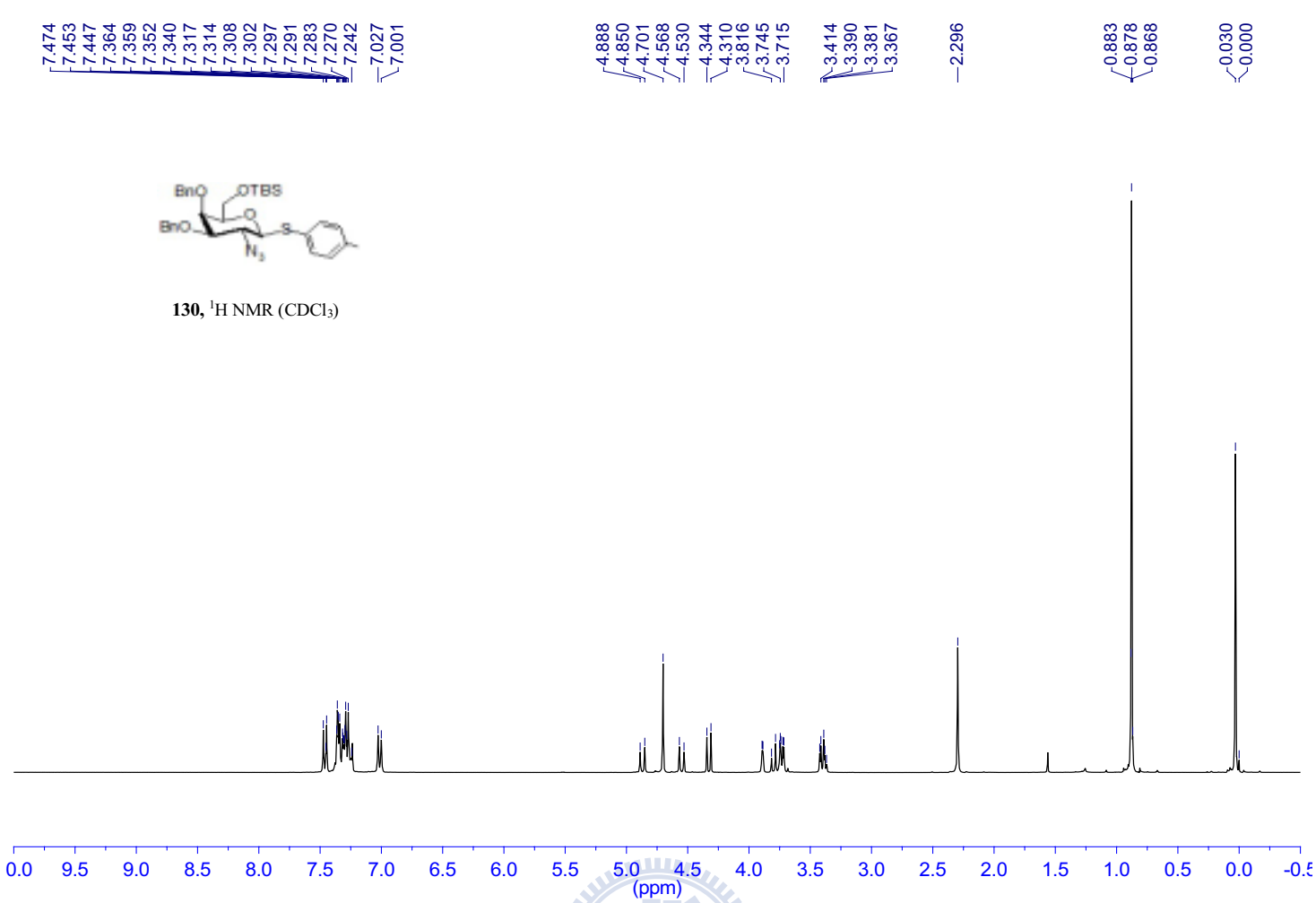


126, ^{1}H NMR ($CDCl_3$)

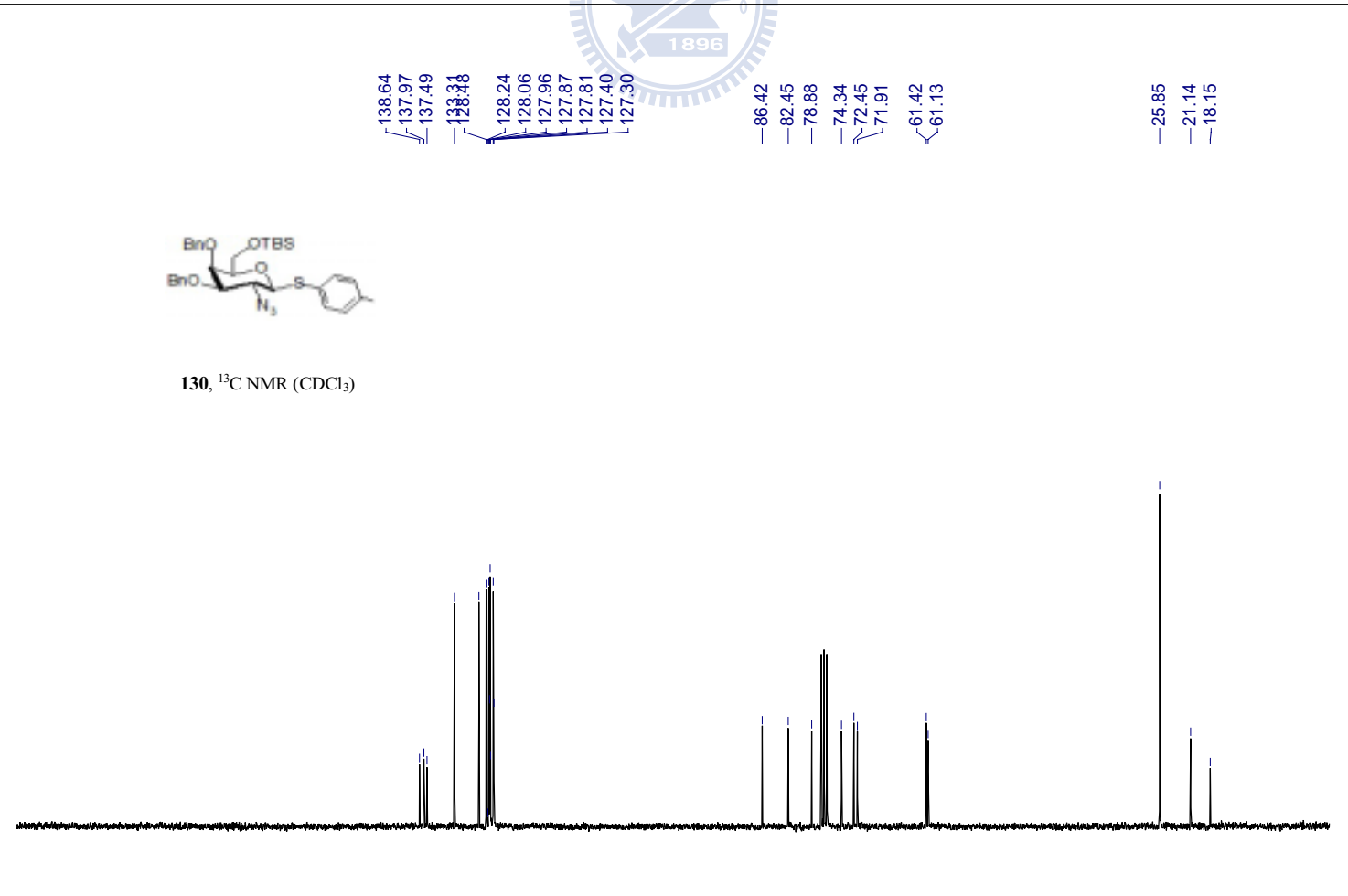


126, ^{13}C NMR ($CDCl_3$)

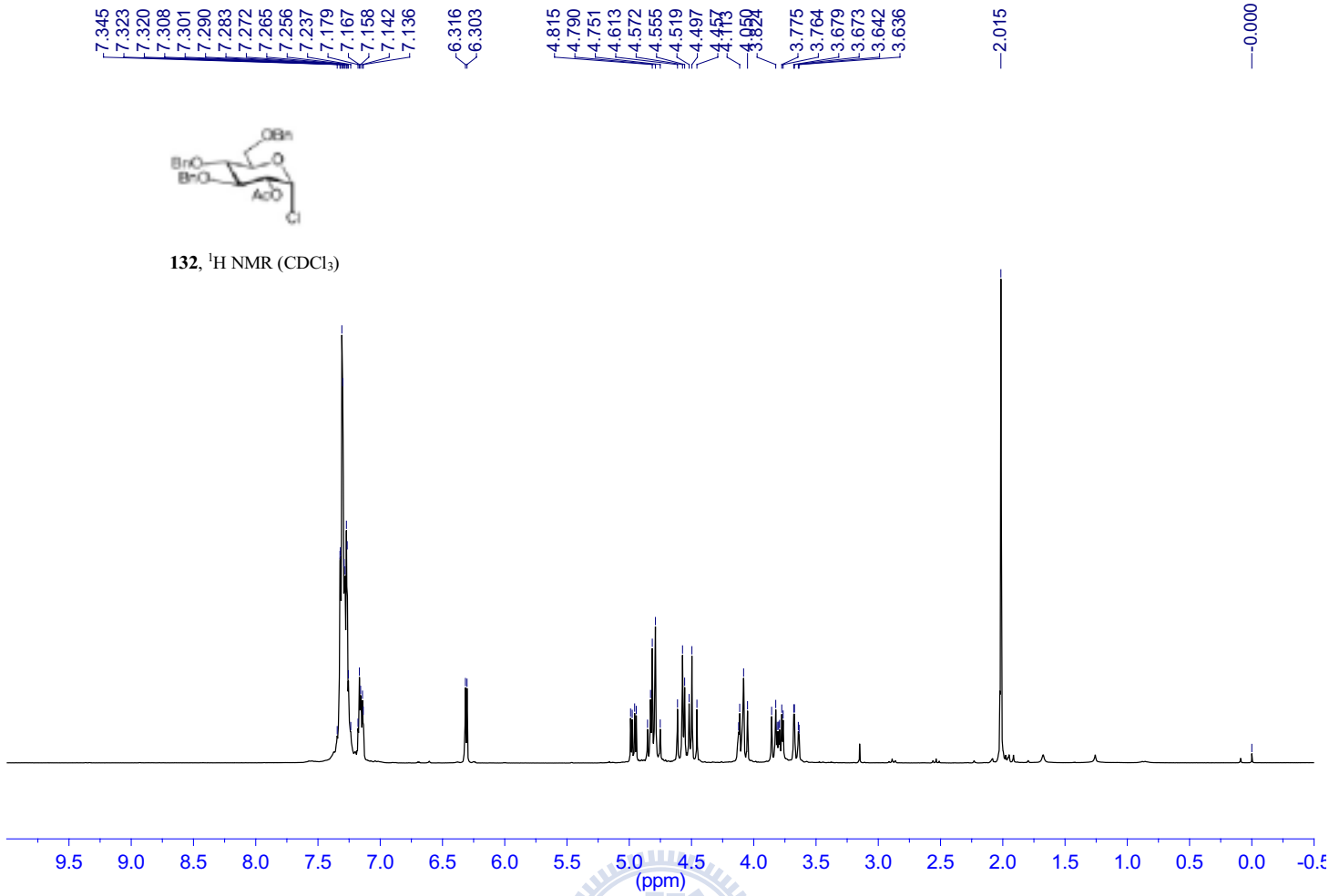
126 , non-decoupling ($CDCl_3$)



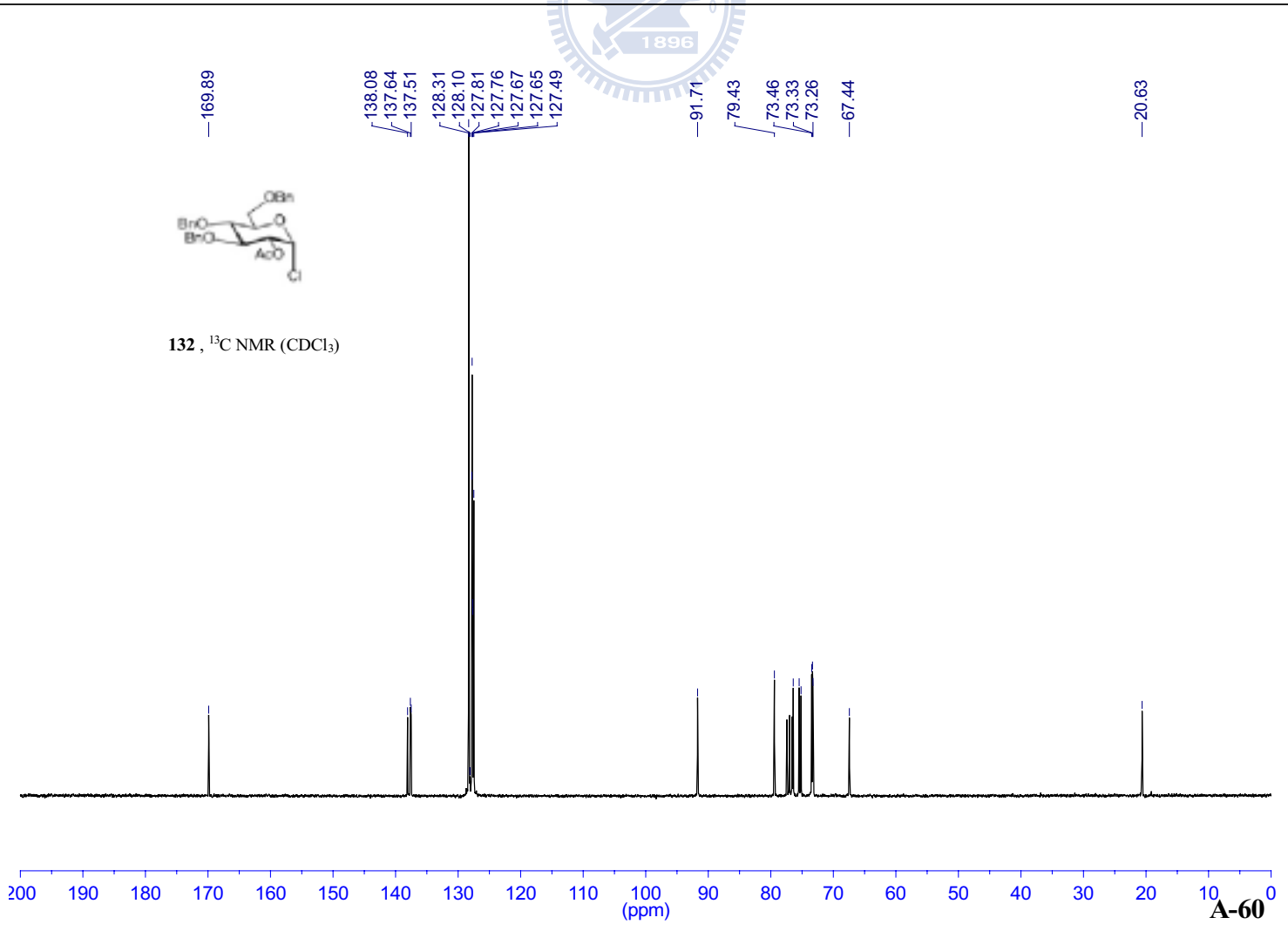
130, ^{1}H NMR ($CDCl_3$)



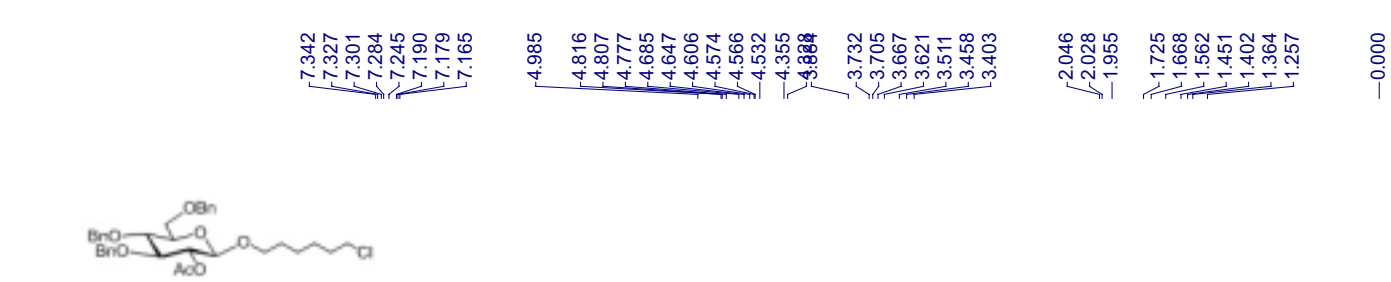
130, ^{13}C NMR ($CDCl_3$)



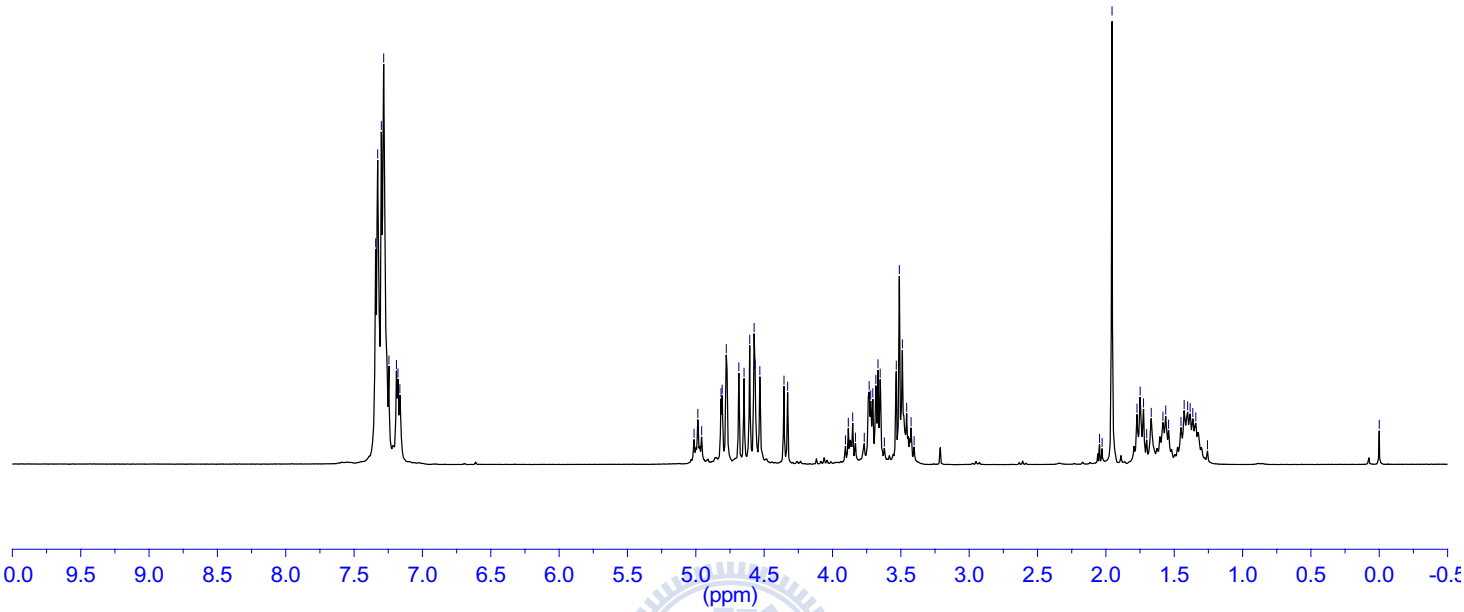
132, ^{1}H NMR ($CDCl_3$)

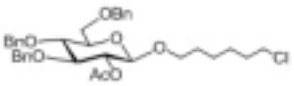


132 , ^{13}C NMR ($CDCl_3$)

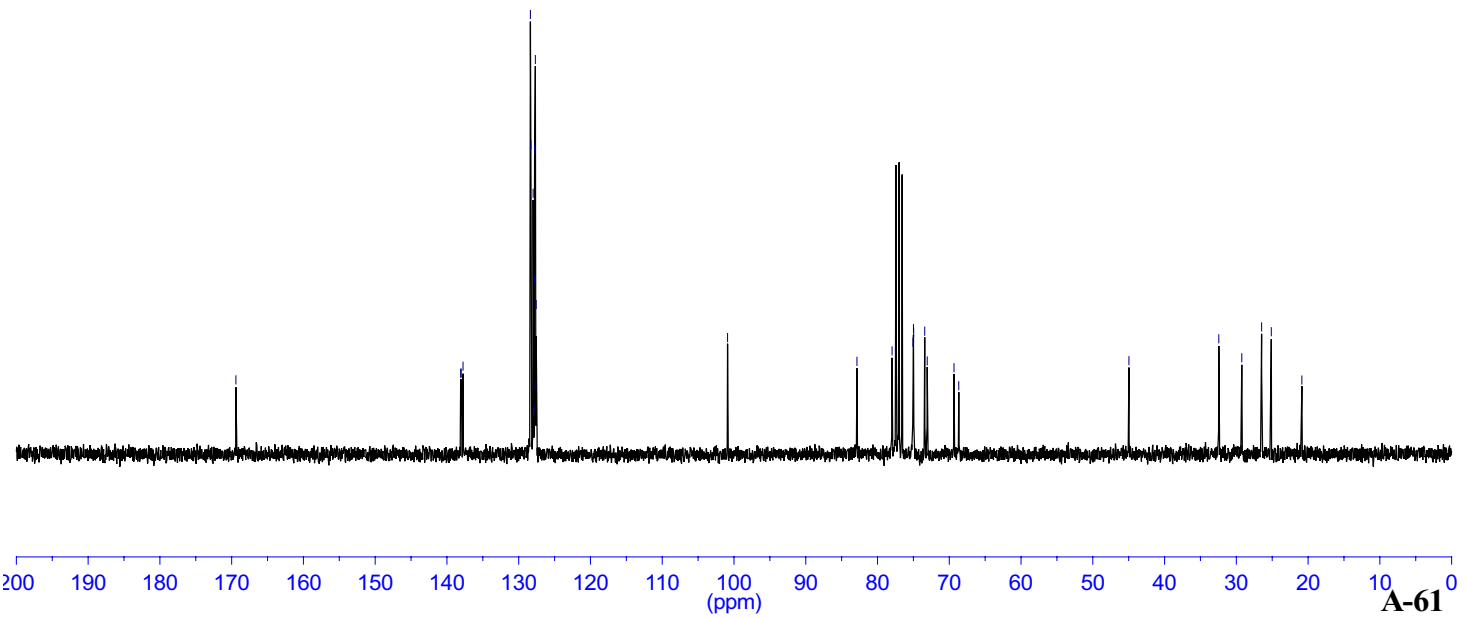


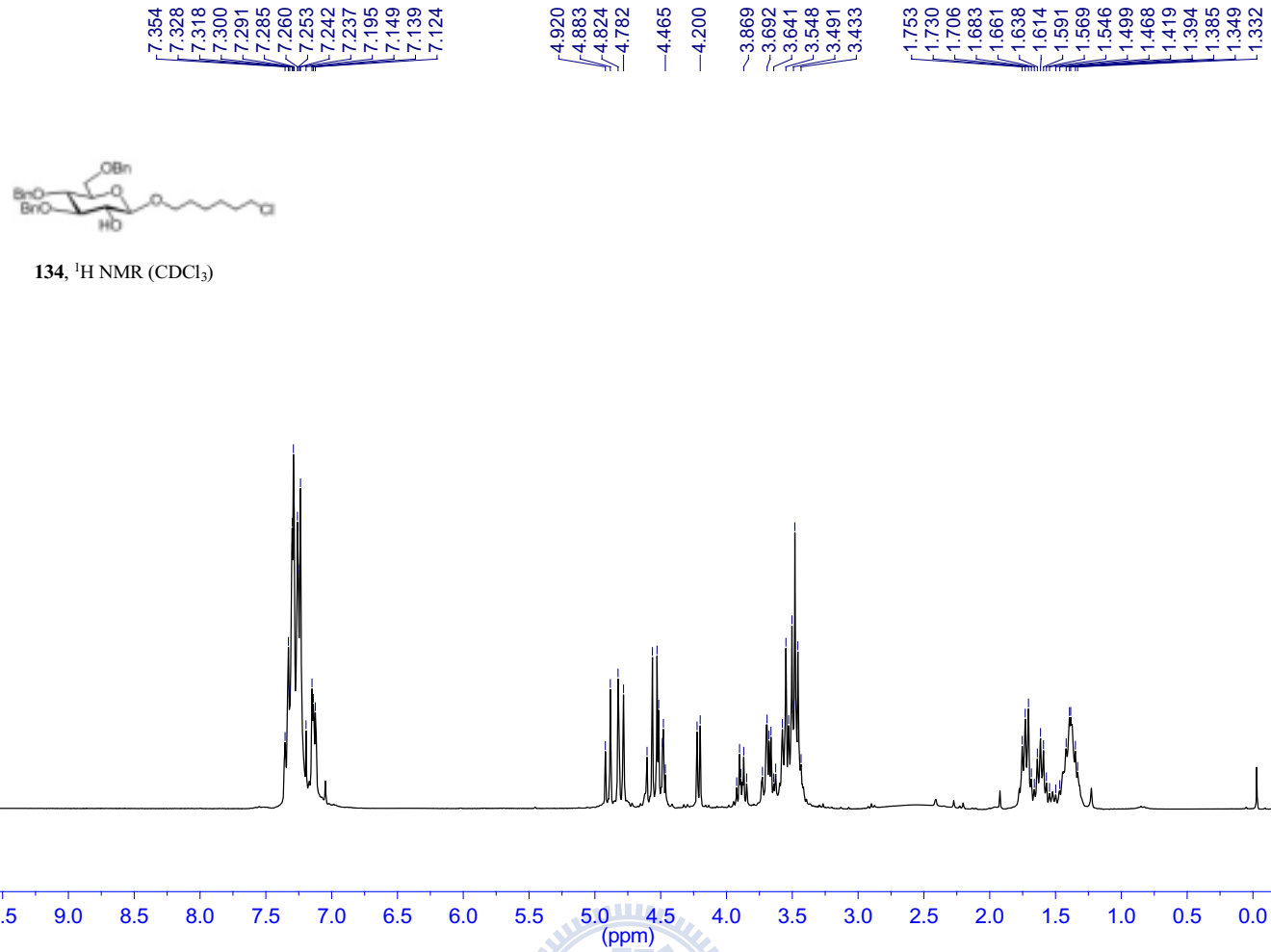
133, ^{1}H NMR ($CDCl_3$)



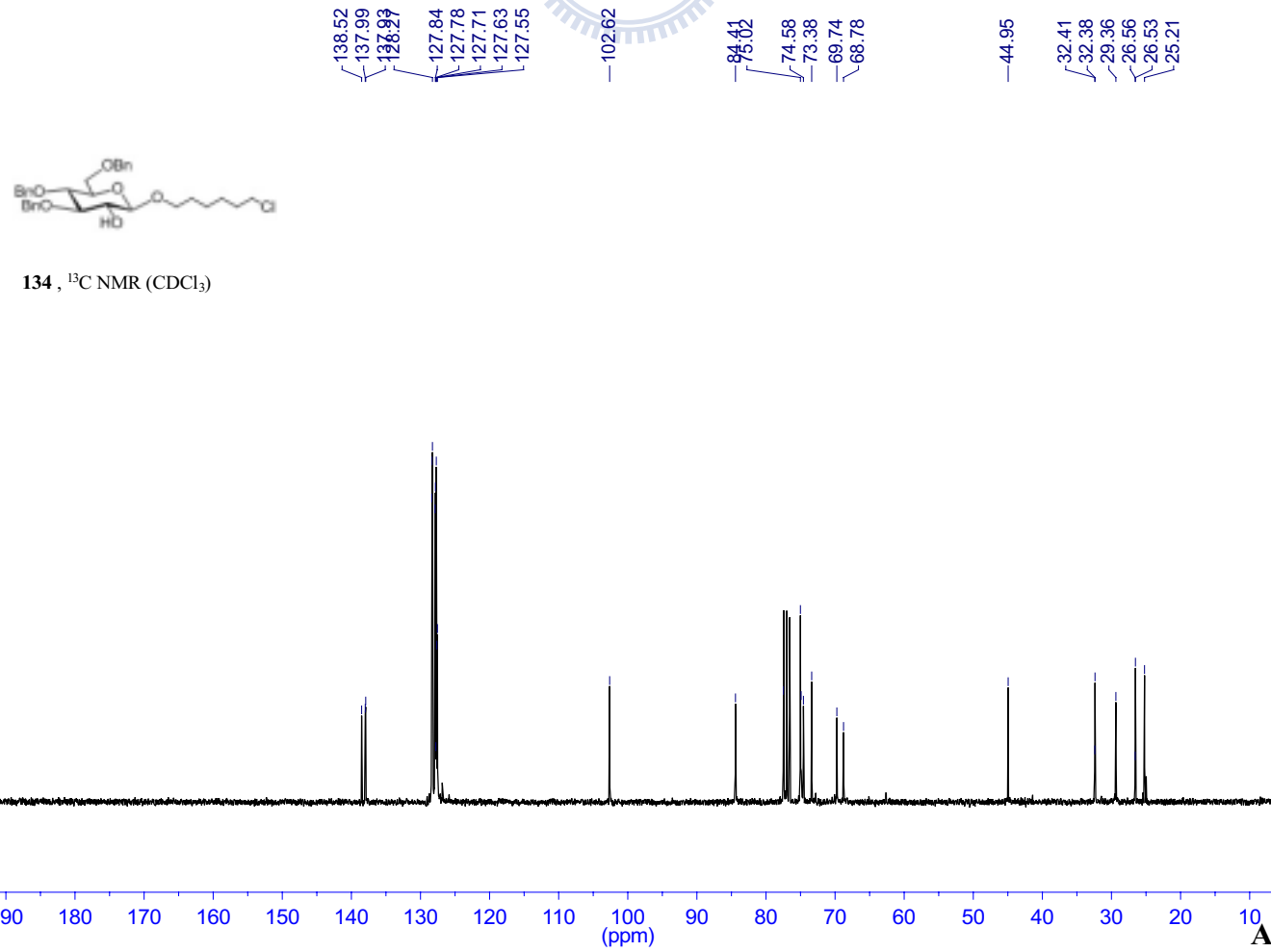


133 , ^{13}C NMR ($CDCl_3$)

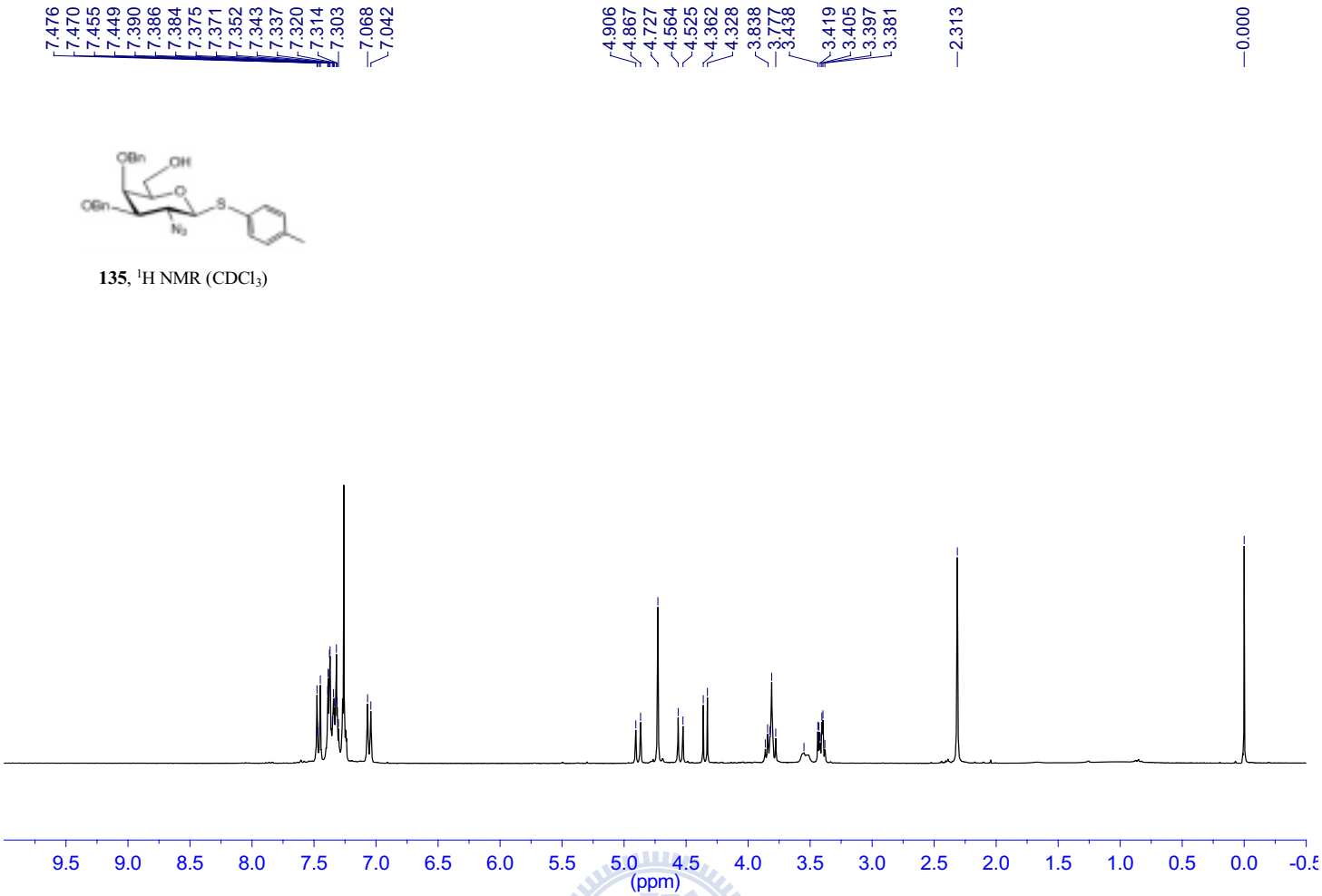




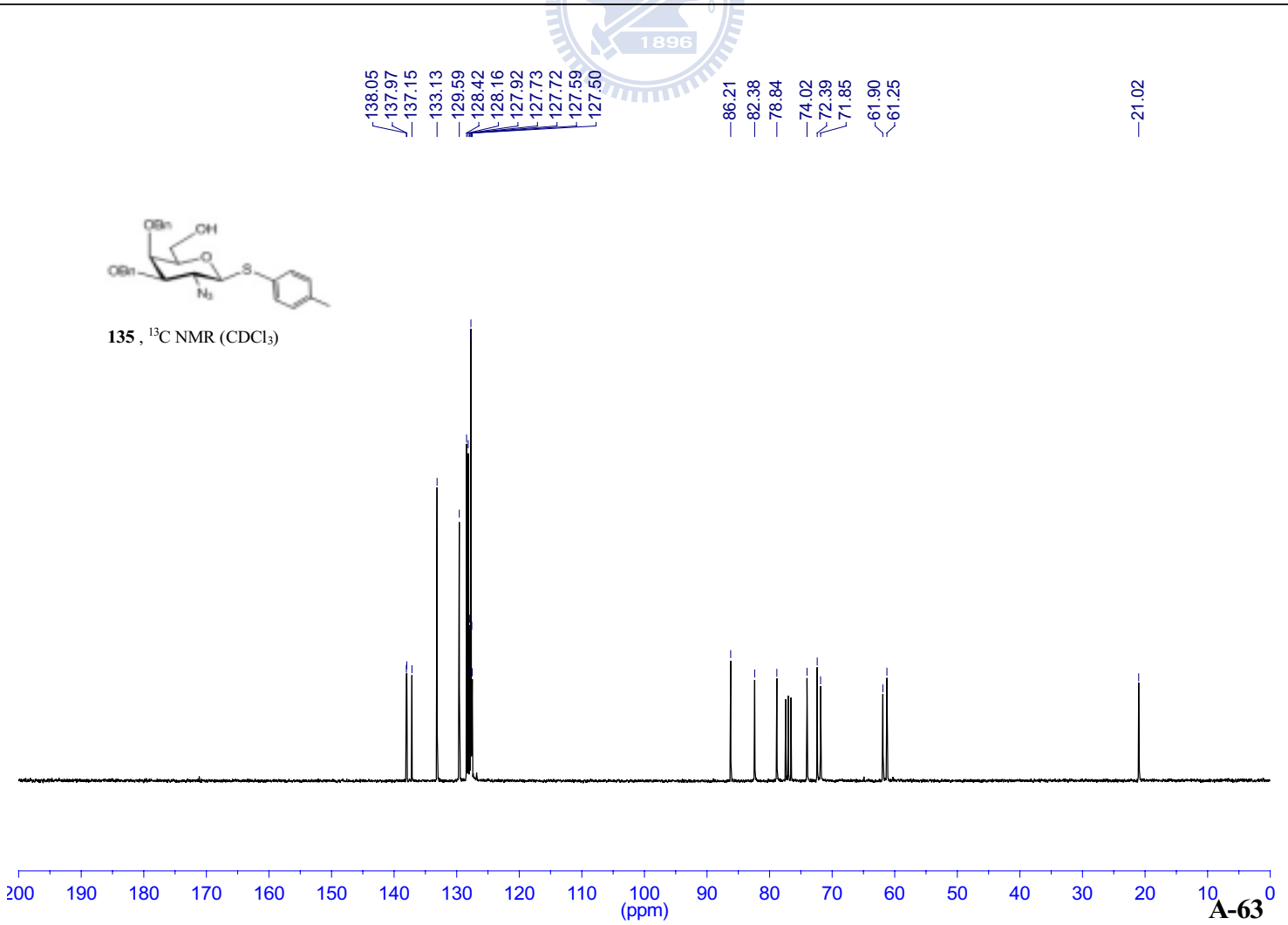
134, ^{1}H NMR ($CDCl_3$)



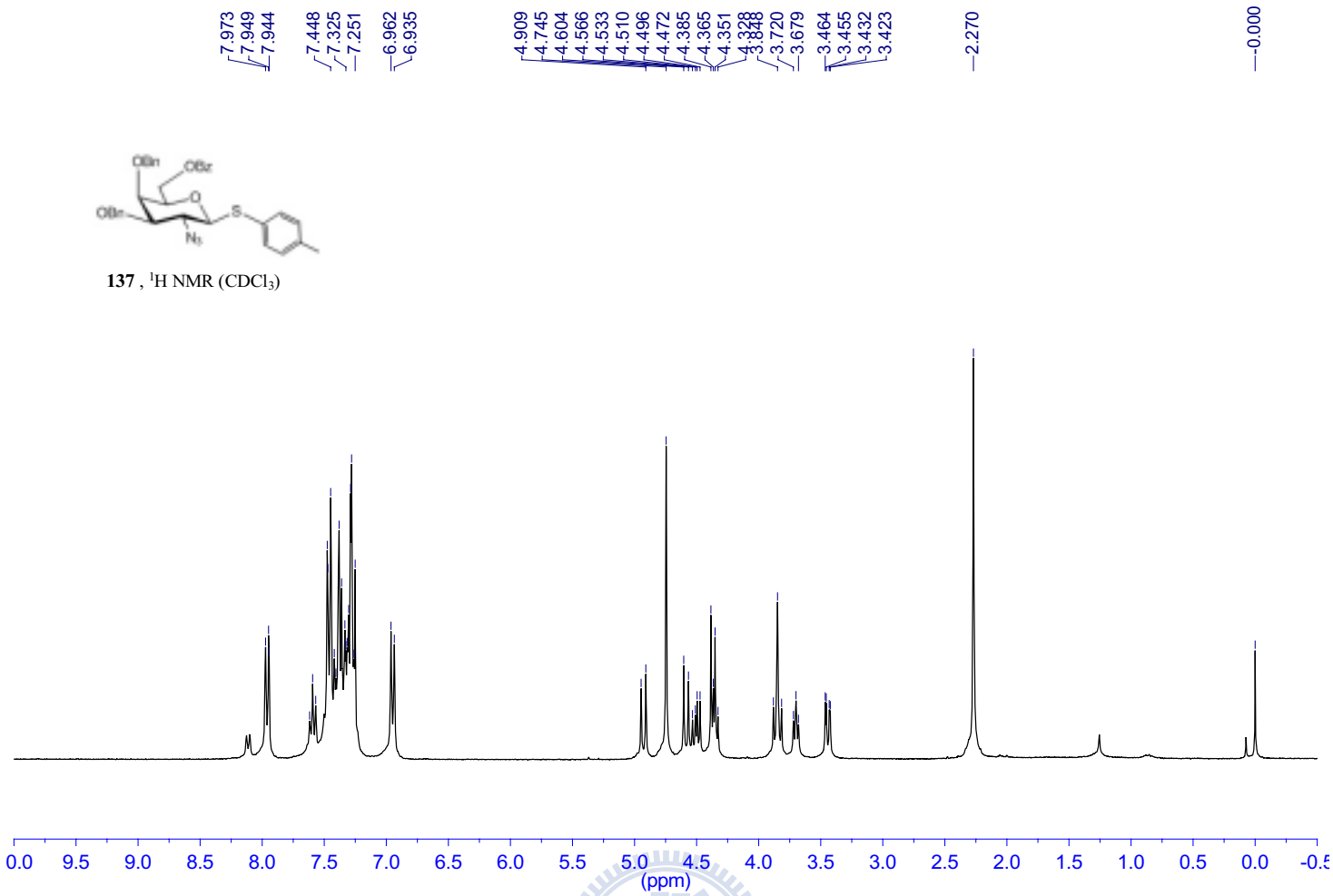
134 , ^{13}C NMR ($CDCl_3$)



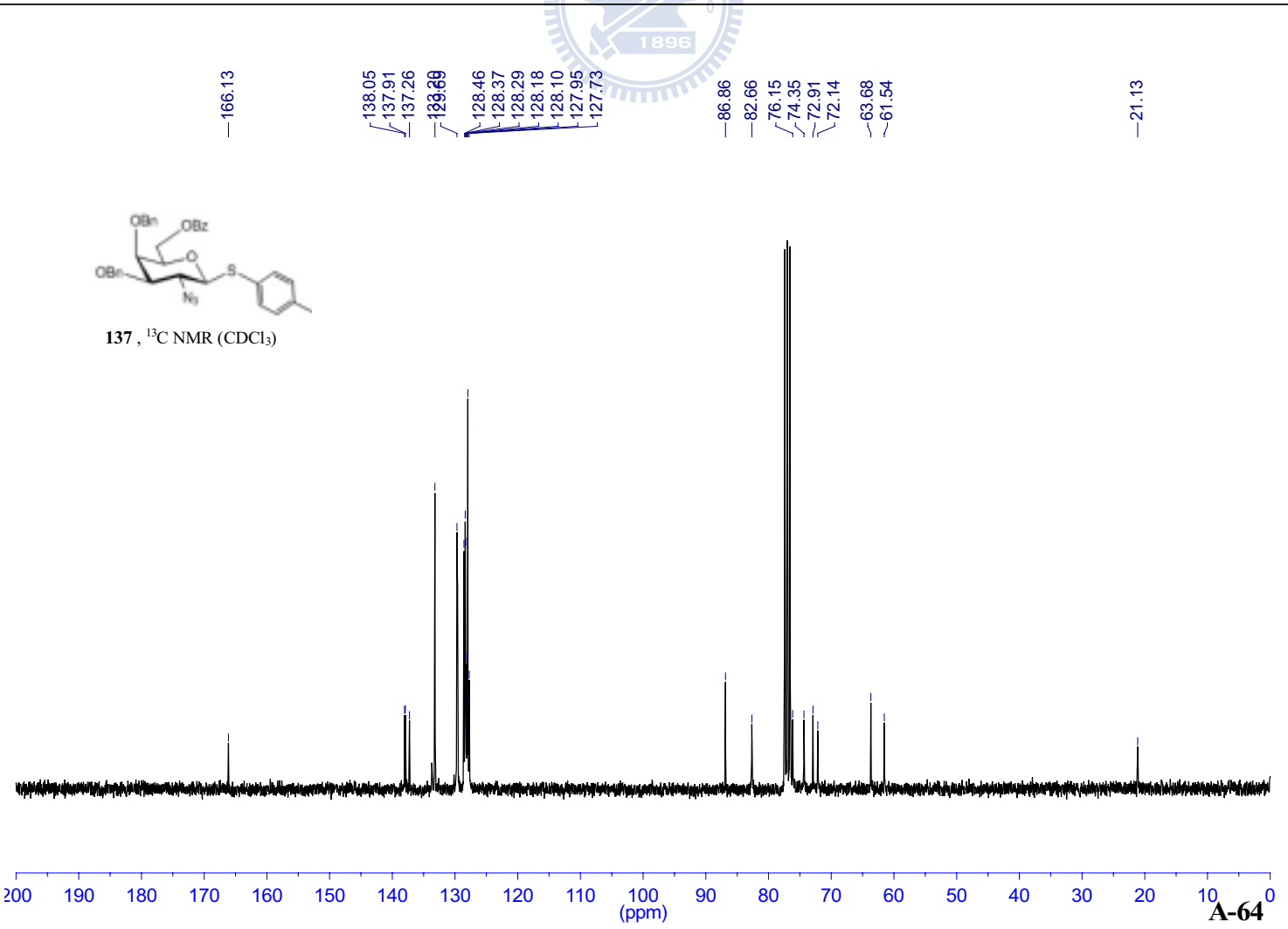
135, ^{1}H NMR ($CDCl_3$)



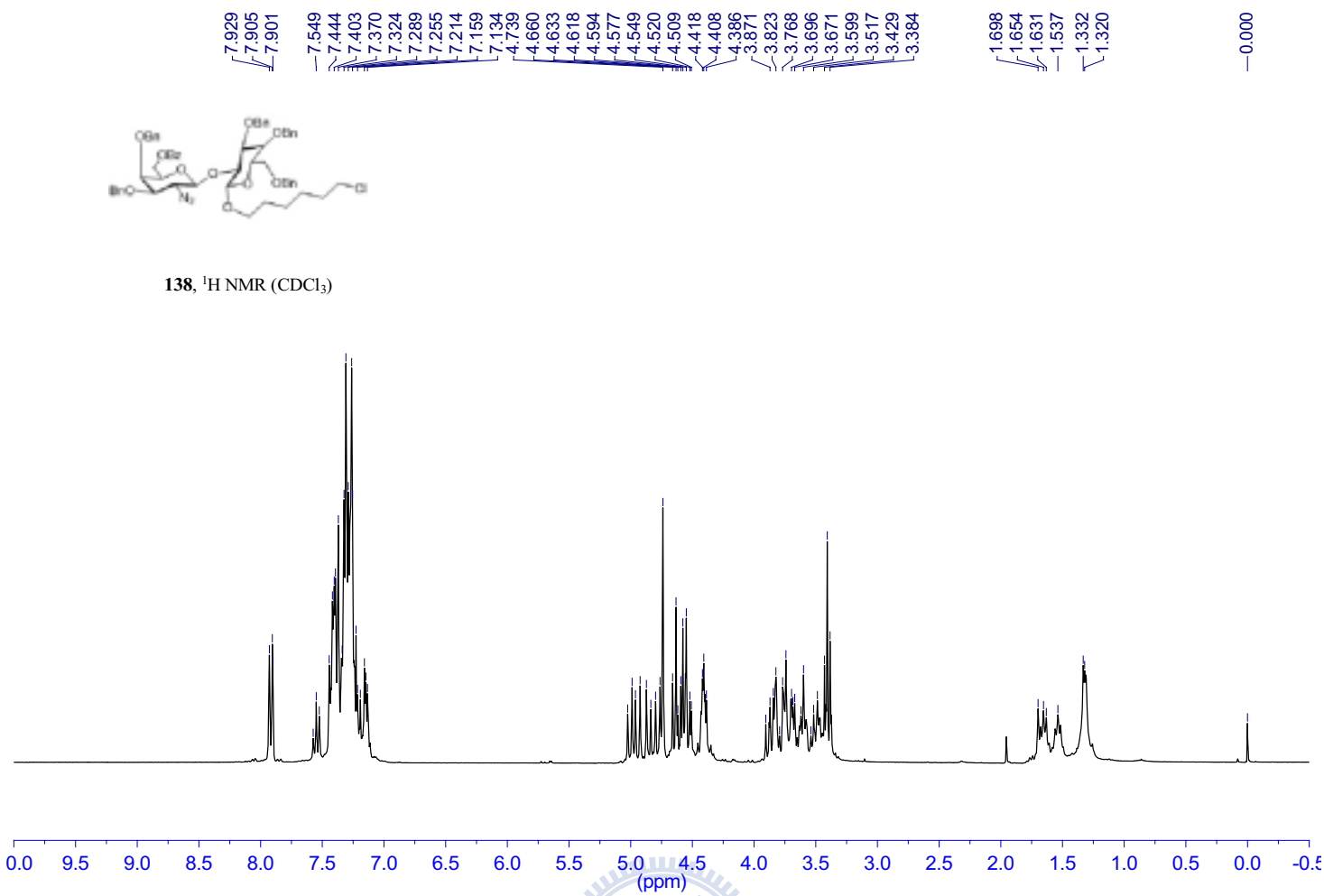
135 , ^{13}C NMR ($CDCl_3$)



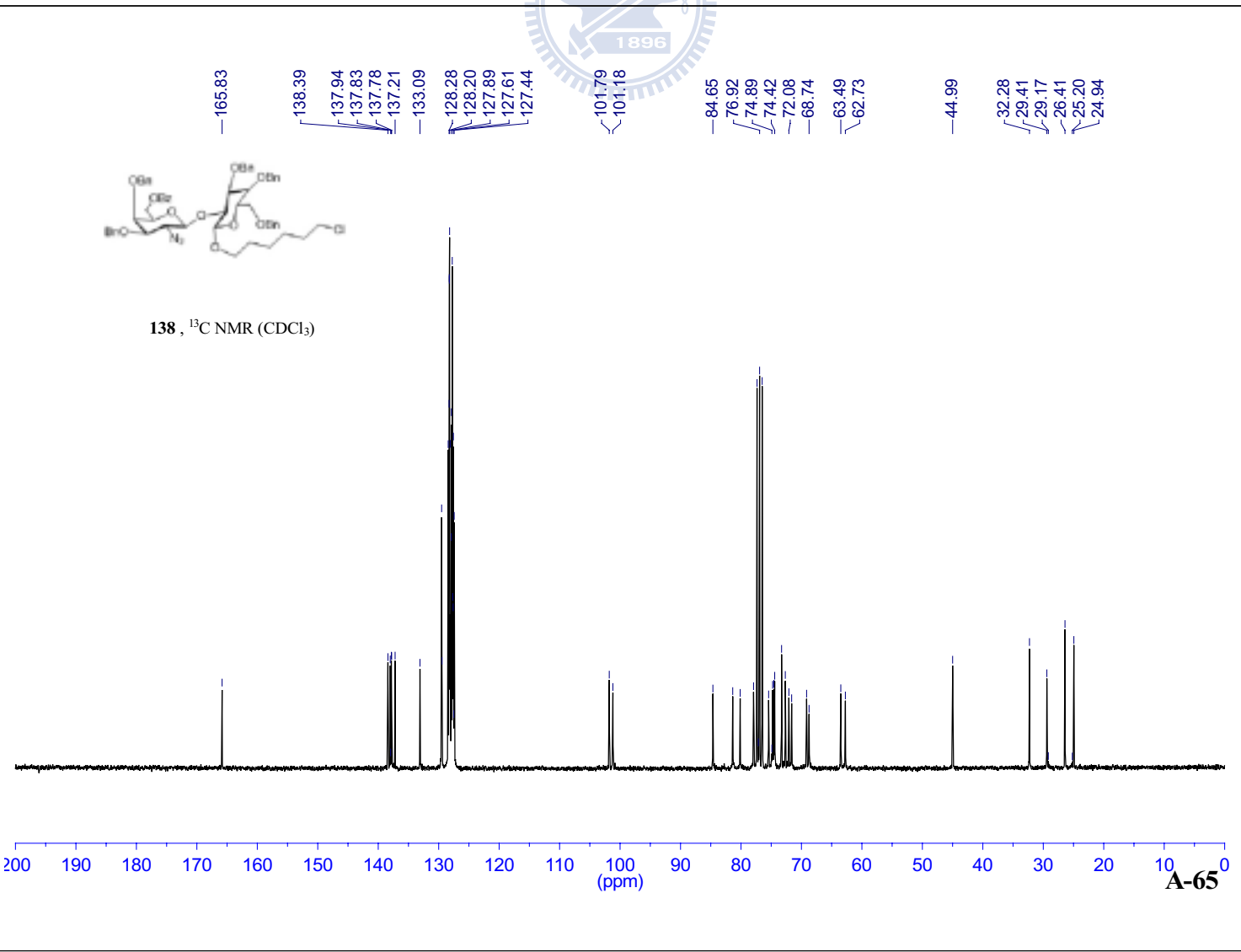
137 , ^{1}H NMR ($CDCl_3$)



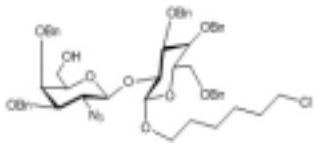
137 , ^{13}C NMR ($CDCl_3$)



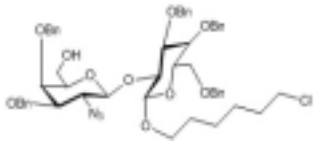
138, ^{1}H NMR ($CDCl_3$)



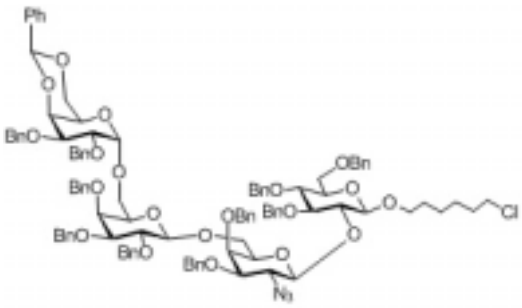
138 , ^{13}C NMR ($CDCl_3$)



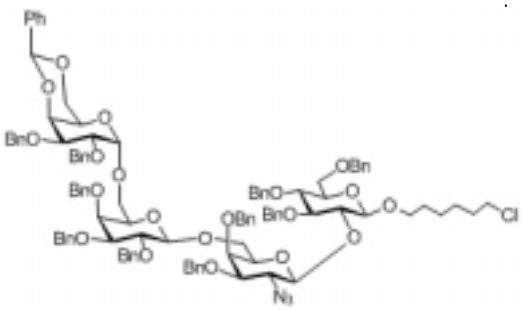
139, ^{1}H NMR ($CDCl_3$)



139 , ^{13}C NMR ($CDCl_3$)

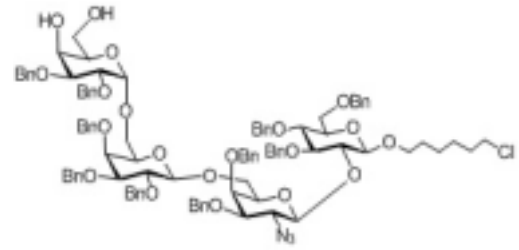


140, ^{1}H NMR ($CDCl_3$)

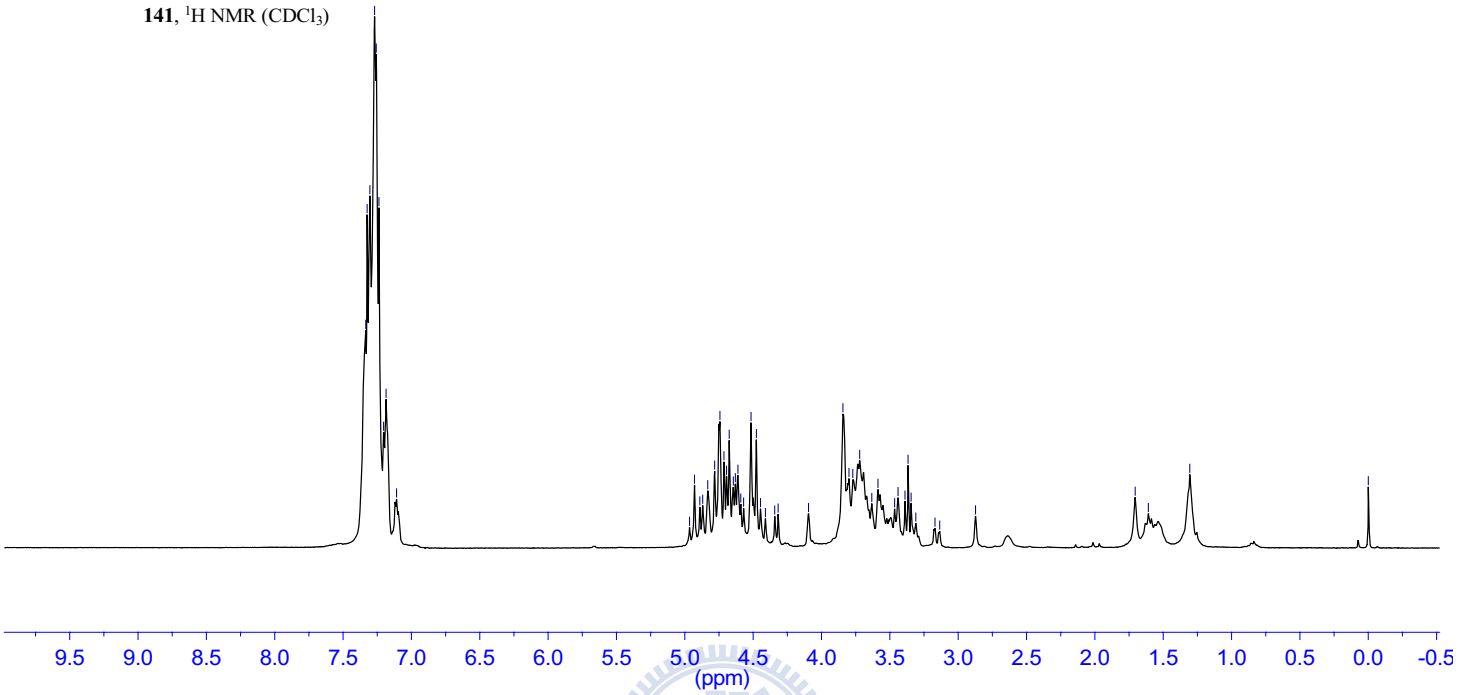


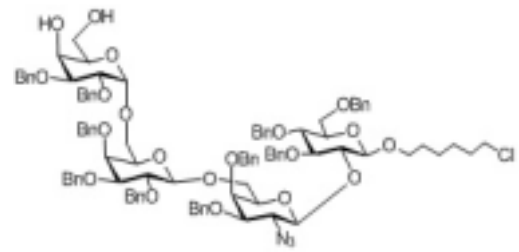
140 , ^{13}C NMR ($CDCl_3$)

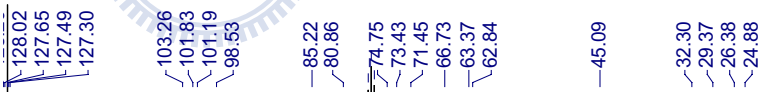
140, non-decoupling ($CDCl_3$)



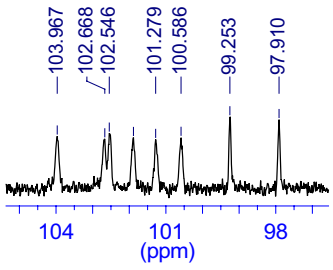
141, ^{1}H NMR ($CDCl_3$)







141, ^{13}C NMR ($CDCl_3$)



140, non-decoupling ($CDCl_3$)