第一章　對甲苯磺酸在醣化學的應用：一個較環保的醣基建構單元合成法

1.1.1 醣分子全乙醯化反應簡介

寡醣分子在生物中扮演許多重要的角色。比如說：黏著、傳訊、辨識等等。[1] 像最近造成世界性疫情的 A 型流感病毒 H1N1 就是利用酵素辨認寡醣分子進出活體細胞，這樣的機制促使科學家們製造了仿糖分子作為藥物，像：Tamiflu、Relenza 或其它藥物；[2] 人類血型抗原中的路易氏抗原 (Lewis antigen) 與 H-抗原則分別與引起胃潰瘍的幽門螺旋菌 (*Helicobacter pylori*)、引起人畜相通腸炎的腸炎弧菌 (*Campylobacter jejuni*) 有關。[3]

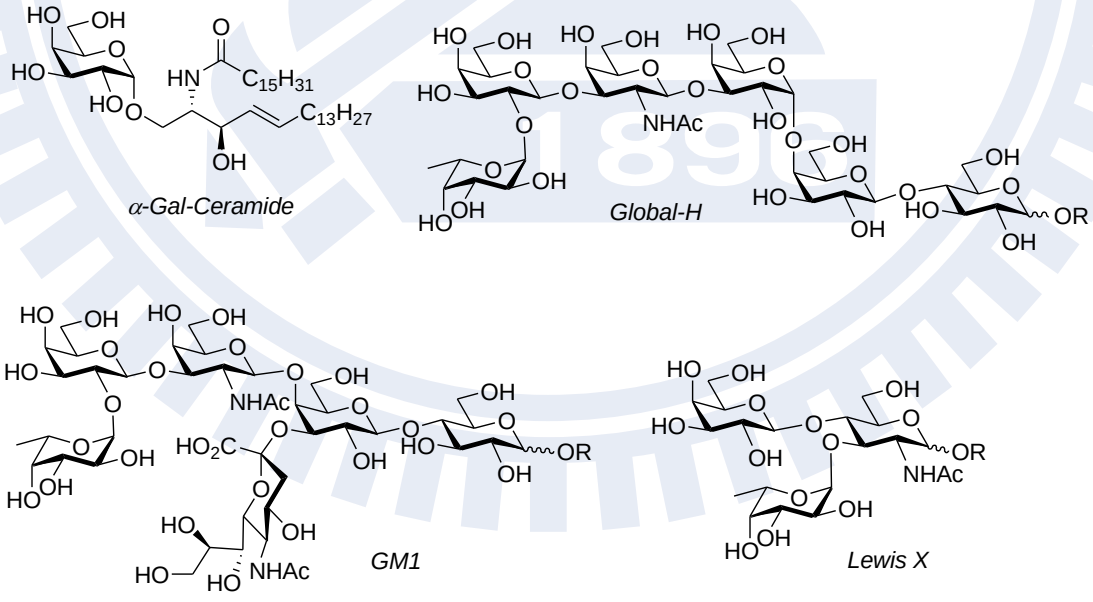


圖 1.1. 一些與人體相關的天然寡醣分子

而人類的健康大敵：癌症，與寡醣息息相關，科學家們嘗試著研

究被癌症細胞大量表現的醣分子，如：Global-H、Gb_3、GM1 等，以治療癌症。[4] 為了研究這些生物作用，首要就是要大量得到各種不同結構、不同單元組合的寡醣。要得到這些寡醣分子，相對於用萃取的方式或酵素進行生物合成的方式，顯然採用有機合成較有效率。

要合成寡糖就必須將醣分子與另一個醣分子或非醣分子進行橋接 (coupling)，這種由酵素或化學方法進行的橋接反應特別命名為「醣基化反應 (glycosylation)」。而用來進行醣基化反應的單醣或多醣分子則被稱為醣基建構單元 (glycosyl building block)，其中又區分為：醣予體 (glycosyl donor) 與醣受體 (glycosyl acceptor)。

顧名思義：醣予體就是醣基的提供者，是醣分子；而醣受體就是醣基的接受者、不一定是醣分子。

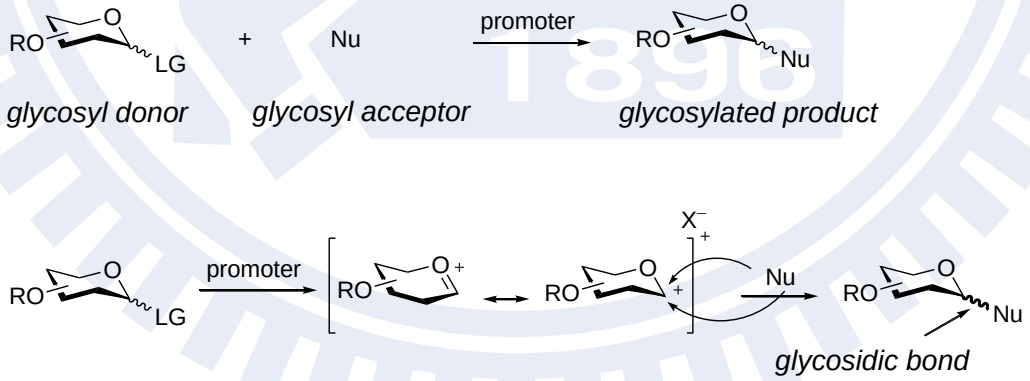


圖 1.2. 較普遍的醣基化反應機構

在醣基化反應的類型中最普遍的一類，往往將醣予體的端點修飾成一離去基，也就是將醣予體修飾成親電子基前驅物 (electrophile

precursor) 後，再以相對應的活化系統將離去基活化離去形成醣基陽離子 (glycosyl cation、glycosyl oxocarbenion)，此時醣受體作為親核基分子由醣基陽離子的 α 或 β 位向攻擊進行橋接，此新生成的化學鍵結又特別被稱做「醣苷鍵 (glycosidic bond)」(圖 1.2)。[5]

在漫長的醣化學歷史中，有許多的端點離去基與活化劑系統被開發出來，除了廣為人知的 Koenigs-Knorr 反應使用的醣基鹵化物 (glycosyl halide) 外，以硫醇做為離去基的硫糖分子 (thioglycoside) 基於其穩定性、高反應性、與其後修飾醣基建構單元的方便性，使硫糖分子在寡醣合成中是相當常見的醣予體。[6]

但要將全羥基的醣分子 (free sugar substrate) 修飾為可以進行有機反應與管柱純化的醣基建構單元，必須先改善醣分子對常用有機溶液 (二氯甲烷、四氫呋喃、乙腈等等) 的低溶解度，要達到此目的就需先對醣分子上的羥基以有機保護基團進行修飾，此官能基需要兩個特點：1. 容易接駁、2. 容易除去 (以進行進一步修飾)；較常見到的選擇有三甲矽烷基 (trimethyl silyl, TMS)，或乙醯基 (acetyl)。[7] 相對來說乙醯基較為穩定，使其普遍被使用於醣分子修飾上。

除了改善溶解度外，乙醯基也容易進行官能基轉換，比如說：在溴化氫的醋酸溶液可以將端點乙醯基轉換為溴，製備醣基溴化物；而在適當的路易士酸試劑活化下，例如：三氟硼烷-乙醚錯合物 (boron

triflouride diethyl etherate，$BF_3.OEt_2$)，端點乙醯基可以被活化離去，與親核基分子進行 Helferich 醣基化反應；若以硫醇或硫酚作為親核基分子，則可以得到硫糖單元，這也是醣分子修飾為硫糖分子的普遍作法。[8] 乙醯基可在較溫和的酸性或鹼性條件下被除去，與其他保護基團做正交選擇性去保護 (orthogonal deprotection)；若在聯胺與二甲基甲醯胺的條件下，則可選擇性除去端點的乙醯基得到醣基半縮醛，並以之進行醣基化反應或進一步修飾半縮醛基團 (圖 1.3)。[9]

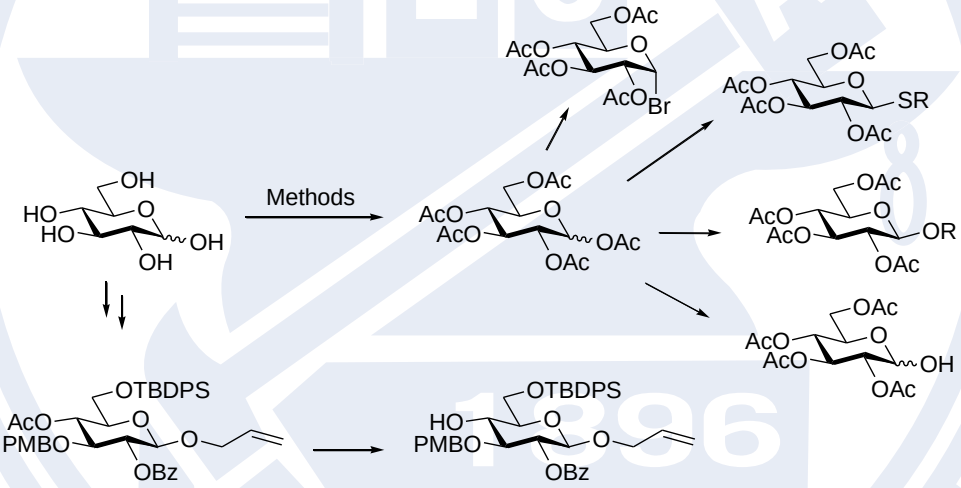


圖 1.3. 乙醯基在醣分子修飾上的應用

此外，講到醣基化反應，C2-乙醯基能提供鄰基效應 (neighboring group participation) 以利建構 1,2-反式醣苷鍵 (這方面的介紹將在本論文第二章節：低濃度醣基化反應有更多的論述)。

除了在合成的應用，在核磁共振圖譜分析上，因為乙醯基的簡單結構與拉電子性質，接駁乙醯基的鄰近碳氫訊號會往 downfield 位

移，使訊號不易彼此重疊，較容易判斷寡醣分子結構 (圖 1.4)。[10]

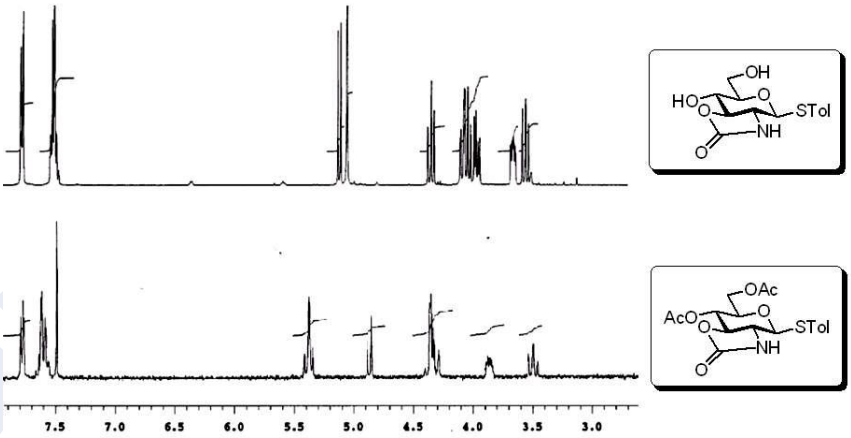


圖 1.4. 乙醯基在核磁共振上的 down field 效果

1.1.2 乙醯化反應的方法與研究動機

市面上已經有許多的乙醯化反應方法發表，簡單可分類為弱鹼性試劑、酸性催化劑、與酸性非勻相試劑等等。

使用弱鹼性試劑進行乙醯化反應，比如說吡啶 (pyridine)、三乙胺 (triethyl amine)、4-二甲基胺吡啶 (*N*,*N*-dimethyl 1,4-amino pyridine，DMAP)、嘧唑 (imidazole)、1,4-二氮二環[2,2,2]辛烷 (1,4-diazabicyclo[2.2.2]octane，DABCO) 等等，此類方法往往在室溫下將糖分子、等當量或過量的有機鹼、與過量醋酸酐一起反應，大量毒性試劑使得後續處理相對困難。[11]

若改用酸性試劑催動反應，則有更多的選項，比如說在室溫下可使用醋酸酐配合催化量的碘、或三氟甲矽烷銅、三氟甲矽烷釩與其他

三氟甲矽烷鹽類、或是氯化鈷與其它滷化鹽類、溴化氫的醋酸溶液、三氟硼烷等等。[12] 若採用醋酸鈉、過氯酸鋰、氧化鋁等有機鹽試劑，就需要適當的加熱配合醋酸酐或醋酸醯氯進行乙醯化反應。[13] 相對來說，使用酸性條件進行乙醯化反應可以使用催化劑量的酸，甚至近於等當量的醋酸酐 (對應糖分子每個羥基使用一當量)，與鹼性試劑條件相比則較為經濟、環保；甚至可以接著在一鍋反應中對全乙醯化產物進行更進一步的修飾、節省純化的步驟。

而 Misra 教授、Chakraborti 教授與 Mukhopadhtay 教授將過氯酸或硫酸吸附在矽膠上，可以當作非勻相酸性試劑用以催動乙醯化反應；[14] 類似的非勻相試劑尚有酸性黏土 K-10 或酸性樹脂等等。[15] 此類試劑的優點在反應後只需過濾掉試劑便可終止反應，較不方便的是需自行製備酸吸附的矽膠，有爆炸性風險。而酸性黏土或樹脂則需要較長的反應時間；且不易估算試劑的實際用量。

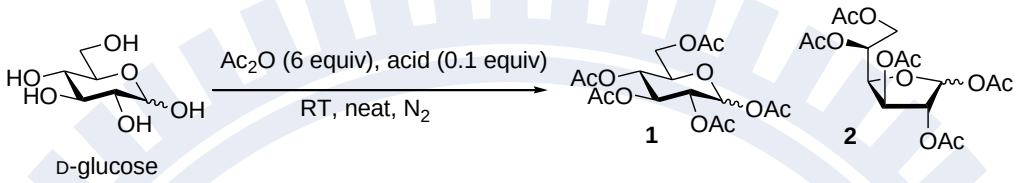
綜合以上的瀏覽，我們想要找尋一個方便使用、成本便宜、對環境相對友善、較無毒性且效率不差的方法，似乎以磺酸催動乙醯化反應的報導較少，於是我們決定先由此著手探討。

1.2 結果與討論

1.2.1 全乙醯化反應的探討

首先我們嘗試以不同的酸與醋酸酐催化乙醯化反應 (表 1.1)，先以葡萄糖為起始物、醋酸酐進行測試。

表 1.1. 催化劑的酸度與乙醯化反應速率關係



entry	Acid	*p*Ka of acid	Time (h)	yield (%)	pyranose **1** : furanose **2**[a]
1	TFA	2	48	0	No reaction
2	CSA	-1	12	90	>95% pyranose
3	TsOH	-6.5	6	95	>95% pyranose
4	H_2SO_4	-10	0.2	70	Not determined
5[b]	H_2SO_4	-10	0.2	94[b]	90 : 10

[a] 由核磁共振氫譜判斷 (ref.16)，[b] 在 0 °C 加入硫酸 (99.99%) 於反應中。

由實驗結果發現乙醯化反應的反應速率與所使用的催化劑酸度正相關，*p*Ka 越小的酸反應速率越快、反之越慢。但採用 *p*Ka 較小的硫酸做為催化劑有兩個缺點：其一是在室溫下將硫酸加入反應中，只需十分鐘就可完成反應，但會伴隨有糖分子脫水碳化的現象、降低產率 (表 1.1，實驗 4)；若將反應先降溫到 0 °C 再加入硫酸，碳化現象相對減少許多。硫酸的另一個缺點是在全乙醯化的過程中會容易產生五員環異構物，經由核磁共振氫譜分析與文獻比對判斷，約產生有

10% 的五員環產物 **2** (表 1.1，實驗 5)。[16]

於是我們選定使用較少引起五員環化且反應速率較快的對甲苯磺酸 (*p*-tolyl sulfonic acid monohydrate，TsOH. H_2O) 做為催化劑，而醋酸酐與催化劑的用量則以糖分子的 OH 基數量做計算標準，嘗試對不同糖分子進行全乙醯化反應測試 (表 1.2)。

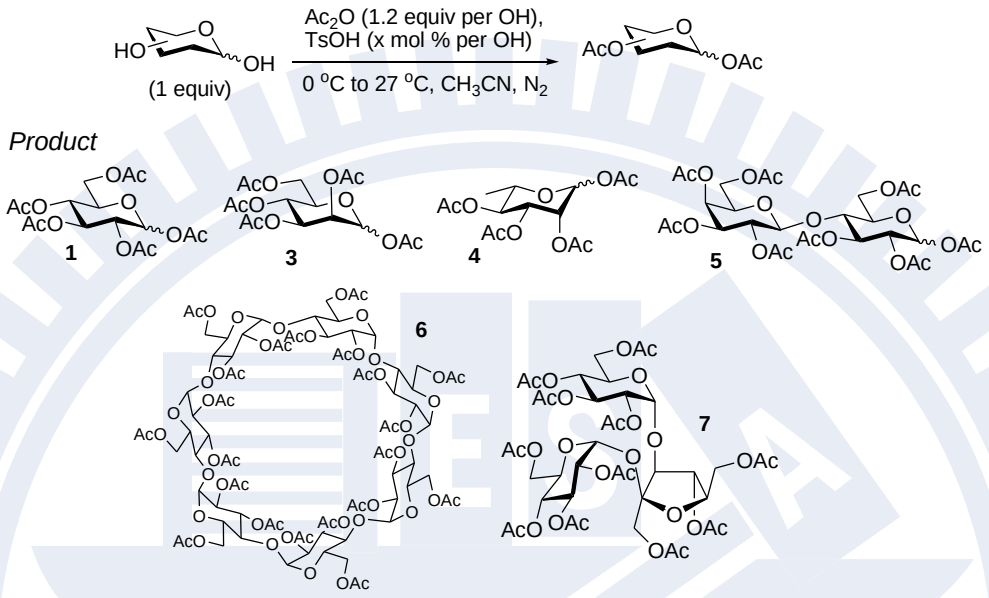
由實驗結果觀察，不論 D-式或 L-式單糖 (甘露糖、鼠李糖) 甚至寡糖分子：乳糖 (lactose)、β-環糊精 (β-cyclodextrin) 可得到 90%以上的全乙醯化產物 **3**、**4**、**5**、**6** (表 1.2，實驗 2、3、4、5)。

但以此條件作用在松三糖 (melezitose) 上會伴隨產生醣甘鍵被破壞的現象，需將催化劑的當量降低，則可得到 75%的產率、醣甘鍵被破壞的情形也較少 (表 1.3，實驗 6)。多數的糖分子可在無溶劑下 (neat) 進行乙醯化反應，而 β-環糊精與松三糖因分子量較大使相對應的醋酸酐體積較小，故需加入適量乙腈作為溶劑幫助攪動。

在以較活潑的糖分子，例如：半乳糖、岩藻糖，進行全乙醯化反應時，我們觀察到即使採用對甲苯磺酸進行反應，卻會明顯產生五員環異構物 (表 1.3，實驗 1、5)。若是將半乳糖保持在 0 °C 下進行乙醯化反應，雖然反應時間拉長到 16 小時，但五原環異構物比例相對降低；依此原則，我們嘗試在 –20 °C 下進行乙醯化反應，希望可再降低旁產物的比例，卻發現就算提高催化劑的當量數，反應經過 72 小

時仍無法得到可接受的產率 (表 1.3，實驗 2、3)。

表 1.2 以 TsOH 對不同結構的醣分子進行全乙醯化測試



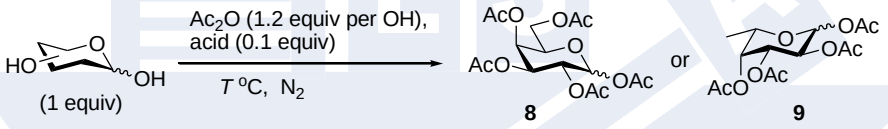
entry	S.M. (0.5 g)	Product	TsOH (mol% per OH)	CH_3CN (mL)	Time (h)	yield (%), α : β
1	D-glucose	**1**	2	0	6	95 (71 : 29)
2	D-mannose	**3**	2	0	5	94 (89 : 11)
3	L-rhamnose	**4**	2	0	4	92 (58 : 42)
4	D-lactose.H_2O	**5**	2	0	4	90 (60 : 40)
5	D-β-cyclodextrin	**6**	1	1.5	10	90
6	D-melezitose	**7**	1	0.5	6	75[a]

[a] 發現有醣鍵被切斷.

配合之前的觀察 (表 1.1)，我們改用酸度較高、反應速率較快的硫酸做為催化劑，在 –20 °C 下將硫酸配製成乙腈溶液後，以注射針

筒滴入半乳糖與醋酸酐的混合液中，再使反應溫度在一小時內緩緩回到 0 °C，經過 18 小時後可得到 92%的全乙醯化半乳糖 **8**，其中僅產生不到 5% 五員環產物 (表 1.3，實驗 4)；以此硫酸 (乙腈溶液) 做為催化劑，改由活性更高的岩藻糖進行反應，則改在–30 °C 下加入催化劑、並讓反應溫度升到–20 °C，在 6 到 8 小時後可得到產率 93%的全乙醯化六員環岩藻糖 **9** (表 1.3，實驗 6)。

表 1.3. 針對活性較大的糖分子的全乙醯化反應測試



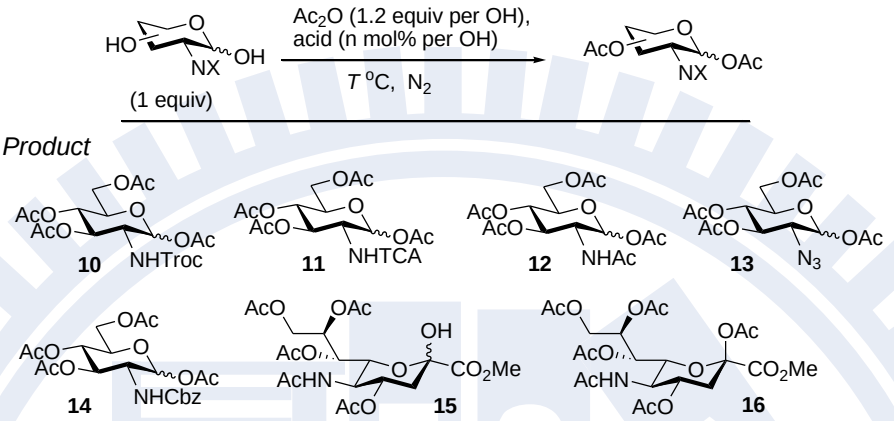
entry	S.M. (0.5 g)	acid (mol% per OH)	T (°C)	Time (h)	yield (%), pyranose : furanose
1	D-galactose	TsOH, 2	0 to 25	8	92, 70 : 30
2	D-galactose	TsOH, 2	0	16	80, 89 : 11
3	D-galactose	TsOH, 5	-20	72	<20
4	D-galactose	H_2SO_4, 2	-20 to 0	18	92, >95 : 5[a]
5	L-fucose	TsOH, 2	0 to 25	0.5	90, 74 : 26
6[a]	L-fucose	H_2SO_4, 2	-30 to -20	8	93, >95 : 5[a]

[a]：將 H_2SO_4 (純度>99.99%, Aldrich) 溶於 CH_3CN 後做為試劑使用.

我們接著嘗試將磺酸催化乙醯化反應推展到糖胺分子上，首先以胺基具有不同保護基的葡萄糖胺分子進行實驗，並以碘催化之乙醯化

反應做對照實驗比較 (表 1.4)。

表 1-4 比較不同路易氏酸對糖胺進行乙醯化反應



entry	S.M. (0.5 g)	Product	Acid (mol% per OH)	T (°C)	Time (h)	yield (%), α : β [a]
1	D-Glc*N*-Troc	**10**	TsOH, 2	0 to 27	5	90, 27 : 73 [b]
2	D-Glc*N*-TCA	**11**	TsOH, 2	0 to 55	5	93, 77 : 23[b]
3	D-Glc*N*-Ac	**12**	TsOH, 2	0 to 50	5	90, 61 : 39
4	D-Glc*N*-azido	**13**	TsOH, 2	0 to 27	4	85, 23 : 77[b]
5	D-Glc*N*-Z	**14**	TsOH, 2	0 to 40	3	94, 58 : 42[b]
6	NANA methyl ester	**15**	TsOH, 2	0 to 27	4	90, 20 : 80[b]
7	NANA methyl ester	**16**	TsOH, 2	0 to 45	12	80, β only [c]
8	D-Glc*N*-Troc	**10**	I_2, 9	0 to 27	26	No reaction[b,c]
9	D-Glc*N*-Ac	**12**	I_2, 5	0 to 27	48	98, 72 : 28 [c,d]
10	NANA methyl ester	**15**	I_2, 6.5	0 to 27	sluggish	-- [c,d]
11	NANA methyl ester	**15**	I_2, 13	0 to 27	0.3	70, -- [c,d]
12	NANA methyl ester	**16**	I_2, 13	0 to 35	1	90, 22 : 78 [c,d]

[a] 由核磁共振氫譜與文獻 (附錄於 1.4 實驗部分 Table s1) 判斷異構物比例，[b] 加入適量乙腈 (0.5 mL per 0.5 g S.M.) 幫助反應攪動，[c] 醋酸酐為每羥基使用 3 ~ 4

當量，[d] 參考文獻 ref.12a。

發現 C2-胺基以 Troc (2,2,2-trichloroethoxycarbonyl) 保護的葡萄糖胺，在室溫下、接近等當量的醋酸酐試劑，3 ~ 5 小時可得到 90% 的全乙醯化產物 **10** (表 1.4，實驗 1)。以此基礎，我們將葡萄糖胺分子的 C2-胺基分別以三氯乙醯基 (trichloroacetamyl，TCA)、乙醯基 (acetamyl，Ac)、疊氮基 (azido，N_3)、與胺甲酸苄酯 (benzyl carbamate，Cbz) 進行保護，再以 TsOH 測試乙醯化反應。

因葡萄糖胺分子量較大，需加入少量的乙腈溶劑幫助磁石攪動；之後在適當溫度下，以催化量的 TSOH (每個羥基使用 2 mol%) 與接近等當量 (每個羥基使用 1.2 當量) 的醋酸酐，3 ~ 5 小時即得到 85 ~ 98% 的預計產物 **11**、**12**、**13**、**14** (表 1.4，實驗 2、3、4、5)。

若改用碘做為路易士酸對 Troc 保護葡萄糖胺進行全乙醯化反應，即使將碘當量增加為每羥基使用 9 mol%，醋酸酐增加為每個羥基使用 3 當量，在室溫下經過 24 小時後反應仍未進行 (表 1.4，實驗 8)。[12a] 而以 *N*-Ac 葡萄糖胺為例，則需增加碘的劑量為每羥基使用 5 mol%，在室溫下經過 48 小時後方得到 98%的預計產物 (表 1.4，實驗 9)；兩相比較顯見 TsOH 催化糖胺進行乙醯化反應的高效率。

此外我們也嘗試以 TsOH 對唾液酸甲酯進行全乙醯化反應，實驗發現，以催化量的 TsOH 與接近等當量的醋酸酐，在室溫下經過四

小時可以得到四乙醯基產物 **15**，C2 上的三級羥基由於立體障礙與鄰近的酯基拉電子效應而未進行乙醯化轉換，於是我們改變反應條件在過量 (每個羥基使用 2 當量醋酸酐) 與適當的溫度下可以得到約 80%的全乙醯化唾液酸 **16**。同樣再與文獻做比較，使用碘與唾液酸甲酯進行乙醯化反應時，需使用每羥基 3 當量的醋酸酐，若在每羥基 6.5 mol% 的碘作用下，乙醯化反應速率相對緩慢，需將碘量為每羥基 13 mol% 則在室溫下可以得到四乙醯基半縮醛 **15**，在 35 ^{o}C 的反應溫度下可得到全乙醯化唾液酸 **16**。

由以上的實驗我們驗證了磺酸催化乙醯化反應，特別是在糖胺分子上的高效率性。在實驗完成後也只需要做萃取的動作就可得到純度頗高的產物，可直接進行進一步反應。值得一提的是此方法可以放大數量到 30 ~ 50 克起始物，如：葡萄糖、半乳糖、甘露糖、*N*-Troc 葡萄糖胺等，而效率未見衰退；而利用溫度與醋酸酐當量的控制更可以得到唾液酸半縮醛分子 **15** 或全乙醯化唾液酸 **16**。

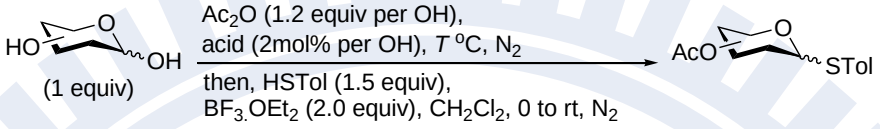
接著我們想到在糖分子建構單元的修飾上，往往需要許多耗時費力的步驟，包括合成與純化，如果可以將其中一些步驟改為一鍋化操作，不就可以省下一些純化的時間嗎？

1.2.2 一鍋化全乙醯化-硫醇化反應

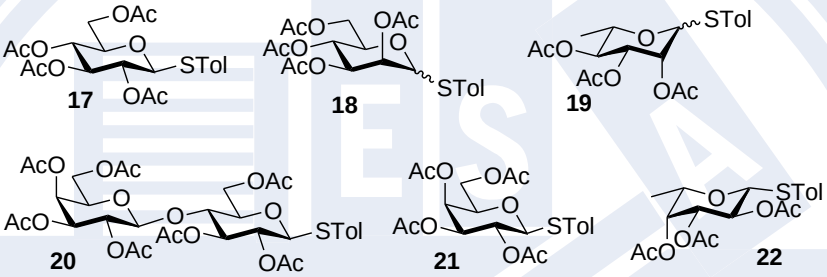
基於之前的成功，我們嘗試將此反應做延伸應用，嘗試進行全乙

醯化反應與硫醇化反應的一鍋化操作實驗，對非胺基糖分子 (non-amino sugar) 進行測試 (表 1.5)。

表 1.5. 一鍋化製備全乙醯化非胺基硫糖分子



Product



entry	S.M. (0.5 g)	Product	Time (h)[a]	yield (%), α : β [b]
1[c]	D-glucose	**17**	24	90, β only
2[c]	D-mannose	**18**	19	81, 9 : 1
3[c]	L-rhamnose	**19**	11	84, 6 : 1
4[c]	D-lactose	**20**	23	75, β only
5[d,e]	D-galactose	**21**	30	68, β only
6[d,e]	L-fucuse	**22**	14	75, β only

[a] 全乙醯化與硫醇化時間，[b] 以核磁共振氫譜判斷，[c] 直接抽乾溶劑，[d] 不抽乾溶劑，直接加入二氯甲烷、硫醇、$BF_3.OEt_2$。

首先以葡萄糖作為起始物，先以 TsOH 催化得到全乙醯化產物後，先減壓濃縮除去反應中產生的醋酸，再將適量二氯甲烷加入反

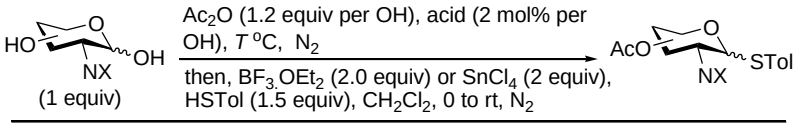
應，之後於 0 °C 下對反應投入對甲苯硫酚 (*p*-thiocresol，HSTol) 與三氟硼烷-乙醚錯合物 ($BF_3.OEt_2$) 後回到室溫進行硫醇化反應，可得到 90%的預計產物 **17** (表 1.5，實驗 1)；以此條件對應甘露糖 (mannose)、鼠李糖 (rhamnose) 與乳糖 (lactose) 進行操作，分別順利得到 75~84% 的預計產物 **18**、**19**、**20** (表 1.5，實驗 2、3、4)。而以硫酸催化的半乳糖與岩藻糖在全乙醯化反應完成後，則不需除去醋酸 (以免有碳化現象發生)，直接在 0 °C 下加入二氯甲烷與其它試劑進行第二步反應，如此操作可分別得到 68%、75%的預期產物 **21**、**22** (表 1.5，實驗 5、6)。

實驗結果發現除了甘露糖與鼠李糖的 C2-羥基位向導致產生 α/β-異構物外，其餘的糖分子都得到 β-位向的硫糖；而屬於去氧糖 (deoxy sugar) 的鼠李糖與岩藻糖則相對有較快的反應速率。

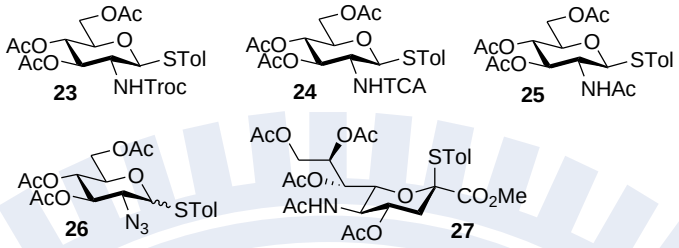
接著我們將在非胺基糖分子的經驗延伸，應用在不同胺基保護的葡萄糖胺分子上 (表 1.6)。

一開始以 *N*-Troc 葡萄糖胺進行實驗，在乙醯化反應完成後以減壓濃縮除去醋酸，再加入二氯甲烷、對甲硫酚與 $BF_3.OEt_2$，在室溫下順利得到 72% 的 β-硫糖產物 **23** (表 1.6，實驗 1)。同樣的操作，在 *N*-TCA 葡萄糖胺分子也得到 65%的 β-硫糖 **24** (表 1.6，實驗 2)。

表 1.6. 一鍋化製備全乙醯化硫糖分子



Product



entry	S.M. (0.5 g)	Product	*T* (°C)	Time (h)	yield (%), α : β [a]
1	D-Glc*N*-Troc	**23**	0 to rt	20	72, β only
2	D-Glc*N*-TCA	**24**	0 to rt	32	65, β only
3	D-Glc*N*-Ac	**25**	0 to 40	48	0[b]
4[c]	D-Glc*N*-Ac	**25**	0 to 40	22	65, β only
5	D-Glc*N*-CbZ	--	0	8	0[d]
6	D-Glc*N*-azido	**26**	-20 to 0	8	0[d]
7	D-Glc*N*-azido	**26**	-20 to rt	72	63, 3 : 1[e]
8	NANA methyl ester	**27**	0 to rt	20	65, β only

[a]α/β 比例由核磁共振氫譜決定，[b]回收化合物 **9**，[c]使用一當量 $SnCl_4$ 做為路易士酸，[d]化合物 **11** 崩解，[e]採用純化合物 **10** 測試，非一鍋化。

當採用 *N*-Ac 葡萄糖胺為起始物時，在 $BF_3.OEt_2$ 的作用下，即使升高反應溫度到 40 °C 仍未進行硫醇化反應，此時終止反應可回收到全乙醯化的葡萄糖胺分子 **12**；若改變路易氏酸為四氯化錫，在 40 °C 的溫度下可以得到 65% 的 β-預計硫糖產物 **25** (表 1.6，實驗 3、4)。

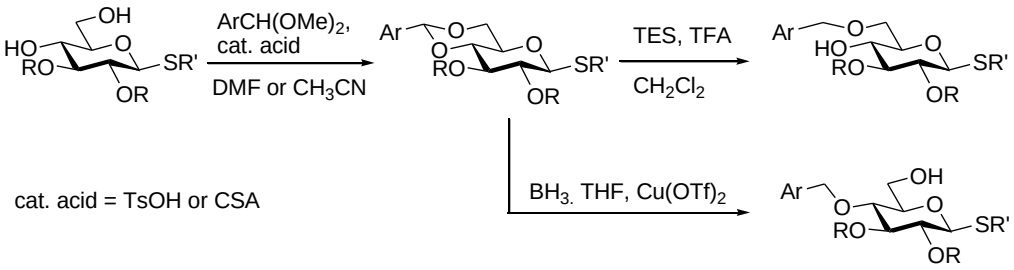
之後再嘗試以 *N*-Cbz 葡萄糖胺進行測試，但觀察到乙醯化產物

14 在一鍋化的條件下崩解的現象 (表 1.6，實驗 5)；之後 N_3 保護的葡萄糖胺也有類似的現象 (表 1.6，實驗 6)，有趣的是當我們以純化過的乙醯化產物 **13** 進行硫醇化反應時，就可以得到約 65% 的預計產物 **26**，但因為缺乏 C2-鄰基效應，反應得到 α/β = 3/1 的混合物 (表 1.6，實驗 7)。最後我們嘗試以唾液酸甲酯進行操作，在乙醯化反應完成後不抽去醋酸，而直接進行硫醇化反應，經過 8 小時後得到 65% 的 β-硫糖分子 **27** (表 1.6，實驗 8)。

由以上的實驗我們測試了一鍋化製備全乙醯硫糖分子的可能，同時也將此反應放大數量，比如說：可以從 10 克的 *N*-Troc 葡萄糖胺分子出發，經過一鍋化的操作而得到 60 ~ 67% 的硫糖分子 **23**。我們相信這樣的操作能使製備初級醣基建構單元變得更加方便。

1.2.3 一鍋化縮醛基化-乙醯化反應

在合成硫醣分子後，我們開始想嘗試將 TsOH 應用到進一步的醣分子修飾上。我們注意到縮醛基是醣化學中經常使用的保護基團，除了可以一次保護兩個羥基以外，縮醛基可做為暫時性的保護基團，酯要使用適當的環原試劑就可以進行選擇性的開環，得到出六號羥基產物或四號羥基產物做進一步反應 (流程 1.1)。[17]

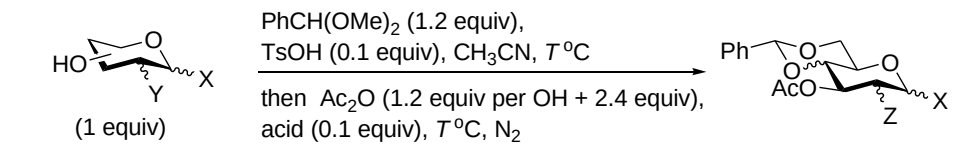


流程 1.1 縮醛基在醣化學的應用舉例 [17]

要在醣分子上引入縮醛基，可在酸性催化條件下，比如說：TsOH 或 CSA (camphor sulfonic acid)，使二甲基縮醛分子 (dimethyl acetal) 與醣分子進行縮合；於是我們嘗試將縮醛基化反應與乙醯化反應結合，進行一鍋化操作測試 (表 1.7)。

首先以市售的 α-甲基葡萄糖分子 **28** 進行實驗，於室溫下葡萄糖分子 **28** 與苯甲醛二甲基縮醛 (benzaldehyde dimethyl acetal) 混合於乙腈溶劑中，再加入 0.1 當量的 TsOH，待縮醛化反應完成後，直接加入醋酸酐進行乙醯化反應。需注意到縮醛化反應會伴隨產生 2 當量的甲醇，因此要多加 2.4 當量的醋酸酐消耗掉此旁產物。此外，因為縮醛化反應在酸濃度較低的條件下進行 (~ 30 mM)，故在乙醯化時需略微提高反應溫度至 35 ~ 50 °C，如此可得到 90% 的預計產物 **37** (表 1.7，實驗 1)。

表 1.7. 縮醛基化與乙醯化的一鍋化測試

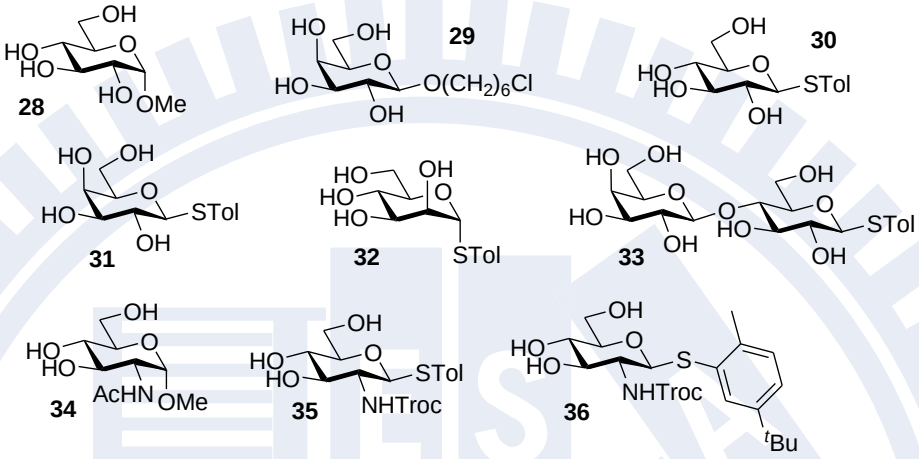


X = SR, α-OMe, Glc, β-$(CH_2)_6$Cl

Y = OH, NHAc, NHTroc

Z = OAc, NHAc, NHTroc

S.M.



entry	S.M. (0.5 g)	Product	TsOH (mol%, mM)	*T* (°C) acetalation, acetylation	Time (h)	yield (%)
1	**28**	**37**	10, 31	25, 35	4	90
2	**29**	**38**	10, 31	25, 40	4	81
3	**30**	**39**	10, 32	25, 40	4.5	75
4	**31**	**40**	10, 32	25, 40	5	77
5	**32**	**41**	10, 10	25, 50	6	70
6	**33**	**42**	10, 14	50, 50	10	82
7	**34**	**43**	10, 41	25, 40	4	76
8	**35**	**44**	32, 19	25, 40	4	70
9	**36**	**45**	10, 27	25, 40	6	75

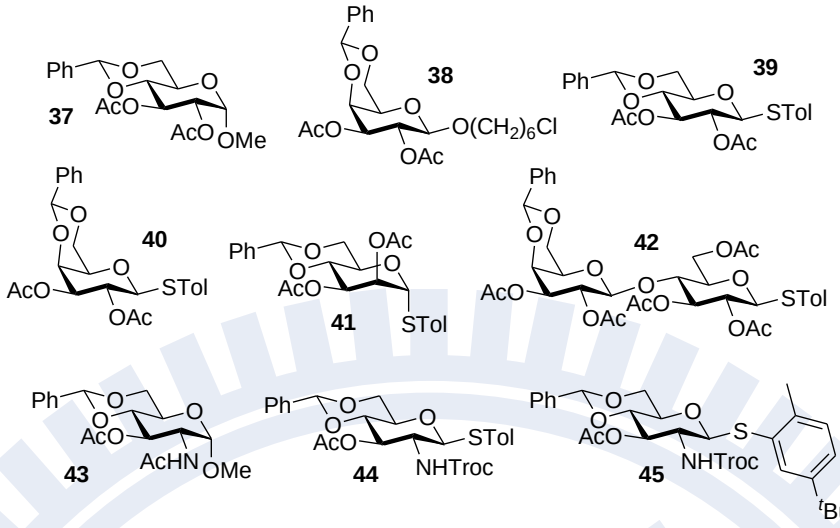
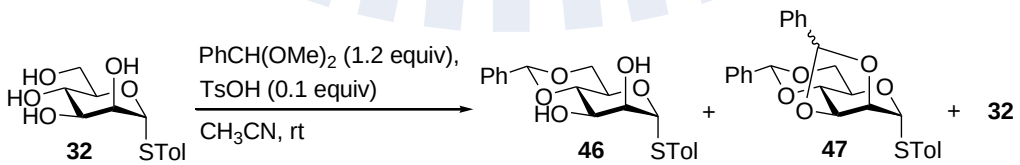


圖 1.5. 縮醛化與乙醯化的產物

接著，我們將此條件應用到端點為 *O*-link 的半乳糖分子 **29** 上，並順利得到 81%的預計產物 **38** (表 1.7，實驗 2)。之後我們再以硫醣醣予體分子進行反應，分別在葡萄糖 **30**、半乳糖 **31**、甘露醣 **32**、乳糖 **33** 上得到 70 ~ 80%的預計硫醣分子 **39**、**40**、**41**、**42** (表 1.7，實驗 3、4、5、6)。

值得一提的是因為甘露醣分子的二號與三號羥基是順式結構，在縮醛化反應時容易競爭進行縮醛化反應，產生 4,6-2,3-二縮醛化副產物 **47**，接連影響之後的一鍋化乙醯化反應 (流程 1.2)。



流程 1.2. 甘露醣在縮醛化反應時的副反應

張世聖學長發現如果將甘露糖的反應濃度由 30 mM 調低到 10 mM 時，可以控制反應就不產生副產物 **47**，但在乙醯化反應時需要將反應溫度調高到 50 ^{o}C 以加快反應速率 (表 1.7，實驗 5)。

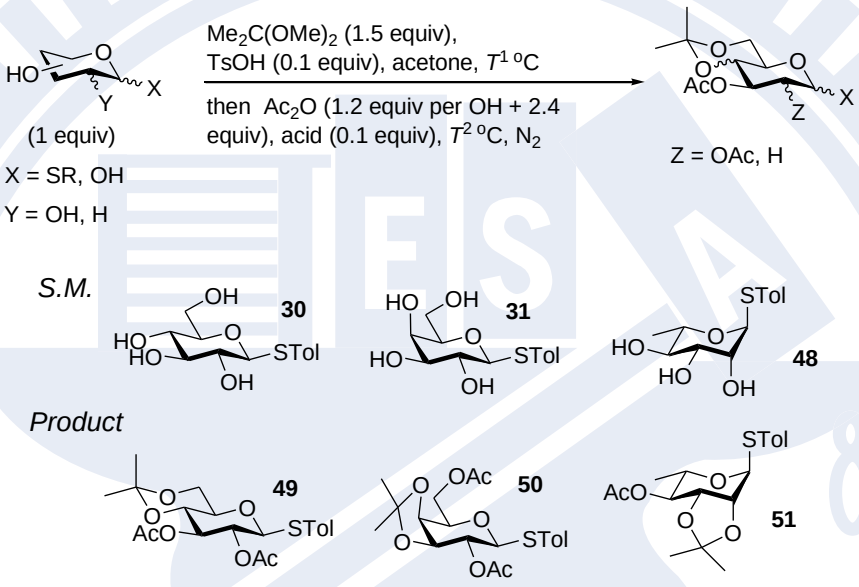
接著我們嘗試在葡萄糖胺分子測試一鍋化反應，當以 *N*-Ac 葡萄糖胺 **34** 做為起始物，反應順利得到 76% 的預計產物 **43** (表 1.7，實驗 7)。改以 *N*-Troc 硫葡萄糖胺 **35** 進行反應，發現因為其縮醛化產物的對乙腈的溶解度很低，影響到之後的乙醯化反應，故需以較多的溶劑以幫助反應進行，而 TsOH 的劑量也相對需要提高到 0.3 當量，可得到約 70% 的硫糖產物 **44** (表 1.7，實驗 8)。若將對甲苯硫酚基改為 5-叔丁基-2-甲苯硫酚基團 (5-tertbutyl-2-methyl thiophenolyl) 則可以提高縮醛化產物的溶解度，不需多加溶劑與催化劑，反應順利得到 75% 的縮醛保護硫葡萄糖胺分子 **45** (表 1.7，實驗 9)。

接著我們也嘗試改變 benzylidine 官能基為耐酸性稍低的 isopropylidine，看看 TsOH 在 isopropylidination-acetylation 一鍋化反應的效果，依慣例先採用非胺基糖分子進行一鍋化測試(表 1.8)。

在室溫下將 1.5 當量的 2,2-二甲氧基丙烷 (2,2-dimethoxyl propane) 、硫葡萄糖 **30** 與 0.1 當量的 TsOH 混合於乾燥的丙酮溶劑中，而 TsOH 的濃度因應 isopropylidine 的穩定性而需要稍微降低 (20 mM)，在室溫下約 10 分鐘左右即完成縮醛化反應；此時再直接加

入適量的醋酸酐 (仍需多加 2.4 當量消耗縮醛化反應產生的甲醇) 並升高反應溫度到 40 °C 進行乙醯化保護，如此操作結果可得到 52 %的預計硫糖產物 **49** (表 1.8，實驗 1)。

表 1.8. 縮醛化與乙醯化的一鍋化測試

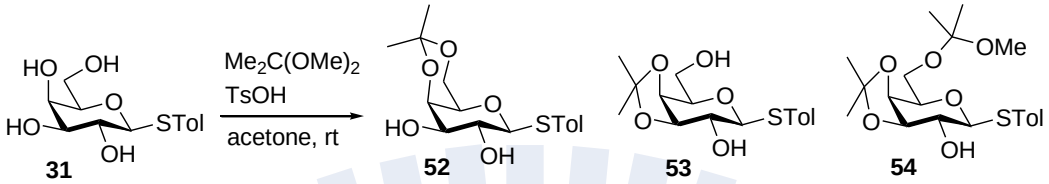


entry	S.M. (0.5 g)	Product	TsOH (mol%, mM)	T^1(°C), T^2(°C)	Time (h)	yield (%)
1	**30**	**49**	10, 23	25, 40	7	52
2[a]	**31**	**50**	10, 23	40, 40	12	70
3	**48**	**51**	5, 18	25, 40	4	75

[a]：以乙腈為溶劑。

接著我們嘗試以硫半乳糖分子 **31** 做為起始物。一般來說半乳糖分子在進行 isopropylation 時，如流程 1.3 所示，可能得到位向異構物

52、**53** 或進一步的二縮醛化產物 **54**。

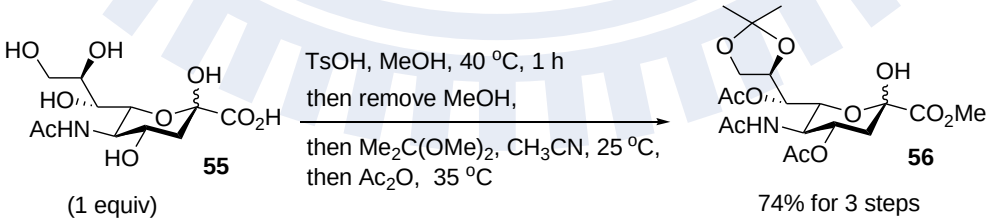


流程 1.3. 半乳糖分子進行 isopropylidination 的可能產物。

於是我們改用乙腈做為溶劑並嘗試在較高溫度進行縮醛化反應，待反應完成後直接再加入適量的醋酸酐進行乙醯化修飾，有趣的是我們僅得到約 70% 的 3,4-縮醛硫糖產物 **50** (表 1.8，實驗 2)。

之後我們以活性較高的鼠李醣嘗試一鍋化操作，採用 5 mol% 的 TsOH、1.5 當量的 2,2-二甲氧基丙烷與硫鼠李醣分子 **48** 於乾燥的丙酮系統中進行縮醛基的保護，待起始物耗盡後再加入醋酸酐做乙醯化修飾，實驗順利得到 75% 的硫鼠李醣分子 **51** (表 1.8，實驗 3)。

最後我們試著將以上的經驗歸整，以一鍋化步驟將天然的唾液酸分子 **55** 修飾為唾液酸半縮醛分子 **56** (流程 1.4)。



流程 1.4. 一鍋化合成唾液酸半縮醛 **56**。

首先在乾燥的甲醇中以 0.1 當量的 TsOH 做酯化反應，在 40 °C 下攪拌約 1 ~ 2 小時完成，然後抽乾溶劑，改加入乙腈溶劑與 1.5 當量的 2,2-二甲氧基丙烷，在室溫下約 5 分鐘完成縮醛基修飾，之後再加入適量的醋酸酐 (5 當量相對於唾液酸 **55**) 於反應中，在微微加熱的條件下 (35 °C) 可得到預計的唾液酸半縮醛分子 **56**，如此經過一鍋化三步驟的操作，只需要一次管柱純化即可得到 74%的產率。

1.3 結論

經由以上的努力，本實驗室成功地將對甲苯磺酸應用到醣基建構單元的修飾上，除了方便操作、成本便宜外，在全乙醯化反應與一鍋化全乙醯硫糖反應上更可以做到大量製備的效果。我們希望除了可以讓醣基建構單元的合成更加便利外，更可重新認識 TsOH 在合成上的應用廣度。以上的工作成果分別發表在 *Carbohydr. Res.* **2008**, *343*, 957–964，與 *Synlett* **2009**, 603–606。

1.4 實驗部份

1.4.1 一般實驗方法敘述

1. 反應試劑均購自試藥等級，使用前無進一步純化。
2. 所有用於實驗的有機溶劑，如：二氯甲烷、甲醇、乙腈、丙腈、1,2-二氯乙烷、*N*,*N*-二甲基甲醯胺、甲苯等等，均依照參考文獻 (*Purification of Laboratory Chemicals*, 4th Edition, Perrin, D.D.; Armarego, W.L.F. Butterworth Heinemann-1997, ISBN 7-5062-4739-9/O.315.) 以氫化鈣 (calcium hydride, CaH_2) 在氮氣下加熱乾燥，待蒸餾冷卻後立即使用、或保存於置有預先活化之 4Å 分子篩的密封燒瓶以供數日內使用。
3. TsOH 在使用前可先與五氧化二磷 (phosphorus pentoxide, P_2O_5) 在真空下乾燥數小時以除去其吸附之水氣。硫酸試劑的純度為 99.99% (購自 Aldrich)，存放於密封乾燥箱中，在氮氣下以注射針筒取用。酸性樹脂 IR-120H 在使用前先以去離子水與甲醇清洗後再於真空下乾燥數小時。$BF_3.OEt_2$、*N*-碘化丁二醯胺、與三甲矽基三氟甲磺酸均購自 98 ~ 99.5% 純度，儲放於–20 °C 冷藏櫃中。醣基化反應使用的 4Å-AW300 分子篩粉末，在使用前須以微波或火燄在真空下加熱活化備用。所有反應 (除了氫化反應外) 均在一大氣壓氮氣下進行，反應物在進行操作前均經過數小時真空乾燥。反應使用之微量針筒、反應燒瓶在使用前須於真空下乾

燥數小時。

4. 低溫反應在杜耳碗或 EYELA-xxtype 電動低溫反應槽中進行，反應槽中以工業級異丙醇 (isopropanol) 作為冷凝劑。

5. 薄層色層分析 (thin layer chromatography，TLC) 採用矽膠 60 F254 (0.25 mm, E. Merck co.) 的鋁片薄膜或玻璃片薄膜為分析材料，展開後以 UV-254 紫外光偵測。並以 4-茴香醛 (*p*-anisaldehyde) 的酸性乙醇溶液，或是混合 $Ce(NH_4)_2(NO_3)_6$ 與 $(NH_4)_6Mo_7O_{24}$ 的硫酸水溶液作為顯色劑。將已展開好的 TLC 片蔭乾、再浸入顯色劑後加熱顯像以佐助反應偵測。

6. 快速管柱色層分析 (flash column chromatography) 採用矽膠 60 (70 ~ 230 mesh, E. Merck co.) 為固相填充物。所有用於管柱色層分析的有機溶劑，如：己烷、乙酸乙酯、甲苯、乙醚、二氯甲烷、甲醇等等，均購自試藥等級或分析等級，並在簡易蒸餾後取用。管柱層析的沖提液若由兩種或兩種以上的成份配製，則以混合前的體積比例為記錄標準。

7. 逆向管柱層析 (reverse phase column chromatography) 採用 C-18 包覆之矽膠 (Cosmosil 75C18-OPN) 作為固相填充物，甲醇與去離子水作為沖提液進行純化操作。

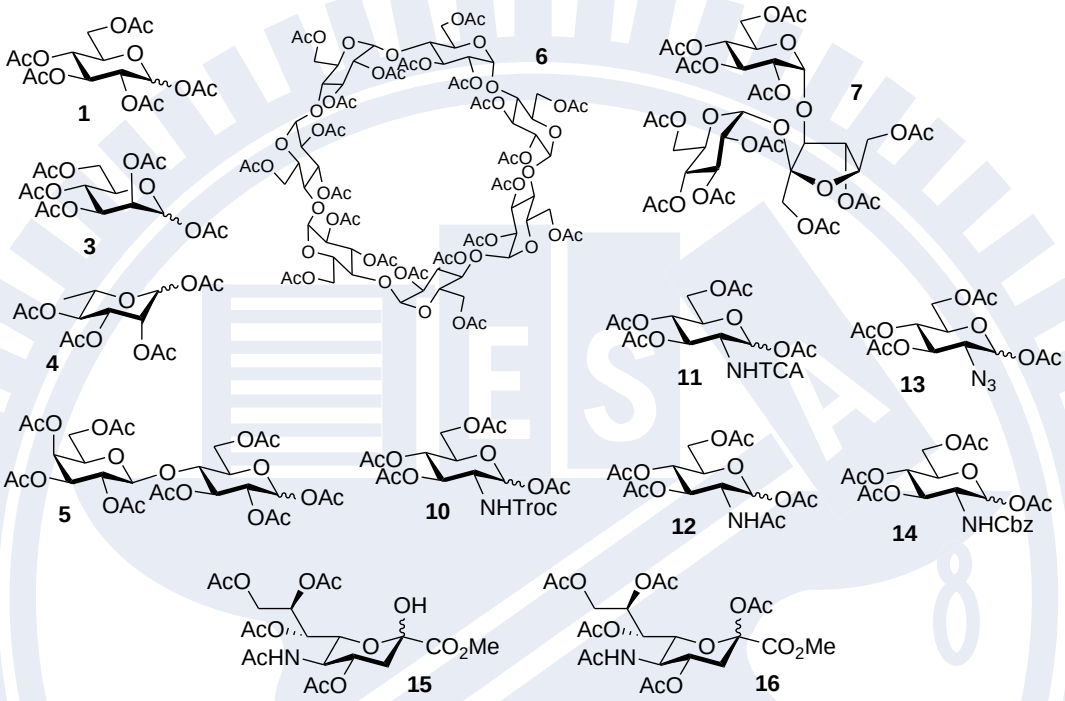
8. 核磁共振光譜，包含：一維氫譜 (^{1}H NMR)、一維碳譜 (^{13}C NMR)

與二維光譜等等實驗，係使用 Varian Unity-300 (300 MHz)、Brüker DRX-300 (300 MHz)或 Varian UI-500 (500 MHz) 進行偵測記錄。化學位移單位 (δ) 為 ppm，以氘氯仿、氘甲醇、二氘化氧等作為溶劑。以四甲基矽烷 (tetramethyl silane, TMS) 的訊號訂為原點 (δ = 0 ppm)，或以未氘化的溶劑，例如：三氯甲烷 ($CHCl_3$, δ = 7.26 ppm for ^1H NMR; 77 ppm for ^{13}C NMR)，作為內標。氫譜吸收峰分裂形式 (splitting pattern, multiplicity) 的定義如下：s 表示單重峰 (singlet)，d 表示雙重峰 (doublet)，t 表示三重峰 (triplet)，q 表示四重峰 (quartet)，quin 表示五重峰 (quintet)，m 表示多重峰 (multiplet)。偶合常數 (coupling constant) 以 *J* 表示，單位為 Hz。

9. 旋光度 (optical rotations) 採用 JASCO DIP-1000 自動旋光儀於 27 °C 下進行測定。
10. 高效能液相層析儀 (HPLC) 使用 Hitachi L-2130 梯度高壓幫浦、Hitachi L-2300 UV 偵測器、與 Mightysil 管柱進行實驗，並使用 xx 軟體進行積分分析。
11. 高解析質譜 (HRMS) 係以 BioTOF Ultraflex II (Bruker Daltonics, Billeriaca, MA 01821, USA) 進行分析 (委託操作)。

1.4.2 化合物的製備方法與各項物理數據

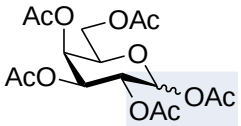
TsOH-catalyzed acetylation procedure for preparation of per-*O*-acetyl glycosyl acetates 1, 3–7 and 10–16



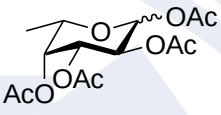
To a 0.5 g mono-, di-, tri- or hepta-saccharides was added Ac_2O (or a mixture of Ac_2O and CH_3CN) in which catalytic amount of TsOH was dissolved. The mixture was firstly stirred at 0 ^oC for 1 h and the stirred at the specified temperature. Exact amount of reagents used and specific reaction conditions were tabulated in Table S1. Upon complete acetylation, the mixture was diluted with EtOAc (20 mL), which was washed with cold satd $NaHCO_3$ (20 mL×2), water (20 mL×1), brine (20 mL×1), dried over $MgSO_4$, filtered, and then concentrated. Except for melezitose and *N*-protected amino sugars, the crude concentrate of peraetyl glycosyl acetates was directly characterized with NMR spectroscopy. For per-*O*-acetyl melezitose and *N*-protected amino

sugars, flash chromatography purification with EtOAc/hexane elution was performed before NMR characterization.

H_2SO_4-catalyzed acetylation procedure for preparation of per-*O*-acetyl glycosyl acetates 8 and 9



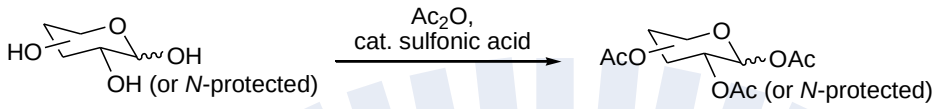
8



9

To a suspension of 0.5 g D-galactose (or L-fucose) in Ac_2O/CH_3CN at –20 ˚C (–30 ˚C for L-fucose) was added H_2SO_4 in CH_3CN solution (neat H_2SO_4 was diluted with CH_3CN to a 10 % v/v solution). Exact amount of reagents used and specific reaction conditions were tabulated in Table S1. After stirring for 1 h at –20 ˚C (–30 ˚C for L-fucose), the reaction mixture was gradually warmed up to the specific temperature (Table S1) and the stirring was continued till the end of reaction. The workup procedure was processed as described above.

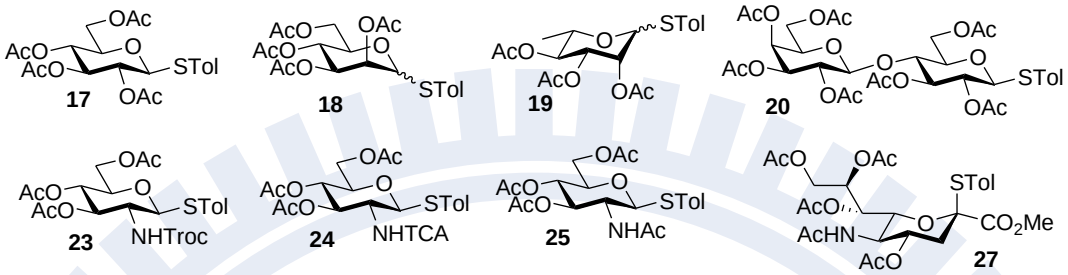
Table S1: Specific experimental conditions for preparation of per-*O*-acetyl glycosyl acetates **1, 3–16**



0.5 g saccharide (mmol)	Ac_2O (mL, mmol)	acid catalyst (mg, mmol)	temp (℃)	CH_3CN (mL)	ref. for spectra
D-glucose, 2.78	1.58, 16.68	53, 0.28[a]	27	0	21, 23
D-mannose, 2.78	1.58, 16.68	53, 0.28[a]	27	0	21, 23
L-rhamnose·H_2O, 2.72	1.54, 16.32	42, 0.22[a]	27	0	22
D-galactose, 2.78	1.58, 16.68	27, 0.28[b]	–20 to 0	0.5	23
L-fucose, 3.05	1.38, 14.64	24, 0.24[b]	–30 to –20	0.5	24
N-Troc D-glucosamine, 1.42	0.64, 6.80	22, 0.11[a]	27	0.5	25
N-TCA D-glucosamine, 2.26	0.70, 7.43	24, 0.13[a]	55	0.5	26
N-Ac D-glucosamine, 2.44	1.03, 10.86	34, 0.18[a]	50	0	27
N-azido D-glucosamine, 1.60	1.11, 11.71	37, 0.20[a]	27	0.5	28
N-Cbz D-glucosamine, 1.55	0.72, 7.67	24, 0.13[a]	40	0.5	29
NANA methyl ester, 1.55[c]	0.88, 9.29	29, 0.15[a]	27	0.5	30
NANA methyl ester, 1.55[d]	1.46, 15.48	29, 0.15[a]	45	0.5	30
D-lactose·H_2O, 1.39	1.42, 15.0	42, 0.22[a]	27	0	31
D-*β*-cyclodextrin, 0.44	0.89, 9.24	17, 0.09[a]	40	1.5	32
D-melezitose·H_2O, 0.96	1.09, 11.49	20, 0.11[a]	30	0.5	33

[a]TsOH.[b]H_2SO_4.[c]Conditions for the synthesis of tetra-*O*-acetyl-2-*β*-D-NANA methyl ester acetate **15**.[d]Conditions for the synthesis of penta-*O*-acetyl-2-*β*-D-NANA methyl ester acetate **16**.

One-pot TsOH-catalyzed acetylation–thioglycosidation procedure for preparation of per-*O*-acetyl thioglycosides of 17–27

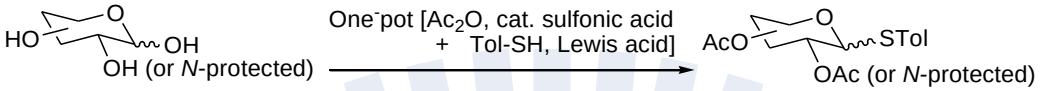


Peracetylation procedure was performed as described above at 0.5 g sugar substrate scale. Upon complete acetylation, the reaction solvent was removed and co-evaporated twice with equal volume of toluene by rotary evaporator. Thiocresol (1.5 mol equiv) in CH_2Cl_2 was added to the crude residue at 0 ˚C, and was followed the addition of Lewis acid (either 2 mol equiv for $BF_3{\cdot}Et_2O$ and 1.1 or mol equiv for $SnCl_4$). Exact amount of reagents used and specific reaction conditions were tabulated in Table S2. The mixture was stirred initially at 0 ˚C and then stirred at room temperature (27 ˚C) under N_2 except for *N*-acetyl-D-glucosmine **8**, which was stirred at 40 ˚C. Upon completion of the reaction, the mixture was diluted with cold EtOAc (50 mL) and sequentially washed with cold satd $NaHCO_3$ (30 mL×2), brine (30 mL×1), dried over $MgSO_4$, filtered, and then concentrated for flash column chromatography to furnish the per-*O*-acetyl thioglycosides **17–20** and **23–27**

One-pot H_2SO_4-catalyzed acetylation–thioglycosidation procedure for preparation of per-*O*-acetyl thioglycosides 21 and 22

The peracetylation procedure of D-galactose (or L-fucose) was performed as described above at 0.5 g scale. Upon complete acetylation, 1.2 equiv of MeOH was added to the mixture and stirred for 1 h at 0 ˚C; subsequent addition of thiocresol (2.0 mol equiv) in CH_2Cl_2 and $BF_3{\cdot}Et_2O$ were followed. The mixture was stirred initially at 0 ˚C and then stirred at room temperature (27 ˚C) under N_2. Exact amount of reagents used and specific reaction conditions were tabulated in Table S2. Upon completion of the reaction, the mixture was diluted with cold EtOAc (50 mL) and washed with cold satd $NaHCO_3$ (30 mL×2), brine (30 mL×1), dried over $MgSO_4$, filtered, and then concentrated for flash column chromatography to furnish the per-*O*-acetyl thioglycosides **21** and **22**.

Table S2: Specific experimental conditions for preparation of per-*O*-acetyl thioglycosides **17–27**



0.5 g saccharide (mmol)	Lewis acid (mL, mmol)	Tol–SH (g, mmol)	CH_2Cl_2 (mL)	temp.[a] (°C)	reaction time (h)	ref. for spectra
D-glucose, 2.78	0.70, 5.5[b]	0.52, 4.2	1.5	0 to 27	24	34
D-mannose, 2.78	0.70, 5.5[b]	0.52, 4.2	1.5	0 to 27	19	35
L-rhamnose·H_2O, 2.72	0.68, 5.4[b]	0.51, 4.1	1.5	0 to 27	11	36, 23
D-galactose, 2.78	1.40, 11.1[b]	0.69, 5.6	3	0 to 27	30	34
L-fucose, 3.05	1.52, 12.2[b]	0.76, 6.1	3	0 to 27	14	34
N-Troc-D-glucosamine, 1.42	0.36, 2.8[b]	0.26, 2.1	1.5	0 to 27	20	35
N-TCA-D-glucosamine,2.26	0.80, 6.2[b]	0.29, 2.3	1.5	0 to 27	32	37
N-Ac-D-glucosamine,2.44	0.57, 4.5[c]	0.42, 3.4	5	0 to 40	22	38
D-NANA methyl ester, 1.55	0.39, 3.1[b]	0.29, 2.3	1.5	0 to 27	20	39
D-lactose·H_2O, 1.39	0.35, 2.8[b]	0.26, 2.1	1.5	0 to 27	23	40

[a]Referring to thioglycosidation temperature.[b]$BF_3{\cdot}Et_2O$ was used.[c]$SnCl_4$ was used.

Peak assignment for ^{1}H and ^{13}C NMR data of pure per-*O*-acetyl thioglycosides 17–27

For *p*-tolyl per-*O*-acetyl-thio-1-*β*-D-glucopyranoside **17**: ^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 7.39 (d, *J* = 8.1 Hz, 2H, Ar-H in STol), 7.10 (d, *J* = 7.9 Hz, 2H, Ar-H in STol), 5.20 (dd, *J* = 9.3, 9.4 Hz, 1H, H-3), 5.01 (dd, *J* = 9.3, 9.9 Hz, 1H, H-4), 4.92 (dd, *J* = 9.3, 10.0 Hz, 1H, H-2), 4.63 (d, *J* = 9.9 Hz, 1H, H-1), 4.20–4.14 (m, 2H, H-6, H-6), 3.70 (ddd, *J* = 2.6, 4.7, 10.1 Hz, 1H, H-5), 2.33 (s, 3H, CH_3 in STol), 2.09 (s, 3H, Ac), 2.08 (s, 3H, Ac), 2.01 (s, 3H, Ac), 1.98 (s, 3H, Ac); ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 170.97, 170.58, 169.78, 169.63, 139.2, 134.2, 130.2, 130.1, 127.9, 86.2, 76.1, 70.3, 68.6, 62.5, 21.58, 21.16, 21.12, 20.97.

For *p*-tolyl per-*O*-acetyl-thio-1-α -D-mannopyranoside **18**: ^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 7.40 (d, *J* = 8.1 Hz, 2H, Ar-H in STol), 7.12 (d, *J* = 8.1 Hz, 2H, Ar-H in STol), 5.50 (dd, *J* = 1.5, 2.5 Hz, 1H), 5.42 (d, *J* = 1.0 Hz, 1H, H-1), 5.34–5.32 (m, 2H), 4.57–4.56 (m, 1H), 4.30 (dd, *J* = 12.3, 6.0 Hz, 1H), 4.10 (dd, *J* = 12.5, 2.6 Hz, 1H), 2.34 (s, 3H, CH_3-STol), 2.15 (s, 3H, Ac), 2.11 (s, 3H, Ac), 2.08 (s, 3H, Ac), 1.99 (s, 3H, Ac); ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 171.0, 170.3, 170.22, 170.16, 138.8, 122.0, 130.4, 129.2, 86.4, 69.8, 69.7, 66.8, 62.9, 21.52, 21.27, 21.09, 21.03.

For ***p*-tolyl per-*O*-acetyl-thio-1-α -L-rhamnopyranoside 19**: ^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 7.36 (d, *J* = 8.1 Hz, 2H, Ar-H in STol), 7.11

(d, *J* = 8.1 Hz, 2H, Ar-H in STol), 5.48 (dd, *J* = 3.3, 1.5 Hz, 1H, H-2), 5.32 (d, *J* = 1.5 Hz, 1H, H-1), 5.27 (dd, *J* = 3.3, 9.9 Hz, 1H, H-2), 5.13 (t, *J* = 9.9 Hz, 1H, H-4), 4.41–4.32 (m, H-5), 2.32 (s, 3H, CH_3-STol), 2.14 (s, 3H, Ac), 2.07 (s, 3H, Ac), 2.00 (s, 3H, Ac), 1.24 (s, 3H, CH_3-R); ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 170.4, 170.3, 138.6, 132.8, 130.4, 130.3, 129.7, 86.4, 71.6 × 2, 69.7, 68.1, 21.52, 21.30, 21.21, 21.08, 17.7.

For ***p*-tolyl per-*O*-acetyl-thio-1-*β*-D-lactoside 20**: ^1H NMR (300 MHz, $CDCl_3$) *δ*: 7.33 (d, *J* = 8.2 Hz, 2H, Ar-H in STol), 7.07 (d, *J* = 8.0 Hz, 2H, Ar-H in STol), 5.30 (dd, *J* = 1.0, 3.3 Hz, H-4'), 5.16 (t, *J* = 9.9 Hz, 1H), 5.05 (dd, *J* = 9.3, 9.4 Hz), 5.03 (dd, *J* = 9.3, 9.9 Hz, 1H), 4.93 (dd, *J* =3.3, 10.0 Hz, 1H), 4.82 (dd, *J* = 9.3, 10.0 Hz, 1H), 4.57 (d, *J* = 10.0 Hz), 4.47–4.43 (m, 2H), 4.09–4.03 (m, 3H), 3.84 (t, *J* = 6.3 Hz, 1H), 3.70 (t, *J* = 7.7 Hz, 1H), 3.64(m, 1H), 2.29 (s, 3H, CH_3-STol), 2.13–1.92 (m, 21H, 7 × Ac); ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 170.69, 170.64, 170.52, 170.42, 170.09, 169.92, 169.41, 138.9, 134.1, 132.8, 130.3, 130.0, 128.1, 101.3, 85.9, 77.0, 74.3, 71.3, 71.2, 71.1, 70.0, 66.9, 62.4, 61.2, 21.55, 21.22, 21.17, 21.01, 20.99, 20.88.

For ***p*-tolyl per-*O*-acetyl-thio-1-*β*-D-galactopyranoside 21**: ^1H NMR (300 MHz, $CDCl_3$) *δ*: 7.39 (d, *J* = 8.1 Hz, 2H, Ar-H in STol), 7.10 (d, *J* = 7.8 Hz, 2H, Ar-H in STol), 5.4 (dd, *J* = 1.0, 3.3 Hz, 1H, H-4), 5.22 (t, *J* = 9.9 Hz, 1H, H-2), 5.03 (dd, *J* = 3.3, 10.0 Hz, H-3), 4.64 (d, *J* = 10.0 Hz, H-1), 4.19 (dd, *J* = 7.0, 11.3 Hz, 1H, H-6), 4.11 (dd, *J* = 6.3, 11.3 Hz,

H-6), 3.92 (dt, *J* = 1.0, 6.1 Hz, H-5), 2.34 (s, 3H, CH_3-STol), 2.12 (s, 3H, Ac), 2.10 (s, 3H, Ac), 2.04 (s, 3H, Ac), 1.97 (s, 3H, Ac); ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 170.8, 170.62, 170.48, 169.84, 138.8, 133.5, 130.0, 129.0, 87.3, 72.4, 67.7, 67.6, 61.9, 21.56, 21.27, 21.07, 21.04, 20.99.

For ***p*-tolyl per-*O*-acetyl-thio-1-*β*-L-fucopyranoside 22**: ^1H NMR (300 MHz, $CDCl_3$) *δ*: 7.42 (d, *J* = 8.1 Hz, 2H, Ar-H in STol), 7.13 (d, *J* = 7.9 Hz, 2H, Ar-H in STol), 5.25 (dd, *J* = 0.7, 3.2 Hz, 1H, H-4), 5.19 (t, *J* = 9.9 Hz, 1H, 1H, H-2), 5.03 (dd, *J* = 3.3, 9.9 Hz, 1H, H-3), 4.64 (d, *J* = 9.8 Hz, 1H, H-1), 3.80 (q, *J* = 6.4 Hz, 1H, H-5), 2.33 (s, 3H, CH_3-STol), 2.14 (s, 3H, Ac), 2.10 (s, 3H, Ac), 1.98 (s, 3H, Ac), 1.24 (d, *J* = 6.4 Hz, 3H, CH_3-R); ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 171.1, 170.6, 169.9, 138.6, 133.3, 130.3, 130.0, 129.5, 8.2, 72.8, 70.7, 67.8, 21.6, 21.3, 21.09, 21.06, 16.8.

For ***p*-tolyl 3,4,6-tri-*O*-acetyl-2-trichloroethoxycarbamyl-2-deoxy-2-thio-1-*β*-D-glucopyranoside (23)**: ^1H NMR (300 MHz, $CDCl_3$) *δ*: 7.42 (d, *J* = 8.1 Hz, 2H, Ar-H in STol), 7.13 (d, *J* = 7.8 Hz, 2H, Ar-H in STol), 5.29-5.26 (m, 2H), 5.03 (t, *J* = 9.8 Hz, 1H, H-4), 4.79 (d, *J* = 10.8 Hz, 1H), 4.75 (d, *J* = 10.9 Hz, 1H), 4.23–4.17 (m, 2H), 3.74-3.65 (m, 2H), 2.36 (s, 3H, CH_3-STol), 2.10 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.01 (s, 3H, Ac); ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 171.4, 171.0, 170.5, 169.8, 154.7, 139.0, 138.7, 134.1, 133.2, 130.3, 130.2, 95.8, 86.7, 75.1, 71.4, 63.8, 63.5, 55.7, 52.6, 21.5, 21.1, 21.07, 20.95.

For ***p*-tolyl 3,4,6-tri-*O*-acetyl-2-trichloroacetamido-2-deoxy-1-*β*-D-thioglucopyranoside (24)**: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.50 (br m, 1H, N-H), 7.39 (d, J = 8.1 Hz, 2H, Ar-H in STol), 7.10 (d, J = 7.9 Hz, 2H, Ar-H in STol), 5.41 (dd, J = 9.3, 11 Hz, 1H, H-3), 5.03 (t, J = 9.6 Hz, 1H, H-4), 4.75 (d, J = 9.8 Hz, 1H, H-1), 4.23–4.01 (m, 3H, H-6, H-2), 3.76 (ddd, J = 2.5, 4.6, 10.0 Hz, 1H, H-5), 2.32 (s, 3H, CH_3-STol), 2.06 (s, 3H, Ac), 1.98 (s, 3H, Ac), 1.78 (s, 3H, Ac); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 171.8, 171.0, 170.0, 162.2, 139.4, 134.5, 130.1, 128.2, 92.8, 87.2, 76.2, 73.9, 68.9, 62.8, 54.5, 21.6, 21.1, 21.0, 20.6.

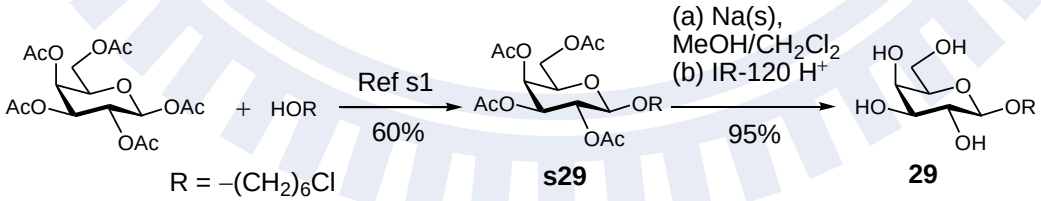
For ***p*-tolyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-*β*-D-thioglucopyranoside (25)**: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.39 (d, J = 8.1 Hz, 2H, Ar-H in STol), 7.10 (d, J = 7.9 Hz, 2H, Ar-H in STol), 5.92 (br d, J = 12 Hz, 1H, N-H), 5.23 (dd, J = 9.3, 10.9 Hz, 1H, H-3), 5.03 (dd, J = 9.3, 9.9 Hz, 1H, H-4), 4.79 (d, J = 9.9 Hz, 1H, H-1), 4.20–4.17 (m, 2H, H-6, H-6’), 4.00 (dd, J = 9.3, 10.0 Hz, 1H, H-2), 3.71 (ddd, J = 2.6, 4.7, 10.1 Hz, 1H, H-5), 2.34 (s, 3H, CH_3-STol), 2.09 (s, 3H, Ac), 2.01 (s, 3H, Ac), 1.99 (s, 3H, Ac); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 171.4, 171.0, 170.5, 169.8, 162.4, 139.4, 134.5, 130.1, 128.2, 87.2, 76.2, 73.9, 68.9, 62.8, 54.5, 21.5, 21.1, 21.07, 20.95.

For ***p*-tolyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-1-β-D-thioglucopyranoside (26β):** $[\alpha]^{27}_{D}$= –53.28 (c = 2.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ7.50–7.47 (m, 2H, ArH), 7.17–7.15 (m, 2H, ArH), 5.07 (t, J = 9.6 Hz, 1H), 4.90 (t, J = 10.2 Hz, 1H), 4.44 (d, J = 10.2 Hz, 1H, H-1), 4.16–4.14 (m, 2H), 3.68 (ddd, J = 2.7, 4.8, 10.2 Hz,, 1H), 3.37

(t, J = 9.9 Hz, 1H), 2.38 (s, 3H, $ArCH_3$), 2.09 (s, 3H, CH_3CO), 2.06 (s, 3H, CH_3CO), 2.01 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ170.9, 170.3, 170.8, 139.7, 135.1, 130.3, 126.5, 86.1, 76.1, 74.8, 68.4, 62.8, 62.4, 21.6, 21.1, 21.03, 20.96; HRMS (Bio-ToFII): calcd for $C_{19}H_{23}N_3O_7SNa$ requires 460.1149; found: m/z = 460.1149 $[M+Na]^+$.

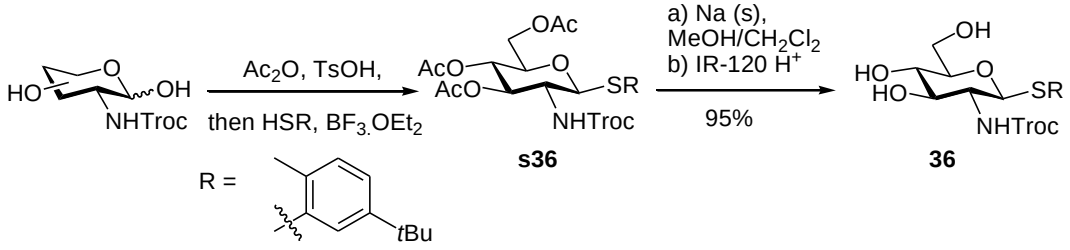
For ***p*-tolyl thio-2-*β*-D-NANA methyl ester (27)**: ^1H NMR (300 MHz, $CDCl_3$) δ: 7.33 (d, J = 12.8 Hz, 2H, Ar-H in STol), 7.12 (d, J = 7.9 Hz, 2H, Ar-H in STol), 5.92 (br d, 1H, N-H), 5.48 (s, 1H), 5.39 (td, J = 1.1, 4.2 Hz, H-4), 4.96 (d, J = 13.9 Hz, 1H), 4.64 (dd, J = 2.3, 10.5 Hz, 1H), 4.50 (dd, J = 1.9, 12.2 Hz, 1H), 4.13 (dd, J = 4.3, 13.4 Hz, 1H), 4.03 (dd, J = 8.7, 7.2 Hz, 1H), 3.59 (s, 3H, CH_3-O), 2.64 (dd, J = 9.1, 4.7 Hz, 1H), 2.32 (s, 3H, CH_3-STol), 2.14 (s, 3H, Ac), 2.12 (s, 3H, Ac), 2.08 (s, 3H, Ac), 1.95 (s, 3H, Ac), 1.89 (s, 3H, Ac); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 171.66, 171.40, 170.66, 170.63, 168.66, 140.5, 136.6, 130.2, 125.6, 89.3, 73.6, 73.5, 69.2, 69.1, 63.2, 52.9, 49.7, 37.8, 23.5, 21.69, 21.49, 21.30, 21.13, 21.09.

Preparation of (6-chlorohexyl) β-D-galactopyranoside (29).



A suspension of penta-*O*-acetyl-β-D-galactopyranosyl acetate (4.0 g, 10 mmol), 6-chlorohexanol (4.0 g, 4.0 mmol) and MS-AW300 (4.0 g) in dried CH_2Cl_2 (20 mL) was treated with $BF_3{\cdot}Et_2O$ (6.4 mL, 52 mmol) at –5 ℃ under N_2. The reaction mixture was stirred from –5 ℃ to rt for

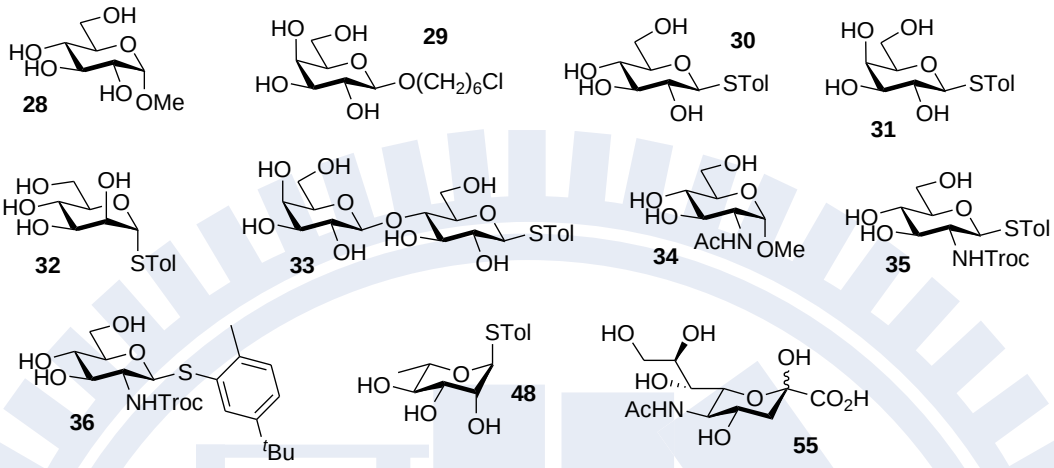
18 h. Upon completion of reaction, Et_3N was added. The reaction crude was diluted with CH_2Cl_2 (50 mL), filtered, and then sequentially washed with sat.$NaHCO_3$ (1 ×50 mL), H_2O (1 × 50 mL), brine (1 × 50 mL), then dried over $MgSO_4$, concentrated for column chromatography over silica gel to afford (6-chlorohexyl) 2,3,4,6-penta-*O*-acetyl β-D-galactopyranoside **s29** as white glassy solid (2.8 g, 59%). The prepared galactoside **s29** was then dissolved in a solution of 2:1 MeOH/CH_2Cl_2 (20 mL) with $Na_{(s)}$ (ca. 100 mg) at room temperature. Upon completion of deacetylation, the reaction was neutralized with IR-120 H^+ resin, filtered, and concentrated to afford the crude compound **29** as white amorphous solid (1.32 g, 95%). For (6-chlorohexyl) 2,3,4,6-penta-*O*-acetyl-β-D-galactopyranoside **s29**. ^{1}H-NMR (300 MHz; $CDCl_3$): δ = 5.39 (dd, *J* = 0.75 and 3.45 Hz, 1 H), 5.20 (dd, *J* = 7.8 and 10.5 Hz, 1 H), 5.02 (dd, *J* = 3.6 and 10.5 Hz, 1 H), 4.46 (d, *J* = 8.1 Hz, 1 H, H-1), 4.22–4.10 (m, 2 H), 3.93–3.86 (m, 2H), , 3.56–3.45 (m, 3 H), 2.15 (s, 3 H), 2.06 (s, 3 H), 2.05 (s, 3 H), 1.99 (s, 3 H), 1.82–1.72 (m, 3 H), 1.66–1.55 (m, 2 H), 1.50–1.26 (m, 3 H). ^{13}C-NMR (75 MHz; $CDCl_3$): δ = 170.8, 170.7, 170.6, 169.8, 101.7, 71.3, 70.9, 70.4, 69.3, 67.5, 61.7, 45.4, 32.9, 29.6, 26.9, 25.5, 21.16, 21.07, 20.99. HRMS–ES *m*/z: $[M + Na]^+$ calcd. for $C_{20}H_{31}ClO_{10}$, 489.1503. Found, 489.1498.



Preparation of (2-methyl-5-*t*butyl-phenyl) 2-(2,2,2-trichloro-ethoxycarbamyl)-2-deoxy-1-thio-β-D-glucopyranoside (36). TsOH (5.4 mg, 0.28 mmol) was added into a stirring mixture of 2-(2,2,2-trichloroethoxycarbamyl)-2-deoxy-D-glucopyranose (1.0 g, 2.8 mmol), Ac_2O (1.28 mL, 13.6 mmol) in CH_3CN (1 mL) at r.t. under N_2. Upon completion of acetylation as assessed by TLC, the solvent was removed by co-evaporation with toluene (2 × 2 mL) by rotary evaporator to furnish the crude per-*O*-acetyl *N*-Troc glucosaminyl acetate. After then, $BF_3.OEt_2$ (0.71 mL, 5.6 mmol) was added into a stirring CH_2Cl_2 solution (6 mL) of crude per-*O*-acetyl *N*-Troc glucosaminyl acetate, 2-methyl-5-*t*butyl-thiophenol (0.78 mL, 0.42 mmol) at 0 ˚C under N_2. After 15h, the reaction mixture was diluted with 30 mL CH_2Cl_2, washed with sat.$NaHCO_3$ (1 × 20 mL), brine (1 × 20 mL), dried over $MgSO_4$, filtered, and concentrated for column chromatography over silica gel to afford thioglycoside **s36** as white glassy solid (1.16 g, 71% over 2 steps). A piece of freshly cut Na(s) (ca. 50 mg) was then added in a stirring solution of the aforementioned crude thioglycoside **s36** in 2:1 MeOH/CH_2Cl_2 (20 mL) at 0 ˚C for 2h. The resulting mixture was neutralized with IR-120 H^+ resin at 0 ˚C, filtered, and concentrated to afford the crude unrprotected thioglycoside **36** as white glassy solid (860 mg, 96%). For thioglycoside **s36**: ^{1}H-NMR (300 MHz; $CDCl_3$): δ = 7.55 (d, *J* = 1.65 Hz, 1 H), 7.22 (dd, *J* = 1.8 and 7.8 Hz, 1 H), 7.09

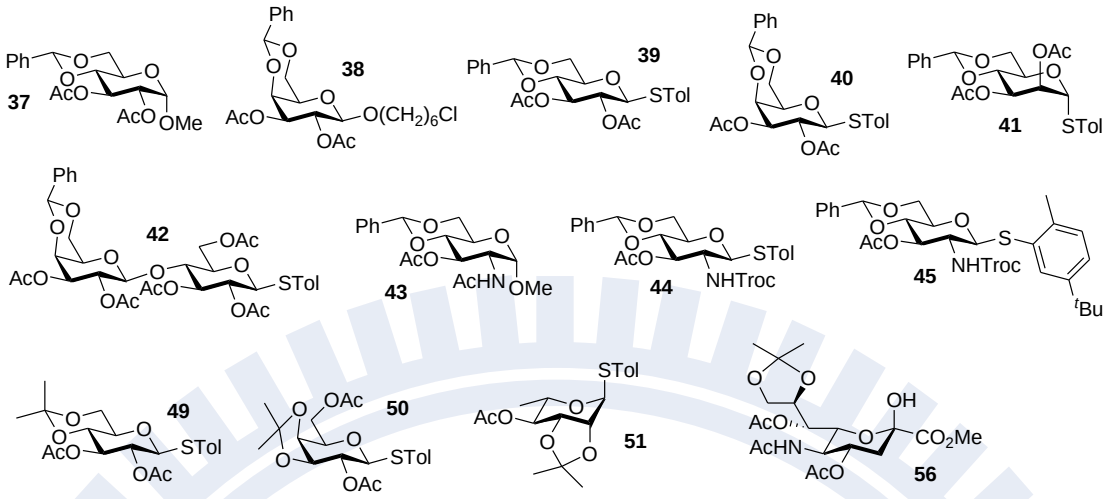
(dd, *J* = 1.75 and 7.75 Hz, 1 H), 5.87 (bs, *J* = 9.3 Hz, 1 H, N-*H*), 5.27 (t, *J* = 9.6 Hz, 1 H), 5.02 (t, *J* = 9.6 Hz, 1 H), 4.78–4.65 (m, 3H), 4.20 (dd, *J* = 4.0 and 9.6 Hz, 1 H), 4.09–4.05 (m, 1H), 3.81–3.69 (m, 2H), 2.33 (s, 3H), 2.01 (s, 3H), 1.98 (s, 3H), 1.86 (s, 3H), 1.27 (s, 9H). ^{13}C-NMR (75 MHz; $CDCl_3$): δ = 171.3, 171.1, 169.9, 169.8, 155.5, 154.5, 152.1, 150.0, 137.9, 131.8, 131.2, 130.5, 127.9, 126.0, 124.0, 95.9, 87.8, 74.9, 69.3, 69.1, 62.9, 60.9, 55.4, 34.8, 31.7, 31.6, 21.1, 21.0, 20.8. MS–ES *m*/z: $[M + Na]^+$ calcd. for $C_{26}H_{34}Cl_3NO_9S$, 664.1. Found, 664.1.

Table S1. Stoichiometric and exact amounts of reagents used in one-pot acetalation−acetylation of carbohydrate substrates **28**–**36, 48, 55**



1 g carbohydrate substrate (mmol)[1,2]	product	solvent (mL)	TsOH (mg, M)	Ac_2O (mL, mmol)
28 (5.0)	**37**	CH_3CN, 10	96, 0.049	2.8, 30
29 (3.4)	**38**	CH_3CN, 10	65, 0.031	1.9, 20
30 (3.5)	**39**	CH_3CN, 10	67, 0.032	2.0, 21
31 (3.5)	**40**	CH_3CN, 10	67, 0.032	2.0, 21
32 (3.5)	**41**	CH_3CN, 35	67, 0.010	2.0, 21
33 (2.2)	**42**	CH_3CN, 15	42, 0.014	2.2, 23
34 (4.3)	**43**	CH_3CN, 10	81, 0.041	2.4, 26
35 (2.2)	**44**	CH_3CN, 35	133, 0.019	1.2, 13
36 (1.9)[a]	**45**	CH_3CN, 7	37, 0.027	1.1, 12
30 (3.5)	**49**	acetone, 13	67, 0.023	2.0, 21
31 (3.5)	**50**	CH_3CN, 13	67, 0.023	2.0, 21
48 (3.7)	**51**	acetone, 10	67, 0.018	2.1, 22
55 (3.1)	**56**	CH_3CN, 15	59, 0.021	1.8, 19

[a] 0.5 g of unprotected thioglycoside was used.



Methyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-1-α-D-glucopyranoside (37). Yield: white glassy solid, 1.69 g, 90%. ^{1}H-NMR (300 MHz; $CDCl_3$): δ = 7.48–7.43 (m, 2 H), 7.39–7.34 (m, 3 H), 5.60 (t, *J* = 9.0 Hz, 1 H), 5.52 (s, 1 H), 4.97–4.90 (m, 2 H), 4.32 (dd, *J* = 3.0 and 9.0 Hz, 2 H), 3.99–3.90 (m, 1 H), 3.79 (t, *J* = 10 Hz, 1 H), 3.67 (t, *J* = 10 Hz, 1 H), 3.43 (s, 3 H), 2.08 (s, 3 H), 2.06 (s, 3 H). ^{13}C-NMR (75 MHz; $CDCl_3$): δ = 170.8, 170.2, 137.3, 129.5, 128.6, 126.6, 101.9, 98.0, 79.6, 72.0, 69.4, 69.3, 62.7, 55.8, 21.2, 21.1.

6-Chlorohexyl

2,3-di-*O*-acetyl-4,6-*O*-benzylidene-1-β-D-galactopyranoside (38) Yield: white amorphous solid, 1.28 g, 81%. ^{1}H-NMR (300 MHz; $CDCl_3$): δ = 7.55–7.52 (m, 2 H), 7.43–7.37 (m, 3 H), 5.52 (s, 1 H), 5.39 (dd, *J* = 8.1 and 10.5 Hz, 1 H,), 4.97 (dd, *J* = 3.6 and 10.5 Hz, 1 H), 4.50 (d, *J* = 8.1 Hz, 1 H, H-1), 4.39–4.32 (m, 2 H), 4.08 (dd, *J* = 1.5 and 12.3 Hz, 1 H), 3.96–3.90 (m, 1 H), 3.56–3.44 (m, 4 H), 2.09 (s, 3 H), 2.07 (s, 3 H), 1.87–1.73 (m, 2 H), 1.53–1.69 (m, 2 H), 1.51–1.30 (m, 4 H). ^{13}C-NMR (75 MHz; $CDCl_3$): δ = 171.3, 169.8, 130.9, 130.9, 129.5, 128.6, 126.8,

101.5, 101.4, 73.8, 72.5, 69.5, 69.3, 69.0, 66.7, 45.5, 32.9, 29.7, 26.9, 25.6, 21.3, 21.2. HRMS–FAB (*m*/z): $[M]^+$ calcd. for $C_{23}H_{31}ClO_8$, 470.1707; found, 470.1731.

***p*-Tolyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (39)** Yield: white glassy solid, 1.20 g, 75%. ^{1}H-NMR (300 MHz; $CDCl_3$): δ = 7.44–7.33 (m, 7 H), 7.15 (d, *J* = 7.8 Hz, 2 H), 5.49 (s, 1 H), 5.33 (t, *J* = 9 Hz, 1 H), 4.97 (dd, *J* = 9.0 and 9.9 Hz, 1 H), 4.74 (d, *J* = 10.2 Hz, 1 H), 4.38 (dd, *J* = 4.7 and 10.4 Hz, 1 H), 3.78 (t, *J* = 10.1 Hz, 1 H), 3.64 (t, *J* = 9.5 Hz, 1 H), 3.59–3.51 (m, 1 H), 2.36 (s, 3 H), 2.11 (s, 3 H), 2.03 (s, 3 H). ^{13}C-NMR (75 MHz; $CDCl_3$): δ = 170.5, 169.9, 137.2, 134.1, 130.2, 129.5, 128.6, 128.1, 126.5, 101.9, 87.2 , 78.5, 73.4, 71.2, 71.0, 68.9, 21.6, 21.2, 21.1.

***p*-Tolyl**

2,3-di-*O*-acetyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (40) Yield: white amorphous solid, 1.24 g, 77%. ^{1}H-NMR (300 MHz; $CDCl_3$): δ = 7.52 (d, *J* = 8.1 Hz, 2 H), 7.42–7.36 (m, 5 H), 7.09 (d, *J* = 8.1 Hz, 2 H), 5.48 (s, 1 H), 5.31 (t, *J* = 9.9 Hz, 1 H), 5.00 (dd, *J* = 3.3 and 9.9 Hz, 1 H), 4.67 (d, *J* = 9.6 Hz, 1 H, H-1), 4.41–4.37 (m, 2 H), 4.03 (d, *J* = 1.5 and 12.6 Hz, 1 H), 3.60 (d, *J* = 1.2 Hz,1 H), 2.36 (s, 3 H), 2.11 (s, 3 H), 2.04 (s, 3 H). ^{13}C-NMR (75 MHz; $CDCl_3$): δ = 171.2, 169.5, 138.8, 137.9, 134.7, 130.0, 129.6, 128.5, 127.5, 101.6, 85.6, 73.8, 73.6, 70.0, 69.5, 67.2, 21.7, 21.34, 21.3. HRMS–FAB (*m*/z): $[M + H]^+$ calcd. for $C_{24}H_{26}O_7S$, 459.1472; found, 459.1473.

***p*-Tolyl**

2,3-di-*O*-acetyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (41) Yield: white amorphous solid, 1.12 g, 70%. ^{1}H-NMR (300 MHz;

$CDCl_3$): δ = 7.52–7.49 (m, 2 H), 7.43–7.38 (m, 5 H), 7.15 (d, *J* = 8.1 Hz, 2 H), 5.63 (dd, *J* = 1.35 and 3.5 Hz, 1 H), 5.61 (s, 1 H), 4.53–4.47 (m, 1 H), 4.27 (dd, *J* = 4.8 and 10.2 Hz, 1 H), 4.15 (t, *J* = 10.0 Hz, 1 H), 3.88 (t, *J* = 10.2 Hz, 1 H), 2.35 (s, 3 H), 2.17 (s, 3 H), 2.05 (s, 3 H). ^{13}C-NMR (75 MHz; $CDCl_3$): δ = 169.7, 169.7, 138.4, 136.9, 132.8, 129.9, 129.1, 128.8, 128.2, 126.1, 101.9, 87.0, 76.1, 71.3, 68.4, 68.3, 65.0, 21.1, 20.8, 20.7. HRMS–FAB (*m*/z): $[M + H]^+$ calcd. for $C_{24}H_{26}O_7$ S, 459.1472; found, 459.1468.

***p*-Tolyl**

2,3,6-tri-*O*-acetyl-4-*O*-(2′,3′-di-*O*-acetyl-4′,6′-*O*-benzylidene-β-D-galactopyranosyl)-1-thio-β-D-glucopyranoside (42) Yield: white amorphous solid, 1.36 g, 82%. ^{1}H-NMR (300 MHz; $CDCl_3$): δ = 7.48–7.37 (m, 7 H), 7.12 (d, *J* = 7.12 Hz, 2 H), 5.47 (s, 1 H), 5. 30–5.22 (m, 2 H), 4.93–4.87 (m, 2 H), 4.64–4.56 (m, 2 H), 4.46 (bs, 1 H), 4.36–4.26 (m, 2 H), 4.14–4.01 (m, 2 H), 3.73 (t, *J* = 9.36 Hz, 1 H), 3.66–3.60 (m, 1 H), 3.46 (s, 1 H), 2.35 (s, 3 H), 2.12 (s, 3 H), 2.09 (s, 3 H), 2.04 (m, 9 H). ^{13}C-NMR (75 MHz; $CDCl_3$): δ = 171.1, 170.7, 170.6, 170.0, 169.3, 139.0, 137.8, 134.1, 130.0, 129.6, 128.7, 128.2, 126.9, 101.7, 101.4, 86.1, 77.3, 76.2, 74.0, 73.5, 72.4, 70.4, 69.4, 68.8, 66.8, 62.5, 21.6, 21.3, 21.2, 21.1. HRMS–FAB (*m*/z): $[M + H]^+$ calcd. for $C_{36}H_{42}O_{15}$ S, 747.2317; found, 747.2336.

Methyl

2-acetamido-3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (43) Yield: white amorphous solid, 1.18 g, 76%. ^{1}H-NMR (300 MHz; $CDCl_3$): δ = 7.86–7.45 (m, 2 H), 7.39–7.36 (m, 3 H), 5.83 (d, *J* = 9.6 Hz, 1 H), 5.55 (s, 1 H), 5.32 (t, *J* = 10.0 Hz, 1 H), 4.74 (d, *J* = 3.6 Hz,

1 H), 3.90–3.70 (m, 3 H), 3.43 (s, 3 H), 2.08 (s, 3 H), 1.99 (s, 3 H). ^{13}C-NMR (75 MHz; $CDCl_3$): δ = 171.9, 170.5, 137.4, 129.5, 128.6, 126.6, 102.0, 99.4, 79.4, 70.7, 69.3, 63.2, 55.7, 53.0, 23.7, 21.3. HRMS–FAB (*m*/z): $[M + H]^+$ calcd. for $C_{18}H_{23}NO_7$ = 366.1547; found, 366.1541.

***p*-Tolyl-3-*O*-acetyl-4,6-*O*-benzylidene-2-(2,2,2-trichloroethoxycarbamyl)-2-deoxy-1-thio-β-D-glucopyranoside (44)** Yield: white glassy solid, 0.9 g, 70%. ^{1}H-NMR (300 MHz; $CDCl_3$): δ = 7.46–7.35 (m, 7 H), 7.16 (d, *J* = 7.8 Hz, 2 H), 5.52 (s, 1 H), 5.37–5.28 (m, 2 H), 4.87–4.74 (m, 3 H), 4.37 (dd, *J* = 4.8 and 10.5 Hz, 1 H), 3.82 (t, *J* = 10.4 Hz, 2 H), 3.69 (t, *J* = 9.3 Hz, 1 H), 3.59–3.50 (m, 1 H), 2.37 (s, 3 H), 2.07 (s, 3 H). ^{13}C-NMR (75 MHz; $CDCl_3$): δ = 171.2, 154.6, 139.1, 137.2, 133.9, 130.3, 129.6, 128.7, 126.6, 101.9, 95.8, 88.7, 78.8, 75.0, 72.8, 71.1, 68.9, 56.0, 21.6, 21.2.

(2-methyl-5-*t*butylphenyl) 3-*O*-acetyl-4,6-*O*-benzylidene-2-(2,2,2-trichloroethoxycarbamyl)-2-deoxy-1-thio-β-D-glucopyranoside (45) Yield: white glassy solid, 940 mg, 75%. ^{1}H-NMR (300 MHz; $CDCl_3$): δ = 7.56 (s, 1 H), 7.43 (d, *J* = 6.0 Hz, 2 H), 7.36–7.23 (m, 4 H), 7.16 (d, *J* = 6.1 Hz, 2 H), 5.58–5.50 (m, 2 H), 5.43–5.30 (dd, *J* = 3.1 and 8.9 Hz, 1 H), 4.83 (d, *J* = 3.2 Hz, 2 H), 4.72 (d, *J* = 8.2 Hz, 1 H), 4.31–4.26 (m, 1 H), 3.93 (dd, *J* = 4.7 and 10.5 Hz, 1 H), 3.79 (t, *J* = 10.2 Hz, 2 H), 3.71 (t, *J* = 9.3 Hz, 1 H), 3.56–3.48 (m, 1 H), 2.33 (s, 3 H), 2.07 (s, 3 H), 1.34 (s, 9 H). ^{13}C-NMR (75 MHz; $CDCl_3$): δ = 171.4, 154.8, 150.1, 137.3, 137.2, 132.3, 130.5, 130.3, 130.1, 129.6, 129.0, 128.7, 126.5, 125.8, 101.7, 95.9, 88.9, 78.9, 75.0, 72.9, 70.8, 68.9, 56.0, 34.9, 31.7, 21.3, 20.7. HRMS–ES (*m*/z): $[M + Na]^+$ calcd. for $C_{29}H_{34}Cl_3NO_7S$, 668.1014; found, 668.1070.

***p*-Tolyl**

2,3-di-*O*-acetyl-4,6-*O*-isopropylidene-1-thio-β-D-glucopyranoside (49) Yield: white amorphous solid, 740 mg, 52%. ^{1}H-NMR (300 MHz; $CDCl_3$): δ = 7.33 (d, *J* = 8.1 Hz, 2 H), 7.11 (d, *J* = 7.9 Hz, 2 H), 5.14 (td, *J* = 6.1 and 9.0 Hz, 1 H), 4.90 (dd, *J* = 6.3 and 9.1 Hz, 1 H), 4.67 (d, *J* = 9 Hz, 1 H), 3.96 (dd, *J* = 6.4 and 8.9 Hz, 1 H), 3.77 (dd, *J* = 6.2 and 9.9 Hz, 1 H), 3.67 (dd, *J* = 6.3 and 9.0 Hz, 1 H), 3.37 (td, *J* = 5.4 and 10.0 Hz, 1 H), 2.34 (s, 3 H), 2.08 (s, 3 H), 2.02 (s, 3 H), 1.44 (s, 3 H), 1.36 (s, 3 H). ^{13}C-NMR (75 MHz; $CDCl_3$): δ = 170.6, 169.9, 139.0, 133.9, 130.2, 128.2, 100.1, 87.0, 73.8, 72.0, 71.4, 71.3, 62.3, 29.3, 21.6, 21.22, 21.2, 19.3. HRMS–ES (*m*/z): $[M + Na]^+$ calcd. for $C_{20}H_{26}NaO_7S$, 433.1291; found, 433.1288.

***p*-Tolyl**

2,6-di-*O*-acetyl-3,4-*O*-isopropylidene-1-thio-β-D-galactopyranoside (50) Yield: white amorphous solid, 1.0 g, 70%. ^{1}H-NMR (300 MHz; $CDCl_3$): δ = 7.42 (d, *J* = 8.1 Hz, 2 H), 7.11 (d, *J* = 7.9 Hz, 2 H), 5.07–5.00 (m, 1 H), 4.54 (d, *J* = 10.1 Hz, 1 H), 4.37 (d, *J* = 6 Hz, 2 H), 4.23–4.20 (m, 2 H), 3.97 (dt, *J* = 1.5 and 6.0 Hz, 1 H), 2.31 (s, 3 H), 2.15 (s, 3 H), 2.10 (s, 3 H), 1.53 (s, 3 H), 1.34 (s, 3 H). ^{13}C-NMR (75 MHz; $CDCl_3$): δ = 171.2, 170.1, 138.4, 133.0, 130.0, 129.9, 111.3, 86.5, 74.5, 73.9, 71.8, 64.0, 28.0, 26.7, 21.5, 21.4, 21.2. HRMS–FAB (*m*/z): $[M + H]^+$ calcd. for $C_{20}H_{26}O_7S$, 411.1472; found, 411.1477.

***p*-Tolyl**

4-*O*-acetyl-2,3-*O*-isopropylidene-1-thio-α-L-rhamnopyranoside (51) Yield: white glassy solid, 980 mg, 75%. ^{1}H-NMR (300 MHz; $CDCl_3$): δ = 7.38 (d, *J* = 8.1 Hz, 2 H), 7.15 (d, *J* = 7.8 Hz, 2 H), 5.70 (s, 1 H), 4.90

(dd, *J* = 8.0 and 10.1 Hz, 1 H), 4.37 (dd, *J* = 0.5 and 5.3 Hz, 1 H), 4.25–4.18 (m, 2 H), 2.35 (s, 3 H), 2.14 (s, 3 H), 1.59 (s, 3 H), 1.38 (s, 3 H), 1.14 (d, *J* = 6.3 Hz, 3 H). ^{13}C-NMR (75 MHz; $CDCl_3$): δ = 170.5, 138.4, 132.9, 130.3, 129.7, 110.4, 84.4, 76.8, 75.9, 75.0, 65.9, 28.1, 26.9, 21.5, 21.4, 17.2.

Methyl 5-acetamido-4,7-di-*O*-acetyl-3,5-dideoxy-8,9-*O*-isopropylidene-β-D-glycero-D-galacto-2-nonulopyranosonate (56) Yield: colorless oily liquid, 1.02 g, 74%. ^{1}H-NMR (500 MHz; $CDCl_3$): δ = 6.13 (d, *J* = 10.0 Hz, 1 H), 5.35–5.34 (m, 1 H, H-7), 5.22–5.18 (m, 1 H, H-4), 4.18 (dd, *J* = 2.0 and 11.0 Hz, 1 H, H-6), 4.13–4.10 (m, 1 H, H-8), 4.07–4.01 (m, 1 H, H-5), 3.91–3.88 (m, 3 H, H-9), 3.81–3.78 (m, 1 H, H-9), 3.76 (s, 3 H, OCH_3), 2.15–2.09 (m, 2 H, H-3), 2.05 (s, 3 H), 1.95 (s, 3 H), 1.82 (s, 3 H), 1.24 (s, 6 H). ^{13}C-NMR (125 MHz; $CDCl_3$): δ = 171.1, 170.5, 170.3, 169.2, 108.3, 94.7, 75.3, 70.9, 69.3, 68.6, 65.3, 53.1, 49.2, 35.9, 26.2, 25.3, 22.8, 20.78, 20.76. HRMS–FAB (*m*/z): $[M + H]^+$ calcd. for $C_{19}H_{29}NO_{11}$, 448.1813; found, 448.1821.

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Note

Versatile acetylation of carbohydrate substrates with bench-top sulfonic acids and application to one-pot syntheses of peracetylated thioglycosides

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Abstract—Inexpensive and readily available sulfonic acids, *p*-toluenesulfonic acid, and sulfuric acid are versatile and efficient catalysts for the peracetylation of a broad spectrum of carbohydrate substrates in good yield and in a practical time frame. Three appealing features in sulfonic acid-catalyzed acetylation of free sugars were explored including (1) suppression of furanosyl acetate formation for D-galactose and L-fucose; (2) high yielding chemoselective acetylation of sialic acid under appropriate conditions; and (3) peracetylation of amino sugars with different amino protecting functions. Simple one-pot two step acetylation–thioglycosidation methods for the expeditious synthesis of *p*-tolyl per-*O*-acetyl thioglycosides were also delineated.

Keywords: *p*-Toluenesulfonic acid; Acid-catalyzed acetylation; Amino sugars; One-pot protecting group manipulation

Chemical synthesis of oligosaccharides is a two-stage process that comprises the preparation of glycosyl building blocks followed by their assemblage.[1] Different synthetic strategies have emerged to speed up the assembling process, which include the armed-disarmed approach,[2] orthogonal glycosylation,[3] reactivity-based one-pot glycosylation,[4] sequential iterative glycosylation,[5] and automated solid phase oligosaccharide synthesis.[6] The implementation of these strategies relies heavily on the facile synthesis of glycosyl building blocks. Thioglycoside derivatives constitute a major class of glycosyl building blocks for oligosaccharide synthesis,[7] which are mainly derived from per-*O*-acetyl thioglycosides.

Conventional preparation of per-*O*-acetyl thioglycosides involves peracetylation and subsequent thioglycosidation.[8] However, classical carbohydrate acetylation uses excess pyridine; not only is pyridine highly toxic, but the presence of excess basic reagent makes the one-pot operation impossible.[9] Provided that the first peracetylation is an acid-catalyzed process, which is compatible to the second thioglycosidation; a one-pot acetylation–thioglycosidation is foreseeable. Thus, various one-pot strategies for the preparation of per-*O*-acetyl thioglycosides have been developed, although most of the existing methods have pitfalls originating from the peracetylation process.[10] For example, the formation of undesired furanosyl acetates for some sugars in acid-catalyzed acetylation compromises the yield in the subsequent thioglycosidation.[10c,e,11] The strong Lewis acid character of some acids makes them less suitable for the peracetylation of N-protected amino sugars and thus limits the scope of application to carbohydrate substrates without amino functions.[10d,e,12–14] Herein, we report a versatile and high yielding (75–95%) carbohydrate peracetylation protocol that can overcome the above drawbacks by using common sulfonic acids in the appropriate reaction conditions. Subsequent development of the simple one-pot two step acetylation–thioglycosidation protocols for the expeditious syntheses of *p*-tolyl per-*O*-acetyl thioglycosides was also delineated.

p-Toluenesulfonic acid monohydrate (TsOH)[15] and sulfuric acid (H_2SO_4)[16,17] are known catalysts for

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hydroxyl acetylation in the presence of excess acetic anhydride, however their efficiency with near stoichiometric acetic anhydride has not been explored. In addition, TsOH has never been used for the acetylation of free sugar substrates. Although silica-supported H_2SO_4 and $HClO_4$ have been used for carbohydrate acetylation, the additional immobilization step makes these protocols less convenient and the use of potentially explosive $HClO_4$ is also discouraged.[14a,b] Our initial observations showed that both neat H_2SO_4 and TsOH exhibited sufficient catalytic efficiency (1 mol % per OH group of the sugar) for the acetylation of D-glucose with a near stoichiometric amount of Ac_2O. Peracetylation of D-glucose with H_2SO_4 was completed in 0.2 h while with TsOH the reaction needed 8 h; such a difference should be useful for the selective peracetylation of sugars under different reaction conditions.

In the standard protocol, TsOH (1 or 2 mol % per OH group of the sugar) in Ac_2O (Table 1, entries a–c, h, and m) or in a mixture of Ac_2O and acetonitrile (CH_3CN) (Table 1, entries d–g, i–l, and n–o) was added to the carbohydrate substrate with stirring at 0 °C for 1 h. Subsequently, the reaction mixture was warmed to the optimal reaction temperature; detailed experimental conditions are given in Table S1 of Supplementary data. In general, a near stoichiometric amount of Ac_2O (1.2 mol equiv per OH group of the sugar) was employed. For carbohydrate substrates without amino functions, the desired peracetylated glycosyl acetates were furnished in good to excellent (75–95%) yield (Table 1, entries a, b, c, m, n, and o).

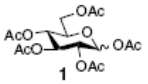
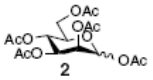
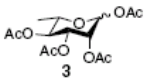
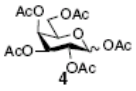
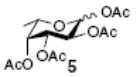
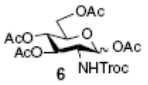
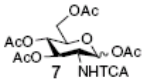
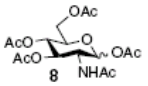
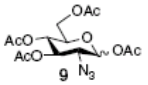
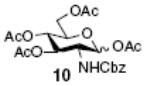
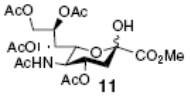
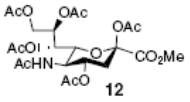
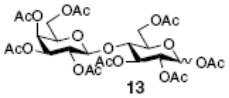
Acetylation of D-galactose and L-fucose requires special attention as both are prone to form undesired furanosyl acetates. Such furanosyl isomers were also formed from the sugars with our standard TsOH-catalyzed acetylation protocol (30% relative to total peracetyl acetates for D-galactose, 26% relative to total peracetyl acetates for L-fucose).[10c,e,11] Gratifyingly, the furanosyl isomer derived from D-galactose was gradually reduced by decreasing the reaction temperatures and nearly complete elimination was accomplished at 0 °C.[10e,15b] At such low reaction temperature, the more reactive H_2SO_4 was needed. However for the acetylation of L-fucose, 7% of furanosyl isomer was formed at 0 °C and thus further decrease in the temperature to −20 °C was required. Under the optimal reaction conditions, the formation of furanosyl isomer was reduced to less than 2% (see spectroscopic in Supplementary data page S23).

After examining the simple carbohydrate substrates, we turned to the amino sugars, which occur in the majority of natural oligosaccharides. Although pyridine-catalyzed acetylation works well for the acetylation of amino sugars, the less toxic TsOH-catalyzed protocol should provide a desirable alternative.[9] As different amino protecting functions have been used in oligosaccharide synthesis, it would be worthy knowing the compatibility of our protocol to such protecting functions. To this end, D-glucosamines with trichloroethoxycarbonyl (Troc), trichloroacetyl (TCA), acetyl (Ac), azido (N_3), and benzylethoxycarbonyl (Cbz) functions were prepared and acetylated with the standard TsOH-catalyzed protocol (Table 1, entries f–j).[18] To our delight, the desired peracetylated products **6**–**10** were furnished within 3–6 h in respectable 85–94% yield. For the acetylation of *N*-acetyl neuraminic acid methyl ester (NANA methyl ester), 4,7,8,9-tetra-*O*-acetyl NANA methyl ester **11** was obtained exclusively in 90% yield without any trace of the pentaacetylated product (Table 1, entry k). Such chemoselectivity is superior to the conventional $HClO_4$-catalyzed protocol.[19] To obtain the pentaacetyl product, a higher reaction temperature (45 °C) and excess Ac_2O were required, and 2,4,7,8,9-pentacetyl NANA methyl ester **12** was furnished in 80% yield along with 5% elimination product (Table 2, entry l). As **11** and **12** are valuable precursors for the synthesis of sialic acid-containing oligosaccharides, our new procedure should provide a more convenient alternative.

It should also be mentioned that the facile acetylation of amino sugars with the TsOH-catalyzed protocol was in sharp contrast to the reaction using I_2.[12] As a comparison, the amount of acid catalyst (in mol % per OH group), reaction time, and product yield for the acetylation of *N*-Troc glucosamine, *N*-acetyl glucosamine, and NANA methyl ester with I_2-catalyzed and TsOH-catalyzed protocols are provided in Table 1 (entries f, h, k and l). For the acetylation of *N*-Troc glucosamine with I_2, the reported experimental procedure was followed and 250 mg I_2 per g of *N*-Troc glucosamine (9 mol % per OH group) was used.[12] For the I_2-catalyzed acetylation of GlcNAc and NANA methyl ester, a much higher catalyst loading was applied (either 5 or 13 mol % per OH group for the I_2-catalyzed acetylation versus 2 mol % per OH group for the TsOH-catalyzed acetylation) (Table 1, entries, h, k, and l). Even at such a high I_2 concentration, it still took two days for the complete acetylation of *N*-acetyl glucosamine (Table 1, entry h). In addition, no significant acetylation was observed for *N*-Troc glucosamine when I_2 catalyst was used (Table 1, entry f).

Regarding the α-/β-selectivity of the process, α-glycosyl acetates were formed preferentially in majority of the cases, which can be explained by thermodynamics (Table 1, entries a–e, g–h, and m). Nevertheless, for the acetylation of *N*-Troc and 2-azido-2-deoxy-glucosamines, β-glycosyl acetates were the major anomers obtained (Table 1, entries f and i). Apparently, the strong participatory effect of trichloroethoxylcarbamyl function outweighed the anomeric effect in *N*-Troc glucosamine, while the reason for β-selectivity in 2-azido-2-dexoy-glucosoamine is not clear.

Table 1. Sulfonic acid-catalyzed acetylation for carbohydrate substrates with different complexities

Entry	Carbohydrate substrate	Per-*O*-acetyl glycosyl acetate	Acid (mol % per OH)	Temp (°C)	Time (h)	Yield % (α:β)
a	D-Glucose	 **1**	TsOH (2)	0–27	6	95 (71:29)
b	D-Mannose	 **2**	TsOH (2)	0–27	5	94 (45:11)
c	L-Rhamnose·H_2O	 **3**	TsOH (2)	0–27	4	92 (58:42)
d	D-Galactose	 **4**	H_2SO_4 (2)	−20 to 0	18	92 (76:24)[a]
e	L-Fucose	 **5**	H_2SO_4 (2)	−30 to −20	8	93 (9:1)[a]
f	2-Trichloroethoxy carbamyl-2-deoxy-D-glucopyranose	 **6**	TsOH (2) I_2 (9)	0–27 0–27	6 >24	90 (27:73)[a] No reaction[b]
g	2-Trichloroacetamido-2-deoxy-D-glucopyranose	 **7**	TsOH (2)	0–55	5	93 (77:23)[a]
h	2-Acetamido-2-deoxy-D-glucopyranose	 **8**	TsOH (2) I_2 (5)	0–50 0–27	5 48	90 (61:39) 98 (2.5:1)[b,c]
i	2-Azido-2-deoxy-D-glucopyranose	 **9**	TsOH (2)	0–27	4	85 (23:77)[a]
j	2-Benzyloxycarbamyl-2-deoxy-D-glucopyranose	 **10**	TsOH (2)	0–40	3	94 (not determined)[a]
k	*N*-Acetyl neuraminic acid methyl ester	 **11**	TsOH (2) I_2 (6.5) I_2 (13)	0–27 0–27 0–27	4 Sluggish 20 min	90 (1:4)[a] Not determined[b,c] 70 (not determined)[b,c]
l	*N*-Acetyl neuraminic acid methyl ester	 **12**	TsOH (2) I_2 (13)	0–45 0–35	12 60 min	80 (β only)[a,b] 90 (1:3.5)[b,c]
m	D-Lactose·H_2O	 **13**	TsOH (2)	0–40	4	90 (3:2)

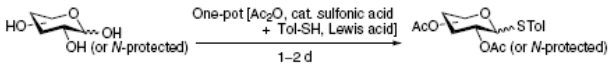
(*continued on next page*)

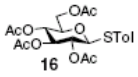
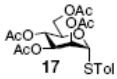
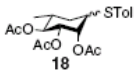
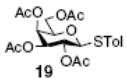
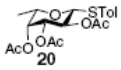
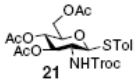
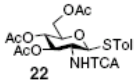
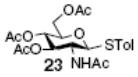
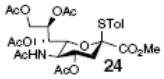
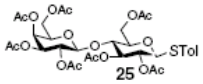
Table 1 (*continued*)

Entry	Carbohydrate substrate	Per-*O*-acetyl glycosyl acetate	Acid (mol % per OH)	Temp (°C)	Time (h)	Yield % (α:β)
n	β-Cylcodextrin	per-*O*-acetyl-β-cylcodextrin **14**	TsOH (1)	0–40	10	90[a]
o	D(+)-Melezitose·H_2O	per-*O*-acetyl-D(+)-Melezitose **15**	TsOH (1)	0–30	6	75[a]

[a] CH_3CN was added to the reaction mixture.
[b] Excess Ac_2O was used.
[c] Ref. 12.

Table 2. One-pot syntheses of per-*O*-acetyl thioglycosides



Entry	Carbohydrate substrate	Thioglycoside	Yield % (α:β)
a	D-Glucose	 **16**	75 (β only)[a]
b	D-Mannose	 **17**	80 (α only)[a]
c	L-Rhamnose·H_2O	 **18**	84 (5:1)[a]
d	D-Galactose	 **19**	68 (β only)[a]
e	L-Fucose	 **20**	75 (β only)[a]
f	2-Trichloroethoxy carbamyl-2-deoxy-D-glucopyranose	 **21**	72 (β only)[a]
g	2-Trichloroacetamido-deoxy-D-glucopyranose	 **22**	65 (β only)[a]
h	2-Acetamido-2-deoxy-D-glucopyranose	 **23**	65 (β only)[b]
i	*N*-Acetyl neuraminic acid methyl ester	 **24**	72 (β only)[a]
k	D-Lactose·H_2O	 **25**	75 (β only)[a]

[a] $BF_3{\cdot}Et_2O$ was used for thioglycosidation.
[b] $SnCl_4$ was used for thioglycosidation.

With the sulfonic acid-catalyzed acetylation protocols in hand, we next explored a simple one-pot two step acetylation–thioglycosidation approach for the preparation of per-*O*-acetyl thioglycosides. In the one-pot TsOH-catalyzed acetylation–thioglycosidation, the sugar substrate was firstly peracetylated with the

described TsOH-catalyzed acetylation, followed by the solvent removal, and the addition of *p*-thiocresol (1.5 mol equiv) in dichloromethane (CH_2Cl_2) and the appropriate Lewis acid catalyst ($BF_3{\cdot}Et_2O$ or $SnCl_4$) (Table 2, entries a–c and f–k). The optimal reaction conditions and exact amount of reagents used are detailed in Table S2 of Supplementary data. In the one-pot H_2SO_4-catalyzed acetylation–thioglycosidation of D-galactose and L-fucose, complete removal of solvent led to undesired dehydration; thus 1.2 equiv of methanol was added to quench the remaining Ac_2O, followed by the addition of thiocresol (2 mol equiv) in CH_2Cl_2 and $BF_3{\cdot}Et_2O$ (2 mol equiv). Simple hexopyranoses (Table 2, entries a–e), glucosamines with different amino protecting functions (Table 2, entries f–h), NANA methyl ester (Table 2, entry i), and lactose (Table 2, entry k) were smoothly converted to the expected per-*O*-acetyl thioglycosides **15**–**25** in respectable yields (65–84%) within 1–2 days. Both *N*-Cbz and N_3 protecting functions were dismantled under these thioglycosidation conditions. Due to the participation of the group at C2, the 1,2-*trans* thioglycosidic bond was formed exclusively in most cases, whereas for L-rhamnose, a 5:1 α- to β-thioglycosides mixture was furnished, which also agreed with previous finding (Table 2, entry c).[10e] For NANA methyl ester, the β-thioglycoside **24** was formed exclusively, which could be attributed to the anomeric effect (Table 2, entry i).

In conclusion, cheap and readily available sulfonic acids, TsOH, and H_2SO_4 are versatile and efficient catalysts for the acetylation of carbohydrates. Contrary to most acid catalysts, which are mainly restricted to the acetylation of simple carbohydrates without amino functions,[10d,e,12–14] our versatile protocol can be applied to different carbohydrate substrates including mono-, di-, tri-, and hepta-saccharides, amino sugars with different amino protecting functions, and oligosaccharides containing fragile furanosyl glycosidic bonds. Additional features include the chemoselective formation of tetra-*O*-acetyl- and penta-*O*-acetyl-NANA esters. In addition, the simple one-pot two step acetylation–thioglycosidation protocols were also developed for the direct access of a panel of *p*-tolyl per-*O*-acetyl thioglycosides including the first one-pot preparation of a sialyl thioglycoside.

1. Experimental

1.1. General methods

All chemicals were purchased as reagent grade and used without further purification. TsOH was dried over P_2O_5 under vacuum and stored in desiccators. 99.99% H_2SO_4 used was purchased from a known chemical vendor. CH_3CN and CH_2Cl_2 were distillated over calcium hydride under N_2 before use. Flash column chromatography was performed on silica gel 60 (70–230 mesh, E. Merck). ^1H and ^{13}C NMR spectra of the prepared compounds were recorded with 300 MHz and 75 MHz Bruker spectrometers. Chemical shift (δ ppm) was measured against TMS, generated from the residual $CHCl_3$ lock signal at δ 7.26 ppm against the residual proton signal of deuterated chloroform, and the ^{13}C resonance signal is calibrated against the ^{13}C signal of deuterated chloroform. Coupling constant(s) in Hertz (Hz) were obtained from ^1H NMR spectra.

1.2. TsOH-catalyzed acetylation procedure for the preparation of per-*O*-acetyl glycosyl acetates 1–3 and 6–15

To 0.5 g of mono-, di-, tri-, or hepta-saccharides was added Ac_2O (or a mixture of Ac_2O and CH_3CN) in which a catalytic amount of TsOH was dissolved. The mixture was firstly stirred at 0 °C for 1 h and then stirred at the optimal reaction temperature.[19] Upon complete acetylation, the mixture was diluted with EtOAc (20 mL), which was washed with cold satd $NaHCO_3$ (20 mL × 2), water (20 mL × 1), brine (20 mL × 1), dried over $MgSO_4$, filtered, and then concentrated. Except for melezitose and N-protected amino sugars, the crude concentrate after work-up was directly characterized with NMR spectroscopy. For the peracetylated products of melezitose and N-protected amino sugars, flash chromatography purification with EtOAc–hexane elution was performed.

1.3. H_2SO_4-catalyzed acetylation procedure for the preparation of per-*O*-acetyl glycosyl acetates 4 and 5

To a suspension of 0.5 g of D-galactose in a mixture of Ac_2O–CH_3CN at −20 °C (or −30 °C for L-fucose) was added catalytic amount of H_2SO_4 in CH_3CN (neat H_2SO_4 was diluted with CH_3CN to a 10% v/v solution). The exact amount of reagents used and specific reaction conditions were detailed in Supplementary data.[19] After stirring for 1 h at −20 °C (or −30 °C for L-fucose), the temperature was gradually warmed up to 0 °C (−20 °C for L-fucose) and the stirring was continued till the end of the reaction. The workup procedure was processed as described above.

1.4. One-pot TsOH-catalyzed acetylation–thioglycosidation procedure for the preparation of per-*O*-acetyl thioglycosides of 16–18 and 21–25

The peracetylation procedure was performed at 0.5 g sugar substrate scale as described above. Upon complete acetylation, the reaction solvent was removed and co-evaporated twice with an equal volume of toluene on a rotary evaporator. Thiocresol (1.5 mol equiv) in CH_2Cl_2 was added to the crude residue at 0 °C, followed

by the addition of Lewis acid (either 2 mol equiv of $BF_3{\cdot}Et_2O$ or 1.1 mol equiv of $SnCl_4$), and the mixture was stirred initially at 0 °C under N_2. After the addition of the reagents, the reaction temperature was raised to 27 °C and the reaction mixture stirred until the end of the reaction, except for *N*-acetyl-D-glucosamine **8**, in which the reaction mixture was warmed up to 40 °C. Upon completion of the reaction, the mixture was diluted with cold EtOAc (50 mL), which was sequentially washed with cold satd $NaHCO_3$ (30 mL × 2), brine (30 mL × 1), dried over $MgSO_4$, filtered, and then concentrated for flash column chromatography to furnish the per-*O*-acetyl thioglycosides **16**–**18** and **21**–**25**.

1.5. One-pot H_2SO_4-catalyzed acetylation–thioglycosidation procedure for the preparation of per-*O*-acetyl thioglycosides 19 and 20

The peracetylation procedure of D-galactose or L-fucose was performed at 0.5 g scale as described above. Upon complete acetylation, 1.2 equiv of methanol was added and the mixture was stirred for 1 h at 0 °C; subsequent addition of thiocresol (1.5 mol equiv) in CH_2Cl_2 and $BF_3{\cdot}Et_2O$ followed. The mixture was stirred initially at 0 °C and then gradually warmed to room temperature (27 °C) under N_2. Upon completion of the reaction, the mixture was diluted with cold EtOAc (50 mL), which was washed with cold satd $NaHCO_3$ (30 mL × 2), brine (30 mL × 1), dried over $MgSO_4$, filtered, and then concentrated for flash column chromatography to furnish the per-*O*-acetyl thioglycosides **19** and **20**.

1.6. *p*-Tolyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (16)

^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.39 (d, $J = 8.1$ Hz, 2H, ArH), 7.10 (d, $J = 7.9$ Hz, 2H, ArH), 5.20 (dd, $J = 9.3$, 9.4 Hz, 1H, H-3), 5.01 (dd, $J = 9.3$, 9.9 Hz, 1H, H-4), 4.92 (dd, $J = 9.3$, 10.0 Hz, 1H, H-2), 4.63 (d, $J = 9.9$ Hz, 1H, H-1), 4.20–4.14 (m, 2H, H-6, H-6), 3.70 (ddd, $J = 2.6$, 4.7, 10.1 Hz, 1H, H-5), 2.33 (s, 3H, STol CH_3), 2.09 (s, 3H, Ac), 2.08 (s, 3H, Ac), 2.01 (s, 3H, Ac), 1.98 (s, 3H, Ac); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.97, 170.58, 169.78, 169.63, 139.2, 134.2, 130.2, 130.1, 127.9, 86.2, 76.1, 70.3, 68.6, 62.5, 21.58, 21.16, 21.12, 20.97.

1.7. *p*-Tolyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranoside (17)

^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.40 (d, $J = 8.1$ Hz, 2H, ArH), 7.12 (d, $J = 8.1$ Hz, 2H, ArH), 5.50 (dd, $J = 1.5$, 2.5 Hz, 1H), 5.42 (d, $J = 1.0$ Hz, 1H, H-1), 5.34–5.32 (m, 2H), 4.57–4.56 (m, 1H), 4.30 (dd, $J = 12.3$, 6.0 Hz, 1H), 4.10 (dd, $J = 12.5$, 2.6 Hz, 1H), 2.34 (s, 3H, STol CH_3), 2.15 (s, 3H, Ac), 2.11 (s, 3H, Ac), 2.08 (s, 3H, Ac), 1.99 (s, 3H, Ac); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 171.0, 170.3, 170.22, 170.16, 138.8, 122.0, 130.4, 129.2, 86.4, 69.8, 69.7, 66.8, 62.9, 21.52, 21.27, 21.09, 21.03.

1.8. *p*-Tolyl 2,3,4-tri-*O*-acetyl-1-thio-α-L-rhamnopyranoside (18)

^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.36 (d, $J = 8.1$ Hz, 2H, ArH), 7.11 (d, $J = 8.1$ Hz, 2H, ArH), 5.48 (dd, $J = 3.3$, 1.5 Hz, 1H, H-2), 5.32 (d, $J = 1.5$ Hz, 1H, H-1), 5.27 (dd, $J = 3.3$, 9.9 Hz, 1H, H-2), 5.13 (t, $J = 9.9$ Hz, 1H, H-4), 4.41–4.32 (m, H-5), 2.32 (s, 3H, STol CH_3), 2.14 (s, 3H, Ac), 2.07 (s, 3H, Ac), 2.00 (s, 3H, Ac), 1.24 (s, 3H, CH_3-R); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.4, 170.3, 138.6, 132.8, 130.4, 130.3, 129.7, 86.4, 71.6 × 2, 69.7, 68.1, 21.52, 21.30, 21.21, 21.08, 17.7.

1.9. *p*-Tolyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (19)

^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.39 (d, $J = 8.1$ Hz, 2H, ArH), 7.10 (d, $J = 7.8$ Hz, 2H, ArH), 5.4 (dd, $J = 1.0$, 3.3 Hz, 1H, H-4), 5.22 (t, $J = 9.9$ Hz, 1H, H-2), 5.03 (dd, $J = 3.3$, 10.0 Hz, H-3), 4.64 (d, $J = 10.0$ Hz, H-1), 4.19 (dd, $J = 7.0$, 11.3 Hz, 1H, H-6), 4.11 (dd, $J = 6.3$, 11.3 Hz, H-6), 3.92 (dt, $J = 1.0$, 6.1 Hz, H-5), 2.34 (s, 3H, STol CH_3), 2.12 (s, 3H, Ac), 2.10 (s, 3H, Ac), 2.04 (s, 3H, Ac), 1.97 (s, 3H, Ac); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.8, 170.62, 170.48, 169.84, 138.8, 133.5, 130.0, 129.0, 87.3, 72.4, 67.7, 67.6, 61.9, 21.56, 21.27, 21.07, 21.04, 20.99.

1.10. *p*-Tolyl 2,3,4-tri-*O*-acetyl-1-thio-β-L-fucopyranoside (20)

^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.42 (d, $J = 8.1$ Hz, 2H, ArH), 7.13 (d, $J = 7.9$ Hz, 2H, ArH), 5.25 (dd, $J = 0.7$, 3.2 Hz, 1H, H-4), 5.19 (t, $J = 9.9$ Hz, 1H, 1H, H-2), 5.03 (dd, $J = 3.3$, 9.9 Hz, 1H, H-3), 4.64 (d, $J = 9.8$ Hz, 1H, H-1), 3.80 (q, $J = 6.4$ Hz, 1H, H-5), 2.33 (s, 3H, STol CH_3), 2.14 (s, 3H, Ac), 2.10 (s, 3H, Ac), 1.98 (s, 3H, Ac), 1.24 (d, $J = 6.4$ Hz, 3H, CH_3-R); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 171.1, 170.6, 169.9, 138.6, 133.3, 130.3, 130.0, 129.5, 8.2, 72.8, 70.7, 67.8, 21.6, 21.3, 21.09, 21.06, 16.8.

1.11. *p*-Tolyl 3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-2-trichloroethoxycarbamyl-β-D-glucopyranoside (21)

^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.42 (d, $J = 8.1$ Hz, 2H, ArH), 7.13 (d, $J = 7.8$ Hz, 2H, ArH), 5.29–5.26 (m, 2H), 5.03 (t, $J = 9.8$ Hz, 1H, H-4), 4.79 (d, $J = 10.8$ Hz, 1H), 4.75 (d, $J = 10.9$ Hz, 1H), 4.23–4.17 (m, 2H), 3.74–3.65 (m, 2H), 2.36 (s, 3H, STol CH_3), 2.10 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.01 (s, 3H, Ac); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 171.4, 171.0, 170.5, 169.8, 154.7, 139.0,

138.7, 134.1, 133.2, 130.3, 130.2, 95.8, 86.7, 75.1, 71.4, 63.8, 63.5, 55.7, 52.6, 21.5, 21.1, 21.07, 20.95.

1.12. *p*-Tolyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-1-thio-β-D-glucopyranoside (22)

^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.50 (br m, 1H, N–H), 7.39 (d, $J = 8.1$ Hz, 2H, ArH), 7.10 (d, $J = 7.9$ Hz, 2H, ArH), 5.41 (dd, $J = 9.3$, 11 Hz, 1H, H-3), 5.03 (t, $J = 9.6$ Hz, 1H, H-4), 4.75 (d, $J = 9.8$ Hz, 1H, H-1), 4.23–4.01 (m, 3H, H-6, H-2), 3.76 (ddd, $J = 2.5$, 4.6, 10.0 Hz, 1H, H-5), 2.32 (s, 3H, STol CH_3), 2.06 (s, 3H, Ac), 1.98 (s, 3H, Ac), 1.78 (s, 3H, Ac); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 171.8, 171.0, 170.0, 162.2, 139.4, 134.5, 130.1, 128.2, 92.8, 87.2, 76.2, 73.9, 68.9, 62.8, 54.5, 21.6, 21.1, 21.0, 20.6.

1.13. *p*-Tolyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-β-D-glucopyranoside (23)

^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.39 (d, $J = 8.1$ Hz, 2H, ArH), 7.10 (d, $J = 7.9$ Hz, 2H, ArH), 5.92 (br d, $J = 12$ Hz, 1H, N–H), 5.23 (dd, $J = 9.3$, 10.9 Hz, 1H, H-3), 5.03 (dd, $J = 9.3$, 9.9 Hz, 1H, H-4), 4.79 (d, $J = 9.9$ Hz, 1H, H-1), 4.20–4.17 (m, 2H, H-6, H-6′), 4.00 (dd, $J = 9.3$, 10.0 Hz, 1H, H-2), 3.71 (ddd, $J = 2.6$, 4.7, 10.1 Hz, 1H, H-5), 2.34 (s, 3H, STol CH_3), 2.09 (s, 3H, Ac), 2.01 (s, 3H, Ac), 1.99 (s, 3H, Ac); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 171.4, 171.0, 170.5, 169.8, 162.4, 139.4, 134.5, 130.1, 128.2, 87.2, 76.2, 73.9, 68.9, 62.8, 54.5, 21.5, 21.1, 21.07, 20.95.

1.14. *p*-Tolyl 2-thio-β-D-*N*-acetyl-neuraminic acid methyl ester (24)

^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.33 (d, $J = 12.8$ Hz, 2H, ArH), 7.12 (d, $J = 7.9$ Hz, 2H, ArH), 5.92 (br d, 1H, N–H), 5.48 (s, 1H), 5.39 (td, $J = 1.1$, 4.2 Hz, H-4), 4.96 (d, $J = 13.9$ Hz, 1H), 4.64 (dd, $J = 2.3$, 10.5 Hz, 1H), 4.50 (dd, $J = 1.9$, 12.2 Hz, 1H), 4.13 (dd, $J = 4.3$, 13.4 Hz, 1H), 4.03 (dd, $J = 8.7$, 7.2 Hz, 1H), 3.59 (s, 3H, CH_3O), 2.64 (dd, $J = 9.1$, 4.7 Hz, 1H), 2.32 (s, 3H, STol CH_3), 2.14 (s, 3H, Ac), 2.12 (s, 3H, Ac), 2.08 (s, 3H, Ac), 1.95 (s, 3H, Ac), 1.89 (s, 3H, Ac); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 171.66, 171.40, 170.66, 170.63, 168.66, 140.5, 136.6, 130.2, 125.6, 89.3, 73.6, 73.5, 69.2, 69.1, 63.2, 52.9, 49.7, 37.8, 23.5, 21.69, 21.49, 21.30, 21.13, 21.09.

1.15. *p*-Tolyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside (25)

^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.33 (d, $J = 8.2$ Hz, 2H, ArH), 7.07 (d, $J = 8.0$ Hz, 2H, ArH), 5.30 (dd, $J = 1.0$, 3.3 Hz, H-4′), 5.16 (t, $J = 9.9$ Hz, 1H), 5.05 (dd, $J = 9.3$, 9.4 Hz), 5.03 (dd, $J = 9.3$, 9.9 Hz, 1H), 4.93 (dd, $J = 3.3$, 10.0 Hz, 1H), 4.82 (dd, $J = 9.3$, 10.0 Hz, 1H), 4.57 (d, $J = 10.0$ Hz), 4.47–4.43 (m, 2H), 4.09–4.03 (m, 3H), 3.84 (t, $J = 6.3$ Hz, 1H), 3.70 (t, $J = 7.7$ Hz, 1H), 3.64 (m, 1H), 2.29 (s, 3H, STol CH_3), 2.13–1.92 (m, 21H, 7 × Ac); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.69, 170.64, 170.52, 170.42, 170.09, 169.92, 169.41, 138.9, 134.1, 132.8, 130.3, 130.0, 128.1, 101.3, 85.9, 77.0, 74.3, 71.3, 71.2, 71.1, 70.0, 66.9, 62.4, 61.2, 21.55, 21.22, 21.17, 21.01, 20.99, 20.88.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2008.01.014.

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Tandem One-Pot Acetalation–Acetylation for Direct Access to Differentially Protected Thioglycosides and *O*-Glycosides with *p*-Toluenesulfonic Acid

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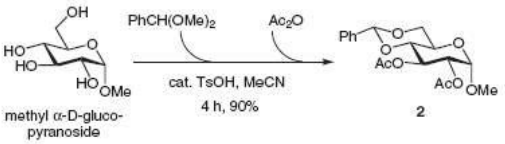
Abstract: A new tandem one-pot acetalation–acetylation procedure is reported which streamlines routine protecting-group manipulation of carbohydrate molecules in production of differentially protected *O*- and thioglycosides. This new procedure eliminates the use of highly toxic pyridine, and *p*-toluenesulfonic acid is employed as catalyst for acetalation and acetylation. Synthetic utility of the new procedure is demonstrated in the expeditious preparation of differentially protected glycosides from a wide variety of carbohydrate substrates including unprotected *O*-glycosides, thioglycosides, and *N*-acetyl neuraminic acid ester.

Key words: tandem, acetals, glycosides, oligosaccharides, protecting group

Preparation of carbohydrate building blocks has always been necessary for oligosaccharide synthesis; thus expeditious synthesis of such building blocks is highly desired. A logical approach toward this goal is to merge two or three sequential reactions into a tandem one-pot operation, which was realized in the preparation of peracetyl glycosyl bromide,[1] peracetyl glycosyl azide,[2] peracetyl glycosyl iodide,[3] peracetyl thioglycoside,[4] and regioselective one-pot protection of carbohydrates.[5]

Acetalation and acetylation are routine synthetic steps for protection of hydroxy function. Generally the former is effected with a free[6] or masked carbonyl function[7–10] in the presence of acid catalyst,[11–17] while the latter is usually promoted in the presence of excess pyridine.[18] However, owing to malodorous and toxic nature of pyridine, such acetylation procedure is gradually superseded by various acid-catalyzed protocols.[19, 20] Sequential acetalation and acetylation of unprotected glycoside substrates are widely employed for production of differentially protected *O*-glycosides and thioglycosides, and the latter constitutes a major class of useful building blocks in oligosaccharide synthesis.[21] As both acetalation and acetylation are catalyzed by acid, it is reasonable for us to merge them into a tandem one-pot operation. Such strategy has been demonstrated in one-pot acetalation–acetylation with immobilized $HClO_4$[22] and H_2SO_4.[23] Though both reported methods are found useful for production of differentially protected *O*-glycosides, preparation of synthetically useful thioglycoside was less discussed in their investigations. In addition, immobilization of acid on silica requires extra procedure rendering them less convenient in practice. Recently, iodine-catalyzed tandem acetonide formation–acetylation was reported, but it is not useful for making benzylidene acetal function.[10] To overcome such limitations and to develop a pyridine-free process, a new one-pot procedure is required. Herein we report the development of a versatile tandem one-pot acetalation–acetylation, which expedites the preparation of differentially protected *O*-glycosides and thioglycosides.

The key to effect a one-pot procedure is to explore the appropriate reaction conditions so that a practical acetylation rate is retained in the presence of the acid-labile acetal



Scheme 1 One-pot acetalation–acetylation of methyl α-D-glucopyranoside

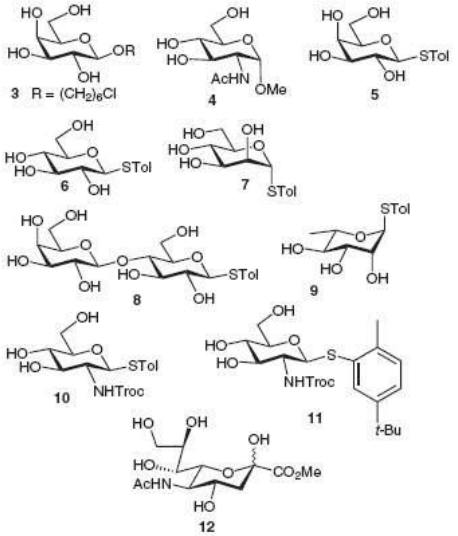


Figure 1 *O*-Glycosides **3** and **4**, thioglycosides **5**–**11**, and NANA ester **12**

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function. Referring to the reported procedures, the amount of acid used in acetalation spans from 5–20 mol%.[11,14,15] Thus in our model study, a suspension of methyl α-D-glucopyranoside and benzaldehyde dimethyl acetal in acetonitrile were treated with 10 mol% of TsOH (0.05 M, Scheme 1). The reaction proceeded smoothly at room temperature and upon complete conversion into acetal intermediate, acetic anhydride (Ac_2O, 1.5 equiv per OH) was added to give the expected 2,3-di-*O*-acetyl-4,6-*O*-benzylidene α-D-gluco-pyranoside **2** in excellent 90% yield.

Being encouraged by the preliminary result, we next examined the one-pot acetalation–acetylation of unprotected *O*-glycosides **3**, **4**,[24] thioglycosides **5–11**,[25] and *N*-acetyl neuraminic acid ester (NANA ester) **12**[26] (Figure 1), and the results were detailed in Table 1.[27]

Table 1 One-Pot Acetalation–Acetylation of *O*-Glycosides **3** and **4**, Thioglycosides **5–11**, and NANA ester **12**

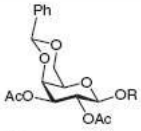
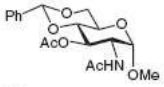
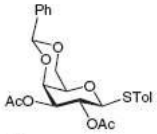
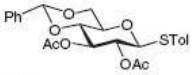
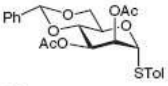
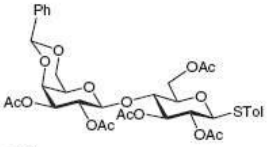
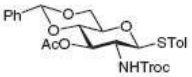
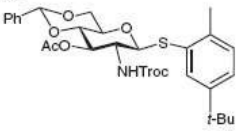
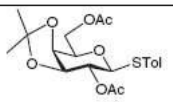
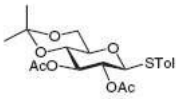
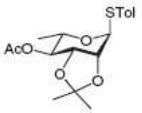
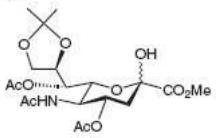
Entry	Substrate	Product	TsOH (mol%, M)	Acetalation/acetylation temp (°C)	Time (h)	Yield (%)
1	3	 **13**	10 (0.031)	25/40	4	81
2	4	 **14**	10 (0.041)	25/40	4	76
3	5	 **15**	10 (0.032)	25/40	5	77
4	6	 **16**[23]	10 (0.032)	25/40	4.5	75
5	7	 **17**	10 (0.01)	25/50	6	70
6	8	 **18**[28]	10 (0.014)	50/50	10	82
7	10	 **19**[29]	32 (0.019)	25/40	4	70
8	11	 **20**	10 (0.027)	25/40	6	75

Table 1 One-Pot Acetalation–Acetylation of *O*-Glycosides **3** and **4**, Thioglycosides **5–11**, and NANA ester **12** (continued)

Entry	Substrate	Product	TsOH (mol%, M)	Acetalation/acetylation temp (°C)	Time (h)	Yield (%)
9	5	 **21**	10 (0.023)	40/40	12	70
10	6	 **22**	10 (0.023)	25/40	7	52
11	9	 **23**[22]	5 (0.018)	25/40	4	75
12	12	 **24**	10 (0.021)	25/35	8	74

One-pot benzylidenation–acetylation of unprotected glycoside substrates **3–8** and **10–11** produced the expected glycosyl acetal derivatives **13–20** in 70–82% yield within 4–10 hours (Table 1, entries 1–8).[30] Owing to variation in physicochemical properties, optimization of reaction conditions was required for particular glycoside substrates. A point in case is the acetalation of mannopyranoside, which suffers from competitive formation of 2,3:4,6-*O*-bis(acetal) derivative. As a consequence, previous one-pot acetalation–acetylation of methyl α-D-mannopyranoside met with difficulty.[22] In our optimized one-pot procedure, a 'diluted' suspension of thiomannopyranoside **7** (1 g in 35 mL of MeCN) and a stoichiometric amount of acetalating agent [1.05 equiv of $PhCH(OMe)_2$] were used. Gratifyingly, formation of bis(acetal) derivative was largely suppressed and the desired 3-*O*-acetyl-4,6-*O*-benzylidene thiomannopyranoside **17** was obtained in satisfactory 70% yield (Table 1, entry 5).

One-pot isopropylidenation–acetylation of carbohydrate substrates **5**, **6**, **9**, and **12** was conducted in acetone,[31] from which the corresponding glycosyl ketal derivatives **21–24** were furnished in 52–75% yield at reasonable time frames (Table 1, entries 9–12). Isopropylidenation of thiogalactopyranoside **5** at room temperature gave a mixture of 3,4-*O*- and 4,6-*O*-isopropylidene ketal derivatives, while elevating the reaction temperature to 40 °C led to the exclusive formation of 3,4-*O*-isopropylidene ketal derivative **21** (Table 1, entry 9). Close examination revealed that 4,6-*O*-isopropylidene ketal derivative was formed preferentially in the beginning, but was gradually isomerized to **21**. Thus, longer reaction time and higher reaction temperature would favor the formation of 3,4-*O*-isopropylidene galactopyranosyl derivative. It should be mentioned that camphorsulfonic acid (CSA)[32] and trimethylsilyl chloride (TMSCl)[33] were also employed as catalyst, though the results were inferior to those obtained by using TsOH catalyst. Isopropylidenation of thioglucopyranoside **6** gave 4,6-*O*-isopropylidene ketal derivative **22** as the single regioisomer in a modest 52% yield along with 15% per-*O*-acetyl thioglucopyranoside. Formation of the latter is attributed to the cleavage of inherently less stable 4,6-*O*-isopropylidene ketal function in conjunction with acetylation (Table 1, entry 10).[34,35]

Other than glycoside substrates, the present procedure is also applicable to carbohydrate hemiacetals as exemplified in the one-pot isopropylidenation–acetylation of NANA ester **12**, which gave NANA ester ketal derivative **24** in 74% yield (Table 1, entry 12). As previously reported, the tertiary C-2 hydroxy function of **12** was unacetylated.[20] To prove the suitability of the procedure for a larger scale operation, 10 g of per-*O*-acetyl thiolactoside was deacetylated to thiolactoside **8**, which after neutralization and purification, underwent the now routine one-pot procedure to give lactosyl benzylidene acetal **18** in reproducible yield.[36]

In summary, an unprecedented TsOH-catalyzed one-pot acetalation–acetylation was developed, which streamlines the routine protecting-group manipulation procedures and obviate the use of toxic pyridine in preparation of a wide diversity of differentially protected thioglycosides.

Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synlett.

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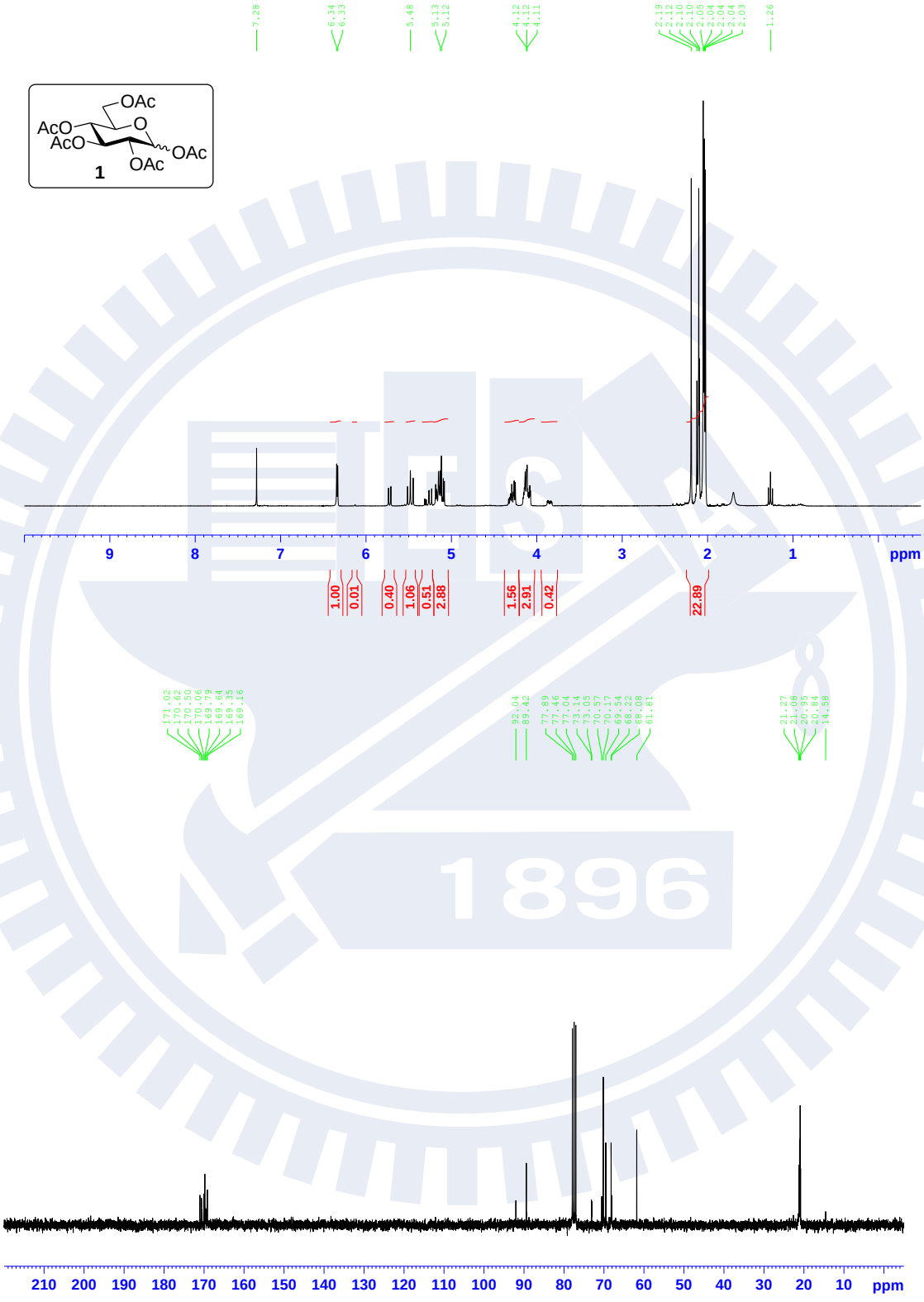
(30) **General One-Pot Benzylidenation–Acetylation Procedure for the Preparation of 2 and 13–20**
TsOH (10–32 mol%, Table 1) was added into a stirring mixture of carbohydrate substrate (1.0 equiv of methyl α-D-glucopyranoside, **3–8**, **10**, or **11**) and $PhCH(OMe)_2$ (1.5 equiv) in MeCN under N_2. Upon complete conversion into benzylidene acetal intermediate as assessed by TLC, Ac_2O (1.5 equiv per OH, total OH equals to the sum of OH of acetal intermediate plus MeOH released from acetalating reagent) was added, and the reaction temperature was brought up to 40 or 50 °C (Table 1). Specific reaction conditions are detailed in the supporting information. Upon complete acetylation as assessed by TLC, excess EtOAc (4 × volume of MeCN used) was added to the mixture, which was then washed with sat. $NaHCO_3$, brine, dried over $MgSO_4$, and concentrated for purification by flash column chromatography over SiO_2. Elution with EtOAc–hexane mixture afforded the compounds **2** and **13–20**.
(31) **General One-Pot Isopropylidenation–Acetylation Procedure for Preparation of 21–24**
TsOH (5–10 mol%, Table 1) was added into a stirring mixture of unprotected thioglycoside (1.0 equiv of **5**, **6**, **9**, or NANA ester **12**), and $Me_2CH(OMe)_2$ (1.5 equiv) in acetone. The mixture was stirred at r.t. or 40 °C under N_2. Upon complete conversion into glycosyl ketal intermediate as assessed by TLC, Ac_2O (1.5 equiv per OH, total OH equals to OH from glycosyl acetal intermediate plus MeOH released from acetalating reagent) was added, and the reaction temperature was brought up to 35 or 40 °C (Table 1). Specific reaction conditions for preparation of compounds **21–24** were detailed in the supporting information. Upon complete acetylation as assessed by TLC, excess EtOAc (4 × volume of MeCN used) was added to the mixture, which was then washed with sat. $NaHCO_3$, brine, dried over $MgSO_4$, and concentrated for purification by flash column chromatography over SiO_2. Elution with EtOAc–hexane mixture afforded compounds **21–24**.
(32) Ziegler, T.; Herold, G. *Liebigs Ann. Chem.* **1994**, 859.
(33) Nishida, Y.; Dohi, H.; Uzawa, H.; Kobayashi, K. *Tetrahedron Lett.* **1998**, *39*, 8681.
(34) In *Monosaccharides: Their Chemistry and their Roles in Natural Products*; Collins, P. M.; Ferrier, R. J., Eds.; John Wiley and Sons: New York, **1996**, 389–405.
(35) Lu, K.-C.; Hseih, S.-Y.; Patkar, L. N.; Chen, C.-T.; Lin, C.-C. *Tetrahedron* **2004**, *60*, 8967.
(36) For acid-catalyzed one-pot acetalation–acetylation, unnecessary prolonged reaction time would compromise the yield of reaction due to the cleavage of the acetal function.

附錄 核磁共振圖譜

1H


7.28
6.34
6.33
5.48
5.13
5.12
4.12
4.12
4.11
2.19
2.12
2.10
2.10
2.05
2.04
2.04
2.04
2.03
1.26
OAc
AcO
O
AcO
OAc
OAc
1
9
8
7
6
5
4
3
2
1
ppm
1.00
0.01
0.40
1.06
0.51
2.88
1.56
2.91
0.42
22.89
171.02
170.62
170.36
170.16
169.79
169.64
169.35
169.16
92.04
89.42
77.89
77.46
77.04
73.14
73.05
70.57
70.17
69.54
68.22
68.08
61.81
21.27
21.08
20.95
20.84
14.58
210
200
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150
140
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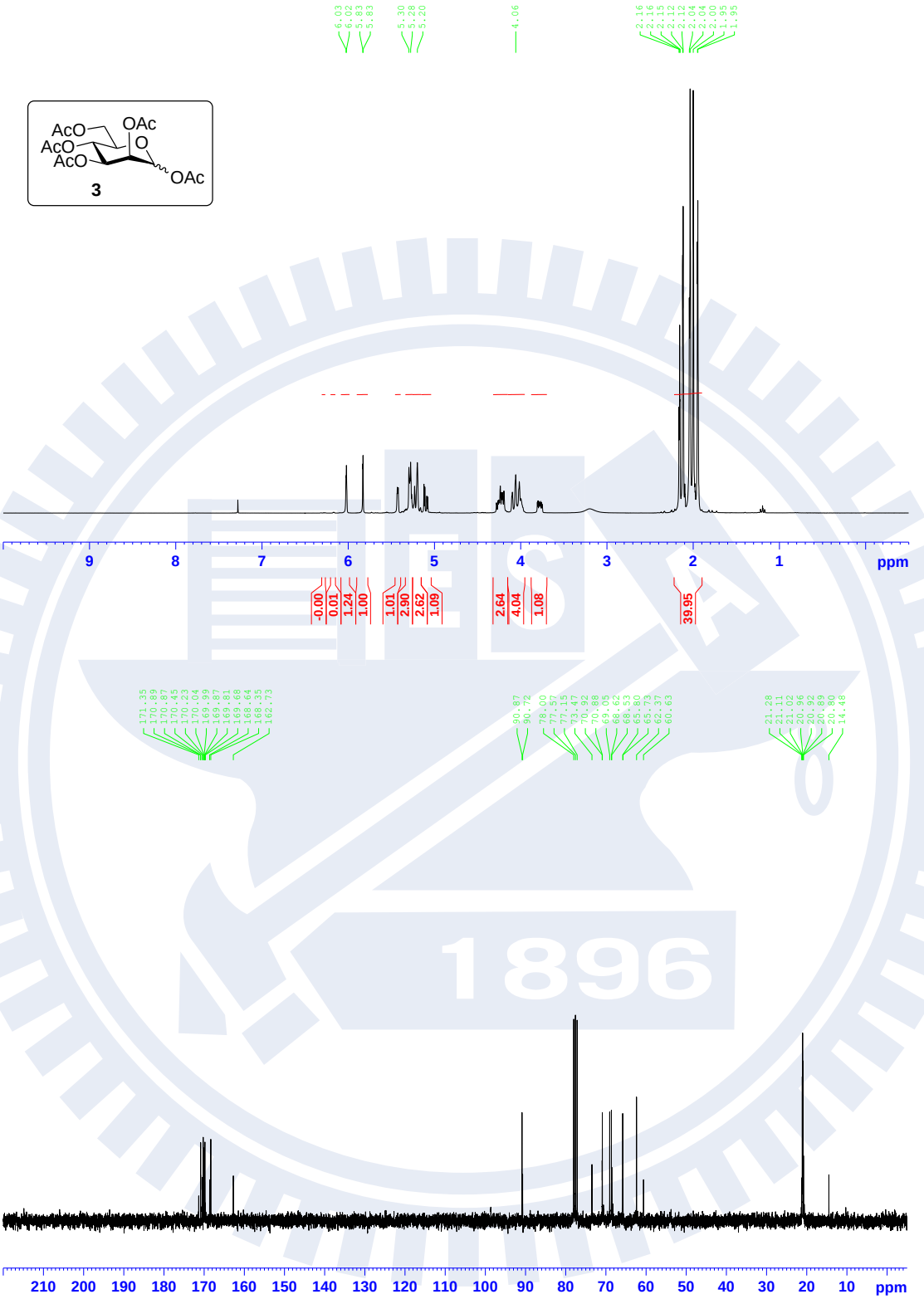
120
110
100
90
80
70
60
50
40
30
20
10
ppm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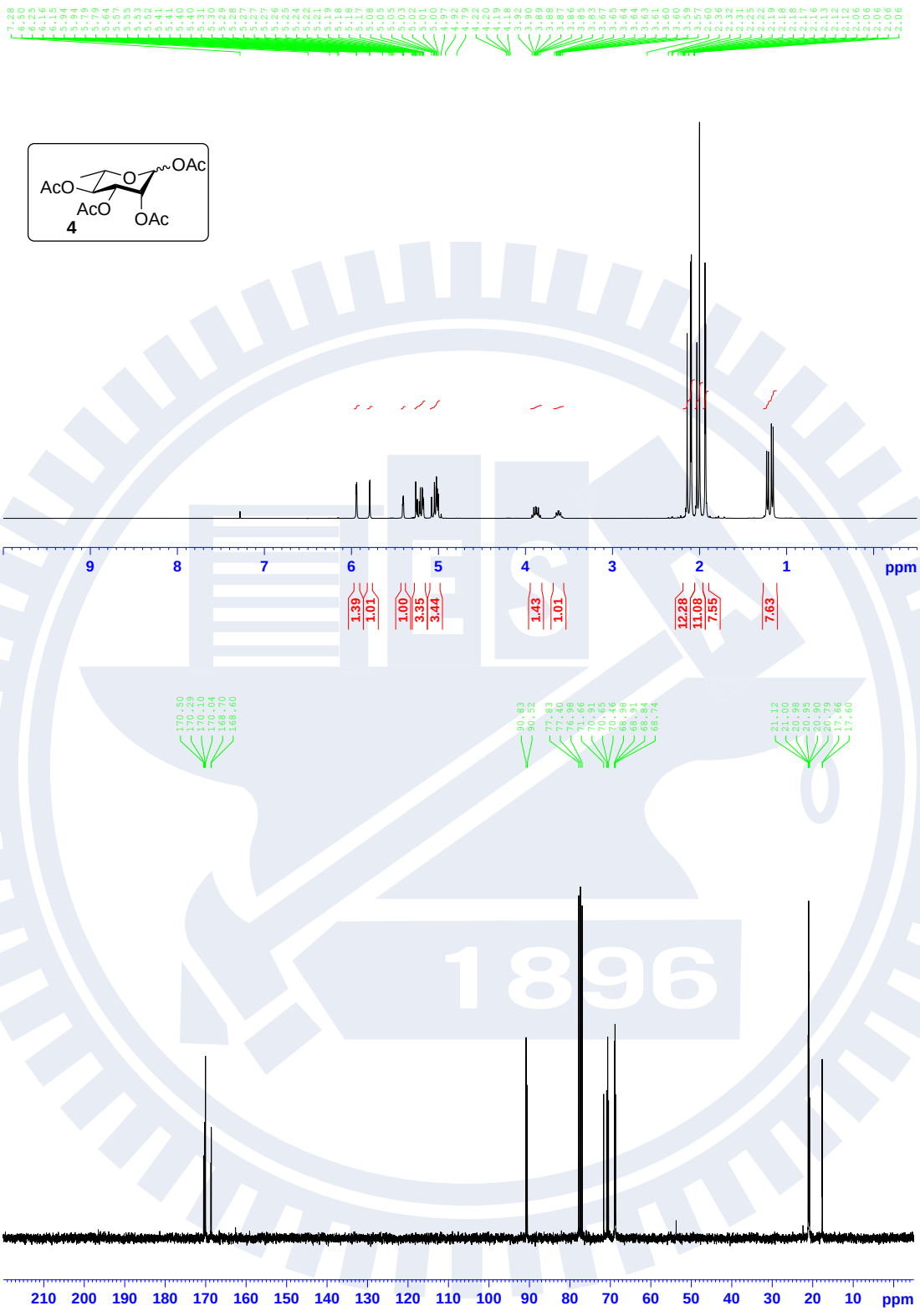
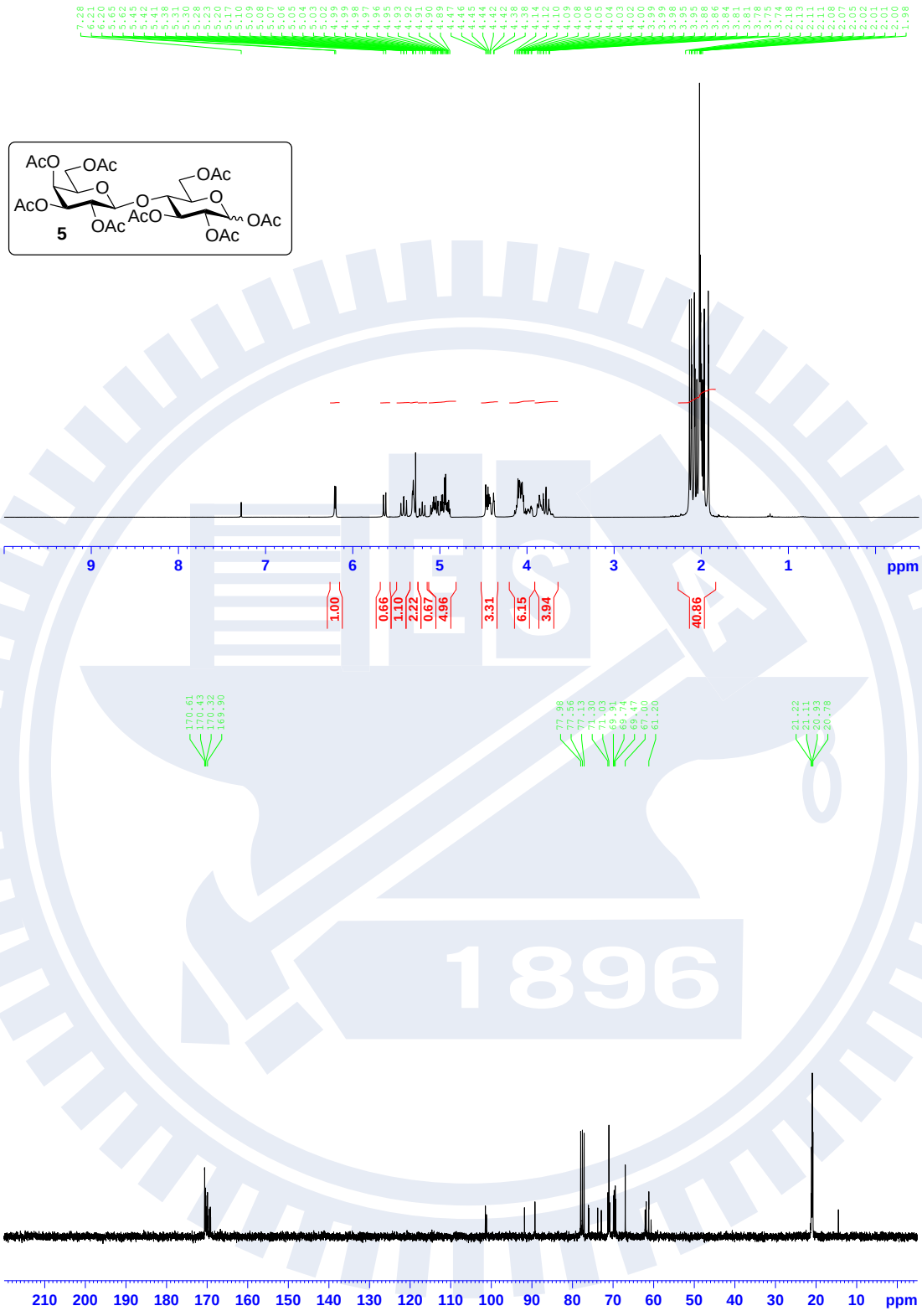
OAc
AcO
O
AcO
OAc
3
6.03
6.02
5.83
5.83
5.30
5.28
5.20
4.06
2.16
2.16
2.15
2.12
2.12
2.04
2.04
2.00
1.95
1.95
9
8
7
6
5
4
3
2
1
ppm
-0.00
0.01
1.24
1.00
1.01
2.90
2.62
1.09
2.64
4.04
1.08
39.95
171.35
170.89
170.87
170.45
170.23
170.04
169.99
169.87
169.81
169.68
168.64
168.35
162.73
90.87
90.72
78.00
77.57
77.15
73.47
70.92
70.88
69.05
68.62
68.53
65.80
65.73
62.37
60.63
21.28
21.11
21.02
20.96
20.92
20.89
20.80
14.48
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


AcO
AcO
O
OAc
OAc
4
9
8
7
6
5
4
3
2
1
ppm
1.39
1.01
1.00
3.35
3.44
1.43
1.01
12.28
11.08
7.55
7.63
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


AcO
OAc
O
AcO
OAc
O
AcO
OAc
OAc
OAc
5
ppm
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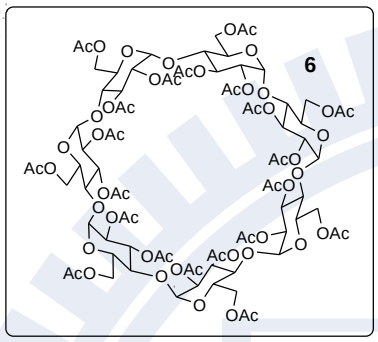
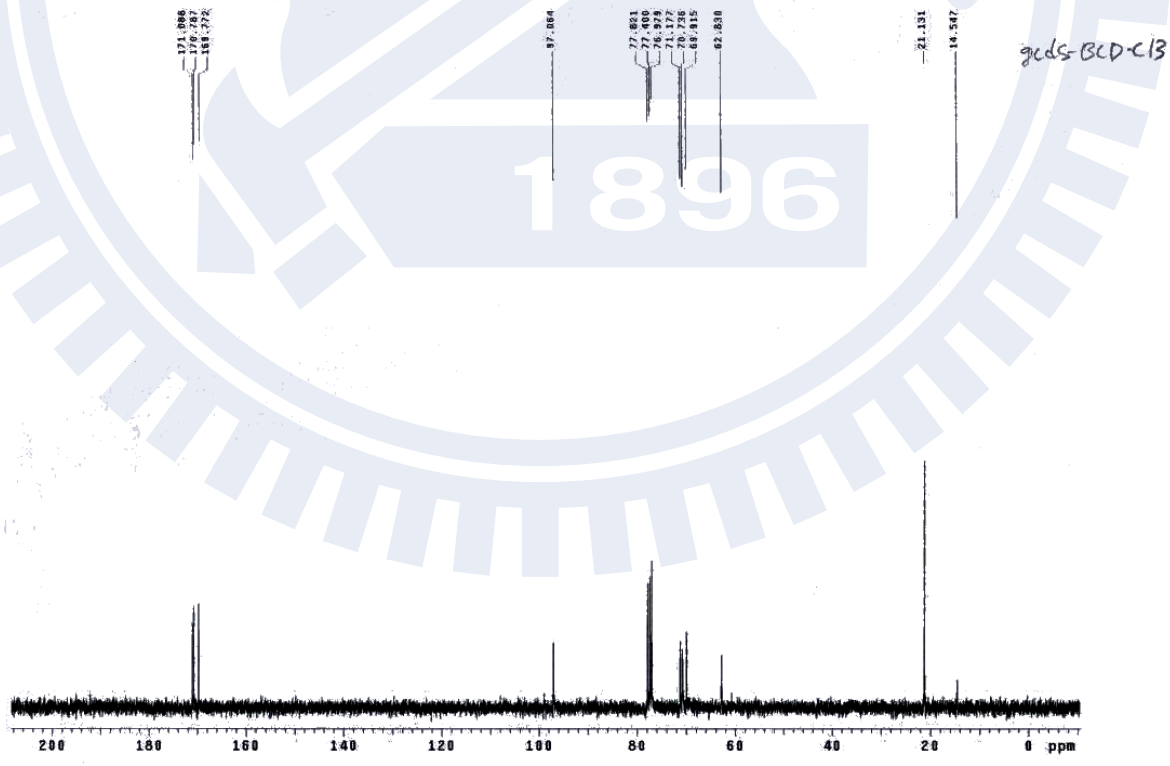
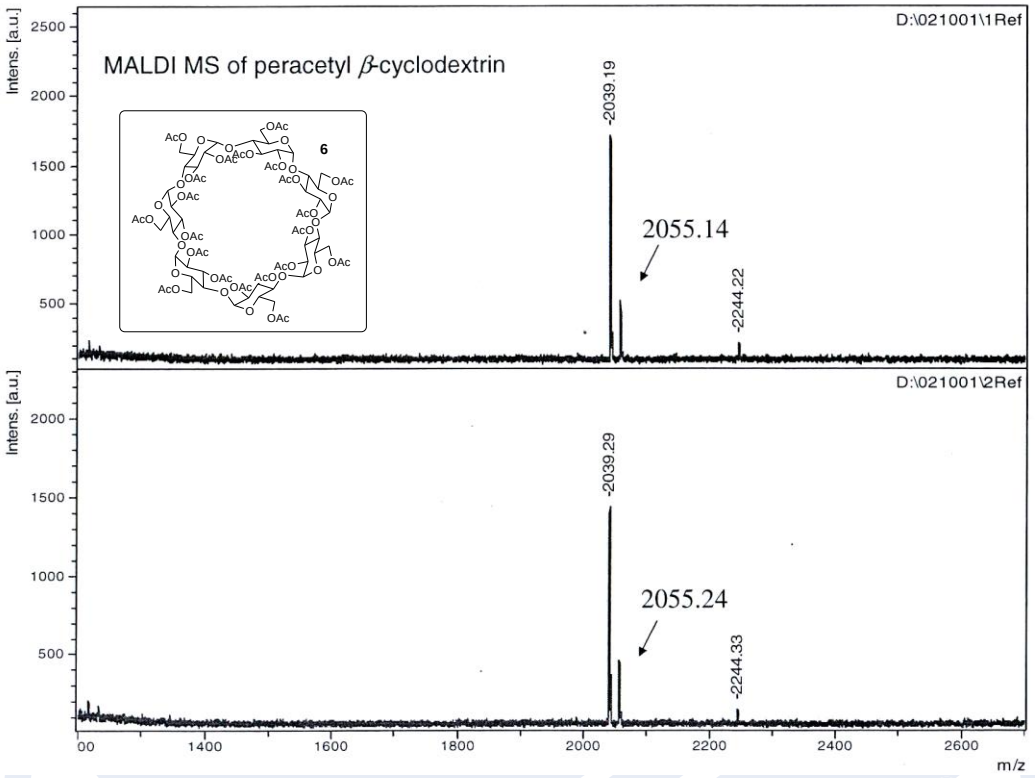
0.66
1.10
2.22
0.67
4.96
3.31
6.15
3.94
40.86

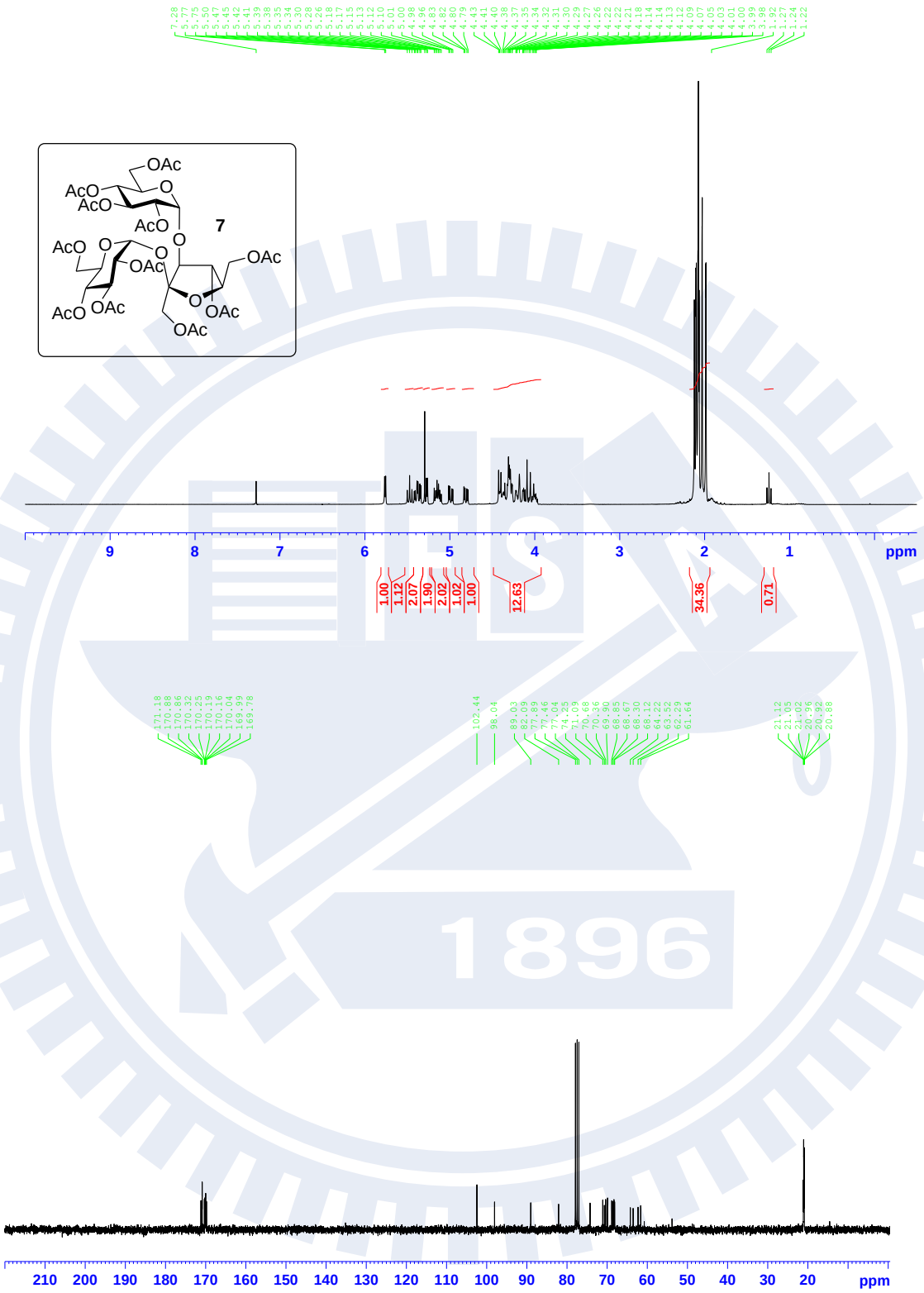

6
OAc
AcO
O

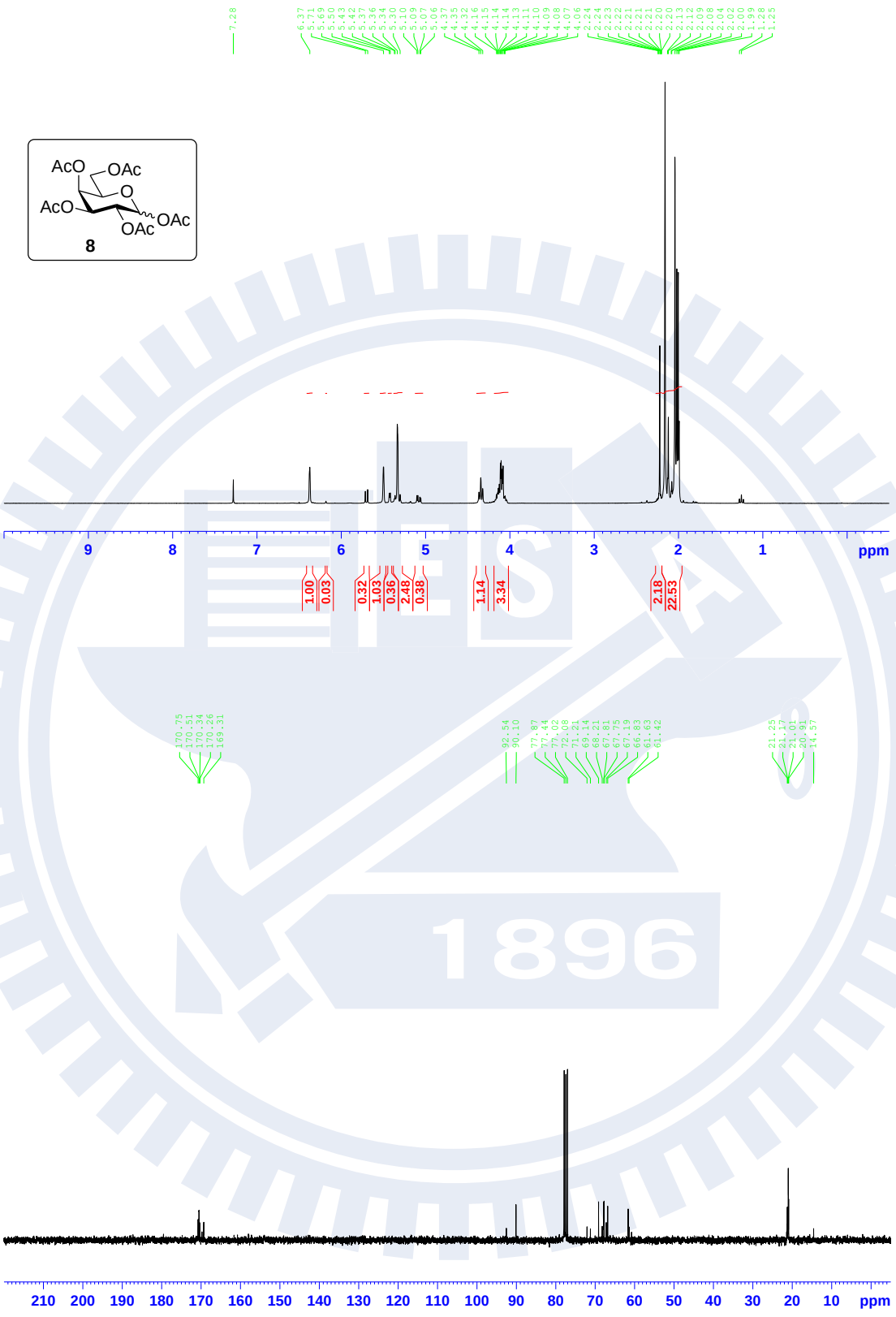
9 8 7 6 5 4 3 2 1 0 ppm
1.00 1.02 1.00 1.05 1.09 1.40 1.08 9.68 0.58

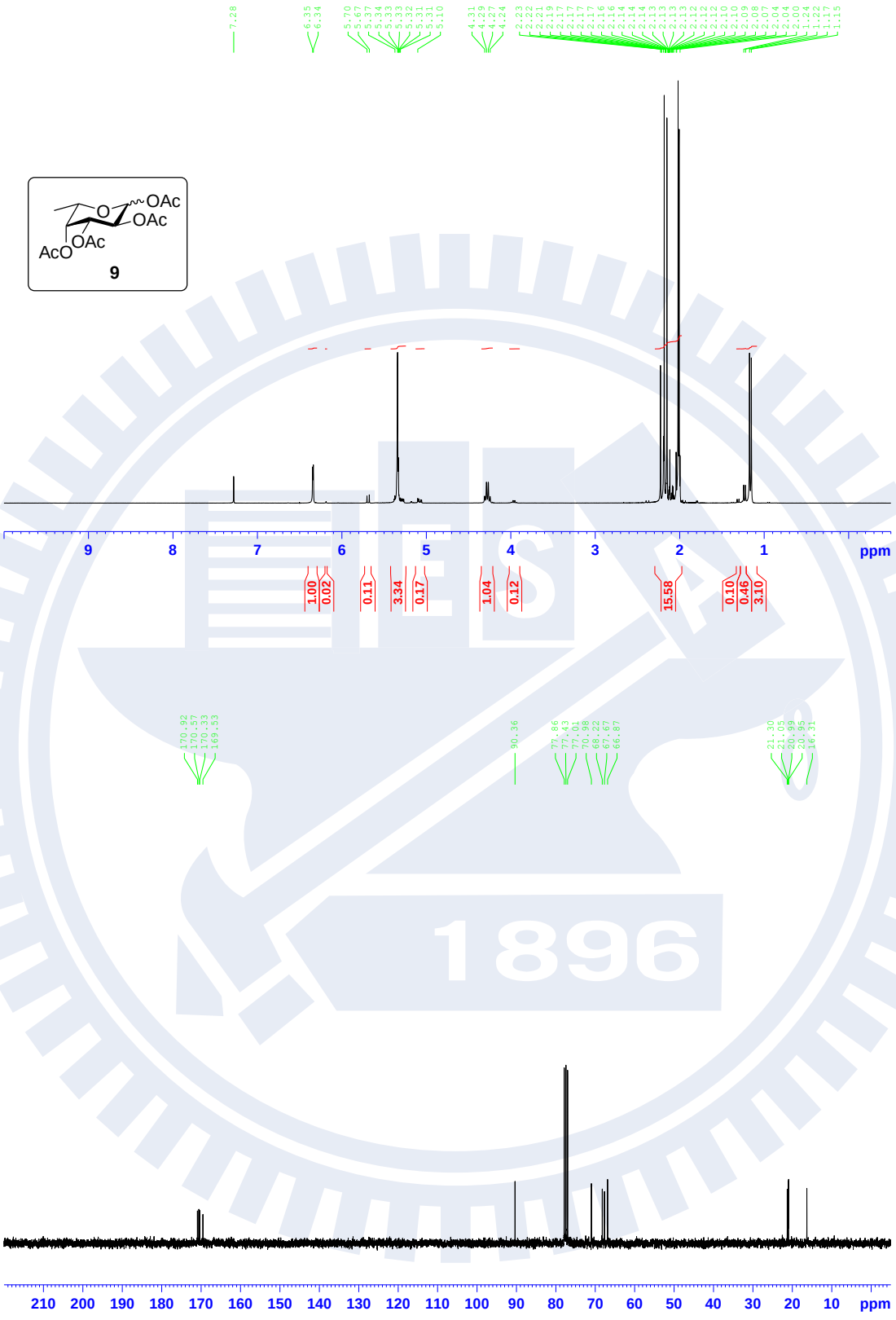

gcds-BCD-C13
200 180 160 140 120 100 80 60 40 20 0 ppm


MALDI MS of peracetyl β-cyclodextrin
6
D:\021001\1Ref
Intens. [a.u.]
2500
2000
1500
1000
500
-2039.19
2055.14
-2244.22
D:\021001\2Ref
Intens. [a.u.]
2000
1500
1000
500
-2039.29
2055.24
-2244.33
00
1400
1600
1800
2000
2200
2400
2600
m/z

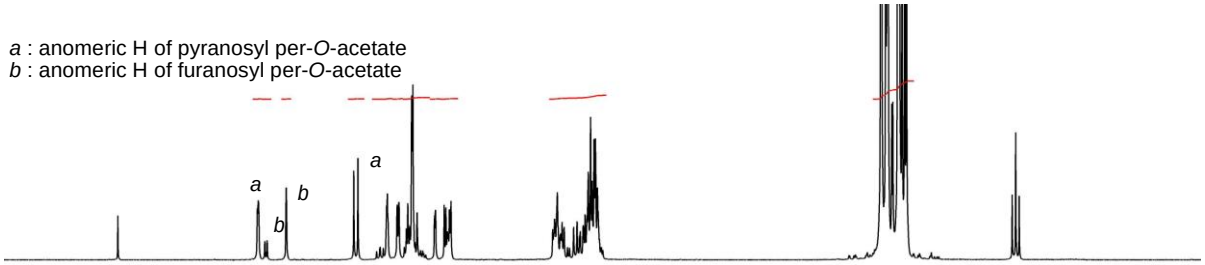
1H


7.28 5.77 5.75 5.50 5.47 5.45 5.42 5.41 5.39 5.38 5.35 5.34 5.30 5.28 5.26 5.18 5.17 5.15 5.13 5.12 5.10 5.01 5.00 4.98 4.96 4.83 4.82 4.80 4.79 4.43 4.41 4.40 4.38 4.37 4.35 4.34 4.32 4.31 4.30 4.29 4.27 4.26 4.22 4.22 4.21 4.18 4.14 4.14 4.13 4.12 4.09 4.07 4.05 4.03 4.01 4.00 3.99 3.98 1.92 1.27 1.24 1.22
OAc
AcO
AcO
O
AcO
7
AcO
O
O
OAc
OAc
AcO
OAc
O
OAc
OAc
9 8 7 6 5 4 3 2 1 ppm
1.00 1.12 2.07 1.90 2.02 1.02 1.00 12.63 34.36 0.71
171.18 170.88 170.86 170.32 170.25 170.19 170.16 170.04 169.99 169.78
102.44 98.04 89.03 82.09 82.00 77.46 77.04 74.25 71.09 70.68 70.36 69.90 68.65 68.57 68.30 68.12 64.22 63.52 62.29 61.64
21.12 21.05 21.02 20.96 20.92 20.88
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

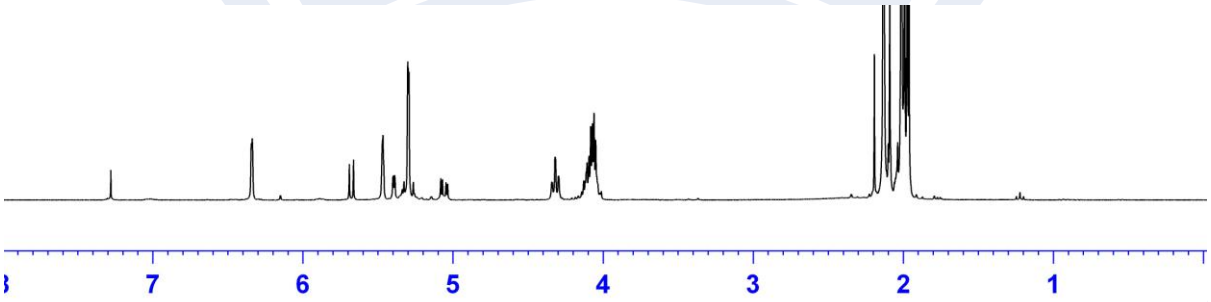

AcO
OAc
O
AcO
OAc
OAc
8
ppm
ppm


OAc
OAc
AcO
OAc
9
ppm
ppm

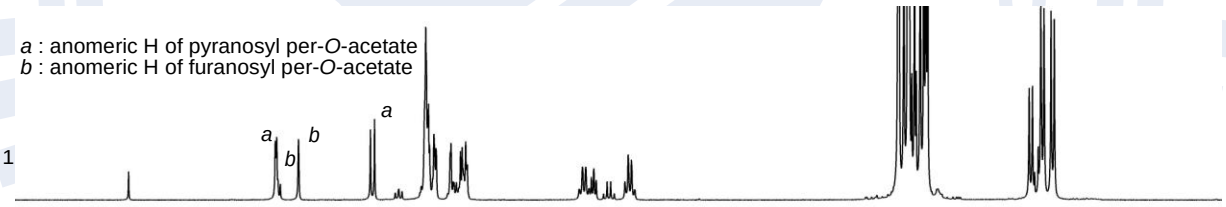
^{1}H NMR of galactosyl peracetate obtained with 1 mol% TsOH/OH and at 25 ^{o}C (ref. 3)



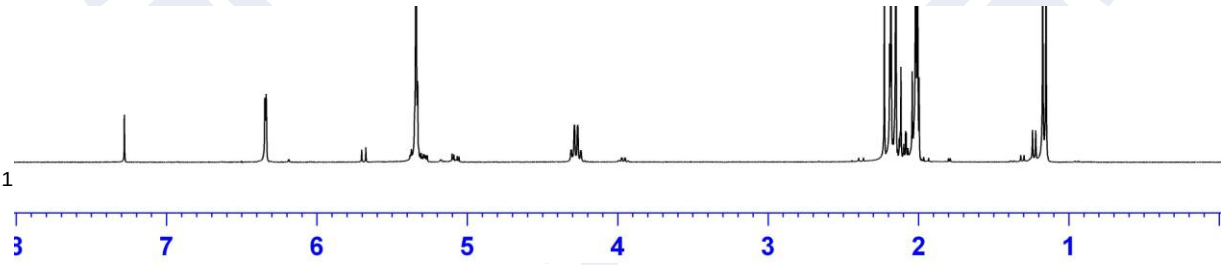
^{1}H NMR of galactosyl peracetate obtained with 3 mol% H_2SO_4/OH and at –20 ^{o}C to 0^{o}C



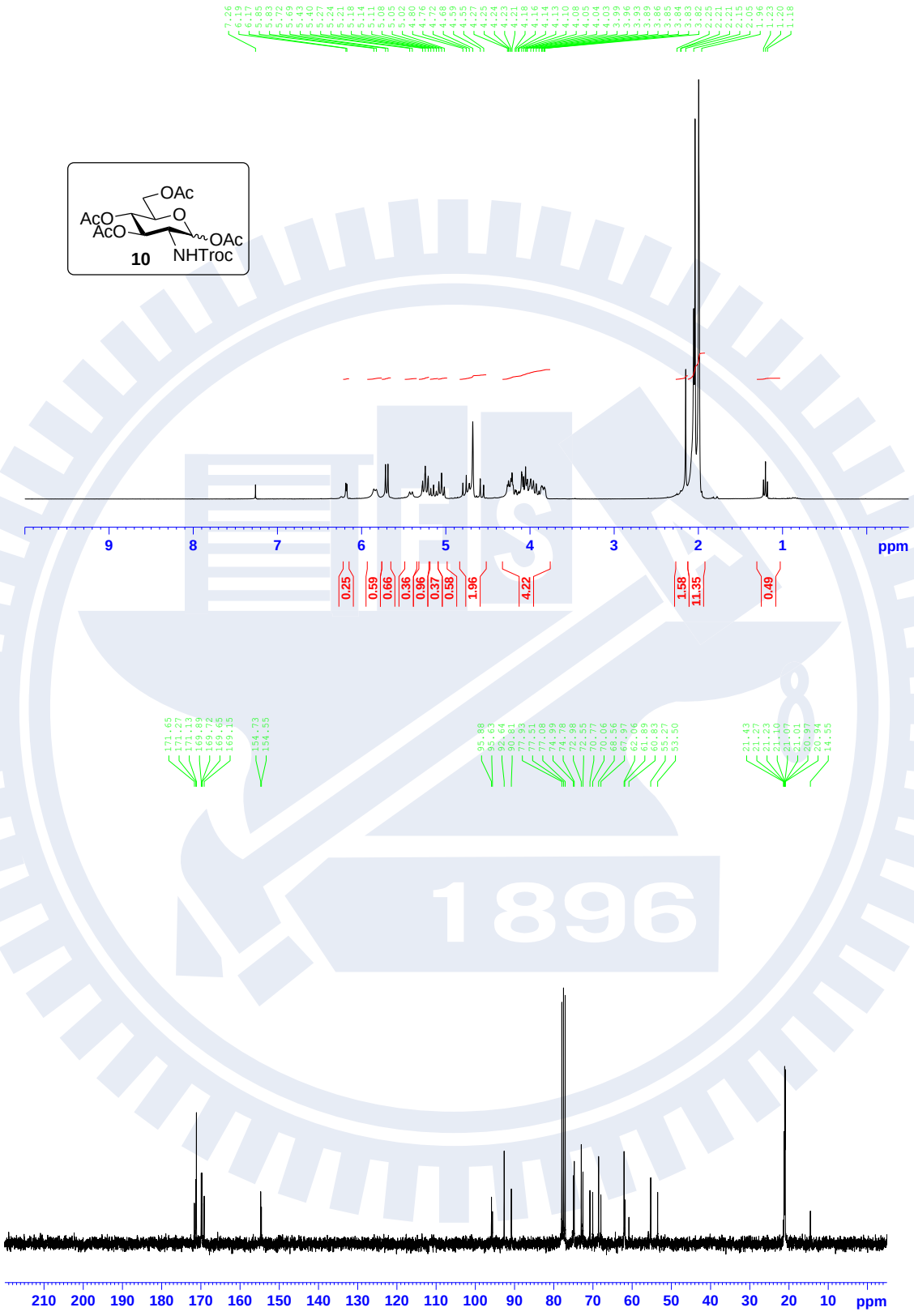
^{1}H NMR of fucosyl peracetate obtained with 1 mol% TsOH/OH and at 25 ^{o}C (ref. 4)

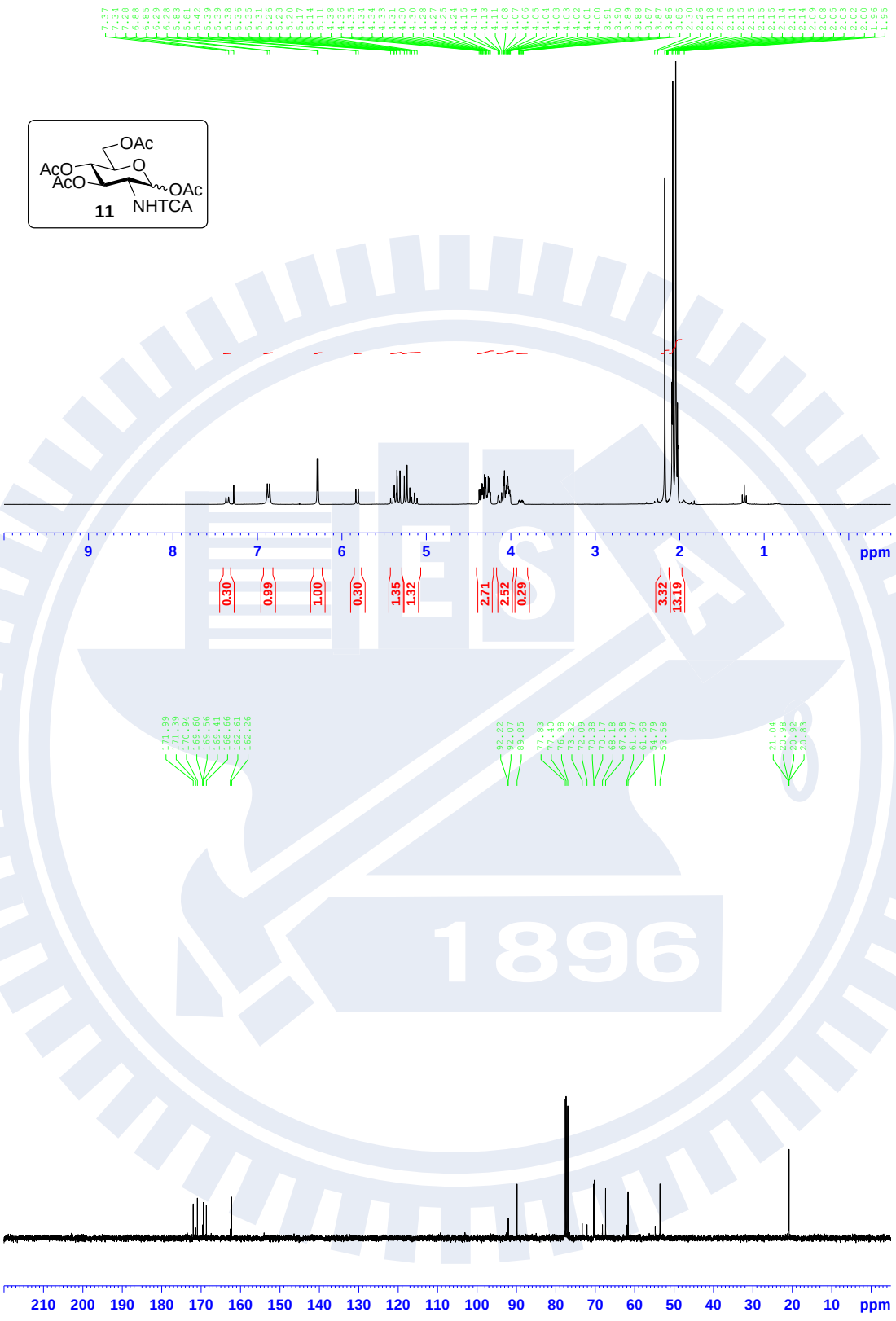


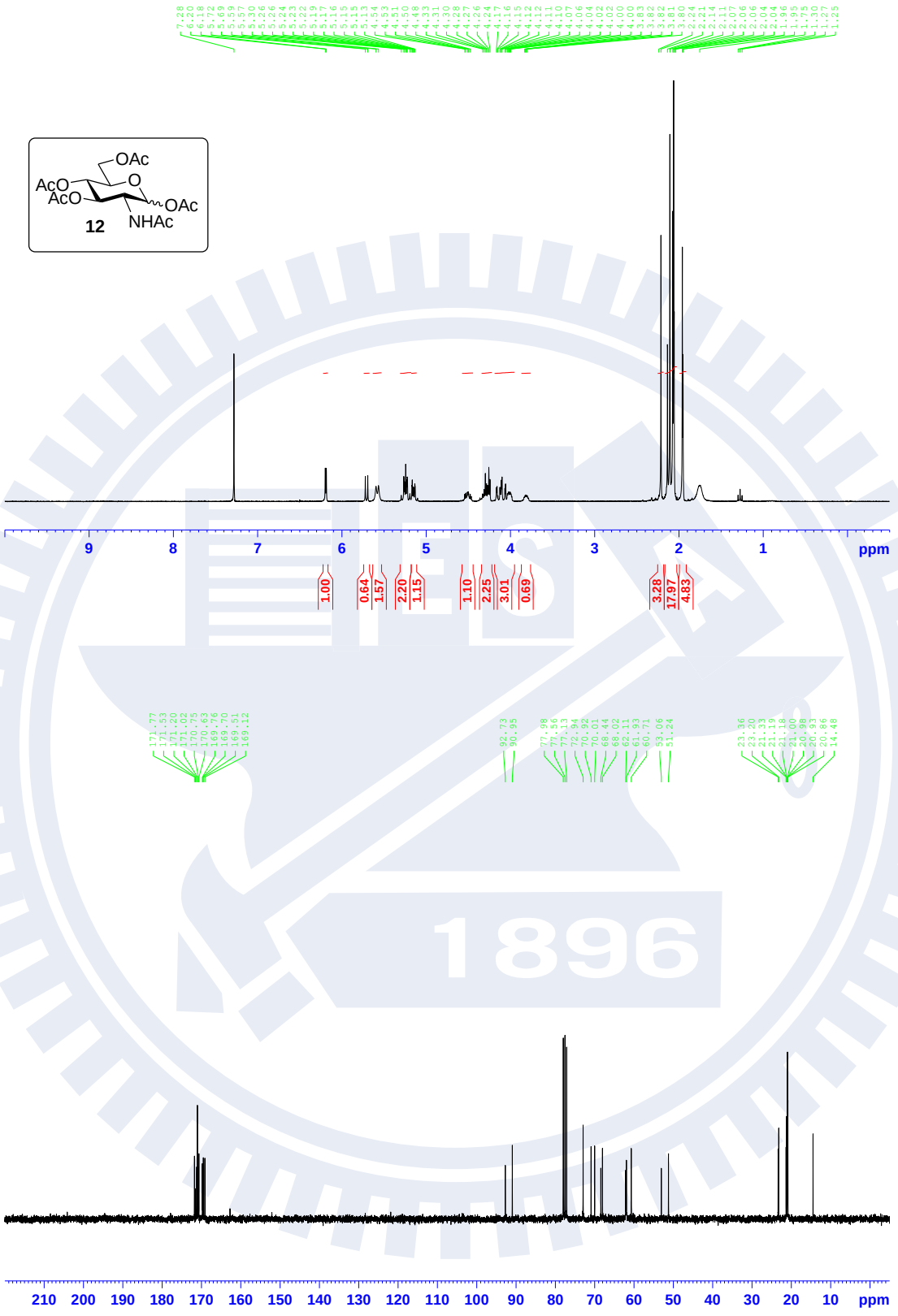
^{1}H NMR of fucosyl peracetate obtained with 2 mol% H_2SO_4/OH and at –30^{o}C to –20 ^{o}C



1H


OAc
AcO
AcO
O
OAc
NHTroc
10
9
8
7
6
5
4
3
2
1
ppm
0.25
0.59
0.66
0.36
0.96
0.37
0.58
1.96
4.22
1.58
11.35
0.49
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


OAc
AcO
O
AcO
OAc
NHTCA
11
9
8
7
6
5
4
3
2
1
ppm
0.30
0.99
1.00
0.30
1.35
1.32
2.71
2.52
0.29
3.32
13.19
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


OAc
AcO
AcO
O
OAc
NHAc
12
ppm
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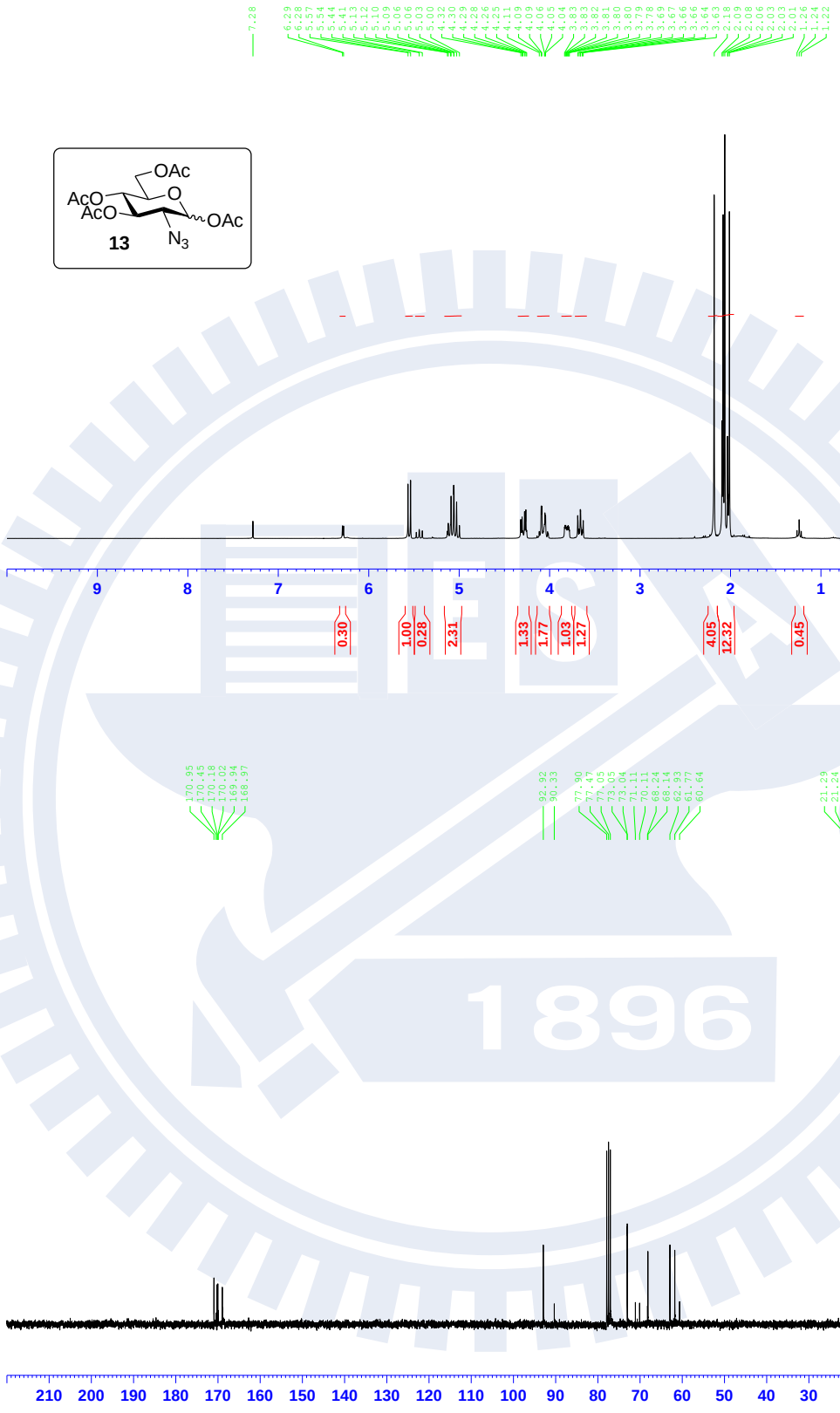
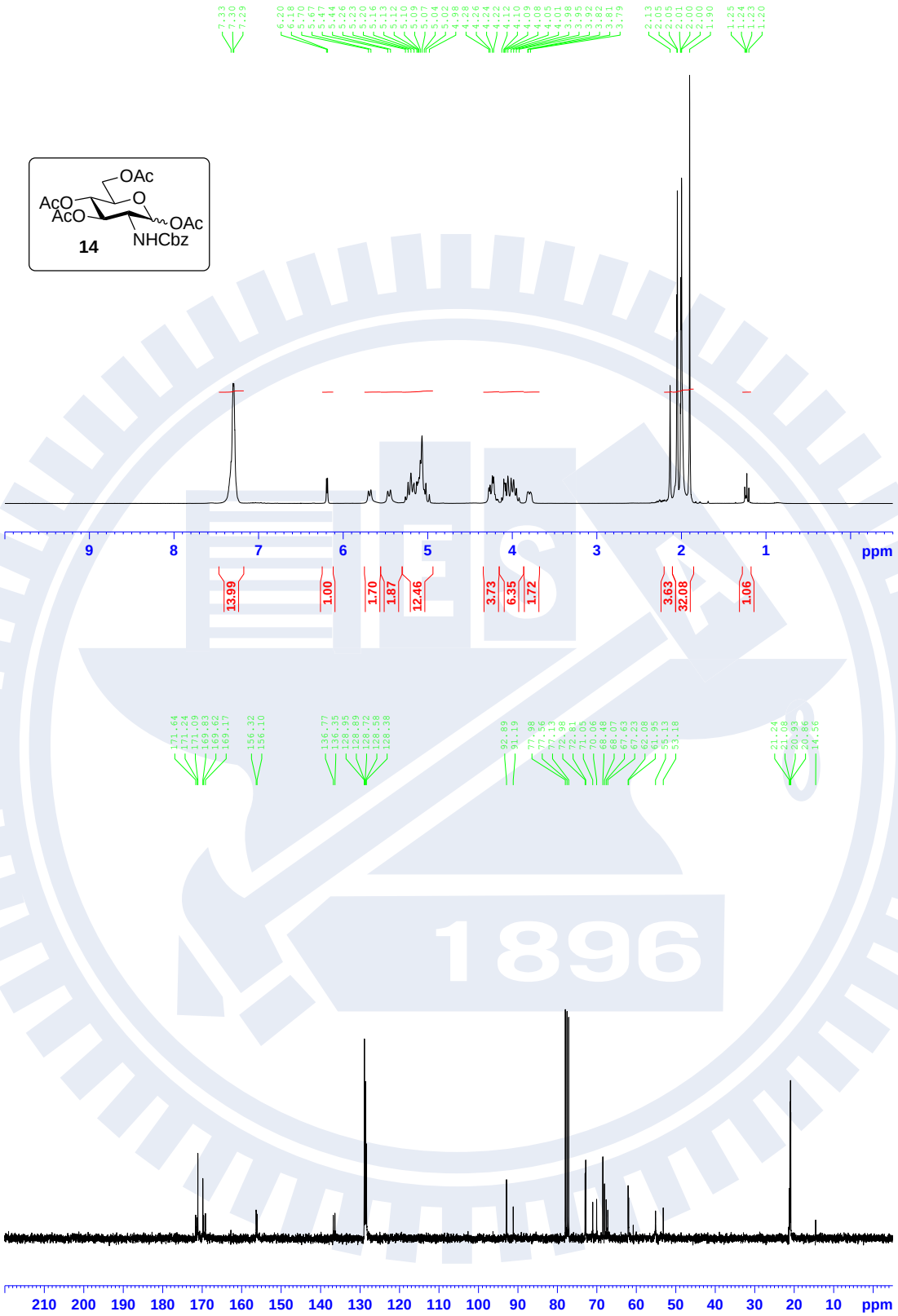
0.64
1.57
2.20
1.15
1.10
2.25
3.01
0.69
3.28
17.97
4.83


OAc
AcO
O
AcO
OAc
13
N3
ppm
0.30
1.00
0.28
2.31
1.33
1.77
1.03
1.27
4.05
12.32
0.45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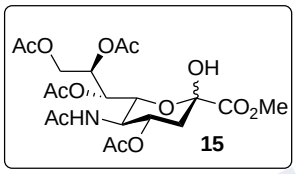
OAc
NHCbz
14
ppm
ppm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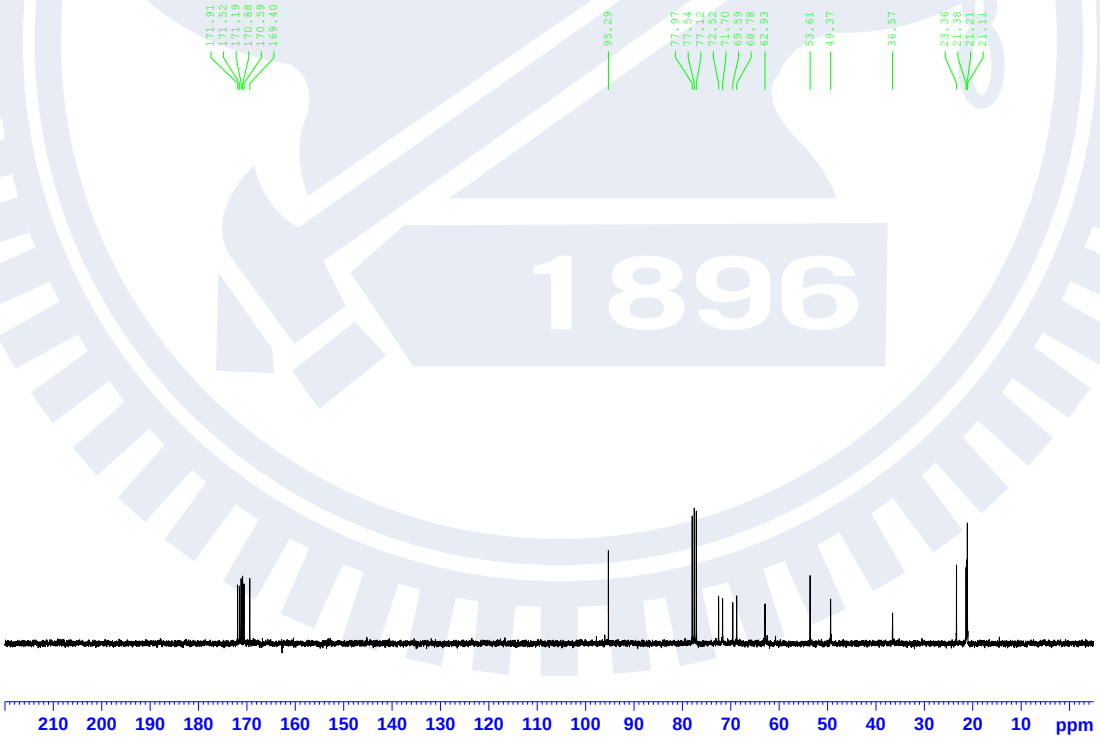
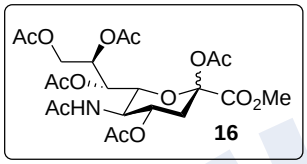
OAc
OH
AcO
AcHN
O
CO2Me
AcO
15


9
8
7
6
5
4
3
2
1
ppm
1.00
1.16
3.55
1.04
1.05
2.79
3.83
1.88
4.47
3.76
8.63
4.06

210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm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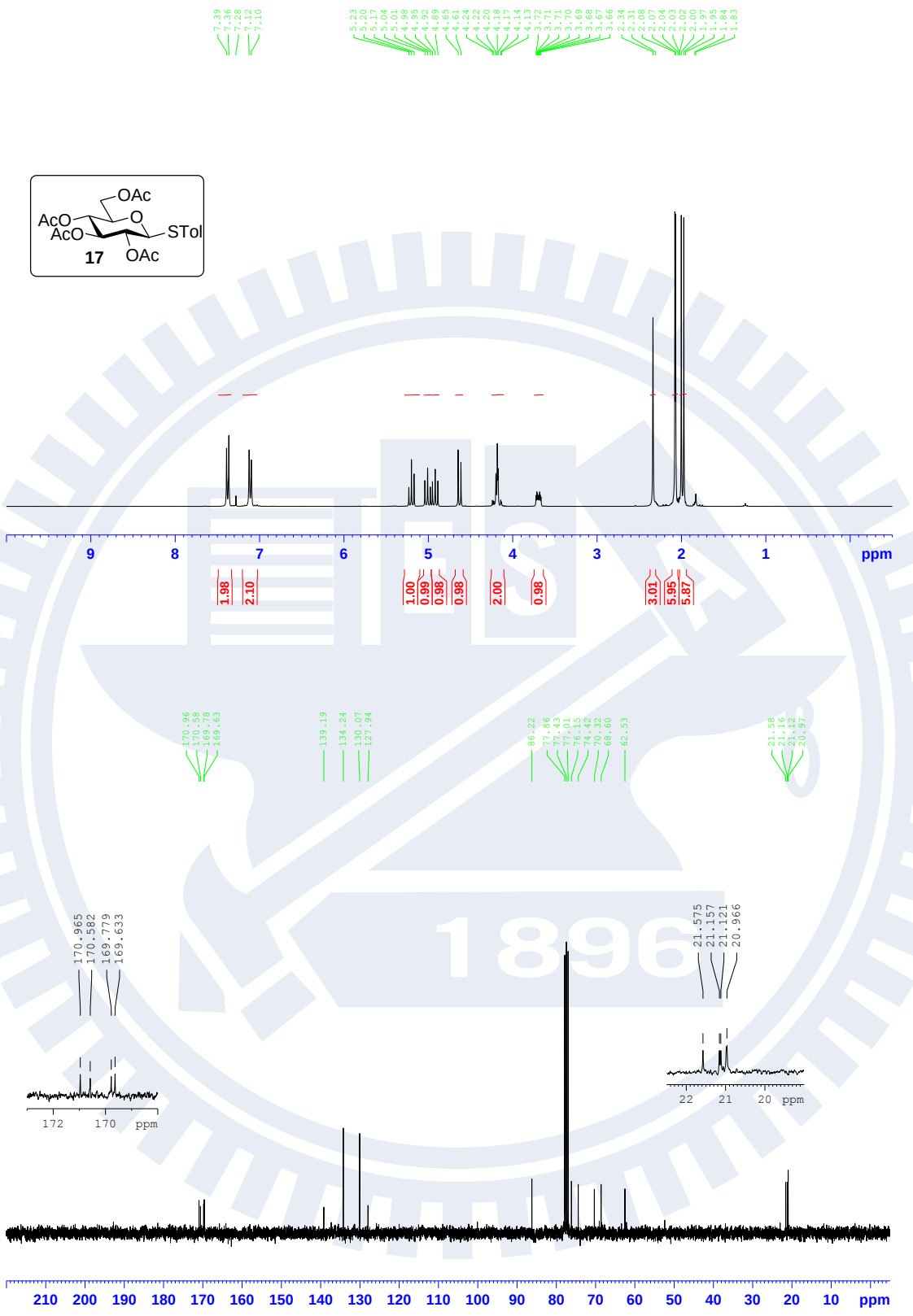
OAc
OAc
AcO
AcHN
O
CO2Me
AcO
16

ppm
ppm


OAc
AcO
AcO
O
STol
17
OAc
ppm
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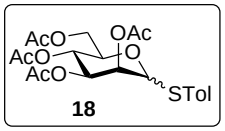
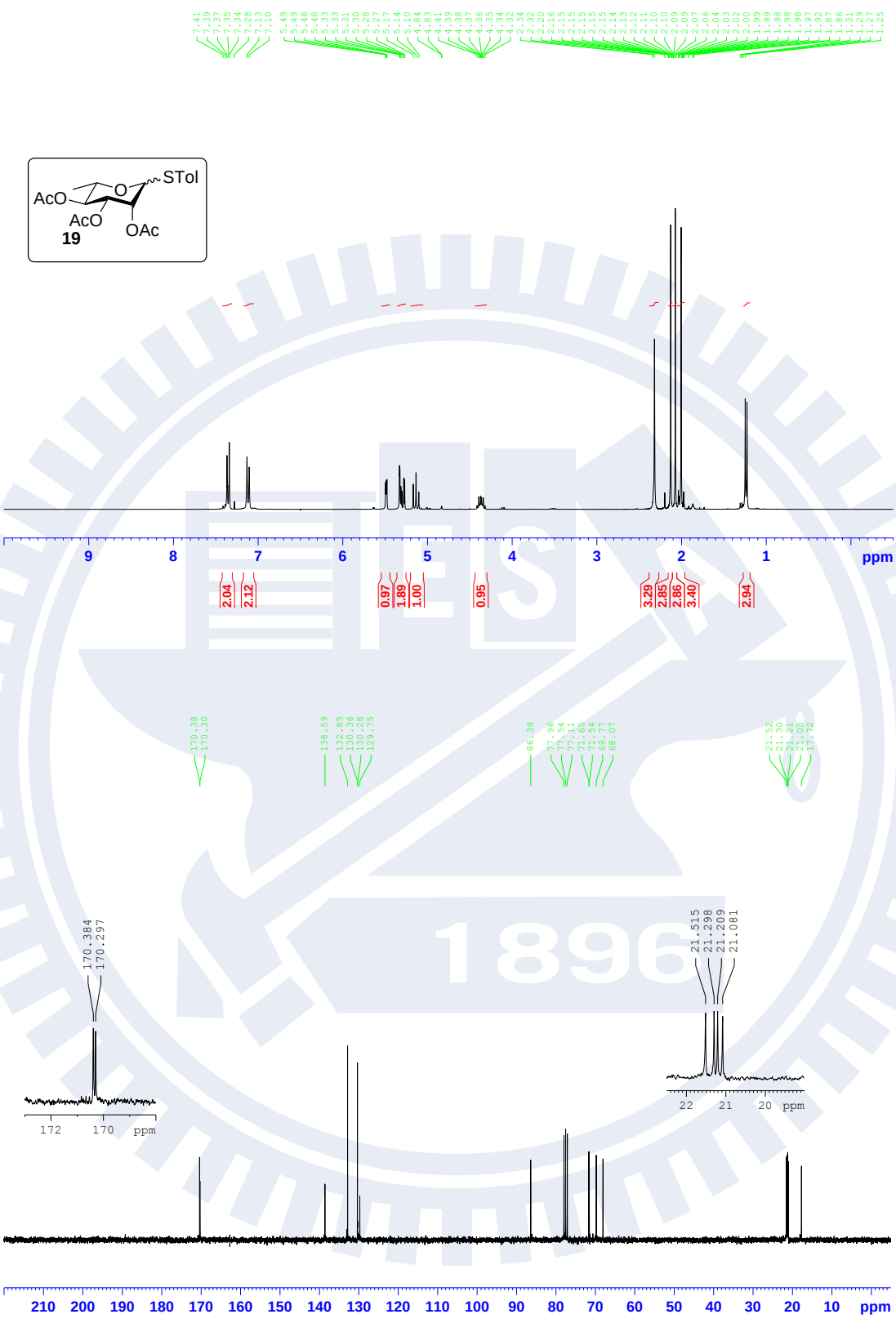
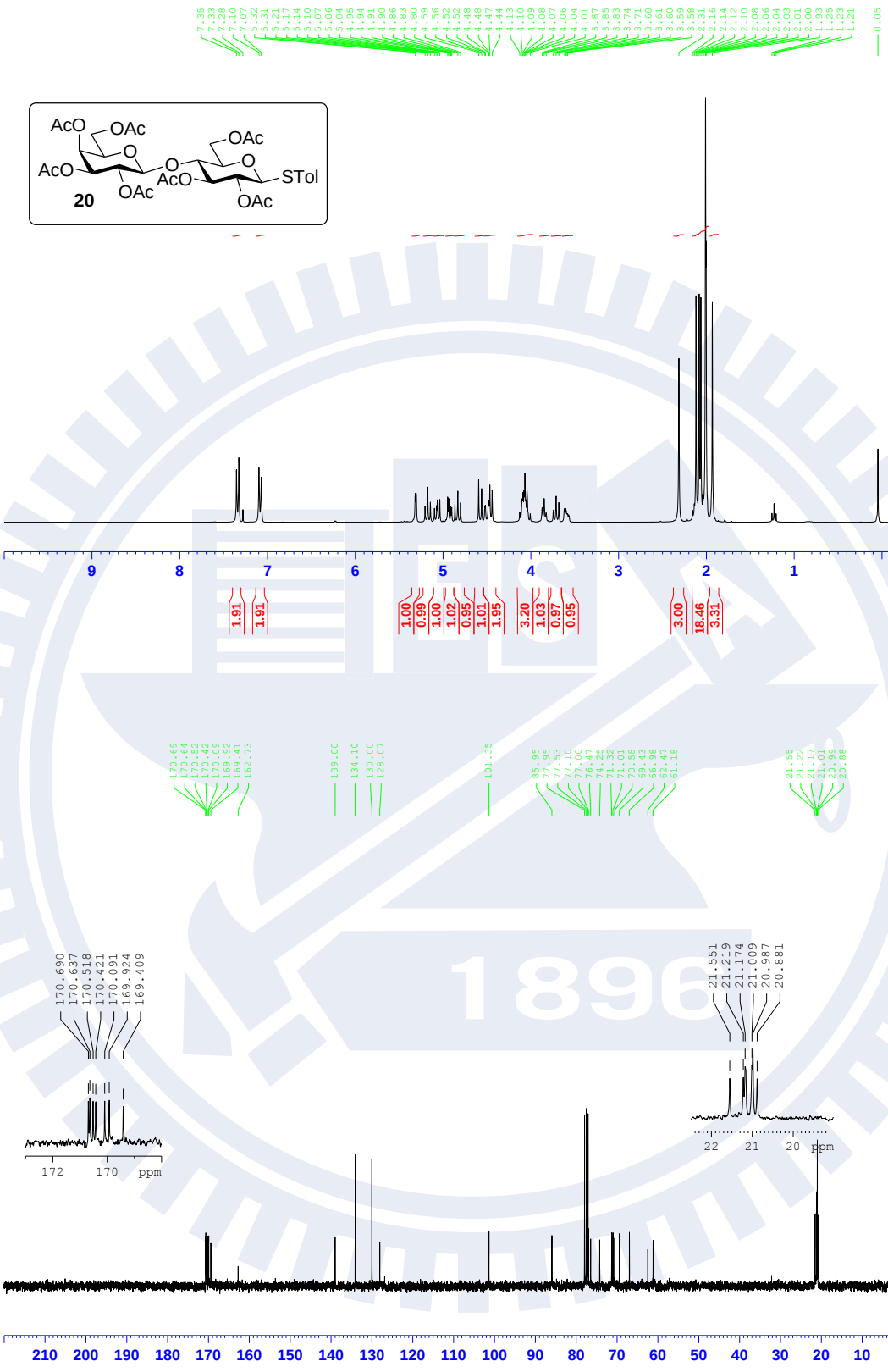
170.582
169.779
169.633
21.575
21.157
21.121
20.966


AcO
OAc
AcO
O
AcO
STol
18

ppm
2.16
2.20
1.00
0.99
1.92
1.00
1.10
1.05
3.30
2.97
9.90
ppm
ppm
ppm


AcO
AcO
O
STol
OAc
19
ppm
2.04
2.12
0.97
1.89
1.00
0.95
3.29
2.85
2.86
3.40
2.94
170.384
170.297
21.515
21.298
21.209
21.081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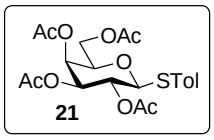
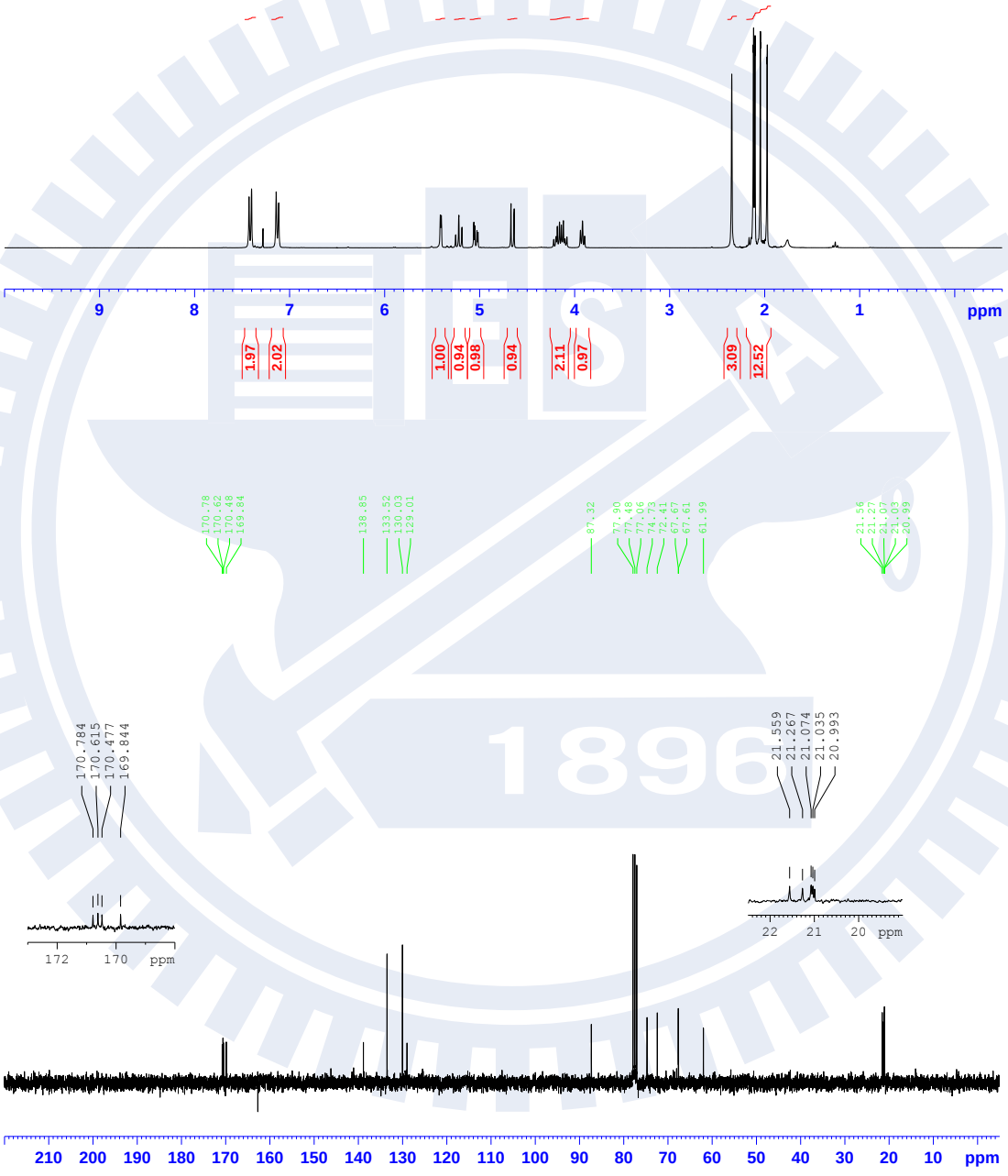
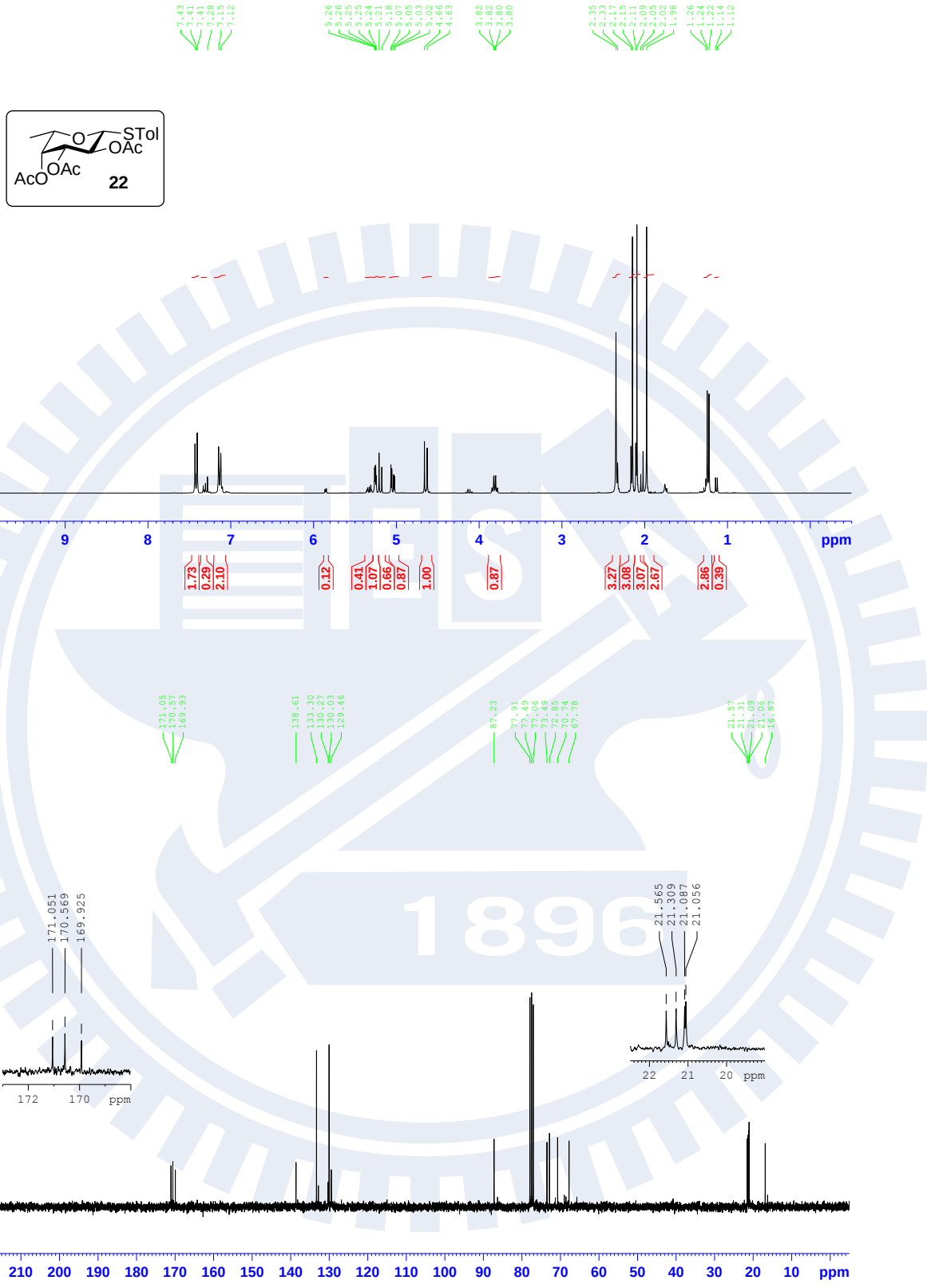
O
AcO
O
AcO
OAc
OAc
O
STol
OAc
20
ppm
ppm
ppm
ppm


AcO
OAc
O
AcO
STol
OAc
21


ppm
1.97
2.02
1.00
0.94
0.98
0.94
2.11
0.97
3.09
12.52
170.784
170.615
170.477
169.844
172
170
ppm
21.559
21.267
21.074
21.035
20.993
22
21
20
ppm
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


7.43
7.41
7.41
7.28
7.15
7.12
5.26
5.26
5.25
5.25
5.24
5.21
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5.07
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5.02
4.66
4.63
3.82
3.82
3.80
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2.35
2.33
2.17
2.15
2.11
2.09
2.05
2.02
1.98
1.26
1.24
1.22
1.14
1.12
STol
OAc
AcO
OAc
22
9
8
7
6
5
4
3
2
1
ppm
1.73
0.29
2.10
0.12
0.41
1.07
0.66
0.87
1.00
0.87
3.27
3.08
3.07
2.67
2.86
0.39
171.05
170.57
169.93
138.61
133.30
130.27
130.03
129.46
87.23
77.91
77.49
77.06
73.49
72.85
70.74
67.78
21.57
21.31
21.09
21.06
16.97
171.051
170.569
169.925
172
170
ppm
21.565
21.309
21.087
21.056
22
21
20
ppm
210
200
190
180
170
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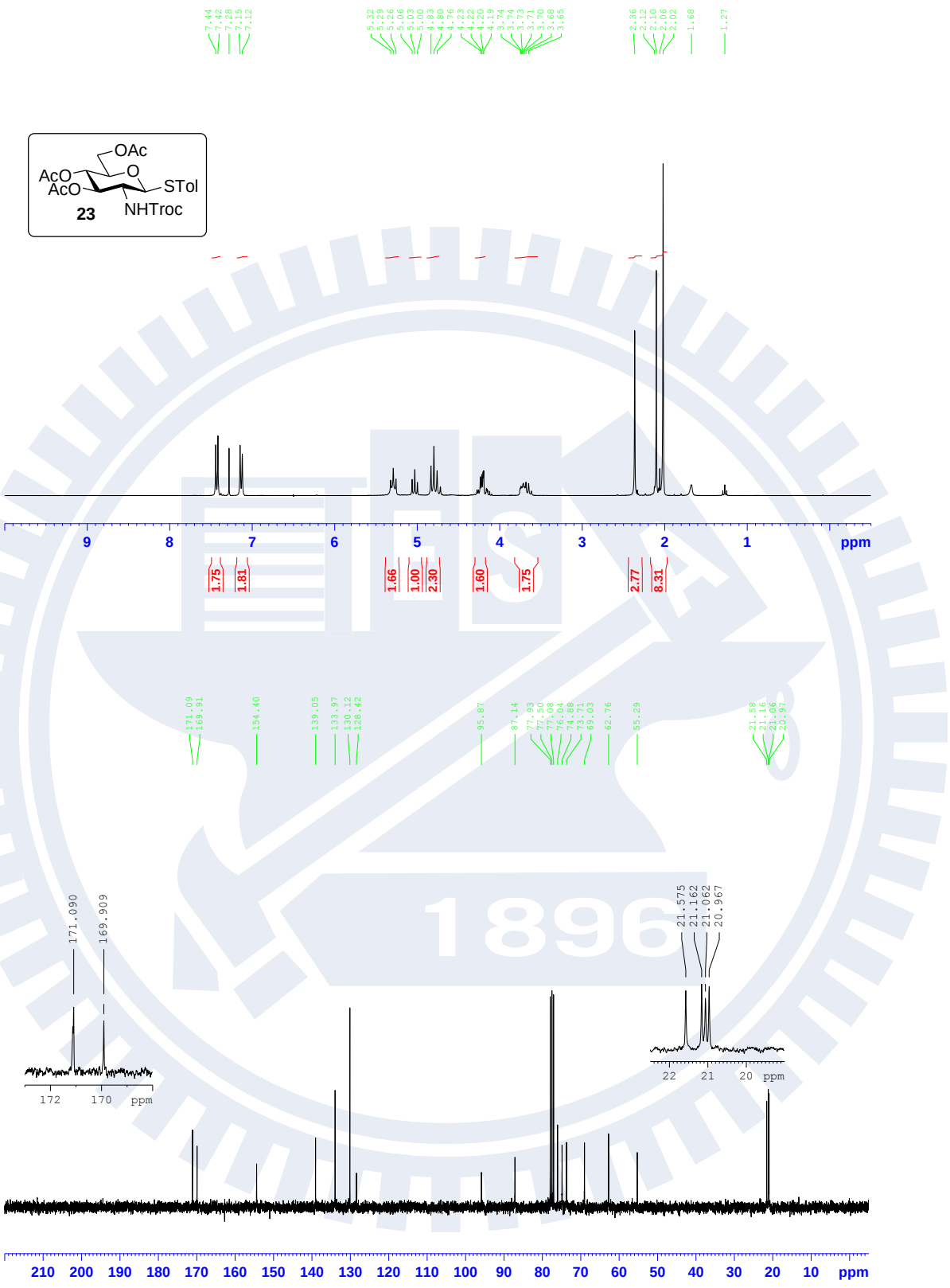
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm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7.42
7.28
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7.12
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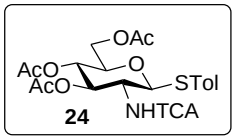
5.29
5.26
5.06
5.03
5.00
4.83
4.80
4.76
4.23
4.22
4.20
4.19
3.74
3.74
3.73
3.71
3.70
3.68
3.65
2.36
2.12
2.10
2.06
2.02
1.68
1.27
OAc
AcO
O
AcO
STol
NHTroc
23
9
8
7
6
5
4
3
2
1
ppm
1.75
1.81
1.66
1.00
2.30
1.60
1.75
2.77
8.31
171.09
169.91
154.40
139.05
133.97
130.12
128.42
95.87
87.14
77.93
77.50
77.08
76.04
74.88
73.71
69.03
62.76
55.29
21.58
21.16
21.06
20.97
171.090
169.909
172
170
ppm
21.575
21.162
21.062
20.967
22
21
20
ppm
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


OAc
AcO
AcO
O
STol
NHTCA
24

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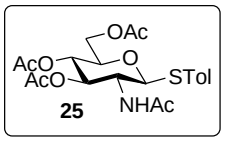
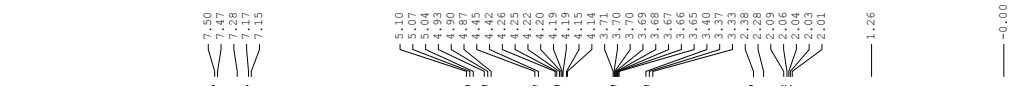
170.992
169.531
21.576
21.121
21.015
20.587


OAc
AcO
AcO
O
STol
NHAc
25

ppm
ppm
ppm
ppm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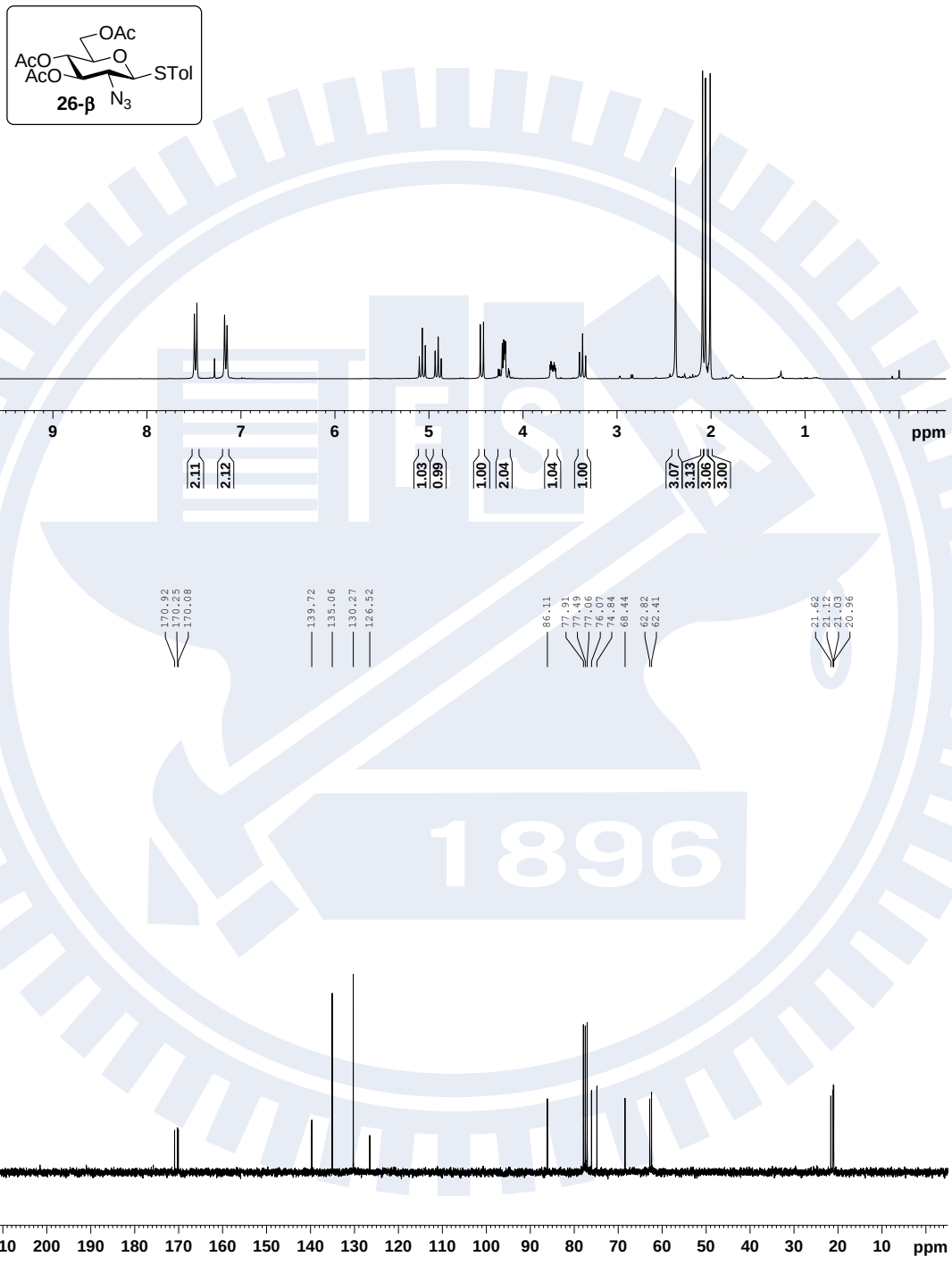
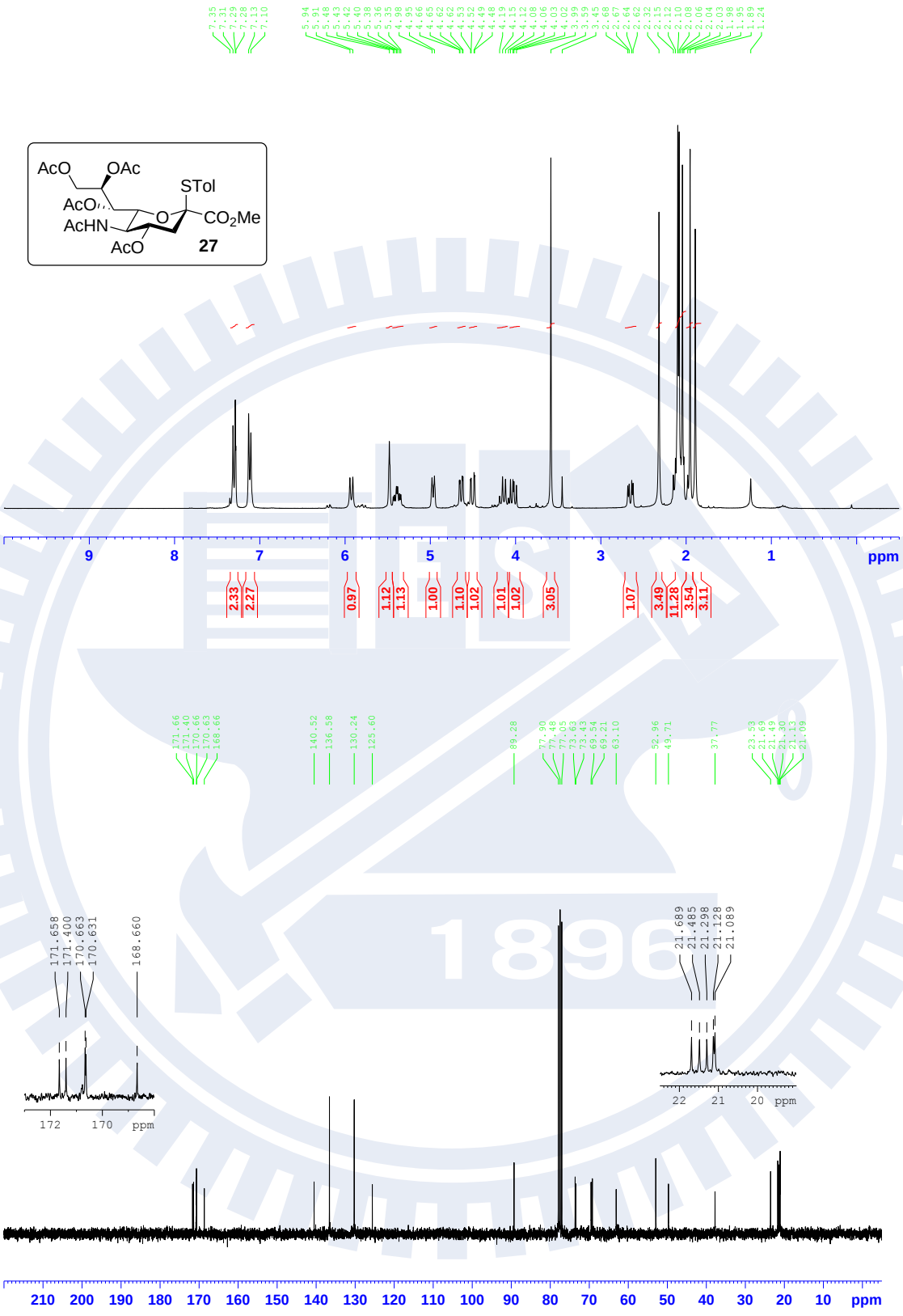
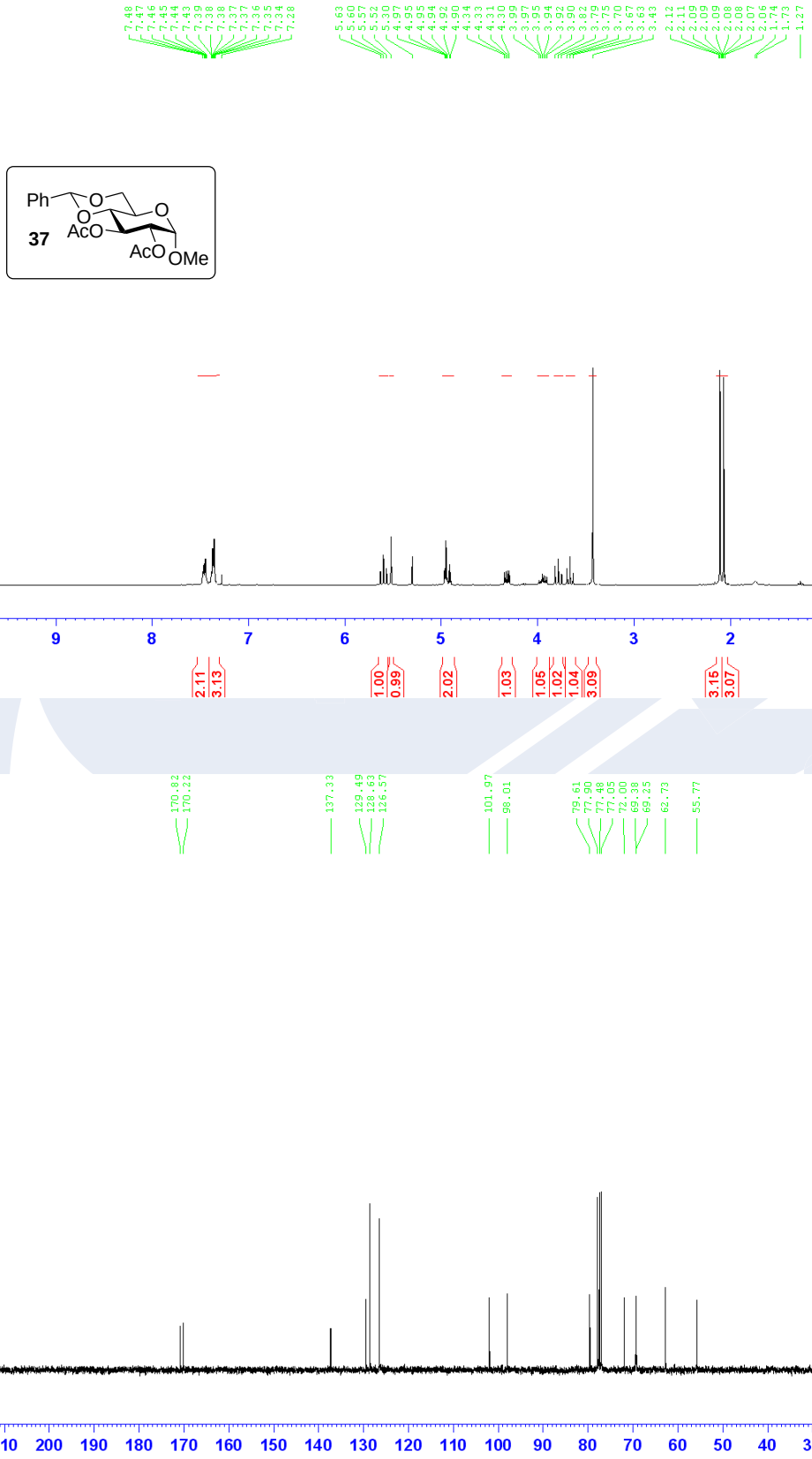
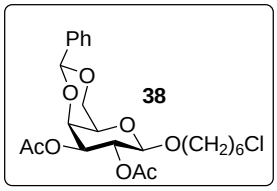
7.47
7.28
7.17
7.15
5.10
5.07
5.04
4.93
4.90
4.87
4.45
4.42
4.26
4.25
4.22
4.20
4.19
4.19
4.15
4.14
3.71
3.70
3.70
3.69
3.68
3.67
3.66
3.65
3.40
3.37
3.33
3.33
2.28
2.09
2.06
2.04
2.03
2.01
1.26
-0.00

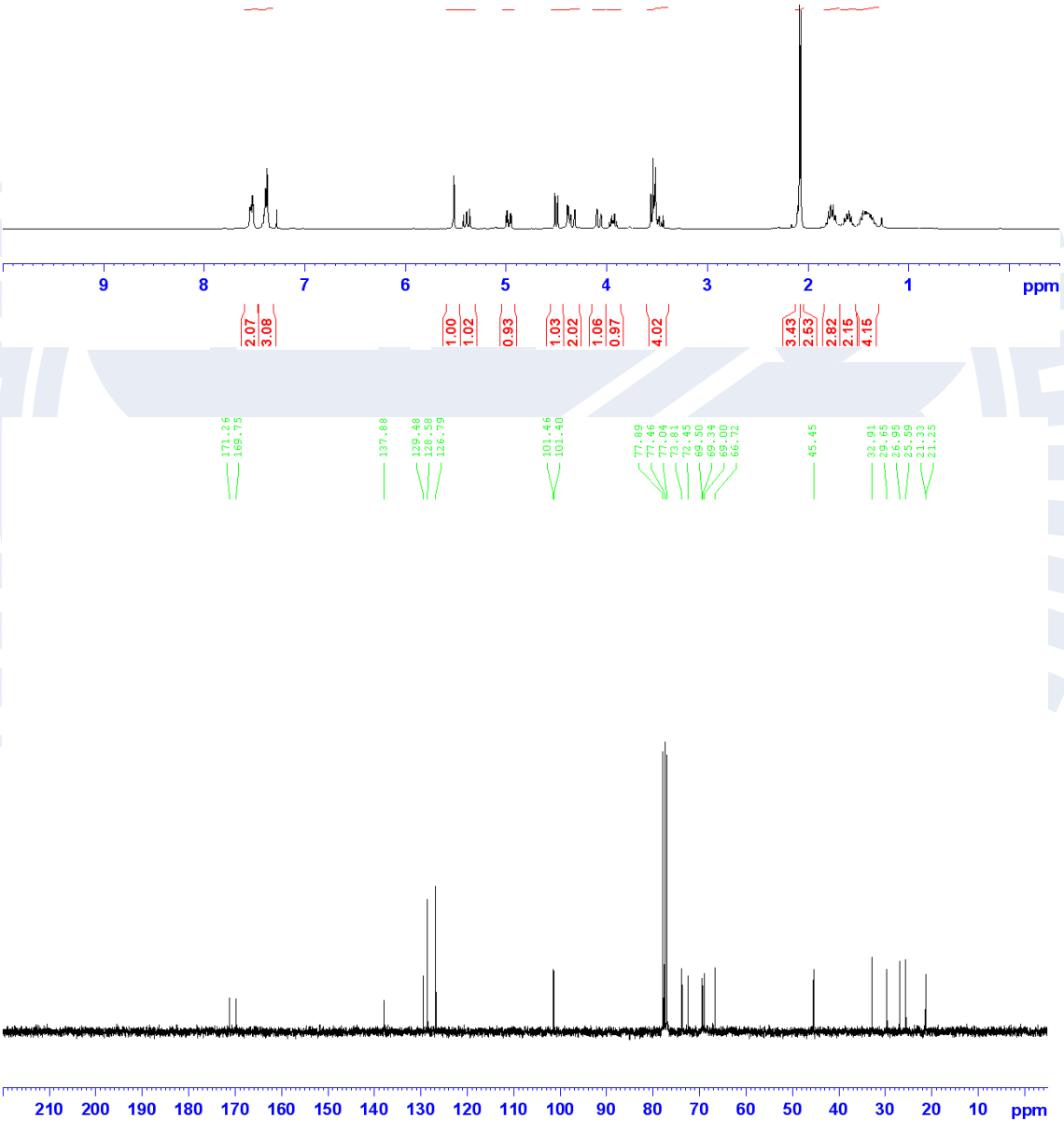

OAc
AcO
AcO
O
STol
26-β
N3
9
8
7
6
5
4
3
2
1
ppm
2.11
2.12
1.03
0.99
1.00
2.04
1.04
1.00
3.07
3.13
3.06
3.00

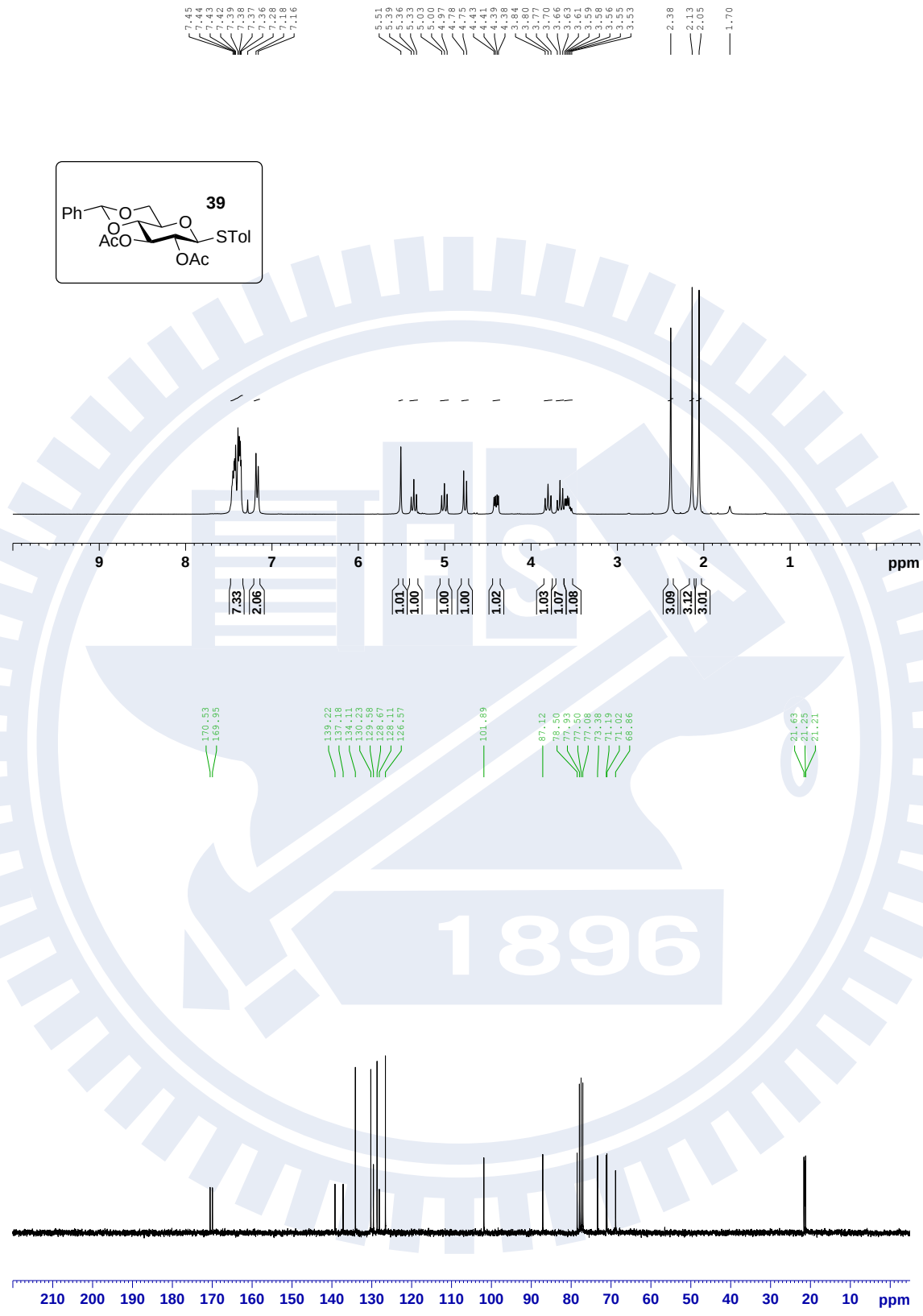
170.92
170.25
170.08
139.72
135.06
130.27
126.52
86.11
77.91
77.49
77.06
76.07
74.84
68.44
62.82
62.41
21.62
21.12
21.03
20.96
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

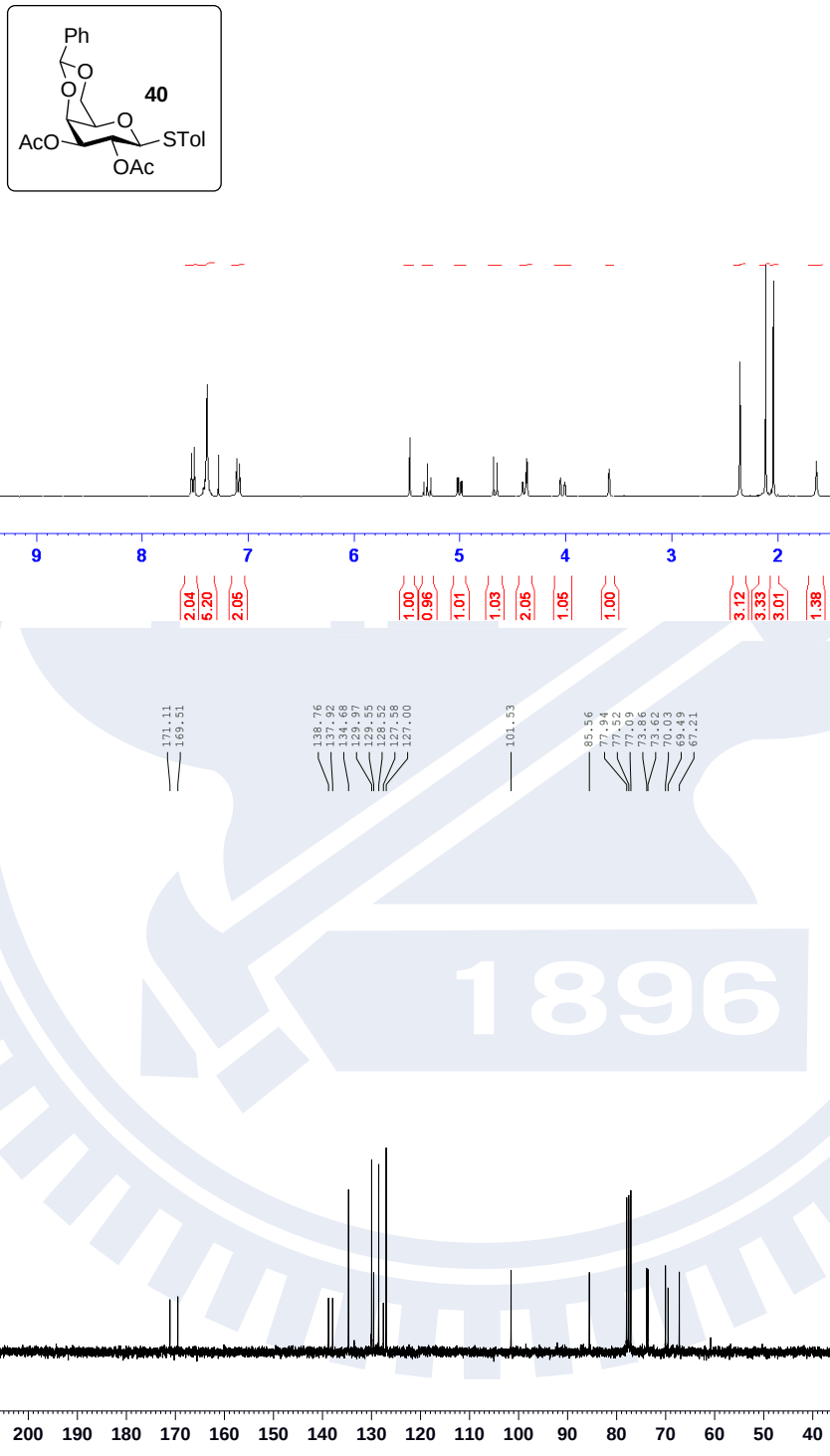

AcO
OAc
STol
AcO
AcHN
O
CO2Me
AcO
27
9
8
7
6
5
4
3
2
1
ppm
2.33
2.27
0.97
1.12
1.13
1.00
1.10
1.02
1.01
1.02
3.05
1.07
3.49
11.28
3.54
3.11
171.658
171.400
170.663
170.631
168.660
172
170
ppm
21.689
21.485
21.298
21.128
21.089
22
21
20
ppm
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


Ph
O
O
O
AcO
AcO
OMe
37
ppm
ppm


Ph
O
O
38
O
AcO
O(CH2)6Cl
OAc


9
8
7
6
5
4
3
2
1
ppm
2.07
3.08
1.00
1.02
0.93
1.03
2.02
1.06
0.97
4.02
3.43
2.53
2.82
2.15
4.16
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


7.45 7.44 7.43 7.42 7.39 7.38 7.37 7.36 7.28 7.18 7.16
5.51 5.39 5.36 5.33 5.03 5.00 4.97 4.78 4.75 4.43 4.41 4.39 4.38 3.84 3.80 3.77 3.70 3.66 3.63 3.61 3.59 3.58 3.56 3.55 3.53
2.38 2.13 2.05 1.70
39
Ph
O
O
O
AcO
STol
OAc
9 8 7 6 5 4 3 2 1 ppm
7.33 2.06 1.01 1.00 1.00 1.00 1.02 1.03 1.07 1.08 3.09 3.12 3.01
170.53 169.95 139.22 137.18 134.11 130.23 129.58 128.67 128.11 126.57 101.89 87.12 78.50 77.93 77.50 77.08 73.38 71.19 71.02 68.86 21.63 21.25 21.21
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm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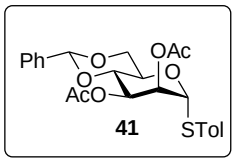
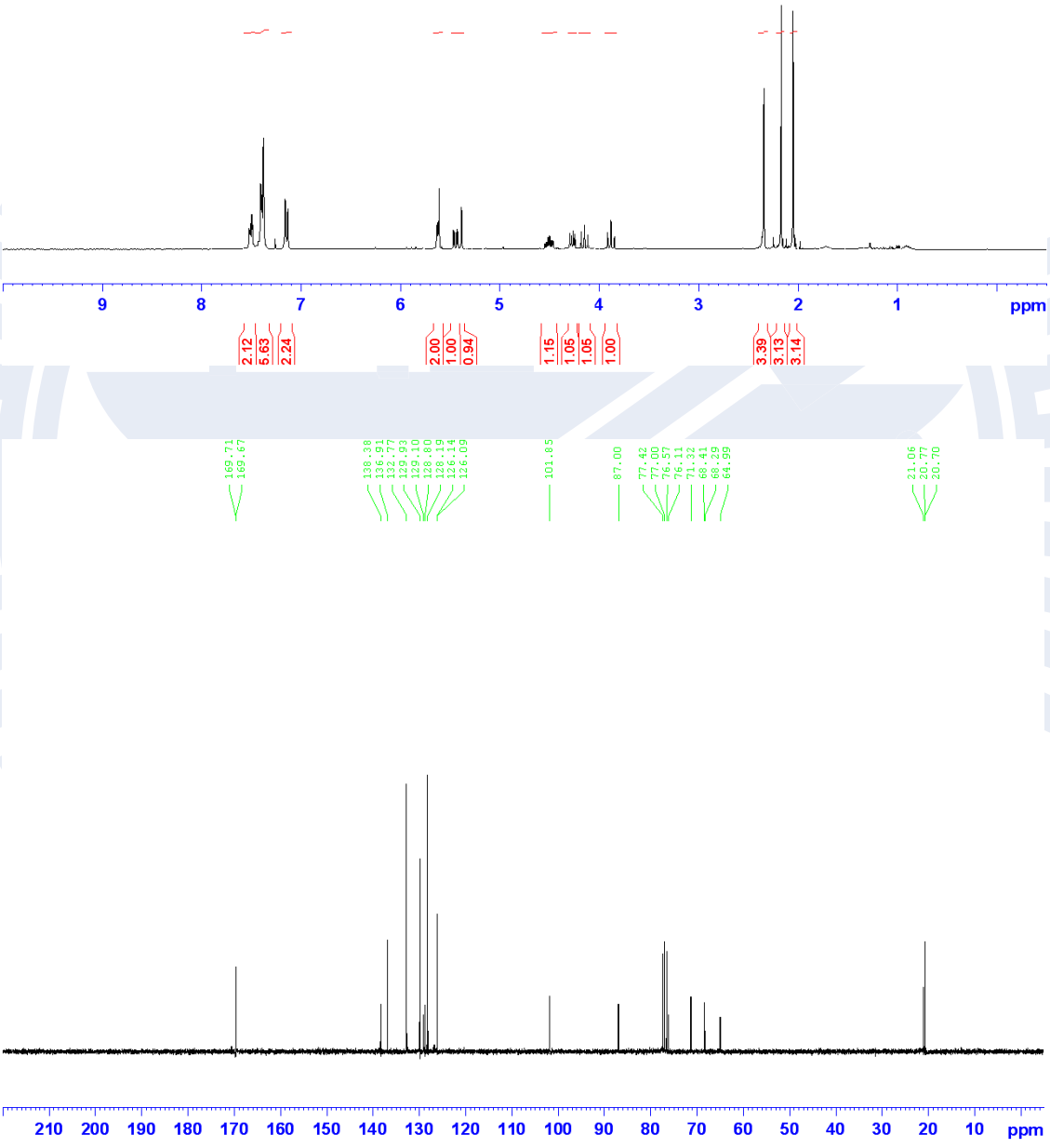
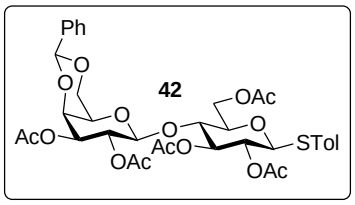
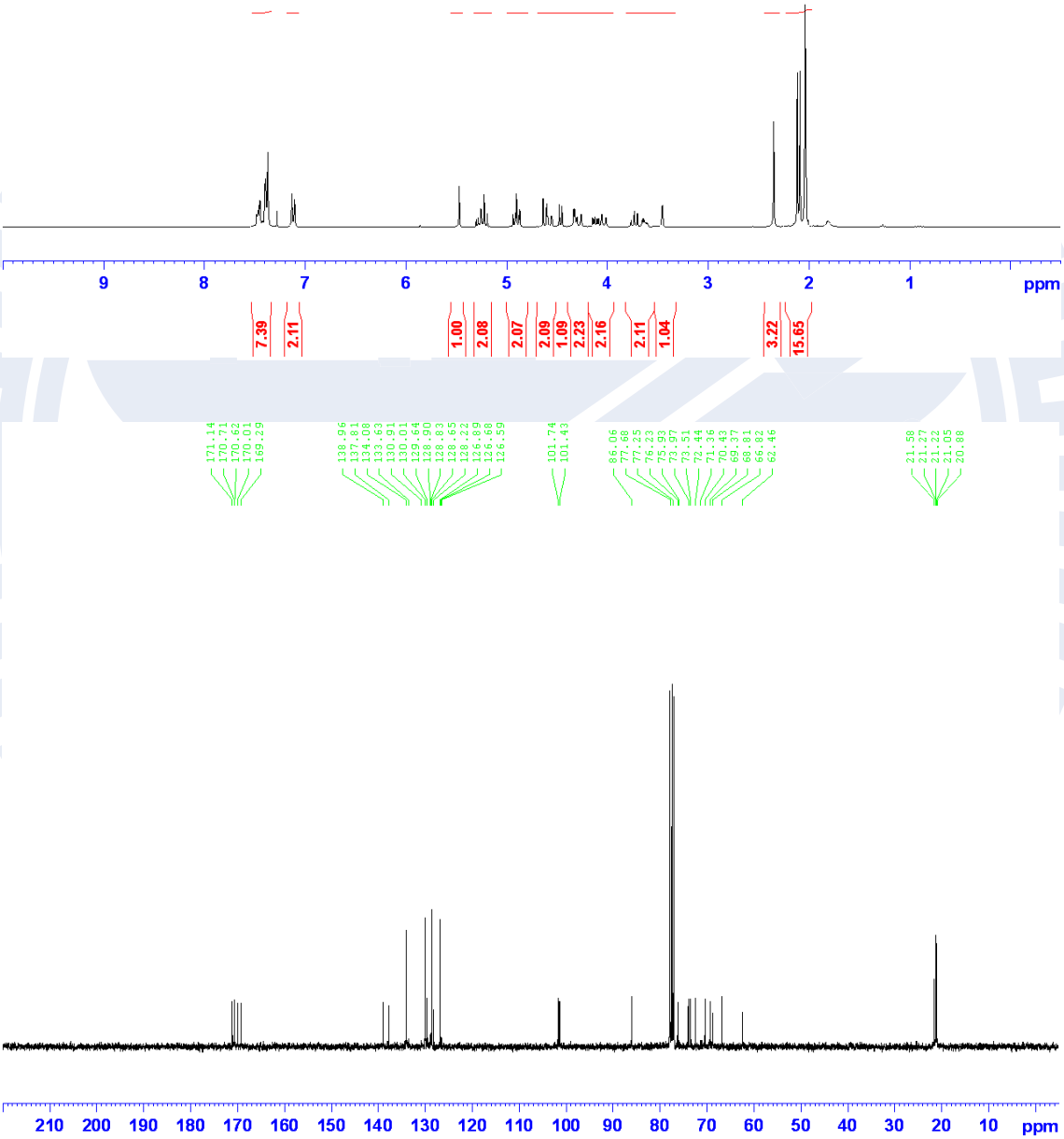
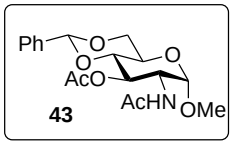
7.51
7.51
7.42
7.41
7.39
7.39
7.38
7.36
7.28
7.11
7.08
5.47
5.34
5.31
5.28
5.03
5.02
4.99
4.98
4.68
4.65
4.41
4.40
4.38
4.37
4.05
4.05
4.01
4.01
3.59
3.59
2.36
2.11
2.07
2.04
1.64
Ph
O
O
O
40
AcO
STol
OAc
9
8
7
6
5
4
3
2
1
ppm
2.04
5.20
2.05
1.00
0.96
1.01
1.03
2.05
1.05
1.00
3.12
3.33
3.01
1.38
171.11
169.51
138.76
137.92
134.68
129.97
129.55
128.52
127.58
127.00
101.53
85.56
77.94
77.52
77.09
73.86
73.62
70.03
69.49
67.21
21.69
21.34
21.31
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

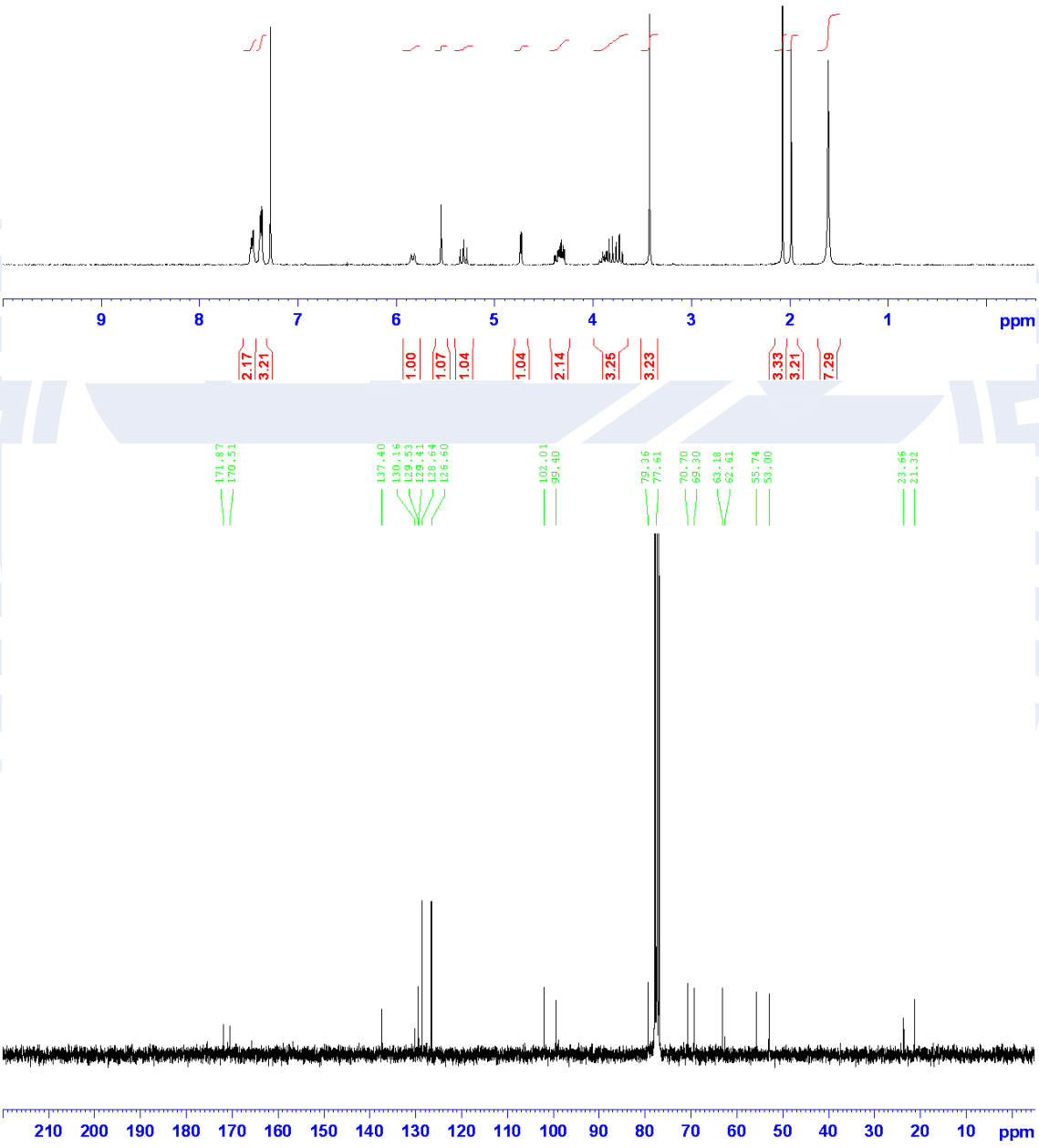

Ph
O
OAc
O
AcO
41
STol


ppm
ppm


Ph
O
O
O
AcO
OAc
O
AcO
OAc
O
OAc
OAc
STol
42


9
8
7
6
5
4
3
2
1
ppm
7.39
2.11
1.00
2.08
2.07
2.09
1.09
2.23
2.16
2.11
1.04
3.22
15.65
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


Ph
O
O
AcO
AcHN
OMe
43


ppm
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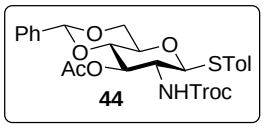
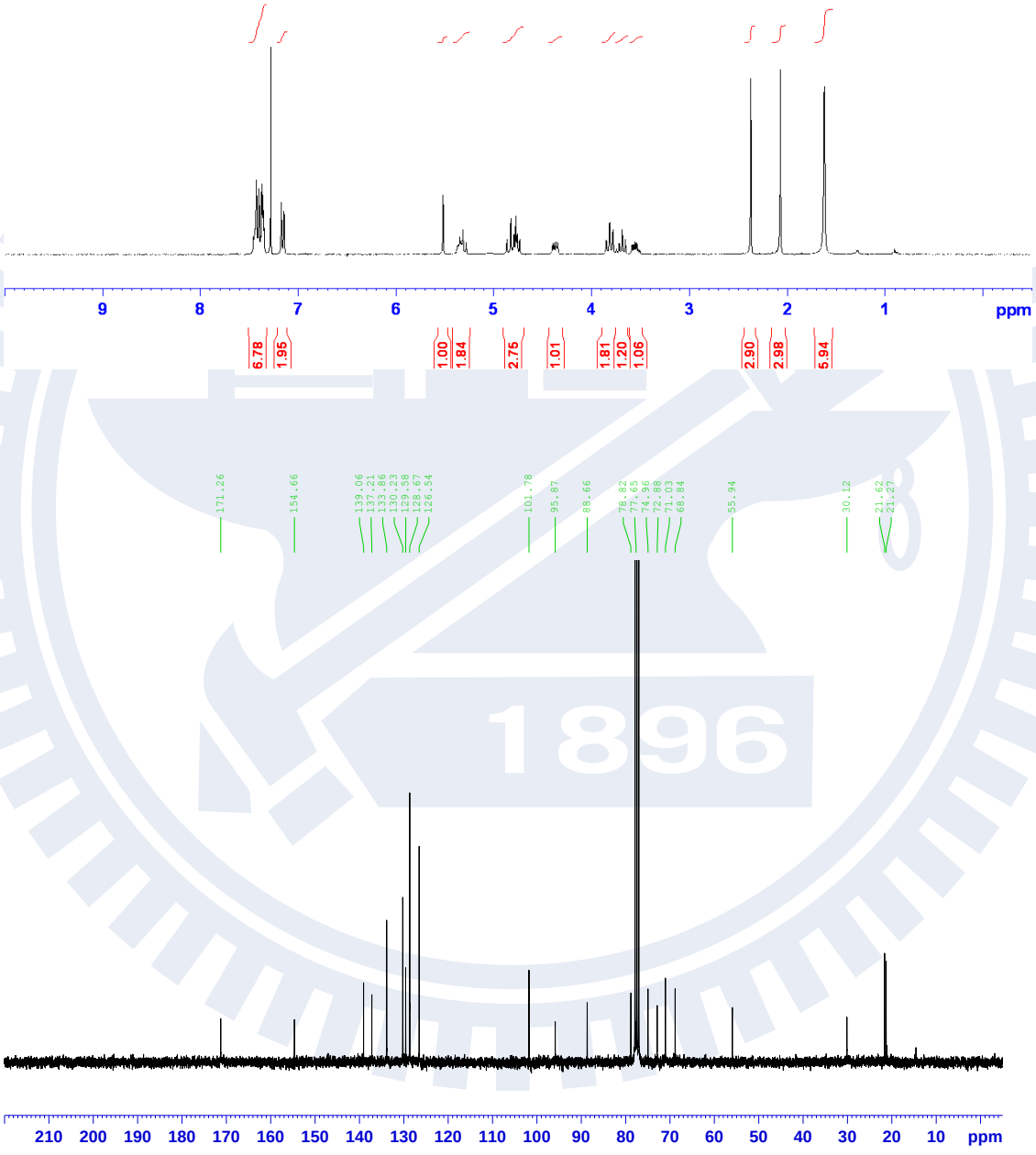
1.00
1.07
1.04
1.04
2.14
3.25
3.23
3.33
3.21
7.29
ppm


Ph
O
O
O
AcO
STol
NHTroc

44


9
8
7
6
5
4
3
2
1
ppm
6.78
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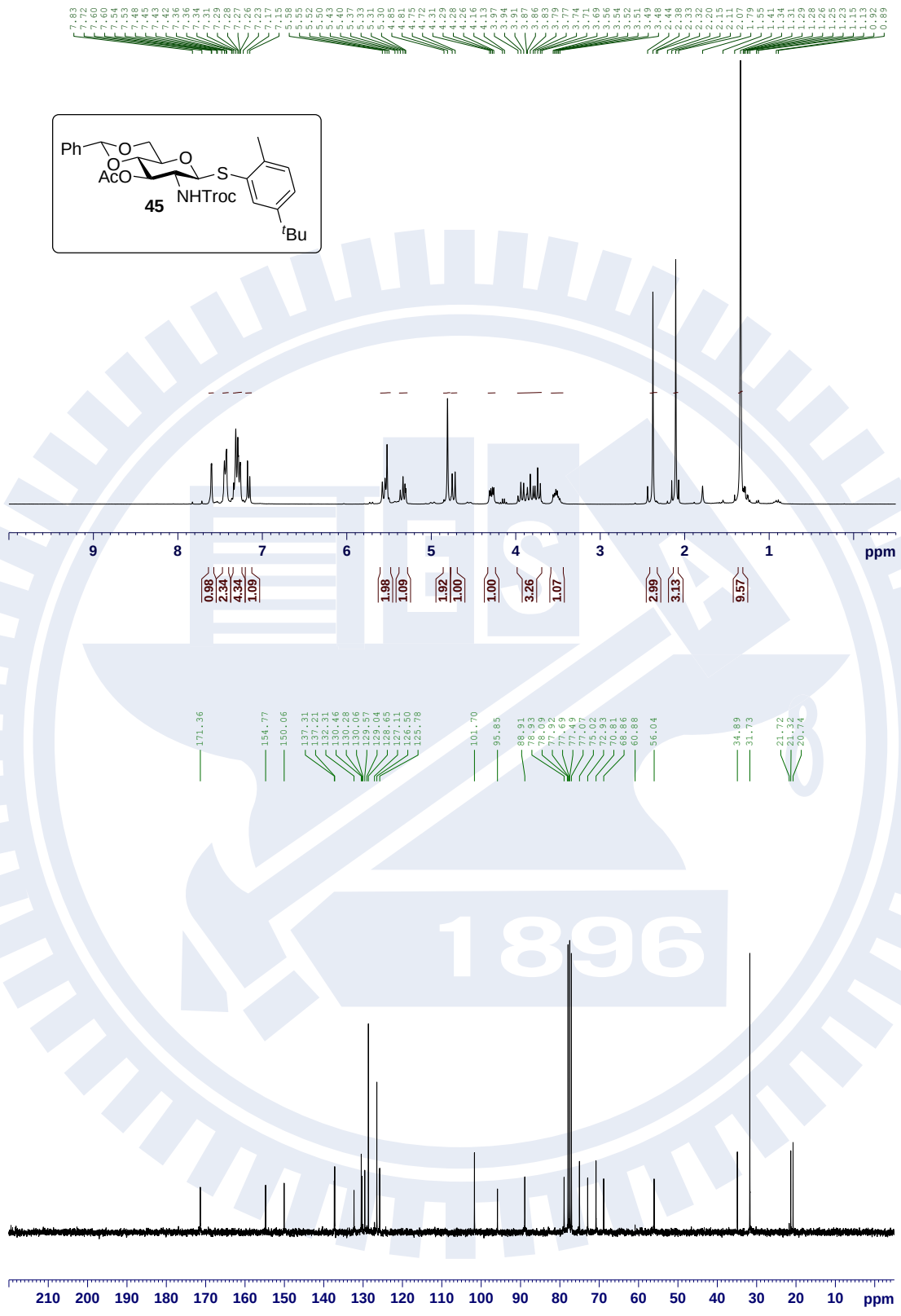
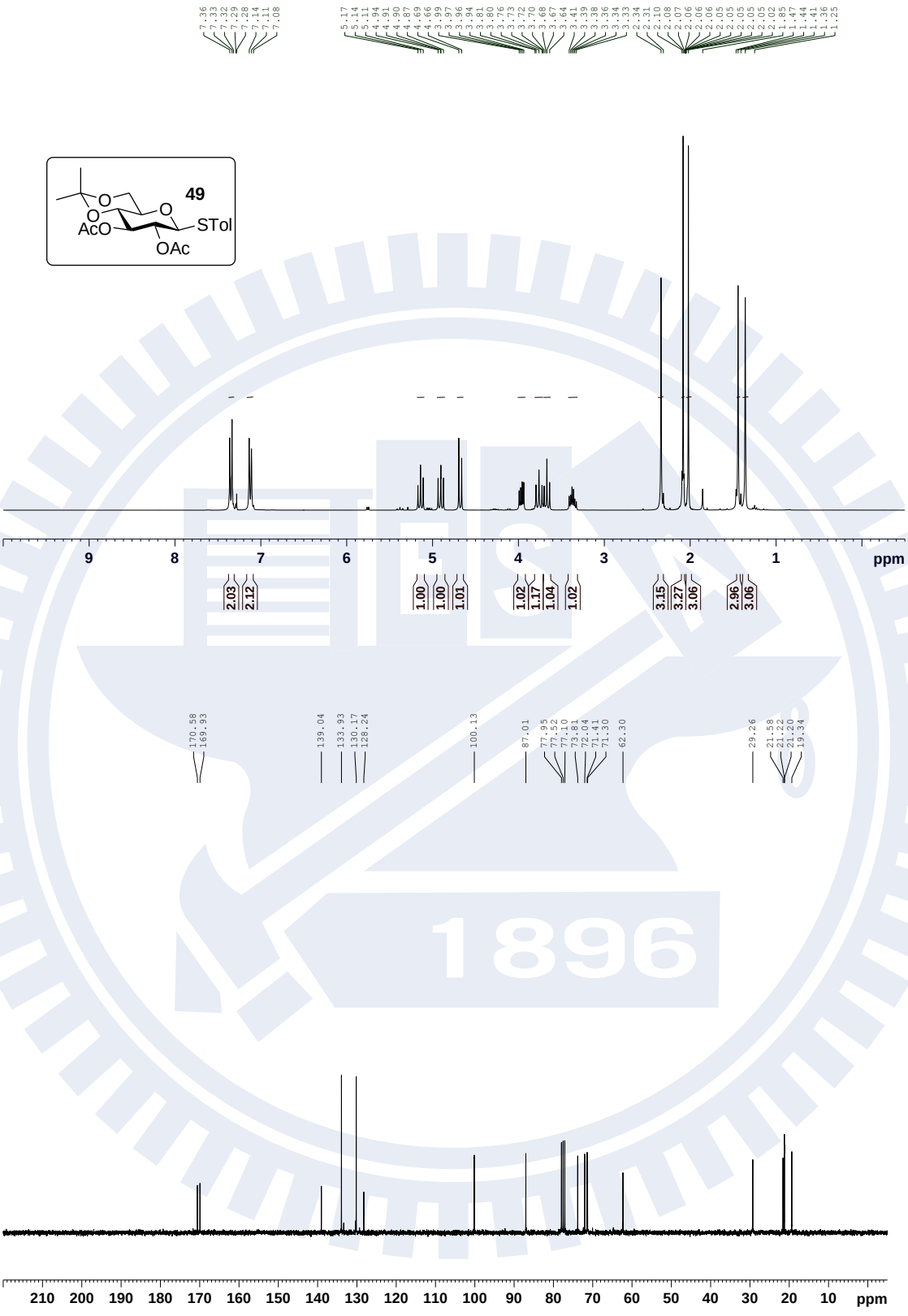
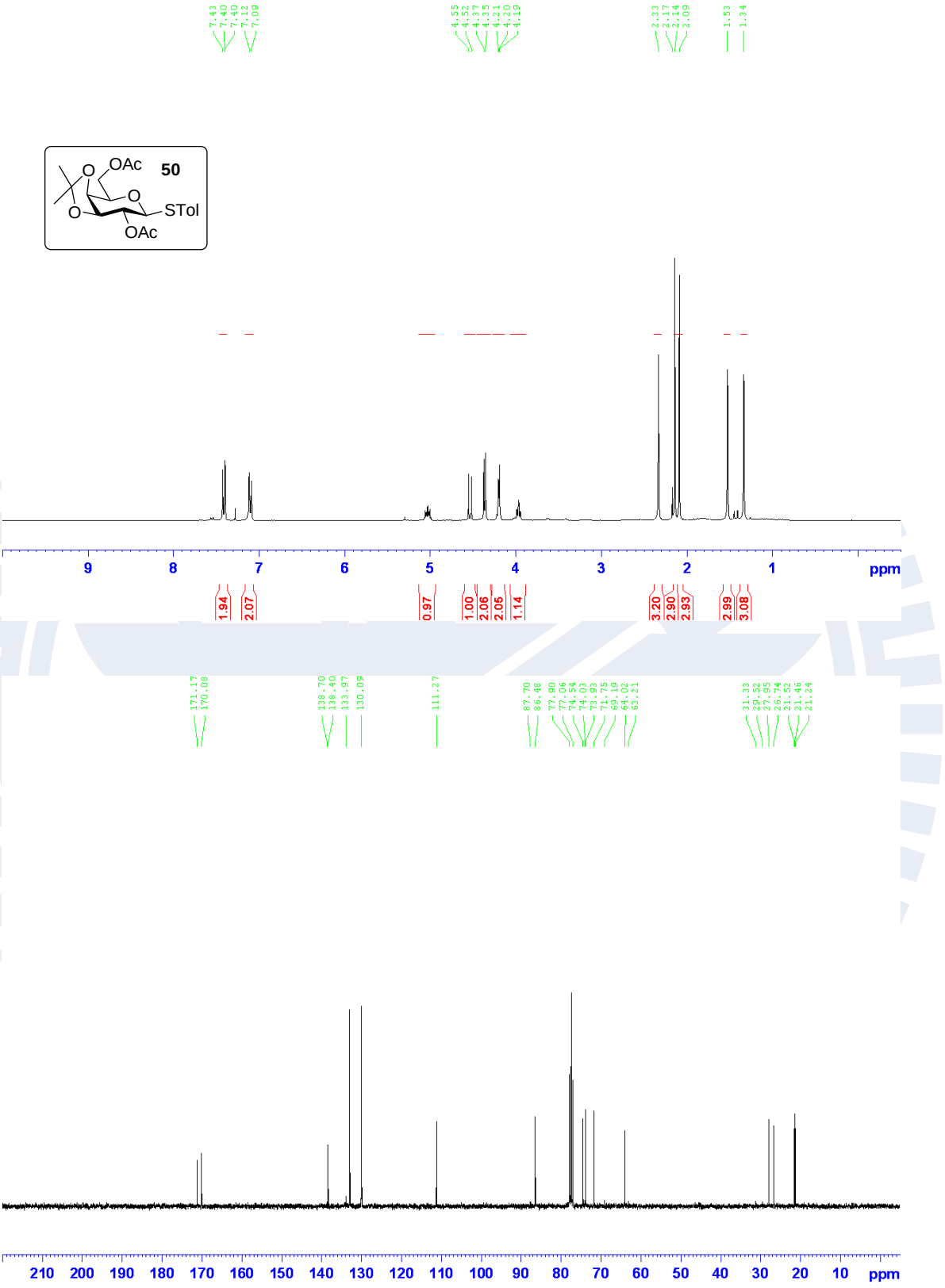
1.00
1.84
2.75
1.01
1.81
1.20
1.06
2.90
2.98
5.94
171.26
154.66
139.06
137.21
133.86
130.23
129.58
128.67
126.54
101.78
95.87
88.66
78.82
77.65
74.96
72.88
71.03
68.84
55.94
30.12
21.62
21.27
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


Ph
O
O
AcO
O
S
NHTroc
45
tBu
9
8
7
6
5
4
3
2
1
ppm
0.98
2.34
4.34
1.09
1.98
1.09
1.92
1.00
1.00
3.26
1.07
2.99
3.13
9.57
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


49
STol
AcO
OAc
ppm
2.03
2.12
1.00
1.00
1.01
1.02
1.17
1.04
1.02
3.15
3.27
3.06
2.96
3.06
170.58
169.93
139.04
133.93
130.17
128.24
100.13
87.01
77.95
77.52
77.10
73.81
72.04
71.41
71.30
62.30
29.26
21.58
21.22
21.20
19.34
ppm


7.43
7.40
7.40
7.22
7.09
4.55
4.52
4.37
4.35
4.21
4.20
4.19
2.33
2.17
2.14
2.09
1.53
1.34
OAc
50
O
O
O
STol
OAc
9
8
7
6
5
4
3
2
1
ppm
1.94
2.07
0.97
1.00
2.06
2.05
1.14
3.20
2.90
2.93
2.99
3.08
171.17
170.08
138.70
138.40
133.97
130.09
111.27
87.70
86.48
77.90
77.06
74.54
74.03
73.93
71.75
69.19
64.02
63.21
31.33
29.52
27.95
26.75
21.52
21.46
21.24
210
200
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150
140
130
120
110
100
90
80
70
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50
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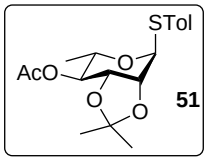
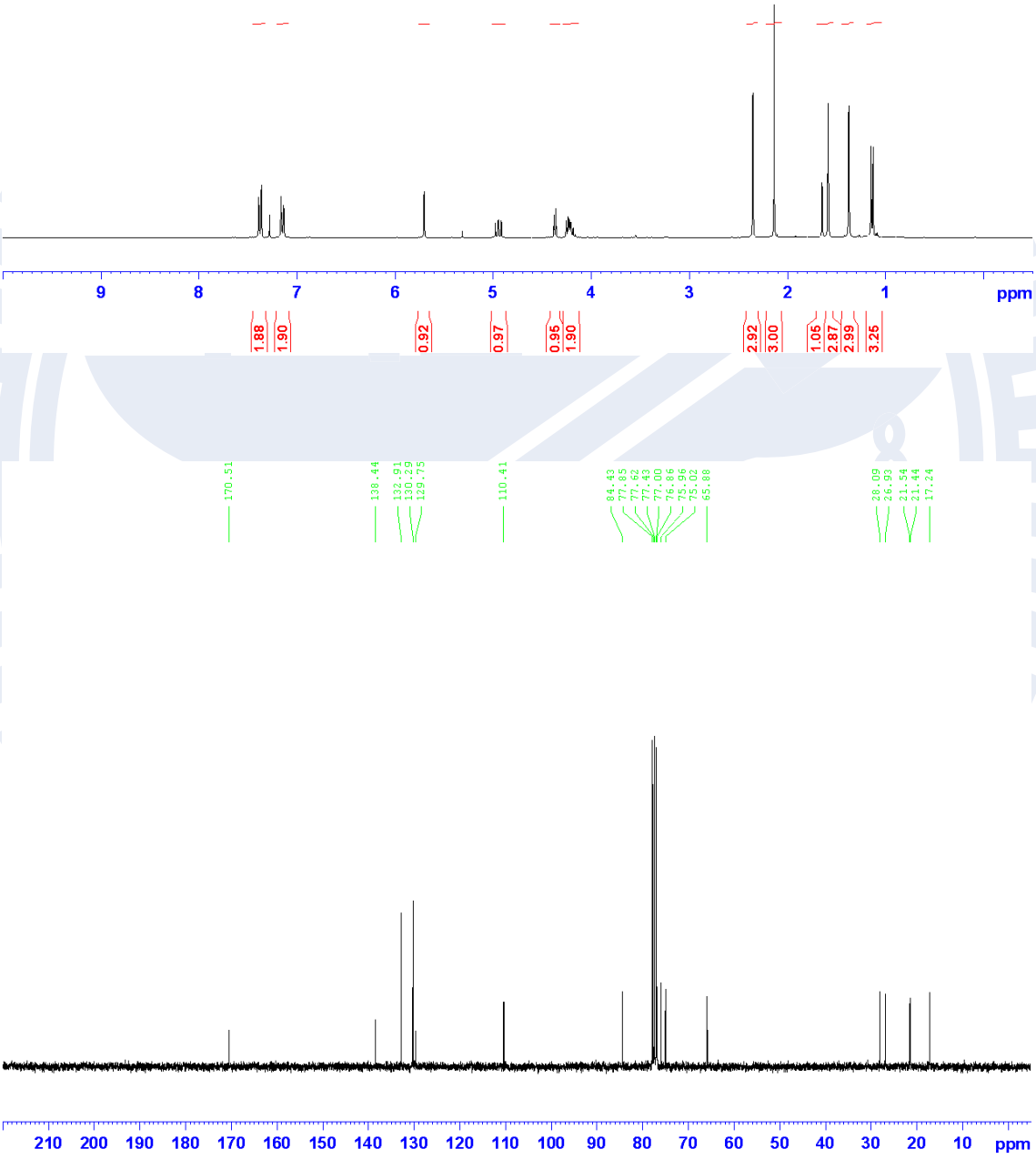
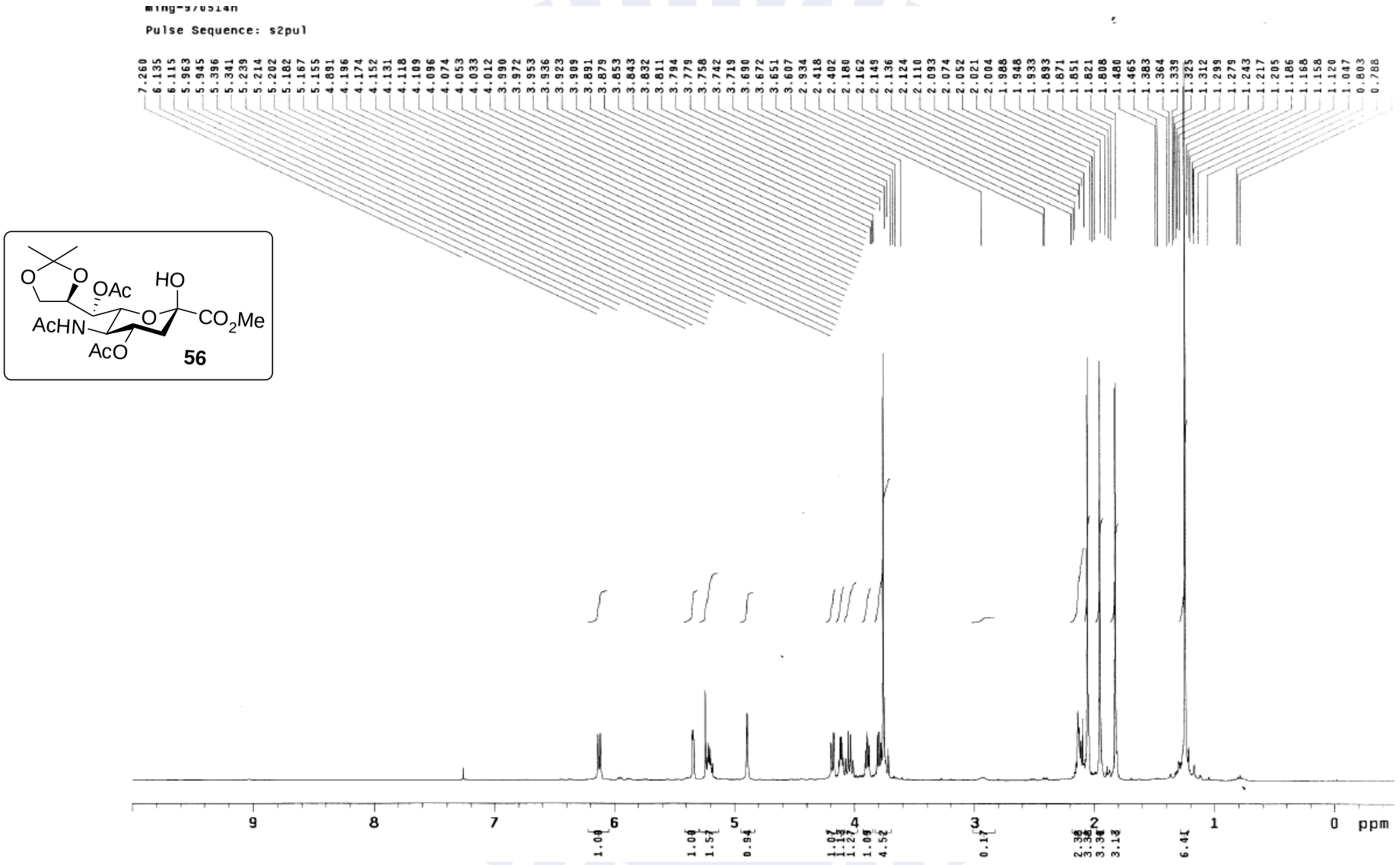
30
20
10
ppm


STol
AcO
O
O
O
51


ppm
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm


Pulse Sequence: s2pul
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5.945
5.396
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5.239
5.214
5.202
5.182
5.167
5.155
4.891
4.196
4.174
4.152
4.131
4.118
4.109
4.096
4.074
4.053
4.033
4.012
3.990
3.972
3.953
3.936
3.923
3.909
3.891
3.879
3.853
3.843
3.832
3.811
3.794
3.779
3.758
3.742
3.719
3.690
3.672
3.651
3.607
2.934
2.418
2.402
2.180
2.162
2.149
2.136
2.124
2.110
2.093
2.074
2.052
2.021
2.004
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1.948
1.933
1.893
1.871
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1.821
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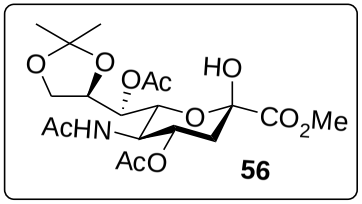
1.465
1.383
1.364
1.339
1.325
1.312
1.299
1.279
1.243
1.217
1.205
1.186
1.168
1.158
1.120
1.047
0.803
0.788
OAc
HO
AcHN
AcO
CO2Me
56
9
8
7
6
5
4
3
2
1
0
ppm

ming-970514c

Pulse Sequence: s2pul

171.054
170.505
170.258
169.213
108.307
94.694
77.257
77.000
76.743
75.292
70.857
69.344
68.600
65.272
53.084
49.154
35.930
26.176
25.299
22.821
20.785
20.768



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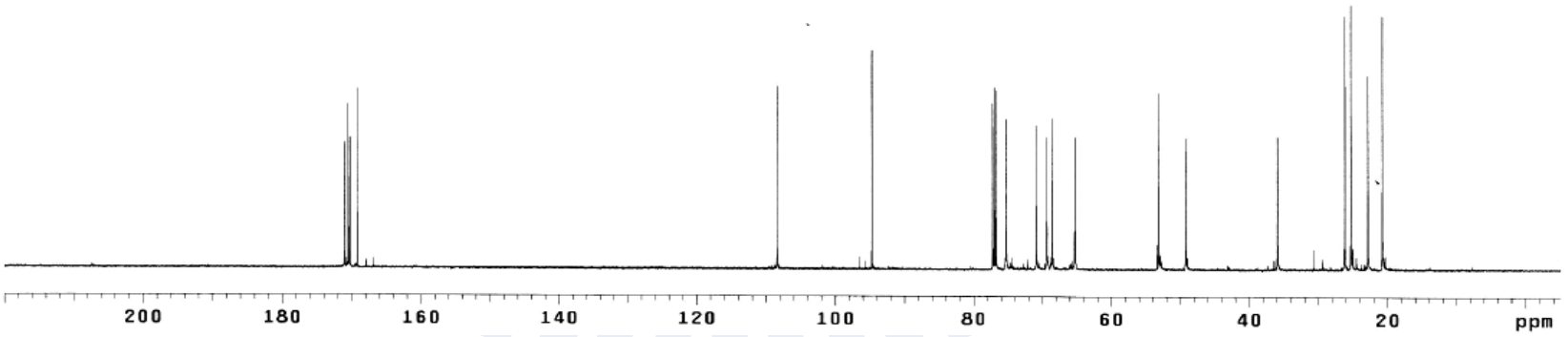

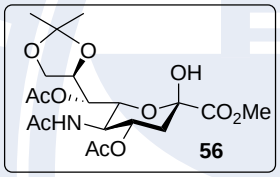

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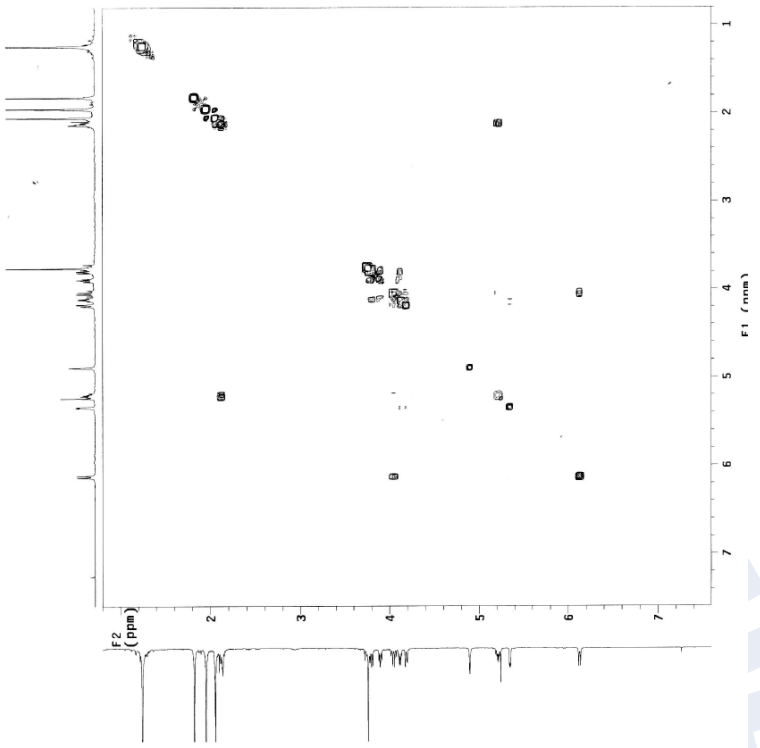
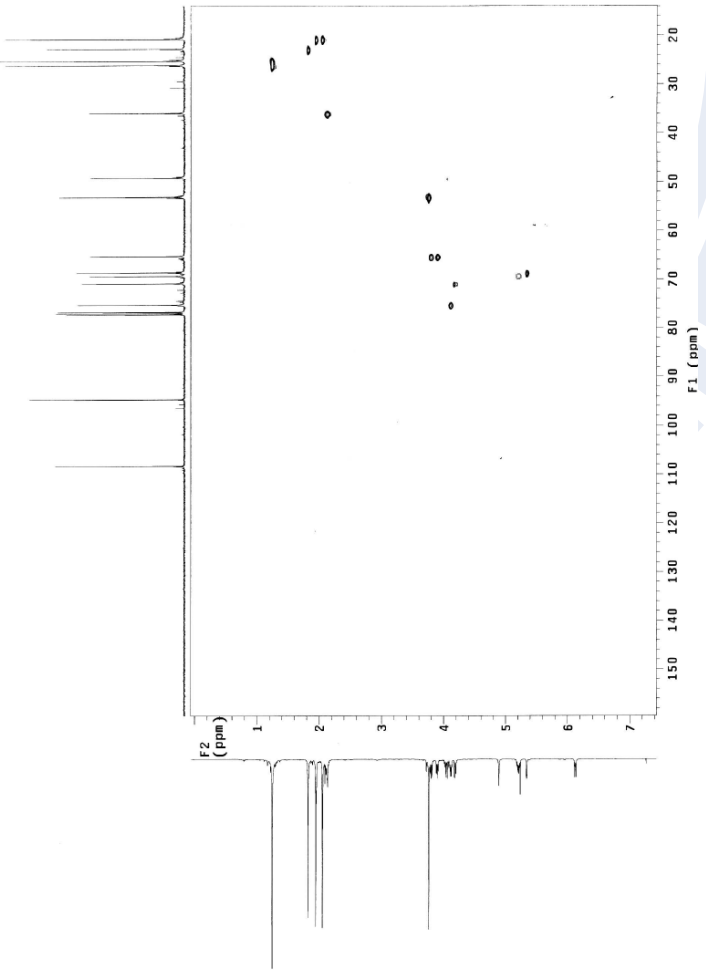




第二章　低濃度腈類溶劑系統醣基化反應建構 1,2-反式 β-醣苷鍵與其反應機構探討

2.1. 緒論: 文獻回顧

2.1.1. 醣基化反應簡介

在第一章中瞭解到醣分子在生物體中的重要性後；接著回歸基本，我們探討醣苷鍵的建立與其立體化學。

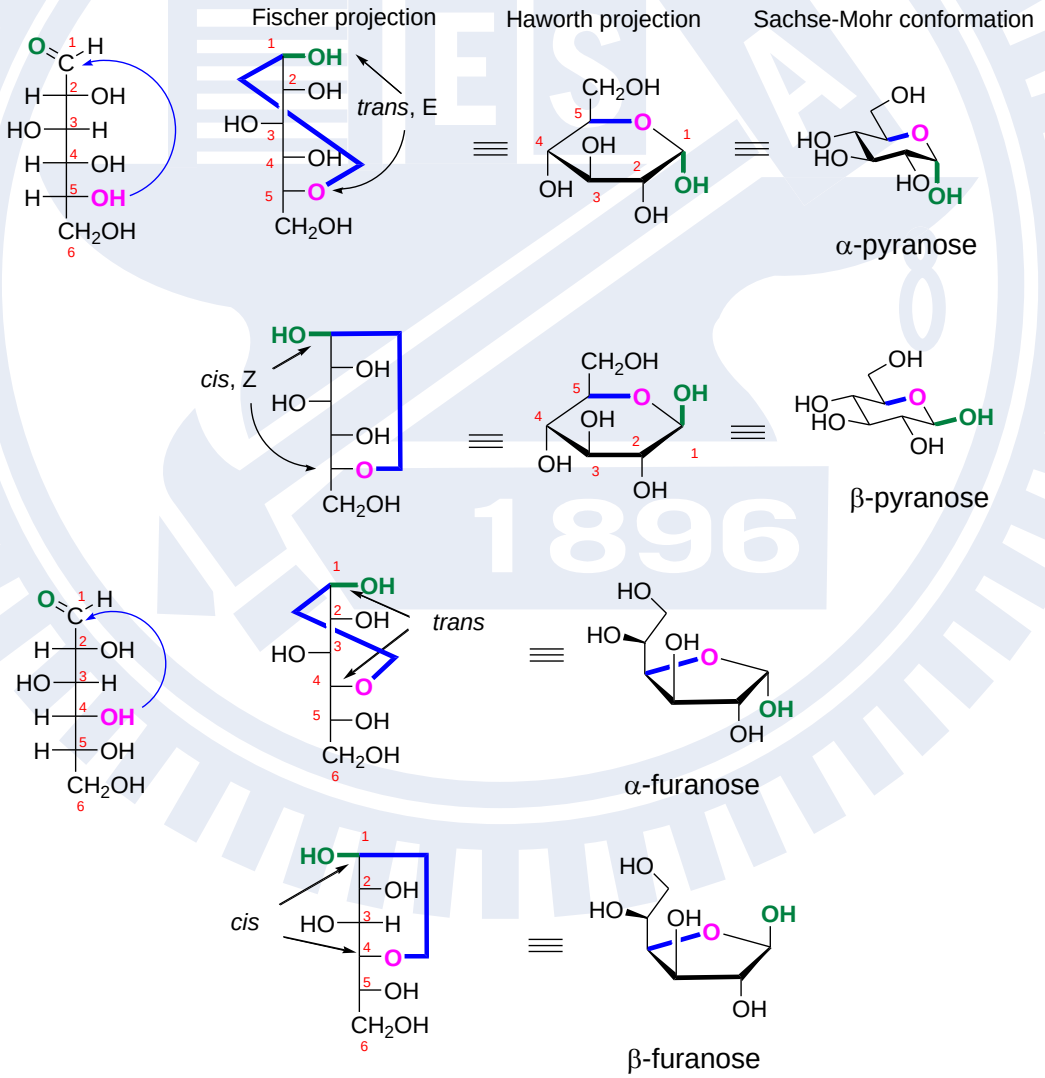


圖 2.1 決定 D-式葡萄糖的 α-或 β-異構物

根據 Emil Fischer 教授提出的命名系統，我們以六員環的醣分子舉例：以六員環醣分子離一號端點位置最遠的立體中心的羥基與醣基一號端點位置的羥基做判斷，若此二羥基是順式則為 β-異構物、反式則是 α-異構物；此定義亦適用於五員環醣分子 (圖 2.1)。

以合環結構的醣分子來說，又可用一號碳與二號碳相接羥基的順反描述醣苷鍵的位向，但此描述與 α-或 β- 並非絕對關係，例如：D-式葡萄糖的 1,2-反式鍵結為 β-醣苷鍵；但 D-式甘露糖的 1,2-反式鍵結卻為 α-醣苷鍵 (圖 2.2)。

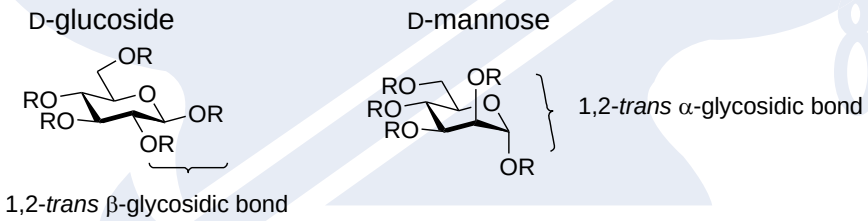


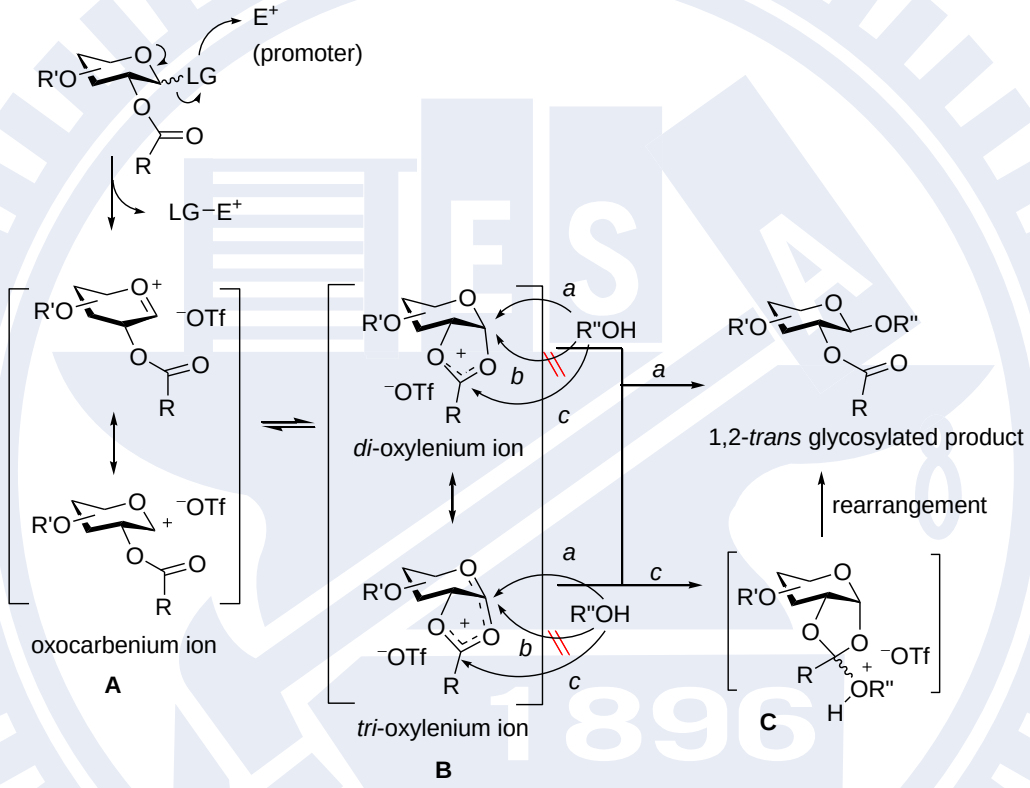
圖 2.2. 決定 D-式糖的 α-或 β-異構物與 1,2-順反鍵結的關係

2.1.2. 腈類溶劑影響反應立體選擇性

那麼，如何能控制得到 1,2-反式醣基化產物呢？有一個傳統而重要的反應機構：鄰基效應。鄰基效應又分兩種，一個由 C2-保護基團的羰基引起，[1] 另一個則是由 C2-原子作用。[2]

醣分子端點的離去基活化後會形成帶有正電荷的醣基陽離子 **A** (glycosyl oxocarbenium ion)，若 C2-保護基團具有羰基，[1] 則羰基上的

氧原子的孤對電子會靠近醣基端點的碳陽離子，形成順式-五員環陽離子 **B** 的過渡狀態 (acetoxocarbenium ion)，此時因 C-2 氧原子、羰基的氧原子與醣基環醚氧原子三者可以提供孤對電子藉由非定域化而穩定醣基端點正電荷 (流程 2.1)。

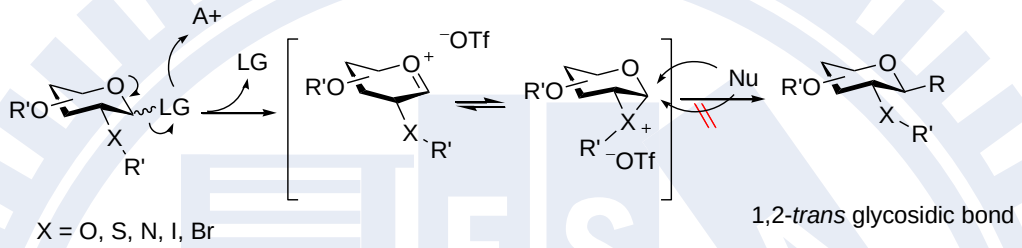


流程 2.1. C2-羰基保護基的鄰基效應可能機構

此時親核基分子則可由 *a*、*b*、*c* 三個方向進入，*a* 方向進入則形成 1,2-反式鍵結；*b* 方向因為順式五員環的立體障礙而被遮蔽；*c* 方向則會產生原酸酯陽離子 **C** (orthoester cation)，再經過重排反應後形成 1,2-反式鍵結產物 (流程 2.1)。

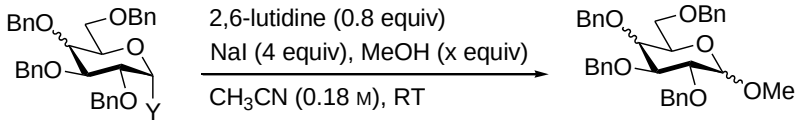
若是 C2-異原子進行鄰基效應，則此異原子 (氧、[2a] 硫、[2b,2c] 氮、

[2d] 鹵素) [2e] 可能提供其孤對電子與醣基端點的碳陽離子形成三員環結構 **B** 來穩定醣基正電荷。此時親核基將由三員環反側攻擊，開環後生成 1,2-反式鍵結的動力學產物，此相近概念的體現就是 Danishefsky 教授的 glycal 化學延伸 (流程 2.2)。[3]



流程 2.2. C2-異原子的鄰基效應

除了利用鄰基效應建構 1,2-反式醣苷鍵，自 1970 年代起，科學家們開始注意探討利用溶劑分子影響醣基化反應的立體選擇性。1974 年，Schuerch 教授使用葡萄糖基碘化物與半乳糖基碘化物、2,6-二甲基吡啶 (2,6-lutidine) 與簡單的非醣基醇類，在乙腈、室溫下進行醣基化反應可得到 1,2-順式 α-醣基化產物；此外 Schuerch 教授發現當使用 40 當量的醇 (醣受體) 進行反應時，醣基化產物的立體選擇性相對下降，這也是首次提到醣基化反應中反應物的濃度 (當量) 會影響到產物的立體選擇性 (流程 2.3)。[4]



For galactosyl halide :

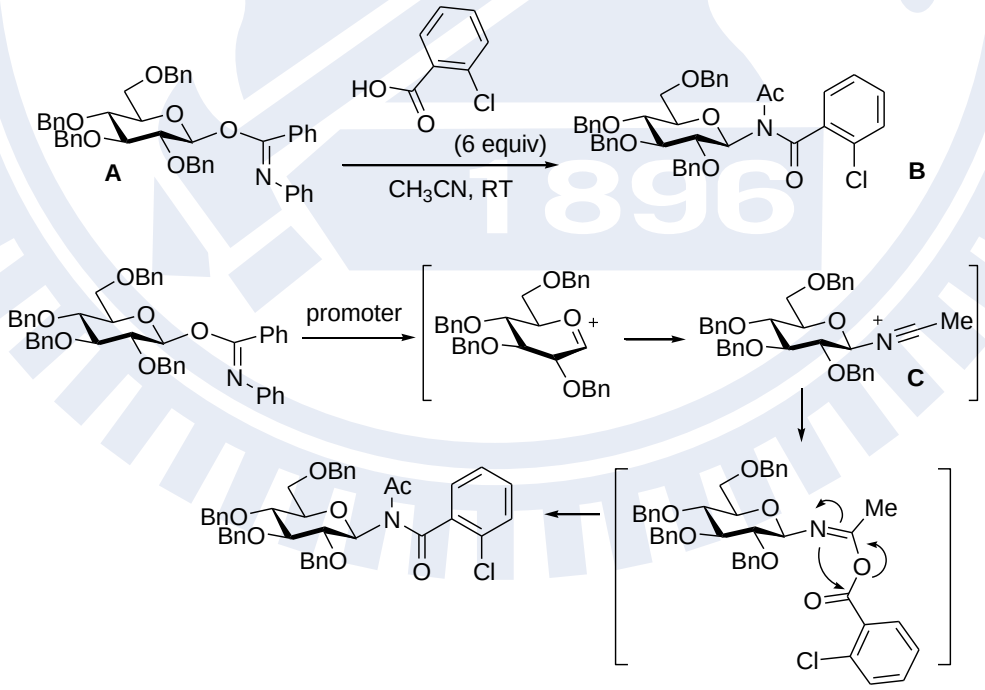
MeOH = 2 equiv, α/ β = 8/ 1 ; MeOH = 40 equiv, α/ β = 3.1/ 1

For glucosyl halide :

MeOH = 2 equiv, α/ β = 12/ 1 ; MeOH = 40 equiv, α/ β = 3/ 1

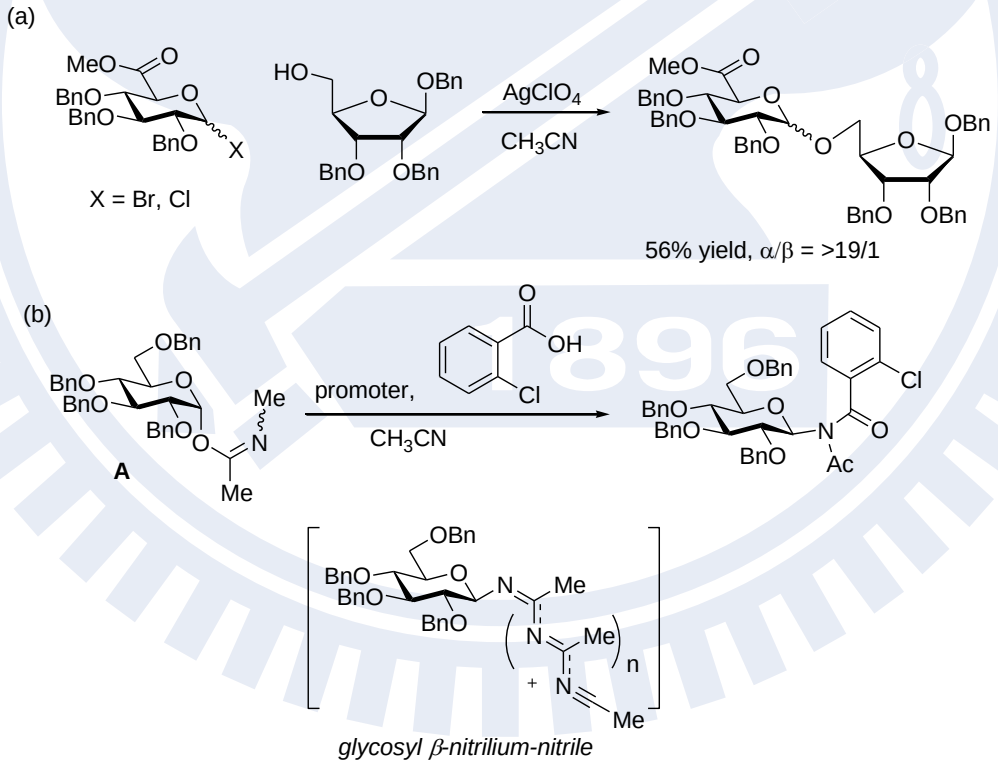
流程 2.3. 醣受體當量數對選擇性的影響

Sinaÿ 教授則在 1976 年報導使用葡萄糖基乙醯胺酯 **A** 與鄰氯苯甲酸在乙腈、室溫下進行醣基化反應時，判斷得到了 1,2-反式 β-位向的 Ritter 類型產物 **B**，推測反應機構可能是離去基活化後先形成醣基 β-nitrilium 過渡態 **C**，再進行重排反應 (流程 2.4)。[5]



流程 2.4. 葡萄糖基乙醯胺酯與鄰氯苯甲酸的反應機構推測

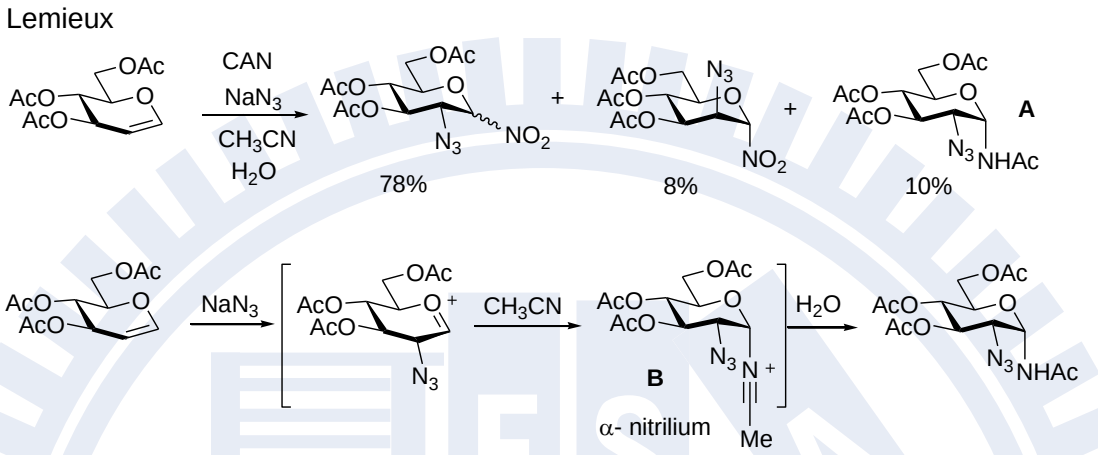
Schmidt 教授在 1980 年用醣基鹵化物，在乙腈、過氯酸銀、–15 ^{o}C 下預先活化醣予體後，再加入醣受體可得到 1,2-順式 α-位向為主的醣基化產物 (流程 2.5-a)。[6a] Schmidt 教授並以醣基乙醯胺酯 **A** 重復了 Sinaÿ 教授的實驗得到相同的醯胺產物。[6b] 此時 Schmidt 教授推測這些反應中乙腈分子因為逆端點效應 (reverse anomeric effect)，而接在醣基陽離子的 β- 位向，並與其它乙腈分子產生了 β-nitrilium-nitrile 過渡態穩定醣基陽離子，此時醣受體只能由 α-位向進入，使得醣基化反應得到 1,2-順式產物 (流程 2.5-b)。[7]



流程 2.5. Schmidt 教授在乙腈中的醣基化反應與中間體推測

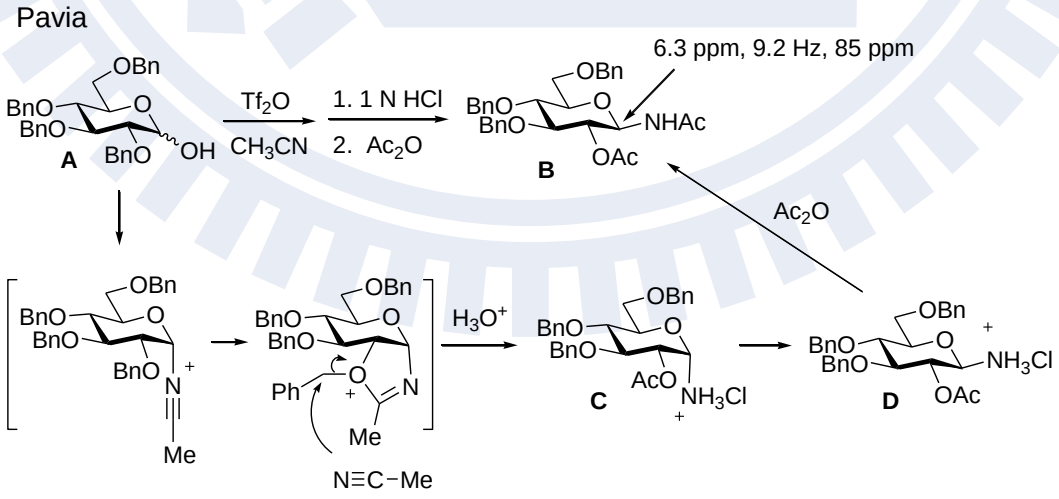
直到 1979 年，Lemieux 教授從 glucal 製備 C-2 疊氮基的實驗中

發現到 10% 的 1,2-順式 α-乙醯胺產物 **A**，因此他預測應該有 glycosyl α-nitrilium **B** 中間體的存在 (流程 2.6)。[8]



流程 2.6. Lemieux 教授預測 glycosyl α-nitrilium 中間體

而 Pavia 教授嘗試將醣基半縮醛 **A** 於乙腈中以三氟甲磺酸酐脫去醣基端點羥基，再以 1 N HCl 處理後對此粗產物進行乙醯化反應，結果得到 β-乙醯胺產物 **B**。



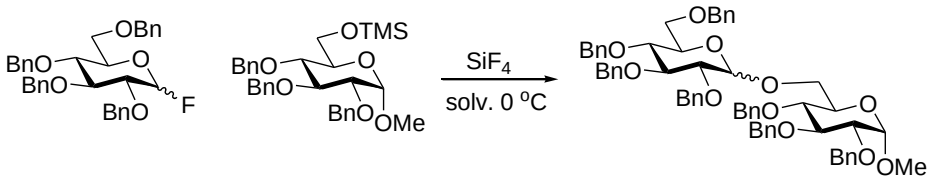
流程 2.7. Pavia 教授推測 glycosyl α-nitrilium 的存在

Pavia 教授推論此產物可能由 glycosyl α-nitrilium 脫去苄基後形成 α- 6,5 氮氧雜環 (α-oxazoline)，再水解成 α-NH_2 產物 **C**，因逆端點效應進行端點異構化 (anomerize) 產生 1,2-反式 β-NH_2 產物 **D**，以醋酸酐進行乙醯化反應後得到 β-乙醯胺產物 **B** (流程 2.7)[9]

爾後在 1980 年代，Noyori 教授採用醣基氟化物分別在乙腈得到 1,2-反式 β-位向醣基化產物為主、在乙醚中得到 1,2-順式 α-位向醣基化產物為主的結果 (流程 2.8-a)；[10] Ogawa 教授用硫糖予體在乙腈、–35 °C 下也得到 1,2-反式 β-位向為主的醣基化產物（流程 2.8-b)；[11] Ikegami 教授採用醣基磷酸酯為醣予體，在低溫的腈類溶劑系統中也有極佳的 1,2-反式 β-選擇性 (流程 2.8-c)。[12]

這些結果讓人提出疑問：直觀來說，glycosyl α-nitrilium 行 S_N2-like 類型反應可得到 β-醣基化產物，那在醣基化反應中的 glycosyl nitrilium 應該是 α-位向還是 β-位向呢？

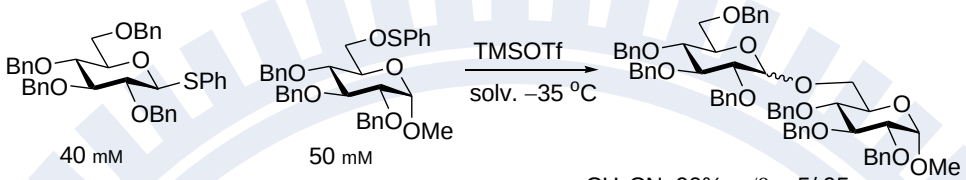
(a) Noyori, 1984



CH_3CN, 90%, α/β = 9/ 91

ether, 68%, α/β = 78/ 22

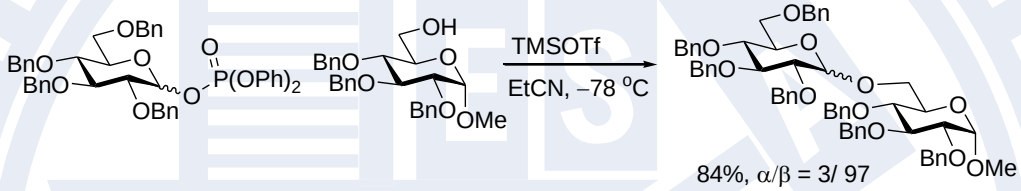
(b) Ogawa, 1987



CH_3CN, 90%, α/β = 5/ 95

ether, 65%, α/β = 67/ 33

(c) Ikegami, 1989

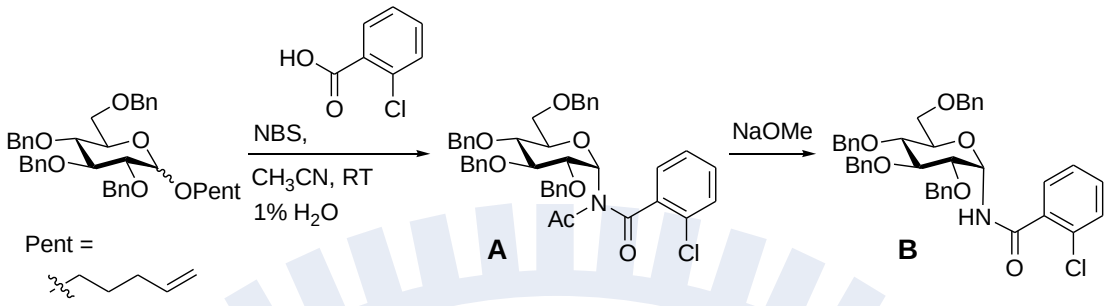


84%, α/β = 3/ 97

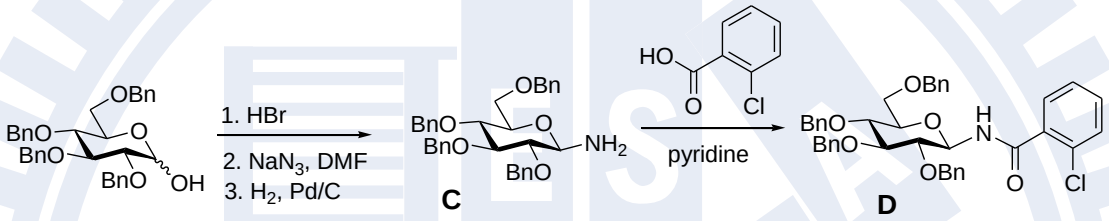
流程 2.8. 摘錄不同醣予體在腈類溶劑中的醣基化反應結果

於是，Fraser-Reid 教授以 4-戊烯基醣於乙腈、室溫下重新檢驗了 Sinaÿ 教授與 Schmidt 教授的實驗 (流程 2.9-a)，並經由另一條以醣基半縮醛出發的路徑取得 1,2-反式 β-位向醯胺產物進行比對 (流程 2.9-b)。[13] 發現當年應該是得到 α-位向的醯胺產物，而非原本判斷的 β-位向的醯胺產物，Fraser-Reid 教授以這樣的結果建議可能有 glycosyl α-nitrilium 過渡態的存在，而且當 α-*N*-醣苷鍵的氮具雙取代基時，會有大於 3 Hz 的非典型 ^3J 氫偶合常數訊號。(表 2.1)

(a) 類似 Sinay 與 Schmidt 教授的合成路徑



(b) Fraiser-Reid 教授的驗證路徑



流程 2.9. Fraser-Reid 教授的驗證實驗步驟

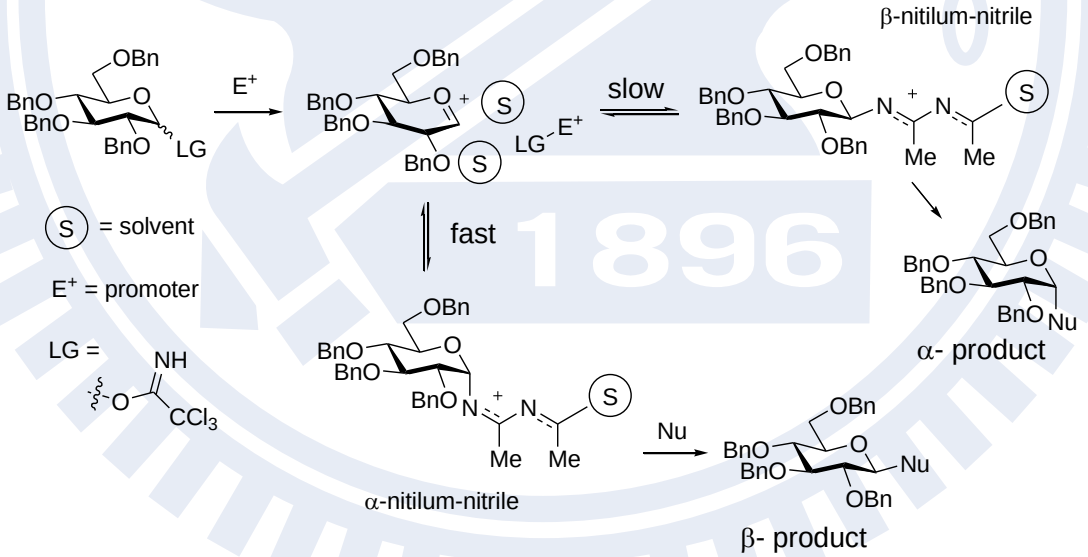
表 2.1 Fraser-Reid 教授的驗證實驗數據

Compound	H-1 signal	Reference
	Chemical shift (ppm), ^3J (Hz), multiplicity	
A	6.18, 7.2, d	Sinaÿ 5
A	6.09, 7, d	Schmidt, 6b
A	5.97, 7, d	Fraser-Reid, 13a
B	6.06, 5.35, 9, dd	Sinaÿ 5
B	5.99, 5.1, t	Fraser-Reid, 13a
C	4.12, 8.8, d	Fraser-Reid, 13a
D	5.31, 9, t	Fraser-Reid, 13a

接著，Schmidt 教授在 1990 年利用醣基三氯乙醯胺酯、三甲矽

基三氟甲磺酸、在不同的腈類溶劑系統與低溫條件下進行醣基化反應，同樣得到主要是 β-位向產物，[14a] 並進而利用這樣的條件合成路易士抗原中的寡醣分子 (Lewis-X antigen oligosaccharides)。[14b]

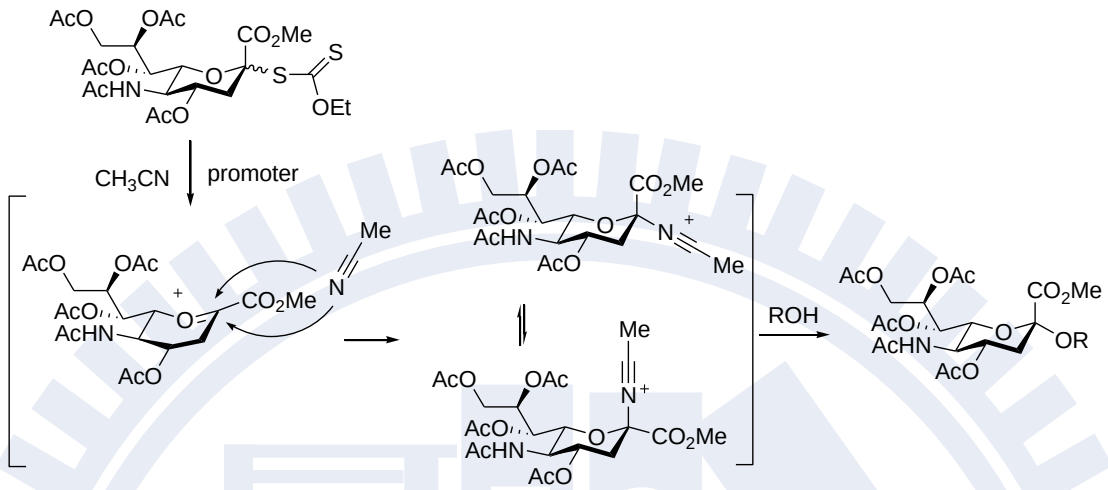
Schmidt 教授提出假設嘗試解釋高溫與低溫反應產物的選擇性來源，他認為：在低溫下 glycosyl α-nitrilium-nitrile 過渡態的生成會比 glycosyl β-nitrilium-nitrile 過渡態快，若在高溫時則會以 β-nitrilium-nitrile 過渡態為主，形成過渡態後、親核基再以 S_N2-like 的機構相對應形成 β-位向醣基化產物或 α-位向醣基化產物，如流程 2.10 所示。



流程 2.10. Schmidt 教授對醣基化反應立體選擇性的解釋

在 1991 年，Birberg 教授使用黃原膠 (xanthate) 修飾為離去基的唾液酸糖予體，嘗試使用乙腈與 1,2-二氯乙烷的混合溶劑、–70 °C 下

控制醣苷鍵的立體選擇性 (流程 2.11)。[15]



流程 2.11. Nitrile 溶劑效應對唾液酸醣基化反應的影響

Sinaÿ 教授在腈類溶劑中測試了幾種不同的醣予體，並得到 1,2-反式 β-雙糖的主要產物；[17] 接著 Sinaÿ 教授嘗試用 enol ether 修飾為離去基的醣予體 **A** 在低溫核磁共振實驗偵測 α-nitrilium 的訊號，經由圖譜與理論計算推測，建議醣基 1,2-順式 α-nitrilium 中間體 **B** 應以 $^{4}C_{1}$ 構型存在 (圖 2.3)。[17c]

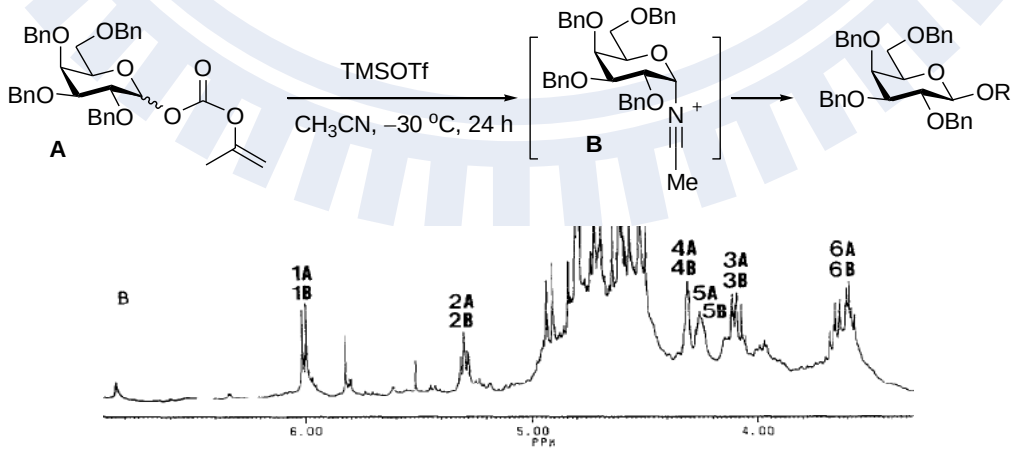
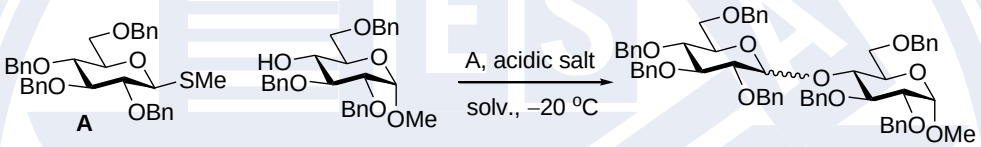


圖 2.3. 低溫核磁共振實驗推測 glycosyl α-nitrilium 的存在證據

之後，Kusumoto 教授利用硫葡萄糖予體**A**、*N*-溴化丁二醯胺 (*N*-bromosuccinimide, NBS) 或 PhIO 作為活化試劑，與酸性有機鹽（例如：過氯酸銀、過氯酸鋰）或有機酸 (例如：三甲矽烷三氟甲磺酸) 搭配，與不同的醣受體於 –20 °C 下進行醣基化反應，分別在腈類溶劑中得到 1,2-反式 β-醣基化產物，而在乙醚中得到 1,2-順式 α-醣基化產物 (流程 2.12)。[18]



solv. = CH_3CN, 70~ 85%, α/β = 1:2 ~ 1:8

solv. = ether or THF, 30~ 80%, α/β = 7:1 ~ 10:1

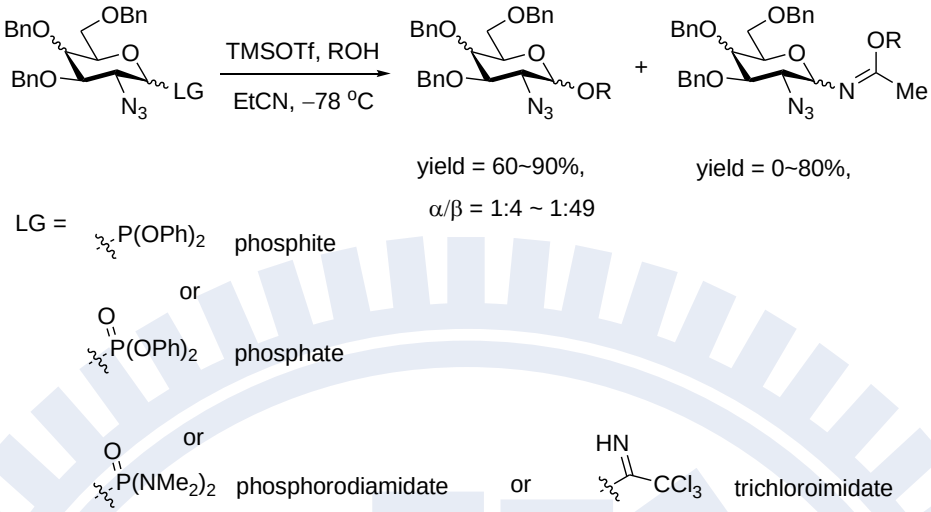
A = NBS or PhIO

acidic salt = TBAOTf, $TBAClO_4$, Ph_2IOTf, $LiClO_4$, $AgClO_4$....etc

acid = TMSOTf, TfOH,...etc

流程 2.12. 溶劑效應對硫醣的醣基化反應探討

Ikegami 教授與 Hashimoto 教授則專注於利用亞磷酸酯基 (phosphite)[19]、磷二醯胺 (phosphorodiamidate)[20]、磷酸酯基 (phosphate)[21]、或三氯亞醯胺酯基 (trichloroacetimidate)[21b] 等官能基做為離去基，修飾成葡萄糖、半乳糖、葡萄糖胺、半乳糖胺醣予體，在低溫腈類溶劑中進行醣基化反應，得到 1,2-反式 β-位向為主的醣基化產物，而 Hashimoto 教授並嘗試對醣予體活性與其旁反應的相關性進行探討 (流程 2.13)。[21b]



流程 2.13. 不同離去基的醣予體於腈類溶劑系統中的醣基化反應

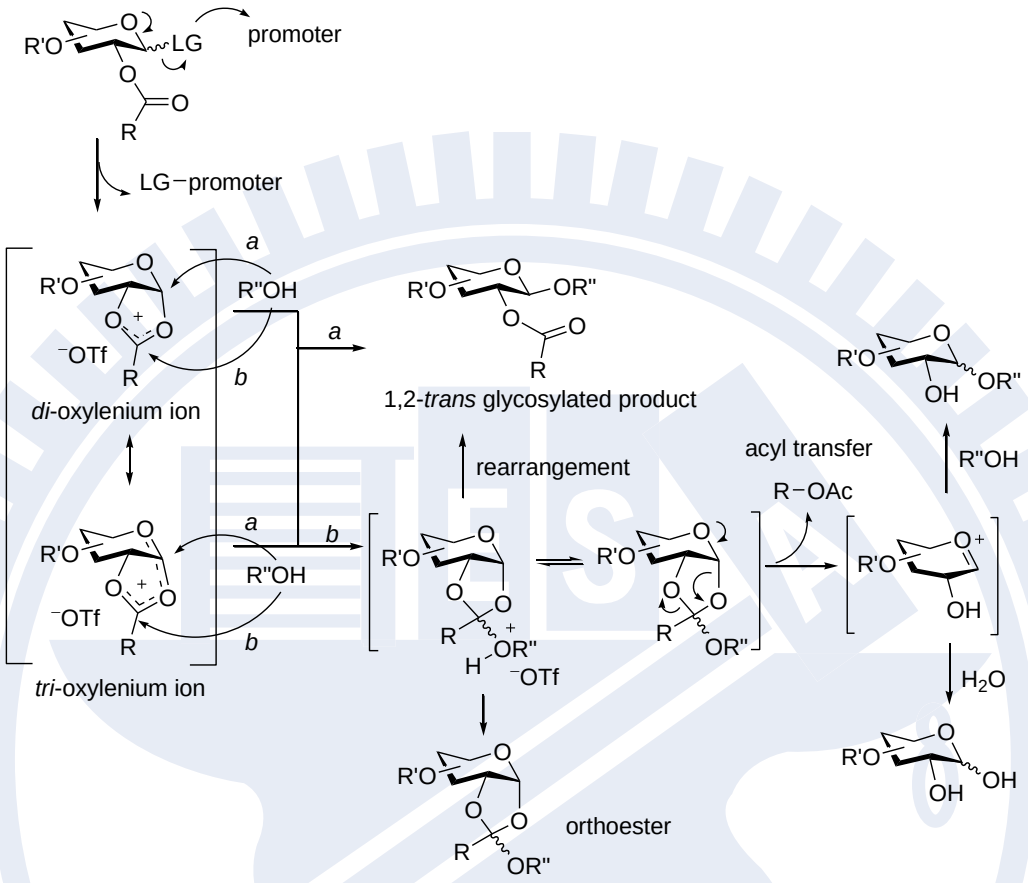
至此，科學家們普遍接受在低溫的腈類溶劑系統中的醣基化反應，將因 α-nitrilium 而控制產生 1,2-反式 β-位向產物。

2.1.3 研究動機與設計

要在醣基化反應中，控制生成單一立體異構物會受到許多因素的影響，比如說：醣予體的離去基與活化系統、醣分子保護基、反應溶劑、溫度、壓力、添加物、加藥方式等等。而本章節要探討的則是較少被討論的部分：反應物濃度效應。

翻看文獻，醣上保護基對醣分子的活性改變將影響醣基建構單元的活性。C2-鄰基效應雖然可以控制產生 1,2-反式產物；[22] 但常會有旁反應伴隨出現，[23] 像：醯基轉移 (acyl transfer)，[23a] 原酸酯化反應 (orthoester formation) 等等。[23b,23c,23d] 這些旁反應往往會降低醣基化反

應的效率 (流程 2.14)。



流程 2.14.　C2-保護基鄰基效應的旁反應

要解決這樣的問題，一是改變乙醯基為苄醯基 (benzyl) 或其他官能基。[23d,24,26,27] 但這樣的做法在醣予體保護基設計與合成上也憑添難度並限制了其應用廣度。另一個做法則是將 C2-羥基改為醚類保護，再利用方法控制形成 1,2-反式醣苷鍵，例如：活化試劑、[25] 掌性輔助基、[26] 異原子長距鄰基效應、[27] 或溶劑等等。

Shuerch 教授提到過多的反應物對立體選擇性的不良影響，那降低反應濃度又會有什麼樣的影響呢？[4] 再回顧文獻記載，醣基化反應

的反應物濃度似乎並非固定不變，明顯在當代研究報導中較少見到相關探討；若進行歸納整理，一般的醣基化反應其濃度多介於 30 mM 到 200 mM 之間 (表 2.1)，其中 Fairbanks 教授利用電化學進行醣基化反應，因為電解質溶解度的問題而需在約 10 mM 反應物濃度進行電導醣質化實驗，值得注意的是此反應得到很好的 β-選擇性。[29a]

表 2.2. 部份文獻記載的醣基化反應濃度

Entry	Donor (mM)	Acceptor (mM)	solvent	Reference
1	90	76	CH_3CN	18a
2	67	73	EtCN	21b
4	100	150	CH_2Cl_2	27a
5	200	160	CH_2Cl_2 or CH_3CN	27b
6	64	32	EtCN	27c
7	80	37	CH_2Cl_2	27d
8	6	13	CH_2Cl_2	28a

基於這些觀察，針對「研究濃度效應對醣基化反應的影響」便成了本研究一開始的動機。

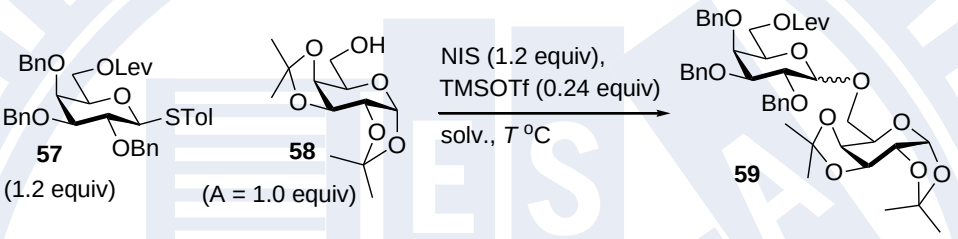
2.2 結果與討論

2.2.1 醣基化反應條件優化

起初由實驗室陳敏君學妹與李振瑋學弟在腈類溶劑系統中的醣基

化反應，其產率與立體選擇性並不固定。比較各個記錄與文獻記載後發現到實驗的反應濃度不盡相同 (醣受體濃度 = 50 mM ~ 190 mM)。於是設計採用 100% 乙腈作為反應溶劑系統，半乳糖基對甲硫酚 **57** 為醣予體、市售半乳糖衍生物 **58** 為醣受體進行醣基化反應 (表 2.3)。

表 2.3. 單一溶劑成份系統的探討：起始觀察、濃度效應、溫度效應



entry	solv., [A] (mM)	T (°C)	time (h)	yield (%), α/β
1	CH_3CN, 190	-18	4	20[a]
2	CH_3CN, 100	-25	1	67, 1:8[a]
3	CH_3CN, 50	-35	0.5	75, 1:10[a]
4	EtCN, 190	-75	1	70, 1:3
5	EtCN, 100	-75	0.5	71, 1:8
6	CH_2Cl_2, 100	-55	0.5	75, 1:1.6
7	CH_2Cl_2, 100	-70	0.5	78, 1:4

[a] 在磁石可以攪動的溫度下進行反應

由表 2.3，實驗 1、2、3 的結果得知：在醣受體濃度 190 mM 下、不到 –20 °C 時便發生類似「結凍」的現象，使磁石難以攪動，反應僅有不到 20% 的預計產物 **59**；若在醣受體濃度 100 mM 時、約 –25

°C 時發生「結凍」現象，此時可得到 67% 預計產物、α/β 選擇性 = 1:8；若將醣受體濃度降低為 50 mM，則可以降溫到約 –35 °C、而磁石仍可攪動，此時可以得到約 75% 預計產物、α/β 選擇性 = 1:10。顯示當濃度降低時，此「結凍」現象發生的溫度相對降低，而醣基化反應的產率與立體選擇性相對提升。

暫且將這個結凍現象視為結冰。我們且先討論影響選擇性的因素究竟是溫度還是濃度呢？

為了釐清這個問題，參考文獻設計在 100% 的丙腈 (propionitrile，凝固點 = –93 °C) 溶劑系統下，分別以醣受體濃度為 190 mM 與 100 mM 的條件下、於 –75 °C 進行測試。[14a] 發現在此溫度下並未觀察到結凍現象；而在 190 mM 下的反應，其立體選擇性明顯比 100 mM 的反應為低，此一結果顯示：反應物濃度越低、β-醣苷鍵選擇性越高。(表 2.3，實驗 4、5)

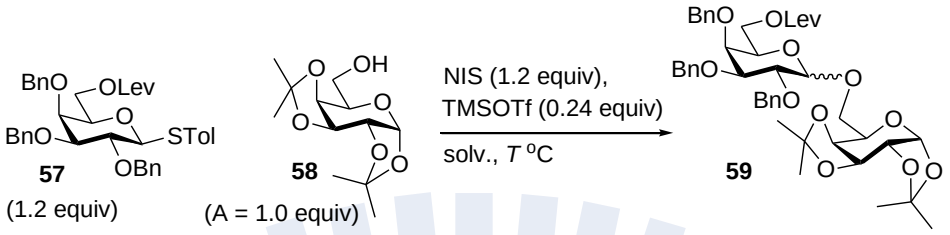
除了檢驗濃度效應，溫度效應有多大的影響呢？我們設計了兩個實驗：在 100% 二氯甲烷，醣受體濃度 = 100 mM 的系統下，分別在 –55 °C、–75 °C 進行測試。發現 –55 °C 時反應可得到 75% 的產率，α/β 選擇性 = 1 : 1.6，–75 °C 得到 78% 的產率 α/β 選擇性 = 1 : 4；因此，溫度越低、β-醣苷鍵的比例越高，這點與文獻記載相符合：1,2-反式醣苷鍵是動力學產物，反應溫度越低、1,2-反式醣苷鍵產物越多

(表 2.3，實驗 6、7)。[2]

由以上的實驗結果得知反應物質濃度降低、反應溫度降低，醣基化反應可得到較好的 β-選擇性。但此時尚未得到可稱為「實用」的結果 (β-產物＞95%)。此外丙腈雖然不發生「結凍」的現象，但需要降低反應溫度到 –75 °C，才可以得到跟乙腈相近的 1,2-反式 β-選擇性。而其他腈類分子的效果就其凝固點過高與其後續處理問題亦暫先不考慮使用。[14a] 因此選擇乙腈作為溶劑選擇；但考慮醣類分子在低溫對腈類溶劑的溶解度問題，加上「結凍」現象，單獨使用一種溶劑的反應系統又顯得不甚通用。若將對醣分子有較好溶解度且凝固點較低的溶劑，例如：二氯甲烷，與乙腈作一混和混合，利用類似共沸點的觀念，產生一個介於兩種溶劑個別凝固點的「共凝固點」來解決結凍的問題？若是可以因此將反應溫度下降，是否可以得到更好的立體選擇性？於是嘗試設計以混和溶劑系統，進行不同比例、濃度、溫度的相關測試 (表 2.4)。

首先參考本實驗室陳敏君同學的實驗 (二氯甲烷：乙腈 = 1/3，醣受體濃度 = 190 mM)，同時設計增加二氯甲烷比例為 50%、75%，在醣受體濃度 100 mM 下進行測試 (表 2.4、實驗 1、2、3，未發表的結果)。

表 2.4. 混和溶劑系統的測試



entry	Solvent component			[A] (mM)	T (°C)	yield (%), α/β[a]
	CH_2Cl_2 (%)	CH_3CN (%)	EtCN (%)			
1	25	75	0	100	-55	86, 1:9
2	50	50	0	100	-55	83, 1:6
3	75	25	0	100	-70	84, 1:5
4	25	75	0	50	-55	84, 1:10
5	25	75	0	10	-55	83, 1:13
6	25	0	75	100	-75	75, 1:6
7	25	0	75	50	-75	81, 1:12
8	25	0	75	10	-75	79, 1:16
9	25	75	0	10	-70	84, 1:19
10	25	50	25	10	-70	81, 1:19

[a] 以 HPLC 與對照實驗 (100% CH_2Cl_2) 判斷 α/β 比例。

發現加入 25%的二氯甲烷可以讓反應降溫到–55 °C，若再更增加二氯甲烷比例，則可以降溫到更低的溫度，但相對 α/β-選擇性會下降，這也說明腈類溶劑分子對於 α/β-選擇性的重要性；於是再以 25% 二氯甲烷、75% 乙腈的混合溶劑為模型系統測試濃度效應，發現當反應濃度降低時，1,2-反式 β-選擇性也相對提升，驗證在單一溶劑系

統中的觀察假設 (表 2.4、實驗 1、4、5)。除了乙腈外，也採用二氯甲烷與丙腈的混合系統測試濃度效應，發現有一樣的趨勢 (表 2.4、實驗 6、7、8)。

基於 1,2-反式 β-醣苷鍵的建立趨向動力學產物，是否可以將乙腈系統的反應溫度再降低溫度呢？於是在醣受體濃度 = 10 mM 下將溫度降低到 –70 °C，反應可得到 84% 的雙糖產物，而選擇性也提升為 α/β = 1/19 (表 2.4、實驗 9)。但在此實驗中觀察到仍有少許「結凍」的現象，於是在維持選擇性與幫助攪動的考量下，將此混合溶劑成份改為 25% 二氯甲烷、50% 乙腈、25% 丙腈的系統，此時在產率與選擇性上可得到最佳效果 (81%產率、α/β = 1/19 ~ 1/20)。於是確定之後的醣基化反應便以醣受體濃度 = 10 mM、溫度在 –70 °C 的條件進行實驗 (表 2.4、實驗 10)。

2.2.2 測試低濃度醣基化反應的廣泛性

在確定低濃度醣基化反應的條件之後，開始嘗試將此方法應用於不同的醣基建構單元上，首先針對不同的醣予體進行測試。

2.2.2.1 不同醣予體的測試

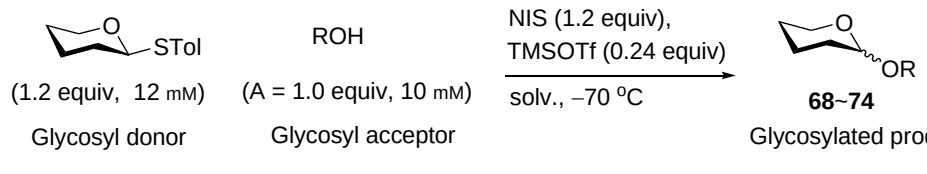
翻看文獻，當醣分子羥基的保護基團具有羰基時，可能造成長距離鄰基效應 (long range participation) 的效果影響 α/β-選擇性。[30] 此

外、酯基或碳酸酯基等保護基往往會降低醣予體的活性，間接影響到醣基化反應的效率。[22] 因此設計在醣予體不同位置的羰基，以酯基或碳酸酯基保護後進行醣基化反應測試；同時以二氯甲烷為單一溶劑作對照實驗，分別以 HPLC 分析結果交叉比對，此也是本人預口試的口試委員洪上程教授的建議 (表 2.5)。

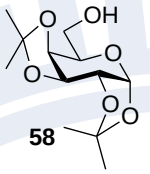
首先，就半乳糖結構，包括全苄基保護與部分四號或六號位置以酯基或碳酸酯基的醣予體 **61**、**62**、**63**，都得到 1,2-反式 β-選擇性 (表 2.5，實驗 1、2、3)；而 4,6-縮醛保護的半乳糖分子 **64** 的 α/β-選擇性較低，但當反應物濃度再次降低，卻可以得到接近 95% 的 β-產物 **71**，與單純使用二氯甲烷溶劑的對照實驗相比較，的確對 β-選擇性有顯提升的效果 (表 2.5，實驗 4、5)。

接著改用活性較半乳糖低的葡萄糖糖予體做測試，以酯基保護不同位置羥基的葡萄糖醣予體 **65**、**66** 也有很好的反應產率與 β-選擇性 (表 2.5，實驗 5、6)。[22b] 除了降低醣予體的活性，我們也嘗試以活性相對較高的去氧醣 (deoxy-sugar) 進行測試，比如說：市售可得的 L-岩藻糖，全苄基保護的岩藻糖醣予體 **67** 與六碳鍊一級醇分子 **60** 得到 80% 的產率與 98% 的 α/β-選擇性，此反應並沒有發現到端點異構化的情形 (表 2.5，實驗 10)。[31]

表 2.5. 不同醣予體的測試



Glycosyl acceptros

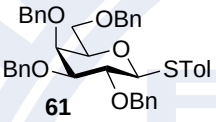


58

or $HO(CH_2)_6Cl$

60

Glycosyl donors



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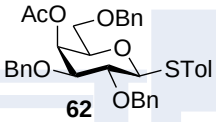
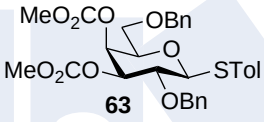


62



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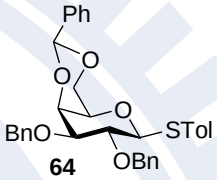

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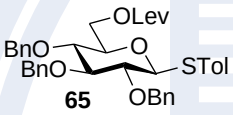

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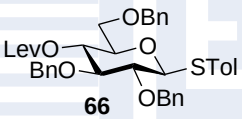

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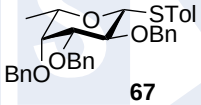


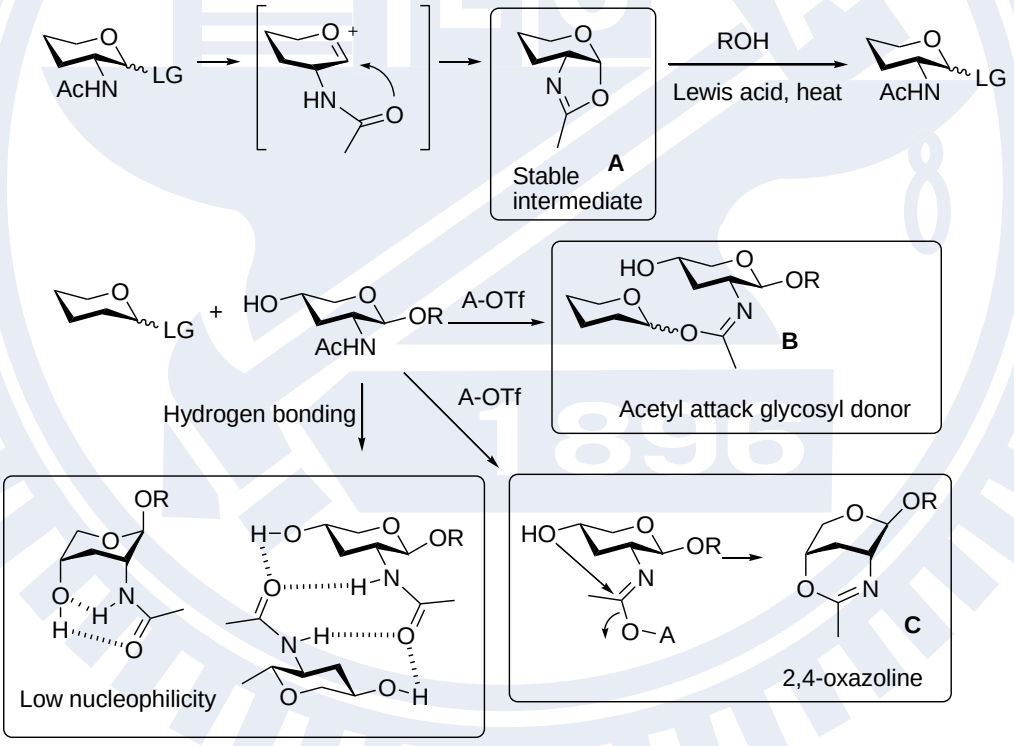
67

entry	Donor	Acceptor	Product	time (h)	yield (%), α : β	yield (%), α : β [a]
1	**61**	**60**	**68**	0.5	85, 1 : >49	88, 1 : 4[b]
2	**62**	**58**	**69**	0.5	85, 1 : 33	83, 1 : 2
3	**63**	**58**	**70**	0.75	70, 1 : 49	67, 1 : 2
4	**64**	**58**	**71**	0.5	75, 1 : 9	75, 1 : 6
5[c]	**64**	**58**	**71**	0.75	83, 1 : 18	Not done
6	**65**	**58**	**72**	0.5	77, 1 : 49	73, 1 : 1
7	**66**	**58**	**73**	0.5	83, 1 : 19	77, 1 : 8
8	**67**	**60**	**74**	0.33	80, 1 : 49	80, 1 : 4

[a] 對照實驗 (100% CH_2Cl_2, –55 °C)，[b] 醣受體為 1.5 莫耳當量，[c] 醣受體濃度 [A] = 5 mM。

此外，糖胺 β-醣苷鍵是許多生物活性相關分子的重要鍵結，而天然糖胺 (aminosugar) 的 C2-胺基多半以乙醯胺基的型式存在。[32]

但若直接以具有乙醯胺基的醣予體進行醣基化反應，則會有許多潛在的問題，比如說：醣予體會形成穩定的中間產物 **A** 使醣受體難以橋接。[33] 此外，乙醯胺基容易以其羰基攻擊醣基陽離子產生亞醯胺產物 **B**。[34] 乙醯胺基上的羰基也容易被路易氏酸活化，與自身的羥基進行分子內合環，產生醣基氮氧雜環產物 **C**；[35] 再者，乙醯胺基本身的高極性也容易產生分子內或分子間的氫鍵，影響到胺糖的醣基化反應效率 (流程 2.15)。[36]



流程 2.15.　NHAc 官能基在醣基化反應中潛在的問題

因此科學家們設計、測試了許多官能基團將 C2-胺基做保護修飾以利醣基化反應的進行。[2d,33,34,37] 鑒於將官能基引入、修飾醣基建構

單元的方便性、全體去保護 (global deprotection) 操作 (與可能的消旋後果),[33] 如果使用疊氮基修飾的醣基胺單元,又能取得 1,2-反式 β-醣苷鍵似乎是好的選項。[38]

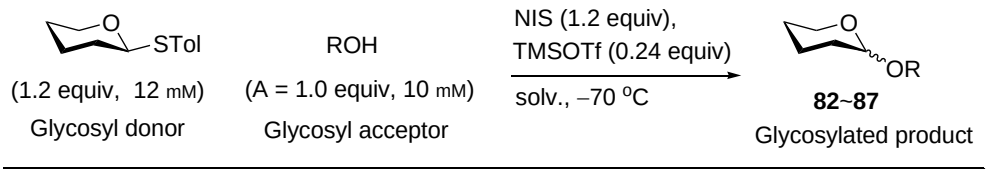
但是由於疊氮基缺乏 C2-羰基鄰基效應，以 C2-疊氮基修飾的醣予體往往難以控制醣苷鍵形成的位向。在此我們嘗試測試低濃度醣基化反應是否可以解決這個問題 (表 2.6)。

一開始以全苄基保護的葡萄糖胺醣予體 **76** 進行反應，結果得到 83% 的產率與 98% 的 β-產物 **82**，反應時間稍微延長，此現象可能因為疊氮基修飾的醣予體其活性較低之故 (表 2.6，實驗 1)。[39]

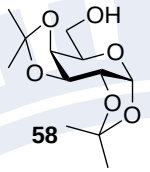
而張世聖學長則嘗試以端點離去基修飾為 2,6-二甲基硫酚的部分苄基保護葡萄糖胺醣予體 **77** 與一級醇分子 **60** 進行低濃度化學選擇性醣基化反應，雖然 β-醣基 2,6-二甲基硫酚的活性較差，使反應溫度需略為提升，但可得到 60% 的產率與 98% 的 β-產物 **83**，在此反應中並未發現到自身偶合的雙糖產物 (表 2.6，實驗 2)。[40]

若以苯縮醛保護 4,6-羥基、2-Nap (2-naphthylmethyl) 保護 3-羥基的硫半乳糖胺醣予體 **78** 與三碳鍊一級醇分子 **75** 進行反應,則可在−70 °C 下得到 79% 的醣基化產物 **84**，其中 β-產物佔了 94% 以上 (表 2.6，實驗 3)。

表 2.6. 胺醣與雙糖醣予體的測試



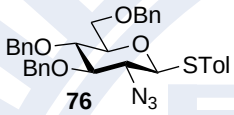
Glycosyl acceptros



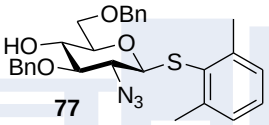
58

or $HO(CH_2)_6Cl$ **60** or $HO(CH_2)_3Cl$ **75**

Glycosyl donors



76



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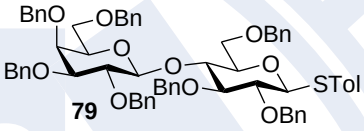

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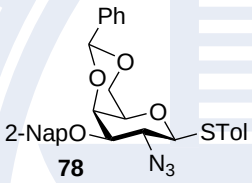

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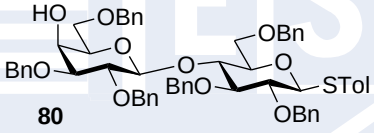
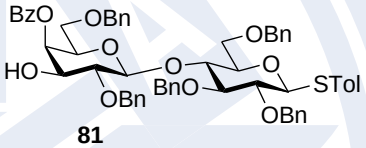


80



81

entry	Donor	Acceptor	Product	time (h)	yield (%), α : β	yield (%), α : β [a]
1	**76**	**58**	**82**	1	83, 1 : 49	85, 1 : 2.5 [b]
2	**77**	**60**	**83**	2	60, 1 : >49	Not done [b,c]
3	**78**	**75**	**84**	0.75	79, 1 : 16	Not done
4	**79**	**58**	**85**	0.5	75, β only	75, 1 : 6
5	**80**	**60**	**86**	0.5	80, β only	77, 1 : 3 [c]
6	**81**	**60**	**87**	0.5	83, 1 : >49	80, 1 : 4 [c]

[a] 對照實驗 (100% CH_2Cl_2, –55 °C)，[b] 反應溫度為 –65 °C，[c] 醣受體為 1.5 莫耳當量。

除了單醣醣予體之外，再嘗試是否可以將方法應用到多醣醣予體上。首先將市售的乳糖分子修飾成雙糖醣予體 **79** 與半乳糖醣受體 **58** 進行測試，發現得到只有單一異構物 (表 2.6，實驗 4)，可見低濃度

醣基化反應可以應用到雙糖醣予體上。而李振瑋學弟也嘗試以部分保護的乳糖醣予體 **80**、**81** 與一級醇分子 **60** 進行化學選擇性醣基化反應，分別都有很好的效果 (表 2.6，實驗 5、6)。

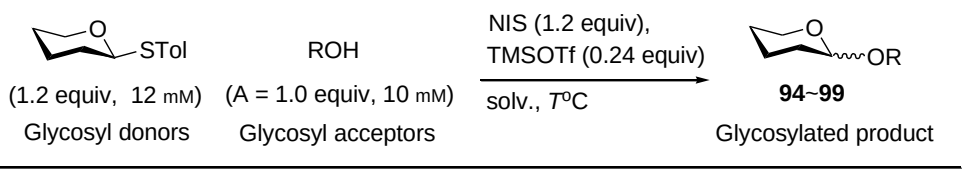
2.2.2.2 不同醣受體與醣予體的配對測試

除了醣予體外，也嘗試探討不同醣受體的應用性。設計鼠李糖、葡萄糖、半乳糖，與絲胺酸修飾為醣受體進行測試 (表 2.7)。

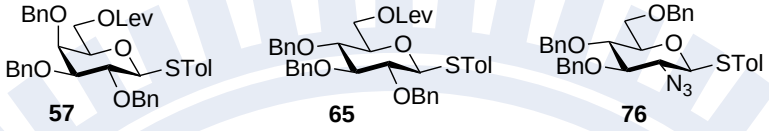
一開始先以半乳糖醣予體 **57** 與二級羥基的醣受體，例如：四號羥基鼠李糖 **88**、四號羥基葡萄糖 **89** 與四號羥基半乳糖 **90**，進行低濃度醣基化反應，雖然產率稍微降低，但仍然可以選擇性得到 98% 以上的 β-立體異構產物 **94**、**95**、**96** (表 2.7，實驗 1、2、3)。接著更換為葡萄糖醣受體 **65** 與六號羥基葡萄糖 **91** 進行橋接，則得到 83%的產率與 98% 的 β-產物 **97** (表 2.7，實驗 4)。

除了醣分子作為醣受體以外，我們也嘗試將半乳糖醣予體 **57** 與胺基酸醣受體 **92** 進行橋接，在使用二氯甲烷溶劑系統時，可得到約 95% 的 α-產物 **98-α**；有趣的是當我們改用腈類混合溶劑系統進行反應，卻得到了 73% 的產率與大於 98% 的 β-產物 **98-β**；我們僅利用溶劑系統的改變卻可以反轉立體選擇性 (表 2.7，實驗 5)。

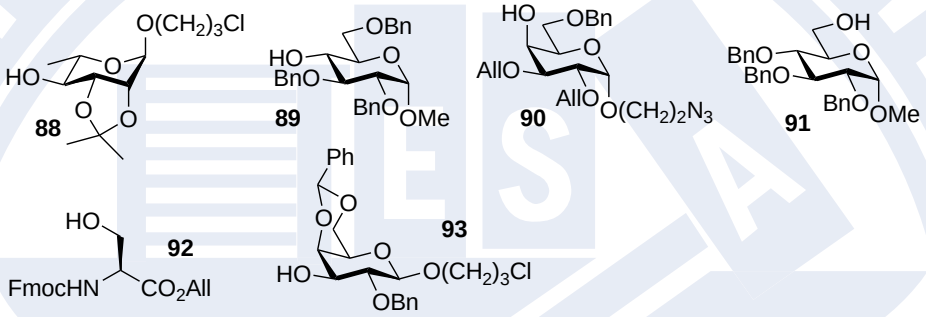
表 2.7 不同醣予體與醣受體的測試



Glycosyl donors



Glycosyl acceptors



entry	Donor	Acceptor	Product	time (h)	yield (%), α : β	yield (%), α : β [a]
1	**57**	**88**	**94**	0.5	65, 1 : 49	64, 1 : 1
2	**57**	**89**	**95**	0.75	63, 1 : 49	60, 1 : 8
3	**57**	**90**	**96**	0.5	60, β only	Not done
4	**65**	**91**	**97**	0.5	83, 1 : >49	80, 1 : 2
5	**57**	**92**	**98**	0.5	73, 1 : >49	75, 19 : 1
6	**76**	**93**	**99**	0.75	77, 1 : 32	78, 1 : 7

[a] 對照實驗 (100% CH_2Cl_2, –55 °C)。

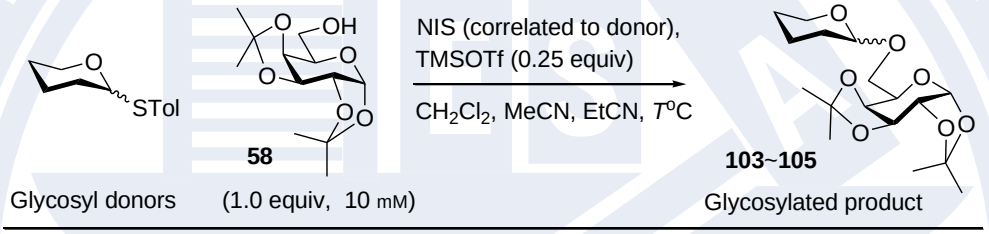
除此之外，在生物體中葡萄糖胺與半乳糖的 β(1→3) 鍵結是一個很重要的鍵結，[32] 因此我們也嘗試以疊氮基修飾的葡萄糖胺醣予體 **76** 與三號羥基半乳糖 **93** 橋接，得到了 77% 的產率與 97% 的 β-產

物 **99** (表 2.7，實驗 6)，這些實驗結果證明了低濃度腈類醣基化反應的應用廣度。

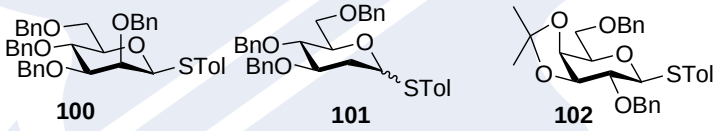
2.2.2.3　醣基化反應效果較差的配對

在這些測試當中，我們也發現到並非是每個醣予體與醣受體的配對都有很好的結果。

表 2.8. 醣基化反應效果較差的配對



Glycosyl donors



entry	Donor	Product	T (°C)	time (h)	yield(%), α : β
1	**100**	**103**	–70	0.5	85, 3:1
2	**101**	**104**	–70	0.5	80, 1:1.2
3[a]	**101**	**104**	-55	0.5	82, 1.2:1
4	**102**	**105**	-70 to 0	18	<10, --

[a]：以 CH_2Cl_2 作為溶劑

當採用甘露糖醣予體 **100** 進行低濃度醣基化反應時，得到 85% 的醣基化產物 **103**，但卻是 α-產物為主的結果 (表 2.8，實驗 1)。

接著使用張智為博士提供的二號去氧葡萄糖醣予體 **101** 與半乳

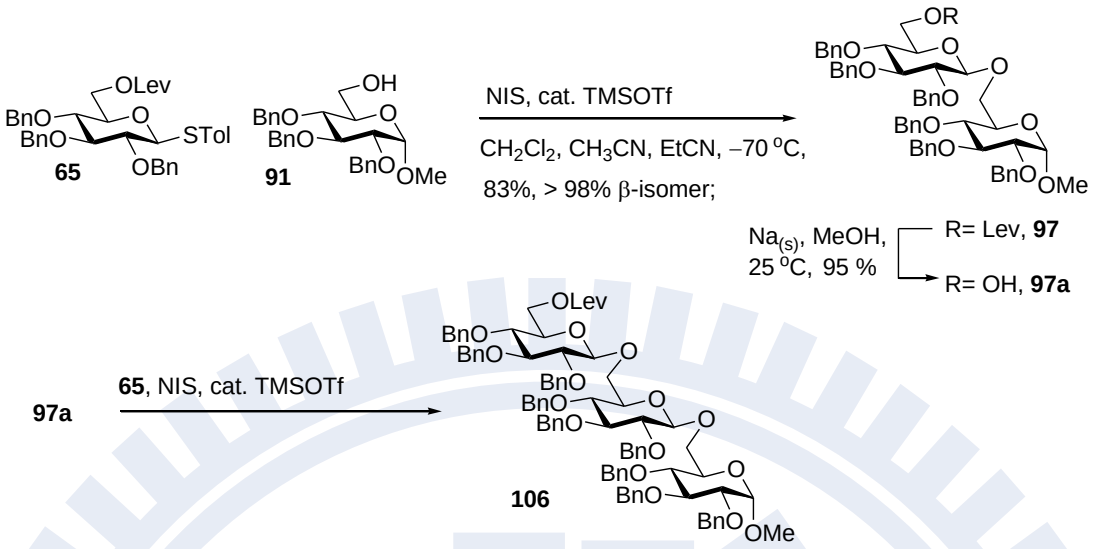
糖醣受體 **104** 進行測試，不論在腈類溶劑或二氯甲烷，雖然得到 80% 的產率，但選擇性均接近 α : β = 1 : 1 (表 2.8，實驗 2、3)。

若是回歸到半乳糖醣予體，我們發現到使用 3,4-縮酮保護的半乳糖醣予體 **102** 進行測試，反應速率相當緩慢，即使升高溫度到 0 °C 也無法得到令人滿意的產率 (表 2.8，實驗 4)，此類 3,4-縮酮保護之半乳糖醣予體在腈類系統的低反應性跟文獻記載相符。[28c]

2.2.3 寡糖合成的應用

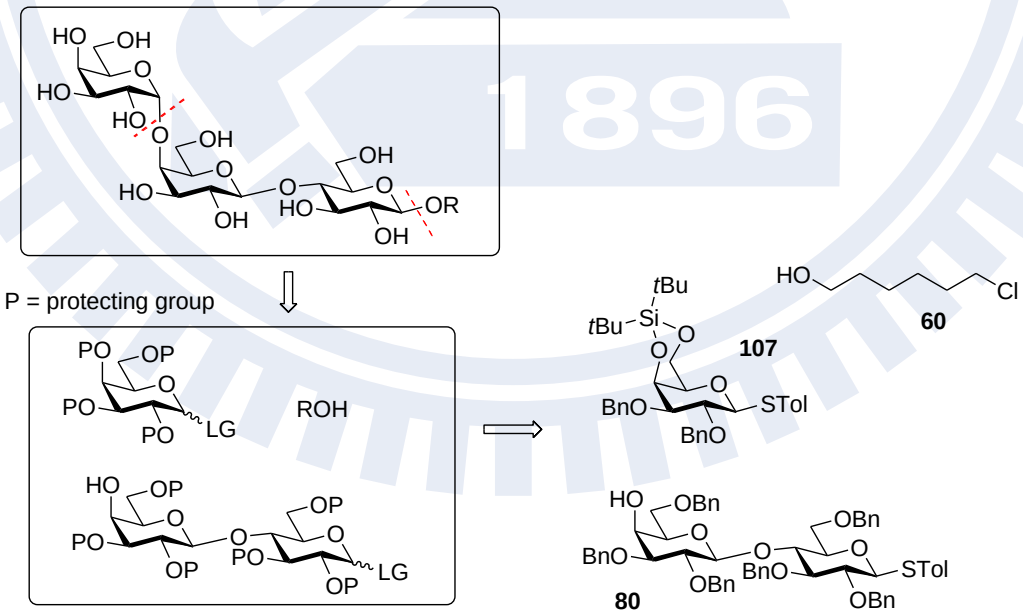
接著嘗試將低濃度醣基化反應應用於寡醣合成，根據之前的測試結果，我們挑選了幾個具生物活性的寡醣分子：β(1→6) glucan，[41] Gb_3，[42] 及 $isoGb_3$，[43] 嘗試應用低濃度醣基化反應合成寡醣結構中的 1,2-反式 β-醣苷鍵。

以 β (1→6) glucan 為起始，在得到雙糖分子 **97** 後，以 Zemplén 鹼性條件除去六號位置的酯基形成雙糖醣受體 **97a**，接著再以葡萄糖糖予體 **65** 與此雙糖醣受體 **97a** 進行低濃度醣基化反應，實驗結果可得到約 70% 的三醣分子 **106** 與近乎單一的立體異構物 (HPLC 只有一組訊號) (流程 2.16)。



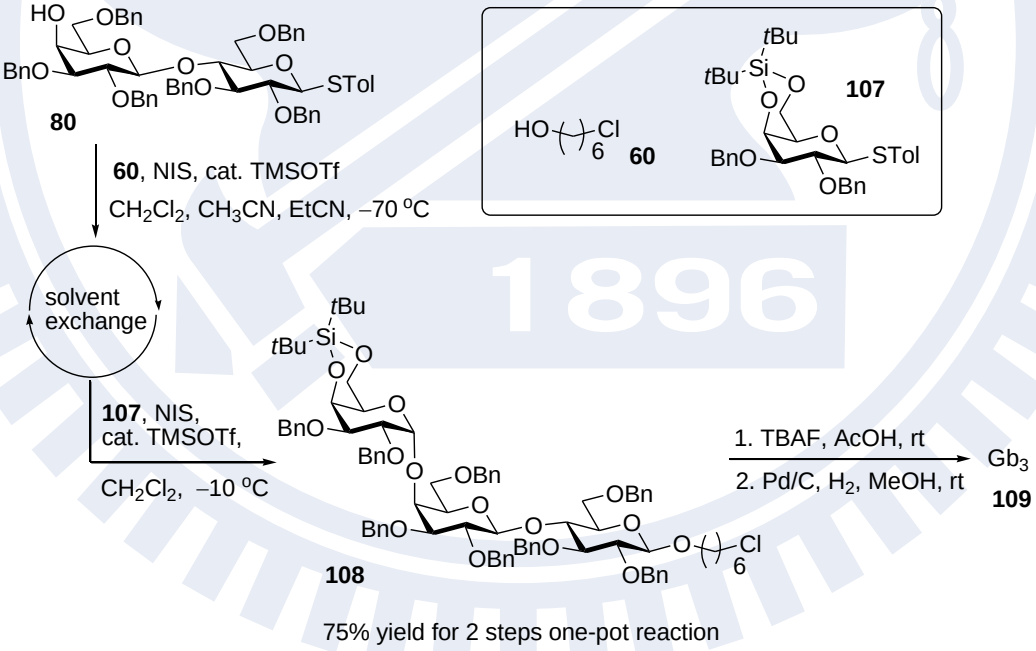
流程 2.16. β (1→6) glucan 衍生物的合成。

以此初步結果，鼓勵我們嘗試合成 Gb_3 分子，首先將此三糖分子拆解，設計使用乳糖分子先與具有一級醇的碳鏈形成 β-醣苷鍵，之後再以半乳糖與此乳糖分子受體建立 α-醣苷鍵。(流程 2.17)



流程 2.17. 逆合成拆解 Gb_3 衍生物

根據之前的實驗結果，乳糖分子 **80** 與長碳鏈醇 **60** 在低濃度醣基化反應的條件下可合成 β-異構物 **86**，在 TLC 確認得到雙糖分子 **86** 後，加入適量三乙基胺 (約 0.4 當量) 終止反應。接著以簡單的萃取除去鹽類、除水、過濾，再將此反應粗產物移除所有溶劑並真空乾燥數小時，然後加入二氯甲烷、半乳糖醣予體 **107** 與活化試劑在 −10 °C 下進行第二次醣基化反應。基於半乳糖上 4,6-矽烷保護基 DTBS (di-*tert*-butyl silyl ether) 上叔丁基的立體障礙，使得醣受體 **86** 只能由 α-方向攻擊醣基陽離子，進而建立 α-醣苷鍵而得到全保護的 Gb_3 的衍生物 **108** (流程 2.18)。

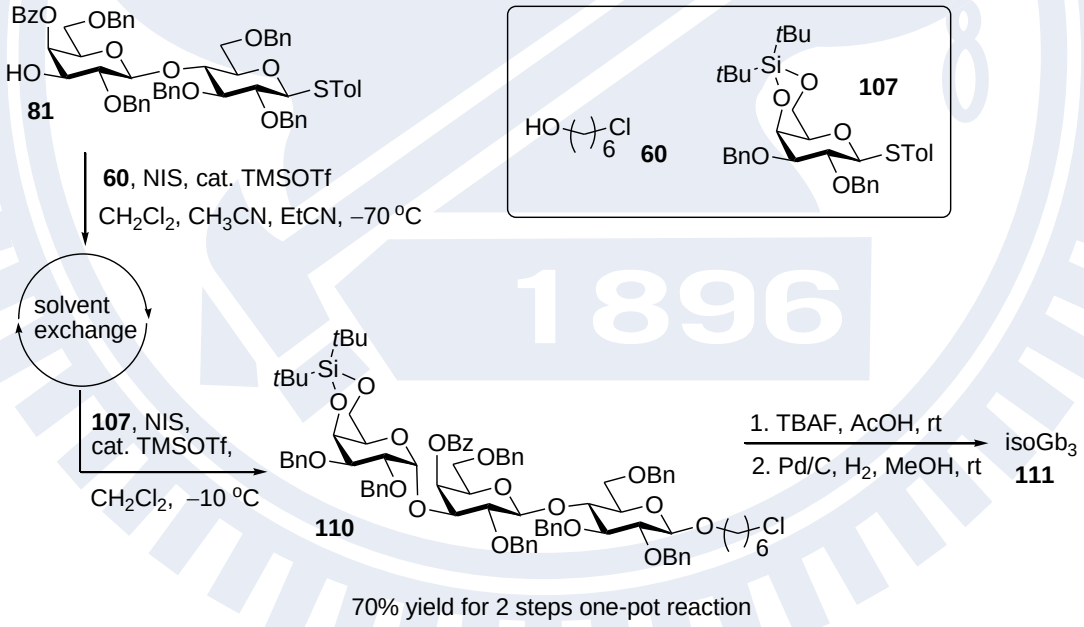


流程 2.18. 三糖分子 Gb_3**109** 的合成

連續兩步合成僅經過一次管柱純化，也不用修飾醣基端點官能基或乳糖分子的四號羥基，如此操作可得到 75%的產率。之後再以醋酸

與四丁基胺氟 (tetrabutylammonium fluoride，TBAF) 除去化合物 **108** 的矽烷保護基，並以 Pd/C 與一大氣壓氫氣氫化還原所有苄基即可得到去保護的 Gb_3 三糖分子 **109**，兩步去保護後純化產率可達 60%。

仿照相似的操作過程，改用乳糖分子 **87** 進行合成，可以得到 $isoGb_3$ 的全保護衍生物 **110**，連續兩步的產率有 70%；再經過三步去保護的操作後，即可得到預期產物 $isoGb_3$ 的三糖分子 **111**，全體去保護後純化產率約 60%。而 Gb_3 與 $isoGb_3$ 的合成工作主要由本實驗室李振瑋同學完成。(流程 2.19)



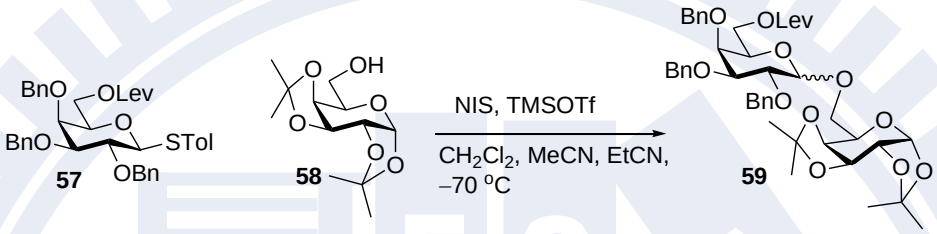
流程 2.19. 三糖分子 $isoGb_3$**111** 的合成

2.2.4 低濃度醣基化反應的劑量影響探討

除了將低濃度醣基化反應應用於寡醣合成外，我們也好奇為什麼

低濃度腈類溶劑系統進行醣基化反應可以提升立體選擇性？於是嘗試設計使用不同計量的反應物種進行比較，究竟哪個因素對立體選擇性的影響較大 (表 2.9)？

表 2.9. 改變劑量對醣基化反應的影響



entry	1, equiv, mM	2, equiv, mM	NIS, equiv, mM	TMSTOf, equiv, mM	yield%, α : β
1	1.2, 12	1.0, 10	1.2, 12	0.24, 2.4	81, 1 : 19
2	1.2, 12	2.5, 25	1.2, 12	0.24, 2.4	80, 1 : 18
3	2.4, 24	1.0, 10	2.4, 24	0.24, 2.4	82, 1 : 15
4	2.4, 24	1.0, 10	6.0, 60	0.24, 2.4	85, 1 : 15
5[a]	2,4, 24	1.0, 10	1.2, 12	0.24, 2.4	80, 1 : 17
6	1.2, 12	1.0, 10	1.2, 12	0.48, 4.8	77, 1 : 17
7	1.2, 1.2	1.0, 10	1.2, 12	0.96, 9.6	75, 1 : 15
8	4.8, 48	1.0, 10	4.8, 48	0.24, 2.4	84, 1 : 12
9	6.0, 60	1.0, 10	6.0, 60	0.24, 2.4	80, 1 : 10
10	1.2, 12	1.0, 10	1.2, 12	0.24, 2.4	80, 1 : 18[b]

[a]0.8 equiv **1** was recovered，[b] 將 **1**，**2** 加入 NIS 與 TMSOTf，5 mL/20 min

由表 2.9 的實驗結果 1、2、3 發現，當醣受體 **58** 增加為 2.5 倍時，

α/β 選擇性會下降為 1/18；增加醣予體、醣受體、*N*-碘化丁二醯胺、三甲矽烷三氟甲磺酸為原本的兩倍時，α/β 選擇性下降為 1/15；也就是說醣受體的濃度對選擇性的影響可能不如醣予體、*N*-碘化丁二醯胺、三甲矽烷三氟甲磺酸來得大。

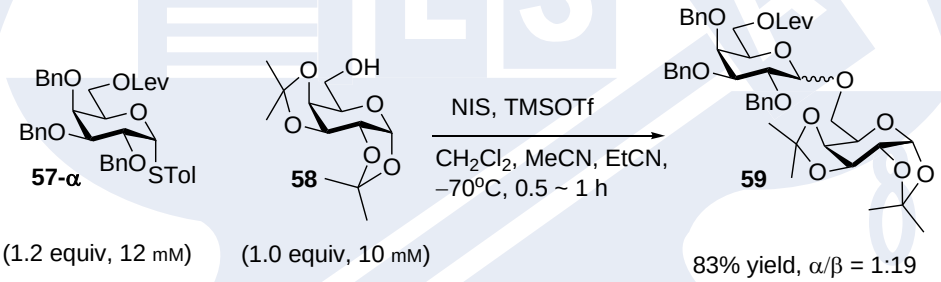
接著我們單純增加 *N*-碘化丁二醯胺的計量為 6 當量，發現到 α/β 選擇性與實驗 3 一樣為 1/15。由實驗 3、5 的比較可推論改變 *N*-碘化丁二醯胺的計量對立體選擇性的影響較小 (表 2.9，實驗 4)。

接著我們嘗試降低 *N*-碘化丁二醯胺的計量為醣予體 **57** 的一半，卻發現到選擇性為 1/17，並且回收了 0.8 當量的醣予體 **57**。我們推論當 *N*-碘化丁二醯胺比醣予體少時，反應中被活化產生的醣基陽離子相對較少，由選擇性的下降多寡推測，判斷醣基陽離子對反應選擇性的影響較其他物種大 (表 2.9，實驗 5)。

若改以實驗 1 的條件單純增加三甲矽烷三氟甲磺酸為原本的兩倍與四倍時，選擇性也下降為 1/17 與 1/15 (表 2.9，實驗 6、7)，與實驗 2、3 相比較，我們推論三甲矽烷三氟甲磺酸的增加對選擇性與產率均有負面影響。接著我們嘗試增加醣予體為原本的四倍、五倍並將之活化，果然反應選擇性相對下降，由其下降的幅度來說，增加任一反應物種的劑量都會降低產物的選擇性，但是以醣基陽離子為選擇性的影響主因 (表 2.9，實驗 8、9)。基於這樣的推測，我們推測是否可

以將 *N*-碘化丁二醯胺與三甲矽烷三氟甲磺酸先在低溫下混合，然後把醣予體與醣受體溶於混合溶液中緩慢加入反應中 (表 2.9，實驗 10)，但這樣的稀釋方法並沒有得到更好的選擇性。

在確定醣予體對選擇性的重要性之後，我們懷疑醣予體的離去基位向對反應是否有影響呢？於是設計了 α-位向的硫醣予體 **57-α** 進行測試，結果發現依然得到 95%的 β-產物，由此斷定反應選擇性的來源只與醣基陽離子有關，與離去基的位向無關。(流程 2.20)



流程 2.20. 化合物 **57-α**的醣基化反應測試

2.2.5 變溫核磁共振實驗

那麼為什麼降低反應濃度可以得到較好的選擇性呢？回顧最初的實驗，我們發現到反應結凍的現象是因為溶劑結冰了嗎？反應溫度並未達到乙腈的凝固點 (–41 °C)；若根據拉午爾定律 (Raoult's law) 的描述，溶液會因非揮發溶質濃度的提升而使溶劑的蒸氣壓下降，進而使沸點上升與凝固點下降，這與我們觀察到現象不一致 (在溶質濃度較高時，反應會在溶劑凝固點以上的溫度結凍，但溶質濃度較低時

卻會降低結凍發生的溫度)；有趣的是當我們以純粹只有混合溶劑、沒有溶質的空白實驗進行降溫測試，反應需要降溫到約 –72 °C 才有明顯結冰的現象。

另外 Ito 教授的報導則指出，在結冰的系統中進行醣基化反應時，會產生因反應實際濃度提升，使反應速率加速、但立體選擇性下降的效果，這也不能解釋在低濃度腈類醣基化反應的實驗結果。[44]

於是我們嘗試利用低溫核磁共振實驗追蹤反應過程，找尋線索解釋濃度效應對於 α/β 選擇性的影響原因。

首先我們先以氘化甲醇進行溫度變化與水訊號位移的溫度定標實驗。之後再設計採用 25%氘化二氯甲烷與 75%氘化乙腈作為溶劑系統，並加入固定量的未氘化丙腈作為內標，並分別配製兩個不同受質濃度 (100 mM、10 mM) 的半乳糖基醣予體或醣受體作為樣品，進行變溫核磁共振實驗。

由氫譜分析中發現到：沒有醣分子的溶劑樣品，溫度的變化只有讓水的訊號有位移現象 (圖 2.4-a、2.4-b)。接著改用溶於氘化二氯甲烷與氘化乙腈混合系統的 100 mM 的硫糖 **57** 進行測試，卻發現當降溫到 –51 °C 時，勻場與鎖定的動作 (shiming、locking) 變得很困難，所得到的圖譜訊號變寬 (broaden) 至難以辨認 (圖 2.4-c、2.4-d)。而 10 mM 的樣品則可以降溫到 –56 °C 才發生訊號寬化現象 (圖

2.4-e、2.4-f)，若是將樣品單純溶於氘化二氯甲烷或是未氘化的丙腈中時，卻沒有發現到這種訊號寬化現象。(圖 2.4-g、2.4-h)

我們推測這種訊號寬化的現象可能代表樣品發生了凝膠化的現象 (gelation)，而不是單純的結冰現象。推測因為氘化乙腈的強介電性質 (dielectric property) 與較高的凝固點，使得溶劑分子緊緊包覆住溶質分子，並聚集 (aggregate) 在一起形成凝膠。這也只有在具有溶質的狀況下才會發生。

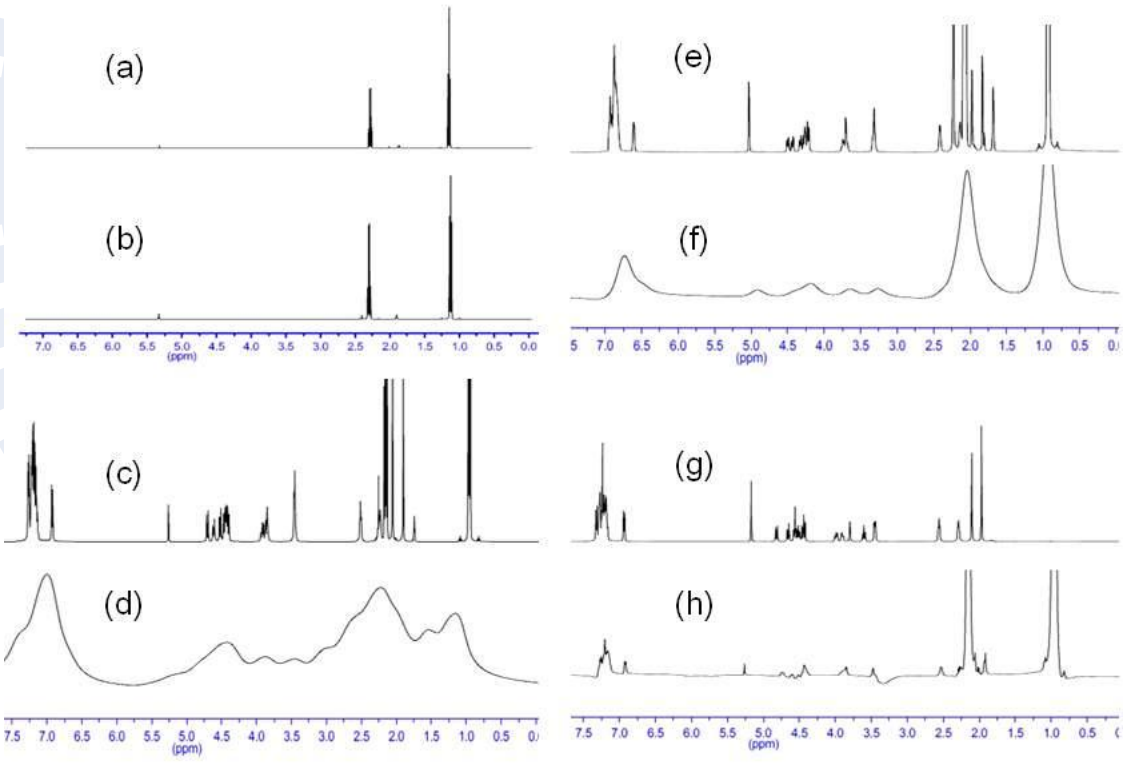


圖 2.4. 變溫核磁共振氫譜，(a) CD_2Cl_2、CD_3CN 與 EtCN 在 25 °C；(b) CD_2Cl_2、CD_3CN 與 EtCN 在–60 °C；(c) 100 mM **57** 於混合溶劑、–50 °C；(d) 100 mM **57** 於混合溶劑、–51 °C；(e) 10 mM **57** 於混合溶劑、

–55 °C；(f) 10 mM **57** 於混合溶劑、–56 °C；(g) 100 mM **57** 於 CD_2Cl_2、–70 °C；(h) 100 mM **57** 於 EtCN、–70 °C。

因此低濃度醣基化反應的結凍現象與單純的結冰是不太一樣的，經由核磁共振圖譜的分析，也暗示現在這個條件可能是一個非傳統的反應系統。此外，在不具有甲苯基團的醣受體 **58** 上也有相同的現象，推測這現象與受質濃度相關，但與芳香環關係不大。(圖 2.5)

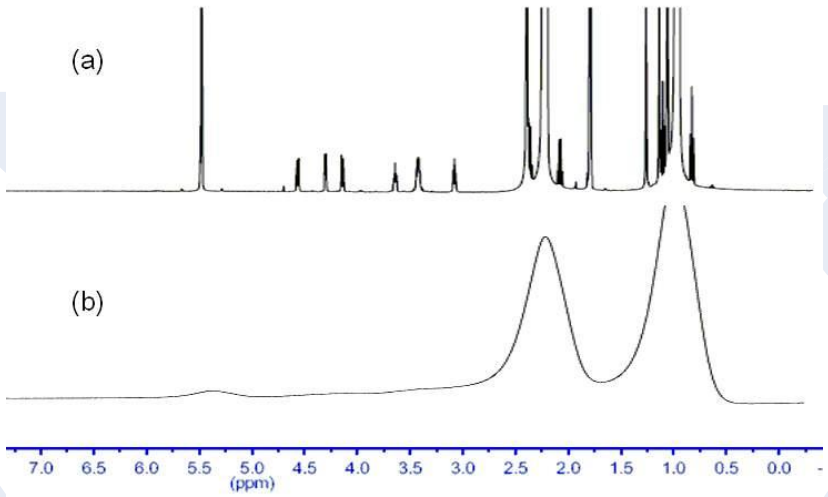


圖 2.5. 10 mM 化合物 **58** 於 CD_2Cl_2、CD_3CN 與 EtCN 混合溶劑的氫譜：(a) –55 °C；(b) –56 °C。

接著，我嘗試將 0.2 當量的三甲矽烷三氟甲基磺酸在低溫下加入樣品之中，發現在 100 mM 的樣品中，訊號寬化現象的發生溫度提升到 –46 °C，而 –45 °C 時測得的圖譜產生了一些新的訊號。至於 10 mM 的樣品在加入三甲矽烷三氟甲基磺酸後，訊號寬化現象的發生溫

度也從 –56 °C 提升到 –51 °C。而 10 mM 樣品的圖譜更出現與 100 mM 樣品不同的訊號。(圖 2.6)

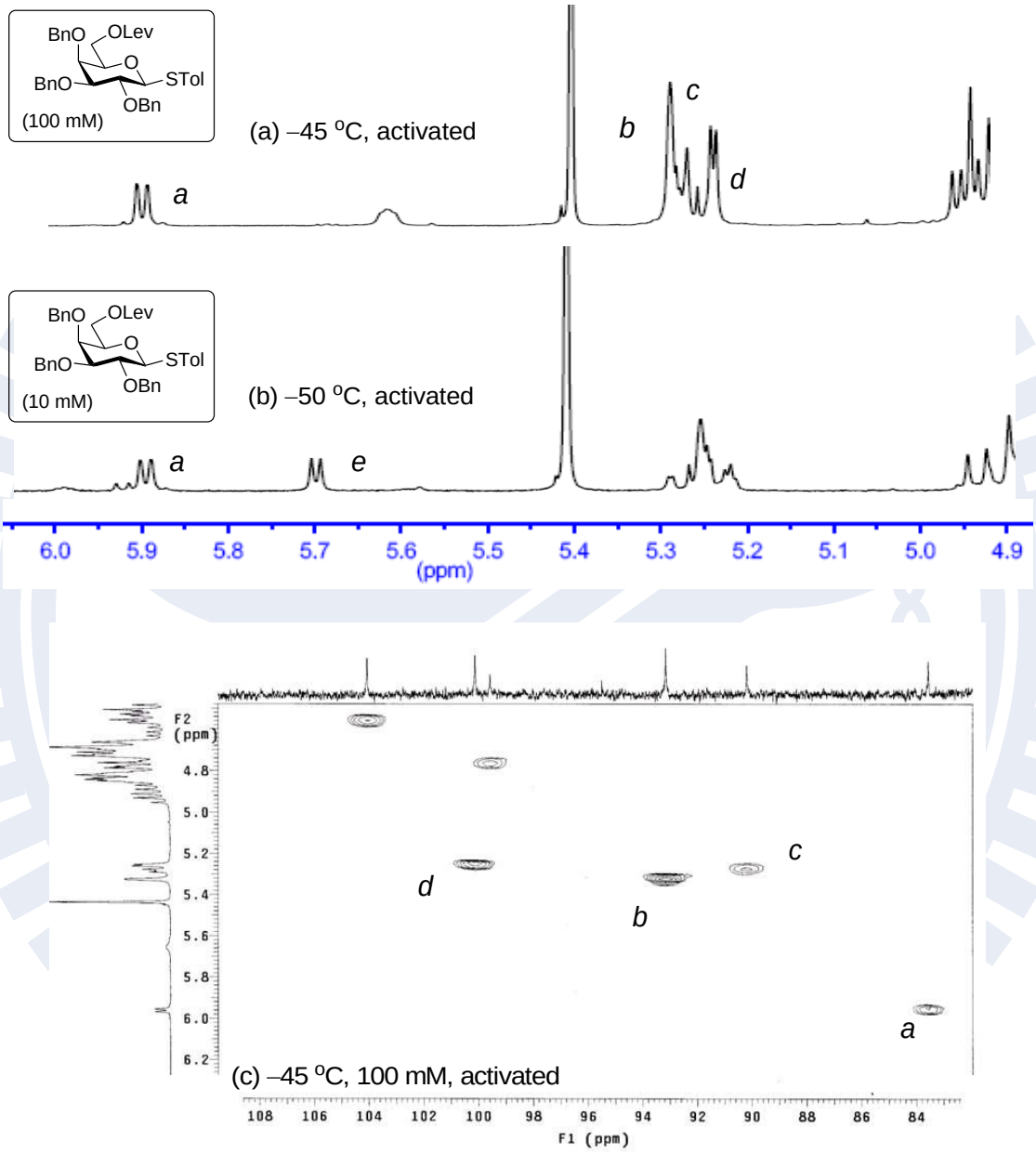
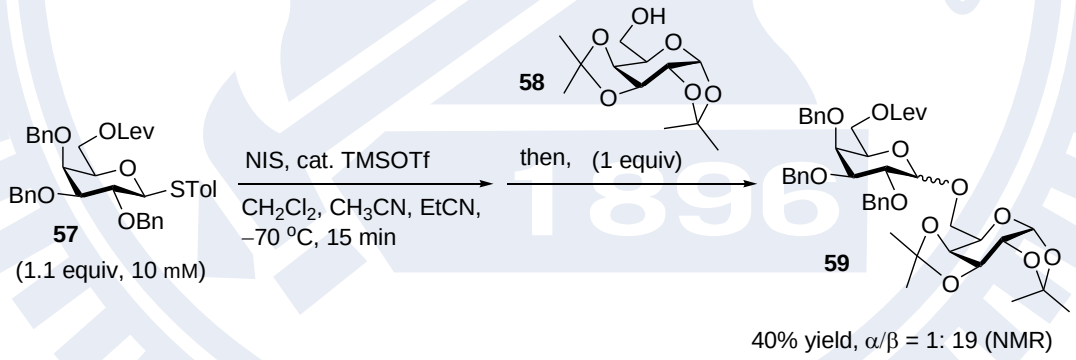


圖 2.6. 化合物 **57** 活化後的部份擷取圖譜:(a) 100 mM 樣品於 –45 °C 的氫譜;(b) 10 mM 樣品於 –50 °C 的氫譜;(c) 100 mM 於 –45 °C 的 HMQC 圖譜。

得到變溫核磁共振實驗的圖譜後，不禁要問：加入三甲矽烷三氟甲基磺酸後出現的新訊號代表甚麼？是醣基化反應的中間產物還是其他的旁反應產品？

於是，設計將半乳糖基醣予體 **57** 在乙腈混合溶劑中以 *N*-碘化丁二醯胺與三甲矽烷三氟甲基磺酸活化，待起始物消失後再將醣受體 **58** 加入反應，結果得到 β-位向為主的醣基化產物。因此推論，醣予體在乙腈系統中活化後的確產生了可進行醣基化反應的中間產物。這個結果也讓我們猜測是否可在腈類溶劑進行預先活化策略(preactivation)(流程 2.21)。



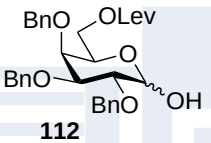
流程 2.21. 於低濃度腈類溶劑系統預先活化測試

確認了變溫圖譜屬於醣基化反應的中間產物，接著我們嘗試解釋訊號寬化溫度為甚麼會上升，推測因為在樣品中添加了強電解質或出現了強帶電物質，比如說醣基陽離子，使溶劑分子更加凝聚。

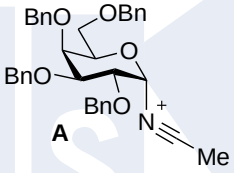
那麼，這些圖譜訊號又代表什麼意義呢？

一般醣分子端點的碳氫訊號，氫譜化學位移多介於 5~7 ppm、而碳譜介於 80~110 ppm 間，我們以此原則與文獻數據進行初步的判斷與比對。由實驗結果，首先確認醣基半縮醛的圖譜訊號，訊號 *b*、*d* 可能與水解產物 **112** 相關，但其它訊號又屬於甚麼物種呢 (表 2.10)？

表 2.10. 文獻比對可能物種訊號



112



A

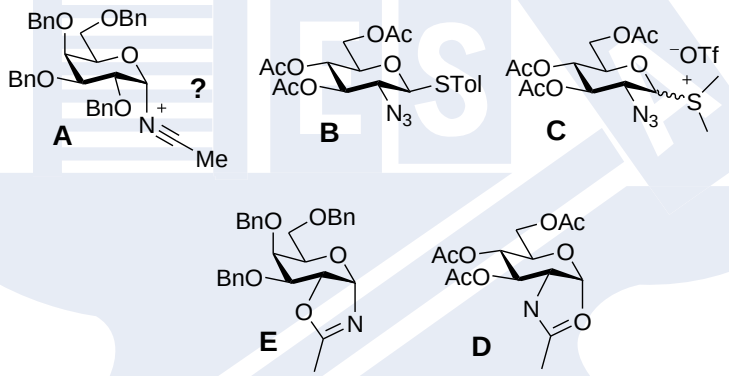
signal	^{1}H (ppm, Hz)	^{13}C (ppm)	Comound	H-1 (ppm, Hz)	C-1 (ppm)	Ref
a	5.94, 7, d	83	**112-**α	5.24, 3.5, d	93	--
b	5.33, 2, d	93	**112-**β	4.7, 10, d	98	--
c	5.27, 5, d	90	**A**	6.02, 6.8, d	83	17c
d	5.24, 3.5, d	100				
e	5.73, 5.5, d	--				

由 Sinaÿ 教授利用 enol ether 醣予體與三甲矽烷三氟甲磺酸在 –30 °C 下混合，在 24 小時後進行了變溫核磁共振實驗，並重複這樣的操作過程得到一系列圖譜訊號，他判斷這些訊號來自於醣基 α-nitrilium 陽離子 **A**。[17c]

假設訊號 *a* 就是文獻中提到的陽離子 **A** 的端點訊號。然由化合物 **K**、**J** 卻暗示我們當化合物帶電荷時，其化學位移與中性分子將

有所不同，其氫譜訊號將往左側 down field 位移、碳譜訊號將往右側 up field 位移、但其氫偶合常數並沒有太大改變；此外在反應溶液中很難存在單獨的電荷，必需要有一個相反電性的離子 (counter ion) 來維持電中性；比如說：在二氯甲烷溶劑系統中，三甲矽基三氟甲磺酸根陰離子 (triflate anion) 往往被認為扮演這樣的角色 (表 2.11)。

表 2.11. 文獻比對可能物種訊號



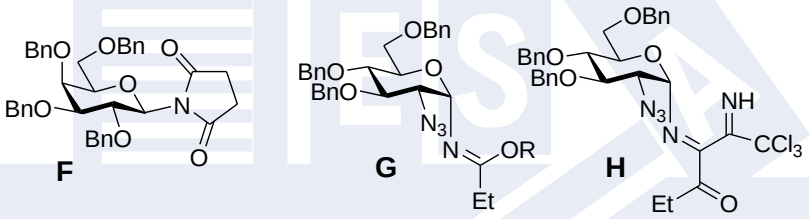
signal	^{1}H (ppm, Hz)	^{13}C (ppm)	Comound	H-1 (ppm, Hz)	C-1 (ppm)	Ref
a	5.94, 7, d	83	**A**	6.02, 6.8, d	83	17c
b	5.33, 2, d	93	**B**	4.4, 10, d	86	---
c	5.27, 5, d	90	**C-β**	5.42, 10.3, d	81	47
d	5.24, 3.5, d	100	**D**	5.98, 7.3, d	99.7	37k
e	5.73, 5.5, d	--	**E**	5.75, 6.6, d	(93.5)	16

那麼在腈類系統中，最可能的 α-nitrilium 結構會是什麼呢？又有甚麼樣的結構可以得到 7 Hz 的偶合常數呢？翻查文獻，我發現了六員環的氧氮雜環分子 **D** 的端點偶合常數是 7.3 Hz！這個數據觸

動我再搜尋文獻，發現 Danishefsky 教授曾經由醣基三員環醚的開環反應得到一個化合物 **E**，此物種端點的氫偶合常數很接近訊號 *a*，其碳氫訊號的位移差異也很接近觀察比對，那麼這是不是暗示我們醣予體在乙腈中活化時可能有類似的結構產生呢？[47]

接著再探討訊號 *e* 又是什麼物種的訊號呢 (表 2.12)？

表 2.12. 文獻比對可能物種訊號



signal	^{1}H (ppm, Hz)	^{13}C (ppm)	Comound	H-1: ppm, Hz	C-1: ppm	Ref
a	5.94, 7, d	83	**F**	6.2, 10, d	80	48
b	5.33, 2, d	93	**G**	5.2, 4.1, d	83	20b
c	5.27, 5, d	90	**H**	5.46, 4.9, d	81	20b
d	5.24, 3.5, d	100				
e	5.73, 5.5, d	--				

就文獻上我們搜尋到相關的化合物的數據進行比對，先排除訊號 *e* 是丁二醯胺產物 **F** 的可能；接著比對 Ritter 反應產物 **G**，就偶合常數判斷也不相像。在 Hashimoto 教授的報導中，有一個有趣的重排反應產物 **H**，其端點訊號卻有點接近訊號 *e* ，這是否暗示我們醣基 α-nitrilium 若接上一個龐大的官能基團，比如說：亞胺 (imine)，

其圖譜訊號就顯得跟訊號 *e* 很相近。[20b]

這讓我回想起 Schmidt 教授曾經提到 α-nitrilium-nitrile 過渡態可以遮蔽醣基陽離子的 α-位向，並且分散、穩定醣基陽離子的正電荷；這個 nitrilie-oligomer 不就是一個龐大的官能基團嗎？[14a]

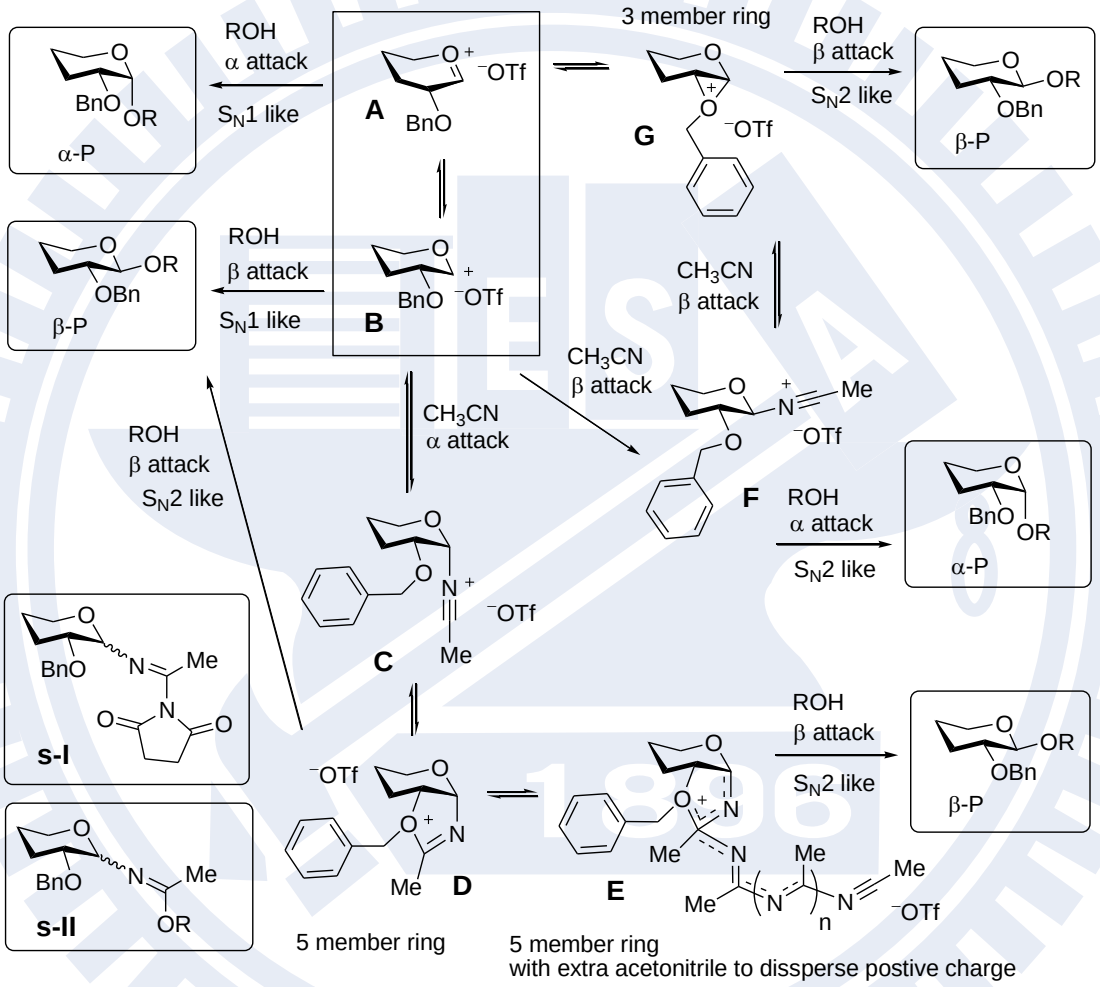
2.2.6 低濃度醣基化的反應機構推測

基於以上的觀察與推論，於是我大膽地假設了一個反應機構，嘗試解釋為何降低受質濃度會有提升 β-選擇性的效果，而此反應恰又只有在葡萄糖、葡萄糖胺、半乳糖、半乳糖胺、乳糖、岩藻糖等結構的醣予體有較好的 β-選擇性 (流程 2.22)。

基於離去基位向並不影響反應結果，因此假設影響立體選擇性的動作將由離去基離去後的醣基陽離子決定。綜合以上實驗觀察推論：醣分子會先被溶劑分子包圍形成類似微胞 (micelle) 的結構。[49] 然後離去基被活化離去而形成醣基陽離子的過渡狀態 **A**、**B**；此時有幾個可能的潛在親核試劑: 丁二醯胺、醣受體、雙硫酚分子、腈類分子。

就親核性上考量，先排除雙硫酚，再論丁二醯胺的氮形成 *N*-TMS 可以增加親核性，但因為亞胺基團 (imide) 的拉電子性質讓丁二醯胺分子不易有強親核性，因此醣受體與腈類溶劑分子會是較可能的親核試劑。再以數量與相對位置上考慮，腈類溶劑分子應優先攻擊醣基陽離子；最後再考慮到腈類分子的強介電常數性質，使得腈類分子很容

易包覆帶電物種，分攤電荷或分開反性離子 (counter ion)，所以假設此反應中產生的三甲矽基三氟甲磺酸根陰離子應該被溶劑分子包圍遠離醣基陽離子，而非與醣基陽離子有緊密連接。



流程 2.22. 低濃度醣基化反應的機構推測

以下將可能的路徑做解釋，第一條路徑：(**A**、**B** → β-**P**、α-**P**)；過渡態 **A**、**B** 可能進行分子內或分子外反應，若是進行類似 S_N1 的分子間反應，醣受體將不受控制，形成 α-或 β-產物。

第二條路徑：(**A**、**B** → **G** → β-**P**)；過渡態 **A**、**B** 先行分子內反應，α-C2 異原子可能提供孤對電子形成 α-醣基三員環醚陽離子 **G** (oxiranium-OTf)，醣受體再由三員環醚 **G** 的反向進入形成 β-產物。

第三條路徑：(**A**、**B** → **G** → **F** → **G** → β-**P**)；若由腈類分子由三員環醚 **G** 反向進入，則可能形成物種 **F** (β-nitrilium-OTf)。此時再次由 α-C2 氧原子與醣受體競爭，進行分子內反應則再次形成 α-三員環醚 **G**，之後再被醣受體攻擊開環形成 β-產物。

第四條路徑：(**A**、**B** → **F** → α-**P**)；若物種 **F** 進行分子間反應，則醣受體會反向取代 β-nitrilium 產生 α-醣基化產物，推論當低濃度時，分子內反應較分子間反應快，故 **F** 較可能再轉換為三員環醚 **G**，而較不易直接產生 α-醣基化產物。

第五條路徑：(**A**、**B**→ **F**→ **G**)；腈類分子因其強介電性質由 α-位向或 β-位向接近醣基陽離子 **A**、**B** 的端點位置；若形成物種 **F** (β-nitrilium) 則有機會被 α-C-2 氧原子攻擊離去，形成分子內 α-三員環醚 **G**，之後進入上述路徑循環。

第六條路徑：(**A**、**B** → **C** → **D** → β-**P**)；若腈類分子由 α-位向與過渡態 **A**、**B** 形成物種 **C** (α-nitrilium)，**C** 也有機會被 α-C2 氧原子攻擊產生 α-6,5-氧氮雜環陽離子 **D** (oxazolinium-OTf)，若 **D** 與醣受體分子橋接，將形成 β-產物。依照核磁共振實驗分析得到的訊號 *a*

與文獻對照，相信氮氧雜環 **D** 會是 α-nitrilium 的真貌。

第七條路徑：(**A**、**B** → **C** → **D** → **E** → β-**P**)；此雜環陽離子 **D** 可能再與其他的腈類溶劑分子相互靠近以穩定正電荷，形成 Schmidt 教授提到的 α-nitrilium-nitrile 過渡態 **E**。[14a] 基於變溫核磁共振實驗觀察到的訊號寬化現象，與實際醣基化反應溫度遠在乙腈的凝固點之下，猜測醣基化反應中可能形成了「凝膠態」，此種非傳統溶液系統令過渡態 **E** 成為相對穩定的物種，也就是在低濃度變溫核磁共振實驗中觀察到的訊號 *e*；之後醣受體再由 β-位向攻擊醣基端點位置形成 β-產物。因為訊號 *e* 只在低濃度出現，相信第七路徑與前六個路徑相比，可能就是低濃度提升 α/β-選擇性的原因。

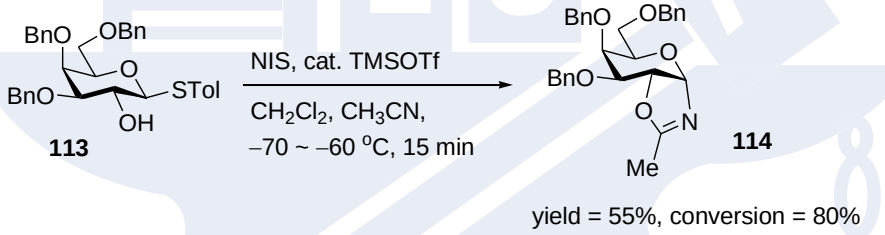
丁二醯胺或醣受體也有機會與氧氮雜環陽離子形成亞胺產物 **s-I** 或 6,5-氧氮雜環-丁二醯胺產物 **s-II** (oxazolinium-succimide)，但這些產物均未觀察到。推測因為溶劑分子相對數量較多，使得 α-nitrilium-nitrile 過渡態遮蔽了其他潛在親核基分子的攻擊機會。

歸納推測路徑：腈類溶劑中得到 β-選擇性的因素，一個是藉由 α-C2 原子的鄰基效應形成分子內三員環醚，但此結構的環張力較大，這樣的反應路徑應在低溫較有機會發生，相信這也是在二氯甲烷溶劑系統中，溫度降低、β-產物增加的原因之一。

另一個重要因素便是醣分子 α-C2 異原子利用其孤對電子穩定

α-nitrilium，形成α-6,5-氮氧雜環 **D** ，或是形成 α-nitrilium-nitrile 過渡態 **E**，並因為採用乙腈混合作為溶劑，在低溫下產生類似凝態的效果，加強了狀態的存在，強化了 α/β-選擇性。

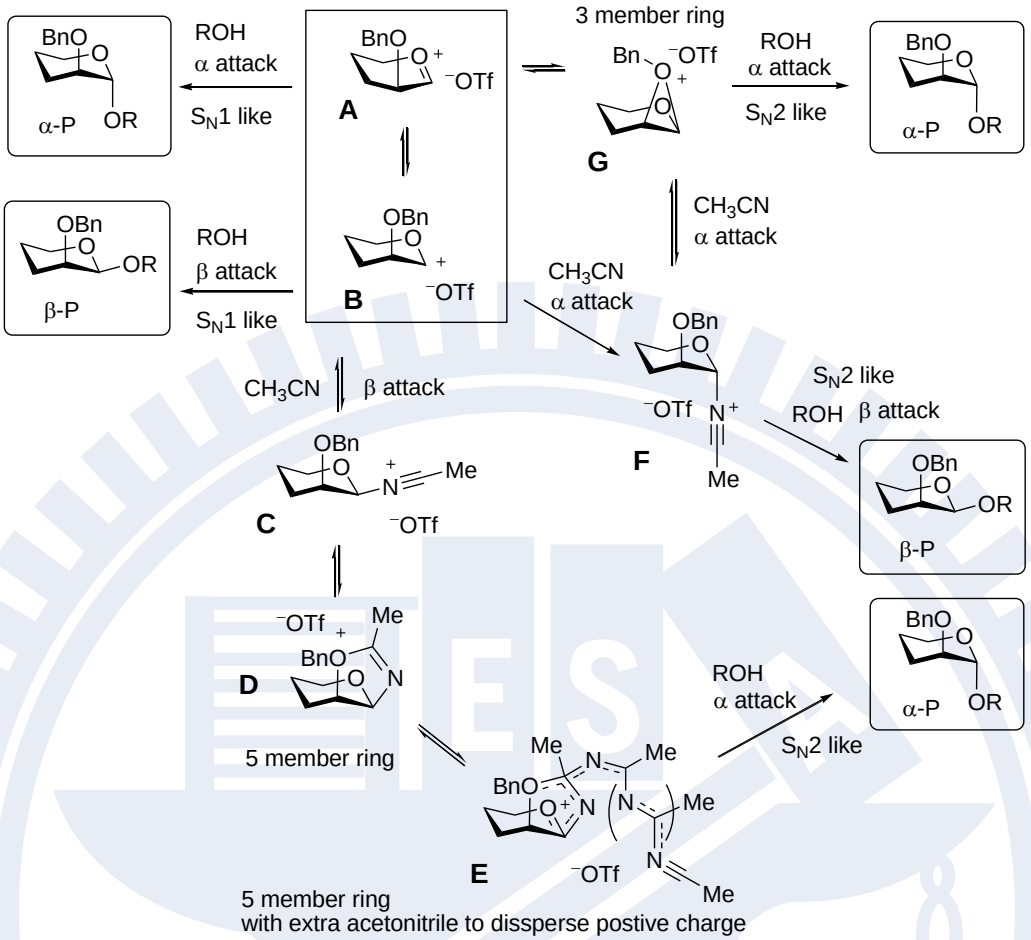
為了驗證這個假設，於是設計了一個實驗，利用 C2-羥基半乳糖對甲基硫酚 **113** 在 −70 °C、低濃度的乙腈與二氯甲烷混合溶劑系統中進行活化，結果並未得到醣基化反應雙糖產物，而是得到 55%的 α-6,5 氮氧雜環化合物 **114** (TLC conversion = 80%)(流程 2.23)。



流程 2.23. 乙腈混合溶劑中得到 α-6,5 氮氧雜環化合物

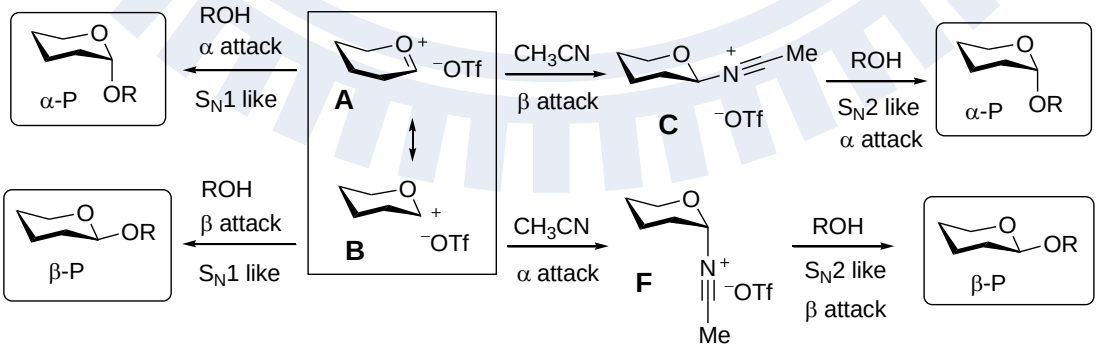
以上的推測著重在醣分子具有 α-C2 異原子，若醣分子沒有 α-C2 異原子，而具有 β-C2 異原子 (比如說：甘露糖 mannose、鼠李糖 rhamnose) 或根本沒有 C2-異原子 (C2-deoxysugar) 呢？

仿照之前推測 α-C2 異原子的推測，β-C2 異原子會形成 β-醣基三員環醚過渡態 **G**、或是 β-6,5 氧氮雜環過渡態 **D**；從而得到 α-為主的醣基化產物，根據之前的實驗結果 (表 2.8，實驗 1) 也支持這樣的假設 (流程 2.24)。



流程 2.24. β-C2 異原子的醣基化反應機構推測

若 C-2 異原子換成氫，則因為氫原子並沒有孤對電子可提供穩定效果，其醣苷鍵的立體選擇性相對趨近 1 : 1。(流程 2.25)



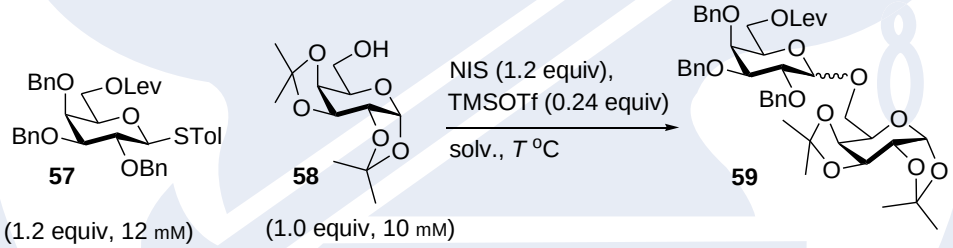
流程 2.25. C2-deoxy sugar 的醣基化反應機構推測

除了氧原子可以引導產生立體選擇性，若是 α-C2-疊氮基，一樣可以得到很好的 β-選擇性，而其他的異原子是否也可以有相同的表現，則需要再進一步的確認。

2.2.7 針對丙腈與乙腈的比較

當在變溫核磁共振中發現到疑似「凝膠化」的特殊物理現象後，再次比較丙腈與乙腈混合溶劑的差別。

表 2.13. 丙腈與乙腈混合溶劑的測試



entry	Solvent component			T (°C)	yield (%), α/β
	CH_2Cl_2 (%)	CH_3CN (%)	EtCN (%)		
1	25	75	0	–30	82, 1 : 7
2	25	0	75	–30	84, 1 : 7
3	25	75	0	–70	80, 1:19
4	25	0	75	–70	82, 1:13

當反應溫度在 –30 °C 時，兩者的反應性與選擇性並無明顯差異(表 2.13，實驗 1、2)，但當溫度下降到 –70 °C，兩者出現差異。乙

腈可以達到 1:19 的選擇性、但丙腈卻得到 1:13 的選擇性 (表 2.13，實驗 3、4)。

這些實驗結果間接支持凝膠化對於反應立體選擇性的正面影響。

2.3 結論

以上的工作針對腈類溶劑系統中的醣基化反應的濃度效應做一初步探討，在搜尋出有效反應條件後再將之應用於不同的醣予體與醣受體配對，摸索其適用範；並嘗試將低濃度醣基化反應推廣至簡單的寡糖分子合成。這部份的工作已發表在 *Chem. Eur. J.* **2009**, *15*, 10972–10982。除了應用以外，我們嘗試以計量變化、變溫核磁共振、中間產物等的實驗推測腈類溶劑系統中的醣基化反應的可能機構，這方面的工作與低濃度醣基化反應的延伸應用將繼續進行。

2.4 實驗部分

2.4.1 一般實驗方法敘述

請參考 1.4.1 的描述。

2.4.2 化合物合成方法與物理數據

General procedure of low concentration 1,2-*trans* β-selective glycosylation: A mixture of thioglycoside (1.2 mol equiv), acceptor (1.0 mol equiv) and flame-dried molecular sieve (AW300) were suspended in 1:3 v/v CH_2Cl_2-CH_3CN or 1: 2:1 v/v CH_2Cl_2-CH_3CN-EtCN solvent mixture such that the final concentrations of thioglycoside and acceptor were 12 and 10 mM respectively (for particular details see Table S1 in ESI). The resulting mixture was stirred at RT for 10 min and at –70˚C cooling bath for additional 20 min under N_2, followed by addition of NIS (1.2 mol equiv) and TMSOTf (0.24 mol equiv). Glycosylation coupling was monitored by TLC with either EtOAc/Hexane or EtOAc/CH_2Cl_2/Hexane mixture as developing solvent. Upon complete glycosylation, 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 MS was removed by filtration over celite. The filtrate was dried (over $MgSO_4$), filtered, and concentrated for flash chromatography purification to furnish glycosylation products for NMR characterization. For α:β-anomeric ratio determination, a small portion of crude reaction mixture was filtered through a short pad of silica gel (ca. 10 cm) to give crude product mixture for HPLC analysis as described in table 3.4.1.

Table 3.4.1: Eexperimental details and exact amounts of thiolgycosyl donor, acceptor, NIS and TMSOTf used in glycosylations

Thioglycosyl donor (mg, mmol, mM)	acceptor (mg, mmol, mM)	NIS (mg, mmol, mM)	TMSOTf (μL, mmol, mM)	CH_2Cl_2, CH_3CN, EtCN (mL)	product
57, 150, 0.23, 12	**58**, 0.19, 10	54, 0.24, 12	8, 0.046, 2.3	5, 11, 4	**59**[a]
61, 162, 0.25, 10	**60**, 51, 0.38, 16	60, 0.26, 11	9, 0.050, 2.3	6, 13, 5	**68**[a]
62, 137, 0.23, 12	**58**, 50, 0.19, 10	54, 0.24, 12	8, 0.046, 2.3	5, 11, 4	**69**[a]
63, 134, 0.23, 12	**58**, 50, 0.19, 10	54, 0.24, 12	8, 0.046, 2.3	5, 15, 0	**70**[a]
64, 128, 0.23, 6	**58**, 50, 0.19, 5	54, 0.24, 6	8, 0.046, 1.2	10, 21, 9	**71**[a]
65, 150, 0.23, 12	**58**, 50, 0.19, 10	54, 0.24, 12	8, 0.046, 2.3	5, 15, 0	**72**[b]
66, 150, 0.23, 12	**58**, 50, 0.19, 10	54, 0.24, 12	8, 0.046, 2.3	5, 11, 4	**73**[a]
67, 113, 0.21, 10	**60**, 43, 0.32, 16	50, 0.22, 11	8, 0.042, 2.2	5, 11, 4.5	**74**[a]
76, 134, 0.23, 12	**58**, 50, 0.19, 10	54, 0.24, 12	8, 0.046, 2.3	5, 11, 4	**82**[a]
77, 75, 0.15, 10	**60**, 41, 0.30, 20	35, 0.16, 10	5, 0.029, 1.9	4, 7, 4	**83**[a]
78, 110, 0.20, 12	**75**, 29, 0.30, 18	46, 0.20, 12	7, 0.038, 2.3	4, 7, 4	**84**[a]
79, 248, 0.23, 12	**58**, 50, 0.19, 10	54, 0.24, 12	8, 0.046, 2.3	5, 11, 4	**85**[a]
80, 207, 0.21, 11	**60**, 57, 0.42, 21	50, 0.22, 11	8, 0.042, 2.1	5, 11, 4	**86**[a]
81, 281, 0.28, 10	**60**, 76, 0.56, 20	67, 0.29, 10	10, 0.056, 2.0	7, 14, 7	**87**[a]
57, 175, 0.26, 12	**88**, 60, 0.21, 10	61, 0.27, 12	10, 0.053, 2.4	5, 12, 5	**94**[a]
57, 149, 0.23, 12	**89**, 90, 0.19, 10	54, 0.24, 12	8, 0.046, 2.3	5, 11, 4	**95**[b]
57, 159, 0.24, 12	**90**, 85, 0.20, 10	57, 0.25, 13	9, 0.050, 2.5	5, 11, 4	**96**[a]
65, 149, 0.23, 12	**91**, 90, 0.19, 10	54, 0.24, 12	8, 0.046, 2.3	5, 11, 4	**97**[a]
57, 150, 0.23, 115	**92**, 70, 0.19, 95	54, 0.24, 120	8, 0.046, 23	2, 0, 0	**98a**[b]
57, 150, 0.23, 12	**92**, 70, 0.19, 10	54, 0.24, 12	8, 0.046, 2.3	5, 11, 4	**98b**[a]
76, 128, 0.22, 12	**93**, 80, 0.18, 10	52, 0.23, 13	8, 0.044, 2.4	4, 10, 4	**99**[a]
100, 149, 0.23, 12	**58**, 50, 0.19, 10	54, 0.24, 12	8, 0.046, 2.3	5, 11, 4	**103**[a]
113, 150, 0.27, 10	**(Non)**	67, 0.29, 11	10, 0.05, 1.85	7, 20, 0	**114**

[a] Glycosylation at –70˚C; [b] Glycosylation at –55˚C

VT-NMR experiments for thiogalactopyranoside (57): To thioglycoside **57** (33 mg for 100 mM or 3.3 mg for 10 mM) in an oven-dried NMR tube was added 500 μL of 1:3 v/v CD_2Cl_2-CD_3CN (with 25 μL of EtCN added as internal reference) at RT under N_2. The resulting mixture was then analyzed by NMR spectroscopy at 25 to –60˚C.

VT-NMR experiments for NIS/TMSOTf activated thiogalactopyranoside (57): To thioglycoside **57** (33 mg) in an oven-dried NMR tube was added 500 μL of 1:3 v/v CD_2Cl_2-CD_3CN (with 25 μL of EtCN added as internal reference) at RT under N_2. The resulting solution was cooled to –80˚C for ca.10 min, followed by addition of 2 μL TMSOTf in CD_2Cl_2 solution (10 μL). The resulting mixture was then

analyzed by NMR spectroscopy at −50 to −30˚C.

For **1,2,3,4-di-*O*-isopropylidene-6-*O*-(2',3',4'-tri-*O*-benzyl-6'-*O*-levulinoyl-1-β-D-galactopyranosyl)-α-D-galactopyranose (59)**: Synthesis of **59** was referred to the general procedure of low concentration 1,2-*trans* β-selective glycosylation. **9** (128 mg, 84 %) was obtained as glassy solid; $[\alpha]^{27}_{D}$ = −35.34 (*c* = 0.9, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.50–7.47 (d, *J* = 9.3 Hz, 2H, ArH), 7.46–7.28 (m, 13H, ArH), 5.59 (d, *J* = 4.95 Hz, 1H, H-1), 5.09 (d, *J* = 11.1 Hz, 1H), 4.88 (d, *J* = 12 Hz, 1H), 4.80–4.75 (m, 2H), 4.68 (d, *J* = 11.4 Hz, 1H), 4.61 (dd, *J* = 2.4, 7.8 Hz, 1H), 4.45 (d, *J* = 7.8 Hz, 1H), 4.33 (dd, *J* = 2.4, 5.1 Hz, 1H), 4.28–4.22 (m, 2H), 4.18–4.10 (m, 3H), 3.91–3.83 (m, 2H), 3.7 (dd, *J* = 6.9, 9.9 Hz, 1H), 3.58–3.54 (m, 2H), 2.76–2.71 (m, 2H, Lev-CH_2), 2.53–2.48 (m, 2H, Lev-CH_2), 2.20 (s, 3H, CH_3CO), 1.52 (s, 3H, CH_3C), 1.48 (s, 3H, CH_3C), 1.35 (s, 3H, CH_3C), 1.34 (s, 3H, CH_3C); ^{13}C NMR (75 MHz, $CDCl_3$): δ 206.9, 172.74, 139.4, 139.0, 138.7, 129.0, 128.9, 128.8, 128.6, 128.5, 128.1, 128.0, 127.8, 109.7, 109.0, 105.1, 96.8, 82.3, 79.3, 75.2, 74.8, 72.3, 71.9, 71.2, 70.9, 7.1, 67.8, 63.5, 38.3, 30.2, 28.2, 26.44, 26.41, 25.5, 24.8; HRMS (FAB): calcd for $C_{44}H_{54}O_{13}$ requires 790.3564; found: *m*/*z* = 790.3561 $[M]^+$.

For **6-chlorohexyl 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranoside (68)**: Synthesis of **68** was referred to the general procedure of low concentration 1,2-*trans* β-selective glycosylation. **68** (140 mg, 85%) was obtained as colourless oil. $[\alpha]^{27}_{D}$ = −4.24 (*c* = 1.90, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.46–7.31 (m, 20H, ArH), 5.59 (d, *J* = 4.8 Hz, 1H), 5.05–4.99 (m, 2H), 4.88–4.77 (m, 33, 3H), 4.71 (d, *J* = 11.7 Hz, 1H), 4.56–4.47 (m, 2H), 4.44 (d, *J* = 7.5 Hz, 1H), 1.85–1.72 (m, 4H), 1.52–1.45 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 139.3, 139.1, 139.0,

138.4, 128.9, 128.84, 128.75, 128.64, 128.50, 128.36, 128.01, 127.93, 104.4, 82.7, 80.1, 75.6, 74.96, 74.01, 73.98, 73.50, 70.22, 69.38, 45.50, 32.99, 30.06, 27.17, 25.96; HRMS (Bio-ToFII): calcd for $C_{40}H_{47}ClO_6Na$ requires 681.2959; found: *m*/*z* = 681.2953 $[M+Na]^+$.

For **1,2,3,4-di-*O*-isopropylidene-6-*O*-(4'-*O*-acetyl-2',3',6'-tri-*O*-benzyl-β-D-galactopyranosyl)-1-α-D-galactopyranose (69)**: Synthesis of **69** was referred to the general procedure of low concentration 1,2-*trans* β-selective glycosylation. **69** (120 mg, 85%) was obtained as colourless oil; $[\alpha]^{27}_D = -6.31$ (*c* = 1.2, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.50–7.48 (m, 2H, ArH), 7.42–7.28 (m, 13H, ArH), 5.63–5.61 (m, 2H), 5.08 (d, *J* = 11.1 Hz, 1H), 4.8 (d, *J* = 11.4 Hz, 1H), 4.72 (d, *J* = 11.1 Hz, 1H), 4.64–4.49 (m, 5H), 4.36 (dd, *J* = 2.4, 5.1 Hz, 1H), 4.27 (dd, *J* = 1.5, 7.8 Hz, 1H), 4.22–4.14 (m, 2H), 3.82–3.73 (m, 2H), 3.68–3.53 (m, 4H), 2.11 (s, 3H, CH_3CO), 1.54 (s, 3H, CH_3C), 1.49 (s, 3H, CH_3C), 1.36 (s, 6H, 2 × CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.8, 139.3, 138.4, 138.0, 128.91, 128.9, 128.7, 128.6, 128.52, 128.51, 128.3, 128.1, 127.8, 109.8, 109.0, 105.0, 96.8, 79.4, 79.0, 75.4, 74.1, 72.5, 72.4, 71.9, 71.2, 70.9, 70.4, 68.4, 67.8, 67.3, 26.4, 25.5, 24.9, 21.4; HRMS (Bio-ToFII): calcd for $C_{41}H_{50}O_{12}Na$ requires 757.3194; found: *m*/*z* = 757.3194 $[M+Na]^+$.

For **1,2,3,4-di-*O*-isopropylidene-6-*O*-(2',6'-di-*O*-benzyl-3',4'-di-*O*-methoxycarbonyl-β-D-galactopyranosyl)-α-D-galactopyranose (70)**: Synthesis of **70** was referred to the general procedure of low concentration 1,2-*trans* β-selective glycosylation. **70** (98 mg, 71%) was obtained as light yellow oil. $[\alpha]^{27}_D = -8.39$ (*c* = 0.53 , $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.39–7.26 (m, 10H, ArH), 5.57 (d, *J* = 5.1 Hz, 1H, H-1), 5.37 (d, *J* = 3.0 Hz, 1H), 5.02 (d, *J* = 11.4 Hz, 1H), 4.85 (dd, *J* = 3.3, 10.2 Hz, 1H), 4.65–4.47 (m, 5H), 4.33 (dd, *J* = 2.4, 4.8 Hz, 1H), 4.23 (dd,

J = 1.8, 8.1 Hz,1H), 4.16–4.07 (m, 2H), 3.82 (s, 3H), 3.80–3.58 (m, 8H), 1.52 (s, 3H, CH_3C), 1.46 (s, 3H, CH_3C), 1.33 (s, 6H, 2 × CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 155.9, 155.3, 138.9, 138.1, 128.8, 128.5, 128.4, 128.22, 128.2, 127.8, 109.8, 109.0, 104.5, 96.8, 76.9, 75.1, 74.0, 72.1, 71.9, 71.8, 71.1, 70.9, 0.1, 68.0, 67.8, 55.53, 55.51, 26.43, 26.38, 25.4, 24.8; HRMS (Bio-ToFII): calcd $C_{36}H_{46}O_{15}Na$ requires for 741.2729; found: *m*/*z* = 741.2729 $[M+Na]^+$.

For **1,2,3,4-di-*O*-isopropylidene-6-*O*-(2',3'-di-*O*-benzyl-4',6'-*O*-benzylidene-β-D-galactopyranosyl)-α-D-galactopyranose (71)**: Synthesis of **71** was generally based on the general procedure of low concentration 1,2-*trans* β-selective glycosylation, but lower concentrations (5 mM for acceptor **58** and 6 mM for donor **64**). **71** (110 mg, 83%) was obtained as white solid. $[\alpha]^{27}_D$ = –12.59 (*c* = 1.4, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.62–7.58 (m, 2H, ArH), 7.55–7.52 (m, 2H, ArH), 7.45–7.28 (m, 11H, ArH), 5.62 (d, *J* = 5.1 Hz, 1H, H-1), 5.54 (s, 1H, PhCH), 5.13 (d, *J* = 11.1 Hz, 1H), 4.84–4.77 (m, 3H), 4.63 (dd, *J* = 2.4, 7.8 Hz, 1H), 4.49 (d, *J* = 7.8 Hz, 1H), 4.36 (dd, *J* = 2.4, 5.1 Hz, 1H), 4.34–4.21 (m, 3H), 4.17–4.13 (m, 2H), 4.04 (dd, *J* = 1.2, 12.3 Hz, 1H), 3.91 (dd, *J* = 7.5, 9.6 Hz, 1H), 3.77 (dd, *J* = 7.2, 10.5 Hz, 1H), 3.60 (dd, *J* = 3.6, 9.6 Hz, 1H), 3.32 (s, 1H), 1.55 (s, 3H, CH_3C), 1.50 (s, 3H, CH_3C), 1.37 (s, 6H, CH_3C); ^{13}C NMR (75 MHz, $CDCl_3$): δ 139.6, 138.9, 138.3, 129.3, 128.9, 128.8, 128.7, 128.6, 128.5, 128.2, 128.1, 127.8, 126.9, 109.7, 109.1, 104.8, 101.6, 96.8, 79.3, 78.6, 75.4, 74.6, 72.5, 71.9, 71.2, 70.9, 69.9, 69.6, 67.8, 66.9, 26.5, 26.4, 25.5, 24.9; HRMS (ESI): calcd for $C_{39}H_{46}O_{11}Na$ requires 713.2932; found: *m*/*z* = 713.2933 $[M+Na]^+$.

For **1,2,3,4-di-*O*-isopropylidene-6-*O*-(2',3',4'-tri-*O*-benzyl-6'-*O*-levulinoyl-β-D-glucopyranosyl)-α-D-galactopyranose (72)**: Synthesis

of **72** was referred to the general procedure of low concentration 1,2-*trans* β-selective glycosylation. **72** (117 mg, 77%) was obtained as white solid. $[\alpha]^{27}_{D}$ = −14.80 (*c* = 0.84, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.46–7.42 (m, 2H, ArH), 7.36–7.25 (m, 13H, ArH), 5.59 (d, *J* = 5.1 Hz, 1H, H-1), 5.08 (d, *J* = 11.1 Hz, 1H), 5.00 (d, *J* = 10.8 Hz, 1H), 4.88 (d, *J* = 10.8 Hz, 1H), 4.81 (d, *J* = 11.1 Hz, 1H), 4.74 (d, *J* = 11.1 Hz, 1H), 4.63–4.56 (m, 2H), 4.49 (d, *J* = 7.8 Hz, 1H), 4.35–4.25 (m, 4H), 4.15–4.10 (m, 2H), 3.77–3.65 (m, 2H), 3.54–3.44 (m, 3H), 2.78–2.72 (m, 2H, Lev-CH_2), 2.62–2.58 (m, 2H, Lev-CH_2), 2.20 (s, 3H, CH_3CO), 1.52 (s, 3H, CH_3C), 1.48 (s, 3H, CH_3C), 1.35 (s, 3H, CH_3C), 1.33 (s, 3H, CH_3C); ^{13}C NMR (75 MHz, $CDCl_3$): δ 206.8, 172.9, 139.0, 138.9, 138.2, 129.0, 128.9, 128.8, 128.6, 128.5, 128.3, 128.0, 127.9, 109.8, 108.9, 104.9, 96.8, 84.9, 81.9, 77.8, 77.6, 76.4, 75.4, 74.8, 73.2, 71.2, 71.8, 71.1, 70.8, 70.2, 67.7, 63.7, 38.3, 30.3, 28.3, 26.43, 26.4, 25.4, 24.8; HRMS (FAB): calcd for $C_{44}H_{54}O_{13}$ requires 790.3564; found: *m*/*z* = 790.3578 $[M]^+$.

For **1,2,3,4-di-*O*-isopropylidene-6-*O*-(2',3',6'-tri-*O*-benzyl-4'-*O*-levulinoyl-β-D-glucopyranosyl)-α-D-galactopyranose (73)**: Synthesis of **73** was referred to the general procedure of low concentration 1,2-*trans* β-selective glycosylation. **73** (126 mg, 83%) was obtained as colourless oil. $[\alpha]^{27}_{D}$ = −22.42 (*c* = 0.35, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.46–7.43 (m, 2H, ArH), 7.37–7.27 (m, 13H, ArH), 5.6 (d, *J* = 5.1 Hz, 1H), 5.09–4.99 (m, 2H), 4.86 (d, *J* = 11.7 Hz, 1H), 4.73 (d, *J* = 11.1 Hz, 1H), 4.67–4.59 (m, 2H), 4.55–4.51 (m, 3H), 4.35 (dd, *J* = 2.4, 5.1 Hz, 1H), 4.26 (dd, *J* = 1.5, 7.8 Hz, 1H),. 4.21–4.12 (m, 2H), 3.78 (dd, *J* = 7.2, 10.2 Hz, 1H), 3.66–3.50 (m, 5H), 2.62–2.54 (m, 2H, Lev-CH_2), 2.45–2.25 (m, 2H, Lev-CH_2), 2.13 (s, 3H, CH_3CO), 1.53 (s, 3H, CH_3C), 1.48 (s, 3H, CH_3C), 1.35 (s, 6H, CH_3C); ^{13}C NMR (75 MHz, $CDCl_3$):

δ206.6, 172.0, 139.0, 138.9, 138.5, 129.1, 128.71, 128.68, 128.64, 128.3, 128.0, 127.9, 109.8, 109.0, 104.7, 96.8, 81.9, 81.7, 75.4, 74.9, 74.0, 73.7, 71.8, 71.4, 71.2, 70.9, 69.9, 67.9, 38.2, 30.2, 28.3, 26.5, 26.4, 25.4, 24.9; HRMS (ESI): calcd for $C_{44}H_{54}O_{13}$ requires 790.3564; found: *m*/*z* = 790.3568 $[M]^+$.

For **6-chlorohexyl 2,3,4-tri-*O*-benzyl-β-L-fucopyranoside (74)**: Synthesis of **74** was referred to the general procedure of low concentration 1,2-*trans* β-selective glycosylation. **74** (93 mg, 80%) was obtained as colourless oil. $[\alpha]^{27}_D$ = +5.52 (*c* = 0.32, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ7.41–7.27 (m, 15H, ArH), 5.01 (d, *J* = 11.7 Hz, 1H), 4.96 (d, *J* = 11.1 Hz, 1H), 4.85–4.47 (m, 4H), 4.34 (d, *J* = 7.5 Hz, 1H), 3.97 (dt, *J* = 6.3, 9.6 Hz, 1H), 3.83 (dd, *J* = 7.8, 9.6 Hz, 1H), 3.59–3.44 (m, 6H), 1.81–1.64 (m, 4H), 1.51–1.37 (m, 4H), 1.20 (d, *J* = 6.3 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ139.33, 139.08, 139.02, 128.95, 128.79, 128.68, 128.53, 128.43, 127.97, 127.91, 104.2, 82.97, 79.89, 77.69, 76.66, 75.49, 74.34, 73.58, 70.69, 69.99, 45.5, 32.95, 30.0, 27.1, 25.9; HRMS (Bio-ToFII): calcd for $C_{33}H_{41}ClO_5Na$ requires 575.2540; found: *m*/*z* = 575.2535 $[M+Na]^+$.

For **1,2,3,4-di-*O*-isopropylidene-6-*O*-(2'-azido-3',4',6'-tri-*O*-benzyl-2'-deoxy-β-D-glucopyranosyl)-α-D-galactopyranose (82)**: Synthesis of **82** was referred to the general procedure of low concentration 1,2-*trans* β-selective glycosylation. **82** (114 mg, 83%) was obtained as colourless oil. $[\alpha]^{27}_D$ = –35.30 (*c* = 1.08, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ7.42–7.30 (m, 13H, ArH), 7.22–7.19 (m, 2H, ArH), 5.6 (d, *J* = 5.1 Hz, 1H), 4.95 (d, *J* = 10.8 Hz, 1H), 4.84 (dd, *J* = 5.7, 10.8 Hz, 1H), 4.67–4.55 (m, 4H), 4.48–4.46 (m, 1H), 4.36 (dd, *J* = 2.4, 4.8 Hz, 1H), 4.32 (dd, *J* = 1.2, 8.1 Hz, 1H), 4.1 (m, 2H), 3.88–3.66 (m, 4H),

3.49–3.45 (m, 3H), 1.59 (s, 3H, CH_3C), 1.49 (s, 3H, CH_3C), 1.38 (s, 3H, CH_3C), 1.37 (s, 3H, CH_3C); ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.49, 138.46, 138.39, 128.88, 128.85, 128.81, 128.47, 128.3, 128.1, 109.7, 109.1, 102.9, 96.7, 83.5, 78.1, 75.9, 75.4, 74.0, 71.7, 71.1, 70.9, 69.2, 68.9, 68.0, 66.8, 26.5, 26.4, 25.4, 24.8; HRMS (Bio-ToFII): calcd for $C_{39}H_{47}N_3O_{10}Na$ requires 740.3159; found: *m*/*z* = 740.3154 $[M+Na]^+$.

For **6-chlorohexyl 2-azido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (83)**: Synthesis of **83** was referred to the general procedure of low concentration 1,2-*trans* β-selective glycosylation. **83** (44 mg, 60%) was obtained as colourless oil. $[\alpha]^{27}_D$ = –32.28 (*c* = 0.3, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.43–7.29 (m, 10H, ArH), 4.96 (d, *J* = 11.4 Hz, 1H), 4.81 (d, *J* = 11.4 Hz, 1H), 4.65 (dd, *J* = 12.0, 4.8 Hz, 1H), 4.31 (d, *J* = 7.8 Hz, 1H), 3.98–3.90 (m, 1H), 3.76 (d, *J* = 6.6 Hz, 1H), 3.68–3.52 (m, 4H), 3.46–3.29 (m, 2H), 3.26 (t, *J* = 9.0 Hz, 1H), 2.72 (br, 1H), 1.83–1.76 (m, 2H, CH_2), 1.72–1.64 (m, 2H, CH_2), 1.51–1.43 (m, 4H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.5, 138.0, 129.0, 128.8, 128.5, 128.4, 128.2, 128.1, 102.6, 82.8, 75.4, 74.3, 74.1, 72.4, 70., 70.4, 66.1, 45.4, 32.9, 29.7, 27.0, 25.6; HRMS (FAB): calcd for $C_{26}H_{34}N_3O_5ClNa$ requires 504.2260; found: *m*/*z* = 504.2260 $[M+Na]^+$.

For **3-chlorohexyl 2-azido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (84)**: Synthesis of **84** was referred to the general procedure of low concentration 1,2-*trans* β-selective glycosylation. **84** (83 mg, 79%) was obtained as colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.61–7.57 (m, 4H, ArH), 7.55–7.50 (m, 6H, ArH), 7.45–7.40 (m, 6H, ArH), 4.31 (dd, *J* = 1.2, 12.3 Hz, 1H), 4.26 (d, *J* = 8.1 Hz, 1H), 4.15–4.08 (m, 2H), 3.98 (dd, *J* = 1.2, 12.3 Hz, 1H), 3.92 (d, *J* = 8.7 Hz, 1H), 3.82–3.66 (m, 2H), 3.42 (dd, *J* = 3.6, 10.2 Hz, 1H), 3.25 (m, 1H),

2.21–2.03 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 129.5, 128.8, 128.7, 128.4, 128.2, 127.1, 126.8, 126.7, 126.56, 126.2, 102.8, 101.5, 72.8, 72.1, 69.5, 66.9, 66.8, 62.6, 42.3, 32.9; LRMS (ESI): calcd for $C_{27}H_{28}N_3O_5ClNa$ requires 532; found: m/z = 532 $[M+Na]^+$.

For **1,2,3,4-di-*O*-isopropylidene-6-*O*-[2',3',6'-tri-*O*-benzyl-4'-*O*-(2",3",4",6"-tetra-*O*-benzyl-β-D-galactopyranosyl)-β-D-gulcopyranosyl)]-α-D-galactopyranose (85)**: Synthesis of **85** was referred to the general procedure of low concentration 1,2-*trans* β-selective glycosylation. **85** (175 mg, 75%) was obtained as colourless oil. $[\alpha]^{27}_D$ = –15.38 (c = 2.07, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.49–7.18 (m, 35H, ArH), 5.62 (d, J = 5.1 Hz, 1H), 5.08–5.00 (m, 3H), 4.84–4.74 (m, 7H), 4.64–4.57 (m, 3H), 4.50–4.44 (m, 2H), 4.41–4.35 (m, 2H), 4.31–4.27 (m, 2H), 4.21–4.17 (m, 2H), 4.03–3.96 (m, 2H), 3.88–3.72 (m, 4H), 3.63–3.56 (m, 2H), 3.49–3.37 (m, 5H), 1.55 (s, 3H, CH_3C), 1.51 (s, 3H, CH_3C), 1.37 (s, 6H, 2 × CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 139.6, 139.5, 139.4, 139.2, 139.0, 138.8, 138.5, 128.9, 128.81, 128.79, 128.68, 128.66, 128.52, 128.38, 128.30, 128.17, 128.11, 127.96, 127.86, 127.80, 127.46, 109.76, 109.02, 104.80, 103.17, 83.18, 82.98, 81.68, 80.40, 77.71, 75.79, 75.70, 75.51, 75.13, 74.97, 74.02, 73.83, 73.52, 73.36, 72.97, 71.83, 71.18, 70.93, 69.91, 68.62, 68.51, 67.74, 26.50, 26.45, 25.49, 24.88; HRMS (FAB): calcd for $C_{73}H_{82}O_{16}$ requires 1214.5603; found: m/z = 1214.5608 $[M]^+$.

For **6-chlorohexyl 2,3,6-tri-*O*-benzyl-4-*O*-(2',3',6'-tri-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (86)**: Synthesis of **86** was referred to the general procedure of low concentration 1,2-*trans* β-selective glycosylation. **86** (168 mg, 80%) was obtained as white glassy solid; $[\alpha]^{27}_D$ = +15.23 (c = 0.52, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$):

δ7.26–7.13 (m, 30 H, ArH), 4.90 (d, *J* = 10.8 Hz, 1 H), 4.80 (d, *J* = 11.1 Hz, 1 H), 4.70–4.55 (m, 6 H), 4.47 (d, *J* = 12.3 Hz, 1 H), 4.39–4.27 (m, 5 H), 3.94–3.81 (m, 3 H), 3.74–3.22 (m, 14 H), 2.36 (br, 1 H), 1.70–1.55 (m, 4 H), 1.37–1.30 (m, 4 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ139.44, 139.00, 138.95, 138.65, 138.48, 138.25, 128.80, 128.71, 128.63, 128.61, 128.40, 128.27, 128.20, 128.13, 128.11, 128.01, 127.96, 127.89, 127.87, 127.81, 127.58, 103.93, 102.89, 83.22, 82.11, 81.43, 79.70, 77.60, 75.67, 75.57, 75.42, 75.25, 73.83, 73.45, 73.06, 72.33, 70.09, 68.76, 68.63, 66.46, 45.38, 32.84, 29.93, 27.01, 25.81; HRMS (FAB): calcd for $C_{60}H_{69}ClO_{11}$ requires 1000.4528; found: *m*/*z* =1000.4529 $[M]^+$.

For **6-chlorohexyl 2,3,6-tri-*O*-benzyl-4-*O*-(3'-*O*-benzoyl-2',6'-di-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (87)**:
Synthesis of **87** was referred to the general procedure of low concentration 1,2-*trans* β-selective glycosylation. **87** (235 mg, 83%) was obtained as glassy solid; $[\alpha]^{27}_D$ = −17.10 (*c* = 0.74, $CHCl_3$) ^{1}H NMR (300 MHz, $CDCl_3$): δ7.90 (d, *J* = 7.2 Hz, 2 H), 7.44 (t, *J* = 7.5 Hz, 1H), 7.30–7.18 (m, 19 H, ArH), 7.09–6.96 (m, 8 H, ArH), 5.50 (d, *J* = 3.0 Hz, 1 H), 5.00 (d, *J* = 10.5 Hz, 1 H), 4.83–4.45 (m, 8 H), 4.38–4.30 (m, 3 H), 4.13 (d, *J* = 12.0 Hz, 1 H), 3.96 (t, *J* = 9.3 Hz, 1 H), 3.88–3.85 (m, 1 H), 3.76 (dd, *J* = 3.6, 11.1 Hz, 1 H), 3.69–3.64 (m, 2 H), 3.58–3.32 (m, 10 H), 2.45 (br, 1 H), 1.66–1.56 (m, 4 H), 1.36–1.31 (m, 4 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ166.79, 139.36, 138.98, 138.54, 138.49, 138.11, 133.50, 130.19, 130.00, 128.78, 128.71, 128.63, 128.60, 128.37, 128.29, 128.23, 128.18, 128.15, 128.01, 127.98, 127.92, 127.87, 127.60, 103.99, 102.85, 83.14, 81.97, 80.49, 77.60, 77.04, 73.78, 73.51, 73.02, 72.65, 70.61, 70.12, 68.48, 67.75, 63.06, 45.36, 32.84, 30.03, 29.92, 27.00, 25.81, 25.39; HRMS (FAB): calcd for $C_{60}H_{68}ClO_{12}$ requires 1015.4399; found: *m*/*z* = 1015.4394 $[M+H]^+$.

For **3-chloropropyl 2,3-*O*-isopropylidene-4-*O*-(2',3',4'-tri-*O*-benzyl-6'-*O*-levulinoyl-β-D-galactopyranosyl)-β-L-rhamnopyranoside (94)**: Synthesis of **94** was referred to the general procedure of low concentration 1,2-*trans* β-selective glycosylation. **94** (114 mg, 65%) was obtained as yellow oil. $[\alpha]^{27}_{D} = -16.32$ (*c* = 1.16, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ7.46–7.29 (m, 15H, ArH), 5.03–4.95 (m, 3H), 4.87–4.75 (m, 4H), 4.69 (d, *J* = 11.7 Hz, 1H), 4.29–4.23 (m, 2H), 4.18–4.09 (m, 2H), 3.93–3.77 (m, 3H), 3.69–3.55 (m, 7H), 2.76–2.71 (m, 2H, Lev-CH_2), 2.51–2.47 (m, 2H, Lev-CH_2), 2.20 (s, 3H, CH_3CO), 2.11–2.03 (m, 2H), 1.55 (s, 3H, CH_3), 1.39–1.29 (m, 7H); ^{13}C NMR (75 MHz, $CDCl_3$): δ206.9, 172.8, 139.4, 139.0, 138.8, 128.8, 128.72, 128.66, 128.62, 128.5, 128.1, 128.0, 127.9, 127.8, 109.6, 102.6, 97.4, 82.7, 79.99, 79.3, 18.6, 76.3, 75.5, 74.8, 73.94, 73.87, 72.43, 42.1, 38.3, 32.7, 30.3, 28.3, 28.1, 26.8, 18.3; HRMS (ESI): calcd for $C_{44}H_{55}ClO_{12}Na$ requires 833.3274; found: *m*/*z* = 833.3274 $[M+Na]^+$.

For **methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2',3',4'-tri-*O*-benzyl-6'-*O*-levulinoyl-β-D-galactopyranosyl)-α-D-glucopyranoside (95)**: Synthesis of **95** was referred to the general procedure of low concentration 1,2-*trans* β-selective glycosylation. **95** (122 mg, 63%) was obtained as white solid. $[\alpha]^{27}_{D} = +2.49$ (*c* = 0.98, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ7.47–7.22 (m, 30H, ArH), 5.06 (m, 2H), 4.88–4.77 (m, 5H), 4.75–4.59 (m, 5H), 4.37 (d, *J* = 12 Hz, 1H), 4.31(d, *J* = 7.8 Hz, 1H), 4.12 (d, *J* = 6.6 Hz, 1H), 3.99-3.70 (m, 6H), 3.65–3.52 (m, 3H), 3.47–3.32 (m, 5H), 2.73 (m, 2H, Lev-CH_2), 2.53–2.47 (m, 2H, Lev-CH_2), 2.19 (s, 3H, CH_3CO); ^{13}C NMR (75 MHz, $CDCl_3$): δ206.9, 172.6, 139.7, 139.3, 139.1, 138.94, 138.93, 138.5, 128.92, 128.82, 128.81, 128.78, 128.67, 128.59, 128.52, 128.45, 128.41, 128.36, 128.20, 128.16, 128.06, 128.02, 127.91, 127.88,

127.82, 127.55, 103.05, 98.85, 82.79, 80.6, 80.3, 77.7, 76.9, 75.9, 75.6, 75.0, 73.7, 73.6, 73.2, 72.0, 70.4, 68.3, 63.1, 55.7, 38.3, 30.3, 28.3; HRMS (ESI): calcd for $C_{60}H_{66}O_{13}$ requires 994.4503; found: *m*/*z* = 994.4506 $[M]^+$.

For **2-azidoethyl 2,3-di-*O*-allyl-4-*O*-(2',3',4'-tri-*O*-benzyl-6'-*O*-levulnoyl-β-D-galactopyranosyl)-β-D-galactopyranoside (96)**: Synthesis of **96** was referred to the general procedure of low concentration 1,2-*trans* β-selective glycosylation. **96** (117 mg, 60%) was obtained as yellow oil. $[\alpha]^{27}_D = -1.98$ (*c* = 0.74, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.50–7.48 (m, 2H, ArH), 7.38–7.28 (m, 18H, ArH), 5.97–5.86 (m, 2H), 5.39 (d, *J* = 1.2 Hz, 1H), 5.24–5.02 (m, 5H), 4.88–4.85 (m, 2H), 4.78–4.74 (m, 2H), 4.65–4.53 (m, 3H), 4.37 (d, *J* = 7.8 Hz, 1H), 4.25–3.99 (m, 8H), 3.87–3.67 (m, 5H), 3.64–3.37 (m, 7H), 2.75–2.70 (m, 2H, Lev-CH_2), 2.51–2.47 (m, 2H, Lev-CH_2), 2.19 (s, 3H, CH_3CO); ^{13}C NMR (75 MHz, $CDCl_3$): δ 206.8, 172.8, 139.5, 139.1, 138.8, 135.6, 135.5, 128.77, 128.76, 128.67, 128.6, 128.4, 128.2, 128.0, 127.7, 116.91, 116.88, 104.17, 103.4, 82.4, 81.6, 80.1, 79.6, 77.7, 75.3, 74.8, 74.5, 74.4, 74.3, 74.1, 73.9, 72.3, 72.1, 71.8, 70.7, 68.6, 63.7, 51.4, 38.2, 30.2, 28.1; HRMS (Bio-ToFII): calcd for $C_{53}H_{63}N_3O_{13}Na$ requires 972.4253; found: *m*/*z* = 972.4253 $[M+Na]^+$.

For **methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2',3',4'-tri-*O*-benzyl-6'-*O*-levulinoyl-β-D-glucopyranosyl)-α-D-glucopyranoside (97)**: Synthesis of **98** was referred to the general procedure of low concentration 1,2-*trans* β-selective glycosylation. **97** (162 mg, 83%) was obtained as white solid. $[\alpha]^{27}_D = +20.57$ (*c* = 1.03, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.41–7.32 (m, 25H, ArH), 7.28–7.24 (m, 5H, ArH), 5.06–4.77 (m, 9H), 4.74–4.56 (m, 4H), 4.43–4.39 (m, 2H), 4.30 (dd, *J* = 4.2, 12 Hz, 1H),

4.22 (d, *J* = 10.5 Hz, 1H), 4.07 (t, *J* = 9.3 Hz, 1H), 3.90 (dd, *J* = 3, 9.9 Hz, 1H), 3.77–3.69 (m, 2H), 3.63–3.51 (m, 5H), 3.41 (s, 3H), 2.78–2.73 (m, 2H, Lev-CH_2), 2.63–2.58 (m, 2H, Lev-CH_2), 2.21 (s, 3H, CH_3CO); ^{13}C NMR (75 MHz, $CDCl_3$): δ206.8, 173.0, 139.3, 138.8, 138.7, 138.6, 138.2, 128.95, 128.93, 128.88, 128.84, 128.81, 128.63, 128.58, 128.44, 128.40, 128.36, 128.32, 128.17, 128.10, 128.04, 104.2, 98.5, 85.2, 82.4, 80.2, 78.4, 77.9, 76.2, 76.1, 75.5, 75.4, 75.3, 73.8, 73.3, 70.2, 69.1, 63.7, 55.7, 38.3, 30.3, 30.2, 28.3; HRMS (ESI): calcd for $C_{60}H_{66}O_{13}Na$ requires 1017.4401; found: *m*/*z* = 1017.4396 $[M+Na]^+$.

For **(2',3',4'-tri-*O*-benzyl-6'-*O*-levulinoyl-α-D-galactopyranosyl) N^{α}-fluoren-9-yl methoxycarbonyl-L-serine allyl ester (98a)**: Synthesis of **98a** was referred to the general procedure of low concentration 1,2-*trans* β-selective glycosylation, but use only CH_2Cl_2 as reaction solvent. **98a** (128 mg, 75%) was obtained as colourless oil. $[\alpha]^{27}_D$ = +8.63 (*c* = 0.90, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ7.80–7.78 (m, 2H, ArH), 7.68–7.56 (m, 3H, ArH), 7.44–7.28 (m, 18H, ArH), 5.96–5.84 (m, 1H), 5.40–5.22 (m, 2H), 5.05–4.80 (m, 7H), 4.74–4.59 (m, 4H), 4.51–4.39 (m, 2H), 4.34–4.04 (m, 5H), 4.02–3.81 (m, 3H), 2.79–2.64 (m, 2H, Lev-CH_2), 2.60–2.45 (m, 2H, Lev-CH_2), 2.16 (s, 3H, CH_3CO); ^{13}C NMR (75 MHz, $CDCl_3$): δ207.1, 172.95, 170.2, 156.5, 144.3, 141.7, 139.0, 138.8, 138.6, 132.0, 128.89, 128.82, 128.74, 128.3, 128.2, 128.14, 128.09, 127.95, 127.87, 127.52, 125.62, 125.59, 125.29, 120.41, 119.21, 99.8, 78.99, 77.67, 76.70, 75.09, 73.79, 73.73, 70.53, 69.73, 67.57, 66.70, 66.63, 64.22, 55.1, 47.5, 38.25, 38.19, 30.29, 30.23, 28.1; HRMS (ESI): calcd for $C_{53}H_{55}NO_{12}Na$ requires 920.3616; found: *m*/*z* = 920.3609 $[M+Na]^+$.

For **(2',3',4'-tri-*O*-benzyl-6'-*O*-levulinoyl-β-D-galactopyranosyl)**

***N*$^{\alpha}$-fluoren-9-yl methoxycarbonyl-L-serine allyl ester (98b)**: Synthesis of **98b** was referred to the general procedure of low concentration 1,2-*trans* β-selective glycosylation. **98b** (124 mg, 73%) was obtained as white solid. $[\alpha]^{27}_{D}$ = +1.37 (*c* = 0.85, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.80–7.77 (m, 2H, ArH), 7.62–7.57 (m, 2H, ArH), 7.47–7.25 (m, 19H, ArH), 5.99–5.87 (m, 2H), 5.37 (d, *J* = 17.1 Hz, 1H), 5.25 (d, *J* = 10.5 Hz, 1H), 5.01 (d, *J* = 11.7 Hz, 1H), 4.93 (d, *J* = 10.8 Hz, 1H), 4.87–4.78 (m, 3H), 4.73–4.57 (m, 4H), 4.49–4.11 (m, 7H), 3.95–3.85 (m, 3H), 3.61–3.54 (m, 3H), 2.77–2.73 (m, 2H, Lev-CH_2), 2.54–2.41 (m, 2H, Lev-CH_2), 2.2 (s, 3H, CH_3CO); ^{13}C NMR (75 MHz, $CDCl_3$): δ 206.97, 172.9, 170.1, 156.5, 144.36, 144.21, 141.66, 138.74, 138.62, 138.59, 131.94, 128.90, 128.86, 128.82, 128.77, 128.66, 128.22, 128.19, 128.13, 128.04, 127.55, 127.50, 125.74, 125.64, 120.36, 119.19, 104.8, 82.6, 79.3, 77.7, 75.8, 74.9, 73.61, 73.37, 72.6, 70.4, 67.7, 66.7, 63.4, 55.1, 47.5, 38.3, 30.3, 28.2; HRMS (ESI): calcd for $C_{53}H_{55}NO_{12}Na$ requires 920.3616; found: *m*/*z* = 920.3619 $[M+Na]^+$.

For **3-chloropropyl 2-*O*-benzyl-4,6-*O*-benzylidiene-3-*O*-(2'-azido-3',4',6'-tri-*O*-benzyl-2'-deoxy-β-D-glucopyranosyl)-β-D-galactopyranoside (99)**: Synthesis of **99** was referred to the general procedure of low concentration 1,2-*trans* β-selective glycosylation. **99** (126 mg, 77%) was obtained as colourless oil. $[\alpha]^{27}_{D}$ = +7.42 (*c* = 1.22, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.64–7.61 (m, 2H, ArH), 7.53–7.51 (m, 2H, ArH), 7.46–7.35 (m, 19H, ArH), 7.28–7.21 (m, 2H, ArH), 5.60 (s, 1H, PhCH), 5.00–4.96 (m, 2H, =CH), 4.93–4.81 (m, 4H), 4.67–4.53 (m, 3H), 4.47 (d, *J* = 7.5 Hz, 1H), 4.36–4.33 (m, 2H), 4.17–3.98 (m, 3H), 3.9 (dd, *J* = 3.3, 9.9 Hz, 1H), 3.82–3.57 (m, 7H), 3.48–3.40 (m, 3H), 2.18–2.09 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.9, 138.40, 138.38, 138.23, 129.13, 128.93, 128.88, 128.82, 128.78,

128.53, 128.47, 128.45, 128.39, 128.35, 128.25, 128.20, 126.7, 104.2, 103.2, 100.98, 83.6, 78.96, 78.33, 38.21, 76.52, 75.94, 75.59, 75.56, 75.14, 73.88, 69.43, 69.30, 66.96, 66.77, 66.70, 42.35, 33.2; HRMS (ESI): calcd for $C_{50}H_{54}ClN_3O_{10}Na$ requires: 914.3395; found: *m*/*z* = 914.3390 $[M+Na]^+$.

For **1,2:3,4-di-*O*-isopropylidene-6-*O*-(2',3',4',6'-tetra-*O*-benzyl-α-D-mannopyranosyl)-α-D-galactopyranose (103)**: Synthesis of **103** was referred to the general procedure of low concentration 1,2-*trans* β-selective glycosylation.**103** (128 mg, 85%) was obtained as colourless oil. $[\alpha]^{27}_D = -46.11$ (*c* = 0.75, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.42–7.25 (m,18H, ArH), 7.19–7.16 (m, 2H, ArH), 5.55 (d, *J* = 5.1 Hz, 1H, H-1), 5.05 (d, *J* = 1.8 Hz, 1H), 4.90 (d, *J* = 10.5 Hz, 1H), 4.75–4.69 (m, 3H), 4.63–4.51 (m, 5H), 4.35 (dd, *J* = 7.5, 5.1 Hz, 1H), 4.19 (dd, *J* = 1.8, 8.1 Hz, 1H), 4.08–3.91 (m, 3H), 3.86–3.69 (m, 6H), 1.52 (s, 3H, CH_3C), 1.45 (s, 3H, CH_3C), 1.35 (s, 6H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.9, 138.88, 138.82, 138.75, 128.7, 128.4, 128.2, 128.0, 127.98, 127.94, 127.88, 127.84, 109.7, 108.9, 97.6, 96.7, 80.4, 77.6, 75.5, 75.2, 74.9, 73.7, 72.7, 72.4, 71.3, 71.0, 70.9, 69.4, 65.7, 65.6, 26.5, 26.3, 25.3, 24.9; HRMS (ESI): calcd for $C_{46}H_{54}O_{11}Na$ requires 805.3564, found: *m*/*z* = 805.3558 $[M+Na]^+$.

For **methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2′,3′,4′-tri-*O*-benzyl-β-D-glucopyranosyl)-α-D-glucopyranoside (97a)**: Disaccharide **97a** (410 mg, 0.4 mmol) in 1:2 v/v CH_2Cl_2-MeOH solution (8 mL) was treated with a piece of freshly cut sodium (ca. 5 mg) at RT. Upon completion of deacetylation, the mixture was neutralized with resin IR-120H^+, filtered, and concentrated for column chromatography (elution: hexane/EtOAc/toluene = 1/1/1) to furnish the disaccharide **97a** as white

glassy solid (350 mg, 95%); $[\alpha]^{27}_{D}$ = +17.13 (*c* = 0.78, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.41–7.34 (m, 25H, ArH), 7.28–7.22 (m, 5H, ArH), 5.06–4.93 (m, 4H), 4.90–4.80 (m, 5H), 4.76–4.67 (m, 4H), 4.57 (d, *J* = 11.4 Hz, 1H), 4.45 (d, *J* = 7.8 Hz, 1H), 4.17 (d, *J* = 10.5 Hz, 1H), 4.06 (t, *J* = 9.3 Hz, 1H), 3.92–3.85 (m, 2H), 3.79–3.50 (m, 8H), 3.42–3.40 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 139.2, 138.84, 138.76, 138.65, 138.54, 138.39, 128.96, 128.93, 128.86, 128.83, 128.62, 128.51, 128.46, 128.41, 128.35, 128.27, 128.10, 128.05, 104.2, 98.6, 85.0, 82.43, 82.41, 80.2, 78.2, 76.2, 75.56, 75,54, 75.42, 75.36, 73.84, 70.2, 69.1, 62.4, 55.7; HRMS (ESI): calcd for $C_{60}H_{66}O_{13}Na$ requires 919.4033; found: *m*/*z* = 919.4030 $[M+Na]^+$.

For **methyl 2,3,4-tri-*O*-benzyl-6-*O*-[2′,3′,4′-tri-*O*-benzyl-6′-*O*-(2”,3”,4”-tri-*O*-benzyl-6-*O*-levulinoyl-β-D-glucopyranosyl)-β-D-glucopyranosyl]-1-α-D-glucopyranoside, protected β-(1→6) derivative (106)**: Disaccharide acceptor **97a** (derived from **97** by Zemplén deacylation, 120 mg, 0.13 mmol), glycosyl donor **65** (105 mg, 0.16 mmol) and flame-dried molecular sieve (AW300, 300 mg) were suspended in a 1:2:1 v/v CH_2Cl_2-CH_3CN-EtCN (13 mL) solution under N_2. The resulting mixture was stirred at RT for 5 min and at −70˚C for another 20 min; then, NIS (38 mg, 0.17 mmol) and TMSOTf (6 μL, 0.032 mmol) were added. Upon completion of glycosylation as monitored by TLC, saturated $NaHCO_3$ (0.1 mL) and slumps of $Na_2S_2O_{3(s)}$ were added to the mixture, followed by vigorous stirring at RT until the deep red color of the solution turned to pale yellow. MS was then removed by filtration over celite, and the filtrate was dried (over $MgSO_4$), filtered, and concentrated for flash chromatography purification (elution: hexane/CH_2Cl_2/EtOAc = 6/3/1) to furnish desired trisaccharide **106** as colorless oil (134 mg, 70%); $[\alpha]^{27}_{D}$ = +16.92 (*c* = 2.84, $CHCl_3$); ^{1}H NMR

(300 MHz, $CDCl_3$): δ 7.38–7.21 (m, 43H, ArH), 7.19–7.16 (m, 2H, ArH), 5.08–4.75 (m, 16H), 4.74–4.45 (m, 9H), 4.39–4.12 (m, 6H), 4.00 (t, J = 9 Hz, 1H), 3.80–3.35 (m, 17H), 3.2 (s, 3H, OCH_3), 2.75–2.70 (m, 2H, Lev-CH_2), 2.62–2.56 (m, 2H, Lev-CH_2), 2.19 (s, 3H, CH_3CO); ^{13}C NMR (75 MHz, $CDCl_3$): δ 206.7, 172.96, 139.3, 138.86, 138.84, 138.77, 138.70, 138.57, 138.42, 138.27, 128.90, 128.86, 128.84, 128.80, 128.76, 128.58, 128.52, 128.44, 128.38, 128.34, 128.27, 128.14, 128.10, 128.08, 127.99, 127.96, 104.3, 103.9, 98.5, 85.2, 85.1, 82.4, 82.3, 80.0, 78.6, 78.2, 77.8, 77.7, 76.14, 76.09, 75.6, 75.42, 75.37, 75.23, 75.11, 73.75 73.26, 70.1, 69.0, 68.6, 63.7, 55.7, 38.2, 30.3, 28.3. HRMS (Bio-ToFII): calcd for $C_{87}H_{94}O_{18}Na$ requires 1449.6332; found: m/z = 1449.6332 $[M+Na]^+$.

For **6-chlorohexyl 2,3,6-tri-*O*-benzyl-4-*O*-[2,3,6-tri-*O*-benzyl-4-*O*-(2,3-di-*O*-benzyl-4,6-*O*-di-*tert*-butylsilylidene-α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside, protected Gb_3 derivative (108)**: Thiolactosyl donor **80** (330 mg, 0.33 mmol), 6-chlorohexanol **60** (90 mg, 0.66 mmol) and flame-dried molecular sieves (AW300, 400 mg) was suspended in a 1:2:1 v/v CH_2Cl_2-CH_3CN-EtCN (32 mL) solution at RT. The resulting mixture was stirred at RT for 10 min and at −70˚C for another 20 min; then, NIS (77 mg, 0.34 mmol) and TMSOTf (12 μL, 0.066 mmol) were added separately. Upon completion of glycosylation as judged by TLC, saturated $NaHCO_3$ (1 v/v% of reaction volume) and a small lumps of $Na_2S_2O_3$(s) were added to the reaction mixture, followed by vigorous stirring at RT until the red color of solution turned to pale yellow. The resulting crude was filtered over celite, and diluted with CH_2Cl_2 (40 mL × 1). The CH_2Cl_2 solution was washed with H_2O (15 mL × 1), brine (15 mL × 1), dried (over $MgSO_4$), filtered, concentrated and dried under reduced *vacuo* for > 2 h. The resulting residue was dissolved in CH_2Cl_2 (5 mL),

followed by addition of thiogalactopyranosyl donor **107** (500 mg, 0.82 mmol) and flame-dried molecular sieves (AW300, 900 mg). The resulting mixture was stirred at RT for min and at −10˚C for additional 20 min, followed by addition of NIS (1.88 mg, 0.83 mmol) and TMSOTf (30 μL, 0.17 mmol). The mixture was stirred at −10˚C under N_2 and reaction was monitored by TLC. Upon completion of glycosylation as assessed by TLC, saturated $NaHCO_3$ (1 v/v% of reaction volume) and a small lumps of $Na_2S_2O_{3\,(s)}$ were added, followed by similar workup procedure as described in preparation of compound **106**. The resulting CH_2Cl_2 solution was concentrated for flash column chromatography over silica gel (elution: hexane/CH_2Cl_2/EtOAc = 7/2/1) to furnish the expected trisaccharide **108** as yellow oil (248 mg, 75% from **80**); $[\alpha]^{27}_D$ = +46.47 (*c* = 1.06, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.11−7.03 (m, 40 H, ArH), 4.97 (d, *J* = 11.4 Hz, 1H), 4.86 (d, *J* = 3.0 Hz, 1H), 4.80−4.14 (m, 19H), 4.02−3.62 (m, 11H), 3.54−3.14 (m, 10H), 1.64−1.53 (m, 4H, 2 × CH_2), 1.33−1.32 (m, 4H, 2 × CH_2), 0.91 (s, 9H, *t*-Bu), 0.88 (s, 9H, *t*-Bu); ^{13}C NMR (75 MHz, $CDCl_3$): δ 139.60, 139.43, 139.01, 138.98, 138.88, 138.83, 138.69, 138.54, 128.80, 128.72, 128.67, 128.63, 128.58, 128.49, 128.16, 128.07, 128.03, 127.97, 127.94, 127.85, 127.78, 127.66, 127.61, 127.56, 103.86, 100.33, 82.75, 82.05, 81.35, 79.31, 78.36, 77.60, 75.39, 75.22, 74.34, 73.98, 73.59, 73.51, 73.45, 73.40, 72.45, 71.33, 70.75, 70.06, 68.70, 67.88, 67.78, 67.40, 45.36, 32.83, 30.03, 29.91, 28.06, 27.96, 27.86, 27.75, 26.85, 27.65, 26.99, 25.80, 23.66, 21.00; HRMS (ESI): calcd $C_{88}H_{108}ClO_{16}Si$ requires 1483.7095; found: *m*/*z* = 1483.7090 $[M+H]^+$.

For **6-chlorohexyl 4-*O*-[4-*O*-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside, Gb_3 derivative, (109)**:
Protected Gb_3 **108** (270 mg, 1.82 mmol) was treated with TBAF (1 M in

THF, 1.8 mL), AcOH (95%, 0.25 mL), and THF (5 mL). After stirring at RT for 13 h, the reaction was diluted with CH_2Cl_2 (40 mL), washed with H_2O (15 mL × 2), dried (over $MgSO_4$), filtered, concentrated for next steps.[xxx] The resulting residue (ca.200 mg) rom previous step was dissolved in a mixture of 5% Pd/C (100 mg), formic acid (0.35 mL), and MeOH (7 mL) at RT. The reaction mixture was then stirred under H_2 for 14 h at RT. Upon completion of hydrogenolysis as assessed by TLC, the mixture was filtered over celite, concentrated and then purified by flash column chromatography over C-18 coated reverse phase gel (cosmosil 75C18-OPN) (elution: H_2O/MeOH gradient from 1/0 to 4/1) to furnish the desired trisaccharide**109** as white glassy solid (67 mg, 60% from **108**); $[\alpha]^{27}_D$ = +33.5 (*c* = 0.20, MeOH); ^{1}H NMR (300 MHz, CD_3OD): δ 4.90 (d, *J* = 3.9 Hz, 1H), 4.45 (t, *J* = 8.3 Hz, 2H), 4.32 (t, *J* = 6.5 Hz, 1H), 3.99–3.50 (m, 20H), 3.25 (t, *J* = 8.4 Hz, 1H), 1.79–1.70 (m, 2H, CH_2), 1.65–1.56 (m, 2H, CH_2), 1.46–1.35 (m, 4H, 2 × CH_2); ^{13}C NMR (75 MHz, CD_3OD): δ 103.56, 102.28, 100.60, 78.89, 77.62, 75.73, 75.10, 74.23, 72.43, 71.19, 71.09, 70.84, 69.62, 69.20, 68.85, 60.76, 60.67, 60.32, 45.93, 32.07, 28.87, 26.09, 24.62; HRMS (ESI): calcd $C_{24}H_{43}ClNaO_{16}$ requires 645.2132; found: *m*/*z* = 645.2132 $[M+Na]^+$.

For **6-chlorohexyl 2,3,6-tri-*O*-benzyl-4-*O*-[2,6-di-*O*-benzyl-4-*O*-benzoyl-3-*O*-(2,3-di-*O*-benzyl-4,6-*O*-di-*tert*-butylsilylidene-α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside, protected isoGb$_3$ (110)**: A mixture of thiolactosyl donor **81** (280 mg, 0.28 mmol), 6-chlorohexanol **60** (76 mg, 0.56 mmol) and flame-dried molecular sieves (AW300, 330 mg) was suspended in 1:2:1 CH_2Cl_2-MeCN-EtCN (28 mL) at RT under N_2. The mixture was stirred at RT for 10 min under N_2 and at −70˚C for additional 20 min; then, followed by addition of NIS (67 mg, 0.29 mmol) and TMSOTf (10 μL,

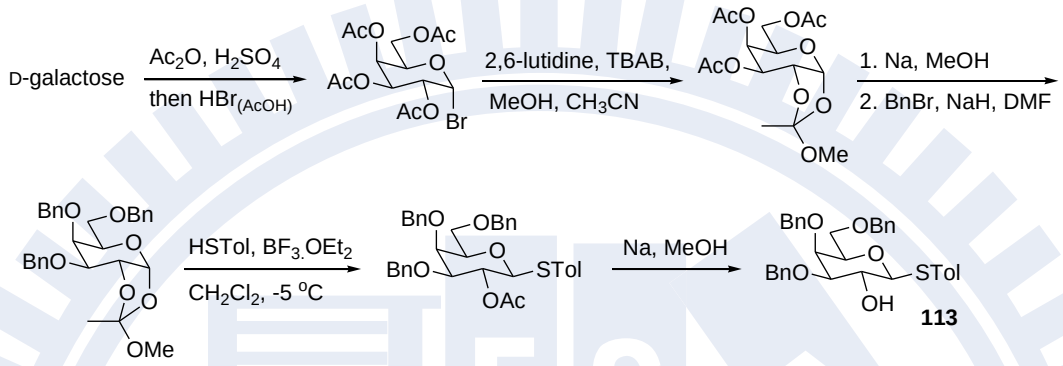
0.056 mmol). Upon completion of glycosylation, saturated $NaHCO_3$ (1 v/v% of reaction volume) and a small lumps of $Na_2S_2O_{3\,(s)}$ were added to the reaction mixture, followed by similar workup procedure as described in preparation of compound **106**. The crude residue obtained was dried under *vacuo* for >2 h and was then dissolved in CH_2Cl_2 (4 mL). To the CH_2Cl_2 solution was added thiogalactopyranosyl donor **107** (436 mg, 0.72 mmol) and flame-dried molecular sieves (AW300, 700 mg) under N_2. The resulting mixture was stirred at −10˚C for 20 min, and then followed by addition of NIS (180 mg, 0.79 mmol) and TMSOTf (29 μL, 0.16 mmol). Upon completion of reaction as assessed by TLC, saturated $NaHCO_3$ (1 v/v% of reaction volume) and a small lumps of $Na_2S_2O_{3\,(s)}$, followed by similar workup procedure as described in preparation of compound **106**. The crude residue was purified by flash column chromatography on silica gel (elution: hexane/CH_2Cl_2/EtOAc = 15/5/1) to furnish expected trisaccharide **110** as yellow oil (292 mg, 70%); $[\alpha]^{27}_D$ = +56.13 (*c* = 0.62, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.95 (d, *J* = 7.2 Hz, 2H, ArH), 7.46 (t, *J* = 7.5 Hz, 1H, ArH), 7.35–7.19 (m, 29H, ArH), 7.14–7.08 (m, 6H, ArH), 7.04–7.00 (m, 2H, ArH), 5.81 (d, *J* = 2.7 Hz, 1H), 5.28 (d, *J* = 3.3 Hz, 1H), 5.09 (d, *J* = 10.8 Hz, 1H), 4.93–4.60 (m, 8H), 4.55–4.36 (m, 8H), 4.24 (d, *J* = 12.0 Hz, 1H), 4.11–4.07 (m, 1H), 3.97–3.83 (m, 6H), 3.73–3.59 (m, 6H), 3.49-3.37 (m, 6H), 1.74–1.65 (m, 4H, 2 × CH_2), 1.43–1.41 (m, 4H, 2 × CH_2), 0.95 (s, 9H, *t*-Bu), 0.92 (s, 9H, *t*-Bu); ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.90, 139.41, 139.24, 138.95, 138.90, 138.66, 138.44, 138.12, 133.18, 130.25, 130.09, 128.73, 128.63, 128.58, 128.49, 128.34, 128.33, 128.25, 128.24, 128.17, 128.07, 128.01, 127.91, 127.87, 127.81, 127.80, 127.75, 127.61, 127.57, 127.33, 103.94, 103.10, 94.00, 83.11, 81.91, 79.71, 77.60, 77.31, 75.67, 75.49, 75.27, 73.85, 73.63, 73.58, 73.26, 72.79, 70.74, 70.51, 70.06, 68.62, 67.91, 67.09, 66.97, 66.19, 45.30, 32.80, 29.89, 27.91, 27.53, 26.96, 25.77, 23.62,

20.87; HRMS (ESI): calcd $C_{88}H_{105}ClNaO_{17}Si$ requires 1519.6707; found: *m*/*z* = 1519.6702 $[M+Na]^+$.

For **6-chlorohexyl 4-*O*-[3-*O*-(α-D-galactopyranosyl)-β-D-galactopyranosyl-β-D-glucopyranoside, isoGb$_3$ derivative (111)**: Protected isoGb$_3$ **110** (280 mg, 1.87 mmol) was treated with TBAF (1 M in THF, 1.8 mL), AcOH (95%, 0.25 mL), and THF (5 mL). After stirring at RT for 13 h, the reaction was diluted with CH_2Cl_2 (40 mL), washed with H_2O (15 mL × 2), dried over $MgSO_4$, filtered, and concentrated for next steps.[s20] The resulting crude (ca.200 mg) dissolved in 1:2 v/v MeOH-THF (3 mL) was treated with 2 N NaOH (2 mL), followed by stirring at 60˚C for 36 h. Upon completion of benzoyl ester removal, the mixture was diluted with CH_2Cl_2 (40 mL), and the solution was then washed with 1 N HCl (15 mL × 1), H_2O (15 mL × 1), brine (15 mL × 1), dried over $MgSO_4$, filtered, concentrated for the next hydrogenolysis. The resulting slurry was dissolved in a mixture of 5% Pd/C (100 mg), formic acid (0.35 mL), and MeOH (7 mL) at RT and was stirred under H_2 for 16 h. Upon completion of hydrogenolysis as assessed by TLC, the reaction mixture was filtered over celite, concentrated and then purified by column chromatography on C-18 coated reverse phase silica gel (Cosmosil 75C18-OPN) (elution: H_2O/MeOH gradient from 1/0 to 4/1) to furnish desired trisaccharide **111** as glassy solid (64 mg, 55% from **110**); $[\alpha]^{27}_D$ = +53.00 (*c* = 0.80, MeOH); ^{1}H NMR (300 MHz, CD_3OD): δ 5.07 (s, 1H), 4.45 (d, *J* = 6.6 Hz, 1H), 4.32 (d, *J* = 7.8 Hz, 1H), 4.24 (t, *J* = 6.3 Hz, 1H), 4.07 (s, 1H), 3.96–3.53 (m, 18H), 3.44–3.42 (m, 1H), 3.34–3.32 (m, 1H), 3.27 (t, *J* = 8.1 Hz, 1H), 1.84–175 (m, 2H, CH_2), 1.71–1.62 (m, 2H, CH_2), 1.48–1.43 (m, 4H, 2 × CH_2), ^{13}C NMR (75 MHz, CD_3OD): δ 105.03, 104.16, 97.66, 80.99, 79.83, 76.57, 76.42, 76.35, 74.67, 72.22, 71.30, 71.08, 70.98, 70.73, 70.11, 66.60, 62.71, 62.42, 61.96, 45.71,

33.71, 30.56, 27.69, 26.32; HRMS (ESI): calcd $C_{24}H_{43}ClO_{16}Na$ requires 645.2132; found: *m*/*z* = 645.2132 $[M + Na]^+$.

For ***p*-Tolyl 3,4,6-tri-*O*-benzyl-1-β-D-thiogalactopyranoside (113)**:



D-Galactose (5 g, 27.8 mmol) is treated with Ac_2O (16 mL, 0.174 mmol) and H_2SO_4 (0.18 mL, 3.3 mmol, dissolved in 5 mL CH_3CN) as described in Chapter-1. Upon completed, HBr (33% in AcOH,) is added into the reaction crude at 0 °C under N_2. After completion, the reaction crude is diluted with CH_2Cl_2 (50 mL), and then washed with saturated icy $NaHCO_{3(aq)}$ (50 mL×4) and brine (30 mL). The neutralized crude is dried ($MgSO_4$), filtered, and concentrated under vacuum ar RT for 3 hours to afford per-*O*-acetyl galactosyl bromide (9.7 g, 23.6 mmol) under one-pot acetylation-bromination operation.

The resulting bromide (9.5 g, 23.2 mmol) is then treated with 2,6-lutidine (6.7 mL, 58 mmol), TBAB (tetra-butyl ammonium bromide, 8 g, 23.2 mmol), and MeOH (3.8 mL, 93 mmol) in CH_3CN (60 mL) at RT under N_2 for 12 h. Upon completion, the crude is treated extracted with EtOAc (80 mL) and sat. icy $NaHCO_{3(aq)}$ (50 mL), dried over $NaSO_4$, filtered, concentrated, and then purified with column chromatography (SiO_2, hexane/AcOEt = 5:1 to 1:1, 2% Et_3N) to afford galactosyl oethoester product as colourless syrup (7.2 g, 19.9 mmol).

The per-*O*-acetyl galactosyl orthoester (7g, 19.3 mmol) is following

treated with MeOH (40 mL) and $Na_{(s)}$ (45 mg, 1.93 mmol) at RT to remove all acetyl groups. After reaction completed, the crude is concentrated under vacuum for 3 hours. The resulting mixture is then dissolved in dry DMF (50 mL). NaH (60% in mineral oil, 4.5 g, 112.5 mmol) and BnBr (8 mL, 67 mmol) are added into the solution described above at 0 ^{o}C under N_2. (*Beware of H_2 releasing!*) After completed, the crude is poured into a icy mixture of ether (120 mL) and sat. $NaHCO_{3(aq)}$ (30 mL). After extraction, the separated organic layer is then collected, dried over $MgSO_4$, filtered, concentrated, and then purified with column chromatography (SiO_2, hexane/AcOEt = 7:1 to 2:1, 2% Et_3N) to afford 3,4,6-tri-*O*-benzyl-D-galactosyl orthoester as white solid (7.7g, 15.2 mmol).

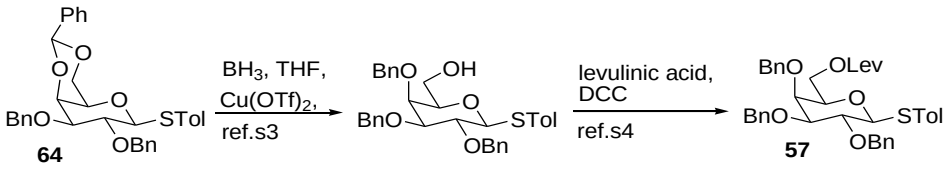
The purified galactosyl orthoester (7 g, 13.8 mmol) is then dissolved in dry CH_2Cl_2 (18 mL) under N_2. HSTol (*p*-thiocresol, 2.2 g, 18 mmol) and $BF_3.Et_2O$ (3 mL, 23.5 mmol) are added into the solution at −5 ^{o}C under N_2. Upon completion judged by TLC, the crude is diluted with CH_2Cl_2 (30 mL), and then washed with sat. $NaHCO_{3(aq)}$ (20 mL×2), brine (20 mL), dried ($MgSO_4$), filtered, concentrated, purified with column chromatography (SiO_2, hexane/AcOEt/CH_2Cl_2 = 8:1:1 to 2:1:1) to afford *p*-tolyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-thiogalactopyranoside (4.96 g, 8.3 mmol). The thiogalactoside is treated with MeOH (30 mL) and $Na_{(s)}$ (20 mg, 0.19 mmol) at RT to remove acetyl group. Upon completed, the reaction crude is neutralized with resin IR-120H, filtered, concentrated as white solid (4.3 g, 7.9 mmol, 30% from D-galactose); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.51–7.49 (d, J = 7.8 Hz, 2H, ArH), 7.44–7.30 (m, 15H, ArH), 7.07–7.05 (d, J = 7.8 Hz, 2H, ArH), 4.96–4.92 (d, J = 11.7 Hz, 1H), 4.80–4.70 (m, 2H), 4.64–4.60 (d, J = 11.7 Hz, 1H), 4.56–4.46 (m, 3H), 4.05–3.99 (m, 2H), 3.73–3.66 (m, 3H), 3.54–3.49 (dd, J = 2.7, 9.3 Hz, 1H), 2.58 (brs, 1H, OH), 2.34 (s, 3H, $ArCH_3$); ^{13}C NMR (75 MHz,

$CDCl_3$): δ139.1, 138.5, 138.3, 138.2, 129.0, 128.97, 128.88, 128.74, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 127.9, 89.2, 83.6, 78.0, 74.8, 74.0, 73.7, 72.8, 69.5, 69.1, 21.6.

For **1-*N*-2-*O*-3,4,6-tri-*O*-benzyl-1-α-D-galatopyranosyl oxazoline (114)**: Add NIS (67 mg, 0.29 mmol) and TMSOTf (10 μL, 0.05 mmol) into a cooled solution of **113** (150 mg, 0.27 mmol) in a mixed solvent system of CH_2Cl_2 and CH_3CN (1:3 v/v, 27 mL) at –70 ^oC under N_2. After completed, add NEt_3 (0.1 mL, 0.78 mmol) into reaction. The crude is extracted with EtOAc (10 mL) and sat. $NaHCO_{3(aq)}$ (10 mL), dried ($MgSO_4$), filtered, concentrated, and then purified with column chromatography (SiO_2 gel, hexane/AcOEt = 4:1 to 2:1, 2% Et_3N) tp afford the galactosyl oxazoline as colourless oil (55%); ^{1}H NMR (300 MHz, $CDCl_3$): δ7.51–7.49 (d, *J* = 7.8 Hz, 2H, ArH), 7.40–7.26 (m, 15H, ArH), 5.75 (d, *J* = 5.7 Hz, 1H), 4.95 (d, *J* = 11.5 Hz, 1H), 4.79–4.61 (m, 4H), 4.64–4.60 (d, *J* = 11.7 Hz, 1H), 4.53 (d, *J* = 11.8 Hz, 1H), 4.46 (d, *J* = 11.85.7 Hz, 1H), 4.11–4.09 (m, 1H, H-4), 4.03–4.00 (m, 1H, H-5), 3.77–3.59 (m, 3H), 2.04 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ169.4, 138.64, 138.25, 138.08, 128.67, 128.62, 128.54, 128.11, 128.02, 127.95, , 127.90, 127.83, 93.47, 82.51, 79.76, 74.8, 73.99, 73.70, 73.42, 71.55, 68.20, 14.92.

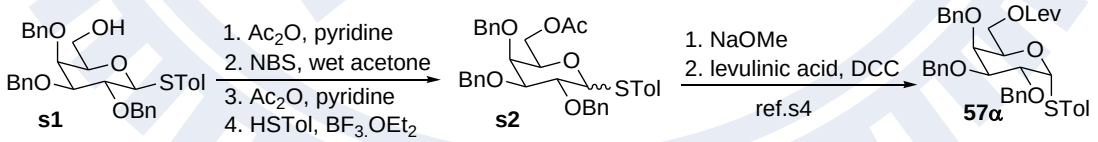
Preparation and spectroscopic data of glycosyl substrates 57, 57α, 61–66, 74, 76–81, 88–93, 100 and 107:

For *p*-tolyl 2,3,4-tri-*O*-benzyl-6-*O*-levulinoyl-1-β-D-thiogalactopyranoside (57):



White solid (82% over 2 steps); $[\alpha]^{27}_{D} = -3.90$ (c = 1.3, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 7.51 (d, J = 8.1 Hz, 2 H, ArH), 7.45–7.28 (m, 15 H, ArH), 7.05 (d, J = 8.1 Hz, 2 H, ArH), 5.02 (d, J = 11.4 Hz, 1H), 4.87–4.76 (m, 4H), 4.67 (d, J = 11.4 Hz, 1H), 4.50 (d, J = 9.6 Hz, 1H, H-1), 4.30 (dd, J = 4.2, 11.1 Hz, 1H, H-6), 4.20 (dd, J = 5.1, 11.1 Hz, 1H, H-6), 3.98–3.92 (m, 2H), 3.66–3.60 (m, 2H), 2.74 (t, J = 6.3 Hz, 2H, Lev-CH_2), 2.54 (t, J = 6.3 Hz, 2H, Lev-CH_2), 2.34 (s, 3H, $ArCH_3$), 2.20 (s, CH_3CO); ^{13}C NMR (75 MHz, $CDCl_3$): δ 206.9, 172.8, 138.8, 138.7, 138.6, 132.7, 130.5, 129.9, 128.9, 128.8, 128.7, 128.5, 128.2, 128.1, 88.4, 88.5, 77.7, 76.2, 76.1, 74.7, 73.6, 73.4, 38.3, 30.3, 28.2, 21.6; HRMS (Bio-ToFII): calcd for $C_{39}H_{42}O_7SNa$ requires 677.2543; found: m/z = 677.2543 $[M+Na]^+$.

For ***p*-tolyl 2,3,4-tri-*O*-benzyl-6-*O*-levulinoyl-1-α-D-thiogalactopyranoside (57α):**



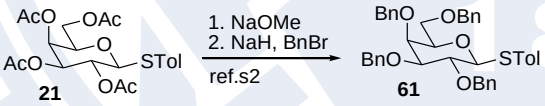
The thiogalactoside **s1** (800 mg, 1.44 mmol) was treat with Ac_2O (0.16 mL, 1.73 mmol) and pyridine (0.18 mL, 2.2 mmol) at RT. Upon acetylation completed, the reaction crude was diluted with EtOAc (10 mL) , then sequentially washed with saturated $NaHCO_3$ (10 mL × 2), 0.5 N $HCl_{(aq)}$(10 mL × 2), brine (10 mL × 1), dried ($MgSO_4$), filtered and concentrated as colorless oil. Then the resulting product was treated

with NBS (700 mg, 3.9 mmol) in 10:1 v/v acetone-water mixture (6 mL) at RT for 30 min. Upon complition, thereaction was neutralized with NEt_3, concentrated, extracted with saturated $NaHCO_3$ (10 mL) and CH_2Cl_2 (20 mL). The organic layer was washed with brine (10 mL × 1), dried ($MgSO_4$), filtered and concentrated to afford the galactosyl hemiacetal as oil. The resulting hemiacetal was treated with Ac_2O (0.13 mL, 1.18 mmol) and pyridine (0.14 mL, 1.71 mmol) at RT. Upon completion of acetylation, the reaction crude was diluted with EtOAc (10 mL), then sequentially washed with saturated $NaHCO_3$ (10 mL × 2), 0.5 N $HCl_{(aq)}$(10 mL × 2), brine (10 mL × 1), dried ($MgSO_4$), filtered, concentrated, and then purified with column chromatography (hexane/EtOAc = 5/1 to 2/1) to afford the protected galactoside as colorless oil (510 mg, 66% from **s1**).

Purified galactopyranosyl acetate **s2** (500 mg, 0.94 mmol) from previous step was treated with *p*-thiocresol (140 mg, 1.13 mmol), boron triflouride diethyl etherate ($BF_3.OEt_2$, 0.2 mL, 1.59 mmol), and CH_2Cl_2 (3 mL) under N_2 at −10˚C. Upon completion of thioglycosidation, the reaction was quenched with NEt_3, concentrated for column chromatography (hexane/EtOAc = 5/1 to 2/1) to afford the thiogalactoside **s3** (77% yield, α-anomer: 174 mg, β-anomer: 255 mg). α-Thiogalactopyranoside (385 mg, 0.64 mmol) was treated with $Na_{(s)}$(8 mg), MeOH (1 mL) and CH_2Cl_2 (4 mL) at RT. Upon complete deacetylation, the reaction ws neutralized with acid resin IR-120H^+, filtered, concentrated under *vacuo* for 2 h to afford oily crude. The crude residue was then treated with levulinic acid (0.08 mL, 0.77 mmol), *N*,*N'*-dicyclohexylcarcodiimide (DCC, 166 mg, 0.80 mmol), 4-(dimethylamino) pyridine (DMAP, 15 mg, 0.12 mmol), and CH_2Cl_2 (5 mL) under N_2 at RT. Upon completion of coupling, the reaction crude was neutralized with a drop of NEt_3, and then filtered over celite. The

filtrate was concentrated for column purification to afford the expected thiogalactopyranoside **57α** as white solid (358 mg, 85% from **s2**). For tolyl 2,3,4-tri-*O*-benzyl-6-*O*-levulinoyl-1-α-D-thiogalactopyranoside **(57α)**: $[\alpha]^{27}_{D}$ = +127.9 (*c* = 1.2, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.51–7.32 (m, 17H, ArH), 7.18 (AB, *J* = 8.1 Hz, 2H, ArH), 5.77 (d, *J* = 5.4 Hz, 1H, H-1), 5.07 (d, *J* = 11.4 Hz, 1H), 4.99 (d, *J* = 11.7 Hz, 1H), 4.90–4.77 (m, 3H), 4.72 (d, *J* = 11.4 Hz, 1H), 4.54 (t, *J* = 6 Hz, 1H), 4.46 (dd, *J* = 5.4, 9.9 Hz, 1H), 4.28 (dd, *J* = 7.2, 11.1 Hz, 1H), 4.31(dd, *J* = 5.1, 11.4 Hz, 1H), 4.00 (s, 1H), 3.93 (dd, *J* = 2.7, 9.9 Hz, 1H), 2.73–2.68 (m, 2H, Lev-CH_2), 2.53–2.49 (m, 2H, Lev-CH_2), 2.39 (s, 3H, $ArCH_3$), 2.19 (s, 3H, CH_3CO); ^{13}C NMR (75 MHz, $CDCl_3$): δ206.9, 172.8, 139.1, 138.7, 138.5, 137.7, 132.7, 130.9, 130.2, 128.9, 128.86, 128.84, 128.80, 128.5, 128.3, 128.1, 128.06, 88.0, 79.7, 76.9, 75.2, 75.1, 74.1, 73.1, 69.8, 63.9, 38.3, 30.3, 28.2, 21.6; HRMS (ESI): calcd for $C_{39}H_{42}O_7SNa$ requires 677.2543; found: *m*/z = 677.2543 $[M+Na]^+$.

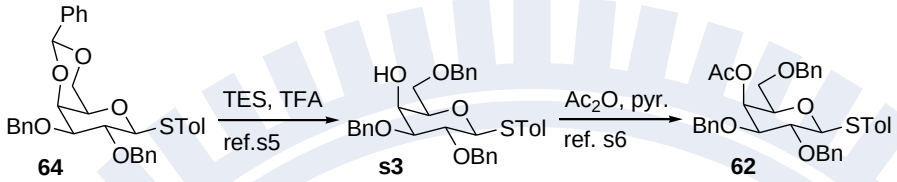
For ***p*-tolyl 2,3,4,6-tetra-*O*-benzyl-1-β-D-thiogalactopyranoside (61):**[s2]



White glassy soild (84% from **21**); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.49–7.39 (m, 34 H, ArH), 7.54–7.50 (m, 2H, ArH), 7.46–7.43 (m, 2H, ArH), 7.37–7.31 (m, 18H, ArH), 7.05–7.02 (m, 2H, ArH), 5.02 (d, *J* = 11.4 Hz, 1H), 4.87–4.73 (m, 4H), 4.67–4.63 (m, 2H), 4.52 (d, *J* = 11.7 Hz, 1H), 4.46 (d, *J* = 11.7 Hz, 1H), 4.04–4.01 (m, 1H), 3.95 (dt, *J* = 1.8, 9.6 Hz, 1H), 3.72–3.70 (m, 2H), 3.67–3.64 (m, 2H), 2.33 (s, 3H, $ArCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ139.2, 138.83, 138.72, 138.33, 137.6, 132.6, 130.5, 130.0, 128.9, 128.8, 128.6, 128.4, 128.3, 128.22, 128.15, 128.1, 128.0, 127.9, 88.5, 84.7, 77.8, 77.7, 76.1, 74.8, 74.0, 73.2, 69.2,

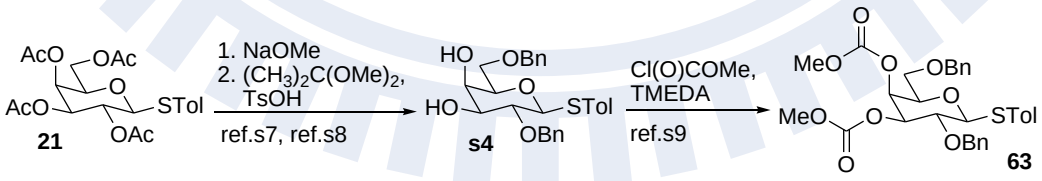
21.5.

For ***p*-tolyl 2,3,6-tri-*O*-benzyl-4-*O*-acetyl-1-β-D-thiogalactopyranoside (62):**



Colorless oil (73% from **64**); $[\alpha]^{27}_{D}$ = +4.76 (*c* = 0.84, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.50 (d, *J* = 8.1 Hz, 2H, ArH), 7.45–7.28 (m, 15 H, ArH), 7.08 (d, *J* = 8.1 Hz, 2H, ArH), 5.66 (d, *J* = 1.2 Hz, 1H, H-4), 4.82–4.74 (m, 3H), 4.66 (d, *J* = 9.0 Hz, 1H), 4.60–4.47 (m, 3H), 3.77 (t, *J* = 6.0 Hz, 1H), 3.70–3.62 (m, 3H), 3.55 (dd, *J* = 6.9, 9.6 Hz, 1H), 2.34 (s, 3H, $ArCH_3$), 2.12 (s, 3H, CH_3CO); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.8, 138.6, 138.10, 138.06, 138.01, 132.9, 130.2, 130.0, 128.88, 128.85, 128.79, 128.71, 128.67, 128.49, 128.28, 128.23, 88.6, 81.7, 77.2, 76.3, 76.2, 74.1, 72.4, 68.6, 67.3, 21.5, 21.4; HRMS (ESI): calcd for $C_{36}H_{38}O_6SNa$ requires 621.2281; found: *m*/z = 621.2281 $[M+Na]^+$.

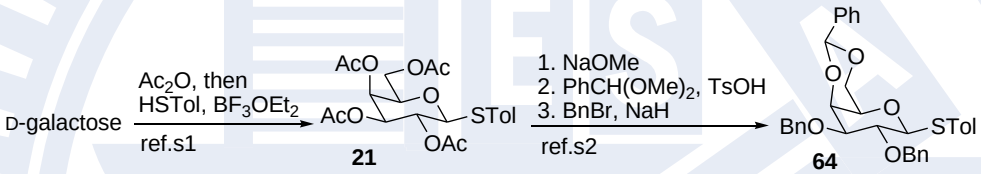
For ***p*-tolyl 2,6-di-*O*-benzyl-3,4-di-*O*-methoxycarbonyl-1-β-D-thiogalactopyranoside (63):**



White solid (43% form **21**); $[\alpha]^{27}_{D}$ = +2.59 (*c* = 0.98, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.51 (d, *J* = 8.1 Hz, 2H, ArH), 7.42–7.30 (m, 10H, ArH), 7.13–7.10 (d, *J* = 8.1 Hz, 2H, ArH), 5.44 (d, *J* = 2.7 Hz, 1H, H-4), 4.92 (dd, *J* = 3.0, 9.3 Hz, 1H), 4.87 (d, *J* = 10.8 Hz, 1H), 4.7 (d, *J* =

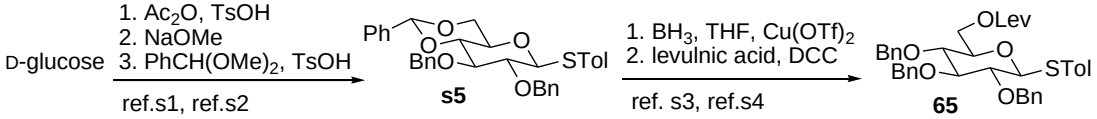
10.5 Hz, 1H), 4.64 (d, *J* = 10.8 Hz, 1H), 4.57 (d, *J* = 11.7 Hz, 1H), 4.50 (d, *J* = 11.7 Hz, 1H), 3.88–3.78 (m, 8H), 3.72–3.62 (m, 2H), 2.36 (s, 3H, $ArCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ155.9, 155.1, 138.3, 138.1, 132.96, 130.2, 129.95, 128.83, 128.76, 128.36, 128.23, 128.2, 88.6, 79.0, 76.14, 75.97, 75.93, 74.0, 72.4, 68.3, 55.65, 55.61, 21.6; HRMS (Bio-ToFII): calcd for $C_{31}H_{34}O_9SNa$ requires 605.1816; found: *m*/z = 605.1816 $[M+Na]^+$.

For ***p*-tolyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-β-D-thiogalactopyranoside (64):**[s1,s2]



White solid (49% from D-galactose); ^1H NMR (300 MHz, $CDCl_3$): δ7.66–7.64 (m, 2H, ArH), 7.58–7.55 (m, 2H, ArH), 7.49–7.31 (m, 13H, ArH), 7.04 (AB, *J* = 8.1 Hz, 2H, ArH), 5.52 (s, 1H, PhCH), 4.76–4.75 (m, 4H), 4.61 (d, *J* = 9.6 Hz, 1H), 4.40 (dd, *J* = 1.5, 12.3 Hz, 1H), 4.18 (d, *J* = 2.7 Hz, 1H), 4.01 (dd, *J* = 1.8, 12.6 Hz, 1H), 3.88 (t, *J* = 9.3 Hz, 1H), 3.66 (dd, *J* = 3.3, 9 Hz, 1H), 3.43 (d, *J* = 0.9 Hz, 1H), 2.34 (s, 3H, $ArCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ139.0, 138.5, 138.4, 138.1, 133.9, 130.1, 129.5, 129.1, 128.84, 128.78, 128.6, 128.3, 128.1, 127.1, 101.8, 87.0, 81.9, 75.8, 75.7, 74.1, 72.2, 70.2, 69.9, 21.6.

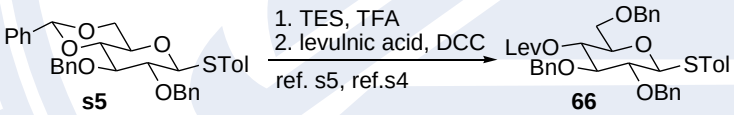
For ***p*-tolyl 2,3,4-tri-*O*-benzyl-6-*O*-levulinoyl-1-β-D-thioglucopyranoside (65):**



White solid (62% over 4 steps); $[\alpha]^{27}_{D}$ = +22.45 (*c* = 0.94, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.51–7.48 (m, 2H, ArH), 7.45–7.42 (m, 2H, ArH), 7.40–7.28 (m, 13H, ArH), 7.15–7.13 (m, 2H, ArH), 4.98–4.86 (m, 4H), 4.77 (d, *J* = 10.2 Hz, 1H), 4.65–4.60 (m, 2H), 4.41 (d, *J* = 11.4 Hz, 1H), 4.27 (d, *J* = 4.5, 11.7 Hz, 1H), 3.75 (t, *J* = 8.7 Hz, 1H), 3.59–3.47 (m, 3H), 2.81–2.76 (m, 2H, Lev-CH_2), 2.66–2.60 (m, 2H, Lev-CH_2), 2.37 (s, 3H, $ArCH_3$), 2.22 (s, 3H, CH_3CO); ^{13}C NMR (75 MHz, $CDCl_3$): δ 206.80, 172.86, 138.65, 138.38, 138.33, 138.06, 130.08, 128.94, 128.92, 128.88, 128.64, 128.53, 128.41, 128.34, 128.21, 88.2, 87.1, 81.2, 77.2, 76.3, 75.9, 76.5, 63.8, 38.3, 30.3, 28.3, 21.6; HRMS (Bio-ToFII): calcd for $C_{39}H_{42}O_7SNa$ requires 677.2543; found: *m*/z = 677.2543 $[M+Na]^+$.

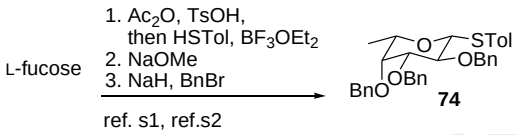
For ***p*-tolyl**

2,3,6-tri-*O*-benzyl-4-*O*-levulinoyl-1-β-D-thioglucopyranoside (66):



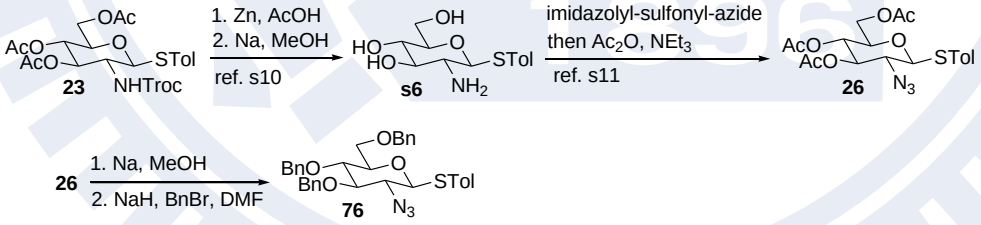
White solid (80% from **s5**); $[\alpha]^{27}_{D}$ = −18.68 (*c* = 1.22, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.51 (d, *J* = 8.1 Hz, 2H, ArH), 7.47–7.27 (m, 15H, ArH), 7.06 (d, *J* = 8.1 Hz, 2H, ArH), 5.05 (t, *J* = 9.3 Hz, 1H, H-4), 4.93 (d, *J* = 10.2 Hz, 1H), 4.84 (d, *J* = 11.4 Hz, 1H), 4.72 (t, *J* = 10.2 Hz, 2H), 4.68 (d, *J* = 9.9 Hz, 1H), 4.55 (s, 2H), 3.71 (t, *J* = 9.0 Hz, 1H), 3.67–3.60 (m, 3H), 3.55 (t, *J* = 9.6 Hz, 1H), 2.70–2.26 (m, 7H), 2.15 (s, 3H, CH_3CO); ^{13}C NMR (75 MHz, $CDCl_3$): δ 206.6, 172.1, 138.66, 138.64, 138.33, 138.25, 133.0, 130.15, 129.97, 128.88, 128.82, 128.72, 128.37, 128.26, 128.22, 128.12, 127.97, 88.2, 84.4, 81.0, 75.90, 75.82, 73.93, 71.4, 70.1, 38.1, 30.2, 28.3, 21.6; HRMS (Bio-ToFII): calcd for $C_{39}H_{42}O_7SNa$ requires 677.2543; found: *m*/z = 677.2543 $[M+Na]^+$.

For ***p*-tolyl 2,3,4-tri-*O*-benzyl-β-L-thiofucopyranoside (74):**[s2]



White glassy soild (52% over 3 steps); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.53–7.50 (m, 2H, ArH), 7.44–7.26 (m, 15H, ArH), 7.05–7.02 (m, 2H, ArH), 5.03 (d, J = 11.7 Hz, 1H), 4.83 (d, J = 10.2 Hz, 1H), 4.77–4.73 (m, 3H), 4.69 (d, J = 11.7 Hz, 1H), 4.57 (d, J = 9.6 Hz, 1H), 3.92 (t, J = 9.0 Hz, 1H), 3.65–3.59 (m, 2H), 3.53 (dd, J = 6.3, 12.9 Hz, 1H), 2.32 (s, 3H, $ArCH_3$), 1.28 (d, J = 6.3 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 139.2, 138.91, 138.82, 137.5, 132.6, 130.9, 129.9, 128.87, 128.78, 128.75, 128.57, 128.39, 128.11, 128.0, 127.87, 88.3, 85.0, 77.57, 75.97, 74.98, 73.3, 21.5, 17.7.

For ***p*-tolyl**
2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-1-β-D-thioglucopyranoside (76):

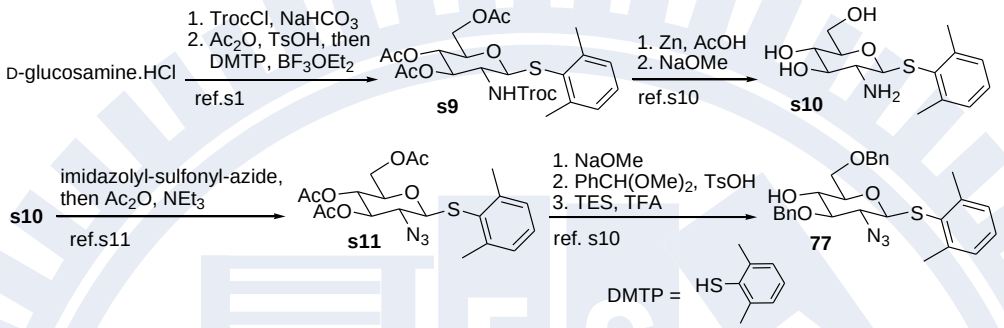


Colorless oil (72% from **23**); $[\alpha]^{27}_{D}$ = –65.94 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.60–7.57 (m, 2H, ArH), 7.43–7.35 (m, 13H, ArH), 7.30–7.27 (m, 2H, ArH), 4.92 (m, 2H), 4.86 (d, J = 10.8 Hz, 1H), 4.72–4.59 (m, 3H), 4.40 (d, J = 10.2 Hz, 1H, H-1), 3.85–3.83 (m, 2H), 3.71–3.51 (m, 3H), 3.38 (t, J = 9.9 Hz, 1H), 2.39 (s, 3H, $ArCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 139.2, 138.7, 138.4, 138.1, 134.8, 130.2, 129.0, 128.95, 128.83, 128.71, 128.49, 128.35, 128.31, 128.04, 127.41,

86.3, 85.5, 79.8, 76.4, 75.5, 73.9, 69.2, 65.3, 21.6; HRMS (ESI): calcd for $C_{34}H_{35}N_3O_4SNa$ requires 604.2240; found: *m*/z = 604.2240 $[M+Na]^+$.

For **(2,6-dimethylphenyl)**

3,6-di-*O*-benzyl-2-azido-2-deoxy-1-β-D-thioglucopy- ranoside (77):



A solution of D-glucosamine hydrochloride (10 g, 46.4 mmol) and $NaHCO_3$ (5.8 g, 116 mmol) in H_2O (150 mL) was treated with 2,2,2-trichloroethyl chloroformate (9.4 mL, 69 mmol) at RT for 12 h. The resulting suspension was filtered and washed with water (30 mL × 2) and cold ether (30 mL × 2) to furnish *N*-Troc protected glucosamine as white solid (16.1 g, quant.). Upon drying under *vacuo* for hours, *N*-Troc protected glucosamine (5 g, 14.2 mmol) was treated with Ac_2O (6.7 mL, 70 mmol) and TsOH (27 mg, 7.0 mmol) in dry CH_3CN (5 mL) at RT under N_2. After completion of acetylation, the reaction solvent was removed by rotary evaporator and dried under *vacuo* for 2 h, followed by the addition of 2,6-dimethyl thiophenol (DMTP, 2.9 g, 21.2 mmol), and $BF_3{\cdot}OEt_2$ (3.6 mL, 28.3 mmol) in CH_2Cl_2 (18 mL) under N_2. The mixture was stirred at RT and upon completion of thioglycosidation, the reaction cude was diluted with EtOAc (40 mL), and washed with $NaHCO_3$ (30 mL × 2), brine (20 mL × 1), dried (over $MgSO_4$), filtered and concentrated for chromatography purification over silica gel (elution: hexane/EtOAc = 4/1 to 1/1) to furnish desired (2,6-dimethylphenyl) 3,4,6-tri-*O*-acetyl -2-(2,2,2-trichloroethoxycarbamyl)-2-deoxy-1-β-D-thioglucopyranoside

s9 as white solid (5.8 g, 68% over 3 steps).

Compound **s9** (2.4 g, 4.0 mmol) was treated with zinc powder (1.3 g, 20.0 mmol) in a mixture of AcOH (0.2 mL, 3.6 mmol) and CH_2Cl_2 (20 mL) at RT. Upon completion of Troc removal, the resulting crude was filtered over a layer of celite, concentrated and then purified with column chromatography over neutralized SiO_2 (elution: hexane/EtOAc = 1/1 to 0/1) to furnish (2,6-dimethylphenyl) 3,4,6-tri-*O*-acetyl-2-deoxy-2-*N*-1-β-D-thioglucopyranoside (1.52 g, 89%). The furnished product (1.52 g, 3.6 mmol) was then disolved in a 1:2 CH_2Cl_2-MeOH mixture (15 mL), and to which was added a piece of freshly cut sodium (ca. 10 mg) at RT. Upon completion of reaction, the mixture was neutralized with conc.HCl and then concentrated to furnish crude thioglucoside **s10** as yellow slurry.

Crude thioglucopyranoside **s10** was then treated with K_2CO_3 (2.4 g, 17.4 mmol), imidazole-1-sulfonyl azide hydrochloride (1.0 g, 4.8 mmol), $CuSO_4.5H_2O$ (1 mg, 4.0 mmol), and MeOH (35 mL) at RT. Upon completion of azide transfer, the solvent was removed by rotary evaporator and co-evaporated with toluene (5 mL × 2); after then, the residue was treated with Ac_2O (2 mL, 21.4 mmol) and NEt_3 (4 mL, 28.8 mmol) at RT. As acetylation completed, the reaction mixture was diluted with EtOAc (20 mL), washed with saturated $NaHCO_3$ (15 mL × 2), brine (10 mL × 1) and dried over $MgSO_4$, filtered, concentrated for column chromatography over silica gel (elution: hexane/EtOAc = 3/1 to 1/1) to furnish the desired (2,6-dimethylphenyl) 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-1-β-D-thio-glucopyranoside **s11** as colorless oil (1.1 g, 63% from **s10**).

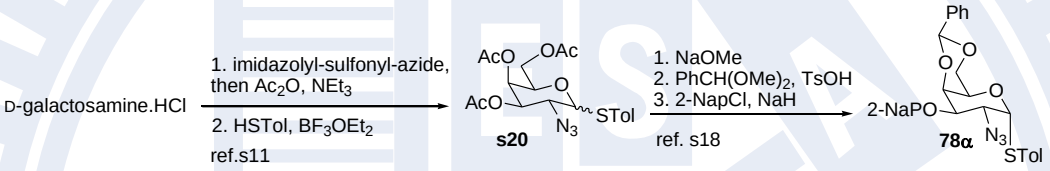
A solution of thioglucoside **s11** (800 mg, 1.8 mmol) in a 1:2 CH_2Cl_2-MeOH mixture (10 mL) was treated with a piece of freshly cut sodium (ca. 5 mg) at RT. Upon completion of deacetylation, the mixture

was neutralized with resin IR-120H^+, filtered, concentrated and dried under *vacuo* for 3 h. The resulting residue from previous deacetylation was suspended in CH_3CN (10 mL), to which $C_6H_5CH(OMe)_2$ (0.32 mL, 2.1 mmol) and TsOH (34 mg, 0.18 mmol) were added at RT under N_2. Upon completion of acetalation, the mixture was neutralized with NEt_3 (0.1 mL), concentrated under *vacuo* for 3 h to furnish crude (2,6-dimethylphenyl)2-azido-4,6-*O*-benzylidene-2-deoxy-1-β-D-thiogluc opyranoside as yellow foam. Crude 4,6-*O*- benzylidene thioglucoside was then dissolved in DMF (5 mL) at 0˚C under N_2, which was treated with 60% NaH (95 mg, 2.4 mmol) and BnBr (0.23 mL, 1.9 mmol). Upon completion of benzylation, the reaction mixture was diluted with CH_2Cl_2 (10 mL), washed with NH_4Cl (5 mL × 2), brine (5 mL × 1), dried (over $MgSO_4$), filtered, concentrated, and purified by column chromatography (elution: hexane/EtOAc = 4/1 to 3/2) over silica gel to furnish desired (2,6-dimethylphenyl) 2-azido-3-*O*-benzyl-4,6- *O*-benzylidene-2-deoxy-1-β-D-thioglucopyranoside as colorless glassy solid (670 mg, 75% from **s11**).

The resulting benzylidene thioglucopyranoside (200 mg, 0.4 mmol) was suspended in TES (0.3 mL, 2.0 mmol) and CH_2Cl_2 (3 mL), to which dry TFA (0.2 mL, 2.4 mmol) was added and the mixture was stirred at –5˚C. Upon completion of reductive ring opening, the mixture was diluted with EtOAc (10 mL), which was then washed with saturated $NaHCO_3$ (10 mL × 2), brine (10 mL × 1), dried (over $MgSO_4$), filtered, concentrated for chromatography purification (elution: hexane/EtOAc = 5/1 to 2/1) to furnish desired thioglucopyranoside **(77)** as colorless oil (172 mg, 85%). For (2,6-dimethylphenyl) 3,6-di-*O*-benzyl-2-azido-2-deoxy-4-*O*-1-β-D-thioglucopyrano side **(77)**: $[\alpha]^{27}_D$ = + 2.42 (*c* = 2.1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.41–7.31 (m, 10H, ArH), 7.24–7.15 (m, 3H, ArH), 4.99 (dd, *J* = 11.1, 16.8 Hz, 2H), 4.06 (dd, *J* =

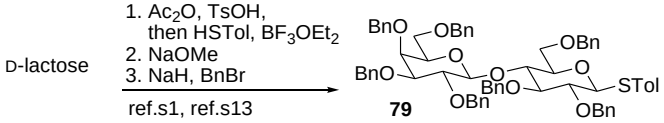
3.6, 12.0 Hz, 2H), 4.28 (d, *J* = 10.2 Hz, 1H), 3.75–3.70 (m, 3H), 3.50 (t, *J* = 9.3 Hz, 1H), 3.37–3.25 (m, 2H), 2.84 (br, 1H), 2.64 (2 × CH_3, 6H, CH_3CO); ^{13}C NMR (75 MHz, CDCl3) δ 144.7, 138.4, 138.1, 131.3, 129.7, 129.0, 128.9, 128.7, 128.6, 128.5, 128.2, 128.1, 89.5, 85.2, 78.1, 75.8, 74.1, 72.3, 70.8, 66.5, 22.9; LRMS (ESI): calcd for $C_{28}H_{31}N_3O_4SNa$ requires 528; found: *m*/*z* = 528 $[M+Na]^+$.

For ***p*-tolyl 2-azido-6-*O*-benzyl-2-deoxy-3-*O*-(2-naphthylmethyl)-1-α-D-thioglucopyranoside (78)**:



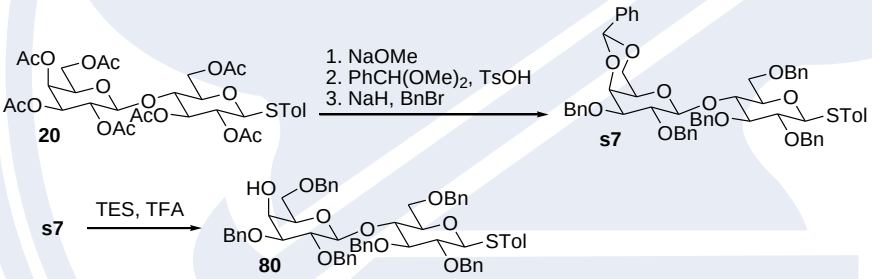
Colourless oil (32% from D-galacosamine hydrochloride) ^1H NMR (300 MHz, $CDCl_3$): δ7.91–7.82 (m, 4H), 7.62–7.49 (m, 5H, ArH), 7.43–7.37 (m, 5H), 7.13 (d, *J* = 9 Hz, 2H, ArH), 5.74 (d, *J* = 5.4 Hz, 1H), 5.5 (s, 1H, $(O)_2CHPh$), 4.96 (d, *J* = 11.8 Hz, 1H), 4.93 (d, *J* = 11.8 Hz, 1H), 4.54 (d, *J* = 10.5 Hz, 1H), 4.52 (d, *J* = 10.5 Hz, 1H), 4.27 (d, *J* = 3 Hz, 1H), 4.20 (dd, *J* = 1.2, 12.3 Hz, 1H), 4.15 (d, *J* = 6.9 Hz, 1H), 4.13 (d, *J* = 6.9 Hz, 1H), 4.05 (dd, *J* = 1.2, 12.3 Hz, 1H), 3.93 (dd, *J* = 3.3, 10.5 Hz, 1H), 2.35 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ138.0, 137.9, 135.6, 133.6, 133.5, 132.0, 130.3, 130.26, 129.5, 128.7, 128.67, 128.4, 128.2, 126.9, 126.7, 126.5, 126.1, 101.5, 88.3, 73.4, 72.2, 69.7, 64.1, 59.8, 21.5.

For ***p*-tolyl 2,3,6-tri-*O*-benzyl-4-*O*-(2′,3′,4′,6′-tetra-*O*-benzyl-β-D-galactopyranosyl)-1-β-D-thioglucopyranoside (79):**[s13]



White glassy soild (57% over 3 steps); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.59–7.19 (m, 37H, ArH), 7.12–7.09 (m, 2H, ArH), 5.21 (d, J = 10.5 Hz, 1H), 5.09 (d, J = 8.7 Hz, 1H), 4.95–4.77 (m, 7H), 4.71–4.33 (m, 7H), 4.10–4.04 (m, 2H), 3.98–3.86 (m, 3H), 3.72 (t, J = 8.7 Hz, 1H), 3.67–3.44 (m, 6H), 2.39 (s, 3H, $ArCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 139.55, 139.44, 139.23, 139.05, 139.02, 138.89, 138.59, 138.12, 133.3, 130.1, 128.89, 128.86, 128.74, 128.66, 128.54, 128.40, 128.33, 128.21, 128.03, 128.97, 128.92, 127.88, 127.84, 127.70, 103.3, 88.0, 85.5, 83.1, 80.5, 79.9, 76.9, 76.1, 76.0, 75.8, 75.2, 74.1, 73.9, 73.5, 73.1, 68.9, 68.5, 21.6.

For *p*-tolyl 2,3,6-tri-*O*-benzyl-4-*O*-(2′,3′,6′-tri-*O*-benzyl-β-D-galactopyranosyl)-1-β-D-thioglucopyranoside (80):

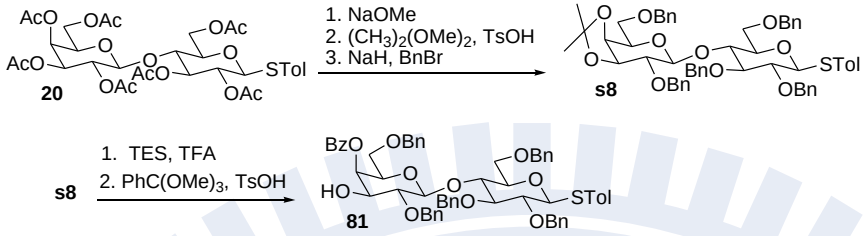


To a solution of per-*O*-acetyl thiolactoside **20** (2.8 g, 3.8 mmol) in 1:2 CH_2Cl_2- MeOH mixture (20 mL) was added a piece of freshly cut sodium (ca. 20 mg) at RT. Upon completion of deacetylation, the mixture was neutralized with resin IR-120H^{+}, filtered, concentrated and dried under *vacuo* for 3 h. The crude residue was suspended in a mixture of $C_6H_5CH(OMe)_2$ (0.7 mL, 4.6 mmol) and CH_3CN (13 mL) at RT, followed by the addition of TsOH (72 mg, 0.38 mmol) and the temperature was then raised to 45˚C. Upon completion of acetalation as assessed by TLC, the reaction temperature was brought to RT, and the reaction mixture was neutralized with Et_3N (0.2 mL), concentrated and dried under *vacuo* for few hours. The resulting residue from previous reaction was dissolved in

a solution of BnBr (2.7 mL, 22.6 mmol), DMF (13 mL) at 0˚C under N_2, to which 60% NaH (1.14 g, 28.5 mmol,) was added, followed by vigorous stirring. After completion of benzylation, the mixture was diluted with excess EtOAc (50 mL), and the resulting EtOAc solution was washed with NH_4Cl (40 mL × 1), dried ($MgSO_4$), filtered, and concentrated for chromatography purification over silica gel to afford the benzylidene protected thiolactoside **s7** as white glassy solid (2.81 g, 75% from **20**).

Thiolactoside **s7** (2.7 g, 2.74 mmol) was suspended in a mixture of triethyl silane (8.6 mL, 54.8 mmol) and CH_2Cl_2 (5 mL), and stirred at −10˚C under N_2. Thereafter, dry TFA (4.2 mL, 54.8 mmol) was added to the suspension and the reaction mixture was stirred at 0˚C. Upon completion of reaction, the mixture was diluted with CH_2Cl_2 (30 mL), and the resulting solution was washed with saturated $NaHCO_3$ (30 mL × 2), brine (20 mL × 1), dried ($MgSO_4$), filtered, and concentrated for chromatography purification over silica gel (elution: hexane/EtOac = 3/1 to 3/2) to furnish the desired thiolactoside **80** as white glassy solid (2.22 g, 80%). For tolyl 2,3,6-tri-*O*-benzyl-4-*O*-(2′,3′,6′-tri-*O*-benzyl-β-D-galactopyranosyl)-1-β-D-thioglucopyranoside **(80)**: $[\alpha]^{27}_D$ = +3.35 (*c* = 0.83, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.89–6.39 (m, 34 H, ArH), 4.96 (d, *J* = 9.0 Hz, 1 H), 4.74–4.15 (m, 13 H), 3.92–3.26 (m, 12 H), 2.35 (br, 1 H), 2.19 (s, 3 H, $ArCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 139.17, 138.83, 138.74, 138.61, 138.47, 138.19, 137.99, 133.12, 129.93, 129.88, 128.77, 128.67, 128.57, 128.48, 128.44, 128.17, 128.06, 127.96, 127.89, 127.81, 127.72, 127.69, 102.87, 87.85, 85.26, 81.40, 80.38, 79.70, 79.63, 77.82, 77.60, 77.40, 76.97, 76.59, 75.94, 75.73, 75.63, 73.80, 73.36, 73.09, 72.34, 68.64, 66.35, 21.42; HRMS (ESI): calcd for $C_{61}H_{64}NaO_{10}S$ requires 1011.4112; found: *m*/z = 1011.4112 $[M+Na]^+$.

For *p*-tolyl 2,3,6-tri-*O*-benzyl-4-*O*-(4′-*O*-benzoyl-2′,6′-di-*O*-benzyl-β-D-galactopy- ranosyl)-1-β-D-thioglucopyranoside (81):

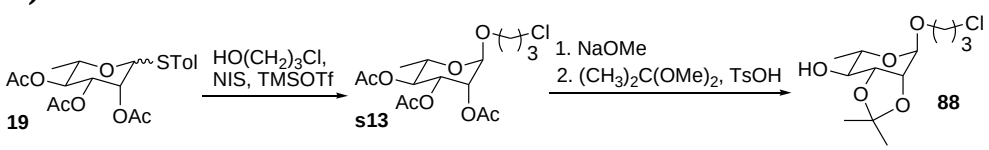


To a solution of per-*O*-acetyl thiolactoside **20** (3.0 g, 4.0 mmol) in 1:2 CH_2Cl_2-MeOH mixture (30 mL) was added a piece of freshly cut sodium (ca. 20 mg) at RT. Upon completion of deacetylation, the mixture was neutralized with resin IR-120H$^+$, filtered, concentrated and dried under *vacuo* for 3 h. The deacetylated crude thiolactoside was resuspended in a mixture of 2,2-diemethoxypropane (0.88 mL, 8.0 mmol) and TsOH (76 mg, 0.4 mmol) in dry acetone (20 mL) at 45˚C under N_2. After completion of ketalation, the reaction mixture was cooled to 0˚C, followed by neutralization with Et_3N (0.1 mL). The resulting solution was concentrated and dried under *vacuo* for 3 h. The crude product from above reaction was dissolved in a mixture of benzyl bromide BnBr (2.9 mL, 24.0 mmol) and DMF solution (18 mL), which was cooled to 0˚C. Followed by addition of 60% NaH (1.2 g, 30.0 mmol), the mixture was stirred vigorously stirring from 0˚C to RT. Upon completion of benzylation, the mixture was diluted with EtOAc (50 mL), which was washed with NH_4Cl (40 mL × 1), dried ($MgSO_4$), filtered and concentrated to **s8** for next deacetalation.

Thiolactoside **s8** was stirred in a mixture of TFA (1 mL), CH_3CN (7 mL), H_2O (1 mL) at RT for 2 h, and the mixture was was diluted with EtOAc (20 mL). The EtOAc solution was washed with saturated $NaHCO_3$ (10 mL), dried ($MgSO_4$), filtered, and concentrated for chromatography purification to afford thiolactosyl diol derivative as white glassy solid

(2.2 g, 61% from **s10**). Thiolactosyl diol derivative (2.2 g, 2.45 mmol) was treated with triethyl orthobenzoate (0.85 mL, 3.7 mmol) and TsOH (47 mg, 0.25 mmol) in a stirring solution of CH_2Cl_2 (8 mL) at RT. Upon completion of 3,4-di-*O*-orthoester formation, a 1:1 TFA-H_2O mixture (0.4 mL) was added to cleave the orthoester function at RT. The resulting mixture was diluted with CH_2Cl_2 (30 mL), and washed with saturated $NaHCO_3$ (15 mL × 2), brine (20 mL × 1), dried ($MgSO_4$), filtered and concentrated for chromatography purification over silica gel (elution: hexane/EtOAc = 3/1 to 3/2) to furnish desired thiolactoside **81** as white glassy solid (1.84 g, 75% from **s8**). For *p*-tolyl 2,3,6-tri-*O*-benzyl -4-*O*-(4′-*O*-benzoyl-2′,6′-di-*O*-benzyl-β-D-galactopyranosyl)-1-β-D-thiog lucopyranoside **(81)**: $[\alpha]^{27}_{D} = -35.96$ (*c* = 1.09, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ7.95 (d, *J* = 7.8 Hz, 2 H), 7.52–7.46 (m, 3 H, ArH), 7.41–7.25 (m, 19 H, ArH), 7.18–7.01 (m, 10 H, ArH), 5.60 (d, *J* = 3.0 Hz, 1 H), 5.16 (d, *J* = 9.0 Hz, 1 H), 4.83–4.68 (m, 5 H), 4.62–4.42 (m, 4 H), 4.32–4.30 (m, 2 H), 4.05 (t, *J* = 9.6 Hz, 1 H), 3.89–3.86 (m, 2 H), 3.81–3.75 (m, 2 H), 3.69–3.63 (m, 2 H), 3.55–3.39 (m, 5 H), 2.54 (br, 1 H), 2.29 (s, 1 H, $ArCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ166.75, 139.14, 138.61, 138.46, 138.09, 133.47, 133.22, 130.15, 129.98, 129.82, 128.78, 128.69, 128.60, 128.54, 128.43, 128.28, 128.25, 128.18, 128.10, 128.00, 127.89, 127.71, 102.90, 87.93, 85.29, 80.49, 80.32, 79.62, 76.79, 75.97, 75.84, 75.54, 73.79, 73.50, 73.01, 72.66, 70.57, 68.53, 67.68, 21.45; HRMS (ESI): calcd for $C_{61}H_{62}O_{11}SNa$ requires 1025.3905; found: *m*/*z* = 1025.3905 $[M+Na]^+$.

For **3-chloropropyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside (88):**

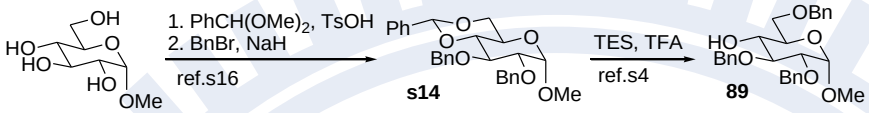


A mixture of L-thiorhamnopyranoside **19** (1.0 g, 2.5 mmol),[s1] 3-chloropropanol (0.32 mL, 3.75 mmol), and flame-dried molecular sieves (AW300, 1 g) were stirred in CH_2Cl_2 (3 mL) at −30˚C under N_2 for 15 min. After then, NIS (600 mg, 2.6 mmol) and TMSOTf (10 μL, 0.6 mmol) were added, and upon completion of glycosylation, the mixture was diluted with CH_2Cl_2 (10 mL), treated with saturated $NaHCO_3$ (0.1 mL), $Na_2S_2O_3$ (s) (2 g), dried (over $MgSO_4$), filtered, and concentrated for chromatography purification over silica gel (elution: hexane/EtOAc = 3/1 to 1/1) to furnish desired 3-chloropropyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranoside **s13** as colorless oil (757 mg, 81%).

Rhamnopyranoside **s13** (750 mg, 2.04 mmol) dissolving in 1:2 CH_2Cl_2-MeOH (10 mL) was treated with a piece of freshly cut sodium (ca. 10 mg) at RT. Upon completion of deacetylation, the reacting solution was neutralized with resin IR-120H^+, filtered, concentrated and dried under *vacuo* for 3 h. The resulting deacetylated product was then suspended in dried acetone (10 mL), to which was added 2,2-dimethoxypropane (0.4 mL, 3.67 mmol) and TsOH (39 mg, 0.2 mmol) at RT under N_2. As acetal formation was complete, the mixture was neutralized with Et_3N (0.1 mL), concentrated for chromatography purification over short pad of pre-neutralized silica gel (elution: hexane/EtOAc = 3/1 to 1/1) to furnish (3-chloro propyl) 2,3-*O*-isopropylidene-α- L-rhamnopyranoside **88** as colorless oil (413 mg, 80% from **s13**). For (3-chloropropyl) 2,3-*O*-isopropylidene-α-L-rhamnopyranoside **(88)**: $[\alpha]^{27}_D$ = −42.75 (*c* = 1.09, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.97 (s, 1H), 4.12 (d, *J* = 6 Hz, 1H), 4.06 (dd, *J* = 5.7, 7.2 Hz, 1H), 3.91−3.84 (m, 1H), 3.68−3.60 (m, 3H), 3.59−3.52 (m, 1H), 3.37 (dt, *J* = 3.6, 7.5 Hz, 1H), 3.1 (br, 1H), 2.08−1.98 (m, 2H), 1.53 (s, 3H, CH_3C), 1.36 (s, 3H, CH_3C), 1.29 (d, *J* = 6.3 Hz, 3H, CH_3CO);

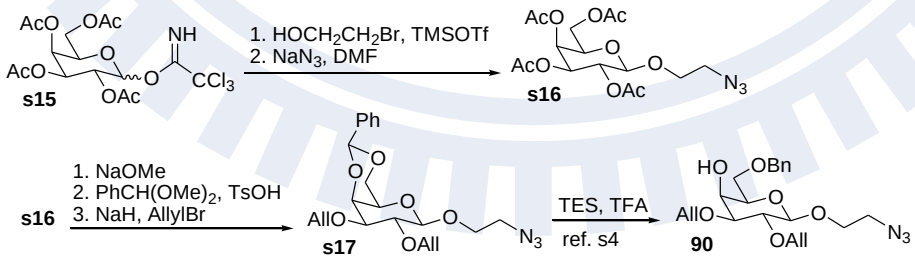
^{13}C NMR (75 MHz, $CDCl_3$): δ109.9, 97.4, 78.9, 76.2, 74.8, 66.1, 64.1, 42.0, 32.6, 28.4, 26.6, 17.7; HRMS (ESI): calcd for $C_{12}H_{21}ClO_5Na$ requires 303.0970; found: *m*/*z* = 303.0970 $[M + Na]^+$.

For **methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (89):**[s4]



Methyl α-glucopyranoside **89** (56% yield over 3 steps) was obtained as colorless oil from literature procedure.[s4] ^{1}H NMR (300 MHz, $CDCl_3$): δ7.42–7.29 (m, 15H, ArH), 5.04 (d, *J* = 11.4 Hz, 1H), 4.81 (d, *J* = 10.2 Hz, 1H), 4.77 (d, *J* = 9.3 Hz, 1H), 4.71–4.67 (m, 2H), 4.63 (d, *J* = 12.0 Hz, 1H), 4.57 (d, *J* = 12.0 Hz, 1H), 3.83 (t, *J* = 9.0 Hz, 1H), 3.78-3.68 (m, 3H), 3.64 (t, *J* = 9.0 Hz, 1H), 3.57 (dd, *J* = 3.6, 9.6 Hz, 1H), 3.42 (s, 3H, OCH_3), 2.2 (br, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ139.2, 138.47, 138.42, 129.0, 128.9, 128.8, 128.6, 128.43, 128.39, 128.27, 128.06, 98.6, 81.9, 80.0, 75.9, 74.0, 73.6, 71.1, 70.3, 69.9, 55.7.

For **2-azidoethyl 2,3-di-*O*-allyl-6-*O*-benzyl-β-D-galactopyranoside (90):**

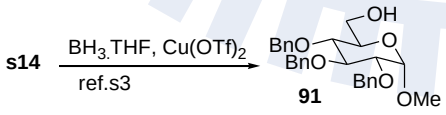


D-Galactopyranosyl imidate **s15** (1.3 g, 2.6 mmol),[s15] 2-bromoethanol (0.28 mL, 3.97 mmol), flame-dried molecular sieves (AW300, 1.5 g) in CH_2Cl_2 (26 mL) were stirred for 30 min at RT under

N_2. The suspension was then cooled at −30˚C bath for 15 min, and then treated with TMSOTf (10 μL, 0.6 mmol). Upon completion of glycosylation, the reaction mixture was washed with saturated $NaHCO_3$ (20 mL × 1), brine (20 mL × 1), dried (over $MgSO_4$), filtered, and concentrated for column chromatography purification over silica gel (elution: hexane/EtOAc = 3/1 to 3/2) to furnish 2-bromoethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside as colorless oil (782 mg, 65%). The resulting oily galactopyranoside (780 mg, 1.7 mmol) was heated in a mixture of NaN_3 (560 mg, 8.6 mmol) and DMF (10 mL) at 90˚C for 16 h. After cooling to RT, the mixture was diluted with CH_2Cl_2 (20 mL), and washed with H_2O (20 mL × 1), brine (10 mL × 1),dried (over $MgSO_4$), and concentrated for column chromatography purification over silica gel (elution: hexane/EtOAc = 3/1 to 3/2) to furnish 2-azidoethyl 2,3,4,6-tetra-*O*-aceyl-β-D-galactopyranoside **s16** as glassy solid (616 mg, 86%). Galactopyranoside **s16** (610 mg, 1.46 mmol) dissolving in 1:2 CH_2Cl_2-MeOH mixture (15 mL) was treated with a piece of freshly cut sodium (ca. 10 mg) at RT. Upon completion of deacetylation, the solution was neutralized with resin IR-120H^+, filtered to remove resin, concentrated and dried under *vacuo* for 3 h. The resulting product from previous deacetylation was treated with $C_6H_5CH(OMe)_2$ (0.27 mL, 1.75 mmol) and TsOH (28 mg, 0.15 mmol) in CH_3CN (5 mL) at RT under N_2. Upon completion of acetalation, the mixture was neutralized with Et_3N (0.1 mL), concentrated and dried under *vacuo* for hours. The resulting product was then treated with 60% NaH (175 mg, 4.4 mmol) and allyl bromide (0.3 mL, 3.5 mmol) in DMF (5 mL) at 0˚C under N_2. Upon completion of allylation, the mixture was diluted with CH_2Cl_2 (20 mL), and the solution was washed with H_2O (20 mL × 1), brine (10 mL × 1), dried (over $MgSO_4$), concentrated for column chromatography purification over silica gel (elution: hexane/EtOAc = 4/1

to 2/1) to furnish 2-azidoethyl 2,3-di-*O*-allyl- 4,6-*O*-benzylidene-β-D-galactopyranoside **s17** as glassy solid (445 mg, 73% from **s16**). The resulting galactopyranoside **s17** (440 mg, 1.06 mmol) dissolving in a mixture of triethylsilane (0.3 mL, 2.0 mmol) and CH_2Cl_2 (3 mL) was treated with dry TFA (0.2 mL, 2.4 mmol) at −20˚C under N_2. The reaction temperature was raised to 0 ˚C slowly. Upon completion of reductive ring opening, the resulting mixture was diluted with EtOAc (20 mL), washed with saturated $NaHCO_3$ (10 mL × 2), brine (10 mL × 1), dried (over $MgSO_4$), filtered and concentrated for chromatography purification over silica gel (elution: hexane/EtOAc = 4/1 to 2/1) to furnish the galactopyranoside **90** (336 mg, 76%) as colorless oil. For 2-azidoethyl 2,3-di-*O*-allyl-6-*O*-benzyl-β-D-galactopyranoside **(90)**: $[\alpha]^{27}_{D} = -12.11$ (*c* = 1.78, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.37−7.25 (m, 5H, ArH), 6.00−5.87 (m, 2H, =CH), 5.35−5.32 (m, 2H, $=CH_2$), 5.22−5.14 (m, 2H, $=CH_2$), 4.58 (s, 2H), 4.41−4.32 (m, 2H), 4.23−4.16 (m, 3H), 4.08−3.99 (m, 2H), 3.82−3.64 (m, 3H), 3.60−3.43 (m, 3H), 3.41−3.33 (m, 2H), 2.72 (br, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.4, 135.6, 135.1, 128.8, 128.2, 117.7, 117.2, 103.9, 80.7, 78.7, 74.3, 74.1, 73.7, 71.9, 69.6, 68.5, 67.4, 51.4; HRMS (Bio-ToFII): calcd for $C_{21}H_{29}N_3O_6Na$ requires 442.1949; found: *m*/z = 442.1949 $[M+Na]^+$.

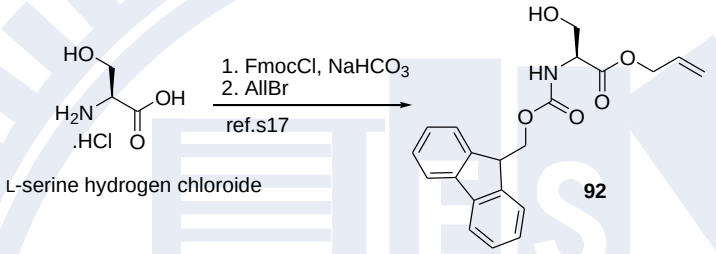
For **methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (91):**[s3]



Colorless oil (87% from **s14**); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.44−7.28 (m, 15H, ArH), 5.01 (d, *J* = 10.8 Hz, 1H), 4.93−4.79 (m, 3H), 4.71−4.64 (m, 2H), 4.60 (d, *J* = 3.6 Hz, 1H), 4.04 (t, *J* = 9.3 Hz, 1H), 3.83−3.66 (m, 3H), 3.59−3.49 (m, 2H), 3.39 (s, 3H, OCH_3); ^{13}C NMR

(75 MHz, $CDCl_3$): δ138.8, 138.45, 138.32, 135.44, 135.93, 128.86, 128.84, 128.81, 128.74, 128.37, 128.30, 128.22, 128.20, 128.12, 128.09, 128.01, 128.0, 122.5, 122.4, 98.1, 80.81, 80.46, 80.32, 80.09, 74.89, 74.08, 74.0, 73.93, 73.68, 73.48, 73.27, 73.21, 72.77, 71.73, 70.8, 68.5, 68.3, 49.99, 25.0, 24.9.

For ***N*$^{\alpha}$-(fluoren-9-ylmethoxycarbonyl)-L-serine allyl ester (92):**[s17]

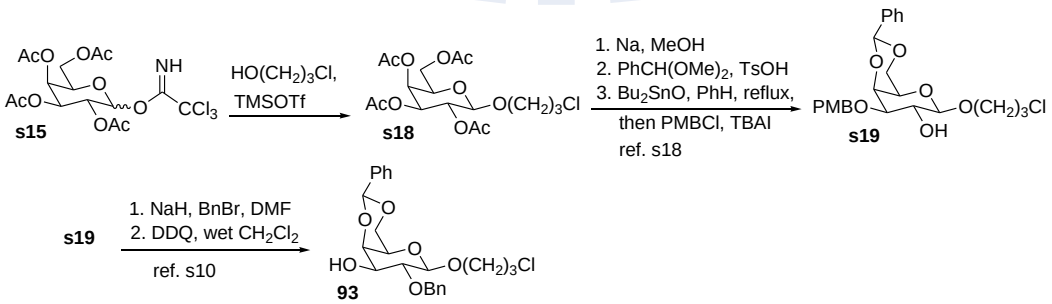


L-serine hydrogen chloroide

White glassy solid (63% from L-serine.HCl); ^{1}H NMR (300 MHz, $CDCl_3$): δ7.79 (d, J = 7.5 Hz, 2H, ArH), 7.63 (d, J = 7.2 Hz, 2H, ArH), 7.43 (t, J = 7.5 Hz, 2H, ArH), 7.34 (t, J = 7.5 Hz, 2H, ArH), 5.98-5.87 (m, 1H, =CH), 5.77 (br, 1H. NH), 5.37 (d, J = 17.1 Hz, 1H, =CH_2), 5.29 (dd, J = 0.9, 10.5 Hz, 1H, =CH_2), 4.39 (d, J = 5.4 Hz, 2H), 4.52–4.40 (m, 3H), 4.25 (t, J = 6.9 Hz, 1H), 4.06 (dd, J = 3.6, 11.4 Hz, 1H), 3.97 (dd, J = 3.0, 11.1 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ144.1, 141.76, 141.71, 131.69, 128.18, 127.53, 127.50, 125.51, 120.45, 119.50, 77.64, 67.62, 66.8, 63.7, 56.5, 47.5.

For **3-chloropropyl**

2-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside (93):[s10]



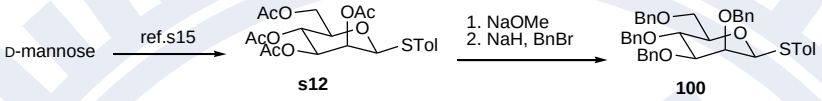
A mixture of peracetyl D-galactopyranosyl imidate **s15** (1.5 g, 3.1 mmol),[s15] 3-chloropropanol (0.38 mL, 4.6 mmol), and flame-dried (AW300, 1.9 g) in CH_2Cl_2 (32 mL) were stirred at RT under N_2. The resulting suspension was stirred at –40˚ for 15 min, and then treated with TMSOTf (10 μL, 0.6 mmol). Upon completion of glycosylation, the reaction mixture was washed with saturated $NaHCO_3$ (20 mL ×2), dried (over $MgSO_4$), filtered, and concentrated for chromatography purification over silica gel (elution: hexane/EtOAc = 4/1 to 3/2) to furnish 3-chloropropyl 2,3,4,6- tetra-*O*-acetyl-β-D-galactopyranoside **s18** as colorless oil (892 g, 68%). The resulting galactopyranoside **s18** from the above reaction (880 mg, 2.08 mmol) was dissolved in 1:2 CH_2Cl_2-MeOH (9.0 mL). A piece of freshly cut sodium (ca. 10 mg) was added into the reaction CH_2Cl_2-MeOH mixture, which was stirred at RT. Upon completion of deacetylation, the mixture was neutralized with resin IR-120H^+, filtered, concentrated and dried under *vacuo* for 3 h. The decatylated product was treated with $C_6H_5CH(OMe)_2$ (0.38 mL, 2.5 mmol) and TsOH (40 mg, 0.21 mmol) in CH_3CN (7 mL) at RT under N_2. As benzylidenation was complete, the mixture was then neutralized with Et_3N (0.1 mL), co-evaporated with toluene (5 mL × 2), concentrated and dried under *vacuo*. The resulting residue was re-suspended in 4:1 benzene-toluene mixture (25 mL), to which Bu_2SnO (820 mg, 3.3 mmol) was added, followed by reflux at 103˚C with Dean-Stark apparatus for 18 h. The temperature was then brought to 75˚C, which was followed by addition of *p*-methoxybenzyl chloride (PMBCl, 0.42 mL, 3.12 mmol) and *n*-Bu_4NI (390 mg, 1.05 mmol). The resulting reaction was stirred at 75˚C for 6 h under N_2. The reaction mixture was cooled to RT, and poured into a mixture of CH_2Cl_2 (40 mL) and saturated $NaHCO_3$ (20 mL). The highly flocculated suspension was then filtered through a pad of sea sand/silica

gel for removal of residual Bu_2SnO. The filtrate was extracted with CH_2Cl_2 (40 mL × 2) and the CH_2Cl_2 solution was dried over $MgSO_4$, filtered, and concentrated for chromatography purification over silica gel (elution: hexane/ether = 4/1 to 1/1) to furnish 3-chloropropyl 4,6-*O*-benzylidiene-3-*O*-(4-methoxybenzyl)-β-D-galactopyranoside **s19** as yellow oil (655 mg, 68% from **s18**).

Purified galactopyranoside **s19** (650 mg, 1.4 mmol) and BnBr (0.2 mL, 1.7 mmol) was dissolved in DMF (5 mL) at 0˚C under N_2, and the mixture was treated with 60% NaH (84 mg, 2.1 mmol). Upon completion of benzylation, the mixture was diluted with CH_2Cl_2 (20 mL), and then washed with NH_4Cl (20 mL × 1), brine (15 mL × 1), dried (over $MgSO_4$), filtered, and concentrated for chromatography purification over silica gel to furnish 3-chloropropyl 4,6-*O*-benzylidene-2-*O*-benzyl-3-*O*-(4-methoxy benzyl)-β-D-galactopyranoside. The resulting galactopyranoside (644 mg, 1.16 mmol) was then dissolved in a mixture of CH_2Cl_2 (7.6 mL) and H_2O (0.4 mL) at 0˚C, to which 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (610 mg, 2.7 mmol) was added. Upon completion of PMB ether removal, the mixture was diluted with CH_2Cl_2 (40 mL), and then washed with saturated $NaHCO_3$ (10 mL × 1), brine (10 mL × 1), dried (over $MgSO_4$), filtered, and concentrated for chromatography purification over silica gel (elution: hexane/EtOAc = 2/1 to 1/1) to furnish galactopyranoside **93** as colorless oil (429 mg, 71% from **s19**). For 3-chloropropyl 2-*O*-benzyl-4,6-*O*-benzy- lidene-β-D- galactopyranoside **(32)**: $[\alpha]^{27}_D$ = +14.60 (*c* = 0.73, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ7.54–7.53 (m, 2H, ArH), 7.41–7.28 (m, 8H, ArH), 5.58 (s, 1H, PhCH), 4.95 (d, *J* = 11.1 Hz, 1H), 4.75 (d, *J* = 11.1 Hz, 1H), 4.44–4.32 (m, 2H), 4.24 (d, *J* = 2.7 Hz, 1H), 4.15–4.04 (m, 2H), 3.81–3.3.61 (m, 5H), 3.47–3.39 (m, 1H), 2.55 (br, 1H), 2.19–2.01 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ138.96, 138.1, 129.6, 129.4, 128.85, 128.65, 128.35,

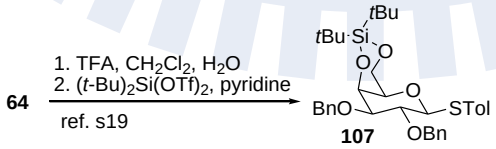
128.17, 126.87, 126.76, 103.93, 101.72, 79.8, 75.95, 75.4, 72.8, 69.5, 66.89, 66.76, 42.3, 33.1; LRMS (ESI): calcd for $C_{23}H_{27}ClO_6Na$ requires 457; found: m/z = 457 $[M+Na]^+$.

For ***p*-tolyl 2,3,4,6-tetra-*O*-benzyl-1-β-D-thiomannopyranoside (100):**



White glassy solid (55% over 4 steps): $[\alpha]^{27}_D$ = –47.14 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.55–7.52 (m, 4H, ArH), 7.48–7.31 (m, 16H, ArH), 7.26–7.24 (m, 2H, ArH), 7.07 (d, J = 7.8 Hz, 2H, ArH), 5.10 (d, J = 11.4 Hz, 1H), 4.95 (dd, J = 3.6, 11.1 Hz, 2H), 4.79–4.70 (m, 3H), 4.65–4.56 (m, 3H), 4.19 (d, J = 2.4 Hz, 1H), 4.01 (t, J = 9.6 Hz, 1H), 3.90 (dd, J = 1.8, 10.8 Hz, 1H), 3.81 (dd, J = 6.3, 10.8 Hz, 1H), 3.68 (dd, J = 2.7, 9.3 Hz, 1H), 3.57–3.51 (m, 1H), 2.32 (s, 3H, CH_3CO); ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.5, 138.2, 138.1, 138.0, 137.1, 131.7, 131.2, 129.6, 128.4, 128.3, 128.2, 128.19, 128.13, 128.0, 127.76, 127.72, 127.6, 127.5, 127.3, 87.9, 84.3, 80.1, 77.3, 75.1, 75.0, 74.8, 73.3, 72.5, 69.7, 21.0; HRMS (Bio-ToFII): calcd for $C_{41}H_{42}O_5SNa$ requires 669.2645; found: m/z = 669.2645 $[M+Na]^+$.

For ***p*-tolyl 2,3-di-*O*-benzyl-4,6-*O*-di-*tert*-butylsilylidene-β-D-galactopyranoside (107):**[s19]



To a solution of thiogalactopyranoside **64**[s2] (0.9 g, 1.6 mmol) CH_2Cl_2 (7 mL) was added a mixture of TFA (1 mL) and H_2O (1 mL) at RT. After completion of debenzylidenation, the reaction mixture was

diluted with CH_2Cl_2 (25 mL), which was washed with saturated $NaHCO_3$ (10 mL × 2), brine (10 mL × 1), dried (over $MgSO_4$), filtered, and concentrated to furnish tolyl 2,3-di-*O*-benzyl-β-D-thiogalactopyranosyl diol. Crude thiogalactopyranosyl diol (640 mg, 1.37 mmol) dissolving in a mixture of CH_2Cl_2 (10 mL) and pyridine (12 mL) was treated with di-*tert*-butylsilyl bis(trifluoromethane sulfonate) (0.59 mL, 1.65 mmol) at 0˚C under N_2. After completion of silylation, the resulting mixture was diluted with CH_2Cl_2 (25 mL), and was then washed with saturated Na_2CO_3 (10 mL × 2), brine (10 mL × 1), dried (over $MgSO_4$), filtered and concentrated for column chromatography purification over silica gel (elution: hexane/EtOAc = 4/1 to 2/1) to furnish thiogalactopyranoside **107** as white glassy solid (686 mg, 71% from **64**);[s19] $[\alpha]^{27}_D$ = +8.42 (*c* = 1.00, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.37–7.13 (m, 12 H, ArH), 6.98 (d, *J* = 3.0 Hz, 2 H, ArH), 4.82 (s, 2 H), 4.50 (d, *J* = 12.0 Hz, 1 H), 4.64 (q, *J* = 12.0, 3.0 Hz, 2 H), 4.08 (ddd, *J* = 18.6, 12.0, 1.2 Hz, 2 H), 3.75 (t, *J* = 12.0 Hz, 1 H), 3.37 (dd, *J* = 7.2 Hz, *J* = 2.7 Hz, 1 H), 3.14 (s, 1 H), 2.22 (s, 3 H, $ArCH_3$), 1.06 (s, 9 H, *t*-Bu), 0.98 (s, 9 H, *t*-Bu); ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.76, 138.71, 133.19, 131.28, 129.85, 128.81, 128.77, 128.65, 128.15, 128.07, 89.39, 83.16, 77.64, 76.26, 75.04, 71.34, 70.31, 67.73, 28.05, 27.99, 23.77, 21.48, 21.07; HRMS (FAB): calcd for $C_{35}H_{46}O_5SSiNa$ requires 606.2835; found: *m*/*z* = 606.2827 $[M+Na]^+$.

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Low-Concentration 1,2-*trans* β-Selective Glycosylation Strategy and Its Applications in Oligosaccharide Synthesis

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Dedicated to Professor Hak-Fun Chow

Abstract: This study develops an operationally easy, efficient, and general 1,2-*trans* β-selective glycosylation reaction that proceeds in the absence of a C2 acyl function. This process employs chemically stable thioglycosyl donors and low substrate concentrations to achieve excellent β-selectivities in glycosylation reactions. This method is widely applicable to a range of glycosyl substrates irrespective of their structures and hydroxyl-protecting functions. This low-concentration 1,2-*trans* β-selective glycosylation in carbohydrate chemistry removes the restriction of using highly reactive thioglycosides to construct 1,2-*trans* β-glycosidic bonds. This is beneficial to the design of new strategies for oligosaccharide synthesis, as illustrated in the preparation of the biologically relevant β-(1→6)-glucan trisaccharide, β-linked Gb_3 and $isoGb_3$ derivatives.

Keywords: carbohydrates · glycosylation · neighboring-group effects · stereoselectivity · substrate concentration

Introduction

Though researchers developed the first glycosylation more than a century ago, the seamless union of the glycosyl donor and acceptor in *O*-glycosidic bond formation remains a formidable challenge.[1] A point in case is the construction of 1,2-*trans* β-glycosidic bonds, which have long been dominated by the C2 neighboring-group participation strategy.[2] Because of the participation mechanism, this strategy occasionally suffers from side effects such as orthoester formation,[3] C2 acyl migration to the hydroxy function,[4] and aglycon transfer reaction.[4b,5] Under these circumstances, the glycosylation yield is reduced. These issues can be alleviated by employing benzoyl[6] and pivaloyl functions.[7] An alternative approach to address this problem is to use 1,2-*trans* β-selective glycosylation methods, which do not need the C2 acyl functions. Though researchers have developed various 1,2-*trans* β-selective glycosylation methods and some of these methods exhibit excellent β-selectivities in glycosylation reactions,[8–13] their use in oligosaccharide synthesis remains scarce.[12c] This is not surprising because most of these methods suffer from stringent requirements for the structures and hydroxy-protecting functions of the glycosyl donors. This lack of flexibility severely limits the scope of the application of such methods. Therefore a general, high-yielding and operationally easy 1,2-*trans* β-selective glycosylation that does not invoke neighboring-group participation is highly desired.

In the light of the discussion above, this study describes, for the first time, the low-concentration 1,2-*trans* β-selective glycosylation process. This method employs easily available and stable thioglycosides as glycosyl donors for glycosylation and does not require any C2 participating groups or the use of special anomeric functions. This study also demonstrates the proposed glycosylation method in the facile preparation of β-(1→6)-glucan trisaccharide,[14] Gb3,[15] and $isoGb_3$ derivatives.[16]

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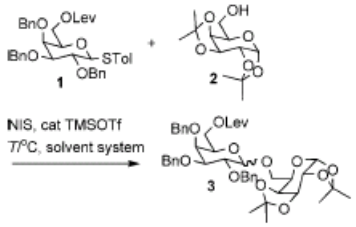
Results and Discussion

Invention of the low-concentration 1,2-*trans* β-selective glycosylation: In an experiment investigating the nitrile solvent effect on the stereochemical outcomes of glycosylation,

commercially available galactopyranose acceptor **2** was coupled with 2,3,4-tri-*O*-benzyl-6-*O*-levulinoyl β-D-thiogalactopyranoside (**1**)[17] in CH_2Cl_2 (solvent A), 1:3 CH_2Cl_2–CH_3CN (solvent B) and 1:3 CH_2Cl_2–EtCN (solvent C; Scheme 1 and Table 1). *N*-Iodosuccinimide and trimethylsilyl trifluoromethanesulfonate were employed as promoters for the activation of thioglycoside.[18]



Scheme 1. Glycosylations of galactopyranose acceptor **2** with thiogalactopyranoside **1**.

Table 1. Glycosylations of galactopyranose acceptor **2** with thiogalactopyranosyl donor **1** under different reaction conditions.

Entry	[1] mM	[2] mM	Solvent[a]	*T* [°C]	Yield [%] (α/β)[b]
1	120	100	A	−55	75 (1:1.6)
2	240	120	B	−55	83 (1: 6)
3	120	100	B	−55	80 (1:9)
4	60	50	B	−55	84 (1:10)
5	12	10	B	−55	83 (1:13)
6	12	10	B	−70	84 (1:19)
7	120	100	A	−75	78 (1:4)
8	240	120	C	−75	75 (1: 6)
9	120	100	C	−75	81 (1:9)
10	60	50	C	−75	81 (1:12)
11	12	10	C	−75	70 (1:16)
12	12	10	D	−70	81 (1:19)
13	12	10	D[c]	−70	81 (1:19)

[a] Solvent system: A = CH_2Cl_2; B = 1:3 v/v CH_2Cl_2–CH_3CN; C = 1:3 v/v CH_2Cl_2–EtCN and D = 1:2:1 v/v CH_2Cl_2–CH_3CN–EtCN. [b] α/β Anomeric ratio was determined by HPLC analysis of crude product mixture. [c] Inverse addition of **1** and **2** to NIS and TMSOTf in 1:2:1 CH_2Cl_2–CH_3CN–EtCN solution at −70°C. Bn = benzyl, Lev = levulinoyl, TMSOTf = trimethylsilyltriflate, Tol = toluene.

As expected, glycosylations in both the CH_2Cl_2–CH_3CN and CH_2Cl_2–EtCN solvent mixture afforded a 1:9 α/β–anomeric ratio of glycosylation product **3** based on the presumably nitrile solvent effect.[12b-d] However, this level of β-selectivity in glycosylation is insufficient for state-of-the art sequential glycosylation technologies, including the automated solid phase synthesis, various one-pot glycosylation strategies, and others.[1c,19–21] These technologies demand highly efficient couplings in each single step. A review of the literature revealed that electrochemical glycosylations in diluted solutions (10 mM of thioglycoside) yield nearly exclusive formation of β-glycosylating products.[22] On the other hand, routine glycosylations are normally performed at a glycosyl substrate concentration between 30 and 100 mM (Table 2).[8d,9a,12e,23,24] Though a preliminary study concerned with the concentration effect on α-selective glycosylation has been reported, there have been no empirical studies concerning the effect of substrate concentration on β-selective glycosylation.[25] This lack in the literature motivated the current study to investigate this unexplored area.

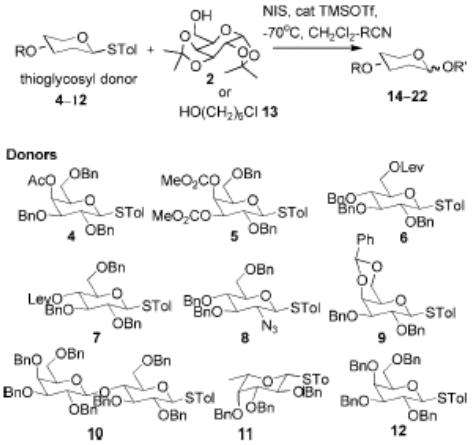
Table 2. Concentration of glycosyl donor and acceptor used in routine glycosylations.

Entry	Donor [mM]	Acceptor [mM]	Solvent	Reference
1	70	75	CH_2Cl_2 or CH_3CN	[8a]
2	66	73	EtCN	[11e]
3	220	170	CH_2Cl_2 or CH_3CN	[22]
4	100	150	CH_2Cl_2	[24]
5	27	80	CH_2Cl_2–CH_3CN	[9e]
6	64	32	EtCN	[23]
7	150	195	CH_2Cl_2	[12]

Thus, this study repeated glycosylations of **2** with **1** at different substrate concentrations. All glycosylations were completed cleanly within 15–20 min. This fast glycosylation can be attributed to the absence of the disarming C2 acyl function.[26] Interestingly, a decrease in substrate concentration accompanies an increase in the formation of the β-anomer. To be specific, when the concentrations of **1** decreased from 240 to 12 mM in 1:3 CH_2Cl_2–CH_3CN mixture (solvent B), the α/β–anomeric ratios of **3** increased from 1:6 to 1:13 (Table 1, entries 2–5). Further improvement in the β-selectivity of glycosylation was achieved by simply lowering the reaction temperature to −70°C, which produced a 1:19 α/β-anomeric ratio (Table 1, entry 6). Considering that the eutectic temperature of the 1:3 CH_2Cl_2–CH_3CN solvent mixture (ca. −72°C) is close to the reaction temperature (−70°C), the reaction solutions may freeze.[27] Therefore, this study repeated the glycosylation reaction in 1:2:1 CH_2Cl_2–CH_3CN/EtCN mixture (solvent D),[28] which to our delight, did not erode the β selectivity observed in glycosylation (Table 1, entry 12).

In typical glycosylation reactions, reaction promoters are added to a mixture of glycosyl donor and acceptors. Schmidt and Toepfer and the others adopted an inverse addition procedure in which glycosyl donor is added to a mixture of acceptor and promoters.[29a] It was reasoned that adding a mixture of donor and acceptor to promoters should presumably provide lower initial substrate concentrations, which may further improve the β-selectivity in glycosylation. To this end, a mixture of donor **1** and acceptor **2** was added to the promoters over 30 minutes at −70°C, however, an improvement in β-selectivity was not observed (Table 1, entry 12). This result is different from subsequent cases.

Scope and limitations of the low-concentration 1,2-*trans* β-selective glycosylation: After exploring the low-concentration 1,2-*trans* β-selective glycosylation process, this study next examined the scope and limitations of this method. Thioglycosides **4**–**12** were employed as glycosyl donors for the glycosylation of acceptors **2** and **13** (Scheme 2 and Table 3).[17] For comparison, the glycosylation reaction was



Scheme 2. Thioglycosyl donors **4–12** for the study of low-concentration 1,2-*trans* β-selective glycosylation.

Table 3. Results of the low-concentration 1,2-*trans* β-selective glycosylations of acceptors **2** and **3** with different thioglycosyl donors **4–12**.

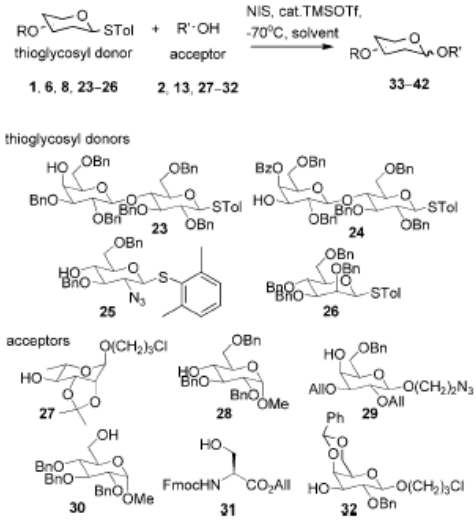
Entry	12 mM donor	10 mM acceptor	Product	Yield [%] (α/β)[a]	Yield [%] (α/β)[a,b]
1	**4**	**2**	**14**	85 (1:33)[c]	83 (1:2)
2	**5**	**2**	**15**	70 (1:≥49)[c]	67 (1:2)
3	**6**	**2**	**16**	77 (1:≥49)[d]	73 (1:1)
4	**7**	**2**	**17**	83 (1:19)[d]	77 (1:8)
5	**8**	**2**	**18**	83 (1:≥49)[c]	85 (1:2.5)
6	**9**	**2**	**19**	75 (1:9)[c]	75 (1:6)
7	**9**	**2**	**19**	83 (1:19)[c,e]	not done
8	**10**	**2**	**20**	75 (β only)[c]	75 (1:6)
9	**11**	**13**	**21**	80 (1:49)[c]	80 (1:4)
10	**12**	**13**	**22**	85 (1:≥49)[c]	88 (1:4)

[a] α/β-Anomeric ratio was determined by HPLC analysis. [b] Glycosylations in CH_2Cl_2 with 120 mM of thioglycosyl donor and 100 mM of acceptor at −55°C. [d] Glycosylations in 1:3 v/v CH_2Cl_2–CH_3CN with 12 mM of thioglycosyl donor and 10 mM of acceptor at −70°C. [c] Glycosylations in 1:2:1 v/v CH_2Cl_2–CH_3CN–EtCN with 12 mM of thioglycosyl donor and 10 mM of acceptor at −70°C. [e] For this particular reaction, 6 mM of thioglycosyl donor **9** and 5 mM of acceptor **2** were used.

performed under both low-concentration 1,2-*trans* β-selective and conventional glycosylation conditions.

Remarkably, the low-concentration β-selective glycosylation for thioglycosyl donors in the D-*gluco* (**6**, **7**, **8**), D-*galacto* (**4**, **5**, **9**, and **12**), D-*lacto* (**10**), and L-*fuco* (**11**) series produced excellent β-selectivities and the resulting α/β-anomeric ratios ranged from 1:9 to 1:≥49. An additional advantage of this method is its tolerance to different hydroxy-protecting functions, as evidenced by the glycosylation reactions with thiogalactopyranosides **1**, **4**, **5**, **9**, and **12** (Table 1 and Table 3, entries 1,2, 6–7, and 10). Although 4,6-*O*-benzylidene thiogalactopyranosyl acetal **9** is generally considered as an α-directing glycosyl donor,[30] the glycosylation of acceptor **2** with **9** under low-concentration conditions resulted in the predominant formation of the β-anomer (Table 3, entries 6, 7).

Encouraged by these preliminary results, we extended the scope of this study to more diverse glycosyl substrates. Thus β-D-thiolactosides (**23**, **24**), 2-azido-2-deoxy-β-D-thioglucopyranoside **25**, and β-D-thiomannopyranoside **26** prepared by standard methods were used for glycosylation of acceptors (Scheme 3 and Table 4).[17,31] With the exception of glycosylation with thiomannopyranoside **26**, all reactions produced excellent β-selectivities in the glycosylation reactions, with α/β-anomeric ratios ranging from 1:32 to β-exclusive. The glycosylations of acceptors **27**, **28**, **29**, and **32** are typical examples (Table 4, entries 1–3 and 6). Though all these acceptors contain the sterically hindered hydroxyl function,



Scheme 3. Glycosyl donors **23–26** and acceptors **27–32** for the extended investigation of the low-concentration 1,2-*trans* β-selective glycosylation.

Table 4. Results of the extended investigation of low-concentration 1,2-*trans* β-selective glycosylation.

Entry	12 mM donor	10 mM acceptor	Product	Yield [%] (α/β)[a,c]	Yield [%] (α/β)[b,c]
1	**1**	**27**	**33**	65 (1:49)	64 (1:1)
2	**1**	**28**	**34**	63 (1:≥49)	60 (1:8)
3	**1**	**29**	**35**	60 (β only)	not done
4	**6**	**30**	**36**	83 (1:≥49)	80 (1:2)
5	**1**	**31**	**37**	73 (1:≥49)	75 (19:1)
6	**8**	**32**	**38**	77 (1:32)	78 (1:7)
7	**23**	**13**	**39**	80 (β only)[d]	77 (1:3)
8	**24**	**13**	**40**	83 (1:≥49)[d]	not done
9	**25**	**13**	**41**	60 (1:≥49)	not done
10	**26**	**2**	**42**	85 (3:1)	not done

[a] Glycosylation in 1:2:1 v/v CH_2Cl_2–CH_3CN–EtCN mixture at −70°C with 12 mM of thioglycosyl donor and 10 mM of acceptor. [b] Glycosylation in CH_2Cl_2 at −55°C with 120 mM of thioglycosyl donor and 100 mM of acceptor. [c] α/β-Anomeric ratio was determined by HPLC analysis. [d] Two equivalents of acceptor was used to prevent self-condensation.

the corresponding glycosylations yielded β-anomers as the sole isomeric products.[32] These results reveal the synthetic utility of this novel method because disaccharides **36** and **38** constitute the essential components in the cell wall of *Saccharomyces cerevisiae*[14] and the type-2 backbone in blood-group determinants, respectively.[33]

Figure 1 presents a dramatic set of data for the glycosylations of N^{α}-Fmoc serine allyl ester **31** (Fmoc=9-fluorenylmethoxycarbonyl) with thiogalactopyranoside **1**. The glycosylation of aglycon acceptor **31** with thioglycoside **1** in CH_2Cl_2 under conventional conditions produced the α-anomer of **37** as the major isomer (Figure 1, HPLC trace a). Even when the same glycosylation was performed in a 1:2:1 CH_2Cl_2/CH_3CN/EtCN mixture, only marginal β-selectivity in glycosylation was observed under conventional glycosylation conditions (Figure 1, HPLC trace b). In sharp contrast, the application of low-concentration 1,2-*trans* β-selective glycosylation led to nearly exclusive formation of the β-anomer (Figure 1, HPLC trace c).

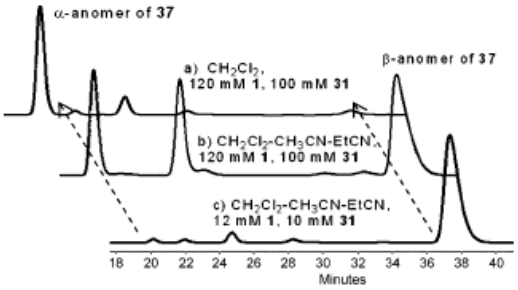


Figure 1. HPLC traces for glycosylations of *N*-Fmoc serine ester **31** with thiogalactopyranoside **1** under different conditions: a) conventional glycosylation conditions in CH_2Cl_2 and b) conventional glycosylation conditions in 1:2:1 v/v CH_2Cl_2–CH_3CN–EtCN mixture; and c) low-concentration 1,2-*trans* β-selective glycosylation conditions in 12:1 v/v CH_2Cl_2–CH_3CN–EtCN mixture.

Mechanistic insight of the low-concentration 1,2-*trans* β-selective glycosylation: To gain insight into the mechanisms of the present glycosylation, this study further investigates which particular substrate concentration is most closely related to the β-selectivity observed. Thus, 10 mM of acceptor **2** was glycosylated with different concentrations of thiogalactopyranoside **1** and *N*-iodosuccinimide (NIS; concentration range of 12–60 mM). Plotting the percentage of the α-anomer of **3** obtained against the concentration of donor **1** revealed a concentration-dependent relationship (Figure 2). Meanwhile, no significant change in β-selectivity was noted for glycosylation of 60 mM of acceptor **2** with 10 mM of donor **1**. This finding confirmed the substantial role of low thioglycoside concentration that is present in 1,2-*trans* β-selective glycosylation.

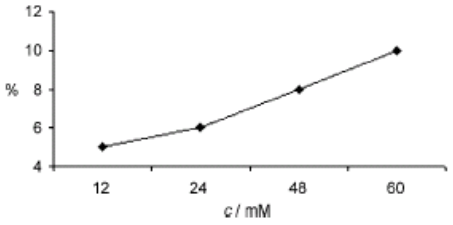
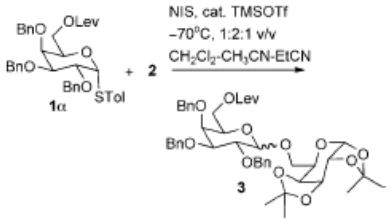


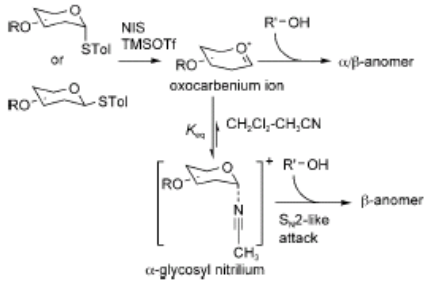
Figure 2. The plot of the percentage of α anomer of **3** against the concentrations (mM) of thiogalactopyranoside **1**.

After confirming the importance of thioglycoside concentration, we next examine the effect of the anomeric configuration of the glycosyl donor on low-concentration 1,2-*trans* β-selective glycosylation. Poletti and co-workers reported that the anomeric configuration of thioglycosyl donors affects the stereoselectivities of glycosylation.[32] However, these results differ from those observed for glycosylations with glycosyl imidate[12d] or phosphite donors.[12f] To clarify the role of anomeric configuration of thioglycoside, α-thiogalactopyranoside **1**α was prepared and used for the glycosylation of **2**. No significant change in β-selectivity was observed (Scheme 4). Empirically, anomeric configuration of thioglycoside is immaterial to β-stereoselectivity in glycosylation, which implicates that the stereochemical course of low-concentration 1,2-*trans* β-selective glycosylation probably involves a common intermediate.

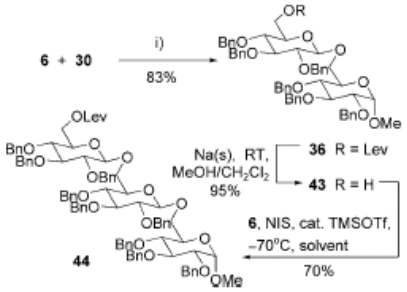


Scheme 4. Low-concentration 1,2-*trans* β-selective glycosylation of galactopyranose acceptor **2** with α-thiogalactopyranoside **1**α.

Based on experimental data and literature review, the aforementioned intermediate should be the free oxocarbenium ion arising from activation of thioglycoside,[12b,c,g] and thereof a mechanism is proposed as shown in Scheme 5. Activation of thioglycoside gives rise to free oxocarbenium ion. Owing to the high dielectric constant of the CH_2Cl_2/nitrile solvent mixture,[34] the formation α-glycosyl triflate is unlikely.[35] Instead, the free oxocarbenium ion reacts with the hydroxy acceptor to produce a α/β-anomeric mixture. Alternately, it might couple with the nitrile solvent molecule to generate the kinetically favoured α-glycosyl nitrilium.[12,36,37] Further S_N2-like reaction of the α-glycosyl nitrilium with the acceptor leads to the formation of the β-anomer.



Scheme 5. Proposed mechanism of the low-concentration1,2-*trans* β-selective glycosylation.



Scheme 6. Stepwise synthesis of β-(1→6)-glucan trisaccharide **44**. i) See Table 3, entry 4 for conditions.

Apparently, low substrate concentrations, low reaction temperature, and appropriate CH_2Cl_2/nitrile solvent mixture act synchronously to enhance α-glycosyl nitrilium formation, which depletes the free oxocarbenium ion through an equilibrium process. The argument agrees with that proposed by Crich and co-workers in β-selective rhamnosylation.[10b] However, attempts to detect the α-glycosyl nitrilium with NMR spectroscopy have so far been unsuccessful owing to substantial peak broadening in low-temperature NMR spectroscopy (below approximately −50°C).[38] Additional experimentations are currently underway to address this issue.

Synthetic utilities of the low-concentration 1,2-*trans* β-selective glycosylation: Regarding synthetic utility, the direct impact of low-concentration 1,2-*trans* β-selective glycosylation is to provide an alternative 1,2-*trans* β-selective glycosylation strategy. This strategy does not require a C2-participation protecting function and therefore allows the use of more-reactive thioglycosyl donors in the formation of 1,2-*trans* β-glycosidic bonds. This is useful in the design of new strategies in oligosaccharide synthesis as the following examples illustrate.

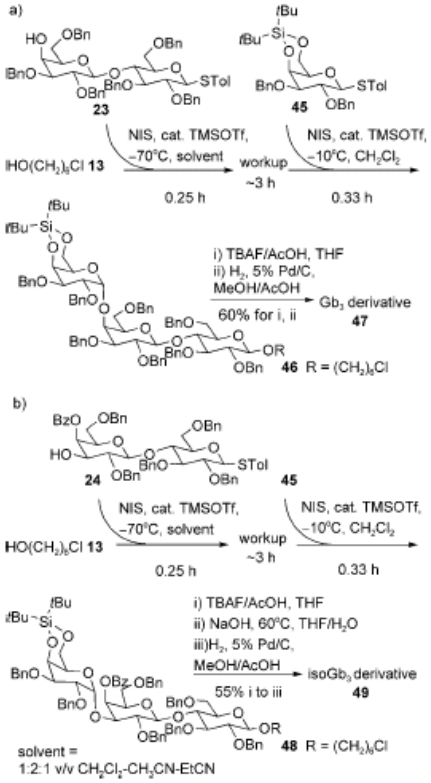
β-(1→6)-Glucan is the essential fungal-specific carbohydrate component found in the cell wall of *Saccharomyces cerevisiae*.[14] Previous attempts to synthesize β-(1→6)-glucan trisaccharide without a C2 acyl function have been reported, resulting in a 1:9 α/β-anomeric mixture.[13] Low-concentration 1,2-*trans* β-selective glycosylation might enable a highly stereoselective synthesis of β-(1→6)-glucan trisaccharide (Scheme 6). The glycosylation of methyl α-D-glucopyranoside **30** with thiogalactopyranoside **6** produced disaccharide glycoside **36** as mentioned above, and the treatment of **36** with standard Zemplén deacylation produced disaccharide acceptor **43**. The glycosylation of **43** with donor **6** afforded protected β-(1→6)-glucan trisaccharide **44** with a high yield and excellent β-anomeric purity.

Further to the stepwise synthesis, the low-concentration 1,2-*trans* β-selective glycosylation is useful for sequential glycosylation, as illustrated in the preparation of globotriaosylceramide (Gb_3) and isoGb$_3$ derivatives. Both the trisaccharide derivatives carry the β-linked functionalizable spacer; with such a spacer, further chemical elaboration is possible. Conventional preparation of Gb_3 and isoGb$_3$ derivatives involves construction of perbenzoyl lactoside, which undergoes further protection–deprotection reactions and additional glycosylation to furnish the target.[39] It was envisaged that the application of low-concentration 1,2-*trans* β-selective glycosylation should enable the development of a reverse sequential glycosylation strategy for the preparation of Gb_3 and isoGb$_3$ derivatives.

Acceptor **13** was chemoselectively glycosylated with thiolactosides **23** and **24** with low-concentration β-selective glycosylations to afford the corresponding β-linked lactoside acceptors (Scheme 7a and b). No traces of self-condensation products were detected, which is attributed to the higher reactivity of the hydroxy function in aglycon acceptor **13** compared with the hindered hydroxy functions in thiolactosides **23** and **24**. Without isolation of the glycosylation product and deprotection of the protected hydroxy function, the crude β-linked lactosides was used directly as acceptors for glycosylation with thiogalactopyranoside **45** in CH_2Cl_2. This reverse sequential glycosylation strategy produces the protected Gb_3 and isoGb$_3$ derivatives **46** and **48** in high overall yields (75% for **46** and 70% for **48** based on two glycosylation steps) with extremely good anomeric purities, as proven in the ^{13}C NMR spectra in Figure 3. Subsequent standard global deprotection reactions afforded Gb_3 and isoGb$_3$ trisaccharide derivatives, **47** and **49**.

Conclusions

The study explores, for the first time, the effect of substrate concentrations on 1,2-*trans* β-selective glycosylation. This study proposes a low-concentration 1,2-*trans* β-selective glycosylation method, which is applicable to a wide range of glycosyl substrates and enables the development of new synthetic routes for biologically relevant β-(1→6)-glucan trisaccharide, Gb_3 and isoGb$_3$. As β-linked glycosidic bonds occur frequently in natural oligosaccharides, this new β-selective glycosylation strategy should prove useful for their preparation.



Scheme 7. a) Sequential glycosylation strategy for synthesis of Gb_3 derivative **47** and b) $isoGb_3$ derivative **49**. TBAF = tetrabutylammonium fluoride, TMSOTf = trimethylsilyltriflate.

Experimental Section

General: Reagent-grade chemicals were purchased and used without further purification. N,N-dimethylformamide (DMF) was distilled over calcium hydride and stocked with flame-dried molecular sieves(MS) under N_2. The progress of reactions was monitored by thin-layer chromatography on silica gel 60 F-254 plate and visualized under UV illumination and by staining with acidic ceric ammonium molybdate or *p*-anisaldehyde. Optical rotations were measured with polarimeter at 27°C. HPLC analysis was performed over Mightysil column with elution of EtOAc/hexane mixture at a 1.2 mL min^{-1} flow rate by the gradient pump (L-2130) and UV detector (L-2400) from Hitachi. NMR spectra were recorded at 300 MHz and 75 MHz spectrometers in a Brüker console or 500 MHz and 125 MHz in a Varian console as specified. The chemical shift was calibrated against the residual proton signal and the ^{13}C signal of deuterated chloroform or deuterated methanol. Coupling constants in Hz was calculated from chemical shift of ^1H NMR or spectra.

General procedure for low-concentration 1,2-*trans* β-selective glycosylation: A mixture of thioglycoside (1.2 mol equiv), acceptor (1.0 mol equiv), and flame-dried MS (AW300) were suspended in 1:3 v/v CH_2Cl_2–CH_3CN or 1:2:1 v/v CH_2Cl_2–CH_3CN–EtCN solvent mixture such that the final concentrations of thioglycoside and acceptor were 12 and 10 mM, respectively (for particular details see Table S1 in the Supporting Information). The resulting mixture was stirred at room temperature for 10 min and at −70°C for an additional 20 min under N_2, and followed by the addition of NIS (1.2 mol equiv) and trimethylsilyltriflate (TMSOTf; 0.24 mol equiv). Glycosylation coupling was monitored by TLC with either EtOAc–hexane or EtOAc–CH_2Cl_2–hexane mixture as the developing solvent. Upon completion of glycosylation, 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 MS were removed by filtration over celite. The filtrate was dried (over $MgSO_4$), filtered, and concentrated for flash-chromatography purification over silica gel to furnish glycosylation products for NMR characterization. A small portion of crude reaction mixture was filtered through a short pad of silica gel (approximately 10 cm) and concentrated for determination of the α/β-anomeric ratio by HPLC analysis by using conditions as described in the general experimental section. Preparation of glycosyl substrates **1**, **4–12**, **23–32** for glycosylation studies and NMR spectra of compounds **1**, **3–12**, **14–49** are presented in the Supporting Information.

Low-temperature NMR spectroscopy for thiogalactopyranoside (1): Thiogalactopyranoside **1** (33 mg for 100 mM or 3.3 mg for 10 mM) in a pre-dried NMR tube was dried under vacuum ovemight and then 1:3 v/v CD_2Cl_2–CD_3CN with 5 vol% EtCN (500 μL) was added under N_2. The resulting mixture was then analyzed by NMR spectroscopy at a temperature range of 25°C to −60°C (selected spectra are given in the Supporting Information).

Low-temperature NMR spectroscopy for NIS-/TMSOTf-activated thiogalactopyranoside (1): Thiogalactopyranoside **1** (33 mg) in a pre-dried NMR tube was dried under vacuum overnight and then 500 μL of 1:3 v/v CD_2Cl_2–CD_3CN (with 25 μL of EtCN added as internal reference) was added under N_2. The resulting solution was cooled at −80°C bath for approximately 10 min, followed by the addition of TMSOTf (2 μL) in CD_2Cl_2 solution (10 μL). The resulting mixture was then analyzed by NMR spectroscopy at temperature range of −50 to −30°C (selected spectra for VT-NMR are given in the Supporting Information).

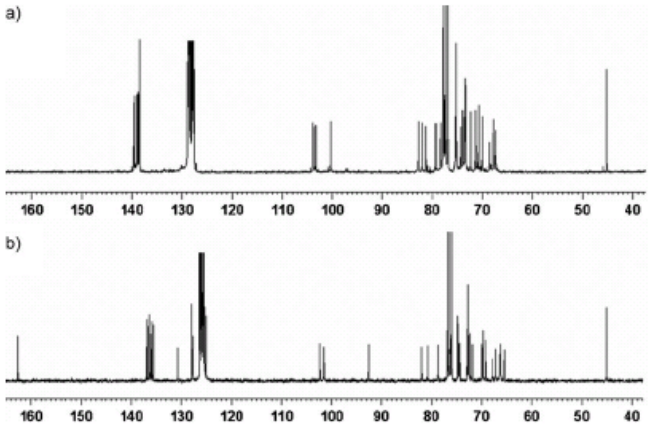


Figure 3. ^{13}C NMR spectra of a) protected Gb_3 **46** and b) protected $isoGb_3$ **48**.

1,2:3,4-di-*O*-isopropylidene-6-*O*-(2′,3′,4′-tri-*O*-benzyl-6′-*O*-levulinoyl-β-D-galactopyranosyl)-1-α-D-galactopyranose (3): Synthesis of **3** was performed as per the general procedure of low-concentration 1,2-*trans* β-selective glycosylation. **3** (128 mg, 84%) was obtained as glassy solid. $[\alpha]_D^{27}=-35.34$ ($c=0.9$, in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, 25°C, TMS): $\delta=7.50$–7.47 (d, $J=9.3$ Hz, 2H; ArH), 7.46–7.28 (m, 13H; ArH), 5.59 (d, $J=4.95$ Hz, 1H, H-1), 5.09 (d, $J=11.1$ Hz, 1H), 4.88 (d, $J=12$ Hz, 1H), 4.80–4.75 (m, 2H), 4.68 (d, $J=11.4$ Hz, 1H), 4.61 (dd, $J=2.4$, 7.8 Hz, 1H), 4.45 (d, $J=7.8$ Hz, 1H), 4.33 (dd, $J=2.4$, 5.1 Hz, 1H), 4.28–4.22 (m, 2H), 4.18–4.10 (m, 3H), 3.91–3.83 (m, 2H), 3.7 (dd, $J=6.9$, 9.9 Hz, 1H), 3.58–3.54 (m, 2H), 2.76–2.71 (m, 2H; CH_2C=O), 2.53–2.48 (m, 2H; CH_2C=O), 2.20 (s, 3H; CH_3C=O), 1.52 (s, 3H; CH_3C), 1.48 (s, 3H; CH_3C), 1.35 (s, 3H; CH_3C), 1.34 ppm (s, 3H; CH_3C); ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): $\delta=206.9$, 172.74, 139.4, 139.0, 138.7, 129.0, 128.9, 128.8, 128.6, 128.5, 128.1, 128.0, 127.8, 109.7, 109.0, 105.1, 96.8, 82.3, 79.3, 75.2, 74.8, 72.3, 71.9, 71.2, 70.9, 7.1, 67.8, 63.5, 38.3, 30.2, 28.2, 26.44, 26.41, 25.5, 24.8 ppm; HRMS (FAB): *m/z*: calcd for $C_{44}H_{54}O_{13}$: 790.3564 $[M]^+$; found: 790.3561.

1,2:3,4-Di-*O*-isopropylidene-6-*O*-(4′-*O*-acetyl-2′,3′,6′-tri-*O*-benzyl-β-D-galactopy- ranosyl)-1-α-D-galactopyranose (14): Synthesis of **14** was performed as per to the general procedure of low-concentration 1,2-*trans* β-selective glycosylation. **14** (120 mg, 85%) was obtained as colorless oil. $[\alpha]_D^{27}=-6.31$ ($c=1.2$, in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, 25°C, TMS): $\delta=7.50$–7.48 (m, 2H; ArH), 7.42–7.28 (m, 13H; ArH), 5.63–5.61 (m, 2H), 5.08 (d, $J=11.1$ Hz, 1H), 4.8 (d, $J=11.4$ Hz, 1H), 4.72 (d, $J=11.1$ Hz, 1H), 4.64–4.49 (m, 5H), 4.36 (dd, $J=2.4$, 5.1 Hz, 1H), 4.27 (dd, $J=1.5$, 7.8 Hz, 1H), 4.22–4.14 (m, 2H), 3.82–3.73 (m, 2H), 3.68–3.53 (m, 4H), 2.11 (s, 3H; CH_3C=O), 1.54 (s, 3H; CH_3C), 1.49 (s, 3H; CH_3C), 1.36 ppm (s, 6H; CH_3C); ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): $\delta=170.8$, 139.3, 138.4, 138.0, 128.91, 128.9, 128.7, 128.6, 128.52, 128.51, 128.3, 128.1, 127.8, 109.8, 109.0, 105.0, 96.8, 79.4, 79.0, 75.4, 74.1, 72.5, 72.4, 71.9, 71.2, 70.9, 70.4, 68.4, 67.8, 67.3, 26.4, 25.5, 24.9, 21.4 ppm; HRMS (ESI): *m/z*: calcd for $C_{41}H_{50}O_{12}Na$: 757.3194 $[M+Na]^+$; found: 757.3194.

1,2:3,4-Di-*O*-isopropylidene-6-*O*-(2′,6′-di-*O*-benzyl-3′,4′-di-*O*-methoxycarbonyl-β-D-galactopyranosyl)-1-α-D-galactopyranose (15): Synthesis of **15** was performed as described in the general procedure for low-concentration 1,2-*trans* β-selective glycosylation. **15** (98 mg, 71%) was obtained as light-yellow oily liquid. $[\alpha]_D^{27}=-8.39$ ($c=0.53$, in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, 25°C, TMS): $\delta=7.39$–7.26 (m, 10H; ArH), 5.57 (d, $J=5.1$ Hz, 1H; H-1), 5.37 (d, $J=3.0$ Hz, 1H), 5.02 (d, $J=11.4$ Hz, 1H), 4.85 (dd, $J=3.3$, 10.2 Hz, 1H), 4.65–4.47 (m, 5H), 4.33 (dd, $J=2.4$, 4.8 Hz, 1H), 4.23 (dd, $J=1.8$, 8.1 Hz,1H), 4.16–4.07 (m, 2H), 3.82 (s, 3H), 3.80–3.58 (m, 8H), 1.52 (s, 3H; CH_3C), 1.46 (s, 3H; CH_3C), 1.33 ppm (s, 6H; CH_3C); ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): $\delta=155.9$, 155.3, 138.9, 138.1, 128.8, 128.5, 128.4, 128.22, 128.2, 127.8, 109.8, 109.0, 104.5, 96.8, 76.9, 75.1, 74.0, 72.1, 71.9, 71.8, 71.1, 70.9, 0.1, 68.0, 67.8, 55.53, 55.51, 26.43, 26.38, 25.4, 24.8 ppm; HRMS (ESI): *m/z*: calcd $C_{36}H_{46}O_{15}Na$: 741.2729 $[M+Na]^+$; found: 741.2729.

1,2:3,4-Di-*O*-isopropylidene-6-*O*-(2′,3′,4′-tri-*O*-benzyl-6′-*O*-levulinoyl-β-D-gluco- pyranosyl)-1-α-D-galactopyranose (16): Synthesis of **16** was prepared according to the general procedure of low-concentration 1,2-*trans* β-selective glycosylation. **16** (117 mg, 77%) was obtained as white glassy solid. $[\alpha]_D^{27}=-14.80$ ($c=0.84$, in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, 25°C, TMS): $\delta=7.46$–7.42 (m, 2H; ArH), 7.36–7.25 (m, 13H; ArH), 5.59 (d, $J=5.1$ Hz, 1H; H-1), 5.08 (d, $J=11.1$ Hz, 1H), 5.00 (d, $J=10.8$ Hz, 1H), 4.88 (d, $J=10.8$ Hz, 1H), 4.81 (d, $J=11.1$ Hz, 1H), 4.74 (d, $J=11.1$ Hz, 1H), 4.63–4.56 (m, 2H), 4.49 (d, $J=7.8$ Hz, 1H), 4.35–4.25 (m, 4H), 4.15–4.10 (m, 2H), 3.77–3.65 (m, 2H), 3.54–3.44 (m, 3H), 2.78–2.72 (m, 2H; CH_2C=O), 2.62–2.58 (m, 2H; CH_2C=O), 2.20 (s, 3H; CH_3C=O), 1.52 (s, 3H; CH_3C), 1.48 (s, 3H; CH_3C), 1.35 (s, 3H; CH_3C), 1.33 ppm (s, 3H; CH_3C); ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): $\delta=206.8$, 172.9, 139.0, 138.9, 138.2, 129.0, 128.9, 128.8, 128.6, 128.5, 128.3, 128.0, 127.9, 109.8, 108.9, 104.9, 96.8, 84.9, 81.9, 77.8, 77.6, 76.4, 75.4, 74.8, 73.2, 71.2, 71.8, 71.1, 70.8, 70.2, 67.7, 63.7, 38.3, 30.3, 28.3, 26.43, 26.4, 25.4, 24.8 ppm; HRMS (FAB): *m/z*: calcd for $C_{44}H_{54}O_{13}$: 790.3564 $[M]^+$; found: 790.3578.

1,2:3,4-Di-*O*-isopropylidene-6-*O*-(2′,3′,6′-tri-*O*-benzyl-4′-*O*-levulinoyl-β-D-glucopyranosyl)-1-α-D-galactopyranose (17): Synthesis of **17** was prepared according to the general procedure of low-concentration 1,2-*trans* β-selective glycosylation. **17** (126 mg, 83%) was obtained as colorless oily liquid. $[\alpha]_D^{27}=-22.42$ ($c=0.35$, in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, 25°C, TMS): $\delta=7.46$–7.43 (m, 2H; ArH), 7.37–7.27 (m, 13H; ArH), 5.6 (d, $J=5.1$ Hz, 1H), 5.09–4.99 (m, 2H), 4.86 (d, $J=11.7$ Hz, 1H), 4.73 (d, $J=11.1$ Hz, 1H), 4.67–4.59 (m, 2H), 4.55–4.51 (m, 3H), 4.35 (dd, $J=2.4$, 5.1 Hz, 1H), 4.26 (dd, $J=1.5$, 7.8 Hz, 1H), 4.21–4.12 (m, 2H), 3.78 (dd, $J=7.2$, 10.2 Hz, 1H), 3.66–3.50 (m, 5H), 2.62–2.54 (m, 2H; CH_2 C=O), 2.45–2.25 (m, 2H; CH_2C=O), 2.13 (s, 3H; CH_3 C=O), 1.53 (s, 3H; CH_3C), 1.48 (s, 3H; CH_3C), 1.35 ppm (s, 6H; CH_3C); ^{13}C NMR (75 MHz, $CDCl_3$, 25°C, TMS): $\delta=206.6$, 172.0, 139.0, 138.9, 138.5, 129.1, 128.71, 128.68, 128.64, 128.3, 128.0, 127.9, 109.8, 109.0, 104.7, 96.8, 81.9, 81.7, 75.4, 74.9, 74.0, 73.7, 71.8, 71.4, 71.2, 70.9, 69.9, 67.9, 38.2, 30.2, 28.3, 26.5, 26.4, 25.4, 24.9 ppm; HRMS (ESI): *m/z*: calcd for $C_{44}H_{54}O_{13}$: 790.3564 $[M]^+$; found: 790.3568.

1,2:3,4-Di-*O*-isopropylidene-6-*O*-(2′-azido-3′,4′,6′-tri-*O*-benzyl-2′-deoxy-β-D-glucopyranosyl)-1-α-D-galactopyranose (18): Synthesis of **18** was performed according to the general procedure of low-concentration 1,2-*trans* β-selective glycosylation. **18** (114 mg, 83%) was obtained as a colorless oily liquid. $[\alpha]_D^{27}=-35.30$ ($c=1.08$, in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, 25°C, TMS): $\delta=7.42$–7.30 (m, 13H; ArH), 7.22–7.19 (m, 2H; ArH), 5.6 (d, $J=5.1$ Hz, 1H), 4.95 (d, $J=10.8$ Hz, 1H), 4.84 (dd, $J=5.7$, 10.8 Hz, 1H), 4.67–4.55 (m, 4H), 4.48–4.46 (m, 1H), 4.36 (dd, $J=2.4$, 4.8 Hz, 1H), 4.32 (dd, $J=1.2$, 8.1 Hz, 1H), 4.1 (m, 2H), 3.88–3.66 (m, 4H), 3.49–3.45 (m, 3H), 1.59 (s, 3H; CH_3C), 1.49 (s, 3H; CH_3C), 1.38 (s, 3H; CH_3C), 1.37 ppm (s, 3H; CH_3C); ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): $\delta=138.49$, 138.46, 138.39, 128.88, 128.85, 128.81, 128.47, 128.3, 128.1, 109.7, 109.1, 102.9, 96.7, 83.5, 78.1, 75.9, 75.4, 74.0, 71.7, 71.1, 70.9, 69.2, 68.9, 68.0, 66.8, 26.5, 26.4, 25.4, 24.8 ppm; HRMS (ESI): *m/z*: calcd for $C_{39}H_{47}N_3O_{10}Na$: 740.3159 $[M+Na]^+$; found: 740.3154.

1,2:3,4-Di-*O*-isopropylidene-6-*O*-(2′,3′-di-*O*-benzyl-4′,6′-*O*-benzylidene-β-D-galactopyranosyl)-1-α-D-galactopyranose (19): Synthesis of **19** was generally based on the general procedure of low-concentration 1,2-*trans* β-selective glycosylation, but lower substrate concentrations (5 mM of glycosyl acceptor **2** and 6 mM of glycosyl donor **9**) were used to obtain a 1:19 α/β anoemric ratio of **19**. Disaccharide **19** (110 mg, 83%) was obtained as white amorphous solid. $[\alpha]_D^{27}=-12.59$ ($c=1.4$, in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, 25°C, TMS): $\delta=7.62$–7.58 (m, 2H, ArH), 7.55–7.52 (m, 2H, ArH), 7.45–7.28 (m, 11H, ArH), 5.62 (d, $J=5.1$ Hz, 1H; H-1), 5.54 (s, 1H; PhCH), 5.13 (d, $J=11.1$ Hz, 1H), 4.84–4.77 (m, 3H), 4.63 (dd, $J=2.4$, 7.8 Hz, 1H), 4.49 (d, $J=7.8$ Hz, 1H), 4.36 (dd, $J=2.4$, 5.1 Hz, 1H), 4.34–4.21 (m, 3H), 4.17–4.13 (m, 2H), 4.04 (dd, $J=1.2$, 12.3 Hz, 1H), 3.91 (dd, $J=7.5$, 9.6 Hz, 1H), 3.77 (dd, $J=7.2$, 10.5 Hz, 1H), 3.60 (dd, $J=3.6$, 9.6 Hz, 1H), 3.32 (s, 1H), 1.55 (s, 3H; CH_3C), 1.50 (s, 3H; CH_3C), 1.37 ppm (s, 6H; CH_3C); ^{13}C NMR (75 MHz, $CDCl_3$, 25°C, TMS): $\delta=139.6$, 138.9, 138.3, 129.3, 128.9, 128.8, 128.7, 128.6, 128.5, 128.2, 128.1, 127.8, 126.9, 109.7, 109.1, 104.8, 101.6, 96.8, 79.3, 78.6, 75.4, 74.6, 72.5, 71.9, 71.2, 70.9, 69.9, 69.6, 67.8, 66.9, 26.5, 26.4, 25.5, 24.9 ppm; HRMS (ESI): *m/z*: calcd for $C_{39}H_{46}O_{11}Na$: 713.2932 $[M+Na]^+$; found: 713.2933.

1,2:3,4-Di-*O*-isopropylidene-6-*O*-[2′,3′,6′-tri-*O*-benzyl-4′-*O*-(2″,3″,4″,6″-tetra-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-1-α-D-galactopyranose (20): Synthesis of **20** was performed according to the general procedure of low-concentration 1,2-*trans* β-selective glycosylation. **20** (175 mg, 75%) was obtained as a colorless oily liquid. $[\alpha]_D^{27}=-15.38$ ($c=2.07$, in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, 25°C, TMS): $\delta=7.49$–7.18 (m, 35H; ArH), 5.62 (d, $J=5.1$ Hz, 1H), 5.08–5.00 (m, 3H), 4.84–4.74 (m, 7H), 4.64–4.57 (m, 3H), 4.50–4.44 (m, 2H), 4.41–4.35 (m, 2H), 4.31–4.27 (m, 2H), 4.21–4.17 (m, 2H), 4.03–3.96 (m, 2H), 3.88–3.72 (m, 4H), 3.63–3.56 (m, 2H), 3.49–3.37 (m, 5H), 1.55 (s, 3H; CH_3C), 1.51 (s, 3H; CH_3C), 1.37 ppm (s, 6H; CH_3); ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): $\delta=139.6$, 139.5, 139.4, 139.2, 139.0, 138.8, 138.5, 128.9, 128.81, 128.79, 128.68, 128.66, 128.52, 128.38, 128.30, 128.17, 128.11, 127.96, 127.86, 127.80, 127.46, 109.76, 109.02, 104.80, 103.17, 83.18, 82.98, 81.68, 80.40, 77.71, 75.79, 75.70, 75.51, 75.13, 74.97, 74.02, 73.83, 73.52, 73.36, 72.97, 71.83, 71.18, 70.93, 69.91, 68.62, 68.51, 67.74, 26.50, 26.45, 25.49, 24.88 ppm; HRMS (FAB): *m/z*: calcd for $C_{75}H_{82}O_{16}$: 1214.5603 $[M]^+$; found: 1214.5608.

6-Chlorohexyl 2,3,4-tri-*O*-benzyl-1-β-L-fucopyranoside (21): Synthesis of **21** was performed according to the general procedure of low-concentration 1,2-*trans* β-selective glycosylation. **21** (93 mg, 80 %) was obtained as colorless oily liquid. $[\alpha]_D^{27}=+5.52$ ($c=0.32$, in $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$, 25°C, TMS): $\delta=7.41$–7.27 (m, 15H; ArH), 5.01 (d, $J=11.7$ Hz, 1H), 4.96 (d, $J=11.1$ Hz, 1H), 4.85–4.47 (m, 4H), 4.34 (d, $J=7.5$ Hz, 1H), 3.97 (dt, $J=6.3$, 9.6 Hz, 1H), 3.83 (dd, $J=7.8$, 9.6 Hz, 1H), 3.59–3.44 (m, 6H), 1.81–1.64 (m, 4H; CH_2), 1.51–1.37 (m, 4H; CH_2), 1.20 ppm (d, $J=6.3$ Hz, 3H; CH_3); ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): $\delta=$ 139.33, 139.08, 139.02, 128.95, 128.79, 128.68, 128.53, 128.43, 127.97, 127.91, 104.2, 82.97, 79.89, 77.69, 76.66, 75.49, 74.34, 73.58, 70.69, 69.99, 45.5, 32.95, 30.0, 27.1, 25.9 ppm; HRMS (ESI): *m/z*: calcd for $C_{33}H_{41}ClO_5Na$: 575.2540 $[M+Na]^+$; found: 575.2535.

6-Chlorohexyl 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranoside (22): Synthesis of **22** was referred to the general procedure of low-concentration 1,2-*trans* β-selective glycosylation. **22** (140 mg, 85 %) was obtained as colourless oily liquid. $[\alpha]_D^{27}=-4.24$ ($c=1.90$, in $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$, 25°C, TMS): $\delta=7.46$–7.31 (m, 20H; ArH), 5.05–4.99 (m, 2H), 4.88–4.77 (m, 3H), 4.71 (d, $J=11.7$ Hz, 1H), 4.56–4.47 (m, 2H), 4.44 (d, $J=7.5$ Hz, 1H), 4.06–4.03 (m, 1H), 3.98 (m, 1H), 3.90 (t, $J=9$ Hz, 1H), 3.69–3.53 (m, 7H), 1.85–1.72 (m, 4H; CH_2), 1.52–1.45 ppm (m, 4H; CH_2); ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): $\delta=$139.3, 139.1, 139.0, 138.4, 128.9, 128.84, 128.75, 128.64, 128.50, 128.36, 128.01, 127.93, 104.4, 82.7, 80.1, 75.6, 74.96, 74.01, 73.98, 73.50, 70.22, 69.38, 45.50, 32.99, 30.06, 27.17, 25.96 ppm; HRMS (ESI): *m/z*: calcd for $C_{40}H_{47}ClO_6Na$: 681.2953 $[M+Na]^+$; found: 681.2953.

3-Chloropropyl 2,3-*O*-isopropylidene-4-*O*-(2′,3′,4′-tri-*O*-benzyl-6′-*O*-levulinoyl-β-D-galactopyranosyl)-α-L-rhamnopyranoside (33): Synthesis of **33** was performed according to the general procedure of low-concentration 1,2-*trans* β-selective glycosylation. **33** (114 mg, 65 %) was obtained as yellow oily liquid. $[\alpha]_D^{27}=-16.32$ ($c=1.16$, in $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$, 25°C, TMS): $\delta=7.46$–7.29 (m, 15H; ArH), 5.03–4.95 (m, 3H), 4.87–4.75 (m, 4H), 4.69 (d, $J=11.7$ Hz, 1H), 4.29–4.23 (m, 2H), 4.18–4.09 (m, 2H), 3.93–3.77 (m, 3H), 3.6–3.55 (m, 7H), 2.76–2.71 (m, 2H; CH_2C=O), 2.51–2.47 (m, 2H; CH_2C=O), 2.20 (s, 3H; CH_3C=O), 2.11–2.03 (m, 2H; CH_2), 1.55 (s, 6H; CH_3C), 1.39–1.29 ppm (m, 7H); ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): $\delta=$206.9, 172.8, 139.4, 139.0, 138.8, 128.8, 128.72, 128.66, 128.62, 128.5, 128.1, 128.0, 127.9, 127.8, 109.6, 102.6, 97.4, 82.7, 79.99, 79.3, 18.6, 76.3, 75.5, 74.8, 73.94, 73.87, 72.43, 42.1, 38.3, 32.7, 30.3, 28.3, 28.1, 26.8, 18.3 ppm; HRMS (ESI): *m/z*: calcd for $C_{46}H_{55}ClO_{12}Na$: 833.3274 $[M+Na]^+$; found: 833.3274.

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2′,3′,4′-tri-*O*-benzyl-6′-*O*-levulinoyl-β-D-galacto pyranosyl)-1-α-D-glucopyranoside (34): Synthesis of **34** was performed according to the general procedure of low-concentration 1,2-*trans* β-selective glycosylation. **34** (122 mg, 63 %) was obtained as white solid. $[\alpha]_D^{27}=+2.49$ ($c=0.98$, in $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$, 25°C, TMS): $\delta=7.47$–7.22 (m, 30H; ArH), 5.06 (m, 2H), 4.88–4.77 (m, 5H), 4.75–4.59 (m, 5H), 4.37 (d, $J=12$ Hz, 1H), 4.31 (d, $J=7.8$ Hz, 1H), 4.12 (d, $J=6.6$ Hz, 1H), 3.99–3.70 (m, 6H), 3.65–3.52 (m, 3H), 3.47–3.32 (m, 5H), 2.73 (m, 2H; CH_2C=O), 2.53–2.47 (m, 2H; CH_2C=O), 2.19 ppm (s, 3H; CH_3C=O); ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): $\delta=$206.9, 172.6, 139.7, 139.3, 139.1, 138.94, 138.93, 138.5, 128.92, 128.82, 128.81, 128.78, 128.67, 128.59, 128.52, 128.45, 128.41, 128.36, 128.20, 128.16, 128.06, 128.02, 127.91, 127.88, 127.82, 127.55, 103.05, 98.85, 82.79, 80.6, 80.3, 77.7, 76.9, 75.9, 75.6, 75.0, 73.7, 73.6, 73.2, 72.0, 70.4, 68.3, 63.1, 55.7, 38.3, 30.3, 28.3 ppm; HRMS (ESI): *m/z*: calcd for $C_{60}H_{66}O_{13}$: 994.4503 $[M]^+$; found: 994.4506.

2-Azidoethyl 2,3-di-*O*-allyl-4-*O*-(2′,3′,4′-tri-*O*-benzyl-6′-*O*-levulinoyl-β-D-galacto pyranosyl)-1-β-D-galactopyranoside (35): Synthesis of **35** was performed according to the general procedure of low-concentration 1,2-*trans* β-selective glycosylation. **35** (117 mg, 60 %) was obtained as a yellow oily liquid. $[\alpha]_D^{27}=-1.98$ ($c=0.74$, in $CHCl_3$); ^1HNMR (300 MHz, $CDCl_3$, 25°C, TMS): $\delta=7.50$–7.48 (m, 2H; ArH), 7.38-7.28 (m, 18H; ArH), 5.97–5.86 (m, 2H), 5.39 (d, $J=1.2$ Hz, 1H), 5.24–5.02 (m, 5H), 4.88–4.85 (m, 2H), 4.78–4.74 (m, 2H), 4.65–4.53 (m, 3H), 4.37 (d, $J=7.8$ Hz, 1H), 4.25–3.99 (m, 8H), 3.87–3.67 (m, 5H), 3.64–3.37 (m, 7H), 2.75–2.70 (m, 2H; CH_2C=O), 2.51–2.47 (m, 2H; CH_2C=O), 2.19 ppm (s, 3H; CH_3C=O); ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): $\delta=$206.8, 172.8, 139.5, 139.1, 138.8, 135.6, 135.5, 128.77, 128.76, 128.67, 128.6, 128.4, 128.2, 128.0, 127.7, 116.91, 116.88, 104.17, 103.4, 82.4, 81.6, 80.1, 79.6, 77.7, 75.3, 74.8, 74.5, 74.4, 74.3, 74.1, 73.9, 72.3, 72.1, 71.8, 70.7, 68.6, 63.7, 51.4, 38.2, 30.2, 28.1 ppm; HRMS (ESI): *m/z*: calcd for $C_{53}H_{63}N_3O_{13}Na$: 972.4253 $[M+Na]^+$; found: 972.4253.

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2′,3′,4′-tri-*O*-benzyl-6′-*O*-levulinoyl-β-D-glucopy ranosyl)-1-α-D-glucopyranoside (36): Synthesis of **36** was referred to the general procedure of low-concentration 1,2-*trans* β-selective glycosylation. **36** (162 mg, 83 %) was obtained as white solid. $[\alpha]_D^{27}=+20.57$ ($c=1.03$, in $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$, 25°C, TMS): $\delta=7.41$–7.32 (m, 25H; ArH), 7.28–7.24 (m, 5H; ArH), 5.06–4.77 (m, 9H), 4.74–4.56 (m, 4H), 4.43–4.39 (m, 2H), 4.30 (dd, $J=4.2$, 12 Hz, 1H), 4.22 (d, $J=10.5$ Hz, 1H), 4.07 (t, $J=9.3$ Hz, 1H), 3.90 (dd, $J=3$, 9.9 Hz, 1H), 3.77–3.69 (m, 2H), 3.63–3.51 (m, 5H), 3.41 (s, 3H), 2.78–2.73 (m, 2H; CH_2C=O), 2.63–2.58 (m, 2H; CH_2C=O), 2.21 ppm (s, 3H; CH_3C=O); ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): $\delta=$206.8, 173.0, 139.3, 138.8, 138.7, 138.6, 138.2, 128.95, 128.93, 128.88, 128.84, 128.81, 128.63, 128.58, 128.44, 128.40, 128.36, 128.32, 128.17, 128.10, 128.04, 104.2, 98.5, 85.2, 82.4, 80.2, 78.4, 77.9, 76.2, 76.1, 75.5, 75.4, 75.3, 73.8, 73.3, 70.2, 69.1, 63.7, 55.7, 38.3, 30.3, 30.2, 28.3 ppm; HRMS (ESI): *m/z*: calcd for $C_{55}H_{60}O_{11}Na$: 994.4503 $[M+Na]^+$; found: 994.4506.

2′,3′,4′-Tri-*O*-benzyl-6′-*O*-levulinoyl-α-D-galactopyranosyl N^{α}-fluoren-9-yl methoxycarbonyl-L-serine allyl ester (37a): Synthesis of **37a** was performed according to the general procedure of low-concentration 1,2-*trans* β-selective glycosylation, but use only CH_2Cl_2 as reaction solvent. **37a** (128 mg, 75 %) was obtained as colorless oil. $[\alpha]_D^{27}=+8.63$ ($c=0.90$, in $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$, 25°C, TMS): $\delta=7.80$–7.78 (m, 2H; ArH), 7.68–7.56 (m, 3H; ArH), 7.44–7.28 (m, 18H; ArH), 5.96–5.84 (m, 1H), 5.40–5.22 (m, 2H), 5.05–4.80 (m, 7H), 4.74–4.59 (m, 4H), 4.51–4.39 (m, 2H), 4.34–4.04 (m, 5H), 4.02–3.81 (m, 3H), 2.79–2.64 (m, 2H; CH_2C=O), 2.60–2.45 (m, 2H; CH_2C=O), 2.16 ppm (s, 3H; CH_3C=O); ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): $\delta=$207.1, 172.95, 170.2, 156.5, 144.3, 141.7, 139.0, 138.8, 138.6, 132.0, 128.89, 128.82, 128.74, 128.3, 128.2, 128.14, 128.09, 127.95, 127.87, 127.52, 125.62, 125.59, 125.29, 120.41, 119.21, 99.8, 78.99, 77.67, 76.70, 75.09, 73.79, 73.73, 70.53, 69.73, 67.57, 66.70, 66.63, 64.22, 55.1, 47.5, 38.25, 38.19, 30.29, 30.23, 28.1 ppm; HRMS (ESI): *m/z*: calcd for $C_{53}H_{55}NO_{12}Na$: 920.3616 $[M+Na]^+$; found: 920.3609.

2′,3′,4′-Tri-*O*-benzyl-6′-*O*-levulinoyl-β-D-galactopyranosyl N^{α}-fluoren-9-yl methoxycarbonyl-L-serine allyl ester (37b): Synthesis of **37b** was performed according to the general procedure of low-concentration 1,2-*trans* β-selective glycosylation. **37b** (124 mg, 73 %) was obtained as white solid. $[\alpha]_D^{27}=+1.37$ ($c=0.85$, in $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$, 25°C, TMS): $\delta=7.80$–7.77 (m, 2H; ArH), 7.62–7.57 (m, 2H; ArH), 7.47–7.25 (m, 19H; ArH), 5.99–5.87 (m, 2H), 5.37 (d, $J=17.1$ Hz, 1H), 5.25 (d, $J=10.5$ Hz, 1H), 5.01 (d, $J=11.7$ Hz, 1H), 4.93 (d, $J=10.8$ Hz, 1H), 4.87–4.78 (m, 3H), 4.73–4.57 (m, 4H), 4.49–4.11 (m, 7H), 3.95–3.85 (m, 3H), 3.61–3.54 (m, 3H), 2.77–2.73 (m, 2H; CH_2C=O), 2.54–2.41 (m, 2H; CH_2C=O), 2.2 ppm (s, 3H; CH_3C=O); ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): $\delta=$206.97, 172.9, 170.1, 156.5, 144.36, 144.21, 141.66, 138.74, 138.62, 138.59, 131.94, 128.90, 128.86, 128.82, 128.77, 128.66, 128.22, 128.19, 128.13, 128.04, 127.55, 127.50, 125.74, 125.64, 120.36, 119.19, 104.8, 82.6, 79.3, 77.7, 75.8, 74.9, 73.61, 73.37, 72.6, 70.4, 67.7, 66.7, 63.4, 55.1, 47.5, 38.3, 30.3, 28.2 ppm; HRMS (ESI): *m/z*: calcd for $C_{53}H_{55}NO_{12}Na$: 920.3616 $[M+Na]^+$; found: 920.3619.

3-Chloropropyl 2-*O*-benzyl-4,6-*O*-benzylidiene-3-*O*-(2′-azido-3′,4′,6′-tri-*O*-benzyl −2′-deoxy-β-D-glucopyranosyl)-1-β-D-galactopyranoside (38): Synthesis of **38** was referred to the general procedure of low-concentration 1,2-*trans* β-selective glycosylatin. **38** (126 mg, 77 %) was obtained as a colorless oily liquid. $[\alpha]_D^{27}=+7.42$ ($c=1.22$, in $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$, 25°C, TMS): $\delta=7.64$–7.61 (m, 2H; ArH), 7.53–7.51 (m, 2H; ArH), 7.46–7.35 (m, 19H; ArH), 7.28–7.21 (m, 2H; ArH), 5.60 (s, 1H; PhCH), 5.00–4.96 (m, 2H; vinyl-CH), 4.93–4.81 (m, 4H), 4.67–4.53 (m, 3H), 4.47 (d, $J=7.5$ Hz, 1H), 4.36–4.33 (m, 2H), 4.17–3.98 (m, 3H), 3.9 (dd, $J=3.3$, 9.9 Hz, 1H), 3.82–3.57 (m, 7H), 3.48–3.40 (m, 3H), 2.18–2.09 ppm (m, 2H; CH_2); ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): $\delta=$ 138.9, 138.40, 138.38, 138.23, 129.13, 128.93, 128.88, 128.82, 128.78, 128.53, 128.47, 128.45, 128.39, 128.35, 128.25, 128.20, 126.7, 104.2, 103.2, 100.98,

83.6, 78.96, 78.33, 38.21, 76.52, 75.94, 75.59, 75.56, 75.14, 73.88, 69.43, 69.30, 66.96, 66.77, 66.70, 42.35, 33.2 ppm; HRMS (ESI): *m/z*: calcd for $C_{60}H_{56}ClN_3O_{10}Na$: 914.3395 $[M+Na]^+$; found: 914.3390.

6-Chlorohexyl 2,3,6-tri-*O*-benzyl-4-*O*-(2′,3′,6′-tri-*O*-benzyl-β-D-galactopyranosyl)-1-β-D-glucopyranoside (39): Synthesis of **39** was prepared according to the general procedure of low-concentration 1,2-*trans* β-selective glycosylation. **39** (168 mg, 80 %) was obtained as white glassy solid. $[\alpha]_D^{27}=+15.23$ ($c=0.52$, in $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$, 25°C, TMS): $\delta=7.26$–7.13 (m, 30 H; ArH), 4.90 (d, $J=10.8$ Hz, 1 H), 4.80 (d, $J=11.1$ Hz, 1 H), 4.70–4.55 (m, 6 H), 4.47 (d, $J=12.3$ Hz, 1 H), 4.39–4.27 (m, 5 H), 3.94–3.81 (m, 3 H), 3.74–3.22 (m, 14 H), 2.36 (br, 1 H), 1.70–1.55 (m, 4 H; CH_2), 1.37–1.30 ppm (m, 4 H; CH_2); ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): $\delta=139.44$, 139.00, 138.95, 138.65, 138.48, 138.25, 128.80, 128.71, 128.63, 128.61, 128.40, 128.27, 128.20, 128.13, 128.11, 128.01, 127.96, 127.89, 127.87, 127.81, 127.58, 103.93, 102.89, 83.22, 82.11, 81.43, 79.70, 77.60, 75.67, 75.57, 75.42, 75.25, 73.83, 73.45, 73.06, 72.33, 70.09, 68.76, 68.63, 66.46, 45.38, 32.84, 29.93, 27.01, 25.81 ppm; HRMS (FAB): *m/z*: calcd for $C_{60}H_{69}ClO_{11}$: 1000.4528 $[M]^+$; found: 1000.4529.

6-Chlorohexyl 2,3,6-tri-*O*-benzyl-4-*O*-(3′-*O*-benzoyl-2′,6′-di-*O*-benzyl-β-D-galactopyranosyl)-1-β-D-glucopyranoside (40): Synthesis of **40** was performed according to the general procedure of low-concentration 1,2-*trans* β-selective glycosylation. **40** (235 mg, 83 %) was obtained as glassy solid. $[\alpha]_D^{27}=-17.10$ ($c=0.74$, in $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$, 25°C, TMS): $\delta=7.90$ (m, 2 H; ArH), 7.44 (m, 3 H; ArH), 7.30–7.18 (m, 19 H; ArH), 7.09–6.96 (m, 8 H; ArH), 5.50 (d, $J=3.0$ Hz, 1 H), 5.00 (d, $J=10.5$ Hz, 1 H), 4.83–4.45 (m, 8 H), 4.38–4.30 (m, 3 H), 4.13 (d, $J=12.0$ Hz, 1 H), 3.96 (t, $J=9.3$ Hz, 1 H), 3.88–3.85 (m, 1 H), 3.76 (dd, $J=3.6$, 11.1 Hz, 1 H), 3.69–3.64 (m, 2 H), 3.58–3.32 (m, 10 H), 2.45 (br, 1 H), 1.66–1.56 (m, 4 H; CH_2), 1.36–1.31 ppm (m, 4 H; CH_2); ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): $\delta=166.79$, 139.36, 138.98, 138.54, 138.49, 138.11, 133.50, 130.19, 130.00, 128.78, 128.71, 128.63, 128.60, 128.37, 128.29, 128.23, 128.18, 128.15, 128.01, 127.98, 127.92, 127.87, 127.60, 103.99, 102.85, 83.14, 81.97, 80.49, 77.60, 77.04, 73.78, 73.51, 73.02, 72.65, 70.61, 70.12, 68.48, 67.75, 63.06, 45.36, 32.84, 30.03, 29.92, 27.00, 25.81, 25.39 ppm; HRMS (FAB): *m/z*: calcd for $C_{67}H_{68}ClO_{12}$: 1015.4399 $[M+H]^{++}$; found: 1015.4394.

6-Chlorohexyl 2-azido-3,6-di-*O*-benzyl-2-deoxy-1-β-D-glucopyranoside (41): Synthesis of **41** was performed according to the general procedure of low-concentration 1,2-*trans* β-selective glycosylation. **41** (44 mg, 60 %) was obtained as colorless oil. $[\alpha]_D^{27}=-32.28$ ($c=0.3$, in $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$, 25°C, TMS): $\delta=7.43$–7.29 (m, 10 H; ArH), 4.96 (d, $J=11.4$ Hz, 1 H), 4.81 (d, $J=11.4$ Hz, 1 H), 4.65 (dd, $J=12.0$, 4.8 Hz, 1 H), 4.31 (d, $J=7.8$ Hz, 1 H), 3.98–3.90 (m, 1 H), 3.76 (d, $J=6.6$ Hz, 1 H), 3.68–3.52 (m, 4 H), 3.46–3.29 (m, 2 H), 3.26 (t, $J=9.0$ Hz, 1 H), 2.72 (br, 1 H), 1.83–1.76 (m, 2 H; CH_2), 1.72–1.64 (m, 2 H; CH_2), 1.51–1.43 ppm (m, 4 H; CH_2); ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): $\delta=138.5$, 138.0, 129.0, 128.8, 128.5, 128.4, 128.2, 128.1, 102.6, 82.8, 75.4, 74.3, 74.1, 72.4, 70., 70.4, 66.1, 45.4, 32.9, 29.7, 27.0, 25.6 ppm; HRMS (FAB): *m/z*: calcd for $C_{26}H_{35}N_3O_5ClNa$: 504.2260 $[M+H]^+$; found: 504.2260.

1,2:3,4-Di-*O*-isopropylidene-6-*O*-(2′,3′,4′,6′-tetra-*O*-benzyl-α-D-mannopyranosy)-1-α-D-galactopyranose (42): Synthesis of **42** was performed according to the general procedure of low-concentration 1,2-*trans* β-selective glycosylation. **42** (128 mg, 85 %) was obtained as colorless oily liquid. $[\alpha]_D^{27}=-46.11$ ($c=0.75$, in $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$, 25°C, TMS): $\delta=7.42$–7.25 (m,18 H; ArH), 7.19–7.16 (m, 2 H; ArH), 5.55 (d, $J=5.1$ Hz, 1 H; H-1), 5.05 (d, $J=1.8$ Hz, 1 H), 4.90 (d, $J=10.5$ Hz, 1 H), 4.75–4.69 (m, 3 H), 4.63–4.51 (m, 5 H), 4.35 (dd, $J=7.5$, 5.1 Hz, 1 H), 4.19 (dd, $J=1.8$, 8.1 Hz, 1 H), 4.08–3.91 (m, 3 H), 3.86–3.69 (m, 6 H), 1.52 (s, 3 H; CH_3C), 1.45 (s, 3 H; CH_3C), 1.35 ppm (s, 6 H; CH_3C); ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): $\delta=138.9$, 138.88, 138.82, 138.75, 128.7, 128.4, 128.2, 128.0, 127.98, 127.94, 127.88, 127.84, 109.7, 108.9, 97.6, 96.7, 80.4, 77.6, 75.5, 75.2, 74.9, 73.7, 72.7, 72.4, 71.3, 71.0, 70.9, 69.4, 65.7, 65.6, 26.5, 26.3, 25.3, 24.9 ppm; HRMS (ESI): *m/z*: calcd for $C_{48}H_{54}O_{11}Na$: 805.3564 $[M+Na]^+$, found: =805.3558.

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2′,3′,4′-tri-*O*-benzyl-β-D-glucopyranosyl)-1-α-D-glucopyranoside (43): Disaccharide **36** (410 mg, 0.4 mmol) in 1:2 v/v CH_2Cl_2–MeOH solution (8 mL) was treated with a piece of freshly cut sodium (approximately 10 mg) at room temperature. Upon completion of deacetylation, the mixture was neutralized with resin IR-120H$^+$, filtered, and concentrated for purification with column chromatography over silica gel (elution:hexane/EtOAc/toluene=1/1/1) to furnish the disaccharide **43** as white glassy solid (350 mg, 95 %). $[\alpha]_D^{27}=+17.13$ ($c=0.78$, in $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$, 25°C, TMS): $\delta=7.41$–7.34 (m, 25 H; ArH), 7.28–7.22 (m, 5 H; ArH), 5.06–4.93 (m, 4 H), 4.90–4.80 (m, 5 H), 4.76–4.67 (m, 4 H), 4.57 (d, $J=11.4$ Hz, 1 H), 4.45 (d, $J=7.8$ Hz, 1 H), 4.17 (d, $J=10.5$ Hz, 1 H), 4.06 (t, $J=9.3$ Hz, 1 H), 3.92–3.85 (m, 2 H), 3.79–3.50 (m, 8 H), 3.42–3.40 ppm (m, 4 H); ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): $\delta=139.2$, 138.84, 138.76, 138.65, 138.54, 138.39, 128.96, 128.93, 128.86, 128.83, 128.62, 128.51, 128.46, 128.41, 128.35, 128.27, 128.10, 128.05, 104.2, 98.6, 85.0, 82.43, 82.41, 80.2, 78.2, 76.2, 75.56, 75,54, 75.42, 75.36, 73.84, 70.2, 69.1, 62.4, 55.7 pm; HRMS (ESI): *m/z*: calcd for $C_{55}H_{60}O_{11}Na$: 919.4033 $[M+Na]^+$; found: =919.4030.

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-[2′,3′,4′-tri-*O*-benzyl-6′-*O*-(2″,3″,4″-tri-*O*-benzyl-6-*O*-levulinoyl-β-D-glucopyranosyl)-β-D-glucopyranosyl]-1-β-D-glucopyranoside (44): Disaccharide acceptor **43** (derived from 36 by Zemplén deacylation, 120 mg, 0.13 mmol), thioglucopyranoside 6 (105 mg, 0.16 mmol) and flame-dried MS (AW300, 300 mg) were suspended in a 1:2:1 v/v CH_2Cl_2–CH_3CN–EtCN (13 mL) solution under N_2. The resulting mixture was stirred at room temperature for 5 min and at −70°C for an additional 20 min, followed by addition of NIS (38 mg, 0.17 mmol) and TMSOTf (6 μL, 0.032 mmol). Upon completion of glycosylation as monitored by TLC, saturated $NaHCO_3$ (0.1 mL) and pieces of $Na_2S_2O_3$(s) were added to the mixture, followed by vigorously stirring at room temperature until the deep-red color of the solution changed to pale yellow. MS was then removed by filtration over celite, and the filtrate was dried (over $MgSO_4$), filtered, and concentrated for flash-chromatography purification over silica gel (elution: hexane/CH_2Cl_2/EtOAc=6:3:1) to furnish the desired trisaccharide **41** as colorless oily liquid (134 mg, 70 %). $[\alpha]_D^{27}=+16.92$ ($c=2.84$, in $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$, 25°C, TMS): $\delta=7.38$–7.21 (m, 43 H; ArH), 7.19–7.16 (m, 2 H; ArH), 5.08–4.75 (m, 16 H), 4.74–4.45 (m, 9 H), 4.39–4.12 (m, 6 H), 4.00 (t, $J=9$ Hz, 1 H), 3.80–3.35 (m, 17 H), 3.2 (s, 3 H; OCH_3), 2.75–2.70 (m, 2 H; $CH_2C=O$), 2.62–2.56 (m, 2 H; $CH_2C=O$), 2.19 ppm (s, 3 H; $CH_3C=O$); ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): $\delta=206.7$, 172.96, 139.3, 138.86, 138.84, 138.77, 138.70, 138.57, 138.42, 138.27, 128.90, 128.86, 128.84, 128.80, 128.76, 128.58, 128.52, 128.44, 128.38, 128.34, 128.27, 128.14, 128.10, 128.08, 127.99, 127.96, 104.3, 103.9, 98.5, 85.2, 85.1, 82.4, 82.3, 80.0, 78.6, 78.2, 77.8, 77.7, 76.14, 76.09, 75.6, 75.42, 75.37, 75.23, 75.11, 73.75 73.26, 70.1, 69.0, 68.6, 63.7, 55.7, 38.2, 30.3, 28.3 ppm. HRMS (ESI): *m/z*: calcd for $C_{87}H_{96}O_{18}Na$: 1449.6332 $[M+Na]^+$; found: =1449.6332.

6-Chlorohexyl 2,3,6-tri-*O*-benzyl-4-*O*-[2,3,6-tri-*O*-benzyl-4-*O*-(2,3-di-*O*-benzyl-4,6-*O*-di-*tert*-butylsilylidene-α-D-galactopyranosyl)-β-D-galactopyranosyl]-1-β-D-glucopyranoside (46): Thiolactoside **23** (330 mg, 0.33 mmol), 6-chlorohexanol 13 (90 mg, 0.66 mmol), and flame-dried MS (AW300, 400 mg) were suspended in a 1:2:1 v/v CH_2Cl_2–CH_3CN–EtCN (32 mL) solution at room temperature. The resulting mixture was stirred at room temperature for 10 min and at −70°C for additional 20 min, followed by addition of NIS (77 mg, 0.34 mmol) and TMSOTf (12 μL, 0.066 mmol). Upon completion of glycosylation as judged by TLC, saturated $NaHCO_3$ (1 v/v % of reaction volume) and pieces of $Na_2S_2O_3$(s) were added to the reaction mixture, followed by vigorously stirring at room temperatuer until the red color of the solution turned to pale yellow. The resulting crude mixture was filtered over celite and diluted with CH_2Cl_2 (40 mL). The CH_2Cl_2 solution was washed with H_2O (15 mL), brine (15 mL), dried (over $MgSO_4$), filtered, concentrated, and dried under vacuum for approximately 2 h. The resulting residue was dissolved in CH_2Cl_2 (5 mL), followed by addition of thiogalactopyranoside 45 (500 mg, 0.82 mmol) and flame-dried MS (AW300, 900 mg). The mixture was stirred at room temperature for 15 min and at −10°C for an additional 20 min, followed by addition of NIS (1.88 mg, 0.83 mmol) and TMSOTf (30 μL, 0.17 mmol). The mixture was stirred at −10°C under N_2 and the glycosylation reaction was monitored by TLC. Upon completion of glycosylation as judged by TLC, saturated $NaHCO_3$ (1 v/v % of reaction volume) and small lumps of $Na_2S_2O_3$(s) were added, followed by the workup procedure described in the preparation of compound **44**. The resulting CH_2Cl_2 solution was concentrated for flash column chromatography over silica gel (elution: hexane/CH_2Cl_2/EtOAc=7:2:1) to furnish

target trisaccharide **46** as yellow oily liquid (248 mg, 75% from 23). $[\alpha]_D^{27}=+46.47$ ($c=1.06$, in $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$, 25°C, TMS): $\delta=7.11$–7.03 (m, 40H; ArH), 4.97 (d, $J=11.4$ Hz, 1H), 4.86 (d, $J=3.0$ Hz, 1H), 4.80–4.14 (m, 19H), 4.02–3.62 (m, 11H), 3.54–3.14 (m, 10H), 1.64–1.53 (m, 4H; CH_2), 1.33–1.32 (m, 4H; CH_2), 0.91 (s, 9H; *t*Bu), 0.88 ppm (s, 9H; *t*Bu); ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): $\delta=$ 139.60, 139.43, 139.01, 138.98, 138.88, 138.83, 138.69, 138.54, 128.80, 128.72, 128.67, 128.63, 128.58, 128.49, 128.16, 128.07, 128.03, 127.97, 127.94, 127.85, 127.78, 127.66, 127.61, 127.56, 103.86, 100.33, 82.75, 82.05, 81.35, 79.31, 78.36, 77.60, 75.39, 75.22, 74.34, 73.98, 73.59, 73.51, 73.45, 73.40, 72.45, 71.33, 70.75, 70.06, 68.70, 67.88, 67.78, 67.40, 45.36, 32.83, 30.03, 29.91, 28.06, 27.96, 27.86, 27.75, 26.85, 27.65, 26.99, 25.80, 23.66, 21.00 ppm; HRMS (ESI): *m/z*: calcd $C_{88}H_{108}ClO_{16}Si$: 1483.7095 $[M+H]^+$; found: =1483.7090.

6-Chlorohexyl 4-*O*-[4-*O*-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-1-β-D-glucopyranoside, Gb_3 derivative (47): Protected Gb_3 **46** (270 mg, 1.82 mmol) dissolved in a mixture of AcOH (95%, 0.25 mL) and THF (5 mL) was treated with tetrabutylammonium fluoride (TBAF; 1M in THF, 1.8 mL).[40] After stirring at room temperature for 13 h, the reaction was diluted with CH_2Cl_2 (40 mL), which was washed with H_2O (2× 15 mL), dried (over $MgSO_4$), filtered, and concentrated for the next hydrogenolysis. The resulting residue from desilylation (approximately 200 mg) was dissolved in a mixture of formic acid (0.35 mL) and MeOH (7 mL) and treated with 5% Pd/C (100 mg). The reaction mixture was stirred at room temperature under H_2 for 14 h. Upon completion of hydrogenolysis as judged by TLC, Pd/C was removed by filtration over celite, and the filtrate was concentrated for column chromatography purification on C18 coated reverse-phase gel (Cosmosil 75C18-OPN) (elution: H_2O/MeOH gradient from 1:0 to 4:1) to furnish desired trisaccharide **47** as white glassy solid (67 mg, 60% from **46**). $[\alpha]_D^{27}=+33.5$ ($c=$ 0.20, in MeOH); ^1H NMR (300 MHz, CD_3OD, 25°C): $\delta=4.90$ (d, $J=$ 3.9 Hz, 1H), 4.45 (t, $J=8.3$ Hz, 2H), 4.32 (t, $J=6.5$ Hz, 1H), 3.99–3.50 (m, 20H), 3.25 (t, $J=8.4$ Hz, 1H), 1.79–1.70 (m, 2H; CH_2), 1.65–1.56 (m, 2H; CH_2), 1.46–1.35 ppm (m, 4H, CH_2); ^{13}C NMR (75 MHz, CD_3OD, 25°C): $\delta=$103.56, 102.28, 100.60, 78.89, 77.62, 75.73, 75.10, 74.23, 72.43, 71.19, 71.09, 70.84, 69.62, 69.20, 68.85, 60.76, 60.67, 60.32, 45.93, 32.07, 28.87, 26.09, 24.62 ppm; HRMS (ESI): *m/z*: calcd $C_{24}H_{45}ClNaO_{16}$: 645.2132 $[M+Na]^+$; found: =645.2132.

6-Chlorohexyl 2,3,6-tri-*O*-benzyl-4-*O*-[2,6-di-*O*-benzyl-4-*O*-benzoyl-3-*O*-(2,3-di-*O*-benzyl-4,6-*O*-di-*tert*-butylsilylidene-α-D-galactopyranosyl)-β-D-galactopyranosyl]-1-β-D-glucopyranoside (48): A mixture of thiolactoside **24** (280 mg, 0.28 mmol), 6-chlorohexanol 13 (76 mg, 0.56 mmol) and flame-dried MS (AW300, 330 mg) were suspended in 1:2:1 CH_2Cl_2–MeCN–EtCN (28 mL) at room temperature under N_2. The mixture was stirred at room temperature for 10 min under N_2 and at −70°C for additional 20 min, followed by addition of NIS (67 mg, 0.29 mmol) and TMSOTf (10 μL, 0.056 mmol). Upon completion of glycosylation, saturated $NaHCO_3$ (1 v/v% of reaction volume) and small lumps of $Na_2S_2O_3$(s) were added to the reaction mixture, followed by the workup procedure as described in the preparation of compound **44**. The crude residue obtained was dried under vacuum for aproximately 2 h and made ready for next glycosylation. The crude residue from the previous step was then dissolved in CH_2Cl_2 (4 mL), to which thiogalactopyranoside 45 (436 mg, 0.72 mmol) and flame-dried MS (AW300, 700 mg) were added under N_2. The resulting mixture was stirred at −10°C for 20 min, and then followed by addition of NIS (180 mg, 0.79 mmol) and TMSOTf (29 μL, 0.16 mmol). Upon completion of glycosylation as judged by TLC, saturated $NaHCO_3$ and small lumps of $Na_2S_2O_3$(s) were added and then the workup procedure as described in the preparation of compound **44** was performed. The crude residue was purified by flash column chromatography over silica gel (elution: hexane/CH_2Cl_2/EtOAc=15:5:1) to furnish expected trisaccharide **48** as yellow oily liquid (292 mg, 70%). $[\alpha]_D^{27}=+56.13$ ($c=0.62$, in $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$, 25°C, TMS): $\delta=7.95$ (d, $J=7.2$ Hz, 2H; ArH), 7.46 (t, $J=7.5$ Hz, 1H; ArH), 7.35–7.19 (m, 29H; ArH), 7.14–7.08 (m, 6H; ArH), 7.04–7.00 (m, 2H; ArH), 5.81 (d, $J=2.7$ Hz, 1H), 5.28 (d, $J=3.3$ Hz, 1H), 5.09 (d, $J=$ 10.8 Hz, 1H), 4.93–4.60 (m, 8H), 4.55–4.36 (m, 8H), 4.24 (d, $J=12.0$ Hz, 1H), 4.11–4.07 (m, 1H), 3.97–3.83 (m, 6H), 3.73–3.59 (m, 6H), 3.49–3.37 (m, 6H), 1.74–1.65 (m, 4H; CH_2), 1.43–1.41 (m, 4H; CH_2), 0.95 (s, 9H; *t*Bu), 0.92 ppm (s, 9H; *t*Bu); ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): $\delta=$ 165.90, 139.41, 139.24, 138.95, 138.90, 138.66, 138.44, 138.12, 133.18, 130.25, 130.09, 128.73, 128.63, 128.58, 128.49, 128.34, 128.33, 128.25, 128.24, 128.17, 128.07, 128.01, 127.91, 127.87, 127.81, 127.80, 127.75, 127.61, 127.57, 127.33, 103.94, 103.10, 94.00, 83.11, 81.91, 79.71, 77.60, 77.31, 75.67, 75.49, 75.27, 73.85, 73.63, 73.58, 73.26, 72.79, 70.74, 70.51, 70.06, 68.62, 67.91, 67.09, 66.97, 66.19, 45.30, 32.80, 29.89, 27.91, 27.53, 26.96, 25.77, 23.62, 20.87 ppm; HRMS (ESI): *m/z*: calcd $C_{88}H_{108}ClNaO_{17}Si$: 1519.6707 $[M+Na]^+$; found: 1519.6702.

6-Chlorohexyl 4-*O*-[3-*O*-(α-D-galactopyranosyl)-β-D-galactopyranosyl-1-β-D-glucopyranoside, isoGb$_3$ derivative (49): Protected isoGb$_3$ **48** (280 mg, 1.87 mmol) in a mixture of AcOH (95%, 0.25 mL) and THF (5 mL) was treated with TBAF (1M in THF, 1.8 mL).[40] After stirring at room temperature for 13 h, the mixture was diluted with CH_2Cl_2 (40 mL), washed with H_2O (2×15 mL), dried over $MgSO_4$, filtered, and concentrated for the next debenzoylation. The resulting crude product from desilylation (approximately 200 mg) was dissolved in 1:2 v/v MeOH/THF (3 mL) and treated with 2N NaOH (2 mL), followed by stirring at 60°C for 36 h. Upon completion of benzoyl ester removal, the mixture was diluted with CH_2Cl_2 (40 mL), and the solution was then washed with 1N HCl (15 mL), H_2O (15 mL), brine (15 mL), dried over $MgSO_4$, filtered, and concentrated for next hydrogenolysis. The crude residue from debenzoylation step was dissolved in a mixture of formic acid (0.35 mL) and MeOH (7 mL), followed by addition of 5% Pd/C (100 mg). The mixture was stirred at room temperature under H_2 for 16 h. Upon completion of hydrogenolysis as judged by TLC, Pd/C was removed by filtration over celite, and the filtrate was concentrated for column chromatography purification on C18 coated reverse phase silica gel (Cosmosil 75C18-OPN; elution: H_2O/MeOH gradient from 1:0 to 4:1) to furnish desired trisaccharide **49** as a colorless glassy solid (64 mg, 55% from **48**). $[\alpha]_D^{27}=+53.00$ ($c=0.80$, in MeOH); ^1H NMR (300 MHz, CD_3OD, 25°C): $\delta=5.07$ (s, 1H), 4.45 (d, $J=6.6$ Hz, 1H), 4.32 (d, $J=$ 7.8 Hz, 1H), 4.24 (t, $J=6.3$ Hz, 1H), 4.07 (s, 1H), 3.96–3.53 (m, 18H), 3.44–3.42 (m, 1H), 3.34–3.32 (m, 1H), 3.27 (t, $J=8.1$ Hz, 1H), 1.84–1.75 (m, 2H; CH_2), 1.71–1.62 (m, 2H; CH_2), 1.48–1.43 ppm (m, 4H; CH_2); ^{13}C NMR (75 MHz, CD_3OD, 25°C): $\delta=105.03$, 104.16, 97.66, 80.99, 79.83, 76.57, 76.42, 76.35, 74.67, 72.22, 71.30, 71.08, 70.98, 70.73, 70.11, 66.60, 62.71, 62.42, 61.96, 45.71, 33.71, 30.56, 27.69, 26.32 ppm; HRMS (ESI): *m/z*: calcd $C_{24}H_{45}ClO_{16}Na$: 645.2132 $[M+Na]^+$; found: 645.2132.

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