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Glycosylation of 'basic' alcohols: methyl 6-(hydroxymethyl)picolinate as a case study

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ABSTRACT

Glycosylation is promoted by acid promoters rendering the reactions with basic acceptors challenging. This report presents an in depth study involving methyl 6-(hydroxymethyl)picolinate as the model acceptor and 22 glycosyl donors to afford the desired glycosides in good yields ranging from 46% to 85%. Several parameters were evaluated, including the protecting groups of the glycosyl donor, the leaving group at the anomeric center, and the promoter. The influence of the pyridine ring was evident with a benzene-based acceptor affording high yields of glycoside (79%) in comparison to the pyridine-based acceptor (46%). The present work provides a general and reliable access to pyridine-containing glycosides.

1. Introduction

Carbohydrates are present in nature as monomers (e.g., glucose is a source of energy) and oligomers (i.e., oligosaccharides) which are interconnected through glycosidic bonds. Glycosylation[1–9] is therefore one of the most important reactions in carbohydrate chemistry, challenged by the control of regioselectivity commonly achieved by protecting group strategies but also by the stereoselectivity of the glycosidic bond created requiring additional strategies based not only on protecting groups but also on specific reaction conditions.

Initially, picolinyl ethers have been identified as reactivity-enhancing replacements for benzyl ethers as exemplified for the glycosylation at the 4-position of a *N*-acetylglucosamine acceptor bearing a picolinyl group at the 3-position.[10] More recently, picolinyl and picoloyl substituents have been identified as protecting groups for the stereocontrol of glycosylation.[11–13] Nevertheless, this approach is usually using excess of promoters in order to balance the intrinsic basicity of the pyridine moiety and then catalyze the formation of the oxocarbenium intermediate. Surprisingly, a careful survey of the literature for the glycosylation of glycosyl acceptors with pyridine-containing acceptors did not afford many results and most of these were poor in terms of isolated yields of glycosides and are discussed below. Such glycosides containing a pyridine aglycone will find applications for the design of neutral nucleotide-diphosphate sugar analogues in the search for neutral inhibitors of glycosyltransferases.[14–17] After determining the optimal glycosylation conditions for these glycosides, the ester moiety will then be conjugated to a 5′-aminonucleoside to afford a neutral NDP-sugar analogue.

Pyridoxine can be glycosylated on the hydroxymethyl groups at positions 4′ or 5′, or both, and is better known as vitamin B_6. The 4′-glucosylated derivative is less stable to hydrolysis in the liver than its 5′-regioisomer. In an initial report, the hydrolysis of the glycosidic bond of glycosylated derivatives of pyridoxine by glycosidases has been studied (Table 1, entry 1).[18] In another series of experiments, the influence of borate was evaluated in order to obtain a regioselective glucosylation of pyridoxine at the 4′- or 5′-positions by microorganisms.[19,20] Enzymatic glycosylation could also be performed using α- and β-glucosidases (Table 1, entry 2).[21,22] Introduction of an additional fluorine atom at the 6-position of the pyridine ring afforded reporter molecules for the identification of genes encoding for β-galactosidase in the search for efficient molecules for the assessment of location, magnitude, and persistence of gene expression.[23] The glycosyl donors used in these reports are natural (oligo)saccharides, sucrose, or dextrins[19–22] but also chemically modified carbohydrates such as *p*-nitrophenyl[18] (PNP) or α-bromo[23] activated glycosides. Nevertheless, under the aforementioned conditions using enzymatic or microbial glycosylations, a maximum yield of 40% was achieved. In a similar enzymatic approach, the PNP-activated glucose was used as a donor

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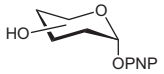
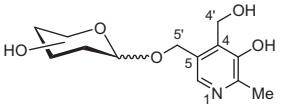
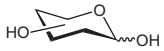
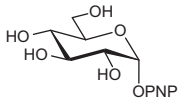
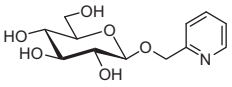
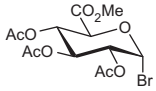
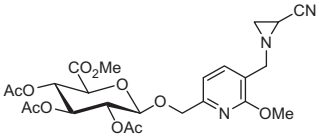
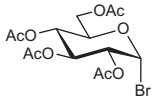
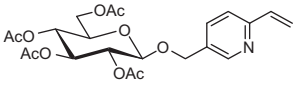
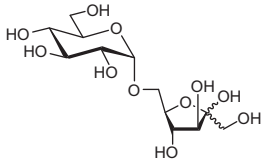
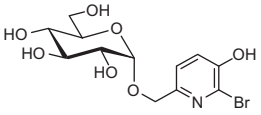
Table 1
Enzymatic and chemical approaches to the synthesis of glycosylated hydroxymethylpyridine derivatives

Entry	Donor	Glycoside	Method	Yield (%)	Ref.
1			Enzymatic	32[a]	18
2				44[b]	22
3				24	24
4				45[c]	25
5			Chemical	24[c]	26
6				<5[d]	27

[a] Yield reported for galactose derivatives.
[b] The 4′-regioisomer was also isolated in a 28:72 mixture with the desired 5′-derivative.
[c] Yield calculated over two steps from the data reported through the orthoester intermediate.
[d] Yield calculated from the reported data for the multi-step process.

for the glucosylation of a series of aliphatic and benzylic alcohols in the presence of a glucosidase providing the desired β-D-glucosides in less than 35% yield (Table 1, entry 3).[24] While these synthetic strategies, inspired by natural enzymes or microorganisms did not require extensive protecting group chemistry and provided the desired glycosides in high anomeric stereoselectivities, the yields obtained remained quite low and did not call for applications in organic synthesis.

In an organic chemistry approach, a handful of reports have been identified in the literature in which chemical synthesis has been applied. The glucuronylation of ciamexon (an immunomodulating agent with application in diabetes) was achieved from the corresponding α-glucuronopyranosyl bromide donor using silver(I) salt as a promoter leading to the desired glucuronide in 45% yield (Table 1, entry 4).[25] The reaction conditions were quite sensitive and the use of basic medium (*sym*-collidine) influenced the reaction affording a large portion of undesired orthoester. This problem could be overcome by using excess of silver triflate (AgOTf, 3 equiv) most probably in order to balance the basicity of the pyridine acceptor. The formation of the orthoester intermediate could not be prevented in the glycosylation between acetobromoglucose and 2-vinyl-5-hydroxymethylpyridine derivative using AgOTf as the promoter[26] in a study for the design of peptide functionalizing agents (Table 1, entry 5). Again, an excess of promoter (1.5 equiv) was used but led to exclusive formation of the orthoester intermediate in 73% yield since the same amount of acceptor was used and its basicity could therefore not be totally balanced. A subsequent rearrangement in the presence of trimethylsilyl triflate (TMSOTf) afforded the desired glycoside in 32% yield corresponding to an overall yield of only 24% over two steps.

Finally, isomaltulose was used as a precursor for α-glucosylated hydroxymethylpyridine derivatives (Table 1, entry 6).[27] Acid dehydration of isomaltulose afforded a glucosylated furfural which could be transformed into a pyridine moiety through two additional synthetic steps. The overall process did not afford the desired compounds in good yields (<5%) but represents yet another possibility for the construction of the pyridine scaffold from a furane moiety intermediate.

2. Results and discussion

Methyl 6-(hydroxymethyl)picolinate **2a** was chosen as the glycosyl acceptor. After reductive desymmetrization of a diester precursor,[28] the subsequent glycosylation was then investigated with a large series of glycosyl donors and glycosylation conditions to address the scope and limitation of such chemical glycosylation of hydroxymethylpyridine derivative. This general investigation of the glycosylation of hydroxymethylpyridine was therefore undertaken based on the literature overview presented above leading to at least two observations: (1) enzymatic or chemical glycosylations are possible, but (2) the yields obtained are rather poor. The chemical glycosylation was studied here with 22 donors and under 43 glycosylation conditions in order to provide a general and useful methodology for the synthesis of a large series of glycosylated hydroxymethylpyridine derivatives.

2.1. Galactosyl donors

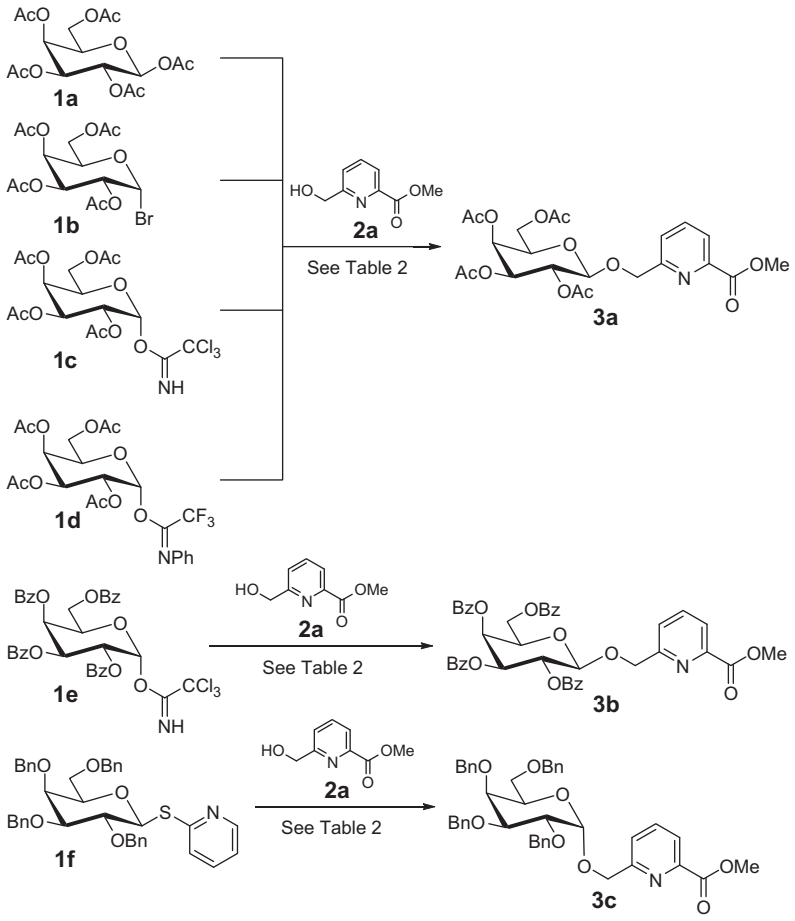
The first type of glycosylation investigated employed galactosyl donors in order to address the influence of protecting groups on the carbohydrate moiety as well as the different leaving groups at the anomeric position on the outcome of the glycosylation reaction. Galactosylation is usually simpler to be studied in comparison to glucosylation providing in most cases higher reactivities of the glycosyl donors, improved yields, and better selectivities.[29–31] In all cases, the promoter was a Lewis acid used in excess in the

reaction in order to balance the basicity of the pyridine-based acceptor. In almost all cases, an excess of glycosyl donor was used to ensure maximal functionalization of the acceptor. The yields reported are based on at least two experiments providing similar reproducible results. All reactions were performed in dichloromethane unless stated otherwise in the Tables.

A series of six different galactosyl donors **1a–f** were synthesized and used in the present study (Scheme 1). Peracetylated galactose **1a** was the simplest donor tested being readily available commercially without requiring additional synthetic steps for its activation at the anomeric center. We have previously reported an efficient synthetic methodology for the glycosylation using silver and tin salts.[32] Nevertheless, in the present work, the same protocol used for the galactosylation of alcohol **2a**[28] with the peracetylated donor **1a** did not afford the desired galactoside **3a** even though a small amount of the product could be detected by TLC analysis of the crude mixture (Table 2, entry 7). We then turned to the Koenigs–Knorr glycosylation system using the galactopyranosyl bromide donor **1b** and insoluble silver salts.[5,33] Later, the Helferich modification[34] used mercury(II) cyanide (Table 2, entry 8) as the promoter for this glycosylation and under these conditions the reaction afforded the desired glycoside **3a** with complete stereoselectivity in favor of the expected β-anomer due to anchimeric participation of the acetyl group at the 2-position of the saccharide. A mixture of acetonitrile and dichloromethane was used for maximal solubility of the reagents. Nevertheless, the poor yield obtained prompted the evaluation of a more common silver triflate promoter (Table 2, entries 9 and 10) but the excess of either donor or acceptor did not improve the yield of isolated galactoside. Our attention then turned to trichloroacetimidate donors such as derivative **1c**[35,36] as powerful glycosylation agents.[5,8,37,38] Boron trifluoride etherate was used as promoter and provided the best results in this respect. Molecular sieves were used to keep the reaction mixture anhydrous and the initial attempts under these standard conditions provided improved yields (up to 34%) in comparison to the previous attempts (Table 2, entries 11 and 12). Molecular sieves could also be avoided and the reaction outcome improved to reach 46% yield (Table 2, entry 13). Changing the solvent to acetonitrile (Table 2, entry 14) or using excess of acceptor (Table 2, entry 15) or larger proportions of donor (Table 2, entry 16) did not improve the yields. Trimethylsilyl trifluoromethanesulfonate is also a general promoter used for these glycosylations but the glycosylation yield was not improved (Table 2, entry 17) and the main product obtained was the silylated acceptor **2b**.

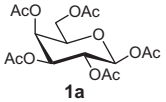
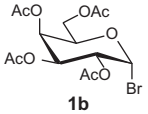
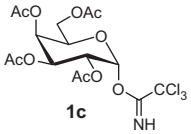
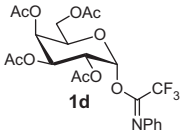
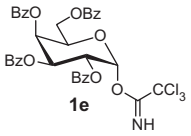
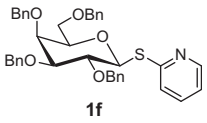
More recently, Schmidt et al.[39,40] have introduced the use of neutral glycosylation conditions with difluoro(phenyl)borane ($PhBF_2$) as the promoter. Since the basicity of the acceptor appeared as a major problem in our glycosylation study, this new methodology was also evaluated (Table 2, entries 18 and 19) but proved rather unsuccessful providing poor yields even at higher temperatures than initially required. Although less stable than trichloroacetimidates, we also turned our attention to *N*-phenyl-trifluoroacetimidate donor **1d** which was recently shown to provide better yields than for standard trichloroacetimidates[41] but the yield of galactoside **3b** was not improved (Table 2, entry 20).

Acetates are the most common protecting groups in carbohydrate chemistry, but benzoates are also used in some cases for stability issues or even possible detection using UV analysis due to



Scheme 1. Galactosylation of methyl 6-(hydroxymethyl)picolinate **2a** with galactosyl donors **1a–f**.

Table 2
Galactosylation of methyl 6-(hydroxymethyl)picolinate **2a** with galactosyl donors **1a–f**

Entry	Donor	Ratio Donor:acceptor	Temp Time	Promoter	Yield[a] (%)	Product Ratio α:β
7	 **1a**	1.5:1	0 °C to rt 16 h	$SnCl_4$ (5 equiv)/CF_3CO_2Ag (2.5 equiv)	<5[b]	–/–
8	 **1b**	1.5:1	rt 16 h	$Hg(CN)_2$ (4 equiv)	11[c]	**3a** β only
9		1:1.5	rt 18 h	AgOTf (1.5 equiv)	8	**3a** β only
10		2.5:1	rt 26 h	AgOTf (1.5 equiv)	18	**3a** β only
11	 **1c**	1.25:1	−20 °C 4 h	$BF_3{\cdot}Et_2O$ (2 equiv) 4 Å MS	34	**3a** β only
12		1.5:1	−20 °C 16 h	$BF_3{\cdot}Et_2O$ (2 equiv) 4 Å MS	32	**3a** β only
13		1.5:1	−20 °C 2 h	$BF_3{\cdot}Et_2O$ (2 equiv)	46	**3a** β only
14		1.5:1	−20 °C 2 h	$BF_3{\cdot}Et_2O$ (2 equiv)	36[d]	**3a** β only
15		1:3	−20 °C 2.5 h	$BF_3{\cdot}Et_2O$ (2 equiv)	<5[b]	–/–
16		3:1	−20 °C 16 h	$BF_3{\cdot}Et_2O$ (3 equiv)	42	**3a** β only
17		1.5:1	−20 °C 1 h	TMSOTf (0.5 equiv) 4 Å MS	5[e]	β only
18		2:1	−78 °C 3 h	$PhBF_2$ (2 equiv)	16	**3a** β only
19		1.5:1	−20 °C 2 h	$PhBF_2$ (2 equiv)	14	**3a** β only
20	 **1d**	1.5:1	−20 °C 2 h	$BF_3{\cdot}Et_2O$ (2 equiv)	32	**3a** β only
21	 **1e**	1.3:1	−20 °C 22 h	TMSOTf (2 equiv) 4 Å MS	33	**3b** β only
22		1.3:1	−20 °C 20 h	$BF_3{\cdot}Et_2O$ (3 equiv)	42	**3b** β only
23	 **1f**	1:1	50 °C 78 h	MeI (4.5 equiv) 4 Å MS	75	**3c** α only[f]

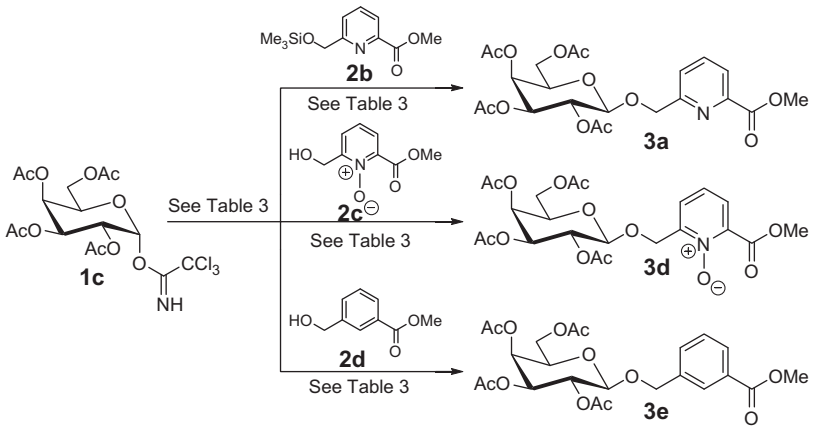
[a] Values are isolated yields.
[b] Product could not be isolated but was detected by TLC.
[c] The glycosylation was performed in CH_3CN/CH_2Cl_2 (1/1, v/v).
[d] The glycosylation was performed in CH_3CN.
[e] The main product isolated was methyl 6-(trimethylsilyloxymethyl)picolinate **2b**.
[f] Even if the β-anomer was formed during the reaction, it was not isolated by column chromatography.

their aromatic rings. The benzoylated trichloroacetimidate donor **1e**[42,43] was therefore used under the best conditions obtained (Table 2, entry 13) although with only 1.3 equiv of donor excess and prolonged reaction time (Table 2, entries 21 and 22). The yield obtained was acceptable, but not improved, remaining below 42%.

The reactivity of the donor can be tuned by choosing the appropriate protecting groups on the carbohydrate scaffold.[44,45] While ester protected glycosyl donors (e.g., acetates or benzoates) would be considered as disarmed and lead to poorer reactivity (i.e., longer reactions and need for stronger promoters) in the glycosylation reaction, the ether protected donors (e.g., benzyl) are armed and would provide higher reactivities and better yields for the glycosylation. The 2-thiopyridyl perbenzylated donor **1f**[46] was therefore investigated providing a good yield (75%) of the desired galactoside **3c** (Table 2, entry 23) although with opposite stereochemistry at the anomeric center, the α-anomer being favored in this case due to the benzyl ether as a non-participating group at the 2-position.

As a conclusion for the galactosylation of 6-(hydroxymethyl)picolinate **2a**, the best conditions obtained after investigating four ester-protected galactosyl donors **1a–d** under 16 different reaction conditions are using the trichloroacetimidate donor with boron trifluoride etherate as a promoter in excess (1.5 equiv) and with only 2 h reaction time at low temperature (−20 °C) to afford the desired β-anomer. Better yields, albeit with the opposite configuration at the anomeric center, can be obtained with benzylated armed donors such as **1f**.

We then investigated the influence of the pyridine-based acceptors in order to address the influence of the basicity of the pyridine ring under acid-promoted glycosylation conditions (Scheme 2).



Scheme 2. Influence of the pyridine-based acceptor on the galactosylation reaction.

In the first attempt the silylated acceptor **2b** was reintroduced for galactosylation with the best donor identified in the previous experiments, namely the trichloroacetimidate **1c**. The acidic promoter can trigger the cleavage of the trimethylsilyl group allowing the glycosylation to proceed with the alcohol function newly generated.[47–50] Nevertheless, only a very small amount of the desired galactoside **3a** (<5%) could be observed by TLC (but not isolated) although the donor **1c** was totally consumed while the silylated acceptor **2b** was largely unreacted (Table 3, entry 24). A common method for masking the basicity of a pyridine ring is the oxidation to the *N*-oxide derivative such as in **2c**.[51] Excess of promoter should not be required under these conditions and while an excess of glycosyl donor **1c** was used, only very limited amounts of the desired galactoside **3d** could be isolated (Table 3, entry 25).

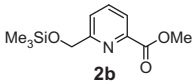
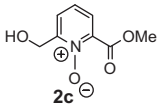
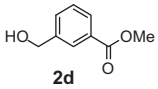
Finally, the influence of the nitrogen atom of the pyridine ring could be clearly demonstrated by studying the galactosylation of the methyl 3-hydroxymethylbenzoate acceptor **2d**.[52] When the reaction was performed under the best conditions determined earlier, a similar yield of 44% was obtained (Table 3, entry 26). Interestingly, we could isolate and identify the acetylated acceptor, namely methyl 3-acetoxymethylbenzoate, in 37% yield. Therefore, using a threefold excess of acceptor provided a very good yield of 79% (Table 3, entry 27). While this last result appeared promising, similar conditions and more specifically, the use of excess of donor did not improve the yield when the pyridine-based acceptor **2a** was used (Table 2, entry 15)

2.2. *N*-Acetylglucosaminyl donors

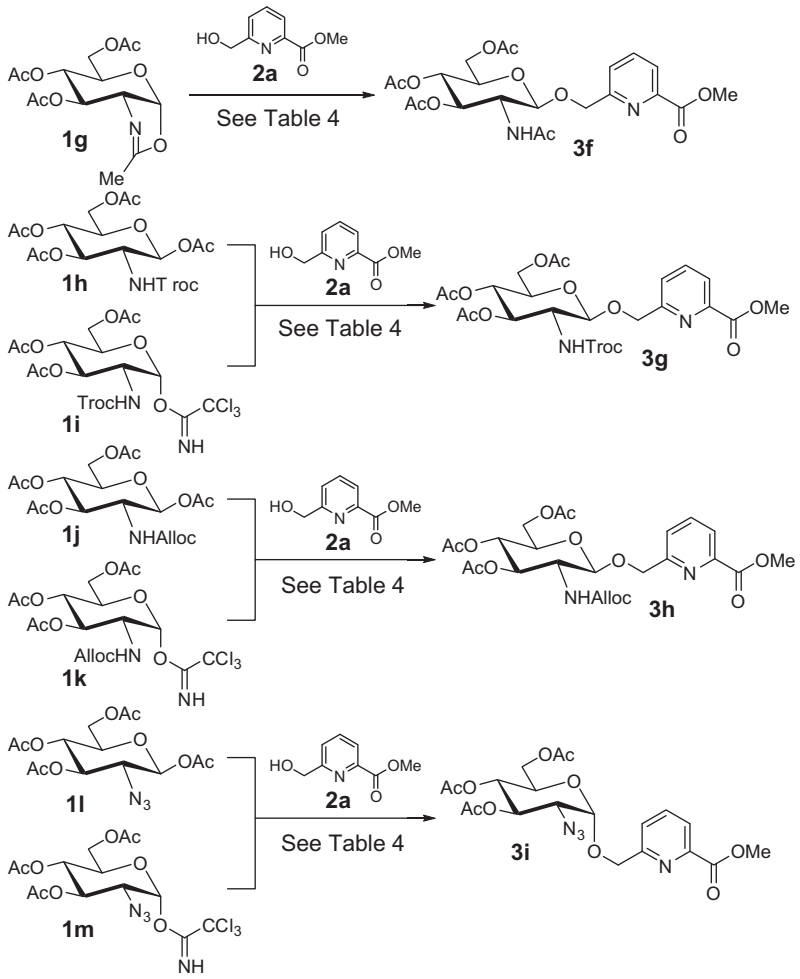
After assessing the parameters influencing the glycosylation reaction in the galactose series, we then studied the same reaction for other donors **1g–m** in the *N*-acetylglucosamine (GlcNAc) series[53–55] (Scheme 3).

The isoxazoline donor **1g**[56] is commonly employed for glycosylation in the GlcNAc series (also referred as GlcNAcylation). Even though two different promoters were used, only moderate yields could be obtained (Table 4, entries 28 and 29). The influence of the protecting group at the 2-position of GlcNAc donors is largely influencing the outcome of the reaction in terms of yields and stereocontrol. Carbamates, such as donors **1h–k**, are usually invoked for their good stereocontrol in favor of β-anomers through anchimeric participation while 2-azido-2-deoxy-derivatives **1l–m** would lead to α-anomers. The 2,2,2-trichloroethyl-carbamate (Troc) protecting group associated with an anomeric acetate leaving group in donor **1h**[57,58] improved slightly the yield of GlcNAcylation (Table 4, entry 30) to 25%. Much better results were then obtained with the more sophisticated donor **1i**[59] bearing a trichloroacetimidate as leaving group and more specifically under

Table 3
Influence of the pyridine-based acceptors **2b–d** with the galactosyl donor **1c**

Entry	Acceptor	Ratio Donor:acceptor	Temp Time	Promoter	Yield[a] (%)	Product Ratio α:β
24		1.5:1	−20 °C 3 h	TMSOTf (1 equiv) 4 Å MS	−/−[b]	**3a**
25		2.25:1	−20 °C 3 h	$BF_3{\cdot}Et_2O$ (0.6 equiv)	7	**3d**[c] 32:68
26		1.5:1	−20 °C 2 h	$BF_3{\cdot}Et_2O$ (0.2 equiv)	44[d]	**3e** β only
27		1:3	−20 °C 1 h	$BF_3{\cdot}Et_2O$ (0.2 equiv)	79	**3e** β only

[a] Values are isolated yields.
[b] Product was not isolated but identified by TLC in a very small proportion.
[c] Product was isolated as an inseparable mixture of hydroxylated donor, unreacted acceptor, α- and β-anomers. Yield was calculated excluding the unreacted **2c** and **1c** based on ^{1}H NMR data.
[d] Acetylated acceptor (methyl 6-acetoxymethylbenzoate) was also isolated in 37% yield. This observation was not made for other glycosylation conditions.



Scheme 3. Glycosylation of methyl 6-(hydroxymethyl)picolinate **2a** with donors **1g**–**m**.

activation with boron trifluoride etherate as a promoter (Table 4, entries 31 and 32) leading to an isolated yield of 66%. The introduction of an allyl-carbamate (Alloc) in donors **1j**–**k**[60,61] did not provide better yields (Table 4, entries 33 and 34).

The azido functionality is a general precursor of *N*-acetyl moieties and its use in GlcNAcylation has two consequences. The azido group is a non-participating group and, therefore, the glycoside obtained is usually the α-anomer, although some discrepancies can be observed according to specific glycosylation conditions. The need for the 2-acetamido substituent requires a subsequent reduction/acetylation process which must be included in the synthetic strategy in terms of steps but also in terms of stability to further elaboration of the target molecule. Nevertheless, these types of 2-azido-2-deoxy donors are quite useful and the peracetylated donor **1l**[62–64] was used but provided only moderate yields (Table 4, entries 35 and 36). Interestingly, a transfer of acetyl group was observed from the glycosyl donor **1l** to the acceptor **2a** affording the methyl 6-(acetoxymethyl)picolinate in 53% isolated yield (Table 4, entries 35). This result explains in part the poor yields obtained under these conditions since a good proportion of the acceptor was trapped by this acetyl transfer, along with some decomposition of the donor losing one acetate protecting group. The 2-azido-2-deoxy-glucopyranosyl trichloroacetimidate donor **1m**[65] was treated with TMSOTf as a promoter but only small amounts of the desired glycoside could be detected (Table 4, entry 37). A very good yield of 82% could then finally be obtained using boron trifluoride etherate as the promoter (Table 4, entry 38) but with a partial erosion of stereoselectivity.

The GlcNAcylation was performed using seven different donors with various protecting groups at the 2-position and under a total of eleven synthetic methodologies. The best conditions found in this series involved the use 2-azido-2-deoxy derivatives to obtain the α-anomer while the Troc carbamate was preferred for preparation of the β-anomer.

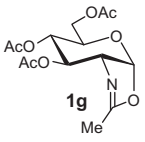
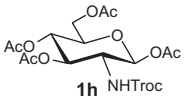
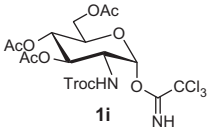
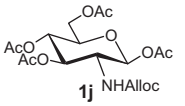
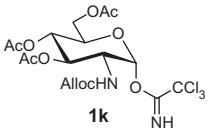
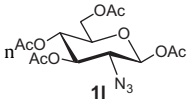
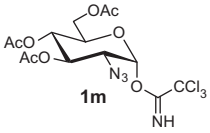
2.3. Other glycosyl donors

In order to broaden the scope of this glycosylation methodology of pyridine-based acceptors, the reaction was studied with several other glycosyl donors of monosaccharides (glucose, mannose, fucose, and rhamnose) and one disaccharide (lactose). The reactions were performed in dichloromethane usually involving a slight excess of the glycosyl donor in order to ensure maximal conversion of the acceptor to the desired glycoside (Scheme 4).

2.3.1. Glucose

Glucosyl donors were evaluated as acetylated or benzoylated trichloroacetimidates **1n**[66] and **1o**[67] respectively, and the yields obtained were good (Table 5, entries 39 and 40) as previously observed in the galactose series (Table 2, entry 13) with a slightly better yield observed in the glucose series which was somewhat unexpected. An improved yield of 85% was obtained as expected when the more reactive armed benzylated donor **1p**[68] was used although the promoter was changed to TMSOTf (Table 5, entries 41 and 42). The more sophisticated donor **1q**[69] can be used to adjust the reactivity due to the bicyclic system involving the

Table 4
Glycosylation in the GlcNAc series with methyl 6-(hydroxymethyl)picolinate **2a**

Entry	Donor	Ratio Donor:acceptor	Temp Time	Promoter	Yield[a] (%)	Product Ratio α:β
28	 **1g**	1.1:1	60 °C 5.5 h	CSA (1.2 equiv) 4 Å MS	17	**3f** β only
29		1.5:1	20 °C 22 h	$BF_3{\cdot}Et_2O$ (3 equiv) 4 Å MS	20	**3f** β only
30	 **1h**	1.1:1	−20 °C 22 h	TMSOTf (3 equiv)	25	**3g** β only
31	 **1i**	1.6:1	−2 °C 4.5 h	TMSOTf (2 equiv)	48	**3g** β only
32		1.1:1	−20 °C 3 h	$BF_3{\cdot}Et_2O$ (2 equiv)	66	**3g** β only
33	 **1j**	1.5:1	−20 °C, 3.5 h rt, 15.5 h	TMSOTf (3 equiv)	27	**3h** β only
34	 **1k**	1.1:1	−20 °C 17 h	$BF_3{\cdot}Et_2O$ (3 equiv)	48	**3h**[b] β only
35	 **1l**	1.5:1	−20 °C, 2.5 h rt, 16 h	TMSOTf (4 equiv)	<5	**3i**[c]
36		1.5:1	−20 °C, 2.5 h rt, 16 h	$BF_3{\cdot}Et_2O$ (4 equiv)	35	**3i** α only[d]
37	 **1m**	1.5:1	−20 °C 2 h	TMSOTf (2 equiv)	17	**3i** α only[d]
38		1.5:1	−20 °C, 2 h rt, 16 h	$BF_3{\cdot}Et_2O$ (4 equiv)	82	**3i** 72:28

[a] Values are isolated yields.
[b] Product was isolated as a mixture of β-anomer **3h** and 0.3 equiv unreacted acceptor **2a** and could not be separated due to similar polarity. Yield was calculated excluding the unreacted acceptor **2a** based on ^{1}H NMR data.
[c] Product could not be isolated but detected by TLC. A byproduct identified as methyl 6-(acetoxymethyl)picolinate was isolated in 53% yield.
[d] Even if the β-anomer was formed during the reaction, it was not isolated by column chromatography.

benzylidene ring. Thus, donor **1q** was reacted with 1-benzenesulfinyl piperidine (BSP) and triflic anhydride as the activating agents in the presence of 2,4,6-tri-*tert*-butylpyrimidine (TTBP) used as a base.[70] The reaction afforded in high yield (89%) the corresponding orthoester **3m** resulting from the attack of the nucleophilic alcohol moiety of acceptor **2a** on the resonating carbocation of the activated donor **1q** (Table 5, entry 43).

2.3.2. Mannose

Mannosylation is a challenging reaction, especially the formation of 1,2-*cis* mannosides with β-configuration at the anomeric center.[2,71,72] The benzoylated trichloroacetimidate mannosyl donor **1r**[73] afforded the desired α-mannoside **3n** in good yield (Table 5, entries 44 and 45). When a bicyclic and more reactive armed donor **1s**[74] was used, the β-mannoside **3o** was isolated in 68% yield (Table 5, entry 46) and with an optimal isolated of 80% when using a slight excess of acceptor **2a** although with a poorer stereoselectivity (Table 5, entry 47). The configurations at the anomeric carbon of mannosides **3n** and **3o** were clearly identified from the ^1J coupling constants measured between the anomeric carbon (C-1) and the anomeric proton (H-1) with values above 170 Hz as expected for α-mannosides versus values below 160 Hz for β-mannosides.[75]

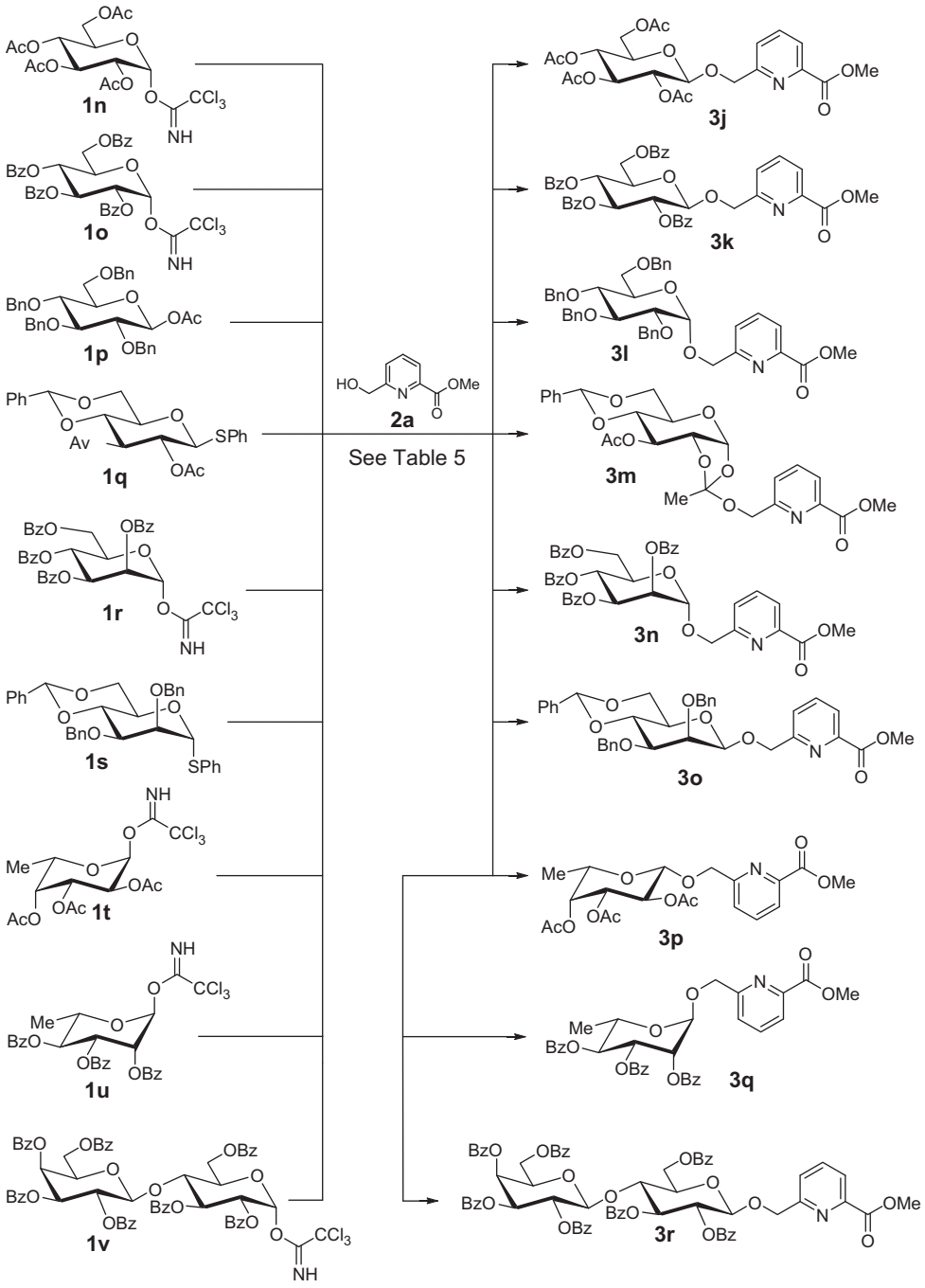
2.3.3. Fucose and rhamnose

Glycosylation using 6-deoxyhexoses such as L-fucose and L-rhamnose is also of prime importance due to the relevant biological implications of such carbohydrates in cancer and bacterial infections.[76,77] The peracetylated fucosyl trichloroacetimidate donor **1t**[78] was therefore reacted under the best conditions elaborated in the galactose series leading to a moderate isolated yield of 39% of fucoside **3p** (Table 5, entry 48). Similarly, the perbenzoylated rhamnosyl trichloroacetimidate donor **1u**[79] afforded the desired rhamnoside **3q** in 66% isolated yield using TMSOTf as a promoter (Table 5, entry 49).

2.3.4. Lactose

Finally, the perbenzoylated lactosyl trichloroacetimidate donor **1v**[80] was reacted with the acceptor **2a** using boron trifluoride etherate as a promoter and afforded the desired lactoside **3r** in 51% isolated yield (Table 5, entry 50).

Glycosylation of a pyridine-based acceptor has been investigated for a series of 22 glycosyl donors derived from glucose, galactose, mannose, fucose, rhamnose, and lactose and using a large number of activating groups at the anomeric center (e.g., acetate, bromide, tricholoracetimidate, and thiophenyl) and with a complete set of activating conditions and numerous promoters.



Scheme 4. Glycosylation of methyl 6-(hydroxymethyl)picolinate **2a** with mono- and disaccharidic glycosyl donors **1n**–**v**.

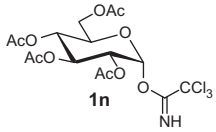
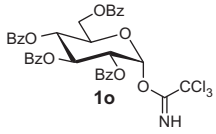
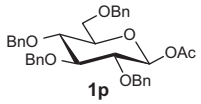
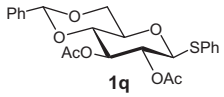
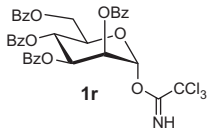
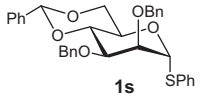
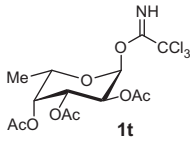
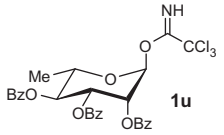
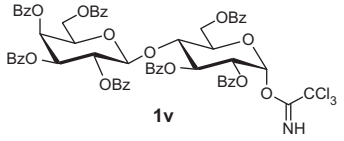
The pyridine moiety was demonstrated to have a negative influence on the reaction since glycosylation with a 'benzene-based' donor **2d** afforded high yields in comparison to the pyridine donor **2a**. Therefore, the basicity of the pyridine ring could be identified as a limitation to the outcome of the glycosylation reaction. The best conditions for the glycosylation of the model donor methyl 6-(hydroxymethyl)picolinate **2a** could therefore be identified for each type of glycosyl donor and provided isolated yields of glycosides from 46% to 85%.

3. Conclusion

The realm of glycomics is now a challenging domain of science at the interface of chemistry, biology, medical sciences, and physics or nanotechnology. A main preoccupation for chemists is the design of powerful and reliable glycosylation methodologies which are still required in order to better control the stereoselectivity of this reaction. Nevertheless, another main feature of glycosylation reaction is the fact that the reaction is catalyzed by acid promoters being Lewis or Brønsted acids. We have identified the glycosylation of pyridine-based donors as challenging. Only very few earlier studies have been reported, mainly focusing on biochemical approaches and the chemical syntheses were always quite poor in terms of yields.

Since glycosylation is usually an acid-promoted reaction, pyridine-containing acceptors are therefore competing with the acid promoter due to the intrinsic basicity of the pyridine moiety. In order to achieve good glycosylation yields, the promoter was

Table 5
Glycosylation of methyl 6-(hydroxymethyl)picolinate **2a** with mono- and disaccharidic glycosyl donors **1n–v**

Entry	Donor	Ratio Donor:acceptor	Temp Time	Promoter	Yield[a] (%)	Product Ratio α:β
39	 **1n**	1.5:1	−20 °C 22 h	$BF_3{\cdot}Et_2O$ (3 equiv)	53	**3j** β only
40	 **1o**	1.3:1	−20 °C 22.5 h	TMSOTf (2 equiv) 4 Å MS	61	**3k**[b] β only
41	 **1p**	1.3:1	−20 °C 20.5 h	$BF_3{\cdot}Et_2O$ (4 equiv)	60	**3l** α only
42		1.3:1	−20 °C 22.5 h	TMSOTf (2 equiv)	85	**3l** α only
43	 **1q**	1:1.15	−60 °C 0.5 h −78°C 3 h	BSP (1.2 equiv) TTBP (1.5 equiv) Tf_2O (1.3 equiv) 1-octene (2 equiv)	89	**3m**
44	 **1r**	1.3:1	−20 °C 22.5 h	$BF_3{\cdot}Et_2O$ (2 equiv)	48	**3n**[c] α only
45		1.3:1	−20 °C 22.5 h	TMSOTf (2 equiv)	22	**3n**[c] α only
46	 **1s**	1.3:1	−40 °C 5.5 h	TMSOTf (2 equiv) NIS (1.56 equiv)	68	**3o**[d] β only[e]
47		1:1.15	−60 °C 0.5 h −78°C 3 h	BSP (1.2 equiv) TTBP (1.5 equiv) Tf_2O (1.3 equiv) 1-octene (2 equiv)	80	3o[f] 19:81
48	 **1t**	1.5:1	−20 °C 2 h	$BF_3{\cdot}Et_2O$ (2 equiv) CH_2Cl_2	39	**3p** β only
49	 **1u**	1.3:1	−20 °C 4 h	TMSOTf (0.8 equiv)	66	**3q** α only
50	 **1v**	1:1	−20 °C 4 h	$BF_3{\cdot}Et_2O$ (2 equiv)	51	**3r** β only

[a] Values are isolated yields.
[b] Product was isolated as a mixture of β-anomer **3k** and 0.2 equiv unreacted acceptor **2a** and could not be separated due to similar polarity. Yield was calculated excluding the unreacted acceptor **2a** based on ^1H NMR data.
[c] Configuration at the α-anomeric center was confirmed by the $^1J_{C\text{-}1,H\text{-}1}$ = 172.4 Hz value.
[d] Configuration at the β-anomeric center was confirmed by the $^1J_{C\text{-}1,H\text{-}1}$ = 155.5 Hz value.
[e] Even if the α-anomer was formed during the reaction, it was not isolated by column chromatography.
[f] Configuration at the α-anomeric center was confirmed by the $^1J_{C\text{-}1,H\text{-}1}$ = 171.5 Hz value.

systematically used in excess (>1.1 equiv). Some very good results could then be obtained for the glycosylation of methyl 6-(hydroxymethyl)picolinate chosen as a model substrate for the present study. A detailed and in depth study was therefore performed in order to determine the influence of the glycosyl donor being galactose, glucose, or mannose but also the nature of the leaving group at the anomeric carbon such as halogens or trichloroacetimidates. A series of 22 glycosyl donors were tested and a reliable and efficient glycosylation could in all cases be obtained for each type of glycosylation. The present study provides a general and reliable access to 'pyridine'-containing glycosides. The best conditions for β-galactosylation were obtained using an acetylated trichloroacetimidate donor in the presence of boron trifluoride etherate, while α-galactosylation involved a benzylated 2-thiopyridyl donor. The 2-trichloroethoxycarbamate protecting group was identified as optimal for the β-glycosylation of *N*-acetylglucosamine. β-Glucosylation was readily achieved from the benzoylated trichloroacetimidate donor while α-glucosides were obtained from the benzylated 1-*O*-acetyl donor. β-Mannosylation was achieved using the benzylated thiophenyl 4,6-*O*-benzylidene-α-D-mannopyranoside donor and proved easier to perform than α-mannosylation.

The glycosides prepared here will next be conjugated with 5-aminonucleoside for the design of neutral glycosyltransferase inhibitors. Their pyridine moiety will be considered as a chelating agent for binding to the cation involved in most of the enzymatic reactions catalyzed by such enzymes while their neutral character should allow them to penetrate cells and find applications for in vivo studies. This strategy is now under investigation in our laboratory and the results will be reported in due course.

4. Experimental section

4.1. Materials and methods

All reagents were obtained from commercial sources and used without further purification. Dichloromethane and acetonitrile were distilled over CaH_2. All reactions were performed under an argon atmosphere. Thin-layer chromatography (TLC) was carried out on aluminum sheets coated with silica gel 60 F_{254} (Merck). TLC plates were inspected by UV light (λ = 254 nm) and developed by treatment with a mixture of 10% H_2SO_4 in EtOH/H_2O (1:1 v/v) followed by heating. Silica gel column chromatography was performed with silica gel Si 60 (40–63 μm). NMR spectra were recorded at 293 K, unless otherwise stated, using a Bruker 400 or 500 MHz spectrometers. Chemical shifts are referenced relative to deuterated solvent residual peaks. The following abbreviations are used to explain the observed multiplicities: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet and bs, broad singlet. Complete signal assignments were based on 1D and 2D NMR (COSY, HSQC, and HMBC correlations). High resolution (HR-ESI-QToF) mass spectra were recorded using a Bruker MicroToF-Q II XL spectrometer. Optical rotation was measured using a Perkin Elmer polarimeter at 20 °C and values are given in 10^{-1} deg cm^2 g^{-1}.

A selection of the best glycosylation conditions is reported below while the complete set of experimental procedures for Tables 2–5 and complete characterization of new compounds and copies of the ^{1}H and ^{13}C NMR data is reported in the Supporting Information of this manuscript.

4.2. (6-Methoxycarbonylpyrid-2-yl)methyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (3a)

$BF_3{\cdot}Et_2O$ (62 μL, 0.488 mmol, 2 equiv) was added dropwise to a stirred solution of **2a** (41 mg, 0.244 mmol, 1 equiv) and **1c** (169 mg, 0.366 mmol, 1.5 equiv) in freshly distilled CH_2Cl_2 (3 mL) at −20 °C. The reaction mixture was stirred at −20 °C for 2 h. Et_3N (0.2 mL) was injected to quench the reaction. The reaction mixture was concentrated and the residue was purified by silica gel column chromatography (PE/EtOAc, 9:1 to 3:2) to afford **3a** as a white foam (56 mg, 46%). R_f = 0.26 (PE/EtOAc = 1:2); $[\alpha]_D^{20}$ −14.0 (CH_2Cl_2, *c* 0.5); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 8.04 (d, *J* = 7.7 Hz, 1H, H_{Pyr}), 7.86 (t, *J* = 7.8 Hz, 1H, H_{Pyr}), 7.65 (d, *J* = 7.6 Hz, 1H, H_{Pyr}), 5.41 (d, *J* = 3.3 Hz, 1H, H_4), 5.34 (dd, *J* = 10.5, 7.9 Hz, 1H, H_2), 5.15 (d, *J* = 13.9 Hz, 1H, OCH_{2a}), 5.04 (dd, *J* = 10.4, 3.4 Hz, 1H, H_3), 4.86 (d, *J* = 13.7 Hz, 1H, OCH_{2b}), 4.65 (d, *J* = 7.9 Hz, 1H, H_1), 4.16–4.12 (m, 2H, H_{6a}, H_{6b}), 4.00 (s, 3H, OMe), 3.95 (t, *J* = 6.4 Hz, 1H, H_5), 2.16 (s, 3H, $COCH_3$), 2.05 (s, 6H, 2 × $COCH_3$), 1.99 (s, 3H, $COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 170.6 (C=O), 170.4 (C=O), 170.2 (C=O), 169.7 (C=O), 165.7 (CO_2Me), 158.3 (C_{Pyr}), 147.2 (C_{Pyr}), 138.0 (C_{PyrH}), 124.6 (C_{PyrH}), 124.2 (C_{PyrH}), 101.2 (C_1), 71.8 (OCH_2), 71.1 (C_5), 70.9 (C_3), 69.0 (C_2), 67.2 (C_4), 61.6 (C_6), 53.2 (OMe), 20.9 ($COCH_3$), 20.8 ($COCH_3$), 20.8 ($COCH_3$), 20.7 ($COCH_3$); HRMS *m*/*z*: Calcd for $C_{22}H_{28}NO_{12}$, $[M+H]^+$ 498.1606, found 498.1604.

4.3. (6-Methoxycarbonylpyrid-2-yl)methyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranoside (3c)

To a dried round bottom flask containing **1f** (618 mg, 0.896 mmol, 1 equiv), **2a** (164 mg, 0.896 mmol, 1 equiv) in CH_2Cl_2 (1 mL) was added methyl iodide (0.18 mL, 3 equiv). Then, the reaction mixture was stirred at 50 °C in the presence of 4 Å MS. After 22 h, another portion of methyl iodide (0.09 mL, 1.5 equiv) was added. After another 55 h, the reaction mixture was filtered through celite, washed with CH_2Cl_2 and evaporated to dryness. The residue was purified by silica gel column chromatography (PE/EtOAc, 4:1 to 3:1) to afford **3c** as a white foam (506 mg, 75%). R_f = 0.24 (PE/EtOAc = 3:1); $[\alpha]_D^{20}$ +46.0 (CH_2Cl_2, *c* 0.2); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 8.01 (d, *J* = 7.4 Hz, 1H, H_{Pyr}), 7.79 (d, *J* = 7.7 Hz, 1H, H_{Pyr}), 7.71 (t, *J* = 7.7 Hz, 1H, H_{Pyr}), 7.44–7.27 (m, 20H, H_{Ar}), 4.99–4.73 (m, 7H, H_1, 4 × OCH_{2a}, 2 × OCH_{2b}), 4.67 (d, *J* = 11.9 Hz, 1H, OCH_{2b}), 4.59 (d, *J* = 11.4 Hz, 1H, OCH_{2b}), 4.46 and 4.39 (2d, AB system, *J* = 11.7 Hz, 2H, OCH_{2a}, OCH_{2b}), 4.13 (dd, *J* = 10.3, 3.1 Hz, 1H, H_2), 4.05 (m, 3H, H_3, H_4, H_5), 3.99 (s, 3H, OMe), 3.62–3.55 (m, 1H, H_{6a}), 3.48 (dd, *J* = 9.1, 5.5 Hz, 1H, H_{6b}); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 165.9 (CO_2Me), 159.2, 147.3, 138.84, 138.79 (C_{PyrH}), 138.7, 138.0, 137.8, 128.6, 128.51, 128.49, 128.36, 128.27, 128.05, 128.00, 127.9, 127.8, 127.69, 127.65, 127.60, 124.9 (C_{PyrH}), 124.0 (C_{PyrH}), 98.0 (C_1), 79.2 (C_3 or C_4 or C_5), 76.7 (C_2), 75.0 (s, 2C, C_3 or C_4 or C_5, OCH_2), 73.9 (OCH_2), 73.7 (OCH_2), 73.1 (OCH_2), 70.0 (OCH_2), 69.8 (C_3 or C_4 or C_5), 68.7 (C_6), 53.1 (OMe); HRMS *m*/*z*: Calcd for $C_{42}H_{44}NO_8$, $[M+H]^+$ 690.3061, found 690.3052.

4.4. (6-Methoxycarbonylpyrid-2-yl)methyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroethoxycarbonylamino-β-D-glucopyranoside (3g)

$BF_3{\cdot}Et_2O$ (38 μL, 0.3 mmol, 1 equiv) was added dropwise into a stirred solution of **2a** (50 mg, 0.30 mmol, 1 equiv) and **1i** (206 mg, 0.33 mmol, 1.1 equiv) in freshly distilled CH_2Cl_2 (3 mL) at −20 °C under argon. After 2 h, additional $BF_3{\cdot}Et_2O$ (38 μL, 0.3 mmol, 1 equiv) was added into the reaction. After another 1 h, Et_3N (0.2 mL) was injected into the reaction mixture at −20 °C to quench the reaction. Then, the solvent was evaporated and the residue was purified by silica gel column chromatography (PE/EtOAc, 9:1 to 1:1) to afford **3g** as a white foam (124 mg, 66%). R_f = 0.32 (PE/EtOAc = 1:1); $[\alpha]_D^{20}$ −10.2 (CH_2Cl_2, *c* 1.2); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 8.03 (d, *J* = 7.4 Hz, 1H, H_{pyr}), 7.82 (t, *J* = 7.8 Hz, 1H, H_{pyr}), 7.47 (d, *J* = 7.8 Hz, 1H, H_{pyr}), 6.34 (d, *J* = 9.1 Hz, 1H, NH), 5.25–5.19 (m, 1H, H_3), 5.12 (t, *J* = 9.6 Hz, 1H, H_4), 5.06–5.03 (m, 2H, H_1, OCH_{2a}), 4.92 (d, *J* = 15.0 Hz, 1H, OCH_{2b}), 4.63 (2d, AB system, *J* = 12.1 Hz, 2H, CH_2CCl_3), 4.26 (dd, *J* = 12.3, 4.9 Hz, 1H, H_{6a}), 4.15–4.12 (m, 1H, H_{6b}), 4.02 (s, 3H, OMe), 3.94 (m, 1H, H_2), 3.72 (ddd, *J* = 9.8, 4.7, 2.2 Hz, 1H, H_5), 2.03 (s, 3H, $COCH_3$), 2.03 (s, 3H, $COCH_3$), 2.02 (s, 3H, $COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 170.9 (C=O), 170.8 (C=O), 169.5 (C=O), 165.5 (C_{pyr}), 156.0 (C=O), 154.9 (C=O), 147.2 (C_{pyr}), 137.9 (C_{pyrH}), 124.6 (C_{pyrH}), 124.2 (C_{pyrH}), 101.5 (C_1), 95.6 (CCl_3), 74.5 (CH_2CCl_3), 72.9 (C_3), 72.3 (C_5), 70.3 (OCH_2), 68.5 (C_4), 62.2 (C_6), 56.3 (C_2), 53.2 (OMe), 20.9 ($COCH_3$), 20.8 ($COCH_3$), 20.7 ($COCH_3$); HRMS *m*/*z*: Calcd for $C_{23}H_{28}Cl_3N_2O_{12}$, $[M+H]^+$ 629.0702, found 629.0697.

4.5. (6-Methoxycarbonylpyrid-2-yl)methyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside (3k)

The trichloroacetimidate **1o** (87 mg, 0.117 mmol, 1.3 equiv) was dissolved in freshly distilled CH_2Cl_2 (5 mL) under argon in the pres-

ence of 4 Å MS. Then, the mixture was cooled down to −20 °C. TMSOTf (3.3 μL, 0.018 mmol, 0.2 equiv) was added at −20 °C, then **2a** (15 mg, 0.090 mmol, 1 equiv) dissolved in 1.5 mL of freshly distilled CH_2Cl_2 was added. The reaction mixture was stirred at −20 °C for 30 min. Another 3.3 μL of TMSOTf (0.2 equiv) was added. After 1 h, another portion of TMSOTf (3.3 μL, 0.018 mmol, 0.2 equiv) was added. After 2.5 h, TMSOTf (6.6 μL, 0.039 mmol, 0.4 equiv) was added. Then, after 2 h, another 1 equiv of TMSOTf (16.5 μL) was added and the reaction mixture was stirred at −20 °C for another 16 h. Et_3N (0.2 mL) was injected at −20 °C to quench the reaction. The reaction mixture was filtered and washed with CH_2Cl_2. The solvent was evaporated, then, the residue was purified by silica gel column chromatography (PE/EtOAc, 9:1 to 7:3) to afford **3k** as a white foam (41 mg, 61%). R_f = 0.29 (PE/EtOAc = 2:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 7.98–7.15 (m, 23H, H_{Ar}), 5.85 (t, J = 9.7 Hz, 1H, H_3), 5.69–5.55 (m, 2H, H_2, H_4), 5.07 (d, J = 14.3 Hz, 1H, OCH_{2a}), 4.94 (d, J = 7.8 Hz, 1H, H_1), 4.87 (d, J = 14.3 Hz, 1H, OCH_{2b}), 4.55 (dd, J = 12.2, 2.9 Hz, 1H, H_{6a}), 4.41 (dd, J = 12.2, 5.2 Hz, 1H, H_{6b}), 4.16–4.08 (m, 1H, H_5), 3.90 (s, 3H, OMe); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 166.2, 165.9, 165.6, 165.3, 165.2, 158.3, 147.0, 137.7, 133.6, 133.5, 133.4, 133.3, 129.92, 129.91, 129.86, 129.6, 129.2, 128.82, 128.79, 128.53, 128.51, 128.4, 124.9, 124.1, 101.2 (C_1), 72.9, 72.5, 72.2, 72.0, 69.6, 63.1, 53.1; HRMS m/z: Calcd for $C_{42}H_{36}NO_{12}$, $[M+H]^+$ 746.2232, found 746.2221.

4.6. (6-Methoxycarbonylpyrid-2-yl)methyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside (3l)

To a solution of **1p** (136 mg, 1.3 equiv) and **2a** (30 mg, 1 equiv) in 10 mL freshly distilled CH_2Cl_2 at −20 °C was added TMSOTf (6.5 μL, 0.2 equiv). The reaction was stirred for a total of 22.5 h, and the addition of TMSOTf was according to the following: 6.5 μL (0.2 equiv) after 0.5 h, 19.5 μL (0.6 equiv) after 2 h, 33 μL (1 equiv) after 3.5 h, and finally another 33 μL (1 equiv) after 6 h for a total of 2 equiv. After 16.5 h the reaction was complete, the reaction mixture was diluted with CH_2Cl_2 (25 mL) and washed with satd aq $NaHCO_3$ (2 × 25 mL). The aqueous layers were combined and extracted with CH_2Cl_2 (2 × 25 mL) after which the combined organic layers were washed with brine (30 mL), dried (Na_2SO_4) and concentrated. The crude product was purified by column chromatography (PE/EtOAc, 2:1) to yield **3l** as a colorless oil (105 mg, 85%). R_f = 0.31 (PE/EtOAc = 2:1); $[\alpha]_D^{20}$ +18.2 (CH_2Cl_2, c 3.6); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 8.05 (dd, J = 7.7 Hz, 1.1 Hz, 1H, H_{Pyr}), 7.82 (dd, J = 7.9 Hz, 1.1 Hz, 1H, H_{Pyr}), 7.79 (dd, J = 7.9 Hz, 7.7 Hz, 1H, H_{Pyr}), 7.45–7.10 (m, 20 H, H_{Ar}), 5.05 (d, J = 10.9 Hz, 1H, OCH_2Ph), 4.98 (d, J = 3.6 Hz, 1H, H_1), 4.96 (d, J = 14.5 Hz, 1H, OCH_{2aPyr}), 4.90 (d, J = 10.9 Hz, 1H, OCH_2Ph), 4.84 (d, J = 11.9 Hz, 1H, OCH_2Ph), 4.80 (d, J =14.5 Hz, 1H, OCH_{2bPyr}), 4.67 (d, J = 10.7 Hz, 1H, OCH_2Ph), 4.64 (d, J = 12.1 Hz, 1H, OCH_2Ph), 4.52 (d, J =10.7 Hz, 1H, OCH_2Ph), 4.52 (d, J = 11.9 Hz, 1H, OCH_2Ph), 4.49 (d, J = 12.1 Hz, 1H, OCH_2Ph), 4.11 (dd, J = 9.7 Hz, 9.0 Hz, 1H, H_3), 4.01 (s, 3H, OCH_3), 3.86 (ddd, J = 10.1 Hz, 3.1 Hz, 2.0 Hz, 1H, H_5), 3.77 (dd, J = 10.7 Hz, 3.1 Hz, 1H, H_{6a}), 3.77 (dd, J = 10.1 Hz, 9.0 Hz, 1H, H_4), 3.68 (dd, J = 9.7 Hz, 3.6 Hz, 1H, H_2), 3.64 (dd, J = 10.7 Hz, 2.0 Hz, 1H, H_{6b}); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 165.7 (CO_2Me), 158.8 (C_{Pyr}), 147.3 (C_{Pyr}), 137.6 (C_{Pyr}), 138.8, 138.2, 137.7, 138.8, 128.5, 128.4, 128.1, 128.0, 127.9, 127.8, 127.7, 124.7 (C_{Pyr}), 124.0 (C_{Pyr}), 97.2 (C_1), 82.1 (C_3), 80.1 (C_2), 77.6 (C_4), 75.8 (OCH_2Ph), 75.2 (OCH_2Ph), 73.6 (s, 2C, OCH_2Ph, OCH_2Ph), 70.8 (C_5), 69.8 (OCH_2Pyr), 68.3 (C_6), 53.0 (OCH_3); HRMS m/z: Calcd for $C_{42}H_{44}NO_8$ $[M+H]^+$ 690.3061, found 690.3047; $C_{42}H_{43}NO_8Na$ $[M+Na]^+$ 712.2861, found 712.2881; $C_{42}H_{43}NO_8K$ $[M+K]^+$ 728.2620, found 728.2615.

4.7. (6-Methoxycarbonylpyrid-2-yl)methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranoside (3o)

To a solution of **1s** (126 mg, 1.3 equiv) and **2a** (30 mg, 1 equiv) in 10 mL freshly distilled CH_2Cl_2 at −40 °C was added NIS (63 mg, 1.56 equiv) and TMSOTf (6.5 μL, 0.2 equiv). The reaction was stirred for a total of 5.5 h, and the addition of TMSOTf was according to the following: 6.5 μL (0.2 equiv) after 1 h, 19.5 μL (0.2 equiv) after 2 h, and finally another 33 μL (1 equiv) after 3 h, for a total of 2 equiv. After 2.5 h the reaction was complete, the reaction was quenched by adding satd aq $NaHCO_3$ (10 mL). The reaction mixture was diluted with CH_2Cl_2 (50 mL), warmed to room temperature and washed with satd aq $NaHCO_3$ (2 × 50 mL). The aqueous layers were combined and extracted with CH_2Cl_2 (2 × 50 mL), after which the combined organic layers were washed with brine (50 mL), dried (Na_2SO_4) and concentrated. The crude product was purified by column chromatography (PE/EtOAc, 2:1) to yield **3o** as a slightly yellow amorphous solid (73 mg, 68%). R_f = 0.38 (hexane/EtOAc = 1:1); $[\alpha]_D^{20}$ −111.1 (CH_2Cl_2, c 1.8); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 8.00 (dd, J = 7.7 Hz, 1.0 Hz, 1H, H_{Pyr}), 7.79 (dd, J = 7.8 Hz, 7.7 Hz, 1H, H_{Pyr}), 7.56 (dd, J = 7.8 Hz, 1.0 Hz, 1H, H_{Pyr}), 7.50–7.20 (m, 15 H, H_{Ar}), 5.58 (s, 1H, 4,6-O*CH*Ph), 5.10(d, J = 13.9 Hz, 1H, O*CH*$_2$Pyr), 4.97 (d, J = 12.2 Hz, 1H, O*CH*$_2$Ph), 4.90 (d, J = 12.2 Hz, 1H, O*CH*$_2$Ph), 4.82 (d, J = 13.9 Hz, 1H, O*CH*$_2$Pyr), 4.70 (d, J = 12.4 Hz, 1H, O*CH*$_2$Ph), 4.59 (d, J = 12.4 Hz, 1H, O*CH*$_2$Ph), 4.58 (s, 1H, H_1), 4.24 (dd, J = 10.5 Hz, 4.8 Hz, 1H, H_{6a}), 4.21 (dd, J = 9.9 Hz, 9.3 Hz, 1H, H_4), 4.00 (d, J = 3.0 Hz, 1H, H_2), 3.95 (s, 3H, OCH_3), 3.88 (dd, J = 10.5 Hz, 10.1 Hz, 1H, H_{6b}), 3.60 (dd, J = 9.9 Hz, 3.0 Hz, 1H, H_3), 3.32 (ddd, J = 10.1 Hz, 9.3 Hz, 4.8 Hz, 1H, H_5). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 165.6 (CO_2Me), 158.6 (C_{Pyr}), 147.2 (C_{Pyr}), 137.8 (C_{Pyr}), 138.4, 138.3, 137.6, 128.9, 128.7, 127.7, 127.6, 126.1, 124.7 (C_{Pyr}), 124.0 (C_{Pyr}), 101.8 (C_1, $^1J_{C\text{-}1,\ H\text{-}1}$ = 155.5 Hz), 101.5 (4,6-O*C*HPh), 78.7 (C_4), 78.0 (C_3), 76.1 (C_2), 75.0 (OCH_2Ph), 72.6 (OCH_2Ph), 72.0 (OCH_2Pyr), 68.2 (C_6), 67.7 (C_5), 53.0(OCH_3); HRMS m/z: Calcd for $C_{35}H_{36}NO_8$ $[M+H]^+$ 598.2441, found 598.2432; $C_{35}H_{35}NO_8Na$ $[M+Na]^+$ 620.2260, found 620.2264; $C_{35}H_{35}NO_8K$ $[M+K]^+$ 636.2000, found 636.2042.

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Supplementary data

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