Journal Pre-proof

Chemical synthesis of 5'-*β*-glycoconjugates of vitamin B_6

Thomas Bachmann, Christian Schnurr, Laura Zainer, Michael Rychlik

PII: S0008-6215(19)30722-0

DOI: https://doi.org/10.1016/j.carres.2020.107940

Reference: CAR 107940

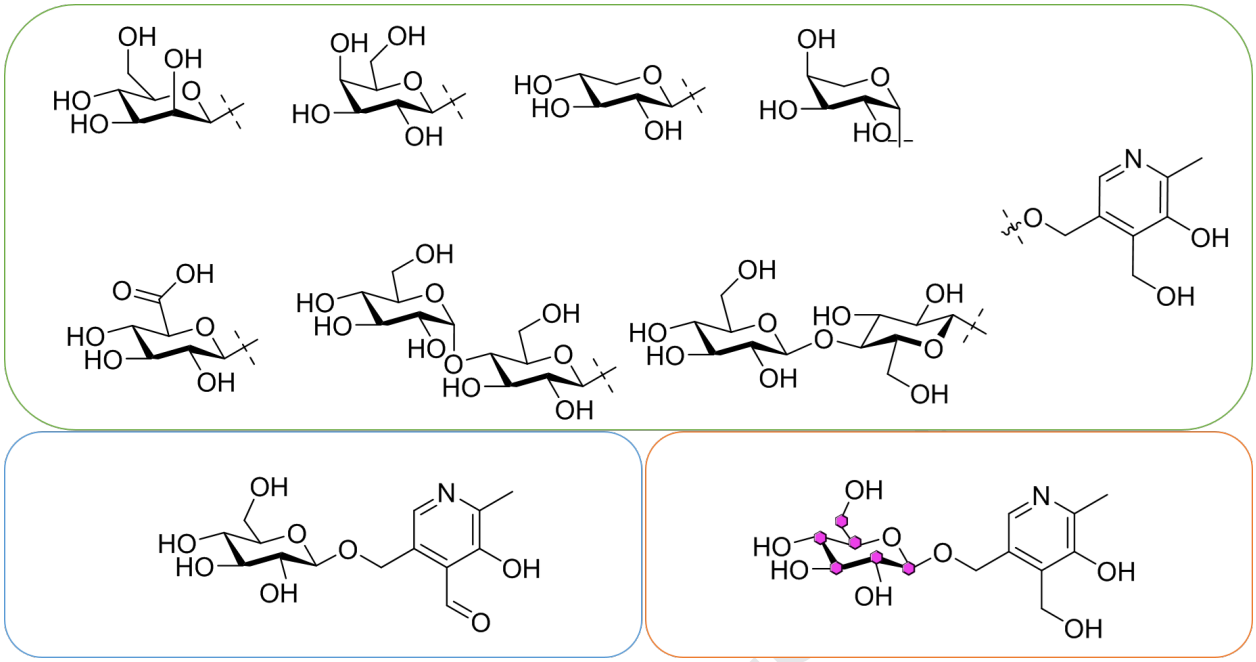
To appear in: *Carbohydrate Research*

Received Date: 10 December 2019

Revised Date: 3 February 2020

Accepted Date: 4 February 2020

Please cite this article as: T. Bachmann, C. Schnurr, L. Zainer, M. Rychlik, Chemical synthesis of 5'-*β*-glycoconjugates of vitamin B_6, *Carbohydrate Research* (2020), doi: https://doi.org/10.1016/j.carres.2020.107940.



Chemical synthesis of 5'-β-glycoconjugates of vitamin B_6

Thomas Bachmann [1,*], Christian Schnurr [1], Laura Zainer [1] and Michael Rychlik [1]

[1] Chair of Analytical Food Chemistry, Technical University of Munich, Maximus-von-Imhof-Forum 2, 85354 Freising, Germany; thomas.bachmann@tum.de; michael.rychlik@tum.de; laura-zainer@online.de; christian.schnurr@tum.de

*correspondence: thomas.bachmann@tum.de

Abstract: Various 5'-β-saccharides of pyridoxine, namely the mannoside, galactoside, arabinoside, maltoside, cellobioside and glucuronide, were synthesized chemically according to KOENIGS-KNORR conditions using α4,3-*O*-isopropylidene pyridoxine and the respective acetobromo glycosyl donors with AgOTf (3.0 eq.) and NIS (3.0 eq.) as promoters at 0 °C. Furthermore, 5'-β-[$^{13}C_6$]-labeled pyridoxine glucoside (PNG) was prepared starting from [$^{13}C_6$]-glucose and pyridoxine. Additionally, two strategies were examined for the synthesis of 5'-β-pyridoxal glycoside (PLG).

Keywords: Glycosylation, Chemical Synthesis, Labeled, Glycoconjugates, Pyridoxine, Pyridoxal, Vitamin B_6, PNG, PLG.

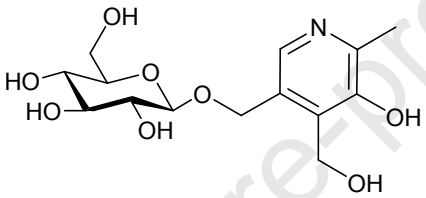
1. Introduction

The formation of glycoconjugates belongs to the most important mechanisms of post-translational modification and takes place in various species and cell types.[1-9] Hence, it is highly relevant to a number of medical fields, *e.g.* topics regarding the nervous[10, 11] or immune system[12-14], alcoholism[15], inflammation[16], apoptosis[17] and cancer[12, 18, 19]. Fascinatingly, merely ten nucleotides suffice as building blocks in the manufacturing of the mammalian glycome in its plethora of substrates.[2, 20]

Although glycoconjugates find purpose in a manifold of different aspects of biological processes, the knowledge about the full spectrum of their impact still resides in its infancy. In plants, glycosylation fulfills not only a function of storage, but shows a significant influence on the signaling processes in cells, where the complexity of the information transfer results from the structural variety of glycans.[21-29]

When glycosylations are carried out in plants that contribute to the human diet, glycoconjugates become a subject of food-related consumers' health.[30-33] Wheat, infected with fungi of the genus *Fusarium*, was found to detoxify the mycotoxin deoxynivalenol (DON) by converting it into the less toxic deoxynivalenol-3-glucoside (D3G).[34, 35] The

assessment of the mycotoxin contamination in food, therefore, must include the modified mycotoxin D3G, since cleavage of the glycosidic bond during digestions releases the free toxin DON.[31-33, 36-41] What is crucial for the analytics of toxic substances also applies to beneficial secondary metabolites. Micronutrients like vitamins occur in glycosylated forms, as well, which affects the bioavailability in the human body.[30] The group of vitamin B_6 constitutes an interesting example hereby, since it consists of six *in vivo* interconvertible[42, 43] vitamers, namely pyridoxine (PN), pyridoxal (PL), pyridoxamine (PM) and their respective phosphorylated compounds pyridoxine 5'-phosphate (PNP), pyridoxal 5'-phosphate (PLP) and pyridoxamine 5'-phosphate (PMP), and an additional glucosylated derivative, pyridoxine-5'-*β*-D-glucoside (5'-*β*-PNG, Figure 1), which can be found as a major part of the B_6 content (5 - 70 %) in plants.[44-49]



Pyridoxine-5'-*β*-glucoside (PNG)

Figure 1. Plants store vitamin B_6 as pyridoxine-5'-*β*-D-glucoside.

PNG was first identified in 1977 after its isolation from rice bran and later prepared by chemical synthesis according to KOENIGS-KNORR conditions implementing *α*4,3-*O*-isopropylidene pyridoxine, 2,3,4,6-tetra-*O*-acetyl-*α*-D-glucopyranosyl bromide and silver carbonate.[50] Other strategies regarding the chemical preparation of this molecule are scarce and numerous methods rely on biotechnological techniques. Here, the two main paths being followed comprise either the assistance of isolated enzymes[47, 51, 52] or the utilization of seeds.[53-58] Regarding the latter, alfalfa seeds present a convenient way to produce unlabeled PNG with yields ranging from 35 to 60 % and also allow the synthesis of [^{2}H]- and [^{3}H]-*β*-PNG.[57, 59-62]

B_6 in its active form (PLP) functions as a cofactor in a variety of processes in the human metabolism.[63] Consequently, the relation between the amount of modified vitamin in food and the total B_6 intake includes important information regarding the impact on the nutrition. Studies with deuterated PNG suggested, that the bioavailability is substantially higher in humans (50 - 58 % relative to PN)[64, 65] than in rats (20 - 30 %).[57, 66]

Although PNG remains in the spotlight of research because of its abundant occurrence[49], other glycoconjugated derivatives of PN were also found in nature. More than 50 % of the glycosylated compounds in rice bran were found to be composed of

5'-*O*-(*β*-cellobiosyl) pyridoxine, 4'-*O*-(*β*-D-glucosyl)-5'-*O*-(*β*-cellobiosyl) pyridoxine and 5'-*O*-(*β*-glucotriosyl) pyridoxine.[67] Additionally, 38 % of the total PN-content in rice bran were assigned to 5'-*O*-[6-*O*-((+)-5-hydroxy-dioxindole-3-acetyl)-*β*-cellobiosyl] pyridoxine after enzymatic and chemical hydrolysis procedures.[68] In wheat bran, popped pea and soybeans, another compound, namely B_6X, exists in small amounts. Although the structure could not be derived from the isolate, treatment of the latter with alkaline solution and *β*-glycosidase released vitamin B_6, which indicates a glycosylated derivate.[69, 70] Further studies revealed, that podded pea *Pisum sativum L*[71, 72] produces 5'-*O*-[6-*O*-(3-hydroxy-3-methyl-4-carboxybutanoyl)-*β*-D-glucopyranosyl)]-pyridoxine (HM GPNG) and 5‘-*O*-(6-*O*-malonyl-*β*-D-glucopyranosyl)-pyridoxine (MalonylPNG) during germination. Furthermore, the potential occurrence of PN-oligosaccharides in potatoes was proposed, but not further specified.[73]

Identification of new PN-saccharides proceeded not only through the process of isolation, but was also accomplished *via* biotechnological approaches. Experiments with an isolated marine exo-*α*-glucosidase (EC 3.2.1.20) from the anaspidean mollusk *Aplysia fasciata* showed transglycosylating properties and were successfully utilized in the preparation of 4’-/5’-*α*-PNG and the respective isomaltosides.[74] Suzuki et al. found a glucuronic acid-like derivative while incubating a mixture of uridine 5’-diphosphoglucuronyltranserfase from rabbit liver, uridine phosphoglucuronic acid and pyridoxine.[75] The three galactosyl conjugates 5'-*O*-(*β*-D-galactopyranosyl)-pyridoxine, 4'-*O*-(*β*-D-galactopyranosyl)-pyridoxine and 4'-*O*-(*β*-*O*-galactopyranosyl-(1→4)-*β*-D-galactopyranosyl)-pyridoxine were reportedly produced in a culture of *Sporobolomyces singularis* growing on a medium containing 5 % lactose, 0.75 % yeast extract and 2 % pyridoxine.[76, 77] Moreover, two fructose derivatives, 5'-*O*-(*β*-D-fructofuranosyl)-pyridoxine and 5'-*O*-[*β*-D-fructofuranosyl-(2→l)-*β*-D-fructofuranosyl]-pyridoxine, were detected in a culture medium of *Aspergillus niger* and *A. sydowi* containing sucrose and pyridoxine.[78] While examining a series of glycosidases for their synthetic potential regarding pyridoxine as a substrate, 4’-/5’-*O*-(2-acetamido-2-deoxy-*β*-D-glucopyranosyl) pyridoxine, 5’-*α*- and 4’-/5’-*O*-(*β*-D-galactopyranosyl) pyridoxine and 4’-/5’-*O*-(*α*-D-mannopyranosyl) pyridoxine were prepared using 4-nitrophenyl-2-acetamido2-deoxy-*β*-D-glucopyranoside, 4-nitrophenyl-*β*-D-galactopyranoside and mannose as sugar donors.[79]

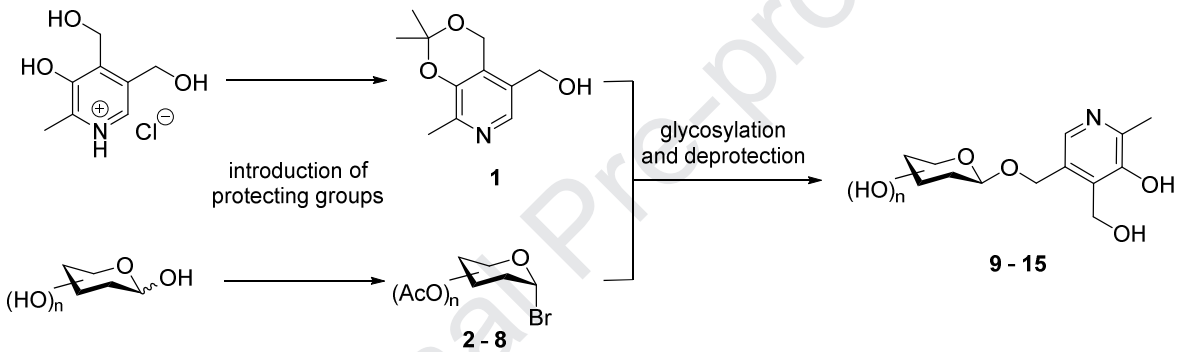
Although a variety of studies were dedicated to the isolation and identification of a manifold of glycoconjugated derivatives of PN, indications about their related content in

food, in particular when PN-glucoside is excluded, are sparsely sown. Therefore, with raising awareness of the relevance of glycosylated natural compounds in the scientific field, the present study pursued to expand the catalogue of known PN-saccharides *via* chemical synthesis and pave the way for further analytical studies revolving around their occurrence in food.

2. Results and discussion

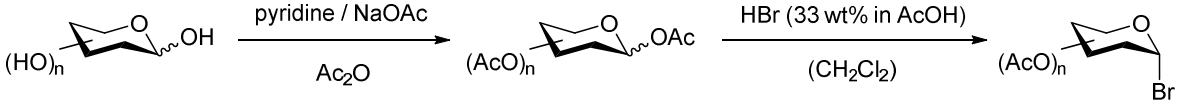
2.1. Synthesis of the starting materials

In order to fulfill this task, glycosyl donors and the acceptor had to be prepared as starting materials, first, before implementing them into glycosylation reactions (Scheme 1).



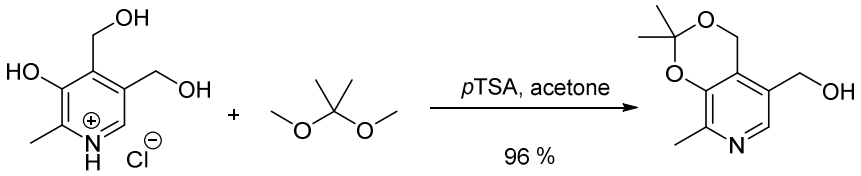
Scheme 1. General synthetic strategy for the preparation of the PN-saccharides.

The preparation of the glycosyl donors started with the installation of protection groups. Hereby, acetate groups were chosen because of their neighbouring group effect.[80-82] The anomeric outcome of the acetylation reaction was directed using either pyridine (py, α-Glc) or sodium acetate (β-Glc) (Scheme 2).[83] Since glucuronidation constitutes an important phase II metabolic pathway for a broad spectrum of substances, the preparation of glucuronides was enclosed in this study.[84-91] Acetylated glucuronic methyl ester was prepared from glucuronic acid γ-lactone.[92] Installation of bromine as leaving group was accomplished with hydrogen bromide (HBr).[93-95]



Scheme 2. General depiction of the preparation process for the saccharide donors involving an acetylation followed by a bromination step.

The selective protection of pyridoxine was undertaken with 2,2-dimethoxypropane and *p*-toluenesulfonic acid (*p*TSA) in order to increase the regioselectivity towards the desired 5'-position and to allow the solubility of PN in organic solvents (**1**, 96 %, Scheme 3).

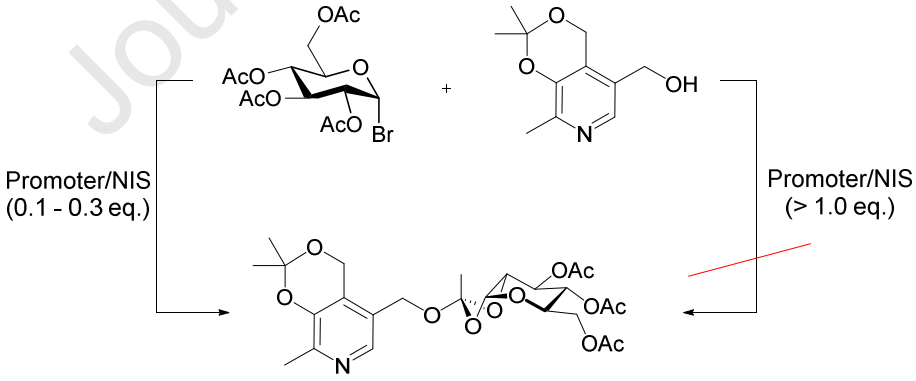


1

Scheme 3. The protection of pyridoxine was accomplished with 2,2-dimethoxypropane and *p*TSA in acetone.

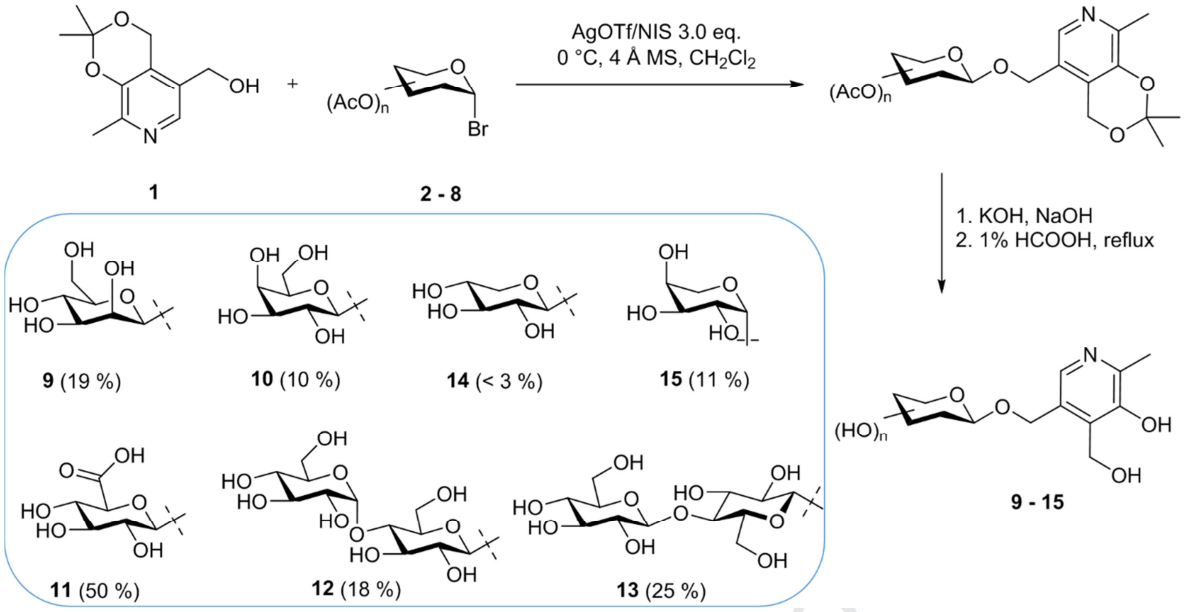
2.2. Glycosylation reactions

The preparation of the glycosides followed the guidelines of KOENIGS-KNORR conditions[96], but was performed with an increased amount of promoter AgOTf/NIS (3.0 eq.). The application of NIS, although presenting a well-established additive with regards to the reaction of thioglycosides, usually doesn't find utilization under KOENIGS-KNORR conditions. Preliminary experiments revolving around the glucosylation of PN under these conditions, however, resulted exclusively in the formation of orthoester-structures when low amounts (0.1 - 0.3 eq.) of promoter (AgOTf, Ag_2CO_3, Ag_2O, $Cu(OTf)_2$, TMSOTf, $BF_3 \cdot OEt_2$) were used in combination with NIS.



Scheme 4. Preliminary experiments resulted exclusively in the orthoester formation, when low amounts of promoter were added.

Additionally, no conversion of the starting material was observed when NIS was missing from the reaction, suggesting an NIS-mediated formation of the orthoester. Interestingly, an increased amount of promoter/NIS (3.0 eq.) resulted both in a complete shift from orthoester structure to the desired glycoconjugate and furthermore in the selective formation of the *β*-epimer. Hence, the glycosylation of PN with various glycosyl donors was performed using 3.0 eq. of AgOTf/NIS in order to obtain the protected glycosides (Scheme 4).



Scheme 5. The glycosylation reaction for the preparation of the PN-saccharides was undertaken based on KOENIGS-KNORR conditions followed by a two-step deprotection procedure. The listed yields refer to the deprotected glycosides obtained after three steps (glycosylation and deprotection).

The robustness of the acetonide group in basic milieu and stability of the acetate groups towards acidic conditions demanded a two-step procedure for the subsequent removal of the protection groups. Thus, the acetate groups were cleaved by application of 1 N KOH/NaOH first, followed by reflux in 1 % HCOOH in order to deprotect the acetonide functionality. Following this procedure, PN-saccharides containing mannose **9**, galactose **10**, glucuronic acid **11**, maltose **12**, cellobiose **13**, xylose **14** and arabinose **15** were synthesized with a selective anomeric (β) outcome, which was verified through NMR experiments.

The deprotected glycosides were obtained after three steps (glycosylation and deprotection) in approximately the same range of yields (10 - 25 %) except for PN-xyloside (< 3 %) and PN-glucuronide (50 %). The low yield of the xyloside arose from a stronger tendency to decomposition resulting from a higher instability of the molecule compared to the other saccharides. This aspect was particularly salient in NMR measurements of the glycoconjugate (which was purified by preparative HPLC and confirmed by ESI-MS beforehand), where the signals of pyridoxine and xylose overshadowed those of the glycoside. Storing the substrate at lowered temperatures (− 28 °C) did not improve this aspect. By contrast, the preparation of the glucuronide worked well and resulted in the highest yield among the reacted sugar donors. Overall, most losses occurred during the glycosylation reaction rather than the deprotection steps. Preliminary experiments including the variation of reaction conditions, next to promoter quantity and type, such as temperature (− 78, − 30, 0 °C or rt) and time, although successfully shifting the formation of orthoester

structures completely towards the *β*-epimer of the desired product, didn't manage to improve the reaction outcome over a certain point, qualifying the chosen reaction conditions (3.0 eq. AgOTf/NIS; 0 °C; CH_2Cl_2) as best variant using PN as substrate under KOENIGS-KNORR conditions. On the other hand, there consequently lies much potential for improvement with regards to aspects like increased reaction outcome in the variation of the glycosylation method and exploration of further leaving groups.

The literature mainly focuses on the synthesis of the abundantly present PN-glucoside. Only few preparations of other PN-saccharides are reported, among them mostly *via* biotechnological methods. Charles and Divakar optimized reaction conditions for the preparation of PN-glucoside (36 ± 10 %), -mannoside (23 ± 10 % of which being 4'*α*/5'*α*/5'*β* = 24/34/42) and -galactoside (40 ± 10 % of which being 4'*α*/5'*α*/5'*β* = 47/27/26) with the aid of *β*-glucosidase from sweet almonds starting from the respective saccharides, but struggled with bad regio- and anomeric selectivity resulting in a mixture of 4'/5'-saccharides and *α*/*β*-anomers.[52] Weignerova et al. utilized specific glycosidases and could improve the regioselectivity. Here, 4'/5'-*β*-PN-galactosides were obtained in 32 % yield (related to the sugar donor) utilizing *β*-galactosidase from *A. oryzae* and 4'/5'-*α*-PN-mannosides were received in 2 % yield (also related to the sugar donor) with mannosidase from *Canavalia ensiformis* (jack beans).[79] Tadera et al. isolated 53 mg of 5'-*β*-PN-cellobioside from 10 kg defatted rice.[67] The yields of the chemical synthesis proposed in the study presented here aligned with the results from the literature, but laborious separation of regio-isomers could be omitted since 5'-selectivity was promoted through the acetonide protecting group. Furthermore, contrary to many biotechnological strategies, upscaling constitutes a simple and non-time-consuming operation because the starting materials are inexpensive and easily prepared. The remaining PN-saccharides (glucuronide, maltoside, xyloside and arabinoside) have not been reported in the literature to date.

In order to evaluate fragmentation patterns of the PN-saccharides and to affirm their structure additionally to NMR-experiments, the deproteced PN-saccharides were measured *via* ESI-MS (Figure 2). Protonated PN-maltoside (**12**, m/z 494, **A**) dissociated through loss of a glucose moiety (m/z 314), followed by loss of another glucose fragment (m/z 170) and water (m/z 152). Protonated PN-cellobioside (**13**, m/z 494, **B**) showed a similar pattern, confirming the disaccharidic structure of the product. Fragmentation of protonated PN-glucuronide (**11**, m/z 346, **C**) resulted in the loss of water (m/z 328) and loss of the glucuronic moiety (m/z 152). Further fragmentation steps of PN (m/z 108) are detailed elsewhere.[97] The ESI-MS spectrum of protonated PN-arabinoside (**15**, m/z 302, **D**)

showed fragments after the loss of water (m/z 284), the sugar moiety (m/z 152) and further fragmentations (m/z 108).

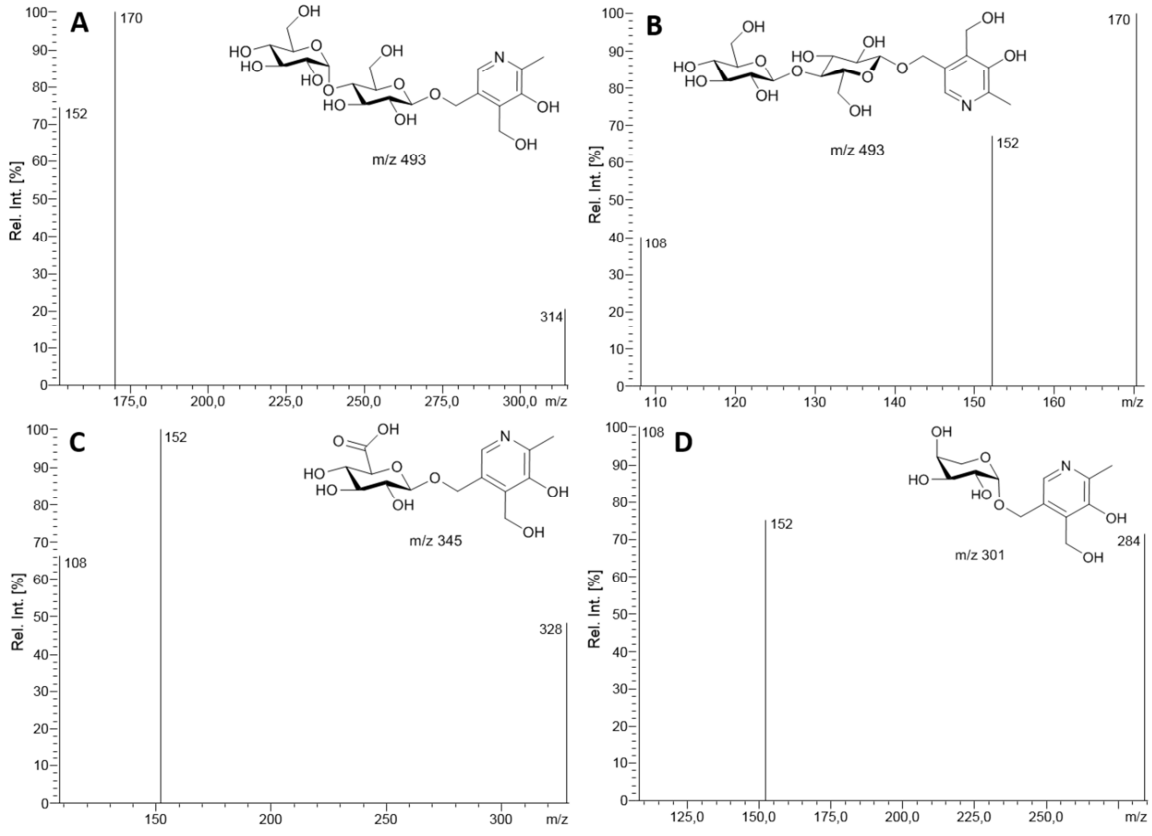


Figure 2. LC-ESI(+)-MS/MS spectra of PN-maltoside (**12**, **A**, collision energy (CE) = − 20 V), PN-cellobioside (**13**, **B**, CE = − 21 V), PN-glucuronide (**11**, **C**, CE = − 25 V) and PN-arabinoside (**15**, **D**, CE = − 23 V).

2.3. Synthesis of 5'-β-pyridoxal glucoside (5'-β-PLG)

An additional branch of this study was directed towards the question, whether other vitamers of the B_6 group occur as glycosylated derivatives in nature. Since PLP plays an important role as a cofactor in many enzymatic reactions in the human metabolism[63, 98], PL was chosen as the target substrate. The vitamer shows the same solubility properties as PN, thus being insoluble in organic liquids. For solving this problem, the strategy outlined above involving the installation of an acetonide group was not available due to the aldehyde group. Masking the latter with imine functionalities[99, 100] enhanced the solubility of the molecule in dichloromethane, but did not result in the formation of product during glycosylation reactions further down the road. Hence, the first step revolved around a solubility study, where the hydrochloric salt (HCl) of PL was suspended in various solvents suitable for glycosylation reactions. In accordance to unprotected PN, no dissolution of the white solid was observed, and the thought arose, whether and to what extent the HCl-salt was inhibiting a proper dissolution in certain solvents. Since vitamin B_6 is based on a pyridine

moiety, a stronger non-nucleophilic base was employed in order to test this hypothesis. Upon addition of imidazole (1.1 eq.), the solid completely dissolved in dimethyl sulfoxide and dimethylformamide (DMF), mediocrely in dichloromethane (DCM) and no dissolution was observed in diethyl ether (Et_2O), tetrahydrofuran (THF) and toluene (Tol) (Figure 3). Additional imidazole did not result in an improved solubility in the other solvents.

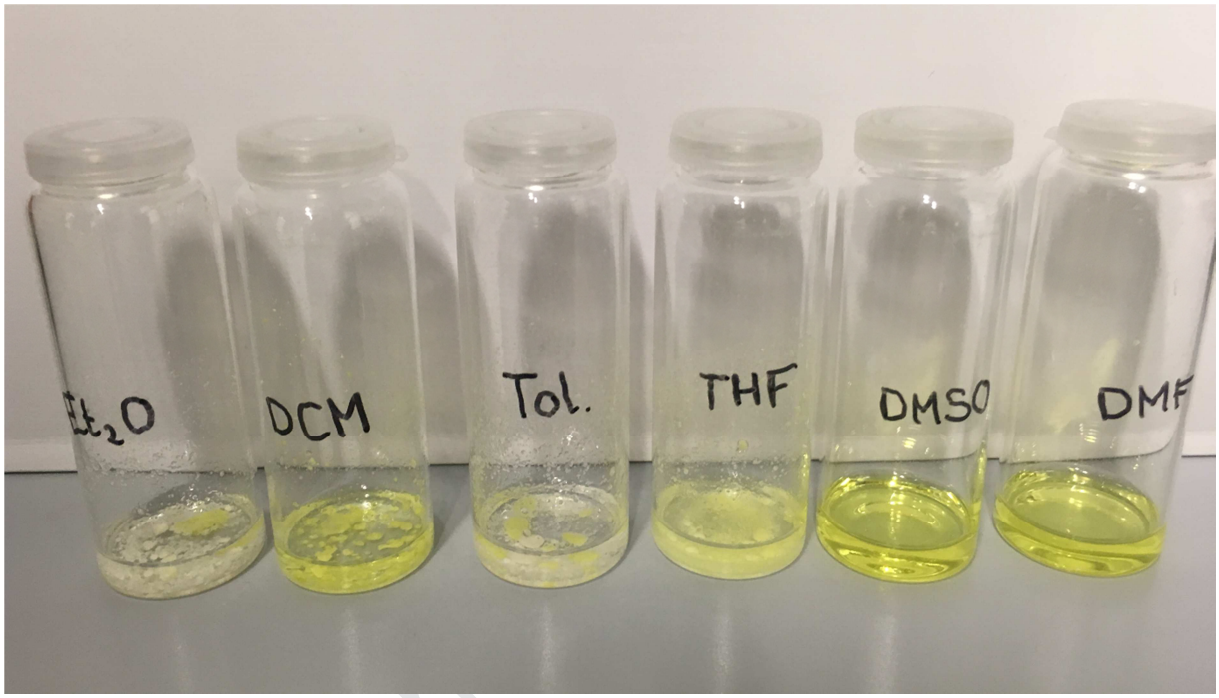
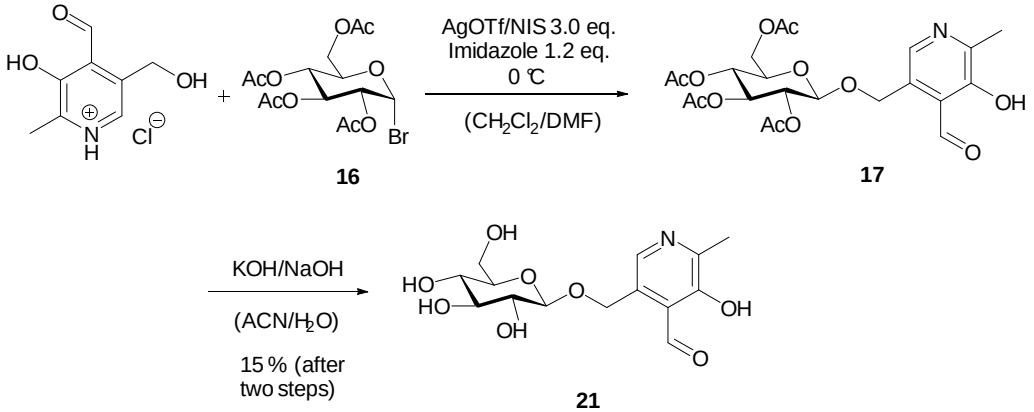


Figure 3. Solubility screening of PL·HCl in various solvents with the addition of imidazole (1.1 eq.).

After finishing the solubility experiments, a preliminary glycosylation was undertaken in DMSO and DMF according to KOENIGS-KNORR conditions (AgOTf/NIS 3.0 eq., 0 °C). Hereby, no product formation was detected *via* ESI-MS in aliquots taken during the reaction and after work-up. Since the synthesis of the PN-saccharides was successful in dichloromethane, but an unsatisfying solubility of PL was observed in that solvent during the screening, a mixture of CH_2Cl_2 and DMF (v/v = 1/1) was tested in the next approach (Scheme 5).



Scheme 6. The glycosylation of PL·HCl was undertaken according to KOENIGS-KNORR conditions with an increased amount of promoter in a solvent mixture.

With this procedure, the product (**17**) was verified in the reaction mixture *via* ESI-MS (m/z 498.1 $[M + H]^+$). After the work-up, the crude substance was deprotected as described for the PN-saccharides to obtain the desired PLG (m/z 330.0 $[M + H]^+$) in 15 % yield after two steps starting from PL·HCl. Hereby, in accordance to the reaction outcome of the PN-saccharides, most losses occurred during the glycosylation step rather than the deprotection steps. A crucial part influencing the reactivity of this particular glucosylation lies in the solvent mixture, that had to be applied in order to guarantee the complete dissolution of the starting materials. Since DMSO and DMF, when used solely as solvent, didn't lead to a conversion of starting material, they could have an impact on the overall reactivity and yield. Furthermore, as results from preliminary experiments stated, pyridine-derivatives with basic properties exhibit an influence on the reactivity of the promoter. Imidazole, in the same way as PN, could inhibit the formation of product due to interaction with the promoter. A furtherly increased addition of promoter didn't lead to an improvement regarding the reaction outcome. The mild treatment with NaOH/KOH did not have an impact on the aldehyde group, leaving it intact during the deprotection procedure.

A singlet at δ = 10.3 ppm and absence of a signal group correlating to a third CH_2-group in the NMR-spectra affirmed the integrity of the aldehyde-group. A doublet at 4.55 ppm (J = 8.0 Hz) confirmed the selective formation of the β-anomer. The fragmentation pattern of the product in LC-ESI(+)-MS/MS showed the loss of the sugar moiety (m/z 168) affirming the structure as well (Figure 4).

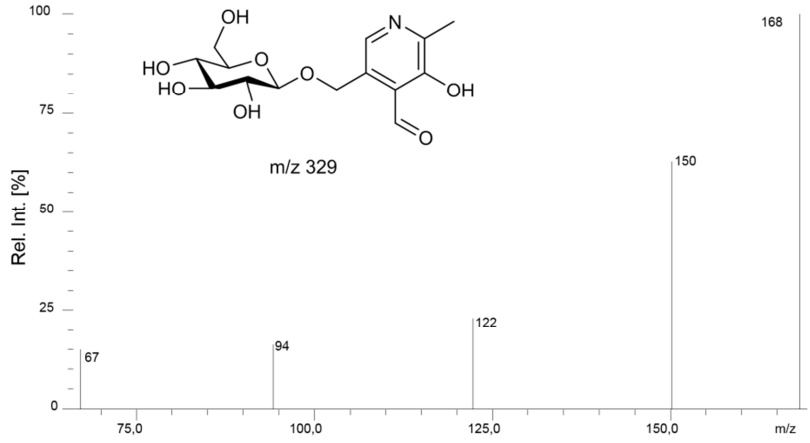
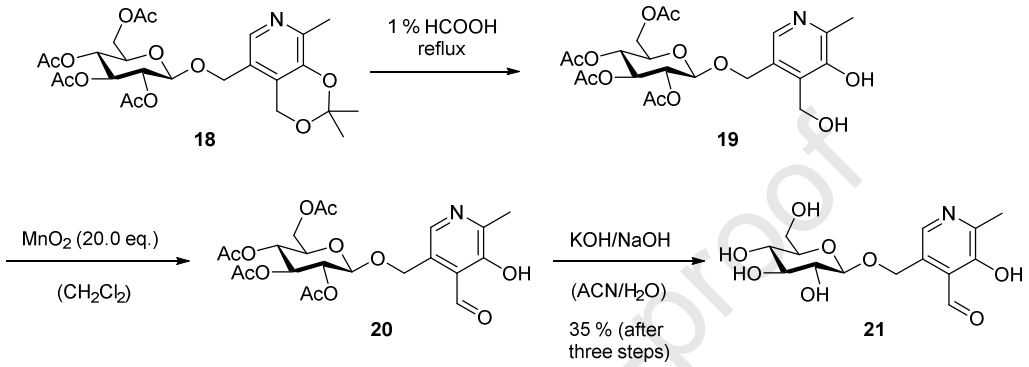


Figure 4. LC-ESI(+)-MS/MS spectrum of PLG (**21**, CE = − 26 V).

This method presents a strategy for the direct synthesis of PLG starting from the commercially available hydrochloric salt of the vitamer while simultaneously proposing a way to dissolve the substrate.

A further conceivable route for the preparation of PLG, beside the preparation starting from the hydrochloric salt, involves the assignment of PNG (**18**) as the starting substrate (Scheme 6).



Scheme 7. An alternative route for the synthesis of PLG led through an additional oxidation step during the deprotection procedure.

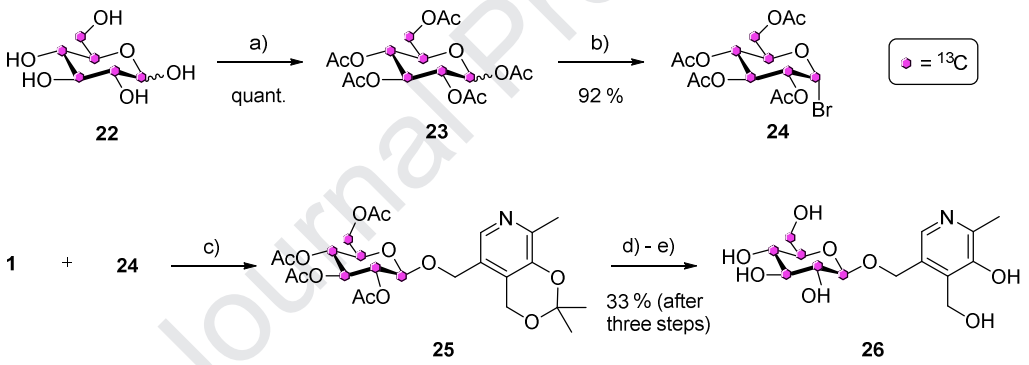
Because the aldehyde functionality in 4'-position constitutes the sole difference between PNG and PLG, the implementation of an oxidation step into the deprotection procedure presents a convenient way for the preparation of the desired glycoconjugate. Hereby, the acetonide group was removed by reflux in accordance to the procedure for PN-saccharides described above unveiling the hydroxyl functionality (**19**) in 4'-position. Oxidation with manganese oxide (MnO_2) in dichloromethane led to the selective formation of the aldehyde group (**20**), whose integrity was assured by ESI-MS measurements (m/z 498.1 $[M + H]^+$). Lastly, the acetate groups were cleaved in basic milieu to obtain PLG (**21**) in 35 % yield after three steps starting from the protected PNG. Although the yield increased compared to the synthesis starting from PL at first glance, it has to be noted, that a multistep procedure for the preparation of PNG preceded the proposed strategy, increasing the overall working effort and hampering the accessibility of the method. Additionally, the oxidation of PN reportedly poses a challenging reaction, often accompanied by the transformation into an oxime and subsequent acid hydrolysis in order to obtain the aldehyde.[101-103] Both methods - direct glycosylation of PL and oxidation of PNG - were carried out under non-optimized reaction conditions, and comprise the potential for higher yields. In this regard, other glycosylation methods involving different protecting- and leaving groups or promoters should be tested for

the direct glycosylation. Additionally, other oxidation reagents like potassium permanganate[104] can be examined for the multistep method.

So far, the glycosylation of PL was only accomplished at the 3‘-position *via* KOENIGS-KNORR synthesis utilizing pyridoxal-monoethylacetal hydrochloride, acetobromoglucose and silver carbonate.[105]

2.4. Synthesis of [$^{13}C_6$]-5’-β-PNG

Stable isotope dilution analysis (SIDA) marks a unique way of quantifying substrates in food, since, among other positive features, the loss of analyte during sample work-up can be exactly compensated for.[106, 107] Although being a promising method, SIDA demands isotopically labeled substrates, which are not always commercially available and thus have to be synthesized. Since this study revolved around the search for glycoconjugated derivatives of vitamin B_6, the last chapter involved the chemical synthesis of isotopically labeled 5’-*β*-PNG for its application in SIDA.



Scheme 8. The synthesis of isotopically labeled 5’-*β*-PNG (**26**) was accomplished according to KOENIGS-KNORR conditions. a) Ac_2O, Py, DMAP; b) HBr; c) AgOTf/NIS; d) NaOH/KOH; e) 1 % HCOOH.

The synthesis followed the strategy for the PN-saccharides mentioned above. Isotopic labeling was introduced through the glucose moiety starting from [$^{13}C_6$]-glucose (Scheme 7). The preparation of the sugar donor proceeded through acetylation (quant.) using acetic anhydride (Ac_2O), pyridine (Py) and 4-dimethylaminopyridine (DMAP) and subsequent bromination (92 %), undertaken with hydrogen bromide (HBr). The glycosidic bond between the sugar donor and *α*4,3-*O*-isopropylidene pyridoxine was established with AgOTf/NIS (3.0 eq.) as promoter in dichloromethane at 0 °C. The following two-step deprotection involved the application of NaOH/KOH and 1 % HCOOH to obtain the desired 5’-*β*-[$^{13}C_6$]-PNG in 33 % yield after the three steps including glycosylation and deprotection. The structural validity was confirmed through NMR spectroscopy - a doublet of doublets at

4.26 ppm with a J = 7.7 Hz affirmed the β conformation - and ESI-MS (m/z 338.1 $[M + H]^+$). Cleaving the glycosidic bond with β-glycosidase from almonds verified the β-selectivity of the reaction.

The currently favored preparation of pyridoxine glycosides involves the addition of saccharides to sprouting alfalfa seeds and allows the isolation of [^{2}H]- (47 %) and [^{3}H]-β-PNG beside unlabeled PNG (35 - 60 %).[57, 59-62, 64] Although the yield seems comparingly higher by 10 % at first glance, the utilization of seeds involves the synthesis of deuterated PN *via* a five step route[62, 104] beforehand in order to provide the starting material. By utilization of commercially available [$^{13}C_6$]-glucose and the simple strategy of acetylation/bromination in order to prepare the starting materials, the overall costs in preparation in the presented method are reduced. Additionally, utilizing seeds for the preparation of glycosides is rather optimized at this point and losses occur mostly during handling and chromatography[62], whereas the chemical synthesis promises high potential for optimization through variation of the reaction conditions. Furthermore, [$^{13}C_6$]-glucose introduces a higher amount of labelling into the final molecule and hence reduces analytical errors as a mass spectrometric overlap due to naturally occurring isotopologues is avoided.

3. Materials and Methods

3.1. General information

Reactions sensitive to air or moisture were carried out in dried glassware under a positive pressure of argon using standard *Schlenk* techniques. Solvents were distilled and stored over molecular sieves prior to use. Chemicals received from commercial sources (*Acros, Sigma-Aldrich, Fluka, Fisher Scientific*) were used without further purification unless stated otherwise. D-[$^{13}C_6$]-glucose was purchased from Sigma-Aldrich. Detailed preparations of the sugar donors and glycosides can be found in the supplementary information. The optical rotation was measured on a P3000 polarimeter (Fa. Kruess).

3.2. Column chromatography/TLC

Column chromatography was performed on silica gel 60 (Merck, 230 - 240 mesh) with the eluent mixtures given for the corresponding procedures. Thin-layer chromatography (TLC) was performed using silica-coated aluminium plates (silica gel 60). The substances were detected by UV (λ = 254 nm, 366 nm) or after visualization with CAM (cerium ammonium molybdate)/potassium permanganate ($KMnO_4$) solution.

3.3. Nuclear Magnetic Resonance

NMR spectra were recorded either on a *Bruker* AV III system (400 MHz, Bruker, Rheinstetten, Germany) or on a Bruker AV III system (500 MHz, Bruker, Rheinstetten, Germany). ^{1}H- and ^{13}C NMR spectra were recorded at 400 or 500 MHz and at 101 or 126 MHz, respectively. ^{1}H and ^{13}C NMR spectroscopic chemical shifts δ are reported in parts per million (ppm) relative to residual proton signal. All coupling constants (*J*) are reported in Hertz (Hz). The following abbreviations were used to explain multiplicities: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, m = multiplet.

3.4. LC-MS/MS

LC-MS/MS was carried out on a Shimadzu Nexera X2 UHPLC system (Shimadzu, Kyoto, Japan) with the mobile phase combinations water/acetonitrile or water/methanol. The injection volume was 1 µL. The LC was interfaced with a triple quadrupole ion trap mass spectrometer (LCMS-8050, Shimadzu, Kyoto, Japan). Data acquisition was performed with LabSolutions software 5.80 (Shimadzu, Kyoto, Japan).

4. Conclusions

The motivation to understand the full extent of the involvement of glycoconjugates in nature has become the driving force to a variety of research fields. Especially in terms of vitamins, the influence of the respective glycosidic conjugates on the human metabolism remains not thoroughly explored to this day. Studies revolving around these frontiers in research show a demand for respective standards, but commercial availability is often the decisive road block. Hence, this study focused on the chemical synthesis of various 5'-*β*-saccharides of PN and isotopically labeled 5'-*β*-[$^{13}C_6$]-PNG. Furthermore, strategies to create other B_6 glycosides (*e.g.* PLG) were illuminated. So far, the main focus in literature lies on the isolation and preparation of PN-saccharides, but data on their content in food is scarce. The synthesized substrates herein can find their purpose in future experiments involving analytical surveys of natural samples.

Supplementary Materials: Experimental procedures and NMR-spectra of the new substances are available in the supplementary information.

Author Contributions: T.B. and M.R. conceived and designed the experiments; T.B. performed the experiments and analyzed the data; L.Z. contributed to the synthesis of PLG; C.S. contributed to the evaluation of the NMRs; T.B. wrote the manuscript; M.R. revised the manuscript. All authors contributed to the revision of the manuscript.

Funding: The study was supported by a grant from the Deutsche Forschungsgemeinschaft (RY 19/17–1).

Acknowledgments: The authors gratefully acknowledge the support by the Chair of Food Chemistry and Molecular Science, Technical University Munich, Germany, for the NMR measurements

Conflicts of Interest: The authors declare no competing financial interests.

References

[1] R.S. Haltiwanger, J.B. Lowe, Role of glycosylation in development, Annu. Rev. Biochem. *73* (**2004)** 491-537. 10.1146/annurev.biochem.73.011303.074043.

[2] K.W. Moremen, M. Tiemeyer, A.V. Nairn, Vertebrate protein glycosylation: diversity, synthesis and function, Nat. Rev. Mol. Cell. Biol. *13* (**2012)** 448-462. 10.1038/nrm3383.

[3] F. Li, C. Li, J. Revote, Y. Zhang, G.I. Webb, J. Li, J. Song, T. Lithgow, *GlycoMine*^{struct}: a new bioinformatics tool for highly accurate mapping of the human N-linked and O-linked glycoproteomes by incorporating structural features, Sci. Rep. *6* (**2016)** 34595. 10.1038/srep34595.

[4] C. Reily, T.J. Stewart, M.B. Renfrow, J. Novak, Glycosylation in health and disease, Nat. Rev. Nephrol. *15* (**2019)** 346-366. 10.1038/s41581-019-0129-4.

[5] M. Marradi, M. Martín-Lomas, S. Penadés, Glyconanoparticles: Polyvalent tools to study carbohydrate-based interactions **2010**; pp 211-290.

[6] M. Mammen, S.-K. Choi, G.M. Whitesides, Polyvalent Interactions in Biological Systems: Implications for Design and Use of Multivalent Ligands and Inhibitors., Angew. Chem. Int. Ed. Engl. *37* (**1998)** 2754-2794. 10.1002/(SICI)1521-3773(19981102)37:20<2754::AID-ANIE2754>3.0.CO;2-3.

[7] H.H. Freeze, Genetic defects in the human glycome, Nat. Rev. Genet. *7* (**2006)** 537-551. 10.1038/nrg1894.

[8] K. Ohtsubo, J.D. Marth, Glycosylation in cellular mechanisms of health and disease, Cell *126* (**2006)** 855-867. 10.1016/j.cell.2006.08.019.

[9] A. Varki, Evolutionary Forces Shaping the Golgi Glycosylation Machinery: Why Cell Surface Glycans Are Universal to Living Cells, Cold Spring Harb. Perspect. Biol. *3* (**2011)**. 10.1101/cshperspect.a005462.

[10] R. Kleene, M. Schachner, Glycans and neural cell interactions, Nat. Rev. Neurosci. *5* (**2004)** 195-208. 10.1038/nrn1349.

[11] S. Schedin-Weiss, B. Winblad, L.O. Tjernberg, The role of protein glycosylation in Alzheimer disease, FEBS J *281* (**2014)** 46-62. 10.1111/febs.12590.

[12] T. Ahmad, H. Ahmad, B. Ashfaq, Z. Khan, F. Ullah Khan, S. Hussain, T. Wang, Y. Tian, B. Shi, H. Haider Khan, Glycan regulation in cancer, nervous and immune system: A narrative review, Biomedical Research and Therapy *6* (**2019)** 3113-3120. 10.15419/bmrat.v6i4.536.

[13] R.D. Astronomo, D.R. Burton, Carbohydrate vaccines: developing sweet solutions to sticky situations?, Nat. Rev. Drug Discov. *9* (**2010)** 308-324. 10.1038/nrd3012.

[14] H. Wan, J. Gao, H. Yang, S. Yang, R. Harvey, Y.Q. Chen, N.Y. Zheng, J. Chang, P.J. Carney, X. Li, E. Plant, L. Jiang, L. Couzens, C. Wang, S. Strohmeier, W.W. Wu, R.F. Shen, F. Krammer, J.F. Cipollo, P.C. Wilson, J. Stevens, X.F. Wan, M.C. Eichelberger, Z. Ye, The neuraminidase of A(H3N2) influenza

viruses circulating since 2016 is antigenically distinct from the A/Hong Kong/4801/2014 vaccine strain, Nat Microbiol (**2019)**. 10.1038/s41564-019-0522-6.

[15] D.S. Park, R.D. Poretz, S. Stein, R. Nora, P. Manowitz, Association of alcoholism with the *N*-glycosylation polymorphism of pseudodeficient human arylsulfatase A., Alcohol Clin. Exp. Res. *20* (**1996)** 228-233. 10.1111/j.1530-0277.1996.tb01634.x.

[16] D.H. Dube, C.R. Bertozzi, Glycans in cancer and inflammation - potential for therapeutics and diagnostics, Nat. Rev. Drug Discov. *4* (**2005)** 477-488. 10.1038/nrd1751.

[17] K. Seyrek, M. Richter, I.N. Lavrik, Decoding the sweet regulation of apoptosis: the role of glycosylation and galectins in apoptotic signaling pathways, Cell Death Differ. *26* (**2019)** 981-993. 10.1038/s41418-019-0317-6.

[18] S.S. Pinho, C.A. Reis, Glycosylation in cancer: mechanisms and clinical implications, Nat. Rev. Cancer *15* (**2015)** 540-555. 10.1038/nrc3982.

[19] S. Rachagani, M.P. Torres, N. Moniaux, S.K. Batra, Current status of mucins in the diagnosis and therapy of cancer, Biofactors *35* (**2009)** 509-527. 10.1002/biof.64.

[20] J.D. Marth, A unified vision of the building blocks of life, Nat. Cell Biol. *10* (**2008)** 1015–1016. 10.1038/ncb0908-1015.

[21] R. Strasser, Biological significance of complex *N*-glycans in plants and their impact on plant physiology, Front. Plant Sci. *5* (**2014)** 363. 10.3389/fpls.2014.00363.

[22] P.J. Winterburn, C.F. Phelps, The Significance of Glycosylated Proteins, Nature *236* (**1972)** 147-151. 10.1038/236147a0.

[23] A. Sturm, *N*-Glycosylation of Plant Proteins. In *Glycoproteins*, **1995**; pp 521-541.

[24] K.J. Yarema, C.R. Bertozzi, Characterizing glycosylation pathways, Genome Biol. *2* (**2001)**. 10.1186/gb-2001-2-5-reviews0004.

[25] N. Lannoo, E.J. Van Damme, C. Albenne, E. Jamet, Plant Glycobiology - a diverse world of lectins, glycoproteins, glycolipids and glycans, Front. Plant Sci. *5* (**2014)** 604. 10.3389/fpls.2014.00604.

[26] J. Le Roy, B. Huss, A. Creach, S. Hawkins, G. Neutelings, Glycosylation Is a Major Regulator of Phenylpropanoid Availability and Biological Activity in Plants, Front. Plant Sci. *7* (**2016)** 735. 10.3389/fpls.2016.00735.

[27] C. Rayon, P. Lerouge, L. Faye, The protein *N*-glycosylation in plants, J. Exp. Bot. *49* (**1998)** 1463–1472. 10.1093/jxb/49.326.1463.

[28] H.J. Gabius, S. Andre, J. Jimenez-Barbero, A. Romero, D. Solis, From lectin structure to functional glycomics: principles of the sugar code, Trends Biochem. Sci. *36* (**2011)** 298-313. 10.1016/j.tibs.2011.01.005.

[29] D. Solis, N.V. Bovin, A.P. Davis, J. Jimenez-Barbero, A. Romero, R. Roy, K. Smetana, Jr., H.J. Gabius, A guide into glycosciences: How chemistry, biochemistry and biology cooperate to crack the sugar code, Biochim. Biophys. Acta *1850* (**2015)** 186-235. 10.1016/j.bbagen.2014.03.016.

[30] J.F. Gregory, Accounting for differences in the bioactivity and bioavailability of vitamers, Food Nutr. Res. *56* (**2012)**. 10.3402/fnr.v56i0.5809.

[31] S.B. Binder, H.E. Schwartz-Zimmermann, E. Varga, G. Bichl, H. Michlmayr, G. Adam, F. Berthiller, Metabolism of Zearalenone and Its Major Modified Forms in Pigs, Toxins *9* (**2017)**. 10.3390/toxins9020056.

[32] K. Mastanjevic, J. Lukinac, M. Jukic, B. Sarkanj, V. Krstanovic, K. Mastanjevic, Multi-(myco)toxins in Malting and Brewing By-Products, Toxins *11* (**2019)**. 10.3390/toxins11010030.

[33] M. Zachariasova, M. Vaclavikova, O. Lacina, L. Vaclavik, J. Hajslova, Deoxynivalenol Oligoglycosides: New "Masked" Fusarium Toxins Occurring in Malt, Beer, and Breadstuff, J. Agric. Food Chem. *60* (**2012)** 9280-9291. 10.1021/jf302069z.

[34] P. Jones, T. Vogt, Glycosyltransferases in secondary plant metabolism: tranquilizers and stimulant controllers, Planta *213* (**2001)** 164-174. 10.1007/s004250000492.

[35] M. Rychlik, H.U. Humpf, D. Marko, S. Danicke, A. Mally, F. Berthiller, H. Klaffke, N. Lorenz, Proposal of a comprehensive definition of modified and other forms of mycotoxins including "masked" mycotoxins, Mycotoxin Res. *30* (**2014)** 197-205. 10.1007/s12550-014-0203-5.

[36] K. Habler, O. Frank, M. Rychlik, Chemical Synthesis of Deoxynivalenol-3-β-D-[$^{13}C_6$]-glucoside and Application in Stable Isotope Dilution Assays, Molecules *21* (**2016)**. 10.3390/molecules21070838.

[37] K. Habler, M. Rychlik, Multi-mycotoxin stable isotope dilution LC-MS/MS method for *Fusarium* toxins in cereals, Anal. Bioanal. Chem. *408* (**2016)** 307-317. 10.1007/s00216-015-9110-7.

[38] M. Gareis, J. Bauer, J. Thiem, G. Plank, S. Grabley, B. Gedek, Cleavage of Zearalenone-Glycoside, a "Masked" Mycotoxin, during Digestion in Swine, J. Vet. Med. B *37* (**1990)** 236-240. 10.1111/j.1439-0450.1990.tb01052.x.

[39] F. Berthiller, C. Crews, C. Dall'Asta, S.D. Saeger, G. Haesaert, P. Karlovsky, I.P. Oswald, W. Seefelder, G. Speijers, J. Stroka, Masked mycotoxins: A review, Mol. Nutr. Food Res. *57* (**2013)** 165-186. 10.1002/mnfr.201100764.

[40] H.S. Schmidt, M. Schulz, C. Focke, S. Becker, B. Cramer, H.U. Humpf, Glucosylation of T-2 and HT-2 toxins using biotransformation and chemical synthesis: Preparation, stereochemistry, and stability, Mycotoxin Res. *34* (**2018)** 159-172. 10.1007/s12550-018-0310-9.

[41] V. Nagl, G. Schatzmayr, Deoxynivalenol and its masked forms in food and feed, Curr. Opin. Food Sci. *5* (**2015)** 43-49. 10.1016/j.cofs.2015.08.001.

[42] S. Huang, H. Zeng, J. Zhang, S. Wei, L. Huang, Interconversions of different forms of vitamin B_6 in tobacco plants, Phytochemistry *72* (**2011)** 2124-2129. 10.1016/j.phytochem.2011.07.019.

[43] Y. Nagahashi, M. Tazoe, T. Hoshino, Cloning of the Pyridoxine 5'-Phosphate Phosphatase Gene (*pdxP*) and Vitamin B_6 Production in *pdxP* Recombinant *Sinorhizobium meliloti*, Biosci. Biotechnol. Biochem. *72* (**2008)** 421-427. 10.1271/bbb.70539.

[44] IUPAC-IUB, Nomenclature for Vitamins B-6 and Related Compounds, Eur. J. Biochem. *40* (**1973)** 325-327. 10.1111/j.1432-1033.1973.tb03200.x.

[45] L. Siegel, D. Melnick, B.L. Oser, Bound Pyridoxine (Vitamin B_6) in Biological Materials, J. Biol. Chem. *149* (**1943)** 361-367.

[46] J.C. Rabinowitz, E.E. Snell, The Vitamin B_6 Group, Distribution of Pyridoxal, Pyridoxamine, and Pyridoxine in some natural Products, J. Biol. Chem. *176* (**1948)** 1157-1167.

[47] K. Tadera, F. Yagi, A. Kobayashi, Specificity of a particulate glucosyltransferase in seedlings of *Pisum sativum* L. which catalyzes the formation of 5'-*O*-(β-D-glucopyranosyl)pyridoxine, J. Nutr. Sci. Vitaminol. *28* (**1982)** 359-366. 10.3177/jnsv.28.359.

[48] H. Tsuge, M. Maeno, T. Hayakawa, Y. Suzuki, Comparative Study of Pyridoxine-α,β-Glucosides, and Phosphopyridoxyl-Lysine as a Vitamin B_6 Nutrient, J. Nutr. Sci. Vitaminol. *42* (**1996)** 377-386. 10.3177/jnsv.42.377.

[49] J.F. Gregory, S.L. Ink, Identification and Quantification of Pyridoxine-β-Glucoside as a Major Form of Vitamin B_6 in Plant-Derived Foods, J. Agric. Food Chem. *35* (**1987)** 76-82. 10.1021/jf00073a018.

[50] K. Yasumoto, H. Tsuji, K. Iwami, H. Mitsuda, Isolation from Rice Bran of a Bound Form of Vitamin B_6 and Its Identification as 5′-*O*-(*β*-D-Glucopyranosyl) Pyridoxine, Agric. Biol. Chem. *41* (**1977)** 1061-1067. 10.1080/00021369.1977.10862620.

[51] K. Iwami, K. Yasumoto, Synthesis of pyridoxine-*β*-glucoside by rice bran *β*-glucosidease and its in situ absorption in rat small intestine, Nutr. Res. *6* (**1986)** 407-414. 10.1016/S0271-5317(86)80181-6.

[52] R.E. Charles, S. Divakar, *β*-Glucosidase Catalyzed Syntheses of Pyridoxine Glycosides, Biosci. Biotechnol. Biochem. *73* (**2009)** 233–236. 10.1271/bbb.80463.

[53] K. Tadera, M. Nakamura, F. Magi, A. Kobayashi, A particulate glucosyltransferase catalyzing the formation of 5'-*O*-(*β*-D-glucopyranosyl)pyridoxine from pyridoxine: The occurrence in the seedlings of *Pisum sativum* L., J. Nutr. Sci. Vitaminol. *25* (**1979)** 347-350. 10.3177/jnsv.25.347.

[54] K. Tadera, M. Nakamura, A. Kobayashi, Vitamins *52* (**1978)** 17-23.

[55] K. Yasumoto, K. Iwami, J. Okada, H. Mitsuda, Eiyoto Shokuryo *32* (**1979)** 105-110.

[56] Y. Suzuki, Y. Inada, K. Uchida, *β*-Glucosylpyridoxines in germinating Seeds cultured in the Presence of Pyridoxine, Phytochemistry *25* (**1986)** 2049-2051. 10.1016/0031-9422(86)80064-4.

[57] S.L. Ink, J.F. Gregory, D.B. Sartain, Determination of Pyridoxine *β*-Glucoside Bioavailability Using Intrinsic and Extrinsic Labeling in the Rat, J. Agric. Food Chem. *34* (**1986)** 857-862. 10.1021/jf00071a024.

[58] Y. Suzuki, K. Uchida, A. Tsuboi, Nippon NogeiKagaku Kaishi *53* (**1979)** 189-196.

[59] P.R. Trumbo, J.F. Gregory, D.B. Sartain, Incomplete Utilization of Pyridoxine-*β*-Glucoside as Vitamin B-6 in the Rat., J. Nutr. *118* (**1988)** 170-175. 10.1093/jn/118.2.170.

[60] P.R. Trumbo, J.F. Gregory, Metabolic Utilization of Pyridoxine-*β*-Glucoside in Rats: Influence of Vitamin B-6 Status and Route of Administration., J. Nutr. *118* (**1988)** 1336-1342. 10.1093/jn/118.11.1336.

[61] P.R. Trumbo, J.F. Gregory, The Fate of Dietary Pyridoxine-*β*-Glucoside in the Lactating Rat, J. Nutr. *119* (**1989)** 36-39. 10.1093/jn/119.1.36.

[62] J.F. Gregory, H. Nakano, [7] Preparation of Nonlabeled, Tritiated, and Deuterated Pyridoxine 5′-*β*-D-Glucoside and Assay of Pyridoxine-5′-*β*-D-glucoside Hydrolase, Methods Enzymol. *280* (**1997)** 58-65. 10.1016/S0076-6879(97)80101-2.

[63] G. Schneider, The manifold of vitamin B_6 dependent enzymes, Structure *8* (**2000)** R1-R6.

[64] J.F. Gregory, P.R. Trumbo, L.B. Bailey, J.P. Toth, T.G. Baumgartner, J.J. Cerda, Bioavailability of Pyridoxine-5′-*β*-D-Glucoside Determined in Humans by Stable-Isotopic Methods, J. Nutr. *121* (**1991)** 177-186. 10.1093/jn/121.2.177.

[65] H. Nakano, L.G. McMahon, J.F. Gregory, Pyridoxine-5′-*β*-D-glucoside Exhibits Incomplete Bioavailability as a Source of Vitamin B-6 and Partially Inhibits the Utilization of Co-Ingested Pyridoxine in Humans, J. Nutr. *127* (**1997)** 1508–1513. 10.1093/jn/127.8.1508.

[66] A.D. Mackey, S.O. Lieu, C. Carman, J.F. Gregory, Hydrolytic Activity toward Pyridoxine-5'-*β*-D-Glucoside in Rat Intestinal Mucosa Is Not Increased by Vitamin B-6 Deficiency: Effect of Basal Diet Composition and Pyridoxine Intake., J. Nutr. *133* (**2003)** 1362-1367. 10.1093/jn/133.5.1362.

[67] K. Tadera, T. Kaneko, F. Yagi, Isolation and Structural Elucidation of Three New Pyridoxine-Glycosides in Rice Bran, J. Nutr. Sci. Vitaminol. *34* (**1988)** 167-177. 10.3177/jnsv.34.167.

[68] K. Tadera, K. Orite, Isolation and Structure of a New Vitamin B_6 Conjugate in Rice Bran, J. Food Sci. *56* (**1991)** 268-269. 10.1111/j.1365-2621.1991.tb08030.x.

[69] K. Tadera, K. Oda, C. Nakahara, Occurrence in Soybeans of a Novel Vitamin B_6 Conjugate that Liberates Pyridoxine by β-Glucosidase Action after Alkali Treatment, Biosci. Biotechnol. Biochem. *63* (**1999)** 213-215. 10.1271/bbb.63.213.

[70] K. Tadera, T. Kaneko, F. Yagi, Evidence for the Occurrence and Distribution of a New Type of Vitamin B_6 Conjugate in Plant Foods, Agric. Biol. Chem. *50* (**1986)** 2933-2934. 10.1080/00021369.1986.10867856.

[71] K. Tadera, K. Nagano, F. Yagi, A. Kobayashi, K. Imada, S. Tanaka, Isolation and Structure of a New Metabolite of Pyridoxine in Seedlings of *Pisum sativum* L., Agric. Biol. Chem. *47* (**1983)** 1357-1359. 10.1080/00021369.1983.10863889.

[72] K. Tadera, E. Mori, F. Yagi, A. Kobayashi, K. Imada, M. Imabeppu, Isolation and Structure of a Minor Metabolite of Pyridoxine in Seedlings of *Pisum sativum* L., J. Nutr. Sci. Vitaminol. *31* (**1985)** 403-408. 10.3177/jnsv.31.403.

[73] C. Addo, J. Augustin, Changes in the Vitamin B-6 Content in Potatoes During Storage, J. Food Sci. *53* (**1988)** 749-752. 10.1111/j.1365-2621.1988.tb08947.x.

[74] A. Tramice, A. Giordano, G. Andreotti, E. Mollo, A. Trincone, High-Yielding Enzymatic α-Glucosylation of Pyridoxine by Marine α-Glucosidase from *Aplysia fasciata*, Mar. Biotechnol. *8* (**2006)** 448-452. 10.1007/s10126-005-6144-4.

[75] Y. Suzuki, K. Uchida, Enzymatic formation of Pyridoxine β-glucuronic acid-like substance, Vitamins *51* (**1977)** 507-511.

[76] Y. Suzuki, K. Uchida, Formation of β-galactosyl compounds of pyridoxine in growing culture of *Sporobolomyces singularis*, Biochim. Biophys. Acta. *1116* (**1992)** 67-71. 10.1016/0304-4165(92)90129-I.

[77] Y. Suzuki, K. Uchida, [9] Formation of β-Galactosides of Pyridoxine using *Sporobolomyces singularis*, Methods Enzymol. *280* (**1997)** 71-77. 10.1016/S0076-6879(97)80103-6.

[78] Y. Suzuki, K. Uchida, Formation of β-Fructosyl Compounds of Pyridoxine in Growing Culture of *Aspergillus niger*, Biosci. Biotechnol. Biochem. *57* (**1993)** 875-880. 10.1271/bbb.57.875.

[79] L. Weignerova, Y. Suzuki, Z. Hunkova, P. Sedmera, V. Havlicek, R. Marek, V. Kren, Pyridoxine as a substrate for screening synthetic potential of glycosidases Collect. Czech. Chem. Commun. *64* (**1999)** 1325-1334. 10.1135/cccc19991325

[80] W. Günter, R. Gerhard, Results and Problems of *O*-Glycoside Synthesis, Angew. Chem. Int. Ed. Engl. *13* (**1974)** 157-216. 10.1002/anie.197401571.

[81] S. Manabe, Y. Ito, Optimizing Glycosylation Reaction Selectivities by Protecting Group Manipulation, Curr. Bioact. Compd. *4* (**2008)** 258-281. 10.2174/157340708786847861.

[82] R.A. Mensink, T.J. Boltje, Advances in Stereoselective 1,2-*cis* Glycosylation using C-2 Auxiliaries, Chemistry *23* (**2017)** 17637-17653. 10.1002/chem.201700908.

[83] R. Šardzík, G.T. Noble, M.J. Weissenborn, Andrew Martin, S.J. Webb, S.L. Flitsch, Preparation of aminoethyl glycosides for glycoconjugation, Beilstein J. Org. Chem. *6* (**2010)** 699-703. 10.3762/bjoc.6.81.

[84] W.E. Evans, M.V. Relling, Pharmacogenomics: translating functional genomics into rational therapeutics., Science *286* (**1999)** 487-491. 10.1126/science.286.5439.487

[85] B.K. Park, A. Boobis, S. Clarke, C.E. Goldring, D. Jones, J.G. Kenna, C. Lambert, H.G. Laverty, D.J. Naisbitt, S. Nelson, D.A. Nicoll-Griffith, R.S. Obach, P. Routledge, D.A. Smith, D.J. Tweedie, N. Vermeulen, D.P. Williams, I.D. Wilson, T.A. Baillie, Managing the challenge of chemically reactive metabolites in drug development, Nat. Rev. Drug Discov. *10* (**2011)** 292-306. 10.1038/nrd3408.

[86] C. Guillemette, Pharmacogenomics of human UDP-glucuronosyltransferase enzymes, Pharmacogenomics J *3* (**2003)** 136-158. 10.1038/sj.tpj.6500171.

[87] Q. Gao, M. Yang, Z. Zuo, Overview of the anti-inflammatory effects, pharmacokinetic properties and clinical efficacies of arctigenin and arctiin from *Arctium lappa* L, Acta Pharmacol. Sin. *39* (**2018)** 787-801. 10.1038/aps.2018.32.

[88] G. Yang, S. Ge, R. Singh, S. Basu, K. Shatzer, M. Zen, J. Liu, Y. Tu, C. Zhang, J. Wei, J. Shi, L. Zhu, Z. Liu, Y. Wang, S. Gao, M. Hu, Glucuronidation: Driving Factors and Their Impact on Glucuronide Disposition, Drug Metab. Rev. *49* (**2017)** 105-138. 10.1080/03602532.2017.1293682.

[89] G.J. Mulder, Glucuronidation and its Role in Regulation of Biological Activity of Drugs, Annu. Rev. Pharmacol. Toxicol. *32* (**1992)** 25-49. 10.1146/annurev.pa.32.040192.000325.

[90] H.K. Kroemer, U. Klotz, Glucuronidation of Drugs. A Re-Evaluation of the Pharmacological Significance of the Conjugates and Modulating Factors, Clin. Pharmacokinet. *23* (**1992)** 292-310. 10.2165/00003088-199223040-00005.

[91] R.E. Hodges, D.M. Minich, Modulation of Metabolic Detoxification Pathways Using Foods and Food-Derived Components: A Scientific Review with Clinical Application, J. Nutr. Metab. *2015* (**2015)** 760689. 10.1155/2015/760689.

[92] Y. Chen, Y. Li, H. Yu, G. Sugiarto, V. Thon, J. Hwang, L. Ding, L. Hie, X. Chen, Tailored Design and Synthesis of Heparan Sulfate Oligosaccharide Analogues Using Sequential One-Pot Multienzyme Systems, Angew. Chem. Int. Ed. Engl. *52* (**2013)** 11852-11856. 10.1002/anie.201305667.

[93] P. Bonomi, M.D. Attieh, C. Gonzato, K. Haupt, A New Versatile Water-Soluble Iniferter Platform for the Preparation of Molecularly Imprinted Nanoparticles by Photopolymerisation in Aqueous Media, Chemistry *22* (**2016)** 10150-10154. 10.1002/chem.201600750.

[94] C. Liang, W. Ju, S. Ding, H. Sun, G. Mao, Effective Synthesis of Nucleosides Utilizing *O*-Acetyl-Glycosyl Chlorides as Glycosyl Donors in the Absence of Catalyst: Mechanism Revision and Application to Silyl-Hilbert-Johnson Reaction, Molecules *22* (**2017)**. 10.3390/molecules22010084.

[95] Q. Wang, J. Fu, J. Zhang, A facile preparation of peracylated α-aldopyranosyl chlorides with thionyl chloride and tin tetrachloride, Carbohydr. Res. *343* (**2008)** 2989-2991. 10.1016/j.carres.2008.08.037.

[96] P.J. Garegg, P. Konradsson, I. Kvarnström, T. Norberg, S.C.T. Svensson, B. Wigilius, Studies on Koenigs-Knorr Glycosidations., Acta Chem. Scand. Ser. B *39* (**1985)** 569-577. 10.3891/acta.chem.scand.39b-0569.

[97] D.C. Dejongh, W. Korytnyk, Mass Spectrometry of Vitamin B_6, Methods Enzymol. *18* (**1970)** 483-488. 10.1016/0076-6879(71)18348-6.

[98] J.S. Casas, M.D. Couce, J. Sordo, Coordination chemistry of vitamin B_6 and derivatives: A structural overview, Coord. Chem. Rev. *256* (**2012)** 3036-3062. 10.1016/j.ccr.2012.07.001.

[99] M. Chan-Huot, C. Niether, S. Sharif, P.M. Tolstoy, M.D. Toney, H.-H. Limbach, NMR studies of the protonation states of pyridoxal-5′-phosphate in water, J. Mol. Struct. *976* (**2010)** 282-289. 10.1016/j.molstruc.2010.03.032.

[100] V.L. Florentiev, V.I. Ivanov, M.Y. Karpeisky, Synthesis and Physicochemical and Coenzyme Properties of Alkyl-Substituted Analogs of the B_6 Vitamins and Pyridoxal Phosphate, Methods Enzymol. *18* (**1970)** 567-598. 10.1016/0076-6879(71)18357-7.

[101] M.Viscontini, C. Ebnother, P. Karrer, 218. Konstitution und Synthese der Codecarboxylase, Helvetica Chimica Acta *34* (**1951)** 1834-1839.

[102] T. Bachmann, M. Rychlik, Synthesis of [$^{13}C_3$]-B6 Vitamers Labelled at Three Consecutive Positions Starting from [$^{13}C_3$]-Propionic Acid, Molecules *23* (**2018)** 2117. 10.3390/molecules23092117.

[103] S. Sharif, D. Schagen, M.D. Toney, H.-H. Limbach, Coupling of Functional Hydrogen Bonds in Pyridoxal-5‘-phosphate−Enzyme Model Systems Observed by Solid-State NMR Spectroscopy, J. Am. Chem. Soc. *129* (**2007)** 4440-4455. 10.1021/ja066240h.

[104] S.P. Coburn, C.C. Lin, W.E. Schaltenbrand, J.D. Mahuren, Synthesis of deuterated vitamin B_6 compounds J. Label. Compd. Radiopharm. *19* (**1982)** 703-716. 10.1002/jlcr.2580190510.

[105] K. Sakamoto, K. Wada, H. Ito, N. Take, H. Morimoto, F. Maniwa, Y. Shimoto Stable Vitamin B6 Derivative **2006**.

[106] M. Rychlik, S. Asam, Stabilisotopenverdünnungsanalysen zur Quantifizierung organischer Spurenkomponenten in der Lebensmittelanalytik, UWSF-Z. *21* (**2009)** 470-482. 10.1007/s12302-009-0082-0.

[107] M. Rychlik, S. Asam, Stable isotope dilution assays in mycotoxin analysis, Anal. Bioanal. Chem. *390* (**2008)** 617-628. 10.1007/s00216-007-1717-x.

Chemical synthesis of 5'-*β*-glycoconjugates of vitamin B_6

Highlights

- The chemical synthesis of the 5'-*β*-saccharides of pyridoxine, namely the mannoside, galactoside, arabinoside, maltoside, cellobioside and glucuronide, was accomplished.
- New strategies for the chemical glucosylation of pyridoxal were investigated.
- The chemical synthesis of 5'-*β*-[$^{13}C_6$]-labeled pyridoxine glucoside (PNG) was done according to KOENIGS-KNORR conditions.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: