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The "Natural Strategy" for the glycosidase-assisted synthesis of simple glycosides

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Abstract

Anomeric product mixtures obtained from simple acid-catalyzed *Fischer* glycosidation are conveniently resolved by application of glycosidase hydrolysis in aqueous medium to provide single diastereomers that are easy to isolate. Choosing from an array of inexpensive glycosidases, the hydrolytic strategy offers a flexible access to unprotected anomerically pure α- or β-glycopyranosides in good overall yields. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Simple glycopyranosides with specific anomeric protection groups are important as chiral pool-based building blocks for asymmetric synthesis [1,2] and as activated components for oligosaccharide synthesis [3]. Particularly, the anomeric allyl group is a useful terminal functionality in the course of the preparation of glycoconjugates and -polymers [4–8] because it is easily cleaved via isomeric propen-1-yl intermediates, in addition to other methods [9,10]. Furthermore, stereoselective chemical modification of the alkene leads to interesting functionalized derivatives [11–13]. Similarly, 4-pentenyl glycosides have proved their utility for the synthesis of glycosides, oligosaccharides, and glyco-polymers from developments by Fraser-Reid et al [14,15]. The benzyl moiety is another important anomeric protecting group that can be selectively cleaved under mild conditions [10].

The primary chemical routes for the stereoselective synthesis of simple glycopyranosides are based on the Koenigs–Knorr method and its numerous variants [16–18]. However, the glycosyl halides or related activated intermediates are typically generated under harsh reaction conditions and are frequently thermally unstable and difficult to handle. Furthermore, expensive, corrosive, or toxic reagents are required for their activation. This is particularly inconvenient when the glycosides are subsequently applied in the synthesis of products to be used for pharmaceutical purposes.

There are several protocols for glycoside synthesis by enzymatic catalysis using specific glycosidases. Anomerically pure glycosides can be obtained

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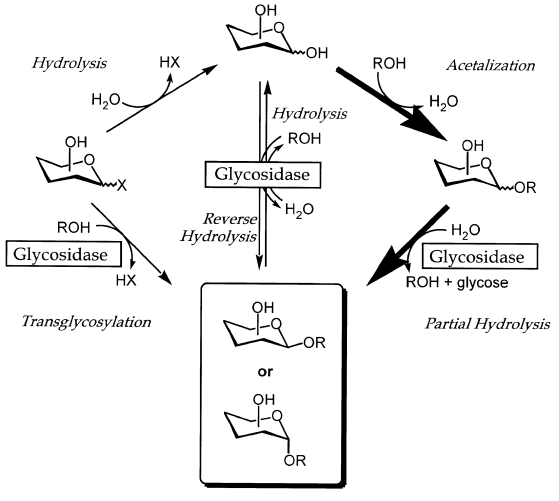
either by kinetically controlled transglycosylation from other glycoside precursors, or by thermodynamically controlled reverse hydrolysis from free sugars [19–21]. Frequently, these approaches suffer from low productivity and from difficulties to separate the target glycosides from starting materials or from isomeric contaminants. Higher productivity can be achieved for glycosides of simple alcohols where the latter can be used at high concentration [22–24]. For both methods, hydrolysis is difficult to compete with — whereas water is essential for the preservation of enzymatic activity, it becomes a problem in the context of glycoside synthesis because the reaction equilibrium is in favor of the natural hydrolytic function of glycosidases (Scheme 1).

The acid-catalyzed synthesis of glycosides by the *Fischer* protocol is a powerful tool to produce simple aliphatic glycopyranosides [4,25]. The reaction needs only one step, it is clean, and it offers the potential to recover the excess of an aliphatic aglycon, either by simple distillation or by extraction for those alcohols having high boiling points. However, the *Fischer* glycosidation is difficult to be kept under kinetic control and thus generally results in a mixture of constitutional (furanosides, pyranosides) and configurational (α and β anomers) isomers.



Scheme 1. Alternative synthetic pathways for the glycosidase-assisted stereoselective preparation of glycosides: transglycosylation, reverse hydrolysis (left and center, plain arrows), and partial hydrolysis (right; bold arrows).

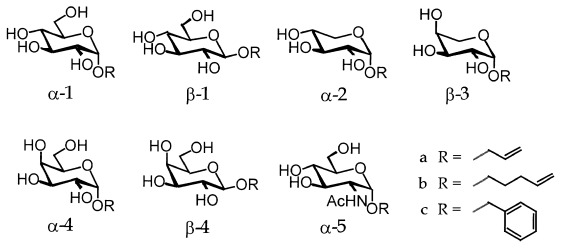
Most often, such components have very similar physical properties and thus, the separation of individual reaction products from the mixtures is rather difficult by traditional methods such as recrystallization or chromatography, which frequently give only poor yields [4,9,26].

Separation of crude product mixtures from *Fischer* type glycosidations should be greatly facilitated if the undesired glycosidic components could be selectively converted back to starting materials because the latter have very distinct physical properties. Here, we present our studies directed at using a specific glycosidase-assisted hydrolysis for the simplification of such separation problems (Scheme 1), a process design that is oriented at the natural capacity of glycosidase function.

2. Results and discussion

According to the *Fischer* method, a glycose is treated with acid in an excess of the appropriate alcohol as the solvent at elevated temperature for acetalization. In practice, the application of ion exchange resins or volatile Lewis acid (BF_3-etherate) greatly facilitates product purification [4]. Due to the thermodynamic nature of the equilibrating process, pyranoid products and α-anomeric isomers often predominate, but adjusting the reaction conditions in favor of kinetic control may influence product composition. Composition of product mixture is analyzed conveniently by ^{13}C NMR spectroscopy, e.g. on the distinct anomeric carbon signals.

Initially, the feasibility of the hydrolytic approach was studied by using allyl glucosides **1a** (Scheme 2) as a typical case. Under thermodynamically controlled conditions, the product mixture consists predominantly of α/β anomeric pyranosides (> 98%; for anomer ratio, see Table 1) with little contamination by α/β furanosides. For the specific hydrolysis of α-D-glucopyranosides (α-**1**) or β-D-glucopyranosides (β-**1**), α-glucosidase from bakers yeast and β-glucosidase from almonds were selected because the enzymes (Table 1, entries 1, 4) are inexpensive and well characterized [21,27]. Hydrolytic reactions were performed in phosphate-buffered (pH 6.8 and 5.7) aqueous solution at ambient temperature, and



Scheme 2. Compounds prepared in anomerically pure form by the stereoselective glycosidase-catalyzed hydrolysis of the opposite anomers from mixtures generated upon *Fischer* type glycosidations.

conversion was monitored by TLC control. Endpoints were verified by ^{1}H NMR spectroscopic analysis on crude concentrates. Work-up consisted of simple solvent evaporation followed by recrystallization of the crude residue from ethanol and from acetone. Pure allyl β-D-glucoside β-**1a** or α-D-glucoside α-**1a** were isolated from reactions catalyzed by α-glucosidase or β-glucosidase, respectively, in absolute isolated yields of 35% and 64% based on the total starting material used. Thus, with respect to their prevalence in the initial substrate mixture, relative yields of isolated materials amount to near quantitative recovery of the corresponding non-reactive diastereoisomers.

Using 4-pentenyl (**1b**) or benzyl D-glucoside (**1c**) mixtures, work-up conditions were modified to allow for a (partial) recovery of the aglycon moiety. Thus, corresponding reaction mixtures obtained from glucosidase treatment were first extracted with ether, and the extracts concentrated to recover the alcohols in good yields (ca. 91% and 90%). Particularly, in case of the expensive 4-pentenol [28] this allows for a cost-efficient recycling. Purification of β- or α-pentenyl (30% β-**1b**, 66% α-**1b**) and β- or α-benzyl D-glucosides (42% β-**1c**, or 55% α-**1c**) from free glucose again proved to be straightforward by crystallization of the crude residues from ethanol. A further simplification of the isolation protocol was found in the adsorption of less polar glucosides that contain a hydrophobic aglycon moiety (**1b**,**1c**) to reversed-phase silica in order to facilitate removal of the polar free sugar (> 95% recovery), followed by ethanol elution and crystallization (quantitative yield of unreactive isomer). This variation should prove particularly useful for small-scale preparation of glycosides where fractional crystallization is much less convenient.

For a broader scope of development, the pairwise availability of glycosidases having identical sugar specificity but complementary anomeric specificity is a prerequisite. In fact, for common sugars that occur naturally as constituents of a manifold of glycoconjugates, such as galactose, mannose, xylose, glucuronic acid or aminohexoses etc., a number of corresponding glycosidases have been identified with appropriate specificities for hydrolytic activity, many of which are commercially available [21,27]. In this respect, α-galactosidase from green coffee beans and β-galactosidase from *Aspergillus oryzae* were tested first with allyl α/β-D-galactosides (**4a**) in buffered solution at pH 4.5 and room temperature (Table 1, entries 16, 17). The reaction course was monitored by TLC which indicated complete conversion of the reactive diastereomers after 48 h. The solution was concentrated and the glycosides were isolated by recrystallization from ethanol or by chromatography over silica gel to furnish allyl β-D-galactoside β-**4a** in 21% and α-D-galactoside α-**4a** in 66% absolute yield. Similarly, 4-pentenyl and benzyl α-D-galactosides were produced in absolute yields of 66% (α-**4b**) and 69% (α-**4c**), respectively, and 4-pentenol was recovered by extraction in 98% yield (Table 1, entries 18, 19).

It has been established by an extensive work of Crout et al. [20] that the crude preparation of *A. oryzae* β-galactosidase can be utilized as a rich but inexpensive source of various other glycosidases with different sugar and anomeric specificity that can be purified along routine procedures. For our purpose, however, purification of individual enzymes with high specificity seemed unnecessary as long as enzymes with competing activity for anomeric pairs of glycosides were absent (or at least would contribute only at very minor relative activities; in fact, this proved true for the highly selective cleavage of β-galactosides although the presence of contaminating α-galactosidase had been reported). Since such enzymatic side activities only constitute minor contaminants in the crude preparation, their deliberate utilization required that larger total protein quantities had to be supplied for the synthetic runs. Still, this approach proved highly cost efficient due to the low

Table 1
Hydrolysis of α/β-glycosides using α-glucosidase from bakers yeast (type A), β-glucosidase from almonds (type B), crude β-galactosidase from *A. oryzae* (type C), and α-galactosidase from green coffee beans (type D)

Entry	Substrate α/β-Mixture of glycopyranoside	Anomer ratio α:β	Scale (mmol)	Glycosidase		Glycoside	Total yield (%)	Relative yield[a] (%)
				Type	(U/mmol)			
1	Allyl-D-gluco-**1a**	4.5:2.5	1.3	A	250	β-**1a**	35	100
2	Pent-4-enyl-D-gluco-**1b**	2:1	0.8	A	250	β-**1b**	30	100
3	Benzyl-D-gluco-**1c**	4:3	0.7	A	370	β-**1c**	42	99
4	Allyl-D-gluco-**1a**	4.5:2.5	31.8	B	6	α-**1a**	64	100
5	Pent-4-enyl-D-gluco-**1b**	2:1	6.9	B	6	α-**1b**	66	100
6	Benzyl-D-gluco-**1c**	4:3	29.6	B	6	α-**1c**	55	97
7	Allyl-D-gluco-**1a**	4.5:2.5	31.8	C	560[b]	α-**1a**	64	100
8	Pent-4-enyl-D-gluco-**1b**	2:1	4.8	C	560[b]	α-**1b**	66	100
9	Benzyl-D-gluco-**1c**	4:3	7.8	C	560[b]	α-**1c**	53	92
10	Allyl-D-xylo-**2a**	2:1	36.8	C	560[b]	α-**2a**	65	100
11	Pent-4-enyl-D-xylo-**2b**	2:1	5.7	C	560[b]	α-**2b**	65	98
12	Benzyl-D-xylo-**2c**	4.3:2.7	31.8	C	560[b]	α-**2c**	61	100
13	Allyl-L-arabino-**3a**	1.5:3.2	5.8	C	605[b]	β-**3a**	46	100
14	Pent-4-enyl-L-arabino-**3b**	1.8:3.1	6.2	C	605[b]	β-**3b**	45	100
15	Benzyl-L-arabino-**3c**	1.8:3.1	3.1	C	605[b]	β-**3c**	45	100
16	Allyl-D-galacto-**4a**	2:1	2.8	D	21	β-**4a**	21	64
17	Allyl-D-galacto-**4a**	2:1	31.8	C	72	α-**4a**	66	100
18	Pent-4-enyl-D-galacto-**4b**	2:1	10.5	C	72	α-**4b**	66	100
19	Benzyl-D-galacto-**4c**	4.9:2.1	19.3	C	72	α-**4c**	69	100
20	Allyl-2-acetamido-2-deoxy-D-gluco-**5a**	4.3:2.7	38.3	C	560[b]	α-**5a**	60	98
21	Pent-4-enyl-2-acetamido-2-deoxy-D-gluco-**5b**	6:1	4.1	C	560[b]	α-**5b**	76	91
22	Benzyl-2-acetamido-2-deoxy-D-gluco-**5c**	2:1	24.5	C	560[b]	α-**5c**	64	98

[a]Calculated yield based on the corresponding anomer fraction present in the starting mixture.
[b]Amount corresponding to nominal crude β-galactosidase activity.

overall expense of the crude protein and, the fact that no additional purification step was required. In order to further enhance the productivity of contaminant enzymes with low specific activities on a preparative scale, hydrolysis reactions were preferentially performed at elevated temperature (37°C). In practice, the D-xylose, L-arabinose, D-hexosamine, and D-glucose glycosidation mixtures from acidic equilibration with the abovementioned alcohols were tested with the crude β-galactosidase preparation from *A. oryzae* (Table 1, entries 7–15, 20–22). The hydrolytic reactions were conducted under standard conditions (pH 4.5), and work-up included aglycon recovery by extraction and glycoside purification by recrystallization. Allyl (α-**2a**), 4-pentenyl (α-**2b**), and benzyl α-D-xylosides (α-**2c**) were obtained in $>60\%$ absolute yields, and recovery of 4-pentenol was near quantitative (98% yield). Although acetalization of L-arabinose produced α/β pyranosides and α/β furanosides in a ratio of 71% and 29%, from selective hydrolysis of the α-L-arabinopyranoside the desired allyl (β-**3a**), 4-pentenyl (β-**3b**), and benzyl β-L-arabinopyranosides (β-**3c**) were cleanly separated from remaining furanosides and from free sugar by crystallization and were isolated in ca. 45% absolute yield (94% recovery of 4-pentenol). For these conversions it is remarkable that the β-galactosidase is specifically cleaving the α-configured arabinopyranosides, however, which is simply due to a change in the stereochemical reference between D- and L-series in carbohydrate nomenclature (cf. Scheme 2). Again, with the aid of reversed phase chromatography in the purification of 4-pentenyl and benzyl L-arabinosides, water elution allowed to efficiently recover the released free sugar ($\geq 97\%$). Representative for β-hexosaminidase activity in the same protein preparation, the allyl (α-**5a**), 4-pentenyl (α-**5b**), and benzyl 2-acetamido-2-deoxy-α-D-glucopyranosides (α-**5c**) were produced in better than 60% absolute yield. Also, β-glucosidase activity proved sufficient and selective enough to furnish pure allyl (α-**1a**), 4-pentenyl (α-**1b**), and benzyl α-D-glucosides (α-**1c**) in good absolute yields.

Initially, a commercial preparation of the crude *A. oryzae* galactosidase was applied in the synthetic studies, which was later realized to be discontinued by the supplier (Sigma; prep G-7138). Gratifyingly, subsequent experimentation verified the replacement product (Sigma; prep G-5160) to be fully equivalent and compatible with all of the above applications.

α-Fucosidase activities were reported to be present in inexpensive, partially purified preparations of β-glucuronidase from bovine liver (Sigma, B-1) and from scallops (Sigma, S-1) [20]. However, all our attempts for the resolution of α/β-fucosides with unpurified protein failed so far, inspite of highly increased catalyst loading. Similarly, the reported α-galactosidase activity in crude commercial ficin [20] could not be used on a preparative scale.

3. Conclusions

The glycosidase-catalyzed hydrolytic differentiation of product mixtures from *Fischer*-type glycosidations has proved to be a straightforward and highly practical alternative to established enzymatic routes for anomer selective glycoside synthesis. The well-distinguished physical properties of glycosides and the corresponding free glycose strongly facilitate their separation by simple fractional crystallization from appropriate solvents. Given the availability of the corresponding glycosidases, the technique gives rise to diastereomerically pure α- or β-glycopyranosides in good overall yields. If desired, both the glycose and aglycon synthetic components can be easily recycled to render the overall process highly scalable and cost-efficient. In comparison to other glycosidase-based methods of glycoside synthesis (transglycosylation, reverse hydrolysis), the hydrolytic differentiation of glycoside mixtures — as outlined here — makes more efficient usage of enzymatic activities due to their involvement for a task that is shaped after their natural function, and due to a process design that is assisted by thermodynamic considerations.

4. Experimental

4.1. Materials and methods

NMR spectra were recorded on Bruker AC-300 and Bruker ARC-300 spectrometers; chemical shifts

are referenced to internal TMS (DMSO) or TSP (D_2O; 0.00 ppm). Mass spectra were recorded on a Bruker Esquire-LC system and elemental analyses were performed on a Heraeus CHN-O-Rapid system. Column chromatography was performed on Merck 60 silica gel (0.063–0.200 mesh), and analytical thin-layer chromatography was performed on Merck silica gel plates 60 GF_{254} using anisaldehyde stain for detection. All R_f values were determined using CH_2Cl_2/CH_3OH (85:15) as eluent. C_{18}-Silica gel 100 (Fluka, 0.040–0.063 mm) was used for reversed phase adsorption. Analytical grade ion exchange media (100–200 mesh) were purchased from Bio-Rad. α-Glucosidase from bakers yeast (Fluka, 63412), β-glucosidase from almonds (Fluka, 49290), α-galactosidase from green coffee beans (Sigma, G 8507), and β-galactosidase from *A. oryzae* (Sigma, G-5160 or G-7138) were used as commercially supplied.

4.2. Synthesis of glycosides

The glycosides **1**–**5** were prepared by heating of a mixture of the free sugar and the alcohol in the presence of ion exchange resin or BF_3-etherate according to the published method [4] (**2** and **3** analogous to the procedure for allyl-D-glucopyranosides). Acetal side-products arising upon glycosidations for **1a**, **4a**, and **5a** were removed by extraction with diethyl ether or ethyl acetate as described [29].

4.3. General procedure for enzymatic hydrolysis

A buffered solution containing the glycoside anomeric mixture (0.4 M) was incubated with the appropriate glycosidase (see Table 1) at room temperature with slow agitation. Reactions using β-glucosidase, α-glucosidase, and β-galactosidase were conducted in phosphate buffers (0.2 M) at pH 5.7, 6.8, and 4.5, respectively, and those using α-galactosidase were performed in a citrate buffer (5 mM) at pH 4.5. Reactions with crude β-galactosidase for contaminant activities was incubated with 0.1 M glycosides at 37°C. Conversion of the reactive anomer, as monitored by NMR analysis of aliquots withdrawn periodically (or by TLC for certain cases), reached completion within 24–48 h. The mixture was extracted three times with diethyl ether (10 ml each), and the ether was removed by rotatory evaporation at 100 mbar and at room temperature for recovery of the aglycon alcohol. The aqueous reaction phase was concentrated by rotatory evaporation under vacuum, and the residue was dried by azeotropic concentration from acetone. The solid residue was crystallized from appropriate solvents to furnish colorless crystals of the anomerically pure unreactive glycoside (for yields, see Table 1). Glycosides β-**1a-c** and β-**4a** were purified by flash chromatography (CH_2Cl_2/CH_3OH, 85/15) prior to crystallization.

Crystallization was performed from ethanol (α-**1a**); ethanol, then acetone (α-**1a-b**, α-**4b**); ethanol, then CH_2Cl_2 (α-**1c**, β-**3a-c**, α-**4c**); acetone (α-**5c**); acetone, then CH_2Cl_2 (α-**2b-c**, α-**5b**); acetone, then 1-propanol/Et_2O (α-**2a**, α-**5a**). Glycosides β-**1a-c**, β-**4a**, and α-**4b** were identified by comparison of their physical and spectroscopic data to those described in the literature [4,22,23,26].

For an alternative work-up of glycosides with pent-4-enyl and benzyl aglycon moieties, the reaction mixtures were passed directly through reversed phase silica gel. Free sugars were recovered by water elution followed by roto-evaporation, and the remaining glycosides were eluted with ethanol, followed by solvent evaporation and crystallization.

α-Allyl-D-glucopyranoside (α-**1a**): $R_f = 0.25$.- ^{1}H NMR (300 MHz, D_2O): $\delta = 6.0$ (dddd, 2-H), 5.37 (dd, 3-H_E), 5.27 (dd, 3-H_Z), 4.96 (d, 1′-H), 4.24 (dd, 1-H_a), 4.08 (dd, 1-H_b), 3.87-3.66 (m, 6′-H_{ab}, 3′-H, 5′-H), 3.56 (dd, 2′-H), 3.42 (dd, 4′-H); $J_{1a,1b} = 12.8$, $J_{1a,2} = 6.0$, $J_{1b,2} = 5.6$, $J_{2,3E} = 17.2$, $J_{2,3Z} = 10.4$, $J_{3E,3Z} = 0.8$, $J_{1',2'} = 3.7$, $J_{2',3'} = 9.7$, $J_{3',4'} = J_{4',5'}$ 9.3 Hz.- ^{13}C NMR (75.4 MHz, D_2O): $\delta = 136.3$ (C-2), 121.0 (C-3), 100.1 (C-1′), 75.9 (C-3′), 74.6 (C-5′), 74.0 (C-2′), 72.4 (C-4′), 71.2 (C-1), 63.3 (C-6′).- MS (ESI): m/z (%) = 242.9 (100) $[M + Na]^+$.- $C_9H_{16}O_6$: 220.09.

α-Pent-4-enyl-D-glucopyranoside (α-**1b**): $R_f = 0.30$.- ^{1}H NMR (300 MHz, D_2O): $\delta = 5.93$ (dddd, 4-H), 5.10 (m, 5-H_E), 5.03 (m, 5-H_Z), 4.91 (d, 1′-H), 3.85 (dd, 6′-H_a), 3.8-3.65 (m, 6′-H_b, 3′-H, 5′-H, 2′-H), 3.58–3.50 (m, 1-H_{ab}), 3.41 (t, 4′-H), 2.15 (m, 3-H_{ab}), 1.75 (m, 2-H_{ab}); $J_{3a,4} = 6.7$, $J_{3b,4} = 6.5$, $J_{4,5Z} = 10.3$, $J_{4,5E} = 17.1$, $J_{1',2'} = 3.6$, $J_{3',4'} = 9.0$,

$J_{4',5'} = 9.4$, $J_{5',6'} = 2.0$, $J_{6a',6b'} = 12.0$ Hz.- ^{13}C NMR (75.4 MHz, D_2O): $\delta = 141.8$ (C-4), 117.6 (C-5), 100.9 (C-1′), 76.0 (C-3′), 74.6 (C-5′), 74.2 (C-2′), 72.4 (C-4′), 70.4 (C-1), 63.4 (C-6′), 32.5 (C-3), 30.7 (C-2).- MS (ESI): m/z (%) = 271.3 (100) $[M + Na]^+$.- $C_{11}H_{20}O_6$: 248.13.

α-Benzyl-D-glucopyranoside (α-**1c**): $R_f = 0.34$. ^{1}H NMR (300 MHz, D_2O): $\delta = 7.49$-7.38 (m, H_{Ar}), 5.03 (d, 1′-H), 4.74 (d, 1-H_a), 4.62 (d, 1-H_b), 3.77-3.66 (m, 6′-H_{ab}, 3′-H, 5′-H), 3.56 (dd, 2′-H), 3.43 (t, 4′-H); $J_{1a,1b} = 11.7$, $J_{1',2'} = 3.8$, $J_{2',3'} = 9.8$, $J_{3',4'} = J_{4',5'} = 9.3$ Hz.- ^{13}C NMR (75.4 MHz, D_2O): δ = 139.9 (C_{Ar}), 131.6 (C_{Ar}), 131.4 (C_{Ar}), 131.1 (C_{Ar}), 100.5 (C-1′), 75.9 (C-3′), 74.8 (C-5′), 74.1 (C-2′), 72.5 (C-1), 72.4 (C-4′), 63.2 (C-6′).- MS (ESI): m/z (%) = 293 (100) $[M + Na]^+$.- $C_{13}H_{18}O_6$: 270.11.

α-Allyl-D-xylopyranoside (α-**2a**): $R_f = 0.4$.- ^{1}H NMR (300 MHz, D_2O): $\delta = 5.97$ (dddd, 2-H), 5.38 (dd, 3-H_E), 5.27 (d, 3-H_Z), 4.93 (d, 1′-H), 4.23 (dd, 1-H_a), 4.06 (dd, 1-H_b), 3.71–3.52 (m, 5′-H_{ab}, 2′-H, 3′-H, 4′-H); $J_{1a,1b} = 12.9$, $J_{1a,2} = 5.8$, $J_{1b,2} = 5.7$, $J_{2,3E} = 16.9$, $J_{2,3Z} = 10.4$, $J_{3E,3Z} = 1.4$, $J_{1',2'} = 3.5$ Hz.- ^{13}C NMR (75.4 MHz, D_2O): $\delta = 136.4$ (C-2), 121.2 (C-3), 100.3 (C-1′), 76.077 (C-2′), 74.1 (C-3′), 72.3 (C-4′), 71.5 (C-1), 64.1 (C-5′).- MS (ESI): m/z (%) = 212.6 (100) $[M + Na]^+$.- $C_8H_{14}O_5$: 190.08.

α-Pent-4-enyl-D-xylopyranoside (α-**2b**): R_f = 0.45.- ^{1}H NMR (300 MHz, D_2O/DMSO): $\delta = 5.91$ (dddd, 4-H), 5.15–5.04 (m, 5-H_{EZ}), 4.79 (d, 1′-H), 3.72-3.39 (m, 5′-H_{ab}, 2′-H, 3′-H, 4′-H, 1-H_{ab}), 2.21–2.14 (m, 3-H_{ab}), 1.77–1.69 (m, 2-H_{ab}); $J_{3a,4} = J_{3b,4}$ = 6.5, $J_{4,5Z} = 10.3$, $J_{4,5E} = 17.0$, $J_{1',2'} = 3.5$ Hz.- ^{13}C NMR (75.4 MHz, D_2O/DMSO): $\delta = 141.8$ (C-4), 118.5 (C-5), 101.7 (C-1′), 76.4 (C-2′), 74.7 (C-3′), 72.7 (C-4′), 70.5 (C-1), 64.5 (C-5′), 33.0 (C-3), 31.2 (C-2).- MS (ESI): m/z (%) = 240.7 (100) $[M + Na]^+$.- $C_{10}H_{18}O_5$: 218.12.

α-Benzyl-D-xylopyranoside (α-**2c**): $R_f = 0.45$.- ^{1}H NMR (300 MHz, D_2O/DMSO): $\delta = 7.43$-7.36 (m, H_{Ar}), 4.88 (d, 1′-H), 4.73 (d, 1-H_a), 4.52 (d, 1-H_b), 3.61–3.43 (m, 5′-H_{ab}, 2′-H, 3′-H, 4′-H); $J_{1a,1b} = 11.9$, $J_{1',2'} = 3.3$ Hz.- ^{13}C NMR (75.4 MHz, D_2O/DMSO): $\delta = 40.5$ (C_{Ar}), 131.9 (C_{Ar}), 131.3 (C_{Ar}), 100.9 (C-1′), 76.4 (C-2′), 74.6 (C-3′), 72.7 (C-4′), 72.2 (C-1), 64.6 (C-5′).- MS (ESI): m/z (%) = 262.8 (100) $[M + Na]^+$.- $C_{12}H_{16}O_5$: 240.10.

β-Allyl-L-arabinopyranoside (β-**3a**): $R_f = 0.37$.- ^{1}H NMR (300 MHz, D_2O): $\delta = 5.99$ (dddd, 2-H), 5.38 (ddd, 3-H_E), 5.28 (ddd, 3-H_Z), 4.99 (d, 1′-H), 4.22 (dddd, 1-H_a), 4.07 (dddd, 1-H_b), 4.0 (dd, 4′-H), 3.92 (dd, 5′-H_a), 3.87-3.85 (m, 2′-H, 3′-H), 3.65 (dd, 5′$_b$); $J_{1a,1b} = 12.9$, $J_{1a,2} = 5.5$, $J_{1a,3E} = 1.1$, $J_{1a,3Z}$ = 1.3, $J_{1b,2} = 6.3$, $J_{1b,3E} = J_{1b,3Z} = 1.1$, $J_{2,3Z} = 10.8$, $J_{2,3E} = 17.0$, $J_{3E,3Z} = 2.7$, $J_{1',2'} = 3.0$, $J_{3',4'} = 3.9$, $J_{4',5a'} = 1.1$, $J_{4',5b'} = 2.1$, $J_{5a',5b'} = 12.7$ Hz.- ^{13}C NMR (75.4 MHz, D_2O): $\delta = 136.5$ (C-2), 121.0 (C-3), 100.8 (C-1′), 71.8 (C-4′), 71.7 (C-2′), 71.6 (C-1), 71.0 (C-3′), 65.6 (C-5′).- MS (ESI): m/z (%) = 213.2 (100) $[M + Na]^+$.- $C_8H_{14}O_5$: 190.08.

β-Pent-4-enyl-L-arabinopyranoside (β-**3b**): R_f = 0.41.- ^{1}H NMR (300 MHz, D_2O): δ = 5.91 (dddd, 4-H), 5.09 (m, 5-H_E), 5.02 (m, 5-H_Z), 4.91 (d, 1′-H), 3.98 (m, 4′-H), 3.90 (d, 5′-H_a), 3.84 (dd, 2′-H), 3.82 (m, 3′-H), 3.70 (m, 1-H_a), 3.63 (dd, 5′-H_b), 3.51 (m, 1-H_b), 2.19-2.12 (m, 3-H_{ab}), 1.78-1.68 (m, 2-H_{ab}); $J_{3a,4} = J_{3b,4} = 6.6$, $J_{4,5Z} = 10.4$, $J_{4,5E} = 17.1$, $J_{1',2'}$ = 3.1, $J_{2',3'} = 5.5$, $J_{4',5a'} = J_{4',5b'} = 2.0$, $J_{5a',5b'} = 12.6$ Hz.- ^{13}C NMR (75.4 MHz, D_2O): $\delta = 141.7$ (C-4), 117.5 (C-5), 101.5 (C-1′), 71.7 (C-4′), 71.7 (C-2′), 71.1 (C-3′), 70.5 (C-1), 65.5 (C-5′), 32.5 (C-3), 30.6 (C-2).- MS (ESI): m/z (%) = 241.2 (100) $[M + Na]^+$.- $C_{10}H_{18}O_5$: 218.12.

β-Benzyl-L-arabinopyranoside (β-**3c**): $R_f = 0.41$.- ^{1}H NMR (300 MHz, DMSO): $\delta = 7.40$-7.25 (m, H_{Ar}), 4.76 (d, 1′-H), 4.66 (d, 1-H_a), 4.45 (d, 1-H_b), 3.72 (m, 4′-H), 3.68 (dd, 5′-H_a), 3.64-3.60 (m, 2′-H, 3′-H), 3.47 (dd, 5′-H_b); $J_{1a,1b} = 12.3$, $J_{1',2'} = 1.4$, $J_{4',5a'} = 1.8$, $J_{4',5b'} = 2.8$, $J_{5a',5b'} = 11.9$ Hz.- ^{13}C NMR (75.4 MHz, DMSO): $\delta = 138.1$ (C_{Ar}), 128.1 (C_{Ar}), 127.4 (C_{Ar}), 127.3 (C_{Ar}), 98.8 (C-1′), 68.8 (C-4′), 68.4 (C-2′), 68.3 (C-1), 68.1 (C-3′), 63.2 (C-5′). MS (ESI): m/z (%) = 263.2 (100) $[M + Na]^+$.- $C_{12}H_{16}O_5$: 240.10.

α-Allyl-D-galactopyranoside (α-**4a**): $R_f = 0.27$.- ^{1}H-NMR (300 MHz, D_2O): $\delta = 6.0$ (dddd, 2-H), 5.38 (dd, 3-H_E), 5.28 (dd, 3-H_Z), 4.99 (d, 1′-H), 4.23 (ddd, 1-H_a), 4.07 (ddd, 1-H_b), 3.99 (d, 4′-H), 3.97 (t, 5′-H) 3.84 (dd, 3′-H, m, 6′-H_a) 3.72 (dd, 2′-H, m, 6′-H_b); $J_{1a,1b} = 12.8$, $J_{1a,2} = 5.5$, $J_{1b,2}$ = 6.3, $J_{1a,3E} = J_{1b,3E} = 0.9$, $J_{2,3E} = 17.4$, $J_{2,3Z} = 10.4$, $J_{3E,3Z} = 1.5$, $J_{1',2'} = 3.1$, $J_{2',3'} = 10.4$, $J_{3',4'} = 3.0$, $J_{5',6a'} = J_{5',6b'} = 6.4$ Hz.- ^{13}C-NMR (75.4 MHz, D_2O): $\delta = 136.5$ (C-2), 121.1 (C-3), 100.4 (C-1′), 73.8 (C-5′), 72.4 (C-3′), 72.1 (C-4′), 71.4 (C-1) 71.1 (C-2′), 64.1 (C-6′).- MS (ESI): m/z (%) = 243.1 (100) $[M + Na]^+$.- $C_9H_{16}O_6$: 220.09.

α-Benzyl-D-galactopyranoside (α-**4c**): $R_f = 0.28$.- ^{1}H NMR (300 MHz, D_2O): $\delta = 7.46–7.34$ (m, H_{Ar}), 5.04 (d, 1′-H), 4.73 (d, 1-H_a), 4.57 (d, 1-H_b), 3.93 (d, 4′-H), 3.90 (dd, 5′-H) 3.85-3.80 (m, 3′-H, 6′-H_a), 3.73-3.65 (m, 2′-H, 6′-H_b); $J_{1a,1b} = 11.6$, $J_{1',2'} = 2.9$, $J_{3',4'} = 2.6$, $J_{5',6a'} = J_{5',6b'} = 6.1$ Hz.- ^{13}C NMR (75.4 MHz, D_2O): $\delta = 140.0$ (C_{Ar}), 131.6 (C_{Ar}), 131.4 (C_{Ar}), 131.2 (C_{Ar}), 100.7 (C-1′), 73.8 (C-5′), 72.5 (C-1), 72.4 (C-3′), 72.1 (C-4′), 71.2 (C-2′), 63.9 (C-6′).- MS (ESI): m/z (%) = 293 (100) $[M + Na]^+$.- $C_{13}H_{18}O_6$: 270.11.

Allyl-2-acetamido-2-desoxy-α-D-glucopyranoside (α-**5a**): $R_f = 0.34$.- ^{1}H NMR (300 MHz, D_2O): $\delta =$ 5.90 (dddd, 2-H), 5.35 (dddd, 3-H_E), 5.26 (dd, 3-H_Z), 4.92 (d, 1′-H), 4.22 (dddd, 1-H_a), 4.03 (dddd, 1-H_b), 3.93 (dd, 2′-H), 3.87 (dd, 6′-H_a), 3.83–3.70 (m, 6′-H_b, 3′-H, 5′-H), 3.50 (dd, 4′-H), 2.05 (s, CH_3); $J_{1a,1b} = 13.2$, $J_{1a,2} = 5.2$, $J_{1a,3E} = 1.3$, $J_{1a,3Z} = 1.4$, $J_{1b,2} = 6.0$, $J_{1b,3E} = J_{1b,3Z} = 1.3$, $J_{2,3E} = 17.2$, $J_{2,3Z} = 10.4$, $J_{3E,3Z} = 1.6$, $J_{1',2'} = 3.6$, $J_{2',3'} = 10.7$, $J_{3',4'} = 9.4$, $J_{4',5'} = 9.2$, $J_{5',6a'} = 1.8$, $J_{6a',6b'} = 12.0$ Hz.- ^{13}C NMR (75.4 MHz, D_2O): $\delta = 177.3$ (C═O), 136.5 (C-2), 120.7 (C-3), 98.9 (C-1′), 74.8 (C-5′), 73.8 (C-3′), 72.8 (C-4′), 71.3 (C-1), 63.4 (C-6′), 56.5 (C-2′), 24.7 (CH_3).- MS (FD): m/z (%): 261 (100) $[M]^+$.- $C_{11}H_{19}NO_6$: 261.12.

Pent-4-enyl-2-acetamido-2-desoxy-α-D-glucopyranoside (α-**5b**): $R_f = 0.46$.- ^{1}H NMR (300 MHz, D_2O): $\delta = 5.89$ (dddd, 4-H), 5.09–5.00 (m, 5-H_{EZ}), 4.85 (d, 1′-H), 3.83 (dd, 2′-H), 3.78-3.68 (m, 3′-H, 6′-H_{ab}, 5′-H, 1-H_{ab}), 3.48 (dd, 4′-H), 2.18–2.11 (m, 3-H_{ab}), 2.0 (s, CH_3), 1.74–1.67 (m, 2-H_{ab}); $J_{3a,4} = J_{3b,4} = 6.6$, $J_{4,5Z} = 10.3$, $J_{4,5E} = 17.1$, $J_{1',2'} = 3.5$, $J_{2',3'} = 10.6$ $J_{3',4'} = J_{4',5'} = 8.8$ Hz.- ^{13}C NMR (75.4 MHz, D_2O): $\delta = 177.0$ (C═O), 141.7 (C-4), 117.6 (C-5), 99.5 (C-1′), 74.5 (C-5′), 73.7 (C-3′), 72.8 (C-4′), 70.1 (C-1), 63.3 (C-6′), 56.6 (C-2′), 32.4 (C-3), 30.5 (C-2), 24.7 (CH_3).- MS (ESI): m/z (%) = 311.9 (100) $[M + Na]^+$.- $C_{13}H_{23}NO_6$: 289.15.

Benzyl-2-acetamido-2-desoxy-α-D-glucopyranoside (α-**5c**): $R_f = 0.51$.- ^{1}H NMR (300 MHz, D_2O/DMSO): $\delta = 7.44$ (m, H_{Ar}), 4.92 (d, 1′-H), 4.75 (d, 1-H_a), 4.53 (d, 1-H_b), 3.93-3.74 (m, 2′-H, 3′-H, 6′-H_{ab}, 5′-H), 3.53 (t, 4′-H), 1.96 (s, CH_3); $J_{1a,1b} = 11.9$, $J_{1',2'} = 3.4$, $J_{3',4'} = J_{4',5'} = 9.2$ Hz.- ^{13}C NMR (75.4 MHz, D_2O/DMSO): $\delta = 177.1$ (C═O), 139.8 (C_{Ar}), 131.6 (C_{Ar}), 131.3 (C_{Ar}), 131.2 (C_{Ar}), 98.7 (C-1′), 74.9 (C-5′), 73.7 (C-3′), 72.8 (C-4′), 72.4 (C-1), 63.3 (C-6′), 56.5 (C-2′), 24.6 (CH_3).- MS (ESI): m/z (%) = 334.1 (100) $[M + Na]^+$.- $C_{15}H_{21}NO_6$: 311.14.

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