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Facile synthesis of pseudo-*C*-glycosyl *p*-amino-DL-phenylalanine building blocks via Amadori rearrangement

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ABSTRACT

We studied the synthesis of pseudo-*C*-glycosyl amino acid via an Amadori rearrangement in aqueous solution using unprotected D-lactose and a tyrosine analogue: the *p*-amino-DL-phenylalanine. Two steps were necessary. In the first step, the N-glycosylation of D-lactose was carried out in aqueous conditions. The synthesized *N*-glycosylamine was stabilized in a second step by the formation of Amadori compound, the *N*-[β-D-galactosyl-1-4-(1-deoxyfructos-1-yl)]-*p*-amino-DL-phenylalanine. Products were purified and characterized by mass spectrometry and by ^{1}H and ^{13}C NMR. The influence of the temperature, the pH, the nature of acid and the concentration of the acid on the synthesis yield was examined in order to determine the optimum conditions of Amadori rearrangement. In the best conditions, 35% of *p*-amino-DL-phenylalanine was converted into *N*-[β-D-galactosyl-1-4-(1-deoxyfructos-1-yl)]-*p*-amino-DL-phenylalanine. For the N-glycosylation, a specific base catalysis took place in the media whereas a general acid catalysis was observed for the Amadori rearrangement using weak acids and with a temperature close to 75 °C. The Amadori compound from glucose [*N*-(1-deoxyfructopyranos-1-yl)-*p*-amino-DL-phenylalanine] was also synthesized and characterized by mass spectrometry and by ^{1}H and ^{13}C NMR.

1. Introduction

O-Linked glycopeptides represent an extensive and critically important group of glycoconjugates with the O-glycosylation of a variety of hydroxylated α-amino acids, in particular serine and threonine, and play a significant role in a number of key cellular processes.[1] To probe and understand the mechanisms of these processes, access to effective molecular tools is a prerequisite, and the emergence of *C*-glycosides[2] as chemically and metabolically stable carbohydrate analogues has led to interest in the potential of the corresponding *C*-glycosyl amino acids. *C*-Glycosides have already been employed to mimic both the conformation (structure)[3] and biological profile (function)[4] of a variety of *O*-glycoconjugates. *C*-Glycosyl amino acids, which could be readily incorporated into larger and biologically more relevant molecular frameworks (using established peptide methodologies), would provide novel tools to study those carbohydrate-based interactions associated with *O*-glycopeptides. As a consequence, the synthesis of a series of different *C*-glycosyl amino acids has been described,[5] and more recently, the development and exploitation of these units as components for glycopeptide synthesis has been reported.[6] Unfortunately, the few reported syntheses of natural products containing *C*-glycosyl α-amino acids are usually complicated, require long protection–deprotection steps of amino acids and sugars for selectivity control and give low final yields.

The Amadori reaction, which is the first step in 'Maillard browning',[7] is a potential non-enzymatic way to link reducing carbohydrates to complex biomolecules with reactive amino groups. The Amadori rearrangement involves the reaction of a α-hydroxy-aldehyde with a suitable amine leading to the corresponding *N*-glycosylamine, which is rearranged into the corresponding ketosamine, the so-called Amadori rearrangement product. An early mechanism for the Amadori rearrangement, which is still deemed to be the most acceptable was first suggested by Hodge[8] (Scheme 1) in 1953. The initial reaction between the anomeric position of aldose **1** and an amino group leads to the formation of *N*-glycosylamine **2**. After ring opening and protonation, the cationic form of the Schiff base **3** is formed, which is in equilibrium with the enol **4**. This enol is stabilized by the formation of 1-amino-1-deoxyketohexose **5**, which undergoes ring closure to the corresponding hemiketal **6**.

In this paper, we report the first facile synthesis of pseudo-*C*-glycosyl α-amino acid building blocks for glycopeptide synthesis using the Amadori rearrangement strategy from an unprotected tyrosine analogue called *p*-amino-DL-phenylalanine (pAmP) and unprotected sugars, especially D-lactose.

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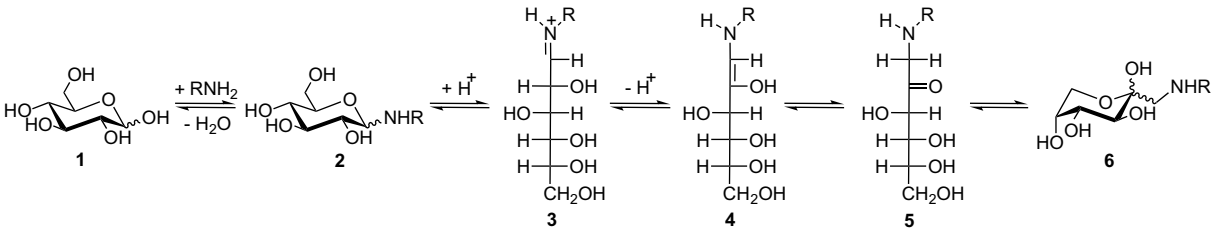


Scheme 1. Amadori rearrangement suggested by Hodge.[8]

2. Results and discussion

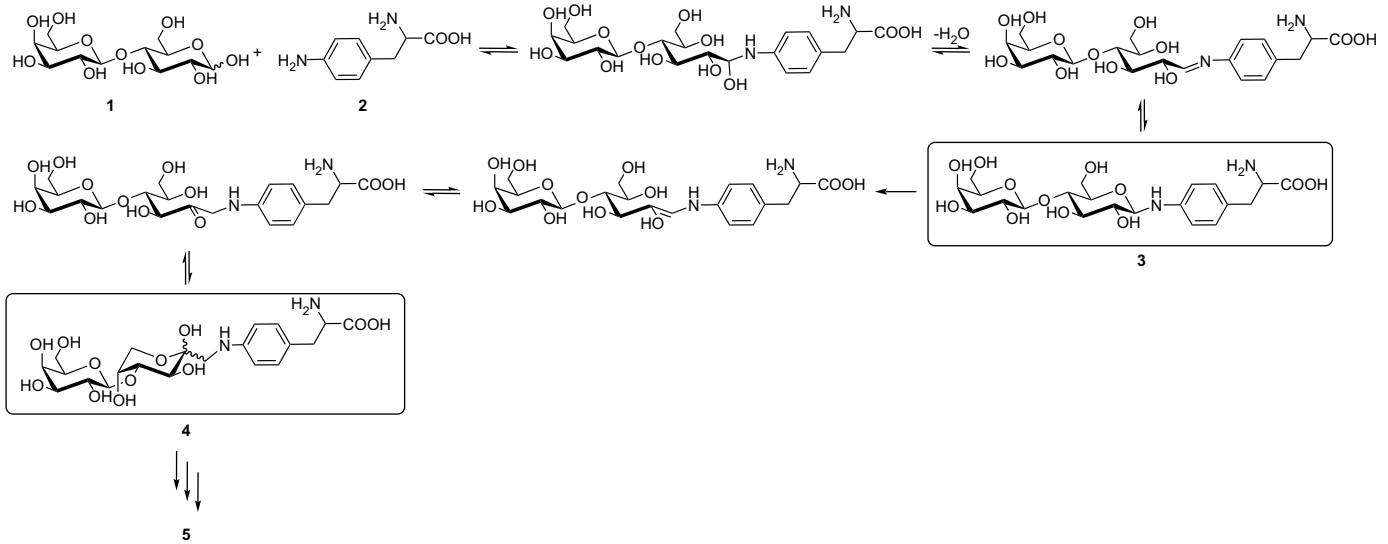
Recently, we have studied the stereoselective synthesis of β-*N*-aryl-glycosides and notably of several β-*N*-glycosyl-*p*-amino-DL-phenylalanine building blocks using unprotected carbohydrates in aqueous citrate/phosphate buffer pH 8 and at 40 °C.[9] Analysis of products by ^{1}H and ^{13}C NMR indicated that the Amadori rearrangement had not occurred after formation of the stereoselective β-*N*-glycosidic bond in the above mentioned experimental conditions. The study of the chemical and enzymatic stability in aqueous media of synthesized β-*N*-aryl-glycosides was also investigated. We have shown that the *N*-glycosidic linkage was relatively stable at pH close to 7 and more stable than the *O*-glycosidic bond to enzymatic hydrolysis. In order to increase the stability of these synthesized building blocks, the synthesis of pseudo-*C*-glycosyl α-amino acids using the Amadori rearrangement strategy from unprotected *p*-amino-DL-phenylalanine and unprotected sugars was investigated.

In a first reaction we decided to carry out the condensation of *p*-amino-DL-phenylalanine **2** (250 mM) and D-lactose **1** (1 M) in a mixture of water/acetic acid (30:1, v/v) and at 75 °C (Scheme 2). The HPLC analysis indicated that the rapid decrease of **2** concentration was proportional to the synthesis of several products (Fig. 1). After 30 min of reaction, 50% of *p*-amino-DL-phenylalanine **2** was converted into a major product **3**. Once formed, this compound **3** was purified by preparative HPLC and analyzed by mass spectrometry, ^{1}H and ^{13}C NMR, confirming that it corresponds to β-*N*-lactosyl-*p*-amino-DL-phenylalanine. Then, this kinetic compound produced during the first 30 min of the reaction disappeared. At the same time, one secondary compound **4** was synthesized. This one was slowly transformed into several thermodynamic products **5**. Once formed, the product **4** was purified and analyzed as previously. Analysis of product **4** confirmed that this compound corresponds to Amadori rearrangement product (*N*-[β-D-galactosyl-1-4-(1-deoxyfructos-1-yl)]-*p*-amino-DL-phenylalanine). The most probable form of the 1-deoxyfructos-1-yl carbohydrate moiety is the β-D-pyranose ring with a lowest energy $^{2}C_{5}$ chair conformation, according to the few crystal X-ray studies of 1-deoxy-1-substituted fructose derivatives, especially sugar-amino acid Amadori compounds.[10] After 4 h of reaction, 33% of *p*-amino-DL-phenylalanine **2** was converted into **4**. This Amadori compound is the first and initial step of the Maillard reaction cascade leading to products **5**.

To optimize the synthesis of **4** and to find conditions allowing the product **4** to be sufficiently stable to be purified, we performed the reaction under different experimental conditions.

2.1. Effect of temperature

Figure 2 shows the effect of reaction temperature on the relative synthesis yield of **3** and **4**. Reactions were carried out in a mixture of water/acetic acid (30:1, v/v) and in pure water without acetic acid addition, using the same substrates' concentrations as previously. We observed that whatever the temperature is, the major compound produced after 4 h is **3** in pure water without acetic acid addition. The optimal reaction temperature is about 50 °C for the synthesis of **3**. When the temperature increases, above 50 °C, the relative synthesis yield of **3** decreases and the synthesis of **4** slightly increases. In contrast, in a mixture of water/acetic acid (30:1, v/v), we observed that an increase of the temperature mainly helps the synthesis of **4**. The optimal reaction temperature is 75 °C for synthesis of **4** (close to 33%). When the temperature is over 75 °C, the relative yield of synthesis of **3** and **4** is not enhanced but decreases. The present studies indicate that Amadori rearrangement is carried



Scheme 2. Formation of *N*-lactosyl-*p*-amino-DL-phenylalanine **3** and Amadori rearrangement product **4** from D-lactose **1** and *p*-amino-DL-phenylalanine **2** in aqueous conditions.

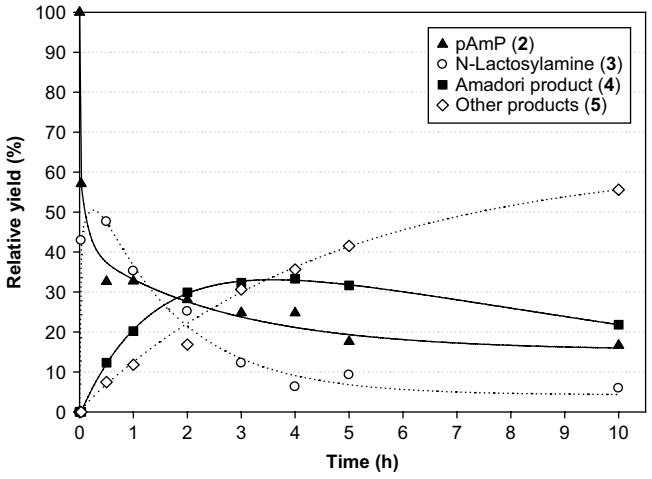


Figure 1. Time course of the condensation of *p*-amino-DL-phenylalanine **2** (250 mM) with D-lactose **1** (1 M) in a mixture of water/acetic acid (30:1, v/v) and at 75 °C; 100% corresponds to 250 mM.

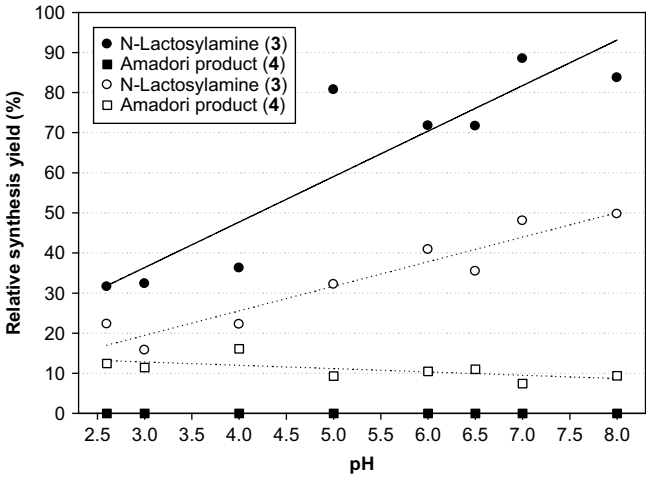


Figure 3. Effect of pH on the relative synthesis yield of **3** and **4** after 4 h of reaction. Reactions were carried out at 40 (●, ■) and 75 °C (○, □) in aqueous buffer pH 2.6–5 (citrate/phosphate) and pH 6–8 (phosphate); 100% corresponds to 250 mM.

out in acidic conditions and with an optimal temperature of 75 °C. With temperature over 75 °C, the decomposition of the Amadori compound into other products **5** is favoured only in acidic conditions.

2.2. Effect of pH

The effect of pH on the relative synthesis yield of **3** and **4** was studied at 40 and 75 °C in aqueous buffer pH 2.6–5 (citrate/phosphate) and pH 6–8 (phosphate), using the same substrates' concentrations as previously (Fig. 3). Compound **3** was shown to be the only product formed at 40 °C whatever the pH is, its synthesis yield being favoured when the pH increases. At 75 °C, compounds **3** and **4** were synthesized. As well as at 40 °C, the synthesis yield of **3** increases when the pH increases. On the other hand, the synthesis yield of compound **4** (close to 15%) is not influenced by the pH. These results confirm the favourable effect of heating for the synthesis of **4**, and clearly show that the N-glycosylation is controlled by the solution pH, contrarily to the Amadori rearrangement.

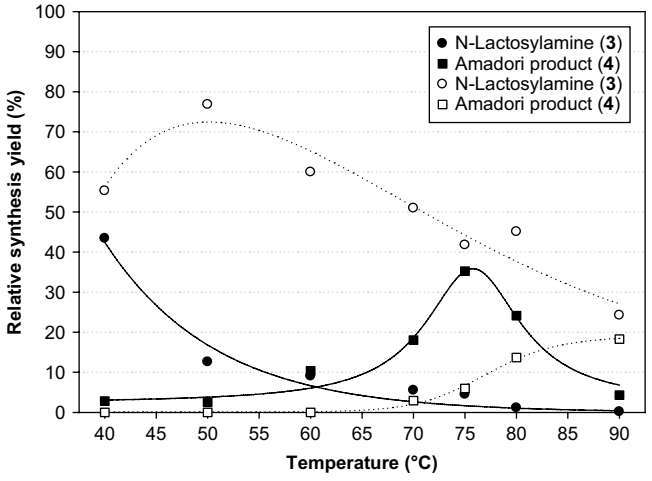


Figure 2. Effect of temperature on the relative synthesis yield of **3** and **4** after 4 h of reaction in a mixture of water/acetic acid (30:1, v/v) (●, ■) and in pure water (○, □); 100% corresponds to 250 mM.

2.3. Effect of the acid nature

The influence of the acid nature on the synthesis is shown in Table 1. Reactions were carried out at 75 °C with *p*-amino-DL-phenylalanine **2** (250 mM), D-lactose **1** (1 M) in an aqueous solution of acid (0.563 M) for 4 h. It was shown that the synthesis of **3** and **4** is not favoured when using strong acids (pK_a lower than 1) such as H_2SO_4, HCl and TFA. On the other hand, the weak acids are more effective (acetic acid, propionic acid and citrate/phosphate buffer) and allow the synthesis of **3** and **4** to be performed. The best synthesis yield of compound **4** (close to 35%) is obtained with propionic acid (the weakest acid, pK_a 4.87).

2.4. Effect of the acid concentration

The effect of the acetic acid concentration on the synthesis yield is shown in Table 2. Reactions were carried out at 75 °C with *p*-amino-DL-phenylalanine **2** (250 mM), D-lactose **1** (1 M) in a mixture of water/acetic acid for 4 h. The conversion yield of **2** was shown to increase in conjunction with an increase of the acid concentration, the selectivity of the synthesis depending on the concentration of acid used. The best synthesis yield of compound **4** (close to 33%) is obtained with a concentration of 0.563 M of acetic acid. Moreover, when the acid concentration is higher than 0.563 M, the decomposition of the Amadori compound in other products **5** increases.

Table 1
Influence of the acid nature on the synthesis

Acid	pK_a	*p*-Amino-DL-phenylalanine **2** conversion (%)	Relative synthesis yield		
			3 (%)	**4** (%)	**5** (%)
Sulfuric acid	−3; 1.9	69.3±0.5	0±0	0±0	69.3±0.5
Chloridric acid	−3	42.5±11.8	0.6±0.9	2.2±1.3	39.7±9.6
Trifluoroacetic acid	0.3	68.9±4.9	1.1±0.9	3.8±4.4	64±0.4
Acetic acid	4.76	75.2	6.3	33.3	35.6
Propionic acid	4.87	70.9±10.3	4.7±0.7	34.7±7.7	31.5±3.4
Citrate/phosphate buffer pH 2.6	Citrate (3.13; 4.76; 6.4); phosphate (2.12; 7.21; 12.67)	66.7	22.3	12.4	32

Reactions were carried out with *p*-amino-DL-phenylalanine **2** (250 mM), D-lactose **1** (1 M) in an aqueous solution of acid (0.563 M) or in a 50 mM citrate/phosphate buffer pH 2.6, at 75 °C for 4 h; 100% corresponds to 250 mM.

Table 2
Influence of the acetic acid concentration on the synthesis

Acetic acid concentration (M)	*p*-Amino-DL-phenylalanine **2** conversion (%)	Relative synthesis yield		
		3 (%)	**4** (%)	**5** (%)
0.0563	54.4	23.1	2.5	28.8
0.2815	61.6	23.3	3.3	35
0.563	75.2	6.3	33.3	35.6
1.126	73.5	1.4	25.1	47
5.63	94.6	0.4	8.7	85.5
11.26	93.0	0.06	5.6	87.3

Reactions were carried out with *p*-amino-DL-phenylalanine **2** (250 mM), D-lactose **1** (1 M) in a mixture of water/acetic acid, at 75 °C for 4 h; 100% corresponds to 250 mM.

Table 3
Influence of the sugar moiety on the synthesis

Sugar **1**	*p*-Amino-DL-phenylalanine **2** conversion (%)	Relative synthesis yield		
		3 (%)	**4** (%)	**5** (%)
D-Lactose	75.2	6.3	33.3	35.6
D-Glucose	55.9±0.5	8.1±1.9 (**3b**)	18.1±1 (**4b**)	29.7±3.5
N-Acetyl-D-glucosamine	33.2±0.1	13.3±0.2	0	19.9±0.1
D-(+)-Glucosamine hydrochloride	55.9±4.7	29.5±3.1	0	26.4±1.6

Reactions were carried out with *p*-amino-DL-phenylalanine **2** (250 mM), sugar **1** (1 M) in an aqueous solution of acetic acid (0.563 M), at 75 °C for 4 h; 100% corresponds to 250 mM.

2.5. Effect of sugar nature

In order to evaluate the likely versatility of this approach, reactivity of other sugars was attempted in optimal conditions. Results reported in Table 3 show that N-glycosylation is carried out with all sugars, whereas the Amadori compound is obtained only with D-lactose and D-glucose. Indeed, the Amadori compound synthesis is not realized using *N*-acetyl-D-glucosamine and D-glucosamine because the enol formation (Scheme 1, **4**) cannot occur.[8a] Both products formed from D-glucose (Table 3, **3b** and **4b**) were purified by preparative HPLC and were analyzed by mass spectrometry, ^{1}H and ^{13}C NMR. Analysis confirmed that compound **3b** corresponds to β-*N*-glucosyl-*p*-amino-DL-phenylalanine and compound **4b** corresponds to *N*-(1-deoxyfructopyranos-1-yl)-*p*-amino-DL-phenylalanine. Unlike compound **4**, the ^{1}H NMR spectra of **4b** allowed us to distinguish the protons H6$_a$′ and H6$_b$′ as two double doublets centred at 3.63 and 3.56 ppm, respectively. These protons were shown to present a high value ^{2}J coupling constant ($^{2}J_{6a',6b'}$=10.8 Hz, $^{2}J_{6b',6a'}$=12.8 Hz) and a low value ^{3}J coupling constant ($^{3}J_{6a',5'}$=1.2 Hz, $^{3}J_{6b',5'}$=1.9 Hz), proving that they are not identical. As a consequence, there is no free rotation of the C5′–C6′ linkage, which means that the 1-deoxyfructos-1-yl carbohydrate moiety is in the pyran form (Scheme 3). A furanose ring would have revealed on the contrary a free rotation of the C5′–C6′ linkage, characterized by the absence of ^{2}J coupling. Moreover, the ^{3}J coupling constant between H6$_a$′ and H5′ is specific of an equatorial–equatorial configuration for these protons while the ^{3}J coupling constant between H6$_b$′ and H5′ indicates an axial–equatorial interaction, implicating that the proton H5′ and the hydroxyl 5′ adopt, respectively, an equatorial and an axial orientation. These observations strengthen the hypothesis made about the structure of product **4** and strongly suggest that the 1-deoxyfructos-1-yl carbohydrate moiety of compounds **4** and **4b** may probably be a β-D-pyranose ring. Indeed, the pyranose ring of the Amadori rearrangement product is known to be represented in solution at 93% by the β anomer.[10a]

3. Conclusion

A short and efficient method has been developed in aqueous media, for pseudo-*C*-selective glycosylation of *p*-amino-DL-phenylalanine from unprotected sugars (D-lactose and D-glucose). After the formation of the stereoselective β-*N*-glycosidic bond, the Amadori rearrangement occurred in acidic conditions. For the N-glycosylation, a specific base catalysis takes place in the media. The reaction yield of N-glycosylation depends on the pH of the system. For the Amadori rearrangement, a general acid catalysis is observed. All species capable of donating protons contribute to reaction yield. The synthesis yield of Amadori compound depends on the nature and concentration of different acids of the system (Tables 1 and 2) but not on the pH (Fig. 3). In the best conditions, 35% of *p*-amino-DL-phenylalanine is converted into *N*-[β-D-galactosyl-1-4-(1-deoxyfructos-1-yl)]-*p*-amino-DL-phenylalanine (Amadori compound). The Amadori compound from D-glucose [*N*-(1-deoxyfructopyranos-1-yl)-*p*-amino-DL-phenylalanine] has also been synthesized and characterized.

4. Experimental

4.1. Chemical materials

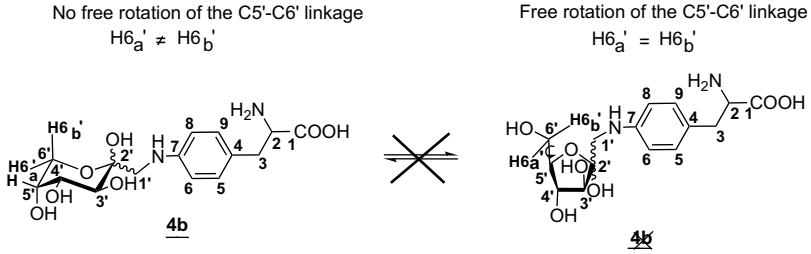
All chemicals were purchased from Sigma Co. (USA). Deionized water was obtained via a Milli-Q system (Millipore, France).

4.2. General procedure for the synthesis

p-Amino-DL-phenylalanine (pAmP) (250 mM) and sugar (1 M) were suspended in an aqueous solution (pure water, water/acid or citrate/phosphate buffer). The mixture was stirred at 40 or 75 °C and glycosylation was carried out until the formation of a steady state obtained after about 4 h. These standard conditions were used except when otherwise stated in the text.

4.3. HPLC analysis and structural analysis

Quantitative and structural analysis of reactants and products were conducted using an LC/MS-ES system from Agilent (1100 LC/MSD Trap mass spectrometer VL and differential refractometer, Waters, model 410), with an Interchim Uptisphere 6 Diol (250×4 mm, 6 μm) normal phase column eluted with acetonitrile/water/acetic acid (80:20:0.1, v/v/v) at room temperature and at



Scheme 3. Structure of the *N*-(1-deoxyfructopyranos-1-yl)-*p*-amino-DL-phenylalanine **4b**.

a flow rate of 1 mL/min. Quantification was carried out at 280 nm using HP Chemstation software off-line for the processing.

Products formed were characterized by ^{1}H and ^{13}C NMR (DEPT) after purification via preparative HPLC, using an Interchim Uptisphere 6 Diol (250 mm×21.2 mm, 6 μm) normal phase column eluted with acetonitrile/water (80:20, v/v) at room temperature and at a flow rate of 10 mL/min. Evaporation of acetonitrile under reduced pressure followed by lyophilization of water gave cream-coloured crystalline products.

^{1}H and ^{13}C NMR (DEPT, Distortionless Enhancement by Polarization Transfer) were recorded on a JEOL-JNM LA400 spectrometer (400 MHz) (Laboratoire Commun d'Analyse, Université de La Rochelle, France), with tetramethylsilane as an internal reference. Samples were studied as solutions in D_2O.

Low-resolution mass spectral analyses were obtained by electrospray in the positive and negative detection modes. Nitrogen was used as the drying gas at 15 L/min and 350 °C at a nebulizer pressure of 4 bar. The scan range was *m*/*z* 50–1000 using five averages and *m*/*z* 13,000 per second resolution. The capillary voltage was −4000 V for negative ion detection. Processing was done off-line using LC-MSD Trap software 6.0.

4.4. Libraries characterization of products

*4.4.1. Compound (**3**)*

m/*z* (LR-ESI$^+$) $C_{21}H_{32}N_2O_{12}Na$ (M+Na$^+$), found: 527.4, calcd: 527.4758. ^{1}H NMR (400 MHz, D_2O): δ 7.01 (d, 2H, $^3J_{5,6}=^3J_{9,8}$=8 Hz, H-5, H-9), 6.79 (d, 2H, $^3J_{6,5}=^3J_{8,9}$=7.6 Hz, H-6, H-8), 4.51 (d, 1H, $^3J_{1',2'}$=7.6 Hz, H-1′), 4.32 (d, 1H, $^3J_{1'',2''}$=7.6 Hz, H-1″), 3.77–3.32 (m, 13H, H-2, H-2′, H-3′, H-4′, H-5′, H-6$_a$′, H-6$_b$′, H-2″, H-3″, H-4″, H-5″, H-6$_a$″, H-6$_b$″), 3.16–2.83 (m, 2H, H-3$_a$, H-3$_b$). ^{13}C NMR (400 MHz, D_2O): δ 175.24 (C-1), 145.74 (C-7), 131.09 (C-5, C-9), 126.62 (C-4), 115.61 (C-6, C-8), 103.75 (C-1″), 85.52 (C-1′), 79.28 (C-5′), 76.25 (C-5″), 76.17 (C-3′), 76.12 (C-3″), 73.37 (C-2′), 73.16 (C-2″), 71.81 (C-4″), 69.39 (C-4′), 61.87 (C-6′), 60.92 (C-6″), 56.98 (C-2), 36.50 (C-3).

*4.4.2. Compound (**4**)*

m/*z* (LR-ESI$^+$) $C_{21}H_{32}N_2O_{12}Na$ (M+Na$^+$), found: 527.4, calcd: 527.4758. ^{1}H NMR (400 MHz, D_2O): δ 6.98 (d, 2H, $^3J_{5,6}=^3J_{9,8}$=7.6 Hz, H-5, H-9), 6.68 (d, 2H, $^3J_{6,5}=^3J_{8,9}$=8 Hz, H-6, H-8), 4.39 (d, 1H, $^3J_{1'',2''}$=7.2 Hz, H-1″), 4.08–3.11 (m, 14H, H-2, H-1$_a$′, H-1$_b$′, H-3′, H-4′, H-5′, H-6$_a$′, H-6$_b$′, H-2″, H-3″, H-4″, H-5″, H-6$_a$″, H-6$_b$″), 3.01 (dd, 1H, $^2J_{3a,3b}$=14.4 Hz, $^3J_{3a,2}$=4.4 Hz, H-3$_a$), 2.80 (dd, 1H, $^2J_{3b,3a}$=14.8 Hz, $^3J_{3b,2}$=7.6 Hz, H-3$_b$). ^{13}C NMR (400 MHz, D_2O): δ 174.8 (C-1), 148.45 (C-7), 130.98 (C-5, C-9), 125.06 (C-4), 114.94 (C-6, C-8), 101.46 (C-1″), 99.03 (C-2′), 78.14 (C-5′), 75.99 (C-5″), 73.21 (C-3″), 71.38 (C-2″), 69.31 (C-4″), 67.75 (C-4′), 67.40 (C-3′), 63.66 (C-6′), 61.79 (C-6″), 56.75 (C-2), 50.22 (C1′), 36.09 (C-3).

*4.4.3. Compound (**3b**)*

m/*z* (LR-ESI$^+$) $C_{15}H_{22}N_2O_7Na$ (M+Na$^+$), found: 365.4, calcd: 365.3358. ^{1}H NMR (400 MHz, D_2O): δ 7.03 (d, 2H, $^3J_{5,6}=^3J_{9,8}$=7.6 Hz, H-5, H-9), 6.73 (d, 2H, $^3J_{6,5}=^3J_{8,9}$=8.4 Hz, H-6, H-8), 4.61 (d, 1H, $^3J_{1',2'}$=9.2 Hz, H-1′), 3.76–3.73 (m, 2H, H-2, H-4′), 3.58 (m, 1H, H5′), 3.48–3.40 (m, 2H, H-2′, H-3′), 3.30 (m, 2H, H-6$_a$′, H-6$_b$′), 3.04 (dd, 1H, $^2J_{3a,3b}$=14.4 Hz, $^3J_{3a,2}$=3.6 Hz, H-3$_a$), 2.87 (dd, 1H, $^2J_{3b,3a}$=13.6 Hz, $^3J_{3b,2}$=7.2 Hz, H-3$_b$). ^{13}C NMR (400 MHz, D_2O): δ 175.21 (C-1), 145.79 (C-7), 131.24 (C-5, C-9), 126.59 (C-4), 115.59 (C-6, C-8), 85.66 (C-1′), 77.67 (C-5′), 77.29 (C-3′), 73.47 (C-2′), 70.53 (C-4′), 61.56 (C-6′), 56.97 (C-2), 36.48 (C-3).

*4.4.4. Compound (**4b**)*

m/*z* (LR-ESI$^+$) $C_{15}H_{22}N_2O_7Na$ (M+Na$^+$), found: 365.4, calcd: 365.3358. ^{1}H NMR (400 MHz, D_2O): δ 6.98 (d, 2H, $^3J_{5,6}=^3J_{9,8}$=8 Hz, H-5, H-9), 6.69 (d, 2H, $^3J_{6,5}=^3J_{8,9}$=8.4 Hz, H-6, H-8), 3.90 (m, 1H, H-3′), 3.85 (m, 1H, H-4′), 3.77 (m, 2H, H-2, H-5′), 3.63 (dd, 1H, $^2J_{6a',6b'}$=10.8 Hz, $^3J_{6a',5'}$=1.2 Hz, H-6$_a$′), 3.56 (dd, 1H, $^2J_{6b',6a'}$=12.8 Hz, $^3J_{6b',5'}$=1.9 Hz, H-6$_b$′), 3.33 (d, 1H, $^2J_{1a',1b'}$=13.2 Hz, H-1$_a$′), 3.13 (d, 1H, $^2J_{1b',1a'}$=13.6 Hz, H-1$_b$′), 3.01 (dd, 1H, $^2J_{3a,3b}$=14.4 Hz, $^3J_{3a,2}$=4.8 Hz, H-3$_a$), 2.86 (dd, 1H, $^2J_{3b,3a}$=15.2 Hz, $^3J_{3b,2}$=8.4 Hz, H-3$_b$). ^{13}C NMR (400 MHz, D_2O): δ 174.93 (C-1), 148.68 (C-7), 131.09 (C-5, C-9), 125.15 (C-4), 115.13 (C-6, C-8), 99.16 (C-2′), 70.53 (C-4′), 69.93 (C-5′), 69.46 (C-3′), 64.21 (C-6′), 56.90 (C-2), 50.39 (C1′), 36.24 (C-3).

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