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One-pot stereoselective synthesis of β-*N*-aryl-glycosides by N-glycosylation of aromatic amines: application to the synthesis of tumor-associated carbohydrate antigen building blocks

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Abstract—We studied the stereoselective synthesis of several β-*N*-aryl-glycosides by glycosylation of aromatic primary amines using unprotected carbohydrates in aqueous solution. This was the first report showing an efficient method for the synthesis with one step of β-*N*-glycosyl-*para*-amino-phenyl alanine building blocks for the tumor-associated carbohydrate antigen (TACA) glycopeptides synthesis. 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-glycoside bond (natural N-glycoprotein linkage). The study of the chemical and enzymatic stability in aqueous media of β-*N*-aryl-glycosides synthesized was also investigated. For the first time we have shown that the N-glycosidic bond was relatively stable at pH near to 7 and more stable than the O-glycosidic bond to enzymatic hydrolysis. This higher enzymatic and chemical stability of the N-glycosidic bond is essential to envisage further development of stable TACA building blocks.

1. Introduction

It is well established that oncological transformations are accompanied by the change of cell surface glycosylation patterns.[1,2] The abnormal glycans expressed on tumor cells are known as tumor-associated carbohydrate antigens (TACAs). Since the early 1970s, many TACAs have been identified[2–6] and become useful molecular templates and targets in the design and development of therapeutic cancer vaccines.[4–10]

Traditionally, cancer vaccines are created by coupling TACAs to a carrier protein, such as keyhole limpet hemocyanin (KLH). Resultant glycoconjugates can be employed to immunize cancer patients to fight tumors that bear the same antigens. This method has witnessed success with some TACAs, and a few cancer vaccines thus developed are now in clinical trials.[11]

Generally, TACAs such as T_N (αGalNAc-), T (Thomsen-Friedenreich, βGal-1,3-αGalNAc-), sialyl-T_N (αNeuNAc-2,6-αGalNAc-), and sialyl-T (αNeuNAc-2,3-βGal-1,3-αGalNAc- and βGal-1,3-[αNeuNAc-2,6-]αGalNAc-) are α-O-glycosidically linked to a serine or a threonine in the peptide backbone. Others, such as sialyl Lewis *x* (αNeuNAc-2,3-βGal-1,4-[αFuc-1,3-]βGlcNAc-) and sialyl Lewis *a* (αNeuNAc-2,3-βGal-1,3-[αFuc-1,4-]βGlcNAc-) antigens, are β-N-glycosidically linked to an asparagine.[12]

Our laboratory has actively been engaged in the chemo-enzymatic synthesis of molecules with biological activities using green conditions and particularly in the synthesis of complex oligosaccharides and glycoconjugates by hydrolases.[13–18] As a result of our advances in this area, we began a program to explore the synthesis of N-linked glycosyl amino acid building blocks for TACA glycopeptides elaboration.

2. Results and discussion

2.1. Synthesis of β-*N*-aryl-glycosides

As a first step toward this goal, we chose to study the N-glycosylation of aniline. Few synthetic methods have been reported for the N-glycosylation of aromatic compounds starting from aniline derivatives and sugars.[19–25] Usually the reaction could be accomplished by heating at reflux in protic solvents such as MeOH. The *N*-aryl-glycosylamines were obtained in variable yields depending on the reactivity of the aniline derivatives. Moreover, the reaction time required was very long (about 6 days) and the stereochemistry at the anomeric carbon depended on the sugar and on the steric features of the amine and the sugar. In some cases,

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a mixture of epimers α/β was obtained. Optimization of the N-glycosylation reaction in terms of yield, reaction time, and diastereoselection represented a significant challenge.

In this context, we chose to study the N-glycosylation reaction in aqueous media as a continuation of our research devoted to the development of green organic chemistry. It is well accepted that the reaction between an amine such as aniline **2a** and aldoses such as D-glucose **1a** in water, at high temperature and in slightly acid conditions first proceeds through the corresponding N-glycoside, **3a**, which is, however, not stable and can be rearranged to produce the so-called Amadori product, **4a** (Scheme 1), as the first relatively stable glycoconjugate.[26]

Therefore, in a preliminary experiment, we decided to carry out the condensation of aniline **2a** (250 mM) and an unprotected D-glucose **1a** (250 mM) in aqueous phosphate buffer pH 8 and at 40 °C. By HPLC analysis, the decrease of **2a** concentration (12% after 5 h) was proportional with the synthesis of only one product. Products formed were purified by semi-preparative HPLC. Analysis of products by ^{1}H and ^{13}C NMR like the mass spectrometry analysis indicated that the Amadori rearrangement had not occurred after formation of the desired product **3a**. In the ^{1}H NMR spectra of **3a**, the anomeric hydrogen is a doublet with a coupling constant of 8.8 Hz. Such coupling constant is in the range for those resulting from an axial–axial configuration of H_1 and H_2 and therefore, for these sugar, a β-N-glycosidic linkage.

The preference for formation of the β isomer can be rationalized in terms of the competition between substitution at the sterically least hindered position (β) versus substitution at a position which incurs stabilization from the anomeric effect (α). The substitution is driven predominantly by sterics and the reaction is stereoselective for the β configuration.

Although these reaction conditions permit the desired product (**β-3a**) to be obtained, they are not particularly efficient, consequently other pHs and temperatures were therefore examined. We investigated the effect of pH and temperature on the N-glycosylation reaction yield. When the reaction was carried out at 40 °C for 5 h with the same concentrations of D-glucose **1a** and aniline **2a**, we observed that the optimum pH of the N-glycosylation was close to 6.5 (55%) and that the reaction was slightly favored in alkaline conditions (Fig. 1). When the effect of the temperature was studied with the same concentrations, we observed that the reaction was favored with temperatures lower than 50 °C (Fig. 2). In fact, in alkaline conditions and at higher temperatures, several others products were obtained, probably resulting of the Amadori rearrangement and characterized by the formation of brown pigments.

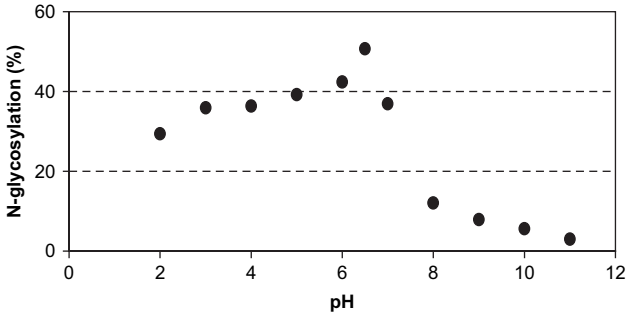


Figure 1. Effect of pH on the N-glycosylation yield. Reaction was carried out at 40 °C in aqueous buffers pH 2–5 (citrate phosphate), pH 6–8 (phosphate), and pH 9–11 (100 mM, glycine NaOH).

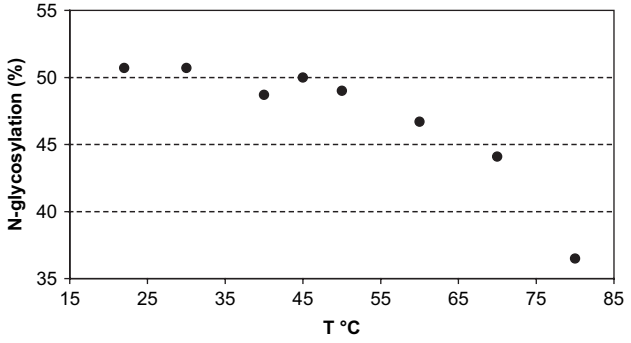
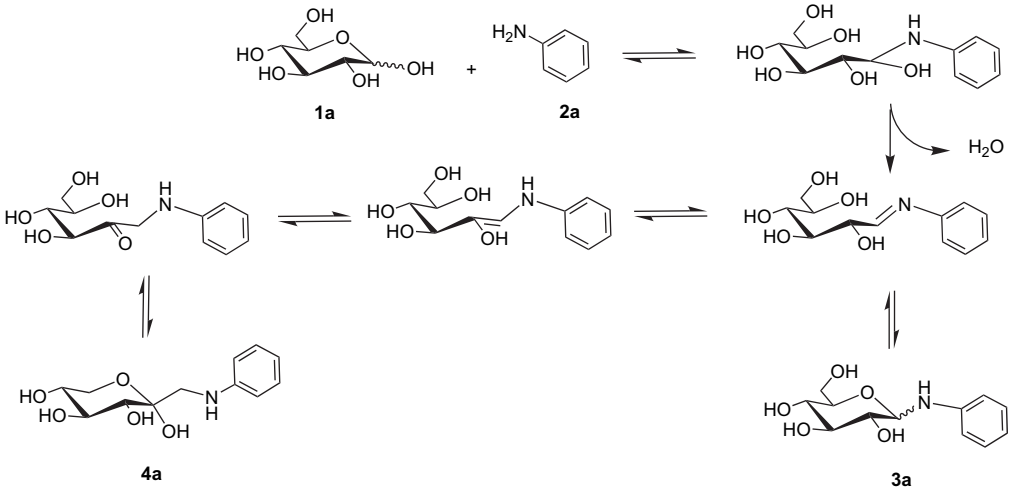


Figure 2. Effect of temperature on the N-glycosylation yield. Reaction was carried out in aqueous buffer pH 6.5 (phosphate).



Scheme 1. Formation of *N*-glucosyl-aniline **3a** and Amadori product **4a** from D-glucose **1a** and aniline **2a** in aqueous alkaline conditions.

Using a temperature of 40 °C and a pH of 6.5, we investigated the preparation in aqueous media of several *N*-aryl-glycosides, **3b**–**3m** using D-glucose (**1a**), D-galactose (**1b**), lactose (**1c**), *N*-acetyl-D-glucosamine (**1d**), *N*-acetyl-D-galactosamine (**1e**), and *N*-acetyl-D-lactosamine (**1f**). In all reactions tested under equimolar conditions, acceptable yields from 30% to 55% were obtained with complete β-selectivity (Table 1). These yields can be increased using a higher sugar concentration (**3j** and **3k** footnote b, **3m**).

The product **3k** can be considered as an analog of T_N antigen and as a precursor of T (Thomsen-Friedenreich), sialyl-T_N, and sialyl-T antigen synthesis. The products **3j** and **3l** can be considered as precursors of sialyl Lewis *x* and sialyl Lewis *a* antigens.

The product **2d** is an intermediary of the synthesis of thiazoloquinazolinones with a glycogen synthase kinase-3 inhibitory activity.[27]

2.2. Chemical hydrolysis of β-*N*-aryl-glycosides

To investigate the kinetics of the chemical hydrolysis of synthesized β-*N*-aryl-glycosides, **3a** was maintained at 40 °C under different pH conditions and the hydrolysis was monitored by HPLC over time. As shown in Figure 3, **3a** was hydrolyzed relatively slowly under alkaline conditions. After 5 h at pH 8, β-N-glycoside **3a** was still the major constituent with some glucose and aniline liberated. The stability of **3a** at pH 8 is well reflected in the long half-life, $\tau_{1/2}$, of about 26 h and in the poor initial rate of hydrolysis. In contrast, hydrolysis of **3a** was found to be faster under neutral and slightly acidic conditions. The low pH of 2 induced a spontaneous hydrolysis of **3a**, which did not allow the measurement of the half-life and the initial rate of hydrolysis.

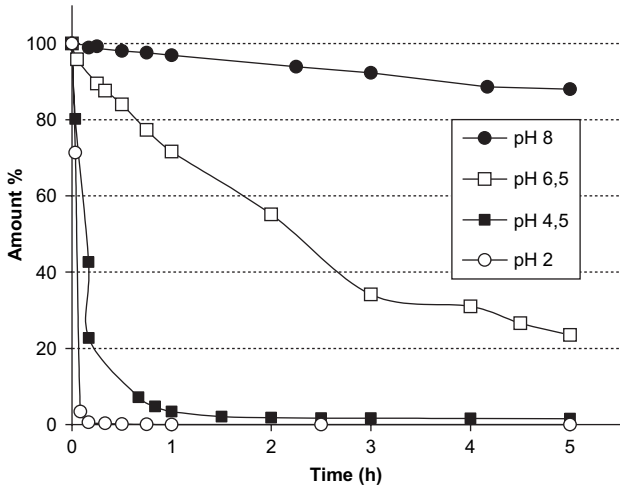
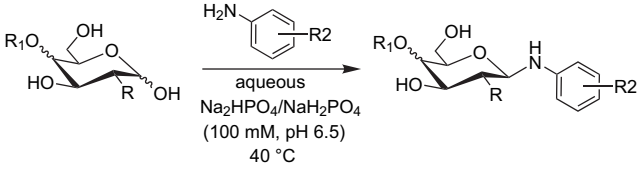


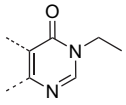
Figure 3. Influence of the pH on the time course of *N*-glucosyl-aniline (**3a**) decomposition. Reactions were carried out at 40 °C in aqueous buffer pH 2 (○) to 4.5 (■) (citrate phosphate), pH 6.5 (□) to 8 (●) (phosphate).

Between these extreme pH values, the rate of decomposition increased with acidity; for example, the $\tau_{1/2}$ of **3a** was about 2 h at pH 6.5 and about 8 min at pH 4.5. A similar comportment was observed with β-*N*-galactoside **3b** (Table 2).

The hydrolytic instability of the β-N-glycosidic bond in β-*N*-glucosyl-aniline (**3a**) and β-*N*-galactosyl-aniline (**3b**) under acidic and neutral pH conditions is well in line with earlier observations on the stability of N-glycosides of amino acids in aqueous solutions.[28,29] In contrast to β-*N*-glucosyl-aniline (**3a**) and β-*N*-galactosyl-aniline (**3b**), we observed that products **3j** and **3k** were more stable under pH near to the neutrality (Table 2).

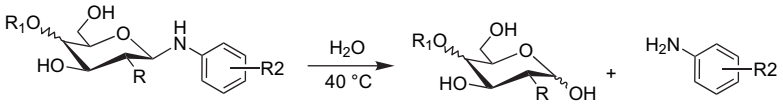
Table 1. N-Glycosylation of aromatic compounds



Sugars	R	R1	Amines	R2	Product (yield %)[a]
1a	OH	H equatorial	**2a**	H	**3a** (55)
1b	OH	H axial	**2a**	H	**3b** (51)
1c	OH	Gal (β-1)	**2a**	H	**3c** (52)
1d	NHAc	H equatorial	**2a**	H	**3d** (37)
1a	OH	H equatorial	**2b**	*m*CH_2OH	**3e** (40)
1b	OH	H axial	**2b**	*m*CH_2OH	**3f** (38)
1a	OH	H equatorial	**2c**	*p*CH_2–CH–(COOH)NH_2	**3g** (54)
1b	OH	H axial	**2c**	*p*CH_2–CH–(COOH)NH_2	**3h** (34)
1c	OH	Gal (β-1)	**2c**	*p*CH_2–CH–(COOH)NH_2	**3i** (40)
1d	NHAc	H equatorial	**2c**	*p*CH_2–CH–(COOH)NH_2	**3j** (41), **3j** (74)[b]
1e	NHAc	H axial	**2c**	*p*CH_2–CH–(COOH)NH_2	**3k** (41), **3k** (75)[b]
1f	NHAc	Gal (β-1)	**2c**	*p*CH_2–CH–(COOH)NH_2	**3l** (30)
1c	OH	Gal (β-1)	**2d**		**3m** (80)[b]

[a] Yields were determined by HPLC analysis after 5 h of reaction.
[b] Reaction was carried out with 4 equiv of sugar.

Table 2. Stability of β-*N*-aryl-glycosides in aqueous media



Product	R	R1	R2	pH	v_i (10^3 μmol min^{-1})	$\tau_{1/2}$
3a	OH	H equatorial	H	8	0.28	~26.5 h
3a	OH	H equatorial	H	6.5	2.53	~2.25 h
3a	OH	H equatorial	H	4.5	29.5	~8.5 min
3a	OH	H equatorial	H	2	nd	Less of 3 min
3b	OH	H axial	H	8	1.22	~4.45 h
3b	OH	H axial	H	6.5	10.90	~24 min
3b	OH	H axial	H	4.5	nd	~2 min
3b	OH	H axial	H	2	nd	Less of 1 min
3j	NHAc	H equatorial	*p*CH_2–CH–(COOH)NH_2	8	0.031	~8 days
3j	NHAc	H equatorial	*p*CH_2–CH–(COOH)NH_2	7.35[a]	0.08	~3 days
3j	NHAc	H equatorial	*p*CH_2–CH–(COOH)NH_2	6.5	0.27	~22 h
3k	NHAc	H axial	*p*CH_2–CH–(COOH)NH_2	8	0.089	~2 days
3k	NHAc	H axial	*p*CH_2–CH–(COOH)NH_2	7.35[a]	0.486	~13 h
3k	NHAc	H axial	*p*CH_2–CH–(COOH)NH_2	6.5	1.763	~3 h

[a] pH physiologique, $\tau_{1/2}$: half-life, v_i: initial rate of chemical hydrolysis, nd: not determined.

2.3. Enzymatic hydrolysis of β-*N*-aryl-glycosides

With an aim of knowing if a O-glycosidic bond is more stable than a N-glycosidic bond to enzymatic hydrolysis, we compared the hydrolysis rates of β-*O*-phenyl-glucopyranoside and β-*O*-phenyl-galactopyranoside with the hydrolysis rate of β-*N*-phenyl-glucopyranoside **3a** and β-*N*-phenyl-galactopyranoside **3a** by using a commercial enzymatic preparation of glucosidase (Novarom G) and galactosidase (Lactozym). Table 3 clearly demonstrates that a N-glycosidic bond is very stable in the presence of glycosidase in comparison to a similar compound with a O-glycosidic bond.

This higher chemical and enzymatic stability is essential to envisage further development of TACA building blocks, especially by chemo-enzymatic synthesis. This strategy is currently in progress in the laboratory for the synthesis of TACA building blocks from products **3j**, **3k**, and **3l**. These TACA building blocks could be incorporated into target glycopeptides using solid-phase peptide synthesis on the Wang resin.

3. Conclusion

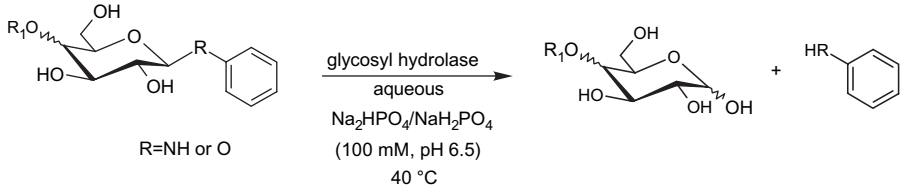
A short (one step) and efficient method was developed in aqueous media for β-N-selective glycosylation of aromatic primary amines such as aniline from unprotected sugar. The Amadori rearrangement had not occurred after formation of the stereoselective β-N-glycoside bond. This method allows the synthesis of various stable β-N-aryl-glycosides, which can be considered as analogs or precursors of TACAs. The study of the chemical and enzymatic stability in aqueous media was also investigated. We have shown that the N-glycosidic bond was relatively stable in pH near to 7 and more stable than the O-glycosidic bond to enzymatic hydrolysis.

4. Experimental

4.1. Biological and chemical materials

All chemicals were purchased from Sigma Co. (USA) except compound **2d**, which was previously synthesized in the

Table 3. Comparison of stability of β-*N*-aryl-glycosides and β-*O*-aryl-glycosides to enzymatic hydrolysis



Product	pH	Enzyme	v_i (μmol min^{-1} g^{-1})	$\tau_{1/2}$
β-*N*-Phenyl-glucopyranoside **3a**	6.5	β-Glucosidase (Novarom G)	14	~2 h
β-*O*-Phenyl-glucopyranoside			323	~1 h 10 min
β-*N*-Phenyl-galactopyranoside **3b**		β-Galactosidase (Lactozym)	0	~26 min
β-*O*-Phenyl-galactopyranoside			18	Less of 1 min

Reactions were carried out at 40 °C with 15 g L^{-1} of Novarom G or Lactozym in aqueous buffer pH 6.5. $\tau_{1/2}$: half-life, v_i: initial rate of enzymatic hydrolysis.

laboratory.[27] Deionized water was obtained via a Milli-Q system (Millipore, France).

Lactozym 3000 L HP-G is a soluble commercial preparation of β-galactosidase from *Kluyveromyces lactis* manufactured by Novo Nordisk A/S (Bagsvaerd, Denmark), Batch DKN08649 with an activity of 3530 U ml^{-1}. One unit is defined as the amount of enzyme which releases 1 mmol glucose per minute under the following standard conditions: 4.75% (w/w) lactose, milk buffer system pH 6.5, 37 °C, reaction time 30 min.

Novarom G is a dry commercial preparation of β-1-4-glucosidase derived from *Aspergillus niger* manufactured by Novo Nordisk A/S (Bagsvaerd, Denmark) with an activity of 80 BGDU g^{-1}. One unit (BGDU) is defined as the amount of enzyme, which releases 1 μmol of *p*-nitrophenol from *p*-nitrophenyl-β-D-glucopyranoside (*p*NPG) per minute at 23 °C and pH 3.5.

4.2. Synthesis of *N*-aryl-glycosides

In standard conditions, aromatic amine (250 mM) was suspended in phosphate buffer (100 mM, pH 6.5). Sugar (250 mM) was added to the suspension. The mixture was stirred at 40 °C. The reactions were carried out until the formation of a steady state obtained around 5 h. These conditions were used except when otherwise stated in the text.

4.3. Chemical and enzymatic stability of *N*-aryl-glycosides

Solution of *N*-aryl-glycosides in water (5 mM) was maintained at 40 °C under different pH conditions with or without glycosylhydrolases (15 g L^{-1}). The hydrolysis was monitored by HPLC over time.

4.4. HPLC and structural analyses

Quantitative and structural analyses 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 a Prontosyl C18 reversed-phase column (250×4 mm, 5 μm) eluted with acetonitrile/water (15/85, v/v) at room temperature and at a flow rate of 0.2 ml/min. Quantification was carried out at 280 nm.

Products formed were characterized by ^1H and ^{13}C NMR (DEPT) after purification via preparative HPLC, using a Prontosyl C18 (250×20 mm, 10 μm) reversed-phase column, eluted with acetonitrile/water (15/85, v/v) at room temperature and at a flow rate of 5 ml/min. Lyophilization of the solvent gave white crystalline products.

^1H 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 bars. The scan range was 50–1000 *m/z* using five averages and 13,000 *m/z* per second resolution. The capillary voltage was −4000 V for negative ion detection. Processing was done off-line using HP Chemstation.

4.5. Libraries characterization of β-*N*-aryl-glycosides

4.5.1. Compound 3a. *m/z* (LR-ESI$^+$) $C_{12}H_{17}NO_5H$ ($M+H^+$), found: 255.9, calcd: 256.2809. ^1H NMR (400 MHz, D_2O): δ 7.10 (dd, 2H, H-3, H-5), 6.72–6.69 (m, 3H, H-2, H-4, H-6), 4.58 (d, 1H, *J*=8.8 Hz, H-1′), 3.69 (dd, 1H, H-4′), 3.52 (dd, 1H, H-5′), 3.42–3.35 (m, 2H, H-2′, H-3′), 3.25 (dd, 2H, H-6′). ^{13}C NMR (D_2O): δ 146.39 (C-1), 130.38 (C-3, C-5), 120.38 (C-4), 115.16 (C-2, C-6), 85.62 (C-1′), 77.63 (C-5′), 77.24 (C-3′), 73.44 (C-2′), 70.50 (C-4′), 61.53 (C-6′).

4.5.2. Compound 3b. *m/z* (LR-ESI$^+$) $C_{12}H_{17}NO_5H$ ($M+H^+$), found: 255.9, calcd: 256.2809. ^1H NMR (400 MHz, D_2O): δ 7.10 (dd, 2H, H-3, H-5), 6.72–6.68 (m, 3H, H-2, H-4, H-6), 4.53 (d, 1H, *J*=8.4 Hz, H-1′), 3.81 (dd, 1H, H-4′), 3.62 (dd, 1H, H-5′), 3.56 (m, 1H, H-3′), 3.53 (dd, 2H, H-6′), 3.49 (dd, 2H, H-2′). ^{13}C NMR (D_2O): δ 146.48 (C-1), 130.37 (C-3, C-5), 120.28 (C-4), 115.12 (C-2, C-6), 86.06 (C-1′), 76.27 (C-5′), 74.50 (C-3′), 71.08 (C-2′), 69.61 (C-4′), 61.65 (C-6′).

4.5.3. Compound 3c. *m/z* (LR-ESI$^+$) $C_{18}H_{27}NO_{10}H$ ($M+H^+$), found: 417.9, calcd: 418.4245. ^1H NMR (400 MHz, D_2O): δ 7.13 (dd, 2H, H-3, H-5), 6.78–6.66 (m, 3H, H-2, H-4, H-6), 4.61 (d, 1H, *J*=9.2 Hz, H-1′), 4.27 (dd, 1H, *J*=8 Hz, H-1″), 3.76–3.28 (m, 12H, H-2′, H-3′, H-4′, H-5′, H-6′, H-2″, H-3″, H-4″, H-5″, H-6″). ^{13}C NMR (D_2O): δ 146.35 (C-1), 130.40 (C-3, C-5), 120.42 (C-4), 115.18 (C-2, C-6), 103.70 (C-1″), 85.48 (C-1′), 79.24 (C-5′), 76.22 (C-5″), 76.13 (C-3′), 76.08 (C-3″), 73.31 (C-2′), 73.14 (C-2″), 71.76 (C-4″), 69.33 (C-4′), 61.83 (C-6′), 60.89 (C-6″).

4.5.4. Compound 3d. *m/z* (LR-ESI$^+$) $C_{14}H_{20}N_2O_5Na$ ($M+Na^+$), found: 318.9, calcd: 319.3087. ^1H NMR (400 MHz, D_2O): δ 7.09 (dd, 2H, H-3, H-5), 6.72 (dd, 1H, H-4), 6.65 (d, 2H, H-2, H-6), 4.62 (d, 1H, *J*=8.4 Hz, H-1′), 3.69 (dd, 2H, H-6′), 3.55 (dd, 1H, H-4′), 3.45 (dd, 1H, H-5′), 3.37 (m, 1H, H-3′), 3.31 (dd, 1H, H-2′), 1.81 (s, 3H, H-8′). ^{13}C NMR (D_2O): δ 175.73 (C-7′), 146.12 (C-1), 130.39 (C-3, C-5), 120.56 (C-4), 115.20 (C-2, C-6), 85.24 (C-1′), 77.27 (C-5′), 75.41 (C-3′), 70.74 (C-4′), 61.51 (C-6′), 55.87 (C-2′), 22.81 (C-8′).

4.5.5. Compound 3e. *m/z* (LR-ESI$^+$) $C_{13}H_{19}NO_6Na$ ($M+Na^+$), found: 307.9, calcd: 308.2893. ^1H NMR (500 MHz, D_2O): δ 7.19 (dd, 1H, H-5), 6.78–6.72 (m, 3H, H-2, H-4, H-6), 4.67 (d, 1H, *J*=8.9 Hz, H-1′), 4.47 (s, 1H, H-7), 3.78 (d, 1H, H-4′), 3.61 (m, 1H, H-5′), 3.51–3.45 (m, 2H, H-2′, H-3′), 3.35 (dd, 2H, H-6′). DEPT ^{13}C NMR (D_2O): δ 129.81 (C-5), 118.38 (C-6), 113.64 (C-2), 113 (C-4), 84.76 (C-1′), 76.81 (C-5′), 76.44 (C-3′), 72.64 (C-2′), 69.71 (C-4′), 63.86 (C-7), 60.76 (C-6′).

4.5.6. Compound 3f. *m/z* (LR-ESI$^+$) $C_{13}H_{19}NO_6Na$ ($M+Na^+$), found: 308.27, calcd: 308.2893. ^1H NMR (500 MHz, D_2O): δ 7.20 (dd, 1H, H-5), 6.79–6.75 (m, 3H,

H-2, H-4, H-6), 4.64 (d, 1H, J=8.6 Hz, H-1′), 4.49 (s, 1H, H-7), 3.91 (dd, 1H, H-4′), 3.71 (m, 1H, H-5′), 3.68–3.58 (m, 3H, H-2′, H-3′, H-6′). DEPT ^{13}C NMR (D_2O): δ 129.79 (C-5), 118.28 (C-6), 113.61 (C-2), 112.95 (C-4), 85.18 (C-1′), 75.47 (C-5′), 73.67 (C-3′), 70.25 (C-2′), 68.80 (C-4′), 63.87 (C-7), 60.87 (C-6′).

4.5.7. Compound 3g. m/z (LR-ESI$^+$) $C_{15}H_{22}N_2O_7H$ ($M+H^+$), found: 342.9, calcd: 343.3597.

4.5.8. Compound 3h. m/z (LR-ESI$^+$) $C_{15}H_{22}N_2O_7H$ ($M+H^+$), found: 342.9, calcd: 343.3597.

4.5.9. Compound 3i. m/z (LR-ESI$^+$) $C_{21}H_{32}N_2O_{12}H$ ($M+H^+$), found: 504.9, calcd: 505.5033.

4.5.10. Compound 3j. m/z (LR-ESI$^+$) $C_{17}H_{25}N_3O_7H$ ($M+H^+$), found: 405.9, calcd: 406.3945. ^{1}H NMR (400 MHz, D_2O): δ 7.02 (d, 2H, H-5, H-9), 6.69 (d, 2H, H-6, H-8), 4.57 (d, 1H, J=8.4 Hz, H-1′), 3.80–3.31 (m, 7H, H-2, H-2′, H-3′, H-4′, H-5′, H-6′), 2.97 (2dd, 2H, H-3), 1.87 (s, 3H, H-8′). ^{13}C NMR (D_2O): δ 175.85 (C-7′), 174.88 (C-1), 145.53 (C-7), 131.25 (C-5, C-9), 126.67 (C-4), 115.70 (C-6, C-8), 85.19 (C-1′), 77.32 (C-5′), 75.44 (C-3′), 70.77 (C-4′), 61.52 (C-6′), 56.91 (C-2), 55.86 (C-2′), 36.31 (C-3), 22.88 (C-8′).

4.5.11. Compound 3k. m/z (LR-ESI$^+$) $C_{17}H_{25}N_3O_7H$ ($M+H^+$), found: 405.9, calcd: 406.3945. ^{1}H NMR (400 MHz, D_2O): δ 6.97 (d, 2H, H-5, H-9), 6.69 (d, 2H, H-6, H-8), 4.46 (d, 1H, J=8.3 Hz, H-1′), 3.96–3.46 (m, 7H, H-2, H-2′, H-3′, H-4′, H-5′, H-6′), 2.91 (2dd, 2H, H-3), 1.81 (s, 3H, H-8′). ^{13}C NMR (D_2O): δ 186.97 (C-7′), 176.08 (C-1), 145.61 (C-7), 131.23 (C-5, C-9), 115.19 (C-6, C-8), 126.30 (C-4), 85.61 (C-1′), 76.35 (C-5′), 72.48 (C-3′), 70.23 (C-4′), 61.67 (C-6′), 56.94 (C-2), 54.47 (C-2′), 36.38 (C-3), 22.91 (C-8′).

4.5.12. Compound 3l. m/z (LR-ESI$^+$) $C_{23}H_{35}N_3O_{12}Na$ ($M+Na^+$), found: 567.9, calcd: 568.5381.

4.5.13. Compound 3m. m/z (LR-ESI$^+$) $C_{23}H_{35}N_3O_{12}Na$ ($M+Na^+$), found: 514.0, calcd: 514.5138.

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References and notes

1. Hakomori, S. *Curr. Opin. Immunol.* **1991**, *3*, 646–653.
2. Hakomori, S.; Zhang, Y. *Chem. Biol.* **1997**, *3*, 97–104.
3. Reisfeld, R. A.; Cheresh, D. A. H. *Adv. Immunol.* **1987**, *40*, 323–377.
4. Ben-Efraim, S. *Tumor Biol.* **1999**, *20*, 1–24.
5. Ragupathi, G. *Cancer Immunol. Immunother.* **1998**, *46*, 82–87.
6. Danishefsky, S. J.; Allen, J. R. *Angew. Chem., Int. Ed.* **2000**, *39*, 837–863.
7. Hellstrom, K. E.; Gladstone, P.; Hellstrom, I. *Mol. Med. Today* **1997**, 286–290.
8. Chen, C.-H.; Wu, T.-C. *J. Biomed. Sci.* **1998**, *5*, 231–252.
9. Livingston, P. O. *Curr. Opin. Immunol.* **1992**, *4*, 624–629.
10. Toyokuni, T.; Singhal, A. K. *Chem. Soc. Rev.* **1995**, 231–242.
11. Stipp, D. *Fortune* **1998**, *138*, 168–176.
12. Brocke, C.; Kunz, H. *Bioorg. Med. Chem.* **2002**, *10*, 3085–3112 (Review).
13. Maugard, T.; Gaunt, D.; Legoy, M. D.; Besson, T. *Biotechnol. Lett.* **2003**, *25*, 623–629.
14. Smaali, M. I.; Gargouri, M.; Legoy, M. D.; Maugard, T.; Liman, F.; Marzouki, N. *Appl. Biochem. Biotechnol.* **2004**, *112*, 63–78.
15. Smaali, M. I.; Michaud, N.; Marzouki, N.; Legoy, M. D.; Maugard, T. *Biotechnol. Lett.* **2004**, *26*, 675–679.
16. Gargouri, M.; Smaali, M. I.; Maugard, T.; Legoy, M. D.; Marzouki, N. *J. Mol. Catal.* **2004**, *29*, 89–94.
17. Abid, I.; Gargouri, M.; Smaali, I.; Limam, F.; Maugard, T.; Legoy, M. D.; Marzouki, N. *Int. J. Pure Appl. Chem.* **2006**, *1*, 83–90.
18. Bridiau, N.; Taboubi, S.; Marzouki, N.; Legoy, M. D.; Maugard, T. *Biotechnol. Prog.* **2006**, *22*, 326–330.
19. Bognar, R.; Nanasi, P. *J. Chem. Soc.* **1953**, 1703–1708.
20. Wang, L.; Maniglia, C. A.; Mella, S. L.; Sartorelli, A. *J. Med. Chem.* **1983**, *26*, 1323–1326.
21. Qian, X.; Li, Z.; Liu, Z.; Song, G.; Li, Z. *Carbohydr. Res.* **2001**, *336*, 79–82.
22. Das, Y. M.; Rao, C. P.; Kolehmainen, E. *Carbohydr. Res.* **2001**, *334*, 261–269.
23. Kublashvili, R. *Chem. Nat. Compd.* **2003**, *39*, 586–588.
24. Du, W.; Hu, Y. *Synth. Commun.* **2004**, *34*, 2987–2992.
25. Winum, J. Y.; Casini, A.; Mincione, F.; Starnotti, M.; Montero, J. L.; Scozzafava, A.; Supuran, C. Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 225–229.
26. Ledl, F.; Schleicher, E. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 565–594.
27. Testard, A.; Picot, L.; Lozach, O.; Blairvacq, M.; Meijer, L.; Murillo, L.; Piot, J. M.; Thiéry, V.; Besson, T. *J. Enzyme Inhib. Med. Chem.* **2005**, *20*, 557–568.
28. Von Euler, H.; Brunius, E. *Chem. Ber.* **1926**, *59B*, 1581–1585.
29. Paulsen, H.; Pflughaupt, H. Glycosylamines. In *The Carbohydrates: Chemistry and Biochemistry*; Pigman, W., Horton, D., Eds.; Academic: New York, NY, 1980; Vol. 1B, pp 881–927.