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Interaction between lectins and neoglycoproteins containing new sialylated glycosynthons

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Neoglycoconjugates are useful tools to study carbohydrate/protein interactions. In order to discover new lectins, to define their fine specificity or to study their intracellular trafficking, there is a need for neoglycoconjugates containing complex oligosaccharides. We recently set up a simple way to transform native oligosaccharides into glycosynthons. The present paper describes i) the synthesis of such glycosynthons starting with sialylated oligosides, ii) the preparation of sialylated neoglycoproteins and iii) their binding to sialic acid-specific lectins assessed by surface plasmon resonance experiments.

Keywords: ***N*-acetyl neuraminic acid, lectin, glycosynthon, oligosaccharide, sialic acid**

Abbreviations: **BOP, benzotriazol-1-yloxi-tris-(dimethylamino) phosphonium hexafluorophosphate; BSA, bovine serum albumin; CM, carboxymethyl; DMF, *N*-dimethyl-formamide; DSS, sodium 4,4-dimethyl-4-silipentane-1-sulfonate, $(CH_3)_3Si(CH_2)_3SO_3Na$; ESI, electrospray ionization; Glp, pyroglutamate; Glu, glutamate; HBS, 0.15 M NaCl, 10 mM Hepes buffer at pH 7.4 containing 50 μL/L of surfactant P20; HPAEC, high pressure anion-exchange chromatography; HPLC, high pressure liquid chromatography; $k_a = k_{ass}$, $k_d = k_{diss}$, kinetic constant for association and dissociation, respectively; K_A, affinity constant. k_{diss} / k_{ass}, L: mol^{-1}; MaA, *Maackia amurensis* lectin; MS, mass spectrometry; PAD, pulsed amperometric detection; PITC, phenylisothiocyanate; *p*NA, *p*-nitroanilide; RU, relative unit; SA, streptavidin-coated (sensor chip); Slac, sialyllactose; 3Slac, Neu5Acα 3Galβ 4Glc; 6Slac, Neu5Acα 6Galβ 4Glc; SnA, *Sambucus nigra* lectin; TEA, triethylammonium acetate; TCBI, *N,N'*-thiocarbamyl bisimidazole.**

Introduction

Oligosaccharide moieties of glycoproteins and glycolipids are involved in numerous biological processes such as cell infection by pathogens, cell-cell recognition and intracellular traffic [1, 2]. They act as ligands for endogenous lectins which are involved in endocytosis and/or intracellular traffic of specific glycoconjugates [3]. According to their diversity and complexity, the investigation on the role of oligosaccharides as recognition signals requires powerful tools. Synthetic glycoconjugates termed neoglycoconjugates, including neoglycoproteins (bovine serum albumin substituted with about 25 simple sugar residues) or neoglycolipids, have been largely used for the study of carbohydrate receptors [see for reviews 4–6 and references therein]. However, a precise study on the role and specificity of lectins would need the use of neoglycoconjugates elaborated from native oligosaccharides.

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Oligosaccharides may be prepared from biological fluids or released from glycoproteins by enzymatic or chemical means. According to the limited amount available, there is a need to substitute oligosaccharides with a reactive device in a simple and efficient way. Recently we described a novel one-pot two-step reaction allowing the synthesis of neoglycoconjugates in a very high yield starting from reducing complex neutral oligosaccharides [7–9]. This reaction consists of the coupling of α-glutamyl-*p*-nitroanilide (Glu-*p*NA) [9] to the reducing oligosaccharide leading to a glycosylamine; this glycosylamine is then stabilized by intramolecular acylation upon adding a carboxylic group activator, acting on the side chain of the glutamyl moiety. Based on the presence of the carboxylic group of sialic acid, which can lead to secondary reactions during the intramolecular acylation step, the experimental procedure needed to be adapted for the case of sialylated derivatives. Using 3-sialyllactose and 6-sialyllactose as models we show that: i) in the case of the 3-sialylated derivative, a minor adaptation is required to prevent the formation of a bis-

Glu-*p*NA derivative; ii) in the case of the 6-sialylated derivative, the 6-sialyllactosyl-Glu-*p*NA conjugate spontaneously reacted to give an unexpected cyclic by-product. This side effect can be avoided thanks to a simple modification of the intramolecular acylation step. Products were characterized both by ^{1}H-NMR spectroscopy and by electrospray ionisation mass spectrometry. Sialyllactosyl-Glp-*p*NA was reduced to an aminophenyl derivative; this intermediate compound was then transformed into sialyllactosyl-Glp-*p*-amidophenylisothiocyanate (Sialyllactosyl-Glp-*p*-amido-PITC); and finally, neoglycoproteins were prepared by allowing sialyllactosyl-Glp-*p*-amido-PITC to react with BSA. The capacity of lectins (*Maackia amurensis*, α-3 specific, and *Sambucus nigra,* α-6 specific) to bind these neoglycoproteins was qualitatively and quantitatively assessed by surface plasmon resonance.

Materials and methods

Materials

α-Glutamyl-*p*-nitroanilide (Glu-*p*NA) was purchased from Bachem (Bubendorf, Switzerland). Imidazole was from Merck (Darmstadt, Germany). Benzotriazol-1-yloxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (BOP) was from Richelieu Biotechnologies (Saint Hyacinthe, Canada). *N*-dimethylformamide (DMF) was freshly distilled in the presence of 0.2 mg/mL benzyloxycarbonyl-glycyl-*p*-nitrophenyl ester. 3-Sialyllactose and 6-sialyllactose were isolated from human milk (G. Strecker, unpublished data) or purchased from Dextra Laboratories (Valbiotech, Paris, France). Lectins were from Sigma (St Louis, MO, USA), biotinylated lectins were from Vector (Burlingame, CA, USA).

Chromatography

Conjugates were purified by gel filtration on a column (2.1 × 100 cm) of Trisacryl GF05 (Biosepra, Villeneuve-la-Garenne, France) stabilized in and eluted (flow rate, 7 mL/h) with 50 mM *N*-methylimidazole (Aldrich, WI, USA).

High performance anion-exchange chromatography (HPAEC) was performed on a Dionex DX-300 chromatography system (Sunnyvale, CA, USA) which includes a quaternary gradient pump, an eluent degas (He) module, a (4 × 250 mm) CarboPac PA1 column with a matching guard column and a pulsed amperometric detector (PAD). The flow rate was 1 mL/min. The gradient was obtained by using two solutions, solvent A: 0.1 N sodium hydroxide and solvent B: 1 M sodium acetate in 0.1 N sodium hydroxide. The column was equilibrated and then eluted with solution A for 5 min and with a double linear gradient increasing B from 0 to 15% over 10 min and from 15% to 100% over 15 min.

High performance liquid chromatography (HPLC) analysis was performed on a reverse phase matrix (Alltima C18 5U, Altech, Deerfield, IL, USA) in a 4.6 × 150 mm column. The HPLC system included a dual pump system monitored by a Waters 600 Controller linked to a Waters 996 photodiode array detector. Two solvents were used: solvent C: 0.1 M triethylammonium acetate (TEAA) + 5% acetonitrile and solvent D: acetonitrile + 5% 0.1 M TEAA. The flow rate was 1 mL/min. The column was equilibrated with a mixture of solvents C and D (95 : 5, per volume). After injecting the sample, the column was eluted for 5 min with the 95:5 mixture; then solvent D was increased linearly from 5% up to 40% over 30 min.

Synthesis and purification of sialyllactosyl-Glp-*p*NA

3-Sialyllactosyl-Glp-*p*NA and 6-sialyllactosyl-Glp-*p*NA were synthesized by the one-pot two-step method adapted from our previous report [9]. In a typical experiment 10 mg (15 μmoles) of sialyllactose, 10 mg (37.5 μmoles) of Glu-*p*NA and 5 mg (75 μmoles) of imidazole were dissolved in 0.5 mL of freshly distilled DMF and the solution was kept at 50°C for 4 hours. The glycosylamine derivative was stabilized by intramolecular *N*-acylation. In the case of 3-sialyllactosyl-Glp-*p*NA the reaction mixture was diluted tenfold (up to 5 mL) with DMF, then 27 mg (60 μmoles) of BOP and 7 mg (105 μmoles) of imidazole were added, the solution was kept for 1 hour at room temperature. For 6-sialyllactosyl-Glp-*p*NA on the one hand, the reaction mixture was not diluted, and on the other hand, BOP (27 mg, 60 μmoles) and imidazole (7 mg, 105 μmoles) in 300 μL of DMF were added in six aliquots leaving 15 min between each addition. The formation of sialyllactosyl-Glp-*p*NA monitored by HPAEC was found to be complete within 1 hour. Finally, in order to hydrolyze any remaining sialic acid ester, 500 μL or 80 μL of water was added to the reaction mixture containing 3-sialyllactosyl-Glp-*p*NA or 6-sialyllactosyl-Glp-*p*NA, respectively.

Both sialyllactosyl-Glp-*p*NA were purified by gel filtration. Reaction mixture was reduced to 0.5 mL under reduced pressure at 0°C. After dilution up to 10 mL with 50 mM *N*-methylimidazole, this solution was purified on a Trisacryl GF05 column (2.1 × 100 cm) equilibrated in 50 mM *N*-methylimidazole and eluted with the same eluent at a flow rate of 7 mL/h. The eluate was monitored by measuring the absorbance at 280 nm and the sugar concentration was determined in each fraction by using the resorcinol sulfuric acid micromethod [10]. Fractions of interest were pooled and freeze-dried. The glycosynthon, dissolved in the minimum of water (about 0.2 mL) was made free of *N*-methylimidazole by precipitation upon adding 20 volumes of isopropanol.

Characterization of sialyllactosyl-Glp-*p*NA

Both sialyllactosyl-Glp-*p*NA were characterized by ^{1}H-NMR spectroscopy and by electrospray ionization mass spectrometry (ESI-MS).

^{1}H-NMR spectroscopy

For ^{1}H-NMR analysis, compounds were dissolved at room temperature in deuterated water at 5 mg/mL, once with D_2O containing 99.9% D, and, after freeze drying, a second time

with D_2O containing 99.96% D (Sigma, Saint-Quentin-Fallavier, France). ^{1}H-NMR spectroscopy was performed at 300 MHz on a Bruker AM-300 spectrometer (Wissembourg, France). Chemical shifts were measured by reference to acetone ($\delta = 2.225$ ppm in D_2O) used as an internal reference and were expressed in ppm downfield from internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS).

Electrospray ionization mass spectrometry (ESI-MS)

The positive ion electrospray mass spectra were obtained using a Platform quadrupole mass spectrometer (VG Biotech, Fisons Instruments, Altrincham, UK) equipped with an electrospray atmospheric pressure ionization source. The capillary voltage was set at + 3 kV and the cone voltage was adjusted at 25 V. The analyzer was calibrated for m/z in the 400–1200 range in positive-ion mode using a caesium iodide solution. The sample was dissolved in an acetonitrile : 0.1% formic acid mixture (1/1 per volume) at a 100 ng/μL concentration.

Preparation of neoglycoproteins

Neoglycoproteins were prepared by allowing sialyllactosyl-Glp-*p*-amidophenylisothiocyanate (SLac-Glp-*p*amido-PITC) to react on BSA. SLac-Glp-*p*amido-PITC was obtained as previously described [9,11]. Briefly, sialyllactosyl-Glp-*p*NA was reduced to its amino form by reaction with palladium charcoal under hydrogen (1 atm). Then, sialyllactosyl-Glp-*p*-aminoanilide was converted to SLac-Glp-*p*amido-PITC by treatment with *N*, *N*'-thiocarbamylbisimidazole (TCBI). SLac-Glp-*p*amido-PITC (11.4 μmoles) was added to BSA (25 mg, 0.37 μmole) dissolved in 2 mL of 100 mM sodium bicarbonate buffer pH 9.5 containing 100 mM sodium acetate. Mixture was stirred overnight at room temperature. Neoglycoproteins were purified on GF05 (2 × 50 cm) column equilibrated in 100 mM sodium acetate, followed by a second GF 05 (2 × 50 cm) equilibrated in 50 mM *N*-methylimidazole. After freeze-drying neoglycoproteins were dissolved in a minimum of water (about 1 mL) and precipitated by adding ten volumes of absolute ethanol. The average number of sugar residues bound per BSA molecule was determined using the resorcinol sulfuric acid micromethod [10].

Surface plasmon resonance spectroscopy

All experiments were performed on a BIAcore 2000 biosensor (Pharmacia biosensor, Uppsala) at 25°C in HBS buffer : 10 mM Hepes, pH 7.4 containing 0.15 M NaCl and 50 μL/L of surfactant P20 (Pharmacia biosensor), or in complete HBS buffer (HBS supplemented with 1 mM $CaCl_2$ and 0.5 mM $MgCl_2$). Lectins (*Sambucus nigra and Maackia amurensis*) were immobilized on CM sensor chips (Pharmacia biosensor) by using the amino coupling kit (Pharmacia biosensor) or on SA (streptavidin-coated) sensor chips (Pharmacia biosensor). For CM chips, the flow rate was 5 μL/min, lectins were injected on activated surface at 100 μg/mL (*Sambucus nigra*) or 200 μg/mL (*Maackia amurensis*) for 5 min. For SA chips, the flow rate was 7 μL/min, lectins were injected at 10 μg/mL for 3 min.

In all steps, the flow rate was 10 μL/min. Neoglycoproteins in complete HBS buffer, pH 7.4 were used at 6.25, 12.5, 25 and 50 μg/mL (around 10^{-7} M). An automatic binding assay cycle was performed as follows : a neoglycoprotein solution was applied for 5 min, then the neoglycoprotein-free buffer for 5 min in order to initiate the dissociation reaction. Sensor chip was regenerated by washing with 50 mM HCl for 1 min when using the chemically bound lectins or for 3 min when using the biotinylated lectins; finally the chips were equilibrated in the buffer for 3 min. Kinetic constants were determined by using the BIA-evaluation software.

Results and discussion

Recently we described a simple and efficient method of preparing glycosynthons from neutral complex oligosaccharides [7–9]. The synthesis takes place in two steps: spontaneous linkage of a reducing sugar to the α-NH_2 of a glutamate derivative, followed by an intramolecular acylation upon activation of the glutamate γ-COOH. Due to the presence of carboxylic group(s) in sialylated oligosides the previously described method required slight modifications. In the present

Table 1. Retention time (min) of oligosides, peptides and glycoaminoacids on both HPAEC and HPLC

Component	*HPAEC (Amperometric detection)*	*HPLC (Spectrometric detection at 316 nm)*
6-sialyllactose	14.2 (14.7)[1]	nr
Glu-*p*NA	nr	12.8
6-sialyllactosyl-Glu-*p*NA	31 (33.5)	nr
6-sialyllactosyl-Glp-*p*NA	26.5 (27.7)	21.5 (21.7)
Glp-*p*NA	nr	26.5

[1] Times for 3-sialyllactose and derivatives are given in brackets
nr: not relevant

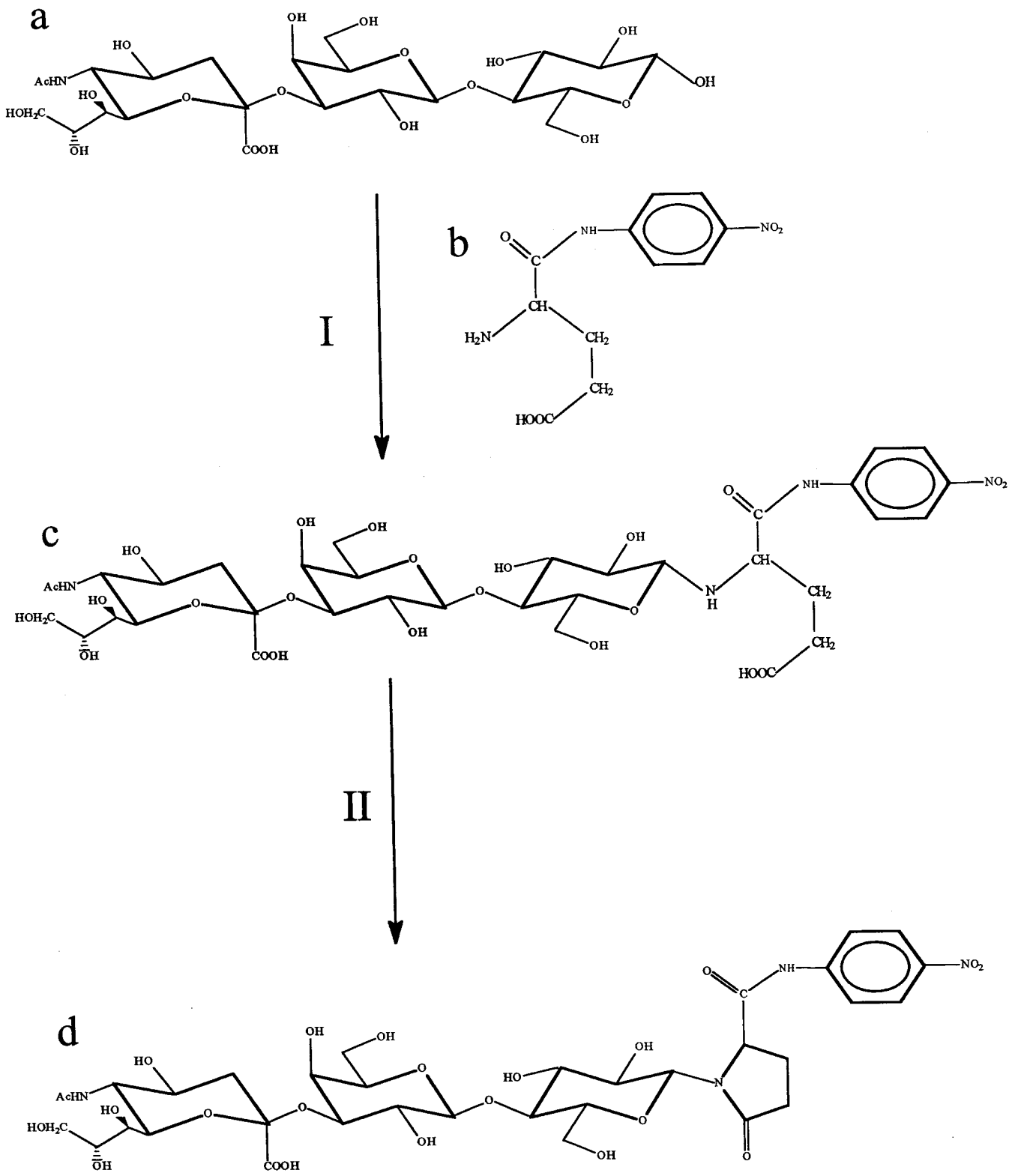

Figure 1. Scheme of the 3-sialyllactosyl-Glp-*p*NA synthesis. I, condensation, II, cyclization; a, 3-sialyllactose, b, Glu-*p*NA, c, 3-sialyllactosyl-Glu-*p*NA and d, 3-sialyllactosyl-Glp-*p*NA.

paper, we show that sialylated oligosides can be used as starting material for glycosynthon synthesis on condition that the relevant adaptations are made. 3-Sialyllactose and 6-sialyllactose were chosen as models. Reactions were monitored both by using HPAEC (amperometric detection) and HPLC (absorbance detection at 316 nm), (Table 1). Glycosynthons were further used to prepare sialylated-neoglycoproteins. Such sialylated-neoglycoproteins are shown to be good ligands of sialic acid-specific lectins as assessed by surface plasmon resonance.

Synthesis of 3-sialyllactosyl-Glp-*p*NA

The glycosynthon synthesis is schematically shown in Figure 1. Upon incubation at 50°C, 3-sialyllactose (a) was condensed (step I) to Glu-*p*NA (b) leading to 3-sialyllactosyl-Glu-*p*NA (c) in an almost quantitative way within 3 h. In order to avoid the formation of a bis-Glu-*p*NA conjugate (data not shown), reaction mixture had to be diluted ten-fold before the intramolecular acylation step (step II). Stabilization of the conjugate was achieved within 1 hour leading to 3-sialyllactosyl-Glp-*p*NA (d). This compound $C_{34}H_{48}O_{22}N_4$ (M+H) + has an expected mass of: 865.28. The experimental mass obtained by an electrospray ionization spectrometer was found to be 865.4. On the basis of ^{1}H-NMR analysis, the glucose moiety was shown to be in β configuration. The assignment of the 3-sialyllactosyl-Glp-*p*NA ^{1}H-NMR spectrum in D_2O was as follows: δ 8.27 and 7.74 (4H, H aromatic), 5.20 (1H, H-1 β-D-Glc), 4.62 (1H, α-CH Glp), 4.43 (1H, H-1 β-D-Gal), 4.07 (1H, H-3 β-D-Gal), 3.94 (1H, H-4 β-D-Gal), 2.80 (1H, γ_e CH_2 Glp), 2.73 (1H, H-3$_e$ Neu5Ac), 2.54 (1H, γ_a CH_2 Glp), 2.44 (1H, β_e CH_2 Glp), 2.25 (1H, β_a CH_2 Glp), 2.02 (3H, CH_3-CO Neu5Ac), 1.77 (1H, H-3$_a$ Neu5Ac) ppm.

Synthesis of 6-sialyllactosyl-Glp-*p*NA.

Using 6-sialyllactose as starting material, because of the high reactivity of the sialic acid carboxylic group, the intramolecular acylation protocol had to be modified. 6-Sialyllactose (Figure 2, peak 1 at 15 min) was conjugated to Glu-*p*NA, under the conditions described for the 3-sialyllactose, leading to 6-sialyllactosyl-Glu-*p*NA (peak 2, at 31 min). The intramolecular cyclisation was induced by adding the carboxylic group activator in small aliquots over 90 min and gave two products (peak 3 and 4 eluted respectively at 20 and 26 min). It must be noted that peak 3 was preferentially formed when cyclisation was conducted by adding the carboxylic group activator at once.

Under treatment with 50 mM H_2SO_4 for 1 hour at 80°C (Figure 3), peak 4 was converted into *N*-acetyl neuraminic acid (peak 5) and lactosyl-Glp-*p*NA (peak 6) while peak 3 was unaffected suggesting that peak 4 corresponded to the expected product i.e. 6-sialyllactosyl-Glp-*p*NA. Peak 4 was purified by gel filtration on GF05 equilibrated in 50 mM *N*-methylimidazole. The mass of this compound, calculated for $C_{34}H_{48}O_{22}N_4$ (M+H)+ : 865.28, was found to be 865.4 using

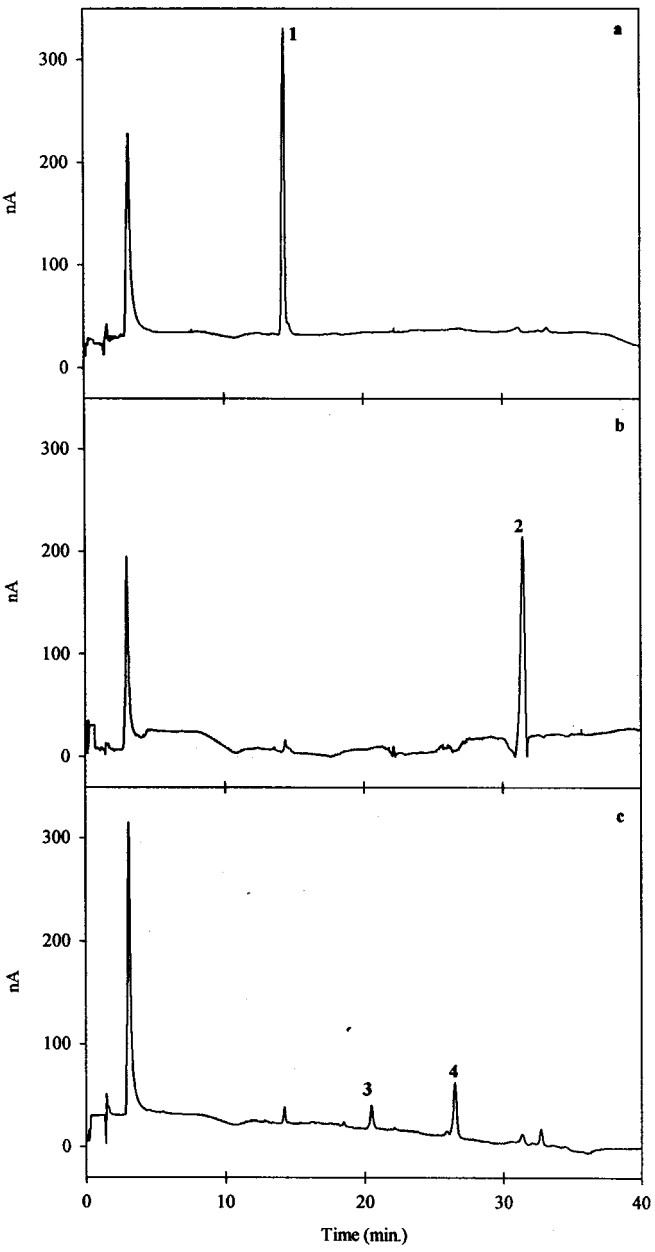

Figure 2. Conversion of 6-sialyllactose to 6-sialyllactosyl-Glp-*p*NA monitored by HPAE chromatography (amperometric detection) after (a) 5 min, (b) 3 h, and (c) the cyclization step. 1, 6-sialyllactose; 2, 6-sialyllactosyl-Glu-*p*NA; 3, by-product; 4, 6-sialyllactosyl-Glp-*p*NA. The first peak, eluted at about 3 min, is imidazole.

an electrospray ionization spectrometer. On the basis of ^{1}H-NMR analysis, it was found that the glucose moiety was in β configuration. The assigment of the 6-sialyllactosyl-Glp-*p*NA ^{1}H-NMR spectrum in D_2O was as follows: δ 8.3 and 7.74 (4H, H aromatic), 5.20 (1H, H-1 β-D-Glc), 4.63 (1H, α-CH Glp), 4.33 (1H, H-1 β-D-Gal), 3.92 (1H, H-4 β-D-Gal), 3.75 (1H, H-4 β-D-Glc), 3.72 (1H, H4 Neu5Ac), 3.51 (1H, H-2 β-D-Gal), 2.82 (1H, γ_e CH_2 Glp), 2.73 (1H, H-3$_e$ Neu5Ac),

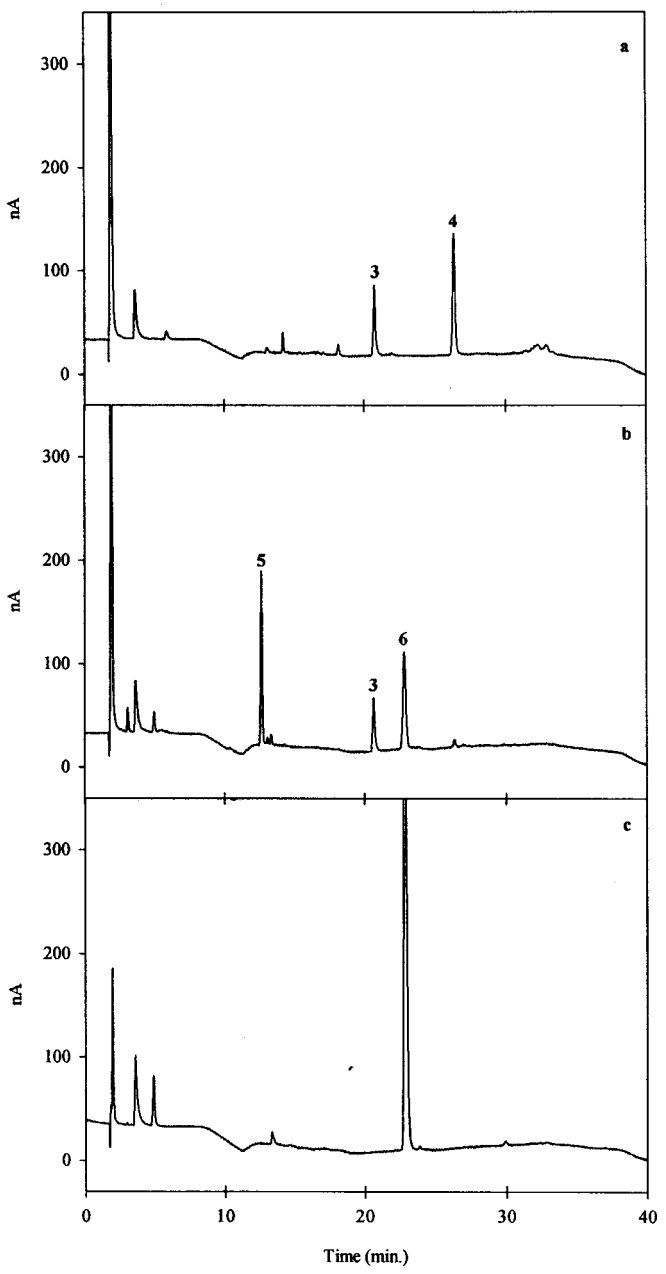

Figure 3. Treatment of the mixture of peak 3 (by-product) and peak 4 (6-sialyllactosyl-Glp-*p*NA) with 50 mM H_2SO_4 monitored by HPAE chromatography: (a) treated for 1 h at 4°C, (b) treated for 1 h at 80°C, (c) lactosyl-Glp-*p*NA as a control. 3, by-product; 4, 6-sialyllactosyl-Glp-*p*NA; 5, Neu5Ac; 6, lactosyl-Glp-*p*NA.

2.57 (1H, γ_a CH_2 Glp), 2.50 (1H, β_e CH_2 Glp), 2.25 (1H, β_a CH_2 Glp), 2.03 (3H, CH_3-CO Neu5Ac), 1.82 (1H, H-3_a Neu5Ac) ppm.

Preparation of neoglycoproteins.

Sialylated glycosynthons were conjugated to BSA using the previously described protocol [9]. Sialyllactosyl-Glp-*p*NA was reduced to its amino form and then, upon addition of TCBI, was converted to sialyllactosyl-Glp-*p*-amidoPITC. The coupling of sialyllactosyl-Glp-*p*-amidoPITC with BSA, according to the protocol described under Materials and Methods, led to neoglycoproteins (3Slac-BSA and 6Slac-BSA) bearing 8 ± 1 sugar residues as determined by the resorcinol micromethod [10]. As a control, we prepared under the same conditions, a lactosylated-BSA (Lac-BSA) bearing an average of 15 lactosyl moieties.

Study of neoglycoprotein binding to lectins by surface plasmon resonance.

In order to test the capacity of sialylated neoglycoproteins to specifically bind sialic acid-specific lectins, neoglycoproteins were injected on sensor chips coated with a 3-sialylactose specific lectin (*Maackia amurensis,* MaA) or a 6-sialylactose specific lectin (*Sambucus nigra,* SnA).

We chose to immobilize the lectins instead of the neoglycoproteins for two main reasons: i) according to Shinohara *et al.*, [12] the binding constants obtained with immobilized lectins are close to those obtained in solution, whilst the binding constants obtained with immobilized glycoconjugates depend on the glycoconjugate density on the chips and ii) sialylated neoglycoproteins are quite susceptible to acidic hydrolysis which could occur during the regeneration step.

We compared two procedures of immobilization: i) immobilization of biotinylated lectins on streptavidin-coated sensor chips and ii) immobilization of lectins on activated carboxylic groups on the sensor chips. Typical sensorgrams obtained with chemically bound lectins are shown in Figure 4.

In preliminary experiments, various parameters were modulated in order to obtain reproducible data: i) the charge of the sensor chip was satisfactory when lectin concentrations were adjusted in order to obtain an increase of the response higher than 1200 RU; ii) Hepes buffer or phosphate buffer gave similar results as long as they contained Ca^{++} and Mg^{++} ions; iii) regeneration is a crucial step: neither basic solution (50 mM glycine/NaOH buffer, pH 9.2; 100 mM NaOH; 100 mM ethanolamine) nor a slightly acidic solution (10 or 20 mM HCl) were satisfactory. Finally, 50 mM HCl was found to be convenient since this solution was able to remove the bound neoglycoprotein and because the regenerated chips led to identical binding data under identical conditions, for more than 10 cycles.

As expected, 3SLac-BSA and 6SLac-BSA strongly bind immobilized MaA and SnA, respectively. In both cases, no significant binding was observed with Lac-BSA, BSA itself or irrelevant sialylated neoglycoprotein(s). 3SLac-BSA, as expected, did not bind to MaA in the absence of divalent cations.

Kinetics constants (Table 2) were determined by using the data obtained with neoglycoproteins used at 4 concentrations from 5 to 50 µg/mL. The kinetics data indicated that the lectins immobilized by chemical means were more accessible

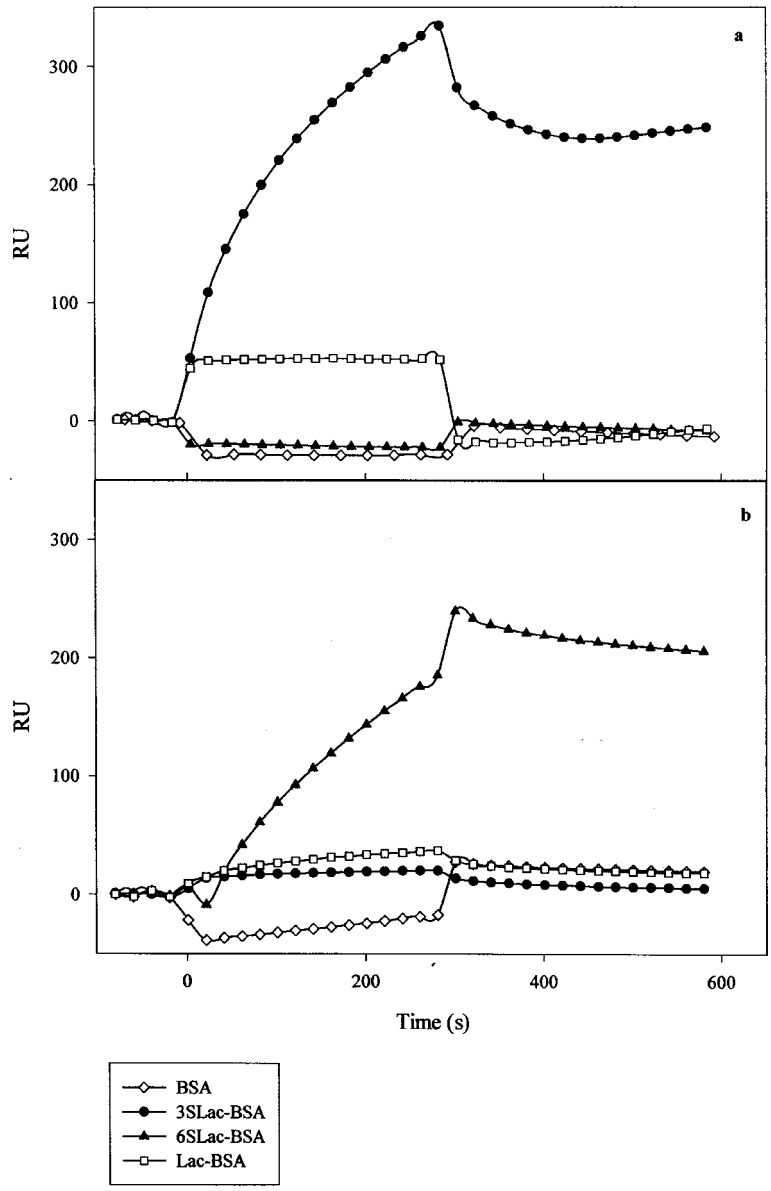

Figure 4. Sensorgrams showing the interactions between neoglycoproteins and immobilized lectins: (a) *Maackia amurensis* and (b) *Sambucus nigra*. Experiments were conducted as described under Materials and Methods. Neoglycoprotein concentration was 50 μg/ml.

than the biotinylated lectins immobilized on streptavidin-coated sensor chips. Both the k_{ass} and k_{dis} had higher values in the case of the chemically immobilized lectins. However, in both cases the binding constants were roughly in the 10^7 L.mole^{-1} range. The values obtained with *Sambucus nigra* are close to those obtained by Okazaki *et al.* [13] using immobilized fetuin and the *Sambucus sieboldiana* lectin; the authors obtained a k_{ass} of 5.4 10^3 L.mole^{-1}.s^{-1}, a k_{dis} of 4 10^{-4} s^{-1} leading to a K_A of 1.4 10^7 L.mole^1. The specificity of the α3 and α6 sialylated neoglycoproteins are quite good and are very similar to an engineered glycoprotein such as the rat thymus CD45. Van der Merwe *et al.* [14] showed that the native CD45 was able to bind both *Maackia amurensis* and *Sambucus nigra* lectins. However, upon desialylation and resialylation with a specific galβ-4GlcNAc α-6 sialyltransferase, the engineered CD45 interacted poorly with *Maackia amurensis* lectin but strongly with *Sambucus nigra* lectin.

Concluding remarks.

The simple one-pot two-step procedure set up for the preparation of glycosynthons containing neutral sugars can be easily adapted to obtain sialylated glycosynthons without any protection-deprotection step. Neoglycoproteins prepared by using the two classical types of sialyllated oligosaccharides i.e. Neu5Ac α-3-galactoside and Neu5Ac α-6-galactoside are

Table 2. Neoglycoprotein-lectin interactions: kinetics and binding constants

A. Lectins bound to chips by chemical means.

Neoglycoprotein	*Lectin*	k_a *(1/Ms)*	K_d *(1/s)*	K_A (M^{-1})
6SLac-BSA	SnA	$6.0\ 10^3$	$3.0\ 10^{-4}$	$2.0\ 10^7$
3SLac-BSA	MaA	$7.5\ 10^3$	$7.7\ 10^{-4}$	$9.7\ 10^6$

B. Biotinylated lectins bound through immobilized streptavidin on chips.

Neoglycoprotein	Lectin	k_a (1/Ms)	K_d (1/s)	K_A (M^{-1})
6SLac-BSA	SnA	$2.9\ 10^3$	$4.2\ 10^{-5}$	$6.9\ 10^7$
3SLac-BSA	MaA	$2.0\ 10^3$	$2.8\ 10^{-5}$	$7.1\ 10^6$

The standard deviation was lower than 10%

quite suitable tools: indeed they are recognized by specific lectins with both a high selectivity and a high affinity.

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