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Synthesis of a water-soluble serine-based neoglycolipid which can be covalently linked to solid phases

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Abstract

N^{α}-Fmoc-serine pentafluorophenyl ester was glycosylated with perbenzoylated lactosyl bromide. The resulting product was coupled to a resin functionalized with 6-aminohexanoic acid and then N^{α}-acylated to give a serine-based analogue of lactosylceramide. The water-soluble neoglycolipid was covalently linked to microtiter plates via its carboxyl group and was recognized by a lactose-binding lectin in an ELISA.

1. Introduction

Glycosphingolipids are key constituents of cell membranes and mediate many important functions involving molecular recognition in vivo [1,2]. Thus, many glycolipids are tumor-associated antigens that can be recognized by the immune system [3]; bacteria and viruses use glycolipids as attachment sites when causing infections [4]; glycolipids mediate cell–cell interactions, for instance in inflammatory responses [1]. Access to pure glycosphingolipids is essential for detailed investigations of these and related phenomena. However, glycosphingolipids are often only available in small amounts and in heterogeneous form from natural sources, and considerable efforts have therefore been made to develop synthetic routes towards both naturally occurring glycosphingolipids [5,6] and neoglycolipids [6].

Glycosphingolipids typically have β-lactose carrying various saccharidic substituents linked to the lipid ceramide, which consists of sphingosine acylated by a

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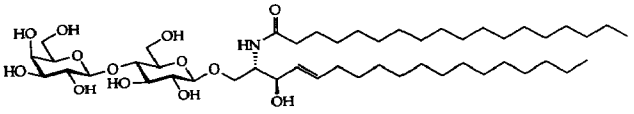
long-chain fatty acid (cf. lactosylceramide, **1**). The two most common strategies for synthesis of glycosphingolipids involve glycosylation either of ceramide or of protected derivatives of 2-azido-2-deaminosphingosine which require subsequent reduction of the azide and *N*-acylation to reach the desired glycolipid [5,6]. Recently, an approach based on Schiff base-protection of the 2-amino group in sphingosine was also reported [7]. Glycosylation of 2-azido-2-deaminosphingosine [8] or Schiff base-protected sphingosine generally gives higher yields than glycosylation of ceramide. This can be explained by favourable hydrogen bonding, resulting in enhanced nucleophilicity [7] of the first two acceptors, as compared to ceramide, and by unfavourable complexation between ceramide and the glycosylation promoter [9,10]. In addition, orthoester formation may lead to decreased yields in the glycosylation step, but this side reaction can be suppressed by protection of the glycosyl donor with benzoyl or pivaloyl groups instead of acetyl groups [9,10].



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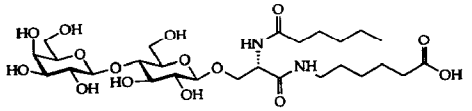

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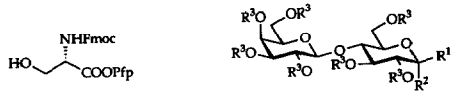
Synthesis of sphingosine [11] is a multistep procedure and access to neoglycolipids with simplified lipid moieties is therefore desirable. However, recent investigations have revealed the importance of the polar ceramide head in binding of bacteria and viruses to glycosylceramides [12]. Neoglycolipids should therefore preferably mimic this part of the ceramide, a criterion which to date is displayed by very few neoglycolipids [6]. Since serine is used as starting material in the biosynthesis of ceramide [13], it occurred to us that glycosylated derivatives of L-serine could be used for synthesis of such neoglycolipids as **2**. The lipid part of **2** resembles ceramide with respect to the stereochemistry for the *N*-acyl group on C-2, and by having an adjacent oxygen functionality. In the present study, we describe the glycosylation of N^{α}-(9-fluorenylmethoxycarbonyl)-L-serine pentafluorophenyl ester [14] (**3**) with different lactosyl donors and the use of one of the resulting building blocks, **10**, for synthesis of the neoglycolipid **2** according to standard solid-phase glycopeptide procedures [15,16]. Previously, asparagine-based neoglycolipids with the carbohydrate moiety located on the side chain of Asn and with long-chain aliphatic amines linked to the α-carboxyl group have been reported [17].

2. Results and discussion

We investigated the glycosylation of N^{α}-(9-fluorenylmethoxycarbonyl)-L-serine pentafluorophenyl ester [14] (Fmoc-Ser-OPfp, **3**) with a panel of lactosyl donors

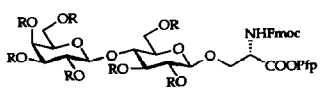
and various promoters since glycosylation of *N*-acylated β-amino alcohols such as **3**, and the structurally related ceramide, is known to be difficult [6–10,18]. Glycosylation of **3** with β-lactose octaacetate [19] (**4**) under boron trifluoride etherate promotion [20] gave **9** contaminated by the corresponding α anomer (α:β, 13:87 according to ^{1}H NMR spectroscopy, 42% total yield). The two anomers could not be separated by flash column chromatography or reversed-phase HPLC. Similarly, glycosylation of **3** with per-*O*-acetylated ethyl 1-thio-β-lactoside under methylsulfenyl triflate promotion [21] gave **9** (35% yield) containing a small amount of the α anomer (< 5% according to ^{1}H NMR spectroscopy). Activation of the thioethyl lactoside with *N*-iodosuccinimide and triflic acid [22,23] led to the formation of the orthoester **11** (~ 85% yield) and attempts to rearrange **11** into the desired glycoside **9** failed. When the per-*O*-acetylated trichloroacetimidate [24] of lactose was used as donor under boron trifluoride etherate promotion, a complex mixture of products was formed and not analyzed further. Silver triflate(AgOTf)-promoted glycosylation of **3** with the per-*O*-acetylated lactosyl bromide **5** [25] gave the pure building block **9** even though the yield was modest (24%). The presence of 1,1,3,3-tetramethylurea in this glycosylation again resulted in formation of the orthoester **11** (53% yield). Improved yields are often obtained in glycosylations with benzoyl, instead of acetyl, protection of the glycosyl donor [26]. In agreement with this, glycosylation of **3** (2 equiv) with the per-*O*-benzoylated lactosyl bromide **6** [27], using AgOTf as promoter, gave the glycoside **10** in a high yield (72%) without competing orthoester or α-glycoside formation. Recently, benzoyl protective groups were also successfully employed [28] in β-D-glucosylation of **3**. During the completion of the present investigation, glycosylation of esters of Schiff base-protected L-serine with the lactosyl bromide **5** in excellent yields was also reported [18].

The building block **10** was used for the synthesis of the neoglycolipid **2** according to standard procedures for solid-phase glycopeptide synthesis [15,16].

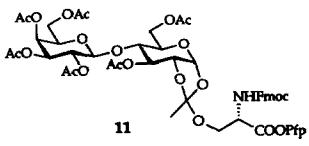


3

4	R^1=OAc	R^2=H	R^3=Ac
5	R^1=H	R^2=Br	R^3=Ac
6	R^1=H	R^2=Br	R^3=Bz
7	R^1=SCH_2CH_2COOH	R^2=H	R^3=Ac
8	R^1=SCH_2CH_2COOH	R^2=H	R^3=H



9 R=Ac
10 R=Bz



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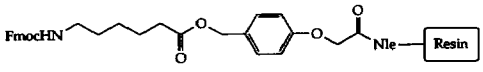
The solid-phase procedure was chosen since approaches in solution gave low overall yields in our hands, and also in order to minimize workup and purification procedures. The synthesis was performed in a mechanically agitated reactor on a polystyrene resin grafted with poly(ethylene glycol) [29]. N^{α}-Fmoc-Norleucine, activated as its benzotriazolyl ester [30], was first coupled as an internal reference amino acid to the amino group of the resin. The level of incorporation of norleucine on the resin was determined to be 0.23 mmol/g (0.25 mmol/g calculated on original resin mass) according to spectrophotometric analysis [31] of *N*-(9-fluorenylmethyl)piperidine formed on Fmoc deprotection of an aliquot of the resin. After cleavage of the Fmoc group with 20% piperidine in DMF, the linker 4-hydroxyphenoxyacetic acid [32] was coupled as its benzotriazolyl ester to the resin. Then *N*-Fmoc-6-aminohexanoic acid was coupled [33] to the linker with 1-mesitylenesulfonyl-3-nitro-1,2,4-triazole (MSNT) and 1-methylimidazole to give the functionalized resin **12**. The level of incorporation of *N*-Fmoc-6-aminohexanoic acid on **12** was determined to be 0.21 mmol/g (0.25 mmol/g calculated on original resin mass), indicating quantitative incorporation of both the linker and 6-aminohexanoic acid. At this stage, the resin had to be washed thoroughly with DMF, toluene, and dichloromethane in order to remove adsorbed *N*-Fmoc-6-aminohexanoic acid. After Fmoc deprotection, the glycosylated building block **10** (1 equiv with respect to the 6-aminohexanoic acid incorporated on the resin **12**) was coupled to the resin followed by Fmoc deprotection with 50% morpholine in DMF and incorporation of hexanoic acid as its benzotriazolyl ester. All *N*-acylations were monitored by addition of Bromophenol Blue [34] to the reactor, and by the ninhydrin test [35]. The protected neoglycolipid **13** was obtained in 63% yield by cleavage from the resin with 9:1 trifluoroacetic acid–water, followed by purification by reversed-phase HPLC.

In a preliminary study, the building block **9**, which contained 13% of the corresponding α anomer, was used in a solid-phase synthesis of **14** essentially as described above. To establish suitable conditions for deacylation, **14** was treated separately with sodium methoxide, potassium cyanide, methanolic ammonia, and hydrazine, and the reactions were monitored by HPLC. *O*-Deacetylation with



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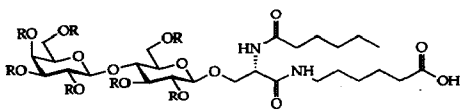

13 R=Bz
14 R=Ac
2 R=H

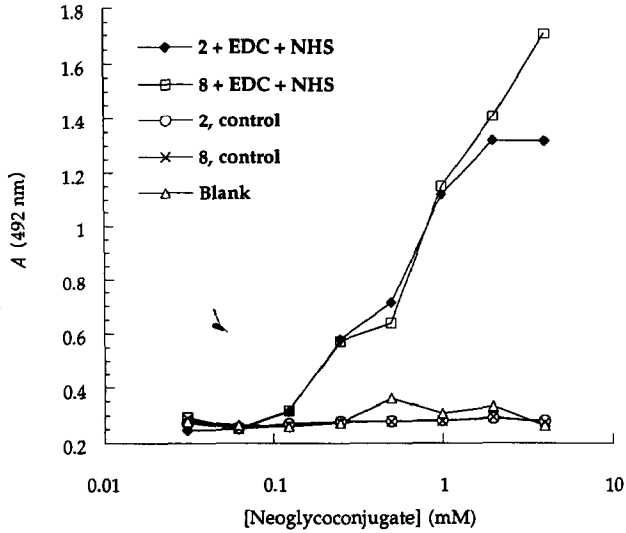

Fig. 1. Binding of the lectin from *Erythrina corallodendron* [38] to the neoglycoconjugates **2** and **8** covalently linked in microtiter plate wells. Compounds **2** and **8** were coupled to amine-functionalized microtiter plates with 1-(3-dimethylaminopropyl)-1-ethylcarbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS). EDC and NHS were omitted in the controls. No neoglycoconjugate was added in the blank. Absorbance data points represent averages of duplicate runs and details are given in the experimental.

sodium methoxide reached completion within 10 min, whereas longer reaction times were required in the treatments with hydrazine (30 min), as well as ammonia and potassium cyanide (> 10 h). Since benzoyl groups are less readily cleaved than acetyl groups under basic conditions [36], sodium methoxide was used to deprotect **13**. The deprotection required 13 h to reach completion and purification by reversed-phase HPLC gave the neoglycolipid **2** in 34% overall yield based on the lactosyl bromide **6** (74% from **13**). Side reactions such as elimination and racemization were not observed in debenzoylation of **13**, as determined by HPLC and NMR spectroscopy. The synthesis of **2** illustrates that neoglycolipids with a close resemblance to glycosylceramides can be obtained in as few as five synthetic steps from suitable glycosyl donors and with few chromatographic purifications.

The use of the serine-based neoglycolipid **2** was illustrated by coupling to microtiter plate wells which had primary amino groups grafted to the polystyrene surface. Compound **2**, and for comparison the mercaptopropionic acid lactoside **8** (prepared from **7** [37]), were covalently linked via their carboxyl groups to the plates using *N*-hydroxysuccinimide and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in aqueous solution. Addition of the biotin-labelled lactose-binding lectin from *Erythrina corallodendron* [38] and detection with avidin–horseradish peroxidase conjugate in an ELISA revealed that both **2** and **8** were bound to the wells and recognized by the lectin (Fig. 1). Controls performed in

wells treated with neoglycoconjugates **2** and **8** in the absence of coupling reagents revealed that adsorption of the conjugates to the wells did not occur. Previously, peptides have been covalently bound to microtiter plates and detected with antibodies using an ELISA procedure [39]. We anticipate that such neoglycolipids as **2** should find further use in preparation of affinity columns, and as soluble inhibitors since the solubility of **2** in water was determined to be > 25 mg/mL.

3. Experimental

General.—TLC was performed on Silica Gel 60 F_{254} (Merck) with detection by UV light and charring with H_2SO_4. Flash column chromatography was performed on Silica Gel 60 (Grace Amicon, 35–70 μm) with distilled solvents. Immediately before being used, CH_2Cl_2 was dried by distillation from CaH_2. Organic solutions were dried over Na_2SO_4.

The ^1H and ^{13}C NMR spectra were recorded with a Varian XL-300 or a Bruker ARX-500 spectrometer for solutions in $CDCl_3$ [residual $CHCl_3$ (δ_H 7.26) and $CDCl_3$ (δ_C 77.0) as internal standards], CD_3OD [residual CHD_2OD (δ_H3.35) and CD_3OD (δ_C 49.0)], or D_2O [residual HDO (δ_H 4.63)]. First-order chemical shifts and coupling constants were obtained from one-dimensional spectra, and proton resonances were assigned from COSY and ROESY experiments. Proton resonances that could not be assigned are not reported. Optical rotations were measured with a Perkin–Elmer 141 polarimeter. Positive ion fast-atom bombardment mass spectra were recorded on a Jeol SX 102 A mass spectrometer. Ions were produced by a beam of Xe atoms (6 keV).

N^{α}-(9-Fluorenylmethoxycarbonyl)-L-serine pentafluorophenyl ester [14] (Fmoc-Ser-OPfp, **3**), 1,2,3,6-tetra-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranose [19] (**4**), 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl bromide [25] (**5**), 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-β-D-glucopyranosyl bromide [27] (**6**), and 3-[2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosylthio]propionic acid [37] (**7**) were prepared according to the indicated literature methods.

Preparative HPLC separations were performed on a Beckman System Gold HPLC, using a Kromasil C-8 column (1000 Å, 20 × 250 mm) with a flow rate of 12 mL/min, detection at 214 nm, and the following solvent systems: *A*, aq 0.1% CF_3CO_2H; and *B*, 0.1% CF_3CO_2H in MeCN.

Satisfactory elemental analyses could not be obtained for the hygroscopic amorphous compounds **2**, **8**, and **13**, but their purity was established by TLC, HPLC, and NMR spectroscopy.

3-(4-O-β-D-Galactopyranosyl-β-D-glucopyranosylthio)propionic acid (**8**).—Compound **7** [37] (360 mg, 0.50 mmol) was dissolved in dry MeOH (50 mL) and methanolic 0.1 M NaOMe was added until the solution became alkaline. The solution was stirred overnight at room temperature, neutralized by addition of Amberlite IR-120 H^+-resin, filtered, and concentrated. Flash column chromato-

graphy ($CHCl_3$–MeOH–H_2O, 65:35:10 + 0.1% HOAc) of the residue gave **8** (200 mg, 96%), $[\alpha]_D^{25}$ + 2.8° (*c* 0.9, D_2O). ^1H NMR data (D_2O): δ 4.53 (d, 1 H, *J* 9.9 Hz, H-1), 4.41 (d, 1 H, *J* 7.7 Hz, H-1'), 3.93 (m, 1 H, H-6 or H-6'), 3.88 (d, 1 H, *J* 3.3 Hz, H-4'), 3.50 (dd, 1 H, *J* 9.9 and 7.7 Hz, H-2'), 3.33 (t, 1 H, H-2), 2.96 and 2.85 (dABd, each 1 H, *J* 13.6, 13.2, 7.5, and 6.6 Hz, SCH_2), 2.50 (t, 2 H, *J* 7.2 Hz, SCH_2CH_2).

N^{α}-(9-Fluorenylmethoxycarbonyl)-3-O-[2,3,6-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-L-serine pentafluorophenyl ester (**9**).—Silver triflate (87 mg, 0.34 mmol) was added at 0°C to a solution of **3** [14] (240 mg, 0.486 mmol) and **5** [25] (170 mg, 0.243 mmol) in dry CH_2Cl_2 (5.5 mL) containing 4A molecular sieves (140 mg). After stirring for 30 min, the mixture was allowed to attain room temperature during 30 min, then diluted with CH_2Cl_2 (70 mL), filtered through Celite, and washed with satd aq $NaHCO_3$ (70 mL). The aqueous phase was extracted with CH_2Cl_2 (2 × 30 mL), and the combined organic phases were dried, filtered, and concentrated. Flash column chromatography (1:1 heptane–EtOAc) of the residue gave **9** (65 mg, 24%), $[\alpha]_D^{25}$ − 7.4° (*c* 0.72, $CHCl_3$). NMR data ($CDCl_3$): ^1H (500 MHz), δ 5.65 (d, 1 H, *J* 8.5 Hz, NH), 5.35 (dd, 1 H, *J*3.5 and 1.0 Hz, H-4'), 5.21 (t, 1 H, *J* 9.2 Hz, H-3), 5.11 (dd, 1 H, *J* 10.4 and 7.8 Hz, H-2'), 4.96 (dd, 1 H, *J* 10.4 and 3.4 Hz, H-3'), 4.88 (dd, 1 H, *J* 9.1 and 8.0 Hz, H-2), 4.85 (1 H, Ser-Hα), 4.51 (d, 1 H, *J* 8.0 Hz, H-1), 4.48 (d, 1 H, *J* 7.9 Hz, H-1'), 4.39 (dd, 1 H, *J* 10.4 and 3.2 Hz, Ser-Hβ), 4.24 (t, 1 H, *J* 6.8 Hz, $OCOCH_2CH$), 4.13 (dd, 1 H, *J* 11.2 and 6.3 Hz, H-6'), 4.08 (dd, 1 H, *J* 11.2 and 7.4 Hz, H-6'), 4.05 (dd, 1 H, *J* 12.0 and 5.0 Hz, H-6), 3.94 (dd, 1 H, *J* 10.2 and 3.2 Hz, Ser-Hβ), 3.87 (t, 1 H, *J* 7.3 Hz, H-5'), 3.80 (t, 1 H, *J* 9.4 Hz, H-4), 3.59 (m, 1 H, H-5), 2.15, 2.07, 2.06, 2.05, 2.04, 1.97, and 1.96 (7 s, each 3 H, 7 Ac); ^{13}C (125 MHz), δ 101.1 and 100.5 (each 1 C, C-1 and C-1'), and 54.2 (C-α). FABMS: $(M + H)^+$ 1112 (Calcd 1112). Anal. Calcd for $C_{50}H_{50}F_5NO_{22}$: C, 54.0; H, 4.5; N, 1.3. Found; C, 53.1; H, 4.4; N, 1.0.

N^{α}-(9-Fluorenylmethoxycarbonyl)-3-O-[2,3,6-tri-O-benzoyl-4-O-(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-L-serine pentafluorophenyl ester (**10**).—Glycosylation of **3** [14] (264 mg, 0.534 mmol) with **6** [27] (303 mg, 0.267 mmol) as described for **9**, but with reaction for 2 h at 0°C, and flash column chromatography (30:1 toluene–acetone) of the residue gave **10** (297 mg, 72%); $[\alpha]_D^{25}$ +27° (*c* 0.75, $CHCl_3$). NMR data ($CDCl_3$): ^1H (500 MHz), δ 5.84 (t, 1 H, *J*9.4 Hz, H-3), 5.75 (dd, 1 H, *J* 10.4 and 7.9 Hz, H-2'), 5.75 (d, 1 H, *J* 3.2 Hz, H-4'), 5.62 (d, 1 H, *J* 8.3 Hz, NH), 5.44 (dd, 1 H, *J* 9.4 and 8.0 Hz, H-2), 5.41 (dd, 1 H, *J* 10.3 and 3.4 Hz, H-3'), 4.91 (d, 1 H, *J* 7.9 Hz, H-1'), 4.82 (1 H, Ser-Hα), 4.71 (d, 1 H, *J* 7.8 Hz, H-1), 4.62 (dd, 1 H, *J* 12.1 and 1.6 Hz, H-6), 4.48 (dd, 1 H, *J* 12.2 and 4.2 Hz, H-6), 4.42 (dd, 1 H, *J* 10.3 and 3.1 Hz, Ser-Hβ), 4.37 (dd, 1 H, *J* 10.6 and 7.2 Hz, $OCOCH_2CH$), 4.25 (H-4), 4.12 (t, 1 H, *J* 7.0 Hz, $OCOCH_2CH$), 3.92 (t, 1 H, *J* 7.0 Hz, H-5'), 3.90 (dd, 1 H, *J* 3.6 Hz, Ser-Hβ), 3.86 (m, 1 H, H-5), and 3.76 (dd, 2 H, *J* 6.6 and 2.6 Hz, H-6',6'); ^{13}C (125 MHz), δ 101.0 and 100.9 (each 1 C, *J* 163 and 160 Hz, respectively, C-1 and C-1'), and 54.1 (C-α). FABMS: $(M + H)^+$ 1546 (Calcd 1546). Anal. Calcd for $C_{85}H_{64}F_5NO_{22}$: C, 66.0, H, 4.2; N, 0.9. Found; C, 65.6; H, 4.3; N, 0.9.

3,6-Di-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-glucopyranose 1,2-[N$^{\alpha}$-(9-fluorenylmethoxycarbonyl)-L-alanin-3-yl orthoacetate] pentafluorophenyl ester (**11**).—Silver triflate (22 mg, 86 μmol) was added at −10°C to a solution of **3** [14] (38 mg, 77 μmol), **5** [25] (50 mg, 71 μmol), and 1,1,3,3-tetramethylurea (12.9 μL, 0.107 mmol) in dry CH_2Cl_2 (0.75 mL) containing 4A molecular sieves (25 mg). After stirring for 25 min, *N,N*-diisopropylethylamine (18.2 μL, 0.106 mmol) was added and the mixture was worked up as described for **9**. Flash column chromatography (1:1 heptane–EtOAc) of the residue gave **11** (42 mg, 53%); $[\alpha]_D^{25}$ − 1.7° (*c* 0.73, $CHCl_3$). NMR data ($CDCl_3$): ^{1}H (500 MHz), δ 5.66 (d, 1 H, *J* 9.0 Hz, NH), 5.62 (d, 1 H, *J* 5.1 Hz, H-1), 5.56 (dd, 1 H, *J* 2.2 and 1.4 Hz, H-3), 5.38 (dd, 1 H, *J* 3.4 and 0.8 Hz, H-4′), 5.19 (dd, 1 H, *J* 10.2 and 8.1 Hz, H-2′), 5.01 (dd, 1 H, *J* 10.4 and 3.4 Hz, H-3′), 4.93 (bd, 1 H, *J* 9.0 Hz, Ser-Hα), 4.61 (d, 1 H, *J* 8.0 Hz, H-1′), 4.49 (dd, 1 H, *J* 10.6 and 7.1 Hz, OCOCH_2CH), 4.41 (dd, 1 H, *J* 10.6 and 7.2 Hz, OCOCH_2CH), 4.33 (bdd, 1 H, *J* 4.2 and 2.2 Hz, H-2), 4.18 (dd, 1 H, *J* 9.5 and 2.2 Hz, Ser-Hβ), 3.95 (bt, 1 H, *J* 6.7 Hz, H-5′), 3.85 (dd, 1 H, *J* 9.5 and 3.4 Hz, Ser-Hβ), 3.82 (m, 1 H, H-5), 3.64 (bd, 1 H, *J* 9.4 Hz, H-4), 2.16, 2.12, 2.09, 2.06, 2.03, 1.98 (6 s, each 3 H, 6 Ac), and 1.75 (s, 3 H, CH_3CO_3); ^{13}C (125 MHz), δ 102.6 and 96.9 (each 1 C, C-1 and C-1′), and 53.8 (C-α). FABMS: $(M + H)^+$ 1112 (Calcd 1112).

Resin functionalized with reference amino acid (Nle), linker, and N-*Fmoc-6-aminohexanoic acid* (**12**).—The reference amino acid N$^{\alpha}$-Fmoc-norleucine (134 mg, 0.38 mmol) and the linker 4-hydroxymethylphenoxyacetic acid [32] (69 mg, 0.38 mmol) were coupled to a poly(ethylene glycol) polystyrene resin [29] (Rapp polymere, Tenta Gel® S NH_2, 0.37 g, 92 μmol), in that order, as benzotriazolyl esters [30]. The benzotriazolyl esters were prepared, in situ, by addition of 1,3-diisopropylcarbodiimide (56 μL, 0.36 mmol) to a solution of the appropriate acid and 1-hydroxybenzotriazole (HOBt; 77 mg, 0.57 mmol) in DMF (1.0 mL). After 30 min, the solution was added to the resin in a mechanically agitated reactor. The *N*-acylations were monitored by addition of Bromophenol Blue [34] to the reactor and by the ninhydrin test [35] N$^{\alpha}$-Fmoc Deprotection of Nle was effected with piperidine (20% in DMF, 2 + 7 min).

N-Fmoc-6-Aminohexanoic acid (Novabiochem, 90 mg, 0.253 mmol) was coupled [33] to the linker with 1-mesitylenesulfonyl-3-nitro-1,2,4-triazole (MSNT, 75 mg, 0.25 mmol) in the presence of 1-methylimidazole (MeIm, 40 μL, 0.51 mmol), in CH_2Cl_2 (2.5 mL). The acylation with *N*-Fmoc-6-aminohexanoic acid was repeated once. The resin was then thoroughly washed with DMF (3 × 5 min + 7 × 5 mL), toluene (3 × 5 min + 7 × 5 mL), and CH_2Cl_2 (5 min + 7 × 5 mL), and dried to give **12** (441 mg). The level of incorporation of *N*-Fmoc-6-aminohexanoic acid on the resin **12** was determined to be 0.21 mmol/g by spectrophotometric determination [31] of *N*-(9-fluorenylmethyl)piperidine formed after Fmoc deprotection of an aliquot of **12**.

N$^{\alpha}$-*Hexanoyl-3-O-[2,3,6-tri-O-benzoyl-4-O-(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-L-seryl-6-aminohexanoic acid* (**13**).—The functionalized resin **12** (200 mg, 42 μmol) was Fmoc-deprotected with piperidine (20% in DMF, 2 + 2 min) in a reactor and washed with DMF. Compound **10** (65 mg, 42

μmol) and HOBt (23 mg, 168 μmol) in DMF (0.8 mL) were added to the resin together with Bromophenol Blue (52 μL of a 2 mM solution in DMF, 105 nmol). After agitation for 23 h, the resin was washed with DMF (8 × 5 mL), N^{α}-Fmoc-deprotected with morpholine (50% in DMF, 2 + 2 × 15 min), and again washed with DMF (8 × 5 mL). Hexanoic acid (15.8 μL, 126 μmol) was coupled as a benzotriazolyl ester during 19 h, as described for **12**, and the resin was washed with DMF (8 × 5 mL) and CH_2Cl_2 (8 × 5 mL), then dried under vacuum. The dried resin was treated with 9:1 CF_3CO_2H–H_2O (25 mL) for 2.5 h and then removed by filtration. Concentration of the filtrate and purification of the residue by HPLC (73% *B* in *A*; retention time, 37 min) gave **13** (36 mg, 63%). NMR data ($CDCl_3$): ^1H (300 MHz), δ 6.53 (bt, 1 H, NH), 6.42 (d, 1 H, *J*6.2 Hz, $^{\alpha}$NH), 5.79 (t, 1 H, *J* 9.3 Hz, H-3), 5.76 (1 H, H-4'), 5.73 (dd, 1 H, *J* 10.4 and 8.0 Hz, H-2'), 5.49 (dd, 1 H, *J* 9.7 and 7.9 Hz, H-2), 5.43 (dd, 1 H, *J* 10.1 and 3.4 Hz, H-3'), 4.96 (d, 1 H, *J* 7.9 Hz, H-1'), 4.88 (d, 1 H,*J* 7.9 Hz, H-1), 4.59 (H-α), 4.33 (t, 1 H, *J* 9.5 Hz, H-4), 3.59 (bt, 1 H, *J* 9.8 Hz, Ser-Hβ), and 0.85 (t, 3 H, *J* 7.0 Hz, CH_3); ^{13}C (75 MHz), δ 102.6 and 101.2 (each 1 C, C-1 and C-1') and 52.0 (C-α). FABMS: $(M + H)^+$ 1369 (Calcd 1369).

3-O-(4-O-β-D-Galactopyranosyl-β-D-glucopyranosyl)-N^{α}*-hexanoyl-L-seryl-6-aminohexanoic acid* (**2**). — Methanolic 2 mM NaOMe was added dropwise to a solution of **13** (15.6 mg, 11 μmol) in MeOH (8 mL) until pH ~ 11 according to wet pH paper. After 13 h, the solution was neutralized with AcOH and concentrated. Purification of the residue by HPLC (15.5% *B* in *A*; retention time, 38 min) gave **2** (5.4 mg, 74%). NMR data (CD_3OD): ^1H (500 MHz), δ 4.59 (t, 1 H, *J* 5.0 Hz, Ser-Hα), 4.39 (d, 1 H, *J* 7.6 Hz, H-1'), 4.37 (d, 1 H, *J* 7.8 Hz, H-1), 4.18 (dd, 1 H, *J* 10.3 and 4.9 Hz, Ser-Hβ), 3.97 (dd, 1 H, *J* 12.1 and 2.5 Hz, H-6), 3.87 (dd, 1 H, *J* 12.1 and 4.6 Hz, H-6), 3.86 (d, 1 H, *J* 2.7 Hz, H-4'), 3.82 (dd, 1 H, *J* 11.5 and 7.4 Hz, H-6'), 3.78 (dd, 1 H, *J* 10.3 and 5.2 Hz, Ser-Hβ), 3.75 (dd, 1 H, *J* 11.7 and 4.7 Hz, H-6'), 3.63 (bdd, 1 H, *J* 7.2 and 4.9 Hz, H-5'), 3.52 (dd, 1 H, *J* 9.7 and 3.3 Hz, H-3'), 3.48 (ddd, 1 H, *J* 9.1, 4.6, and 2.4 Hz, H-5), 3.30 (dd, 1 H, *J* 8.7 and 8.1 Hz, H-2), 3.25 (bt, 2 H, *J* 6.8 Hz, $NHCH_2$), 2.35–2.31 (m, 4 H,$COCH_2$), 0.96 (t, 3 H, *J* 7.0 Hz, CH_3); ^{13}C (125 MHz), δ 106.7 and 105.9 (each 1 C, C-1 and C-1'). FABMS: $(M + H)^+$ 641 (Calcd 641).

Immobilization of neoglycoconjugates **2** *and* **8** *in microtiter plate wells and binding by a lectin.*—Aliquots (50 μL) of aq 16 mM solutions of the neoglycoconjugates **2** and **8** were serially diluted in microtiter plate wells containing 50 μL of water (i.e., after dilution, the first well in each lane contained 50 μL of an 8 mM solution). The microtiter plates were kindly provided for research purposes by Dr. Svend-Erik Rasmussen (A/S Nunc, Copenhagen, Denmark) and had primary amino groups grafted to their polystyrene surfaces via a short spacer. An aqueous solution (50 μL) of *N*-hydroxysuccinimide (NHS, 12.3 mM) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, 37.1 mM) was then added to each well. Control experiments where the aqueous NHS–EDC solution was replaced by water were run in parallel. The plates were shaken at room temperature for 120 min, emptied, and blocked by incubation overnight at 4°C with bovine serum albumin [BSA; 1% w/v in phosphate-buffered saline (PBS), 200 μL/well], then

washed with PBS containing BSA (1%) and Triton X-100 (0.05% v/v). The biotin-labelled, lactose-binding lectin from *Erythrina corallodendron* [38] (Sigma Chemical Co.; 10 μg/mL in PBS containing 0.5% BSA; 100 μL/well) was added, and the plates were incubated at room temperature for 120 min and washed with Covabuffer [PBS containing $MgSO_4 \cdot 7H_2O$ (10 g/L), Tween 20 (0.05%, v/v), and NaCl (117 g/L)]. Avidin–horseradish peroxidase conjugate (Dakopatts, Copenhagen, Denmark; diluted 1/1000 in PBS containing 0.5% BSA; 100 μL/well) was added and, after incubation for 1 h, the plates were washed with Covabuffer. Substrate solution [100 μL/well of a solution of aq 30% H_2O_2 (50 μL) and *O*-phenylenediamine dihydrochloride (60 mg) in 0.1 M citrate–phosphate buffer (pH 5, 100 mL)] was added to the wells followed after 15 min by M aq H_2SO_4 (100 μL/well), and the absorbance (A) was measured at 492 nm.

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