Glycoconjugate Journal 21, 251–256, 2004

Synthesis of the Lewis b hexasaccharide and HSA-conjugates thereof

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An efficient and short route has been elaborated for the aminopropyl spacer equipped Le^b hexasaccharide. For the preparation of HSA-conjugates of this oligosaccharide, the use of disuccinimidyl suberate (DSS) and disuccinimidyl glutarate (DSG) as cross-linker reagents has been evaluated. This conjugation method emerged as being faster and easier to monitor by standard MALDI-TOF spectrometry than squarate ester based conjugations of similar efficiency if DSS is used as cross-linker.

Published in 2004.

Keywords: **suberic acid, DSS-conjugation, neoglycoconjugate, glycosylation, MALDI-TOF**

Introduction

Our interest in the Lewis b (Le^b) structure derived from its association with *Helicobacter pylori* infections. This gastric pathogen induces chronic inflammation, which may progress to severe forms of gastroduodenal diseases such as peptic ulceration and gastric adenocarcinoma [1]. *H. pylori* express adhesins that bind to carbohydrate antigens in the gastric mucosa [2]. Binding of *H. pylori* to the fucosylated blood group antigens H-1 and Le^b is mediated by the **B**lood group **a**ntigen **b**inding **A**dhesin, BabA, which belongs to a large and divergent family of *H. pylori* outer membrane proteins with conserved N- and C-terminal domains [3]. It has been found that most disease associated strains express BabA [1]. To investigate these binding events in more detail and to aid in the purification of the BabA protein through Lewis b-based affinity chromatography, substantial amount of Lewis b hexasaccharide conjugates were needed. The Le^b-tetrasaccharide, where a good and practical large scale synthesis has been available for several years [4], has been found to have a too low affinity to be beneficial in affinity chromatography. Our earlier synthesis of the hexasaccharide contained features that made scaling-up a problem [5]. Herein, we report an improved synthesis of the spacer-equipped Le^b-hexasaccharide **1**. This approach is simple to carry out and allows preparation on a larger scale. Furthermore, the conjugation step to human serum albumin (HSA) with different linking agents is performed and discussed.

Result and discussion

Block synthesis is a common strategy for preparing larger and branched oligosaccharides. In our earlier approach [5], we favoured a $4 + 2$ block synthesis for the preparation of the Le^b hexasaccharide, to enable variation in the spacer glycoside acceptor. The synthesis of the tetrasaccharide donor block proceeded smoothly, but this thioglycoside was found to be quite labile, eliminating ethyl mercaptan even during storage in a freezer. This 1,2-elimination was also a major side reaction in the couplings to the spacer acceptor if a large excess (20–30 equivalents) of promoter was not used. Also, the deprotection sequence contained problems, especially the removal of the phthalimido group, which was difficult to monitor and low-yielding. A change of the amino protecting group was considered, but as the phthalimido protecting group in the donor unit considerably improves the solubility and ensures excellent β-selectivity during the glycosylation reaction, we instead decided to change the block approach but to keep our established building blocks (Scheme 1). A tetrasaccharide acceptor block was designed, allowing transformation of the phthalimido group to the acetamido group already at this stage. Furthermore, a suitable protecting group pattern permitted introduction of the two fucose residues to furnish the hexasaccharide.

Thus, coupling experiments between the disaccharide donor **2** and acceptor **5** [6], both intermediates in the earlier synthesis,

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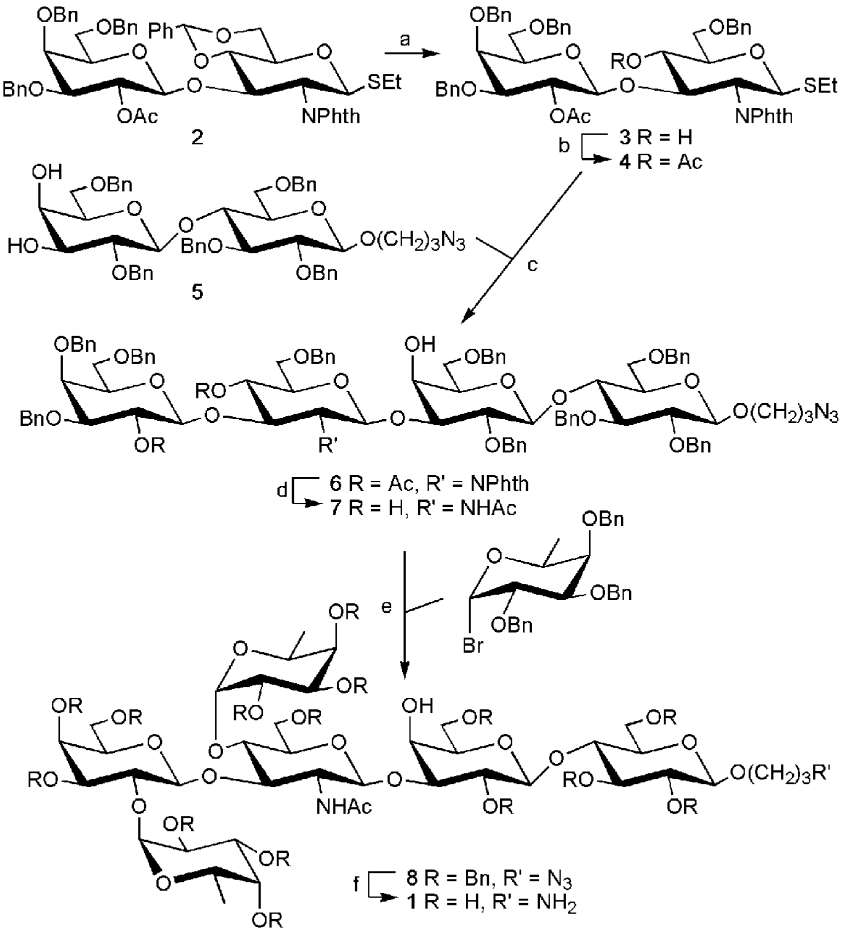

(a) $NaCNBH_3$, HCl/Et_2O, 82 %; (b) Ac_2O, pyridine, 96 %; (c) **4**, NIS, AgOTf, CH_2Cl_2, 78 %; (d) 1. EDA, EtOH, 70 °C; 2. NaOMe; 3. Ac_2O, MeOH, 74 %; (e) Et_4NBr,DMF/CH_2Cl_2, 79 %; (f) 1. Pd/C, THF; 2. Pd/C, 1M HCl (1eq), THF, EtOH, H_2O, H_2, 1 atm, RT, 71 %.

Scheme 1. Synthesis of the spacer-equipped Le^b-hexasaccharide **1**.

were carried out. However, it was found that in this coupling, not only the elimination reaction of the donor was a problem but also the regioselectivity. For example, the use of *N*-iodosuccinimide (NIS)/silver triflate (AgOTf) as promoter system in CH_2Cl_2, both at low (-75°C to -15°C) and ambient temperature, produced from smaller amounts of the 1,2-elimination product of donor **2** as well as an equal amount of the β-1→3 and the β-1→4 glycosylation products (as estimated by TLC). Assuming that conformational strain originating from the 4,6-*O*-benzylidene acetal was liable for the loss of regioselectivity during the glycosylation reaction, this acetal was opened under reductive conditions to form the 6-*O*-benzyl ether **3** (82%). The subsequent acetylation under standard conditions gave new donor **4** (96%). Now, glycosylation with **4** (aprox. 1.2 equiv.) and diol acceptor **5** promoted by NIS/AgOTf proceeded smoothly and regioselectively to afford the desired tetrasaccharide **6** (78%). The phthalimido group was removed using ethylenediamine in ethanol at elevated temperature. The cleavage of the acetates was incomplete under these conditions, therefore sodium methoxide was added, before *N*-acetylation was accomplished with acetic anhydride in methanol to produce the triol-tetrasaccharide **7** (74%). Inversion of this reaction sequence, *i.e.* cleavage of the acetates prior to the phthalimido group, was tried as well but with a lower overall yield due to partial opening of the phthalimido ring during the deacetylation. The regioselective introduction of two fucose residues at the 2‴- and the 4″-positions in triol **7** was carried out using halide-assisted conditions yielding protected Le^b-hexasaccharide **8** (79%). Glycosylation at the unprotected 4′-position, *i.e.* assembly of the trifucosylated heptasaccharide, could successfully be avoided by addition of dimethyl formamide as co-solvent. Hydrogenolysis over Pd/C in different solvent mixtures with and without addition of acetic acid rendered the unprotected aminopropyl Le^b-hexasaccharide **1**, but these reactions were slow and sluggish. Addition of one equivalent of 1M $HCl_{aq.}$ instead of acetic acid to the reaction mixture, significantly improved the deprotection. Complete removal of all benzyl groups and concomitant reduction of the azido group without notable cleavage of fucosidic linkages was accomplished at atmospheric pressure overnight producing **1** (71%) ready for conjugation to a protein.

Since the affinity to the carbohydrate recognition domain of a protein is dependent on the efficient and fine-tuned presentation of the carbohydrate portion, the whole conjugate—comprising the receptor-saccharide, a spacer and a carrier—has to be carefully considered [7]. Squaric diester chemistry is well established for the preparation of glycoconjugates, but monitoring with standard MALDI-TOF methodology of an ongoing conjugation is difficult due to the high buffer-salt concentration to achieve. SELDI-TOF MS, in combination with ProteinChip®, is an option to circumvent this difficulty [8].

Since disuccinimidyl suberate (DSS, Figure 1) and disuccinimidyl glutarate (DSG, Figure 1) have been successfully employed for the conjugation of mannose to BSA in a comparative study [9] on a number of commercially available cross-linkers, we decided to examine these linkers for use in two-step conjugations of Le^b-hexasaccharide **1** to HSA. The disuccinimidyl diesters of the diacids were prepared according to standard DCC methodology and obtained as crystalline solids. The first conjugation step, addition of the activated diester to hexasaccharide **1**, was carried out in DMSO in the presence of triethylamine using a tenfold excess of the activated diester (DSS or DSG) to avoid dimerisation. Complete conversion was usually observed after 30 to 60 min reaction time. Excess spacer reagent was removed either by precipitation and filtration or by solid phase extraction. The conjugation of the purified activated hexasaccharide to HSA was performed in a buffer solution (pH 10) and reaction progress monitored by analysis of withdrawn samples using MALDI-TOF spectrometry. Initial conjugation experiments using large excess of the substrate oligosaccharides produced a generally higher incorporation of the carbohydrate portion when DSS was employed as cross-linking agent. However, an incorporation of 25 carbohydrate units per HSA, with 59 theoretically available amino groups, was not exceeded. In similarity to the investigation mentioned above [9], the efficiency of the conjugation was studied with different ratios of DSS activated **1** to HSA (Table 1).

After 45 min the conjugation was almost complete and only a small increase was observed with elongated reaction time (2 d). Interestingly, use of larger excess (more than 40 equiv.) of HSA seemed to have only slight effect on the hapten incorporation.

Table 1. Degree of incorporated Le^b-hexaoligosaccharides per HSA

Hapten/HSA	*10:1*	*20:1*	*40:1*	*60:1*	*120:1*
Incorporation confirmed by MALDI-TOF	3.9 (2 h)	11.7 (45 min)	16.4 (45 min)	16.5 (45 min)	16.5 (45 min)
	4.3 (24 h)	11.4 (2 d)	17.3 (2 d)	19.0 (2 d)	20.4 (2 d)

In summary, we have established a short and easy approach to the synthesis of the Le^b-hexasaccharide. The use of DSS as cross-linker in a two-step conjugation procedure for the preparation of Le^b-hexasaccharide-HSA conjugates has been shown to be similar in efficiency to the squaric acid diester chemistry but advantageous if a fast incorporation of receptor saccharide to the carrier protein is desired. Further, both conjugations steps of the DSS conjugation chemistry were easy to monitor by standard MALDI-TOF spectrometry.

Experimental

General methods

Methylene chloride was distilled before use and dry DMSO was kept over molecular sieves 3 Å, all other chemicals were used as purchased. Organic solutions were dried over MgSO$_4$ before concentration, which was performed under reduced pressure at <40°C (bath temperature). NMR spectra were recorded at 300 MHz (Varian) or 400 MHz (Varian or Jeol) (^{1}H) or at 75 MHz or 100 MHz (^{13}C) respectively, in CDCl$_3$, D$_2$O or CD$_3$OD at ambient temperature if not other stated. TMS was used as internal standard ($\delta = 0$) for ^{1}H-spectra in chloroform. ^{13}C-spectra were referenced to the chloroform signal ($\delta = 77.17$). NMR experiments in methanol were referenced to the solvent signal (^{1}H: $\delta = 3.31$, ^{13}C: $\delta = 49.15$). Acetone (^{1}H: $\delta = 2.21$, ^{13}C: $\delta = 31.0$) was applied as internal standard for spectra in D$_2$O. Silica gel Normasil (40–63 μm, Prolabo, VWR International) was used for flash chromatography. TLC was performed on silica gel 60 F$_{254}$ (Merck) glass plates with detection by UV-light and/or charring with 8% sulfuric acid, ninhydrin (0.1% in ethanol) or AMC-solution (ammonium molybdate, cerium (IV) sulfate, 10% sulfuric acid [5: 0.1: 100, w/w/v]). MALDI-TOF spectra were recorded on a Bruker Biflex III using 2′,4′,6′-trihydroxy-acetophenone monohydrate (THAP) as matrix.

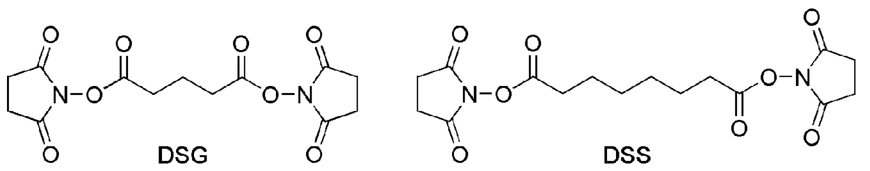

Figure 1. Linkers tested for the conjugation of Le^b-hexasaccharide **1** to HSA.

Ethyl (2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**4**)

NaCNBH$_3$ (1.5 g, 23.9 mmol) and powdered molecular sieves (3 Å) were added to a solution of ethyl (2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**2** [5], 3.6 g, 3.9 mmol) in THF (60 mL). After 30 min, HCl/Et$_2$O was added dropwise until evolution of gas ceased. The reaction was stirred for an additional 30 min and then quenched with Et$_3$N (2 mL). The mixture was filtered through Celite, concentrated and purified on a silica gel column (toluene → toluene/EtOAc 3:1) to give ethyl (2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1 → 3)-6-O-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**3**) (2.9 g, 3.2 mmol, 82%); NMR (CDCl$_3$): ^{13}C 15.1, 20.2, 24.1, 54.0, 68.7, 70.0, 70.1, 71.1, 72.2, 72.3, 73.6, 73.8, 74.0, 74.5, 80.1, 80.6, 81.1, 82.8, 101.4, 123.5, 123.7, 127.3-128.6, 131.6, 134.3, 134.5, 137.8, 137.9, 138.1, 138.6, 167.2, 168.5, 169.0. ^{1}H , δ 1.17 (t, 3H, SEt), 2.67 (m, 2H, SEt), 3.32 (dd, 1H, H-3′, $J_{3,4} = 2.5$ Hz, $J_{2,3} = 10.2$ Hz), 3.45 (m, 1H), 3.62 (m, 4H), 3.79 (d, 1H, H-4′), 3.88 (d, 1H, $J = 10.8$ Hz), 4.30–4.54 (m, 8H), 4.60 (s, 1H), 4.85 (d, 1H, $J = 11.8$ Hz), 5.15 (d, 1H, $J = 10.2$ Hz), 5.27 (dd, 1H, $J = 8.0$ Hz, $J = 10.0$ Hz), 7.08–7.40 (m, 20H), 7.75 (m, 2H), 7.86 (m, 2H). Acetylation of **3** (2.5 g, 2.7 mmol) with Ac$_2$O (1.3 mL, 13.8 mmol) and a catalytic amount of DMAP in pyridine (5 mL) provided, after purification on a silica gel column (toluene → toluene/EtOAc 3:1), the title compound **4** (2.5 g, 2.6 mmol, 96%); $[\alpha]_D + 17°$ (c1.3, CH$_2$Cl$_2$); NMR (CDCl$_3$): ^{13}C 14.9, 20.7, 20.8, 23.8, 54.7, 67.9, 69.8, 70.4, 71.5, 71.6, 72.4, 73.3, 73.5, 73.6, 74.4, 75.4, 78.0, 80.3, 81.0, 100.5, 123.5, 123.7, 127.2–128.6, 131.5, 131.6, 134.3, 134.5, 137.8, 137.9, 138.1, 138.6, 167.1, 168.7, 169.3, 169.6. ^{1}H , δ 1.2 (t, 3H, SEt), 1.85 (s, 6H, OAc), 2.67 (m, 2H, SEt), 3.19 (dd, 1H, H-3′, $J_{3,4} = 2.5$ Hz, $J_{2,3} = 10$ Hz), 3.40 (m, 1H), 3.58 (m, 4H), 3.77 (m, 1H), 3.86 (d, 1H, H-4′), 4.16 (d, 1H, H-1, $J_{1,2} = 7.8$ Hz), 4.30–4.58 (m, 8H), 4.72 (t, 1H), 4.88 (d, 1H), 4.97 (t, 1H), 5.08 (dd, 1H), 5.21 (d, 1H), 7.08–7.40 (m, 20H), 7.79 (m, 2H), 7.83 (m, 2H). Anal. Calcd. for $C_{54}H_{57}NO_{13}S$: C 67.55, H 5.98; Found: C 67.71, H 6.05.

3-Azidopropyl (2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1 → 3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1 → 3)-(2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**6**)

A solution of **4** (2.1 g, 2.2 mmol) and 3-azidopropyl-(2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**5** [6], 1.5 g, 1.8 mmol) in dry CH$_2$Cl$_2$ (20 mL) was stirred with powdered molecular sieves (4 Å) under argon for 1 h when NIS (1.0 g, 4.6 mmol) and a catalytic amount of TfOH (5 μL) were added. After 3 h stirring at room temperature, the reaction was quenched by addition of Et$_3$N (200 μL), and the mixture diluted with CH$_2$Cl$_2$ (20 mL), filtered through Celite and concentrated. The residue was applied onto a silica gel column and eluted (toluene (0.5% Et$_3$N)→toluene/EtOAc 6:1) to give **6** (2.5 g, 1.4 mmol, 78%); $[\alpha]_D$ +14° (c1.40, CH$_2$Cl$_2$); NMR (CDCl$_3$): ^{13}C 20.7, 20.8, 29.3, 48.4, 55.6, 66.4, 67.7, 67.9, 68.9, 69.7, 70.4, 71.4, 71.6, 72.3, 73.1, 73.2, 73.3, 73.5, 73.6, 73.7, 74.1, 74.4, 74.8, 75.1, 75.4, 75.9, 78.1, 80.2, 81.7, 82.9, 83.7, 98.6, 100.5, 101.9, 103.5, 123.3, 126.2-128.6, 137.8, 138.3, 138.6, 138.7, 139.1, 169.2, 169.6; ^{1}H, δ 1.77 (s, 3H, OAc), 1.81 (m, 2H), 1.84 (s, 6H, OAc), 2.76 (d, 1H), 2.80 (m, 1H), 3.10 (dd, 1H, $J = 2.5$ Hz, $J = 10$ Hz), 3.25–3.64 (m, 17H), 3.83 (m, 4H), 4.03 (m, 2H), 4.12–4.47 (m, 16H), 4.66 (m, 3H), 4.75 (d, 1H), 4.88 (m, 3H), 5.02 (dd, 1H), 5.27 (d, 1H), 7.08–7.29 (m, 45H), 7.50 (sb, 4H). Anal. Calcd. for $C_{102}H_{108}N_4O_{24}$: C 69.06, H 6.14; Found: C 69.15, H 6.06.

3-Azidopropyl (3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1 → 3)-(6-*O*-benzyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 3)-(2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-benzyl-2-β-D-glucopyranoside (**7**)

A solution of **6** (2.0 g, 1.1 mmol) and ethylenediamine (4 mL) in EtOH (99.5%, 40 mL) was brought to reflux. After 6 h, NaOMe (1 M, 200 μL) was added to cleave remaining acetates. The mixture was left over night at ambient temperature, then diluted with toluene and concentrated. After co-evaporation with toluene, the residue was dissolved in MeOH/toluene (2:1, 30 mL) and Ac$_2$O (3 mL) was added. After an additional 30 min, the mixture was concentrated and co-evaporated with toluene. The residue was treated with water (100 mL) and extracted with ethyl acetate (2 × 150 mL), dried, filtered and concentrated. Purification on a silica gel column (toluene → toluene/EtOAc 1:1) gave **7** (1.3 g, 0.81 mmol, 74%); $[\alpha]_D + 8.5°$ (c1.3, CH$_2$Cl$_2$); NMR (CDCl$_3$): ^{13}C 23.3, 29.4, 48.4, 56.5, 66.5, 67.8, 68.8, 69.0, 69.8, 70.0, 70.8, 72.7,72.9, 73.3, 73.5, 73.6, 73.7, 74.1, 74.7, 75.1, 75.2, 75.4, 76.5, 77.3, 77.6, 79.3, 81.6, 81.9, 82.9, 85.5, 100.4, 102.3, 103.6, 104.7, 126.8–128.7, 137.6, 138.1, 138.2, 138.3, 138.6, 138.7, 139.1, 139.2, 171.8; ^{1}H, δ 1.55 (s, 3H, NHAc), 1.86 (m, 2H), 2.87 (m, 1H), 3.29–3.64 (m, 19H), 3.72 (dd, 1H), 3.80 (m, 3H), 3.95 (m, 3H), 4.08 (m, 2H), 4.26–4.45 (m, 8H), 4.54 (m, 4H), 4.63 (d, 1H), 4.69–4.87 (m, 7H), 4.92 (m, 1H), 5.01 (d, 1H), 5.40 (sb, 1H), 7.18–7.41 (m, 45H). Anal. Calcd. for $C_{92}H_{104}N_4O_{21}Si$: C 68.98, H 6.54; Found: C 69.10, H 6.62.

3-Azidopropyl (2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1 → 2)-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1 → 3)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1 → 4)]-(6-*O*-benzyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 3)-(2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**8**)

Ethyl 2,3,4-tri-*O*-benzyl-1-thio-α-L-fucopyranoside [10], (1.05 g, 2.2 mmol) in CH$_2$Cl$_2$ (10 mL) was treated with bromine (140 μL, 2.73 mmol) at 0°C for 10 min. After concentration and co-evaporation with dry toluene, the crude 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide [11], was dissolved in CH_2Cl_2 (2 mL) and added to a mixture of **7** (1.0 g, 0.63 mmol), Et_4NBr (145 mg, 0.69 mmol) and powdered molecular sieves (4 Å, 1.5 g) in CH_2Cl_2/DMF (3:2, 5 mL), which had been stirred under argon for 1 h. After 48 h, MeOH (2 mL) was added, followed by filtration through Celite. Purification on silica gel (two columns: toluene → toluene/EtOAc 2:1) gave **8** (1.2 g, 0.49 mmol, 79%); NMR ($CDCl_3$): ^{13}C 16.4, 16.5, 23.4, 29.4, 48.4, 56.9, 66.6, 66.9, 67.0, 67.6, 68.2, 68.3, 68.5, 68.8, 71.6, 71.8, 72.3, 72.9, 73.1, 73.2, 73.3, 73.4, 73.6, 73.7, 73.9, 74.5, 74.8, 74.9, 75.2, 75.4, 75.6, 75.7, 76.4, 77.4, 77.8, 78.2, 79.6, 79.9, 80.5, 81.7, 81.9, 82.9, 84.1, 98.1, 98.2, 101.5, 102.2, 102.3, 103.6, 126.2–128.8, 137.8, 138.1, 138.2, 138.3, 138.5, 138.6, 138.7 (2C), 138.8, 138.9, 139.0, 139.1, 139.3, 139.4 (2C), 170.0; ^1H, δ 1.14, 1.22 (d, 2H, 2 × CH_3)1.61 (s, 3H, NHAc), 1.86 (m, 2H), 3.22 (m, 1H), 3.27 (m, 1H), 3.34–4.09 (m), 4.25–4.82 (m), 4.86 (t, 1H), 4.95 (d, 1H), 4.99 (d, 1H), 5.63 (d, 1H), 7.02–7.43 (m). MALDI-Tof: 2456.1 $[M + Na]^+$, found: 2456.6 $[M + Na]^+$. Anal. Calcd. for $C_{146}H_{160}N_4O_{29}$: C 72.02, H 6.62; Found: C 71.88, H 6.54.

3-Aminopropyl (α-L-fucopyranosyl)-(1 → 2)-(β-D-galactopyranosyl)-(1 → 3)-[α-L-fucopyranosyl-(1 → 4)]-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 3)-(β-D-galactopyranosyl)-(1 → 4)-β-D-glucopyranoside (**1**)

Hexasaccharide **8** (535 mg, 220 μmol) was dissolved in THF (5 mL) and Pd/C (5%, 10 mg) added. The slurry was stirred for 2 h, filtered through a sandwich of filters (10 μm on top of a 5 μm filter pellet) and concentrated. The residue was dissolved in a mixture of THF (11 mL)/EtOH (95%, 11 mL)/H_2O (330 μL)/HCl (1M, 220 μL) and Pd/C (5%, 300 mg) was added. The reaction mixture was set under an hydrogen atmosphere at atmospheric pressure. After 6 h, water (4.4 mL) was added to keep the material in solution. An extra portion Pd/C (5%, 50 mg) was added and stirring under hydrogen was continued for an additional 6 h. The suspension was buffered with $NaHCO_3$ (10% aq., 200 μL), filtered through a sandwich of filters (as above), diluted with water (50 mL) and washed with Et_2O (2 × 50 mL). The water phase was collected and concentrated. Purification by reverse phase chromatography (C18, water) gave compound **1** (165 mg, 156 μmol, 71%). $[\alpha]_D$ −43° (*c* 1.0, H_2O); NMR (D_2O): ^{13}C 16.1, 16.2, 22.9, 27.4, 38.3, 56.5, 60.2, 60.7, 61.7, 62.3, 66.9, 67.7, 68.5, 68.8, 69.0, 69.3, 69.4, 69.8, 70.1, 70.8, 72.5, 72.7, 73.4, 74.2, 74.9, 75.2, 75.4, 75.5, 75.9, 77.3, 78.9, 82.4, 98.5, 100.3, 101.3, 102.8, 103.7, 103.9, 174.4; ^1H (25.0°C), δ 1.26 (2 × t, 6H, 2 × CH_3), 2.00 (m, 2H), 2.05 (s, 3H, NHAc), 3.31 (m, 1H), 3.51–4.09 (m), 4.12 (m), 4.31 (q, 1H), 4.41 (d, 1H, $J_{1,2} = 7.8$ Hz), 4.51 (d, 1H, $J_{1,2} = 7.8$ Hz), 4.60 (d, 1H, $J_{1,2} = 8.0$ Hz), 4.66 (d, 1H, $J_{1,2} = 8.6$ Hz), 4.86 (t, 1H), 5.02 (d, 1H, $J_{1,2} = 3.9$ Hz), 5.15 (d, 1H, $J_{1,2} = 3.9$ Hz). MALDI-Tof: 2456.1 $[M + Na]^+$, found: 2456.6 $[M + Na]^+$.

3-Aminopropyl (α-L-fucopyranosyl)-(1 → 2)-(β-D-galactopyranosyl)-(1 → 3)-[(α-L-fucopyranosyl)-(1 → 4)]-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 3)-(β-D-galactopyranosyl)-(1 → 4)-β-D-glucopyranoside conjugates

General procedure for conjugation with DSS

1 (7.0 mg, 6.6 μmol) and DSS[1] (21 mg, 57 μmol) were dissolved in dry DMSO (175 μl) and Et_3N (1.5 μl) was added. The mixture was gently swirled and monitored by MALDI-TOF. When all **1** was conversed into the activated ester (approx. 30 min), the product (7.7 to 7.8 mg, 5.9 μmol, 89%) was isolated by one of the following procedures.

Work-up 1: The mixture was poured into ice-cold water (2.5 mL). Unused active ester precipitated and was removed by filtration. The precipitate was washed with ice-cold water (3 × 2.5 mL) and the pooled water fractions freeze-dried to remove traces of DMSO.

Work-up 2: The reaction mixture was transferred onto a C18 column (3 g, prewashed with 10 mL DCM). DMSO was washed off with DCM (8 mL) and then the DCM was removed from the column with a stream of nitrogen. The activated ester was released from the column with cold water (double distilled, 10 mL) and product containing fractions were pooled and freeze-dried.

Conjugation with HSA

HSA (18.3 mg, 0.27 μmol, Sigma) was treated with a Labassco buffer (pH 10, 600 μL) and added to an aqueous solution of the DSS-active ester conjugate (21.5 mg, 16.4 μmol, in 3 mL double dest. water). The progress of the conjugation was monitored by MALDI-TOF. After 18 h the conjugation mixture was transferred to a centrifugal tube (30 kD, omega membrane, Microsep^{TM}, Pall) and centrifuged (3 × 1 h, addition of 3 × 2 mL double dest. water). The filter residue was taken up in a small amount of water (3 × 500 μL) and freeze-dried to yield the conjugate (22.0 mg, 93% calc. with respect to the protein). MALDI-TOF: 86500 (corresponds to 16 incorporated receptor saccharides).

Acknowledgment

Financial support from the Swedish Research Council (Vetenskapsrådet) is gratefully acknowledged.

Note

1. DSS was prepared from suberic acid (430 mg, 2.5 mmol) and *N*-hydroxysuccinimide (630 mg, 5.5 mmol) with DCC (1.1 g, 5.5 mmol) in DCM/THF (1 : 1) at 0°C. The stirred reaction mixture was allowed to attain ambient temperature, filtered trough a pad of Celite with additional DCM. The solvent was removed under reduced pressure and the crystalline residue recrystallised from EtOH (99.5%) to yield DSS (635 mg, 1.7 mmol, 70%).

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