ELSEVIER

Carbohydrate Research 298 (1997) 153–161

CARBOHYDRATE RESEARCH

Synthesis of α- and β-biotinylated T-antigen

Sylvie Bay [a], Odile Berthier-Vergnes [b], Danièle Cantacuzene [a,*]

[a] *Institut Pasteur, Département de Biochimie et Génétique Moléculaire, Unité de Chimie Organique, 28 rue du Dr. Roux, 75724 Paris Cedex 15, France*

[b] *INSERM U346, Pavillon R, Hôpital Ed. Herriot, 69437 Lyon 03, France*

Received 13 March 1996; accepted 30 October 1996

Abstract

The T-antigen [β-D-Gal-(1 → 3)-D-GalNAc] has been linked to biotin through a C_6 spacer arm for the detection of a specific 'T-antigen-lectin' complex at the surface and/or on the migration pathway of melanoma cells. When 4,6-di-*O*-acetyl-2-azido-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α- or -β-D-galactopyranosyl halides were treated with *N*-benzyloxycarbonyl or *N*-fluorenylmethoxycarbonyl protected aminohexanols (used as the spacer arm), unusual stereoselectivities were observed for the synthesis of the α and β anomers. The synthesis of the α anomer could only be achieved, in reasonable yields, with the Schiff base of aminohexanol.

Keywords: T-antigen; Chemoenzymatic synthesis; α- and β-Biotinylated T-antigen; Stereoselective synthesis; Aminohexanol as a spacer arm; Aminohexanol Schiff base

1. Introduction

Cell-surface glycoconjugates play an important rôle in biological processes [1]. Neoplastic transformation is generally accompanied by structural alterations in cell-surface oligosaccharides [2,3]; this results in the accumulation of unusual glycosidic structures such as the tumor-associated antigens or tumor markers. T antigen [Thomsen–Friedenreich antigen, β-D-Gal-(1 → 3)-D-GalNAc] and Tn antigen (α-D-GalNAc-Ser/Thr) are two well-known examples of these markers, frequently present in carcinomas but not usually in normal tissues [3,4]. We have shown recently that human melanoma cells from variants selected for high metastatic potential bind peanut agglutinin lectin [PNA, specific for β-D-Gal-(1 → 3)-D-GalNAc] and that human cell populations enriched for PNA-binding cells generate a higher frequency of metastasis [5]. Consequently, it has been postulated that PNA-reactive molecules (T-antigen) on tumor cells may represent a carbohydrate marker for invasiveness and metastatic capacity of these melanoma cells. It is also well established that changes in the expression of endogenous lectins occur in malignant cells compared with normal cells [2,6], and the T antigen could be involved in the formation of a specific disaccharide–lectin complex at the surface and/or on the migration pathway of melanoma cells.

In order to identify an eventual specific lectin for the T-antigen in melanoma cells we needed a labelled T-antigen. Because the avidin/biotin system is a powerful tool for bioanalytical applications, we chose to prepare a biotinylated derivative of this T-antigen.

* Corresponding author.

0008-6215/97/$17.00
PII S0008-6215(96)00309-6

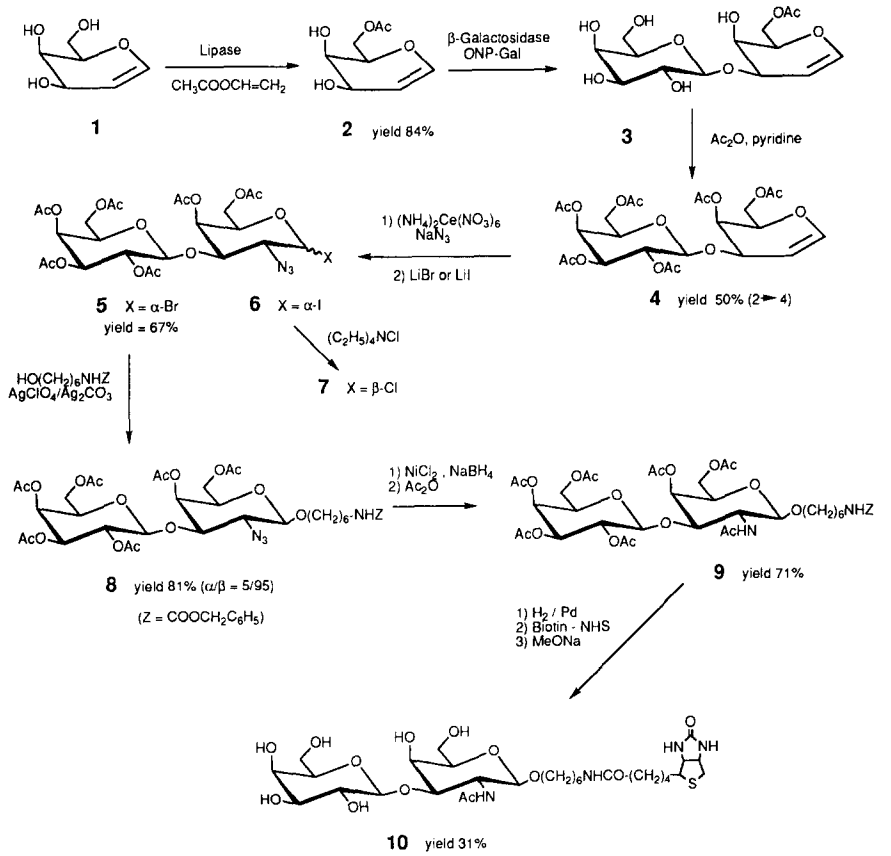
The normally cryptic T-antigen is present on different types of glycoconjugates (mucin, glycoprotein, glycolipid). However, the exact nature of the region anchoring the antigen is not clearly established and the anomeric position of the glycosidic linkage (α- or β-D-GalNAc-glycoconjugate) is still ambiguous [7]. Both α and β anomers of the biotinylated T-antigen (with a C_6 spacer arm) were therefore needed for testing.

In this paper we describe the preparation of the two derivatives, the unusually high stereospecificity obtained for the β anomer, and the unexpected difficulties encountered for the α anomer.

2. Results and discussion

Synthesis of the β-biotinylated T-antigen (Scheme 1).—We chose a chemo-enzymatic method for the preparation of the precursor disaccharide **4**, based on the synthesis developed by Wong and co-workers [8]. D-Galactal (**1**) was acetylated in the 6-position in order to avoid the formation of the β-(1 → 6) disaccharide regioisomer that could be formed in the next transglycosylation step using β-D-galactosidase; this was achieved by a lipase-catalyzed transesterification with vinyl acetate according to Holla's procedure [9]. 6-*O*-Acetyl-D-galactal (**2**) [9] was glycosylated using *o*-nitrophenyl β-D-galactopyranoside and β-D-galactosidase from *E. coli*, to afford the β-(1 → 3)-linked disaccharide **3**. The conditions had to be optimized and they differ appreciably from those given in the literature [8]. Peracetylation of the crude mixture gave the hexaacetate **4** which was ready for the next step of azidonitration using classical methods [10,11].

In fact, azidonitration of the disaccharide **4** worked as well as azidonitration of per-*O*-acetyl-D-galactal itself, although the conditions had to be optimized [8]. Bromination of the 2-azido, 1-nitro intermediate gave the α-bromodisaccharide **5** [10]. Similar disaccharide glycosyl donors are known; they have been prepared by classical methods with comparable overall yields [12,13]. Bromide **5** reacted with *N*-benzyloxycarbonyl (N-Z) protected aminohexanol, in the presence of silver carbonate/silver perchlorate, to provide the β derivative **8**, with a low proportion of the α anomer (**8**α/**8**β = 5/95). This high stereoselectivity is quite unexpected with a non-participating



Scheme 1. Synthesis of the β-biotinylated T-antigen.

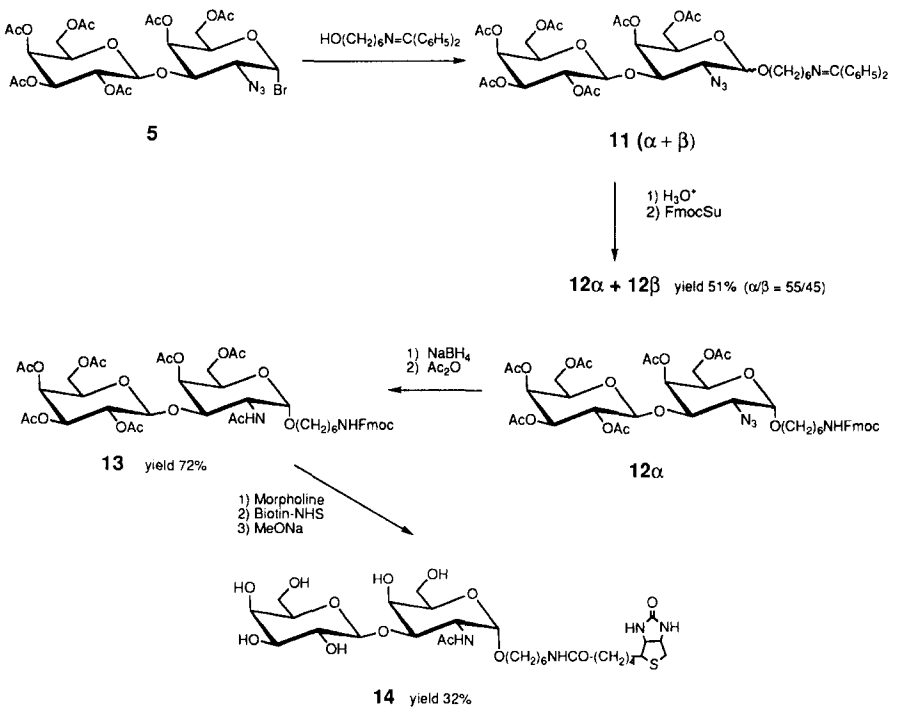
group in the 2-position, although it has been observed in some cases with 2-azido-glucosyl donors [13,14]. Reduction of the azido group and acetylation gave **9**. Deprotection of the amino group of the spacer arm, biotinylation with biotin–*N*-hydroxysuccinimide ester, and final deacetylation of the peracetylated disaccharide gave the expected β-biotinylated T-antigen **10** (Scheme 1).

Synthesis of the α-biotinylated T-antigen (Scheme 2).—Starting from the same peracetylated disaccharide **4**, azidonitration gave, after reaction with lithium iodide, the α-iodo derivative **6**. In situ anomerization with tetraethylammonium chloride gave the β-chloro anomer **7**. This β-chloro anomer is generally used to introduce aglycons in the α position and works very well for serine derivatives [11,15] as well as for other alcohols. For example, the α derivative of the T-antigen with a long-chain alcohol bearing an ester group at one end [$HOCH_2(CH_2)_7CO_2Me$] has been prepared in good yield and high stereoselectivity [16,17]. After many attempts and using our N-Z protected aminohexanol it was impossible to obtain the α anomer in reasonable amounts, the ratio being always of the order **8**α/**8**β = 20/80. Changing the protecting group on the amine or the length of the spacer arm (C_4) did not improve the percentage of the α anomer (see Table 1) except when the amine was replaced by an azido group (**8**α/**8**β = 50/50). However, the azido group on the spacer arm was excluded for the introduction of biotin because of the presence of the other azido functionality on the 2-position of galactose.

All the reactions were performed in dichloromethane or dichloromethane/toluene. However, donor solvents are known to favor the α anomer [19]. We therefore tried acetonitrile [20] but in our hands the percentage of the α anomer was still very low (10%).

Another strategy was considered starting from peracetylgalactal and performing the azidonitration first, to obtain the desired peracetylated monosaccharide with the spacer arm in the α position (donor **15** or **16**, see Table 1 and Scheme 3): this derivative could have been elongated further by the classical methods of deprotection–protection to leave a free 3-OH group. However, here again all the attempts to obtain the α anomer as the major product were unsuccessful. At best we obtained equal amounts of the α and β anomers which were practically impossible to separate. Surprisingly, starting from tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl bromide (**15**) or from the β-chloro derivative **16** does not substantially change the proportion of the α anomer in the glycosylation reaction (see Table 1).

Direct condensation of the biotinylated spacer arm with the appropriate β-chlorodisaccharide **7** was also



Scheme 2. Synthesis of the α-biotinylated T-antigen.

Table 1
Condensation of the spacer arm with 2-azido, 1-halogeno, peracetylated mono- and di-saccharides as donors

Acceptor	Donor [a]	Catalyst	Yield [b] (%)	α/β ratio
$HO(CH_2)_6NHZ$	**5**	$AgClO_4$, Ag_2CO_3	81	5/95
	5	$ZnBr_2$ [c]	58	20/80
$HO(CH_2)_6NHZ$	**7**	$AgClO_4$, Ag_2CO_3	54	30/70
	7	$AgClO_4$ [d]	59	10/90
	7	AgOTf	78	25/75
$HO(CH_2)_6NHZ$	**15**	$AgClO_4$, Ag_2CO_3	56	50/50
	15	$ZnBr_2$ [c]	55	45/55
$HO(CH_2)_6NHZ$	**16**	$AgClO_4$, Ag_2CO_3	56	40/60
$HO(CH_2)_4NHZ$	**7**	$AgClO_4$, Ag_2CO_3	62	25/75
$HO(CH_2)_6NHFmoc$	**7**	$AgClO_4$, Ag_2CO_3	58	20/80
$HO(CH_2)_6N_3$	**7**	$AgClO_4$, Ag_2CO_3	[e]	[e]
	7	AgOTf	70	50/50
$HO(CH_2)_6Br$	**7**	$AgClO_4$, Ag_2CO_3	[e]	[e]
	7	AgOTf	45	$\alpha \ll \beta$
$HO(CH_2)_6NHBiotin$	**7**	$AgClO_4$	[e]	[e]
$HO(CH_2)_6N{=}C(C_6H_5)_2$	**5**	$AgClO_4$	51 [f]	55/45
	7	AgOTf	30 [f]	25/75
	7	$AgClO_4$, Ag_2CO_3	30 [f]	55/45
	15	$AgClO_4$	32 [f]	35/65

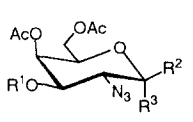
[a] Scheme 3.
[b] The yields (< 60%) were not optimized because we were mainly interested in the α/β stereoselectivity.
[c] Ref. [18].
[d] Condensation performed in MeCN; the other reactions were run in CH_2Cl_2 or toluene/CH_2Cl_2.
[e] No condensation observed.
[f] Overall yield after hydrolysis of the imine and protection of the amine by the Fmoc group to obtain **12** (3 steps).

unsuccessful; no condensation was observed, probably due to solubility problems.

Of the other published methods, those using thioglycosides [21] did not seem very promising so far as stereoselectivity was concerned. A recent example was published of a high α-stereoselectivity using trichloroacetimidates [22] and acetyl protecting groups for the sugar. However, many examples show rather poor selectivity [23]. Consequently we chose to investigate further the use of glycosyl halides as donors.

Without understanding clearly why we could not prepare this α anomer in reasonable amounts we noticed that the best results were obtained when the amino group of aminohexanol was replaced by an azido group. On the other hand Polt and his colleagues have been using, for several years now, an imine instead of an acyl protecting group for serine or threonine derivatives in glycosylation reactions,

5 : R^1 = Per-Ac-β-D-Gal, R^2 = H, R^3 = Br
7 : R^1 = Per-Ac-β-D-Gal, R^2 = Cl, R^3 = H
15 : R^1 = Ac, R^2 = H, R^3 = Br
16 : R^1 = Ac, R^2 = Cl, R^3 = H



Scheme 3. Structure of the glycosyl donors in the reaction with amino alcohol derivatives.

with good yields and high stereoselectivity [24]. We therefore prepared the benzophenone imine of 6-amino-1-hexanol and used it for glycosylations with the peracetylated α-glycosyl bromide **5** under the conditions described by Polt for the α anomer. In fact, the condensation took place and after hydrolysis of the imine and protection of the amine by a fluorenylmethoxycarbonyl (Fmoc) protective group the two anomers **12**α and **12**β were obtained in an overall yield of 50% and could be easily separated by chromatography. The ratio of **12**α/**12**β was 55/45, the best selectivity that we could get. It is noteworthy that with the β-chlorodisaccharide **7**, obtained from the α-iodo derivative **6**, the selectivity was not better but the yields were lower (see Table 1). The α anomer was submitted to the usual reduction of the azido group and acetylation to give **13**. Removal of the Fmoc protective group and condensation with biotin–*N*-hydroxysuccinimide ester gave the expected α-biotinylated T-antigen **14** after the final removal of the acetyl groups of the disaccharide.

The biological tests to detect an endogenous specific lectin on melanocytes were performed with the α- and β-biotinylated T-antigen on highly metastatic clones as well as on poorly metastatic ones. So far,

only negative results were obtained. This could be due to the fact that the aglyconic part of the natural glycoconjugate is needed for the interaction with lectins, and that the T-antigen, exposed at the surface of melanocytes, could be linked to a serine (or threonine) residue on a glycoprotein, as is the case for the Tn-antigen. Alternatively, the interaction with the lectin is too weak and multimeric T-antigen structures might be more appropriate [25]. The synthesis of other derivatives of the T-antigen is therefore in progress.

3. Experimental

D-Galactal (**1**), prepared from β-D-galactose pentaacetate (Sigma) following a known procedure [26], was selectively monoacetylated in the 6-position using Holla's procedure [9] (H_2O, lipase, vinyl acetate) to give 6-*O*-acetyl-D-galactal (**2**) in 84% yield. The protected derivatives were purified either by flash chromatography on silica gel (E. Merck, 0.040–0.063 mm) using petroleum ether–EtOAc or CH_2Cl_2–MeOH, or on a chromatotron (Harrison Research) when the products could be detected by UV. The final deprotected biotinylated T-antigens **10** and **14** were first purified by reversed-phase chromatography on C_{18} using water–MeCN as the mobile phase. They were repurified by reversed-phase high-performance chromatography (HPLC) using a Perkin–Elmer pump system with a UV detector (230 nm). A column (250 × 10 mm) of Nucleosil C_{18} (5 μm) was used and the solvent system was *A*, 0.1% CF_3CO_2H in water; *B*, MeCN; the products were eluted with 10 to 28% *B* in *A*, flow rate 6 mL/min. Biotin was obtained from Aldrich and biotin–*N*-hydroxysuccinimide ester was freshly prepared by coupling biotin and the succinimide in DMF in the presence of dicyclohexylcarbodiimide [27]. All the solvents used for the condensations were high grade and dry; CH_2Cl_2 was distilled over CaH_2, and toluene over Na with benzophenone, before use; $(NH_4)_2Ce(NO_3)_6$, NaN_3, LiBr, LiI, $(C_2H_5)_4NCl$, $AgClO_4$, Ag_2CO_3, and AgOTf were thoroughly dried over P_2O_5 under vacuum before use. Lipase from *Candida cylindracea* (type VII) was purchased from Sigma. β-D-Galactosidase from *E. coli* was obtained from Boehringer (100 units per mg). A solution of β-D-galactosidase was prepared by dissolving the enzyme powder in 0.03 M sodium phosphate buffer (pH 6.5) (17 mg/mL) containing mM $MgCl_2$ and 5 mM 1,4-dithiothreitol. ^1H NMR spectra (300.134 MHz, sodium 4,4-dimethyl-4-silapentanoate as standard for spectra in D_2O) were recorded on a Bruker instrument. Chemical shifts for ^{13}C NMR (75.47 MHz) are given relative to Me_4Si with 1,4-dioxane as spectral reference (δ versus Me_4Si, 67.86 ppm). COSY spectra (proton–proton shift correlations) as well as DEPT experiments were performed for the assignments of the peaks. Mass spectra were measured by electrospray.

4,6-Di-O-acetyl-1,5-anhydro-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-D-lyxo-hex-1-enitol (**4**).—This derivative was prepared by a modification of the conditions given by Wong and co-workers [8] since poor yields were obtained by following the described procedure. In a typical experiment *o*-nitrophenyl β-D-galactopyranoside (5.4 g, 18 mmol) was incubated, at room temperature, with 6-*O*-acetyl-D-galactal (**2**) [9] (4.9 g, 26 mmol, 1.5 equiv) in sodium phosphate buffer pH 6.5 containing mM $MgCl_2$ and 5 mM 1,4-dithiothreitol (35 mL) and the β-D-galactosidase solution (1.4 mL, 2360 units). After 7 h the water was evaporated under vacuum (0.5 mm; water bath temperature < 30 °C). After drying the residue was treated directly with Ac_2O (25 mL) and pyridine (50 mL), and heated at 50 °C for 4 h. The solvents were then evaporated and the disaccharide **4** purified by flash chromatography (petroleum ether–EtOAc); 5 g of a white solid (yield 50%); mp 132 °C; R_f 0.24 (CH_2Cl_2/MeOH 3%); $[\alpha]_D$ −19° (*c* 1.2, CH_2Cl_2), lit. [8] −13°.

4,6-Di-O-acetyl-2-azido-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-galactopyranosyl bromide (**5**).—Following the procedure of Lemieux and Ratcliffe [10], modified by Wong and co-workers [8], ammonium cerium(IV) nitrate (2.9 g, 5.3 mmol) and NaN_3 (174 mg, 2.7 mmol) were added under Ar and at −25 °C to a solution of **4** (1 g, 1.8 mmol) in dry MeCN (50 mL). The mixture was left for 18 h at −10 °C and treated as described [8]. Purification by flash chromatography on a short column (CH_2Cl_2/Et_2O/1% Et_3N) gave a mixture of the expected nitro derivatives (790 mg). The mixture (790 mg, 1.2 mmol) was dissolved in MeCN (5 mL) and treated directly with LiBr (522 mg, 6 mmol) as described for the monosaccharide [10]. After 3 h, CH_2Cl_2 (50 mL) and H_2O (50 mL) were added. The water phase was extracted with CH_2Cl_2, dried over Na_2SO_4, and evaporated to give **5** as a syrup (810 mg, overall yield from **4**, 67%) which was used without further purification; R_f 0.3 (CH_2Cl_2/MeOH 2%); ^1H NMR ($CDCl_3$): δ 6.45 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.70 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1').

4,6-Di-O-acetyl-2-azido-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-galactopyranosyl iodide (**6**) *and chloride* (**7**).—Compounds **6** and **7** were synthesized from the 2-azido,1-nitro intermediate according to known procedures [10,11]: **6** (syrup, crude product; 95% yield): ^{1}H NMR ($CDCl_3$): δ 6.85 (d, 1 H, $J_{1,2}$ 3.9 Hz, H-1), 4.75 (d, 1 H, $J_{1',2'}$ 8 Hz, H-1'); **7** (white solid, crude product; 95% yield from **6**); ^{1}H NMR ($CDCl_3$): δ 4.95 (d, 1 H, $J_{1,2}$ 8.9 Hz, H-1), 4.7 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1').

6-(Benzyloxycarbonylamino)hexyl 4,6-di-O-acetyl-2-azido-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-galactopyranoside (**8**).—To a solution of 6-benzyloxycarbonylamino-1-hexanol (315 mg, 1.25 mmol) in CH_2Cl_2 (15 mL) and toluene (15 mL) were added Ag_2CO_3 (351 mg), $AgClO_4$ (36 mg), and powdered 4 Å molecular sieves (1.75 g). The mixture, protected from light, was stirred for 2 h. A solution of the bromodisaccharide **5** (1 g, 1.5 mmol) in CH_2Cl_2 (7.5 mL) and toluene (7.5 mL) was then added dropwise; the mixture was stirred for an additional 18 h (TLC: CH_2Cl_2/MeOH 2%), diluted with CH_2Cl_2, filtered through Celite, and evaporated. The residue was redissolved in CH_2Cl_2, and the solution was washed with water, dilute aq $NaHCO_3$, and water. After evaporation of the solvent the product was purified by flash chromatography (CH_2Cl_2/MeOH) to give the pure anomer **8**β as a syrup [862 mg, yield 81%; the α anomer could not be isolated since such a small amount of it was present, as estimated by NMR (α/β = 5/95)]; R_f 0.19 (1:1 cyclohexane–EtOAc); ^{1}H NMR ($CDCl_3$): δ 7.18 (5 H, Ar), 5.3 (d, 1 H, $J_{3',4'}$ 3.3 Hz, H-4'), 5.25 (d, 1 H, $J_{3,4}$ 3.2 Hz, H-4), 5.05–5.18 (3 H, $J_{1',2'}$ 7.7, $J_{2',3'}$ 10.4 Hz, H-2', CH_2C$_6$H$_5$), 4.95 (dd, 1 H, $J_{3',4'}$ 3.3, $J_{2',3'}$ 10.4 Hz, H-3'), 4.7 (NH), 4.6 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1'), 4.2 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1), 4.15–3.4 (8 H, H-5, 2 H-6, H-5', 2 H-6', CH_2O), 3.47 (H-2), 3.43 (H-3), 3.15 (CH_2NH), 2.0–1.8 (Ac), 1.5–1.1 (CH_2 spacer); ^{13}C NMR ($CDCl_3$): δ 170–169 (Me*C*O), 156.4 (OCONH), 136.6, 128.5, 128.1 (C$_6$H$_5$), 102.3, 101.5 (C-1, C-1'), 77.1, 71.3, 70.8, 70.6, 68.8, 68.1, 66.8 (C-2', C-3, C-3', C-4, C-4', C-5, C-5'), 70.4 (OCH_2), 66.6 (CH_2 benzyl), 63.4 (C-2), 62.3, 61.0 (C-6, C-6'), 41.0 (CH_2N), 29.9, 29.4, 26.4, 25.6 (CH_2 spacer), 20.7–20.6 (*Me*CO).

6-(Benzyloxycarbonylamino)hexyl 2-acetamido-4,6-di-O-acetyl-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-galactopyranoside (**9**).—Reduction of the azido group of **8** and acetylation was performed as described by Paulsen and Paal [17]. Compound **8**β (862 mg, 1 mmol) was dissolved in EtOH (100 mL) containing $NiCl_2 \cdot 6H_2O$ (3.9 g) and H_3BO_4 (2 g). Sodium borohydride was added portionwise until the solution remained black for at least 1 h. Acetic anhydride (4 mL) was then added and the mixture stirred for 2 h (TLC: CH_2Cl_2/MeOH 4%). Cold water was added to the reaction mixture and the water phase extracted with CH_2Cl_2. After the usual washings, the organic solvent was evaporated and the product purified by flash chromatography (CH_2Cl_2/MeOH) to give **9** as a syrup (614 mg, yield 71%); R_f 0.25 (CH_2Cl_2/MeOH 3%); ^{1}H NMR ($CDCl_3$): δ 7.3 (5 H, C$_6$H$_5$), 6.0 (d, 1 H, *J* 6.9 Hz, N*H*Ac), 5.35 (d, 1 H, $J_{3,4}$ 3.2 Hz, H-4), 5.28 (d, 1 H, $J_{3',4'}$ 3.0 Hz, H-4'), 5.1–5.0 (dd, 3 H, $J_{1',2'}$ 7.8, $J_{2',3'}$ 10.4 Hz, H-2', CH_2C$_6$H$_5$), 4.98 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 4.9 (dd, 1 H, $J_{3',4'}$ 3.4 Hz, H-3'), 4.75 (NH), 4.62 (dd, 1 H $J_{2,3}$ 10.8, $J_{3,4}$ 3.4 Hz, H-3), 4.53 (d, 1 H, H-1'), 4.18–3.9 (2 H-6, 2 H-6'), 3.8 (m, 3 H, H-5, H-5', CH_2O), 3.4 (1 H, CH_2O), 3.22 (1 H, H-2), 3.1 (2 H, CH_2NH), 2.1–1.8 (7 Ac), 1.5–1.1 (CH_2 spacer); ^{13}C NMR ($CDCl_3$): δ 171.1–169.5 (Me*C*O), 156.6 (OCONH), 136.5, 128.6, 128.2, 128.1 (Ar), 100.3 (C-1'), 99.1 (C-1), 74.7 (C-3), 71.1, 70.8 (C-5, C-5'), 71.0 (C-3'), 69.8 (CH_2O), 69.2 (C-2'), 68.1 (C-4), 66.7 (C-4'), 66.6 (CH_2 benzyl), 62.4, 60.9 (C-6, C-6'), 55.4 (C-2), 40.7 (CH_2N), 29.6, 29.0, 26.0, 25.3 (CH_2 spacer), 23.6 (NHCO*Me*), 20.8 (*Me*CO).

6-(Biotinylamino)hexyl 2-acetamido-2-deoxy-3-O-β-D-galactopyranosyl-β-D-galactopyranoside (**10**).—Hydrogenolysis of the benzyloxycarbonyl group was performed on **9** (614 mg) dissolved in MeOH (80 mL) with Pd–C (700 mg). After completion of the reaction the catalyst was filtered off on Celite and the solvent removed. The crude amine (520 mg, 0.71 mmol) was dissolved in DMF (4 mL), and biotin–*N*-hydroxysuccinimide (NHS) ester [27] (290 mg, 0.85 mmol), dissolved in the same solvent (4 mL), was added dropwise at 0 °C. The reaction mixture was stirred for an additional 18 h at −4 °C (TLC: 90:10:0.1 CH_2Cl_2–MeOH–AcOH). DMF was evaporated and the residue dissolved in CH_2Cl_2; the organic phase was washed with dilute aq $NaHCO_3$, dilute aq citric acid, and water, and dried. Purification was performed by flash chromatography (90:5:0.2 CH_2Cl_2–MeOH–AcOH). The product (307 mg, yield 45%) showed small impurities by NMR and was therefore directly treated with NaOMe in MeOH for deacetylation: the peracetylated biotinyl derivative (307 mg, 0.32 mmol) was dissolved in MeOH (8 mL) with Na (17 mg) and stirred for 1 h. The medium was neutralized by Dowex 50W-X8 (H^+) resin and the

final product purified on a reversed-phase column (C_{18}) using a gradient of water/MeCN to give **10** (156 mg, yield 69%); mp 210 °C (dec); R_f 0.33 (7:1:2 *i*PrOH–20% NH_3–H_2O); $[\alpha]_D$ +6° (*c* 0.55, water); ^{1}H NMR (D_2O): δ 4.58 (dd, 1 H, ABX system, J_{AX} 4.8, J_{BX} 0 Hz, $J_{CH,CHNH}$ 8.1 Hz, NHCHC*H*CH_2S), 4.45 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 4.42, 4.38 (2 H, H-1′, NHC*H*CHS), 4.15 (d, 1 H, $J_{3,4}$ 2.9 Hz, H-4), 3.95 (H-2), 3.85 (2 H, H-3, H-4′), 3.8–3.5 (m, 9 H, H-3′, H-5, H-5′, 2 H-6, 2 H-6′, CH_2O), 3.5 (m, 1 H, H-2′), 3.3 (m, 1 H, *J* 4.9 Hz, CH_2C*H*S), 3.12 (t, *J* 6.6 Hz, CH_2NHCO), 3.0–2.75 (2 H, ABX system, J_{AB} 13 Hz, CH_2S), 2.2 (t, 2 H, *J* 7.2 Hz, CH_2CO), 2.0 (NHAc), 1.7–1.2 (CH_2 spacer + CH_2 biotin); ^{13}C NMR (D_2O): δ 177.5–175.5 (CONH, Me*C*O), 166.2 (NHCONH), 105.7, 102.3 (C-1, C-1′), 80.7, 75.8, 75.5, 73.3, 71.4, 69.4, 68.8 (C-2′, C-3, C-3′, C-4, C-4′, C-5, C-5′), 71.1 (CH_2O), 62.9, 61.1 (CHNH), 61.8, 61.7 (C-6, C-6′), 56.2 (CH-S), 52.1 (C-2), 40.5–40.0 (CH_2S, CH_2N), 36.3 (CH_2CO), 29.3, 29.2, 28.7, 28.5, 26.6, 26.0, 25.6 (spacer + CH_2 biotin), 23.1 (NHCO*Me*). MS: Calcd for $[M-H]^-$: *m/z* 707.325; Found: 707.34. Anal. Calcd for $C_{30}H_{52}N_4O_{13}S \cdot 3H_2O$: C, 47.23; H, 7.66; N, 7.34. Found: C, 47.53; H, 7.53; N, 7.05.

*6-(Fluorenylmethoxycarbonylamino)hexyl 4,6-di-*O*-acetyl-2-azido-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-α- and -β-*D*-galactopyranoside* (**12α** *and* **12β**).—A mixture of the disaccharide bromide **5** (1 g, 1.5 mmol), 6-[(diphenylmethylene)-amino]-1-hexanol (309 mg, 1.1 mmol) [28], and 2,4,6-collidine (246 μL, 1.7 equiv) in CH_2Cl_2 (6 mL) was added dropwise to a suspension of $AgClO_4$ (420 mg, 1.7 equiv) in CH_2Cl_2 (12 mL) previously stirred vigorously for 2 h with 1 g of powdered 4 Å molecular sieves. After 6 h the mixture was quenched with triethylamine (280 mL). Dichloromethane was added and the mixture filtered through Celite. The filtrate was washed with aq 5% $Na_2S_2O_3$ and water. The solution was dried and evaporated. The crude mixture **11α** + **11β** (1.3 g) was dissolved in THF (9 mL), trifluoroacetic acid (1 mL), and water (0.2 mL). Hydrolysis of the imine was complete after 10 min. The solution was diluted with CH_2Cl_2 and washed with dilute aq $NaHCO_3$, then water. Evaporation of the solvent gave a crude mixture (1.3 g) which was used without purification for the next step. This crude amino derivative was dissolved in CH_2Cl_2 (12 mL) with *N*-(9-fluorenylmethoxycarbonyl)succinimide (FmocSu) (337 mg, 1 mmol) and *N*,*N*-diisopropylethylamine (174 μL, 1 equiv). The mixture was stirred for 2 h. The organic phase was washed with dilute aq HCl and water, and dried. A first purification by flash chromatography (CH_2Cl_2/MeOH) gave a clean mixture of the α and β anomers **12α** + **12β** (527 mg, yield 51%, α/β 55/45 as estimated by NMR). A second purification on a chromatotron (1:1 petroleum ether–EtOAc) gave the pure α anomer of **12** which was eluted first (290 mg) followed by the β anomer (237 mg). **12α** (syrup): R_f 0.26 (1:1 cyclohexane–EtOAc); ^{1}H NMR ($CDCl_3$): δ 7.7, 7.5, 7.38, 7.25 (8 H, Fmoc), 5.4 (d, 1 H, $J_{3,4}$ 3.1 Hz, H-4), 5.25 (d, 1 H $J_{3',4'}$ 3.1 Hz, H-4′), 5.1 (dd, 1 H, $J_{1',2'}$ 7.9, $J_{2',3'}$ 10.4 Hz, H-2′), 4.9 (dd, 1 H, H-3′), 4.88 (d, $J_{1,2}$ 3.5 Hz, H-1), 4.78 (NH), 4.6 (d, 1 H, H-1′), 4.38 (CH_2 Fmoc), 4.12 (CH Fmoc), 4.1–3.8 (7 H, H-3, H-5, H-5′, 2 H-6, 2 H-6′), 3.52 (dd, 1 H, $J_{1,2}$ 3.5, $J_{2,3}$ 10.6 Hz, H-2), 3.55–3.4 (CH_2O), 3.15 (q, 2 H, CH_2NH), 2.1–1.95 (Ac), 1.7–1.3 (8 H, CH_2 spacer); ^{13}C NMR ($CDCl_3$): δ 170.4–169.4 (Me*C*O), 156.3 (OCONH), 143.8, 141.2 (Fmoc), 127.5, 126.9, 124.9, 119.8 (Fmoc), 101.4 (C-1′), 97.8 (C-1), 74.5, 70.6, 70.5, 69.3, 68.6, 67.2, 66.6 (C-2′, C-3, C-3′, C-4, C-4′, C-5, C-5′), 68.3 (OCH_2), 66.3 (CH_2 Fmoc), 62.6, 60.8 (C-6, C-6′), 59.3 (C-2), 47.1 (CH Fmoc), 40.7 (CH_2N), 29.7, 29.0, 26.3, 25.6 (CH_2 spacer), 20.6 (*Me*CO). **12β** (syrup): R_f 0.17 (1:1 cyclohexane–EtOAc); NMR: same characteristics as for **8β** except for the signals of the Fmoc protecting group: ^{1}H NMR ($CDCl_3$): δ 7.7, 7.5, 7.4, 7.25 (8 H, Ar), 4.4 (d, *J* 6.8 Hz, CH_2 Fmoc), 4.1 (CH Fmoc); ^{13}C NMR ($CDCl_3$): δ 143.9, 141.2 (C Ar), 127.6, 126.9, 124.9, 119.9 (CH Ar), 66.5 (CH_2 Fmoc), 47.3 (CH Fmoc).

*6-(Fluorenylmethoxycarbonylamino)hexyl 2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-α-*D*-galactopyranoside* (**13**).—Reduction of the azido group of **12α** and acetylation was performed as described for **8β**. From 380 mg (0.4 mmol) of **12α**, 278 mg (syrup, yield 72%) of the 2-acetamido derivative **13** was obtained after purification on a chromatotron (CH_2Cl_2/MeOH); R_f 0.38 (19:1 CH_2Cl_2–MeOH); ^{1}H NMR ($CDCl_3$): δ 7.7, 7.5, 7.38, 7.25 (Fmoc), 5.8 (d, 1 H, *J* 8.9 Hz, NHAc), 5.3 (m, 2 H, H-4, H-4′), 5.05 (dd, 1 H, $J_{1',2'}$ 7.9, $J_{2',3'}$ 10.3 Hz, H-2′), 4.8 (m, 3 H, H-3′, N*H*Fmoc, H-1), 4.5 (d, 1 H, H-1′), 4.4 (m, 1 H, H-2), 4.3 (CH_2 Fmoc), 4.1 (CH Fmoc), 4.1–3.7 (m, 6 H, H-5, H-5′, 2 H-6, 2 H-6′), 3.7 (H-3), 3.55–3.35 (2 H, CH_2O), 3.1 (2 H, CH_2NH), 2.1–1.8 (7 Ac), 1.5–1.1 (8 H, CH_2 spacer); ^{13}C NMR ($CDCl_3$): δ 171.1–169.5 (Me*C*O), 156.6 (OCONH), 143.9–141.3, 127.8–127.1–124.9–120.1 (Fmoc), 100.6 (C-1′), 97.5 (C-1), 73.2 (C-3), 70.8, 70.7, 68.9,

68.7, 67.3, 66.7 (C-2′, C-3′, C-4, C-4′, C-5, C-5′), 68.0 (CH_2O), 66.6 (CH_2 Fmoc), 62.8, 61.0 (C-6, C-6′), 49.0 (C-2), 47.3 (CH Fmoc), 40.7 (CH_2N), 29.7, 29.0, 26.0, 25.5 (CH_2 spacer), 23.4 (NHCO*Me*), 20.8 (*Me*CO).

6-(Biotinylamino)hexyl 2-acetamido-2-deoxy-3-O-β-D-galactopyranosyl-α-D-galactopyranoside (**14**).— Compound **13** (328 mg, 0.34 mmol) was dissolved in DMF (7 mL) with morpholine (7 mL). The mixture was stirred for 1 h and DMF was evaporated under high vacuum. The crude amine was dissolved in DMF (2 mL) and the mixture cooled to 0 °C. Biotin–NHS (130 mg, 0.38 mmol) dissolved in DMF (2 mL) was added dropwise and stirred for 18 h at −4 °C. The same workup as described for **10** was followed and the product was purified by flash chromatography (96:4:0.2 CH_2Cl_2–MeOH–AcOH) to give 180 mg (yield 55%) of the expected peracetylated biotinylated derivative. Deacetylation with NaOMe in MeOH as above and purification by reversed-phase chromatography gave 80 mg (yield 60%) of the α-biotinylated T-antigen **14** as a white solid after lyophilization; mp 210 °C (dec); R_f 0.46 (7:1:2 *i*PrOH–20% NH_3–H_2O); $[\alpha]_D$ +88° (*c* 1.2, water); ^{1}H NMR (D_2O): δ 4.89 (H-1), 4.62 (dd, 1 H, ABX system, J_{AX} 4.9, J_{BX} 0, $J_{CH,CHNH}$ 7.9 Hz, NHCHC*H*CH_2S), 4.49 (d, 1 H, $J_{1',2'}$ 7.7 Hz, H-1′), 4.43 (dd, 1 H, $J_{CH,CHS}$ 4.4 Hz, NH–C*H*–CHS), 4.32 (dd, 1 H, $J_{1,2}$ 3.7, $J_{2,3}$ 11 Hz, H-2), 4.24 (d, 1 H, $J_{3,4}$ 2.8 Hz, H-4), 4.03 (H-3), 3.9 (d, 1 H, $J_{3',4'}$ 3.2 Hz, H-4′), 4.03–3.46 (m, 7 H, H-3′, H-5, H-5′, 2 H-6, 2 H-6′), 3.6 (H-2′), 3.37 (m, 1 H, *J* 4.9 Hz, CH_2C*H*S), 3.2 (CH_2NHCO), 3.0–2.8 (2 H, ABX system, J_{AB} 13.1 Hz, CH_2S), 2.2 (t, 2 H, *J* 7 Hz, CH_2CO), 2.08 (NHAc), 1.8–1.3 (spacer + CH_2 biotin); ^{13}C NMR (D_2O): δ 177.4, 175.5 (CONH, *C*OMe), 166.1 (NHCONH), 105.5 (C-1′), 97.8 (C-1), 77.9, 75.6, 73.1, 71.2, 71.0, 69.3, 69.2 (C-2′, C-3′, C-3, C-4, C-4′, C-5, C-5′), 68.6 (CH_2O), 62.7 (CHNH), 61.8–61.6 (C-6, C-6′), 60.8 (CHNH), 56.0 (CHS), 49.3 (C-2), 40.3, 39.8 (CH_2N, CH_2S), 36.1 (CH_2CO), 28.9–28.3, 26.4–25.6 (CH_2 spacer + CH_2 biotin), 22.6 (NHCO*Me*). MS: Calcd for $[M+H]^+$: *m/z* 709.325; Found: 709.32. Anal. Calcd for $C_{30}H_{52}N_4O_{13}S \cdot 2H_2O$: C, 48.22; H, 7.57; N, 7.25. Found C, 48.22; H, 7.50; N, 7.48.

References

[1] J. Montreuil, *Adv. Carbohydr. Chem. Biochem.*, 37 (1980) 157–223; K. Olden, J.B. Parent, and S.L. White, *Biochim. Biophys. Acta*, 650 (1982) 209–232.

[2] E. Hébert and M. Monsigny, *Biol. Cell.*, 79 (1993) 97–109.

[3] S. Hakomori, *Adv. Cancer Res.*, 52 (1989) 257–331.

[4] G.F. Springer, *Science*, 224 (1984) 1198–1206; S.H. Itzkowitz, M. Yuan, C.K. Montgomery, T. Kjeldsen, H.K. Takahashi, W.L. Bigbee, and Y.S. Kim, *Cancer Res.*, 49 (1989) 197–204.

[5] O. Berthier-Vergnes, N. Zebda, M. Bailly, C. Bailly, and J.-F. Doré, *Lancet*, 341 (1993) 1292; N. Zebda, M. Bailly, S. Brown, J.-F. Doré, and O. Berthier-Vergnes, *J. Cell. Biochem.*, 54 (1994) 161–173; J.-F. Doré, O. Berthier-Vergnes, N. Zebda, M. Bailly, L. Thomas, C. Bailly, and A. Cocheran, *Pigment Cell Res.*, 7 (1994) 461–464.

[6] N. Sharon and H. Lis, *Science*, 246 (1989) 227–234; R. Lotan and A. Raz, *J. Cell. Biochem.*, 37 (1988) 107–117.

[7] B.M. Longenecker, D.J. Willans, G.D. MacLean, S. Selvaraj, M.R. Suresh, and A.A. Noujaim, *J. Natl. Cancer Inst.*, 78 (1987) 489–492; H.-J. Gabius, C. Schröter, S. Gabius, U. Brinck, and L.-F. Tietze, *J. Histochem. Cytochem.*, 38 (1990) 1625–1631.

[8] G.C. Look, Y. Ichikawa, G.-J. Shen, P.-W. Cheng, and C.-H. Wong, *J. Org. Chem.*, 58 (1993) 4326–4330.

[9] E.W. Holla, *Angew. Chem. Int. Ed. Engl.*, 28 (1989) 220–221.

[10] R.U. Lemieux and R.M. Ratcliffe, *Can. J. Chem.*, 57 (1979) 1244–1251.

[11] B. Ferrari and A.A. Pavia, *Carbohydr. Res.*, 79 (1980) C1–C7.

[12] V.V. Bencomo, J.-C. Jacquinet, and P. Sinaÿ, *Carbohydr. Res.*, 110 (1982) C9–C11; H. Paulsen and J.-P. Hölck, *Liebigs Ann. Chem.*, (1982) 1121–1131.

[13] L.-X. Wang, N. Sakairi, and H. Kuzuhara, *Carbohydr. Res.*, 219 (1991) 133–148.

[14] R. Schaubach, J. Hemberger, and W. Kinzy, *Liebigs Ann. Chem.*, (1991) 607–614.

[15] H. Kunz and S. Birnbach, *Angew. Chem. Int. Ed. Engl.*, 25 (1986) 360–362; H. Paulsen and J.-P. Hölck, *Carbohydr. Res.*, 109 (1982) 89–107.

[16] R.M. Ratcliffe, D.A. Baker, and R.U. Lemieux, *Carbohydr. Res.*, 93 (1981) 35–41.

[17] H. Paulsen and M. Paal, *Carbohydr. Res.*, 135 (1984) 71–84.

[18] K. Higashi, K. Nakayama, T. Soga, E. Shioya, K. Uoto, and T. Kusama, *Chem. Pharm. Bull.*, 38 (1990) 3280–3282.

[19] R.R. Schmidt, *Synthesis of Glycosides*, in B.M. Trost and I. Fleming (Eds.), *Comprehensive Organic Synthesis*, Vol. 6, Pergamon, Oxford, 1991, pp 33–64.

[20] R.R. Schmidt and E. Rücker, *Tetrahedron Lett.*, 21 (1980) 1421–1424.

[21] H. Paulsen, W. Rauwald, and U. Weichert, *Liebigs Ann. Chem.*, (1988) 75–86; P. Braun, H. Waldmann, and H. Kunz, *Bioorg. Med. Chem.*, 1 (1993) 197–207.

[22] J. Rademann and R.R. Schmidt, *Carbohydr. Res.*, 269 (1995) 217–225.

[23] H. Iijima and T. Ogawa, *Carbohydr. Res.*, 172 (1988) 183–193; 186 (1989) 107–118.

[24] L. Szabó, J. Ramza, C. Langdon, and R. Polt, *Carbo-*

hydr. Res., 274 (1995) 11–28, and references cited therein.
[25] T. Feizi, M.S. Stoll, C.-T. Yuen, W. Chai, and A.M. Lawson, *Methods Enzymol.*, 230 (1994) 484–519.
[26] F. Shafizadeh, *Methods Carbohydr. Chem.*, 2 (1963) 409–410.
[27] M. Wilchek and E.A. Bayer, *Methods Enzymol.*, 184 (1990) 123–138.
[28] M.J. O'Donnell and R.L. Polt, *J. Org. Chem.*, 47 (1982) 2663–2666.