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Unusual lactam formation occurring in the synthesis of a biotinylated T-antigen–serine derivative

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Abstract

Synthesis of the biotinylated T-antigens, linked to a serine by an α (**7α**) or a β (**7β**) 2-acetamido-2-deoxy-D-galactoside bond, is described. These derivatives were needed for the detection of a specific endogenous lectin at the surface and/or on the migration pathway of melanoma cells. In the course of the synthesis, an unusual lactam formation was observed with the β anomer of the azido-disaccharide **5β**. © 1997 Elsevier Science Ltd.

Keywords: T-Antigen; Biotinylated T-antigen–serine; Chemoenzymatic synthesis; Lactam formation

1. Introduction

Alterations in glycosylation and accumulation of unusual glycosidic structures have often been described in cancer cells [1,2]. The T-antigen [β-D-Gal-(1 → 3)-D-GalNAc] is one of these tumor-associated carbohydrate antigens present in a cryptic form in normal tissues, whereas it is exposed at the cell surface in carcinomas [2,3].

We have recently shown that this disaccharide is a marker for invasiveness and metastatic capacity in human melanoma cells [4,5]. Furthermore, it is now well established that the expression of lectins can correlate with the metastatic potential of tumor cells [1,6]. Therefore, a specific endogenous lectin could be involved, *via* a 'disaccharide–lectin' complex, in the metastatic process of the melanoma cells.

In order to detect the presence of such lectin, a labelled T-antigen was needed as a probe. The avidin–biotin system being a powerful tool for bioanalytical applications, it was decided to prepare biotinylated derivatives of the T-antigen.

α- And β-biotinylated T-antigen derivatives directly linked to biotin through a spacer arm [7] were previously prepared in our laboratory; however, neither of them could allow the detection of an endogenous lectin in melanoma tissues. To date, the importance of the region anchoring the T-antigen (structure

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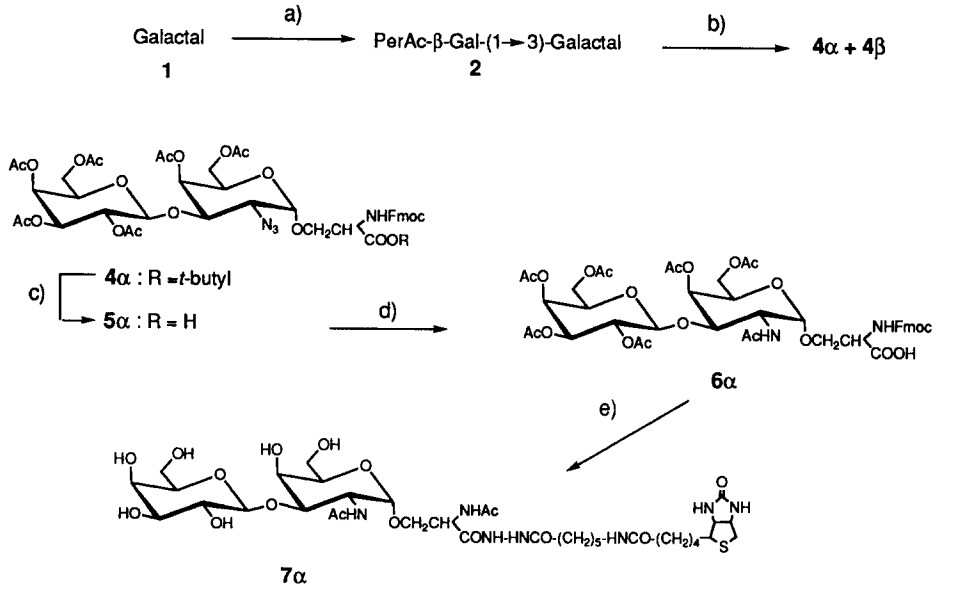


Scheme 1. Synthesis of the α-biotinylated T-antigen–serine derivative. a) i: Lipase, vinyl acetate [19]; ii: β-galactosidase, ONP-Gal; iii: Ac_2O, pyridine [7,12]; b) i: CAN, NaN_3 [12]; ii: LiBr [13]; iii: AgOTf, FmocSerO'Bu [14]; c) HCOOH [15]; d) i: $NaBH_4$, $NiCl_2$; ii: Ac_2O [16]; e) i: biotinaminocaproyl–hydrazide, DCC/HOBT [17]; ii: morpholine [20]; iii: Ac_2O; iv: MeONa [18].

and anomeric configuration) in the interaction with antibodies or lectins is not clear [8]. However, in the case of melanoma cells, the disaccharide [β-D-Gal-(1 → 3)-D-GalNAc] is part of two glycoproteins [5]. Since, in natural *O*-glycoproteins, the connection between GalNAc and the peptide backbone involves an α glycosidic linkage with serine or threonine (α-D-GalNAc–Ser/Thr), we postulated that serine (or threonine) might participate to the lectin combining sites.

We now report the synthesis of the biotinylated α- and β-T-antigen–serine derivatives **7α** and **7β**. The α anomer, being close to the biological structures, was of interest as a probe for the detection of an endogenous lectin in melanoma cells. Derivative **7β** was further prepared in order to study the influence of the anomeric configuration of the glycosidic bond. The unexpected formation of a cyclic by-product (**8**) observed in the case of the β anomer is discussed.

2. Results and discussion

Various α-T-antigen–serine derivatives have already been prepared by chemical synthesis [9,10]. A chemoenzymatic method for the synthesis of **7**, starting from 1,5-anhydro-2-deoxy-D-*lyxo*-hex-1-enitol (D-galactal) **1** [11] is now used. As described previously [7,12], stereo- and regio-specific transgalactosylation was achieved by use of a β-galactosidase from *E. coli*, followed by peracetylation of the resulting disaccharide to give the key intermediate β-Gal-(1 → 3)-D-galactal **2**. After azidonitration [12], compound **2** was converted into the α-bromide **3** [13]. Treatment of **3** with *N*-(9-fluorenylmethoxycarbonyl) serine *tert*-butyl ester, in the presence of silver triflate [14], gave **4** as a mixture of α and β anomers with an overall yield of 65% (**4α/4β** 6/4) [1]. The anomeric mixture was easily separated by column chromatography on silica gel, and the α derivative submitted to the following treatments: i) deprotection of the carboxyl group by formic acid [15]; ii) reduction of the azido group and acetylation of the amine to give perAc-[β-Gal-(1 → 3)-α-GalNAc-OSer(Fmoc,OH)] **6α** [16]; iii) conjugation of **6α** to biotinaminocaproyl hydrazide in the presence of DCC and HOBT [17]; iv) replacement of the Fmoc protective group by the biocompatible *N*-acetyl group; v) deacetylation of the disaccharide using sodium methanolate [18] to give the expected biotinylated T-antigen **7α** with an overall yield of 30% from **6α** (Scheme 1).

For the synthesis of **7β**, a similar reaction sequence was initially used; however, we noticed an

[1] We did not try to improve the selectivity since both anomers were needed. However, with the analogous β-chloroglycosyl disaccharide, we observed that the α-selectivity is improved (α / β 7/3) but is still, however, less as compared with the β-chloro monosaccharide (α / β 9/1).

unusual cyclisation which was not observed with the α anomer (Scheme 2). Treated with formic acid as above, **4β** gave the desired acid **5β**. However, when **5β** was reacted successively with sodium borohydride and acetic anhydride, the cyclic derivative **8** was obtained in high yield in addition to the expected compound **6β** (overall yield 75%: **8/6β** 5/5). This reaction was unexpected since the reduction of the azido group seemed to take place as usual. A mixed anhydride could have been formed via an intermediate betaïne; this explanation is, however, in contradiction with the results observed when the amount of acetic anhydride was increased (with or without pyridine), resulting in a decrease in the formation of the cyclic product. Furthermore, the difference in reactivity between the two anomers is difficult to explain.

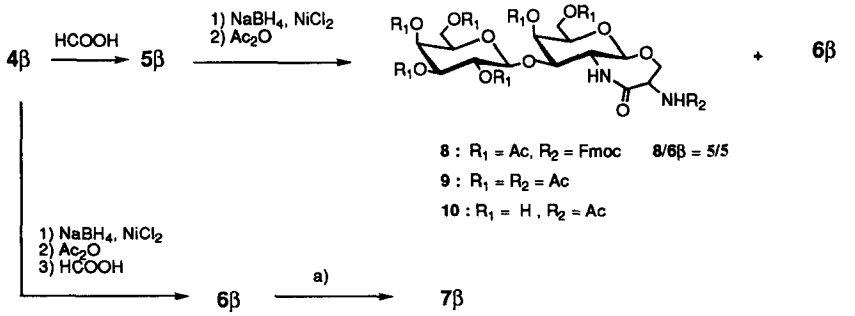
The structure of the lactam derivative **8** was determined unambiguously by NMR and confirmed by mass spectra. As compared to **6β**, shifts in the resonance signals of the CH α of serine and of the H-2 proton of the sugar moiety were observed with δ values of 3.4 ppm (H-2) and 4.3 ppm (CH α) for **8** and 3.7 ppm (H-2), 4.1 ppm (CH α) for **6β**. ^{13}C NMR (Me_2SO) clearly showed a shift of the C-2 resonance in the cyclic compound with δ values of 54.1 ppm for **8** (or 54.7 ppm) and 50.5 ppm for **6β**. Furthermore, no signal for the acetamido group was observed. The mass spectrum revealed a loss of CH_3COOH, and therefore agreed with the cyclic structure **8**. In order to confirm this structure, further derivatisation was accomplished. Removal of the Fmoc protective group and acylation of the resulting primary amine functionality gave the *N*-acylated cyclic compound **9**. The ^1H NMR of **9** revealed a change in the coupling constant of the anomeric proton H-1 ($J_{1,2}$ 6.6 Hz as compared to $J_{1,2}$ 8.2 Hz observed for **6β**). Final deacetylation of the sugar moiety of **9** gave **10**, the NMR and mass spectra of which were in agreement with the proposed structure.

To circumvent this side-reaction, the reduction-acetylation of the azido group had to be performed before the deprotection step of the carboxyl group. The resulting perO-Ac-β-D-Gal-(1 → 3)-β-D-GalNAc-OSer(Fmoc,OH) **6β** was then submitted to the same series of reactions as **6α** and the anomer **7β** was easily obtained (Scheme 2).

The synthesis of labelled T-antigens, either with an α anomeric linkage mimicking the natural *O*-glycoproteins, or with a β linkage was then achieved. The biological tests are in progress. However, it could be expected that the lectin might have too low intrinsic affinity for such monovalent carbohydrate ligands. Since the presentation of the epitope in a clustered state seems to be an important requirement for interaction [21], multimeric T-antigen structures should therefore be more suitable for the detection of a lectin. Such syntheses are under further investigation in our laboratory.

3. Experimental

General methods.—1,5-Anhydro-2-deoxy-D-*lyxo*-hex-1-enitol (D-galactal **1**), prepared from β-D-galactose pentaacetate [11], was acetylated in the 6-position using Holla's procedure (H_2O, lipase, vinyl acetate) to give 6-*O*-acetyl-1,5-anhydro-2-deoxy-D-*lyxo*-hex-1-enitol in 84% yield [19]. 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1 → 3)-4,6-di-*O*-acetyl-1,5-anhydro-2-deoxy-D-*lyxo*-hex-1-enitol **2** was obtained from 6-*O*-acetyl-1,5-anhydro-2-deoxy-D-*lyxo*-hex-1-enitol and *o*-nitrophenyl β-D-galactopyranoside using β-galactosidase from *E. coli* as already described [7,12]. Azidonitration [7,12], bromination [13], and glycosylation [14] were performed according to known procedures. Deprotection of the *tert*-butylester and reduction-acetylation of the azido group were achieved as described in the literature [15,16].



Scheme 2. Synthesis of the β-biotinylated T-antigen–serine derivative. a) i: Biotinamidocapropyl hydrazide, DCC/HOBT [17]; ii: morpholine [20]; iii: Ac_2O; iv: MeONa [18].

The intermediate 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 protected and deprotected biotinylated T-antigens were purified by reverse-phase chromatography on C_{18} using water–MeCN as the mobile phase. The final products **7α** and **7β** were repurified by reverse-phase HPLC using a Perkin–Elmer pump system with a UV detector (230 nm). A column (250 × 10 mm) of Nucleosil C_{18} (5 mm) was used and the solvent system was: A: 0.1% TFA in water; B: MeCN; the products were eluted with 8–15% B in A, flow rate 6 mL/mn. Biotinaminocaproyl hydrazide was obtained from Fluka. All the solvents used for the condensations were high grade and dry; CH_2Cl_2 was distilled over P_2O_5, and toluene over sodium with benzophenone, before use; the reactants [$(NH_4)_2Ce(NO_3)_6$, NaN_3, LiBr] were dried over P_2O_5 under vacuum and AgOTf dried at 120 °C before use. Fmoc-serine *tert*-butylester was prepared from Fmoc-serine and *tert*-butanol in the presence of dicyclohexylcarbodiimide and CuCl according to a known procedure [22]; its characteristics are in agreement with the literature data [15]. ^{1}H NMR spectra [300.134 MHz, 3-(trimethylsilyl)propionic acid sodium salt 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 vs. 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 in the positive mode on a ES/MS Platform (VG Biotech-Micromass): the solvents used for analysis were 1:1 0.2% formic acid–MeCN for **7α** and **7β** and MeOH for **8**, **9**, and **10**.

*(2,3,4,6-Tetra-*O*-acetyl-β-*D*-galactopyranosyl-(1 → 3)-4,6-di-*O*-acetyl-2-azido-2-deoxy-α- and β-*D*-galactopyranosyl)-*N*-(fluoren-9-ylmethoxycarbonyl)-3-*O*-*L*-serine* tert-*butyl ester* (**4α** *and* **4β**).—A mixture of 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1 → 3)-4,6-di-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl bromide **3** (510 mg, 0.75 mmol) [13], Fmoc-serine *tert*-butylester [22] (191 mg, 0.5 mmol), and 2,4,6-collidine (99 μL, 1.5 equiv) in CH_2Cl_2 (8 mL) was added with stirring at −20 °C to a suspension of AgOTf (256 mg, 1 mmol) in CH_2Cl_2 (4 mL) [14]. After 6 h at room temperature, the reaction mixture was filtered through Celite. The filtrate was washed with a mixture of aq 5% $NaHCO_3$ and 5% $Na_2S_2O_3$. The organic phase was dried and concd. Purification on a chromatotron (6:4, petroleum ether–ETOAc) gave **4α** (192 mg, eluted first, yield 39%) and **4β** (128 mg, yield 26%). For **4α** (literature data for similar compounds: refs [10,16]); ^{1}H NMR ($CDCl_3$): δ 1.45 [9 H, $C(CH_3)_3$], 1.8–2.0 (5 s, 18 H, COMe), 3.5 (dd, 1 H, $J_{1,2}$ 3.9, $J_{2,3}$ 10.3 Hz, H-2), 3.6–4.0 (H-3, H-5, H-5′, H-6, H-6′, CH_2 Ser), 4.1 (CH Fmoc), 4.2 (3 H, CH α Ser, CH_2 Fmoc), 4.4 (d, 1 H, $J_{1',2'}$ 7.7 Hz, H-1′), 4.7 (d, 1 H, H-1), 4.8 (dd, 1 H, $J_{2',3'}$ 10.4, $J_{3',4'}$ 3.4 Hz, H-3′), 5.0 (dd, 1 H, H-2′), 5.15 (d, 1 H, H-4′), 5.25 (d, 1 H, $J_{3,4}$ 3.0 Hz, H-4), 6.65 (d, 1 H, NH Fmoc), 7.1, 7.25, 7.4, 7.6 (8 H, Fmoc); ^{13}C NMR: δ 20.6 (CO*Me*), 26.8 (C*Me*$_3$), 47.0 (CH Fmoc), 54.7 (CH α Ser), 59.4 (C-2), 60.9, 62.6 (C-6, C-6′), 67.0, 69.4 (CH_2 Fmoc, CH_2 Ser), 66.7, 67.9, 68.6, 69.2, 70.7, 74.6 (C-2′, C-3, C-3′, C-4, C-4′, C-5, C-5′), 98.8 (C-1), 101.4 (C-1′), 120.0, 125.0, 127.0, 127.7 (CH Fmoc), 141.3, 143.7 (C Fmoc), 155.8 (OCONH), 168.5–170.4 (*CO*Me). For **4β** ^{1}H NMR: same as **4α** except δ 3.48–3.58 (m, 2 H, H-2, H-3), 4.1 (H-1), 4.5 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1′), 5.15 (d, 1 H, $J_{3,4}$ 3.4 Hz), 5.18 (d, 1 H, $J_{3',4'}$ 3.2 Hz, H-4′); ^{13}C NMR: same as for **4α** except δ 63.4 (C-2), 66.8, 68.0, 68.7, 70.6, 70.8, 71.5, 77.1 (C-2′, C-3, C-3′, C-4, C-4′, C-5, C-5′), 101.6–101.9 (C-1, C-1′).

*(2,3,4,6-Tetra-*O*-acetyl-β-*D*-galactopyranosyl-(1 → 3)-4,6-di-*O*-acetyl-2-acetamido-2-deoxy-α-*D*-galactopyranosyl)-*N*-(fluoren-9-ylmethoxycarbonyl)-3-*O*-*L*-serine* (**6α**).—Compound **4α** (200 mg, 0.2 mmol) was dissolved in formic acid (5 mL) [16]. The mixture was stirred for 6 h and formic acid was evaporated under high vacuum to give **5α** (188 mg). Reduction of the azido group of **5α** and acetylation was performed as described [16]. Compound **5α** (188 mg, 0.2 mmol) was dissolved in EtOH (20 mL) containing $NiCl_2 \cdot 6H_2O$ (760 mg) and H_3BO_3 (380 mg). Sodium borohydride (110 mg) was added portionwise until the soln remained black for at least 1 h. Acetic anhydride (1.9 mL) was then added and the mixture stirred for 2 h. 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 expected product **6α** purified on a chromatotron (CH_2Cl_2–0.2% HOAc–MeOH) (139 mg, 74%); R_f (90:10:0.6 CH_2Cl_2–MeOH–AcOH) 0.25; ^{1}H NMR (Me_2SO): δ 1.9–2.1 (21 H, COMe, NHCO*Me*), 3.6–4.4 (H-2, H-3, H-6, H-6′, H-5, H-5′, CH α Ser, CH_2 Ser, CH_2 Fmoc, CH Fmoc), 4.7 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.8 (2 H, H-1′, H-2′), 5.0 (dd, 1 H, $J_{2',3'}$ 10.1, $J_{3',4'}$ 3.6 Hz, H-3′),

5.2 (2 H, H-4, H-4′), 7.25, 7.35, 7.62, 7.8 (10 H, NHCOMe, NH Fmoc, CH Fmoc); ^{13}C NMR: δ 20.3–20.9 (CO*Me*), 22.5 (NHCO*Me*), 46.5 (CH Fmoc), 48.1 (C-2), 54.4 (CH α Ser), 60.7, 62.7 (C-6, C-6′), 65.6, 66.9 (CH_2 Fmoc, CH_2 Ser), 67.1, 67.4, 68.2, 69.8, 69.5, 70.1, 72.9 (C-2′, C-3, C-3′, C-4, C-4′, C-5, C-5′), 98.1 (C-1), 99.9 (C-1′), 120.1, 125.1, 127.0, 127.6 (CH Fmoc), 140.6, 143.6 (C Fmoc), 155.8 (OCONH), 169.0–171.9 (*CO*Me).

(β-D-Galactopyranosyl-(1 → 3)-2-acetamido-2-deoxy-α-D-galactopyranosyl)-N-acetyl-3-O-L-seryl)biotinaminocaproyl hydrazide (**7α**).—In a typical experiment, **6α** (100 mg, 0.1 mmol) was dissolved in Me_2NCHO (4 mL) containing biotinaminocaproyl hydrazide (43 mg, 0.11 mmol) and hydroxybenzotriazole (29 mg, 0.21 mmol). Dicyclohexylcarbodiimide (24 mg, 0.11 mmol) and DIEA (45 μL) were added at 0 °C. The mixture was stirred for 18 h at room temperature. The solvent was then evaporated and the product purified (roughly) on a C_{18} reverse-phase column (elution with 70% MeCN, 93 mg, yield 68%). Removal of the Fmoc group of this biotinylated derivative was achieved with morpholine in Me_2NCHO (1:1, 1.5 mL). After 1 h, the solvent was evaporated and the crude mixture treated with Ac_2O (10 μL) in Me_2NCHO (1 mL). The solvent again was removed to give 95 mg of a crude product which was dissolved in MeOH (5 mL). Sodium methanolate (1% soln in MeOH) was added until pH 11.2 was reached. After 4 h, neutralisation was performed (using dry ice) and a rough purification was accomplished using a C_{18} column (elution with 25% MeCN): **7α** was eluted first (27 mg, overall yield from **6α** 30%) followed by a fraction containing a mixture of **7α** and an unidentified product (11 mg). The highly pure product was finally obtained after HPLC as a white solid: mp 205 °C (dec); $[\alpha]_D$ +68° (*c* 0.7, water); R_f (7:1:2 ^{i}PrOH–20% NH_3–H_2O) 0.42; ^{1}H NMR (D_2O): δ 1.2–1.8 (12 H, spacer + CH_2 biotin), 2.01, 2.1 (NHCO*Me*), 2.25, 2.35 (2 t, 4 H, *J* 7.3 Hz, CH_2CO), 2.78, 3.0 (2 H, ABX system, J_{AB} 13.1 Hz, C*H*$_2$S), 3.18 (2 H, C*H*$_2$NHCO), 3.35 (m, 1 H, *J* 4.5 Hz, CH_2–C*H*S), 3.5 (1 H, H-2′), 3.58 (d, $J_{3',4'}$ 3.3 Hz, H-3′), 3.6–4.0 (m, 7 H, H-4′, H-5, H-5′, 2 H-6, 2 H-6′), 3.9–4.0 (CH_2 serine), 4.08 (dd, 1 H, $J_{3,4}$ 2.9, $J_{2,3}$ 11 Hz, H-3), 4.22 (d, 1 H, H-4), 4.35 (dd, 1 H, $J_{1,2}$ 3.6 Hz, H-2), 4.45 (dd, J_{CH-CHS} 4.4 Hz, NH–C*H*–CHS), 4.55 (d, *J* 7.6 Hz, H-1′), 4.6 (ABX system, J_{AX} 4.9, J_{BX} 0, $J_{CH-CHNH}$ 7.9 Hz, NHCH–C*H*–CH_2S), 4.7 (CH α serine), 4.95 (d, 1 H, *J* 3.7 Hz, H-1); ^{13}C NMR (Me_2SO): δ 22.6, 23.0 (2 NHCO*Me*), 24.8, 25.4, 26.1, 28.1, 28.3, 29.0 (CH_2 biotin + CH_2 spacer), 33.2, 35.3 (CH_2CO), 38.4, 40.1 (CH_2N, CH_2S), 48.1 (C-2), 50.9 (CH α serine), 55.5 (CHS), 60.5, 60.7 (C-6, C-6′), 59.3, 61.1 (CHNH), 67.0 (CH_2 serine), 67.7, 68.1, 70.7, 71.6, 73.4, 75.4, 76.8 (C-2, C-3, C-3′, C-4, C-4′, C-5, C-5′), 98.1 (C-1), 104.5 (C-1′), 162.7 (CO biotin), 168.7, 169.3, 170.1, 171.4, 171.8 (CO). FABMS: *m/z* 866.2, $[M + H]^+$. Anal. Calcd for $C_{35}H_{59}N_7O_{16}S \cdot 3H_2O$: C, 45.69%; H, 7.12%; N, 10.66%. Found: C, 45.59%; H, 6.98%; N, 10.75%.

(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl-(1 → 3)-4,6-di-O-acetyl-2-acetamido-2-deoxy-β-D-galactopyranosyl)-N-(fluoren-9-ylmethoxycarbonyl)-3-O-L-serine (**6β**).—Starting from **4β**, the reduction ($NaBH_4$–$NiCl_2$) and acetylation (Ac_2O) step had to be performed before removal of the *tert*-butylgroup of the ester. Derivative **4β** (580 mg, 0.59 mmol) was dissolved in EtOH (56 mL) containing $NiCl_2 \cdot 6H_2O$ (2.2 g) and H_3BO_3 (1.1 g). Sodium borohydride (324 mg) was added portionwise. After 1 h, Ac_2O (5.6 mL) was added and the mixture stirred for 2 h. The solvent was removed and the residue treated as above; purification of the expected product was performed on a chromatotron (1:1 to 1:9 cyclohexane–EtOAc, 436 mg, 74%). This intermediate was dissolved in formic acid (9 mL) and stirred for 6 h; the acid was evaporated and, after coevaporation with toluene, the derivative **6β** was obtained (411 mg) and used without further purification; R_f (90:10:0.6 CH_2Cl_2–MeOH–HOAc) 0.25; ^{1}H NMR (Me_2SO): same as **6α** except δ 4.35 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 5.08 (dd, 1 H, $J_{2',3'}$ 9.8, $J_{3',4'}$ 3.6 Hz, H-3′); ^{13}C NMR: same as **6α** except δ 50.5 (C-2), 67.2, 68.6, 69.7, 70.4, 70.9, 76.4 (C-2′, C-3, C-3′, C-4, C-4′, C-5, C-5′), 100.1, 100.9 (C-1, C-1′).

(β-D-Galactopyranosyl-(1 → 3)-2-acetamido-2-deoxy-β-D-galactopyranosyl)-N-acetyl-3-O-L-seryl)biotinaminocaproyl hydrazide (**7β**).—From **6β** (150 mg, 0.16 mmol), the same procedure as above was followed to give **7β** (53 mg, overall yield 36% from **6β**): mp 215 °C (dec); $[\alpha]_D$ +14° (*c* 0.6, water); R_f (7:1:2 ^{i}PrOH–20% NH_3–H_2O) 0.32; ^{1}H NMR (D_2O): δ 1.3–1.8 (12 H, spacer + CH_2 biotin), 2.02, 2.1 (NHCO*Me*), 2.27, 2.35 (2 t, 4 H, *J* 3.7 Hz, CH_2CO), 2.78, 3.01 (2 H, ABX system, J_{AB} 13.1 Hz, CH_2S), 3.18 (2 H, C*H*$_2$NHCO), 3.35 (m, 1 H, *J* 4.5 Hz, CH_2–C*H*S), 3.54 (d, 1 H, H-2′), 3.64 (d, 1 H, $J_{3',4'}$ 3.3 Hz, H-3′), 3.62–3.88 (H-5, H-5′, 2 H-6, 2 H-6′), 3.89 (H-3), 3.92 (H-4′), 4.01 (2 H, H-2, 1 CH_2 serine), 4.09 (1 CH_2 serine), 4.2 (d, $J_{3,4}$ 3 Hz, H-4), 4.43 (NH–C*H*–CH), 4.46 (H-1′), 4.55 (d, $J_{1,2}$

8.4 Hz, H-1), 4.63 (m, NHCH–C*H*–CH_2S + CH α serine); ^{13}C NMR (Me_2SO): δ 22.6, 23.2 (2 NHCO*Me*), 24.8, 25.4, 26.0, 28.1, 28.3, 29.0 (CH_2 biotin + CH_2 spacer), 33.2, 35.3 (CH_2CO), 38.4, 39.9 (CH_2N, CH_2S), 50.6, 51.4 (C-2, CH α serine), 55.5 (CHS), 60.5 (C-6, C-6′), 59.3, 61.1 (CHNH), 67.6 (CH_2 serine), 67.2, 68.1, 70.7, 73.2, 75.4, 79.3 (C-2, C-3, C-3′, C-4, C-4′, C-5, C-5′), 100.7 (C-1β), 104.7 (C-1′β), 162.8 (CO biotin), 168.6, 169.4, 170.6, 171.2, 171.9 (CO). ESMS: *m/z* 866.3, $[M + H]^+$. Anal. Calcd for $C_{35}H_{59}N_7O_{16}S \cdot 4H_2O$: C, 44.82%; H, 7.2%; N, 10.45%. Found: C, 44.79; H, 7.35%; N, 10.45%.

2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl-(1 → 3)-2H, 3-N-fluoren-9-ylmethoxycarbonyl amino, 4-one-(4,6-di-O-acetyl-1,2-dideoxy-β-D-galactopyranoso)[1,2]perhydro-1,5-oxazepine (**8**).—Compound **4β** (285 mg, 0.29 mmol) was dissolved in formic acid (8 mL) and stirred for 6 h [15]. The acid was evaporated and the residue coevaporated with toluene to give **5β** (270 mg, 0.29 mmol). This derivative was dissolved in EtOH (27 mL) and treated with $NiCl_2 \cdot 6H_2O$ (1.1 g), H_3BO_3 (550 mg), $NaBH_4$ (159 mg), and Ac_2O (2.7 mL) as described above. Purification of the reaction mixture on a chromatotron (97:7:1.5 CH_2Cl_2–MeOH–HOAc) gave **8** (95 mg, first eluted, 37%) and the expected derivative **6β** (101 mg, 37%); **8**: mp 138 °C; $[\alpha]_D$ +45° (*c* 0.56, MeOH); R_f (95:5 CH_2Cl_2–MeOH) 0.55; ^1H NMR (in $CDCl_3$ since the resolution is better than in Me_2SO): δ 1.9, 2.02, 2.1, 2.15 (18 H, CO*Me*), 3.55, 4.18 (CH_2 serine), 3.7 (H-2, H-3), 3.8–4.3 (H-5, H-5′, 2 H-6, 2 H-6′, CH_2 Fmoc), 4.35 (H-1, CH Fmoc), 4.5 (CH α serine), 4.7 (d, $J_{1',2'}$ 8.3 Hz, H-1′), 4.95 (dd, $J_{3',4'}$ 3.4, $J_{2',3'}$ 10.2 Hz, H-3′), 5.18 (dd, H-2′), 5.28 (H-4), 5.33 (d, H-4′), 5.85 (d, *J* 4.6 Hz, N*H*–CH α), 6.4 (s, NH), 7.25, 7.35, 7.55, 7.7 (Fmoc); ^{13}C NMR (Me_2SO): δ 20.6, 20.7, 20.8, 21.2 (CO*Me*), 46.8 (CH Fmoc), 54.1, 54.7 (CH α serine, C-2), 61.0, 63.0 (C-6, C-6′), 65.7, 66.0 (CH_2 serine, CH_2 Fmoc), 67.2, 68.5, 69.9, 70.3, 71.6, 76.6 (C-2′, C-3, C-3′, C-4, C-4′, C-5, C-5′), 100.2, 100.7 (C-1, C-1′), 120.3, 125.5, 127.3, 127.8, (CH Fmoc), 140.9, 144.0 (C Fmoc), 155.9 (OCONH), 169.6, 169.7, 169.9, 170.1, 170.3, 172.2 (*C*OMe). FABMS: *m/z* 885.3, $[M + H]^+$. Anal. Calcd for $C_{42}H_{48}N_2O_{19} \cdot H_2O$: C, 55.87%; H, 5.58%; N, 3.1%. Found: C, 55.90%; H, 5.64%; N, 2.95%.

2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl-(1 → 3)-2H, 3-N-acetylamino, 4-one-(4,6-di-O-acetyl-1,2-dideoxy-β-D-galactopyranoso)[1,2]perhydro-1,5-oxazepine (**9**).—Compound **8** (40 mg, 0.045 mmol) was treated with morpholine in Me_2NCHO (1:1, 1.5 mL). After 1 h, the solvents were evaporated. The residue was dissolved in MeOH (1.5 mL) with DIEA (16 μL) and Ac_2O (4 μL). The mixture was stirred 30 mn and concd. Purification on a chromatotron (CH_2Cl_2–0.1% AcOH–MeOH) gave **9** (21 mg, 66%); R_f (95:5 CH_2Cl_2–MeOH) 0.2; ^1H NMR ($CDCl_3$): same as **8** except for δ 6.38 (s, NH), 6.48 (d, *J* 5.1 Hz, NH), 4.7 (CH α), 1.85–2.1 (7 COMe); ^{13}C NMR: δ 23.1 (NHCO*Me*); FABMS: *m/z* 705.3, $[M + H]^+$.

β-D-Galactopyranosyl-(1 → 3)-2H, 3-N-acetylamino, 4-one-(1,2-dideoxy-β-D-galactopyranoso)[1,2]perhydro-1,5-oxazepine (**10**).—The peracetylated derivative **9** (21 mg, 0.03 mmol) was dissolved in MeOH (0.5 mL), and MeONa (0.5% in MeOH) was added as described before. After 1 h, neutralisation was performed and purification accomplished on a C_{18} reverse-phase column as usual. Product **10** was obtained as a white powder (10 mg, 74%): mp 175 °C (dec); R_f (7:1:2 ^{i}PrOH–20% NH_3–H_2O) 0.2; ^1H NMR (D_2O): δ 2.0 (3 H, NHCO*Me*), 3.58 (H-2), 3.6–3.9 (H-4, H-5, H-5′, 2 H-6, 2 H-6′, 1 CH_2 Ser), 4.0 (dd, 1 H, $J_{2',3'}$10.5, $J_{3',4'}$ 3.0 Hz, H-3′), 4.2 (ABX system, J_{AB} 12.7 Hz, 1 CH_2 Ser), 4.58 (d, $J_{1,2}$ 7.3 Hz, H-1), 4.62 (d, $J_{1',2'}$ 7.3 Hz, H-1′), 4.9 (m, 1 H, $J_{AX} = J_{BX} = J_{CH-NH}$ 3.7 Hz, CH α); ^{13}C NMR: δ 22.4 (NHCO*Me*), 54.4, 54.7 (C-2, CH α Ser), 61.4, 61.6 (C-6, C-6′), 66.5 (CH_2 Ser), 67.2, 69.2, 71.4, 73.3, 75.9, 76.0, 80.4 (C-2′, C-3, C-3′, C-4, C-4′, C-5, C-5′), 101.2, 104.8 (C-1, C-1′), 174.3, 175.4 (NH*C*OMe, NH*C*OCH). FABMS: *m/z* 453.2, $[M + H]^+$.

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