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# SYNTHESIS OF GLYCOPEPTIDES OF THE MUCIN TYPE CONTAINING A β-D-GlcpNAc-(1→3)-D-GalpNAc UNIT*

WILLY KINZY† AND RICHARD R. SCHMIDT‡
*Fakultät Chemie, Universität Konstanz, Postfach 5560, D-7750 Konstanz (F.R.G.)*

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## ABSTRACT

Mucin-type *O*-glycopeptides containing the β-D-GlcpNAc-(1→3)-D-GalpNAc core unit were synthesised from 2-azido-2-deoxy-D-glucose, 2-azido-2-deoxy-D-galactose, 2-azido-2-deoxylactose, and L-serine precursors, using the trichloroacetimidate method. Thus, β-D-GlcpNAc-(1→3)-α-D-GalpNAc-(1→3)-Ser (**1**) and a derivative of β-D-Galp-(1→4)-β-D-GlcpNAc-(1→6)-[β-D-GlcpNAc-(1→3)]-α-D-GalpNAc-(1→3)-Ser (**2**) were obtained. A precursor of **1** having HO-4,6 of the 2-azido-2-deoxy-D-galactose residue unsubstituted was a valuable intermediate for the synthesis of **2** due to regioselective glycosylation at O-6. Regioselectivity in this reaction was not observed for precursors having a *tert*-butyldimethylsilyl group at O-1 instead of a protected L-serine moiety.

## INTRODUCTION

Epithelial mucous secretions consist mainly of glycoproteins in which the oligosaccharide moiety is *O*-glycosidically linked to L-serine and L-threonine through 2-acetamido-2-deoxy-α-D-galactopyranose[2]. In one core type, a β-D-Galp-(1→3)-α-D-GalpNAc-(1→3)-Ser glycoside unit is present[3], which is also the determinant of the T-antigen, an *O*-glycoprotein closely related to glycophorin A and considered to be a tumor-associated antigen[4]. Several methods of glycosylation, including the trichloroacetimidate method, have been used for the synthesis of this core unit and derived higher oligosaccharides α-linked to L-serine[5–9].

However, from stomach and bronchial mucins, glycoproteins of another core type were isolated, where the GalpNAc unit carries a GlcpNAc residues as in **1**[10]. This and related structures are of special interest with respect to diseases of the bronchial tract. Thus, from bronchial mucins of patients suffering from chronic

*Glycosylimidates, Part 39. For Part 38, see ref. 1.
†Present address: E. Merck Darmstadt, Abteilung Immunochemie, Postfach 4119, D-6100 Darmstadt 1, F.R.G.
‡Author for correspondence.

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bronchitis and cystic fibrosis, amongst other oligosaccharides, the L-serine-linked tetrasaccharide **2** was identified[10d].

β-D-Glc*p*NAc-(1→3)-α-D-Gal*p*NAc-(1→3)-Ser

**1**

β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→6)
α-D-Gal*p*NAc-(1→3)-Ser
β-D-Glc*p*NAc-(1→3)

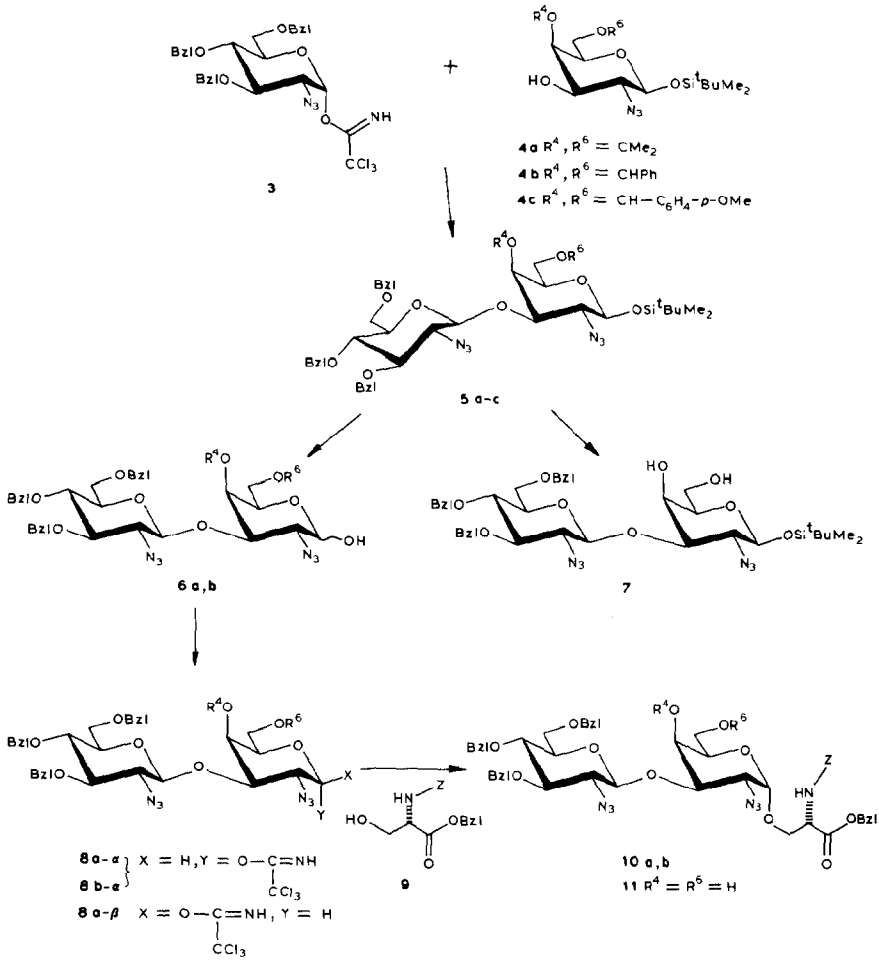
**2**

As part of a programme on the synthesis of mucin-related glycopeptides[9,11–14], we have developed syntheses for these core types, which demonstrate the efficiency of the trichloroacetimidate method in general and for the α-glycosidation of L-serine[11].

## RESULTS AND DISCUSSION

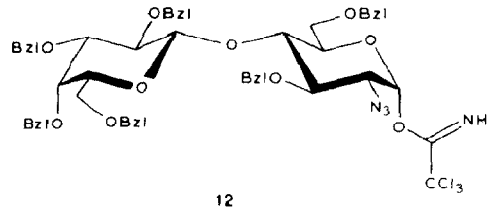
For the synthesis of **1**, the 2-azido-2-deoxy-α-D-glucosyl trichloroacetimidate derivative **3**, obtained by a known procedure[15], was used as the GlcNAc donor. Acceptors were the 2-azido-2-deoxygalactose derivatives **4a–c** with HO-3 unsubstituted, prepared according to known methods (**4a**[9], **4b**[14]) or by reaction of *tert*-butyldimethylsilyl 2-azido-2-deoxy-β-D-galactopyranoside[14,16] with *p*-methoxybenzaldehyde (→ **4c**). The formation of the disaccharide derivatives involved boron trifluoride etherate as a catalyst in dichloromethane–hexane and afforded exclusively the β-anomers **5a–c** in good yields, although **3** had no participating group at position 2.

New glycosyl donors and acceptors were readily obtained from the disaccharide derivatives **5a–c**. For instance, with trifluoroacetic acid, each compound gave the acceptor **7** with HO-4,6 unsubstituted. Removal of the $^t$BuMe$_2$Si groups from **5a** and **5b** with tetrabutylammonium fluoride afforded αβ-mixtures of the disaccharide derivatives **6a** and **6b**, respectively. Treatment of **6a** and **6b** with trichloroacetonitrile in the presence of potassium carbonate–sodium hydride or sodium hydride alone gave, exclusively, the respective α-trichloroacetimidates **8a**-α and **8b**-α in excellent yields. However, as previously observed[9], with α-trichloroacetimidates as donors, the L-serine derivative[17] **9** as acceptor, and trimethylsilyl trifluoromethanesulfonate (triflate) as catalyst at −50°, **8a**-α gave 78% of a 4:1 mixture of **10a** and **10a**-β. At room temperature (under forcing conditions), the thermodynamically more stable α-isomer **10b** was obtained exclusively from **8b**-α, but in lower yield (52%).

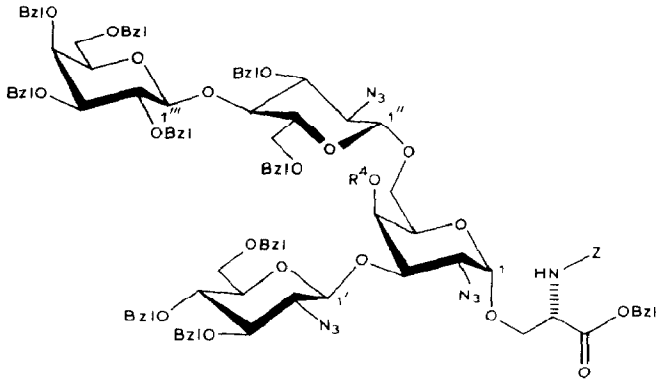



Results with other systems indicated that much better α-selectivities can be obtained with the corresponding β-trichloroacetimidates[11,18,19]. Therefore, the β-trichloroacetimidate **8a**-β was generated from **6a**, trichloroacetonitrile, and potassium carbonate. Small amounts of the α-anomer were removed by flash chromatography. The reaction of **8a**-β with the L-serine derivative **9** and trimethylsilyl triflate as catalyst gave exclusively the desired α-glycoside derivative **10a** (88%). Subsequent treatment of **10a** and **10b** with trifluoroacetic acid gave the glycosyl acceptor **11** with HO-4,6 unsubstituted.

For the synthesis of protected derivatives of the L-serine-linked tetrasaccharide **2**, a 2-amino-2-deoxylactose donor was required. Thus, lactal[20] was transformed into the α-trichloroacetimidate **12** following a known procedure[9]. The excellent donor properties[9] of **12** were exhibited in the reaction with **11** catalysed by boron trifluoride etherate in dichloromethane–hexane, which gave 80% of the



12



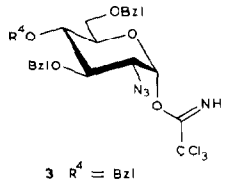
13 $R^4$ = H

14 $R^4$ = $CCl_3$—CO—NH—CO

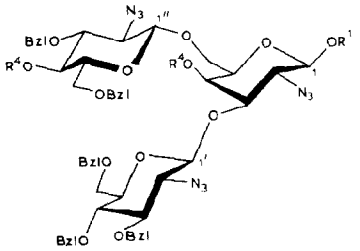
tetrasaccharide-peptide derivative **13** as the sole product. The regioselectivity of the reaction was shown by the addition of trichloroacetyl isocyanate[21] to a solution of **13** in $CDCl_3$. The signal for H-4 in the $^1$H-n.m.r. spectrum of the product **14** was shifted to $\delta$ 5.40 ($J_{3,4}$ 2.0, $J_{4,5}$ 0.5 Hz), indicating carbamoylation of HO-4.

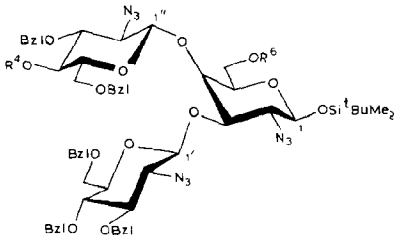
An alternative route for the synthesis of this type of compound, namely, oligosaccharide formation first and then connection to L-serine, gave an unexpected result. Glycosylation of **7** with **3** and **12**, using boron trifluoride etherate as the catalyst, afforded high yields of the $\beta$-glycoside derivatives. However, presumably due to the bulky 1-*O*-$^t$BuMe$_2$Si group, the regioselectivity for reaction at positions 6 and 4 was low (90% of a 2:1 mixture of **15** and **16**; 84% of a 1:1 mixture of **20** and **21**). This result indicates that 3,4-branching from 2-azido-2-deoxygalactose should be accessible via this approach. The sites of glycosylation of **15, 16, 20**, and **21** were assigned by *O*-carbamoylation[21] with trichloroacetyl isocyanate or by *O*-acetylation with acetic anhydride–pyridine, affording **17, 18, 22**, and **23**, respectively. Compounds **22** and **23** were separated readily and treatment with methanolic sodium methoxide then gave **20** and **21**. Removal of the $^t$BuMe$_2$Si groups from **15** and **20** with tetrabutylammonium fluoride aforded $\alpha\beta$-mixtures of **19** and **24**, respectively.

For the synthesis of **1**, the derivative **11** was treated first with nickel chloride–sodium borohydride[22] and then immediately with acetic anhydride to provide the acetamido derivative **25**, acetylation of which with acetic anhydride–pyridine



**3** $R^4$ = Bzl
**12** $R^4$ = TBGA

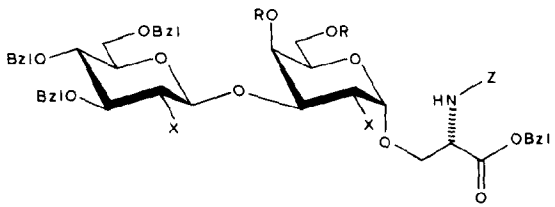




**15** $R^1$ = $Si^tBuMe_2$, $R^4$ = H, $R''^4$ = Bzl
**17** $R^1$ = $Si^tBuMe_2$, $R^4$ = $CCl_3CONHCO$, $R''^4$ = Bzl
**19** $R^1$ = $R^4$ = H, $R''^4$ = Bzl (α,β-anomers)
**20** $R^1$ = $Si^tBuMe_2$, $R^4$ = H, $R''^4$ = TBGA
**22** $R^1$ = $Si^tBuMe_2$, $R^4$ = Ac, $R''^4$ = TBGA
**24** $R^1$ = $R^4$ = H, $R''^4$ = TBGA (α,β-anomers)

**16** $R^6$ = H, $R''^4$ = Bzl
**18** $R^6$ = Ac, $R''^4$ = Bzl
**21** $R^6$ = H, $R''^4$ = TBGA
**23** $R^6$ = Ac, $R''^4$ = TBGA

TBGA = 2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl



**11** R = H, X = $N_3$
**25** R = H, X = NHAc
**26** R = Ac, X = NHAc

afforded **26**. Hydrogenolysis of the *O*-benzyl groups from compound **25** in the presence of acetic acid gave, as expected[9], **1** in high yield (90%). The structure of **1** was assigned on the basis of $^1$H-n.m.r. data [δ 4.68 (d, $J_{1,2}$ 3.9 Hz, $H^{-1}$), 4.39 (d, $J_{1',2'}$ 8.3 Hz, H-1')].

## EXPERIMENTAL

*General methods.* — Melting points are uncorrected. Optical rotations were determined with a Perkin–Elmer 241 MC polarimeter for solutions in $CHCl_3$ at 20°, unless noted otherwise. Column chromatography was performed on silica gel

(Merck, 70–230 mesh ASTM and 230–400 mesh ASTM for flash chromatography). Medium-pressure liquid chromatography (m.p.l.c.) was performed at 10–15 bar on columns of LiChroprep Si-60 (Merck, 40–60 μm) with light petroleum (b.p. 40–60°)–ethyl acetate (LP–EA) mixtures. T.l.c. and h.p.t.l.c. were performed on silica gel 60 $F_{254}$ (Merck); $R_F$ values are for t.l.c. $^1$H-N.m.r. spectra were recorded with a Bruker WM 250 Cryospec or Jeol JNM-GX 400 instrument. $^{13}$C-N.m.r. spectra were recorded with a Bruker WM 250 Cryospec instrument operated at 62.97 MHz. The values of $\delta_C$ and $\delta_H$ are expressed in p.p.m. downwards from the signal for internal $Me_4Si$, for solutions in $CDCl_3$, unless noted otherwise. Values of $\delta_H$ ($D_2O$) and $\delta_C$ ($D_2O$) are expressed in p.p.m. downwards from $Me_4Si$ by reference to internal standards of $Me_2CO$ (2.225), $Me_3COH$ (1.230) and 1,4-dioxane (67.4) or MeOH (49.8), respectively.

tert-*Butyldimethylsilyl 2-azido-2-deoxy-4,6-*O-p-*methoxybenzylidene-β-*D-*galactopyranoside* (**4c**). — To a solution of *tert*-butyldimethylsilyl 2-azido-2-deoxy-β-D-galactopyranoside[14,16] (150 mg, 0.47 mmol) and *p*-methoxybenzaldehyde dimethyl acetal (365 mg, 2 mmol) in dry *N,N*-dimethylformamide (20 mL) was added *p*-toluenesulfonic acid (10 mg). The mixture was stirred for 1 h at 25°, diluted with dichloromethane (100 mL), washed successively with aqueous $NaHCO_3$ and water, dried ($MgSO_4$), and concentrated *in vacuo*. M.p.l.c. (1:1 LP–EA) of the residue afforded **4c** (150 mg, 75%), $[\alpha]_D$ −6.2° (*c* 1, dichloromethane); $R_F$ 0.24. $^1$H-N.m.r. data (250 MHz): δ 7.18 (m, 4 H, $C_6H_4$), 5.51 (s, 1 H, PhC*H*), 4.55 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1), 4.02–4.24 (m, 2 H, $J_{6a,6e}$ 12.5, $J_{5,6e}$ 1.8 Hz, H-6*a,e*), 4.13 (dd, 1 H, $J_{4,5}$ 1.2, $J_{3,4}$ 2.1 Hz, H-4), 3.81 (s, 3 H, Me), 3.50 (dd, 1 H, H-3), 3.40 (ddd, 1 H, H-5), 2.54 (d, 1 H, exchangeable by $D_2O$, HO-4), 0.95 (s, 9 H, $CMe_3$), 0.20 and 0.18 (2 s, 6 H, $SiMe_2$).

*Anal.* Calc. for $C_{20}H_{31}N_3O_6Si$ (437.57): C, 54.9; H, 7.1; N, 9.6. Found: C, 55.1; H, 7.2; N, 9.3.

tert-*Butyldimethylsilyl 2-azido-3-*O-*(2-azido-3,4,6-tri-*O-*benzyl-2-deoxy-β-*D-*glucopyranosyl)-2-deoxy-4,6-*O-*isopropylidene-β-*D-*galactopyranoside* (**5a**). — To a solution of **4a**[9] (10.7 g, 30.0 mmol) and **3**[15] (22.0 g, 35.0 mmol) in a small amount of dry dichloromethane at −30°, with exclusion of moisture, was added dry hexane until precipitation of **4a** began ($CH_2Cl_2$–hexane 4:1). 0.1M Boron trifluoride etherate (2 mL) in anhydrous 4:1 dichloromethane–hexane (5 mL) was added during 1 h under nitrogen at −15°. Stirring was continued for 2 h at −10°. T.l.c. (3:1 LP–EA) then revealed one major product. Sodium hydrogencarbonate was added, and the mixture was stirred for 15 min, filtered, diluted with $CH_2Cl_2$ (200 mL), washed with aqueous NaCl and water, dried ($MgSO_4$), and concentrated to dryness. Flash chromatography (3:1 LP–EA) of the oily residue gave **6** (22.0 g, 90%), isolated as a colourless syrup, $[\alpha]_D$ +25° (*c* 1); $R_F$ 0.49. $^1$H-N.m.r. data (250 MHz): δ 7.38–7.15 (m, 15 H, 3 Ph), 4.94–4.77 (m, 3 H, 1.5 PhC$H_2$), 4.55–4.44 (m, 5 H, 1.5 PhC$H_2$, H-1,1'), 4.25 (dd, 1 H, $J_{3,4}$ 3.4 Hz, H-4), 3.89–3.41 (m, 9 H), 3.33 (d, 1 H, $J_{2,3}$ 10.7 Hz, H-3), 3.15 (ddd, 1 H, H-5), 1.46, 1.39 (2 s, 6 H, $CMe_2$), 0.94 (s, 9 H, $CMe_3$), 0.18 and 0.16 (s, 3 H, $SiMe_2$).

*Anal.* Calc. for $C_{42}H_{56}N_6O_9Si$ (817.02): C, 61.7; H, 6.9; N, 10.3. Found: C, 61.3; H, 6.6; N, 9.7.

tert-*Butyldimethylsilyl 2-azido-3-*O*-(2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-β-*D*-glucopyranosyl-4,6-*O*-benzylidene-2-deoxy-β-*D*-galactopyranoside* (**5b**). — A solution of **4b** (2.36 g, 5.80 mmol) and **3** (3.60 g, 5.81 mmol) in 1:2 dichloromethane–hexane (15 mL) was stirred under nitrogen at −18°. A 0.1M solution of boron trifluoride etherate (0.2 mL) in dichloromethane (2 mL) was added dropwise. The mixture was stirred for 3 h at −10°, sodium hydrogencarbonate was added, and the mixture was diluted with hexane (50 mL), filtered, washed with saturated aqueous NaCl, dried ($MgSO_4$), and concentrated *in vacuo*. Flash chromatography (45:3 toluene–acetone) of the residue gave **5b** (3.0 g, 60%), isolated as a syrup, $[\alpha]_D$ +25° (*c* 1); $R_F$ 0.31 (4:1 LP–EA). N.m.r. data: $^1$H (250 MHz), δ 7.54–7.16 (m, 20 H, 4 Ph), 5.48 (s, 1 H, PhC*H*), 4.92, 4.82, 4.79 (3 d, 3 H, 1.5 PhC$H_2$), 4.60–4.45 (m, 3 H, 1.5 PhC$H_2$), 4.57 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1'), 4.53 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1), 4.22 (dd, 1 H, $J_{3,4}$ <0.5 Hz, H-4), 4.19 (dd, 1 H, H-6*a*), 3.90 (dd, 1 H, H-6*e*), 3.82 (dd, 1 H, $J_{2,3}$ 10.6 Hz, H-2), 3.75–3.40 (m, 7 H), 3.26 (bs, 1 H, H-5), 0.95 (s, 9 H, $CMe_3$), 0.18 and 0.17 (2 s, 6 H, $SiMe_2$); $^{13}$C, δ 103.20, 100.59 (C-1', PhCH), 97.46 (C-1). The compound was used directly in the next step.

tert-*Butyldimethylsilyl 2-azido-3-*O*-(2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-β-*D*-glucopyranosyl)-2-deoxy-4,6-*O*-p-methoxybenzylidene-β-*D*-galactopyranoside* (**5c**). — As described for **5b**, a solution of **4c** (100 mg, 0.23 mmol) and **3** (186 mg, 0.30 mmol) in dichloromethane–hexane (10 mL, 1:3) was treated at −15°, with exclusion of moisture, with 0.1M boron trifluoride etherate (0.2 mL) in dry hexane (3 mL). T.l.c. (2:1 LP–EA) showed the reaction to be complete after 6 h. After work-up as described for **5b** and m.p.l.c. (3:1 LP–EA), **5c** (144 mg, 70%) was isolated as a colourless syrup, $[\alpha]_D$ +30° (*c* 1); $R_F$ 0.51. $^1$H-N.m.r. data: (250 MHz): δ 7.36–7.15 (m, 19 H, 3 Ph, $C_6H_4$), 5.44 (s, 1 H, PhC*H*), 4.94–4.76 (m, 3 H, 1.5 PhC$H_2$), 4.59–4.51 (m, 5 H, 1.5 PhC$H_2$, H-1,1'), 4.20 (dd, 1 H, $J_{3,4}$ 2.75 Hz, H-4), 4.16–3.42 (m, 10 H), 3.80 (s, 3 H, Me), 3.26 (m, 1 H, H-5), 0.94 (s, 9 H, $CMe_3$), 0.18, 0.17 (2 s, 6 H, $SiMe_2$). The compound contained minor traces of impurity.

*2-Azido-3-*O*-(2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-β-*D*-glucopyranosyl)-2-deoxy-4,6-*O*-isopropylidene-*D*-galactopyranose* (**6a**). — A solution of **5a** (2.10 g, 2.50 mmol) in dry tetrahydrofuran (20 mL) was treated at −30° with M acetic acid in tetrahydrofuran (0.4 mL) and dropwise addition of M tetrabutylammonium fluoride (10 mL). Stirring was continued for 4 h at 0°, ether (100 mL) was added, and the solution was washed with saturated aqueous ammonium chloride and water, dried ($MgSO_4$), and concentrated *in vacuo*. Flash chromatography (1:1 LP–EA) of the residue gave **6a**, isolated as a colourless syrup (1.50 g, 85%), $[\alpha]_D$ +51.5° (*c* 1); $R_F$ 0.24 and 0.17. $^1$H-N.m.r. data (250 MHz): δ 7.38–7.14 (m, 15 H, 3 Ph), 5.40 (dd, 1 H, H-1α), 4.90–4.45 (m, 7 H, 3 PhC$H_2$, H-1'), 4.30 (dd, 1 H, $J_{3,4}$ 3.0, $J_{4,5}$ <0.5 Hz, H-4), 4.16–3.40 (m, 13 H), 3.21 (bs, 1 H, H-5β), 1.47, 1.39 (2 s, 6 H, $CMe_2$).

*Anal.* Calc. for $C_{36}H_{42}N_6O_9$ (702.76): C, 61.5; H, 6.0; N, 12.0. Found: C, 61.1; H, 6.5; N, 11.4.

*2-Azido-3-O-(2-azido-3,4,6-tri-O-benzyl-2-deoxy-β-D-glucopyranosyl)-4,6-O-benzylidene-2-deoxy-D-galactopyranose* (**6b**). — A solution of **5b** (130 mg, 0.15 mmol) in dry tetrahydrofuran (8 mL) was stirred at room temperature with exclusion of moisture. Silica (3.0 g) was added followed by a solution of tetrabutylammonium fluoride (80 mg, 0.25 mmol) in tetrahydrofuran (5 mL) dropwise at 40°. After 5 h, saturated aqueous ammonium chloride (5 mL) was added, the mixture was diluted with ether (50 mL) and washed successively with water (3 × 20 mL), and the ether layer was dried ($MgSO_4$) and concentrated *in vacuo*. M.p.l.c. (1:1 LP–ether) of the residue gave **5b** (70 mg, 63%), isolated as a syrup, $R_F$ 0.53 and 0.42. The compound was used immediately for the synthesis of **8b**-α.

tert-*Butyldimethylsilyl 2-azido-3-O-(2-azido-3,4,6-tri-O-benzyl-β-D-glucopyranosyl)-2-deoxy-β-D-galactopyranoside* (**7**). — To a solution of **5a** (10.0 g, 12.2 mmol) in dichloromethane (200 mL) was added aqueous 60% trifluoroacetic acid (2 mL). The solution was stirred overnight at room temperature, diluted with dichloromethane (200 mL), washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried ($MgSO_4$), and concentrated *in vacuo*. Flash chromatography (2:1 LP–EA) of the residue gave **7** (7.20 g, 76%), m.p. 69–72° (from MeOH), $[\alpha]_D$ +9.6° (*c* 1); $R_F$ 0.31. N.m.r. data: $^1H$ (250 MHz), δ 7.36–7.18 (m, 15 H, 3 Ph), 4.92–4.78 (m, 3 H, 1.5 PhC$H_2$), 4.55–4.43 (m, 5 H, 1.5 PhC$H_2$, H-1,1'), 4.00 (m, 1 H, H-4), 3.90–3.40 (m, 11 H), 2.83 (s, 1 H exchangeable, HO-4), 1.90 (bs, 1 H, exchangeable, HO-6), 0.94 (s, 9 H, $CMe_3$), 0.16 (s, 6 H, $SiMe_2$); $^{13}C$, 137.84–137.70 (PhC-1), 128.49–127.62 ($C_6H_5$), 102.80 (C-1'), 97.50 (C-1), 25.66, 17.98, −4.12, −4.98.

*Anal.* Calc. for $C_{39}H_{52}N_6O_9Si$ (776.96): C, 60.3; H, 6.8; N, 10.8. Found: C, 60.5; H, 6.9; N, 10.5.

The same procedure was applied for the transformation of **5b** and **5c** into **7**.

*2-Azido-3-O-(2-azido-3,4,6-tri-O-benzyl-2-deoxy-β-D-glucopyranosyl)-2-deoxy-4,6-O-isopropylidene-α-D-galactopyranosyl trichloroacetimidate* (**8a**-α). — To a solution of **6a** (5.0 g, 7.11 mmol) in dry dichloromethane (150 mL) was added trichloroacetonitrile (5 mL) and dry potassium carbonate (5.0 g). After 2 h of rigorous stirring, more potassium carbonate (2.0 g) was added. When **6a** had disappeared completely, sodium hydride (200 mg) was added, and the mixture was stirred for 3 h, then filtered, and concentrated to dryness. Short-column chromatography (2:1 LP–EA) of the residue gave **8a**-α (4.5 g, 75%), isolated as a syrup, $[\alpha]_D$ +57° (*c* 1, dichloromethane); $R_F$ 0.50. $^1H$-N.m.r. data (250 MHz): δ 8.71 (s, 1 H, NH), 7.38–7.15 (m, 15 H, 3 Ph), 6.52 (d, 1 H, $J_{1,2}$ 3.3 Hz, H-1), 4.94–3.49 (m, 19 H), 1.47, 1.40 (2 s, 6 H, $CMe_2$). The compound was used directly in the next step.

*2-Azido-3-O-(2-azido-3,4,6-tri-O-benzyl-2-deoxy-β-D-glucopyranosyl)-4,6-O-benzylidene-2-deoxy-α-D-galactopyranosyl trichloroacetimidate* (**8b**-α). — To a solution of **6b** (1.30 g, 1.73 mmol) in dry dichloromethane (5 mL) were added trichloroacetonitrile (1.5 mL) and a catalytic amount of sodium hydride. After t.l.c. (3:1 LP–EA) showed complete formation of the β-trichloroacetimidate, 4 equiv. of sodium hydride were added and the suspension was stirred until complete

anomerisation of the β-trichloroacetimidate was reached. The mixture was filtered through silica to remove excess of sodium hydride, the filter cake was washed several times with dry dichloromethane, and the combined filtrate and washings were concentrated *in vacuo*. Flash chromatography (3:1 LP–EA) of the residue gave **8b**-α (1.30 g, 84%), isolated as a practically pure, colourless syrup; $R_F$ 0.43. $^1$H-N.m.r. data (250 MHz): δ 8.73 (s, 1 H, NH), 7.52–7.18 (m, 20 H, 4 Ph), 6.56 (d, 1 H, $J_{1,2}$ 3.2 Hz, H-1), 5.46 (s, 1 H, PhC*H*), 4.94–3.53 (m, 19 H). The product was used directly in the next step.

*2-Azido-3-*O*-(2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-β-*D*-glucopyranosyl)-2-deoxy-4,6-*O*-isopropylidene-β-*D*-galactopyranosyl trichloroacetimidate* (**8a**-β). — As described for **8a**-α, **6a** (5.0 g, 7.11 mmol) gave, after treatment with potassium carbonate (without sodium hydride) and flash chromatography (2:1 LP–EA), **8a**-β (4.6 g, 76%) and **8a**-α (0.8 g, 13%), each isolated as a colourless oil. The reaction was followed by t.l.c. and, after **6a** had disappeared, the reaction was stopped. Compound **8a**-β had $[\alpha]_D$ −10.5° (*c* 1); $R_F$ 0.26. $^1$H-N.m.r. data (250 MHz): δ 8.69 (s, 1 H, NH), 7.38–7.15 (m, 15 H, 3 Ph), 5.55 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 4.93–3.45 (m, 17 H), 4.34 (dd, 1 H, $J_{3,4}$ 3.1, $J_{4,5}$ <0.5 Hz, H-4), 3.35 (bs, 1 H, H-5), 1.39, 1.47 (2 s, 6 H, $CMe_2$).

*Anal.* Calc. for $C_{38}H_{42}Cl_3N_7O_9$ (847.15): C, 53.9; H, 5.0; N, 11.6. Found: C, 54.0; H, 4.9; N, 11.3.

*3-*O*-[2-Azido-3-*O*-(2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-β-*D*-glucopyranosyl)-2-deoxy-4,6-*O*-isopropylidene-α-* (**10a**) *and -β-*D*-galactopyranosyl]-*N*-benzyloxycarbonyl-*L*-serine benzyl ester* (**10a**-β). — (*a*) *From* **8a**-α. A solution of **8a**-α (800 mg, 0.94 mmol) and *N*-benzyloxycarbonyl-L-serine benzyl ester[17] (**9**; 165 mg, 0.50 mmol) in dry dichloromethane (10 mL) was treated dropwise under $N_2$ at −50° with a freshly prepared solution of trimethylsilyl trifluoromethanesulfonate (0.4 mL) in dichloromethane (3 mL). The mixture was stirred for 3 h at −40°. T.l.c. (1:1 LP–EA) then indicated the absence of **8a**-α. The solution was neutralised with $NaHCO_3$, filtered, and concentrated to dryness. M.p.l.c. (2:1 LP–EA) of the residue gave **10a** (314 mg, 63%) and **10a**-β (77 mg, 15%).

Compound **10a** had $[\alpha]_D$ +51° (*c* 1, dichloromethane); $R_F$ 0.45. $^1$H-N.m.r. data (250 MHz): δ 7.35–7.14 (m, 25 H, 5 Ph), 5.87 (d, 1 H, NH), 5.21 (bs, 2 H, PhC$H_2$), 5.12 (bs, 2 H, PhC$H_2$), 4.91 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1), 4.94–4.78 (m, 3 H), 4.57 (m, 1 H, Ser α-CH), 4.54–4.44 (m, 3 H), 4.39 (d, 1 H, $J_{1',2'}$ 7.0 Hz, H-1′), 4.40–4.16 (m, 11 H), 4.38 (bs, 1 H, *J* <0.5 Hz, H-4), 3.75 (dd, 1 H, H-2), 3.35 (bs, 1 H, H-5), 1.34, 1.43 (2 s, 6 H, $CMe_2$).

*Anal.* Calc. for $C_{54}H_{59}N_7O_{13}\cdot 0.5\ H_2O$ (1023.10): C, 63.4; H, 5.9; N, 9.6. Found: C, 63.3; H, 6.0; N, 9.3.

Compound **10a**-β had $[\alpha]_D$ +17° (*c* 1, dichloromethane); $R_F$ 0.18. $^1$H-N.m.r. data (250 MHz): δ 7.36–7.15 (m, 25 H, 5 Ph), 5.87 (d, 1 H, NH), 5.21, 5.13 (2 s, 4 H, 2 PhC$H_2$), 4.58 (m, 1 H, Ser α-CH), 4.40 (m, 1 H, Ser β-CHa), 4.24 (dd, 1 H, $J_{3,4}$ 4.0 Hz, $J_{4,5}$ <0.5 Hz, H-4), 4.22 (m, 1 H, 1-H′), 4.17 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 3.90 (m, 1 H, Ser β-CHb), 3.83–3.40 (m, 7 H), 3.77 (dd, 1 H, $J_{2,3}$ 10.4 Hz, H-2),

3.60 (dd, 1 H, H-2'), 3.31 (dd, 1 H, H-3), 3.06 (bs, 1 H, H-5), 1.43, 1.38 (2 s, 6 H, $CMe_2$).

*(b) From* **8a**-*β*. As described above, a solution of **8a**-*β* (3.60 g, 4.25 mmol) and **9** (1.50 g, 4.50 mmol) in dry dichloromethane (20 mL) was treated at −20° with trimethylsilyl trifluoromethanesulfonate (0.1 mL) in dry dichloromethane (2 mL). After 3 h, t.l.c. (2:1 LP–EA) revealed complete formation of **10a**; **10a**-*β* could not be detected. Purification of the product gave **10a** (3.80 g, 88%).

*3-O-[2-Azido-3-O-(2-azido-3,4,6-tri-O-benzyl-2-deoxy-β-D-glucopyranosyl)-4,6-O-benzylidene-2-deoxy-α-D-galactopyranosyl]-N-benzyloxycarbonyl-L-serine benzyl ester* (**10b**). — A solution of **8b**-*α* (1.30 g, 1.45 mmol) and **9** (460 mg, 1.40 mmol) in dry dichloromethane–hexane (1:1, 10 mL) was treated at ambient temperature with trimethylsilyl trifluoromethanesulfonate (0.2 mL) in dichloromethane (2 mL). After 10 min, the reaction was finished and the mixture was treated as described for **10a**, to give **10b** (772 mg, 52%), isolated as a syrup, $[\alpha]_D$ +59.5° (*c* 1); $R_F$ 0.60 (2:1 LP–EA). $^1$H-N.m.r. data (250 MHz): *δ* 7.48–7.15 (m, 30 H, 5 Ph), 5.92 (d, 1 H, *J* 8.2 Hz, NH), 5.39 (s, 1 H, PhC*H*), 5.21, 5.13 (2 s, 4 H, 2 Ph$CH_2$), 4.94 (d, 1 H, $J_{1,2}$ 3.0 Hz, H-1), 4.92, 4.82, 4.83 (3 d, 3 H, 1.5 Ph$CH_2$), 4.60 (m, 1 H, Ser *α*-CH), 4.47 (d, 1 H, $J_{1',2'}$ 7.3 Hz, H-1'), 4.60–4.39 (m, 3 H, 1.5 Ph$CH_2$), 4.33 (dd, 1 H, $J_{3,4}$ 2.4 Hz, H-4), 4.20–3.45 (m, 11 H), 3.95 (dd, 1 H, 3-H), 3.82 (dd, 1 H, Ser *β*-CH).

*Anal.* Calc. for $C_{58}H_{59}N_7O_{13}$ (1061.5): C, 65.6; H, 5.6; N, 9.2. Found: C, 65.3; H, 5.4; N, 8.9.

*3-O-[2-Azido-3-O-(2-azido-3,4,6-tri-O-benzyl-2-deoxy-β-D-glucopyranosyl)-2-deoxy-α-D-galactopyranosyl]-N-benzyloxycarbonyl-L-serine benzyl ester* (**11**). — A solution of **10a** (6.8 g, 6.70 mmol) in dichloromethane (200 mL) was stirred with aqueous 60% trifluoroacetic acid (1 mL) at room temperature for 5 h. T.l.c. then showed complete reaction. Conventional work-up and flash chromatography (1:1 LP–EA) of the product gave **11** (6.5 g, ~100%), isolated as a colourless oil, $[\alpha]_D$ +51° (*c* 1); $R_F$ 0.23. $^1$H-N.m.r. data (250 MHz): *δ* 7.35–7.18 (m, 25 H, 5 Ph), 5.95 (d, 1 H, *J* 8.24 Hz, NH), 5.32–5.22 (m, 2 H, Ph$CH_2$), 5.11 (s, 2 H, Ph$CH_2$), 4.93–4.80 (m, 3 H, 1.5 Ph$CH_2$), 4.87 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.60 (m, 1 H, Ser *α*-CH), 4.56–4.47 (m, 3 H, 1.5 Ph$CH_2$), 4.37 (m, 1 H, $J_{1',2'}$ 7.3 Hz, H-1'), 4.14–3.48 (m, 15 H), 2.96 (d, 1 H, exchangeable, $J_{4,OH}$ 1.5 Hz, HO-4), 1.67 (m, 1 H, exchangeable, HO-6).

*Anal.* Calc. for $C_{51}H_{55}N_7O_{13}$ (974.03): C, 62.9; H, 5.7; N, 10.1. Found: C, 62.7; H, 5.7; N, 10.1.

Application of the procedure to **10b** gave **11** (65%).

*3-O-{2-Azido-6-O-[2-azido-3,6-di-O-benzyl-2-deoxy-4-O-(2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-3-O-(2-azido-3,4,6-tri-O-benzyl-2-deoxy-β-D-glucopyranosyl-2-deoxy-α-D-galactopyranosyl}-N-benzyloxycarbonyl-L-serine benzyl ester* (**13**). — As described for **5a**, a solution of **11** (487 mg, 0.5 mmol) and 2-azido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-benzyl-*β*-D-galactopyranosyl)-*α*-D-glucopyranosyl trichloroacetimidate[9] (**12**; 520 mg, 0.5

mmol) in dry hexane–dichloromethane (5 mL, 3:2) was treated at −15° with 0.1M boron trifluoride etherate (0.5 mL). After 3 h, t.l.c. showed complete conversion of **11**. Work-up and flash chromatography (45:3 toluene–acetone) of the product gave **13** (715 mg, 80%), $[\alpha]_D$ +15° (*c* 1); $R_F$ 0.57 (2:1 LP–EA). N.m.r. data: $^1H$ (250 MHz), $\delta$ 7.40–7.15 (m, 55 H, 11 Ph), 5.72 (d, 1 H, $J_{NH,H\text{-}2}$ 8.0 Hz, NH, exchangeable), 5.20–3.25 (m, 53 H), 2.90 (d, 1 H, $J_{4,OH}$ 1.2 Hz, HO-4, exchangeable); $^{13}C$ (62.97 MHz), $\delta$ 169.38 (*C*OOBzl), 155.6 (NH*C*OOBzl), 102.36, 101.84, 101.67 (C-1′,1″,1‴), 98.81 (C-1).

*Anal.* Calc. for $C_{105}H_{110}N_{10}O_{22}$ (1864.10): C, 67.7; H, 6.0; N, 7.5. Found: C, 67.5; H, 6.0; N, 7.7.

*3-O-{2-Azido-6-O-[2-azido-3.6-di-O-benzyl-2-deoxy-4-O-(2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-3-O-(2-azido-3,4,6-tri-O-benzyl-2-deoxy-β-D-glucopyranosyl)-2-deoxy-4-O-trichloroacetylcarbamoyl-α-D-galactopyranosyl}-N-benzyloxycarbonyl-L-serine benzyl ester* (**14**). — Trichloroacetyl isocyanate (20 μg) was added to a solution of **13** (10 mg) in $CDCl_3$ (0.4 mL). The mixture was immediately used for n.m.r. spectroscopy. $^1H$-N.m.r. data (250 MHz): $\delta$ 8.40 (s, 1 H, NH), 7.40–7.10 (m, 55 H, 11 Ph), 5.65 (d, 1 H, *J* 8.0 Hz, NH), 5.40 (d, 1 H, $J_{3,4}$ 2.0 Hz, H-4), 5.10–3.20 (m, 53 H).

tert-*Butyldimethylsilyl 2-azido-3,6-* (**15**) *and -3,4-di-O-(2-azido-3,4,6-tri-O-benzyl-β-D-glucopyranosyl)-2-deoxy-β-D-galactopyranoside* (**16**). — A solution of **7** (1.10 g, 1.41 mmol) and **3** (0.91 g, 1.47 mmol) in dichloromethane (6 mL) was treated dropwise at −45° with a solution of 0.1M boron trifluoride etherate (0.5 mL diluted with 3 mL of dichloromethane). Stirring was continued for 8 h. T.l.c. then showed complete conversion of the starting material. After standard work-up, the crude mixture was purified by m.p.l.c. (4:1 LP–EA) to give **15** (1040 mg, 60%) and **16** (520 mg, 30%) isolated as syrups.

Compound **15** had $R_F$ 0.23. N.m.r. data: $^1H$ (400 MHz), $\delta$ 7.35–7.11 (m, 30 H, 5 Ph), 4.93–4.74 (m, 6 H, 3 PhC$H_2$), 4.56–4.44 (m, 8 H, H-1,1′, 3 PhC$H_2$), 4.35 (d, 1 H, $J_{1'',2''}$ 7.3 Hz, H-1″), 4.03 (dd, 1 H, $J_{3,4}$ 4.0 Hz, H-4), 4.00–3.92 (m, 2 H, H-6*a*,6*e*), 3.66–3.35 (m, 15 H), 2.86 (d, 1 H, $J_{4,OH}$ 2.1 Hz, HO-4), 0.94 (s, 9 H, $Me_3$), 0.18 (s, 6 H, 2 Me); $^{13}C$ (62.97 MHz), $\delta$ 137.9–137.8 ($C_6H_5$), 128.4–127.6 ($C_6H_5$), 102.74 (C-1′), 102.34 (C-1″), 97.39 (C-1), 25.71, 17.98, −4.09, −5.20.

*Anal.* Calc. for $C_{66}H_{78}N_9O_{13}Si$ (1233.48): C, 64.3; H, 6.4. Found: C, 64.8; H, 6.5.

Compound **16** had $R_F$ 0.17, $[\alpha]_D$ +45° (*c* 1, dichloromethane). N.m.r. data: $^1H$ (400 MHz), $\delta$ 7.41–7.06 (m, 30 H, 6 Ph), 4.96–4.32 (m, 15 H, H-1,1′,1″, 6 PhC$H_2$), 4.24 (dd, 1 H, $J_{3,4}$ 2.8 Hz, H-4), 3.93–3.22 (m, 18 H), 0.93 (s, 9 H, $CMe_3$), 0.16 (s, 6 H, 2 Me); $^{13}C$ (62.97 MHz), $\delta$ 138.06–137.20 ($C_6H_5$), 128.34–127.37 ($C_6H_5$), 103.65 (C-1″), 102.78 (C-1′), 97.60 (C-1), 59.13 (C-6), 25.69, 18.02, −4.18, −4.86.

tert- *Butyldimethylsilyl 2-azido-3,6-di-O-(2-azido-3,4,6-tri-O-benzyl-β-D-glucopyranosyl)-2-deoxy-4-O-trichloroacetylcarbamoyl-β-D-galactopyranoside* (**17**). — To a solution of **15** (10 mg) in $CDCl_3$ (0.4 mL) was added $CCl_3CONCO$ (20 μL).

The product was immediately used for n.m.r. spectroscopy. $^1$H-N.m.r. data (250 MHz): $\delta$ 8.50 (s, 1 H, NH), 7.33–7.12 (m, 30 H, 6 Ph), 5.35 (d, 1 H, $J_{3,4}$ 2.1 Hz, H-4), 4.89–4.74 (m, 6 H), 4.62–4.43 (m, 8 H), 4.27 (d, 1 H, $J_{1'',2''}$ 7.6 Hz, H-1''), 4.00–3.93 (m, 2 H, H-6*a*,6*e*), 3.78–3.37 (m, 15 H), 0.96 (s, 9 H, $CMe_3$), 0.21 (s, 6 H, 2 Me).

tert-*Butyldimethylsilyl 6-O-acetyl-2-azido-3,4-di-O-(2-azido-3,4,6-tri-O-benzyl-β-*D*-glucopyranosyl)-2-deoxy-β-*D*-galactopyranoside* (**18**). — A mixture of **16** (300 mg, 0.24 mmol) and $Ac_2O$ (4 mL) containing pyridine (6 mL) was left at ambient temperature overnight and then concentrated *in vacuo*. After repeated distillations of toluene from the residue, it was crystallised from hexane–ether to give **18** (300 mg, 97%), m.p. 140–142°, $[\alpha]_D$ +42° (*c* 1), $R_F$ 0.46 (3:2 LP–ether). N.m.r. data: $^1$H (250 MHz), $\delta$ 7.40–7.15 (m, 30 H, 6 Ph), 4.93, 4.85, 4.78, 4.75 (4 d, 4 H, 2 PhC$H_2$), 4.67 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.55–4.40 (m, 8 H, H-1',1'', 3 PhC$H_2$), 4.33 (m, 2 H, H-6*a*,6*e*), 4.31 (dd, 1 H, $J_{3,4}$ 4.0, $J_{4,5}$ <0.5 Hz, H-4), 3.83–3.25 (m, 15 H), 2.04 (s, 3 H, Me), 0.94 (s, 9 H, $CMe_3$), 0.17, 0.16 (2 s, 6 H, 2 Me); $^{13}$C, $\delta$ 170.35 (C=O), 138.25–137.77 (PhC-1), 103.65, 102.37 (C-1',1''), 97.56 (C-1), 25.71 ($CH_3$), 20.75 ($CH_3$), 18.04 (C-Si), −4.28, −4.97 ($CH_3$-Si).

*Anal.* Calc. for $C_{68}H_{81}N_9O_{14}Si$ (1276.54): C, 64.0; H, 6.4; N, 9.9. Found: C, 63.9; H, 6.4; N, 9.8.

*2-Azido-3,6-di-O-(2-azido-2-deoxy-3,4,6-tri-O-benzyl-β-*D*-glucopyranosyl)-2-deoxy-β-*D*-galactopyranose* (**19**). — To a solution of **15** (330 mg, 0.26 mmol) in dry tetrahydrofuran was added M acetic acid (1 mL) at −20°, followed by M tetrabutylammonium fluoride (1 mL). T.l.c. (2:1 LP–EA) showed, after 8 h, complete conversion of the starting material. The mixture was diluted with ether (70 mL), washed extensively with water and saturated aqueous NaCl, and dried ($MgSO_4$); after m.p.l.c. (2:1 LP–EA), **19** (260 mg, 87%) was obtained, m.p. 101–104°, $R_F$ 0.36–0.28. $^1$H-N.m.r. data (250 MHz): $\delta$ 7.37–7.09 (m, 30 H, 6 Ph), 5.37 (m, 0.6 H, H-1$\alpha$), 4.93–3.30 (m, 33.3 H), 3.00 (d, 0.3 H, HO-4), 2.89 (d, 0.6 H, HO-4).

*Anal.* Calc. for $C_{60}H_{65}N_9O_{13}$ (1120.23): C, 64.3; H, 5.9; N, 11.3. Found: C, 64.1; H, 6.0; N, 11.1.

tert-*Butyldimethylsilyl 2-azido-4-O-* (**21**) *and -6-O-[2-azido-3,6-di-O-benzyl-2-deoxy-4-O-(2,3,4,6-tetra-O-benzyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranosyl]-3-O-(2-azido-3,4,6-tri-O-benzyl-2-deoxy-β-*D*-glucopyranosyl)-2-deoxy-β-*D*-galactopyranoside* (**20**). — As described for **5b**, **7** (3.8 g, 4.89 mmol) and 2-azido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-α-D-glucopyranosyl trichloroacetimide (**12**; 4.60 g, 4.30 mmol) were treated with boron trifluoride etherate and worked-up after 4 h. Flash chromatography (4:1 LP–EA) of the product gave a 1:1 mixture (6.0 g, 84%) of **20** and **21** as indicated by the relative intensities of the $^1$H-n.m.r. signals of the SiMe-group. An analytical sample was isolated by m.p.l.c. (90:2 toluene–acetone).

Compound **21** had $[\alpha]_D$ +20° (*c* 1), $R_F$ 0.29 (45:3 toluene–acetone). $^1$H-N.m.r. data (250 MHz): $\delta$ 7.38–7.08 (m, 45 H, 9 Ph), 5.0–4.18 (m, 22 H, H-1,1',1'',1''', 6 PhC$H_2$), 3.92–3.85 (m, 2 H), 3.29–3.32 (m, 22 H), 1.11 (bs, 1 H, exchangeable, HO-6), 0.92 (s, 9 H, $CMe_3$), 0.13 (s, 6 H, 2 Me).

*Anal.* Calc. for $C_{93}H_{107}N_9O_{18}Si$ (1667.0): C, 67.0; H, 6.5; N, 7.6. Found: C, 67.1; H, 6.4; N, 7.5.

Compound **20** had $R_F$ 0.22. $^1$H-N.m.r. data (250 MHz): δ 7.37–7.12 (m, 45 H, 9 Ph), 5.04–3.31 (m, 46 H), 2.78 (d, 1 H, $J_{OH,4}$ 2.1 Hz, HO-4), 0.94 (s, 9 H, $CMe_3$), 0.19, 0.18 (2 s, 6 H, 2 Me).

*Anal.* Calc. for $C_{93}H_{107}N_9O_{18}Si$ (1667.0): C, 67.0; H, 6.5; N, 7.6. Found: C, 66.7; H, 6.3; N, 7.4.

*Compound* **20** *from* **22**. — A solution of **22** (280 mg, 0.164 mmol) in methanol (30 mL) and M NaOMe (0.1 mL) was stirred for 20 h at 20°, then neutralised with Amberlite IR-120 ($H^+$) resin, filtered, and subjected to chromatography using a short column of $SiO_2$ (1:1 LP–ether) to afford **20** (240 mg, 88%), $R_F$ 0.33 (4:1 LP–ether).

tert-*Butyldimethylsilyl 4-O-acetyl-2-azido-6-O-[2-azido-3,6-di-O-benzyl-2-deoxy-4-O-(2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-3-O-(2-azido-3,4,6-tri-O-benzyl-2-deoxy-β-D-glucopyranosyl)-2-deoxy-β-D-galactopyranoside* (**22**) *and* tert-*butyldimethylsilyl 6-O-acetyl-2-azido-4-O-[2-azido-3,6-di-O-benzyl-2-deoxy-4-O-(2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-3-O-(2-azido-3,4,6-tri-O-benzyl-2-deoxy-β-D-glucopyranosyl)-2-deoxy-β-D-galactopyranoside* (**23**). — The above mixture of **20** and **21** (6.0 g, 3.60 mmol) was acetylated overnight in pyridine (50 mL) and acetic anhydride (10 mL) at 20°. The mixture was then concentrated and toluene was distilled from the residue. M.p.l.c. (4:1 LP–EA) then gave **22** (3.0 g, 49%) and **23** (2.0 g, 33%) as syrups.

Compound **22** had $[\alpha]_D$ +0.1° (*c* 1), $R_F$ 0.30. N.m.r. data: $^1$H (250 MHz), δ 7.40–7.15 (m, 45 H, 9 Ph), 5.30 (d, 1 H, $J_{3,4}$ 3.4, $J_{4,5} < 0.5$ Hz, H-4), 5.10–3.30 (m, 46 H), 2.05 (s, 3 H, Ac), 0.92 (s, 9 H, $CMe_3$), 0.18, 0.14 (2 s, 6 H, 2 Me); $^{13}$C (62.97 MHz), δ 169.50 (C=O), 138.9–137.9 (9 PhC-1), 102.6, 102.3, 101.6 (C-1′,1″,1‴), 97.2 (C-1), 25.6 ($CH_3C$), 20.6 ($CH_3CO$), 17.9 (CSi), −4.3, −5.3 ($CH_3Si$).

Compound **23** had $[\alpha]_D$ +24° (*c* 1), $R_F$ 0.25. $^1$H-N.m.r. data (250 MHz): δ 7.33–7.02 (m, 45 H, 9 Ph), 4.96–3.18 (m, 47 H), 1.92 (s, 3 H, Ac), 0.86 (s, 9 H, $CMe_3$), 0.09, 0.07 (2 s, 6 H, 2 Me).

*Anal.* Calc. for $C_{95}H_{109}N_9O_{19}Si$ (1709.04): C, 66.8; H, 6.4; N, 7.4. Found for **22**: C, 66.4; H, 6.4; N, 7.1. Found for **23**: C, 67.0; H, 6.6; N, 7.0.

*2-Azido-6-O-[2-azido-3,6-di-O-benzyl-2-deoxy-4-O-(2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-3-O-(2-azido-3,4,6-tri-O-benzyl-2-deoxy-β-D-glucopyranosyl)-2-deoxy-β-D-galactopyranose* (**24**). — As described for **6b**, a solution of **20** (2.30 g, 1.38 mmol) in tetrahydrofuran (200 mL) was treated at 0° with M tetrabutylammonium fluoride and afterwards, at room temperature, more fluoride was added (total of 5 equiv.). After work-up and chromatography (2:1 LP–EA), **24** (1.50 g, 70%) was obtained, $R_F$ 0.35. $^1$H-N.m.r. data (250 MHz): δ 7.37–7.11 (m, 45 H, 9 Ph), 5.35 (bs, 0.5 H, H-1α), 5.03–3.29 (m, 46.5 H), 2.83 (bs, 1 H, HO-4).

*Anal.* Calc. for $C_{87}H_{93}N_9O_{18} \cdot H_2O$ (1570.76): C, 66.5; H, 6.1; N, 8.0. Found: C, 66.7; H, 6.2; N, 7.8.

*3-O-[2-Acetamido-3-O-(2-acetamido-3,4,6-tri-O-benzyl-2-deoxy-β-D-galacto-pyranosyl)-2-deoxy-α-D-galactopyranosyl]-N-benzyloxycarbonyl-L-serine benzyl ester* (**25**). — To a solution of **11** (500 mg, 0.51 mmol), $NiCl_2 \cdot 6\ H_2O$ (4%), and boric acid (2%) in ethanol (50 mL) was added dropwise a filtered solution of sodium borohydride in ethanol until the black color remained for 1 h. After t.l.c. (95:5 $CHCl_3$–methanol) showed complete conversion of the starting material, acetic anhydride (2 mL) was added, and the mixture was stirred for 4 h, then poured into ice–water (100 mL), and extracted several times with dichloromethane. The combined extracts were neutralised, dried ($MgSO_4$), and concentrated *in vacuo*. Flash chromatography (20:1 chloroform–methanol) of the residue gave **25** (410 mg, 80%) as an amorphous solid, $[\alpha]_D$ +57° (*c* 1, *N,N*-dimethylformamide); $R_F$ 0.38. $^1$H-N.m.r. data (250 MHz): δ 7.84, 7.80 (2 d, 2 H, *J* 8.0 Hz, 2 NH), 7.77–7.11 (m, 26 H, NH, 5 Ph), 5.13–3.33 (m, 29 H), 1.82, 1.76 (2 s, 6 H, 2 Me).

*Anal.* Calc. for $C_{55}H_{63}N_3O_{15}$ (1006.11): C, 65.7; H, 6.3; N, 4.2. Found: C, 65.3; H, 6.2; N, 4.1.

*3-O-[2-Acetamido-3-O-(2-acetamido-3,4,6-tri-O-benzyl-2-deoxy-β-D-gluco-pyranosyl)-4,6-di-O-acetyl-2-deoxy-α-D-galactopyranosyl]-N-benzyloxycarbonyl-L-serine benzyl ester* (**26**). — A solution of **25** (20 mg, 19.9 μmol) in pyridine (4 mL) and acetic anhydride (2 mL) was left overnight at room temperature. M.p.l.c. (1:1 LP–EA) of the product gave **26** (92%) as a syrup, $[\alpha]_D$ +71° (*c* 1). $^1$H-N.m.r. data (250 MHz): δ 7.34–7.15 (m, 25 H, 5 Ph), 6.14 (d, 1 H, *J* 7.3 Hz, exchangeable, Ser-NH), 5.86 (d, 1 H, *J* 8.5 Hz, exchangeable, NH), 5.68 (d, 1 H, *J* 7.0 Hz, exchangeable, NH), 5.35 (dd, 1 H, $J_{3,4}$ 2.4, $J_{4,5}$ <0.5 Hz, H-4), 5.24–5.07 (m, 4 H, 2 PhC$H_2$), 4.96 (d, 1 H, $J_{1,2}$ 2.5 Hz, H-1), 4.94 (m, 1 H, Ser-β-CHa), 4.81, 4.75, 4.62, 4.58 (4 d, 4 H, 2 PhC$H_2$), 4.57 (d, 1 H, H-1′), 4.55 (bs, 2 H, PhC$H_2$), 4.52 (m, 1 H, Ser-β-CHb), 4.28 (m, 1 H, Ser-α-CH), 4.00 (dd, 1 H, H-3), 4.10–3.85 (m, 4 H), 3.86 (m, 1 H, H-5), 3.71 (bs, 2 H), 3.55 (bs, 2 H), 3.29 (ddd, 1 H, H-2), 2.06, 1.98, 1.93, 1.84 (4 s, 12 H, 4 Ac).

*Anal.* Calc. for $C_{59}H_{67}N_3O_{17} \cdot H_2O$ (1108.19): C, 64.0; H, 6.3; N, 3.8. Found: C, 63.9; H, 6.2; N, 3.9.

*3-O-[2-Acetamido-3-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-α-D-galactopyranosyl]-L-serine* (**1**). — A solution of **25** (180 mg, 0.18 mmol) in AcOH–MeOH–1,4-dioxane (4:1:1, 20 mL) was hydrogenated in the presence of 10% Pd/C (40 mg) for 5 h at room temperature. Filtration, concentration *in vacuo*, and co-distillation with toluene afforded, after chromatography (LiChroprep $NH_2$; 1:1 acetonitrile–water) and lyophilisation, **1** (82.6 mg, 90%) as an amorphous solid, $[\alpha]_D$ +47° (*c* 0.8, water); $R_F$ 0.61 (LiChroprep $NH_2$; 1:1 acetonitrile–water), 0.42 ($SiO_2$; 1:2:1 chloroform–methanol–water). $^1$H-N.m.r. data (400 MHz, $D_2O$): δ 4.68 (d, 1 H, $J_{1,2}$ 3.9 Hz, H-1), 4.39 (d, 1 H, $J_{1',2'}$ 8.3 Hz, H-1′), 4.08 (dd, 1 H, $J_{2,3}$ 11.0 Hz, H-2), 4.03 (dd, 1 H, $J_{3,4}$ 2.7, $J_{4,5}$ <0.5 Hz, H-4), 3.86 (m, 1 H, Ser-α-CHa), 3.83 (dd, 1 H, 3 H), 3.78 (m, 1 H, Ser-α-CHb), 3.72–3.55 (m, 6 H, H-5,6*a*,6*e*,6*a*′,6*e*′), 3.50 (dd, 1 H, $J_{2',3'}$ 10.3 Hz, H-2′), 3.36 (dd, 1 H, $J_{3',4'}$ 8.5 Hz, H-3′), 3.28 (dd, 1 H, $J_{4',5'}$ 9.5 Hz, H-4′), 3.24 (m, 1 H, H-5′), 1.87, 1.83 (2 s, 6 H, 2 Me).

*Anal.* Calc. for $C_{19}H_{33}N_3O_{13}\cdot 2.5\ H_2O$ (556.53): C, 41.0; H, 6.9; N, 7.6. Found: C, 40.8; H, 6.5; N, 7.3.

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