Chemoenzymic synthesis of oligosaccharide fragments of the capsular polysaccharide of *Streptococcus pneumoniae* type 14

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The chemoenzymic synthesis is described of β-D-Gal*p*-(1⟶4)-β-D-Glc*p*-(1⟶6)-[(β-D-Gal*p*)-(1⟶4)]-β-D-Glc*p*NAc-(1⟶3)-β-D-Gal*p*-(1⟶O$(CH_2)_6NH_2$) **20** and β-D-Gal*p*-(1⟶4)-β-D-Glc*p*-(1⟶6)-[(β-D-Gal*p*)-(1⟶4)]-β-D-Glc*p*NAc-(1⟶3)-β-D-Gal*p*-(1⟶4)-β-D-Glc*p*-(1⟶O$(CH_2)_6NH_2$) **21**, representing penta- and hexasaccharide fragments of the *Streptococcus pneumoniae* type 14 capsular polysaccharide. In a chemical approach the intermediate tetra- and pentasaccharide fragments **18** and **19**, respectively, were synthesised, wherein the non-terminal *N*-acetyl-β-D-glucosamine residues were not yet galactosylated. Both intermediates were found to be good acceptor substrates for bovine milk β-1,4-galactosyltransferase. The title oligosaccharides form suitable compounds for conjugation with carrier proteins, to be tested as potential vaccines in animal models.

Introduction

Streptococcus pneumoniae is a pathogenic bacterium causing infections of the lung (pneumonia), middle ear (otitis media) and meninges (meningitis). Pneumococcal infections are still significant causes of morbidity and mortality all over the world. The initial success of antibiotic treatment is now negatively influenced by a growing resistance against antibiotics.[1] Immunocompetent people can be protected efficiently by vaccination with the available 23-valent capsular polysaccharide (CPS) vaccines.[2] The highest incidence of pneumococcal infections is, however, in young children, the elderly and immunocompromised patients. People in these groups do not respond adequately to the T-cell independent polysaccharides as antigens.[3] Conjugation of carbohydrate antigens to protein carriers represents an approach to overcome these problems by converting T-cell independent antigens into more immunogenic T-cell dependent antigens.[4]

Currently, neoglycoconjugate vaccines have been introduced, amongst others against *S. pneumoniae*.[5] These neoglycoproteins are prepared by conjugation of isolated capsular polysaccharides or a mixture of polysaccharide-derived oligosaccharides to a protein carrier.[6] Recent studies with *S. pneumoniae* type 6B neoglycoproteins showed that a synthetic tetrasaccharide fragment, *i.e.* one repeating unit of the 6B CPS, coupled to keyhole limpet hemocyanin (KLH) was sufficient to generate a protective antibody response in mice.[7] Similar results were shown for *S. pneumoniae* type 3 neoglycoproteins, consisting of di-, tri- and tetrasaccharides coupled to the cross-reacting material (CRM_{197}) of modified diphtheria toxin in different molar carbohydrate–protein ratios.[8]

In earlier reports we have described the chemoenzymic synthesis of a spacered branched tetrasaccharide fragment of the CPS of *S. pneumoniae* type 14, corresponding with one repeating unit,[9] as well as of alkyl-bridged hexa- and octasaccharide mimics of fragments of the CPS.[10] Especially, the tetrasaccharide-containing CRM_{197} conjugate showed promising immunological data when tested in mice models.[11] Based on these findings, it was decided to synthesise longer intact oligosaccharide fragments of the CPS of *S. pneumoniae* type 14.

Here, we report on the chemoenzymic synthesis of spacered oligosaccharides, representing penta- and hexasaccharide fragments of the CPS of *S. pneumoniae* type 14 (Fig.1).

[→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→6)-[β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→]$_n$

Fig. 1 Repeating unit of the capsular polysaccharide of *Streptococcus pneumoniae* type 14.

Results and discussion

For the preparation of the 6-aminohexyl glycosides of the tetra- (**18**) and penta- (**19**) saccharide, the acceptors for the enzymic galactosylation to obtain the corresponding spacered title penta- (**20**) and hexa- (**21**) saccharides, three building blocks were designed: the trisaccharide donor **13**, the monosaccharide acceptor **6**, and the disaccharide acceptor **11**. In the case of the acceptors, benzyl groups were introduced to increase the solubility of the generated protected tetra- and pentasaccharides in the generally applied organic solvents, which is of importance in the deprotection protocols.

The synthesis of monosaccharide acceptor **6** involved as the first step the coupling of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate (**1**)[12] to the spacer 6-azidohexan-1-ol using boron trifluoride–diethyl ether as a catalyst (Scheme 1). After deacetylation (⟶**2**, 37%), the formed product was isopropylidenated at O-3 and O-4 with 2,2-dimethoxypropane in the presence of a catalytic amount of camphorsulfonic acid to give **3** (42%).[13,14] Subsequent benzylation of the remaining two hydroxy groups in **3** with benzyl bromide in *N*,*N*-dimethylformamide using sodium hydride as base gave the fully protected derivative **4** (95%). Deisopropylidenation of **4** with aqueous trifluoroacetic acid in dichloromethane yielded **5** (99%), which was selectively acetylated at O-4 *via* orthoester formation using trimethyl orthoacetate and toluene-*p*-sulfonic acid, and subsequent ring opening with aqueous acetic acid (⟶**6**, 98%).[15]

For the synthesis of disaccharide acceptor **11**, (2,6-di-*O*-acetyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-(1⟶4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (**7**)[16] was coupled to the spacer 6-azidohexan-1-ol (Scheme 2)

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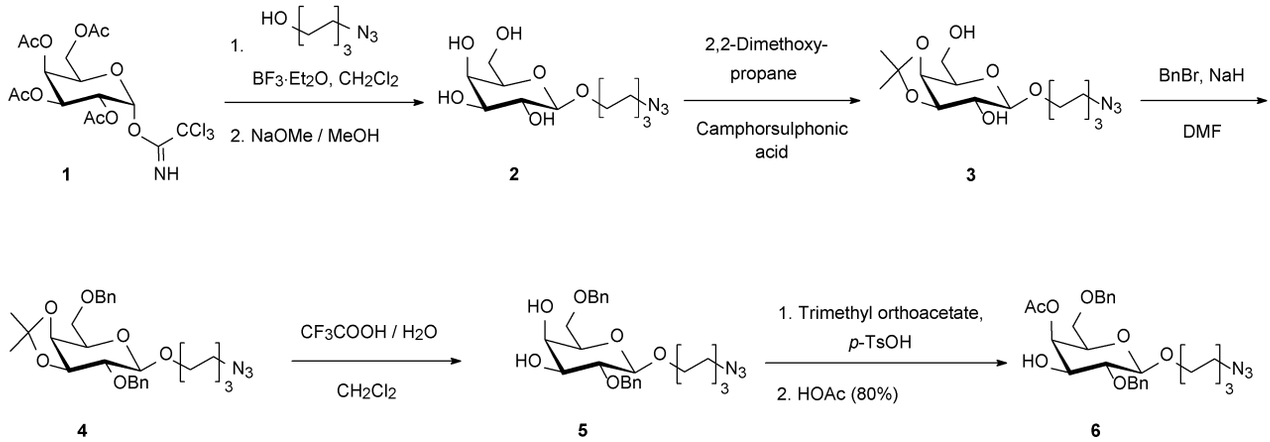
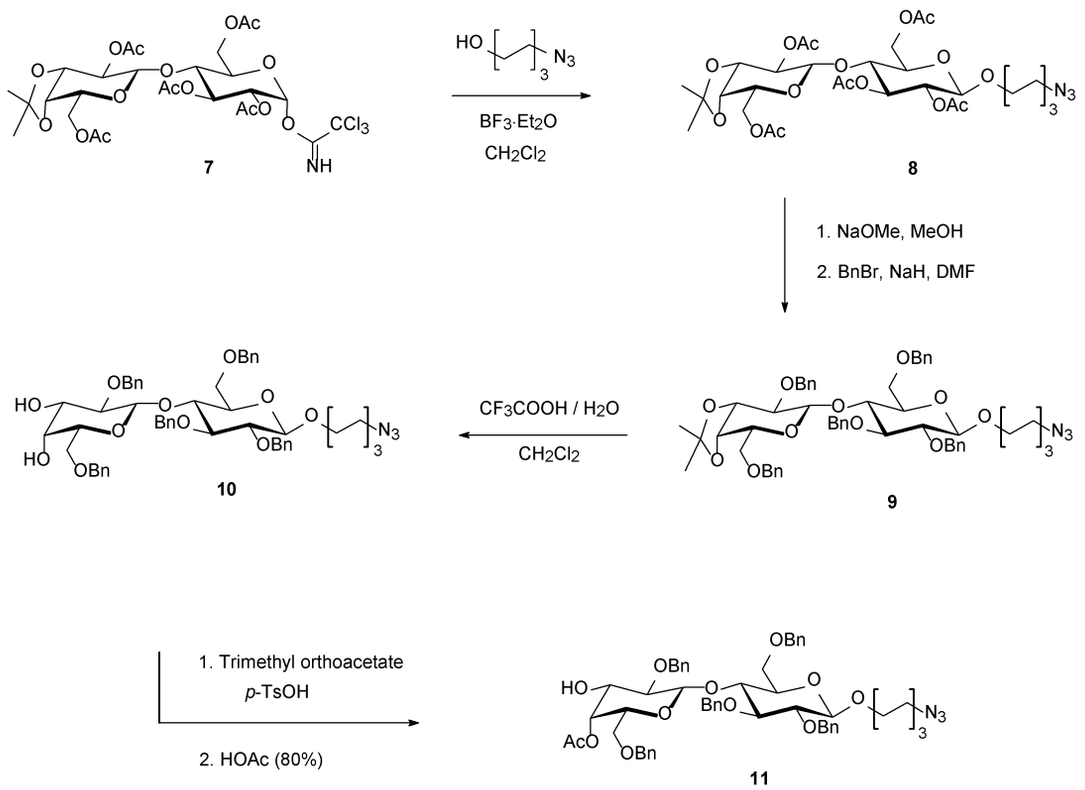


Scheme 1



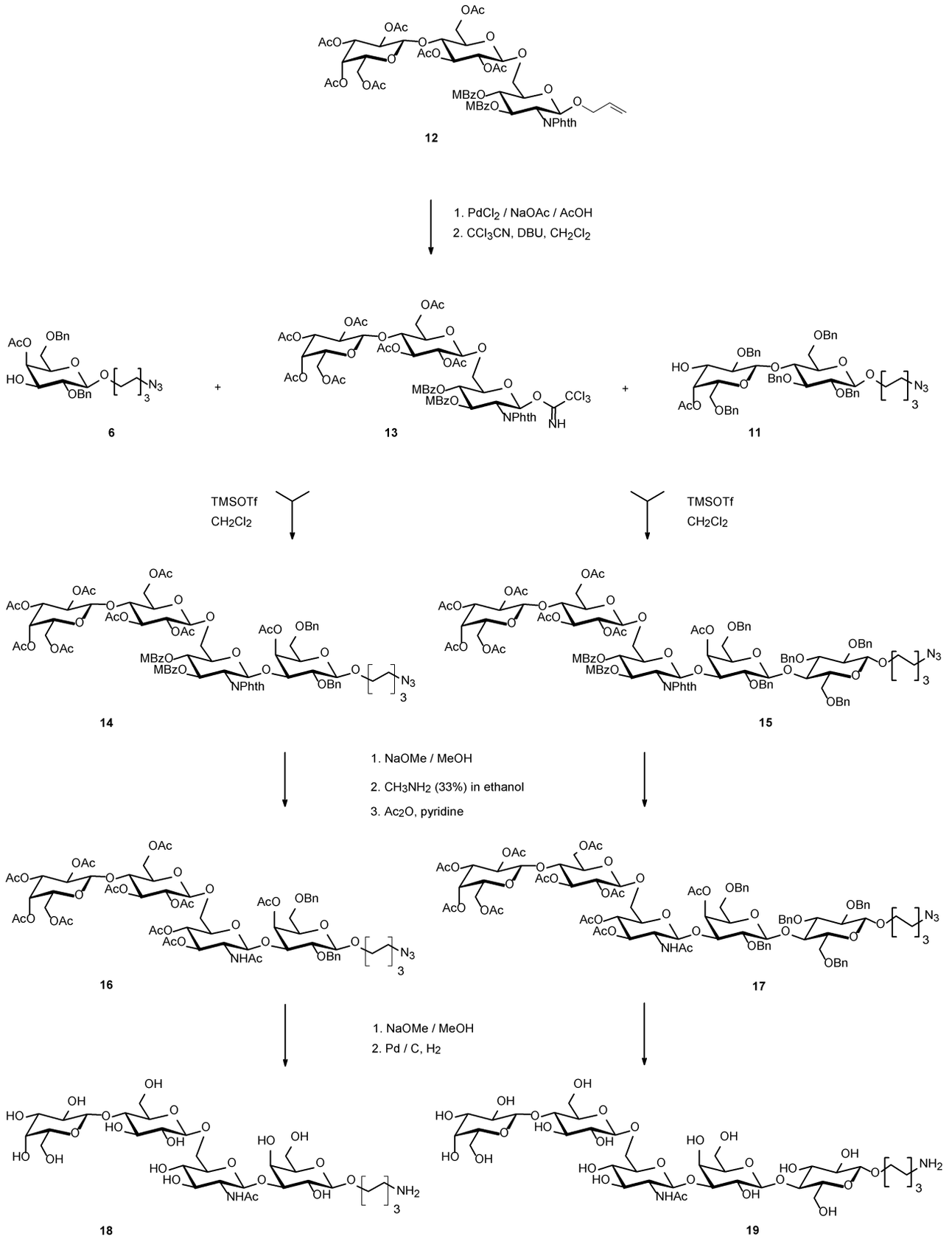
Scheme 2

using boron trifluoride–diethyl ether as a catalyst (⟶**8**, 36%). Deacetylation of **8** and subsequent benzylation with benzyl bromide in *N*,*N*-dimethylformamide using sodium hydride as base gave the fully protected derivative **9** (64%). Deisopropylidenation of **9** with aqueous trifluoroacetic acid in dichloromethane (⟶**10**, 90%), followed by selective acetylation at O-4 of the galactose residue as described above for **6**, gave **11** (95%).

Both the synthesis of tetrasaccharide **14** (leading to **18**) and pentasaccharide **15** (leading to **19**) required the trisaccharide donor **13**. The latter compound was obtained from allyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1⟶4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1⟶6)-2-deoxy-3,4-bis(*O*-*p*-methylbenzoyl)-2-phthalimido-β-D-glucopyranoside (**12**)[9] by subsequent deallylation with $PdCl_2$ and imidation with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene as a catalyst[17,18] (⟶**13**, 52%) (Scheme 3). Compound **14** was prepared by coupling of **13** with **6** in dichloromethane using trimethylsilyl trifluoromethanesulfonate as a catalyst (49%). Under similar conditions **15** was obtained by coupling of **13** with **11** (38%).

In the deprotection sequence the fully protected oligosaccharides **14** and **15** were deacylated and dephthaloylated (33% ethanolic methylamine) followed by re-*N*,*O*-acetylation to yield the *N*-acetyl protected derivatives **16** (65%) and **17** (57%), respectively. Complete conventional de-*O*-acetylation of both products required long reaction times (>24 h), as indicated by NMR spectroscopy. Finally, debenzylation (Pd/C, H_2, alkaline conditions, ammonia[19]) followed by hydrogenation of the azido group (Pd/C, H_2, weak acid conditions, acetic acid), and subsequent purification on Toyopearl HW-40S afforded **18** (96%) and **19** (82%), respectively. Verification of the formed products was carried out by high-resolution MS and NMR (2D ^{1}H COSY, TOCSY and ROESY) (Tables 1 and 2).

The β-1,4-galactosyltransferase-catalysed syntheses of the branched-chain oligosaccharides **20** and **21** was achieved by galactosyl transfer from UDP-galactose to O-4 of the *N*-acetyl-β-D-glucosamine residues of **18** and **19** (Scheme 4).[20–23] Alkaline phosphatase was added to the incubation mixtures to prevent feedback inhibition by released UDP[24,25] and to promote a high



Scheme 3

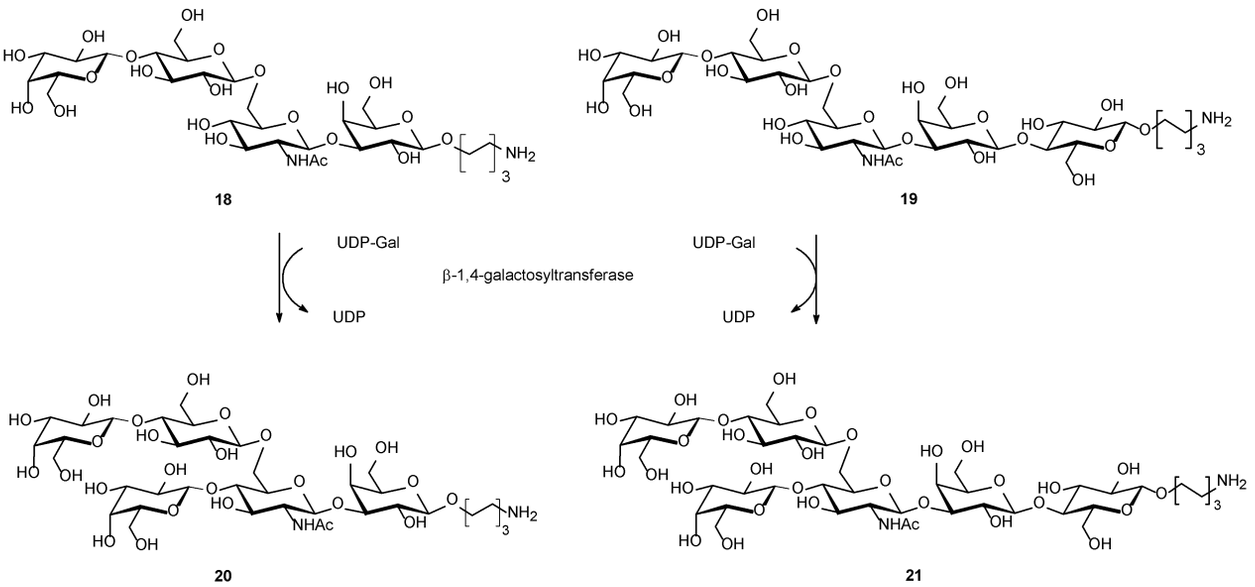
conversion of the acceptors. Final products were isolated after purification on Toyopearl HW-40S (⟶**20**, 80%; ⟶**21**, 96%). Verification of the formed products was carried out by high-resolution MS and NMR (2D ^{1}H COSY, TOCSY and ROESY) (Tables 3 and 4).

Conjugation of the oligosaccharides to CRM_{197} (cross-reactive material) and immunological studies are in progress.

Experimental

General procedures

Reactions were monitored by TLC on Silica Gel 60 F_{254} (Merck) with detection either by UV light or charring with either 10% H_2SO_4 in EtOH or 0.2% orcinol in 20% methanolic H_2SO_4. Solutions were concentrated under reduced pressure at



Scheme 4

Table 1 ^{1}H NMR data (COSY, TOCSY, ROESY) of compound **18**

	δ_H			
Proton	Gal a[a]	GlcNAc b	Glc c	Gal d[b]
H-1	4.37	4.70	4.54	4.45
H-2	3.55	3.75	3.36	3.55
H-3	3.70	3.56	3.66	3.67
H-4	4.14	3.56	3.66	3.93
H-5	n.d.[c]	3.61	3.56	n.d.
H-6	n.d.	4.21	3.99	n.d.
H-6′	n.d.	3.89	3.81	n.d.
$OCH_2(CH_2)_5NH_2$		3.92, 3.67		
CH_2NH_2		2.99		
$OCH_2CH_2(CH_2)_2CH_2CH_2NH_2$		1.70–1.60 (4 H)		
$O(CH_2)_2(CH_2)_2(CH_2)_2NH_2$		1.45–1.35 (4 H)		
$NHCOCH_3$		2.04		

[a] Gal a: Gal(β1-O$(CH_2)_6NH_2$). [b] Gal d: Gal(β1–4)Glc. [c] n.d., not determined.

Table 2 ^{1}H NMR data (COSY, TOCSY, ROESY) of compound **19**

	δ_H				
Proton	Glc a[a]	Gal b[b]	GlcNAc c	Glc d[c]	Gal e[d]
H-1	4.48	4.43	4.69	4.53	4.45
H-2	3.29	3.60	3.76	3.36	3.55
H-3	3.63	3.76	3.56	3.66	3.67
H-4	n.d.[e]	4.16	3.56	n.d.	3.93
H-5	n.d.	n.d.	3.61	n.d.	n.d.
H-6	3.97	n.d.	4.21	3.99	n.d.
H-6′	3.79	n.d.	3.88	3.81	n.d.
$OCH_2(CH_2)_5NH_2$			3.92, 3.68		
CH_2NH_2			2.99		
$OCH_2CH_2(CH_2)_2CH_2CH_2NH_2$			1.71–1.60 (4 H)		
$O(CH_2)_2(CH_2)_2(CH_2)_2NH_2$			1.46–1.36 (4 H)		
$NHCOCH_3$			2.04		

[a] Glc a: Glc(β1-O$(CH_2)_6NH_2$). [b] Gal b: Gal(β1–4)Glc(β1-O$(CH_2)_6NH_2$). [c] Glc d: Glc(β1–6)GlcNAc. [d] Gal e: Gal(β1–4)Glc(β1–6)GlcNAc. [e] n.d., not determined.

Table 3 ^{1}H NMR data (COSY, TOCSY, ROESY) of compound **20**

	δ_H				
Proton	Gal a[a]	GlcNAc b	Glc c	Gal d[b]	Gal e[c]
H-1	4.38	4.73	4.56	4.46	4.54
H-2	3.55	3.75	3.37	3.55	3.54
H-3	3.70	3.73	3.67	3.67	3.67
H-4	4.16	3.86	3.67	3.94	3.93
H-5	n.d.[d]	3.73	3.61	n.d.	n.d.
H-6	n.d.	4.28	3.99	n.d.	n.d.
H-6′	n.d.	3.96	3.82	n.d.	n.d.
$OCH_2(CH_2)_5NH_2$		3.92, 3.67			
CH_2NH_2		2.99			
$OCH_2CH_2(CH_2)_2CH_2CH_2NH_2$		1.70–1.60 (4 H)			
$O(CH_2)_2(CH_2)_2(CH_2)_2NH_2$		1.43–1.37 (4 H)			
$NHCOCH_3$		2.03			

[a] Gal a: Gal(β1-O$(CH_2)_6NH_2$). [b] Gal d: Gal(β1–4)Glc. [c] Gal e: Gal-(β1–4)GlcNAc. [d] n.d., not determined.

Table 4 ^{1}H NMR data (COSY, TOCSY, ROESY) of compound **21**

	δ_H					
Proton	Glc a[a]	Gal b[b]	GlcNAc c	Glc d[c]	Gal e[d]	Gal f[e]
H-1	4.48	4.43	4.70	4.56	4.45	4.54
H-2	3.29	3.58	3.72	3.37	3.54	3.54
H-3	3.63	3.72	3.81	3.67	3.67	3.66
H-4	3.62	4.12	3.88	n.d.	3.92	3.92
H-5	n.d.[f]	n.d.	n.d.	3.61	n.d.	n.d.
H-6	3.98	n.d.	4.28	3.99	n.d.	n.d.
H-6′	3.79	n.d.	3.96	3.82	n.d.	n.d.
$OCH_2(CH_2)_5NH_2$			3.93, 3.68			
CH_2NH_2			2.99			
$OCH_2CH_2(CH_2)_2CH_2CH_2NH_2$			1.71–1.60 (4 H)			
$O(CH_2)_2(CH_2)_2(CH_2)_2NH_2$			1.45–1.37 (4 H)			
$NHCOCH_3$			2.03			

[a] Glc a: Glc(β1-O$(CH_2)_6NH_2$). [b] Gal b: Gal(β1–4)Glc(β1-O-$(CH_2)_6NH_2$). [c] Glc d: Glc(β1–6)GlcNAc. [d] Gal e: Gal(β1–4)Glc(β1–6)-GlcNAc. [e] Gal f: Gal(β1–4)GlcNAc. [f] n.d., not determined.

40 °C. Column chromatography was performed on Silica Gel 60 (0.063–0.200 mm, Merck). Gel-permeation chromatography was performed on Toyopearl® HW-40S (Supelco) (2.0 × 100 cm). Optical rotations were measured with a Perkin-Elmer 241 polarimeter. $[\alpha]_D$-Values are given in 10^{-1} deg cm^2 g^{-1}. ^{1}H NMR spectra (300 MHz) were recorded with a Bruker AC 300

spectrometer; only selected NMR data are reported. 2D DQF ^{1}H–^{1}H COSY spectra, 2D TOCSY spectra with 7 ms and 100 ms mixing times, and 2D ^{1}H ROESY spectra (300 ms mixing times) were recorded at 300 K using a Bruker AMX 500 spectrometer. δ_H(ppm)-Values are given relative to the signal for internal Me_4Si (δ 0, $CDCl_3$) or acetone (δ 2.225, D_2O). *J*-Values are given in Hz. ^{13}C NMR spectra (75.5 or 125.7 MHz) were recorded with a Bruker AC 300 or AMX 500 spectrometer. δ_C(ppm)-Values are given relative to the signal for $CDCl_3$ (δ_C76.9) or internal acetone (δ_C 31.08). Elemental analyses were carried out with a CHNS-932 (Fa. Leco). Exact masses of final products were measured by nano ES-TOF MS using a Micromass LCTOF mass spectrometer at a resolution of 5000 FWHM. Gold-coated capillaries were loaded with 1 μl of sample (conc. 20 μM) dissolved in 1 : 1 acetonitrile–water with 0.1% formic acid. Pentaphenylalanine was added as internal standard. The capillary voltage was set at 1500 V and the cone voltage was set at 30 V.

6-Azidohexyl β-D-galactopyranoside 2

To a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate **1** [12] (1.9 g, 3.86 mmol) and 6-azidohexan-1-ol (1.08 g, 7.56 mmol) in dry CH_2Cl_2 (40 ml) was added powdered 4 Å molecular sieves (1 g), and the suspension was stirred for 2 h at room temperature. Then, at 0 °C $BF_3{\cdot}Et_2O$ (0.3 ml) was added. After stirring for 2 h at room temperature, the mixture was neutralised with Et_3N, diluted with CH_2Cl_2, filtered through Celite, and the filtrate was concentrated. Column chromatography (CH_2Cl_2–MeOH, 50 : 1) of the residue gave a mixture of the intermediate product (CH_2Cl_2–MeOH, 20 : 1, R_f 0.78) and 6-azidohexan-1-ol (R_f 0.44), which was dissolved in MeOH (60 ml). A solution of NaOMe (100 mg) in MeOH (10 ml) was added, and the mixture was stirred for 2.5 h at room temperature, then neutralised with Dowex 50W-X8 (H^+-form), filtered, and concentrated. Column chromatography (CH_2Cl_2–MeOH, 20 : 1) of the residue gave **2**, isolated as an amorphous solid (433 mg, 37%); TLC (CH_2Cl_2–MeOH, 5 : 1) R_f 0.37; $[\alpha]_D^{24}$ −23 (*c* 0.3, $CHCl_3$) (Found: C, 47.02; H, 7.38; N, 13.40. $C_{12}H_{23}N_3O_6$ requires C, 47.21; H, 7.59; N, 13.76%); δ_H(300 MHz; CD_3OD) 4.18 (1 H, d, $J_{1,2}$ 7.2, H-1), 3.87 (1 H, dt, OC*H*HCH_2), 3.80 (1 H, dd, $J_{3,4}$ 3.0, $J_{4,5}$ 0.8, H-4), 3.52 (1 H, dt, OCH*H*CH_2), 3.25 (2 H, t, CH_2N_3), 1.66–1.52 (4 H, m, $OCH_2CH_2(CH_2)_2CH_2$), 1.43–1.32 (4 H, m, $OCH_2CH_2(CH_2)_2CH_2$); δ_C(75.5 MHz; CD_3OD) 105.0 (C-1), 76.5, 75.0, 72.6 and 70.3 (C-2,-3,-4,-5), 70.5 (OCH_2CH_2), 62.4 (C-6), 52.4 (CH_2N_3), 30.6, 29.8, 27.6 and 26.6 [$CH_2(CH_2)_4CH_2N_3$].

6-Azidohexyl 3,4-*O*-isopropylidene-β-D-galactopyranoside 3

To a solution of **2** (433 mg, 1.25 mmol) in 2,2-dimethoxypropane (15 ml) and acetonitrile (15 ml) was added camphorsulfonic acid (20 mg), and the mixture was stirred for 48 h at room temperature. After neutralisation with Et_3N and co-concentration with toluene, a mixture of MeOH–H_2O (10 : 1) (50 ml) was added to the residue, and the solution was boiled under reflux for 3 h, then concentrated. Column chromatography (toluene–EtOAc, 1 : 1) of the residue gave **3**, isolated as a colourless syrup (206 mg, 42%); TLC (EtOAc) R_f 0.53; $[\alpha]_D^{22}$ +9 (*c* 1.1, $CHCl_3$) (Found: C, 52.36; H, 7.69; N, 11.98. $C_{15}H_{27}N_3O_6$ requires C, 52.16; H, 7.88; N, 12.17%); δ_H(300 MHz; $CDCl_3$) 4.18 (1 H, d, $J_{1,2}$ 8.2, H-1), 4.16 (1 H, dd, $J_{3,4}$ 5.5, $J_{4,5}$ 2.0, H-4), 4.10 (1 H, dd, $J_{2,3}$ 7.3, H-3), 3.93 (1 H, dt, OC*H*-HCH_2), 3.56 (1 H, dd, H-2), 3.51 (1 H, dt, OCH*H*CH_2), 3.27 (2 H, t, CH_2N_3), 2.48 and 2.18 (each 1 H, 2 br s, 2 OH), 1.68–1.55 (4 H, m, $OCH_2CH_2(CH_2)_2CH_2$), 1.47–1.34 (4 H, m, $OCH_2CH_2(CH_2)_2CH_2$), 1.52 and 1.35 (each 3 H, 2 s, 2 Me); δ_C(75.5 MHz; $CDCl_3$) 110.3 [$C(CH_3)_2$], 102.2 (C-1), 78.8, 73.8, 73.6 and 73.4 (C-2,-3,-4,-5), 69.7 (OCH_2CH_2), 62.3 (C-6), 51.2 (CH_2N_3), 29.3, 28.6, 26.3 and 25.4 [$CH_2(CH_2)_4CH_2N_3$], 28.0 and 26.2 [$C(CH_3)_2$].

6-Azidohexyl 2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranoside 4

To a solution of **3** (195 mg, 0.56 mmol) in DMF (10 ml) was added sodium hydride (80 mg, 80% suspension in oil). The mixture was stirred for 10 min, benzyl bromide (0.2 ml, 1.65 mmol) was added, and the stirring was continued for 2.5 h at room temperature. Then, the mixture was poured onto ice–water (300 ml). After extraction with CH_2Cl_2, the combined organic layers were washed with water, dried (Na_2SO_4), filtered, and concentrated. Column chromatography (toluene–EtOAc, 10 : 1) gave **4**, isolated as a colourless syrup (281 mg, 95%); TLC (toluene–EtOAc, 2 : 1) R_f 0.79; $[\alpha]_D^{22}$ +9 (*c* 1.1, $CHCl_3$) (Found: C, 66.52; H, 7.49; N, 7.72. $C_{29}H_{39}N_3O_6$ requires C, 66.27; H, 7.48; N, 7.99%); δ_H(300 MHz; $CDCl_3$) 7.40–7.20 (10 H, m, 2 Ph), 4.82 and 4.79 (each 1 H, 2 d, PhC*H*$_2$O), 4.63 and 4.56 (each 1 H, 2 d, PhC*H*$_2$O), 4.29 (1 H, d, $J_{1,2}$ 8.1, H-1), 3.94 (1 H, dt, OC*H*HCH_2), 3.89 (1 H, dd, $J_{3,4}$ 6.7, $J_{4,5}$ 1.4, H-4), 3.51 (1 H, dt, OCH*H*CH_2), 3.38 (1 H, m, H-5), 3.23 (2 H, t, CH_2N_3), 1.68–1.53 (4 H, m, $OCH_2CH_2(CH_2)_2CH_2$), 1.47–1.32 (4 H, m, $OCH_2CH_2(CH_2)_2CH_2$), 1.36 and 1.32 (each 3 H, 2 s, 2 Me); δ_C(75.5 MHz; $CDCl_3$) 138.3 and 138.1 (2 *i*-Ph), 128.3–127.3 (C-ar), 109.8 [$C(CH_3)_2$], 102.8 (C-1), 79.6, 79.0, 73.8 and 72.1 (C-2,-3,-4,-5), 73.5 (2 C) (2 PhCH_2O), 69.5 (2 C) (C-6, OCH_2CH_2), 51.3 (CH_2N_3), 29.5, 28.7, 26.4 and 25.6 [$CH_2(CH_2)_4CH_2N_3$], 27.7 and 26.3 [$C(CH_3)_2$].

6-Azidohexyl 2,6-di-*O*-benzyl-β-D-galactopyranoside 5

To a cooled (0 °C) solution of **4** (281 mg, 0.53 mmol) in CH_2Cl_2 (15 ml) was added 90% aq. trifluoroacetic acid (1 ml). The mixture was stirred for 1.5 h at 0 °C, then diluted with CH_2Cl_2, and washed with cold water, saturated aq. Na_2CO_3, and water. The organic layer was dried (Na_2SO_4), filtered, and concentrated. Column chromatography (toluene–EtOAc, 2 : 1) of the residue gave **5**, isolated as a colourless syrup (257 mg, 99%); TLC (toluene–EtOAc, 2 : 1) R_f 0.29; $[\alpha]_D^{24}$ +3 (*c* 0.5, $CHCl_3$) (Found: C, 64.53; H, 7.24; N, 8.44. $C_{26}H_{35}N_3O_6$ requires C, 64.31; H, 7.27; N, 8.65%); δ_H(300 MHz; $CDCl_3$) 7.37–7.25 (10 H, m, 2 Ph), 4.96 and 4.68 (each 1 H, 2 d, PhC*H*$_2$O), 4.59 (2 H, s, PhC*H*$_2$O), 4.36 (1 H, d, $J_{1,2}$ 7.5, H-1), 3.99 (1 H, m, H-4), 3.96 (1 H, dt, OC*H*HCH_2), 3.23 (2 H, t, CH_2N_3), 2.58 and 2.52 (each 1 H, 2 d, 2 OH), 1.69–1.53 (4 H, m, $OCH_2CH_2(CH_2)_2CH_2$), 1.46–1.35 (4 H, m, $OCH_2CH_2(CH_2)_2CH_2$); δ_C(75.5 MHz; $CDCl_3$) 138.4, 137.9, 128.5 (4 C), 128.0 (2 C), 127.9, 127.8 and 127.7 (2 C) (2 Ph), 103.7 (C-1), 79.1, 74.6, 73.7, 73.3, 73.2, 69.7, 69.4 and 69.0 (C-2,-3,-4,-5,-6, OCH_2CH_2, 2 PhCH_2O), 51.4 (CH_2N_3), 29.6, 28.8, 26.5 and 25.8 [$CH_2(CH_2)_4CH_2N_3$].

6-Azidohexyl 4-*O*-acetyl-2,6-di-*O*-benzyl-β-D-galactopyranoside 6

To a solution of **5** (190 mg, 0.39 mmol) in acetonitrile (5 ml) were added trimethyl orthoacetate (0.2 ml) and toluene-*p*-sulfonic acid monohydrate in catalytic amounts, and the mixture was stirred for 30 min. Then, 80% aq. HOAc (6.0 ml) was added and stirring was continued for another 30 min. The mixture was diluted with CH_2Cl_2 and washed with cold water, saturated aq. Na_2CO_3, and water. The organic layer was dried (Na_2SO_4), filtered, and concentrated. Column chromatography (toluene–EtOAc, 2 : 1) gave **6**, isolated as a colourless syrup (202 mg, 98%); TLC (toluene–EtOAc, 2 : 1) R_f 0.50; $[\alpha]_D^{22}$ −4 (*c* 1.2, $CHCl_3$) (Found: C, 63.94; H, 7.04; N, 7.79. $C_{28}H_{37}N_3O_7$ requires C, 63.74; H, 7.07; N, 7.96%); δ_H(300 MHz; $CDCl_3$) 7.37–7.25 (10 H, m, 2 Ph), 5.40 (1 H, dd, $J_{3,4}$ 3.5, $J_{4,5}$ 1.0, H-4), 4.96 and 4.67 (2 H, 2 d, PhC*H*$_2$O), 4.54 and 4.47 (2 H, 2 d, PhC*H*$_2$O), 4.39 (1 H, d, $J_{1,2}$ 7.7, H-1), 3.97 (1 H, dt, OC*H*-HCH_2), 3.22 (2 H, t, CH_2N_3), 2.32 (1 H, d, OH), 2.06 (3 H, s, Ac), 1.69–1.52 (4 H, m, $OCH_2CH_2(CH_2)_2CH_2$), 1.48–1.35 (4 H, m, $OCH_2CH_2(CH_2)_2CH_2$); δ_C(75.5 MHz; $CDCl_3$) 170.9

($COCH_3$), 138.3 and 137.8 (2 *i*-Ph), 128.5–127.7 (C-ar), 103.7 (C-1), 79.2, 72.5, 72.0 and 69.6 (C-2,-3,-4,-5), 74.7 and 73.6 (2 PhCH_2O), 70.1 (OCH_2CH_2), 68.3 (C-6), 51.3 (CH_2N_3), 29.5, 28.7, 26.5 and 25.7 [$CH_2(CH_2)_4CH_2N_3$], 20.8 ($COCH_3$).

6-Azidohexyl (2,6-di-*O*-acetyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-(1⟶4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside 8

To a solution of (2,6-di-*O*-acetyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-(1⟶4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate **7**[16] (2.5 g, 3.4 mmol) and 6-azidohexan-1-ol (1.02 g, 7.1 mmol) in dry CH_2Cl_2 (50 ml) was added powdered 4 Å molecular sieves (2 g). After stirring for 2 h at room temperature, $BF_3{\cdot}Et_2O$ (0.25 ml) was added to the suspension at 0 °C, and stirring was continued for another 2 h at room temperature. Then, the mixture was neutralised with Et_3N, diluted with CH_2Cl_2, filtered through Celite, and concentrated. Column chromatography (toluene–EtOAc, 3 : 1) of the residue gave **8**, isolated as a colourless syrup (872 mg, 36%); TLC (toluene–EtOAc, 2 : 1) R_f 0.20; $[\alpha]_D^{20}$ +8 (*c* 1, $CHCl_3$) (Found: C, 52.06; H, 6.53; N, 6.06. $C_{31}H_{47}N_3O_{16}$ requires C, 51.88; H, 6.60; N, 5.85%); δ_H(300 MHz; $CDCl_3$) 5.18 (1 H, t, $J_{2a,3a}$ 9.6, $J_{3a,4a}$ 9.6, H-3a), 4.89 (1 H, dd, $J_{1a,2a}$ 8.0, H-2a), 4.85 (1 H, dd, $J_{1b,2b}$ 7.4, $J_{2b,3b}$ 6.3, H-2b), 4.44 (1 H, d, H-1a), 4.35 (1 H, d, H-1b), 3.93 (1 H, m, H-5b), 3.83 (1 H, dt, $OCHHCH_2$), 3.74 (1 H, t, H-4a), 3.60 (1 H, ddd, $J_{4a,5a}$ 9.9, $J_{5a,6a}$ 1.9, $J_{5a,6a'}$ 5.0, H-5a), 3.46 (1 H, dt, $OCHHCH_2$), 3.25 (2 H, t, CH_2N_3), 2.12, 2.10, 2.06, 2.04 and 2.03 (each 3 H, 5 s, 5 Ac), 1.67–1.53 (4 H, m, $OCH_2CH_2(CH_2)_2CH_2$), 1.43–1.32 (4 H, m, $OCH_2CH_2(CH_2)_2CH_2$), 1.53 and 1.31 (each 3 H, 2 s, 2 Me); δ_C(75.5 MHz; $CDCl_3$) 170.6, 170.3, 169.9, 169.4 and 169.0 ($COCH_3$), 110.7 [$C(CH_3)_2$], 100.5 and 100.4 (C-1a,-1b), 76.7, 76.0, 72.9, 72.7 (2 C), 72.3, 71.6 and 70.8 (C-2a,-2b,-3a,-3b,-4a,-4b,-5a,-5b), 69.7 (OCH_2CH_2), 63.0 and 62.1 (C-6a,-6b), 51.2 (CH_2N_3), 29.1, 28.6, 26.2 and 25.3 [$CH_2(CH_2)_4CH_2N_3$], 27.2 and 26.0 [$C(CH_3)_2$].

6-Azidohexyl (2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-(1⟶4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside 9

A solution of **8** (770 mg, 1.07 mmol) in MeOH (70 ml) was stirred with NaOMe at pH 8–9 for 24 h at room temperature. Then, the solution was neutralised with Dowex 50W-X8 (H^+ form), filtered, and concentrated. Column chromatography (CH_2Cl_2–MeOH, 5 : 1) of the residue gave an amorphous solid (530 mg) that was dissolved in DMF (25 ml). Sodium hydride (171 mg, 80% suspension in oil) was added, and the mixture was stirred for 30 min at room temperature. After adding benzyl bromide (0.8 ml, 6.6 mmol), the mixture was stirred overnight, then poured onto ice–water. The organic layer was separated and the water phase was extracted (3×) with CH_2Cl_2. The combined organic layers were collected, washed with water, dried (Na_2SO_4), filtered, and concentrated. Column chromatography (toluene–EtOAc, 10 : 1) of the residue gave **9**, isolated as a colourless syrup (657 mg, 64%); TLC (toluene–EtOAc, 10 : 1) R_f 0.40; $[\alpha]_D^{20}$ +15 (*c* 1, $CHCl_3$) (Found: C, 70.49; H, 7.04; N, 4.39. $C_{56}H_{67}N_3O_{11}$ requires C, 70.20; H, 7.05; N, 4.39%); δ_H(300 MHz; $CDCl_3$) 7.40–7.19 (25 H, m, 5 Ph), 4.93 and 4.74 (each 1 H, 2 d, PhCH_2O), 4.87 and 4.71 (each 1 H, 2 d, PhCH_2O), 4.79 and 4.66 (each 1 H, 2 d, PhCH_2O), 4.57 and 4.42 (each 1 H, 2 d, PhCH_2O), 4.50 and 4.31 (each 1 H, 2 d, PhCH_2O), 4.41 and 4.37 (each 1 H, 2 d, $J_{1a,2a/1b,2b}$ 8.5/7.7, H-1a,-1b), 4.10 (1 H, dd, $J_{3b,4b}$ 5.6, $J_{4b,5b}$ 1.5, H-4b), 4.03 (1 H, t, $J_{2b,3b}$ 5.9, H-3b), 3.92 and 3.51 (each 1 H, 2 dt, OCH_2CH_2), 3.21 (2 H, t, CH_2N_3), 1.68–1.52 (4 H, m, $OCH_2CH_2(CH_2)_2CH_2$), 1.47–1.36 (4 H, m, $OCH_2CH_2(CH_2)_2CH_2$), 1.40 and 1.34 (each 3 H, 2 s, 2 Me); δ_C(75.5 MHz; $CDCl_3$) 138.9, 138.6, 138.5, 138.4 and 138.2 (5 *i*-Ph), 128.2–127.1 (C-ar), 109.6 [$C(CH_3)_2$], 103.5 and 101.8 (C-1a,-1b), 82.9, 81.8, 80.6, 79.3, 76.3, 75.0, 73.5 and 71.9 (C-2a,-2b,-3a,-3b,-4a,-4b,-5a,-5b), 75.3, 74.8, 73.3 and 73.1 (2 C) (5 PhCH_2O), 69.6 (OCH_2CH_2), 68.8 and 68.2 (C-6a,-6b), 51.2 (CH_2N_3), 29.5, 28.7, 26.4 and 25.6 [$CH_2(CH_2)_4CH_2N_3$], 27.8 and 26.3 [$C(CH_3)_2$].

6-Azidohexyl (2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1⟶4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside 10

To a cooled (0 °C) solution of **9** (576 mg, 0.6 mmol) in CH_2Cl_2 (15 ml) was added 90% aq. trifluoroacetic acid (1 ml). The mixture was stirred for 1.5 h at 0 °C, then diluted with CH_2Cl_2 and washed with cold water, saturated aq. Na_2CO_3, and water. The organic layer was dried (Na_2SO_4), filtered, and concentrated. Column chromatography (toluene–EtOAc, 3 : 1) of the residue gave **10**, isolated as a colourless syrup (497 mg, 90%); TLC (toluene–EtOAc, 2 : 1) R_f 0.40; $[\alpha]_D^{20}$ +15 (*c* 1, $CHCl_3$) (Found: C, 69.47; H, 6.70; N, 4.69. $C_{53}H_{63}N_3O_{11}$ requires C, 69.34; H, 6.92; N, 4.58%); δ_H(300 MHz; $CDCl_3$) 7.40–7.19 (25 H, m, 5 Ph), 4.99–4.57 (7 H, m, 3 PhCH_2O, PhCHHO), 3.20 (2 H, t, CH_2N_3), 2.49 and 2.43 (each 1 H, 2 d, 2 OH), 1.70–1.50 (4 H, m, $OCH_2CH_2(CH_2)_2CH_2$), 1.46–1.32 (4 H, m, $OCH_2CH_2(CH_2)_2CH_2$); δ_C(75.5 MHz; $CDCl_3$) 139.1, 138.6, 138.3, 138.1 and 137.9 (5 *i*-Ph), 128.4–127.1 (C-ar), 103.5 and 102.4 (C-1a,-1b), 82.7, 81.7, 79.9, 76.5, 75.0, 73.4, 72.8 and 68.6 (C-2a,-2b,-3a,-3b,-4a,-4b,-5a,-5b), 75.1, 74.7 (2 C), 73.3 and 73.1 (5 PhCH_2O), 69.6 (OCH_2CH_2), 68.7 and 68.2 (C-6a,-6b), 51.2 (CH_2N_3), 29.5, 28.6, 26.4 and 25.6 [$CH_2(CH_2)_4CH_2N_3$].

6-Azidohexyl (4-*O*-acetyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1⟶4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside 11

To a solution of **10** (390 mg, 0.42 mmol) in acetonitrile (5 ml) were added trimethyl orthoacetate (0.2 ml) and toluene-*p*-sulfonic acid monohydrate (catalytic amounts), and the mixture was stirred for 30 min. Then, 80% aq. HOAc (6.6 ml) was added, and stirring was continued for 30 min. After dilution with CH_2Cl_2, the mixture was washed with cold water, saturated aq. Na_2CO_3, and water. The organic layer was dried (Na_2SO_4), filtered, and concentrated. Column chromatography (toluene–EtOAc, 2 : 1) of the residue gave **11**, isolated as a colourless syrup (389 mg, 95%); TLC (toluene–EtOAc, 2 : 1) R_f 0.51; $[\alpha]_D^{20}$ −4 (*c* 1, $CHCl_3$) (Found: C, 68.74; H, 6.68; N, 4.39. $C_{55}H_{65}N_3O_{12}$ requires C, 68.80; H, 6.82; N, 4.38%); δ_H(300 MHz; $CDCl_3$) 7.40–7.15 (25 H, m, 5 Ph), 5.33 (1 H, d, $J_{3b,4b}$ 3.3, $J_{4b,5b}$ 0, H-4b), 4.98 and 4.76 (each 1 H, 2 d, PhCH_2O), 4.85 and 4.73 (each 1 H, 2 d, PhCH_2O), 4.80 and 4.67 (each 1 H, 2 d, PhCH_2O), 4.60 and 4.42 (each 1 H, 2 d, PhCH_2O), 4.45 and 4.24 (each 1 H, 2 d, PhCH_2O), 4.47 and 4.36 (each 1 H, 2 d, $J_{1a,2a/1b,2b}$ 7.7/8.0, H-1a,-1b), 3.21 (2 H, t, CH_2N_3), 2.29 (1 H, d, OH), 2.02 (3 H, s, Ac), 1.70–1.51 (4 H, m, $OCH_2CH_2(CH_2)_2CH_2$), 1.48–1.30 (4 H, m, $OCH_2CH_2(CH_2)_2CH_2$); δ_C(75.5 MHz; $CDCl_3$) 170.8 ($COCH_3$), 139.0, 138.6, 138.2, 138.1 and 137.8 (5 *i*-Ph), 128.2–127.2 (C-ar), 103.5 and 102.2 (C-1a,-1b), 82.6, 81.6, 80.0, 76.3, 75.0, 72.3, 71.9 and 69.5 (C-2a,-2b,-3a,-3b,-4a,-4b,-5a,-5b), 75.1, 74.9, 74.7, 73.3 and 73.1 (5 PhCH_2O), 69.6 (OCH_2CH_2), 68.1 and 67.1 (C-6a,-6b), 51.2 (CH_2N_3), 29.5, 28.6, 26.4 and 25.6 [$CH_2(CH_2)_4CH_2N_3$], 20.6 ($COCH_3$).

(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1⟶4)-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1⟶6)-2-deoxy-3,4-bis-(*O*-*p*-methylbenzoyl)-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate 13

To a solution of allyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1⟶4)-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1⟶6)-2-deoxy-3,4-bis(*O*-*p*-methylbenzoyl)-2-phthalimido-β-D-glucopyranoside (**12**)[9] (3.47 g, 2.9 mmol) in 96% aq. HOAc (75 ml) were added $PdCl_2$ (2.3 g) and NaOAc (2.0 g), and the mixture was sonicated in an ultrasonic bath for 24 h at room temperature. After filtration through Celite, CH_2Cl_2 was added

to the filtrate, and the organic layer was washed with cold water, dried (Na_2SO_4), filtered, and concentrated. Column chromatography (toluene–EtOAc, 2 : 1) of the residue gave a colourless syrup (2.93 g) that was dissolved in CH_2Cl_2 (40 ml), and trichloroacetonitrile (0.92 ml, 9.15 mmol) was added. Then, at 0 °C 1,8-diazabicyclo[5.4.0]undec-7-ene (100 µl) was added, and the mixture was stirred for 5 h at room temperature. After concentration, column chromatography (toluene–EtOAc, 2 : 1) of the residue gave **13**, isolated as an amorphous solid (1.70 g, 52%); TLC (toluene–EtOAc, 1 : 1) R_f 0.51; $[\alpha]_D^{20}$ +11 (*c* 1, $CHCl_3$) (Found: C, 53.38; H, 4.79. $C_{58}H_{61}Cl_3N_2O_{26}$ requires C, 53.24; H, 4.70%); δ_H(300 MHz; $CDCl_3$) 8.78 (1 H, s, OCN*H*CCl_3), 7.81–7.05 (12 H, m, 2 $COC_6H_4CH_3$ and Phth), 6.78 (1 H, d, $J_{1a,2a}$ 8.8, H-1a), 6.28 (1 H, dd, $J_{2a,3a}$ 9.3, $J_{3a,4a}$ 9.3, H-3a), 5.49 (1 H, t, $J_{4a,5a}$ 9.3, H-4a), 5.35 (1 H, d, $J_{3c,4c}$ 3.1, $J_{4c,5c}$ 0, H-4c), 4.69 (1 H, d, $J_{1b,2b}$ 7.6, H-1b), 4.47 (1 H, d, $J_{1c,2c}$ 7.9, H-1c), 2.36 and 2.29 (each 3 H, 2 s, 2 $COC_6H_4CH_3$), 2.17–1.63 (21 H, m, 7 Ac); δ_C(75.5 MHz; $CDCl_3$) 170.3–168.9 (*C*OCH_3), 165.4 and 165.1 (2 *C*$OC_6H_4CH_3$), 160.3 (O*C*NH-CCl_3), 101.0 and 99.9 (C-1b,-1c), 93.5 (C-1a), 68.2, 61.2, 60.7 (C-6a,-6b,-6c), 55.3 (C-2a), 21.5–20.5 ($COC_6H_4CH_3$ and $COCH_3$).

6-Azidohexyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-(2-deoxy-3,4-bis(*O*-*p*-methylbenzoyl)-2-phthalimido-β-D-glucopyranosyl)-(1→3)-4-*O*-acetyl-2,6-di-*O*-benzyl-β-D-galactopyranoside 14

To a solution of **13** (325 mg, 0.25 mmol) and **6** (128 mg, 0.24 mmol) in dry CH_2Cl_2 (10 ml) was added powdered 4 Å molecular sieves (500 mg), and the suspension was stirred for 2 h at room temperature. Then, at 0 °C trimethylsilyl trifluoromethanesulfonate (50 µl, 0.27 mmol) was added, and the mixture was stirred for 2 h at room temperature, neutralised with Et_3N (40 µl, 0.29 mmol), diluted with CH_2Cl_2, filtered through Celite, and the filtrate was concentrated. Column chromatography (toluene–EtOAc, 3 : 1) of the residue gave **14**, isolated as an amorphous solid (201 mg, 49%); TLC (toluene–EtOAc, 2 : 1) R_f 0.42; $[\alpha]_D^{23}$ −2 (*c* 1, $CHCl_3$) (Found: C, 60.05; H, 5.69; N, 3.27. $C_{84}H_{96}N_4O_{32}$ requires C, 60.28; H, 5.78; N, 3.35%); δ_H(300 MHz; $CDCl_3$) 7.78 and 7.61 (each 2 H, 2 m, $COC_6H_4CH_3$), 7.52 (4 H, br s, Phth), 7.35–7.22 and 7.08 (8 and 2 H, 2 m, 2 Ph), 7.15 and 7.04 (each 2 H, 2 m, $COC_6H_4CH_3$), 6.24 (1 H, dd, $J_{2b,3b}$ 10.8, $J_{3b,4b}$ 9.1, H-3b), 5.67 (1 H, d, $J_{1b,2b}$ 8.2, H-1b), 5.54 (1 H, dd, $J_{3a,4a}$ 3.8, $J_{4a,5a}$ 1.0, H-4a), 5.36 (1 H, dd, $J_{4b,5b}$ 10.2, H-4b), 5.33 (1 H, dd, $J_{3d,4d}$ 3.4, $J_{4d,5d}$ 1.2, H-4d), 5.24 (1 H, dd, $J_{2c,3c}$ 9.5, $J_{3c,4c}$ 9.0, H-3c), 5.07 (1 H, dd, $J_{2d,3d}$ 10.4, $J_{1d,2d}$ 7.7, H-2d), 4.92 (1 H, dd, $J_{3d,4d}$ 3.4, H-3d), 4.89 (1 H, dd, $J_{1c,2c}$ 7.8, H-2c), 4.69 (1 H, d, H-1c), 4.54 and 4.37 (each 1 H, 2 d, PhCH_2O), 4.53 and 4.05 (each 1 H, 2 d, PhCH_2O), 4.46 (1 H, d, H-1d), 4.35 (1 H, d, $J_{1a,2a}$ 7.8, H-1a), 4.42 (1 H, dd, $J_{2b,3b}$ 10.8, H-2b), 3.98 (1 H, dd, $J_{2a,3a}$ 9.7, H-3a), 3.74 (1 H, dd, $J_{4c,5c}$ 9.9, H-4c), 3.35 (1 H, dd, $J_{2a,3a}$ 9.7, H-2a), 3.10 (2 H, t, CH_2N_3), 2.33 and 2.26 (each 3 H, 2 s, 2 $COC_6H_4CH_3$), 2.11, 2.06, 2.05, 2.04, 2.03, 2.02, 1.93 and 1.91 (each 3 H, 8 s, 8 Ac), 1.55–1.35 (4 H, m, OCH_2CH_2-$(CH_2)_2CH_2$), 1.30–1.20 (4 H, m, $OCH_2CH_2(CH_2)_2CH_2$); δ_C(75.5 MHz; $CDCl_3$) 170.3 (2 C), 170.1, 170.0 (2 C), 169.8, 169.5 and 169.0 (8 *C*OCH_3), 167.7 (br s, *C*OPhth), 165.5 and 165.2 (2 *C*$OC_6H_4CH_3$), 103.6 (C-1a), 101.2 (C-1d), 100.9 (C-1c), 98.4 (C-1b), 78.6 (C-2a), 77.4 (C-3a), 76.4 (C-4c), 74.6 (C-5b), 74.0 and 73.3 (2 Ph*C*H_2O), 72.8 (C-3c), 72.3 (C-5c), 72.1 (C-2c), 71.8 (C-5a), 71.1 (C-3d), 70.6 (C-5d), 70.4 (C-3b), 69.9 (2 C) (C-4b, O*C*H_2CH_2), 69.7 (C-4a), 69.0 (C-2d), 68.2 (C-6b), 67.6 (C-6a), 66.7 (C-4d), 62.1 (C-6c), 60.7 (C-6d), 55.3 (C-2b), 51.2 (CH_2N_3), 29.4 [$CH_2CH_2(CH_2)_4N_3$], 28.6 [$CH_2(CH_2)_3$-$CH_2CH_2N_3$], 26.4 and 25.6 [$CH_2CH_2(CH_2)_2CH_2CH_2N_3$], 21.6 and 21.5 (2 $COC_6H_4CH_3$), 20.9, 20.8, 20.7 (2 C), 20.6 (2 C) and 20.4 (2 C) (8 $COCH_3$).

6-Azidohexyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-(2-deoxy-3,4-bis(*O*-*p*-methylbenzoyl)-2-phthalimido-β-D-glucopyranosyl)-(1→3)-(4-*O*-acetyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside 15

To a solution of **13** (327 mg, 0.25 mmol) and **11** (302 mg, 0.31 mmol) in dry CH_2Cl_2 (10 ml) was added powdered 4 Å molecular sieves (500 mg), and the suspension was stirred for 1 h at room temperature. Then, at 0 °C trimethylsilyl trifluoromethanesulfonate (63 µl, 0.35 mmol) was added, and the mixture was stirred for 2 h at room temperature, neutralised with Et_3N, diluted with CH_2Cl_2, filtered through Celite, and the filtrate was concentrated. Column chromatography (toluene–EtOAc, 5 : 1) of the residue gave **15**, isolated as a white crystalline powder (202 mg, 38%); Mp 93–94 °C; TLC (toluene–EtOAc, 2 : 1) R_f 0.47; $[\alpha]_D^{22}$ −0.4 (*c* 1, $CHCl_3$) (Found: C, 63.14; H, 5.92; N, 2.73. $C_{111}H_{124}N_4O_{37}$ requires C, 63.30; H, 5.93; N, 2.66%); δ_H(300 MHz; $CDCl_3$) 7.79 and 7.60 (each 2 H, 2 m, $COC_6H_4CH_3$), 7.47 (4 H, br s, Phth), 7.37–6.96 (29 H, m, 5 Ph, $COC_6H_4CH_3$), 6.25 (1 H, dd, $J_{2c,3c}$ 10.8, $J_{3c,4c}$ 9.1, H-3c), 5.66 (1 H, d, $J_{1c,2c}$ 8.2, H-1c), 5.56 (1 H, dd, $J_{3b,4b}$ 3.6, $J_{4b,5b}$ 0.8, H-4b), 5.40 (1 H, dd, $J_{4c,5c}$ 10.2, H-4c), 5.32 (1 H, dd, $J_{3e,4e}$ 3.5, $J_{4e,5e}$ 1.2, H-4e), 5.23 (1 H, dd, $J_{2d,3d}$ 9.5, $J_{3d,4d}$ 9.0, H-3d), 5.07 (1 H, dd, $J_{2e,3e}$ 10.7, $J_{1e,2e}$ 7.8, H-2e), 4.91 (1 H, dd, $J_{3e,4e}$ 3.5, H-3e), 4.88 (1 H, dd, $J_{1d,2d}$ 7.9, H-2d), 4.87 and 4.62 (each 1 H, 2 d, PhCH_2O), 4.83 and 4.68 (each 1 H, 2 d, PhCH_2O), 4.65 (d, 1 H, H-1d), 4.52–4.40 (6 H, m, 3 PhCH_2O), 4.43 (1 H, dd, H-2c), 4.42 (2 H, m, H-1a,-1e), 4.25 (1 H, d, $J_{1b,2b}$ 7.5, H-1b), 4.02 (1 H, H-5c), 3.92 (1 H, H-3b), 3.87 (1 H, H-5b), 3.85 and 3.47 (each 1 H, OCH_2CH_2), 3.84 (1 H, H-5e), 3.82 (1 H, H-4a), 3.69 (1 H, dd, $J_{4d,5d}$ 9.8, H-4d), 3.55 (1 H, H-5d), 3.41 (1 H, H-3a), 3.34 (1 H, H-2a), 3.31 (1 H, H-2b), 3.19 (2 H, t, CH_2N_3), 3.15 (1 H, H-5a), 2.34 and 2.26 (each 3 H, 2 s, 2 $COC_6H_4CH_3$), 2.13, 2.04, 2.03, 2.01, 1.94 and 1.90 (3,6,3,3,6,3 H, 6 s, 8 Ac), 1.61–1.47 (4 H, m, $OCH_2CH_2(CH_2)_2CH_2$), 1.40–1.30 (4 H, m, $OCH_2CH_2(CH_2)_2CH_2$); δ_C(75.5 MHz; $CDCl_3$) 170.3, 170.2, 170.1, 169.9 (2 C), 169.6, 169.4 and 168.9 (8 *C*OCH_3), 167.2 (br s, *C*OPhth), 165.5 and 165.2 (2 *C*$OC_6H_4CH_3$), 103.5 (C-1b), 102.2 (C-1a), 101.1 (C-1e), 101.0 (C-1d), 98.5 (C-1c), 82.6 (C-3a), 81.7 (C-2b), 79.1 (C-2a), 78.0 (C-3b), 76.4 (C-4d), 76.3 (C-4a), 75.2 (Ph*C*H_2O), 75.1 (C-5a), 74.9 (Ph*C*H_2O), 74.5 (C-5c), 74.5, 73.1 and 73.0 (3 Ph*C*H_2O), 72.7 (C-3d), 72.3 (C-5d), 72.0 (C-2d), 71.8 (C-5b), 71.1 (C-3e), 70.6 (C-5e), 70.4 (C-3c), 69.9 (C-4c), 69.8 (C-4b), 69.6 (O*C*H_2CH_2), 69.1 (C-2e), 68.4 (C-6c), 68.0 (C-6a), 67.2 (C-6b), 66.6 (C-4e), 62.1 (C-6d), 60.7 (C-6e), 55.3 (C-2c), 51.3 (CH_2N_3), 29.6 [CH_2CH_2-$(CH_2)_4N_3$], 28.7 [$CH_2(CH_2)_3CH_2CH_2N_3$], 26.5 and 25.7 [$CH_2CH_2(CH_2)_2CH_2CH_2N_3$], 21.6 and 21.5 (2 $COC_6H_4CH_3$), 20.8, 20.7 (3 C), 20.6, 20.5 and 20.4 (2 C) (8 $COCH_3$).

6-Azidohexyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-(2-acetamido-3,4-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-4-*O*-acetyl-2,6-di-*O*-benzyl-β-D-galactopyranoside 16

A solution of **14** (201 mg, 0.12 mmol) in MeOH (50 ml) was stirred with NaOMe at pH 8–9 for 24 h at room temperature. Then, the solution was neutralised with Dowex 50W-X8 (H^+ form), filtered, and concentrated. Column chromatography (CH_2Cl_2–MeOH, 5 : 1) of the residue gave an amorphous solid (112 mg); TLC (CH_2Cl_2–MeOH, 5 : 1) R_f 0.14. A solution of the residue in 33% ethanolic methylamine (10 ml) was stirred for 24 h at room temperature, then concentrated and co-concentrated with toluene; TLC (CH_2Cl_2–MeOH, 2 : 1) R_f 0.41. A solution of the residue in pyridine (12 ml) and Ac_2O (7 ml) was stirred overnight at room temperature, then concentrated and co-concentrated with toluene. Column chromatography (CH_2Cl_2–MeOH, 50 : 1) of the residue gave **16**, isolated as an amorphous solid (112 mg, 65%); TLC (CH_2Cl_2–MeOH, 20 : 1)

R_f 0.23; $[\alpha]_D^{24}$ −17 (*c* 0.25, $CHCl_3$) (Found: C, 55.13; H, 6.35; N, 3.93. $C_{66}H_{88}N_4O_{31}$ requires C, 55.30; H, 6.19; N, 3.91%); δ_H(300 MHz; $CDCl_3$) 7.43–7.21 (10 H, m, 2 Ph), 5.43 (1 H, dd, $J_{3a,4a}$ 3.8, $J_{4a,5a}$ 1.0, H-4a), 5.34 (1 H, dd, $J_{3d,4d}$ 3.4, $J_{4d,5d}$ 1.3, H-4d), 5.23 (1 H, dd, $J_{2c,3c}$ 9.3, $J_{3c,4c}$ 9.0, H-3c), 5.08 (1 H, dd, $J_{2d,3d}$ 10.4, $J_{1d,2d}$ 7.8, H-2d), 5.02 and 4.54 (each 1 H, 2 d, PhCH_2O), 4.95 (1 H, dd, H-3d), 4.87 (1 H, H-3b), 4.86 (1 H, dd, $J_{1c,2c}$ 7.7, H-2c), 4.79 (1 H, H-4b), 4.76 (1 H, d, NH), 4.72 (1 H, d, $J_{1b,2b}$ 8.4, H-1b), 4.62 (1 H, d, H-1c), 4.53 and 4.39 (each 1 H, 2 d, PhCH_2O), 4.50 (1 H, d, H-1d), 4.40 (1 H, d, $J_{1a,2a}$ 7.8, H-1a), 3.94 (1 H, OCHHCH$_2$), 3.93 (1 H, H-5c), 3.86 (1 H, H-5d), 3.84 (1 H, H-2b), 3.84 (1 H, H-3a), 3.78 (1 H, H-4c), 3.61 (1 H, H-5a), 3.54 (1 H, H-2a), 3.53 (1 H, H-5b), 3.52 (1 H, OCHHCH$_2$), 3.18 (2 H, t, CH_2N_3), 2.12, 2.05, 2.04, 2.02, 1.98 and 1.94 (6,6,3,3,3,9 H, 6 s, 10 Ac), 1.65–1.46 (4 H, m, OCH$_2$CH_2-(CH$_2$)$_2$CH_2), 1.59 (3 H, s, NHAc), 1.39–1.31 (4 H, m, OCH$_2$-CH$_2$(CH_2)$_2$CH$_2$); δ_C(75.5 MHz; $CDCl_3$) 170.8, 170.3 (2 C), 170.2, 170.1, 170.0, 169.7, 169.5 (2 C), 169.3 and 169.0 (11 *C*OCH$_3$), 103.6 (C-1a), 101.4 (C-1b), 101.2 (C-1d), 100.5 (C-1c), 80.0 (C-2a), 77.0 (C-3a), 76.3 (C-4c), 74.5 (Ph*C*H$_2$O), 73.8 (C-5b), 73.4 (Ph*C*H$_2$O), 72.8 (C-3c), 72.6 (C-3b), 72.6 (C-5a), 72.3 (C-5c), 72.1 (C-2c), 71.1 (C-3d), 70.7 (C-5d), 69.9 (O*C*H$_2$CH$_2$), 69.6 (C-4a), 69.1 (C-4b), 69.1 (C-2d), 68.1 (C-6a), 68.1 (C-6b), 66.7 (C-4d), 62.1 (C-6c), 60.8 (C-6d), 54.2 (C-2b), 51.3 (CH_2N_3), 29.5 [CH$_2$*C*H$_2$(CH$_2$)$_4$N$_3$], 28.7 [CH$_2$(CH$_2$)$_3$*C*H$_2$-CH$_2$N$_3$], 26.5 and 25.7 [CH$_2$CH$_2$(*C*H$_2$)$_2$CH$_2$CH$_2$N$_3$], 22.9 (NHCO*C*H$_3$), 20.9 (2 C), 20.8, 20.7, 20.6 (4 C) and 20.5 (2 C) (10 CO*C*H$_3$).

6-Azidohexyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-(2-acetamido-3,4-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-(4-*O*-acetyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside 17

A solution of **15** (105 mg, 0.05 mmol) in MeOH (25 ml) was stirred with NaOMe at pH 8–9 for 24 h at room temperature. Then, the solution was neutralised with Dowex 50W-X8 (H^+ form), filtered, and concentrated. Column chromatography (CH_2Cl_2–MeOH, 5 : 1) of the residue gave an amorphous solid (64 mg); TLC (CH_2Cl_2-MeOH, 5 : 1) R_f 0.20. A solution of the residue in 33% ethanolic methylamine (10 ml) was stirred for 24 h at room temperature, then concentrated and co-concentrated with toluene; TLC (CH_2Cl_2–MeOH, 2 : 1) R_f 0.14. A solution of the residue in pyridine (10 ml) and Ac_2O (5 ml) was stirred for 20 h at room temperature, then concentrated and co-concentrated with toluene. Column chromatography (CH_2Cl_2–MeOH, 40 : 1) of the residue gave **17**, isolated as an amorphous solid (53 mg, 57%); TLC (CH_2Cl_2–MeOH, 20 : 1) R_f 0.43; $[\alpha]_D^{22}$ −114 (*c* 1, $CHCl_3$) (Found: C, 59.59; H, 6.22; N, 2.94. $C_{93}H_{116}N_4O_{36}$ requires C, 59.86; H, 6.27; N, 3.00%); δ_H(300 MHz; $CDCl_3$) 7.41–7.08 (25 H, m, 5 Ph), 5.43 (1 H, dd, $J_{3b,4b}$ 3.5, $J_{4b,5b}$ 0.8, H-4b), 5.33 (1 H, dd, $J_{3e,4e}$ 3.5, $J_{4e,5e}$ 1.2, H-4e), 5.21 (1 H, t, $J_{2d,3d}$ 9.1, $J_{3d,4d}$ 9.1, H-3d), 5.07 (1 H, dd, $J_{2e,3e}$ 10.4, $J_{1e,2e}$ 7.8, H-2e), 4.94 (1 H, dd, H-3e), 4.93 (1 H, H-3c), 4.88 (1 H, H-4c), 4.87 (1 H, H-2d), 4.85 (1 H, NH), 4.68 (1 H, d, $J_{1c,2c}$ 8.2, H-1c), 4.57 (1 H, H-1d), 4.51 (1 H, H-1a), 4.47 (1 H, d, H-1e), 4.46 and 4.21 (each 1 H, 2 d, PhCH_2O), 4.32 (1 H, d, $J_{1b,2b}$ 7.7, H-1b), 3.94 (1 H, H-4a), 3.88 (1 H, OCHHCH$_2$), 3.86 (1 H, H-5e), 3.84 (1 H, H-2c), 3.80 (1 H, H-5d), 3.77 (1 H, H-3b), 3.72 (1 H, H-4d), 3.60 (1 H, H-5c), 3.51 (1 H, H-3a), 3.50 (1 H, H-5b), 3.49 (1 H, OCHHCH$_2$), 3.47 (1 H, H-2a), 3.31 (1 H, H-5a), 3.20 (2 H, t, CH_2N_3), 2.13, 2.08, 2.03, 2.02, 1.99, 1.98, 1.97, 1.94 and 1.93 (3,3,3,3,3,3,3,6,3 H, 9 s, 10 Ac), 1.65–1.50 (4 H, m, OCH$_2$CH_2(CH$_2$)$_2$CH_2), 1.55 (3 H, s, NHAc), 1.44–1.33 (4 H, m, OCH$_2$CH$_2$(CH_2)$_2$CH$_2$); δ_C(75.5 MHz; $CDCl_3$) 170.9, 170.3 (2 C), 170.1, 170.0 (2 C), 169.6, 169.5, 169.4, 169.3 and 169.0 (11 *C*OCH$_3$), 103.6 (C-1b), 102.2 (C-1a), 101.5 (C-1c), 101.2 (C-1e), 100.6 (C-1d), 82.5 (C-3a), 81.8 (C-2b), 80.6 (C-2a), 77.7 (C-3b), 76.4 (C-4a), 76.2 (C-4d), 75.3 (Ph*C*H$_2$O), 75.2 (C-5a), 74.9 and 74.7 (2 Ph*C*H$_2$O), 73.6 (C-5c), 73.4 (2 Ph*C*H$_2$O), 72.7 (3 C) (C-3c,-3d,-5b), 72.3 (C-5d), 72.0 (C-2d), 71.1 (C-3e), 70.7 (C-5e), 69.9 (C-4b), 69.7 (O*C*H$_2$CH$_2$), 69.2 (C-4c), 69.2 (C-2e), 68.5 (C-6c), 68.2 (C-6a), 67.6 (C-6b), 66.7 (C-4e), 62.1 (C-6d), 60.8 (C-6e), 54.3 (C-2c), 51.4 (CH_2N_3), 29.6 [CH$_2$*C*H$_2$-(CH$_2$)$_4$N$_3$], 28.8 [CH$_2$(CH$_2$)$_3$*C*H$_2$CH$_2$N$_3$], 26.5 and 25.7 [CH$_2$CH$_2$(*C*H$_2$)$_2$CH$_2$CH$_2$N$_3$], 22.9 (NHCO*C*H$_3$), 20.8, 20.7 (3 C), 20.6 (3 C), 20.5, 20.4 (2 C) (10 CO*C*H$_3$).

6-Aminohexyl (β-D-galactopyranosyl)-(1→4)-(β-D-glucopyranosyl)-(1→6)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-β-D-galactopyranoside 18

To a solution of **16** (56 mg, 39 μmol) in dry MeOH (5 ml) was added NaOMe (45 mg), and the solution was stirred overnight at room temperature. Then, water (2 ml) was added, and after stirring overnight at room temperature, the mixture was neutralised with Dowex 50W-X8 (H^+ form), filtered, and concentrated. To a solution of the crude de-*O*-acetylated intermediate (42 mg) in a mixture of 2-methylpropan-2-ol (8 ml) and water (8 ml) were added 3 drops of ammonia and Pd/C (100 mg, 10% Pd). The suspension was stirred for 3 h at room temperature in a hydrogen atmosphere, after which ammonia was removed by bubbling nitrogen through the solution. Then, two drops of HOAc and fresh Pd/C (50 mg) were added. The suspension was stirred for another 24 h at room temperature in a hydrogen atmosphere, neutralised with ammonia, and filtered through Celite. The filtrate was concentrated, and the residue was applied to a Toyopearl HW-40S column, eluted with 0.1 M NH_4OAc at a flow rate of 40 ml h^{-1}. The appropriate fractions were lyophilised to give **18** (30 mg, 96%); TLC (HOAc–butanol–H_2O, 2 : 1 : 1) R_f 0.25; $[\alpha]_D^{25}$ −2.3 (*c* 1.2, H_2O); δ_C(125.7 MHz; D_2O) 175.8 (*C*OCH$_3$), 103.8, 103.6 (2 C) and 103.5 (C-1a,-1b,-1c,-1d), 83.2, 79.3, 76.2, 75.6 (2 C), 75.5, 75.2, 74.4, 73.6, 73.4, 71.8, 71.2, 70.7, 70.5, 69.5, 69.4 and 69.1 (C-2a,-2c,-2d,-3a,-3b,-3c,-3d,-4a,-4b,-4c,-4d,-5a,-5b,-5c,-5d,-6b,O*C*H$_2$-CH$_2$), 61.9, 61.6 and 61.0 (C-6a,-6c,-6d), 56.5 (C-2b), 40.3 (CH$_2$NH$_2$), 29.3, 27.6, 26.1 and 25.4 [CH$_2$(*C*H$_2$)$_4$CH$_2$NH$_2$], 23.0 (CO*C*H$_3$). For ^{1}H NMR data (500 MHz, D_2O), see Table 1. High-resolution MS data of $C_{32}H_{58}N_2O_{21}$ (M, 806.3532): M + H found 807.3596, calculated 807.3610.

6-Aminohexyl (β-D-galactopyranosyl)-(1→4)-(β-D-glucopyranosyl)-(1→6)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-β-D-gluco-pyranoside 19

To a solution of **17** (49 mg, 26 μmol) in dry MeOH (5 ml) was added NaOMe (55 mg), and the solution was stirred overnight at room temperature. Then, water (1 ml) was added, and after stirring for 20 h at room temperature, the mixture was neutralised with Dowex 50W-X8 (H^+ form), filtered, and concentrated. To a solution of de-*O*-acetylated intermediate (40 mg) in a mixture of 2-methylpropan-2-ol (8 ml) and water (6 ml) were added 3 drops of ammonia and Pd/C (90 mg, 10% Pd). The suspension was stirred for 2 h at room temperature in a hydrogen atmosphere, after which ammonia was removed by bubbling nitrogen through the solution. Then, two drops of HOAc and fresh Pd/C (90 mg) were added. The suspension was stirred for another 24 h at room temperature in a hydrogen atmosphere, neutralised with ammonia, and filtered through Celite. The filtrate was concentrated, and the residue was applied to a Toyopearl HW-40S column, eluted with 0.1 M NH_4OAc at a flow rate of 40 ml h^{-1}. The appropriate fractions were lyophilised to give **19** (21 mg, 82%); TLC (HOAc–butanol–H_2O, 2 : 1 : 1) R_f 0.18; $[\alpha]_D^{25}$ −0.5 (*c* 0.9, H_2O); δ_C(125.7 MHz; D_2O) 175.8 (*C*OCH$_3$), 103.8 (2 C), 103.7 (2 C) and 102.9 (C-1a,-1b,-1c,-1d,-1e), 82.9, 79.3 (2 C), 76.2, 75.8, 75.7, 75.6, 75.5, 75.3, 75.2, 74.4, 73.7 (2 C), 73.4, 71.8, 71.3, 70.9, 70.5, 69.6, 69.4, 69.2 (C-2a,-2b,-2d,-2e,-3a,-3b,-3c,-3d,-3e,-4a,-4b,-4c,-4d,-4e,-5a,-5b,-5c,-5d,-5e,-6c, O*C*H$_2$CH$_2$), 61.9 (2 C) and

61.0 (2 C) (C-6a,-6b,-6d,-6e), 56.5 (C-2c), 40.3 (CH_2NH_2), 29.3, 27.5, 26.1 and 25.4 [$CH_2(CH_2)_4CH_2NH_2$], 23.0 ($COCH_3$). For ^{1}H NMR data (500 MHz, D_2O), see Table 2. High-resolution MS data of $C_{38}H_{68}N_2O_{26}$ (M, 968.4060): M + H found 969.4154, calculated 969.4138.

6-Aminohexyl (β-D-galactopyranosyl)-(1→4)-(β-D-glucopyranosyl)-(1→6)-[(β-D-galactopyranosyl)-(1→4)]-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-β-D-galacto- pyranoside 20

To a solution of **18** (10 mg, 12.3 μmol) in 50 mM sodium cacodylate buffer pH 7.5 (600 μl) containing $MnCl_2$ (5 mmol), bovine serum albumin (0.8 mg) and NaN_3 (0.02%), were added alkaline phosphatase (7 U), UDP-galactose (10 mg, 16.3 μmol) and β-1,4-galactosyltransferase (2.5 U). The mixture was incubated for 19 h at 37 °C. Then, water (100 μl) was added, and UDP-galactose was removed using a Dowex 1X8 (Cl^- form) column with water as eluent. The eluate was concentrated, and the residue was applied to a Toyopearl HW-40S column, eluted with 0.1 M NH_4OAc at a flow rate of 40 ml h^{-1}. The appropriate fractions were lyophilised to give **20** (9.6 mg, 80%); TLC (HOAc–butanol–H_2O, 2 : 1 : 1) R_f 0.16; $[\alpha]_D^{25}$ −2.7 (*c* 0.6, H_2O); δ_C (125.7 MHz; D_2O) 175.8 ($COCH_3$), 103.8, 103.7, 103.6 (2 C) and 103.5 (C-1a,-1b,-1c,-1d,-1e), 83.4, 79.3, 78.6, 76.2, 76.1, 75.6, 75.5, 75.1, 74.2, 73.5, 73.4 (2 C), 73.0, 71.8 (2 C), 71.2, 70.6, 69.4 (2 C), 69.1 and 68.4 (C-2a,-2c,-2d,-2e,-3a,-3b,-3c, -3d,-3e,-4a,-4b,-4c,-4d,-4e,-5a,-5b,-5c,-5d,-5e,-6b, OCH_2CH_2), 61.9 (2 C), 61.8 and 61.0 (C-6a,-6c,-6d,-6e), 56.1 (C-2b), 40.3 (CH_2NH_2), 29.3, 27.5, 26.1 and 25.4 [$CH_2(CH_2)_4CH_2NH_2$], 23.1 ($COCH_3$). For ^{1}H NMR data (500 MHz, D_2O), see Table 3. High-resolution MS data of $C_{38}H_{68}N_2O_{26}$ (M, 968.4060): M + H found 969.4164, calculated 969.4138.

6-Aminohexyl (β-D-galactopyranosyl)-(1→4)-(β-D-glucopyranosyl)-(1→6)-[(β-D-galactopyranosyl)-(1→4)]-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside 21

To a solution of **19** (11.9 mg, 12.3 μmol) in 50 mM sodium cacodylate buffer pH 7.5 (600 μl) containing $MnCl_2$ (5 mmol), bovine serum albumin (0.5 mg) and NaN_3 (0.02%), were added alkaline phosphatase (11.5 U), UDP-galactose (10 mg, 16.3 μmol) and β-1,4-galactosyltransferase (2.5 U). The mixture was incubated for 20 h at 37 °C. Then, water (100 μl) was added, and UDP-galactose was removed using a Dowex 1X8 (Cl^- form) column with water as eluent. The eluate was concentrated, and the residue was applied to a Toyopearl HW-40S column, eluted with 0.1 M NH_4OAc at a flow rate of 40 ml h^{-1}. The appropriate fractions were lyophilised to give **21** (13.4 mg, 96%); TLC (HOAc–butanol–H_2O, 2 : 1 : 1) R_f 0.14; $[\alpha]_D^{25}$ −3.2 (*c* 0.8, H_2O); δ_C(125.7 MHz; D_2O) 175.8 ($COCH_3$), 103.8 (3 C), 103.6, 103.5 and 102.8 (C-1a,-1b,-1c,-1d,-1e,-1f), 82.0, 79.2, 76.2, 76.1, 75.8, 75.6, 75.3, 75.1, 73.7, 73.5, 73.4, 72.9, 72.4 (3 C), 71.8, 71.3 and 69.4 (C-2a,-2b,-2d,-2e,-2f,-3a,-3b,-3c,-3d,-3e,-3f,-4a,-4b,-4c, -4d,-4e,-4f,-5a,-5b,-5c,-5d,-5e,-5f,-6c, OCH_2CH_2), 62.5, 61.9, 61.3 (2 C) and 60.9 (C-6a,-6b,-6d,-6e,-6f), 56.0 (C-2c), 40.3 (CH_2NH_2), 29.3, 27.5, 26.1 and 25.4 [$CH_2(CH_2)_4CH_2NH_2$], 23.0 ($COCH_3$). For ^{1}H NMR data (500 MHz, D_2O), see Table 4. High-resolution MS data of $C_{44}H_{78}N_2O_{31}$ (M, 1130.4588): M + H found 1131.4506, calculated 1131.4666.

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