Available online at www.sciencedirect.com
SCIENCE DIRECT®
Carbohydrate Research 338 (2003) 2611–2627
CARBOHYDRATE RESEARCH
www.elsevier.com/locate/carres

Chemo-enzymatic synthesis of a tetra- and octasaccharide fragment of the capsular polysaccharide of *Streptococcus pneumoniae* type 14

John A.F. Joosten, Johannis P. Kamerling,* Johannes F.G. Vliegenthart

Bijvoet Center, Department of Bio-Organic Chemistry, Section of Glycoscience and Biocatalysis, Utrecht University, Padualaan 8, NL-3584 CH Utrecht, The Netherlands

Received 16 April 2003; accepted 17 June 2003

Abstract

The chemo-enzymatic synthesis is described of tetrasaccharide β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→6)-[β-D-Gal*p*-(1→4)]-β-D-Glc*p*NAc-(1→O(CH$_2$)$_6$NH$_2$ (**1**) and octasaccharide 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→6)-[β-D-Gal*p*-(1→4)]-β-D-Glc*p*NAc-(1→O(CH$_2$)$_6$NH$_2$ (**2**), representing one and two tetrasaccharide repeating units of *Streptococcus pneumoniae* serotype 14 capsular polysaccharide. In a chemical approach, the intermediate linear trisaccharide **3** and hexasaccharide **4** were synthesized. Galactose residues were β-(1→4)-connected to the internal *N*-acetyl-β-D-glucosamine residues by using bovine milk β-1,4-galactosyltransferase. Both title oligosaccharides will be conjugated to carrier proteins to be tested as potential vaccines in animal models.

Keywords: Carbohydrates; *Streptococcus pneumoniae*; Oligosaccharide synthesis

1. Introduction

Gram-positive *Streptococcus pneumoniae* are members of the normal microflora of the human nasopharynx. Carriage of *S. pneumoniae* is more common in young children than in adults, and varies by geographic region and time.[1,2] *S. pneumoniae* is still a leading cause of life-threatening diseases such as otitis media, pneumonia, meningitis, bacteraemia, and septicaemia,[3,4] mainly due to a growing resistance towards antibiotics.[5,6] Vaccination with the available 23-valent capsular polysaccharide (CPS) vaccines[7] offers protection for healthy adults to invasive pneumococcal diseases.[8] However, these vaccines are ineffective in the most important high-risk groups, such as infants, small children, immuno-compromised patients, and the elderly,[9] because they do not respond adequately to the T-cell independent polysaccharides as antigens.[10] Conjugation of *S. pneumoniae* carbohydrate antigens to a protein carrier results in a T-cell dependent neoglycoconjugate antigen of which it has been proven that it gives an efficient immune response in the high-risk groups.[11] Currently, neoglycoconjugate vaccines against *S. pneumoniae* serotypes, prepared by conjugation of isolated CPSs or of a mixture of polysaccharide-derived oligosaccharides to a protein carrier, have been introduced.[11] The presence of oligosaccharide mixtures can complicate the product analysis, especially in the case of pneumococcal conjugate vaccines where many serotypes have to be included.

By using well-defined oligosaccharides for conjugation, it is possible to investigate the influence of different parameters originating from the carbohydrate part of the neoglycoproteins on their immunogenicity. Studies with oligosaccharide–protein conjugates related to the CPS of *S. pneumoniae* serotypes 3 and 6B showed that, via this approach, the minimal size of the carbohydrate antigen could be determined.[12,13]

Recently, we have reported the chemo-enzymatic synthesis of (spacered mimics of) fragments of the CPS of *S. pneumoniae* type 14, comprising a branched tetrasaccharide and alkyl-bridged hexa- and octasaccharides.[14,15] These oligosaccharides, together with

* Corresponding author. Tel.: +31-30-2533479; fax: +31-30-2540980.
E-mail address: j.p.kamerling@chem.uu.nl (J.P. Kamerling).

0008-6215/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0008-6215(03)00313-6

tetra- and octasaccharide fragments of the type 14 CPS obtained by partial N-deacetylation/deamination, were investigated for their immunological behavior.[16] Based on the promising results of the branched tetrasaccharide, representing one repeating unit, it was decided to synthesize longer intact oligosaccharide fragments of the type 14 CPS. So far, we have reported the synthesis of 6-aminohexyl-spacered penta- and hexasaccharide fragments,[17] and here we report the chemo-enzymatic synthesis of 6-aminohexyl-spacered tetra- and octasaccharide fragments of the CPS (Fig. 1).

2. Results and discussion

2.1. Retrosynthetic strategy

The CPS of *S. pneumoniae* type 14 is built up from the tetrasaccharide repeating unit →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→.[18] The title oligosaccharides (Fig. 1) represent either one (**1**) or two (**2**) tetrasaccharide-repeating units in which each repeating unit is built up from a linear backbone and a branched galactose residue. In a chemical approach to **1** and **2**, first the intermediate linear trisaccharide **3** (Scheme 1) and hexasaccharide **4** (Scheme 2), respectively, were synthesized. Galactose residues were β-(1→4)-attached to the internal *N*-acetyl-β-D-glucosamine residues by using bovine milk β-1,4-galactosyltransferase (EC 2.4.1.22) and UDP-galactose.

2.2. Synthesis of tetrasaccharide fragment 1

In an earlier report,[17] we have shown that by using the trisaccharide donor (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-di-*O*-*p*-methylbenzoyl-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (**20**) (Scheme 2) a linear tetra- and pentasaccharide could be synthesized. However, coupling of donor **20** with the spacer 6-azido-1-hexanol (**6**) could not be successfully realized. Therefore, an alternative route was followed to synthesize trisaccharide **3** (Scheme 1). To this end, two building blocks, 6-azidohexyl 2-deoxy-3,4-di-*O*-*p*-methylbenzoyl-2-phthalimido-β-D-glucopyranoside acceptor (**11**) and (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl

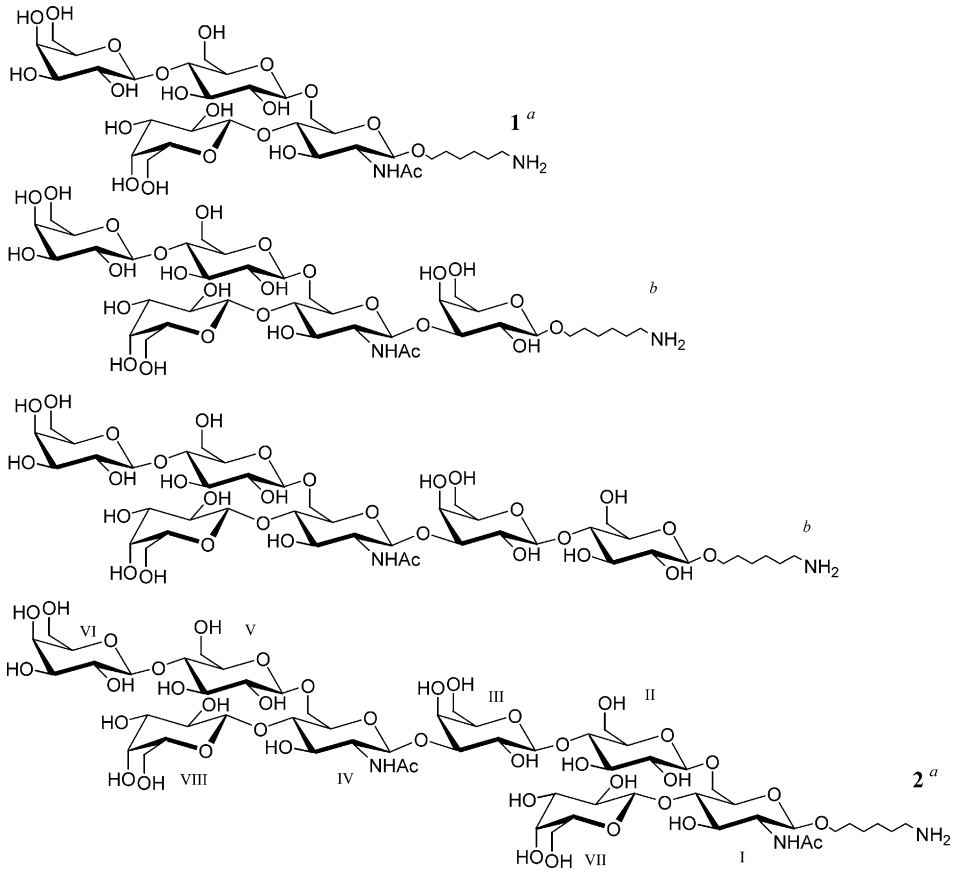
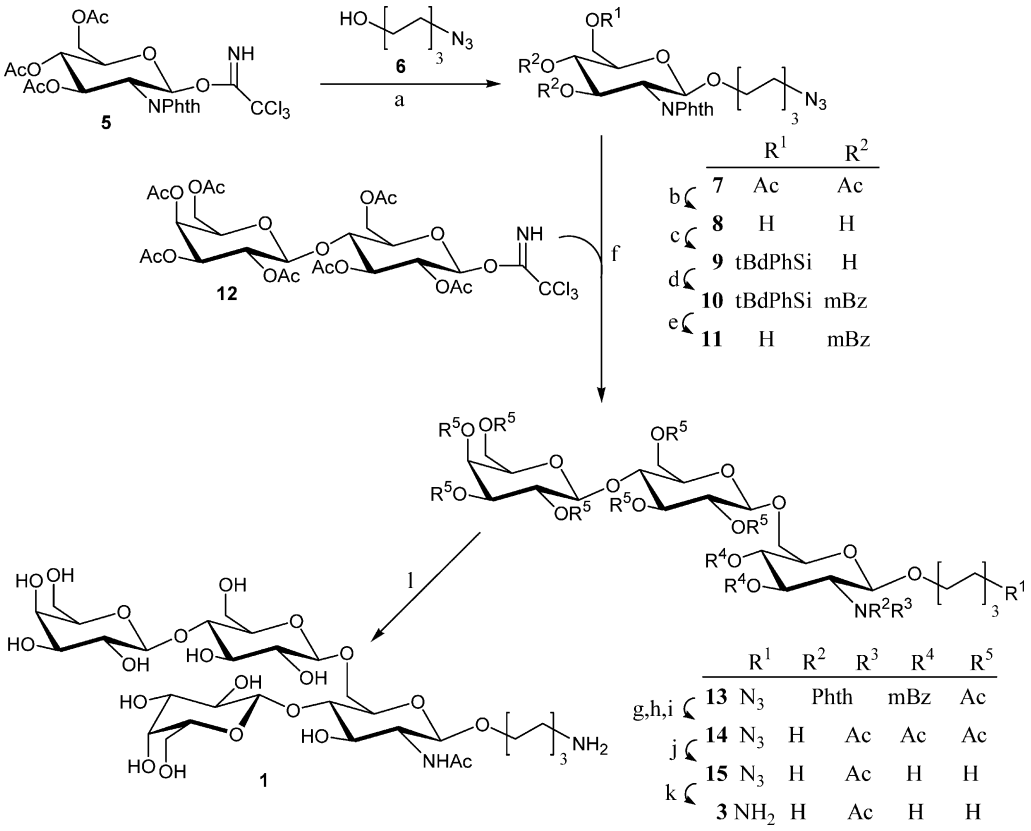
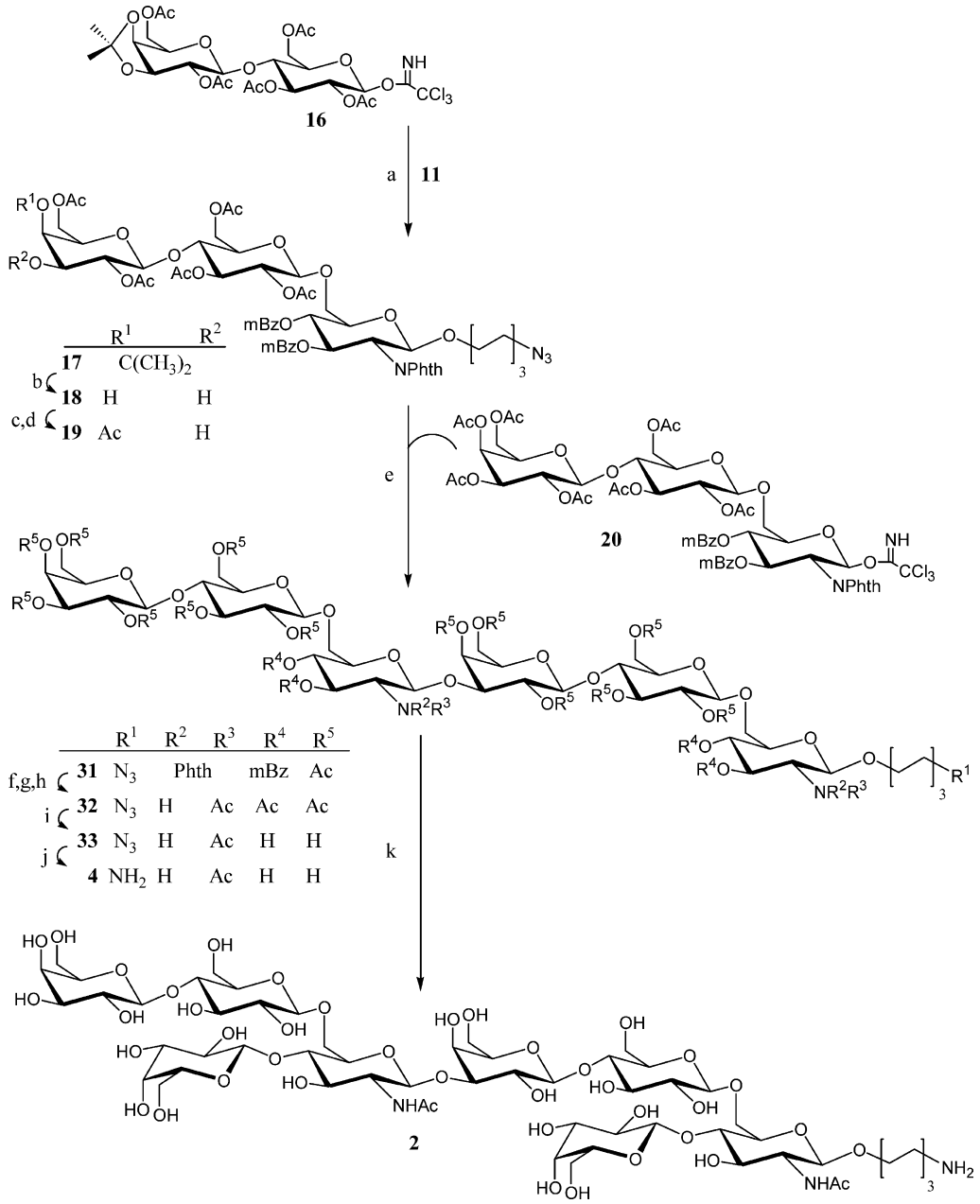


Fig. 1. Overview of 6-aminohexyl-spacered oligosaccharides, representing fragments varying in length between one and two repeating units of the capsular polysaccharide of *S. pneumoniae* type 14. (a) Synthesis described in this paper (compounds **1** and **2**). (b) Synthesis described by Michalik and co-workers.[17]



Scheme 1. Synthesis of tetrasaccharide **1**: (a) 0.5 equiv $BF_3 \cdot Et_2O$, 0 °C, CH_2Cl_2, 89%; (b) NaOMe (pH 8), MeOH, CH_2Cl_2, quantitative; (c) *t*-BdPhSiCl, DMAP, Et_3N, pyridine, CH_2Cl_2, 88%; (d) *p*-mBzCl, 0 °C, pyridine, CH_2Cl_2, 69%; (e) AcCl, 0 °C, MeOH, toluene, 75%; (f) 10% TMSOTf, 0 °C, CH_2Cl_2, 45%; (g) NaOMe (pH 10), MeOH, CH_2Cl_2; (h) $NH_2CH_2CH_2NH_2$, 90 °C, 1-BuOH; (i) pyridine, Ac_2O, 86% over three steps; (j) NaOMe (pH 10), MeOH, CH_2Cl_2, 90%; (k) 10% Pd–C, H_2, aq 25% NH_3, *t*-BuOH, water, 71%; (l) 1.8 equiv UDP-Gal, aq 50 mM sodium cacodylate buffer (pH 7.5), 2.5 U β-1,4-galactosyltransferase, 12 U alkaline phosphatase, 37 °C, 91%.

trichloroacetimidate donor (**12**)[19] were designed (Scheme 1). As a first step in the synthesis of **11**, 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (**5**)[20] was coupled to 6-azido-1-hexanol (**6**) using 0.5 equiv boron trifluoride ethyl etherate ($BF_3 \cdot Et_2O$) as a catalyst at 0 °C in dry dichloromethane, giving **7** in 89% yield. After O-deacetylation of **7** using sodium methoxide at pH 8 (→**8**), a *tert*-butyldiphenylsilyl group (*t*BdPhSi) was selectively introduced at the primary hydroxyl function of **8** using *tert*-butyldiphenylsilyl chloride in pyridine in the presence of a catalytic amount of 4-dimethylaminopyridine (DMAP), affording **9** in 88% yield over two steps. The remaining free hydroxyl functions at O-3 and O-4 of **9** were protected with a toluoyl group (mBz) by using *p*-methylbenzoyl chloride in pyridine at 0 °C (→**10**, 69%). The toluoyl group was chosen to avoid migration of an ester group from O-4 to O-6 after removal of the silyl group at O-6.[21] Finally, removal of the *tert*-butyldiphenylsilyl ether group by using acetyl chloride in dry methanol yielded monosaccharide acceptor **11** (75%). Coupling of **12** and **11**, at 0 °C, using 10% trimethylsilyl trifluoromethanesulfonate (TMSOTf) as a catalyst gave trisaccharide **13** in a moderate yield (45%). The main side-product was the 6-O-acetylated acceptor, which was isolated in 47% yield. This product, probably formed via the orthoester intermediate, could be converted back into acceptor **11** by treatment with acetyl chloride in dry methanol (data not shown). No attempts were made to improve the yield of this condensation reaction. O-Deacylation of **13** using sodium methoxide at pH 10, followed by N-dephthaloylation by treatment with 1,2-diaminoethane in 1-butanol at 90 °C, and subsequent N,O-acetylation using acetic anhydride in pyridine afforded **14** in 86% yield over three steps. The O-acetylation step was carried out to facilitate chromatographic purification. O-Deacetylation of **14** using sodium methoxide at pH 10 gave **15** (90%). Finally, catalytic hydrogenation of the azido group of **15** using 10% palladium on charcoal and H_2 in the presence of ammonia, yielded linear backbone **3** (71%). Tetrasaccharide **1** was synthesized in 91% yield by transfer of galactose from UDP-galactose to O-4 of the *N*-acetyl-β-D-glucosamine residue of **3** by using bovine milk β-1,4-galactosyltransferase as a catalyst (Scheme 1). Alkaline phosphatase was added to the incubation mixture to



Scheme 2. Synthesis of octasaccharide **2**: (a) 66% TMSOTf, 0 °C, CH_2Cl_2, 62%; (b) TFA, 0 °C, water, CH_2Cl_2, 84%; (c) trimethyl orthoacetate, *p*-TsOH, CH_3CN; (d) AcOH, water, 85% over two steps; (e) 10% TMSOTf, −20 °C, CH_2Cl_2, 12%; (f) NaOMe (pH 10), MeOH, CH_2Cl_2; (g) $NH_2CH_2CH_2NH_2$, 80 °C, 1-BuOH; (h) pyridine, Ac_2O, 71% over three steps; (i) NaOMe (pH 10), MeOH, 89%; (j) 10% Pd–C, H_2, aq 25% NH_3, *t*-BuOH, water, 67%; (k) 3 equiv UDP-Gal, aq 50 mM sodium cacodylate buffer (pH 7.5), 1 U β-1,4-galactosyltransferase, 10 U alkaline phosphatase, 37 °C, 22%.

prevent feedback inhibition by released UDP, thereby facilitating a high conversion of **3**. Chemical and chemoenzymatic syntheses of structure **1**, containing other functionalities at the anomeric center have been described and reviewed by Kamerling.[22] ^{1}H NMR data of **15**, **3**, and **1** are presented in Tables 1–3, respectively.

2.3. Synthesis of octasaccharide fragment 2

Coupling of (2,6-di-*O*-acetyl-3,4-di-*O*-isopropylidene-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl trichloroacetimidate donor (**16**)[23] to glucosamine acceptor **11** (Scheme 2) at 0 °C, using 66%

Table 1
500 MHz ^{1}H NMR data (TOCSY, ROESY) of **15** at 300 K (in ppm)

Proton	δ_H		
	GlcNAcI	GlcII	GalIII
H-1	4.51	4.55	4.45
H-2	3.68	3.38	3.53
H-3	3.52	3.67	3.66
H-4	3.52	3.67	3.93
H-5	3.62	3.61	n.d. [a]
H-6a	4.21	3.98	n.d.
H-6b	3.89	3.82	n.d.
$O(CH_2)_2(CH_2)_2(CH_2)_2N_3$			1.34–1.37 (4 H)
$OCH_2CH_2(CH_2)_2CH_2CH_2N_3$			1.54–1.61 (4 H)
CH_2N_3			3.32
$OCH_2(CH_2)_5N_3$			3.61, 3.89
$NDCOCH_3$			2.03

[a] n.d., not determined.

trimethylsilyl trifluoromethanesulfonate, gave trisaccharide **17** (62%). *O*-Deisopropylidenation of **17** with aqueous 90% trifluoroacetic acid (→**18**, 84%), followed by selective acetylation at O-4 of the galactose residue via orthoester formation using trimethyl orthoacetate and *p*-toluenesulfonic acid, and subsequent ring opening with aqueous 80% acetic acid afforded **19** (85% over two steps).[24] A small amount of side-product (6%) was formed in which O-3 of the galactose residue was acetylated, either due to acetyl migration or ring opening to O-3. Coupling of **19** with (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-di-*O*-*p*-methylbenzoyl-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (**20**)[17] in dichloromethane at −20 °C, using 10% trimethylsilyl trifluoromethanesulfonate as a catalyst, gave hexasaccharide **31** in only 12% yield (Scheme 2). The low yield is probably due to the low reactivity of the free O-3 position, since the acceptor could be isolated after the reaction. Other coupling attempts, at different temperatures and with different catalysts, did not improve the yield of the reaction.

Therefore, in a second approach, an attempt was made to synthesize hexasaccharide **31** via a slightly different route in which acetyl ester groups were replaced by benzyl ether functions. O-Deacylation of **17**, followed by benzylation with benzyl bromide in dry *N*,*N*-dimethylformamide in the presence of sodium hydride resulted also in the removal of the phthalimido protective group. Introduction of benzyl groups via

Table 2
500 MHz ^{1}H NMR data (TOCSY, ROESY) of **3** at 300 K (in ppm)

Proton	δ_H		
	GlcNAcI	GlcII	GalIII
H-1	4.51	4.55	4.45
H-2	3.68	3.37	3.55
H-3	3.53	3.67	3.67
H-4	3.52	3.67	3.93
H-5	3.62	3.62	n.d. [a]
H-6a	4.21	3.99	n.d.
H-6b	3.89	3.82	n.d.
$O(CH_2)_2(CH_2)_2(CH_2)_2ND_2$			1.32–1.34 (4 H)
$OCH_2CH_2(CH_2)_2CH_2CH_2ND_2$			1.53–1.56 (2 H), 1.62–1.68 (2 H)
CH_2ND_2			2.98
$OCH_2(CH_2)_5ND_2$			3.58, 3.92
$NDCOCH_3$			2.03

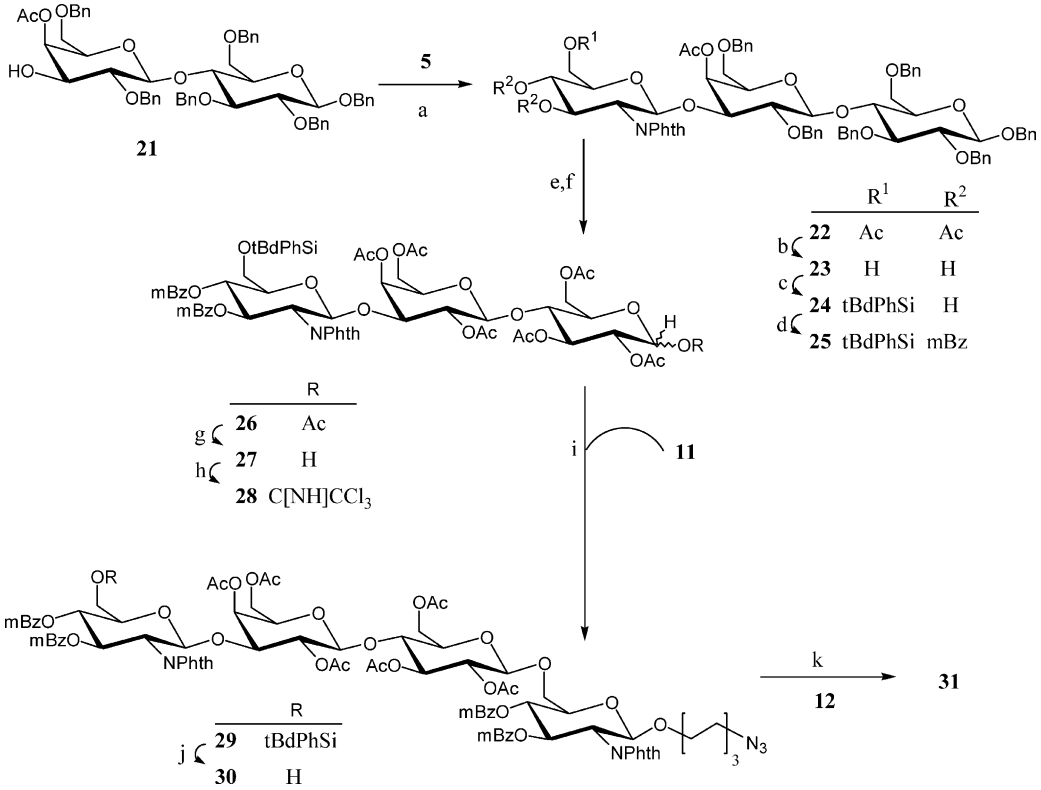
[a] n.d., not determined.

Table 3
500 MHz ^{1}H NMR data (TOCSY, ROESY) of **1** at 300 K (in ppm)

Proton	δ_H			
	GlcNAcI	GlcII	GalIII [a]	GalVII [b]
H-1	4.54	4.56	4.45	4.53
H-2	3.74	3.38	3.55	3.53
H-3	3.69	3.67	3.68	3.68
H-4	3.82	3.67	3.92	3.92
H-5	3.72	3.62	n.d. [c]	n.d.
H-6a	4.29	3.98	3.73	3.73
H-6b	3.96	3.82	3.78	3.78
$O(CH_2)_2(CH_2)_2(CH_2)_2ND_2$			1.36–1.38 (4 H)	
$OCH_2CH_2(CH_2)_2CH_2CH_2ND_2$			1.55–1.58 (2 H), 1.62–1.67 (2 H)	
CH_2ND_2			2.98	
$OCH_2(CH_2)_5ND_2$			3.60, 3.92	
$NDCOCH_3$			2.03	

[a] Gal(β1-4)Glc.
[b] Gal(β1-4)GlcNAc.
[c] n.d., not determined.

coupling of the benzyl-protected lactose donor, with acceptor **11**, yielded α/β-mixtures, which could not be fractionated. In an alternative approach to obtain **31**, trisaccharide donor **28** was designed to be combined with **11** and **12** (Scheme 3). Coupling of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl tri-



Scheme 3. Alternative synthesis of hexasaccharide backbone **31**: (a) 8% TMSOTf, −70 °C, CH_2Cl_2, 86%; (b) NaOMe (pH 9), MeOH, CH_2Cl_2, 82%; (c) *t*-BdPhSiCl, DMAP, Et_3N, pyridine, CH_2Cl_2, 89%; (d) *p*-mBzCl, 0 °C, pyridine, CH_2Cl_2, 74%; (e) 10% Pd–C, H_2, EtOH, EtOAc, 90%; (f) pyridine, Ac_2O, quantitative; (g) hydrazinium acetate, DMF, 65%; (h) CCl_3CN, DBU, 0 °C, CH_2Cl_2, 69%; (i) 10% TMSOTf, −40 °C, CH_2Cl_2, 38%; (j) 1.0 M TBAF in THF, AcOH, 0 °C, 90%; (k) TMSOTf, −40 °C, CH_2Cl_2, 60%.

chloroacetimidate (**5**)[20] to (4-*O*-acetyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-1,2,3,6-tetra-*O*-benzyl-β-D-glucopyranose (**21**)[25] in dichloromethane at −70 °C, using 8% trimethylsilyl trifluoromethanesulfonate as a catalyst gave **22** (86%). Mild O-deacetylation of **22**, using sodium methoxide (pH 9), afforded **23** (82%), with retention of the acetyl group at galactose O-4. Selective *tert*-butyldiphenylsilylation of O-6 of the glucosamine residue of **23** using *tert*-butyldiphenylsilyl chloride and a catalytic amount of 4-dimethylaminopyridine in pyridine (→**24**, 89%), followed by toluoylation of O-3 and O-4 using *p*-methylbenzoyl chloride in pyridine, gave **25** (74%). O-Debenzylation of **25** using 10% palladium on charcoal and H_2 and subsequent O-acetylation using acetic anhydride in pyridine yielded **26** in 90% over two steps. Selective removal of the anomeric *O*-acetyl group by treatment with hydrazinium acetate in dry *N*,*N*-dimethylformamide (→**27**, 65%), followed by imidation using trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) gave donor **28** in an $\alpha:\beta$ ratio of 9:1 (69%). Coupling of **28** to glucosamine acceptor **11** (Scheme 3) at −40 °C using 10% trimethylsilyl trifluoromethanesulfonate as a catalyst afforded tetrasaccharide **29** (38%). O-De-*tert*-butyldiphenylsilylation of **29** in a 1:1 mixture of 1.0 M tetrabutylammonium fluoride (TBAF) in tetrahydrofuran and acetic acid (pH 6) yielded **30** (90%). It should be noted that removal of the *tert*-butyldiphenylsilyl group by using acetyl chloride in dry methanol at 0 °C also lead to the removal of acetyl groups. Coupling of tetrasaccharide acceptor **30** with 3 equiv of (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl trichloroacetimidate (**12**),[19] using 10% trimethylsilyl trifluoromethanesulfonate as a catalyst at −40 °C, gave hexasaccharide **31** (60%). O-Deacylation of **31** with sodium methoxide in a methanol–dichloromethane mixture, followed by N-dephthaloylation by treatment with 1,2-diaminoethane in 1-butanol at 80 °C, and subsequent N,O-acetylation using acetic anhydride in pyridine gave **32** (71% over three steps). *O*-Deacetylation of **32** using sodium methoxide at pH 10 (→**33**; 89%), and catalytic hydrogenation of the azido group using 10% palladium on charcoal and H_2 gave **4** in 67% yield. Finally, octasaccharide **2** was synthesized in 22% yield by transfer of galactose from UDP-galactose to O-4 of the two *N*-acetyl-β-D-glucosamine residues of **4** by using bovine milk β-1,4-galactosyltransferase as a catalyst (Scheme 2). The low yield of the reaction was due to purification problems. ^{1}H NMR data of **33**, **4**, and **2** are presented in Tables 4–6, respectively.

Conjugation of the oligosaccharides **1** and **2** to CRM_{197} (cross reactive material) and immunological studies are in progress.

3. Experimental

3.1. General methods

All chemicals were of reagent grade, and were used without further purification. Reactions were monitored by TLC on Silica Gel 60 F_{254} (E. Merck); after examination under UV light, compounds were visualized by heating with 10% (v/v) ethanolic H_2SO_4, orcinol (2 mg/mL) in 20% (v/v) methanolic H_2SO_4, or ninhydrin (1.5 mg/mL) in 1-BuOH–water–AcOH (38:1.75:0.25). In the work-up procedures of reaction mixtures, organic solns were washed with appropriate amounts of the indicated aq solns, then dried ($MgSO_4$), and concentrated under diminished pressure at 40 °C. Column chromatography was performed on Silica Gel 60 (E. Merck, 0.063–0.200 mm). Optical rotations were measured with a Perkin–Elmer 241 polarimeter, using a 10 cm, 1 mL cell. ^{1}H NMR spectra were recorded at 300 K with a Bruker AC 300 (300 MHz) or a Bruker AMX 500 (500 MHz) spectrometer; the δ_H values are given in ppm relative to the signal for internal Me_4Si (δ_H 0, $CDCl_3$ and CD_3OD) or internal acetone (δ_H 2.225, D_2O). ^{13}C NMR spectra (APT, 75 MHz) were recorded at 300 K with a Bruker AC 300 spectrometer; δ_C values are given in ppm relative to the signal of $CDCl_3$ (δ_C 76.9, $CDCl_3$) or for internal acetone (δ_C 30.89, D_2O). Two-dimensional ^{1}H–^{1}H TOCSY (mixing times 7 and 100 ms), ROESY (mixing time 300 ms), and ^{1}H–^{13}C correlated HSQC NMR spectra (500 MHz) were recorded at 300 K with a Bruker AMX 500 spectrometer. Exact masses were measured by nano electrospray time-of-flight mass spectrometry (positive-ion mode) 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 a 1:1 (v/v) mixture of MeCN–water with 0.1% formic acid. Pentafluorophenylalanine was added as internal standard. The capillary voltage was set at 1500 V and the cone voltage was set at 30 V. Elemental analyses were carried out at the Department of Organic Chemistry of the University of Nijmegen (The Netherlands).

3.2. 6-Azidohexyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose (7)

A soln of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (**5**)[20] (2.45 g, 4.23 mmol) and 6-azido-1-hexanol (**6**) (1.3 g, 9.08 mmol) in dry CH_2Cl_2 (50 mL), containing molecular sieves 4 Å (400 mg), was stirred for 2 h under Ar. After cooling to 0 °C, $BF_3{\cdot}Et_2O$ (0.28 mL, 2.19 mmol) was added and the mixture was stirred for 1.5 h, after which a second portion of $BF_3{\cdot}Et_2O$ (0.1 mL, 0.79 mmol) was added, and the stirring was continued for 1.5 h. The mixture was neutralized with Et_3N, washed with aq satd

Table 4
500 MHz ^{1}H NMR data (TOCSY, ROESY) of **33** at 300 K (in ppm)

Proton	δ_H					
	GlcNAcI [a]	GlcII [b]	GaIII [c]	GlcNAcIV [d]	GlcV [e]	GalVI [f]
H-1	4.52	4.55	4.44	4.70	4.55	4.46
H-2	3.68	3.37	3.59	3.76	3.37	3.57
H-3	3.52	3.66	3.72	3.56	3.66	3.68
H-4	n.d. [g]	n.d.	4.16	n.d.	n.d.	3.92
H-5	3.60	n.d.	n.d.	3.61	n.d.	n.d.
H-6a	4.21	3.98	n.d.	4.22	3.98	n.d.
H-6b	3.88	3.81	n.d.	3.89	3.81	n.d.
$O(CH_2)_2(CH_2)_2(CH_2)_2N_3$				1.33–1.37 (4 H)		
$OCH_2CH_2(CH_2)_2CH_2CH_2N_3$				1.53–1.60 (4 H)		
CH_2N_3				3.32		
$OCH_2(CH_2)_5N_3$				3.61, 3.89		
$NDCOCH_3$				2.03 (2)		

[a] GlcNAc(β1-O$(CH_2)_6N_3$).
[b] Glc(β1-6)GlcNAc(β1-O$(CH_2)_6N_3$).
[c] Gal(β1-4)Glc(β1-6)GlcNAc(β1-O$(CH_2)_6N_3$).
[d] GlcNAc(β1-3)Gal.
[e] Glc(β1-6)GlcNAc(β1-3)Gal.
[f] Gal(β1-4)Glc(β1-6)GlcNAc(β1-3)Gal.
[g] n.d., not determined.

$NaHCO_3$ and water, dried, filtered, and concentrated. Column chromatography (9:1 CH_2Cl_2–EtOAc) of the residue gave **7**, isolated as a slightly yellow syrup (2.10 g, 89%); R_f 0.66 (9:1 CH_2Cl_2–EtOAc); $[\alpha]_D^{20}$ +19° (*c* 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.12–1.17 (m, 4 H, 2 CH_2), 1.23–1.29 (m, 2 H, CH_2), 1.43–1.45 (m, 2 H, CH_2), 1.86, 2.03, and 2.11 (3 s, each 3 H, 3 $COCH_3$), 3.06 (t, 2 H, CH_2N_3), 3.44 (m, 1 H, OC*H*H), 4.17 (dd, 1

Table 5
500 MHz ^{1}H NMR data (TOCSY, ROESY) of **4** at 300 K (in ppm)

Proton	δ_H					
	GlcNAcI [a]	GlcII [b]	GalIII [c]	GlcNAcIV [d]	GlcV [e]	GalVI [f]
H-1	4.51	4.56	4.43	4.70	4.56	4.45
H-2	3.68	3.37	3.60	3.76	3.37	3.55
H-3	3.52	3.67	3.72	3.55	3.67	3.67
H-4	n.d. [g]	n.d.	4.16	n.d.	n.d.	3.93
H-5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
H-6a	4.21	n.d.	n.d.	4.22	n.d.	n.d.
H-6b	3.88	n.d.	n.d.	3.89	n.d.	n.d.
$O(CH_2)_2(CH_2)_2(CH_2)_2ND_2$				1.35–1.39 (4 H)		
$OCH_2CH_2(CH_2)_2CH_2CH_2ND_2$				1.55–1.57 (2 H), 1.64–1.66 (2 H)		
CH_2ND_2				2.99		
$OCH_2(CH_2)_5ND_2$				3.59, 3.91		
$NDCOCH_3$				2.03 (2)		

[a] GlcNAc(β1-O$(CH_2)_6NH_2$).
[b] Glc(β1-6)GlcNAc(β1-O$(CH_2)_6NH_2$).
[c] Gal(β1-4)Glc(β1-6)GlcNAc(β1-O$(CH_2)_6NH_2$).
[d] GlcNAc(β1-3)Gal.
[e] Glc(β1-6)GlcNAc(β1-3)Gal.
[f] Gal(β1-4)Glc(β1-6)GlcNAc(β1-3)Gal.
[g] n.d., not determined.

Table 6
500 MHz ^{1}H NMR data (TOCSY, ROESY) of **2** at 300 K (in ppm)

Proton	δ_H							
	GlcNAcI [a]	GlcII [b]	GalIII [c]	GlcNAcIV [d]	GlcV [e]	GalVI [f]	GalVII [g]	GalVIII [h]
H-1	4.53	4.56	4.43	4.70	4.56	4.46	4.54	4.54
H-2	3.72	3.37	3.59	3.81	3.37	3.55	3.54	3.54
H-3	n.d. [i]	3.67	3.72	n.d.	3.67	n.d.	3.67	3.67
H-4	n.d.	n.d.	4.16	n.d.	n.d.	3.94	3.93	3.93
H-5	n.d.	3.62	n.d.	3.73	3.62	n.d.	n.d.	n.d.
H-6a	4.29	3.99	n.d.	4.28	3.99	n.d.	n.d.	n.d.
H-6b	3.95	3.83	n.d.	3.97	3.83	n.d.	n.d.	n.d.
$O(CH_2)_2(CH_2)_2(CH_2)_2ND_2$				1.38–1.42 (4 H)				
$OCH_2CH_2(CH_2)_2CH_2CH_2ND_2$				1.54–1.57 (2 H), 1.63–1.66 (2 H)				
CH_2ND_2				2.98				
$OCH_2(CH_2)_5ND_2$				3.68, 3.92				
$NDCOCH_3$				2.03 (2)				

[a] GlcNAc(β1-O$(CH_2)_6NH_2$).
[b] Glc(β1-6)GlcNAc(β1-O$(CH_2)_6NH_2$).
[c] Gal(β1-4)Glc(β1-6)GlcNAc(β1-O$(CH_2)_6NH_2$).
[d] GlcNAc(β1-3)Gal.
[e] Glc(β1-6)GlcNAc(β1-3)Gal.
[f] Gal(β1-4)Glc(β1-6)GlcNAc(β1-3)Gal.
[g] Gal(β1-4)GlcNAc(β1-O$(CH_2)_6NH_2$).
[h] Gal(β1-4)GlcNAc(β1-3)Gal.
[i] n.d., not determined.

H, $J_{5,6a}$ 2.3, $J_{6a,6b}$ 12.3 Hz, H-6a), 4.31 (dd, 1 H, $J_{1,2}$ 8.5, $J_{2,3}$ 10.8 Hz, H-2), 4.33 (dd, 1 H, $J_{5,6b}$ 4.7 Hz, H-6b), 5.17 (dd, 1 H, $J_{3,4}$ 9.1, $J_{4,5}$ 9.8 Hz, H-4), 5.36 (d, 1 H, H-1), 5.79 (dd, 1 H, H-3), 7.75–7.89 (2 m, 4 H, Phth); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.2, 20.4, and 20.5 ($COCH_3$), 25.1, 26.0, 28.4, and 28.8 (4 CH_2), 50.9 (CH_2N_3), 54.5 (C-2), 61.9 and 69.6 (C-6, OCH_2), 68.9, 70.6, and 71.6 (C-3, C-4, C-5), 98.0 (C-1), 123.3, 131.1, and 134.2 (Phth), 169.3, 169.9, and 170.5 ($COCH_3$); HRMS of $C_{26}H_{32}N_4O_{10}$ (M, 560.211): $[M+NH_4]^+$ found 578.250, calcd 578.254.

3.3. 6-Azidohexyl 2-deoxy-2-phthalimido-β-D-glucopyranoside (8)

To a soln of **7** (2.60 g, 4.64 mmol) in MeOH (20 mL) and CH_2Cl_2 (5 mL) was added NaOMe (pH 8). The mixture was stirred for 5 h, then neutralized with Dowex 50 × 8 (H^+), filtered, and concentrated giving **8** as a white solid (2.01 g, quantitative); R_f 0.42 (4:1 CH_2Cl_2–MeOH); $[\alpha]_D^{20}$ −15° (*c* 1, $CHCl_3$); ^{1}H NMR (300 MHz, CD_3OD): δ 1.08–1.10 (m, 4 H, 2 CH_2), 1.14–1.20 (m, 2 H, CH_2), 1.38–1.42 (m, 2 H, CH_2), 3.01 (t, 2 H, CH_2N_3), 3.73 (dd, 1 H, $J_{5,6b}$ 4.9, $J_{6a,6b}$ 11.8 Hz, H-6b), 3.84 (m, 1 H, OCH*H*), 3.92 (dd, 1 H, $J_{5,6a}$ 1.5 Hz, H-6a), 3.96 (dd, 1 H, $J_{1,2}$ 8.5, $J_{2,3}$ 10.6 Hz, H-2), 4.25 (dd, 1 H, $J_{3,4}$ 7.7 Hz, H-3), 5.13 (d, 1 H, H-1), 7.81–7.90 (m, 4 H, Phth); ^{13}C NMR (75.5 MHz, CD_3OD): δ 26.2, 26.9, 29.3, and 29.8 (4 CH_2), 51.8 (CH_2N_3), 58.2 (C-2), 62.4 and 70.0 (C-6, OCH_2), 72.2, 72.3, and 77.9 (C-3, C-4, C-5), 99.3 (C-1), 123.8, 132.6, and 135.3 (Phth); HRMS of $C_{20}H_{26}N_4O_7$ (M, 434.180): $[M+NH_4]^+$ found 452.212, calcd 452.214.

3.4. 6-Azidohexyl 6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (9)

To a soln of **8** (2.01 g, 4.64 mmol) in CH_2Cl_2 (30 mL) were added Py (3.0 mL), a catalytic amount of 4-dimethylaminopyridine, Et_3N (0.86 mL), and *tert*-butyldiphenylsilylchloride (1.7 mL, 6.54 mmol). The mixture was stirred for 16 h, then poured into cold water, washed with aq satd $NaHCO_3$, dried, filtered, and concentrated. Column chromatography (1:1 toluene–EtOAc) of the residue gave **9**, isolated as a white foam [3.12 g, 88% (starting from **7**)]; R_f 0.53 (1:1 toluene–EtOAc); $[\alpha]_D^{20}$ −16° (*c* 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.06 [s, 9 H, $(CH_3)_3CSi$], 1.06–1.13 (m, 4 H, 2 CH_2), 1.25–1.27 (m, 2 H, CH_2), 1.39–1.41 (m, 2 H, CH_2), 3.02 (t, 2 H, CH_2N_3), 3.38 (m, 1 H, OC*H*H), 3.58 (m, 1 H, H-5), 3.67 (t, 1 H, H-4), 3.76 (m, 1 H, OCH*H*), 3.96 (d, 2 H, $J_{5,6}$ 4.9 Hz, H-6a, H-6b), 4.09 (dd, 1 H, $J_{1,2}$

8.4, $J_{2,3}$ 10.9 Hz, H-2), 4.36 (dd, 1 H, $J_{3,4}$ 8.4 Hz, H-3), 5.17 (d, 1 H, H-1); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 19.1 [$(CH_3)_3CSi$], 25.3, 26.0, 28.4, and 29.0 (4 CH_2), 26.7 [$(CH_3)_3CSi$], 51.0 (CH_2N_3), 56.3 (C-2), 64.8 and 69.1 (C-6, OCH_2), 71.7, 74.1, and 74.4 (C-3, C-4, C-5), 98.0 (C-1); HRMS of $C_{36}H_{44}N_4O_7Si$ (M, 672.297): $[M+NH_4]^+$ found 690.332, calcd 690.330.

3.5. 6-Azidohexyl 6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-3,4-di-*O*-*p*-methylbenzoyl-2-phthalimido-β-D-glucopyranoside (10)

To a soln of **9** (2.75 g, 4.09 mmol) in Py (35 mL) was added, at 0 °C, dropwise a soln of *p*-methylbenzoyl chloride (1.35 mL, 10.2 mmol) in dry CH_2Cl_2 (10 mL). The mixture was stirred for 20 h, then, washed with cold water and aq satd $NaHCO_3$, dried, filtered, and concentrated. Column chromatography (19:1 toluene–EtOAc) of the residue gave **10**, isolated as a colourless syrup (3.25 g, 69%); R_f 0.65 (7:1 toluene–EtOAc); $[\alpha]_D^{20}$ −6° (*c* 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.03 [s, 9 H, $(CH_3)_3CSi$], 1.16–1.19 (m, 4 H, 2 CH_2), 1.25–1.29 (m, 2 H, CH_2), 1.49–1.55 (m, 2 H, CH_2), 2.27 and 2.35 (2 s, each 3 H, 2 CH_3C$_6$H$_4$CO), 3.05 (t, 2 H, CH_2N_3), 3.48 (m, 1 H, OC*H*H), 4.51 (dd, 1 H, $J_{1,2}$ 8.5, $J_{2,3}$ 10.8 Hz, H-2), 5.48 (d, 1 H, H-1), 5.57 (t, 1 H, H-4), 6.17 (dd, 1 H, $J_{3,4}$ 9.3 Hz, H-3), 7.04 and 7.13 (2 d, each 2 H, Phth); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 19.1 [$(CH_3)_3CSi$], 21.5 (2 $CH_3C_6H_4CO$), 25.4, 26.2, 28.5, and 29.1 (4 CH_2), 26.6 [$(CH_3)_3CSi$], 51.1 (CH_2N_3), 55.0 (C-2), 62.9 and 69.3 (C-6, OCH_2), 69.7, 71.3, and 75.1 (C-3, C-4, C-5), 98.0 (C-1), 164.9 and 165.0 (2 $CH_3C_6H_4CO$); Anal. Calcd for $C_{52}H_{56}N_4O_9Si$: C, 68.70; H, 6.21; N, 6.16. Found: C, 68.58; H, 6.26; N, 6.02.

3.6. 6-Azidohexyl 2-deoxy-3,4-di-*O*-*p*-methylbenzoyl-2-phthalimido-β-D-glucopyranoside (11)

To a soln of **10** (0.72 g, 0.79 mmol) in dry toluene (5.0 mL) was added, at 0 °C, a soln of acetyl chloride (0.93 mL, 13.05 mmol) in dry MeOH (25 mL). The mixture was stirred under Ar for 4 h, then neutralized with Et_3N, washed with water, dried, filtered, and concentrated. Low-pressure column chromatography (5:1 toluene–EtOAc) of the residue gave **11**, isolated as a colourless syrup (0.40 g, 75%); R_f 0.28 (4:1 toluene–EtOAc); $[\alpha]_D^{20}$ −3° (*c* 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.15–1.13 (m, 4 H, 2 CH_2), 1.24–1.28 (m, 2 H, CH_2), 1.43–1.49 (m, 2 H, CH_2), 2.23 and 2.30 (2 s, each 3 H, 2 CH_3C$_6$H$_4$CO), 3.04 (t, 2 H, CH_2N_3), 3.51 (m, 1 H, OC*H*H), 3.75 (dd, 1 H, $J_{5,6b}$ 4.0, $J_{6a,6b}$ 12.7 Hz, H-6b), 4.55 (dd, 1 H, $J_{1,2}$ 8.5, $J_{2,3}$ 10.7 Hz, H-2), 5.50 (t, 1 H, H-4), 5.55 (d, 1 H, H-1), 6.31 (dd, 1 H, $J_{3,4}$ 9.3 Hz, H-3), 7.02 and 7.13 (2 d, each 2 H, Phth), 7.66 and 7.83 (2 d, each 4 H, 2 $CH_3C_6H_4CO$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 21.3 and 21.4 (2 $CH_3C_6H_4CO$), 25.2, 26.0, 28.4, and 28.9 (4 CH_2), 50.9 (CH_2N_3), 54.7 (C-2), 61.2 and 69.5 (C-6, OCH_2), 69.8, 70.5, and 74.3 (C-3, C-4, C-5), 98.1 (C-1), 165.4 and 165.9 (2 $CH_3C_6H_4CO$); HRMS of $C_{36}H_{38}N_4O_9$ (M, 670.263): $[M+NH_4]^+$ found 688.294, calcd 688.298.

3.7. 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-di-*O*-*p*-methylbenzoyl-2-phthalimido-β-D-glucopyranoside (13)

A soln of (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl trichloroacetimidate (**12**)[19] (0.26 g, 0.33 mmol) and **11** (0.17 g, 0.26 mmol) in dry CH_2Cl_2 (5 mL), containing molecular sieves 4 Å (0.2 g), was stirred for 1 h under Ar. The mixture was cooled to 0 °C and TMSOTf (6 μL, 33 μmol) was added. The mixture was stirred for 1 h, during which period the temperature was allowed to reach room temperature (rt), then neutralized with Et_3N, washed with aq satd $NaHCO_3$ and water, dried, filtered, and concentrated. Column chromatography (1:2 toluene–EtOAc) of the residue afforded **13**, isolated as a white foam (148 mg, 45%); R_f 0.55 (1:2 toluene–EtOAc); $[\alpha]_D^{20}$ −6° (*c* 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.14–1.16 (m, 4 H, 2 CH_2), 1.22–1.30 (m, 2 H, CH_2), 1.45–1.51 (m, 2 H, CH_2), 1.95, 2.04, 2.05, 2.07, and 2.14 (5 s, 3,3,9,3,3 H, 7 $COCH_3$), 2.27 and 2.34 (2 s, each 3 H, 2 CH_3C$_6$H$_4$CO), 3.05 (t, 2 H, CH_2N_3), 3.49 (m, 1 H, OC*H*H), 3.60 (m, 1 H, H-5^{II}), 4.64 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1^{II}), 4.90 (t, 1 H, $J_{2,3}$ 9.2 Hz, H-2^{II}), 4.95 (dd, 1 H, $J_{2,3}$ 10.3, $J_{3,4}$ 3.4 Hz, H-3^{III}), 5.10 (dd, 1 H, $J_{1,2}$ 7.8 Hz, H-2^{III}), 5.15 (t, 1 H, H-4^{I}), 5.46 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1^{I}), 6.17 (dd, 1 H, $J_{2,3}$ 10.7, $J_{3,4}$ 9.2 Hz, H-3^{I}), 7.04 and 7.15 (2 d, each 2 H, Phth), 7.61 and 7.78 (2 d, each 4 H, 2 $CH_3C_6H_4CO$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.3–20.6 ($COCH_3$), 21.4 (2 $CH_3C_6H_4CO$), 25.3, 26.0, 28.4, and 28.9 (4 CH_2), 50.9 (CH_2N_3), 54.8 (C-2^{I}), 60.6, 62.0, 68.1, and 69.6 (C-6^{I}, C-6^{II}, C-6^{III}, OCH_2), 66.5, 68.9, 69.8, 70.5, 70.7, 70.8, 71.5, 72.4, 72.7, 74.0, and 76.2 (C-3^{I}, C-4^{I}, C-5^{I}, C-2^{II}, C-3^{II}, C-4^{II}, C-5^{II}, C-2^{III}, C-3^{III}, C-4^{III}, C-5^{III}), 98.0, 100.3, and 100.9 (C-1^{I}, C-1^{II}, C-1^{III}), 165.1 and 165.4 (2 $CH_3C_6H_4CO$), 168.8–170.1 ($COCH_3$); Anal. Calcd for $C_{62}H_{72}N_4O_{26}$: C, 57.76; H, 5.63; N, 4.35. Found: C, 57.65; H, 5.81; N, 4.19.

3.8. 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-glucopyranoside (14)

To a soln of **13** (141 mg, 0.11 mmol) in MeOH (20 mL) and CH_2Cl_2 (3 mL) was added NaOMe (pH 10), and the mixture was stirred for 24 h. After neutralization with Dowex 50 × 8 (H^+) and filtration, the solution was concentrated. To a solution of the residue in 1-BuOH

(45 mL) was added 1,2-diaminoethane (1 mL, 15 mmol), and the mixture was stirred overnight at 90 °C, then, co-concentrated with toluene. A soln of the residue in Py (50 mL) and Ac_2O (40 mL) was stirred overnight, then co-concentrated with toluene, EtOH and CH_2Cl_2. Column chromatography (3:1 CH_2Cl_2–acetone) of the residue yielded **14**, isolated as a colourless glass (98 mg, 86%); R_f 0.61 (9:1 CH_2Cl_2–MeOH); $[\alpha]_D^{20}$ −12° (*c* 1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$; 2D TOCSY, ROESY, HSQC): δ 1.37–1.38 (m, 4 H, 2 CH_2), 1.58–1.59 (m, 4 H, 2 CH_2), 1.93 (s, 3 H, NHCOC*H*$_3$), 1.96, 2.01, 2.03, 2.04, 2.06, 2.12, and 2.14 (7 s, 3,6,3,6,3,3,3 H, 9 $COCH_3$), 3.26 (t, 2 H, CH_2N_3), 3.46 (m, 1 H, OC*H*H), 3.70 (m, 1 H, H-5^{I}), 4.13 (dd, 1 H, $J_{5,6b}$ 6.4, $J_{6a,6b}$ 11.3 Hz, H-6b^{III}), 4.47 (dd, 1 H, $J_{5,6a}$ 2.2, $J_{6a,6b}$ 12.2 Hz, H-6a^{II}), 4.49 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{III}), 4.58 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1^{II}), 4.65 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1^{I}), 4.95 (dd, 1 H, $J_{2,3}$ 10.4, $J_{3,4}$ 3.4 Hz, H-3^{III}), 5.10 (dd, 1 H, H-2^{III}), 5.14 (t, 1 H, $J_{2,3}$ 9.2, $J_{3,4}$ 9.2 Hz, H-3^{II}), 5.26 (dd, 1 H, $J_{2,3}$ 10.4, $J_{3,4}$ 9.1 Hz, H-3^{I}), 5.34 (d, 1 H, $J_{4,5}$ < 1 Hz, H-4^{III}), 5.79 (d, 1 H, $J_{2,NH}$ 8.9 Hz, N*H*COCH$_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.2–20.5 (CO*C*H$_3$), 22.9 (NHCO*C*H$_3$), 25.2, 26.1, 28.5, and 29.0 (4 CH_2), 51.0 (CH_2N_3), 54.5 (C-2^{I}), 60.6 (C-6^{III}), 61.8 (C-6^{II}), 66.4 (C-4^{III}), 67.9 (C-6^{I}), 68.8 (C-2^{III}), 69.1 (C-4^{I}), 69.2 (OCH_2), 70.3 (C-5^{III}), 70.7 (C-3^{III}), 71.3 (C-2^{II}), 72.1 (C-3^{I}), 72.4 (C-5^{II}), 72.6 (C-3^{II}), 73.1 (C-5^{I}), 75.9 (C-4^{II}), 100.0 (C-1^{II}), 100.2 (C-1^{I}), 100.8 (C-1^{III}), 168.7–170.4 (*C*OCH$_3$, NH*C*OCH$_3$); Anal. Calcd for $C_{44}H_{64}N_4O_{25}$: C, 50.38; H, 6.15; N, 5.34. Found: C, 50.24; H, 6.06; N, 5.35; HRMS of $C_{44}H_{64}N_4O_{25}$ (M, 1048.386): $[M+H]^+$ found 1049.397, calcd 1049.393.

3.9. 6-Azidohexyl β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranoside (15)

To a soln of **14** (50 mg, 47.6 μmol) in MeOH (8 mL) and CH_2Cl_2 (1 mL) was added NaOMe (pH 10), and the mixture was stirred for 2.5 h. After neutralization with Dowex 50 × 8 (H^+) and filtration, the solution was concentrated. Column chromatography (2:1 MeOH–CH_2Cl_2) of the residue yielded **15**, isolated as a colourless glass (29 mg, 90%); R_f 0.63 (4:1 MeOH–CH_2Cl_2); $[\alpha]_D^{20}$ −8° (*c* 1, water); ^{13}C NMR (75.5 MHz, D_2O): δ 22.8 (NDCO*C*H$_3$), 25.3, 26.2, 28.6, and 29.1 (4 CH_2), 51.8 (CH_2N_3), 56.2 (C-2^{I}), 60.7 (C-6^{II}), 61.7 (C-6^{III}), 69.3 (C-6^{I}), 71.1 (OCH_2), 69.2 (C-4^{III}), 70.4, 71.6, 73.2, 73.4, 74.4, 75.0, 75.4, 75.5, 76.0, and 79.1 (C-3^{I}, C-4^{I}, C-5^{I}, C-2^{II}, C-3^{II}, C-4^{II}, C-5^{II}, C-2^{III}, C-3^{III}, C-5^{III}), 101.8 (C-1^{I}), 103.2 (C-1^{II}), 103.6 (C-1^{III}), 175.0 (ND*C*OCH$_3$); HRMS of $C_{26}H_{46}N_4O_{16}$ (M, 670.290): $[M+NH_4]^+$ found 688.330, calcd 688.333. For ^{1}H NMR data, see Table 1.

3.10. 6-Aminohexyl β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranoside (3)

To a soln of **15** (60 mg, 89.4 μmol) in *t*-BuOH (25 mL) and water (20 mL) were added 10% Pd–C (240 mg) and three drops aq 25% NH_3, and the mixture was stirred for 24 h under H_2, then filtered over hyflo, and co-concentrated with toluene. Column chromatography of the residue on a Toyopearl HW-40S column, eluted with aq 0.1 M NH_4OAc, and subsequent lyophilization gave **3**, isolated as a white powder (41 mg, 71%); R_f 0.30 (2:1:1 AcOH–1-BuOH–water); $[\alpha]_D^{20}$ −2° (*c* 1, water); ^{13}C NMR (75.5 MHz, D_2O): δ 22.8 (NDCO*C*H$_3$), 25.2, 25.8, 27.3, and 29.0 (4 CH_2), 40.0 (CH_2ND_2), 56.1 (C-2^{I}), 60.7 (C-6^{II}), 61.6 (C-6^{III}), 69.1 (C-4^{III}), 69.2 (C-6^{I}), 71.5 (OCH_2), 73.4 (C-2^{II}), 70.3, 71.5, 73.1, 74.3, 74.9, 75.4, 75.5, and 75.9 (C-3^{I}, C-4^{I}, C-5^{I}, C-3^{II}, C-5^{II}, C-2^{III}, C-3^{III}, C-5^{III}), 79.0 (C-4^{II}), 101.8 (C-1^{I}), 103.2 (C-1^{II}), 103.5 (C-1^{III}), 175.0 (ND*C*OCH$_3$); HRMS of $C_{26}H_{48}N_2O_{16}$ (M, 644.300): $[M+H]^+$ found 645.306, calcd 645.308. For ^{1}H NMR data, see Table 2.

3.11. 6-Aminohexyl β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-[β-D-galactopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranoside (1)

To a soln of **3** (8.4 mg, 13.0 μmol) in aq 50 mM sodium cacodylate buffer pH 7.5 (600 μL), containing 5 mM $MnCl_2$, bovine serum albumin (BSA) (0.5 mg), and NaN_3 (0.02%), were added alkaline phosphatase (12 U), UDP-galactose (15 mg, 24.5 μmol), and β-1,4-galactosyltransferase (2.5 U). The reaction mixture was incubated for 20 h at 37 °C, then, water (150 μL) was added. UDP-Galactose was removed on a Dowex 1 × 8 (Cl^-) column, eluted with water. The eluate was concentrated, and the residue applied on a Bio Gel P-2 column, eluted with aq 0.1 M NH_4HCO_3 at a flow rate of 40 mL/h. The appropriate fractions were freeze-dried to give **1** (9.6 mg, 91%); R_f 0.15 (2:1:1 AcOH–1-BuOH–water); $[\alpha]_D^{20}$ −3° (*c* 0.8, water); ^{13}C NMR (75.5 MHz, D_2O): δ 22.7 (NDCO*C*H$_3$), 25.2, 25.9, 27.3, and 29.0 (4 CH_2), 40.1 (CH_2ND_2), 55.8 (C-2^{I}), 60.7, 61.7 (2 C), 68.1, and 71.2 (C-6^{I}, C-6^{II}, C-6^{III}, C-6^{IV}, OCH_2), 69.2 (2 C), 71.6 (3 C), 73.0, 73.2, 73.3, 74.2, 75.0, 75.4, 75.9, 76.0, 78.7, and 79.1 (C-3^{I}, C-4^{I}, C-5^{I}, C-2^{II}, C-3^{II}, C-4^{II}, C-5^{II}, C-2^{III}, C-3^{III}, C-4^{III}, C-5^{III}, C-2^{IV}, C-3^{IV}, C-4^{IV}, C-5^{IV}), 101.8, 103.0, 103.4, and 103.6 (C-1^{I}, C-1^{II}, C-1^{III}, C-1^{IV}), 175.0 (ND*C*OCH$_3$); HRMS of $C_{32}H_{58}N_2O_{21}$ (M, 806.353): $[M+H]^+$ found 807.359, calcd 807.361. For ^{1}H NMR data, see Table 3.

3.12. 6-Azidohexyl (2,6-di-*O*-acetyl-3,4-di-*O*-isopropylidene-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-2-deoxy-3,4-di-*O*-*p*-methylbenzoyl-2-phthalimido-β-D-glucopyranoside (17)

A soln of (2,6-di-*O*-acetyl-3,4-di-*O*-isopropylidene-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl trichloroacetimidate donor (**16**)[23] (0.51 g, 0.69 mmol) and **11** (0.31 g, 0.48 mmol) in dry CH_2Cl_2 (5 mL), containing powdered molecular sieves 4 Å (1 g), was stirred for 1 h under Ar. After cooling to 0 °C, TMSOTf (83 μL, 0.46 mmol) was added. The mixture was stirred for 20 min, during which period the temperature was allowed to reach rt, then neutralized with Et_3N, filtered over hyflo, and concentrated. Column chromatography (3:1 toluene–EtOAc with 1% Et_3N) of the residue afforded **17**, isolated as a white foam (0.36 g, 62%); R_f 0.57 (1:1 toluene–EtOAc); $[\alpha]_D^{20}$ +3° (*c* 1, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 1.15–1.16 (m, 4 H, 2 CH_2), 1.26–1.30 (m, 2 H, CH_2), 1.30 and 1.52 [2 s, each 3 H, $C(CH_3)_2$], 1.47–1.52 (m, 2 H, CH_2), 2.04, 2.05, 2.07, and 2.11 (4 s, 3,3,6,3 H, 5 $COCH_3$), 2.25 and 2.32 (2 s, each 3 H, 2 $CH_3C_6H_4CO$), 3.04 (t, 2 H, CH_2N_3), 3.49 (m, 1 H, OC*H*H), 3.60 (m, 1 H, H-5^{II}), 4.47 (dd, 1 H, $J_{1,2}$ 8.4, $J_{2,3}$ 10.7 Hz, H-2^{I}), 4.63 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{II}), 4.85 (t, 1 H, H-2^{III}), 4.93 (t, 1 H, H-2^{II}), 5.15 (t, 1 H, H-3^{II}), 5.36 (t, 1 H, H-4^{I}), 5.47 (d, 1 H, H-1^{I}), 6.18 (dd, 1 H, $J_{3,4}$ 9.2 Hz, H-3^{I}), 7.03 and 7.13 (2 d, each 2 H, Phth), 7.62 and 7.78 (2 d, each 4 H, 2 $CH_3C_6H_4CO$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.5–20.6 ($COCH_3$), 21.3 and 21.4 (2 $CH_3C_6H_4CO$), 25.2 and 26.0 (2 CH_2), 25.9 and 27.1 [$(CH_3)_2C$], 28.4 and 28.9 (2 CH_2), 50.9 (CH_2N_3), 54.7 (C-2^{I}), 62.1, 62.9, 68.1, and 69.6 (C-6^{I}, C-6^{II}, C-6^{III}, OCH_2), 69.6, 70.7 (2 C), 71.4, 72.3, 72.6 (2 C), 72.9, 74.0, 75.9, and 76.7 (C-3^{I}, C-4^{I}, C-5^{I}, C-2^{II}, C-3^{II}, C-4^{II}, C-5^{II}, C-2^{III}, C-3^{III}, C-4^{III}, C-5^{III}), 97.9, 100.3, and 100.5 (C-1^{I}, C-1^{II}, C-1^{III}), 110.6 [$(CH_3)_2C$], 165.1 and 165.4 (2 $CH_3C_6H_4CO$), 168.9–170.5 ($COCH_3$); HRMS of $C_{61}H_{72}N_4O_{24}$ (M, 1244.453): $[M+NH_4]^+$ found 1262.475, calcd 1262.488.

3.13. 6-Azidohexyl (2,6-di-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-2-deoxy-3,4-di-*O*-*p*-methylbenzoyl-2-phthalimido-β-D-glucopyranoside (18)

To a soln of **17** (155 mg, 0.13 mmol) in CH_2Cl_2 (2.5 mL) at 0 °C, was added aq 90% TFA (0.25 mL). The mixture was stirred for 1.5 h, then co-concentrated with toluene to give **18**, isolated as a colourless glass (127 mg, 84%); R_f 0.20 (1:2 toluene–EtOAc); $[\alpha]_D^{20}$ −6° (*c* 1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$; 2D TOCSY, ROESY): δ 1.11–1.17 (m, 4 H, 2 CH_2), 1.25–1.30 (m, 2 H, CH_2), 1.44–1.50 (m, 2 H, CH_2), 2.04, 2.07, 2.10, and 2.12 (4 s, 6,3,3,3 H, 5 $COCH_3$), 2.28 and 2.34 (2 s, each 3 H, 2 $CH_3C_6H_4CO$), 2.81 (bs, 1 H, OH), 3.04 (t, 2 H, CH_2N_3), 3.24 (bs, 1 H, OH), 3.48 (m, 1 H, OC*H*H), 3.72 (dd, 1 H, $J_{3,4}$ 9.3, $J_{4,5}$ 9.7 Hz, H-4^{II}), 3.78 (dd, 1 H, $J_{5,6b}$ 7.7, $J_{6a,6b}$ 11.6 Hz, H-$6b^{I}$), 3.83 (bs, 1 H, H-4^{III}), 4.05 (m, 1 H, H-5^{I}), 4.13 (dd, 1 H, $J_{5,6b}$ 5.7, $J_{6a,6b}$ 11.9 Hz, H-$6b^{II}$), 4.22 (dd, 1 H, $J_{5,6a}$ 6.4, $J_{6a,6b}$ 11.4 Hz, H-$6b^{III}$), 4.42 (dd, 1 H, $J_{5,6a}$ 1.5 Hz, H-$6a^{II}$), 4.46 (dd, 1 H, $J_{1,2}$ 8.5, $J_{2,3}$ 10.7 Hz, H-2^{I}), 4.62 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1^{II}), 4.81 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.1 Hz, H-2^{III}), 4.92 (dd, 1 H, $J_{2,3}$ 9.2 Hz, H-2^{II}), 5.13 (t, 1 H, H-3^{II}), 5.34 (dd, 1 H, $J_{3,4}$ 9.3, $J_{4,5}$ 9.9 Hz, H-4^{I}), 5.45 (d, 1 H, H-1^{I}), 6.16 (dd, 1 H, H-3^{I}), 7.04 and 7.15 (2 d, each 2 H, Phth), 7.62 and 7.78 (2 d, each 4 H, 2 $CH_3C_6H_4CO$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.4–20.6 ($COCH_3$), 21.2 and 21.3 (2 $CH_3C_6H_4CO$), 25.1, 25.9, 28.3, and 28.8 (4 CH_2), 50.8 (CH_2N_3), 54.6 (C-2^{I}), 62.0, 62.3, 68.1, and 69.4 (C-6^{I}, C-6^{II}, C-6^{III}, OCH_2), 68.3, 69.8, 70.7, 71.2, 72.1, 72.2, 72.5, 72.7, 73.7, 76.1, and 77.1 (C-3^{I}, C-4^{I}, C-5^{I}, C-2^{II}, C-3^{II}, C-4^{II}, C-5^{II}, C-2^{III}, C-3^{III}, C-4^{III}, C-5^{III}), 97.8, 100.4, and 100.7 (C-1^{I}, C-1^{II}, C-1^{III}), 165.0 and 165.3 (2 $CH_3C_6H_4CO$), 169.1–170.7 ($COCH_3$); HRMS of $C_{58}H_{68}N_4O_{24}$ (M, 1204.422): $[M+H]^+$ found 1205.399, calcd 1205.430.

3.14. 6-Azidohexyl (2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-2-deoxy-3,4-di-*O*-*p*-methylbenzoyl-2-phthalimido-β-D-glucopyranoside (19)

To a soln of **18** (86 mg, 72.8 μmol) in dry MeCN (1 mL) were added trimethyl orthoacetate (23 μL, 182 μmol) and a catalytic amount of *p*-toluenesulfonic acid. After stirring for 3.5 h, aq 80% AcOH (50 μL) was added, and the stirring was continued for 1 h. The mixture was diluted with CH_2Cl_2, washed with cold aq satd $NaHCO_3$ and cold water, dried, filtered, and concentrated. Column chromatography (1:4 toluene–EtOAc) of the residue afforded **19**, isolated as a white foam (78 mg, 85%); R_f 0.49 (1:4 toluene–EtOAc); $[\alpha]_D^{20}$ −10° (*c* 1, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 1.14–1.16 (m, 4 H, 2 CH_2), 1.23–1.28 (m, 2 H, CH_2), 1.45–1.53 (m, 2 H, CH_2), 2.03, 2.04, 2.06, 2.07, 2.11, and 2.15 (6 s, each 3 H, 6 $COCH_3$), 2.27 and 2.34 (2 s, each 3 H, 2 $CH_3C_6H_4CO$), 2.76 (bd, 1 H, OH), 3.05 (t, 2 H, CH_2N_3), 3.49 (m, 1 H, OC*H*H), 3.61 (m, 1 H, H-5^{II}), 4.47 (dd, 1 H, $J_{1,2}$ 8.4, $J_{2,3}$ 10.7 Hz, H-2^{I}), 4.63 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{II}), 4.85 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 9.9 Hz, H-2^{III}), 4.92 (dd, 1 H, $J_{2,3}$ 9.3 Hz, H-2^{II}), 5.14 (t, 1 H, H-3^{II}), 5.28 (d, 1 H, $J_{3,4}$ 3.3, $J_{4,5}$ < 1 Hz, H-4^{III}), 5.35 (t, 1 H, H-4^{I}), 5.46 (d, 1 H, H-1^{I}), 6.18 (dd, 1 H, $J_{3,4}$ 9.1 Hz, H-3^{I}), 7.04 and 7.14 (2 d, each 2 H, Phth), 7.62 and 7.78 (2 d, each 4 H, 2 $CH_3C_6H_4CO$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.5–20.6 ($COCH_3$), 21.4 (2 $CH_3C_6H_4CO$), 25.3, 26.0, 28.4, and 28.9 (4 CH_2), 50.9 (CH_2N_3), 54.8 (C-2^{I}), 61.3, 62.2, 68.2, and 69.6 (C-6^{I}, C-6^{II}, C-6^{III}, OCH_2), 69.1, 69.9, 70.7, 70.8, 71.4 (2 C), 72.5, 72.6, 72.8, 74.0, and 76.2 (C-3^{I}, C-4^{I}, C-5^{I}, C-2^{II}, C-3^{II}, C-4^{II}, C-5^{II}, C-2^{III}, C-3^{III}, C-4^{III}, C-5^{III}), 165.1 and 165.4 (2

$CH_3C_6H_4CO$), 169.2–170.8 ($COCH_3$); HRMS of $C_{60}H_{70}N_4O_{25}$ (M, 1246.432): $[M+NH_4]^+$ found 1264.443, calcd 1264.467.

3.15. Benzyl (3,4,6-tri-*O*-acetyl-2-deoxy-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 (22)

A soln of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (**5**)[20] (2.62 g, 4.52 mmol) and (4-*O*-acetyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-1,2,3,6-tetra-*O*-benzyl-β-D-glucopyranose (**21**)[25] (3.39 g, 3.66 mmol) in dry CH_2Cl_2 (80 mL), containing powdered molecular sieves 4 Å (2 g), was stirred for 30 min under Ar. The mixture was cooled to −70 °C, and TMSOTf (0.1 mL, 0.54 mmol) was added. The mixture was stirred for 2.5 h, during which period the temperature was allowed to reach rt, then neutralized with Et_3N, and filtered over hyflo. The filtrate was washed with water, dried, filtered, and concentrated. Low-pressure column chromatography (9:1 toluene–EtOAc) of the residue gave **22**, isolated as a white solid (4.26 g, 86%); R_f 0.37 (2:1 toluene–EtOAc); $[\alpha]_D^{20}$ +12° (*c* 1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$; 2D TOCSY, ROESY, HSQC): δ 1.81, 2.01, 2.02, and 2.06 (4 s, each 3 H, 4 $COCH_3$), 3.02 (m, 1 H, H-5^I), 3.46 (t, 1 H, H-5^{II}), 3.52 (dd, 1 H, $J_{5,6a}$ 3.6, $J_{6a,6b}$ 10.9 Hz, H-6^I), 3.60 (dd, 1 H, $J_{2,3}$ 9.6, $J_{3,4}$ 3.3 Hz, H-3^{II}), 3.81 (m, 1 H, H-5^{III}), 3.92 (m, 1 H, H-4^I), 4.22 (dd, 1 H, $J_{5,6b}$ 4.1, $J_{6a,6b}$ 12.3 Hz, H-6b^{III}), 4.32 (d, 1 H, $J_{1,2}$ 7.4 Hz, H-1^{II}), 4.33 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1^I), 4.15 and 4.34 (2 d, each 1 H, $OCH_2C_6H_5$), 4.42 (d, 1 H, $OCHHC_6H_5$), 4.51 (d, 1 H, $OCHHC_6H_5$), 4.68 and 4.84 (2 d, each 1 H, $OCH_2C_6H_5$), 4.57 and 4.86 (2 d, each 1 H, $OCH_2C_6H_5$), 4.65 and 4.89 (2 d, each 1 H, $OCH_2C_6H_5$), 5.17 (t, 1 H, $J_{4,5}$ 9.5 Hz, H-4^{III}), 5.40 (d, 1 H, $J_{4,5}<1$ Hz, H-4^{II}), 5.55 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1^{III}), 5.80 (dd, 1 H, $J_{2,3}$ 10.7, $J_{3,4}$ 9.5 Hz, H-3^{III}); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 19.9–20.2 ($COCH_3$), 54.5 (C-2^{III}), 61.1 (C-6^{III}), 67.3 and 67.9 (C-6^I, C-6^{II}), 70.4, 72.7, 73.0, 74.0, 74.5, and 74.7 (6 $OCH_2C_6H_5$), 68.4, 69.3, 70.1, 71.3, 72.2, 74.3, 75.2, 78.4, 78.9, 81.2, and 82.2 (C-2^I, C-3^I, C-4^I, C-5^I, C-2^{II}, C-3^{II}, C-4^{II}, C-5^{II}, C-3^{III}, C-4^{III}, C-5^{III}), 97.8 (C-1^{III}), 101.3 and 101.9 (C-1^I, C-1^{II}), 168.9, 169.1, 169.5, and 170.1 (4 $COCH_3$); HRMS of $C_{76}H_{79}NO_{21}$ (M, 1341.514): $[M+NH_4]^+$ found 1359.546, calcd 1359.549.

3.16. Benzyl (2-deoxy-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 (23)

To a soln of **22** (6.88 g, 5.12 mmol) in CH_2Cl_2 (13.5 mL) and MeOH (54 mL) was added NaOMe (pH 9). The mixture was stirred for 2 h, then neutralized with Dowex 50 × 8 (H^+), filtered, and concentrated. Low-pressure column chromatography (9:1→1:1 toluene–EtOAc) of the residue gave **23**, isolated as a white solid (5.1 g, 82%); R_f 0.10 (1:1 toluene–EtOAc); $[\alpha]_D^{20}$ +1° (*c* 1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$; 2D TOCSY, ROESY, HSQC): δ 2.07 (s, 3 H, $COCH_3$), 2.97 (m, 1 H, H-5^I), 3.23 (dd, 1 H, $J_{5,6b}$ 7.4, $J_{6a,6b}$ 9.6 Hz, H-6b^{II}), 3.68 (t, 1 H, H-4^{III}), 3.81 (dd, 1 H, $J_{5,6b}$ 3.1, $J_{6a,6b}$ 12.3 Hz, H-6b^{III}), 3.87 (dd, 1 H, H-4^I), 3.92 (dd, 1 H, $J_{5,6a}$ 2.0 Hz, H-6a^{III}), 4.04 (dd, 1 H, $J_{1,2}$ 8.1, $J_{2,3}$ 10.6 Hz, H-2^{III}), 4.08 and 4.22 (2 d, each 1 H, $OCH_2C_6H_5$), 4.24 and 4.38 (2 d, each 1 H, $OCH_2C_6H_5$), 4.24 and 4.45 (2 d, each 1 H, $OCH_2C_6H_5$), 4.66 and 4.83 (2 d, each 1 H, $OCH_2C_6H_5$), 4.55 and 4.85 (2 d, each 1 H, $OCH_2C_6H_5$), 4.63 and 4.87 (2 d, each 1 H, $OCH_2C_6H_5$), 5.39 (d, 1 H, H-1^{III}), 5.53 (d, 1 H, $J_{3,4}$ 3.5, $J_{4,5}<1$ Hz, H-4^{II}); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.6 ($COCH_3$), 56.6 (C-2^{III}), 61.0 (C-6^{III}), 67.3 and 67.7 (C-6^I, C-6^{II}), 70.4, 72.7, 73.1, 74.0, 74.6, and 74.7 (6 $OCH_2C_6H_5$), 70.2 (2 C), 70.6, 71.8, 74.3, 75.5 (2 C), 78.2, 80.1, 81.2, and 82.3 (C-2^I, C-3^I, C-4^I, C-5^I, C-2^{II}, C-3^{II}, C-4^{II}, C-5^{II}, C-3^{III}, C-4^{III}, C-5^{III}), 99.0 (C-1^{III}), 101.4 and 101.9 (C-1^I, C-1^{II}), 170.9 ($COCH_3$); HRMS of $C_{70}H_{73}NO_{18}$ (M, 1215.482): $[M+NH_4]^+$ found 1233.497, calcd 1233.517.

3.17. Benzyl (6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-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 (24)

To a soln of **23** (4.25 g, 3.49 mmol) in dry CH_2Cl_2 (35 mL) was added dry Py (1.67 mL), 4-dimethylaminopyridine (0.14 g, 1.1 mmol), *tert*-butyldiphenylsilylchloride (0.61 mL, 4.35 mmol), and Et_3N (1.20 mL). The mixture was stirred for 48 h, washed with cold water, cold aq satd $NaHCO_3$, and cold water, dried, filtered, and concentrated. Low-pressure column chromatography (17:3→3:1 toluene–EtOAc) of the residue gave **24**, isolated as a white solid (4.51 g, 89%); R_f 0.49 (1:1 toluene–EtOAc); $[\alpha]_D^{20}$ −15° (*c* 1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$; 2D TOCSY, ROESY, HSQC): δ 1.05 [s, 9 H, $(CH_3)_3CSi$], 1.97 (s, 3 H, $COCH_3$), 3.03 (m, 1 H, H-5^I), 3.51 (dd, 1 H, $J_{5,6b}$ 4.0, $J_{6a,6b}$ 10.8 Hz, H-6b^I), 3.63 (dd, 1 H, $J_{2,3}$ 9.6, $J_{3,4}$ 3.5 Hz, H-3^{II}), 3.89 (m, 1 H, H-4^I), 3.98 (d, 2 H, H-6a^{III}, H-6b^{III}), 4.05 (dd, 1 H, $J_{1,2}$ 8.3, $J_{2,3}$ 11.0 Hz, H-2^{III}), 4.28 (d, 1 H, $J_{1,2}$ 7.2 Hz, H-1^{II}), 4.12 and 4.28 (2 d, each 1 H, $OCH_2C_6H_5$), 4.34 (d, 1 H, $J_{1,2}$ 7.4 Hz, H-1^I), 4.16 and 4.36 (2 d, each 1 H, $OCH_2C_6H_5$), 4.40 (dd, 1 H, $J_{3,4}$ 8.2 Hz, H-3^{III}), 4.25 and 4.46 (2 d, each 1 H, $OCH_2C_6H_5$), 4.66 and 4.83 (2 d, each 1 H, $OCH_2C_6H_5$), 4.56 and 4.86 (2 d, each 1 H, $OCH_2C_6H_5$), 4.61 and 4.87 (2 d, each 1 H, $OCH_2C_6H_5$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 19.0 [$(CH_3)_3CSi$], 20.5 ($COCH_3$), 26.6 [$(CH_3)_3CSi$], 56.6 (C-2^{III}), 64.8 (C-6^{III}), 67.6 and 68.1 (C-6^I, C-6^{II}), 70.7, 72.9, 73.2, 74.3, 74.8, and 74.9 (6 $OCH_2C_6H_5$), 70.1, 70.9, 72.5, 74.0, 74.4,

74.7, 75.5, 77.2, 79.1, 81.5, and 82.5 (C-2^{I}, C-3^{I}, C-4^{I}, C-5^{I}, C-2^{II}, C-3^{II}, C-4^{II}, C-5^{II}, C-3^{III}, C-4^{III}, C-5^{III}), 98.3 (C-1^{III}), 101.7 and 102.2 (C-1^{I}, C-1^{II}), 169.7 ($COCH_3$); HRMS of $C_{86}H_{91}NO_{18}Si$ (M, 1453.600): $[M+NH_4]^+$ found 1471.598, calcd 1471.635.

3.18. Benzyl (6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-3,4-di-*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 (25)

To a soln of **24** (1.17 g, 0.80 mmol) in dry Py (115 mL) was added dropwise at 0 °C, a soln of *p*-methylbenzoyl chloride (0.45 mL, 3.40 mmol) in dry CH_2Cl_2 (15 mL). The mixture was stirred under Ar for 20 h at rt, diluted with CH_2Cl_2 and poured into cold water. The organic layer was washed with aq satd $NaHCO_3$, dried, filtered, and concentrated. Low-pressure column chromatography (24:1 toluene–EtOAc) of the residue gave **25**, isolated as a white solid (1.01 g, 74%); R_f 0.57 (9:1 toluene–EtOAc); $[\alpha]_D^{20}$ +11° (*c* 1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$; 2D TOCSY, ROESY, HSQC): δ 1.03 [s, 9 H, $(CH_3)_3CSi$], 2.06 (s, 3 H, $COCH_3$), 2.21 and 2.31 (2 s, each 3 H, 2 $CH_3C_6H_4CO$), 3.08 (m, 1 H, H-5^{I}), 3.33 (dd, 1 H, $J_{5,6b}$ 6.4, $J_{6a,6b}$ 9.9 Hz, H-$6b^{II}$), 3.37 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.5 Hz, H-2^{II}), 3.46 (dd, 1 H, $J_{5,6a}<1$, $J_{6a,6b}$ 11.0 Hz, H-$6a^{I}$), 3.53 (t, 1 H, H-5^{II}), 3.58 (dd, 1 H, $J_{5,6b}$ 4.0 Hz, H-$6b^{I}$), 4.13 and 4.36 (2 d, each 1 H, $OCH_2C_6H_5$), 4.21 and 4.43 (2 d, each 1 H, $OCH_2C_6H_5$), 4.31 and 4.50 (2 d, each 1 H, $OCH_2C_6H_5$), 4.51 (dd, 1 H, $J_{1,2}$ 8.1, $J_{2,3}$ 10.7 Hz, H-2^{III}), 4.70 and 4.87 (2 d, each 1 H, $OCH_2C_6H_5$), 4.59 and 4.88 (2 d, each 1 H, $OCH_2C_6H_5$), 4.65 and 4.93 (2 d, each 1 H, $OCH_2C_6H_5$), 5.74 (d, 1 H, H-1^{III}), 6.29 (dd, 1 H, $J_{3,4}$ 9.2 Hz, H-3^{III}); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 18.9 [$(CH_3)_3CSi$], 20.5 ($COCH_3$), 21.3 and 21.4 (2 $CH_3C_6H_4CO$), 26.5 [$(CH_3)_3CSi$], 55.4 (C-2^{III}), 62.8 (C-6^{III}), 67.6 (C-6^{I}), 68.1 (C-6^{II}), 70.6, 72.9, 73.2, 74.3, 74.8, and 74.9 (6 $OCH_2C_6H_5$), 69.7 (C-4^{III}), 70.2 (C-4^{II}), 70.7 (C-3^{III}), 72.7 (C-5^{II}), 74.7 (C-5^{I}), 74.8 and 75.4 (C-4^{I}, C-5^{III}), 77.2 (C-3^{II}), 79.2 (C-2^{II}), 81.5 and 82.5 (C-2^{I}, C-3^{I}), 98.4 (C-1^{III}), 101.8 and 102.2 (C-1^{I}, C-1^{II}), 164.8 and 165.4 (2 $CH_3C_6H_4CO$), 169.3 ($COCH_3$); HRMS of $C_{102}H_{103}NO_{20}Si$ (M, 1689.684): $[M+NH_4]^+$ found 1707.713, calcd 1707.718.

3.19. (6-*O*-*Tert*-butyldiphenylsilyl-2-deoxy-3,4-di-*O*-*p*-methylbenzoyl-2-phthalimido-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-α,β-D-glucopyranose (27)

To a soln of **25** (1.46 g, 0.87 mmol) in EtOH (45 mL) and EtOAc (45 mL) was added 10% Pd–C (1.78 g), and the mixture was stirred for 8 h, while H_2 was bubbled through. After filtration over hyflo and concentration, column chromatography (19:1 CH_2Cl_2–MeOH) of the residue gave a colourless syrup (0.94 g, 90%). A soln of the syrup in Py (75 mL) and Ac_2O (75 mL) was stirred overnight, then co-concentrated with toluene, EtOH, and CH_2Cl_2. Low-pressure column chromatography (9:1 toluene–EtOAc) of the residue gave **26**, isolated as a colourless syrup (1.08 g, quantitatively). To a soln of **26** (0.11 g, 78 μmol) in dry DMF (5 mL) was added hydrazinium acetate (9 mg, 118 μmol). The mixture was stirred for 4 h, washed with aq satd NaCl, dried, filtered, and co-concentrated with toluene. Low-pressure column chromatography (1:1→2:3 toluene–EtOAc) of the residue gave **27**, isolated as a white solid (69 mg, 65%); R_f 0.42 (1:1 toluene–EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$; 2D TOCSY, ROESY): δ 1.05 [s, 9 H, $(CH_3)_3CSi$], 1.88, 1.96, 2.03, 2.10, and 2.14 (5 s, 3,3,6,3,3 H, 6 $COCH_3$), 2.25 and 2.34 (2 s, each 3 H, 2 $CH_3C_6H_4CO$), 3.48 (m, 1 H, H-5^{II}), 3.54 (m, 0.5 H, H-$5^{I\beta}$), 3.83 (dd, 1 H, $J_{5,6a}$ 1.3, $J_{6a,6b}$ 11.0 Hz, H-$6a^{III}$), 4.25 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1^{II}), 4.38 (dd, 1 H, $J_{1,2}$ 8.3, $J_{2,3}$ 10.8 Hz, H-2^{III}), 4.66 (d, 0.5 H, $J_{1\beta,2\beta}$ 7.9 Hz, H-$1^{I\beta}$), 4.76 (dd, 0.5 H, $J_{2\beta,3\beta}$ 9.6 Hz, H-$2^{I\beta}$), 4.77 (dd, 0.5 H, $J_{1\alpha,2\alpha}$ 3.5, $J_{2\alpha,3\alpha}$ 10.1 Hz, H-$2^{I\alpha}$), 4.84 (dd, 1 H, $J_{2,3}$ 9.8 Hz, H-2^{II}), 5.08 (t, 0.5 H, H-$3^{I\beta}$), 5.31 (d, 0.5 H, H-$1^{I\alpha}$), 5.38 (t, 0.5 H, H-$3^{I\alpha}$), 5.44 (t, 1 H, H-4^{III}), 6.20 (m, 1 H, H-3^{III}), 7.02 and 7.12 (2 d, each 2 H, Phth); HRMS of $C_{70}H_{77}NO_{25}Si$ (M, 1359.455): $[M+H]^+$ found 1360.424, calcd 1360.463.

3.20. (6-*O*-*Tert*-butyldiphenylsilyl-2-deoxy-3,4-di-*O*-*p*-methylbenzoyl-2-phthalimido-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-α,β-D-glucopyranosyl trichloroacetimidate (28)

To a soln of **27** (75 mg, 55 μmol) in dry CH_2Cl_2 (0.5 mL) were added, at 0 °C under Ar, trichloroacetonitrile (29 μL, 0.29 μmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (0.90 μL). After stirring at rt for 1.5 h, followed by concentration, low-pressure column chromatography (1:1 toluene–EtOAc with 1% Et_3N) of the residue gave **28**, isolated as a slightly yellow foam (58 mg, 69%); R_f 0.63 (1:1 toluene–EtOAc); ^{1}H NMR α-product (300 MHz, $CDCl_3$): δ 1.06 [s, 9 H, $(CH_3)_3CSi$], 1.88, 1.97, 1.98, 2.05, 2.10, and 2.15 (6 s, each 3 H, 6 $COCH_3$), 2.24 and 2.34 (2 s, each 3 H, 2 $CH_3C_6H_4CO$), 3.50 (m, 1 H, H-5^{II}), 3.75 (t, 1 H, H-4^{I}), 4.27 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{II}), 4.38 (dd, 1 H, $J_{1,2}$ 8.1, $J_{2,3}$ 10.8 Hz, H-2^{III}), 4.44 (dd, 1 H, $J_{5,6a}$ 1.5, $J_{6a,6b}$ 11.7 Hz, H-$6a^{I}$), 4.85 (dd, 1 H, $J_{2,3}$ 9.8 Hz, H-2^{II}), 5.02 (dd, 1 H, $J_{1,2}$ 3.8, $J_{2,3}$ 10.2 Hz, H-2^{I}), 6.24 (dd, 1 H, $J_{3,4}$ 9.2 Hz, H-3^{III}), 6.45 (d, 1 H, H-1^{I}), 7.01 and 7.11 (2 d, each 2 H, Phth), 8.63 (s, 1 H, $OC[NH]CCl_3$).

3.21. 6-Azidohexyl (6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-3,4-di-*O*-*p*-methylbenzoyl-2-phthalimido-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-2-deoxy-3,4-di-*O*-*p*-methylbenzoyl-2-phthalimido-β-D-glucopyranoside (29)

A soln of **28** (0.36 g, 0.24 mmol) and **11** (0.18 g, 0.29 mmol) in dry CH_2Cl_2 (5 mL), containing molecular sieves 4 Å (0.1 g), was stirred for 30 min under Ar. The mixture was cooled to −40 °C, and TMSOTf (4 μL, 24 μmol) was added. The mixture was stirred for 2 h, during which period the temperature was allowed to reach rt, then neutralized with Et_3N, washed with aq satd $NaHCO_3$ and water, dried, filtered, and concentrated. Column chromatography (3:1 toluene–EtOAc) of the residue gave crude **29** after concentration. Gel-filtration of the residue on a LH-20 column eluted with CH_2Cl_2–MeOH (1:1) gave **29**, isolated as a white solid (0.18 g, 38%); R_f 0.88 (1:1 toluene–EtOAc); $[\alpha]_D^{20}$ +4° (*c* 1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$; 2D TOCSY, ROESY): δ 1.05 [s, 9 H, $(CH_3)_3CSi$], 1.11–1.16 (m, 4 H, 2 CH_2), 1.25–1.29 (m, 2 H, CH_2), 1.42–1.50 (m, 2 H, CH_2), 1.88, 1.94, 2.02, 2.03, 2.04, and 2.12 (6 s, each 3 H, 6 $COCH_3$), 2.25, 2.26, and 2.33 (3 s, 3,3,6 H, 4 $CH_3C_6H_4CO$), 3.02 (t, 2 H, CH_2N_3), 3.62 (t, 1 H, H-4^{II}), 3.76 (dd, 1 H, $J_{5,6b}$ 7.7, $J_{6a,6b}$ 11.6 Hz, H-6b^{IV}), 3.83 (dd, 1 H, $J_{5,6a}$ 1.5, $J_{6a,6b}$ 11.2 Hz, H-6a^{I}), 4.18 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1^{III}), 4.46 (dd, 1 H, $J_{1,2}$ 8.5, $J_{2,3}$ 10.8 Hz, H-2^{IV}), 4.57 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1^{II}), 4.81 (dd, 1 H, $J_{2,3}$ 9.8 Hz, H-2^{III}), 4.87 (dd, 1 H, $J_{2,3}$ 9.0 Hz, H-2^{II}), 5.01 (t, 1 H, H-3^{II}), 5.33 (t, 1 H, H-4^{IV}), 5.47 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1^{I}); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 19.1 [$(CH_3)_3CSi$], 20.4–20.6 ($COCH_3$), 21.4 and 21.5 ($CH_3C_6H_4CO$), 25.3, 26.1, 28.4, and 29.0 (4 CH_2), 26.6 [$(CH_3)_3CSi$], 51.0 (CH_2N_3), 54.8 and 55.0 (C-2^{I}, C-2^{IV}), 61.3, 62.1, 63.0, 68.3, and 69.8 (C-6^{I}, C-6^{II}, C-6^{III}, C-6^{IV}, OCH_2), 68.8, 69.8, 69.9, 70.3, 70.8, 70.9, 71.4, 72.4, 72.7, 74.1, 74.4 (2 C), 75.3, and 75.6 (C-3^{I}, C-4^{I}, C-5^{I}, C-2^{II}, C-3^{II}, C-4^{II}, C-5^{II}, C-2^{III}, C-3^{III}, C-4^{III}, C-5^{III}, C-3^{IV}, C-4^{IV}, C-5^{IV}), 97.9, 98.0, 100.5, and 100.6 (C-1^{I}, C-1^{II}, C-1^{III}, C-1^{IV}), 164.9, 165.2, 165.4, and 165.5 (4 $CH_3C_6H_4CO$), 168.7, 169.3, 169.4, 169.7, 170.1, and 170.2 (6 $COCH_3$); HRMS of $C_{106}H_{113}N_5O_{33}Si$ (M, 2011.708): $[M+H]^+$ found 2012.795, calcd 2012.717.

3.22. 6-Azidohexyl (2-deoxy-3,4-di-*O*-*p*-methylbenzoyl-2-phthalimido-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-2-deoxy-3,4-di-*O*-*p*-methylbenzoyl-2-phthalimido-β-D-glucopyranoside (30)

A soln of **29** (60 mg, 29.8 μmol) in 1.0 M TBAF in THF (0.7 mL) and AcOH (0.7 mL) was stirred for 1 h at pH 6, at 0 °C, then for 3 h at rt. The mixture was washed with water and aq 10% NaCl, dried, filtered, and concentrated. Column chromatography (1:1 toluene–EtOAc) of the residue gave **30**, isolated as a white solid (48 mg, 90%); R_f 0.42 (1:1 toluene–EtOAc); $[\alpha]_D^{20}$ +2° (*c* 1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$; 2D TOCSY, ROESY): δ 1.10–1.16 (m, 4 H, 2 CH_2), 1.22–1.29 (m, 2 H, CH_2), 1.42–1.46 (m, 2 H, CH_2), 1.96, 1.97, 2.01, 2.04, 2.07, and 2.21 (6 s, each 3 H, 6 $COCH_3$), 2.27 and 2.33 (2 s, each 6 H, 4 $CH_3C_6H_4CO$), 3.03 (t, 2 H, CH_2N_3), 3.96 (dd, 1 H, $J_{5,6b}$ 5.7, $J_{6a,6b}$ 11.9 Hz, H-6b^{II}), 4.27 (dd, 1 H, $J_{5,6a}$ 1.9 Hz, H-6a^{II}), 4.28 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1^{III}), 4.39 (dd, 1 H, $J_{1,2}$ 8.3, $J_{2,3}$ 10.9 Hz, H-2^{IV}), 4.44 (dd, 1 H, $J_{1,2}$ 8.5, $J_{2,3}$ 10.8 Hz, H-2^{I}), 4.57 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1^{II}), 4.86 (dd, 1 H, $J_{2,3}$ 9.4 Hz, H-2^{II}), 4.90 (dd, 1 H, $J_{2,3}$ 9.9 Hz, H-2^{III}), 5.04 (t, 1 H, H-3^{II}), 5.31 (dd, 1 H, $J_{3,4}$ 9.0, $J_{4,5}$ 10.1 Hz, H-4^{I}), 5.43 (d, 1 H, H-1^{I}), 5.46 (t, 1 H, H-4^{IV}), 5.56 (d, 1 H, $J_{3,4}$ 3.9, $J_{4,5}$ < 1 Hz, H-4^{III}), 5.60 (d, 1 H, H-1^{IV}), 6.12 (dd, 1 H, $J_{3,4}$ 9.2 Hz, H-3^{IV}), 6.16 (dd, 1 H, H-3^{I}); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 19.5–20.8 ($COCH_3$), 21.4 ($CH_3C_6H_4CO$), 25.3, 26.1, 28.4, and 28.9 (4 CH_2), 51.0 (CH_2N_3), 54.6 and 54.8 (C-2^{I}, C-2^{IV}), 61.2, 62.0, 68.2, and 69.6 (2 C) (C-6^{I}, C-6^{II}, C-6^{III}, C-6^{IV}, OCH_2), 68.9, 69.0, 69.9, 70.2, 70.3, 70.8 (2 C), 71.4, 72.5 (2 C), 74.1, 74.9, 75.6, and 78.2 (C-3^{I}, C-4^{I}, C-5^{I}, C-2^{II}, C-3^{II}, C-4^{II}, C-5^{II}, C-2^{III}, C-3^{III}, C-4^{III}, C-5^{III}, C-3^{IV}, C-4^{IV}, C-5^{IV}), 98.0, 98.8, 100.4, and 100.5 (C-1^{I}, C-1^{II}, C-1^{III}, C-1^{IV}), 165.1 and 165.4 ($CH_3C_6H_4CO$), 168.1–170.9 ($COCH_3$); HRMS of $C_{90}H_{95}N_5O_{33}$ (M, 1773.591): $[M+H]^+$ found 1774.675, calcd 1774.599.

3.23. 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-di-*O*-*p*-methylbenzoyl-2-phthalimido-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-2-deoxy-3,4-di-*O*-*p*-methylbenzoyl-2-phthalimido-β-D-glucopyranoside (31)

(a) A soln of **19** (129 mg, 103 μmol) and (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-di-*O*-*p*-methylbenzoyl-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (**20**)[17] (202 mg, 155 μmol) in dry CH_2Cl_2 (2 mL), containing powdered molecular sieves 4 Å (0.2 g), was stirred for 30 min under Ar, then cooled to −20 °C, and TMSOTf (3 μL, 16 μmol) was added. The mixture was stirred for 1 h, during which period the temperature was allowed to reach rt, then neutralized with Et_3N, filtered over hyflo, and concentrated. Column chromatography (1:2 toluene–EtOAc) of the residue yielded after concentration crude **31**. Gel-filtration of the residue on a LH-20 column, eluted with CH_2Cl_2–MeOH (1:1), gave **31**, isolated as white foam (30 mg, 12%).

(b) A soln of **30** (84 mg, 47 μmol) and (**12**)[19] (125 mg, 160 μmol) in dry CH_2Cl_2 (1 mL), containing powdered

molecular sieves 4 Å (0.1 g), was stirred for 0.5 h under Ar, then cooled to −40 °C, and TMSOTf (3 μL, 16 μmol) was added. The mixture was stirred for 30 min at −40 °C, then at rt for 3 h, and concentrated. Column chromatography (1:1 toluene–EtOAc) of the residue yielded crude **31**. Gel-filtration of the residue on a LH-20 column, eluted with 1:1 CH_2Cl_2–MeOH, gave **31**, isolated as a white foam (68 mg, 60%); R_f 0.74 (1:2 toluene–EtOAc); $[\alpha]_D^{20}$ −4° (*c* 1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$; 2D TOCSY, ROESY, HSQC): δ 1.08–1.17 (m, 4 H, 2 CH_2), 1.23–1.30 (m, 2 H, CH_2), 1.41–1.49 (m, 2 H, CH_2), 1.96, 2.01, 2.03, 2.05, 2.06, 2.07, 2.12, 2.15, and 2.16 (9 s, 9,3,6,3,3,6,3,3,3 H, 13 $COCH_3$), 2.25, 2.27, 2.33, and 2.34 (4 s, each 3 H, 4 CH_3C$_6$H$_4$CO), 3.02 (t, 2 H, CH_2N_3), 3.75 (dd, 1 H, $J_{5,6a}$ 7.9, $J_{6a,6b}$ 11.8 Hz, H-6a^{I}), 3.80 (t, 1 H, H-4^{V}), 4.45 (dd, 1 H, $J_{1,2}$ 8.5, $J_{2,3}$ 10.7 Hz, H-2^{I}), 4.52 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1^{VI}), 4.57 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1^{II}), 4.63 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1^{V}), 4.95 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 3.3 Hz, H-3^{VI}), 5.00 (t, 1 H, $J_{2,3}$ 9.6, $J_{3,4}$ 9.2 Hz, H-3^{II}), 5.41 (d, 1 H, $J_{3,4}$ 3.7, $J_{4,5}$ < 1 Hz, H-4^{III}), 5.43 (d, 1 H, H-1^{I}), 5.44 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1^{VI}); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.4–20.6 (CO*C*H$_3$), 21.4 and 21.5 (*C*H$_3$C$_6$H$_4$CO), 25.3, 26.1, 28.4, and 28.9 (4 CH_2), 51.0 (CH_2N_3), 54.8 (2 C) (C-2^{I}, C-2^{IV}), 60.4 (C-6^{III}), 61.6 (C-6^{VI}), 62.2 (C-6^{V}), 62.4 (C-6^{II}), 68.3 (C-6^{I}), 68.7 (C-6^{IV}), 69.6 (OCH_2), 66.4 (C-4^{VI}), 68.9 (2 C) (C-4^{III}, C-2^{VI}), 69.8 (2 C) (C-4^{I}, C-4^{IV}), 69.9 (C-3^{IV}), 70.5 (C-5^{VI}), 70.7 (C-3^{I}), 70.8 (2 C) (C-3^{III}, C-3^{VI}), 71.0 (C-2^{III}), 71.3 (C-2^{II}), 71.8 (C-2^{V}), 72.3 (C-3^{II}), 72.7 (C-5^{V}), 72.8 (C-5^{II}), 72.9 (C-3^{V}), 74.1 (2 C) and 74.9 (C-5^{I}, C-5^{III}, C-5^{IV}), 75.7 (C-4^{II}), 76.1 (C-4^{V}), 97.6 and 98.0 (C-1^{I}, C-1^{IV}), 100.3 (C-1^{III}), 100.5 (C-1^{II}), 100.8 (C-1^{V}), 100.9 (C-1^{VI}), 165.1, 165.2, 165.3, and 165.4 (4 $CH_3C_6H_4CO$), 168.6–170.9 (*C*OCH$_3$); HRMS of $C_{116}H_{129}N_5O_{50}$ (M, 2391.770): $[M+H]^+$ found 2392.742, calcd 2392.778.

3.24. 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)-(2,4,6-tri-*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-glucopyranoside (32)

To a soln of **31** (30 mg, 12.5 μmol) in MeOH (2 mL) and CH_2Cl_2 (0.3 mL) was added NaOMe (pH 10), and the mixture was stirred for 24 h. After neutralization with Dowex 50 × 8 (H^+) and filtration, the mixture was concentrated. To a solution of the residue in 1-BuOH (5 mL) was added 1,2-diaminoethane (1 mL), and the mixture was stirred overnight at 80 °C, then, co-concentrated with toluene. A solution of the residue in Py (10 mL) and Ac_2O (10 mL) was stirred overnight, then co-concentrated with toluene. Column chromatography (9:1 CH_2Cl_2–MeOH) of the residue afforded **32**, isolated as a colourless glass (17 mg, 71%); R_f 0.30 (9:1 CH_2Cl_2–MeOH); $[\alpha]_D^{20}$ −2° (*c* 1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$; 2D TOCSY, ROESY): δ 1.37–1.38 (m, 4 H, 2 CH_2), 1.58–1.61 (m, 4 H, 2 CH_2), 1.90, 1.93, 1.96, 1.98, 1.99, 2.01, 2.02, 2.03, 2.06, 2.07, 2.08, 2.09, 2.10, 2.12, 2.13, 2.14 s and 2.16 (17 s, 3,3,3,3,3,6,3,3,3,6,3,3,3,3,3,3,3 H, 2 NHCOC*H*$_3$, 17 $COCH_3$), 3.26 (t, 2 H, CH_2N_3), 3.31 (dd, 1 H, $J_{1,2}$ 8.2, $J_{2,3}$ 10.6 Hz, H-2^{IV}), 3.44 (m, 1 H, OC*H*H), 3.97 (dd, 1 H, $J_{2,3}$ 9.8, $J_{3,4}$ 3.8 Hz, H-3^{III}), 4.53 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1^{IV}), 4.56 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{II}), 4.57 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1^{V}), 4.60 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1^{I}), 4.97 (dd, 1 H, $J_{2,3}$ 10.4, $J_{3,4}$ 3.4 Hz, H-3^{IV}), 5.02 (dd, 1 H, $J_{1,2}$ 8.0 Hz, H-2^{III}), 5.08 (dd, 1 H, H-2^{IV}), 5.12 (t, 1 H, $J_{2,3}$ 9.3, $J_{3,4}$ 9.3 Hz, H-3^{II}), 5.12 (dd, 1 H, $J_{2,3}$ 9.4, $J_{3,4}$ 9.5 Hz, H-3^{V}), 5.25 (dd, 1 H, $J_{2,3}$ 10.6, $J_{3,4}$ 9.3 Hz, H-3^{I}), 5.40 (d, 1 H, $J_{2,NH}$ 7.8 Hz, N*H*COCH$_3^{IV}$), 5.40 (dd, 1 H, $J_{3,4}$ 9.1 Hz, H-3^{IV}), 5.45 (d, 1 H, $J_{2,NH}$ 8.7 Hz, N*H*COCH$_3^{I}$); HRMS of $C_{80}H_{113}N_5O_{48}$ (M, 1911.655): $[M+H]^+$ found 1912.644, calcd 1912.663.

3.25. 6-Azidohexyl 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-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranoside (33)

To a soln of **32** (17 mg, 8.88 μmol) in MeOH (0.7 mL) was added NaOMe (pH 10). The mixture was stirred for 4 h, then neutralized with Dowex 50 × 8 (H^+), filtered, and concentrated. Gel-filtration of the residue on a Bio Gel P-2 column, eluted with aq 0.1 M NH_4HCO_3 at a flow rate of 40 mL/h, gave **33** after freeze-drying as a white powder (9.4 mg, 89%); R_f 0.19 (1:3 CH_2Cl_2–MeOH); $[\alpha]_D^{20}$ −1° (*c* 0.5, water); HRMS of $C_{46}H_{79}N_5O_{31}$ (M, 1197.475): $[M+H]^+$ found 1198.508, calcd 1198.483. For ^{1}H NMR data, see Table 4.

3.26. 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-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranoside (4)

To a soln of **33** (8 mg, 6.67 μmol) in *t*-BuOH (3 mL) and water (2 mL) were added 10% Pd–C (30 mg) and 2 drops of aq 25% NH_3, and the mixture was stirred for 24 h under H_2, then filtered over cotton, and concentrated. Gel-filtration of the residue on a Bio Gel P-2 column, eluted with aq 0.1 M NH_4HCO_3 at a flow rate of 40 mL/h, gave **4** after freeze-drying as a white powder (5.3 mg, 67%); R_f 0.16 (2:1:1 AcOH–1-BuOH–water); $[\alpha]_D^{20}$ −1° (*c* 0.3, H_2O); HRMS of $C_{46}H_{81}N_3O_{31}$ (M, 1171.485): $[M+H]^+$ found 1172.486, calcd 1172.492. For ^{1}H NMR data, see Table 5.

3.27. 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-glucopyranosyl-(1→6)-[β-D-galactopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranoside (2)

To a soln of **4** (5.3 mg, 4.52 μmol) in aq 50 mM sodium cacodylate buffer pH 7.5 (500 μL), containing 5 mM $MnCl_2$, BSA (0.5 mg), and NaN_3 (0.02%), were added alkaline phosphatase (10 U), UDP-galactose (8.2 mg, 13.4 μmol), and β-1,4-galactosyltransferase (1 U). The reaction mixture was incubated for 20 h at 37 °C, then, water (100 μL) was added. UDP-Galactose was removed on a Dowex 1 × 8 (Cl^-) column, eluted with water. The eluate was concentrated, and the residue applied on a Bio Gel P-2 column eluted with aq 0.1 M NH_4HCO_3 at a flow rate of 40 mL/h. The appropriate fractions were freeze-dried to give **2** (1.5 mg, 22%); R_f 0.07 (2:1:1 AcOH–1-BuOH–water); HRMS of $C_{58}H_{101}N_3O_{41}$ (M, 1495.591): $[M+H]^+$ found 1496.614, calcd 1496.598. For ^1H NMR data, see Table 6.

Acknowledgements

We thank H.I.V. Amatdjais-Groenen (Nijmegen University) for measuring the elemental analysis data and C. Versluis (Utrecht University) for recording the HRMS spectra. This work was financially supported by the EU, project BIO CT 95-0138.

References

1. Hausdorff, W. P.; Bryant, J.; Paradiso, P. R.; Siber, G. R. *Clin. Infect. Dis.* **2000**, *30*, 100–121.
2. Sniadack, D. H.; Schwartz, B.; Lipman, H.; Bogaerts, J.; Butler, J. C.; Dagan, R.; Echanizaviles, G.; Lloydevans, N.; Fenoll, A.; Girgis, N. I.; Henrichsen, J.; Klugman, K.; Lehmann, D.; Takala, A. K.; Vandepitte, J.; Gove, S.; Breiman, J. F. *Pediatr. Infect. Dis. J.* **1995**, *14*, 503–510.
3. Centers for Disease Control and Prevention. Prevention of pneumococcal disease: recommendations of the Advisory Committee on Immunization Practices (ACIP), *MMWR Morb. Mortal. Wkly. Rep.* **1997**, *46*, 1–24.
4. Fine, M. J.; Smith, M. A.; Carson, C. A.; Mutha, S. S.; Sankey, S. S.; Weissfeld, L. A.; Kapoor, W. N. *J. Am. Med. Assoc.* **1996**, *275*, 134–141.
5. Doern, G. V.; Brueggemann, A. B.; Huynh, H.; Wingert, E.; Rhomberg, P. *Emerg. Infect. Dis.* **1999**, *5*, 757–765.
6. Klugman, K. P. *Clin. Microbiol. Rev.* **1990**, *3*, 171–196.
7. Robbins, J. B.; Austrian, R.; Lee, C. J.; Rastogi, S. C.; Schiffman, G.; Henrichsen, J.; Mäkalä, P. H.; Broome, C. V.; Facklam, R. R.; Tiesjema, R. H.; Parke, J. C. J., Jr. *J. Infect. Dis.* **1983**, *148*, 1136–1159.
8. Butler, J.; Breiman, R.; Campbell, J.; Lipman, H.; Broome, C.; Facklam, R. *J. Am. Med. Assoc.* **1993**, *270*, 1826–1831.
9. Örtqist, Å.; Hedlund, J.; Burman, L.-Å.; Elbel, E.; Höfer, M.; Leinonen, M.; Lindblad, I.; Sundelöf, B.; Kalin, M. *Lancet* **1998**, *351*, 399–403.
10. Beuvery, E. C.; Van Rossem, F.; Nagel, J. *Infect. Immun.* **1982**, *37*, 15–22.
11. Rennels, M. B.; Edwards, K. M.; Keyserling, H. L.; Reisinger, K. S.; Hogerman, D. A.; Madore, D. V.; Chang, I.; Paradiso, P. R.; Malinoski, F. J.; Kimura, A. *Pediatrics* **1998**, *101*, 604–611.
12. Jansen, W. T. M.; Hogenboom, S.; Thijssen, M. J. L.; Kamerling, J. P.; Vliegenthart, J. F. G.; Verhoef, J.; Snippe, H.; Verheul, A. F. M. *Infect. Immun.* **2001**, *69*, 787–793.
13. Benaissa-Trouw, B.; Lefeber, D. J.; Kamerling, J. P.; Vliegenthart, J. F. G.; Kraaijeveld, K.; Snippe, H. *Infect. Immun.* **2001**, *69*, 4698–4701.
14. Niggemann, J.; Kamerling, J. P.; Vliegenthart, J. F. G. *Bioorg. Med. Chem.* **1998**, *6*, 1605–1612.
15. Niggemann, J.; Kamerling, J. P.; Vliegenthart, J. F. G. *J. Chem. Soc. Perkin Trans. 1* **1998**, 3011–3020.
16. Mawas, F.; Niggemann, J.; Jones, C.; Corbel, M. J.; Kamerling, J. P.; Vliegenthart, J. F. G. *Infect. Immun.* **2002**, *70*, 5107–5114.
17. Michalik, D.; Vliegenthart, J. F. G.; Kamerling, J. P. *J. Chem. Soc. Perkin Trans. 1* **2002**, 1973–1981.
18. Lindberg, B.; Lönngren, J.; Powell, D. A. *Carbohydr. Res.* **1977**, *58*, 177–186.
19. Koeman, F. A. W.; Meissner, J. W. G.; van Ritter, H. R. P.; Kamerling, J. P.; Vliegenthart, J. F. G. *J. Carbohydr. Chem.* **1994**, *13*, 1–25.
20. Grundler, G.; Schmidt, R. R. *Carbohydr. Res.* **1985**, *135*, 203–218.
21. Slaghek, T. M.; Nakahara, N.; Ogawa, T.; Kamerling, J. P.; Vliegenthart, J. F. G. *Carbohydr. Res.* **1994**, *255*, 61–85.
22. Kamerling, J. P. In *Streptococcus pneumoniae*, Molecular Biology & Mechanisms of Disease; Thomasz, A., Ed. Pneumococcal polysaccharides: a chemical view; Mary Ann Liebert: New York, 2000; pp 81–114.
23. Zhou, W.; Jennings, H. J. *J. Carbohydr. Chem.* **1996**, *15*, 279–295.
24. Lemieux, R. U.; Driguez, H. *J. Am. Chem. Soc.* **1975**, *97*, 4063–4069.
25. Yoshino, T.; Sadamitsu, M.; Minagawa, M.; Reuter, G.; Schauer, R. *Glycoconjugate J.* **1988**, *5*, 377–384.