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Chemo-enzymatic synthesis of tetra-, penta-, and hexasaccharide fragments of the capsular polysaccharide of *Streptococcus pneumoniae* type 14

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Abstract

The chemo-enzymatic synthesis is described of 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$_2$)$_6$NH$_2$ (**1**), 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$_2$)$_6$NH$_2$ (**2**), 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$_2$)$_6$NH$_2$ (**3**), and 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$_2$ (**4**), representing fragments of the repeating unit of the *Streptococcus pneumoniae* serotype 14 capsular polysaccharide. Linear intermediate oligosaccharides **5**–**8** were synthesized via chemical synthesis, followed by enzymatic galactosylation using bovine milk β-1,4-galactosyltransferase as a catalyst. The title oligosaccharides form suitable compounds for conjugation with carrier proteins, to be tested as potential vaccines in animal models.

Keywords: Carbohydrates; *Streptococcus pneumoniae*; Oligosaccharide synthesis

1. Introduction

The encapsulated bacterium *Streptococcus pneumoniae* is a major cause of life-threatening diseases such as otitis media, pneumonia, meningitis, bacteraemia, and septicaemia,[1,2] mainly due to a growing resistance towards antibiotics.[3,4] Vaccination with the available 23-valent capsular polysaccharide (CPS) vaccines[5] offers protection in healthy adults, but they are ineffective in the most important high-risk groups, such as infants, small children, immuno-compromised patients, and the elderly.[6] Conjugation of *S. pneumoniae* carbohydrate antigens to a protein carrier results in a T-cell dependent neoglycoconjugate antigen that gives an efficient immune response in the high-risk groups,[7] as have been shown for other bacteria.[8,9] Currently, neoglycoconjugate vaccines against *S. pneumoniae* serotypes have been introduced.[7]

The CPS of *S. pneumoniae* type 14 is built up from the tetrasaccharide repeating unit →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→.[10] In earlier reports we have described the chemo-enzymatic synthesis of a spacer-containing tetrasaccharide fragment of the CPS of *S. pneumoniae* type 14, corresponding with one repeating unit {β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→6)-[β-D-Gal*p*-(1→4)]-β-D-Glc*p*NAc}, as well as mimics of the CPS.[11,12] The tetrasaccharide-containing neoglycoconjugate (CRM$_{197}$ as carrier protein) showed particularly promising immunological data when tested in mice models.[13] Based on these results, it was decided to synthesize a series of longer oligosaccharide fragments of the CPS for more detailed immunological studies. Recently, we have described the chemo-enzymatic synthesis of four oligosaccharides with a 6-aminohexyl spacer, varying in length between one and two repeating units.[14,15] Here, we report the chemo-enzymatic synthesis of another four oligosac-

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charides, containing the same spacer. The whole series of eight spacered oligosaccharides will form an excellent panel to investigate the structural parameters influencing immunogenicity.

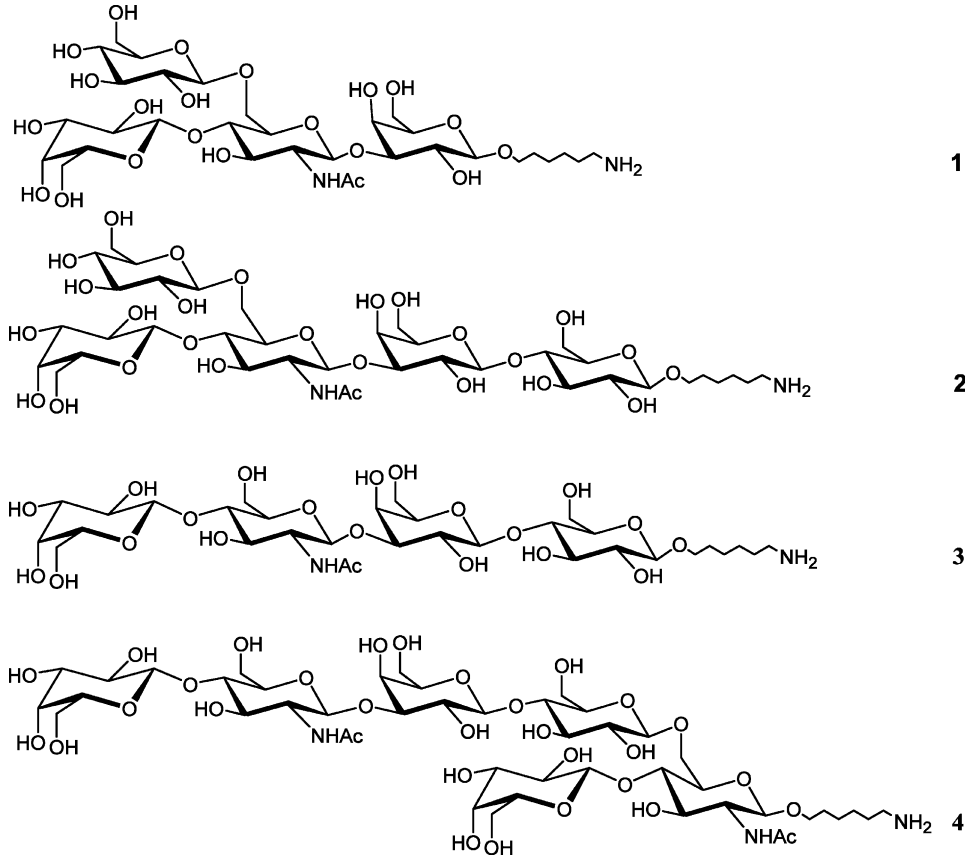
2. Results and discussion

For the organic synthesis of the structurally closely related compounds **5**–**8**, a series of broadly applicable building blocks, **9**, **13**, **24**, and **37**, were designed. Condensation of these key building blocks with appropriate acceptor or donor building blocks gave, after deprotection, the aimed linear oligosaccharides (**5**–**8**), which were used as acceptor substrates for β-1,4-galactosyltransferase (EC 2.4.1.22) from bovine milk to give the desired title compounds **1**–**4** (Scheme 1).

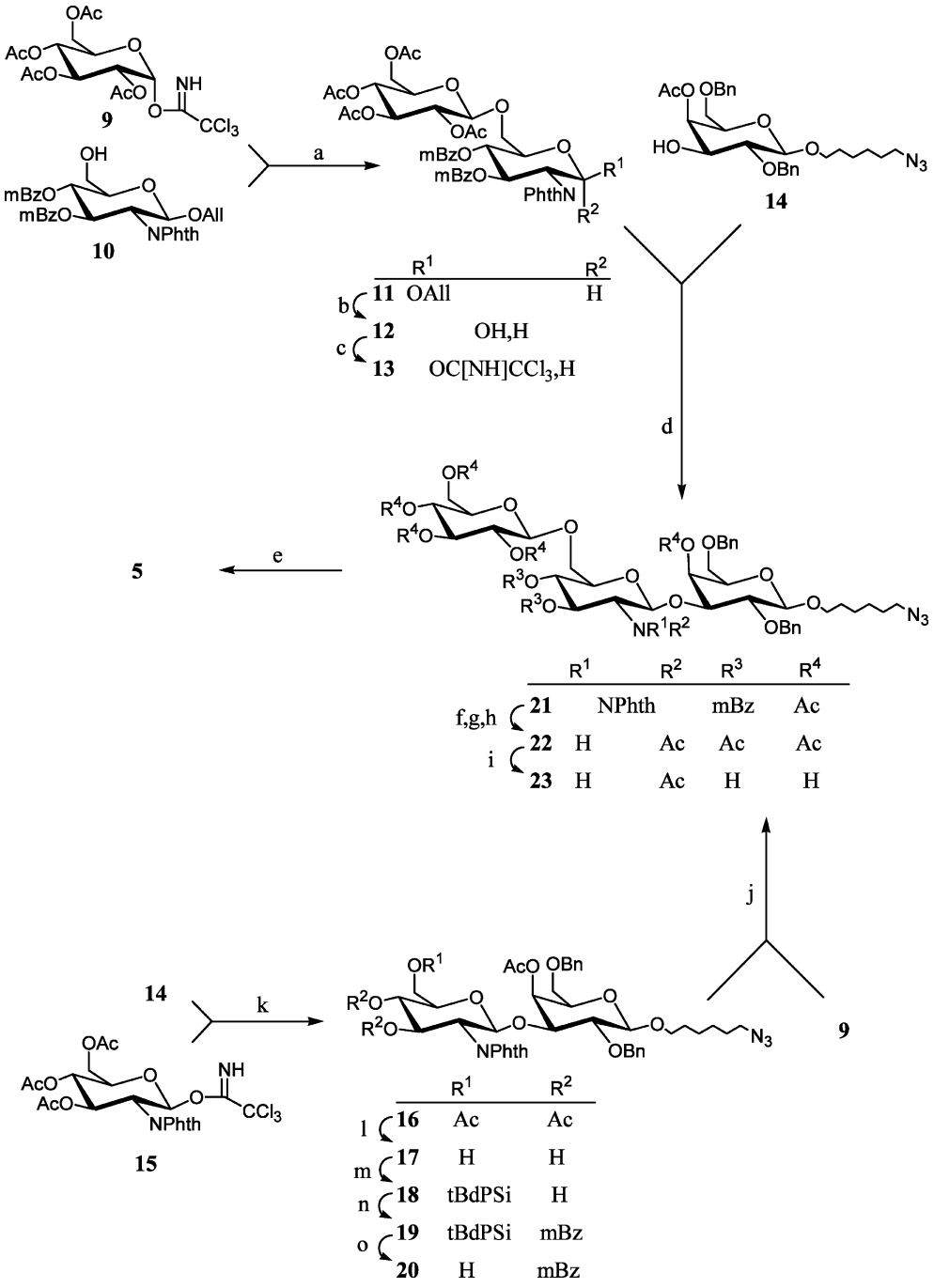
2.1. Synthesis of tetrasaccharide fragment 1

The linear trisaccharide backbone **5** was prepared via two different routes (Scheme 2). For the first route, disaccharide donor **13** was needed. Coupling of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (**9**)[16] to allyl 2-deoxy-3,4-di-*O*-*p*-methylbenzoyl-2-phthalimido-β-D-glucopyranoside (**10**)[11] in dichloromethane at 0 °C, using 1 equivalent silver trifluoromethanesulfonate (AgOTf) as a catalyst, gave disaccharide **11** in 62% yield. A small amount of a side product, the acetylated acceptor, was also isolated. Side product formation was much higher when using 10% trimethylsilyl trifluoromethanesulfonate (TMSOTf) as a catalyst (0 °C: 34% **11**, 53% side product; −40 °C: 50% **11**, 25% side product). De-allylation of **11** by using palladium(II)chloride, sodium acetate, and acetic acid in an ultrasonic bath (→**12**, 70%), followed by imidation of the anomeric center, using trichloroacetonitrile with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as a catalyst, gave disaccharide donor **13** (76%). Coupling of **13** to 6-azidohexyl 4-*O*-acetyl-2,6-di-*O*-benzyl-β-D-galactopyranoside (**14**)[14] at −70 °C in dichloromethane, using 10% trimethylsilyl trifluoromethanesulfonate as a catalyst, gave trisaccharide **21** (73%).

In the first step of an alternative route to trisaccharide **21**, 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (**15**)[17]was coupled to galactose acceptor **14**, at −70 °C in dichloromethane, using 10% trimethylsilyl trifluoromethanesulfonate as a catalyst, to give disaccharide **16** in 86% yield. Mild de-O-acetylation of **16**, using sodium methoxide at pH 8,



Scheme 1. Overview of 6-aminohexyl-spacered oligosaccharides **1**–**4**, representing fragments of the repeating unit of the *S. pneumoniae* serotype 14 capsular polysaccharide.



Scheme 2. Synthesis of the linear trisaccharide backbone **5**: (a) 1 equiv AgOTf, CH_2Cl_2, 0 °C, 62%; (b) $Pd(II)Cl_2$, NaOAc, AcOH, 70%; (c) Cl_3CCN, DBU, CH_2Cl_2, 76%; (d) 10% TMSOTf, CH_2Cl_2, −70 °C, 73%; (e) 10% Pd–C, H_2, water, *tert*-BuOH, aq 25% NH_3/10% Pd–C, AcOH, 69%; (f) NaOMe (pH 10), MeOH, CH_2Cl_2; (g) $NH_2CH_2CH_2NH_2$, 1-BuOH, 80 °C; (h) pyridine, Ac_2O, 80% over three steps; (i) NaOMe (pH 9), MeOH, CH_2Cl_2, 97%; (j) 10% TMSOTf, CH_2Cl_2, −40 °C, 56%; (k) 10% TMSOTf, CH_2Cl_2, −70 °C, 86%; (l) NaOMe (pH 8), MeOH, CH_2Cl_2, 91%; (m) *t*-BdPSiCl, DMAP, Et_3N, CH_2Cl_2, pyridine, 83%; (n) *p*-mBzCl, pyridine, CH_2Cl_2, 90%; (o) AcCl, MeOH, toluene, 0 °C, 81%.

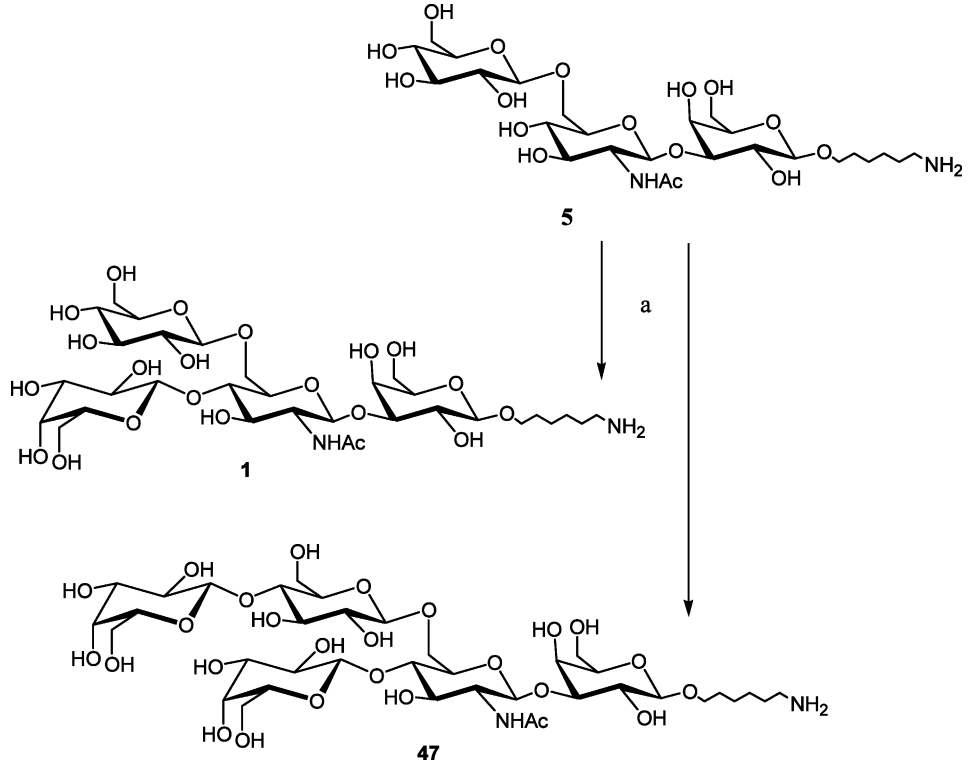
gave **17** (91%) with a retention of the acetyl group at galactose O-4. Selective introduction of a *tert*-butyldiphenylsilyl group (*t*BdPSi) at the primary hydroxyl function of **17** using *tert*-butyldiphenylsilyl chloride and a catalytic amount of 4-dimethylaminopyridine (DMAP) (→**18**, 83%), followed by *p*-methylbenzoylation (mBz) of the two remaining hydroxyl groups, using *p*-methylbenzoyl chloride in pyridine, gave **19** (90%). Finally, removal of the *tert*-butyldiphenylsilyl group under mild acidic conditions afforded disaccharide acceptor **20** in 81% yield. A minor side product, the O-de-acetylated analogue of **20**, was isolated also (14%

yield). Coupling of glucose donor **9** to disaccharide **20** in dichloromethane at −40 °C, using 10% trimethylsilyl trifluoromethanesulfonate as a catalyst, gave trisaccharide **21** in 56% yield. O-De-acylation of **21** using sodium methoxide at pH 10, followed by N-de-phthaloylation using 1,2-diaminoethane in 1-butanol at 80 °C, and subsequent N,O-acetylation using acetic anhydride in pyridine yielded **22** in 80% yield over three steps. The O-acetylation step was carried out to facilitate chromatographic purification. O-De-acetylation of **22** with sodium methoxide at pH 9 gave crude **23**, and after reduction of the azido function using 10% Pd–C and H_2 in the presence of ammonia, and subsequent debenzylation using 10% Pd–C and H_2 in the presence of acetic acid, linear trisaccharide backbone **5** was obtained in a yield of 69%. Tetrasaccharide **1** was synthesized in 54% yield by the transfer of galactose from UDP-galactose to O-4 of the *N*-acetyl-β-D-glucosamine residue of **5** by using bovine milk β-1,4-galactosyltransferase as a catalyst (Scheme 3). As a result of the enzymatic galactosylation of the terminal glucose residue, the previously described pentasaccharide 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-galactopyranoside (**47**)[14] was obtained as a side product (35%) (Scheme 3). So far, the enzymatic galactosylation of glucose by β-1,4-galactosyltransferase has only been shown to proceed in the presence of α-lactalbumin (lactose synthase complex).[18] ^{1}H NMR data of **5** and **1**, derived from 2D TOCSY and ROESY measurements, are presented in Tables 1 and 2, respectively.

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

Proton	δ_H		
	Gal I	GlcNAc II	Glc III
H-1	4.37	4.70	4.50
H-2	3.57	3.76	3.31
H-3	3.72	3.56	3.50
H-4	4.15	n.d. [a]	3.39
H-5	n.d.	3.61	3.48
H-6a	n.d.	4.22	3.92
H-6b	n.d.	3.89	3.72
$O(CH_2)_2(CH_2)_2(CH_2)_2ND_2$		1.39–1.41 (4 H)	
$OCH_2CH_2(CH_2)_2CH_2CH_2ND_2$		1.64–1.65 (4 H)	
CH_2ND_2		2.96	
$OCH_2(CH_2)_5ND_2$		3.68, 3.92	
$NDCOCH_3$		2.04	

[a] n.d., not determined.



Scheme 3. Synthesis of tetrasaccharide **1** and pentasaccharide **47**: (a) 1.4 equiv UDP-Gal, aq 50 mM sodium cacodylate buffer (pH 7.5), 3 U β-1,4-galactosyltransferase, 14 U alkaline phosphatase, 37 °C, 54% (**1**); 35% (**47**).

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

Proton	δ_H			
	Gal I [a]	GlcNAc II	Glc III	Gal IV [b]
H-1	4.38	4.72	4.52	4.54
H-2	3.55	3.81	3.32	3.54
H-3	3.70	3.73	3.50	3.67
H-4	4.16	3.87	3.39	3.92
H-5	n.d. [c]	3.73	3.47	n.d.
H-6a	n.d.	4.28	3.86	n.d.
H-6b	n.d.	3.95	3.72	n.d.
$O(CH_2)_2(CH_2)_2(CH_2)_2ND_2$			1.39–1.41 (4 H)	
$OCH_2CH_2(CH_2)_2CH_2CH_2ND_2$			1.63–1.64 (4 H)	
CH_2ND_2			2.96	
$OCH_2(CH_2)_5ND_2$			3.67, 3.92	
$NDCOCH_3$			2.03	

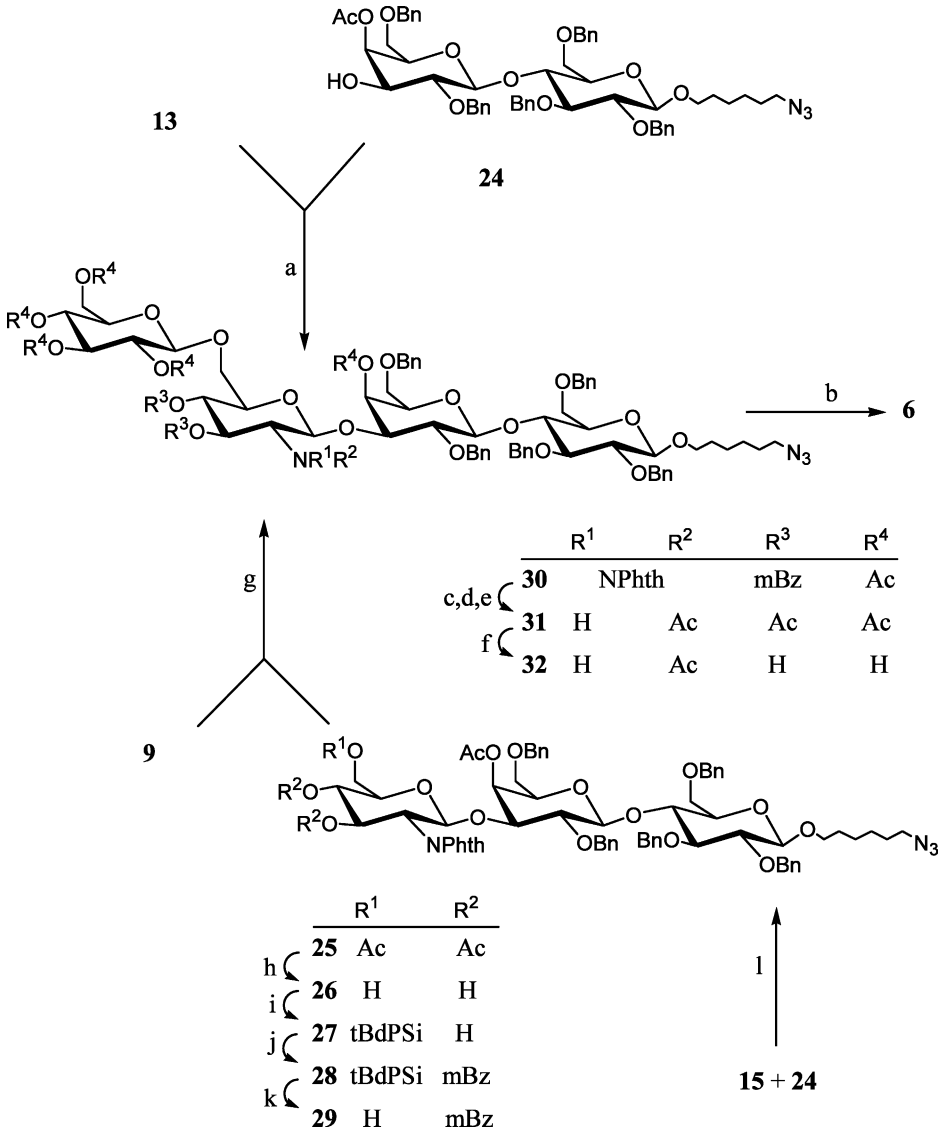
[a] Gal(β1-$O(CH_2)_6NH_2$).
[b] Gal(β1-4)GlcNAc.
[c] n.d., not determined.

2.2. Synthesis of pentasaccharide fragment 2

The linear tetrasaccharide backbone **6** was prepared via two different routes (Scheme 4). In the first route, condensation of disaccharide donor **13** with 6-azidohexyl (4-*O*-acetyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**24**)[14] in dichloromethane at −70 °C, using 10% trimethylsilyl trifluoromethanesulfonate as a catalyst, afforded tetrasaccharide **30** in 83% yield. An alternative route to tetrasaccharide **30** involved the condensation of glucose donor **9** (see Scheme 2) with trisaccharide acceptor 6-azidohexyl (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 (**29**). As a first step in the synthesis of **29**, glucosamine donor **15**[17] (see Scheme 2) was coupled to lactose acceptor **24** in dichloromethane at −70 °C, using 10% trimethylsilyl trifluoromethanesulfonate as a catalyst, to give trisaccharide **25** in 97% yield. Mild O-de-acetylation of **25**, using sodium methoxide at pH 8, gave **26** (78%), with a retention of the acetyl group at galactose O-4. Selective introduction of a *tert*-butyldiphenylsilyl group at the primary hydroxyl function of **26** using *tert*-butyldiphenylsilyl chloride and a catalytic amount of 4-dimethylaminopyridine (→**27**, 84%), followed by *p*-methylbenzoylation of the two remaining hydroxyl groups, using *p*-methylbenzoyl chloride in pyridine gave **28** (91%). Finally, selective removal of the silyl group, using a 1:1 mixture of 1.0 M TBAF in THF and AcOH at pH 6, gave **29** in 89% yield. Coupling of donor **9** to acceptor **29** in dichloromethane at −40 °C, using 10% trimethylsilyl trifluoromethanesulfonate as a catalyst gave tetrasaccharide **30** (58%). O-De acylation of **30** using sodium methoxide at pH 10, followed by N-dephthaloylation using 1,2-diaminoethane in 1-butanol at 80 °C, and subsequent N,O-acetylation using acetic anhydride in pyridine yielded **31** in 88% yield over three steps. O-De-acetylation of **31** with sodium methoxide at pH 10 gave crude **32**. After reduction of the azido function using 10% Pd–C and H_2 in the presence of ammonia, and subsequent debenzylation using 10% Pd–C and H_2 in the presence of acetic acid, the linear tetrasaccharide backbone **6** was obtained in a yield of 81%. Pentasaccharide **2** was synthesized in 65% yield by the transfer of galactose from UDP-galactose to O-4 of the *N*-acetyl-β-D-glucosamine residue of **6** by using bovine milk β-1,4-galactosyltransferase as a catalyst (Scheme 5). As a digalactosylated side product the previously described hexasaccharide 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 (**48**)[14] (6%; Scheme 5) was obtained. ^{1}H NMR data of **6** and **2**, derived from 2D TOCSY and ROESY measurements, are presented in Tables 3 and 4, respectively.

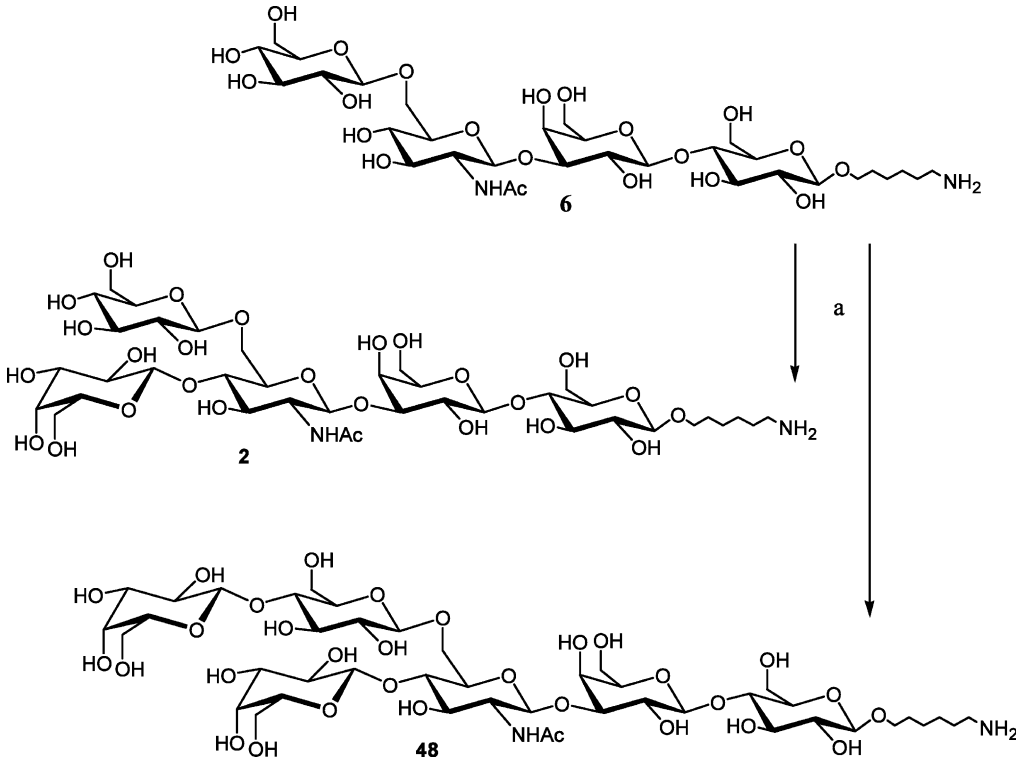
2.3. Synthesis of tetrasaccharide fragment 3

The linear trisaccharide backbone **7** was prepared via two different routes (Scheme 6). For the first route, trisaccharide donor **37** was needed. Debenzylation of (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)-1,2,3,6-tetra-*O*-benzyl-β-D-glucopyranoside (**34**),[15] using 10% Pd–C and H_2 in the



Scheme 4. Synthesis of the linear tetrasaccharide backbone **6**: (a) 10% TMSOTf, CH_2Cl_2, −70 °C, 83%; (b) 10% Pd–C, H_2, water, *tert*-BuOH, aq 25% NH_3/10% Pd–C, AcOH, 81%; (c) NaOMe (pH 10), MeOH, CH_2Cl_2; (d) $NH_2CH_2CH_2NH_2$, 1-BuOH, 80 °C; (e) pyridine, Ac_2O, 88% over three steps; (f) NaOMe (pH 10), MeOH, CH_2Cl_2, quantitative; (g) 10% TMSOTf, CH_2Cl_2, −40 °C, 58%; (h) NaOMe (pH 8), MeOH, CH_2Cl_2, 78%; (i) *t*-BdPSiCl, DMAP, Et_3N, CH_2Cl_2, pyridine, 84%; (j) *p*-mBzCl, pyridine, CH_2Cl_2, 91%; (k) 1.0 M TBAF in THF, AcOH (pH 6), 89%; (l) 10% TMSOTf, CH_2Cl_2, −70 °C, 97%.

presence of acetic acid, followed by O-acetylation using acetic anhydride in pyridine afforded **35** in 66% yield over two steps. Selective O-de-acetylation of the anomeric center of **35**, using hydrazinium acetate in *N*,*N*-dimethylformamide (→**36**, 89%), and subsequent imidation using trichloroacetonitrile with 1,8-diazabicyclo[5.4.0]undec-7-ene as a catalyst gave trisaccharide donor **37** in 69% yield. Condensation of **37** with 6-azido-1-hexanol (**33**) in dichloromethane at 0 °C, using 1 equivalent silver trifluoromethanesulfonate as a catalyst, gave trisaccharide **38** in 22% yield only. The low yield was due to orthoester formation, and coupling attempts at different temperatures and/or applying trimethylsilyl trifluoromethanesulfonate as a catalyst did not improve the yield. O-De-acetylation of **38**, using sodium methoxide (pH 10), followed by N-de-phthaloylation using 1,2-diaminoethane in 1-butanol at 80 °C, and subsequent N,O-acetylation using acetic anhydride in pyridine yielded **39** (77% over three steps). De-O-acetylation of **39** with sodium methoxide at pH 10 (→**40**, 55%), and reduction of the azido function using 10% Pd–C and H_2 in the presence of ammonia, gave the linear trisaccharide backbone **7** (83%). ^{1}H NMR data of **40** and **7**, derived from 2D TOCSY and ROESY measurements, are presented in Tables 5 and 6, respectively. In an alternative route to linear backbone **7**, trisaccharide **25** was O-de-acetylated using sodium methoxide (pH 10), followed by N-de-phthaloylation using 1,2-diami-



Scheme 5. Synthesis of pentasaccharide **2** and hexasaccharide **48**: (a) 1.4 equiv UDP-Gal, aq 50 mM sodium cacodylate buffer (pH 7.5), 3 U β-1,4-galactosyltransferase, 14 U alkaline phosphatase, 37 °C, 65% (2); 6% (**48**).

Table 3
500 MHz ^{1}H NMR data (TOCSY, ROESY) of **6** at 300 K (in ppm)

Proton	δ_H			
	Glc I [a]	Gal II	GlcNAc III	Glc IV [b]
H-1	4.47	4.43	4.69	4.49
H-2	3.29	3.58	3.76	3.30
H-3	3.63	3.72	3.57	3.49
H-4	3.63	4.15	n.d. [c]	3.38
H-5	3.60	n.d.	3.61	3.47
H-6a	3.97	n.d.	4.20	3.92
H-6b		n.d.	3.88	3.72
$O(CH_2)_2(CH_2)_2(CH_2)_2ND_2$			1.39–1.41 (4 H)	
$OCH_2CH_2(CH_2)_2CH_2CH_2ND_2$			1.64–1.65 (4 H)	
CH_2ND_2			2.95	
$OCH_2(CH_2)_5ND_2$			3.68, 3.92	
$NDCOCH_3$			2.03	

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

noethane in 1-butanol at 80 °C, and subsequent N,O-acetylation using acetic anhydride in pyridine afforded **41** (78% over three steps). De-O-acetylation of **41** with sodium methoxide (pH 10) gave crude **42**, and after reduction of the azido function of **42** using 10% Pd–C and H_2 in the presence of ammonia, and subsequent debenzylation using 10% Pd–C and H_2 in the presence of acetic acid, linear trisaccharide backbone **7** was obtained in a yield of 68%. Tetrasaccharide **3** was synthesized in 94% yield by the transfer of galactose from UDP-galactose to O-4 of the *N*-acetyl-β-D-glucosamine residue of **7** by using bovine milk β-1,4-galactosyltransferase as a catalyst (Scheme 7). 2D TOCSY and ROESY measurements confirmed the structure of **3** (Table 7). Chemical- and chemo-enzymatic syntheses of **7** containing other functionalities at the anomeric center have been described earlier.[19]

2.4. Synthesis of hexasaccharide fragment 4

To obtain the linear tetrasaccharide backbone **8**, as a first step trisaccharide donor **37** (see Scheme 6) was coupled with 6-azidohexyl 2-deoxy-3,4-di-*O*-*p*-methylbenzoyl-2-phthalimido-β-D-glucopyranoside (**43**)[15] (Scheme 8) in dichloromethane at 0 °C, using 15% trimethylsilyl trifluoromethanesulfonate as a catalyst, to give tetrasaccharide **44** in 34% yield. O-De-acylation of **44** using sodium methoxide (pH 10), followed by N-de-phthaloylation using 1,2-diaminoethane in 1-butanol at 90 °C, and subsequent N,O-acetylation using acetic

Table 4
500 MHz ^{1}H NMR data (TOCSY, ROESY) of **2** at 300 K (in ppm)

Proton	δ_H				
	Glc I [a]	Gal II [b]	GlcNAc III	Glc IV [c]	Gal V [d]
H-1	4.48	4.43	4.71	4.53	4.52
H-2	3.29	3.59	3.81	3.33	3.54
H-3	3.64	3.72	3.73	3.50	3.67
H-4	n.d. [e]	4.16	3.87	3.40	3.93
H-5	n.d.	n.d.	3.73	3.46	n.d.
H-6a	3.95	n.d.	4.27	3.86	n.d.
H-6b	3.78	n.d.	3.95	3.73	n.d.
$O(CH_2)_2(CH_2)_2(CH_2)_2ND_2$			1.39–1.41 (4 H)		
$OCH_2CH_2(CH_2)_2CH_2CH_2ND_2$			1.64–1.65 (4 H)		
CH_2ND_2			2.96		
$OCH_2(CH_2)_5ND_2$			3.67, 3.92		
$NDCOCH_3$			2.03		

[a] Glc(β1-O(CH$_2$)$_6$NH$_2$).
[b] Gal(β1-4)Glc.
[c] Glc(β1-6)GlcNAc.
[d] Gal(β1-4)GlcNAc.
[e] n.d., not determined.

anhydride in pyridine afforded **45** (86% over three steps). O-De-acetylation of **45** using sodium methoxide (pH 10) (→**46**, 79%), and reduction of the azide function using 10% Pd–C and H_2 in the presence of ammonia, gave the linear tetrasaccharide backbone **8** in 82% yield. Hexasaccharide **4** was synthesized in 76% yield by the transfer of galactose from UDP-galactose to O-4 of the *N*-acetyl-β-D-glucosamine residues of **8** by using bovine milk β-1,4-galactosyltransferase as a catalyst (Scheme 9). ^{1}H NMR data of **46**, **8**, and **4**, derived from 2D TOCSY and ROESY measurements, are presented in Tables 8–10, respectively.

Conjugation of the oligosaccharides **1**–**4** to CRM_{197} (cross reactive material) and immunological studies are in progress.

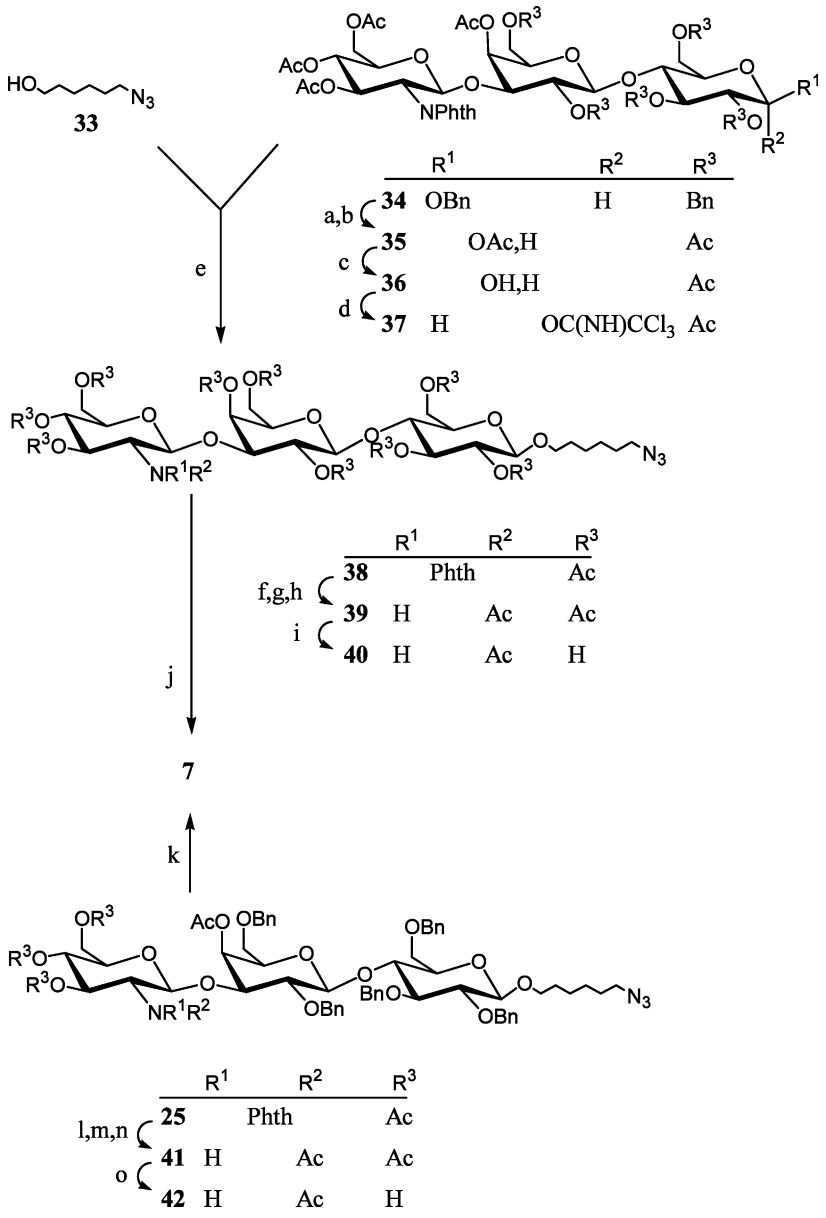
3. Experimental

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 38:1.75:0.25 1-BuOH–water–AcOH. In the work-up procedures of reaction mixtures, organic solutions were washed with appropriate amounts of the indicated aqueous solutions, then dried ($MgSO_4$ or Na_2SO_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, $CDCl_3$) or internal acetone (δ_H 2.225, D_2O). ^{13}C NMR spectra (APT, 75.5 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 internal acetone (δ_C 30.89, D_2O). Two-dimensional ^{1}H–^{1}H TOCSY (mixing times 7 and 100 ms) and ROESY (mixing time 300 ms), and ^{1}H–^{13}C correlated HSQC NMR spectra 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 1:1 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.

3.2. Allyl (2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-2-deoxy-3,4-di-*O*-*p*-methylbenzoyl-2-phthalimido-β-D-glucopyranoside (11)

A soln of allyl 2-deoxy-3,4-di-*O*-*p*-methylbenzoyl-2-phthalimido-β-D-glucopyranoside (**10**)[11] (0.15 g, 0.26 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl



Scheme 6. Synthesis of the linear trisaccharide backbone **7**: (a) 10% Pd–C, H_2, EtOH, EtOAc, AcOH; (b) pyridine, Ac_2O, 66% over two steps; (c) hydrazinium acetate, DMF, 89%; (d) Cl_3CCN, DBU, CH_2Cl_2, 69%; (e) 1 equiv AgOTf, CH_2Cl_2, 0 °C, 22%; (f) NaOMe (pH 10), MeOH, CH_2Cl_2; (g) $NH_2CH_2CH_2NH_2$, 1-BuOH, 80 °C; (h) pyridine, Ac_2O, 77% over three steps; (i) NaOMe (pH 10), MeOH, CH_2Cl_2, 55%; (j) 10% Pd–C, H_2, water, *tert*-BuOH, aq 25% NH_3, 83%; (k) 10% Pd–C, H_2, water, *tert*-BuOH, aq 25% NH_3/10% Pd–C, AcOH, 68%; (l) NaOMe (pH 10), MeOH, CH_2Cl_2; (m) $NH_2CH_2CH_2NH_2$, 1-BuOH, 80 °C; (n) pyridine, Ac_2O, 78% over three steps; (o) NaOMe (pH 10), MeOH, CH_2Cl_2, 99%.

trichloroacetimidate (**9**)[16] (0.16 g, 0.32 mmol) in dry CH_2Cl_2 (7.5 mL), containing 4 Å molecular sieves (0.1 g), was stirred under Ar for 0.5 h. After cooling to 0 °C, AgOTf (77 mg, 0.3 mmol) was added and the mixture was stirred for 1.5 h. After filtration, the soln was washed with aq 10% $NaHSO_3$, aq satd $NaHCO_3$, and water, dried ($MgSO_4$), filtered, and concentrated. Low-pressure column chromatography (10:1→4:1 toluene–EtOAc) of the residue gave **11**, isolated as a white foam (0.14 g, 62%); R_f 0.65 (1:1 toluene–EtOAc); $[\alpha]_D^{20}$ −4° (*c* 1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$; 2D TOCSY, ROESY): δ 2.00, 2.01, and 2.08 (3 s, 6,3,3 H, 4 $COCH_3$), 2.23 and 2.31 (2 s, each 3 H, 2 $COC_6H_4CH_3$), 3.71 (m, 1 H, H-5^{II}), 3.83 (dd, 1 H, $J_{5,6b}$ 7.9, $J_{6a,6b}$ 10.8 Hz, H-6b^{I}), 4.23 (dd, 1 H, $J_{5,6b}$ 4.6, $J_{6a,6b}$ 12.1 Hz, H-6b^{II}), 4.36 (m, 1 H, OCH*H*CH=CH_2), 4.54 (dd, 1 H, $J_{1,2}$ 8.4, $J_{2,3}$ 10.5 Hz, H-2^{I}), 4.69 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1^{II}), 5.39 (t, 1 H, H-4^{I}), 5.57 (d, 1 H, H-1^{I}), 5.77 (m, 1 H, OCH_2CH=CH_2), 6.22 (dd, 1 H, $J_{3,4}$ 9.4 Hz, H-3^{I}), 7.02 and 7.14 (2 d, each 2 H, Phth), 7.61–7.80 (2 m, 8 H, 2 $COC_6H_4CH_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.2–20.4 ($COCH_3$), 21.2 and 21.3 (2 $COC_6H_4CH_3$), 54.6 (C-

Table 5
500 MHz ^{1}H NMR data (TOCSY, ROESY) of **40** at 300 K (in ppm)

Proton	δ_H		
	Glc I	Gal II	GlcNAc III
H-1	4.48	4.43	4.68
H-2	3.29	3.60	3.76
H-3	3.65	3.72	3.58
H-4	n.d. [a]	4.15	3.47
H-5	3.60	n.d.	3.47
H-6a	3.97	n.d.	3.89
H-6b	3.80	n.d.	3.76
$O(CH_2)_2(CH_2)_2(CH_2)_2N_3$			1.39–1.42 (4 H)
$OCH_2CH_2(CH_2)_2CH_2CH_2N_3$			1.63–1.65 (4 H)
CH_2N_3			3.32
$OCH_2(CH_2)_5N_3$			3.62, 3.92
$NDCOCH_3$			2.04

[a] n.d., not determined.

2^I), 53.2, 61.6, and 69.8 (C-6^I, C-6^{II}, $OCH_2CH{=}CH_2$), 68.1, 69.9, 70.7, 71.0, 71.6, 72.6, and 73.6 (C-3^I, C-4^I, C-5^I, C-2^{II}, C-3^{II}, C-4^{II}, C-5^{II}), 96.9 and 100.5 (C-1^I, C-1^{II}), 117.5 ($OCH_2CH{=}CH_2$), 165.0 and 165.2 (2 $COC_6H_4CH_3$), 168.9–170.2 ($COCH_3$); HRMS data of $C_{47}H_{49}NO_{18}$ (M, 915.295): $[M+NH_4]^+$ found 933.335, calcd 933.329.

Table 6
500 MHz ^{1}H NMR data (TOCSY, ROESY) of **7** at 300 K (in ppm)

Proton	δ_H		
	Glc I	Gal II	GlcNAc III
H-1	4.47	4.43	4.68
H-2	3.29	3.59	3.74
H-3	3.64	3.73	3.56
H-4	3.64	4.15	3.46
H-5	3.59	n.d. [a]	3.46
H-6a	3.98	n.d.	3.90
H-6b	3.80	n.d.	3.77
$O(CH_2)_2(CH_2)_2(CH_2)_2ND_2$		1.39–1.41 (4 H)	
$OCH_2CH_2(CH_2)_2CH_2CH_2ND_2$		1.63–1.65 (4 H)	
CH_2ND_2		2.95	
$OCH_2(CH_2)_5ND_2$		3.58, 3.83	
$NDCOCH_3$		2.03	

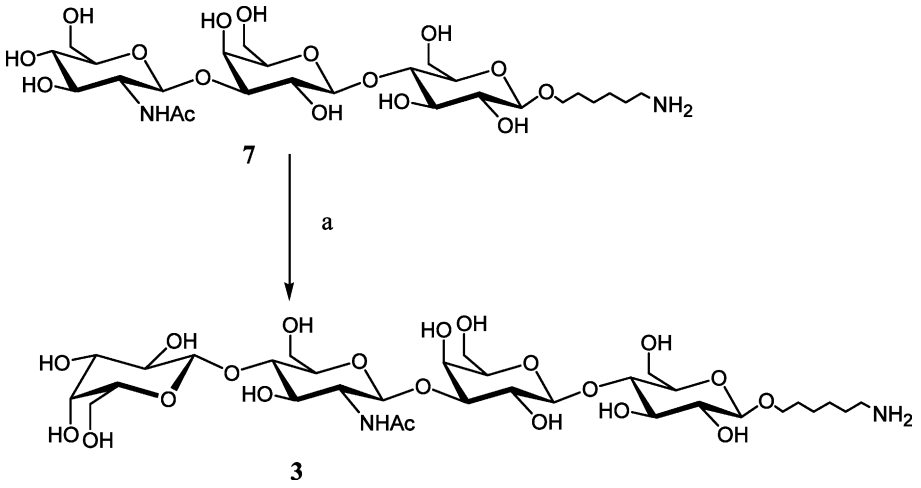
[a] n.d., not determined.

3.3. (2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1 → 6)-2-deoxy-3,4-di-*O*-*p*-methylbenzoyl-2-phthalimido-α,β-D-glucopyranose (12)

To a soln of **11** (0.41 g, 0.45 mmol) in AcOH (15 mL) were added $Pd(II)Cl_2$ (0.36 g, 2.03 mmol) and NaOAc (0.31 g, 3.78 mmol), and the mixture was kept overnight in an ultrasonic bath. After filtration over hyflo, the soln was diluted with CH_2Cl_2 then washed with water, aq satd $NaHCO_3$, and water, dried ($MgSO_4$), filtered, and concentrated. Low-pressure column chromatography (4:1 → 1:1 toluene–EtOAc) of the residue gave **12**, isolated as a slightly yellow foam (0.27 g, 70%); R_f 0.45 (1:1 toluene–EtOAc); ^{1}H NMR β-product (300 MHz, $CDCl_3$): δ 2.02, 2.04, 2.05, and 2.12 (4 s, each 3 H, 4 $COCH_3$), 2.27 and 2.34 (2 s, each 3 H, 2 $COC_6H_4CH_3$), 4.02 (dd, 1 H, $J_{5,6a}$ 1.9, $J_{6a,6b}$ 11.2 Hz, H-6a^I), 4.20 (dd, 1 H, $J_{5,6b}$ 4.7, $J_{6a,6b}$ 12.2 Hz, H-6b^{II}), 4.43 (dd, 1 H, $J_{1,2}$ 8.4, $J_{2,3}$ 10.7 Hz, H-2^I), 4.65 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1^{II}), 4.96 (dd, 1 H, $J_{2,3}$ 8.6 Hz, H-2^{II}), 5.42 (t, 1 H, H-4^I), 5.74 (d, 1 H, H-1^I), 6.23 (dd, 1 H, $J_{3,4}$ 9.2 Hz, H-3^I), 7.04 and 7.15 (2 d, each 2 H, Phth), 7.60–7.81 (2 m, 8 H, 2 $COC_6H_4CH_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.3–20.4 ($COCH_3$), 21.2 and 21.3 (2 $COC_6H_4CH_3$), 56.1 (C-2^I), 61.7 and 68.3 (C-6^I, C-6^{II}), 68.1, 69.7, 70.7, 71.2, 71.5, 72.5, and 73.3 (C-3^I, C-4^I, C-5^I, C-2^{II}, C-3^{II}, C-4^{II}, C-5^{II}), 92.4 and 100.5 (C-1^I, C-1^{II}), 165.0 and 165.2 (2 $COC_6H_4CH_3$), 169.2–170.4 ($COCH_3$); HRMS of $C_{44}H_{45}NO_{18}$ (M, 875.263): $[M+H]^+$ found 876.273, calcd 876.271.

3.4. (2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1 → 6)-2-deoxy-3,4-di-*O*-*p*-methylbenzoyl-2-phthalimido-α,β-D-glucopyranosyl trichloroacetimidate (13)

To a soln of **12** (0.37 g, 0.42 mmol) in dry CH_2Cl_2 (15 mL) were added, at 0 °C, trichloroacetonitrile (0.2 mL, 2.1 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (60 μL, 0.041 mmol), and the mixture was stirred for 1.5 h, then concentrated. Column chromatography (2:1 toluene–EtOAc) of the residue gave **13**, isolated as a slightly yellow foam (0.33 g, 76%); R_f 0.64 (1:1 toluene–EtOAc); ^{1}H NMR β-product (300 MHz, $CDCl_3$): δ 1.99, 2.03, and 2.09 (3 s, 6,3,3 H, 4 $COCH_3$), 2.27 and 2.34 (2 s, each 3 H, 2 $COC_6H_4CH_3$), 3.62 (m, 1 H, H-5^{II}), 3.86 (dd, 1 H, $J_{5,6b}$ 6.8, $J_{6a,6b}$ 11.8 Hz, H-6b^I), 4.70 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{II}), 5.15 (t, 1 H, H-3^{II}), 5.50 (t, 1 H, H-4^I), 6.32 (t, 1 H, H-3^I), 6.79 (d, 1 H, $J_{1,2}$ 8.8 Hz, H-1^I), 7.05 and 7.15 (2 d, each 2 H, Phth), 7.63–7.81 (2 m, 8 H, 2 $COC_6H_4CH_3$), 8.78 (s, 1 H, NH); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.4–20.5 ($COCH_3$), 21.3 and 21.4 (2 $COC_6H_4CH_3$), 53.7 (C-2^I), 61.7 and 67.3 (C-6^I, C-6^{II}), 68.2, 69.3, 70.5, 70.9, 71.6, 72.7, and 75.0 (C-3^I, C-4^I, C-5^I, C-2^{II}, C-3^{II}, C-4^{II}, C-5^{II}), 93.4 and 100.2 (C-1^I, C-1^{II}), 160.2 ($OC(NH)CCl_3$), 165.0 and 165.3 (2 $COC_6H_4CH_3$), 169.1–170.3 ($COCH_3$).



Scheme 7. Synthesis of tetrasaccharide **3**: (a) 1.5 equiv UDP-Gal, aq 50 mM sodium cacodylate buffer (pH 7.5), 2.5 U β-1,4-galactosyltransferase, 14 U alkaline phosphatase, 37 °C, 94%.

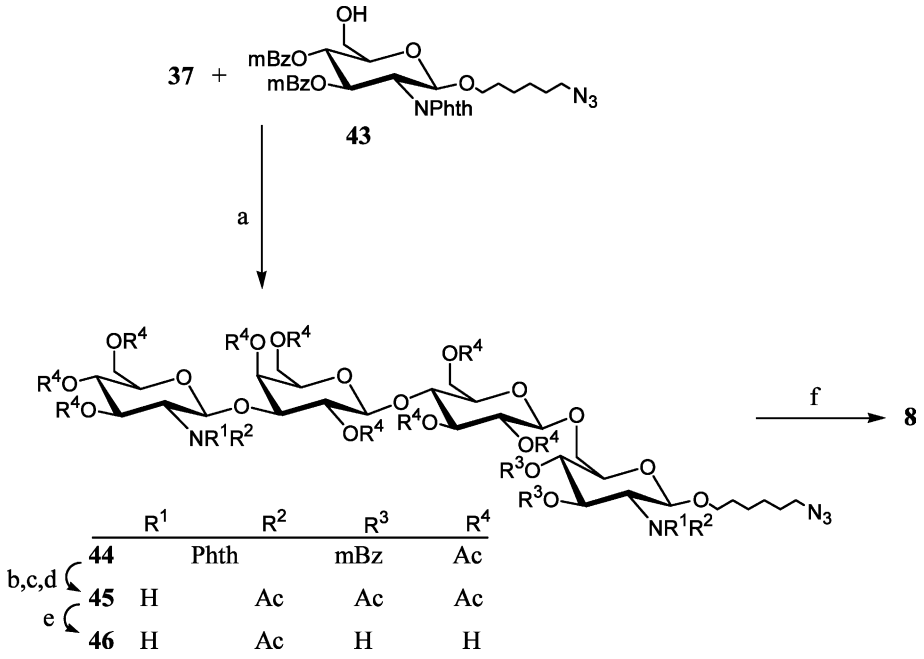
3.5. 6-Azidohexyl (3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-4-*O*-acetyl-2,6-di-*O*-benzyl-β-D-galactopyranoside (16)

A soln of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (**15**)[17] (1.09 g, 1.88 mmol) and 6-azidohexyl 4-*O*-acetyl-2,6-di-*O*-benzyl-β-D-galactopyranoside (**14**)[14] (0.84 g, 1.47 mmol) in dry CH_2Cl_2 (50 mL), containing 4 Å molecular sieves (1 g), was stirred under Ar for 1 h. After cooling to −70 °C, TMSOTf (34 μL, 0.19 mmol) was added and the mixture was stirred for 2 h, during which period the temperature was allowed to reach room temperature (rt). The mixture was neutralized with Et_3N, filtered, washed with water, dried ($MgSO_4$), filtered, and concentrated. Low-pressure column chromatography (10:1→4:1 toluene–EtOAc) of the residue gave **16**, isolated as a white foam (1.28 g, 86%); R_f 0.44 (2:1 toluene–EtOAc); $[\alpha]_D^{20}$ +3° (*c* 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.22–1.24 (m, 4 H, 2 CH_2), 1.37–1.42 (m, 2 H, CH_2), 1.48–1.52 (m, 2 H, CH_2), 1.82, 2.00, 2.03, and 2.08 (4 s, each 3 H, 4 $COCH_3$), 3.07 (t, 2 H, CH_2N_3), 3.84 (m, 1 H, OCH*H*), 4.48 (d, 2 H, $OCH_2C_6H_5$), 4.56 (d, 1 H, $OCHHC_6H_5$), 5.16 (t, 1 H, H-4^{II}), 5.38 (d, 1 H, $J_{3,4}$ 3.5, $J_{4,5}<1$ Hz, H-4^{I}), 5.59 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1^{II}), 5.77 (dd, 1 H, $J_{2,3}$ 10.7, $J_{3,4}$ 9.1 Hz, H-3^{II}); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.2–20.5 ($COCH_3$), 25.3, 26.2, 28.4, and 29.2 (4 CH_2), 51.0 (CH_2N_3), 54.7 (C-2^{II}), 61.2, 68.9, 69.8, 73.4, and 73.9 (C-6^{I}, C-6^{II}, 2 $OCH_2C_6H_5$, OCH_2), 68.6, 69.5, 70.4, 71.6, 72.6, 78.1, and 78.5 (C-2^{I}, C-3^{I}, C-

Table 7
500 MHz ^{1}H NMR data (TOCSY, ROESY) of **3** at 300 K (in ppm)

Proton	δ_H			
	Glc I	Gal II [a]	GlcNAc III	Gal IV [b]
H-1	4.48	4.44	4.71	4.48
H-2	3.29	3.58	3.78	3.54
H-3	3.63	3.72	3.73	3.67
H-4	3.60	4.15	3.73	3.93
H-5	3.62	n.d. [c]	3.58	n.d.
H-6a	3.96	n.d.	3.93	n.d.
H-6b	3.78	n.d.	3.85	n.d.
$O(CH_2)_2(CH_2)_2(CH_2)_2ND_2$		1.39–1.42 (4 H)		
$OCH_2CH_2(CH_2)_2CH_2CH_2ND_2$		1.63–1.70 (4 H)		
CH_2ND_2		2.99		
$OCH_2(CH_2)_5ND_2$		3.68, 3.92		
$NDCOCH_3$		2.03		

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

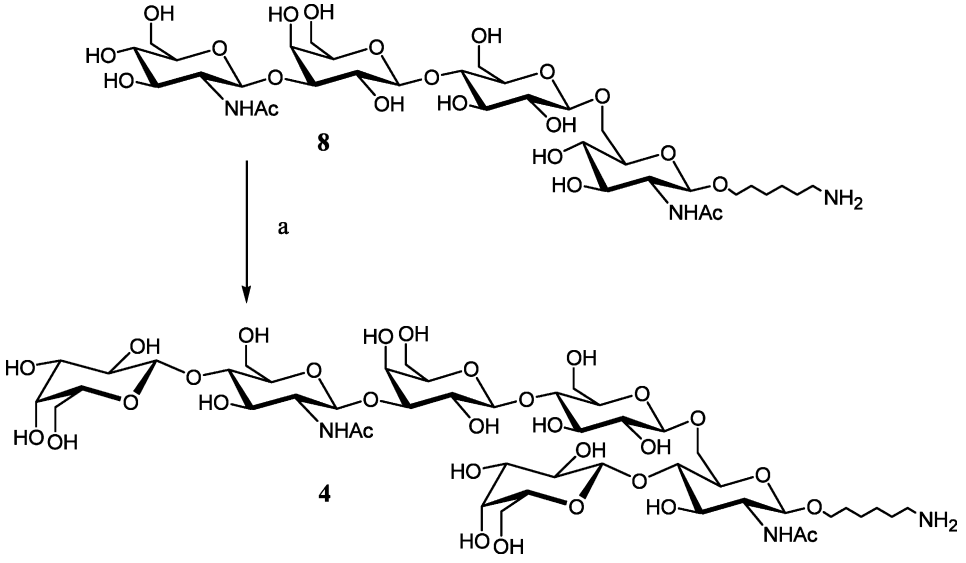


Scheme 8. Synthesis of the linear tetrasaccharide backbone **8**: (a) 15% TMSOTf, CH_2Cl_2, 0 °C, 34%; (b) NaOMe (pH 10), MeOH, CH_2Cl_2; (c) $NH_2CH_2CH_2NH_2$, 1-BuOH, 90 °C; (d) pyridine, Ac_2O, 86% over three steps; (e) NaOMe (pH 10), MeOH, CH_2Cl_2, 79%; (f) 10% Pd–C, H_2, water, *tert*-BuOH, aq 25% NH_3, 82%.

4^{I}, C-5^{I}, C-3^{II}, C-4^{II}, C-5^{II}), 97.9 and 103.3 (C-1^{I}, C-1^{II}), 169.1–170.4 ($COCH_3$); HRMS of $C_{48}H_{56}N_4O_{16}$ (M, 944.369): $[M+NH_4]^+$ found 962.399, calcd 962.403.

3.6. 6-Azidohexyl (2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-4-*O*-acetyl-2,6-di-*O*-benzyl-β-D-galactopyranoside (17)

To a soln of **16** (1.28 g, 1.35 mmol) in CH_2Cl_2 (5 mL) and MeOH (2 mL) was added NaOMe (pH 8). The mixture was stirred for 3 h, then neutralized with Dowex 50 × 8 (H^+), filtered, and concentrated. Column chromatography (1:2 toluene–EtOAc) of the residue gave **17**, isolated as a colourless glass (1.01 g, 91%); R_f 0.36 (1:3 toluene–EtOAc); $[\alpha]_D^{20}$ +5° (*c* 1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$; 2D TOCSY, ROESY): δ 1.13–1.17 (m, 4 H, 2 CH_2), 1.31–1.37 (m, 2 H, CH_2), 1.39–1.44 (m, 2 H, CH_2), 2.06 ($COCH_3$), 3.06 (t, 2 H, CH_2N_3), 4.00 (dd, 1 H, $J_{1,2}$ 8.5, $J_{2,3}$ 10.5 Hz, H-2^{II}), 4.21 (t, 1 H, H-3^{II}), 4.24 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1^{I}), 4.10 and 4.41 (2



Scheme 9. Synthesis of hexasaccharide **4**: (a) 3.2 equiv UDP-Gal, aq 50 mM sodium cacodylate buffer (pH 7.5), 5 U β-1,4-galactosyltransferase, 30 U alkaline phosphatase, 37 °C, 76%.

Table 8
500 MHz ^{1}H NMR data (TOCSY, ROESY) of **46** at 300 K (in ppm)

Proton	δ_H			
	GlcNAc I [a]	Glc II	Gal III	GlcNAc IV [b]
H-1	4.52	4.56	4.44	4.69
H-2	3.69	3.38	3.59	3.56
H-3	3.54	3.65	3.72	3.59
H-4	n.d. [c]	3.65	4.16	n.d.
H-5	3.62	3.62	n.d.	3.48
H-6a	4.22	3.99	n.d.	3.90
H-6b	3.89	3.81	n.d.	3.77
$O(CH_2)_2(CH_2)_2(CH_2)_2N_3$			1.35–1.37 (4 H)	
$OCH_2CH_2(CH_2)_2CH_2CH_2N_3$			1.57–1.61 (4 H)	
CH_2N_3			3.33	
$OCH_2(CH_2)_5N_3$			3.62, 3.91	
$NDCOCH_3$			2.04 (2)	

[a] GlcNAc(β1-O$(CH_2)_6N_3$).
[b] GlcNAc(β1-3)Gal.
[c] n.d., not determined.

d, each 1 H, $OCH_2C_6H_5$), 4.46 (s, 2 H, $OCH_2C_6H_5$), 5.36 (d, 1 H, H-1^{II}), 5.49 (d, 1 H, $J_{3,4}$ 3.3, $J_{4,5} < 1$ Hz, H-4^I); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.9 ($COCH_3$), 25.2, 26.1, 28.3, and 29.1 (4 CH_2), 51.1 (CH_2N_3), 56.6 (C-2^{II}), 61.3, 68.7, 69.8, 73.4, and 73.8 (C-6^I, C-6^{II}, 2 $OCH_2C_6H_5$, OCH_2), 70.4, 70.7, 71.0, 72.1, 75.8, 77.7, and 79.9 (C-2^I, C-3^I, C-4^I, C-5^I, C-3^{II}, C-4^{II}, C-5^{II}), 99.2 and 103.3 (C-1^I, C-1^{II}), 171.4 ($COCH_3$); HRMS of $C_{42}H_{50}N_4O_{13}$ (M, 818.337): $[M+Na]^+$ found 841.332, calcd 841.327.

3.7. 6-Azidohexyl (6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-4-*O*-acetyl-2,6-di-*O*-benzyl-β-D-galactopyranoside (18)

To a soln of **17** (0.18 g, 0.23 mmol) in CH_2Cl_2 (5 mL) and Py (0.4 mL) were added 4-dimethylaminopyridine (20 mg, 0.18 mmol), Et_3N (70 μL), and *tert*-butyldiphenylsilyl chloride (70 μL, 0.27 mmol). The mixture was stirred for 4 h, then poured into ice water, extracted with CH_2Cl_2, washed with aq satd $NaHCO_3$, dried ($MgSO_4$),

Table 9
500 MHz ^{1}H NMR data (TOCSY, ROESY) of **8** at 300 K (in ppm)

Proton	δ_H			
	GlcNAc I [a]	Glc II	Gal III	GlcNAc IV [b]
H-1	4.49	4.54	4.44	4.69
H-2	3.68	3.38	3.60	3.76
H-3	3.53	3.66	3.72	3.57
H-4	3.52	3.66	4.15	3.46
H-5	3.60	3.59	n.d. [c]	3.46
H-6a	4.21	3.97	n.d.	3.90
H-6b	3.88	3.81	n.d.	3.77
$O(CH_2)_2(CH_2)_2(CH_2)_2ND_2$			1.32–1.36 (4 H)	
$OCH_2CH_2(CH_2)_2CH_2CH_2ND_2$			1.52–1.55 (2 H), 1.62–1.65 (2 H)	
CH_2ND_2			2.97	
$OCH_2(CH_2)_5ND_2$			3.56, 3.83	
$NDCOCH_3$			2.02, 2.04	

[a] GlcNAc(β1-O$(CH_2)_6NH_2$).
[b] GlcNAc(β1-3)Gal.
[c] n.d., not determined.

Table 10
500 MHz ^{1}H NMR data (TOCSY, ROESY) of **4** at 300 K (in ppm)

Proton	δ_H					
	GlcNAc I [a]	Glc II	Gal III [b]	GlcNAc IV [c]	Gal V [d]	Gal VI [e]
H-1	4.53	4.56	4.44	4.71	4.53	4.48
H-2	3.72	3.37	3.59	3.80	3.55	3.55
H-3	n.d. [f]	3.67	3.72	3.73	3.67	3.67
H-4	3.83	3.67	4.16	3.73	3.93	3.92
H-5	3.72	3.62	n.d.	3.58	n.d.	n.d.
H-6a	4.29	4.00	n.d.	3.95	n.d.	n.d.
H-6b	3.95	3.83	n.d.	3.85	n.d.	n.d.
$O(CH_2)_2(CH_2)_2(CH_2)_2ND_2$			1.36–1.38 (4 H)			
$OCH_2CH_2(CH_2)_2CH_2CH_2ND_2$			1.54–1.57 (2 H), 1.64–1.67 (2 H)			
CH_2ND_2			2.99			
$OCH_2(CH_2)_5ND_2$			3.68, 3.92			
$NDCOCH_3$			2.03 (2)			

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

filtered, and concentrated. Low-pressure column chromatography (5:1 → 1:1 toluene–EtOAc) of the residue gave **18**, isolated as a colourless glass (0.19 g, 83%); R_f 0.53 (1:1 toluene–EtOAc); $[\alpha]_D^{20}$ +9° (*c* 1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$; 2D TOCSY, ROESY): δ 1.05 [s, 9 H, $SiC(CH_3)_3$], 1.19–1.22 (m, 4 H, 2 CH_2), 1.37–1.40 (m, 2 H, CH_2), 1.46–1.48 (m, 2 H, CH_2), 1.96 (s, 3 H, $COCH_3$), 3.07 (t, 2 H, CH_2N_3), 3.33 (dd, 1 H, $J_{1,2}$ 7.9, $J_{2,3}$ 9.8 Hz, H-2^I), 3.37 (m, 1 H, OC*H*H), 3.78 (dd, 1 H, $J_{3,4}$ 3.5 Hz, H-3^I), 3.82 (m, 1 H, OCH*H*), 4.04 (dd, 1 H, $J_{1,2}$ 8.3, $J_{2,3}$ 11.0 Hz, H-2^{II}), 4.17 and 4.53 (2 d, each 1 H, $OCH_2C_6H_5$), 4.20 (d, 1 H, H-1^I), 4.37 (t, 1 H, H-3^{II}), 4.38 and 4.43 (2 d, each 1 H, $OCH_2C_6H_5$), 5.33 (d, 1 H, $J_{4,5} < 1$ Hz, H-4^I), 5.41 (d, 1 H, H-1^{II}); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 19.0 [$SiC(CH_3)_3$], 20.5 ($COCH_3$), 25.3, 26.2, 28.4, and 29.2 (4 CH_2), 26.6 [$SiC(CH_3)_3$], 51.0 (CH_2N_3), 56.6 (C-2^{II}), 64.5, 68.9, 69.8, 73.3, and 73.9 (C-6^I, C-6^{II}, 2 $OCH_2C_6H_5$, OCH_2), 70.1, 71.0, 72.5, 73.6, 74.8, 76.4, and 78.6 (C-2^I, C-3^I, C-4^I, C-5^I, C-3^{II}, C-4^{II}, C-5^{II}), 98.0 and 103.4 (C-1^I, C-1^{II}), 170.0 ($COCH_3$); HRMS of $C_{58}H_{68}N_4O_{13}Si$ (M, 1056.455): $[M+NH_4]^+$ found 1074.496, calcd 1074.489.

3.8. 6-Azidohexyl (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-galactopyranoside (19)

To a soln of **18** (0.19 g, 0.19 mmol) in dry Py (4 mL) was added dropwise, at 0 °C, a soln of *p*-methylbenzoyl chloride (60 μL, 0.48 mmol) in dry CH_2Cl_2 (1 mL). The mixture was stirred for 4 h at rt, then poured into ice water, extracted with CH_2Cl_2, washed with aq satd $NaHCO_3$, dried ($MgSO_4$), filtered, and concentrated. Column chromatography (5:1 toluene–EtOAc) of the residue gave **19**, isolated as a colourless syrup (0.21 g, 90%); R_f 0.39 (5:1 toluene–EtOAc); $[\alpha]_D^{20}$ +1° (*c* 1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$; 2D TOCSY, ROESY): δ 1.06 [s, 9 H, $SiC(CH_3)_3$], 1.23–1.26 (m, 4 H, 2 CH_2), 1.38–1.41 (m, 2 H, CH_2), 1.52–1.54 (m, 2 H, CH_2), 2.04 (s, 3 H, $COCH_3$), 2.18 and 2.28 (2 s, each 3 H, 2 $COC_6H_4CH_3$), 3.07 (t, 2 H, CH_2N_3), 3.64 (t, 1 H, H-6a^I), 3.83 (d, 1 H, $J_{6a,6b}$ 9.4 Hz, H-6b^{II}), 3.89 (m, 1 H, OCH*H*), 4.00 (dd, 1 H, $J_{2,3}$ 9.6, $J_{3,4}$ 3.5 Hz, H-3^I), 4.24 and 4.65 (2 d, each 1 H, $OCH_2C_6H_5$), 4.30 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1^I), 4.43 and 4.49 (2 d, each 1 H, $OCH_2C_6H_5$), 4.53 (dd, 1 H, $J_{1,2}$ 8.3, $J_{2,3}$ 10.7 Hz, H-2^{II}), 5.56 (t, 1 H, H-4^{II}), 5.59 (d, 1 H, $J_{4,5} < 1$ Hz, H-4^I), 5.84 (d, 1 H, H-1^{II}), 6.31 (dd, 1 H, $J_{3,4}$ 9.2 Hz, H-3^{II}), 6.97 and 7.08 (2 d, each 2 H, Phth); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 19.1 [$SiC(CH_3)_3$], 20.5 ($COCH_3$), 21.4 and 21.5 (2 $COC_6H_4CH_3$), 25.5, 26.3, 28.5, and 29.4 (4 CH_2), 26.6 [$SiC(CH_3)_3$], 51.1 (CH_2N_3), 55.5 (C-2^{II}), 63.0, 69.1, 69.8, 73.4, and 73.9 (C-6^I, C-6^{II}, 2 $OCH_2C_6H_5$, OCH_2), 69.9, 70.3, 70.8, 72.8, 75.1, 76.4, and 78.7 (C-2^I, C-3^I, C-4^I, C-5^I, C-3^{II}, C-4^{II}, C-5^{II}), 98.2 and 103.6 (C-1^I, C-1^{II}), 164.9 and 165.5 (2 $COC_6H_4CH_3$), 169.7 ($COCH_3$); HRMS of $C_{74}H_{80}N_4O_{17}Si$ (M, 1292.538): $[M+NH_4]^+$ found 1310.584, calcd 1310.573.

3.9. 6-Azidohexyl (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-galactopyranoside (20)

To a soln of acetyl chloride (0.80 mL, 9.70 mmol) in dry MeOH (15 mL) was added, at 0 °C, a soln of **19** (0.88 g, 0.71 mmol) in dry toluene (3 mL). The mixture was stirred for 4 h at 0 °C, then co-concentrated with toluene. Column chromatography (4:1 toluene–EtOAc) of the residue gave **20**, isolated as a colourless syrup (0.61 g, 81%); R_f 0.44 (2:1 toluene–EtOAc); $[\alpha]_D^{20}$ +9° (*c* 1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$; 2D TOCSY, ROESY): δ 1.12–1.17 (m, 4 H, 2 CH_2), 1.32–1.35 (m, 2 H, CH_2), 1.40–1.44 (m, 2 H, CH_2), 2.18 (s, 3 H, $COCH_3$), 2.26 and 2.33 (2 s, each 3 H, 2 $COC_6H_4CH_3$), 3.05 (CH_2N_3), 3.34 (m, 1 H, OC*H*H), 3.49 (dd, 1 H, $J_{1,2}$ 7.7, $J_{2,3}$ 9.4 Hz, H-2^I), 3.51 (d, 2 H, $J_{6a,6b}$ 5.8 Hz, H-6a^I, H-6b^I), 3.82 (m, 1 H, OCH*H*), 3.90 (m, 1 H, H-5^{II}), 4.21 and 4.45 (2 d, each 1 H, $OCH_2C_6H_5$), 4.27 (d, 1 H, H-1^I), 4.48 (s, 2 H, $OCH_2C_6H_5$), 4.51 (dd, 1 H, $J_{1,2}$ 8.3, $J_{2,3}$ 10.7 Hz, H-2^{II}), 5.43 (t, 1 H, H-4^{II}), 5.60 (d, 1 H, $J_{3,4}$ 3.5, $J_{4,5}<1$ Hz, H-4^I), 5.72 (d, 1 H, H-1^{II}), 6.13 (dd, 1 H, $J_{3,4}$ 9.2 Hz, H-3^{II}); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 21.0 ($COCH_3$), 21.4 and 21.5 (2 $COC_6H_4CH_3$), 25.3, 26.2, 28.4, and 29.2 (4 CH_2), 51.1 (CH_2N_3), 55.0 (C-2^{II}), 61.5, 70.0, 73.6, 68.8, and 73.9 (C-6^I, C-6^{II}, 2 $OCH_2C_6H_5$, OCH_2), 69.3, 70.1, 70.8, 72.4, 75.2, 77.5, and 81.5 (C-2^I, C-3^I, C-4^I, C-5^I, C-3^{II}, C-4^{II}, C-5^{II}), 99.4 and 103.5 (C-1^I, C-1^{II}), 165.2 and 165.4 (2 $COC_6H_4CH_3$), 171.4 ($COCH_3$); HRMS of $C_{58}H_{62}N_4O_{15}$ (M, 1054.421): $[M+NH_4]^+$ found 1072.461, calcd 1072.455.

3.10. 6-Azidohexyl (2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-(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-galactopyranoside (21)

(a) A soln of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (**9**)[16] (0.40 g, 0.82 mmol) and **20** (0.48 g, 0.45 mmol) in dry CH_2Cl_2 (20 mL), containing 4 Å molecular sieves (0.5 g), was stirred under Ar for 0.5 h. After cooling to −40 °C, TMSOTf (14 μL, 0.077 mmol) was added, and the mixture was stirred for 2.5 h, during which period the temperature was allowed to reach rt, then neutralized with Et_3N, filtered, and concentrated. Column chromatography (4:1 toluene–EtOAc) of the residue gave **21**, isolated as white foam (0.35 g, 56%).

(b) A soln of **13** (0.13 g, 0.13 mmol) and 6-azidohexyl 4-*O*-acetyl-2,6-di-*O*-benzyl-β-D-galactopyranoside (**14**)[14] (56 mg, 0.11 mmol) in dry CH_2Cl_2 (3 mL), containing 4 Å molecular sieves (0.1 g), was stirred under Ar for 0.5 h. After cooling to −70 °C, TMSOTf (2.2 μL, 0.013 mmol) was added, and the mixture was stirred for 2.5 h, during which period the temperature was allowed to reach rt, then neutralized with Et_3N, filtered, and concentrated. Column chromatography (4:1 toluene–EtOAc) of the residue gave **21**, isolated as a white foam (0.13 g, 73%); R_f 0.54 (2:1 toluene–EtOAc); $[\alpha]_D^{20}$ +8° (*c* 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.23–1.25 (m, 4 H, 2 CH_2), 1.39–1.43 (m, 2 H, CH_2), 1.47–1.52 (m, 2 H, CH_2), 1.95, 1.98, 2.02, 2.05, and 2.09 (5 s, each 3 H, 5 $COCH_3$), 2.27 and 2.34 (2 s, each 3 H, 2 $COC_6H_4CH_3$), 3.11 (t, 2 H, CH_2N_3), 3.38 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 9.5 Hz, H-2^I), 3.42 (m, 1 H, OC*H*H), 4.07 (dd, 1 H, $J_{5,6b}$ 4.4, $J_{6a,6b}$ 12.3 Hz, H-6b^{III}), 4.10 and 4.55 (2 d, each 1 H, $OCH_2C_6H_5$), 4.36 (d, 1 H, H-1^I), 4.44 (dd, 1 H, $J_{1,2}$ 8.2, $J_{2,3}$ 10.8 Hz, H-2^{II}), 4.47 and 4.56 (2 d, each 1 H, $OCH_2C_6H_5$), 4.70 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{III}), 4.95 (dd, 1 H, $J_{2,3}$ 9.6 Hz, H-2^{III}), 5.01 (t, 1 H, H-4^{III}), 5.21 (t, 1 H, H-3^{III}), 5.36 (t, 1 H, H-4^{II}), 5.55 (d, 1 H, $J_{3,4}$ 3.5, $J_{4,5}<1$ Hz, H-4^I), 5.68 (d, 1 H, H-1^{II}), 6.24 (dd, 1 H, $J_{3,4}$ 9.1 Hz, H-3^{II}); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.4–20.8 ($COCH_3$), 21.4 and 21.5 (2 $COC_6H_4CH_3$), 25.5, 26.3, 28.5, and 29.3 (4 CH_2), 51.2 (CH_2N_3), 55.3 (C-2^{II}), 61.5, 68.0 (2 C), 69.9, 73.4, and 73.9 (C-6^I, C-6^{II}, C-6^{III}, 2 $OCH_2C_6H_5$, OCH_2), 67.9, 69.7, 69.9, 70.3, 71.5, 71.6, 71.8, 72.7, 74.5, 77.2, and 78.5 (C-2^I, C-3^I, C-4^I, C-5^I, C-3^{II}, C-4^{II}, C-5^{II}, C-2^{III}, C-3^{III}, C-4^{III}, C-5^{III}), 98.4, 100.9, and 103.6 (C-1^I, C-1^{II}, C-1^{III}), 165.1 and 165.4 (2 $COC_6H_4CH_3$), 169.1–170.5 ($COCH_3$); HRMS of $C_{72}H_{80}N_4O_{24}$ (M, 1384.516): $[M+Na]^+$ found 1407.495, calcd 1407.506.

3.11. 6-Azidohexyl (2,3,4,6-tetra-*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 (22)

To a soln of **21** (90 mg, 64.9 μmol) in MeOH (8 mL) and CH_2Cl_2 (2 mL) was added NaOMe (pH 10), and the mixture was stirred for 4 h, then neutralized with Dowex 50 × 8 (H^+), filtered, and concentrated. To a soln of the residue in 1-BuOH (30 mL) was added 1,2-diaminoethane (6 mL), and the mixture was stirred overnight at 80 °C, then co-concentrated with toluene, EtOH, and CH_2Cl_2. A soln of the residue in Py (30 mL) and Ac_2O (30 mL) was stirred overnight, then co-concentrated with toluene, EtOH and CH_2Cl_2. Column chromatography (1:2 toluene–EtOAc) of the residue gave **22**, isolated as a colourless syrup (60 mg, 80%); R_f 0.27 (1:2 toluene–EtOAc); $[\alpha]_D^{20}$ −8° (*c* 1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$; 2D TOCSY, ROESY): δ 1.32–1.39 (m, 4 H, 2 CH_2), 1.47–1.54 (m, 2 H, CH_2), 1.59–1.63 (m, 2 H, CH_2), 1.59, 1.95, 1.96, 1.99, 2.06 and 2.07 (6 s, 3,3,3,6,3,6 H, 7 $COCH_3$, $NHCOCH_3$), 3.18 (t, 2 H, CH_2N_3), 3.63 (m, 1 H, H-5^{III}), 3.71 (dd, 1 H, $J_{5,6b}$ 6.8, $J_{6a,6b}$ 12.3 Hz, H-6b^{II}), 3.80 (dd, 1 H, $J_{5,6a}$ 1.5 Hz, H-6a^{II}), 4.19 (dd, 1 H, $J_{5,6b}$ 4.2, $J_{6a,6b}$ 12.5 Hz, H-6b^{III}), 4.43 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1^{III}), 4.47 and 4.56 (2 d, each 1 H, $OCH_2C_6H_5$), 4.55 and 5.04 (2 d, each 1 H,

$OCH_2C_6H_5$), 4.65 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1^{I}), 4.73 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1^{II}), 4.83 (t, 1 H, H-4^{II}), 4.89 (t, 1 H, H-2^{II}), 4.99 (t, 1 H, H-2^{III}), 5.09 (t, 1 H, H-4^{III}), 5.25 (t, 1 H, H-3^{III}), 5.47 (d, 1 H, $J_{3,4}$ 3.5, $J_{4,5} < 1$ Hz, H-4^{I}); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.4–20.7 ($COCH_3$), 22.8 ($NHCOCH_3$), 25.5, 26.3, 28.6, and 29.4 (4 CH_2), 51.2 (CH_2N_3), 54.1 (C-2^{II}), 61.5, 68.2 (2 C), 69.8, 73.4, and 74.4 (C-6^{I}, C-6^{II}, C-6^{III}, 2 $OCH_2C_6H_5$, OCH_2), 68.0, 69.0, 69.5, 71.6 (2 C), 72.1, 72.4, 72.6, 73.8, 76.9, and 79.9 (C-2^{I}, C-3^{I}, C-4^{I}, C-5^{I}, C-3^{II}, C-4^{II}, C-5^{II}, C-2^{III}, C-3^{III}, C-4^{III}, C-5^{III}), 100.6, 101.2, and 103.4 (C-1^{I}, C-1^{II}, C-1^{III}), 169.1–170.6 ($COCH_3$); HRMS of $C_{54}H_{72}N_4O_{23}$ (M, 1144.458): $[M+NH_4]^+$ found 1162.489, calcd 1162.492.

3.12. 6-Aminohexyl β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranoside (5)

To a soln of **22** (50 mg, 43.6 μmol) in CH_2Cl_2 (1 mL) and MeOH (1 mL) was added NaOMe (pH 9). The mixture was stirred for 3 h, then neutralized with Dowex 50 × 8 (H^+), filtered, and concentrated, giving crude **23** as a white solid (36 mg). To a soln of **23** in *tert*-BuOH (8 mL) and water (8 mL) were added 10% Pd–C (100 mg) and 3 drops of aq 25% NH_3. The mixture was stirred for 3 h under H_2 after which NH_3 was removed by bubbling with N_2, then 10% Pd–C (60 mg) and 3 drops of AcOH were added, and the stirring under H_2 was continued overnight. The mixture was loaded on a short Dowex 50 × 8 (H^+) column, which was first eluted with water to remove contaminations, then with aq 10% NH_4OH to give **5**, isolated as a white solid after lyophilization (19 mg, 69%); R_f 0.34 (2:1:1 AcOH–1-BuOH–water); $[\alpha]_D^{20}$ −2° (*c* 1, water); ^{13}C NMR (75.5 MHz, D_2O): δ 22.9 ($NDCOCH_3$), 25.3, 26.0, 27.9, and 29.2 (4 CH_2), 40.3 (CH_2ND_2), 56.4 (C-2^{II}), 61.5, 61.7, 69.4, and 71.1 (C-6^{I}, C-6^{II}, C-6^{III}, OCH_2), 69.0, 70.4 (2 C), 70.5, 73.8, 74.3, 75.4 (2 C), 76.5, 76.7, and 83.1 (C-2^{I}, C-3^{I}, C-4^{I}, C-5^{I}, C-3^{II}, C-4^{II}, C-5^{II}, C-2^{III}, C-3^{III}, C-4^{III}, C-5^{III}), 103.3, 103.5, and 103.7 (C-1^{I}, C-1^{II}, C-1^{III}), 175.7 ($NDCOCH_3$); 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 1.

3.13. 6-Aminohexyl β-D-glucopyranosyl-(1→6)-[β-D-galactopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranoside (1)

To a soln of **5** (9.9 mg, 15.35 μmol) in aq 50 mM sodium cacodylate buffer pH 7.5 (700 μL), containing 5 mM $MnCl_2$, bovine serum albumin (BSA) (0.5 mg), and NaN_3 (0.02%), were added alkaline phosphatase (14 U), UDP-galactose (13 mg, 21.3 μmol), and β-1,4-Galactosyltransferase (3 U). The mixture was incubated for 20 h at 37 °C, then water (100 μL) was added. UDP-galactose was removed using a Dowex 1 × 8 (Cl^-) column with water as eluent. The eluate was concentrated, and the residue applied to 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** (7.2 mg, 54%) and **47** (5.2 mg, 35%); R_f 0.20 (2:1:1 AcOH–1-BuOH–water); $[\alpha]_D^{20}$ −3° (*c* 0.5, water); HRMS of $C_{32}H_{58}N_2O_{21}$ (M, 806.353): $[M+H]^+$ found 807.352, calcd 807.361. For ^{1}H NMR data, see Table 2.

3.14. 6-Azidohexyl (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 (25)

A soln of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (**15**)[17] (0.48 g, 0.77 mmol) and 6-azidohexyl (4-*O*-acetyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**24**)[14] (0.62 g, 0.64 mmol) in dry CH_2Cl_2 (15 mL), containing 4 Å powdered molecular sieves (0.5 g), was stirred under Ar for 0.5 h. After cooling to −70 °C, TMSOTf (14 μL, 0.077 mmol) was added, and the mixture was stirred for 2.5 h, during which period the temperature was allowed to reach rt, then neutralized with Et_3N, filtered over hyflo, washed with water, dried ($MgSO_4$), filtered, and concentrated. Column chromatography (2:1 toluene–EtOAc) of the residue gave **25**, isolated as a colourless foam (0.87 g, 97%); R_f 0.50 (2:1 toluene–EtOAc); $[\alpha]_D^{20}$ −2° (*c* 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.33–1.37 (m, 4 H, 2 CH_2), 1.51–1.54 (m, 2 H, CH_2), 1.58–1.60 (m, 2 H, CH_2), 1.82, 2.02, and 2.06 (3 s, 3,6,3 H, 4 $COCH_3$), 3.03 (m, 1 H, H-5^{I}), 3.18 (t, 2 H, CH_2N_3), 3.49 (dd, 1 H, $J_{5,6a}$ 4.1, $J_{6a,6b}$ 11.0 Hz, H-$6a^{I}$), 3.58 (dd, 1 H, $J_{2,3}$ 9.5, $J_{3,4}$ 3.5 Hz, H-3^{II}), 3.87 (t, 1 H, H-4^{I}), 4.15 (d, 1 H, $OCHHC_6H_5$), 4.41 (d, 1 H, $OCHHC_6H_5$), 4.48 (d, 1 H, $OCHHC_6H_5$), 4.65 and 4.88 (2 d, each 1 H, $OCH_2C_6H_5$), 4.67 and 4.81 (2 d, each 1 H, $OCH_2C_6H_5$), 5.16 (t, 1 H, H-4^{III}), 5.38 (d, 1 H, $J_{4,5} < 1$ Hz, H-4^{II}), 5.53 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1^{III}), 5.78 (dd, 1 H, $J_{2,3}$ 10.7, $J_{3,4}$ 9.2 Hz, H-3^{III}); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.0–20.3 ($COCH_3$), 25.3, 26.1, 28.4, and 29.2 (4 CH_2), 50.9 (CH_2N_3), 54.6 (C-2^{III}), 61.3, 67.4, 67.9, 69.3, 72.2, 73.1, 74.0, 74.5, and 74.7 (C-6^{I}, C-6^{II}, C-6^{III}, 5 $OCH_2C_6H_5$, OCH_2), 68.6, 69.5, 70.2, 71.4, 72.2, 74.3, 75.3, 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}), 97.9, 101.4, and 103.1 (C-1^{I}, C-1^{II}, C-1^{III}), 169.0, 169.3, 169.7, and 170.3 (4 $COCH_3$); HRMS of $C_{75}H_{84}N_4O_{21}$ (M, 1376.562): $[M+NH_4]^+$ found: 1394.548, calcd 1394.597.

3.15. 6-Azidohexyl (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 (26)

To a soln of **25** (0.85 g, 0.62 mmol) in CH_2Cl_2 (10 mL) and MeOH (20 mL) was added NaOMe (pH 8). The mixture was stirred for 3 h, then neutralized with Dowex 50 × 8 (H^+), filtered, and concentrated. Column chromatography (3:1→1:1 toluene–EtOAc) of the residue gave **26**, isolated as a colourless syrup (0.60 g, 78%); R_f 0.35 (1:3 toluene–EtOAc); $[\alpha]_D^{20}$ +3° (*c* 1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$; 2D TOCSY, ROESY): δ 1.33–1.36 (m, 4 H, 2 CH_2), 1.50–1.53 (m, 2 H, CH_2), 1.57–1.60 (m, 2 H, CH_2), 2.07 (s, 3 H, $COCH_3$), 2.98 (m, 1 H, H-5^I), 3.17 (t, 2 H, CH_2N_3), 4.02 (dd, 1 H, $J_{1,2}$ 8.3, $J_{2,3}$ 10.8 Hz, H-2^{III}), 4.08 and 4.21 (2 d, each 1 H, $OCH_2C_6H_5$), 4.20 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1^I), 4.22 and 4.42 (2 d, each 1 H, $OCH_2C_6H_5$), 4.24 and 4.37 (2 d, each 1 H, $OCH_2C_6H_5$), 4.31 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1^{II}), 4.64 and 4.81 (2 d, each 1 H, $OCH_2C_6H_5$), 4.66 and 4.87 (2 d, each 1 H, $OCH_2C_6H_5$), 5.37 (d, 1 H, H-1^{III}), 5.52 (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 20.9 ($COCH_3$), 25.5, 26.3, 28.6, and 29.4 (4 CH_2), 51.2 (CH_2N_3), 56.7 (C-2^{III}), 61.0, 67.7, 67.8, 69.4, 72.9, 73.4, 74.1, 74.7, and 74.9 (C-6^I, C-6^{II}, C-6^{III}, 5 $OCH_2C_6H_5$, OCH_2), 70.5 (2 C), 70.9, 71.9, 74.5, 75.7, 75.8, 78.4, 80.4, 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}), 99.2, 101.7, and 103.4 (C-1^I, C-1^{II}, C-1^{III}), 171.1 ($COCH_3$); HRMS of $C_{69}H_{78}N_4O_{18}$ (M, 1250.531): $[M+NH_4]^+$ found 1268.537, calcd 1268.565.

3.16. 6-Azidohexyl (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 (27)

To a soln of **26** (0.30 g, 0.24 mmol) in CH_2Cl_2 (3 mL) and Py (0.2 mL) were added 4-dimethylaminopyridine (9 mg, 0.072 mmol), Et_3N (0.2 mL), and *tert*-butyldiphenylsilyl chloride (73 μL, 0.29 mmol). The mixture was stirred for 20 h, then poured into ice water, extracted with CH_2Cl_2, washed with aq satd $NaHCO_3$, dried ($MgSO_4$), filtered, and concentrated. Column chromatography (1:1 toluene–EtOAc) of the residue gave **27**, isolated as a colourless syrup (0.30 g, 84%); R_f 0.42 (1:1 toluene–EtOAc); $[\alpha]_D^{20}$ +8° (*c* 1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$; 2D TOCSY, ROESY, HSQC): δ 1.04 [s, 9 H, $SiC(CH_3)_3$], 1.33–1.38 (m, 4 H, 2 CH_2), 1.48–1.55 (m, 2 H, CH_2), 1.58–1.59 (m, 2 H, CH_2), 1.97 (s, 3 H, $COCH_3$), 3.05 (m, 1 H, H-5^I), 3.16 (t, 2 H, CH_2N_3), 3.51 (t, 1 H, H-6b^I), 3.63 (dd, 1 H, $J_{2,3}$ 9.8, $J_{3,4}$ 3.2 Hz, H-3^{II}), 4.04 (dd, 1 H, $J_{1,2}$ 8.3, $J_{2,3}$ 11.0 Hz, H-2^{III}), 4.12 (d, 1 H, $OCHC_6H_5$), 4.16 and 4.35 (2 d, each 1 H, $OCH_2C_6H_5$), 4.40 (m, 1 H, H-3^{III}), 4.43 (d, 1 H, $OCHC_6H_5$), 4.62 and 4.87 (2 d, each 1 H, $OCH_2C_6H_5$), 4.66 and 4.81 (2 d, each 1 H, $OCH_2C_6H_5$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 19.0 [$SiC(CH_3)_3$], 20.5 ($COCH_3$), 25.5, 26.3, 28.5, and 29.4 (4 CH_2), 26.6 [$SiC(CH_3)_3$], 51.1 (CH_2N_3), 56.6 (C-2^{III}), 64.8 (C-6^{III}), 67.7 (C-6^I), 68.1 (C-6^{II}), 69.4 (OCH_2), 70.1 (C-4^{II}), 70.9 (C-3^{III}), 72.5 (C-5^{II}), 73.9 (C-4^{III}), 74.5 (C-5^{III}), 74.6 (C-5^I), 72.9, 73.2, 74.2, 74.7, and 74.9 (5 $OCH_2C_6H_5$), 75.5 (C-4^I), 77.2 (C-3^{II}), 79.1 (C-2^{II}), 81.4 (C-2^I), 82.4 (C-3^I), 98.3 (C-1^{III}), 101.7 (C-1^{II}), 103.3 (C-1^I), 169.7 ($COCH_3$); HRMS of $C_{85}H_{96}N_4O_{18}$ (M, 1488.648): $[M+H]^+$ found 1489.638, calcd 1489.657.

3.17. 6-Azidohexyl (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 (28)

To a soln of **27** (0.12 g, 0.083 mmol) in dry Py (5 mL) was added dropwise, at 0 °C, a soln of *p*-methylbenzoyl chloride (27 μL, 0.21 mmol) in dry CH_2Cl_2 (1 mL). The mixture was stirred for 18 h at rt, then poured into ice water, extracted with CH_2Cl_2, washed with aq satd $NaHCO_3$, dried ($MgSO_4$), filtered, and concentrated. Column chromatography (8:1 toluene–EtOAc) of the residue gave **28**, isolated as a white solid (0.13 g, 91%); R_f 0.68 (4:1 toluene–EtOAc); $[\alpha]_D^{20}$ −3° (*c* 1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$; 2D TOCSY, ROESY, HSQC): δ 1.06 [$SiC(CH_3)_3$], 1.32–1.36 (m, 4 H, 2 CH_2), 1.48–1.51 (m, 2 H, CH_2), 1.58–1.62 (m, 2 H, CH_2), 2.10 ($COCH_3$), 2.17 and 2.34 (2 s, each 3 H, 2 $COC_6H_4CH_3$), 3.13 (t, 2 H, CH_2N_3), 3.16 (m, 1 H, H-5^I), 3.43 (dd, 1 H, $J_{1,2}$ 8.3, $J_{2,3}$ 9.2 Hz, H-2^{II}), 3.59 (m, 1 H, H-5^{II}), 3.63 (dd, 1 H, $J_{5,6b}$ 3.5, $J_{6a,6b}$ 10.8 Hz, H-6b^I), 4.18 (d, 1 H, $OCHC_6H_5$), 4.24 (d, 1 H, $OCHC_6H_5$), 4.31 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1^I), 4.32 and 4.52 (2 d, each 1 H, $OCH_2C_6H_5$), 4.58 (dd, 1 H, $J_{1,2}$ 8.3, $J_{2,3}$ 10.7 Hz, H-2^{III}), 4.73 and 4.87 (2 d, each 1 H, $OCH_2C_6H_5$), 4.69 and 4.97 (2 d, each 1 H, $OCH_2C_6H_5$), 5.81 (d, 1 H, H-1^{III}), 6.37 (t, 1 H, H-3^{III}); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 18.7 [$SiC(CH_3)_3$], 20.3 ($COCH_3$), 21.0 and 21.3 ($COC_6H_4CH_3$), 25.3, 26.0, 28.3, and 29.2 (4 CH_2), 26.3 [$SiC(CH_3)_3$], 50.9 (CH_2N_3), 55.2 (C-2^{III}), 62.6 (C-6^{III}), 67.4 (C-6^I), 67.9 (C-6^{II}), 69.2 (OCH_2), 69.5 (C-4^{III}), 70.1 (C-4^{II}), 70.6 (C-3^{III}), 72.5 (C-5^{II}), 72.7, 73.0, 74.1, 74.4, and 74.5 (5 $OCH_2C_6H_5$), 74.5 (C-5^I), 75.2 (2 C) (C-4^I, C-5^{III}), 77.0 (C-3^{II}), 78.9 (C-2^{II}), 81.3 (C-2^I), 82.2 (C-3^I), 98.2 (C-1^{III}), 101.6 (C-1^{II}), 103.1 (C-1^I), 164.6 and 165.2 (2 $COC_6H_4CH_3$), 169.2 ($COCH_3$); HRMS of $C_{101}H_{108}N_4O_{20}Si$ (M, 1724.736): $[M+H]^+$ found 1725.741, calcd 1725.740.

3.18. 6-Azidohexyl (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 (29)

A soln of **28** (0.10 g, 60.2 μmol) in 1.0 M TBAF in THF (1 mL) and AcOH (1 mL) (pH 6) was stirred for 1 h at 0 °C followed by 4 h at rt. After the addition of CH_2Cl_2, the soln was washed with water and aq satd NaCl, dried (Na_2SO_4), filtered, and concentrated. Column chromatography (4:1 toluene–EtOAc) of the residue gave **29**, isolated as a colourless syrup (80 mg, 89%); R_f 0.34 (4:1 toluene–EtOAc); $[\alpha]_D^{20}$ −2° (*c* 1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$; 2D TOCSY, ROESY): δ 1.31–1.33 (m, 4 H, 2 CH_2), 1.49–1.52 (m, 2 H, CH_2), 1.55–1.59 (m, 2 H, CH_2), 2.14 (s, 3 H, $COCH_3$), 2.25 and 2.33 (2 s, each 1 H, 2 $COC_6H_4CH_3$), 2.95 (m, 1 H, H-5^I), 3.18 (t, 2 H, CH_2N_3), 3.55 (dd, 1 H, $J_{2,3}$ 9.6, $J_{3,4}$ 3.5 Hz, H-3^{II}), 3.66 (dd, 1 H, $J_{5,6b}$ 5.5, $J_{6a,6b}$ 10.8 Hz, H-6b^{III}), 3.81 (m, 1 H, OCH*H*), 3.85 (t, 1 H, H-4^I), 3.92 (m, 1 H, H-5^{III}), 4.18 and 4.23 (2 d, each 1 H, $OCH_2C_6H_5$), 4.19 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1^I), 4.23 and 4.45 (2 d, each 1 H, $OCH_2C_6H_5$), 4.29 and 4.38 (2 d, each 1 H, $OCH_2C_6H_5$), 4.34 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1^{II}), 4.49 (dd, 1 H, $J_{1,2}$ 8.5, $J_{2,3}$ 10.6 Hz, H-2^{III}), 4.66 and 4.81 (2 d, each 1 H, $OCH_2C_6H_5$), 4.67 and 4.90 (2 d, each 1 H, $OCH_2C_6H_5$), 5.49 (t, 1 H, H-4^{III}), 5.59 (d, 1 H, $J_{4,5}$ < 1 Hz, H-4^{II}), 5.70 (d, 1 H, H-1^{III}), 6.16 (dd, 1 H, $J_{3,4}$ 9.4 Hz, H-3^{III}); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.3 ($COCH_3$), 21.0 and 21.5 ($COC_6H_4CH_3$), 25.6, 26.4, 28.6, and 29.4 (4 CH_2), 51.2 (CH_2N_3), 55.0 (C-2^{III}), 61.3, 67.7, 68.0, 69.5, 72.9, 73.5, 74.2, 74.7, and 74.8 (C-6^I, C-6^{II}, C-6^{III}, 5 $OCH_2C_6H_5$, OCH_2), 69.0, 70.1, 70.7, 72.0, 74.5, 75.0, 75.7, 78.2, 81.5, 81.8, 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}), 99.3, 101.7, and 103.4 (C-1^I, C-1^{II}, C-1^{III}), 165.2 and 165.5 ($COC_6H_4CH_3$), 171.0 ($COCH_3$); HRMS of $C_{85}H_{90}N_4O_{20}$ (M, 1486.614): $[M+H]^+$ found 1487.621, calcd 1487.623.

3.19. 6-Azidohexyl (2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-(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 (30)

(a) A soln of **13** (0.27 g, 0.26 mmol) and **24**[14] (0.23 g, 0.24 mmol) in dry CH_2Cl_2 (8 mL), containing 4 Å molecular sieves (0.2 g), was stirred under Ar for 0.5 h. After cooling to −70 °C, TMSOTf (5 μL, 0.026 mmol) was added, and the mixture was stirred for 3 h, during which period the temperature was allowed to reach rt. The mixture was neutralized with Et_3N, filtered, washed with water, dried ($MgSO_4$), filtered, and concentrated. Column chromatography (3:1 toluene–EtOAc) of the residue gave **30**, isolated as colourless syrup (0.20 g, 83%).

(b) A soln of **9**[16] (36.5 mg, 74.2 μmol) and **29** (65 mg, 43.6 μmol) in dry CH_2Cl_2 (1 mL), containing 4 Å molecular sieves (40 mg), was stirred under Ar for 0.5 h. After cooling to −40 °C, TMSOTf (1.4 μL, 7.7 μmol) was added, and the mixture was stirred for 3 h, during which period the temperature was allowed to reach rt. The mixture was neutralized with Et_3N, filtered, washed with water, dried ($MgSO_4$), filtered, and concentrated. Column chromatography (3:1 toluene–EtOAc) of the residue gave **30**, isolated as a colourless syrup (46 mg, 58%); R_f 0.56 (2:1 toluene–EtOAc); $[\alpha]_D^{20}$ +1° (*c* 1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$; 2D TOCSY, ROESY): δ 1.34–1.37 (m, 4 H, 2 CH_2), 1.52–1.55 (m, 2 H, CH_2), 1.60–1.63 (m, 2 H, CH_2), 1.85, 1.94, 2.00, 2.01, and 2.11 (5 s, each 3 H, 5 $COCH_3$), 2.25 and 2.33 (2 s, each 3 H, 2 $COC_6H_4CH_3$), 3.17 (m, 1 H, H-5^I), 3.18 (t, 2 H, CH_2N_3), 3.37 (dd, 1 H, $J_{1,2}$ 8.1, $J_{2,3}$ 9.7 Hz, H-2^{II}), 3.52 (ddd, 1 H, $J_{4,5}$ 10.1, $J_{5,6a}$ 2.4, $J_{5,6b}$ 4.2 Hz, H-5^{IV}), 4.09 and 4.38 (2 d, each 1 H, $OCH_2C_6H_5$), 4.24 and 4.35 (2 d, each 1 H, $OCH_2C_6H_5$), 4.27 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1^I), 4.28 and 4.50 (2 d, each 1 H, $OCH_2C_6H_5$), 4.64 and 4.89 (2 d, each 1 H, $OCH_2C_6H_5$), 4.68 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1^{IV}), 4.69 and 4.84 (2 d, each 1 H, $OCH_2C_6H_5$), 4.94 (dd, 1 H, $J_{2,3}$ 9.9 Hz, H-2^{IV}), 4.98 (t, 1 H, H-3^{IV}), 5.19 (t, 1 H, H-4^{IV}), 5.40 (dd, 1 H, $J_{4,5}$ 9.9, $J_{3,4}$ 9.2 Hz, H-4^{III}), 5.59 (d, 1 H, $J_{3,4}$ 3.7, $J_{4,5}$ < 1 Hz, H-4^{II}), 5.68 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1^{III}), 6.28 (dd, 1 H, $J_{2,3}$ 10.8 Hz, H-3^{III}); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.3, 20.4, 20.5, and 20.6 (2 C) (5 $COCH_3$), 21.4 (2 C) (2 $COC_6H_4CH_3$), 25.6, 26.3, 28.6, and 29.4 (4 CH_2), 51.2 (CH_2N_3), 55.3 (C-2^{III}), 61.4, 67.4, 67.9, 68.2, 69.5, 72.8, 73.2, 74.4, 74.7, and 75.1 (C-6^I, C-6^{II}, C-6^{III}, C-6^{IV}, 5 $OCH_2C_6H_5$, OCH_2), 62.5, 67.8, 69.7, 69.8, 70.2, 71.4, 71.6, 72.5, 74.5, 75.0, 76.3, 77.8, 79.0, 81.6, 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}, C-2^{IV}, C-3^{IV}, C-4^{IV}, C-5^{IV}), 98.4, 101.0, 102.1, and 103.3 (C-1^I, C-1^{II}, C-1^{III}, C-1^{IV}), 165.1 and 165.3 ($COC_6H_4CH_3$), 169.0–169.8 ($COCH_3$); HRMS of $C_{99}H_{108}N_4O_{29}$ (M, 1816.709): $[M+NH_4]^+$ found 1834.719, calcd 1834.743.

3.20. 6-Azidohexyl (2,3,4,6-tetra-*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 (31)

To a soln of **30** (0.20 g, 0.11 mmol) in MeOH (3.5 mL) and CH_2Cl_2 (1.5 mL) was added NaOMe (pH 10), and the mixture was stirred for 4 h, then neutralized with Dowex 50 × 8 (H^+), filtered, and concentrated. To a soln of the residue in 1-BuOH (50 mL) was added 1,2-diaminoethane (10 mL), and the mixture was stirred overnight at 80 °C, then co-concentrated with toluene,

EtOH, and CH_2Cl_2. A soln of the residue in Py (30 mL) and Ac_2O (30 mL) was stirred for 4 h, then co-concentrated with toluene, EtOH, and CH_2Cl_2. Column chromatography (1:1 toluene–EtOAc) of the residue gave **31**, isolated as a colourless glass (0.16 g, 88%); R_f 0.45 (1:3 toluene–EtOAc); $[\alpha]_D^{20}$ −5° (*c* 1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$; 2D TOCSY, ROESY): δ 1.36–1.38 (m, 4 H, 2 CH_2), 1.52–1.56 (m, 2 H, CH_2), 1.63–1.65 (m, 2 H, CH_2), 1.91, 1.94, 1.95, 1.98, 2.00, 2.04, 2.07, and 2.12 (8 s, each 3 H, 7 $COCH_3$, $NHCOCH_3$), 3.19 (t, 2 H, CH_2N_3), 3.73 (dd, 1 H, $J_{5,6b}$ 7.5, $J_{6a,6b}$ 11.6 Hz, H-6b^{III}), 3.95 (t, 1 H, H-4^{I}), 3.99 (dd, 1 H, $J_{5,6a}$ 1.8, $J_{6a,6b}$ 12.3 Hz, H-6a^{IV}), 4.16 (dd, 1 H, $J_{5,6b}$ 4.2 Hz, H-6b^{IV}), 4.27 and 4.48 (2 d, each 1 H, $OCH_2C_6H_5$), 4.34 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1^{I}), 4.42 and 4.55 (2 d, each 1 H, $OCH_2C_6H_5$), 4.55 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1^{II}), 4.56 (d, 1 H, $OCHHC_6H_5$), 4.63 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1^{IV}), 5.21 (t, 1 H, H-3^{IV}), 5.48 (d, 1 H, $J_{3,4}$ 3.5, $J_{4,5}<1$ Hz, H-4^{II}), 7.14–7.37 (m, 25 H, 5 $OCH_2C_6H_5$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.3–20.5 ($COCH_3$), 22.6 ($NHCOCH_3$), 25.4, 26.2, 28.5, 29.3 (4 CH_2), 51.0 (CH_2N_3), 54.1 (C-2^{III}), 61.4, 67.5, 67.9, 68.1, 69.4, 73.0, 73.5, 74.6 (2 C), and 74.9 (C-6^{I}, C-6^{II}, C-6^{III}, C-6^{IV}, 5 $OCH_2C_6H_5$, OCH_2), 67.8, 69.3, 69.9, 71.3, 71.4, 71.9, 72.3, 72.4, 73.5, 75.0, 76.2, 77.4, 80.3, 81.5, 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}, C-2^{IV}, C-3^{IV}, C-4^{IV}, C-5^{IV}), 100.6, 101.2, 101.8, and 103.2 (C-1^{I}, C-1^{II}, C-1^{III}, C-1^{IV}), 168.9–170.5 ($COCH_3$, $NHCOCH_3$); HRMS of $C_{81}H_{100}N_4O_{28}$ (M, 1576.652): $[M+NH_4]^+$ found 1594.647, calcd 1594.686.

3.21. 6-Aminohexyl β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (6)

To a soln of **31** (155 mg, 98.2 μmol) in CH_2Cl_2 (4 mL) and MeOH (2 mL) was added NaOMe (pH 10). The mixture was stirred for 2 h, then neutralized with Dowex 50 × 8 (H^+), filtered, and concentrated, giving crude **32** as a white solid (126 mg). To a soln of **32** in *tert*-BuOH (4 mL) and water (2 mL) were added 10% Pd–C (200 mg) and 3 drops of aq 25% NH_3. The mixture was stirred for 3 h under H_2 after which NH_3 was removed by bubbling with N_2, then 10% Pd–C (100 mg) and 3 drops of AcOH were added, and the stirring under H_2 was continued overnight. The mixture was loaded on a short Dowex 50 × 8 (H^+) column, which was first eluted with water to remove contaminations, then with aq 10% NH_4OH to give **6**, isolated as a white solid after lyophilization (65 mg, 81%); R_f 0.26 (2:1:1 AcOH–1-BuOH–water); $[\alpha]_D^{20}$ −2° (*c* 1, water); ^{13}C NMR (75.5 MHz, D_2O): δ 23.6 ($NDCOCH_3$), 26.0, 26.7, 28.5, and 29.9 (4 CH_2), 41.0 (CH_2ND_2), 57.1 (C-2^{III}), 61.6, 62.2, 62.5, 70.2, and 71.9 (C-6^{I}, C-6^{II}, C-6^{III}, C-6^{IV}, OCH_2), 69.7, 71.1 (2 C), 71.5, 74.3, 74.6, 75.0, 75.9, 76.1, 76.2, 76.4, 77.2, 77.4, 79.9, and 83.4 (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}, C-2^{IV}, C-3^{IV}, C-4^{IV}, C-5^{IV}), 103.4, 104.2, 104.3, and 104.4 (C-1^{I}, C-1^{II}, C-1^{III}, C-1^{IV}), 176.3 ($NDCOCH_3$); HRMS of $C_{32}H_{58}N_2O_{21}$ (M, 806.353): $[M+H]^+$ found 807.367, calcd 807.361. For ^1H NMR data, see Table 3.

3.22. 6-Aminohexyl 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 (2)

To a soln of **6** (11.4 mg, 14.12 μmol) in aq 50 mM sodium cacodylate buffer pH 7.5 (700 μL), containing 5 mM $MnCl_2$, BSA (0.5 mg), and NaN_3 (0.02%), were added alkaline phosphatase (14 U), UDP-galactose (12 mg, 19.66 μmol), and β-1,4-galactosyltransferase (3 U). The mixture was incubated for 20 h at 37 °C then water (100 μL) was added. UDP-Galactose was removed using a Dowex 1 × 8 (Cl^-) column with water as eluent. The eluate was concentrated, and the residue applied to 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** (9.0 mg, 65%) and **48** (1.0 mg, 6%); R_f 0.23 (2:1:1 AcOH–1-BuOH–water); $[\alpha]_D^{20}$ −1° (*c* 0.5, water); HRMS of $C_{38}H_{68}N_2O_{26}$ (M, 968.406): $[M+H]^+$ found 969.418, calcd 969.414. For ^1H NMR data, see Table 4.

3.23. (3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-1,2,3,6-tetra-*O*-acetyl-α,β-D-glucopyranose (35)

To a soln of (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)-1,2,3,6-tetra-*O*-benzyl-β-D-glucopyranose (**34**)[15] (1.5 g, 1.12 mmol) in EtOH (45 mL) and EtOAc (45 mL) was added 10% Pd–C (1.78 g) and 2 drops of AcOH, and the mixture was stirred under H_2 for 8 h, then filtered over hyflo, and concentrated. Column chromatography (7:1 CH_2Cl_2–MeOH) of the residue gave a white solid which was dissolved in Py (75 mL) and Ac_2O (75 mL), and the soln was stirred overnight, then co-concentrated with toluene, EtOH and CH_2Cl_2. Low-pressure column chromatography (7:1→1:1 toluene–EtOAc) of the residue gave **35**, isolated as a white foam (0.95 g, 66%); R_f 0.45 (1:2 toluene–EtOAc); $[\alpha]_D^{20}$ −17° (*c* 1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$; 2D TOCSY, ROESY): δ 4.46 (t, 1 H, H-6b^{III}), 4.80 (m, 1 H, H-2^{II}), 4.93 (dd, 0.5 H, $J_{1,2}$ 3.6, $J_{2,3}$ 10.3 Hz, H-2$^{I\alpha}$), 4.98 (t, 0.5 H, H-2$^{I\beta}$), 5.13 (t, 0.5 H, H-3$^{I\beta}$), 5.18 (m, 1 H, H-4^{III}), 5.63 (d, 0.5 H, $J_{1,2}$ 8.3 Hz, H-1$^{I\beta}$), 5.74 (m, 1 H, H-3^{III}), 6.19 (d, 0.5 H, H-1$^{I\alpha}$), 7.77–7.84 (m, 4 H, Phth); HRMS of $C_{46}H_{55}NO_{27}$ (M, 1053.296): $[M+NH_4]^+$ found 1071.329, calcd 1071.330.

3.24. (3,4,6-Tri-*O*-acetyl-2-deoxy-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-D-glucopyranosyl trichloroacetimidate (37)

To a soln of **35** (714 mg, 0.68 mmol) in dry DMF (10 mL) was added hydrazinium acetate (67 mg, 0.73 mmol). The mixture was stirred for 3 h, then co-concentrated with toluene, EtOH and CH_2Cl_2. Low-pressure column chromatography (3:1→1:1 toluene–EtOAc) of the residue gave **36**, isolated as a white solid (609 mg, 89%). To a soln of **36** (519 mg, 0.51 mmol) in dry CH_2Cl_2 (20 mL) was added, at 0 °C, trichloroacetonitrile (4.9 mL, 49 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (0.26 mL, 1.25 mmol). The mixture was stirred under Ar for 1.5 h at rt, then concentrated. Low-pressure column chromatography (7:1→2:1 toluene–EtOAc) of the residue gave **37**, isolated as a slightly yellow solid (407 mg, 69%); R_f 0.28 (1:2 toluene–EtOAc); $[\alpha]_D^{20}$ −8° (*c* 1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$; 2D TOCSY, ROESY): δ 1.79, 1.84, 1.97, 1.99, 2.03, 2.08, 2.10, 2.12, and 2.15 (9 s, each 3 H, 9 $COCH_3$), 3.96 (m, 1 H, H-5^I), 4.14 (dd, 1 H, $J_{1,2}$ 8.3, $J_{2,3}$ 11.0 Hz, H-2^{III}), 4.30 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1^{II}), 4.36 (dd, 1 H, $J_{5,6a}$ 1.8, $J_{6a,6b}$ 12.1 Hz, H-6a^I), 4.51 (dd, 1 H, $J_{5,6a}$ 2.6, $J_{6a,6b}$ 12.3 Hz, H-6a^{III}), 4.81 (dd, 1 H, $J_{2,3}$ 9.9 Hz, H-2^{II}), 5.00 (dd, 1 H, $J_{1,2}$ 3.5, $J_{2,3}$ 10.1 Hz, H-2^I), 5.19 (dd, 1 H, $J_{3,4}$ 9.2, $J_{4,5}$ 9.9 Hz, H-4^{III}), 5.41 (t, 1 H, H-3^I), 5.76 (dd, 1 H, H-3^{III}), 6.43 (d, 1 H, H-1^I), 7.73–7.75 (m, 4 H, Phth), 8.65 (s, 1 H, OC(NH)CCl_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.3–20.6 ($CO\mathit{C}H_3$), 54.5 (C-2^{III}), 60.8, 61.5, and 62.0 (C-6^I, C-6^{II}, C-6^{III}), 68.6, 68.7, 69.2, 69.8 (2 C), 70.0, 70.7, 71.0, 71.8, 74.9, and 75.5 (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}), 92.8, 97.6, and 100.6 (C-1^I, C-1^{II}, C-1^{III}), 161.8 (O*C*(NH)CCl_3), 168.5–171.6 ($\mathit{C}OCH_3$).

3.25. 6-Azidohexyl (3,4,6-tri-*O*-acetyl-2-deoxy-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-glucopyranoside (38)

A soln of **37** (0.16 g, 0.14 mmol) and 6-azido-1-hexanol **33** (23 mg, 0.16 mmol) in dry CH_2Cl_2 (4 mL), containing 4 Å powdered molecular sieves (0.1 g), was stirred under Ar for 0.5 h. After cooling to 0 °C, AgOTf (35 mg, 0.14 mmol) was added, and the mixture was stirred for 1.5 h at 0 °C, then at rt for 2 h. After neutralization with Et_3N, the mixture was filtered over hyflo, washed with water, dried ($MgSO_4$), filtered, and concentrated. Column chromatography (1:2 toluene–EtOAc) of the residue gave **38**, isolated as a white solid (34 mg, 22%); R_f 0.41 (1:2 toluene–EtOAc); $[\alpha]_D^{20}$ +2° (*c* 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.26–1.40 (m, 6 H, 3 CH_2), 1.52–1.60 (m, 2 H, CH_2), 1.58, 1.78, 1.84, 1.97, 1.99, 2.03, 2.09, 2.10, and 2.15 (9 s, each 3 H, 9 $COCH_3$), 3.24 (t, 2 H, CH_2N_3), 3.67 (t, 1 H, H-4^I), 4.09 (dd, 1 H, $J_{5,6b}$ 3.5, $J_{6a,6b}$ 12.3 Hz, H-6b^{III}), 4.14 (dd, 1 H, $J_{1,2}$ 8.3, $J_{2,3}$ 10.9 Hz, H-2^{III}), 4.26 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{II}), 4.48 (dd, 1 H, $J_{5,6a}$ 2.6 Hz, H-6a^{III}), 5.06 (dd, 1 H, H-3^I), 5.19 (dd, 1 H, $J_{3,4}$ 9.1 Hz, H-4^{III}), 5.74 (dd, 1 H, H-3^{III}), 7.72–7.75 and 7.79–7.81 (2 m, each 2 H, Phth); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.3–20.6 ($CO\mathit{C}H_3$), 25.3, 26.3, 28.6, and 29.1 (4 CH_2), 51.2 (CH_2N_3), 54.5 (C-2^{III}), 60.8, 61.5, 62.0, and 69.7 (C-6^I, C-6^{II}, C-6^{III}, OCH_2), 68.7, 68.8, 70.0, 70.6, 71.0, 71.5, 71.8, 72.4, 72.6, and 75.5 (2 C) (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}), 97.6 and 100.4 (2 C) (C-1^I, C-1^{II}, C-1^{III}), 168.5–171.6 ($\mathit{C}OCH_3$); HRMS of $C_{50}H_{64}N_4O_{26}$ (M, 1136.380): $[M+Na]^+$ found 1159.358, calcd 1159.370.

3.26. 6-Azidohexyl (2-acetamido-3,4,6-tri-*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-glucopyranoside (39)

To a soln of **38** (22 mg, 21 μmol) in CH_2Cl_2 (0.5 mL) and MeOH (3 mL) was added NaOMe (pH 10), and the mixture was stirred for 2 h, then neutralized with Dowex 50 × 8 (H^+), filtered, and concentrated. To a soln of the residue in 1-BuOH (10 mL) was added 1,2-diaminoethane (2 mL), and the mixture was stirred overnight at 80 °C, then co-concentrated with toluene, EtOH and CH_2Cl_2. A soln of the residue in Py (10 mL) and Ac_2O (10 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 gave **39**, isolated as a white solid (17 mg, 77%); R_f 0.22 (3:1 CH_2Cl_2–acetone); $[\alpha]_D^{20}$ +6° (*c* 1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$; 2D TOCSY, ROESY): δ 1.25–1.28 (m, 4 H, 2 CH_2), 1.33–1.36 (m, 2 H, CH_2), 1.57–1.59 (m, 2 H, CH_2), 1.90, 2.00, 2.01, 2.02, 2.08, 2.09, 2.10, 2.11, and 2.12 (9 s, 3,3,6,3,3,3,3,3,3 H, 9 $COCH_3$, NHCO$\mathit{C}H_3$), 3.46 (m, 1 H, OC*H*H), 3.59 (m, 1 H, H-5^I), 3.66 (m, 1 H, H-5^{III}), 4.87 (dd, 1 H, $J_{1,2}$ 8.1, $J_{2,3}$ 9.6 Hz, H-2^I), 5.16 (t, 1 H, H-3^I), 5.32 (d, 1 H, $J_{3,4}$ 2.4, $J_{4,5}$ < 1 Hz, H-4^{II}), 5.47 (t, 1 H, H-3^{III}), 5.60 (d, 1 H, $J_{NH,2}$ 7.5 Hz, N*H*$COCH_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.5–20.8 (NHCO$\mathit{C}H_3$, CO$\mathit{C}H_3$), 25.3, 26.3, 28.6, and 29.2 (4 CH_2), 51.2 (CH_2N_3), 56.1 (C-2^{III}), 61.1, 61.5, 62.1, and 69.7 (C-6^I, C-6^{II}, C-6^{III}, OCH_2), 68.7, 68.8, 71.0, 71.1 (2 C), 71.5, 71.7, 72.5, 72.7, 75.7, and 75.9 (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}), 99.5, 100.5, and 100.6 (C-1^I, C-1^{II}, C-1^{III}), 168.5–171.4 ($\mathit{C}OCH_3$, NH$\mathit{C}OCH_3$); HRMS of $C_{44}H_{64}N_4O_{25}$ (M, 1048.386): $[M+Na]^+$ found 1071.397, calcd 1071.375.

3.27. 6-Azidohexyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (40)

To a soln of **39** (15 mg, 14 μmol) in CH_2Cl_2 (0.5 mL) and MeOH (2 mL) was added NaOMe (pH 10). The mixture was stirred for 2 h, then neutralized with Dowex 50 × 8 (H^+), filtered, and concentrated. Column chromatography (1:1 CH_2Cl_2–MeOH) of the residue gave **40**, isolated as a white solid (5.3 mg, 55%); R_f 0.81 (1:2 CH_2Cl_2–MeOH); $[\alpha]_D^{20}$ −4° (*c* 0.5, water); HRMS data of $C_{26}H_{46}N_4O_{16}$ (M, 670.290): $[M+H]^+$ found 671.300, calcd 671.302. For ^1H NMR data, see Table 5.

3.28. 6-Azidohexyl (2-acetamido-3,4,6-tri-*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 (41)

To a soln of **25** (0.21 g, 0.15 mmol) in MeOH (4 mL) and CH_2Cl_2 (2 mL) was added NaOMe (pH 10), and the mixture was stirred for 3 h, then neutralized with Dowex 50 × 8 (H^+), filtered, and concentrated. To a soln of the residue in 1-BuOH (30 mL) was added 1,2-diaminoethane (6 mL), and the mixture was stirred overnight at 80 °C, then co-concentrated with toluene, EtOH, and CH_2Cl_2. A soln of the residue in Py (30 mL) and Ac_2O (30 mL) was stirred for 48 h, then co-concentrated with toluene, EtOH, and CH_2Cl_2. Column chromatography (1:1 toluene–EtOAc) of the residue gave **41**, isolated as a colourless glass (0.15 g, 78%); R_f 0.39 (1:1 toluene–EtOAc); $[\alpha]_D^{20}$ −2° (*c* 1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$; 2D TOCSY, ROESY): δ 1.36–1.42 (m, 4 H, 2 CH_2), 1.51–1.56 (m, 2 H, CH_2), 1.61–1.64 (m, 2 H, CH_2), 1.51, 1.94, 1.99, 2.02, and 2.05 (5 s, each 3 H, 4 $COCH_3$, $NHCOCH_3$), 3.18 (t, 2 H, CH_2N_3), 3.72 (dd, 1 H, $J_{5,6b}$ 4.1, $J_{6a,6b}$ 11.0 Hz, H-6b^I), 3.98 (t, 1 H, H-4^I), 4.26 and 4.42 (2 d, each 1 H, $OCH_2C_6H_5$), 4.33 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1^I), 4.38 (d, 1 H, $OCHHC_6H_5$), 4.49 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1^{II}), 4.72 and 4.86 (2 d, each 1 H, $OCH_2C_6H_5$), 4.74 and 4.98 (2 d, each 1 H, $OCH_2C_6H_5$), 5.01 (t, 1 H, H-4^{III}), 5.09 (t, 1 H, H-3^{III}), 5.25 (d, 1 H, $J_{2,NH}$ 9.2 Hz, $NHCOCH_3$), 5.40 (d, 1 H, $J_{3,4}$ 3.3, $J_{4,5}<1$ Hz, H-4^{II}), 7.18–7.36 (m, 25 H, 5 $OCH_2C_6H_5$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.3–20.5 ($COCH_3$), 22.5 ($NHCOCH_3$), 25.4, 26.2, 28.5, and 29.3 (4 CH_2), 51.0 (CH_2N_3), 54.3 (C-2^{III}), 61.6, 67.9, 68.1, 69.4, 73.0, 73.2, 74.4, 74.6, and 74.8 (C-6^I, C-6^{II}, C-6^{III}, 5 $OCH_2C_6H_5$, OCH_2), 68.3, 71.4 (2 C), 72.3, 72.6, 74.7, 75.8, 78.9, 79.5, 81.4, 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}), 100.9, 101.7, and 103.3 (C-1^I, C-1^{II}, C-1^{III}), 168.9, 169.4, 169.5, 170.3, and 170.4 (4 $COCH_3$, $NHCOCH_3$); HRMS of $C_{69}H_{84}N_4O_{20}$ (M, 1288.567): $[M+NH_4]^+$ found 1306.629, calcd 1306.600.

3.29. 6-Aminohexyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (7)

(a) To a soln of **41** (75 mg, 58.5 μmol) in CH_2Cl_2 (1 mL) and MeOH (2 mL) was added NaOMe (pH 10). The mixture was stirred for 1.5 h, then neutralized with Dowex 50 × 8 (H^+), filtered, and concentrated, giving crude **42** as a white solid (65 mg). To a soln of **42** in *tert*-BuOH (15 mL) and water (10 mL) were added 10% Pd–C (150 mg) and 3 drops of aq 25% NH_3. The mixture was stirred for 3 h under H_2 after which NH_3 was removed by bubbling with N_2, then 10% Pd–C (100 mg) and 3 drops of AcOH were added, and the stirring under H_2 was continued overnight. The mixture was loaded on a short Dowex 50 × 8 (H^+) column, which was first eluted with water to remove contaminations, then with aq 10% NH_4OH to give **7**, isolated as a white solid after lyophilization (25 mg, 68%).

(b) To a soln of **40** (5.3 mg, 7.90 μmol) in *tert*-BuOH (3 mL) and water (2 mL) was added 10% Pd–C (40 mg) and 2 drops of aq 25% NH_3. The mixture was stirred for 20 h under H_2, after which NH_3 was removed by bubbling with N_2, then filtered over cotton and concentrated. Chromatography of the residue on a Bio-Gel P-2 column eluted with 0.1 M NH_4HCO_3, and subsequent lyophilization yielded **7**, isolated as a white solid (4.2 mg, 83%); R_f 0.34 (2:1:1 AcOH–1-BuOH–water); $[\alpha]_D^{20}$ −2° (*c* 1, water); ^{13}C NMR (75.5 MHz, D_2O): δ 23.0 ($NDCOCH_3$), 25.4, 26.1, 28.3, and 29.3 (4 CH_2), 40.4 (CH_2ND_2), 56.5 (C-2^{III}), 60.9, 61.3, 61.7, and 71.2 (C-6^I, C-6^{II}, C-6^{III}, OCH_2), 69.1, 70.5, 70.8, 73.6, 74.4, 75.3, 75.6, 75.7, 76.5, 79.3, and 82.8 (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}), 102.8, 103.6, and 103.7 (C-1^I, C-1^{II}, C-1^{III}), 175.7 ($NDCOCH_3$); HRMS of $C_{26}H_{48}N_2O_{16}$ (M, 644.300): $[M+H]^+$ found 645.311, calcd 645.308. For ^1H NMR data, see Table 6.

3.30. 6-Aminohexyl β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (3)

To a soln of **7** (8.2 mg, 12.71 μmol) in aq 50 mM sodium cacodylate buffer pH 7.5 (600 μL), containing 5 mM $MnCl_2$, BSA (0.5 mg), and NaN_3 (0.02%), were added alkaline phosphatase (14 U), UDP-galactose (11.5 mg, 18.84 μmol), and β-1,4-galactosyltransferase (2.5 U). The mixture was incubated for 20 h at 37 °C then water (200 μL) was added. UDP-Galactose was removed using a Dowex 1 × 8 (Cl^-) column with water as eluent. The eluate was concentrated, and the residue applied to 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 **3** (9.6 mg, 94%); R_f 0.22 (2:1:1 AcOH–1-BuOH–water); $[\alpha]_D^{20}$ −1° (*c* 0.5, water); HRMS data of $C_{32}H_{58}N_2O_{21}$ (M, 806.353): $[M+H]^+$ found 807.352, calcd 807.361. For ^1H NMR data, see Table 7.

3.31. 6-Azidohexyl (3,4,6-tri-*O*-acetyl-2-deoxy-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 (44)

To a soln of **37** (100 mg, 87 μmol) and **43** (70 mg, 104 μmol) in dry CH_2Cl_2 (3 mL), containing 4 Å molecular sieves (200 mg), was added, under Ar at 0 °C, TMSOTf (2.5 μL, 12.96 μmol). The mixture was stirred for 1 h at 0 °C, followed by 1 h at rt, then neutralized with Et_3N, filtered over hyflo, washed with water, dried ($MgSO_4$), filtered, and concentrated. Low-pressure column chromatography (4:1→2:1 toluene–EtOAc) of the residue gave **44**, isolated as a white solid (49 mg, 34%); R_f 0.58 (1:2 toluene–EtOAc); $[\alpha]_D^{20}$ −11° (*c* 1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$; 2D TOCSY, ROESY): δ 1.12–1.20 (m, 4 H, 2 CH_2), 1.25–1.32 (m, 2 H, CH_2), 1.49–1.53 (m, 2 H, CH_2), 1.78, 1.83, 1.96, 2.02, 2.03, 2.04, 2.08, 2.10, and 2.14 (9 s, each 3 H, 9 $COCH_3$), 2.27 and 2.33 (2 s, each 3 H, 2 $COC_6H_4CH_3$), 3.64 (t, 1 H, H-4^{II}), 4.09 (dd, 1 H, $J_{5,6b}$ 3.1, $J_{6a,6b}$ 12.1 Hz, H-$6b^{IV}$), 4.14 (dd, 1 H, $J_{1,2}$ 8.3, $J_{2,3}$ 10.8 Hz, H-2^{IV}), 4.24 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1^{III}), 4.57 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1^{II}), 4.78 (dd, 1 H, $J_{2,3}$ 9.9 Hz, H-2^{III}), 4.85 (dd, 1 H, $J_{2,3}$ 9.2 Hz, H-2^{II}), 5.02 (t, 1 H, H-3^{II}), 5.19 (t, 1 H, H-4^{IV}), 5.44 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1^{I}), 5.75 (dd, 1 H, $J_{3,4}$ 9.2 Hz, H-3^{IV}), 6.16 (dd, 1 H, $J_{2,3}$ 10.7, $J_{3,4}$ 9.2 Hz, H-3^{I}); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.2–20.6 ($COCH_3$), 21.4 (2 C) (2 $COC_6H_4CH_3$), 25.3, 26.0, 28.4, and 28.9 (4 CH_2), 51.0 (CH_2N_3), 54.4 and 54.8 (C-2^{I}, C-2^{IV}), 60.8, 61.5, 62.0, 68.2, and 69.6 (C-6^{I}, C-6^{II}, C-6^{III}, C-6^{IV}, OCH_2), 68.6, 68.7, 69.9, 70.0, 70.5, 70.8, 70.9, 71.3, 71.7, 72.4, 72.6, 74.1, and 75.4 (2 C) (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}), 97.5, 98.0, and 100.5 (2 C) (C-1^{I}, C-1^{II}, C-1^{III}, C-1^{IV}), 165.1 and 165.4 (2 $COC_6H_4CH_3$), 168.4–170.6 ($COCH_3$); HRMS of $C_{68}H_{81}N_5O_{34}$ (M, 1151.476): $[M+NH_4]^+$ found 1169.524, calcd 1169.510.

3.32. 6-Azidohexyl (2-acetamido-3,4,6-tri-*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 (45)

To a soln of **44** (56 mg, 63 μmol) in CH_2Cl_2 (0.45 mL) and MeOH (0.55 mL) was added NaOMe (pH 10), and the mixture was stirred for 2 h, then neutralized with Dowex 50 × 8 (H^+), filtered, and concentrated. To a soln of the residue in 1-BuOH (25 mL) was added 1,2-diaminoethane (5 mL), and the mixture was stirred overnight at 90 °C, then co-concentrated with toluene, EtOH and CH_2Cl_2. A soln of the residue in Py (10 mL) and Ac_2O (10 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 gave **45**, isolated as a white foam (48 mg, 86%); R_f 0.44 (2:1 CH_2Cl_2–acetone); $[\alpha]_D^{20}$ −10° (*c* 1, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 1.25–1.34 (m, 4 H, 2 CH_2), 1.59–1.64 (m, 4 H, 2 CH_2), 1.90, 1.93, 2.00, 2.01, 2.02, 2.08, 2.09, 2.10, 2.11, and 2.12 (10 s, 3,3,3,12,3,3,3,3,3,3 H, 11 $COCH_3$, 2 $NHCOCH_3$), 3.46 (m, 1 H, OC*H*H), 4.12 (dd, 1 H, $J_{5,6b}$ 5.1, $J_{6a,6b}$ 11.7 Hz, H-$6b^{II}$), 4.44 (dd, 1 H, $J_{5,6a}$ < 1 Hz, H-$6a^{II}$), 4.56 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{II}), 4.61 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1^{I}), 5.12 (t, 1 H, H-3^{II}), 5.24 (t, 1 H, H-3^{I}), 5.32 (d, 1 H, $J_{3,4}$ 3.5, $J_{4,5}$ < 1 Hz, H-4^{III}); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.5–20.7 ($COCH_3$), 23.2 (2 C) (2 $NHCOCH_3$), 25.4, 26.3, 28.6, and 29.2 (4 CH_2), 51.2 (CH_2N_3), 54.8 and 56.1 (C-2^{I}, C-2^{IV}), 61.0, 61.5, 61.9, 68.2, and 69.4 (C-6^{I}, C-6^{II}, C-6^{III}, C-6^{IV}, OCH_2), 68.6, 68.8, 69.2, 70.9, 71.1 (2 C), 71.4, 71.7, 72.3, 72.5, 72.8, 73.4, 75.6, and 75.9 (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}), 99.5, 100.4 (2 C), and 100.6 (C-1^{I}, C-1^{II}, C-1^{III}, C-1^{IV}), 169.0–170.7 (*C*OCH_3, NH*C*OCH_3); HRMS of $C_{56}H_{81}N_5O_{32}$ (M, 1335.486): $[M+NH_4]^+$ found 1353.547, calcd 1353.520.

3.33. 6-Azidohexyl 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 (46)

To a soln of **45** (48 mg, 35.9 μmol) in CH_2Cl_2 (1 mL) and MeOH (8 mL) was added NaOMe (pH 10). The mixture was stirred for 3 h, then neutralized with Dowex 50 × 8 (H^+), filtered, and concentrated. Column chromatography (1:2 CH_2Cl_2–MeOH) of the residue gave **46**, isolated as a white solid (25 mg, 79%); R_f 0.62 (1:4 CH_2Cl_2–MeOH); $[\alpha]_D^{20}$ −6° (*c* 1, water); ^{13}C NMR (75.5 MHz, D_2O): δ 22.4 (2 C) ($NDCOCH_3$), 24.9, 25.8, 28.2, and 28.7 (4 CH_2), 51.4 (CH_2N_3), 55.8 (C-2^{I}, C-2^{IV}), 60.3, 60.7, 61.1, 68.8, and 70.7 (C-6^{I}, C-6^{II}, C-6^{III}, C-6^{IV}, OCH_2), 68.6, 70.0 (2 C), 70.2, 73.0, 73.8, 73.9, 74.5, 75.1 (3 C), 75.9, 78.7, and 82.2 (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}), 101.3, 102.8, 103.0, and 103.1 (C-1^{I}, C-1^{II}, C-1^{III}, C-1^{IV}), 174.6 and 175.1 (2 NH*C*OCH_3); HRMS of $C_{34}H_{59}N_5O_{21}$ (M, 873.370): $[M+Na]^+$ found 896.359, calcd 896.360. For ^1H NMR data, see Table 8.

3.34. 6-Aminohexyl 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 (8)

To a soln of **46** (10 mg, 11.44 μmol) in *tert*-BuOH (2 mL) and water (2 mL) was added 10% Pd–C (50 mg) and 1 drop of aq 25% NH_3. The mixture was stirred for 20 h under H_2, then acidified with Dowex 50 × 8 (H^+) (pH 4) and loaded on a short column of Dowex 50 × 8 (H^+). Elution with water, to remove contaminants, followed by 10% NH_4OH, and subsequent lyophilization yielded **8**, isolated as a white solid (8 mg, 82%); R_f 0.27 (2:1:1 AcOH–1-BuOH–water); $[\alpha]_D^{20}$ −2° (*c* 0.5, water); ^{13}C NMR (75.5 MHz, D_2O): δ 22.8 (2 C) ($NDCOCH_3$), 25.3, 25.9, 27.4, and 29.0 (4 CH_2), 40.1 (CH_2ND_2), 56.2 and 56.3 (C-2^I, C-2^{IV}), 60.7, 61.2, 61.6, 69.3, and 71.2 (C-6^I, C-6^{II}, C-6^{III}, C-6^{IV}, OCH_2), 69.0, 70.4 (2 C), 70.7, 73.4, 74.2 (2 C), 75.0, 75.4, 75.6 (2 C), 76.3, 79.1, and 82.6 (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}), 101.9, 103.3, 103.5, and 103.6 (C-1^I, C-1^{II}, C-1^{III}, C-1^{IV}), 175.1 and 175.6 (2 $NDCOCH_3$); HRMS of $C_{34}H_{61}N_3O_{21}$ (M, 847.379): $[M+H]^+$ found 848.390, calcd 848.387. For ^1H NMR data, see Table 9.

3.35. 6-Aminohexyl 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 (4)

To a soln of **8** (10.8 mg, 12.73 μmol) in aq 50 mM sodium cacodylate buffer pH 7.5 (600 μL), containing 5 mM $MnCl_2$, BSA (0.5 mg), and NaN_3 (0.02%), were added alkaline phosphatase (30 U), UDP-galactose (25 mg, 40.96 μmol), and β-1,4-galactosyltransferase (5 U). The mixture was incubated for 20 h at 37 °C, then water (200 μL) was added. UDP-Galactose was removed using a Dowex 1 × 8 (Cl^-) column with water as eluent. The eluate was concentrated, and the residue applied to 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 **4** (11.4 mg, 76%); R_f 0.15 (2:1:1 AcOH–1-BuOH–water); $[\alpha]_D^{20}$ −1° (*c* 1, water); HRMS of $C_{46}H_{81}N_3O_{31}$ (M, 1171.485): $[M+H]^+$ found 1172.503, calcd 1172.492. For ^1H NMR data, see Table 10.

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