

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

John A.F. Joosten, Bart J. Lazet, Johannis P. Kamerling,\* Johannes F.G. Vliegthart

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

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## Abstract

The chemo-enzymatic synthesis is described of  $\beta$ -D-Glcp-(1  $\rightarrow$  6)-[ $\beta$ -D-Galp-(1  $\rightarrow$  4)]- $\beta$ -D-GlcpNAc-(1  $\rightarrow$  3)- $\beta$ -D-Galp-(1  $\rightarrow$  O(CH<sub>2</sub>)<sub>6</sub>NH<sub>2</sub>) (**1**),  $\beta$ -D-Glcp-(1  $\rightarrow$  6)-[ $\beta$ -D-Galp-(1  $\rightarrow$  4)]- $\beta$ -D-GlcpNAc-(1  $\rightarrow$  3)- $\beta$ -D-Galp-(1  $\rightarrow$  4)- $\beta$ -D-Glcp-(1  $\rightarrow$  O(CH<sub>2</sub>)<sub>6</sub>NH<sub>2</sub>) (**2**),  $\beta$ -D-Galp-(1  $\rightarrow$  4)- $\beta$ -D-GlcpNAc-(1  $\rightarrow$  3)- $\beta$ -D-Galp-(1  $\rightarrow$  4)- $\beta$ -D-Glcp-(1  $\rightarrow$  O(CH<sub>2</sub>)<sub>6</sub>NH<sub>2</sub>) (**3**), and  $\beta$ -D-Galp-(1  $\rightarrow$  4)- $\beta$ -D-GlcpNAc-(1  $\rightarrow$  3)- $\beta$ -D-Galp-(1  $\rightarrow$  4)- $\beta$ -D-Glcp-(1  $\rightarrow$  6)-[ $\beta$ -D-Galp-(1  $\rightarrow$  4)]- $\beta$ -D-GlcpNAc-(1  $\rightarrow$  O(CH<sub>2</sub>)<sub>6</sub>NH<sub>2</sub>) (**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  $\beta$ -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.

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**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,<sup>1,2</sup> mainly due to a growing resistance towards antibiotics.<sup>3,4</sup> Vaccination with the available 23-valent capsular polysaccharide (CPS) vaccines<sup>5</sup> 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.<sup>6</sup> 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,<sup>7</sup> as have been shown for other bacteria.<sup>8,9</sup> Currently, neoglycoconju-

gate vaccines against *S. pneumoniae* serotypes have been introduced.<sup>7</sup>

The CPS of *S. pneumoniae* type 14 is built up from the tetrasaccharide repeating unit  $\rightarrow$ 6)-[ $\beta$ -D-Galp-(1  $\rightarrow$  4)]- $\beta$ -D-GlcpNAc-(1  $\rightarrow$  3)- $\beta$ -D-Galp-(1  $\rightarrow$  4)- $\beta$ -D-Glcp-(1  $\rightarrow$  .<sup>10</sup> 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 { $\beta$ -D-Galp-(1  $\rightarrow$  4)- $\beta$ -D-Glcp-(1  $\rightarrow$  6)-[ $\beta$ -D-Galp-(1  $\rightarrow$  4)]- $\beta$ -D-GlcpNAc}, as well as mimics of the CPS.<sup>11,12</sup> The tetrasaccharide-containing neoglycoconjugate (CRM<sub>197</sub> as carrier protein) showed particularly promising immunological data when tested in mice models.<sup>13</sup> 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.<sup>14,15</sup> Here, we report the chemo-enzymatic synthesis of another four oligosac-

\* Corresponding author. Tel.: +31-30-2533479; fax: +31-30-2540980.

E-mail address: [j.p.kamerling@chem.uu.nl](mailto:j.p.kamerling@chem.uu.nl) (J.P. Kamerling).

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

## 2. Results and discussion

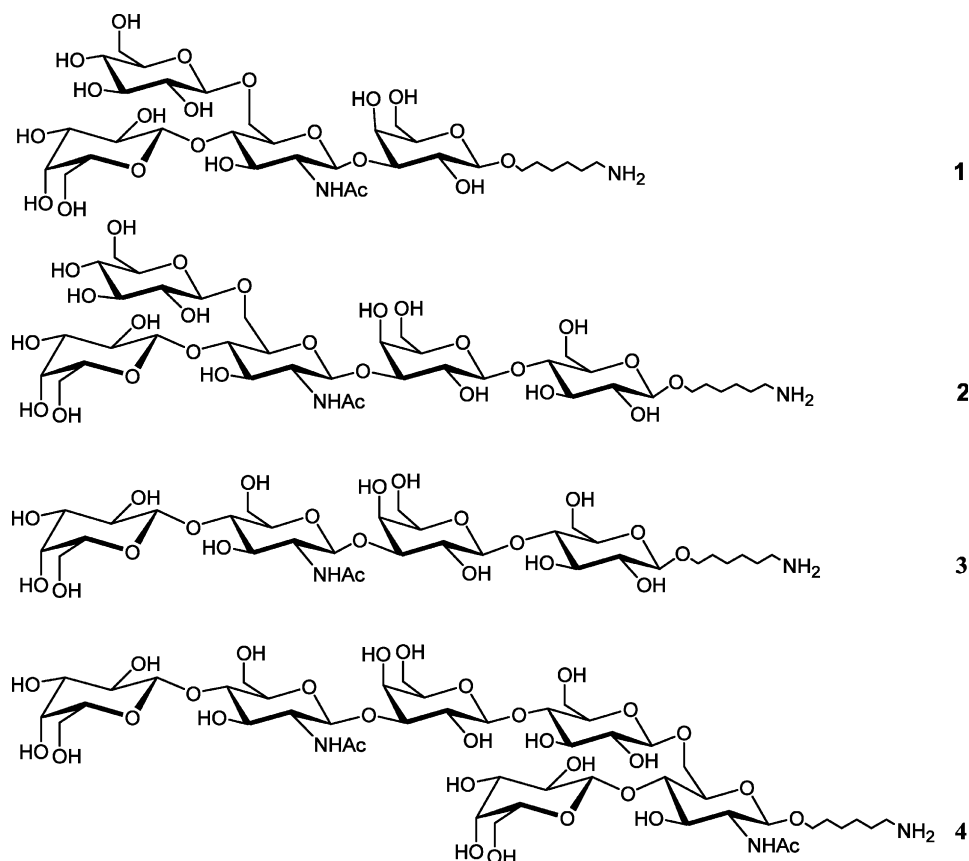
For the organic synthesis of the structurally closely related compounds **5–8**, a series of broadly applicable building blocks, **9**, **13**, **24**, and **37**, were designed. Condensation of these key building blocks with appropriate acceptor or donor building blocks gave, after deprotection, the aimed linear oligosaccharides (**5–8**), which were used as acceptor substrates for  $\beta$ -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

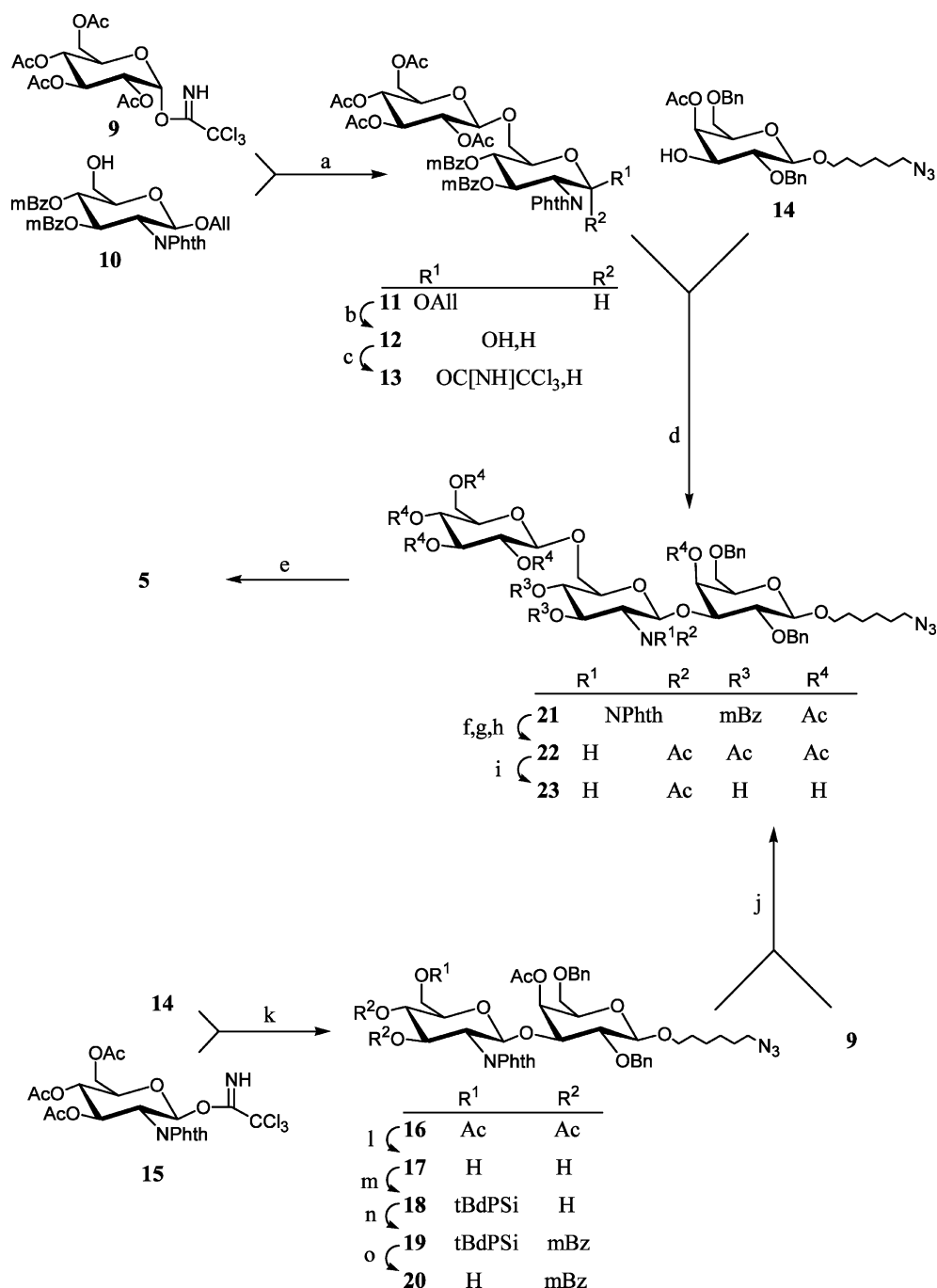
The linear trisaccharide backbone **5** was prepared via two different routes (Scheme 2). For the first route, disaccharide donor **13** was needed. Coupling of 2,3,4,6-tetra-*O*-acetyl- $\alpha$ -D-glucopyranosyl trichloroacetimidate (**9**)<sup>16</sup> to allyl 2-deoxy-3,4-di-*O*-*p*-methylbenzoyl-2-

phthalimido- $\beta$ -D-glucopyranoside (**10**)<sup>11</sup> 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 ( $\rightarrow$  **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-azidoheptyl 4-*O*-acetyl-2,6-di-*O*-benzyl- $\beta$ -D-galactopyranoside (**14**)<sup>14</sup> 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- $\beta$ -D-glucopyranosyl trichloroacetimidate (**15**)<sup>17</sup> 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-aminoheptyl-spaced 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<sub>2</sub>Cl<sub>2</sub>, 0 °C, 62%; (b) Pd(II)Cl<sub>2</sub>, NaOAc, AcOH, 70%; (c) Cl<sub>3</sub>CCN, DBU, CH<sub>2</sub>Cl<sub>2</sub>, 76%; (d) 10% TMSOTf, CH<sub>2</sub>Cl<sub>2</sub>, –70 °C, 73%; (e) 10% Pd–C, H<sub>2</sub>, water, *tert*-BuOH, aq 25% NH<sub>3</sub>/10% Pd–C, AcOH, 69%; (f) NaOMe (pH 10), MeOH, CH<sub>2</sub>Cl<sub>2</sub>; (g) NH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>, 1-BuOH, 80 °C; (h) pyridine, Ac<sub>2</sub>O, 80% over three steps; (i) NaOMe (pH 9), MeOH, CH<sub>2</sub>Cl<sub>2</sub>, 97%; (j) 10% TMSOTf, CH<sub>2</sub>Cl<sub>2</sub>, –40 °C, 56%; (k) 10% TMSOTf, CH<sub>2</sub>Cl<sub>2</sub>, –70 °C, 86%; (l) NaOMe (pH 8), MeOH, CH<sub>2</sub>Cl<sub>2</sub>, 91%; (m) *t*-BdPSiCl, DMAP, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, pyridine, 83%; (n) *p*-mBzCl, pyridine, CH<sub>2</sub>Cl<sub>2</sub>, 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) ( $\rightarrow$ **18**, 83%), followed by *p*-methylbenzoyla-

tion (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%

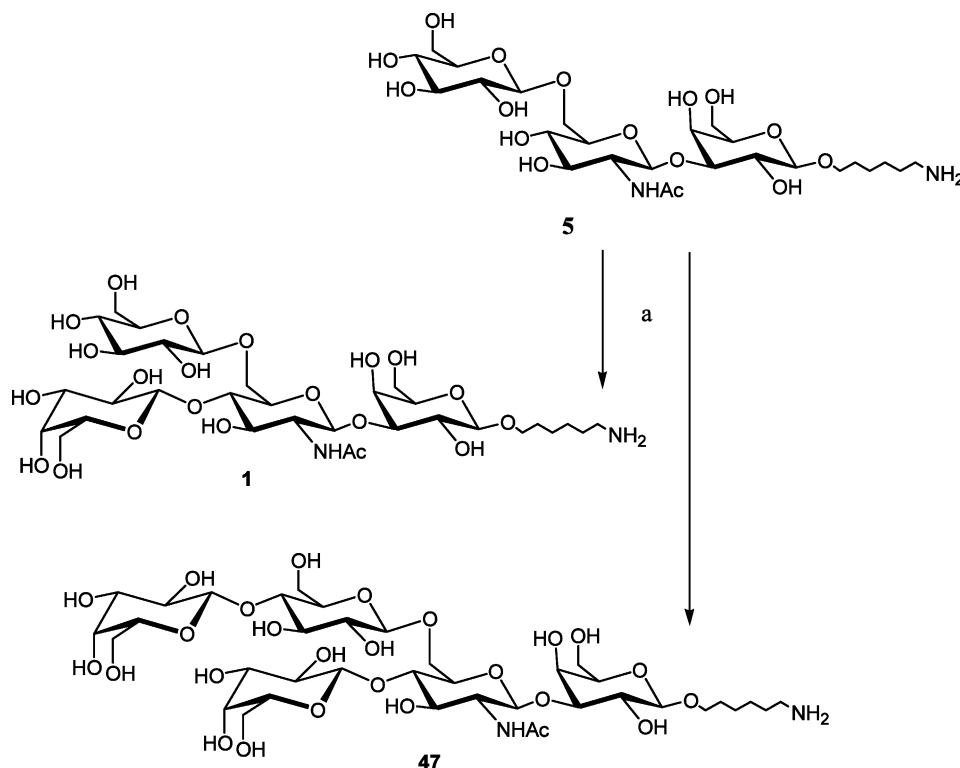
yield). Coupling of glucose donor **9** to disaccharide **20** in dichloromethane at  $-40^{\circ}\text{C}$ , using 10% trimethylsilyl trifluoromethanesulfonate as a catalyst, gave trisaccharide **21** in 56% yield. O-De-acylation of **21** using sodium methoxide at pH 10, followed by N-de-phthaloylation using 1,2-diaminoethane in 1-butanol at  $80^{\circ}\text{C}$ , 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  $\text{H}_2$  in the presence of ammonia, and subsequent debenzoylation using 10% Pd–C and  $\text{H}_2$  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- $\beta$ -D-glucosamine residue of **5** by using bovine milk  $\beta$ -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-aminoethyl  $\beta$ -D-galactopyranosyl-(1  $\rightarrow$  4)- $\beta$ -D-glucopyranosyl-(1  $\rightarrow$  6)-[ $\beta$ -D-galactopyranosyl-(1  $\rightarrow$  4)]-2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl-(1  $\rightarrow$  3)- $\beta$ -D-galactopyranoside (**47**)<sup>14</sup> was obtained as a side product (35%) (Scheme 3). So far, the enzymatic galactosylation of glucose by  $\beta$ -1,4-galactosyltransferase has only been shown to proceed

in the presence of  $\alpha$ -lactalbumin (lactose synthase complex).<sup>18</sup>  $^1\text{H}$  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  $^1\text{H}$  NMR data (TOCSY, ROESY) of **5** at 300 K (in ppm)

Proton	$\delta_{\text{H}}$		
	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. <sup>a</sup>	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 <sub>2</sub> ) <sub>2</sub> (CH <sub>2</sub> ) <sub>2</sub> (CH <sub>2</sub> ) <sub>2</sub> ND <sub>2</sub>		1.39–1.41 (4 H)	
OCH <sub>2</sub> CH <sub>2</sub> (CH <sub>2</sub> ) <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> ND <sub>2</sub>		1.64–1.65 (4 H)	
CH <sub>2</sub> ND <sub>2</sub>		2.96	
OCH <sub>2</sub> (CH <sub>2</sub> ) <sub>5</sub> ND <sub>2</sub>		3.68, 3.92	
NDCOCH <sub>3</sub>		2.04	

<sup>a</sup> 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  $\beta$ -1,4-galactosyltransferase, 14 U alkaline phosphatase,  $37^{\circ}\text{C}$ , 54% (**1**); 35% (**47**).

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

Proton	$\delta_{\text{H}}$			
	Gal I <sup>a</sup>	GlcNAc II	Glc III	Gal IV <sup>b</sup>
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. <sup>c</sup>	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 <sub>2</sub> ) <sub>2</sub> (CH <sub>2</sub> ) <sub>2</sub> (CH <sub>2</sub> ) <sub>2</sub> ND <sub>2</sub>			1.39–1.41 (4 H)	
OCH <sub>2</sub> CH <sub>2</sub> (CH <sub>2</sub> ) <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> ND <sub>2</sub>			1.63–1.64 (4 H)	
CH <sub>2</sub> ND <sub>2</sub>			2.96	
OCH <sub>2</sub> (CH <sub>2</sub> ) <sub>5</sub> ND <sub>2</sub>			3.67, 3.92	
NDCOCH <sub>3</sub>			2.03	

<sup>a</sup> Gal(β1-O(CH<sub>2</sub>)<sub>6</sub>NH<sub>2</sub>).

<sup>b</sup> Gal(β1-4)GlcNAc.

<sup>c</sup> n.d., not determined.

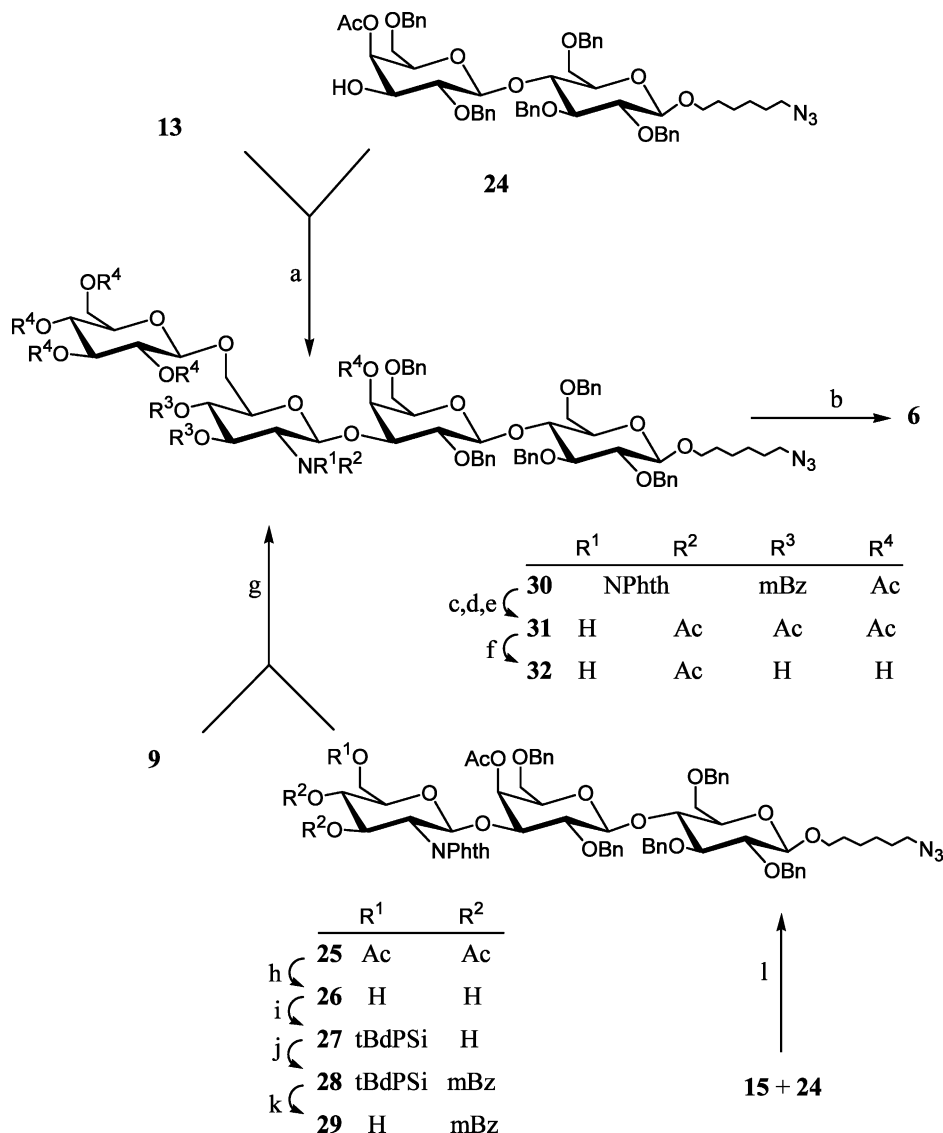
## 2.2. Synthesis of pentasaccharide fragment 2

The linear tetrasaccharide backbone **6** was prepared via two different routes (Scheme 4). In the first route, condensation of disaccharide donor **13** with 6-azido-hexyl (4-*O*-acetyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**24**)<sup>14</sup> 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**<sup>17</sup> (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 cata-

lyst gave tetrasaccharide **30** (58%). *O*-De acylation of **30** 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 **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<sub>2</sub> in the presence of ammonia, and subsequent debenzoylation using 10% Pd–C and H<sub>2</sub> 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-amino-6-deoxy-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**)<sup>14</sup> (6%; Scheme 5) was obtained.  $^1\text{H}$  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

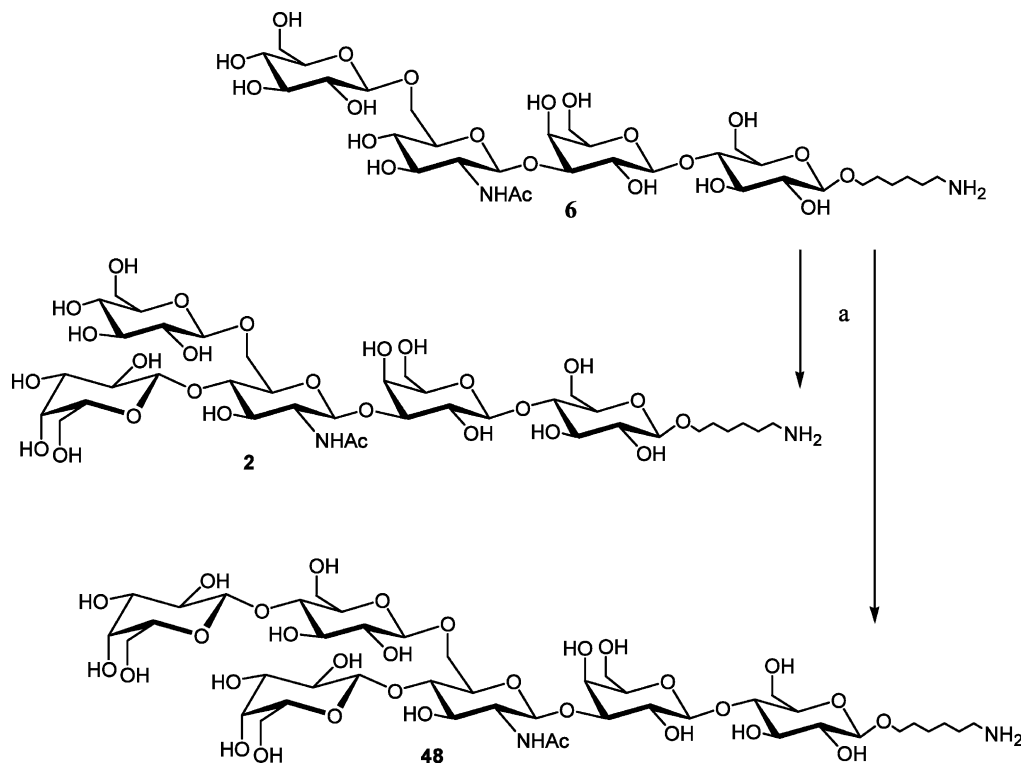
The linear trisaccharide backbone **7** was prepared via two different routes (Scheme 6). For the first route, trisaccharide donor **37** was needed. Debzoylation of (3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1 → 3)-(4-*O*-acetyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1 → 4)-1,2,3,6-tetra-*O*-benzyl-β-D-glucopyranoside (**34**),<sup>15</sup> using 10% Pd–C and H<sub>2</sub> in the



Scheme 4. Synthesis of the linear tetrasaccharide backbone **6**: (a) 10% TMSOTf, CH<sub>2</sub>Cl<sub>2</sub>, –70 °C, 83%; (b) 10% Pd–C, H<sub>2</sub>, water, *tert*-BuOH, aq 25% NH<sub>3</sub>/10% Pd–C, AcOH, 81%; (c) NaOMe (pH 10), MeOH, CH<sub>2</sub>Cl<sub>2</sub>; (d) NH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>, 1-BuOH, 80 °C; (e) pyridine, Ac<sub>2</sub>O, 88% over three steps; (f) NaOMe (pH 10), MeOH, CH<sub>2</sub>Cl<sub>2</sub>, quantitative; (g) 10% TMSOTf, CH<sub>2</sub>Cl<sub>2</sub>, –40 °C, 58%; (h) NaOMe (pH 8), MeOH, CH<sub>2</sub>Cl<sub>2</sub>, 78%; (i) *t*-BdPSiCl, DMAP, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, pyridine, 84%; (j) *p*-mBzCl, pyridine, CH<sub>2</sub>Cl<sub>2</sub>, 91%; (k) 1.0 M TBAF in THF, AcOH (pH 6), 89%; (l) 10% TMSOTf, CH<sub>2</sub>Cl<sub>2</sub>, –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<sub>2</sub> in the presence of ammonia, gave the linear trisaccharide backbone **7** (83%). <sup>1</sup>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  $\beta$ -1,4-galactosyltransferase, 14 U alkaline phosphatase, 37 °C, 65% (**2**); 6% (**48**).

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

Proton	$\delta_{\text{H}}$			
	Glc I <sup>a</sup>	Gal II	GlcNAc III	Glc IV <sup>b</sup>
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. <sup>c</sup>	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
$\text{O}(\text{CH}_2)_2(\text{CH}_2)_2(\text{CH}_2)_2\text{ND}_2$			1.39–1.41 (4 H)	
$\text{OCH}_2\text{CH}_2(\text{CH}_2)_2\text{CH}_2\text{CH}_2\text{ND}_2$			1.64–1.65 (4 H)	
$\text{CH}_2\text{ND}_2$			2.95	
$\text{OCH}_2(\text{CH}_2)_5\text{ND}_2$			3.68, 3.92	
$\text{NDCOCH}_3$			2.03	

<sup>a</sup> Glc( $\beta$ 1- $\text{O}(\text{CH}_2)_6\text{NH}_2$ ).

<sup>b</sup> Glc( $\beta$ 1-6)GlcNAc.

<sup>c</sup> 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  $\text{H}_2$  in the presence of ammonia, and subsequent debenzoylation using 10% Pd–C and  $\text{H}_2$  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- $\beta$ -D-glucosamine residue of **7** by using bovine milk  $\beta$ -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.<sup>19</sup>

#### 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- $\beta$ -D-glucopyranoside (**43**)<sup>15</sup> (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  $^1\text{H}$  NMR data (TOCSY, ROESY) of **2** at 300 K (in ppm)

Proton	$\delta_{\text{H}}$				
	Glc I <sup>a</sup>	Gal II <sup>b</sup>	GlcNAc III	Glc IV <sup>c</sup>	Gal V <sup>d</sup>
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. <sup>e</sup>	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 <sub>2</sub> ) <sub>2</sub> (CH <sub>2</sub> ) <sub>2</sub> (CH <sub>2</sub> ) <sub>2</sub> ND <sub>2</sub>	1.39–1.41 (4 H)				
OCH <sub>2</sub> CH <sub>2</sub> (CH <sub>2</sub> ) <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> ND <sub>2</sub>	1.64–1.65 (4 H)				
CH <sub>2</sub> ND <sub>2</sub>	2.96				
OCH <sub>2</sub> (CH <sub>2</sub> ) <sub>5</sub> ND <sub>2</sub>	3.67, 3.92				
NDCOCH <sub>3</sub>	2.03				

<sup>a</sup> Glc( $\beta$ 1-O(CH<sub>2</sub>)<sub>6</sub>NH<sub>2</sub>).

<sup>b</sup> Gal( $\beta$ 1-4)Glc.

<sup>c</sup> Glc( $\beta$ 1-6)GlcNAc.

<sup>d</sup> Gal( $\beta$ 1-4)GlcNAc.

<sup>e</sup> n.d., not determined.

anhydride in pyridine afforded **45** (86% over three steps). O-De-acetylation of **45** using sodium methoxide (pH 10) ( $\rightarrow$ **46**, 79%), and reduction of the azide function using 10% Pd–C and H<sub>2</sub> 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- $\beta$ -D-glucosamine residues of **8** by using bovine milk  $\beta$ -1,4-galactosyltransferase as a catalyst (Scheme 9).  $^1\text{H}$  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<sub>197</sub> (cross reactive material) and immunological studies are in progress.

### 3. Experimental

#### 3.1. General methods

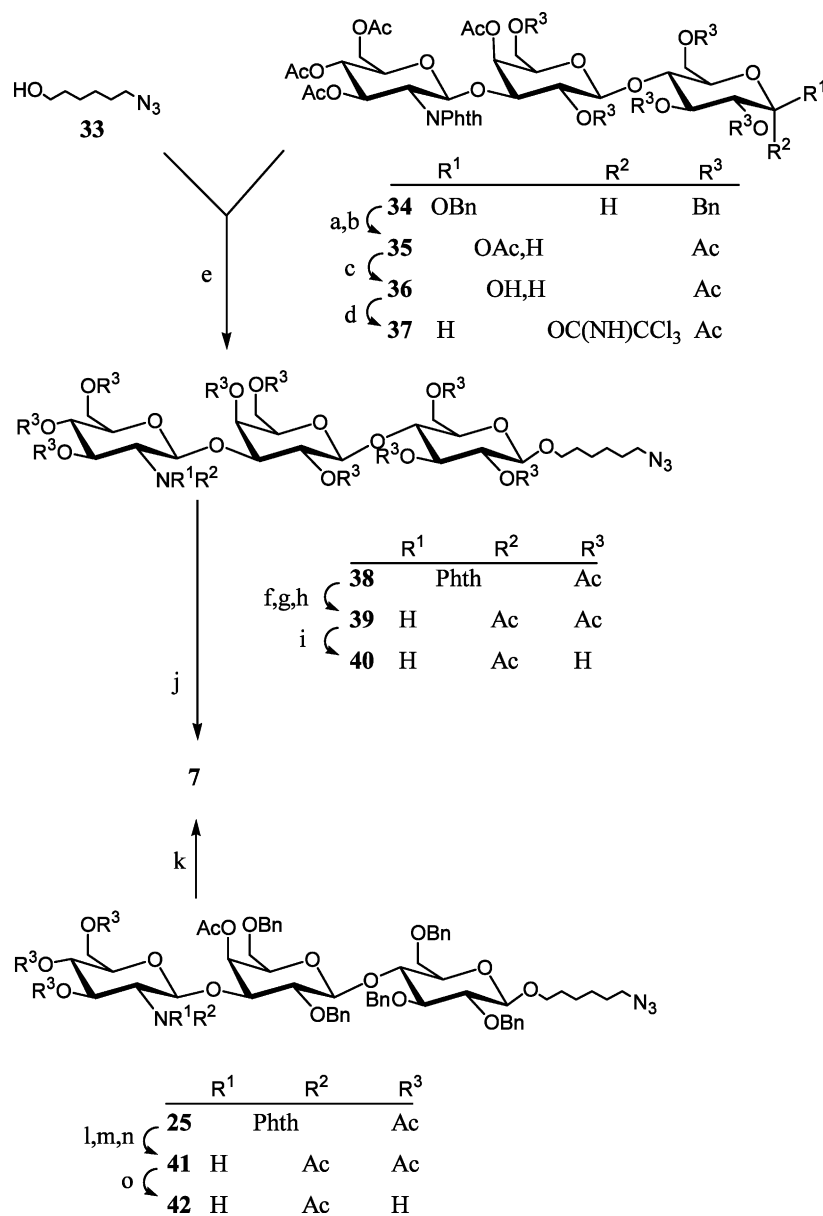
All chemicals were of reagent grade, and were used without further purification. Reactions were monitored by TLC on Silica Gel 60 F<sub>254</sub> (E. Merck); after examination under UV light, compounds were visualized by heating with 10% (v/v) ethanolic H<sub>2</sub>SO<sub>4</sub>, orcinol (2 mg/mL) in 20% (v/v) methanolic H<sub>2</sub>SO<sub>4</sub>, 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<sub>4</sub> or Na<sub>2</sub>SO<sub>4</sub>), 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.  $^1\text{H}$  NMR spectra were recorded at 300 K with a Bruker AC 300 (300 MHz) or a Bruker AMX 500 (500 MHz) spectrometer; the  $\delta_{\text{H}}$  values are given in ppm relative to the signal for internal Me<sub>4</sub>Si ( $\delta_{\text{H}}$  0, CDCl<sub>3</sub>) or internal acetone ( $\delta_{\text{H}}$  2.225, D<sub>2</sub>O).  $^{13}\text{C}$  NMR spectra (APT, 75.5 MHz) were recorded at 300 K with a Bruker AC 300 spectrometer;  $\delta_{\text{C}}$  values are given in ppm relative to the signal of CDCl<sub>3</sub> ( $\delta_{\text{C}}$  76.9, CDCl<sub>3</sub>) or internal acetone ( $\delta_{\text{C}}$  30.89, D<sub>2</sub>O). Two-dimensional  $^1\text{H}$ – $^1\text{H}$  TOCSY (mixing times 7 and 100 ms) and ROESY (mixing time 300 ms), and  $^1\text{H}$ – $^{13}\text{C}$  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  $\mu\text{L}$  of sample (conc 20  $\mu\text{M}$ ) 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- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 6)-2-deoxy-3,4-di-*O*-*p*-methylbenzoyl-2-phthalimido- $\beta$ -D-glucopyranoside (**11**)

A soln of allyl 2-deoxy-3,4-di-*O*-*p*-methylbenzoyl-2-phthalimido- $\beta$ -D-glucopyranoside (**10**)<sup>11</sup> (0.15 g, 0.26 mmol) and 2,3,4,6-tetra-*O*-acetyl- $\alpha$ -D-glucopyranosyl





Scheme 6. Synthesis of the linear trisaccharide backbone **7**: (a) 10% Pd–C, H<sub>2</sub>, EtOH, EtOAc, AcOH; (b) pyridine, Ac<sub>2</sub>O, 66% over two steps; (c) hydrazinium acetate, DMF, 89%; (d) Cl<sub>3</sub>CCN, DBU, CH<sub>2</sub>Cl<sub>2</sub>, 69%; (e) 1 equiv AgOTf, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, 22%; (f) NaOMe (pH 10), MeOH, CH<sub>2</sub>Cl<sub>2</sub>; (g) NH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>, 1-BuOH, 80 °C; (h) pyridine, Ac<sub>2</sub>O, 77% over three steps; (i) NaOMe (pH 10), MeOH, CH<sub>2</sub>Cl<sub>2</sub>, 55%; (j) 10% Pd–C, H<sub>2</sub>, water, *tert*-BuOH, aq 25% NH<sub>3</sub>, 83%; (k) 10% Pd–C, H<sub>2</sub>, water, *tert*-BuOH, aq 25% NH<sub>3</sub>/10% Pd–C, AcOH, 68%; (l) NaOMe (pH 10), MeOH, CH<sub>2</sub>Cl<sub>2</sub>; (m) NH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>, 1-BuOH, 80 °C; (n) pyridine, Ac<sub>2</sub>O, 78% over three steps; (o) NaOMe (pH 10), MeOH, CH<sub>2</sub>Cl<sub>2</sub>, 99%.

trichloroacetimidate (**9**)<sup>16</sup> (0.16 g, 0.32 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (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<sub>3</sub>, aq satd NaHCO<sub>3</sub>, and water, dried (MgSO<sub>4</sub>), 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*<sub>f</sub> 0.65 (1:1 toluene–EtOAc); [α]<sub>D</sub><sup>20</sup> –4° (*c* 1, CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>; 2D TOCSY,

ROESY): δ 2.00, 2.01, and 2.08 (3 s, 6,3,3 H, 4 COCH<sub>3</sub>), 2.23 and 2.31 (2 s, each 3 H, 2 COC<sub>6</sub>H<sub>4</sub>CH<sub>3</sub>), 3.71 (m, 1 H, H-5<sup>II</sup>), 3.83 (dd, 1 H, *J*<sub>5,6b</sub> 7.9, *J*<sub>6a,6b</sub> 10.8 Hz, H-6b<sup>I</sup>), 4.23 (dd, 1 H, *J*<sub>5,6b</sub> 4.6, *J*<sub>6a,6b</sub> 12.1 Hz, H-6b<sup>II</sup>), 4.36 (m, 1 H, OCHHCH=CH<sub>2</sub>), 4.54 (dd, 1 H, *J*<sub>1,2</sub> 8.4, *J*<sub>2,3</sub> 10.5 Hz, H-2<sup>I</sup>), 4.69 (d, 1 H, *J*<sub>1,2</sub> 7.9 Hz, H-1<sup>II</sup>), 5.39 (t, 1 H, H-4<sup>I</sup>), 5.57 (d, 1 H, H-1<sup>I</sup>), 5.77 (m, 1 H, OCH<sub>2</sub>CH=CH<sub>2</sub>), 6.22 (dd, 1 H, *J*<sub>3,4</sub> 9.4 Hz, H-3<sup>I</sup>), 7.02 and 7.14 (2 d, each 2 H, Phth), 7.61–7.80 (2 m, 8 H, 2 COC<sub>6</sub>H<sub>4</sub>CH<sub>3</sub>); <sup>13</sup>C NMR (75.5 MHz, CDCl<sub>3</sub>): δ 20.2–20.4 (COCH<sub>3</sub>), 21.2 and 21.3 (2 COC<sub>6</sub>H<sub>4</sub>CH<sub>3</sub>), 54.6 (C-

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

Proton	$\delta_{\text{H}}$		
	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. <sup>a</sup>	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
$\text{O}(\text{CH}_2)_2(\text{CH}_2)_2(\text{CH}_2)_2\text{N}_3$			1.39–1.42 (4 H)
$\text{OCH}_2\text{CH}_2(\text{CH}_2)_2\text{CH}_2\text{CH}_2\text{N}_3$			1.63–1.65 (4 H)
$\text{CH}_2\text{N}_3$			3.32
$\text{OCH}_2(\text{CH}_2)_5\text{N}_3$			3.62, 3.92
$\text{NDCOCH}_3$			2.04

<sup>a</sup> n.d., not determined.

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  53.2, 61.6, and 69.8 (C-6<sup>I</sup>, C-6<sup>II</sup>,  $\text{OCH}_2\text{CH}=\text{CH}_2$ ), 68.1, 69.9, 70.7, 71.0, 71.6, 72.6, and 73.6 (C-3<sup>I</sup>, C-4<sup>I</sup>, C-5<sup>I</sup>, C-2<sup>II</sup>, C-3<sup>II</sup>, C-4<sup>II</sup>, C-5<sup>II</sup>), 96.9 and 100.5 (C-1<sup>I</sup>, C-1<sup>II</sup>), 117.5 ( $\text{OCH}_2\text{CH}=\text{CH}_2$ ), 165.0 and 165.2 (2  $\text{COC}_6\text{H}_4\text{CH}_3$ ), 168.9–170.2 ( $\text{COCH}_3$ ); HRMS data of  $\text{C}_{47}\text{H}_{49}\text{NO}_{18}$  (M, 915.295):  $[\text{M}+\text{NH}_4]^+$  found 933.335, calcd 933.329.

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

Proton	$\delta_{\text{H}}$		
	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. <sup>a</sup>	3.46
H-6a	3.98	n.d.	3.90
H-6b	3.80	n.d.	3.77
$\text{O}(\text{CH}_2)_2(\text{CH}_2)_2(\text{CH}_2)_2\text{ND}_2$			1.39–1.41 (4 H)
$\text{OCH}_2\text{CH}_2(\text{CH}_2)_2\text{CH}_2\text{CH}_2\text{ND}_2$			1.63–1.65 (4 H)
$\text{CH}_2\text{ND}_2$			2.95
$\text{OCH}_2(\text{CH}_2)_5\text{ND}_2$			3.58, 3.83
$\text{NDCOCH}_3$			2.03

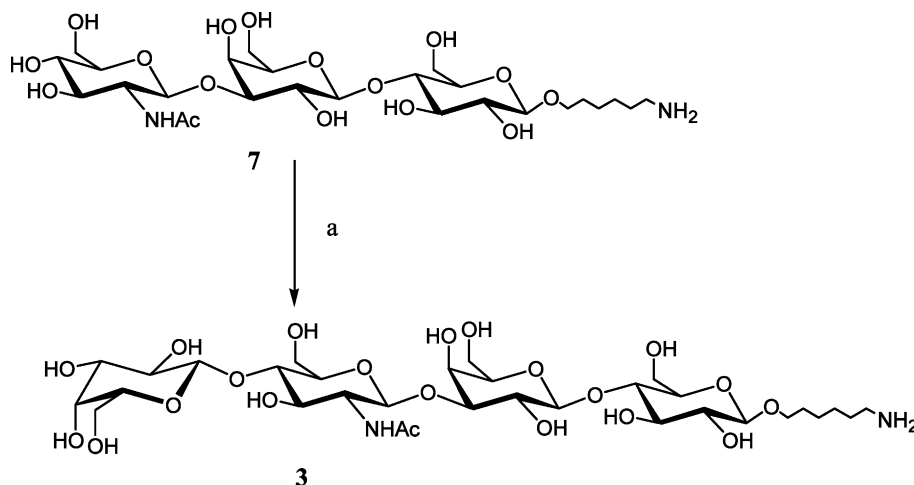
<sup>a</sup> n.d., not determined.

### 3.3. (2,3,4,6-Tetra-*O*-acetyl- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 6)-2-deoxy-3,4-di-*O*-*p*-methylbenzoyl-2-phthalimido- $\alpha$ , $\beta$ -D-glucopyranose (**12**)

To a soln of **11** (0.41 g, 0.45 mmol) in AcOH (15 mL) were added  $\text{Pd}(\text{II})\text{Cl}_2$  (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  $\text{CH}_2\text{Cl}_2$  then washed with water, aq satd  $\text{NaHCO}_3$ , and water, dried ( $\text{MgSO}_4$ ), filtered, and concentrated. Low-pressure column chromatography (4:1  $\rightarrow$  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);  $^1\text{H}$  NMR  $\beta$ -product (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  2.02, 2.04, 2.05, and 2.12 (4 s, each 3 H, 4  $\text{COCH}_3$ ), 2.27 and 2.34 (2 s, each 3 H, 2  $\text{COC}_6\text{H}_4\text{CH}_3$ ), 4.02 (dd, 1 H,  $J_{5,6a}$  1.9,  $J_{6a,6b}$  11.2 Hz, H-6a<sup>I</sup>), 4.20 (dd, 1 H,  $J_{5,6b}$  4.7,  $J_{6a,6b}$  12.2 Hz, H-6b<sup>II</sup>), 4.43 (dd, 1 H,  $J_{1,2}$  8.4,  $J_{2,3}$  10.7 Hz, H-2<sup>I</sup>), 4.65 (d, 1 H,  $J_{1,2}$  7.5 Hz, H-1<sup>II</sup>), 4.96 (dd, 1 H,  $J_{2,3}$  8.6 Hz, H-2<sup>II</sup>), 5.42 (t, 1 H, H-4<sup>I</sup>), 5.74 (d, 1 H, H-1<sup>I</sup>), 6.23 (dd, 1 H,  $J_{3,4}$  9.2 Hz, H-3<sup>I</sup>), 7.04 and 7.15 (2 d, each 2 H, Phth), 7.60–7.81 (2 m, 8 H, 2  $\text{COC}_6\text{H}_4\text{CH}_3$ );  $^{13}\text{C}$  NMR (75.5 MHz,  $\text{CDCl}_3$ ):  $\delta$  20.3–20.4 ( $\text{COCH}_3$ ), 21.2 and 21.3 (2  $\text{COC}_6\text{H}_4\text{CH}_3$ ), 56.1 (C-2<sup>I</sup>), 61.7 and 68.3 (C-6<sup>I</sup>, C-6<sup>II</sup>), 68.1, 69.7, 70.7, 71.2, 71.5, 72.5, and 73.3 (C-3<sup>I</sup>, C-4<sup>I</sup>, C-5<sup>I</sup>, C-2<sup>II</sup>, C-3<sup>II</sup>, C-4<sup>II</sup>, C-5<sup>II</sup>), 92.4 and 100.5 (C-1<sup>I</sup>, C-1<sup>II</sup>), 165.0 and 165.2 (2  $\text{COC}_6\text{H}_4\text{CH}_3$ ), 169.2–170.4 ( $\text{COCH}_3$ ); HRMS of  $\text{C}_{44}\text{H}_{45}\text{NO}_{18}$  (M, 875.263):  $[\text{M}+\text{H}]^+$  found 876.273, calcd 876.271.

### 3.4. (2,3,4,6-Tetra-*O*-acetyl- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 6)-2-deoxy-3,4-di-*O*-*p*-methylbenzoyl-2-phthalimido- $\alpha$ , $\beta$ -D-glucopyranosyl trichloroacetimidate (**13**)

To a soln of **12** (0.37 g, 0.42 mmol) in dry  $\text{CH}_2\text{Cl}_2$  (15 mL) were added, at 0  $^\circ\text{C}$ , trichloroacetonitrile (0.2 mL, 2.1 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (60  $\mu\text{L}$ , 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);  $^1\text{H}$  NMR  $\beta$ -product (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.99, 2.03, and 2.09 (3 s, 6,3,3 H, 4  $\text{COCH}_3$ ), 2.27 and 2.34 (2 s, each 3 H, 2  $\text{COC}_6\text{H}_4\text{CH}_3$ ), 3.62 (m, 1 H, H-5<sup>II</sup>), 3.86 (dd, 1 H,  $J_{5,6b}$  6.8,  $J_{6a,6b}$  11.8 Hz, H-6b<sup>I</sup>), 4.70 (d, 1 H,  $J_{1,2}$  7.8 Hz, H-1<sup>II</sup>), 5.15 (t, 1 H, H-3<sup>II</sup>), 5.50 (t, 1 H, H-4<sup>I</sup>), 6.32 (t, 1 H, H-3<sup>I</sup>), 6.79 (d, 1 H,  $J_{1,2}$  8.8 Hz, H-1<sup>I</sup>), 7.05 and 7.15 (2 d, each 2 H, Phth), 7.63–7.81 (2 m, 8 H, 2  $\text{COC}_6\text{H}_4\text{CH}_3$ ), 8.78 (s, 1 H, NH);  $^{13}\text{C}$  NMR (75.5 MHz,  $\text{CDCl}_3$ ):  $\delta$  20.4–20.5 ( $\text{COCH}_3$ ), 21.3 and 21.4 (2  $\text{COC}_6\text{H}_4\text{CH}_3$ ), 53.7 (C-2<sup>I</sup>), 61.7 and 67.3 (C-6<sup>I</sup>, C-6<sup>II</sup>), 68.2, 69.3, 70.5, 70.9, 71.6, 72.7, and 75.0 (C-3<sup>I</sup>, C-4<sup>I</sup>, C-5<sup>I</sup>, C-2<sup>II</sup>, C-3<sup>II</sup>, C-4<sup>II</sup>, C-5<sup>II</sup>), 93.4 and 100.2 (C-1<sup>I</sup>, C-1<sup>II</sup>), 160.2 ( $\text{OC}(\text{NH})\text{CCl}_3$ ), 165.0 and 165.3 (2  $\text{COC}_6\text{H}_4\text{CH}_3$ ), 169.1–170.3 ( $\text{COCH}_3$ ).



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

### 3.5. 6-Azidoheptyl (3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 3)-4-*O*-acetyl-2,6-di-*O*-benzyl- $\beta$ -D-galactopyranoside (**16**)

A soln of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido- $\beta$ -D-glucopyranosyl trichloroacetimidate (**15**)<sup>17</sup> (1.09 g, 1.88 mmol) and 6-azidoheptyl 4-*O*-acetyl-2,6-di-*O*-benzyl- $\beta$ -D-galactopyranoside (**14**)<sup>14</sup> (0.84 g, 1.47 mmol) in dry  $\text{CH}_2\text{Cl}_2$  (50 mL), containing 4 Å molecular sieves (1 g), was stirred under Ar for 1 h. After cooling to  $-70^\circ\text{C}$ , TMSOTf (34  $\mu\text{L}$ , 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  $\text{Et}_3\text{N}$ , filtered, washed with water, dried ( $\text{MgSO}_4$ ), filtered, and concentrated.

Low-pressure column chromatography (10:1  $\rightarrow$  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);  $[\alpha]_D^{20} + 3^\circ$  ( $c$  1,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.22–1.24 (m, 4 H, 2  $\text{CH}_2$ ), 1.37–1.42 (m, 2 H,  $\text{CH}_2$ ), 1.48–1.52 (m, 2 H,  $\text{CH}_2$ ), 1.82, 2.00, 2.03, and 2.08 (4 s, each 3 H, 4  $\text{COCH}_3$ ), 3.07 (t, 2 H,  $\text{CH}_2\text{N}_3$ ), 3.84 (m, 1 H,  $\text{OCHH}$ ), 4.48 (d, 2 H,  $\text{OCH}_2\text{C}_6\text{H}_5$ ), 4.56 (d, 1 H,  $\text{OCHHC}_6\text{H}_5$ ), 5.16 (t, 1 H,  $\text{H-4}^{\text{II}}$ ), 5.38 (d, 1 H,  $J_{3,4}$  3.5,  $J_{4,5} < 1$  Hz,  $\text{H-4}^{\text{I}}$ ), 5.59 (d, 1 H,  $J_{1,2}$  8.3 Hz,  $\text{H-1}^{\text{II}}$ ), 5.77 (dd, 1 H,  $J_{2,3}$  10.7,  $J_{3,4}$  9.1 Hz,  $\text{H-3}^{\text{II}}$ );  $^{13}\text{C}$  NMR (75.5 MHz,  $\text{CDCl}_3$ ):  $\delta$  20.2–20.5 ( $\text{COCH}_3$ ), 25.3, 26.2, 28.4, and 29.2 (4  $\text{CH}_2$ ), 51.0 ( $\text{CH}_2\text{N}_3$ ), 54.7 ( $\text{C-2}^{\text{II}}$ ), 61.2, 68.9, 69.8, 73.4, and 73.9 ( $\text{C-6}^{\text{I}}$ ,  $\text{C-6}^{\text{II}}$ , 2  $\text{OCH}_2\text{C}_6\text{H}_5$ ,  $\text{OCH}_2$ ), 68.6, 69.5, 70.4, 71.6, 72.6, 78.1, and 78.5 ( $\text{C-2}^{\text{I}}$ ,  $\text{C-3}^{\text{I}}$ ,  $\text{C-}$

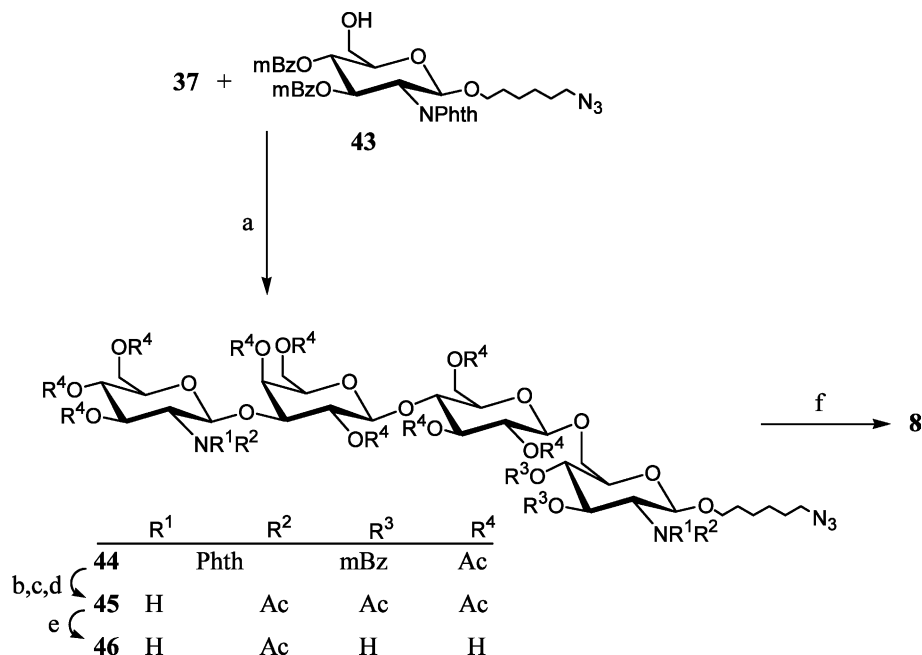
Table 7  
500 MHz  $^1\text{H}$  NMR data (TOCSY, ROESY) of **3** at 300 K (in ppm)

Proton	$\delta_{\text{H}}$			
	Glc I	Gal II <sup>a</sup>	GlcNAc III	Gal IV <sup>b</sup>
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. <sup>c</sup>	3.58	n.d.
H-6a	3.96	n.d.	3.93	n.d.
H-6b	3.78	n.d.	3.85	n.d.
$\text{O}(\text{CH}_2)_2(\text{CH}_2)_2(\text{CH}_2)_2\text{ND}_2$		1.39–1.42 (4 H)		
$\text{OCH}_2\text{CH}_2(\text{CH}_2)_2\text{CH}_2\text{CH}_2\text{ND}_2$		1.63–1.70 (4 H)		
$\text{CH}_2\text{ND}_2$		2.99		
$\text{OCH}_2(\text{CH}_2)_5\text{ND}_2$		3.68, 3.92		
$\text{NDCOCH}_3$		2.03		

<sup>a</sup> Gal( $\beta$ 1-4)Glc.

<sup>b</sup> Gal( $\beta$ 1-4)GlcNAc.

<sup>c</sup> n.d., not determined.



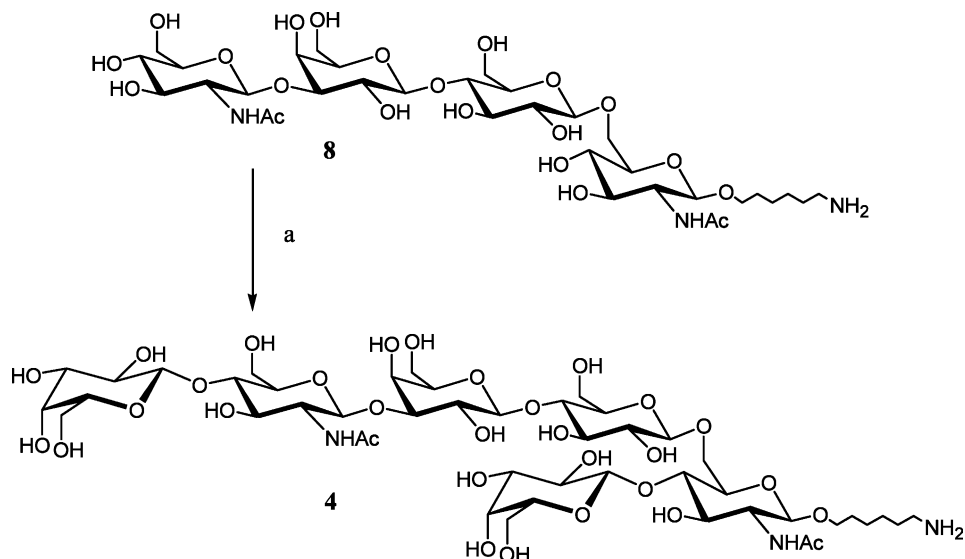
Scheme 8. Synthesis of the linear tetrasaccharide backbone **8**: (a) 15% TMSOTf, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, 34%; (b) NaOMe (pH 10), MeOH, CH<sub>2</sub>Cl<sub>2</sub>; (c) NH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>, 1-BuOH, 90 °C; (d) pyridine, Ac<sub>2</sub>O, 86% over three steps; (e) NaOMe (pH 10), MeOH, CH<sub>2</sub>Cl<sub>2</sub>, 79%; (f) 10% Pd–C, H<sub>2</sub>, water, *tert*-BuOH, aq 25% NH<sub>3</sub>, 82%.

4<sup>I</sup>, C-5<sup>I</sup>, C-3<sup>II</sup>, C-4<sup>II</sup>, C-5<sup>II</sup>), 97.9 and 103.3 (C-1<sup>I</sup>, C-1<sup>II</sup>), 169.1–170.4 (COCH<sub>3</sub>); HRMS of C<sub>48</sub>H<sub>56</sub>N<sub>4</sub>O<sub>16</sub> (M, 944.369): [M+NH<sub>4</sub>]<sup>+</sup> found 962.399, calcd 962.403.

### 3.6. 6-Azidoheptyl (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<sub>2</sub>Cl<sub>2</sub> (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<sup>+</sup>), filtered, and concentrated. Column chromatography (1:2 toluene–EtOAc) of the residue gave **17**, isolated as a colourless glass (1.01 g, 91%); *R*<sub>f</sub> 0.36 (1:3 toluene–EtOAc); [α]<sub>D</sub><sup>20</sup> +5° (*c* 1, CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>; 2D TOCSY, ROESY): δ 1.13–1.17 (m, 4 H, 2 CH<sub>2</sub>), 1.31–1.37 (m, 2 H, CH<sub>2</sub>), 1.39–1.44 (m, 2 H, CH<sub>2</sub>), 2.06 (COCH<sub>3</sub>), 3.06 (t, 2 H, CH<sub>2</sub>N<sub>3</sub>), 4.00 (dd, 1 H, *J*<sub>1,2</sub> 8.5, *J*<sub>2,3</sub> 10.5 Hz, H-2<sup>II</sup>), 4.21 (t, 1 H, H-3<sup>II</sup>), 4.24 (d, 1 H, *J*<sub>1,2</sub> 7.7 Hz, H-1<sup>I</sup>), 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  $^1\text{H}$  NMR data (TOCSY, ROESY) of **46** at 300 K (in ppm)

Proton	$\delta_{\text{H}}$			
	GlcNAc I <sup>a</sup>	Glc II	Gal III	GlcNAc IV <sup>b</sup>
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. <sup>c</sup>	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 <sub>2</sub> ) <sub>2</sub> (CH <sub>2</sub> ) <sub>2</sub> (CH <sub>2</sub> ) <sub>2</sub> N <sub>3</sub>			1.35–1.37 (4 H)	
OCH <sub>2</sub> CH <sub>2</sub> (CH <sub>2</sub> ) <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> N <sub>3</sub>			1.57–1.61 (4 H)	
CH <sub>2</sub> N <sub>3</sub>			3.33	
OCH <sub>2</sub> (CH <sub>2</sub> ) <sub>5</sub> N <sub>3</sub>			3.62, 3.91	
NDCOCH <sub>3</sub>			2.04 (2)	

<sup>a</sup> GlcNAc( $\beta$ 1-O(CH<sub>2</sub>)<sub>6</sub>N<sub>3</sub>).

<sup>b</sup> GlcNAc( $\beta$ 1-3)Gal.

<sup>c</sup> n.d., not determined.

d, each 1 H, OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 4.46 (s, 2 H, OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 5.36 (d, 1 H, H-1<sup>II</sup>), 5.49 (d, 1 H,  $J_{3,4}$  3.3,  $J_{4,5}$  < 1 Hz, H-4<sup>I</sup>);  $^{13}\text{C}$  NMR (75.5 MHz, CDCl<sub>3</sub>):  $\delta$  20.9 (COCH<sub>3</sub>), 25.2, 26.1, 28.3, and 29.1 (4 CH<sub>2</sub>), 51.1 (CH<sub>2</sub>N<sub>3</sub>), 56.6 (C-2<sup>II</sup>), 61.3, 68.7, 69.8, 73.4, and 73.8 (C-6<sup>I</sup>, C-6<sup>II</sup>, 2 OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>, OCH<sub>2</sub>), 70.4, 70.7, 71.0, 72.1, 75.8, 77.7, and 79.9 (C-2<sup>I</sup>, C-3<sup>I</sup>, C-4<sup>I</sup>, C-5<sup>I</sup>, C-3<sup>II</sup>, C-4<sup>II</sup>, C-5<sup>II</sup>), 99.2 and 103.3 (C-1<sup>I</sup>, C-1<sup>II</sup>), 171.4 (COCH<sub>3</sub>); HRMS of C<sub>42</sub>H<sub>50</sub>N<sub>4</sub>O<sub>13</sub> (M, 818.337): [M + Na]<sup>+</sup> found 841.332, calcd 841.327.

### 3.7. 6-Azidoheptyl (6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-2-phthalimido- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 3)-4-*O*-acetyl-2,6-di-*O*-benzyl- $\beta$ -D-galactopyranoside (**18**)

To a soln of **17** (0.18 g, 0.23 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) and Py (0.4 mL) were added 4-dimethylaminopyridine (20 mg, 0.18 mmol), Et<sub>3</sub>N (70  $\mu$ L), and *tert*-butyldiphenylsilyl chloride (70  $\mu$ L, 0.27 mmol). The mixture was stirred for 4 h, then poured into ice water, extracted with CH<sub>2</sub>Cl<sub>2</sub>, washed with aq satd NaHCO<sub>3</sub>, dried (MgSO<sub>4</sub>),

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

Proton	$\delta_{\text{H}}$			
	GlcNAc I <sup>a</sup>	Glc II	Gal III	GlcNAc IV <sup>b</sup>
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. <sup>c</sup>	3.46
H-6a	4.21	3.97	n.d.	3.90
H-6b	3.88	3.81	n.d.	3.77
O(CH <sub>2</sub> ) <sub>2</sub> (CH <sub>2</sub> ) <sub>2</sub> (CH <sub>2</sub> ) <sub>2</sub> ND <sub>2</sub>			1.32–1.36 (4 H)	
OCH <sub>2</sub> CH <sub>2</sub> (CH <sub>2</sub> ) <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> ND <sub>2</sub>			1.52–1.55 (2 H), 1.62–1.65 (2 H)	
CH <sub>2</sub> ND <sub>2</sub>			2.97	
OCH <sub>2</sub> (CH <sub>2</sub> ) <sub>5</sub> ND <sub>2</sub>			3.56, 3.83	
NDCOCH <sub>3</sub>			2.02, 2.04	

<sup>a</sup> GlcNAc( $\beta$ 1-O(CH<sub>2</sub>)<sub>6</sub>NH<sub>2</sub>).

<sup>b</sup> GlcNAc( $\beta$ 1-3)Gal.

<sup>c</sup> n.d., not determined.

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

Proton	$\delta_{\text{H}}$					
	GlcNAc I <sup>a</sup>	Glc II	Gal III <sup>b</sup>	GlcNAc IV <sup>c</sup>	Gal V <sup>d</sup>	Gal VI <sup>e</sup>
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. <sup>f</sup>	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 <sub>2</sub> ) <sub>2</sub> (CH <sub>2</sub> ) <sub>2</sub> (CH <sub>2</sub> ) <sub>2</sub> ND <sub>2</sub>			1.36–1.38 (4 H)			
OCH <sub>2</sub> CH <sub>2</sub> (CH <sub>2</sub> ) <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> ND <sub>2</sub>			1.54–1.57 (2 H), 1.64–1.67 (2 H)			
CH <sub>2</sub> ND <sub>2</sub>			2.99			
OCH <sub>2</sub> (CH <sub>2</sub> ) <sub>5</sub> ND <sub>2</sub>			3.68, 3.92			
NDCOCH <sub>3</sub>			2.03 (2)			

<sup>a</sup> GlcNAc( $\beta$ 1-O(CH<sub>2</sub>)<sub>6</sub>NH<sub>2</sub>).

<sup>b</sup> Gal( $\beta$ 1-4)Glc.

<sup>c</sup> GlcNAc( $\beta$ 1-3)Gal.

<sup>d</sup> Gal( $\beta$ 1-4)GlcNAc( $\beta$ 1-O(CH<sub>2</sub>)<sub>6</sub>NH<sub>2</sub>).

<sup>e</sup> Gal( $\beta$ 1-4)GlcNAc( $\beta$ 1-3)Gal.

<sup>f</sup> n.d., not determined.

filtered, and concentrated. Low-pressure column chromatography (5:1  $\rightarrow$  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);  $[\alpha]_{\text{D}}^{20} +9^\circ$  ( $c$  1, CHCl<sub>3</sub>);  $^1\text{H}$  NMR (500 MHz, CDCl<sub>3</sub>; 2D TOCSY, ROESY):  $\delta$  1.05 [s, 9 H, SiC(CH<sub>3</sub>)<sub>3</sub>], 1.19–1.22 (m, 4 H, 2 CH<sub>2</sub>), 1.37–1.40 (m, 2 H, CH<sub>2</sub>), 1.46–1.48 (m, 2 H, CH<sub>2</sub>), 1.96 (s, 3 H, COCH<sub>3</sub>), 3.07 (t, 2 H, CH<sub>2</sub>N<sub>3</sub>), 3.33 (dd, 1 H,  $J_{1,2}$  7.9,  $J_{2,3}$  9.8 Hz, H-2<sup>I</sup>), 3.37 (m, 1 H, OCHH), 3.78 (dd, 1 H,  $J_{3,4}$  3.5 Hz, H-3<sup>I</sup>), 3.82 (m, 1 H, OCHH), 4.04 (dd, 1 H,  $J_{1,2}$  8.3,  $J_{2,3}$  11.0 Hz, H-2<sup>II</sup>), 4.17 and 4.53 (2 d, each 1 H, OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 4.20 (d, 1 H, H-1<sup>I</sup>), 4.37 (t, 1 H, H-3<sup>II</sup>), 4.38 and 4.43 (2 d, each 1 H, OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 5.33 (d, 1 H,  $J_{4,5} < 1$  Hz, H-4<sup>I</sup>), 5.41 (d, 1 H, H-1<sup>II</sup>);  $^{13}\text{C}$  NMR (75.5 MHz, CDCl<sub>3</sub>):  $\delta$  19.0 [SiC(CH<sub>3</sub>)<sub>3</sub>], 20.5 (COCH<sub>3</sub>), 25.3, 26.2, 28.4, and 29.2 (4 CH<sub>2</sub>), 26.6 [SiC(CH<sub>3</sub>)<sub>3</sub>], 51.0 (CH<sub>2</sub>N<sub>3</sub>), 56.6 (C-2<sup>II</sup>), 64.5, 68.9, 69.8, 73.3, and 73.9 (C-6<sup>I</sup>, C-6<sup>II</sup>, 2 OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>, OCH<sub>2</sub>), 70.1, 71.0, 72.5, 73.6, 74.8, 76.4, and 78.6 (C-2<sup>I</sup>, C-3<sup>I</sup>, C-4<sup>I</sup>, C-5<sup>I</sup>, C-3<sup>II</sup>, C-4<sup>II</sup>, C-5<sup>II</sup>), 98.0 and 103.4 (C-1<sup>I</sup>, C-1<sup>II</sup>), 170.0 (COCH<sub>3</sub>); HRMS of C<sub>58</sub>H<sub>68</sub>N<sub>4</sub>O<sub>13</sub>Si (M, 1056.455): [M+NH<sub>4</sub>]<sup>+</sup> found 1074.496, calcd 1074.489.

### 3.8. 6-Azidoheptyl (6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-3,4-di-*O*-*p*-methylbenzoyl-2-phthalimido- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 3)-4-*O*-acetyl-2,6-di-*O*-benzyl- $\beta$ -D-galactopyranoside (**19**)

To a soln of **18** (0.19 g, 0.19 mmol) in dry Py (4 mL) was added dropwise, at 0  $^\circ\text{C}$ , a soln of *p*-methylbenzoyl

chloride (60  $\mu\text{L}$ , 0.48 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (1 mL). The mixture was stirred for 4 h at rt, then poured into ice water, extracted with CH<sub>2</sub>Cl<sub>2</sub>, washed with aq satd NaHCO<sub>3</sub>, dried (MgSO<sub>4</sub>), 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);  $[\alpha]_{\text{D}}^{20} +1^\circ$  ( $c$  1, CHCl<sub>3</sub>);  $^1\text{H}$  NMR (500 MHz, CDCl<sub>3</sub>; 2D TOCSY, ROESY):  $\delta$  1.06 [s, 9 H, SiC(CH<sub>3</sub>)<sub>3</sub>], 1.23–1.26 (m, 4 H, 2 CH<sub>2</sub>), 1.38–1.41 (m, 2 H, CH<sub>2</sub>), 1.52–1.54 (m, 2 H, CH<sub>2</sub>), 2.04 (s, 3 H, COCH<sub>3</sub>), 2.18 and 2.28 (2 s, each 3 H, 2 COC<sub>6</sub>H<sub>4</sub>CH<sub>3</sub>), 3.07 (t, 2 H, CH<sub>2</sub>N<sub>3</sub>), 3.64 (t, 1 H, H-6a<sup>I</sup>), 3.83 (d, 1 H,  $J_{6a,6b}$  9.4 Hz, H-6b<sup>II</sup>), 3.89 (m, 1 H, OCHH), 4.00 (dd, 1 H,  $J_{2,3}$  9.6,  $J_{3,4}$  3.5 Hz, H-3<sup>I</sup>), 4.24 and 4.65 (2 d, each 1 H, OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 4.30 (d, 1 H,  $J_{1,2}$  7.7 Hz, H-1<sup>I</sup>), 4.43 and 4.49 (2 d, each 1 H, OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 4.53 (dd, 1 H,  $J_{1,2}$  8.3,  $J_{2,3}$  10.7 Hz, H-2<sup>II</sup>), 5.56 (t, 1 H, H-4<sup>II</sup>), 5.59 (d, 1 H,  $J_{4,5} < 1$  Hz, H-4<sup>I</sup>), 5.84 (d, 1 H, H-1<sup>II</sup>), 6.31 (dd, 1 H,  $J_{3,4}$  9.2 Hz, H-3<sup>II</sup>), 6.97 and 7.08 (2 d, each 2 H, Phth);  $^{13}\text{C}$  NMR (75.5 MHz, CDCl<sub>3</sub>):  $\delta$  19.1 [SiC(CH<sub>3</sub>)<sub>3</sub>], 20.5 (COCH<sub>3</sub>), 21.4 and 21.5 (2 COC<sub>6</sub>H<sub>4</sub>CH<sub>3</sub>), 25.5, 26.3, 28.5, and 29.4 (4 CH<sub>2</sub>), 26.6 [SiC(CH<sub>3</sub>)<sub>3</sub>], 51.1 (CH<sub>2</sub>N<sub>3</sub>), 55.5 (C-2<sup>II</sup>), 63.0, 69.1, 69.8, 73.4, and 73.9 (C-6<sup>I</sup>, C-6<sup>II</sup>, 2 OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>, OCH<sub>2</sub>), 69.9, 70.3, 70.8, 72.8, 75.1, 76.4, and 78.7 (C-2<sup>I</sup>, C-3<sup>I</sup>, C-4<sup>I</sup>, C-5<sup>I</sup>, C-3<sup>II</sup>, C-4<sup>II</sup>, C-5<sup>II</sup>), 98.2 and 103.6 (C-1<sup>I</sup>, C-1<sup>II</sup>), 164.9 and 165.5 (2 COC<sub>6</sub>H<sub>4</sub>CH<sub>3</sub>), 169.7 (COCH<sub>3</sub>); HRMS of C<sub>74</sub>H<sub>80</sub>N<sub>4</sub>O<sub>17</sub>Si (M, 1292.538): [M+NH<sub>4</sub>]<sup>+</sup> found 1310.584, calcd 1310.573.



### 3.9. 6-Azidoheptyl (2-deoxy-3,4-di-*O*-*p*-methylbenzoyl-2-phthalimido- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 3)-4-*O*-acetyl-2,6-di-*O*-benzyl- $\beta$ -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);  $[\alpha]_D^{20} +9^\circ$  ( $c$  1, CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>; 2D TOCSY, ROESY):  $\delta$  1.12–1.17 (m, 4 H, 2 CH<sub>2</sub>), 1.32–1.35 (m, 2 H, CH<sub>2</sub>), 1.40–1.44 (m, 2 H, CH<sub>2</sub>), 2.18 (s, 3 H, COCH<sub>3</sub>), 2.26 and 2.33 (2 s, each 3 H, 2 COC<sub>6</sub>H<sub>4</sub>CH<sub>3</sub>), 3.05 (CH<sub>2</sub>N<sub>3</sub>), 3.34 (m, 1 H, OCHH), 3.49 (dd, 1 H,  $J_{1,2}$  7.7,  $J_{2,3}$  9.4 Hz, H-2<sup>I</sup>), 3.51 (d, 2 H,  $J_{6a,6b}$  5.8 Hz, H-6a<sup>I</sup>, H-6b<sup>I</sup>), 3.82 (m, 1 H, OCHH), 3.90 (m, 1 H, H-5<sup>II</sup>), 4.21 and 4.45 (2 d, each 1 H, OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 4.27 (d, 1 H, H-1<sup>I</sup>), 4.48 (s, 2 H, OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 4.51 (dd, 1 H,  $J_{1,2}$  8.3,  $J_{2,3}$  10.7 Hz, H-2<sup>II</sup>), 5.43 (t, 1 H, H-4<sup>II</sup>), 5.60 (d, 1 H,  $J_{3,4}$  3.5,  $J_{4,5} < 1$  Hz, H-4<sup>I</sup>), 5.72 (d, 1 H, H-1<sup>II</sup>), 6.13 (dd, 1 H,  $J_{3,4}$  9.2 Hz, H-3<sup>II</sup>); <sup>13</sup>C NMR (75.5 MHz, CDCl<sub>3</sub>):  $\delta$  21.0 (COCH<sub>3</sub>), 21.4 and 21.5 (2 COC<sub>6</sub>H<sub>4</sub>CH<sub>3</sub>), 25.3, 26.2, 28.4, and 29.2 (4 CH<sub>2</sub>), 51.1 (CH<sub>2</sub>N<sub>3</sub>), 55.0 (C-2<sup>II</sup>), 61.5, 70.0, 73.6, 68.8, and 73.9 (C-6<sup>I</sup>, C-6<sup>II</sup>, 2 OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>, OCH<sub>2</sub>), 69.3, 70.1, 70.8, 72.4, 75.2, 77.5, and 81.5 (C-2<sup>I</sup>, C-3<sup>I</sup>, C-4<sup>I</sup>, C-5<sup>I</sup>, C-3<sup>II</sup>, C-4<sup>II</sup>, C-5<sup>II</sup>), 99.4 and 103.5 (C-1<sup>I</sup>, C-1<sup>II</sup>), 165.2 and 165.4 (2 COC<sub>6</sub>H<sub>4</sub>CH<sub>3</sub>), 171.4 (COCH<sub>3</sub>); HRMS of C<sub>58</sub>H<sub>62</sub>N<sub>4</sub>O<sub>15</sub> (M, 1054.421):  $[M + NH_4]^+$  found 1072.461, calcd 1072.455.

### 3.10. 6-Azidoheptyl (2,3,4,6-tetra-*O*-acetyl- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 6)-(2-deoxy-3,4-di-*O*-*p*-methylbenzoyl-2-phthalimido- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 3)-4-*O*-acetyl-2,6-di-*O*-benzyl- $\beta$ -D-galactopyranoside (**21**)

(a) A soln of 2,3,4,6-tetra-*O*-acetyl- $\alpha$ -D-glucopyranosyl trichloroacetimidate (**9**)<sup>16</sup> (0.40 g, 0.82 mmol) and **20** (0.48 g, 0.45 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (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  $\mu$ L, 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<sub>3</sub>N, 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-azidoheptyl 4-*O*-acetyl-2,6-di-*O*-benzyl- $\beta$ -D-galactopyranoside (**14**)<sup>14</sup> (56 mg, 0.11 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (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  $\mu$ L, 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<sub>3</sub>N, 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);  $[\alpha]_D^{20} +8^\circ$  ( $c$  1, CHCl<sub>3</sub>); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  1.23–1.25 (m, 4 H, 2 CH<sub>2</sub>), 1.39–1.43 (m, 2 H, CH<sub>2</sub>), 1.47–1.52 (m, 2 H, CH<sub>2</sub>), 1.95, 1.98, 2.02, 2.05, and 2.09 (5 s, each 3 H, 5 COCH<sub>3</sub>), 2.27 and 2.34 (2 s, each 3 H, 2 COC<sub>6</sub>H<sub>4</sub>CH<sub>3</sub>), 3.11 (t, 2 H, CH<sub>2</sub>N<sub>3</sub>), 3.38 (dd, 1 H,  $J_{1,2}$  7.8,  $J_{2,3}$  9.5 Hz, H-2<sup>I</sup>), 3.42 (m, 1 H, OCHH), 4.07 (dd, 1 H,  $J_{5,6b}$  4.4,  $J_{6a,6b}$  12.3 Hz, H-6b<sup>III</sup>), 4.10 and 4.55 (2 d, each 1 H, OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 4.36 (d, 1 H, H-1<sup>I</sup>), 4.44 (dd, 1 H,  $J_{1,2}$  8.2,  $J_{2,3}$  10.8 Hz, H-2<sup>II</sup>), 4.47 and 4.56 (2 d, each 1 H, OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 4.70 (d, 1 H,  $J_{1,2}$  8.0 Hz, H-1<sup>III</sup>), 4.95 (dd, 1 H,  $J_{2,3}$  9.6 Hz, H-2<sup>III</sup>), 5.01 (t, 1 H, H-4<sup>III</sup>), 5.21 (t, 1 H, H-3<sup>III</sup>), 5.36 (t, 1 H, H-4<sup>II</sup>), 5.55 (d, 1 H,  $J_{3,4}$  3.5,  $J_{4,5} < 1$  Hz, H-4<sup>I</sup>), 5.68 (d, 1 H, H-1<sup>II</sup>), 6.24 (dd, 1 H,  $J_{3,4}$  9.1 Hz, H-3<sup>II</sup>); <sup>13</sup>C NMR (75.5 MHz, CDCl<sub>3</sub>):  $\delta$  20.4–20.8 (COCH<sub>3</sub>), 21.4 and 21.5 (2 COC<sub>6</sub>H<sub>4</sub>CH<sub>3</sub>), 25.5, 26.3, 28.5, and 29.3 (4 CH<sub>2</sub>), 51.2 (CH<sub>2</sub>N<sub>3</sub>), 55.3 (C-2<sup>II</sup>), 61.5, 68.0 (2 C), 69.9, 73.4, and 73.9 (C-6<sup>I</sup>, C-6<sup>II</sup>, C-6<sup>III</sup>, 2 OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>, OCH<sub>2</sub>), 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<sup>I</sup>, C-3<sup>I</sup>, C-4<sup>I</sup>, C-5<sup>I</sup>, C-3<sup>II</sup>, C-4<sup>II</sup>, C-5<sup>II</sup>, C-2<sup>III</sup>, C-3<sup>III</sup>, C-4<sup>III</sup>, C-5<sup>III</sup>), 98.4, 100.9, and 103.6 (C-1<sup>I</sup>, C-1<sup>II</sup>, C-1<sup>III</sup>), 165.1 and 165.4 (2 COC<sub>6</sub>H<sub>4</sub>CH<sub>3</sub>), 169.1–170.5 (COCH<sub>3</sub>); HRMS of C<sub>72</sub>H<sub>80</sub>N<sub>4</sub>O<sub>24</sub> (M, 1384.516):  $[M + Na]^+$  found 1407.495, calcd 1407.506.

### 3.11. 6-Azidoheptyl (2,3,4,6-tetra-*O*-acetyl- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 6)-(2-acetamido-3,4-di-*O*-acetyl-2-deoxy- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 3)-4-*O*-acetyl-2,6-di-*O*-benzyl- $\beta$ -D-galactopyranoside (**22**)

To a soln of **21** (90 mg, 64.9  $\mu$ mol) in MeOH (8 mL) and CH<sub>2</sub>Cl<sub>2</sub> (2 mL) was added NaOMe (pH 10), and the mixture was stirred for 4 h, then neutralized with Dowex 50  $\times$  8 (H<sup>+</sup>), 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<sub>2</sub>Cl<sub>2</sub>. A soln of the residue in Py (30 mL) and Ac<sub>2</sub>O (30 mL) was stirred overnight, then co-concentrated with toluene, EtOH and CH<sub>2</sub>Cl<sub>2</sub>. 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);  $[\alpha]_D^{20} -8^\circ$  ( $c$  1, CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>; 2D TOCSY, ROESY):  $\delta$  1.32–1.39 (m, 4 H, 2 CH<sub>2</sub>), 1.47–1.54 (m, 2 H, CH<sub>2</sub>), 1.59–1.63 (m, 2 H, CH<sub>2</sub>), 1.59, 1.95, 1.96, 1.99, 2.06 and 2.07 (6 s, 3,3,3,6,3,6 H, 7 COCH<sub>3</sub>, NHCOCH<sub>3</sub>), 3.18 (t, 2 H, CH<sub>2</sub>N<sub>3</sub>), 3.63 (m, 1 H, H-5<sup>III</sup>), 3.71 (dd, 1 H,  $J_{5,6b}$  6.8,  $J_{6a,6b}$  12.3 Hz, H-6b<sup>II</sup>), 3.80 (dd, 1 H,  $J_{5,6a}$  1.5 Hz, H-6a<sup>II</sup>), 4.19 (dd, 1 H,  $J_{5,6b}$  4.2,  $J_{6a,6b}$  12.5 Hz, H-6b<sup>III</sup>), 4.43 (d, 1 H,  $J_{1,2}$  8.1 Hz, H-1<sup>III</sup>), 4.47 and 4.56 (2 d, each 1 H, OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 4.55 and 5.04 (2 d, each 1 H,

OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 4.65 (d, 1 H,  $J_{1,2}$  8.1 Hz, H-1<sup>I</sup>), 4.73 (d, 1 H,  $J_{1,2}$  8.3 Hz, H-1<sup>II</sup>), 4.83 (t, 1 H, H-4<sup>II</sup>), 4.89 (t, 1 H, H-2<sup>II</sup>), 4.99 (t, 1 H, H-2<sup>III</sup>), 5.09 (t, 1 H, H-4<sup>III</sup>), 5.25 (t, 1 H, H-3<sup>III</sup>), 5.47 (d, 1 H,  $J_{3,4}$  3.5,  $J_{4,5}$  < 1 Hz, H-4<sup>I</sup>); <sup>13</sup>C NMR (75.5 MHz, CDCl<sub>3</sub>): δ 20.4–20.7 (COCH<sub>3</sub>), 22.8 (NHCOCH<sub>3</sub>), 25.5, 26.3, 28.6, and 29.4 (4 CH<sub>2</sub>), 51.2 (CH<sub>2</sub>N<sub>3</sub>), 54.1 (C-2<sup>II</sup>), 61.5, 68.2 (2 C), 69.8, 73.4, and 74.4 (C-6<sup>I</sup>, C-6<sup>II</sup>, C-6<sup>III</sup>, 2 OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>, OCH<sub>2</sub>), 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<sup>I</sup>, C-3<sup>I</sup>, C-4<sup>I</sup>, C-5<sup>I</sup>, C-3<sup>II</sup>, C-4<sup>II</sup>, C-5<sup>II</sup>, C-2<sup>III</sup>, C-3<sup>III</sup>, C-4<sup>III</sup>, C-5<sup>III</sup>), 100.6, 101.2, and 103.4 (C-1<sup>I</sup>, C-1<sup>II</sup>, C-1<sup>III</sup>), 169.1–170.6 (COCH<sub>3</sub>); HRMS of C<sub>54</sub>H<sub>72</sub>N<sub>4</sub>O<sub>23</sub> (M, 1144.458): [M+NH<sub>4</sub>]<sup>+</sup> 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<sub>2</sub>Cl<sub>2</sub> (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<sup>+</sup>), 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<sub>3</sub>. The mixture was stirred for 3 h under H<sub>2</sub> after which NH<sub>3</sub> was removed by bubbling with N<sub>2</sub>, then 10% Pd–C (60 mg) and 3 drops of AcOH were added, and the stirring under H<sub>2</sub> was continued overnight. The mixture was loaded on a short Dowex 50 × 8 (H<sup>+</sup>) column, which was first eluted with water to remove contaminations, then with aq 10% NH<sub>4</sub>OH to give **5**, isolated as a white solid after lyophilization (19 mg, 69%);  $R_f$  0.34 (2:1:1 AcOH–1-BuOH–water); [α]<sub>D</sub><sup>20</sup> –2° (c 1, water); <sup>13</sup>C NMR (75.5 MHz, D<sub>2</sub>O): δ 22.9 (NDCOCH<sub>3</sub>), 25.3, 26.0, 27.9, and 29.2 (4 CH<sub>2</sub>), 40.3 (CH<sub>2</sub>ND<sub>2</sub>), 56.4 (C-2<sup>II</sup>), 61.5, 61.7, 69.4, and 71.1 (C-6<sup>I</sup>, C-6<sup>II</sup>, C-6<sup>III</sup>, OCH<sub>2</sub>), 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<sup>I</sup>, C-3<sup>I</sup>, C-4<sup>I</sup>, C-5<sup>I</sup>, C-3<sup>II</sup>, C-4<sup>II</sup>, C-5<sup>II</sup>, C-2<sup>III</sup>, C-3<sup>III</sup>, C-4<sup>III</sup>, C-5<sup>III</sup>), 103.3, 103.5, and 103.7 (C-1<sup>I</sup>, C-1<sup>II</sup>, C-1<sup>III</sup>), 175.7 (NDCOCH<sub>3</sub>); HRMS of C<sub>26</sub>H<sub>48</sub>N<sub>2</sub>O<sub>16</sub> (M, 644.300): [M+H]<sup>+</sup> found 645.306, calcd 645.308. For <sup>1</sup>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<sub>2</sub>, bovine serum albumin (BSA) (0.5 mg), and NaN<sub>3</sub> (0.02%), were added alkaline phosphatase (14 U), UDP-galactose (13 mg, 21.3 μmol), and β-1,4-Galacto-

syltransferase (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<sup>–</sup>) 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<sub>4</sub>HCO<sub>3</sub> 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); [α]<sub>D</sub><sup>20</sup> –3° (c 0.5, water); HRMS of C<sub>32</sub>H<sub>58</sub>N<sub>2</sub>O<sub>21</sub> (M, 806.353): [M+H]<sup>+</sup> found 807.352, calcd 807.361. For <sup>1</sup>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**)<sup>17</sup> (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**)<sup>14</sup> (0.62 g, 0.64 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (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<sub>3</sub>N, filtered over hyflo, washed with water, dried (MgSO<sub>4</sub>), 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); [α]<sub>D</sub><sup>20</sup> –2° (c 1, CHCl<sub>3</sub>); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 1.33–1.37 (m, 4 H, 2 CH<sub>2</sub>), 1.51–1.54 (m, 2 H, CH<sub>2</sub>), 1.58–1.60 (m, 2 H, CH<sub>2</sub>), 1.82, 2.02, and 2.06 (3 s, 3,6,3 H, 4 COCH<sub>3</sub>), 3.03 (m, 1 H, H-5<sup>I</sup>), 3.18 (t, 2 H, CH<sub>2</sub>N<sub>3</sub>), 3.49 (dd, 1 H,  $J_{5,6a}$  4.1,  $J_{6a,6b}$  11.0 Hz, H-6a<sup>I</sup>), 3.58 (dd, 1 H,  $J_{2,3}$  9.5,  $J_{3,4}$  3.5 Hz, H-3<sup>II</sup>), 3.87 (t, 1 H, H-4<sup>I</sup>), 4.15 (d, 1 H, OCHHC<sub>6</sub>H<sub>5</sub>), 4.41 (d, 1 H, OCHHC<sub>6</sub>H<sub>5</sub>), 4.48 (d, 1 H, OCHHC<sub>6</sub>H<sub>5</sub>), 4.65 and 4.88 (2 d, each 1 H, OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 4.67 and 4.81 (2 d, each 1 H, OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 5.16 (t, 1 H, H-4<sup>III</sup>), 5.38 (d, 1 H,  $J_{4,5}$  < 1 Hz, H-4<sup>II</sup>), 5.53 (d, 1 H,  $J_{1,2}$  8.3 Hz, H-1<sup>III</sup>), 5.78 (dd, 1 H,  $J_{2,3}$  10.7,  $J_{3,4}$  9.2 Hz, H-3<sup>III</sup>); <sup>13</sup>C NMR (75.5 MHz, CDCl<sub>3</sub>): δ 20.0–20.3 (COCH<sub>3</sub>), 25.3, 26.1, 28.4, and 29.2 (4 CH<sub>2</sub>), 50.9 (CH<sub>2</sub>N<sub>3</sub>), 54.6 (C-2<sup>III</sup>), 61.3, 67.4, 67.9, 69.3, 72.2, 73.1, 74.0, 74.5, and 74.7 (C-6<sup>I</sup>, C-6<sup>II</sup>, C-6<sup>III</sup>, 5 OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>, OCH<sub>2</sub>), 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<sup>I</sup>, C-3<sup>I</sup>, C-4<sup>I</sup>, C-5<sup>I</sup>, C-2<sup>II</sup>, C-3<sup>II</sup>, C-4<sup>II</sup>, C-5<sup>II</sup>, C-3<sup>III</sup>, C-4<sup>III</sup>, C-5<sup>III</sup>), 97.9, 101.4, and 103.1 (C-1<sup>I</sup>, C-1<sup>II</sup>, C-1<sup>III</sup>), 169.0, 169.3, 169.7, and 170.3 (4 COCH<sub>3</sub>); HRMS of C<sub>75</sub>H<sub>84</sub>N<sub>4</sub>O<sub>21</sub> (M, 1376.562): [M+NH<sub>4</sub>]<sup>+</sup> found: 1394.548, calcd 1394.597.

### 3.15. 6-Azidoheptyl (2-deoxy-2-phthalimido- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 3)-(4-O-acetyl-2,6-di-O-benzyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-2,3,6-tri-O-benzyl- $\beta$ -D-glucopyranoside (26)

To a soln of **25** (0.85 g, 0.62 mmol) in  $\text{CH}_2\text{Cl}_2$  (10 mL) and MeOH (20 mL) was added NaOMe (pH 8). The mixture was stirred for 3 h, then neutralized with Dowex 50  $\times$  8 ( $\text{H}^+$ ), filtered, and concentrated. Column chromatography (3:1  $\rightarrow$  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);  $[\alpha]_D^{20} +3^\circ$  ( $c$  1,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ; 2D TOCSY, ROESY):  $\delta$  1.33–1.36 (m, 4 H, 2  $\text{CH}_2$ ), 1.50–1.53 (m, 2 H,  $\text{CH}_2$ ), 1.57–1.60 (m, 2 H,  $\text{CH}_2$ ), 2.07 (s, 3 H,  $\text{COCH}_3$ ), 2.98 (m, 1 H,  $\text{H}-5^{\text{I}}$ ), 3.17 (t, 2 H,  $\text{CH}_2\text{N}_3$ ), 4.02 (dd, 1 H,  $J_{1,2}$  8.3,  $J_{2,3}$  10.8 Hz,  $\text{H}-2^{\text{III}}$ ), 4.08 and 4.21 (2 d, each 1 H,  $\text{OCH}_2\text{C}_6\text{H}_5$ ), 4.20 (d, 1 H,  $J_{1,2}$  8.1 Hz,  $\text{H}-1^{\text{I}}$ ), 4.22 and 4.42 (2 d, each 1 H,  $\text{OCH}_2\text{C}_6\text{H}_5$ ), 4.24 and 4.37 (2 d, each 1 H,  $\text{OCH}_2\text{C}_6\text{H}_5$ ), 4.31 (d, 1 H,  $J_{1,2}$  7.7 Hz,  $\text{H}-1^{\text{II}}$ ), 4.64 and 4.81 (2 d, each 1 H,  $\text{OCH}_2\text{C}_6\text{H}_5$ ), 4.66 and 4.87 (2 d, each 1 H,  $\text{OCH}_2\text{C}_6\text{H}_5$ ), 5.37 (d, 1 H,  $\text{H}-1^{\text{III}}$ ), 5.52 (d, 1 H,  $J_{3,4}$  3.5,  $J_{4,5} < 1$  Hz,  $\text{H}-4^{\text{II}}$ );  $^{13}\text{C}$  NMR (75.5 MHz,  $\text{CDCl}_3$ ):  $\delta$  20.9 ( $\text{COCH}_3$ ), 25.5, 26.3, 28.6, and 29.4 (4  $\text{CH}_2$ ), 51.2 ( $\text{CH}_2\text{N}_3$ ), 56.7 ( $\text{C}-2^{\text{III}}$ ), 61.0, 67.7, 67.8, 69.4, 72.9, 73.4, 74.1, 74.7, and 74.9 ( $\text{C}-6^{\text{I}}$ ,  $\text{C}-6^{\text{II}}$ ,  $\text{C}-6^{\text{III}}$ , 5  $\text{OCH}_2\text{C}_6\text{H}_5$ ,  $\text{OCH}_2$ ), 70.5 (2 C), 70.9, 71.9, 74.5, 75.7, 75.8, 78.4, 80.4, 81.5, and 82.5 ( $\text{C}-2^{\text{I}}$ ,  $\text{C}-3^{\text{I}}$ ,  $\text{C}-4^{\text{I}}$ ,  $\text{C}-5^{\text{I}}$ ,  $\text{C}-2^{\text{II}}$ ,  $\text{C}-3^{\text{II}}$ ,  $\text{C}-4^{\text{II}}$ ,  $\text{C}-5^{\text{II}}$ ,  $\text{C}-3^{\text{III}}$ ,  $\text{C}-4^{\text{III}}$ ,  $\text{C}-5^{\text{III}}$ ), 99.2, 101.7, and 103.4 ( $\text{C}-1^{\text{I}}$ ,  $\text{C}-1^{\text{II}}$ ,  $\text{C}-1^{\text{III}}$ ), 171.1 ( $\text{COCH}_3$ ); HRMS of  $\text{C}_{69}\text{H}_{78}\text{N}_4\text{O}_{18}$  (M, 1250.531):  $[\text{M} + \text{NH}_4]^+$  found 1268.537, calcd 1268.565.

### 3.16. 6-Azidoheptyl (6-O-*tert*-butyldiphenylsilyl-2-deoxy-2-phthalimido- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 3)-(4-O-acetyl-2,6-di-O-benzyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-2,3,6-tri-O-benzyl- $\beta$ -D-glucopyranoside (27)

To a soln of **26** (0.30 g, 0.24 mmol) in  $\text{CH}_2\text{Cl}_2$  (3 mL) and Py (0.2 mL) were added 4-dimethylaminopyridine (9 mg, 0.072 mmol),  $\text{Et}_3\text{N}$  (0.2 mL), and *tert*-butyldiphenylsilyl chloride (73  $\mu\text{L}$ , 0.29 mmol). The mixture was stirred for 20 h, then poured into ice water, extracted with  $\text{CH}_2\text{Cl}_2$ , washed with aq satd  $\text{NaHCO}_3$ , dried ( $\text{MgSO}_4$ ), 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);  $[\alpha]_D^{20} +8^\circ$  ( $c$  1,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ; 2D TOCSY, ROESY, HSQC):  $\delta$  1.04 [s, 9 H,  $\text{SiC}(\text{CH}_3)_3$ ], 1.33–1.38 (m, 4 H, 2  $\text{CH}_2$ ), 1.48–1.55 (m, 2 H,  $\text{CH}_2$ ), 1.58–1.59 (m, 2 H,  $\text{CH}_2$ ), 1.97 (s, 3 H,  $\text{COCH}_3$ ), 3.05 (m, 1 H,  $\text{H}-5^{\text{I}}$ ), 3.16 (t, 2 H,  $\text{CH}_2\text{N}_3$ ), 3.51 (t, 1 H,  $\text{H}-6\text{b}^{\text{I}}$ ), 3.63 (dd, 1 H,  $J_{2,3}$  9.8,  $J_{3,4}$  3.2 Hz,  $\text{H}-3^{\text{II}}$ ), 4.04 (dd, 1 H,  $J_{1,2}$  8.3,  $J_{2,3}$  11.0 Hz,  $\text{H}-2^{\text{III}}$ ), 4.12 (d, 1 H,  $\text{OCHHC}_6\text{H}_5$ ), 4.16 and 4.35 (2 d,

each 1 H,  $\text{OCH}_2\text{C}_6\text{H}_5$ ), 4.40 (m, 1 H,  $\text{H}-3^{\text{III}}$ ), 4.43 (d, 1 H,  $\text{OCHHC}_6\text{H}_5$ ), 4.62 and 4.87 (2 d, each 1 H,  $\text{OCH}_2\text{C}_6\text{H}_5$ ), 4.66 and 4.81 (2 d, each 1 H,  $\text{OCH}_2\text{C}_6\text{H}_5$ );  $^{13}\text{C}$  NMR (75.5 MHz,  $\text{CDCl}_3$ ):  $\delta$  19.0 [ $\text{SiC}(\text{CH}_3)_3$ ], 20.5 ( $\text{COCH}_3$ ), 25.5, 26.3, 28.5, and 29.4 (4  $\text{CH}_2$ ), 26.6 [ $\text{SiC}(\text{CH}_3)_3$ ], 51.1 ( $\text{CH}_2\text{N}_3$ ), 56.6 ( $\text{C}-2^{\text{III}}$ ), 64.8 ( $\text{C}-6^{\text{III}}$ ), 67.7 ( $\text{C}-6^{\text{I}}$ ), 68.1 ( $\text{C}-6^{\text{II}}$ ), 69.4 ( $\text{OCH}_2$ ), 70.1 ( $\text{C}-4^{\text{II}}$ ), 70.9 ( $\text{C}-3^{\text{III}}$ ), 72.5 ( $\text{C}-5^{\text{II}}$ ), 73.9 ( $\text{C}-4^{\text{III}}$ ), 74.5 ( $\text{C}-5^{\text{III}}$ ), 74.6 ( $\text{C}-5^{\text{I}}$ ), 72.9, 73.2, 74.2, 74.7, and 74.9 (5  $\text{OCH}_2\text{C}_6\text{H}_5$ ), 75.5 ( $\text{C}-4^{\text{I}}$ ), 77.2 ( $\text{C}-3^{\text{II}}$ ), 79.1 ( $\text{C}-2^{\text{II}}$ ), 81.4 ( $\text{C}-2^{\text{I}}$ ), 82.4 ( $\text{C}-3^{\text{I}}$ ), 98.3 ( $\text{C}-1^{\text{III}}$ ), 101.7 ( $\text{C}-1^{\text{II}}$ ), 103.3 ( $\text{C}-1^{\text{I}}$ ), 169.7 ( $\text{COCH}_3$ ); HRMS of  $\text{C}_{85}\text{H}_{96}\text{N}_4\text{O}_{18}$  (M, 1488.648):  $[\text{M} + \text{H}]^+$  found 1489.638, calcd 1489.657.

### 3.17. 6-Azidoheptyl (6-O-*tert*-butyldiphenylsilyl-2-deoxy-3,4-di-O-*p*-methylbenzoyl-2-phthalimido- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 3)-(4-O-acetyl-2,6-di-O-benzyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-2,3,6-tri-O-benzyl- $\beta$ -D-glucopyranoside (28)

To a soln of **27** (0.12 g, 0.083 mmol) in dry Py (5 mL) was added dropwise, at  $0^\circ\text{C}$ , a soln of *p*-methylbenzoyl chloride (27  $\mu\text{L}$ , 0.21 mmol) in dry  $\text{CH}_2\text{Cl}_2$  (1 mL). The mixture was stirred for 18 h at rt, then poured into ice water, extracted with  $\text{CH}_2\text{Cl}_2$ , washed with aq satd  $\text{NaHCO}_3$ , dried ( $\text{MgSO}_4$ ), 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);  $[\alpha]_D^{20} -3^\circ$  ( $c$  1,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ; 2D TOCSY, ROESY, HSQC):  $\delta$  1.06 [ $\text{SiC}(\text{CH}_3)_3$ ], 1.32–1.36 (m, 4 H, 2  $\text{CH}_2$ ), 1.48–1.51 (m, 2 H,  $\text{CH}_2$ ), 1.58–1.62 (m, 2 H,  $\text{CH}_2$ ), 2.10 ( $\text{COCH}_3$ ), 2.17 and 2.34 (2 s, each 3 H, 2  $\text{COC}_6\text{H}_4\text{CH}_3$ ), 3.13 (t, 2 H,  $\text{CH}_2\text{N}_3$ ), 3.16 (m, 1 H,  $\text{H}-5^{\text{I}}$ ), 3.43 (dd, 1 H,  $J_{1,2}$  8.3,  $J_{2,3}$  9.2 Hz,  $\text{H}-2^{\text{II}}$ ), 3.59 (m, 1 H,  $\text{H}-5^{\text{II}}$ ), 3.63 (dd, 1 H,  $J_{5,6\text{b}}$  3.5,  $J_{6\text{a},6\text{b}}$  10.8 Hz,  $\text{H}-6\text{b}^{\text{I}}$ ), 4.18 (d, 1 H,  $\text{OCHHC}_6\text{H}_5$ ), 4.24 (d, 1 H,  $\text{OCHHC}_6\text{H}_5$ ), 4.31 (d, 1 H,  $J_{1,2}$  8.3 Hz,  $\text{H}-1^{\text{I}}$ ), 4.32 and 4.52 (2 d, each 1 H,  $\text{OCH}_2\text{C}_6\text{H}_5$ ), 4.58 (dd, 1 H,  $J_{1,2}$  8.3,  $J_{2,3}$  10.7 Hz,  $\text{H}-2^{\text{III}}$ ), 4.73 and 4.87 (2 d, each 1 H,  $\text{OCH}_2\text{C}_6\text{H}_5$ ), 4.69 and 4.97 (2 d, each 1 H,  $\text{OCH}_2\text{C}_6\text{H}_5$ ), 5.81 (d, 1 H,  $\text{H}-1^{\text{III}}$ ), 6.37 (t, 1 H,  $\text{H}-3^{\text{III}}$ );  $^{13}\text{C}$  NMR (75.5 MHz,  $\text{CDCl}_3$ ):  $\delta$  18.7 [ $\text{SiC}(\text{CH}_3)_3$ ], 20.3 ( $\text{COCH}_3$ ), 21.0 and 21.3 ( $\text{COC}_6\text{H}_4\text{CH}_3$ ), 25.3, 26.0, 28.3, and 29.2 (4  $\text{CH}_2$ ), 26.3 [ $\text{SiC}(\text{CH}_3)_3$ ], 50.9 ( $\text{CH}_2\text{N}_3$ ), 55.2 ( $\text{C}-2^{\text{III}}$ ), 62.6 ( $\text{C}-6^{\text{III}}$ ), 67.4 ( $\text{C}-6^{\text{I}}$ ), 67.9 ( $\text{C}-6^{\text{II}}$ ), 69.2 ( $\text{OCH}_2$ ), 69.5 ( $\text{C}-4^{\text{III}}$ ), 70.1 ( $\text{C}-4^{\text{II}}$ ), 70.6 ( $\text{C}-3^{\text{III}}$ ), 72.5 ( $\text{C}-5^{\text{II}}$ ), 72.7, 73.0, 74.1, 74.4, and 74.5 (5  $\text{OCH}_2\text{C}_6\text{H}_5$ ), 74.5 ( $\text{C}-5^{\text{I}}$ ), 75.2 (2 C) ( $\text{C}-4^{\text{I}}$ ,  $\text{C}-5^{\text{III}}$ ), 77.0 ( $\text{C}-3^{\text{II}}$ ), 78.9 ( $\text{C}-2^{\text{II}}$ ), 81.3 ( $\text{C}-2^{\text{I}}$ ), 82.2 ( $\text{C}-3^{\text{I}}$ ), 98.2 ( $\text{C}-1^{\text{III}}$ ), 101.6 ( $\text{C}-1^{\text{II}}$ ), 103.1 ( $\text{C}-1^{\text{I}}$ ), 164.6 and 165.2 (2  $\text{COC}_6\text{H}_4\text{CH}_3$ ), 169.2 ( $\text{COCH}_3$ ); HRMS of  $\text{C}_{101}\text{H}_{108}\text{N}_4\text{O}_{20}\text{Si}$  (M, 1724.736):  $[\text{M} + \text{H}]^+$  found 1725.741, calcd 1725.740.

**3.18. 6-Azidoheptyl (2-deoxy-3,4-di-*O*-*p*-methylbenzoyl-2-phthalimido- $\beta$ -D-glucopyranosyl)-(1  $\rightarrow$  3)-(4-*O*-acetyl-2,6-di-*O*-benzyl- $\beta$ -D-galactopyranosyl)-(1  $\rightarrow$  4)-2,3,6-tri-*O*-benzyl- $\beta$ -D-glucopyranoside (29)**

A soln of **28** (0.10 g, 60.2  $\mu$ mol) 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<sub>2</sub>Cl<sub>2</sub>, the soln was washed with water and aq satd NaCl, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. Column chromatography (4:1 toluene–EtOAc) of the residue gave **29**, isolated as a colourless syrup (80 mg, 89%); *R*<sub>f</sub> 0.34 (4:1 toluene–EtOAc);  $[\alpha]_D^{20}$   $-2^\circ$  (*c* 1, CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>; 2D TOCSY, ROESY):  $\delta$  1.31–1.33 (m, 4 H, 2 CH<sub>2</sub>), 1.49–1.52 (m, 2 H, CH<sub>2</sub>), 1.55–1.59 (m, 2 H, CH<sub>2</sub>), 2.14 (s, 3 H, COCH<sub>3</sub>), 2.25 and 2.33 (2 s, each 1 H, 2 COC<sub>6</sub>H<sub>4</sub>CH<sub>3</sub>), 2.95 (m, 1 H, H-5<sup>I</sup>), 3.18 (t, 2 H, CH<sub>2</sub>N<sub>3</sub>), 3.55 (dd, 1 H, *J*<sub>2,3</sub> 9.6, *J*<sub>3,4</sub> 3.5 Hz, H-3<sup>II</sup>), 3.66 (dd, 1 H, *J*<sub>5,6b</sub> 5.5, *J*<sub>6a,6b</sub> 10.8 Hz, H-6b<sup>III</sup>), 3.81 (m, 1 H, OCHH), 3.85 (t, 1 H, H-4<sup>I</sup>), 3.92 (m, 1 H, H-5<sup>III</sup>), 4.18 and 4.23 (2 d, each 1 H, OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 4.19 (d, 1 H, *J*<sub>1,2</sub> 7.7 Hz, H-1<sup>I</sup>), 4.23 and 4.45 (2 d, each 1 H, OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 4.29 and 4.38 (2 d, each 1 H, OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 4.34 (d, 1 H, *J*<sub>1,2</sub> 7.7 Hz, H-1<sup>II</sup>), 4.49 (dd, 1 H, *J*<sub>1,2</sub> 8.5, *J*<sub>2,3</sub> 10.6 Hz, H-2<sup>III</sup>), 4.66 and 4.81 (2 d, each 1 H, OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 4.67 and 4.90 (2 d, each 1 H, OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 5.49 (t, 1 H, H-4<sup>III</sup>), 5.59 (d, 1 H, *J*<sub>4,5</sub> < 1 Hz, H-4<sup>II</sup>), 5.70 (d, 1 H, H-1<sup>III</sup>), 6.16 (dd, 1 H, *J*<sub>3,4</sub> 9.4 Hz, H-3<sup>III</sup>); <sup>13</sup>C NMR (75.5 MHz, CDCl<sub>3</sub>):  $\delta$  20.3 (COCH<sub>3</sub>), 21.0 and 21.5 (COC<sub>6</sub>H<sub>4</sub>CH<sub>3</sub>), 25.6, 26.4, 28.6, and 29.4 (4 CH<sub>2</sub>), 51.2 (CH<sub>2</sub>N<sub>3</sub>), 55.0 (C-2<sup>III</sup>), 61.3, 67.7, 68.0, 69.5, 72.9, 73.5, 74.2, 74.7, and 74.8 (C-6<sup>I</sup>, C-6<sup>II</sup>, C-6<sup>III</sup>, 5 OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>, OCH<sub>2</sub>), 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<sup>I</sup>, C-3<sup>I</sup>, C-4<sup>I</sup>, C-5<sup>I</sup>, C-2<sup>II</sup>, C-3<sup>II</sup>, C-4<sup>II</sup>, C-5<sup>II</sup>, C-3<sup>III</sup>, C-4<sup>III</sup>, C-5<sup>III</sup>), 99.3, 101.7, and 103.4 (C-1<sup>I</sup>, C-1<sup>II</sup>, C-1<sup>III</sup>), 165.2 and 165.5 (COC<sub>6</sub>H<sub>4</sub>CH<sub>3</sub>), 171.0 (COCH<sub>3</sub>); HRMS of C<sub>85</sub>H<sub>90</sub>N<sub>4</sub>O<sub>20</sub> (M, 1486.614):  $[M+H]^+$  found 1487.621, calcd 1487.623.

**3.19. 6-Azidoheptyl (2,3,4,6-tetra-*O*-acetyl- $\beta$ -D-glucopyranosyl)-(1  $\rightarrow$  6)-(2-deoxy-3,4-di-*O*-*p*-methylbenzoyl-2-phthalimido- $\beta$ -D-glucopyranosyl)-(1  $\rightarrow$  3)-(4-*O*-acetyl-2,6-di-*O*-benzyl- $\beta$ -D-galactopyranosyl)-(1  $\rightarrow$  4)-2,3,6-tri-*O*-benzyl- $\beta$ -D-glucopyranoside (30)**

(a) A soln of **13** (0.27 g, 0.26 mmol) and **24**<sup>14</sup> (0.23 g, 0.24 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (8 mL), containing 4 Å molecular sieves (0.2 g), was stirred under Ar for 0.5 h. After cooling to  $-70^\circ\text{C}$ , TMSOTf (5  $\mu$ L, 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<sub>3</sub>N, filtered, washed with water, dried (MgSO<sub>4</sub>), 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**<sup>16</sup> (36.5 mg, 74.2  $\mu$ mol) and **29** (65 mg, 43.6  $\mu$ mol) in dry CH<sub>2</sub>Cl<sub>2</sub> (1 mL), containing 4 Å molecular sieves (40 mg), was stirred under Ar for 0.5 h. After cooling to  $-40^\circ\text{C}$ , TMSOTf (1.4  $\mu$ L, 7.7  $\mu$ mol) 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<sub>3</sub>N, filtered, washed with water, dried (MgSO<sub>4</sub>), filtered, and concentrated. Column chromatography (3:1 toluene–EtOAc) of the residue gave **30**, isolated as a colourless syrup (46 mg, 58%); *R*<sub>f</sub> 0.56 (2:1 toluene–EtOAc);  $[\alpha]_D^{20}$   $+1^\circ$  (*c* 1, CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>; 2D TOCSY, ROESY):  $\delta$  1.34–1.37 (m, 4 H, 2 CH<sub>2</sub>), 1.52–1.55 (m, 2 H, CH<sub>2</sub>), 1.60–1.63 (m, 2 H, CH<sub>2</sub>), 1.85, 1.94, 2.00, 2.01, and 2.11 (5 s, each 3 H, 5 COCH<sub>3</sub>), 2.25 and 2.33 (2 s, each 3 H, 2 COC<sub>6</sub>H<sub>4</sub>CH<sub>3</sub>), 3.17 (m, 1 H, H-5<sup>I</sup>), 3.18 (t, 2 H, CH<sub>2</sub>N<sub>3</sub>), 3.37 (dd, 1 H, *J*<sub>1,2</sub> 8.1, *J*<sub>2,3</sub> 9.7 Hz, H-2<sup>II</sup>), 3.52 (ddd, 1 H, *J*<sub>4,5</sub> 10.1, *J*<sub>5,6a</sub> 2.4, *J*<sub>5,6b</sub> 4.2 Hz, H-5<sup>IV</sup>), 4.09 and 4.38 (2 d, each 1 H, OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 4.24 and 4.35 (2 d, each 1 H, OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 4.27 (d, 1 H, *J*<sub>1,2</sub> 7.7 Hz, H-1<sup>I</sup>), 4.28 and 4.50 (2 d, each 1 H, OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 4.64 and 4.89 (2 d, each 1 H, OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 4.68 (d, 1 H, *J*<sub>1,2</sub> 8.1 Hz, H-1<sup>IV</sup>), 4.69 and 4.84 (2 d, each 1 H, OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 4.94 (dd, 1 H, *J*<sub>2,3</sub> 9.9 Hz, H-2<sup>IV</sup>), 4.98 (t, 1 H, H-3<sup>IV</sup>), 5.19 (t, 1 H, H-4<sup>IV</sup>), 5.40 (dd, 1 H, *J*<sub>4,5</sub> 9.9, *J*<sub>3,4</sub> 9.2 Hz, H-4<sup>III</sup>), 5.59 (d, 1 H, *J*<sub>3,4</sub> 3.7, *J*<sub>4,5</sub> < 1 Hz, H-4<sup>II</sup>), 5.68 (d, 1 H, *J*<sub>1,2</sub> 8.1 Hz, H-1<sup>III</sup>), 6.28 (dd, 1 H, *J*<sub>2,3</sub> 10.8 Hz, H-3<sup>III</sup>); <sup>13</sup>C NMR (75.5 MHz, CDCl<sub>3</sub>):  $\delta$  20.3, 20.4, 20.5, and 20.6 (2 C) (5 COCH<sub>3</sub>), 21.4 (2 C) (2 COC<sub>6</sub>H<sub>4</sub>CH<sub>3</sub>), 25.6, 26.3, 28.6, and 29.4 (4 CH<sub>2</sub>), 51.2 (CH<sub>2</sub>N<sub>3</sub>), 55.3 (C-2<sup>III</sup>), 61.4, 67.4, 67.9, 68.2, 69.5, 72.8, 73.2, 74.4, 74.7, and 75.1 (C-6<sup>I</sup>, C-6<sup>II</sup>, C-6<sup>III</sup>, C-6<sup>IV</sup>, 5 OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>, OCH<sub>2</sub>), 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<sup>I</sup>, C-3<sup>I</sup>, C-4<sup>I</sup>, C-5<sup>I</sup>, C-2<sup>II</sup>, C-3<sup>II</sup>, C-4<sup>II</sup>, C-5<sup>II</sup>, C-3<sup>III</sup>, C-4<sup>III</sup>, C-5<sup>III</sup>, C-2<sup>IV</sup>, C-3<sup>IV</sup>, C-4<sup>IV</sup>, C-5<sup>IV</sup>), 98.4, 101.0, 102.1, and 103.3 (C-1<sup>I</sup>, C-1<sup>II</sup>, C-1<sup>III</sup>, C-1<sup>IV</sup>), 165.1 and 165.3 (COC<sub>6</sub>H<sub>4</sub>CH<sub>3</sub>), 169.0–169.8 (COCH<sub>3</sub>); HRMS of C<sub>99</sub>H<sub>108</sub>N<sub>4</sub>O<sub>29</sub> (M, 1816.709):  $[M+NH_4]^+$  found 1834.719, calcd 1834.743.

**3.20. 6-Azidoheptyl (2,3,4,6-tetra-*O*-acetyl- $\beta$ -D-glucopyranosyl)-(1  $\rightarrow$  6)-(2-acetamido-3,4-di-*O*-acetyl-2-deoxy- $\beta$ -D-glucopyranosyl)-(1  $\rightarrow$  3)-(4-*O*-acetyl-2,6-di-*O*-benzyl- $\beta$ -D-galactopyranosyl)-(1  $\rightarrow$  4)-2,3,6-tri-*O*-benzyl- $\beta$ -D-glucopyranoside (31)**

To a soln of **30** (0.20 g, 0.11 mmol) in MeOH (3.5 mL) and CH<sub>2</sub>Cl<sub>2</sub> (1.5 mL) was added NaOMe (pH 10), and the mixture was stirred for 4 h, then neutralized with Dowex 50  $\times$  8 (H<sup>+</sup>), 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<sub>2</sub>Cl<sub>2</sub>. A soln of the residue in Py (30 mL) and Ac<sub>2</sub>O (30 mL) was stirred for 4 h, then co-concentrated with toluene, EtOH, and CH<sub>2</sub>Cl<sub>2</sub>. Column chromatography (1:1 toluene–EtOAc) of the residue gave **31**, isolated as a colourless glass (0.16 g, 88%); *R<sub>f</sub>* 0.45 (1:3 toluene–EtOAc); [ $\alpha$ ]<sub>D</sub><sup>20</sup> – 5° (*c* 1, CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>; 2D TOCSY, ROESY):  $\delta$  1.36–1.38 (m, 4 H, 2 CH<sub>2</sub>), 1.52–1.56 (m, 2 H, CH<sub>2</sub>), 1.63–1.65 (m, 2 H, CH<sub>2</sub>), 1.91, 1.94, 1.95, 1.98, 2.00, 2.04, 2.07, and 2.12 (8 s, each 3 H, 7 COCH<sub>3</sub>, NHCOCH<sub>3</sub>), 3.19 (t, 2 H, CH<sub>2</sub>N<sub>3</sub>), 3.73 (dd, 1 H, *J*<sub>5,6b</sub> 7.5, *J*<sub>6a,6b</sub> 11.6 Hz, H-6b<sup>III</sup>), 3.95 (t, 1 H, H-4<sup>I</sup>), 3.99 (dd, 1 H, *J*<sub>5,6a</sub> 1.8, *J*<sub>6a,6b</sub> 12.3 Hz, H-6a<sup>IV</sup>), 4.16 (dd, 1 H, *J*<sub>5,6b</sub> 4.2 Hz, H-6b<sup>IV</sup>), 4.27 and 4.48 (2 d, each 1 H, OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 4.34 (d, 1 H, *J*<sub>1,2</sub> 7.7 Hz, H-1<sup>I</sup>), 4.42 and 4.55 (2 d, each 1 H, OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 4.55 (d, 1 H, *J*<sub>1,2</sub> 7.7 Hz, H-1<sup>II</sup>), 4.56 (d, 1 H, OCHHC<sub>6</sub>H<sub>5</sub>), 4.63 (d, 1 H, *J*<sub>1,2</sub> 7.9 Hz, H-1<sup>IV</sup>), 5.21 (t, 1 H, H-3<sup>IV</sup>), 5.48 (d, 1 H, *J*<sub>3,4</sub> 3.5, *J*<sub>4,5</sub> < 1 Hz, H-4<sup>II</sup>), 7.14–7.37 (m, 25 H, 5 OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>); <sup>13</sup>C NMR (75.5 MHz, CDCl<sub>3</sub>):  $\delta$  20.3–20.5 (COCH<sub>3</sub>), 22.6 (NHCOCH<sub>3</sub>), 25.4, 26.2, 28.5, 29.3 (4 CH<sub>2</sub>), 51.0 (CH<sub>2</sub>N<sub>3</sub>), 54.1 (C-2<sup>III</sup>), 61.4, 67.5, 67.9, 68.1, 69.4, 73.0, 73.5, 74.6 (2 C), and 74.9 (C-6<sup>I</sup>, C-6<sup>II</sup>, C-6<sup>III</sup>, C-6<sup>IV</sup>, 5 OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>, OCH<sub>2</sub>), 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<sup>I</sup>, C-3<sup>I</sup>, C-4<sup>I</sup>, C-5<sup>I</sup>, C-2<sup>II</sup>, C-3<sup>II</sup>, C-4<sup>II</sup>, C-5<sup>II</sup>, C-3<sup>III</sup>, C-4<sup>III</sup>, C-5<sup>III</sup>, C-2<sup>IV</sup>, C-3<sup>IV</sup>, C-4<sup>IV</sup>, C-5<sup>IV</sup>), 100.6, 101.2, 101.8, and 103.2 (C-1<sup>I</sup>, C-1<sup>II</sup>, C-1<sup>III</sup>, C-1<sup>IV</sup>), 168.9–170.5 (COCH<sub>3</sub>, NHCOCH<sub>3</sub>); HRMS of C<sub>81</sub>H<sub>100</sub>N<sub>4</sub>O<sub>28</sub> (M, 1576.652): [M+NH<sub>4</sub>]<sup>+</sup> found 1594.647, calcd 1594.686.

### 3.21. 6-Aminohexyl $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 6)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 3)- $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-glucopyranoside (**6**)

To a soln of **31** (155 mg, 98.2  $\mu$ mol) in CH<sub>2</sub>Cl<sub>2</sub> (4 mL) and MeOH (2 mL) was added NaOMe (pH 10). The mixture was stirred for 2 h, then neutralized with Dowex 50  $\times$  8 (H<sup>+</sup>), 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<sub>3</sub>. The mixture was stirred for 3 h under H<sub>2</sub> after which NH<sub>3</sub> was removed by bubbling with N<sub>2</sub>, then 10% Pd–C (100 mg) and 3 drops of AcOH were added, and the stirring under H<sub>2</sub> was continued overnight. The mixture was loaded on a short Dowex 50  $\times$  8 (H<sup>+</sup>) column, which was first eluted with water to remove contaminations, then with aq 10% NH<sub>4</sub>OH to give **6**, isolated as a white solid after lyophilization (65 mg, 81%); *R<sub>f</sub>* 0.26 (2:1:1 AcOH–1-BuOH–water); [ $\alpha$ ]<sub>D</sub><sup>20</sup> – 2° (*c* 1, water); <sup>13</sup>C NMR (75.5 MHz, D<sub>2</sub>O):  $\delta$  23.6 (NDCOCH<sub>3</sub>), 26.0, 26.7, 28.5, and 29.9 (4 CH<sub>2</sub>), 41.0 (CH<sub>2</sub>ND<sub>2</sub>), 57.1 (C-2<sup>III</sup>), 61.6, 62.2, 62.5, 70.2, and 71.9 (C-6<sup>I</sup>, C-6<sup>II</sup>, C-6<sup>III</sup>, C-6<sup>IV</sup>, OCH<sub>2</sub>), 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<sup>I</sup>, C-3<sup>I</sup>, C-4<sup>I</sup>, C-5<sup>I</sup>, C-

2<sup>II</sup>, C-3<sup>II</sup>, C-4<sup>II</sup>, C-5<sup>II</sup>, C-3<sup>III</sup>, C-4<sup>III</sup>, C-5<sup>III</sup>, C-2<sup>IV</sup>, C-3<sup>IV</sup>, C-4<sup>IV</sup>, C-5<sup>IV</sup>), 103.4, 104.2, 104.3, and 104.4 (C-1<sup>I</sup>, C-1<sup>II</sup>, C-1<sup>III</sup>, C-1<sup>IV</sup>), 176.3 (NDCOCH<sub>3</sub>); HRMS of C<sub>32</sub>H<sub>58</sub>N<sub>2</sub>O<sub>21</sub> (M, 806.353): [M+H]<sup>+</sup> found 807.367, calcd 807.361. For <sup>1</sup>H NMR data, see Table 3.

### 3.22. 6-Aminohexyl $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 6)- $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 4)]-2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 3)- $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-glucopyranoside (**2**)

To a soln of **6** (11.4 mg, 14.12  $\mu$ mol) in aq 50 mM sodium cacodylate buffer pH 7.5 (700  $\mu$ L), containing 5 mM MnCl<sub>2</sub>, BSA (0.5 mg), and NaN<sub>3</sub> (0.02%), were added alkaline phosphatase (14 U), UDP-galactose (12 mg, 19.66  $\mu$ mol), and  $\beta$ -1,4-galactosyltransferase (3 U). The mixture was incubated for 20 h at 37 °C then water (100  $\mu$ L) was added. UDP-Galactose was removed using a Dowex 1  $\times$  8 (Cl<sup>–</sup>) 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<sub>4</sub>HCO<sub>3</sub> 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<sub>f</sub>* 0.23 (2:1:1 AcOH–1-BuOH–water); [ $\alpha$ ]<sub>D</sub><sup>20</sup> – 1° (*c* 0.5, water); HRMS of C<sub>38</sub>H<sub>68</sub>N<sub>2</sub>O<sub>26</sub> (M, 968.406): [M+H]<sup>+</sup> found 969.418, calcd 969.414. For <sup>1</sup>H NMR data, see Table 4.

### 3.23. (3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 3)-(2,4,6-tri-*O*-acetyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-1,2,3,6-tetra-*O*-acetyl- $\alpha$ , $\beta$ -D-glucopyranose (**35**)

To a soln of (3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido- $\beta$ -D-glucopyranosyl)-(1  $\rightarrow$  3)-(4-*O*-acetyl-2,6-di-*O*-benzyl- $\beta$ -D-galactopyranosyl)-(1  $\rightarrow$  4)-1,2,3,6-tetra-*O*-benzyl- $\beta$ -D-glucopyranose (**34**)<sup>15</sup> (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<sub>2</sub> for 8 h, then filtered over hyflo, and concentrated. Column chromatography (7:1 CH<sub>2</sub>Cl<sub>2</sub>–MeOH) of the residue gave a white solid which was dissolved in Py (75 mL) and Ac<sub>2</sub>O (75 mL), and the soln was stirred overnight, then co-concentrated with toluene, EtOH and CH<sub>2</sub>Cl<sub>2</sub>. Low-pressure column chromatography (7:1  $\rightarrow$  1:1 toluene–EtOAc) of the residue gave **35**, isolated as a white foam (0.95 g, 66%); *R<sub>f</sub>* 0.45 (1:2 toluene–EtOAc); [ $\alpha$ ]<sub>D</sub><sup>20</sup> – 17° (*c* 1, CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>; 2D TOCSY, ROESY):  $\delta$  4.46 (t, 1 H, H-6b<sup>III</sup>), 4.80 (m, 1 H, H-2<sup>II</sup>), 4.93 (dd, 0.5 H, *J*<sub>1,2</sub> 3.6, *J*<sub>2,3</sub> 10.3 Hz, H-2<sup>I $\alpha$</sup> ), 4.98 (t, 0.5 H, H-2<sup>I $\beta$</sup> ), 5.13 (t, 0.5 H, H-3<sup>I $\beta$</sup> ), 5.18 (m, 1 H, H-4<sup>III</sup>), 5.63 (d, 0.5 H, *J*<sub>1,2</sub> 8.3 Hz, H-1<sup>I $\beta$</sup> ), 5.74 (m, 1 H, H-3<sup>III</sup>), 6.19 (d, 0.5 H, H-1<sup>I $\alpha$</sup> ), 7.77–7.84 (m, 4 H, Phth); HRMS of C<sub>46</sub>H<sub>55</sub>NO<sub>27</sub> (M, 1053.296): [M+NH<sub>4</sub>]<sup>+</sup> found 1071.329, calcd 1071.330.

**3.24. (3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido- $\beta$ -D-glucopyranosyl)-(1  $\rightarrow$  3)-(2,4,6-tri-*O*-acetyl- $\beta$ -D-galactopyranosyl)-(1  $\rightarrow$  4)-2,3,6-tri-*O*-acetyl- $\alpha$ -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  $\text{CH}_2\text{Cl}_2$ . Low-pressure column chromatography (3:1  $\rightarrow$  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  $\text{CH}_2\text{Cl}_2$  (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  $\rightarrow$  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);  $[\alpha]_D^{20} - 8^\circ$  ( $c$  1,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ; 2D TOCSY, ROESY):  $\delta$  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  $\text{COCH}_3$ ), 3.96 (m, 1 H, H-5<sup>I</sup>), 4.14 (dd, 1 H,  $J_{1,2}$  8.3,  $J_{2,3}$  11.0 Hz, H-2<sup>III</sup>), 4.30 (d, 1 H,  $J_{1,2}$  8.1 Hz, H-1<sup>II</sup>), 4.36 (dd, 1 H,  $J_{5,6a}$  1.8,  $J_{6a,6b}$  12.1 Hz, H-6a<sup>I</sup>), 4.51 (dd, 1 H,  $J_{5,6a}$  2.6,  $J_{6a,6b}$  12.3 Hz, H-6a<sup>III</sup>), 4.81 (dd, 1 H,  $J_{2,3}$  9.9 Hz, H-2<sup>II</sup>), 5.00 (dd, 1 H,  $J_{1,2}$  3.5,  $J_{2,3}$  10.1 Hz, H-2<sup>I</sup>), 5.19 (dd, 1 H,  $J_{3,4}$  9.2,  $J_{4,5}$  9.9 Hz, H-4<sup>III</sup>), 5.41 (t, 1 H, H-3<sup>I</sup>), 5.76 (dd, 1 H, H-3<sup>III</sup>), 6.43 (d, 1 H, H-1<sup>I</sup>), 7.73–7.75 (m, 4 H, Phth), 8.65 (s, 1 H,  $\text{OC}(\text{NH})\text{CCl}_3$ );  $^{13}\text{C}$  NMR (75.5 MHz,  $\text{CDCl}_3$ ):  $\delta$  20.3–20.6 ( $\text{COCH}_3$ ), 54.5 (C-2<sup>III</sup>), 60.8, 61.5, and 62.0 (C-6<sup>I</sup>, C-6<sup>II</sup>, C-6<sup>III</sup>), 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<sup>I</sup>, C-3<sup>I</sup>, C-4<sup>I</sup>, C-5<sup>I</sup>, C-2<sup>II</sup>, C-3<sup>II</sup>, C-4<sup>II</sup>, C-5<sup>II</sup>, C-3<sup>III</sup>, C-4<sup>III</sup>, C-5<sup>III</sup>), 92.8, 97.6, and 100.6 (C-1<sup>I</sup>, C-1<sup>II</sup>, C-1<sup>III</sup>), 161.8 ( $\text{OC}(\text{NH})\text{CCl}_3$ ), 168.5–171.6 ( $\text{COCH}_3$ ).

**3.25. 6-Azidohexyl (3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido- $\beta$ -D-glucopyranosyl)-(1  $\rightarrow$  3)-(2,4,6-tri-*O*-acetyl- $\beta$ -D-galactopyranosyl)-(1  $\rightarrow$  4)-2,3,6-tri-*O*-acetyl- $\beta$ -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  $\text{CH}_2\text{Cl}_2$  (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  $\text{Et}_3\text{N}$ , the mixture was filtered over hyflo, washed with water, dried ( $\text{MgSO}_4$ ), 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);  $[\alpha]_D^{20} + 2^\circ$  ( $c$  1,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.26–1.40 (m, 6 H, 3  $\text{CH}_2$ ), 1.52–1.60 (m, 2 H,  $\text{CH}_2$ ), 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  $\text{COCH}_3$ ), 3.24 (t, 2 H,  $\text{CH}_2\text{N}_3$ ), 3.67 (t, 1 H, H-4<sup>I</sup>), 4.09 (dd, 1 H,  $J_{5,6b}$  3.5,  $J_{6a,6b}$  12.3 Hz, H-6b<sup>III</sup>), 4.14 (dd, 1 H,  $J_{1,2}$  8.3,  $J_{2,3}$  10.9 Hz, H-2<sup>III</sup>), 4.26 (d, 1 H,  $J_{1,2}$  8.0 Hz, H-1<sup>II</sup>), 4.48 (dd, 1 H,  $J_{5,6a}$  2.6 Hz, H-6a<sup>III</sup>), 5.06 (dd, 1 H, H-3<sup>I</sup>), 5.19 (dd, 1 H,  $J_{3,4}$  9.1 Hz, H-4<sup>III</sup>), 5.74 (dd, 1 H, H-3<sup>III</sup>), 7.72–7.75 and 7.79–7.81 (2 m, each 2 H, Phth);  $^{13}\text{C}$  NMR (75.5 MHz,  $\text{CDCl}_3$ ):  $\delta$  20.3–20.6 ( $\text{COCH}_3$ ), 25.3, 26.3, 28.6, and 29.1 (4  $\text{CH}_2$ ), 51.2 ( $\text{CH}_2\text{N}_3$ ), 54.5 (C-2<sup>III</sup>), 60.8, 61.5, 62.0, and 69.7 (C-6<sup>I</sup>, C-6<sup>II</sup>, C-6<sup>III</sup>,  $\text{OCH}_2$ ), 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<sup>I</sup>, C-3<sup>I</sup>, C-4<sup>I</sup>, C-5<sup>I</sup>, C-2<sup>II</sup>, C-3<sup>II</sup>, C-4<sup>II</sup>, C-5<sup>II</sup>, C-3<sup>III</sup>, C-4<sup>III</sup>, C-5<sup>III</sup>), 97.6 and 100.4 (2 C) (C-1<sup>I</sup>, C-1<sup>II</sup>, C-1<sup>III</sup>), 168.5–171.6 ( $\text{COCH}_3$ ); HRMS of  $\text{C}_{50}\text{H}_{64}\text{N}_4\text{O}_{26}$  (M, 1136.380):  $[\text{M} + \text{Na}]^+$  found 1159.358, calcd 1159.370.

**3.26. 6-Azidohexyl (2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- $\beta$ -D-glucopyranosyl)-(1  $\rightarrow$  3)-(2,4,6-tri-*O*-acetyl- $\beta$ -D-galactopyranosyl)-(1  $\rightarrow$  4)-2,3,6-tri-*O*-acetyl- $\beta$ -D-glucopyranoside (39)**

To a soln of **38** (22 mg, 21  $\mu\text{mol}$ ) in  $\text{CH}_2\text{Cl}_2$  (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  $\times$  8 ( $\text{H}^+$ ), 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  $\text{CH}_2\text{Cl}_2$ . A soln of the residue in Py (10 mL) and  $\text{Ac}_2\text{O}$  (10 mL) was stirred overnight, then co-concentrated with toluene, EtOH, and  $\text{CH}_2\text{Cl}_2$ . Column chromatography (3:1  $\text{CH}_2\text{Cl}_2$ –acetone) of the residue gave **39**, isolated as a white solid (17 mg, 77%);  $R_f$  0.22 (3:1  $\text{CH}_2\text{Cl}_2$ –acetone);  $[\alpha]_D^{20} + 6^\circ$  ( $c$  1,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ; 2D TOCSY, ROESY):  $\delta$  1.25–1.28 (m, 4 H, 2  $\text{CH}_2$ ), 1.33–1.36 (m, 2 H,  $\text{CH}_2$ ), 1.57–1.59 (m, 2 H,  $\text{CH}_2$ ), 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 H, 9  $\text{COCH}_3$ ,  $\text{NHCOCH}_3$ ), 3.46 (m, 1 H,  $\text{OCHH}$ ), 3.59 (m, 1 H, H-5<sup>I</sup>), 3.66 (m, 1 H, H-5<sup>III</sup>), 4.87 (dd, 1 H,  $J_{1,2}$  8.1,  $J_{2,3}$  9.6 Hz, H-2<sup>I</sup>), 5.16 (t, 1 H, H-3<sup>I</sup>), 5.32 (d, 1 H,  $J_{3,4}$  2.4,  $J_{4,5} < 1$  Hz, H-4<sup>II</sup>), 5.47 (t, 1 H, H-3<sup>III</sup>), 5.60 (d, 1 H,  $J_{\text{NH},2}$  7.5 Hz,  $\text{NHCOCH}_3$ );  $^{13}\text{C}$  NMR (75.5 MHz,  $\text{CDCl}_3$ ):  $\delta$  20.5–20.8 ( $\text{NHCOCH}_3$ ,  $\text{COCH}_3$ ), 25.3, 26.3, 28.6, and 29.2 (4  $\text{CH}_2$ ), 51.2 ( $\text{CH}_2\text{N}_3$ ), 56.1 (C-2<sup>III</sup>), 61.1, 61.5, 62.1, and 69.7 (C-6<sup>I</sup>, C-6<sup>II</sup>, C-6<sup>III</sup>,  $\text{OCH}_2$ ), 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<sup>I</sup>, C-3<sup>I</sup>, C-4<sup>I</sup>, C-5<sup>I</sup>, C-2<sup>II</sup>, C-3<sup>II</sup>, C-4<sup>II</sup>, C-5<sup>II</sup>, C-3<sup>III</sup>, C-4<sup>III</sup>, C-5<sup>III</sup>), 99.5, 100.5, and 100.6 (C-1<sup>I</sup>, C-1<sup>II</sup>, C-1<sup>III</sup>), 168.5–171.4 ( $\text{COCH}_3$ ,  $\text{NHCOCH}_3$ ); HRMS of  $\text{C}_{44}\text{H}_{64}\text{N}_4\text{O}_{25}$  (M, 1048.386):  $[\text{M} + \text{Na}]^+$  found 1071.397, calcd 1071.375.



### 3.27. 6-Azidohexyl 2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 3)- $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-glucopyranoside (40)

To a soln of **39** (15 mg, 14  $\mu$ mol) in  $\text{CH}_2\text{Cl}_2$  (0.5 mL) and MeOH (2 mL) was added NaOMe (pH 10). The mixture was stirred for 2 h, then neutralized with Dowex 50  $\times$  8 ( $\text{H}^+$ ), filtered, and concentrated. Column chromatography (1:1  $\text{CH}_2\text{Cl}_2$ –MeOH) of the residue gave **40**, isolated as a white solid (5.3 mg, 55%);  $R_f$  0.81 (1:2  $\text{CH}_2\text{Cl}_2$ –MeOH);  $[\alpha]_{\text{D}}^{20} -4^\circ$  ( $c$  0.5, water); HRMS data of  $\text{C}_{26}\text{H}_{46}\text{N}_4\text{O}_{16}$  (M, 670.290):  $[\text{M}+\text{H}]^+$  found 671.300, calcd 671.302. For  $^1\text{H}$  NMR data, see Table 5.

### 3.28. 6-Azidohexyl (2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 3)-(4-*O*-acetyl-2,6-di-*O*-benzyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-2,3,6-tri-*O*-benzyl- $\beta$ -D-glucopyranoside (41)

To a soln of **25** (0.21 g, 0.15 mmol) in MeOH (4 mL) and  $\text{CH}_2\text{Cl}_2$  (2 mL) was added NaOMe (pH 10), and the mixture was stirred for 3 h, then neutralized with Dowex 50  $\times$  8 ( $\text{H}^+$ ), 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  $^\circ\text{C}$ , then co-concentrated with toluene, EtOH, and  $\text{CH}_2\text{Cl}_2$ . A soln of the residue in Py (30 mL) and  $\text{Ac}_2\text{O}$  (30 mL) was stirred for 48 h, then co-concentrated with toluene, EtOH, and  $\text{CH}_2\text{Cl}_2$ . 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);  $[\alpha]_{\text{D}}^{20} -2^\circ$  ( $c$  1,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ; 2D TOCSY, ROESY):  $\delta$  1.36–1.42 (m, 4 H, 2  $\text{CH}_2$ ), 1.51–1.56 (m, 2 H,  $\text{CH}_2$ ), 1.61–1.64 (m, 2 H,  $\text{CH}_2$ ), 1.51, 1.94, 1.99, 2.02, and 2.05 (5 s, each 3 H, 4  $\text{COCH}_3$ ,  $\text{NHCOCH}_3$ ), 3.18 (t, 2 H,  $\text{CH}_2\text{N}_3$ ), 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,  $\text{OCH}_2\text{C}_6\text{H}_5$ ), 4.33 (d, 1 H,  $J_{1,2}$  7.9 Hz, H-1 $^I$ ), 4.38 (d, 1 H,  $\text{OCHHC}_6\text{H}_5$ ), 4.49 (d, 1 H,  $J_{1,2}$  7.7 Hz, H-1 $^{II}$ ), 4.72 and 4.86 (2 d, each 1 H,  $\text{OCH}_2\text{C}_6\text{H}_5$ ), 4.74 and 4.98 (2 d, each 1 H,  $\text{OCH}_2\text{C}_6\text{H}_5$ ), 5.01 (t, 1 H, H-4 $^{III}$ ), 5.09 (t, 1 H, H-3 $^{III}$ ), 5.25 (d, 1 H,  $J_{2,\text{NH}}$  9.2 Hz,  $\text{NHCOCH}_3$ ), 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  $\text{OCH}_2\text{C}_6\text{H}_5$ );  $^{13}\text{C}$  NMR (75.5 MHz,  $\text{CDCl}_3$ ):  $\delta$  20.3–20.5 ( $\text{COCH}_3$ ), 22.5 ( $\text{NHCOCH}_3$ ), 25.4, 26.2, 28.5, and 29.3 (4  $\text{CH}_2$ ), 51.0 ( $\text{CH}_2\text{N}_3$ ), 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  $\text{OCH}_2\text{C}_6\text{H}_5$ ,  $\text{OCH}_2$ ), 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  $\text{COCH}_3$ ,  $\text{NHCOCH}_3$ ); HRMS of  $\text{C}_{69}\text{H}_{84}\text{N}_4\text{O}_{20}$  (M, 1288.567):  $[\text{M}+\text{NH}_4]^+$  found 1306.629, calcd 1306.600.

### 3.29. 6-Aminohexyl 2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 3)- $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-glucopyranoside (7)

(a) To a soln of **41** (75 mg, 58.5  $\mu$ mol) in  $\text{CH}_2\text{Cl}_2$  (1 mL) and MeOH (2 mL) was added NaOMe (pH 10). The mixture was stirred for 1.5 h, then neutralized with Dowex 50  $\times$  8 ( $\text{H}^+$ ), 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%  $\text{NH}_3$ . The mixture was stirred for 3 h under  $\text{H}_2$  after which  $\text{NH}_3$  was removed by bubbling with  $\text{N}_2$ , then 10% Pd–C (100 mg) and 3 drops of AcOH were added, and the stirring under  $\text{H}_2$  was continued overnight. The mixture was loaded on a short Dowex 50  $\times$  8 ( $\text{H}^+$ ) column, which was first eluted with water to remove contaminations, then with aq 10%  $\text{NH}_4\text{OH}$  to give **7**, isolated as a white solid after lyophilization (25 mg, 68%).

(b) To a soln of **40** (5.3 mg, 7.90  $\mu$ mol) in *tert*-BuOH (3 mL) and water (2 mL) was added 10% Pd–C (40 mg) and 2 drops of aq 25%  $\text{NH}_3$ . The mixture was stirred for 20 h under  $\text{H}_2$ , after which  $\text{NH}_3$  was removed by bubbling with  $\text{N}_2$ , then filtered over cotton and concentrated. Chromatography of the residue on a Bio-Gel P-2 column eluted with 0.1 M  $\text{NH}_4\text{HCO}_3$ , 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);  $[\alpha]_{\text{D}}^{20} -2^\circ$  ( $c$  1, water);  $^{13}\text{C}$  NMR (75.5 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  23.0 ( $\text{NDCOCH}_3$ ), 25.4, 26.1, 28.3, and 29.3 (4  $\text{CH}_2$ ), 40.4 ( $\text{CH}_2\text{ND}_2$ ), 56.5 (C-2 $^{III}$ ), 60.9, 61.3, 61.7, and 71.2 (C-6 $^I$ , C-6 $^{II}$ , C-6 $^{III}$ ,  $\text{OCH}_2$ ), 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 ( $\text{NDCOCH}_3$ ); HRMS of  $\text{C}_{26}\text{H}_{48}\text{N}_2\text{O}_{16}$  (M, 644.300):  $[\text{M}+\text{H}]^+$  found 645.311, calcd 645.308. For  $^1\text{H}$  NMR data, see Table 6.

### 3.30. 6-Aminohexyl $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 4)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 3)- $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-glucopyranoside (3)

To a soln of **7** (8.2 mg, 12.71  $\mu$ mol) in aq 50 mM sodium cacodylate buffer pH 7.5 (600  $\mu$ L), containing 5 mM  $\text{MnCl}_2$ , BSA (0.5 mg), and  $\text{NaN}_3$  (0.02%), were added alkaline phosphatase (14 U), UDP-galactose (11.5 mg, 18.84  $\mu$ mol), and  $\beta$ -1,4-galactosyltransferase (2.5 U). The mixture was incubated for 20 h at 37  $^\circ\text{C}$  then water (200  $\mu$ L) was added. UDP-Galactose was removed using a Dowex 1  $\times$  8 ( $\text{Cl}^-$ ) 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  $\text{NH}_4\text{HCO}_3$  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);  $[\alpha]_D^{20} -1^\circ$  ( $c$  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- $\beta$ -D-glucopyranosyl)-(1  $\rightarrow$  3)-(2,4,6-tri-*O*-acetyl- $\beta$ -D-galactopyranosyl)-(1  $\rightarrow$  4)-(2,3,6-tri-*O*-acetyl- $\beta$ -D-glucopyranosyl)-(1  $\rightarrow$  6)-2-deoxy-3,4-di-*O*-*p*-methylbenzoyl-2-phthalimido- $\beta$ -D-glucopyranoside (**44**)**

To a soln of **37** (100 mg, 87  $\mu$ mol) and **43** (70 mg, 104  $\mu$ mol) in dry  $CH_2Cl_2$  (3 mL), containing 4 Å molecular sieves (200 mg), was added, under Ar at 0 °C, TMSOTf (2.5  $\mu$ L, 12.96  $\mu$ mol). 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  $\rightarrow$  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);  $[\alpha]_D^{20} -11^\circ$  ( $c$  1,  $CHCl_3$ );  $^1H$  NMR (500 MHz,  $CDCl_3$ ; 2D TOCSY, ROESY):  $\delta$  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<sup>II</sup>), 4.09 (dd, 1 H,  $J_{5,6b}$  3.1,  $J_{6a,6b}$  12.1 Hz, H-6b<sup>IV</sup>), 4.14 (dd, 1 H,  $J_{1,2}$  8.3,  $J_{2,3}$  10.8 Hz, H-2<sup>IV</sup>), 4.24 (d, 1 H,  $J_{1,2}$  8.1 Hz, H-1<sup>III</sup>), 4.57 (d, 1 H,  $J_{1,2}$  7.9 Hz, H-1<sup>II</sup>), 4.78 (dd, 1 H,  $J_{2,3}$  9.9 Hz, H-2<sup>III</sup>), 4.85 (dd, 1 H,  $J_{2,3}$  9.2 Hz, H-2<sup>II</sup>), 5.02 (t, 1 H, H-3<sup>II</sup>), 5.19 (t, 1 H, H-4<sup>IV</sup>), 5.44 (d, 1 H,  $J_{1,2}$  8.5 Hz, H-1<sup>I</sup>), 5.75 (dd, 1 H,  $J_{3,4}$  9.2 Hz, H-3<sup>IV</sup>), 6.16 (dd, 1 H,  $J_{2,3}$  10.7,  $J_{3,4}$  9.2 Hz, H-3<sup>I</sup>);  $^{13}C$  NMR (75.5 MHz,  $CDCl_3$ ):  $\delta$  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<sup>I</sup>, C-2<sup>IV</sup>), 60.8, 61.5, 62.0, 68.2, and 69.6 (C-6<sup>I</sup>, C-6<sup>II</sup>, C-6<sup>III</sup>, C-6<sup>IV</sup>,  $OCH_2$ ), 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<sup>I</sup>, C-4<sup>I</sup>, C-5<sup>I</sup>, C-2<sup>II</sup>, C-3<sup>II</sup>, C-4<sup>II</sup>, C-5<sup>II</sup>, C-2<sup>III</sup>, C-3<sup>III</sup>, C-4<sup>III</sup>, C-5<sup>III</sup>, C-3<sup>IV</sup>, C-4<sup>IV</sup>, C-5<sup>IV</sup>), 97.5, 98.0, and 100.5 (2 C) (C-1<sup>I</sup>, C-1<sup>II</sup>, C-1<sup>III</sup>, C-1<sup>IV</sup>), 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- $\beta$ -D-glucopyranosyl)-(1  $\rightarrow$  3)-(2,4,6-tri-*O*-acetyl- $\beta$ -D-galactopyranosyl)-(1  $\rightarrow$  4)-(2,3,6-tri-*O*-acetyl- $\beta$ -D-glucopyranosyl)-(1  $\rightarrow$  6)-2-acetamido-3,4-di-*O*-acetyl-2-deoxy- $\beta$ -D-glucopyranoside (**45**)**

To a soln of **44** (56 mg, 63  $\mu$ mol) 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  $\times$  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);  $[\alpha]_D^{20} -10^\circ$  ( $c$  1,  $CHCl_3$ );  $^1H$  NMR (300 MHz,  $CDCl_3$ ):  $\delta$  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,  $OCHH$ ), 4.12 (dd, 1 H,  $J_{5,6b}$  5.1,  $J_{6a,6b}$  11.7 Hz, H-6b<sup>II</sup>), 4.44 (dd, 1 H,  $J_{5,6a} < 1$  Hz, H-6a<sup>II</sup>), 4.56 (d, 1 H,  $J_{1,2}$  7.8 Hz, H-1<sup>II</sup>), 4.61 (d, 1 H,  $J_{1,2}$  8.3 Hz, H-1<sup>I</sup>), 5.12 (t, 1 H, H-3<sup>II</sup>), 5.24 (t, 1 H, H-3<sup>I</sup>), 5.32 (d, 1 H,  $J_{3,4}$  3.5,  $J_{4,5} < 1$  Hz, H-4<sup>III</sup>);  $^{13}C$  NMR (75.5 MHz,  $CDCl_3$ ):  $\delta$  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<sup>I</sup>, C-2<sup>IV</sup>), 61.0, 61.5, 61.9, 68.2, and 69.4 (C-6<sup>I</sup>, C-6<sup>II</sup>, C-6<sup>III</sup>, C-6<sup>IV</sup>,  $OCH_2$ ), 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<sup>I</sup>, C-4<sup>I</sup>, C-5<sup>I</sup>, C-2<sup>II</sup>, C-3<sup>II</sup>, C-4<sup>II</sup>, C-5<sup>II</sup>, C-2<sup>III</sup>, C-3<sup>III</sup>, C-4<sup>III</sup>, C-5<sup>III</sup>, C-3<sup>IV</sup>, C-4<sup>IV</sup>, C-5<sup>IV</sup>), 99.5, 100.4 (2 C), and 100.6 (C-1<sup>I</sup>, C-1<sup>II</sup>, C-1<sup>III</sup>, C-1<sup>IV</sup>), 169.0–170.7 ( $COCH_3$ ,  $NHCOCH_3$ ); 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- $\beta$ -D-glucopyranosyl-(1  $\rightarrow$  3)- $\beta$ -D-galactopyranosyl-(1  $\rightarrow$  4)- $\beta$ -D-glucopyranosyl-(1  $\rightarrow$  6)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranoside (**46**)**

To a soln of **45** (48 mg, 35.9  $\mu$ mol) 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  $\times$  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);  $[\alpha]_D^{20} -6^\circ$  ( $c$  1, water);  $^{13}C$  NMR (75.5 MHz,  $D_2O$ ):  $\delta$  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<sup>I</sup>, C-2<sup>IV</sup>), 60.3, 60.7, 61.1, 68.8, and 70.7 (C-6<sup>I</sup>, C-6<sup>II</sup>, C-6<sup>III</sup>, C-6<sup>IV</sup>,  $OCH_2$ ), 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<sup>I</sup>, C-4<sup>I</sup>, C-5<sup>I</sup>, C-2<sup>II</sup>, C-3<sup>II</sup>, C-4<sup>II</sup>, C-5<sup>II</sup>, C-2<sup>III</sup>, C-3<sup>III</sup>, C-4<sup>III</sup>, C-5<sup>III</sup>, C-3<sup>IV</sup>, C-4<sup>IV</sup>, C-5<sup>IV</sup>), 101.3, 102.8, 103.0, and 103.1 (C-1<sup>I</sup>, C-1<sup>II</sup>, C-1<sup>III</sup>, C-1<sup>IV</sup>), 174.6 and 175.1 (2  $NHCOCH_3$ ); 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- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 3)- $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 6)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranoside (8)

To a soln of **46** (10 mg, 11.44  $\mu$ mol) in *tert*-BuOH (2 mL) and water (2 mL) was added 10% Pd–C (50 mg) and 1 drop of aq 25% NH<sub>3</sub>. The mixture was stirred for 20 h under H<sub>2</sub>, then acidified with Dowex 50  $\times$  8 (H<sup>+</sup>) (pH 4) and loaded on a short column of Dowex 50  $\times$  8 (H<sup>+</sup>). Elution with water, to remove contaminants, followed by 10% NH<sub>4</sub>OH, and subsequent lyophilization yielded **8**, isolated as a white solid (8 mg, 82%); *R*<sub>f</sub> 0.27 (2:1:1 AcOH–1-BuOH–water); [ $\alpha$ ]<sub>D</sub><sup>20</sup> –2° (*c* 0.5, water); <sup>13</sup>C NMR (75.5 MHz, D<sub>2</sub>O):  $\delta$  22.8 (2 C) (NDCOCH<sub>3</sub>), 25.3, 25.9, 27.4, and 29.0 (4 CH<sub>2</sub>), 40.1 (CH<sub>2</sub>ND<sub>2</sub>), 56.2 and 56.3 (C-2<sup>I</sup>, C-2<sup>IV</sup>), 60.7, 61.2, 61.6, 69.3, and 71.2 (C-6<sup>I</sup>, C-6<sup>II</sup>, C-6<sup>III</sup>, C-6<sup>IV</sup>, OCH<sub>2</sub>), 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<sup>I</sup>, C-4<sup>I</sup>, C-5<sup>I</sup>, C-2<sup>II</sup>, C-3<sup>II</sup>, C-4<sup>II</sup>, C-5<sup>II</sup>, C-2<sup>III</sup>, C-3<sup>III</sup>, C-4<sup>III</sup>, C-5<sup>III</sup>, C-3<sup>IV</sup>, C-4<sup>IV</sup>, C-5<sup>IV</sup>), 101.9, 103.3, 103.5, and 103.6 (C-1<sup>I</sup>, C-1<sup>II</sup>, C-1<sup>III</sup>, C-1<sup>IV</sup>), 175.1 and 175.6 (2 NDCOCH<sub>3</sub>); HRMS of C<sub>34</sub>H<sub>61</sub>N<sub>3</sub>O<sub>21</sub> (M, 847.379): [M+H]<sup>+</sup> found 848.390, calcd 848.387. For <sup>1</sup>H NMR data, see Table 9.

### 3.35. 6-Aminohexyl $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 4)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 3)- $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 6)- $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 4)]-2-acetamido-2-deoxy- $\beta$ -D-glucopyranoside (4)

To a soln of **8** (10.8 mg, 12.73  $\mu$ mol) in aq 50 mM sodium cacodylate buffer pH 7.5 (600  $\mu$ L), containing 5 mM MnCl<sub>2</sub>, BSA (0.5 mg), and NaN<sub>3</sub> (0.02%), were added alkaline phosphatase (30 U), UDP-galactose (25 mg, 40.96  $\mu$ mol), and  $\beta$ -1,4-galactosyltransferase (5 U). The mixture was incubated for 20 h at 37 °C, then water (200  $\mu$ L) was added. UDP-Galactose was removed using a Dowex 1  $\times$  8 (Cl<sup>–</sup>) 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<sub>4</sub>HCO<sub>3</sub> at a flow rate of 40 mL/h. The appropriate fractions were freeze-dried to give **4** (11.4 mg, 76%); *R*<sub>f</sub> 0.15 (2:1:1 AcOH–1-BuOH–water); [ $\alpha$ ]<sub>D</sub><sup>20</sup> –1° (*c* 1, water); HRMS of C<sub>46</sub>H<sub>81</sub>N<sub>3</sub>O<sub>31</sub> (M, 1171.485): [M+H]<sup>+</sup> found 1172.503, calcd 1172.492. For <sup>1</sup>H NMR data, see Table 10.

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