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Note

Synthesis of a fluorescence-labeled K30 antigen repeating unit using click chemistry

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Abstract—An *N*-dansyl-labeled K30 antigen repeating unit, {4-[5-(*N*,*N*′-dimethylamino)naphthalene-1-sulfonamine]-1*H*-1,2,3-triazol-1-yl}hexyl β-D-glucopyranosyluronate-(1→3)-α-D-galactopyranosyl-α-D-mannopyranosyl-(1→3)-β-D-galactopyranoside, was synthesized using click chemistry, the copper(I)-catalyzed 1,3-dipolar cycloaddition reaction of an azide and an alkyne. The target compound could further facilitate the studies of interactions among K30 oligosaccharides and proteins.

Keywords: Glycosylation; Click chemistry; K30 Antigen; Fluorescence; Thioglycosides

Bacterial cells are capable of secreting a range of large molecules, including both complex polysaccharides and proteins. Some of these macromolecules are required for cell viability, whereas others are involved in interactions between a bacterial pathogen and its host. Among these cell-surface polysaccharides, K30 antigen[1] is an interesting one. The K30 capsular antigen is a member of the group I, or heat-stable, capsules.[2] It may be responsible for resistance against phagocytosis.[3] Unlike most of the K-antigen, formation of the K30 capsule in *Escherichia coli* does not require attachment to a lipopolysaccharide lipid A-core.[4] A mutation in *wza* (K30), an integral membrane lipoprotein, severely restricts the formation of the K30 capsular structure on the cell surface, but does not interfere with biosynthesis or polymerization of the K30 repeating unit.[5]

Generally, carbohydrates do have not effective absorption and fluorescence for sensitive detection. Furthermore, the available amounts in most analyses are minute, and the direct detection of weak interaction between carbohydrate chains and their conjugates in real samples is very difficult.[6] Fluorescence-labeling of enzyme substrates is a valuable tool, as it enables the sensitive detection of the small quantities of material involved in an enzymatic assay.[7] This technique has been used in this capacity for single sugar glycosyltransferase systems.[8]

In a collaborative project for an investigation into the biological functions of the K30 antigen, we were required to prepare a fluorescence-labeled K30 repeating unit, compound **1**, as shown in Figure 1.

Initially, we thought the α-linkage between sugar units II and III (Scheme 1) could be prepared based on our previous results.[9] Accordingly, disaccharide donor A and acceptor B were prepared. We expected that the β-bond in donor A, although it had an acetyl group for neighboring-group participation at C-2, would predominantly give a 1,2-cis outcome in glycosylation with acceptor B.[10] Unfortunately, the desired α product was obtained in less than 30% yield, and we had to turn our attention on a new strategy. We describe herein a step-by-step, but more efficient synthesis of a fluorescence-labeled K30 repeating unit.

As outlined in Scheme 2, building blocks isopropyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (**3**), 6-azidohexyl 2-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranoside (**8**), isopropyl 2,4,6-tri-*O*-acetyl-3-*O*-benzyl-1-thio-α-D-mannopyranoside (**9**), and

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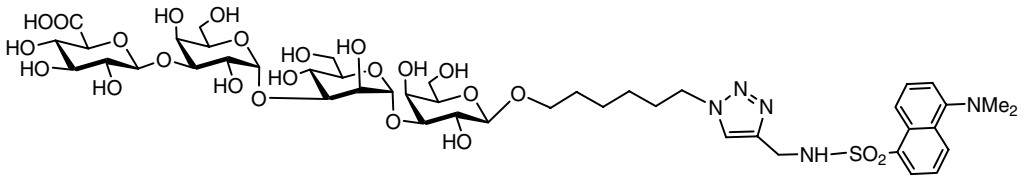
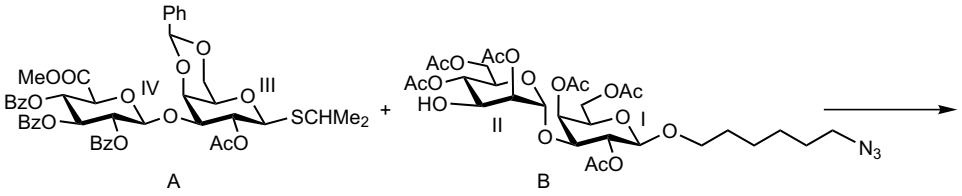
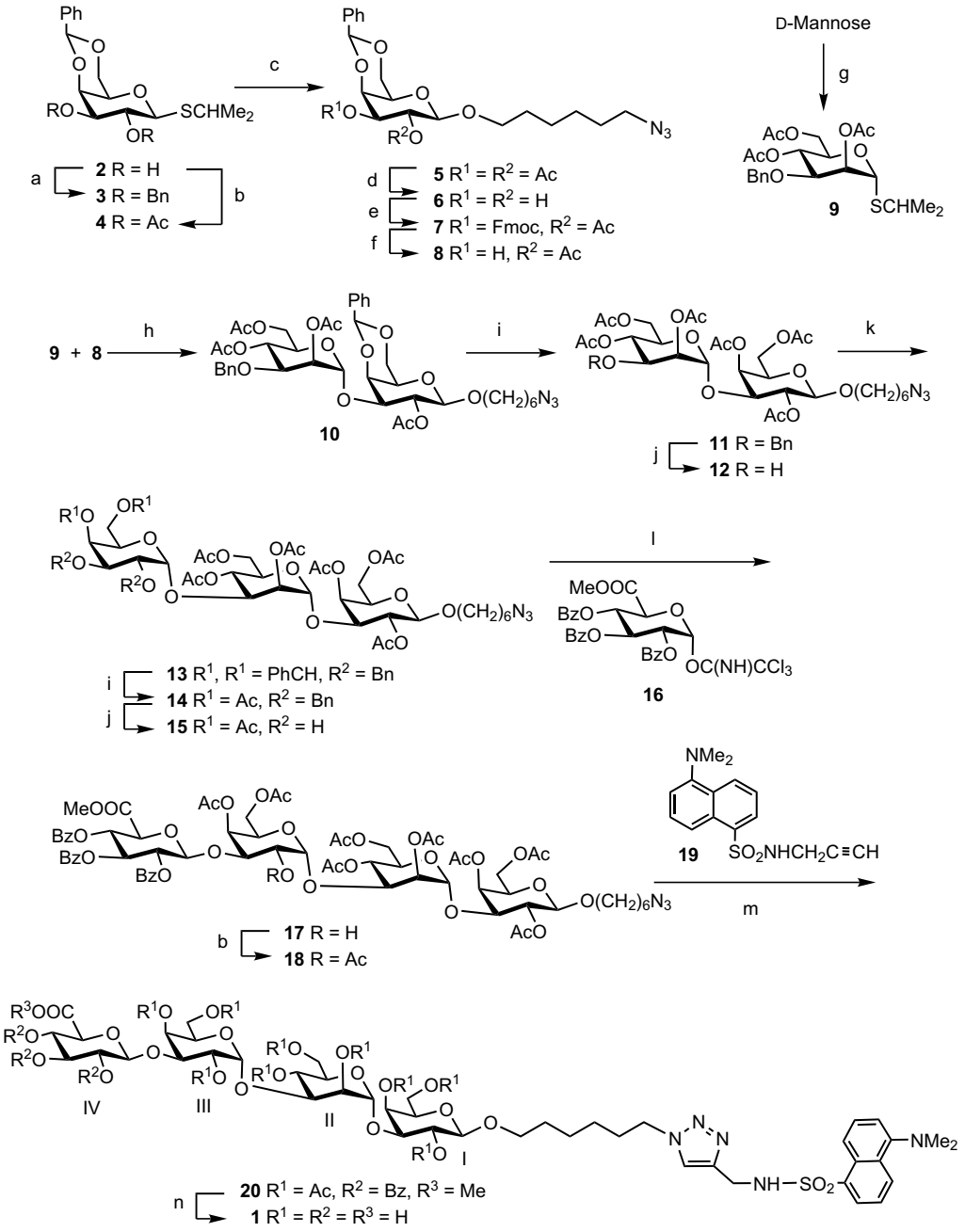


Figure 1. Structure of a fluorescence-labeled K30 repeating unit, compound **1**.



Scheme 1. Attempted preparation of K30 tetrasaccharide.

methyl 2,3,4-tri-*O*-benzoyl-α-D-glucuronosyl trichloroacetimidate (**16**)[11] were selected for the synthesis of target compound **1**. Thus, isopropyl 4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (**2**)[12] was benzylated with benzyl bromide and sodium hydride in DMF at 0 °C obtaining synthon **3** in a yield of 95%. Selection of benzyl protecting group on C-2 is to favor the α-glycoside bond formation in the ongoing coupling reaction. Meanwhile, **2** was acetylated with acetic anhydride in pyridine to give isopropyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (**4**), which was reacted with 6-azido-1-hexanol[13] in the presence of NIS and TMSOTf in CH_2Cl_2 at −20 °C for 4 h affording 6-azidohexyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranoside (**5**) in good yield (85%). Deacetylation of **5** (→**6**) with NaOMe in MeOH, followed by regioselective protection of C-3 with 9-fluorenylmethyloxycarbonyl chloride (FmocCl) in pyridine,[14] and acetylation of the C_2–OH with Ac_2O in a single step gave 6-azidohexyl 2-*O*-acetyl-3-*O*-(9-fluorenylmethyloxycarbonyl)-4,6-*O*-benzylidene-β-D-galactopyranoside (**7**). Removal of Fmoc from **7** with triethylamine in dichloromethane at rt afforded synthon **8** in an overall yield of 83% for four steps. Isopropyl 2,4,6-tri-*O*-acetyl-3-*O*-benzyl-1-thio-α-D-mannopyranoside (**9**) was prepared according to the literature procedures.[15] Coupling of **9** and **8** in dry CH_2Cl_2 in the presence of TMSOTf and NIS at −20 °C for 30 min afforded 6-azidohexyl 2,4,6-tri-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranoside (**10**). Hydrolysis of 4,6-*O*-benzylidene of **10** with 80% aqueous acetic acid, followed by acetylation with Ac_2O in pyridine, gave 6-azidohexyl 2,4,6-tri-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (**11**) in 72% yield for three steps. $NaBrO_3/Na_2S_2O_4$ catalyzed debenzylation[16] under two-phase reaction conditions carried out smoothly to generate disaccharide acceptor, 6-azidohexyl 2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (**12**, 80%). To favor the α glycosyl bond as required in the target molecule, donor **3** was prepared and condensed with **12**, using the same method as described in the preparation of **10**, to form 6-azidohexyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (**13**) in 78% isolated yield. Debenzylidenation and acetylation of **13** as described in the preparation of **11**, followed by debenzylation, afforded trisaccharide acceptor 6-azidohexyl 4,6-di-*O*-acetyl-α-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (**15**, 70% yield over three steps). Regioselective glycosylation of **15** and **16** in dry dichloromethane at −20 °C, in the presence of a catalytic amount of TMSOTf, gave tetrasaccharide 6-azidohexyl (methyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyluronate)-(1→3)-2,4,6-tri-*O*-acetyl-α-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galacopyranoside (**17**) in 89% yield. The desired (1→3)-linkage of **17** was further confirmed by 2D NMR spectral analysis of its acetylated compound **18**, showing a downfield-shifted doublet of doublets at δ 4.86 ppm ($J_{1,2}$ 3.7 Hz, $J_{2,3}$ 10.4 Hz) corresponding to the H-2^{III} unit. On the basis of the copper(I)-catalyzed 1,3-dipolar cycloaddition reaction of the azide and alkyne,[17] **18** was condensed with 5-(dimethylamino)-*N*-(2-propynyl)-1-naphthalene-sulfonamide (**19**)[18] to obtain {4-[5-(*N*,*N*′-dimethylamino)naphthalene-1-sulfonamine]-1*H*-1,2,3-triazol-1-yl}hexyl (methyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyluronate)-(1→3)-2,4,6-tri-*O*-acetyl-α-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (**20**) in 90% yield. Global deprotection of **20** in 0.5 N NaOH



Scheme 2. Regents and conditions: (a) BnBr, NaH, DMF, 95%; (b) Ac_2O, Py; (c) 6-azido-hexanol, NIS, TMSOTf, CH_2Cl_2, −20 °C, 85%; (d) NaOMe, MeOH; (e) (i) FmocCl, Py, overnight; (ii) Ac_2O, Py; (f) Et_3N, CH_2Cl_2, 83% for four steps; (g) (i) Ac_2O, Py; (ii) $HSCHMe_2$, $BF_3{\cdot}Et_2O$, CH_2Cl_2; (iii) NaOMe, MeOH; (iv) Bu_2SnO, MeOH, reflux, 4 h; (v) Bu_4NI, BnBr, toluene; (vi) Ac_2O, Py, 46% for six steps; (h) NIS, TMSOTf, CH_2Cl_2, −20 °C; (i) (i) 80% HOAc, 60 °C, 6 h; (ii) Ac_2O, Py, 72% for three steps; (j) $NaBrO_3$, $Na_2S_2O_4$, EtOAc/H_2O, rt, 80% for **12**; 70% for **15** for three steps; (k) **3**, NIS, TMSOTf, CH_2Cl_2, −20 °C, 78%; (l) TMSOTf, CH_2Cl_2, −20 °C, 89%; (m) $CuSO_4{\cdot}5H_2O$, sodium ascorbate, 1:1 THF–H_2O, 50–60 °C, 90%; (n) 0.5 N NaOH, 1:1 CH_2Cl_2–MeOH, 0 °C–rt, 2.5 h, 95%.

aqueous solution at 0 °C for 2.5 h furnished fluorescence-labeled K30 unit **1**.

In conclusion, we have synthesized a *N*-dansyl fluorescence-labeled K30 antigen repeating unit, {4-[5-(*N*,*N*′-dimethylamino)naphthalene-1-sulfonamine]-1*H*-1,2,3-triazol-1-yl}hexyl β-D-glucopyranosyluronate-(1→3)-α-D-galactopyranosyl-α-D-mannopyranosyl-(1→3)-β-D-galactopyranoside, using isopropyl thioglycosides as donors in NIS/TMSOTf-catalyzed glycosylations and Cu(I)-catalyzed 1,3-dipolar cycloaddition reaction of azide and alkyne. The compound thus prepared could be further used for the studies of interactions among K30 oligosaccharides and proteins, and the results will be reported in due course.

1. Experimental

1.1. General methods

Optical rotations were determined at 25 °C with a Perkin–Elmer Model 241-Mc automatic polarimeter, and $[\alpha]_D$-values are in units of 10^{-1} deg cm^2 g^{-1}. ^{1}H NMR, ^{13}C NMR and ^{1}H–^{1}H, ^{1}H–^{13}C COSY spectra were recorded with a Bruker ARX 400 spectrometer for solutions in $CDCl_3$ or D_2O. Chemical shifts are given in parts per million downfield from internal Me_4Si. Mass spectra were measured using a MALDI-TOF-MS with α-cyano-4-hydroxycinnamic acid (CCA) as the matrix. Thin-layer chromatography (TLC) was performed on silica gel HF_{254} with detection by charring with 30% (v/v) H_2SO_4 in MeOH or in some cases by UV detection. Column chromatography was conducted by the elution of a column of silica gel (100–200 mesh) with EtOAc–petroleum ether (60–90 °C) as the eluent. Solutions were concentrated at <60 °C under reduced pressure.

1.2. Isopropyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (3)

To a vigorously stirred solution of **2** (1.273 g, 3.90 mmol) in DMF (15 mL) at 0 °C was added NaH (50% content, 0.75 g, 15.6 mmol). BnBr (1.00 mL, 8.58 mmol) was added 20 min later, and allowed to stir at rt for 2 h. It was then poured into ice-cold water (50 mL) and extracted with EtOAc (3 × 50 mL). The combined organic phases were dried over anhyd Na_2SO_4 and concentrated. Purification of the residue by silica gel column chromatography (3:1 petroleum ether–EtOAc) gave **3** (1.87 g, 95%) as an amorphous solid: $[\alpha]_D^{25}$ −70 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.33, 1.37 (2s, 6H, 2C*H*$_3$), 3.26–3.33 (m, 2H, H-5, SC*H*), 3.58 (dd, 1H, *J* 3.5, 9.2 Hz, H-3), 3.86 (t, 1H, *J* 9.2 Hz, H-2), 3.94 (dd, 1H, *J* 1.7, 12.2 Hz, H-6a), 4.14 (d, 1H, *J* 3.5 Hz, H-4), 4.28 (br d, 1H, *J* 1.7, 12.2 Hz, H-6b), 4.49 (d, 1H, *J* 9.2 Hz, H-1), 4.74 (s, 2H, PhC*H*$_2$), 4.80, 4.89 (2d, 2H, PhC*H*$_2$), 5.46 (s, 1H, PhC*H*), 7.27–7.56 (m, 15H, Ph). Anal. Calcd for $C_{30}H_{34}O_5S$: C, 71.12; H, 6.76. Found: 71.44; H, 6.57.

1.3. 6-Azidohexyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranoside (5)

To a solution of compound **4** (5.70 g, 13.8 mmol) and 6-azido-1-hexanol (1.80 g, 12.58 mmol) in dry CH_2Cl_2 (60 mL) was added 4 Å molecular sieves (2 g). The mixture was stirred at −20 °C for 20 min under an N_2 atmosphere, then NIS (3.70 g, 16.44 mmol) and TMSOTf (125 μL, 0.69 mmol) were added. The mixture was stirred under these conditions for 30 min, quenched by Et_3N, diluted with CH_2Cl_2 (100 mL), and washed with aq $Na_2S_2O_3$. The organic phase was dried over Na_2SO_4. The solvents were evaporated in vacuo to give a residue, which was purified by silica gel column chromatography (2:1 petroleum ether–EtOAc) to give compound **5** as a syrup (5.64 g, 85%): $[\alpha]_D^{25}$ +20 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.35–1.39 (m, 4H), 1.57–1.61 (m, 4H), 2.06, 2.07 (2s, 2 × 3H, 2C*H*$_3$CO), 3.26 (t, 2H, *J* 6.9 Hz, C*H*$_2$N$_3$), 3.45–3.51 (m, 2H, one proton of OC*H*$_2$ and H-5), 3.89–3.95 (m, 1H, one proton of OC*H*$_2$), 4.06 (dd, 1H, *J* 1.6, 12.4 Hz, H-6a), 4.33 (dd, 1H, *J* 1.6, 12.4 Hz, H-6b), 4.37 (br d, 1H, *J* 3.6 Hz, H-4), 4.49 (d, 1H, *J* 8.0 Hz, H-1), 4.96 (dd, 1H, *J* 3.6, 10.4 Hz, H-3), 5.38 (dd, 1H, *J* 8.0, 10.4 Hz, H-2), 5.51 (s, 1H, PhC*H*), 7.36–7.53 (m, 5H, Ph). Anal. Calcd for $C_{23}H_{31}N_3O_8$: C, 57.85; H, 6.54. Found: C, 58.09; H, 6.39.

1.4. 6-Azidohexyl 2-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranoside (8)

A solution of **5** (5.10 g, 10.7 mmol) in MeOH (100 mL) was treated with NaOMe (2.0 mL, 1 M in MeOH) at rt for 6 h. The mixture was neutralized with IR-120 (H^+) resin, and filtered, and the filtrate was evaporated. The syrup was dissolved in pyridine (20 mL), and FmocCl (3.04 g, 11.77 mmol) was added to the solution in an ice-water bath. The mixture was stirred at rt for 15 h, then Ac_2O (5 mL) and a catalytic amount of DMAP (50 mg) were added. The mixture was stirred at rt for 3 h, then co-evaporated with toluene to dryness under reduced pressure. The residue was subsequently dissolved in CH_2Cl_2 (80 mL), to which was added Et_3N (2 mL) at rt and stirred under these conditions for 10 h. The solvents were evaporated in vacuo to give a residue, which was purified by silica gel column chromatography (3:2 petroleum ether–EtOAc) to give compound **8** as a syrup (3.86 g, 83%): $[\alpha]_D^{25}$ +24 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.37–1.40 (m, 4H), 1.57–1.61 (m, 4H), 2.04 (s, 3H, C*H*$_3$CO), 3.26 (t, 2H, *J* 6.9 Hz, C*H*$_2$N$_3$), 3.47–3.51 (m, 2H, one proton of OC*H*$_2$ and H-5), 3.73 (dd, 1H, *J* 3.8, 9.9 Hz, H-3), 3.88–3.94 (m, 1H, one proton of OC*H*$_2$), 4.07 (dd, 1H, *J* 1.7, 12.4 Hz, H-6a), 4.21 (d, 1H, *J* 3.8 Hz, H-4), 4.31 (dd, 1H, *J* 1.7, 12.4 Hz, H-6b), 4.41 (d, 1H, *J* 8.0 Hz, H-1), 5.08 (dd, 1H, *J* 8.0, 9.9 Hz, H-2), 5.55 (s, 1H, PhC*H*), 7.36–7.52 (m, 5H, Ph). Anal. Calcd for $C_{21}H_{29}N_3O_7$: C, 57.92; H, 6.71. Found: C, 58.27; H, 6.60.

1.5. Isopropyl 2,4,6-tri-*O*-acetyl-3-*O*-benzyl-1-thio-α-D-mannopyranoside (9)

The mixtures of isopropyl 1-thio-α-D-mannopyranoside (7.60 g, 31.9 mmol) and dibutyltin oxide (8 g, 32 mmol) were dissolved into anhyd MeOH (300 mL).[15] The reaction mixture was refluxed for 4 h, concentrated to dryness under reduced pressure. The syrup was suspended into anhyd toluene (250 mL), and Bu_4NI (11.8 g, 31.9 mmol) and BnBr (5.73 mL, 47.9 mmol) were added. The mixture was stirred at 70 °C for 16 h, then concen-

trated to dryness. The residue was treated with Ac_2O (15 mL) in pyridine (40 mL) at rt for 6 h, and co-evaporated with toluene under diminished pressure. Purification of the residue by silica-gel column chromatography (3:1 petroleum ether–EtOAc) gave compound **9** as a syrup (8.12 g, 56%): $[\alpha]_D^{25}$ −37 (*c* 1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 1.30, 1.32 (d, 6H, $2CH_3$), 2.03, 2.07, 2.16 (3s, 3×3H, $3CH_3CO$), 3.02–3.10 (m, 1H, SC*H*), 3.75 (dd, 1H, *J* 3.3, 9.6 Hz, H-3), 4.05–4.09 (m, 1H, H-6a), 4.23–4.30 (m, 2H, H-5, H-6b), 4.40, 4.60 (2d, 2H, *J* 12.1 Hz, PhCH_2), 5.23 (t, 1H, *J* 9.7 Hz, H-4), 5.36 (d, 1H, *J* 1.4 Hz, H-1), 5.42 (dd, 1H, *J* 1.4, 3.3 Hz, H-2), 7.24–7.35 (m, 5H, Ph). Anal. Calcd for $C_{22}H_{30}O_8S$: C, 58.13; H, 6.65. Found: C, 58.29; H, 6.51.

1.6. 6-Azidohexyl 2,4,6-tri-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (11)

To a solution of compounds **9** (2.25 g, 4.95 mmol) and **8** (1.96 g, 4.50 mmol) in dry CH_2Cl_2 (30 mL) was added 4 Å molecular sieves (1 g). The mixture was stirred at −20 °C for 10 min under an N_2 atmosphere, then NIS (1.34 g, 5.95 mmol) and TMSOTf (80 μL, 0.5 mmol) were added. The mixture was stirred under these conditions for 30 min, quenched by Et_3N, diluted with CH_2Cl_2 (40 mL), and washed with aq $Na_2S_2O_3$. The organic phase was dried over Na_2SO_4 and concentrated. The residue was purified by silica-gel column chromatography (2:1 petroleum ether–EtOAc) to give syrupy compound **10**, which was treated with aq 80% HOAc (60 mL) at 60 °C for 6 h, then co-evaporated with toluene. The residue was reacted with Ac_2O (5 mL) in pyridine (15 mL) at rt overnight, and co-evaporated with toluene under diminished pressure. Purification of the residue by silica-gel column chromatography (2:1 petroleum ether–EtOAc) gave **11** as a syrup (2.62 g, 72%): $[\alpha]_D^{25}$ +21 (*c* 1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 1.36–1.62 (m, 8H), 1.91, 1.98, 2.06, 2.10, 2.14, 2.19 (6s, 6×3H, $6CH_3CO$), 3.27 (t, 2H, *J* 6.8 Hz, CH_2N_3), 3.45–3.48 (m, 1H, one of OCH_2), 3.65 (dd, 1H, *J* 3.3, 9.8 Hz, H-3^I), 3.79–3.90 (m, 4H, one of OCH_2, H-5^I, H-3^{II}, H-$6a^{II}$), 4.13–4.22 (m, 4H, H-5^{II}, H-$6b^{II}$, H-$6a^I$, H-$6b^I$), 4.33 (d, H, *J* 11.9 Hz, PhCH_2), 5.08 (d, 1H, *J* 1.5 Hz, H-1^{II}), 5.13–5.19 (m, 3H, H-2^I, H-2^{II}, H-4^{II}), 5.38 (d, 1H, *J* 2.9 Hz, H-4^I), 7.24–7.34 (m, 5H, Ph). Anal. Calcd for $C_{37}H_{51}N_3O_{17}$: C, 54.88; H, 6.35. Found: C, 54.58; H, 6.40.

1.7. 6-Azidohexyl 2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (12)

Compound **11** (1.28 g, 1.55 mmol) was dissolved in EtOAc (25 mL) and then a solution of $NaBrO_3$ (2.30 g, 15.5 mmol) in water (60 mL) was added. To the well-stirred two-phase system aq $Na_2S_2O_4$ (2.70 g, 15.5 mmol, dissolved in 60 mL water) was added dropwise over 10 min at rt. After completion of the reaction (TLC), the mixture was diluted with EtOAc, and the organic phase was washed with aq $Na_2S_2O_3$. The crude product was then purified by silica-gel chromatography (1:1 petroleum ether–EtOAc) to give syrupy compound **12** (0.91 g, 80%): $[\alpha]_D^{25}$ +24 (*c* 1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 1.36–1.38 (m, 4H), 1.58–1.60 (m, 4H), 2.05, 2.09, 2.11, 2.12, 2.16, 2.18 (6s, 6×3H, $6CH_3CO$), 3.27 (t, 2H, *J* 6.8 Hz, CH_2N_3), 3.43–3.49 (m, 1H, one proton of OCH_2), 3.79–3.90 (m, 4H, H-5^I, H-5^{II}, one proton of OCH_2, H-$6a^I$), 3.92 (dd, 1H, *J* 3.5, 10.0 Hz, H-3^{II}), 4.12 (dd, 1H, *J* 7.1, 11.2 Hz, H-$6a^{II}$), 4.17–4.26 (m, 3H, H-3^I, H-$6b^I$, H-$6b^{II}$), 4.37 (d, 1H, *J* 7.9 Hz, H-1^I), 4.96 (dd, 1H, *J* 2.9, 3.5 Hz, H-2^{II}), 5.06 (t, 1H, *J* 10.0 Hz, H-4^{II}), 5.08 (d, 1H, *J* 1.0 Hz, H-4^I), 5.14 (dd, 1H, *J* 7.9, 10.2 Hz, H-2^I), 5.39 (d, 1H, *J* 2.9 Hz, H-1^{II}). Anal. Calcd for $C_{30}H_{45}N_3O_{17}$: C, 50.07; H, 6.30. Found: C, 49.75; H, 6.23.

1.8. 6-Azidohexyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (13)

To a solution of compounds **3** (260 mg, 0.51 mmol) and **12** (310 mg, 0.43 mmol) in dry CH_2Cl_2 (10 mL) was added NIS (270 mg, 0.60 mmol) and TMSOTf (15 μL, 0.08 mmol) at −20 °C under an N_2 atmosphere. The mixture was stirred under these conditions for 45 min, quenched by Et_3N, diluted with CH_2Cl_2 (40 mL), and washed with aq $Na_2S_2O_3$. The organic phase was dried over Na_2SO_4, the solvents were evaporated in vacuo, and the residue was purified by silica-gel column chromatography (1:1 petroleum ether–EtOAc) to give compound **13** (386 mg, 78%) as a syrup: $[\alpha]_D^{25}$ +52 (*c* 1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 1.16–1.52 (m, 8H), 1.68, 2.04, 2.05, 2.08, 2.09, 2.16 (6s, 6×3H, $6CH_3CO$), 3.26 (t, 2H, *J* 6.8 Hz, CH_2N_3), 3.44–3.48 (m, 1H, one proton of OCH_2), 3.61 (br s, 1H, H-5^{III}), 3.79–3.94 (m, 6H, one proton of OCH_2, H-5^I, H-5^{II}, H-$6a^{II}$, and 2H-6^{III}), 4.01 (dd, 1H, *J* 3.5, 10.2 Hz, H-3^{II}), 4.09–4.20 (m, 6H, H-2^{III}, H-3^{III}, H-3^I, 2H-6^I, H-$6b^{II}$), 4.37 (d, 1H, *J* 8.0 Hz, H-1^I), 4.63, 4.75 (2d, 2H, *J* 11.6 Hz, PhCH_2), 4.69, 4.78 (2d, 2H, *J* 11.9 Hz, PhCH_2), 4.99 (d, 1H, *J* 1.5 Hz, H-1^{II}), 5.08–5.11 (m, 2H, H-1^{III}, H-2^{II}), 5.14 (dd, 1H, *J* 8.0, 10.4 Hz, H-2^I), 5.26 (t, 1H, *J* 10.0 Hz, H-4^{II}), 5.37 (d, 1H, *J* 2.9 Hz, H-4^I), 5.43 (s, 1H, PhC*H*), 7.25–7.47 (m, 15H, Ph). Anal. Calcd for $C_{57}H_{71}N_3O_{22}$: C, 59.52; H, 6.22. Found: C, 59.39; H, 6.06.

1.9. 6-Azidohexyl 4,6-di-*O*-acetyl-α-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (15)

Compound **13** (385 mg, 0.33 mmol) in 80% HOAc–H_2O (10 mL) was heated at 60 °C for 6 h, and then

co-evaporated with the help of toluene. The residue was acetylated with Ac_2O (1 mL) in pyridine (4 mL), then purified by silica-gel column chromatography (2:1 petroleum ether–EtOAc). The residue was dissolved in EtOAc (10 mL), and then a solution of $NaBrO_3$ (0.99 g, 6.6 mmol) in water (30 mL) was added. To the well-stirred two-phase system aq $Na_2S_2O_4$ (1.15 g, 6.6 mmol, dissolved in 25 mL water) was added dropwise over 10 min at rt. The reaction was monitored by TLC until all starting material was consumed. The mixture was diluted with EtOAc, and the organic phase was washed with aq $Na_2S_2O_3$. The crude product was then purified by silica-gel column chromatography (1:2 petroleum ether–EtOAc) to give compound **15** as a syrup (228 mg, 70%): $[\alpha]_D^{25}$ +27 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.37–1.62 (m, 8H), 2.05 (s, 3H, CH_3CO), 2.09 (s, 9H, 3CH_3CO), 2.11 (s, 6H, 2CH_3CO), 2.15 (s, 3H, CH_3CO), 2.17 (s, 3H, CH_3CO), 3.27 (t, 2H, *J* 6.8 Hz, CH_2N$_3$), 3.45–3.47 (m, 1H, one proton of OCH_2), 3.69 (dd, 1H, *J* 3.6, 11.9 Hz, H-6), 3.77 (dd, 1H, *J* 3.3, 11.9 Hz, H-6), 3.81 (br t, 1H, *J* 7.0 Hz, H-5^{II}), 3.85–3.91 (m, 3H, one proton of OCH_2, H-5^{I}, H-5^{III}), 3.95 (dd, 1H, *J* 2.8, 10.2 Hz, H-3^{I}), 4.05–4.10 (m, 3H, 3H-6), 4.19–4.23 (m, 4H, H-2^{III}, H-3^{II}, H-3^{III}, H-6), 4.37 (d, 1H, *J* 7.9 Hz, H-1^{I}), 4.99 (d, 1H, *J* 3.7 Hz, H-1^{III}), 5.04–5.05 (m, 2H, H-2^{II}, H-1^{II}), 5.12 (dd, 1H, *J* 7.9, 10.2 Hz, H-2^{I}), 5.27 (t, 1H, *J* 10.0 Hz, H-4^{II}), 5.34 (d, 1H, *J* 2.2 Hz, H-4^{III}), 5.39 (d, 1H, *J* 2.8 Hz, H-4^{I}); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.4, 20.5, 20.6, 20.7, 20.8, 25.3, 26.3, 28.7, 29.2, 51.2, 61.3, 61.5, 62.2, 65.1, 67.5, 67.6, 68.9, 69.1, 69.7, 69.75, 69.82, 70.5, 73.4, 75.0, 77.2, 94.7, 101.1, 101.2, 168.8, 169.5, 170.1, 170.3, 170.4, 170.5, 170.6, 170.8. Anal. Calcd for $C_{40}H_{59}N_3O_{24}$: C, 49.74; H, 6.16. Found: C, 49.52; H, 6.30.

1.10. 6-Azidohexyl (methyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyluronate)-(1→3)-2,4,6-tri-*O*-acetyl-α-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (18)

To a solution of **16** (45 mg, 0.067 mmol) and **15** (54 mg. 0.056 mmol) in dry CH_2Cl_2 (3 mL) was added TMSOTf (1.8 μL, 0.01 mmol) at −20 °C under an N_2 atmosphere. The mixture was stirred under these conditions for 30 min, then quenched with Et_3N. The solvent was evaporated, and the residue was dissolved into pyridine (4 mL). To this mixture was added Ac_2O (1 mL) and then co-evaporated with toluene after 6 h. Purification of the residue by silica-gel column chromatography (2:3 petroleum ether–EtOAc) obtained compound **18** as a syrup (75 mg, 89%): $[\alpha]_D^{25}$ +18 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.35–1.60 (m, 8H), 1.65, 1.97, 2.03, 2.04, 2.09, 2.11, 2.12, 2.13, 2.17 (9s, 9 × 3H, 9CH_3CO), 3.26 (t, 2H, *J* 6.8 Hz, CH_2N$_3$), 3.41–3.45 (m, 1H, one proton of OCH_2), 3.74 (s, 3H, CH_3), 3.75–3.90 (m, 4H), 3.95 (dd, 1H, *J* 2.1, 9.8 Hz, H-3^{I}), 3.98 (dd, 1H, *J* 4.0, 11.2 Hz, H-6), 4.05 (dd, 1H, *J* 2.2, 9.6 Hz, H-3^{III}), 4.10–4.28 (m, 5H), 4.30 (d, 1H, *J* 9.9 Hz, H-5^{IV}), 4.30–4.33 (m, 2H, *J* 7.9 Hz, H-1^{I}, H-6), 4.86 (dd, 1H, *J* 3.7, 10.4 Hz, H-2^{III}), 4.96 (d, 1H, *J* 7.5 Hz, H-1^{IV}), 5.02–5.05 (m, 3H, H-2^{I}, H-1^{II}, H-4^{I}), 5.14 (t, 1H, *J* 9.3 Hz, H-4^{II}), 5.20 (d, 1H, *J* 3.7 Hz, H-1^{III}), 5.38 (br d, 1H, *J* 3.1 Hz, H-2^{II}), 5.42 (dd, 1H, *J* 7.5, 9.3 Hz, H-2^{IV}), 5.49 (d, 1H, *J* 3.0 Hz, H-4^{III}), 5.71 (t, 1H, *J* 9.3 Hz, H-4^{IV}), 5.83 (t, 1H, *J* 9.3 Hz, H-3^{IV}), 7.27–7.92 (m, 15H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.1, 20.4, 20.5, 20.55, 20.58, 20.66, 20.68, 20.7, 20.8, 25.3, 26.3, 28.7, 29.1, 51.2, 52.8, 60.3, 61.1, 62.1, 62.4, 64.8, 67.6, 68.4, 69.0, 69.2, 69.6, 69.7, 69.8, 69.9, 70.1, 70.4, 70.5, 71.7, 72.2, 72.8, 73.0, 94.6, 96.0, 100.8, 101.3, 128.2, 128.3, 128.6, 128.8, 129.0, 129.5, 129.7, 133.3, 164.8, 165.6, 166.7, 168.5, 169.6, 169.7, 170.0, 170.2, 170.29, 170.31, 170.4, 170.6. Anal. Calcd for $C_{70}H_{83}N_3O_{34}$: C, 55.66; H, 5.54. Found: C, 55.29; H, 5.35. MALDITOF-MS: calcd for $C_{70}H_{83}N_3O_{34}$: 1509.5 $[M]^+$; found: 1532.4 $[M+Na]^+$.

1.11. {4-[5-(*N*,*N*′-Dimethylamino)naphthalene-1-sulfonamine]-1*H*-1,2,3-triazol-1-yl}hexyl (methyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyluronate)-(1→3)-2,4,6-tri-*O*-acetyl-α-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (20)

To a mixture of **18** (50 mg, 0.033 mmol) and **19** (15 mg, 0.04 mmol) in 1:1 H_2O–THF (6 mL) were added freshly prepared 1 M aq sodium ascorbate (2 mg, 0.01 mmol) and $CuSO_4{\cdot}5H_2O$ (1 mg, 0.0033 mmol). The heterogeneous mixture was stirred vigorously in a dark room at 50 °C until complete consumption of the reactants was indicated by TLC analysis. After removal of THF under reduced pressure, water (6 mL) was added, and the product was extracted with EtOAc (3 × 15 mL). The combined organic layers were dried over anhyd Na_2SO_4 and evaporated in vacuo. The crude product was subjected to column chromatography (1:1 petroleum ether–EtOAc) to give **20** as a foamy solid (54 mg, 90%): $[\alpha]_D^{25}$ +16 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.50–1.55 (m, 3H), 1.75–1.83 (m, 5H), 1.64, 1.96, 2.04, 2.05, 2.09, 2.11, 2.12, 2.13, 2.17 (9s, 9 × 3H, 9CH_3CO), 2.91 (s, 6H, 2CH_3), 3.38–3.43 (m, 1H, one proton of OCH_2), 3.74 (s, 3H, CH_3), 3.80–3.88 (m, 2H), 3.90 (dd, 1H, *J* 2.3, 9.8 Hz, H-3^{I}), 3.97 (dd, 1H, *J* 2.4, 9.6 Hz, H-3^{III}), 4.00 (dd, 1H, *J* 4.0, 10.9 Hz, H-6), 4.05 (dd, 1H, *J* 3.6, 11.3 Hz, H-6), 4.10–4.30 (m, 8H), 4.31–4.38 (m, 3H, H-1^{I}, H-5^{IV}, H-6), 4.86 (dd, 1H, *J* 3.6, 10.5 Hz, H-2^{III}), 4.97 (d, 1H, *J* 7.5 Hz, H-1^{IV}), 5.00–5.05 (m, 3H, H-2^{I}, H-1^{II}, H-4^{I}), 5.15 (t, 1H, *J* 9.2 Hz, H-4^{II}), 5.20 (d, 1H, *J* 3.7 Hz, H-1^{III}), 5.38 (br d, 1H, *J* 2.9 Hz, H-2^{II}), 5.41 (dd, 1H, *J* 7.5, 9.5 Hz, H-2^{IV}), 5.48 (d, 1H, *J* 3.2 Hz, H-4^{III}),

5.50–5.55 (m, 1H, N*H*SO_2), 5.70 (t, 1H, *J* 9.5 Hz, H-4^{IV}), 5.86 (t, 1H, *J* 9.5 Hz, H-3^{IV}), 7.26–8.27 (m, 22H, Ph and C=C*H*); Selected ^{13}C NMR (100 MHz, $CDCl_3$): δ 50.0, 52.8, 60.3, 61.1, 62.2, 62.4, 64.9, 67.6, 68.4, 68.9, 69.2, 69.6, 69.8, 69.9, 70.0, 70.4, 71.7, 72.1, 72.8, 73.0, 94.6, 96.0, 100.8, 101.3, 115.3, 118.7, 164.5, 164.9, 165.6, 166.7, 168.6, 169.7, 169.8, 170.0, 170.3, 170.6. Anal. Calcd for $C_{85}H_{99}N_5O_{36}S$: C, 56.76; H, 5.55. Found: C, 57.02; H, 5.41. MALDITOF-MS: calcd for $C_{85}H_{99}N_5O_{36}S$: 1797.6 $[M]^+$; found: 1820.5 $[M+Na]^+$.

1.12. {4-[5-(*N*,*N*′-Dimethylamino)naphthalene-1-sulfonamine]-1*H*-1,2,3-triazol-1-yl}hexyl β-D-glucopyranosyluronate-(1→3)-α-D-galactopyranosyl-α-D-mannopyranosyl-(1→3)-β-D-galactopyranoside (1)

To a mixture of compound **20** (50 mg, 0.027 mmol) in 1:1 MeOH–CH_2Cl_2 (40 mL) was added aq 0.5 N NaOH at rt. After stirring for 2.5 h, the mixture was neutralized with IR-120 (H^+) resin, and filtered, and the filtrate was concentrated to dryness under diminished pressure to give compound **1** (28 mg, 95%) as a syrup: $[\alpha]_D^{25}$ +21 (*c* 0.5, H_2O); ^{1}H NMR (400 MHz, D_2O): δ 0.90–0.92 (m, 2H), 1.10–1.17 (m, 2H), 1.30–1.45 (m, 4H), 3.37 (s, 6H, 2CH_3), 3.40–4.20 (m, 31H), 4.93 (s, 1H, H-1^{III}), 5.20 (s, 1H, H-1^{II}), 7.31 (s, 1H, C=C*H*), 7.67–7.74 (m, 2H), 7.93 (d, 1H, *J* 7.2 Hz, Ph), 8.14 (d, 1H, *J* 7.2 Hz, Ph), 8.23 (d, 1H, *J* 8.4 Hz, Ph), 8.44 (d, 1H, *J* 8.4 Hz, Ph); ^{13}C NMR (100 MHz, D_2O): δ 13.2, 19.3, 20.5, 24.3, 25.0, 28.3, 29.0, 36.9, 46.8, 49.7, 60.8, 60.9, 61.3, 61.7, 62.5, 64.3, 65.9, 67.7, 69.1, 69.2, 69.9, 70.2, 71.2, 71.3, 72.8, 72.9, 74.9, 75.1, 76.3, 78.4, 79.3, 95.9, 100.4, 102.6, 103.7, 119.3, 123.9, 125.2, 125.5, 126.2, 126.9, 128.0, 128.4, 131.0, 135.6, 138.5, 142.6, 172.8. MALDI-TOF-MS: calcd for $C_{45}H_{67}N_5O_{24}S$: 1093.3897 $[M]^+$; found: 1116.3843 $[M+Na]^+$.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2007.01.015.

References

1. Chakraborty, A. K.; Friebolin, H.; Stirm, S. *J. Bacteriol.* **1980**, *141*, 971–972.
2. Keenleyside, W. J.; Jayaratne, P.; MacLachlan, P. R.; Whitfield, C. *J. Bacteriol.* **1992**, *174*, 8–16.
3. Whitfield, C.; Paiment, A. *Carbohydr. Res.* **2003**, *338*, 2491–2502.
4. MacLachlan, P. R.; Keenleyside, W. J.; Dodgson, C.; Whitfield, C. *J. Bacteriol.* **1993**, *175*, 7515–7522.
5. Drummelsmith, J.; Whitfield, C. *EMBO J.* **2000**, *19*, 57–66.
6. (a) Min, J. Z.; Toyo'oka, T.; Kawanishi, H.; Fukushima, T.; Kato, M. *Anal. Chim. Acta* **2005**, *550*, 173–181; (b) Araki, Y.; Andoh, A.; Fujiyama, Y.; Hata, K.; Makino, J.; Okuno, T.; Nakanura, F.; Bamba, T. *J. Chromatogr., B* **2001**, *753*, 209–216.
7. Shibaev, V. N.; Veselovsky, V. V.; Lozanova, A. V.; Maltsev, S. D.; Danilov, L. L.; Forsee, W. T.; Xing, J.; Cheung, H. C.; Jedrzejas, M. J. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 189–192.
8. (a) Cumpstey, I.; Butters, T. D.; Tennant-Eyles, R. J.; Fairbanks, A. J.; France, R. R.; Wormald, M. R. *Carbohydr. Res.* **2003**, *338*, 1937–1949; (b) Kotani, N.; Takasaki, S. *Anal. Biochem.* **1998**, *264*, 66–73; (c) Kinoshita, A.; Sugahara, K. *Anal. Biochem.* **1999**, *269*, 367–378; (d) Watanabe, T.; Inoue, N.; Kutsukake, T.; Matsuki, S.; Takeuchi, M. *Biol. Pharm. Bull.* **2000**, *23*, 269–273.
9. (a) Yang, F.; He, H.; Du, Y.; Lu, M. *Carbohydr. Res.* **2002**, *337*, 1165–1169; (b) He, H.; Gu, G.; Du, Y. *J. Carbohydr. Chem.* **2003**, *22*, 275–283.
10. (a) Zeng, Y.; Kong, F. *Tetrahedron Lett.* **2002**, *43*, 3129–3733; (b) Zeng, Y.; Kong, F. *Carbohydr. Res.* **2003**, *338*, 307–311; (c) Zeng, Y.; Kong, F. *Carbohydr. Res.* **2003**, *338*, 2047–2056.
11. Allen, J. G.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1999**, *121*, 468–469.
12. (a) Du, Y.; Gu, G.; Wei, G.; Hua, Y.; Linhardt, R. J. *Org. Lett.* **2003**, *5*, 3627–3630; (b) Gu, G.; Du, Y.; Linhardt, R. J. *J. Org. Chem.* **2004**, *69*, 5497–5500.
13. Chen, Q.; Yang, F.; Du, Y. *Carbohydr. Res.* **2005**, *340*, 2476–2482.
14. Gioeli, C.; Chattopadhyaya, J. B. *J. Chem. Soc., Chem. Commun.* **1982**, 672–674.
15. (a) Yang, F.; He, H.; Du, Y. *Tetrahedron Lett.* **2002**, *43*, 7561–7563; (b) Zhang, Z.; Magnusson, G. *J. Org. Chem.* **1995**, *60*, 7304–7315.
16. Adinolfi, M.; Barone, G.; Guariniello, L.; Iadonisi, A. *Tetrahedron Lett.* **1999**, *40*, 8439–8441.
17. (a) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596–2599; (b) Temelkoff, D. P.; Zeller, M.; Norris, P. *Carbohydr. Res.* **2006**, *341*, 1081–1090.
18. Bolletta, F.; Fabbri, D.; Lombardo, M.; Prodi, L.; Trombini, C.; Zaccheroni, N. *Organometallics* **1996**, *15*, 2415–2417.