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The synthesis of a multivalent heterobifunctional ligand for specific interaction with Shiga toxin 2 produced by *E. coli* O157:H7

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This paper is dedicated to the memory of Dr. Malcolm Perry and Porfessor Lennart Kenne

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ABSTRACT

Hemolytic uremic syndrome is a potentially fatal complication of food poisoning caused by *Escherichia coli* O157:H7, especially those strains that produce the Stx2 Shiga toxin. Multivalent inhibitors based on the P^k trisaccharide are most effective against Stx1 the less dangerous of the two Shiga toxins. Inhibitors containing a terminal 2-acetamido-2-deoxy-α-D-galactopyranosyl residue in place of the terminal α-D-galactopyranosyl residue of P^k trisaccharide have been shown to exhibit preferential binding to Stx2. A multivalent heterobifunctional P^k analog containing 2-acetamido-2-deoxy-α-D-galactopyranose has been synthesized in a format that facilitates the ablation of toxin activity via supramolecular complex formation between Stx and the endogenous protein, Human serum amyloid P component (HuSAP).

1. Introduction

Hemolytic uremic syndrome (HUS) is a potentially fatal kidney disease caused by Shiga toxin producing *E. coli* (STEC), such as *E. coli* O157:H7 and *E. coli* O104:H4.[1,2] *E. coli* O157:H7 produces two types of phage encoded Shiga toxins, Shiga toxin 1 (Stx1) and Shiga toxin 2 (Stx2) which share approximately 56% sequence identity.[3] Shiga toxins are AB_5 holotoxins consisting of a catalytic A subunit, responsible for the cleavage of a single nucleotide residue on rRNA resulting in cell death, and the cell surface binding B_5 homopentameric subunit.[4–6] The natural ligand for Stx1 is Globotriaosyl ceramide (Gb_3) consisting of P^k trisaccharide (αGal(1→4)-βGal(1→4)-βGlc) (Fig. 1).[7] It has been shown that Stx1 binds Gb_3 with greater affinity than Stx2, an apparent anomaly to the observation that Stx2 producing bacteria are more likely to result in the development of HUS than those producing Stx1.[8] However, an alternative receptor for Stx2 other than Gb_3, is yet to be elucidated. Recently Kale et al. have shown that a modified P^k trisaccharide derivative with a terminal 2-acetamido-2-deoxy-D-galactose moiety (Fig. 1) binds Stx2 selectively over Stx1.[9]

The intrinsic affinity of proteins for carbohydrate ligands is low and disassociation constants, K_d, often fall in the mM range. This can be overcome through the use of multivalency or supramolecular interaction.[10] We have previously reported the effective inhibition of Stx1 by a decavalent dendrimer, STARFISH.[11] This molecule has a pentameric display of bivalent ligands, which was found to bring together two molecules of Stx1 in a face-to-face manner to form a supramolecular complex.[11] Expanding upon the principle of face-to-face aggregation of protein, we later reported the synthesis of a heterobifunctional ligand, BAIT-P^k, and its polymeric counterpart, PolyBAIT-P^k, which allowed for the simultaneous binding of Stx1 and an endogenous protein of the innate immune system, Human serum amyloid P component (HuSAP).[12,13] HuSAP acts as a supramolecular template protein. Like Stx it is a pentameric, radially symmetric protein. Additionally, analysis of both the Stx1-B_5 subunit and HuSAP indicated that when the two proteins are overlaid, their respective binding sites can be brought into register. The ligand PolyBAIT-P^k was designed to incorporate the Stx1 binding P^k trisaccharide and the HuSAP binding pyruvate moiety[14] to enable the formation of a supramolecular complex (Fig. 2). In vitro and in vivo studies of PolyBAIT-P^k showed sequestering of the toxin and its removal via the liver, thereby affording protection of HuSAP transgenic mice in vivo after challenge with a lethal dose of Shiga toxin.[13]

As Stx2 is found to be the most clinically relevant of the toxins,[15] we applied our previously established heterobifunctional ligand strategy to the development of a Stx2 specific ligand. We present here the synthesis of a Stx2 specific multivalent heterobifunctional ligand.

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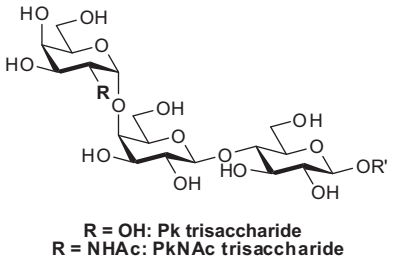


Figure 1. P^k trisaccharide; P^kNAc trisaccharide.

2. Results and discussion

A retrosynthetic analysis of **1** requires the incorporation of four necessary moieties (Scheme 1); a linker **4** that allows for conjugation to a polymeric scaffold and three monosaccharide building blocks, **5**, **6**, and **7**.

The synthesis of **1** was accomplished by click reaction between BAIT-P^kNAc, **2** and an azido-polyacrylamide. BAIT-P^kNAc **2** was synthesized via coupling of the amine linker **4** and a P^k trisaccharide derivative obtainable from **3**. The P^k trisaccharide derivative is synthesized via the coupling of the thioglycoside donor **6** with acceptor **7**, which after glycosylation with trichloroacetimidate donor **5** provides the P^k trisaccharide derivative **3**.

2.1. Synthesis of acceptor 7

The synthesis of the acceptor **7** was accomplished via the formation of the known cyanoethylidene derivative **8** from the corresponding glycosyl bromide according to the literature procedures (Scheme 2).[16] The fully acetylated cyanoethylidene **8** underwent global deprotection using $NaOCH_3$ in CH_3OH to first remove all the acetate groups and to aid in the formation of the methoxy(ethylidene) moiety, by forming the corresponding methoxy imidate species **8a**. Treatment of **8a** with glacial CH_3COOH results in the formation of **9** in 75% yield. The triol **9** was treated with $PhCH(OCH_3)_2$ and camphorsulfonic acid (CSA) to form the 4,6-*O*-benzylidene intermediate and subsequent acetylation of the remaining 3-OH gave the benzylidene derivative **10** in 85% yield over two steps.[17] Lastly, the benzylidene acetal **10** was regioselectively opened using Et_3SiH and trifluoroacetic acid (TFA) to provide the selectively protected glycosyl acceptor **7** in 85% yield.[18]

2.2. Synthesis of donor 6

The synthesis of the thiophenyl galactoside donor **6** was achieved through the synthesis of the known thiogalactoside **12** from the peracetylated derivative **11**[19] using $NaOCH_3$ in CH_3OH to give **12** in 97% yield (Scheme 3). The deprotected galactoside **12** was treated with the dimethyl acetal of anisaldehyde and CSA to provide a *para*-methoxybenzylidene intermediate which was subsequently treated with benzoyl chloride in pyridine to provide the intermediate **13** in 88% over two steps.[17] The *para*-methoxybenzylidene acetal of **13** was regioselectively opened using BH_3·THF and TMSOTf to provide the 6-OH, 4-*O*-*p*-methoxybenzyl intermediate which was acetylated to provide **6** in 84% yield over two steps.[20]

2.3. Trisaccharide formation

The construction of the trisaccharide framework was completed by glycosidation of thiophenyl donor **6** and the glucosyl acceptor **7** using *N*-iodosuccinimide (NIS) and AgOTf at −20 °C to give the lactosyl derivative **14** in 90% yield (Scheme 4).[21] The selective deprotection of the 4′-OPMB ether was done by the addition of one equivalent of TfOH at −20 °C to give the lactosyl 4′-OH acceptor **15** in 81% yield. Subsequent glycosylation of acceptor **15** by trichloroacetimidate donor **5**[22] was performed using TMSOTf in Et_2O at room temperature to provide the trisaccharide **16** in a modest yield, 48%. Decomposition of the donor was observable before the formation of the glycosidic linkage and often resulted in no yield of the product. This problem was circumvented by the slow drop-wise addition of donor to a mixture of acceptor and activator.[23] The azide **16** was reduced to the corresponding amine using dithiothreitol (DTT) in a mixture of CH_3CN:Et_3N (6:1)[24] and converted to the *N*-acetyl derivative after treatment with Ac_2O and pyridine to give **17** in 87% yield. The benzyl ether was selectively removed by hydrogenation to give **3** in 97% yield.

2.4. Completion of PolyBAIT-P^kNAc 1

To complete the synthesis of **1**, intermediate **3** was combined with 4-nitrophenyl chloroformate in the presence of pyridine to make the 4-nitrophenyl carbonate **18** in 99% yield (Scheme 5). Treatment of **18** with amine **4** under basic conditions gave **19** in 95% yield. Global deprotection of **19** was achieved through the use of one equivalent of 1 M $NaOCH_3$ in CH_3OH to remove ester groups. Subsequent treatment with water, converted the methyl ester of the pyruvate moiety to the corresponding carboxylic acid, to form **2** in 88% yield over two steps. Lastly, a Cu catalyzed [3+2]-Huisgen cycloaddition[25,26] was performed to conjugate the monomeric ligand **2** to the previously synthesized poly[acrylamide-co-(3-azidopropylmethacrylamide)][27] with polydispersity index (PDI) of 1.29 to provide the desired PolyBAIT-P^kNAc product **1**.

2.5. Conclusion

We have described the synthesis of a Stx2 specific multivalent heterobifunctional ligand, PolyBAIT-P^kNAc. This approach differs from previous syntheses of PolyBAIT-P^k where our approach was to employ a linking arm terminated with a pentenyl group for co polymerization with acrylamide. The adoption of the azide/alkyne

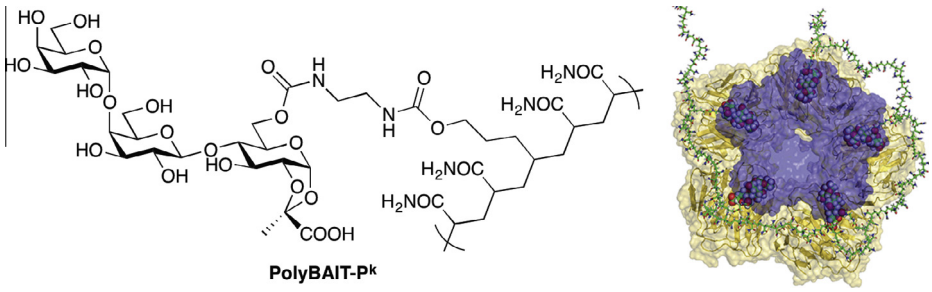
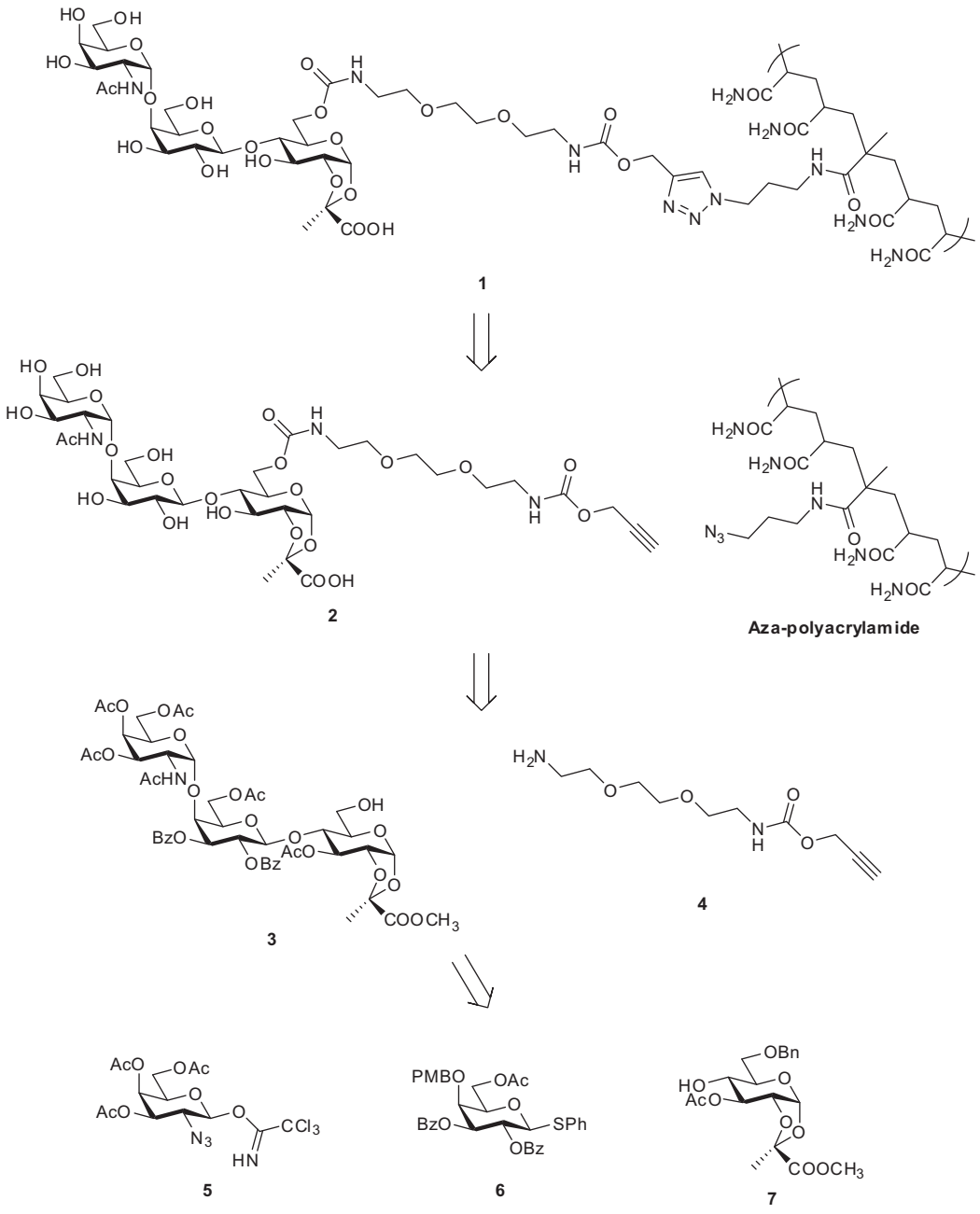


Figure 2. Left: PolyBAIT-P^k. Right: Graphical representation of a supramolecular complex: Yellow = HuSAP, Blue = Stx1, and Polymer = PolyBAIT-P^k.



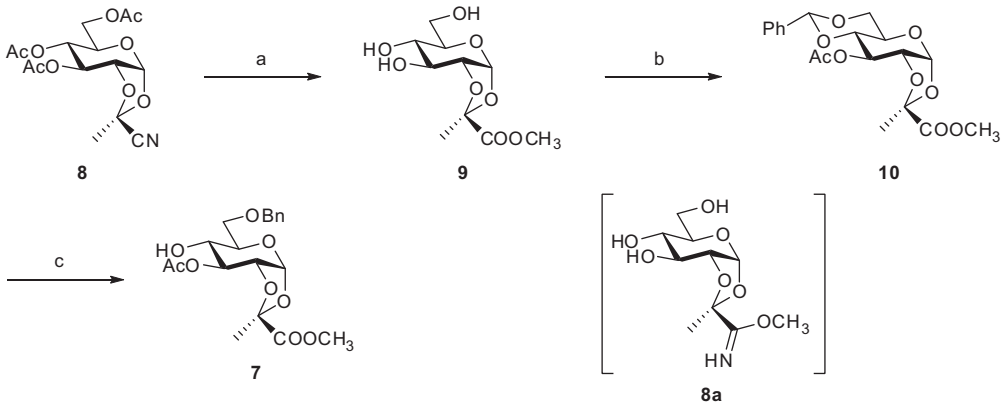
Scheme 1. Retrosynthetic pathway to achieve synthesis of PolyBAIT-P^{k}NAc **1**.

cycloaddition provides a more flexible approach to the creation of polymeric inhibitors, which we have also applied to other polymeric scaffolds that display Stx ligands. *In vitro* and *in vivo* results are currently being obtained and will be presented elsewhere.

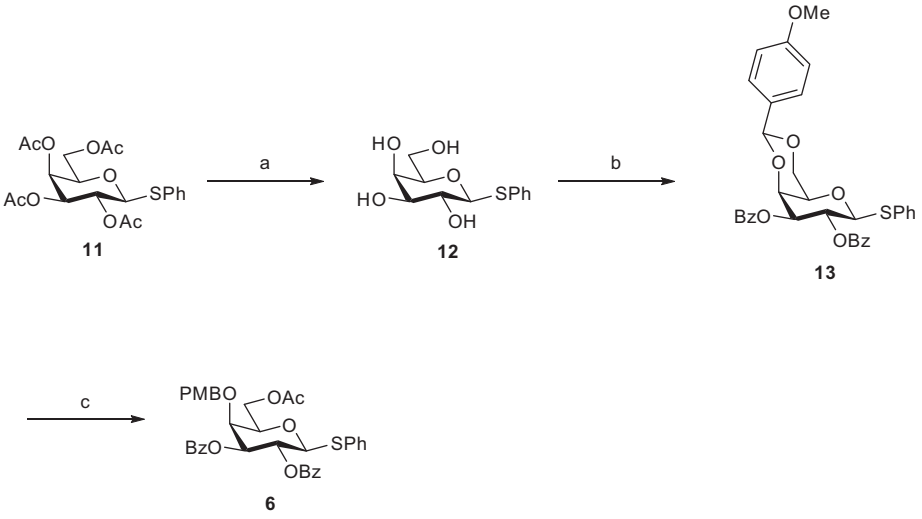
3. Experimental

3.1. General methods

All chemical reagents obtained were of analytical grade and used as obtained from commercial sources unless otherwise indicated. Solvents used in water-sensitive reactions were purified by passage through columns of alumina and copper under nitrogen atmosphere except for methanol, which was distilled prior to use over magnesium methoxide and collected as needed. Unless otherwise stated, all reactions were performed at room temperature and under argon atmosphere. Molecular sieves were dried in an oven maintaining an internal temperature of 350 °C to ensure dryness and were allowed to cool under vacuum or under argon atmosphere at room temperature. Reactions were monitored by analytical thin-layered chromatography (TLC) on Silica gel 60-F254 (E. Merck). Plates were visualized under UV light and/or by treatment with either 5% sulfuric acid in ethanol, potassium permanganate solution, ninhydrin solution, or molybdate solution followed by charring 250 °C. All solvents were removed by rotary evaporation at <40 °C unless otherwise stated. Flash column chromatography was performed using silica gel (230–400 mesh, Silicycle, Montreal) at flow rates between 6 and 18.5 mL min^{-1}. ^{1}H NMR spectra were recorded at 500, 600, or 700 MHz. Chemical shifts are reported in ppm (δ) and were referenced to internal residual protonated solvent signals or to external acetone in the case of D_2O (0.1% external acetone δ 2.225 ppm). ^{13}C NMR spectra were recorded at 125 MHz



Scheme 2. Reagents and conditions: (a) (i) $NaOCH_3$, CH_3OH, (ii) CH_3COOH, 75%; (b) (i) $PhCH(OCH_3)_2$, CSA, CH_3CN, (ii) Ac_2O, Pyr, 83%; and (c) Et_3SiH, TFA, CH_2Cl_2, 0 °C, 85%.



Scheme 3. Reagents and conditions: (a) $NaOCH_3$, CH_3OH, 97%; (b) (i) $CH_3OPhCH(OCH_3)_2$, CSA, CH_3CN, 30 °C, (ii) BzCl, pyridine, 88%; (c) (i) BH_3·THF, TMSOTf, (ii) Ac_2O, pyridine, 84%.

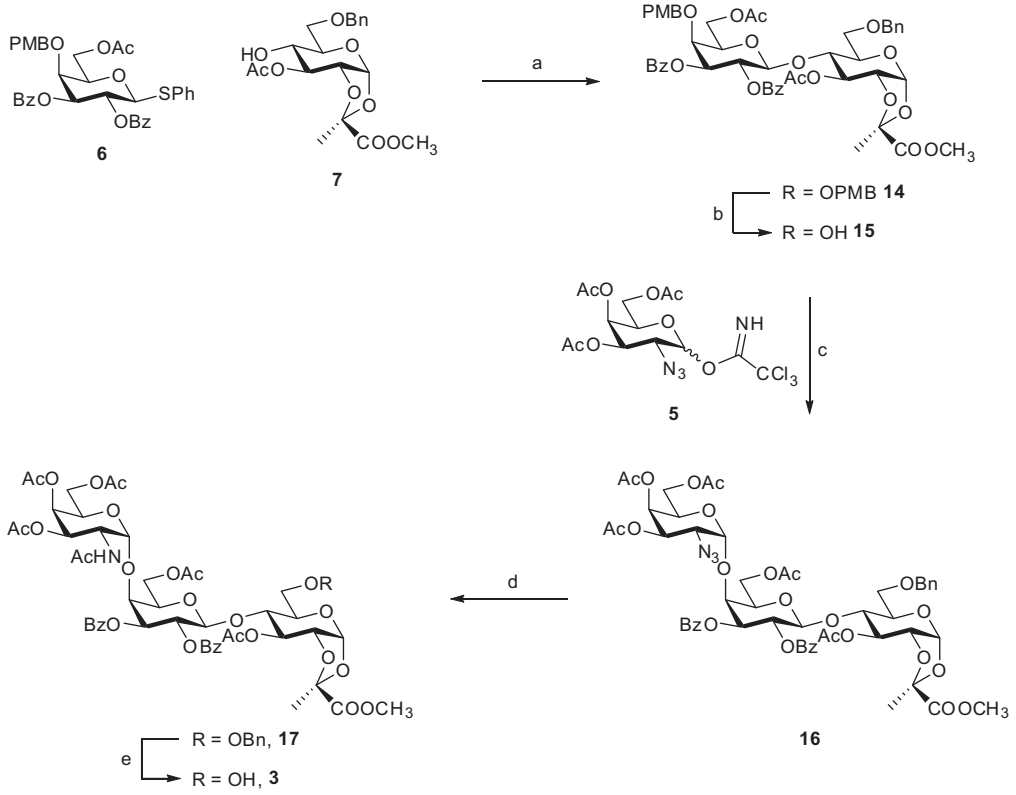
and chemicals shifts are referenced to internal $CDCl_3$ (77.23 ppm) or external acetone (31.07 ppm). High resolution mass spectra were obtained on a Micromass Zabspec TOF-mass spectrometer by the analytical services of this Department. Optical rotations were determined with a Perkin–Elmer model 241 polarimeter at room temperature using the sodium D-line and are reported in deg mL g^{-1} dm^{-1}. Combustion analysis was performed by analytical services of this Department.

3.1.1. 3,4,6-Tri-*O*-acetyl-1,2-*O*-[(*S*)-1-(cyano)ethylidene]-α-D-glucopyranoside (8)

Per-*O*-acetyl-α-D-glucopyranosyl bromide (19.9 g, 0.0485 mol) was combined with ground up KCN (15.8 g 0.242 mol) and TBAB (5.10 g, 0.0242 mol) in a round bottomed flask to which dry acetonitrile (120 mL) was added. The vessel was placed under argon atmosphere and the reaction proceeded at room temperature for 3 days. The reaction turned a dark brown color, indicating a completed reaction. Completion of the reaction was confirmed using ^{1}H NMR spectroscopy, which showed no remaining anomeric proton signal for the glucosyl bromide. The reaction mixture was then filtered through Celite and concentrated to dryness. The crude reaction mixture was then subjected to flash column chromatography on silica gel using 2:1, hexanes–ethyl acetate. Fractions were collected and found to contain both *exo*-CN and *endo*-CN products. The *exo*-CN isomer was recrystalized from ethyl acetate and hexanes to provide the pure *exo*-CN product **8** (7.26 g, 42%) as a white needle solid; R_f 0.52 (1:1, hexanes–ethyl acetate); $[\alpha]_D$ +12.9° (*c* 1.01, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.80 (d, 1H, $J_{1,2}$ 5.1 Hz, H-1), 5.21 (vt, 1H, $J_{2,3}$ 2.8 Hz, $J_{3,4}$ 2.8 Hz, H-3), 4.91 (ddd, 1H, $J_{2,4}$ 0.8 Hz, $J_{2,4}$ 2.6 Hz, $J_{4,5}$ 9.6 Hz, H-4), 4.39 (ddd, 1H, $J_{2,4}$ 0.9 Hz, $J_{2,3}$ 2.9 Hz, $J_{1,2}$ 5.2 Hz, H-2), 4.20 (m, 1H, H-6a), 4.19 (m, 1H, H-6b), 3.90 (ddd, 1H, $J_{5,6a}$ 4.3 Hz, $J_{5,6b}$ 4.3 Hz, $J_{4,5}$ 9.3 Hz, H-5), 2.14 (s, 3H, $CH_3C(O)O$), 2.09 (s, 3H, $CH_3C(O)O$), 2.09 (s, 3H, $CH_3C(O)O$), 1.92 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.6 ($CH_3C(O)O$), 169.5 ($CH_3C(O)O$), 168.9 ($CH_3C(O)O$), 116.4 (C≡N), 98.8 (quat. C pyruvate), 97.4 (C-1), 74.2 (C-2), 69.3 (C-3), 67.8 (C-4), 67.3 (C-5), 62.8 (C-6), 24.3 (–CH_3), 20.7 ($CH_3C(O)O$); ESI HRMS: calcd for $C_{15}H_{19}NO_{19}Na$ 380.0952. Found 380.0947; Anal. Calcd for $C_{15}H_{19}NO_{19}$: C, 50.42; H, 5.36; N, 3.92. Found: C, 50.59; H, 5.33; N, 3.93.

3.1.2. 1,2-*O*-[(*S*)-1-(Methoxycarbonyl)ethylidene]-α-D-glucopyranoside (9)

Cyanoethylidene **8** (6.56 g, 18.4 mmol) was dissolved in anhydrous methanol (60 mL). The flask was placed under argon atmosphere and sodium metal (0.20 g) was added and allowed to dissolve. The reaction was allowed to proceed for 2 days at which point TLC analysis (20%, methanol–dichloromethane) showed no remaining starting material. Glacial acetic acid (40 mL) was added and the reaction proceeded overnight. The reaction mixture was



Scheme 4. Reagents and conditions: (a) NIS, AgOTf, 4 Å MS, CH_2Cl_2, −20 °C, 90%; (b) TfOH, CH_2Cl_2, −20 °C, 81%; (c) 15, TMSOTf, 4 Å MS, Et_2O, 48%; (d) (i) DTT, CH_3CN:Et_3N (6:1), (ii) Ac_2O, Pyr 87%; and (e) H_2, $Pd(OH)_2$/C, 97%.

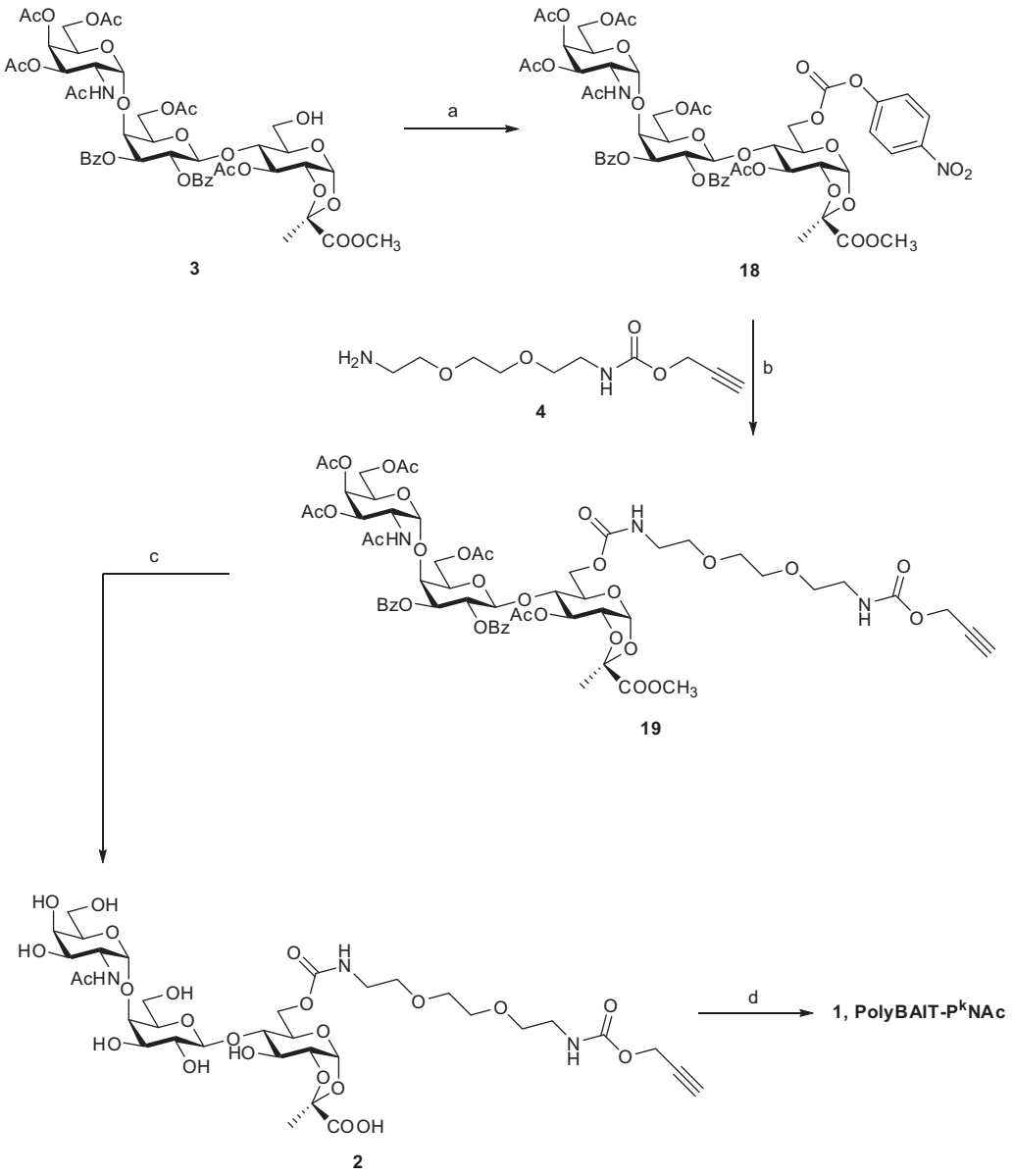
subsequently co-evaporated with toluene. Once dry, the solid was dissolved in methanol, and adsorbed onto silica gel and purified by flash column chromatography (5%, methanol–dichloromethane) to give the product **9** (3.48 g, 72%) as a white solid; $[\alpha]_D$ +34.5° (*c* 1.10, CH_3OH); ^{1}H NMR (500 MHz, D_2O) δ 5.77 (d, 1H, $J_{1,2}$ 4.9 Hz, H-1), 4.27 (ddd, 1H, $J_{2,4}$ 0.8 Hz, $J_{2,3}$ 2.2 Hz, $J_{1,2}$ 5.0 Hz, H-2), 3.99 (vt, 1H, $J_{2,3}$ 4.0 Hz, $J_{3,4}$ 4.0 Hz, H-3), 3.85 (m, 1H, H-6a), 3.80 (s, 3H, COO*CH*$_3$), 3.72 (m, 2H, H-5, H-6b), 3.58 (ddd, 1H, $J_{2,4}$ 0.7 Hz, $J_{3,4}$ 3.9 Hz, $J_{4,5}$ 9.1 Hz, H-4), 1.72 (s, 3H, CH_3 (pyruvate)); ^{13}C NMR (125 MHz, D_2O): δ 172.2 (*C*(O)OCH$_3$), 105.7 (quart. C pyruvate), 98.6 (C-1), 76.9 (C-2), 73.7 (C-5), 72.4 (C-3), 69.0 (C-4), 62.2 (C-6), 54.3 (C(O)O*CH*$_3$), 22.2 (*CH*$_3$ pyruvate); ESI HRMS Calcd for $C_{10}H_{16}O_8Na$ 287.0737. Found 287.0733.

3.1.3. 3-*O*-Acetyl-4,6-*O*-benzylidene-1,2-*O*-[(*S*)-1-(methoxycarbonyl)ethylidene]-α-D-glucopyranoside (10)

Glucoside **9** (2.58 g, 9.76 mmol) was dissolved in anhydrous acetonitrile (30 mL). α,α-Dimethoxytoluene (1.97 mL, 13.6 mmol) was then added followed by a catalytic amount of CSA (20 mg). The reaction was carried out at reduced pressure at 30 °C to remove methanol generated during reaction. After 2.5 h, TLC analysis (5%, methanol–dichloromethane) showed that the reaction was complete. The reaction was quenched with five drops of Et_3N and concentrated. The crude acetal product was dissolved in pyridine (50 mL) and acetic anhydride (50 mL) and the reaction allowed to proceed overnight under argon atmosphere after which TLC analysis (2:1, hexanes–ethyl acetate) showed maximum product formation. The mixture was co-evaporated with toluene and purified by flash column chromatography on silica gel (3:1 → 1:1, hexanes–ethyl acetate) to provide the product **10** (3.18 g, 83%) as a white solid; R_f 0.56 (1:1, hexanes–ethyl acetate); $[\alpha]_D$ +27.1° (*c* 1.02, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.47 (m, 2H, ArH), 7.37 (m, 3H, ArH), 5.84 (d, 1H, $J_{1,2}$ 5.1 Hz, H-1), 5.53 (s, 1H, C–H benzylidene), 5.24 (dd, 1H, $J_{2,3}$ 3.5 Hz, $J_{3,4}$ 3.5 Hz, H-3), 4.41 (dd, 1H, $J_{5,6a}$ 5.2 Hz, $J_{6a,6b}$ 10.6 Hz, H-6a), 4.33 (dd, 1H, $J_{2,3}$ 3.4 Hz, $J_{1,2}$ 5.1 Hz, H-2), 3.94 (ddd, $J_{5,6a}$ 5.3 Hz, $J_{4,5}$ 9.8 Hz, H-5), 3.77 (s, 3H, C*H*$_3$OC(O)), 3.72 (m, 2H, H-4, H-6b), 2.12 (s, 3H, C*H*$_3$C(O)O), 1.76 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.8 (C=O), 169.5 (C=O), 136.8 (Ar), 129.2 (Ar), 128.3 (Ar), 126.1 (Ar), 104.0 (quaternary C-pyruvate), 101.6 (PhCH benzylidene), 98.8 (C-1), 77.7 (C-4), 77.2 (C-2), 73.1 (C-3), 68.8 (C-6), 62.3 (C-5), 52.7 (C(O)O*CH*$_3$), 22.4 (*CH*$_3$C(O)O), 20.9 (CH_3 pyruvate); ESI HRMS calcd for $C_{19}H_{22}O_9Na$ 417.1156. Found 417.1154; Anal. Calcd for $C_{19}H_{22}O_9$: C, 57.86; H, 5.62. Found: C, 58.01; H, 5.77.

3.1.4. 3-*O*-Acetyl-6-*O*-benzyl-1,2-*O*-[(*S*)-1-(methoxycarbonyl)ethylidene]-α-D-glucopyranoside (7)

Benzylidene acetal **10** (3.89 g, 9.88 mmol) was dissolved in dry dichloromethane (20 mL) and the reaction vessel placed under an argon atmosphere. Et_3SiH (16 mL, 98.8 mmol) was added and the mixture cooled to 0 °C using an ice-water bath. TFA (7.6 mL, 98.8 mmol) was added and the reaction allowed to proceed at 0 °C. After 1 h, TLC analysis (15%, ethyl acetate–toluene) showed no remaining starting material. The reaction mixture was diluted with dichloromethane and transferred to a separatory funnel where the organic layer was washed with saturated aqueous sodium bicarbonate, distilled water, and saturated aqueous sodium chloride. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated to dryness. The crude product was purified by flash column chromatography on silica gel (15% → 30%, ethyl acetate–toluene) to provide the product **7** (3.35 g, 85%) as a white solid; R_f 0.18 (15%, ethyl acetate–toluene); $[\alpha]_D$ +12.9° (*c* 1.03, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35–7.26 (m, 5H, ArH), 5.81 (d, 1H, $J_{1,2}$ 5.1 Hz, H-1), 5.03 (vt, 1H, $J_{2,3}$ 3.4 Hz, $J_{3,4}$ 3.4 Hz, H-3), 4.62 (d, 1H, J_{gem} 12.2 Hz, PhC*H*$_2$O), 4.57 (d, 1H, J_{gem} 12.1 Hz, PhC*H*$_2$O), 4.38 (ddd, 1H, $J_{2,4}$ 0.8 Hz, $J_{2,3}$ 3.1 Hz, $J_{1,2}$ 5.1 Hz,



Scheme 5. Reagents and conditions: (a) 4-nitrophenyl chloroformate, pyr, CH_2Cl_2, 99%; (b) amine **4**, Et_3N, CH_2Cl_2, 95%; (c) (i) 1 M $NaOCH_3$, CH_3OH, (ii) H_2O, 88%; (d) azapolyacrylamide, sodium ascorbate, $CuSO_4$, and H_2O.

H-2), 3.83 (m, 1H, H-5), 3.76 (m, 4H, H-4, C*H*$_3$OC(O)), 3.71 (m, 2H, H-6a, H-6b), 2.83 (br s, 1H, O-H), 2.09 (s, 3H, C*H*$_3$C(O)O), 1.77 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.7 (C═O), 169.4 (C═O), 137.7 (Ar), 128.4 (Ar), 127.8 (Ar), 104.9 (quaternary C pyruvate), 98.1 (C-1), 74.8, 74.5 (C-2, C-3), 73.7 (PhCH_2O), 70.2 (C-5), 69.6 (C-6), 69.2 (C-4), 52.7 (C(O)OCH_3), 21.7 (*C*H_3C(O)O), 20.9 (CH_3 pyruvate); ESI HRMS calcd for $C_{19}H_{24}O_9Na$ 419.1313. Found 419.1311; Anal. Calcd for $C_{19}H_{24}O_9$: C, 57.57; H, 6.10. Found: C, 57.29; H, 6.45.

3.1.5. Phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (11)

α,β-Galactose pentaacetate (40.6 g, 0.104 mol) was dissolved in dry dichloromethane (60 mL) and placed under argon atmosphere. PhSH (16.0 mL, 0.156 mol) was added and the solution was cooled to 0 °C in an ice-water bath while stirring. Once cool $BF_3{\cdot}OEt_2$, (19.6 mL, 0.156 mol) was added and the reaction allowed to proceed while warming to room temperature. After 2 h TLC analysis (1:1, hexanes–ethyl acetate) showed maximum product formation. The reaction was quenched with Et_3N until neutral and the mixture was diluted with dichloromethane (20 mL) and concentrated to dryness. The crude product was purified by flash column chromatography on silica gel (2:1, hexanes–ethyl acetate) to obtain the product **11** (45.1 g, 98%) as a white foam; R_f 0.40 (1:1, hexanes–ethyl acetate); $[\alpha]_D$ + 3.5° (*c* 1.06, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.52 (m, 2H, ArH), 7.32 (m, 3H, ArH), 5.42 (dd, 1H, $J_{4,5}$ 1.0 Hz, $J_{3,4}$ 3.4 Hz, H-4), 5.24 (dd, 1H, $J_{2,3}$ 9.9, $J_{1,2}$ 9.9 Hz, H-2), 5.06 (dd, 1H, $J_{3,4}$ 3.3 Hz, $J_{2,3}$ 9.9 Hz, H-3), 4.72 (d, 1H, $J_{1,2}$ 9.9 Hz, H-1), 4.20 (dd, 1H, $J_{5,6a}$ 7.0 Hz, $J_{6a,6b}$ 11.3 Hz, H-6a), 4.12 (dd, 1H, $J_{5,6b}$ 6.2 Hz, $J_{6a,6b}$ 11.4 Hz, H-6b), 3.94 (ddd, 1H, $J_{4,5}$ 1.0, $J_{5,6b}$ 6.1 Hz, $J_{5,6a}$ 7.1 Hz, H-5), 2.12 (s, 3H, C*H*$_3$C(O)O), 2.10 (s, 3H, C*H*$_3$C(O)O), 2.05 (s, 3H, C*H*$_3$C(O)O), 1.98 (s, 3H, C*H*$_3$C(O)O); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.3 (C═O), 170.2 (C═O), 170.0 (C═O), 169.4 (C═O), 132.6 (Ar), 132.4 (Ar), 128.9 (Ar), 128.1 (C═O), 86.6 (C-1), 74.4 (C-5), 72.0 (C-3), 67.3, 67.2 (C-2, C-4), 61.6 (C-6), 20.8 (*C*H_3C(O)O), 20.6 (*C*H_3C(O)O), 20.5 (*C*H_3C(O)O);

ESI HRMS calcd for $C_{20}H_{24}O_9SNa$ 463.10332. Found 463.10322; Anal. Calcd for $C_{20}H_{24}O_9S$: C, 54.54; H, 5.49; S, 7.28. Found: C, 54.38; H, 5.56; S, 7.29.

3.1.6. Phenyl 1-thio-β-D-galactopyranoside (12)

The acetylated thiogalactoside **11** (11.84 g, 26.9 mmol) was dissolved in 60 mL anhydrous methanol to which a catalytic amount of sodium metal (0.045 g) was added. The reaction was allowed to proceed overnight at room temperature and under argon atmosphere. Subsequent TLC analysis (20%, methanol–dichloromethane) showed no remaining starting material The reaction was neutralized with Dowex H^+ ion exchange resin, filtered, and concentrated to dryness. The crude product was recrystalized from anhydrous ethanol and filtered to give the pure product **12** (7.10 g, 97%) as a white solid; R_f 0.79 (20%, methanol–dichloromethane); $[\alpha]_D$ −51.6° (*c* 1.00, CH_3OH); ^1H NMR (500 MHz, D_2O) δ 7.65 (m, 2H, ArH), 7.47 (m, 3H, ArH), 4.84 (d, 1H, $J_{1,2}$ 13.2 Hz, H-1), 4.06 (d, 1H, $J_{3,4}$ 3.2 Hz, H-4), 3.85–3.75 (m, 4H, H-3, H-5, H-6ab), 3.70 (m, 1H, H-2); ^{13}C NMR (125 MHz, D_2O): δ 133.7 (Ar), 132.1 (Ar), 130.3 (Ar), 128.8 (Ar), 89.0 (C-1), 79.9 (C-5), 74.9 (C-3), 70.2 (C-2), 69.7 (C-4), 61.9 (C-6); ESI HRMS calcd for $C_{12}H_{16}O_5Na$ 295.0611. Found 295.0607; Anal. Calcd for $C_{12}H_{16}O_5$: C, 52.93; H, 5.92; S, 11.77. Found: C, 52.67; H, 5.99; S, 11.76.

3.1.7. Phenyl 2,3-di-*O*-benzoyl-4,6-*O*-*para*-methoxybenzylidenyl-1-thio-β-D-galactopyranoside (13)

To a solution of dry phenyl 1-thio-β-D-galactopyranoside **12** (5.43 g, 19.9 mmol) in anhydrous acetonitrile (50 mL), $CH_3OPhCH(OCH_3)_2$ (4.1 mL, 23.9 mmol) was added followed by a catalytic amount of CSA (100 mg). The reaction was done at reduced pressure at 30 °C to remove methanol generated during the reaction. After 1.5 h, TLC analysis (10%, methanol–dichloromethane) showed the reaction to be complete. The reaction was quenched with five drops of Et_3N and concentrated. The crude *para*-methoxybenzylidene product was dissolved in pyridine (50 mL) and BzCl (11.6 mL, 99.7 mmol) was added slowly and the reaction proceeded overnight. Once again TLC analysis (2:1, hexanes–ethyl acetate) showed maximum product formation and no remaining starting material. The mixture was concentrated to dryness while co-evaporating with toluene (3 × 20 mL). The crude product was purified by flash column chromatography on silica gel (3:1 → 1:1, hexanes–ethyl acetate) to provide the product **13** (10.5 g, 88%) as a white foam; R_f 0.71 (1:1, hexanes–ethyl acetate); $[\alpha]_D$ +81.4° (*c* 1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.97 (m, 2H, ArH), 7.92 (m, 2H, ArH), 7.62 (m, 2H, ArH), 7.52 (m, 1H, ArH), 7.46 (m, 1H, ArH), 7.39 (m, 2H, ArH), 7.31 (m, 7H, ArH), 6.87 (m, 2H, ArH), 5.80 (t, 1H, $J_{1,2}$ 9.9 Hz, $J_{2,3}$ 9.9 Hz, H-2), 5.46 (s, 1H, benzylidene C–H), 5.35 (dd, 1H, $J_{3,4}$ 3.4 Hz, $J_{3,2}$ 10.0 Hz, H-3), 4.96 (d, 1H, $J_{1,2}$ 9.8 Hz, H-1), 4.57 (d, 1H, $J_{3,4}$ 3.3 Hz, H-4), 4.43 (dd, 1H, $J_{5,6a}$ 1.3 Hz, $J_{6a,6b}$ 12.3 Hz, H-6a), 4.08 (dd, 1H, $J_{5,6b}$ 1.4 Hz, $J_{6a,6b}$ 12.3, H-6b), 3.82 (s, 3H, CH_3OPh), 3.74 (s, 1H, H-5); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.2 (C═O), 164.9 (C═O), 160.1 (Ar), 133.9 (Ar), 133.3 (Ar), 133.1 (Ar), 131.1 (Ar), 130.2 (Ar), 129.9 (Ar), 129.8 (Ar), 129.7 (Ar), 129.1 (Ar), 128.8 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (Ar), 127.8 (Ar), 114.7 (Ar), 113.5 (Ar), 100.9 (benzylidene C–H), 85.3 (C-1), 74.1 (C-3), 73.7 (C-4), 69.9 (C-5), 69.1 (C-6), 67.1 (C-2), 55.3 (OCH_3); ESI HRMS Calcd for $C_{34}H_{30}O_8SNH_4$ 616.2000. Found 616.1998; Anal. Calcd for $C_{34}H_{30}O_8S$: C, 68.21; H, 5.05; S, 5.36. Found: C, 68.25; H, 5.28; S, 5.36.

3.1.8. Phenyl 6-*O*-acetyl-2,3-di-*O*-benzoyl-4-*O*-*para*-methoxybenzyl-1-thio-β-D-galactopyranoside (6)

The *p*-methoxylbenzylidene acetal **13** (0.540 g, 0.902 mmol) was dissolved in dry dichloromethane (30 mL) to which 1.0 M BH_3·THF (4.51 mL, 4.51 mmol) was added followed by TMSOTf (24 μL, 0.135 mmol). The reaction was allowed to proceed at room temperature for 1.5 h at which point TLC (1:1, hexanes–ethyl acetate) showed no remaining starting material. The reaction was quenched with two drops of Et_3N followed by very slow addition of methanol until bubbling ceased. The reaction mixture was then concentrated to dryness and subsequently dissolved in a 1:1 mixture of pyridine and acetic anhydride (10 mL) and allowed to react overnight. TLC analysis (1:1, hexanes–ethyl acetate) showed no remaining intermediate. The mixture was co-evaporated with toluene (3 × 20 mL) until dry. The crude product was purified by flash column chromatography on silica gel (3:1 → 2:1, hexanes–ethyl acetate) and recrystalized from ethyl acetate and hexanes to give the product **6** (0.486 g, 84%) as a white powder; R_f 0.58 (1:1, hexanes–ethyl acetate); $[\alpha]_D$ +66.7° (*c* 1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.96 (m, 4H, ArH), 7.51 (m, 4H, ArH), 7.38 (m, 4H, ArH), 7.26 (m, 3H, ArH), 7.13 (m, 2H, ArH), 6.76 (m, 2H, ArH), 5.87 (vt, 1H, $J_{1,2}$ 10.0 Hz, $J_{2,3}$ 10.0 Hz, H-2), 5.34 (dd, 1H, $J_{3,4}$ 2.9 Hz, $J_{2,3}$ 10.0 Hz, H-3), 4.91 (d, 1H, $J_{1,2}$ 10.0 Hz, H-1), 4.68 (d, 1H, J_{gem} 11.4 Hz, CH_3OPhCH_2O), 4.46 (d, 1H, J_{gem} 11.4 Hz, OCH_2PhOCH_3), 4.35 (dd, 1H, $J_{5,6a}$ 6.8 Hz, $J_{6a,6b}$ 11.3 Hz, H-6a), 4.16 (d, 1H, $J_{3,4}$ 2.8 Hz, H-4), 4.11 (dd, 1H, $J_{5,6b}$ 6.2 Hz, $J_{6a,6b}$ 11.3 Hz, H-6b), 3.91 (m, 1H, H-5), 3.76 (s, 3H, OCH_2PhOCH_3), 2.03 (s, 3H, $CH_3C(O)O$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.4 (C═O), 165.9 (C═O), 165.2 (C═O), 159.3 (ArC-O), 133.5 (Ar), 133.2 (Ar), 132.8 (Ar), 132.5 (Ar), 129.9 (Ar), 129.8 (Ar), 129.5 (Ar), 129.4 (Ar), 128.9 (Ar), 128.8 (Ar), 128.5 (Ar), 128.4 (Ar), 127.9 (Ar), 113.8 (Ar), 86.7 (C-1), 76.1 (C-5), 75.9 (C-3), 74.3 (CH_3OPhCH_2O), 72.9 (C-4), 68.3 (C-2), 62.7 (C-6), 55.2 (CH_3OPhCH_2O), 20.8 ($CH_3C(O)O$); ESI HRMS calcd for $C_{36}H_{34}O_9SNa$ 665.1816. Found 665.1806; Anal. Calcd for $C_{36}H_{34}O_9S$: C, 67.27; H, 5.33; S, 4.99. Found: C, 67.37; H, 5.33; S, 4.78.

3.1.9. 6-*O*-acetyl-2,3-di-*O*-benzoyl-4-*O*-*para*-methoxybenzyl-β-D-galactopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-1,2-*O*-[(*S*)-1-(methoxycarbonyl)ethylidene]-α-D-glucopyranoside (14)

The acceptor **7** (0.518 g, 1.31 mmol) and donor **6** (1.26 g, 1.96 mmol) were combined with pre-activated 4 Å molecular sieves (0.500 g) and dissolved in anhydrous dichloromethane (25 mL). The mixture was stirred for 1 h at room temperature and under argon atmosphere. The contents were cooled to −20 °C after which the reaction was activated by the addition of NIS (0.209 g, 1.57 mmol) and AgOTf (0.050 g, 0.196 mmol). Reaction progress was monitored by TLC analysis (1:1, hexanes–ethyl acetate) and after 1 h the reaction was found to be complete. The reaction was quenched by the addition of Et_3N (1 drop) and the mixture was diluted with dichloromethane. This mixture was filtered through Celite and subsequently washed with saturated aqueous sodium bicarbonate, saturated aqueous sodium thiosulfate, distilled water, and saturated aqueous sodium chloride. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated to dryness. The crude product was purified by flash column chromatography on silica gel (1:1, hexanes–ethyl acetate) to give the product **14** (1.09 g, 90%) as a white foam; R_f 0.38 (1:1, hexanes–ethyl acetate); $[\alpha]_D$ +23.9° (*c* 1.08, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.96 (m, 2H, ArH), 7.89 (m, 2H, ArH), 7.50 (m, 2H, ArH), 7.35 (m, 7H, ArH), 7.19 (m, 2H, ArH), 7.15 (m, 2H, ArH), 6.75 (m, 2H, ArH), 5.77 (m, 2H, $J_{1,2}$ 4.9 Hz, $J_{1',2'}$ 7.9 Hz, $J_{2',3'}$ 10.4 Hz, H-1, H-2′), 5.52 (vt, 1H, $J_{2,3}$ 2.5 Hz, $J_{3,4}$ 2.5 Hz, H-3), 5.22 (dd, 1H, $J_{3',4'}$ 3.1 Hz, $J_{2',3'}$ 10.5 Hz, H-3′), 4.65 (m, 2H, J_{gem} 11.3 Hz, $J_{1',2'}$ 7.9 Hz, CH_3OPhCH_2O, H-1′), 4.45 (d, 1H, J_{gem} 11.5 Hz, CH_3OPhCH_2O), 4.36 (d, 1H, J_{gem} 12.2 Hz, $PhCH_2O$), 4.31 (ddd, 1H, $J_{2,4}$ 1.0 Hz, $J_{2,3}$ 2.9 Hz, $J_{1,2}$ 5.2 Hz, H-2), 4.27 (dd, 1H, $J_{5',6a'}$ 5.9 Hz, $J_{6a',6b'}$ 11.0 Hz, H-6a′), 4.15 (d, 1H, J_{gem} 12.2 Hz, $PhCH_2O$), 4.11 (m, 2H, H-4′, H-6b′), 3.89 (m, 1H, H-4), 3.82 (m, 1H, H-5′), 3.74 (s, 3H, CH_3OPhCH_2O), 3.74 (s, 3H, $C(O)OCH_3$), 3.70 (m, 1H, H-5), 3.39 (m, 2H, $J_{5,6a}$ 2.3 Hz, $J_{6a,6b}$ 10.9 Hz, $J_{5,6b}$ 3.5 Hz, $J_{6a,6b}$ 10.9 Hz, H-6a, H-6b), 2.07 (s, 3H, $CH_3C(O)O$), 2.01 (s, 3H, $CH_3C(O)O$), 1.67 (s, 3H,

CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.4 (C=O), 169.5 (C=O), 169.2 (C=O), 165.9 (C=O), 164.9 (C=O), 159.4 ($ArOCH_3$), 137.9 (Ar), 133.5 (Ar), 133.2 (Ar), 130.1 (Ar), 129.8 (Ar), 129.6 (Ar), 129.5 (Ar), 129.4 (Ar), 128.9 (Ar), 128.5 (Ar), 128.4 (Ar), 128.4 (Ar), 127.7 (Ar), 113.8 (Ar), 105.3 (C, pyruvate), 102.6 (C-1′), 98.1 (C-1), 76.1 (C-4), 74.6 (C-2), 74.5 (CH_3OPhCH_2O), 74.1 (C-3′), 73.1 ($PhCH_2O$), 72.6, 72.3 (C-4′, C-5′), 70.6 (C-3), 69.9 (C-2′), 68.6 (C-5), 68.3 (C-6), 62.0 (C-6′), 55.2 (CH_3OPhCH_2O), 52.6 ($C(O)OCH_3$), 21.2 ($CH_3C(O)O$), 20.9 ($CH_3C(O)O$), 20.8 (CH_3, pyruvate); ESI HRMS calcd for $C_{55}H_{58}O_{18}Na$ 917.3871. Found 917.3864; Anal. Calcd for $C_{55}H_{58}O_{18}$: C, 63.36; H, 5.64. Found: C, 63.02; H, 5.73.

3.1.10. 6-*O*-Acetyl-2,3-di-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-1,2-*O*-[(*S*)-1-(methoxycarbonyl)ethylidene]-α-D-glucopyranoside (15)

Compound **14** (0.685 g, 0.737 mmol) was dissolved in anhydrous dichloromethane (24 mL) and placed under argon atmosphere. The stirred solution was cooled to −20 °C in a dry ice/acetone bath. TfOH (0.065 mL, 0.737 mmol) was added and the reaction instantly turned purple indicating a complete reaction. TLC (20%, ethyl acetate–toluene) confirmed the reaction to be complete. The reaction mixture was neutralized with Et_3N until neutral and the contents were diluted with dichloromethane. This crude mixture was washed with saturated aqueous sodium bicarbonate, distilled water, and saturated aqueous sodium chloride. The organic phase was dried over anhydrous sodium sulfate, filtered, and concentrated. The crude product was purified by flash column chromatography on silica gel (2:1, hexanes–ethyl acetate) to give the product **15** (0.483 g, 81%) as a white foam; R_f 0.22 (20%, ethyl acetate–toluene); $[\alpha]_D$ +41.8° (*c* 1.08, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.99 (m, 2H, ArH), 7.93 (m, 2H, ArH), 7.54 (m, 2H, ArH), 7.37 (m, 7H, ArH), 7.22 (m, 2H, ArH), 5.80 (d, 1H, $J_{1,2}$ 5.1 Hz, H-1), 5.71 (dd, 1H, $J_{1',2'}$ 7.9 Hz, $J_{2',3'}$ 10.4 Hz, H-2′), 5.57 (vt, 1H, $J_{2,3}$ 2.8 Hz, $J_{3,4}$ 2.8 Hz, H-3), 5.24 (dd, 1H, $J_{3',4'}$ 3.3 Hz, $J_{2',3'}$ 10.4 Hz, H-3′), 4.70 (d, 1H, $J_{1',2'}$ 7.9 Hz, H-1′), 4.43 (dd, 1H, $J_{5',6a'}$ 7.1 Hz, $J_{6a',6b'}$ 11.5 Hz, H-6a′), 4.40 (d, 1H, J_{gem} 12.2 Hz, $PhCH_2O$), 4.36 (ddd, 1H, $J_{2,4}$ 1.2 Hz, $J_{2,3}$ 3.0 Hz, $J_{1,2}$ 5.2 Hz, H-2), 4.33 (dd, 1H, $J_{5',6b'}$ 5.9 Hz, $J_{6a',6b'}$ 11.4 Hz, H-6b′), 4.27 (m, 1H, $J_{3',4'}$ 3.1 Hz, H-4′), 4.18 (d, 1H, J_{gem} 12.3 Hz, $PhCH_2O$), 3.94 (m, 1H, H-4), 3.90 (m, 1H, H-5′), 3.77 (s, 3H, $C(O)OCH_3$), 3.73 (m, 1H, H-5), 3.43 (dd, 1H, $J_{5,6a}$ 2.4 Hz, $J_{6a,6b}$ 10.9 Hz, H-6a), 3.40 (dd, 1H, $J_{5,6b}$ 3.5 Hz, $J_{6a,6b}$ 10.9 Hz, H-6b), 2.45 (d, 1H, $J_{4',OH}$ 6.0 Hz, 4′-OH), 2.12 (s, 3H, $CH_3C(O)O$), 2.11 (s, 3H, $CH_3C(O)O$), 1.71 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.9 (C=O), 169.5 (C=O), 169.3 (C=O), 165.8 (C=O), 164.9 (C=O), 137.9 (Ar), 133.5 (Ar), 133.3 (Ar), 129.9 (Ar), 129.7 (Ar), 129.3 (Ar), 129.0 (Ar), 128.5 (Ar), 128.4 (Ar), 127.8 (Ar), 105.4 (C, pyruvate), 102.6 (C-1′), 98.2 (C-1), 76.1 (C-4), 74.0 (C-2, C-3′), 73.2 ($PhCH_2O$), 72.2 (C-5′), 70.5 (C-3), 69.5 (C-2′), 68.7 (C-5), 68.3 (C-6), 67.1 (C-4′), 61.9 (C-6′), 52.6 ($C(O)OCH_3$), 21.2 ($CH_3C(O)O$), 20.9 ($CH_3C(O)O$), 20.8 (CH_3, pyruvate); ESI HRMS Calcd for $C_{41}H_{44}O_{17}Na$ 831.2471. Found 831.2473; Anal. Calcd for $C_{41}H_{44}O_{17}$: C, 60.89; H, 5.48; O, 33.63. Found: C, 61.06; H, 5.54.

3.1.11. 3,4,6-tri-*O*-Acetyl-2-azido-2-deoxy-α-D-galactopyranosyl-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-1,2-*O*-[(*S*)-1-(methoxycarbonyl)ethylidene]-α-D-glucopyranoside (16)

The acceptor **15** (0.203 g, 0.251 mmol) and 4 Å MS (100 mg) were combined in a dry flask and dissolved in anhydrous diethyl ether (2 mL). The reaction mixture was stirred for 1 h under argon atmosphere, after which the activator TMSOTf (6.8 mL, 0.038 mmol) was added. The trichloroacetimidate donor **5** (0.478 g, 1.00 mmol) was dissolved in anhydrous diethyl ether (2 mL) and added dropwise at room temperature to the stirred solution. The reaction was allowed to proceed for 2 h and reaction progress was monitored by TLC analysis (60%, ethyl acetate–hexanes) at which point the donor was consumed. The reaction was quenched with one drop of Et_3N, filtered through Celite, and concentrated to dryness. The crude reaction mixture was purified by flash column chromatography in silica gel (1:1, ethyl acetate–hexanes) to provide the product **16** (0.134 g, 48%) as a white foam; R_f 0.44 (60%, ethyl acetate–toluene); $[\alpha]_D$ +77.2° (*c* 1.04, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.95 (m, 2H, ArH), 7.89 (m, 2H, ArH), 7.49 (m, 2H, ArH), 7.34 (m, 7H, ArH), 7.20 (m, 2H, ArH), 5.78 (d, 1H, $J_{1,2}$ 5.2 Hz, H-1), 5.62 (dd, 1H, $J_{1',2'}$ 7.9 Hz, $J_{2',3'}$ 10.7 Hz, H-2′), 5.54 (vt, 1H, $J_{2,3}$ 2.5 Hz, $J_{3,4}$ 2.5 Hz, H-3), 5.43 (dd, 1H, $J_{3'',4''}$ 3.1 Hz, $J_{2'',3''}$ 11.1 Hz, H-3″), 5.21 (dd, 1H, $J_{3',4'}$ 3.1 Hz, $J_{2',3'}$ 10.7 Hz, H-3′), 5.01 (d, 1H, $J_{1'',2''}$ 3.5 Hz, H-1″), 4.72 (d, 1H, $J_{1',2'}$ 7.8 Hz, H-1′), 4.50 (m, 1H, H-5″), 4.46 (m, 2H, H-6ab′), 4.38 (m, 2H, H-4′, $PhCH_2O$), 4.34 (ddd, 1H, $J_{2,4}$ 0.9 Hz, $J_{2,3}$ 3.1 Hz, $J_{1,2}$ 5.2 Hz, H-2), 4.18 (d, 1H, J_{gem} 12.1 Hz, $PhCH_2O$), 3.92 (m, 2H, H-4, H-5′), 3.81 (m, 2H, H-2″, H-6a″), 3.75 (m, 4H, H-5, $C(O)OCH_3$), 3.43 (m, 3H, H-6ab, H-6b″), 2.12 (s, 3H, $CH_3C(O)O$), 2.11 (s, 3H, $CH_3C(O)O$), 2.07 (s, 3H, $CH_3C(O)O$), 1.85 (s, 3H, $CH_3C(O)O$), 1.70 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.4 (C=O), 170.1 (C=O), 169.9 (C=O), 169.6 (C=O), 169.3 (C=O), 166.0 (C=O), 164.7 (C=O), 137.9 (Ar), 133.6 (Ar), 133.3 (Ar), 129.8 (Ar), 129.6 (Ar), 129.2 (Ar), 128.7 (Ar), 128.4 (Ar), 128.3 (Ar), 127.7 (Ar), 127.6 (Ar), 105.3 (quaternary C, pyruvate), 102.4 (C-1′), 99.2 (C-1″), 98.1 (C-1), 76.2 (C-5′), 74.8 (C-4′), 74.3 (C-2), 73.5 (C-3′), 73.2 ($PhCH_2O$), 72.1 (C-4), 70.7 (C-3), 69.5 (C-2′), 68.6, 68.5 (C-5, C-3″), 68.3 (C-6), 67.1, 67.0 (C-4″, C-5″), 61.4 (C-6′), 60.5 (C-6″), 58.2 (C-2″), 52.6 ($CH_3OC(O)$), 21.3 ($CH_3C(O)O$), 20.9 ($CH_3C(O)O$), 20.8 ($CH_3C(O)O$), 20.6 ($CH_3C(O)O$), 20.5(5) ($CH_3C(O)O$), 20.5(1) ($CH_3C(O)O$); ESI HRMS Calc′d. for $C_{53}H_{59}N_3O_{24}$-Na 1144.3381. Found 1144.3376; Anal. Calcd for $C_{53}H_{59}N_3O_{24}$: C, 56.73; H, 5.30; N, 3.74. Found: C, 56.86; H, 5.42; N, 3.44.

3.1.12. 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-1,2-*O*-[(*S*)-1-(methoxycarbonyl)ethylidene]-α-D-glucopyranoside (17)

Azide **16** (0.297 g, 0.265 mmol) was placed in a dry flask dissolved in a 6:1 mixture of acetonitrile:triethylamine (8 mL) and placed under an argon atmosphere. Dithiothreitol (0.163 g, 1.06 mmol) was added and the reaction proceeded at room temperature overnight. Subsequent TLC analysis (60%, ethyl acetate–hexanes) showed no remaining starting material. A 1:1 mixture of acetic anhydride:pyridine was added to the reaction flask and again allowed to proceed overnight. The reaction mixture was then concentrated to dryness while co-evaporating with toluene. The crude product was then subjected to flash column chromatography on silica gel (40%, acetone–hexanes) to provide the product **17** (0.262 g, 87%) as a white foam; R_f 0.30 (1:1, acetone–hexanes); $[\alpha]_D$ +77.2° (*c* 1.12, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.94 (m, 2H, ArH), 7.91 (m, 2H, ArH), 7.51 (m, 2H, ArH), 7.41–7.31 (m, 7H, ArH), 7.21 (m, 2H, ArH), 6.05 (d, 1H, $J_{2'',NH}$ 9.5 Hz, N–H), 5.80 (d, 1H, $J_{1,2}$ 5.1 Hz, H-1), 5.69 (dd, 1H, $J_{1',2'}$ 7.9 Hz, $J_{2',3'}$ 10.5 Hz, H-2′), 5.61 (m, 1H, H-3), 5.35 (m, 1H, H-4″), 5.29 (dd, 1H, $J_{3'',4''}$ 3.1 Hz, $J_{2'',3''}$ 11.5 Hz, H-3″), 5.24 (dd, 1H, $J_{3',4'}$ 3.0 Hz, $J_{2',3'}$ 10.6 Hz, H-3′), 5.06 (d, 1H, $J_{1'',2''}$ 3.9 Hz, H-1″), 4.71 (d, 1H, $J_{1',2'}$ 7.8 Hz, H-1′), 4.69 (ddd, 1H, $J_{1'',2''}$ 3.9 Hz, $J_{2'',NH}$ 9.7 Hz, $J_{2'',3''}$ 11.6 Hz, H-2″), 4.47 (dd, 1H, $J_{5',6a'}$ 6.2 Hz, $J_{6a',6b'}$ 11.2 Hz, H-6a′), 4.43 (d, 1H, $J_{3',4'}$ 3.0 Hz, H-4′), 4.39 (m, 2H, J_{gem} 12.2 Hz, H-5″, $PhCH_2O$), 4.33 (ddd, 1H, $J_{2,4}$ 1.0 Hz, $J_{2,3}$ 2.5 Hz, $J_{1,2}$ 5.1 Hz, H-2), 4.19 (d, 1H, J_{gem} 12.1 Hz, $PhCH_2O$), 4.02 (dd, 1H, $J_{5',6b'}$ 8.2 Hz, $J_{6a',6b'}$ 11.2 Hz, H-6b′), 3.94 (m, 2H, H-4, H-5′), 3.77 (s, 3H, $C(O)OCH_3$), 3.74 (m, 1H, H-5), 3.68 (dd, 1H, $J_{5'',6a''}$ 8.5 Hz, $J_{6a'',6b''}$ 10.9 Hz, H-6a″), 3.46 (dd, 1H, $J_{5,6a}$ 2.2 Hz, $J_{6a,6b}$ 10.9 Hz, H-6a), 3.40 (dd, 1H, $J_{5,6b}$ 3.5 Hz, $J_{6a,6b}$ 10.8 Hz, H-6b), 3.20 (dd, 1H, $J_{5'',6b''}$ 6.1 Hz, $J_{6a'',6b''}$ 10.9 Hz, H-6b″), 2.14 (s, 3H, $CH_3C(O)O$), 2.12 (s, 3H, $CH_3C(O)O$), 2.09 (s, 3H, $CH_3C(O)O$), 2.08 (s, 3H, $CH_3C(O)O$), 2.04 (s, 3H, $CH_3C(O)O$), 1.77 (s, 3H, $CH_3C(O)NH$), 1.73 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$)

δ 170.7 (C═O), 170.2 (C═O), 169.7 (C═O), 169.4 (C═O), 169.2 (C═O), 165.8 (C═O), 164.9 (C═O), 137.9 (Ar), 133.6 (Ar), 133.4 (Ar), 129.7 (Ar), 129.6 (Ar), 129.1 (Ar), 128.8 (Ar), 128.6 (Ar), 128.5 (Ar), 128.4 (Ar), 127.8 (Ar), 127.7 (Ar), 105.5 (quaternary C, pyruvate), 102.6 (C-1′), 98.2 (C-1″), 97.9 (C-1), 76.4 (C-4), 74.1 (C-2), 73.2 (PhCH$_2$O), 73.1 (C-3′), 72.2 (C-4′), 72.1 (C-5′), 70.0 (C-3), 69.6 (C-2′), 68.3 (C-5), 68.2 (C-6), 68.1 (C-3″), 66.8, 66.7 C-4″, -5″), 60.6, 60.5 (C-6′, C-6″), 52.7 (*C*H$_3$OC(O)), 47.6 (C-2″), 23.2 (*C*H$_3$C(O)O), 21.2 (*C*H$_3$C(O)O), 20.9 (*C*H$_3$C(O)O), 20.8 (*C*H$_3$C(O)O), 20.7 (*C*H$_3$, pyruvate), 20.5 (*C*H$_3$C(O)NH); ESI HRMS calcd for $C_{55}H_{63}NO_{25}Na$ 1160.3581. Found 1160.3573; Anal. Calcd for $C_{55}H_{63}NO_{25}$: C, 58.04; H, 5.58; N, 1.23. Found: C, 57.76; H, 5.57; N, 1.28.

3.1.13. 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-3-*O*-acetyl-1,2-*O*-[(*S*)-1-(methoxycarbonyl)ethylidene]-α-D-glucopyranoside (3)

The 6-*O*-benzyl trisaccharide derivative **17** (0.738 g, 0.648 mmol) was combined with 20% wt. Pd(OH)$_2$ on charcoal (0.250 g) in the reaction flask and the starting material was dissolved in ethyl acetate (14 mL). The reaction vessel was placed under a hydrogen atmosphere and the hydrogenation reaction was allowed to proceed at room temperature for 3 h. TLC analysis (1:1, acetone–hexanes) showed the reaction to be complete. The reaction mixture was filtered through a Millipore filter to remove the solid catalyst and the filtrate was subsequently concentrated to dryness to provide the product **3** (0.660 g, 97%) as a white foam; R_f 0.24 (1:1, acetone–hexanes); $[\alpha]_D$ +81.6° (*c* 1.11, CHCl$_3$); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.93 (m, 2H, ArH), 7.90 (m, 2H, ArH), 7.48 (m, 2H, ArH), 7.36 (m, 4H, ArH), 6.28 (d, 1H, $J_{2'',NH}$ 10.4 Hz, N–H), 5.74 (m, 2H, H-1, H-2′), 5.50 (m, 1H, H-3), 5.33 (m, 1H, H-4″), 5.30 (dd, 1H, $J_{3'',4''}$ 3.2 Hz, $J_{2'',3''}$ 11.4 Hz, H-3″), 5.24 (dd, 1H, $J_{3',4'}$ 2.9 Hz, $J_{2',3'}$ 10.5 Hz, H-3′), 5.01 (d, 1H, $J_{1'',2''}$ 3.8 Hz, H-1″), 4.93 (d, 1H, $J_{1',2'}$ 8.0 Hz, H-1′), 4.65 (ddd, 1H, $J_{1'',2''}$ 3.8 Hz, $J_{2'',NH}$ 9.6 Hz, $J_{2'',3''}$ 11.4 Hz, H-2″), 4.46 (dd, 1H, $J_{5',6a'}$ 4.2 Hz, $J_{6a',6b'}$ 9.0 Hz, H-6a′), 4.42 (d, 1H, $J_{3',4'}$ 2.9 Hz, H-4′), 4.38 (m, 1H, H-5″), 4.32 (ddd, 1H, $J_{2,4}$ 1.0 Hz, $J_{2,3}$ 2.6 Hz, $J_{1,2}$ 5.1 Hz, H-2), 4.00 (m, 3H, H-4, H-5′, H-6b′), 3.74 (s, 3H, C(O)OC*H*$_3$), 3.66 (m, 3H, H-5, H-6a, H-6a″), 3.49 (m, 1H, H-6b), 3.10 (dd, 1H, $J_{5'',6b''}$ 6.0 Hz, $J_{6a'',6b''}$ 10.9 Hz, H-6b″), 2.10 (s, 3H, C*H*$_3$C(O)O), 2.09 (s, 3H, C*H*$_3$C(O)O), 2.06 (s, 3H, C*H*$_3$C(O)O), 2.05 (s, 3H, C*H*$_3$C(O)O), 2.01 (s, 3H, C*H*$_3$C(O)O), 1.76 (s, 3H, C*H*$_3$C(O)NH), 1.72 (s, 3H, CH$_3$); ^{13}C NMR (125 MHz, CDCl$_3$) δ 170.8 (C═O), 170.7 (C═O), 170.2 (C═O), 169.7 (C═O), 169.3 (C═O), 169.2 (C═O), 165.9 (C═O), 165.1 (C═O), 133.7 (Ar), 133.5 (Ar), 129.7 (Ar), 129.6 (Ar), 128.9 (Ar), 128.7 (Ar), 128.5 (Ar), 105.6 (quaternary C, pyruvate), 102.3 (C-1′), 98.6 (C-1″), 97.8 (C-1), 75.3 (C-4), 73.9 (C-2), 73.5 (C-3′), 72.6 (C-4′), 72.3 (C-5′), 70.2 (C-3), 69.2, 68.9 (C-5, C-2′), 67.9 (C-3″), 66.8, 66.7 (C-4″, C-5″), 61.5 (C-6″), 60.6, 60.4 (C-6, C-6′), 52.7 (C(O)OCH$_3$), 47.7 (C-2″), 23.1 (*C*H$_3$C(O)O), 21.2 (*C*H$_3$C(O)O), 20.9 (*C*H$_3$C(O)O), 20.8 (*C*H$_3$C(O)O), 20.6 (*C*H$_3$C(O)NH), 20.5 (CH$_3$–pyruvate); ESI HRMS calcd for $C_{48}H_{57}NO_{25}Na$ 1070.3112. Found 1070.3105; Anal. Calcd for $C_{48}H_{57}NO_{25}$: C, 55.01; H, 5.48; N, 1.34. Found: C, 55.04; H, 5.65; N, 1.38.

3.1.14. 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-3-*O*-acetyl-6-(4-nitrophenylcarbonate)-1,2-*O*-[(*S*)-1-(methoxycarbonyl)ethylidene]-α-D-glucopyranoside (18)

The primary alcohol **3** (0.144 g, 0.137 mmol) was combined with 4-nitrophenyl chloroformate (0.033 g, 0.165 mmol) and dissolved in dry dichloromethane (3 mL). Pyridine (0.022 mL, 0.274 mmol) was then added and the reaction allowed to proceed at room temperature for 10 min. TLC analysis (1:1, acetone–hexanes) showed that no starting material remained. The reaction was diluted with dichloromethane and washed with saturated aqueous sodium bicarbonate, distilled water, and saturated aqueous sodium chloride. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated to dryness. The crude product was purified by flash column chromatography on silica gel (1:1, acetone–hexanes) to give the product **18** (0.165 g, 99%) was a white foam; R_f 0.26 (1:1, acetone–hexanes); $[\alpha]_D$ +92.2° (*c* 1.08, CHCl$_3$); ^{1}H NMR (600 MHz, CDCl$_3$) δ 8.27 (m, 2H, ArH), 7.93 (m, 4H, ArH), 7.48 (m, 2H, ArH), 7.38 (m, 2H, ArH), 7.32 (m, 2H, ArH), 7.28 (m, 2H, ArH), 6.02 (d, 1H, $J_{2'',NH}$ 9.5 Hz, N–H), 5.79 (d, 1H, $J_{1,2}$ 5.2 Hz, H-1), 5.72 (dd, 1H, $J_{1',2'}$ 7.7 Hz, $J_{2',3'}$ 10.5 Hz, H-2′), 5.64 (m, 1H, H-3), 5.34 (m, 1H, H-4″), 5.32 (dd, 1H, $J_{3',4'}$ 3.1 Hz, $J_{2',3'}$ 10.6 Hz, H-3′), 5.29 (dd, 1H, $J_{3'',4''}$ 3.2 Hz, $J_{2'',3''}$ 11.6 Hz, H-3″), 5.06 (d, 1H, $J_{1'',2''}$ 3.9 Hz, H-1″), 4.94 (d, 1H, $J_{1',2'}$ 7.8 Hz, H-1′), 4.66 (ddd, 1H, $J_{1'',2''}$ 3.8 Hz, $J_{2'',NH}$ 9.6 Hz, $J_{2'',3''}$ 11.5 Hz, H-2″), 4.50 (m, 1H, H-6a′), 4.44 (d, 1H, $J_{3',4'}$ 3.1 Hz, H-4′), 4.37 (m, 2H, H-2, H-5″), 4.32 (dd, 1H, $J_{5,6a}$ 2.2 Hz, $J_{6a,6b}$ 11.7 Hz, H-6a), 4.16 (dd, 1H, $J_{5,6b}$ 4.9 Hz, $J_{6a,6b}$ 11.8 Hz, H-6b), 4.00 (m, 3H, $J_{5,6a}$ 2.2 Hz, $J_{5,6b}$ 4.9 Hz, $J_{4,5}$ 9.5 Hz, H-5, H-5′, H-6b′), 3.83 (m, 1H, $J_{4,5}$ 9.5 Hz, H-4), 3.78 (s, 3H, C(O)OC*H*$_3$), 3.67 (m, 1H, $J_{5'',6a''}$ 8.4 Hz, $J_{6a'',6b''}$ 10.9 Hz, H-6a″), 3.21 (dd, 1H, $J_{5'',6b''}$ 6.1 Hz, $J_{6a'',6b''}$ 11.0 Hz, H-6b″), 2.13 (s, 3H, C*H*$_3$C(O)O), 2.12 (s, 3H, C*H*$_3$C(O)O), 2.08 (s, 3H, C*H*$_3$C(O)O), 2.07 (s, 3H, C*H*$_3$C(O)O), 2.03 (s, 3H, C*H*$_3$C(O)O), 1.78 (s, 3H, C*H*$_3$C(O)NH), 1.76 (s, 3H, CH$_3$); ^{13}C NMR (125 MHz, CDCl$_3$) δ 170.7 (C═O), 170.6 (C═O), 170.2 (C═O), 169.7 (C═O), 169.2 (C═O), 165.8 (C═O), 165.0 (Ar), 155.3 (Ar), 151.8 (Ar), 145.4 (Ar), 133.7 (Ar), 133.5 (Ar), 129.7 (Ar), 129.6 (Ar), 128.8 (Ar), 128.7 (Ar), 128.5 (Ar), 128.4 (Ar), 105.7 (quaternary C, pyruvate), 102.5 (C-1′), 98.3 (C-1″), 97.7 (C-1), 77.0 (C-4), 74.2 (C-2), 73.1 (C-3′), 72.3, 72.2 (C-4′, C-5′), 69.6, 69.5(9) (C-3, C-2′), 68.0 (C-3″), 67.4 (C-5), 66.8 (C-6), 66.6, 66.5 (C-4″, C-5″), 60.6 (C-6″), 60.5 (C-6′), 52.7 (*C*H$_3$OC(O)), 47.7 (C-2″), 23.1 (*C*H$_3$C(O)O), 21.2 (*C*H$_3$C(O)O), 20.9 (*C*H$_3$C(O)O), 20.7(8) (*C*H$_3$C(O)O), 20.7(6) (*C*H$_3$C(O)O), 20.6 (*C*H$_3$C(O)NH), 20.5 (CH$_3$, pyruvate); ESI HRMS calcd for $C_{55}H_{60}N_2O_{29}Na$ 1235.3174. Found 1235.3167; Anal. Calcd for $C_{55}H_{60}N_2O_{29}$: C, 54.46; H, 4.99; N, 2.31. Found: C, 54.45; H, 5.22; N, 2.19.

3.1.15. 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-(10-oxo-3,6,11-trioxa-9-azatetradec-13-ynyl)carbamoyl-1,2-*O*-[(*S*)-1-(methoxycarbonyl)ethylidene]-α-D-glucopyranoside (19)

The *p*-nitrophenylcarbamoyl derivative **18** (0.656 g, 0.541 mmol) was combined with the amine **4** (0.186 g, 0.811 mmol) and dissolved in dry CH_2Cl_2 (10 mL). Et_3N (0.151 mL, 1.08 mmol) was added and the reaction proceeded at room temperature for 2 h. The reaction mixture was concentrated to dryness and the crude product purified by flash column chromatography on silica gel (1:1, acetone–hexanes) to provide the product **19** (0.706 g, 95%) as a white foam; R_f 0.34 (1:1, acetone–hexanes); $[\alpha]_D$ +70.7° (*c* 1.00, CHCl$_3$); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.95 (m, 2H, ArH), 7.92 (m, 2H, ArH), 7.49 (m, 2H, ArH), 7.37 (m, 4H, ArH), 6.07 (d, 1H, $J_{2'',NH}$ 9.3 Hz, N–H), 5.75 (d, 1H, $J_{1,2}$ 5.2 Hz, H-1), 5.70 (dd, 1H, $J_{1',2'}$ 7.8 Hz, $J_{2',3'}$ 10.5 Hz, H-2′), 5.64 (br s, 1H, H-3), 5.40 (br s, 1H, N–H linker), 5.34 (m, 1H, $J_{3'',4''}$ 2.8 Hz, H-4″), 5.29 (t, 1H, $J_{3'',4''}$ 2.8 Hz, H-3″), 5.26 (m, 1H, H-3′), 5.04 (d, 1H, $J_{1'',2''}$ 3.9 Hz, H-1″), 4.89 (d, 1H, $J_{1',2'}$ 7.8 Hz, H-1′), 4.66 (m, 3H, $J_{1'',2''}$ 3.8 Hz, H-2″, OC*H*$_2$C≡CH), 4.48 (dd, 1H, $J_{5',6a'}$ 9.7 Hz, $J_{6a',6b'}$ 14.3 Hz, H-6a′), 4.43 (d, 1H, $J_{3',4'}$ 3.1 Hz, H-4′), 4.37 (m, 1H, H-5″), 4.31 (dd, 1H, $J_{2,3}$ 2.5 Hz, $J_{1,2}$ 5.2 Hz, H-2), 4.18 (dd, 1H, $J_{5,6a}$ 4.2 Hz, $J_{6a,6b}$ 11.8 Hz, H-6a), 4.03 (dd, 1H, $J_{5,6b}$ 1.2 Hz, $J_{6a,6b}$ 11.6 Hz, H-6b), 3.98 (m, 2H, H-5′, H-6b′), 3.82 (m, 1H, H-5), 3.75 (s, 3H, C(O)OC*H*$_3$), 3.72 (m, 1H, H-4), 3.66 (dd, 1H, $J_{5'',6a''}$ 8.5 Hz, $J_{6a'',6b''}$ 10.8 Hz, H-6a″), 3.60 (s, 4H, OC*H*$_2$C*H*$_2$O linker), 3.55 (m, 4H, HNCH$_2$C*H*$_2$O linker), 3.36 (m, 4H, HNC*H*$_2$CH$_2$O), 3.15 (dd, 1H,

$J_{5'',6b''}$ 6.0 Hz, $J_{6a'',6b''}$ 10.8 Hz, H-6b″), 2.48 (t, 1H, $J_{CH,CH2}$ 2.1 Hz, $OCH_2C{\equiv}CH$), 2.10 (s, 3H, $CH_3C(O)O$), 2.09 (s, 3H, $CH_3C(O)O$), 2.07 (s, 3H, $CH_3C(O)O$), 2.06 (s, 3H, $CH_3C(O)O$), 2.02 (s, 3H, $CH_3C(O)O$), 1.75 (s, 3H, CH_3 pyruvate), 1.72 (s, 3H, $CH_3C(O)NH$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.7 (C═O), 170.6 (C═O), 170.2 (C═O), 170.1 (C═O), 169.7 (C═O), 169.3 (C═O), 168.9 (C═O), 165.8 (C═O), 165.1 (C═O), 155.8 (C═O), 155.5 (C═O), 133.6 (Ar), 133.3 (Ar), 129.7 (Ar), 129.7 (Ar), 129.6 (Ar), 128.9 (Ar), 128.7 (Ar), 128.5 (Ar), 128.4 (Ar), 105.6 (quaternary C, pyruvate), 102.3 (C-1′), 98.4 (C-1″), 97.7 (C-1), 76.4 (C-4), 74.7 ($OCH_2C{\equiv}CH$), 73.9 (C-2), 73.3 (C-3″), 72.3, 72.1 (C-4′, C-5′), 70.3, (CH_2, linker), 70.0 (CH_2, linker), 69.9 CH_2 linker), 69.8 (C-3), 69.5 (C-2′), 68.0 (C-3′), 67.5 (C-5), 66.8, 66.7 (C-4″, C-5″), 63.4 (C-6), 60.4 (C-6, C-6′), 52.7 ($C(O)OCH_3$), 52.4 ($OCH_2C{\equiv}CH$), 47.6 (C-2″), 40.9 (CH_2 linker), 23.1 ($CH_3C(O)O$), 21.1 ($CH_3C(O)O$), 20.9 ($CH_3C(O)O$), 20.8 ($CH_3C(O)O$), 20.6 ($CH_3C(O)NH$), 20.4 (CH_3, pyruvate); ESI HRMS calcd for $C_{59}H_{73}N_3O_{30}Na$ 1326.4171. Found 1326.4159; Anal. Calcd for $C_{59}H_{70}N_3O_{30}$: C, 54.33; H, 5.64; N, 3.22. Found: C, 54.43; H, 5.79; N, 3.31.

3.1.16. 2-Acetamido-2-deoxy-α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-6-O-(10-oxo-3,6,11-trioxa-9-azatetradec-13-ynyl)carbamoyl-1,2-*O*-[(*S*)-1-(carboxy)ethylidene]-α-D-glucopyranoside (2)

The acetylated trisaccharide **19** (0.410 g, 0.314 mmol) was placed in a round bottomed flask and dissolved in anhydrous methanol (20 mL). A 1 M solution of CH_3ONa in methanol was prepared and added (0.314 mL, 0.314 mmol) to the reaction flask. The reaction was allowed to proceed for 2 days at which point TLC (10%, methanol–dichloromethane) showed the reaction was complete. The reaction mixture was concentrated to dryness and placed under high vacuum for 2 h. The residue was subsequently dissolved in 20 mL MilliQ water and the pH checked and adjusted to ensure a pH ~8/9. The hydrolysis of the methyl ester was allowed to proceed for 15 min at which point TLC analysis (4:5:1:0.1, dichloromethane–methanol–water–acetic acid) showed no remaining starting material. The reaction was quenched by dropwise addition of TFA until the pH was ~7. The reaction mixture was concentrated and lyophilized. The crude product was purified by HPLC on an X-Bridge C18 reverse-phase column (100% water (0.1%, TFA) → 100% acetonitrile) and was once again lyophilized to provide the product **2** (0.242 g, 88%) was a white powder; R_f 0.58 (4:5:1:0.1, dichloromethane–methanol–water–acetic acid); $[\alpha]_D$ +79.4° (*c* 1.20, H_2O); ^1H NMR (700 MHz, D_2O) δ 5.77 (d, 1H, $J_{1,2}$ 4.9 Hz, H-1), 4.90 (d, 1H, $J_{1'',2''}$ 3.9 Hz, H-1″), 4.62 (br s, 1H, $OCH_2C{\equiv}CH$), 4.52 (d, 1H, $J_{1',2'}$ 7.7 Hz, H-1′), 4.42 (m, 3H, $J_{5,6a}$ 6.4 Hz, H-3, H-6a, H-5″), 4.37 (t, 1H, $J_{1,2}$ 4.1 Hz, H-2), 4.24 (dd, 1H, $J_{5,6b}$ 5.4 Hz, $J_{6a,6b}$ 11.9 Hz, H-6b), 4.21 (dd, 1H, $J_{1'',2''}$ 3.9 Hz, $J_{2'',3''}$ 11.2 Hz, H-2″), 4.04 (m, 2H, $J_{3'',4''}$ 3.1 Hz, H-5, H-4″), 3.98 (m, 2H, $J_{3',4'}$ 3.1 Hz, $J_{3'',4''}$ 3.2 Hz, $J_{2'',3''}$ 11.3 Hz, H-4′, H-3″), 3.82 (d, 1H, $J_{4,5}$ 9.4 Hz, H-4), 3.76 (m, 2H, H-5′, H-6a′), 3.72 (m, 4H, $J_{3',4'}$ 3.1 Hz, $J_{2'3'}$ 10.4 Hz, H-3′, H-6b′, H-6ab″), 3.68 (s, 4H, OCH_2CH_2O), 3.61 (m, 5H, $J_{1',2'}$ 7.7 Hz, $J_{2',3'}$ 10.2 Hz, H-2′, $HNCH_2CH_2O(X2)$), 3.34 (m, 4H, $HNCH_2CH_2O(X2)$), 2.91 (br s, 1H, $OCH_2C{\equiv}CH$), 2.09 (s, 3H, $CH_3C(O)NH$), 1.76 (s, 3H, CH_3, pyruvate); ^{13}C NMR (125 MHz, D_2O) δ 175.4 (C═O), 173.8 (C═O), 158.9 (C═O), 158.6 (C═O), 106.4 (quaternary C, pyruvate), 106.3 (C-1′), 99.3 (C-1″), 98.0 (C-1), 79.6 ($OCH_2C{\equiv}CH$), 79.5 (C-4), 77.4 ($OCH_2C{\equiv}CH$), 76.7 (C-4′), 76.6 (C-5′), 76.2, (C-2), 73.1 (C-3′), 71.6 (C-2′, C-5″), 70.4 (CH_2-linker), 70.2 (CH_2-linker), 70.1 (CH_2-linker), 69.8, 69.5 (C-3, C-5), 69.2 (C-4″), 68.2 (C-3″), 65.1 (C-6), 61.5, 61.3 (C-6′, C-6″), 53.8 ($OCH_2C{\equiv}CH$), 51.0 (C-2″), 41.1 (CH_2-linker), 22.9 ($CH_3C(O)NH$), 21.8 (CH_3, pyruvate); and ESI HRMS calcd for $C_{34}H_{52}N_3O_{23}[M-H]^-$ 870.2997. Found 870.3005.

3.1.17. PolyBAIT-P^{k}NAc (1)

The heterobivalent monomer **2** (0.113 g, 0.130 mmol) was combined with poly[acrylamide-co-(2-azidopropylmethacrylamide)] (27 kDa, PDI 1.29,[27] 0.139 g, and 0.0865 mmol) and the contents dissolved in degassed MilliQ water (0.5 mL). A 1.0 M solution of sodium ascorbate was prepared and added to the solution (125 μL) followed by the addition of a 0.005 M solution of $CuSO_4$ (250 μL). The reaction proceeded for 2 days with stirring and subsequent TLC (4:5:1:0.1, dichloromethane:methanol:water:acetic acid) showed the reaction to be incomplete. The pH of the reaction was checked and found to be neutral. Sodium bicarbonate was added until the pH was ~8 and the reaction was allowed to continue for 24 h at which point TLC analysis showed the reaction to be complete. The reaction product was isolated and purified via dialysis and the pure product lyophilized from water to give the PolyBAIT-P^{k}NAc **1** product as a white solid; Characterization of PolyBAIT-PkNAc required comparison of the spectrum of starting monomer with that obtained for conjugated polymer (Supplementary data). The percent incorporation of monomer to polymer, was established by integrating the resonance of an anomeric proton with resonances of the polymer backbone. Specifically, the integration of peak A (δ 5.61, d, 1H, and H-1 ligand) was compared to resonances labeled peak B (d 2.35–1.28, br m, 67H, $-CH_2-$, –CH– polymer backbone, $-CH_3$ pyruvate, and $-CH_3$ NHAc). Since this group included six protons from ligand pyruvate and acetamido groups, 6H were subtracted from the total of peak B, leaving 61H. As each acrylamide monomer has 3H, we have a total of approximately 20 acrylamide residues to 1 carbohydrate residue. This corresponds to a 5% incorporation of the carbohydrate ligand to the polymer.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carres.2013.05.010.

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