RSC Advances

PAPER

View Article Online
View Journal | View Issue

Cite this: *RSC Adv.*, 2017, **7**, 49903

Concise synthesis of the pyruvic acid acetal containing pentasaccharide repeating unit of the cell wall O-antigen of *Escherichia coli* O156†

Anshupriya Si and Anup Kumar Misra *

Received 10th July 2017
Accepted 17th October 2017

DOI: 10.1039/c7ra07567g

rsc.li/rsc-advances

An elegant convergent synthetic strategy has been developed for the preparation of the 4,6-*O*-(*R*)-pyruvate acetal containing pentasaccharide repeating unit of the cell wall O-antigen of *Escherichia coli* O156 using stereoselective [2 + 3] block glycosylation. Stereoselective 1,2-*cis* glycosylation of the judiciously functionalized monosaccharide intermediates led to the formation of the desired pentasaccharide in satisfactory yield.

Introduction

Diarrhoeal epidemic due to bacterial infections is a serious health concern worldwide.[1] A significant number of people are suffering from gastrointestinal infections in developing countries where adequate sanitation is lacking.[2] Diarrhoea is one of the leading causes of death in children, elderly people and patients with insufficient immunity.[3,4] Enteric infections and colitis are also commonly found in developed countries due to the consumption of uncooked or semi cooked foods.[5] Enteropathogenic *Escherichia coli* (*E. coli*) strains are the predominant bacteria causing gastrointestinal disorder among the various microorganisms responsible for the diarrhoea.[6] *E. coli* is a commensal organism present in the gastrointestinal microflora in human.[7] In an immunocompromised state of the host they become virulent and cause a number of infections such as diarrhoea,[8] urinary tract infection,[9] hemorrhagic colitis (HC),[10] hemolytic uremic syndrome (HUS),[11] septicaemia[12] *etc.* *E. coli* strains causing enteric infections are classified into several subclasses based on their mode of infections,[13] which include (a) enteropathogenic *E. coli* (EPEC); (b) enterohemorrhagic *E. coli* (EHEC); (c) enteroinvasive *E. coli* (EIEC); (d) enterotoxigenic *E. coli* (ETEC); (e) enteroaggregative *E. coli* (EAEC); (f) diffusely adherent *E. coli* (DAEC). EHEC strains are also termed as Shiga-toxin producing *E. coli* (STEC) because of their capability to secrete Shiga-like toxin during the early stage of infections.[14] *E. coli* strains belong to the subclass EHEC or STEC have been associated with a number of deadly gastrointestinal outbreaks in the developed countries.[15] One of the frequently found EHEC strain is *E. coli* O157, which was the causative agent for several diarrhoeal outbreaks in Europe and America.[16] Besides, *E. coli* O157, several *E. coli* strains associated with diarrheal diseases have been identified and characterized which belong to the EHEC category such as *E. coli* O4, O26, O55, O103, O111, O145, O150, O156 *etc.*[17] The cell walls of the pathogenic bacteria are highly associated with their virulent properties. The *O*-polysaccharides or O-antigens, a component of bacterial cell wall endotoxins play vital role at the initial stage of bacterial infections to the host.[18] Therefore, the O-antigen of the bacterial cell wall is useful tool for the development of therapeutics for the eradication of the bacterial infections. Conventionally, several polysaccharide vaccines have been developed in the past using cell wall polysaccharides of pathogenic bacterial strains.[19] Later the polysaccharide vaccines have been replaced by more efficacious glycoconjugate vaccines based on the cell wall O-antigens.[20] Duan *et al.*[21] reported the structure of the pyruvic acid acetal containing pentasaccharide repeating unit of the cell wall O-antigen of *E. coli* O156, which belongs to STEC class and responsible for a number of EHEC associated diseases in humans. Therefore, it would be pertinent to develop vaccine candidates against *E. coli* O156 using the O-antigen. However, isolation of polysaccharides from the natural bacterial sources is quite troublesome and suffers from a number of shortcomings such as, handling of live bacterial strain, cannot produce substantial quantities of polysaccharides at a time, lack of adequate purity and free from biological impurities, lack of homogeneity in the isolated polysaccharide fragments *etc.* Therefore, it is preferable to develop concise synthetic strategies for the chemical synthesis of the oligosaccharide repeating units with precise structures devoid of above mentioned shortcomings.[22] Recently, glycoconjugate vaccines developed using synthetic oligosaccharides have been proved to be equally or better immunogenic than the conventional polysaccharide conjugate vaccines.[23] The synthetic oligosaccharides can be conveniently functionalized according to the requirement of the conjugation with appropriate proteins to furnish glycoconjugates with higher better homogeneity. In this context, it

Bose Institute, Division of Molecular Medicine, P-1/12, C.I.T. Scheme VII M, Kolkata 700054, India. E-mail: akmisra69@gmail.com; Fax: +91-33-2355-3886

† Electronic supplementary information (ESI) available: Copies of the NMR spectra of compounds **1**, **7**, **8**, **9**, **11**, **12**, **13**, **15**. See DOI: 10.1039/c7ra07567g

was decided to develop a convergent synthetic strategy for the synthesis of the pyruvic acid acetal containing pentasaccharide repeating unit of the O-antigen of *E. coli* O156 as its 2-aminoethyl glycoside. The 2-aminoethyl group at the reducing end of the pentasaccharide could be useful in conjugating the glycan moiety with an appropriate protein or aglycon to prepare glycoconjugate derivatives. A concise chemical synthesis of the pentasaccharide repeating unit of the O-antigen of *E. coli* O156 is reported herein.

Results and discussion

The target pentasaccharide containing a pyruvic acid acetal has been synthesized as its 2-aminoethyl glycoside using a stereoselective convergent [2 + 3] block glycosylation strategy. Presence of a number of 1,2-*cis* glycosidic linkages present pentasaccharide poses extra challenges for its synthesis. The key features of the synthetic strategy are (a) stereoselective 1,2-*cis* glycosylation using either thioglycoside or glycosyl trichloroacetimidate derivatives as glycosyl donors; (b) use of perchloric acid supported over silica gel ($HClO_4$–SiO_2)[24] as a solid acid catalyst; (c) activation of thioglycosides using a combination of *N*-iodosuccinimide (NIS) and $HClO_4$–SiO_2;[25] (d) activation of glycosyl trichloroacetimidate using $HClO_4$–SiO_2;[26] (e) preparation of pyruvate acetal using pyruvate dithioacetal;[27] (f) achievements of 1,2-*cis* glycosylated products in high yield. A set of suitably functionalized monosaccharide intermediates **2**,[28] **3**,[29] **4**,[30] **5** (ref. 31) and **6** (ref. 32) were prepared from the commercially available reducing sugars using literature reported reaction methodologies (Fig. 1).

Stereoselective 1,2-*cis*-glycosylation of compound **2** and L-fucosyl thioglycoside **3** in the presence of a combination[25] of NIS and $HClO_4$–SiO_2 in a mixed solvent of CH_2Cl_2–Et_2O (1 : 5) furnished disaccharide derivative **7** in 70% yield, which was characterized by its NMR spectral analysis [signals at δ 5.34 (d, $J = 8.5$ Hz, H-1_A), 4.84 (d, $J = 3.5$ Hz, H-1_B) in ^{1}H NMR and δ 99.1 (C-1_A), 98.7 (C-1_B) in ^{13}C NMR spectra]. Treatment of compound **7** with sodium methoxide[33] resulted in the formation of the disaccharide acceptor **8** after de-*O*-acetylation in 93% yield. Stereoselective 1,2-*cis* glycosylation of compound **8** with D-galactosyl thioglycoside **4** using NIS and $HClO_4$–SiO_2 combination[25] in a mixed solvent of CH_2Cl_2–Et_2O (1 : 3) furnished trisaccharide derivative **9** in 76% yield together with the minor quantity (~5%) of its 1,2-*trans* glycoside, which was separated by column chromatography. The newly formed glycosyl linkage in compound **9** was confirmed by its NMR spectral analysis [signals at δ 5.33 (d, $J = 8.5$ Hz, H-1_A), 4.85 (d, $J = 3.5$ Hz, H-1_B), 4.65 (d, $J = 3.0$ Hz, H-1_C) in ^{1}H NMR and δ 99.5 (C-1_C), 99.0 (C-1_A), 98.4 (C-1_B) in ^{13}C NMR spectra]. Treatment of compound **9** with acetic anhydride in the presence of $HClO_4$–SiO_2 resulted in the formation of compound **10** in 88% yield by direct conversion of the benzylidene acetal into di-*O*-acetylated derivative.[34] The allyl ether of compound **10** was removed by the treatment with palladium chloride[35] to furnish trisaccharide acceptor **11** in 72% yield. NMR spectral analysis of compound **11** supported its formation [signals at δ 5.44 (d, $J = 8.0$ Hz, H-1_A), 4.50 (d, $J = 3.0$ Hz, H-1_B), 4.30 (d, $J = 3.0$ Hz, H-1_C) in ^{1}H NMR and δ 101.3 (C-1_C), 98.6 (C-1_A), 95.9 (C-1_B) in ^{13}C NMR spectra] (Scheme 1).

In another experiment, stereoselective orthogonal 1,2-*cis*-glycosylation of L-fucosyl thioglycoside acceptor **5** with D-galactosyl trichloroacetimidate donor **6** in the presence of $HClO_4$–SiO_2 (ref. 26) in CH_2Cl_2–Et_2O (1 : 3) resulted in the formation of compound **12** in 74% yield. The anomeric thioether present in the acceptor was unaffected under the reaction condition maintaining the orthogonality[36] of the reaction. The formation the 1,2-*cis*-glycosylated disaccharide thioglycoside derivative **12** was confirmed from its NMR spectral analysis [signals at δ 5.44 (d, $J = 3.5$ Hz, H-1_E), 4.44 (d, $J = 9.5$ Hz, H-1_D) in ^{1}H NMR and δ 100.0 (C-1_E), 85.2 (C-1_D) in ^{13}C NMR spectra] (Scheme 2).

The disaccharide thioglycoside donor **12** and trisaccharide acceptor **11** was allowed to couple in a 1,2-*cis* stereoselective manner in the presence of a combination[25] of NIS and $HClO_4$–SiO_2 in CH_2Cl_2–Et_2O (1 : 4) to furnish the pentasaccharide derivative **13** in 72% yield. The formation of compound **13** was confirmed from its NMR spectral analysis [signals at δ 5.57 (d, $J = 3.5$ Hz, H-1_D), 5.42 (d, $J = 7.5$ Hz, H-1_A), 4.86 (d, $J = 3.0$ Hz, H-1_E), 4.65 (d, $J = 3.0$ Hz, H-1_B), 4.23 (d, $J = 3.0$ Hz, H-1_C) in ^{1}H NMR and δ 102.4 ($J_{C\text{-}1/H\text{-}1} = 168$ Hz, C-1_C), 99.03 ($J_{C\text{-}1/H\text{-}1} = 170$ Hz, C-1_D), 98.3 ($J_{C\text{-}1/H\text{-}1} = 168$ Hz, C-1_E), 98.2 ($J_{C\text{-}1/H\text{-}1} = 170$ Hz, C-1_B), 97.5 ($J_{C\text{-}1/H\text{-}1} = 158$ Hz, C-1_A) ^{13}C NMR spectra]. The presence of four α-glycosyl linkages and one β-glycosyl linkage in the molecule was also unambiguously confirmed from the C-1/H-1 coupling constants ($J_{C\text{-}1/H\text{-}1}$) of the monosaccharide moieties in ^{1}H coupled ^{13}C NMR spectrum.[37,38] The benzylidene acetal in the terminal D-galactosyl moiety in compound **13** was smoothly removed by the treatment[34] with $HClO_4$–SiO_2 at room temperature to give the diol derivative **14** in 82% yield. Compound **14** was allowed to react with methyl 2,2-di(ethylthio)propionate[39] in the presence of a combination of NIS and triflic acid (TfOH)[27] to furnish compound **15** in 68% yield containing the desired 4,6-(*R*)-pyruvate acetal in the D-galactosyl moiety, which was confirmed from its NMR spectral analysis [signals at δ 5.55 (d, $J = 3.5$ Hz, H-1_D), 5.42 (d, $J = 8.0$ Hz, H-1_A), 4.89 (d, $J = 3.0$ Hz, H-1_E), 4.67 (d, $J = 3.5$ Hz, H-1_B),

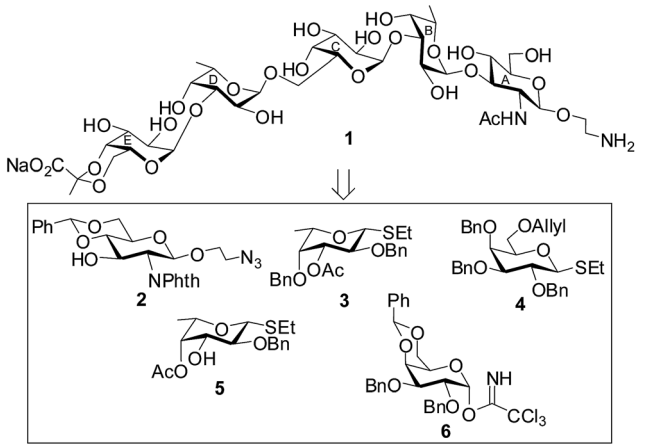
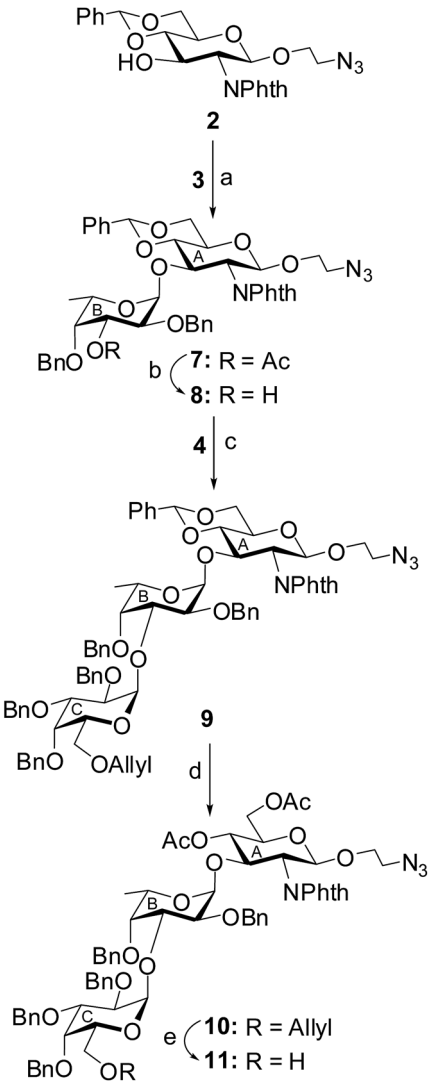
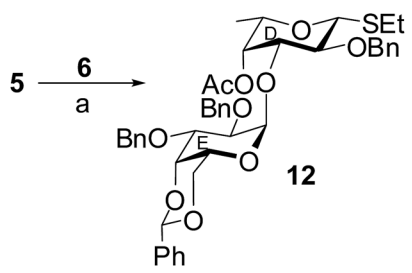


Fig. 1 Structure of the synthesized pentasaccharide containing pyruvic acid acetal and its synthetic intermediates.

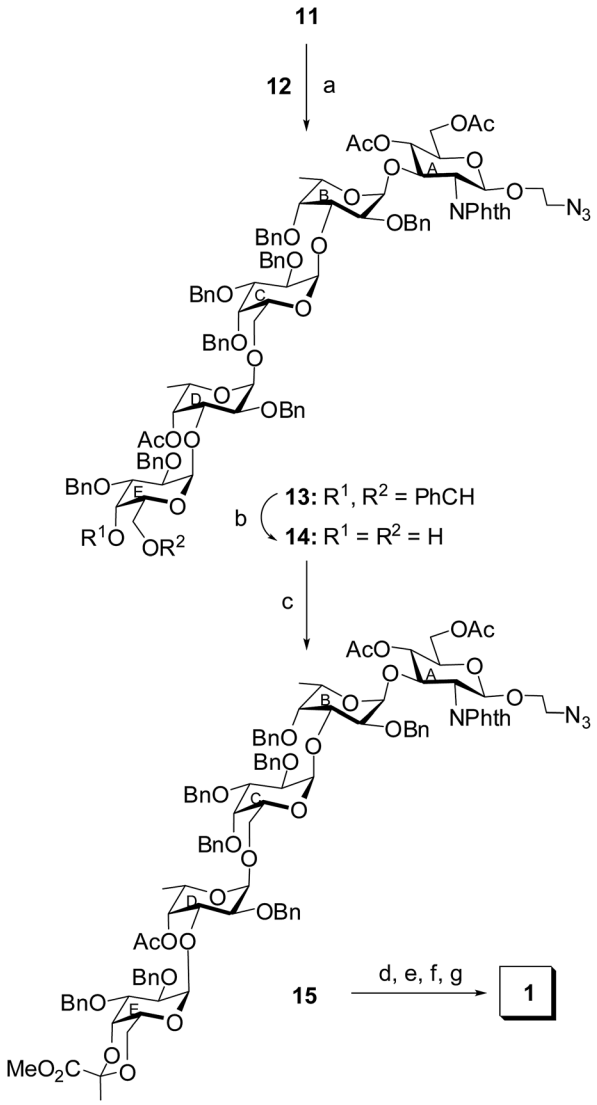


Scheme 1 Reagents: (a) NIS, $HClO_4$–SiO_2, MS 4 Å, CH_2Cl_2–Et_2O (1 : 5, v/v), 0 °C, 15 min, 70%; (b) CH_3ONa, CH_3OH, room temperature, 3 h, 93%; (c) NIS, $HClO_4$–SiO_2, MS 4 Å, CH_2Cl_2–Et_2O (1 : 3, v/v), −10 °C, 25 min, 76%; (d) acetic anhydride, $HClO_4$–SiO_2, room temperature, 20 min, 88%; (e) $PdCl_2$, CH_3OH, 0 °C, 1 h, 76%.

4.28 (d, $J = 3.5$ Hz, H-1_C) in ^{1}H NMR and δ 102.4 (C-1_C), 99.08 (C-1_D), 98.8 (CCH_3), 98.3 (C-1_E), 98.1 (C-1_B), 97.5 (C-1_A), 26.0 (CCH_3) in ^{13}C NMR spectra]. Appearance of the methyl carbon of the pyruvate acetal at δ 26 ppm in ^{13}C NMR spectrum confirmed the formation of the 4,6-(*R*)-pyruvate acetal.[40] Finally, compound **15** was subjected to a sequence of reactions involving (a) treatment with ethylenediamine to remove the *N*-phthaloyl group;[41] (b) acetylation of the newly generated amine; (c) removal of the benzyl ethers using hydrogenolysis over $Pd(OH)_2$–C;[42] (d) removal of the *O*-acetyl group followed by hydrolysis of the methyl ester in the pyruvate moiety using sodium methoxide to furnish the desired pentasaccharide **1** in 54% over all yield. The NMR spectral analysis of compound **1** supported the formation of the desired compound [signals at δ 5.08 (d, $J = 3.5$ Hz, H-1_E), 5.05 (d, $J = 4.0$ Hz, H-1_C), 4.89 (d, $J = 3.5$ Hz, H-1_B), 4.78 (d, $J = 4.0$ Hz, H-1_D), 4.41 (d, $J = 8.5$ Hz, H-1_A) in ^{1}H NMR and δ 101.5



Scheme 2 Reagents: (a) $HClO_4$–SiO_2, MS 4 Å, CH_2Cl_2–Et_2O (1 : 3, v/v), −10 °C, 2 h, 74%.



Scheme 3 Reagents: (a) NIS, $HClO_4$–SiO_2, MS 4 Å, CH_2Cl_2–Et_2O (1 : 4, v/v), −10 °C, 30 min, 72%; (b) $HClO_4$–SiO_2, CH_3CN, room temperature, 20 min, 82%; (c) methyl 2,2-di(ethylthio)propionate, NIS, TfOH, 0 °C, 30 min, 68%; (d) $(CH_2NH_2)_2$, ^{n}BuOH, 90 °C, 10 h; (e) acetic anhydride, pyridine, room temperature, 1 h; (f) H_2, 20% $Pd(OH)_2$–C, CH_3OH, room temperature, 15 h; (g) CH_3ONa, CH_3OH, room temperature, 3 h, then H_2O, 8 h, 54% in four steps.

($C\text{-}1_A$), 100.7 (2C, $C\text{-}1_C$, $C\text{-}1_E$), 100.6 (*C*COOH), 99.8 ($C\text{-}1_D$), 98.4 ($C\text{-}1_B$) in ^{13}C NMR spectra] (Scheme 3).

Experimental

General methods

All reactions were monitored by thin layer chromatography over silica gel coated TLC plates. The spots on TLC were visualized by warming ceric sulphate (2% $Ce(SO_4)_2$ in 2 N H_2SO_4) sprayed plates in hot plate. Silica gel 230–400 mesh was used for column chromatography. NMR spectra were recorded on Bruker Avance 500 MHz using $CDCl_3$ as solvent and TMS as internal reference unless stated otherwise. Chemical shift value is expressed in δ ppm. The complete assignment of proton and carbon spectra was carried out by using a standard set of NMR experiments, *e.g.* ^1H NMR, ^{13}C NMR, ^{13}C DEPT 135, 2D COSY and 2D HSQC *etc.* MALDI-MS were recorded on a Bruker Daltonics mass spectrometer. Optical rotations were recorded in a Jasco P-2000 spectrometer. Commercially available grades of organic solvents of adequate purity are used in all reactions. $HClO_4$–SiO_2 was prepared following the procedure reported by Chakraborti *et al.*[24]

2-Azidoethyl *O*-(3-*O*-acetyl-2,4-di-*O*-benzyl-α-L-fucopyranosyl)-(1 → 3)-2-*N*-phthalimido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (7)

To a solution of compound **2** (1.5 g, 3.22 mmol) and compound **3** (1.5 g, 3.48 mmol) in anhydrous CH_2Cl_2–Et_2O (18 mL, 1 : 5 v/v) was added MS 4 Å (2 g) and it was cooled to 0 °C under argon. To the cold reaction mixture were added NIS (945 mg, 4.19 mmol) and $HClO_4$–SiO_2 (40 mg) and it was allowed to stir at same temperature for 15 min. The reaction mixture was filtered and washed with CH_2Cl_2 (100 mL). The combined filtrate was successively washed with 5% $Na_2S_2O_3$ (50 mL), satd aq. $NaHCO_3$ (50 mL) and water (50 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified over SiO_2 using hexane–EtOAc (2 : 1) as eluant to furnish pure compound **7** (1.9 g, 70%). Colorless oil; $[\alpha]_D^{25}$ −50 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 7.68–6.94 (m, 15H, Ar-H), 5.56 (s, 1H, PhC*H*), 5.34 (d, J = 8.5 Hz, 1H, $H\text{-}1_A$), 4.97 (dd, J = 10.5, 3.0 Hz, 1H, $H\text{-}3_B$), 4.84 (d, J = 3.5 Hz, 1H, $H\text{-}1_B$), 4.72 (t, J = 9.0 Hz, 1H, $H\text{-}3_A$), 4.46–4.40 (m, 4H, 2PhC*H*, $H\text{-}2_A$, $H\text{-}6_{aA}$), 4.14–4.11 (m, 2H, PhC*H*, $H\text{-}5_B$), 4.01 (m, 1H, OC*H*), 3.97 (d, J = 11.5 Hz, 1H, PhC*H*), 3.87 (t, J = 10.0 Hz, 1H, $H\text{-}6_{bA}$), 3.76 (t, J = 9.0 Hz, 1H, $H\text{-}4_A$), 3.74–3.70 (m, 2H, $H\text{-}2_C$, $H\text{-}5_A$), 3.68–3.63 (m, 1H, OC*H*), 3.61 (d, J = 2.0 Hz, 1H, $H\text{-}4_B$), 3.41–3.35 (m, 1H, NC*H*), 3.23–3.19 (m, 1H, NC*H*), 1.64 (s, 3H, $COCH_3$), 0.74–0.73 (m, 3H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.1 (*C*OCH_3), 168.2, 167.4 (Phth*C*O), 138.7–123.5 (Ar-C), 101.8 (Ph*C*H), 99.1 ($C\text{-}1_A$), 98.7 ($C\text{-}1_B$), 81.6 ($C\text{-}4_A$), 78.6 ($C\text{-}4_B$), 77.3 (Ph*C*H), 75.6 ($C\text{-}2_B$), 75.5 ($C\text{-}3_A$), 74.7 ($C\text{-}3_B$), 73.5 (Ph*C*H), 68.7 (O*C*H), 68.5 ($C\text{-}6_A$), 66.6 ($C\text{-}5_A$), 66.5 ($C\text{-}5_B$), 55.6 ($C\text{-}2_A$), 50.5 (N*C*H), 21.1 ($COCH_3$), 16.3 (CCH_3); MALDI-MS: 857.3 $[M + Na]^+$; anal. calcd for $C_{45}H_{46}N_4O_{12}$ (834.86): C, 64.74; H, 5.55%; found: C, 64.60; H, 5.70%.

2-Azidoethyl *O*-(2,4-di-*O*-benzyl-α-L-fucopyranosyl)-(1 → 3)-2-*N*-phthalimido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (8)

A solution of compound **7** (1.7 g, 2.04 mmol) in 0.1 M CH_3ONa in CH_3OH (20 mL) was allowed to stir at room temperature for 3 h. The reaction mixture was neutralized with Dowex 50W X8 (H^+) resin, filtered and concentrated under reduced pressure. The crude mass was passed through a short pad of SiO_2 using hexane–EtOAc (1 : 1) as eluant to give pure compound **8** (1.5 g, 93%). White solid; mp 174–175 °C [EtOH]; $[\alpha]_D^{25}$ −97 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 7.75–6.97 (m, 15H, Ar-H), 5.55 (s, 1H, PhC*H*), 5.36 (d, J = 3.0 Hz, $H\text{-}1_B$), 4.70 (t, J = 9.0 Hz, 1H, $H\text{-}3_A$), 4.57 (d, J = 11.5 Hz, 1H, PhC*H*), 4.49 (d, J = 11.5 Hz, 1H, PhC*H*), 4.40 (m, 1H, $H\text{-}6_{aA}$), 4.39 (t, J = 9.0 Hz, 1H, $H\text{-}2_A$), 4.30 (d, J = 12.5 Hz, 1H, PhC*H*), 4.12–4.09 (m, 1H, $H\text{-}5_B$), 4.01–3.95 (m, 1H, $H\text{-}6_{bA}$), 3.87 (d, J = 10.0 Hz, 1H, PhC*H*), 3.85–3.79 (m, 2H, $H\text{-}3_B$, OC*H*), 3.75–3.60 (m, 3H, $H\text{-}4_A$, $H\text{-}5_A$, OC*H*), 3.45 (d, J = 3.0 Hz, 1H, $H\text{-}4_B$), 3.43 (s, 1H, $H\text{-}2_B$), 3.73–3.33 (m, 1H, NC*H*), 3.21–3.17 (m, 1H, NC*H*), 0.87–0.85 (m, 3H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 168.2, 167.4 (Phth*C*O), 134.0–125.3 (Ar-*C*), 101.6 (Ph*C*H), 99.0 ($C\text{-}1_A$), 97.8 ($C\text{-}1_B$), 81.6 ($C\text{-}4_A$), 79.9 ($C\text{-}4_B$), 78.6 ($C\text{-}2_B$), 77.3 (Ph*C*H), 76.2 ($C\text{-}3_A$), 75.5 (Ph*C*H), 70.1 ($C\text{-}3_B$), 68.7 (OCH_2), 68.6 ($C\text{-}6_A$), 67.0 ($C\text{-}5_A$), 66.4 ($C\text{-}5_B$), 55.7 ($C\text{-}2_A$), 50.5 (NCH_2), 15.8 (CCH_3); MALDI-MS: 815.3 $[M + Na]^+$; anal. calcd for $C_{43}H_{44}N_4O_{11}$ (792.83): C, 65.14; H, 5.59%; found: C, 64.95; H, 5.75%.

2-Azidoethyl *O*-(6-*O*-allyl-2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl)-(1 → 3)-(2,4-di-*O*-benzyl-α-L-fucopyranosyl)-(1 → 3)-2-*N*-phthalimido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (9)

A solution of compound **8** (1.4 g, 1.77 mmol), compound **4** (1.1 g, 2.06 mmol) and MS 4 Å (2 g) in anhydrous CH_2Cl_2–Et_2O (15 mL, 1 : 3 v/v) was cooled to −10 °C under argon. NIS (560 mg, 2.5 mmol) and $HClO_4$–SiO_2 (20 mg) were added to the cold reaction mixture and it was allowed to stir at same temperature for 25 min. The reaction mixture was filtered and washed with CH_2Cl_2 (100 mL). The combined filtrate was successively washed with 5% $Na_2S_2O_3$ (50 mL), satd aq. $NaHCO_3$ (50 mL) and water (50 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified over SiO_2 using hexane–EtOAc (3 : 1) as eluant to furnish pure compound **9** (1.7 g, 76%). Colorless oil; $[\alpha]_D^{25}$ +30 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 7.70–6.84 (m, 30H, Ar-*H*), 5.90–5.80 (m, 1H, $OCH{=}CH_2$), 5.53 (s, 1H, PhC*H*), 5.33 (d, J = 8.5 Hz, 1H, $H\text{-}1_A$), 5.28–5.15 (m, 2H, $CH{=}CH_2$), 4.92 (d, J = 11.5 Hz, 1H, PhC*H*), 4.85 (d, J = 3.5 Hz, 1H, $H\text{-}1_B$), 4.72 (d, J = 11.5 Hz, 1H, PhC*H*), 4.65 (d, J = 3.0 Hz, 1H, $H\text{-}1_C$), 4.63–4.59 (m, 2H, $H\text{-}3_A$, OCH_2–CH═), 4.56–4.51 (m, 5H, 5PhC*H*), 4.41–4.37 (m, 2H, $H\text{-}6_{aA}$, OCH_2–CH═), 4.35 (t, J = 10.5 Hz, 1H, $H\text{-}2_A$), 4.27 (d, J = 12.5 Hz, 1H, PhC*H*), 4.08–4.03 (m, 1H, $H\text{-}5_B$), 4.02–3.92 (m, 3H, PhC*H*, $H\text{-}3_B$, $H\text{-}6_{bA}$), 3.91–3.80 (m, 2H, $H\text{-}2_B$, OC*H*), 3.78–3.60 (m, 7H, $H\text{-}2_C$, $H\text{-}3_C$, $H\text{-}4_A$, $H\text{-}4_C$, $H\text{-}5_A$, $H\text{-}5_C$, OC*H*), 3.59–3.51 (m, 1H, $H\text{-}6_{aC}$), 3.50 (s, 1H, $H\text{-}4_B$), 3.49–3.41 (m, 1H, $H\text{-}6_{bC}$), 3.38–3.31 (m, 1H, NC*H*), 0.85–0.84 (m, 3H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 167.8, 168.4 (Phth*C*O), 138.9–125.5 (Ar-C, $CH_2{=}CH$),

117.0 (CH_2=CH), 101.4 (Ph*C*H), 99.5 (C-1_C), 99.0 (C-1_A), 98.4 (C-1_B), 81.9 (C-4_A), 81.6 (C-4_B), 78.6 (C-4_C), 75.8 (C-2_B), 75.5 (Ph*C*H), 74.9 (3C, C-2_C, C-3_C, C-5_C), 73.1 (C-3_A), 72.9 (Ph*C*H), 72.7 (Ph*C*H), 72.4 (Ph*C*H), 72.3 (O*C*H_2–CH=CH_2), 70.4 (C-3_B), 69.4 (C-6_C), 68.7 (O*C*H_2), 68.5 (C-6_A), 68.1 (C-5_A), 67.5 (C-5_B), 55.6 (C-2_A), 50.5 (N*C*H_2), 16.3 (C*C*H_3); MALDI-MS: 1287.5 $[M + Na]^+$; anal. calcd for $C_{73}H_{76}N_4O_{16}$ (1265.40): C, 69.29; H, 6.05%; found: C, 69.10; H, 6.20%.

2-Azidoethyl *O*-(2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl)-(1 → 3)-(2,4-di-*O*-benzyl-α-L-fucopyranosyl)-(1 → 3)-4,6-di-*O*-acetyl-2-*N*-phthalimido-2-deoxy-β-D-glucopyranoside (11)

To a solution of compound **9** (1.7 g, 1.34 mmol) in acetic anhydride (5 mL) was added $HClO_4$–SiO_2 (200 mg) and the reaction mixture was stirred at room temperature for 20 min. The reaction mixture was filtered and washed with EtOAc (50 mL). The combined filtrate was concentrated and co-evaporated with toluene (3 × 20 mL) under reduced pressure. The crude product was passed through a short pad of SiO_2 using hexane–EtOAc (2 : 1) as eluant to give pure compound **10** (1.49 g, 88%). To a solution of compound **10** (1.4 g, 1.11 mmol) in dry CH_3OH (25 mL) was added $PdCl_2$ (100 mg, 0.56 mmol) and the reaction mixture was stirred at 0 °C for 1 h. Then the reaction mixture was filtered through a Celite bed and washed with CH_3OH (50 mL). The combined filtrate was concentrated under reduced pressure and the crude product was purified over SiO_2 using hexane–EtOAc (1 : 1) as eluant to give pure compound **11** (980 mg, 72%). Yellowish solid; mp 154–155 °C [EtOH]; $[\alpha]_D^{25}$ +100 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.71–6.95 (m, 25H, Ar-*H*), 5.44 (d, J = 8.0 Hz, 1H, H-1_A), 5.03 (d, J = 10.0 Hz, 1H, H-4_A), 4.99 (d, J = 11.5 Hz, 1H, PhC*H*), 4.75 (d, J = 11.5 Hz, 1H, PhC*H*), 4.73 (d, J = 11.5 Hz, 1H, PhC*H*), 4.63 (d, J = 11.5 Hz, 1H, PhC*H*), 4.60 (d, J = 11.5 Hz, 1H, PhC*H*), 4.53 (d, J = 12.0 Hz, 1H, PhC*H*), 4.50 (d, J = 3.0 Hz, 1H, H-1_B), 4.42 (d, J = 12.0 Hz, 1H, PhC*H*), 4.40 (d, J = 11.5 Hz, 1H, PhC*H*), 4.30 (d, J = 3.0 Hz, 1H, H-1_C), 4.28–4.25 (m, 2H, H-2_A, H-4_C), 4.20–4.01 (m, 6H, 2PhC*H*, H-3_A, H-6_{abA}, OC*H*), 3.98–3.90 (m, 1H, H-5_B), 3.85–3.78 (m, 2H, H-2_B, H-3_C), 3.71–3.69 (m, 3H, H-5_A, H-5_C, OC*H*), 3.68–3.58 (m, 2H, H-2_C, H-3_B), 3.55–3.39 (m, 4H, H-4_B, H-6_{abC}, NC*H*), 3.20–3.15 (m, 1H, NC*H*), 2.1, 1.9 (s, 6H, 2COCH_3), 1.01–1.00 (m, 3H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.8, 169.7 (*C*OCH$_3$), 168.2, 167.4 (PhthN*C*O), 138.6–122.3 (Ar-*C*), 101.3 (C-1_C), 98.6 (C-1_A), 95.9 (C-1_B), 80.9 (C-4_B), 78.7 (C-4_C), 78.4 (C-2_B), 76.4 (C-2_C), 76.3 (C-3_C), 75.8 (Ph*C*H), 75.5 (C-5_C), 74.3 (Ph*C*H), 73.6 (Ph*C*H), 72.9 (Ph*C*H), 72.7 (Ph*C*H), 72.5 (C-3_A), 72.1 (C-3_B), 71.2 (C-5_A), 71.1 (C-4_A), 68.6 (O*C*H_2), 68.4 (C-5_B), 54.8 (C-2_A), 50.4 (N*C*H_2), 21.2, 20.9 (2CO*C*H_3), 15.7 (C*C*H_3); MALDI-MS: 1243.4 $[M + Na]^+$; anal. calcd for $C_{67}H_{72}N_4O_{18}$ (1221.30): C, 65.89; H, 5.94%; found: C, 65.74; H, 6.06%.

Ethyl (2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl)-(1 → 3)-4-*O*-acetyl-2-*O*-benzyl-1-thio-β-L-fucopyranoside (12)

To a solution of compound **5** (500 mg, 1.47 mmol) and compound **6** (1.1 g, 1.86 mmol) in anhydrous CH_2Cl_2–Et_2O (10 mL, 1 : 3 v/v) was added MS 4 Å (4 g) and the reaction mixture was allowed to stir at room temperature for 10 min under argon then cooled to −10 °C. To the cooled reaction mixture was added $HClO_4$–SiO_2 (50 mg) and it was allowed to stir at the same temperature for 2 h. The reaction was quenched with Et_3N (0.1 mL), filtered and washed with CH_2Cl_2 (100 mL). The organic layer was washed with satd aq. $NaHCO_3$ (50 mL) and water (50 mL), dried (Na_2SO_4) and concentrated. The crude product was purified over SiO_2 using hexane–EtOAc (2 : 1) as eluant to give pure compound **12** (840 mg, 74%). White solid; 109–110 °C [EtOH]; $[\alpha]_D^{25}$ +44.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.55–7.12 (m, 20H, Ar-*H*), 5.52 (s, 1H, PhC*H*), 5.44 (d, J = 3.5 Hz, 1H, H-1_E), 5.12 (d, J = 3.5 Hz, 1H, H-4_D), 4.90 (d, J = 12.0 Hz, 1H, PhC*H*), 4.83–4.71 (4d, J = 12 Hz, 4H, 4PhC*H*), 4.57 (d, 1H, J = 12.5 Hz, PhC*H*), 4.44 (d, J = 9.5 Hz, 1H, H-1_D), 4.27 (d, J = 2.5 Hz, 1H, H-4_E), 4.25 (d, J = 12.5 Hz, 1H, H-6_{aE}), 4.12 (dd, J = 9.0, 3.0 Hz, 1H, H-2_E), 4.09 (d, J = 11.5 Hz, H-6_{bE}), 3.98–3.91 (m, 2H, H-3_D, H-3_E), 3.86 (s, 1H, H-5_E), 3.78–3.73 (m, 1H, H-5_D), 3.71 (t, J = 8.5 Hz, 1H each, H-2_D), 2.85–2.70 (m, 2H, S*C*H_2CH$_3$), 2.06 (s, 3H, COCH_3), 1.35 (t, J = 7.5 Hz, 3H, SCH$_2$CH_3), 1.19–1.74 (m, 3H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.3 (*C*OCH$_3$), 138.6–126.4 (Ar-*C*), 100.9 (Ph*C*H), 100.0 (C-1_E), 85.2 (C-1_D), 78.3 (C-2_D), 77.6 (C-3_D), 75.6 (C-3_E), 75.1 (Ph*C*H), 74.8 (C-2_E), 74.5 (C-4_E), 73.8 (Ph*C*H), 73.0 (C-5_D), 72.9 (C-4_D), 71.4 (Ph*C*H), 69.6 (Ph*C*H), 68.9 (C-6_E), 63.4 (C-5_E), 25.6 (S*C*H_2CH$_3$), 22.7 (CO*C*H_3), 16.6 (C*C*H_3), 16.1 (SCH$_2$*C*H$_3$); MALDI-MS: 793.3 $[M + Na]^+$; anal. calcd for $C_{44}H_{50}O_{10}S$ (770.92): C, 68.55; H, 6.54%; found: C, 68.40; H, 6.70%.

2-Azidoethyl *O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl)-(1 → 3)-(4-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl)-(1 → 6)-(2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl)-(1 → 3)-(2,4-di-*O*-benzyl-α-L-fucopyranosyl)-(1 → 3)-4,6-di-*O*-acetyl-2-*N*-phthalimido-2-deoxy-β-D-glucopyranoside (13)

A solution of compound **11** (900 mg, 0.72 mmol), compound **12** (660 mg, 0.86 mmol) and MS 4 Å (1 g) in anhydrous CH_2Cl_2–Et_2O (15 mL, 1 : 4 v/v) was cooled to −10 °C under argon. NIS (230 mg, 1.03 mmol) and $HClO_4$–SiO_2 (10 mg) were added to the cold reaction mixture and it was allowed to stir at same temperature for 30 min. The reaction mixture was filtered and washed with CH_2Cl_2 (50 mL). The combined filtrate was successively washed with 5% $Na_2S_2O_3$ (25 mL), satd aq. $NaHCO_3$ (25 mL) and water (25 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified over SiO_2 using hexane–EtOAc (3 : 2) as eluant to give pure compound **13** (1.0 g, 72%). White solid; mp 167–168 °C [EtOH]; $[\alpha]_D^{25}$ +46 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.92–6.87 (m, 45H, Ar-*H*), 5.57 (d, J = 3.5 Hz, 1H, H-1_D), 5.50 (s, 1H, PhC*H*), 5.42 (d, J = 7.5 Hz, 1H, H-1_A), 5.12 (d, J = 12.0 Hz, 1H, PhC*H*), 5.02 (d, J = 3.0 Hz, 1H, H-4_D), 4.95 (t, J = 9.0 Hz each, 1H, H-4_A), 4.86 (d, J = 3.0 Hz, 1H, H-1_E), 4.65 (d, J = 3.0 Hz, 1H, H-1_B), 4.74–4.42 (m, 11H, 9PhC*H*, H-6_{abA}), 4.38–4.03 (m, 13H, H-2_A, H-2_D, H-3_A, H-3_D, H-4_E, H-6_{aE}, H-6_{bE}, 6PhC*H*), 4.23 (d, J = 3.0 Hz, 1H, H-1_C), 4.09–3.98 (m, 1H, OC*H*), 3.95–3.78 (m, 10H, H-2_B, H-2_E, H-3_B, H-3_C, H-3_E, H-4_C, H-5_B, H-5_C, H-5_D, H-6_{aC}), 3.79–3.63 (m, 2H, H-5_A, OC*H*), 3.58–3.50 (m, 2H, H-2_C, H-6_{bC}), 3.41–3.33 (m,

2H, H-4$_B$, NC*H*), 3.25–3.15 (m, 2H, H-5$_E$, NC*H*), 2.08, 2.02, 1.90 (3s, 9H, 3COCH_3), 0.92–0.98 (m, 6H, 2CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.3, 170.8, 169.8 (3*C*OCH$_3$), 168.2, 167.4 (Phth*C*O), 139.2–122.7 (Ar-*C*), 102.4 (C-1$_C$), 101.1 (Ph*C*H), 99.03 (C-1$_D$), 98.3 (C-1$_E$), 98.2 (C-1$_B$), 97.5 (C-1$_A$), 81.9 (C-4$_B$), 79.4 (C-4$_C$), 79.0 (C-2$_B$), 76.3 (2C, C-2$_C$, C-2$_D$), 75.9 (Ph*C*H), 75.3 (C-3$_C$), 75.1 (C-5$_C$), 74.9 (C-4$_E$), 74.7 (C-3$_D$), 74.5 (2C, C-2$_E$, C-3$_E$), 73.3 (C-3$_B$), 72.8 (Ph*C*H), 72.7 (Ph*C*H), 72.5 (Ph*C*H), 72.1 (C-3$_A$), 71.9 (Ph*C*H), 71.8 (C-4$_D$), 71.4 (Ph*C*H), 71.1 (C-5$_D$), 69.6 (C-6$_E$), 69.3 (C-4$_A$), 68.6 (O*C*H$_2$), 68.3 (C-5$_A$), 66.2 (C-6$_C$), 64.9 (C-5$_E$), 63.3 (C-5$_B$), 62.3 (C-6$_A$), 54.8 (C-2$_A$), 50.4 (N*C*H$_2$), 21.3, 20.9 (2C) (3CO*C*H$_3$), 15.9, 15.8 (2C*C*H$_3$); MALDI-MS: 1951.8 [M + Na]$^+$; anal. calcd for $C_{109}H_{116}N_4O_{28}$ (1930.09): C, 67.83; H, 6.06%; found: C, 67.70; H, 6.20%.

2-Azidoethyl *O*-(2,3-di-*O*-benzyl-4,6-*O*-[(*R*)-(1-carboxymethyl) ethylidene]-α-D-galactopyranosyl)-(1 → 3)-(4-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl)-(1 → 6)-(2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl)-(1 → 3)-(2,4-di-*O*-benzyl-α-L-fucopyranosyl)-(1 → 3)-4,6-di-*O*-acetyl-2-*N*-phthalimido-2-deoxy-β-D-glucopyranoside (15)

To a solution of compound **13** (900 mg, 0.47 mmol) in CH_3CN (15 mL) was added $HClO_4$–SiO_2 (100 mg) and it was stirred at room temperature for 20 min. The reaction mixture was filtered and washed with EtOAc (50 mL). The solvents were removed under reduced pressure and the crude product was purified over SiO_2 using hexane–EtOAc (1 : 1) as eluant to give pure compound **14** (705 mg, 82%). To a solution of compound **14** (500 mg, 0.27 mmol) in dry CH_3CN (15 mL) was added methyl 2,2-di(ethylthio)propionate (170 mg, 0.81 mmol) and NIS (460 mg, 2.03 mmol) and the mixture was stirred at 0 °C under argon for 10 min. To the cold reaction mixture was added TfOH (15 μL) and it was stirred at 0 °C for a further 20 min. After completion of the reaction (TLC), 10% aq. $Na_2S_2O_3$ (50 mL) was added to the reaction mixture and the solvents were removed and the crude mass was diluted with CH_2Cl_2 (50 mL). The organic solution was washed with satd aq. $NaHCO_3$ (50 mL) and water (50 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified over SiO_2 using hexane–EtOAc (3 : 1) to give the pure compound **15** (360 mg, 68%). White solid; mp 144–145 °C [EtOH]; $[\alpha]_D^{25}$ +22.8 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.92–6.93 (m, 40H, Ar-H), 5.55 (d, J = 3.5 Hz, 1H, H-1$_D$), 5.42 (d, J = 8.0 Hz, 1H, H-1$_A$), 5.15 (d, J = 12.0 Hz, 1H, PhC*H*), 5.13–4.95 (m, 2H, H-4$_A$, H-4$_D$), 4.89 (d, J = 3.0 Hz, 1H, H-1$_E$), 4.87 (d, J = 12.0 Hz, 1H, PhC*H*), 4.79–4.45 (m, 9H, 9PhC*H*), 4.67 (d, J = 3.5 Hz, 1H, H-1$_B$), 4.41–4.30 (m, 7H, H-2$_A$, H-3$_A$, H-4$_E$, H-6$_{aE}$, 3PhC*H*), 4.28 (d, J = 3.5 Hz, 1H, H-1$_C$), 4.25–4.13 (m, 5H, H-2$_D$, H-3$_D$, H-6$_{bE}$, 2PhC*H*), 4.08–3.92 (m, 8H, H-2$_B$, H-2$_E$, H-3$_B$, H-4$_C$, H-6$_{abA}$, H-6$_{aC}$, OC*H*), 3.90 (s, 3H, CO_2CH_3), 3.88–3.78 (m, 5H, H-3$_C$, H-3$_E$, H-5$_B$, H-5$_C$, H-5$_D$), 3.76–3.68 (m, 2H, H-5$_A$, OC*H*), 3.62–3.55 (m, 2H, H-2$_C$, H-6$_{bC}$), 3.47–3.38 (m, 2H, NC*H*, H-4$_B$), 3.28–3.19 (m, 2H, NC*H*, H-5$_E$), 2.08, 2.00, 1.95 (3s, 9H, 3COCH_3), 1.61 (s, 3H, CCH_3), 0.97–0.89 (m, 6H, 2CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.3, 170.8, 170.7 (3*C*OCH$_3$), 169.8 (*C*O$_2$CH$_3$), 168.2, 167.4 (Phth*C*O), 139.2–122 (Ar-*C*), 102.4 (C-1$_C$), 99.08 (C-1$_D$), 98.8 (*C*CH$_3$), 98.3 (C-1$_E$), 98.1 (C-1$_B$), 97.5 (C-1$_A$), 81.9 (C-4$_B$), 79.4 (C-4$_C$), 78.9 (C-2$_B$), 76.3 (2C, C-2$_C$, C-2$_D$), 75.9 (Ph*C*H), 75.3 (2C, C-3$_C$, C-5$_C$), 75.0 (C-4$_E$), 74.9 (Ph*C*H), 74.7 (C-3$_D$), 74.5 (2C, C-2$_E$, C-3$_E$), 74.0 (Ph*C*H), 73.3 (C-3$_B$), 72.8 (2C, 2Ph*C*H), 72.7 (Ph*C*H), 72.1 (C-3$_A$), 71.9 (Ph*C*H), 71.1 (C-4$_D$), 70.5 (Ph*C*H), 69.4 (C-5$_D$), 68.6 (C-6$_E$), 68.3 (C-4$_A$), 66.1 (O*C*H$_2$), 65.8 (C-6$_C$), 64.6 (C-5$_A$), 64.9 (C-6$_A$), 63.3 (C-5$_E$), 62.3 (C-5$_B$), 54.8 (C-2$_A$), 50.4 (N*C*H$_2$), 26.0 (C*C*H$_3$), 21.2, 20.9, 20.8 (3CO*C*H$_3$), 15.9, 15.8 (2C*C*H$_3$); MALDI-MS: 1947.7 [M + Na]$^+$; anal. calcd for $C_{106}H_{116}N_4O_{30}$ (1926.06): C, 66.10; H, 6.07%; found: C, 66.00; H, 6.20%.

2-Aminoethyl *O*-(4,6-*O*-[(*R*)-(1-sodium carboxylate)ethylidene]-α-D-galactopyranosyl)-(1 → 3)-(α-L-fucopyranosyl)-(1 → 6)-(α-D-galactopyranosyl)-(1 → 3)-(α-L-fucopyranosyl)-(1 → 3)-2-acetamido-2-deoxy-β-D-glucopyranoside (1)

To a solution of compound **15** (300 mg, 0.23 mmol) in ^{n}butanol (15 mL) was added ethylene diamine (0.3 mL) and the mixture was stirred at 90 °C for 10 h. The solvents were removed under reduced pressure, the crude product was dissolved in acetic anhydride (2 mL) and pyridine (2 mL) and the solution was kept at room temperature for 1 h. The solvents were removed under reduced pressure to give the acetylated product that was passed through a short pad of silica gel with EtOAc (50 mL) as eluent. To a solution of the acetylated product in CH_3OH (10 mL) was added 20% Pd(OH)$_2$–C (100 mg) and the reaction mixture was stirred under a positive pressure of hydrogen at room temperature for 15 h. The reaction mixture was filtered through a Celite bed, washed with CH_3OH (30 mL) and concentrated. A solution of the crude product in 0.1 M CH_3ONa (10 mL) was stirred at room temperature for 3 h, then few drops of water was added and further stirred for another 8 h. The reaction mixture was neutralized with Dowex 50W X8 (H^+), filtered, and concentrated to give the crude product that was purified by gel chromatography using Sephadex LH-20 using CH_3OH–H_2O (3 : 1) as eluant to give 1 (120 mg, 54%). White powder; $[\alpha]_D^{25}$ +10.2 (*c* 1.0, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 5.08 (d, J = 3.5 Hz, 1H, H-1$_E$), 5.05 (d, J = 4.0 Hz, 1H, H-1$_C$), 4.89 (d, J = 3.5 Hz, 1H, H-1$_B$), 4.78 (d, J = 4.0 Hz, 1H, H-1$_D$), 4.41 (d, J = 8.5 Hz, 1H, H-1$_A$), 4.18 (d, J = 7.0 Hz, 1H, H-5$_B$), 4.12 (t, J = 6.0 Hz each, 1H, H-5$_C$), 4.09 (d, J = 3.5 Hz, 1H, H-4$_E$), 4.00–3.85 (m, 4H, H-3$_E$, H-4$_C$, H-4$_B$, H-5$_D$), 3.84–3.78 (m, 5H, H-2$_D$, H-2$_E$, H-3$_D$, H-5$_E$, OC*H*), 3.77–3.70 (m, 10H, H-2$_A$, H-2$_B$, H-2$_C$, H-3$_B$, H-3$_C$, H-4$_D$, H-6$_{abE}$, H-6$_{aA}$, H-6$_{aC}$), 3.69–3.58 (m, 2H, H-4$_A$, OC*H*), 3.57–3.50 (m, 1H, H-3$_A$), 3.48–3.32 (m, 3H, H-5$_A$, H-6$_{bA}$, H-6$_{bC}$), 3.12–3.0 (m, 2H, NCH_2), 1.88 (s, 3H, NHCOCH_3), 1.43 (s, 3H, CCH_3), 1.03–1.01 (m, 6H, 2CCH_3); ^{13}C NMR (125 MHz, D_2O): δ 176.09 (*C*OOH), 174.0 (NH*C*OCH$_3$), 101.5 (C-1$_A$), 100.7 (2C, C-1$_C$, C-1$_E$), 100.6 (*C*COOH), 99.8 (C-1$_D$), 98.4 (C-1$_B$), 80.0 (C-3$_A$), 78.9 (C-3$_B$), 78.2 (C-4$_D$), 75.8 (C-5$_A$), 71.7 (2C, C-3$_D$, C-4$_E$), 71.4 (2C, C-4$_B$, C-4$_C$), 69.7 (C-5$_C$), 69.4 (C-4$_A$), 69.2 (C-3$_C$), 68.6 (C-2$_C$), 68.5 (C-2$_E$), 68.4 (C-3$_E$), 67.9 (C-2$_D$), 66.8 (2C, C-5$_B$, C-5$_D$), 66.6 (C-2$_B$), 66.5 (C-6$_C$), 65.9 (C-6$_E$), 64.9 (C-6$_A$), 62.9 (C-5$_E$), 60.6 (O*C*H$_2$), 55.0 (C-2$_A$), 39.4 (N*C*H$_2$), 25.1 (C*C*H$_3$), 22.2 (CO*C*H$_3$), 15.5, 15.2 (C*C*H$_3$); MALDI-MS: 972.3 [M]$^+$; anal. calcd for $C_{37}H_{61}N_2NaO_{26}$ (972.86): C, 45.68; H, 6.32%; found: C, 45.50; H, 6.45%.

Conclusions

In conclusion, a concise convergent strategy has been developed for the synthesis of the pyruvate acetal containing pentasaccharide repeating unit of the cell wall O-antigen of *E. coli* O156 in excellent yield. Application of stereoselective [2 + 3] block glycosylation, achievements of several α-glycosidic linkages, use of $HClO_4$–SiO_2, a cheap and stable solid acid in the glycosylation, preparation of pyruvic acid acetal using pyruvate dithioacetal under glycosylation condition, attachment of 2-aminoethyl linker at the reducing end for easy connectivity with an appropriate protein or aglycone are important features of the synthetic scheme. All glycosylation steps are high yielding with excellent stereoselectivity.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

A. S. thanks CSIR, New Delhi for providing Senior Research Fellowship. The work is supported by SERB, New Delhi (Project No. EMR/2015/000282 dated 17.09.2015) (AKM) and Bose Institute.

Notes and references

1 J. T. Watson, M. Gayer and M. A. Connolly, *Emerging Infect. Dis.*, 2007, **13**, 1–5.
2 V. Curtis, S. Cairncross and R. Yonli, *Trop. Med. Int. Health*, 2000, **5**, 22–32.
3 G. Gavazzi, F. Herrmann and K.-H. Krause, *Clin. Infect. Dis.*, 2004, **39**, 83–91.
4 O. Müller and M. Krawinkel, *Can. Med. Assoc. J.*, 2005, **173**, 279–286.
5 B. M. Lund and S. J. O'Brien, *Foodborne Pathog. Dis.*, 2011, **8**, 961–973.
6 F. Jafari, L. Shokrzadeh, M. Hamidian, S. Salmanzadeh-Ahrabi and M. R. Ali, *Jpn. J. Infect. Dis.*, 2008, **61**, 269–273.
7 G. O. Canny and B. A. McCormick, *Infect. Immun.*, 2008, **76**, 3360–3373.
8 J. P. Nataro and J. B. Kaper, *Clin. Microbiol. Rev.*, 1998, **11**, 142–201.
9 B. Köves and B. Wullt, *Eur. Urol., Suppl.*, 2016, **15**, 88–94.
10 J. M. Rhodes, *Gut*, 2007, **56**, 610–612.
11 R. E. Besser, S. M. Lett, J. T. Weber, M. P. Doyle, T. J. Barrett, J. G. Wells and P. M. Griffin, *J. Am. Med. Assoc.*, 1993, **269**, 2217–2220.
12 F. E. Preston, R. G. Malia, M. J. Sworn and E. K. Blackburn, *J. Clin. Pathol.*, 1973, **26**, 120–125.
13 M. A. Croxen, R. J. Law, R. Scholz, K. M. Keeney, M. Wlodarska and B. B. Finlay, *Clin. Microbiol. Rev.*, 2013, **26**, 822–880.
14 J. C. Paton and A. W. Paton, *Clin. Microbiol. Rev.*, 1998, **11**, 450–479.
15 J. Y. Lim, J. W. Yoon and C. J. Hovde, *J. Microbiol. Biotechnol.*, 2010, **20**, 5–14.
16 N. Allocati, M. Masulli, M. F. Alexeyev and C. D. Ilio, *Int. J. Environ. Res. Public Health*, 2013, **10**, 6235–6254.
17 J. M. Bosilevac and M. Koohmaraie, *Appl. Environ. Microbiol.*, 2011, **77**, 2103–2112.
18 I. Lerouge and J. Vanderleyden, *FEMS Microbiol. Rev.*, 2002, **26**, 17–47.
19 (*a*) *Carbohydrate based vaccines*, ed. R. Roy, Oxford University Press, Oxford, 2008; (*b*) J. J. Mond, A. Lees and C. M. Snapper, *Annu. Rev. Immunol.*, 1995, **13**, 655–692.
20 (*a*) R. D. Astronomo and D. R. Burton, *Nat. Rev. Drug Discovery*, 2010, **9**, 308–324; (*b*) M. Cavallari and G. De Libero, *Vaccines*, 2017, **5**, 4, DOI: 10.3390/vaccines5010004.
21 Z. Duan, S. N. Senchenkova, X. Guo, A. V. Perepelov, A. S. Shashkov, B. Liu and Y. A. Knirel, *Carbohydr. Res.*, 2016, **430**, 24–28.
22 A. Chakkumkal, S. Benjamin, L. P. Claney and P. H. Seeberger, *Chem. Biol.*, 2014, **21**, 38–50.
23 (*a*) V. Pozsgay, *Curr. Top. Med. Chem.*, 2008, **8**, 126–140; (*b*) A. Hölemann and P. H. Seeberger, *Curr. Opin. Biotechnol.*, 2004, **15**, 522–555.
24 A. K. Chakraborti and R. Gulhane, *Chem. Commun.*, 2003, 1896–1897.
25 C. Mukherjee and A. K. Misra, *Synthesis*, 2007, 683–692.
26 B. Mukhopadhyay, S. V. Maurer, N. Rudolph, R. M. van Well, D. A. Russell and R. A. Field, *J. Org. Chem.*, 2005, **70**, 9059–9062.
27 G. Agnihotri and A. K. Misra, *Tetrahedron Lett.*, 2006, **47**, 8493–8497.
28 P. K. Mandal, *Synthesis*, 2015, **47**, 836–844.
29 T. Ziegler, *Carbohydr. Res.*, 1994, **262**, 195–212.
30 J. Lindberg, P. Stralfors and P. Konradsson, *Tetrahedron*, 2002, **58**, 4245–4254.
31 Y. Hua, Y. Du, G. Yu and S. Chu, *Carbohydr. Res.*, 2004, **339**, 2083–2090.
32 S. Figueroa-Perez and R. R. Schmidt, *Carbohydr. Res.*, 2000, **328**, 95–102.
33 G. Zemplen, *Ber. Dtsch. Chem. Ges.*, 1926, **59**, 1254–1266.
34 G. Agnihotri and A. K. Misra, *Tetrahedron Lett.*, 2006, **47**, 3653–3658.
35 T. Ogawa and H. Yamamoto, *Agric. Biol. Chem.*, 1985, **49**, 475–482.
36 O. Kanie, Y. Ito and T. Ogawa, *J. Am. Chem. Soc.*, 1994, **116**, 12073–12074.
37 K. Bock and C. Pederson, *J. Chem. Soc., Perkin Trans. 2*, 1974, 293–297.
38 D. Crich and H. Li, *J. Org. Chem.*, 2002, **67**, 4640–4646.
39 A. Liptak, I. Bajza, J. Kerekgyarto, J. Hajko and L. Szilagyi, *Carbohydr. Res.*, 1994, **253**, 111–120.
40 P. A. J. Gorin, M. Mazurek, H. S. Duarte, M. Iacomini and J. H. Duarte, *Carbohydr. Res.*, 1982, **100**, l–15.
41 J. S. Debenham, R. Madsen, C. Roberts and B. Fraser-Reid, *J. Am. Chem. Soc.*, 1995, **117**, 3302–3303.
42 W. M. Pearlman, *Tetrahedron Lett.*, 1967, **8**, 1663–1664.