Bioorganic & Medicinal Chemistry 20 (2012) 3898–3914

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis of β(1,3) oligoglucans exhibiting a Dectin-1 binding affinity and their biological evaluation

Hiroshi Tanaka [a,*], Tetsuya Kawai [a], Yoshiyuki Adachi [b], Shinya Hanashima [c], Yoshiki Yamaguchi [c], Naohito Ohno [b], Takashi Takahashi [a,*]

[a] Department of Applied Chemistry, Graduate School of Science and Engineering, Tokyo Institute of Technology, 2-12-1-S1-35 Ookayama, Meguro, Tokyo 152-8552, Japan
[b] Laboratory for Immunopharmacology of Microbial Products, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, 1432-1 Horinouchi Hachioji, Tokyo 192-0392, Japan
[c] Chemical Biology Department, RIKEN Advanced Research Institute, 2-1 Hirosawa Wako, Saitama 351-0198, Japan

ARTICLE INFO

Article history:
Received 11 February 2012
Revised 6 April 2012
Accepted 7 April 2012
Available online 16 April 2012

Keywords:
Innate immunity
Polysaccharides
STD-NMR
Chemoselecitve glycosylation
β-Glucan

ABSTRACT

In this report, we describe the synthesis and biological evaluation of β(1,3) oligosaccharides that contain an aminoalkyl group and their biological evaluation. A 2,3 diol glycoside with a 4,6 benzylidene protecting group was used as an effective glycosyl acceptor for the synthesis of some β(1,3) linked glycosides. The use of a combination of a linear tetrasaccharide and a branched pentasaccharide as glycosyl donors led to the preparation of β(1,3) linear octa- to hexadecasaccharides and branched nona- to heptadecasaccharides in good total yields. Measurements of the competitive effects of the oligosaccharides on the binding of a soluble form of Dectin-1 to a solid-supported Schizophyllan (SPG) revealed that the branched heptadecasaccharide and the linear hexadecasaccharides also have binding activity for Dectin-1. In addition, the two oligosaccharides, both of which contain a β(1,3) hexadecasaccharide backbone, exhibited agonist activity in a luciferase-assisted NF-κB assay. STD-NMR analyses of complexes of Dectin-1 and the linear hexadecasaccharides clearly indicate Dectin-1 specifically recognizes the sugar part of the oligosaccharides and not the aminoalkyl chain.

1. Introduction

Certain types of β-glucans containing β(1,3) and β(1,6) linkages exhibit biological activity, in the sense that they stimulate innate immunity. Dectin-1, originally identified as a dendritic cell receptor in the mouse,[1] is a β-glucan receptor that mediates phagocytosis and the production of inflammatory mediators that are involved in the development of immunity to fungal pathogens.[2] The elucidation of the minimum structure required for binding to Dectin-1 and for exhibiting Dectin-1 agonist or antagonist activity is an important issue, in terms of our understanding of β-glucan-induced biological events and would strongly assist in the development of artificial modulators for innate immunity. A previous study using naturally occurring β-glucans and hydrolysates derived from them revealed that oligosaccharides containing more than 10 glucose units are required for binding to Dectin-1.[3] In addition, particulate β-glucans would need to activate Dectin-1 signalling.[4] However, for structure–activity relationship studies, the use of chemically synthesized β-glucans as biochemical probes would be highly desirable, because it is difficult to purify structurally defined oligosaccharides from hydrolysates and the possibility that isolated glucan derivatives could be heterogeneous and/or be contaminated with antigenic compounds cannot be excluded. Williams and co-workers recently elucidated the importance of a β(1,6) branching glucosyl unit for binding to Dectin-1 based on the experiments using a purely synthetic nonasaccharide containing β(1,3) and β(1,6) branching sugar units.[5]

β(1,3) oligosaccharides are, in practice, challenging targets owing to the considerable steric hindrance that results in their frequent adoption of non-chair conformers.[6] The synthesis of β(1,3) glucan related oligosaccharides have been investigated by several research groups.[7] The 4,6-benzylidene and 2-benzoyl protected glucosides have been reported to be effective blocking groups for the elongation of β(1,3) glucosides.[8] The 4,6-benzylidene acetal effectively blocks the C4 hydroxyl group with minimum steric hindrance to the C3 hydroxyl group. The C2 acyl protecting group assists in β-selective glycosidation reactions, but slightly reduces the reactivity of the C3 hydroxyl group towards glycosylation. Takeo et al. successfully prepared a β(1,3) octasaccharide using a disaccharide donor in a blockwise synthesis.[8a] We also investigated the combinatorial synthesis of branched β(1,3) glucan oligosaccharides.[9] However most of the established methodologies are directed at the synthesis of oligosaccharides composed of less than 10 glucose units. In addition, coupling of the complex branched

* Corresponding authors. Tel.: +81 3 5734 2471; fax: +81 3 5734 2884.
E-mail addresses: thiroshi@apc.titech.ac.jp (H. Tanaka), ttak@apc.titech.ac.jp (T. Takahashi).

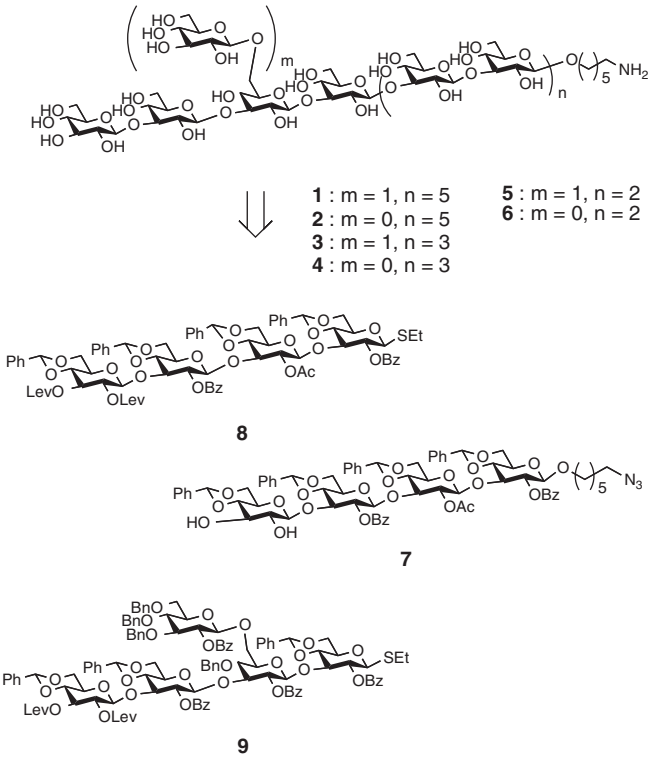
0968-0896/$ - see front matter © 2012 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.bmc.2012.04.017

oligosaccharide building blocks at the C3 position resulted in the unexpected production of β-isomers.[10] Therefore, an effective method for the synthesis of complex or large β(1,3) glucans continues to be needed. Herein, we report on the biological evaluation of the β(1,3) heptadeca to octasaccharides that were synthesized based on a convergent strategy. We also report on an analysis of saturated transfer difference (STD)-NMR spectra of complexes of a synthetic β(1,3) hexadecasaccharide and Dectin-1.[11]

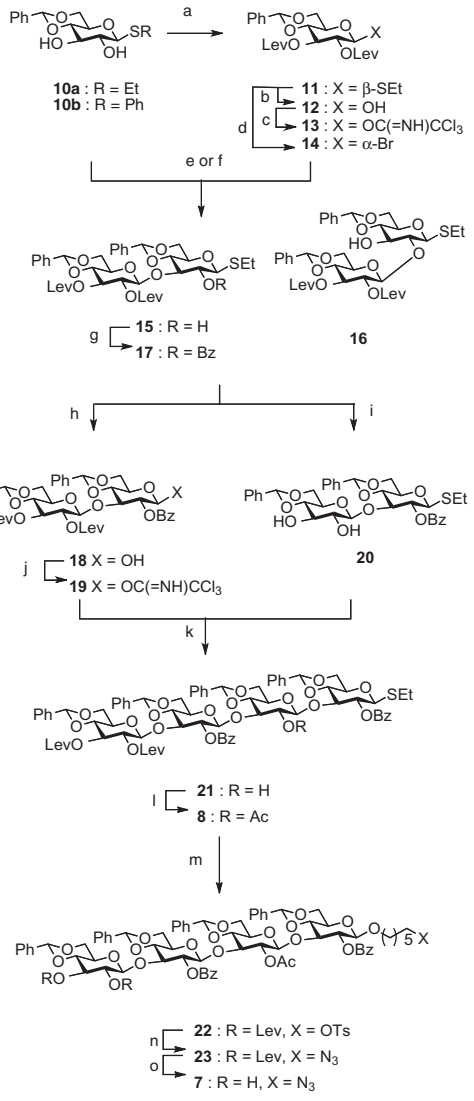
2. Results and discusstion

We originally planned to synthesize the linear β(1,3) hexadeca-, dodeca- and octasaccharides **2**, **4**, and **6** and branched heptadeca-, trideca- and nonasaccharides **1**, **3**, and **5** bearing an alkylamino group at the reducing end (Scheme 1). The presence of an amino group enhances the solubility of the compounds and serves as a connecting group for a wide variety of functionalized molecules. The synthesis of β(1,3) glucan oligosaccharides **2**, **4**, and **6** involved the regio- and stereoselecitve glycosylation of the 4,6-benzyliden-protected 2,3 diol acceptors **7** at the 3 position with the linear tetrasaccharide donor **8**. Although 4,6-benzylidene-protected 2,3 diol S-glycosides are known to undergo regioselecitve glycosylation at the 3 position, the corresponding methyl and allyl O-glycoside provided a significant amount of the undesired O2 glyosylated product.[12] In our experience, the steric hindrance of the sugar moiety at the anomeric position can prevent glycosylation at the 2 position.[13] The resulting coupling product can be converted into the 2,3 diol acceptor by protecting the remaining hydroxyl group followed by the removal of the levulinyl (Lev) protecting group for further glycosylation. Repetition of the coupling of the diol with the linear tetrasaccharide **8** and deprotection of the Lev group provided the linear oligosaccharides **2** and **4**. The use of the branched pentasaccharide **9** as a glycosyl donor enabled the preparation of the branched oligosaccharides **1**, **3** and **5**. Because of the large difference in molecular weights, the pure coupling products could be easily separated from the donors and accepters by gel permeation chromatography.[11]

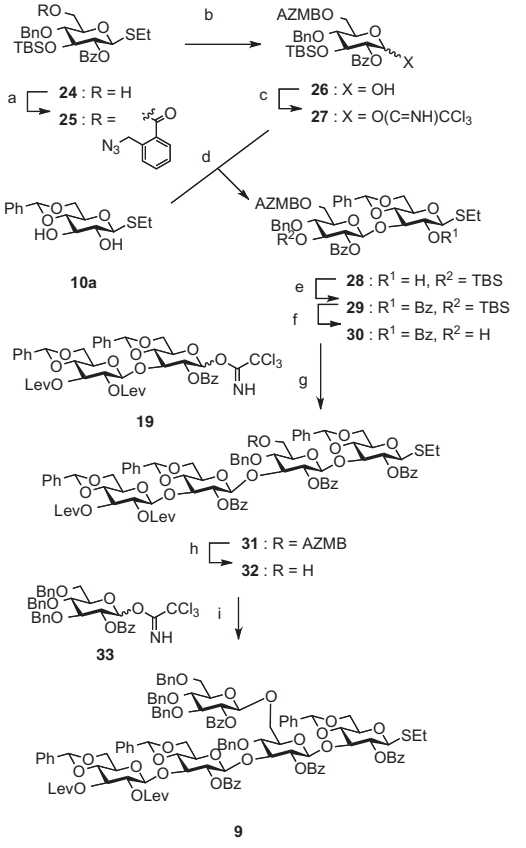
Scheme 2 shows the synthesis of the tetrasaccharide building blocks **7** and **8**. The thioglycoside **10a** was converted to the diester **11** in quantitative yield. Treatment of the thioglycoside **11** with benzenesulfinyl morpholine and Tf_2O under basic conditions,[14] followed by quenching the reaction mixture with water provided the hemiacetal **12** in 96% yield. Hydrolysis of the thioglcyoside **11** under oxidative conditions with NBS in acetone containing water resulted in an incomplete reaction, in which the removal of the benzyliden acetal group occurred. The hemiacetal **12** was converted to the glycosyl trichloroimidate **13** in 96% yield. The imidate **13** was sufficiently stable that it could be purified by column chromatography on silica gel. Chemo- and regio-selective glycosylation of thioglycoside **10a** with the glycosyl imidate **13** was examined. Treatment of the glycosyl imidate **13** and a 1.1 equiv of acceptor



Scheme 1. Strategy for the synthesis of β-glucan oligosaccahrides **1–6**.



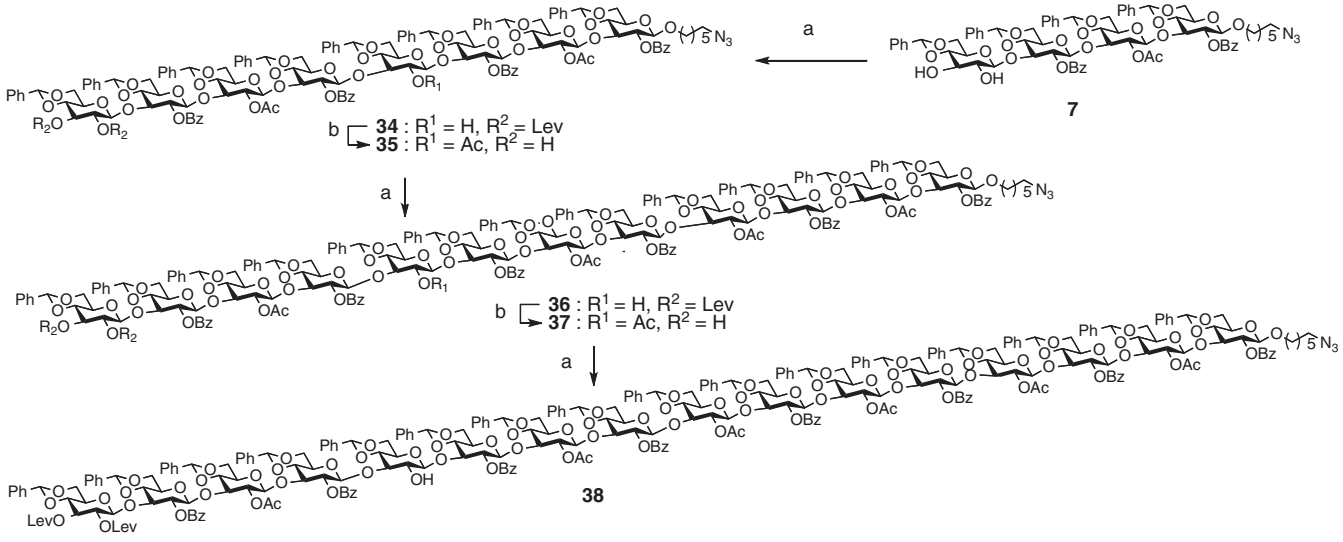
Scheme 2. Reagents and conditions: (a) levulinic acid, EDC-HCl, DMAP, CH_2Cl_2, quant.; (b) benzenesulfinyl morpholine, Tf_2O, CH_2Cl_2, −50 °C then NEt_3, 96%, α:β = 61:39; (c) CCl_3CN, Cs_2CO_3, CH_2Cl_2, 96%, α:β = 50:50; (d) IBr, MS-4A, CH_2Cl_2; (e) cat. TMSOTf, CH_2Cl_2, MS4A, −40 °C, 69% for **15**, 6% for **16**; (f) AgOTf, MS-4A, CH_2Cl_2, 28%; (g) BzCl, cat. DMAP, Py., 120 °C, 4 h, 96%; (h) benzenesulfinyl morpholine, Tf_2O, CH_2Cl_2, −50 °C then NEt_3, 86%, α:β = 66.34; (i) $NH_2NH_2{\cdot}H_2O$, AcOH, THF, 99%; (j) CCl_3CN, Cs_2CO_3, CH_2Cl_2, 96%, α:β = 84:16; (k) cat. TMSOTf, CH_2Cl_2, MS4A, −50 °C, 90%; (l) Ac_2O, cat. DMAP, Py., 96%; (m) $HO(CH_2)_6OTs$, NIS, cat. TfOH, MS4A, −35 °C, 31%; (n) NaN_3, DMF; (o) $NH_2NH_2{\cdot}H_2O$, AcOH, THF, 73%.



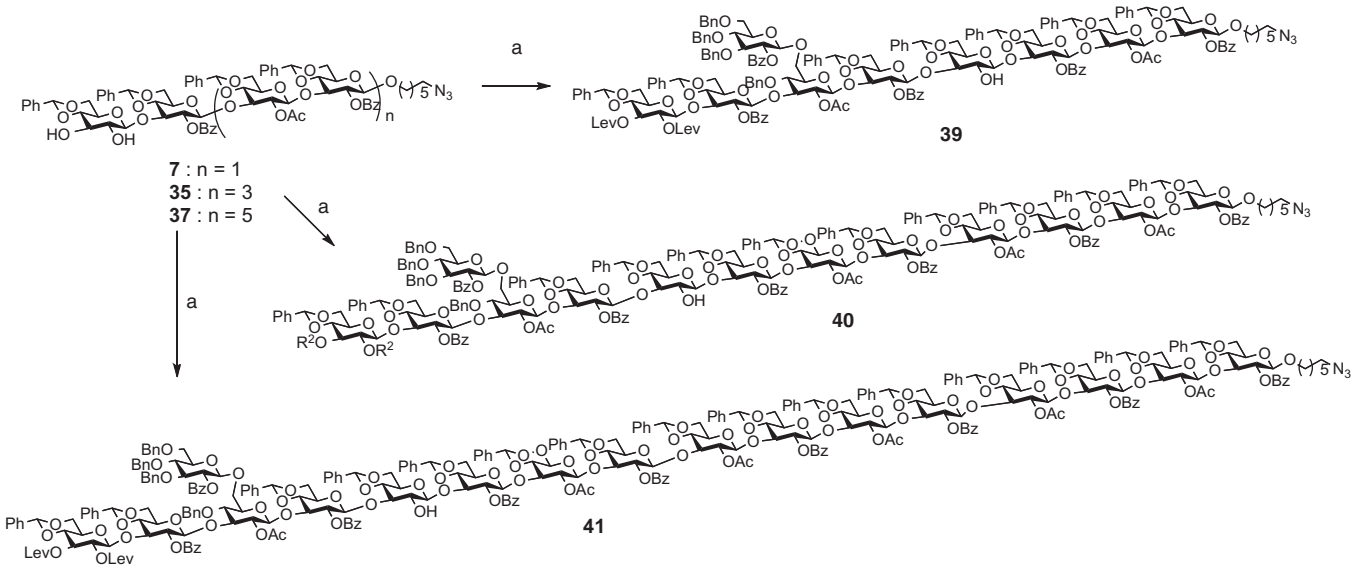
Scheme 3. Reagents and conditions: (a) 2-(azidomethyl)benzoyl chloride, DMAP, CH_2Cl_2, rt, 92%; (b) NBS, acetone, H_2O, rt, 10 min, then NEt_3, rt, 1 h, 76%; (c) CCl_3CN, Cs_2CO_3, CH_2Cl_2, rt, 1 h. 93%; (d) **10a**, cat. TMSOTf, CH_2Cl_2, MS4A, −15 °C, 94%; (e) BzCl, cat. DMAP, Py., 120 °C, 7 h; (f) HF·Py, Py, 100 °C, 12 h, 84% (g) cat. TMSOTf, CH_2Cl_2, MS4A, rt, 2 h, 59%; (h) nBu3P, THF, H_2O, rt, 30 min, 93%. (i) cat. TMSOTf, CH_2Cl_2, MS4A, 0 °C, 3 h, 91%.

10a in the presence of a catalytic amount of TMSOTf provided the C3 glycosylated product **15** in 69% yield along with the C2 glycosylated product **16** in 6% yield.[12] This method permitted us to use 39.7 g of donor for the glycosylation of diol **10a** without a significant reduction in the coupling yield. The use of phenylthioglycoside **10b** as a diol acceptor instead of ethylthioglycoside **10a** reduced the yield of the coupling product due to the reduced solubility of the acceptor **10b** to the reaction solvent. In addition, glycosyl bromide **14** did not worked well in the chemoselective glycosylation. Treatment of the remaining alcohol **15** with benzoyl chloride in the presence of a catalytic amount of DMAP in pyridine at 120 °C for 4 h provided the fully protected thioglycoside **17** in 84% yield. Removal of the Lev groups by treatment with NH_2NH_2 provided the disaccharide acceptor **20** in 99% yield. Conversion of the thioglycoside **17** to the glycosyl imidate **19** via the hemiacatal **18** was achieved by hydrolysis of thioglycoside (96%) and acylation of the resulting hydroxyl group with trichloroacetonitrile (94%). Glycosylation of the disaccharide acceptor **20** with the disaccharide donor **19** with a catalytic amount of TMSOTf provided the tetrasaccharide **21** in 90% yield. The remaining hydroxyl group was acylated by treatment with acetic anhydride. Glycosylation of the 1,6-hexanediol monotosylate with the tetrasaccharide donor **8** provided tosylate **22** in 31% yield. Treatment of tosylate **22** with sodium azide, followed by hydrolysis of the Lev esters afforded the diol acceptor **7** in 73% yield based on **22**.

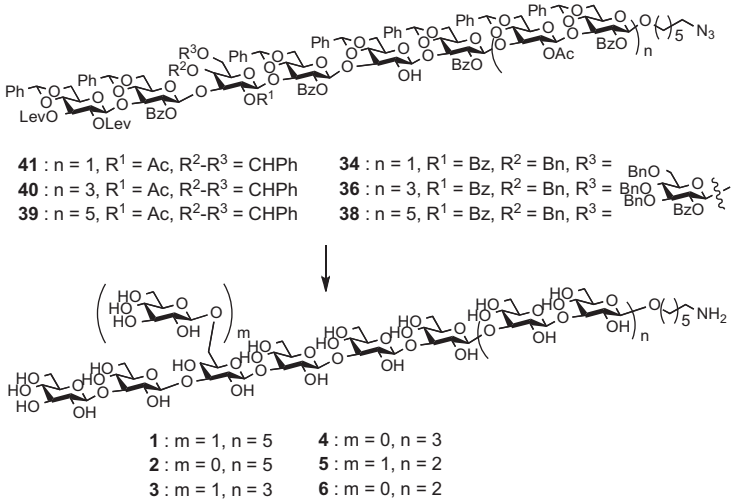
Scheme 3 shows the procedure used for the synthesis of the branched pentasaccharide **9**. Acylation of the primary alcohol with 2-(azidomethyl)benzoyl chloride provided 2-(azidomethyl)benzoate (AZMB) ester **25** in 92% yield. Oxidative hydrolysis of the thioglycoside **25** to the hemiacetal **26** (76%), followed by reaction with trichloroacetonitrile provided the glycosyl imidate **27** in 93% yield. The chemo- and regio-selecitve glycosylation of diol **10a** with the glycosyl imidate **27** provided the disaccharide **28** in 94% yield. The remaining hydroxyl group at the C2 position was protected by reaction with benzoyl chloride to afford the protected disaccharide **29**. Removal of the TBS group at the 3 position of **29**, provided the disaccharide acceptor **30** in 84% yield based on **28**. Glycosylation of the C3 hydroxyl group of **30** with the glycosyl imdiate **19** provided the linear tetrasaccharide **31** in 59% yield. Treatment of the tetrasaccharide **31** with tetra-*n*-butylphosphine at room temperature resulted in the removal of the AZMB ester to provide the primary alcohol **32** in 93% yield. Glycosylation of the resulting primary alcohol was achieved by treatment with the super-armed glycosyl imitate[15] **33** in the presence of a catalytic amount of TMSOTf at 0 °C for 3 h provided the pentasaccharide **9** in 91% yield.



Scheme 4. Reagents and conditions: (a) **8**, NIS, cat. TfOH, MS4A, −35 °C, 97% for **34**, 88% for **36**, 82% for **38**; (b) (i) Ac_2O, cat. DMAP, Py.; (ii) $NH_2NH_2{\cdot}H_2O$, AcOH, THF, 93% for **35**, 92% for **37**.



Scheme 5. Reagents and conditions: (a) **9**, NIS, cat. TfOH, MS4A, −35 °C, 89% for **39**, 79% for **40**, 82% for **41**.



Scheme 6. Strategy for the synthesis of β-glucan oligosaccahrides **1–6**.

Table 1
Deprotection of the protected oligosaccharides **34**, **36** and **38–41**

Substrate	Products	Yield
39	1	51
38	2	80
40	3	81
36	4	90
41	5	Quant.
34	6	43

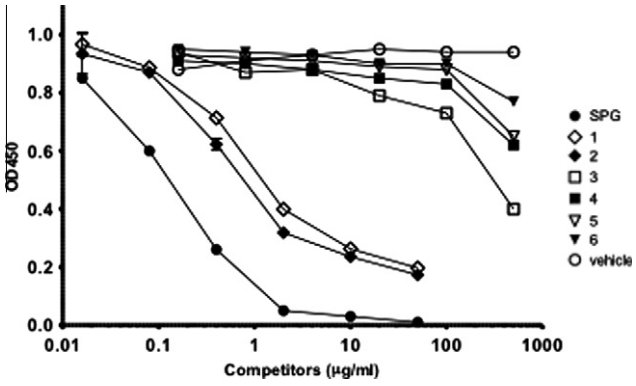


Figure 1. Competitive effects of the oligosaccharides **1–6** on binding of Dectin-1 to SPG.

The synthesis of the linear β(1,3) hexadeca- dodeca- and octasaccharides **38**, **36** and **34** from the tetrasaccharide donor **8** and acceptor **7** is shown in Scheme 4. The glycosylation of the acceptor **7** with thioglycoside **8** provided the octasaccharide **34** in 97% yield as a single isomer. The octasaccharide **34** was converted to diol **35** by acetylation of the remaining unprotected hydroxyl group, followed by removal of the Lev groups. Glycosylation of the acceptor **35** with thioglycoside **8** provided the dodecasaccharide **36** in 88% yield as a single isomer. The dodecasaccharide **33** was converted to diol **37** using the same procedure as was used for **34** to **35**. Glycosylation of dodecasaccharide **37** with the linear tetrasaccahride **8**

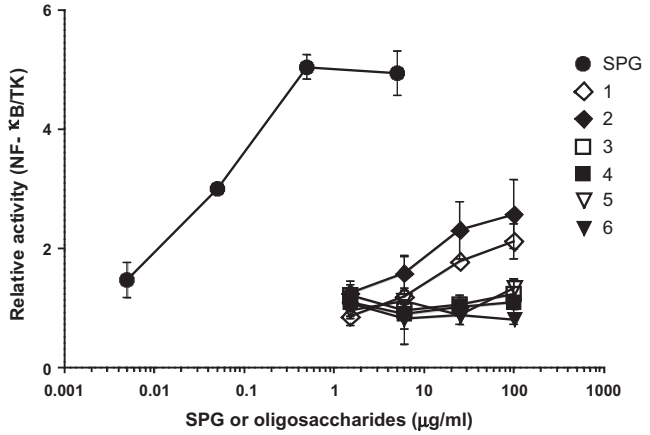


Figure 2. Activation of luciferase-assisted NF-κB in Dectin-1 transfectant by the oligosaccharides **1–6**.

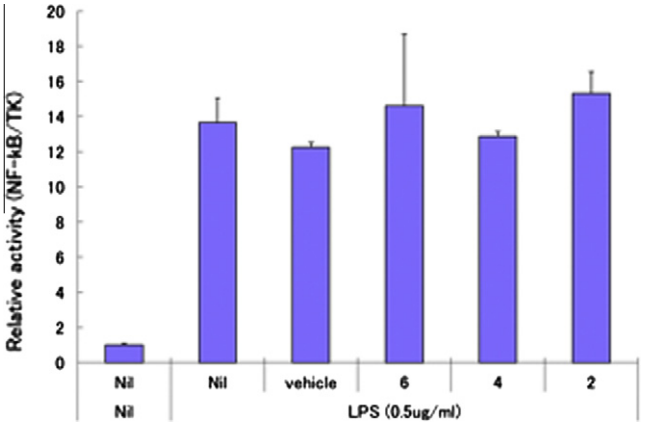


Figure 3. Activation of luciferase-assisted NF-κB in TLR4 transfectant by combination with the oligosaccharides **2**, **4**, **6** and LPS.

provided the linear hexadecasaccharide **38** in 82% yield (Scheme 5). In addition, Glycosylation of the diol acceptors **7**, **35** and **37** with the branched pentasaccharide **9** provided branched oligosaccharides **39**, **40** and **41** in 89%, 79% and 82% yields, respectively. The regioselective glycosylation at the C3 position was confirmed by 2D NMR analysis of coupling products **34**, **36**, **38** and **39–41** indicating that the remaining hydroxyl groups were located at the C2 position. Deprotection of the protected oligosaccharides **34**, **36**, **38** and **39–41** was achieved by exposure of the protected oligosaccharides **34**, **36**, **38** and **39–41** to Birch reduction conditions, followed by reaction with LiOMe at room temperature for 12 h to provide the corresponding oligosaccharides **1–6** in good to moderate yields (Scheme 6 and Table 1).

Competitive effects of the synthetic glucans **1–6** on binding to a soluble form of Dectin-1 attached to a solid-supported Schizophyllan (SPG) were examined (Fig. 1). The branched heptadecasaccharide **1** and the linear hexadecasaccharide **2** inhibited the binding of the soluble form of Dectin-1 to the solid-supported SPG. The binding activity of **1** and **2** was only 10-fold weaker than that of natural SPG. No significant differences in affinity between the branched and linear oligosaccharides **1** and **2** were observed. The inhibitory activities of the remaining oligosaccharides **3–6**, which contained less than tridecasaccharide units were dramatically reduced. These results indicate that the affinity of the oligosaccharide probes to the soluble Dectin-1 is clearly dependent on the length of the β(1,3) glucose sequences of **1** and **2**.

In another series of experiments, a luciferase-assisted NF-κB assay was conducted for oligosacchrides **1–6** (Fig. 2). 293T cells, transfected with a plasmid DNA mixture, encoding Dectin-1 and signaling molecules including CARD9 and Bcl10, were stimulated with oligosaccharides **1–6**. Luciferase activity, expressed as the ratio of NF-κB-assisted firefly luciferase activity to thymdine kinase (TK) promoter-assisted renillaluciferase activity, was measured using a Dual-Luciferase Reporter Assay System. NF-κB activation was observed during the stimulation of the 293T cells with the branched heptadecasacccharide **1** and the linear hexadecasacccharide **2**. On the other hand, the low-affinity ligands **3–6** failed to stimulate NF-κB activation. However, the difference in the activities of the synthetic oligosaccharides and SPG in promoting NF-kB activation cannot be completely explained by the differences in their binding affinity to Dectin-1. In addition, the linear oligosaccharides **2**,**4**,**6** failed to enhance LPS-mediated NF-κB activation through TLR4 (Fig. 3). These results strongly support the view that

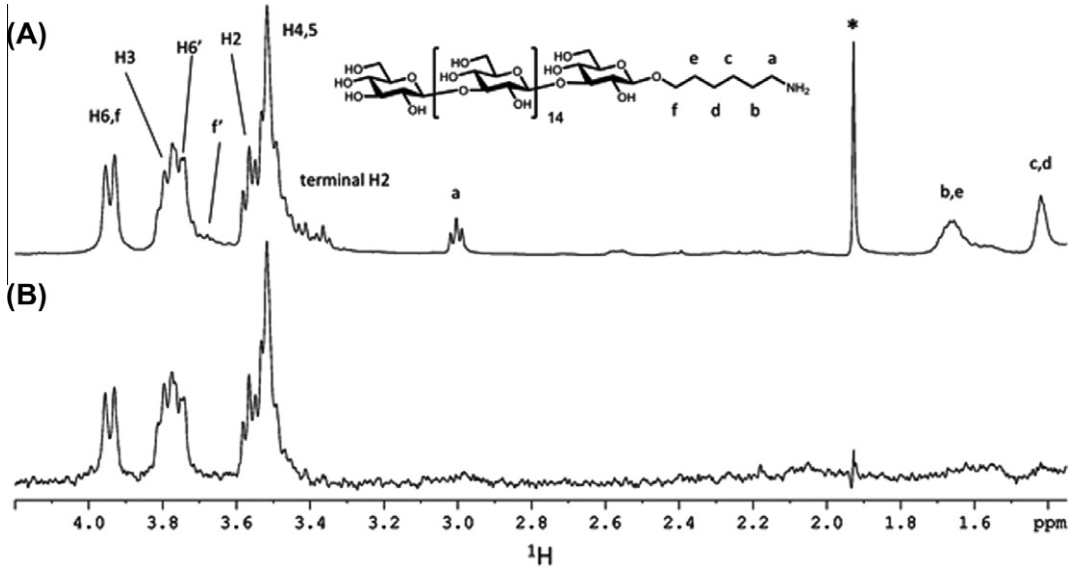


Figure 4. Saturation transfer difference (STD)-NMR spectra of **2** with Dectin-1. (A) ^{1}H NMR of **2** with Dectin-1, which was off-resonance spectrum irradiated at 40 ppm. (B) STD-NMR spectrum subtracts the on-resonance spectrum irradiated at 7.5 ppm from the off-resonance spectrum. Experiments were performed at 5 °C in PBS (pH 7.4) including 10% D_2O, and the molar ratio of Dectin-1 (10 μM) to **2** was 1:25. Spectra were obtained with 500 MHz spectrometer. *: impurity.

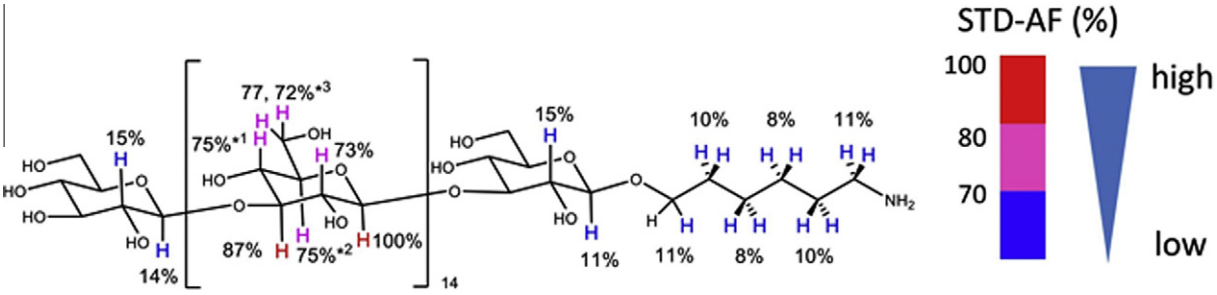


Figure 5. Binding epitope of **2** for Dectin-1. H-1 of inner glucose residue is calculated as 100%, and relative signal intensities were determined based on the STD amplification factors (STD-AF). *1: Averaged value of all 4-positon signals including the sugar units at reducing and non-reducing ends. *2: The average value of all 5-positon signals including sugar units at reducing and non-reducing ends, and 3-positon of the non-reducing end sugar unit.*3: Averaged value of all 6-positon signals including sugar units at reducing and non-reducing ends, and one proton at ϕ-position of alkyl-chain.

the stimulation of innate immunity by a β-glucan is likely initiated by the recognition of Dectin-1.

The binding between the extracellular C-type lectin-like domain (CTLD) of Dectin-1 and the linear hexadecasaccharide **2** was analyzed in detail by NMR spectroscopy. CTLD of Dectin-1 was prepared by means of an *E. coli* expression system as an insoluble fraction, and the refolded protein was purified prior to use (see Experimental procedure). Titration with the linear hexadecasaccharide **2** into the protein solution was initially performed from 0 to 25 M equiv. The results showed that the ^{1}H NMR signals originating from Dectin-1 were slightly perturbed and dependent on the molar ratio (data not shown). Based on the findings from the titration experiments as well as ELISA assays, we further analyzed the binding event using a saturation transfer difference (STD)-NMR technique (Fig. 4).[16] The CTLD of Dectin-1 (10 μM) with the linear hexadecasaccharide **2** (25 equiv) in PBS with 10% D_2O was used in the STD-NMR experiment. The saturation pulse was irradiated to the protein signal at 7.5 ppm, which consists of aromatic and amide protons. STD spectrum (Fig. 4B) was obtained by subtraction from a reference spectrum irradiated at 40 ppm. The spectrum clearly indicated the binding epitope of Dectin-1 (Fig. 5). The protons contained by the inner residues of the glucose-chain showed higher signal intensities, whereas the 6-aminohexyl-chain at the reducing termini did not receive a significant saturation transfer effect from Dectin-1. Further, the H-1 and H-3 signals of the inner glucose residues provided especially higher STD-amplification factors (100% and 87%) than the H-2, H-4 and H-6 signals (72–75%). On the other hand, glucose residues at both reducing and non-reducing termini appeared to include a small contribution to the binding, based on their STD results originating from H-1 and H-2 (11–15%). The above spectral evidence suggests that Dectin-1 specifically recognizes H-1 and H-3 at the α-face of the inner glucose residues. Daniellou's group recently reported comparable results using a laminarine polysaccharide as a probe. They also reported that significant STD effects were not observed when laminarinhexaose was used as a probe.[17] An earlier mutagenesis study indicated that Trp221 and His223 of Dectin-1 are key amino acids for β(1,3)-glucan binding.[18] The indole moiety of Trp221 may interact around the α-face of the inner type stacking interactions, because this type of glucose residues by CH-π binding has frequently been identified in lectin–glycan interactions.

3. Conclusion

In conclusion, we describe an effective convergent synthesis of β(1,3) oligosaccharides using linear tetra- and the branched pentasaccharides. The 2,3 diol glycoside with a 4,6 benzylidene protecting group acted as an effective glycosyl acceptor for the synthesis of a β(1,3) linked glycosides. The use of a combination of a linear tetrasaccharide and a branched pentasaccharide as glycosyl donors permitted β(1,3) linear octa- to hexadecasaccharides and branched nona- to heptadecasaccharides to be prepared in good total yields. Measurements of the competitive effects of oligosaccharides on the binding of a soluble form of Dectin-1 to a solid-supported Schizophyllan (SPG) revealed that the branched heptadecasaccharide **1** and the linear hexadecasaccharides **2** also have binding activity for Dectin-1. The binding affinity of the trideca- and dodecasaccharides were dramatically reduced compared to **1** and **2**. In addition, oligosaccharides **1** and **2**, containing a β(1,3) hexadecasaccharide backbone, exhibited agonist activity in a luciferase-assisted NF-κB assay. An STD-NMR analysis of complexes of Dectin-1 and the linear hexadecasacccharides was also examined and the results clearly show that Dectin-1 specifically recognizes the sugar part of the oligosaccharides and not the aminoalkyl chain. Further studies of the nature of the interactions between β(1,3)-glucans and Dectin-1 are ongoing.

4. Experimental section

4.1. Chemistry

4.1.1. Ethylthio 4,6-*O*-benzylidene-2,3-di-*O*-levulinyl-β-D-glucopyranoside (11)

To a stirred solution of ethylthio 4,6-*O*-benzylidene-β-D-glucopyranoside (**10a**) (9.50 g, 30.4 mmol) in CH_2Cl_2 (60.8 mL) was added EDCI-HCl (12.8 g, 66.9 mmol,), levulinic acid (6.85 mL, 66.9 mmol) and a catalytic amount of DMAP (371 mg, 3.04 mmol) at 0 °C. After being stirred at room temperature for 3 h, the reaction mixture was poured into 1 M HCl. The aqueous layer was extracted with two portions of ethyl acetate. The combined extract was washed with saturated aq $NaHCO_3$ and brine, and dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by chromatography on silica gel with 80:20 toluene:ethyl acetate to give diester **11** (15.4 g, 30.3 mmol, quant.). $[\alpha]_D^{24}$ −74.7 (c = 1.20, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42–7.45 (m, 2H), 7.33–7.36 (m, 3H), 5.50 (s, 1H), 5.36 (dd, 1H, J = 9.2, 9.7 Hz), 5.06 (dd, 1H, J = 9.2, 9.7 Hz), 4.56 (d, 1H, H-1, J = 9.7 Hz), 4.36 (dd, 1H, J = 4.8, 10.6 Hz), 3.77 (dd, 1H, J = 9.7, 10.6 Hz), 3.68 (dd, 1H, J = 9.2, 9.7 Hz), 3.55 (ddd, 1H, J = 4.8, 9.2, 9.7 Hz), 2.52–2.87 (m, 10H), 2.17 (s, 3H), 2.13 (s, 3H), 1.26 (t, 3H, J = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.3, 206.2, 171.8, 171.5, 136.8, 129.0, 128.2, 126.2, 101.4, 84.2, 78.4, 72.6, 70.8, 70.5, 68.5, 37.8 × 2, 29.7 × 2, 27.9, 24.3, 23.5, 14.9; IR (solid): 2971, 2873, 1746, 1718, 1369, 1208, 1152, 1080, 1026, 918, 753, 701, 544 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{25}H_{36}NO_9S$ $[M+NH_4]^+$ m/z = 526.2111, found: 526.2112.

4.1.2. 4,6-*O*-Benzylidene-2,3-di-*O*-levulinyl-D-glucopyranose (12)

To a stirred solution of thioglycoside **11** (102 mg, 0.201 mmol) in CH_2Cl_2 (2.01 mL) was added benzenesulfinyl morpholine (46.7 mg, 0.221 mmol) and Tf_2O (40.6 μL, 0.241 mmol) at −40 °C. After being stirred at the same temperature for 5 min, the reaction mixture was poured into a mixture of saturated aq $NaHCO_3$ and 10% aq $Na_2S_2O_3$. The aqueous layer was extracted with two portions of ethyl acetate. The combined extract was washed with a mixture of saturated aq $NaHCO_3$ and 10% aq $Na_2S_2O_3$, and brine, and dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was used for the next reaction without further purification. To a stirred solution of the residue in CH_2Cl_2 (2.00 mL) was added triethylamine (0.500 mL) at room temperature. After being stirred at the same temperature for 1 h, the reaction mixture was concentrated in vacuo. The residue was purified by chromatography on silica gel with 40:60 hexane:ethyl acetate to give hemiacetal **12** (89.9 mg, 0.194 mmol, 2 steps 96%, α:β = 61:39). The α:β ratio was determined by ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34–7.46 (m, 5H), 5.63 (dd, 0.61H, J = 9.2, 9.7 Hz), 5.50 (s, 1H), 5.40 (br-d, 0.61H, J = 2.4 Hz), 5.37 (dd, 0.39H, J = 9.7, 9.7 Hz), 4.89–4.93 (m, 1H), 4.82 (br-d, 0.39H, J = 8.2 Hz), 4.37 (dd, 0.39H, J = 4.8, 10.6 Hz), 4.30 (dd, 0.61H, J = 4.8, 10.1 Hz), 4.18 (ddd, 0.61H, J = 4.8, 9.7, 10.1 Hz), 3.95 (br-s, 0.39H), 3.52–3.83 (m, 3H), 2.50–2.84 (m, 8H), 2.14–2.19 (m, 6H); IR (solid): 3457, 2920, 2871, 1758, 1391, 974, 919, 769, 648, 509 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{23}H_{32}NO_{10}$ $[M+NH_4]^+$ m/z = 482.2026, found: 482.2027.

4.1.3. O-(4,6-*O*-Benzylidene-2,3-di-*O*-levulinyl-D-glucopyranosyl)trichloroacetimidate (13)

To a stirred solution of hemiacetal **12** (31.7 g, 68.3 mmol) in CH_2Cl_2 (68.3 mL) was added trichloroacetonitrile (20.5 mL, 205 mmol) and a catalytic amount of Cs_2CO_3 (222 mg, 0.683 mmol) at room temperature. After being stirred at the same temperature for 1 h, the reaction mixture was filtered through a pad of Celite® and concentrated in vacuo. The residue was purified by chromatography on silica gel with 60:40 hexane:ethyl acetate to give imidate **13** (39.7 g, 65.2 mmol, 96%, α:β = 50:50). The α:β ratio was determined by ^{1}H NMR analysis. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.73 (s, 0.50H), 8.67 (s, 0.50H), 7.35–7.47 (m, 5H), 6.50 (d, 0.50H, J = 3.9 Hz), 5.96 (d, 0.50H, J = 7.7 Hz), 5.69 (dd, 0.50H, J = 9.2, 9.7 Hz), 5.54 (s, 0.50H), 5.52 (s, 0.50H), 5.42 (dd, 0.50H, J = 9.2, 9.7 Hz), 5.32 (dd, 0.50H, J = 7.7, 9.2 Hz), 5.19 (dd, 0.50H, J = 3.9, 9.7 Hz), 4.43 (dd, 0.50H, J = 4.8, 10.1 Hz), 4.35 (dd, 0.50H, J = 4.8, 10.1 Hz), 4.12 (ddd, 0.50H, J = 4.8, 9.7, 10.1 Hz), 3.70–3.85 (m, 2.5H), 2.49–2.84 (m, 8H), 2.14–2.16 (m, 6H); IR (solid): 3316, 2920, 1751, 1718, 1677, 1364, 1146, 921, 797, 648 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{25}H_{32}N_2O_{10}$ $[M+NH_4]^+$ m/z = 625.1123, found: 625.1124.

4.1.4. Ethylthio 4,6-*O*-benzylidene-3-*O*-(4,6-*O*-benzylidene-2,3-di-*O*-levulinyl-β-D-glucopyranosyl)-β-D-glucopyranoside (15)

A mixture of thioglycoside **10** (6.99 g, 22.4 mmol), imidate **13** (12.4 g, 20.4 mmol) (azeotroped twice with dry toluene) and pulverized activated MS-4A (20.0 g) in dry CH_2Cl_2 (407 mL) was stirred at room temperature for 30 min under argon to remove a trace amount of water. Then the reaction mixture was cooled to −40 °C. A catalytic amount of trimethylsilyl trifluoromethanesulfonate (0.369 mL, 2.04 mmol) was added to the reaction mixture. After being stirred at the same temperature for 30 min, the reaction mixture was neutralized with triethylamine, filtered through a pad of Celite® and concentrated in vacuo. The residue was purified by chromatography on silica gel with 48:52 hexane:ethyl acetate to give the β(1,3) isomer **15** (10.6 g, 14.0 mmol, 69%) and the β(1,2) isomer **16** (0.994 g, 1.31 mmol, 6%). β(1,3) isomer **15**: $[\alpha]_D^{24}$ −54.0 (c = 1.03, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31–7.51 (m, 10H), 5.55 (s, 1H), 5.44 (s, 1H), 5.33 (dd, 1H, J = 9.7, 9.7 Hz), 5.10 (dd, 1H, J = 7.7, 9.7 Hz), 4.85 (d, 1H, J = 7.7 Hz), 4.47 (d, 1H, J = 9.7 Hz), 4.36 (dd, 1H, J = 4.8, 10.6 Hz), 4.28 (d, 1H, J = 4.8 Hz), 4.20 (dd, 1H, J = 4.8, 10.6 Hz), 3.73–3.79 (m, 3H), 3.68 (dd, 1H, J = 9.7, 9.7 Hz), 3.59–3.64 (m, 2H), 3.41–3.49 (m, 2H), 2.44–3.01 (m, 10H), 2.22 (s, 3H), 2.13 (s, 3H), 1.32 (t, 3H, J = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 208.9, 206.3, 171.7 × 2, 137.4, 136.9, 129.1 × 2, 128.3, 128.2, 126.3, 126.1, 103.1, 101.5, 101.0, 87.0, 84.7, 79.2, 78.3, 72.8, 72.4, 71.9, 70.9, 68.7, 66.3, 37.8, 30.1, 29.8, 27.9, 27.7, 24.3, 15.1; IR (solid): 3488, 2868, 2457, 2272, 1719, 1387, 1147, 1106, 917, 693, 524 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{38}H_{50}NO_{14}S$ $[M+NH_4]^+$ m/z = 776.2952, found: 776.2944. β(1,2) isomer **16**: $[\alpha]_D^{26}$ −65.8 (c = 1.14, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34–7.52 (m, 10H), 5.53 (s, 1H), 5.49 (s, 1H), 5.35 (dd, 1H, J = 9.2, 9.2 Hz), 5.04–5.11 (m, 2H), 4.52 (d, 1H, J = 9.7 Hz), 4.31–4.38 (m, 2H), 3.93 (ddd, 1H, J = 3.9, 8.7, 8.7 Hz), 3.81–3.87 (m, 2H), 3.71–3.76 (m, 2H), 3.65 (dd, 1H, J = 8.7, 9.7 Hz), 3.48–3.54 (m, 2H), 3.44 (ddd, 1H, J = 4.8, 9.7, 9.7 Hz), 2.46–2.84 (m, 10H), 2.19 (s, 3H), 2.13 (s, 3H), 1.28 (t, 3H, J = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 208.0, 206.3, 171.8, 171.5, 137.2, 136.9, 129.2, 129.1, 128.3, 128.2, 126.4, 126.2, 101.8, 101.7, 101.5, 84.1, 80.4, 80.3, 78.2, 75.2, 72.7, 71.9, 70.3, 68.7, 68.5, 66.3, 37.8 × 2, 29.9, 29.8, 27.9, 24.4, 14.7; IR (solid): 3425, 2856, 2351, 1742, 1706, 1368, 1307, 1154, 1097, 971, 749, 698, 547 cm^{-1}.

4.1.5. Ethylthio 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(4,6-*O*-benzylidene-2,3-di-*O*-D-glucopyranosyl)-β-levulinyl-β-D-glucopyranoside (17)

To a stirred solution of **15** (1.02 g, 1.34 mmol) in pyridine (6.70 mL) was added benzoyl chloride (0.471 mL, 4.02 mmol) and a catalytic amount of DMAP (16.4 mg, 0.134 mmol) at room temperature. After being stirred at 120 °C for 4 h, the reaction mixture was poured into 1 M HCl. The aqueous layer was extracted with two portions of ethyl acetate. The combined extract was washed with 1 M HCl, saturated aq $NaHCO_3$ and brine, and dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by chromatography on silica gel with 97:3 toluene:acetone to give benzoate **17** (1.11 g, 1.28 mmol, 96%). $[\alpha]_D^{24}$ −42.7 (c = 1.05, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (d, 2H, J = 7.7 Hz), 7.32–7.65 (m, 13H), 5.57 (s, 1H), 5.38 (s, 1H), 5.31 (dd, 1H, J = 9.2, 9.7 Hz), 5.08 (dd, 1H, J = 8.7, 9.2 Hz), 5.01 (dd, 1H, J = 7.7, 8.7 Hz), 4.70 (d, 1H, J = 7.7 Hz), 4.60 (d, 1H, J = 9.7 Hz), 4.38 (dd, 1H, J = 4.8, 10.6 Hz), 4.24 (dd, 1H, J = 4.8, 10.1 Hz), 4.18 (dd, 1H, J = 8.7, 9.2 Hz), 3.81 (dd, 1H, J = 10.1, 10.6 Hz), 3.75 (dd, 1H, J = 8.7, 9.7 Hz), 3.68 (dd, 1H, J = 10.1, 10.1 Hz), 3.63 (dd, 1H, J = 9.2, 9.7 Hz), 3.56 (ddd, 1H, J = 4,8, 9.7, 10.1 Hz), 3.37 (ddd, 1H, J = 4.8, 9.7, 10.1 Hz), 2.33–2.76 (m, 10H), 2.08 (s, 6H), 1.20 (t, 3H, J = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.4 × 2, 171.8, 171.5, 165.1, 137.3, 136.9, 133.6, 129.9, 129.6, 129.2, 129.1, 128.8, 128.3, 128.2, 126.2, 126.1, 101.4, 101.2, 101.1, 84.4, 79.6, 78.9, 78.4, 72.2, 71.9, 71.8, 71.2, 68.6, 66.3, 37.9, 37.8, 29.7 × 2, 28.0, 27.5, 24.1, 14.8; IR (solid): 2972, 1753, 1717, 1401, 1364, 1149, 1095, 1015, 917, 751, 698, 540 cm^{-1}.

4.1.6. 2-*O*-Benzoyl-4,6-*O*-benzylidene-3-*O*-(4,6-*O*-benzylidene-2,3-di-*O*-levulinyl-β-D-glucopyranosyl)-D-glucopyranose (18)

To a stirred solution of thioglycoside **17** (103 mg, 0.119 mmol) in CH_2Cl_2 (1.19 mL) was added benzenesulfinyl morpholine (27.8 mg, 0.131 mmol) and Tf_2O (24.0 μl, 0.143 mmol) at −50 °C. After being stirred at the same temperature for 5 min, the reaction mixture was poured into a mixture of saturated aq $NaHCO_3$ and 10% aq $Na_2S_2O_3$. The aqueous layer was extracted with two portions of ethyl acetate. The combined extract was washed with a mixture of saturated aq $NaHCO_3$ and 10% aq $Na_2S_2O_3$, and brine, and dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was used for the next reaction without further purification. To

a stirred solution of the residue in CH_2Cl_2 (2.00 mL) was added triethylamine (0.500 mL) at room temperature. After being stirred at the same temperature for 1 h, the reaction mixture was concentrated in vacuo. The residue was purified by chromatography on silica gel with 91:9 toluene:acetone to give hemiacatal **18** (84.2 mg, 0.103 mmol, 2 steps 86%, α:β = 66:34). The α:β ratio was determined by ^{1}H NMR analysis. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04–8.07 (m, 2H), 7.31–7.64 (m, 13H), 5.55 (s, 0.66H), 5.53 (s, 0.34H), 5.46 (br-s, 0.66H), 5.37 (s, 0.66H), 5.35 (s, 0.34H), 5.06–5.17 (m, 2H), 4.98–5.03 (m, 1H), 4.85 (d, 0.66H, J = 7.7 Hz), 4.78 (dd, 0.34H, J = 8.2, 8.7 Hz), 4.75 (d, 0.34H, J = 7.7 Hz), 4.44 (dd, 0.66H, J = 9.2, 9.7 Hz), 4.34 (dd, 0.34H, J = 4.8, 10.1 Hz), 4.11–4.30 (m, 3H), 3.89 (d, 0.66H, J = 2.4 Hz), 3.59–3.81 (m, 4H), 3.38–3.54 (m, 1.34H), 2.01–2.71 (m, 14H); IR (solid): 3493, 2921, 2862, 2056, 1895, 1709, 1601, 1413, 1265, 975, 772, 672, 534 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{43}H_{50}NO_{16}$ $[M+NH_4]^+$ m/z = 836.3130, found: 836.3127.

4.1.7. O-(2-*O*-Benzoyl-4,6-*O*-benzylidene-3-*O*-(4,6-*O*-benzylidene-2,3-di-*O*-levulinyl-β-D-glucopyranosyl)-D-glucopyranosyl) trichloroacetimidate (19)

To a stirred solution of hemiacatal **18** (4.34 mg, 5.30 mmol) in CH_2Cl_2 (26.5 mL) was added trichloroacetonitrile (2.66 mL, 26.5 mmol) and a catalytic amount of Cs_2CO_3 (173 mg, 0.530 mmol) at room temperature. After being stirred at the same temperature for 1 h, the reaction mixture was filtered through a pad of Celite® and concentrated in vacuo. The residue was purified by chromatography on silica gel with 94:6 toluene:acetone to give imidate **19** (4.86 g, 5.06 mmol, 96%, α:β = 84:16). The α:β ratio was determined by ^{1}H NMR analysis. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (s, 0.16H), 8.57 (s, 0.84H), 7.99–8.03 (m, 2H), 7.33–7.64 (m, 13H), 6.61 (d, 0.84H, J = 4.3 Hz), 6.05 (d, 0.16H, J = 7.2 Hz), 5.62 (s, 0.84H), 5.60 (s, 0.16H), 5.56 (dd, 0.16H, J = 7.2, 7.7 Hz), 5.37–5.40 (m, 1.84H), 5.13–5.18 (m, 1H), 5.02–5.08 (m, 1H), 4.84 (d, 0.84H, J = 7.7 Hz), 4.77 (d, 0.16H, J = 7.7 Hz), 4.44–4.48 (m, 1H), 4.37 (dd, 0.84H, J = 4.8, 10.6 Hz), 4.33 (dd, 0.84H, J = 4.8, 10.6 Hz), 4.22–4.26 (m, 0.32H), 4.11 (ddd, 0.84H, J = 4.8, 9.7, 10.1 Hz), 3.96 (dd, 0.16H, J = 9.2, 9.2 Hz), 3.63–3.88 (m, 4H), 3.51 (ddd, 0.84H, J = 4.8, 9.7, 9.7 Hz), 3.42 (ddd, 0.16H, J = 4.8, 9.7, 10.6 Hz), 2.06–2.75 (m, 14H); IR (solid): 2886, 1720, 1368, 1142, 959, 793, 664, 530 cm^{-1}.

4.1.8. Ethylthio 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(4,6-*O*-benzylidene-β-D-glucopyranosyl)-β-D-glucopyranoside (20)

To a stirred solution of the levulinyl ester **17** (4.03 g, 4.67 mmol) in THF (50.0 mL) was added acetic acid (5.00 mL) and hydrazine monohydrate (2.00 mL) at 0 °C. After being stirred at the same temperature for 20 min, the reaction mixture was poured into 1 M HCl. The aqueous layer was extracted with two portions of ethyl acetate. The combined extract was washed with saturated aq $NaHCO_3$ and brine, and dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by chromatography on silica gel with 89:11 toluene:acetone to give diol **20** (3.09 g, 4.63 mmol, 99%). $[\alpha]_D^{24}$ $[\alpha]_D^{24}$ −49.6 (c = 1.05, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, 2H, J = 7.7 Hz), 7.31–7.61 (m, 13H), 5.61 (s, 1H), 5.41 (s, 1H), 5.35 (dd, 1H, J = 9.2, 9.7 Hz), 4.72 (d, 1H, J = 9.7 Hz), 4.46 (d, 1H, J = 7.7 Hz), 4.42 (dd, 1H, J = 4.8, 10.1 Hz), 4.20 (dd, 1H, J = 8.7, 9.2 Hz), 3.77–3.86 (m, 3H), 3.57–3.63 (m, 2H), 3.42–3.47 (m, 3H), 3.28 (ddd, 1H, J = 4.8, 9.7, 9.7 Hz), 2.92 (d, 1H, J = 2.4 Hz), 2.66–2.80 (m, 2H), 2.54 (d, 1H, J = 1.0 Hz), 1.25 (t, 3H, J = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.9, 137.0, 136.7, 133.6, 130.0, 129.7, 129.5, 129.3, 128.6, 128.4, 128.3, 126.4, 126.2, 103.1, 101.9, 101.7, 84.4, 80.3, 79.4, 79.1, 73.5, 72.8, 72.0, 71.0, 68.6, 68.4, 66.7, 24.2, 14.9; IR (solid): 3488, 2980, 2868, 1721, 1452, 1388, 1287, 1183, 1071, 971, 922, 751, 710, 572 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{35}H_{42}NO_{11}S$ $[M+NH_4]^+$ m/z = 684.2479, found: 684.2487.

4.1.9. Ethylthio 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(4,6-*O*-benzylidene-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(4,6-*O*-benzylidene-2,3-di-*O*–*O*-levulinyl-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranoside (21)

A mixture of diol **20** (46.8 mg, 0.0702 mmol), imidate **19** (56.1 mg, 0.0600 mmol) (azeotroped twice with dry toluene) and pulverized activated MS-4A (120 mg) in dry CH_2Cl_2 (2.40 mL) was stirred at room temperature for 30 min under argon to remove a trace amount of water. Then the reaction mixture was cooled to −50 °C. A catalytic amount of trimethylsilyl trifluoromethanesulfonate (1.09 μL, 6.00 μmol) was added to the reaction mixture. After being stirred at the same temperature for 30 min, the reaction mixture was neutralized with triethylamine, filtered through a pad of Celite® and concentrated in vacuo. The residue was purified by chromatography on silica gel with 94:6 toluene:acetone to give tetrasaccharide **21** (79.2 mg, 0.0540 mmol, 90%). $[\alpha]_D^{24}$ −32.8 (c = 1.11, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00–8.02 (m, 4H), 7.20–7.64 (m, 26H), 5.56 (s, 1H), 5.44 (s, 1H), 5.42 (s, 1H), 5.23–5.29 (m, 2H,), 5.11 (dd, 1H, J = 4.3, 4.8 Hz), 5.06 (dd, 1H, J = 7.7, 9.2 Hz), 5.01 (d, 1H, J = 4.8 Hz), 4.88 (d, 1H, J = 7.7 Hz), 4.85 (s, 1H), 4.66 (d, 1H, J = 9.7 Hz), 4.39–4.41 (m, 2H), 4.23 (dd, 1H, J = 4.8, 10.1 Hz), 4.18 (dd, 1H, J = 8.7, 9.2 Hz), 4.03–4.08 (m, 2H), 3.93 (dd, 1H, J = 4.3, 8.2 Hz), 3.39–3.86 (m, 13H), 3.25 (ddd, 1H, J = 4.8, 9.2, 10.1 Hz), 3.07 (d, 1H, J = 3.4 Hz), 2.49–2.80 (m, 10H), 2.14 (s, 3H, Lev), 2.12 (s, 3H), 1.23 (t, 3H, J = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.0, 206.4, 171.9, 171.3, 165.7, 165.2, 137.5, 137.3, 137.0, 136.7, 133.6, 133.4, 129.9, 129.8, 129.6, 129.4, 129.1, 128.9, 128.7, 128.5, 128.3 × 3, 128.0, 126.4, 126.2, 126.1, 126.0, 103.1, 101.9, 101.6, 101.5, 100.5 × 2, 99.2, 84.4, 79.1, 78.9, 78.4, 77.7, 77.2, 74.2, 73.9, 72.0, 70.8, 68.8, 68.6 × 2, 68.5, 66.9, 66.3, 65.5, 37.9 × 2, 29.8 × 3, 29.7, 28.1, 27.8, 24.3, 14.9; IR (solid): 3523, 2923, 2853, 2362, 1720, 1380, 1069, 917, 734, 672, 507 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{78}H_{86}NO_{26}S$ $[M+NH_4]^+$ m/z = 1484.5159, found: 1484.5149.

4.1.10. Ethylthio 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(4,6-*O*-benzylidene-2,3-di-*O*-levulinyl-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranoside (8)

To a stirred solution of **21** (3.53 g, 2.40 mmol) in pyridine (12.0 mL) was added acetic anhydride (2.25 mL, 24.0 mmol) and a catalytic amount of DMAP (29.3 mg, 0.240 mmol) at room temperature. After being stirred at the same temperature for 30 min, the reaction mixture was poured into 1 M HCl. The aqueous layer was extracted with two portions of ethyl acetate. The combined extract was washed with 1 M HCl, saturated aq $NaHCO_3$ and brine, and dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by chromatography on silica gel with 95:5 toluene:acetone to give benzoate **8** (3.48 g, 2.30 mmol, 96%). $[\alpha]_D^{24}$ −33.5 (c = 1.13, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (d, 2H, J = 7.2 Hz), 7.98 (d, 2H, J = 7.2 Hz), 7.29–7.68 (m, 25H), 7.21 (t, 1H, J = 7.2 Hz), 5.53 (s, 1H), 5.38 (s, 1H), 5.17–5.21 (m, 2H), 5.11 (dd, 1H, J = 8.7, 9.2 Hz), 4.99 (dd, 1H, J = 7.7, 8.7 Hz), 4.93 (dd, 1H, J = 9.2, 9.7 Hz), 4.90 (s, 1H), 4.88 (d, 1H, J = 6.8 Hz), 4.83 (dd, 1H, J = 5.3, 5.8 Hz), 4.70 (d, 1H, J = 7.7 Hz), 4.64 (d, 1H, J = 5.3 Hz), 4.50 (d, 1H, J = 9.7 Hz), 4.34 (dd, 1H, J = 4.8, 10.6 Hz), 4.22 (dd, 1H, J = 4.8, 10.6 Hz), 4.09–4.18 (m, 3H), 4.05 (dd, 1H, $J_{2,3}$ = 8.7, 9.2 Hz), 3.90 (dd, 1H, J = 9.2, 9.2 Hz), 3.84 (dd, 1H, J = 9.2, 9.2 Hz), 3.54–3.77 (m, 6H), 3.32–3.50 (m, 5H), 2.34–2.72 (m, 10H), 2.09 (s, 3H), 2.05 (s, 3H), 1.73 (s, 3H, Ac), 1.20 (t, 3H, J = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.5, 206.4, 171.8, 171.4, 169.2, 164.9, 164.8, 137.4, 137.3, 137.2, 136.9, 133.8, 133.4, 129.9, 129.8, 129.7, 129.3, 129.1, 129.0, 128.9, 128.6, 128.3, 128.2 × 2, 126.3 × 2, 126.2, 126.1, 101.6, 101.4, 101.1, 100.9, 100.7, 99.0, 98.4, 84.4, 78.6, 78.4 × 2, 78.1, 76.7, 76.2, 73.9, 72.8, 72.5, 71.8 × 2, 71.2, 68.8, 68.6 × 2, 66.2, 66.1, 65.7, 37.9, 37.8, 29.7 × 2,

28.0, 27.6, 24.3, 20.5, 14.8; IR (solid): 2983, 2884, 1753 1719, 1374, 1267, 1095, 1006, 746, 696, 515 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{80}H_{88}NO_{27}S$ $[M+NH_4]^+$ m/z = 1526.5264, found: 1526.5272.

4.1.11. 6-(((4-Methylphenyl)sulfonyl)oxy)hexyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(4,6-*O*-benzylidene-2,3-di-*O*-levulinyl-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranoside (22)

A mixture of 1,6-hexanediol mono-*p*-toluenesulfonate (21.7 mg, 79.5 μmol), thioglycoside **8** (100 mg, 66.2 μmol) (azeotroped twice with dry toluene) and pulverized activated MS-4A (265 mg) in dry CH_2Cl_2 (2.65 mL) was stirred at room temperature for 30 min under argon to remove a trace amount of water. Then the reaction mixture was cooled to −35 °C. *N*-Iodosuccinimide (17.9 mg, 79.5 μmol) and a catalytic amount oftrifluoromethanesulfonic acid (2.93 μL, 33.1 μmol) were added to the reaction mixture. After being stirred at the same temperature for 30 min, the reaction mixture was neutralized with triethylamine and filtered through a pad of Celite®. The filtrate was poured into a mixture of saturated aq $NaHCO_3$ and 10% aq $Na_2S_2O_3$ with cooling. The aqueous layer was extracted with two portions of ethyl acetate. The combined extract was washed with a mixture of saturated aq $NaHCO_3$ and 10% aq $Na_2S_2O_3$, and brine, and dried over $MgSO_4$, filtered, and evaporated in vacuo in vacuo. The residue was purified by chromatography on silica gel with 92:8 toluene:acetone to give tosyalte **22** (35.2 mg, 20.5 μmol, 31%). $[\alpha]_D^{24}$ −31.9 (c = 0.290, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, 2H, J = 8.2 Hz), 7.97 (d, 2H, J = 7.7 Hz), 7.76 (d, 2H, J = 7.7 Hz), 7.24–7.64 (m, 28H), 5.51 (s, 1H), 5.38 (s, 1H), 5.09–5.18 (m, 3H), 4.94–5.01 (m, 3H), 4.87 (d, 1H, J = 6.3 Hz), 4.84 (dd, 1H, J = 6.3, 6.3 Hz), 4.70 (d, 1H, J = 7.7 Hz), 4.62 (d, 1H, J = 6.3 Hz), 4.47 (d, 1H, J = 7.7 Hz), 4.32 (dd, 1H, J = 4.8, 10.6 Hz), 4.22 (dd, 1H, J = 4.8, 10.6 Hz), 4.17 (dd, 1H, J = 4.8, 10.6 Hz), 4.01–4.14 (m, 3H), 3.57–3.87 (m, 11H), 3.32–3.47 (m, 6H), 2.38–2.76 (m, 11H), 2.09 (s, 3H), 2.05 (s, 3H), 1.70 (s, 3H), 1.07–1.56 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.5, 206.4, 171.9, 171.5, 169.1, 164.8, 144.7, 137.4, 137.3, 133.7, 133.5, 133.4, 129.9, 129.8, 129.7, 129.6, 129.3, 129.2, 129.1, 128.9, 128.7, 128.3, 128.2 × 2, 127.9, 126.3, 126.2, 126.1, 101.7, 101.5, 101.4, 101.3, 100.8, 100.7, 99.4, 98.6, 78.7 × 2, 78.4 × 2, 77.7, 76.3, 76.1, 74.3, 73.9, 72.6, 71.8 × 2, 70.5, 69.8, 68.8, 68.7 × 2, 68.6, 66.7, 66.3, 66.1, 65.9, 38.0, 37.8, 29.8, 29.7, 29.2, 28.7, 28.0, 27.6, 25.3, 25.0, 21.7, 20.5; IR (solid): 2873, 2350, 2111, 1720, 1450, 1366, 1266, 1084, 916, 752, 634, 494 cm^{-1}.

4.1.12. 6-Azidohexyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(4,6-*O*-benzylidene-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranoside (7)

To a stirred solution of **22** (52.2 mg, 30.4 μmol) in DMF (1.52 mL) was added sodium azide (3.95 mg, 60.7 μmol) at room temperature. After being stirred at the same temperature for 1 h, the reaction mixture was poured into water. The aqueous layer was extracted with two portions of ethyl acetate. The combined extract was washed with water and brine, and dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was used for the next reaction without further purification. To a stirred solution of the residue in THF (1.00 mL) was added acetic acid (0.300 mL) and hydrazine monohydrate (0.100 mL) at 0 °C. After being stirred at the same temperature for 20 min, the reaction mixture was poured into 1 M HCl. The aqueous layer was extracted with two portions of ethyl acetate. The combined extract was washed with saturated aq $NaHCO_3$ and brine, and dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by chromatography on silica gel with 88:12 toluene:acetone to give diol **7** (31.0 mg, 22.2 μmol, 2 steps 73%). $[\alpha]_D^{24}$ −34.6 (c = 1.10, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, 2H, J = 8.2 Hz), 8.00 (d, 2H, J = 8.2 Hz), 7.25–7.62 (m, 26H), 5.51 (s, 1H), 5.42 (s, 1H), 5.27 (s, 1H), 5.23 (dd, 1H, J = 6.3, 6.3 Hz), 5.06 (dd, 1H, J = 8.2, 8.7 Hz), 5.02 (d, 1H, J = 6.3 Hz), 4.96 (s, 1H), 4.90 (dd, 1H, J = 5.3, 5.8 Hz), 4.69 (d, 1H, J = 5.3 Hz), 4.53 (d, 1H, J = 8.2 Hz), 4.50 (d, 1H, J = 8.2 Hz), 4.32 (dd, 1H, J_a = 3.4, 9.7 Hz), 4.05–4.17 (m, 4H), 3.35–3.92 (m, 17H), 3.31 (ddd, 1H, J = 4.8, 9.2, 9.7 Hz), 3.03–3.06 (m, 3H), 2.60 (br-s, 1H), 1.74 (s, 3H), 1.14–1.50 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 208.3, 169.5, 165.5, 164.8, 137.3, 137.2, 137.1, 136.9, 133.7, 133.4, 130.0, 129.8, 129.7, 129.6, 129.3 × 2, 128.8, 128.7, 128.3 × 2, 126.4 × 2, 126.3, 126.1, 102.5, 101.9, 101.6, 101.4 × 2, 101.3, 99.1, 98.4, 80.3, 78.8, 78.7, 78.6, 78.5, 78.2, 76.1, 74.4, 73.7, 73.5, 72.9, 72.3, 69.9, 68.8, 68.7 × 2, 68.5, 66.7, 65.8, 65.6, 51.3, 29.3, 28.6, 26.3, 25.4, 20.5; IR (solid): 3625, 2974, 2884, 2831, 2089, 1760, 1719, 1380, 1275, 1220, 1095, 765, 697, 515 cm^{-1}.

4.1.13. 6-*O*-(2-(azidomethyl)benzoyl)-2-*O*-benzoyl-4-*O*-benzyl-3-*O*-tert-butyldimethylsilyl-β-D-glucopyranoside (25)

To a stirred solution of ethylthio 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranoside (**24**) (778 mg, 1.46 mmol) in CH_2Cl_2 (7.26 mL) was added 2-(azidomethyl)benzoyl chloride (947 mg, 4.84 mmol) and DMAP (1.48 g, 12.1 mmol) at room temperature. After being stirred at the same temperature for 30 min, the reaction mixture was poured into saturated aq $NaHCO_3$. The aqueous layer was extracted with two portions of ethyl acetate. The combined extract was washed with saturated aq $NaHCO_3$ and brine, and dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was chromatographed on silica gel with 90:10 hexane:ethyl acetate to give 2-(azidomethyl)benzoate ester **25** (927 mg, 1.34 mmol, 92%). $[\alpha]_D^{20}$ = +24.6 (c = 0.855, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01–8.06 (m, 3H), 7.30–7.61 (m, 11H), 5.25 (dd, 1H, J = 9.2, 9.7 Hz), 4.89 (d, 1H, J = 11.1 Hz), 4.80 (s, 2H), 4.63 (dd, 1H, J = 2.4, 12.1 Hz), 4.58 (d, 1H, J = 11.1 Hz), 4.56 (d, 1H, J = 9.7 Hz), 4.35 (dd, 1H, J = 4.8, 12.1 Hz), 4.01 (dd, 1H, J = 8.7, 9.2 Hz), 3.74 (ddd, 1H, J = 2.4, 4.8, 9.2 Hz), 3.63 (dd, 1H, J = 8.7, 9.2 Hz), 2.60–2.78 (m, 2H), 1.18 (t, 3H, J = 7.2 Hz), 0.80 (s, 9H), 0.04 (s, 3H), -0.13 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2, 165.6, 137.6, 137.5, 133.2, 133.0, 121.4, 130.3, 130.0, 129.8, 128.5, 128.4, 128.2, 128.0, 127.8, 83.7, 78.8, 77.0, 75.4, 72.9, 63.9, 53.1, 25.9, 25.7, 23.9, 17.9, 14.9, -3.9, -4.1; IR (solid): 2959, 2928, 2856, 2104, 1724, 1452, 1262, 1070, 838, 711, 519 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{36}H_{49}N_4O_7SiS$ $[M+NH_4]^+$ m/z = 709.3091, found: 709.3082.

4.1.14. 6-*O*-(2-(Azidomethyl)benzoyl)-2-*O*-benzoyl-4-*O*-benzyl-3-*O*-tert-butyldimethylsilyl-D-glucopyranose (26)

To a stirred solution of ethylthio 6-*O*-(2-(azidomethyl)benzoyl)-2-*O*-benzoyl-4-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranoside (**25**) (682 mg, 0.986 mmol) in acetone (4.93 L) and water (49.3 mL) was added *N*-bromosuccinimide (211 mg, 1.18 mmol) at 0 °C. After being stirred at the same temperature for 10 min, the reaction mixture was added triethylamine (1.00 mL). After being stirred at room temperature for 1 h, the reaction mixture was poured into a mixture of saturated aq $NaHCO_3$ and 10% aq $Na_2S_2O_3$. The aqueous layer was extracted with two portions of ethyl acetate. The combined extract was washed with a mixture of saturated aq $NaHCO_3$ and 10% aq $Na_2S_2O_3$, and brine, and dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was chromatographed on silica gel with 85:15 hexane:ethyl acetate to give hemiacetal **26** (488 mg, 0.753 mmol, 76%, α:β = 77:23). The α:β ratio was determined by ^{1}H NMR analysis. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00–8.10 (m, 3H), 7.22–7.66 (m, 11H), 5.41 (br-s, 0.77H), 5.14 (dd, 0.77H, J = 3.4, 9.2 Hz), 5.10 (dd, 0.23H, J = 8.7, 8.7 Hz), 4.88–4.93 (m, 1H), 4.73–4.83 (m, 2.23H), 4.57–4.65 (m, 2H), 4.37–4.42 (m, 1.77H), 4.30 (ddd, 0.77H, J = 1.9, 9.7, 4.3 Hz), 4.06 (dd, 0.23H, J = 8.7, 8.7 Hz), 3.77 (ddd, 0.23H, J = 2.4, 4.3, 9.7 Hz), 3.58–3.67

(m, 1H), 3.34 (d, 0.23H, J = 9.7 Hz), 2.97 (br-s, 0.77H), 0.79 (s, 9H), -0.05–0.08 (m, 6H); IR (solid): 3427, 2928, 2856, 2411, 2101, 1724, 1453, 1264, 1097, 838, 713, 520 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{34}H_{45}N_4O_8Si$ $[M+NH_4]^+$ m/z = 665.3007, found: 665.3002.

4.1.15. O-(6-*O*-(2-(Azidomethyl)benzoyl)-2-*O*-benzoyl-4-*O*-benzyl-3-*O*-tert-butyldimethylsilyl-D-glucopyranosyl)trichloroacetimidate (27)

To a stirred solution of 6-*O*-(2-(azidomethyl)benzoyl)-2-*O*-benzoyl-4-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-D-glucopyranose (**26**) (960 mg, 1.48 mmol) in CH_2Cl_2 (2.96 mL) was added trichloroacetonitrile (0.742 mL, 7.40 mmol) and a catalytic amount of Cs_2CO_3 (48.0 mg, 0.148 mmol) at room temperature. After being stirred at the same temperature for 1 h, the reaction mixture was filtered through a pad of Celite and concentrated in vacuo. The residue was chromatographed on silica gel with 90:10 hexane:ethyl acetate to give imidate **27** (1.09 g, 1.38 mmol, 93%, α:β = 59:41). The α:β ratio was determined by ^{1}H NMR analysis. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (s, 0.41H), 8.51 (s, 0.59H), 8.00–8.05 (m, 3H), 7.22–7.61 (m, 11H), 6.55 (d, 0.59H, J = 3.4 Hz), 5.97 (d, 0.41H, J = 7.2 Hz), 5.51 (dd, 0.41H, J = 7.2, 8.2 Hz), 5.40 (dd, 0.59H, J = 3.4, 9.7 Hz), 4.93 (d, 0.59H, J = 11.1 Hz), 4.89 (d, 0.41H, J = 11.6 Hz), 4.74–4.83 (m, 2H), 4.56–4.65 (m, 2H), 4.38–4.49 (m, 1.59H), 4.25 (ddd, 0.59H, J = 1.9, 3.4, 10.1 Hz), 4.12 (dd, 0.41H, J = 8.2, 8.7 Hz), 3.96 (ddd, 0.41H, J = 2.4, 4.8, 9.2 Hz), 3.72–3.79 (m, 1H), 0.80–0.83 (m, 9H), -0.08–0.11 (m, 6H); IR (solid): 2959, 2855, 2105, 1725, 1672, 1452, 1255, 1070, 712, 519 cm^{-1}.

4.1.16. Ethylthio 3-*O*-(6-*O*-(2-(azidomethyl)benzoyl)-2-*O*-benzoyl-4-*O*-benzyl-3-*O*-tert-butyldimethylsilyl-β-D-glucopyranosyl)-4,6-*O*-benzylidene-β-D-glucopyranoside (28)

A mixture of **10a** (5.64 g, 18.1 mmol), **27** (11.9 g, 15.1 mmol) (azeotroped twice with dry toluene) and pulverized activated MS-4A (15.1 g) in dry CH_2Cl_2 (151 mL) was stirred at room temperature for 30 min under argon to remove a trace amount of water. Then the reaction mixture was cooled to −15 °C. A catalytic amount of trimethylsilyltrifluoromethanesulfonate (0.544 mL, 3.01 mmol) was added to the reaction mixture. After being stirred at the same temperature for 1 h, the reaction mixture was neutralized with triethylamine, filtered through a pad of Celite® and concentrated in vacuo. The residue was purified by chromatography on silica gel with 90:10 hexane:ethyl acetate to give **28** (13.3 g, 14.2 mmol, 94%). $[\alpha]_D^{19}$ = -3.94 (c = 1.05, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, 2H, J = 7.2 Hz), 7.97 (d, 1H, J = 7.7 Hz), 7.15–7.60 (m, 16H), 5.48 (s, 1H), 5.23 (dd, 1H, J = 7.7, 8.7 Hz), 4.93 (d, 1H, J = 7.7 Hz), 4.83 (d, 1H, J = 11.1 Hz), 4.74 (d, 1H, J = 14.5 Hz), 4.67 (d, 1H, J = 14.5 Hz), 4.50 (d, 1H, J = 11.1 Hz), 4.46 (dd, 1H, J = 1.9, 11.6 Hz), 4.25–4.31 (m, 3H), 4.00 (dd, 1H, J = 8.7, 9.2 Hz), 3.79 (dd, 1H, J = 8.7, 8.7 Hz), 3.54–3.72 (m, 4H), 3.31–3.42 (m, 2H), 2.48–2.64 (m, 2H), 2.42 (d, 1H, J = 1.9 Hz), 1.18 (t, 3H, J = 7.2 Hz), 0.79 (s, 9H), 0.03 (s, 3H), -0.11 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.3, 165.9, 137.5, 137.3, 137.1, 133.3, 132.8, 131.6, 130.2, 129.9, 129.7, 129.0, 128.6, 128.5 × 2, 128.2 × 2, 127.9, 127.8, 126.0, 101.4, 101.3, 86.2, 81.8, 79.2, 78.7, 75.4, 75.3, 72.9, 72.8, 71.0, 68.6, 63.7, 53.1, 25.7, 24.0, 17.9, 15.2, -4.0, -4.2; IR (solid): 3478, 2928, 2855, 2102, 1724, 1600, 1452, 1264, 1071, 985, 837, 700, 518 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{49}H_{63}N_4O_{12}S$ $[M+NH_4]^+$ m/z = 959.3932, found: 959.3979.

4.1.17. Ethylthio 3-*O*-(6-*O*-(2-(azidomethyl)benzoyl)-2-*O*-benzoyl-4-*O*-benzyl-β-D-glucopyranosyl)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranoside (30)

To a stirred solution of **28** (3.05 g, 3.23 mmol) in pyridine (16.2 mL) was added benzoyl chloride (1.14 mL, 9.69 mmol) and a catalytic amount of DMAP (39.5 mg, 0.323 mmol) at room temperature. After being stirred at 120 °C for 7 h, the reaction mixture was poured into 1 M HCl. The aqueous layer was extracted with two portions of ethyl acetate. The combined extract was washed with 1 M HCl, saturated aq $NaHCO_3$ and brine, and dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was used for the next reaction without further purification. To a stirred solution of the residue in pyridine (16.2 mL) was added HF in pyridine (1.62 mL) at room temperature. After being stirred at 100 °C for 12 h, the reaction mixture was poured into 1 M HCl. The aqueous layer was extracted with two portions of ethyl acetate. The combined extract was washed with 1 M HCl, saturated aq $NaHCO_3$ and brine, and dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by chromatography on silica gel with 78:22 hexane:ethyl acetate to give **30** (2.53 g, 2.72 mmol, 2 steps 84%). $[\alpha]_D^{18}$ −8.44 (c = 1.07, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, 1H, J = 7.7 Hz), 7.83 (d, 2H, J = 8.2 Hz), 7.73 (d, 2H, J = 8.2 Hz), 7.21–7.59 (m, 19H,), 5.55 (s, 1H), 5.30 (dd, 1H, J = 8.7, 9.7 Hz,), 5.01 (dd, 1H, J = 7.2, 8.2 Hz), 4.86 (d, 1H, J = 7.2 Hz), 4.65–4.78 (m, 3H), 4.50–4.60 (m, 3H), 4.32–4.37 (m, 2H), 4.27 (dd, 1H, J = 8.7, 9.2 Hz), 3.71–3.83 (m, 3H), 3.62 (dd, 1H, J = 8.7, 9.2 Hz), 3.49–3.55 (m, 2H), 2.61–2.68 (m, 2H), 2.57 (d, 1H, J = 4.8 Hz), 1.16 (t, 3H, J = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ166.4, 164.8, 137.6, 137.2, 137.1, 133.2, 132.9, 131.6, 130.1, 130.0, 129.8, 129.5, 129.2 × 2, 128.7, 128.6 × 2, 128.5, 128.3 × 3, 128.1, 126.1, 101.6, 99.9, 84.3, 79.4, 78.7, 77.8, 76.5, 75.3, 74.9, 72.8, 72.2, 71.1, 68.7, 63.7, 53.1, 24.1, 14.8; IR (solid): 3499, 2967, 2875, 2105, 1732, 1602, 1452, 1273, 1096, 913, 712, 542 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{50}H_{53}N_4O_{13}S$ $[M+NH_4]^+$ m/z = 949.3330, found: 949.3322.

4.1.18. Ethylthio 3-*O*-(6-*O*-(2-(azidomethyl)benzoyl)-2-*O*-benzoyl-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(4,6-*O*-benzylidene-2,3-di-*O*-levnulinyl-β-D-glucopyranosyl)-β-D-glucopyranosyl)-4-*O*-benzyl-β-D-glucopyranosyl)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranoside (31)

A mixture of **30** (521 mg, 55.9 μmol), **19** (592 mg, 61.5 μmol) (azeotroped twice with dry toluene) and pulverized activated MS-4A (280 mg) in dry CH_2Cl_2 (2.80 mL) was stirred at room temperature for 30 min under argon to remove a trace amount of water. Then the reaction mixture was cooled to −50 °C. A catalytic amount of trimethylsilyl trifluoromethanesulfonate (10.1 μL, 55.9 μmol) was added to the reaction mixture. After being stirred at room temperature for 2 h, the reaction mixture was neutralized with triethylamine, filtered through a pad of Celite® and concentrated in vacuo. The residue was purified by chromatography on silica gel with 85:15 toluene:ethyl acetate and further purified by gel permeation chromatography (GPC) to give **31** (573 mg, 0.330 mmol, 59%). $[\alpha]_D^{18}$ −17.0 (c = 1.19, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (d, 1H, J = 7.7 Hz), 7.92 (d, 2H, J = 7.7 Hz), 7.81 (d, 2H, J = 7.7 Hz), 7.14–7.72 (m, 34H), 5.52 (s, 1H), 5.49 (s, 1H), 5.37 (s, 1H), 5.25 (dd, 1H, J = 8.2, 8.7 Hz), 4.93–5.06 (m, 4H), 4.69–4.81 (m, 4H), 4.63 (d, 1H, J = 10.6 Hz), 4.55 (d, 1H, J = 7.7 Hz), 4.37–4.44 (m, 3H), 4.16–4.32 (m, 4H), 4.02 (dd, 1H, $J_{2,3}$ = 5.8, 7.2 Hz), 3.93–3.98 (m, 2H), 3.77 (dd, 1H, J = 7.2, 10.1 Hz), 3.62–3.70 (m, 5H), 3.58 (dd, 1H, J = 9.2, 9.7 Hz), 3.36–3.44 (m, 2H), 3.25–3.32 (m, 2H), 2.14–2.71 (m, 10H), 2.08 (s, 3H), 2.03 (s, 3H), 1.14 (t, 3H, J = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.4, 206.3, 171.8, 171.4, 166.5, 165.0, 164.8, 164.5, 138.0, 137.3, 137.2, 137.1, 136.9, 133.8, 133.3, 133.2, 132.8, 131.7, 130.0, 129.9, 129.8, 129.6 × 2, 129.3, 129.2, 129.1, 129.0, 128.9, 128.4, 128.3, 128.2, 127.8, 126.5, 126.2, 126.0, 102.0, 101.3, 101.2, 101.0, 99.5, 98.0, 84.3, 79.9, 79.2, 78.8, 78.4 × 2, 76.0, 75.4, 74.3, 73.5, 73.3, 72.9, 72.0, 71.8, 71.6, 70.9, 68.7, 68.6, 66.6, 66.2, 64.6, 53.1, 37.9, 37.7, 29.8, 29.7, 27.9, 27.4, 24.4, 14.8; IR (solid): 2881, 2101, 1716, 1367, 1261, 1096, 994, 879, 702, 517 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{93}H_{97}N_4O_{28}S$ $[M+NH_4]^+$ m/z = 1749.6010, found: 1749.6022.

4.1.19. Ethylthio 2-*O*-benzoyl-3-*O*-(2-*O*-benzoyl-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(4,6-*O*-benzylidene-2,3-di-*O*-levnulinyl-β-D-glucopyranosyl)-β-D-glucopyranosyl)-4-*O*-benzyl-β-D-glucopyranosyl)-4,6-*O*-benzylidene-β-D-glucopyranoside (32)

To a stirred solution of **31** (251 mg, 0.145 mmol) in THF (1.45 μL, 0.725 mmol) and water (13.1 mL) was added $^{n}Bu_3P$ (0.109 mL, 0.435 mmol) at 0 °C. After being stirred at room temperature for 30 min, the reaction mixture was poured into saturated aq $NaHCO_3$. The aqueous layer was extracted with two portions of ethyl acetate. The combined extract was washed with saturated aq $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by chromatography on silica gel with 91:9 toluene:acetone to give **32** (212 mg, 0.134 mmol, 93%). $[\alpha]_D^{17}$ −13.1 (*c* = 1.04, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (d, 2H, *J* = 7.2 Hz), 7.73 (d, 2H, *J* = 7.2 Hz), 7.22–7.69 (m, 31H,), 5.47 (s, 2H), 5.37 (s, 1H), 5.21 (dd, 1H, *J* = 7.7, 8.7 Hz), 5.01 (dd, 1H, $J_{2,3}$ = 9.2, 9.7 Hz), 4.88–4.96 (m, 3H), 4.86 (d, 1H, *J* = 7.7 Hz), 4.67–4.71 (m, 2H), 4.52 (d, 1H, *J* = 7.7 Hz), 4.46 (d, 1H, *J* = 10.1 Hz), 4.32 (dd, 1H, *J* = 4.8, 10.6 Hz), 4.24 (dd, 1H, *J* = 4.8, 10.6 Hz), 4.18 (dd, 1H, *J* = 4.8, 10.6 Hz), 4.12 (d, 1H, *J* = 10.6 Hz), 4.09 (dd, 1H, *J* = 9.2, 9.2 Hz), 4.01 (dd, 1H, *J* = 6.8, 7.7 Hz), 3.88 (dd, 1H, *J* = 8.7, 9.2 Hz), 3.54–3.70 (m, 7H), 3.33–3.51 (m, 5H), 3.26 (ddd, 1H, *J* = 4.8, 9.7, 9.7 Hz), 2.17–2.67 (m, 10H), 2.07 (s, 3H), 2.03 (s, 3H), 1.93 (br-s, 1H), 1.14 (t, 3H, *J* = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.4, 206.3, 171.8, 171.7, 171.4, 165.0, 164.6, 164.2, 143.7, 138.2, 137.1, 136.8, 133.6, 133.2, 133.1, 132.1, 131.8, 129.9, 129.8, 129.7, 129.5, 129.4, 129.2 × 2, 129.0, 128.8, 128.4, 128.3, 128.2 × 3, 128.0, 127.8, 126.3, 126.1, 126.0, 123.8, 123.2, 102.0, 101.3, 101.0, 100.9, 99.7, 98.8, 84.4, 79.5, 79.0, 78.8, 78.4, 78.3, 75.6, 74.5, 74.2, 73.6, 73.4, 72.4, 71.6 × 2, 71.0, 68.6, 68.5, 66.5, 66.1, 63.7, 62.3, 45.6, 37.8, 37.6, 29.7, 29.6, 28.1, 27.9, 27.3, 27.2, 24.3, 24.1 × 2, 24.0 × 2, 18.9, 14.7, 13.7, 13.6; IR (solid): 3531, 2875, 1737, 1602, 1452, 1376, 1264, 984, 750, 703, 484 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{85}H_{89}O_{27}S$ $[M+H]^+$ *m*/*z* = 1573.5312, found: 1573.5287.

4.1.20. Ethylthio 2-*O*-benzoyl-3-*O*-(2-*O*-benzoyl-6-*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl)-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(4,6-*O*-benzylidene-2,3-di-*O*-levnulinyl-β-D-glucopyranosyl)-β-D-glucopyranosyl)-4-*O*-benzyl-β-D-glucopyranosyl)-4,6-*O*-benzylidene-β-D-glucopyranoside (9)

A mixture of **32** (212 mg, 0.134 mmol), **33** (188 mg, 0.269 mmol) (azeotroped twice with dry toluene) and pulverized activated MS-4A (134 mg) in dry CH_2Cl_2 (1.34 mL) was stirred at room temperature for 30 min under argon to remove a trace amount of water. Then the reaction mixture was cooled to −10 °C. A catalytic amount of trimethylsilyl trifluoromethanesulfonate (2.43 μL, 13.4 μmol) was added to the reaction mixture. After being stirred at 0 °C for 3 h, the reaction mixture was neutralized with triethylamine, filtered through a pad of Celite® and concentrated in vacuo. The residue was purified by chromatography on silica gel with 94:6 toluene:acetone to give **9** (259 mg, 0.123 mmol, 91%). $[\alpha]_D^{17}$ −10.4 (*c* = 1.19, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, 2H, *J* = 7.2 Hz), 7.84 (d, 2H, *J* = 7.7 Hz), 7.69 (d, 2H, *J* = 7.2 Hz), 7.10–7.67 (m, 49H,), 5.47 (s, 1H), 5.41 (s, 1H), 5.36 (s, 1H), 5.26 (dd, 1H, *J* = 7.7, 8.7 Hz), 5.15 (dd, 1H, *J* = 7.7, 8.7 Hz), 5.00 (dd, 1H, *J* = 9.2, 9.2 Hz), 4.93 (dd, 1H, *J* = 7.7, 9.2 Hz), 4.88 (dd, 1H, *J* = 6.3, 6.3 Hz), 4.73–4.80 (m, 4H), 4.54–4.66 (m, 6H), 4.51 (d, 1H, *J* = 7.7 Hz), 4.46 (d, 1H, *J* = 12.1 Hz), 4.13–4.26 (m, 4H), 4.05 (d, 1H, *J* = 12.1 Hz), 3.85–3.96 (m, 4H), 3.78 (dd, 1H, *J* = 8.7, 9.2 Hz), 3.69 (dd, 1H, *J* = 10.1 Hz, *J* = 10.1 Hz), 3.45–3.65 (m, 9H), 3.40 (dd, 1H, *J* = 7.7, 9.2 Hz), 3.15–3.32 (m, 5H), 2.22–2.68 (m, 10H), 2.07 (s, 3H), 2.02 (s, 3H), 1.15 (t, 3H, *J* = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.4 × 2, 171.8, 171.4, 165.4, 165.0, 164.7, 164.2, 138.3, 138.2, 137.9, 137.4, 137.1, 136.8, 133.5, 133.1, 133.0, 130.2, 129.9 × 2, 129.8, 129.6 × 2, 129.2, 129.1, 129.0, 128.8, 128.7, 128.6 × 2, 128.4, 128.3 × 2, 128.2 × 3, 128.0 × 2, 127.9, 127.8 × 2, 127.7, 127.6 × 2, 126.6, 126.1, 126.0, 125.4, 101.6, 101.3, 101.0, 100.9, 100.8, 99.4, 98.2, 84.0, 82.7, 79.3 × 2, 79.0, 78.9, 78.4, 78.3, 78.1, 75.7, 75.1, 75.0, 74.8, 74.3, 74.0, 73.8, 73.6, 73.5, 73.4, 72.3, 71.7, 71.6, 71.0, 68.6, 68.5 × 2, 68.4, 66.4, 66.1, 37.9, 37.7, 29.7, 29.6, 27.9, 27.4, 24.1, 14.8; IR (solid): 2967, 2318, 1955, 1730, 1602, 1451, 1265, 1096, 772, 702, 527 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{119}H_{124}NO_{33}S$ $[M+NH_4]^+$ *m*/*z* = 2126.7776, found: 2126.7778.

4.1.21. 6-Azidohexyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(4,6-*O*-benzylidene-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(4,6-*O*-benzylidene-2,3-di-*O*-levnulinyl-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranoside (34)

A mixture of **7** (631 mg, 0.453 mmol), **8** (752 mg, 0.498 mmol, 1.10 equiv) (azeotroped twice with dry toluene) and pulverized activated MS-4A (906 mg) in dry CH_2Cl_2 (18.1 mL) was stirred at room temperature for 30 min under argon to remove a trace amount of water. Then the reaction mixture was cooled to −35 °C. *N*-iodosuccinimide (135 mg, 0.598 mmol) and a catalytic amount of trifluoromethanesulfonic acid (20.0 μL, 0.227 μmol) were added to the reaction mixture. After being stirred at the same temperature for 30 min, the reaction mixture was neutralized with triethylamine and filtered through a pad of Celite®. The filtrate was poured into a mixture of saturated aq $NaHCO_3$ and 10% aq $Na_2S_2O_3$ with cooling. The aqueous layer was extracted with two portions of ethyl acetate. The combined extract was washed with a mixture of saturated aq $NaHCO_3$ and 10% aq $Na_2S_2O_3$, and brine, and dried over $MgSO_4$, filtered, and evaporated in vacuo. The residue was purified by chromatography on silica gel with 92:8 toluene:acetone and further purified by gel permeation chromatography (GPC) to give **34** (1.25 g, 0.441 mmol, 97%). $[\alpha]_D^{24}$ −28.3 (*c* = 0.840, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98–8.03 (m, 8H), 7.18–7.63 (m, 52H), 5.50 (s, 1H), 5.47 (s, 1H), 5.39 (s, 1H), 4.85–5.21 (m, 16H), 4.71–4.74 (m, 2H), 4.67 (d, 1H, *J* = 6.3 Hz), 4.52 (d, 1H, *J* = 7.7 Hz), 4.32–4.37 (m, 2H), 3.22–4.24 (m, 41H), 3.05 (t, 2H, *J* = 7.2 Hz), 2.35–2.74 (m, 9H), 2.09 (s, 3H), 2.01 (s, 3H), 1.80 (s, 3H), 1.77 (s, 3H), 1.10–1.50 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.5 × 2, 171.9, 171.4, 169.6, 169.0, 165.5, 165.2, 164.8 × 3, 137.4 × 3, 137.3 × 2, 137.1, 137.0, 136.9, 133.9, 133.8, 133.6, 133.5, 130.0, 129.9, 129.8 × 2, 129.7 × 2, 129.6, 129.5, 129.4, 129.3, 129.2 × 2, 129.1, 129.0, 128.9, 128.7 × 2, 128.3 × 2, 128.2 × 2, 126.4, 126.3 × 2, 126.2, 126.1, 126.0, 102.9, 101.7, 101.6 × 2, 101.5, 101.4, 101.3, 101.1, 101.0, 100.8, 100.6, 99.8, 99.6, 98.9, 98.7, 98.6, 78.8, 78.7, 78.6 × 2, 78.5, 78.4, 78.3, 78.0, 77.8, 77.7, 77.6, 76.6, 76.5, 76.4, 76.2, 74.5, 74.4, 74.3 × 2, 73.9, 73.8, 72.6, 72.4, 71.8 × 2, 69.9, 68.8 × 2, 68.7 × 2, 68.6 × 2, 68.5 × 2, 66.8, 66.7, 66.2, 66.1, 65.9 × 2, 65.8, 65.7 × 2, 51.3, 38.0, 37.8, 29.8, 29.7, 29.3, 28.6, 28.0, 27.6, 26.3, 25.4, 20.8, 20.5; IR (solid): 2989, 2883, 2822, 2267, 1725, 1386, 1262, 1091, 675, 517 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{152}H_{161}N_4O_{51}$ $[M+NH_4]^+$ *m*/*z* = 2858.0128, found: 2858.0181.

4.1.22. 6-Azidohexyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(4,6-*O*-D-glucopyranosyl)-β-benzylidene-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranoside (35)

To a stirred solution of **34** (57.9 mg, 20.4 μmol) in pyridine (0.408 mL) was added acetic anhydride (19.1 μL, 0.204 mmol)

and a catalytic amount of DMAP (1.25 mg, 10.2 μmol) at room temperature. After being stirred at the same temperature for 1 h, the reaction mixture was poured into 1 M HCl. The aqueous layer was extracted with two portions of ethyl acetate. The combined extract was washed with 1 M HCl, saturated aq $NaHCO_3$ and brine, and dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was used for the next reaction without further purification. To a stirred solution of the residue in THF (1.00 mL) was added acetic acid (0.300 mL) and hydrazine monohydrate (0.100 mL) at 0 °C. After being stirred at the same temperature for 20 min, the reaction mixture was poured into 1 M HCl. The aqueous layer was extracted with two portions of ethyl acetate. The combined extract was washed with saturated aq $NaHCO_3$ and brine, and dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by chromatography on silica gel with 88:12 toluene:acetone to give **35** (50.8 mg, 18.9 μmol, 2 steps 93%). $[\alpha]_D^{23}$ −23.8 (*c* = 1.04, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96–8.06 (m, 8H), 7.17–7.63 (m, 52H), 5.52 (s, 1H), 5.42 (s, 1H), 5.32 (s, 1H), 5.26 (dd, 1H, *J* = 6.8 Hz, 6.8 Hz), 5.12 (s, 2H), 5.06 (d, 1H, *J* = 6.8 Hz), 4.94–5.00 (m, 3H), 4.76–4.88 (m, 8H), 4.70 (d, 1H, *J* = 5.3 Hz), 4.63 (br-d, 2H, *J* = 5.3 Hz), 4.48–4.52 (m, 2H), 4.33 (dd, 1H, *J* = 4.3 Hz, 10.1 Hz), 3.25–4.19 (m, 42H), 3.06 (br-s, 1H), 3.05 (t, 2H, *J* = 7.2 Hz), 2.71 (br-s, 1H), 1.79 (s, 3H), 1.77 (s, 3H), 1.71 (s, 3H), 1.14–1.48 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.2, 169.0, 168.9, 165.5, 164.8, 164.6 × 2, 137.8, 137.2, 137.0, 136.8, 133.9, 133.8, 133.5, 133.3, 129.8, 129.7 × 2, 129.6, 129.5 × 2, 129.4, 129.3, 129.2 × 2, 129.1, 129.0, 128.9, 128.8, 128.7, 128.5, 128.4 × 2, 128.2 × 2, 128.1 × 2, 128.0 × 2, 127.8, 126.3, 126.2, 126.1, 126.0, 125.3, 102.5, 101.7, 101.6, 101.5, 101.4 × 2, 101.3 × 2, 101.0, 100.9, 100.8, 99.1, 98.2 × 2, 98.0, 97.8, 80.2, 78.7, 78.5, 78.2 × 2, 78.0, 77.9, 75.9, 75.7 × 2, 75.4, 75.2, 74.2, 74.1, 73.4, 73.3, 72.7, 72.2, 72.0 × 2, 71.9 × 2, 69.7, 68.6, 68.5, 68.4 × 2, 68.3, 66.5, 65.9, 65.7, 65.5, 65.3, 51.1, 29.1, 28.5, 26.1, 25.3, 21.4, 20.5 × 2, 20.3; IR (solid): 3046, 2883, 2320, 1732, 1373, 1266, 1097, 997, 700, 517 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{144}H_{151}N_4O_{48}$ $[M+NH_4]^+$ *m/z* = 2703.9498, found: 2703.9475.

4.1.23. 6-Azidohexyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzoyl-4,6-*O*-ben zylidene-3-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(4,6-*O*-benzylidene-3-*O*-(2-*O*-benzoyl-4, 6-*O*-benzylidene-3-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(4,6-*O*-benzylidene-2,3-di-*O*-le vnulinyl-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyr anosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyra nosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyran osyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyrano side (36)

A mixture of **35** (125 mg, 46.6 μmol), **8** (84.3 mg, 55.9 μmol) (azeotroped twice with dry toluene) and pulverized activated MS-4A (279 mg) in dry CH_2Cl_2 (2.79 mL) was stirred at room temperature for 30 min under argon to remove a trace amount of water. Then the reaction mixture was cooled to −35 °C. *N*-iodosuccinimide (15.1 mg, 67.0 μmol) and a catalytic amount of trifluoromethanesulfonic acid (2.10 μL, 23.3 μmol) were added to the reaction mixture. After being stirred at the same temperature for 30 min, the reaction mixture was neutralized with triethylamine and filtered through a pad of Celite®. The filtrate was poured into a mixture of saturated aq $NaHCO_3$ and 10% aq $Na_2S_2O_3$ with cooling. The aqueous layer was extracted with two portions of ethyl acetate. The combined extract was washed with a mixture of saturated aq $NaHCO_3$ and 10% aq $Na_2S_2O_3$, and brine, and dried over $MgSO_4$, filtered, and evaporated in vacuo. The residue was purified by chromatography on silica gel with 91:9 toluene:acetone and further purified by gel permeation chromatography (GPC) to give **36** (170 mg, 41.1 μmol, 88%). $[\alpha]_D^{16}$ −24.6 (*c* = 0.950, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97–8.03 (m, 12H), 7.16–7.62 (m, 78H), 5.51 (s, 1H), 5.47 (s, 1H), 5.38 (s, 1H), 5.10–5.21 (m, 6H), 4.91–5.04 (m, 11H), 4.78–4.88 (m, 9H), 4.68–4.72 (m, 3H), 4.63 (br-d, 2H, *J* = 6.3 Hz), 4.48 (d, 1H, *J* = 7.7 Hz), 4.31–4.37 (m, 2H), 3.22–4.24 (m, 61H), 3.05 (t, 2H, *J* = 7.2 Hz), 2.35–2.75 (m, 9H), 2.09 (s, 3H), 2.01 (s, 3H), 1.85 (s, 3H), 1.78 (s, 3H), 1.76 (s, 3H), 1.72 (s, 3H), 1.10–1.43 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.5, 206.4, 171.8, 171.4, 169.6, 169.1, 169.0 × 2, 165.5, 165.1, 164.9, 164.8 × 2, 164.7, 137.4, 137.3, 137.2, 137.0, 136.9, 134.0, 133.9, 133.7, 133.6, 133.4, 129.9, 129.8 × 3, 129.7 × 2, 129.6, 129.5 × 2, 129.4, 129.3 × 2, 129.2 × 2, 129.1, 129.0, 128.9, 128.8 × 2, 128.7, 128.6, 128.3, 128.2 × 3, 128.1, 126.3 × 2, 126.2, 126.1, 126.0, 102.9, 101.7, 101.6, 101.5, 101.4 × 2, 101.3, 101.2, 101.1, 100.9, 100.8, 100.6, 99.9, 99.2, 98.8, 98.5, 98.4, 98.2, 97.9, 78.7, 78.6 × 2, 78.5, 78.4, 78.2, 78.1, 78.0, 76.3, 76.1, 75.9 × 2, 75.7, 75.5, 74.5, 74.4 × 2, 74.2, 74.1, 73.8 × 2, 72.5, 72.4, 72.2, 71.8, 71.7, 69.9, 68.8, 68.7, 68.6, 68.5, 66.8, 66.7, 66.2, 66.0 × 2, 65.8, 65.7, 51.2, 37.9, 37.7, 29.7 × 2, 29.3, 28.6, 28.0, 27.6, 26.3, 25.4, 20.7 × 3, 20.5; IR (solid): 3492, 2971, 2877, 2417, 2097, 1732, 1602, 1452, 1373, 1262, 1222, 1096, 996, 765, 697, 508 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{222}H_{229}N_4O_{75}$ $[M+NH_4]^+$ *m/z* = 4150.4228, found: 4150.4053.

4.1.24. 6-Azidohexyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(4,6-*O*-benzylidene-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranoside (37)

To a stirred solution of **36** (72.6 mg, 17.6 μmol) in pyridine (1.76 mL) was added acetic anhydride (16.4 μL, 0.176 mmol) and a catalytic amount of DMAP (1.07 mg, 8.80 μmol) at room temperature. After being stirred at the same temperature for 1 h, the reaction mixture was poured into 1 M HCl. The aqueous layer was extracted with two portions of ethyl acetate. The combined extract was washed with 1 M HCl, saturated aq $NaHCO_3$ and brine, and dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was used for the next reaction without further purification. To a stirred solution of the residue in THF (1.00 mL) was added acetic acid (0.300 mL) and hydrazine monohydrate (0.100 mL) at 0 °C. After being stirred at the same temperature for 20 min, the reaction mixture was poured into 1 M HCl. The aqueous layer was extracted with two portions of ethyl acetate. The combined extract was washed with saturated aq $NaHCO_3$ and brine, and dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by chromatography on silica gel with 88:12 toluene:acetone to give **37** (65.2 mg, 16.4 μmol, 2 steps 93%). $[\alpha]_D^{16}$ −24.2 (*c* = 1.43, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96–8.06 (m, 12H), 7.17–7.62 (m, 78H), 5.52 (s, 1H), 5.42 (s, 1H), 5.32 (s, 1H), 5.26 (dd, 1H, *J* = 6.8 Hz, 7.2 Hz), 5.16 (s, 2H), 5.13 (s, 1H), 5.12 (s, 1H), 5.06 (d, 1H, *J* = 6.3 Hz), 4.94–4.98 (m, 3H), 4.77–4.88 (m, 16H), 4.71 (d, 1H, *J* = 5.3 Hz), 4.63–4.64 (m, 4H), 4.47–4.52 (m, 2H), 4.33 (dd, 1H, *J* = 4.3 Hz, 10.1 Hz), 3.18–4.19 (m, 62H), 3.03–3.08 (m, 3H), 2.65 (br-s, 1H), 1.79 (s, 3H), 1.77 (s, 3H), 1.76 (br-s, 6H), 1.72 (s, 3H), 1.14–1.46 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.4, 169.1 × 3, 165.6, 164.9 × 2, 164.8 × 2, 137.4 × 3, 137.3 × 2, 137.1, 136.9, 134.0 × 2, 133.6, 129.9, 129.8 × 2, 129.7, 129.6, 129.5, 129.3 × 4, 129.2 × 2, 129.1 × 5, 129.0 × 3, 128.9, 128.8 × 2, 128.6 × 2, 128.4, 128.3 × 3, 128.2 × 4, 128.1 × 2,

126.4 × 2, 126.3, 126.2, 102.7, 101.9, 101.7, 101.6 × 3, 101.5, 101.4, 101.2, 101.1, 101.0 × 2, 98.5, 98.4, 98.3 × 3, 97.9, 78.9, 78.7, 78.4, 78.3 × 3, 78.2 × 3, 78.1, 76.6, 75.9, 75.8, 75.7, 74.4, 74.3 × 2, 74.2 × 3, 73.6, 72.8, 72.1 × 3, 69.9, 68.8, 68.7, 68.6 × 4, 68.4, 66.7, 66.1, 66.0 × 2, 65.8, 65.6, 51.3, 29.8, 29.3, 28.6, 26.3, 25.4, 20.7, 20.6 × 2, 20.5; IR (solid): 2971, 2877, 2825, 1733, 1374, 1262, 1222, 1091, 695, 505 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{214}H_{219}N_4O_{72}$ $[M+NH_4]^+$ m/z = 3996.3598, found: 3996.3635.

4.1.25. 6-Azidohexyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(4,6-*O*-benzylidene-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(4,6-*O*-benzylidene-2,3-di-*O*-levnulinyl-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranoside (38)

A mixture of **37** (20.4 mg, 5.12 μmol), **8** (9.30 mg, 6.15 μmol) (azeotroped twice with dry toluene) and pulverized activated MS-4A (51.2 mg) in dry CH_2Cl_2 (0.512 mL) was stirred at room temperature for 30 min under argon to remove a trace amount of water. Then the reaction mixture was cooled to −35 °C. *N*-iodosuccinimide (1.70 mg, 7.37 μmol) and a catalytic amount of trifluoromethanesulfonic acid (0.317 μL, 3.58 μmol) were added to the reaction mixture. After being stirred at the same temperature for 30 min, the reaction mixture was neutralized with triethylamine and filtered through a pad of Celite®. The filtrate was poured into a mixture of saturated aq $NaHCO_3$ and 10% aq $Na_2S_2O_3$ with cooling. The aqueous layer was extracted with two portions of ethyl acetate. The combined extract was washed with a mixture of saturated aq $NaHCO_3$ and 10% aq $Na_2S_2O_3$, and brine, and dried over $MgSO_4$, filtered, and evaporated in vacuo. The residue was purified by chromatography on silica gel with 91:9 toluene:acetone and further purified by gel permeation chromatography (GPC) to give **38** (22.8 mg, 4.20 μmol, 82%). $[\alpha]_D^{24}$ −17.6 (c = 2.02, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97–8.03 (m, 16H), 7.14–7.62 (m, 104H), 5.51 (s, 1H), 5.47 (s, 1H), 5.38 (s, 1H), 4.63–5.21 (m, 43H), 4.48 (d, 1H, J = 7.7 Hz), 4.31–4.37 (m, 2H), 3.17–4.24 (m, 81H), 3.05 (t, 2H, J = 7.2 Hz), 2.35–2.70 (m, 9H, Lev, OH), 2.08 (s, 3H), 2.00 (s, 3H), 1.86 (s, 3H), 1.78 (s, 3H), 1.76 (s, 9H), 1.71 (s, 3H), 1.08–1.50 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.5 × 2, 171.8, 171.4, 169.6, 169.1 × 3, 169.0, 165.6 165.2, 164.9 × 2, 164.8 × 3, 137.4, 137.3, 137.2, 137.1, 137.0, 136.9, 134.1 × 2, 134.0 × 3, 133.9 × 3, 133.7, 133.6, 133.4, 129.9, 129.8 × 2, 129.7 × 2, 129.6, 129.5 × 2, 129.4, 129.3 × 2, 129.2, 129.1, 129.0, 128.9, 128.8 × 2, 128.7, 128.6, 128.3 × 2, 128.2 × 3, 128.1, 128.0, 126.4, 126.3 × 3, 126.2, 126.1, 126.0, 125.4, 102.9, 102.8, 101.7, 101.6, 101.5 × 2, 101.4, 101.3 × 2, 101.2 × 2, 101.1 × 3, 101.0, 100.9, 100.8, 100.6, 99.9, 99.2, 98.8, 98.4 × 2, 98.2, 98.0 × 2, 97.8 × 2, 78.7 × 3, 78.6, 78.5 × 2, 78.4, 78.2 × 2, 78.1, 78.0 × 3, 77.9, 77.8, 77.7, 76.6 × 2, 76.4, 76.3 × 2, 76.0, 75.9, 75.8, 75.7 × 2, 75.6 × 3, 75.4, 75.3, 74.5 × 2, 74.4 × 2, 74.3 × 2, 74.2 × 2, 74.1, 73.9, 73.8, 72.5, 72.3, 72.2 × 2, 72.1 × 2, 71.8 × 2, 71.7, 69.9, 68.9 × 3, 68.8, 68.7, 68.6, 68.5, 68.4 × 2, 68.2, 66.8, 66.7, 66.2, 66.1, 66.0 × 2, 65.9, 65.8, 65.7, 65.6, 51.2, 38.8, 37.9, 37.7, 32.0, 29.8, 29.7, 29.6 × 3, 29.5 × 2, 29.4, 29.3, 29.0, 28.6, 28.0, 27.6, 26.3, 25.4, 23.0, 22.7, 21.5, 20.7 × 2, 20.6, 20.5; IR (solid): 3536, 2926, 2093, 1720, 1369, 1256, 1093, 909, 647, 496 cm^{-1}.

4.1.26. 6-Azidohexyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(3-*O*-(2-*O*-benzoyl-3-*O*-(2-*O*-benzoyl-6-*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-D-glucopyranosyl)-3-β-benzyl-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(4,6-*O*-benzylidene-2,3-di-*O*-levnulinyl-β-D-glucopyranosyl)-β-D-glucopyranosyl)-4-*O*-benzyl-β-D-glucopyranosyl)-4,6-*O*-benzylidene-β-D-glucopyranosyl)-4,6-*O*-benzylidene-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranoside (39)

A mixture of **7** (87.0 mg, 62.4 μmol), **9** (158 mg, 74.9 μmol) (azeotroped twice with dry toluene) and pulverized activated MS-4A (374 mg) in dry CH_2Cl_2 (3.74 mL) was stirred at room temperature for 30 min under argon to remove a trace amount of water. Then the reaction mixture was cooled to −35 °C. *N*-iodosuccinimide (20.2 mg, 89.9 μmol) and a catalytic amount of trifluoromethanesulfonic acid (1.66 μL, 18.7 μmol) were added to the reaction mixture. After being stirred at the same temperature for 30 min, the reaction mixture was neutralized with triethylamine and filtered through a pad of Celite®. The filtrate was poured into a mixture of saturated aq $NaHCO_3$ and 10% aq $Na_2S_2O_3$ with cooling. The aqueous layer was extracted with two portions of ethyl acetate. The combined extract was washed with a mixture of saturated aq $NaHCO_3$ and 10% aq $Na_2S_2O_3$, and brine, and dried over $MgSO_4$, filtered, and evaporated in vacuo. The residue was purified by chromatography on silica gel with 91:9 toluene:acetone and further purified by gel permeation chromatography (GPC) to give **39** (191 mg, 55.5 μmol, 89%). $[\alpha]_D^{21}$ −5.37 (c = 0.980, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98–8.01 (m, 4H), 7.91 (d, 2H, J = 7.2 Hz), 7.81 (d, 2H, J = 7.2 Hz), 7.76 (d, 2H, J = 7.7 Hz), 7.63 (d, 2H, J = 7.2 Hz), 7.03–7.61 (m, 73H), 5.48 (s, 1H), 5.41 (s, 1H), 5.37 (s, 1H), 5.30 (s, 1H), 5.28 (s, 1H), 5.25 (dd, 1H, J = 8.7 Hz, 8.7 Hz), 5.09–5.17 (m, 2H), 4.88–5.05 (m, 8H), 4.76–4.84 (m, 5H), 4.64–4.69 (m, 3H), 4.54–4.61 (m, 3H), 4.50 (br-d, 2H, J = 7.7 Hz), 4.40–4.46 (m, 2H), 4.29–4.34 (m, 2H), 4.05–4.23 (m, 7H), 3.98 (dd, 1H, J = 4.8 Hz, 10.1 Hz), 3.20–3.94 (m, 36H), 3.14 (ddd, 1H, J = 4.8 Hz, 9.7 Hz, 10.1 Hz), 3.01–3.06 (m, 3H), 2.25–2.72 (m, 10H), 2.07 (s, 3H), 2.01 (s, 3H), 1.72 (s, 3H), 1.10–1.48 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.3 × 2, 171.7, 171.3, 169.3, 165.4, 165.2, 165.1, 164.8, 164.7, 163.9, 138.7, 138.4, 138.3, 138.0, 137.6, 137.2 × 2, 136.9, 136.8, 133.7, 133.4, 133.3, 133.2 × 2, 133.0 × 2, 129.8 × 2, 129.7, 129.6, 129.5 × 2, 129.2 × 3, 129.1 × 2, 129.0, 128.9, 128.8 × 2, 128.7, 128.6, 128.5 × 2, 128.4, 128.3, 128.2 × 2, 128.1 × 2, 128.0, 127.9, 127.8, 127.6, 127.5 × 2, 127.4, 126.4, 126.3, 126.2 × 2, 126.1, 126.0 × 2, 102.6, 101.6 × 2, 101.5, 101.4, 101.3, 101.2, 101.0, 100.9, 100.8, 100.6, 99.9, 99.2, 98.7, 98.5, 97.7, 82.8, 79.5, 78.8, 78.6, 78.5, 78.4, 78.3, 78.0, 77.8, 76.3, 76.0, 75.9, 75.7, 75.2, 74.8, 74.3 × 2, 74.1 × 2, 74.0, 73.9, 73.8, 73.4, 73.3, 72.1, 71.6, 69.8, 69.2, 68.7 × 3, 68.6 × 2, 68.5 × 2, 68.4 × 3, 68.3, 66.6 × 2, 66.3, 66.1, 65.9, 65.6, 65.2, 51.2, 37.8, 37.6, 29.6 × 2, 29.2, 28.5, 27.9, 27.3, 26.2, 25.3, 20.4; IR (solid): 3513, 2869, 2092, 1734, 1451, 1374, 1264, 1069, 752, 696, 654, 501 cm^{-1}.

4.1.27. 6-Azidohexyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(3-*O*-(2-*O*-benzoyl-3-*O*-(2-*O*-benzoyl-6-*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl)-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(4,6-*O*-benzylidene-2,3-di-*O*-levnulinyl-β-D-glucopyranosyl)-4-β-D-glucopyranosyl)-*O*-benzyl-β-D-glucopyranosyl)-4,6-*O*-benzylidene-β-D-glucopyrano syl)-4,6-*O*-benzylidene-β-D-glucopyranosyl)-β-D-glucopyranos yl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranos yl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyrano side (40)

A mixture of **35** (73.0 mg, 27.2 μmol), **9** (86.0 mg, 40.8 μmol) (azeotroped twice with dry toluene) and pulverized activated

MS-4A (163 mg) in dry CH_2Cl_2 (1.63 mL) was stirred at room temperature for 30 min under argon to remove a trace amount of water. Then the reaction mixture was cooled to −35 °C. *N*-iodosuccinimide (11.0 mg, 48.9 μmol) and a catalytic amount of trifluoromethanesulfonic acid (1.20 μL, 13.6 μmol) were added to the reaction mixture. After being stirred at the same temperature for 30 min, the reaction mixture was neutralized with triethylamine and filtered through a pad of Celite®. The filtrate was poured into a mixture of saturated aq $NaHCO_3$ and 10% aq $Na_2S_2O_3$ with cooling. The aqueous layer was extracted with two portions of ethyl acetate. The combined extract was washed with a mixture of saturated aq $NaHCO_3$ and 10% aq $Na_2S_2O_3$, and brine, and dried over $MgSO_4$, filtered, and evaporated in vacuo. The residue was purified by chromatography on silica gel with 91:9 toluene:acetone and further purified by gel permeation chromatography (GPC) to give **40** (102 mg, 21.5 μmol, 79%). $[\alpha]_D^{21} = -9.17$ ($c = 0.960$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96–8.00 (m, 8H), 7.90 (d, 2H, $J = 7.2$ Hz), 7.81 (d, 2H, $J = 7.2$ Hz), 7.75 (d, 2H, $J = 7.2$ Hz), 6.99–7.63 (m, 101H), 5.51 (s, 1H), 5.40 (s, 1H), 5.36 (s, 1H), 5.33 (s, 1H), 5.30 (s, 1H), 5.24 (dd, 1H, $J = 8.2$ Hz, 8.7 Hz), 5.11–5.16 (m, 4H), 4.76–5.02 (m, 21H), 4.54–4.70 (m, 8H), 4.49 (d, 1H, $J = 6.8$ Hz), 4.48 (d, 1H, $J = 7.7$ Hz), 4.40–4.45 (m, 2H), 4.31–4.34 (m, 2H), 3.22–4.22 (m, 64H), 3.14 (ddd, 1H, $J = 4.8$ Hz, 8.7 Hz, 9.2 Hz), 3.00–3.07 (m, 3H), 2.22–2.67 (m, 10H), 2.07 (s, 3H), 2.04 (s, 3H), 1.76 (s, 3H), 1.75 (s, 3H), 1.71 (s, 3H), 1.10–1.48 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 208.1, 206.4, 171.8, 171.4, 169.3, 169.1, 169.0, 165.3, 165.1, 164.9, 164.8 × 2, 138.7, 138.4, 138.3, 138.0, 137.7, 137.4, 137.3 × 3, 137.0, 136.9, 133.4 × 2, 133.3 × 2, 133.2, 133.1, 130.0 × 2, 129.8 × 2, 129.7 × 2, 129.6 × 2, 129.5 × 2, 129.4, 129.3 × 2, 129.2 × 3, 129.1 × 2, 129.0 × 2, 128.9 × 2, 128.8, 128.7, 128.6 × 2, 128.5 × 2, 128.3, 128.2 × 2, 128.1 × 2, 128.0, 127.9 × 2, 127.8, 127.7, 127.6, 127.5, 127.4, 126.5, 126.4, 126.3, 126.2, 126.1 × 3, 102.7, 101.8, 101.7, 101.6 × 2, 101.5 × 2, 101.4, 101.3, 101.2 × 2, 101.1 × 2, 101.0, 100.8, 100.6, 100.0, 99.2, 98.6 × 2, 98.5 × 2, 98.2, 98.0, 97.8, 82.9, 78.8, 78.7, 78.5 × 2, 78.4, 78.3, 78.2 × 2, 78.0, 77.9, 76.5, 76.1, 76.0, 75.9 × 2, 75.7, 75.2, 74.9, 74.4, 74.3, 74.2 × 2, 74.1, 73.9 × 2, 73.8, 73.5, 73.4, 72.4, 72.1, 71.6, 69.9, 68.8, 68.7 × 3, 68.6 × 3, 68.5, 68.4 × 2, 66.7, 66.6, 66.4, 66.1 × 2, 66.0 × 2, 65.8, 65.6 × 2, 65.2, 53.5, 51.2, 37.9, 37.7, 29.7, 29.6, 29.3, 28.6, 28.0, 27.4, 26.3, 25.4, 20.6 × 2, 20.5; IR (solid): 3631, 2964, 2093, 1734, 1412, 1258, 1089, 773, 696, 500 cm^{-1}.

4.1.28. 6-Azidohexyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(3-*O*-(2-*O*-benzoyl-3-*O*-(2-*O*-benzoyl-6-*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl)-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(4,6-*O*-benzylidene-2,3-di-*O*-levnulinyl-β-D-glucopyranosyl)-β-D-glucopyranosyl)-4-*O*-benzyl-β-D-glucopyranosyl)-4,6-*O*-benzylidene-β-D-glucopyranosyl)-4,6-*O*-benzylidene-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranoside (41)**

A mixture of **37** (70.0 mg, 17.6 μmol,), **9** (55.7 mg, 26.4 μmol) (azeotroped twice with dry toluene) and pulverized activated MS-4A (176 mg) in dry CH_2Cl_2 (1.76 mL) was stirred at room temperature for 30 min under argon to remove a trace amount of water. Then the reaction mixture was cooled to −35 °C. *N*-iodosuccinimide (7.13 mg, 31.7 μmol) and a catalytic amount of trifluoromethanesulfonic acid (0.467 μL, 5.28 μmol) were added to the reaction mixture. After being stirred at the same temperature for 30 min, the reaction mixture was neutralized with triethylamine and filtered through a pad of Celite®. The filtrate was poured into a mixture of saturated aq $NaHCO_3$ and 10% aq $Na_2S_2O_3$ with cooling. The aqueous layer was extracted with two portions of ethyl acetate. The combined extract was washed with a mixture of saturated aq $NaHCO_3$ and 10% aq $Na_2S_2O_3$, and brine, and dried over $MgSO_4$, filtered, and evaporated in vacuo. The residue was purified by chromatography on silica gel with 85:15 toluene:ethyl acetate and further purified by gel permeation chromatography (GPC) to give **41** (87.0 mg, 14.4 μmol, 82%). $[\alpha]_D^{21} = -10.6$ ($c = 2.16$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96–8.01 (m, 14H), 7.90 (d, 2H, $J = 7.7$ Hz), 7.81 (d, 2H, $J = 7.7$ Hz), 7.74 (d, 2H, $J = 7.7$ Hz), 6.99–7.63 (m, 125H), 5.52 (s, 1H), 5.41 (s, 1H), 5.36 (s, 1H), 5.33 (s, 1H), 5.30 (s, 1H), 5.25 (dd, 1H, $J = 8.7$ Hz, 8.7 Hz), 5.09–5.17 (m, 6H), 4.76–5.03 (m, 29H), 4.54–4.70 (m, 10H), 4.48 (br-d, 2H, $J = 7.7$ Hz), 4.40–4.45 (m, 2H), 4.31–4.35 (m, 2H), 3.12–4.22 (m, 85H), 3.00–3.07 (m, 3H), 2.17–2.67 (m, 10H), 2.07 (s, 3H), 2.00 (s, 3H), 1.76 (s, 12H), 1.72 (s, 3H), 1.10–1.48 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.4 × 2, 171.8, 171.4, 169.3, 169.1 × 2, 165.7 × 3, 165.3, 165.1, 165.0, 164.9 × 2, 164.8, 164.0, 138.7, 138.5, 138.4, 138.1, 137.7, 137.5 × 2, 137.4, 137.3 × 2, 137.1, 136.9, 134.1, 134.0 × 3, 133.7, 133.6, 133.4 × 2, 133.3 × 3, 133.2, 133.1 × 2, 130.3, 130.2 × 2, 130.1, 129.8 × 2, 129.7, 129.6, 129.5 × 2, 129.4, 129.3, 129.2, 129.1, 128.9, 128.8, 128.7, 128.6 × 2, 128.5, 128.4, 128.3, 128.2 × 2, 128.1, 128.0, 127.9, 127.7, 127.6 × 2, 127.4, 126.6 × 3, 126.4 × 2, 126.3, 126.2, 126.1, 102.7 × 2, 102.5, 101.8 × 3, 101.7 × 2, 101.6 × 2, 101.5 × 3, 101.4 × 3, 101.3 × 2, 101.2 × 2, 101.1 × 3, 101.0, 100.9, 100.7 × 2, 100.1, 100.0, 99.2, 98.7, 98.6, 98.5, 98.4 × 3, 98.3 × 2, 98.2, 98.1, 98.0 × 3, 97.9 × 2, 82.9, 79.6, 79.5, 79.0, 78.9, 78.8, 78.7, 78.5, 78.4, 78.3, 78.2, 78.1, 77.9, 77.8 × 2, 77.7 × 3, 76.5 × 2, 76.4, 76.0 × 3, 75.9, 75.8, 75.7, 75.6 × 2, 75.5 × 2, 75.3 × 2, 75.2 × 2, 74.9, 74.4 × 2, 74.2 × 3, 73.9 × 2, 73.8, 73.5, 73.4, 72.4, 72.1, 71.7, 70.6, 69.9, 68.9, 68.7, 68.6 × 2, 68.4, 66.7, 66.6, 66.4, 66.1 × 2, 66.0, 65.8, 65.7, 65.6, 51.3, 37.9, 37.7, 29.7, 29.6, 29.3, 28.6, 28.0, 27.4, 26.3, 25.4, 20.7, 20.6, 20.5; IR (solid): 3729, 2863, 2344, 2138, 1968, 1734, 1496, 1368, 1218, 1023, 876, 770, 654, 508 cm^{-1}.

4.1.29. 6-Aminohexyl 3-*O*-(3-*O*-(3-*O*-(3-*O*-(3-*O*-(6-*O*-(β-D-glucopyranosyl)-3-*O*-(3-*O*-(β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranoside (5)

To a stirred solution of the protected nonasaccharide **39** (33.9 mg, 9.85 μmol) in NH_3/THF/EtOH (8.50 mL/1.00 mL/0.500 mL) was added a large amount of lithium (100 mg) at −78 °C. After being stirred under reflux for 1.5 h, the reaction mixture was added methanol (1.00 mL). After being stirred at room temperature for 12 h, the reaction mixture was concentrated in vacuo. The residue was purified by size exclusion column chromatography on Sephadex LH-20 eluted with water and further purified by revese-phase column chromatography (Bond Elut-C18) with 90:10 water:methanol to give nonasaccharide **5** (15.5 mg, 9.83 μmol, quant.). $[\alpha]_D^{24} = -24.2$ ($c = 0.335$, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 4.70–4.80 (m, 7H), 4.49 (d, 1H, $J = 7.7$ Hz), 4.44 (d, 1H, $J = 7.7$ Hz), 4.18 (br-d, 1H, $J = 10.6$ Hz), 3.27–3.91 (m, 55H), 2.80 (t, 2H, $J = 7.2$ Hz), 1.51–1.63 (m, 4H), 1.35–1.36 (m, 4H); ^{13}C NMR (100 MHz, D_2O) δ 103.6 × 2, 103.4 × 2, 102.7, 85.7, 85.5, 85.2, 85.1, 84.8, 76.8, 76.7, 76.5, 76.4, 75.4, 74.3, 74.1, 74.0 × 2, 73.9, 73.8, 73.7, 71.2, 70.4, 69.6, 69.0 × 2, 68.9, 61.5, 40.5, 29.3, 28.5, 26.2, 25.4; IR (solid): 3481, 2884, 1846, 1669, 1438, 1314, 1153, 1032, 855, 664, 526 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{60}H_{106}NO_{46}$ $[M+H]^+$ $m/z = 1576.5986$, found: 1576.5981.

4.1.30. 6-Aminohexyl 3-*O*-(3-*O*-(3-*O*-(3-*O*-(3-*O*-(3-*O*-(3-*O*-(3-*O*-(3-*O*-(6-*O*-(β-D-D-glucopyranosyl)-3-*O*-(3-*O*-(β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranoside (3)

To a stirred solution of the protected tridecasaccharide **40** (16.1 mg, 3.40 μmol) in NH_3/THF/EtOH (8.50 mL/1.00 mL/0.500 mL) was added a large amount of lithium (100 mg) at −78 °C. After being stirred under reflux for 1.5 h, the reaction mixture was added methanol (1.00 mL). After being stirred at room temperature for 12 h, the reaction mixture was concentrated in vacuo. The residue was purified by size exclusion column chromatography on Sephadex LH-20 eluted with water and further purified by revese-phase column chromatography (Bond Elut-C18) with 80:20 water:methanol to give tridecasaccharide **3** (6.10 mg, 2.74 μmol, 81%). $[\alpha]_D^{24}$ −22.9 (*c* = 0.135, H_2O); ^1H NMR (400 MHz, D_2O) δ 4.70–4.76 (m, 11H), 4.49 (d, 1H, *J* = 7.7 Hz), 4.45 (d, 1H, *J* = 8.7 Hz), 4.18 (br-d, 1H, *J* = 11.1 Hz), 3.27–3.91 (m, 79H), 2.79 (t, 2H, *J* = 7.2 Hz), 1.52–1.63 (m, 4H), 1.35–1.37 (m, 4H); ^{13}C NMR (100 MHz, D_2O) δ 103.6, 103.4 × 2, 102.5, 85.1, 76.5, 76.4, 74.1, 74.0, 70.4, 69.0, 68.9, 61.5, 40.6, 29.3, 29.0, 26.2, 25.4; IR (solid): 3413, 2885, 1821, 1648, 1591, 1368, 1154, 1077, 1042, 652, 519 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{84}H_{146}NO_{66}$ $[M+H]^+$ *m/z* = 2224.8099, found: 2224.8054.

4.1.31. 6-Aminohexyl 3-*O*-(3-*O*-(3-*O*-(3-*O*-(3-*O*-(3-*O*-(3-*O*-(3-*O*-(3-*O*-(3-*O*-(3-*O*-(3-*O*-(3-*O*-(6-*O*-(β-D-D-glucopyranosyl)-3-*O*-(3-*O*-(β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranoside (1)

To a stirred solution of the protected heptadecasacchairde **41** (40.7 mg, 6.75 μmol) in NH_3/THF/EtOH (8.50 mL/1.00 mL/0.500 mL) was added a large amount of lithium (100 mg) at −78 °C. After being stirred under reflux for 1.5 h, the reaction mixture was added methanol (1.00 mL). After being stirred at room temperature for 12 h, the reaction mixture was concentrated in vacuo. The residue was purified by size exclusion column chromatography on Sephadex LH-20 eluted with water and further purified by revese-phase column chromatography (Bond Elut-C18) with 70:30 water:methanol to give heptadecasacchairde (**1**) (9.80 mg, 3.41 μmol, 51%). $[\alpha]_D^{24}$ −31.9 (*c* = 0.125, H_2O); ^1H NMR (400 MHz, D_2O) δ 4.70–4.76 (m, 15H), 4.49 (d, 1H, *J* = 7.7 Hz), 4.44 (d, 1H, *J* = 7.7 Hz), 4.18 (br-d, 1H, *J* = 11.1 Hz), 3.26–3.95 (m, 103H), 2.82 (br-s, 1H), 2.50 (br-s, 1H), 1.55–1.64 (m, 4H), 1.36 (br-s, 4H); ^{13}C NMR (100 MHz, D_2O) δ 103.6, 103.3, 85.1, 85.0 × 2, 76.5, 74.2, 74.1, 74.0 × 2, 70.4, 69.3, 68.9, 61.6, 61.5, 20.9; IR (solid): 3308, 2897, 1982, 1811, 1656, 1373, 1043, 891, 649, 526 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{108}H_{186}NO_{86}$ $[M+H]^+$ *m/z* = 2873.0212, found: 2873.0244.

4.1.32. 6-Aminohexyl 3-*O*-(3-*O*-(3-*O*-(3-*O*-(3-*O*-(3-*O*-(3-*O*-(β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranoside (6)

To a stirred solution of the protected octasaccharide **34** (28.5 mg, 10.6 μmol) in NH_3/THF/EtOH (8.50 mL/1.00 mL/0.500 mL) was added a large amount of lithium (100 mg) at −78 °C. After being stirred under reflux for 1.5 h, the reaction mixture was added methanol (1.00 mL). After being stirred at room temperature for 12 h, the reaction mixture was concentrated in vacuo. The residue was purified by size exclusion column chromatography on Sephadex LH-20 eluted with water and further purified by revese-phase column chromatography (Bond Elut-C18) with 90:10 water:methanol to give octasaccharide **6** (7.00 mg, 4.58 μmol, 43%). $[\alpha]_D^{24}$ −33.7 (*c* = 0.255, H_2O); ^1H NMR (400 MHz, D_2O) δ 4.70–4.76 (m, 7H), 4.44 (d, 1H, *J* = 7.7 Hz), 3.88–3.91 (m, 9H), 3.70–3.85 (m, 16H), 3.33–3.56 (m, 27H), 2.99 (t, 2H, *J* = 7.2 Hz), 1.59–1.68 (m, 4H), 1.37–1.40 (m, 4H); ^{13}C NMR (100 MHz, D_2O) δ 103.7, 103.6, 103.5, 103.4, 102.8, 85.5 × 2, 85.4, 85.3, 85.2 × 2, 85.0, 76.8, 76.6, 76.5, 76.4, 74.3, 74.2, 74.1, 74.0, 73.7, 71.2, 70.4, 69.0, 68.9, 61.5, 40.5, 29.3, 28.7, 26.2, 25.4; IR (solid): 3307, 2897, 2341, 1591, 1438, 1307, 1154, 1042, 669, 525 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{54}H_{96}NO_{41}$ $[M+H]^+$ *m/z* = 1414.5458, found: 1414.5460.

4.1.33. 6-Aminohexyl 3-*O*-(3-*O*-(3-*O*-(3-*O*-(3-*O*-(3-*O*-(3-*O*-(3-*O*-(3-*O*-(3-*O*-(3-*O*-(β-D-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranoside (4)

To a stirred solution of the protected decasaccharide **36** (30.8 mg, 7.45 μmol) in NH_3/THF/EtOH (8.50 mL/1.00 mL/0.500 mL) was added a large amount of lithium (100 mg) at −78 °C. After being stirred under reflux for 1.5 h, the reaction mixture was added methanol (1.00 mL). After being stirred at room temperature for 12 h, the reaction mixture was concentrated in vacuo. The residue was purified by size exclusion column chromatography on Sephadex LH-20 eluted with water and further purified by revese-phase column chromatography (Bond Elut-C18) with 90:10 water:methanol to give dodecasaccharide **4** (13.8 mg, 6.69 μmol, 90%). $[\alpha]_D^{24}$ −13.9 (*c* = 0.315, H_2O); ^1H NMR (400 MHz, D_2O) δ 4.70–4.76 (m, 11H), 4.44 (d, 1H, *J* = 8.2 Hz), 3.88–3.91 (m, 13H), 3.63–3.81 (m, 24H), 3.32–3.56 (m, 37H), 2.77 (t, 2H, *J* = 7.2 Hz), 1.49–1.63 (m, 4H), 1.28–1.36 (m, 4H); ^{13}C NMR (100 MHz, D_2O) δ 103.6, 103.4, 102.8, 85.5, 85.2, 85.0, 76.8, 76.5, 76.4, 74.3, 74.1, 73.7, 71.2, 70.4, 69.0, 68.9, 61.5, 40.4, 30.9, 30.7, 30.5, 30.3, 30.1, 29.9, 29.7, 29.3, 28.1, 26.1, 25.4; IR (solid): 3341, 2912, 2352, 2014, 1836, 1592, 1439, 1309, 1154, 1077, 1044, 911, 671, 526 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{78}H_{136}NO_{61}$ $[M+H]^+$ *m/z* = 2062.7571, found: 2062.7598.

4.1.34. 6-Aminohexyl 3-*O*-(3-*O*-(3-*O*-(3-*O*-(3-*O*-(3-*O*-(3-*O*-(3-*O*-(3-*O*-(3-*O*-(3-*O*-(3-*O*-(3-*O*-(3-*O*-(3-*O*-(β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranoside (2)

To a stirred solution of the protected hexadecasaccharide **38** (50.7 mg, 9.34 μmol) in NH_3/THF/EtOH (8.50 mL/1.00 mL/0.500 mL) was added a large amount of lithium (100 mg) at −78 °C. After being stirred under reflux for 1.5 h, the reaction mixture was added methanol (1.00 mL). After being stirred at room temperature for 12 h, the reaction mixture was concentrated in vacuo. The residue was purified by size exclusion column chromatography on Sephadex LH-20 eluted with water and further purified by revese-phase column chromatography (Bond Elut-C18) with 80:20 water:methanol to give hexadecasaccharide **2** (20.2 mg, 7.45 μmol, 80%). $[\alpha]_D^{23}$ −22.5 (*c* = 0.130, H_2O); ^1H NMR (400 MHz, D_2O) δ 4.71–4.77 (m, 14H), 4.58 (d, 1H, *J* = 7.7 Hz), 4.45 (d, 1H, *J* = 7.7 Hz), 3.88–3.92 (m, 17H), 3.67–3.78 (m, 32H), 3.32–3.56 (m, 49H), 2.96 (t, 2H, *J* = 7.7 Hz), 1.57–1.63 (m, 4H), 1.38 (br-s, 4H); ^{13}C NMR (100 MHz, D_2O) δ 103.4, 85.1, 76.5, 74.1, 70.4, 69.0, 61.5; IR (solid): 3341, 2898, 2327, 2155, 1597, 1458, 1313, 1154, 1072, 1042, 806, 670, 526 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{102}H_{176}NO_{81}$ $[M+H]^+$ *m/z* = 2710.9684, found: 2710.9724.

4.2. Biological experiment

4.2.1. Binding assay to soluble Dectin-1

Binding ability of various oligosaccharides to dectin-1 was assessed by competitive enzyme linked immunosorbent assay (ELISA). Briefly, (1,6)-monoglucosyl branched (1,3)-β-D-glucan from *Schizophyllum commune*, SPG (1 μg/ml), dissolved in phosphate buffer pH 6.9, was coated on ELISA plate (NUNC) by overnight incubation at 4 °C. The unbound SPG was washed with phosphate-buffered saline (PBS) containing 0.05% Tween20 (PBST), and the plate was blocked with PBS containing 0.5% BSA (BPBS) by 2 h incubation at room temperature. Various oligosaccharides or SPG samples were diluted with BPBS to prepare 0 to 500 μg/ml and mixed with soluble Dectin-1-Fc (1 μg/ml) for 30 min before adding to the SPG-coated ELISA plate. The plate containing soluble Dectin-1-Fc (sDectin-1) was incubated for 1 h at room temperature, washed with PBST, and further incubated with 2000-fold diluted peroxidase-conjugated anti-HA IgG (SantaCruz). The binding of Dectin-1 to solid-phase SPG was monitored by reaction of peroxidase substrate TMB (KPL Inc., MD) and color development was stopped with 1 m phosphoric acid. The absorbance at 450 nm was measured by microplate reader, MTP450 (Corona electric, Japan) The data are presented as means for duplicate samples.

4.2.2. Luciferase-assisted NF-B assay

On the day prior to transfection, 293T cells were plated (1×10^4 cells/well) in 96-well plates cultured in DMEM containing 10% FBS. The cells incubated for 20 h at 37 °C under 5% CO_2 atmospheres. Then, transfection was performed in the 96-well plates using Lipofectamine LTX (Invitrogen) and PLUS Reagent (Invitrogen) with Plasmid DNA mixtures for Dectin-1 (p3x-FLAG-CMV 14/Dectin-1A, p3x-FLAG-CMV 14/CARD9, pBud-CE4.1/Bcl10, pGL4.32[luc2P/NF-κB-RE/Hygro], and pGL4.74[hRluc/TK]) or for TLR4 (p3xFLAG-CMV9/TLR4, pBud-CE4.1/MD-2, pGL4.32[luc2P/NF-κB-RE/Hygro], and pGL4.74[hRluc/TK]). The transfection mixture was added dropwise to the cells and incubated for 18 h in DMEM containing 10% FBS. At 18 h after transfection, the cells were stimulated with the oligosaccharides, SPG, or combination of the oligosaccharides and LPS for 6 h. The cells were then lysed with Passive Lysis Buffer (Promega). Luciferase activity was measured using the Dual-Luciferase Reporter Assay System (Promega). The luciferase assay reagents were added to 20 μL of the lysate with an infector, and the results were read immediately with Microplate Luminometer (Berthold Technologies GmbH, KG, Bad Wildbad, Germany). Luciferase activity was expressed as the ratio of the NF-κB luciferase (firefly) activity to the RL-TK (renilla) activity. The data are presented as means ± standard deviation for triplicate samples.

4.3. NMR experiments

NMR spectra were recorded with 500 MHz and 600 MHz spectrometers (DRX-500 and DRX-600, BrukerBioSpin) equipped with either a triple resonance inverse cryogenic (cryo-TXI) probe or a triple resonance inverse (TXI) probe. Extracellular C-type lectin-like domain (CTLD) Dectin-1 (10 μM, 500 μL) in PBS (8.1 mM Na_2HPO_4, 1.5 mM, KH_2PO_4, 137 mM NaCl and 2.7 mM KCl (pH 7.4) was prepared for NMR experiments, and 10% D_2O was added to obtain lock signal. (1,3)Glc 16-merβ **2** was added to the corresponding NMR samples to be the molar ratio of 1:25.

In saturation transfer difference (STD)-NMR experiments, the protein signal at 7.5 ppm was saturated with Gaussian 25 Hz pulse train with 60 times (on-resonance). Reference spectra were obtained with irradiation at 40 ppm (off-resonance). The on-resonance and off-resonance spectra were collected in an interleaved manner, and accumulated into two different data sets. Water suppression was achieved using WATERGATE pulse sequence with 3-9-19 pulse train, and probe temperature was set to 5 °C. For STD-NMR experiments, either 128 scans (cryogenic probe) or 1024 scans (normal probe) with 4 repetition loops were required to obtain good signal to noise ratio, and protein signals were suppressed using T2rho filter. ^1H and ^{13}C chemical shifts indicated with parts per million (ppm) were calibrated based on outer standards of the chemical shift of 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS) at 0 ppm. Assignments of ^1H and ^{13}C NMR signals of β(1,3)Glc 16-mer **2** were obtained by ^1H–^1H DQF-COSY, ^1H–^1H NOESY, ^1H-^{13}C HSQC and ^1H–^{13}C HSQC-TOCSY experiments measured at 25 °C. NMR data were processed with XWIN-NMR (ver.3.5) and the spectra were displayed using XWIN-PLOT (ver.3.5).

4.3.1. Expression of Dection-1

Mouse Dectin-1 CTLD (Gly113–Leu244) was produced as inclusion bodies in *Escherichia coli,* and the protein was refolded as described following. We constructed pET28a-Dectin-1 CTLD vector, which composed of an N-terminal hexahistidine-tag and the Dectin-1 CTLD coding sequence with a thrombin cleavage site (Novagen). The plasmid was then transformed into *E. coli* BL21(DE3)codonplus. Cells were cultured in LB medium and harvested after 6 h induction with isopropylthio-β-D-galactopyranoside at 37 °C, and then sonicated in PBS with BugBuster (Novagen). The resultant inclusion bodies were solubilized with 8 M urea including 50 mM Tris–HCl (pH 8.0) and 50 mM NaCl. The solubilized protein was refolded by dilution into 200 mM Tris–HCl (pH 8.0), 0.4 M L-arginine, 5.0 mM reduced glutathione and 0.5 mM oxidized glutathione at 4 °C for 16 h. After refolding, the protein solution was passed through a Ni-Sepharose 6 Fast Flow column (GE Healthcare) and desired protein was eluted with 500 mM imidazole in PBS. Yielded protein was treated with thrombin to remove the hexahistidine-tag moiety, and then concentrated by ultrafiltration using Amicon Ultra 10 K (Millipore). Finally, the protein was dissolved in appropriate buffer for NMR analysis.

Acknowledgments

We sincerely thank Dr. Yukishige Ito (RIKEN, ERATO JST) and Dr. Osamu Kanie (ERATO JST) for use of the NMR spectrometer. We thank Ms. Akemi Ikeda for expression and purification of Dectin-1.This work was supported by Grant-in-Aid for Scientific Research (B) (Grant-in-aid No. 23310150) and Research (C) (Grant-in-aid No. 20550149) from the Ministry of Education, Culture, Sports, Science, and Technology. We thank EIWEISS Chemical Corporation for generous gift of EDCI and Central Glass Co., Ltd. for their gift of trifluoromethanesufonic acid anhydride.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.04.017.

References and notes

1. Ariizumi, K.; Shen, G.-L.; Shikano, S.; Xu, S.; Ritter, R., III; Kumamoto, T.; Edelbaum, D.; Morita, A.; Bergstresser, P. R.; Takashima, A. *J. Biol. Chem.* **2000**, *275*, 20157.
2. (a) Brown, G. D.; Gordon, S. *Nature* **2001**, *413*, 36; (b) Goodridge, H. S.; Wolf, A. J.; Underhill, D. M. *Immunol. Rev.* **2009**, *230*, 38; (c) Adachi, Y. *Trends Glycosci. Glyctechnol.* **2007**, *19*, 195; (d) Saijo, S.; Fujikado, N.; Furuta, T.; Chung, S.; Kotaki, H.; Seki, K.; Sudo, K.; Akira, S.; Adachi, Y.; Ohno, N.; Kinjo, T.; Nakamura, K.; Kawakami, K.; Iwakura, Y. *Nature Immunol.* **2007**, *8*, 39.
3. Palma, A. S.; Feizi, T.; Zhang, Y.; Stoll, S. M.; Lawson, A. M.; Diaz-Rodriguez, E.; Campanero-Rhodes, M. A.; Costa, J.; Gordon, S.; Brown, G. D.; Chai, W. *J. Biol. Chem.* **2006**, *281*, 5771.
4. Goodridge, H. S.; Reyes, C. N.; Becker, C. A.; Katsumoto, T. R.; Ma, J.; Wolf, A. J.; Bose, N.; Chan, A. S. H.; Magee, A. S.; Danielson, M. E.; Weiss, A.; Vasialkos, J. P.; Underhill, D. M. *Nature* **2011**, *472*, 471.

5. Adams, E. L.; Rice, P. J.; Graves, B.; Ensley, H. E.; Yu, H.; Brown, G. D.; Gordon, S.; Monteiro, M. A.; Papp-Szabo, E.; Lowman, D. W.; Power, T. D.; Wempe, M. F.; Williams, D. L. *J. Pharmacol. Exp. Ther.* **2008**, *325*, 115.
6. (a) Jamois, F.; Ferrières, V.; Guégan, J.-P.; Yvin, J.-C.; Plusquellec, D.; Vetvicka, V. *Glycobiology* **2005**, *15*, 393; (b) Mo, K.-F.; Li, H.; Mague, J. T.; Ensley, H. E. *Carbohydr. Res.* **2009**, *344*, 439.
7. Descroix, K.; Ferrières, V.; Jamois, F.; Yvin, J.-C.; Plusquellec, D. *Mini.-Rev. Med. Chem.* **2006**, *6*, 1341.
8. (a) Takeo, K.; Maki, K.; Wada, Y.; Kitamura, S. *Carbohydr. Res.* **1993**, *245*, 81; (b) Yang, G.; Kong, F. *Synlett* **2000**, 1423; (c) Huang, G.-L.; Mei, X.-Y.; Liu, M.-X. *Carbohydr. Res.* **2005**, *340*, 603.
9. (a) Amaya, T.; Tanaka, H.; Yamaguchi, T.; Shibuya, N.; Takahashi, T. *Tetrahedron Lett.* **2001**, *42*, 9191; (b) Takahashi, T.; Okano, A.; Amaya, T.; Tanaka, H.; Doi, T. *Synlett* **2002**, 911; (c) Tanaka, H.; Amaya, T.; Takahashi, T. *Tetrahedron Lett.* **2003**, *44*, 3053.
10. (a) Yang, F.; He, H.; Du, Y.; Lu, M. *Carbohydr. Res.* **2002**, *337*, 1165; (b) Zeng, Y.; Ning, J.; Kong, F. *Tetrahedron Lett.* **2002**, *43*, 3729; (c) Zeng, Y.; Ning, J.; Kong, F. *Carbohydr. Res.* **2003**, *338*, 307.
11. A preliminary result has been published as a following paper: Tanaka, H.; Kawai, T.; Adachi, Y.; Ohno, N.; Takahashi, T. *Chem. Commun* **2010**, *46*, 8249.
12. (a) Zeng, Y.; Kong, F. *Carbohydr. Res.* **2003**, *338*, 843; (b) Zeng, Y.; Kong, F. *Carbohydr. Res.* **2003**, *338*, 2359.
13. He, H.; Yang, F.; Du, Y. *Carbohydr. Res.* **2002**, *337*, 1673.
14. (a) Crich, D.; Smith, M. *J. Am. Chem. Soc.* **2001**, *123*, 9015; (b) Wang, C.; Wang, H.; Huang, X.; Zhang, L.-H.; Ye, X.-S. *Synlett* **2006**, 2846.
15. (a) Mydock, L. K.; Demchenko, A. V. *Org. Lett.* **2008**, *10*, 2103; (b) Mydock, L. K.; Demchenko, A. V. *Org. Lett.* **2008**, *10*, 2107; Premathilake, H. D.; Mydock, L. K.; Demchenko, A. V. *J. Org. Chem.* **2010**, *75*, 1095.
16. (a) Mayer, M.; Meyer, B. *J. Am. Chem. Soc.* **2001**, *123*, 6108; (b) Haselhorst, T.; Weimar, T.; Peters, T. *J. Am. Chem. Soc.* **2001**, *123*, 10705; (c) Mikkelsen, L. M.; Hernaiz, M. J.; Martin-Pastor, M.; Skrydstrup, T.; Jimenez-Barbero, J. *J. Am. Chem. Soc.* **2002**, *124*, 14940; (d) Lycknert, K.; Edblad, M.; Imberty, A.; Widmalm, G. *Biochemistry* **2004**, *43*, 9647; (e) Castro, S.; Duff, M.; Snyder, N. L.; Morton, M.; Kumar, C. V.; Peezuh, M. W. *Org. Biomol. Chem.* **2005**, *3*, 3869; (f) Clavel, C.; Canales, A.; Gupta, G.; Santos, J. I.; Canada, F. J.; Penades, S.; Surolia, A.; Jimenez-Barbero, J. *Jesus Glycoonj. J.* **2008**, *24*, 449; (g) Reina, J. J.; Diaz, I.; Nieto, P. M.; Campillo, N. E.; Paez, J. A.; Tabarani, G.; Fieschi, F.; Rojo, J. *Org. Biomol. Chem.* **2008**, *6*, 2743; (h) Timpano, G.; Tabarani, G.; Anderluh, M.; Invernizzi, D.; Vasile, F.; Potenza, D.; Nieto, P. M.; Rojo, J.; Fieschi, F.; Bernardi, A. *ChemBioChem* **2008**, *9*, 1921; (i) Weber, E.; Hetenyi, A.; Vaczi, B.; Szolnoki, E.; Roberta, R.; Tubak, V.; Monostori, E.; Martinek, T. A. *ChemBioChem* **2011**, *11*, 228.
17. Sylla, B.; Guégan, J.-P.; Wieruszeski, J.-M.; Nugier-Chauvin, C.; Legentil, L.; Daniellou, R.; Ferrières, V. *Carbohydrate Res.* **2011**, *346*, 1490.
18. Tada, R.; Adachi, Y.; Ishibashi, K.; Tsubaki, K.; Ohno, N. *J. Agric. Food Chem.* **2008**, *56*, 1442.