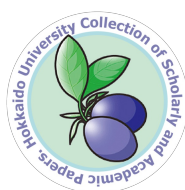




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Chemical synthesis of oligosaccharide derivatives with partial structure of β 1-3/1-6 glucan, using monomeric units for the formation of β 1-3 and β 1-6 glucosidic linkages

Author information:

Tomoya Ota,¹ Wataru Saburi,¹ Shiro Komba,² and Haruhide Mori^{1†}

¹*Research Faculty of Agriculture, Hokkaido University (Kita 9 Nishi 9, Sapporo 060-8589, Japan)*

²*Institute of Food Research, National Agriculture and Food Research Organization (2-1-12 Kannondai, Tsukuba 305-8642, Japan)*

[†]Corresponding Author (Postal address: Research Faculty of Agriculture, Hokkaido University, Kita 9 Nishi 9, Sapporo 060-8589, Japan; E-mail address: hmori@agr.hokudai.ac.jp)

Running head: Synthesis of β 1-3/1-6 glucooligosaccharide derivatives

Abstract

β 1-3/1-6 Glucans, known for their diverse structures, comprise a β 1-3-linked main chain and β 1-6-linked short branches. Laminarin, a β 1-3/1-6 glucan extracted from brown seaweed, for instance, includes β 1-6 linkages even in the main chain. The diverse structures provide various beneficial functions of the glucan. To investigate the relationship between structure and functionality, and to enable the characterization of β 1-3/1-6 glucan-metabolizing enzymes, oligosaccharides containing exact structures of β 1-3/1-6 glucans are required. We synthesized the monomeric units for the synthesis of β 1-3/1-6 mixed-linked glucooligosaccharides. 2-(Trimethylsilyl)ethyl 2-*O*-benzoyl-4,6-*O*-benzylidene- β -D-glucopyranoside served as an acceptor in the formation of β 1-3 linkages. Phenyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(*tert*-butyldiphenylsilyl)-1-thio- β -D-glucopyranoside and phenyl 2,3-di-*O*-benzoyl-4,6-di-*O*-levulinyl-1-thio- β -D-glucopyranoside acted as donors, synthesizing acceptors suitable for the formation of β 1-3- and β 1-6-linkages, respectively. These were used to synthesize a derivative of Glc β 1-6Glc β 1-3Glc β 1-3Glc, demonstrating that the proposed route can be applied to synthesize the main chain of β -glucan, with the inclusion of both β 1-3 and β 1-6 linkages.

Keywords: β 1-3/1-6 glucan, oligosaccharide, chemical route, laminarin, monomeric unit

1 **Introduction**

2 β 1-3/1-6 Glucan is a polysaccharide, abundant in nature as a storage
3 polysaccharide of seaweeds and as a cell wall and extracellular polysaccharide of fungi
4 (Chen and Seviour 2007). Fungal β 1-3/1-6 glucans such as *Schizophyllum commune*
5 schizophyllan and *Lentinula edodes* lentinan have a main chain comprising only β 1-3
6 glucosidic linkages and side chains comprising β 1-6 glucosides (Zhang *et al.* 2011;
7 Zhang *et al.* 2013). β 1-3/1-6 Glucans extracted from brown algae such as *Eisenia*
8 *bicyclis* are known as laminarins. *E. bicyclis* laminarins contain β 1-6 glucosidic linkages
9 in the β 1-3 glucan main chain and single glucosyl as well as gentiooligosaccharyl
10 residues in their side chain (Menshova *et al.* 2014; Liu *et al.* 2018).

11 β 1-3/1-6 Glucans have various health benefits and are known to act as
12 antitumor, anti-inflammatory, and immunostimulatory agents (Vetvicka and Yvin 2004;
13 Brown and Gordon 2005; Chen and Seviour 2007; Zhang *et al.* 2011; Zhang *et al.* 2013;
14 Menshova *et al.* 2014; Liu *et al.* 2018). Their functionality is related to their structure
15 (Pang *et al.* 2005; Chen and Seviour 2007; Zhang *et al.* 2013; Menshova *et al.* 2014;
16 Liu *et al.* 2018), a relationship that is not yet fully understood. Furthermore, due to their
17 structural diversity, the degradation scheme of β 1-3/1-6 glucans is complex and still
18 needs to be determined. To investigate their structure-dependent bioactivity and
19 enzymatic degradation scheme, oligosaccharides are needed that accurately represent
20 portions of the β 1-3/1-6 glucan structure.

21 Laminarioligosaccharides have been synthesized through chemical routes,
22 containing β 1-6-linked glucosyl branches (Takeo and Tei 1986; Du *et al.* 2000; Yang
23 and Kong 2000; Ning *et al.* 2003; Tanaka *et al.* 2003; Yang and Kong 2005; Adamo *et*
24 *al.* 2011; Tanaka *et al.* 2012; Miyagawa *et al.* 2015), laminaribiosyl branches, and
25 gentiotetraosyl branches (Zeng and Kong 2003; Zhao *et al.* 2003; Zhou *et al.* 2021). Of

these, 6^I,6^{III}-di-*O*-glucosyl laminaritetraose has been used to demonstrate its antitumor activity (Ning *et al.* 2003). Conversely, the synthesis of β 1-3-linked glucooligosaccharides containing β 1-6 linkages in the main chain (β 1-3/1-6 mixed-linked glucooligosaccharide) is limited (Takeo *et al.* 1986; Adamo *et al.* 2011; Miyagawa *et al.* 2015), and none of the synthetic routes included the synthesis of internal β 1-6 linkages or the development of monomeric units suitable for it. The enzymatic synthesis is also limited to 6-*O*-glucosylation of laminarioligosaccharides at the non-reducing end (Kawai *et al.* 2004; Smaali *et al.* 2004). To synthesize the oligosaccharides with the partial structure of *E. bicyclis* laminarin main chain, another method is required. The synthesis of β 1-3 and β 1-6 linkages in desired order requires suitable monomeric units. In this study, we designed three monomeric units, and show that these units function effectively for the sequential synthesis of β 1-3 and β 1-6 linkages. One serves as a β 1-3-specific acceptor for the first glycosylation, and the other two units as donors for glycosylation to produce β 1-3- and β 1-6-specific acceptors. The 3-specific introduction of the orthogonal protecting group proceeded highly efficiently. The monomeric unit for the synthesis of β 1-6 linkage was synthesized in short steps. These three units were derived from the same starting compound. Using the three units, a derivative of the tetrasaccharide Glc β 1-6Glc β 1-3Glc β 1-3Glc (compound **1**) was synthesized.

Results and Discussion

Synthesis of monomeric units for the formation of β 1-3 glucosidic linkage

2-(Trimethylsilyl)ethyl 2,4,6-tri-*O*-benzoyl- β -D-glucopyranoside (compound **2**), 1,2:5,6-di-*O*-isopropylidene- α -D-glucofuranose (compound **3**) and 2-(trimethylsilyl)ethyl 2-*O*-benzoyl-4,6-*O*-benzylidene- β -D-glucopyranoside (compound

4) were tested as monomeric acceptors in the formation of β 1-3 glucosidic linkages (Fig. 1). Compounds **2** and **4** were synthesized, while compound **3** was commercially available. The phenylthio (SPh) group, being more stable than the frequently used acid-sensitive trichloroacetimidate group (Du *et al.* 2000; Ning *et al.* 2003; Zhao *et al.* 2003; Zeng and Kong 2003; Yang and Kong 2005; Tanaka *et al.* 2012; Miyagawa *et al.* 2015), was selected as the leaving group of the donor units.

To synthesize compound **2**, first, the reaction conditions were investigated for the 3-position-specific introduction of a levulinyl (Lev) group as an orthogonal protecting group (Fig. 2). Regioselectivity was controlled by using acid anhydride as an active ester for levulinylation to promote acylation of the secondary hydroxy group (Kattnig and Albert 2004). Phenylthio β -D-glucopyranoside (compound **5**), prepared from D-glucose as reported previously (Roy *et al.* 2008), was exposed to reaction with levulinic acid anhydride, under three conditions: a) 0.1 equivalent (eq.) 4-(dimethylamino)pyridine (DMAP) and *N,N*-dimethylformamide (DMF) as nucleophile and solvent, respectively; b) pyridine as nucleophile and solvent; and c) 1.1 eq. pyridine and DMF as nucleophile and solvent, respectively. The reactions were terminated upon reaching a mole percent of 44–51% of products containing the single Lev group. Reaction times increased in the order of c, b, and a. Compound **6** (6-Lev, 18%) was the most produced under conditions b, but compound **8** (3-Lev, 16%) was produced as much as compound **6** (6-Lev, 16%) under conditions a. Under conditions c, compound **8** reached the highest yield (3-Lev, 28%), although the reaction proceeded slowly (Fig. 2), with the 3-position-specific acylation seemingly due to enhanced formation of bidentate hydrogen bonds between carboxylate ions and the 6- and 3-OH groups (Kattnig and Albert 2004). Compound **8** was prepared from compound **5** (14 g, 51.4 mmol) under conditions c. The remaining hydroxy groups were protected by benzoyl (Bz) groups,

and compound **10** (5.17 g, 3.24 mmol) was obtained in 14% yield (two steps) (Scheme 1). Compound **9**, a byproduct, was neither clearly removed from compound **8** nor from compound **10** after benzylation, resulting in a low yield for compound **10**. Compound **10** (1.00 g, 1.43 mmol) was partially subjected to the conversion from the SPh group to a 2-(trimethylsilyl)ethyl (TMSEt) group by using the method (Komba and Tsuzuki 2017; Tanaka *et al.*, 2018), and compound **11** (449 mg, 629 μ mol) was obtained in 44% yield. The unreacted portion of compound **10** (269 mg, 385 μ mol) was subjected to the same conversion to obtain additional compound **11** (95 mg, 135 μ mol) in 35% yield. The combined compound **11** (528 mg, 747 μ mol) was subjected to the removal of its 3-Lev group, which provided compound **2** (397 mg, 650 μ mol) in 87% yield.

Compound **4** was synthesized according to Scheme 2. This entailed the initial, 4-step synthesis of compound **12** from D-glucose by the method (Roy *et al.* 2008). Compound **13** was then obtained quantitatively (3.17 g, 5.30 mmol) by introducing a *tert*-butyldiphenylsilyl (TBDPS) group at the 3-position of compound **12** (1.90 g, 5.27 mmol). In compound **12**, 3-OH was previously reported to possess higher reactivity than 2-OH (Tanaka *et al.* 2018). Due to the size of the TBDPS group, and the steric hindrance caused by the SPh group at the anomeric position of compound **12**, the difference of reactivity in 3-OH and 2-OH was enhanced, and the reaction proceeded at the 3-position rather than at the 2-position. Compound **14** (1.46 g, 2.07 mmol) was obtained from compound **13** (2.43 g, 4.05 mmol) in 51% yield due to the protecting of the unmodified 2-OH by the Bz group. This reaction was able to proceed as the Bz group is smaller than the TBDPS group. The Bz group helps β -anomeric glycosylation of the donor unit *via* neighboring group participation (Nukada *et al.* 1998). Compound **14** (858 mg, 1.22 mmol) was partially subjected the conversion from the SPh group to the TBDPS group by using the method (Komba and Tsuzuki 2017; Tanaka *et al.*, 2018),

and compound **15** (505 mg, 720 μmol) was obtained in 59% yield. Compound **4** (210 mg, 445 μmol) was obtained in 73% yield by removal of the TBDPS group from compound **15** (433 mg, 609 μmol). Due to the high stability of the TBDPS group bound to secondary hydroxy group, this removal reaction was proceeded slowly (5 days). Although each reaction takes a long time, the high efficiency of introduction and removal of TBDPS group at 3-position is the advantage of the monomeric unit in β 1-3 linkage synthesis.

Glycosylation of compounds 2, 3, and 4 as acceptors

The formation of β 1-3 linkage by glycosylation was tested using the three monomeric acceptors, compounds **2**, **3**, and **4** (Scheme 3). For the efficiency of chemical route, compounds **10** and **14**, which were obtained as intermediates of compounds **2** (Scheme 1) and **4** (Scheme 2), respectively, were used as donors for the glycosylation (Scheme 3). The β 1-3-linked disaccharide derivative (compound **16**) was synthesized only by combining compounds **4** (190 mg, 402 μmol) and **14** (339 mg, 482 μmol), resulting in a mixture of compound **16** and the remaining donor (compound **14**), while the acceptor (compound **4**) was completely consumed. The TBDPS group was removed from the 3-position of the non-reducing-end sugar residue of compound **16**, synthesizing compound **17** (220 mg, 266 μmol) in 66% yield (two steps). During glycosylation using compounds **2** (140 mg, 236 μmol) and **3** (20 mg, 76.8 μmol) as acceptors, compound **18** (54 mg, 47.2 μmol), a derivative of α 1-3-linked disaccharide, and compound **19** (32 mg, 38.4 μmol), a derivative of β 1-6-linked disaccharide, were produced in 20% and 50% yields, respectively (Scheme 3). This suggests that the bulky and electron-withdrawing Bz group at the 4-position of compound **2** prevents β -glycosylation of the neighboring 3-OH while compound **4** possesses enough space to

1 receive D-glucosyl at the 3-position, thereby forming a β 1-3 glucosidic linkage.
2 Although the 2-position of the donor (compound **10**) is protected by Bz group, α -
3 glycosylation proceeded. This was considered that the anchimeric assistance (Nishimura
4 *et al.* 1972; Zhang *et al.* 2021) caused by the Lev group at 3-position of compound **10**
5 was prioritized than the neighboring group participation of the Bz group at 2-position as
6 the reactivity of 3-OH of the acceptor (compound **2**) was decreased by neighboring Bz
7 groups. Compound **3** is often used for 3-position-specific glycosylation, using
8 trimethylsilyl trifluoromethanesulfonate as an acid catalyst (Ning *et al.* 2003; Zhao *et*
9 *al.* 2003). However, a β 1-6 disaccharide derivative (compound **19**) was produced,
10 indicating that 3-OH glycosylation was prevented due to the rearrangement of the
11 isopropylidene group to the 3, 5-position. This is attributed to severe acidity caused by
12 *N*-iodosuccinimide (NIS)/ trifluoromethanesulfonic acid (TfOH), used for SPh
13 activation (Scheme 4). Similar rearrangements have been reported for boron
14 trifluoride·diethyl ether complex-catalyzed reactions (Just *et al.* 1988; Cruz-Gregorio *et*
15 *al.* 2005). In this case, although the donor (compound **10**) has the Lev group at 3-
16 position, β -glycosylation was proceeded. The reason of this was considered that the
17 effect of the neighboring group participation of the Bz group at 2-position was greater
18 than the anchimeric assistance of Lev group at 3-position due to the high reactivity of 6-
19 OH in compound **20**.

20 21 *Synthesis of the monomeric unit for the formation of β 1-6 linkages and Glc β 1-6Glc β 1- 22 3Glc β 1-3Glc derivative*

23 Compound **21**, required for β 1-6 linkage synthesis, was synthesized from
24 compound **12** in three steps (Scheme 5). The 2- and 3-positions of compound **12** (310
25 mg, 860 μ mol) were protected with Bz groups. Subsequently, the 4- and 6-positions

were protected by converting the benzylidene group to Lev groups, providing compound **21** (462 mg, 679 μmol) in 79% yield. Glycosylation of compounds **21** (215 mg, 318 μmol) and **17** (217 mg, 262 μmol) was performed (Scheme 6). Compounds **21** and **17** acted as donor and 3-position-specific acceptor, respectively, to form a β 1-3 linkage, obtaining the laminaritriose derivative compound **22** (170 mg, 123 μmol) in 47% yield. The Lev groups were removed from compound **22** (70 mg, 50.2 μmol), providing a 6-position-specific acceptor, compound **23** (47 mg, 39.2 μmol), in 78% yield. In the reaction of compounds **14** (27.8 mg, 39.5 μmol) and **23** (39.4 mg, 32.9 μmol), a β 1-6 glucosidic linkage was freshly formed, resulting in compound **1** (12.5 mg, 7.57 μmol), in 23% yield. The results indicate that compound **21**, which can be synthesized from compound **12** in short steps, acts as a donor in the synthesis of compounds that serve as acceptors toward the formation of β 1-6-glucosidic linkages. 4^{III} -*O*-glycosylation was not observed, even though the 4-OH group of the non-reducing-end sugar residue of compound **23** was unprotected because the primary hydroxy group is more reactive than the secondary hydroxy group. Additionally, the reactivity of the 4-OH group was decreased due to the bulky, electron-withdrawing Bz group at the 3-position, promoting the effective formation of the β 1-6 linkage in the β 1-3/1-6 glucan main chain. Compound **1**, with its non-reducing end-unit derived from compound **14**, presumably acts as an acceptor, to form another β 1-3 linkage in the subsequent glycosylation. The deprotection of compound **1** can be performed at high yield as reported previously (Komba and Tsuzuki 2017).

Conclusion

Two monomeric units, phenyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(*tert*-butyldiphenylsilyl)-1-thio- β -D-glucopyranoside (compound **14**) and phenyl 2,3-di-*O*-

1 benzoyl-4,6-di-*O*-levuliny-1-thio- β -D-glucopyranoside (compound **21**) were chemically
2 synthesized. These served as donors in the synthesis of acceptor compounds, which can
3 be used to introduce β 1-3 and β 1-6 linkages, respectively, to their non-reducing ends.
4 By the successive glycosylation reactions using the two compounds (compounds **14** and
5 **21**), starting with a β 1-3-specific acceptor (compound **4**), the tetrasaccharide derivative
6 (compound **1**) was synthesized. Thus, we conclude that the monomeric units
7 synthesized in this study is suitable and effective for the sequential synthesis of β 1-3
8 and β 1-6 linkages. β 1-3/1-6 Mixed-linked glucooligosaccharides with desired linkage
9 sequences can be synthesized by selective use of the monomeric units in each
10 glycosylation reaction.

12 **Experimental**

13 *General*

14 Wakogel C-200 (Fujifilm Wako Pure Chemical, Osaka, Japan) or Silica gel
15 60N (Kanto Chemical, Tokyo, Japan) was used for silica gel column chromatography.
16 The reaction progress and collected fractions were monitored and checked by thin-layer
17 chromatography (TLC) (TLC Silica gel 60 F₂₅₄ plates [Merck, Darmstadt, Germany]).
18 Plates were visualized by UV detection, treated with a detection reagent (acetic
19 acid/sulfuric acid/anisaldehyde [100/2/1 (v/v/v)], or 20% (v/v) sulfuric acid in EtOH),
20 and heated. For NMR analysis, samples were dissolved in CDCl₃ and analyzed with an
21 Avance Neo spectrometer (Bruker, Billerica, MA, USA). An Exactive Plus mass
22 spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) was used for ESI-FT-MS
23 analysis. The data were collected in positive and negative ion modes. A JASCO P-2200
24 polarimeter (JASCO, Tokyo, Japan) was used for optical rotation analysis.

1 *Introduction of a 3-position-specific Lev group to compound 5 under three conditions*
2 *(a, b, and c)*

3 Preparation of levulinic acid anhydride (for use in a, b, and c): A solution of
4 levulinic acid (4.4 eq., 188 mg, 1.61 mmol) in dry dichloromethane (2 mL) was mixed
5 with *N,N'*-diisopropylcarbodiimide (DIC) (2.2 eq., 102 mg, 807 μ mol), stirred at 0 °C
6 for 40 min, and concentrated in vacuo. The residue was dissolved in dry DMF (4 mL).

7 Conditions a: Compound **5** (1 eq., 100 mg, 367 μ mol), which was synthesized
8 from D-glucose as reported previously (Roy *et al.* 2008), dissolved in DMF (2 mL), was
9 mixed with DMAP (0.1 eq., 4.48 mg, 36.7 μ mol) and levulinic acid anhydride in DMF
10 (2 mL), and stirred at 0 °C for 3.5 h.

11 Conditions b: Compound **5** (1 eq., 1 g, 3.67 mmol), dissolved in pyridine (2
12 mL), was mixed with the levulinic acid anhydride in DMF (20 mL), and stirred at 0 °C
13 for 15.5 h.

14 Conditions c: Compound **5** (1 eq., 100 mg, 367 μ mol), dissolved in DMF (2
15 mL), was mixed with pyridine (1.1 eq., 32.7 μ L, 2.02 mmol) and levulinic acid
16 anhydride in DMF (2 mL), and stirred at 0 °C for 21.5 h, and for further 74.5 h at 27 °C.

17 In all the above conditions (a, b, and c), the reaction was terminated by adding
18 crushed ice, and the solution was concentrated in vacuo. The reaction solution of
19 conditions b was separated *via* silica gel column chromatography using CHCl₃/MeOH
20 (19/1, v/v) as mobile phase. The eluted compounds were further separated *via* silica gel
21 column chromatography using ethyl acetate as mobile phase, obtaining pure compounds
22 **6**, **8** and **9** and the mixture of compounds **7** and **8**. Compounds **6**, **8** and **9** were
23 confirmed by NMR. These were used as standards in following HPLC analysis.

24 Compound **6**; ¹H NMR (400 MHz, CDCl₃): δ 2.15 (s, 3H, Me of Lev), 2.57 (t, 2H, *J* 6.3
25 Hz, COCH₂CH₂COCH₃), 2.72 (t, 2H, *J* 6.4 Hz, COCH₂CH₂COCH₃), 3.38 (t, 1H, *J*_{1,2} =

$J_{2,3}$ 9.4 Hz, H-2), 3.41 (t, 1H, $J_{3,4} = J_{4,5}$ 9.8 Hz, H-4), 3.50 (ddd, 1H, $J_{4,5}$ 9.7 Hz, $J_{5,6}$ 5.2 Hz, $J_{5,6'}$ 2.5 Hz, H-5), 3.56 (t, 1H, $J_{2,3} = J_{3,4}$ 8.8 Hz, H-3), 4.30 (dd, 1H, $J_{5,6}$ 5.1 Hz, J_{gem} 12.2 Hz, H-6), 4.35 (dd, 1H, $J_{5,6'}$ 2.2 Hz, J_{gem} 11.9 Hz, H-6'), 4.59 (d, 1H, $J_{1,2}$ 9.7 Hz, H-1), 7.22–7.51 (m, 5H, Ph); ^{13}C NMR (100 MHz, CDCl_3): δ 27.9 ($\text{COCH}_2\text{CH}_2\text{COCH}_3$), 29.9 (Me of Lev), 38.0 ($\text{COCH}_2\text{CH}_2\text{COCH}_3$), 63.8 (C-6), 69.7 (C-4), 72.1 (C-2), 77.5 (C-5), 77.7 (C-3), 87.7 (C-1), 127.7, 128.9, 132.0, 132.9, 173.1 ($\text{COCH}_2\text{CH}_2\text{COCH}_3$), 207.8 ($\text{COCH}_2\text{CH}_2\text{COCH}_3$); HMBC 2D-NMR (400 MHz, CDCl_3), δ 4.30 (H-6) and 4.35 (H-6') $\rightarrow$ 173.1 ($\text{COCH}_2\text{CH}_2\text{COCH}_3$), indicates 6-OH was protected by Lev.

Compound **8**; ^1H NMR (400 MHz, CDCl_3): δ 2.18 (s, 3H, Me of Lev), 2.60 (m, 2H, $\text{COCH}_2\text{CH}_2\text{COCH}_3$), 2.82 (m, 2H, $\text{COCH}_2\text{CH}_2\text{COCH}_3$), 3.47 (ddd, 1H, $J_{4,5}$ 9.6 Hz, $J_{5,6}$ 4.7 Hz, $J_{5,6'}$ 3.3 Hz, H-5), 3.47 (t, 1H, $J_{1,2}$ 9.6 Hz, $J_{2,3}$ 9.3 Hz, H-2), 3.66 (t, 1H, $J_{3,4} = J_{4,5}$ 9.5 Hz, H-4), 3.81 (dd, 1H, $J_{5,6}$ 4.8 Hz, J_{gem} 12.0 Hz, H-6), 3.94 (dd, 1H, $J_{5,6'}$ 3.3 Hz, J_{gem} 12.0 Hz, H-6'), 4.65 (d, 1H, $J_{1,2}$ 9.8 Hz, H-1), 5.01 (t, 1H, $J_{2,3} = J_{3,4}$ 9.2 Hz, H-3), 7.25–7.59 (m, 5H, Ph); ^{13}C NMR (100 MHz, CDCl_3): δ 28.3 ($\text{COCH}_2\text{CH}_2\text{COCH}_3$), 29.8 (Me of Lev), 38.5 ($\text{COCH}_2\text{CH}_2\text{COCH}_3$), 62.5 (C-6), 69.2 (C-4), 70.4 (C-2), 79.6 (C-5), 79.6 (C-3), 88.1 (C-1), 128.3, 129.1, 131.6, 132.8, 173.7 ($\text{COCH}_2\text{CH}_2\text{COCH}_3$), 208.4 ($\text{COCH}_2\text{CH}_2\text{COCH}_3$); HMBC 2D-NMR (400 MHz, CDCl_3), δ 5.01 (H-3) $\rightarrow$ 173.7 ($\text{COCH}_2\text{CH}_2\text{COCH}_3$), indicates 3-OH was protected by Lev.

Compound **9**; ^1H NMR (400 MHz, CDCl_3): δ 2.21 (s, 3H, Me of Lev), 2.61 (m, 2H, $\text{COCH}_2\text{CH}_2\text{COCH}_3$), 2.89 (m, 2H, $\text{COCH}_2\text{CH}_2\text{COCH}_3$), 3.47 (m, 1H, H-5), 3.61 (t, 1H, $J_{3,4} = J_{4,5}$ 9.4 Hz, H-4), 3.72 (t, 1H, $J_{2,3} = J_{3,4}$ 8.8 Hz, H-3), 3.81 (dd, 1H, $J_{5,6}$ 5.0 Hz, J_{gem} 12.2 Hz, H-6), 3.94 (m, 1H, H-6'), 4.74 (d, 1H, $J_{1,2}$ 10.1 Hz, H-1), 4.82 (t, 1H, $J_{1,2} = J_{2,3}$ 9.4 Hz, H-2), 6.98–7.54 (m, 5H, Ph); δ 4.82 (H-2) indicates 2-OH was protected by Lev.

HPLC analysis: A portion of each sample was dissolved in acetonitrile and analyzed by HPLC, employing a COSMOSIL Cholester column (4.6 mm I.D. $\times$ 250

mm; Nacalai Tesque, Kyoto, Japan), with elution at a linear gradient of 5–100% acetonitrile for 40 min at room temperature, at a flow rate of 1 mL/min, and detection at A_{254} .

Phenyl 3-O-levulinyl-1-thio-β-D-glucopyranoside (compound **8**) and *phenyl 2,4,6-tri-O-benzoyl-3-O-levulinyl-1-thio-β-D-glucopyranoside* (compound **10**)

Compound **5** (1 eq., 14 g, 51.4 mmol), dissolved in DMF (280 mL), was mixed with pyridine (1.1 eq., 4.6 mL, 56.6 mmol) and levulinic acid anhydride in DMF (280 mL), and stirred at 23 °C for 110 h. The reaction was terminated by adding ice water.

The solution was concentrated in vacuo and separated *via* silica gel column chromatography using CHCl₃/MeOH (19/1, v/v) as the mobile phase. The eluted compounds were further separated *via* the same column chromatography, obtaining a mixture of compounds **8** and **9**. This mixture (1 eq., 8.71 g, 22.5 mmol) in pyridine (26 mL) was mixed with benzoyl chloride (BzCl) (6 eq., 15.6 mL, 135 mmol), and stirred for 1 h. The reaction was terminated with ice water, and the solution was extracted with 2 M HCl and chloroform. The organic layer obtained was concentrated in vacuo, and compound **10** (5.17 g, 14%) was obtained by purification *via* silica gel column

chromatography using toluene/ethyl acetate (10/1, v/v) as the mobile phase. $[\alpha]_D^{27} +14.0$ (c 1.25, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ 1.86 (s, 3H, Me of Lev), 2.32 (m, 2H, COCH₂CH₂COCH₃), 2.41 (m, 2H, COCH₂CH₂COCH₃), 4.12 (ddd, 1H, $J_{4,5}$ 10.0 Hz, $J_{5,6}$ 6.0 Hz, $J_{5,6'}$ 2.8 Hz, H-5), 4.44 (dd, 1H, J_{gem} 12.2 Hz, $J_{5,6}$ 6.0 Hz, H-6), 4.65 (dd, 1H, J_{gem} 12.2 Hz, $J_{5,6'}$ 2.8 Hz, H-6'), 4.98 (d, 1H, $J_{1,2}$ 10.0 Hz, H-1), 5.34 (t, 1H, $J_{1,2} = J_{2,3}$ 9.8 Hz, H-2), 5.46 (t, 1H, $J_{3,4} = J_{4,5}$ 9.8 Hz, H-4), 5.68 (t, 1H, $J_{2,3} = J_{3,4}$ 9.5 Hz, H-3), 7.10–8.13 (m, 20H, 4Ph); ¹³C NMR (100 MHz, CDCl₃): δ 28.0 (COCH₂CH₂COCH₃), 29.2 (Me of Lev), 37.8 (COCH₂CH₂COCH₃), 63.2 (C-6), 69.3

(C-4), 70.4 (C-2), 73.8 (C-3), 76.2 (C-5), 86.1 (C-1), 128.3, 128.4, 128.49, 128.52, 128.54, 128.8, 128.9, 129.3, 129.6, 129.8, 129.97, 130.00, 130.2, 131.8, 133.1, 133.2, 133.5, 133.6, 133.7, 165.1 (C=O of Bz), 165.2 (C=O of Bz), 166.1 (C=O of Bz), 171.8 ($\underline{\text{C}}\text{OCH}_2\text{CH}_2\text{COCH}_3$), 205.4 ($\text{COCH}_2\text{CH}_2\underline{\text{C}}\text{OCH}_3$); HMBC 2D-NMR (400 MHz, CDCl_3), δ 5.68 (H-3) $\rightarrow$ 171.8 ($\underline{\text{C}}\text{OCH}_2\text{CH}_2\text{COCH}_3$), indicates 3-OH was protected by Lev, 5.34 (H-2) $\rightarrow$ 165.1 (C=O of Bz), 5.46 (H-4) $\rightarrow$ 165.1 (C=O of Bz), and 4.44 (H-6) and 4.65 (H-6') $\rightarrow$ 166.1 (C=O of Bz) indicate 2-, 4- and 6-OH were protected by Bz; ESI-FT-MS, calculated for $\text{C}_{38}\text{H}_{34}\text{O}_{10}\text{SNa}^+$ ($\text{M} + \text{Na}$) $^+$: 705.1765, found m/z : 705.1769.

2-(Trimethylsilyl)ethyl 2,4,6-tri-O-benzoyl-3-O-levulinyl- β -D-glucopyranoside
(compound **11**)

Compound **10** (1 eq., 1.00 g, 1.43 mmol) was mixed with activated 4Å molecular sieves (MS4Å) (1.0 g) and NIS (3 eq., 0.966 g, 4.29 mmol), and dissolved in dry dichloromethane/acetonitrile (1/1, v/v) (20 mL) under argon gas. After adding 2-(trimethylsilyl)ethanol (TMSEtOH) (2 eq., 410 μL , 2.86 mmol), the solution was cooled to -40°C . TfOH (1 eq., 127 μL , 1.43 mmol) was then added, and the solution was stirred at -40°C for 2 h. The reaction was terminated with 700 μL triethylamine (Et_3N). The reaction mixture was filtered through Celite, extracted with sodium thiosulfate and chloroform, and the obtained chloroform layer was concentrated in vacuo. Compound **11** (449 mg, 44%) was obtained *via* silica gel column chromatography using ethyl acetate/n-hexane (1/2, v/v) as the mobile phase.

The unreacted portion of compound **10** (1 eq., 269 mg, 385 μmol) was mixed with MS4Å (269 mg) and NIS (3 eq., 260 mg, 1.16 mmol), and dissolved in dry dichloromethane/acetonitrile (1/1, v/v) (5.4 mL) under argon gas. After adding TMSEtOH (2 eq., 110 μL , 770 μmol), the solution was cooled to -40°C . TfOH (1 eq.,

34 μL , 385 μmol) was then added, and the solution was stirred at $-40\text{ }^{\circ}\text{C}$ for 2 h. The reaction was terminated with 190 μL Et_3N . Compound **11** (95 mg, 35%) was obtained by purification according to the same procedure described above. $[\alpha]_{\text{D}}^{27} +17.4$ (c 1.03, CHCl_3); ^1H NMR (400 MHz, CDCl_3): δ -0.04 (s, 9H, 3Me of TMSEt), 0.91 (ddd, 1H, J_{gem} 13.9 Hz, $J_{\text{H-2,H-1}}$ 10.2 Hz, $J_{\text{H-2,H-1'}}$ 5.8 Hz, H-2 of TMSEt), 0.98 (ddd, 1H, J_{gem} 13.9 Hz, $J_{\text{H-2',H-1'}}$ 10.5 Hz, $J_{\text{H-2',H-1}}$ 6.6 Hz, H-2' of TMSEt), 1.93 (s, 3H, Me of Lev), 2.39 (m, 2H, $\text{COCH}_2\text{CH}_2\text{COCH}_3$), 2.49 (m, 2H, $\text{COCH}_2\text{CH}_2\text{COCH}_3$), 3.66 (td, 1H, $J_{\text{gem}} = J_{\text{H-1,H-2}}$ 10.1 Hz, $J_{\text{H-1,H-2'}}$ 6.6 Hz, H-1 of TMSEt), 4.04 (ddd, 1H, J_{gem} 10.5 Hz, $J_{\text{H-1',H-2'}}$ 9.6 Hz, $J_{\text{H-1',H-2}}$ 5.8 Hz, H-1' of TMSEt), 4.13 (ddd, 1H, $J_{4,5}$ 9.0 Hz, $J_{5,6}$ 5.6 Hz, $J_{5,6'}$ 3.3 Hz, H-5), 4.52 (dd, 1H, J_{gem} 12.1 Hz, $J_{6,5}$ 5.6 Hz, H-6), 4.65 (dd, 1H, J_{gem} 12.0 Hz, $J_{6',5}$ 3.3 Hz, H-6'), 4.83 (d, 1H, $J_{1,2}$ 7.9 Hz, H-1), 5.40 (dd, 1H, $J_{2,3}$ 9.7 Hz, $J_{2,1}$ 7.9 Hz, H-2), 5.55 (t, 1H, $J_{3,4} = J_{4,5}$ 9.6 Hz, H-4), 5.70 (t, 1H, $J_{2,3} = J_{3,4}$ 9.6 Hz, H-3), $7.42\text{--}8.08$ (m, 15H, 3Ph); ^{13}C NMR (100 MHz, CDCl_3): δ -1.5 (3Me of TMSEt), 17.9 (C-2 of TMSEt), 28.1 ($\text{COCH}_2\text{CH}_2\text{COCH}_3$), 29.3 (Me of Lev), 37.8 ($\text{COCH}_2\text{CH}_2\text{COCH}_3$), 63.3 (C-6), 67.6 (C-1 of TMSEt), 69.9 (C-4), 71.9 (C-2), 72.1 (C-5), 72.8 (C-3), 100.5 (C-1), 128.35 , 128.40 , 128.5 , 129.0 , 129.6 , 129.65 , 129.71 , 129.89 , 129.94 , 133.1 , 133.2 , 133.5 , 165.1 (C=O of Bz), 165.3 (C=O of Bz), 166.1 (C=O of Bz), 171.9 ($\text{COCH}_2\text{CH}_2\text{COCH}_3$), 205.3 ($\text{COCH}_2\text{CH}_2\text{COCH}_3$); HMBC 2D-NMR (400 MHz, CDCl_3), δ 3.66 (H-1 of TMSEt) and 4.04 (H-1' of TMSEt) $\rightarrow$ 100.5 (C-1), indicates 1-OH was protected by TMSEt; ESI-FT-MS, calculated for $\text{C}_{37}\text{H}_{42}\text{O}_{11}\text{SiNa}^+$ ($\text{M} + \text{Na}$) $^+$: 713.2389, found m/z : 713.2372.

2-(Trimethylsilyl)ethyl 2,4,6-tri-O-benzoyl- β -D-glucopyranoside (compound **2**)

Compound **11** (528 mg, 747 μmol) was dissolved in pyridine (10 mL), mixed with 1 M hydrazine monohydrate in pyridine/acetic acid (3/2, v/v) (10 mL), and stirred

at room temperature for 5 min. Ethyl acetate (100 mL) and 2 M HCl (150 mL) were added at 0 °C, and the mixture was stirred at 0 °C for 10 min. The samples were then extracted using ethyl acetate. Compound **2** (397 mg, 87%) was obtained from the ethyl acetate layer *via* silica gel column chromatography using toluene/ethyl acetate (10/1, v/v) as the mobile phase. ¹H NMR (400 MHz, CDCl₃): δ -0.08 (s, 9H, 3Me of TMSEt), 0.87 (ddd, 1H, J_{gem} 13.9 Hz, $J_{\text{H-2,H-1}}$ 10.3 Hz, $J_{\text{H-2,H-1'}}$ 5.7 Hz, H-2 of TMSEt), 0.95 (ddd, 1H, J_{gem} 13.9 Hz, $J_{\text{H-2',H-1'}}$ 10.4 Hz, $J_{\text{H-2',H-1}}$ 6.4 Hz, H-2' of TMSEt), 2.85 (d, 1H, $J_{\text{OH,3}}$ 6.0 Hz, 3-OH), 3.62 (td, 1H, $J_{\text{gem}} = J_{\text{H-1,H-2}}$ 9.9 Hz, $J_{\text{H-1,H-2'}}$ 6.5 Hz, H-1 of TMSEt), 4.00 (m, 1H, H-1' of TMSEt), 4.04 (m, 1H, H-5), 4.11 (td, 1H, $J_{2,3} = J_{3,4}$ 9.2 Hz, $J_{3,\text{OH}}$ 6.0 Hz, H-3), 4.45 (dd, 1H, J_{gem} 12.0 Hz, $J_{5,6}$ 5.8 Hz, H-6), 4.64 (dd, 1H, J_{gem} 12.0 Hz, $J_{5,6'}$ 3.0 Hz, H-6'), 4.75 (d, 1H, $J_{1,2}$ 7.7 Hz, H-1), 5.19 (dd, 1H, $J_{2,3}$ 9.3 Hz, $J_{1,2}$ 7.8 Hz, H-2), 5.38 (t, 1H, $J_{3,4} = J_{4,5}$ 9.4 Hz, H-4), 7.37–8.08 (m, 15H, 3Ph); ¹³C NMR (100 MHz, CDCl₃): δ -1.5 (3Me of TMSEt), 18.0 (C-2 of TMSEt), 63.5 (C-6), 67.5 (C-1 of TMSEt), 71.9 (C-5), 72.6 (C-4), 74.2 (C-3), 75.1 (C-2), 100.3 (C-1), 128.4, 128.5, 129.2, 129.6, 129.67, 129.72, 129.9, 133.1, 133.3, 133.5, 166.1 (C=O of Bz), 166.2 (C=O of Bz), 166.3 (C=O of Bz).

Phenyl 4,6-O-benzylidene-3-O-(tert-butyldiphenylsilyl)-1-thio-β-D-glucopyranoside
(compound **13**)

Compound **12** (1 eq., 1.90 g, 5.27 mmol), which was synthesized from D-glucose as reported previously (Roy *et al.* 2008), was dissolved in DMF (20 mL), then mixed with *tert*-butyldiphenylchlorosilane (TBDPS-Cl) (1.5 eq., 2.06 mL, 7.91 mmol), and stirred at 0 °C. Imidazole (3 eq., 1.08 g, 15.8 mmol) was added, and the solution was kept at 4 °C for 8 days. The reaction was terminated by adding ice water. After stirring at room temperature for a few minutes, the supernatant was removed by

decantation. The precipitate was washed with 2 M HCl (twice), then with water, and finally dissolved in MeOH. This solution was then washed with water, forming a precipitate. Compound **13**, contained in the supernatant, was extracted twice with n-hexane and mixed with the precipitate. Compound **13** (3.17 g, quant.) was yielded. $[\alpha]_D^{27} -13.7$ (c 1.00, CHCl₃); ¹H NMR (500 MHz, CDCl₃): δ 0.99 (s, 9H, 3Me of TBDPS), 2.33 (d, 1H, $J_{2,OH}$ 2.6 Hz, 2-OH), 3.30 (td, 1H, $J_{4,5} = J_{5,6}$ 9.7 Hz, $J_{5,6'}$ 4.9 Hz, H-5), 3.55 (t, 1H, $J_{3,4} = J_{4,5}$ 9.3 Hz, H-4), 3.58 (ddd, $J_{2,OH}$ 2.4 Hz, $J_{1,2}$ 10.9 Hz, $J_{2,3}$ 8.0 Hz, H-2), 3.68 (t, 1H, $J_{5,6} = J_{gem}$ 10.3 Hz, H-6), 3.82 (dd, 1H, $J_{2,3}$ 8.2 Hz, $J_{3,4}$ 9.1 Hz, H-3), 4.26 (dd, 1H, $J_{5,6'}$ 5.0 Hz, J_{gem} 10.4 Hz, H-6'), 4.53 (d, 1H, $J_{1,2}$ 9.8 Hz, H-1), 5.24 (s, 1H, O₂CHPh), 7.01–7.72 (m, 20H, 4Ph); ¹³C NMR (126 MHz, CDCl₃): δ 19.6 (C of TBDPS), 26.9 (3Me of TBDPS), 68.5 (C-6), 70.5 (C-5), 73.9 (C-2), 76.8 (C-3), 80.7 (C-4), 88.6 (C-1), 101.4 (O₂CHPh), 126.2, 127.3, 127.5, 127.7, 127.8, 128.1, 128.7, 129.0, 129.5, 129.6, 132.2, 132.6, 133.2, 133.6, 134.8, 135.2, 135.8, 136.0, 136.9; ESI-FT-MS, calculated for C₃₅H₃₈O₅SSiNa⁺ (M + Na)⁺: 621.2101, found m/z : 621.2088.

Phenyl 2-O-benzoyl-4,6-O-benzylidene-3-O-(tert-butyldiphenylsilyl)-1-thio-β-D-glucopyranoside (compound **14**)

Compound **13** (1 eq., 2.43 g, 4.05 mmol) in pyridine (50 mL) was mixed with BzCl (3 eq., 1.42 mL, 12.2 mmol), and stirred at 60 °C for 7 days. The reaction was terminated by adding ice water, and the solution was concentrated in vacuo. The remaining pyridine was removed by co-evaporation with toluene. The sample was dissolved in chloroform and separated *via* silica gel column chromatography. Benzoic acid was removed using ethyl acetate/n-hexane/acetic acid (5/95/2, v/v/v) as the mobile phase, and compound **14** (1.46 g, 51%) was obtained by elution with toluene. $[\alpha]_D^{27} -2.93$ (c 1.00, CHCl₃); ¹H NMR (500 MHz, CDCl₃): δ 0.75 (s, 9H, 3Me of TBDPS),

3.34 (td, 1H, $J_{4,5} = J_{5,6}$ 9.7 Hz, $J_{5,6'}$ 4.9 Hz, H-5), 3.68 (t, 1H, $J_{3,4} = J_{4,5}$ 9.3 Hz, H-4),
 3.70 (t, 1H, $J_{5,6} = J_{\text{gem}}$ 10.3 Hz, H-6), 4.04 (t, 1H, $J_{2,3} = J_{3,4}$ 8.8 Hz, H-3), 4.26 (dd, 1H,
 J_{gem} 10.4 Hz, $J_{5,6'}$ 4.9 Hz, H-6'), 4.74 (d, 1H, $J_{1,2}$ 10.1 Hz, H-1), 5.12 (s, 1H, O₂CHPh),
 5.42 (t, 1H, $J_{1,2} = J_{2,3}$ 8.7 Hz, H-2), 6.81–7.98 (m, 25H, 5Ph); ¹³C NMR (126 MHz,
 CDCl₃): δ 19.3 (OSi(Ph)₂CMe₃), 26.6 (3Me of TBDPS), 68.4 (C-6), 70.5 (C-5), 73.6
 (C-2), 74.9 (C-3), 80.9 (C-4), 87.5 (C-1), 101.6 (O₂CHPh), 126.3, 127.1, 127.2, 127.7,
 127.8, 128.18, 128.21, 128.7, 128.9, 129.0, 129.3, 129.4, 129.8, 130.1, 132.1, 133.0,
 133.2, 134.0, 135.6, 136.2, 136.6, 165.4 (C=O of Bz); HMBC 2D-NMR (500 MHz,
 CDCl₃), δ 5.42 (H-2) → 165.4 (C=O of Bz), indicates 2-OH was protected by Bz; ESI-
 FT-MS, calculated for C₄₂H₄₂O₆SSiNa⁺ (M + Na)⁺: 725.2364, found *m/z*: 725.2356.

2-(Trimethylsilyl)ethyl 2-O-benzoyl-4,6-O-benzylidene-3-O-(tert-butylidiphenylsilyl)-β-D-glucopyranoside (compound **15**)

Compound **14** (1 eq., 858 mg, 1.22 mmol) was mixed with MS4Å (860 mg)
 and NIS (3 eq., 824 mg, 3.66 mmol) and dissolved in dry dichloromethane/acetonitrile
 (1/1, v/v) (20 mL) under nitrogen. After adding TMSEtOH (2 eq., 350 μL, 2.44 mmol),
 the solution was cooled to −40 °C. TfOH (1 eq., 108 μL, 1.22 mmol) was added to the
 solution, which was kept at −40 °C for 1 h. The reaction was terminated with 858 μL
 Et₃N. Compound **15** (505 mg, 59%) was obtained by purification according to the same
 procedure as applied to compound **11**, however, using toluene as the mobile phase for
 the silica gel column chromatography. $[\alpha]_D^{27}$ −36.0 (c 1.00, CHCl₃); ¹H NMR (500
 MHz, CDCl₃): δ −0.12 (s, 9H, 3Me of TMSEt), 0.73–0.85 (m, H-2 and H-2' of TMSEt),
 0.78 (s, 9H, 3Me of TBDPS), 3.29 (td, 1H, $J_{4,5} = J_{5,6}$ 9.7 Hz, $J_{5,6'}$ 4.9 Hz, H-5), 3.47 (td,
 1H, J_{gem} 10.1 Hz, J 6.4 Hz, H-1 of TMSEt), 3.67 (t, 1H, $J_{3,4} = J_{4,5}$ 9.1 Hz, H-4), 3.71 (t,
 1H, $J_{5,6} = J_{\text{gem}}$ 10.1 Hz, H-6), 3.89 (ddd, 1H, J_{gem} 10.4 Hz, J 5.7 Hz, J 10.6 Hz, H-1' of

TMSEt), 4.04 (t, 1H, $J_{2,3} = J_{3,4}$ 9.0 Hz, H-3), 4.25 (dd, 1H, $J_{5,6'}$ 4.9 Hz, J_{gem} 10.4 Hz, H-6'), 4.48 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 5.12 (s, 1H, O₂C $\underline{\text{H}}$ Ph), 5.36 (dd, 1H, $J_{1,2}$ 8.1 Hz, $J_{2,3}$ 8.8 Hz, H-2), 6.84–8.10 (m, 20H, 4Ph); ¹³C NMR (126 MHz, CDCl₃): δ –1.6 (3Me of TMSEt), 18.0 (C-2 of TMSEt), 19.3 (C of TBDPS), 26.6 (3Me of TBDPS), 66.2 (C-5), 67.6 (C-1 of TMSEt), 68.6 (C-6), 73.8 (C-3), 75.1 (C-2), 81.2 (C-4), 101.3 (C-1), 101.5 (O₂C $\underline{\text{H}}$ Ph), 126.2, 127.1, 127.2, 127.4, 127.69, 127.72, 128.1, 128.2, 128.5, 128.6, 129.0, 129.2, 129.3, 129.6, 129.7, 129.8, 129.9, 130.0, 130.2, 130.3, 132.4, 132.76, 132.82, 133.6, 134.1, 134.35, 134.44, 134.8, 134.9, 135.3, 135.6, 136.2, 136.3, 136.7, 165.1 (C=O of Bz); ESI-FT-MS, calculated for C₄₁H₅₀O₇Si₂Na⁺ (M + Na)⁺: 733.2987, found m/z : 733.2966.

2-(Trimethylsilyl)ethyl 2-O-benzoyl-4,6-O-benzylidene- β -D-glucopyranoside (compound **4**)

Compound **15** (1 eq., 433 mg, 609 μ mol) in DMF (4 mL) was mixed with acetic acid (2.11 eq., 73.4 μ L, 1.28 mmol) and cooled to 0 °C. A 1.0 M TBAF in THF (2 eq., 1.22 mL, 1.22 mmol) was added, and the solution was stirred at room temperature for 5 days. The samples were extracted with water and ethyl acetate, and the ethyl acetate layer was concentrated in vacuo. The DMF was eliminated by co-evaporation with toluene. Compound **4** (210 mg, 73%) was obtained by purification *via* silica gel column chromatography, using ethyl acetate/toluene (1/9, v/v) as the mobile phase.

$[\alpha]_{\text{D}}^{27}$ –41.7 (c 1.00, CHCl₃); ¹H NMR (500 MHz, CDCl₃): δ –0.08 (s, 9H, 3Me of TMSEt), 0.83 (ddd, 1H, $J_{\text{H-1}', \text{H-2}}$ 5.5 Hz, $J_{\text{H-1}, \text{H-2}}$ 10.4 Hz, J_{gem} 13.9 Hz, H-2 of TMSEt), 0.90 (ddd, 1H, $J_{\text{H-1}, \text{H-2}'}$ 6.5 Hz, $J_{\text{H-1}', \text{H-2}'}$ 10.8 Hz, J_{gem} 13.9 Hz, H-2' of TMSEt), 2.70 (d, 1H, $J_{\text{OH}, 3}$ 3.2 Hz, 3-OH), 3.51 (td, 1H, $J_{4,5} = J_{5,6}$ 9.7 Hz, $J_{5,6'}$ 4.9 Hz, H-5), 3.57 (ddd, 1H, $J_{\text{H-1}, \text{H-2}'}$ 6.4 Hz, $J_{\text{H-1}, \text{H-2}}$ 10.3 Hz, J_{gem} 9.7 Hz, H-1 of TMSEt), 3.66 (t, 1H, $J_{3,4} = J_{4,5}$

9.3 Hz, H-4), 3.82 (t, 1H, $J_{5,6} = J_{\text{gem}}$ 10.3 Hz, H-6), 3.96 (ddd, 1H, $J_{\text{H-1'}, \text{H-2}}$ 5.5 Hz, $J_{\text{H-1'}, \text{H-2'}}$ 10.8 Hz, J_{gem} 9.6 Hz, H-1' of TMSEt), 4.02 (td, 1H, $J_{2,3} = J_{3,4}$ 9.2 Hz, $J_{\text{OH},3}$ 2.9 Hz, H-3), 4.38 (dd, 1H, $J_{5,6'}$ 5.0 Hz, J_{gem} 10.5 Hz, H-6'), 4.68 (d, 1H, $J_{1,2}$ 7.9 Hz, H-1), 5.15 (dd, 1H, $J_{1,2}$ 7.9 Hz, $J_{2,3}$ 9.1 Hz, H-2), 5.56 (s, 1H, O_2CHPh), 7.23–8.09 (m, 10H, 2Ph); ^{13}C NMR (126 MHz, CDCl_3): δ –1.5 (3Me of TMSEt), 18.1 (C-2 of TMSEt), 66.2 (C-5), 67.8 (C-1 of TMSEt), 68.7 (C-6), 72.6 (C-3), 75.0 (C-2), 81.0 (C-4), 101.0 (C-1), 101.9 (O_2CHPh), 126.3, 128.33, 128.34, 129.3, 129.7, 129.9, 133.2, 136.9, 165.9 (C=O of Bz); ESI-FT-MS, calculated for $\text{C}_{25}\text{H}_{32}\text{O}_7\text{SiNa}^+$ ($\text{M} + \text{Na}$) $^+$: 495.1810, found m/z : 495.1801.

2-(Trimethylsilyl)ethyl O-(2-O-benzoyl-4,6-O-benzylidene-3-O-(tert-butyldiphenylsilyl)- β -D-glucopyranosyl)-(1 $\rightarrow$ 3)-2-O-benzoyl-4,6-O-benzylidene- β -D-glucopyranoside
(compound **16**)

Compound **14** (1.2 eq., 339 mg, 482 μmol), compound **4** (1 eq., 190 mg, 402 μmol), $\text{MS4}\text{\AA}$ (530 mg), and NIS (2.4 eq., 217 mg, 965 μmol) were dissolved in dry dichloromethane (6 mL), under nitrogen. After cooling the solution to $-30\text{ }^\circ\text{C}$, TfOH (0.48 eq., 17 μL , 193 μmol) was added. This reaction solution was kept at $-30\text{ }^\circ\text{C}$ for 2 h, and the reaction was terminated with 160 μL Et_3N . Compound **16** (458 mg) was obtained by purification according to the same procedure as applied to compound **11**, however, using ethyl acetate/toluene (1/19, v/v) as the mobile phase for the silica gel column chromatography. $[\alpha]_{\text{D}}^{27}$ –9.09 (c 1.00, CHCl_3); ^1H NMR (500 MHz, CDCl_3): δ –0.15 (s, 9H, 3Me of TMSEt), 0.70 (s, 9H, 3Me of TBDPS), 0.68–0.77 (m, 2H, H-2, H-2' of TMSEt), 3.18 (td, 1H, $J_{4,5} = J_{5,6}$ 9.7 Hz, $J_{5,6'}$ 4.8 Hz, H-5 $^{\text{II}}$), 3.40 (ddd, 1H, J 6.4 Hz, J 6.8 Hz, J 10.3 Hz, H-1 of TMSEt), 3.51 (td, 1H, $J_{4,5}$ 9.7 Hz, $J_{5,6}$ 4.8 Hz, 5-H $^{\text{I}}$), 3.56 (t, 1H, $J_{5,6} = J_{\text{gem}}$ 10.2 Hz, H-6 $^{\text{II}}$), 3.70 (t, 1H, $J_{3,4} = J_{4,5}$ 9.2 Hz, H-4 $^{\text{II}}$), 3.77–3.86

(m, 3H, H-1' of TMSEt, H-6^I, and H-4^I), 3.89 (t, 1H, $J_{2,3} = J_{3,4}$ 8.3 Hz, H-3^{II}), 3.82 (dd, 1H, $J_{5,6'}$ 4.9 Hz, J_{gem} 10.4 Hz, 6-H^{II}), 4.13 (t, 1H, $J_{2,3} = J_{3,4}$ 8.8 Hz, H-3^I), 4.35 (dd, 1H, $J_{5,6'}$ 4.9 Hz, J_{gem} 10.4 Hz, H-6^I), 4.55 (d, 1H, $J_{1,2}$ 7.5 Hz, H-1^I), 4.77 (d, 1H, $J_{1,2}$ 7.2 Hz, H-1^{II}), 4.85 (s, 1H, O₂CHPh^{II}), 5.19 (dd, 1H, $J_{1,2}$ 7.7 Hz, $J_{2,3}$ 8.3 Hz, H-2^I), 5.31 (t, 1H, $J_{1,2} = J_{2,3}$ 7.5 Hz, H-2^{II}), 5.55 (s, 1H, O₂CHPh^I), 6.76–7.80 (m, 30H, 6Ph); ¹³C NMR (126 MHz, CDCl₃): δ -1.6 (3Me of TMSEt), 18.0 (C-2 of TMSEt), 19.1 (OSi(Ph)₂CMe₃), 26.5 (3Me of TBDPS), 65.8 (C-5^{II}), 66.4 (C-5^I), 67.4 (C-1 of TMSEt), 68.5 (C-6^{II}), 68.8 (C-6^I), 73.77 (C-2^{II}), 73.84 (C-3^{II}), 75.4 (C-2^I), 77.8 (C-3^I), 79.4 (C-4^I), 80.7 (C-4^{II}), 100.4 (C-1^{II}), 100.9 (C-1^I), 101.2 (O₂CHPh^{II}), 101.4 (O₂CHPh^I), 125.3, 126.0, 126.1, 126.2, 127.0, 127.1, 127.5, 127.6, 127.8, 128.16, 128.20, 128.53, 129.00, 129.03, 129.1, 129.2, 129.68, 129.73, 129.8, 129.9, 132.36, 132.37, 132.7, 134.2, 135.5, 136.0, 136.7, 137.2, 137.8, 164.4 (C=O of Bz^I), 164.6 (C=O of Bz^{II}); HMBC 2D-NMR (500 MHz, CDCl₃), δ 4.77 (H-1^{II}) $\rightarrow$ 77.8 (C-3^I), indicates 1 $\rightarrow$ 3 linkage; ESI-FT-MS, calculated for C₆₁H₆₈O₁₃Si₂Na⁺ (M + Na)⁺: 1087.4091, found m/z : 1087.4080.

2-(Trimethylsilyl)ethyl O-(2-O-benzoyl-4,6-O-benzylidene- β -D-glucopyranosyl)-(1 $\rightarrow$ 3)-2-O-benzoyl-4,6-O-benzylidene- β -D-glucopyranoside (compound **17**)

Compound **16** (1 eq., 429 mg, 403 μ mol), dissolved in DMF (4 mL), was mixed with acetic acid (2.11 eq., 49 μ L, 850 μ mol). The solution was cooled to 0 °C, and 1.0 M TBAF in THF (2 eq., 806 μ L, 806 μ mol) was added. The reaction mixture was stirred at room temperature for 14 days. The sample was extracted with water and ethyl acetate, and the ethyl acetate layer was concentrated in vacuo. This mixture was co-evaporated with toluene to eliminate the DMF. Compound **17** (220 mg, 66% [two steps]) was obtained by purification *via* silica gel column chromatography using ethyl acetate/toluene (1/9, v/v) as the mobile phase. $[\alpha]_D^{27}$ -20.8 (c 1.00, CHCl₃); ¹H NMR

(500 MHz, CDCl₃): δ -0.14 (s, 9H, 3Me of TMSEt), 0.67–0.80 (m, 2H, H-2 and H-2' of TMSEt), 2.53 (d, 1H, $J_{\text{OH},3}$ 2.7 Hz, 3-OH), 3.37 (td, 1H, $J_{4,5} = J_{5,6}$ 10.1 Hz, $J_{5,6'}$ 5.1 Hz, H-5^{II}), 3.43 (td, 1H, J 6.7 Hz, J 9.9 Hz, H-1 of TMSEt), 3.59 (td, 1H, $J_{4,5}$ 9.8 Hz, $J_{5,6} = J_{5,6'}$ 4.9 Hz, H-5^I), 3.67 (t, 1H, $J_{3,4} = J_{4,5}$ 9.2 Hz, H-4^{II}), 3.68 (t, 1H, $J_{5,6} = J_{\text{gem}}$ 10.5 Hz, H-6^{II}), 3.78–3.87 (m, 4H, H-4^I, H-6^I, H-3^{II}, and H-1' of TMSEt), 4.17 (dd, $J_{5,6'}$ 5.1 Hz, J_{gem} 10.4 Hz, H-6^{II}), 4.19 (t, 1H, $J_{2,3} = J_{3,4}$ 8.8 Hz, H-3^I), 4.36 (dd, 1H, $J_{5,6'}$ 4.9 Hz, J_{gem} 10.5 Hz, H-6^I), 4.59 (d, 1H, $J_{1,2}$ 7.5 Hz, H-1^I), 4.94 (d, 1H, $J_{1,2}$ 7.2 Hz, H-1^{II}), 5.08 (dd, 1H, $J_{1,2}$ 7.2 Hz, $J_{2,3}$ 7.8 Hz, H-2^{II}), 5.22 (dd, 1H, $J_{1,2}$ 7.6 Hz, $J_{2,3}$ 8.4 Hz, H-2^I), 5.34 (s, 1H, O₂CHPh^{II}), 5.55 (s, 1H, O₂CHPh^I), 7.11–7.92 (m, 20H, 4Ph); ¹³C NMR (126 MHz, CDCl₃): δ -1.59 (3Me of TMSEt), 18.0 (C-2 of TMSEt), 66.0 (C-5^{II}), 66.4 (C-5^I), 67.5 (C-1 of TMSEt), 68.6 (C-6^{II}), 68.8 (C-6^I), 72.7 (C-3^{II}), 73.7 (C-2^I), 75.4 (C-2^{II}), 78.0 (C-3^I), 79.4 (C-4^I), 80.5 (C-4^{II}), 100.2 (C-1^{II}), 100.8 (C-1^I), 101.5 (O₂CHPh^I), 101.7 (O₂CHPh^{II}), 125.3, 126.1, 126.2, 128.16, 128.21, 128.24, 128.26, 128.27, 128.4, 128.5, 129.0, 129.18, 129.24, 129.3, 129.6, 129.7, 129.8, 132.9, 136.9, 137.2, 137.9, 164.6 (C=O of Bz^{II}), 165.6 (C=O of Bz^I); ESI-FT-MS, calculated for C₄₅H₅₀O₁₃SiNa⁺ (M + Na)⁺: 849.2913, found m/z : 849.2894.

2-(Trimethylsilyl)ethyl O-(2,4,6-tri-O-benzoyl-3-levulinyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 3)-2,3,4-tri-O-benzoyl- β -D-glucopyranoside (compound **18**)

Compounds **10** (1.5 eq., 242 mg, 354 μ mol), compound **2** (1 eq., 140 mg, 236 μ mol), MS4Å (382 mg), and NIS (3 eq., 159 mg, 708 μ mol) were dissolved in dry dichloromethane (2 mL), under argon gas. After cooling this solution to -30 °C, TfOH (0.6 eq., 12.5 μ L, 142 μ mol) was added, and the reaction solution was stirred at -30 °C for 40 h. The reaction was terminated with 154 μ L Et₃N. Compound **18** (54 mg, 20%) was obtained by purification according to the same procedure as applied to compound **11**.

¹H NMR (400 MHz, CDCl₃): δ -0.12 (s, 9H, 3Me of TMSEt), 0.99–1.04 (m, 2H, H-2 and H-2' of TMSEt), 1.92 (s, 3H, Me of Lev), 2.29 (m, 2H, COCH₂CH₂COCH₃), 2.40 (m, 2H, COCH₂CH₂COCH₃), 3.64 (dd, 1H, *J*_{5,6} 3.1 Hz, *J*_{gem} 12.6 Hz, H-6^{II}), 3.69 (td, 1H, *J* 6.1 Hz, *J* 10.0 Hz, H-1 of TMSEt), 3.99–4.05 (m, 1H, H-5^I), 4.06 (td, 1H, *J* 5.9 Hz, *J* 10.2 Hz, H-1' of TMSEt), 4.28 (dt, 1H, *J*_{4,5} 10.2 Hz, *J*_{5,6} = *J*_{5,6'} 2.8 Hz, H-5^{II}), 4.33 (dd, 1H, *J*_{5,6'} 2.6 Hz, *J*_{gem} 12.4 Hz, H-6^{II}), 4.46 (dd, 1H, *J*_{5,6} 6.0 Hz, *J*_{gem} 11.9 Hz, H-6^I), 4.54 (dd, 1H, *J*_{5,6'} 4.0 Hz, *J*_{gem} 11.8 Hz, H-6'), 4.57 (t, 1H, *J*_{2,3} = *J*_{3,4} 9.0 Hz, H-3^I), 4.82 (d, 1H, *J*_{1,2} 7.7 Hz, H-1^I), 5.13 (dd, 1H, *J*_{1,2} 3.8 Hz, *J*_{2,3} 10.4 Hz, H-2^{II}), 5.47 (t, 1H, *J*_{3,4} = *J*_{4,5} 9.8 Hz, H-4^{II}), 5.61 (dd, 1H, *J*_{1,2} 7.6 Hz, *J*_{2,3} 9.3 Hz, H-2^I), 5.62 (d, 1H, *J*_{1,2} 3.4 Hz, H-1^{II}), 5.63 (dd, 1H, *J*_{3,4} 8.5 Hz, *J*_{4,5} 10.9 Hz, H-4^I), 5.81 (t, 1H, *J*_{2,3} = *J*_{3,4} 10.0 Hz, H-3^{II}), 7.33–8.20 (m, 30H, 6Ph); ¹³C NMR (100 MHz, CDCl₃): δ 0.25 (3Me of TMSEt), 19.5 (C-2 of TMSEt), 29.4 (COCH₂CH₂COCH₃), 30.8 (Me of Lev), 39.3 (COCH₂CH₂COCH₃), 63.2 (C-6^{II}), 65.2 (C-6^I), 69.0 (C-1 of TMSEt), 69.5 (C-5^{II}), 70.3 (C-4^{II}), 71.2 (C-3^{II}), 72.0 (C-2^{II}), 73.7 (C-5^I), 74.0 (C-2^I or C-4^I), 74.1 (C-2^I or C-4^I), 78.2 (C-3^I), 98.2 (C-1^{II}), 102.0 (C-1^I), 129.7, 129.8, 129.92, 129.99, 130.04, 130.1, 130.4, 131.07, 131.16, 131.24, 131.3, 131.5, 131.7, 134.5, 134.6, 134.91, 134.96, 135.02, 135.1, 166.1 (C=O of Bz), 166.4 (C=O of Bz), 166.5 (C=O of Bz), 166.8 (C=O of Bz), 167.5 (C=O of Bz), 167.6 (C=O of Bz), 172.9 (COCH₂CH₂COCH₃), 205.3 (COCH₂CH₂COCH₃); HMBC 2D-NMR (400 MHz, CDCl₃), δ 4.57 (H-3^I) → 98.2 (C-1^{II}), indicates 1→3 linkage.

O-(2,4,6-tri-*O*-benzoyl-3-levulinyl-β-*D*-glucopyranosyl)-(1→6)-1,2:3,5-di-*O*-isopropylidene-α-*D*-glucopyranoside (compound **19**)

Compound **3** (1 eq., 20 mg, 76.8 μmol; Tokyo Chemical Industry, Tokyo, Japan), compound **10** (1.1 eq., 58 mg, 85.0 μmol), MS4Å (78 mg), and NIS (2.2 eq., 38 mg, 169 μmol) were dissolved in dry dichloromethane (1 mL) under argon gas. After

cooling this solution to $-30\text{ }^{\circ}\text{C}$, TfOH (0.44 eq., 3.0 μL , 33.8 μmol) was added. This reaction solution was stirred at $-30\text{ }^{\circ}\text{C}$ for 1 h, and the reaction was terminated with 36 μL Et_3N . Compound **19** (32 mg, 50%) was obtained by purification according to the same procedure as applied to compound **11**. $[\alpha]_{\text{D}}^{27} +18.7$ (c 0.603, CHCl_3); ^1H NMR (400 MHz, CDCl_3): δ 1.08 (s, 3H, Me of isopropylidene at 3- and 5-OH), 1.15 (s, 3H, Me of isopropylidene at 3- and 5-OH), 1.27 (s, 3H, Me of isopropylidene at 1- and 2-OH), 1.36 (s, 3H, Me of isopropylidene at 1- and 2-OH), 1.87 (s, 3H, Me of Lev), 2.33 (m, 2H, $\text{COCH}_2\text{CH}_2\text{COCH}_3$), 2.43 (m, 2H, $\text{COCH}_2\text{CH}_2\text{COCH}_3$), 3.63–3.73 (m, 2H, H-4^I and H-6^I), 4.01–4.16 (m, 4H, H-3^I, H-5^I, H-6^I and H-5^{II}), 4.42–4.49 (m, 2H, H-2^I and H-6^{II}), 4.60 (dd, 1H, J 3.4 Hz, J 8.3 Hz, H-6^{II}), 4.87 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1^{II}), 5.37 (dd, 1H, $J_{1,2}$ 7.9 Hz, $J_{2,3}$ 9.7 Hz, H-2^{II}), 5.51 (t, 1H, $J_{3,4} = J_{4,5}$ 9.7 Hz, H-4^{II}), 5.64 (t, 1H, $J_{2,3} = J_{3,4}$ 9.6 Hz, H-3^{II}), 5.87 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1^I), 7.35–8.07 (m, 15H, 3Ph); ^{13}C NMR (100 MHz, CDCl_3): δ 23.6 (Me of isopropylidene at 3- and 5-OH), 23.7 (Me of isopropylidene at 3- and 5-OH), 26.5 (Me of isopropylidene at 1- and 2-OH), 27.1 (Me of isopropylidene at 1- and 2-OH), 28.0 ($\text{COCH}_2\text{CH}_2\text{COCH}_3$), 29.3 (Me of Lev), 37.8 ($\text{COCH}_2\text{CH}_2\text{COCH}_3$), 63.1 (C-6^{II}), 69.7 (C-4^{II}), 70.3 (C-6^I), 71.6 (C-4^I), 71.8 (C-2^{II}), 72.1 (C-3^I), 72.5 (C-3^{II}), 74.9 (C-5^{II}), 79.1 (C-5^I), 84.0 (C-2^I), 100.9 (C of isopropylidene at 3- and 5-OH), 101.6 (C-1^{II}), 106.3 (C-1^I), 112.1 (C of isopropylidene at 1- and 2-OH), 128.3, 128.4, 128.5, 129.5, 129.6, 129.8, 129.90, 129.93, 133.1, 133.2, 133.5, 165.0 (C=O of Bz), 165.2 (C=O of Bz), 166.1 (C=O of Bz), 171.8 ($\text{COCH}_2\text{CH}_2\text{COCH}_3$), 205.3 ($\text{COCH}_2\text{CH}_2\text{COCH}_3$); HMBC 2D-NMR (400 MHz, CDCl_3), δ 4.87 (H-1^{II}) $\rightarrow$ 70.3 (C-6^I), indicates 1 $\rightarrow$ 6 linkage; ESI-FT-MS, calculated for $\text{C}_{44}\text{H}_{48}\text{O}_{16}\text{Na}^+$ ($\text{M} + \text{Na}$)⁺: 855.2835, found m/z : 855.2826.

Phenyl 2,3-di-O-benzoyl-4,6-di-O-levulinyl-1-thio- β -D-glucopyranoside (compound **21**)

Compound **12** (1 eq., 310 mg, 860 μ mol) in pyridine (15 mL) was mixed with BzCl (3 eq., 300 μ L, 2.58 mmol), and stirred at room temperature for 2 h. The reaction was terminated by adding ice water. The precipitated sample was washed with water and 2 M HCl, and dissolved in chloroform. The pyridine was removed by co-evaporation with toluene. Acetic acid aqueous solution (80%, v/v) (100 mL) was added to the reaction mixture, and the resulting solution was stirred at 60 °C for 3 h. The sample was concentrated in vacuo, washed with water, and reconcentrated in vacuo. Levulinic acid anhydride, prepared from levulinic acid (6 eq., 600 mg, 5.17 mmol) as described above, was dissolved in pyridine/dichloromethane (1/1, v/v) (6 mL) and mixed with the concentrated sample. DMAP (1 eq., 105 mg, 859 μ mol) was added, and the solution was stirred at room temperature for 2 h. The reaction was terminated by adding ice water. The sample was extracted with 2 M HCl and chloroform. Compound **21** (462 mg, 79%) was obtained from the chloroform layer *via* silica gel column chromatography using ethyl acetate/toluene (1/4, v/v) as the mobile phase. $[\alpha]_D^{27} +76.7$ (c 1.00, CHCl₃); ¹H NMR (500 MHz, CDCl₃): δ 2.00 (s, 3H, Me of Lev), 2.18 (s, 3H, Me of Lev), 2.30–2.90 (m, 8H, CH₂ of Lev), 3.89 (ddd, 1H, $J_{4,5}$ 10.0 Hz, $J_{5,6}$ 5.5 Hz, $J_{5,6'}$ 2.4 Hz, H-5), 4.26 (dd, 1H, $J_{5,6}$ 5.5 Hz, J_{gem} 12.2 Hz, H-6), 4.32 (dd, 1H, $J_{5,6'}$ 2.4 Hz, J_{gem} 12.1 Hz, H-6'), 4.93 (d, 1H, $J_{1,2}$ 10.0 Hz, H-1), 5.25 (t, 1H, $J_{3,4} = J_{4,5}$ 9.8 Hz, H-4), 5.37 (t, 1H, $J_{1,2} = J_{2,3}$ 9.7 Hz, H-2), 5.66 (t, 1H, $J_{2,3} = J_{3,4}$ 9.5 Hz, H-3), 7.21–7.99 (m, 15H, 3Ph); ¹³C NMR (126 MHz, CDCl₃): δ 27.77 (CH₂ of Lev), 27.84 (CH₂ of Lev), 29.4 (Me of Lev), 29.82 (Me of Lev), 37.76 (CH₂ of Lev), 37.9 (CH₂ of Lev), 62.5 (C-6), 68.50 (C-4), 70.4 (C-2), 74.2 (C-3), 76.1 (C-5), 86.1 (C-1), 127.8, 128.2, 128.31, 128.33, 128.38, 128.5, 128.75, 128.83, 128.89, 128.99, 129.08, 129.10, 129.13, 129.81, 129.83, 130.0, 131.8, 132.0, 132.5, 133.0, 133.3, 133.5, 165.0 (C=O of Bz), 165.7 (C=O of Bz), 171.3 (COCH₂CH₂COCH₃), 172.2 (COCH₂CH₂COCH₃), 205.7

(COCH₂CH₂COCH₃), 206.36 (COCH₂CH₂COCH₃); HMBC 2D-NMR (500 MHz, CDCl₃), δ 5.37 (H-2) $\rightarrow$ 165.0 (C=O of Bz) and δ 5.66 (H-3) $\rightarrow$ 165.7 (C=O of Bz), indicate 2-OH and 3-OH were protected by Bz, respectively, and δ 5.25 (H-4) $\rightarrow$ 171.3 (COCH₂CH₂COCH₃), 4.26 (H-6) $\rightarrow$ 172.2 (COCH₂CH₂COCH₃) and 4.32 (H-6') $\rightarrow$ 172.2 (COCH₂CH₂COCH₃) indicate 4-OH and 6-OH were protected by Lev, respectively; ESI-FT-MS, calculated for C₃₆H₃₆O₁₁SNa⁺ (M + Na)⁺: 699.1871, found *m/z*: 699.1860.

2-(Trimethylsilyl)ethyl O-(2,3-di-O-benzoyl-4,6-di-O-levulinyl- β -D-glucopyranosyl)-(1 $\rightarrow$ 3)-O-(2-O-benzoyl-4,6-O-benzylidene- β -D-glucopyranosyl)-(1 $\rightarrow$ 3)-2-O-benzoyl-4,6-O-benzylidene- β -D-glucopyranoside (compound **22**)

Compound **21** (1.2 eq., 215 mg, 318 μ mol), compound **17** (1 eq., 217 mg, 262 μ mol), MS4Å (432 mg), and NIS (2.4 eq., 142 mg, 0.629 mmol) were dissolved in dry dichloromethane (5 mL), under nitrogen. After cooling this solution to -30 °C, TfOH (0.48 eq., 11 μ L, 0.126 mmol) was added and kept at -40 °C for 2 h. The reaction was terminated with 104 μ L Et₃N. Compound **22** (170 mg, 47%) was obtained according to the same procedure as applied to compound **11**, however, using ethyl acetate/toluene (1/4, v/v) as the mobile phase for the silica gel column chromatography. $[\alpha]_D^{27} +29.7$ (c 0.59, CHCl₃); ¹H NMR (500 MHz, CDCl₃): δ -0.13 (s, 1H, 3Me of TMSEt), 0.65–0.77 (m, 2H, H-2 and H-2' of TMSEt), 1.99 (s, 3H, Me of Lev), 2.07 (s, 3H, Me of Lev), 2.36–2.49 (m, 4H, COCH₂CH₂COCH₃), 2.49–2.81 (m, 4H, COCH₂CH₂COCH₃), 3.19 (t, 1H, $J_{3,4} = J_{4,5}$ 9.4 Hz, H-4^I), 3.35–3.44 (m, 2H, H-5^I and H-1 of TMSEt), 3.50–3.57 (m, 2H, H-5^{II} and H-6^{II}), 3.61 (t, 1H, $J_{5,6} = J_{\text{gem}}$ 10.2 Hz, H-6^I), 3.73 (ddd, 1H, $J_{4,5}$ 10.0 Hz, $J_{5,6}$ 2.4 Hz, $J_{5,6'}$ 4.5 Hz, H-5^{III}), 3.80 (td, 1H, J 6.0 Hz, J 10.2 Hz, 1-H' of TMSEt), 4.01 (dd, 1H, $J_{2,3}$ 4.2 Hz, $J_{3,4}$ 8.2 Hz, H-3^{II}), 4.05–4.15 (m, 4H, H-3^I, H-4^{II}, H-6^{III}, H-

6^{III}), 4.18 (dd, 1H, $J_{5,6'}$ 4.5 Hz, J_{gem} 12.2 Hz, H-6'^{III}), 4.27 (dd, 1H, $J_{5,6'}$ 4.9 Hz, J_{gem} 10.4 Hz, H-6'^I), 4.45 (d, 1H, $J_{1,2}$ 7.7 Hz, H-1^I), 4.70 (s, 1H, O₂CHPh^{II}), 4.83 (t, 1H, $J_{1,2} = J_{2,3}$ 8.2 Hz, H-2^I), 4.90 (d, 1H, $J_{1,2}$ 4.6 Hz, H-1^{II}), 5.11 (t, 1H, $J_{1,2} = J_{2,3}$ 4.4 Hz, H-2^{II}), 5.13 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1^{III}), 5.26 (t, 1H, $J_{3,4} = J_{4,5}$ 9.8 Hz, H-4^{III}), 5.41 (s, 1H, O₂CHPh^I) 5.42 (dd, 1H, $J_{1,2}$ 7.9 Hz, $J_{2,3}$ 9.7 Hz, H-2^{III}), 5.55 (t, 1H, $J_{2,3} = J_{3,4}$ 9.6 Hz, H-3^{III}), 7.12–8.06 (m, 30H, 6Ph); ¹³C NMR (126 MHz, CDCl₃): δ –1.5 (3Me of TMSEt), 18.0 (C-2 of TMSEt), 27.8 (COCH₂CH₂COCH₃), 27.9 (COCH₂CH₂COCH₃), 29.5 (COCH₂CH₂COCH₃), 29.8 (COCH₂CH₂COCH₃), 37.8 (COCH₂CH₂COCH₃), 37.9 (COCH₂CH₂COCH₃), 62.0 (C-6^{III}), 65.4 (C-5^{II}), 66.4 (C-5^I), 67.5 (C-1 of TMSEt), 68.7 (C-6^I), 68.8 (C-6^{II} and C-4^{III}), 71.6 (C-5^{III}), 71.7 (C-2^{III}), 72.6 (C-2^{II}), 73.3 (C-3^{III}), 74.4 (C-2^I), 74.7 (C-4^{II}), 77.0 (C-3^{II}), 77.8 (C-3^I), 78.9 (C-4^I), 98.0 (C-1^{II}), 98.3 (C-1^{III}), 100.5 (O₂CHPh^{II}), 100.8 (C-1^I), 101.8 (O₂CHPh^I), 126.1, 126.3, 128.0, 128.2, 128.32, 128.38, 128.41, 128.6, 128.8, 129.02, 129.04, 129.1, 129.2, 129.3, 129.67, 129.70, 129.74, 129.8, 133.00, 133.04, 133.07, 133.2, 133.3, 137.2, 137.3, 164.5 (C=O of Bz), 164.7 (C=O of Bz), 165.0 (C=O of Bz), 165.7 (C=O of Bz), 171.3 (COCH₂CH₂COCH₃), 172.3 (COCH₂CH₂COCH₃), 205.8 (COCH₂CH₂COCH₃), 206.4 (COCH₂CH₂COCH₃); HMBC 2D-NMR (500 MHz, CDCl₃), δ 5.13 (H-1^{III}) → 77.0 (C-3^{II}), indicates 1→3 linkage; ESI-FT-MS, calculated for C₇₅H₈₀O₂₄SiNa⁺ (M + Na)⁺: 1415.4701, found m/z : 1415.4669.

*2-(Trimethylsilyl)ethyl O-(2,3-di-O-benzoyl- β -D-glucopyranosyl)-(1→3)-O-(2-O-benzoyl-4,6-O-benzylidene- β -D-glucopyranosyl)-(1→3)-2-O-benzoyl-4,6-O-benzylidene- β -D-glucopyranoside (compound **23**)*

Compound **22** (70 mg, 50.2 μ mol) in pyridine (1 mL) was mixed with 1 M hydrazine monohydrate in pyridine/acetic acid (3/2, v/v) (1 mL). This solution was

stirred at room temperature for 1 h and concentrated in vacuo. After co-evaporation with toluene, silica gel column chromatography using ethyl acetate/toluene (1/9, v/v) as the mobile phase was performed to purify compound **23** (47 mg, 78%). $[\alpha]_D^{27} +29.2$ (c 0.57, CHCl₃); ¹H NMR (500 MHz, CDCl₃): δ -0.14 (s, 9H, 3Me of TMSEt), 0.65–0.77 (m, 2H, H-2 and H-2' of TMSEt), 2.06–2.17 (m, 1H, 6-OH), 3.06 (d, 1H, $J_{4,OH}$ 4.1 Hz, 4-OH), 3.33 (t, 1H, $J_{3,4} = J_{4,5}$ 9.3 Hz, H-4^I), 3.36–3.43 (m, 2H, H-5^I and H-1 of TMSEt), 3.46 (ddd, 1H, $J_{5,6}$ 9.4 Hz, J 5.1 Hz, J 3.4 Hz, H-5^{III}), 3.50 (dd, 1H, $J_{5,6}$ 9.6 Hz, $J_{5,6'}$ 4.6 Hz, H-5^{II}), 3.57 (t, 1H, $J_{5,6} = J_{gem}$ 10.1 Hz, H-6^{II}), 3.65 (t, 2H, $J_{5,6} = J_{gem}$ 10.1 Hz, H-6^I and H-6^{III}), 3.76–3.86 (m, 3H, H-4^{III}, H-6^{III} and H-1' of TMSEt), 3.96–4.03 (m, 2H, H-3^{II} and H-4^{II}), 4.09 (t, 1H, $J_{2,3} = J_{3,4}$ 8.9 Hz, H-3^I), 4.15 (dd, 1H, $J_{5,6'}$ 4.6 Hz, J_{gem} 10.1 Hz, H-6^{III}), 4.28 (dd, 1H, $J_{5,6'}$ 4.7 Hz, J_{gem} 10.5 Hz, H-6^I), 4.47 (d, 1H, $J_{1,2}$ 7.7 Hz, H-1^I), 4.86 (d, 1H, $J_{1,2}$ 5.4 Hz, H-1^{II}), 4.89 (s, 1H, O₂CHPh^{II}), 4.92 (dd, 1H, $J_{1,2}$ 7.9 Hz, $J_{2,3}$ 8.6 Hz, H-2^I), 5.00 (d, 1H, $J_{1,2}$ 7.9 Hz, H-1^{III}), 5.16 (t, 1H, $J_{1,2} = J_{2,3}$ 5.3 Hz, H-2^{II}), 5.21 (t, 1H, $J_{2,3} = J_{3,4}$ 9.3 Hz, H-3^{III}), 5.37 (dd, 1H, $J_{1,2}$ 7.9 Hz, $J_{2,3}$ 9.6 Hz, H-2^{III}), 5.43 (s, 1H, O₂CHPh^I), 7.10–7.98 (m, 30H, 6Ph); ¹³C NMR (126 MHz, CDCl₃): δ -1.6 (3Me of TMSEt), 18.0 (C-2 of TMSEt), 62.0 (C-6^{III}), 65.7 (C-5^{II}), 66.4 (C-5^I), 67.5 (C-1 of TMSEt), 68.7 (C-6^I), 68.8 (C-6^{II}), 70.1 (C-4^{III}), 71.3 (C-2^{III}), 72.9 (C-2^{II}), 74.2 (C-2^I), 75.65 (C-3^I), 75.72 (C-5^{III}), 77.3 (C-3^{II}), 77.8 (C-3^{III}), 77.9 (C-4^{II}), 78.9 (C-4^I), 98.4 (C-1^{III}), 98.8 (C-1^{II}), 100.8 (C-1^I), 101.0 (O₂CHPh^{II}), 101.7 (O₂CHPh^I), 126.0, 126.3, 128.1, 128.2, 128.3, 128.4, 128.5, 128.8, 129.0, 129.1, 129.3, 129.67, 129.69, 239.74, 129.76, 129.84, 130.0, 132.8, 132.99, 133.03, 133.2, 133.6, 137.2, 164.56 (C=O of Bz), 164.60 (C=O of Bz), 165.3 (C=O of Bz), 167.7 (C=O of Bz); ESI-FT-MS, calculated for C₆₅H₆₇O₂₀Si (M – H)[–]: 1195.4000, found m/z : 1195.4029.

2-(Trimethylsilyl)ethyl O-(2-O-benzoyl-4,6-O-benzylidene-3-O-(tert-butyldiphenylsilyl)-

β-D-glucopyranosyl)-(1→6)-O-(2,3-di-O-benzoyl-β-D-glucopyranosyl)-(1→3)-O-(2-O-benzoyl-4,6-O-benzylidene-β-D-glucopyranosyl)-(1→3)-2-O-benzoyl-4,6-O-benzylidene-β-D-glucopyranoside (compound **1**)

Compound **14** (1.2 eq., 27.8 mg, 39.5 μmol), compound **23** (1 eq., 39.4 mg, 32.9 μmol), MS4Å (67.2 mg), and NIS (2.4 eq., 17.8 mg, 79.1 μmol) were dissolved in dry dichloromethane (2 mL), under nitrogen. After cooling this solution to −30 °C, TfOH (0.48 eq., 1.4 μL, 15.8 μmol) was added, and this solution was kept at −30 °C for 2 h. The reaction was terminated with 13.2 μL Et₃N. Compound **1** (12.5 mg, 23%) was obtained according to the same procedure as applied to compound **11**, however, using ethyl acetate/toluene (1/18, v/v) as the mobile phase in the silica gel column chromatography. $[\alpha]_D^{27}$ −6.05 (c 0.61, CHCl₃); ¹H NMR (500 MHz, CDCl₃): δ −0.14 (s, 9H, 3Me of TMSEt), 0.67–0.74 (m, 2H, H-2 and H-2' of TMSEt), 0.77 (s, 9H, 3Me of TBDPS), 2.82 (d, 1H, *J*_{4,OH} 3.3 Hz, 4-OH), 3.22 (td, 1H, *J*_{4,5} = *J*_{5,6} 9.7 Hz, *J*_{5,6'} 4.9 Hz, H-5^{IV}), 3.32–3.46 (m, 5H, H-4^I, H-5^I, H-5^{II}, H-5^{III} and H-1 of TMSEt), 3.59–3.70 (m, 4H, H-6^I, H-6^{II}, H-6^{III} and H-6^{IV}), 3.75–3.83 (m, 2H, H-4^{III} and H-1' of TMSEt), 3.88–3.95 (m, 2H, H-3^{II} and H-6^{III}), 4.00 (t, 1H, *J*_{2,3} = *J*_{3,4} 8.9 Hz, H-3^{IV}), 4.01 (t, 1H, *J*_{2,3} = *J*_{3,4} 9.2 Hz, H-3^{II}), 4.09 (t, 1H, *J*_{2,3} = *J*_{3,4} 8.9 Hz, H-3^I), 4.11 (dd, 1H, *J* 4.8 Hz, *J* 10.1 Hz, H-6^{III}), 4.21 (dd, 1H, *J*_{5,6} 4.9 Hz, *J*_{gem} 10.3 Hz, H-6^{IV}), 4.28 (dd, 1H, *J* 5.2 Hz, *J* 10.8 Hz, H-6^I), 4.37 (d, 1H, *J*_{1,2} 7.9 Hz, H-1^{IV}), 4.47 (d, 1H, *J*_{1,2} 7.7 Hz, H-1^I), 4.85 (d, 1H, *J*_{1,2} 5.4 Hz, H-1^{II}), 4.86 (s, 1H, O₂CHPh^{II}), 4.89 (d, 1H, *J*_{1,2} 7.8 Hz, H-1^{III}), 4.91 (dd, 1H, *J*_{1,2} 7.9 Hz, *J*_{2,3} 8.4 Hz, H-2^I), 5.04 (t, 1H, *J*_{1,2} = *J*_{2,3} 5.2 Hz, H-2^{II}), 5.14 (s, 1H, O₂CHPh^{IV}), 5.15 (t, 1H, *J*_{2,3} = *J*_{3,4} 9.3 Hz, H-3^{III}), 5.22 (dd, 1H, *J*_{1,2} 7.9 Hz, *J*_{2,3} 9.6 Hz, H-2^{III}), 5.33 (dd, 1H, *J*_{1,2} 7.9 Hz, *J*_{2,3} 8.6 Hz, H-2^{IV}), 5.45 (s, 1H, O₂CHPh^I), 6.82–7.95 (m, 50H, 10Ph); ¹³C NMR (126 MHz, CDCl₃): δ −1.6 (3Me of TMSEt), 18.0 (C-2 of TMSEt), 19.3 (C of TBDPS), 26.7 (3Me of TBDPS), 65.6 (C-5^{II}), 66.2 (C-5^{IV}), 66.4 (C-

1 5^I), 67.5 (C-1 of TMSEt), 68.4 (C-6^{IV}), 68.6 (C-6^{II}), 68.7 (C-6^I), 69.5 (C-6^{III}), 70.3 (C-
 2 4^{III}), 71.4 (C-2^{III}), 72.7 (C-2^{II}), 73.5 (C-3^{IV}), 73.8 (C-5^{III}), 74.2 (C-2^I), 75.0 (C-2^{IV}), 75.3
 3 (C-3^I), 76.7 (C-3^{III}), 77.4 (C-3^{II}), 78.1 (C-4^{II}), 79.0 (C-4^I), 81.1 (C-4^{IV}), 98.46 (C-1^{II}),
 4 98.51 (C-1^{III}), 100.5 (O₂C $\overline{\text{C}}$ HPh^{II}), 100.8 (C-1^I), 101.5 (O₂C $\overline{\text{C}}$ HPh^{IV}), 101.7 (O₂CHPh^I),
 5 102.0 (C-1^{IV}), 126.25, 126.27, 127.2, 127.3, 127.8, 127.96, 128.06, 128.19, 128.24,
 6 128.31, 128.34, 128.6, 128.7, 128.87, 128.91, 129.0, 129.16, 129.23, 129.32, 129.36,
 7 129.37, 129.57, 129.65, 129.70, 129.87, 129.91, 129.95, 131.0, 132.2, 132.3, 132.7,
 8 132.88, 132.94, 133.03, 133.2, 133.9, 135.7, 136.1, 136.7, 137.1, 137.4, 164.3 (C=O of
 9 Bz), 164.6 (C=O of Bz), 164.9 (C=O of Bz), 165.4 (C=O of Bz), 166.9 (C=O of Bz);
 10 HMBC 2D-NMR (500 MHz, CDCl₃), δ 4.37 (H-1^{IV}) $\rightarrow$ 69.5 (C-6^{III}), indicates 1 $\rightarrow$ 6
 11 linkage; ESI-FT-MS, calculated for C₁₀₁H₁₀₄O₂₆Si₂Na⁺ (M + Na)⁺: 1811.6247, found
 12 *m/z*: 1811.6232.

Conflicts of interest

The authors declare that there is no conflict of interest.

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Author contribution

T.O. designed and conducted the experiments, and wrote the paper. W.S. designed the experiments and contributed to the paper. S.K. designed and conducted the experiments, and contributed to the paper. H.M. designed the experiments and contributed to the paper.

Data availability

1 Sharing of the study data will be considered on request.

2

3 **Supplementary Material**

4 Supplementary material is available at *Bioscience, Biotechnology, and*

5 *Biochemistry* online.

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10

Graphical abstract caption:

Three monomeric units were chemically synthesized for the formation of β 1-3/1-6 glucan and synthesis of a tetrasaccharide derivative.

Figure captions:

Fig. 1. Tested monomeric acceptors for forming β 1-3 linkage

2: 2-(trimethylsilyl)ethyl 2,4,6-tri-*O*-benzoyl- β -D-glucopyranoside. **3:** 1,2:5,6-di-*O*-isopropylidene- α -D-glucofuranose. **4:** 2-(trimethylsilyl)ethyl 2-*O*-benzoyl-4,6-*O*-benzylidene- β -D-glucopyranoside.

Fig. 2. The yield of regioselective levulinylation under each reaction conditions

The yield was calculated by analytical HPLC. Reagents and conditions: (a) levulinic acid anhydride, DMAP, DMF, 0 °C, 3.5 h. (b) levulinic acid anhydride, pyridine, 0 °C, 15.5 h. (c) levulinic acid anhydride, pyridine, DMF, 0 °C for 21.5 h and 27 °C for 74.5 h.

Scheme 1. Synthesis of monomeric units modified with levulinyl and benzoyl groups

Reagents and conditions: (a) levulinic acid anhydride, pyridine, DMF, 23 °C for 109.5 h. (b) BzCl, pyridine, room temperature, 1 h, 14% (2 steps). (c) TMSEtOH, NIS, TfOH, CH₂Cl₂, CH₃CN, -40 °C, 2 h, 44%. (d) hydrazine monohydrate, acetic acid, pyridine, room temperature, 5 min, 87%.

Scheme 2. Synthesis of monomeric units modified with benzylidene and silyl groups

Reagents and conditions: (a) TBDPS-Cl, imidazole, DMF, 4 °C, 8 days, quantitatively.
(b) BzCl, pyridine, 60 °C, 7 days, 51%. (c) TMSEtOH, NIS, TfOH, CH₂Cl₂, CH₃CN,
–40 °C, 1 h, 59%. (d) TBAF in THF, acetic acid, DMF, room temperature, 5 days, 73%.

Scheme 3. Glycosylation reactions occurring with three acceptor units

Reagents and conditions: (a) TfOH, NIS, CH₂Cl₂, –30 °C, 2 h. (b) TBAF in THF, acetic
acid, DMF, room temperature, 14 days, 66% (2 steps). (c) TfOH, NIS, CH₂Cl₂, –30 °C,
40 h, 20%. (d) TfOH, NIS, CH₂Cl₂, CH₃CN, –30 °C, 1 h, 50%.

Scheme 4. Conversion of compound 3 in reaction d in Scheme 3

20: 1,2:3,5-di-*O*-isopropylidene- α -D-glucofuranose. This conversion occurred during
the synthesis of compound **19**.

Scheme 5. Synthesis of monomeric unit for forming β 1-6 linkage

(a) 1) BzCl, pyridine, room temperature, 2 h, 2) 80% (v/v) acetic acid aqueous solution,
60 °C, 3 h, 3) levulinic acid anhydride, DMAP, pyridine, CH₂Cl₂, room temperature, 2
h, 79% (3 steps).

Scheme 6. Synthesis of compound 1 by using three monomeric units

Reagents and conditions: (a) TfOH, NIS, CH₂Cl₂, –40 °C, 2 h, 47%. (b) hydrazine
monohydrate, acetic acid, pyridine, room temperature, 1 h, 78%. (c) TfOH, NIS,
CH₂Cl₂, –30 °C, 2 h, 23%.

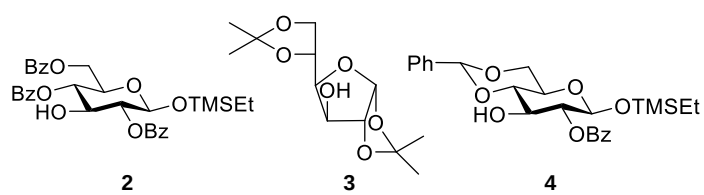


Fig. 1, Ota *et al.*

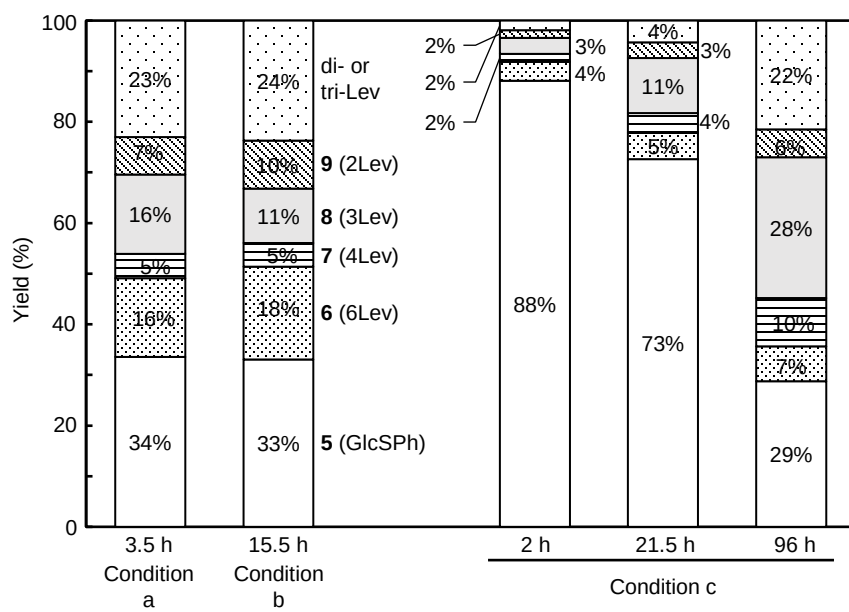
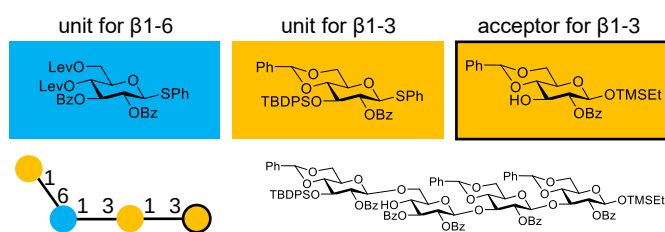
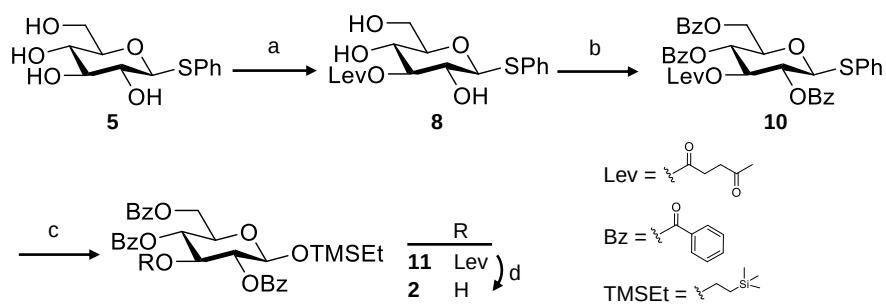


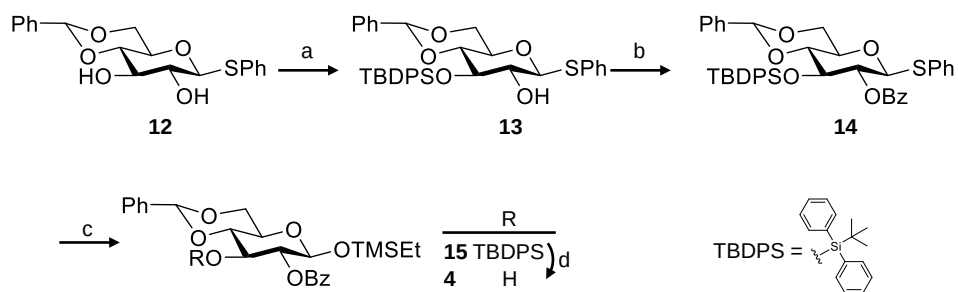
Fig. 2, Ota *et al.*



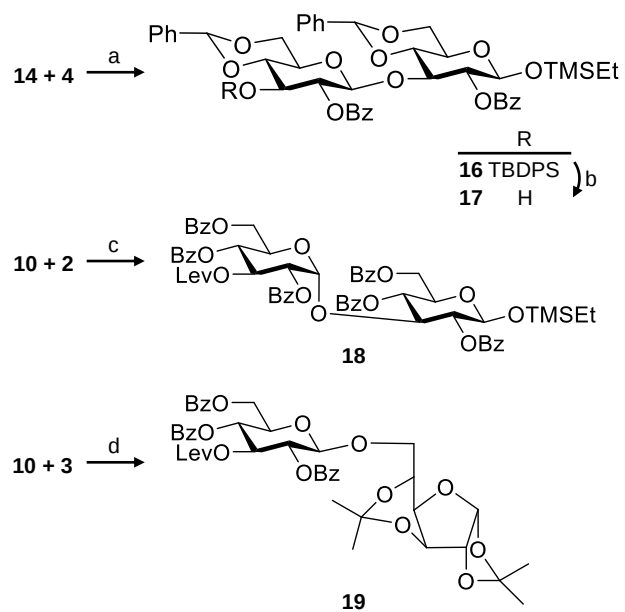
Graphical abstract, Ota *et al.*



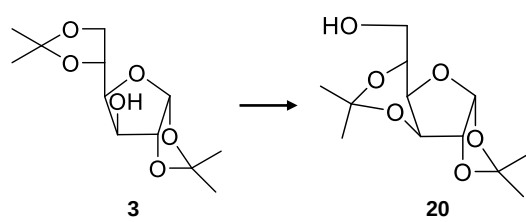
Scheme 1, Ota *et al.*



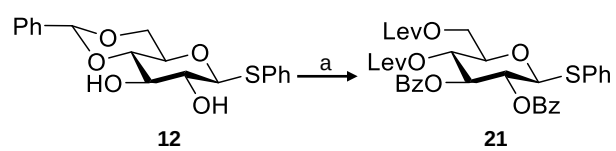
Scheme 2, Ota *et al.*



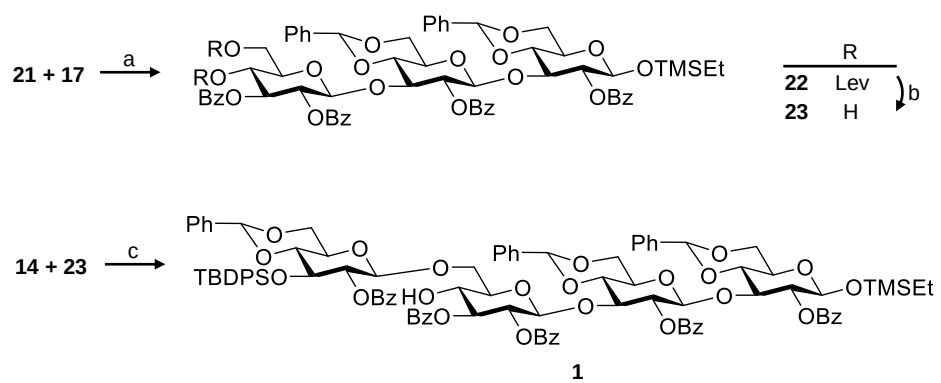
Scheme 3, Ota *et al.*



Scheme 4, Ota *et al.*



Scheme 5, Ota *et al.*



Scheme 6, Ota *et al.*