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Chemical Syntheses of Inulin and Levan Structures

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A fructofuranosyl thiglycoside donor, ethyl 6-*O*-acetyl-3-*O*-benzyl-1,4-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2-thio-β-D-fructofuranoside (**11**), designed to yield stereospecifically β-linkages and also to allow subsequent elongation in the 6- and/or 1-positions, was prepared and used in syntheses of levan and inulin structures. DMTST-promoted glycosylation between **11** (1.3 mol equiv) and methyl β-D-fructofuranoside 6-OH and 1-OH acceptors (**3** and **6**) gave stereospecifically the protected methyl levanobioside **12** and inulinobioside **17** in excellent yields (80 and 86%), respectively. Protecting group manipulations on these afforded new disaccharide 6′-OH and 1′-OH acceptors (**13** and **19**), which were coupled again with donor **11** (1.0 mol equiv) to yield methyl levanotrioside **14** and inulinotrioside **20** in high yields, 65 and 67%, respectively. These were transformed into new acceptors and also fully deprotected to afford the methyl glycosides of levanotriose and inulinotriose, all structures that have earlier not been accessible by chemical synthesis.

Introduction

Fructofurans built up from β-linked D-fructofuranoside units are common in plants and also bacteria.[1−3] Two major types are found, 2→1-linked (inulin-type) and 2→6-linked (levan-type) (Figure 1). Although there are several reports on enzymatic syntheses of part structures of these fructans,[3] chemical syntheses have never been reported, since the methodology to synthesize β-fructofuranosides in a stereospecific way has not been available. Recently, we developed a new concept for a β-directing fructofuranosyl donor (Figure 1) and used this successfully in the first stereospecific coupling to yield sucrose.[4] In this paper this concept is further investigated and utilized for the synthesis of inulin and levan structures.

Results and Discussion

Methyl β-D-fructofuranosides were chosen as model acceptors, the 6-OH acceptor **3** for levan structures and the 1-OH acceptor **6** for inulin structures, and synthesized according to Scheme 1. Methyl β-D-fructofuranoside[5] (**1**) was regioselectively tritylated (→**2**) or silylated (→**4**) at the 6-position. Benzylation of **2** and consecutive detritylation[6] yielded acceptor **3**, whereas regioselective tritylation of **4** followed by desilylation, benzylation, and detritylation afforded acceptor **6**.

To be able to synthesize levan and inulin structures, the protecting group pattern in the earlier used[4] donor (Figure 1) had to be changed, since a differentiation between positions 1 and 6 is necessary, and thus, these position require orthogonal protecting groups. Investigations had shown that the disiloxane group as a bridge is almost a necessity, considering efficiency both in the formation and function as a donor.[7] Diacyl bridges, e.g., are formed in low yields and are not stable during the glycosylation conditions. It was therefore decided to change the 6-*O*-protecting group, from a silyl group to an acetate (Scheme 2). The TBDMS group in **7**[4] was cleaved off and the resulting diol **8** regioselectively acetylated[8] in the primary position (→**9**). Detritylation (→**10**) and treatment with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (TIPS chloride) then afforded the desired donor **11**.

Earlier we found that the 6-*O*-TBDMS donor derivative was more efficient as a donor than the corresponding 6-*O*-TBDPS analogue regarding the yields.[4] Both donors were, however, stereospecific for β-fructofuranoside formation. The 6-*O*-acetyl analogue **11** also proved to be a most efficient and stereoselective donor. Coupling between acceptor **3** and donor **11** (1.3 mol equiv) yielded the levanobiose derivative **12** in 80% yield using dimethyl(methylthio)sulfonium trifluoromethanesulfonate (DMTST)[9] as promoter. In this coupling also two other commonly used thiophilic promoters, MeOTf[10] and NIS/TfOH,[11] were tried. The results (Table 1) showed that all

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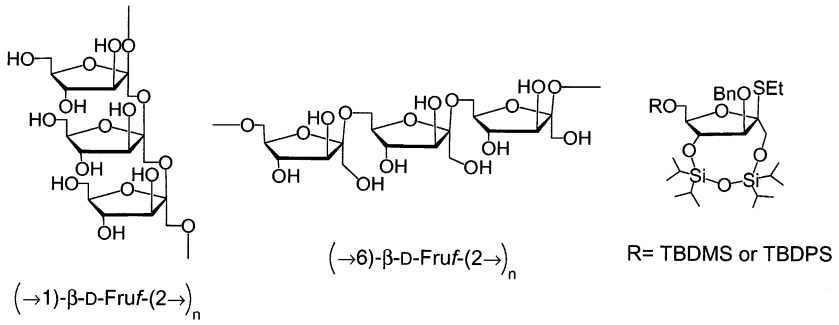
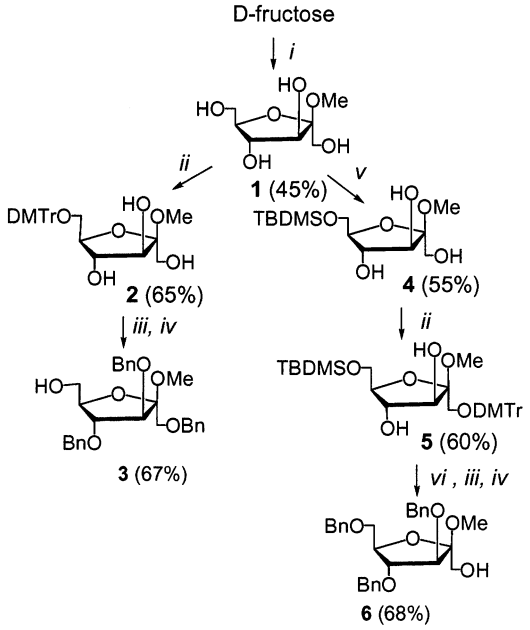


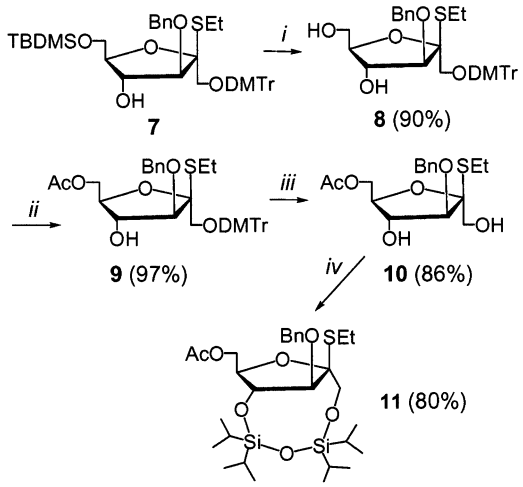
FIGURE 1. Structures of inulin and levan and the β-directing fructofuranosyl donor.

SCHEME 1. Synthesis of the Fructofuranosyl Acceptors[a]



[a] Key: (i) MeOH, H_2SO_4; (ii) DMTrCl, pyridine; (iii) BnBr, NaH, DMF; (iv) 1% TFA in CH_2Cl_2, TES; (v) TBDMSCl, pyridine; (vi) 1 M TBAF in THF.

SCHEME 2. Synthesis of the Fructofuranosyl Donor[a]



[a] Key: (i) TBAF, THF; (ii) AcCl, 2,4,6-collidine, CH_2Cl_2, −70 °C; (iii) 1% TFA in CH_2Cl_2, TES; (iv) $TIPSCl_2$, imidazole, DMF.

TABLE 1. Testing of Different Promoters in the Coupling of Acceptor 3 and Donor 11 To Give Disaccharide 12

promoter	base	solvent	temp	% yield
MeOTf	DTBMP	CH_2Cl_2	rt	75
NIS/TfOH		CH_2Cl_2	rt	63
DMTST	DTBMP	CH_2Cl_2	rt	80

three promoters gave high yields and stereospecificity, but that the first tried promoter, DMTST, was the best one.

Built into the synthetic pathway is the possibility to do an iterative repetition of these reactions to obtain levanoligomers with increasing and exact chain length. Deacetylation of compound **12** afforded a new acceptor **13**, which could be glycosylated again with donor **11** (1 mol equiv) to give the levanotrioside **14** in 65% yield (Scheme 3). Due to some hydrolysis of the donor, not all acceptor is consumed; therefore, the coupling yield can probably be raised by using an excess of the donor. Removal of the acetate in **14** would afford a new 6-OH acceptor. Complete deprotection of **14** gave the β-methyl glycoside **16** of levanotriose. As was found in the earlier paper,[4] β-fructofuranosides containing the 1,4-TIPS group show "abnormal" anomeric ^{13}C NMR chemical shifts, around 110 ppm. However, after removal of the TIPS group (e.g. with TASF[12]), they all show the characteristic shifts of β-fructofuranosides, i.e., between 103 and 105 ppm as opposed to 107−109 ppm for α-linked fructofuranosides.[13] Also the rest of the NMR spectra was in accordance with literature data on levan structures.[14] The change in chemical shift of the anomeric carbon due to the introduction of the TIPS bridge is also noticed in the formation of the thioglycoside donor. C-2 of compound **11** gives a signal at 98 ppm as compared to 94 ppm in **10**.

For the synthesis of inulin structures acceptor **6** was coupled with donor **11** (1.3 mol equiv) to yield the inulinobiose derivative **17** in 86% yield (Scheme 4). Removal of the disiloxane bridge gave the 1′,4′-diol **18**, which was tried as acceptor in an attempted regioselective glycosylation to give inulinotriose. However, the selectivity of donor **11** for the primary hydroxyl group

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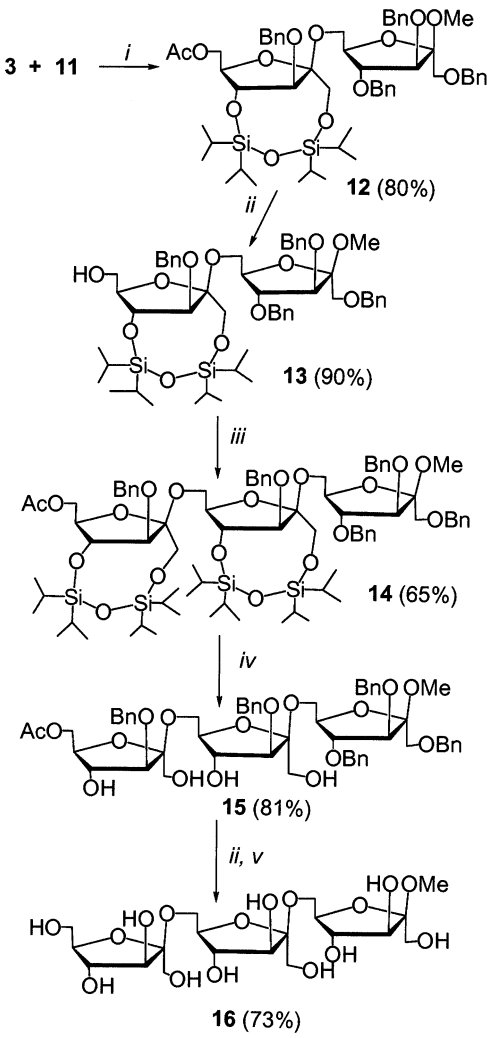
SCHEME 3. Synthesis of Levan Derivatives[a]



[a] Key: (i) DMTST, DTBMP, CH_2Cl_2, 4 Å MS; (ii) 1 M MeONa in MeOH; (iii) **11**, DMTST, DTBMP, CH_2Cl_2, 4 Å MS; (iv) TASF, DMF; (v) H_2, Pd/C, Amberlite IR-45 (OH^-) resin, MeOH−EtOAc.

was low and only a ratio of 1.3:1 between the 1′-*O*-linked and the 4′-*O*-linked trisaccharides was obtained in an inefficient glycosylation reaction (40% total yield). Hence, the 1′-OH acceptor **19** was prepared by dimethoxytritylation, benzoylation, and detritylation of diol **18** in an overall yield of 60%. DMTST-promoted glycosylation of **19** with donor **11** (1 mol equiv) then gave the inulinotrioside **20** in good yield (67%), proving a working iterative approach also to inulin oligomers. Removal of the protecting groups gave **22**, which also had NMR data in accordance with the literature.[15,16]

In conclusion, thiofructofuranoside donors containing the 1,4-TIPS group seem to be a general solution to the stereospecific formation of β-fructofuranoside-containing oligosaccharides, making possible the formation of structures earlier not available by chemical synthesis.

Experimental Section

General Methods. CH_2Cl_2 and pyridine were distilled from calcium hydride. Thin-layer chromatography was performed using silica gel 60 F-254 (Merck) plates with detection by UV and charring with 8% H_2SO_4 or AMC [ammonium molybdate−cerium(IV) sulfate−10% sulfuric acid 100 g:2 g:2 L]. Organic solutions were dried over $MgSO_4$ before concentrations, which were performed under reduced pressure at 40°C (water bath). Silica gel (Si 60A, 35−70 μm, Amicon) was used for column chromatography. NMR spectra were recorded in $CDCl_3$ at 25 °C (internal standard Me_4Si, $\delta = 0.00$) unless otherwise stated, using a 270 MHz or a 400 MHz instrument. MALDI-TOF spectra were recorded using 2′,4′,6′-trihydroxyacetophenone monohydrate (THAP) as matrix.

Methyl 1,3,4-Tri-*O*-benzyl-β-D-fructofuranoside (3). A solution of 4,4′-dimethoxytrityl chloride (1.02 g, 4.47 mmol) in pyridine (6 mL) was added, under Ar, to a cooled (0 °C) solution of methyl β-D-fructofuranoside[5] (**1**,789 mg, 4.06 mmol) in pyridine (15 mL). The mixture was stirred for 24 h at 6 °C. MeOH (2 mL) was then added and the mixture concentrated. Column chromatography ($CHCl_3$−MeOH 14:1 + 1% Et_3N) of the residue afforded methyl 6-*O*-(4,4′-dimethoxytriphenyl)-methyl-β-D-fructofuranoside (**2**) (1.35 g, 2.64 mmol, 65%). ^{13}C NMR: δ 49.2, 55.2, 61.1, 64.2, 79.9, 78.8, 80.7, 86.0, 103.3, 113.1−158.4. Compound **2** (0.4 g, 0.78 mmol) in DMF (3 mL) was added dropwise, under N_2, to a suspension of NaH−60% oil dispersion (0.37 g, 9.25 mmol) in DMF (0.5 mL). The mixture was stirred for 1 h and benzyl bromide (1 mL, 8.44 mmol) was then added. After stirring for 2 h, the reaction was quenched by the addition of MeOH (3 mL). The mixture was diluted with toluene (40 mL), washed twice with water (15 mL), dried, concentrated, and dried in a vacuum overnight. Triethylsilane (0.124 mL, 0.78 mmol), followed by TFA (15 mL, 1% solution in CH_2Cl_2), was added to the concentrate. The reaction mixture was stirred for 1 h and then diluted with CH_2Cl_2 (30 mL), washed with aqueous saturated $NaHCO_3$ and water, dried, and concentrated. The crude product was subjected to flash chromatography (toluene−EtOAc 1.5:1) to yield **3** (0.24 g, 0.52 mmol, 67%). $[\alpha]_D$ −8 (*c* 1.06, $CHCl_3$). NMR ($CDCl_3$): ^1H, δ 3.37 (s, 3H), 3.57 (d, 2H), 3.60−3.76 (m, 2H), 3.96 (m, 1H), 4.15 (t, 1H), 4.31 (d, 1H, $J = 6.87$ Hz), 4.46 (d, 1H, $J = 11.81$ Hz), 4.52−4.63 (m, 4H), 4.71 (d, 1H, $J = 11.81$ Hz); ^{13}C, δ 50.12, 64.13, 70.28, 72.86, 73.00, 73.87, 80.87, 82.99, 84.98, 104.50, 127.96−138.36. Anal. Calcd for $C_{28}H_{32}O_6$: C, 72.39; H, 6.94. Found: C, 72.21; H, 7.03.

Methyl 3,4,6-Tri-*O*-benzyl-β-D-fructofuranoside (6). A solution of *tert*-butyldimethylsilyl chloride (93 mg, 0.62 mmol) in pyridine (0.7 mL) was added, at −10 °C and under Ar, to **1** (100 mg, 0.515 mmol) in pyridine (1 mL). The reaction was allowed to attain room temperature and stirring was continued for an additional 3 h. MeOH (2 mL) was added and the reaction mixture was concentrated. Column chromatography ($CHCl_3$−MeOH 7:1) of the residue gave methyl 6-*O*-*tert*-butyldimethylsilyl-β-D-fructofuranoside (**4**) (86 mg, 0.28 mmol, 55%). NMR: ^1H, δ 0.09 (s, 6H), 0.91 (s, 9H), 3.31 (s, 3H), 3.61 (m, 5H), 4.01 (m, 2H); ^{13}C, δ -5.37, −5.32, 18.44, 25.96, 49.10, 61.24, 64.17, 76.23, 78.75, 81.78, 103.39. Compound **4** (77 mg, 0.25 mmol) was dissolved in pyridine (0.5 mL) and 4,4′-dimethoxytrityl chloride (93 mg, 0.27 mmol) in pyridine (0.5 mL) was added. The reaction mixture was stirred overnight and then concentrated and the residue was applied to a silica gel column. Elution (toluene−EtOAc 4:1 + 1% Et_3N) gave methyl 6-*O*-*tert*-butyldimethylsilyl-1-*O*-(4,4′-dimethoxytriphenyl)methyl-β-D-fructofuranoside (**5**) (94 mg, 0.15 mmol, 60%). NMR:^1H, δ 0.09 (d, 6H), 0.91 (s, 9H), 3.18 (s, 3H), 3.19 (m, 2H), 3.7 (s, 6H), 3.82 (m, 3H), 4.12 (m, 2); ^{13}C, δ -5.41, −5.37, 18.33, 25.90, 49.01, 55.13, 62.71, 64.16, 77.37, 80.42, 81.49, 86.18, 102.99, 113.06−135.72. To a solution of compound **5** (145 mg, 0.23 mmol) in THF (2 mL) was added tetrabutylammonium fluoride (TBAF) (0.46 mL, 1 M in THF). The mixture was stirred for 1 h and then diluted with CH_2Cl_2 (20 mL), washed with brine, dried, and concentrated. The product was purified by flash chromatography ($CHCl_3$−MeOH 9:1 + 1% Et_3N) and dried

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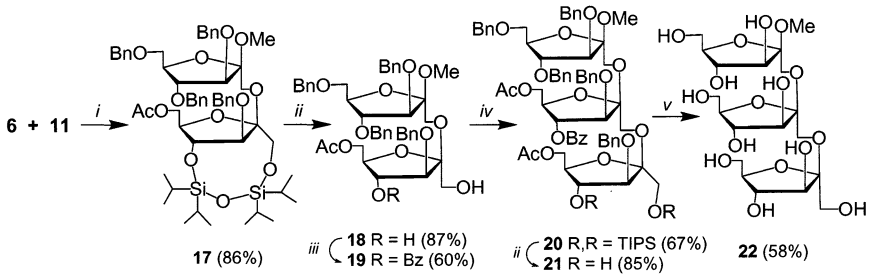
SCHEME 4. Synthesis of Inulin Derivatives[a]



[a] Key: (i) DMTST, DTBMP, CH_2Cl_2, 4 Å MS; (ii) TASF, DMF; (iii) (a) DMTrCl, pyridine, (b) BzCl, pyridine−CH_2Cl_2 (1:1), 4-DMAP, (c) 1%TFA in CH_2Cl_2, Et_3SiH, CH_2Cl_2; (iv) **11**, DMTST, DTBMP, CH_2Cl_2, 4 Å MS; (v) (a) TBAF, THF, (b) NaOMe, MeOH:CH_2Cl_2, (c) H_2, Pd/C, Amberlite IR-45 (OH^-) resin, MeOH−EtOAc−H_2O.

overnight on the oil pump. The residue was dissolved in DMF (1 mL) and this solution was added to a suspension of sodium NaH−60% oil dispersion (111 mg, 2.77 mmol) in DMF (3 mL). After 1 h, benzyl bromide (0.35 mL, 2.08 mmol) was added and the stirring was continued for an additional 1 h. MeOH (2 mL) was added dropwise followed by addition of toluene (40 mL). The reaction mixture was washed with water (15 mL), dried, concentrated, and dried under reduced pressure. Triethylsilane (37 μL, 0.23 mmol), followed by TFA (10 mL, 1% solution in CH_2Cl_2), was added to the concentrate. After stirring for 1 h, CH_2Cl_2 (30 mL) was added and the mixture was washed with aqueous saturated $NaHCO_3$, water, dried, and concentrated. Flash chromatography (toluene−EtOAc 3:1) yielded **6** (47 mg, 0.102 mmol, 68%). $[\alpha]_D$ +6 (*c* 0.43, $CHCl_3$). NMR ($CDCl_3$): ^{1}H, δ 3.30 (s, 3H), 3.53 (m, 3H), 3.69 (d, 1H, J = 11.72 Hz), 4.06 (m, 1H), 4.13 (t, 1H), 4.26 (d, 1H, J = 6.96 Hz), 4.50 (m, 4H), 4.65 (d, 1H, J = 11.72 Hz), 4.76 (d, 1H, J = 11.71 Hz); ^{13}C, δ 49.61, 62.65, 71.33, 72.55, 72.98, 73.41, 79.69, 83.71, 84.33, 104.38, 127.53−137.94. Anal. Calcd for $C_{28}H_{32}O_6$: C, 72.39; H, 6.94. Found: C, 72.25; H, 7.05.

Ethyl 6-*O*-Acetyl-3-*O*-benzyl-1,4-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2-thio-*β*-D-fructofuranoside (11). To a solution of **7**[4] (351 mg, 0.47 mmol) in THF (2 mL) was added TBAF (0.7 mL, 1M in THF). The reaction mixture was stirred for 30 min and then diluted with CH_2Cl_2 (20 mL), washed with brine, dried, and concentrated. Column chromatography of the residue (toluene−EtOAc 1:1) gave ethyl 3-*O*-benzyl-1-*O*-(4,4'-dimethoxytriphenyl)methyl-2-thio-*β*-D-fructofuranoside (**8**, 263 mg, 0.43 mmol, 90%). NMR: ^{1}H, δ 1.12 (t, 3H), 2.42 (m, 2H), 3.24 (d, 1H, J = 10.25 Hz), 3.62 (d, 1H, J = 10.26 Hz), 3.76 (s, 6H), 3.79 (m, 3H), 4.11 (m, 1H), 4.34 (d, 1H, J = 11.72 Hz), 4.48 (m, 1H), 4.56 (d, 1H, J = 11.9 Hz), 6.76−7.46 (m, 18H); ^{13}C, δ 14.89, 21.20, 55.20, 62.44, 66.61, 72.83, 75.36, 82.63, 85.83, 86.43, 93.98, 113.17−130.15. Acetyl chloride (23 μL, 0.32 mmol) in CH_2Cl_2 (0.1 mL) was added dropwise, at −70 °C, to a solution of **8** (183 mg, 0.29 mmol) and 2,4,6-collidine (0.19 mL, 1.45 mmol) in CH_2Cl_2 (5 mL). The reaction was stirred for 2 h and then quenched with MeOH (2 mL) and concentrated. The residue was applied to a silica gel column and eluted with (toluene−EtOAc 4:1) to give ethyl 6-*O*-acetyl-3-*O*-benzyl-1-*O*-(4,4'-dimethoxytriphenyl)methyl-2-thio-*β*-D-fructofuranoside (**9**) (187 mg, 0.28 mmol, 97%). NMR: ^{1}H, δ 1.11 (t, 3H), 2.09 (s, 3H), 2.42 (m, 2H), 3.20 (d, 1H, J = 10.17 Hz), 3.60 (d, 1H, J = 10.17 Hz), 3.74 (s, 6H), 4.16 (m, 1H), 4.33 (m, 4H), 4.34 (d, 1H, J = 11.81 Hz), 4.53 (d, 1H, J = 11.81 Hz), 6.78−7.47 (m, 13H); ^{13}C, δ 14.76, 20.87, 21.25, 55.16, 64.19, 66.93, 72.83, 76.14, 79.92, 85.28, 86.36, 93.47, 113.14−158.49, 171.17. TFA (2 mL, 1% solution in CH_2Cl_2) was added to a mixture of compound **9** (136 mg, 0.20 mmol) and triethylsilane (38 μL, 0.24 mmol). The reaction mixture was stirred for 30 min and then diluted with CH_2Cl_2 (25 mL), washed with aqueous saturated $NaHCO_3$ and water, dried, and concentrated. Chromatography (toluene−EtOAc 1:1) of the residue gave ethyl 6-*O*-acetyl-3-*O*-benzyl-2-thio-*β*-D-fructofuranoside (**10**) (62 mg, 0.173 mmol, 86%). NMR: ^{1}H, δ 1.2 (t, 3H), 2.07 (s, 3H), 2.5 (m, 3H), 3.60 (d, 1H, J = 12.09 Hz), 3.77 (d, 1H, J = 12.09 Hz), 4.02 (m, 1H), 4.27−4.46 (m, 4H), 4.62 (d, 1H, J = 11.81 Hz), 4.78 (d, 1H, J = 11.82 Hz), 7.28−7.39 (m, 5H); ^{13}C, δ 14.96, 20.78, 20.88, 64.75, 65.34, 73.15, 76.64, 80.57, 85.19, 94.31, 127.81−137.82, 171.25. 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane−$TIPSCl_2$ (66 μL in 0.1 mL DMF) was added, at −30 °C and under argon, to a solution of compound **10** (58 mg, 0.16 mmol) and imidazole (44 mg, 0.65 mmol) in DMF (2 mL). The cooling bath was removed after 30 min and the mixture was allowed to attain room temperature and to stir for 6 h. MeOH (1 mL) was then added and the mixture concentrated and coconcentrated three times with toluene (2 mL). The residue was applied to a silica gel column and eluted (toluene−EtOAc 4:1) to give **11** (76 mg, 0.13 mmol, 81%). $[\alpha]_D$ −4 (*c* 0.81, $CHCl_3$). NMR: ^{1}H, δ 0.89−1.03 (m, 28H), 1.23 (t, 3H), 2.06 (s, 3H), 2.72 (q, 2H), 3.90 (d, 1H, J = 11.72 Hz), 4.16 (d, 1H, J = 11.72 Hz), 4.26−4.36 (m, 5H), 4.64 (d, 1H, J = 12.09 Hz), 4.76 (d, 1H, J = 12.08 Hz), 7.26−7.37 (m, 5H); ^{13}C, δ 12.67, 13.33, 13.61, 13.91, 14.86, 17.19, 17.23, 17.29, 17.35, 17.45, 20.88, 22.18, 64.82, 65.07, 73.15, 79.82, 85.92, 86.57, 98.14, 127.52−137.66, 170.81. Anal. Calcd for $C_{29}H_{50}O_7SSi_2$: C, 58.15; H, 8.41. Found: C, 58.15; H, 8.53.

Methyl *β*-D-Fructofuranosyl-(2→6)-*β*-D-fructofuranosyl-(2→6)-*β*-D-fructofuranoside (16). The acceptor **3** (40 mg, 67 μmol), the donor **11** (40 mg, 86 μmol) and 2,6-di-*tert*-butyl-4-methylpyridine (31 mg, 67 μmol) were dissolved in CH_2Cl_2 (2 mL) containing crushed molecular sieves (4 Å, 0.3 g). The mixture was stirred at room temperature and under an argon atmosphere. After 10 min, dimethyl(methylthio)sulfonium triflate (DMTST) (52 mg, 0.2 mmol) was added and the stirring was continued another 30 min, after which Et_3N (0.1 mL) was added. The mixture was diluted with CH_2Cl_2, filtered through Celite, and concentrated, and the residue was purified on silica gel (toluene−EtOAc 10:1) to yield methyl 6-*O*-acetyl-3-*O*-benzyl-1,4-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-*β*-D-fructofuranosyl-(2→6)-1,3,4-tri-*O*-benzyl-*β*-D-fructofuranoside (**12**) (54 mg, 53 μmol, 80%). NMR: ^{1}H, δ 0.96−1.05 (m, 28H), 2.00 (s, 3H), 3.34 (s, 3H), 3.55 (s, 2H), 3.91−4.03 (m, 2H), 3.95 (s, 2H), 4.06−4.15 (m, 2H), 4.24−4.29 (m, 5H), 4.31(d, 1H, J = 6.96 Hz), 4.44 (d, 1H, J = 12.09 Hz), 4.53−4.67 (m, 5H), 4.71 (d, 1H, J = 12.08 Hz), 4.83 (d, 1H, J = 12.09 Hz), 7.23−7.30 (m, 20H); ^{13}C, δ 13.64, 13.23, 13.58, 13.70, 17.12, 17.14, 17.16, 17.28, 17.39, 20.82, 49.60, 64.44, 64.55, 64.76, 69.92, 72.45, 72.62, 73.15, 73.53, 79.22, 79.74, 82.65, 84.22, 84.63 (2C), 103.99, 109.59, 127.48−138.42, 170.81. Compound **12** (40 mg, 39 μmol) was dissolved in CH_2Cl_2−MeOH (1:1, 2 mL) and treated with methanolic sodium methoxide (3 drops, 1 M in MeOH). After stirring for 1 h, the reaction was diluted with CH_2Cl_2 (25 mL), washed with brine, dried, and concentrated. Purification of the residue on silica gel (toluene−EtOAc 6:1) yielded methyl 3-*O*-benzyl-1,4-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-*β*-D-fructofuranosyl-(2→6)-1,3,4-tri-*O*-benzyl-*β*-D-fructofuranoside (**13**) (34 mg, 36 μmol, 90%). NMR: ^{1}H, δ 0.8−1.05 (m, 28H), 3.35 (s, 3H), 3.52−3.58(s, 2H), 3.66−3.81 (m, 2H), 3.92−3.98 (m, 6H), 4.07 (m, 2H), 4.24−4.32 (m, 4H), 4.46 (d, 1H, J = 12.09 Hz), 4.55−4.61 (m, 4H), 4.72 (d, 1H, J = 12.09 Hz), 4.83 (d, 1H, J = 12.08

Hz); ^{13}C, δ 12.7, 13.3, 13.6, 13.8, 17.2, 17.3, 17.4, 49.8, 63.3, 63.5, 64.4, 70.4, 72.3, 72.6, 73.2, 73.6, 78.9, 79.7, 82.6, 84.1, 84.7, 87.7, 104.2, 109.2, 127.5−138.4. The acceptor **13** (32 mg, 33 μmol), the donor **11** (20 mg, 34 μmol), and 2,6-di-*tert*-butyl-4-methylpyridine (6 mg, 34 μmol) were dissolved in CH_2Cl_2 (2 mL) at room temperature and under argon atmosphere. Crushed molecular sieves (4 Å, 0.3 g) were added, and the mixture was stirred for 10 min before DMTST (35 mg, 0.136 mmol) was added. The stirring was continued for 1 h, whereafter Et_3N (0.1 mL) was added. The mixture was diluted with CH_2Cl_2, filtered through Celite, and concentrated, and the residue was purified on silica gel (toluene−EtOAc 12:1) to yield methyl 6-*O*-acetyl-3-*O*-benzyl-1,4-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-fructofuranosyl-(2→6)-3-*O*-benzyl-1,4-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-fructofuranosyl-(2→6)-1,3,4-tri-*O*-benzyl-β-D-fructofuranoside (**14**) (32 mg, 22 μmol, 65%). NMR: ^{1}H, δ 0.75−0.99 (m, 56H), 1.95 (s, 3H), 3.32 (s, 3H), 3.54 (s, 2H), 3.86−3.98 (s, 7H), 4.02−4.31 (m, 11H), 4.44 (d, 1H, J = 12.08 Hz), 4.51−4.68 (m, 7H), 4.71 (d, 1H, J = 12.09 Hz), 4.76 (d, 1H, J = 12.08 Hz), 4.86 (d, 1H, J = 12.81 Hz), 7.19−7.33 (m, 25H); ^{13}C, δ 12.6, 12.7, 13.1, 13.3, 13.6, 13.67, 13.71, 17.14, 17.17, 17.22, 17.26, 17.32, 17.39, 20.79, 49.68, 62.89, 63.31, 64.27, 65,02, 70.24, 72.52, 72.61, 72.73, 73.14, 73.56, 78.65, 79.69, 79.73, 81.99, 82.64, 84.19, 84.69, 84.85, 86.18, 103.87, 109.53, 109.79, 127.39−138.59, 170.71. Tris(dimethylamino)sulfonium difluorotrimethylsilicate (TASF) (0.123 mL, 1.3 M in DMF) was added to a solution of compound **14** (40 mg, 27 μmol) in DMF (1.5 mL). After stirring for 1 h the reaction was diluted with EtOAc (50 mL) and washed with water buffer solution (pH = 7, 30 mL). The aqueous layer was extracted with EtOAc (3 × 10 mL), and the combined organic layers were dried and concentrated, and the residue was purified by silica gel chromatography ($CHCl_3$−MeOH 18:1) to afford methyl 6-*O*-acetyl-3-*O*-benzyl-β-D-fructofuranosyl-(2→6)-3-*O*-benzyl-β-D-fructofuranosyl-(2→6)-1,3,4-tri-*O*-benzyl-β-D-fructofuranoside (**15**) (22 mg, 24 μmol, 81%. NMR: ^{1}H, δ 2.05 (s, 3H), 3.32 (s, 3H), 3.36 (d, 1H), 3.46−3.60 (m, 6H), 3.73 (m, 3H), 3.87 (m, 4H), 4.05 (m, 2H), 4.17 (m, 3H), 4.29−4.43 (d, 3H), 4.46−4.58 (m, 5H), 4.61 (d, 1H, J = 12.08 Hz), 4.67 (m, 2H), 4.75 (d, 1H, J = 11.72 Hz), 7.19−7.39 (m, 25H); ^{13}C, δ 20.80, 49.79, 61.79, 63.82, 63.84, 64.41, 65.36, 69.76, 72.12, 72.66, 72.74, 72.92, 73.56, 75.32, 75.38, 77.71, 79.21, 79.54, 84.21, 84.69, 84.85, 85.56, 104.09, 104.38, 104.62, 127.54−138.20, 171.25. Sodium methoxide (3 drops, 1 M in MeOH) was added to a solution of compound **15** (22 mg, 24 μmol) in CH_2Cl_2−MeOH 1:1 (1 mL). After stirring for 1 h, the reaction was carefully neutralized with Dowex 50 (H^+) ion-exchange resin, filtered, and concentrated. The residue was dissolved in MeOH−EtOAc (5:1, 4 mL). Amberlite resin IR-45 (OH^-) resin (30 mg) and palladium on activated carbon were added. The mixture was hydrogenolyzed at 110 psi overnight, filtered twice through Celite, and concentrated. Lyophilization of the concentrate gave methyl β-D-fructofuranosyl-(2→6)-β-D-fructofuranosyl-(2→6)-β-D-fructofuranoside (**16**) (9 mg, 17 μmol, 73%). $[\alpha]_D$ −35 (*c* 0.97, CH_3OH). NMR (CD_3OD + 1 drop D_2O): ^{13}C, δ 49.99, 61.51, 61.95, 62.25, 64.03, 64.48, 64.52, 76.53, 77.37, 77.62, 78.55, 78.61, 78.76, 81.72, 81.92, 83.07, 105.23, 105.50 (double intensity); ^{1}H, δ 3.34 (s, 3H), 3.58−3.95 (15H), 4.03 (t, J = 8 Hz, 1H), 4.04 (t, J = 8 Hz, 1H), 4.06 (t, J = 8 Hz, 1H), 4.14 (d, J = 8 Hz, 1H), 4.15 (2d, J = 8 Hz, 2H). Anal. Calcd for $C_{19}H_{34}O_{16}\cdot 3H_2O$: C, 39.9; H, 7.04. Found: C, 40,1; H, 7.18.

Methyl 6-*O*-Acetyl-3-*O*-benzyl-β-D-fructofuranosyl-(2→1)-4-*O*-benzoyl-3-*O*-benzyl-β-D-fructofuranosyl-(2→1)-3,4,6-tri-*O*-benzyl-β-D-fructofuranoside (21). The acceptor **6** (60 mg, 0.10 mmol), the donor **11** (77 mg, 0.13 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (26 mg, 0.13 mmol) were dissolved in CH_2Cl_2 (2 mL) together with crushed molecular sieves (4 Å, 0.3 g). The mixture was stirred at room temperature and under argon atmosphere for 10 min before DMTST (83 mg, 0.32 mmol) was added. The mixture was additionally stirred for 40 min, quenched by adding triethylamine (0.15 mL), diluted with CH_2Cl_2, filtered through Celite, and concentrated. The residue was purified on silica gel (toluene−EtOAc 12:1) to afford methyl 6-*O*-acetyl-3-*O*-benzyl-1,4-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-fructofuranosyl-(2→1)-3,4,6-tri-*O*-benzyl-β-D-fructofuranoside (**17**) (92 mg, 93 μmol, 86%). NMR ($CDCl_3$): ^{1}H, δ 0.76−1.10 (m, 28H), 1.19 (s, 3H), 3.38 (s, 3H), 3.52 (m, 2H), 3.83−4.32 (m, 10H), 4.49−4.59 (m, 7H), 4.67 (d, 1H, J = 12.08 Hz), 4.85 (d, 1H, J = 12.44 Hz), 4.29 (d, 1H, J = 11.71 Hz), 7.18−7.37 (m, 20H); ^{13}C, δ 12.67, 13.18, 13.59, 13.62, 17.12, 17.16, 17.19, 17.22, 17.29, 17.36, 20.81, 49.32, 62.62, 63.17, 64.95, 71.46, 72.32, 73.02, 73.12, 73.28, 78.86, 79.22, 81.99, 84.09, 85.04, 86.40, 103.94, 109.72, 127.43−138.64, 170.71. To a solution of compound **17** (82 mg, 82 μmol) in DMF (1.5 mL) was added TASF in DMF (1.3 M, 0.18 mL, 0.24 mmol). After stirring for 1 h, the reaction was diluted with EtOAc (50 mL) and washed with water buffer solution (pH = 7, 30 mL). The aqueous layer was extracted with EtOAc (3 × 10 mL), and the combined organic layers were dried and concentrated, and the residue was purified by silica gel chromatography (chloroform−MeOH 18:1) to afford methyl 6-*O*-acetyl-3-*O*-benzyl-β-D-fructofuranosyl-(2→1)-3,4,6-tri-*O*-benzyl-β-D-fructofuranoside (**18**) (57 mg, 74 μmol, 93%). NMR ($CDCl_3$): ^{1}H, δ 2.03 (s, 3H), 3.36 (s, 3H), 3.57−3.60 (m, 4H), 3.78 (s, 2H), 3.82−3.91 (m, 1H), 3.99 (m, 2H), 4.10 (m, 2H), 4.19(m, 1H), 4.26 (m, 2H), 4.51−4.61 (m, 6H), 4.75 (t, 2H), 7.20−7.32 (m, 20 H); ^{13}C, δ 20.79, 49.96, 63.09, 64.17, 64.37, 70.82, 72.44, 72.84, 72.93, 73.29, 75.72, 78.43, 78.74, 83.55, 85.32, 85.55, 103.91, 104.18, 127.61−138.30, 171.08. 4,4'-Dimethoxytrityl chloride (24 mg, 76 μmol) was added to a solution of **18** (30 mg, 40 μmol) and 4-DMAP (2−4 mg, 16 μmol) in pyridine (1.5 mL). The reaction mixture was stirred overnight at 60 °C. MeOH (3 mL) was then added, the mixture was concentrated and coevaporated with toluene, and the crude product was dried overnight at reduced pressure. The residue was dissolved in pyridine−CH_2Cl_2 (1:1, 4 mL) under argon. The solution was cooled (0 °C) and benzoyl chloride (20 μL, 0.173 mmol) in CH_2Cl_2 (0.1 mL) was added. The mixture was stirred for 3 h at room temperature, diluted with CH_2Cl_2 (20 mL), washed with aqueous saturated $NaHCO_3$ and water, dried, concentrated, and dried at reduced pressure. TFA (1 mL, 1% in CH_2Cl_2) was added dropwise to a solution of the residue and triethylsilane (8 μL, 50 μmol) in CH_2Cl_2 (0.8 mL). After 20 min aqueous saturated $NaHCO_3$ (2 mL) and then CH_2Cl_2 (10 mL) were added. The organic phase was separated, dried and concentrated. The residue was purified on a silica gel column (toluene−EtOAc 8:1) to afford methyl 6-*O*-acetyl-4-*O*-benzoyl-3-*O*-benzyl-β-D-fructofuranosyl-(2→1)-3,4,6-tri-*O*-benzy l-β-D-fructofuranoside (**19**) (20 mg, 24 μmol, 60% after three steps). NMR ($CDCl_3$): ^{1}H, δ 2.01 (s, 3H), 3.36 (s, 3H), 3.62−3.72 (m, 2H), 3.88−4.04 (m, 4H); 4.17−4.34 (m, 6H), 4.38−4.60 (m, 7H), 4.67 (d, 1H, J = 12.45 Hz), 4.74 (d, 1H, J = 12.45 Hz), 5.59 (m, 1H), 7.19−7.99 (m, 25H); ^{13}C, δ 20.85, 49.96, 62.90, 63.14, 65.37, 70.30, 71.84, 72.04, 72.62, 73.50, 78.69, 79.63, 81.76, 82.08, 83.78, 88.21, 103.84, 104.24, 127.58−138.26, 162.50, 170.69. The acceptor **19** (8 mg, 9.27 μmol), the donor **11** (5.5 mg, 9.27 μmol), and 2,6-di-*tert*-butyl-4-methylpyridine (1−2 mg, 9.27 μmol) were dissolved in CH_2Cl_2 (1 mL) together with crushed molecular sieves. The mixture was stirred at room temperature and under argon atmosphere. After 10 min, DMTST (20 mg, 77 μmol) was added and the mixture was additionally stirred for 40 min and then quenched by adding triethylamine (3−5 drops). The mixture was diluted with CH_2Cl_2, filtered through Celite, and concentrated, and the residue was purified on silica gel (toluene−EtOAc 8:1) to yield methyl 6-*O*-acetyl-3-*O*-benzyl-1,4-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-fructofuranosyl-(2→1)-6-*O*-acetyl-4-*O*-benzoyl-3-*O*-benzyl-β-D-fructofuranosyl-(2→1)-3,4,6-tri-*O*-benzyl-β-D-fructofuranoside (**20**) (8.7 mg, 6.21 μmol, 67%). NMR ($CDCl_3$): ^{1}H, δ 0.97−1.08 (m, 28H), 1.97 (s, 3H), 2.00 (s, 3H), 3.35 (s, 3H), 3.47 (d, 1H, J = 11.72 Hz), 3.55−3.75 (m, 3H), 3.89−4.06 (m, 9H), 4.18−4.37 (m, 5H), 4.38−4.46 (m, 3H), 4.47−4.60 (m, 5H), 4.65−4.87 (m, 4H), 5.61 (m, 1H), 7.29−

8.13 (m, 30H); ^{13}C, δ 12.92, 13.49, 13.77, 13.87, 14.40, 17.34−17.61, 20.94, 20.99, 49.79, 62.48, 63.58, 63.72, 64.19, 64.95, 71.28, 72.27, 72.59, 72.94, 73.10, 73.42, 73.72, 77.85, 79.01, 79.85, 81.99, 82.29, 82.78, 83.75, 85.34, 104.10, 104.47, 109.91, 127.80−138.30, 165.52, 170.61, 171.01. Trisaccharide **20** (8.7 mg, 6.21 μmol) was dissolved in DMF (1 mL), and TASF (25 μL, 1.3 M in DMF) was added. The reaction was stirred for 30 min, diluted with EtOAc (20 mL), and washed with water buffer solution (pH = 7, 10 mL). The aqueous layer was extracted with EtOAc (3 × 5 mL), and the combined organic layers were dried and concentrated. Column chromatography of the residue (toluene−EtOAc 1:1) gave **21** (6 mg, 5.3 μmol, 85%). $[\alpha]_D$ −48° (*c* 0.47, CH_3OH). NMR ($CDCl_3$): ^{1}H, δ 1.98 (s, 3H), 2.02 (s, 3H), 3.34 (s, 3H), 3.55 (m, 4H), 3.77−4.63 (m, 23H), 4.70−4.83 (m, 3H), 5.58 (t, 1H), 7.12−8.12 (m, 30H); ^{13}C, δ 20.66, 20.75, 49.63, 62.36, 63.81, 64.02, 64.24, 71.11, 72.42, 72.49, 72.94, 72.97, 73.25, 75.96, 76.48, 76.70, 77.47, 78.46, 78.96, 82.42, 83.58, 84.77, 85.23, 103.84, 104.24, 104.29, 127.20−138.50, 165.52, 170.61, 170.98. Anal. Calcd for $C_{65}H_{72}O_{19}$: C, 67.46; H, 6.27. Found: C, 67.22; H, 6.41.

Methyl *β*-D-Fructofuranosyl-(2→1)-*β*-D-fructofuranosyl-(2→1)-*β*-D-fructofuranoside (22). Compound **20** (15.6 mg, 13.7 μmol) was dissolved in THF (0.5 mL) and treated with TBAF (50 μL, 50 μmmol) for 1 h. CH_2Cl_2 (10 mL) was added and the organic phase was washed with water, dried, and concentrated. The product was dissolved in MeOH:CH_2Cl_2 (4:1, 1 mL) and NaOMe (1M in MeOH) was added (pH = 10−11). After stirring for 15 min, the reaction was carefully neutralized with Dowex 50 (H^+) ion-exchange resin, filtered, and concentrated to yield methyl 3-*O*-benzyl-*β*-D-fructofuranosyl-(2→1)-3-*O*-benzyl-*β*-D-fructofuranosyl-(2→1)-3,4,6-tri-*O*-benzyl-*β*-D-fructofuranoside. NMR ($CDCl_3$): ^{13}C, δ 49.72, 61.05, 62.18, 62.30, 62.93, 63.41, 65.27, 72.56, 72.69, 73.00, 73.03, 73.21, 73.74, 75.02, 79.08, 81.76, 83.12, 83.21, 84.28, 84.55, 84.82, 103.44, 103.74, 104.01. The residue was dissolved in MeOH−EtOAc−water (5:1:1, 2 mL). Amberlite resin IR-45 (OH^-) resin (30 mg) and palladium on activated carbon were added. The mixture was hydrogenolyzed at 110 psi overnight, filtered twice through Celite, concentrated, and purified using Cartridges max-clean C18, 600 mg, from Alltech. Lyophilization of the concentrate gave **22** (4 mg, 8 μmol, 58%). $[\alpha]_D$ −19 (*c* 0.25, H_2O). NMR (D_2O): ^{1}H, δ 3.34 (s, 3H), 3.62−3.89 (15H), 4.05 (t, J = 8 Hz, 1H), 4.10 (2t, J = 8 Hz, 2H), 4.19 (d, J = 8 Hz, 1H), 4.20 (d, J = 8 Hz, 1H), 4.22 (d, J = 8 Hz, 1H). MALDI-TOF MS: calcd for $C_{19}H_{34}O_{16}$ 518.2, found 541.0 [M + Na], 556.9 [M + K].

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Supporting Information Available: ^{13}C NMR spectra of compounds **2**−**5**, **9**−**18**, and **21** and ^{1}H NMR spectra of compounds **6**, **11**, **12**, **15**, **16**, **21**, and **22**. This material is available free of charge via the Internet at http://pubs.acs.org.

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