DOI: 10.1002/asia.200600210

A Method of Orthogonal Oligosaccharide Synthesis Leading to a Combinatorial Library Based on Stationary Solid-Phase Reaction

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Abstract: A new, efficient synthesis of oligosaccharides, which involves solid-phase reactions without mixing in combination with an orthogonal-glycosylation strategy, is described. Despite a great deal of biological interest, the combinatorial chemistry of oligosaccharides is an extremely difficult subject. The problems include 1) lengthy synthetic protocols required for the synthesis and 2) the variety of glycosylation conditions necessary for individual reactions. These issues were addressed and solved by using the orthogonal-coupling protocol and the application of a temperature gradient to provide appropriate conditions for individual reactions. Furthermore, we succeeded in carrying out solid-phase reactions with neither mechanical mixing nor flow. In this report, the synthesis of a series of trisaccharides, namely, α/β-L-Fuc-(1→6)-α/β-D-Gal-(1→2/3/4/6)-α/β-D-Glc-octyl, is reported to demonstrate the eligibility of the synthetic method in combinatorial chemistry.

Keywords: anomers · combinatorial chemistry · oligosaccharides · orthogonal glycosylation · solid-phase synthesis

Introduction

Oligosaccharides are a class of diverse molecules synthesized through a series of glycosyl-transfer reactions in the Golgi apparatus. Thus, the majority of proteins are post-translationally modified with oligosaccharides. Together with glycolipids, another important family, the surfaces of all mammalian cells are decorated with a variety of glycoconjugates. Furthermore, there is rapidly expanding information regarding important functions of oligosaccharides,[1] some of which relates to cancer metastasis,[2] immune responses,[3] and infectious diseases.[4,5] The importance of synthetic oligosaccharides in functional investigations and in the generation of anti-oligosaccharide antibodies in connection with vaccine development can be readily understood.[6] Therefore, synthetic methods to access oligosaccharide structures are extremely important.[7] Furthermore, a combinatorial oligosaccharide library is potentially important in terms of diagnoses and providing potential seeds for new pharmaceuticals.[8]

Solid-phase synthesis of oligosaccharides is a focus area because it relates to rapid access to such molecules, and important achievements have been reported.[9,10] One of the goals of this area of research is the establishment of a solid-phase-based synthetic method, which is considered to be of fundamental importance in providing the chemical and technological basis for an automated oligosaccharide synthesizer.[9c] Such technology is important for further biological investigations as it allows a rapid supply of required molecules.

On the other hand, important issues that need to be addressed in the development of solid-phase oligosaccharide synthesis are high-yielding stereoselective glycosylation reactions and the overall efficiency of the chemical processes.[11–13] Regarding the overall efficiency throughout the synthesis, chemoselective activation of anomeric leaving groups has been intensively investigated.[14] The majority of these methods allows iterative glycosylation reactions without the tedious protecting-group manipulations usually required in carbohydrate chemistry, thus minimizing the number of reaction steps. Among them, an extremely efficient method, orthogonal glycosylation, was reported.[15] This methodology relies on a set of distinct chemical reactivities of anomeric leaving groups, which has also proven useful in polymer (MPEG: monomethyl polyethyleneglycol)-bound oligosaccharide synthesis[15d,e] and in solid-phase synthesis to some extent.[16] The usefulness of the system has been demonstrated not only in oligosaccharide synthesis, but also in the

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synthesis of polymers, dendrimers, and oligomeric catechins.[17]

Despite advances in synthetic chemistry, the synthesis of combinatorial oligosaccharide libraries is a difficult subject. The carbohydrate-related combinatorial libraries reported thus far can be divided into six categories, namely, 1) a simple oligosaccharide library,[8,18] 2) a natural-product-like library,[19] 3) an oligosaccharide library with modifications of functional groups,[20] 4) a combinatorial glycopeptide library,[21] 5) a glycolipid library,[22] and 6) a chiral scaffold of a library.[23] We have been interested in the stand-alone oligosaccharide library (category 1) and attempted to synthesize oligosaccharides in the solid phase, leading to an automated synthesizer and combinatorial library.

It is readily anticipated that an active oligosaccharide that has been identified to play an important biological role is susceptible to existing hydrolases that degrade the molecule, leading to rapid loss of activity. For this reason, one might conclude that synthetic oligosaccharides may serve as important tools for biological investigations but not as therapeutic agents. Although this is true on the one hand, a potential piece of research was reported in which the investigation of synthetic inhibitors of *N*-acetylglucosaminyl transferase V was exploited. All monosaccharide units of a synthetic substrate of the enzyme β-D-GlcNAc-(1→2)-α-D-Man-(1→6)-β-D-Man-octyl were successfully replaced to give an inhibitor with other monosaccharide residues, namely, β-D-Glc, α-D-Rha, and β-D-Glc, respectively.[24] Although activities against degrading enzymes have not been investigated, the transferase inhibitor should be resistant to the β-*N*-acetylglucosaminidase. A combinatorial library may be considered as a source of such compounds for both a better understanding of the basis of carbohydrate–protein interactions and as potential therapeutic agents.

Another potential role of a combinatorial oligosaccharide library is the source of physical information contained in the structures of individual molecules. With such a library in hand, data related to chemical bond energies may be extracted by using energy-resolved mass spectrometry.[25] This type of information will be extremely important in elucidating the structure of an unknown oligosaccharide isolated from natural sources. A method of estimating such a structure may also be established after finding structural characteristics.

We report herein an extremely efficient method for oligosaccharide synthesis applicable to combinatorial synthesis. Our method is based on the combined use of a stationary solid-phase reaction (SSPR), in which no mechanical mixing is applied, an orthogonal glycosylation strategy, and solid-phase extraction (SPE). The synthesis of a series of trisaccharides, namely, α/β-L-Fuc-(1→6)-α/β-D-Gal-(1→2/3/4/6)-α/β-D-Glc-octyl, is reported to show the potential usefulness of the method in the synthesis of a combinatorial oligosaccharide library.

Abstract in Japanese:
オルトゴナルグリコシル化反応と無撹拌固相法に基づく新たな高効率糖鎖合成を報告する。生物学的な興味とは裏腹に糖鎖のコンビナトリアル合成は極めて困難な課題である。問題の一部として、1）糖鎖の合成経路が長いこと、2）各々のグリコシル化反応に対する多様な反応条件の必要性が挙げられる。これらの問題についてオルトゴナルグリコシル化法、および、個々の反応条件について適当な条件を与えるための昇温率の適応により取り組み解決した。さらに我々は、物理的撹拌や送 液をおこなうことなく固相合成を行うことを達成した。これらからなる方法論の糖 鎖のコンビナトリアル化学における有効性を明らかにすべく、一連の糖鎖 、すなわち、α/β-L-Fuc-(1→6)-α/β-D-Gal-(1→2/3/4/6)-α/β-D-Glc-octylの合成を行ったので報告する。

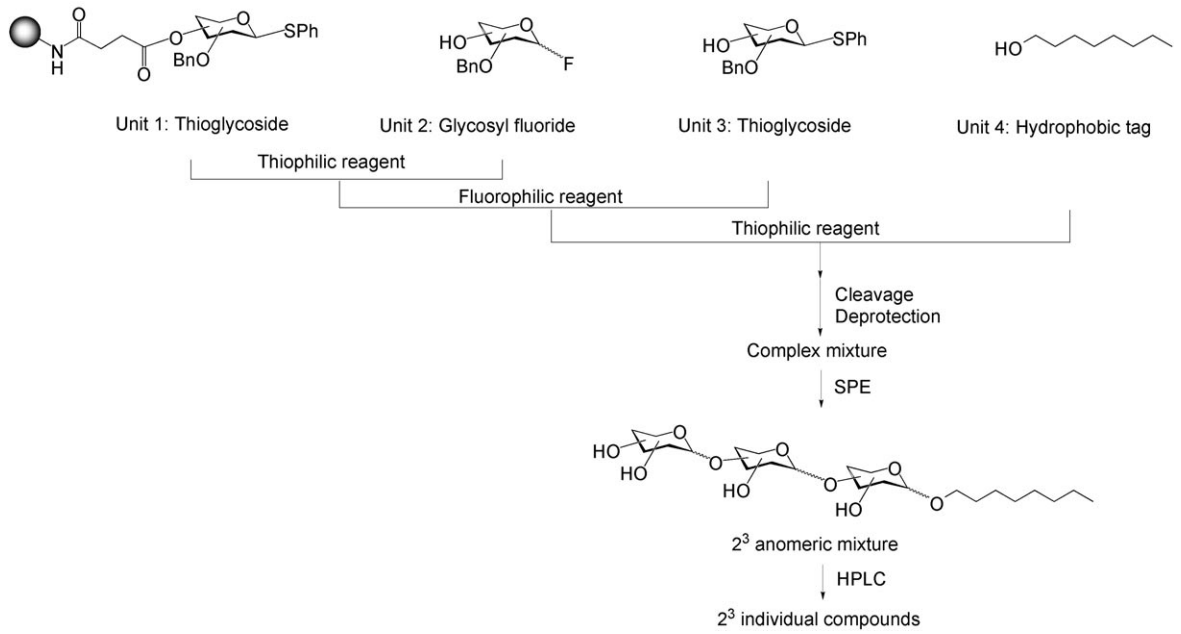
Results and Discussion

Successful oligosaccharide synthesis requires careful selection of a strategy, suitable coupling methods, and protecting groups.[9] At first, a decision regarding the direction of synthesis, that is, whether to start from the reducing or nonreducing terminus, has to be made. For the current investigation, we selected the latter, which is suited to an orthogonal-glycosylation strategy.[15a] We also decided to synthesize the possible stereoisomers regarding anomeric configurations.[8,26]

The current trend in the chemical synthesis of oligosaccharides, including solid-phase synthesis, aims primarily to synthesize a "desired" product, which is isolated, structurally defined, and/or found to play an important biological role, and thus becomes a target for the synthesis.[9] Stereoselective synthesis is required for the synthesis of a target molecule. Alternatively, it may be possible to identify useful structures in nonnatural oligosaccharides from a combinatorial library.

Rationale for Not Performing Stereoselective Glycosylations

Our intension was to synthesize all anomers as a member of a library (Scheme 1; focus on protecting group).[8,26] It is possible to accomplish such a goal by using nonneighboring and neighboring participating groups at the position next to the anomeric center to obtain α and β glycosides, respectively.[7] However, tremendous effort has to be made to achieve stereospecific synthesis, not only for stereocontrolled synthetic methods but also for the preparation of a series of synthetic units, especially with glycosyl donors equipped with a participating or nonparticipating protecting group. In combinatorial synthesis, the addition of a second set of reactions indicates that all the reactions have to be carried out twice. Here, one of the important considerations in carbohydrate chemistry is that the glycosylation proceeds basically by an S_N1 reaction mechanism, especially when nonneighboring groups are used as protecting groups at O2. In this case, a mixture of anomers is formed, and thus isolation of both anomers is necessary. This was the method for obtaining α glycosides. Therefore, we decided to perform the synthesis



Scheme 1. Overall synthetic plan. Four individual synthetic equivalents prepared before the synthesis were sequentially coupled by means of the orthogonal-glycosylation strategy. The last component introduced acts as a hydrophobic tag that facilitates rapid isolation of the products carrying it after cleavage of the substances accumulated on the support and the deprotection reactions. The trisaccharides obtained were finally isolated by using reverse-phase HPLC. Bn = benzyl.

of oligosaccharide in a nonstereospecific manner and to isolate the obtained mixture by chromatographic techniques. Regardless of this consideration, however, one can perform stereoselective glycosylation reactions by using neighboring participating groups as they are also used in solution chemistry.

Considerations for Efficient Oligosaccharide Synthesis

How would one access oligosaccharides in a minimum number of chemical steps? One may choose either of the following techniques: 1) chemoselective activation of anomeric leaving groups[14] and 2) solid-phase chemistry. Despite successful expansion of one-pot syntheses based on chemoselective glycosylations in solution,[11] however, combined use of this technique with solid-phase reaction usually does not give satisfactory results because the less-reactive species has to be exposed to the activating agent before addition to the solid carrying the other more-reactive species. As chemoselective reactions rely on subtle differences in reactivities of chemical species, less-reactive species would react slowly in the absence of more-reactive species. Thus, chemoselective reaction would give rise to a mixture of products consisting of deletion sequences. Therefore, complicated purification is inevitable when such reactions are used. To address the problem, we decided to use the orthogonal-glycosylation strategy.[15a] In this system, the chemical species involved is chemically independent. This is thus considered best-suited for solid-phase reaction, especially for oligosaccharide synthesis. Of course, one of the advantages of solid-phase organic reactions is rapid operation due to exclusion of column chromatography. We also planned to incorporate a hydrophobic tag at the end of the synthesis. This facilitates rapid isolation of the desired products by SPE after cleavage of the accumulated products from resin beads and deprotection (Scheme 1; focus on solid-phase orthogonal glycosylation and SPE).[27]

Objectives

To determine the eligibility of our synthetic method based on orthogonal glycosylation and the proposed SSPR, a series of linear trisaccharides consisting of L-fucose, D-galactose, and D-glucose was chosen as targets. L-Fucose-containing oligosaccharides were found to be part of glycoproteins and glycolipids and are known to play important biological roles, including involvement of Le^b antigen as a ligand of *Helicobacter pylori*.[28] Furthermore, the selected trisaccharides contain the α-L-Fuc-(1→6)-β-D-Gal structure, which was identified to be a weak substrate of α-fucosidase.[8] One of our goals is to find a potential therapeutic agent that consists basically of carbohydrates only. Such a molecule has to be recognized by a targeted binding protein and has to be slowly decomposed by endogenous hydrolases. Therefore, the series of trisaccharides was selected for future screening purposes as well. A conceptual summary of our library synthesis is depicted in Figure 1. A nonreducing end unit, fucose unit in this case (see also Scheme 1), was selected as a first monosaccharide. The second unit, for example, galactose unit with a 6-hydroxy group, was then coupled to afford anomer mixtures. The individual mixtures were coupled with the third unit, and so on. At each coupling cycle, new

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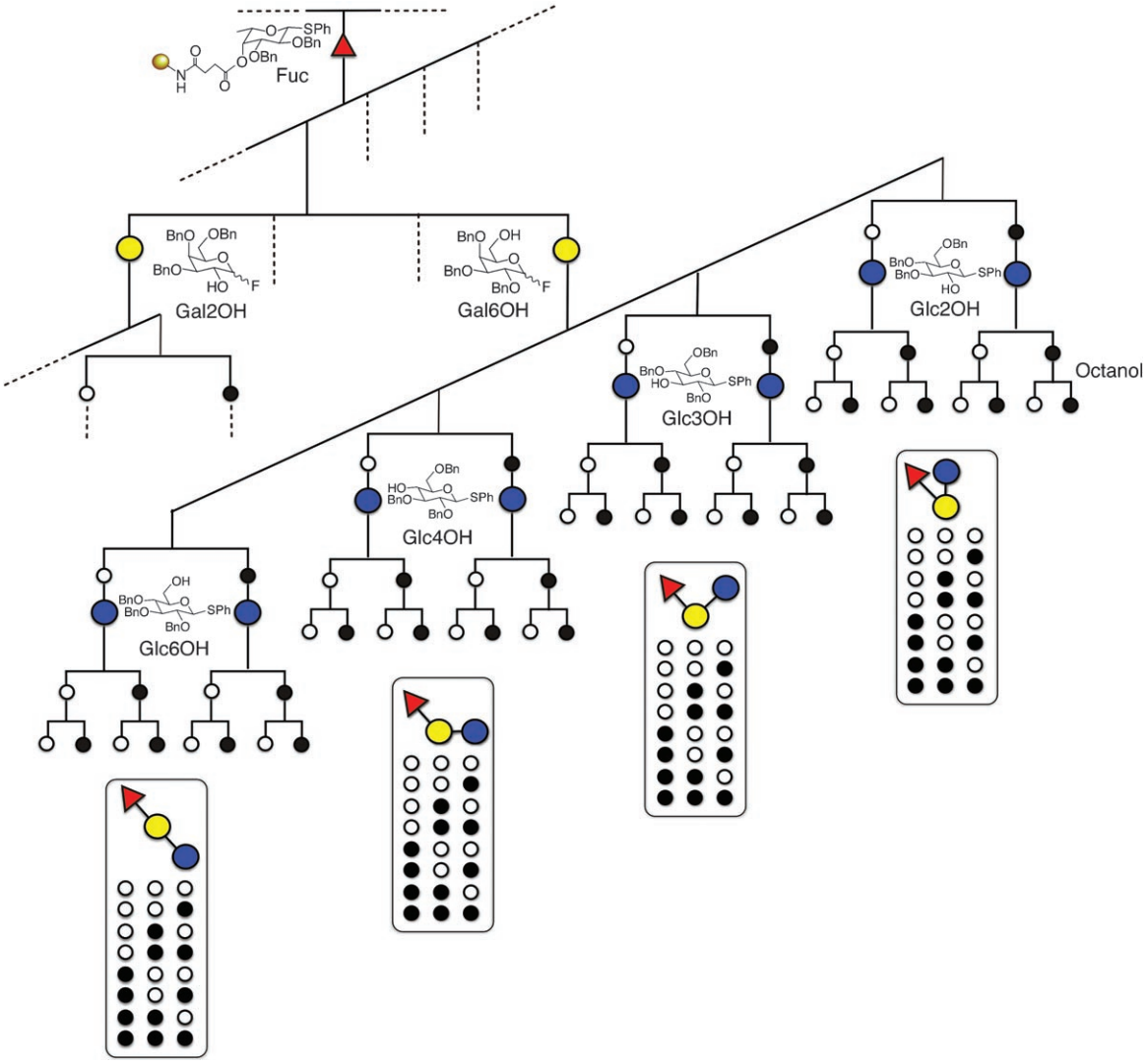
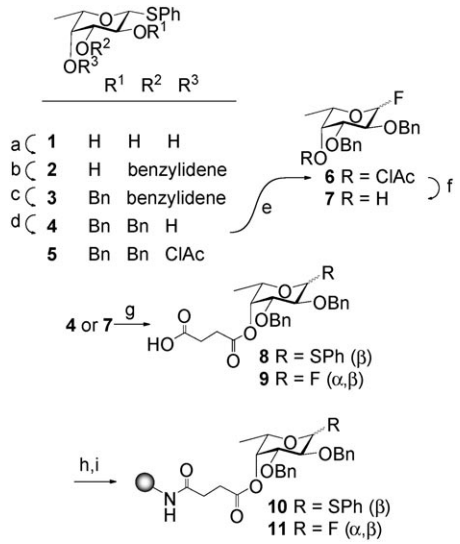


Figure 1. Concept of trisaccharide combinatorial library. ○ = α anomer, ● = β anomer. Isolated or interconnected objects indicate either individual monosaccharides or trisaccharides. Glycan structures are represented as suggested by the Nomenclature Committee, the Consortium for Functional Glycomics (http://www.functionalglycomics.org). ▲ = L-Fucose, ● = D-galactose, ● = D-glucose.

anomers were formed; thus, a series of compounds with a sequence consisting of 2^3 anomeric combinations were formed in each sequence.

Synthesis of Fucose Derivatives

To perform solid-phase orthogonal-glycosylation reactions, a nonreducing-end monosaccharide unit has to be attached to a resin prior to the synthesis. To begin with, the phenylthioglycoside of L-fucose derivative **4**,[29] of which the 2- and 3-OH groups were protected as benzyl ethers, was synthesized from compound **1** by benzylidenation (benzaldehyde dimethylacetal, *p*TsOH), benzylation (BnBr, NaH), acid hydrolysis (80% AcOH), and selective benzylation (Bu_2SnO, BnBr) (Scheme 2). Compound **4** was converted into the succinyl esters **8** and **9** as follows. Compound **5** obtained by protection of the 4-OH group with a chloroacetyl group (90%) was converted into glycosyl fluoride **6** in 91% yield by using DAST. The formation of α- and β-glycosyl fluorides was evident from the typical anomeric ^{1}H NMR signals at 5.57 ppm ($J_{1\text{-H},2\text{-H}} = 2.7$, $J_{1\text{-H,F}} = 53.2$ Hz) for the α anomer and 5.18 ppm ($J_{1\text{-H},2\text{-H}} = 6.8$, $J_{1\text{-H,F}} = 52.4$ Hz) for the β anomer. The reaction did not require any of the additives usually used, such as *N*-bromosuccinimide (NBS). It is believed that an electrophilic sulfiminium cation species is the active agent in the reaction.[8b] Although a higher reaction temperature (40 °C in this case) was required, the reaction was very much cleaner than the one with NBS, which usually produces glycosyl bromide as a by-product. Compound **6** was then treated with DABCO to remove the chloroacetyl group and give **7** (92%). Position 4 of compounds **4** and **7** was functionalized as succinyl esters **8** (97%) and **9** (60%), respectively.



Scheme 2. Synthesis of resin-bound fucosyl donors. a) Benzaldehyde dimethylacetal, *p*TsOH, DMF, 87%; b) BnBr, NaH, DMF, 58%; c) i) 80% AcOH; ii) Bu_2SnO, toluene; iii) BnBr, CsF, DMF, 89% over 3 steps; d) chloroacetyl chloride, DCM, Pyr, 90%; e) DAST, DCM, 40°C, 91%; f) DABCO, EtOH, Pyr, 70°C, 92%; g) succinic anhydride, DMAP, DCM, Pyr; h) Amino TentaGel, DIC, HOBt, DMF; i) Ac_2O, Pyr, DCM. ClAc = chloroacetyl, DABCO = 1,4-diazabicyclo[2.2.2]octane, DAST = *N*,*N*-diethylaminosulfur trifluoride, DCM = dichloromethane, DIC = diisopropylcarbodiimide, DMF = *N*,*N*-dimethylformamide, HOBt = 1-hydroxybenzotriazole, *p*Ts = tosyl, Pyr = pyridine.

Synthesis of Resin-Bound Fucosyl Donor under Stationary Conditions (SSPR)

In solid-phase synthesis, chemical reactions take place inside the pores of resins. We often mix such beads mechanically in a reaction slurry with the intention of aiding diffusion of heat and the chemical entities involved so as to accelerate the reaction. The governing factors of chemical reactions that occur inside resins, however, are 1) temperature, 2) concentration, and 3) the diffusion constants of the reactants; mixing is therefore not needed for factors 2 and 3. Thus, mechanical mixing may be avoided, especially in a relatively small-scale synthesis such as in the case of combinatorial chemistry. If possible, the mixing mechanism, which often makes the machine bulky, costly, brittle, and wasteful of solvents, can be eliminated from the organic synthesizer.

For the SSPR to be effective, the following conditions have to be fulfilled whenever possible: 1) the reactants required should be dissolved in the minimum volume of selected solvent necessary to swell the resin used; 2) resin beads should be predried to allow not only successful anhydrous reaction conditions but also efficient introduction of a solution into the resin pores based on solvation effect;[30b] and 3) soluble reagents should be used to avoid clogging of the pores. The amount of solvent required to swell a certain quantity of resin beads can be obtained by slowly adding the solvent(s) to the beads. Polystyrene-based resins swell within 10 min for nonpolar organic solvents, thus addition is performed during this period. The "liquefaction" phenomenon of the beads is used to judge the amount ($\approx$5 mL dichloroethane (DCE) or DMF per g TentaGel).[30] Thus, the resin beads (Amino TentaGel; 1.00 g, 0.29 mmol) were swollen with a solution containing **8** (2 equiv), DIC (3 equiv), and HOBt (2.5 equiv) in DMF (5.0 mL). The vessel containing the resulting calluslike resin beads was left to stand at room temperature for 24 h. The resins were thoroughly washed and subjected to acetylation conditions to cap the existing amine. According to the Kaiser test and weight gain of the resin, the yield of **10** from this amide-bond formation was 67% (Scheme 2). In a similar manner, resin-bound fucosyl fluoride donor **11** was synthesized in 60% yield by using **9**. The rate of introduction of these first monosaccharide units was controlled at 60–70% based on the preliminary examination, which indicated that this loading level resulted in the best overall yield. One of the reasons for this result is fewer donor-to-donor cross-reactions. When higher loading (quasiquantitative yield) was achieved, formation of fucosyl dimer was observed. Also, regarding the reaction efficiency relative to that with vortex mixing, similar yields were obtained with identical quantities of individual materials (data not shown, but details of SSPR are given below for glycosylation reactions).

With a successful example of amide-bond formation under SSPR conditions, we turned our attention to the glycosylation reaction. We first carried out experiments to compare conditions with and without mixing. Experiments were terminated after 15 min, before completion of the reaction, to compare quasi-initial rates. In both cases, compound **10** was used as a glycosyl donor. For the SSPR, a minimum amount of solvent containing *n*-octanol and dimethylmethylthiosulfonium trifluoromethanesulfonate (DMTST)[31] was used and the resins were left at −30°C. Excess solvent has to be used to stir the reaction mixture in a traditional reaction. For this, twofold dilution of the reagents was used to allow the standard reaction. It was shown that the reaction rate in the SSPR was 1.4 times greater than that of a standard reaction. This result is believed to reflect the effect of the higher concentration in the former conditions, which is similar to a phenomenon observed for acceleration of chemical reaction in frozen solution.[32] A homogenous, high concentration of the reaction solution to be sucked into the resin in our experiment was achieved because the glycosyl acceptor and the promoter can be mixed in the orthogonal system. Furthermore, this is advantageous over other glycosylation strategies because time is not required for the diffusive mixing of solutions of acceptor and promoter. Regardless of the mechanism that accelerates the solid-phase reaction, it is clear that mechanical mixing is not needed for this reaction. Also, the result indicates that the method can eliminate the need for unnecessary amounts of reagents and solvents. The SSPR is thus considered best-suited to the automated synthesizer because of minimum solvent consumption and space occupancy, except for large-scale synthesis.

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Examination of Solid-Phase Glycosylation Conditions

Before initiating solid-phase oligosaccharide synthesis, we first examined the reaction conditions with thioglycoside and glycosyl fluoride as glycosyl donors in the orthogonal coupling.[15a,b,d] To determine each reaction condition, resin-bound fucosyl donors carrying phenylthio (**10**) and fluoro groups (**11**) at anomeric centers were prepared and used in the glycosylation reactions to obtain **12**; other conditions were varied (Tables 1 and 2). DMTST and hafnocene bistrifluoromethanesulfonate $[Cp_2Hf(OTf)_2]$[33] were used as the activating agents for the respective functional groups. One of the objectives of these experiments was to identify the condition that yields a mixture of glycosides in which the desired α/β ratio is 1, which is important as we want all possible anomers.

Activation of Phenylthioglycoside

Table 1 shows the results of using phenylthiofucoside **10** as the glycosyl donor; the quantities of acceptor and DMTST were fixed at 2 and 3 equivalents, respectively, based on our preliminary experiments. The results clearly show that the yields of octyl glycosides **12** were very high (≈97%), as confirmed by ^{1}H NMR analyses. It seems that application of a temperature gradient from lower to higher temperature, to facilitate reactions for which the optimal temperature is not known, was effective (Table 1, entries 1–8), especially in terms of reaction time. This is clear when the results of entries 1 and 9 in Table 1 are compared. The reaction was successfully completed in the former but not the latter case. We do not have an explanation for the results obtained for the reactions carried out at higher temperatures that did not go to completion (Table 1, entries 10–12). Also, the formation of the β glycoside tended to increase at lower temperatures. Regarding the α/β ratio, the higher the temperature, the higher the rate of formation of the α glycoside (Table 1, entries 7, 8, 10–12). Despite the use of nonneighboring participating protecting groups (benzyl in this case) at O2, the result indicates that β glycoside formation is preferred under kinetic control. On the other hand, the proportion of the thermodynamically stable α glycoside increased at higher temperatures as expected. In the presence of CH_3CN, the α/β ratio did not change dramatically, whereas the preferred α/β ratio (closest to one) was obtained in the absence of CH_3CN. Notably, glycosyl fluoride **13** was found to gradually decompose at reaction temperatures over 0°C (data not shown). Also, a longer reaction time was necessary to complete the reaction (Table 1, entries 13 and 14). As the difference in the α/β ratio was not affected significantly (Table 1, entry 7 vs. 8 and entry 13 vs. 14), we decided to use the reaction conditions (activation of phenylthioglycosides) shown in Table 1, entry 15 for further experiments.

Table 1. Glycosylation reactions of resin-bound phenylthiofucoside **10**.[a]

Entry	Acceptor[b]	Solvent[c]	T [°C] initial/final	t [h]	**12** formed Yield [%][d]	α/β[d]	**4** [%][d]
1	*n*-octanol	S^1	−30/0	1	≈97	1:2.5	N.D.[e]
2	*n*-octanol	S^1	−30/0	2	≈97	1:2.6	N.D.[e]
3	*n*-octanol	S^1	−30/0	3	≈97	1:2.5	N.D.[e]
4	*n*-octanol	S^2	−30/0	1	≈97	1:2.5	N.D.[e]
5	*n*-octanol	S^3	−30/0	1	≈97	1:2.3	N.D.[e]
6	*n*-octanol	S^4	−30/0	1	≈97	1:1.8	N.D.[e]
7	*n*-octanol	S^1	−20/0	1	≈97	1:2.1	N.D.[e]
8	*n*-octanol	S^1	−10/0	1	≈97	1:1.7	N.D.[e]
9	*n*-octanol	S^1	−30	1	≈66	1:2.9	≈31
10	*n*-octanol	S^1	0	1	≈70	1:1.4	≈27
11	*n*-octanol	S^1	10	1	≈70	1:1.6	≈27
12	*n*-octanol	S^1	20	1	≈70	1:1.3	≈27
13	**13**	S^1	−30/0	1	≈85	1:3.9	≈12
14	**13**	S^1	−15/0	1	≈93	1:3.2	≈4
15	**13**	S^1	−30/0	12	≈97	1:2.3	N.D.[e]

[a] Reaction conditions: 1) DMTST (3 equiv), DCE/CH_3CN; 2) NaOMe, MeOH/DCE (1:4). [b] 2 equiv acceptor was used. [c] Solvents: DCE/CH_3CN = 1:1 (S^1), 2:1 (S^2), 3:1 (S^3); S^4: DCE. [d] Yields and ratio were determined by ^{1}H NMR spectroscopy. [e] Not detected.

Activation of Glycosyl Fluoride

Next, we examined the glycosylation reaction with resin-bound fucosyl fluoride as a glycosyl donor (Table 2). We found that DCM was the superior solvent because of its potent solvating ability. Our initial choice of DCE was based on the consideration that solvents with relatively high boiling points and/or viscosity were preferred in terms of liquid handling when using a synthesizer. The ratio of

Table 2. Glycosylation reactions of resin-bound fucosyl fluoride **11**.[a]

Entry	$[Cp_2HfCl_2]$ [equiv]	AgOTf [equiv]	Solvent[b]	T [°C] initial/final	t [h]	**12** formed Yield [%][c]	α/β[c]	Remaining **11** [%][c]
1	3	3	S^1	−30/0	2	≈30	1:2.4	67
2	3	6	S^1	−30/0	2	67	1:2.4	≈30
3	3	6	S^2	−30/0	12	≈97	1:3.3	<1.0
4	3	6	S^2	−30/0	1	≈97	1:2.3	<1.0
5	3	3	S^2	−30	0.25	<1.0	N.D.[d]	98
6	3	6	S^2	−30	0.25	≈36	1:2.4	61

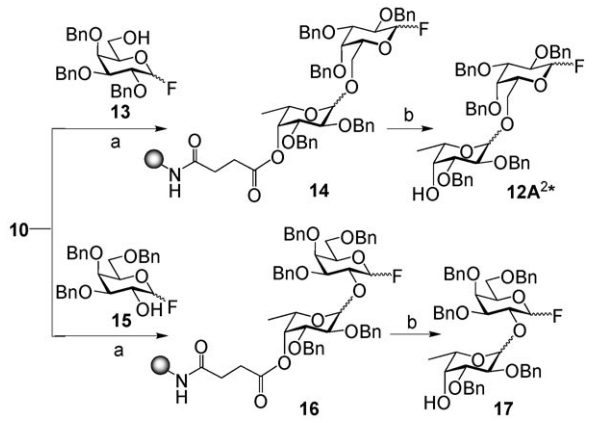
[a] Reaction conditions: 1) Acceptor = *n*-octanol (2 equiv), $[Cp_2HfCl_2]$, DCE/CH_3CN; 2) NaOMe, MeOH/DCE (1:4). [b] Solvents: DCE/CH_3CN = 1:1 (S^1), DCM (S^2). [c] Yields and ratios were determined by ^{1}H NMR spectroscopy. [d] Not determined.

$[Cp_2HfCl_2]$ to AgOTf was determined to be 1:2 (Table 2, entry 1 vs. 2).[33a] Again, the efficiency of the application of a temperature gradient was confirmed (Table 2, entries 1–4 vs. 5 and 6). A steeper increment rate resulted in an excellent yield (Table 2, entry 4). The result indicated no clear effect on the ratio of the glycosides formed under these conditions.

Orthogonal Glycosylation in the Solid Phase

With the results of the activation conditions of phenylthioglycoside and glycosyl fluoride, we examined the synthesis of trisaccharides by using an orthogonal-glycosylation reaction based on the newly introduced SSPR (Schemes 3–5). The orthogonal-glycosylation reaction relies on the chemical distinctiveness of two independent functional groups at anomeric positions. In this system, 1) phenylthioglycoside is selectively activated by DMTST in the presence of glycosyl fluoride carrying a hydroxy group and 2) glycosyl fluoride is selectively activated by $[Cp_2Hf(OTf)_2]$ in the presence of thioglycoside. In this method, the reaction starts from the nonreducing terminus, where formation of the deletion sequence can be suppressed without capping reactions as described for glycal chemistry, because the by-products cannot serve as glycosylating agents in the next cycle.[9a]

The resin-bound fucose derivative **10** was used as a glycosyl donor (Scheme 3). To achieve a "mixing-free" SSPR, a



Scheme 3. Synthesis of disaccharides. a) DMTST, DCE/CH_3CN (1:1); b) NaOMe, MeOH/DCE (1:4). *For a detailed examination of the reaction conditions, see Table 1.

selected galactosyl fluoride **13**, in which the 2-, 3-, and 4-hydroxy groups were protected with benzyl groups, was prepared as an acceptor[8b] and mixed with a solution of DMTST. The mixture was added to a resin at −15 °C. The swelled resin was left while the reaction temperature was raised to 0 °C over 12 h (temperature increment rate: 1.25 °C h^{-1}) to give resin-bound disaccharide **14**. Hereafter we use "TF" to describe the temperature factor of the reaction conditions, for example, (TF −15, 0, 1.25) in this case. The resin was thoroughly washed, and aliquots were taken to obtain the coupling yield after Zemplén deacetylation. Analysis by quadrupole ion-trap mass spectrometry (QITMS) revealed that disaccharide **12** (where R in Table 1 is A^2) ($m/z = 801.3$) was formed with excellent efficiency (Figure 2a). Furthermore, glycosylation of **10** with compound **15** (2-OH form)[34] was also carried out to yield resin-bound **16**. An extremely high yield was shown again by QITMS analysis (Figure 2b).

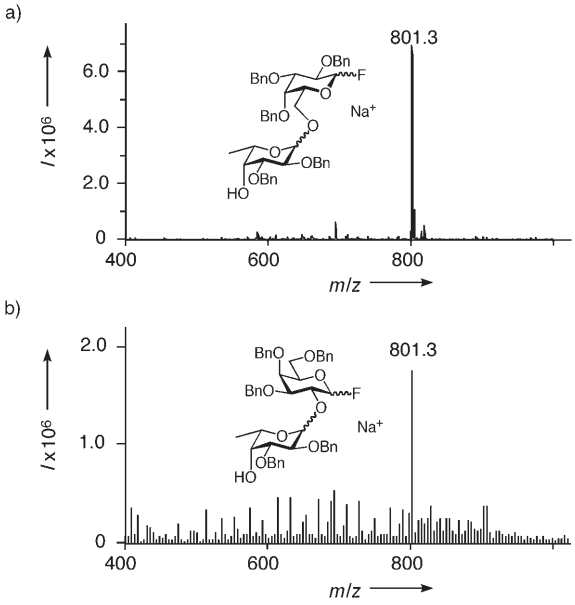


Figure 2. Mass spectra of compounds **12** ($R = A^2$) and **17**.

The compounds that accumulated on the solid support consisted of anomers; however, we proceeded with the synthesis because our intention was to synthesize all possible anomers. Thus, resin-bound disaccharide fluorides **14** were activated by $[Cp_2Hf(OTf)_2]$ and coupled with phenylthioglucoside carrying a 6-OH functionality (**18**)[35] (Scheme 4). The reaction was carried out under SSPR conditions (TF −30, 0, 2.5). The conditions of Table 2, entry 3 were used based on the consideration that glycosylation of a monosaccharide unit may require a longer reaction time according to the glycosylation of phenylthioglycoside as shown above. QITMS analysis after cleavage of **19** from the resin again indicated a successful reaction. The resin-bound trisaccharide thioglycoside **20** was then coupled with *n*-octanol in the presence of DMTST (TF −30, 0, 2.5) without mixing to give compound **21**. The trisaccharides were cleaved from the resin under Zemplén conditions, and the mixture consisting of 2^3 anomers (**22**, Figure 3a) was hydrogenated over $Pd(OH)_2$ on charcoal to yield **23** (Figure 3b) as an anomeric mixture. In this mixture, only the desired products have a hydrophobic tag (octyl in this case), as potential by-products such as hydrolysis and elimination products were not involved in further glycosylation reactions. The final compounds were thus easily separated from the by-products by means of a reverse-phase short column (SPE) (33%, 5 steps).

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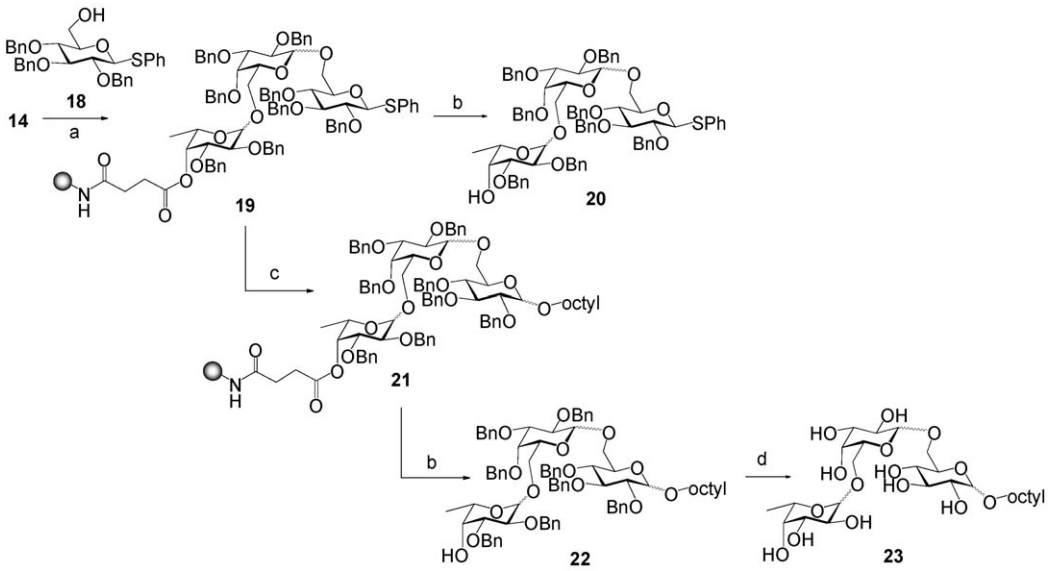


Scheme 4. Synthesis of trisaccharides. a) $[Cp_2Hf(OTf)_2]$, DCE/CH_3CN (1:1); b) NaOMe, MeOH/DCE (1:4); c) *n*-octanol, DMTST, DCE/CH_3CN (2:1); d) H_2, $Pd(OH)_2$/C, MeOH/EtOAc (1:1).

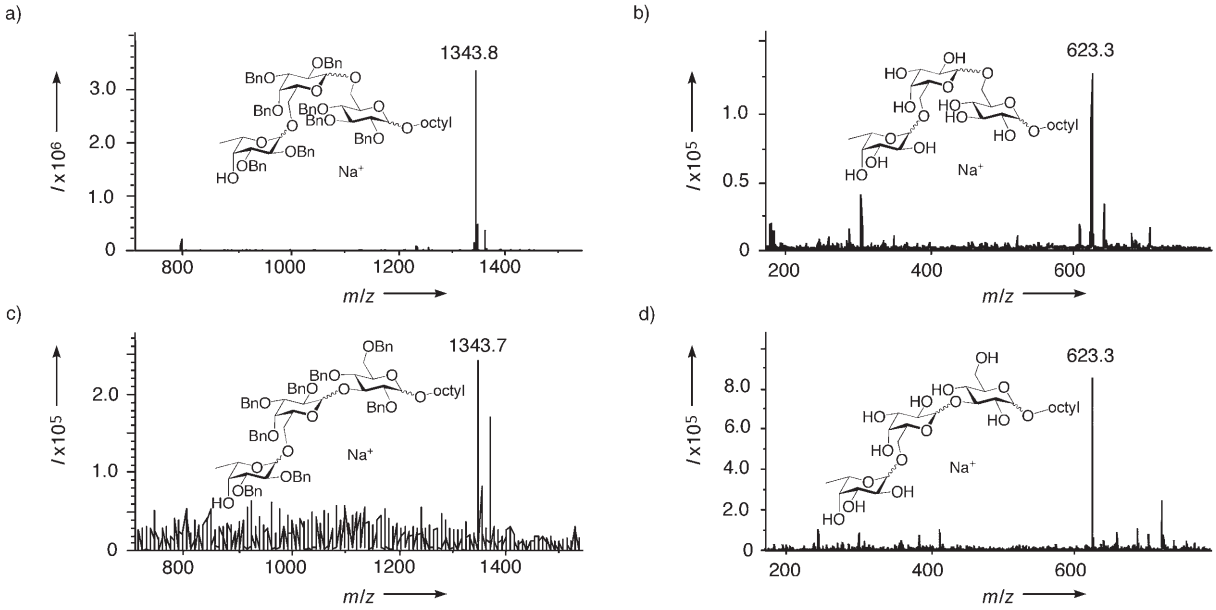


Figure 3. Mass spectra of compounds **22**, **23**, **27**, and **28**.

Trisaccharides obtained in this manner consist of a series of anomers. Each anomer was isolated by using liquid chromatography (LC) MS with a reverse-phase monolithic column. As a demonstration, the separation of α/β-Fuc-(1→6)-α/β-Gal-(1→6)-α/β-Glc-octyl **23** was carried out, the chromatograph of which is shown in Figure 4a. Anomeric configurations were then characterized by ^{1}H NMR spectroscopy and, when necessary, ^{1}H–^{1}H COSY and heteronuclear single-quantum correlation (HSQC) experiments. The assigned chemical shifts and coupling constants of these anomers are listed in Table 3.

Having shown a solid-phase orthogonal system in which a glycosylation reaction of disaccharide glycosyl fluoride with a primary hydroxy group was achieved, we then examined a glycosylation reaction with a secondary hydroxy group by using the 3-hydroxy free-glucose derivative **24**[36] (Scheme 5). Thus, the coupling of **24** and resin-bound **14** was carried out to give trisaccharide **25**, which was then coupled with *n*-octanol to give **26**. Compound **27** (Figure 3c) obtained after cleavage from the resin was finally subjected to hydrogenolysis to yield **28** (Figure 3d) (5.2 mg, 17.8%, 5 steps). Individual compounds were isolated by using

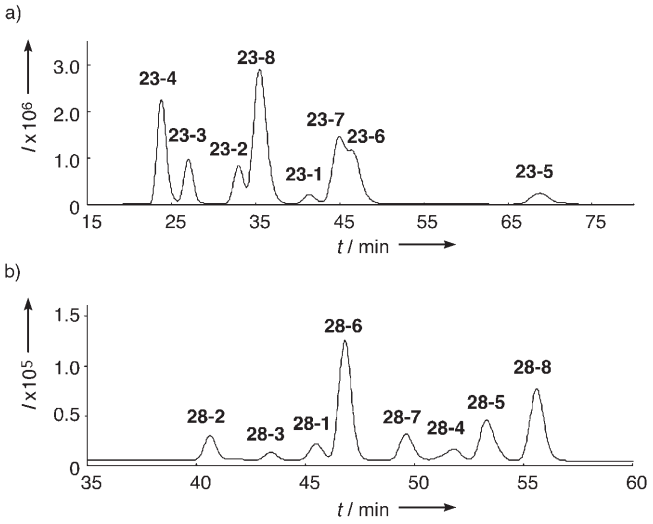


Figure 4. HPLC profile of a) **23** and b) **28**. Positive *m*/*z* in both cases = 623. For anomeric configurations, retention times, and the ratio of individual compounds, see Tables 3 and 4.

Table 3. Assignments of ^{1}H NMR spectra of the trisaccharides **23**, their retention times, and anomer ratio.

Compounds	Anomeric configurations[a]	δ [ppm] (*J* [Hz]) Fuc	Gal	Glc	t_R [min]	Ratio [%][c]
23–1	ααα	4.91[b] (3.8)	4.88[b] (2.7)	4.98[b] (4.0)	41.0	1.4
23–2	ααβ	4.94 (3.6)	5.02 (2.7)	4.48 (8.0)	32.9	6.5
23–3	αβα	4.94 (3.9)	4.43 (7.8)	4.90 (3.6)	26.6	6.4
23–4	αββ	4.97 (3.8)	4.50 (7.9)	4.48 (8.2)	23.4	17.7
23–5	βαα	4.43 (7.8)	4.99 (3.2)	4.94 (3.8)	68.3	2.8
23–6	βαβ	4.45 (7.9)	5.01 (3.4)	4.50 (8.0)	46.3	14.9
23–7	ββα	4.43 (7.9)	4.45 (8.1)	4.92 (3.6)	44.2	14.2
23–8	βββ	4.43 (7.8)	4.49 (7.8)	4.48 (8.0)	35.1	36.1

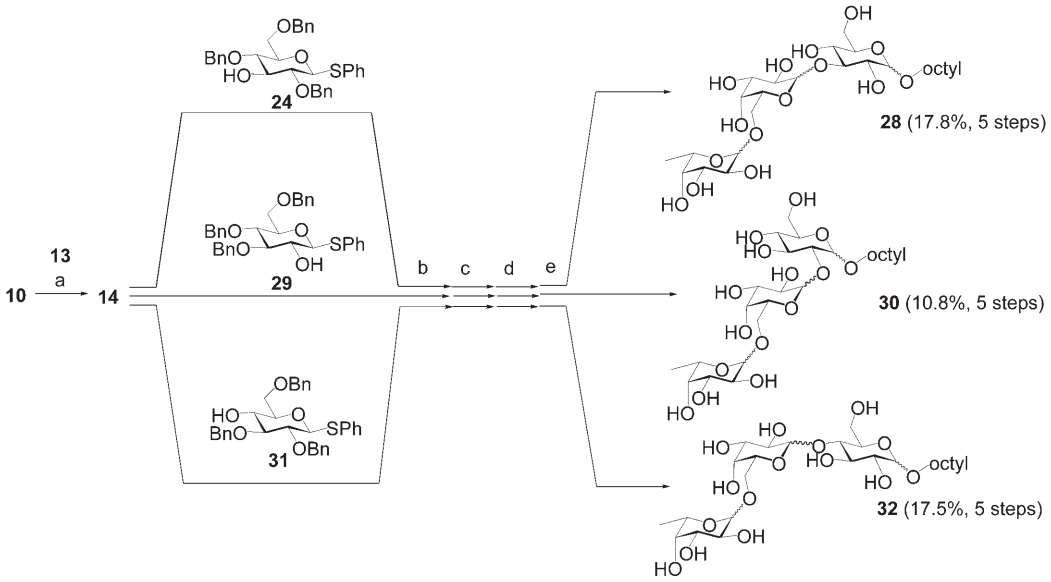
[a] Anomeric configurations are shown as starting from the nonreducing and proceeding to the reducing terminus. [b] Assignments are tentative. [c] Ratio of anomers was estimated from the LCMS spectrum based on the assumption that these structural isomers were ionized similarly.

HPLC (Figure 4b), and the anomeric configurations were characterized by NMR spectroscopy. The assigned chemical shifts and coupling constants of the anomers are shown in Table 4.

In the same manner, α/β-Fuc-(1→6)-α/β-Gal-(1→2/4)-α/β-Glc-octyl **30** and **32** were synthesized from disaccharide **14** and corresponding glucose derivatives (Scheme 5).[37] Formation of trisaccharides were confirmed by mass spectral analysis as shown in Figure 5a (**30**) and b (**32**).

Conclusions

We have demonstrated successful oligosaccharide synthesis by using an orthogonal glycosylation strategy based on SSPR. An overall improvement of the synthetic protocol was achieved, which will be effectively used in the upcoming combinatorial synthesis of oligosaccharides. The usefulness of our method was shown through the synthesis of a small combinatorial library of linear trisaccharides with the general sequence L-Fuc-(1→6)-D-Gal-(1→2/3/4/6)-D-Glc as an octyl glycoside. Furthermore, we have shown that all anomers with the sequence can be isolated by simple reverse-phase HPLC. The anomeric configurations of compounds thus isolated were successfully assigned by using ^{1}H–^{1}H COSY and HSQC. The SSPR may also affect the future design of synthesizers. Investigations directed toward 1) finding potential



Scheme 5. Synthesis of trisaccharides **28**, **30**, and **32**. a) DMTST, DCE/CH_3CN; b) [$Cp_2Hf(OTf)_2$], DCE/CH_3CN; c) *n*-octanol, DMTST, DCE/CH_3CN; d) NaOMe, MeOH/DCE; e) H_2, $Pd(OH)_2$/C, MeOH/EtOAc.

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Table 4. Assignments of ^{1}H NMR spectra of the trisaccharides **28**, their retention times, and anomer ratio.

Compounds	Anomeric configurations[a]	δ [ppm] (*J* [Hz]) Fuc	Gal	Glc	t_R [min]	Ratio [%][c]
28–1	ααα	4.92[b]	5.32[b] (3.5)	4.92[b]	45.2	4.8
28–2	ααβ	4.90 (3.69)	5.34 (3.8)	4.47 (8.1)	40.4	6.5
28–3	αβα	4.93 (3.8)	4.54 (7.3)	4.93 (3.8)	43.2	2.0
28–4	αββ	4.92 (3.8)	4.59[b] (7.5)	4.48[b] (8.2)	51.5	4.4
28–5	βαα	4.38 (7.9)	5.33 (3.9)	4.91 (3.7)	53.1	14.1
28–6	βαβ	4.41 (8.0)	5.37 (3.6)	4.48 (8.1)	46.6	35.9
28–7	ββα	4.39 (7.9)	4.58 (7.8)	4.92 (3.7)	49.3	8.2
28–8	βββ	4.40 (8.5)	4.62[b] (7.9)	4.49[b] (7.8)	55.3	24.0

[a] Anomeric configurations are shown as starting from the nonreducing and proceeding to the reducing terminus. [b] Assignments are tentative due to signal overlap. [c] Ratio of anomers was estimated from the LCMS spectrum based on the assumption that these structural isomers were ionized similarly.

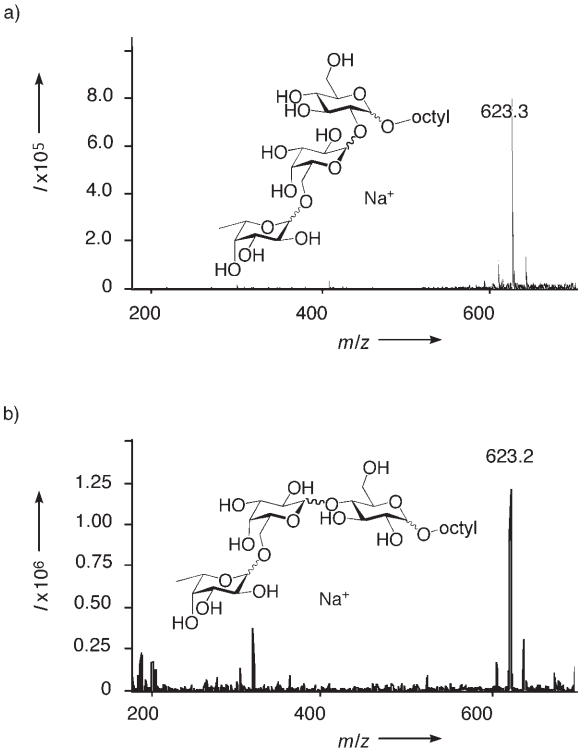

Figure 5. Mass spectra of a) **29** and b) **30**.

agents for some therapeutic treatments[8] and 2) establishing a new method for structural elucidation by extracting physical data based on energy-resolved mass spectrometry[25] are underway using an oligosaccharide library.[38]

Experimental Section

General Methods

NovaSyn TG amino resin was purchased from Merck. Bohdan Miniblock™ was equipped with a heat-transfer layer. Dried solvents were used for all reactions. Solutions were evaporated under reduced pressure at a bath temperature not exceeding 50 °C. Analytical TLC was performed on a Merck Art 5715 chromatograph with Kieselgel 60 F_{254} 0.25-mm thick plates. Visualization was performed with UV light and phosphomolybdic acid and/or sulfuric acid followed by heating. Column chromatography was performed with a Merck Art 7734 chromatograph with silica gel 60 (70–230 mesh). Optical rotations were measured in a 1.0-dm tube with a Horiba SEPA-200 polarimeter. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded with an AVANCE 500 spectrometer (Bruker Biospin Inc.) in deuterated solvents with tetramethylsilane as an internal standard. ESI QIT mass spectra were recorded on an Esquire 3000 plus mass spectrometer (Bruker Daltonics Inc.). High-resolution mass spectra were obtained on a Q-star pulsar-*i* with ESI interface (Triple Q TOF mass spectrometer with reflectron; mass accuracy: 5 ppm for 150–900; Applied Biosystems Inc.) and internal standards Fucα(1→2)Galβ(1→4)Glc (theoretical monoisotopic mass as $[C_{18}H_{32}O_{15}Na]^+$: 511.1633) and lacto-*N*-neotetraose (theoretical monoisotopic mass as $[C_{26}H_{45}NO_{21}Na]^+$: 730.2376), which were purchased from Dextra Laboratories (Reading, UK).

Compounds **1**,[29] **13**,[8b] and **15**[34] were prepared according the procedure reported. Compounds **18**[35] and **24**[36] were prepared according to procedures described previously[37].

In the assignment of ^{1}H NMR spectra for the mixture of α and β anomers, individual protons were counted separately regardless of the ratio.

Syntheses

2: Benzaldehyde dimethylacetal (1.33 mL, 8.9 mmol) and *p*TsOH (112 mg, 0.59 mmol) were added to a solution of **1**[29] (2.0 g, 5.9 mmol) dissolved in DMF (7.0 mL), and the resulting mixture was stirred at 45 °C for 1 h. After completion of the reaction, Et_3N was added to the mixture, which was then concentrated. The residue was purified by a column of silica gel (hexane/EtOAc = 3:1) to yield **2** (1.84 g, 87 %). **2a**: R_f = 0.52 (toluene/EtOAc = 4:1); ^{1}H NMR ($CDCl_3$): δ = 7.54–7.26 (m, 10H, aromatic H), 6.19 (s, 1H, PhC*H*), 5.17 (dd, *J* = 6.8, 10.4 Hz, 1H, 2-H), 4.67 (d, *J* = 10.0 Hz, 1H, 1-H), 4.48 (t, *J* = 5.7 Hz, 1H, 4-H), 4.11 (dd, *J* = 2.9, 5.3 Hz, 1H, 3-H), 3.87 (q, 1H, 5-H), 1.38 ppm (d, *J* = 6.5 Hz, 3H, 6-H). **2b**: R_f = 0.49 (toluene/EtOAc = 4:1); ^{1}H NMR ($CDCl_3$): δ = 7.89–7.27 (m, 10H, aromatic H), 5.89 (s, 1H, PhC*H*), 5.06 (dd, *J* = 6.7, 10.0 Hz, 1H, 2-H), 4.69 (d, *J* = 10.1 Hz, 1H, 1-H), 4.36 (t, *J* = 6.3 Hz, 1H, 4-H), 4.15 (dd, *J* = 2.1, 5.8 Hz, 1H, 3-H), 3.98 (q, 1H, 5-H), 1.38 ppm (d, *J* = 6.3 Hz, 3H, 6-H).

3: NaH (8.8 mg, 0.61 mmol) was added to a solution of **2** (1.1 g, 3.0 mmol) in DMF (20 mL) containing BnBr (760 μL, 6.1 mmol) at about 0 °C. The reaction mixture was stirred at room temperature for 1.5 h and diluted with EtOAc. The mixture was washed with water, dried over $MgSO_4$, and concentrated. The resulting residue was purified on a silica-gel column chromatograph (hexane/EtOAc = 4:1) to give **3** (783 mg, 58 %). **3a**: ^{1}H NMR ($CDCl_3$): δ = 7.59–7.22 (m, 15H, aromatic H), 5.99 (s, 1H, PhC*H*), 4.88, 4.78 (d, *J* = 11.4 Hz, 2H, PhCH_2), 4.67 (d, *J* = 9.5 Hz, 1H, 1-H), 4.55 (t, *J* = 6.0 Hz, 1H, 4-H), 4.07 (dd, *J* = 9.5, 1.8 Hz, 1H, 3-H), 3.77 (q, 1H, 5-H), 3.64 (dd, *J* = 5.6, 9.8 Hz, 1H, 2-H), 1.41 ppm (d, *J* = 6.7 Hz, 3H, 6-H). **3b**: ^{1}H NMR ($CDCl_3$): δ = 7.58–7.22 (m, 15H, aromatic H), 5.89 (s, 1H, PhC*H*), 4.73, 4.56 (d, *J* = 11.4 Hz, 2H, PhCH_2), 4.65 (d, *J* = 10.1 Hz, 1H, 1-H), 4.37 (t, *J* = 5.8 Hz, 1H, 4-H), 4.11 (dd, *J* = 6.5, 2.5 Hz, 1H, 3-H), 3.91 (q, 1H, 5-H), 3.52 (dd, *J* = 5.6, 9.8 Hz, 1H, 2-H), 1.46 ppm (d, *J* = 6.5 Hz, 3H, 6-H).

4:[29] Compound **3** (2.7 g, 6.1 mmol) was treated with AcOH (80 mL) and water (20 mL) at 45 °C. After 5 h, the solution was concentrated to leave a residue, which was purified by silica-gel column chromatography (CH_2Cl_2/MeOH = 100:1) to give a 3,4-diol (1.9 g, 5.6 mmol, 91 %; R_f = 0.22 (toluene/EtOAc = 4:1)). The diol was dissolved in toluene (30 mL), and Bu_2SnO (1.66 g, 6.6 mmol) was added to the solution. The mixture was stirred for 3 h under reflux conditions and concentrated to dryness. The residue was dissolved in DMF (30 mL), CsF (1.1 g, 6.6 mmol) and

BnBr (820 μL, 6.6 mmol) were added, and the resulting mixture was stirred for 3 h, diluted with EtOAc, washed with $NaHCO_3$, dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography (toluene/EtOAc = 10:1) to yield **4** (1.9 g, 89 %). R_f = 0.51 (toluene/EtOAc = 4:1); ^{1}H NMR ($CDCl_3$): δ = 7.58–7.23 (m, 15H, aromatic protons), 4.84–4.68 (m, 4H, 2 × PhCH_2), 4.60 (d, J = 9.8 Hz, 1H, 1-H), 3.82 (dd, J = 3.2, 2.8 Hz, 1H, 4-H), 3.69 (t, J = 9.5 Hz, 1H, 2-H), 3.59–3.55 (m, J = 3.2 Hz, 2H, 3-H, 5-H), 1.37 ppm (d, J = 6.5 Hz, 3H, 6-H); ^{13}C NMR ($CDCl_3$): δ = 131.9–127.3 (aromatic C), 87.5 (C1), 82.8 (C3), 76.8 (C2), 75.7 (PhCH_2), 74.2 (C5), 72.1 (PhCH_2), 69.4 (C4), 16.7 ppm (C6); HRMS: m/z calcd for $[C_{26}H_{28}O_4SNa]^+$: 459.1600; found: 459.1604.

5: Chloroacetyl chloride (300 μL, 3.73 mmol) was added dropwise to a solution of **4** (811 mg, 1.86 mmol) in DCM/pyridine (18:1, 19 mL) in an ice-cooled bath with mixing. The resulting solution was stirred at room temperature for 1 h and diluted with EtOAc. The mixture was washed with saturated $NaHCO_3$ and saturated NaCl. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The resulting residue was purified on a column of silica gel (hexane/EtOAc = 5:1) to give **5** (858 mg, 90.0 %) as a syrup. R_f = 0.16 (hexane/EtOAc = 6:1); $[\alpha]_D^{26} = -14.2°$ (c = 0.38, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ = 7.60–7.26 (m, 15H, aromatic H), 5.43 (d, J = 3.0 Hz, 1H, 4-H), 4.76 (d, J = 10.2 Hz, 1H, PhCH_2), 4.73 (d, J = 9.9 Hz, 1H, PhCH_2), 4.71 (d, J = 9.8 Hz, 1H, PhCH_2), 4.64 (d, $J_{1\text{-H},2\text{-H}}$ = 9.5 Hz, 1H, 1-H), 4.51 (d, J = 11.1 Hz, 1H, PhCH_2), 4.20 (d, J = 15.0 Hz, 1H, ClCH_2CO), 4.10 (d, J = 15.1 Hz, 1H, ClCH_2CO), 3.73 (q, $J_{5\text{-H},6\text{-H}}$ = 6.4 Hz, 1H, 5-H), 3.68 (dd, $J_{3\text{-H},4\text{-H}}$ = 3.2 Hz, 1H, 3-H), 3.62 (t, $J_{2\text{-H},3\text{-H}}$ = 9.2 Hz, 1H, 2-H), 1.28 ppm (d, J = 6.4 Hz, 3H, 6-H); ^{13}C NMR ($CDCl_3$): δ = 167.4 (ClCH_2CO), 138.1–127.6 (aromatic C), 87.4 (C1), 81.0 (C3), 76.3 (C2), 75.7 (PhCH_2), 72.7 (C5), 72.2 (PhCH_2), 71.9 (C4), 40.8 (ClCH_2CO), 16.8 ppm (C6); HRMS: m/z calcd for $[C_{28}H_{29}ClO_5SNa]^+$: 535.1316; found: 535.1316.

6: DAST (360 μL, 2.91 mmol) was added dropwise to a solution of **5** (737 mg, 1.44 mmol) in DCM (14 mL) at 0 °C under N_2 atmosphere. The reaction mixture was stirred at 40 °C for 13 h and then diluted with EtOAc. The solution was washed with saturated $NaHCO_3$ and saturated NaCl. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The resulting residue was purified on a column of silica gel (hexane/EtOAc = 5:1–4:1) to give **6** (555 mg, 91.4 %, α/β = 17:1) as a pale-yellow syrup. R_f = 0.22 (α), 0.14 (β) (hexane/EtOAc = 5:1); $[\alpha]_D^{26} = -42.1°$ (α/β = 17:1, c = 0.20, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ = 7.51–7.05 (m, 20H, aromatic H), 5.57 (dd, $J_{1\text{-H},2\text{-H}}$ = 2.7 Hz, $^2J_{1\text{-H},F}$ = 53.2 Hz, 1H, 1-Hα), 5.48 (brd, J = 2.6 Hz, 1H, 4-Hα), 5.38 (brs, 1H, 4-Hβ), 5.18 (dd, $J_{1\text{-H},2\text{-H}}$ = 6.8 Hz, $^2J_{1\text{-H},F}$ = 52.4 Hz, 1H, 1-Hβ), 4.85 (d, J = 11.8 Hz, 1H, PhCH_2-α), 4.83 (d, J = 9.2 Hz, 1H, PhCH_2-β), 4.77–4.68 (m, 4H, PhCH_2-α,β), 4.59 (d, J = 11.2 Hz, 1H, PhCH_2-α), 4.55 (d, J = 11.4 Hz, 1H, PhCH_2-β), 4.25 (q, J = 6.5, 13.1 Hz, 1H, 5-Hα), 4.20 (dd, J = 15.3 Hz, 2H, ClCH_2CO-β), 4.15 (t, J = 15.2 Hz, 2H, ClCH_2CO-α), 4.00 (dd, $J_{3\text{-H},4\text{-H}}$ = 3.3 Hz, 1H, 3-Hα), 3.81 (q, 1H, 5-Hβ), 3.74 (ddd, $J_{2\text{-H},3\text{-H}}$ = 10.0 Hz, $^3J_{2\text{-H},F}$ = 12.7 Hz, 1H, 2-Hα), 3.67 (ddd, $J_{2\text{-H},3\text{-H}}$ = 9.7 Hz, $^3J_{2\text{-H},F}$ = 12.9 Hz, 1H, 2-Hβ), 3.62 (dd, $J_{3\text{-H},4\text{-H}}$ = 3.2 Hz, 1H, 3-Hβ), 1.30 (d, J = 6.4 Hz, 3H, 6-Hβ), 1.20 ppm (d, J = 6.5 Hz, 3H, 6-Hα); ^{13}C NMR ($CDCl_3$): δ = 167.1 (ClCH_2CO-α), 137.8–127.9 (aromatic C), 106.1 (d, $J_{C1,F}$ = 226.3 Hz, C1α), 75.4 (C3α), 74.4 (d, $^2J_{C2,F}$ = 23.8 Hz, C2α), 73.8 (PhCH_2-α), 72.2 (C4α, PhCH_2-α), 67.0 (d, $^3J_{C5,F}$ = 3.6 Hz, C5α), 40.7 (ClCH_2CO-α), 16.0 ppm (C6α); HRMS: m/z calcd for $[C_{22}H_{24}ClFO_5Na]^+$: 445.1188; found: 445.1181.

7: DABCO (447 mg, 3.90 mmol) was added to a solution of **6** (550 mg, 1.30 mmol) in EtOH/pyridine (5:1, 13 mL). The resulting mixture was stirred at 70 °C for 15 h and concentrated. The resulting residue was dissolved in EtOAc, and the mixture was washed with saturated $NaHCO_3$ and saturated NaCl. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The resulting residue was purified on a column of silica gel (hexane/EtOAc = 3:1) to give **7** (415 mg, 92.1 %, α/β = 25:1) as a pale-yellow syrup. R_f = 0.22 (α/β mixture) (hexane/EtOAc = 3:1); $[\alpha]_D^{26} = -26.4°$ (α/β = 25:1, c = 0.45, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ = 7.53–7.28 (m, 20H, aromatic H), 5.55 (dd, $J_{1\text{-H},2\text{-H}}$ = 2.7 Hz, $^2J_{1\text{-H},F}$ = 53.7 Hz, 1H, 1-Hα), 5.15 (dd, $J_{1\text{-H},2\text{-H}}$ = 7.3 Hz, $^2J_{1\text{-H},F}$ = 52.8 Hz, 1H, 1-Hβ), 4.87–4.65 (m, 8H, PhCH_2-α,β), 4.09 (q, $J_{5\text{-H},6\text{-H}}$ = 6.6 Hz, 1H, 5-Hα), 3.89 (dd, $J_{3\text{-H},4\text{-H}}$ = 3.1 Hz, 1H, 3-Hα), 3.88 (d, J = 2.3 Hz, 1H, 4-Hα), 3.82 (ddd, $J_{2\text{-H},3\text{-H}}$ = 9.4 Hz, $^3J_{2\text{-H},F}$ = 25.3 Hz, 1H, 2-Hα), 3.77–3.71 (m, 2H, 2-Hβ, 4-Hβ), 3.64 (q, $J_{5\text{-H},6\text{-H}}$ = 6.5 Hz, 1H, 5-Hβ), 3.53 (dd, $J_{2\text{-H},3\text{-H}}$ = 9.2 Hz, $J_{3\text{-H},4\text{-H}}$ = 3.2 Hz, 1H, 3-Hβ), 2.63 (brs, 1H, 4-OHβ), 2.43 (brs, 1H, 4-OHα), 1.40 (d, J = 6.5 Hz, 3H, 6-Hβ), 1.31 ppm (d, J = 6.6 Hz, 3H, 6-Hα); ^{13}C NMR ($CDCl_3$): δ = 137.8–127.8 (aromatic C), 106.0 (d, $J_{C1,F}$ = 225.0 Hz, C1α), 77.3 (C3α), 74.8 (d, $^2J_{C2,F}$ = 24.5 Hz, C2α), 73.6 (PhCH_2-α), 72.7 (PhCH_2-α), 69.6 (C4α), 68.0 (d, $^3J_{C5,F}$ = 3.6 Hz, C5α), 16.0 ppm (C6α); HRMS: m/z calcd for $[C_{20}H_{23}FO_4Na]^+$: 369.1473; found: 369.1485.

8: DMAP (2.98 g, 24.1 mmol) and succinic anhydride (2.44 g, 24.1 mmol) were added to a solution of **4** (3.10 g, 7.10 mmol) in DCM/pyridine (1:1, 69 mL). The mixture was stirred at 50 °C for 12 h and diluted with EtOAc. The mixture was washed with saturated $NaHCO_3$ and saturated NaCl. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The resulting residue was purified on a column of silica gel (hexane/EtOAc = 1:1) to give **8** (3.71 g, 97.4 %) as a syrup. R_f = 0.33 (hexane/EtOAc = 2:3); $[\alpha]_D^{26} = -21.3°$ (c = 1.03, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ = 7.59–7.24 (m, 15H, aromatic H), 5.39 (d, J = 2.1 Hz, 1H, 4-H), 4.77–4.68 (m, 3H, PhCH_2), 4.63 (d, $J_{1\text{-H},2\text{-H}}$ = 9.0 Hz, 1H, 1-H), 4.49 (d, 1H, PhCH_2), 3.71–3.60 (m, 3H, 5-H, 3-H, 2-H), 2.79–2.62 (m, 4H, OCOCH_2CH_2COOH), 1.24 ppm (d, J = 6.4 Hz, 3H, 6-H); ^{13}C NMR ($CDCl_3$): δ = 177.5, 171.9 (2 × C=O), 138.2–127.5 (aromatic C), 87.4 (C1), 81.1 (C3), 76.5 (C2), 75.7 (PhCH_2), 73.0 (C5), 71.8 (PhCH_2), 70.2 (C4), 28.9, 28.8 (OCOCH_2CH_2COOH), 16.7 ppm (C6); HRMS: m/z calcd for $[C_{30}H_{32}O_7SNa]^+$: 559.1761; found: 559.1751.

9: Compound **9** was synthesized according to the procedure described for the synthesis of **8** by using **7** (384 mg, 1.11 mmol), DCM/pyridine (1:1, 11 mL), DMAP (412 mg, 3.34 mmol), and succinic anhydride (341 mg, 3.34 mmol). The reaction was carried out at 50 °C for 18 h. Column chromatography (hexane/EtOAc = 2:3) yielded **9** (297 mg, 60.0 %, α/β = 50:1 based on ^{1}H NMR) as a pale-yellow syrup. R_f = 0.25 (hexane/EtOAc = 2:3); $[\alpha]_D^{26} = -21.3°$ (α/β = 50:1, c = 1.03, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ = 7.49–7.26 (m, 10H, aromatic H), 5.56 (dd, $J_{1\text{-H},2\text{-H}}$ = 2.7 Hz, $^2J_{1\text{-H},F}$ = 53.3 Hz, 1H, 1-Hα), 5.46 (d, J = 2.5 Hz, 1H, 4-Hα), 5.17 (dd, $J_{1\text{-H},2\text{-H}}$ = 7.1 Hz, $^2J_{1\text{-H},F}$ = 52.7 Hz, 1H, 1-Hβ), 4.85 (d, J = 11.8 Hz, 1H, PhCH_2-α), 4.72 (d, J = 11.3 Hz, 1H, PhCH_2-α), 4.70 (d, J = 11.7 Hz, 1H, PhCH_2-α), 4.56 (d, J = 11.3 Hz, 1H, PhCH_2-α), 4.21 (q, $J_{5\text{-H},6\text{-H}}$ = 6.5 Hz, 1H, 5-Hα), 3.97 (dd, $J_{3\text{-H},4\text{-H}}$ = 3.3 Hz, 1H, 3-Hα), 3.76 (ddd, $J_{2\text{-H},3\text{-H}}$ = 10.0 Hz, $^3J_{2\text{-H},F}$ = 25.4 Hz, 1H, 2-Hα), 3.69 (ddd, $J_{2\text{-H},3\text{-H}}$ = 9.4 Hz, $^3J_{2\text{-H},F}$ = 23.4 Hz, 1H, 2-Hβ), 3.59 (dd, $J_{3\text{-H},4\text{-H}}$ = 3.2 Hz, 1H, 3-Hβ), 2.77–2.58 (m, 4H, OCOCH_2CH_2COOH), 1.16 ppm (d, J = 6.5 Hz, 3H, 6-Hα); ^{13}C NMR ($CDCl_3$): δ = 176.9, 171.7 (2 × C=Oα), 137.9–127.8 (aromatic C), 106.2 (d, $J_{C1,F}$ = 226.0 Hz, C1α), 75.4 (C3α), 74.6 (d, $^2J_{C2,F}$ = 23.8 Hz, C2α), 73.8, 71.8 (2 × PhCH_2-α), 70.5 (C4α), 67.3 (d, $^3J_{C5,F}$ = 3.5 Hz, C5α), 28.9, 28.9 (OCOCH_2CH_2COOH-α), 16.0 ppm (C6α); HRMS: m/z calcd for $[C_{24}H_{27}FO_7Na]^+$: 469.1633; found: 469.1637.

10: A solution of **8** (314 mg, 0.585 mmol), HOBt (100 mg, 0.725 mmol), and DIC (140 μL, 0.867 mmol) in DMF (5.0 mL) was soaked into NovaSyn TG amino resin (1.00 g, 0.29 mmol, 0.29 mmol g^{-1} loading) under N_2 atmosphere, and the swelled resin was allowed to stand at room temperature for 24 h without mixing. The reaction progress was monitored by the Kaiser and TNBS tests. After completion of the reaction, the resin was washed with DMF, DCM, MeOH, and DCM, and dried under vacuum to afford **10**. The reaction yield was estimated by weight gain (1.10 g, 67.4 %). Finally, any remaining amine functionality was capped by using Ac_2O/pyridine/DCM (1:1.5:1 $v/v/v$, 5.0 mL) in the same manner to afford resin-bound **10** (0.177 mmol g^{-1} loading).

11: Compound **11** was prepared according to the procedure described for the synthesis of **10** by using **9** (288 mg, 645 μmol), HOBt (260 mg, 1.89 mmol), DIC (295 μL, 1.83 mmol), and NovaSyn TG amino resin (2.09 g, 627 μmol, 0.3 mmol g^{-1} loading). Yield: 2.25 g (60.2 %, 0.166 mmol g^{-1} loading).

Comparison of Solid-Phase Reaction With and Without Mixing (SSPR) using 10

12: To confirm the effect of mixing, **10** was selected as a glycosyl donor and coupled with *n*-octanol in the presence of DMTST as a promoter. In the following experiments, identical reaction conditions, except for the

concentrations, were used to determine any differences in the solid-phase reactions with or without mixing.

Preparation of reagent stock solution containing an acceptor and DMTST: MeOTf (11.6 μL, 101 μmol) was added to MeSSMe (9.2 μL, 101 μmol) and stirred at room temperature for 5 min, and then the mixture was diluted with DCE (300 μL) and CH_3CN (300 μL) to give a stock solution of DMTST (0.168 M). *n*-Octanol (10.4 μL, 65.4 μmol) was added to this solution to afford a stock solution of *n*-octanol (0.109 M)/DMTST (0.168 M).

Procedure for SSPR: A stock solution of acceptor (0.109 M)/DMTST (0.168 M) (200 μL, acceptor: 21.8 μmol, DMTST: 33.6 μmol) was soaked into resin-bound **10** (40.5 mg, 11.2 μmol) at −30 °C under N_2 atmosphere. The reaction was stopped after 15 min, and the resin was washed successively with DCM, DMF, and DCM. The resulting resin was swollen with NaOMe/MeOH (4 drops) in DCE (0.5 mL) and allowed to stand for 1 h. The resin was washed with DCM, the eluent was concentrated, and the residue containing **12** was analyzed directly by ^{1}H NMR spectroscopy.

Solid-phase reaction procedure with mixing: A stock solution of acceptor (0.109 M)/DMTST (0.168 M) was diluted with DCE/CH_3CN (1:1) to form a solution of acceptor (0.055 M)/DMTST (0.084 M). The solution (400 μL, acceptor: 22 μmol, DMTST: 34 μmol) was added to **10** (40.4 mg, 11.2 μmol) at −30 °C under N_2 atmosphere. The reaction slurry was stirred at that temperature for 15 min. The resin was treated as described in the diffusive-reaction procedure.

Analysis of the results: Reaction yields were estimated by ^{1}H NMR analysis. Some of the signals are summarized as follows. Doublets observed at 4.31 and 1.34 ppm were assigned to be anomeric proton and 6-H of **12** (β). Doublets observed at 4.28 and 1.18 ppm belong to a hydrolysis product, which was formed during a quenching reaction after a given reaction time. The starting material, phenylthiofucoside, disappeared after quenching. Under these conditions, formation of an α glycoside could be negligible. Based on these analyses, we concluded that the reaction rates could be estimated as the ratio of the integrals of the two major products, octyl β-fucopyranoside and the hydrolysis product, which revealed that the SSPR is 1.43 times faster.

Some of the chemical shifts of the assigned protons for the glycosides and the hydrolysis product are given below.

12α (R = A[1]): ^{1}H NMR ($CDCl_3$): δ = 4.75 (d, $J_{1\text{-H},2\text{-H}}$ = 3.6 Hz, 1H, 1-H), 3.93 (m, 1H, 5-H), 3.88 (dd, $J_{3\text{-H},4\text{-H}}$ = 3.3 Hz, 1H, 3-H), 3.83 (br d, J = 3.3 Hz, 1H, 4-H), 3.80 (dd, $J_{2\text{-H},3\text{-H}}$ = 9.8 Hz, 1H, 2-H), 3.64 (m, 1H, $OCH_2CH_2(CH_2)_6CH_3$), 3.43 (dt, J = 6.7, 9.8 Hz, 1H, $OCH_2CH_2(CH_2)_6CH_3$), 1.27 ppm (6-H); ^{13}C NMR ($CDCl_3$): δ = 97.1 (C1), 78.1 (C3), 75.7 (C2), 70.2 (C4), 68.2 ($OCH_2CH_2(CH_2)_6CH_3$), 65.1 (C5), 16.2 ppm (C6).

12β (R = A[1]): ^{1}H NMR ($CDCl_3$): δ = 4.31 (d, $J_{1\text{-H},2\text{-H}}$ = 7.7 Hz, 1H, 1-H), 3.94 (dt, J = 6.4, 9.4 Hz, 1H, $OCH_2CH_2(CH_2)_6CH_3$), 3.74 (br d, J = 4.2 Hz, 1H, 4-H), 3.59 (dd, $J_{2\text{-H},3\text{-H}}$ = 9.2 Hz, 1H, 2-H), 3.53–3.46 (m, 3H, 3-H, 5-H, $OCH_2CH_2(CH_2)_6CH_3$), 1.34 ppm (d, J = 6.5 Hz, 3H, 6-H); ^{13}C NMR ($CDCl_3$): δ = 103.6 (C1), 81.0 (C3), 79.0 (C2), 70.0 ($OCH_2CH_2(CH_2)_6CH_3$), 69.9 (C5), 69.5 (C4), 16.6 ppm (C6).

2,3-Di-*O*-benzyl-α-L-fucopyranose: ^{1}H NMR ($CDCl_3$): δ = 5.51 (d, J = 2.2 Hz, 1H, 1-H), 4.28 (br s, 1H, 4-H), 3.84–3.78 (m, 3H, 2-H, 3-H, 5-H), 1.18 ppm (d, J = 6.2 Hz, 3H, 6-H); ^{13}C NMR ($CDCl_3$): δ = 99.3 (C1), 87.3, 84.2 (C4), 72.9, 70.6, 20.2 ppm (C6).

Glycosylation using Fucosyl Fluoride and [$Cp_2Hf(OTf)_2$] Based on SSPR

Preparation of reagent stock solution containing acceptor and [$Cp_2Hf(OTf)_2$]: DCE (0.9 mL) was added to [Cp_2HfCl_2] (57.7 mg, 149 μmol) and AgOTf (77.4 mg, 304 μmol) under N_2 atmosphere in the dark, and the mixture was stirred for 2 h then centrifuged at about 2000 rpm for 10 min. The supernatant solution was used as a stock solution of [$Cp_2Hf(OTf)_2$] (0.166 M). A stock solution of [$Cp_2Hf(OTf)_2$] (181 μL, 30.0 μmol) was added to a solution of *n*-octanol (3.2 μL, 20.1 μmol) dissolved in CH_3CN (181 μL) under N_2 atmosphere to afford a solution of acceptor (0.055 M)/[$Cp_2Hf(OTf)_2$] (0.083 M).

Procedure for SSPR: Resin-bound **11** (20.0 mg, 3.32 μmol) was swollen with this stock solution (120 μL, acceptor: 6.64 μmol, [$Cp_2Hf(OTf)_2$]: 9.96 μmol) at −30 °C under N_2 atmosphere. After completion of the reaction, the resin was washed successively with DCM, DMF, and DCM. The resulting resin was swollen with NaOMe/MeOH (4 drops) in DCE (0.5 mL) and allowed to stand for 1 h. The resin was washed with DCM, and the eluent was concentrated. The residue containing **12** was analyzed directly by ^{1}H NMR spectroscopy.

Resin-bound **14**: A stock solution of DMTST (0.168 M) prepared as described above (530 μL, 89.0 μmol) was mixed with **13**[32] (26.6 mg, 58.8 μmol, α/β = 21:1) at −15 °C to give a solution of acceptor (0.111 M)/DMTST (0.168 M). Compound **10** (100.4 mg, 27.9 μmol) was swollen by addition of the DMTST/acceptor solution (500 μL, acceptor: 55.5 μmol, DMTST: 84.0 μmol) at −15 °C, and the reaction was set (TF −15, 0, 1.25) under SSPR conditions. Formation of **12** (R = A[2]; see Table 1) was monitored to follow the progress of the reaction by QITMS after aliquots of resin were treated with NaOMe/MeOH (4 drops) in DCE (0.5 mL) at room temperature for 1 h. After completion of the reaction, the resin was washed successively with DCM, DMF, and DCM to afford **14. 12** (R = A[2]; see Table 1): MS (ESI-QIT): *m*/*z* calcd for $[C_{47}H_{51}FO_9Na]^+$: 801.3; found: 801.3.

Resin-bound **16**: A stock solution of DMTST (0.168 M, 278 μL, 46.7 μmol) was mixed with **15**[34] (14.0 mg, 30.9 μmol, α/β = 17:1) at −15 °C to give a solution of acceptor (0.111 M)/DMTST (0.168 M). Compound **10** (55.6 mg, 15.5 μmol) was swollen by addition of the acceptor/DMTST solution (278 μL, acceptor: 30.9 μmol, DMTST: 46.7 μmol) at −30 °C, and the reaction was set (TF −30, 0, 2.5) under SSPR conditions. Formation of **17** was monitored to follow the progress of the reaction by QITMS after aliquots of resin were treated with NaOMe/MeOH (4 drops) in DCE (0.5 mL) at room temperature for 1 h. After completion of the reaction, the resin was washed successively with DCM, DMF, and DCM to afford **16. 17**: MS (ESI-QIT): *m*/*z* calcd for $[C_{47}H_{51}FO_9Na]^+$: 801.3; found: 801.3.

Resin-bound **19**: A stock solution of [$Cp_2Hf(OTf)_2$] was prepared prior to the reaction. DCE (0.9 mL) was added to [Cp_2HfCl_2] (79.1 mg, 206 μmol) and AgOTf (107.1 mg, 412.7 μmol) under N_2 atmosphere in the dark, and the mixture was stirred for 2 h then centrifuged at about 2000 rpm for 10 min. The supernatant solution was used as a stock solution of [$Cp_2Hf(OTf)_2$] (0.229 M).

A stock solution of [$Cp_2Hf(OTf)_2$] (362 μL, 82.9 μmol) was added to a solution of **18** (32.7 mg, 60.3 μmol) dissolved in CH_3CN (181 μL) under N_2 atmosphere to afford a solution of acceptor (0.111 M)/[$Cp_2Hf(OTf)_2$] (0.153 M). Resin-bound **14** (90.4 mg, 25.1 μmol) was swollen with this stock solution (450 μL, acceptor: 50.0 μmol, [$Cp_2Hf(OTf)_2$]: 68.9 μmol) at −30 °C under N_2 atmosphere, and the reaction mixture was maintained under SSPR conditions (TF−30, 0, 2.5). Formation of **20** was monitored to follow the progress of the reaction by QITMS after aliquots of resin were treated with NaOMe/MeOH (4 drops) in DCE (0.5 mL) at room temperature for 1 h. After completion of the reaction, the resin was washed successively with DCM, DMF, and DCM to afford **19. 20**: MS (ESI-QIT): *m*/*z* calcd for $[C_{80}H_{84}O_{14}SNa]^+$: 1323.5; found: 1323.5.

Resin-bound **21**: A was prepared prior to the reaction. MeSSMe (22.8 μL, 251 μmol) was added to MeOTf (28.7 μL, 251 μmol) under N_2 atmosphere, and the mixture was stirred for 5 min. The mixture was diluted with DCE (1.0 mL) and CH_3CN (0.5 mL) to afford a stock solution of DMTST (0.167 M).

n-Octanol (26.5 μL, 167 μmol) was added to DMTST to give a solution of acceptor (0.111 M)/DMTST (0.167 M). Resin-bound **19** (25.1 μmol) was swollen by addition of the acceptor/DMTST solution (450 μL, acceptor: 50.0 μmol, DMTST: 75.2 μmol) at −30 °C, and the reaction was set (TF −30, 0, 2.5) under SSPR conditions. Formation of **22** was monitored to follow the progress of the reaction by QITMS after aliquots of resin were treated with NaOMe/MeOH (4 drops) in DCE (0.5 mL) at room temperature for 1 h. After completion of the reaction, the resin was washed successively with DCM, DMF, and DCM to afford **21. 22**: MS (ESI-QIT): *m*/*z* calcd for $[C_{82}H_{96}O_{15}Na]^+$: 1343.7; found: 1343.7.

23: Resin-bound **21** (25.1 μmol) was treated with NaOMe/MeOH (100 μL) in DCE (1.0 mL) at room temperature for 1 h. After completion of the reaction, the resin was filtered and washed with DCM. The eluent was neutralized with Dowex 50W H^+ form, filtered, and concentrated.

The residue was hydrogenolized with 20% $Pd(OH)_2$ on charcoal in MeOH/EtOAc (1:1 *v*/*v*, 6.0 mL) under H_2 atmosphere at room temperature for 2.5 days. The mixture was filtered thorough celite and the eluent was concentrated. The obtained residue was then dissolved in water and purified by using a reverse-phase short column (Sep-Pak C18). The MeOH fraction was concentrated to afford **23** as an anomeric mixture (5.0 mg, 33.1% over 5 steps). MS (ESI-QIT): *m*/*z* calcd for $[C_{26}H_{48}O_{15}Na]^+$: 623.3; found: 623.2.

Separation of all anomers of **23** with LCMS: Separation of eight sets of anomers was achieved by sequential HPLC steps (Table 3). First HPLC was carried out by using a reverse-phase monolithic column (condition 1), which resulted in the isolation of compounds **23–4**, **23–3**, **23–2**, **23–8**, **23–1**, and **23–5** (Figure 5a). Unresolved peaks that appeared at 44.2 and 46.3 min (**23–7** and **23–6**) were further separated on another reverse-phase column (condition 2). The purity and anomeric assignment of each peak was achieved based on NMR analysis. The HPLC conditions were as follows: Condition 1: column: Chromolith Performance RP-18 (100 mm × 4.6 mm); flow rate: 1.0 mL min^{-1}; mobile phase: CH_3CN/H_2O (15:85); column temperature: 30 °C. Condition 2: column: Xterra C18 (250 mm × 4.6 mm); flow rate: 1.0 mL min^{-1}; mobile phase: CH_3CN/H_2O (20:80); column temperature: 30 °C. Details of the monitoring conditions for mass spectrometry are as follows: Capillary: 3.0 kV; cone: 45 V; source temperature: 110 °C; desolvation temperature: 310 °C; monitoring: single-ion; gas flow (desolvation): 400 L h^{-1}; gas flow (cone): 50 L h^{-1}.

Determination of anomeric configurations of **23**: Determination of the anomeric configuration of each glycosidic linkage was based on ^{1}H NMR, COSY, and HSQC (in D_2O) experiments. Chemical shifts for ^{13}C were obtained by HSQC experiments. Chemical shifts of the anomeric protons are listed in Table 3.

23–1: HPLC (condition 1): t_R: 41.0 min; yield estimated from the overall yield and HPLC profile: see Table 3; ^{1}H NMR (D_2O): δ = 4.98 (d, $J_{1\text{-H},2\text{-H}}$ = 4.0 Hz, 1H, 1-H), 4.91 (d, $J_{1''\text{-H},2''\text{-H}}$ = 3.8 Hz, 1H, 1″-H), 4.88 ppm (d, $J_{1'\text{-H},2'\text{-H}}$ = 2.7 Hz, 1H, 1′-H); HRMS: *m*/*z* calcd for $[C_{26}H_{48}O_{15}Na]^+$: 623.2885; found: 623.2886.

23–2: HPLC (condition 1): t_R: 32.9 min; ^{1}H NMR (D_2O): δ = 5.02 (d, $J_{1'\text{-H},2'\text{-H}}$ = 2.7 Hz, 1H, 1′-H), 4.94 (d, $J_{1''\text{-H},2''\text{-H}}$ = 3.6 Hz, 1H, 1″-H), 4.48 (d, $J_{1\text{-H},2\text{-H}}$ = 8.0 Hz, 1H, 1-H), 4.21 (dd, $J_{4'\text{-H},5'\text{-H}}$ = 4.4 Hz, $J_{5'\text{-H},6'\text{-H}}$ = 8.3 Hz, 1H, 5′-H), 4.11 (q, *J* = 7.0 Hz, 1H, 5″-H), 4.01 (br d, 1H, 4′-H), 3.95 (dd, $J_{5\text{-H},6a\text{-H}}$ = 5.6 Hz, $J_{6a\text{-H},6b\text{-H}}$ = 10.2 Hz, 1H, 6a-H), 3.91–3.84 (m, 5H, 2′-H, 3′-H, 3″-H, 6a′-H, OCH_2CH$_2$(CH$_2$)$_6$CH$_3$), 3.82–3.77 (m, 3H, 2″-H, 4″-H, 6b-H), 3.73–3.66 (m, 3H, 5-H, 6b′-H, OCH_2CH$_2$(CH$_2$)$_6$CH$_3$), 3.50 (t, $J_{3\text{-H},4\text{-H}}$ = 9.1 Hz, 1H, 3-H), 3.45 (t, $J_{4\text{-H},5\text{-H}}$ = 9.5 Hz, 1H, 4-H), 3.25 (t, $J_{2\text{-H},3\text{-H}}$ = 8.7 Hz, 1H, 2-H), 1.64 (m, 2H, OCH$_2$CH_2(CH$_2$)$_5$CH$_3$), 1.35–1.26 (m, 10H, OCH$_2$CH$_2$(CH_2)$_5$CH$_3$), 1.24 (d, *J* = 6.5 Hz, 3H, 6″-H), 0.88 ppm (t, 3H, OCH$_2$CH$_2$(CH$_2$)$_5$CH_3); HRMS: *m*/*z* calcd for $[C_{26}H_{48}O_{15}Na]^+$: 623.2885; found: 623.2886.

23–3: HPLC (condition 1): t_R: 26.6 min; ^{1}H NMR (D_2O): δ = 4.94 (d, $J_{1''\text{-H},2''\text{-H}}$ = 3.9 Hz, 1H, 1″-H), 4.90 (d, $J_{1\text{-H},2\text{-H}}$ = 3.6 Hz, 1H, 1-H), 4.43 (d, $J_{1'\text{-H},2'\text{-H}}$ = 7.8 Hz, 1H, 1′-H), 4.14 (br d, *J* = 11.1 Hz, 1H, 6a-H), 4.09 (q, *J* = 6.8, 13.7 Hz, 1H, 5″-H), 3.94 (br d, *J* = 3.2 Hz, 1H, 4′-H), 3.92–3.76 (m, 8H, 2″-H, 3″-H, 4″-H, 5-H, 5′-H, 6a′-H, 6b-H, 6b′-H), 3.74–3.62 (m, 3H, 3-H, 3′-H, OCH_2CH$_2$(CH$_2$)$_6$CH$_3$), 3.58–3.43 (m, $J_{4\text{-H},5\text{-H}}$ = 8.4 Hz, 4H, 2-H, 2′-H, 4-H, OCH_2CH$_2$(CH$_2$)$_6$CH$_3$), 1.62 (m, 2H, OCH$_2$CH_2(CH$_2$)$_5$CH$_3$), 1.32–1.27 (m, 10H, OCH$_2$CH$_2$(CH_2)$_5$CH$_3$), 1.23 (d, *J* = 6.6 Hz, 3H, 6″-H), 0.86 ppm (t, 3H, OCH$_2$CH$_2$(CH$_2$)$_5$CH_3); ^{13}C NMR (D_2O): δ = 103.1 (C1′), 99.2 (C1″), 97.8 (C1), 73.4 (C5″), 72.6 (C3′), 71.8 (C3), 71.6 (C4″), 71.0 (C2), 70.7 (C2′), 70.5 (C5), 69.4 (C3″), 69.2 (C4), 68.6 (C4′), 68.2 (OCH$_2$CH$_2$(CH$_2$)$_6$CH$_3$), 68.1 (C6), 67.9 (C2″), 67.2 (C6′), 66.7 (C5″), 15.5 ppm (C6″); HRMS: *m*/*z* calcd for $[C_{26}H_{48}O_{15}Na]^+$: 623.2885; found: 623.2885.

23–4: HPLC (condition 1); t_R: 23.4 min; ^{1}H NMR (D_2O): δ = 4.97 (d, $J_{1''\text{-H},2''\text{-H}}$ = 3.8 Hz, 1H, 1″-H), 4.50 (d, $J_{1'\text{-H},2'\text{-H}}$ = 7.9 Hz, 1H, 1′-H), 4.48 (d, $J_{1\text{-H},2\text{-H}}$ = 8.2 Hz, 1H, 1-H), 4.22 (br d, *J* = 1.5 Hz, 1H, 6a-H), 4.12 (q, *J* = 6.7, 13.4 Hz, 1H, 5″-H), 3.96 (br d, *J* = 3.2 Hz, 1H, 4′-H), 3.95–3.86 (m, 5H, 3″-H, 5′-H, 6a-H, 6b′-H, OCH_2CH$_2$(CH$_2$)$_6$CH$_3$), 3.83–3.79 (m, $J_{2''\text{-H},3''\text{-H}}$ = 10.3 Hz, 3H, 2″-H, 4″-H), 3.75–3.63 (m, 3H, 3′-H, 5-H, OCH_2CH$_2$(CH$_2$)$_6$CH$_3$), 3.59 (dd, $J_{2'\text{-H},3'\text{-H}}$ = 10.0 Hz, 1H, 2′-H), 3.49 (m, $J_{3\text{-H},4\text{-H}}$ = 9.0 Hz, $J_{4\text{-H},5\text{-H}}$ = 9.1 Hz, 2H, 3-H, 4-H), 3.28 (t, $J_{2\text{-H},3\text{-H}}$ = 8.5 Hz, 1H, 2-H), 1.64 (m, 2H, OCH$_2$CH_2(CH$_2$)$_5$CH$_3$), 1.39–1.30 (m, 10H, OCH$_2$CH$_2$(CH_2)$_5$CH$_3$), 1.28 (d, *J* = 6.6 Hz, 3H, 6″-H), 0.89 ppm (t, 3H, OCH$_2$CH$_2$(CH$_2$)$_5$CH_3); ^{13}C NMR (D_2O): δ = 103.4 (C1′), 102.2 (C1), 99.2 (C1″), 75.6 (C3), 74.9 (C5), 73.5 (C5′), 73.0 (C2), 72.4 (C3′), 71.6 (C4″), 70.7 (OCH$_2$CH$_2$(CH$_2$)$_6$CH$_3$), 70.5 (C2′), 69.4 (C3″), 69.3 (C4), 68.6 (C4″), 68.3 (C6), 68.0 (C2″), 67.3 (C6′), 66.7 (C5″), 15.1 ppm (C6″); HRMS: *m*/*z* calcd for $[C_{26}H_{48}O_{15}Na]^+$: 623.2885; found: 623.2885.

23–5: HPLC (condition 1): t_R: 68.3 min; ^{1}H NMR (D_2O): δ = 4.99 (d, $J_{1'\text{-H},2'\text{-H}}$ = 3.2 Hz, 1H, 1′-H), 4.94 (d, $J_{1\text{-H},2\text{-H}}$ = 3.8 Hz, 1H, 1-H), 4.43 (d, $J_{1''\text{-H},2''\text{-H}}$ = 7.8 Hz, 1H, 1″-H), 4.18 (dd, 1H, 5′-H), 4.06–4.02 (m, 3H, 4′-H, 6a-H, 6a′-H), 3.90–3.64 (m, 10H, 2′-H, 3-H, 3′-H, 3″-H, 4″-H, 5-H, 5″-H, 6b-H,6b′-H, OCH_2CH$_2$(CH$_2$)$_6$CH$_3$), 3.59–3.49 (m, 4H, 2-H, 2″-H, 4-H, OCH_2CH$_2$(CH$_2$)$_6$CH$_3$), 1.65 (m, 2H, OCH$_2$CH_2(CH$_2$)$_5$CH$_3$), 1.35–1.27 (m, 13H, 6″-H, OCH$_2$CH$_2$(CH_2)$_5$CH$_3$), 0.89 ppm (t, 3H, OCH$_2$CH$_2$(CH$_2$)$_5$CH_3); ^{13}C NMR (D_2O): δ = 102.6 (C1″), 98.3 (C1), 98.2 (C1′), 73.4 (C3), 72.8 (C3″), 71.4 (C4″), 71.2 (C2), 70.8 (C5″), 70.4 (C2″), 70.2 (C2′), 69.5 (C4), 69.4 (C3′), 69.3 (C5), 69.1 (C4′), 68.9 (C5′), 68.6 (OCH$_2$CH$_2$(CH$_2$)$_6$CH$_3$), 68.3 (C6 or C6′), 65.7 (C6 or C6′), 15.4 ppm (C6″); HRMS: *m*/*z* calcd for $[C_{26}H_{48}O_{15}Na]^+$: 623.2885; found: 623.2885.

23–6: HPLC (condition 2); t_R: 38.6 min; ^{1}H NMR (D_2O): δ = 5.01 (d, $J_{1'\text{-H},2'\text{-H}}$ = 3.4 Hz, 1H, 1′-H), 4.50 (d, $J_{1\text{-H},2\text{-H}}$ = 8.0 Hz, 1H, 1-H), 4.45 (d, $J_{1''\text{-H},2''\text{-H}}$ = 7.9 Hz, 1H, 1″-H), 4.20 (dd, $J_{4'\text{-H},5'\text{-H}}$ = 4.9 Hz, $J_{5'\text{-H},6'\text{-H}}$ = 7.2 Hz, 1H, 5′-H), 4.07–4.00 (m, 3H, 4′-H, 6a-H, 6a′-H), 3.95–3.65 (m, 10H, 2′-H, 3′-H, 3″-H, 4″-H, 5-H, 5″-H, 6b-H, 6b′-H, OCH_2CH$_2$(CH$_2$)$_6$CH$_3$), 3.56–3.49 (m, 3H, 2′-H, 3-H, 4-H), 3.28 (t, $J_{2\text{-H},3\text{-H}}$ = 8.2 Hz, 1H, 2-H), 1.62 (m, 2H, OCH$_2$CH_2(CH$_2$)$_5$CH$_3$), 1.35–1.28 (m, 13H, 6″-H, OCH$_2$CH$_2$(CH_2)$_5$CH$_3$), 0.89 ppm (t, 3H, OCH$_2$CH$_2$(CH$_2$)$_5$CH_3); ^{13}C NMR (D_2O): δ = 102.3 (C1), 102.2 (C1″), 98.0 (C1′), 76.1 (C3), 74.2 (C5), 73.2 (C2), 72.8 (C3″), 71.3 (C4″), 70.9 (C5″), 70.9 (OCH$_2$CH$_2$(CH$_2$)$_6$CH$_3$), 70.5 (C2′), 69.5 (C3′, C4), 69.3 (C4′), 68.6 (C5′), 68.4 (C2′), 68.1 (C6′), 65.6 (C6), 15.4 ppm (C6″); HRMS: *m*/*z* calcd for $[C_{26}H_{48}O_{15}Na]^+$: 623.2885; found: 623.2885.

23–7: HPLC (condition 2); t_R: 36.6 min; ^{1}H NMR (D_2O): δ = 4.92 (d, $J_{1\text{-H},2\text{-H}}$ = 3.6 Hz, 1H, 1-H), 4.45 (d, $J_{1'\text{-H},2'\text{-H}}$ = 8.1 Hz, 1H, 1′-H), 4.43 (d, $J_{1''\text{-H},2''\text{-H}}$ = 7.9 Hz, 1H, 1″-H), 4.17 (br d, *J* = 10.3 Hz, 1H, 6a-H), 4.06 (dd, $J_{5'\text{-H},6a'\text{-H}}$ = 8.7 Hz, $J_{6a'\text{-H},6b'\text{-H}}$ = 12.0 Hz, 1H, 6a′-H), 4.00 (br d, *J* = 3.2 Hz, 1H, 4′-H), 3.95–3.65 (m, 10H, 3-H, 3′-H, 3″-H, 4″-H, 5-H, 5′-H, 5″-H, 6b-H, 6b′-H, OCH_2CH$_2$(CH$_2$)$_6$CH$_3$), 3.58–3.49 (m, 5H, 2-H, 2′-H, 2″-H, 4-H, OCH_2CH$_2$(CH$_2$)$_6$CH$_3$), 1.64 (m, 2H, OCH$_2$CH_2(CH$_2$)$_5$CH$_3$), 1.35–1.26 (m, 13H, 6″-H, OCH$_2$CH$_2$(CH_2)$_5$CH$_3$), 0.88 ppm (t, 3H, OCH$_2$CH$_2$(CH$_2$)$_5$CH_3); HRMS: *m*/*z* calcd for $[C_{26}H_{48}O_{15}Na]^+$: 623.2885; found: 623.2887.

23–8: HPLC (condition 1); t_R: 35.1 min; ^{1}H NMR (D_2O): δ = 4.49 (d, $J_{1'\text{-H},2'\text{-H}}$ 7.8 Hz, 1H, 1′-H), 4.48 (d, $J_{1\text{-H},2\text{-H}}$ = 8.0 Hz, 1H, 1-H), 4.43 (d, >$J_{1''\text{-H},2''\text{-H}}$ = 7.8 Hz, 1H, 1″-H), 4.22 (br d, *J* = 11.3 Hz, 1H, 6a-H), 4.06 (q, $J_{5'\text{-H},6'\text{-H}}$ = 8.8 Hz, $J_{6a'\text{-H},6b'\text{-H}}$ = 12.1 Hz, 1H, 6a′-H), 3.99 (br d, *J* = 3.1 Hz, 1H, 4′-H), 3.95–3.86 (m, 4H, 5′-H, 6b-H, 6b′-H, OCH_2CH$_2$(CH$_2$)$_6$CH$_3$), 3.81 (q, *J* = 6.2, 12.8 Hz, 1H, 5″-H), 3.76 (br d, *J* = 3.3 Hz, 1H, 4″-H), 3.73–3.63 (m, 4H, 3′-H, 3″-H, 5-H, OCH_2CH$_2$(CH$_2$)$_6$CH$_3$), 3.58 (dd, $J_{2'\text{-H},3'\text{-H}}$ = 9.6 Hz, 1H, 2′-H), 3.53–3.46 (m, 3H, 2″-H, 3-H, 4-H), 3.28 (t, $J_{2\text{-H},3\text{-H}}$ = 8.4 Hz, 1H, 2-H), 1.64 (m, 2H, OCH$_2$CH_2(CH$_2$)$_5$CH$_3$), 1.37–1.27 (m, 13H, 6″-H, OCH$_2$CH$_2$(CH_2)$_5$CH$_3$), 0.88 ppm (t, 3H, OCH$_2$CH$_2$(CH$_2$)$_5$CH_3); ^{13}C NMR (D_2O): δ = 103.2 (C1′), 102.8 (C1″), 102.1 (C1), 75.8 (C3), 74.8 (C5), 73.0 (C2, C5′), 72.7 (C3′, C3″), 71.2 (C4″), 71.0 (C5″), 70.8 (OCH$_2$CH$_2$(CH$_2$)$_6$CH$_3$), 70.7 (C2′), 70.4 (C2″), 69.5 (C4), 68.5 (C4, C6, C6′), 15.4 ppm (C6″); HRMS: *m*/*z* calcd for $[C_{26}H_{48}O_{15}Na]^+$: 623.2885; found: 623.2889.

28: Compound **28** was synthesized according to the procedure described for the synthesis of **23**. A solution containing **24** (82.6 μmol) and $[Cp_2Hf(OTf)_2]$ (145 μmol) in DCM (1.1 mL) was added to resin-bound **14** (250 mg, 48.5 μmol) under N_2 atmosphere at −30 °C, and the resulting mixture was kept (TF −30, 0, 10) under SSPR conditions. After completion of the reaction, the resin was washed with DCM, DMF, and DCM and dried under vacuum to give **25**. The resin-bound **25** (48.5 μmol) was added to a solution containing *n*-octanol (96.0 μmol) and DMTST (144 μmol) in DCE/MeCN (1:1, 1.1 mL) under N_2 atmosphere at −30 °C, and the resulting mixture was kept (TF −30, 0, 5) under SSPR conditions.

After completion of the reaction, the resin was washed with DCM, DMF, and DCM and dried under vacuum to give **26**. The eluent was neutralized with Dowex 50W H^+ form, filtered, and concentrated to give **27**. Hydrogenolysis (20% $Pd(OH)_2/C$) afforded a mixture, which was purified with Sep-Pak C-18 (MeOH) to afford **28**. Yield: 5.2 mg, 17.8% over 5 steps (including synthesis of **14**).

Determination of the anomeric configuration of each glycosidic linkage was based on ^1H NMR, COSY, and HSQC (in D_2O) experiments. Chemical shifts for ^{13}C were obtained by HSQC experiments. Chemical shifts of the anomeric protons are listed in Table 4.

Separation of all anomers of **28** with LCMS: Separation of eight sets of anomers was achieved by sequential HPLC steps. First HPLC was carried out by using a reverse-phase monolithic column, which resulted in the isolation of compounds **28–2**, **28–3**, **28–1**, **28–6**, **28–7**, **28–5**, **28–4**, and **28–8**. The purity and anomeric assignment of each peak was achieved based on NMR analysis. The HPLC conditions were as follows: column: Imtakt Cadenza CD-C18 (240 mm × 4.6 mm) and two columns of Chromolith Performance RP-18 (100 mm × 4.6 mm) were connected and used; flow rate: 1.0 mL min^{-1}; mobile phase: CH_3CN/H_2O (23:77); column temperature: 40 °C. Details of the monitoring conditions for mass spectrometry are as follows: capillary: 3.0 kV; cone: 45 V; source temperature: 110 °C; desolvation temperature: 310 °C; monitoring: Single-ion; gas flow (desolvation): 400 L h^{-1}; gas flow (cone): 50 L h^{-1}.

28–1: HPLC: t_R: 45.2 min; ^1H NMR (D_2O): δ = 5.32 (d, J = 3.5 Hz, 1H), 4.92 (m, 2H), 4.41 (m, 1H), 4.03 (brs, 1H), 3.92–3.81 (m, 7H), 3.81–3.73 (m, 4H), 3.72–3.67 (m, 2H), 3.66–3.60 (m, 3H), 3.55 (m, 1H), 1.63 (m, 2H, $OCH_2CH_2(CH_2)_5CH_3$), 1.39–1.23 (m, 10H, $OCH_2CH_2(CH_2)_5CH_3$), 1.22 (d, J = 6.6 Hz, 3H, 6″-H), 0.86 ppm (t, 3H, $OCH_2CH_2(CH_2)_5CH_3$); HRMS: m/z calcd for $[C_{26}H_{48}O_{15}Na]^+$: 623.2885; found: 623.2877.

28–2: HPLC: t_R: 40.4 min; ^1H NMR (D_2O): δ = 5.34 (d, $J_{1'\text{-H},2'\text{-H}}$ = 3.8 Hz, 1H, 1′-H), 4.90 (d, $J_{1'\text{-H},2'\text{-H}}$ = 3.9 Hz, 1H, 1″-H), 4.47 (m, 1H, 5′-H), 4.47 (d, $J_{1\text{-H},2\text{-H}}$ = 8.1 Hz, 1H, 1-H), 4.09 (q, J = 6.8 Hz, 1H, 5′-H), 4.01 (brd, J = 1.7 Hz, 1H, 4′-H), 3.94–3.81 (m, 6H, 2′-H, 3′-H, 3″-H, 6a-H, 6a′-H, $OCH_2CH_2(CH_2)_6CH_3$), 3.80–3.74 (m, $J_{2''\text{-H},3''\text{-H}}$ = 10.4 Hz, 2H, 2-H, 4″-H), 3.73–3.56 (m, 5H, 3-H, 4-H, 6b-H, 6b′-H, $OCH_2CH_2(CH_2)_6CH_3$), 3.44 (m, 1H, 5-H), 3.33 (t, $J_{2\text{-H},3\text{-H}}$ = 8.4 Hz, 1H, 2-H), 1.61 (m, 2H, $OCH_2CH_2(CH_2)_5CH_3$), 1.37–1.22 (m, 10H, $OCH_2CH_2(CH_2)_5CH_3$), 1.21 (d, J = 6.6 Hz, 3H, 6″-H), 0.85 ppm (t, 3H, $OCH_2CH_2(CH_2)_5CH_3$); ^{13}C NMR (D_2O): δ = 102.5 (C1), 99.4 (C1′), 98.3 (C1″), 82.2 (C3), 75.6 (C5), 71.9 (C2), 71.8 (C4″), 70.8 ($OCH_2CH_2(CH_2)_6CH_3$), 69.9 (C4), 69.5 (C3″), 69.3 (C3′), 69.1 (C4′), 69.0 (C5′), 68.3 (C2′), 68.1 (C2″), 66.7 (C5″), 66.1 (C6′), 60.6 (C6), 15.3 ppm (C6″); HRMS: m/z calcd for $[C_{26}H_{48}O_{15}Na]^+$: 623.2885; found: 623.2890.

28–3: HPLC: t_R: 43.2 min; ^1H NMR (D_2O): δ = 4.93 (d, J = 3.8 Hz, 2H, 1-H, 1″-H), 4.54 ppm (d, $J_{1'\text{-H},2'\text{-H}}$ = 7.3 Hz, 1H, 1′-H); HRMS: m/z calcd for $[C_{26}H_{48}O_{15}Na]^+$: 623.2885; found: 623.2880.

28–4: HPLC: t_R: 51.5 min; ^1H NMR (D_2O): δ = 4.92 (d, $J_{1''\text{-H},2''\text{-H}}$ = 3.8 Hz, 1H, 1″-H), 4.59 (d, J = 7.5 Hz, 1H), 4.48 (d, J = 8.2 Hz, 1H), 3.96–3.84 (m, 6H), 3.81–3.65 (m, 8H), 3.62 (dd, J = 7.9, 9.5 Hz, 1H), 3.52 (t, J = 9.1 Hz, 1H), 3.48–3.43 (m, 2H), 1.62 (m, 2H, $OCH_2CH_2(CH_2)_5CH_3$), 1.39–1.17 (m, 10H, $OCH_2CH_2(CH_2)_5CH_3$), 1.22 (d, J = 6.3 Hz, 3H, 6″-H), 0.86 ppm (t, 3H, $OCH_2CH_2(CH_2)_5CH_3$); HRMS: m/z calcd for $[C_{26}H_{48}O_{15}Na]^+$: 623.2885; found: 623.2891.

28–5: HPLC: t_R: 53.1 min; ^1H NMR (D_2O): δ = 5.33 (d, $J_{1'\text{-H},2'\text{-H}}$ = 3.9 Hz, 1H, 1′-H), 4.91 (d, $J_{1\text{-H},2\text{-H}}$ = 3.7 Hz, 1H, 1-H), 4.39 (m, 1H, 5′-H), 4.38 (d, $J_{1''\text{-H},2''\text{-H}}$ = 7.9 Hz, 1H, 1″-H), 4.04 (brd, 1H, 4′-H), 4.00 (dd, $J_{5'\text{-H},6a'\text{-H}}$ = 6.9 Hz, $J_{6a'\text{-H},6b'\text{-H}}$ = 10.3 Hz, 1H, 6a′-H), 3.90 (dd, $J_{2'\text{-H},3'\text{-H}}$ = 10.4 Hz, $J_{3'\text{-H},4'\text{-H}}$ = 3.2 Hz, 1H, 3′-H), 3.85–3.66 (m, 9H, 2′-H, 3-H, 4″-H, 5-H, 5″-H, 6a-H, 6b-H, 6b′-H, $OCH_2CH_2(CH_2)_6CH_3$), 3.65–3.60 (m, 3H, 2-H, 3″-H, 4-H), 3.53 (m, 1H, $OCH_2CH_2(CH_2)_6CH_3$), 3.48 (dd, $J_{2''\text{-H},3''\text{-H}}$ = 9.7 Hz, 1H, 2″-H), 1.63 (m, 2H, $OCH_2CH_2(CH_2)_5CH_3$), 1.40–1.23 (m, 10H, $OCH_2CH_2(CH_2)_5CH_3$), 1.25 (d, J = 6.5 Hz, 3H, 6″-H), 0.85 ppm (t, 3H, $OCH_2CH_2(CH_2)_5CH_3$); ^{13}C NMR (D_2O): δ = 103.0 (C1″), 99.4 (C1′), 98.3 (C1), 80.5 (C3), 72.9 (C3″), 71.2 (C4″, C5), 71.0 (C5″), 70.4 (C2″), 70.0 (C2, C4), 69.2 (C3′, C5′), 69.1 (C4′), 68.6 (C2′, C6′), 68.4 ($OCH_2CH_2(CH_2)_6CH_3$), 60.2 (C6), 15.5 ppm (C6″); HRMS: m/z calcd for $[C_{26}H_{48}O_{15}Na]^+$: 623.2885; found: 623.2889.

28–6: HPLC: t_R: 46.6 min; ^1H NMR (D_2O): δ = 5.37 (d, $J_{1'\text{-H},2'\text{-H}}$ = 3.6 Hz, 1H, 1′-H), 4.48 (d, $J_{1\text{-H},2\text{-H}}$ = 8.1 Hz, 1H, 1-H), 4.42 (m, 1H, 5′-H), 4.41 (d, $J_{1''\text{-H},2''\text{-H}}$ = 8.0 Hz, 1H, 1″-H), 4.05 (brs, 1H, 4′-H), 4.00–3.87 (m, 4H, 3′-H, 6a-H, 6a′-H, $OCH_2CH_2(CH_2)_6CH_3$), 3.85–3.75 (m, 3H, 2′-H, 5″-H, 6b′-H), 3.75–3.58 (m, 6H, 3-H, 3″-H, 4-H, 4″-H, 6b-H, $OCH_2CH_2(CH_2)_6CH_3$), 3.51–3.43 (m, 2H, 2″-H, 5-H), 3.34 (t, $J_{2\text{-H},3\text{-H}}$ = 9.0 Hz, 1H, 2-H), 1.62 (m, 2H, $OCH_2CH_2(CH_2)_5CH_3$), 1.39–1.23 (m, 13H, 6″-H, $OCH_2CH_2(CH_2)_5CH_3$), 0.86 ppm (t, 3H, $OCH_2CH_2(CH_2)_5CH_3$); ^{13}C NMR (D_2O): δ = 102.8 (C1″), 102.2 (C1), 99.1 (C1′), 82.1 (C3), 75.6 (C5), 72.9 (C3″), 72.0 (C2), 71.4 (C4″), 71.0 (C5″, $OCH_2CH_2(CH_2)_6CH_3$), 70.6 (C2″), 70.1 (C4), 69.1 (C3′), 69.0 (C4′), 68.6 (C2′, C5′), 68.1 (C6′), 60.6 (C6), 15.6 ppm (C6″); HRMS: m/z calcd for $[C_{26}H_{48}O_{15}Na]^+$: 623.2885; found: 623.2881.

28–7: HPLC: t_R: 49.3 min; ^1H NMR (D_2O): δ = 4.92 (d, $J_{1\text{-H},2\text{-H}}$ = 3.7 Hz, 1H, 1-H), 4.58 (d, $J_{1'\text{-H},2'\text{-H}}$ = 7.8 Hz, 1H, 1′-H), 4.39 (d, $J_{1''\text{-H},2''\text{-H}}$ = 7.9 Hz, 1H, 1″-H), 4.05 (dd, $J_{5'\text{-H},6a'\text{-H}}$ = 7.9 Hz, $J_{6a'\text{-H},6b'\text{-H}}$ = 10.7 Hz, 1H, 6a′-H), 3.96 (brd, J = 3.0 Hz, 1H, 4′-H), 3.92 (m, 1H, 5′-H), 3.86–3.81 (m, 3H, 3-H, 6a-H, 6b′-H), 3.80–3.75 (m, 2H, 5″-H, 6b-H), 3.75–3.67 (m, 5H, 2-H, 3′-H, 4″-H, 5-H, $OCH_2CH_2(CH_2)_6CH_3$), 3.63 (dd, $J_{3''\text{-H},4''\text{-H}}$ = 3.5 Hz, 1H, 3″-H), 3.60 (dd, $J_{2'\text{-H},3'\text{-H}}$ = 9.9 Hz, 1H, 2′-H), 3.53 (m, 2H, 4-H, $OCH_2CH_2(CH_2)_6CH_3$), 3.48 (dd, $J_{2''\text{-H},3''\text{-H}}$ = 9.8 Hz, 1H, 2″-H), 1.62 (m, 2H, $OCH_2CH_2(CH_2)_5CH_3$), 1.38–1.23 (m, 10H, $OCH_2CH_2(CH_2)_5CH_3$), 1.25 (d, J = 6.5 Hz, 3H, 6″-H), 0.85 ppm (t, 3H, $OCH_2CH_2(CH_2)_5CH_3$); ^{13}C NMR (D_2O): δ = 104.0 (C1′), 102.6 (C1″), 98.0 (C1), 84.0 (C3), 73.2 (C5′), 72.7 (C3″), 72.3 (C3′), 71.3 (C4″, C5), 71.1 (C2′), 71.0 (C5″), 70.4 (C2, C2″), 68.5 (C4′), 68.4 (C6′, $OCH_2CH_2(CH_2)_6CH_3$), 68.1 (C4), 60.5 (C6), 15.5 ppm (C6″); HRMS: m/z calcd for $[C_{26}H_{48}O_{15}Na]^+$: 623.2885; found: 623.2880.

28–8: HPLC: t_R: 55.3 min; ^1H NMR (D_2O): δ = 4.62 (d, J = 7.9 Hz, 1H), 4.49 (d, J = 7.8 Hz, 1H), 4.40 (d, J = 8.5 Hz, 1H), 4.06 (t, J = 9.9 Hz, 1H), 3.96 (brs, 1H), 3.94–3.89 (m, 3H), 3.84 (dd, J = 3.1 Hz, 1H), 3.81–3.58 (m, 8H), 3.54–3.42 (m, 4H), 1.62 (m, 2H, $OCH_2CH_2(CH_2)_5CH_3$), 1.38–1.23 (m, 10H, $OCH_2CH_2(CH_2)_5CH_3$), 1.25 (d, J = 6.8 Hz, 3H, 6″-H), 0.85 ppm (t, 3H, $OCH_2CH_2(CH_2)_5CH_3$); HRMS: m/z calcd for $[C_{26}H_{48}O_{15}Na]^+$: 623.2885; found: 623.2889.

30: A solution of $[Cp_2Hf(OTf)_2]$ (145 μmol), **29**[37] (84.7 μmol), and resin-bound **14** (253 mg, 49.1 μmol) were mixed in DCM (1.10 mL) and swollen to allow the reaction under N_2 atmosphere, and the reaction mixture was maintained (TF −30, 0, 10) under SSPR conditions. After completion of the reaction, the resin was washed successively with DCM, DMF, and DCM to afford resin-bound phenyl 2,3-di-*O*-benzyl-α,β-L-fucopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α,β-D-galactopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside. MS (ESI-QIT) (after cleavage from the resin): m/z calcd for $[C_{80}H_{84}O_{14}SNa]^+$: 1323.5; found: 1323.5. *n*-Octanol (145 μmol), DMTST solution (145 μmol), and resin-bound phenylthioglycoside (49.1 μmol) were then mixed and swollen in DCE/CH_3CN (1:1, 1.10 mL) at −30 °C, and the reaction was set (TF −30, 0, 5) under SSPR conditions. After completion of the reaction, the resin was washed successively with DCM, DMF, and DCM to afford resin-bound octyl 2,3-di-*O*-benzyl-α,β-L-fucopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α,β-D-galactopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α,β-D-glucopyranoside. MS (ESI-QIT) (after cleavage from the resin): m/z calcd for $[C_{82}H_{96}O_{15}Na]^+$: 1343.7; found: 1343.8. Resin-bound octyl trisaccharide (49.1 μmol) was then treated with NaOMe/MeOH (200 μL) in CH_3CN (0.8 mL) at room temperature for 1 h. After completion of the reaction, the resin was filtered and washed with DCM. The eluent was neutralized with Dowex 50W H^+ form, filtered, and concentrated. The residue was hydrogenolized with 20% $Pd(OH)_2$ on charcoal in MeOH/EtOAc (1:1 *v*/*v*, 7.0 mL) under H_2 atmosphere at room temperature for 20 h. The mixture was filtered thorough celite, and the eluent was concentrated. The obtained residue was then dissolved in water and purified by using a reverse-phase short column (Sep-Pak C18). The MeOH fraction was concentrated to afford **30** as an anomeric mixture (3.2 mg, 10.8% over 5 steps). MS (ESI-QIT): m/z calcd for $[C_{26}H_{48}O_{15}Na]^+$: 623.3; found: 623.3.

32: A solution of $[Cp_2Hf(OTf)_2]$ (91.8 μmol), **31**[37] (33.1 mg, 61.0 μmol), and resin-bound **14** (110 mg, 30.5 μmol) were mixed in DCE and CH_3CN and swollen to allow the reaction under N_2 atmosphere, and the reaction mixture was maintained without mixing (SSPR) (TF −30, 0, 2.5). After

completion of the reaction, the resin was washed successively with DCM, DMF, and DCM to afford resin-bound phenyl 2,3-di-*O*-benzyl-α,β-L-fucopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α,β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside. MS (ESI-QIT) (after cleavage from the resin): *m*/*z* calcd for $[C_{80}H_{84}O_{14}SNa]^+$: 1323.5; found: 1323.6. *n*-Octanol (61.1 μmol), DMTST solution (91.9 μmol), and resin-bound phenylthioglycoside (30.5 μmol) were then mixed and swollen in DCE/CH_3CN (1:2, 0.5 mL) at −30 °C, and the reaction was set (TF −30, 0, 2.5) under SSPR conditions. After completion of the reaction, the resin was washed successively with DCM, DMF, and DCM to afford resin-bound octyl 2,3-di-*O*-benzyl-α,β-L-fucopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α,β-D-galactopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α,β-D-glucopyranoside. MS (ESI-QIT) (after cleavage from the resin): *m*/*z* calcd for $[C_{82}H_{96}O_{15}Na]^+$: 1343.7; found: 1343.7. Resin-bound octyl trisaccharide (30.5 μmol) was then treated with NaOMe/MeOH (100 μL) in DCE (1.0 mL) at room temperature for 1 h. After completion of the reaction, the resin was filtered and washed with DCM. The eluent was neutralized with Dowex 50W H^+ form, filtered, and concentrated. The residue was hydrogenolized with 20% $Pd(OH)_2$ on charcoal in MeOH/EtOAc (1:1 *v*/*v*, 7.0 mL) under H_2 atmosphere at room temperature for 2.5 days. The mixture was filtered thorough celite, and the eluent was concentrated. The obtained residue was then dissolved in water and purified by using a reverse-phase short column (Sep-Pak C18). The MeOH fraction was concentrated to afford **32** as an anomeric mixture (3.2 mg, 17.5% over 5 steps). MS (ESI-QIT): *m*/*z* calcd for $[C_{26}H_{48}O_{15}Na]^+$: 623.3; found: 623.2.

Acknowledgements

Financial support provided by Mitsubishi Chemical Co. (MCC) and the Key Technology Research Promotion Program of the New Energy and Industrial Development Organization (NEDO), Ministry of Economy, Trade, and Industry (METI) of Japan is gratefully acknowledged.

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Received: July 5, 2006
Revised: September 27, 2006
Published online: November 22, 2006

