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Synthesis of Bisdesmosidic Oleanolic Acid Saponins via a Glycosylation-Deprotection Sequence under Continuous Microfluidic/Batch Conditions

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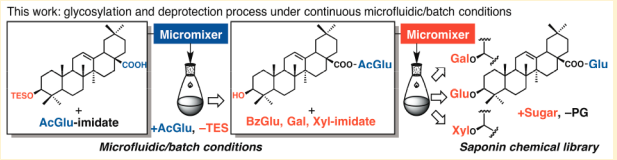
Supporting Information

ABSTRACT: We report the first synthesis of a series of bisdesmosidic oleanolic acid saponins using microflow reactor Comet X-01 via a continuous flow glycosylation-batch deprotection sequence. The main results of this study can be summarized as follows: (1) The microfluidic glycosylation of oleanolic acid at C-28 was achieved in quantitative yield and was applied to the synthesis of six C-28-monoglycosidic saponins. (2) The microfluidic glycosylation of oleanolic acid at C-3 was achieved in good yield without orthoester byproduct formation and was applied to the synthesis of three bisdesmosidic saponins. (3) The continuous synthesis of saponins via a microfluidic glycosylation-batch deprotection sequence was achieved in four steps involving two purifications. Thus, the continuous microfluidic glycosylation-deprotection process is expected to be suitable for the preparation of a library of bisdesmosidic oleanolic acid saponins for in vivo pharmacological studies.



INTRODUCTION

Naturally occurring saponins, found in plants and echinoderms, have several biological activities including cytotoxic, antitumor, anti-inflammatory, and interfacial properties.[1] Thus, they have been widely used as detergents, emulsifiers, and medicinal drugs. During our studies on the biological effects of saponins, several onjisaponins isolated from the rhizomes of *Polygala tenuifolia* Willd were found to be active as nasal vaccine adjuvants.[2] However, the development of a pure saponin-based drug has been hampered by the low yields and high costs associated with their isolation from natural sources. Moreover, the total synthesis of saponins[3] based on the regio- and stereoselective formation of a glycosidic linkage using a variety of sugars in the presence of many hydroxyl groups on the aglycon and sugar moieties is a challenging task. Recently, we synthesized a series of simplified oleanolic acid saponins glycosylated at the C-28 carboxyl, which revealed that the introduction of a 4-*O*-cinnamoyl glucosyl ester moiety enhanced the vaccine adjuvant activity;[4] nevertheless, the adjuvant activity was much lower than that of onjisaponins.

For improving mucosal adjuvant activity and gaining a deeper understanding of the structure−activity relationship, a library of glycoforms differing in carbohydrate composition, linkages, and position is required. In particular, we focused on the synthesis of the simplified saponin oleanolic acid 3,28-bisdesmoside[5] because some bisdesmosidic saponins with a complex carbohydrate structure, such as QS-21,[6] have shown adjuvant activity[7] (Figure 1). A sequential one-pot method has been reported for the efficient synthesis of bisdesmosidic saponins;[5] however, in the one-pot approach, only one saponin compound is obtained. On the other hand, in a stepwise approach, isolation of a monosaccharide glycosyl acceptor as an intermediate after the first glycosylation followed by glycosylation with different glycosyl donors would result in the generation of a saponin library (Figure 2). Unfortunately, this stepwise method disturbs the continuity of the reaction sequence. Moreover, glycosylation reactions usually require extensive protecting group manipulations, and the additional protection/deprotection steps by conventional procedures leads to lengthy and impractical synthetic schemes (e.g., synthesis of QS-21).[6] Thus, the construction of a saponin library is a laborious and time-consuming task.

To solve this problem, we turned our attention to continuous flow synthesis[8] including flow microreactor synthesis.[9] Microfluidic synthesis has various advantages compared to conventional batch synthesis strategies: (1) strict control of temperature, stirring, and reaction time, (2) easy scale-up, (3) safe and reproducible operations, and (4) possibility of reactions involving short-lived reactive intermediates.[10]

Microflow reactors[11] (e.g., silicon microfluidic reactor, PFA reactor,[12] Comet X-01[13]) have been widely used in carbohydrate synthesis for glycosylation reactions (Figure 3A). However, microfluidic glycosylations have not been

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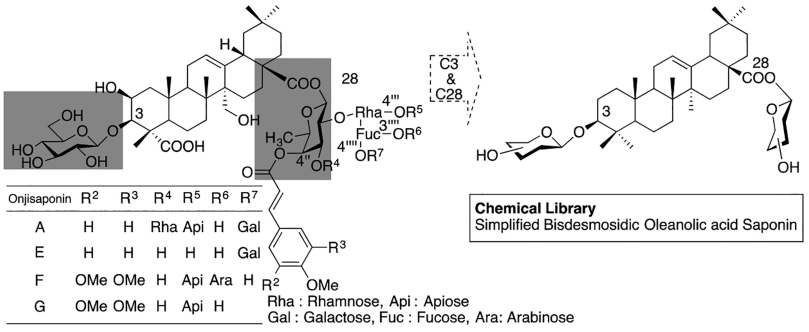

Figure 1. Structure of onjisaponins and simplified synthetic bisdesmosidic oleanolic acid saponins.

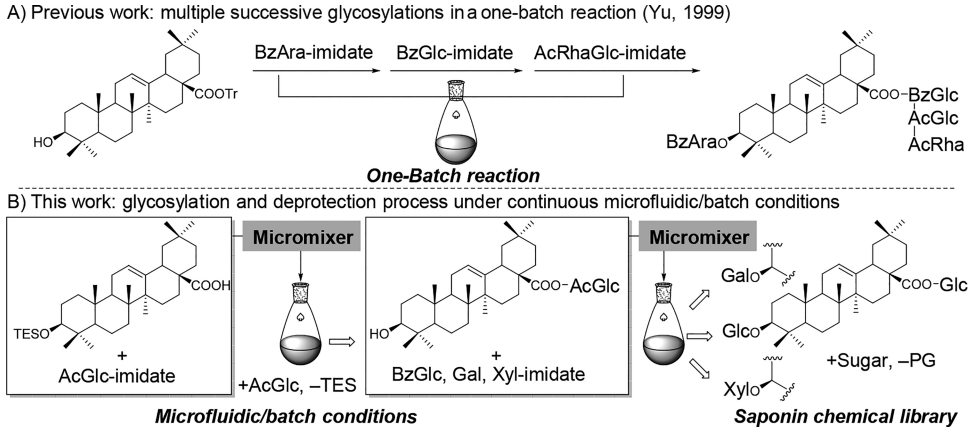

Figure 2. Synthesis of bisdesmosidic oleanolic acid saponins to construct a saponin chemical library.

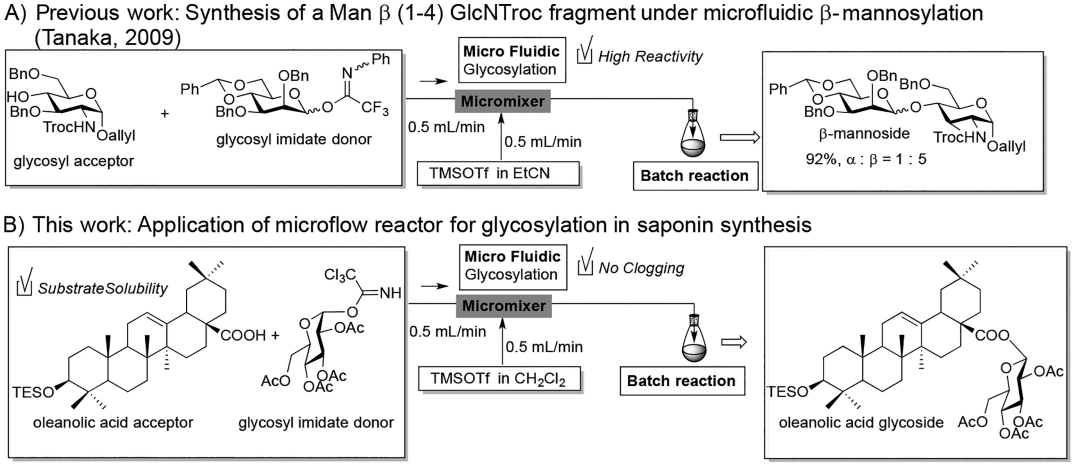

Figure 3. Applications of the microflow reactor for glycosylation.

extensively studied for saponin synthesis because of solubility and reactivity limitations of medium-sized triterpenoid acceptors.

In this study, it was expected that microfluidic glycosylation conditions would benefit from the advantages of microflow reactors. Thus, the glycosylation step of the synthesis of a simplified saponin was carried out for the first time in a microflow reactor (Figure 3B). In addition, continuous glycosylation and deprotection processes for the synthesis of saponins, such as bisdesmosides, can be efficiently performed in microfluidic reactors, which would allow for shorter synthetic routes without the need for purification steps and to generate the saponin chemical library (Figure 2B).

In this paper, we describe the glycosylation of oleanolic acid at C-28 and C-3 in a microfluidic system and a continuous flow glycosylation-batch deprotection process for the synthesis of bisdesmosidic oleanolic acid saponins with different sugar moieties.

■ RESULTS AND DISCUSSION

Glycosylation at C-28. We initially investigated the glycosylation of oleanolic acid at C-28 in a microfluidic system. The Schmidt conditions[14] were considered for the microfluidic glycosylation, where C-28 glycosylation has been reported, such as Koenigs–Knorr[15] glycosylation and phase-transfer catalyst

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conditions,[16] which are not suitable for microfluidic systems due to clogging of the silver salts.

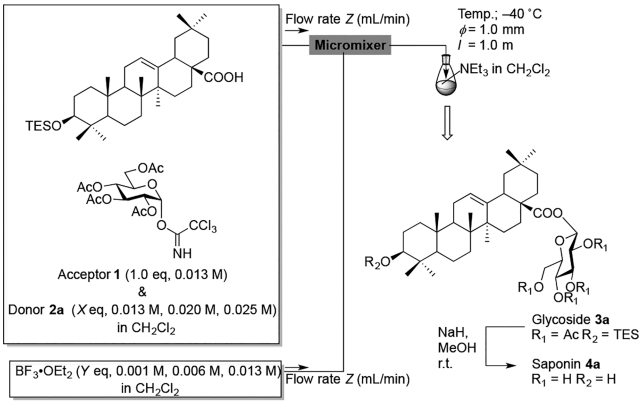
Substrate solubility in the reaction medium is an important requirement for successful microfluidic reactions; thus, a key feature of this study is the solubility of oleanolic acid derivatives in CH_2Cl_2, which is the solvent used for Schmidt glycosylation. We decided to synthesize oleanolic acid acceptor **1**[4] with a triethylsilyl (TES) protecting group at C-3 following Gin's method, which showed improved solubility in CH_2Cl_2 as well as different reactivities at C-3 and C-28 allowing for orthogonal deprotection. Accordingly, acceptor **1** was prepared from commercially available oleanolic acid via 3,28-double protection followed by treatment with silica gel to remove the 28-*O*-TES group. On the other hand, selective protection of C-3-OH with an acetyl (Ac) group was attempted by a stepwise protection−deprotection sequence, resulting in only 32% yield of the desired C-3 Ac-protected acceptor.[16]

Next, we attempted the microfluidic β-selective glycosylation of oleanolic acid at C-28[17] after preparation of acceptor **1** and imidate donor **2a**,[18] and the reaction conditions were optimized by changing the following parameters: (1) the amount of glycosyl imidate donor **2a**, (2) the amount of $BF_3{\cdot}OEt_2$, used as an activator (catalytic or stoichiometric), and (3) the flow rate in the microreactor. A solution of acceptor **1** and donor **2a** in CH_2Cl_2 and a solution of $BF_3{\cdot}OEt_2$ in CH_2Cl_2 were prepared and mixed using a Comet X-01 micromixer at −40 °C (see Table S1). As shown in Table 1, in our initial attempts, the microfluidic glycosylation was carried out at different flow rates (0.4−0.7 mL/min) and ratios of donor **2a** to $BF_3{\cdot}OEt_2$. The use of 1.0 equiv of donor **2a** slightly promoted the glycosylation except at a flow rate of 0.6 mL/min (entries 5−12), whereas the use of 2.0 equiv of **2a** significantly improved the reaction efficiency to provide glycoside **3a**[4] (entries 13−20). In an effort to examine the possibility of decreasing the required amount of **2a**, additional conditions were tested; however, the glycosylation did not proceed effectively when 1.5 equiv of **2a** was used (entries 21−23). Moreover, a catalytic amount (0.1 equiv) of $BF_3{\cdot}OEt_2$ did not promote the reaction even at an efficient flow rate of 0.6 mL/min (entry 24). Interestingly, with 2.0 equiv of **2a**, a lower yield was obtained at a flow rate of 0.8 mL/min as compared with those at 0.4−0.6 mL/min (entry 25), whereas the glycosylation at the low flow rate of 0.1 mL/min proceeded in excellent yield (entry 26). These results indicate that, in the presence of an excess amount of **2a**, a sufficient residence time was required for complete glycosylation. In contrast to the batch reaction in the presence of MS4Å,[19] under microfluidic conditions, the glycosylation proceeded to completion even when the amount of $BF_3{\cdot}OEt_2$ was decreased (entry 27). These results suggest that the efficient mixing of reactants in the microflow reactor resulted in the efficient activation of **2a** by $BF_3{\cdot}OEt_2$ followed by rapid coupling with **1**.

The scope and limitation of the microfluidic glycosylation at C-28 was examined with various sugar moieties (Table 2). Acetylated galactosyl-, xylosyl-, mannosyl-, rhamnosyl-, glucuronyl-, and cinnamoyl-type (**2b**−**2g**, respectively) imidate donors were prepared according to previous reports,[20] and the results of their microfluidic glycosylation reactions are summarized in Table 2. Protected glycosides **3c** and **3f** were obtained in moderate yields because of the low reactivity of the corresponding donors due to the electron-withdrawing 5′-methylene or carboxyl moiety, which decreased the nucleophilicity of the 1′-anomeric hydroxyl group (entries 2 and 5). On the other hand, **3b**, **3d**, **3e**, and **3g** were obtained in high yields (entries 1,3,4, and 6). In particular, the glycosylation of **1** (1.0 equiv) with 3,4-dimethoxycinnamoyl (3,4-DMC) donor **2g** (1.0 equiv) in the presence of $BF_3{\cdot}OEt_2$ (10.0 equiv) at a flow rate of 0.1 mL/min proceeded smoothly to produce protected cinnamoyl glucoside **3g**[4] in quantitative yield (entry 6) in contrast to that of the corresponding batch reaction[4] (61% yield).

With protected C-28 glycosides in hand, Ac and TES deprotection of **3a**−**3e** was performed by treatment with NaH (60% dispersion in mineral oil) and MeOH at room temperature followed by acidification to pH 4 with Dowex resin to afford saponins **4a**,[4] **4b**,[16a] **4c**,[16a] **4d**, and **4e**,[16a] respectively, in excellent yields (entries 1−4). On the other

Table 1. Synthesis of Glycoside 3a in a Microfluidic System with Various Reaction Conditions and Saponin 4a by Deprotection



entry	donor **2a** *X* [eq]	$BF_3{\cdot}OEt_2$ *Y* [eq]	flow rate *Z* [mL/min]	yield[c] [%]
1	1.0	0.1	0.4	no reaction
2	1.0	0.1	0.5	no reaction
3	1.0	0.1	0.6	no reaction
4	1.0	0.1	0.7	no reaction
5	1.0	0.5	0.4	4.9
6	1.0	0.5	0.5	17
7	1.0	0.5	0.6	48
8	1.0	0.5	0.7	3.6
9	1.0	1.0	0.4	2.6
10	1.0	1.0	0.5	4.5
11	1.0	1.0	0.6	51
12	1.0	1.0	0.7	4.5
13	2.0	0.5	0.4	quant.
14	2.0	0.5	0.5	quant.
15	2.0	0.5	0.6	quant.
16	2.0	0.5	0.7	quant.
17	2.0	1.0	0.4	76
18	2.0	1.0	0.5	quant.
19	2.0	1.0	0.6	quant.
20	2.0	1.0	0.7	quant.
21[a]	1.5	0.1	0.6	0.9
22[a]	1.5	0.5	0.6	59
23[a]	1.5	1.0	0.6	66
24[a]	2.0	0.1	0.6	11
25[a]	2.0	0.5	0.8	75
26[a]	2.0	0.5	0.1	quant.
27[b]	2.0	1.0		86

[a]Additional experiments were performed except entries **1**−**20**. [b]Reaction performed using a flask apparatus. [c]Isolated yield.

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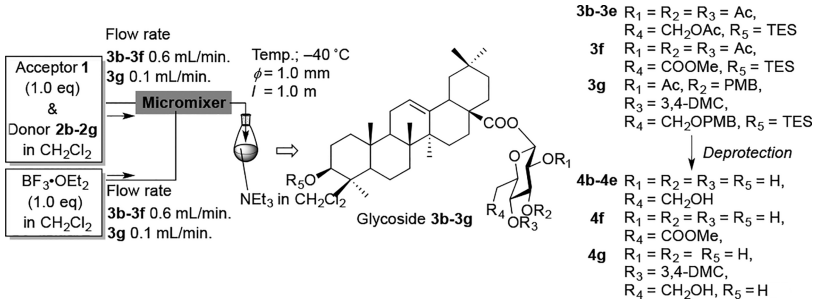
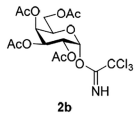
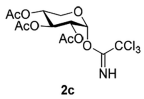
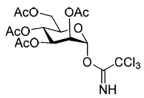
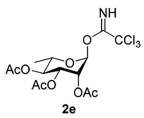
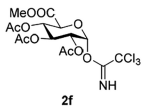
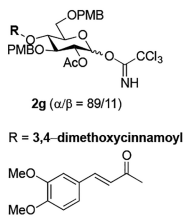
Table 2. Application of Glycosylation at C-28 in a Microfluidic System and Synthesis of Various C-28 Monoglycoside Saponins



Entry	Donor equiv	Donor Structure	Deprotection conditions	Glycoside Product	Glycoside Yield[a] (%)	Saponin Product	Saponin Yield[a] (%)
1	2.5	 2b	NaH (0.1 eq.), MeOH (0.05 M), r.t., 1 h	**3b** (β only)	quant.	**4b** (β only)	quant.
2	2.0	 2c	NaH (0.1 eq.), MeOH (0.1 M), r.t., 1 h	**3c** (β only)	66	**4c** (β only)	99
3	2.0	 2d	NaH (0.1 eq.), MeOH (0.05 M), r.t., 40 min	**3d** (α only)	93	**4d** (α only)	quant.
4	2.0	 2e	NaH (0.1 eq.), MeOH (0.1 M), r.t., 30 min	**3e** (α only)	94	**4e** (α only)	quant.
5	2.0	 2f	NaH (0.1 eq.), MeOH (0.05 M), r.t., 3.5 h	**3f** (β only)	74	**4f** (β only)	27
6	1.0	 2g (α/β = 89/11) R = 3,4-dimethoxycinnamoyl	1) NaH (6.4 eq.), MeOH (0.02 M), CH_2Cl_2 (0.08 M), r.t., 1.5 h 2) DDQ (2.0 eq.), H_2O (0.2 M) CH_2Cl_2 (0.01 M), r.t., 17 h	**3g** (β only)	quant.	**4g** (β only)	23

[a]Isolated yield.

hand, glucuronyl saponin **4f**[21] was obtained in low yield because of the unhydrolyzed methyl ester at the 6′ position. Other deprotection conditions, such as alkaline hydrolysis with KOH[22] and dealkylation with dodecyl methyl sulfide,[23] resulted in the undesired simultaneous cleavage of the 6′-methyl and 28-glycosyl ester moieties in **3f** (entry 5). As for compound **3g**, the deprotection was conducted according to our previous report[4] (entry 6).

The structures of the C-28-glycosidic saponins were determined by ^{1}H NMR, ^{13}C NMR, ^{1}H–^{1}H COSY, HMQC, and HMBC analyses, which confirmed the glycosidic linkage at C-28, except for mannosyl glycoside **4d** and rhamnosyl glycoside **4e**.[24] The anomeric configurations in **4d** and **4e** were determined by GATE-1 decoupling experiments[25] to give α conformation (see SI IV-2; J_{C-H} = 173.4 Hz for **4d**, J_{C-H} = 173.0 Hz for **4e**).

Encouraged by the results of the oleanolic acid glycosylation at C-28 in a flow microreactor, acceptor **5** was prepared from glycoside **3a** by deprotection of the TES ether at C-3 to attempt the microfluidic glycosylation at C-3 (Table 3). Acidic (AcOH-THF-H_2O 5:11:3[26]) and mildly acidic (TBAF/THF-AcOH[27]) conditions gave selectively deprotected product **5** in moderate yield along with nonselectively deprotected **4a** (entries 1 and 2). Selective removal of the TES group at C-3 was not achieved under the conditions described above because of the steric hindrance of the dimethyl groups at C-23 and C-

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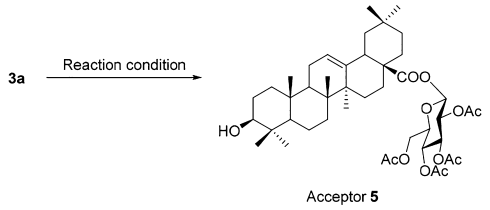
Table 3. Synthesis of Acceptor 5



Acceptor **5**

entry	reaction conditions	yield[a] (%)
1	AcOH-THF-H_2O (5:11:3) (0.1 M), 16 h	65
2	TBAF, THF (5.0 equiv), AcOH (5.0 equiv), 24 h	68
3	$ZnBr_2$ (5.0 equiv), H_2O (5.0 equiv), CH_2Cl_2 (0.25 M), 16 h	97

[a]Isolated yield.

24. On the other hand, treatment with excess $ZnBr_2$ in aqueous CH_2Cl_2[28] afforded acceptor **5** in excellent yield (entry 3). Thus, we were able to achieve microfluidic C-28 glycosylation of oleanolic acid in quantitative yield without byproduct formation as well as efficient conditions for the subsequent selective C-3 TES deprotection, which was applied to a continuous glycosylation-deprotection reaction.

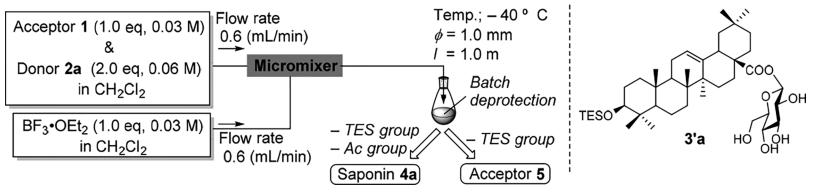
Continuous Reaction of Microfluidic C-28 Glycosylation Deprotection. The advantages of the microfluidic glycosylation reactions of oleanolic acid at C-28 are beneficial for the development of a continuous glycosylation-deprotection process by avoiding the purification of the protected glycoside intermediate. Thus, we attempted the continuous C-28 glycosylation-C-3 selective TES deprotection process of oleanolic acid (Table 4). In light of the changes in physical properties required for saponin synthesis, the continuous reaction system was designed based on safety and solubility criteria. In the continuous system, the microfluidic glycosylation at C-28 was performed under previously optimized conditions, whereas the subsequent protecting group removal required the addition of a suitable deprotecting reagent instead of the quenching reagent triethylamine. Using small amounts of NaH (60% dispersion in mineral oil, 1.5 or 3.0 equiv) in MeOH at room temperature, the deprotection reaction did not proceed in the continuous system (entries 1 and 2), indicating that NaH was quenched by $BF_3{\cdot}OEt_2$. On the other hand, in the batch deprotection reaction, when excess NaH (10.0 equiv) was used, only byproduct **3′a** was obtained by neutralization with Dowex resin after methanolysis (entry 3). Acidification to pH 4 with Dowex resin after methanolysis resulted in TES deprotection to afford saponin **4a** in excellent yield over two steps (entry 4).

For the synthesis of glycosyl acceptor **5** in the continuous system, regioselective TES deprotection was investigated. Treatment with TBAF/THF-AcOH afforded **5** in only 24% yield over two steps because of the use of different solvents in the two reactions (entry 5). When 5.0 equiv of $ZnBr_2$ and H_2O were used at −40 °C to room temperature, acceptor **5** was obtained in 55% yield over two steps (entry 6). In contrast to the batch synthesis of acceptor **5**, the deprotection conditions in the continuous system were dependent on the concentration of acceptor **5** in CH_2Cl_2 solvent used for the microfluidic C-28 glycosylation. Hence, we increased the amount of $ZnBr_2$ and H_2O (10.0 equiv), which provided acceptor **5** in 78% yield over two steps (entry 7). These results indicate that it is important to carefully select the type and concentration of the deprotecting reagent so that the deprotection step is not affected by the glycosylation conditions used in the microfluidic system.

Glycosylation at C-3. The glycosylation of oleanolic acid at C-3 was performed with the microfluidic system (Table 5), as well as with the batch system for comparison (see Table S2). Glycosylation using acetylated **2a** did not give the protected diglycoside **7a**; instead, the acyloxonium ion intermediate derived from the acetyl imidate donor produced orthoester **8a**,[29] which then rearranged to acetylated oleanolic acid **9**. The structure of orthoester **8a** was determined by a chemical shift in the ^1H NMR at the anomeric proton compared to the β glycosidic bond. The batch glycosylation using benzoylated glucosyl imidate donor **6a**[30] minimizes orthoester formation (see Supporting Information for details).

As shown in Table 5, the C-3 glycosylation in a microfluidic system using the same amount of **6a** (1.5 equiv) used for the batch experiment. Glycosyl acceptor **5** and donor **6a** were dissolved in CH_2Cl_2 and mixed with various amounts of activator in CH_2Cl_2 at various temperatures using a Comet X-01 microreactor at a flow rate of 0.6 mL/min. After flowing

Table 4. Continuous Synthesis for Preparation of Saponin 4a and Acceptor 5 with Microfluidic Glycosylation at C-28 and Batch Deprotection



entry	deprotection conditions with batch apparatus	results: product	results: yield[a] (two steps, %)
1	NaH (1.5 equiv), MeOH, rt, 1 h	**3a**	quant.
2	NaH (3.0 equiv), MeOH, rt, 1 h	**3a**	quant.
3	NaH (4.5−10 equiv), MeOH, rt, 40 min, neutralized to pH 7	**3′a**	40−89
4	NaH (10 equiv), MeOH, rt, 40 min, neutralized to pH 4	**4a**	95
5	TBAF,THF (5.0 equiv), AcOH (2.5 equiv), rt, 2 days	**5**	24
6	$ZnBr_2$ (5.0 equiv) H_2O (5.0 equiv), CH_2Cl_2 (6 mL), rt, 30 min	**5**	55
7	$ZnBr_2$ (10 equiv) H_2O (10 equiv), CH_2Cl_2 (6 mL), rt, 30 min	**5**	78

[a]Isolated yield.

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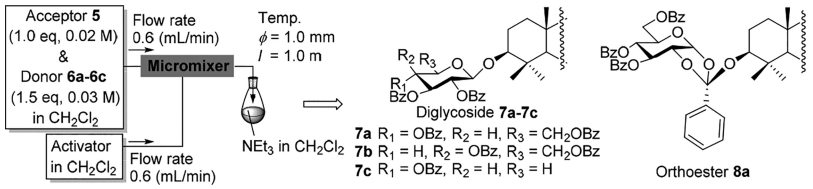
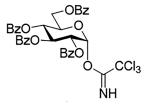
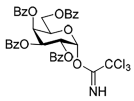
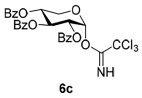
Table 5. Study of Glycosylation at C-3 in a Microfluidic System toward the Synthesis of Diglycosides 7a–7c[a]



Entry	Donor (1.5 equiv)	Activator (equiv)	Temp (°C)	**7a-7c** (%)	**8a** (%)
1		$BF_3 \cdot OEt_2$ (1.0)	r.t.	0	0
2		TMSOTf (0.1)	-40	20[b]	65[b]
3	 **6a**	TMSOTf (0.1)	-20	22[b]	54[b]
4		TMSOTf (0.1)	0	24[b]	66[b]
5		TMSOTf (0.1)	r.t.	27[b]	34[b]
6		TMSOTf (1.0)	0	47[c]	0
7		TMSOTf (1.0)	r.t.	83[c]	0
8	 **6b**	TMSOTf (1.0)	r.t.	71[c]	-[d]
9	 **6c**	TMSOTf (1.0)	r.t.	81[c]	-[d]

[a]Results under the batch conditions; see Table S2. [b]Yield based on the **7a**/**8a** ratio by ^{1}H NMR measurements. [c]Isolated yield. [d]Corresponding orthoester not observed.

through the reactor tube (1.0 m; i.d.: 1.0 mm), the mixture was quenched by adding a triethylamine solution in CH_2Cl_2 in the batch apparatus.

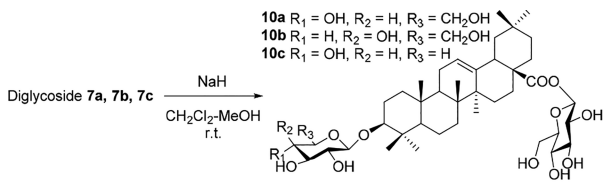
Micromixing of **5**, benzoyl donor **6a**, and 1.0 equiv of $BF_3 \cdot OEt_2$ did not afford **7a** at all (entry 1), and analytical TLC showed incomplete reaction of acceptor **5**. To improve the reaction, we tested TMSOTf as an activator. Micromixing of **5**, **6a**, and a catalytic amount of TMSOTf at different temperatures provided diglycoside **7a** in only ~20% yield (entries 2–5), and orthoester **8a** was observed as a byproduct in ~60% yield. Mild reaction conditions utilizing catalytic TMSOTf in lower temperature caused the preferential formation of **8a**.[29] On the other hand, a stoichiometric amount of TMSOTf promoted the C-3 glycosylation at room temperature, and diglycoside **7a** was obtained in 83% yield without the formation of orthoester **8a** (entry 7), whereas at 0 °C, the yield of **7a** was decreased (entry 6). These results indicate that rapid activation by an appropriate amount of TMSOTf at a suitable temperature was critical for C-3 glycosylation. Notably, microfluidic C-3 glycosylation required a lower amount of donor **6a** and resulted in an increased yield of **7a** as compared to that of the batch reaction.

For the versatility of this approach to be examined, microfluidic C-3 glycosylation was applied to different carbohydrates. Benzoylated galactosyl- and xylosyl-type (**6b** and **6c**, respectively) imidate donors were prepared from the corresponding commercially available monosaccharides by procedures described in the literature.[30] Under the optimized conditions, the microfluidic glycosylation at C-3 afforded protected diglycosides **7b** and **7c** in 71 and 81% yields, respectively (entries 8 and 9), which are ~10% higher than the yields of the corresponding batch reactions.

Next, deprotection of all acyl groups in **7a–7c** by treatment with NaH (60% dispersion in mineral oil) and MeOH at room temperature afforded known bisdesmosidic saponins **10a**,[31] **10b**,[16a,32] and **10c**[16b] in 93, 74, and 87% yields, respectively (Table 6).

On the basis of these results, we envisioned the continuous synthesis of saponins by a consecutive glycosylation-deprotection process. Moreover, suitable conditions were found for the glycosylation at C-3 without orthoester byproduct formation. In addition, it should be noted that the efficiency

Table 6. Synthesis of Bisdesmosidic Saponins 10a–10c



entry	diglycoside	bisdesmosidic saponin	yield[a]
1	**7a**	**10a**	93
2	**7b**	**10b**	74
3	**7c**	**10c**	87

[a]Isolated yield.

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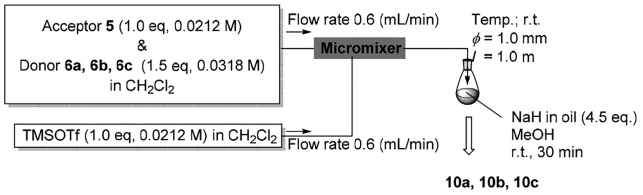
of these transformations, with trace amounts of unreacted substrates and byproducts, allows for the easy isolation of the desired bisdesmosidic saponins by column chromatography.

Continuous Reaction of Microfluidic C-3 Glycosylation Deprotection. We attempted the continuous C-3 glycosylation-acyl deprotection sequence (Table 7). Gratifyingly, bisdesmosidic saponins **10a**–**10c** were obtained in ∼40% yield over two steps by treatment with 4.5 equiv of NaH (60% dispersion in mineral oil) in MeOH at room temperature. Thus, bisdesmosidic saponins could be readily prepared from acceptor **1** in ∼30% total yield over four steps involving two purification operations.

Table 7. Continuous Synthesis of Bisdesmosidic Saponins with Microfluidic Glycosylation at C-3 and Batch Deprotection of Acyl Groups



		saponin	
entry	donor (1.5 equiv)	product	yield[a] (2 steps, %)
1	**6a**	**10a**	47
2	**6b**	**10b**	43
3	**6c**	**10c**	47

[a]Isolated yield.

Assignments of Bisdesmosidic Acid Saponins. In this work, we determined for the first time the detailed structure of synthetic bisdesmosidic saponins **10a**–**10c**, and the spectral data of their sugar moieties are shown[33] in Table 8. Because of overlap of the sugar proton signals, the chemical structures of the synthesized saponins could not be identified by ^{1}H NMR spectroscopy even by comparison with previous reports,[16a] and it was therefore necessary to resort to TOCSY experiments.[34] The ^{1}H NMR chemical shifts, multiplicities, and coupling constants of the sugar signals were assigned by ^{1}H–^{1}H COSY and 1D TOCSY spectra; HSQC and HSQC-TOCSY experiments were used to assign the chemical shifts and multiplicities in the ^{13}C NMR spectra and the corresponding sugar proton signals (see SI IV-3). The *β*-anomeric configurations of the 28-*O*-sugar and 3-*O*-sugar were demonstrated by the large *J* values (7.5–8.5 Hz). The HMBC correlations between the anomeric proton (1′-H or 1″-H) and C-28 or C-3 confirmed the presence of glycosidic linkages. Interestingly, the ^{1}H NMR spectra of diglycosidic saponins showed differences in the multiplicities of the 2′-H and 3′-H signals of the 28-*O*-sugar moiety, suggesting that the nature of the 3-*O*-sugar moiety affected the conformation of the saponin.

CONCLUSIONS

In conclusion, we have achieved the first C-28 and C-3 glycosylations of oleanolic acid in a microfluidic system in quantitative and good yields, respectively. Six C-28-monoglycosidic and three bisdesmosidic saponins were synthesized, and their detailed structures were determined. The advantages of the microfluidic glycosylation were exploited for the development of a continuous glycosylation-deprotection sequence for saponin synthesis. This simple continuous method is based on the addition of a deprotecting reagent, instead of a quenching reagent, after the glycosylation step. Three bisdesmosidic saponins could be easily prepared from oleanolic acid via continuous microfluidic glycosylation-batch deprotection in 28–31% total yield. By reducing the number of purification steps, this synthetic approach using a microflow reactor could readily provide a wide variety of bisdesmosidic

Table 8. ^{1}H- and ^{13}C-NMR Spectral Assignments for Sugar Moieties of Bisdesmosidic Saponins 10a–10c

	28-*O*-*β*-Glc	δH	δC	3-*O*-*β*-Glc	δH	δC
10a	1′	6.30 (d, 8.0)	95.9	1″	4.91 (d, 8.0)	107.0
	2′	4.18 (t, 8.5)	74.2	2″	4.01 (t, 8.0)	75.9
	3′	4.26 (dd, 9.0, 8.5)	78.9	3″	4.22 (dd, 8.5, 8.0)	78.8
	4′	4.34 (dd, 9.5, 9.0)	71.2	4″	4.20 (dd, 9.0, 8.5)	71.9
	5′	4.00 (ddd, 9.5, 4.0, 2.0)	79.5	5″	3.98 (ddd, 9.0, 5.0, 2.0)	78.4
	6′	4.43 (dd, 12.0, 2.0)	62.2	6″	4.56 (dd, 12.0, 2.0)	63.1
		4.38 (dd, 12.0, 5.0)			4.37 (dd, 12.0, 5.0)	
	28-*O*-*β*-Glc	δH	δC	3-*O*-*β*-Gal	δH	δC
10b	1′	6.33 (d, 8.0)	95.9	1″	4.87 (d, 8.0)	107.0
	2′	4.22 (dd, 8.5, 8.0)	74.2	2″	4.46 (dd, 9.0, 8.0)	75.9
	3′	4.30 (dd, 9.0, 8.5)	78.9	3″	4.19 (dd, 9.0, 3.0)	73.2
	4′	4.37 (dd, 9.5, 9.0)	71.1	4″	4.60 (dd, 3.0, 1.0)	71.9
	5′	4.04 (ddd, 9.5, 4.0, 3.0)	79.5	5″	4.13 (td, 6.0, 1.0)	70.3
	6′	4.48 (dd, 12.0, 3.0)	62.2	6″	4.50 (dd, 12.0, 6.0)	62.5
		4.41 (dd, 12.0, 4.0)			4.45 (dd, 12.0, 6.0)	
	28-*O*-*β*-Glc	δH	δC	3-*O*-*β*-Xyl	δH	δC
10c	1′	6.33 (d, 8.5)	95.9	1″	4.84 (d, 7.5)	107.8
	2′	4.21 (dd, 8.5, 8.0)	74.2	2″	4.03 (dd, 8.5, 7.5)	75.6
	3′	4.30 (t, 9.0)	78.9	3″	4.18 (t, 8.5)	78.7
	4′	4.38 (dd, 9.5, 9.0)	71.1	4″	4.24 (ddd, 10.0, 8.5, 5.0)	71.2
	5′	4.04 (ddd, 9.5, 4.0, 2.0)	79.5	5″	4.39 (dd, 11.0, 5.0)	67.3
	6′	4.47 (dd, 12.0, 2.0)	62.2		3.79 (dd, 11.0, 5.0)	
		4.41 (dd, 12.0, 4.0)				

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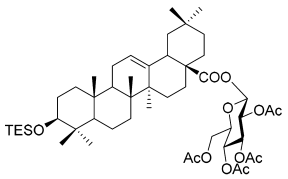
saponins and find application in the development of a saponin library for biological assays.

EXPERIMENTAL SECTION

General Information. All reactions were carried out under an argon atmosphere in dried glassware, unless otherwise noted. All reagents were purchased from Tokyo Kasei Kogyo, Kanto Chemical, Wako Pure Chemical Industries, Fluka, or Aldrich companies and used without further purification unless otherwise noted. Dry DMF, THF, and CH_2Cl_2 were purchased from Kanto Chemical Co. Precoated silica gel plates with a fluorescent indicator (Merck 60 F254) were used for analytical and preparative thin layer chromatography. Flash column chromatography was carried out with Kanto Chemical silica gel (Kanto Chemical, silica gel 60N, spherical neutral, 0.040−0.050 mm, Cat. No. 37 563-84). Powdered and predried 4 Å molecular sieves were used in glycosylation. Detection was carried out by staining with phosphomolybdic acid, resulting in blue spots and application of UV (254 nm). ^{1}H NMR spectra were recorded on Agilent Technologies 400-MR (400 MHz), 400-MR DD2 (400 Hz), and NMR DD2 400NB (400 MHz) spectrometers. ^{13}C NMR spectra were recorded on Agilent Technologies 400-MR (400 MHz), 400-MR DD2 (400 Hz), and NMR DD2 400NB (400 MHz) spectrometers. The chemical shifts are expressed in ppm downfield from the internal solvent peaks for $CDCl_3$ (7.26 ppm, ^{1}H NMR), CD_3OD (3.31 ppm, ^{1}H NMR), pyridine-d_5 (8.73 ppm, ^{1}H NMR), $CDCl_3$ (77.0 ppm, ^{13}C NMR), CD_3OD (49.0 ppm, ^{13}C NMR), or pyridine-d_5 (150.0 ppm, ^{13}C NMR), and J values are given in hertz. The coupling patterns are denoted s (singlet), d (doublet), dd (double doublet), ddd (double double doublet), t (triplet), dt (double triplet), q (quartet), m (multiplet), or br (broad). All infrared spectra were measured on a JASCO FT/IR-460 spectrometer, and absorbance bands are reported in wavenumber (cm^{-1}). High- and low-resolution mass spectra were measured on a JEOL JMS-T100 LP, JMS-700 MStation, and JEOL JMS-AX505 HA spectrometer. Optical rotations were measured using JASCO DIP-370 and a P-2200 polarimeter.

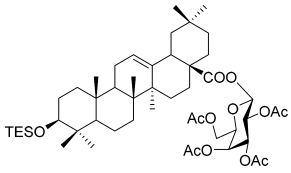
Olean-12-en-28-oic Acid, 3-*O*-Triethylsilyl-, 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl Ester (3a).



3a

MicroFlow. A solution of $BF_3{\cdot}OEt_2$ (6.0 μL, 0.0478 mmol, 0.006 M) dissolved in CH_2Cl_2 (7.5 mL) was injected into the micromixer using a syringe pump at a flow rate of 0.6 mL/min. Then, a solution of donor **2a** (94.2 mg, 0.1912 mmol, 0.025 M) and acceptor **1** (54.6 mg, 0.0956 mmol, 0.013 M) dissolved in CH_2Cl_2 (7.5 mL) was injected into the micromixer by the same syringe pump at a flow rate of 0.6 mL/min and mixed at −40 °C. After the reaction mixture was allowed to flow at −40 °C for an additional 100 s through a Teflon reactor tube (ϕ = 1.0 mm, l = 1.0 m), the reaction was quenched by adding triethylamine (3.0 μL) diluted in CH_2Cl_2. The mixture was concentrated under reduced pressure to give the crude product. Purification by flash column chromatography (silica gel, 20 g; hexane/AcOEt = 6:1) afforded **3a** (90.5 mg, 0.100 mmol, quant.) as a white foamy solid. R_f = 0.43 (hexane/AcOEt = 2:1); $[\alpha]_D^{26}$ +25.1 (c 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.58 (d, J = 8.0 Hz, 1H, 1′-H), 5.31 (dd, J = 4.8 Hz, 3.2 Hz, 1H, 12-H), 5.25 (dd, J = 10.0 Hz, 9.1 Hz, 1H, 4′-H), 5.18 (dd, J = 9.1 Hz, 8.0 Hz, 1H, 2′-H), 5.13 (t, J = 9.1 Hz, 1H, 3′-H), 4.27 (dd, J = 12.5 Hz, 4.3 Hz, 1H, 6′-H), 4.04 (dd, J = 12.5 Hz, 2.2 Hz, 1H, 6′-H), 3.79 (ddd, J = 10.0 Hz, 4.3 Hz, 2.2 Hz, 1H, 5′-H), 3.19 (m, 1H, 3-H), 2.78 (m, 1H, 18-H), 2.06 (s, 3H, -OCOC$\underline{H}_3$), 2.02 (s, 3H, -OCOC$\underline{H}_3$), 2.01 (s, 6H, -OCOC$\underline{H}_3$ ×2), 1.95 (m, 1H, 11-H), 1.84 (m, 2H, 16-H), 1.60 (m, 1H, 19-H), 1.58 (m, 2H, 22-H), 1.56 (m, 1H, 15-H), 1.54 (m, 1H, 1-H), 1.53 (m, 1H, 11-H), 1.48 (m, 2H, 6-H, 9-H), 1.44 (m, 2H, 2-H), 1.40 (m, 1H, 7-H), 1.31 (m, 1H, 6-H), 1.29 (m, 1H, 21-H), 1.18 (m, 1H, 21-H), 1.15 (m, 1H, 7-H), 1.14 (m, 1H, 19-H), 1.10 (s, 3H, 27-H), 1.01 (m, 1H, 15-H), 0.93 (m, 9H, -Si(CH$_2$C$\underline{H}_3$)$_3$), 0.89 (m, 1H, 1-H), 0.88 (s, 6H, 23-H, 30-H), 0.87 (s, 6H, 25-H, 29-H), 0.72 (s, 3H, 24-H), 0.69 (s, 3H, 26-H), 0.65 (m, 1H, 5-H), 0.56 (m, 6H, -Si(C$\underline{H}_2$CH$_3$)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.6 (C-28), 170.6 (-O$\underline{C}$OCH$_3$), 170.2 (-O$\underline{C}$OCH$_3$), 169.4 (-O$\underline{C}$OCH$_3$), 169.0 (-O$\underline{C}$OCH$_3$), 142.8 (C-13), 122.9 (C-12), 91.5 (C-1′), 79.4 (C-3), 72.8 (C-4′), 72.3 (C-5′), 69.8 (C-2′), 68.0 (C-3′), 61.5 (C-6′), 47.5 (C-5), 46.7 (C-9), 45.7 (C-17), 41.6 (C-19), 41.0 (C-14), 39.2 (C-18), 38.4 (C-8), 38.4 (C-4), 36.8 (C-1), 33.7 (C-10), 32.9 (C-21), 32.8 (C-29), 32.8 (C-7), 31.7 (C-22), 30.6 (C-20), 28.4 (C-23), 27.6 (C-15), 27.6 (C-2), 25.6 (C-27), 23.4 (C-30), 23.4 (C-16), 22.8 (C-11), 20.7 (-OCO$\underline{C}$H$_3$), 20.7 (-OCO$\underline{C}$H$_3$), 20.6 (-OCO$\underline{C}$H$_3$), 20.6 (-OCO$\underline{C}$H$_3$), 18.5 (C-6), 16.9 (C-26), 16.0 (C-24), 15.3 (C-25), 7.0 (-Si(CH$_2\underline{C}$H$_3$)$_3$), 5.2 (-Si($\underline{C}$H$_2$CH$_3$)$_3$); IR (NaCl) cm^{-1} ν 2950 (=C−H), 1692 (-C=O), 1075 (-C−O-); HRMS (ESI-TOF) m/z [M + Na]$^+$ calcd for $C_{50}H_{80}O_{12}SiNa$ 923.5317, found 923.5326.

Olean-12-en-28-oic Acid, 3-*O*-Triethylsilyl-, 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl Ester (3b).



3b

MicroFlow. A solution of $BF_3{\cdot}OEt_2$ (55 μL, 0.4379 mmol, 0.03 M) dissolved in CH_2Cl_2 (14.6 mL) was injected into the micromixer using a syringe pump at a flow rate of 0.6 mL/min. Then, a solution of donor **2b** (539 mg, 1.095 mmol, 0.075 M) and acceptor **1** (250 mg, 0.4379 mmol, 0.03 M) dissolved in CH_2Cl_2 (16.4 mL) was injected into the micromixer by the same syringe pump at a flow rate of 0.6 mL/min and mixed at −40 °C. After the reaction mixture was allowed to flow at −40 °C for an additional 100 s through a Teflon reactor tube (ϕ = 1.0 mm, l = 1.0 m), the reaction was quenched by adding triethylamine (0.24 mL) diluted in CH_2Cl_2. The mixture was concentrated under reduced pressure to give the crude product. Purification by flash column chromatography (silica gel, 80 g; hexane/AcOEt = 5:1) afforded **3b** (413 mg, 0.458 mmol, quant.) as a white solid. R_f = 0.40 (hexane/AcOEt = 2:1); $[\alpha]_D^{22}$ +44.1 (c 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.55 (d, J = 8.5 Hz, 1H, 1′-H), 5.40 (dd, J = 3.5 Hz, 1.0 Hz, 1H, 4′-H), 5.35 (dd, J = 10.4 Hz, 8.4 Hz, 1H, 2′-H), 5.32 (dd, J = 3.3 Hz, 3.3 Hz, 1H, 12-H), 5.07 (dd, J = 10.4 Hz, 3.5 Hz, 1H, 3′-H), 4.10 (m, 2H, 6′-H), 4.00 (ddd, J = 7.2 Hz, 5.9 Hz, 1.2 Hz, 1H, 5′-H), 3.20 (dd, J = 11.1 Hz, 4.3 Hz, 1H, 3-H), 2.80 (dd, J = 13.9 Hz, 4.0 Hz, 1H, 18-H), 2.16 (s, 3H, -OCOC$\underline{H}_3$), 2.03 (s, 3H, -OCOC$\underline{H}_3$), 2.02 (s, 3H, -OCOC$\underline{H}_3$), 1.99 (s, 3H, -OCOC$\underline{H}_3$), 1.95 (m, 1H, 11-H), 1.86 (m, 2H, 16-H), 1.65 (m, 2H, 22-H), 1.61 (m, 1H, 19-H), 1.58 (m, 1H, 15-H), 1.54 (m, 1H, 1-H), 1.49 (m, 1H, 6-H), 1.49 (m, 2H, 9-H), 1.45 (m, 2H, 2-H), 1.40 (m, 1H, 7-H), 1.33 (m, 1H, 6-H), 1.30 (m, 1H, 21-H), 1.22 (m, 1H, 21-H), 1.18 (m, 1H, 7-H), 1.15 (m, 1H, 19-H), 1.12 (s, 3H, 27-H), 1.02 (m, 1H, 15-H), 0.95 (t, J = 7.6 Hz, 9H, -Si(CH$_2$C$\underline{H}_3$)$_3$), 0.91 (m, 1H, 1-H), 0.90 (s, 12H, 23-H, 25-H, 29-H, 30-H), 0.74 (s, 3H, 24-H), 0.73 (s, 3H, 26-H), 0.68 (m, 1H, 5-H), 0.58 (m, 6H, -Si(C$\underline{H}_2$CH$_3$)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.6 (C-28), 170.3 (-O$\underline{C}$OCH$_3$), 170.2 (-O$\underline{C}$OCH$_3$), 169.9 (-O$\underline{C}$OCH$_3$), 169.1 (-O$\underline{C}$OCH$_3$), 142.9 (C-13), 122.8 (C-12), 92.0 (C-1′), 79.4 (C-3), 71.4 (C-5′), 70.9 (C-3′), 67.5 (C-2′), 66.8 (C-4′), 60.8 (C-6′), 55.3 (C-5), 47.6 (C-9), 46.8 (C-17), 45.8 (C-19), 41.7 (C-14), 41.0 (C-18), 39.5 (C-1), 39.3 (C-8), 39.3 (C-4), 36.9 (C-10), 33.8 (C-21), 33.0 (C-7), 33.0 (C-29), 31.6 (C-22), 30.6 (C-20), 28.4 (C-23), 27.8 (C-15), 27.7 (C-2), 25.6 (C-27), 23.5 (C-11), 23.4 (C-16), 22.8 (C-30), 20.7 (-OCO$\underline{C}$H$_3$), 20.6 (-OCO$\underline{C}$H$_3$), 20.6 (-OCO$\underline{C}$H$_3$), 20.5 (-OCO$\underline{C}$H$_3$), 18.5 (C-6), 17.0 (C-26), 16.0 (C-24), 15.4 (C-25), 7.0 (-Si(CH$_2\underline{C}$H$_3$)$_3$), 5.3 (-Si($\underline{C}$H$_2$CH$_3$)$_3$); IR (KBr) cm^{-1} ν 2957 (=C−H), 1752 (-C=O), 1067 (-C−O-); HRMS (ESI-TOF) m/z [M + Na]$^+$ calcd for $C_{50}H_{80}O_{12}SiNa$ 923.5317, found 923.5307.

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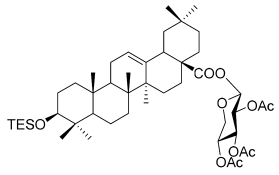
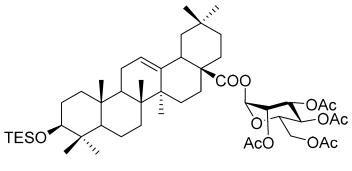
Olean-12-en-28-oic Acid, 3-*O*-Triethylsilyl-, 2,3,4-Tri-*O*-acetyl-β-D-xylopyranosyl Ester (3c).



3c

MicroFlow. A solution of $BF_3{\cdot}OEt_2$ (72.0 μL, 0.5705 mmol, 0.0316 M) dissolved in CH_2Cl_2 (18 mL) was injected into the micromixer using a syringe pump at a flow rate of 0.6 mL/min. Then, a solution of donor **2c** (480 mg, 1.1411 mmol, 0.0634 M) and acceptor **1** (326 mg, 0.5705 mmol, 0.0317 M) dissolved in CH_2Cl_2 (18 mL) was injected into the micromixer by the same syringe pump at a flow rate of 0.6 mL/min and mixed at −40 °C. After the reaction mixture was allowed to flow at −40 °C for an additional 100 s through a Teflon reactor tube (ϕ = 1.0 mm, l = 1.0 m), the reaction was quenched by adding triethylamine (79.4 μL) diluted in CH_2Cl_2. The mixture was concentrated under reduced pressure to give the crude product. Purification by flash column chromatography (silica gel, 25 g; hexane/AcOEt = 6:1) afforded **3c** (312 mg, 0.0941 mmol, 66%) as a white foamy solid. R_f = 0.43 (hexane/AcOEt = 2:1); $[\alpha]_D^{22}$ +28.2 (*c* 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.63 (d, *J* = 6.8 Hz, 1H, 1′-H), 5.31 (t, *J* = 3.5 Hz, 1H, 12-H), 5.20 (t, *J* = 8.1 Hz, 3′-H), 5.05 (dd, *J* = 8.1 Hz, 6.8 Hz, 1H, 2′-H), 5.13 (m, *J* = 9.1 Hz, 1H, 4′-H), 4.12 (m, 1H, 5′-H), 3.49 (dd, *J* = 13.6 Hz, 8.4 Hz, 1H, 5′-H), 3.20 (m, 1H, 3-H), 2.84 (m, 1H, 18-H), 2.06 (s, 3H, -OCOC$\underline{H}_3$), 2.05 (s, 3H, -OCOC$\underline{H}_3$), 2.04 (s, 3H, -OCOC$\underline{H}_3$), 1.95 (m, 1H, 11-H), 1.84 (m, 2H, 16-H), 1.60 (m, 1H, 19-H), 1.58 (m, 2H, 22-H), 1.56 (m, 1H, 15-H), 1.54 (m, 1H, 1-H), 1.53 (m, 1H, 11-H), 1.48 (m, 2H, 6-H, 9-H), 1.44 (m, 2H, 2-H), 1.40 (m, 1H, 7-H), 1.31 (m, 1H, 6-H), 1.29 (m, 1H, 21-H), 1.18 (m, 1H, 21-H), 1.15 (m, 1H, 7-H), 1.14 (m, 1H, 19-H), 1.10 (s, 3H, 27-H), 1.01 (m, 1H, 15-H), 0.93 (m, 9H, -Si($CH_2C\underline{H}_3$)$_3$), 0.89 (m, 1H, 1-H), 0.88 (s, 6H, 23-H, 30-H), 0.87 (s, 6H, 25-H, 29-H), 0.72 (s, 3H, 24-H), 0.69 (s, 3H, 26-H), 0.65 (m, 1H, 5-H), 0.56 (m, 6H, -Si($C\underline{H}_2CH_3$)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.6 (C-28), 169.8 (-O$\underline{C}$OCH$_3$ ×2), 169.0 (-O$\underline{C}$OCH$_3$), 143.0 (C-13), 123.0 (C-12), 91.7 (C-1′), 79.5 (C-3), 71.0 (C-3′), 69.3 (C-2′), 68.3 (C-4′), 62.5 (C-5′), 55.2 (C-5), 47.6 (C-9), 46.8 (C-17), 45.7 (C-19), 41.7 (C-14), 39.2 (C-18), 38.7 (C-8), 38.4 (C-4), 37.0 (C-1), 33.7 (C-10), 32.9 (C-21), 32.9 (C-29), 32.8 (C-7), 31.9 (C-22), 30.6 (C-20), 28.0 (C-23), 27.7 (C-15), 27.1 (C-2), 25.6 (C-27), 23.5 (C-30), 23.4 (C-16), 22.8 (C-11), 20.7 (-OCO$\underline{C}$H$_3$), 20.7 (-OCO$\underline{C}$H$_3$), 20.6 (-OCO$\underline{C}$H$_3$), 18.3 (C-6), 16.9 (C-26), 15.5 (C-24), 15.3 (C-25); IR (KBr) cm^{-1} ν 2933 (=C−H), 1760 (-C=O); HRMS (ESI-TOF) *m*/*z* [M + Na]$^+$ calcd for $C_{47}H_{76}O_{10}SiNa$ 851.5074, found 851.5078.

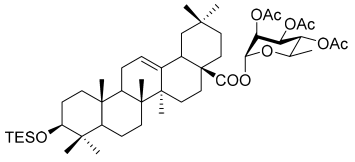
Olean-12-en-28-oic Acid, 3-*O*-Triethylsilyl-, 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl Ester (3d).



3d

A solution of $BF_3{\cdot}OEt_2$ (62 μL, 0.5070 mmol, 0.0035 M) dissolved in CH_2Cl_2 (16.4 mL) was injected into the micromixer using a syringe pump at a flow rate of 0.6 mL/min. Then, a solution of donor **2d** (500 mg, 1.0148 mmol, 0.0619 M) and acceptor **1** (289.5 mg, 0.5070 mmol, 0.0309 M) dissolved in CH_2Cl_2 (16.4 mL) was injected into the micromixer by the same syringe pump at a flow rate of 0.6 mL/min and mixed at −40 °C. After the reaction mixture was allowed to flow at −40 °C for an additional 3 min through a Teflon reactor tube (ϕ = 1.0 mm, l = 1.0 m), the reaction was quenched by adding triethylamine (0.26 mL) diluted in CH_2Cl_2. The mixture was concentrated under reduced pressure to give the crude product. Purification by flash column chromatography (silica gel, 40 g; hexane/AcOEt = 9:1) afforded **3d** (414.7 mg, 0.4601 mmol, 93%) as a white solid. R_f = 0.40 (hexane/AcOEt = 2:1); $[\alpha]_D^{22}$ +51.6 (*c* 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.06 (d, 2.0 Hz, 1H, 1′-H), 5.36 (t, *J* = 3.5 Hz, 1H, 12-H), 5.32 (t, *J* = 10.0 Hz, 1H, 4′-H), 5.26 (dd, *J* = 10.0 Hz, 3.2 Hz, 1H, 3′-H), 5.17 (dd, *J* = 3.2 Hz, 2.0 Hz, 1H, 2′-H), 4.29 (dd, *J* = 12.3 Hz, 5.0 Hz, 1H, 6′-H), 4.07 (dd, *J* = 12.3 Hz, 2.3 Hz, 1H, 6′-H), 3.99 (ddd, *J* = 10.0 Hz, 5.0 Hz, 2.3 Hz, 1H, 5′-H), 3.18 (dd, *J* = 11.0 Hz, 4.5 Hz, 1H, 3-H), 2.83 (dd, *J* = 13.8 Hz, 4.0 Hz, 1H, 18-H), 2.14 (s, 3H, -OCOC$\underline{H}_3$), 2.06 (s, 3H, -OCOC$\underline{H}_3$), 2.05 (s, 3H, -OCOC$\underline{H}_3$), 1.98 (s, 3H, -OCOC$\underline{H}_3$), 1.93 (m, 2H, 11-H), 1.86 (m, 2H, 16-H), 1.62 (m, 1H, 19-H), 1.60 (m, 2H, 22-H), 1.57 (m, 1H,15-H), 1.53 (m, 1H, 1-H), 1.51 (m, 1H, 9-H), 1.50 (m, 1H, 6-H), 1.47 (m, 2H, 2-H), 1.42 (m, 2H, 7-H), 1.31 (m, 1H, 21-H), 1.31 (m, 1H, 6-H), 1.20 (m, 1H, 21-H), 1.16 (m, 1H, 19-H), 1.12 (s, 3H, 27-H), 1.08 (m, 1H, 15-H), 0.97 (t, *J* = 7.9 Hz, 9H, -Si($CH_2C\underline{H}_3$)$_3$), 0.92 (s, 6H, 29-H, 30-H), 0.90 (s, 3H, 23-H), 0.90 (m, 1H, 1-H), 0.89 (s, 3H, 25-H), 0.73 (s, 3H, 24-H), 0.71 (s, 3H, 26-H), 0.68 (m, 1H, 5-H), 0.58 (m, 6H, -Si($C\underline{H}_2CH_3$)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.7 (C-28), 170.6 (-O$\underline{C}$OCH$_3$), 169.8 (-O$\underline{C}$OCH$_3$), 169.6 (-O$\underline{C}$OCH$_3$), 169.6 (-O$\underline{C}$OCH$_3$), 142.7 (C-13), 123.4 (C-12), 90.2 (C-1′), 79.5 (C-3), 71.0 (C-5′), 68.9 (C-3′), 68.3 (C-2′), 65.5 (C-4′), 62.2 (C-6′), 55.3 (C-5), 47.6 (C-9), 47.2 (C-17), 45.7 (C-19), 41.6 (C-14), 41.2 (C-18), 39.3 (C-8), 38.4 (C-1,C-4), 36.9 (C-10), 33.7 (C-21), 33.0 (C-7), 32.7 (C-29), 32.2 (C-22), 30.6 (C-20), 28.4 (C-23), 27.7 (C-15), 27.5 (C-2), 25.8 (C-27), 23.5 (C-30), 23.3 (C-16), 23.3 (C-11), 20.7 (-OCO$\underline{C}$H$_3$), 20.7 (-OCO$\underline{C}$H$_3$), 20.7 (-OCO$\underline{C}$H$_3$), 20.6 (-OCO$\underline{C}$H$_3$), 18.5 (C-6), 16.9 (C-26), 16.0 (C-24), 15.3 (C-25), 7.0 (-Si($CH_2\underline{C}H_3$)$_3$), 5.3 (-Si($\underline{C}H_2CH_3$)$_3$); IR (KBr) cm^{-1} ν 2952 (=C−H), 1755 (-C=O), 1697 (-C=C-), 1057 (-C−O-); HRMS (ESI-TOF) *m*/*z* [M + Na]$^+$ calcd for $C_{50}H_{80}O_{12}SiNa$ 923.5317, found 923.5303.

Olean-12-en-28-oic Acid, 3-*O*-Triethylsilyl-, 2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl Ester (3e).



3e

MicroFlow. A solution of $BF_3{\cdot}OEt_2$ (12.5 μL, 0.1002 mmol, 0.0334 M) dissolved in CH_2Cl_2 (3.0 mL) was injected into the micromixer using a syringe pump at a flow rate of 0.6 mL/min. Then, a solution of donor **2e** (87.1 mg, 0.2004 mmol, 0.0668 M) and acceptor **1** (57.2 mg, 0.1002 mmol, 0.0334 M) dissolved in CH_2Cl_2 (3.0 mL) was injected into the micromixer by the same syringe pump at a flow rate of 0.6 mL/min and mixed at −40 °C. After the reaction mixture was allowed to flow at −40 °C for an additional 100 s through a Teflon reactor tube (ϕ = 1.0 mm, l = 1.0 m), the reaction was quenched by adding triethylamine (13.9 μL) diluted in CH_2Cl_2. The mixture was concentrated under reduced pressure to give the crude product. Purification by flash column chromatography (silica gel, 30 g; hexane/AcOEt = 10:1 → 8:1 → 7:1 → 5:1) afforded **3e** (79.3 mg, 0.0941 mmol, 94%) as a white solid. R_f = 0.56 (hexane/AcOEt = 2:1); $[\alpha]_D^{22}$ +16.0 (*c* 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.02 (d, *J* = 2.0 Hz, 1H, 1′-H), 5.32 (t, *J* = 3.2 Hz, 1H, 12-H), 5.27 (dd, *J* = 10.0 Hz, 3.3 Hz, 1H, 3′-H), 5.20 (dd, *J* = 3.3 Hz, 2.0 Hz, 1H, 2′-H), 5.13 (t, *J* = 10.0 Hz, 1H, 4′-H), 3.94 (m, 1H, 5′-H), 3.20 (m, 1H, 3-H), 2.90 (m, 1H, 18-H), 2.16 (s, 3H, -OCOC$\underline{H}_3$), 2.08 (s, 3H, -OCOC$\underline{H}_3$), 2.00 (s, 3H, -OCOC$\underline{H}_3$), 1.95 (m, 1H, 11-H), 1.84 (m, 2H, 16-H), 1.60 (m, 1H, 19-H), 1.58 (m, 2H, 22-H), 1.56 (m, 1H, 15-H), 1.54 (m, 1H, 1-H), 1.53 (m, 1H, 11-H), 1.48 (m, 2H, 6-H, 9-H), 1.44 (m, 2H, 2-H), 1.40 (m, 1H, 7-H), 1.31 (m, 1H, 6-H), 1.29 (m, 1H, 21-H), 1.20 (d, *J* = 6.2 Hz, 3H, 6′-C$\underline{H}_3$), 1.18 (m, 1H, 21-H), 1.15 (m, 1H, 7-H), 1.14 (m, 1H, 19-H), 1.10 (s, 3H, 27-H), 1.01 (m, 1H, 15-H), 0.93 (m, 9H, -Si($CH_2C\underline{H}_3$)$_3$), 0.89 (m, 1H, 1-H), 0.88 (s, 6H, 23-H, 30-H), 0.87 (s, 6H, 25-H, 29-H), 0.72 (s, 3H, 24-H), 0.69 (s, 3H, 26-H), 0.65 (m, 1H, 5-H), 0.56 (m, 6H, -Si($C\underline{H}_2CH_3$)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.7 (C-28), 169.9 (-O$\underline{C}$OCH$_3$), 169.8 (-O$\underline{C}$OCH$_3$), 169.8

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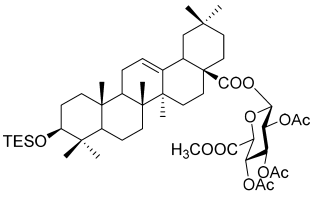
(-O<u>C</u>OCH$_3$), 143.6 (C-13), 122.9 (C-12), 90.1 (C-1′), 78.9 (C-3), 70.3 (C-4′), 69.0 (C-3′), 68.9 (C-5′), 68.7 (C-2′), 55.2 (C-5), 47.5 (C-9), 47.3 (C-17), 45.6 (C-19), 41.8 (C-14), 41.7 (C-18), 39.4 (C-8), 38.7 (C-4), 38.4 (C-1), 37.0 (C-10), 33.7 (C-21), 33.0 (C-29), 32.7 (C-7), 32.5 (C-22), 30.7 (C-20), 28.1 (C-23), 27.5 (C-15), 27.1 (C-2), 25.8 (C-27), 23.5 (C-30), 23.4 (C-16), 22.9 (C-11), 20.8 (-OCO<u>C</u>H$_3$), 20.8 (-OCO<u>C</u>H$_3$), 20.6 (-OCO<u>C</u>H$_3$), 18.3 (C-6), 17.5 (6′-<u>C</u>H$_3$) 17.0 (C-26), 15.6 (C-24), 15.3 (C-25), 7.0 (-Si(CH$_2$<u>C</u>H$_3$)$_3$), 5.3 (-Si(<u>C</u>H$_2$CH$_3$)$_3$); IR (KBr) cm^{-1} ν 2924 (=C−H), 1761 (-C=O); HRMS (ESI-TOF) m/z [M + Na]$^+$ calcd for $C_{48}H_{78}O_{10}SiNa$ 865.5231, found 865.5237.

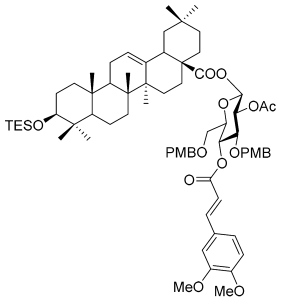
Olean-12-en-28-oic Acid, 3-*O*-Triethylsilyl, Methyl 2,3,4-Tri-*O*-acetyl-β-D-glucopyranuronosyl Ester (3f).



3f

MicroFlow. A solution of $BF_3{\cdot}OEt_2$ (39 μL, 0.3097 mmol, 0.03 M) dissolved in CH_2Cl_2 (10.3 mL) was injected into the micromixer using a syringe pump at a flow rate of 0.6 mL/min. Then, a solution of donor **2f** (296.5 mg, 0.6194 mmol, 0.0601 M) and acceptor **1** (176.8 mg, 0.3097 mmol, 0.03 M) dissolved in CH_2Cl_2 (10.3 mL) was injected into the micromixer by the same syringe pump at a flow rate of 0.6 mL/min and mixed at −40 °C. After the reaction mixture was allowed to flow at −40 °C for an additional 3 min through a Teflon reactor tube (ϕ = 1.0 mm, l = 1.0 m), the reaction was quenched by adding triethylamine (0.17 mL) diluted in CH_2Cl_2. The mixture was concentrated under reduced pressure to give the crude product. Purification by flash column chromatography (silica gel, 40 g; hexane/AcOEt = 6:1) afforded **3f** (203.9 mg, 0.2298 mmol, 74%) as a white solid. R_f = 0.40 (hexane/AcOEt = 2:1); $[\alpha]_D^{24}$ +30.9 (c 0.98, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.63 (d, J = 8.1 Hz, 1H, 1′-H), 5.31 (dt, J = 3.7 Hz, 1H, 12-H), 5.30 (t, J = 9.5 Hz, 1H, 3′-H), 5.23 (t, J = 9.5 Hz, 1H, 4′-H), 5.20 (dd, J = 9.5 Hz, 8.1 Hz, 1H, 2′-H), 4.12 (d, J = 9.5 Hz, 1H, 5′-H), 3.72 (s, 3H, -COOC<u>H</u>$_3$), 3.20 (dd, J = 11.1 Hz, 4.4 Hz, 1H, 3-H), 2.81 (dd, J = 14.0 Hz, 4.0 Hz, 1H, 18-H), 2.03 (s, 3H, -OCOC<u>H</u>$_3$), 2.02 (s, 3H, -OCOC<u>H</u>$_3$), 2.02 (s, 3H, -OCOC<u>H</u>$_3$), 1.96 (m, 2H, 11-H), 1.87 (m, 1H, 16-H), 1.64 (m, 2H, 22-H), 1.62 (m, 1H, 19-H), 1.60 (m, 1H, 15-H), 1.58 (m, 1H, 2-H), 1.56 (m, 1H, 1-H), 1.54 (m, 1H, 16-H), 1.52 (m, 1H, 6-H), 1.50 (m, 1H, 9-H), 1.49 (m, 1H, 2-H), 1.40 (m, 1H, 7-H), 1.35 (m, 1H, 6-H), 1.31 (m, 1H, 21-H), 1.21 (m, 1H, 21-H), 1.18 (m, 1H, 19-H), 1.12 (s, 3H, 27-H), 1.03 (m, 1H, 15-H), 0.95 (t, J = 8.0 Hz, 9H, -Si(CH$_2$C<u>H</u>$_3$)$_3$), 0.90 (s, 3H, 23-H), 0.90 (s, 3H, 25-H), 0.89 (m, 1H, 1-H), 0.89 (s, 3H, 29-H), 0.89 (s, 3H, 30-H), 0.74 (s, 3H, 24-H), 0.71 (s, 3H, 26-H), 0.68 (m, 1H, 5-H), 0.58 (m, 6H, -Si(C<u>H</u>$_2$CH$_3$)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.5 (C-28), 169.9 (-O<u>C</u>OCH$_3$), 169.4 (-O<u>C</u>OCH$_3$), 168.9 (-O<u>C</u>OCH$_3$), 166.7 (C-6′), 142.7 (C-13), 123.0 (C-12), 91.2 (C-1′), 79.5 (C-3), 72.9 (C-5′), 72.0 (C-3′), 69.7 (C-2′), 69.3 (C-4′), 55.3 (C-5), 52.9 (-COO<u>C</u>H$_3$), 47.6 (C-9), 46.8 (C-17), 45.7 (C-19), 41.7 (C-14), 41.0 (C-18), 39.3 (C-4), 39.3(C-10), 38.5 (C-1), 36.9 (C-8), 33.7 (C-21), 33.7 (C-29), 33.0 (C-7), 31.6 (C-22), 30.6 (C-20), 28.4 (C-23), 27.7 (C-2), 27.7 (C-15), 25.7 (C-27), 23.4 (C-30), 23.4 (C-16), 22.8 (C-11), 20.6 (-OCO<u>C</u>H$_3$), 20.6 (-OCO<u>C</u>H$_3$), 20.4 (-OCO<u>C</u>H$_3$), 18.5 (C-6), 16.9 (C-26), 16.4 (C-24), 15.4 (C-25), 7.0 (-Si(CH$_2$<u>C</u>H$_3$)$_3$), 5.3 (-Si(<u>C</u>H$_2$CH$_3$)$_3$); IR (KBr) cm^{-1} ν 2952 (=C−H), 1759 (-C=O), 1698 (-C=C-), 1068 (-C−O-); HRMS (ESI-TOF) m/z [M + Na]$^+$ calcd for $C_{49}H_{78}O_{12}SiNa$ 909.5160, found 909.5147.

Olean-12-en-28-olic Acid, 3-*O*-Triethylsilyl-, 2′-*O*-Acetyl-, 3′,6′-*O*-(4-Methoxybenzyl)-, 4-*O*-[(*E*)-3,4-Dimethoxycinnamoyl]-β-D-glucopyranosyl Ester (3g).



3g

MicroFlow. A solution of $BF_3{\cdot}OEt_2$ (12.0 μL, 0.0976 mmol, 0.0976 M) dissolved in CH_2Cl_2 (1.0 mL) was injected into the micromixer using a syringe pump at a flow rate of 0.1 mL/min. Then, a solution of donor **2g** (7.8 mg, 9.76 μmol) and acceptor **1** (5.6 mg, 9.76 μmol) dissolved in CH_2Cl_2 (7.5 mL) was injected into the micromixer by the same syringe pump at a flow rate of 0.1 mL/min and mixed at −40 °C. After the reaction mixture was allowed to flow at −40 °C for an additional 18 min through a Teflon reactor tube (ϕ = 1.0 mm, l = 1.0 m), the reaction was quenched by adding triethylamine (3.0 μL) diluted in CH_2Cl_2. The mixture was concentrated under reduced pressure to give the crude product. Purification by flash column chromatography (silica gel, 7.5 g; hexane/AcOEt = 5:1) afforded **3g** (12.3 mg, 9.76 μmol, quant.) as a colorless oil. R_f = 0.29 (hexane/AcOEt = 2:1); $[\alpha]_D^{27}$ +21.1 (c 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (d, J = 16.0 Hz, 1H, PhCH=C<u>H</u>), 7.21−6.73 (m, 11H, Ph-H), 6.14 (d, J = 16.0 Hz, 1H, PhC<u>H</u>=CH), 5.56 (d, J = 7.8 Hz, 1H, 1′-H), 5.33 (m, 1H, 12-H), 5.27 (t, J = 9.2 Hz, 1H, 4′-H), 5.22 (dd, J = 9.4 Hz, 7.8 Hz, 1H, 2′-H), 4.54 (m, 2H, -C<u>H</u>$_2$Ph), 4.40 (m, 2H, -C<u>H</u>$_2$Ph), 3.93 (s, 6H, -OC<u>H</u>$_3$ ×2), 3.79 (t, J = 9.4 Hz, 1H, 3′-H), 3.71 (ddd, J = 9.4 Hz, 4.7 Hz, 3.7 Hz, 1H, 5′-H), 3.68 (s, 3H, -OC<u>H</u>$_3$), 3.67 (s, 3H, -OC<u>H</u>$_3$), 3.57 (dd, J = 11.0 Hz, 3.7 Hz, 1H, 6′-H), 3.51 (dd, J = 11.0 Hz, 4.7 Hz, 1H, 6′-H), 3.21 (m, 1H, 3-H), 2.84 (m, 1H, 18-H), 2.00 (s, 3H, -OCOC<u>H</u>$_3$), 1.99 (m, 1H, 11-H), 1.87 (m, 2H, 16-H), 1.63 (m, 2H, 22-H), 1.62 (m, 1H, 19-H), 1.59 (m, 1H, 15-H), 1.58 (m, 1H, 11-H), 1.56 (m, 1H, 1-H), 1.52 (m, 1H, 6-H), 1.51 (m, 1H, 9-H), 1.47 (m, 2H, 2-H) 1.34 (m, 1H, 6-H), 1.33 (m, 2H, 21-H) 1.18 (m, 2H, 7-H), 1.15 (m, 1H, 19-H), 1.13 (s, 3H, 27-H), 1.04 (m, 1H, 15-H), 0.98 (m, 9H, -Si(CH$_2$C<u>H</u>$_3$)$_3$), 0.91 (s, 3H, 23-H), 0.90 (m, 4H, 1-H, 30-H), 0.89 (s, 3H, 29-H), 0.88 (s, 3H, 25-H), 0.75 (s, 6H, 24-H, 26-H), 0.69 (m, 1H, 5-H), 0.59 (m, 6 H, -Si(C<u>H</u>$_2$CH$_3$)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.9 (C-28), 168.8 (-O<u>C</u>OCH$_3$), 165.6 (4′-O<u>C</u>O-), 159.2, 159.0, 151.3, 149.2, 145.6 (PhCH=<u>C</u>H), 143.0 (C-13), 129.9, 129.9, 129.9, 129.7, 129.6, 129.4, 129.3, 127.1, 122.9, 122.8 (C-12), 114.8 (Ph<u>C</u>H=CH), 113.7, 113.6, 113.6, 110.9, 109.5, 91.9 (C-1′), 79.5 (C-3′), 79.4 (C-3), 74.4 (C-5′), 73.2 (-<u>C</u>H$_2$Ph), 73.1 (-<u>C</u>H$_2$Ph), 71.3 (C-2′), 70.3 (C-4′), 68.7 (C-6′), 56.0 (-O<u>C</u>H$_3$), 55.8 (-O<u>C</u>H$_3$), 55.3 (C-5), 55.1 (-O<u>C</u>H$_3$), 55.0 (-O<u>C</u>H$_3$), 47.6 (C-9), 46.8 (C-17), 45.8 (C-19), 41.7 (C-14), 40.9 (C-18), 39.3 (C-8), 39.3 (C-4), 38.5 (C-1), 36.9 (C-10), 33.8 (C-21), 33.0 (C-29), 32.9 (C-7), 31.7 (C-22), 30.6 (C-20), 28.4 (C-23), 27.8 (C-15), 27.7 (C-2), 25.6 (C-27), 23.5 (C-30), 23.4 (C-16), 22.7 (C-11), 20.9 (-OCO<u>C</u>H$_3$), 18.5 (C-6), 17.0 (C-26), 16.0 (C-24), 15.4 (C-25), 7.0 (-Si-(CH$_2$<u>C</u>H$_3$)$_3$), 5.2 (-Si(<u>C</u>H$_2$CH$_3$)$_3$); IR (NaCl) cm^{-1} ν 2951 (=C−H), 1753 (-C=O), 1631 (-C=C−), 1514 (-C=C−), 1066 (-C−O-); HRMS (ESI-TOF) m/z [M + Na]$^+$ calcd for $C_{71}H_{100}O_{14}SiNa$ 1227.6780, found 1227.6772.

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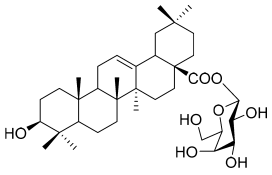
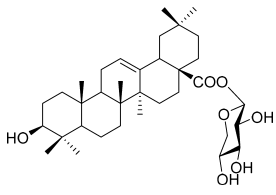
Olean-12-en-28-oic Acid, 3-Hydroxy-, β-D-Galactopyranosyloxy Ester (4b).



4b

To a solution of **3b** (200 mg, 0.2219 mmol) in MeOH (4.4 mL) at ambient temperature was added NaH (0.88 mg, 0.0222 mmol, 60% disp.). The reaction mixture was stirred at ambient temperature for 1 h, neutralized with Dowex H^+ resin to pH 4, and filtered. The filtrate was concentrated under reduced pressure to give pure saponin **4b** (144.4 mg, 0.2333 mmol, quant.) as a white solid without further purification. R_f = 0.43 ($CHCl_3$/MeOH = 5:1); $[\alpha]_D^{22}$ +74.2 (*c* 0.25, MeOH); ^{1}H NMR (400 MHz, pyridine-d_5) δ 6.29 (d, *J* = 8.2 Hz, 1H, 1′-H), 5.46 (dd, *J* = 3.4 Hz, 3.4 Hz, 1H, 12-H), 4.68 (m, 1H, 3′-H), 4.65 (m, 1H, 2′-H), 4.51 (ddd, *J* = 10.9 Hz, 6.5 Hz, 6.5 Hz, 1H, 6′-H), 4.41 (ddd, *J* = 10.8 Hz, 5.4 Hz, 5.4 Hz, 1H, 6′-H), 4.25 (m, 1H, 4′-H), 4.21 (m, 1H, 5′-H), 3.45 (m, 1H, 3-H), 3.22 (dd, *J* = 4.2 Hz, 4.2 Hz, 1H, 18-H), 2.35 (m, 1H, 2-H), 2.06 (m, 2H, 11-H), 1.94 (m, 2H, 16-H), 1.83 (m, 2H, 2-H, 22-H), 1.78 (m, 1H, 22-H), 1.77 (m, 1H, 19-H), 1.68 (m, 1H, 9-H), 1.58 (m, 1H, 1-H), 1.54 (m, 1H, 6-H), 1.48 (m, 1H, 7-H), 1.40 (m, 1H, 6-H), 1.38 (m, 1H, 7-H), 1.35 (m, 1H, 21-H), 1.30 (m, 1H, 19-H), 1.24 (s, 6H, 23-H, 27-H), 1.16 (s, 3H, 26-H), 1.13 (m, 2H, 15-H), 1.09 (m, 1H, 21-H), 1.04 (s, 3H, 24-H), 0.99 (m, 1H, 1-H), 0.94 (s, 3H, 25-H), 0.91 (s, 3H, 29-H), 0.88 (s, 3H, 30-H), 0.85 (m, 1H, 5-H); ^{13}C NMR (100 MHz, pyridine-d_5) δ 176.6 (C-28), 144.3 (C-13), 123.0 (C-12), 96.4 (C-1′), 78.2 (C-3), 77.9 (C-5′), 75.9 (C-4′), 71.6 (C-3′), 70.2 (C-2′), 62.0 (C-6′), 56.0 (C-5), 48.3 (C-9), 47.1 (C-17), 46.4 (C-19), 42.3 (C-14), 41.9 (C-18), 40.1 (C-8), 39.5 (C-4), 39.1 (C-1), 37.5 (C-10), 34.1 (C-21), 33.3 (C-7), 33.3 (C-29), 32.7 (C-22), 30.9 (C-20), 28.9 (C-23), 28.4 (C-15), 28.3 (C-2), 26.2 (C-27), 24.0 (C-30), 23.8 (C-16), 23.5 (C-11), 19.0 (C-6), 17.7 (C-26), 16.7 (C-24), 15.8 (C-25).; IR (KBr) cm^{-1} ν 3435 (-O−H), 2949 (=C−H), 1736 (-C=O), 1719 (-C=C-), 1070 (-C−O-); HRMS (ESI-TOF) *m*/*z* $[M + Na]^+$ calcd for $C_{36}H_{58}O_8Na$ 641.4029, found 641.4016.

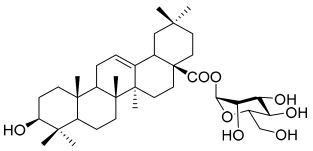
Olean-12-en-28-oic Acid, 3-Hydroxy-, β-D-Xylopyranosyloxy Ester (4c).



4c

To a solution of **3c** (200 mg, 0.241 mmol) in MeOH (2.4 mL) at ambient temperature was added NaH (0.96 mg, 0.024 mmol, 60% disp.). The reaction mixture was stirred at ambient temperature for 1 h, neutralized with Dowex H^+ resin to pH 4, filtered, and rinsed with MeOH (20 mL). The filtrate was concentrated under reduced pressure to give pure **4c** (141 mg, 0.239 mmol, 99%) as a white solid without further purification. R_f = 0.59 ($CHCl_3$/MeOH = 5:1); $[\alpha]_D^{22}$ +41.7 (*c* 0.31, MeOH); ^{1}H NMR (400 MHz, pyridine-d_5) δ 6.25 (d, *J* = 7.0 Hz, 1H, 1′-H), 5.48 (t, *J* = 3.3 Hz, 1H, 12-H), 4.40 (dd, *J* = 11.0 Hz, 4.0 Hz, 1H, 5′-H), 4.23−4.19 (m, 3H, 2′-H, 3′-H, 4′-H), 3.85 (dd, *J* = 11.0 Hz, 10.0 Hz, 1H, 5′-H), 3.45 (dd, 10.7 Hz, 5.7 Hz, 1H, 3-H), 3.27 (dd, *J* = 14.2 Hz, 3.8 Hz, 1H, 18-H), 2.32 (m, 1H, 15-H), 2.10 (m, 2H, 16-H), 2.05 (m, 1H, 22-H), 1.97 (m, 2H, 11-H), 1.85 (m, 2H, 15-H, 22-H), 1.80 (m, 1H, 19-H), 1.68 (m, 1H, 9-H), 1.54 (m, 2H, 1-H, 6-H), 1.51 (m, 2H, 7-H), 1.37 (m, 2H, 6-H, 21-H), 1.30 (m, 1H, 19-H), 1.25 (s, 3H, 27-H), 1.24 (s, 3H, 23-H), 1.18 (m, 1H, 2-H), 1.15 (m, 1H, 21-H), 1.12 (s, 3H, 26-H), 1.05 (s, 3H, 24-H), 1.00 (m, 1H, 1-H), 0.95 (s, 3H, 30-H), 0.94 (s, 6H, 25-H, 29-H), 0.86 (m, 1H, 5-H); ^{13}C NMR (100 MHz, pyridine-d_5) δ 176.7 (C-28), 144.3 (C-13), 123.1 (C-12), 96.4 (C-1′), 78.4 (C-2′), 78.2 (C-3), 73.8 (C-3′), 71.0 (C-4′), 67.9 (C-5′), 56.0 (C-5), 48.3 (C-9), 47.3 (C-17), 46.4 (C-19), 42.3 (C-14), 41.9 (C-18), 40.0 (C-8), 39.5 (C-4), 39.1 (C-1), 37.5 (C-10), 34.2 (C-21), 33.4 (C-7), 33.3 (C-29), 32.9 (C-22), 31.0 (C-20), 29.0 (C-23), 28.4 (C-15), 28.3 (C-2), 26.2 (C-27), 24.0 (C-11), 23.8 (C-30), 23.5 (C-16), 19.0 (C-6), 17.7 (C-26), 16.7 (C-24), 15.8 (C-25); IR (KBr) cm^{-1} ν 3407 (-O−H), 2940 (=C−H), 1744 (-C=O), 1032 (-C−O-); HRMS (ESI-TOF) *m*/*z* $[M + Na]^+$ calcd for $C_{35}H_{56}O_7Na$ 611.3924, found 611.3908.

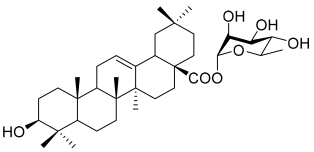
Olean-12-en-28-oic Acid, 3-Hydroxy-, α-D-Mannopyranosyloxy Ester (4d).



4d

To a solution of **3d** (200 mg, 0.2219 mmol) stirring in dry MeOH (4.4 mL) was added sodium hydride (0.88 mg, 0.0222 mmol). The mixture was stirred at ambient temperature for 40 min, neutralized with Dowex H^+ resin to pH 4, and filtered. The filtrate was concentrated under reduced pressure to give pure saponin **4d** (150.5 mg, 0.2432 mmol, quant.) as a white solid. R_f = 0.53 ($CHCl_3$/MeOH= 5:1); $[\alpha]_D^{22}$ +67.9 (*c* 0.24, MeOH); ^{1}H NMR (400 MHz, pyridine-d_5) δ 6.89 (d, *J* = 1.6 Hz, 1H, 1′-H), 5.40 (t, *J* = 3.5 Hz, 1H, 12-H), 4.86 (dt, 9.5 Hz, 5.0 Hz, 1H, 4′-H), 4.68 (ddd, *J* = 10.0 Hz, 9.5 Hz, 6.5 Hz, 1H, 3′-H), 4.65 (ddd, *J* = 11.5 Hz, 6.0 Hz, 2.0 Hz, 1H, 6′-H), 4.63 (ddd, *J* = 10.0 Hz, 4.5 Hz, 1.6 Hz, 2′-H), 4.56 (ddd, *J* = 11.5 Hz, 6.5 Hz, 5.0 Hz, 1H, 6′-H), 4.51 (ddd, *J* = 9.5 Hz, 5.0 Hz, 2.0 Hz, 1H, 5′-H), 3.44 (ddd, *J* = 10.9 Hz, 5.4 Hz, 5.4 Hz, 1H, 3-H), 3.17 (dd, *J* = 13.8 Hz, 4.0 Hz, 1H, 18-H), 2.05 (m, 1H, 11-H), 2.02 (m, 2H, 2-H), 1.88 (m, 1H, 22-H), 1.85 (m, 2H, 16-H), 1.83 (m, 1H, 15-H), 1.78 (m, 1H, 11-H), 1.75 (m, 1H, 19-H), 1.64 (m, 1H, 9-H), 1.58 (m, 1H, 22-H), 1.53 (m, 1H, 1-H), 1.53 (m, 1H, 6-H), 1.47 (m, 1H, 7-H), 1.39 (m, 1H, 21-H), 1.34 (m, 1H, 7-H), 1.31 (m, 1H, 6-H), 1.24 (s, 3H, 23-H), 1.23 (m, 1H, 19-H), 1.21 (s, 3H, 27-H), 1.14 (m, 1H, 21-H), 1.11 (m, 1H, 15-H), 1.04 (s, 3H, 24-H), 1.00 (m, 1H, 1-H), 0.95 (s, 3H, 30-H), 0.93 (s, 3H, 26-H), 0.91 (s, 3H, 29-H), 0.86 (s, 3H, 25-H), 0.83 (m, 1H, 5-H); ^{13}C NMR (100 MHz, pyridine-d_5) δ 176.1 (C-28), 144.2 (C-13), 123.2 (C-12), 95.5 (C-1′), 78.5 (C-5′), 78.2 (C-3), 73.2 (C-3′), 71.4 (C-2′), 68.5 (C-4′), 62.8 (C-6′), 55.9 (C-5), 48.2 (C-9), 47.5 (C-17), 46.2 (C-19), 42.2 (C-14), 42.0 (C-18), 39.9 (C-8), 39.5 (C-4), 39.1 (C-1), 37.4 (C-10), 34.1 (C-21), 33.3 (C-7), 33.3 (C-29), 32.9 (C-22), 31.0 (C-20), 28.9 (C-23), 28.2 (C-15), 28.1 (C-2), 26.1 (C-27), 24.0 (C-30), 23.8 (C-16), 23.6 (C-11), 18.9 (C-6), 17.6 (C-26), 16.7 (C-24), 15.8 (C-25); IR (KBr) cm^{-1} ν 3382 (-O−H), 2933 (=C−H), 1697 (-C=O); HRMS (ESI-TOF) *m*/*z* $[M + Na]^+$ calcd for $C_{36}H_{58}O_8Na$ 641.4029, found 641.4014. GATEI (400 MHz, pyridine-d_5) δ 95.5 (C-1′), $^1J_{C,H}$ = 173.4 Hz.

Olean-12-en-28-oic Acid, 3-Hydroxy-, α-L-Rhamnopyranosyloxy Ester (4e).



4e

To a solution of **3e** (200 mg, 0.237 mmol) in MeOH (2.3 mL) at ambient temperature was added NaH (0.948 mg, 0.024 mmol, 60% disp.). The reaction mixture was stirred at ambient temperature for 30 min, neutralized with Dowex H^+ resin to pH 4, filtered, and rinsed with MeOH (20 mL). The filtrate was concentrated under reduced pressure to give pure **4e** (150 mg, 0.248 mmol, quant.) as a white solid without further purification. R_f = 0.57 ($CHCl_3$/MeOH = 5:1); $[\alpha]_D^{22}$ +25.7 (*c* 1.00, MeOH); ^{1}H NMR (400 MHz, pyridine-d_5); δ 6.83 (d, *J* = 1.5 Hz, 1H, 1′-H), 5.46 (t, J = 3.3 Hz, 1H, 12-H), 4.61 (dd, *J* = 3.3 Hz, 1.5 Hz, 1H, 2′-H), 4.54 (dd, *J* = 8.8 Hz, 3.3 Hz, 1H, 3′-H), 4.39 (t, *J* = 8.8 Hz, 1H, 4′-H), 4.39 (m, 1H, 5′-H), 3.45 (dd, 10.7 Hz, 5.7 Hz,

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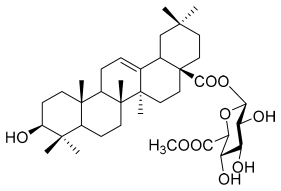
1H, 3-H), 3.17 (dd, J = 14.2 Hz, 3.8 Hz, 1H, 18-H), 2.05 (m, 1H, 16-H), 2.01 (m, 1H, 2-H), 1.95 (m, 2H, 11-H), 1.86 (m, 2H, 2-H, 22-H), 1.75 (m, 2H, 16-H, 19-H), 1.72 (d, J = 5.5 Hz, 3H, 6′-C$\underline{H}_3$), 1.64 (m, 1H, 9-H), 1.61 (m, 1H, 22-H), 1.57 (m, 2H, 1-H, 6-H), 1.46 (m, 1H, 7-H), 1.41 (m, 1H, 21-H), 1.36 (m, 1H, 6-H), 1.30 (m, 2H, 7-H, 19-H), 1.25 (s, 3H, 23-H), 1.22 (s, 3H, 27-H), 1.17 (m, 1H, 21-H), 1.12 (m, 2H, 15-H), 1.06 (s, 3H, 26-H), 1.00 (m, 1H, 1-H), 0.92 (s, 3H, 24-H), 0.92 (s, 3H, 25-H), 0.92 (s, 3H, 29-H), 0.89 (s, 3H, 30-H), 0.87 (m, 1H, 5-H); ^{13}C NMR (100 MHz, pyridine-d_5) δ 176.0 (C-28), 143.6 (C-13), 123.5 (C-12), 95.5 (C-1′), 78.2 (C-3), 73.6 (C-4′), 73.0 (C-3′), 72.7 (C-5′), 71.6 (C-2′), 55.9 (C-5), 48.1 (C-9), 47.5 (C-17), 46.1 (C-19), 42.2 (C-14), 42.1 (C-18), 39.9 (C-8), 39.5 (C-1), 39.0 (C-4), 37.5 (C-10), 34.1 (C-21), 33.4 (C-7), 33.2 (C-22), 33.1 (C-29), 31.0 (C-20), 29.0 (C-23), 28.2 (C-15), 28.1 (C-2), 26.1 (C-27), 24.0 (C-11), 23.7 (C-30), 23.4 (C-16), 18.9 (6′-$\underline{C}H_3$), 18.9 (C-6), 17.2 (C-26), 16.7 (C-24), 15.7 (C-25); IR (KBr) cm^{-1} ν 3431 (-O−H), 2927 (=C−H), 1743 (-C=O), 1061 (-C−O-); HRMS (ESI-TOF) m/z [M + Na]$^+$ calcd for $C_{36}H_{58}O_7Na$ 625.4080, found 625.4070. GATEI (400 MHz, pyridine-d_5) δ 95.5 (C-1′), $^1J_{C,H}$ = 173.0 Hz.

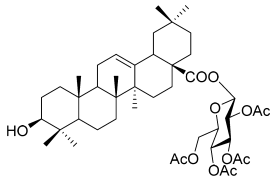
Olean-12-en-28-oic Acid, 3-Hydroxy-, Methyl-β-D-glucopyranuronosyl Ester (4f).



4f

To a solution of **3f** (143.3 mg, 0.1615 mmol) stirring in dry MeOH (3.23 mL) was added sodium hydride (0.53 mg, 0.0162 mmol). The mixture was stirred at ambient temperature for 3.5 h, neutralized with Dowex H$^+$ resin to pH 4, and filtered. The filtrate was concentrated under reduced pressure to give the crude product. Purification by flash column chromatography (silica gel, 20 g; $CHCl_3$/MeOH = 20:1) afforded **4f** (28.2 mg, 0.0436 mmol, 27%) as a white solid. R_f = 0.53 ($CHCl_3$/MeOH= 5:1); $[\alpha]_D^{23}$ +61.1 (c 0.19, MeOH); ^{1}H NMR (400 MHz, pyridine-d_5) δ 6.37 (d, J = 7.9 Hz, 1H, 1′-H), 5.45 (t, J = 3.5 Hz, 1H, 12-H), 4.70 (d, J = 9.6 Hz, 1H, 5′-H), 4.52 (dt, J = 9.6 Hz, 5.5 Hz, 1H, 4′-H), 4.34 (m, 1H, 3′-H), 4.28 (m, 1H, 2′-H), 3.64 (s, 3H, -COOC$\underline{H}_3$), 3.45 (ddd, J = 10.0 Hz, 5.0 Hz, 5.0 Hz, 1H, 3-H), 3.21 (dd, J = 13.8 Hz, 4.2 Hz, 1H, 18-H), 2.32 (m, 1H, 2-H), 2.08 (m, 1H, 11-H), 1.95 (m, 1H, 11-H), 1.95 (m, 2H, 16-H), 1.89 (m, 1H, 22-H), 1.85 (m, 1H, 2-H), 1.84 (m, 1H, 15-H), 1.76 (m, 1H, 19-H), 1.76 (m, 1H, 22-H), 1.67 (dd, J = 10.5 Hz, 7.2 Hz, 1H, 9-H), 1.57 (m, 1H, 1-H), 1.54 (m, 1H, 6-H), 1.51 (m, 1H, 7-H), 1.39 (m, 1H, 6-H), 1.37 (m, 1H, 7-H), 1.34 (m, 1H, 21-H), 1.26 (m, 1H, 19-H), 1.24 (s, 3H, 23-H), 1.24 (s, 3H, 27-H), 1.17 (m, 1H, 15-H), 1.12 (s, 3H, 26-H), 1.08 (m, 1H, 21-H), 1.05 (s, 3H, 24-H), 1.00 (m, 1H, 1-H), 0.97 (s, 3H, 25-H), 0.89 (s, 3H, 29-H), 0.88 (s, 3H, 30-H), 0.85 (m, 1H, 5-H); ^{13}C NMR (100 MHz, pyridine-d_5) δ 176.6 (C-28), 170.3 (C-6′), 144.1 (C-13), 123.2 (C-12), 95.7 (C-1′), 78.2 (C-3), 78.2 (C-3′), 77.9 (C-5′), 73.8 (C-2′), 73.2 (C-4′), 55.9 (C-5), 52.2 (-COO$\underline{C}H_3$), 48.3 (C-9), 47.2 (C-17), 46.3 (C-19), 42.2 (C-14), 41.9 (C-18), 40.1 (C-8), 39.5 (C-4), 39.1 (C-1), 37.5 (C-10), 34.1 (C-21), 33.3 (C-7), 33.3 (C-29), 32.7 (C-22), 30.9 (C-20), 28.9 (C-23), 28.3 (C-2), 28.3 (C-15), 26,2 (C-27), 24.0 (C-30), 23.7 (C-16), 23.5 (C-11), 19.0 (C-6), 17.6 (C-26), 16.7 (C-24), 15.8 (C-25); IR (KBr) cm^{-1} ν 3455 (-O−H), 2942 (=C−H), 1752 (-C=O), 1719 (-C=C-), 1090 (-C−O-); HRMS (ESI-TOF) m/z [M + Na]$^+$ calcd for $C_{37}H_{58}O_9Na$ 669.3979, found 669.3963.

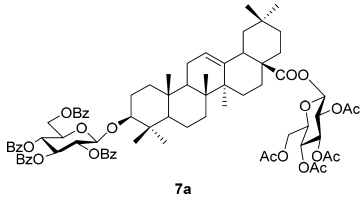
Olean-12-en-28-oic Acid, 3-Hydroxy-, 2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl Ester (5).



5

To a stirred solution of **3a** (100 mg, 0.110 mmol) at ambient temperature in dry CH_2Cl_2 (0.25 M) were added $ZnBr_2$ (125 mg, 0.550 mmol) followed by H_2O (9.9 μL, 0.550 mmol). The reaction mixture was stirred at ambient temperature for 10 min before it was quenched with sat. aq. $NaHCO_3$ (1 mL). The mixture was extracted with CH_2Cl_2 (10 mL). The organic layer was washed with sat. aq. $NaHCO_3$ (5 mL), H_2O (5 × 60 mL), and brine (5 mL). The organic layer was dried with sodium sulfate, filtered, and concentrated under reduced pressure. Purification by flash column chromatography (silica gel, 10 g; hexane/AcOEt = 3:1 → 2:1 → 1:1) afforded **5** (84 mg, 0.107 mmol, 97%) as a white foamy solid. R_f = 0.40 (hexane/AcOEt = 1:1); $[\alpha]_D^{22}$ +35.4 (c 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.58 (d, J = 8.0 Hz, 1H, 1′-H), 5.31 (t, J = 3.5 Hz, 1H, 12-H), 5.25 (t, J = 9.3 Hz, 1H, 3′-H), 5.18 (dd, J = 9.3 Hz, 8.0 Hz, 1H, 2′-H), 5.13 (dd, J = 10.0 Hz, 9.3 Hz, 1H, 4′-H), 4.27 (dd, J = 12.5 Hz, 4.4 Hz, 1H, 6′-H), 4.05 (dd, J = 12.5 Hz, 2.4 Hz, 1H, 6′-H), 3.79 (ddd, J = 10.0 Hz, 4.4 Hz, 2.4 Hz, 1H, 5′-H), 3.19 (m, 1H, 3-H), 2.78 (m, 1H, 18-H), 2.06 (s, 3H, -OCOC$\underline{H}_3$), 2.02 (s, 3H, -OCOC$\underline{H}_3$), 2.01 (s, 6H, -OCOC$\underline{H}_3$ ×2), 1.95 (m, 1H, 11-H), 1.84 (m, 2H, 16-H), 1.60 (m, 1H, 19-H), 1.58 (m, 2H, 22-H), 1.56 (m, 1H, 15-H), 1.54 (m, 1H, 1-H), 1.53 (m, 1H, 11-H), 1.48 (m, 2H, 6-H, 9-H), 1.44 (m, 2H, 2-H), 1.40 (m, 1H, 7-H), 1.31 (m, 1H, 6-H), 1.29 (m, 1H, 21-H), 1.18 (m, 1H, 21-H), 1.15 (m, 1H, 7-H), 1.14 (m, 1H, 19-H), 1.10 (s, 3H, 27-H), 1.01 (m, 1H, 15-H), 0.89 (m, 1H, 1-H), 0.88 (s, 6H, 23-H, 30-H), 0.87 (s, 6H, 25-H, 29-H), 0.72 (s, 3H, 24-H), 0.69 (s, 3H, 26-H), 0.65 (m, 1H, 5-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.6 (C-28), 170.5 (-O$\underline{C}$OCH$_3$), 170.0 (-O$\underline{C}$OCH$_3$), 169.4 (-O$\underline{C}$OCH$_3$), 168.9 (-O$\underline{C}$OCH$_3$), 142.8 (C-13), 122.8 (C-12), 91.5 (C-1′), 78.8 (C-3), 72.8 (C-3′), 72.4 (C-5′), 69.9 (C-2′), 68.0 (C-4′), 61.5 (C-6′), 55.1 (C-5), 47.5 (C-9), 46.8 (C-17), 45.7 (C-19), 41.6 (C-14), 39.3 (C-18), 38.7 (C-8), 38.4 (C-4), 36.8 (C-1), 33.7 (C-10), 32.9 (C-21), 32.8 (C-29), 32.8 (C-7), 31.7 (C-22), 30.5 (C-20), 28.0 (C-23), 27.7 (C-15), 27.1 (C-2), 25.6 (C-27), 23.4 (C-30), 23.3 (C-16), 22.8 (C-11), 20.6 (-OCO$\underline{C}$H$_3$), 20.5 (-OCO$\underline{C}$H$_3$), 20.5 (-OCO$\underline{C}$H$_3$), 20.5 (-OCO$\underline{C}$H$_3$), 18.2 (C-6), 16.9 (C-26), 15.5 (C-24), 15.3 (C-25); IR (KBr) cm^{-1} ν 3445 (-O−H), 2933 (=C−H), 1760 (-C=O), 1036 (-C−O-); HRMS (ESI-TOF) m/z [M + Na]$^+$ calcd for $C_{44}H_{66}O_{12}Na$ 809.4452, found 809.4444. The purity of **5** synthesized by a continuous reaction was compared with the corresponding compound in stepwise synthesis by a proton NMR spectrum (see Supporting Information for details).

Olean-12-en-28-oic Acid, 3-[(2,3,4,6,-Tetra-O-benzoyl-β-D-glucopyranosyl)oxy]-2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl Ester (7a).



7a

MicroFlow. A solution of TMSOTf (12.0 μL, 0.0640 mmol, 0.0213 M) dissolved in dry CH_2Cl_2 (3.0 mL) was injected into the micromixer using a syringe pump at a flow rate of 0.6 mL/min. Then, a solution of donor **6a** (71.0 mg, 0.0941 mmol, 0.0314 M) and acceptor **5** (50.0 mg, 0.0640 mmol, 0.0213 M) dissolved in dry CH_2Cl_2 (3.0 mL) was injected into the micromixer by the same syringe pump at a flow rate of 0.6 mL/min and mixed at ambient temperature. After the reaction mixture was allowed to flow at ambient

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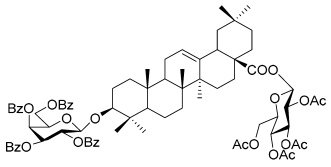
temperature for an additional 100 s through a Teflon reactor tube (ϕ = 1.0 mm, l = 1.0 m), the reaction was quenched by adding triethylamine (9.0 μL, 0.0640 mmol) diluted in CH_2Cl_2. The mixture was concentrated under reduced pressure to give the crude product. Purification by flash column chromatography (silica gel, 20 g; toluene/AcOEt = 10:1 → 6:1 → 4:1) afforded **7a** (72.5 mg, 0.0531 mmol, 83%) as a white solid. R_f = 0.41 (toluene/AcOEt = 4:1); $[\alpha]_D^{22}$ +31.9 (*c* 0.30, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03−7.28 (m, 25H, Ar−H), 5.90 (t, *J* = 9.5 Hz, 1H, 3″-H), 5.58 (t, *J* = 9.5 Hz, 1H, 4″-H), 5.56 (dd, *J* = 9.5 Hz, 8.0 Hz, 1H, 2″-H), 5.55 (d, *J* = 8.0 Hz, 1H, 1′-H), 5.32 (t, *J* = 3.3 Hz, 1H, 12-H), 5.24 (t, *J* = 9.3 Hz, 1H, 3′-H), 5.17 (dd, *J* = 9.3 Hz, 8.0 Hz, 1H, 2′-H), 5.12 (dd, *J* = 10.0 Hz, 9.3 Hz, 1H, 4′-H), 4.85 (d, *J* = 8.0 Hz, 1H, 1″-H), 4.59 (dd, *J* = 12.0 Hz, 3.6 Hz, 1H, 6″-H), 4.54 (dd, *J* = 12.0 Hz, 6.6 Hz, 1H, 6″-H), 4.27 (dd, *J* = 12.5 Hz, 4.4 Hz, 1H, 6′-H), 4.17−4.09 (m, 1H, 5″-H), 4.04 (dd, *J* = 12.5 Hz, 2.4 Hz, 1H, 6′-H), 3.78 (ddd, *J* = 10.0 Hz, 4.4 Hz, 2.4 Hz, 1H, 5′-H), 3.09 (m, 1H, 3-H), 2.82 (m, 1H, 18-H), 2.06 (s, 3H, -OCOC$\underline{H}_3$), 2.02 (s, 3H, -OCOC$\underline{H}_3$), 2.00 (s, 3H, -OCOC$\underline{H}_3$), 1.99 (s, 3H, -OCOC$\underline{H}_3$), 1.97 (m, 1H, 16-H), 1.84 (m, 2H, 2-H, 11-H), 1.82 (m, 1H, 2-H), 1.80 (m, 1H, 19-H), 1.78 (m, 2H, 22-H), 1.70 (m, 1H, 16-H), 1.42 (m, 2H, 1-H, 15-H), 1.39 (m, 1H, 9-H), 1.38 (m, 1H, 6-H), 1.33 (m, 2H, 7-H, 21-H), 1.26 (m, 1H, 6-H), 1.21 (m, 1H, 21-H), 1.16 (m, 1H, 19-H), 1.12 (m, 1H, 7-H), 1.08 (s, 3H, 27-H), 1.00 (m, 1H, 15-H), 0.91 (s, 6H, 29-H, 30-H), 0.83 (s, 3H, 25-H), 0.74 (m, 1H, 1-H), 0.68 (s, 6H, 23-H, 24-H), 0.63 (s, 3H, 26-H), 0.59 (m, 1H, 5-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.5 (C-28), 170.5 (-O$\underline{C}$OCH$_3$), 170.0 (-O$\underline{C}$OCH$_3$), 169.4 (-O$\underline{C}$OCH$_3$), 168.9 (-O$\underline{C}$OCH$_3$), 166.0 (-O$\underline{C}$OPh), 165.8 (-O$\underline{C}$OPh), 165.3 (-O$\underline{C}$OPh), 160.0 (-O$\underline{C}$OPh), 142.8 (C-13), 133.4, 133.2, 133.0, 133.0, 129.8, 129.7, 129.7, 129.7, 129.6, 129.4, 129.0, 128.8, 128.8, 128.4, 128.3, 128.3, 128.2, 128.2, 122.8 (C-12), 103.2 (C-1″), 91.5 (C-1′), 90.7 (C-3), 72.9 (C-3″), 72.8 (C-3′), 72.4 (C-5′), 72.1 (C-4″), 72.0 (C-5″), 70.3 (C-2″), 69.9 (C-2′), 68.0 (C-4′), 63.4 (C-6″), 61.5 (C-6′), 55.4 (C-5), 47.5 (C-9), 46.8 (C-17), 45.8 (C-19), 41.6 (C-14), 40.9 (C-18), 39.2 (C-8), 38.7 (C-1), 38.2 (C-4), 36.6 (C-10), 33.7 (C-21), 33.0 (C-29), 32.8 (C-7), 31.7 (C-22), 30.6 (C-20), 27.7 (C-23), 27.6 (C-15), 25.8 (C-2), 25.6 (C-27), 23.4 (C-30), 23.3 (C-16), 22.8 (C-11), 20.7 (-OCO$\underline{C}$H$_3$), 20.5 (-OCO$\underline{C}$H$_3$), 20.5 (-OCO$\underline{C}$H$_3$), 20.5 (-OCO$\underline{C}$H$_3$), 18.0 (C-6), 16.9 (C-26), 16.2 (C-24), 15.2 (C-25); IR (KBr) cm^{-1} ν 2950 (=C−H), 1737 (-C=O), 1069 (−C−O-); HRMS (ESI-TOF) *m*/*z* [M + Na]$^+$ calcd for $C_{78}H_{92}O_{21}Na$ 1387.6029, found 1387.6001.

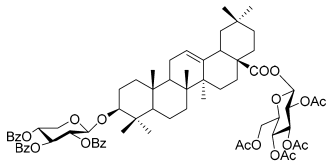
Olean-12-en-28-oic Acid, 3-[(2,3,4,6,-Tetra-*O*-benzoyl-β-D-galactopyranosyl)oxy]-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl Ester (7b).



7b

MicroFlow. A solution of TMSOTf (35.0 μL, 0.1922 mmol, 0.0214 M) dissolved in CH_2Cl_2 (9.0 mL) was injected into the micromixer using a syringe pump at a flow rate of 0.6 mL/min. Then, a solution of donor **6b** (213 mg, 0.2883 mmol, 0.0320 M) and acceptor **5** (150 mg, 0.1922 mmol, 0.0214 M) dissolved in CH_2Cl_2 (9.0 mL) was injected into the micromixer by the same syringe pump at a flow rate of 0.6 mL/min and mixed at ambient temperature. After the reaction mixture was allowed to flow at ambient temperature for an additional 100 s through a Teflon reactor tube (ϕ = 1.0 mm, l = 1.0 m), the reaction was quenched by adding triethylamine (27 μL, 0.1922 mmol) diluted in CH_2Cl_2 (1.0 mL). The mixture was concentrated under reduced pressure to give the crude product. Purification by flash column chromatography (silica gel, 20 g; toluene/AcOEt = 20:1 → 18:1 → 17:1) afforded **7b** (185 mg, 0.1355 mmol, 71%) as a white solid. R_f = 0.54 (toluene/AcOEt = 4:1); $[\alpha]_D^{23}$ +74.2 (*c* 0.35, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12−7.34 (m, 25H, Ar−H), 5.95 (dd, *J* = 3.4 Hz, 1.0 Hz 1H, 4″-H), 5.83 (dd, *J* = 10.0 Hz, 8.0 Hz, 1H, 2″-H), 5.60 (dd, *J* = 10.0 Hz, 3.4 Hz, 1H, 3″-H), 5.57 (d, *J* = 8.0 Hz, 1H, 1′-H), 5.33 (t, *J* = 3.3 Hz, 1H, 12-H), 5.24 (t, *J* = 9.2 Hz, 1H, 3′-H), 5.17 (dd, *J* = 9.2 Hz, 8.0 Hz, 1H, 2′-H), 5.12 (dd, *J* = 10.0 Hz, 9.2 Hz, 1H, 4′-H), 4.82 (d, *J* = 8.0 Hz, 1H, 1″-H), 4.66 (dd, *J* = 11.6 Hz, 7.4 Hz, 1H, 6″-H), 4.43 (dd, *J* = 11.6 Hz, 6.0 Hz, 1H, 6″-H), 4.30 (ddd, *J* = 7.4 Hz, 6.0 Hz, 1.0 Hz, 1H, 5″-H), 4.27 (dd, *J* = 12.5 Hz, 4.4 Hz, 1H, 6′-H), 4.04 (dd, *J* = 12.5 Hz, 2.4 Hz, 1H, 6′-H), 3.78 (ddd, *J* = 10.0 Hz, 4.4 Hz, 2.4 Hz, 1H, 5′-H), 3.13 (m, 1H, 3-H), 2.82 (m, 1H, 18-H), 2.06 (s, 3H, -OCOC$\underline{H}_3$), 2.02 (s, 3H, -OCOC$\underline{H}_3$), 2.01 (s, 3H, -OCOC$\underline{H}_3$), 1.99 (s, 3H, -OCOC$\underline{H}_3$), 1.97 (m, 1H, 16-H), 1.86 (m, 1H, 2-H), 1.80 (m, 2H, 11-H), 1.76 (m, 1H, 2-H), 1.63 (m, 1H, 19-H), 1.62 (m, 2H, 22-H), 1.53 (m, 1H, 16-H), 1.42 (m, 1H, 1-H), 1.38 (m, 2H, 9-H, 15-H), 1.34 (m, 2H, 6-H, 7-H), 1.26 (m, 1H, 6-H), 1.18 (m, 2H, 21-H), 1.14 (m, 1H, 19-H), 1.12 (m, 1H, 7-H), 1.10 (s, 3H, 27-H), 1.01 (m, 1H, 15-H), 0.92 (s, 6H, 29-H, 30-H), 0.85 (s, 3H, 25-H), 0.76 (m, 1H, 1-H), 0.70 (s, 3H, 23-H), 0.69 (s, 3H, 26-H), 0.66 (s, 3H, 24-H), 0.62 (m, 1H, 5-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.6 (C-28), 170.6 (-O$\underline{C}$OCH$_3$), 170.1 (-O$\underline{C}$OCH$_3$), 169.4 (-O$\underline{C}$OCH$_3$), 168.9 (-O$\underline{C}$OCH$_3$), 166.0 (-O$\underline{C}$OPh), 165.7 (-O$\underline{C}$OPh), 165.6 (-O$\underline{C}$OPh), 165.2 (-O$\underline{C}$OPh), 142.8 (C-13), 133.5, 133.2, 133.2, 133.0, 133.0, 129.7, 129.7, 129.5, 129.5, 129.0, 129.0, 128.9, 128.7, 128.5, 128.5, 128.4, 128.2, 122.8 (C-12), 103.8 (C-1″), 91.5 (C-1′), 90.9 (C-3), 72.8 (C-3′), 72.4 (C-5′), 71.8 (C-3″), 71.2 (C-5″), 71.0 (C-2″), 69.9 (C-2′), 68.2 (C-4″), 68.0 (C-4′), 62.1 (C-6″), 61.5 (C-6′), 55.4 (C-5), 47.5 (C-9), 46.8 (C-17), 45.8 (C-19), 41.6 (C-14), 39.3 (C-18), 38.7 (C-8), 38.3 (C-4), 36.6 (C-1), 33.7 (C-10), 33.0 (C-21), 32.8 (C-29), 31.7 (C-7), 30.6 (C-22), 29.7 (C-20), 27.7 (C-23), 27.7 (C-15), 25.9 (C-2), 25.6 (C-27), 23.4 (C-30), 23.3 (C-16), 22.8 (C-11), 20.7 (-OCO$\underline{C}$H$_3$), 20.6 (-OCO$\underline{C}$H$_3$), 20.6 (-OCO$\underline{C}$H$_3$), 20.6 (-OCO$\underline{C}$H$_3$), 18.0 (C-6), 16.9 (C-26), 16.2 (C-24), 15.2 (C-25); IR (KBr) cm^{-1} ν 2952 (=C−H), 1735 (-C=O), 1069 (-C−O-); HRMS (ESI-TOF) *m*/*z* [M + Na]$^+$ calcd for $C_{78}H_{92}O_{21}Na$ 1387.6029, found 1387.6019.

Olean-12-en-28-oic Acid, 3-[(2,3,4,-Tri-*O*-benzoyl-β-D-xylopyranosyl)oxy]-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl Ester (7c).



7c

MicroFlow. A solution of TMSOTf (35.0 μL, 0.1922 mmol, 0.0214 M) dissolved in CH_2Cl_2 (9.0 mL) was injected into the micromixer using a syringe pump at a flow rate of 0.6 mL/min. Then, a solution of donor **6c** (175 mg, 0.2883 mmol, 0.0320 M) and acceptor **5** (150 mg, 0.1922 mmol, 0.0214 M) dissolved in CH_2Cl_2 (9.0 mL) was injected into the micromixer by the same syringe pump at a flow rate of 0.6 mL/min and mixed at ambient temperature. After the reaction mixture was allowed to flow at ambient temperature for an additional 100 s through a Teflon reactor tube (ϕ = 1.0 mm, l = 1.0 m), the reaction was quenched by adding triethylamine (27 μL, 0.1922 mmol) diluted in CH_2Cl_2 (1.0 mL). The mixture was concentrated under reduced pressure to give the crude product. Purification by flash column chromatography (silica gel, 20 g; toluene/AcOEt = 20:1 → 18:1 → 17:1) afforded **7c** (193 mg, 0.1567 mmol, 81%) as a white solid. R_f = 0.54 (toluene/AcOEt = 4:1); $[\alpha]_D^{23}$ +17.5 (*c* 0.30, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10−7.31 (m, 25H, Ar−H), 5.78 (t, *J* = 8.0 Hz, 1H, 3″-H), 5.57 (d, *J* = 8.0 Hz, 1H, 1′-H), 5.44 (dd, *J* = 8.0 Hz, 6.1 Hz, 1H, 2′-H), 5.33 (t, *J* = 3.3 Hz, 1H, 12-H), 5.24 (t, *J* = 9.3 Hz, 1H, 3′-H), 5.17 (dd, *J* = 9.3 Hz, 8.0 Hz, 1H, 2′-H), 5.12 (dd, *J* = 10.0 Hz, 9.2 Hz, 1H, 4′-H), 4.84 (d, *J* = 6.1 Hz, 1H, 1″-H), 4.43 (dd, *J* = 12.0 Hz, 4.6 Hz, 1H, 5″-H), 4.27 (dd, *J* = 12.5 Hz, 4.4 Hz, 1H, 6′-H), 4.04 (dd, *J* = 12.5 Hz, 2.4 Hz, 1H, 6′-H), 3.78 (ddd, *J* = 10.0 Hz, 4.4 Hz, 2.4 Hz, 1H, 5′-H), 3.63 (dd, *J* = 12.0 Hz, 8.0 Hz, 1H, 5″-H), 3.13 (dd, *J* = 11.5 Hz, 4.8 Hz, 1H, 3-H), 2.81 (m, 1H, 18-H), 2.06 (s, 3H, -OCOC$\underline{H}_3$), 2.02 (s, 3H, -OCOC$\underline{H}_3$), 2.01 (s, 3H, -OCOC$\underline{H}_3$), 2.00 (s, 3H, -OCOC$\underline{H}_3$), 1.94 (m, 1H, 16-H), 1.86 (m, 2H, 11-H), 1.79 (m, 2H, 2-H), 1.64 (m, 2H, 1-H, 19-H), 1.61 (m, 2H, 22-H), 1.51 (m, 1H, 16-H), 1.48 (m, 1H, 9-H), 1.42 (m, 1H, 15-H), 1.39 (m, 2H, 6-H, 7-H),

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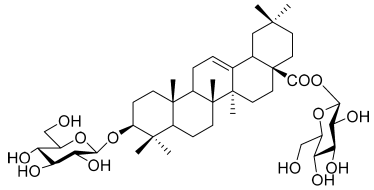
1.26 (m, 2H, 6-H, 21-H), 1.22 (m, 1H, 21-H), 1.18 (m, 1H, 19-H), 1.14 (m, 1H, 7-H), 1.10 (s, 3H, 27-H), 1.03 (m, 1H, 15-H), 0.98 (m, 1H, 1-H), 0.90 (s, 6H, 29-H, 30-H), 0.89 (s, 3H, 25-H), 0.77 (s, 3H, 23-H), 0.70 (s, 3H, 26-H), 0.69 (m, 1H, 5-H), 0.65 (s, 3H, 24-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.6 (C-28), 170.6 (-O$\underline{C}$OCH$_3$), 170.0 (-O$\underline{C}$OCH$_3$), 169.4 (-O$\underline{C}$OCH$_3$), 168.9 (-O$\underline{C}$OCH$_3$), 165.5 (-O$\underline{C}$OPh), 165.5 (-O$\underline{C}$OPh), 165.0 (-O$\underline{C}$OPh), 142.8 (C-13), 135.8, 133.5, 133.3, 133.2, 133.0, 130.1, 129.8, 129.8, 129.4, 129.2, 129.1, 128.9, 128.5, 128.5, 128.4, 128.4, 128.3, 128.2, 122.9 (C-12), 102.7 (C-1″), 91.5 (C-1′), 89.9 (C-3), 72.8 (C-3′), 72.4 (C-5′), 71.0 (C-2″), 69.9 (C-2′), 69.5 (C-4″), 68.0 (C-4′), 61.6 (C-5″), 61.5 (C-6′), 55.4 (C-5), 47.5 (C-9), 46.8 (C-17), 45.7 (C-19), 41.6 (C-14), 41.0 (C-18), 39.3 (C-8), 38.9 (C-4), 38.4 (C-1), 36.7 (C-10), 33.7 (C-21), 33.0 (C-29), 32.8 (C-7), 31.7 (C-22), 30.6 (C-20), 27.7 (C-23), 27.7 (C-15), 25.8 (C-2), 25.6 (C-27), 23.4 (C-30), 23.4 (C-16), 22.8 (C-11), 20.7 (-OCO$\underline{C}$H$_3$), 20.6 (-OCO$\underline{C}$H$_3$), 20.6 (-OCO$\underline{C}$H$_3$), 20.5 (-OCO$\underline{C}$H$_3$), 18.1 (C-6), 16.9 (C-26), 16.2 (C-24), 15.3 (C-25); IR (KBr) cm^{-1} ν 2946 (=C−H), 1759 (-C=O), 1070 (-C−O-); HRMS (ESI-TOF) m/z [M + Na]$^+$ calcd for $C_{70}H_{86}O_{19}Na$ 1253.5661, found 1253.5649.

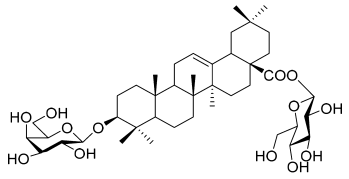
Olean-12-en-28-oic Acid, 3-(β-D-Glucopyranosyloxy)-, β-D-Glucopyranosyl Ester (10a).



10a

To a solution of **7a** (100 mg, 0.0732 mmol) in dry MeOH (1.4 mL) and CH_2Cl_2 (1.4 mL) was added NaH (3.0 mg, 0.0732 mmol 60% disp.). The mixture was stirred at ambient temperature for 1 h, neutralized with Dowex H$^+$ resin to pH 7, and filtered. The filtrate was concentrated under reduced pressure to give the crude product. Purification by flash column chromatography (silica gel, 20 g; $CHCl_3$/MeOH = 5:1 → 4:1 → 3:1) afforded **10a** (53 mg, 0.0679 mmol, 93%) as a white solid. R_f = 0.08 ($CHCl_3$/MeOH = 5:1); $[\alpha]_D^{24}$ +18.4 (*c* 1.00, CH_3OH); ^{1}H NMR (400 MHz, pyridine-d_5) δ 6.30 (d, *J* = 8.0 Hz, 1H, 1′-H), 5.40 (t, J = 3.3 Hz, 1H, 12-H), 4.91 (d, *J* = 8.0 Hz, 1H, 1″-H), 4.56 (dd, *J* = 12.0 Hz, 2.0 Hz, 1H, 6″-H), 4.43 (dd, *J* = 12.0 Hz, 2.0 Hz, 1H, 6′-H), 4.38 (dd, *J* = 12.0 Hz, 5.0 Hz, 1H, 6′-H), 4.37 (dd, *J* = 12.0 Hz, 5.0 Hz, 1H, 6″-H), 4.34 (dd, *J* = 9.5 Hz, 9.0 Hz, 1H, 4′-H), 4.26 (dd, *J* = 9.0 Hz, 8.5 Hz, 1H, 3′-H), 4.22 (dd, *J* = 8.5 Hz, 8.0 Hz, 1H, 3″-H), 4.20 (dd, *J* = 9.0 Hz, 8.5 Hz, 1H, 4″-H), 4.18 (t, *J* = 8.5 Hz, 1H, 2′-H), 4.01 (t, *J* = 8.0 Hz, 1H, 2″-H), 4.00 (ddd, *J* = 9.5 Hz, 4.0 Hz, 2.0 Hz, 1H, 5′-H), 3.98 (ddd, *J* = 9.0 Hz, 5.0 Hz, 2.0 Hz, 1H, 5″-H), 3.39 (dd, *J* = 14.0 Hz, 4.2 Hz, 1H, 3-H), 3.21 (dd, *J* = 14.0 Hz, 3.9 Hz, 1H, 18-H), 2.37 (m, 1H, 15-H), 2.24 (m, 1H, 2-H), 2.10 (m, 2H, 11-H), 1.99 (m, 2H, 16-H), 1.90 (m, 1H, 2-H), 1.85 (m, 2H, 22-H), 1.78 (m, 1H, 19-H), 1.63 (m, 1H, 9-H), 1.49 (m, 2H, 1-H, 6-H), 1.45 (m, 1H, 7-H), 1.41 (m, 2H, 21-H), 1.36 (m, 1H, 6-H), 1.32 (s, 3H, 23-H), 1.28 (s, 3H, 27-H), 1.21 (m, 1H, 19-H), 1.18 (m, 1H, 15-H), 1.11 (s, 3H, 26-H), 1.07 (m, 1H, 7-H), 1.01 (s, 3H, 24-H), 0.96 (m, 1H, 1-H), 0.92 (s, 3H, 29-H), 0.90 (s, 3H, 30-H), 0.86 (s, 3H, 25-H), 0.79 (m, 1H, 5-H); ^{13}C NMR (100 MHz, pyridine-d_5) δ 176.6 (C-28), 144.3 (C-13), 123.2 (C-12), 107.0 (C-1″), 95.9 (C-1′), 89.0 (C-3), 79.5 (C-5′), 78.9 (C-3′), 78.8 (C-3″), 78.4 (C-5″), 75.9 (C-2″), 74.2 (C-2′), 71,9 (C-4″), 71.2 (C-4′), 63.1 (C-6″), 62.2 (C-6′), 56.0 (C-5), 48.2 (C-9), 47.2 (C-17), 46.4 (C-19), 42.3 (C-14), 41.9 (C-18), 40.1 (C-8), 39.7 (C-4), 38.9 (C-1), 37.1 (C-10), 34.1 (C-21), 33.3 (C-7), 33.3 (C-29), 32.7 (C-22), 30.9 (C-20), 28.4 (C-23), 28.4 (C-15), 26.7 (C-2), 26.3 (C-27), 23.9 (C-30), 23.8 (C-16), 23.5 (C-11), 18.7 (C-6), 17.6 (C-26), 17.1 (C-24), 15.7 (C-25); IR (KBr) cm^{-1} ν 3441 (-O−H), 2939 (=C−H), 1735 (-C=O), 1069 (-C−O-); HRMS (ESI-TOF) m/z [M + Na]$^+$ calcd for $C_{42}H_{68}O_{13}Na$ 803.4558, found 803.4558; TOCSY (400 MHz, pyridine-d_5) mixing time τ_m = 150 ms.

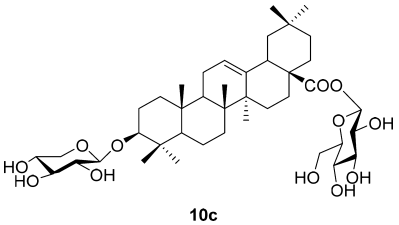
Olean-12-en-28-oic Acid, 3-(β-D-Galactopyranosyloxy)-, β-D-Glucopyranosyl Ester (10b).



10b

To a solution of **7b** (137 mg, 0.1003 mmol) in dry MeOH (1.0 mL) and CH_2Cl_2 (1.0 mL) was added NaH (4.0 mg, 0.1003 mmol 60% disp.). The mixture was stirred at ambient temperature for 1 h, neutralized with Dowex H$^+$ resin to pH 7, and filtered. The filtrate was concentrated under reduced pressure to give the crude product. Purification by flash column chromatography (silica gel, 20 g; $CHCl_3$/MeOH = 5:1 → 4:1 → 3:1) afforded **10b** (58 mg, 0.0743 mmol, 74%) as a white solid. R_f = 0.36 ($CHCl_3$/MeOH = 3:1); $[\alpha]_D^{24}$ +24.2 (*c* 1.00, CH_3OH); ^{1}H NMR (400 MHz, pyridine-d_5) δ 6.33 (d, *J* = 8.0 Hz, 1H, 1′-H), 5.43 (t, J = 3.3 Hz, 1H, 12-H), 4.87 (d, *J* = 8.0 Hz, 1H, 1″-H), 4.60 (dd, *J* = 3.0 Hz, 1.0 Hz, 1H, 4″-H), 4.50 (dd, *J* = 12.0 Hz, 6.0 Hz, 1H, 6″-H), 4.48 (dd, *J* = 12.0 Hz, 3.0 Hz, 1H, 6′-H), 4.46 (dd, *J* = 9.0 Hz, 8.0 Hz, 1H, 2″-H), 4.45 (dd, *J* = 12.0 Hz, 6.0 Hz, 1H, 6″-H), 4.41 (dd, *J* = 12.0 Hz, 4.0 Hz, 1H, 6′-H), 4.37 (dd, *J* = 9.5 Hz, 9.0 Hz, 1H, 4′-H), 4.30 (dd, *J* = 9.0 Hz, 8.5 Hz, 1H, 3′-H), 4.22 (dd, *J* = 8.5 Hz, 8.0 Hz, 1H, 2′-H), 4.19 (dd, *J* = 9.0 Hz, 3.0 Hz, 1H, 3″-H), 4.13 (td, *J* = 6.0 Hz, 1.0 Hz, 1H, 5″-H), 4.04 (ddd, *J* = 9.5 Hz, 4.0 Hz, 3.0 Hz, 1H, 5′-H), 3.37 (dd, *J* = 12.0 Hz, 4.5 Hz, 1H, 3-H), 3.19 (dd, *J* = 14.0 Hz, 3.9 Hz, 1H, 18-H), 2.35 (m, 1H, 15-H), 2.26 (m, 1H, 2-H), 2.08 (m, 2H, 11-H), 1.95 (m, 2H, 16-H), 1.81 (m, 1H, 2-H), 1.76 (m, 2H, 22-H), 1.72 (m, 1H, 19-H), 1.61 (m, 1H, 9-H), 1.40 (m, 2H, 1-H, 6-H), 1.43 (m, 1H, 7-H), 1.34 (m, 2H, 21-H), 1.31 (m, 1H, 6-H), 1.29 (s, 3H, 23-H), 1.26 (s, 3H, 27-H), 1.23 (m, 1H, 19-H), 1.17 (m, 1H, 15-H), 1.09 (s, 3H, 26-H), 1.04 (m, 1H, 7-H), 0.96 (s, 3H, 24-H), 0.93 (m, 1H, 1-H), 0.90 (s, 3H, 29-H), 0.88 (s, 3H, 30-H), 0.82 (s, 3H, 25-H), 0.77 (m, 1H, 5-H); ^{13}C NMR (100 MHz, pyridine-d_5) δ 176.6 (C-28), 144.3 (C-13), 123.2 (C-12), 107.0 (C-1″), 95.9 (C-1′), 88.9 (C-3), 79.5 (C-5′), 78.9 (C-3′), 76.9 (C-5″), 75.5 (C-3″), 74.2 (C-2′), 73.2 (C-2″), 71.1 (C-4′), 70.3 (C-4″), 62.5 (C-6″), 62.2 (C-6′), 56.0 (C-5), 48.2 (C-9), 47.2 (C-17), 46.4 (C-19), 42.3 (C-14), 41.9 (C-18), 40.1 (C-8), 39.7 (C-4), 38.9 (C-1), 37.1 (C-10), 34.2 (C-21), 33.3 (C-29), 33.3 (C-7), 32.7 (C-22), 31.0 (C-20), 28.4 (C-23), 28.4 (C-15), 26.8 (C-2), 26.3 (C-27), 24.0 (C-30), 23.8 (C-16), 23.6 (C-11), 18.7 (C-6), 17.6 (C-26), 17.2 (C-24), 15.7 (C-25); IR (KBr) cm^{-1} ν 3398 (-O−H), 2948 (=C−H), 1735 (-C=O), 1074 (-C−O-); HRMS (ESI-TOF) m/z [M + Na]$^+$ calcd for $C_{42}H_{68}O_{13}Na$ 803.4558, found 803.4551; TOCSY (400 MHz, pyridine-d_5) mixing time τ_m = 150 ms.

Olean-12-en-28-oic Acid, 3-(β-D-Xylopyranosyloxy)-, β-D-Glucopyranosyl Ester (10c).



10c

To a solution of **7c** (159 mg, 0.1291 mmol) in dry MeOH (2.4 mL) and CH_2Cl_2 (1.2 mL) was added NaH (5.16 mg, 0.1291 mmol, 60% disp.). The mixture was stirred at ambient temperature for 50 min, neutralized with Dowex H$^+$ resin to pH 7, and filtered. The filtrate was concentrated under reduced pressure to give the crude product. Purification by flash column chromatography (silica gel, 20 g; $CHCl_3$/MeOH = 10:1) afforded **10c** (77.9 mg, 0.1037 mmol, 87%) as a white solid. R_f = 0.48 ($CHCl_3$/MeOH = 3:1); $[\alpha]_D^{24}$ +20.6 (*c* 1.00, CH_3OH); ^{1}H NMR (400 MHz, pyridine-d_5) δ 6.33 (d, *J* = 8.5 Hz, 1H, 1′-H), 5.44 (t, *J* = 3.3 Hz, 1H, 12-H), 4.84 (d, *J* = 7.5 Hz, 1H, 1″-H), 4.47 (dd, *J* = 12.0 Hz, 2.0 Hz, 1H, 6′-H), 4.41 (dd, *J* = 12.0 Hz, 4.0 Hz,

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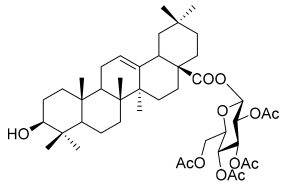
1H, 6′-H), 4.39 (dd, J = 11.0 Hz, 5.0 Hz, 1H, 5″-H), 4.38 (dd, J = 9.5 Hz, 9.0 Hz, 1H, 4′-H), 4.30 (t, J = 9.0 Hz, 1H, 3′-H), 4.24 (ddd, J = 10.0 Hz, 8,5 Hz, 5.0 Hz, 1H, 4″-H), 4.21 (dd, J = 9.0 Hz, 8.5 Hz, 1H, 2′-H), 4.18 (t, J = 8.5 Hz, 1H, 3″-H), 4.04 (ddd, J = 9.5 Hz, 4.0 Hz, 2.0 Hz, 1H, 5′-H), 4.03 (dd, J = 8.5 Hz, 7.5 Hz, 1H, 2″-H), 3.79 (dd, J = 11.0 Hz, 5.0 Hz, 1H, 5″-H), 3.36 (dd, J = 12.0 Hz, 4.5 Hz, 1H, 3-H), 3.21 (dd, J = 14.0 Hz, 3.9 Hz, 1H, 18-H), 2.37 (m, 1H, 15-H), 2.20 (m, 1H, 2-H), 2.13 (m, 2H, 11-H), 2.00 (m, 2H, 16-H), 1.94 (m, 1H, 2-H), 1.92 (m, 2H, 22-H), 1.77 (m, 1H, 19-H), 1.66 (m, 1H, 9-H), 1.58 (m, 1H, 1-H), 1.47 (m, 2H, 6-H, 7-H), 1.37 (m, 2H, 21-H), 1.31 (s, 3H, 23-H), 1.29 (m, 1H, 6-H), 1.27 (s, 3H, 27-H), 1.25 (m, 1H, 19-H), 1.19 (m, 1H, 15-H), 1.12 (s, 3H, 26-H), 1.08 (m, 1H, 7-H), 1.00 (s, 3H, 24-H), 0.98 (m, 1H, 1-H), 0.93 (s, 3H, 29-H), 0.90 (s, 3H, 30-H), 0.88 (s, 3H, 25-H), 0.83 (m, 1H, 5-H); ^{13}C NMR (100 MHz, pyridine-d_5) δ 176.7 (C-28), 144.3 (C-13), 123.1 (C-12), 107.8 (C-1″), 95.9 (C-1′), 88.8 (C-3), 79.5 (C-5′), 78.9 (C-3′), 78.7 (C-3″), 75.6 (C-2″), 74.2 (C-2′), 71.2 (C-4″), 71.1 (C-4′), 67.3 (C-5″), 62.2 (C-6′), 56.0 (C-5), 48.2 (C-9), 47.2 (C-17), 46.4 (C-19), 42.3 (C-14), 41.9 (C-18), 40.1 (C-8), 39.7 (C-4), 39.0 (C-1), 37.2 (C-10), 34.2 (C-21), 33.3 (C-29), 33.3 (C-7), 32.7 (C-22), 31.0 (C-20), 28.4 (C-23), 28.4 (C-15), 26.9 (C-2), 26.3 (C-27), 24.0 (C-30), 23.8 (C-16), 23.6 (C-11), 18.7 (C-6), 17.6 (C-26), 17.1 (C-24), 15.8 (C-25); IR (KBr) cm^{-1} ν 3390 (-O−H), 2959 (=C−H), 1730 (-C=O), 1075 (-C−O-); HRMS (ESI-TOF) m/z [M + Na]$^+$ calcd for $C_{41}H_{66}O_{12}Na$ 773.4452, found 773.4455; TOCSY (400 MHz, pyridine-d_5) mixing time τ_m = 150 ms.

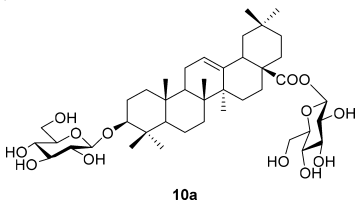
Olean-12-en-28-oic Acid, 3-Hydroxy-, 2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl Ester (5).



5

Continuous Flow Glycosylation and Batch Deprotection. A solution of BF_3·OEt_2 (12.0 μL, 0.0956 mmol, 0.0320 M) dissolved in CH_2Cl_2 (3.0 mL) was injected into the micromixer using a syringe pump at a flow rate of 0.6 mL/min. Then, a solution of donor **2a** (94.2 mg, 0.1912 mmol, 0.0637 M) and acceptor **1** (54.6 mg, 0.0956 mmol, 0.0320 M) dissolved in CH_2Cl_2 (3.0 mL) was injected into the micromixer by the same syringe pump at a flow rate of 0.6 mL/min and mixed in a thermostatic bath at −40 °C. After the reaction mixture was allowed to flow at −40 °C through a Teflon reactor tube (ϕ = 1.0 mm, l = 1.0 m), the crude mixture of **3a** was introduced into a flask, which was cooled to −40 °C and connected to the outlet of a Teflon reactor tube. The reaction mixture was stirred for 30 min at ambient temperature in flask containing $ZnBr_2$ (216 mg, 0.956 mmol) and H_2O (17.2 μL, 0.956 mmol) in advance. After the reaction was completed, the reaction mixture was quenched with NEt_3, filtered by Celite, and rinsed with CH_2Cl_2 (20 mL). The filtrate was concentrated under reduced pressure to give the crude product. Purification by flash column chromatography (silica gel, 20 g; hexane/AcOEt = 3:1 → 2:1 → 3:2) afforded **5** (58.4 mg, 0.0748 mmol, 78%) as a white foamy solid in two steps.

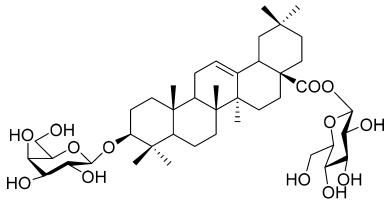
Olean-12-en-28-oic Acid, 3-(β-D-Glucopyranosyloxy)-, β-D-Glucopyranosyl Ester (10a).



10a

Continuous Flow Glycosylation and Batch Deprotection. A solution of TMSOTf (11.5 μL, 0.0635 mmol, 0.0212 M) dissolved in CH_2Cl_2 (3.0 mL) was injected into the micromixer using a syringe pump at a flow rate of 0.6 mL/min. Then, a solution of donor **6a** (71.0 mg, 0.0954 mmol, 0.0318 M) and acceptor **5** (50.0 mg, 0.0635 mmol, 0.0212 M) dissolved in CH_2Cl_2 (3.0 mL) was injected into the micromixer by the same syringe pump at a flow rate of 0.6 mL/min and mixed in a thermostatic bath at ambient temperature. After the reaction mixture was allowed to flow at ambient temperature through a Teflon reactor tube (ϕ = 1.0 mm, l = 1.0 m), the crude mixture of **7a** was introduced into a flask, which was connected to the outlet of a Teflon reactor tube. The reaction mixture was stirred for 30 min at ambient temperature in a flask containing NaH (11.5 mg, 0.2858 mmol, 60% disp.) and MeOH (12 mL) in advance. After the reaction was completed, the reaction mixture was neutralized with Dowex H$^+$ resin to pH 7, filtered, and rinsed with MeOH (20 mL). The filtrate was concentrated under reduced pressure to give the crude product. Purification by flash column chromatography (silica gel, 20 g; $CHCl_3$/MeOH = 10:1 → 8:1 → 6:1) afforded **10a** (23.3 mg, 0.0298 mmol, 47%) as a white solid in two steps.

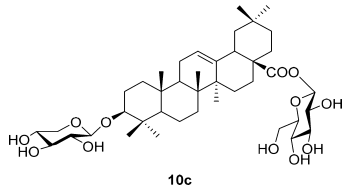
Olean-12-en-28-oic Acid, 3-(β-D-Galactopyranosyloxy)-, β-D-Glucopyranosyl Ester (10b).



10b

Continuous Flow Glycosylation and Batch Deprotection. A solution of TMSOTf (11.5 μL, 0.0635 mmol, 0.0212 M) dissolved in CH_2Cl_2 (3.0 mL) was injected into the micromixer using a syringe pump at a flow rate of 0.6 mL/min. Then, a solution of donor **6b** (71.0 mg, 0.0954 mmol, 0.0318 M) and acceptor **5** (50.0 mg, 0.0635 mmol, 0.0212 M) dissolved in CH_2Cl_2 (3.0 mL) was injected into the micromixer by the same syringe pump at a flow rate of 0.6 mL/min and mixed in a thermostatic bath at ambient temperature. After the reaction mixture was allowed to flow at ambient temperature through a Teflon reactor tube (ϕ = 1.0 mm, l = 1.0 m), the crude mixture of **7b** was introduced into a flask, which was connected to the outlet of a Teflon reactor tube. The reaction mixture was stirred for 30 min at ambient temperature in a flask containing NaH (11.5 mg, 0.2858 mmol, 60% disp.) and MeOH (12 mL) in advance. After the reaction was completed, the reaction mixture was neutralized with Dowex H$^+$ resin to pH 7, filtered, and rinsed with MeOH (20 mL). The filtrate was concentrated under reduced pressure to give the crude product. Purification by flash column chromatography (silica gel, 20 g; $CHCl_3$/MeOH = 10:1 → 8:1 → 6:1) afforded **10b** (21.5 mg, 0.0275 mmol, 43%) as a white solid in two steps.

Olean-12-en-28-oic Acid, 3-(β-D-Xylopyranosyloxy)-, β-D-Glucopyranosyl Ester (10c).



10c

Continuous Flow Glycosylation and Batch Deprotection. A solution of TMSOTf (11.5 μL, 0.0635 mmol, 0.0212 M) dissolved in CH_2Cl_2 (3.0 mL) was injected into the micromixer using a syringe pump at a flow rate of 0.6 mL/min. Then, a solution of donor **6c** (71.0 mg, 0.0954 mmol, 0.0318 M) and acceptor **5** (50.0 mg, 0.0635 mmol, 0.0212 M) dissolved in CH_2Cl_2 (3.0 mL) was injected into the micromixer by the same syringe pump at a flow rate of 0.6 mL/min and mixed in a thermostatic bath at ambient temperature. After the reaction mixture was allowed to flow at ambient temperature through a Teflon reactor tube (ϕ = 1.0 mm, l = 1.0 m), the crude mixture of **7c** was introduced into a flask, which was connected to the outlet of a Teflon reactor tube. The reaction mixture was stirred for 30 min at ambient temperature in a flask containing NaH (11.5 mg, 0.2858 mmol, 60% disp.) and MeOH (12 mL) in advance. After the reaction

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was completed, the reaction mixture was neutralized with Dowex H^+ resin to pH 7, filtered, and rinsed with MeOH (20 mL). The filtrate was concentrated under reduced pressure to give the crude product. Purification by flash column chromatography (silica gel, 20 g; $CHCl_3$/MeOH = 10:1 → 9:1) afforded **10c** (22.5 mg, 0.0300 mmol, 47%) as a white solid in two steps.

The purities of **10a−10c** were compared to the corresponding compounds in stepwise synthesis by a proton NMR spectrum (see Supporting Information for details).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.7b00841.

^1H and ^{13}C NMR spectra for the synthetic compounds (PDF)

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Notes

The authors declare no competing financial interest.

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