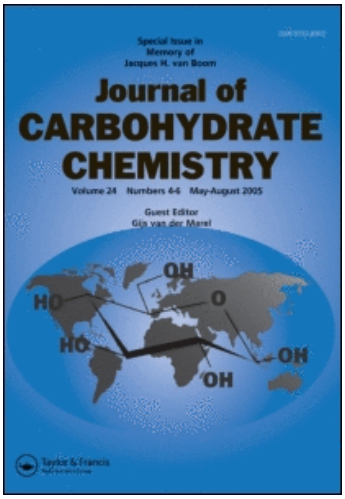

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UCHP Method for Oligosaccharide Combinatorial Library Synthesis

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A new methodology for oligosaccharide combinatorial library synthesis using a special hydroxy protecting group, the uni-chemo hydroxy protection (UCHP) group, was developed. The UCHP group was composed of oligomeric amino acid derivatives. The amino terminals of UCHP groups were protected by either Boc or Fmoc groups. By using these two types of UCHP, five kinds of trigalactoses [Galβ1-3Galβ1-3Gal, Galβ1-3(Galβ1-4)Gal, Galβ1-4Galβ1-3Gal, Galβ1-3Galβ1-4Gal, and Galβ1-4Galβ1-4Gal] were successfully synthesized on a solid support as a model of oligosaccharide combinatorial library. Each step of all reactions was also successfully monitored using a combination of two colorimetric tests, chloranil and methyl red-DIC.

Keywords UCHP; Oligosaccharide combinatorial library; Solid-phase synthesis

INTRODUCTION

Three important bio-functional chain types maintain homeostasis of living organisms: nucleic acids (DNA and RNA), proteins (peptides), and oligosaccharides. In the assembly of both nucleic acids (polynucleotides) and proteins (polypeptides), no stereochemistry exists in the repeating bond constructions (phosphate and amide, respectively), resulting in no need for selection. In contrast, in the assembly of oligosaccharides (polysaccharides), selection

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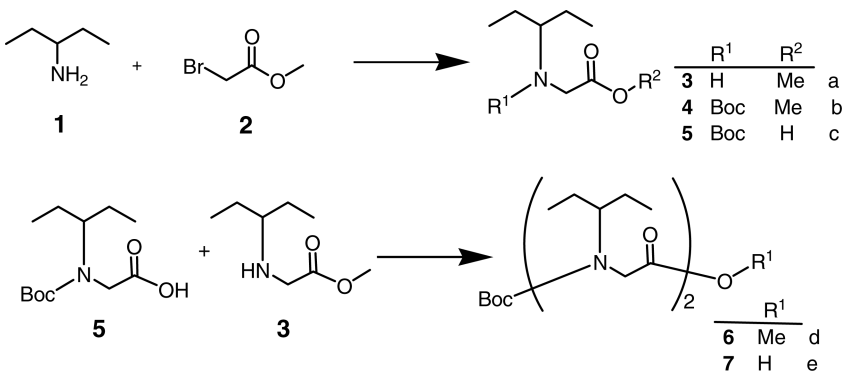
is necessary for desired bond constructions (location of glycoside bond and anomeric configuration). Each glycosidic bond to be fashioned in a growing oligosaccharide constitutes a new stereogenic center and a locus of possible complexity. Because of the branching structure of oligosaccharides, the buildup of the target structure requires complicated orthogonal protecting groups for each hydroxyl group.[1] Recently, we applied the uni-chemo protection (UCP) method[2] to oligosaccharide synthesis.[3,4] The UCP is composed of an amino acid oligomer, which was originally designed to protect the amino group on a peptide. We applied this method for hydroxy protection, naming it uni-chemo hydroxy protection (UCHP). The individual hydroxyl groups on the carbohydrates were protected with a different oligomerization degree of amino acids by ester linkage. Deprotection of this UCHP group was performed using the Edman degradation reaction,[5] which removes only the *N*-terminal mono-amino acid from all UCHP groups. The general Edman degradation reaction for the UCHP method consists of four steps: the first step is deprotection of the amino protecting group (de-Fmoc or de-Boc step); the second step is phenyl isothiocyanate (PITC: Edman reagent) coupling to all free amino terminals (PITC coupling step); the third step is cleaving of the *N*-terminal phenyl thiocarbamoyl mono-amino acid derivative by using trifluoroacetic acid (TFA) to decrease the oligomerization degree (TFA treatment step); and the fourth step is reprotection by the Boc group to the newly exposed *N*-terminal amino group (re-Boc step). However, the first and second steps are sufficient conditions for deprotection of monomeric-UCHP because the phenyl thiocarbamoyl mono-amino acid derivative is removed immediately from the hydroxyl group by the electron withdrawing effect at such basic PITC coupling conditions. Depending on the oligomerization degree of UCHP, each hydroxyl group was characterized.

To demonstrate the efficiency of this UCHP method in the synthesis of an oligosaccharide combinatorial library, five kinds of trigalactoses [Galβ1-3Galβ1-3Gal (**40**), Galβ1-3(Galβ1-4)Gal (**42**), Galβ1-4Galβ1-3Gal (**44**), Galβ1-3Galβ1-4Gal (**46**), and Galβ1-4Galβ1-4Gal (**48**)] have been synthesized on a solid support as a model of oligosaccharide combinatorial library. The glycoside bonds selected were β 1-3 and β 1-4 for the second and third galactoses, respectively, to demonstrate selective deprotection of the UCHP group even if it protected to the adjacent hydroxyl groups. Moreover, each reaction step was monitored by the combination of two colorimetric tests, chloranil[6] and methyl red-DIC.[7] The chloranil test was used for the detection of the amino group. The color of the resin changes to dark blue in the presence of an amino group. The methyl red-DIC test is a newly developed method from our group for the detection of the hydroxyl group. The resin reddens in the presence of a hydroxyl group.

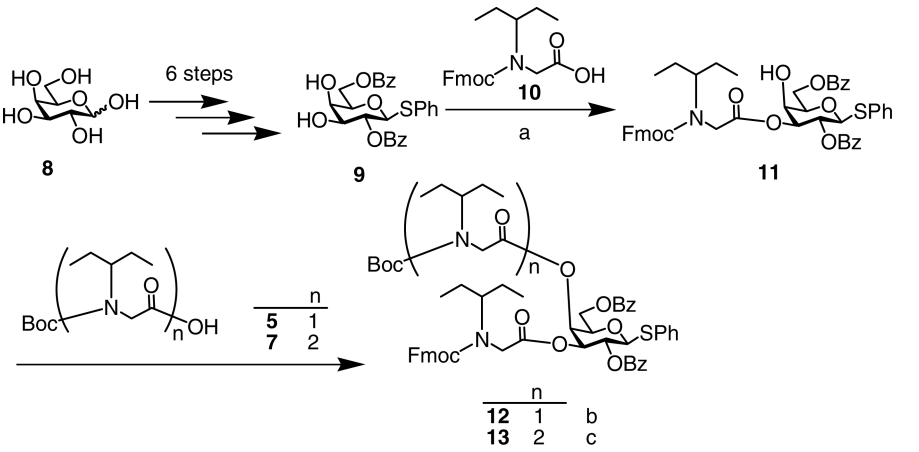
RESULTS AND DISCUSSION

One of the disadvantages of the UCHP method is an increase in steric hindrance. The oligomerization level of UCHP rises as the number of hydroxyl groups that protect UCHP increases. As a result, the steric hindrance increases. To decrease the steric hindrance, two types of UCHP were designed. One is the Fmoc (base-removable amino protecting group)-protected UCHP; the other is the Boc (acid-removable amino protecting group)-protected one. Fmoc and Boc are orthogonal amino protecting groups. If the amino terminal of UCHP is protected by one kind of amino protecting group, it is necessary to protect two hydroxyl groups by a monomeric-UCHP and dimeric-UCHP combination. However, if the UCHP amino terminals are protected by both the Fmoc group and the Boc group, two hydroxyl groups can be protected by two monomeric-UCHP groups of the kind that would create the least steric hindrance. Interestingly, the dimeric-UCHP protected with Fmoc group was removed from the hydroxyl group at the de-Fmoc step (by using 20% piperidine/DMF for 20 min, the dimeric-UCHP removes quantitatively) because dimeric-UCHP with a free amino group cyclized immediately by such a basic condition and cleaved from the hydroxyl group. As a result, the Fmoc protection was applied only for monomeric UCHP. On the other hand, the Boc protection bears no restriction for usage (Sch. 1).

The galactose donors for trigalactose combinatorial library were designated as **12** and **13** in Scheme 2. SPh (thiophenyl) was chosen as the anomeric leaving group for easy activation by either DMTST [dimethyl(methylthio)sulfonium triflate][8] or NIS (*N*-iodosuccinimide)-TfOH (trifluoromethanesulfonic acid).[8] 2-OH on galactose was protected by the

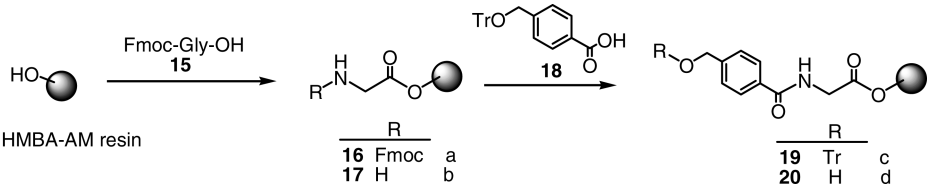


Scheme 1. Preparation of UCHP groups. (a) THF, rt, 2 h, 92%; (b) 1.5 equiv. Boc_2O, 1,4-dioxane/saturated aq $NaHCO_3$, rt, 2 h; (c) 10 N NaOH, MeOH/H_2O, 40°C, 4 h, 94% (two steps); (d) 4.0 equiv. DIC, 0.4 equiv. DMAP, CH_2Cl_2, 6°C, 12 h; (e) 10 N NaOH, MeOH/H_2O, 40°C, 2 h, 57% (two steps).

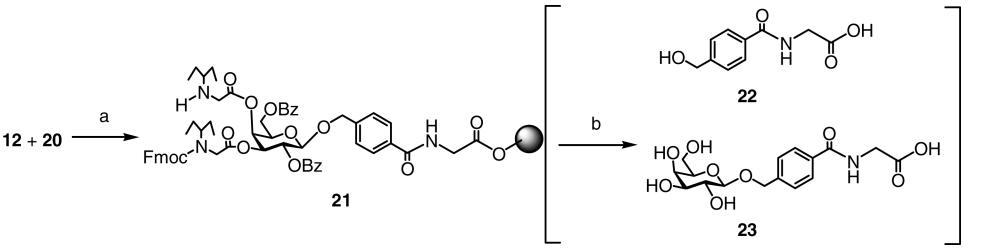


Scheme 2. Preparation of galactose donors. (a) 1.2 equiv. **10**, 1.1 equiv. DIC, 0.1 equiv. DMAP, CH_2Cl_2, -40°C, 2 days, 93%; (b) 1.2 equiv. **5**, 2.0 equiv. DIC, 0.1 equiv. DMAP, CH_2Cl_2, 0°C, 2 h, 99%; (c) 1.2 equiv. **7**, 2.0 equiv. DIC, 0.1 equiv. DMAP, CH_2Cl_2, 0°C, 2 h, 95%.

benzoyl (Bz) group as a neighboring effect group for selective β-glycosidation. 3-OH on galactose was protected by monomeric-UCHP protected with an Fmoc group. 4-OH was protected by either monomeric-UCHP protected with a Boc group or dimeric-UCHP protected with a Boc group. To synthesize these two kinds of galactose donors, phenyl 2,6-di-*O*-benzoyl-1-thio-β-D-galactopyranoside (**9**)[9] was used as the starting compound. The monomeric-UCHP protected with an Fmoc group (**10**)[3,4] selectively protected 3-OH on compound **9** by using DIC and DMAP at –40°C. Subsequently, by using DIC and DMAP at 0°C, its 4-OH was protected by either monomeric-UCHP protected with a Boc group (**5**) or dimeric-UCHP protected with a Boc group (**7**). The solid support used was HMBA-AM resin (**14**, 1.16 mmol/g; Novabiochem, Merck Ltd.), a hydroxy functionalized polystyrene resin (Sch. 3). HMBA-Gly {2-[4-(hydroxymethyl)benzamido]acetic acid} was designed as the linker,[3] which bound to the hydroxyl group on HMBA-AM resin via ester linkage.



Scheme 3. Preparation of linker connected solid support. (a) **14** (1.0 g, 1.16 mmol/g), 3.0 equiv. **15**, 3.0 equiv. DIC, 0.1 equiv. DMAP, CH_2Cl_2, rt, 1 day, then Ac_2O, 0.1 equiv. DMAP, pyr, CH_2Cl_2, rt, 1 day. The loading was determined to be 0.8447 mmol/g from UV absorbance of the eluate of Fmoc group; (b) 20% piperidine in DMF, rt, 18 min; (c) 3.0 equiv. **18**, 6.0 equiv. NEM, 2.9 equiv. TBTU, DMF, rt, 4 h, then Ac_2O, DMF, rt, 20 min; (d) 50% TFA in CH_2Cl_2, rt, 5 min, 6 times.



Scheme 4. Condensation of first galactose. (a) 6.0 equiv. **12**, 18.0 equiv. DMTST, CH_2Cl_2, rt, 1 day; (b) NaOMe, CH_2Cl_2, rt, 1 h, then added H_2O and MeOH, rt, 1 h. The coupling yield was 60%. The respective retention times for **22** and **23** were 5.0 and 20.3 min.

The products of the oligosaccharide linker complex could be cleaved off from the resin at basic conditions and could be detected by UV on HPLC. The theoretical loading value of hydroxyl groups on linker-connected resin (**20**) was calculated as 0.9126 mmol/g from Fmoc loading value of compound **16** (0.8447 mmol/g).[10] The first galactose donor (**12**) was coupled with a hydroxyl group on the resin (**20**) by using DMTST as a promoter in CH_2Cl_2 at rt (Sch. 4). After 1 day of the coupling reaction, an aliquot of the resin was treated with base; next, the eluate was analyzed by analytical normal-phase HPLC. Analytical normal-phase HPLC separation was performed using an HPLC system (Shimadzu Corp.) with a TSK-gel Amide-80 column (4.6 $\times$ 250 mm, 5 μm; Tosoh Co. Ltd.) at a flow rate of 1 mL/min and detection at 254 nm. Both the unglycosylated linker (**22**, retention time of 5.0 min) and glycosylated linker (**23**, retention time of 20.3 min) were detected, and the coupling yield was calculated as 60% (Fig. 1). Because the Boc group was cleaved during the glycosidation reaction, the resultant exposed amino group on terminal UCHP was protected by a Boc group again (re-Boc) (Sch. 5). Re-Boc reaction was monitored by chloranil test for amino detection. This reaction was continued until the chloranil test produced a negative response. After the unglycosylated hydroxyl group had been protected by the benzoyl group, the monomeric-UCHP protected with Fmoc group

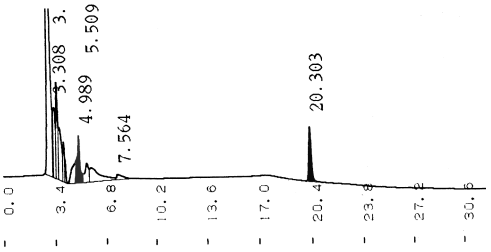
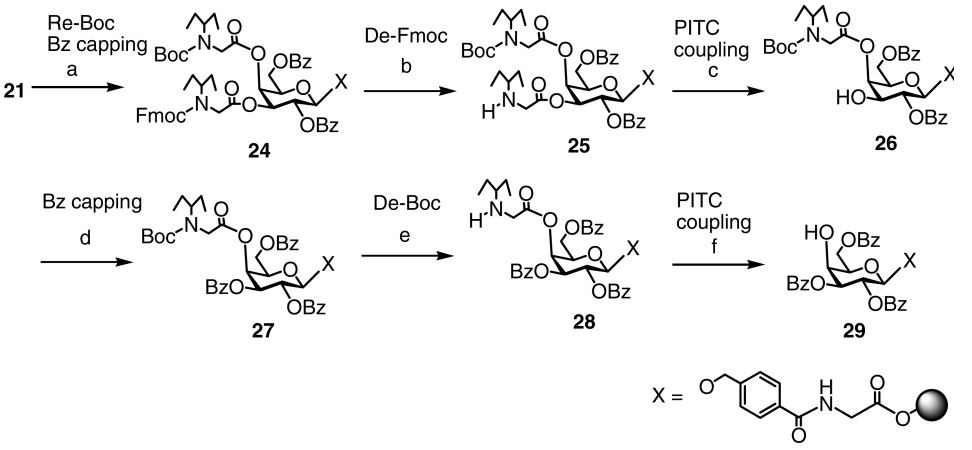


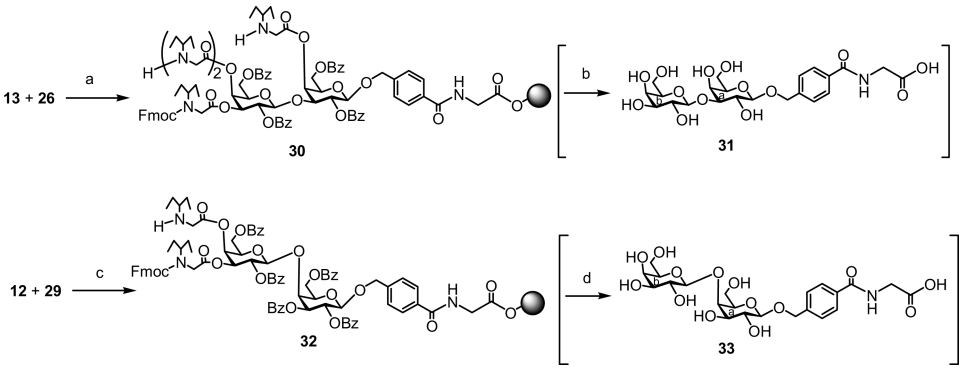
Figure 1. HPLC trace. After the coupling of the first galactose (**12**) to the resin (**20**). The 5.0-min peak indicated the presence of a linker (**22**). The 20.3-min peak indicated compound **23**. The coupling yield was estimated to be 60% based on the peak area. HPLC condition A was used.



Scheme 5. Deprotection of UCHP groups by Edman degradation. (a) Boc_2O, saturated aq $NaHCO_3$, DMF, rt, 8 h, then BzOH, DMAP, DIC, CH_2Cl_2, rt, 12 h; (b) 20% piperidine in DMF, rt, 18 min; (c) PITC, NMM, DMF, rt, 30 min; (d) BzOH, DMAP, DIC, CH_2Cl_2, rt, 12 h; (e) 20% TFA in CH_2Cl_2, rt, 30 min, twice; (f) PITC, NMM, DMF, rt, 30 min.

that bound to the hydroxyl group at the 3-position was selectively removed by one cycle of the Edman degradation reaction (de-Fmoc and PITC coupling). Consequently, the galactose acceptor with a selectively free hydroxyl group at the 3-position (**26**) was selectively obtained. The Bz capping step was monitored by the methyl red-DIC test, and the de-Fmoc step of the first step in the Edman degradation reaction was monitored by the chloranil test. The PITC coupling step of the second step in the Edman degradation reaction was monitored by both the chloranil test and the methyl red-DIC test. All steps were successfully monitored by a combination of chloranil and methyl red-DIC tests. For the synthesis of compounds **40**, **42**, and **44**, the resultant galactose acceptor with a selectively free hydroxyl group at the 3-position (**26**) was coupled with a second galactose donor (**13**). For the synthesis of compounds **46** and **48**, the resin (**26**) was capped with a benzoyl group. Following that, the monomeric-UCHP protected with a Boc group at the 4-position was selectively removed by one cycle of the Edman degradation reaction (de-Boc and PITC coupling). As a result, the galactose acceptor with a selectively free hydroxyl group at the 4-position (**29**) was selectively obtained. All steps were also successfully monitored by the combination of chloranil and methyl red-DIC tests. This galactose acceptor with a selectively free hydroxyl group at the 4-position (**29**) was coupled with a second galactose donor (**12**).

For the coupling of the second galactose, DMTST was used as a coupling promoter (Sch. 6). The second galactose donor (**13**) was coupled with 3-OH of the galactose on the resin (**26**) by using DMTST in CH_2Cl_2 at rt for 1 day, identically to the previously described procedure. However, in this case, the glycosylation yield was sufficiently low to warrant repeating the coupling procedure



Scheme 6. Condensation of second galactose. (a) 6.0 equiv. **13**, 18.0 equiv. DMTST, CH_2Cl_2, rt, 1 day, three times; (b) NaOMe, CH_2Cl_2, rt, 1 h, then added H_2O and MeOH, rt, 1 h; (c) 6.0 equiv. **12**, 18.0 equiv. NIS, 18.0 equiv. TfOH, CH_2Cl_2, -30°C, 1 day; (d) NaOMe, CH_2Cl_2, rt, 1 h, then added H_2O and MeOH, rt, 1 h. The respective retention times for **31** and **33** were 23.7 and 23.2 min. The respective coupling yields for **31** and **33** were 50% (triple coupling) and 53% (single coupling).

three times in all. Using an analytical normal-phase HPLC at the same conditions as previously described, the single, double, and triple coupling yields were estimated as 17%, 34%, and 50%, respectively (Fig. 2i). On the other hand, in the case of coupling the second galactose donor (**12**) with 4-OH of the galactose on the resin (**29**) by using DMTST, as in the previously described procedure, the product of the disaccharide was obtained in less than 10% yield. To increase the coupling yield, the promoter was changed to NIS-TfOH from DMTST and the reaction temperature was changed to –30°C. As a result, the coupling yield dramatically increased, reaching 53% with only a single coupling, based on analysis of analytical normal-phase HPLC at the same conditions as previously described (Fig. 2ii). The disaccharide of Galβ1-3Gal resin (**30**) was subsequently treated by re-Boc, Bz capping, and one cycle of Edman degradation reactions (de-Fmoc and PITC coupling) (Sch. 7). This selectively

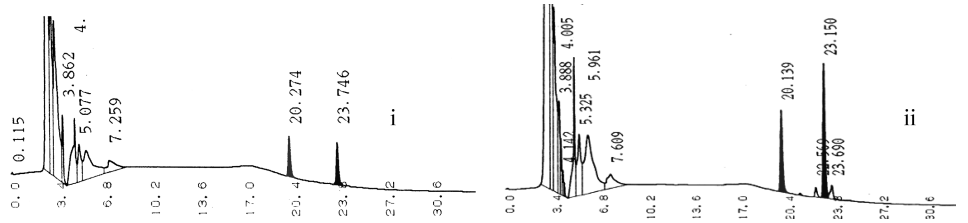
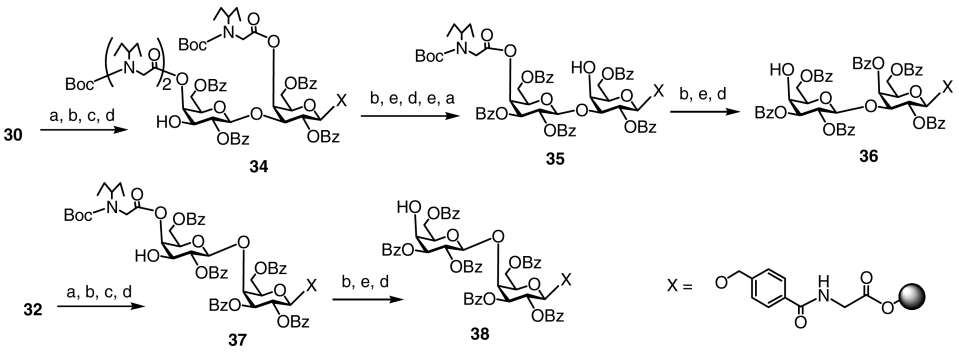


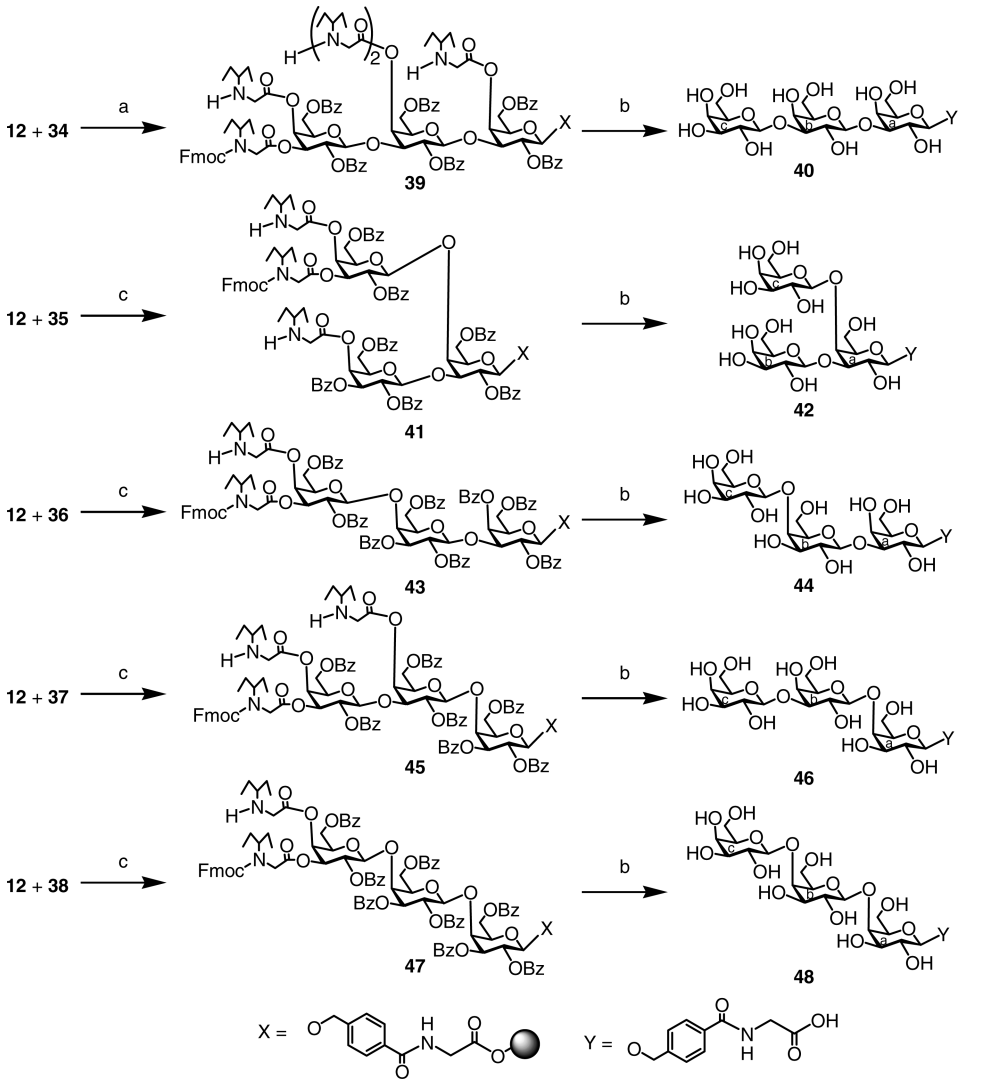
Figure 2. HPLC trace. (i) After the triple coupling of the second galactose (**13**) to the resin (**26**). The 20.3-min peak indicated un-reacted monogalactose (**23**). The 23.7-min peak indicated Galβ1-3Gal (**31**). The coupling yield was estimated to be 50% based on the peak area; (ii) After single coupling of the second galactose (**12**) to the resin (**29**). The 20.1-min peak indicated un-reacted monogalactose (**23**). The 23.2-min peak indicated Galβ1-4Gal (**33**). The coupling yield was estimated to be 53% based on the peak area. HPLC condition A was used.



Scheme 7. Deprotection of UCHP groups by Edman degradation. (a) Boc_2O, saturated aq $NaHCO_3$, DMF, rt, 8 h; (b) BzOH, DMAP, DIC, CH_2Cl_2, rt, 12 h; (c) 20% piperidine in DMF, rt, 18 min; (d) PITC, NMM, DMF, rt, 30 min; (e) 20% TFA in CH_2Cl_2, rt, 30 min, twice.

produced the digalactose acceptor with a selectively free hydroxyl group at the 3′-position (**34**) for the synthesis of **40**. For the synthesis of both **42** and **44**, the above resin (**34**) was then capped by a Bz group. It was then treated by one cycle of Edman degradation reaction (de-Boc, PITC coupling, TFA treating, and re-Boc). Consequently, the digalactose acceptor with a selectively free hydroxyl group at the 4-position (**35**) for the synthesis of **42** was selectively obtained. For the synthesis of **44**, this resin (**35**) was subsequently treated by Bz capping and one cycle of Edman degradation reactions (de-Boc and PITC coupling). As a product, the digalactose acceptor with a selectively free hydroxyl group at the 4′-position (**36**) for the synthesis of **44** was selectively obtained. When the disaccharide of Galβ1-4Gal resin (**32**) was treated with re-Boc, Bz capping, and one cycle of Edman degradation reactions (de-Fmoc and PITC coupling), the digalactose acceptor with a selectively free hydroxyl group at the 3′-position (**37**) for the synthesis of **46** was selectively obtained. In the next step of the synthesis of **48**, this resin (**37**) was treated by Bz capping and one cycle of Edman degradation reactions (de-Boc and PITC coupling). As a result, the digalactose acceptor with a selectively free hydroxyl group at the 4′-position (**38**) for the synthesis of **48** was selectively obtained. All steps were also successfully monitored by the combination of chloranil and methyl red-DIC tests.

The next step was the coupling of the third galactose (Sch. 8). For the synthesis of Galβ1-3Galβ1-3Gal (**40**), the digalactose acceptor with a selectively free hydroxyl group on 3′-postion (**34**) was coupled with a third galactose donor (**12**) by using DMTST, as in the previously described procedure. This coupling procedure was repeated two times in all. For the synthesis of Galβ1-3(Galβ1-4)Gal (**42**), Galβ1-4Galβ1-3Gal (**44**), Galβ1-3Galβ1-4Gal (**46**), and Galβ1-4Galβ1-4Gal (**48**), the digalactose acceptors with selectively free hydroxyl groups at the 4-postion (**35**), 4′-position (**36**), 3′-position (**37**), and 4′-position (**38**), respectively, were coupled with the third galactose donor (**12**) using NIS-TfOH at –30°C, and one use of the coupling procedure was sufficient



Scheme 8. Condensation of third galactose and cleavage from the resin. (a) 6.0 equiv. **12**, 18.0 equiv. DMTST, CH_2Cl_2, rt, 1 day, twice; (b) NaOMe, CH_2Cl_2, MeOH, rt, 1 day, then added H_2O and MeOH, rt, 1 day; (c) 6.0 equiv. **12**, 18.0 equiv. NIS, 18.0 equiv. TfOH, CH_2Cl_2, -30°C, 1 day. The respective retention times for **40**, **42**, **44**, **46**, and **48** were 25.4, 25.3, 25.1, 25.1, and 24.8 min. Coupling yields of the third galactose for **40**, **42**, **44**, **46**, and **48** were, respectively, 46%, 52%, 59%, 70%, and 42%. Total yields from **16** for **40**, **42**, **44**, **46**, and **48** were, respectively, 8%, 6%, 8 %, 14%, and 13%.

to reach satisfactory coupling yields. In the case of the coupling of the third galactose with the resin (**34**) for the synthesis of Galβ1-3Galβ1-3Gal (**40**), the NIS-TfOH procedure was also applicable, and a single coupling yield was almost identical to the double coupling yield of DMTST (data not shown). All trigalactose derivatives were cleaved from the resin by basic conditions, and

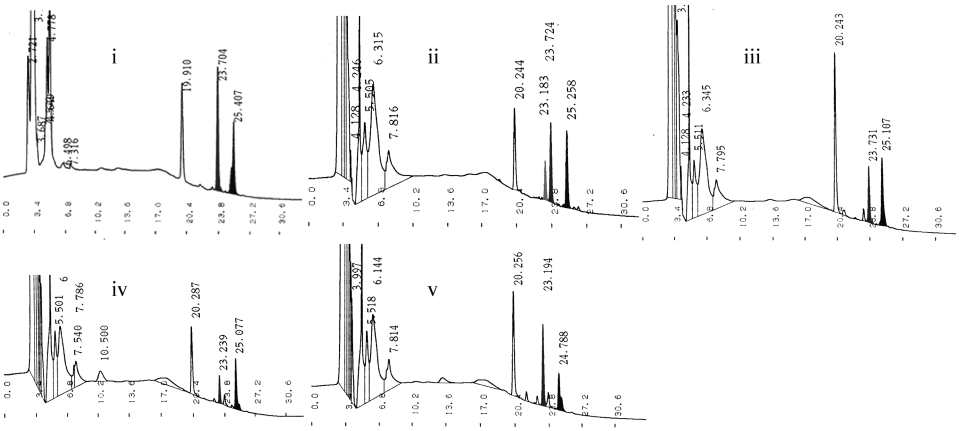


Figure 3. HPLC trace. (i) After the double coupling of the third galactose (**12**) to the resin (**34**). The 19.9-min peak indicated monogalactose (**23**). The 23.7-min peak indicated Galβ1-3Gal (**31**). The 25.4-min peak indicated Galβ1-3Galβ1-3Gal (**40**); (ii) After the single coupling of the third galactose (**12**) to the resin (**35**). The 20.2-min peak indicated monogalactose (**23**). The 23.7-min peak indicated Galβ1-3Gal (**31**). The 25.3-min peak indicated Galβ1-3(Galβ1-4)Gal (**42**). For the synthesis of the branched form, the compound that uncoupled from the second Gal formed 3-OBz-4-Boc-mono-UCHP on mono-Gal after the second Gal coupling and capping. The 4-OH mono-Gal was synthesized selectively when it was treated with the next Edman degradation for the removal of the Boc protected mono-UCHP. This was coupled with the third Gal donor to obtain Galβ1-4Gal disaccharide (**33**, 23.2-min peak); (iii) After the single coupling of the third galactose (**12**) to the resin (**36**). The 20.2-min peak indicated monogalactose (**23**). The 23.7-min peak indicated Galβ1-3Gal (**31**). The 25.1-min peak indicated Galβ1-4Galβ1-3Gal (**44**); (iv) After the single coupling of the third galactose (**12**) to the resin (**37**). The 20.3-min peak indicated monogalactose (**23**). The 23.2-min peak indicated Galβ1-4Gal (**33**). The 25.1-min peak indicated Galβ1-3Galβ1-4Gal (**46**); (v) After the single coupling of the third galactose (**12**) to the resin (**38**). The 20.3-min peak indicated monogalactose (**23**). The 23.2-min peak indicated Galβ1-4Gal (**33**). The 24.8-min peak indicated Galβ1-4Galβ1-4Gal (**48**). HPLC condition A was used.

the eluates were purified by HPLC using the same conditions as those employed for analytical HPLC. All five kinds of trigalactoses as target compounds were obtained as main peaks within subpeaks (Fig. 3). The final glycosidation yields for **40**, **42**, **44**, **46**, and **48** were determined to be 46%, 52%, 59%, 70%, and 42%, respectively. The total yields from **16** for **40**, **42**, **44**, **46**, and **48** were determined to be 8% (11 steps), 6% (16 steps), 8% (19 steps), 14% (14 steps), and 13% (17 steps), respectively. From the HMBC-NMR analysis for **40**, **42**, **44**, **46**, and **48**, all glycoside linkages were confirmed.

In summary, by using the UCHP method, a model of an oligosaccharide combinatorial library was successfully synthesized. NIS-TfOH was a more suitable coupling promoter for solid-phase oligosaccharide synthesis than DMTST, although it required a lower temperature. Through repetition of only one cycle of the Edman degradation reaction and a capping reaction, the hydroxyl groups on carbohydrate were sequentially selectively deprotected. This simple method for selective deprotection of each hydroxyl group facilitated

oligosaccharide library synthesis. To advance oligosaccharide combinatorial library synthesis even more, we will pursue research to increase the reaction yield and to establish selective α-glycosidation.

EXPERIMENTAL SECTION

General

^{1}H NMR and ^{13}C NMR spectra were obtained using a spectrometer (AVANCE 800, AVANCE 500, or AV 400, Bruker BioSpin) in $CDCl_3$ or D_2O. Chemical shifts are expressed in parts per million relative to the signal of either $CHCl_3$ or Me_4Si in $CDCl_3$ solvent, adjusted, respectively, to 7.24 or 0.00 ppm, or MeOH in D_2O solvent, adjusted to 3.30 ppm. ESI-FT-MS spectra were recorded in the positive ion mode (APEX II 70e, Bruker Daltonics). Optical rotations (P-1020-GT, Jasco Inc.) were determined in $CHCl_3$, H_2O, or MeOH at ambient temperature. Both analytical normal-phase HPLC separation and preparative normal-phase HPLC separation were performed using same HPLC system (Shimadzu Corp.) with a TSK-gel Amide-80 column (4.6 $\times$ 250 mm, 5 μm; Tosoh Co. Ltd.) at a flow rate of 1 mL/min and detection at 254 nm. For HPLC condition A, the solvent system was as follows: solvent A, TFA/water (1:1000); solvent B, TFA/acetonitrile/water (1:900:100). Initially, 100% solvent B was performed isocratically for 10 min at a flow rate of 1 mL/min. Then, a linear gradient of 0% to 67% of solvent A was applied for 20 min at a flow rate of 1 mL/min. All reagents and solvents were reagent grade.

Abbreviations used

Ac, acetyl; Ac_2O, acetic anhydride; aq, aqueous; Boc, *tert*-butoxycarbonyl; Boc_2O, di-*tert*-butyl dicarbonate; *t*Bu, *tert*-butyl; Bz, benzoyl; calcd., calculated; DIC, 1,3-diisopropylcarbodiimide; DMAP, 4-(dimethylamino)pyridine; DMF, *N*,*N*-dimethylformamide; DMTST, dimethyl(methylthio)sulfonium triflate; equiv., equivalent; Fmoc, 9*H*-fluoren-9-ylmethoxycarbonyl; Gal, D-galactose; NEM, *N*-ethylmorpholine; NIS, *N*-iodosuccinimide; NMM, *N*-methylmorpholine; PITC, phenyl isothiocyanate; pyr, pyridine; rt, room temperature; SPh, thiophenyl; TBTU, *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate; TFA, trifluoroacetic acid; TfOH, trifluoromethanesulfonic acid; THF, tetrahydrofuran; TMSOTf, trimethylsilyl trifluoromethanesulfonate; Tr, trityl.

Methyl 2-(1-Ethylpropylamino)acetate (3)

A solution of methyl bromoacetate (**2**, 28.53 mL, 0.301 mol) in THF (160 mL) was added drop-wise to a cooled solution of 3-amino pentane (**1**,

77.28 mL, 0.663 mol) in THF (230 mL) over 5 min. The solution was stirred for 2 h at rt and concentrated. The product was suspended in diethyl ether. The residue of the hydrobromide salt was removed by filtration and washed with diethyl ether; the filtrate and washings were collected and concentrated in vacuo to give **3** (44.22 g, 92%) as a colorless oil. $[\alpha]_D$+2.9 (*c* 0.10, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.90$ (t, 6H, $J_{CH3,CH} = J_{CH3,CH'} = 7.5$ Hz, $(\underline{CH_3}CH_2)_2CH$), 1.43 (m, 4H, $(CH_3\underline{CH_2})_2CH$), 2.37 (quintet, 1H, $J_{CH,CH} = J_{CH,CH'} = J_{CH,CH''} = J_{CH,CH'''} = J_{CH,NH} = 6.0$ Hz, $(CH_3CH_2)_2\underline{CH}$), 3.42 (s, 2H, $N\underline{CH_2}CO$), 3.73 (s, 3H, $COO\underline{Me}$); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 9.7 ($(\underline{CH_3}CH_2)_2CH$), 25.7 ($(CH_3\underline{CH_2})_2CH$), 48.4 ($N\underline{CH_2}CO$), 51.8 ($COO\underline{Me}$), 59.8 ($(CH_3CH_2)_2\underline{CH}$); ESI-FT-MS, calcd. for $C_8H_{18}NO_2^+$ $(M + H)^+$: 160.1332, found: 160.1293.

2-[(*tert*-Butoxycarbonyl)(1-ethylpropyl)amino]acetic Acid (5)

A solution of di-*tert*-butyl dicarbonate (96.22 g, 0.441 mol) in 1,4-dioxane (350 mL) was added drop-wise to a cooled solution of **3** (46.8 g, 0.293 mol) in saturated aq $NaHCO_3$ (350 mL) over 5 min. After stirring for 2 h at rt, ethyl acetate was added, and the mixture was washed with water and 2 N HCl, dried (Na_2SO_4), and concentrated. The residue was dissolved in methanol (1000 mL) and water (100 mL), and then 10 N NaOH was added until the mixture reached a pH of 14. After stirring for 4 h at 40°C, the unsolved material was filtered through paper and washed with methanol. The filtrate and washings were collected and concentrated. The residue was purified using column chromatography (chloroform/methanol 10:1) on silica gel to afford **5** (67.60 g, 94%, two steps). $[\alpha]_D$+1.6 (*c* 0.8, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.86$, 0.88 (each t, 6H, $J_{CH3,CH} = J_{CH3,CH'} = 7.4$, 7.5 Hz, $2\underline{CH_3}CH_2$), 1.31–1.43 (m, 4H, $2CH_3\underline{CH_2}$), 1.41, 1.43 (each s, 9H, $\underline{Me_3}O$), 3.61 (s, 2H, $N\underline{CH_2}CO$), 3.72, 3.93 (each m, 1H, $(CH_3CH_2)_2\underline{CH}$), 10.16 (broad s, 1H, COOH); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 11.0$, 11.2 ($\underline{CH_3}CH_2$), 25.8, 26.1 ($CH_3\underline{CH_2}$), 28.3, 28.5 ($\underline{Me_3}C$), 44.3, 45.4 ($N\underline{CH_2}CO$), 58.3, 60.3 ($(CH_3CH_2)_2\underline{CH}$), 79.6, 80.1 ($Me_3\underline{C}O$), 156.1, 157.5 ($N\underline{C}OO$), 175.6, 176.0 (COOH); ESI-FT-MS, calcd. for $C_{12}H_{23}NO_4Na^+$ $(M + Na)^+$: 268.15193, found: 268.14971.

2-[{2-[(*tert*-Butoxycarbonyl)(1-ethylpropyl)amino]acetyl}(1-ethylpropyl)amino]acetic Acid (7)

DIC (52.03 mL, 0.336 mol) and DMAP (4.11 g, 0.034 mol) were added to a mixture of **5** (20.61 g, 0.084 mol) and **3** (13.38 g, 0.084 mol) in CH_2Cl_2 (77.4 mL) at 0°C. The mixture was stirred for 12 h at 6°C. The product was dissolved in chloroform; the solution was washed with 0.5 N HCl and saturated aq $NaHCO_3$, dried (Na_2SO_4), and concentrated. Column chromatography (ethyl acetate/hexane 1:3) of the residue on silica gel gave roughly purified **6**. The roughly purified **6** was dissolved in methanol (508 mL) and water (50.8 mL), following which, 10 N NaOH was added to attain a pH of 14. After stirring for

2 h at 40°C, the undissolved material was filtered through paper and washed with methanol. The filtrate and washings were collected and concentrated. The residue was purified using column chromatography (chloroform/methanol 10:1) on silica gel to afford **7** (17.85 g, 57%, two steps). $[\alpha]_D$+1.0 (*c* 0.7, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.82–0.97 (m, 12H, 2($\underline{CH_3}CH_2)_2CH$), 1.29–1.62 (m, 8H, 2($CH_3\underline{CH_2})_2CH$), 1.41, 1.47 (each s, 9H, $\underline{Me_3}C$), 3.43, 3.56, 3.71, 3.98 (4m, 2H, 2($CH_3CH_2)_2\underline{CH}$), 3.86, 3.88, 3.91, 3.94 (4s, 4H, 2N$\underline{CH_2}$CO); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 11.1, 11.1, 11.4 (2($\underline{CH_3}CH_2)_2CH$), 25.7, 26.1, 26.2, 26.3 (2($CH_3\underline{CH_2})_2CH$), 28.2, 28.5 ($\underline{Me_3}C$), 43.7, 43.9, 44.0, 44.3 (2N$\underline{CH_2}$CO), 58.3, 60.4, 60.7, 61.1 (2($CH_3CH_2)_2\underline{CH}$), 79.8, 80.2 ($Me_3\underline{C}$), 156.0, 156.6 (2N$\underline{C}$O), 171.8, 172.1 ($\underline{C}$OOH); ESI-FT-MS, calcd. for $C_{19}H_{36}N_2O_5Na^+$ $(M + Na)^+$: 395.2516, found: 395.2518.

Phenyl 2,6-Di-*O*-benzoyl-3-*O*-{2-[(1-ethylpropyl)(9*H*-fluoren-9-ylmethoxycarbonyl)amino]acetyl}-1-thio-β-D-galactopyranoside (11)

DIC (4.3 mL, 27.5 mmol) and DMAP (305 mg, 2.5 mmol) were added to a solution of phenyl 2,6-di-*O*-benzoyl-1-thio-β-D-galactopyranoside (**9**, 12.0 g, 25.0 mmol) in CH_2Cl_2 (240 mL) and cooled to –40°C. A solution of 2-[(1-ethylpropyl)(9*H*-fluoren-9-ylmethoxycarbonyl)amino]acetic acid[3,4] (**10**, 11.0 g, 29.9 mmol) in CH_2Cl_2 (100 mL) was added to the mixture at –40°C, and the mixture was stirred for 2 days at –40°C. After methanol (100 mL) was added to stop the reaction, the solution was concentrated. Column chromatography (ethyl acetate/toluene 1:10) of the residue on silica gel gave **11** (19.28 g, 93%). $[\alpha]_D$+20.2 (*c* 1.3, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.56–0.66 (m, 6H, 2$\underline{CH_3}CH_2$), 0.89–1.29 (m, 4H, 2$CH_3\underline{CH_2}$), 3.48–3.56 (m, 1H, ($CH_3CH_2)_2\underline{CH}$), 3.64 (d, 2H, J_{gem} = 2.2 Hz, N$\underline{CH_2}$CO), 4.02 (t, 1H, $J_{5,6} = J_{5,6'}$ = 6.2 Hz, H-5), 4.22 (t, 1H, $J_{9,CH} = J_{9,CH'}$ = 6.0 Hz, H-9 Fmoc), 4.31, 4.35, 4.47, 4.53 (4dd, 2H, $J_{9,CH2}$ = 5.9, 6.7, 6.1, 5.8 Hz, J_{gem} = 10.9, 11.1, 10.6, 10.6 Hz, CH_2 Fmoc), 4.40 (m, 1H, H-4), 4.66 (d, 2H, $J_{5,6}$ = 6.1 Hz, H-6), 4.84, 4.86 (2d, 1H, $J_{1,2}$ = 10.1, 10.1 Hz, H-1), 5.12, 5.21 (2dd, 1H, $J_{2,3}$ = 9.8, 9.7 Hz, $J_{3,4}$ = 3.2, 3.2 Hz, H-3), 5.51, 5.67 (2t, 1H, $J_{1,2} = J_{2,3}$ = 9.9 Hz, H-2), 7.04–8.06 (m, 23H, Ar Fmoc, 2$\underline{Ph}$CO, and $\underline{Ph}$S); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 10.7, 10.7 (2$\underline{CH_3}CH_2$), 25.6, 25.9 (2$CH_3\underline{CH_2}$), 44.4 (N$\underline{CH_2}$CO), 47.2, 47.4 (C-9 Fmoc), 59.3, 59.9 (($CH_3CH_2)_2\underline{CH}$), 64.0 (C-6), 66.3 (C-4), 67.6, 67.7 (CH_2 Fmoc), 67.9, 68.2 (C-2), 76.0 (C-3), 76.2 (C-5), 86.7, 87.2 (C-1), 119.9, 119.9, 124.8, 125.0, 125.3, 126.9, 127.1, 127.2, 127.6, 127.7, 128.1, 128.2, 128.4, 128.5, 128.8, 128.9, 129.0, 129.6, 129.8, 129.9, 131.8, 132.5, 133.2, 133.3, 133.4, 133.7 ($C_{1\text{-}8}$-H Fmoc, 2$\underline{Ph}$COO, and $\underline{Ph}$S), 141.4, 141.5 (C_{4a} and C_{4b} Fmoc), 143.7, 143.9 (C_{8a} and C_{9a} Fmoc), 157.8 (N$\underline{C}$OO), 165.1 (Ph$\underline{C}$OO at C-2), 166.3 (Ph$\underline{C}$OO at C-6), 169.0 ($NCH_2\underline{C}$OO); ESI-FT-MS, calcd. for $C_{48}H_{47}NO_{10}SNa^+$ $(M + Na)^+$: 852.2813, found: 852.2815.

Phenyl 2,6-Di-*O*-benzoyl-4-*O*-{2-[(*tert*-butoxycarbonyl)(1-ethylpropyl)amino]acetyl}-3-*O*-{2-[(1-ethylpropyl)(9*H*-fluoren-9-ylmethoxycarbonyl)amino]acetyl}-1-thio-β-D-galactopyranoside (12)

DIC (3.77 mL, 24.1 mmol) and DMAP (147 mg, 1.2 mmol) were added to a mixture of **11** (10 g, 12.0 mmol) and **5** (3.5 g, 14.3 mmol) in CH_2Cl_2 (50 mL) at 0°C, and the mixture was stirred for 2 h at 0°C. After methanol (50 mL) was added to stop the reaction, the solution was concentrated. Column chromatography (ethyl acetate/hexane 1:4) of the residue on silica gel gave **12** (12.60 g, 99%). $[\alpha]_D$+23.1 (*c* 1.6, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.50–0.96 (m, 12H, 4$\underline{CH_3}CH_2$), 1.09–1.55 (m, 8H, 4$CH_3\underline{CH_2}$), 1.40, 1.42, 1.43, 1.45, (4s, 9H, Boc), 3.50–4.03 (m, 2H, 2$(CH_3CH_2)_2\underline{CH}$), 3.52–4.02 (m, 4H, 2N$\underline{CH_2}$COO), 3.87, 4.12 (each m, 1H, H-9 Fmoc), 4.04, 4.25 (each m, 2H, CH_2 Fmoc), 4.30–4.60 (m, 2H, H-6), 4.90 (m, 1H, H-1), 5.34, 5.44 (m, 1H, H-3), 5.51–5.60 (m, 1H, H-2), 5.62, 5.68 (each d, 1H, $J_{3,4}$ 3.1, 3.2 Hz, H-4), 7.04–8.04 (m, 23H, Ar Fmoc, 2$\underline{Ph}$CO, and $\underline{Ph}$S); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.1, 11.1, 11.2, 11.2, 11.3, 11.3 (4$\underline{CH_3}CH_2$), 25.4, 25.5, 25.7, 25.8, 25.9, 26.0, 26.0, 26.3, 26.3, 26.4, 26.6 (4$CH_3\underline{CH_2}$), 28.3, 28.3, 28.3, 28.4, ($\underline{Me_3}$CO), 43.1, 43.2, 43.5 (2N$\underline{CH_2}$COO), 46.9, 47.2 (C-9 Fmoc), 58.3, 58.5, 59.5, 59.6, 59.8, 59.9, 60.0 (2$(CH_3CH_2)_2\underline{CH}$), 62.1, 62.2, 62.3 (C-6), 67.1, 67.2, 68.4 (CH_2 Fmoc), 67.7, 67.8, 67.9, 68.0 (C-2 and C-4), 72.3, 72.5, 72.8 (C-3), 74.7, 74.7, 74.8 (C-5), 80.0, 80.0, 80.2, 80.2 ($Me_3\underline{C}$O), 87.3, 87.3 (C-1), 119.7, 119.9, 119.9, 124.9, 124.9, 125.1, 125.2, 126.9, 126.9, 127.0, 127.4, 127.5, 127.6, 127.6, 127.9, 128.0, 128.1, 128.2, 128.2, 128.3, 128.4, 128.5, 128.7, 128.8, 128.9, 129.1, 129.3, 129.3, 129.4, 129.5, 129.5, 129.6, 129.8, 129.8, 129.9, 130.0, 132.2, 132.3, 132.5, 132.6, 132.7, 133.0, 133.2, 133.2, 133.2, 133.4, 133.5, 141.1, 141.2, 141.3, 141.4, 143.9, 144.0, 144.1, 144.1, 144.2, 144.3 (Ar Fmoc, 2$\underline{Ph}$COO, and $\underline{Ph}$S), 155.7, 156.2, 156.2, 156.6, 156.7 (2N$\underline{C}$OO), 165.0, 165.4 (Ph$\underline{C}$OO at C-2), 165.9, 166.0 (Ph$\underline{C}$OO at C-6), 169.2, 169.4, 169.5, 169.6, 169.6, 170.0 (2NCH$_2\underline{C}$OO); ESI-FT-MS, calcd. for $C_{60}H_{68}N_2O_{13}SNa^+$ $(M + Na)^+$: 1079.4334, found: 1079.4331.

Phenyl 2,6-Di-*O*-benzoyl-4-*O*-{2-[{2-[(*tert*-butoxycarbonyl)(1-ethylpropyl)amino]acetyl}(1-ethylpropyl)amino]acetyl}-3-*O*-{2-[(1-ethylpropyl)(9*H*-fluoren-9-ylmethoxycarbonyl)amino]acetyl}-1-thio-β-D-galactopyranoside (13)

DIC (1.89 mL, 12.1 mmol) and DMAP (74 mg, 0.6 mmol) were added to a mixture of **11** (5 g, 6.0 mmol) and **7** (2.7 g, 7.2 mmol) in CH_2Cl_2 (25 mL) at 0°C. The mixture was stirred for 2 h at 0°C. After methanol (25 mL) was added to stop the reaction, the solution was concentrated. Column chromatography

(ethyl acetate/toluene 1:10) of the residue on silica gel gave **13** (6.79 g, 95%). $[\alpha]_D$+25.2 (*c* 1.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.65–1.07 (m, 18H, 6$\underline{CH_3}CH_2$), 1.10–1.62 (m, 12H, 6$CH_3\underline{CH_2}$), 1.39, 1.40, 1.43 (each s, 9H, Boc), 3.43–3.97 (m, 3H, 3$(CH_3CH_2)_2\underline{CH}$), 3.56–4.00 (m, 6H, 3N$\underline{CH_2}$CO), 3.89–4.12 (each m, 1H, H-9 Fmoc), 4.03–4.31 (m, 2H, CH_2 Fmoc), 4.15 (m, 1H, H-5), 4.46–4.66 (m, 2H, H-6), 4.84–4.94 (m, 1H, H-1), 5.31–5.37 (m, 1H, H-3), 5.44, 5.63 (each d, 1H, $J_{3,4}$ = 3.0, 3.2 Hz, H-4), 5.48–5.61 (m, 1H, H-2), 6.98–8.06 (m, 23H, Ar Fmoc, 2$\underline{Ph}$CO, and $\underline{Ph}$S); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 10.8, 10.9, 11.2, 11.2, 11.4, 11.5 (6$\underline{CH_3}CH_2$), 25.5, 25.6, 25.7, 25.8, 26.2, 26.5, 26.6, 26.7 (6$CH_3\underline{CH_2}$), 28.3, 28.5, ($\underline{Me_3}$CO), 42.5, 42.8, 43.0, 43.3, 43.5, 43.7 (3N$\underline{CH_2}$CO), 47.0, 47.2 (C-9 Fmoc), 58.3, 59.6, 59.9, 60.0, 60.1, 60.3, 60.7 (3$(CH_3CH_2)_2\underline{CH}$), 62.6, 62.8 (C-6), 67.1, 67.8 (CH_2 Fmoc), 68.0, 68.1, 68.3 (C-2 and C-4), 72.2, 72.3 (C-3), 75.1, 75.4 (C-5), 79.5, 79.6 ($Me_3\underline{CO}$), 87.1, 87.3 (C-1), 119.9, 124.9, 125.1, 125.3, 126.9, 127.0, 127.6, 127.8, 127.9, 128.2, 128.3, 128.4, 128.7, 128.8, 128.9, 129.0, 129.4, 129.6, 129.7, 129.8, 129.9, 130.0, 132.2, 132.2, 132.3, 133.2, 141.3, 144.0, 144.1, 144.1, 144.3 (Ar Fmoc, 2$\underline{Ph}$COO, and $\underline{Ph}$S), 156.1, 156.2, 156.3, 156.5, 156.6, 156.6, 156.8 (2N$\underline{C}$OO), 165.1 (Ph$\underline{C}$OO at C-2), 166.1 (Ph$\underline{C}$OO at C-6), 168.6, 168.9, 168.9, 169.0, 169.1, 169.3 (2N$CH_2\underline{C}$OO); ESI-FT-MS, calcd. for $C_{67}H_{81}N_3O_{14}SNa^+$ $(M + Na)^+$: 1206.5332, found: 1206.5334.

[4-(Carboxymethylcarbamoyl)phenyl]methyl *O*-(β-D-Galactopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→3)-β-D-galactopyranoside (40)

The HMBA-AM resin (**14**, 1.0 g, 1.16 mmol/g; Novabiochem, Merck Ltd.) was packed into a 20-mL disposable chromatography column (Econo-Pac, Bio-Rad Laboratories Inc., Hercules, CA). The column was connected to a suction flask using a Teflon tube with a manual two-way Teflon valve; excess reagents, DMF, etc., were removed by applying a vacuum. The resin was derivatized with the HMBA-Gly linker via the following procedure. First, the resin was swollen with dichloromethane (12 mL). *N*-α-Fmoc-glycine (**15**, 1.03 g, 3.46 mmol), DMAP (14 mg, 0.115 mmol), and DIC (545 μL, 3.48 mmol) were then added and the mixture was agitated. After 1 day, the solution was removed, and the resin was washed thoroughly with MeOH (5 × 10 mL) and dichloromethane (5 × 10 mL). Subsequently, dichloromethane (8 mL), acetic anhydride (4 mL), pyridine (2 mL), and DMAP (14 mg, 0.115 mmol) were added to the resin and the mixture was again agitated. After 1 day, the solution was removed; the resin was then washed thoroughly with MeOH (5 × 10 mL), DMF (5 × 10 mL), dichloromethane (5 × 10 mL), and diethyl ether (3 × 10 mL), and dried in vacuo. The loading was determined as 0.8447 mmol/g from UV absorbance of the eluate of the Fmoc group.[10] The Fmoc group was cleaved by 20% piperidine in DMF (12 mL) for 18 min. Then 4-(trityloxymethyl)benzoic acid[3] (**18**, Tr-HMBA, 1.4 g, 3.549 mmol), NEM (886 μL, 6.962 mmol), and TBTU (1.08 g,

3.364 mmol) were dissolved in DMF. After 5 min, the mixture was added to the resin and agitated. After 4 h, the solution was removed. The resin was washed with DMF (6 × 10 mL) and a solution of acetic anhydride/DMF (2 mL/14 mL) was added and the mixture was agitated. After 20 min, the resin was washed thoroughly with DMF (5 × 10 mL) and dichloromethane (5 × 10 mL). Deprotection of the trityl group from the resin was performed using dichloromethane (6 mL) and TFA (6 mL) at rt. After agitation for 5 min, the resin was washed thoroughly with dichloromethane (1 × 10 mL). This trityl deprotection procedure was repeated six times in all. The resin was then washed thoroughly with DMF (5 × 10 mL), dichloromethane (5 × 10 mL), and diethyl ether (3 × 10 mL) and dried in vacuo. Consequently, hydroxy functionalized HMBA-Gly-HMBA-AM resin (**20**) was obtained (theoretical hydroxyl group loading was 0.9126 mmol/g).

The hydroxy functionalized HMBA-Gly-HMBA-AM resin (**20**, 35 mg, 0.032 mmol, 0.9126 mmol/g) was packed into a disposable chromatography column (mini column, SARSTEDT AG & Co.) with a rubber septum for inert atmosphere. This hydroxy functionalized resin was glycosylated with a galactose donor (**12**, 200 mg, 0.189 mmol) using DMTST (147 mg, 0.569 mmol) in dichloromethane (4 mL) at rt. After agitation for 1 day at rt, the solution was removed. The resin was washed thoroughly with DMF (5 × 10 mL) and dichloromethane (5 × 10 mL). To determine the coupling yield, aliquots of the resin were taken and treated with a solution of dichloromethane/25 wt% NaOMe in MeOH (200 μL/30 μL) for 1 h. Subsequently, with added water/MeOH (30 μL/200 μL), the reaction mixture was agitated for an additional 1 h. The resin was then filtered and neutralized with acetic acid, and the eluate was analyzed using normal-phase HPLC condition A. The peak of monoglycosylated compound **23** was detected at 20.3 min, and the glycosylation yield was determined as 60%, as calculated from the area under the peaks. Unfortunately, the Boc group was removed during the coupling condition. The exposed amino group was protected by the Boc group again (re-Boc) using DMF (2 mL), Boc_2O (100 mg, 0.458 mmol), and saturated aq $NaHCO_3$ (100 μL). After agitation for 8 h, the solution was removed; the resin was washed thoroughly with aq 50% DMF (5 × 2 mL), DMF (5 × 2 mL), dichloromethane (5 × 2 mL), and diethyl ether (3 × 2 mL). Then it was dried in vacuo. The chloranil colorimetric test to detect secondary amines was used to detect the reaction completion. To protect the unreacted hydroxyl group, benzoylation was performed (Bz capping) with benzoic acid (40 mg, 0.328 mmol), DIC (100 μL, 0.646 mmol), and DMAP (10 mg, 0.082 mmol) in CH_2Cl_2 (2.4 mL). After agitation for 12 h, the solution was removed. The resin was washed thoroughly using aq 50% DMF (5 × 2 mL), DMF (5 × 2 mL), dichloromethane (5 × 2 mL), and diethyl ether (3 × 2 mL). Then it was dried in vacuo. The newly developed methyl red-DIC colorimetric test for detecting hydroxyl groups was used to detect the reaction completion. It is very important to protect the unreacted hydroxyl group

completely; otherwise, the synthesized oligosaccharide will be obtained as a complicated mixture. The next step was Edman degradation to selectively deprotect the hydroxyl group at the 3-position of Gal. Selective deprotection of the monomeric-UCHP protected with Fmoc group by one cycle of Edman degradation reaction (de-Fmoc and PITC coupling) was performed: (1) De-Fmoc step: Deprotection of the Fmoc group was performed using 20% piperidine/DMF (4 mL) at rt. After agitation for 18 min, the solution was removed, and the resin was washed thoroughly with DMF (6 × 10 mL). The chloranil test changed to positive because of exposure of the amino group. (2) PITC coupling step: PITC coupling reaction was performed using PITC (1 mL) and NMM (240 μL) in DMF (2 mL). After agitation for 30 min, the solution was removed; the resin was washed thoroughly with DMF (5 × 10 mL), dichloromethane (5 × 2 mL), and diethyl ether (3 × 2 mL). Then it was dried in vacuo. At this time, the results of the chloranil test changed to negative because of PITC coupling to the amino group. Furthermore, the methyl red-DIC test gave a positive result due to the existence of a free hydroxyl group. As a result of this reaction, a Gal acceptor with a selectively free hydroxyl group at the 3-position (**26**) was synthesized selectively.

The next step was coupling with a second galactose. The previous resultant 3-OH resin (**26**) was glycosylated with a galactose donor (**13**, 224 mg, 0.1891 mmol) using DMTST (110 mg, 0.425 mmol) in dichloromethane (3 mL) at rt. After agitation for 1 day at rt, the solution was removed. The resin was washed thoroughly with DMF (5 × 10 mL) and dichloromethane (5 × 10 mL). Galβ1-3Gal (**31**) was detected at 23.7 min, and the glycosylation yield was determined to be 17% by analytical HPLC as described previously. To increase the coupling yield, the same coupling reaction was repeated three times in all: the double coupling yield was estimated as 34%; the triple coupling yield was estimated as 50%. Next, the exposed amino group on digalactose (**30**, di-Gal) was protected by the Boc group (re-Boc) and the unreacted hydroxyl group on monogalactose (**26**, mono-Gal) was capped by benzoyl group (Bz capping). Again, to deprotect monomeric-UCHP protected with Fmoc group, one cycle of Edman degradation (de-Fmoc and PITC coupling) was performed to selectively synthesize the di-Gal acceptor with a selectively free hydroxyl group at the 3′-position (**34**).

The last step was coupling with a third galactose. The previously resultant 3′-OH resin (**34**) was glycosylated with a galactose donor (**12**, 200 mg, 0.189 mmol) using DMTST (147 mg, 0.569 mmol) in dichloromethane (4 mL) at rt. After agitation for 1 day at rt, the solution was removed; the resin was washed thoroughly with DMF (5 × 10 mL), dichloromethane (5 × 10 mL), and diethyl ether (3 × 2 mL). Then it was dried in vacuo. The coupling reaction was repeated once to increase the coupling yield. The resin was treated with a solution of dichloromethane/MeOH/25 wt% NaOMe in MeOH (1 mL/100 μL/50 μL) for 1 day to cleave off the target compound from the resin. Water/MeOH

(300 μL/1 mL) were then added, and the reaction mixture was agitated for an additional 1 day. Subsequently, the mixture was neutralized with acetic acid and the resin filtered, which was washed with MeOH and water. The filtrate and washings were concentrated, and the residue was analyzed and purified using normal-phase HPLC condition A. The target trigalactose Galβ1-3Galβ1-3Gal (**40**, 2.03 mg, retention time of 25.4 min) was synthesized and purified. The glycosylation yield was determined to be 46%, which was calculated from the area under the peaks. The total yield was 8% (11 steps from **16**). At the same time, the monogalactose (**23**, 2.9 mg, retention time of 19.9 min) and digalactose Galβ1-3Gal (**31**, 1.99 mg, retention time of 23.7 min) were also purified.

Compound **40** (Galβ1-3Galβ1-3Galβ1-linker); $[\alpha]_D$+1.5 (*c* 0.07, MeOH); ^{1}H NMR (500 MHz, D_2O): $\delta = 3.50$ (dd, 1H, $J_{1,2} = 7.6$ Hz, $J_{2,3} = 9.9$ Hz, H-2c), 3.56 (dd, 1H, $J_{2,3} = 10.1$ Hz, $J_{3,4} = 3.3$ Hz, H-3c), 3.55–3.62 (m, 3H, H-5a, H-5b, and H-5c), 3.55–3.70 (m, 6H, H-6a, H-6b, and H-6c), 3.64 (m, 1H, H-2a), 3.66 (m, 1H, H-2b), 3.67–3.73 (m, 2H, H-3a and H-3b), 3.81 (d, 1H, $J_{3,4} = 3.2$ Hz, H-4c), 4.07 (s, 2H, N$\underline{CH_2}$CO), 4.09 (d, 2H, $J_{3,4} = 3.1$ Hz, H-4a and H-4b), 4.43 (d, 1H, $J_{1,2} = 7.9$ Hz H-1a), 4.51 (d, 1H, $J_{1,2} = 7.6$ Hz, H-1c), 4.56 (d, 1H, $J_{1,2} = 7.7$ Hz, H-1b), 4.74, 4.92 (each d, 2H, $J_{gem} = 12.3$ Hz, C_6H_4-$\underline{CH_2}$), 7.49, 7.74 (each d, 4H, Ar); ^{13}C NMR (125 MHz, D_2O): $\delta = 41.8$ (N$\underline{CH_2}$CO), 60.8, 60.9 (C-6a, C-6b, and C-6c), 68.3, 68.4, 68.5 (C-4a, C-4b, and C-4c), 69.9 (C-2a), 70.2 (C-2b), 70.5 (C_6H_4-$\underline{CH_2}$), 71.0 (C-2c), 72.5 (C-3c), 74.6, 74.8, 75.0 (C-5a, C-5b, and C-5c), 82.0 (C-3b), 82.2 (C-3a), 101.5 (C-1a), 104.0 (C-1b), 104.3 (C-1c), 127.5, 128.6 (C_{Ar}-H), 132.6, 141.1 (C_{Ar}), 170.8 (CONH), 173.9 (COOH); HMBC NMR (500 MHz, D_2O): H-1a $\rightarrow$ C_6H_4-$\underline{CH_2}$, H-1b $\rightarrow$ C-3a, H-1c $\rightarrow$ C-3b; ESI-FT-MS, calcd. for $C_{28}H_{40}NO_{19}^-$ $(M - H)^-$: 694.2200, found: 694.2203.

Compound **31** (Galβ1-3Galβ1-linker); $[\alpha]_D$ –0.7 (*c* 0.1, MeOH); ^{1}H NMR (500 MHz, D_2O): $\delta = 3.50$ (dd, 1H, $J_{1,2} = 7.6$ Hz, $J_{2,3} = 9.9$ Hz, H-2b), 3.55 (dd, 1H, $J_{2,3} = 10.2$ Hz, $J_{3,4} = 3.3$ Hz, H-3b), 3.55–3.62 (m, 2H, H-5a and H-5b), 3.59–3.65 (m, 1H, H-2a), 3.60–3.71 (m, 4H, H-6a and H-6b), 3.69 (m, 1H, H-3a), 3.81 (d, 1H, $J_{3,4} = 3.3$ Hz, H-4b), 4.06 (s, 2H, N$\underline{CH_2}$CO), 4.09 (d, 1H, $J_{3,4} = 3.2$ Hz, H-4a), 4.43 (d, 1H, $J_{1,2} = 7.7$ Hz, H-1a), 4.49 (d, 1H, $J_{1,2} = 7.6$ Hz, H-1b), 4.74, 4.92 (each d, 2H, $J_{gem} = 12.3$ Hz, C_6H_4-$\underline{CH_2}$), 7.49, 7.74 (each d, 4H, Ar); ^{13}C NMR (125 MHz, D_2O): $\delta = 41.8$ (N$\underline{CH_2}$CO), 60.8, 60.9 (C-6a and C-6b), 68.4 (C-4a), 68.5 (C-4b), 69.9 (C-2a), 70.5 (C_6H_4-$\underline{CH_2}$), 71.0 (C-2b), 72.4 (C-3b), 74.8, 75.0 (C-5a and C-5b), 82.3 (C-3a), 101.5 (C-1a), 104.3 (C-1b), 127.5, 128.6 (C_{Ar}-H), 132.6, 141.1 (C_{Ar}), 170.8 (CONH), 173.9 (COOH); HMBC NMR (500 MHz, D_2O): H-1a $\rightarrow$ C_6H_4-$\underline{CH_2}$, H-1b $\rightarrow$ C-3a; ESI-FT-MS, calcd. for $C_{22}H_{30}NO_{14}^-$ $(M - H)^-$: 532.1672, found: 532.1668.

Compound **23** (Galβ1-linker); $[\alpha]_D$ –6.5 (*c* 0.2, MeOH); ^{1}H NMR (400 MHz, D_2O): $\delta = 3.50$ (dd, 1H, $J_{1,2} = 7.5$ Hz, $J_{2,3} = 9.9$ Hz, H-2), 3.56 (dd, 1H, $J_{2,3} = 9.9$ Hz, $J_{3,4} = 3.3$ Hz, H-3), 3.61 (dddd, 1H, $J_{4,5} = 0.9$ Hz, $J_{5,6} = 4.4$ Hz, $J_{5,6'} = 7.8$ Hz, H-5), 3.68 (dd, 1H, $J_{5,6} = 4.4$ Hz, $J_{gem} = 11.7$ Hz, H-6), 3.74 (dd, 1H,

$J_{5,6'} = 7.8$ Hz, $J_{gem} = 11.7$ Hz, H-6′), 3.86 (dd, 1H, $J_{3,4} = 3.3$ Hz, $J_{4,5} = 0.8$ Hz, H-4), 4.00 (s, 2H, NCH$_2$CO), 4.41 (d, 1H, $J_{1,2} = 7.5$ Hz, H-1), 4.77, 4.94 (each d, 2H, $J_{gem} = 12.3$ Hz, C$_6$H$_4$-CH$_2$), 7.52, 7.77 (each d, 4H, $J_{o,m} = 8.3$ Hz, Ar); ^{13}C NMR (200 MHz, D$_2$O): $\delta = 42.7$ (NCH$_2$CO), 60.9 (C-6), 68.6 (C-4), 70.6 (C$_6$H$_4$-CH$_2$), 70.7 (C-2), 72.7 (C-3), 75.2 (C-5), 101.9 (C-1), 127.4, 128.5 (C$_{Ar}$-H), 132.9, 141.0 (C$_{Ar}$), 170.5 (NCO), 175.3 (COOH); HMBC NMR (800 MHz, D$_2$O): H-1a → C$_6$H$_4$-CH$_2$; ESI-FT-MS, calcd. for $C_{16}H_{21}NO_9Na^+$ $(M + Na)^+$: 394.1109, found: 394.1108.

[4-(Carboxymethylcarbamoyl)phenyl]methyl *O*-(β-D-Galactopyranosyl)-(1→3)-*O*-[(β-D-galactopyranosyl)-(1→3)]-β-D-galactopyranoside (42)

To synthesize compound **42**, similar to the synthesis of compound **40**, the hydroxy functionalized HMBA-Gly-HMBA-AM resin (**20**, 35 mg, 0.032 mmol, 0.9126 mmol/g) was used. The reaction conditions were identical up to the synthesis of the di-Gal acceptor with a selectively free hydroxyl group at the 3′-position (**34**). To cap the hydroxyl group at the 3′-position, benzoylation was performed (Bz capping) with benzoic acid (40 mg, 0.328 mmol), DIC (100 μL, 0.646 mmol), and DMAP (10 mg, 0.082 mmol) in CH_2Cl_2 (2.4 mL). After agitation for 12 h, the solution was removed, and the resin was washed thoroughly with aq 50% DMF (5 × 2 mL), DMF (5 × 2 mL), dichloromethane (5 × 2 mL), and diethyl ether (3 × 2 mL). Then it was dried in vacuo. The methyl red-DIC test was used to detect the reaction completion. The next step was Edman degradation for selective deprotection of the hydroxyl group at the 4-position of di-Gal. To selectively deprotect the monomeric-UCHP protected with Boc group and to simultaneously decrease the polymerization degree of the dimeric-UCHP protected with Boc group to the monomeric-UCHP protected with Boc group, one cycle of Edman degradation was performed: (1) De-Boc: Deprotection of the Boc group was performed using 20% TFA/CH_2Cl_2 (3 mL) at rt. After agitation for 30 min, the solution was removed; the reaction was then repeated once more. Next, the resin was washed thoroughly with dichloromethane (5 × 2 mL) and DMF (5 × 10 mL). The chloranil test changed to positive because of the exposed amino group. (2) PITC coupling step: The PITC coupling reaction was performed similarly to the method previously described, using PITC (1 mL) and NMM (240 μL) in DMF (2 mL). After agitation for 30 min, the solution was removed, and the resin was washed thoroughly with DMF (5 × 10 mL), dichloromethane (5 × 2 mL), and diethyl ether (3 × 2 mL). Then it was dried in vacuo. At this time, the chloranil test changed to negative because of the coupling with PITC to the amino group. As a result of this reaction, the 4-position was selectively deprotected. (3) TFA treatment step: To decrease the polymerization degree of dimeric-UCHP at the 4′-position to monomeric-UCHP, *N*-terminal phenyl thiocarbamoyl mono-amino

acid derivative was selectively cleaved using 20% TFA/CH_2Cl_2 (3 mL) at rt, with identical reaction conditions to those of "de-Boc." After agitation for 30 min, the solution was removed; then the reaction was repeated once more. Next, the resin was washed thoroughly using dichloromethane (5 × 2 mL) and DMF (5 × 10 mL). The chloranil test changed to positive because of the exposure of the amino group on monomeric-UCHP at the 4′-position. (4) Re-Boc step: To protect the newly exposed amino group by the Boc group, the resin was treated with DMF (2 mL), Boc_2O (100 mg, 0.458 mmol), and saturated aq $NaHCO_3$ (100 μL), similarly to the method described previously. After agitation for 8 h, the solution was removed. The resin was washed thoroughly with aq 50% DMF (5 × 2 mL), DMF (5 × 2 mL), dichloromethane (5 × 2 mL), and diethyl ether (3 × 2 mL). It was then dried in vacuo. At this time, the chloranil test changed to negative because the exposed amino group was protected by the Boc group. Furthermore, the methyl red-DIC test gave a positive result due to the free hydroxyl group at the 4-position. As a result of this reaction, a di-Gal acceptor with a selectively free hydroxyl group at the 4-position (**35**) was synthesized selectively.

The next step was coupling with a third galactose. To the above product (**35**), a galactose donor (**12**, 200 mg, 0.189 mmol), NIS (128 mg, 0.569 mmol), and dichloromethane (500 μL) were added. The mixture was then cooled at –30°C and TfOH (50 μL, 0.565 mmol) was added. After agitation for 1 day at –30°C, the solution was removed, and the resin was washed thoroughly with DMF (5 × 10 mL) and dichloromethane (5 × 10 mL). To cleave off the target compound from the resin, the resin was treated with a solution of dichloromethane/MeOH/25 wt% NaOMe in MeOH (1 mL/100 μL/50 μL) for 1 day. Subsequently, with added water/MeOH (300 μL/1 mL), the reaction mixture was agitated for another 1 day. Next, it was neutralized with acetic acid and the reaction mixture was filtered. The resin was washed with MeOH and water; the combined filtrate and washings were concentrated. The residue was analyzed and purified using normal-phase HPLC condition A. The target trigalactose Galβ1-3(Galβ1-4)Galβ1-linker (**42**, 1.41 mg, retention time of 25.3 min) was synthesized and purified. The glycosylation yield was determined to be 52%, which was calculated from the area under the peaks. The total yield was 6% (16 steps from **16**). At the same time, the monogalactose Galβ1-linker (**23**, 1.2 mg, retention time of 20.2 min) and digalactose Galβ1-3Galβ1-linker (**31**, 0.84 mg, retention time of 23.7 min) were also purified.

Compound **42** {Galβ1-3(Galβ1-4)Galβ1-linker}; $[\alpha]_D$+0.5 (*c* 0.2, MeOH); ^{1}H NMR (800 MHz, D_2O): δ = 3.43 (dd, 1H, $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 9.9 Hz, H-2c), 3.49 (dd, 1H, $J_{1,2}$ = 7.8 Hz, $J_{2,3}$ = 9.9 Hz, H-2b), 3.54–3.58 (m, 2H, H-3b and H-3c), 3.53–3.62 (m, 3H, H-5a, H-5b, and H-5c), 3.62–3.74 (m, 6H, H-6a, H-6b, and H-6c), 3.75 (m, 2H, H-2a and H-3a), 3.78, 3.80 (each d, 2H, $J_{3,4}$ = 3.4, 3.4 Hz, H-4b and H-4c), 4.07 (s, 2H, N$\underline{CH_2}$CO), 4.29 (d, 1H, $J_{3,4}$ = 1.8 Hz, H-4a), 4.44 (d, 1H, $J_{1,2,}$ = 7.7 Hz, H-1a), 4.48 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1b), 4.71 (H-1c), 4.72,

4.89 (each d, 2H, J_{gem} = 12.3 Hz, C_6H_4-$\underline{CH_2}$), 7.47, 7.73 (each d, 4H, Ar); ^{13}C NMR (200 MHz, D_2O): δ = 41.6 (N$\underline{CH_2}$CO), 60.2, 61.0, 61.1 (C-6a, C-6b, and C-6c), 68.6, 68.6 (C-4b and C-4c), 70.2 (C-2a), 70.5 (C_6H_4-$\underline{CH_2}$), 71.0 (C-2b), 71.2 (C-2c), 72.4, 72.6 (C-3b and C-3c), 74.1, 74.7, 75.0 (C-5a, C-5b, and C-5c), 75.3 (C-4a), 81.8 (C-3a), 101.5 (C-1a), 102.9 (C-1c), 104.6 (C-1b), 127.5, 128.6 (C_{Ar}-H), 132.6, 141.1 (C_{Ar}), 170.9 (CONH), 173.7 (COOH); HMBC NMR (800 MHz, D_2O): H-1a → C_6H_4-$\underline{CH_2}$, H-1b → C-3a, H-1c → C-4a; ESI-FT-MS, calcd. for $C_{28}H_{40}NO_{19}^-$ $(M - H)^-$: 694.2200, found: 694.2197.

[4-(Carboxymethylcarbamoyl)phenyl]methyl *O*-(β-D-Galactopyranosyl)-(1→4)-*O*-(β-D-galactopyranosyl)-(1→3)-β-D-galactopyranoside (44)

To synthesize compound **44**, similarly to the syntheses of compounds **40** and **42**, the hydroxy functionalized HMBA-Gly-HMBA-AM resin (**20**, 35 mg, 0.032 mmol, 0.9126 mmol/g) was used in the same reaction conditions, up to the synthesis of the di-Gal acceptor with a selectively free hydroxyl group at the 4-position (**35**). To cap the hydroxyl group at the 4-position, benzoylation was performed (Bz capping) similarly to the method described previously. The next step was Edman degradation for selective deprotection of the hydroxyl group at the 4′-position of di-Gal. For selective deprotection of the monomeric-UCHP protected with Boc group, one cycle of the Edman degradation reaction (de-Boc and PITC coupling) was performed to selectively synthesize the di-Gal acceptor with a selectively free hydroxyl group at the 4′-position (**36**), similarly to the method described previously.

The last step was coupling with a third galactose. After adding a galactose donor (**12**, 200 mg, 0.189 mmol), NIS (128 mg, 0.569 mmol), and dichloromethane (500 μL) to the 4′-OH resin (**36**), the mixture was cooled at –30°C, and TfOH (50 μL, 0.565 mmol) was added. Using the same reaction conditions, workup, and purification procedures for the synthesis of compound **42**, the target trigalactose Galβ1-4Galβ1-3Galβ1-linker (**44**, 1.74 mg, retention time of 25.1 min) was synthesized and purified. The glycosylation yield was determined to be 59%, which was calculated from the area under the peaks. The total yield was 8% (19 steps from **16**). At the same time, the monogalactose Galβ1-linker (**23**, 1.2 mg, retention time of 20.2 min) and digalactose Galβ1-3Galβ1-linker (**31**, 0.86 mg, retention time of 23.7 min) were also purified.

Compound **44** (Galβ1-4Galβ1-3Galβ1-linker); $[\alpha]_D$+1.9 (*c* 0.2, MeOH); ^1H NMR (800 MHz, D_2O): δ = 3.46 (dd, 1H, $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 9.9 Hz, H-2c), 3.55 (dd, 1H, $J_{2,3}$ = 9.9 Hz, $J_{3,4}$ = 3.5 Hz, H-3c), 3.55–3.62 (m, 3H, C-5a, C-5b and C-5c), 3.57 (m, 1H, H-2b), 3.62 (m, 1H, H-2a), 3.62–3.73 (m, 6H, H-6a, H-6b, and H-6c), 3.65 (m, 1H, H-3b), 3.68 (m, 1H, H-3a), 3.79 (d, 1H, $J_{3,4}$ = 3.3 Hz, H-4c), 4.05 (d, 1H, $J_{3,4}$ = 3.2 Hz, H-4a), 4.06 (d, 1H, $J_{3,4}$ = 3.1 Hz H-4b),

4.08 (s, 2H, N$\underline{CH_2}$CO), 4.41 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1a), 4.48 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1c), 4.53 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1b), 4.73, 4.90 (each d, 2H, J_{gem} = 12.3 Hz, C_6H_4-$\underline{CH_2}$), 7.48, 7.73 (each d, 4H, Ar); ^{13}C NMR (200 MHz, D_2O): δ = 41.6 (N$\underline{CH_2}$CO), 60.4, 60.8, 60.9 (C-6a, C-6b, and C-6c), 68.4 (C-4a), 68.5 (C-4c), 69.9 (C-2a), 70.5 (C_6H_4-$\underline{CH_2}$), 71.4, 71.4 (C-2b and C-2c), 72.7 (C-3c), 72.8 (C-3b), 74.1, 74.8, 75.1 (C-5a, C-5b, and C-5c), 77.1 (C-4b), 82.2 (C-3a), 101.5 (C-1a), 104.2 (C-1c), 104.3 (C-1b), 127.5, 128.6 (C_{Ar}-H), 132.5, 141.1 (C_{Ar}), 170.9 (CONH), 173.6 (COOH); HMBC-NMR (800 MHz, D_2O): H-1a → C_6H_4-$\underline{CH_2}$, H-1b → C-3a, H-1c → C-4b; ESI-FT-MS, calcd. for $C_{28}H_{40}NO_{19}^-$ $(M - H)^-$: 694.2200, found: 694.2197.

[4-(Carboxymethylcarbamoyl)phenyl]methyl *O*-(*β*-D-Galactopyranosyl)-(1→3)-*O*-(*β*-D-galactopyranosyl)-(1→4)-*β*-D-galactopyranoside (46)

To synthesize compounds **46** and **48**, the reaction was initially performed in a single reaction vessel. At the point of diverging reaction conditions (after synthesis of compound **37**), the resin was divided. The hydroxy functionalized HMBA-Gly-HMBA-AM resin (**20**, 173 mg, 0.158 mmol, 0.9126 mmol/g) was packed into a 20-mL disposable chromatography column (Econo-Pac; Bio-Rad Laboratories Inc., Hercules, CA). This hydroxy functionalized resin was glycosylated with a galactose donor (**12**, 1 g, 0.946 mmol) using DMTST (734 mg, 2.84 mmol) in dichloromethane (15 mL) at rt for 1 day of agitation. Using the same reaction conditions, workup, and HPLC analysis procedure as previously described, the mono-glycosylated compound **23** was detected at 20.3 min, and the glycosylation yield was determined as 59% from HPLC analysis. Using the same reaction conditions as that previously described, the steps of (1) re-Boc, (2) Bz capping, and (3) one cycle of Edman degradation reaction (de-Fmoc and PITC coupling) were performed, and a Gal acceptor with a selectively free hydroxyl group at the 3-position (**26**) was selectively synthesized. Aliquots of the resin (**26**, 70.30 mg) were taken and used in the next reaction. Benzoylation was performed (Bz capping) similarly to the method described previously to cap the hydroxyl group at the 3-position. The reaction was continued until a negative result in the methyl red-DIC test. The next step was the Edman degradation step for selective deprotection of the 4-position of hydroxyl group. For selective deprotection of the monomeric-UCHP protected with Boc group, one cycle of Edman degradation reaction (de-Boc and PITC coupling) was performed to selectively synthesize the mono-Gal acceptor with a selectively free hydroxyl group at the 4-position (**29**), similar to the method described previously. All reactions were also monitored by the combination of the chloranil and methyl red-DIC tests.

The next step was coupling with a second galactose. A galactose donor (**12**, 360 mg, 0.341 mmol), NIS (230 mg, 1.02 mmol), and dichloromethane

(1 mL) were then added to the 4-OH resin (**29**, 89.16 mg), the mixture was cooled at –30°C, and TfOH (90 μL, 1.02 mmol) was added. After agitation for 1 day at –30°C, the solution was removed. The resin was washed thoroughly with DMF (5 × 4 mL) and dichloromethane (5 × 4 mL). The coupling yield was determined using analytical HPLC as in the previously described procedure; Galβ1-4Galβ1-linker (**33**) was detected at 23.2 min, and the glycosylation yield was determined as 53%. Subsequently, the protected exposed amino group on di-Gal by Boc group (re-Boc) and the unreacted hydroxyl group on mono-Gal were capped with benzoyl groups (Bz capping). Again, to deprotect Fmoc-protected monomeric-UCHP, one cycle of Edman degradation reaction (de-Fmoc and PITC coupling) was performed to synthesize a di-Gal acceptor with a selectively free hydroxyl group at the 3′-position (**37**, 117.96 mg). This resin was divided into fractions of 50 mg and 63 mg for the synthesis of compounds **46** and **48**, respectively.

The last step was coupling with a third galactose. A galactose donor (**12**, 200 mg, 0.189 mmol), NIS (128 mg, 0.569 mmol), and dichloromethane (500 μL) were added to the 3′-OH resin (**37**, 50 mg), the mixture was cooled at –30°C, and TfOH (50 μL, 0.565 mmol) was added. Using the same reaction conditions, workup, and purification procedure as for the synthesis of compound **42**, the target trigalactose Galβ1-3Galβ1-4Galβ1-linker (**46**, 3.03 mg, retention time of 25.1 min) was synthesized and purified. The glycosylation yield was determined to be 70%, as calculated from the area under the peaks. The total yield was 14% (14 steps from **16**). At the same time, the monogalactose Galβ1-linker (**23**, 1.2 mg, retention time of 20.3 min) and digalactose Galβ1-4Galβ1-linker (**33**, 0.49 mg, retention time of 23.2 min) were also purified.

Compound **46** (Galβ1-3Galβ1-4Galβ1-linker); $[\alpha]_D$+0.7 (*c* 0.3, MeOH); ^{1}H NMR (500 MHz, D_2O): δ = 3.50 (dd, 1H, $J_{1,2}$ = 7.6 Hz, $J_{2,3}$ = 10.0 Hz, H-2c), 3.55 (dd, 1H, $J_{1,2}$ = 7.1 Hz, $J_{2,3}$ = 10.3 Hz, H-2a), 3.56 (dd, 1H, $J_{2,3}$ = 10.6 Hz, $J_{3,4}$ = 3.3 Hz, H-3c), 3.82 (dd, 1H, $J_{3,4}$ = 3.3 Hz, $J_{4,5}$ = 0.7 Hz, H-4c), 4.07 (broad d, 2H, H-4a, and H-4b), 4.09 (s, 2H, N$\underline{CH_2}$CO), 4.40 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1a), 4.51 (d, 1H, $J_{1,2}$ = 7.6 Hz, H-1c), 4.55 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1b), 4.72, 4.90 (each d, 2H, J_{gem} = 12.3 Hz, C_6H_4-$\underline{CH_2}$), 7.48, 7.74 (each d, 4H, Ar); ^{13}C NMR (125 MHz, D_2O): δ = 41.6 (N$\underline{CH_2}$CO), 60.4, 60.9 (C-6a, C-6b, and C-6c), 68.4 (C-4b), 68.5 (C-4c), 70.5 (C_6H_4-$\underline{CH_2}$), 70.6 (C-2b), 71.0 (C-2c), 71.2 (C-2a), 72.5 (C-3c), 73.2 (C-3a), 74.3, 74.7, 75.0 (C-5a, C-5b, and C-5c), 77.3 (C-4a), 82.1 (C-3b), 101.7 (C-1a), 104.0 (C-1b), 104.3 (C-1c), 127.5, 128.5 (C_{Ar}-H), 132.5, 141.2 (C_{Ar}), 170.9, 173.6 (2C=O); HMBC NMR (500 MHz, D_2O): H-1a → C_6H_4-$\underline{CH_2}$, H-1b → C-4a, H-1c → C-3b; ESI-FT-MS, calcd. for $C_{28}H_{40}NO_{19}^-$ $(M - H)^-$: 694.2200, found: 694.2200.

Compound **33** (Galβ1-4Galβ1-linker); $[\alpha]_D$ –1.5 (*c* 0.3, MeOH); ^{1}H NMR (400 MHz, D_2O): δ = 3.51 (dd, 1H, $J_{1,2}$ = 7.7 Hz, $J_{2,3}$ = 9.9 Hz, H-2b), 3.57 (dd, 1H, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 9.7 Hz, H-2a), 3.58 (m, 1H, H-3b), 3.59–3.67 (m, 2H, H-5a and H-5b), 3.64 (m, 1H, H-3a), 3.64–3.80 (m, 4H, H-6a and H-6b),

3.82 (d, 1H, $J_{3,4}$ = 3.1 Hz, H-4b), 3.91 (s, 2H, N$\underline{CH_2}$CO), 4.09 (d, 1H, $J_{3,4}$ = 2.9 Hz, H-4a), 4.42 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1a), 4.51 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1b), 4.74, 4.92 (each d, 2H, J_{gem} = 12.3 Hz, C_6H_4-$\underline{CH_2}$), 7.49, 7.76 (each d, 4H, $J_{o,m}$ = 8.2 Hz, Ar); ^{13}C NMR (200 MHz, D_2O): δ = 43.4 (N$\underline{CH_2}$CO), 60.4 (C-6a), 60.9 (C-6b), 68.5 (C-4b), 70.5 (C_6H_4-$\underline{CH_2}$), 71.2 (C-2a), 71.3 (C-2b), 72.7 (C-3b), 73.1 (C-3a), 74.2 (C-5a), 75.0 (C-5b), 77.0 (C-4a), 101.7 (C-1a), 104.2 (C-1b), 127.4, 128.5 (C_{Ar}-H), 133.1, 140.8 (C_{Ar}), 170.3 (NCO), 176.4 (COOH); HMBC NMR (800 MHz, D_2O): H-1a → C_6H_4-$\underline{CH_2}$, H-1b → C-4a; ESI-FT-MS, calcd. for $C_{22}H_{31}NO_{14}Na^+$ $(M + Na)^+$: 556.1637, found: 556.1640.

[4-(Carboxymethylcarbamoyl)phenyl]methyl *O*-(β-D-Galactopyranosyl)-(1→4)-*O*-(β-D-galactopyranosyl)-(1→4)-β-D-galactopyranoside (48)

To synthesize compound **48**, the di-Gal acceptor with a selectively free hydroxyl group at the 3′-position (**37**, 63.48 mg) that was synthesized for compound **46** was used. To protect the hydroxyl group at the 3′-position, benzoylation was performed (Bz capping) similarly to the method described previously. The next step was Edman degradation for selective deprotection of the hydroxyl group at the 4′-position of di-Gal. For selective deprotection of the monomeric-UCHP protected with Boc group, one cycle of the Edman degradation reaction (de-Boc and PITC coupling) was performed to selectively synthesize the di-Gal acceptor with a selectively free hydroxyl group at the 4′-position (**38**), similar to the method described previously.

The last step was coupling with a third galactose. A galactose donor (**12**, 200 mg, 0.189 mmol), NIS (128 mg, 0.569 mmol), and dichloromethane (500 μL) were next added to the 4′-OH resin (**38**), the mixture was cooled at –30°C, and TfOH (50 μL, 0.565 mmol) was added. Using the same reaction condition, workup, and purification procedure as for the synthesis of compound **42**, the target trigalactose Galβ1-4Galβ1-4Galβ1-linker (**48**, 2.90 mg, retention time of 24.8 min) was synthesized and purified. The glycosylation yield was determined as 42%, as calculated from the area under the peaks. The total yield was 13% (17 steps from **16**). At the same time, the monogalactose Galβ1-linker (**23**, 1.0 mg, retention time of 20.3 min) and digalactose Galβ1-4Galβ1-linker (**33**, 1.56 mg, retention time of 23.2 min) were also purified.

Compound **48** (Galβ1-4Galβ1-4Galβ1-linker); $[\alpha]_D$+3.9 (*c* 0.3, H_2O); ^{1}H NMR (500 MHz, D_2O): δ = 3.49 (dd, 1H, $J_{1,2}$ = 7.8 Hz, $J_{2,3}$ = 9.9 Hz, H-2c), 3.80 (d, 1H, $J_{3,4}$ = 3.4 Hz, H-4c), 4.07 (broad s, 2H, H-4a and H-4b), 4.09 (s, 2H, N$\underline{CH_2}$CO), 4.40 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1a), 4.49 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1c), 4.54 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1b), 4.72, 4.90 (each d, 2H, J_{gem} = 12.3 Hz, C_6H_4-$\underline{CH_2}$), 7.48, 7.74 (each d, 4H, $J_{o,m}$ = 8.3 Hz, Ar); ^{13}C NMR (125 MHz, D_2O): δ = 41.6 (N$\underline{CH_2}$CO), 60.5, 60.6, 60.9 (C-6a, C-6b, and C-6c), 68.6 (C-4c), 70.6 (C_6H_4-$\underline{CH_2}$), 71.2 (C-2a), 71.3 (C-2c), 71.8 (C-2b), 72.7 (C-3c), 73.2, 73.2 (C-3a

and C-3b), 74.4, 74.4, 75.1 (C-5a, C-5b, and C-5c), 77.1 (C-4b), 77.6 (C-4a), 101.8 (C-1a), 104.3 (C-1c), 104.3 (C-1b), 127.5, 128.6 (C_{Ar}-H), 132.5, 141.2 (C_{Ar}), 170.9, 173.5 (2C=O); HMBC NMR (500 MHz, D_2O): H-1a → C_6H_4-$\underline{CH_2}$, H-1b → C-4a, H-1c → C-4b; ESI-FT-MS, calcd. for $C_{28}H_{40}NO_{19}^-$ $(M - H)^-$: 694.2200, found: 694.2198.

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