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Synthesis of galactose-linked uridine derivatives with simple linkers as potential galactosyltransferase inhibitors☆

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Abstract—Galactose-linked uridine derivatives without charge or dipole contributions in the linker were designed and synthesized via cross metathesis (CM). This strategy would provide a ready access to a range of hybrid compounds linking uridine and galactose derivatives.

1. Introduction

Oligosaccharides on cell surfaces and other glycoconjugates are responsible for several intercellular and intracellular events, including intercellular adhesion in inflammation, tumor metastasis, bacterial or viral infection, or activation of the innate immune system.[1–5] Since diseases such as rheumatoid arthritis, for example, are caused by malfunction of glycoconjugate biosynthesis, the challenge to modulate glycoconjugate biosynthesis is compelling and is being actively undertaken. Glycosyltransferases are key enzymes responsible for oligosaccharide biosynthesis and for catalysis of the sugar-transfer reaction in a stereo- and regiospecific manner. Each individual glycosyltransferase is responsible for each glycosylation step in oligosaccharide biosynthesis. Thus, inhibition of glycosyltransferases leads to the modulation of oligosaccharide biosynthesis, providing us with an opportunity to study their biological functions and to develop new therapeutic agents. Generally, a nucleoside diphosphate sugar (an NDP-sugar) is utilized as the sugar donor and the sugar moiety found in an NDP-sugar is transferred to the hydroxyl group of the acceptor molecule such as a growing oligosaccharide, a protein, or a lipid. Extensive efforts have been devoted to developing inhibitors for these glycosyltransferases with donor analogues.[9–20] However, only limited success has been achieved.[9–11] The diphosphate linkage found in an NDP-sugar is prone to hydrolysis, and its inherent negative charge is disfavored in terms of cell permeability. Some glycosyltransferases are known to require a divalent metal for their activity and form a salt bridge with the NDP-sugar through the metal in their active site called a DXD motif. Thus, modification of a diphosphate found in an NDP-sugar has been conducted primarily with respect to its ability to interact with the divalent metal ion in the acceptor analogue. In addition to the charge or dipole interaction mentioned above, the relative position and orientation of nucleoside and sugar moiety within the enzyme upon binding are likewise important concerns. Here we describe in an initial study the design and synthesis of galactose-linked uridine derivatives without charge or dipole contributions in the linker, which would be useful for the development of novel NDP-sugar analogues. Bovine β1,4-galactosyltransferase (β4GalTI) was chosen as the target enzyme because it is the most well-studied enzyme among the glycosyltransferases, and because X-ray crystal structure analysis data are available.[6–8] β4GalT catalyzes the transfer of galactose from UDP-galactose (Fig. 1, **1**) to the 4-hydroxyl group of *N*-acetylglucosamine found in oligosaccharides. We designed two sets of galactose-linked uridine derivatives, one of which possesses the simple alkane linker (Fig. 1, **2**), the other the alkene (Fig. 1, **3**), with the same number of atoms between the 5′-position of uridine and the anomeric position of galactose. Each set of compounds also consists of diastereomers at the 5′-position of uridine. With the introduction of an olefin and a hydroxyl group, a systematic structure-activity relationship could be conducted in terms of the distance between the uridine and galactose moiety and the relative orientation toward each other. Compounds **3** are also versatile intermediates because a variety of functional groups can be introduced at the olefinic position, which corresponds to the diphosphate in the natural substrate.

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Keywords: Glycosyltransferase inhibitor; β1,4-Galactosyltransferase; Cross metathesis.

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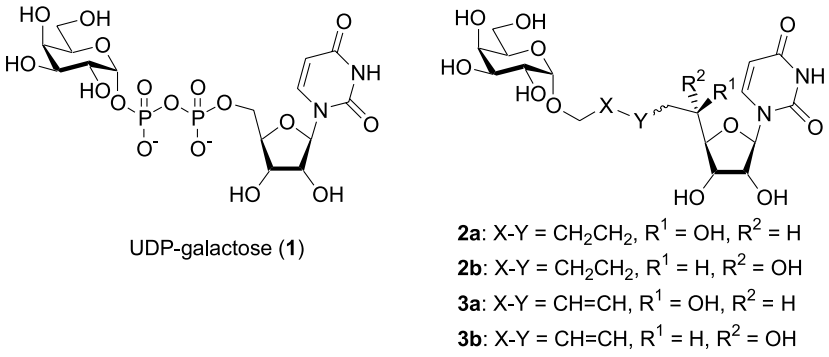
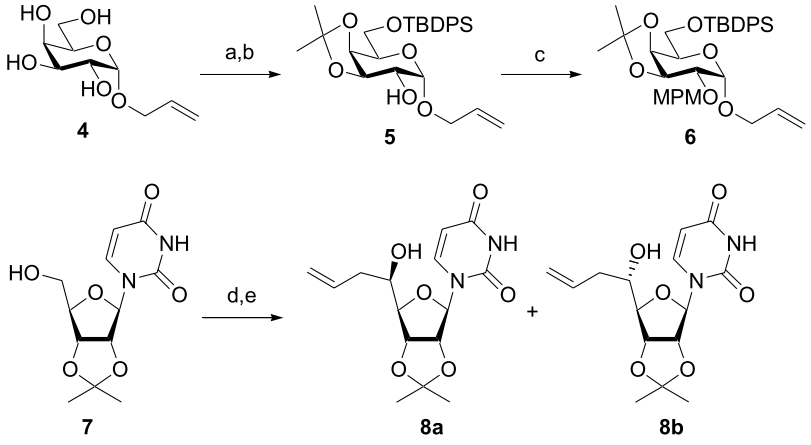


Figure 1. Structures of UDP-Gal (**1**) and designed compounds (**2**, **3**).



Scheme 1. Preparation of **6** and **8**. Reagents and conditions: (a) TBDPSCl, imidazole, DMF, room temperature; (b) 2,2-dimethoxypropane, TsOH, acetone, room temperature (90%, two steps); (c) MPMCl, NaH, DMF, room temperature (87%); (d) IBX, MeCN, 80 °C; (e) allylmagnesium bromide, THF, −78 °C (89%, two steps).

Table 1

Entry	Catalyst	Solvent	Temp. (°C)	Yield (%)		
				9a	**10**	**6**[a] (recovered)
1	**A**	CH_2Cl_2	40	trace	trace	-
2	**B**	CH_2Cl_2	40	35	38	43
3	**B**	toluene	110	15	54	40
4[b]	**B**	CH_2Cl_2	40	89	6	62

[a] Yield was based on the sugar derivative **6**.
[b] The reaction was carried out using 5 equiv of **6**.

2. Results and discussion

Our strategy for the synthesis of compounds **2** and **3** consisted of connecting the suitably protected allyl galactoside and the 5′-*C*-allyluridine derivative via olefin cross metathesis (CM).[21–29]

The CM precursors were prepared as shown in Scheme 1. Allyl α-galactopyranoside **4**[30] was sequentially protected with TBDPS, isopropylidene, and MPM groups to give **6**. 2′,3′-*O*-Isopropylideneuridine (**7**) was oxidized with IBX[31] to give the 5′-aldehyde, which was allylated upon treatment with allylmagnesium bromide to provide the 5′-*C*-allyl uridine[32] **8** in 89% yield (**8a/8b**=5/1) for two steps. Each diastereomer was separated by HPLC to give the pure material.

With these compounds in hand, we next examined the key cross metathesis, and the results are summarized in Table 1 and Scheme 2. When **6** (1 equiv) and **8a** (1 equiv) in a 0.1 M CH_2Cl_2 solution was treated with catalyst **B**[33,34] (Fig. 2) at reflux for 12 h, the desired **9a** was obtained in 35% yield (*E*/*Z*=5/1) as well as the homodimer of the uridine derivative **10** (Fig. 3) in 38% yield (entry 2). The use of catalyst **A**[35,36] gave only a trace amount of **9a** and was not effective for CM, as reported (entry 1). A trace amount of the homodimer of the allyl galactoside **11** was observed in the reaction mixture. Changing the solvent to toluene and the reaction temperature decreased the yield of **9a** (entry 3).

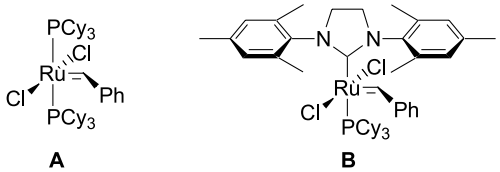
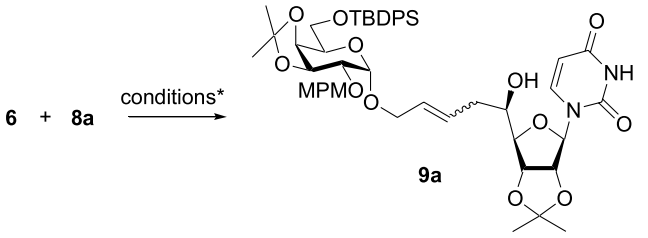


Figure 2. Structure of ruthenium catalysts.

It is known that the ruthenium carbene complex coordinates with the neighboring oxygen, and this coordination affects the selectivity and reactivity.[21] It is suggested that an initial [2+2] cycloaddition of the ruthenium carbene in the catalytic process might selectively proceed with **8a** rather than **6** via coordination with the 5′-hydroxyl group in **8a**. This result prompted us to use an excess of **6** to increase the ratio of the desired cross metathesis product **9a** to the homodimer **10**. Thus, when **6** (1 equiv) and **8a** (5 equiv) in 0.1 M CH_2Cl_2 solution was treated with catalyst **B** at reflux for 12 h, the yield of the desired **9a** was increased to 89% with the decreased amount of homodimer **10** (6% yield, entry 4). A limited amount of dimerized product **11** was also obtained under these conditions and the unreacted **6** was recovered and recycled. The CM with the diastereomer **8b** gave similar results to provide **9b**.

Deprotection of **9a** and **9b** by a two-step sequence afforded the target compounds **3a** and **3b**, respectively. Hydrogenation of **3a** and **3b** gave **2a** and **2b**, respectively (Scheme 3).



Scheme 2. Cross metathesis of **6** and **8a**.

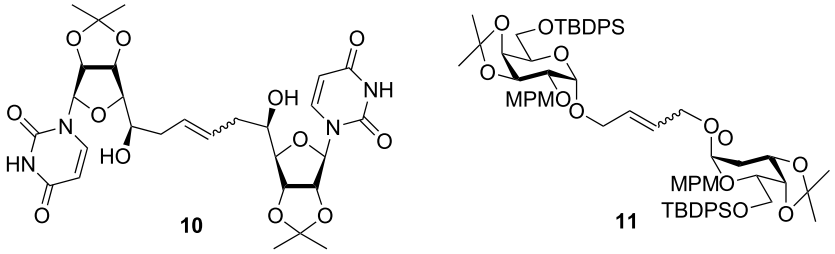
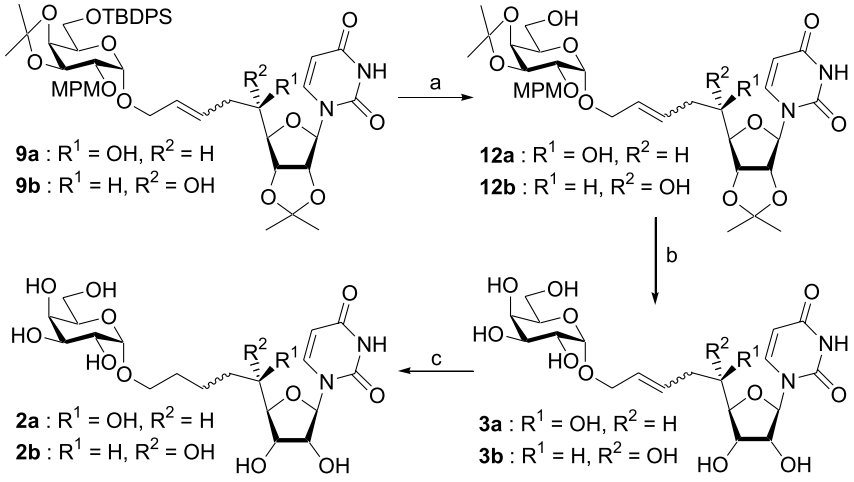


Figure 3. Structures of compound **10** and **11**.



Scheme 3. Synthesis of compound **2** and **3**. Reagents and conditions: (a) TBAF, THF, room temperature (91% for **12a**, 93% for **12b**); (b) 80% aq. TFA, room temperature (quant. for **3a** and **3c**); (c) H_2, $Pd(OH)_2$/C, MeOH, room temperature (quant. for **2a** and **2c**).

All the synthesized target compounds **2a**, **2b**, **3a** and **3b** were tested as inhibitors of bovine β1,4-galactosyltransferase I, according to the previously reported procedure[18,19] using UDP-[^{3}H]Gal as the glycosyl donor. Compounds **2a** and **2b** were tested as a geometric mixture because the geometrical isomers were inseparable. However, none of them exhibited inhibitory activities at concentrations up to 1 mM. Compounds **3** possess neutral and hydrophobic liker between uridine and galactose moieties. Thus, these results suggest that charge and/or dipole interaction with DXD motif found in β4GalTI is important for binding. However, compounds **3** also versatile intermediates because a variety of functional groups can be introduced at the olefinic position, which can interact with DXD motif.

3. Conclusion

Galactose-linked uridine derivatives without charge or dipole contributions in the linker were designed and synthesized via CM. This strategy would provide a ready access to a range of hybrid compounds linking uridine and galactose derivatives. Further transformation of the olefin found in compounds **3** could provide additional functionality, and these studies are in progress.

4. Experimental

4.1. General

NMR spectra were obtained on a JEOL EX270, JEOL

GX270, JEOL AL400 or Bruker ARX-500 and were reported in parts per million (δ) relative to tetramethylsilane (0.00 ppm) as an internal standard otherwise noted. Coupling constant (J) was reported in herz (Hz). Abbreviations of multiplicity were as follows; s: singlet, d; doublet, t: triplet, q: quartet, m: multiplet, br: broad. Data were presented as follows; chemical shift (multiplicity, integration, coupling constant). Assignment was based on ^{1}H–^{1}H COSY, HMBC and HMQC NMR spectra. Optical rotations were recorded on JASCO DIP-370 digital polarimeter or JASCO P-1030 polarimeter. FAB-MS were obtained on a JEOL JMS-HX101 or JEOL JMS-700TZ. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60F_{254} plates. Normal-phase column chromatography was performed on Merck silica gel 5715 or Kanto Chemical silica gel 60N (neutral). Flash column chromatography was performed on Merck silica gel 60. Reverse-phase column chromatography was performed on Waters Preparative C18 125 Å (55–105 μm). HPLC systems were Shimadzu LC-8A, Shimadzu SPD-6A, Shimadzu C-R6A. Dichloromethane and acetonitorile were distilled from P_2O_5 and then CaH_2. Methanol was distilled from sodium metal or directly used HPLC grade solvent from Kanto Chemical Co., Inc. Toluene was distilled from sodium metal/benzophenone ketyl. *N*,*N*-Dimethylformamide and dimethylsulfoxide were distilled from CaH_2 under reduced pressure or purchased dehydrated solvent from Kishida Chemical Co., Ltd. Tetrahydrofuran was purchased dehydrated stabilizer free solvent from Kanto Chemical Co., Inc. Bovine β1,4-galactosyltransferase I (β4GalTI) was purchased from TOYOBO Co. Ltd. Uridine diphosphate-[^{3}H]-galactose was purchased from American Radiolabeled Chemical Inc. Ovalbmin was purchased from nacalai tesque. Scintillation count was performed on PACKARD 1600 TR and Aloka LSC-120.

4.1.1. Allyl 6-*O*-(*tert*-butyldiphenylsilyl)-3,4-*O*-isopropylidene-α-D-galactopyranoside (5). To a solution of **4**[30] (5.00 mmol, 1.10 g) in DMF (40 ml), imidazole (12.0 mmol, 817 mg) and TBDPSCl (6.0 mmol, 1.56 ml) were added and the mixture was stirred for 12 h. The reaction was quenched with MeOH, and the solvent was removed in vacuo. The residue was partitioned between AcOEt (300 ml) and H_2O (100 ml) and the organic layer was washed with H_2O (100 ml×2) and brine (70 ml), dried over Na_2SO_4, filtered and concentrated. To the residue in acetone (100 ml), 2,2-dimethoxypropane (50 mmol, 6.1 ml) and TsOH (0.5 mmol, 86 mg) were added and the mixture was stirred for 12 h. The reaction mixture was neutralized with saturated aqueous $NaHCO_3$ and the solvent was removed in vacuo. The residue was partitioned between AcOEt (200 ml) and H_2O (100 ml) and the organic layer was washed with H_2O (100 ml) and brine (70 ml), dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography (SiO_2, 7/1-6/1-5/1; hexane/AcOEt) to give **5** (4.50 mmol, 2.24 g, 90% for two steps) as a colorless syrup. $[\alpha]_D^{20}$ +62.6 (*c* 1.00, $CDCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (m, 4H), 7.38 (m, 6H), 5.90 (m, 1H), 5.29 (ddd, 1H, J=1.7, 17.2 Hz), 5.21 (ddd, 1H, J=1.7, 10.4 Hz), 4.89 (d, 1H, J=3.9 Hz), 4.26 (m, 3H), 4.11 (ddd, 1H, J=2.3, 6.5, 6.6 Hz), 4.03 (ddd, 1H, J=6.3, 12.8 Hz), 3.94 (m, 2H), 3.80 (m, 1H), 2.30 (d, 1H, J=7.1 Hz, exchangeable with D_2O), 1.48, 1.34 (each s, each 3H), 1.07 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.6, 133.6, 133.4, 133.3, 129.6, 127.6, 117.7, 109.3, 96.4, 76.2, 72.9, 69.7, 68.8, 68.4, 63.0, 27.8, 26.8, 26.0, 19.3; FAB-LRMS *m*/*z* 499 (MH^+); FAB-HRMS calcd for $C_{28}H_{39}O_6Si$ 499.2516, found 499.2520 (MH).

4.1.2. Allyl 6-*O*-(*tert*-butyldiphenylsilyl)-3,4-*O*-isopropylidene-2-*O*-(4-methoxybenzyl)-α-D-galactopyranoside (6). To a solution of **5** (4.56 mmol, 2.27 g) in DMF (40 ml), NaH (5.47 mmol, 131 mg) was added and the mixture was stirred for 1 h. After 1 h stirring, MPMCl (5.47 mmol, 742 μl) was added and the mixture was stirred for further 10 h. The reaction was quenched with MeOH and the solvent was removed in vacuo. The residue was diluted with AcOEt (300 ml), and the organic layer was washed with H_2O (100 ml×4) and brine (70 ml), dried over Na_2SO_4, filtered and concentrated. The resulting residue was purified by column chromatography (SiO_2, 10/1-8/1-7/1; hexane/AcOEt) to give **6** (3.97 mmol, 2.45 g, 87%) as a colorless syrup. $[\alpha]_D^{19}$ +52.3 (*c* 1.44, $CDCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68 (m, 4H), 7.41 (m, 6H), 7.27 (m, 2H), 6.85 (m, 2H), 5.91 (m, 1H), 5.30 (dd, 1H, J=1.4, 17.3 Hz), 5.20 (dd, 1H, J=1.4, 10.6 Hz), 4.76 (d, 1H, J=3.5 Hz), 4.72 (d, 1H, J=12.3 Hz), 4.62 (d, 1H, J=12.3 Hz), 4.33 (m, 1H), 4.25 (dd, 1H, J=2.4, 5.4 Hz), 4.14 (dd, 1H, J=5.1, 13.0 Hz), 4.08 (m, 1H), 3.96 (dd, 1H, J=13.0, 6.4 Hz), 3.90 (m, 1H), 3.83 (m, 1H), 3.79 (s, 3H), 3.49 (dd, 1H, J=3.6, 7.9 Hz), 1.38, 1.35 (each s, each 3H), 1.05 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.1, 135.5, 133.7, 133.5, 133.3, 130.4, 129.7, 129.4, 127.6, 117.8, 113.7, 108.9, 95.8, 76.1, 76.0, 73.3, 71.9, 68.2, 68.0, 63.0, 55.3, 28.3, 26.8, 26.4, 19.3; FAB-LRMS *m*/*z* 619 (MH^+); FAB-HRMS calcd for $C_{36}H_{47}O_7Si$ 619.3091, found 619.3088 (MH^+).

4.1.3. 1-(2′,3′-*O*-Isopropylidene-6′,7′,8′-trideoxy-α-L-*talo*-oct-7′-enofranosyl)uracil (8a) and 1-(2′,3′-*O*-isopropylidene-6′,7′,8′-trideoxy-β-D-*allo*-oct-7′-enofranosyl)-uracil (8b). To a solution of **7** (4.32 mmol, 1.23 g) in MeCN (40 ml), IBX (12.9 mmol, 3.60 g) was added and the mixture was refluxed for 1.5 h. The reaction mixture was cooled to 0 °C and the precipitate was filtered off. The filtrate was concentrated and the residue was coevaporated with toluene (5 ml×2). A solution of the residue in THF (40 ml) was cooled to −78 °C. To the solution, allylmagnesium bromide (1.0 M solution in Et_2O; 13.0 mmol, 13.0 ml) was added dropwise over 10 min and the mixture was stirred for further 10 min. The reaction was quenched with saturated aqueous NH_4Cl and the precipitate was filtered off. The filtrate was diluted with AcOEt (200 ml) and the organic layers were washed with H_2O (70 ml) and brine (70 ml). The aqueous layer was extracted with $CHCl_3$ (70 ml) and combined organic layers were dried over Na_2SO_4, filtered and concentrated. The resulting residue was purified by column chromatography (SiO_2, 0–2% ethanol in $CHCl_3$) to give **8** (3.84 mmol, 1.24 g, 89% for two steps; diastereomixture of 5′*R*/*S*-isomer) as a white foam. Separation of 5′-stereoisomer was accomplished by HPLC (YMC-PACK SIL-06; 1/3; hexane/AcOEt). Physical data for 5′*R*-isomer **8a** were identical with the properties of the previously reported.[32] For 5′*R*-isomer **8a**; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.43 (br s, 1H, exchangeable with D_2O), 7.42 (d, 1H, J=8.0 Hz), 5.84 (m, 1H), 5.72 (d, 1H, J=8.0 Hz), 5.62 (d, 1H, J=2.7 Hz), 5.20 (m, 2H), 4.97 (m,

2H), 4.13 (m, 1H), 3.94 (m, 1H), 2.93 (br s, 1H, exchangeable with D_2O), 2.32 (m, 2H), 1.57, 1.37 (each s, each 3H). For 5′*S*-isomer **8b**; $[\alpha]_D^{21}$ −7.15 (*c* 0.91, $CDCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 8.09 (br s, 1H, exchangeable with D_2O), 7.44 (d, 1H, J=8.3 Hz), 5.83 (m, 1H), 5.73 (d, 1H, J=8.3 Hz), 5.67 (d, 1H, J=3.4 Hz), 5.19 (d, 1H, J=5.8 Hz), 5.15 (s, 1H), 4.96 (dd, 1H, J=3.4, 6.9 Hz), 4.92 (dd, 1H, J=3.4, 6.9 Hz), 4.15 (dd, 1H, J=3.0, 3.4 Hz), 3.83 (m, 1H), 2.65 (d, 1H, J=6.4 Hz, exchangeable with D_2O), 2.36 (m, 2H), 1.55, 1.36 (each s, each 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.3, 142.5, 133.8, 118.8, 114.5, 102.8, 95.0, 87.4, 83.2, 81.3, 71.1, 38.5, 27.4, 25.3; FAB-LRMS *m/z* 325 (MH^+); FAB-HRMS calcd for $C_{15}H_{21}N_2O_6$ 325.1400, found 325.1399 (MH^+).

4.1.4. 1-{9′-*O*-[6″-*O*-(*tert*-Butyldiphenylsilyl)-3″,4″-*O*-isopropylidene-2″-*O*-(4-methoxybenzyl)-α-D-galactopyranosyl]-2′,3′-*O*-isopropylidene-6′,7′,8′-trideoxy-α-L-*talo*-non-7′-enofranosyl}uracil (9a). To a solution of **8a** (59 μmol, 19 mg) and **6** (295 μmol, 182 mg) in CH_2Cl_2 (2 ml), Grubbs second catalyst **B** (10 mol%, 6.0 mg) was added and the mixture was refluxed for 12 h. The solvent was removed in vacuo, and the residue was purified by column chromatography (SiO_2, 0–2% ethanol in $CHCl_3$) to give **9a** (52 μmol, 48 mg, 89%) as a brown foam. ^1H NMR (500 MHz, $CDCl_3$) for *E* geometrical isomer δ 8.13 (br s, 1H, exchangeable with D_2O), 7.67 (m, 3H), 7.41 (m, 7H), 7.36 (m, 3H), 6.85 (m, 2H), 5.71 (m, 3H), 5.58 (d, 1H, J=2.7 Hz), 4.97 (m, 2H), 4.76 (d, 1H, J=3.6 Hz), 4.73 (d, 1H, J=12.1 Hz), 4.61 (d, 1H, J=12.1 Hz), 4.32 (m, 1H), 4.26 (m, 1H), 4.12 (m, 1H), 4.07 (m, 2H), 3.91 (m, 3H), 3.86 (m, 1H), 3.79 (s, 3H), 3.49 (m, 1H), 2.90 (br s, 1H, exchangeable with D_2O), 2.29 (m, 2H), 1.53, 1.37, 1.34, 1.31 (each s, each 3H), 1.05 (s, 9H); FAB-LRMS *m/z* 915 (MH^+); FAB-HRMS calcd for $C_{49}H_{63}N_2O_{13}Si$ 915.4099, found 915.4095 (MH^+).

4.1.5. 1-{9′-*O*-[6″-*O*-(*tert*-Butyldiphenylsilyl)-3″,4″-*O*-isopropylidene-2″-*O*-(4-methoxybenzyl)-α-D-galactopyranosyl]-2′,3′-*O*-isopropylidene-6′,7′,8′-trideoxy-β-D-*allo*-non-7′-enofranosyl}uracil (9b). Compound **9b** (0.14 mmol, 125 mg, 80%) was obtained as a pale brown foam from **8a** (0.17 mmol, 54 mg) and **6** (0.85 mmol, 556 mg) as described above for the synthesis of **9b**, after purification by flash column chromatography (SiO_2, 3/1-2/1-1/1-1/2; hexane/AcOEt). ^1H NMR (500 MHz, $CDCl_3$) for *E* geometrical isomer δ 8.27 (br s, 1H, exchangeable with D_2O), 7.68 (m, 3H), 7.41 (m, 7H), 7.28 (m, 3H), 6.86 (m, 2H), 5.71 (m, 3H), 5.60 (d, 1H, J=3.3 Hz), 4.95 (m, 1H), 4.89 (m, 1H), 4.76 (d, 1H, J=3.3 Hz), 4.71 (d, 1H, J=12.2 Hz), 4.60 (d, 1H, J=12.2 Hz), 4.31 (m, 1H), 4.26 (m, 1H), 4.12 (m, 3H), 4.07 (m, 1H), 3.92 (m, 2H), 3.84 (m, 1H), 3.81 (s, 3H), 3.49 (dd, 1H, J=3.6, 7.9 Hz), 2.87 (d, 1H, J=6.0 Hz, exchangeable with D_2O), 2.33 (m, 2H), 1.56, 1.40, 1.37, 1.35 (each s, each 3H), 1.05 (s, 9H); FAB-LRMS *m/z* 915 (MH^+); FAB-HRMS calcd for $C_{49}H_{63}N_2O_{13}Si$ 915.4099, found 915.4103 (MH^+).

4.1.6. 1-{9′-*O*-[3″,4″-*O*-Isopropylidene-2″-*O*-(4-methoxybenzyl)-α-D-galactopyranosyl]-2′,3′-*O*-isopropylidene-6′,7′,8′-trideoxy-α-L-*talo*-non-7′-enofranosyl}uracil (12a). To a solution of **9a** (140 μmol, 125 mg) in THF (2 ml), TBAF (1 M solution in THF; 210 μmol, 210 μl) was added and stirred for 8 h. The solvent was removed in vacuo, and the residue was purified by flash column chromatography (SiO_2, 0–1–2–3–4% ethanol in $CHCl_3$) to give **12a** (127 μmol, 86 mg, 91%) as a colorless glass. ^1H NMR (500 MHz, $CDCl_3$) for *E* geometrical isomer δ 8.21 (br s, 1H, exchangeable with D_2O), 7.38 (d, 1H, J=8.0 Hz), 7.27 (m, 2H), 6.87 (m, 2H), 5.74 (m, 3H), 5.58 (d, 1H, J=1.5 Hz), 4.97 (m, 2H), 4.83 (d, 1H, J=3.4 Hz), 4.74 (d, 1H, J=12.2 Hz), 4.62 (d, 1H, J=12.2 Hz), 4.35 (dd, 1H, J=5.5, 7.7 Hz), 4.21 (dd, 1H, J=2.7, 5.5 Hz), 4.06 (m, 4H), 3.91 (m, 2H), 3.80 (s, 3H), 3.49 (m, 2H), 3.13 (d, 1H, J=2.5 Hz, exchangeable with D_2O), 2.31 (m, 2H), 1.57, 1.41, 1.36, 1.34 (each s, each 3H); FAB-LRMS *m/z* 677 (MH^+); FAB-HRMS calcd for $C_{33}H_{45}N_2O_{13}$ 677.2922, found 677.2918 (MH^+).

4.1.7. 1-{9′-*O*-[3″,4″-*O*-Isopropylidene-2″-*O*-(4-methoxybenzyl)-α-D-galactopyranosyl]-2′,3′-*O*-isopropylidene-6′,7′,8′-trideoxy-β-D-*allo*-non-7′-enofranosyl}uracil (12b). Compound **12b** (0.10 mmol, 68 mg, 93%) was obtained as a colorless glass from **9b** (0.11 mmol, 100 mg) as described above for the synthesis of **12a**, after purification by flash column chromatography (SiO_2, 0–1–2–3–4% ethanol in $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) for *E* geometrical isomer δ 7.40 (d, 1H, J=8.1 Hz), 7.29 (d, 2H, J=8.5 Hz), 6.87 (d, 2H, J=8.5 Hz), 5.72 (m, 3H), 5.61 (d, 1H, J=3.0 Hz), 4.97 (m, 1H), 4.91 (m, 1H), 4.86 (d, 1H, J=3.5 Hz), 4.72 (d, 1H, J=12.1 Hz), 4.62 (d, 1H, J=12.1 Hz), 4.34 (dd, 1H, J=5.5, 7.7 Hz), 4.19 (dd, 1H, J=2.5, 5.5 Hz), 4.13 (m, 2H), 4.09 (m, 1H), 3.90 (m, 1H), 3.82 (m, 2H), 3.80 (s, 3H), 3.49 (dd, 1H, J=3.6, 8.0 Hz), 3.24 (br s, 1H, exchangeable with D_2O), 1.57, 1.39, 1.35, 1.33 (each s, each 3H); FAB-LRMS *m/z* 677 (MH^+); FAB-HRMS calcd for $C_{33}H_{45}N_2O_{13}$ 677.2922, found 677.2930 (MH^+).

4.1.8. 1-(9′-*O*-α-D-Galactopyranosyl-6′,7′,8′-trideoxy-α-L-*talo*-non-7′-enofranosyl)uracil (3a). 12a (30 μmol, 20 mg) was dissolved in 80% aqueous TFA (200 μl) and the mixture was stirred for 5 min. The solvent was removed in vacuo and the residue was coevaporated with EtOH (1 ml×6). The resulting residue was purified by column chromatography (C18, 5–10–15% MeOH in H_2O) to give **3a** (30 μmol, 14 mg, quant.) as a colorless glass. ^1H NMR (500 MHz, DMSO-d_6, D_2O) for *E* geometrical isomer δ 7.82 (d, 1H, J=8.0 Hz), 5.89 (m, 1H), 5.87 (m, 1H), 5.75 (m, 2H), 4.97 (d, 1H, J=2.3 Hz), 4.18 (m, 2H), 4.20 (dd, 1H, J=5.4, 12.1 Hz), 4.05 (m, 2H), 3.92 (m, 3H), 3.78 (m, 2H), 3.71 (m, 2H), 2.42 (m, 1H), 2.29 (m, 2H); FAB-LRMS *m/z* 477 (MH^+); FAB-HRMS calcd for $C_{19}H_{29}N_2O_{12}$ 477.1720, found 477.1731 (MH^+).

4.1.9. 1-(9′-*O*-α-D-Galactopyranosyl-6′,7′,8′-trideoxy-β-D-*allo*-non-7′-enofranosyl)uracil (3b). Compound **3b** (74 μmol, 35 mg, quant.) was obtained as a colorless glass from **12b** (74 μmol, 50 mg) as described above for the synthesis of **3a**, after purification by column chromatography (C18, 5–10% methanol in H_2O). ^1H NMR (400 MHz, DMSO-d_6, D_2O) δ 7.97 (d, 1H, J=7.8 Hz), 5.77 (d, 1H, J=5.4 Hz), 5.62 (d, 1H, J=5.62 Hz), 4.62 (d, 1H, J=3.4 Hz), 4.00 (m, 3H), 3.53 (m, 7H), 3.44 (m, 2H), 1.48 (m, 6H); FAB-LRMS *m/z* 477 (MH^+); FAB-HRMS calcd for $C_{19}H_{29}N_2O_{12}$ 477.1720, found 477.1735 (MH^+).

4.1.10. 1-(9′-*O*-α-D-Galactopyranosyl-6′,7′,8′-trideoxy-α-L-*talo*-nonofranosyl)uracil (2a). To a solution of **3a** (15 μmol, 7.0 mg) in MeOH (2 ml), palladium hydroxide on carbon (wet. 10%; 5 mg) was added and the mixture was vigorously stirred under H_2 atmosphere for 10 min. The catalyst was filtered off through Celite pad and the filtrate was concentrated. The resulting residue was purified by column chromatography (C18, 5–10–15% methanol in H_2O) to give **2a** (15 μmol, 7.0 mg, quant.) as a colorless glass. $[\alpha]_D^{21}$ +21.6 (*c* 1.09, H_2O); ^{1}H NMR (500 MHz, DMSO-d_6, D_2O) δ 7.82 (d, 1H, J=8.1 Hz), 5.77 (d, 1H, J=6.6 Hz), 5.62 (d, 1H, J=8.1 Hz), 4.61 (d, 1H, J=3.4 Hz), 3.68 (m, 2H), 3.57 (m, 4H), 3.53 (m, 3H), 3.42 (m, 2H), 1.47 (m, 6H); ^{13}C NMR (100 MHz, DMSO-d_6, D_2O) δ 163.2, 151.0, 140.9, 101.9, 98.8, 87.8, 86.7, 73.3, 71.1, 70.2, 69.6, 69.0, 68.8, 68.4, 66.9, 60.6, 32.8, 29.1, 22.1; FAB-LRMS *m/z* 479 (MH^+); FAB-HRMS calcd for $C_{19}H_{31}N_2O_{12}$ 479.1877, found 479.1868 (MH^+).

4.1.11. 1-(9′-*O*-α-D-Galactopyranosyl-6′,7′,8′-trideoxy-β-D-*allo*-nonofranosyl)uracil (2b). Compound **2b** (10 μmol, 7.0 mg, quant.) was obtained as a colorless glass from **3b** (10 μmol, 7.0 mg) as described above for the synthesis of **2a**, after purification by column chromatography (C18, 5–10% methanol in H_2O). $[\alpha]_D^{21}$ +22.2 (*c* 1.02, H_2O); ^{1}H NMR (400 MHz, DMSO-d_6, D_2O) δ 7.97 (d, 1H, J=7.8 Hz), 5.77 (d, 1H, J=5.4 Hz), 5.63 (d, 1H, J=7.8 Hz), 4.62 (d, 1H, J=3.4 Hz), 4.01 (m, 2H), 3.97 (m, 1H), 3.76 (m, 1H), 3.69 (br s, 1H), 3.50 (m, 7H), 1.46 (m, 6H); ^{13}C NMR (100 MHz, DMSO-d_6, D_2O) δ 140.5, 101.7, 98.8, 87.6, 86.6, 73.7, 71.1, 70.7, 69.6, 69.3, 68.9, 68.4, 66.9, 60.6, 33.3, 29.1, 22.2; FAB-LRMS *m/z* 479 (MH^+); FAB-HRMS calcd for $C_{19}H_{31}N_2O_{12}$ 479.1877, found 479.1875 (MH^+); FAB-HRMS calcd for $C_{19}H_{31}N_2O_{12}$ 479.1877, found 479.1878 (MH^+).

4.2. β1,4-Galactosyltransferase assay

Assays were performed in a total volume of 100 μl. The assay medium contained ovalbmin (1 mg/ml), 0.1 M sodium cacodylate buffer, 10 mM $MnCl_2$, 2 mM AMP, inhibitor (varying conc.), 1 μCi of UDP-[^{3}H]-galactose, and β1,4-galactosyltransferase (4 mU). The enzyme assay was incubated for 3 h at 37 °C and the reaction stopped with 1 ml of 10% TCA. The precipitate was washed with 10% TCA and once with ethanol/ether (2/1). The precipitate was dissolved in 200 μl of 2 N NaOH and the solution was counted in 2 ml of scintillation fluid by using β-scintillation counter.

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