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Synthesis and anti-angiogenetic activity evaluation of *N*-(3-aryl acryloyl)aminosaccharide derivatives

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ABSTRACT

In order to find novel potent inhibitors for signal pathways of FGF/FGFR, nineteen *N*-(3-aryl acryloyl)aminosaccharide derivatives were designed and synthesized based on the binding sites of FGF and oligosaccharides of heparin. Their structures were confirmed by IR, ^{1}H NMR, ^{13}C NMR, MS and elemental analysis. The nineteen target compounds were evaluated for biological activity against HUVEC cell. In vitro assays showed that compound **10s** (IC_{50} = 5.3 μM) exhibited comparable inhibitory effects on endothelial cell growth with topotecan (IC_{50} = 2.7 μM). Compound **10s** (10 μg/egg) also showed obvious anti-angiogenetic activity in the in vivo chicken chorio allantoic membrane (CAM) assay, and the potency was similar to topotecan (10 μg/egg).

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1. Introduction

Tumor angiogenesis inhibitors can inhibit the formation of new blood vessels and block tumor growth, metastasis and relapse.[1,2] They showed great advantages when compared to traditional anti-tumor drugs. Fibroblast growth factors (FGFs) are an important class of angiogenic factors. Studies have shown that the FGF signaling pathway dysregulation is closely related to tumor blood vessel growth. This signaling pathway particularly plays an important role in Angiogenic Rescue.[3] For example, animal experiment results showed that the anti-VEGFR-2 antibody therapy can induce the release of FGF-2.[4] Therefore, the FGF signal pathway is a potential target for cancer therapy.

Heparin can regulate FGF signal pathway. Heparin/HS is required to form a ternary complex with FGF and FGFR, which stabilize and activate FGF signaling for a long enough period of time to elicit a specific response.[5] Heparin and its analogs have been found as FGF signal pathway regulating agents. For example, PI-88,[6] a mixture of highly sulfated monophosphorylated mannose oligosaccharides, has entered phase III clinical trial as an anti-tumor agent. However, bleeding complications of these heparin analogs limit their clinical application due to the inhibition of thrombin system.[7] The further studies showed that different signal pathways require different heparin sugar chain lengths. The pentose sequence is required for anticoagulant effect. Researchers have found that disaccharide, trisaccharide, or tetrasaccharide heparanoids could regulate FGF signal pathway and have little influence on the thrombin system in the meantime.[8,9] These results suggest that shorter chain oligosaccharides may avoid the inhibition of the thrombin system while retaining the action of FGF signal pathway.

Mono-saccharide-based glycoconjugates such as compound **1** (Fig. 1) exhibit potent bioactivities in both a FGF-2 binding assay and an endothelial cell survival assay.[10] Further more, Rawe et al. found per-O-acetylated glucosamine derivatives such as compound **2** possessing significantly improved inhibitory potency compared to their corresponding polyhydroxylated derivatives.[11,12] Our group also designed and synthesized a series of *N*-heteroaroyl aminosaccharide derivatives, and found compound **3** could inhibit heparin- and FGF-2-dependent BaF3 cell proliferation.[13]

Cinnamic acid derivatives, such as ferulic acid and sodium caffeate, are known to possess notable inhibitory effect on endothelial cell growth due to their tumor angiogenetic inhibitory activity. Here, we designed and synthesized a series of *N*-(3-aryl acryloyl)aminosaccharide derivatives by combining aminosaccharide with cinnamic acid, and evaluated their inhibitory effects on HUVEC cell growth through the MTT assay and their anti-angiogenetic activities through the in vivo CAM assay.

2. Results and discussion

2.1. Synthesis

2,3,4,6-Tetra-*O*-acetyl-1-deoxy-β-D-galactopyranosamine (**G_1-NH_2**),[14,15] 1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-galactopyranosamine

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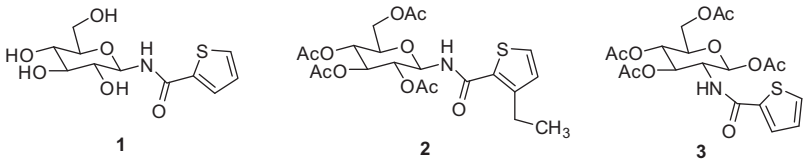


Figure 1. The structures of compound **1**, **2**, and **3**.

($\mathbf{G_2\text{-}NH_2}$), and[16] methyl 6-amino-6-deoxy-α-D-galactopyranoside ($\mathbf{G_3\text{-}NH_2}$)[17–21] were synthesized according to the reported references. 1,3,6-Tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-amino-2-deoxy-β-D-glucopyranose *p*-toluenesulfonate ($\mathbf{G_4\text{-}NH_2}$·TsOH) was synthesized from lactose (**4**) via five steps (Scheme 1).[22–25]

Treatment of hexa-*O*-acetyl-D-lactal (**5**) with cerium(IV) ammonium nitrate and sodium azide gave the azidonitrates **6a**, **6b**, and **6c** (Scheme 1).[22,23] Compound **6c** could be easily removed from the mixture of **6a**, **6b**, and **6c** by column chromatography. However, **6a** and **6b** were difficult to be separated under the same condition because of their similar polarity. When the mixture of **6a** and **6b** (2:1 according to ^{1}H NMR) reacted with LiBr, the compounds **6a** and **6c** were easily converted into bromination product **7**, and the compound **6b** remained unchanged. $\mathbf{G_4\text{-}NH_2}$·TsOH could be obtained from compound **7** by reaction with $Hg(OAc)_2$, followed by hydrogenation in the presence of 10% Pd–C and *p*-toluenesulfonic acid.

Target compounds **10a**–**10f**, **10r**, and **10s** were obtained through the reaction of $\mathbf{G_1\text{-}NH_2}$ or $\mathbf{G_4\text{-}NH_2}$ with the corresponding acyl chloride derived from diverse cinnamic acids. Compounds **10g**–**10p** were obtained by reacting $\mathbf{G_2\text{-}NH_2}$ or $\mathbf{G_3\text{-}NH_2}$ with diverse cinnamic acids using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride/1-hydroxybenzotriazole (EDCI/HOBt) as condensing agent (Scheme 2). In order to avoid undesirable deacetylation, the amount of triethylamine as acid-binding agent should be limited to between 1 and 2 equiv. Compound **10q** was synthesized through acetylation of the compound **10o** (Scheme 3).

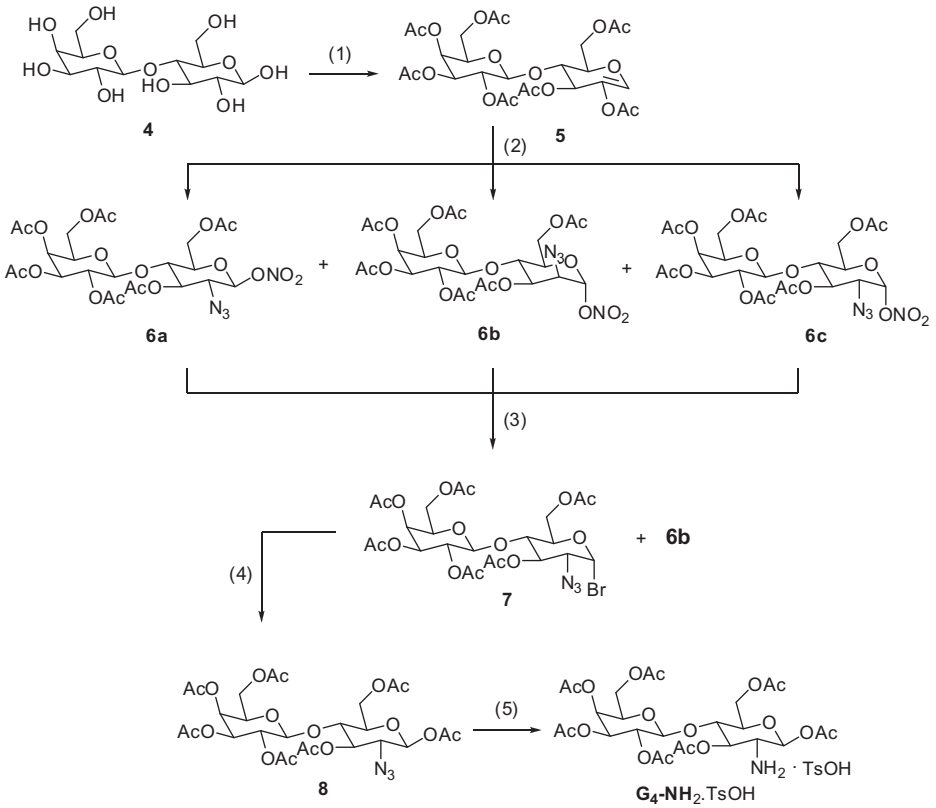
2.2. Biological evaluation and discussion

2.2.1. In vitro MTT assay against HUVEC cell growth

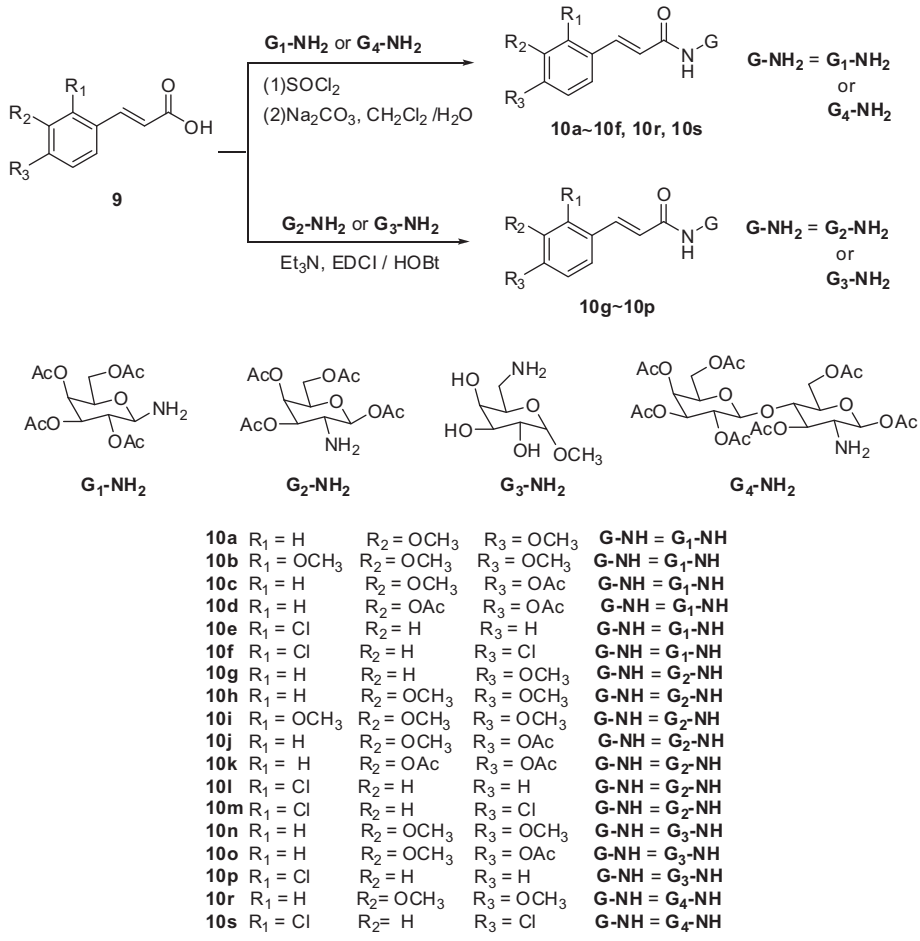
The results of the in vitro MTT assay showed that majority of target compounds exhibited better inhibitory effect on HUVEC cell growth than positive compound **2** except **10d** and **10k** which promoted HUVEC cell proliferation. The IC_{50} value of compounds **10a**, **10j**, **10n**, and **10o** could not be measured due to their poor solubility in DMSO and medium (Table 1).

Among the active compounds, compounds **10l**, **10m**, and **10s** showed good inhibitory effects on HUVEC cell growth. Especially compound **10s** (IC_{50} = 5.3 μM) showed comparable inhibitory activity against HUVEC cell growth with topotecan (IC_{50} = 2.7 μM).

The structure–activity relationship showed that electron-withdrawing group substituted cinnamic acids were more potent than electron-donating group substituted ones when the saccharide portion of conjugates was $\mathbf{G_1\text{-}NH_2}$, the order being 3-methoxy-4-



Scheme 1. Synthesis of compound $\mathbf{G_4\text{-}NH_2}$·**TsOH**. Reagents and conditions: (1) Ac_2O, HBr/HAc, rt, 10 h; $CuSO_4{\cdot}5H_2O$/NaOAc/HAc, 20 °C, 2 h; (2) NaN_3, CAN, CH_3CN, −15 °C, N_2, 16 h; (3) LiBr, CH_3CN, rt, 6 h; (4) $Hg(OAc)_2$/HAc, rt, 17 h; (5) H_2, *p*-TsOH, THF, 10% Pd–C, rt, 6 h.



10a	R_1 = H	R_2 = OCH_3	R_3 = OCH_3	**G-NH = G_1-NH**
10b	R_1 = OCH_3	R_2 = OCH_3	R_3 = OCH_3	**G-NH = G_1-NH**
10c	R_1 = H	R_2 = OCH_3	R_3 = OAc	**G-NH = G_1-NH**
10d	R_1 = H	R_2 = OAc	R_3 = OAc	**G-NH = G_1-NH**
10e	R_1 = Cl	R_2 = H	R_3 = H	**G-NH = G_1-NH**
10f	R_1 = Cl	R_2 = H	R_3 = Cl	**G-NH = G_1-NH**
10g	R_1 = H	R_2 = H	R_3 = OCH_3	**G-NH = G_2-NH**
10h	R_1 = H	R_2 = OCH_3	R_3 = OCH_3	**G-NH = G_2-NH**
10i	R_1 = OCH_3	R_2 = OCH_3	R_3 = OCH_3	**G-NH = G_2-NH**
10j	R_1 = H	R_2 = OCH_3	R_3 = OAc	**G-NH = G_2-NH**
10k	R_1 = H	R_2 = OAc	R_3 = OAc	**G-NH = G_2-NH**
10l	R_1 = Cl	R_2 = H	R_3 = H	**G-NH = G_2-NH**
10m	R_1 = Cl	R_2 = H	R_3 = Cl	**G-NH = G_2-NH**
10n	R_1 = H	R_2 = OCH_3	R_3 = OCH_3	**G-NH = G_3-NH**
10o	R_1 = H	R_2 = OCH_3	R_3 = OAc	**G-NH = G_3-NH**
10p	R_1 = Cl	R_2 = H	R_3 = H	**G-NH = G_3-NH**
10r	R_1 = H	R_2 = OCH_3	R_3 = OCH_3	**G-NH = G_4-NH**
10s	R_1 = Cl	R_2 = H	R_3 = Cl	**G-NH = G_4-NH**

Scheme 2. The structures and synthetic routes of the target compounds **10a**–**10p, 10r**, and **10s**.

Scheme 3. The synthetic route of the target compound **10q**.

Table 1
Inhibitory effects of *N*-(3-aryl acryloyl)aminosaccharide derivatives on endothelial cell proliferation

Compd	IC_{50} (μM)	Compd	IC_{50} (μM)
2	660	**10j**	>1000[a]
Topotecan	2.7	**10k**	*no*[b]
10a	>1000[a]	**10l**	93.1
10b	475.6	**10m**	89
10c	114.3	**10n**	>1000[a]
10d	no[b]	**10o**	>1000[a]
10e	145.71	**10p**	290.1
10f	132.4	**10q**	186.3
10g	1013.9	**10r**	281.2
10h	356.1	**10s**	5.3
10i	312.2		

[a] >1000: Compounds in setting concentrations appeared to different levels of turbidity, and their IC_{50} value could not be measured.
[b] Promote proliferation.

acetoxy > 2,4-dichloro > 2-chloro ≫ 2,3,4-trimethoxy. By comparing compound **10m** versus **10i**, **10p** versus **10n**, and **10s** versus **10r**, coincident conclusion could be obtained that electron-withdrawing group substituted cinnamic acids is beneficial to improve the expected anti-angiogenetic activity when the saccharide portion was substituted with **G_2-NH_2**, **G_3-NH_2**, or **G_4-NH_2**.

On the other hand, electron-withdrawing group substituted cinnamic acids conjugated with different aminosaccharides possessed different levels of inhibitory activities on HUVEC cell growth. Compound **10p** was about two times weaker than **10e** and **10l**. The reason may be the multihydroxy increasing the compounds polarity, which lead to cell membrane impermeability in the in vitro assay although they can be transported into cells through active transportation. The activity of **10s** (IC_{50} = 5.3 μM) was 15–30 times more potent than **10f** and **10m** although they contained the same 3-(2,4-dichlorophenyl) acryloyl group. Thus, we may infer that disaccharide conjugates possessed much stronger proliferation inhibition activities than monosaccharides. It is possible that the

space structure of the disaccharides is better for combination with bFGF than monosacchrides.

2.2.2. In vivo CAM assay

Effects of compounds **10m** and **10s** on angiogenesis of CAM are shown in Figure 2. The anti-angiogenetic activities of compounds **10m** and **10s** were semiquantitatively analyzed using Graph Pad Prism 5.0. (shown in Figure 3). The result showed that both **10m** and **10s** (10 μg, $p < 0.05$) could inhibit the angiogenesis of CAM. The anti-angiogenetic activity of compound **10s** was comparable with topotecan in the in vivo CAM assay at the same dose (10 μg/egg).

3. Conclusions

Nineteen novel *N*-(3-aryl acryloyl)aminosaccharide derivatives were synthesized and evaluated for their anti-angiogenetic activity on HUVEC cells through the MTT assay. The results of bioassay and SAR study showed that disaccharide structure and electron-withdrawing group substituted cinnamic acids are important to improve the expected anti-angiogenetic activity. This conclusion was also corroborated by the results of further CAM study. It pointed out the direction for further modification. What's more, we obtained compound **10s** which had the potential to be developed as novel tumor angiogenesis inhibitor candidate. According to the above conclusion, further investigations will explore the cell biological studies to determine their action mechanism. Since the activity observed is possible in general non-specific inhibition, investigations are also underway to demonstrate whether the aminosaccharide derivatives have the specific modulation of bFGF.

4. Experimental

4.1. General

Melting points were determined on a RDCSY-I capillary apparatus and were uncorrected. The IR spectra (in KBr pellets) were recorded on a Nicolet Impact 410 spectrophotometer. The ^{1}H and ^{13}C

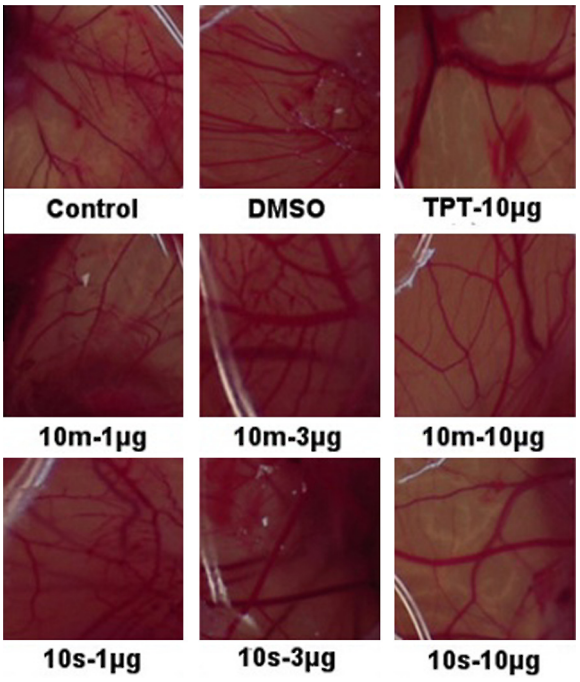


Figure 2. Effects of **10m** and **10s** on angiogenesis of CAM.

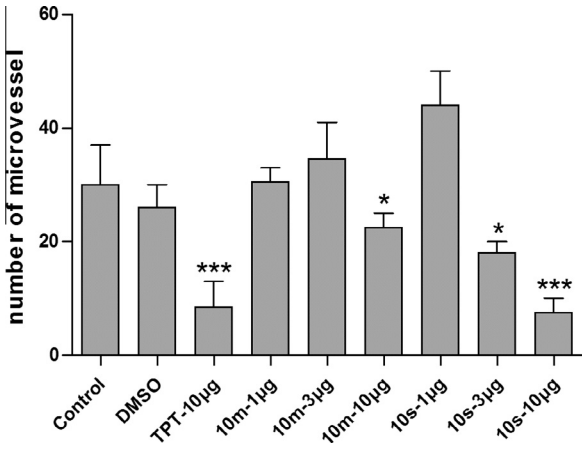


Figure 3. The anti-angiogenetic activity of compounds **10m** and **10s**.

NMR spectra were recorded on a Brucker AV-300 or AV-500 NMR spectrometer using tetramethylsilane (TMS) as an internal standard and chemical shifts were given in d ppm with TMS. Mass spectra were recorded on an Agilent 1100 series LC/MSD Tarp (SL). The Elemental analysis data were obtained using an Elementar Vario EL III instrument. All solvents were purchased from commercial sources and used as received unless otherwise stated.

4.1.1. Hexa-*O*-acetyl-lactal (5)

To a stirring suspension of lactose (5.0 g, 14.6 mmol) in acetic anhydride (13.4 g, 0.13 mol) was added slowly 31% HBr/HAc (5.0 g, 3.5 mL) at room temperature. After stirring for 24 h at room temperature $CuSO_4{\cdot}5H_2O$ (0.91 g, 40 mmol) in 50 mL water and NaOAc (27.4 g, 0.33 mol) in 75 mL acetic acid were added, and the reaction mixture was stirred violently for 2 h keeping temperature at about 20 °C. The mixture was filtered, and the filter cake was washed with ethyl acetate (250 mL), water (250 mL). The organic layer was washed with saturated aqueous $NaHCO_3$ (150 mL) twice and brine (150 mL) twice, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was subjected to flash silica gel column chromatography using petroleum ether–acetic ether (2:1, v/v) to give compound **5** as a clean oil (3.40 g, 41.5%); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 6.42 (1H, d, J = 6.2 Hz, –CH═), 5.41 (1H, dd, J = 3.9 Hz, J = 3.9 Hz, $Glc^{A}H$-3), 5.37 (1H, d, J = 2.7 Hz, $Gal^{B}H$-4), 5.20 (1H, m, $Gal^{B}H$-2), 5.01 (1H, dd, J = 3.3 Hz, J = 10.5 Hz, $Gal^{B}H$-3), 4.84 (1H, dd, J = 3.6 Hz, J = 5.9 Hz, –CH═), 4.67 (1H, d, J = 7.8 Hz, $Gal^{B}H$-1), 4.44 (1H, d, J = 9.6 Hz, $Glc^{A}H$-6a), 4.23–4.09 (4H, m, $Glc^{A}H$-4, $Glc^{A}H$-6b, $Gal^{B}H$-6a, $Gal^{B}H$-6b), 4.00 (1H, m, $Glc^{A}H$-5), 3.98 (1H, dd, J = 6.9 Hz, J = 6.9 Hz, $Gal^{B}H$-5), 2.16, 2.12, 2.09, 2.07, 2.06, 1.98 (each s, each 3H, 6 × OAc).

4.1.2. 3,6-Di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-azido-2-deoxy-α/β-D-glucopyranosyl/mannopyranosyl nitrate (6)

NaN_3 (1.18 g, 18.2 mmol) and ceric ammonium nitrate (16.6 g, 30.3 mmol) were added into 100 mL reaction flask under N_2 protection at temperature of −15 °C. Then **5** (6.80 g, 12.1 mmol) in 68 mL anhydrous acetonitrile was added slowly into reaction flask and the reaction mixture was stirred violently for 16 h. Cold diethyl ether (80 mL) and cold water (80 mL) were added into the reaction solution. The aqueous layer was extracted with cold diethyl ether (60 mL). The combined organic layer was washed with water (80 mL) twice, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was chro-

matographed on a silica gel column by use of petroleum ether–acetic ether (2:1, v/v) to give the compound as follows:

(1) white solid, 4.0 g, R_f = 0.75 (petroleum/ether–acetic ether = 2/1).

4.1.2.1. Compound 6a: 3,6-Di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-azido-2-deoxy-β-D-glucopyranosyl nitrate. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 5.56 (2H, d, J = 8.6 Hz, Glc^{A}H-1).

4.1.2.2. Compound 6b: 3,6-Di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-azido-2-deoxy-α-D-mannopyranosyl nitrate. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 6.13 (1H, d, J = 3.4 Hz, Glc^{A}H-1).

(2) white solid, 1.80 g, R_f = 0.60 (petroleum/ether–acetic ether = 2/1).

4.1.2.3. Compound 6c: 3,6-Di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-azido-2-deoxy-α-D-glucopyranosyl nitrate. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 6.27 (1H, d, J = 4.2 Hz, GlcN^{A}H-1), 5.39–5.36 (2H, m, GlcN^{A}H-3, Gal^{B}H-4), 5.11 (1H, dd, J = 8.0 Hz, J = 10.3 Hz, Gal^{B}H-2), 4.95 (dd, 1H, J = 3.4 Hz, J = 10.4 Hz, Gal^{B}H-3), 4.48 (1H, d, J = 7.9 Hz, Gal^{B}H-1), 4.43 (1H, dd, J = 1.8 Hz, J = 12.3 Hz, GlcN^A H-6a), 4.19–4.15 (2H, m, Gal^{B}H-6a, Gal^{B}H-6b), 4.10–4.06 (2H, m, Gal^B H-5, GlcN^A H-6b), 3.89–3.80 (2H, m, GlcN^A H-4, Gal^{A}H-5), 3.70 (1H, dd, J = 4.2 Hz, J = 10.8 Hz, GlcN^A H-2), 2.17, 2.14, 2.11, 2.06, 2.04, 1.96 (each 3H, each s, 6 × OAc).

4.1.3. 1-Bromo-3,6-di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-azido-2-deoxy-α-D-glucopyranoside (7)

The mixture of **6a** and **6b** (0.5 g, 0.75 mmol) was dissolved in anhydrous acetonitrile (5 mL). Then LiBr (0.5 g) and molecular sieve (1.0 g) were added into above solution. The reaction mixture was stirred at room temperature for 6 h. The filtrate was evaporated under reduced pressure. The residue was chromatographed on a silica gel column by use of petroleum ether–acetic ether (2:1, v/v) to give compound **7** as a white solid (0.26 g, 51.0%), mp 154–155 °C (lit.[26] mp 156–157 °C); ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 6.37 (1H, d, J = 3.9 Hz, GlcN^{A}H-1), 5.49 (1H, d, J = 9.6 Hz, J = 9.6 Hz, GlcN^{A}H-3), 5.37 (1H, d, J = 3.4 Hz, Gal^{B}H-4), 5.13 (1H, m, Gal^{B}H-2), 4.96 (1H, dd, J = 3.4 Hz, J = 10.4 Hz, Gal^{B}H-3), 4.52–4.48 (2H, m, GlcN^{A}H-6a, Gal^{B}H-1), 4.23–4.17 (3H, m, GlcN^A H-6b, Gal^{B}H-6a, Gal^{B}H-6b), 4.10–4.07 (1H, m, Gal^B H-5), 3.91–3.86 (2H, m, GlcN^A H-4, Gal^{A}H-5), 3.68 (1H, dd, J = 10.2 Hz, J = 3.9 Hz, GlcN^A H-2), 2.17, 2.14, 2.13, 2.07, 2.06, 1.97 (each 3H, each s, 6 × OAc); MS(ESI(+)70 V, m/z): 699.1 $[M+NH_4]^+$.

4.1.4. 1,3,6-Tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-azido-2-deoxy-β-D-glucopyranose (8)

Compound **7** (0.3 g, 0.44 mmol) and $Hg(OAc)_2$ (0.28 g, 0.88 mmol) were dissolved in acetic acid (5 mL). The mixture was stirred at room temperature for 17 h. After CH_2Cl_2 (50 mL) was added, the mixture was washed with KBr (40 mL) three times and water (50 mL) twice, dried over anhydrous Na_2SO_4 overnight. The filtrate was evaporated under reduced pressure. The residue was chromatographed on a silica gel column by use of petroleum ether–acetic ether (2:1, v/v) to give **8** as a white solid (0.26 g, 89.7%), mp 72–75 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 5.52 (1H, d, J = 8.5 Hz, GlcN^{A}H-1), 5.38–5.36 (1H, m, Gal^{B}H-4), 5.13–5.07 (2H, m, GlcN^{A}H-3, Gal^{B}H-2), 4.96 (1H, dd, J = 3.4 Hz, J = 10.4 Hz, Gal^{B}H-3), 4.50–4.43 (2H, m, GlcN^{A}H-6a, Gal^{B}H-1), 4.19–4.06 (3H, m, GlcN^A H-6b, Gal^{B}H-6a, Gal^{B}H-6b), 3.89–3.88 (1H, m, Gal^B H-5), 3.77–3.73 (2H, m, GlcN^A H-4, Gal^{A}H-5), 3.58 (1H, d, J = 10.2 Hz, GlcN^A H-2), 2.17, 2.16, 2.13, 2.12, 2.08, 2.03, 1.96 (each 3H, each s, 7 × OAc); IR (cm^{-1}): 2117 (N_3), 1753 (ester, C=O), 1436, 1372, 1219, 1073, 1046, 896; MS(ESI(+)70 V, m/z): 679.1 $[M+NH_4]^+$.

4.1.5. 1,3,6-Tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-amino-2-deoxy-β-D-glucopyranose *p*-toluenesulfonate (G_5-NH_2·TsOH)

Compound **8** (1.3 g, 1.97 mmol) was dissolved in THF (50 mL). Then *p*-TsOH·H_2O (0.37 g, 1.97 mmol) and 10% Pd–C (0.13 g) were added into above solution in turn. The reaction mixture was stirred at room temperature for 6 h. The catalyst was filtered and the filtrate was evaporated under reduced pressure. The residue was chromatographed on a silica gel column by use of dichloromethane methanol (30:1, v/v) to give G_5-NH_2·TsOH as a yellow oil (1.0 g, 61.3%). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.5 (3H, br s, OH, NH_2), 7.71 (2H, d, J = 7.3 Hz, ArH), 7.12 (2H, d, J = 6.5 Hz, ArH), 5.98 (1H, d, J = 8.7 Hz, GlcN^{A}H-1), 5.52 (1H, dd, J = 9.2 Hz, GlcN^A-H-3), 5.31 (1H, d, J = 3.5 Hz, Gal^{B}H-4), 5.04 (1H, dd, J = 7.9 Hz, J = 10.3 Hz, Gal^{B}H-2), 4.92 (1H, dd, J = 3.5 Hz, J = 10.4 Hz, Gal^{B}H-3), 4.48 (1H, d, J = 7.9 Hz, Gal^{B}H-1), 4.43 (1H, d, J = 10.7 Hz, GlcN^A H-6a), 4.12–4.05 (3H, m, Gal^A H-6b, Gal^B H-6a, Gal^B H-6b), 3.84–3.79 (3H, m, GlcN^A H-2, Gal^{A}H-4, Gal^B H-5), 3.35 (1H, m, GlcN^A H-5), 2.36 (3H, s, CH_3), 2.17, 2.14, 2.10, 2.04, 2.01, 1.96, 1.94 (each 3H, each s, 7 × OAc); MS(ESI(+)70 V, m/z): 636.2 $[M+H]^+$;

4.1.6. *N*-(2,3,4,6-Tetra-*O*-acetyl-1-deoxy-β-D-galactopyranosyl)-3-(3,4-dimethoxyphenyl)-2-propenamide (10a·0.5H_2O)

3,4-dimethoxycinnamic acid (0.36 g, 1.73 mmol) was suspended in anhydrous $CHCl_3$ (2 mL). $SOCl_2$ (1 mL) was slowly added to this mixture while cooling in an ice bath, and the resulting solution was stirred at room temperature for 1 h. After the completion of the reaction, the solvent was removed under reduced pressure to give acyl chloride as yellow solid which was used without further purification. The new prepared acyl chloride was dissolved in CH_2Cl_2 (4 mL) and the solution was slowly added into a biphasic mixture of **G_1-NH_2** (0.5 g, 1.44 mmol) in anhydrous CH_2Cl_2 (7 mL) and Na_2CO_3 (0.15 g, 1.4 mmol) in H_2O (7 mL). The reaction mixture was stirred at room temperature for 12 h. Then the organic layer was removed and the aqueous layer was extracted with CH_2Cl_2 (10 mL × 2). The combined organic layer was washed with saturated aqueous $NaHCO_3$ (10 mL × 2) and brine (10 mL × 2), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was chromatographed on a silica gel column by use of petroleum ether–acetic ether (2:1, v/v) to give compound **10a** as a white solid (0.45 g, 58.4%), mp 98–102 °C; $[\alpha]_D^{15.8}$ −9.88 (c 0.2450, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.60 (1H, d, J = 15.6 Hz, –CH=), 7.15 (1H, d, J = 8.4 Hz, ArH), 7.02 (1H, s, ArH), 6.87 (1H, d, J = 8.4 Hz, ArH), 6.40 (1H, d, J = 9.3 Hz, NH), 6.21 (1H, d, J = 15.6 Hz, –CH=), 5.47 (1H, s, H-4), 5.37 (1H, t, J = 9.0 Hz, H-1), 5.23–5.19 (2H, m, H-2, H-3), 4.19–4.10 (3H, m, H-5, H-6a, H-6b), 3.92, 3.88 (each 3H, each s, 2 × OCH_3), 2.17, 2.07, 2.04, 1.95 (each 3H, each s, 4 × OAc); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 171.57, 170.34, 169.98, 169.71 (4C, ester C=O), 166.02 (1C, amide C=O), 151.20, 149.32, 127.29, 122.56, 111.19, 109.84 (6C, ArC), 143.08, 117.19 (2C, CH=CH), 78.84, 72.35, 70.91, 68.52, 67.28, 61.15 (6C, carbohydrate ring carbons), 55.98 (2C, 2 × OCH_3), 20.77, 20.63, 20.56, 20.51 (4C, 4 × CH_3); IR (cm^{-1}): 3466 (NH), 2938 (CH), 1751 (ester, C=O), 1677 (amide, C=O), 1630 (C=C), 1599, 1516, 1466, 1370, 1226, 1083, 1052, 909, 847; MS(ESI(+)70 V, m/z): 538.0 $[M+H]^+$; Anal. Calcd for $C_{25}H_{31}NO_{12}$·0.5H_2O: C, 54.94, H, 5.90, N, 2.56. Found: C, 54.92, H, 6.34, N, 2.64.

4.1.7. *N*-(2,3,4,6-Tetra-*O*-acetyl-1-deoxy-β-D-gluctopyranosyl)-3-(2,3,4-trimethoxyphenyl)-2-propenamide (10b)

3,4,5-Trimethoxycinnamic acid (0.41 g, 1.73 mmol) was suspended in anhydrous $CHCl_3$ (2 mL). $SOCl_2$ (1 mL) was slowly added to this mixture while cooling in an ice bath, and the resulting solu-

tion was stirred at room temperature for 1 h. After the completion of the reaction, the solvent was removed under reduced pressure to give acyl chloride as yellow solid which was used without further purification. The new prepared acyl chloride was dissolved in CH_2Cl_2 (4 mL) and the solution was slowly added into a biphasic mixture of **G_1-NH_2** (0.5 g, 1.44 mmol) in anhydrous CH_2Cl_2 (7 mL) and Na_2CO_3 (0.15 g, 1.4 mmol) in H_2O (7 mL). The reaction mixture was stirred at room temperature for 12 h. Then the organic layer was removed and the aqueous layer was extracted with CH_2Cl_2 (10 mL × 2). The combined organic layer was washed with saturated aqueous $NaHCO_3$ (10 mL × 2) and brine (10 mL × 2), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was chromatographed on a silica gel column by use of petroleum ether–acetic ether (3:2, v/v) to give compound **10b** as a white solid (0.25 g, 30.5%), mp 91–94 °C; $[\alpha]_D^{19.9}$ −1.24 (*c* 0.225, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.80 (1H, d, *J* = 15.9 Hz, –CH═), 7.21 (1H, d, *J* = 8.7 Hz, ArH), 6.69 (1H, d, *J* = 8.7 Hz, ArH), 6.40–6.34 (2H, overlapping, –CH═, NH), 5.47 (1H, s, H-4), 5.39 (1H, s, H-1), 5.23–5.19 (2H, m, H-2, H-3), 4.16–4.10 (3H, m, H-5, H-6a, H-6b), 3.91, 3.87, 3.82 (each 3H, each s, 3 × OCH_3), 2.31 (3H, s, OAc), 2.05, 2.04, 2.00, 1.98 (each 3H, each s, 4 × OAc); IR (cm^{-1}): 3349 (NH), 2941 (CH), 1751 (ester, C═O), 1684 (amide, C═O), 1626 (C═C), 1594, 1536, 1497, 1228, 1096, 1046, 909, 801; MS(ESI(+)70 V, *m*/*z*): 568.0 $[M+H]^+$; Anal. Calcd for $C_{26}H_{33}NO_{13}$: C, 55.02, H, 5.86, N, 2.47. Found: C, 54.70, H, 5.92, N, 2.18.

4.1.8. *N*-(2,3,4,6-Tetra-*O*-acetyl-1-deoxy-β-D-galactopyranosyl)-3-(3-methoxy-4-acetoxyphenyl)-2-propenamide (10c)

Ferulic acid(1.5 g, 7.73 mmol) was dissolved in water (7.7 mL) containing NaOH (0.8 g, 20.6 mmol) in an ice bath. Then Ac_2O was added dropwise and the mixture was stirred for 1.5 h at room temperature. After the reaction completed, the pH was adjusted to 2 with 10% H_2SO_4, and the mixture was stirred for 1 h. The mixture was filtered, and the filter cake was washed three times with water and dried under infrared lamp to give (*E*)-3-(4-acetoxy-3-methoxyphenyl)acrylic acid as gray white solid (1.74 g, 95.35%). (*E*)-3-(4-Acetoxy-3-methoxyphenyl)acrylic acid (0.34 g, 1.44 mmol) was suspended in anhydrous CH_2Cl_2 (10 mL). Oxalyl chloride (0.47 mL, 5.5 mmol) was added dropwise into the solution. Then a drop of DMF was added and the solution was stirred for 24 h at room temperature. The solvent was removed under reduced pressure to give acyl chloride as yellow solid which was used without further purification. The new prepared acyl chloride was dissolved in CH_2Cl_2 (4 mL) and the solution was slowly added into a biphasic mixture of **G_1-NH_2** (0.5 g, 1.44 mmol) in anhydrous CH_2Cl_2 (7 mL) and Na_2CO_3 (0.15 g, 1.4 mmol) in H_2O (7 mL). The reaction mixture was stirred at room temperature for 24 h. Then the organic layer was removed and the aqueous layer was extracted with CH_2Cl_2 (10 mL × 2). The combined organic layer was washed with saturated aqueous $NaHCO_3$ (10 mL × 2) and brine (10 mL × 2), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was chromatographed on a silica gel column by use of petroleum ether–acetic ether (3:2, v/v) to give compound **10c** as a white solid (0.44 g, 54.3%), mp 117–120 °C; $[\alpha]_D^{24}$ −6.53 (*c* 0.095, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.61 (1H, d, *J* = 15.6 Hz, –CH═), 7.12–7.03 (3H, m, ArH), 6.39 (1H, d, *J* = 8.7 Hz, NH), 6.27 (1H, d, *J* = 15.6 Hz, –CH═), 5.47 (1H, d, *J* = 1.5 Hz, H-4), 5.37 (1H, t, *J* = 9.0 Hz, H-1), 5.19–5.17 (2H, m, *J* = 9.6 Hz, H-2, H-3), 4.15–4.08 (3H, m, H-5, H-6a, H-6b), 3.87 (3H, s, OCH_3), 2.32 (3H, s, $COCH_3$), 2.16, 2.06, 2.04, 2.01 (each 3H, each s, 4 × OAc); IR (cm^{-1}): 3357 (NH), 2942 (CH), 1751 (ester, C═O), 1686 (amide, C═O), 1633 (C═C), 1600, 1535, 1512, 1224, 1158, 1123, 1051, 834; MS(ESI(+)70 V, *m*/*z*): 566.0 $[M+H]^+$;-MS(ESI(−)70 V, *m*/*z*): 563.9 $[M-H]^-$; Anal. Calcd for $C_{26}H_{31}NO_{13}$: C, 55.22, H, 5.53, N, 2.48. Found: C, 55.52, H, 5.93, N, 2.17.

4.1.9. *N*-(2,3,4,6-Tetra-*O*-acetyl-1-deoxy-β-D-galactopyranosyl)-3-(3,4-diacetoxyphenyl)-2-propenamide (10d)

Caffeic acid (1.5 g, 8.33 mmol) was dissolved in water (11 mL) containing NaOH (1.15 g, 28.75 mmol) in an ice bath. Then Ac_2O (2.27 mL, 20.82 mmol)was added dropwise and the mixture was stirred for 1.5 h at room temperature. After the reaction completed, the pH was adjusted to 2 with 10% H_2SO_4, and the mixture was stirred for 1 h. The mixture was filtered, and the filter cake was washed three times with water and dried under infrared lamp to give (*E*)-3-(3,4-diacetoxyphenyl)acrylic acid as gray white solid (2.01 g, 91.36%). (*E*)-3-(3,4-Diacetoxyphenyl)acrylic acid (0.46 g, 1.73 mmol) was suspended in anhydrous $CHCl_3$ (2 mL). $SOCl_2$ (1 mL) was added dropwise in an ice bath and the mixture was heated to reflux for 1 h. The solvent was removed under reduced pressure to give acyl chloride as yellow oil which was used without further purification. The new prepared acyl chloride was dissolved in CH_2Cl_2 (4 mL) and the solution was slowly added into a biphasic mixture of **G_1-NH_2** (0.5 g, 1.44 mmol) in anhydrous CH_2Cl_2 (7 mL) and Na_2CO_3 (0.15 g, 1.4 mmol) in H_2O (7 mL). The reaction mixture was stirred at room temperature for 24 h. Then the organic layer was removed and the aqueous layer was extracted with CH_2Cl_2 (10 mL × 2). The combined organic layer was washed with saturated aqueous $NaHCO_3$ (10 mL × 2) and brine (10 mL × 2), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was chromatographed on a silica gel column by use of petroleum ether–acetic ether (3:2, v/v) to give compound **10d** as a white solid (0.48 g, 56.5%), mp 103–105 °C; $[\alpha]_D^{16.5}$ −7.1 (*c* 0.2, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.60 (1H, d, *J* = 15.6 Hz, –CH═), 7.40–7.21 (3H, m, ArH), 6.44 (1H, d, *J* = 8.7 Hz, NH), 6.27 (1H, d, *J* = 15.6 Hz, –CH═), 5.47 (1H, d, *J* = 1.5 Hz, H-4), 5.35 (1H, t, *J* = 9.0 Hz, H-1), 5.18 (2H, m, *J* = 9.6 Hz, H-2, H-3), 4.16–4.10 (3H, m, H-5, H-6a, H-6b), 2.38 (3H, s, $COCH_3$), 2.32 (3H, s, $COCH_3$), 2.17, 2.06, 2.05, 2.01 (each 3H, each s, 4 × OAc); IR (cm^{-1}): 3359 (NH), 3063, 2940 (CH), 1751 (ester, C═O), 1687 (amide, C═O), 1635 (C═C), 1537, 1506, 1221, 1112, 1050, 837; MS(ESI(+)70 V, *m*/*z*): 594.0 $[M+H]^+$; Anal. Calcd for $C_{27}H_{31}NO_{14}$: C, 54.64, H, 5.26, N, 2.36. Found: C, 54.62, H, 5.22, N, 2.17.

4.1.10. *N*-(2,3,4,6-Tetra-*O*-acetyl-1-deoxy-β-D-galactopyranosyl)-3-(2-chlorophenyl)-2-propenamide (10e)

2-Chlorocinnamic acid (0.32 g, 1.73 mmol) was suspended in $SOCl_2$ (1 mL) and the mixture was heated to reflux for 7 h. After the completion of the reaction, the solvent was removed under reduced pressure to give acyl chloride as yellow oil which was used without further purification. The new prepared acyl chloride was dissolved in CH_2Cl_2 (4 mL) and the solution was slowly added into a biphasic mixture of **G_1-NH_2** (0.5 g, 1.44 mmol) in anhydrous CH_2Cl_2 (7 mL) and Na_2CO_3 (0.15 g, 1.4 mmol) in H_2O (7 mL). The reaction mixture was stirred at room temperature for 12 h. Then the organic layer was removed and the aqueous layer was extracted with CH_2Cl_2 (10 mL × 2). The combined organic layer was washed with saturated aqueous $NaHCO_3$ (10 mL × 2) and brine (10 mL × 2), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was chromatographed on a silica gel column by use of petroleum ether–acetic ether (2:1, v/v) to give compound **10e** as a white solid (0.33 g, 44.6%). mp 77–81 °C; $[\alpha]_D^{15.9}$ −9.27 (*c* 0.300, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.59 (1H, d, *J* = 15.6 Hz, –CH═), 7.49 (1H, s, ArH), 7.39–7.29 (3H, m, ArH), 6.49 (1H, d, *J* = 9 Hz, NH), 6.34 (1H, d, *J* = 15.6 Hz, –CH═), 5.47 (1H, s, H-4), 5.37 (1H, t, *J* = 9.0 Hz, H-1), 5.19–5.17 (2H, m, H-2, H-3), 4.16–4.07 (3H, m, H-5, H-6a, H-6b), 2.16, 2.06, 2.04, 2.01 (each 3H, each s, 4 × OAc);IR (cm^{-1}): 3355 (NH), 3066, 2965 (CH), 1751 (ester, C═O), 1688 (amide, C═O), 1636 (C═C), 1594, 1538, 1370, 1225, 1123, 1083, 1052, 790; MS(ESI(+)70 V, *m*/*z*): 512.0 $[M+H]^+$; Anal. Calcd for $C_{23}H_{26}ClNO_{10}$: C, 53.96, H, 5.12, N, 2.74. Found: C, 53.66, H, 5.44, N, 2.43.

4.1.11. *N*-(2,3,4,6-Tetra-*O*-acetyl-1-deoxy-β-D-galactopyranosyl)-3-(2,4-dichlorophenyl)-2-propenamide (10f)

2,4-Dichlorocinnamic acid (0.38 g, 1.73 mmol) was suspended in $SOCl_2$ (1 mL) and the mixture was heated to reflux for 2 h. After the completion of the reaction, the solvent was removed under reduced pressure to give acyl chloride as white oil which was used without further purification. The new prepared acyl chloride was dissolved in CH_2Cl_2 (4 mL) and the solution was slowly added into a biphasic mixture of **G_1-NH_2** (0.5 g, 1.44 mmol) in anhydrous CH_2Cl_2 (7 mL) and Na_2CO_3 (0.15 g, 1.4 mmol) in H_2O (7 mL). The reaction mixture was stirred at room temperature for 12 h. Then the organic layer was removed and the aqueous layer was extracted with CH_2Cl_2 (10 mL × 2). The combined organic layer was washed with saturated aqueous $NaHCO_3$ (10 mL × 2) and brine (10 mL × 2), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was chromatographed on a silica gel column by use of petroleum ether–acetic ether (3:2, v/v) to give compound **10f** as a white solid (0.52 g, 66.3%), mp 95–97 °C; $[\alpha]_D^{16.6}$ −3.06 (*c* 0.085, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.96 (1H, d, *J* = 15.6 Hz, –CH═), 7.52–7.26 (3H, m, ArH), 6.50 (1H, d, *J* = 8.4 Hz, NH), 6.32 (1H, d, *J* = 15.6 Hz, –CH═), 5.47 (1H, d, *J* = 1.5 Hz, H-4), 5.37 (1H, t, *J* = 9.0 Hz, H-1), 5.19–5.17 (2H, m, *J* = 9.6 Hz, H-2, H-3), 4.12–4.10 (3H, m, H-5, H-6a, H-6b), 2.16, 2.07, 2.05, 2.01 (each 3H, each s, 4 × OAc); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 171.60, 170.34, 169.97, 169.69 (4C, ester C═O), 165.07 (1C, amide C═O), 136.32, 135.59, 131.28, 130.64, 128.40, 127.55 (6C, ArC), 137.85, 122.68 (2C, CH═CH), 78.90, 72.48, 70.82, 68.53, 67.26, 61.17 (6C, carbohydrate ring carbons), 20.77, 20.64, 20.56, 20.50 (4C, 4 × CH_3); IR (cm^{-1}): 3369 (NH), 2961, 2935 (CH), 1751 (ester, C═O), 1688 (amide, C═O), 1632 (C═C), 1584, 1537, 1370, 1225, 1123, 1084, 1051, 789; MS(ESI(+)70 V, *m*/*z*): 546.0 $[M+H]^+$; Anal. Calcd for $C_{23}H_{25}Cl_2NO_{10}$: C, 50.56, H, 4.61, N, 2.56. Found: C, 50.27 H, 4.61, N, 2.48.

4.1.12. *N*-(1,3,4,6-Tetra-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-3-(4-methoxyphenyl)-2-propenamide (10g)

G_2-NH_2·HCl (0.5 g, 1.3 mmol) was suspended in anhydrous CH_2Cl_2 (20 mL). Et_3N (0.17 mL, 1.30 mmol) was added to this mixture while cooling in an ice bath, and the resulting solution was stirred at room temperature for 1 h. To this solution, HOBt (0.21 g, 1.56 mmol), 4-Methoxycinnamic acid (0.28 g, 1.56 mmol), and EDCI (0.30 g, 1.56 mmol) were added successively, and the reaction mixture was stirred at room temperature for 36 h. Then the mixture was washed with water (20 mL × 2), saturated aqueous $NaHCO_3$ (20 mL × 2), and brine (20 mL × 2), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was chromatographed on a silica gel column by use of petroleum ether–acetic ether (1:1, v/v) to give compound **10g** as a white solid (0.32 g, 48.6%), mp181–183 °C; $[\alpha]_D^{21}$ +25.68 (*c* 0.19, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.57 (1H, d, *J* = 15.6 Hz, –CH═), 7.44 (2H, d, *J* = 8.7 Hz, ArH), 6.89 (2H, d, *J* = 8.7 Hz, ArH), 6.18 (1H, d, *J* = 15.3 Hz, –CH═), 5.78 (1H, d, *J* = 8.7 Hz, NH), 5.57 (1H, d, *J* = 9.6 Hz, H-1), 5.41 (1H, d, *J* = 2.7 Hz, H-4), 5.17 (1H, dd, *J* = 3.3 Hz, *J* = 11.3 Hz, H-3), 4.63 (1H, dd, *J* = 9.3 Hz, *J* = 10.5 Hz, H-2), 4.23–4.04 (3H, m, H-5, H-6a, H-6b), 3.83 (3H, s, OCH_3), 2.19, 2.11, 2.05, 2.00 (each 3H, each s, 4 × OAc); IR (cm^{-1}): 3329 (NH), 2974, 2941 (CH), 1745 (ester, C═O), 1659 (amide, C═O), 1630 (C═C), 1538, 1451, 1370, 1221, 1080, 1041; MS(ESI(−)70 V, *m*/*z*): 506.0 $[M-H]^-$; Anal. Calcd for $C_{24}H_{29}NO_{11}$: C, 56.80, H, 5.76, N, 2.76. Found: C, 56.95, H, 5.85, N, 2.75.

4.1.13. *N*-(1,3,4,6-Tetra-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-3-(3,4-dimethoxyphenyl)-2-propenamide (10h)

G_2-NH_2·HCl (0.5 g, 1.3 mmol) was suspended in anhydrous CH_2Cl_2 (20 mL). Et_3N (0.17 mL, 1.30 mmol) was added to this mixture while cooling in an ice bath, and the resulting solution was stirred at room temperature for 1 h. To this solution, HOBt (0.21 g, 1.56 mmol), 3,4-dimethoxycinnamic acid (0.33 g, 1.56 mmol), and EDCI (0.30 g, 1.56 mmol) were added successively, and the reaction mixture was stirred at room temperature for 36 h. Then the mixture was washed with water (20 mL × 2), saturated aqueous $NaHCO_3$ (20 mL × 2), and brine (20 mL × 2), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was chromatographed on a silica gel column by use of petroleum ether–acetic ether (1:1, v/v) to give compound **10h** as a white solid (0.44 g, 63.0%), mp 105–108 °C; $[\alpha]_D^{24}$ +23.2 (*c* 0.075, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.55 (1H, d, *J* = 15.5 Hz, –CH═), 7.07 (1H, d, *J* = 8.2 Hz, ArH), 7.01 (1H, d, *J* = 1.88 Hz, ArH), 6.85 (1H, d, *J* = 8.3 Hz, ArH), 6.17 (1H, d, *J* = 15.5 Hz, –CH═), 5.76 (1H, d, *J* = 8.8 Hz, NH), 5.45 (1H, d, *J* = 9.5 Hz, H-1), 5.41 (1H, d, *J* = 2.5 Hz, H-4), 5.15 (1H, dd, *J* = 3.2 Hz, *J* = 11.3 Hz, H-3), 4.63 (1H, dd, *J* = 9.5 Hz, *J* = 10.5 Hz, H-2), 4.22–4.06 (3H, m, H-5, H-6a, H-6b), 3.91 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 2.19, 2.11, 2.05, 2.00 (each 3H, each s, 4 × OAc); IR (cm^{-1}): 3457 (NH), 2965, 2939 (CH), 1750 (ester, C═O), 1662 (amide, C═O), 1626 (C═C), 1516, 1423, 1370, 1264, 1220, 1074, 1040, 846, 810; MS(ESI(+)70 V, *m*/*z*): 538.1 $[M+H]^+$; Anal. Calcd for $C_{25}H_{31}NO_{12}$: C, 55.86, H, 5.81, N, 2.61. Found: C, 55.81, H, 5.87, N, 2.44.

4.1.14. *N*-(1,3,4,6-Tetra-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-3-(2,3,4-trimethoxyphenyl)-2-propenamide (10i)

G_2-NH_2·HCl (0.5 g, 1.3 mmol) was suspended in anhydrous CH_2Cl_2 (20 mL). Et_3N (0.17 mL, 1.30 mmol) was added to this mixture while cooling in an ice bath, and the resulting solution was stirred at room temperature for 1 h. To this solution, HOBt (0.21 g, 1.56 mmol), 2,3,4-trimethoxycinnamic acid (0.37 g, 1.56 mmol), and EDCI (0.30 g, 1.56 mmol) were added successively, and the reaction mixture was stirred at room temperature for 36 h. Then the mixture was washed with water (20 mL × 2), saturated aqueous $NaHCO_3$ (20 mL × 2), and brine (20 mL × 2), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was chromatographed on a silica gel column by use of petroleum ether–acetic ether (2:1, v/v) to give compound **10i** as a white solid (0.38 g, 51.6%), mp 92–94 °C; $[\alpha]_D^{24}$ +21.3 (*c* 0.28, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.75 (1H, d, *J* = 15.8 Hz, –CH═), 7.20 (1H, d, *J* = 8.8 Hz, ArH), 6.67 (1H, d, *J* = 8.8 Hz, ArH), 6.32 (1H, d, *J* = 15.7 Hz, –CH═), 5.77 (1H, d, *J* = 8.8 Hz, NH), 5.51 (1H, d, *J* = 9.6 Hz, H-1), 5.41 (1H, d, *J* = 2.5 Hz, H-4), 5.15 (1H, dd, *J* = 3.2 Hz, *J* = 11.3 Hz, H-3), 4.64 (1H, dd, *J* = 9.4 Hz, *J* = 10.5 Hz, H-2), 4.20–4.07 (3H, m, H-5, H-6a, H-6b), 3.90, 3.89, 3.86 (each 3H, each s, 3 × OCH_3), 2.19, 2.11, 2.05, 2.00 (each 3H, each s, 4 × OAc); IR (cm^{-1}): 3471 (NH), 2942 (CH), 1751 (ester, C═O), 1660 (amide, C═O), 1624 (C═C), 1548, 1437, 1370, 1220, 1096, 1041, 800; MS(ESI(+)70 V, *m*/*z*): 568.2 $[M+H]^+$; MS(ESI(−)70 V, *m*/*z*): 566.1 $[M-H]^-$; Anal. Calcd for $C_{26}H_{33}NO_{13}$: C, 55.02, H, 5.86, N, 2.47. Found: C, 54.71, H, 6.02, N, 2.24.

4.1.15. *N*-(1,3,4,6-Tetra-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-3-(3-methoxy-4-acetoxyphenyl)-2-propenamide (10j)

G_2-NH_2·HCl (0.5 g, 1.3 mmol) was suspended in anhydrous CH_2Cl_2 (20 mL). Et_3N (0.17 mL, 1.30 mmol) was added to this mixture while cooling in an ice bath, and the resulting solution was stirred at room temperature for 1 h. To this solution, HOBt (0.175 g, 1.3 mmol), (*E*)-3-(4-acetoxy-3-methoxyphenyl)acrylic acid (0.31 g, 1.3 mmol), and EDCI (0.25 g, 1.3 mmol) were added successively, and the reaction mixture was stirred at room temperature for 36 h. Then the mixture was washed with water (20 mL × 2), saturated aqueous $NaHCO_3$ (20 mL × 2), and brine (20 mL × 2), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was chromatographed on a silica gel column by use of dichloromethane–methanol (100:1–30:1, v/v)

to give compound **10j** as a white solid (0.17 g, 23.3%), mp 183–185 °C; $[\alpha]_D^{24}$ +20.63 (*c* 0.095, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.56 (1H, d, *J* = 15.6 Hz, –CH═), 7.06 (3H, m, ArH), 6.24 (1H, d, *J* = 15.6 Hz, –CH═), 5.77 (1H, d, *J* = 8.7 Hz, NH), 5.56 (1H, d, *J* = 9.6 Hz, H-1), 5.41 (1H, d, *J* = 3 Hz, H-4), 5.15 (1H, dd, *J* = 3.3 Hz, *J* = 11.3 Hz, H-3), 4.63 (1H, dd, *J* = 9.6 Hz, *J* = 10.5 Hz, H-2), 4.17–4.06 (3H, m, H-5, H-6a, H-6b), 3.86 (3H, s, OCH_3), 2.32 (3H, s, $COCH_3$), 2.19, 2.11, 2.06, 2.00 (each 3H, each s, 4 × OAc); IR (cm^{-1}): 3337 (NH), 2950 (CH), 1745 (ester, C═O), 1663 (amide, C═O), 1630 (C═C), 1601, 1520, 1221, 1126, 1038, 860; MS(ESI(+)70 V, *m*/*z*): 567.0 $[M+H]^+$; MS(ESI(−)70 V, *m*/*z*): 564.0 $[M-H]^-$; Anal. Calcd for $C_{26}H_{31}NO_{13}$: C, 55.22, H, 5.53, N, 2.48. Found: C, 55.32, H, 5.46, N, 2.47.

4.1.16. *N*-(1,3,4,6-Tetra-O-acetyl-2-deoxy-β-D-galactopyranosyl)-3-(3,4-diacetoxyphenyl)-2-propenamide (10k)

G_2-NH_2·HCl (0.5 g, 1.3 mmol) was suspended in anhydrous CH_2Cl_2 (20 mL). Et_3N (0.17 mL, 1.30 mmol) was added to this mixture while cooling in an ice bath, and the resulting solution was stirred at room temperature for 1 h. To this solution, HOBt (0.21 g, 1.56 mmol), (*E*)-3-(3,4-diacetoxyphenyl)acrylic acid (0.41 g, 1.56 mmol), and EDCI (0.30 g, 1.56 mmol) were added successively, and the reaction mixture was stirred at room temperature for 36 h. Then the mixture was washed with water (20 mL × 2), saturated aqueous $NaHCO_3$ (20 mL × 2) and brine (20 mL × 2), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was chromatographed on a silica gel column by use of petroleum ether–acetic ether (1:1, v/v) to give compound **10k** as a white solid (0.34 g, 46.6%), mp 115–119 °C; $[\alpha]_D^{19.8}$ +24.10 (*c* 0.195, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.55 (1H, d, *J* = 15.6 Hz, –CH═), 7.37–7.19 (3H, m, ArH), 6.21 (1H, d, *J* = 15.6 Hz, –CH═), 5.76 (1H, d, *J* = 8.7 Hz, NH), 5.59 (1H, d, *J* = 9.6 Hz, H-1), 5.41 (1H, d, *J* = 3 Hz, H-4), 5.13 (1H, dd, *J* = 3.3 Hz, *J* = 11.4 Hz, H-3), 4.63 (1H, dd, *J* = 9.6 Hz, *J* = 10.5 Hz, H-2), 4.19–4.05 (3H, m, H-5, H-6a, H-6b), 3.49 (3H, s, OCH_3), 2.31 (3H, s, $COCH_3$), 2.19, 2.11, 2.06, 2.00 (each 3H, each s, 4 × OAc); IR (cm^{-1}): 3385 (NH), 2939 (CH), 1751 (ester, C═O), 1668 (amide, C═O), 1632 (C═C), 1544, 1506, 1429, 1219, 1074, 1041; MS(ESI(+)70 V, *m*/*z*): 594.1 $[M+H]^+$; Anal. Calcd for $C_{27}H_{31}NO_{14}·0.5H_2O$: C, 53.82, H, 5.35, N, 2.32. Found: C, 53.91, H, 5.26, N, 1.90.

4.1.17. *N*-(1,3,4,6-Tetra-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-3-(2-chlorophenyl)-2-propenamide (10l)

G_2-NH_2·HCl (0.5 g, 1.3 mmol) was suspended in anhydrous CH_2Cl_2 (20 mL). Et_3N (0.17 mL, 1.30 mmol) was added to this mixture while cooling in an ice bath, and the resulting solution was stirred at room temperature for 1 h. To this solution, HOBt (0.21 g, 1.56 mmol), 2-Chlorocinnamic acid (0.29 g, 1.56 mmol), and EDCI (0.30 g, 1.56 mmol) were added successively, and the reaction mixture was stirred at room temperature for 36 h. Then the mixture was washed with water (20 mL × 2), saturated aqueous $NaHCO_3$ (20 mL × 2), and brine (20 mL × 2), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was chromatographed on a silica gel column by use of petroleum ether–acetic ether (1:1, v/v) to give compound **10l** as a white solid (0.36 g, 54.5%), mp 100–102 °C; $[\alpha]_D^{24}$ +18.4 (*c* 0.075, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.54 (1H, d, *J* = 15.6 Hz, –CH═), 7.47 (1H, s, ArH), 7.35–7.27 (3H, m, ArH), 6.32 (1H, d, *J* = 15.6 Hz, –CH═), 5.81 (2H, d, *J* = 8.8 Hz, overlapping, NH, H-1), 5.42 (1H, d, *J* = 2.7 Hz, H-4), 5.21 (1H, dd, *J* = 3.1 Hz, *J* = 11.2 Hz, H-3), 4.60 (1H, dd, *J* = 9.3 Hz, *J* = 10.5 Hz, H-2), 4.22–4.08 (3H, m, H-5, H-6a, H-6b), 2.20, 2.11, 2.05, 2.00 (each 3H, each s, 4 × OAc); ^{13}C NMR(300 MHz, $CDCl_3$) δ (ppm): 170.75, 170.32, 170.10, 169.50 (4C, ester C═O), 165.48 (1C, amide C═O), 136.19, 134.90, 130.08, 129.91, 127.55, 126.20 (4C, ArC), 140.80, 120.97 (2C, CH═CH), 93.08, 71.99, 70.43, 66.41, 61.28, 50.06 (6C, carbohydrate ring carbons), 20.82, 20.61, 20.60, 20.58 (4C, 4 × CH_3), 15.60 (1C, CH_3); IR (cm^{-1}): 3375 (NH), 3073, 2970 (CH), 1751 (ester, C═O), 1666 (amide, C═O), 1631 (C═C), 1547, 1431, 1369, 1221, 1077, 1041, 788, 745; MS(ESI(−)70 V, *m*/*z*): 509.9 $[M-H]^-$; Anal. Calcd for $C_{23}H_{26}ClNO_{10}$: C, 53.96, H, 5.12, N, 2.74. Found: C, 53.91, H, 5.42, N, 2.44.

4.1.18. *N*-(1,3,4,6-Tetra-O-acetyl-2-deoxy-β-D-galactopyranosyl)-3-(3,4-dichlorophenyl)-2-propenamide (10m)

G_2-NH_2·HCl (0.5 g, 1.3 mmol) was suspended in anhydrous CH_2Cl_2 (20 mL). Et_3N (0.17 mL, 1.30 mmol) was added to this mixture while cooling in an ice bath, and the resulting solution was stirred at room temperature for 1 h. To this solution, HOBt (0.21 g, 1.56 mmol), 2,4-dichlorocinnamic acid (0.34 g, 1.56 mmol), and EDCI (0.30 g, 1.56 mmol) were added successively, and the reaction mixture was stirred at room temperature for 36 h. Then the mixture was washed with water (20 mL × 2), saturated aqueous $NaHCO_3$ (20 mL × 2), and brine (20 mL × 2), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was chromatographed on a silica gel column by use of petroleum ether–acetic ether (1:1, v/v) to give compound **10m** as a white solid (0.38 g, 53.5%), mp 153–156 °C; $[\alpha]_D^{24}$ +16.22 (*c* 0.185, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.91 (1H, d, *J* = 15.6 Hz, –CH═), 7.50 (1H, d, *J* = 8.4 Hz, ArH), 7.43 (1H, s, ArH), 7.24 (1H, d, *J* = 8.7 Hz, ArH), 6.132 (1H, d, *J* = 15.6 Hz, –CH═), 5.87 (1H, d, *J* = 9.3 Hz, NH), 5.81 (1H, d, *J* = 8.7 Hz, H-1), 5.41 (1H, d, *J* = 2.4 Hz, H-4), 5.19 (1H, dd, *J* = 3.3 Hz, *J* = 11.4 Hz, H-3), 4.61 (1H, dd, *J* = 9.3 Hz, *J* = 10.5 Hz, H-2), 4.23–4.07 (3H, m, H-5, H-6a, H-6b), 2.20, 2.12, 2.06, 2.01 (each 3H, each s, 4 × OAc); IR (cm^{-1}): 3342 (NH), 3069, 2973 (CH), 1748 (ester, C═O), 1665 (amide, C═O), 1629 (C═C), 1532, 1471, 1373, 1223, 1081, 1044; MS(ESI(−)70 V, *m*/*z*): 543.9 $[M-H]^-$; Anal. Calcd for $C_{23}H_{25}Cl_2NO_{10}$: C, 50.56, H, 4.61, N, 2.56. Found: C, 50.40, H, 4.64, N, 2.15.

4.1.19. Methyl 6-[3-(3,4-dimethoxyphenyl)-propenamido]-6-deoxy-α-D-galactopyranoside (10n)

3,4-Dimethoxycinnamic acid (0.46 g, 2.2 mmol), HOBt (0.3 g, 2.2 mmol) and EDCI (0.42 g, 2.2 mmol) were dissolved in anhydrous DMF(25 mL). **G_3-NH_2** (0.42 g, 2.2 mmol) dissolved in DMF(3 mL) was slowly added into this solution and the reaction mixture was stirred at room temperature for 24 h. Then the mixture was concentrated under reduced pressure. The residue was chromatographed on a silica gel column by use of dichloromethane–methanol (20:1, v/v) to give compound **10n** as a white solid (0.42 g, 50.0%), mp 113–114 °C; $[\alpha]_D^{20.8}$ +86.2 (*c* 0.0650, CH_3OH); ^1H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.01 (1H, t, *J* = 5.9 Hz, CONH), 7.36 (1H, d, *J* = 15.7, –CH═), 7.15 (1H, d, *J* = 1.9 Hz, ArH), 7.11 (1H, dd, *J* = 2.0 Hz, *J* = 8.4 Hz, ArH), 6.98 (1H, d, *J* = 8.4 Hz, ArH), 6.56 (1H, d, *J* = 15.7 Hz, –CH═), 4.56 (1H, d, *J* = 3.6 Hz, H-1), 4.55–4.47 (3H, m, 3 × OH), 3.79 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 3.68–3.64 (2H, m, H-5, H-2), 3.59–3.39 (3H, m, H-3, H-4, H-6a), 3.24 (3H, s, OCH_3), 3.17 (1H, m, H-6b); IR (cm^{-1}): 3397 (br, NH, CH), 2936, 2838 (CH), 1657, 1614 (amide, C═O), 1599, 1515, 1264, 1141, 1079, 1023, 847; MS(ESI(+)70 V, *m*/*z*): 384.2 $[M+H]^+$; Anal. Calcd for $C_{18}H_{25}NO_8.H_2O$: C, 53.86, H, 6.78, N, 3.49. Found: C, 53.70, H, 6.74, N, 3.65.

4.1.20. Methyl 6-[3-(3-methoxy-4-acetoxyphenyl)-propenamido]-6-deoxy-α-D-galacto-pyranoside (10o)

3-Methoxy-4-acetoxycinnamic acid (1.25 g, 5.3 mmol), HOBt (0.72 g, 5.3 mmol) and EDCI (1.02 g, 5.3 mmol) were dissolved in anhydrous DMF(50 mL). **G_3-NH_2** (1.02 g, 5.3 mmol) dissolved in DMF(6 mL) was slowly added into this solution and the reaction mixture was stirred at room temperature for 24 h. Then the mix-

ture was concentrated under reduced pressure. The residue was chromatographed on a silica gel column by use of dichloromethane–methanol (20:1, v/v) to give compound **10o** as a white solid (1.26 g, 57.8%), mp 119–124 °C; $[\alpha]_D^{22.1}$ +120.25 (*c* 0.0800, CH_3OH); ^1H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.11 (1H, t, *J* = 5.8 Hz, CONH), 7.42 (1H, d, *J* = 15.8 Hz, –CH═), 7.31 (1H, d, *J* = 1.8 Hz, ArH), 7.15 (1H, dd, *J* = 1.8 Hz, *J* = 8.3H, ArH), 7.11 (1H, d, *J* = 8.1 Hz, ArH), 6.68 (1H, d, *J* = 15.8 Hz, –CH═), 4.55 (1H, d, *J* = 3.6 Hz, H-1), 4.56–4.48 (3H, m, 3 × OH), 3.82 (3H, s, OCH_3), 3.68–3.65 (2H, m, H-5, H-2), 3.59–3.52 (3H, m, H-3, H-4, H-6a), 3.24 (3H, s, OCH_3), 3.20 (1H, m, H-6b), 2.27 (3H, s, OAc); ^{13}C NMR(300 MHz, $CDCl_3$) δ (ppm): 168.35 (1C, ester C═O), 165.24 (1C, amide C═O), 151.04, 140.15, 133.88, 123.20, 122.38, 111.60 (6C, ArC), 140.15, 120.00 (2C, CH═CH), 100.11 (1C, C-1), 69.40, 69.33, 68.64, 68.26 (4C, C-2, C-3, C-4, C-5), 40.37 (C-6), 55.78, 54.45 (2C, 2 × OCH_3), 20.32 (1C, CH_3); IR (cm^{-1}):3422 (br, NH, OH), 2939, 2841 (CH), 1762 (ester, C═O), 1659, 1620 (amide, C═O), 1549, 1263, 1199, 1155, 1124, 1033, 788; MS(ESI(−)70 V, *m*/*z*): 410.1 $[M-H]^-$; Anal. Calcd for $C_{19}H_{25}NO_9$: C, 55.47, H, 6.13, N, 3.40. Found: C, 55.28, H, 6.45, N, 3.29.

4.1.21. Methyl 6-[3-(2-chloro)-propenamido]-6-deoxy-α-D-galactopyranoside (10p)

2-Chlorocinnamic acid (0.4 g, 2.2 mmol), HOBt (0.3 g, 2.2 mmol) and EDCI (0.42 g, 2.2 mmol) were dissolved in anhydrous DMF(25 mL). **G_3-NH_2** (0.42 g, 2.2 mmol) dissolved in DMF(3 mL) was slowly added into this solution and the reaction mixture was stirred at room temperature for 24 h. Then the mixture was concentrated under reduced pressure. The residue was chromatographed on a silica gel column by use of dichloromethane–methanol (20:1, v/v) to give compound **10p** as a white solid (0.42 g, 57.8%), mp 105–107 °C; $[\alpha]_D^{21.0}$ +100.5 (*c* 0.1100, CH_3OH); ^1H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.14 (1H, t, *J* = 5.8 Hz, CONH), 7.62 (1H, s, ArH), 7.52 (1H, m, ArH), 7.42 (3H, m, ArH, –CH═), 6.75 (1H, d, *J* = 15.8 Hz, –CH═), 4.55 (1H, d, *J* = 3.6 Hz, H-1), 4.56–4.48 (3H, m, 3 × OH), 3.68–3.64 (2H, m, H-5, H-2), 3.59–3.41 (3H, m, H-3, H-4, H-6a), 3.24 (3H, s, OCH_3), 3.17 (1H, m, H-6b); IR (cm^{-1}):3416 (br, NH, OH), 2935, 2838 (CH), 1660, 1621 (amide, C═O), 1565, 1228, 1193, 1146, 1127, 1044, 785; MS(ESI(+)70 V, *m*/*z*): 358.2 $[M+H]^+$; MS(ESI(−)70 V, *m*/*z*: 356.0 $[M-H]^-$; Anal. Calcd for $C_{16}H_{20}ClNO_6 \cdot 0.75H_2O$: C, 51.76, H, 5.84, N, 3.77. Found: C, 51.70, H, 5.61, N, 4.26.

4.1.22. Methyl 2,3,4-tri-*O*-acetyl-6-[3-(3-meoxy-4-acetoxyphenyl)-propenamido]- 6-deoxy-α-D-galactopyranoside (10q)

In a 100 mL round bottom flask, were placed **10p** (0.3 g, 0.73 mmol), 4-DMAP (0.05 g, 0.37 mmol), Et_3N (0.05 mL, 0.37 mmol), Ac_2O (0.69 mL, 7.3 mmol), and Pyridine (43 mL). The mixture was stirred for 12 h at room temperature. The solvent was removed under reduced pressure and the residue was chromatographed on a silica gel column by use of petroleum ether–acetic ether (1:1, v/v) to give compound **10q** as a white solid (0.29 g, 74.4%), mp 224–226 °C; $[\alpha]_D^{21.9}$ +73.6 (*c* 0.1250, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.58 (1H, d, *J* = 15.6 Hz, –CH═), 7.11–7.02 (3H, m, ArH), 6.31 (1H, d, *J* = 15.6 Hz, –CH═), 5.97 (1H, t, CONH), 5.44 (1H, d, *J* = 3.3 Hz, H-4), 5.36 (1H, dd, *J* = 10.8 Hz, *J* = 3.4 Hz, H-3), 5.17 (1H, dd, *J* = 10.8 Hz, *J* = 3.6 Hz, H-2), 4.99 (1H, d, *J* = 3.6 Hz, H-1), 4.14–4.10 (1H, m, H-5), 3.86 (3H, s, OCH_3), 3.62–3.56 (1H, m, H-6a), 3.40 (3H, s, OCH_3), 3.37–3.31 (1H, m, H-6b), 2.31, 2.19, 2.09, 2.00 (each 3H, each s, 4 × OAc); ^{13}C NMR(300 MHz, $CDCl_3$) δ (ppm): 170.88, 170.36, 169.71, 168.69 (4C, ester C═O), 165.70 (1C, amide C═O), 151.31, 141.06, 133.61, 123.11, 120.76, 111.34 (6C, ArC), 140.94, 120.32 (2C, CH═CH), 97.20 (1C, C-1), 69.26 (1C, C-4), 68.29 (1C, C-2), 67.58 (1C, C-3), 66.58 (1C, C-5), 38.91 (1C, C-6), 55.89, 55.51 (2C, 2 × OCH_3), 20.72, 20.65, 20.54, 20.54 (4C, 4 × CH_3);IR (cm^{-1}):3409 (NH), 2948, 2842 (CH), 1762 (ester, C═O), 1661, 1630 (amide, C═O), 1517, 1227, 1200, 1046, 837; MS(ESI(+)70 V, *m*/*z*): 538.2 $[M+H]^+$; Anal. Calcd for $C_{25}H_{31}NO_{12}$: C, 55.86, H, 5.81, N, 2.61. Found: C, 55.54, H, 6.17, N, 2.47.

4.1.23. *N*-[1,3,6-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-2-amino-2-deoxy-β-D-glucopyranosyl]-3-(3,4-dimethoxyphenyl)-2-propenamide (10r)

3,4-dimethoxycinnamic acid (0.13 g, 0.62 mmol) was suspended in anhydrous $CHCl_3$ (1 mL). $SOCl_2$ (0.5 mL) was slowly added to this mixture while cooling in an ice bath, and the resulting solution was stirred at room temperature for 1 h. After the completion of the reaction, the solvent was removed under reduced pressure to give acyl chloride as yellow solid which was used without further purification. The new prepared acyl chloride was dissolved in CH_2Cl_2 (4 mL) and the solution was slowly added into a biphasic mixture of **G_4-NH_2·TsOH** (0.5 g, 0.62 mmol) in anhydrous CH_2Cl_2 (7 mL) and Na_2CO_3 (0.15 g, 1.4 mmol) in H_2O (7 mL). The reaction mixture was stirred at room temperature for 12 h. Then the organic layer was removed and the aqueous layer was extracted with CH_2Cl_2 (10 mL × 2). The combined organic layer was washed with saturated aqueous $NaHCO_3$ (10 mL × 2) and brine (10 mL × 2), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was chromatographed on a silica gel column by use of petroleum ether–acetic ether (1:1, v/v) to give compound **10r** as a white solid (0.36 g, 58.4%), mp 127–130 °C; $[\alpha]_D^{23}$ +26.6 (*c* 0.1150, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.55 (1H, d, *J* = 15.6 Hz, –CH═), 7.07 (1H, dd, *J* = 8.3 Hz, *J* = 1.8 Hz, ArH), 7.02 (1H, d, *J* = 1.8 Hz, ArH), 6.84 (1H, d, *J* = 8.3 Hz, ArH), 6.25 (1H, d, *J* = 15.6 Hz, –CH═), 6.04 (1H, d, *J* = 9.8 Hz, NH), 5.71 (1H, d, *J* = 7.9 Hz, $GlcN^A$H-1), 5.37 (1H, d, *J* = 3.4 Hz, Gal^BH-4), 5.18–5.12 (2H, m, $GlcN^A$ H-3, Gal^BH-2), 4.99 (1H, dd, *J* = 3.4 Hz, *J* = 10.4 Hz, Gal^BH-3), 4.53 (1H, d, *J* = 7.9 Hz, Gal^B-H-1), 4.49–4.43 (2H, m, $GlcN^A$ H-2, $GlcN^A$ H-6a), 4.19–4.08 (3H, m, Gal^A H-6b, Gal^B H-6a, Gal^B H-6b), 3.92–3.89 (2H, m, Gal^AH-5, Gal^B H-5), 3.88 (6H, s, 2 × OCH_3), 3.83–3.80 (1H, m, $GlcN^A$ H-4), 2.15, 2.13, 2.10, 2.06, 2.04, 1.97, 1.91 (each 3H, each s, 7 × OAc); ^{13}C NMR(500 MHz, $CDCl_3$) δ (ppm): 170.67, 170.31, 170.26, 170.04, 169.96, 169.41, 169.37 (7C, ester C═O), 166.07 (1C, amide C═O), 150.85, 149.14, 127.41, 122.26, 111.07, 109.78 (6C, ArC), 141.98, 117.49 (2C, CH═CH), 100.82, 92.55, 76.75, 74.88, 73.55, 72.20, 70.79, 69.02, 66.63, 62.08, 60.80, 51.93 (12C, carbohydrate ring carbons), 55.89, 55.83 (2C, 2 × OCH_3), 20.84, 20.74, 20.57, 20.54, 20.53, 20.48, 20.41 (7C, 7 × CH_3); IR (cm^{-1}): 3481 (NH), 2960 (CH), 1752 (ester, C═O), 1664 (amide, C═O), 1628 (C═C), 1516, 1424, 1372, 1223, 1064, 897, 846; MS(ESI(+)70 V, *m*/*z*): 826.1 $[M+H]^+$; MS(ESI(−)70 V, *m*/*z*): 824.3 $[M-H]^-$; Anal. Calcd for $C_{37}H_{47}NO_{20}$: C, 53.82, H, 5.74, N, 1.70. Found: C, 53.80, H, 5.73, N, 1.48.

4.1.24. *N*-[1,3,6-Tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-amino-2-deoxy-β-D-glucopyranosyl]-3-(2,4-dichlorophenyl)-2-propenamide (10s)

2,4-Dichlorocinnamic acid (0.14 g, 0.62 mmol) was suspended in $SOCl_2$ (0.5 mL) and the mixture was heated to reflux for 2 h. After the completion of the reaction, the solvent was removed under reduced pressure to give acyl chloride as white solid which was used without further purification. The new prepared acyl chloride was dissolved in CH_2Cl_2 (4 mL) and the solution was slowly added into a biphasic mixture of **G_4-NH_2.TsOH** (0.5 g, 0.62 mmol) in anhydrous CH_2Cl_2 (7 mL) and Na_2CO_3 (0.15 g, 1.4 mmol) in H_2O (7 mL). The reaction mixture was stirred at room temperature for 12 h. Then the organic layer was removed and the aqueous layer was extracted with CH_2Cl_2 (10 mL × 2). The combined organic layer was washed with saturated aqueous $NaHCO_3$ (10 mL × 2) and brine

(10 mL × 2), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was chromatographed on a silica gel column by use of petroleum ether–acetic ether (1:1, v/v) to give compound **10s** as a white solid (0.19 g, 36.5%), mp 109–110 °C; $[\alpha]_D^{23}$ +14.2 (*c* 0.1200, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.92 (1H, d, *J* = 15.7 Hz, –CH=), 7.51 (1H, d, *J* = 8.5 Hz, ArH), 7.41 (1H, d, *J* = 2.1 Hz, ArH), 7.23 (1H, dd, *J* = 2.1 Hz, *J* = 8.4 Hz, ArH), 6.41 (1H, d, *J* = 15.7 Hz, –CH=), 6.30 (1H, d, *J* = 9.7 Hz, NH), 5.73 (1H, d, *J* = 7.5 Hz, $GlcN^AH$-1), 5.37 (1H, d, *J* = 3.4 Hz, Gal^BH-4), 5.18–5.11 (2H, m, $GlcN^A$ H-3, Gal^BH-2), 4.99 (1H, dd, *J* = 3.4 Hz, *J* = 10.5 Hz, Gal^BH-3), 4.52 (1H, d, *J* = 7.8 Hz, Gal^B-H-1), 4.49–4.44 (2H, m, $GlcN^A$ H-2, $GlcN^A$ H-6a), 4.21–4.11 (3H, m, Gal^A H-6b, Gal^B H-6a, Gal^B H-6b), 3.94–3.84 (3H, m, Gal^AH-5, Gal^A-H-4, Gal^B H-5), 2.15, 2.14, 2.10, 2.09, 2.08, 2.01, 1.99 (each 3H, each s, 7 × OAc); ^{13}C NMR(500 MHz, $CDCl_3$) δ (ppm): 170.51, 170.30, 170.25, 170.02, 169.96, 169.56, 169.33 (7C, ester C=O), 165.10 (1C, amide C=O), 135.89, 135.40, 131.44, 129.90, 128.27, 127.38 (6C, ArC), 136.69, 123.05 (2C, CH=CH), 100.67, 92.41, 74.40, 73.48, 71.89, 70.85, 70.72, 69.01, 66.65, 62.20, 60.82, 51.73 (12C, carbohydrate ring carbons), 20.87, 20.75, 20.62, 20.54, 20.43 (7C, 7 × CH_3); IR (cm^{-1}): 3463 (NH), 2938 (CH), 1751 (ester, C=O), 1668 (amide, C=O), 1630 (C=C), 1583, 1548, 1433, 1371, 1225, 1067, 898, 863; MS(ESI(−)70 V, *m*/*z*): 833.9 $[M-H]^-$; Anal. Calcd for $C_{35}H_{41}Cl_2NO_{18}$: C, 50.37, H, 4.95, N, 1.68. Found: C, 50.58, H, 4.95, N, 1.65.

4.2. Bioassay

4.2.1. In vitro MTT assay against HUVEC cell growth

HUVEC cells were seeded at a density of 4.0×10^3 cells/well in 96-well plates and cultured at 37 °C in a 5% CO_2 humidified incubator for 24 h. Compounds were added to the wells at final concentrations of 0.01, 0.1, 1, 10, 100, and 1000 μg/mL. Control wells were prepared by addition of DMEM. Wells containing DMEM without cells were used as blanks. The plates were incubated at 37 °C in a 5% CO_2 incubator for 48 h. Upon completion of the incubation, stock MTT reagent (20 μL, purchased from American Fluka Company) was added to each well. After 4 h of incubation, the supernatant was removed and DMSO (100 μL, 0.5 mol/L) was added to dissolve the MTT. Then, shaken for 15 min on the oscillator at room temperature. OD value was measured with Thermo Multiskan system in the wave length of 570 nm. Data were analyzed with GWBASIC logarithmic methods. The inhibitory activity of compounds was expressed as IC_{50} (concentration of 50% cytotoxicity, which was extrapolated from linear regression analysis of experimental data).

4.2.2. In vivo CAM assay

Fertilized chicken eggs (Guangda Co. Ltd., Zhejiang, China) were positioned in a horizontal position and incubated at 37 °C under 60% relative humidity in an egg incubator equipped with a turner which automatically turned eggs 6 times/day. On day 6, a rectangle window (1.0 cm × 1.0 cm) was cut in the shell as a portal of access for the CAM. Seal the pseudo air chamber with transparent tape. The eggs were returned back to the incubator. On day-10, the eggs were divided into following groups: model group, DMSO group and topotecan group of (10 μg/egg), and the high, medium, low dose group for each candidate compound (10, 3, 1 μg/egg). Each group has three eggs. After adding drugs, the eggs were returned back to the incubator. On day-13, the transparent tape was removed and fixative containing equal methanol and acetone was dropped on the observation window. Then the CAMs were excised, fixed, and photographed.

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