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Synthesis of allose-templated hydroxyornithine and hydroxyarginine analogs

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ABSTRACT

Conformationally constrained amino acid analogs are widely used to probe the bioactive conformation of peptides. In this paper we report on the synthesis of hexafunctional allose-templated L- and D-hydroxyornithine and L- and D-hydroxyarginine analogs in which the allose-based polyol scaffold constrains the side chain of hydroxyornithine and hydroxyarginine in an extended conformation. The partially protected building blocks were selected for future use in solid-phase peptide synthesis using the Fmoc-strategy. The synthesis starts from a previously prepared *C*-glucosyl glycine analog. Multiple chemical protection–deprotection steps and an oxidation are used to prepare 3-keto-*C*-glucosyl analogs that serve as a precursor to install an amino function via reductive amination. Guanidinylation of the amino group provides access to allose-templated hydroxyarginine analogs. Both hexafunctional building blocks are further chemically modified to provide suitable protection for solid-phase peptide synthesis using the Fmoc-strategy.

1. Introduction

Conformationally constrained amino acids have found wide applications as building blocks to probe the bioactive conformation(s) of peptides when binding to receptors.[1–3] Previous work from our laboratory has focused on the synthesis and properties of polyhydroxylated and conformationally constrained amino acids such as carbohydrate-templated (hydroxy)proline analogs,[4–7] carbohydrate-templated lysine analogs,[8,9] and carbohydrate-templated (hydroxy)proline-lysine chimera.[10] Carbohydrate-templated amino acids (CTAAs) form a class of synthetic glycosylamino acids in which the cyclic polyol scaffold of carbohydrates is part of the side chain of naturally occurring amino acids. The relative rigidity of the pyran ring combined with the polyfunctional nature of the carbohydrate scaffold provides unique opportunities to tune the chemical, physical and conformational properties of amino acids. In addition, decoration of the polyol scaffold with hydrophobic or hydrophilic substituents can be used to adjust the pharmacokinetic properties of CTAAs.

Incorporation of CTAAs into bioactive peptides provides access to novel peptidomimetics that may overcome the inherent problems of peptide-based drugs associated with poor pharmacokinetics, bioavailability and tissue absorption, and proteolytic cleavage of the peptide bonds.[11–13] Furthermore, the incorporation of the carbohydrate-based polyol scaffold into the side chain of amino acids may mimic post-translational hydroxylation in peptides and proteins. For instance, lysine-, arginine-, and proline-containing peptides/proteins can undergo post-translational mono- or di-hydroxylation to hydroxylysines,[14] hydroxyarginines,[15] and hydroxyprolines.[14] In some cases hydroxylation is followed by glycosylation as in the case of hydroxylysine and hydroxyproline.[14,16] The biological and conformational implications of this 'double post-translational modification' are currently poorly understood.

In this paper we report on the synthesis of allose-templated hydroxyornithine (AlloTHyOrn) and allose-templated hydroxarginine analogs (AlloTHyArg) in which the cationic side chain is presented in an extended conformation (Fig. 1). The glucose-derived allose scaffold was selected because of the relative stability of the $^4C_1$ chair conformation and expected high reactivity of the equatorial hydroxyl groups. Ornithine and arginine were selected due to their frequent occurrence in many biologically active peptides including antimicrobial peptides[17] and cell penetrating peptides.[18] Several approaches to conformationally constrained arginine analogs have been described.[19–26] However, none of these described analogs can be used to incorporate additional diversity and to probe the effects of single and double post-translational modifications in peptides.

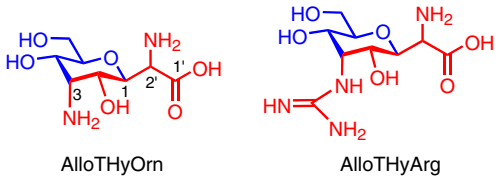

Figure 1. Structure of hexafunctional allose-templated hydroxyornithine (AlloTHyOrn) and allose-templated hydroxyarginine (AlloTHyArg) analogs with extended side-chain conformation.

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2. Results and discussion

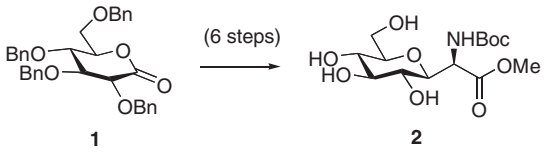
Our strategy was to develop a synthetic route to AlloTHyOrn and AlloTHyArg building blocks suitable for solid-phase peptide synthesis using the Fmoc-strategy.[27] The synthesis started with the known *C*-glucopyranosyl glycine derivative **2** easily obtained in 43% overall yield from commercially available 2,3,4,6-tetra-*O*-benzyl-D-glucono-1,5-lactone **1** in six steps (Scheme 1).[28]

The *C*-glucosyl glycine **2** was converted into the benzylidene-protected derivative **3** in 97% yield by treatment with benzaldehyde dimethyl acetal and catalytic amounts of *para*-toluenesulfonic acid (*p*-TsOH) in *N*,*N*-dimethylformamide. Exposure of **3** to benzoyl chloride in dry pyridine at 0 °C produced benzoate ester **4** and dibenzoate ester **5**, respectively. The major isomer **4** was isolated in 87% yield while **5** was isolated in 12% yield. The remaining hydroxyl group at the C-2 position in **4** was protected as methoxymethyl ether (MOM) **6** in moderate yield using MOMCl and Hünig's base in dichloromethane. Selective cleavage of the benzoate ester in **6** was achieved by employing sodium methoxide in methanol at 0 °C to afford a C-2′ epimeric mixture of compounds **7** and **8** (1:3) in combined 96% yield. Both isomers could be conveniently separated by column chromatography. In order to establish the absolute stereochemistry at C-2′ compounds **7** and **8** were benzoylated separately using benzoyl chloride in dry pyridine at 0 °C to produce previously synthesized **6** thereby confirming the absolute C-2′ (*S*) stereochemistry in **8** (Scheme 2).

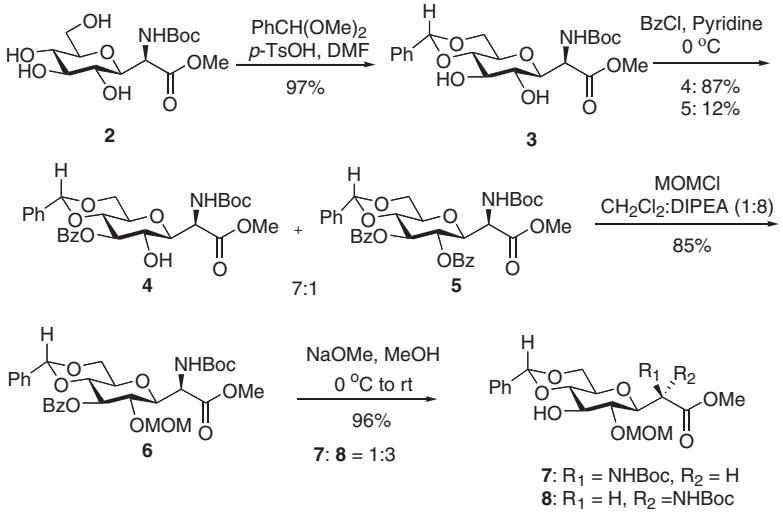
With both C-2′ epimers **7** and **8** in hand, we then explored the conversion of the C-3 hydroxyl group into an amino function. We anticipated that the equatorial position of the C-3 hydroxyl group in both **7** and **8** may complicate nucleophilic substitution reaction of activated esters at this position. Stereoelectronic considerations suggest that nucleophilic substitutions bearing axial leaving groups in conformationally constrained pyranose rings should be preferred when compared to equatorially positioned leaving groups.[32] Moreover, we were interested to develop an approach that would enable access to both *gluco*- and *allo*-configurated amines. As a result, we decided to install the amino function at C-3 via reductive amination. The unprotected hydroxyl group in **7** and **8** was oxidized using pyridinium chlorochromate (PCC) in dichloromethane to afford 3-ulose derivatives **9** and **10** in 93% and 95% yield, respectively (Scheme 3). Subsequently, 3-ulose derivatives **9** and **10** were reductively aminated using benzylamine and titanium(IV) isopropoxide followed by the addition of sodium cyanoborohydride as previously described by Mattson et al.[29] to afford stereoselectively compounds **11** and **12** in 78% and 80% yield, respectively. Using these reductive amination conditions we were unable to isolate *gluco*-configurated benzylamines. During this reaction we also recovered the alcohol **7** and **8** in 12% and 10% yield together with small amounts of (<5%) of transesterification products containing an isopropyl group. Compounds **11** and **12** were selectively debenzylated by catalytic hydrogenation on Pd–C in methanol to afford ornithine analogs **13** and **14** (Scheme 3). The stereochemistry of the newly incorporated amino function was established by 1D and 2D NMR analysis. The coupling constants $J_{H2,H3}$ = 2.9 Hz and $J_{H3,H4}$ = 2.9 Hz for compound **13** and $J_{H2,H3}$ = 3.0 Hz and $J_{H3,H4}$ = 3.0 Hz for **14** are consistent with the axial position of the amino function.

To modify the polyfunctional ornithine analogs **13** and **14** into suitable building blocks for solid-phase peptide synthesis we converted them into Fmoc-protected amino acids **23** and **24**. CTHyOrn analogs **23** and **24** are polyfunctional amino acids that fulfill the requirements for use in solid-phase peptide synthesis using the Fmoc-strategy. The partially protected hexafunctional building blocks **23** and **24** were selected with the vision to reduce the number of steps during global side-chain deprotection in solid-phase peptide synthesis using acidic conditions.

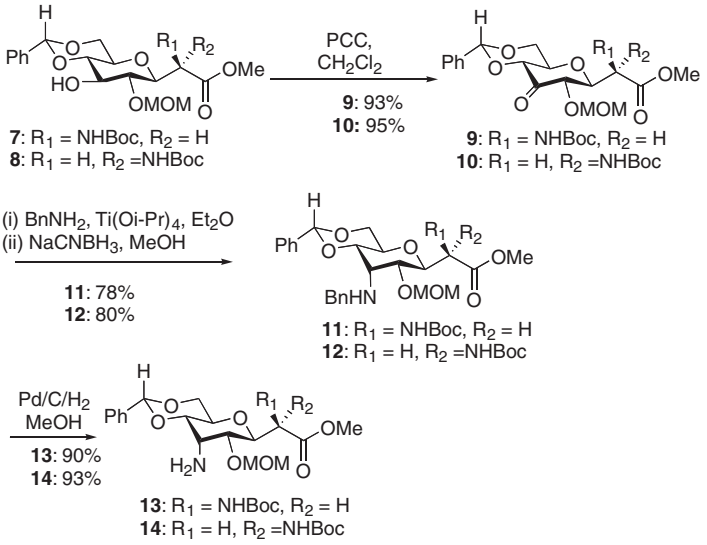
Previous work from our laboratory has shown that solid-phase peptide synthesis can be carried out with polyhydroxylated Fmoc-protected amino acids bearing no secondary hydroxyl protecting group while protection of the primary hydroxyl groups is usually required.[8] For instance, Fmoc-protected hydroxyproline is frequently used as building block in solid-phase peptide synthesis without need of protection.[30] However, the high reactivity of the primary hydroxyl group usually interferes with peptide coupling reaction and usually requires protection. As a result, we decided to protect the primary hydroxyl group in amino acids **13** and **14** with an acid labile triphenylmethyl group (Trt) and protect the side-chain amino function as *t*-butoxycarbonyl group (Boc). This was achieved via a five-step protection–deprotection sequence (Scheme 4).



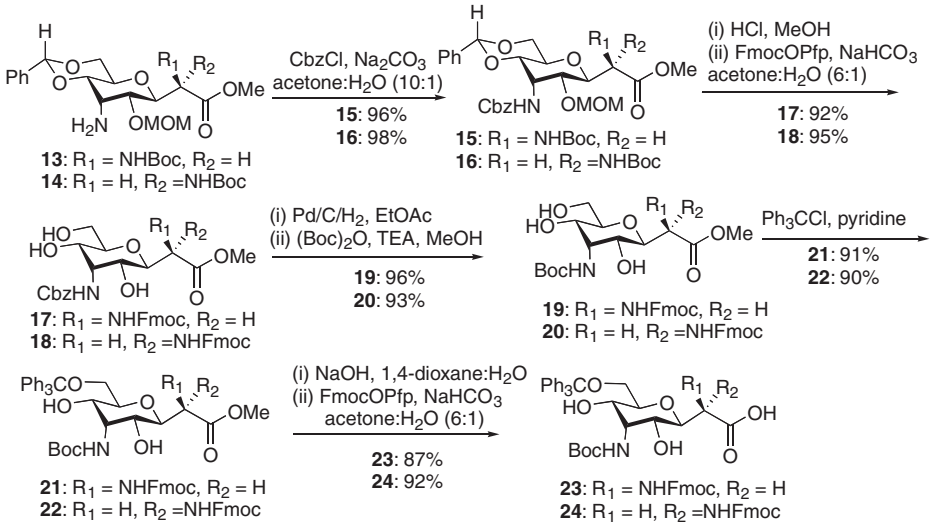
Scheme 1. Previously described synthesis of *C*-glucosyl glycine **2** from **1**.[28]



Scheme 2. Synthesis of suitably protected *C*-glucosyl glycine analogs **7** and **8**.



Scheme 3. Synthesis of amines **13** and **14** from precursors **7** and **8**.



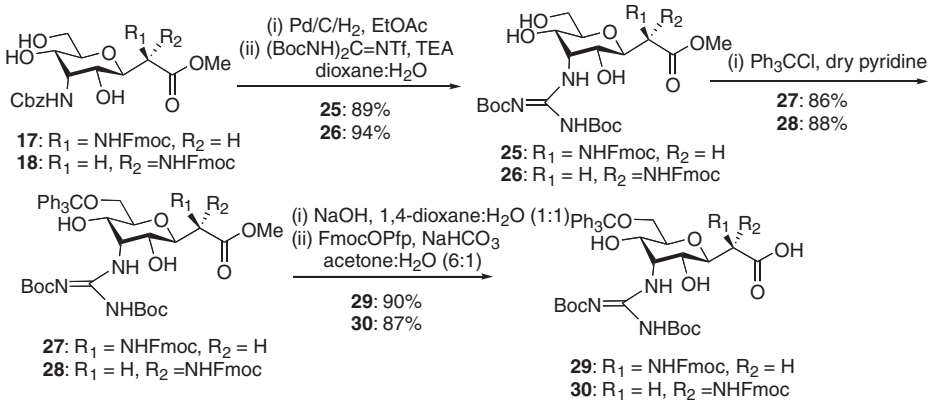
Scheme 4. Synthesis of protected allose-templated hydroxyornithine building blocks **23** and **24** for solid-phase peptide synthesis.

At first, the unprotected amino function in the side chain of **13** and **14** was protected as benzylcarbamate (Cbz) by treatment with benzyloxycarbonyl chloride and sodium carbonate in aqueous acetone to afford *N*-benzyloxy carbamates **15** and **16** in excellent yield, respectively. Exposure of compounds **15** and **16** to methanolic 6 N HCl at room temperature for 12 h resulted in cleavage of the benzylidene acetal and N-terminal *t*-butoxycarbamate group. The N-terminal amino group was reprotected using 9-fluorenylmethyl pentafluorophenyl carbonate (FmocOPfp) and sodium bicarbonate in aqueous acetone to form 9-fluorenylmethyloxy carbamate derivatives **17** and **18** in 92% and 95% isolated yield over two steps. The Cbz-group in **17** and **18** was selectively removed in the presence of 9-fluorenylmethyloxy carbonyl group by catalytic hydrogenation using 10% Pd–C in ethyl acetate followed by *tert*-butyloxy carbamate (Boc) formation of the resulting 5-amino function to provide **19** and **20** in 96% and 93% yields, respectively. The primary hydroxyl group present in **19** and **20** was selectively protected as triphenylmethyl ether using trityl chloride and dry pyridine to produce **21** and **22** in 90–91% isolated yield. Finally, ester saponification using 1 N NaOH in 1,4-dioxane of compounds **21** and **22** followed by reprotection of the N-terminal amine with FmocOPfp generated AlloTHyOrn building blocks **23** and **24** (Scheme 4). No epimerization was observed under this reaction conditions.

In a similar way AlloTHyArg analogs **29** and **30** were prepared from the hydroxyornithine analogs **17** and **18** in a three-step process (Scheme 5). At first, the ornithine analog was converted into the arginine analogs **25** and **26** on treatment with *N*,*N*′-di-*tert*-butoxycarbonyl-*N*″-triflyl-guanidine[31] and triethyl amine in dichloromethane at room temperature. Subsequently, the primary hydroxyl group in guanidinylated analogs **25** and **26** was protected as triphenylmethyl ethers **27** and **28** and ester saponification followed by reprotection with FmocOPfp generated AlloTHyArg building blocks **29** and **30** (Scheme 5).

3. Conclusion

We have developed a synthetic method for the synthesis of suitably protected and hexafunctional allose-templated hydroxyorni-



Scheme 5. Synthesis of suitably protected allose-templated hydroxyarginine building blocks **29** and **30** for solid-phase peptide synthesis.

thine and hydroxyarginine building blocks. It is envisaged that conformationally constrained building blocks **23**, **24**, **29**, and **30** will find application in solid-phase peptide synthesis using the Fmoc-strategy to probe the extended side chain conformation of ornithine and arginine in bioactive peptides. In addition, the presence of the polyol scaffold may mimic post-translational modifications in ornithine- or arginine-containing peptides.

4. Experimental

4.1. General methods

CH_2Cl_2 was distilled from calcium hydride. Organic solutions were concentrated under diminished pressure at <40 °C (bath temperature). NMR spectra were recorded at 300 or 500 MHz for ^1H and at 75 MHz for ^{13}C. Chemical shifts are reported relative to $CHCl_3$ (δ_H 7.26, δ_c (center of triplet) 77.0 ppm) or to CH_3OH (δ_H 3.35, δ_C (center of septet) 49.0 ppm) or to acetone as an internal standard (D_2O). TLC was performed on E. Merck Silica Gel 60 F254 with detection by charring with 8% H_2SO_4 acid. Silica gel (0.040–0.063 mm) was used for column chromatography.

4.2. (2′*R*)-Methyl 2′(*t*-butoxycarbonylamino)-2′-(4,6-*O*-benzylidene-β-D-glucopyranosyl)-acetate (3)

Compound **2** (0.070 g, 0.20 mmol) was dissolved in DMF (2.0 mL). To this mixture, $PhCH(OMe)_2$ (150 μL, 1.0 mmol) and a catalytic amount of toluenesulfonic acid were added. The reaction mixture was stirred at room temperature for 2 days. After completion of the reaction, the reaction mixture was poured into a dilute solution of $NaHCO_3$ (5.0 mL) and extracted with EtOAc (2 × 10.0 mL) and then the solvent was removed with a rotovap. The crude product was subjected to flash column chromatography using hexanes and EtOAc (3:2, v/v) as an eluant to afford the pure product **3** (0.085 g, 97%) as a thick liquid. R_f = 0.20 (EtOAc–hexane 2:3); $[\alpha]_D^{25}$ −44.0 (*c* 1.0, $CHCl_3$); ^1H NMR (300 MHz, MeOH-d_4): δ 1.47 (s, 9H), 3.40 (dd, 1H, *J* = 2.2, 5.6 Hz), 3.46 (dd, 1H, *J* = 4.6, 9.2 Hz), 3.57–3.67 (m, 2H), 3.67–3.73 (m, 2H), 3.75 (s, 3H), 4.29 (dd, 1H, *J* = 4.5, 10.1 Hz), 4.68 (br d, 1H, *J* = 5.6 Hz), 5.56 (s, 1H), 6.91 (d, 1H, *J* = 9.0 Hz), 7.33–7.38 (m, 3H), 7.48–7.53 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.3 (×3), 52.6, 54.0, 68.6, 70.8, 71.2, 74.8, 80.6 (×2), 81.1, 101.8, 126.3–137.0 (aromatic carbons), 155.5, 169.9; MS (ES+): *m/z* 462.26 $[M+Na]^+$. Anal. Calcd for $C_{21}H_{29}NO_9$: C, 57.39; H, 6.65; N, 3.19. Found: C, 57.15; H, 6.29; N, 3.35.

4.3. (2′*R*)-Methyl 2′-(*t*-butoxycarbonylamino)-2′-(3-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-acetate (4); (2′*R*)-methyl 2′-*t*-butoxycarbonylamino)-2′-(2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-acetate (5)

To a stirred solution of the benzylidene compound **3** (0.060 g, 0.17 mmol) in pyridine (1.5 mL) at 0 °C was slowly added benzoyl chloride (30 μL, 0.26 mmol). The reaction was monitored by TLC. At the end, the reaction mixture was diluted with EtOAc (10.0 mL) and washed with a dilute HCl solution, a saturated $NaHCO_3$ solution, and then brine. The organic phase was dried over anhyd Na_2SO_4 and evaporated to dryness. The crude product was subjected to flash column chromatography using hexanes and EtOAc (4:1, v/v) as an eluant to afford the pure product **4** (0.065 g, 87%) then **5** (0.025 g, 12%) as an amorphous solid. Compound **4**: R_f = 0.50 (EtOAc–hexane 2:3); $[\alpha]_D^{25}$ −84.6 (*c* 0.7, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 1.47 (s, 9H), 2.02 (br s, 1H), 3.57 (ddd, 1H, *J* = 4.6, 9.7, 14.3 Hz), 3.72 (t, 2H, *J* = 9.6 Hz), 3.80 (s, 3H), 3.82 (dd, 1H, *J* = 2.5, 10.1 Hz), 4.04 (dd, 1H, *J* = 9.6, 10.2 Hz), 4.37 (dd, 1H, *J* = 4.6, 10.3 Hz), 4.82 (br d, 1H, *J* = 8.1 Hz), 5.37 (dd, 1H, J_1 = 9.6 Hz, J_2 = 9.6 Hz), 5.48 (d, 1H, *J* = 9.0 Hz), 5.52 (s, 1H), 7.29–7.33 (m, 3H), 7.39–7.48 (m, 4H), 7.58 (m, 1H), 8.05–8.11 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.3, 52.6, 54.0, 68.6, 70.5, 71.0, 76.8, 78.5, 80.6, 81.0, 101.4, 126.1–136.9 (aromatic carbons), 155.5, 167.4, 169.9; MS (ES+): *m/z* 566.19 $[M+Na]^+$. Anal. Calcd for $C_{28}H_{33}NO_{10}$: C, 61.87; H, 6.12; N, 2.58. Found: C, 62.08; H, 6.43; N, 2.39. Compound **5**: R_f = 0.60 (EtOAc–hexane 2:3); ^1H NMR (300 MHz, $CDCl_3$): δ 1.47 (s, 9H), 3.54 (s, 3H), 3.73 (ddd, 1H, *J* = 4.0, 10.0, 14.0 Hz), 3.78 (q, 1H, *J* = 10.0 Hz), 3.88 (dd, 1H, *J* = 9.2, 10.0 Hz), 4.27 (br d, 1H, *J* = 10.0 Hz), 4.44 (dd, 1H, *J* = 3.9, 9.8 Hz), 4.8 (br d, 1H, *J* = 9.6 Hz), 5.5 (s, 1H), 5.57 (d, 1H, *J* = 9.8 Hz), 5.65 (d, 1H, *J* = 9.8 Hz), 5.77 (t, 1H, *J* = 9.5 Hz), 7.29–7.43 (m, 6H), 7.44–7.54 (m, 4H), 7.62 (m, 1H), 7.92 (m, 2H), 8.05–8.11 (m, 2H); MS (ES+): *m/z* 670.21 $[M+Na]^+$. Anal. Calcd for $C_{35}H_{37}NO_{11}$: C, 64.91; H, 5.76; N, 2.16. Found: C, 65.18; H, 6.10; N, 2.50.

4.4. (2′*R*)-Methyl 2′-(*t*-butoxycarbonylamino)-2′-(2-*O*-methoxymethyl-3-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-acetate (6)

Methoxymethyl chloride (118.0 μL, 1.55 mmol) was added to a solution of **4** (0.120 g, 0.22 mmol) in diisopropylethylamine (neat, 3.0 mL). The reaction mixture was stirred at room temperature for 24 h and then water (10.0 mL) was added. The reaction mixture was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic extracts were washed with saturated NH_4Cl solution (5.0 mL) and

evaporated in vacuo. Flash chromatography on silica gel with hexane–EtOAc (4:1, v/v) afforded **6** (0.110 g, 85%) as an oil. R_f = 0.50 (EtOAc–hexane 2:3); $[\alpha]_D^{25}$ −60.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.48 (s, 9H), 3.22 (s, 3H), 3.57–3.72 (m, 3H), 3.81 (dd, 1H, *J* = 1.9, 9.9 Hz), 3.82 (s, 3H), 4.19 (dd, 1H, *J* = 9.4, 10.1 Hz), 4.36 (dd, 1H, *J* = 4.3, 10.0 Hz), 4.76 (s, 2H), 4.80 (br d, 1H, *J* = 9.6 Hz), 5.40 (s, 1H), 5.50 (br d, 1H, *J* = 9.6 Hz), 5.57 (t, 1H, *J* = 9.0 Hz), 7.26–7.30 (m, 3H), 7.34–7.39 (m, 2H), 7.41–7.48 (m, 2H), 7.55 (m, 1H), 8.07–8.12 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.3 (×3), 52.6, 53.6, 56.5, 68.6, 70.8, 74.9, 76.6, 78.9, 80.3, 81.4, 98.3, 101.4, 126.1–136.9 (aromatic carbons), 155.3, 165.6, 169.6; MS (ES+): *m/z* 610.17 $[M+Na]^+$. Anal. Calcd for $C_{30}H_{37}NO_{11}$: C, 61.32; H, 6.35; N, 2.38. Found: C, 61.07; H, 6.29; N, 2.70.

4.5. (2′*R*)-Methyl 2′-*t*-butoxycarbonylamino)-2′-(2-*O*-methoxy methyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-acetate (7) and (2′*S*)-methyl 2′-(*t*-butoxycarbonylamino)-2′-(2-*O*-methoxy methyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-acetate (8)

To a solution of **6** (0.050 g, 0.085 mmol) in methanol (1.0 mL) was added 1.7 mL (2.0 equiv) of a freshly prepared methanol solution of sodium methoxide (0.1 M). The solution was stirred at room temperature for 3 h and neutralized with formic acid. After filtration and concentration, the resulting syrup was purified by flash chromatography on silica gel, eluting with hexanes–EtOAc 4:1, to give **7** (30 mg, 72%) and with hexane–EtOAc 3:1, to give **8** (10 mg, 24%) as a colorless liquid. Compound **7**: R_f = 0.40 (EtOAc–hexane 2:3); $[\alpha]_D^{25}$ −12.0 (*c* 0.2, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.47 (s, 9H), 3.39 (m, 1H), 3.44 (m, 1H), 3.48 (s, 3H), 3.64–3.76 (m, 4H), 3.78 (s, 3H), 4.32 (dd, 1H, *J* = 4.4, 10.0 Hz), 4.66 (br s, 1H), 4.74 (br d, 1H, *J* = 10.1 Hz), 4.82 (s, 1H), 4.87 (m, 1H), 5.43 (br d, 1H, *J* = 9.1 Hz), 5.53 (s, 1H), 7.29–7.40 (m, 3H), 7.46–7.52 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.3, 52.5, 53.5, 56.3, 68.6, 70.6, 73.5, 77.2, 80.0, 80.3, 82.0, 98.2, 101.9, 126.4, −136.9 (aromatic carbons), 155.3, 169.6; MS (ES+): *m/z* 506.21 $[M+Na]^+$. Anal. Calcd for $C_{23}H_{33}NO_{10}$: C, 57.13; H, 6.88; N, 2.90. Found: C, 57.48; H, 6.67; N, 3.18. Compound **8**: R_f = 0.45 (EtOAc–hexane 2:3); $[\alpha]_D^{25}$ −12.0 (*c* 0.2, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.47 (s, 9H), 3.32 (t, 1H, *J* = 8.7 Hz), 3.46 (s, 3H), 3.48 (m, 2H), 3.66 (t, 1H, *J* = 9.8 Hz), 3.78 (s, 3H), 3.83 (dd, 1H, *J* = 8.7, 9.0 Hz), 3.97 (dd, 1H, *J* = 1.6, 9.7 Hz), 4.26 (dd, 1H, *J* = 4.4, 10.0 Hz), 4.68 (d, 1H, *J* = 7.4 Hz), 4.76 (br d, 2H, *J* = 10.1 Hz), 4.80 (d, 1H, *J* = 7.6 Hz), 5.17 (br d, 1H, *J* = 10.1 Hz), 5.53 (s, 1H), 7.32–7.36 (m, 3H), 7.46–7.52 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.3, 52.7, 53.1, 56.3, 68.6, 70.1, 73.3, 78.5, 80.3, 80.5, 81.9, 98.7, 101.9, 126.4–136.9 (aromatic carbons), 155.8, 171.0; MS (ES+): *m/z* 506.13 $[M+Na]^+$. Anal. Calcd for $C_{23}H_{33}NO_{10}$: C, 57.13; H, 6.88; N, 2.90. Found: C, 57.55; H, 7.11; N, 2.65.

4.6. (2′*R*)-Methyl 2′-(*t*-butoxycarbonylamino)-2′-(2-*O*-methoxy methyl-4,6-*O*-benzylidene-β-D-gluco-3-ulo-pyranosyl)-acetate (9) and (2′*S*)-methyl 2′-(*t*-butoxycarbonylamino)-2′-(2-*O*-methoxy methyl-4,6-*O*-benzylidene-β-D-gluco-3-ulo-pyranosyl)-acetate (10)

To a stirred solution of alcohol **7** or **8** (110 mg, 0.23 mmol) and ground 4 Å molecular sieves (110 mg) in anhyd CH_2Cl_2 (3.0 mL) was added PCC (198 mg, 0.92 mmol). The mixture was stirred for 14–16 h (TLC monitoring), diluted with CH_2Cl_2–EtOAc mixture (1:1, 10 mL), and poured onto a Celite pad. The solvent was concentrated with a rotovap. Elution with the hexane–EtOAc 4:1 system gave pure ketone **9** or **10** (**9**, 102 mg, 93%; **10**, 105 mg, 95%) as a colorless amorphous solid. Compound **9**: R_f = 0.40 (EtOAc–hexane 2:3); $[\alpha]_D^{25}$ −74.0 (*c* 0.3, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.47 (2*s*, 9H), 3.50 (s, 3H), 3.66 (m, 1H), 3.76 (m, 1H), 3.81 (s, 3H), 3.95 (dd, 1H, *J* = 1.9, 9.7 Hz), 4.24 (dd, 1H, *J* = 1.9, 9.7 Hz), 4.44 (dd, 1H, *J* = 4.6, 10.4 Hz), 4.66 (dd, 1H, *J* = 1.9, 10.4 Hz), 4.76–4.88 (m, 3H), 5.42 (br d, 1H, *J* = 10.0 Hz), 5.53 (s, 1H), 7.32–7.40 (m, 3H), 7.46–7.52 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.3 (×3), 52.7, 54.1, 56.9, 69.0, 73.3, 77.1, 80.3, 82.1, 83.7, 97.0, 101.8, 126.4–136.2 (aromatic carbons), 155.1, 169.2, 197.8; MS (ES+): *m/z* 504.22 $[M+Na]^+$. Anal. Calcd for $C_{23}H_{31}NO_{10}$: C, 57.37; H, 6.49; N, 2.91. Found: C, 57.53; H, 6.72; N, 2.69. Compound **10**: R_f = 0.45 (EtOAc–hexane 2:3); $[\alpha]_D^{25}$ +16.0 (*c* 1.8, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.47 (2*s*, 9H), 3.46 (s, 3H), 3.48 (br d, 1H, *J* = 2.4 Hz), 3.65 (m, 1H), 3.78 (2*s*, 3H), 3.95 (br d, 1H, *J* = 9.7 Hz), 4.20–4.40 (m, 2H), 4.65–4.76 (m, 2H), 4.80–4.84 (m, 2H), 5.18 (dd, 1H, *J* = 10.7, 13.3 Hz), 5.53 (s, 1H), 7.32–7.36 (m, 3H), 7.46–7.52 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.2 (×3), 52.7, 53.1, 56.2, 68.6, 70.1, 73.0, 78.4, 80.3, 81.8, 97.1, 101.8, 126.4–136.2 (aromatic carbons), 155.8, 170.5, 197.8; MS (ES+): *m/z* 504.11 $[M+Na]^+$. Anal. Calcd for $C_{23}H_{31}NO_{10}$: C, 57.37; H, 6.49; N, 2.91. Found: C, 57.01; H, 6.68; N, 3.22.

4.7. (2′*R*)-Methyl 2′-(*t*-butoxycarbonylamino)-2′-(3-amino-3-deoxy-2-*O*-methoxymethyl-4,6-*O*-benzylidene-β-D-allopyranosyl)-acetate (13) and (2′*S*)-methyl 2′-(*t*-butoxycarbonylamino)-2′-(3-amino-3-deoxy-2-*O*-methoxymethyl-4,6-*O*-benzylidene-β-D-allopyranosyl)-acetate (14)

A mixture of the ketone **9** or **10** (50 mg, 0.10 mmol), benzyl amine (11.0 μL, 0.10 mmol), and titanium(IV) isopropoxide (38 μL, 0.13 mmol) was stirred at room temperature in a round-bottomed flask. After 2 h, the viscous solution was diluted with dry methanol (2.0 mL). Sodium cyanoborohydride (5.0 mg, 0.07 mmol) was added, and the solution was stirred for 20 h. Water (2.0 mL) was added with stirring, and the resulting inorganic precipitate was filtered and washed with methanol. The filtrate was then concentrated in vacuo. The crude product was dissolved in ethyl acetate, filtered to remove the remaining inorganic solids, and concentrated in vacuo. The obtained products **11** and **12** (**11**, 48.0 mg, 78%, **12**, 45 mg, 80%) were purified by flash chromatography using with hexane–EtOAc 5:1. Both compounds were directly used for catalytic hydrogenation. Compound **11** or **12** (18.0 mg, 0.032 mmol) was dissolved in MeOH (5.0 mL). Ten percentages of Pd–C (20 mg) were added and the mixture was hydrogenated for 6 h at atmospheric pressure. The mixture was filtered and concentrated. The crude residue was purified with flash silica gel column chromatography (hexane–EtOAc 1:1) to afford **13** or **14** (**13**, 14.0 mg, 90%; **14**, 18 mg, 93%) as a syrup. Compound **13**: R_f = 0.20 (EtOAc–hexane 2:3); $[\alpha]_D^{25}$ −49.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.45 (s, 9H, $-C(CH_3)_3$), 3.34 (dd, 1H, *J* = 2.9, 9.8H-2), 3.43 (s, 3H, $-CH_2OCH_3$), 3.48 (dd, 1H, *J* = 2.9, 9.2 Hz, H-4), 3.56 (t, 1H, *J* = 10.0 Hz, H-6a), 3.75 (s, 3H, $-CO_2Me$), 3.90 (m, 1H, H-3), 4.14 (ddd, 1H, *J* = 5.0, 10.2, 15.2 Hz, H-6b), 4.29 (dd, 1H, *J* = 5.1, 10.3 Hz, H-5), 4.65 (d, 1H, *J* = 9.6 Hz, H-1), 4.76 (ABq, 2H, *J* = 7.0 Hz, $-OCH_2O-$), 4.79 (d, 1H, *J* = 9.6 Hz, *CH*NHBoc), 5.46 (br d, 1H, *J* = 9.6 Hz, NH), 5.48 (s, 1H, *CH*Ph), 7.30–7.39 (m, 3H, Ph), 7.42–7.49 (m, 2H, Ph); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.3 (×3), 50.0, 52.2, 53.8, 58.2, 64.9, 69.2, 74.3, 75.8, 79.3, 80.0, 96.5, 101.9, 126.2–137.3 (aromatic carbons), 155.3, 170.0; MS (ES+): *m/z* 483.25 $[M+H]^+$. Anal. Calcd for $C_{23}H_{34}N_2O_9$: C, 57.25; H, 7.10; N, 5.81. Found: C, 57.55; H, 7.36; N, 5.94. Compound **14**: R_f = 0.25 (EtOAc–hexane 2:3); $[\alpha]_D^{25}$ +5.0 (*c* 0.2, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.47 (s, 9H, $-C(CH_3)_3$), 3.39 (s, 3H, $-CH_2OCH_3$), 3.52 (dd, 1H, *J* = 3.0, 9.2 Hz, H-4), 3.59 (m, 1H, H-6a), 3.65 (dd, 1H, *J* = 3.0, 9.7 Hz, H-2), 3.77 (s, 3H, $-CO_2Me$), 3.92 (m, 1H, H-3), 4.19 (ddd, 1H, *J* = 5.1, 10, 14.6 Hz, H-6b), 4.25 (dd, 1H, *J* = 5.1, 10.0 Hz, H-5),

4.46 (br d, 1H, J = 10.3 Hz, H-1), 4.62 (br d, 1H, J = 10.1 Hz, C*H*NHBoc), 4.70 (ABq, 2H, J = 7.0 Hz, –OCH_2O–), 5.13 (br d, 1H, J = 10.1 Hz, NH), 5.50 (s, 1H, C*H*Ph), 7.34–7.39 (m, 3H, Ph), 7.46–7.49 (m, 2H, Ph); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.7 (×3), 49.9, 52.5, 53.3, 56.2, 64.7, 69.2, 73.7, 73.8, 79.3, 80.2, 96.5, 101.9, 126.2–137.3 (aromatic carbons), 156.0, 171.7; MS (ES+): m/z 483.26 $[M+H]^+$. Anal. Calcd for $C_{23}H_{34}N_2O_9$: C, 57.25; H, 7.10; N, 5.81. Found: C, 56.87; H, 6.73; N, 6.13.

4.8. (2′*R*)-Methyl 2′-(*t*-butoxycarbonylamino)-2′-[3-benzyloxy carbonylamino-3-deoxy-2-*O*-methoxymethyl-4,6-*O*-benzylidene-β-D-allopyranosyl]-acetate (15) and (2′*S*)-methyl 2′-(*t*-butoxycar bonylamino)-2′-[3-benzyloxycarbonylamino-3-deoxy-2-*O*-meth oxymethyl-4,6-*O*-benzylidene-β-D-allopyranosyl]-acetate (16)

Na_2CO_3 (44 mg, 0.416 mmol) followed by benzyl chloroformate (58 μL, 0.416 mmol) was added to a solution of **13** or **14** (50 mg, 0.104 mmol) in a mixture of acetone and water (3 mL, 10:1). The reaction mixture was stirred at room temperature for 12 h and concentrated in vacuo. The crude residue was then extracted with EtOAc (2 × 10 mL). The extract was dried over anhyd Na_2SO_4, filtered, and evaporated. The crude product was purified by flash column chromatography on silica gel (hexane–EtOAc 3:1) affording **15/16** (**15**, 62 mg, 96%; **16**, 64 mg, 98%) as an oil. Compound **15**: R_f = 0.40 (EtOAc–hexane 2:3); $[\alpha]_D^{25}$ −26.0 (c 1.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.46 (s, 9H), 3.39 (s, 3H), 3.54–3.65 (m, 3H), 3.70 (dd, 1H, J = 1.8, 10.5 Hz), 3.77 (s, 3H), 4.10 (dd, 1H, J = 4.5, 10.9 Hz), 4.31 (dd, 1H, J = 3.8, 9.5 Hz), 4.64 (d, 1H, J = 6.9 Hz), 4.70–4.80 (m, 2H), 4.83 (d, 1H, J = 7.0 Hz), 5.07 (m, 1H), 5.12 (ABq, 2H, J = 12.0 Hz), 5.47 (br s, 1H), 5.51 (s, 1H), 7.28–7.36 (m, 8H), 7.40–7.44 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.3 (×3), 49.9, 52.4, 53.9, 56.5, 67.0 (×2), 68.9, 70.3, 76.9, 77.5, 80.4, 95.3, 101.4, 126.1–137.0 (aromatic carbons), 155.3, 156.7, 169.5; MS (ES+): m/z 639.23 $[M+Na]^+$. Anal. Calcd for $C_{31}H_{40}N_2O_{11}$: C, 60.38; H, 6.54; N, 4.54. Found: C, 60.07; H, 6.76; N, 4.19. Compound **16**: R_f = 0.45 (EtOAc–hexane 2:3); $[\alpha]_D^{25}$ +20.0 (c 0.4, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.47 (s, 9H), 3.33 (s, 3H), 3.52–3.62 (m, 3H), 3.77 (s, 3H), 3.82 (dd, 1H, J = 3.9, 10.1 Hz), 4.02 (br d, 1H, J = 10.1 Hz), 4.24 (dd, 1H, J = 3.9, 10.0 Hz), 4.55 (d, 1H, J = 7.1 Hz), 4.67 (d, 1H, J = 10.1 Hz), 4.77–4.86 (m, 2H), 5.07 (m, 1H), 5.12 (ABq, 2H, J = 12.0 Hz), 5.19 (br s, 1H), 5.51 (s, 1H), 7.18–7.36 (m, 8H), 7.40–7.44 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.3 (×3), 49.9, 52.7, 53.3, 56.4, 66.7, 67.0, 68.9, 69.4, 75.3, 76.9, 80.4, 95.3, 101.4, 128.1–137.0 (aromatic carbons), 155.9, 156.7, 171.3; MS (ES+): m/z 639.19 $[M+Na]^+$. Anal. Calcd for $C_{31}H_{40}N_2O_{11}$: C, 60.38; H, 6.54; N, 4.54. Found: C, 60.66; H, 6.34; N, 4.87.

4.9. (2′*R*)-Methyl 2′-(9*H*-fluoren-9-ylmethoxycarbonylamino)-2′-[3-benzyloxycarbonylamino-3-deoxy-β-D-allopyranosyl]-acetate (17) and (2′*S*)-methyl 2′-(9*H*-fluoren-9-ylmethoxycarbony lamino)-2′-[3-benzyloxycarbonylamino-3-deoxy-β-D-allopyranosyl]-acetate (18)

To a solution of **15** or **16** (0.405 g, 0.62 mmol) in methanol (10 mL) was added HCl (1 N, 5 drops) and the reaction mixture was stirred at room temperature for 12 h. The reaction mixture was diluted with saturated $NaHCO_3$ solution (30 mL) and extracted with EtOAc (3 × 30 mL). After removal of solvent, the residue was dissolved in aqueous acetone (3.0 mL, 1:1) and treated with 9-fluorenylmethyl pentafluorophenyl carbonate (91 mg, 0.24 mmol) and sodium bicarbonate (31 mg, 0.37 mmol) for 4 h at room temperature. Water (10.0 mL) was added and the aqueous layer was extracted with EtOAc (6 × 10 mL). Finally, the solvent was dried (Na_2SO_4) and concentrated. The crude product was purified by flash column chromatography (methanol–EtOAc 1:9) to afford compound **17/18** (**17**, 45 mg, 92%; **18**, 48 mg, 95%) as a colorless thick liquid. Compound **17**: R_f = 0.20 (EtOAc); $[\alpha]_D^{25}$ −39.0 (c 0.2, $CHCl_3$); ^{1}H NMR (300 MHz, MeOH-d_4): δ 3.40–3.53 (m, 3H), 3.73 (s, 3H), 3.75 (d, 1H, J = 1.9 Hz), 3.86 (d, 1H, J = 9.9 Hz), 3.92 (dd, 1H, J = 3.9, 10.5 Hz), 4.21 (dd, 1H, J = 6.6, 6.8 Hz), 4.34–4.47 (m, 3H), 4.73 (m, 1H), 5.13 (s, 2H), 7.30–7.46 (m, 9H), 7.64–7.70 (d, 2H, J = 7.6 Hz), 7.78 (d, 2H, J = 7.6 Hz); ^{13}C NMR (75 MHz, MeOD): δ 48.4, 52.7, 55.9, 57.0, 63.7, 67.7, 67.9, 68.0, 68.2, 78.2, 78.3, 120.9–145.3 (aromatic carbons), 156.3, 158.7, 171.7; MS (ES+): m/z 629.10 $[M+Na]^+$. Anal. Calcd for $C_{32}H_{34}N_2O_{10}$: C, 63.36; H, 5.65; N, 4.62. Found: C, 63.70; H, 5.88; N, 4.95. Compound **18**: R_f = 0.30 (EtOAc); $[\alpha]_D^{25}$ +18.0 (c 0.3, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.15 (br s, 1H), 3.23 (m, 1H), 3.47–3.53 (m, 2H), 3.65 (dd, 1H, J = 3.9, 12.0 Hz), 3.77 (s, 3H), 3.78–3.82 (m, 2H), 4.21 (t, 1H, J = 6.1 Hz), 4.28 (m, 1H), 4.41 (br d, 1H, J = 4.5 Hz), 4.60 (dd, 1H, J = 6.0, 10.9 Hz), 4.63 (dd, 1H, J = 2.2, 8.1 Hz), 4.72 (dd, 1H, J = 6.2, 10.9 Hz), 5.13 (ABq, 2H, J = 12.0 Hz), 5.17 (br s, 1H), 5.31 (br d, 1H, J = 2.9 Hz), 5.68 (d, 1H, J = 8.1 Hz), 7.30–7.46 (m, 9H), 7.59 (d, 2H, J = 7.6 Hz), 7.78 (d, 2H, J = 7.6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 47.3, 53.0, 54.2, 55.4, 62.7, 65.4, 67.0, 68.0, 69.5, 75.7, 76.2, 119.9–143.6 (aromatic carbons), 157.7, 160.0, 170.1; MS (ES+): m/z 629.20 $[M+Na]^+$. Anal. Calcd for $C_{32}H_{34}N_2O_{10}$: C, 63.36; H, 5.65; N, 4.62. Found: C, 62.98; H, 6.02; N, 4.27.

4.10. (2′*R*)-Methyl 2′-(9*H*-fluoren-9-ylmethoxycarbonylamino)-2′-[3-*t*-butoxycarbonylamino-3-deoxy-β-D-allopyranosyl]-acetate (19) and (2′*S*)-methyl 2′-(9*H*-fluoren-9-ylmethoxycarbonylamino)-2′-[3-*t*-butoxycarbonylamino-3-deoxy-β-D-allopyranosyl]-acetate (20)

Hydrogenation of **17**/**18** (1.0 mmol) in 10 mL of EtOAc containing 0.5 mL of methanol in the presence of Pd–C was accomplished in 6 h. After removal of the catalyst, the resulting solution was concentrated under vacuum. The amine residue was dissolved in methanol with stirring and reacted with BOC-anhydride (1.5 equiv) and triethylamine (2.0 equiv). The resulting mixture was stirred at room temperature for 3 h. Water is then added and the aqueous layer was extracted with EtOAc (4 × 10 mL). The combined organic layers were dried (sodium sulfate) and concentrated in vacuo. The resulting residue was purified by flash chromatography (methanol–EtOAc 1:8) to provide **19/20** (**19** g, 96%; **20**, 93%) as a colorless oil. Compound **19**: R_f = 0.15 (EtOAc); $[\alpha]_D^{25}$ −63.0 (c 0.1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.47 (s, 9H), 3.10 (m, 1H), 3.50 (m, 1H), 3.72 (m, 2H), 3.76 (s, 3H), 3.80–3.90 (m, 3H), 4.18–4.23 (m, 2H), 4.24 (dd, 1H, J = 3.3, 3.6 Hz, H3), 4.40–4.50 (m, 3H), 4.76 (br d, 1H, J = 7.9 Hz), 5.12 (s, 1H), 5.85 (br d, 1H, J = 7.8 Hz), 7.30–7.46 (m, 4H), 7.59 (m, 2H), 7.75–7.77 (d, 2H, J = 7.6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.2 (×3), 47.3, 52.6, 54.9, 55.2, 62.8, 67.2, 67.3, 67.7, 67.8, 77.2, 81.6, 120.0–143.6 (aromatic carbons), 157.7, 169.8, 169.8; MS (ES+): m/z 595.21 $[M+Na]^+$. Anal. Calcd for $C_{29}H_{36}N_2O_{10}$: C, 60.83; H, 6.34; N, 4.89. Found: C, 61.06; H, 5.97; N, 5.21. Compound **20**: R_f = 0.25 (EtOAc); $[\alpha]_D^{25}$ +30.0 (c 0.15, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.47 (s, 9H), 3.23 (m, 1H), 3.42–3.53 (m, 2H), 3.62 (dd, 1H, J = 4.9, 12.0 Hz), 3.78 (s, 3H), 3.80–3.84 (m, 3H), 4.20 (dd, 1H, J = 3.7, 4.3 Hz, H3), 4.22 (m, 1H), 4.43 (d, 1H, J = 4.8 Hz), 4.47 (dd, 1H, J = 6.0, 10.9 Hz), 4.62 (dd, 1H, J = 2.2, 8.1 Hz), 4.73 (dd, 1H, J = 6.2, 10.9 Hz), 5.07 (br d, 1H, J = 2.0 Hz), 5.56 (br s, 1H), 5.69 (d, 1H, J = 8.2 Hz), 7.30–7.46 (m, 4H), 7.59 (d, 2H, J = 7.6 Hz), 7.78 (d, 2H, J = 7.6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.2 (×3), 47.3, 53.0, 54.2, 55.2, 63.3, 65.2, 67.0, 70.8, 75.7, 76.2, 81.5, 120.0–143.6 (aromatic carbons), 157.7, 160.0, 170.0; MS (ES+): m/z 595.16 $[M+Na]^+$. Anal. Calcd for $C_{29}H_{36}N_2O_{10}$: C, 60.83; H, 6.34; N, 4.89. Found: C, 60.98; H, 6.47; N, 5.21.

4.11. (2′*R*)-Methyl 2′-(9*H*-fluoren-9-ylmethoxycarbonylamino)-2′-[3-*t*-butoxycarbonylamino-3-deoxy-6-*O*-triphenylmethyl-β-D-allopyranosyl]-acetate (21) and (2′*S*)-methyl 2′-(9*H*-fluoren-9-ylmethoxycarbonylamino)-2′-[3-*t*-butoxycarbonylamino-3-deoxy-6-*O*-triphenylmethyl-β-D-allopyranosyl]-acetate (22)

A solution of **19/20** (0.1 g) in dry pyridine (3 mL) was cooled to 0 °C under argon. Triphenylmethyl chloride (10 equiv) was added, and then the mixture was stirred at room temperature overnight. The reaction mixture was diluted with CH_2Cl_2 (10 mL), washed with saturated $NaHCO_3$ solution (5 mL), and dried over $MgSO_4$, and the solvent was removed in vacuo. The residue was purified by column chromatography on silica gel (hexane–EtOAc) 1:3 to afford **21/22** (**21**, 91%; **22**, 90%). Compound **21**: R_f = 0.40 (EtOAc–hexane 2:3); $[\alpha]_D^{25}$ −35.0 (*c* 0.2, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.46 (s, 9H), 3.09–3.20 (m, 2H), 3.32–3.46 (m, 3H), 3.73 (s, 3H), 3.83–3.90 (m, 2H), 4.18–4.28 (m, 2H), 4.32–4.48 (m, 3H), 4.80 (br d, 1H, *J* = 8.7 Hz), 5.04 (br s, 1H), 5.73 (br d, 1H, *J* = 8.2 Hz), 7.28–7.45 (m, 19H), 7.59 (dd, 2H, *J* = 7.6, 10.0 Hz), 7.74 (d, 2H, *J* = 7.6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.2 (×3), 47.2, 52.1, 54.9, 55.4, 63.1, 64.3, 67.9, 71.0, 75.7, 76.0, 79.3, 86.1, 120.0–146.9 (aromatic carbons), 154.9, 161.8, 169.9; MS (ES+): *m/z* 837.39 $[M+Na]^+$. Anal. Calcd for $C_{48}H_{50}N_2O_{10}$: C, 70.74; H, 6.18; N, 3.44. Found: C, 71.13; H, 6.01; N, 3.78. Compound **22**: R_f = 0.50 (EtOAc–hexane 2:3); $[\alpha]_D^{25}$ +15.0 (*c* 0.2, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.47 (s, 9H), 3.23 (m, 1H), 3.30–3.45 (m, 3H), 3.48–3.62 (m, 2H), 3.76 (s, 3H), 3.85–3.90 (m, 1H), 4.18–4.28 (m, 2H), 4.46 (dd, 2H, *J* = 6.0, 10.7 Hz), 4.61–4.72 (m, 2H), 5.00 (m, 1H), 5.63 (d, 1H, *J* = 8.2 Hz), 7.18–7.34 (m, 10H), 7.38–7.45 (m, 9H), 7.59 (dd, 2H, *J* = 7.6, 10.0 Hz), 7.74 (d, 2H, *J* = 7.6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.2, 47.3, 53.0, 54.2, 55.2, 63.3, 65.2, 67.0, 71.0, 75.7, 76.0, 81.6, 86.5, 120.0–146.9 (aromatic carbons), 157.7, 163.5, 170.1; MS (ES+): *m/z* 837.32 $[M+Na]^+$. Anal. Calcd for $C_{48}H_{50}N_2O_{10}$: C, 70.74; H, 6.18; N, 3.44. Found: C, 70.39; H, 6.56; N, 3.08.

4.12. (2′*R*)-2′-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-2′-[3-*t*-butoxycarbonylamino-3-deoxy-6-*O*-triphenylmethyl-β-D-allopyranosyl]-acetic acid (23) and (2′*S*)-2′-(9*H*-fluoren-9-ylmethoxycarbonylamino)-2′-[3-*t*-butoxycarbonylamino-3-deoxy-6-*O*-triphenylmethyl-β-D-allopyranosyl]-acetic acid (24)

The ester **21**/**22** (50 mg) was dissolved in aqueous dioxane and NaOH (4.0 equiv, 1 N) was added at 0 °C. The solution was then stirred until complete by TLC (usually <1 h). Formic acid was added until the mixture was neutral and then diluted with EtOAc. The organic solution was extracted with EtOAc (3 × 10 mL), dried (sodium sulfate), and concentrated in vacuo. The residue was purified by flash chromatography (methanol–EtOAc 1:19) on SiO_2 providing **23/24** (**23**, 87%; **24**, 92%) as a colorless thick oil. Compound **23**: R_f = 0.1 (EtOAc); $[\alpha]_D^{25}$ −56.0 (*c* 0.5, $CHCl_3$); ^{1}H NMR (300 MHz, MeOH-d_4): δ 1.45 (s, 9H), 3.29 (m, 1H), 3.37 (m, 1H), 3.60 (m, 1H), 3.69 (m, 2H, *J* = 10.1 Hz), 3.70 (m, 1H), 4.12 (m, 1H), 4.21–4.32 (m, 2H), 4.42 (d, 1H, *J* = 6.9), 4.58 (m, 1H), 7.18–7.26 (m, 5H), 7.32–7.41 (m, 8H), 7.47–7.53 (m, 6H), 7.65 (dd, 2H, *J* = 7.8, 10.0 Hz), 7.75 (dd, 2H, *J* = 4.0, 7.8 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.8 (×3), 46.2, 56.3, 56.7, 65.3, 67.6, 68.2, 68.3, 76.7, 77.3, 80.7, 87.8, 120.9–130.1 (aromatic carbons), 142.5–147.6 (×7), 159.9, 160.2, 170.9; MS (ES+): *m/z* 823.29 $[M+Na]^+$. Anal. Calcd for $C_{47}H_{48}N_2O_{10}$: C, 70.48; H, 6.04; N, 3.50. Found: C, 70.20; H, 6.25; N, 3.85. Compound **24**: R_f = 0.10 (EtOAc); $[\alpha]_D^{25}$ +36.0 (*c* 0.1, $CHCl_3$); ^{1}H NMR (300 MHz, MeOH-d_4): δ 1.48 (s, 9H), 3.23 (m, 1H), 3.35 (m, 1H), 3.56 (m, 1H), 3.65 (m, 2H), 3.70 (m, 1H), 4.10 (m, 1H), 4.21–4.32 (m, 2H), 4.42 (d, 1H, *J* = 6.8 Hz), 4.56 (m, 1H), 7.20–7.27 (m, 5H), 7.29–7.38 (m, 8H), 7.45–7.50 (m, 6H), 7.63 (dd, 2H, *J* = 7.6, 10.6 Hz), 7.76 (t, 2H, *J* = 7.6); ^{13}C NMR (75 MHz, MeOH-d_4): δ 28.9 (×3), 46.4, 56.4, 56.6, 65.3, 67.7, 68.1, 68.3, 76.8, 77.0, 80.9, 87.9, 121.0–145.7 (aromatic carbons), 159.8, 160.3, 171.8; MS (ES+): *m/z* 823.41 $[M+Na]^+$. Anal. Calcd for $C_{47}H_{48}N_2O_{10}$: C, 70.48; H, 6.04; N, 3.50. Found: C, 70.72; H, 6.36; N, 3.17.

4.13. (2′*R*)-Methyl 2′-(9*H*-fluoren-9-ylmethoxycarbonylamino)-2′-[3-(*N*,*N*′-di-*tert*-butoxycarbonyl-guanidine)-3-deoxy-β-D-allopyranosyl]-acetate (25) and (2′*S*)-methyl 2′-(9*H*-fluoren-9-ylmethoxycarbonylamino)-2′-[3-(*N*,*N*′-di-*tert*-butoxycarbonyl-guanidine)-3-deoxy-β-D-allopyranosyl]-acetate (26)

Hydrogenation of **17/18** (0.607 g, 1.0 mmol) in 10 mL of EtOAc and few drops of methanol in the presence of Pd–C was accomplished in 6 h. After removal of the catalyst, the resulting solution of amine was concentrated under vacuum. To a solution of amine (0.095 g, 0.20 mmol) in dioxane and water (3:1, 2 mL) was added *N*,*N*-diBoc-*N*″-triflylguanidine (157 mg, 0.40 mmol, 2 equiv). After 5 min, NEt_3 (56 μL, 0.40 mmol, 2 equiv) was added at room temperature. After 10 h, the dichloromethane was removed under reduced pressure. The remaining residue product was purified by flash column chromatography (hexane–EtOAc 1:9) on silica gel to give **25/26** (**25**, 89%; **26**, 94%) as an oil. Compound **25**: R_f = 0.20 (EtOAc); $[\alpha]_D^{25}$ −49.0 (*c* 0.4, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.46 (s, 9H), 1.53 (s, 9H), 2.60 (br s, 2H), 3.54 (m, 1H), 3.75 (m, 1H), 3.77 (s, 3H), 3.83–3.90 (m, 4H), 4.23 (dd, 1H, *J* = 7.0, 6.9 Hz), 4.01 (br s, 1H), 4.36–4.47 (m, 2H), 4.66 (br s, 1H), 4.80 (br d, 1H, *J* = 8.6 Hz), 5.92 (br d, 1H, *J* = 8.6 Hz), 7.30–7.42 (m, 4H), 7.59 (d, 2H, *J* = 7.6 Hz), 7.78 (d, 2H, *J* = 7.6 Hz), 8.96 (br d, 1H, *J* = 2.8 Hz), 11.4 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.0 (×3), 28.1 (×3), 47.1, 52.6, 54.9, 56.5, 62.6, 67.3, 67.9, 68.5, 75.8, 76.9, 80.1, 84.1, 120.0–143.8 (aromatic carbons), 152.8, 156.2, 159.1, 161.7, 170.1; MS (ES+): *m/z* 737.14 $[M+Na]^+$. Anal. Calcd for $C_{35}H_{46}N_4O_{12}$: C, 58.81; H, 6.49; N, 7.84. Found: C, 58.98; H, 6.87; N, 7.57. Compound **26**: R_f = 0.25 (EtOAc); $[\alpha]_D^{25}$ +6.0 (*c* 0.4, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.47 (s, 9H), 1.53 (s, 9H), 2.40 (br s, 1H), 3.36 (dd, 1H, *J* = 4.4, 9.9 Hz), 3.49 (m, 1H), 3.58 (dd, 1H, *J* = 2.9, 9.9 Hz), 3.68 (br s, 1H), 3.77 (m, 2H), 3.80 (s, 3H), 3.90 (dd, 1H, *J* = 2.0, 9.9 Hz), 4.00 (br s, 1H), 4.21 (t, 1H, *J* = 6.5 Hz), 4.47 (dd, 1H, *J* = 6.0, 10.8 Hz), 4.59 (m, 1H), 4.66 (dd, 1H, *J* = 6.4, 10.8 Hz), 4.73 (dd, 1H, *J* = 2.0, 8.3 Hz), 5.70 (br d, 1H, *J* = 8.3 Hz), 7.30–7.38 (m, 2H), 7.39–7.47 (m, 2H), 7.59 (d, 2H, *J* = 7.6 Hz), 7.78 (d, 2H, *J* = 7.6 Hz), 8.91 (br d, 1H, *J* = 2.8 Hz), 11.45 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.1 (×6), 47.3, 53.1, 54.4, 56.4, 63.3, 65.6, 67.1, 71.0, 75.6, 76.6, 80.1, 84.0, 120.0–143.6 (aromatic carbons), 152.8, 157.7, 159.0, 161.7, 170.0; MS (ES+): *m/z* 737.37 $[M+Na]^+$. Anal. Calcd for $C_{35}H_{46}N_4O_{12}$: C, 58.81; H, 6.49; N, 7.84. Found: C, 59.08; H, 6.17; N, 8.22.

4.14. (2′*R*)-Methyl 2′-(9*H*-fluoren-9-ylmethoxycarbonylamino)-2′-[3-(*N*,*N*′-di-*tert*-butoxycarbonyl-guanidine)-3-deoxy-6-O-triphenylmethyl-β-D-allopyranosyl]-acetate (27) and (2′*S*)-methyl 2′-(9*H*-fluoren-9-ylmethoxycarbonylamino)-2′-[3-(*N*,*N*′-di-*tert*-butoxycarbonyl-guanidine)-3-deoxy-6-*O*-triphenylmethyl-β-D-allopyranosyl]-acetate (28)

A solution of **25** or **26** (50 mg) in pyridine (3 mL) was cooled to 0 °C under argon. Triphenylmethyl chloride (10 equiv) was added, and then the mixture was stirred at room temperature overnight. The reaction mixture was diluted with CH_2Cl_2 (15 mL), washed with saturated $NaHCO_3$ solution (5 mL), and dried over $MgSO_4$, and the solvent was removed in vacuo. The residue was purified by column chromatography on silica gel (EtOAc–hexane 1:1) to afford **27/28** (**27**, 86%; **28**, 88%) as an oil. Compound **27**: R_f = 0.45 (EtOAc–hexane 2:3); $[\alpha]_D^{25}$ −42.0 (*c* 0.5, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.45 (s, 9H), 1.52 (s, 9H), 2.00 (br s, 2H), 3.06

(s, 1H), 3.38 (d, 2H, J = 4.6 Hz), 3.60 (m, 1H), 3.74 (s, 3H), 3.80 (dd, 1H, J = 2.4, 9.9 Hz), 3.98 (m, 2H), 4.22 (dd, 1H, J = 7.8, 7.9 Hz), 4.34 (dd, 1H, J = 7.6, 10.8 Hz), 4.43 (dd, 1H, J = 7.5, 10.6 Hz), 4.70 (s, 1H), 4.88 (dd, 1H, J = 2.6, 10.0 Hz), 5.80 (br d, 1H, J = 8.9 Hz), 7.20–7.49 (m, 19H), 7.59 (d, 1H, J = 7.9 Hz), 7.74 (d, 2H, J = 7.6 Hz), 8.9 (s, 1H), 11.4 (s 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.0, 28.1, 47.2, 52.1, 52.6, 56.2, 64.0, 67.4, 68.5, 69.9, 74.7, 76.8, 80.3, 84.1, 87.2, 120.0–144.0 (aromatic carbons), 152.9, 156.3, 158.9, 161.9, 170.0; MS (ES+): m/z 979.38 $[M+Na]^+$. Anal. Calcd for $C_{54}H_{60}N_4O_{12}$: C, 67.77; H, 6.32; N, 5.85. Found: C, 67.93; H, 6.00; N, 6.20. Compound **28**: R_f = 0.50 (EtOAc–hexane 2:3); $[\alpha]_D^{25}$ +28.0 (c 0.4, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.43 (s, 9H), 1.55 (s, 9H), 3.16–3.28 (m, 2H), 3.36 (br d, 1H, J = 10.1 Hz), 3.48 (dd, 1H, J = 3.9, 10.1 Hz), 3.54 (m, 1H), 3.74 (m, 2H), 3.77 (s, 3H), 3.96 (dd, 1H, J = 2.0, 9.9 Hz), 4.24 (m, 1H), 4.48 (dd, 1H, J = 6.6, 10.8 Hz), 4.56 (dd, 1H, J = 7.0, 10.6 Hz), 4.62 (m, 1H), 4.80 (dd, 1H, J = 2.0, 8.3 Hz), 5.64 (br d, 1H, J = 8.3 Hz), 7.20–7.34 (m, 10H), 7.37–7.48 (m, 9H), 7.59 (m, 2H), 7.76 (d, 2H, J = 7.6 Hz), 8.87 (br s, 1H), 11.50 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.1 (×6), 47.3, 53.1, 54.4, 56.4, 64.1, 65.9, 67.4, 68.2, 69.8, 76.0, 80.0, 84.0, 86.5, 120.0–144.0 (aromatic carbons), 152.8, 157.7, 159.2, 161.9, 170.0; MS (ES+): m/z 979.41 $[M+Na]^+$. Anal. Calcd for $C_{54}H_{60}N_4O_{12}$: C, 67.77; H, 6.32; N, 5.85. Found: C, 67.36; H, 6.69; N, 6.16.

4.15. (2′*R*)-2′-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-2′-[3-(*N*,*N*′-di-*tert*-butoxycarbonyl-guanidine)-3-deoxy-6-O-triphenyl methyl-β-D-allopyranosyl]-acetic acid (29) and (2′*S*)-2′-(9*H*-fluoren-9-ylmethoxycarbonylamino)-2′-[3-(*N*,*N*′-di-*tert*-butoxy carbonyl-guanidine)-3-deoxy-6-*O*-triphenylmethyl-β-D-allopyranosyl]-acetic acid (30)

Ester **27** or **28** (50 mg, 0.05 mmol) was dissolved in aqueous dioxane and NaOH (4.0 equiv, 1 N) was added at 0 °C. The solution was then stirred until complete by TLC (usually <1 h). Formic acid was added until the mixture is neutral and then diluted with EtOAc. The organic solution was extracted with EtOAc (3 × 10 mL), dried (sodium sulfate), and concentrated in vacuo. The residue was purified by flash chromatography (methanol–EtOAc 1:19) on SiO_2 providing **29**/**30** (**29**, 90%; **30**, 87%) as a colorless thick oil. Compound **29**: R_f = 0.15 (EtOAc); $[\alpha]_D^{25}$ −32.0 (c 0.1, $CHCl_3$); ^{1}H NMR (300 MHz, MeOH-d_4): δ 1.37 (s, 9H), 1.45 (s, 9H), 3.23 (m, 1H), 3.35–3.46 (m, 3H), 3.65 (m, 2H), 3.97 (m, 1H), 4.14 (m, 1H), 4.21–4.32 (m, 2H), 4.56 (m, 1H), 4.72 (dd, 1H, J = 2.8, 3.2 Hz, H-3), 7.09–7.17 (m, 5H), 7.19–7.28 (m, 8H), 7.34–7.40 (m, 6H), 7.57 (dd, 2H, J = 7.6, 10.6 Hz), 7.76 (t, 2H, J = 7.6); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.4, 28.6, 47.3, 57.5, 64.3, 65.1, 67.7, 68.1, 68.3, 71.1, 77.4, 80.9, 85.1, 87.9, 121.0–145.7 (aromatic carbons), 155.0, 159.2, 160.3, 163.8, 171.8; MS (ES+): m/z 965.27 $[M+Na]^+$. Anal. Calcd for $C_{53}H_{58}N_4O_{12}$: C, 67.50; H, 6.20; N, 5.94. Found: C, 67.88; H, 5.93; N, 6.17. Compound **30**: R_f = 0.15 (EtOAc); $[\alpha]_D^{25}$ +16.0 (c 0.1, $CHCl_3$); ^{1}H NMR (300 MHz, MeOH-d_4): δ 1.35 (s, 9H), 1.46 (s, 9H), 3.29 (m, 1H), 3.37–3.45 (m, 3H), 3.60–3.70 (m, 2H), 3.96 (br d, 1H, J = 10.0 Hz), 4.13 (dd, 1H, J = 6.5, 7.4 Hz), 4.24–4.36 (m, 2H), 4.56 (m, 1H), 4.70 (dd, 1H, J = 2.9, 3.3 Hz, H-3), 7.08–7.16 (m, 5H), 7.17–7.29 (m, 8H), 7.32–7.41 (m, 6H), 7.55 (dd, 2H, J = 7.8, 10.0 Hz), 7.65 (dd, 2H, J = 4.0, 7.8 Hz); ^{13}C NMR (75 MHz, MeOH-d_4): δ 28.3, 28.5, 47.2, 57.3, 64.2, 65.0, 67.5, 68.2, 68.3, 71.4, 77.3, 80.7, 85.1, 87.8, 120.9–130.1 (aromatic), 142.5–147.6 (aromatic), 154.3, 159.0, 160.0, 163.6, 170.1; MS (ES+): m/z 965.36 $[M+Na]^+$. Anal. Calcd for $C_{53}H_{58}N_4O_{12}$: C, 67.50; H, 6.20; N, 5.94. Found: C, 67.18; H, 6.53; N, 6.32.

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Supplementary data

Supplementary data (^{1}H NMR and ^{13}C NMR spectra for compounds **3**–**10** and **13**–**24**) associated with this article can be found, in the online version, at doi:10.1016/j.carres.2010.04.017.

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