Synthesis of 2-(*N*-Acetylamino)-2-deoxy-*C*-glucopyranosyl Nucleosides as Potential Inhibitors of Chitin Synthases†

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The *C*-glucopyranosyl nucleosides (**1**−**4**) containing the *N*-acetyl glucosaminyl and uridine units have been synthesized as nonhydrolyzable substrate analogues of UDP-GlcNAc aimed to inhibit the chitin synthases. The key intermediate, 4-(2′-(*N*-acetylamino)-3′,4′,6′-tri-*O*-benzyl-2′-deoxy-α-D-glucopyranosyl)but-2-enoic acid (**5**), was prepared from the perbenzylated (*N*-acetylamino)-α-*C*-allylglucoside (**7**), by successive oxidative cleavage, Wittig olefination, and ester deprotection. The coupling of the acid **5** with the hydroxyl or amine function of the uridine derivatives (**6a** or **6b**) afforded, respectively, the ester **12** and amide **14**. The dihydroxylation of the conjugated double bond in ester **12** or amide **14** was better achieved with osmium tetraoxide/barium chlorate, leading to the expected diols **13** and **15** as a mixture of two diastereoisomers. The desired compounds **1**−**4** were obtained after catalytic hydrogenation of compounds **12**−**15**.

Introduction

Inhibitors of glycosyltransferases, which are involved in the biosynthesis of glycoproteins, glycolipids, and other glycoconjugates, have proven to be useful for studies on biological function of cell-surface carbohydrates. Such compounds present particular interest in the development of potential pharmaceuticals such as antiviral, antitumor, and immunoregulatory agents.[1] For example, tunicamycin, an inhibitor of UDP-GlcNAc dolicyl phosphate GlcNAc-1-phosphotransferase, has been shown to inhibit replication in yeast, fungi, protozoa, enveloped virus, and mammalian cell lines in culture.[2]

Chitin, the β-1→4-linked homopolymer of *N*-acetyl-D-glucosamine (GlcNAc), is one of the most abundant natural polymers and one of the major structural components of the cell wall of most fungi. The biosynthesis of chitin is assumed by chitin synthases (UDP-*N*-acetyl-D-glucosamine:chitin 4-β-*N*-acetylglucosaminyl transferase EC 2.4.1.16) which perform polymerization of *N*-acetyl-D-glucosamine starting from UDP-GlcNAc.[3] Inhibition of chitin synthases represents an attractive approach to the design of effective new antifungal agents. Polyoxines and nikkomycines, a group of peptidyl nucleoside antibiotics produced by some species of *Streptomyces*, have been demonstrated to be competitive inhibitors of chitin synthases and exhibit antifungal, insecticidal, and acarcidal activities.[4] To the best of our knowledge, no synthetic inhibitor of chitin synthases has even been reported and little is known about the biochemical nature of these enzymes.[5] Accordingly, our initial approach in the design and synthesis of competitive inhibitors of chitin synthases was to synthesize nucleosidic *C*-glycosides derivatives designed as nonhydrolyzable analogues of the natural substrate UDP-GlcNAc (Figure 1).

It is assumed that a nonhydrolyzable substrate analogue featuring a *N*-acetyl-D-glucosamine residue linked to uridine through a suitable carbon chain could block the glycosyl transfer. A hydroxylated carbon chain is expected to imitate the diphosphate moiety by chelating the divalent cation present at the active site of the enzyme. Furthermore, because of the carbon linker, such compounds would be resistant to acidic and enzymatic hydrolysis. These molecules, which represent the first nonhydrolyzable substrate analogues of UDP-GlcNAc, are also expected to be useful in glycobiology for probing the details of catalytic mechanism of other *N*-acetylglucosaminyltransferases. Indeed the GlcNAc residue is a common component of natural glycoconjugates that are generated by a large class of *N*-acetylglucosaminyl transferases processing with UDP-GlcNAc as glycosyl donnor.[6] We reported herein the synthesis of compounds **1**−**4**.

Results and Discussion

The synthesis of the target molecules **1**−**4** was planned by coupling the (*N*-acetylamino)-α-*C*-glucopyranoside **5** and the protected uridine derivative **6** (Scheme 1). Compound **5** was prepared from *N*-acetyl-D-glucosamine, which was transformed into amino α-*C*-allylglucopyranoside **7** (containing less than 10% of β anomer) as previously described.[7] Zemplén deacetylation of **7** followed by classical benzylation afforded **8** in 94% yield

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† In the memory of Professor Stanislas Czernecki. Deceased October 20, 1997.

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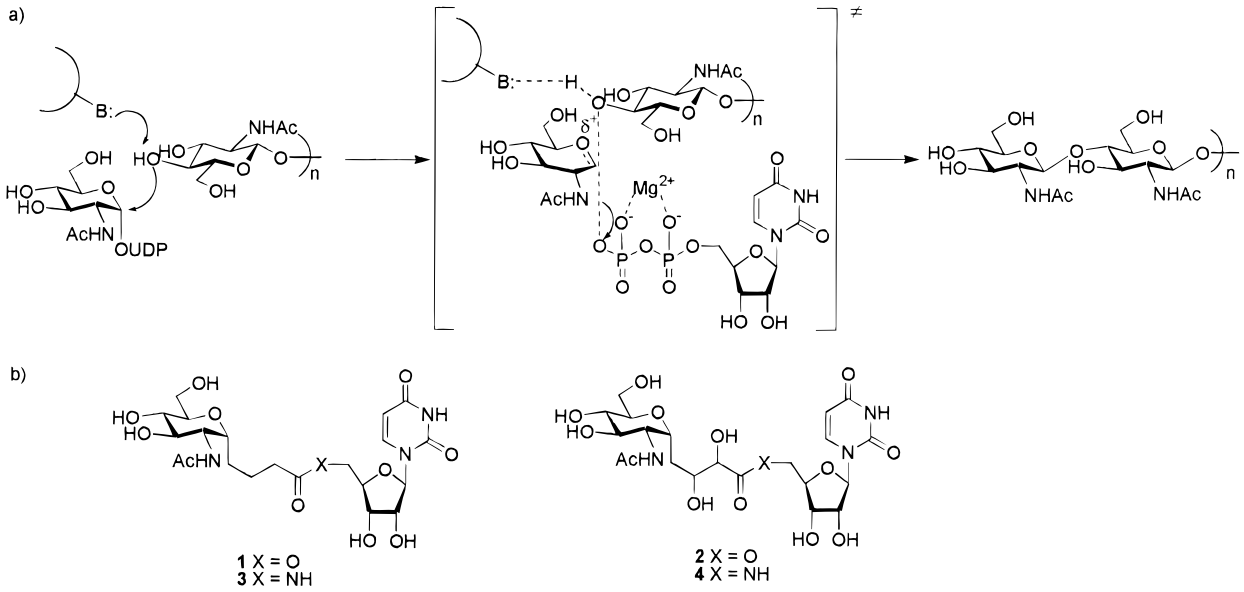
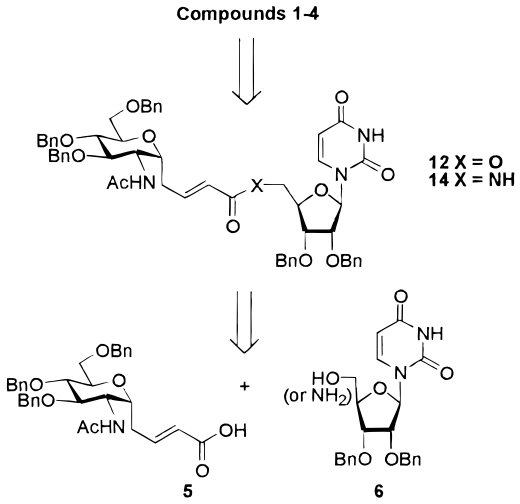
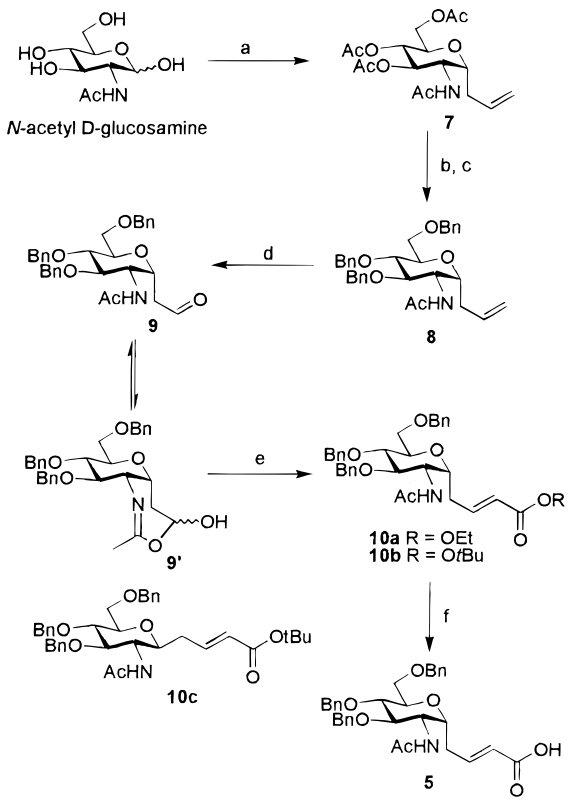


Figure 1. (a) Postulated transition structure for chitin synthases. (b) Designed nonhydrolyzable substrate analogues.

Scheme 1



Scheme 2[a]



[a]Key: (a) see ref 7; (b) MeONa/MeOH, 0 °C to rt, 3 h, quant.; (c) NaH, DMF, BnBr, 0 °C to rt, 94 %; (d) OsO_4, $NaIO_4$, THF/H_2O 99 %; (e) Ph_3P=CHCOOEt (or *t*Bu), toluene (or THF), 69 %; (f) TFA, CH_2Cl_2, 86 %.

(Scheme 2). Oxidative cleavage of the double bond (OsO_4/$NaIO_4$) of **8** yielded the aldehyde **9** which is in equilibrium with the cyclic form **9′** as demonstrated by ^{1}H and ^{13}C NMR spectra (see Experimental Section). Wittig condensation was performed on the mixture of **9** and **9′** with Ph_3P=CHCOOEt in toluene and afforded compound **10a** as the sole isomer (69% yield). The *E* configuration of the so-formed double bond was established by the large coupling constant between the two vinylic protons (J = 16 Hz) on the ^{1}H NMR spectrum. However, the basic deprotection of **10a** to **5** was not effective, leading to less than 50% yield, and was accompanied by partial epimerization to β-anomer (until ~50%), presumably via a retro-Michael pathway (Chart 1).

A similar epimerization of α- to β-*C*-glucopyranosides under basic conditions was observed previously.[8] To avoid this side reaction, we decided to use Ph_3P=CHCOO*t*Bu as the olefination agent. In this case, we were able to separate (in 2% yield) the small amount of β-anomer **10c** arising from the allylation reaction which was used to obtain compound **7**. The α-*tert*-butyl ester **10b** was obtained in 69% and was easily cleaved (CF_3COOH/CH_2Cl_2) to afford the desired acid **5** in good yield (86%).

The required 2′,3′-di-*O*-benzyl-protected uridine **6a** was obtained as previously described.[9] The 5′-amino-5′-deoxy derivative **6b** was easily prepared from **6a**, by a one-pot azidation of the 5′-hydroxyl group ($CBr_4/PPh_3/NaN_3$ in

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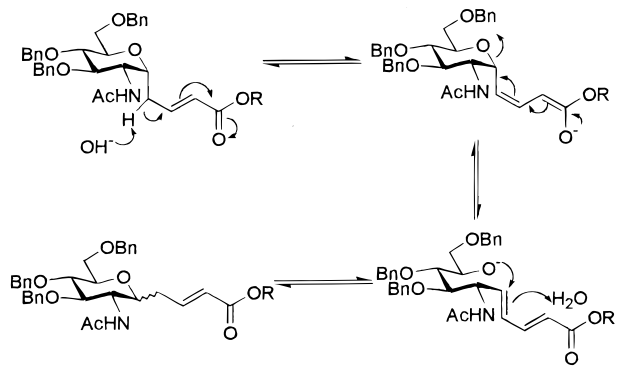
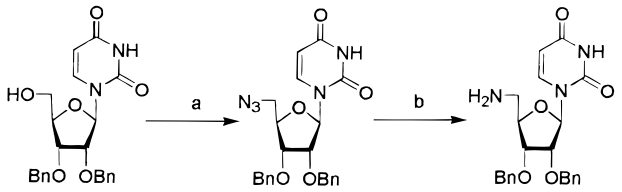
Chart 1



Scheme 3[a]



[a]Key: (a) CBr_4, PPh_3, NaN_3, DMF, 90 °C, 95 %; (b) $NaBH_4$, $HS(CH_2)_3SH$, Et_3N, *i*PrOH, 60 %.

DMF), affording **11**, and consecutive selective reduction (Scheme 3).

The esterification of a primary hydroxyl group is a well-established process. However, the coupling between the acid **5** and the alcohol **6a** was particularly difficult: the activations of the acid function of **5** with $(CF_3CO)_2O$ or Bop-Cl were both inefficient. The use of DCC/DMAP or IIDQ as activating agents afforded **12** in low yield (<30%). The use of the corresponding acid fluoride (**5a**) or pentafluorophenyl ester (**5b**) allowed the esterification together with partial anomerization of the *N*-acetyl glucosaminyl unit due to the presence of base in the reaction mixture. Finally, the best result was obtained by the mixed anhydride ($EtOCOCl/Et_3N$) method, which afforded **12** in 58% yield (Scheme 4). On the contrary, the amide derivative **14** was better obtained by condensation of the pentafluorophenyl ester **5b** with **6b** (92% yield without epimerization).

The tentative asymmetric dihydroxylation of the double bond in compounds **12** and **14** with AD-mix-β[10] proved to be unsuccessful. The basic conditions required by this reaction resulted in the cleavage of the ester or amide bond in **12** or **14**. Classical dihydroxylation of **12** (OsO_4/NMO) afforded less than 10% of the expected diol **13** and was exceedingly slow (one week). Fortunately, the use of $OsO_4/Ba(ClO_3)_2$[11] was much more efficient leading to **13** (75% yield) as an inseparable mixture of two isomers (45:55). Starting from the amide **14**, both systems afforded the diol **15** (84%) as a mixture of two isomers (33:67). The major isomer was obtained in pure form by preparative TLC, but it was not possible until now to obtain this derivative in a crystalline form suitable for X-ray crystallographic studies.

Hydrogenation of compounds **14** and **15** with Pd/C in MeOH led to the target molecules **3** and **4** in excellent yield. However, we have noted that the deprotected esters **1** and **2** were not stable in MeOH: transterification occurred at room temperature leading to the corresponding methyl ester and uridine. Consequently, hydrogenations have to be conducted in dry THF.

In summary, a new class of (*N*-acetylamino)-*C*-glucopyranosyl nucleosides derivatives were prepared. Different functionalizations of compounds **12** and **14** (e.g., epoxidation or monohydroxylation of the double bond) are planned. Compounds **1**−**4** are nonhydrolyzable analogues of the natural substrate of *N*-acetylglucosaminyltransferases including chitin synthases. The biological assays of these compounds toward chitin synthases are under way.

Experimental Section

General. The general methods were previously described.[12] THF was distilled over sodium and benzophenone prior to use. CH_2Cl_2 and pyridine were distilled over CaH_2. Compounds **6a**[9] and **7**[7] were prepared as described in the literature.

3-(2′-(*N*-Acetylamino)-3′,4′,6′-tri-*O*-benzyl-2′-deoxy-α-D-glucopyranosyl)propene (8). To a MeOH (100 mL) solution of compound **7** (7.028 g, 18.9 mmol), at 0 °C under argon atmosphere, was added NaOMe (1 M, 3.2 mL). After 3 h stirring at room temperature, the reaction mixture was neutralized by Amberlite IR-120 (H^+ form), filtered, and concentrated. The deprotected *C*-glucoside in dry DMF (60 mL) was then added to a suspension of NaH (1.3 eq/OH) in DMF (10 mL) at 0 °C. Benzyl bromide (7.5 mL, 6.237 mmol) was introduced dropwise after 30 min further stirring at 0 °C and the mixture stirred for additional 16 h at room temperature. Crushed ice was added, leading to precipitation of a white solid. The precipitate was filtered and washed with water and cold ether to give **8** (9.16 g, 94%): mp 125 °C, $[\alpha]_D$ +11.5 (*c* 0.98, CH_2Cl_2); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.80 (s, 3 H), 2.13−2.23 (m, 2 H), 3.55 (m, 1 H), 3.65−3.69 (m, 1 H), 3.71−3.84 (m, 2 H), 3.89−3.93 (td, 1 H), 4.19 (m, 1 H), 4.20 (m, 1 H), 4.38−4.61 (m, 6 H), 4.99−5.11 (m, 2 H), 5.73−5.84 (m, 1 H), 6.50 (d, 1 H, J = 9.8 Hz), 7.18−7.34 (m, 15 H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 21.3, 33.8, 45.4, 65.8, 65.9, 69.8, 71.2, 74.7, 75.7, 115.1, 125.6, 125.8, 125.9, 126.1, 126.4, 126.5, 132.4, 135.4, 135.6, 136.2, 167.7. Anal. Calcd for $C_{32}H_{37}NO_5$: C, 74.53; H, 7.23; N, 2.71. Found: C, 74.56; H, 7.09; N, 2.69.

2-(2′-(*N*-Acetylamino)-3′,4′,6′-tri-*O*-benzyl-2′-deoxy-α-D-glucopyranosyl)ethanal (9 and 9′). OsO_4 (1% solution in *t*-BuOH, 3 mL) and $NaIO_4$ (2.75 g, 59.58 mmol) were added to a solution of compound **8** (6.138 g, 11.92 mmol) in a mixture of THF/H_2O (1:1, 100 mL). After 15 min stirring, the reaction mixture was extracted with $CHCl_3$ (3 × 50 mL). The organic layer was washed (H_2O), dried ($MgSO_4$), filtered, and concentrated in vacuo to give **9** and **9′** (6.11 g, 99%) as white solid. Column chromatography (2:1 EtOAc−hexane) afforded an analytical sample: mp 104 °C, $[\alpha]_D$ +20.5 (*c* 1, CH_2Cl_2); ^{1}H NMR (250 MHz, $CDCl_3$) δ 2.03 (s, 1.5 H), 2.14 (s, 1.5 H), 1.88−2.39 (m, 2 H), 3.37−4.20 (m, 6.5 H), 4.11 (d, 0.5 H), 4.17−4.21 (m, 0.5 H), 4.29−4.92 (m, 6 H), 5.27 (dd, 0.5 H, J = 5.2, 12.1 Hz), 5.55 (d, 0.02 H), 5.59 (td, 0.5 H, J = 2.4, 6.6 Hz), 7.11−7.29 (m, 15 H), 9.68 (s, 0.02 H). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 20.7, 21.1, 30.2, 39.1, 56.3, 58.8, 64.7, 66.5, 66.9, 67.2, 70.0, 71.0, 71.2, 71.6, 71.7, 72.2, 73.2, 73.4, 74.7, 76.4, 78.2, 81.6, 82.4, 126.2, 126.3, 126.4, 126.5, 126.8, 126.9, 136.0, 136.3, 136.6, 136.7, 169.8, 170.6. M/S (CI, NH_3) *m*/*z* (rel intensity) 535 ($M + NH_4^+$, 6.4), 518 ($M + H^+$, 100). Anal. Calcd for $C_{31}H_{35}NO_6$: C, 71.93; H, 6.81; N, 2.70. Found: C, 71.77; H, 6.88; N, 2.78.

Ethyl 4-(2′-(*N*-Acetylamino)-3′,4′,6′-tri-*O*-benzyl-2′-deoxy-α-D-glucopyranosyl)but-2-enoate (10a). Ph_3P=CHCOOEt (542 mg, 1.56 mmol) was added to a solution of **9** and **9′** (403 mg, 0.779 mmol) in toluene (8 mL). After 15 h stirring at

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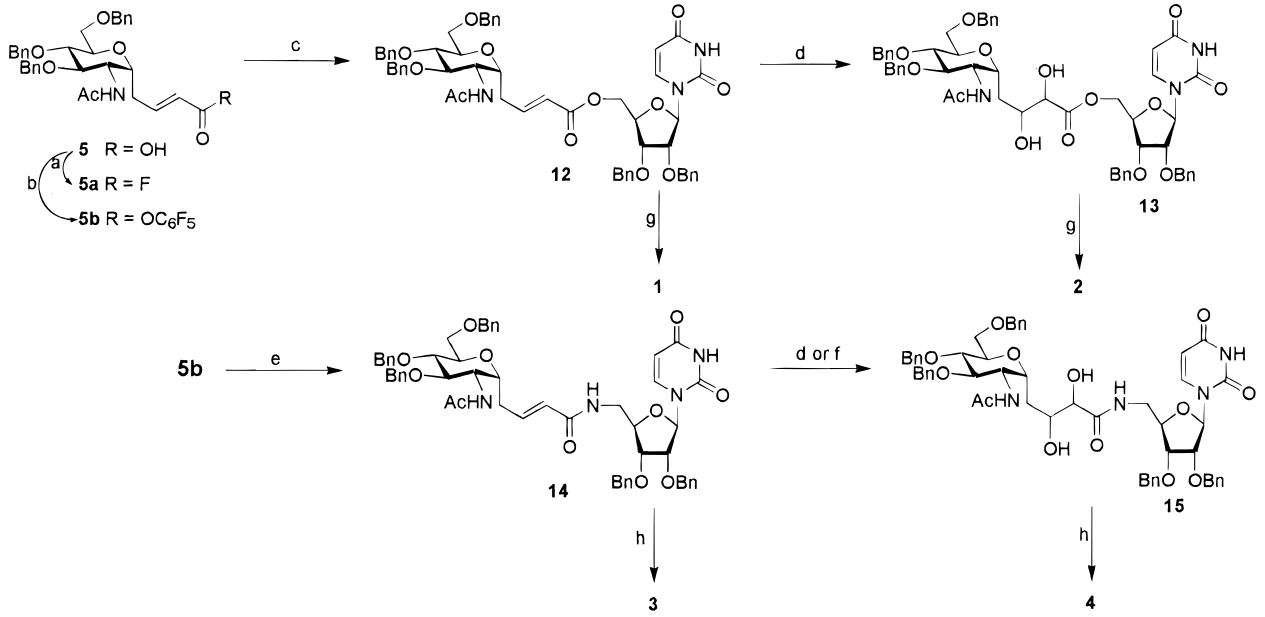
Scheme 4[a]



[a]Key: (a) $(FCN)_3$, CH_2Cl_2, Pyr, 100 %; (b) HOC_6F_5, DCC, DMF, 92 %; (c) EtOCOCl, Et_3N, CH_2Cl_2, **6a**, DMAP cat., 58 %; (d) OsO_4, $Ba(ClO_3)_2$, THF/H_2O, 75 %; (e) **6b**, DMAP cat., DMF, 94 %; (f) OsO_4, NMO, THF/H_2O, 84 %; (g) H_2, Pd/C, dry THF, 93-100 %; (h) H_2, Pd/C, MeOH, 94-100%.

reflux, the mixture was chromatographied (2:1 hexanes−EtOAc) to give **10a** as white solid (421 mg, 92%): mp 92 °C, $[\alpha]_D$ +9.6 (*c* 1.04, CH_2Cl_2); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.36 (t, 3 H, J = 7.1 Hz), 1.94 (s, 3 H), 2.43 (m, 2 H), 3.69−3.96 (m, 5 H), 4.12 (m, 1 H), 4.22−4.38 (m, 3 H), 4.51−4.73 (m, 6 H), 6.02 (d, 1 H, J = 15.7 Hz), 6.74 (d, 1 H, J = 9.5 Hz), 7.09 (td, 1 H, J = 15.7, 7.1 Hz), 7.32−7.41 (m, 15 H). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 13.3, 22.4, 33.4, 46.8, 59.2, 66.2, 66.6, 70.9, 71.2, 71.9, 72.4, 73.3, 74.2, 122.4, 126.6, 126.8, 126.9, 127.0, 127.2, 127.5, 127.6, 136.3, 136.5, 137.2, 144.0, 165.5, 168.9. Anal. Calcd for $C_{35}H_{41}NO_7$: C, 71.53; H, 7.03; N, 2.38. Found: C, 71.44; H, 6.91; N, 2.29.

***tert*-Butyl 4-(2′-(*N*-Acetylamino)-3′,4′,6′-tri-*O*-benzyl-2′-deoxy-α-D-glucopyranosyl)but-2-enoate (10b)**. Treatment of Ph_3P=CHCOO*t*Bu (1.264 g, 3.36 mmol) with **9** and **9′** (868 mg, 1.68 mmol) in THF (25 mL) as described for **10a** followed by chromatography (1:1 hexanes−EtOAc) afforded 712 mg (69%) of α-anomer **10b** and 20 mg (2%) of β-anomer **10c**. α-Anomer **10b**: white solid, mp 70 °C, $[\alpha]_D$ +20.4 (*c* 1.1, MeOH); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.42 (s, 9 H), 1.79 (s, 3 H), 2.26 (m, 2 H), 3.56−3.84 (m, 4 H), 3.99 (m, 1 H), 4.13−4.23 (m, 2 H), 4.36−4.58 (m, 6 H), 5.80 (d, 1 H, J = 15.7 Hz), 6.57 (d, 1 H, J = 9.7 Hz), 6.85 (td, 1 H, J = 7.8, 15.7 Hz), 7.17−7.28 (m, 15 H). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 23.67, 28.55, 34.54, 48.32, 67.54, 67.90, 72.23, 72.55, 72.84, 73.32, 73.70, 74.69, 75.52, 80.47, 125.33, 127.98, 128.12, 128.36, 128.93, 128.94, 137.67, 138.48, 144.10, 166.21, 170.47. Anal. Calcd for $C_{37}H_{45}NO_7$: C, 72.17; H, 7.37; N, 2.27. Found: C, 72.44; H, 7.31; N, 2.39. β-Anomer **10c**: mp 75 °C, $[\alpha]_D$ +14.0 (*c* 0.1, CH_2Cl_2); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.38 (s, 9 H), 1.70 (s, 3 H), 2.36 (m, 2 H), 3.28−3.36 (m, 2 H), 3.45−3.74 (m, 5 H), 4.37−4.80 (m, 6 H), 5.00 (d, 1 H, J = 8.8 Hz), 5.71 (d, 1 H, J = 15.7 Hz), 6.78 (td, 1 H, J = 15.7, 7.2 Hz), 7.13−7.30 (m, 15 H). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 23.84, 28.53, 35.52, 55.47, 69.16, 73.88, 74.81, 75.25, 78.15, 79.23, 79.56, 83.09, 125.21, 127.97, 128.18, 128.41, 128.66, 128.77, 128.86, 129.00, 138.40, 138.59, 138.79, 144.15, 166.30, 176.60. Anal. Calcd for $C_{37}H_{45}NO_7$: C, 72.17; H, 7.37; N, 2.27. Found: C, 72.70; H, 7.22; N, 2.15.

4-(2′-(*N*-Acetylamino)-3′,4′,6′-tri-*O*-benzyl-2′-deoxy-α-D-glucopyranosyl)but-2-enoic Acid (5). To a solution of **10b** (640 mg, 1.04 mmol) in dry CH_2Cl_2 (4 mL) was added TFA (800 μL, 10.4 mmol) at 0 °C. The solution was stirred at room temperature for 1.5 h. After dilution with CH_2Cl_2 (20 mL), the solution was washed with H_2O (3 × 30 mL), dried ($MgSO_4$), filtered, and evaporated to give **5** as white solid (501 mg, 86%): mp 76 °C, $[\alpha]_D$ +6.2 (*c* 0.69, CH_2Cl_2); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.80 (s, 3 H), 2.24 (m, 2 H), 3.53−3.80 (m, 4 H), 3.94 (m, 1 H, H-1′), 4.10−4.20 (m, 2 H), 4.33−4.56 (m, 6 H), 5.81 (d, 1 H, J = 15.7 Hz), 6.61 (d, 1 H, J = 10.0 Hz), 6.79 (m, 1 H), 7.14−7.28 (m, 15 H). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 22.18, 32.94, 46.44, 66.25, 66.53, 70.73, 71.08, 71.91, 72.15, 73.23, 73.82, 125.22, 126.54, 126.67, 126.83, 126.97, 127.33, 127.43, 130.95, 136.26, 136.44, 136.98, 141.57, 168.97, 170.02. Anal. Calcd for $C_{33}H_{37}NO_7$: C, 70.82; H, 6.66; N, 2.50. Found: C, 70.64; H, 6.82; N, 2.33.

4-(2′-(*N*-Acetylamino)-3′,4′,6′-tri-*O*-benzyl-2′-deoxy-α-D-glucopyranosyl)but-2-enoic Acid Fluoride (5a). To a solution of **5** (16 mg, 0.029 mmol) in dry CH_2Cl_2 (0.5 mL) were added cyanuric fluoride (19.3 μL, 0.232 mmol) and Pyr (2.3 μL, 0.029 mmol) under Ar atmosphere.[13] After 16 h at reflux, the solution was diluted with CH_2Cl_2 (50 mL) and washed with H_2O (3 × 30 mL), dried ($MgSO_4$), filtered, and evaporated to give **5a** as an oil (16 mg, 100%): ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.80 (s, 3 H), 2.37 (m, 2 H), 3.52−3.84 (m, 4 H), 4.00 (m, 1 H), 4.13−4.25 (m, 2 H), 4.37−4.60 (m, 6 H), 5.89 (dd, 1 H, J = 15.7, 8.6 Hz), 6.69 (d, 1 H, J = 9.6 Hz), 7.15−7.34 (m, 16 H). IR 1808 cm^{-1}.

Pentafluorophenyl 4-(2′-(*N*-Acetylamino)-3′,4′,6′-tri-*O*-benzyl-2′-deoxy-α-D-glucopyranosyl)but-2-enoate (5b). To a solution of **5** (100 mg, 0.179 mmol) in dry DMF (4 mL) at 0 °C were added pentafluorophenol (33 mg, 0.179 mmol) and DCC (37 mg, 0.179 mmol).[14] The solution was stirred at room temperature for 22 h. After concentration in vacuo, the crude residue was purified by preparative TLC (1:2 hexanes−EtOAc) to give **5b** (120 mg, 92%) as white solid: ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.83 (s, 3 H), 2.43 (m, 2 H), 3.56−3.68 (m, 3 H), 3.90 (t, 1 H, J = 7.7 Hz), 4.07 (m, 1 H), 4.19 (d, 1 H, J = 8.7 Hz), 4.30 (t, 1 H, J = 7.0 Hz), 4.38−4.62 (m, 6 H), 6.16 (d, 1 H, J = 15.7 Hz), 6.75 (d, 1 H, J = 9.7 Hz), 7.18−7.36 (m, 16 H).

5′-Azido-2′ 3′-di-*O*-benzyl-5′-deoxyuridine (11). To a solution of 2′,3′-di-*O*-benzyluridine (**6a**)[9] (887 mg, 2.092 mmol) in dry DMF (4 mL) were added successively Ph_3P (657 mg, 2.510 mmol), CBr_4 (972 mg, 2.929 mmol), and NaN_3 (2.72 g, 41.84 mmol). The mixture was stirred at 90 °C for 15 h. After solvent evaporation in vacuo, the residue was diluted in

(13) Carpino, L. A.; Sadat-Aalacc, D.; Chao, H. G.; Deselons, R. H. *J. Am. Chem. Soc.* **1990**, *112*, 9651−9652.

(14) Kisfaludy, L.; Schön, I. *Synthesis* **1983**, 325−327.

EtOAc, washed (H_2O), dried ($MgSO_4$), filtered, and concentrated. The crude product was chromatographied (1:1 hexanes−EtOAc) to give **11** as an oil (892 mg, 95%): $[\alpha]_D$ +118 (*c* 1, CH_2Cl_2); ^{1}H NMR (250 MHz, $CDCl_3$) δ 3.50 (dd, 1 H, J = 13.6, 2.3 Hz), 3.74 (dd, 1 H, J = 13.6, 2.9 Hz), 3.81 (dd, 1 H, J = 7.7, 5.1 Hz), 3.98 (dd, 1 H, J = 5.1, 2.2 Hz), 4.23 (td, 1 H, J = 7.7, 3.0 Hz), 4.31 (d, 1 H, J = 11.6 Hz), 4.60 (d, 1 H, J = 11.6 Hz), 4.70 (s, 2 H), 5.62 (d, 1 H, J = 8.2 Hz), 5.84 (d, 1 H, J = 2.1 Hz), 7.19−7.32 (m, 10 H), 7.43 (d, 1 H, J = 8.2 Hz), 8.89 (s, 1 H). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 48.89, 70.11, 70.44, 73.25, 76.48, 77.83, 87.86, 100.36, 125.86, 126.11, 126.16, 126.34, 126.50, 127.88, 134.97, 135.16, 137.93, 148.19, 161.80. Anal. Calcd for $C_{23}H_{23}N_5O_5$: C, 61.46; H, 5.16; N, 15.58. Found: C, 61.88; H, 5.31; N, 15.74.

5′-Amino-2′,3′-di-*O*-benzyl-5′-deoxyuridine (6b). To a stirred suspension of **11** (892 mg, 1.987 mmol) in *i*PrOH (3 mL) were added $HS(CH_2)_3SH$ (40 μL, 0.4 mmol), Et_3N (553 μL, 3.974 mmol), and $NaBH_4$ (151 mg, 3.974 mmol) . After stirring at 70 °C for 2 h, solvent was evaporated. The residue was diluted with CH_2Cl_2, washed with H_2O, dried ($MgSO_4$), filtered, and concentrated. The crude product was chromatographied (9:1 to 1:1 CH_2Cl_2−MeOH) to give **6b** as white solid (504 mg, 60%): mp 95 °C, $[\alpha]_D$ +46.5 (*c* 0.65, MeOH), ^{1}H NMR (250 MHz, $CDCl_3$) δ 2.86 (dd, 1 H, J = 14.1, 4.4 Hz), 3.08 (dd, 1 H, J = 14.1, 3.2 Hz), 3.50 (s, 2 H), 3.86 (dd, 1 H, J = 7.4, 5.2 Hz), 4.06 (dd, 1 H, J = 5.2, 2.4 Hz), 4.16 (m, 1 H), 4.36 (d, 1 H, J = 11.8 Hz), 4.55 (d, 1 H, J = 11.8 Hz), 4.73 (s, 2 H), 5.61 (d, 1 H, J = 8.1 Hz), 5.85 (d, 1 H, J = 2.3 Hz), 7.23−7.37 (m, 10 H), 7.69 (d, 1 H, J = 8.1 Hz), 8.89 (s, 1 H). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 41.65, 71.50, 71.62, 76.17, 78.32, 82.07, 89.30, 101.66, 127.30, 127.45, 127.73, 127.91, 136.82, 140.17, 149.88, 163.58. Anal. Calcd for $C_{23}H_{25}N_3O_5$: C, 65.24; H, 5.95; N, 9.92. Found: C, 65.50; H, 6.03; N, 9.70.

Preparation of Ester 12. The acid **5** (40 mg, 0.072 mmol) was dissolved in dry CH_2Cl_2 (1 mL), and to this solution, stirred at − 10 °C under Ar atmosphere, were added EtOCOCl (12 μL, 0.130 mmol) and Et_3N (15 μL, 0.108 mmol). The reaction mixture was stirred at −10 °C for 20 min, and alcohol **6a** (30 mg, 0.072 mmol) and DMAP (cat.) were then introduced. The mixture was stirred 48 h at room temperature and then diluted in CH_2Cl_2, washed with H_2O, dried ($MgSO_4$), filtered, concentrated and purified by preparative TLC (1:3 hexanes−EtOAc) to afford **12** as white solid (40 mg, 58%): mp 65 °C, $[\alpha]_D$ +38.4 (*c* 0.68, CH_2Cl_2); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.82 (s, 3 H), 2.31 (m, 2 H), 3.46−3.66 (m, 4 H), 3.75−3.82 (m, 1 H), 3.86 (dd, 1 H, J = 7.0, 5.2 Hz), 4.00 (m, 1 H), 4.01 (dd, 1 H, J = 5.0, 2.3 Hz), 4.08−4.14 (m, 1 H), 4.24−4.59 (m, 10 H), 4.72 (s, 2 H), 5.65 (d, 1 H, J = 8.3 Hz), 5.82 (d, 1 H, J = 16.4 Hz), 5.83 (d, 1 H, J = 2.4 Hz), 6.66 (d, 1 H, J = 9.7 Hz), 6.94 (td, 1 H, J = 15.5, 7.6 Hz), 7.22−7.29 (m, 26 H). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 23.89, 35.06, 48.22, 62.67, 67.48, 67.94, 72.34, 72.55, 72.73, 72.92, 73.19, 73.72, 74.68, 75.33, 75.52, 79.07, 80.32, 90.14, 102.79, 122.73, 128.16, 128.36, 128.64, 128.96, 129.06, 137.52, 127.70, 137.79, 137.87, 138.54, 140.04, 147.31, 150.57, 163.93, 166.10, 170.53. Anal. Calcd for $C_{56}H_{59}N_3O_{12}$: C, 70.82; H, 6.66; N, 2.50. Found: C, 70.64; H, 6.82; N, 2.33.

Preparation of Diol 13. To a stirred solution of **12** (65 mg, 0.067 mmol) in 2 mL of THF and 1 mL of water were added $Ba(ClO_3)_2 \cdot H_2O$ (32.5 mg, 0.101 mmol) and OsO_4 (1% solution in *t*BuOH, 130 μL), and the mixture was left at room temperature for 15 h. The mixture was concentrated to a residue that was diluted in EtOAc, washed with H_2O, dried ($MgSO_4$), filtered, concentrated, and purified by preparative TLC (19:1 CH_2Cl_2-MeOH) to afford the title compound (50 mg, 75%) as a mixture of two isomers (45:55): mp 70−71 °C, $[\alpha]_D$ +17.5 (*c* 0.4, CH_2Cl_2); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.70 (m, 2 H), 1.90 and 1.91 (2s, 3 H), 3.44 (m, 1 H), 3.48−3.57 (m, 1 H), 3.69 (m, 1 H), 3.98−4.51 (m, 21 H), 5.57 (d, 0.55 H, J = 2.2 Hz), 5.59 (dd, 0.55 H, J = 2.0 and 8.1 Hz), 5.64 (d, 0.45 H, J = 8.1 Hz), 5.68 (d, 0.55 H, J = 4.0 Hz), 5.91 (d, 0.45 H, J = 2.4 Hz), 6.68 (d, 0.45 H, J = 9.6 Hz), 6.91 (d, 0.55 H, J = 9.6 Hz), 7.07 (d, 0.55 H, J = 8.1 Hz), 7.26−7.35 (m, 25 H), 7.47 (d, 0.45 H, J = 8.2 Hz). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 23.43, 34.29, 48.13, 48.50, 61.33, 62.79, 63.12, 65.38, 66.92, 67.51, 69.83, 71.99, 72.15, 72.34, 72.66, 72.99, 73.18, 73.32, 73.81, 74.01, 74.69, 75.00, 75.15, 75.29, 78.86, 79.83, 80.02, 89.78, 91.37, 92.01, 102.54, 127.77, 127.97, 128.20, 128.41, 128.57, 137.41, 140.75, 141.39, 141.88, 150.23, 163.51, 170.39, 170.80, 172.65. Anal. Calcd for $C_{56}H_{59}N_3O_{14}$: C, 67.39; H, 5.96; N, 4.21. Found: C, 67.70; H, 5.84; N, 4.08.

Preparation of Amide 14. To a solution of **5b** (110 mg, 0.152 mmol) in dry DMF (5 mL) were added **6b** (64 mg, 0.152 mmol) and DMAP (1.5 mg). After 2 days stirring at room temperature, the solvent was evaporated and the residue purified by preparative TLC (1:9 MeOH−CH_2Cl_2) to give **14** as white solid (131 mg, 94%): mp 227 °C, $[\alpha]_D$ −39.0 (*c* 1, CH_2Cl_2); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.86 (s, 3 H), 2.30 (m, 2 H), 3.44 (m, 1 H), 3.67 (m, 2 H), 3.85 (m, 1 H), 4.12 (m, 1 H), 4.24 (m, 1 H), 4.34−4.58 (m, 12 H), 4.71 (d, 1 H, J = 12.0 Hz), 5.20 (d, 1 H, J = 4.0 Hz), 5.55 (d, 1 H, J = 8.0 Hz), 5.89 (d, 1 H, J = 15.7 Hz), 6.62 (m, 1 H), 6.76 (d, 1 H, J = 8.4 Hz), 6.79 (d, 1 H, J = 7.9 Hz), 6.90 (m, 1 H), 7.14−7.30 (m, 25 H). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 23.56, 34.41, 40.73, 47.21, 66.25, 67.56, 71.89, 72.52, 73.01, 73.33, 74.25, 75.49, 78.65, 82.07, 96.58, 102.86, 127.76, 127.85, 127.93, 128.21, 128.54, 128.65, 128.83, 137.27, 137.49, 137.81, 138.07, 143.24, 150.72, 163.13, 166.66, 170.59. Anal. Calcd for $C_{56}H_{60}N_4O_{11}$: C, 69.69; H, 6.27; N, 5.81. Found: C, 69.44; H, 6.38; N, 5.89.

Preparation of Diol 15. A solution of compound **14** (140 mg, 0.145 mmol) in THF/H_2O (8:1, 4 mL) was stirred at room temperature and treated with NMO (33 mg, 0.282 mmol) and OsO_4 (1% solution in *t*BuOH, 37 μL). The reaction mixture was left at room temperature for 72 h before being diluted with a 10% aqueous solution of sodium bisulfite. The resulting solution was left under stirring for 10 min before extraction with CH_2Cl_2 (3 × 15 mL). The combined organic layers were washed with H_2O, dried ($MgSO_4$), filtered, concentrated, and purified by preparative TLC (19:1 CH_2Cl_2−MeOH) to afford pure compound **15** as a mixture of two isomers (67:33, 121 mg, 84%). Further purification led to a pure sample of the major isomer: mp 131 °C, $[\alpha]_D$ −11.0 (*c* 1, CH_2Cl_2); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.92 (m, 2 H), 2.02 (s, 3 H), 3.55−3.65 (m, 3 H), 3.81 (m, 2 H), 4.05−4.16 (m, 4 H), 4.34−4.40 (m, 5 H), 4.53−4.83 (m, 10 H), 5.70 (d, 1 H, J = 3.9 Hz), 5.71 (d, 1 H, J = 8.1 Hz), 6.84 (d, 1 H, J = 9.6 Hz), 7.20 (d, 1 H, J = 8.1 Hz), 7.32−7.50 (m, 26 H), 8.82 (s, 1 H). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 23.65, 34.90, 40.02, 48.43, 67.08, 67.52, 71.71, 72.35, 72.61, 72.98, 73.40, 73.50, 73.65, 73.93, 74.33, 75.61, 77.05, 78.89, 81.52, 92.53, 103.07, 128.01, 128.25, 128.41, 128.64, 128.94, 142.33, 150.50, 163.56, 170.99, 173.39. Anal. Calcd for $C_{56}H_{60}N_4O_{13}$: C, 67.46; H, 6.07; N, 5.62. Found: C, 67.06; H, 6.10; N, 5.51.

Experimental Procedure for the Catalytic Hydrogenation of Compounds 12 to 15. Compounds **12** to **15** (∼70 mg) were hydrogenated in dry THF (for compounds **12** and **13**) or MeOH (for compounds **14** and **15**) (2 mL) at atmospheric pressure over 10% palladium on charcoal (20 mg) for 20 h at room temperature. The catalyst was filtered off and washed with THF or MeOH. Concentration of the solution afforded the desired compound.

Ester 1: 93 % yield from **12**; mp 124 °C, $[\alpha]_D$ +35.0 (*c* 1, H_2O); ^{1}H NMR (250 MHz, D_2O) δ 1.47−1.80 (m, 2 H), 1.93 (t, 3 H, J = 6.4 Hz), 2.00 (s, 3 H), 2.49 (t, 2 H, J = 6.4 Hz), 3.38 (dd, 1 H, J = 8.4, 9.7 Hz), 3.45−3.52 (m, 1 H), 3.64−3.73 (m, 1 H), 3.68 (t, 2 H, J = 6.4 Hz), 3.80 (dd, 1 H, J = 2.2, 12.2 Hz), 3.92 (dd, 1 H, J = 5.7, 10.5 Hz), 3.99−4.06 (m, 1H), 4.30−4.32 (m, 2 H), 4.36 (dd, 1 H, J = 3.9, 5.1 Hz), 4.42 (d, 1 H, J = 3.2 Hz), 5.85 (d, 1 H, J = 3.7 Hz), 5.89 (d, 1 H, J = 8.1 Hz), 7.74 (d, 1 H, J = 8.0 Hz), 8.13 (s, 1 H). ^{13}C NMR (62.9 MHz, D_2O) δ 20.60, 22.15, 24.04, 33.42, 53.76, 61.28, 63.80, 69.70, 71.04, 72.81, 73.58, 73.84, 81.58, 90.65, 102.49, 142.08, 151.79, 166.52, 174.68, 176.02. Anal. Calcd for $C_{21}H_{31}N_3O_{12}$: C, 48.74; H, 6.04; N, 8.12. Found: C, 48.55; H, 6.20; N, 8.01.

Ester 2 (two diastereoisomer)**:** 100 % yield from **13**; mp 204 °C, $[\alpha]_D$ + 30.9 (*c* 0.7, H_2O), ^{1}H NMR (250 MHz, D_2O) δ 1.87−2.01 (m, 2 H), 2.23 (s, 3 H), 3.38−3.45 (m, 1 H), 3.58−4.52 (m, 13 H), 5.83 and 5.90 (2d, 1 H, J = 3.6 Hz), 5.88 and 5.89 (2d, 1 H, J = 8.0 Hz), 7.74 and 7.78 (2d, 0.5 H, J = 8.5 Hz), 7.86 (d, 1 H, J = 8.0 Hz), 8.44 (s, 0.25 H). ^{13}C NMR (62.9 MHz, D_2O) δ 22.23, 28.37, 53.49, 61.36, 61.45, 64.38, 64.71,

68.46, 69.74, 70.86, 71.74, 72.35, 73.02, 73.52, 73.98, 75.96, 81.34, 81.57, 84.53, 89.66, 90.31, 102.63, 142.14, 152.03, 166.81, 173.84, 174.79. Anal. Calcd for $C_{21}H_{31}N_3O_{14}$: C, 45.90; H, 5.69; N, 7.65. Found: C, 45.70; H,5.81; N, 7.53.

Amide 3: 94 % yield from **14**; mp 118−120 °C (decomposition), $[\alpha]_D$ +50.4 (*c* 1, MeOH); ^{1}H NMR (250 MHz, CD_3OD) δ 1.31−1.76 (m, 4 H), 1.93 (s, 3 H), 2.22 (m, 2H), 3.31−3.63 (m, 6 H), 3.73−3.77 (m, 1 H), 3.85−3.96 (m, 5 H), 4.21 (m, 1 H), 5.69 (d, 1 H, J = 4.6 Hz), 5.70 (d, 1 H, J = 8.0 Hz), 7.66 (d, 1 H, J = 8.0 Hz), 8.06 (s, 1 H). ^{13}C NMR (62.9 MHz, CD_3OD) δ 22.71, 23.03, 25.70, 36.46, 42.26, 55.04, 63.12, 72.36, 72.48, 72.88, 74.22, 74.58, 74.65, 83.97, 92.60, 102.91, 143.44, 152.35, 166.15, 173.58, 176.39. Anal. Calcd for $C_{21}H_{32}N_4O_{11}$: C, 48.83; H, 6.24; N, 10.85. Found: C, 48.41; H, 6.37; N, 10.76.

Amide 4 (one diastereoisomer)**:** 100% yield from **15**; mp 176 °C (decomposition), $[\alpha]_D$ + 36.2 (*c* 1.1, MeOH), ^{1}H NMR (250 MHz, D_2O) δ 1.97 (s, 3 H), 1.72−2.05 (m, 2 H), 2.67 (t, 1 H, J = 6.5 Hz), 3.37 (t, 1 H, J = 9.1 Hz), 3.48−3.78 (m, 7 H), 3.91 (dd, 1 H, J = 5.8, 10.7 Hz), 4.07−4.22 (m, 4 H), 4.31 (t, 1 H), 5.77 (d, 1 H, J = 4.6 Hz), 5.85 (d, 1 H, J = 8.1 Hz), 7.65 (d, 1 H, J = 8.1 Hz). ^{13}C NMR (62.9 MHz, D_2O) δ 22.18, 28.68, 40.59, 53.59, 61.28, 69.63, 70.41, 70.79, 71.01, 71.36, 72.64, 73.30, 73.50, 82.38, 90.39, 102.69, 142.58, 151.88, 166.51, 174.83, 175.79. Anal. Calcd for $C_{21}H_{32}N_4O_{13}$: C, 45.99; H, 5.88; N, 10.21. Found: C, 45.48; H, 5.99; N, 10.13.

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