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Synthesis of N^4-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-asparagine analogues: Succinamide, L-2-hydroxysuccinamide, and L-2-hydroxysuccinamic acid hydrazide analogues

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Abstract

The syntheses of three analogues of N^4-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-asparagine are described. *N*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)succinamide was synthesized by the reaction of pentafluorophenyl succinamate with 2-acetamido-2-deoxy-β-D-glucopyranosylamine. 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosylamine was synthesized, and the complete assignment of the ^{1}H NMR spectrum is given. Reaction of the protected β-D-glycosylamine with L-malic acid chloralid in the presence of a coupling agent (EEDQ) gave N^4-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-L-malamic acid chloralid that was deprotected two ways: (1) using ammonia, which gave N^4-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-2-hydroxysuccinamide, and (2) using hydrazine, which gave N^4-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-2-hydroxysuccinamic acid hydrazide.

Keywords: β-*N*-Acetylglucosaminyl-L-asparagine analogues; N^4-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)succinamides; Pentafluorophenyl succinamate; L-Malic acid chloralid; Succinamic acid hydrazide

1. Introduction

The synthesis of analogues of N^4-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-asparagine ((GlcNAc-)Asn) has not been widely reported. Two types of analogues containing the *N*-glycosylic bond are possible: analogues of the sugar and analogues of the amino acid. Four analogues of the sugar have been reported: N^4-(β-D-glucopyranosyl)-L-asparagine ((Glc-)Asn) [1–3], N^4-(β-D-mannopyranosyl)-L-asparagine ((Man-)Asn) [2,3], N^4-(β-D-galactopyranosyl)-L-asparagine ((Gal-)Asn) [2,3], and N^4-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-L-asparagine ((GalNAc-)Asn) [2]. Nine analogues of the amino acid have been reported: *N*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)acetamide [4–8], *N*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)chloroacetamide [4,7], *N*-(2-acetam-ido-2-deoxy-β-D-glucopyranosyl)bromoacetamide [4], *N*-(2-acet-

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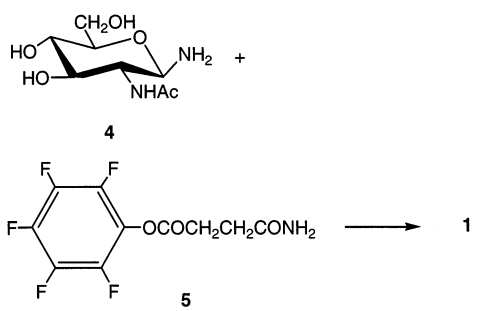
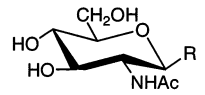
amido - 2 - deoxy - β - D - glucopyranosyl)azidoacetamide [4], N^1-(2-acetamido-2-deoxy-β-D-glucopyranosyl)glycinamide [4,7], N^1-(2-acetamido - 2 - deoxy - β - D - glucopyranosyl) - N^2,N^2-dimethylglycinamide [4], N^1-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-N^2-benzyloxycarbonylglycinamide [4], N-(2-acetamido-2-deoxy-β-D-glucopyranosyl)propionamide [8], N^1-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-3-amino-4-hydroxybutyramide [6], and three that we recently reported [9], N^4-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-2-chlorosuccinamic acid, N^4-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-2-bromosuccinamic acid, and N^4-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D,L-2-methylsuccinamic acid. (Paul et al. [4] reported the synthesis of five additional analogues of the amino acid where the sugar hydroxyl groups were acetylated; however, these analogues were not O-deacetylated and characterized.) The amide bond between *N*-acetylglucosamine and asparagine is the principal linkage in the structure of N-linked glycoproteins [10], and the hydrolysis of this bond by glycosylasparaginase (GA, aspartylglucosaminidase (AGA), N^4-(β-*N*-acetyl-D-glucosaminyl)-L-asparaginase; EC 3.5.1.26) is a key step in the catabolism of N-linked glycoproteins [11]. The synthesis of the GlcNAc(β1-N)-succinamide **1**, L-2-hydroxysuccinamide **2**, and L-2-hydroxysuccinamic acid hydrazide ((3*S*) - 3 - hydrazinocarbonyl - 3 - hydroxypropionamide) **3** analogues of the amino acid of GlcNAc-Asn are described in this paper. In addition, the complete assignment of the ^{1}H NMR spectrum of 2-acetamido-3,4,6-tri-*O*-acetyl - 2 - deoxy - β - D - glucopyranosylamine is given.



Scheme 1.



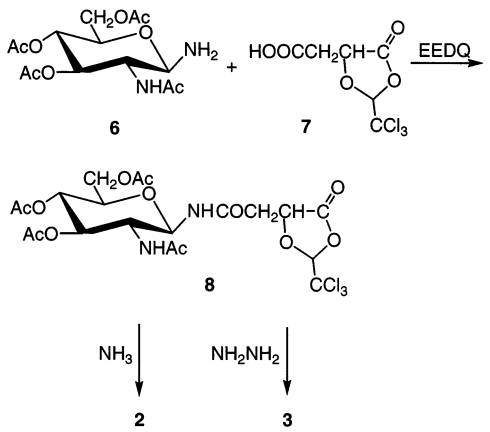
1 R = $NHCOCH_2CH_2CONH_2$

2 R = $NHCOCH_2CHOHCONH_2$

3 R = $NHCOCH_2CHOHCONHNH_2$

2. Results and discussion

The synthesis of the succinamide analogue **1** of GlcNAc-Asn (Scheme 1) required activation of the carboxylic acid group of succinamic acid. While there are many methods to activate a carboxylic acid, we chose the pentafluorophenyl ester [12], which was straightforward and gave crystals of **5** of high purity. The reaction of **5** with the β amino group of **4** in *N*,*N*-dimethylformamide (DMF) occurred exclusively at the amino group to form the *N*-glycosylic bond, which followed the well-established preference of activated carboxylic acids to react with the amino group to form the *N*-glycosylic (amide) bond rather than the sugar hydroxyl groups on carbohydrate molecules [3,9,12,13]. Thus, although **4** used in the reaction contained approximately 24% GlcNAc, reaction of **5** did not occur with a sugar hydroxyl group to form a carboxylate ester between GlcNAc and succinamic acid. We found that the use of aqueous DMF reduced the yield of **1** by approximately 75%. In order to purify **1**, pentafluorophenol released during the reaction was first removed on an anion-exchange column. We thought that it might be possible to oxidize the anomeric carbon of unreacted GlcNAc under very mild conditions using Tollen's reagent [14] for removal of the aldonic acid by anion-exchange chromatography; this was only partially successful (data not shown). Reversed-phase HPLC provided a direct route to the purification of **1**. The properties of **1** indicated the successful synthesis of the analogue. The nomenclature used to assign the NMR signals follows that used for GlcNAc-Asn [15]. The NMR spectra agreed with data reported for GlcNAc-Asn [15] and other analogues [9]. The characteristic doublet for the anomeric proton in the ^{1}H NMR spectrum appears at δ 5.068 with $J_{1,2}$ 9.77 Hz [13,15].



Scheme 2.

The L-2-hydroxysuccinamide analogue **2** and the L-2-hydroxysuccinamic acid hydrazide analogue **3** of GlcNAc-Asn were synthesized (Scheme 2) from a common intermediate, the protected sugar L-malamic acid chloralid **8**. The protected β-D-glycosylamine **6** is an important intermediate in the synthesis of *N*-glycosylic carbohydrates; the procedure most often cited to synthesize **6** is that of Bolton et al. [16,17]. The protected sugar **6** was synthesized in three steps from GlcNAc using well-established procedures: acetylation–chlorination with acetyl chloride to give the glycosyl chloride, azide substitution to give the glycosyl azide and hydrogenation to give the glycosyl amine **6**. Bolton et al. reported that the 60 MHz ^{1}H NMR spectrum of the intermediate glycosyl azide in $CDCl_3$ confirmed the β anomeric configuration because the H-1 proton was a doublet with $J_{1,2}$ 9 Hz, but no additional NMR data were given. In the characterization of the glycosyl amine **6**, no ^{1}H NMR data were reported by Bolton et al. Paul and Korytnyk [18] reported limited, selected ^{1}H NMR data for both the glycosyl azide and **6** in $CDCl_3$ at 100 MHz. The complete ^{1}H NMR assignments for the glycosyl azide were reported by Tropper et al. [19] in 1992, but we were not able to find a report of the complete ^{1}H NMR assignments for **6** despite the many citations to its synthesis. All of the signals for the glycosyl azide are first order, and our results agree with Tropper et al. [19]. The data also generally agree with data reported for similar compounds, such as the *C*-glycosyl compounds ethyl 2-*C*-(2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)acetate [20], 2-*C*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-nitroethene [21], and 4-*C*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-1-methoxybenzene [22]. Upon hydrogenation of the intermediate glycosyl azide to give **6**, significant changes occur in the ^{1}H NMR spectrum for H-1, H-2, H-3, H-4 and H-5. Specifically, H-3 and H-4 shift upfield, but the shift of H-3 is much larger than the shift of H-4 such that the positions of the protons in the spectrum have changed, and importantly the coupling changes from a first-order spectrum in the glycosyl azide to an ABMX (or MABX) second-order spectrum in **6**; the high symmetry arises because $J_{2,3}$ and $J_{4,5}$ are nearly identical. The assignments of the protons in **6** were verified in decoupling experiments. In particular, decoupling of H-2 or H-5 in **6** breaks the symmetry of the second-order spectrum for H-3 and H-4, respectively, in an identical, but opposite, manner to give half of a typical AB spectrum, as expected. To confirm the second-order spectrum for H-3 and H-4, a theoretical computation was done that was identical to the observed spectrum (data not shown). Our assignments differ from the broad, general chemical shifts reported by Paul and Korytnyk [18] only for H-1 and H-4, which are reversed. The properties of **6** were satisfactory.

The α-carboxyl and α-hydroxyl groups of L-malic acid were protected by reaction with chloral hydrate to give L-malic acid chloralid **7**. The chloralid is sufficiently unreactive that the β-carboxyl group may be activated; we activated the β-carboxyl group as the pentafluorophenyl ester and found that reaction with the β amino group of **4** occurred exclusively at the β-carboxyl group, and not with the chloralid (data not shown). However, it was more convenient to couple **7** directly with **6** using the coupling agent, EEDQ, without the intermediate synthesis of an activated ester. Again, we found that the chloralid was unreactive toward the β amino group of **6** under these conditions as the protected sugar L-malamic acid chloralid **8** was synthesized in high purity. Formation of the amide (*N*-glycosylic) bond at the anomeric carbon results in a

shift of the H-3 and H-4 protons in the ^{1}H NMR spectrum to give a first-order spectrum. The properties of the protected sugar L-malamic acid chloralid **8** were satisfactory. The L-2-hydroxysuccinamide analogue **2** was synthesized by deprotecting **8** in ethanol in the presence of ammonia from concentrated ammonium hydroxide. Analogue **2** was insoluble in ethanol and precipitated from solution during the 4 days of the deprotecting reaction. Compound **2** was purified by HPLC, and the eluent with a retention time of approximately 16 min was lyophilized. The properties of **2** were satisfactory and indicate successful synthesis of the analogue.

The L-2-hydroxysuccinamic acid hydrazide analogue **3** was synthesized by deprotecting **8** in ethanol in the presence of hydrazine by addition of hydrazine monohydrate. Analogue **3** was insoluble in ethanol and precipitated from solution during the 2 days of the deprotecting reaction. Compound **3** was purified by HPLC, and the eluent with a retention time of approximately 18 min was lyophilized. The properties of **3** were satisfactory and indicate successful synthesis of the compound. Deprotection of **8** with sodium methoxide gave a mixture of products that included the methyl ester analogue, and dehydration to give the fumaramic and maleamic acid analogues (data not shown)[1].

3. Experimental

Materials.—Chemicals purchased from the following suppliers were: 2-acetamido-2-deoxy-D-glucopyranose, succinamic acid, pentafluorophenol, 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline and hydrazine monohydrate from Aldrich Chemical Co.; D_2O (99.9 atom% ^{2}H), Me_2SO-d_6 (99.9 atom% ^{2}H), and $CHCl_3$-d (99.8 atom% ^{2}H) from Cambridge Isotopes Laboratories; chloral hydrate from Fischer Scientific Co.; *N*,*N*′-dicyclohexylcarbodiimide, Amberlite® IRA-400 (Cl^-) strongly basic gel-type anion-exchange resin, acetyl chloride, L-malic acid from Sigma Chemical Co.; ammonium bicarbonate from Spectrum. All other chemicals were at least analytical grade.

General methods.—A GE 300 spectrometer was used to record NMR spectra. ^{1}H NMR spectra were recorded at 300.2 MHz in a 5-mm probe at ambient temperature with a 2000 Hz sweep width, 30° pulse angle, and an 8K data block; no line-broadening factor was applied to the accumulated FID. Natural abundance ^{13}C NMR spectra were recorded at 75.5 MHz in a 5-mm probe at ambient temperature with a 10,000 Hz sweep width, 30° pulse angle, and an 8K data block; protons were broad-band decoupled and a line-broadening factor of 2.0 Hz was applied to the accumulated FID. The error in the measured chemical shifts is ± 0.002 ppm for ^{1}H NMR and ± 0.033 ppm for ^{13}C NMR; the error in the measured coupling constants is ± 0.50 Hz. Reversed-phase HPLC was done on a Perkin–Elmer Series 2 Liquid Chromatograph equipped with an LC-75 spectrophotometric UV detector and an ODS-3 preparative column. Water was used as the mobile phase at a flow rate of 1.0 mL/min, and the column was monitored at 220 or 240 nm. Infrared spectra were recorded on a Bio-Rad FTS 175C FTIR. Evaporation of solvents was conducted on a rotary evaporator at $\sim$60–70 °C at water aspirator vacuum. Elemental analyses were done at Atlantic Microlabs, Inc. (Norcross, GA).

Preparation of 2-acetamido-2-deoxy-β-D-glucopyranosylamine [6,7,9,12] (**4**).—2-Acetamido-2-deoxy-D-glucopyranose (GlcNAc) was dissolved in satd aq ammonium bicarbonate ($\sim$1.5 M) to give a molar ratio of 5:1 ammonia–sugar. After 6 days at 37 °C, with the intermittent addition of solid ammonium bicarbonate to maintain saturation,

[1] W.M. York, J.M. Risley, unpublished data. The fumaramic acid analogue and the maleamic acid analogue were synthesized independently by reaction of **4** with maleic anhydride and allowed to equilibrate overnight. The two analogues were separated by chromatofocusing on an Amberlite IRA-400 (Cl^-) anion-exchange column: the fumaramic acid analogue eluted at pH 3.9 and the maleamic acid analogue eluted at pH 3.0. The ^{1}H NMR spectrum of the fumaramic acid analogue has three characteristic signals (D_2O): δ 5.12 (d, 1 H, $J_{1,2}$ 9.77 Hz, H-1), 6.00 and 6.50 (d, 1 H each, $J_{\alpha,\beta}$ 15.9 Hz, H-α and H-β (*trans*-C*H*=C*H*–)). The ^{1}H NMR spectrum of the maleamic acid analogue has three characteristic signals (D_2O): δ 5.15 (d, 1 H, $J_{1,2}$ 9.77 Hz, H-1), 6.35 and 6.45 (d, 1 H each, $J_{\alpha,\beta}$ 12.5 Hz, H-α and H-β (*cis*-C*H*=C*H*–)).

solid ammonium bicarbonate was removed by filtration, and excess ammonium bicarbonate was removed by repeated rotary evaporation of aq solns. The white solid contained 76% **4** and 24% α and β anomers of GlcNAc as determined by integration of the ^{1}H NMR signals in D_2O for the anomeric protons at δ 4.14 ($J_{1,2}$ 9.28 Hz), δ 5.19 ($J_{1,2}$ 3.42 Hz), and δ 4.34 ($J_{1,2}$ 9.77 Hz), respectively. The crude compound was used without further purification.

Preparation of pentafluorophenyl succinamate (**5**).—Reaction of succinamic acid (1.43 g, 12.2 mmol), pentafluorophenol (2.25 g, 12.2 mmol), and *N*,*N*′-dicyclohexylcarbodiimide (2.52 g, 12.2 mmol) in DMF (15 mL) followed the general procedure of Urge et al. [12]. The mixture was stirred in an ice bath for 5 h, the precipitated dicyclohexylurea was removed by filtration, and the solvent was evaporated at 70 °C. The slightly yellow solid was recrystallized from 1,4-dioxane overnight at −20 °C. The white crystals were collected by filtration, washed with CH_2Cl_2, and air-dried for 24 h; yield 2.55 g (74%): mp 138–139.5 °C; ^{1}H NMR (Me_2SO-d_6, reference Me_4Si): δ 2.51 (t, 2 H, $J_{\alpha,\beta}$ 6.59 Hz, H-α (CH_2)), 2.93 (t, 2 H, $J_{\alpha,\beta}$ 6.59 Hz, H-β (CH_2)), 6.9 and 7.5 (s, 1 H, NH_2); ^{13}C NMR (Me_2SO-d_6, reference Me_4Si): δ 28.92 (C-β), 29.63 (C-α), 134–140 (pentafluorophenyl, $J_{CF} \sim 250$ Hz), 173.07 ($CONH_2$), 173.94 (COO).

N-*(2-Acetamido-2-deoxy-β-*D*-glucopyranosyl)succinamide hydrate* (**1**).—Compounds **4** (0.60 g, 2.2 mmol) and **5** (2.52 g, 8.9 mmol) were dissolved in DMF (24 mL) and stirred in an ice bath for 72 h [12]. After the solvent was evaporated at 60 °C, the light yellow solid was dissolved in distilled water (4 mL), and the insoluble material was removed by filtration. To remove pentafluorophenol, an Amberlite IRA-400 (Cl^-) anion-exchange column (1 × 25 cm) was activated with 0.1 M NaOH and washed with water. The filtrate was loaded onto the column and washed with distilled water (25 mL) at a rate of 1 mL/min. The eluent was collected and lyophilized to give 150 mg of a white solid, containing approximately 50% of **1**. Portions of the solid were purified by HPLC. The eluent with a retention time of 19.1–20.4 min was collected and lyophilized to give **1** as a white solid: mp 248.5–249.0 °C; IR (KBr): 3300, 2960, 2927, 1656.9, 1635.5, 1539.9, 1426.8 cm^{-1}; ^{1}H NMR (D_2O, reference acetone (2.225 ppm)): δ 2.050 (s, 3 H, $COCH_3$), 2.556 (center of A_2B_2 second order spectrum not resolved, 4 H, $NHCOCH_2CH_2CONH_2$), 3.474 (dd, 1 H, $J_{3,4}$ 10.25, $J_{4,5}$ 8.79 Hz, H-4 (C*H*OH)), 3.518 (m, 1 H, $J_{5,6a}$ 1.46, $J_{5,6b}$ 4.39 Hz, H-5 (CHO–)), 3.611 (dd, 1 H, $J_{2,3}$ 9.77 Hz, H-3 (C*H*OH)), 3.746 (dd, 1 H, $J_{6a,6b}$ −12.21 Hz, H-6b (CH_2OH)), 3.819 (dd, 1 H, $J_{1,2}$ 9.77 Hz, H-2 (C*H*NHAc)), 3.882 (dd, 1 H, H-6a (CH_2OH)), 5.068 (d, 1 H, H-1 (C*H*NH)); ^{13}C NMR (D_2O, reference 1,4-dioxane (66.66 ppm)): δ 22.06 ($COCH_3$), 29.85 (CH_2CONH_2), 30.48 (CH_2CONH), 54.37 (C-2), 60.58 (C-6), 69.57 (C-4), 74.29 (C-3), 77.69 (C-5), 78.43 (C-1), 174.82 ($CONH_2$), 175.62 (CONH), 177.76 ($COCH_3$). Anal. Calcd for $C_{12}H_{21}N_3O_7 \cdot 0.25H_2O$: C, 44.51; H, 6.69; N, 12.98. Found: C, 44.52; H, 6.66; N, 13.26.

*Preparation of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-*D*-glucopyranosyl chloride [23].*—The ^{1}H NMR spectrum in $CDCl_3$ showed a doublet for the anomeric proton at δ 6.197, $J_{1,2}$ 3.42 Hz.

*Preparation of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl azide [16].*—This compound was synthesized from 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (above) and gave crystals: mp 165–166 °C, lit. 166–168 °C [16], lit. 166–167 °C (dec) [19]; ^{1}H NMR ($CDCl_3$, reference Me_4Si): δ 1.993, 2.046, 2.053, 2.115 (s, 3 H each, 4 $COCH_3$), 3.811 (m, 1 H, $J_{4,5}$ 9.77, $J_{5,6a}$ 2.44, $J_{5,6b}$ 4.88 Hz, H-5 (CHO–)), 3.930 (m, 1 H, $J_{1,2}$ 9.28, $J_{2,3}$ 9.77, $J_{2,NH}$ 8.79 Hz, H-2 (C*H*NHAc)), 4.177 (dd, 1 H, $J_{6a,6b}$ −12.21 Hz, H-6a (CH_2OAc)), 4.284 (dd, 1 H, H-6b (CH_2OAc)), 4.782 (d, 1 H, H-1 (CHN_3)), 5.111 (dd, 1 H, $J_{3,4}$ 9.28 Hz, H-4 (CHOAc)), 5.265 (dd, 1 H, H-3 (CHOAc)), 5.779 (d, 1 H, NH (NHAc)).

*Preparation of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosylamine [16,17]* (**6**).—Hydrogenation of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl azide (above) over Adams' platinum oxide catalyst in anhyd EtOH at ambient temperature gave crystals: mp 150–151 °C (dec), lit. 150 °C (dec) [17]; ^{1}H NMR ($CDCl_3$, reference Me_4Si):

δ 1.983, 2.036, 2.051, 2.105 (s, 3 H each, 4 $COCH_3$), 3.650 (m, 1 H, $J_{4,5}$ 9.28, $J_{5,6a}$ 2.44, $J_{5,6b}$ 4.88 Hz, H-5 (CHO–)), 4.018 (m, 1 H, $J_{1,2}$ 9.28, $J_{2,3}$ 9.77, $J_{2,NH}$ 8.79 Hz, H-2 (C*H*NHAc)), 4.114 (dd, 1 H, $J_{6a,6b}$ −12.21 Hz, H-6a (CH_2OAc)), 4.133 (d, 1 H, H-1 (C*H*NH_2)), 4.226 (dd, 1 H, H-6b (CH_2OAc)), 5.042 (MA part of ABMX second-order spectrum, 1 H, $J_{3,4}$ 9.28 Hz, H-3 (CHOAc)), 5.089 (BX part of ABMX second-order spectrum, 1 H, H-4 (CHOAc)), 5.716 (d, 1 H, NH (NHAc)).

Preparation of L-*malic acid chloralid [24]* (**7**).—L-Malic acid (40.2 g, 0.30 mol) and chloral hydrate (59.5 g, 0.36 mol) were mixed with concd H_2SO_4 (30 mL) in an ice bath, stirred for 2 h, and allowed to warm to rt overnight. The mixture was poured onto ice (200 g) and extracted with EtOAc (4 × 90 mL). The extracts were combined, washed with water (3 × 180 mL), and dried over sodium sulfate. The solvent was evaporated to give a light yellow solid that was recrystallized from toluene (300 mL) at 4 °C overnight to give a white solid that was dried to give 13.70 g (52%) of **7**: mp 135–136.5 °C, lit. 140 °C [24]; ^{1}H NMR ($CDCl_3$, reference Me_4Si): δ 3.055 (dd, 1 H, $J_{\alpha,\beta}$ 3.42, $J_{\beta,\beta'}$ −18.07 Hz, H-β (CH_2)), 3.149 (dd, 1 H, $J_{\alpha,\beta'}$ 3.91 Hz, H-β′ (CH_2)), 4.904 (dd, 1 H, H-α (CH_2CH)), 5.903 (s, 1 H, chloralid ($CHCCl_3$)).

N^4-*(2-Acetamido-3,4,6-tri*-O-*acetyl-2-deoxy-β*-D-*glucopyranosyl)*-L-*malamic acid chloralid* (**8**).—Compounds **6** (1.74 g, 5 mmol) and **7** (1.32 g, 5 mmol) were dissolved in $CHCl_3$ (100 mL), to which was added 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) (1.24 g, 5 mmol) [25]. The reaction was stirred at ambient temperature for 22 h, and the solvent was evaporated at a temperature of less than 40 °C. The white solid was dissolved in EtOAc (40 mL), petroleum ether was added to turbidity, and the mixture was allowed to crystallize at rt for 8 h. The crystals were collected and washed with diethyl ether to give 2.13 g (72%) of **8**: mp 199–200 °C; ^{1}H NMR ($CDCl_3$, reference Me_4Si): δ 1.991, 2.054, 2.082, 2.100 (s, 3 H each, 4 $COCH_3$), 2.806 (dd, 1 H, $J_{\alpha,\beta}$ 3.42, $J_{\beta,\beta'}$ −17.09 Hz, H-β (CH_2CH)), 2.968 (dd, 1 H, $J_{\alpha,\beta'}$ 3.91 Hz, H-β′ (CH_2CH)), 3.746 (m, 1 H, $J_{4,5}$ 9.77, $J_{5,6a}$ 1.95, $J_{5,6b}$ 4.39 Hz, H-5 (CHO–)), 4.089 (m, 1 H, $J_{1,2}$ not resolved, $J_{2,3}$ 9.77, $J_{2,NH}$ 7.81 Hz, H-2 (C*H*NHAc)), 4.095 (m, 1 H, $J_{1,NH}$ not resolved, H-1 (C*H*NHCOR)), 4.104 (dd, 1 H, $J_{6a,6b}$ −12.70 Hz, H-6a (CH_2OAc)), 4.323 (dd, 1 H, H-6b (CH_2OAc)), 4.813 (dd, 1 H, H-α (CH_2CH)), 5.033 (dd, 1 H, $J_{3,4}$ 9.28 Hz, H-3 (CHOAc)), 5.138 (dd, 1 H, H-4 (CHOAc)), 5.917 (s, 1 H, chloralid ($CHCCl_3$)), 6.126 (d, 1 H, NH (NHAc)). Anal. Calcd for $C_{20}H_{25}Cl_3N_2O_{12}$: C, 40.67; H, 4.27; Cl, 17.78; N, 4.75. Found: C, 40.73; H, 4.32; Cl, 17.82; N, 4.73.

N^4-*(2-Acetamido-2-deoxy-β*-D-*glucopyranosyl)*-L-*2-hydroxysuccinamide* (**2**).—Compound **8** (0.30 g, 0.51 mmol) was dissolved in EtOH (25 mL), and concd ammonium hydroxide (1.25 mL) was added [26]. The soln was stirred at rt for 4 days. The white precipitate was collected, washed with EtOH and diethyl ether, dissolved in water (2 mL), and purified by HPLC. The eluent with a retention time of 15.1–16.8 min was collected and lyophilized to give 65 mg (38%) of **2** as a white powder: mp 218–218.5 °C; IR (KBr): 3300, 2960, 2940, 2927, 2910, 1671, 1659, 1556 cm^{-1}; ^{1}H NMR (D_2O, reference acetone (2.225 ppm)): δ 2.004 (s, 3 H, $COCH_3$), 2.594 (dd, 1 H, $J_{\alpha,\beta}$ 8.79, $J_{\beta,\beta'}$ −15.14 Hz, H-β (CH_2CH)), 2.773 (dd, 1 H, $J_{\alpha,\beta'}$ 3.91 Hz, H-β′ (CH_2CH)), 3.478 (dd, 1 H, $J_{3,4}$ 10.25, $J_{4,5}$ 8.30 Hz, H-4 (C*H*OH)), 3.536 (m, 1 H, $J_{5,6a}$ 1.46, $J_{5,6b}$ 4.39 Hz, H-5 (CHO–)), 3.612 (dd, 1 H, $J_{2,3}$ 9.77 Hz, H-3 (C*H*OH)), 3.749 (dd, 1 H, $J_{6a,6b}$ −12.21 Hz, H-6b (CH_2OH)), 3.830 (dd, 1 H, $J_{1,2}$ 9.77 Hz, H-2 (C*H*NHAc)), 3.887 (dd, 1 H, H-6a (CH_2OH)), 4.473 (dd, 1 H, H-α (CH_2CH)), 5.097 (d, 1 H, H-1 (C*H*NHCOR)); ^{13}C NMR (D_2O, reference 1,4-dioxane (66.66 ppm)): δ 22.060 (CO*C*H_3), 40.074 (C-β), 54.240 (C-2), 60.515 (C-6), 67.986 (C-α), 69.506 (C-4), 74.228 (C-3), 77.657 (C-5), 78.271 (C-1), 173.363 (NHCOR), 174.848 ($CONH_2$), 178.471 (*C*OCH_3). Anal. Calcd for $C_{12}H_{21}N_3O_8$: C, 42.97; H, 6.32; N, 12.54. Found: C, 42.71; H, 6.28; N, 12.81.

N^4-*(2-Acetamido-2-deoxy-β*-D-*glucopyranosyl)*-L-*2-hydroxysuccinamic acid hydrazide dihydrate* (**3**)[2].—Compound **8** (0.25 g, 0.42

[2] IUPAC name: (3*S*)-*N*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(3-hydrazinocarbonyl-3-hydroxypropionamide).

mmol) was dissolved in EtOH (20 mL), and hydrazine monohydrate (1.25 mL) was added [27]. The soln was stirred at rt for 2 days. The white precipitate was collected, washed with EtOH and diethyl ether, dissolved in water (3 mL), and purified by HPLC. The eluent with a retention time of 16.6–19.3 min was collected and lyophilized to give 106 mg (65%) of **3** as a white powder: mp 207–208 °C; IR (KBr): 3315, 2929, 2902, 1652, 1555, 1423 cm^{-1}; ^1H NMR (D_2O, reference acetone (2.225 ppm)): δ 2.004 (s, 3 H, $COCH_3$), 2.591 (dd, 1 H, $J_{\alpha,\beta}$ 8.30, $J_{\beta,\beta'}$ −15.14 Hz, H-β (CH_2CH)), 2.762 (dd, 1 H, $J_{\alpha,\beta'}$ 3.91 Hz, H-β′ (CH_2CH)), 3.479 (dd, 1 H, $J_{3,4}$ 10.25, $J_{4,5}$ 8.30 Hz, H-4 ($CHOH$)), 3.533 (m, 1 H, $J_{5,6a}$ 1.95, $J_{5,6b}$ 4.88 Hz, H-5 (CHO–)), 3.616 (dd, 1 H, $J_{2,3}$ 9.77 Hz, H-3 ($CHOH$)), 3.747 (dd, 1 H, $J_{6a,6b}$ −12.21 Hz, H-6b (CH_2OH)), 3.827 (dd, 1 H, $J_{1,2}$ 9.77 Hz, H-2 ($CHNHAc$)), 3.887 (dd, 1 H, H-6a (CH_2OH)), 4.518 (dd, 1 H, H-α (CH_2CH)), 5.089 (d, 1 H, H-1 ($CHNHCOR$)); ^{13}C NMR (D_2O, reference 1,4-dioxane (66.66 ppm)): δ 22.12 ($COCH_3$), 40.24 (C-β), 54.31 (C-2), 60.58 (C-6), 67.76 (C-α), 69.57 (C-4), 74.29 (C-3), 77.72 (C-5), 78.37 (C-1), 173.23 ($CONHNH_2$), 173.62 (NHCOR), 174.95 ($COCH_3$). Anal. Calcd for $C_{12}H_{22}N_4O_8 \cdot 2H_2O$: C, 37.30; H, 6.78; N, 14.50. Found: C, 37.10; H, 6.70; N, 14.27.

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