

CARBOHYDRATE RESEARCH

www.elsevier.nl/locate/carres

Carbohydrate Research 329 (2000) 487-493

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

De Hua Huang, John M. Risley \*

Department of Chemistry, University of North Carolina at Charlotte, 9201 University City Blvd., Charlotte, NC 28223, USA

Received 20 June 2000; accepted 21 July 2000

#### Abstract

The syntheses of three analogues of  $N^4$ -(2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl)-L-asparagine are described. N-(2-Acetamido-2-deoxy- $\beta$ -D-glucopyranosyl)succinamide was synthesized by the reaction of pentafluorophenyl succinamate with 2-acetamido-2-deoxy- $\beta$ -D-glucopyranosylamine. 2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy- $\beta$ -D-glucopyranosylamine was synthesized, and the complete assignment of the <sup>1</sup>H NMR spectrum is given. Reaction of the protected  $\beta$ -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- $\beta$ -D-glucopyranosyl)-L-malamic acid chloralid that was deprotected two ways: (1) using ammonia, which gave  $N^4$ -(2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl)-L-2-hydroxysuccinamide, and (2) using hydrazine, which gave  $N^4$ -(2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl)-L-2-hydroxysuccinamic acid hydrazide. © 2000 Elsevier Science Ltd. All rights reserved.

*Keywords:*  $\beta$ -*N*-Acetylglucosaminyl-L-asparagine analogues;  $N^4$ -(2-Acetamido-2-deoxy- $\beta$ -D-glucopyranosyl)succinamides; Penta-fluorophenyl succinamite; L-Malic acid chloralid; Succinamic acid hydrazide

## 1. Introduction

The synthesis of analogues of  $N^4$ -(2-acetamido-2-deoxy- $\beta$ -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-Dglucopyranosyl)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-

<sup>\*</sup> Corresponding author. Tel.: + 1-704-6874844; fax: + 1-704-6873151.

E-mail address: jmrisley@email.uncc.edu (J.M. Risley).

amido - 2 - deoxy -  $\beta$  - D - glucopyranosyl)azidoacetamide [4],  $N^1$ -(2-acetamido-2-deoxy- $\beta$ -Dglucopyranosyl)glycinamide [4,7],  $N^{1}$ -(2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl)- $N^2$ , $N^2$ dimethylglycinamide [4],  $N^1$ -(2-acetamido-2deoxy- $\beta$ -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)-3amino-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- $\beta$ -D-glucopyranosyl)-L-2-bromosuccinamic acid, and  $N^4$ -(2-acetamido-2-deoxy- $\beta$ -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 Nacetylglucosamine 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),  $N^4$ -( $\beta$ -N-acetyl-Dglucosaminyl)-L-asparaginase; EC 3.5.1.26) is a key step in the catabolism of N-linked glycoproteins [11]. The synthesis of the GlcNAc( $\beta$ 1-N)-succinamide 1, L-2-hydroxysuccinamide 2, and L-2-hydroxysuccinamic acid hydrazide ((3S) - 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 <sup>1</sup>H NMR spectrum of 2-acetamido-3,4,6-tri-Oacetyl - 2 - deoxy -  $\beta$  - D - glucopyranosylamine is given.



Scheme 1.



### 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  $\beta$  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 <sup>1</sup>H NMR spectrum appears at  $\delta$  5.068 with J<sub>1.2</sub> 9.77 Hz [13,15].



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  $\beta$ -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: acetylationchlorination 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 <sup>1</sup>H NMR spectrum of the intermediate glycosyl azide in CDCl<sub>3</sub> confirmed the  $\beta$  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 <sup>1</sup>H NMR data were reported by Bolton et al. Paul and Korytnyk [18] reported limited, selected <sup>1</sup>H NMR data for both the glycosyl azide and 6 in CDCl<sub>3</sub> at 100 MHz. The complete <sup>1</sup>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 <sup>1</sup>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-B-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-3,4,6tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-1methoxybenzene [22]. Upon hydrogenation of the intermediate glycosyl azide to give 6, significant changes occur in the <sup>1</sup>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  $\alpha$ -carboxyl and  $\alpha$ -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  $\beta$ -carboxyl group may be activated; we activated the  $\beta$ -carboxyl group as the pentafluorophenyl ester and found that reaction with the  $\beta$  amino group of 4 occurred exclusively at the  $\beta$ -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  $\beta$  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 <sup>1</sup>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)<sup>1</sup>.

# 3. Experimental

*Materials.*—Chemicals purchased from the following suppliers were: 2-acetamido-2-de-oxy-D-glucopyranose, succinamic acid, pen-

2-ethoxy-1-ethoxycarbonyltafluorophenol, 1,2-dihydroquinoline and hydrazine monohydrate from Aldrich Chemical Co.; D<sub>2</sub>O (99.9 atom% <sup>2</sup>H), Me<sub>2</sub>SO- $d_6$  (99.9 atom% <sup>2</sup>H), and CHCl<sub>3</sub>-d (99.8 atom<sup>%</sup> <sup>2</sup>H) from Cambridge Isotopes Laboratories; chloral hydrate from Fischer Scientific Co.; N,N'-dicyclohexylcar-Amberlite<sup>®</sup> bodiimide. 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. <sup>1</sup>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 <sup>13</sup>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  $\pm 0.002$  ppm for <sup>1</sup>H NMR and  $\pm 0.033$  ppm for <sup>13</sup>C NMR; the error in the measured coupling constants is  $\pm 0.50$  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- $\beta$ -Dglucopyranosylamine [6,7,9,12] (4).—2-Acetamido-2-deoxy-D-glucopyranose (GlcNAc) was dissolved in satd aq ammonium bicarbonate (~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,

<sup>&</sup>lt;sup>1</sup> 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<sup>-</sup>) anion-exchange column: the fumaramic acid analogue eluted at pH 3.9 and the maleamic acid analogue eluted at pH 3.0. The <sup>1</sup>H NMR spectrum of the fumaramic acid analogue has three characteristic signals (D<sub>2</sub>O): δ 5.12 (d, 1 H, J<sub>1,2</sub> 9.77 Hz, H-1), 6.00 and 6.50 (d, 1 H each, J<sub>α,β</sub> 15.9 Hz, H-α and H-β (*trans-CH=CH-*)). The <sup>1</sup>H NMR spectrum of the maleamic acid analogue has three characteristic signals (D<sub>2</sub>O): δ 5.15 (d, 1 H, J<sub>1,2</sub> 9.77 Hz, H-1), 6.35 and 6.45 (d, 1 H each, J<sub>α,β</sub> 12.5 Hz, H-α and H-β (*cis-CH=CH-*)).

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%  $\alpha$  and  $\beta$  anomers of GlcNAc as determined by integration of the <sup>1</sup>H NMR signals in D<sub>2</sub>O for the anomeric protons at  $\delta$ 4.14 ( $J_{1,2}$  9.28 Hz),  $\delta$  5.19 ( $J_{1,2}$  3.42 Hz), and  $\delta$ 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<sub>2</sub>Cl<sub>2</sub>, and air-dried for 24 h; yield 2.55 g (74%): mp 138–139.5 °C; <sup>1</sup>H NMR (Me<sub>2</sub>SO- $d_6$ , reference Me<sub>4</sub>Si):  $\delta$  2.51 (t, 2 H,  $J_{\alpha,\beta}$  6.59 Hz, H- $\alpha$  (CH<sub>2</sub>)), 2.93 (t, 2 H,  $J_{\alpha,\beta}$ 6.59 Hz, H- $\beta$  (CH<sub>2</sub>)), 6.9 and 7.5 (s, 1 H, NH<sub>2</sub>); <sup>13</sup>C NMR (Me<sub>2</sub>SO- $d_6$ , reference Me<sub>4</sub>Si): δ 28.92 (C-β), 29.63 (C-α), 134–140 (pentafluorophenyl,  $J_{\rm CF} \sim 250$ Hz), 173.07 (CONH<sub>2</sub>), 173.94 (COO).

 $N-(2-Acetamido-2-deoxy-\beta-D-glucopyran$ osyl)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<sup>-</sup>) 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<sup>-1</sup>; <sup>1</sup>H NMR (D<sub>2</sub>O, reference acetone (2.225 ppm)):  $\delta$  2.050 (s, 3 H, COCH<sub>3</sub>), 2.556 (center of  $A_2B_2$  second order spectrum not resolved, 4 H. NHCOC $H_2$ C $H_2$ CON $H_2$ ), 3.474 (dd, 1 H,  $J_{34}$ 10.25, J<sub>4.5</sub> 8.79 Hz, H-4 (CHOH)), 3.518 (m, 1 H, J<sub>5,6a</sub> 1.46, J<sub>5,6b</sub> 4.39 Hz, H-5 (CHO–)), 3.611 (dd, 1 H, J<sub>2,3</sub> 9.77 Hz, H-3 (CHOH)), 3.746 (dd, 1 H,  $J_{6a,6b}$  – 12.21 Hz, H-6b (CH<sub>2</sub>OH)), 3.819 (dd, 1 H, J<sub>1.2</sub> 9.77 Hz, H-2 (CHNHAc)), 3.882 (dd, 1 H, H-6a (CH<sub>2</sub>OH)), 5.068 (d, 1 H, H-1 (CHNH)); <sup>13</sup>C NMR (D<sub>2</sub>O, reference 1,4-dioxane (66.66 ppm)):  $\delta$  22.06  $(COCH_3),$ 29.85  $(CH_2CONH_2),$ 30.48 (CH<sub>2</sub>CONH), 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<sub>2</sub>), 175.62 (CONH), 177.76 (COCH<sub>3</sub>). Anal. Calcd for  $C_{12}H_{21}N_3O_7$ . 0.25H<sub>2</sub>O: 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-Oacetyl-2-deoxy- $\alpha$ -D-glucopyranosyl chloride [23].—The <sup>1</sup>H NMR spectrum in CDCl<sub>3</sub> showed a doublet for the anomeric proton at  $\delta$  6.197, J<sub>1,2</sub> 3.42 Hz.

Preparation of 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- $\beta$ -D-glucopyranosyl azide [16].— This compound was synthesized from 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-a-Dglucopyranosyl chloride (above) and gave crystals: mp 165–166 °C, lit. 166–168 °C [16], lit. 166–167 °C (dec) [19]; <sup>1</sup>H NMR (CDCl<sub>3</sub>, reference Me<sub>4</sub>Si):  $\delta$  1.993, 2.046, 2.053, 2.115 (s, 3 H each, 4 COCH<sub>3</sub>), 3.811 (m, 1 H,  $J_{45}$ 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 (CHNHAc)), 4.177 (dd, 1 H,  $J_{6a,6b}$ - 12.21 Hz, H-6a (CH<sub>2</sub>OAc)), 4.284 (dd, 1 H, H-6b (CH<sub>2</sub>OAc)), 4.782 (d, 1 H, H-1 (CHN<sub>3</sub>)), 5.111 (dd, 1 H, J<sub>3,4</sub> 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- $\beta$ -D-glucopyranosylamine [16,17] (6).—Hydrogenation of 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- $\beta$ -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]; <sup>1</sup>H NMR (CDCl<sub>3</sub>, reference Me<sub>4</sub>Si): δ 1.983, 2.036, 2.051, 2.105 (s, 3 H each, 4 COCH<sub>3</sub>), 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 (CHN-HAc)), 4.114 (dd, 1 H,  $J_{6a,6b}$  – 12.21 Hz, H-6a (CH<sub>2</sub>OAc)), 4.133 (d, 1 H, H-1 (CHNH<sub>2</sub>)), 4.226 (dd, 1 H, H-6b (CH<sub>2</sub>OAc)), 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 ( $4 \times 90$ mL). The extracts were combined, washed with water  $(3 \times 180 \text{ mL})$ , 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]; <sup>1</sup>H NMR (CDCl<sub>3</sub>, reference Me<sub>4</sub>Si):  $\delta$ 3.055 (dd, 1 H,  $J_{\alpha,\beta}$  3.42,  $J_{\beta,\beta'}$  – 18.07 Hz, H- $\beta$ (CH<sub>2</sub>)), 3.149 (dd, 1 H,  $J_{\alpha,\beta'}$  3.91 Hz, H- $\beta'$ (CH<sub>2</sub>)), 4.904 (dd, 1 H, H- $\alpha$  (CH<sub>2</sub>CH)), 5.903 (s, 1 H, chloralid (CHCCl<sub>3</sub>)).

N<sup>4</sup>-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy- $\beta$ -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<sub>3</sub> (100 mmol)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; <sup>1</sup>H NMR (CDCl<sub>3</sub>, reference  $Me_4Si$ ):  $\delta$  1.991, 2.054, 2.082, 2.100 (s, 3 H each, 4 COCH<sub>3</sub>), 2.806 (dd, 1 H,  $J_{\alpha,\beta}$  3.42,  $J_{\beta,\beta'}$  – 17.09 Hz, H- $\beta$  (CH<sub>2</sub>CH)), 2.968 (dd, 1 H,  $J_{\alpha,\beta'}$  3.91 Hz, H- $\beta'$  (C $H_2$ CH)), 3.746 (m, 1 H,  $J_{4,5}^{,,p}$  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,  $J_{2,\rm NH}$  7.81 Hz, H-2 (CHNHAc)), 4.095 (m, 1 H,  $J_{1,\rm NH}$  not resolved, H-1 (CHN-HCOR)), 4.104 (dd, 1 H,  $J_{6a,6b}$  – 12.70 Hz, H-6a (CH<sub>2</sub>OAc)), 4.323 (dd, 1 H, H-6b (CH<sub>2</sub>OAc)), 4.813 (dd, 1 H, H- $\alpha$  (CH<sub>2</sub>CH)), 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<sub>3</sub>)), 6.126 (d, 1 H, NH (NHAc)). Anal. Calcd for C<sub>20</sub>H<sub>25</sub>Cl<sub>3</sub>N<sub>2</sub>O<sub>12</sub>: 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- $\beta$ -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<sup>-1</sup>; <sup>1</sup>H NMR (D<sub>2</sub>O, reference acetone (2.225 ppm):  $\delta$  2.004 (s, 3 H, COCH<sub>3</sub>), 2.594 (dd, 1 H,  $J_{\alpha,\beta}$  8.79,  $J_{\beta,\beta'}$  – 15.14 Hz, H- $\beta$ (CH<sub>2</sub>CH)), 2.773 (dd, 1 H,  $J_{\alpha,\beta'}$  3.91 Hz, H- $\beta'$  $(CH_2CH)$ , 3.478 (dd, 1 H,  $J_{3,4}^{3,4}$  10.25,  $J_{4,5}$  8.30 Hz, H-4 (CHOH)), 3.536 (m, 1 H, J<sub>5.6a</sub> 1.46, J<sub>5.6b</sub> 4.39 Hz, H-5 (CHO–)), 3.612 (dd, 1 H, J<sub>23</sub>, 9.77 Hz, H-3 (CHOH)), 3.749 (dd, 1 H,  $J_{6a,6b}^{-}$  – 12.21 Hz, H-6b (CH<sub>2</sub>OH)), 3.830 (dd, 1 H,  $J_{1,2}$  9.77 Hz, H-2 (CHNHAc)), 3.887 (dd, 1 H, H-6a (CH<sub>2</sub>OH)), 4.473 (dd, 1 H, H-α (CH<sub>2</sub>CH)), 5.097 (d, 1 H, H-1 (CHNHCOR));  $^{13}$ C NMR (D<sub>2</sub>O, reference 1,4-dioxane (66.66 ppm)):  $\delta$  22.060 (COCH<sub>3</sub>), 40.074 (C- $\beta$ ), 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<sub>2</sub>), 178.471 (COCH<sub>3</sub>). 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<sup>4</sup>-(2-Acetamido-2-deoxy- $\beta$ -D-glucopyranosyl)-L-2-hydroxysuccinamic acid hydrazide dihydrate (3)<sup>2</sup>.—Compound **8** (0.25 g, 0.42

<sup>&</sup>lt;sup>2</sup> IUPAC name: (3*S*)-*N*-(2-acetamido-2-deoxy-β-D-glucopy-ranosyl)-(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<sup>-1</sup>; <sup>1</sup>H NMR (D<sub>2</sub>O, reference acetone (2.225 ppm)):  $\delta$  2.004 (s, 3 H, COCH<sub>3</sub>), 2.591 (dd, 1 H,  $J_{\alpha,\beta}$  8.30,  $J_{\beta,\beta'}$ -15.14 Hz, H- $\beta$  (CH<sub>2</sub>CH)), 2.762 (dd, 1 H,  $J_{\alpha \beta'}$  3.91 Hz, H- $\beta'$  (CH<sub>2</sub>CH)), 3.479 (dd, 1 H, J<sub>3,4</sub> 10.25, J<sub>4,5</sub> 8.30 Hz, H-4 (CHOH)), 3.533 (m, 1 H, J<sub>5,6a</sub> 1.95, J<sub>5,6b</sub> 4.88 Hz, H-5 (CHO-)), 3.616 (dd, 1 H, J<sub>2,3</sub> 9.77 Hz, H-3 (CHOH)), 3.747 (dd, 1 H,  $J_{6a,6b}$  – 12.21 Hz, H-6b (CH<sub>2</sub>OH)), 3.827 (dd, 1 H,  $J_{1,2}$  9.77 Hz, H-2 (CHNHAc)), 3.887 (dd, 1 H, H-6a  $(CH_2OH)), 4.518 (dd, 1 H, H-\alpha (CH_2CH)),$ 5.089 (d, 1 H, H-1 (CHNHCOR)); <sup>13</sup>C NMR (D<sub>2</sub>O, reference 1,4-dioxane (66.66 ppm)):  $\delta$ 22.12 (COCH<sub>3</sub>), 40.24 (C- $\beta$ ), 54.31 (C-2), 60.58 (C-6), 67.76 (C-a), 69.57 (C-4), 74.29 78.37 (C-1), 173.23 (C-3), 77.72 (C-5), 173.62 (NHCOR), (CONHNH<sub>2</sub>), 174.95 (COCH<sub>3</sub>). 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.

#### Acknowledgements

The authors thank Yuan-Qing Xia for his preliminary work on the syntheses of the succinamide analogue 1 and L-malic acid chloralid 7, and Dr Clifford M. Carlin for the computation of the second-order spectrum. This research was supported by a Cottrell College Science Award of Research Corporation. Acknowledgment is made to the Donors of the Petroleum Research Fund, administered by the American Chemical Society, for partial support of this research. This research was supported, in part, by funds provided by the University of North Carolina at Charlotte.

### References

- [1] G.S. Marks, A. Neuberger, J. Chem. Soc. (1961) 4872– 4879.
- [2] M. Tanaka, M. Kohno, I. Yamashina, J. Biochem., 73 (1973) 1285–1289.
- [3] M. Tamura, H. Nishizaki, C. Miyazaki, H. Okai, Bull. Chem. Soc. Jpn., 57 (1984) 3167–3172.
- [4] B. Paul, R.J. Bernacki, W. Korytnyk, Carbohydr. Res., 80 (1980) 99–115.
- [5] J.M. Risley, R.L. Van Etten, J. Biol. Chem., 260 (1985) 15488–15494.
- [6] L.M. Likhosherstov, O.S. Novikova, V.A. Derevitskaja, N.K. Kochetkov, *Carbohydr. Res.*, 146 (1986) C1–C5.
- [7] I.D. Manger, T.W. Rademacher, R.A. Dwek, *Biochemistry*, 31 (1992) 10724–10732.
- [8] A.L. Handlon, B. Fraser-Reid, J. Am. Chem. Soc., 115 (1993) 3796–3797.
- [9] Y.-Q. Xia, J.M. Risley, submitted for publication.
- [10] C.M. Taylor, Tetrahedron, 54 (1998) 11317-11362.
- [11] N.N. Aronson Jr., Biochim. Biophys. Acta, 1455 (1999) 139-154.
- [12] L. Urge, D.C. Jackson, L. Gorbics, K. Wroblewski, G. Graczyk, L. Otvos Jr., *Tetrahedron*, 50 (1994) 2373–2390.
- [13] M. Monsigny, C. Quétard, S. Bourgerie, D. Delay, C. Pichon, P. Midoux, R. Mayer, A.C. Roche, *Biochimie*, 80 (1998) 99–108.
- [14] B.S. Furniss, A.J. Hannaford, P.W.G. Smith, A.R. Tatchell, Vogel's Textbook of Practical Organic Chemistry, fifth ed., Longman, UK, 1989, p. 1219.
- [15] J.F.G. Vliegenthart, L. Dorland, H. van Halbeek, *Adv. Carbohydr. Chem. Biochem.*, 41 (1983) 209–373.
- [16] C.H. Bolton, R.W. Jeanloz, J. Org. Chem., 28 (1963) 3228–3230.
- [17] C.H. Bolton, L. Hough, M.Y. Khan, *Biochem. J.*, 101 (1966) 184–190.
- [18] B. Paul, W. Korytnyk, *Carbohydr. Res.*, 67 (1978) 457–468.
- [19] F.D. Tropper, F.O. Anderson, S. Braun, R. Roy, Synthesis (1992) 618–620.
- [20] K.-I. Kim, R.I. Hollingsworth, *Tetrahedron Lett.*, 35 (1994) 1031–1032.
- [21] M. Petrušová, J.N. BeMiller, L. Petruš, *Tetrahedron Lett.*, 37 (1996) 2341–2344.
- [22] J.C. Castro-Palomino, R.R. Schmidt, *Liebigs Ann.* (1996) 1623–1626.
- [23] D. Horton, Org. Syn. Col. Vol., 5 (1973) 1-4.
- [24] V.H. Eggerer, C. Grunewalder, Justus Liebigs Ann. Chem., 677 (1964) 200–208.
- [25] B. Belleau, G. Malek, J. Am. Chem. Soc., 90 (1968) 1651–1652.
- [26] H. Kunz, Agnew. Chem., Int. Ed. Engl., 26 (1987) 294– 308.
- [27] H. Kunz, H. Waldmann, *Helv. Chim. Acta*, 68 (1985) 618–662.