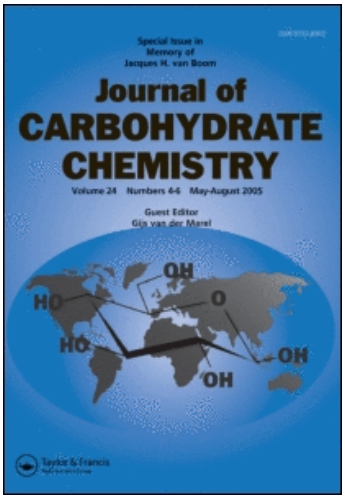

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SYNTHESIS OF N^4-(2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSYL)-L-ASPARAGINE ANALOGUES. L-2-CHLORO-, L-2-BROMO-, AND D,L-2-METHYLSUCCINAMIC ACID ANALOGUES

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ABSTRACT

L-Chlorosuccinic anhydride, L-bromosuccinic anhydride, and D,L-methylsuccinic anhydride react with 2-acetamido-2-deoxy-β-D-glucopyranosylamine to give varying mixtures of N^4-(β-GlcNAc)-2-substituted- and N^4-(β-GlcNAc)-3-substituted-succinamic acid isomers. The two regioisomers are separated by anion exchange chromatography. The N^4-(β-GlcNAc)-2-substituted-succinamic acid isomers are characterized as analogues of N^4-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-asparagine.

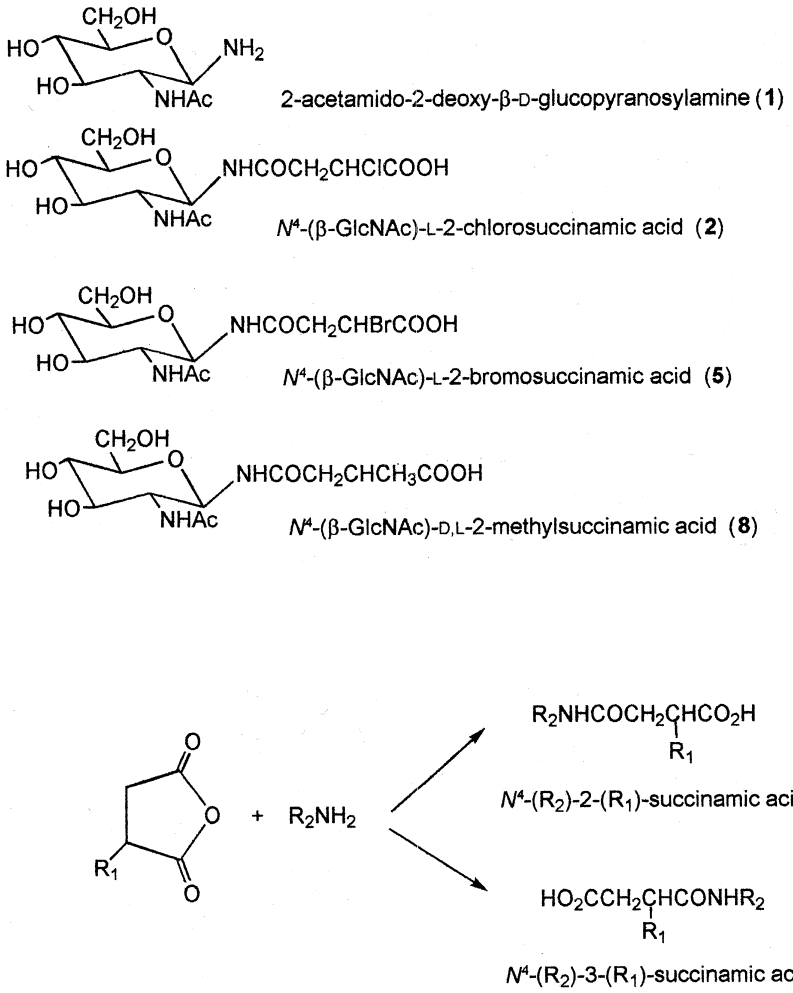
INTRODUCTION

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*-glycosyl 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-acetamido-2-deoxy-β-D-glucopyranosyl)bromoacetamide,[4] *N*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)azidoac

etamide,[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] and N^1-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-3-amino-4-hydroxybutyramide.[6] (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 principle linkage in the structure of N-linked glycoproteins,[9] 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.[10] For a study of the active site fingerprint of glycosylasparaginase, the synthesis of the N^4-(β-GlcNAc)-L-2-chlorosuccinamic acid (**2**), L-2-bromosuccinamic acid (**5**), and D,L-2-methylsuccinamic acid (**8**) analogues of the amino acid of (GlcNAc-)Asn were required, and are described in this paper.

RESULTS AND DISCUSSION

The reaction of monosubstituted succinic anhydrides with amines can occur to give regioselectively an N^4-(R_2)-2-(R_1)-succinamic acid (**I**) or an N^4-(R_2)-3-(R_1)-succinamic acid (**II**), as shown in Scheme 1. While studies of this reaction have been reported for various 2-aryl- and 2-alkylsuccinic anhydrides, the primary focus has been on the reactions of L-aspartic anhydride (L-aminosuccinic anhydride) because of its biochemical significance. Naps and Johns[11] reported that, based on previous work, the reaction of 2-arylsuccinic anhydrides with ammonia in anhydrous ether resulted exclusively in the formation of 2-aryl-succinamic acids (**I**: $R_2 = H$), the reaction of 2-arylsuccinic anhydrides with methylamine in anhydrous ether resulted exclusively in the formation of N^4-methyl-2-aryl-succinamic acids (**I**: $R_2 = CH_3$), and the reaction of 2-aryl- and 2-alkylsuccinic anhydrides with aniline in benzene resulted exclusively in the formation of N^4-phenyl-2-aryl- and N^4-phenyl-2-alkyl-succinamic acids (**I**: R_2 = phenyl). The reactions of L-aspartic anhydride have been studied for *N*-trifluoroacetyl-,[12-14] *N*-benzoxycarbonyl-,[3,15-18] *N*-9-fluorenylmethoxycarbonyl-,[18] and *N-tert*-butoxycarbonyl-L-aspartic anhydride.[18] The following three general conculsions may be drawn from these studies. Reaction of the anhydride with amines results in a mixture of products **I** and **II**; in only one study[12] were single isomers reportedly formed. There is little regioselectivity for the reaction, as the mixture of products ranges from **I**:**II** ≈ 1:1 to **I**:**II** or **II**:**I** up to 6:1; in only one study[16] was the regioselectivity reportedly significant (**I**:**II** ≈ 25:1). There is evidence to indicate in some reactions a regioselectivity that is solvent dependent; in nonpolar aprotic solvents **II** is slightly favored while in polar aprotic solvents **I** is slightly favored,[16-18] but this is not true in all instances.[3,18] (This contrasts with the significant solvent effect on regioselectivity observed in some reactions for L-glutamic anhydride, where **I**:**II** or **II**:**I** up to 100:0 is reported for the



Scheme 1.

comparable regioisomers.[18]) We studied the reaction of L-chloro-, L-bromo-, and D,L-methylsuccinic anhydrides with 2-acetamido-2-deoxy-β-D-glucopyranosylamine (**1**) to give the succinamic acid analogues of GlcNAc.

The syntheses of the sugar-amine, **1**, and the monosubstituted succinic acids, **3** and **6**, and anhydrides, **4** and **7**, were straightforward. The properties of **1**, **3**, **4**, **6**, and **7** were satisfactory. The NMR data for **6** agree with the data obtained in methanol-d_4.[19] Following the well-established preference for the reaction of activated carboxylic acids with the amino group rather than the sugar hydroxyl groups on carbohydrate molecules,[3,20,21] when **1** and the anhydrides were mixed, only the amide (*N*-glycosyl) bond was formed. The reaction of each anhydride with **1** was done in D_2O in order to acquire a ^1H NMR spectrum of each reaction prior to workup. These ^1H NMR spectra are shown in Figure 1, and show that a mixture of

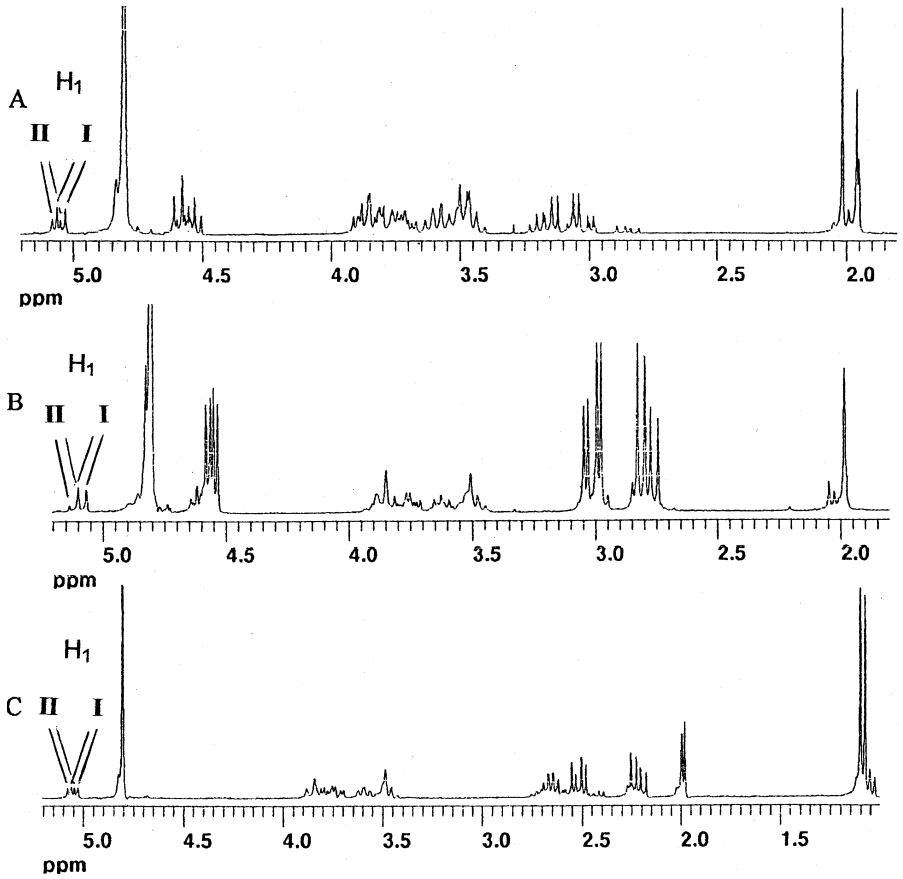


Figure 1. ^{1}H NMR spectra of the (A) 2-bromosuccinamic acid analogue, (B) 2-chlorosuccinamic acid analogue, and (C) 2-methylsuccinamic acid analogue in D_2O. The signals of interest are the doublets at δ 5.05-5.10 for the anomeric proton on the sugar.

I and **II** (R_2 = β-GlcNAc) formed in each reaction. This is evidenced by the ^{1}H NMR signal for the β-anomeric proton which characteristically appears as a doublet at δ 5.05-5.10 ppm with a $J_{1,2} \sim 9.7$ Hz.[21,22] Following separation of the isomers (see below) we were able to assign these NMR signals to specific isomers. The ^{1}H NMR signal for the β-anomeric proton in regioisomer **II** appears slightly downfield from that in regioisomer **I**. The integration of these signals in the ^{1}H NMR spectra shows that isomer **I** was 80% (**I**:**II** ≈ 4:1) for the L-chlorosuccinic anhydride reaction, 65% (**I**:**II** ≈ 2:1) for the L-bromosuccinic anhydride, and 50% (**I**:**II** ≈ 1:1) for the D,L-methylsuccinic anhydride. These results are consistent with those reported previously where a mixture of **I** and **II** is formed and there is little regioselectivity for the reaction. The ^{1}H NMR spectra also indicate that the reactions of the anhydrides with the sugar-amine was greater than 95% with little evidence for unreacted sugar-amine or hydrolyzed sugar produced in the reaction, and there is no evidence for formation of an α-anomeric *N*-glycosyl bond (δ ~ 5.27

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with $J_{1,2} \sim 1$ Hz).[21] The reaction of L-chlorosuccinic anhydride was complete in 10 min at room temperature, L-bromosuccinic anhydride in 20 min at room temperature, and D,L-methylsuccinic anhydride in 30 min at 40 °C.

Tamura et al.[3] showed that the difference in acidities of the carboxylate groups in isoasparagine and asparagine sugar analogues could be used to separate the two regioisomers by ion exchange chromatography. We used this property in the separation of isomer **I** from isomer **II**. The acidity of the carboxylate group in **I** is greater than that of the carboxylate group in **II** due to substitution at the 2-position. Therefore **I** will bind more strongly to an anion exchange resin than will **II**. An Amberlite® IRA-400(Cl) anion exchange column was activated with 1.0 M sodium acetate (25 mL used for a 1 x 20 cm column) and then washed with water. Activation of the resin with sodium hydroxide, ammonium acetate, and sodium chloride were not as successful. The solution of succinamic acid analogue was adjusted to pH 4.0-4.5 and the solution was loaded onto the column. Any free sugar was washed through the column with a water wash. Several eluent solvents were tested: sodium hydroxide, hydrochloric acid, ammonium acetate, sodium acetate, and sodium chloride.[23] Two solvents were found to elute the isomers from the column: 0.1 M acetic acid eluted **II** from the column and subsequent addition of 0.5 M acetic acid eluted **I** from the column. Lyophilization of the sample removed all of the acetic acid in the eluent. Figure 2 shows the ^{1}H NMR spectrum of the 2-chlorosuccinamic acid analogue (**2**) obtained in the second eluent following lyophilization; all of the signals can clearly be identified, utilizing the nomenclature used for (GlcNAc-)Asn.[22]

The N^4-(β-GlcNAc)-2-substituted-succinamic acid isomers (**I**) as analogues of β-*N*-acetylglucosaminyl-L-asparagine were characterized; the N^4-(β-GlcNAc)-

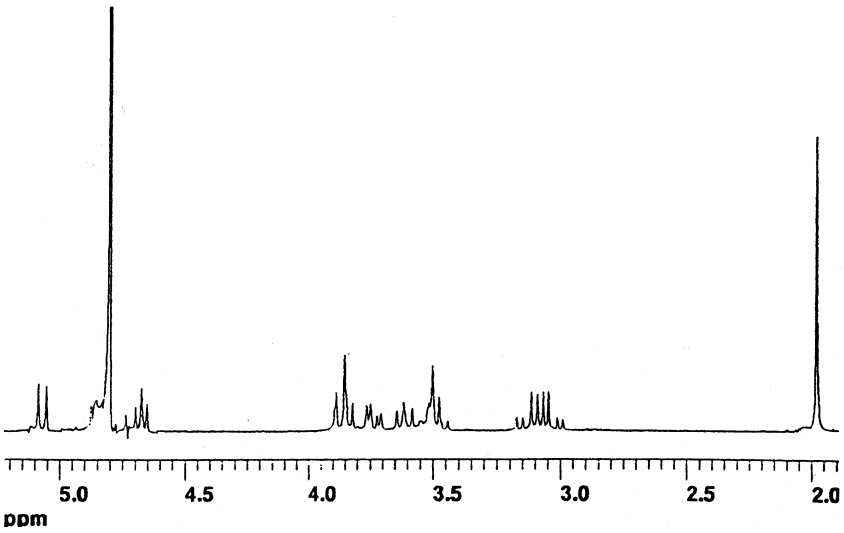

Figure 2. The ^{1}H NMR spectrum of **2** following separation from its isomer by anion exchange chromatography. The NMR signals are clearly resolved and can be assigned.

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3-substituted-succinamic acid isomers (**II**) were not further characterized. The properties of the analogues clearly indicate the successful synthesis of each analogue. Of particular importance is the ^{1}H NMR signal of the anomeric proton that shows a β-*N*-glycosyl bond is present in each analogue. In the ^{1}H NMR spectrum of the D,L-2-methylsuccinamic acid analogue, separate signals for the D and L enantiomers were detected for the α-methyl group, acetamido group, the α, β and β' protons, and the anomeric proton; in the ^{13}C NMR spectrum separate signals were detected for the α-methyl group, both carbons in the acetamido group, the α and β carbons, the anomeric carbon, the carboxyl group, and the *N*-glycosyl carbon.

CONCLUSIONS

We have synthesized and characterized three new amino acid analogues of N^4-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-asparagine. The reactions between the sugar-amine and substituted succinic anhydrides are consistent with previously reported reactions in terms of the formation of regioisomers and the generally poor regioselectivity of these reactions. Separation of the isomers are effected by anion exchange chromatography. While our use of these analogues is in a study of the active site fingerprint of glycosylasparaginase, these analogues have potential for incorporation into new glycopeptides and complex glycoconjugates.

EXPERIMENTAL

Materials and Methods. Chemicals purchased from the following suppliers were: *N*-acetyl-D-glucosamine and Amberlite® CG-120 cation exchange resin from Janssen Chimica; ammonium bicarbonate from Mallinckrodt; L-aspartic acid and Amberlite® IRA-400(Cl) strongly basic gel-type anion exchange resin 16-50 mesh from Spectrum Chemical; acetyl chloride and deuterium oxide (99.9 atom % ^{2}H) from Sigma; thionyl chloride from Fisher Scientific; D,L-methylsuccinic anhydride from Aldrich; acetone-d_6 (99.9 atom % ^{2}H) from Cambridge Isotopes. Acetyl chloride and thionyl chloride were distilled *in vacuo* before use. All other chemicals were at least analytical grade.

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, 90° 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, 90° 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. Infrared spectra were recorded on a Midac Laser FTIR. Elemental analyses were done at Atlanta Microlabs, Inc. (Norcross, Georgia).

2-Acetamido-2-deoxy-β-D-glucopyranosylamine (**1**). The synthesis of **1** was carried out as described with slight modification.[20] *N*-Acetyl-D-glucosamine (0.11 moles) and ammonium bicarbonate (1.01 moles) in water (350 mL) were allowed to equilibrate at 30 °C over five days. Glacial acetic acid (3 mL) was added, the solution filtered, and concentrated on a rotary evaporator *in vacuo.* Excess ammonium bicarbonate was removed by dissolving the product in water (50 mL) and concentrating *in vacuo* for four times. A ^{1}H NMR spectrum showed 80 % **1** and 20 % *N*-acetyl-D-glucosamine. The mixture was applied to an activated column of Amberlite® CG-120 cation exchange resin (1 x 25 cm), *N*-acetyl-D-glucosamine washed through the column with water, and **1** was eluted from the column with 0.05 M NaOH. **1** was recovered by lyophilization and stored at -20 °C. Properties of **1** agree with those reported in the literature.[6,7]

N^4-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-L-2-chlorosuccinamic Acid (**2**)

L-2-Chlorosuccinic acid (**3**). The synthesis of **3** followed the reported procedure.[24] L-Aspartic acid (5 g; 38 mmoles) and urea (0.5 g) were dissolved in a mixture of concentrated hydrochloric acid/concentrated nitric acid (16 mL; 1:1 v/v), stirred at 65 °C for 2 h, and allowed to crystallize at 4 °C overnight. Crude **3** was collected by suction filtration, and recrystallized by dissolving **3** in acetone (15 mL), adding benzene to turbidity, and placed at 4 °C overnight. The yield of **3** (white crystals) was 4.27 g (74.5 %). mp 176-177 °C (lit.[24] 176 °C) ^{1}H NMR (acetone-d_6, internal reference 1,4-dioxane (3.53 ppm)): δ 2.87 (dd, 1H, $J_{\alpha,\beta}$ = 6.83 Hz, $J_{\beta,\beta'}$ = 17.09 Hz, H_β (CH_2)), 3.08 (dd, 1H, $J_{\alpha,\beta'}$ = 7.08 Hz, $J_{\beta,\beta'}$ = 17.09 Hz, $H_{\beta'}$(CH_2)), 4.62 (dd, 1H, $J_{\alpha,\beta}$ = 6.83 Hz, $J_{\alpha,\beta'}$ = 7.08 Hz, H_α (CHCl)). ^{13}C NMR (D_2O, pD = 1.00, internal reference 1,4-dioxane (66.50 ppm)): δ 39.14 (C_3), 52.30 (C_2), 172.16 (C_4), 173.29 (C_1).

L-Chlorosuccinic anhydride (**4**). The synthesis of **4** followed the reported procedure with slight modification.[11] **3** (2.1 g; 14 mmoles) was dissolved in a mixture of acetyl chloride/thionyl chloride (18 mL; 2:1 v/v) and gently refluxed at 70 °C for 3 h. Excess acetyl chloride and thionyl chloride were removed *in vacuo*. The yellow oil was dissolved in chloroform/acetone (4 mL; 1:1 v/v), ligroine was added to turbidity, and kept at 4 °C overnight to crystallize. The yield of **4** (white crystals) was 1.5 g (79 %). mp 75-76 °C ^{1}H NMR (acetone-d_6, internal reference 1,4-dioxane (3.53 ppm)): δ 3.31 (dd, 1H, $J_{\alpha,\beta}$ = 5.38 Hz, $J_{\beta,\beta'}$ = 19.05 Hz, H_β (CH_2)), 3.75 (dd, 1H, $J_{\alpha,\beta'}$ = 9.28 Hz, $J_{\beta,\beta'}$ = 19.05 Hz, $H_{\beta'}$ (CH_2)), 5.27 (dd, 1H, $J_{\alpha,\beta}$ = 5.38 Hz, $J_{\alpha,\beta'}$ = 9.28 Hz, H_α (CHCl)).

N^4-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-L-2-chlorosuccinamic acid (**2**). **1** (50 mg; 0.23 mmoles) was dissolved in D_2O (1 mL), **4** (34 mg; 0.26 mmoles) was added, and vortex-mixed for 10 min at room temperature. The homogeneous solution was adjusted to pH 4.15 with 1.25 M NaOH, and was applied to an Amberlite® IRA-400(Cl) column (1 x 20 cm) activated with 1.0 M sodium acetate. The column was washed with water (10 mL), and eluted with 0.1 M acetic acid (50 mL) and 0.5 M acetic acid (50 mL) at 1 mL/min. **2** eluted in the second eluent and was recovered by lyophilization. mp 168-169 °C ^{1}H NMR (D_2O, pD = 2.47, reference

acetone (2.225 ppm)): δ 1.97 (s, 3H, $COCH_3$), 3.03 (dd, 1H, $J_{\alpha,\beta}$ = 6.84 Hz, $J_{\beta,\beta'}$ = 17.09 Hz, H_β (CH_2)), 3.13 (dd, 1H, $J_{\alpha,\beta'}$ = 6.35 Hz, $J_{\beta,\beta'}$ = 17.09 Hz, $H_{\beta'}$ (CH_2)), 3.47 (dd, 1H, $J_{3,4}$ = 9.04 Hz, $J_{4,5}$ = 8.79 Hz, H_4 (C*H*OH)), 3.53 (m, 1H, $J_{4,5}$ = 8.79 Hz, $J_{5,6a}$ = 1.93 Hz, $J_{5,6b}$ = 4.88 Hz, H_5 (CHO-)), 3.61 (dd, 1H, $J_{2,3}$ = 10.01 Hz, $J_{3,4}$ = 9.04 Hz, H_3 (C*H*OH)), 3.74 (dd, 1H, $J_{5,6b}$ = 4.88 Hz, $J_{6a,6b}$ = 12.45 Hz, H_{6b} (CH_2OH)), 3.85 (dd, 1H, $J_{1,2}$ = 9.76 Hz, $J_{2,3}$ = 10.01 Hz, H_2 (C*H*NH)), 3.87 (dd, 1H, $J_{5,6a}$ = 1.93 Hz, $J_{6a,6b}$ = 12.45 Hz, H_{6a} (CH_2OH)), 4.68 (dd, 1H, $J_{\alpha,\beta}$ = 6.84 Hz, $J_{\alpha,\beta'}$ = 6.35 Hz, H_α (CHCl)), 5.07 (d, 1H, $J_{1,2}$ = 9.76 Hz, H_1 (C*H*NH)). ^{13}C NMR (D_2O, pD = 2.47, internal reference 1,4-dioxane (66.50 ppm)): δ 21.93 (CO*C*H_3), 52.40 (C_β), 54.31 (CHCl), 60.42 (C_2), 65.85 (C_6), 69.48 (C_4), 73.81 (C_3), 77.75 (C_5), 78.82 (C_1), 171.15 (COOH), 173.09 (CONH), 174.61 (*C*OCH_3). IR (cm^{-1}): 3315, 1736, 1665, 1556, 1416, 1368, 1301, 1220, 1168, 1085, 919, 622.

Anal. Calcd for $C_{12}H_{19}ClN_2O_8$: C 40.63, H 5.40, Cl 9.90, N 7.90. Found: C 40.36, H 5.47, Cl 9.89, N 7.83.

N^4-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-L-2-bromosuccinamic Acid Hydrate (**5**)

L-2-Bromosuccinic acid (**6**). The synthesis of **6** followed the reported procedure.[24] L-Aspartic acid (10 g; 75 mmoles) and urea (1.5 g) were dissolved in a mixture of hydrobromic acid/concentrated nitric acid (50 mL; 1:1 v/v) in an icebath, and diethyl ether (100 mL) was added. The reaction was completed in 20 min with stirring. The solution was extracted three times with diethyl ether (100 mL each time) and the aqueous phase was set at 4 °C overnight where crude **6** crystallized (yield 4.0 g; 27 %). The crystals were dissolved in diethyl ether (10 mL) and ligroine (8 mL), and recrystallized at 4 °C overnight. The yield of **6** (white crystals) was 2.0 g (13.5 %). mp 179-180 °C (lit.[19] 171-172 °C, lit.[24] 175 °C, lit.[25] 178-180 °C) ^{1}H NMR (acetone-d_6, internal reference 1,4-dioxane (3.53 ppm)): δ 2.93 (dd, 1H, $J_{\alpha,\beta}$ = 6.34 Hz, $J_{\alpha,\beta'}$ = 17.33 Hz, H_β (CH_2)), 3.17 (dd, 1H, $J_{\alpha,\beta'}$ = 8.79 Hz, $J_{\beta,\beta'}$ = 17.33 Hz, $H_{\beta'}$ (CH_2)), 4.56 (dd, 1H, $J_{\alpha,\beta}$ = 6.34 Hz, $J_{\alpha,\beta'}$ = 8.79 Hz, H_α (CHBr)). ^{13}C NMR (acetone-d_6, internal reference 1,4-dioxane (66.50 ppm)): δ 38.75 (C_3), 39.08 (C_2), 169.86 (C_4), 170.93 (C_1).

L-Bromosuccinic anhydride (**7**). The synthesis of **7** followed the reported procedure with slight modification.[11] **6** (2.1 g; 12 mmoles) was dissolved in a mixture of acetyl chloride/thionyl chloride (24 mL; 1:1 v/v) and gently refluxed at 70 °C for 4 h. Excess acetyl chloride and thionyl chloride were removed *in vacuo*. The yellow oil was dissolved in chloroform (8 mL), ligroine was added to turbidity, and kept at 4 °C overnight to crystallize. The yield of **7** was 1.5 g (71 %). mp 66-68 °C ^{1}H NMR (acetone-d_6, internal reference 1,4-dioxane (3.53 ppm)): δ 3.35 (dd, 1H, $J_{\alpha,\beta}$ = 4.40 Hz, $J_{\beta,\beta'}$ = 19.54 Hz, H_β (CH_2)), 3.87 (dd, 1H, $J_{\alpha,\beta'}$ = 9.03 Hz, $J_{\beta,\beta'}$ = 19.54 Hz, $H_{\beta'}$ (CH_2)), 5.24 (dd, 1H, $J_{\alpha,\beta}$ = 4.40 Hz, $J_{\alpha,\beta'}$ = 9.03 Hz, H_α (CHBr)). ^{13}C NMR (acetone-d_6, internal reference 1,4-dioxane (66.50 ppm)): δ?35.00 (C_3), 39.37 (C_2), 168.31 (C_4), 168.92 (C_1).

N^4-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-L-2-bromosuccinamic acid hydrate (**5**). **1** (52 mg; 0.24 mmoles) was dissolved in D_2O (1 mL), **7** (42 mg; 0.26

mmoles) was added, and vortex-mixed for 20 min at room temperature. The homogeneous solution was adjusted to pH 4.15 with 1.25 M NaOH, and was applied to an Amberlite® IRA-400(Cl) column (1 x 20 cm) activated with 1.0 M sodium acetate. The column was washed with water (10 mL), and eluted with 0.1 M acetic acid (50 mL) and 0.5 M acetic acid (50 mL) at 1 mL/min. **5** eluted in the second eluent and was recovered by lyophilization. mp 160-161 °C ^{1}H NMR (D_2O, pD = 2.94, internal standard acetone (2.225 ppm)): δ 2.00 (s, 3H, $COCH_3$), 3.07 (dd, 1H, $J_{\alpha,\beta}$ = 6.84 Hz, $J_{\beta,\beta'}$ = 17.58 Hz, H_β (CH_2)), 3.24 (dd, 1H, $J_{\alpha,\beta'}$ = 8.79 Hz, $J_{\beta,\beta'}$ = 17.58 Hz, $H_{\beta'}$ (CH_2)), 3.46 (dd, 1H, $J_{3,4}$ = 8.79 Hz, $J_{4,5}$ = 8.55 Hz, H_4 (C*H*OH)), 3.53 (m, 1H, $J_{4,5}$ = 8.55 Hz, $J_{5,6a}$ = 1.96 Hz, $J_{5,6b}$ = 4.88 Hz, H_5 (CHO-)), 3.62 (dd, 1H, $J_{2,3}$ = 10.01 Hz, $J_{3,4}$ = 8.79 Hz, H_3 (C*H*OH)), 3.74 (dd, 1H, $J_{5,6b}$ = 4.88 Hz, $J_{6a,6b}$ = 12.21 Hz, H_{6b} (CH_2OH)), 3.84 (dd, 1H, $J_{1,2}$ = 9.77 Hz, $J_{2,3}$ = 10.01 Hz, H_2 (C*H*NH)), 3.89 (dd, 1H, $J_{5,6a}$ = 1.96 Hz, $J_{6a,6b}$ = 12.21 Hz, H_{6a} (CH_2OH)), 4.63 (dd, 1H, $J_{\alpha,\beta}$ = 6.84 Hz, $J_{\alpha,\beta'}$ = 8.79 Hz, H_α (CHBr)), 5.09 (d, 1H, $J_{1,2}$ = 9.77 Hz, H_1 (C*H*NH)). ^{13}C NMR (D_2O, pD = 2.94, internal reference 1,4-dioxane (66.50 ppm)): δ 22.16 ($COCH_3$), 40.72 (C_β), 54.34 (CHBr), 60.48 (C_2), 65.50 (C_6), 69.48 (C_4), 74.13 (C_3), 77.63 (C_5), 78.14 (C_1), 172.03 (COOH), 173.40 (CONH), 174.61 ($COCH_3$). IR (cm^{-1}): 3265, 2930, 2370, 2341, 1653, 1561, 1540.

Anal. Calcd for $C_{12}H_{19}BrN_2O_8 0.5H_2O$: C 35.31, H 4.94, N 6.86. Found: C 35.58, H 5.53, N 6.67.

N^4-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-D,L-2-methylsuccinamic Acid Hydrate (**8**). **1** (47 mg; 0.21 mmoles) was dissolved in D_2O (1 mL), D,L-2-methylsuccinic anhydride (25 mg; 0.21 mmoles) was added, and stirred at 40 °C for 30 min. The homogeneous solution was adjusted to pH 4.15 with 1.25 M NaOH, and was applied to an Amberlite® IRA-400(Cl) column (1 x 20 cm) activated with 1.0 M sodium acetate. The column was washed with water (10 mL), and eluted with 0.1 M acetic acid (50 mL) and 0.5 M acetic acid (50 mL) at 1 mL/min. **8** eluted in the second eluent and was recovered by lyophilization. mp 161-163 °C ^{1}H NMR (D_2O, pD = 2.98, reference acetone (2.225 ppm)): δ 1.13 and 1.15 (d, 3H, $J_{\alpha,Me}$ = 6.7 Hz, CHCH_3), 2.02 and 2.03 (s, 3H, $COCH_3$), 2.39 (dd, 1H, $J_{\alpha,\beta}$ = 6.7 Hz, $J_{\beta,\beta'}$ = 15.7 Hz, H_β (CH_2)), 2.45 (dd, 1H, $J_{\alpha,\beta}$ = 6.6 Hz, $J_{\beta,\beta'}$ = 15.7 Hz, H_β (CH_2)), 2.60 (dd, 1H, $J_{\alpha,\beta'}$ = 3 Hz, $J_{\beta,\beta'}$ = 15.7 Hz, $H_{\beta'}$ (CH_2)), 2.63 (dd, 1H, $J_{\alpha,\beta'}$ = 2 Hz $J_{\beta,\beta'}$ = 15.7 Hz, $H_{\beta'}$ (CH_2)), 2.8$_1$ (m, 1H, $J_{\alpha,\beta}$ = 6.7 Hz, $J_{\alpha,\beta'}$ = 3 Hz, $J_{\alpha,\beta}$ = 6.6 Hz, $J_{\alpha,\beta'}$ = 2 Hz, $J_{\alpha,Me}$ = 6.7 Hz, H_α (C*H*CH$_3$)), 3.50 (dd, 1H, $J_{3,4}$ = 9.28 Hz, $J_{4,5}$ = 9.03 Hz, H_4 (C*H*OH)), 3.55 (m, 1H, $J_{4,5}$ = 9.03 Hz, $J_{5,6a}$ not resolved, $J_{5,6b}$ = 4.95 Hz, H_5 (CHO-)), 3.64 (dd, 1H, $J_{2,3}$ = 9.77 Hz, $J_{3,4}$ = 9.28 Hz, H_3 (C*H*OH)), 3.77 (dd, 1H, $J_{5,6b}$ = 4.95 Hz, $J_{6a,6b}$ = 12.46 Hz, H_{6b} (CH_2OH)), 3.86 (dd, 1H, $J_{5,6a}$ not resolved, $J_{6a,6b}$ = 12.46 Hz, H_{6a} (CH_2OH)), 3.88 (dd, 1H, $J_{1,2}$ = 9.77 Hz, $J_{2,3}$ = 9.77 Hz, H_2 (C*H*NH)), 5.07 and 5.09 (d, 1H, $J_{1,2}$ = 9.77 Hz, H_1 (C*H*NH)). ^{13}C NMR (D_2O, pD = 3.16, reference 1,4-dioxane (66.50 ppm)): δ 15.92 and 15.98 (CH*C*H$_3$), 21.93 and 22.00 ($COCH_3$), 36.75 and 37.20 (C_β), 38.85 and 38.72 (*C*HCH$_3$), 54.24 (C_2), 60.48 (C_6), 69.48 (C_4), 74.13 (C_3), 77.56 (C_5), 78.21 and 78.34 (C_1), 174.45 and 174.68 (COOH), 175.58 and 175.80 (CONH),

179.01 and 179.50 ($COCH_3$). IR (cm^{-1}): 3270, 2977, 2938, 2372, 1728, 1663, 1592, 1097, 1042, 617, 432.

Anal. Calcd for $C_{13}H_{22}N_2O_8 0.25H_2O$: C 46.08, H 6.69, N 8.27. Found: C 45.85, H 6.68, N 8.21.

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REFERENCES

1. Marks, G.S.; Neuberger, A. Synthetic Studies Relating to the Carbohydrate-Protein Linkage in Egg Albumin. J. Chem. Soc. **1961**, 4872-4879.
2. Tanaka, M.; Kohno, M.; Yamashina, I. Specificity Studies of 4-L-Aspartylglycosylamine Amido Hydrolase. J. Biochem. **1973**, *73*, 1285-1289.
3. Tamura, M.; Nishizaki, H.; Miyazaki, C.; Okai, H. Studies on *N*-Glycopeptides. II. Separation of α- and β-Amide of *N*-(L-Aspartyl)-β-D-glucopyranosylamine Obtained via *N*-(Benzyloxycarbonyl)-L-aspartic Anhydride. Bull. Chem. Soc. Jpn. **1984**, *57*, 3167-3172.
4. Paul, B.; Bernacki, R.J.; Korytnyk, W. Synthesis and Biological Activity of Some 1-*N*-Substituted 2-Acetamido-2-deoxy-β-D-glycopyranosylamine Derivatives and Related Analogs. Carbohydr. Res. **1980**, *80*, 99-115.
5. Risley, J.M.; Van Etten, R.L. ^{1}H NMR Evidence that Almond "Peptide:*N*-Glycosidase" is an Amidase: Kinetic Data and Trapping of the Intermediate. J. Biol. Chem. **1985**, *260*, 15488-15494.
6. Likhosherstov, L.M.; Novikova, O.S.; Derevitskaja, V.A.; Kochetkov, N.K. A New Simple Synthesis of Amino Sugar β-D-Glycosylamines. Carbohydr. Res. **1986**, *146*, C1-C5.
7. Manger, I.D.; Rademacher, T.W.; Dwek, R.A. 1-*N*-Glycyl β-Oligosaccharide Derivatives as Stable Intermediates for the Formation of Glycoconjugate Probes. Biochemistry **1992**, *31*, 10724-10732.
8. Handlon, A.L.; Fraser-Reid, B. A Convergent Strategy for the Critical β-Linked Chitobiosyl-*N*-glycopeptide Core. J. Am. Chem. Soc. **1993**, *115*, 3796-3797.
9. Taylor, C.M. Glycopeptides and Glycoproteins: Focus on the Glycosidic Linkage. Tetrahedron **1998**, *54*, 11317-11362.
10. Aronson, N.N., Jr. Aspartylglycosaminuria: Biochemistry and Molecular Biology. Biochim. Biophys. Acta **1999**, *1455*, 139-154.
11. Naps, M.; Johns, I.B. Optically Active Mono-substituted Succinic Acids and Derivatives. J. Am. Chem. Soc. **1940**, *62*, 2450-2457.
12. Weygand, F.; Klinke, P.; Eigen, I. *N*-Trifluoracetyl-aminosäuren, X. *N*-Trifluoracetyl-L-asparaginsäure-anhydrid und *N*-Trifluoracetyl-L-prolin-anhydrid. Chem. Ber. **1957**, *90*, 1896-1905.

13. Lapidus, M.; Sweeney, M. L-4′-Cyano-3-(2,2,2-trifluoroacetamido)succinanilic Acid and Related Synthetic Sweetening Agents. J. Med. Chem. **1973**, *16*, 163-166.
14. Kawai, M.; Nyfeler, R.; Berman, J.M.; Goodman, M. Peptide Sweeteners. 5. Side-chain Homologs Relating Zwitterionic and Trifluoroacetylated Amino-acid Anilide and Dipeptide Sweeteners. J. Med. Chem. **1982**, *25*, 397-402.
15. Le Quesne, W.J.; Young, G.T. Amino-acids and Peptides. Part VI. Synthesis of L-Aspartyl Peptides from Carbobenzyloxy-L-aspartic anhydride. J. Chem. Soc. **1952**, 24-28.
16. Yang, C.P.; Su, C.S. Effects of Solvents and Additives on the Reaction of *N*-Benzyloxycarbonyl-L-aspartic Anhydride with L-Phenylalanine Methyl Ester (Synthesis of Aspartame). J. Org. Chem. **1986**, *51*, 5186-5191.
17. Chen, F.M.F.; Benoiton, N.L. *N*-Alkoxycarbonyl-glutamic and aspartic Acids — Studies on the Activation and Cyclodehydration and Side-reaction Encountered in Analysis of Glutamic-acid using FMOC-chloride. Int. J. Pept. Protein Res. **1992**, *40*, 13-18.
18. Huang, X.; Luo, X.; Roupioz, Y.; Keillor, J.W. Controlled Regioselective Anilide Formation from Aspartic and Glutamic Acid Anhydrides. J. Org. Chem. **1997**, *62*, 8821-8825.
19. Frick, J.A.; Klassen, J.B.; Bathe, A.; Abramson, J.M.; Rapoport, H. An Efficient Synthesis of Enantiomerically Pure (*R*)-(2-Benzyloxyethyl)oxirane from (*S*)-Aspartic Acid. Synthesis **1992**, 621-623.
20. Urge, L.; Jackson, D.C.; Gorbics, L.; Wroblewski, K.; Graczyk, G.; Otvos, L., Jr. Synthesis and Conformational Analysis of N-glycopeptides that Contain Extended Sugar Chains. Tetrahedron **1994**, *50*, 2373-2390.
21. Monsigny, M.; Quétard, C.; Bourgerie, S.; Delay, D.; Pichon, C.; Midoux, P.; Mayer, R.; Roche, A.C. Glycotargeting: The Preparation of Glyco-amino Acids and Derivatives from Unprotected Reducing Sugars. Biochimie **1998**, *80*, 99-108.
22. Vliegenthart, J.F.G.; Dorland, L.; van Halbeek, H. High-resolution, ^{1}H-nuclear Magnetic Resonance Spectroscopy as a Tool in the Structural Analysis of Carbohydrates Related to Glycoproteins. Adv. Carbohydr. Chem. Biochem. **1983**, *41*, 209-374.
23. Not surprisingly, when 0.1 M sodium hydroxide was tested as an eluent solvent, elimination of HCl and HBr occurred from **2** and **5**, respectively, to give the fumaramic acid analogue. This was verified by purifying the latter analogue (unreported observation) and comparing it to an original sample synthesized by the reaction of **1** with maleic anhydride (W. M. York and J. M. Risley, unpublished results). The ^{1}H NMR spectrum of the fumaramic acid analogue has three characteristic signals (D_2O): δ 5.12 (d, 1H, $J_{1,2}$ = 9.77 Hz, H_1 (C*H*NH)), 6.00 and 6.50 (d, 1H each, $J_{\alpha,\beta}$ = 15.9 Hz, H_α and H_β (*trans* -C*H*=C*H*-)).
24. Karrer, P.; Reschopky, H.; Kaase, W. Bildung von α-Halogencarbonsäuren aus α-Aminosäuren, Salpetersäure und Salzsäure. Helv. Chim. Acta **1947**, *30*, 271-274.
25. Murakami, Y.; Koga, K.; Yamada, S.I. Stereochemical Studies. 51. Stereochemical Courses of Deaminative Bromination of L-Aspartic Acid and its Esters. Chem. Pharm. Bull. **1978**, *26*, 307-309.

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