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Hydrolysis of the GlcNAc oxazoline: deamidation and acyl rearrangement

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Abstract

The specific deamidation of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose is achieved by *p*-toluenesulfonic acid-promoted hydrolysis of 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline **2** to give quantitative formation of the 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranose *p*-toluenesulfonate (**5d**). This two-step procedure provides an amino sugar which may be readily acylated to give novel glycoconjugates. Alternatively, base-catalyzed O-1 → N-2 acyl rearrangement of the amino tosylate **5d** gives the 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-D-glucopyranose **4** as a 9:1 mixture of α and β anomers. Thus, hydrolysis of GlcNAc oxazoline **2** gives the amino-ester **5** as the kinetic product and the amido-alcohol **4** as the thermodynamic product.

Keywords: GlcNAc oxazoline; Deamidation; Acyl rearrangement

1. Introduction

N-Acetylglucosamine (GlcNAc) is a key component of glycoproteins, glycolipids and glycosaminoglycans. Asn-linked glycoproteins have a β1-*N* linkage between an Asn γ-carboxamide and a β-GlcNAc-(1 → 4)-GlcNAc core disaccharide [1], while *O*-linked glycoproteins can have GlcNAc bound to a Ser or Thr β-hydroxyl group [2]. The *rhizobium* growth factor, a glycolipid, has an acylated C-2 glucosamine [3]. Glycosaminoglycan polymers often contain C-2 modified GlcNAc residues [4]. GlcNAc-containing carbohydrates have also been incorporated into neoglycoproteins to probe ligand–receptor interactions [5]. The biological importance of these glycoconjugates

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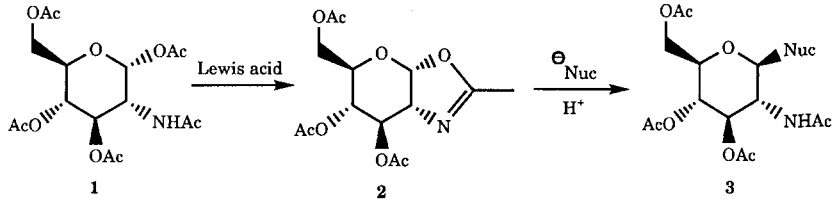
makes it essential to develop methods for selective GlcNAc modification. One useful method for modification would involve the selective deamidation of the C-2 acetamide of a peracetylated GlcNAc, followed by reacylation of the resulting glucosamine.

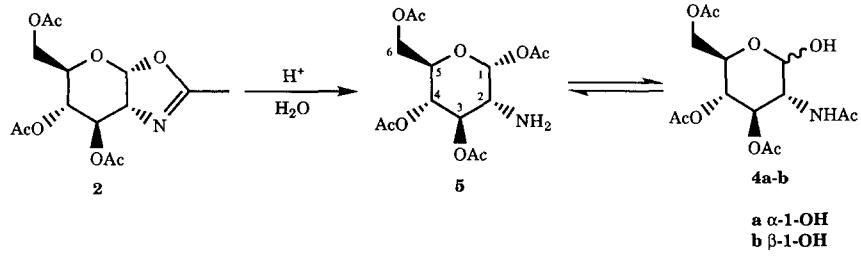
While methods for deamidation of GlcNAc exist [6], the usual protocols involving strong base or hydrazine are too vigorous for selective deamidation if the GlcNAc saccharide also contains *O*-acyl protecting groups. An alternative approach involves *O*-alkylation of the GlcNAc C-2 amide with Meerwein's reagent, $Me_3O^+BF_4^-$, followed by hydrolysis of the resulting imidate salt [7]. While mild and chemospecific, the *O*-alkylation method does not discriminate between different GlcNAc residues in an oligosaccharide. In this paper, we report a convenient and regiospecific method for quantitative deamidation of a GlcNAc peracetate, without hydrolysis of the *O*-acetates of the carbohydrate. Also, since the method involves an oxazoline intermediate **2**, GlcNAc residues at the reducing end of oligosaccharides can be specifically deamidated in the presence of other GlcNAc sugars.

Classically, GlcNAc oligosaccharides are derivatized at the reducing GlcNAc's C-1 anomeric position [8]. For instance, 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline (**2**), which is readily accessible from the GlcNAc peracetate **1** [9], can undergo nucleophilic addition at C-1. Thus, acid-catalyzed glycosylation of the GlcNAc oxazoline peracetate **2** by alcohols [10], and other nucleophiles [11], leads to the exclusive formation of β-glycosides **3** [eq (1)].



(1)

Nucleophilic addition of water to oxazoline **2** also formally occurs at C-1, as the 1-α-OH GlcNAc peracetate **4a** is the major oxazoline hydrolysis product [eq (2)] [12]. While preparing GlcNAc oxazoline **2**, we isolated 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranose (**5**) as a reaction side product. Formation of the amino-ester **5** indicated that hydrolysis did not occur by initial addition of water to the oxazoline's C-1 position. In practice, therefore, acid-catalyzed oxazoline hydrolysis is a useful method for selective deamidation of GlcNAc peracetates.



(2)

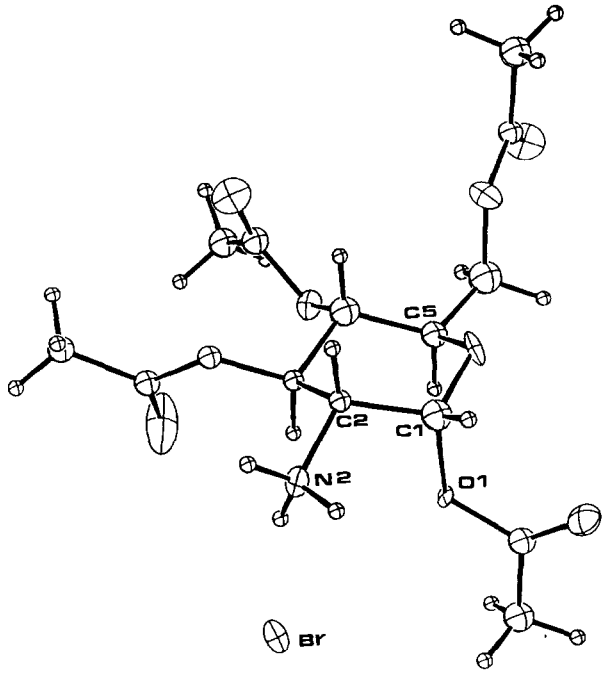
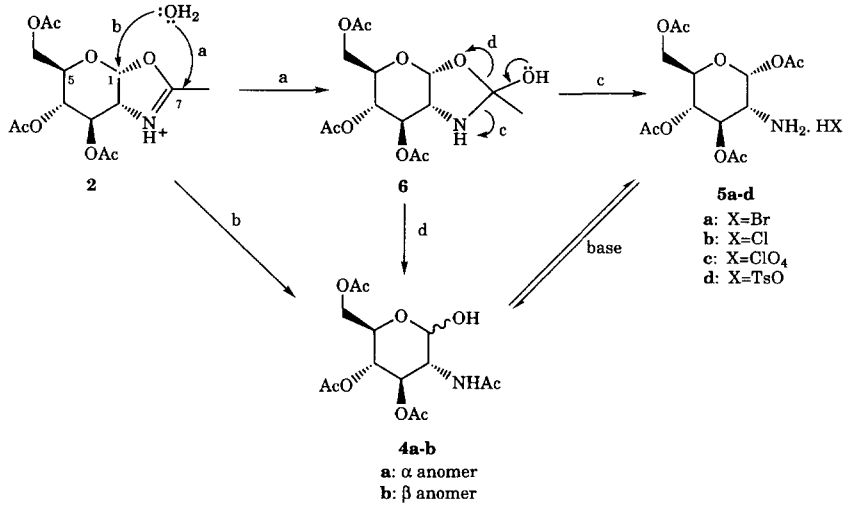


Fig. 1. X-ray crystal structure of 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranose hydrobromide (**5a**).

This paper describes the selective formation of both possible oxazoline hydrolysis products: (1) the kinetic product, 2-amino GlcN tetraacetate **5** and; (2) the thermodynamic product, 1-OH GlcNAc peracetate **4**, which arises from **5** via intramolecular O-1 → N-2 acyl migration. Thus, depending on the conditions, either the aminoester **5** or hydroxy-amide **4** can be obtained in high yield. In addition, the amino-ester **5** can also be acylated to provide novel glycoconjugates which are modified at the GlcNAc's C-2 amino position.

2. Results and discussion

Identification of the 2-amino hydrobromide **5a**.—During the Lewis acid-promoted formation of GlcNAc oxazoline **2** from peracetate **1**, 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranose hydrobromide (**5a**) was obtained as a side product. Thus, treatment of GlcNAc α-peracetate **1** with excess $BF_3 \cdot OEt_2$ (7 equiv) and Me_3SiBr (7 equiv) at room temperature for 24 h gave mainly oxazoline **2**, and small amounts of 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranose (**5**), as determined by TLC and ^1H NMR of the crude reaction mixture. However, the 2-amino hydrobromide **5a** precipitated from the organic layer as the major product during the aqueous work-up. While ^1H and ^{13}C NMR spectroscopic data were consistent with C-2 deamidation, a single crystal X-ray structure confirmed the peracetate hydrobromide's identity (Fig. 1) [13]. The in situ formation of the amine side product **5** is completely suppressed if base

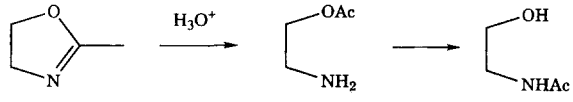


Scheme 1.

is included in the reaction mixture and work-up. Thus, reaction of GlcNAc α-peracetate **1**, $BF_3 \cdot OEt_2$ (7 equiv), Me_3SiBr (7 equiv), and NEt_3 (4 equiv) at room temperature for 24 h gave only oxazoline **2** (> 95%), with no detectable 2-amino hydrobromide **5a** or 1-hydroxy-amide **4**.

We reasoned that the 2-amino hydrobromide **5a** arose from acid-catalyzed hydrolysis of GlcNAc oxazoline **2** during the aqueous work-up (Scheme 1). Protonation of the oxazoline's nitrogen, followed by addition of water to the C-7 acyl carbon, would give orthoimidate intermediate **6** (pathway a). Under acidic conditions, orthoimidate **6** would collapse to the protonated 2-amino GlcN α-tetraacetate **5** (pathway c).

In fact, over 30 years ago, Porter et al. showed that acid hydrolysis of the parent 2-methyl-2-oxazoline gave *O*-acetylethanolamine as the kinetic hydrolysis product [14]. After O → N acyl rearrangement *N*-acetylethanolamine was obtained as the thermodynamic product [eq (3)]. Deslongchamps later showed that orthoimidate hydrolysis is governed by stereoelectronic control to give the amino-ester as the kinetic hydrolysis product under acidic conditions [15]. However, because of possible nucleophilic attack at the anomeric C-1, the hydrolysis of GlcNAc oxazoline **2** may be more complicated than the hydrolysis of the parent 2-methyl-2-oxazoline.



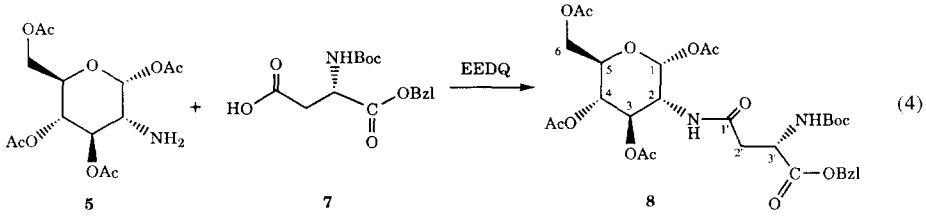
(3)

The GlcNAc C-2 deamidation process has been previously proposed to proceed through an oxazolinium ion intermediate. Inouye and co-workers reported that treatment

of the GlcNAc α-peracetate with HBr in acetic acid effected *N*-deacetylation [16]. Similarly, Horton found that the 1-α-chloro GlcNAc peracetate, when refluxed in acetone–water, gave 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranose hydrochloride (**5b**) [17]. Horton reasoned that this rearrangement had occurred via an oxazoline intermediate. Finally, Sinaÿ reported that hydrolysis of oxazoline **2** in the presence of perchloric acid gave the 2-amino GlcN perchlorate **5c** [18].

Isolation of the 2-amino GlcN hydrobromide **5a** from the initial Me_3SiBr–BF_3 reaction prompted us to develop a procedure that would give 2-amino GlcN peracetates in high yield. Therefore, we first prepared oxazoline and then reacted it with water in the presence of an organic acid. Indeed, reaction of oxazoline **2** with water (6 equiv), in the presence of one equivalent of *p*-toluenesulfonic acid (*p*-TsOH), in either CH_3CN or THF at room temperature gave quantitative formation of 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranose *p*-toluenesulfonate (**5d**) as an insoluble white precipitate. Using this simple hydrolysis protocol, gram quantities of the amino tosylate **5d** can be prepared.

Acylation of the 2-amino GlcN peracetate **5**.—The generation of an amine at the GlcNAc C-2 position, in the presence of acylated hydroxyl groups, should enable the regiospecific modification of GlcNAc at that C-2 amino group. Thus, the protected amino acid *N*-(*tert*-butoxycarbonyl)-L-aspartic acid α-benzyl ester **7** was coupled to the 2-amino GlcN peracetate **5** [eq (4)]. Reaction of 2-amino tosylate **5d** with excess *N*Boc-Asp-α-*O*Bzl **7** (1.5 equiv), NEt_3 (1.5 equiv), and the coupling reagent, EEDQ (1.5 equiv), gave quantitative formation of glycoconjugate **8**, as judged by ^1H NMR. Chromatography provided pure glycoconjugate **8** in 65% yield. Importantly, there was no evidence for competing intramolecular acyl migration under these coupling conditions. The C-2 acylated GlcN derivative **8** is an isostere of the Asn-linked glycopeptide fragment, GlcNAc–β-1-*N*-Asn. This glycopeptide analog may prove useful in the design of glycosyltransferase inhibitors.



(4)

Intramolecular O–N acyl transfer in tosylate **5d**.—Despite our studies that show quantitative formation of the 2-amino GlcNAc **5**, acid-catalyzed hydrolysis of the GlcNAc oxazoline has been reported to give the 1-α-OH GlcNAc peracetate **4a** as the major reaction product [10]. As shown in Scheme 1, the 1-α-OH peracetate **4a** may arise via two possible processes. First, the 1-β-OH product **4b** could be obtained by direct nucleophilic attack of water on the protonated oxazoline's C-1 anomeric position (pathway b). Mutarotation of the 1-β-OH peracetate **4b** would give 1-α-OH GlcNAc

peracetate **4a**. Pathway b is reasonable since acid-catalyzed addition of alcohols to C-1 of the GlcNAc oxazoline gives β-glycosides [8].

Alternatively, addition of water to the protonated oxazoline's center of charge at C-7, followed by collapse of orthoimidate **6**, would give the amino-ester **5** as the kinetic product (pathway a). Amino-ester **5** could then undergo intramolecular acyl O-1 → N-2 transfer, providing the 1-α-OH GlcNAc peracetate **4a**. Both Sinaÿ's [18] and our experiments indicate that oxazoline hydrolysis occurs via pathway *a*, with formation of amino-ester **5** as the kinetic product. In addition, we find that the 2-amino-ester **5** rearranges to the thermodynamic 1-α-OH GlcNAc peracetate **4a**, even under the acidic reaction conditions (catalytic *p*-TsOH in THF).

The number of *p*-TsOH equivalents included in the reaction mixture affects both the reaction kinetics and the final hydrolysis product. When GlcNAc oxazoline **2** was treated with one equivalent of *p*-TsOH and excess water in CH_3CN, the amino tosylate **5d** precipitated immediately in quantitative yield. Because of the tosylate's insolubility in CH_3CN, subsequent O-1 → N-2 acyl migration was not observed with stoichiometric *p*-TsOH. However, when sub-stoichiometric amounts of *p*-TsOH (0.1 equiv) were used in the hydrolysis, TLC and ^1H NMR analysis indicated that the kinetic product, the amino tosylate **5d**, slowly rearranged to a 9:1 mixture of the 1-α-OH tetraacetate **4a** and the 1-β-OH tetraacetate **4b**. Control experiments showed that amino tosylate **5d**, in the presence of 0.1 equiv of *p*-TsOH, but without added oxazoline **2**, did not undergo this O-1 → N-2 acyl migration. The unreacted oxazoline **2**, therefore, is essential for the O-1 → N-2 acyl rearrangement. Oxazolines are weak bases with pK_a of 5.5 [15]. The oxazoline **2** must deprotonate the GlcN ammonium salt **5d** (pK_a 8.5) so that subsequent O-1 → N-2 acyl rearrangement to hydroxy-amide **4** is kinetically feasible.

A number of methods have been reported for the regioselective deacylation of the 1-OAc group in sugar peracetates [19,20]. Our two-step oxazoline hydrolysis method is an attractive alternative to these deacylation protocols. For example, if the 1-OH GlcNAc peracetate **4** is desired, we first isolate the amine tosylate **5d**, and then carry out the O-1 → N-2 acyl rearrangement in a separate step. The base-catalyzed intramolecular O-1 → N-2 acyl transfer gives clean formation of hydroxy-amide **4**, without competing intermolecular acyl transfer.

The acyl migration from O-1 in amino-ester **5d** to N-2 in hydroxy-amide **4** was monitored in both $CDCl_3$ and CD_3CN using 500-MHz ^1H NMR spectroscopy. For example, a solution of the 2-amino tosylate **5d** and NEt_3 (1 equiv) in $CDCl_3$ rearranged cleanly to a 9:1 ratio of the 1-α-OH GlcNAc peracetate **4a** and the 1-β-OH GlcNAc derivative **4b**. The acyl migration was followed by the disappearance of the amine's C-1 acetate CH_3 signal at 2.13 ppm, and the appearance of the 1-hydroxy derivative's (**4**) NHAc CH_3 signal at 1.88 ppm. The integrals of these two methyl peaks were periodically measured to determine the relative amounts of the starting amine-ester **5d** and the rearrangement product **4**. In both CD_3CN and $CDCl_3$, plots of ln $[\mathbf{5}]_o/[\mathbf{5}]_t$ vs. time gave straight lines, consistent with a first-order, intramolecular O-1 → N-2 acyl migration. The acyl rearrangement was faster in CD_3CN ($t_{1/2} = 2.1$ h) than in $CDCl_3$ ($t_{1/2} = 4.6$ h), as would be expected for a reaction which proceeds via a polar transition-state.

Conclusion.—We have described a mild and convenient method for the selective deamidation of the GlcNAc α-peracetate **1**. The method uses stoichiometric *p*-TsOH to promote regiospecific hydrolysis of the GlcNAc oxazoline **2**, giving the insoluble 2-amino GlcN tosylate **5d**. The described deamidation protocol has advantages over existing methods, as it allows deamidation of the C-2 acetamido function in the presence of base-labile OAc protecting groups. In addition, since deamidation is only possible at a reducing-end GlcNAc, the method allows for specific modification of oligosaccharides containing multiple GlcNAc residues. Subsequent reacylation of the GlcNAc 2-amino position may be used to prepare novel glycoconjugates, or isotopically labeled GlcNAc oligosaccharides for biophysical and biochemical experiments. The 2-amino GlcN peracetate **5** also undergoes base-catalyzed, intramolecular rearrangement to the 1-α-OH GlcNAc derivative **4**. Regiospecific formation of a 1-OH GlcNAc peracetate by O-1 → N-2 acyl rearrangement of **5** is a practical alternative to methods that rely on selective hydrolysis of a peracetyl GlcNAc at the C-1 OAc. Thus, depending on the reaction conditions, either hydrolysis product, amine-ester **5** or hydroxy-amide **4** can be obtained in high yields from GlcNAc oxazoline **2**.

3. Experimental

General methods.—All solvents were distilled from drying agents prior to use. Melting points were determined in Kimex glass capillary tubes in a Mel-Temp apparatus. The ^1H and ^{13}C spectra were obtained on a Bruker AF-200 or a Bruker AMX-500 spectrometer, and are reported in ppm relative to the solvent peak. Mass spectral data were obtained on a Finnigan 3200 twin EI and CI quadrupole mass spectrometer. Flash chromatography was performed using Kieselgel 60 silica gel (230–400 mesh).

*1,3,4,6-Tetra-*O*-acetyl-2-amino-2-deoxy-α-*D*-glucopyranose hydrobromide* (**5a**).—To oxazoline **2** (100 mg, 0.30 mmol) in THF (2 mL) at room temperature was added dropwise Me_3SiBr (80 μL, 0.61 mmol), followed by $BF_3 \cdot OEt_2$ (75 μL, 0.61 mmol). The mixture was stirred at room temperature for 10 min, and then water (8 μL, 0.46 mmol) was added. After 15 min, a white precipitate formed. The solid was filtered, washed with diethyl ether, and recrystallized from MeOH to give 118 mg (95%) of the amine hydrobromide **5a**: mp 210–220°C (dec); $[\alpha]_D^{20}$ +56.0° (*c* 0.2, MeOH); ^1H NMR (200 MHz, D_2O): δ 6.19 (d, $J_{1,2}$ 3.9 Hz, H-1), 5.40 (dd, $J_{3,2}$ 10.3, $J_{3,4}$ 9.8 Hz, H-3), 4.99 (dd, $J_{4,3}$ 9.8, $J_{4,5}$ 9.8 Hz, H-4), 4.25–4.17 (m, H-5, H-6), 3.97 (d, J_{gem} 11.0 Hz, H-6′), 3.83 (dd, $J_{2,3}$ 10.3 Hz, $J_{2,1}$ 3.9 Hz, H-2), 2.07–1.92 (4s, 12 H, 4 OAc); ^{13}C NMR (55 MHz, CD_3OD): δ 174.3, 173.6, 173.3, 172.1, 89.8, 70.5, 70.2, 68.8, 62.4, 51.7, 25.9, 21.0, 20.9; MS (CI) *m/z* (%) (Calcd mw of cation = 348), 348 (1), 288 (4), 228 (29), 168 (76), 138 (7), 126 (11), 83 (93), 81 (100), 61(75).

*1,3,4,6-Tetra-*O*-acetyl-2-amino-2-deoxy-α-*D*-glucopyranose p-toluenesulfonate* (**5d**). —To oxazoline **2** (100 mg, 0.31 mmol) in CH_3CN (3.5 mL) at room temperature was added a solution of *p*-toluenesulfonic acid (58 mg, 0.31 mmol) in distilled water (30 μL, 1.82 mmol). A white precipitate formed immediately. The precipitate was filtered and washed with diethyl ether to give 153 mg (97%) of the 2-amino tosylate **5d**: mp 235–240°C (dec); $[\alpha]_D^{20}$ +111.2° (*c* 0.4, MeOH); ^1H NMR (200 MHz, CD_3OD): δ

7.71 (d, 2 H, *J* 7.2 Hz, Ar), 7.24 (d, 2 H, *J* 7.2 Hz, Ar), 6.33 (d, $J_{1,2}$ 3.4 Hz, H-1), 5.43 (dd, $J_{3,4}$ 9.0, $J_{3,2}$ 9.0 Hz, H-3), 5.15 (dd, $J_{4,3}$ 9.0, $J_{4,5}$ 9.0 Hz, H-4), 4.34 (dd, J_{gem} 9.3, $J_{6,5}$ 3.3 Hz, H-6), 4.20 (m, H-5), 4.06 (dd, J_{gem} 9.3 Hz, $J_{6',5}$ 3.3 Hz, H-6'), 3.90 (dd, $J_{2,3}$ 9.0 Hz, $J_{2,1}$ 3.4 Hz, H-2,), 2.36 (s, 3 H, CH_3-Ar), 2.21 (s, 1-OAc), 2.08 (s, 3 H, OAc), 2.02 (s, 3 H, OAc), 1.98 (s, 3 H, OAc); ^{13}C NMR (55 MHz, MeOD): δ 171.8, 171.0, 169.9, 141.6, 129.7, 126.9, 89.9, 70.8, 69.0, 62.5, 52.5, 49.7, 21.2, 20.6, 20.4. MS (FAB) *m/z* (%) (Calcd MW of the cation = 348), 348 (28), 288 (12), 228 (34), 186 (6), 168 (100), 138 (17), 126 (46), 108 (21), 96 (11); HRMS Calcd for $C_{14}H_{22}NO_9$ 348.1295, found 348.1306.

N^2-*(1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-*D*-glucopyranos-2-yl)-*N*-(*tert*-butoxycarbonyl)-*L*-asparagine α-benzyl ester* (**8**).—To a suspension of tosylate **5d** (80 mg, 0.15 mmol) in dichloroethane (1 mL) at room temperature was added NEt_3 (25 μL, 0.18 mmol). The suspension dissolved immediately. A mixture of *N*-(*tert*-butoxycarbonyl)-L-aspartic acid α-benzyl ester **7** (75 mg, 0.23 mmol) and 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) (58 mg, 0.23 mmol) in CH_2Cl_2 (2 mL) was then added dropwise to the reaction mixture. The reaction mixture was stirred at room temperature for 2.5 h, after which time TLC indicated the reaction was complete (R_f of **5** = 0.62, R_f of **8** = 0.52; 10:1 $CHCl_3$–MeOH). The mixture was diluted with CH_2Cl_2 (9 mL), washed with saturated $NaHCO_3$ (2 × 12 mL), 0.1 M HCl (2 × 15 mL), and brine (2 × 15 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated in vacuo to give a yellow oil. The oil was triturated with hexane to give 65 mg (65%) of glycopeptide **8** as a white solid: mp 234–240°C (dec); $[\alpha]_D^{20}$ +98.4° (*c* 1.25, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.35 (s, 5 H, Ar), 6.12 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1), 5.92 (d, 1 H, $J_{NH,2}$ 7.4 Hz, NH-2), 5.57 (d, 1 H, $J_{NH,3'}$ 7.0 Hz, Asp NH), 5.15–5.09 (m, 4 H, H-3, H-4 and Bn CH_2), 4.42 (m, 2 H, H-2 and Asp αH), 4.22 (dd, 1 H, J_{gem} 9.0 Hz, $J_{6,5}$ 2.9 Hz, H-6), 4.05 (dd, 1 H, J_{gem} 9.0 Hz, $J_{6',5}$ 2.9 Hz, H-6'), 3.95 (m, 1 H, H-5), 2.80 (dd, 1 H, J_{gem} 16.5, $J_{2',3'}$ 3.6 Hz, Asn βH), 2.62 (dd, 1 H, J_{gem} 16.5, $J_{2',3'}$ 3.4 Hz, Asn βH'), 2.15–2.00 (4s, 12 H, 4 OAc), 1.50 (s, 9 H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.4, 171.0, 170.6, 170.0, 169.1, 168.6, 155.5, 135.2, 128.5, 128.3, 128.1, 90.3, 80.1, 70.5, 69.6, 67.4, 61.5, 50.8, 50.3, 38.0, 28.2, 20.8, 20.6, 20.5; MS (FAB) (Calcd MW = 652) *m/z* (%) 653 (M + H$^+$, 3), 593 (3), 552 (4), 537 (21), 486 (5), 168 (13), 126 (11), 108 (11), 91 (100), 57 (49), 55 (10); HRMS Calcd for $C_{30}H_{40}N_2O_{14}$ 652.2558, found 652.2549.

O-*1*–N-*2-Acyl rearrangement of amino tosylate* (**5d**) *to 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*D*-glucopyranose* (**4**).—To a $CDCl_3$ (0.5 mL) suspension of amino tosylate **5d** (30 mg, 0.06 mmol) in an NMR tube was added NEt_3 (9 μL, 0.06 mmol). The amino tosylate **5d** dissolved immediately. The O-1 → N-2 acyl rearrangement of amino-ester **5d** to hydroxy-amide **4** was monitored at 25°C by 500 MHz ^{1}H NMR spectroscopy. The acyl migration was followed by the disappearance of the amine's (**5**) C-1 acetate CH_3 signal at 2.13 ppm, and the appearance of the 1-hydroxy derivative's (**4**) NHAc CH_3 signal at 1.88 ppm. The integrals of the two methyl peaks were periodically measured over 24 h to determine the relative amounts of the starting amine **5** and the rearrangement product **4**. The first-order rate constants for O–N rearrangement were determined from a plot of relative concentration of **5**, ln $[\mathbf{5}]_0/[\mathbf{5}]_t$ vs. time. The 500 MHz ^{1}H NMR spectrum of the rearrangement product, hydroxy-amide **4**, was consistent

with published ^{1}H NMR data [13]. The 1-OH GlcNAc peracetate **4** was purified by flash column chromatography to give 20 mg (67%) of a colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.04 (d, $J_{NH,2}$ 9.5 Hz, NH), 5.25 (dd, $J_{3,2}$ 10.0, $J_{3,4}$ 9.5 Hz, H-3), 5.15 (d, $J_{1,2}$ 3.5 Hz, H-1), 5.07 (t, $J_{4,3}$ 9.5 Hz, H-4), 4.73 (d, $J_{1,2}$ 8.5 Hz, H-1 of β-anomer), 4.22 (m, H-5), 4.16 (m, H-2, H-6), 4.02 (m, H-6′), 2.03 (s, 3 H, OAc), 1.97 (s, 3 H, OAc), 1.96 (s, 3 H, OAc), 1.88 (s, 3 H, N-2 Ac); ^{13}C NMR (55 MHz, $CDCl_3$): δ 171.1, 170.8, 170.3, 169.3, 91.45, 71.4, 68.5, 67.2, 62.2, 52.4, 23.1, 21.2, 20.6, 20.5; CI-MS *m/z* (%) (Calcd MW 347): 348 (3), 330 (4), 241 (7), 172 (16), 139 (11), 113 (37), 101 (19), 91 (29), 86 (100), 73 (12), 66 (13); HRMS Calcd for $C_{16}H_{28}N_4O_7$ 347.1295 (M^+), found 347.1279.

Acyl rearrangement of **5d** *under acidic conditions.*—To a suspension of amino tosylate **5d** (23 mg, 0.056 mmol) in CD_3CN (0.40 mL) was added a solution of *p*-TsOH (2.6 mg, 0.014 mmol) in D_2O (0.10 mL). The amine tosylate **5d** dissolved immediately. The ^{1}H NMR analysis indicated no O-1–N-2 acyl rearrangement over a 10 day period.

Acyl rearrangement of **5d** *under neutral conditions.*—Distilled water (0.10 mL) was used to dissolve the tosylate salt **5d** (20 mg, 0.049 mmol). The solution was then added to CD_3CN (0.40 mL) in an NMR tube. The 500 MHz ^{1}H NMR spectra showed no rearrangement after 10 days.

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