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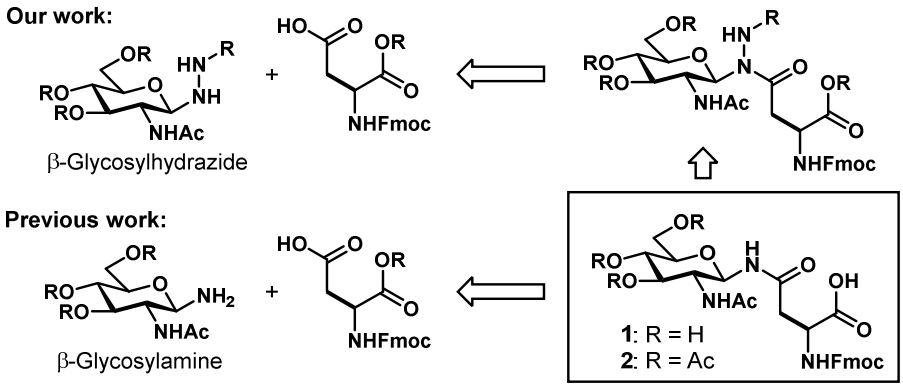

Generation of a glycosylated asparagine residue through chemoselective acylation of a glycosylhydrazide

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Abstract:

Herein, we report the first selective anomeric N-acylation of a glycosylhydrazide. We show that this transformation can be harnessed to generate amino acid building blocks including FmocAsn(GlcNAc)OH (**1**), a residue that has been previously shown to be a competent reagent in the solid-phase peptide synthesis of *N*-linked glycopeptides.

1. Introduction

Post-translational *N*-linked glycosylation can significantly influence protein solubility, folding, and function.[1] Unfortunately, our understanding of the glycoproteome is limited in part by difficulties associated with synthesizing homogeneous *N*-linked glycoproteins. In response, scientists have developed a range of chemical, chemoenzymatic, and biosynthetic methods to synthesize *N*-glycosylated proteins, peptides, and amino acids.[2] Many approaches employ β-glycosylamines with success,[3] although these intermediates can be prone to mutarotation, dimerization, and hydrolysis during their generation and use. Several groups have investigated β-glycosylamine surrogates that circumvent some of these limitations while still reacting with activated aspartic acid side chains to form β-*N*-glycosylasparigines.[4] We hypothesized that β-glycosylhydrazides might be advantageous β-glycosylamine surrogates, reacting selectively with activated aspartic acid residues to form β-*N*-glycosylasparigines following N-N bond cleavage (Fig. 1). β-Glycosylhydrazides are relatively stable to hydrolysis,[5] do not dimerize, and we imagined they could prove more nucleophilic than the corresponding amines due to the alpha effect.[6] Somewhat similar acylations are well precedented, for example the acylation of semicarbazides to form azapeptides.[7] Moreover, analogous β-glycosylamines can be acylated selectively on the anomeric nitrogen atom, even in the presence of unprotected sugar hydroxyl groups.[8] Thus, we set out to determine if we could utilize β-glycosylhydrazides to generate Fmoc-protected β-*N*-glycosylasparigine building blocks (i.e., FmocAsn(GlcNAc)OH (**1**) and O-peracetylated FmocAsn(GlcNAc)OH (**2**)) that have been previously shown[3a,9] to be competent reagents in the solid-phase peptide synthesis of *N*-linked glycopeptides.

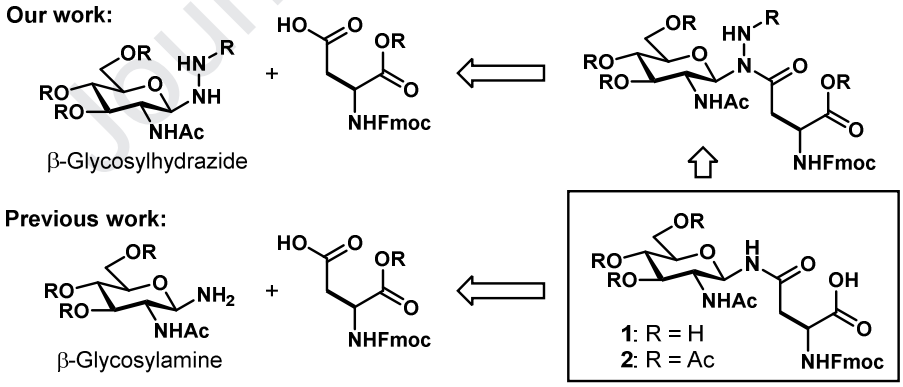


Fig. 1. Two anomeric N-acylation strategies for the generation of β-*N*-glycosylasparigines.

2. Results and discussion

We chose synthetic targets **1** and **2** because they bear the core N-acetylglucosamine residue found in nearly all protein N-glycans. As a first step toward the validation of our novel strategy (Fig. 1), we set out to synthesize β-N-acetylglucosylhydrazides and to identify conditions for their selective anomeric N-acylation. Unprotected, unactivated GlcNAc (**5**) reacted with benzoyl and p-toluenesulfonyl hydrazides

to form closed ring glycosylhydrazides **6**[10] and **7a** predominately in the desired β-pyranose form (Fig. 2). We explored two tactics for the generation of O-peracetylated glycosylhydrazides: acetylating glycosylhydrazides **6** and **7a**, and condensing protected reducing sugar **4**[11] with hydrazides. In both instances, only low yields of product were generated in the form of complex mixtures containing β-pyranose, α-pyranose, and open-chain hydrazone isomers. Because it is not possible to resolve isomeric glycosylhydrazide mixtures due to rapid equilibration between isomeric forms,[12] we narrowed our focus to the synthesis of **1** through derivatives **6** and **7a**.

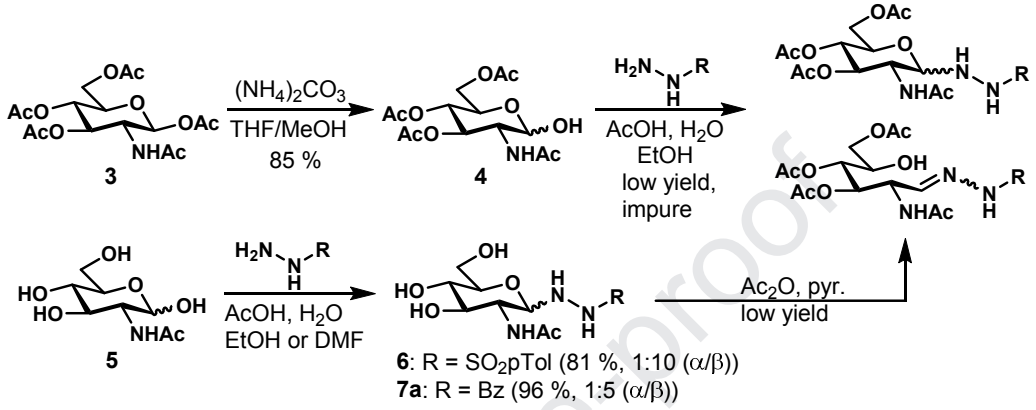


Fig. 2. Generation of glycosylhydrazides; attempts to generate O-peracetylated glycosylhydrazides resulted in inseparable isomeric mixtures.

We next attempted to couple hydrazides **6** and **7a** with a model carboxylic acid using COMU under standard conditions (Fig. 3).[13] Hydrazide **6** provided no traces of desired product **8**, only mixed isolates containing 6′-O-acylated and other mono-O-acylated products. Hydrazide **7a** did result in a small trace of **9a**, but O-acylated products were again predominant. We next screened a representative coupling agent from each remaining major class (BOPCl, EDCI/HOBt, EEDQ, T3P, EEDQ) using HPLC to estimate product and byproduct formation for the conversion of **7a** to **9a**. These agents provided at most traces of desired product **9a**, as well as varying amounts of O-acylated material (data not shown).

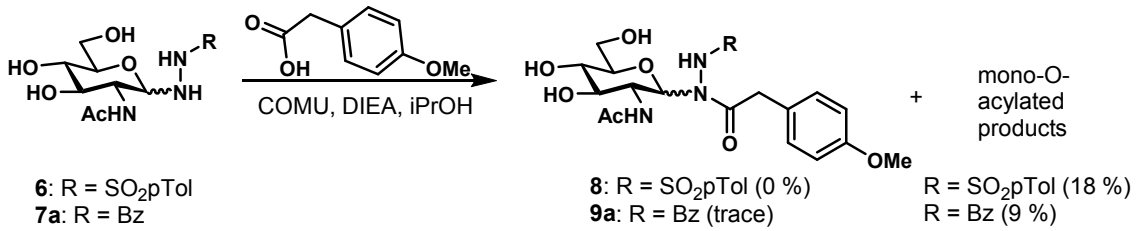


Fig. 3. Attempted anomeric N-acylation of glycosylhydrazides using a uronium-type coupling agent. Other coupling agents provided similar results (data not shown).

Inspired by a report that unprotected O-glycosides of N-hydroxy-N-arylamines can be selectively N-acylated using acyl chlorides,[8b] we attempted the selective anomeric N-acylation of glycosylhydrazides

under similar conditions (Fig. 4), reasoning that an "over activated" agent like an acyl chloride might be required to acylate anomeric hydrazide nitrogen atoms. When we treated **6** and **7a** with 4-methoxyphenylacetyl chloride and $NaHCO_3$ in dioxane, **6** provided only mixtures of O-acylated products, but **7a** provided a 55 % isolated yield of the desired N-acylated product (**9a**). The utilization of alternative solvents (THF, CH_2Cl_2) and bases (DIEA, NMM, TMAP, DMAP) resulted in lower yields. To our knowledge, our successful generation of **9a** represents the first selective anomeric N-acylation of a glycosylhydrazide, producing a novel class of glycoside products that could ultimately prove useful not only in amino acid synthesis, but also efforts to glycorandomize natural products.[14]

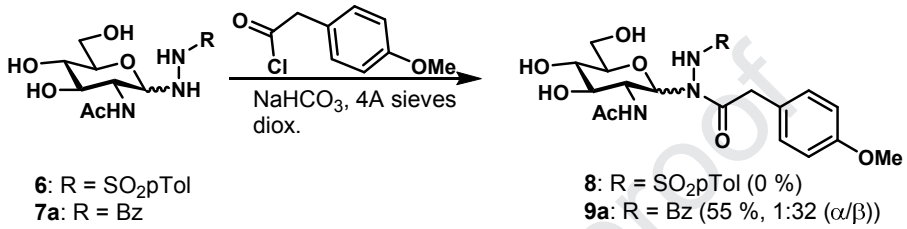
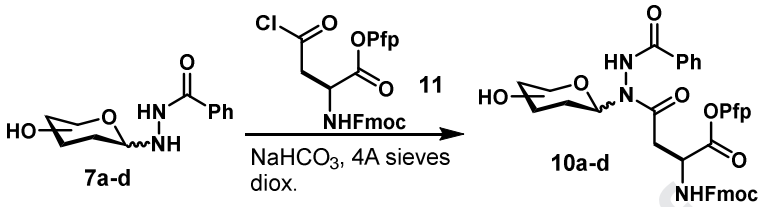


Fig. 4. Successful anomeric N-acylation of a glycosylhydrazide using an acyl chloride.

Given this positive result, we turned our attention to the formation of a suitably protected aspartic acid chloride derivative for use in N-acylations. In our hands, treating FmocAsp(OH)O^{t}Bu and FmocAsp(OH)OBn with thionyl chloride or oxalyl chloride led to the corresponding aspartic anhydrides. However, treating FmocAsp(O^{t}Bu)OPfp with thionyl chloride and TFA resulted in FmocAsp(Cl)OPfp (**11**) as previously reported.[15] We treated hydrazide **7a** with **11** in the presence of $NaHCO_3$ in dioxane. Gratifyingly, we obtained the desired N-acylated glycoside (**10a**), in a 57 % yield. To determine if this methodology was compatible with other sugars to enable the synthesis of rare glycoforms,[16] we worked to generate a few additional representative glycosylhydrazides. While we were unable to cleanly synthesize significant quantities of disaccharide glycosylhydrazides, we were able to make and selectively N-acylate a glucosylhydrazide (**10b**), a xylosylhydrazide (**10c**), and a galactosylhydrazide (**10d**) in modest to low yields (Table 1). The isomer ratios of N-acylated products **10a-d** mirrored those of hydrazides **7a-d**, providing **10a** as a 90:10 mixture (β/α) and **10b-d** exclusively in the β-pyranose form. We observed no evidence for mutarotation of compounds **10a-d**, consistent with the instability of the imminum-like ion that would be required for mutarotation to occur. The small change in anomer ratio observed in the formation of **10a** is likely due to fractionation during chromatography. Analogous Fmoc-protected, Pfp-activated amino acids are suitable for solid-phase peptide synthesis,[17] and thus the generation of **10a-d** provides novel building blocks for the generation of non-natural glycopeptide derivatives. We plan to explore the utility of such building blocks in future work.

Table 1. Anomeric N-acylation of a β-glycosylhydrazides using FmocAsp(Cl)OPfp.



Sugar	Substrate	β-Pyr/α-pyr[a]	Product	Yield (%)	β-Pyr/α-pyr[a]
GlcNAc	**7a**	85:15	**10a**	57	90:10
Glc	**7b**	100:0	**10b**	35	100:0
Xyl	**7c**	100:0	**10c**	55	100:0
Gal	**7d**	100:0	**10d**	29	100:0

[a]Isomer ratios calculated via ^{1}H NMR integration.

To generate our target, FmocAsn(GlcNAc)OH (**1**), we turned our attention to N-N bond cleavage (Fig. 5). Literature precedents suggested that SmI_2 in the presence of a proton source might cleave the N-N bond of **10a** to provide a solid-phase peptide synthesis-ready building block.[18] However, our initial attempts to react **10a** using SmI_2[19] in THF/MeOH resulted in complex product mixtures that appeared to contain N-N cleaved product that had undergone transesterification to the corresponding methyl ester. Attempts to avoid transesterification through the use PfpOH as a proton source resulted in low yields of impure material. Based on these findings, we decided to hydrolyze the Pfp group of **10a**[20] and to instead work with Fmoc amino acid **12**. We were gratified to find that reacting **12** with SmI_2 in THF/MeOH successfully resulted in the formation of product **1a** (51 % yield, 92:8 β/α).

In conclusion, glycosylhydrazides can be used to generate an Fmoc-protected N-glycosylasparigine building block (i.e., **1**) that has seen previous use in solid-phase peptide synthesis (overall yield: 23 % over 4 steps). Our strategy involved the first selective anomeric N-acylation of a glycosylhydrazide; this transformation can be used to provide new non-natural building blocks (**9a**, **10a-d**) and may provide inspiration for the generation of other derivatives containing novel glycosidic linkages.

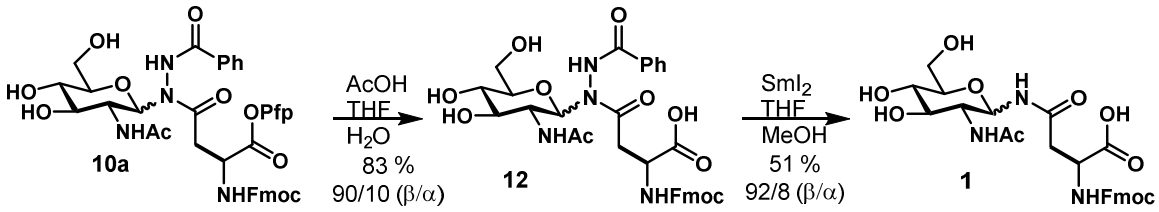


Fig. 5. Hydrolysis and N-N bond cleavage provide an Fmoc-protected β-N-glycosylasparigine building block.

3. Experimental Section

3.1 General methods

Proton nuclear magnetic resonance (^{1}H NMR) spectra and gcosy spectra were recorded in deuterated solvents on a Bruker Avance III 400 MHz spectrometer. Chemical shifts are reported in parts per million (ppm, δ) relative to tetramethylsilane (0.00) for *d*-chloroform, or the residual protic solvent peak for other solvents. ^{1}H NMR splitting patterns with observed first order coupling are designated as singlet (s), doublet (d), triplet (t), or quartet (q). Splitting patterns that could not be interpreted or easily visualized are designated as multiplet (m). The presence of rotamers was confirmed using a NMR-based chemical-exchange experiment.[21] Anomers assignments were deduced using H-1 coupling constants; anomer and rotamer populations were estimated using ^{1}H NMR integration. Carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on a Bruker Avance III 400 MHz spectrometer. Mass spectra (MS) were obtained using electrospray ionization (ESI). Analytical thin layer chromatography (TLC) was carried out on TLC plates pre-coated with silica gel 60 (250 µm layer thickness). Visualization was accomplished using either a UV lamp or potassium permanganate stain (2 g $KMnO_4$, 13.3 g K_2CO_3, 2 mL 2M NaOH, 200 mL H_2O). Flash column chromatography was performed on 40-60 µm silica gel (230-400 mesh). Solvent mixtures used for TLC and flash column chromatography are reported in v/v ratios. Commercially available reagents and solvents were used without further purification. Compound **6** was generated as previously described.[10]

1-(2-Acetamido-2-deoxy-D-glucopyranosyl)-2-benzoylhydrazine (7a). Benzoylhydrazide (2.46 g, 18.2 mmol) and N-acetyl-D-glucosamine (2.0 g, 9.1 mmol) were dissolved in EtOH/H_2O (3:1, 12 mL) and 2 drops of AcOH were added. The resulting mixture was stirred at 60 °C for 48 h then concentrated followed by co-evaporation with toluene then DCM/EtOH. The crude product mixture was purified by SiO_2 column chromatography eluting with 5:1 DCM/MeOH to provide **X** (TLC R_f = 0.10 in 5:1 DCM/MeOH) as an off white foam (2.95 g, 96 % yield). The product was comprised of an inseparable mixture of β-pyranoside (85 %) and α-pyranoside (15 %) isomers. HRMS (ESI, TOF) *m/z* (M + H)

calculated for $C_{15}H_{22}N_3O_6$ 340.1503, observed 340.1501. **β-pyranoside**: ^{1}H NMR (MeOD-*d4*, 400 MHz): δ 7.82 (m, 2H), 7.55 (m, 1H), 7.48 (m, 2H), 4.09 (d, *J* = 9.5, 1H), 3.80 (dd, *J* = 9.4, 9.4, 1H), 3.49 (dd, *J* = 10.2, 8.2, 1H), 3.29 (X of ABX, 1H) 3.25 (m, 1H), 3.86 (A of ABX, *J* = 11.9, 2.1, 1H), 3.62 (B of ABX, *J* = 11.9, 6.1, 1H), 2.07 (s, 3H); ^{13}C NMR (MeOD-*d4*), 100 MHz): δ 173.7, 167.2, 132.7, 131.6, 128.3, 126.8, 90.0, 77.9, 74.9, 70.9, 61.6, 53.3, 21.6. **α-pyranoside**: ^{1}H NMR (MeOD-*d4*, 400 MHz): δ 7.82 (m, 2H), 7.55 (m, 1H), 7.48 (m, 2H), 4.68 (d, *J* = 4.8, 1H), 4.07 (m, 1H), 3.97 (m, 1H), 3.92 (m, 1H), 3.74 (m, 1H), 3.30 (m, 1H), 3.69 (m, 1H), 2.09 (s, 3H).

Attempted peracetylation of 7a. Hydrazide **7a** (153 mg, 452 μmol) was dissolved in pyridine (0.9 mL) and cooled to 0 °C, then acetic anhydride (0.25 mL, 2.6 mmol) was added. The resulting mixture was stirred at rt for 12 h then quenched with MeOH and concentrated. The crude product mixture was purified by SiO_2 column chromatography eluting with 15:1 DCM/MeOH to provide an impure, inseparable mixture (0.032 g) of O-peracetylated isomers (TLC R_f = 0.16 and 0.23 in 15:1 DCM/MeOH); open-chain hydrazide, β-pyranoside, and α-pyranoside (~ 15 : 6 : 1). **Open-chain hydrazide**: ^{1}H NMR (MeOD-*d4*, 400 MHz): δ 7.92 – 7.45 (m, 5H), 7.80 (m, 1H), 5.72 – 5.62 (m, 1H), 5.42 (dd, *J* = 7.6, 3.5, 1H), 5.16 (m, 1H), 4.92 (m, 1H), 4.28 (m, 1H), 4.10 (m, 1H), 2.17 – 1.92 (m, 9H). **β-pyranoside**: ^{1}H NMR (MeOD-*d4*, 400 MHz): δ 7.92 – 7.45 (m, 5H), 5.23 (dd, *J* = 10.3, 9.4, 1H), 5.00 (dd, *J* = 10.0, 9.6, 1H), 4.36 (d, *J* = 9.7, 1H), 4.27 (m, 1H), 4.10 (m, 1H), 4.08 (m, 1H), 3.80 (m, 1H), 2.17 – 1.92 (m, 9H). **α-pyranoside**: ^{1}H NMR (MeOD-*d4*, 400 MHz): δ 7.92 – 7.45 (m, 5H), 5.38 (dd, *J* = 11.4, 9.2, 1H), 4.07 (dd, *J* = 10.2, 9.2, 1H), 4.75 (d, *J* = 4.9, 1H), 4.55 (m, 1H), 4.33 (m, 1H), 4.31 (m, 1H), 4.12 (m, 1H), 2.17 – 1.92 (m, 9H).

1-(2-Acetamido-2-deoxy-D-glucopyranosyl)-1-(4-methoxyphenylacetyl)-2-benzoylhydrazine (9a). Hydrazide **7a** (87.1 mg, 256 μmol), $NaHCO_3$ (43.1 mg, 512 μmol), and 4 Å molecular sieves (91 mg) were suspended in dioxane (7.3 mL) and stirred 5 min. 4-Methoxyphenylacetyl chloride (51.0 μL, 333 μmol) was added. After 2 h, EtOH (120 μL, 2.07 mmol) was added dropwise. The reaction mixture was stirred 4 h, filtered, then concentrated. The crude product mixture was purified by SiO_2 column chromatography eluting with 9:1 DCM/MeOH to provide **9a** (TLC R_f = 0.27 in 9:1 DCM/MeOH) as a white solid (69.1 mg, 55 % yield). HRMS (ESI, TOF) *m/z* (M + H) calculated for $C_{24}H_{30}N_3O_8$ 488.2027, observed 488.2025. The product was comprised of an inseparable mixture of β-pyranoside (97 %) and α-pyranoside (3 %) isomers. **β-pyranoside**: ^{1}H NMR (MeOD-*d4*, 400 MHz) (rotamers, 92:8): δ 8.05 – 7.47 (m, 5H), 7.24 – 6.81 (m, 4H), 5.89 (d, *J* = 10.4, 0.03H), 5.56 (d, *J* = 9.9, 0.97H), 3.84 - 3.52 (m, 2H), 3.81 (m, 1H), 3.76 (s, 3H), 3.54 (m, 2H), 3.52 (m, 1H), 3.39 (m, 1H), 3.11 (dd, 1H, *J* = 9.8, 8.8), 1.93 (s, 0.3), 1.78 (s, 2.7 H); ^{13}C NMR (MeOD-*d4*), 100 MHz): δ 174.6, 174.4, 167.0, 158.8, 132.9, 131.0, 129.8, 128.8, 127.3, 125.9, 113.7, 82.2, 79.6, 74.2, 70.7, 61.7, 54.3, 52.6, 39.1, 21.1. **α-**

pyranoside: ^{1}H NMR (MeOD-*d4*, 400 MHz) (rotamers, 59:41): δ 8.05 – 7.47 (m, 5H), 7.24 – 6.81 (m, 4H), 6.24 (d, *J* = 10.4, 0.41H), 5.93 (d, *J* = 9.9, 0.59H), 3.84 - 3.52 (m, 2H), 3.81 (m, 1H), 3.76 (s, 3H), 3.54 (m, 2H), 3.52 (m, 1H), 3.39 (m, 1H), 3.11 (dd, 1H, *J* = 9.8, 8.8), 1.93 (s, 0.3), 1.78 (s, 2.7 H).

1-(D-Glucopyranosyl)-2-benzoylhydrazine (7b). Benzoylhydrazide (2.3 g, 16.8 mmol) and D-glucose (2.0 g, 11.2 mmol) were dissolved in EtOH (8 mL) and 12 drops of AcOH were added. The resulting mixture was stirred at 80 °C for 3 h then cooled and filtered. The filtrate was washed with hot EtOH (50 mL), then cold EtOH (50 mL), and the resulting white solid **7b** (TLC R_f = 0.14 in 5:1 DCM/MeOH) was used without further purification (3.1 g, 93 % yield). The product was 100 % β-pyranoside. HRMS (ESI, TOF) *m/z* (M + H) calculated for $C_{13}H_{19}N_2O_6$ 299.1238, observed 299.1237. ^{1}H NMR (MeOD-*d4*, 400 MHz): δ 7.82 (m, 2H), 7.57 (m, 1H), 7.48 (m, 2H), 4.01 (d, *J* = 8.9, 1H), 3.91 (m, 1H), 3.63 (m, 1H), 3.42 (dd, 1H, *J* = 9.2, 9.2), 3.35 – 3.20 (m, 3H); ^{13}C NMR (D_2O, 100 MHz): δ 173.3, 135.2, 134.7, 131.5, 130.0, 92.3, 79.7, 79.0, 73.5, 72.3, 63.6.

1-(D-Xylopyranosyl)-2-benzoylhydrazine (7c). Benzoylhydrazide (1.33 g, 9.77 mmol) and D-xylose (0.98 g, 6.51 mmol) were dissolved in EtOH and 4 drops of AcOH were added. The resulting mixture was stirred at 78 °C for 3 h then concentrated. The solid residue was suspended in EtOH and filtered. The filtrate was washed with hot EtOH (20 mL), then cold EtOH (40 mL), and the resulting solid **7c** (TLC R_f = 0.27 in 5:1 DCM/MeOH) was used without further purification (1.01 g, 58 % yield). The product was 100 % β-pyranoside. ^{1}H NMR (MeOD-*d4*, 400 MHz): δ 7.81 (m, 2H), 7.55 (m, 1H), 7.47 (m, 2H), 3.97 (d, 1H, *J* = 8.9), 3.90 (dd, 1H, *J* = 11.7, 5.9), 3.48 (m, 1H), 3.38 (t, 1H, *J* = 8.7), 3.21 (m, 2H); ^{13}C NMR (MeOD-*d4* + DMSO-*d6*), 100 MHz): δ 170.0, 134.0, 133.0, 129.6, 128.5, 93.2, 78.3, 72.6, 71.2, 68.4. HRMS (ESI, TOF) *m/z* (M + H) calculated for $C_{12}H_{17}N_2O_5$ 269.1132, observed 269.1131.

1-(D-Galactopyranosyl)-2-benzoylhydrazine (7d). Benzoylhydrazide (896 mg, 6.6 mmol) and D-galactose (790 mg, 4.4 mmol) were dissolved in 3:1 EtOH/H_2O (3.3 mL) and 4 drops of AcOH were added. The resulting mixture was stirred at 60 °C for overnight then cooled and filtered. The filtrate was washed with hot EtOH (25 mL) and the resulting white solid **7d** (TLC R_f = 0.26 in 5:1 DCM/MeOH) was used without further purification (769 mg, 59 % yield). The product was 100 % β-pyranoside. HRMS (ESI, TOF) *m/z* (M + H) calculated for $C_{13}H_{19}N_2O_6$ 299.1238, observed 299.1236. ^{1}H NMR (D_2O, 400 MHz): δ 7.76 (m, 2H), 7.63 (m, 1H), 7.53 (m, 2H), 4.16 (d, 1H, *J* = 8.9), 3.93 (m, 1H), 3.81 (m, 4H), 3.61 (dd, 1H, *J* = 9.4); ^{13}C NMR (D_2O + *d6*-DMSO), 100 MHz): δ 173.1, 135.1, 134.6, 131.4, 129.9, 93.2, 78.8, 75.8, 71.5, 71.0, 64.0.

1-(2-Acetamido-2-deoxy-D-glucopyranosyl)-1-(N^{α}-(fluoren-9-ylmethoxycarbonyl)-L-asparagine pentafluorophenyl ester))-2-benzoylhydrazine (10a). Hydrazide **7a** (352 mg, 1.04 mmol), $NaHCO_3$

(350 mg, 4.20 mmol), and 4 Å molecular sieves (356 mg) were suspended in dioxane (15 mL) and stirred 5 min. Chloride **10** (560 mg, 1.04 mmol) was added, followed 1 h later by another addition of **10** (560 mg, 1.04 mmol). After 2 h, EtOH (120 µL, 2.07 mmol) was added. The reaction mixture was stirred 20 min., filtered, then concentrated. The crude product mixture was purified by SiO_2 column chromatography eluting with 15:1 DCM/MeOH to provide **9a** (TLC R_f = 0.44 in 9:1 DCM/MeOH) as a white solid (495 mg, 57 % yield). HRMS (ESI, TOF) *m/z* (M + H) calculated for $C_{40}H_{36}F_5N_4O_{11}$ 843.2295, observed 843.2283. The product was comprised of an inseparable mixture of β-pyranoside (90 %) and α-pyranoside (10 %) isomers. **β-pyranoside**: ^{1}H NMR (MeOD-*d4*, 400 MHz) (rotamers, 85:15): δ 8.07 – 7.46 (m, 8H), 7.40 – 7.22 (m, 5H), 5.90 (d, *J* = 9.8, 0.15H), 5.62 (d, *J* = 10.1, 0.85H), 5.03 (m, 1H), 4.51 – 4.31 (m, 2H), 4.23 (m, 1H), 3.90 (m, 1H), 3.81 (m, 1H), 3.61 (m, 1H), 3.52 (m, 1H), 3.41 (m, 1H), 3.25 (m, 1H), 3.16 (m, 1H), 2.99 (m, 1H), 1.99 – 1.91 (m, 3H); ^{13}C NMR (MeOD-*d4*), 100 MHz): δ 173.0, 171.1, 168.2, 164.4, 155.9, 143.7, 140.8, 132.7, 131.7, 129.0, 127.7, 127.2, 127.1, 127.0, 125.2, 120.2, 79.6, 73.7, 70.4, 66.0, 61.0, 54.0, 52.5, 49.9, 46.6, 33.9, 22.4. **α-pyranoside**: ^{1}H NMR (MeOD-*d4*, 400 MHz) (rotamers, 54:46): δ 8.07 – 7.46 (m, 8H), 7.40 – 7.22 (m, 5H), 6.04 (d, *J* = 4.0, 0.54H), 6.28 (d, *J* = 4.0, 0.46H), 5.03 (m, 1H), 4.51 – 4.31 (m, 2.54H), 4.23 (m, 1.46H), 4.01 – 2.87 (m, 7H), 1.99 – 1.91 (m, 3H).

1-(D-Glucopyranosyl)-1-(N^{α}-(fluoren-9-ylmethoxycarbonyl)-L-asparagine pentafluorophenyl ester))-2-benzoylhydrazine (10b). Hydrazide **7b** (315 mg, 1.06 mmol), $NaHCO_3$ (355 mg, 4.22 mmol), and 4 Å molecular sieves (362 mg) were suspended in dioxane (15 mL) and stirred 10 min. Chloride **11** (570 mg, 1.06 mmol) was added, followed 1.5 h later by another addition of **11** (570 mg, 1.06 mmol). After 2 h, EtOH (123 µL, 2.11 mmol) was added. The reaction mixture was stirred 20 min., filtered, then concentrated. The crude product mixture was purified by SiO_2 column chromatography eluting with 15:1 DCM/MeOH to provide **10b** (TLC R_f = 0.08 in 15:1 DCM/MeOH) as a white solid (295 mg, 35 % yield). HRMS (ESI, TOF) *m/z* (M + H) calculated for $C_{38}H_{33}F_5N_3O_{11}$ 802.2030, observed 802.2019. The product was 100 % β-pyranoside. ^{1}H NMR (MeOD-*d4*, 400 MHz) (rotamers, 10:1): δ 8.03 – 7.25 (m, 13H), 5.67 (d, 0.9H, *J* = 9.1), 5.49 (d, 0.1H, *J* = 9.0), 5.01 (m, 1H), 4.52 – 4.21 (m, 3H), 3.85 (m, 1H), 3.72 (m, 1H), 3.55 (t, 1H, *J* = 9.1), 3.44 (m, 1H), 3.37 (m, 1H), 3.27 (m, 1H), 3.20 (t, 1H, *J* = 9.1), 3.06 (m, 1H); ^{13}C NMR (MeOD-*d4*), 100 MHz): δ 172.2, 169.2, 168.3, 156.3, 144.1, 141.2, 133.6, 131.0, 129.3, 128.6, 128.1, 127.5, 125.7, 120.6, 83.0, 80.0, 76.1, 70.1, 70.0, 66.5, 61.3, 55.4, 50.0, 47.0, 35.3.

1-(D-Xylopyranosyl)-1-(N^{α}-(fluoren-9-ylmethoxycarbonyl)-L-asparagine pentafluorophenyl ester))-2-benzoylhydrazine (10c). Hydrazide **7c** (380 mg, 1.41 mmol), $NaHCO_3$ (475 mg, 5.66 mmol), and 4 Å molecular sieves (485 mg) were suspended in dioxane (20 mL) and stirred 10 min. Chloride **11** (764 mg, 1.41 mmol) was added, followed 1.5 h later by another addition of **11** (764 mg, 1.41 mmol). After 1.5 h, EtOH (170 µL, 1.82 mmol) was added. The reaction mixture was stirred 20

min., filtered, then concentrated. The crude product mixture was purified by SiO_2 column chromatography eluting with 15:1 DCM/MeOH to provide **10c** (TLC R_f = 0.37 in 15:1 DCM/MeOH) as a white foam (603 mg, 55 % yield). ^{1}H NMR (MeOD-*d4*, 400 MHz) (rotamers, 76:24): δ 7.99 – 7.24 (m, 13H), 5.57 (d, *J* = 9.1, 0.76H), 5.43 (d, *J* = 8.8, 0.24H), 3.19 (m, 0.76H), 3.47 (m, 0.24H), 5.01 (m, 1H), 3.34 (m, 1H), 3.05 (m, 1H), 3.95 – 3.16 (m, 4H), 4.37 (m, 2H), 4.24 (m, 1H); ^{13}C NMR (DMSO-*d6*), 100 MHz) (rotamers): δ 172.4, 169.1, 168.2, 144.1, 141.1, 133.6, 130.9, 129.3, 129.1, 128.5, 128.1, 127.5, 125.6, 85.0, 83.8, 76.3, 70.0, 69.7, 68.3, 55.4, 50.0, 49.1, 47.0, 35.2. HRMS (ESI, TOF) *m/z* (M + H) calculated for $C_{37}H_{31}F_5N_3O_{10}$ 772.1924, observed 772.1912.

1-(D-Galactopyranosyl)-1-(N$^{\alpha}$-(fluoren-9-ylmethoxycarbonyl)-L-asparagine pentafluorophenyl ester))-2-benzoylhydrazine (10d). Hydrazide **7d** (224 mg, 0.75 mmol), $NaHCO_3$ (253 mg, 3.00 mmol), and 4 Å molecular sieves (258 mg) were suspended in dioxane (10.7 mL) and stirred 10 min. Chloride **11** (406 mg, 0.75 mmol) was added, followed 1.5 h later by another addition of **11** (406 mg, 0.75 mmol). After 2 h, EtOH (88 µL, 1.50 mmol) was added. The reaction mixture was stirred 20 min., filtered, then concentrated. The crude product mixture was purified by SiO_2 column chromatography eluting with 15:1 DCM/MeOH to provide β-pyranoside **10d** (TLC R_f = 0.13 in 15:1 DCM/MeOH) as a white solid (177 mg, 29 % yield). HRMS (ESI, TOF) *m/z* (M + H) calculated for $C_{38}H_{33}F_5N_3O_{11}$ 802.2030, observed 802.2003. ^{1}H NMR (MeOD-*d4*, 400 MHz) (rotamers, 5:1): δ 8.02 – 7.23 (m, 13H), 5.62 (d, *J* = 8.8, 0.8H), 5.50 (d, *J* = 10.0, 0.2H), 5.04 (m, 1H), 4.46 – 4.33 (m, 2H), 4.25 (m, 1H), 3.99 – 3.45 (m, 5.2H), 3.62 (dd, *J* = 9.3, 9.0, 0.8H), 3.37 (m, 1H), 3.10 (m, 1H); ^{13}C NMR (MeOD-*d4*), 100 MHz): δ 172.3, 169.2, 168.3, 156.3, 144.1, 141.2, 133.6, 131.1, 129.2, 128.6, 128.1, 127.5, 125.6, 120.6, 83.8, 78.4, 73.0, 68.3, 67.3, 66.5, 60.8, 55.4, 50.0, 47.0, 35.3.

1-(2-Acetamido-2-deoxy-D-glucopyranosyl)-1-(N$^{\alpha}$-(fluoren-9-ylmethoxycarbonyl)-L-asparagine))-2-benzoylhydrazine (12). Residue **10a** (304 mg, 0.360 mmol) was dissolved in 4:2:1 AcOH/THF/H_2O (2.8 mL) and heated at 60 °C overnight. The reaction mixture was concentrated then co-evaporated with toluene. The crude product mixture was purified by SiO_2 column chromatography eluting with 5:1 DCM/MeOH + 0.5 % AcOH to provide **12** (TLC R_f = 0.09 in 5:1 DCM/MeOH + 0.5 % AcOH) as a white solid (202 mg, 83 % yield). HRMS (ESI, TOF) *m/z* (M + H) calculated for $C_{34}H_{37}N_4O_{11}$ 677.2453, observed 677.2450. The product was comprised of an inseparable mixture of β-pyranoside (90 %) and α-pyranoside (10 %) isomers. **β-pyranoside**: ^{1}H NMR (MeOD-*d4*, 400 MHz) (rotamers, 82:18): δ 8.08 – 7.46 (m, 8H), 7.43 – 7.20 (m, 5H), 5.88 (d, *J* = 9.9, 0.18H), 5.62 (d, *J* = 10.0, 0.82H), 3.87 (m, 0.82H), 3.81 (m, 0.18H), 4.62 – 4.12 (m, 4H), 3.93 – 3.09 (m, 5H), 3.04 (m, 1H), 2.83 (m, 1H), 2.07 – 1.91 (m, 3H); ^{13}C NMR (MeOD-*d4*, 100 MHz): δ 174.8, 173.2, 167.2, 157.0, 143.8, 141.1, 132.6, 131.0, 128.7, 127.4, 127.3, 126.8, 124.9, 119.5, 82.1, 79.6, 74.1, 70.7, 66.7, 61.7, 52.6, 46.9, 34.7, 21.4. **α-pyranoside**: ^{1}H NMR (MeOD-*d4*, 400 MHz) (rotamers, 55:45): δ 8.08 – 7.46 (m, 8H), 7.43 – 7.20 (m, 5H), 6.20 (d, *J* = 3.9, 0.55H), 5.99 (d, *J* = 3.3, 0.45H), 4.62 – 4.12 (m, 4H), 3.93 – 3.09 (m,

6H), 3.04 (m, 1H), 2.83 (m, 1H), 2.07 – 1.91 (m, 3H).

N^{α}-(Fluoren-9-ylmethoxycarbonyl)-N^{γ}-(2-acetamido-2-deoxy-D-glucopyranosyl)-L-asparagine (1). Residue **12** (49.9 mg, 0.074 mmol) was dissolved in MeOH (0.74 mL) under nitrogen. A 0.1 M solution of SmI_2 in THF (1.1 mL, 0.111 mmol) was added dropwise. After 5 min. the reaction mixture was exposed to the atmosphere. After the color had faded from dark blue to yellow, the mixture was concentrated. The material was suspended in 1:1 AcOH/H_2O (2 mL) and loaded onto a 6 mL Resprep C18 disposable solid phase extraction cartridge that had been preconditioned with MeOH then H_2O. Using a vacuum manifold, the cartridge was washed with H_2O, 0.1 M aq. HCl, and H_2O (1 column volume each) then dried for 5 min. The cartridge was then eluted with 2:1 $CHCl_3$/MeOH + 0.5 % AcOH to provide **1** (TLC R_f = 0.08 in 2:1 $CHCl_3$/MeOH + 0.5 % AcOH; mp = 211 °C (decomp.)) as a white solid (20.9 mg, 51 % yield). The product was comprised of an inseparable mixture of β-pyranoside (92 %) and α-pyranoside (8 %) isomers. **β-pyranoside**: ^{1}H NMR (AcOD-*d4*, 400 MHz) (rotamers, 8:2): δ 7.82 – 7.27 (m, 8H), 5.16 (d, *J* = 9.7, 1H), 4.76 (m, 0.8H), 4.60 (m, 0.2H), 4.56 – 4.20 (m, 3H), 4.06 – 3.50 (m, 6H), 2.92 (m, 1.6H), 2.75 (m, 0.4H), 2.01 (s, 2.4H), 2.06 (s, 0.6H); ^{13}C NMR (AcOD-*d4*, 100 MHz): δ 175.6, 174.3, 172.1, 157.0, 143.8, 141.2, 127.6, 127.0, 125.0, 119.8, 78.9, 77.3, 74.2, 70.0, 67.2, 60.8, 54.5, 50.3, 46.9, 37.1, 21.6. **α-pyranoside**: ^{1}H NMR (AcOD-*d4*, 400 MHz): δ δ 7.82 – 7.27 (m, 8H), 5.62 (d, *J* = 2.7, 1H), 4.76 (m, 0.8H), 4.60 (m, 0.2H), 4.56 – 4.20 (m, 3H), 4.06 – 3.50 (m, 6H), 2.92 (m, 1.6H), 2.75 (m, 0.4H), 2.01 (s, 2.4H), 2.06 (s, 0.6H). HRMS (ESI, TOF) *m/z* (M + H) calculated for $C_{27}H_{32}N_3O_{10}$ 558.2082, observed 558.2086.

Associated Content

^{1}H, gcosy, and ^{13}C NMR spectra.

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- Glycosylhydrazides can be selectively acylated at the anomeric nitrogen
- This transformation can be harnessed to generate amino acid building blocks (e.g., FmocAsn(GlcNAc)OH).

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: