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Synthesis of sulfonamide-conjugated glycosyl-amino acid building blocks

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ABSTRACT

The efficient synthesis of novel glycoconjugate amino acid building blocks wherein the amino acid and carbohydrate moieties are linked via a sulfonamide functional group is reported. The general reaction sequence consists of coupling a glycosyl thioacetate to an amino acid methyl ester followed by oxidation and deprotection of the carbohydrate moiety. We demonstrate the synthesis of derivatives from a range of amino acids, with reaction at either the α-amino group of amino acid precursors or the sidechain ε-amino group of lysine precursors.

1. Introduction

The importance of carbohydrates and glycoconjugates in diverse biochemical processes has stimulated the development of neoglycoconjugates as fundamental tools for biological research.[1,2] A central role for carbohydrate–protein conjugates in medicine is now well appreciated with glycoprotein based therapeutics representing more than one third of approved biopharmaceuticals, while glycopeptide antibiotics remain the frontline defense against a wide range of drug-resistant bacterial infections.[3] Glycopeptides are formidable synthetic targets, with synthetic challenges far exceeding those associated with their oligopeptide counterparts. As well, native glycoproteins and glycopeptides comprise *O*-linked and *N*-linked glycosidic bonds and can lack the stability and bioavailability required in a therapeutic setting.[2] Glycopeptide mimetics may confer advantages over their native analogues, including stability toward degrading enzymes, improved bioavailability and reduced clearance rates. In addition, a non-native link between carbohydrate and amino acid moieties may provide added opportunities for interactions with the biological target leading to enhanced affinity and/or improved specificity. The development of glycoconjugate amino acid building blocks with non-native structural features thus opens new possibilities for the synthesis of biologically relevant glycopeptide mimetics.[1] Our group and others recently developed a synthesis for *S*-glycosyl sulfonamides and employed this new methodology to the synthesis of sulfonamide-bridged glycomimetics.[4–6] In this contribution we further elaborate the scope of this chemistry to provide a simple, efficient and novel methodology for tethering sugar moieties to amino acids via a sulfonamide linker with potential for the application to a new class of glycopeptide mimetics.

2. Results and discussion

Our preliminary study was conducted using the simple amino acid glycine. The synthetic procedure toward carbohydrate–glycine glycoconjugates involved the reaction of *S*-acetyl thioglucose **1** with the α-amino group of glycine methyl ester to give the glycoconjugate sulfenamide **2a**, Scheme 1. Oxidation of **2a** provided the sulfonamide glycoconjugate **2b**, wherein the carbohydrate and glycine methyl ester are linked by a sulfonamide group. Standard Zemplén conditions[7] for removal of the acetate protecting groups of the glycosyl moiety of **2b** (0.05 M NaOMe in MeOH, pH ~12) were employed to provide the deprotected glycoconjugate as the methyl ester **2c**, or alternatively using NaOH in place of NaOMe, to provide the deprotected glycoconjugate as the free acid **2d**. All reactions proceeded in high yield and purifications were straightforward, Table 1, entries 1 and 2.

We next extended this preliminary study to a selection of amino acid derivatives that encompassed variable side chain properties including aliphatic (valine), aromatic (phenylglycine), polar/alcohol (serine) and conformationally restricted (proline), Table 1. These amino acids were available enantiomerically pure as their methyl

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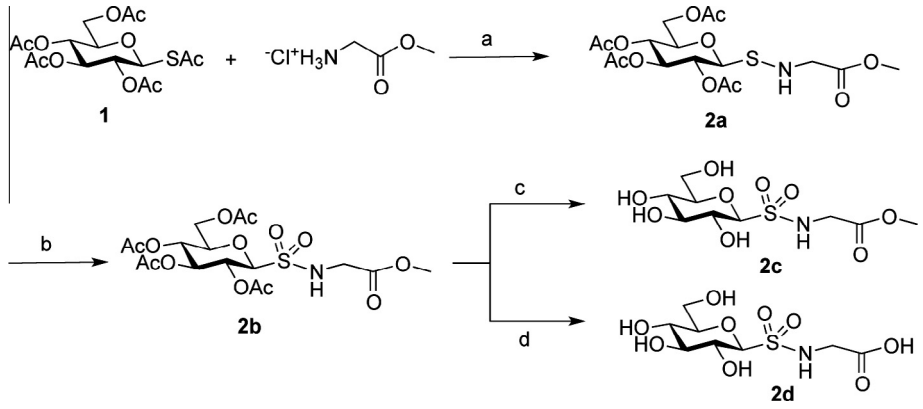

Scheme 1. Synthesis of glycoconjugate glycine building blocks with a sulfonamide linker. Reagents and conditions: (i) (a) 2.5 equiv $BrCH(CO_2Et)_2$, MeOH, rt, 20 min, (b) 3.0 equiv glycine methyl ester, 3.0 equiv DIPEA, rt, 2 h, 96%; (ii) 7.0 equiv *m*CPBA, CH_2Cl_2, rt, 1 h, 87%; (iii) NaOMe, MeOH, rt, 2 h, 94%; (iv) NaOH, MeOH, 0 °C to rt, 1 h; then Amberlite IR120-H^+, quantitative.

ester. The chemistry to form glycoconjugates proceeded as in Scheme 1, however the sugar acetate deprotection step (Scheme 1, step iii) for both the phenylglycine and serine glycoconjugates **4b** and **5b** gave **4c**′ and **5c**′ in high yield but as a mixture of two diastereomers, Table 1 entries 4 and 6. To avoid this racemization alternate non-basic acetate deprotection reaction conditions were sought. We investigated the removal of the acetate protecting groups of **4b** and **5b** under mild acidic conditions (8% HCl in methanol).[8] The acetate groups were cleanly removed with retention of the amino acid α-carbon stereochemistry to give glycoconjugates **4c** (23%) and **5c** (36%) as a single diastereomer, Table 1 entries 5 and 7.

The biological activity of proteins and peptides may be enhanced by conjugation to carbohydrates.[9] Synthetic strategies toward glycoconjugate amino acids may capitalize either on the inherent reactivity of the α-amino, carboxylic acid or side chain functionality of native amino acids or on introduced chemoselective reactivity of a modified side chain in a non-native amino acid.[1,10–12] The glycoconjugate amino acids of our study were linked through reaction of the α-amino group of the amino acid so it is expected that conjugation of a sugar to the free *N*-terminus of a peptide or protein would be possible using this synthetic approach. In order to increase the generality of this method to allow site specific incorporation of a carbohydrate moiety into a peptide or protein it is necessary to apply this chemistry to the side chain of an amino acid. The sulfonamide linker has been previously employed as an amide bond isostere in the synthesis of peptidosulfonamide peptidomimetics.[13] The ε-amino group of lysine is unique among native amino acids and lysine was selected as the candidate to evaluate side chain glycoconjugate formation through a sulfonamide linkage.

From L-lysine methyl ester dihydrochloride the disubstituted glycoconjugates **7a–c** were synthesized, Table 1, entry 9. Reaction occurred without difficulty (monitored by TLC) at the α-amino group while reaction of the sidechain ε-amino appeared relatively less favoured leading to a low yield of the disubstituted compound **7a** (30%). Similarly the reaction of N^{α}-acetyl-L-lysine methyl ester or N^{α}-fluorenylmethyloxycarbonyl-L-lysine with **1** also proceeded to form the sulfenamide-linked glycosyl amino acids **8a** (24%) and **9a** (14%) in low yields Table 2, entries 10 and 11. A reduced yield for the lysine sulfenamides **7a–9a** compared to sulfenamides **2a–6a** (yields >80%) identified a caveat to this methodology, which had so far proven high yielding. To avoid deacetylation of the sugar moiety of both reagent **1** and product sulfenamides it was necessary to maintain the pH value of the reaction (Scheme 1, step i) at ~pH 9. The pK_a value of the side chain of the ε-amino group of lysine is however ~10.5, rendering it predominantly protonated under the reaction conditions. A balance between maximizing the conversion to sulfenamide product while avoiding deacetylation of the carbohydrate moieties resulted in overall reduced yields for reaction of the side chain lysine ε-amino group compared to the α-amino groups (pK_a values ~9–10). Despite this, the bis-sulfenamide **7a** was formed in 30% yield, while the yield for **8a** and **9a** was 24% and 14%, respectively, Table 1, entries 9–11. Given the ready availability and low cost of starting reagents this limitation does not significantly impact quantities of target compounds that may be synthesized.

3. Experimental

3.1. General methods

All starting materials and reagents, including per-*O*-acetylated glucopyranose, were purchased from commercial suppliers. 1-*S*-Acetyl-2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose (**1**) was synthesized as described earlier.[14] All reactions were monitored by TLC. TLC plates were visualized with UV light, ninhydrin stain (1 g of ninhydrin in 100 mL of EtOH containing 3% (v/v) acetic acid) and/or orcinol stain (1 g of orcinol monohydrate in a mixture of EtOH:H_2O:H_2SO_4 72.5:22.5:5 mL). Silica gel flash chromatography was performed using silica gel 60 Å (230–400 mesh). ^{1}H NMR were acquired at 500 MHz and ^{13}C NMR at 125 MHz at 30 °C. For ^{1}H and ^{13}C NMR acquired in $CDCl_3$ chemical shifts (δ) are reported in ppm relative to the solvent residual peak: proton (δ 7.27 ppm) and carbon (δ 77.2 ppm). Chemical shifts for ^{1}H and ^{13}C NMR acquired in DMSO-d_6 are reported in ppm relative to residual solvent proton (δ 2.50 ppm) and carbon (δ 39.5 ppm) signals, respectively. Assignments for ^{1}H NMR were confirmed by ^{1}H–^{1}H gCOSY, while assignments for ^{13}C NMR were confirmed by ^{1}H–^{13}C HSQC. Multiplicity is indicated as follows: s (singlet); d (doublet); t (triplet); m (multiplet); dd (doublet of doublet); ddd (doublet of doublet of doublet); br (broad). Coupling constants are reported in Hertz (Hz). Melting points are uncorrected. High and low resolution electrospray ionization mass spectra were acquired using electrospray as the ionization technique in positive ion and/or negative ion modes as stated. All MS analysis samples were prepared as solutions in MeOH. Optical rotations were measured at 25 °C with Na-589 nm wave length and a 100 mm cell and reported as an average of ten measurements. Purity of all compounds was ⩾95% by NMR. Glycoconjugates are named in accordance with the recommendations of the IUPAC-IUBMB Joint Commission on Biochemical Nomenclature: 'Nomenclature of Carbohydrates (Recommendations 1996)' (http://www.chem.qmul.ac.uk/iupac/2carb/).