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Synthesis and X-ray crystallographic investigation of *N*-(α-D-arabinopyranosyl)alkanamides as *N*-glycoprotein linkage region analogs

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ABSTRACT

N-Glycoprotein linkage region constituents namely 2-deoxy-2-acetamido-β-D-glucopyranose (GlcNAc) and asparagine (Asn) are conserved among all eukaryotes. Earlier crystallographic studies on the linkage region conformation revealed that among all the models and analogs of the *N*-glycoprotein linkage region, XylβNHAc showed maximum deviation in the ϕ_N value as compared to the value reported for the model compound, GlcNAcβNHAc. In order to understand the effect of another pentopyranose, viz., arabinose, on the *N*-glycosidic torsion angles and molecular assembly, three arabinopyranosyl alkanamides were synthesized and their X-ray crystal structures elucidated. A comparative analysis of the *N*-glycosidic torsion, ϕ_N of the three analogs revealed the greater rotational freedom around the C1–N1 bond as compared to the GlcNAc derivatives. Molecular assembly of propionamido and chloroacetamido derivatives is characterized by the presence of anti-parallel bilayers of the molecules. This unique molecular assembly is hitherto unknown in all other models and analogs of *N*-glycoprotein linkage region. This study reveals that *N*-glycosidic torsions are influenced by the glycan as well as molecular packing.

1. Introduction

Glycan part of glycoproteins play vital roles in many biological processes.[1,2] As *N*-linked glycosylation influences the protein stability and function, it is important to gain a complete understanding of how carbohydrates influence the peptide structure and function. To gain a better understanding for nature's choice of GlcNAcβAsn (Fig. 1) as linkage region constituents and inter- & intramolecular carbohydrate–protein interactions, a detailed systemic structural study of linkage region conformation is needed. Due to conformational flexibility of carbohydrates, crystallization of glycoproteins is still a daunting task. In this regard, using smaller models and analogs of the *N*-glycoprotein linkage region would be a valuable approach. A major focus of our laboratory is on the synthesis, X-ray crystallography, and ab initio calculations of the models and analogs of the *N*-glycoprotein linkage region.[3–15] A systematic crystallographic investigation among several *N*-(β-glycopyranosyl)alkanamido derivatives, on the *N*-glycosidic torsion, ϕ_N (O5–C1–N1–C1′) revealed variations up to 31.9° for XylβNHAc from the value observed for the model compound, GlcNAcβNHAc.[6] The ϕ_N value of the propionamido derivative of the xylose differs by 20° from that of the acetamido analog. In the case of fully acetylated XylβNHAc, ϕ_N value (−90.2°) is found to be nearly same as observed for the model compound GlcNAcβNHAc.[11] Further studies on the *N*-(β-D-glycopyranosyl)alkanamides and haloacetamides showed that the *N*-acetyl group at C2 controls the side chain torsion angle χ_2 (N1–C1′–C2′–C3′) at the linkage region and helps in establishing the extended aglycon conformation.[10] Influence of substituents at C2 & C5 and environmental factors mainly inter- and intramolecular interactions involving the hydrogen bonds and C–H···O interactions on the energy preferences of the ϕ_N torsion angle was also examined.[11,12] The present work was initiated with the aim of understanding the conformational preferences of another common pentose, namely, arabinose. Arabinose is found α-linked to hydroxylysine in plant cell wall and attached in β fashion to hydroxyproline in the potato lectin.[16] We report herein the synthesis and X-ray crystallographic investigation of the three *N*-(α-D-arabinopyranosyl)alkanamides, namely, AraαNHAc, AraαNHCOEt, and AraαNHCOCH$_2$Cl (Fig. 1).

2. Results and discussion

2.1. Synthesis of *N*-(α-D-arabinopyranosyl)alkanamides

2,3,4-Tri-*O*-acetyl-α-D-arabinopyranosyl azide (**1**)[17] was chosen as starting material. Azide (**1**) was reduced using Pd/C & hydrogen and acetylated with various acid anhydrides at 0 °C to give the fully acetylated arabinopyranosyl alkanamides (**2**–**4**) in good yield (Scheme 1). De-*O*-acetylation of the fully acetylated compounds

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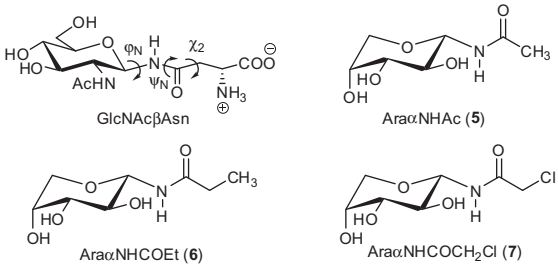


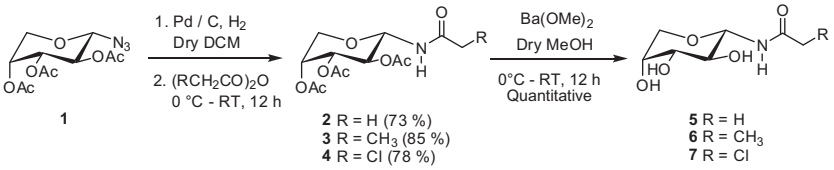
Figure 1. Molecular structures of GlcNAcβAsn and *N*-(α-D-arabinopyranosyl)alkanamides (**5**–**7**).

(**2**–**4**) was done using $Ba(OMe)_2$/MeOH at 0 °C to give the *N*-(α-D-arabinopyranosyl)alkanamides (**5**–**7**) in quantitative yield. These compounds were fully characterized based on the physical and spectral methods including IR, ^1H NMR, ^{13}C NMR & HR-MS. The H-1 proton coupling constant value of ~9.0 Hz confirmed their α-anomeric configuration.

2.2. X-Ray crystallographic investigation

2.2.1. Structure description

Single crystals of the *N*-(α-D-arabinopyranosyl)alkanamides (**5**–**7**) were obtained from aqueous methanol by slow evaporation method at room temperature. All the three compounds, AraαNHAc (**5**), AraαNHCOEt (**6**), and AraαNHCOCH$_2$Cl (**7**) crystallized in $P2_12_12_1$ space group without any solvent molecule in the lattice. The ORTEP representations of their solved structures with atom numbering are shown in Figure 2. The α-anomeric configuration of compounds **5**–**7** is evident from the ORTEP (Fig. 2). The pyranose ring adopts $^1C_4$ conformation in all the three compounds (**5**–**7**) as evident from their puckering parameters (Table 1). List of selected bond lengths and bond angles is provided in Table 2. Geometrical parameters hereafter will be compared among themselves as well as with those already reported for XylβNHAc and XylβNHCOEt. Among the sugar ring C–O distances, there is no general trend observed for the compounds **5**–**7**. In the case of AraαNHAc (**5**), both the bond lengths (C1–O5 and C5–O5) were found to be equal. For AraαNHCOEt (**6**), C1–O5 is longer than the C5–O5 and a similar



Scheme 1. Synthesis of *N*-(α-D-arabinopyranosyl)alkanamides (**5**–**7**).

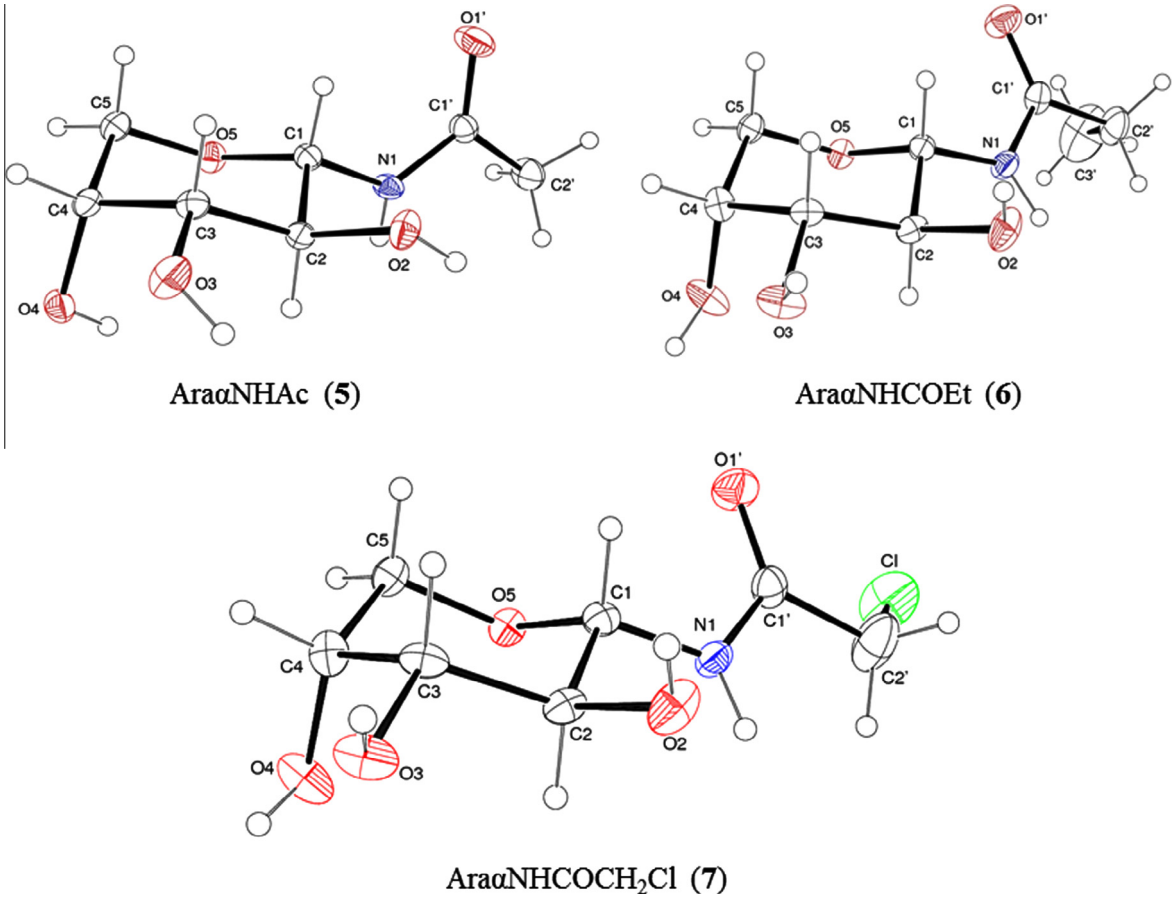


Figure 2. ORTEP representations (probability 30%) of crystal structure of compounds **5**, **6** and **7**.