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Preparation and functional analysis of gossypols having two carbohydrate appendages with enaminooxy linkages

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ABSTRACT

We developed new gossypol (Gos)-based glycoconjugates through dehydration condensation of native Gos and chemically modified glycosides having aminooxy groups. The resultant glycoconjugates (glycoGos) were resistant to hydrolysis, although they were light-sensitive and slowly decomposed even under indoor lighting. The glycoGos also exhibited improved water solubility compared with native Gos, but their saturated concentrations in water were still low (6.4–17 μM), due to their hydrophobic naphthyl rings. We also carried out WST-8 assays to assess the anticancer activity of the glycoGos on DLD-1 and HepG2 cells and found that the glycoGos having β-lactosides and having β-galactosides (specific ligands for asialoglycoprotein receptors) showed enhanced anticancer activity on HepG2 cells.

1. Introduction

Gossypol (Gos), a yellow pigment from cotton plants, exerts antioxidative [1,2], antibacterial [3], and insecticidal activities [4] and it is widely believed to play important roles in biological defense systems in these plants (Chart 1). It has also been reported that Gos has antiproliferative activity against cancer cells [5–8] and antiviral activity against influenza virus [9–11]. All of these bioactivities make Gos attractive as a research target in pharmaceutical/medicinal chemistry. However, the high cytotoxicity and low water solubility of Gos, arising from its two aldehydes and two naphthyl rings, respectively, strongly hinder its clinical applications. Many research groups have attempted to develop chemically modified Gos (Gos derivatives) with amplified bioactivity and reduced cytotoxicity [12]. A series of these works revealed that dehydration condensation between the aldehydes of Gos and various amines (H_2N-R) is the most promising strategy to access Gos derivatives, and such Gos derivatives having imino linkages (-C=N-R) are usually less cytotoxic than native Gos [13–15]. The advantage of dehydration condensation as a synthetic strategy relies on the following. First, it proceeds smoothly in an almost quantitative manner just by mixing the precursors (Gos and the amines). Second, neither catalyst nor additional reagent is required for the reaction. Third, no byproduct except water forms through the reaction. These points are quite advantageous for obtaining pure Gos derivatives without time/labor-consuming purification processes. Considering these advantages, a variety of Gos derivatives have been prepared. For example, Yang et al. reported dehydration condensation between Gos and various dipeptides to afford Gos–peptide conjugates and assessed their anticancer activity [16].

The introduction of carbohydrate units onto a Gos scaffold is also attractive for pharmaceutical applications, since the resultant Gos-based glycoconjugates (glycoGos) would have improved water solubility and cell specificity originating from their carbohydrate appendages. However, to date, only a couple of glycoGos have been reported in the literature [16,17].

One of these preceding works featured glycoGos having D-glucosamine appendages [16]. In this preceding paper, Gos was coupled with D-glucosamine through dehydration condensation, in which aldehydes of the former and an amino group at the C2

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position of the latter reacted with each other to give glycoGos carrying two glucosamine appendages. In this glycoGos, two aldehydes in native Gos were converted into two imino linkages and the resultant glycoGos had reduced cytotoxicity. Its enhanced anti-HIV-1 activity was also reported in this previous paper.

However, this dehydration condensation is still associated with certain problems as a general synthetic strategy to access glycoGos for pharmaceutical use, as follows. (1) Amino groups of carbohydrates are essential for the dehydration condensation and thus carbohydrates without a free amino group (e.g., glucose, galactose, mannose, *N*-acetyl-glucosamine) are not acceptable in this approach. (2) The amino group at the C2 position of D-glucosamine is usually a critical structural motif for binding with its specific lectins; therefore, the glucosamine appendages having imino groups at their C2 position should in most cases have lower affinity for these lectins. (3) In addition, the imino linkages obtained through the dehydration condensation are usually not resistant to hydrolysis (the reverse reaction of the dehydration condensation). The products would thus readily be hydrolyzed to give their precursors (Gos and the amines) under physiological conditions. The resultant Gos liberated from the Gos derivatives would have toxic effects on patients. In this respect, it is highly attractive to design glycoGos with improved water resistance.

Recently, we established an alternative synthetic approach to access glycoGos, in which chemically modified glycosides having aminooxy (-ONH_2) groups at their C1 positions (1-*O*-aminoglycosides) were synthesized and then coupled with Gos to afford new glycoGos, in which two glycosides were attached onto a Gos scaffold with iminooxy (-C=N-O-R) linkages. It is widely recognized by organic chemists that aminooxy groups react with aldehydes in a chemoselective manner to form iminooxy linkages that are robust even in aqueous media [18–21]. Furthermore, these new glycoGos have *O*-linked glycosides and thus the carbohydrate units should retain their inherent affinities towards their specific lectins. We herein report synthetic procedures to access our new glycoGos, their solubility, and their anticancer activity.

2. Results and discussion

2.1. Synthesis of 1-*O*-aminoglycosides

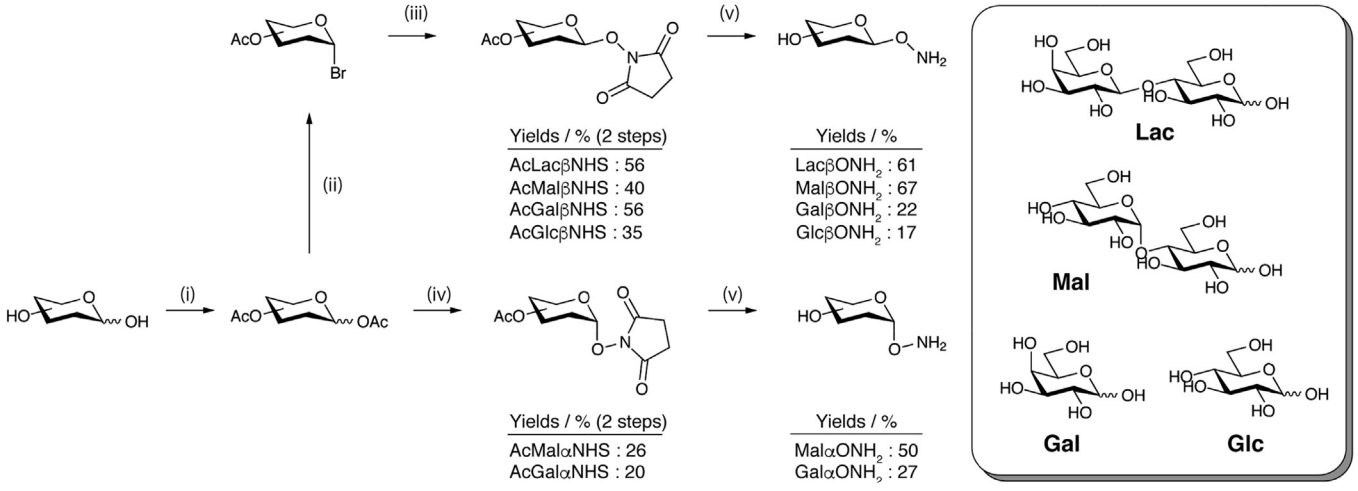
The 1-*O*-aminoglycosides were obtained through synthetic routes reported in the literature (Scheme 1) [22]. In the case of lactose (Lac), for example, commercially available Lac monohydrate was acetylated with acetic anhydride and pyridine, and the resultant *per*-acetylated lactose (AcLac) was then brominated with HBr [23]. The resultant lactosyl bromide (AcLacBr) was glycosylated with *N*-hydroxysuccinimide (NHS) in a biphasic solvent system containing Na_2CO_3 and tetrabutylammonium hydrogen sulfate (TBAHS) to afford *per*-acetylated lactoside having β-linked NHS aglycon (AcLacβNHS) at a 56% yield (two steps from AcLac). The other β-linked NHS-glycosides (AcMalβNHS, AcGalβNHS, and AcGlcβNHS) were also obtained through similar synthetic procedures at moderate yields (40%, 56%, and 35%, respectively).

On the other hand, the corresponding glycosides having α-linked NHS aglycons were prepared through alternative routes. For example, in the case of maltose (Mal), AcMal was subjected to glycosylation with NHS in the presence of BF_3OEt_2 to give *per*-acetylated Mal having α-linked NHS aglycon (AcMalαNHS). In this reaction, the product was a mixture containing AcMalαNHS (major product) and AcMalβNHS (minor product). We carried out repeated column purification processes using various solvent systems and finally retrieved AcMalαNHS in its pure form. This purification process greatly deteriorated the yield of this glycosylation step. *Per*-acetylated galactoside having α-linked NHS aglycon (AcGalαNHS) was also obtained through a similar reaction scheme starting from galactose.

With these six α/β-linked NHS-glycosides in hand, we carried out simultaneous removal of the *O*-acetyl and *N*,*N*-succinimidyl groups from these NHS-glycosides by treating them with hydrazine monohydrate to give the corresponding 1-*O*-aminoglycosides.

2.2. Coupling between Gos and 1-*O*-aminoglycosides

The dehydration condensation of Gos with the 1-*O*-aminoglycosides was achieved by mixing the former (1 eq.) and excess amounts of the latter (3 eq.) in dry MeOH and evaporation of the resultant mixtures (Scheme 2). Removal of the unreacted 1-*O*-aminoglycosides from the residue was achieved by washing them with small amounts of cold water to obtain the glycoGos in their pure forms. Successful syntheses of the desired glycoGos were confirmed through electrospray ionization time-of-flight mass (ESI-TOF-MS) spectral measurements. For example, in the case of the dehydration condensation of Gos with 1-*O*-amino-β-lactoside



Scheme 1. Synthesis of 1-*O*-aminoglycosides: i) Ac_2O, Py, rt, 17–43 h, ii) HBr, acetic acid, Ac_2O, CH_2Cl_2, rt, 1.5–2.0 h, iii) NHS, TBAHS, CH_2Cl_2, Na_2CO_3 aq. rt, 8.5–13 h, iv) NHS, BF_3OEt_2, CH_2Cl_2, under N_2, rt, 22–24 h, v) hydrazine monohydrate, dry MeOH, rt, 2–5 h (see Experimental for the detailed reaction time and yield of each carbohydrate derivative).