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**Research Article**

# Preparation of 1,2,3,4,6-penta-*O*-galloyl-[U-$^{14}$C]-D-glucopyranose

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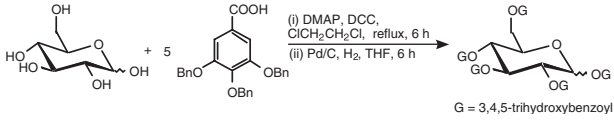
## Summary

The synthesis of 1,2,3,4,6-penta-*O*-galloyl-[U-$^{14}$C]-D-glucopyranose is described. [U-$^{14}$C]glucopyranose was reacted with tri-*O*-benzylgallic acid forming 1,2,3,4,6-penta(tri-*O*-benzylgalloyl)-[U-$^{14}$C]-D-glucopyranose as chromatograpically separable anomers. Removal of the benzyl group by catalytic hydrogenation afforded 1,2,3,4,6-penta-*O*-galloyl-[U-$^{14}$C]-D-glucopyranose in 54% overall yield.






**Key Words:** hydrolyzable tannin; polyphenol; antioxidant; pentagalloylglucose

## Introduction

Hydrolyzable tannins are plant secondary metabolites that are synthesized by a wide variety of plants.[1] Their building blocks are gallic acid and polyols, typically glucose, with ester bonds formed

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between the gallic acid and polyol residues. Hydrolyzable tannins are found in human foods, animal feeds, and medical herbs.[1–4] The fate of hydrolyzable tannin after ingestion is still elusive. The central compound in the biosynthetic pathway for hydrolyzable tannins is 1,2,3,4,6-penta-*O*-galloyl-*β*-D-glucopyranose (PGG), and all the other hydrolyzable tannins with more than five galloyl moieties are derived from it.[1, 5] PGG can bind proteins, inhibit enzymes, and has substantial antioxidant activity.[1–4, 6] Availability of radiolabeled PGG would facilitate studies of protein binding, enzyme inhibition, and antioxidant activities, and would provide a probe for tracing the fate of hydrolyzable tannins after ingestion. *In vivo* incorporation of $^{14}CO_2$ using staghorn sumac leaves has been previously reported for the biosynthesis of [U-$^{14}$C]PGG.[7] We now report adaptation of a previously reported chemical synthesis of PGG to access PGG uniformly [$^{14}$C]-labeled at the hexose.[8] Using chemical synthesis, a higher specific activity radiolabeled product could be obtained versus biochemical methods. Furthermore, modification of our procedure will allow synthesis of structural variants of PGG that are not biosynthetically accessible.

## Results and discussion

In the synthesis of 1,2,3,4,6-penta-*O*-(tri-*O*-benzylgalloyl)-[U-$^{14}$C]-D-glucopyranose (BnPGG, **1**), 1,2-dichloroethane was used as solvent instead of dichloromethane as had previously been reported.[8] This modification reduced the reaction time from 36 to 6 h. Centrifugal thin-layer chromatography (TLC), using a Chromatotron, was an improvement over column chromatography for the purification. Clean separation of the impurities from the products was easily achieved, and less [$^{14}$C]-contaminated solvent waste was produced. The separation of protected PGG anomers could also be achieved using centrifugal TLC with a 2 mm silica plate. In order to do this, the crude sample was first purified and a 20 mg aliquot of the anomeric mixture of **1** was applied to the Chromatotron for resolution of the anomers. After removing the benzyl groups, pure α- and β-[U-$^{14}$C]PGG were obtained (Figure 1). The mass yield and radioactivity recovery from this step were 60% and 55%, respectively.

No purification was required after the catalytic hydrogenation **ii** (Scheme 1). Toluene, the byproduct from the cleavage of the benzyl

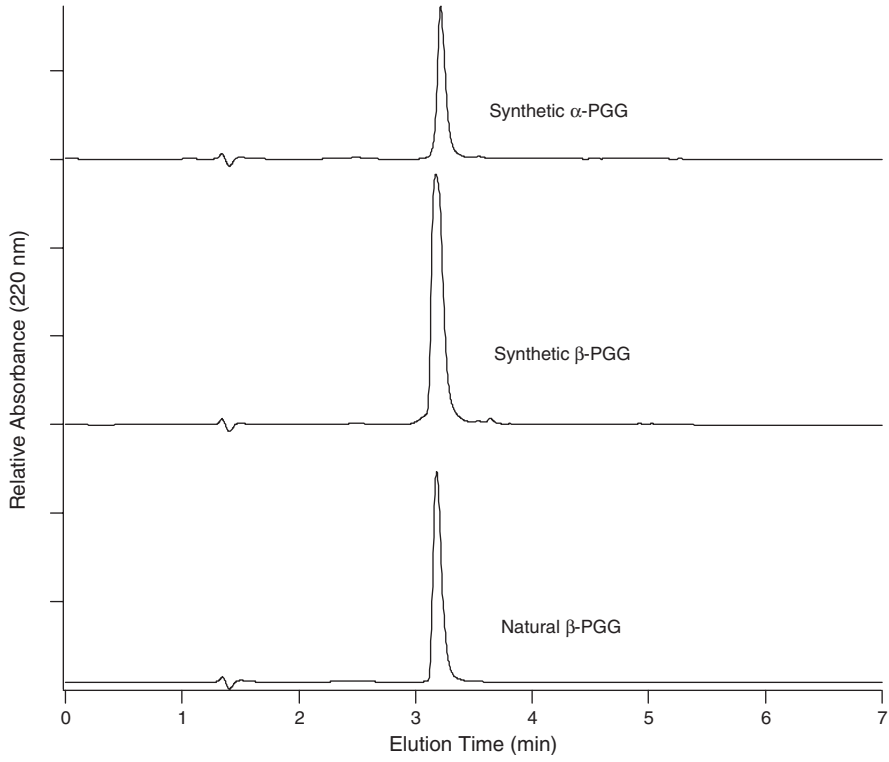
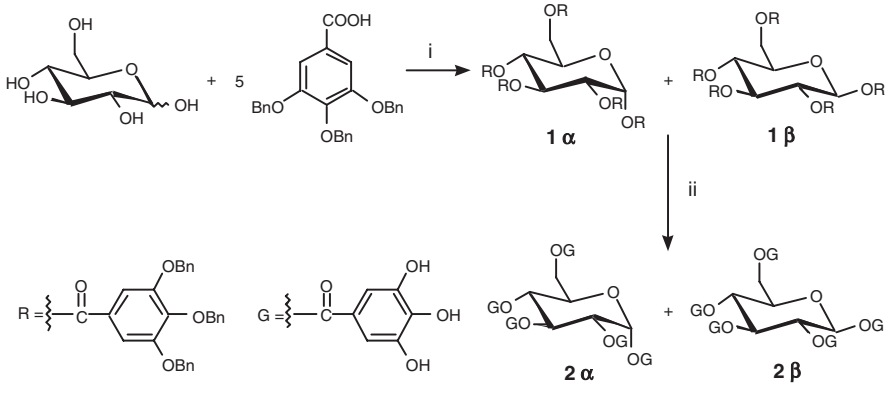



**Figure 1. HPLC of natural PGG, synthetic α- and β-PGG. Between 0.05 and 0.07 g of each sample was injected, resulting in absorbances at 220 nm of 1.6 and 2.7 AU at the peak height**




**Scheme 1. Reagents and conditions: (i) DMAP, DCC, $ClCH_2CH_2Cl$, reflux, 6 h; (ii) $Pd/C, H_2, THF, 6$ h**

groups by hydrogenation, was removed under reduced pressure. A chemical purity of at least 95%, as determined by HPLC, was routinely achieved for the final product **2** (Figure 1). Mass yield and radioactivity recoveries were 90 and 93%, respectively. As unlabeled glucose was added at the outset of the synthesis as a diluent, the specific radioactivity of the final product was 1.19 mCi/mmol, starting with 1 mCi of D-[U-$^{14}$C]glucopyranose (specific activity 4.68 mCi/mmol).

The overall mass yield and radioactivity recovery were 54 and 51%, respectively, providing a direct convenient route to the radiolabeled hydrolyzable tannin **2**.

## Experimental

[U-$^{14}$C]-D-Glucopyranose in an ethanol/$H_2O$ solution (0.1 mCi/ml, 4.68 mCi/mmol) was purchased from Perkin-Elmer Life Science, Inc. (Boston, MA). All other reagents (ACS reagent grade) were purchased from Aldrich (St. Louis, MO). Before use, 1,2-dichloroethane was distilled. All other chemicals were used without further purification.

Radioactivity was quantified with a Wallac 1409 liquid scintillation analyzer (Turku, Finland). UV spectra were taken in acetonitrile solution with an Agilent 8453 UV-visible spectrometer (Agilent Technologies, Palo Alto, CA). The Chromatotron (Harrison Research, CA Model 8924) was equipped with a pump (Model RHSY, Fluid Metering Inc., NY). A Hewlett-Packard 1050 HPLC system with two pumps, an autosampler and UV-detector was equipped with a C-18 column (Adsorbosphere XL C18 90 Å, 3 μm, 100 × 4.6 mm, Alltech, Deerfield, IL). Mobile phase A was water containing 0.1% acetic acid (v/v) and mobile phase B was acetonitrile containing 0.1% acetic acid (v/v). The flow rate was 1 ml/min. The UV detector was set at 220 nm. The gradient started at 5% B, increased to 100% B in 3 min, then returned to 5% B in 3 min, and the column was re-equilibrated for 7 min.

The reaction conditions were optimized using unlabeled reagents. Identities and purities of natural abundance intermediates and products were determined by $^{1}$H and $^{13}$C{$^{1}$H} NMR spectroscopy (Bruker 200 MHz Avance spectrometer), electrospray mass spectrometry (Campus Chemical Instrumentation Laboratory, Department of Chemistry, The Ohio State University), and UV—visible spectra (Figure 2), and were in agreement with previously reported data.[9] The identities of the

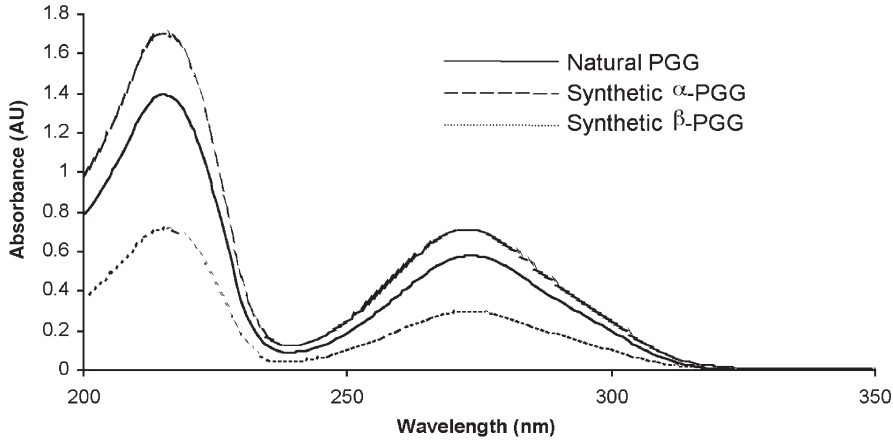



**Figure 2. UV-visible spectra of natural PGG, synthetic α- and β-PGG. Samples were dissolved in MeCN. The concentration was $9 \times 10^{-3}$ g/l for natural PGG, $1.1 \times 10^{-2}$ g/l for synthetic α-PGG, and $4.5 \times 10^{-3}$ g/l for synthetic β-PGG**

[$^{14}$C]-labeled intermediate and final products were established by HPLC co-elution with authentic unlabeled compounds.

## 1,2,3,4,6-Penta-*O*-(tri-*O*-benzylgalloyl)-[U-$^{14}$C]-D-glucopyranose

[U-$^{14}$C]-D-Glucopyranose (0.5 mCi) was transferred into a 50-ml three-necked round-bottom flask. The solvent was removed under reduced pressure using a rotary evaporator followed by azeotropic elimination of residual water using benzene. Anhydrous D-(+)-glucose (31 mg) was added to provide a total of 50 mg glucose (0.28 mmol, 1.0 eq). Tri-*O*-benzylgallic acid (0.915 g, 2.08 mmol, 7.5 eq),[10] 1,3-dicyclohexylcarbodimide (DCC, 0.46 g, 2.22 mmol, 8 eq), and 4-(*N*,*N*-dimethylamino)-pyridine (DMAP, 0.270 g, 2.22 mmol, 8 eq) were added. After the addition of 1,2-dichloroethane (15 ml), the reactants were stirred and refluxed for 6 h under an $N_2$ atmosphere. The reaction mixture was cooled to room temperature, diethyl ether (15 ml) was added, and the mixture was incubated in ice-bath for 1 h. The suspension was vacuum filtered through a Celite pad, and the filtrate was washed with water ($2 \times 30$ ml), saturated $NaHCO_3$ solution ($3 \times 30$ ml) and brine (30 ml). The organic phase was dried over $Na_2SO_4$ and the solvent was removed using a rotary evaporator under reduced pressure. The resulting residue was dissolved in $CH_2Cl_2$ (8 ml) and insoluble impurities were removed

by filtering through a sintered glass funnel. The filtrate was loaded onto the Chromatotron (2 mm plate) and eluted with $CH_2Cl_2$ (7 ml/min). When the first short-wavelength UV-active band eluted, the eluent composition was shifted to $CH_2Cl_2$:acetone (200:1). The second and third bands were collected. Following solvent removal, 0.40 g (60%) of the anomeric mixture of **1** was obtained as an off-white foam. Separation of 20 mg of the anomeric mixture was routinely achieved by a second Chromatotron separation using the same conditions. After purification, washing the Chromatotron plate with 200 ml methanol removed all impurities on the plate.

## 1,2,3,4,6-Penta-*O*-galloyl-[U-$^{14}$C]-D-glucopyranose

In a 50-ml round-bottom flask, **1** (anomeric mixture, 0.40 g, 0.23 mmol) and 10% Pd/C (1.04 g) were suspended in dry THF (10 ml) and the apparatus was flushed with $N_2$ thrice. An $H_2$ atmosphere, provided with a balloon, was attached to the flask. After 6 h, the reaction mixture was flushed with $N_2$ and acetone (5 ml) was added. After vacuum filtration through a Celite pad, evaporation of solvent with a rotary evaporator yielded an anomeric mixture of 1,2,3,4,6-penta-*O*-galloyl-[U-$^{14}$C]-D-glucopyranose (0.36 g, 90%, 1.19 mCi/mmol). An identical procedure was successful with the individual isomers.

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