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Synthesis of polyunsaturated fatty acid-containing glucuronosyldiacylglycerol through direct glycosylation

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ABSTRACT

We describe a total synthesis of a polyunsaturated fatty acid (PUFA)-containing glucuronosyldiacylglycerol (GlcADG), which is a surrogate glycolipid whose synthesis is remarkably upregulated in plant membranes under phosphorus-depleted conditions. Glycosylation between the glucuronide donor bearing 3,4-dimethoxybenzyl (DMPM) protecting groups and di-acylglycerol acceptor proceeded smoothly in the presence of gold(I) catalyst to provide the protected α-isomer of GlcADG as the major product.

Glucuronosyldiacylglycerols (GlcADGs), which are characterized by a 1,2-diacyl-*sn*-glycerol with glucuronic acid at the *sn*-3 position, are unusual acidic glycolipids found in algae,[1] bacteria[2,3] and higher plants such as *Arabidopsis thaliana*,[4] tomato, and soybean.[5] These non-phosphorus glycolipids serve as surrogates for phospholipids during lipid remodeling in phototrophic organisms under conditions of phosphorus depletion stress.[4] Because of the low natural abundance of GlcADGs, as well as lack of commercially available GlcADGs or other related molecules, their functions are poorly understood. Therefore, there is a need for chemical synthesis to provide sufficient quantities of pure glycolipids for further studies.

Two groups have recently reported syntheses of GlcADG or related molecules. Williams and co-workers reported the first synthesis of GlcADG **1** bearing two different acyl groups, (*R*)-tuberculostearic acid ($C_{19:0}$) and palmitic acid ($C_{16:0}$); this compound had been isolated from *Mycobacteria* and *Corynebacteria* (Fig. 1A).[6] They first generated the 1,2-*cis*(α)-glycosidic linkage by glycosylation of glucosyl iodide donor with orthogonally protected glycerol to afford GlcDG **3**. Oxidation of 6-OH to afford the glucuronate, followed by stepwise introduction of acyl groups provided tetra-benzyl-protected GlcADG **2**, which was finally converted to **1** by hydrogenolysis. Colombo and co-workers synthesized GlcADG analogues bearing a glucuronate moiety connected to the *sn*-2 position of glycerol via a 1,2-*trans*(β)-glycosidic linkage[7] by means of indirect synthetic methodologies similar to those used by Williams' group.

These strategies provided the target molecules with high stereoselectivity, but are unsuitable for efficient synthesis of GlcADG containing polyunsaturated fatty acids (PUFAs), such as compound **4**[4] (Fig. 1B). PUFAs, especially α-linolenic acid (α-Lin, 18:3(n-3)), are widely distributed in the glycolipids of plants.[8] However, to our knowledge, chemical synthesis of GlcADG bearing a PUFA has not been reported so far. We speculated that, in contrast to GlcADG derivatives with saturated fatty acids, which would be compatible with oxidative conditions and hydrogenation, it would be possible to synthesize compound **4** by direct glycosylation reaction between protected GlcA donor **5** and diacylglycerol acceptor **6** bearing α-Lin at a late stage. This simple idea should be feasible, if the reactivity of both units is sufficient. The oxocarbenium ion is a key intermediate during glycosylation, and is generated upon departure of the anomeric leaving group assisted by an activator (promoter or catalyst) (Fig. 1C). The electron-withdrawing character of the 6-carboxyl group in the glucuronosyl donor could

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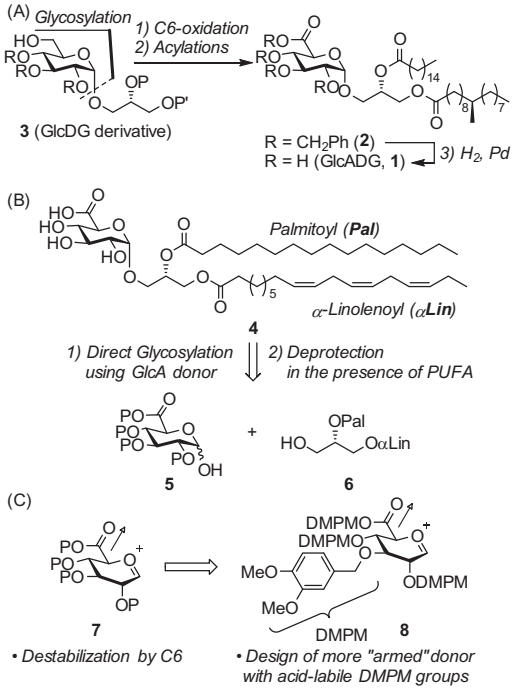
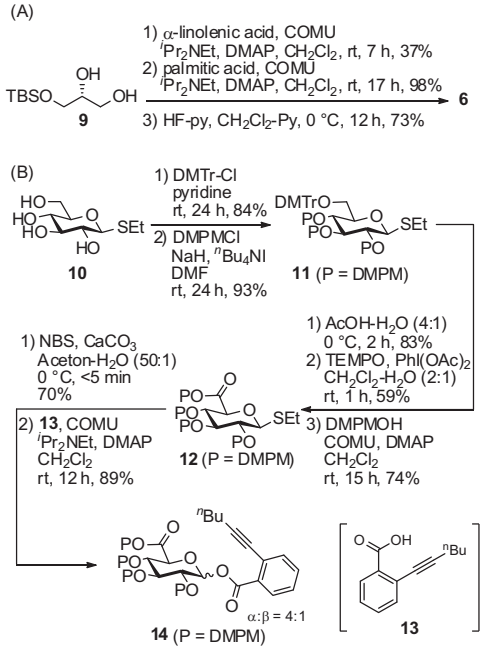


Fig. 1. A) Synthesis of GlcADG **1** bearing saturated fatty acids as reported by Williams et al. see Ref. 6; B) Structure of target GlcAGD **4** bearing a PUFA moiety, and synthetic plan for direct glycosylation using GlcA donor **5** and the diaylglycerol acceptor **6**; C) Design of DMPM-protected glucuronic acid donor **8** to realize direct synthesis of **4**.



Scheme 1. Synthesis of acceptor **6** and donors **12** and **14**.

destabilize the cation, potentially making direct glycosylation problematic. Although a β-linkage can be readily accessed by virtue of neighboring group assistance, the formation of an α-linkage is normally more challenging, despite several reports on the glycosylation of GlcA donor.[9] On the other hand, the nucleophilicity of diacylglycerol acceptor **6** might be poor due to the neighboring electron-withdrawing acyl groups. In this work, we selected 3,4-dimethoxybenzyl (DMPM) protecting groups for the GlcA donor **5** (Fig. 1C), in order to increase the stability of the intermediate **8** as well as to assist the formation of the α-glycoside. Considering the vulnerability of the PUFA moiety,[10] the DMPM group is also favorable as it can be removed under mild conditions.

The synthesis began with preparation of **6** (Scheme 1A). Compound **9** bearing a *tert*-butyldimethylsilyl (TBS) group at the *sn*-3 position was generated from commercially available (*S*)-2,2-dimethyl-1,3-dioxolane-4-methanol in two steps according to a literature procedure.[11] α-Linolenic acid and palmitic acid were then introduced at the *sn*-1 and *sn*-2 positions of glycerol in sequence, using COMU[12] as a condensation reagent. Removal of the TBS group gave the target diacylglycerol **6**, which gradually underwent acyl migration to the corresponding 1,3-diacylglycerol. The optical purity of **6** was confirmed to be >95% ee by condensation with optically pure carboxylic acid[13] (Fig. S1). Namely, the NMR spectrum of the corresponding Mosher ester confirmed that the configuration of the glycerol had been maintained during the conversions.

Given the instability of the PUFA moiety and the acid-lability of the DMPM group, we first employed thioglucuronide **12** as a donor, since it can be activated under mild conditions (Scheme 1B).[14] Selective protection of 6-OH in thioglucoside **10** with a 4,4′-dimethoxytrityl (DMTr) group followed by full protection of the remaining hydroxyl groups with DMPM groups gave **11** in good yield. After acid hydrolysis of the DMTr group in 80% AcOH/H_2O solution,[15] oxidation of the liberated hydroxyl group with 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO) and bis(acetoxy)iodobenzene[9a] afforded glucuronic acid, which was converted to thioglucuronide **12** by esterification in the presence of COMU (1-[(1-(Cyano-2-ethoxy-2-oxoethylideneaminooxy)-dimethylamino-morpholinomethylene)]methanaminium hexafluorophosphate).

Glycosylation of **6** and thioglycoside **12** activated by methyl triflate (MeOTf) resulted in the formation of protected glycolipid **15** in 31% yield with 1.5:1 α:β stereo-selectivity, judged from the ^{1}H NMR spectrum of the crude material (Scheme 2A). Although glycosylation between poor GlcA donor **12** and acceptor **6** proceeded to some extent, probably aided by the DMPM groups, non-selective glycosidic linkage formation occurred. In the literature on glycosylation of thioglucuronide donors,[9b] α-isomers were formed with excellent selectivity in the case of electron-rich acceptors, and with moderate selectivity (α:β = 4:1) in the case of electron-poor acceptors, indicating that an acceptor with high nucleophilicity appeared to be necessary to obtain the α-glucuronide predominantly. Thus, the poor selectivity in our reaction (α:β = 1.5:1) suggested that the reactivity of diacylglycerol acceptor **6** was too low.

We also speculated that low stereoselectivity might be attributable in part to the effect of methylethylsulfide (MeSEt) generated from donor **12** and MeOTf (Scheme 2B). Then, interaction of nucleophilic MeSEt with the oxonium species would afford an interconvertible mixture of **16α** and **16β** (as sulfonium salts, contact ion pairs, or solvent-separated ion pairs), which would be converted to a mixture of **15α** and **15β** by reaction with acceptor **6**.

This speculation prompted us to investigate gold(I)-catalyzed glycosylation, which have emerged as powerful tools for glycosylation.[16] Gold(I) complexes, such as $Ph_3PAuOTf$ or $Ph_3PAuNTf_2$, would activate glycosyl *ortho*-alkynylbenzoates donor **14** without affecting acid-labile DMPM group. We expected that the resulting gold-isocoumarin species **19** would not exert negative impact to the stereo-selectivity, unlike MeSEt shown above, and that gold-catalyzed glycosylation with less reactive acceptor like **6** would proceed through S_N1-like reaction pathway (Scheme 3B).[16b]

In pursuing the synthesis of **14**, thioglucuronide **12** was oxidatively hydrolyzed with *N*-bromosuccinimide (NBS) in acetone-H_2O mixture (Scheme 1). Careful optimization was necessary to reduce the formation of by-products, such as sulfoxide. We found that