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An efficient and versatile chemical synthesis of bioactive glyco-glycerolipids

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ABSTRACT

Synthesis of β-glyco-1,2-diacylglycerols is achieved by a versatile and simple procedure based on trichloro-acetimidate methodology and use of peracetate sugar substrates. The chemical strategy was tested through stereoselective preparation of β-galacto- and β-gluco-lipid derivatives capable to trigger immune system response. The synthetic approach is designed to obtain enantiomerically pure regio- and stereo-isomers including derivatives containing poly-unsaturated fatty acids.

Glycoglycerolipids are ubiquitous structural and signaling molecules of living organisms.[1–3] Within the family, mono- (MGDG) and di- (DGDG) galactosyl diacylglycerols represent 70–80% of membrane lipids in photosynthetic cells[4] and comprise the most abundant lipid class in the biosphere. In the last years, natural and synthetic analogs of MGDGs and DGDGs have attracted the interest of the bio-medical community for the biological properties including antiviral,[5] anti-tumor,[6] anti-inflammatory, and immunosuppressive activities.[7] In particular, innate immune response triggered by glycolipids is emerging as a key process against bacterial and viral infections, as well as tumor immunosurveillance and autoimmunity.[8] Immunogenic properties of lipids depend on the structural characteristics of the molecule. Very recently, Kinjo et al. showed that recognition of MGDG and DGDG by natural killer T cells is strictly dependent on fatty acid composition of the diacylglycerol residue.[9] The high specificity of this process requires accurate purification of the antigenic lipids and, for this reason, the synthetic preparation is regarded as a crucial step for addressing evaluation and development of these immune-modulatory lipids.

Here we discuss an improved and versatile strategy for the synthesis of β-galactosyl- and β-glucosyl-mono- and di-acylglycerols containing both saturated and unsaturated fatty acids.

As shown in Fig. 1, the synthesis starts with acetylation of the sugar unit, followed by selective deacetylation of the anomeric hydroxyl group with benzylamine (compounds **2** and **13**). The coupling with 1,2-*O*-isopropylidene glycerol by trichloroacetimidate methodology[10] gave galactose- (**4**) and glucose- (**15**) derivatives in good yields (about 81% and 82%, respectively). Acetate group at C-2′ of the sugars oriented the equatorial attack with very high stereoselectivity, as proved by ^{1}H NMR analysis that showed only signals for the β-isomers (δ = 4.51, J = 7.7 Hz, H-1′). The two-step strategy proved to be more efficient than the traditional Koenigs–Knorr conditions[11] that are generally employed for the synthesis of glycolipids from acetate-protected pyranose sugars.[12,13] Diols **5** and **16** were quantitatively obtained through selective hydrolysis of the isopropylidene intermediates by zinc nitrate hexahydrate in acetonitrile.[14] DCC-mediated condensation with two or one equivalents of free fatty acids (i.e., linolenic or stearic) yielded alternatively di- and mono-acyl derivatives **6** and **8**, respectively. Notably, equimolar amounts of reagents gave only esterification at C-1′, as indicated by NMR spectra (e.g., **8**) that showed deshielding of H_2-1 signals at δ 4.10, long range correlation of H_2-1 to the carboxylic ester at δ 173.3, and resonances of H-2 at 3.86 ppm. Although esterification of the secondary hydroxyl group of glycerol has been sometimes reported,[15] 2-acyl-glycerol derivatives were not observed under these experimental conditions. The procedure allows step-wise introduction of different acyl residues at C-1 and C-2 of glycerol, thus permitting to control fatty acid regiospecificity of the synthetic glycolipid (e.g., **10** and **10′**). Selective removal of acetyl groups on the sugar unit with hydrazine hydrate led to β-galactosyl-diacylglycerols (**7** and **11**) and β-galactosyl-monoacylglycerols (**9**). Usually this reaction cannot be applied to unsaturated substrates because of partial hydrogenation of double bonds,[12,16–18] but we found that this drawback could be efficiently solved (75–88% yield for the linolenoyl derivatives **7**, **11**, **18,** and **22**) by reducing the amount of hydrazine (2.4 mol per acetate unit) and keeping the temperature reaction below 45 °C.

In conclusion, access to natural and synthetic analogs of β-glyco-1,2-diacyl-glycerols was achieved by a versatile and simple

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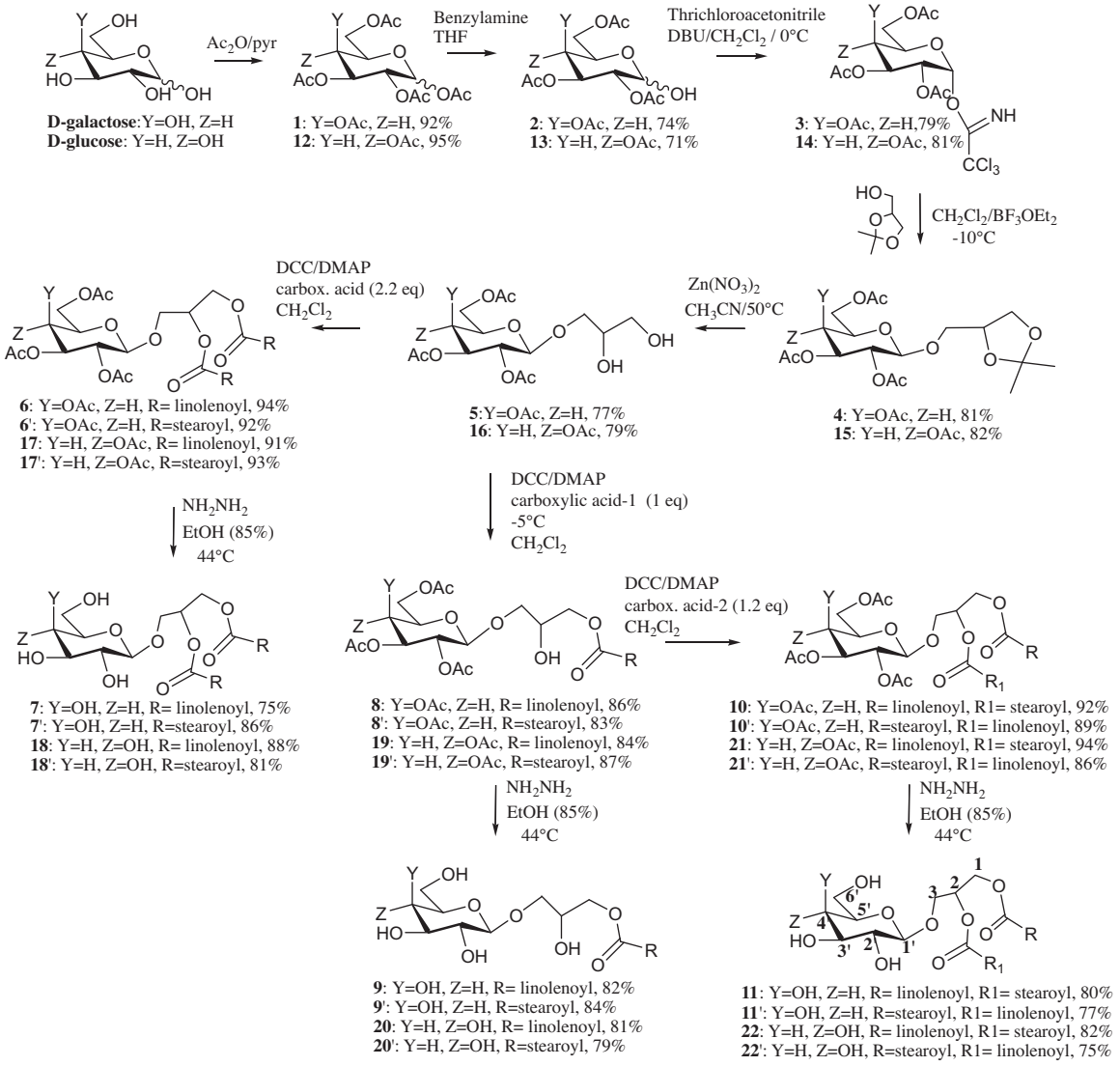

Figure 1. Synthesis of β-galactosyl- and β-glucosyl-1,2-mono- and di-acylglycerols.

chemical synthesis involving stereoselective glycosylation of trichloroacetimidate-sugar donor and 1,2-*O*-isopropylidene glycerol acceptor. Unlike other methods based on benzyl-derivatives,[19,20] the use of acetate as protecting group of the sugar units allows the synthesis of derivatives containing poly-unsaturated fatty acids (e.g., **7** and **11**) in very high yields. The synthetic strategy is also more convenient than methods based on acetobromo derivatives of α-D-galactose or α-D-glucose, which suffers for high toxicity and low stability of bromo glycosides, as well as for the high costs of the promoters associated to the Koenigs–Knorr reaction.[12,13,21] Furthermore, in addition to the simplification of the synthetic steps, the use of acetate determines at very high extent the stereoselectivity of the coupling reaction that leads exclusively to beta-anomers.

The sequence of reactions is of general application and gives access to regio- and stereo-pure compounds. Enantiopure 1,2-*O*-isopropylidene glycerols were not tested in this work, but their use is compatible with the trichloroacetimidate/etherate boron trifluoride coupling step that, unlike Koenigs–Knorr and galactosyl-bromide procedures,[13b,22] avoid epimerization at C-2 of these chiral substrates.[19] We are currently applying the above synthesis for the preparation of natural variants of glycolipid binders to CD1 receptors of natural killer T cells.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.12.030.

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