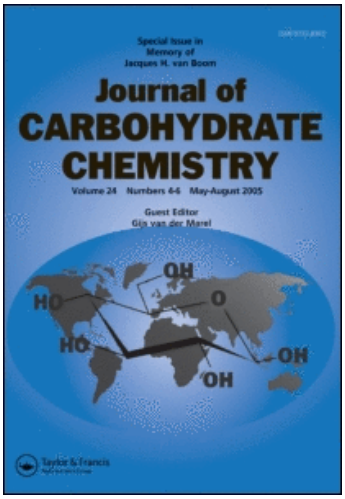

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A Highly Sensitive HPLC Method to Determine the Absolute Configuration of Glycosyl Diacylglycerols Using a Fluorescent Chiral Derivatizing Reagent

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COMMUNICATION

A HIGHLY SENSITIVE HPLC METHOD TO DETERMINE THE ABSOLUTE CONFIGURATION OF GLYCOSYL DIACYLGLYCEROLS USING A FLUORESCENT CHIRAL DERIVATIZING REAGENT

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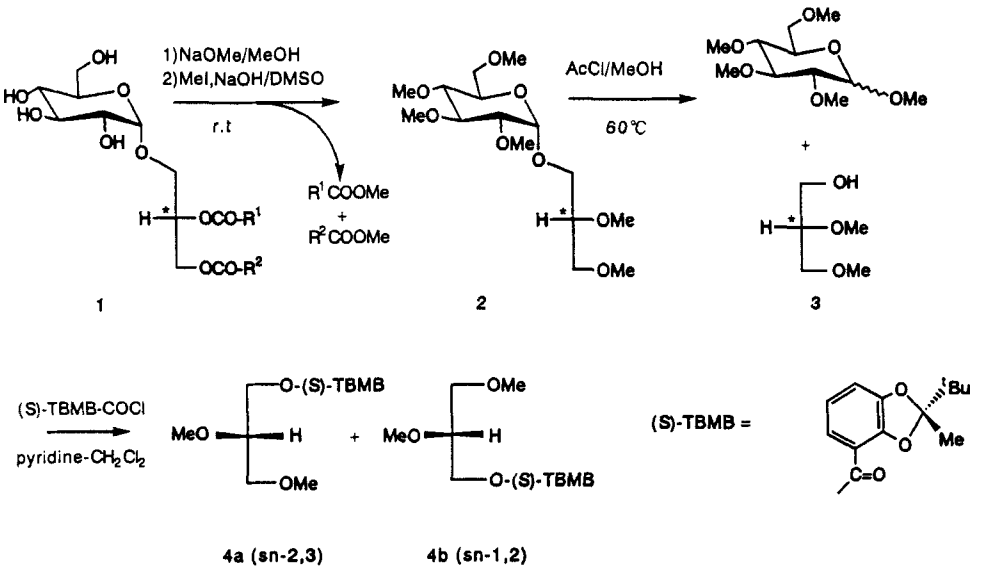
Structural elucidations of naturally occurring glycosyl diacylglycerols have been hampered by the difficulty of the assignment of the absolute configuration at the glycerol moiety. Although several approaches towards solving this problem have been proposed,[1-6] they were not satisfactory in their simplicity or sensitivity. Hence, the absolute configurations have been usually determined through total syntheses starting from chiral glycerols with known configurations.[7-9] Previously,[10] we have developed a fluorescent chiral derivatizing agent, (*S*)-(+)-2-*tert*-butyl-2-methyl-1,3-benzodioxole-4-carboxylic acid [(*S*)-TBMB carboxylic acid, **Scheme**]. This agent or its ester having strong fluorescence with the maximum emission wavelength at ca. 380 nm and the excitation maximum at ca. 310 nm has provided useful ways to discriminate D,L-isomers of amino acids,[10] monosaccharides,[11] and diacylglycerols.[12] In this paper, we report a highly sensitive HPLC way to determine the absolute configuration of glycosyl diacylglycerols using (*S*)-TBMB carboxylic acid.



Scheme

Our strategy involves chemical degradation of glycosyl diacylglycerol **1** into chiral di-*O*-methylglycerol **3** (**Scheme**); deacylation of **1** with sodium methoxide in MeOH to remove fatty acid components as the methyl esters followed by per-*O*-methylation with methyl iodide and NaOH in dimethyl sulfoxide,[13] and cleavage of the glycosidic linkage with HCl in MeOH afforded the per-*O*-methylated sugar and chiral 1,2- (or 2,3-) di-*O*-methyl-sn-glycerol **3**. Then, the glycerol **3** was esterified with (*S*)-TBMB carbonyl chloride[12] to give either **4a** or **4b** according to the configuration of the mother glycosyl diacylglycerol. Finally, **4a** or **4b** as a diastereomer was subjected to HPLC analysis.

Preliminary HPLC studies indicated that **4a** and **4b** could be well separated in 35 minutes by using a silica gel HPLC column and eluting with *tert*-BuOH and *n*-hexane (1:100, w/w) (**Figure**). By comparison with the authentic sample of **4a** prepared from commercially available chiral 1,2-*O*-isopropylidene-sn-glycerol, the first and the second HPLC peaks could be assigned to **4a** and **4b**, respectively.

Being encouraged by the above HPLC study, we employed the strategy illustrated in the Scheme for the present purpose. The reaction conditions for preparing **4a** and **4b** from glycosyl diacylglycerols were determined as described below using the synthetic models 3-

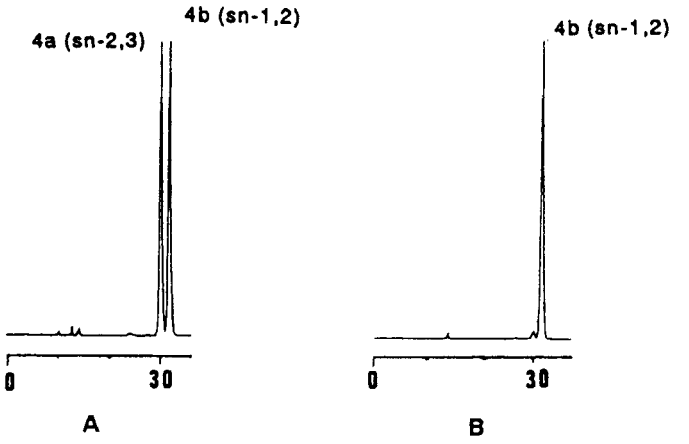


Figure. HPLC profile of **4a** and **4b** under normal-phase conditions. HPLC conditions: silica gel column [Develosil 60-3 (NOMURA CHEMICAL), 25 cm × 4.6 mm, I.D.], elution by *n*-hexane:*tert*-BuOH (100:1, w/w), flow rate=1 mL/min, fluorescence detection at 370 nm (emission) and 310 nm (excitation).

O-(α- and β-D-glucopyranosyl)-1,2-di-*O*-palmitoyl-sn-glycerols prepared from chiral glycerols by reported methods.[9,14]

i) Typical Procedure to Derive Chiral Glycerol 3 from Glycosyl Diacylglycerol 1.

A mixture of glycosyl diacylglycerol (0.01-5 mg) and sodium methoxide (5 mg) in dry MeOH (0.5 ml) was stirred in a reaction vial with a screw cap for 30 min. The mixture was extracted with *n*-hexane (1 mL) and the MeOH phase was evaporated. The residue was treated with ca. 5 mg of NaOH (powdered), methyl iodide (0.3 mL) and 1 mL of dimethyl sulfoxide at room temperature. After 30 min, the solution was diluted with 1 mL of aqueous NaCl (saturated) and extracted with 2 mL of chloroform. The organic layer was washed with water (1 mL × 3) and co-concentrated with toluene (1 mL × 3) to dryness. The residue was treated with 2 mL of 15-20% acetyl chloride in MeOH and the solution warmed at 60 ℃ for 4 h in the reaction vial. The above solution was diluted with MeOH (1 mL) and transferred to a round flask and co-concentrated with toluene (1 mL × 3) to dryness.

ii) Derivatization of 3 with (S)-TBMB-COCl and Preparation of HPLC Samples.

The residue was dissolved in 1 mL of dichloromethane-pyridine (1:1) and 1 mg of *N,N*-dimethylaminopyridine and (*S*)-TBMB carbonyl chloride (1-5 mg) were added. The mixture was stirred for 2-3 h. A small aliquot of the reaction solution was adsorbed onto a thin layer silica gel plate[15] (Merck, DC-Alufolien Kieselgel 60 F_{254}) and developed with *n*-hexane:ethyl acetate (3:1). The light blue fluorescent spot of **4a** and **4b** observed under a UV lamp (Rf = ca. 0.5) was extracted with *tert*-BuOH:*n*-hexane (1:50), and the extract (10 μl) was applied for HPLC analysis under the conditions detailed in the **Figure**.

A test analysis using 3-*O*-β-D-glucopyranosyl-1,2-di-*O*-palmitoyl-sn-glycerol on a preparative scale (10 mg) indicated that each reaction in the **Scheme** proceeded almost quantitatively to afford **4b** totally in more than 80 % yield. Owing to the strong fluorescence of (*S*)-TBMB carboxylic acid, **4a** and **4b** could be detected at a few picomol amount. Thus, a new approach based on the (*S*)-TBMB derivatization and the HPLC separation was proposed to determine the absolute configuration of glycosyl diacylglycerols in a highly sensitive manner. Application of this method to natural products is in progress in our group and will be reported elsewhere.

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15. The TLC purification of **4a** and **4b** prior to the HPLC injection was recommended in oder not only to eliminate reagent peaks but also to remove pyridine and the salts which are not favorable using the silica gel HPLC column.