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Synthesis of fluorescent lactosylceramide stereoisomers

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Abstract

The intracellular distribution of synthetic glycosphingolipids (GSLs) bearing a fluorophore can be monitored in living cells by fluorescence microscopy. We reported previously that variation in the length of the long-chain base and in the structure of the carbohydrate-containing polar head group of (2*S*,3*R*) (or D-*erythro*-)-β-lactosylceramide (LacCer) did not alter the mechanism of endocytic uptake from the plasma membrane of various mammalian cell types [Singh, R.D., Puri, V., Valiyaveettil, J.T., Marks, D.L., Bittman, R., Pagano, R.E., 2003. Selective caveolin-1-dependent endocytosis of glycosphingolipids. Mol. Biol. Cell 14, 3254–3265]. To extend our examination of the molecular features in LacCer that are responsible for its uptake by the caveolar-requiring endocytic pathway, we have synthesized the three unnatural stereoisomers [(2*R*,3*R*)-, (2*S*,3*S*)-, and (2*R*,3*S*)] of dipyrromethene difluoride (BODIPY^{TM})-LacCer. These analogues will be used to probe the role of stereochemistry in the long-chain base of LacCer in the mechanism of endocytic uptake.

Keywords: Fluorescent lipid analogues; Glycosphingolipids; Lipid synthesis

1. Introduction

A boron dipyrromethene difluoride (BODIPY^{TM}) (Johnson et al., 1991) fluorophore linked to the long-chain base of naturally occurring (2*S*,3*R*)-β-lactosylceramide (LacCer) via the ω end of a *N*-pentanoyl moiety (compound **a** in Fig. 1) has been used to examine the intracellular trafficking of this and other glycosphingolipids (GSLs) in normal and disease cell types (Pagano et al., 2000). This GSL was localized in lysosomes of a diseased cell type, but was observed at the Golgi complex in normal fibroblasts (Chen et al., 1998). (2*S*,3*R*)-C_5-BODIPY^{TM}-LacCer (which is available commercially) and a synthetic analogue bearing a maltosyl polar head group (2*S*,3*R*)-C_5-BODIPY^{TM}-MalCer, utilized the same caveolar-dependent endocytic pathway for uptake from the plasma membrane of different cells (Singh et al., 2003; Bittman, 2004). In contrast, BODIPY^{TM}-sphingomyelin utilizes both a clathrin-dependent and a caveolar-dependent pathway in approximately equal extents for internalization (Puri et al., 2001). To examine the role of stereochemistry at C2 and C3 of the sphingosine chain of LacCer in determining the mechanism of endocytosis, we have prepared the following unnatural stereoisomeric analogues: (2*R*,3*R*)-, (2*S*,3*S*)-, and (2*R*,3*S*)-BODIPY^{TM}-LacCer (compounds **b**–**d** in Fig. 1).

2. Experimental

2.1. Materials and analytical procedures

2.1.1. Chemicals

The sources of the chemicals were as follows: BODIPY^{TM}-C_5-*N*-hydroxysuccinimidoyl (NHS) ester,

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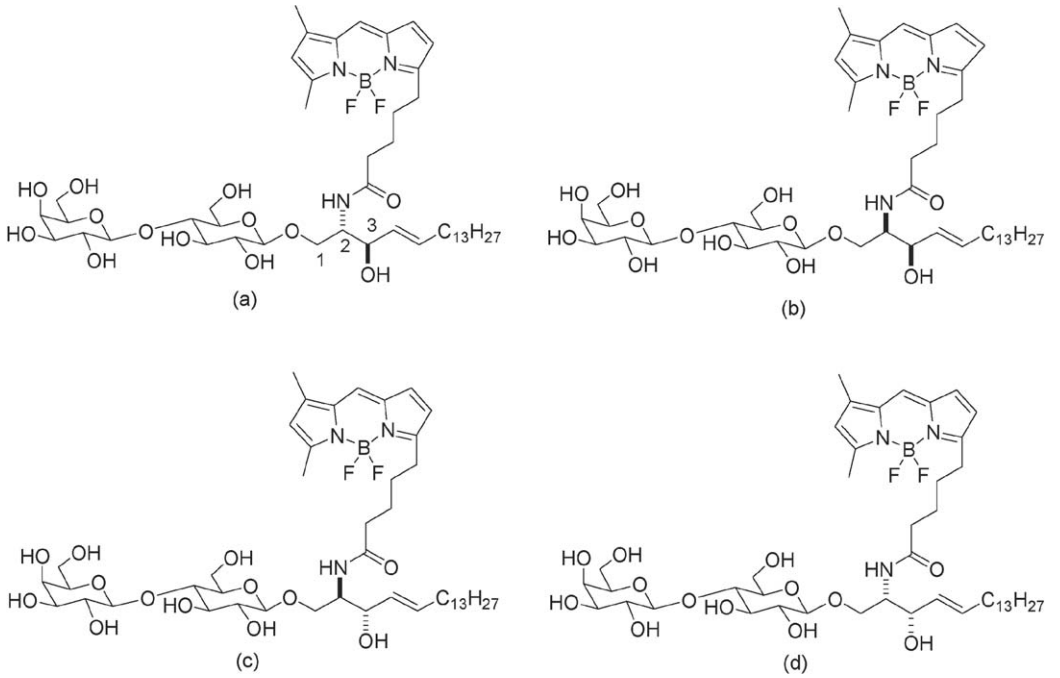

Fig. 1. Structures of (a) (2*S*,3*R*) (or D-*erythro*); (b) (2*R*,3*R*) (or D-*threo*); (c) (2*R*,3*S*) (or L-*erythro*); and (d) (2*S*,3*S*) (or L-*threo*)-BODIPY^{TM}-LacCer.

Invitrogen/Molecular Probes (Eugene, OR); *N*-Boc-D-serine and diisobutylaluminum hydride (DIBAL-H, a 20 wt.% solution in toluene), Acros (Morris Plains, NJ); L-*threo*-sphingosine, Avanti Polar Lipids (Alabaster, AL); 1-pentadecyne, *p*-toluenesulfonic acid monohydrate (*p*-TsOH), and sodium bis(2-methoxyethoxy)aluminum hydride (Red-Al, a 70%, w/w solution in toluene), Alfa Aesar/Lancaster (Pelham, NH); β-D-lactosyl octaacetate, triphenylphosphine, trichloroacetonitrile, *tert*-butyldiphenylsilyl chloride (TBDPSCl), hydrazine acetate, benzoic anhydride, $BF_3{\cdot}OEt_2$, imidazole, 4-(dimethylamino)pyridine (DMAP), and $(n\text{-Bu})_4NF$ (TBAF), Sigma–Aldrich. Trifluoromethanesulfonyl azide (TfN_3) was prepared according to Vasella et al. (1991). Hepta-*O*-acetyllactosyl-1-trichloroacetimidate (compound **13**) was synthesized from per-*O*-acetyllactose as described (Amvam-Zollo and Sinay, 1986). Molecular sieves (300AW) were dried for 5 h at 150 °C and stored under vacuum over P_2O_5.

2.1.2. General methods

Air- and moisture-sensitive reactions were carried out under nitrogen in flame-dried glassware. THF and toluene were distilled from sodium/benzophenone and dichloromethane was distilled from calcium hydride prior to use. DMF was dried over calcium hydride. TLC was performed using aluminum-backed or glass-backed silica gel 60 F254 plates (0.25-mm thick), and the compounds were visualized by charring with 10% H_2SO_4 in EtOH or by UV light. Column chromatography was carried out with silica gel 60 (230–400 mesh) using the elution solvents indicated in the text. Suspended silica gel was removed by filtration through an Osmonics Cameo filter (Fisher Scientific, Pittsburgh, PA). The ^1H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, and were referenced to the residual $CHCl_3$ at δ 7.24 (^1H) and the central line of $CDCl_3$ at δ 77.0 ppm (^{13}C). Optical rotations were measured on a digital polarimeter at room temperature in the solvents stated.

2.2. Synthesis

2.2.1. *N*-[(1,1-Dimethylethoxy)carbonyl]-D-serine methyl ester (**2**)

To a cold solution of *N*-Boc-D-serine (compound **1** in Scheme 1, 3.0 g, 14.6 mmol) in DMF (20 ml) was added potassium carbonate (2.28 g, 16.5 mmol). After the mixture was stirred for 10 min in an ice-water bath, methyl iodide (1.88 ml, 4.26 g, 30 mmol) was added to the white suspension, and stirring was continued at 0 °C for 30 min, whereupon the mixture solidified. The reaction mixture was warmed to room temperature and stirred for an additional hour. The reaction mixture was filtered by suction and the filtrate was partitioned between EtOAc (30 ml) and water (30 ml). The organic phase was washed with brine (2 × 30 ml), dried (Na_2SO_4), filtered, and con-