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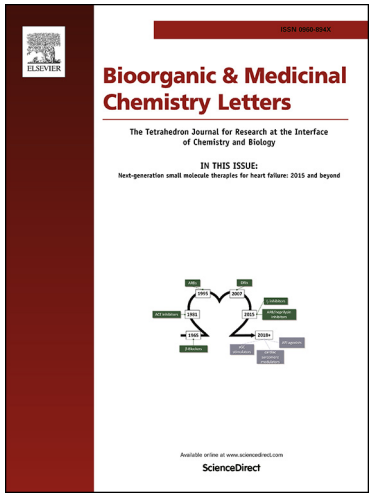

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Development of a New Doubly-Labeled Fluorescent Ceramide Probe for Monitoring the Metabolism of Sphingolipids in Living Cells

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Abstract
A new ceramide analog, **1**, containing two fluorescent dyes, NBD in the *N*-acyl part and KFL5 in the alkyl part, was synthesized. The fluorescence from both NBD and KFL5 was detected in living cells in a time-dependent manner. A multi-wavelength fluorescence detector was used to detect ceramide metabolites including sphingosine, sphingosine-1-phosphate, glucosylceramide, and sphingomyelin, which are connected to the fluorescent dyes, simultaneously in a single TLC plate.

Keywords
Florescent Probe, Ceramide, Sphigolipids, KFL5, NBD

Ceramide (Cer) is a component of sphingolipids and plays a key role in mammalian cells.[1] Cleavage of the *N*-acyl moiety of Cer is a key transformation in the metabolism of sphingolipids and generates sphingosine (Sph), which is also a bioactive molecule that can be converted into various metabolites such as sphingosine-1-phosphate (S1P), an important signaling molecule (Fig. 1).[2] We previously reported the synthesis of a new fluorescent probe, acetyl-C16-ceramide-NBD, which has a core structure of Cer (Fig. 2).[3] This fluorescent ceramide analogue was shown to be a long-term Golgi marker because of its stability in bioconversion compared with the commercially available Golgi marker C6-NBD-ceramide.[4] Here we report a new doubly-labeled fluorescent ceramide analogue **1**, which makes it possible to monitor the dynamic metabolism of Cer in living cells. We also synthesized **2a** and **2b**, singly-labeled fluorescent ceramide probes, to compare their optical properties to those of **1**.

Figure 1. Bioconversion of Ceramide