

## BODIPY-based Fluorescent Labeling Tag for Oligosaccharide and N-Linked Glycan Analysis by High-Performance Liquid Chromatography with Fluorescence Detection

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### Summary

Glycosylation analysis is still challenging, not only because of the extreme structure complexity and conjugation diversity of glycans but also because of instrumental aspects such as the sensitivity limits of analyses. Therefore, glycan analysis by chromatographic methods is very often combined with fluorescence detection in addition to MS. The majority of fluorescent labeling employed before LC separation is based on 2-aminobenzamide, which has several disadvantages such as low labeling yield, poor fluorescence properties, and MS ionization efficiency. Therefore, even after several decades of development of new labels, there is still a need for new labeling tags with improved characteristics.

We present the application of a newly synthesized fluorescent label designed for oligosaccharide and glycan analysis by high-performance liquid chromatography with fluorescence detection (HPLC/FLD). The novel hydrazide derivative of dipyrrometheneboron difluoride (BODIPY) was synthesized from 2,4-dimethylpyrrole, methyl succinyl chloride, and boron trifluoride etherate followed by a reaction with hydrazine. The synthesized label was characterized by several analytical methods including NMR, UV/Vis and fluorescence spectroscopy, and mass spectrometry. The labeling reaction via hydrazone formation chemistry was optimized by labeling of maltooligosaccharide standards. The analysis of maltohexaose labeled by BODIPY-hydrazide followed by HPLC/FLD analysis provided the limit of detection in the low tens of femtomole. The presented method based on fluorescence detection is at least 30 times more sensitive than the standard approach employing labeling by 2-aminobenzamide. In addition, the labeling method by BODIPY-hydrazide was used for N-linked glycan profiling of several glycoproteins (ribonuclease B, immunoglobulin G) by RP-HPLC/FLD as well as HILIC/FLD analysis.