BODIPY® Lipid Probes

Introduction

The intensely fluorescent BODIPY® (4,4-difluoro-3a,4a-diaza-s-indacene) fluorophore is intrinsically lipophilic, unlike most other long-wavelength dyes. Consequently, probes incorporating this fluorophore are more likely to mimic the properties of natural lipids. Molecular Probes prepares BODIPY fatty acid, phospholipid and cholesteryl ester analogs that undergo native-like transport and metabolism in cells.[1] They are therefore effective tracers of lipid trafficking, as well as being useful general-purpose membrane probes. We also offer several nonpolar BODIPY dyes for staining neutral lipids, oils and polymers. Our extensive range of BODIPY dye–labeled sphingolipids is described in Product Information sheet MP01154. The spectroscopic properties of BODIPY lipid probes are summarized in Table 1. These and other data relating to the characterization of BODIPY fatty acids and phospholipids as membrane probes are described in more detail elsewhere.[2]

Product Descriptions

Fatty Acids

Biosynthetic incorporation of BODIPY fatty acids into BHK cells has been characterized by HPLC analysis.[3] Incorporation levels into glycerophosphocholine were found to be greater than 90% for BODIPY 500/510 dodecanoic acid (D-3823). Microscopic examination of biosynthetically labeled cells revealed localized areas of red-shifted fluorescence. This long-wavelength fluorescence results from the accumulation of BODIPY dye–labeled neutral lipids in cytoplasmic droplets at concentrations sufficient to induce the formation of BODIPY excimers. Absorption of BODIPY FL and BODIPY 500/510 is well matched to argon-ion laser excitation at 488 nm, allowing analysis of cellular fatty acid uptake by flow cytometry and confocal microscopy.[4] BODIPY FL dodecanoic acid (D-3822) has been employed to examine the co-transfer of lipids and membrane proteins from human neutrophils to the parasite *Schistosoma mansoni*.[5] Researchers have also used BODIPY fatty acids and phospholipids to visualize compartmentalization of specific lipid classes in this parasite.[6] Binding of BODIPY fatty acids to bovine serum albumin can be monitored by the accompanying fluorescence quenching caused by charge-transfer interactions with aromatic amino acid residues. BODIPY 581/591 C_{11} (D-3861) can be used to measure antioxidant activity in lipid environments by exploiting its loss of fluorescence upon interaction with peroxyl radicals.[7,8]

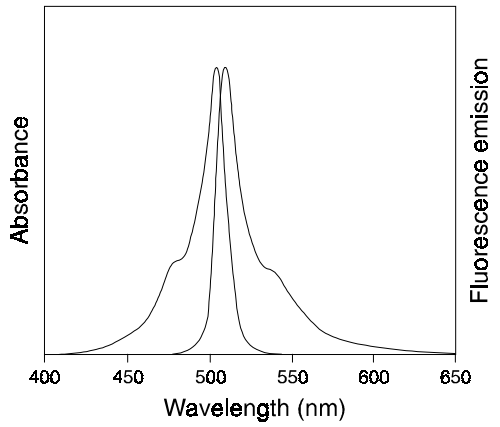

Figure 1. *Absorption and corrected fluorescence emission spectra of C_4-BODIPY 500/510–C_9 (B-3824) in methanol.*

Table 1. *Spectral properties of BODIPY lipid probes.**

Wavelength Range	Fluorescence excitation maxima from 500 nm to ~650 nm. Emission maxima from 510 nm to ~665 nm.
Spectral Bandwidth	Narrow (Figure 1).
Fluorescence Stokes Shift	Small (Figure 1). Spectral overlap results in Förster transfer radius (R_o) = 57 Å for BODIPY FL.
Fluorescence Quantum Yield	High. Typically 0.9 in fluid phase lipid bilayers.
Molar Absorptivity	High. ϵ_{max} typically >80,000 $cm^{-1}M^{-1}$.
Sensitivity to Environment	Generally low. Fluorescence quantum yields of fatty acids are not diminished in water. Fluorescence is quenched by collisional interactions with aromatic amino acids.
Concentration Dependence	Long-wavelength excimer emission detectable at incorporation levels of about 1:10 mole:mole with respect to unlabeled phospholipid in lipid bilayers.

***** Data in this table are compiled, in part, from J Am Chem Soc 116, 7801 (1994) and Anal Biochem 198, 228 (1991).

Glycerophospholipids with Labeled Acyl Chains

The nonpolar BODIPY fluorophore has been used to produce the longest-wavelength phospholipids with fluorescently labeled acyl chains currently available. The optical properties of BODIPY phospholipids are superior in several respects to those of NBD analogs,[2] as shown in Figure 2. The fluorescence quantum yield of BODIPY-PC analogs in fluid-phase diacylglycerophosphocholine liposomes is typically 0.9, around three times that of NBD-PC probes. This, coupled with their higher

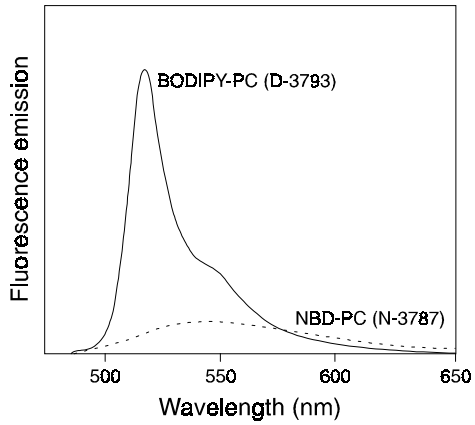

Figure 2. *Fluorescence spectra (excitation at 475 nm) of β-BODIPY 500/510 C_{12}-HPC (solid line) and NBD-C_{12}-HPC (dashed line) incorporated in DOPC (dioctadecenoylglycerophosphocholine) liposomes at molar ratios of 1:400 mole:mole (labeled:unlabeled PC). The integrated intensities of the spectra are proportional to the relative fluorescence quantum yields of the two probes.*

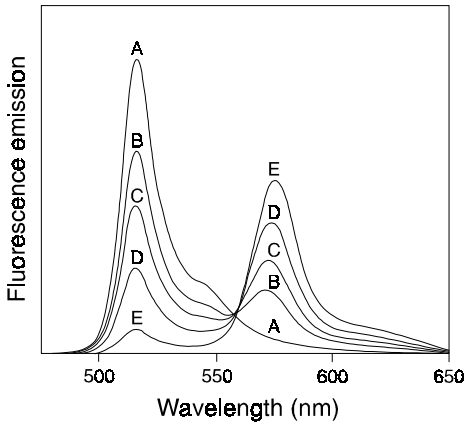

Figure 3. *Energy transfer from β-BODIPY 500/510 C_{12}-HPC (peak at 516 nm) to BODIPY 558/568 C_{12} (peak at 572 nm) in DOPC (dioctadecenoylglycerophosphocholine) lipid bilayers using 475 nm excitation. Ratio of acceptors to donors is: A) 0; B) 0.2; C) 0.4; D) 0.8; E) 2.0.*

photostability and stronger absorptivity, represents a considerable advantage for BODIPY-PC in terms of photometric detectability. BODIPY PC analogs with the fluorophore attached to a short (pentanoyl) acyl chain (D-3803, D-3805) are expected to be more readily incorporated into cells because of higher spontaneous transfer rates.[1] β-BODIPY FL C_5-HPC (D-3803) is a functional analog of antineoplastic 1-*O*-alkyl ether phospholipids[9] and of arachidonyl phospholipids in fluorescence-based assays of cytosolic phospholipase A_2 (cPLA_2).[10] The green-fluorescent BODIPY 500/510 and BODIPY FL analogs are effective fluorescence resonance energy transfer donors to longer-wavelength BODIPY probes (Figure 3) and acceptors from coumarin-labeled phospholipids or proteins.[11,12]

Glycerophospholipids with Labeled Head Groups

Molecular Probes offers two glycerophosphoethanolamine conjugates modified with a BODIPY propionic acid attached to the terminal amine group via an amide linkage (D-3800, D-12656). Attachment of the BODIPY fluorophore to the head group does not produce major changes in its spectroscopic properties, since BODIPY fluorophores are largely insensitive to their environment (Table 1). These probes have obvious potential for studies of molecular recognition interactions at membrane surfaces. D-3800 and D-12656 probably can be used in conjunction with our anti-BODIPY FL antibody (A-5770).

Cholesteryl Esters

Cholesteryl esters are highly nonpolar lipids that are completely resistant to spontaneous intermembrane transfer. They are the major core lipid components of low- and high-density lipoproteins. Our BODIPY cholesteryl ester analog (C-3927) has been used to develop assays for cholesteryl ester transfer protein activity.[13-16] It is also useful as a general nonexchangeable membrane marker and for tracing receptor-mediated endocytosis of lipoproteins and cholesterol transport by fluorescence microscopy.[17,18] Two longer-wavelength BODIPY cholesteryl esters (C-12680, C-12681) extend the available wavelength options.

Neutral Lipid Stains

Molecular Probes offers three nonpolar derivatives of the BODIPY fluorophore with an unusual combination of nonpolar structure and long-wavelength absorption and fluorescence (D-3921, D-3922). Their fluorescence Stokes shifts are generally small (see Figure 1), extinction coefficients are typically greater than 80,000 $cm^{-1}M^{-1}$, and fluorescence quantum yields are very high (0.94 for D-3921 in methanol[2]). The fluorescence quantum yield of the BODIPY dyes is not diminished in water, except at higher concentrations, where aggregation may result in self-quenching. Staining with BODIPY 493/503 (D-3922) has been shown by flow cytometry to be more specific for cellular lipid droplets than staining with nile red.[19] Mark Cooper at University of Washington has found that BODIPY 505/515 (D-3921) rapidly permeates cell membranes of live zebrafish embryos, selectively staining cytoplasmic yolk platelets. This staining provides dramatic contrast enhancement of cytoplasm relative to nucleoplasm and interstitial spaces, enabling dynamic developmental processes in the embryo to be followed using time-lapse confocal microscopy.[20]

References

1. Biochemistry 36, 8840 (1997); **2.** Anal Biochem 198, 228 (1991); **3.** Biochem Biophys Res Commun 187, 1594 (1992); **4.** Cytometry 23, 166 (1996); **5.** J Cell Sci 106, 485 (1993); **6.** J Lipid Res 36, 1 (1995); **7.** Anal Biochem 265, 290 (1998); **8.** FEBS Lett 453, 278 (1999); **9.** Biochim Biophys Acta 1390, 73 (1998); **10.** J Biol Chem 274, 19338 (1999); **11.** Biochem Biophys Res Commun 207, 508 (1995); **12.** Biochemistry 35, 3063 (1996); **13.** Lipids 29, 811 (1994); **14.** J Lipid Res 34, 1625 (1993); **15.** Chem Phys Lipids 77, 51 (1995); **16.** Biochemistry 34, 12560 (1995); **17.** J Biol Chem 271, 16208 (1996); **18.** J Biol Chem 270, 28767 (1995); **19.** Cytometry 17, 151 (1994); **20.** Methods Cell Biol 59, 179 (1999).

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
	Fatty Acids	
B-3824	5-butyl-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene-3-nonanoic acid (C_4-BODIPY® 500/510 C_9)	1 mg
D-3826	5-decyl-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene-3-propionic acid (C_{10}-BODIPY® 500/510 C_3)	1 mg
D-3822	4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-dodecanoic acid (BODIPY® FL C_{12})	1 mg
D-3821	4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-hexadecanoic acid (BODIPY® FL C_{16})	1 mg
D-3834	4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-pentanoic acid (BODIPY® FL C_5)	1 mg
D-3862	4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-undecanoic acid (BODIPY® FL C_{11})	1 mg
D-3832	4,4-difluoro-5,7-diphenyl-4-bora-3a,4a-diaza-s-indacene-3-dodecanoic acid (BODIPY® 530/550 C_{12})	1 mg
D-3823	4,4-difluoro-5-methyl-4-bora-3a,4a-diaza-s-indacene-3-dodecanoic acid (C_1-BODIPY® 500/510 C_{12})	1 mg
D-3825	4,4-difluoro-5-octyl-4-bora-3a,4a-diaza-s-indacene-3-pentanoic acid (C_8-BODIPY® 500/510 C_5)	1 mg
D-3860	4,4-difluoro-5-(4-phenyl-1,3-butadienyl)-4-bora-3a,4a-diaza-s-indacene-3-pentanoic acid (BODIPY® 581/591 C_5)	1 mg
D-3861	4,4-difluoro-5-(4-phenyl-1,3-butadienyl)-4-bora-3a,4a-diaza-s-indacene-3-undecanoic acid (BODIPY® 581/591 C_{11})	1 mg
D-3835	4,4-difluoro-5-(2-thienyl)-4-bora-3a,4a-diaza-s-indacene-3-dodecanoic acid (BODIPY® 558/568 C_{12})	1 mg
	Acyl-Modified Glycerophosphocholines	
D-3792	2-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-dodecanoyl)-1-hexadecanoyl-*sn*-glycero-3-phosphocholine (β-BODIPY® FL C_{12}-HPC)	100 µg
D-3803	2-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-pentanoyl)-1-hexadecanoyl-*sn*-glycero-3-phosphocholine (β-BODIPY® FL C_5-HPC)	100 µg
D-3793	2-(4,4-difluoro-5-methyl-4-bora-3a,4a-diaza-s-indacene-3-dodecanoyl)-1-hexadecanoyl-*sn*-glycero-3-phosphocholine (β-BODIPY® 500/510 C_{12}-HPC)	100 µg
D-3795	2-(4,4-difluoro-5-octyl-4-bora-3a,4a-diaza-s-indacene-3-pentanoyl)-1-hexadecanoyl-*sn*-glycero-3-phosphocholine (β-C_8-BODIPY® 500/510 C_5-HPC)	100 µg
D-3806	2-(4,4-difluoro-5-(4-phenyl-1,3-butadienyl)-4-bora-3a,4a-diaza-s-indacene-3-pentanoyl)-1-hexadecanoyl-*sn*-glycero-3-phosphocholine (β-BODIPY® 581/591 C_5-HPC)	100 µg
	Acyl-Modified Phosphatidic Acid	
D-3805	2-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-pentanoyl)-1-hexadecanoyl-*sn*-glycero-3-phosphate, diammonium salt (β-BODIPY® FL C_5-HPA)	100 µg
	Head-Group Labeled Glycerophosphoethanolamines	
D-3800	N-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-propionyl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine, triethylammonium salt (BODIPY® FL DHPE)	100 µg
D-12656	N-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-propionyl)-1,2-dihexanoyl-*sn*-glycero-3-phosphoethanolamine, triethylammonium salt (BODIPY® FL dicaproyl PE)	100 µg
	Nonpolar BODIPY Probes	
C-3927	cholesteryl 4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-dodecanoate (cholesteryl BODIPY® FL C_{12})	1 mg
C-12680	cholesteryl 4,4-difluoro-5-(4-methoxyphenyl)-4-bora-3a,4a-diaza-s-indacene-3-undecanoate (cholesteryl BODIPY® 542/563 C_{11})	1 mg
C-12681	cholesteryl 4,4-difluoro-5-(2-pyrrolyl)-4-bora-3a,4a-diaza-s-indacene-3-undecanoate (cholesteryl BODIPY® 576/589 C_{11})	1 mg
D-3922	4,4-difluoro-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-s-indacene (BODIPY® 493/503)	10 mg
D-3921	4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene (BODIPY® 505/515)	10 mg

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