

Cat #	Product Name	Unit Size
L-3484	low-density lipoprotein from human plasma, acetylated, Dil complex (Dil AcLDL) *1 mg/mL*	200 µL
L-3483	low-density lipoprotein from human plasma, BODIPY® FL complex (BODIPY® FL LDL) *1 mg/mL*	200 µL
L-3482	low-density lipoprotein from human plasma, Dil complex (Dil LDL) *1 mg/mL*	200 µL
L-453	lucifer yellow CH, lithium salt	25 mg
M-13440	[Nle ⁴ , D-Phe ⁷]-α-melanocyte-stimulating hormone, fluorescein conjugate ([Nle ⁴ , D-Phe ⁷]-α-MSH, fluorescein conjugate)	25 µg
M-23361	mucin from bovine submaxillary gland, Oregon Green® 488 conjugate	5 mg
N-13195	2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose (2-NBDG)	5 mg
N-23106	6-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-6-deoxyglucose (6-NBDG)	5 mg
O-13291	OxyBURST® Green H ₂ HFF BSA *special packaging*	5 x 1 mg
P-3900	N-((4-(6-phenyl-1,3,5-hexatrienyl)phenyl)propyl)trimethylammonium <i>p</i> -toluenesulfonate (TMAP-DPH)	5 mg
S-2860	<i>Staphylococcus aureus</i> BioParticles® opsonizing reagent	1 U
S-23371	<i>Staphylococcus aureus</i> (Wood strain without protein A) BioParticles®, Alexa Fluor® 488 conjugate	2 mg
S-23372	<i>Staphylococcus aureus</i> (Wood strain without protein A) BioParticles®, Alexa Fluor® 594 conjugate	2 mg
S-2854	<i>Staphylococcus aureus</i> (Wood strain without protein A) BioParticles®, BODIPY® FL conjugate	10 mg
S-2851	<i>Staphylococcus aureus</i> (Wood strain without protein A) BioParticles®, fluorescein conjugate	10 mg
S-2859	<i>Staphylococcus aureus</i> (Wood strain without protein A) BioParticles®, unlabeled	100 mg
S-359	sulforhodamine 101	25 mg
T-13342	transferrin from human serum, Alexa Fluor® 488 conjugate	5 mg
T-23364	transferrin from human serum, Alexa Fluor® 546 conjugate	5 mg
T-23365	transferrin from human serum, Alexa Fluor® 568 conjugate	5 mg
T-13343	transferrin from human serum, Alexa Fluor® 594 conjugate	5 mg
T-23362	transferrin from human serum, Alexa Fluor® 633 conjugate	5 mg
T-23366	transferrin from human serum, Alexa Fluor® 647 conjugate	5 mg
T-23363	transferrin from human serum, biotin-XX conjugate	5 mg
T-2873	transferrin from human serum, BODIPY® FL conjugate	5 mg
T-2871	transferrin from human serum, fluorescein conjugate	5 mg
T-13341	transferrin from human serum, Oregon Green® 488 conjugate	5 mg
T-2872	transferrin from human serum, tetramethylrhodamine conjugate	5 mg
T-2875	transferrin from human serum, Texas Red® conjugate	5 mg
T-3163	<i>N</i> -(3-triethylammoniumpropyl)-4-(4-(dibutylamino)styryl)pyridinium dibromide (FM® 1-43)	1 mg
T-1111	<i>N</i> -(3-triethylammoniumpropyl)-4-(4-(diethylamino)phenyl)butadienylpyridinium dibromide (RH 414)	5 mg
T-3166	<i>N</i> -(3-triethylammoniumpropyl)-4-(6-(4-(diethylamino)phenyl)hexatrienyl)pyridinium dibromide (FM® 4-64)	1 mg
T-13320	<i>N</i> -(3-triethylammoniumpropyl)-4-(6-(4-(diethylamino)phenyl)hexatrienyl)pyridinium dibromide (FM® 4-64) *special packaging*	10 x 100 µg
T-7508	<i>N</i> -(3-triethylammoniumpropyl)-4-(4-(diethylamino)styryl)pyridinium dibromide (FM® 2-10)	5 mg
T-204	1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene <i>p</i> -toluenesulfonate (TMA-DPH)	25 mg
T-23360	<i>N</i> -(3-trimethylammoniumpropyl)-4-(6-(4-(diethylamino)phenyl)hexatrienyl)pyridinium dibromide (FM® 5-95)	1 mg
T-23011	trypsin inhibitor from soybean, Alexa Fluor® 488 conjugate	1 mg
V-6694	Vybrant® Phagocytosis Assay Kit *250 assays*	1 kit
Z-2850	zymosan A BioParticles® opsonizing reagent	1 U
Z-23373	zymosan A (<i>S. cerevisiae</i>) BioParticles®, Alexa Fluor® 488 conjugate	2 mg
Z-23374	zymosan A (<i>S. cerevisiae</i>) BioParticles®, Alexa Fluor® 594 conjugate	2 mg
Z-2844	zymosan A (<i>S. cerevisiae</i>) BioParticles®, BODIPY® FL conjugate	10 mg
Z-2841	zymosan A (<i>S. cerevisiae</i>) BioParticles®, fluorescein conjugate	10 mg
Z-2843	zymosan A (<i>S. cerevisiae</i>) BioParticles®, Texas Red® conjugate	10 mg
Z-2849	zymosan A (<i>S. cerevisiae</i>) BioParticles®, unlabeled	100 mg

16.2 Probes for Neurotransmitter Receptors

Because receptor-mediated signal transduction underlies much of what occurs in cellular biochemistry and physiology,^{1,2} fluorescent receptor ligands can provide a sensitive means of identifying and localizing some of the most pivotal molecules in cell biology. Molecular Probes offers fluorescently labeled and unlabeled ligands for various cellular receptors, ion channels and ion carriers. Many of these site-selective fluorescent probes may be used on live or fixed cells, as well as in cell-free extracts. In particular, we would like to highlight those ligands conjugated to the green-fluorescent Alexa Fluor 488, BODIPY FL and Oregon Green 514 dyes and the red-fluorescent Alexa Fluor 594 and Texas Red dyes,

which provide extremely bright signals and superior photostability. The high sensitivity and selectivity of these fluorescent probes make them especially good candidates for measuring low-abundance receptors. Various methods for further amplifying detection of these receptors are discussed in Chapter 6 and Chapter 7.

This section is devoted to our probes for neurotransmitter receptors. Additional fluorescently labeled receptor ligands (including low-density lipoproteins, epidermal growth factors, transferrin and fibrinogen conjugates and chemotactic peptides) are described in Section 16.1, along with other probes for studying receptor-mediated endocytosis, as well as membrane markers of

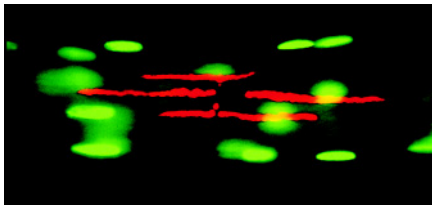


Figure 16.25 Pseudocolored photomicrograph of the synaptic region of fluorescently labeled living muscle fibers from the lumbricalis muscle of the adult frog *Rana pipiens*. Six hours after isolation of the muscle fibers, acetylcholine receptors were stained with the red-fluorescent tetramethylrhodamine α -bungarotoxin (T-1175) and myonuclei were stained with the green-fluorescent SYTO 13 live-cell nucleic acid stain (S-7575).

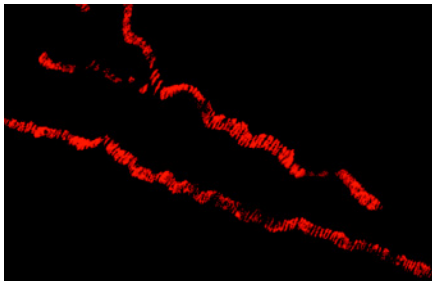


Figure 16.26 Confocal micrograph showing acetylcholine receptors at frog neuromuscular junction labeled with Texas Red-X α -bungarotoxin (B-7489). Image contributed by D. Raciborska and M.P. Charlton, University of Toronto.

The fluorescent α -bungarotoxin probes permit easy labeling of nicotinic acetylcholine receptors. The Alexa Fluor 488 and Alexa Fluor 594 α -bungarotoxin conjugates are recommended for their brightness and enhanced photostability.

endocytosis and exocytosis. Section 16.3 describes a variety of probes for Ca^{2+} , Na^+ , K^+ and Cl^- ion channels and carriers. Chapter 18, entitled “Probes for Signal Transduction,” focuses on reagents for investigating events — such as calcium regulation; kinase, phosphatase and phospholipase activation; nucleotide binding and lipid trafficking — that occur downstream from the receptor–ligand interaction (Figure 18.1).

α -Bungarotoxin Probes for Nicotinic Acetylcholine Receptors

Fluorescent α -Bungarotoxins

Nicotinic acetylcholine receptors (nicotinic AChRs) are neurotransmitter-gated ion channels that produce an increase in Na^+ and K^+ permeability, depolarization and excitation upon activation by acetylcholine³ (Figure 18.1). α -Bungarotoxin, a 74–amino acid peptide extracted from *Bungarus multicinctus* venom, binds with high affinity to the α -subunit of the nicotinic AChR of neuromuscular junctions.⁴ Molecular Probes provides an extensive selection of fluorescent α -bungarotoxin conjugates (Table 16.4) to facilitate visualization of nicotinic AChRs with a variety of instrumentation. We attach approximately one fluorophore to each molecule of α -bungarotoxin, thus retaining optimal binding specificity. The labeled bungarotoxins are then chromatographically separated from unlabeled molecules to ensure adequate labeling of the product.

Alexa Fluor 488 α -bungarotoxin (B-13422) and Oregon Green 514 α -bungarotoxin⁵ (B-7488) have fluorescence spectra (Figure 7.5, Figure 7.18) similar to those of fluorescein α -bungarotoxin (F-1176, Figure 7.23) and are therefore suitable for use with standard fluorescein optical filter sets (Table 24.8). Our Alexa Fluor 488 and Oregon Green 514 dyes are brighter and more photostable than any other green-fluorescent dye (Figure 1.42), making their conjugates of α -bungarotoxin especially well suited to fluorescence and confocal laser-scanning microscopy. Tetramethylrhodamine α -bungarotoxin^{6,7} (T-1175, B-13421) is currently the preferred red-orange–fluorescent probe for staining the nicotinic AChR (Figure 16.25). However, our Alexa Fluor 594 and Texas Red-X conjugates of α -bungarotoxin (B-13423, B-7489; Figure 16.26) have longer-wavelength emission maxima and therefore offer better spectral separation from green-fluorescent dyes in multicolor experiments. Our receptor-grade tetramethylrhodamine α -bungarotoxin (B-13421) has been specially purified by HPLC for use in microscopy.

Fluorescent α -bungarotoxins have been used in a variety of informative investigations of the nicotinic AChR to:

- Correlate receptor clustering during neuromuscular development with tyrosine phosphorylation of the receptor^{8,9}
- Document nicotinic AChR cluster formation after myoblast fusion¹⁰
- Quantitate nicotinic AChRs in a study that showed that several isoforms of agrin, a component of synaptic basal lamina, help trigger the nicotinic AChR aggregation that occurs during neuromuscular junction formation¹¹
- Detect reinnervation of adult muscle after nerve damage and to identify and visualize endplates^{12,13}

Table 16.4 Labeled and unlabeled α -bungarotoxins.

Cat #	Label	Ex/Em (nm)	Notes	Size
F-1176	Fluorescein	494/518	Original green-fluorescent conjugate	500 μg
B-13422	Alexa Fluor 488	495/519	Brightest and most photostable green-fluorescent conjugate	500 μg
B-7488	Oregon Green 514	512/530	Extremely photostable green-fluorescent α -bungarotoxin	500 μg
T-1175	Tetramethylrhodamine	553/577	Currently the most extensively used conjugate; now also available as a highly purified receptor-grade product (B-13421)	500 μg
B-13423	Alexa Fluor 594	590/617	Excellent dye to combine with green-fluorescent probes	500 μg
B-7489	Texas Red-X	593/613	Excellent dye to combine with green-fluorescent probes	500 μg
B-1196	Biotin-XX	NA	Visualized with secondary detection reagents (Section 7.6)	500 μg
B-1601	Unlabeled	NA	Useful as a control, as well as for radioiodination and for preparation of new conjugates	1 mg

NA = not applicable.

Biotinylated α -Bungarotoxin

Nicotinic AChRs can also be labeled with biotinylated α -bungarotoxin (B-1196), which is then localized using enzyme-, fluorophore- or NANOGOLD and Alexa Fluor FluoroNanogold 1.4 nm gold cluster-labeled conjugates of avidin or streptavidin^{9,14-16} (Section 7.6, Table 7.17). In addition, the biotinylated toxin can be employed for affinity isolation of the nicotinic AChR using streptavidin or CaptAvidin agarose (S-951, C-21386; Section 7.6) column¹⁷ or with Captivate ferrofluid streptavidin (C-21476, Section 7.6) for enzyme-linked immunosorbent assays (ELISAs) designed to detect anti-nicotinic AChR antibodies.¹⁸

Unlabeled α -Bungarotoxin

In addition to the fluorescent and biotinylated derivatives, we have unlabeled α -bungarotoxin (B-1601), which has been shown to be useful for radioiodination.^{4,19} Unlabeled α -bungarotoxin has also been employed for ELISA testing of nicotinic AChR binding,²⁰ as well as for investigating the function of the α -bungarotoxin-binding component (α -BgtBC) in vertebrate neurons.²¹

Amplex Red Acetylcholine/Acetylcholinesterase Assay Kit

The action of acetylcholine (ACh) at neuromuscular junctions is regulated by acetylcholinesterase (AChE), the enzyme that hydrolyzes ACh to choline and acetate. The Amplex Red Acetylcholine/Acetylcholinesterase Assay Kit (A-12217) provides an ultrasensitive method for continuously monitoring AChE activity and for detecting ACh in a fluorescence microplate reader or fluorometer. Other potential uses for this kit include screening for AChE inhibitors and measuring the release of ACh from synaptosomes. The Amplex Red Acetylcholine/Acetylcholinesterase Assay Kit can also be used for the ultrasensitive and specific assay of free choline, an “essential nutrient,” in foods.²²

In the assay, AChE activity is monitored indirectly using the Amplex Red reagent, 10-acetyl-3,7-dihydroxyphenoxazine, a highly sensitive and stable fluorogenic probe for H_2O_2 that is useful in numerous assays for enzymes and other analytes (Section 10.5). First, AChE converts the acetylcholine substrate to choline. Choline is in turn oxidized by choline oxidase to betaine and H_2O_2 , the latter of which, in the presence of horseradish peroxidase, reacts with the Amplex Red reagent to generate the red-fluorescent product resorufin (R-363, Section 10.1, Figure 10.50) with excitation/emission maxima of $\sim 570/585$ nm (Figure 10.5). Experiments with purified AChE from electric eel indicate that the Amplex Red Acetylcholine/Acetylcholinesterase Assay Kit can detect AChE levels as low as 0.002 U/mL using a reaction time of only one hour (Figure 16.27). By providing an excess of AChE in the assay, the kit can also be used to detect acetylcholine levels as low as 0.3 μ M, with a range of detection from 0.3 μ M to ~ 100 μ M acetylcholine (Figure 16.28). Each kit contains:

- The Amplex Red reagent
- Horseradish peroxidase (HRP)
- H_2O_2 for use as a positive control
- Concentrated reaction buffer
- Choline oxidase
- Acetylcholine (ACh)
- Acetylcholinesterase (AChE)
- A detailed protocol

Each kit provides sufficient reagents for approximately 500 assays using a fluorescence microplate reader and a reaction volume of 200 μ L per assay.

Pirenzepine Probes for Muscarinic Acetylcholine Receptors

Unlike nicotinic AChRs, muscarinic acetylcholine receptors (muscarinic AChRs) are G-protein-coupled receptors that produce either excitatory or inhibitory responses and are not necessarily associated with changes in ion permeability (Figure 18.1). Fluorescent derivatives of pirenzepine are selective antagonists for the M_1 muscarinic AChR.²³⁻²⁶ The

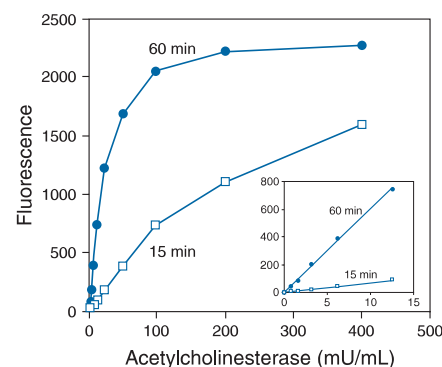


Figure 16.27 Detection of electric eel acetylcholinesterase activity using the Amplex Red Acetylcholine/Acetylcholinesterase Assay Kit (A-12217). Each reaction contained 50 μ M acetylcholine, 200 μ M Amplex Red reagent, 1 U/mL HRP, 0.1 U/mL choline oxidase and the indicated amount of acetylcholinesterase in 1 \times reaction buffer. Reactions were incubated at room temperature. After 15 and 60 minutes, fluorescence was measured in a fluorescence microplate reader using excitation at 560 ± 10 nm and fluorescence detection at 590 ± 10 nm. The inset shows the sensitivity of the 15 min (\square) and 60 min (\bullet) assays at low levels of acetylcholinesterase activity (0–13 mU/mL).

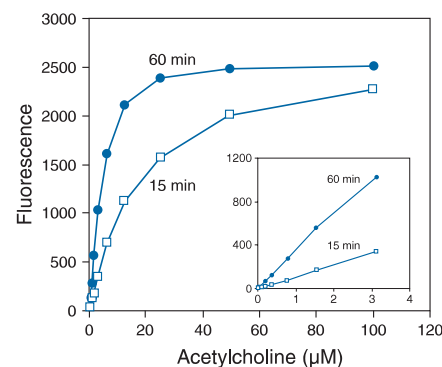


Figure 16.28 Detection of acetylcholine using the Amplex Red Acetylcholine/Acetylcholinesterase Assay Kit (A-12217). Each reaction contained 200 μ M Amplex Red reagent, 1 U/mL HRP, 0.1 U/mL choline oxidase, 0.5 U/mL acetylcholinesterase and the indicated amount of acetylcholine in 1 \times reaction buffer. Reactions were incubated at room temperature. After 15 and 60 minutes, fluorescence was measured with a fluorescence microplate reader using excitation at 560 ± 10 nm and fluorescence detection at 590 ± 10 nm. The inset shows the sensitivity of the 15 min (\square) and 60 min (\bullet) assays at low levels of acetylcholine (0–3 μ M).

Our Amplex Red acetylcholine and acetylcholinesterase assays are fast and ultrasensitive and may be useful for detection of these analytes in cell and tissue preparations.

green-fluorescent BODIPY FL (B-7436, Figure 16.29) and red-fluorescent BODIPY 558/568 (B-7437) derivatives retain pirenzepine's specificity for the M_1 muscarinic receptor and exhibit similar inhibition and displacement profiles. BODIPY FL pirenzepine was employed to study the activity-dependent expression of the M_1 muscarinic receptor in cultured neurons derived from rat visual cortex.²³ Expression of the receptor increased in response to antagonist application and to chronic membrane depolarization. Conversely, receptor expression decreased when an agonist or the Na^+ -channel blocker tetrodotoxin (T-6913, Section 16.3) was applied.

Prazosin Probes for α_1 -Adrenergic Receptors

We have prepared the green-fluorescent BODIPY FL (B-7433, Figure 16.30) and red-fluorescent BODIPY 558/568 (B-7434) derivatives of prazosin, a high-affinity antagonist for the α_1 -adrenergic receptor.^{27,28} These derivatives can be used to localize the α_1 -adrenergic receptor on cultured cortical neurons.^{29,30} BODIPY FL prazosin has been employed for flow cytometric detection of α_1 -adrenergic receptors, including the α_{1a} , α_{1b} and α_{1d} receptor subtypes, on Chinese hamster ovary (CHO) cells.³¹

CGP 12177 Probe for β -Adrenergic Receptors

The most thoroughly studied receptor-modulated ion channel mechanism is the activation of the β -adrenergic receptor upon binding adrenaline, which causes an enhancement of the Ca^{2+} current. Molecular Probes offers the red-orange-fluorescent

BODIPY TMR derivative (B-13420, Figure 16.31) of the hydrophilic β -adrenergic receptor agonist CGP 12177.³² A similar, but not identical, BODIPY CGP 12177 conjugate reportedly gave a strong receptor-specific signal, enabling researchers to measure on-rate and off-rate constants and dissociation constants that agreed well with those determined for tritiated CGP 12177.³³

BODIPY FL Muscimol for the GABA_A Receptor

Muscimol is a powerful agonist of the GABA_A receptor and has been widely used to reversibly inactivate localized groups of neurons.^{34,35} With the introduction of the BODIPY TMR-X muscimol conjugate (M-23400, Figure 16.32), researchers can avoid using radioactive methods to map the distribution of the drug in the central nervous system,³⁶ as well as to detect the presence of GABA_A receptors on cell surfaces.³⁷

Neuropeptide Probes for the Neurokinin Receptors

In response to the increased need for fluorescent peptide analogs for imaging of receptor localization and endocytosis, characterization of ligand-binding interactions and pharmacological screening, we have introduced a variety of fluorescent peptides, including derivatives of substance P, neuromedin C and angiotensin II. All of our fluorescent neuropeptides have been characterized for purity by HPLC and mass spectrometry; however, in most cases, the receptor-binding properties of the ligands have not been completely characterized.

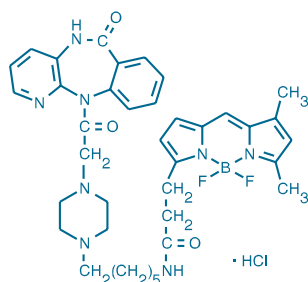


Figure 16.29 B-7436 BODIPY FL pirenzepine, hydrochloride.

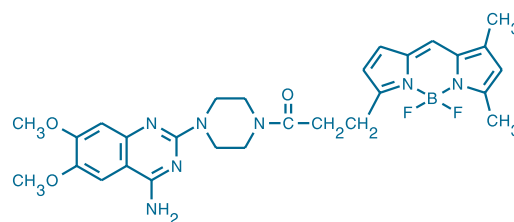


Figure 16.30 B-7433 BODIPY FL prazosin.

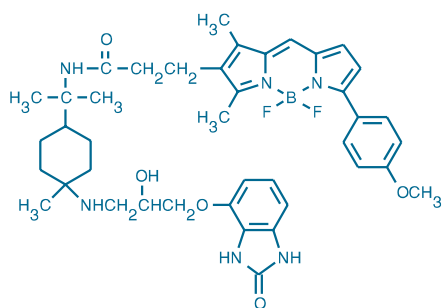


Figure 16.31 B-13420 BODIPY TMR (±) CGP 12177.

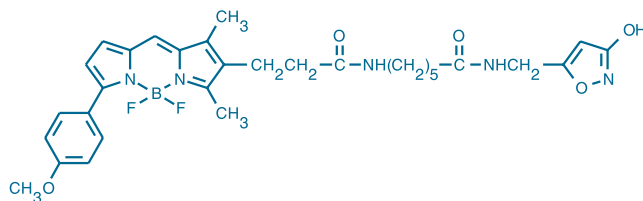


Figure 16.32 M-23400 muscimol, BODIPY TMR-X conjugate.

Substance P Analogs

Substance P is an agonist of the neurokinin 1 (NK1) receptor, a member of the seven-transmembrane G-protein-coupled receptor family.^{38,39} Substance P is a tachykinin — one of a group of neuropeptides that are primarily involved in the mediation of inflammatory responses. Our HPLC-purified substance P analogs are labeled on the ϵ -amino group of Lys-3 using a single-isomer succinimidyl ester of one of four different fluorescent labels. Our NK1 receptor probes include the:

- Alexa Fluor 488 (S-13426) and Oregon Green 488 (S-13427) conjugates of substance P — The Alexa Fluor 488 and Oregon Green 488 dyes virtually match fluorescein's excitation and emission spectra but have the additional benefits of superior photostability and lower pH sensitivity.
- BODIPY FL conjugate of substance P (S-13425) — The BODIPY FL dye usually produces less perturbation of native receptor binding than other dyes used to prepare fluorescent ligands.
- Fluorescein conjugate of substance P (S-13424) — Our fluorescein-labeled substance P analog is very similar to one described by Tota and co-workers.^{40,41}
- Tetramethylrhodamine conjugate of substance P (S-13428) — Substance P labeled with the photostable, orange-fluorescent tetramethylrhodamine dye offers researchers another option for multicolor experiments.

A detailed study of the use of our fluorescent substance P analogs for binding, imaging and receptor activation has been published by Bennett and Simmons.⁴² The Oregon Green 488 and BODIPY FL analogs were found to be the most useful probes for labeling the receptor without affecting its biological activity.

Fluorescent Neuromedin C Analogs

Neuromedin C consists of the C-terminal decapeptide fragment (residues 18–27) of gastrin-releasing peptide (GRP). Its normal function is stimulation of gastrin release in the stomach and amylase release in the pancreas, but it also appears as a mitogen in lung, breast and gastrointestinal cancer cells. Our fluorescent analog of neuromedin C is labeled on the N-terminal amino group with the exceptionally photostable Alexa Fluor 488 dye (N-13437). A fluorescein-labeled neuromedin C is known to be biologically active in amylase-release assays;⁴³ it has also been used to identify specific neuromedin C receptors in rat stomach tissue preparations.⁴³

Fluorescent Angiotensin II Analogs

Angiotensin II (Asp-Arg-Val-Ile-His-Pro-Phe) stimulates smooth muscle contraction and plays an important role in blood pressure control and in water and salt homeostasis. These effects are exerted via two G-protein-coupled receptor subtypes, referred to as AT1 and AT2. Our N-terminal-labeled fluorescein and Alexa Fluor 488 analogs of angiotensin II (A-13438, A-13439) should be useful tools for imaging the distribution of these receptors.^{44–46} It is not known at present whether these fluorescent analogs display selectivity for AT1 versus AT2 binding.

Naloxone and Naltrexone Probes for μ -Opioid Receptors

The μ -opioid receptor plays a critical role in analgesia. Among the antagonists that have been used to define and characterize these receptors is naltrexone, a nonaddictive drug that has been used for the treatment of opioid addiction. The fluorescent derivatives naloxone fluorescein (Figure 16.33) and naltrexone fluorescein (N-1384, N-1385) have been reported to bind to the μ -opioid binding site with high affinity,^{47–50} permitting their visualization in Chinese hamster ovary (CHO) cells containing transfected receptors.⁵¹ Flow cytometric analysis of the binding of fluorescein naloxone to NMDA- and μ -opioid receptors (which was displaced by NMDA and met-enkephalin, respectively) has been used to deduce the effects of operant conditioning on visual cortex receptor pattern.⁵²

Probes for Amino Acid Neurotransmitter Receptors

Caged Amino Acid Neurotransmitters

When illuminated with UV light or by multiphoton excitation,⁵³ caged amino acid neurotransmitters are converted into biologically active amino acids that rapidly initiate neurotransmitter action.^{54–57} Thus, these caged probes provide a means of controlling the release — both spatially and temporally — of agonists for kinetic studies of receptor binding or channel opening.

The different caging groups confer special properties on these photoactivatable probes (Table 17.1). We synthesize several caged versions of L-glutamic acid^{58–66} (C-7122, G-7055), as well as caged carbachol^{67,68} (*N*-(CNB-caged) carbachol, C-13654), caged γ -aminobutyric acid,^{62,69–72} *N*-CNB-caged *N*-methyl-D-aspartic acid (M-7114) and *O*-(CNB-caged) GABA (A-7110), all of which are biologically inactive before photolysis.⁵⁴ *O*-(CNB-caged) GABA (A-7110) and γ -(CNB-caged) L-glutamic acid (G-7055), which exhibit fast uncaging rates and high photolysis quantum yields, have been used to investigate the activation kinetics of GABA receptors⁷² and glutamate receptors,⁶¹ respectively. *N*-(CNB-caged) L-glutamic acid (C-7122) does not hydrolyze in aqueous solution because it is caged on the amino group, thus enabling researchers to use very high concentrations without risk of light-independent glutamic acid production.^{61,63}

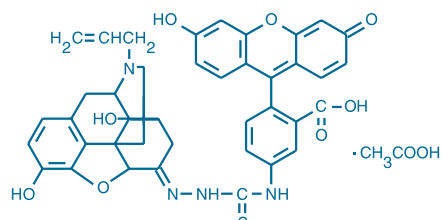


Figure 16.33 N-1384 naloxone fluorescein.

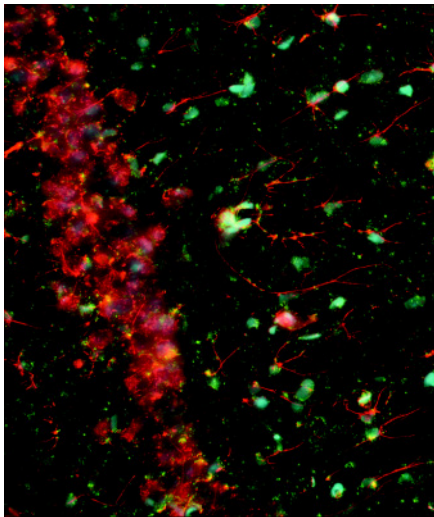


Figure 16.34 Rat brain cryosections labeled with anti-NMDA receptor, subunit 2A (rat), rabbit IgG fraction (A-6473) and detected using Alexa Fluor 488 goat anti-rabbit IgG antibody (A-11008). The tissue was also labeled with Alexa Fluor 594 anti-glial fibrillary acidic protein (A-21295) and counterstained with TOTO-3 iodide (T-3604), which was pseudocolored light blue in this image.

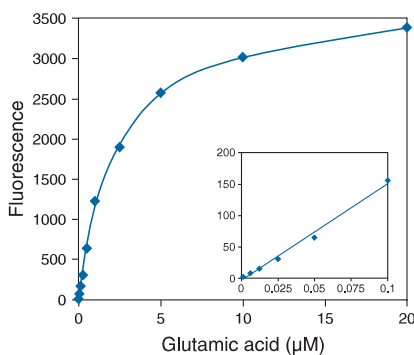


Figure 16.35 Detection of L-glutamic acid using the Amplex Red Glutamic Acid/Glutamate Oxidase Assay Kit (A-12221). Each reaction contained 50 μM Amplex Red reagent, 0.125 U/mL HRP, 0.04 U/mL L-glutamate oxidase, 0.25 U/mL L-glutamate-pyruvate transaminase, 100 μM L-alanine and the indicated amount of L-glutamic acid in 1× reaction buffer. Reactions were incubated at 37°C. After 30 minutes, fluorescence was measured in a fluorescence microplate reader using excitation at 530 ± 12.5 nm and fluorescence detection at 590 ± 17.5 nm.

Our Amplex Red assay for L-glutamate is by far the best and fastest specific assay for this important neurotransmitter. This high-throughput assay can easily quantitate L-glutamate in complex samples, including cell and tissue preparations and brain homogenates.

Anti-NMDA Receptor Antibodies

N-methyl-D-aspartate (NMDA) receptors constitute cation channels of the central nervous system that are gated by the excitatory neurotransmitter L-glutamate.^{73,74} Activation of NMDA receptors is essential for inducing long-term potentiation (LTP), a form of activity-dependent synaptic plasticity that is implicated in the learning process in animal behavioral models.⁷⁵ The biophysical properties of NMDA receptor channels contributing to LTP include Ca²⁺ permeability, voltage-dependent Mg²⁺ block and slow-gating kinetics.^{76–79} NMDA receptor channel activities play a role in neuronal development and in disorders such as epilepsy and ischemic neuronal cell death. As targets for ethanol, NMDA receptors may also function in the pathology of alcoholism.^{80,81}

In vitro reconstitution experiments with the cloned NMDA receptor subunit 1 and any one of the four NMDA receptor subunits 2A, 2B, 2C and 2D revealed that the physical properties of the heteromeric NMDA receptor channel appear to be imparted by the particular NMDA receptor subunit 2.⁸² NMDA receptor subunits 2A and 2B are detected predominantly in the hippocampus and cortex, whereas 2C is found mainly in the cerebellum. Thus, cellular expression profiles of the NMDA receptor subunits 2A, 2B, 2C and 2D may contribute to the biophysical properties of NMDA receptors in specific central neurons.

Molecular Probes offers rabbit polyclonal antibodies to NMDA receptor subunits 2A (A-6473), 2B (A-6474) and 2C (A-6475) that are suitable for immunohistochemistry (Figure 16.34), Western blots, enzyme-linked immunosorbent assays (ELISAs) and immunoprecipitations. The anti-NMDA receptor subunit 2A and 2B antibodies were generated against fusion proteins containing amino acid residues 1253–1391 of subunit 2A and 984–1104 of subunit 2B, respectively. These two antibodies are active against mouse, rat and human forms of the antigens and are specific for the subunit against which they were generated. In contrast, the anti-NMDA receptor subunit 2C antibody was generated against amino acid residues 25–130 of subunit 2C and recognizes the 140,000-dalton subunit 2C, as well as the 180,000-dalton subunit 2A and subunit 2B from mouse, rat and human. The affinity-purified antibodies were fractionated from sera by affinity chromatography in which NMDA receptor subunit fusion proteins were bound to a column matrix.

Amplex Red Glutamic Acid/Glutamate Oxidase Assay Kit

The Amplex Red Glutamic Acid/Glutamate Oxidase Assay Kit (A-12221) provides an ultrasensitive method for continuously detecting glutamic acid or for monitoring glutamate oxidase activity in a fluorescence microplate reader or fluorometer.⁸³ In this assay, L-glutamic acid is oxidized by glutamate oxidase to produce α-ketoglutarate, NH₃ and H₂O₂. L-Alanine and L-glutamate-pyruvate transaminase are also included in the reaction. Thus, the L-glutamic acid is regenerated by transamination of α-ketoglutarate, resulting in multiple cycles of the initial reaction and a significant amplification of the H₂O₂ produced. The hydrogen peroxide reacts with the Amplex Red reagent (10-acetyl-3,7-dihydroxyphenoxazine) in a 1:1 stoichiometry in the reaction catalyzed by horseradish peroxidase (HRP) to generate the highly fluorescent product resorufin^{84,85} (R-363, Section 10.1, Figure 10.50). Because resorufin has absorption/emission maxima of 563/587 nm (Figure 10.5), there is little interference from autofluorescence in most biological samples.

If the concentration of L-glutamic acid is limiting in this assay, then the fluorescence increase is proportional to the initial L-glutamic acid concentration. The Amplex Red Glutamic Acid/Glutamate Oxidase Assay Kit allows detection of as little as 10 nM L-glutamic acid in purified systems using a 30-minute reaction time (Figure 16.35). If the reaction is modified to include an excess of L-glutamic acid, then this kit can be used to continuously monitor glutamate oxidase activity. For example, purified L-glutamate oxidase from *Streptomyces* can be detected at levels as low as 40 μU/mL (Figure 16.36).

Each Amplex Red Glutamic Acid/Glutamate Oxidase Assay Kit contains:

- The Amplex Red reagent
- Dimethylsulfoxide (DMSO)
- Horseradish peroxidase (HRP)
- Hydrogen peroxide
- Concentrated reaction buffer

- L-Glutamate oxidase from *Streptomyces* sp.
- L-Glutamate–pyruvate transaminase from pig heart
- L-Glutamic acid
- L-Alanine
- A detailed protocol

Each kit provides sufficient reagents for approximately 200 assays using a fluorescence microplate reader and a reaction volume of 100 μ L per assay.

Probes for Other Receptors

Molecular Probes offers a diverse array of fluorescent derivatives of:

- Low-density lipoprotein (LDL)
- Epidermal growth factor (EGF)
- Transferrin
- Lactoferrin
- Fibrinogen
- Gelatin and collagen
- Ovalbumin and bovine serum albumin
- Soybean trypsin inhibitor
- Casein
- BioParticles *E. coli*, *S. aureus* and zymosan A conjugates
- Lipopolysaccharides
- Histone H1
- α -Crystallin from bovine eye lens
- Hyaluronic acid
- Mucin
- Subunit B of cholera toxin
- Chemotactic peptides
- Insulin

These ligands are all transported into the cell by receptor-mediated endocytosis. Additional information about these probes as well as probes for following endocytosis and exocytosis can be found in Section 16.1.

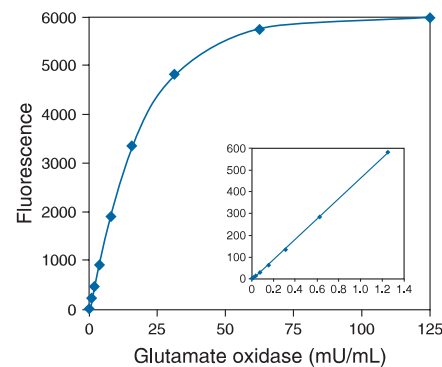


Figure 16.36 Detection of L-glutamate oxidase using the Amplex Red Glutamic Acid/Glutamate Oxidase Assay Kit (A-12221). Each reaction contained 50 μ M Amplex Red reagent, 0.125 U/mL HRP, 0.25 U/mL L-glutamate–pyruvate transaminase, 20 μ M L-glutamic acid, 100 μ M L-alanine and the indicated amount of *Streptomyces* L-glutamate oxidase in 1 \times reaction buffer. Reactions were incubated at 37°C. After 60 minutes, fluorescence was measured in a fluorescence microplate reader using excitation at 530 \pm 12.5 nm and fluorescence detection at 590 \pm 17.5 nm. The inset represents data from a separate experiment for lower L-glutamate oxidase concentrations and incubation time of 60 minutes (0–1.25 mU/mL).

By using an excess of L-glutamate, the Amplex Red substrate–based assay can be used to quantitate the activity of glutamate oxidase, and indirectly the amount of bacteria containing this enzyme, including Streptomyces.

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Data Table — 16.2 Probes for Neurotransmitter Receptors

Cat #	MW	Storage	Soluble	Abs	EC	Em	Solvent	Notes
A-7110	396.28	F,D,LL	H ₂ O	262	4,500	none	pH 7	1, 2
A-13438	1404.50	F,D,L	H ₂ O, DMSO	494	78,000	522	pH 9	3
A-13439	1586.64	F,D,L	H ₂ O, DMSO	491	78,000	516	pH 7	3
B-1196	~8400	F,D	H ₂ O	<300		none		4
B-1601	7984.14	F	H ₂ O	<300		see Notes		5
B-7433	563.41	F,D,L	DMSO, EtOH	504	77,000	511	MeOH	
B-7434	617.48	F,D,L	DMSO, EtOH	560	76,000	569	MeOH	
B-7436	747.09	F,D,L	DMSO, EtOH	504	77,000	511	MeOH	
B-7437	801.16	F,D,L	DMSO, EtOH	560	75,000	569	MeOH	
B-7488	~8500	F,D,L	H ₂ O	512	85,000	530	pH 8	4, 6
B-7489	~8600	F,D,L	H ₂ O	593	95,000	613	H ₂ O	4, 6
B-13420	756.70	F,D,L	DMSO	545	60,000	570	MeOH	
B-13421	~8400	F,D,L	H ₂ O	556	85,000	584	pH 7	4, 6
B-13422	~8500	F,D,L	H ₂ O	495	71,000	519	pH 8	4, 6
B-13423	~8700	F,D,L	H ₂ O	593	85,000	617	pH 7	4, 6
C-7122	440.29	F,D,LL	H ₂ O	266	4,800	none	pH 7	1, 2
C-13654	439.34	F,D,LL	H ₂ O	264	4,200	none	H ₂ O	1, 2
F-1176	~8400	F,D,L	H ₂ O	494	84,000	518	pH 8	4, 6
G-7055	440.29	F,D,LL	H ₂ O, DMSO	262	5,100	none	pH 7	1, 2
M-7114	440.29	F,D,LL	H ₂ O	264	5,100	none	pH 7	1, 2
M-23400	607.46	F,D,L	DMSO	543	60,000	572	MeOH	
N-1384	790.84	D,L	EtOH, DMF	492	79,000	516	pH 9	
N-1385	804.87	D,L	EtOH, DMF	493	87,000	516	pH 9	
N-13437	1658.68	F,D,L	H ₂ O, DMSO	491	78,000	515	pH 7	3
S-13424	1705.95	F,D,L	DMSO	494	78,000	524	pH 9	3
S-13425	1621.72	F,D,L	DMSO	504	83,000	511	MeOH	3
S-13426	1908.06	F,D,L	DMSO	493	78,000	516	pH 7	3
S-13427	1741.93	F,D,L	DMSO	496	86,000	520	pH 9	3
S-13428	1760.08	F,D,L	DMSO	554	85,000	578	pH 7	3
T-1175	~8400	F,D,L	H ₂ O	553	85,000	577	H ₂ O	4, 6

For definitions of the contents of this data table, see "How to Use This Book" on page viii.

Notes

- All photoactivatable probes are sensitive to light. They should be protected from illumination except when photolysis is intended.
- This compound has weaker visible absorption at >300 nm but no discernible absorption peaks in this region.
- The value of EC listed for this peptide conjugate is that of the labeling dye in free solution. Use of this value for the conjugate assumes a 1:1 dye:peptide labeling ratio and no change of EC due to dye-peptide interactions.
- α -bungarotoxin conjugates have approximately one label per peptide.
- This peptide exhibits intrinsic tryptophan fluorescence (Em ~350 nm) when excited at <300 nm.
- The value of EC listed for this α -bungarotoxin conjugate is for the labeling dye in free solution. Use of this value for the conjugate assumes a 1:1 dye:peptide labeling ratio and no change of EC due to dye-peptide interactions.

Product List — 16.2 Probes for Neurotransmitter Receptors

Cat #	Product Name	Unit Size
A-7110	γ -aminobutyric acid, α -carboxy-2-nitrobenzyl ester, trifluoroacetic acid salt (<i>O</i> -(CNB-caged) GABA)	5 mg
A-12217	Amplex [®] Red Acetylcholine/Acetylcholinesterase Assay Kit *500 assays*	1 kit
A-12221	Amplex [®] Red Glutamic Acid/Glutamate Oxidase Assay Kit *200 assays*	1 kit
A-13439	angiotensin II, Alexa Fluor [®] 488 conjugate	25 μ g
A-13438	angiotensin II, fluorescein conjugate	25 μ g
A-6473	anti-NMDA receptor, subunit 2A (rat), rabbit IgG fraction *affinity purified*	10 μ g
A-6474	anti-NMDA receptor, subunit 2B (rat), rabbit IgG fraction *affinity purified*	10 μ g
A-6475	anti-NMDA receptor, subunit 2C (rat), rabbit IgG fraction *affinity purified*	10 μ g
B-7436	BODIPY [®] FL pirenzepine, hydrochloride	100 μ g
B-7433	BODIPY [®] FL prazosin	100 μ g
B-7437	BODIPY [®] 558/568 pirenzepine, hydrochloride	100 μ g
B-7434	BODIPY [®] 558/568 prazosin	100 μ g
B-13420	BODIPY [®] TMR (\pm) CGP 12177	100 μ g
B-1601	α -bungarotoxin *from <i>Bungarus multicinctus</i> *	1 mg
B-13422	α -bungarotoxin, Alexa Fluor [®] 488 conjugate	500 μ g
B-13423	α -bungarotoxin, Alexa Fluor [®] 594 conjugate	500 μ g
B-1196	α -bungarotoxin, biotin-XX conjugate	500 μ g
B-7488	α -bungarotoxin, Oregon Green [®] 514 conjugate	500 μ g
B-13421	α -bungarotoxin, tetramethylrhodamine conjugate *>99% monolabeled*	25 μ g
B-7489	α -bungarotoxin, Texas Red [®] -X conjugate	500 μ g
C-13654	<i>N</i> -(α -carboxy-2-nitrobenzyl)carbamylcholine, trifluoroacetic acid salt (<i>N</i> -(CNB-caged) carbachol)	5 mg
C-7122	<i>N</i> -(α -carboxy-2-nitrobenzyl)-L-glutamic acid, trifluoroacetic acid salt (<i>N</i> -(CNB-caged) L-glutamic acid)	5 mg

Cat #	Product Name	Unit Size
F-1176	fluorescein α -bungarotoxin (α -bungarotoxin, fluorescein conjugate)	500 μ g
G-7055	L-glutamic acid, γ -(α -carboxy-2-nitrobenzyl) ester, trifluoroacetic acid salt (γ -(CNB-caged) L-glutamic acid)	5 mg
M-7114	N-methyl-D-aspartic acid, β -(α -carboxy-2-nitrobenzyl) ester, trifluoroacetic acid salt (β -(CNB-caged) NMDA)	1 mg
M-23400	muscimol, BODIPY [®] TMR-X conjugate	1 mg
N-1384	naloxone fluorescein	5 mg
N-1385	naltrexone fluorescein	5 mg
N-13437	neuromedin C, Alexa Fluor [®] 488 conjugate	25 μ g
S-13426	substance P, Alexa Fluor [®] 488 conjugate	25 μ g
S-13425	substance P, BODIPY [®] FL conjugate	25 μ g
S-13424	substance P, fluorescein conjugate	25 μ g
S-13427	substance P, Oregon Green [®] 488 conjugate	25 μ g
S-13428	substance P, tetramethylrhodamine conjugate	25 μ g
T-1175	tetramethylrhodamine α -bungarotoxin (α -bungarotoxin, tetramethylrhodamine conjugate)	500 μ g

16.3 Probes for Ion Channels and Carriers

This section describes a variety of probes for Ca^{2+} , Na^+ , K^+ and Cl^- ion channels and carriers. Chapter 20, Chapter 21 and Chapter 22 contain our extensive selection of indicators for these physiologically important ions, providing a means of correlating ion channel activation with subsequent changes in intracellular ion concentration. Ion flux also affects the cell's membrane potential, which can be measured with the probes described in Chapter 23.

Probes for Ca^{2+} Channels and Carriers

In both excitable and nonexcitable cells, intracellular Ca^{2+} levels modulate a multitude of vital cellular processes — including gene expression, cell viability, cell proliferation, cell motility and cell shape and volume regulation — thereby playing a key role in regulating cell responses to external activating agents. These dynamic changes in intracellular Ca^{2+} levels are regulated by ligand-gated and G-protein-coupled ion channels in the plasma membrane, as well as by mobilization of Ca^{2+} from intracellular stores. One of the best-studied examples of Ca^{2+} -dependent signal transduction is the depolarization of excitable cells, such as those of neuronal, cardiac, skeletal and smooth muscle tissue, which is mediated by inward Ca^{2+} and Na^+ currents. The Ca^{2+} current is attributed to the movement of ions through N-, L-, P- and T-type Ca^{2+} channels, which are defined both pharmacologically and by their biophysical properties, including conductance and voltage sensitivity. Molecular Probes offers fluorescent analogs of dihydropyridine and verapamil as ligands for L-type Ca^{2+} channels. In addition, we offer unlabeled and fluorescent derivatives of ryanodine, a powerful modulator of the intracellular Ca^{2+} channels found in the sarcoplasmic reticulum and other subcellular organelles.

Fluorescent Dihydropyridines for L-Type Ca^{2+} Channels

The L-type Ca^{2+} channel is readily blocked by the binding of dihydropyridine to the channel's pore-forming α_1 -subunit. To facilitate the study of channel number and distribution in single cells, Molecular Probes has developed fluorescent dihydropyridine derivatives. The high-affinity (–)-enantiomer of dihydropyridine is

available labeled with either the green-fluorescent DM-BODIPY (D-7443, Figure 16.37) or the orange-fluorescent ST-BODIPY (S-7445) fluorophore. Knaus and colleagues have shown that these BODIPY dihydropyridines bind to L-type Ca^{2+} channels with high affinity and inhibit the Ca^{2+} influx in GH_3 cells.¹ DM-BODIPY dihydropyridine has been employed to investigate the molecular mechanism for dihydropyridine binding to L-type channels. Upon binding to the α_1 -subunit, this ligand is reported to exhibit an increase in fluorescence quantum yield, as well as fluorescence resonance energy transfer (FRET, see Section 1.3) between its fluorophore and one or more of the channel's tryptophan residues.² For neuronal L-type Ca^{2+} channels, the (–)-enantiomers of the DM-BODIPY dihydropyridine and ST-BODIPY derivatives each exhibit a K_i of 0.9 nM. Their affinities for skeletal muscle L-type Ca^{2+} channels are somewhat lower. Although DM-BODIPY dihydropyridine exhibits a more intense fluorescence, the particularly high degree of stereoselectivity retained by the ST-BODIPY derivatives has proven useful for the *in vivo* visualization of L-type Ca^{2+} channels.

The spatial distribution and density of L-type Ca^{2+} channels in cultured olfactory neurons were determined by confocal laser-scanning microscopy using the ratio of the site-selective fluorescent staining produced by the (–)-enantiomer of DM-BODIPY dihydropyridine (D-7443, Figure 16.37) to the uniform fluorescent membrane staining by RH 414³ (T-1111, Section 14.4). In this study, RH 414 staining served to control for optical artifacts and

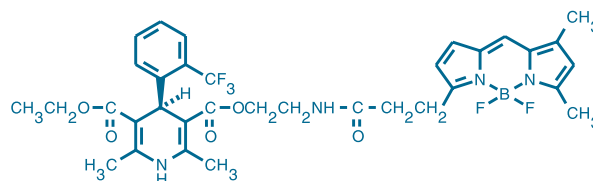


Figure 16.37 D-7443 DM-BODIPY (–)-dihydropyridine.