

5.3 Photoreactive Crosslinking and Labeling Reagents

Nonfluorescent Photoreactive Crosslinking Reagents

In contrast to chemical crosslinking reagents (Section 5.2), which are often used to prepare bioconjugates, photoreactive crosslinking reagents are important tools for determining the proximity of two sites. Thus, these probes can be employed to define relationships between two reactive groups on a protein, on a ligand and its receptor or on separate biomolecules within an assembly. In the lattermost case, photoreactive crosslinking reagents can potentially reveal interactions among proteins, nucleic acids and membranes in live cells. The general scheme for defining spatial relationships usually involves photoreactive crosslinking reagents that contain a chemically reactive group as well as a photoreactive group. These crosslinkers are first chemically reacted with one molecule, for example a receptor ligand, and then this modified molecule is coupled to a second molecule, for example the ligand's receptor, using UV illumination. Depending on the reactive properties of the chemical and photoreactive groups, these crosslinkers can be used to couple like or unlike functional groups.

Molecular Probes offers three types of photoreactive reagents for covalent labeling:

- Simple aryl azides that upon illumination (usually at <360 nm) generate reactive intermediates that form bonds with nucleophilic groups (Figure 5.8)
- Fluorinated aryl azides that upon UV photolysis generate reactive nitrenes, thereby producing more C–H insertion products than the simple aryl azides (Figure 5.9)
- Benzophenone derivatives that can be repeatedly excited at <360 nm until they generate covalent adducts, without loss of reactivity (Figure 5.10)

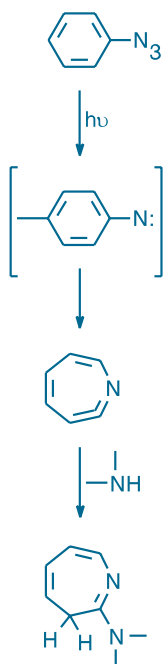


Figure 5.8 Photoreactive crosslinking reaction of a simple aryl azide.

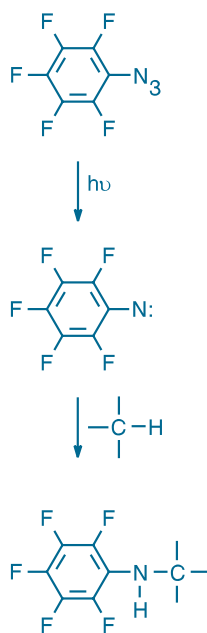


Figure 5.9 Photoreactive crosslinking reaction of a fluorinated aryl azide.

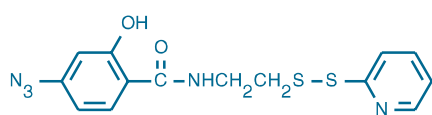


Figure 5.11 P-6317 *N*-((2-pyridyl)dithio)ethyl)-4-azidosalicylamide.

Simple Aryl Azide Crosslinker

The “transferable” aryl azide *N*-((2-pyridyl)dithio)ethyl)-4-azidosalicylamide (PEAS, P-6317; Figure 5.11) is a unique reagent for assessing protein–protein or protein–nucleic acid interactions. This aryl azide undergoes disulfide–thiol interchange of its pyridyldisulfide groups with the thiol groups of biomolecules to form mixed disulfides in the same way as SPDP¹ (S-1531, Section 5.2). UV photolysis induces covalent crosslinking to residues or biomolecules adjacent to the crosslinker. The mixed disulfide can then be cleaved with DTT or TCEP (D-1532, T-2556; Section 5.2). If the phenolic PEAS reagent is radioiodinated before the coupling and photolysis steps, then only the resulting target biomolecule will be radioactive at the conclusion of the reaction.

Fluorinated Aryl Azides: True Nitrene-Generating Reagents

Although the simple aryl azides may be initially photolyzed to electron-deficient aryl nitrenes, it has been shown that these rapidly ring-expand to form dehydroazepines — molecules that tend to react with nucleophiles rather than form C–H insertion products.^{2,3} In contrast, Keana and Cai have shown that the photolysis products of the fluorinated aryl azides are clearly aryl nitrenes⁴ and undergo characteristic nitrene reactions such as C–H bond insertion with high efficiency. Moreover, conjugates prepared from the amine-reactive succinimidyl ester (A-2522) or the more water-soluble STP ester (A-10661) of 4-azido-2,3,5,6-tetrafluorobenzoic acid may have quantum yields for formation of photo-crosslinked products that are superior to those of the nonfluorinated aryl azides. An

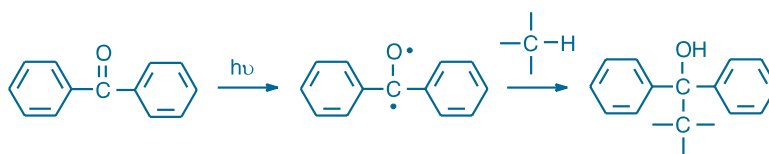


Figure 5.10 Photoreactive crosslinking reaction of a benzophenone derivative.

important application of these reactive derivatives of 4-azido-2,3,5,6-tetrafluorobenzoic acid is the photofunctionalization of polymer surfaces^{5,6} (Figure 5.12). 4-Azido-2,3,5,6-tetrafluorobenzyl amine (A-10662) is a useful building block for other fluorinated photo-reactive reagents.

Benzophenone-Based Photoreactive Reagents

Benzophenones generally have higher crosslinking yields than the aryl azide photoreactive reagents.⁷ Benzophenone maleimide (B-1508) has been used for efficient irreversible protein crosslinking of actin,⁸ calmodulin,^{9,10} myosin,^{11,12} tropomyosin,¹³ troponin,¹⁴⁻¹⁷ ATP synthase^{18,19} and other proteins. The succinimidyl ester of 4-benzoylbenzoic acid (B-1577) and benzophenone isothiocyanate (B-1526) have proven useful for synthesizing photoreactive peptides²⁰⁻²³ and oligonucleotides.^{24,25} Benzophenone-labeled ATP and GTP probes are described below.

Other Photoreactive Reagents

Ethidium Monoazide for Photoreactive Fluorescent Labeling of Nucleic Acids

Ethidium monoazide (E-1374) can be photolyzed in the presence of DNA or RNA to yield fluorescently labeled nucleic acids, both in solution and in cells.²⁶⁻²⁹ The efficiency of the irreversible photolytic coupling of ethidium monoazide, which intercalates into nucleic acids like ethidium bromide, is unusually high³⁰ (>40%). Photolabeling of DNA can be used to follow its transport,³¹ phase transitions³² and diffusion.³³ In addition to its utility for studying DNA dynamics, the membrane-impermeant ethidium monoazide is reported to label only those cells with compromised membranes and can therefore serve as a fixable cell viability probe. A mixed population of live and dead cells labeled with this reagent retains its staining pattern after aldehyde-based fixation,^{34,35} thereby reducing the investigator's exposure to potentially pathogenic cells during cell viability analysis. Also, leukocyte phagocytosis was investigated by flow cytometry using ethidium monoazide-labeled *Candida albicans*.³⁶ Multiphoton-targeted photochemistry of vertebrate cells labeled with ethidium monoazide was used to selectively inactivate gene expression.³⁷ Photolyzed ethidium monoazide is reported to activate topoisomerase II-mediated single- and double-stranded DNA cleavage.³⁸

Photoreactive ATP and GTP Derivatives for Labeling Nucleotide-Binding Proteins

Functional ion channels can be assembled from both homomeric and heteromeric combinations of the seven P2X receptor subunits so far identified (P2X₁₋₇). Due to the lack of specific agonists or antagonists for P2X receptors, it is difficult to determine which receptor subtypes mediate particular cellular responses. One of the most potent and widely used P2X receptor agonists, BzBzATP (2'-(or-3')-O-(4-benzoylbenzoyl)-adenosine 5'-triphosphate, B-22358; Figure 5.13), is available from Molecular Probes,³⁹⁻⁴² along with the corresponding GTP derivative (B-22357). Both BzBzATP and BzBzGTP have more general applications for site-directed irreversible modification of nucleotide-binding proteins via photoaffinity labeling.

References

- Bioconjug Chem 7, 380 (1996);
- Annu Rev Biochem 62, 483 (1993);
- Adv Photochem 17, 69 (1992);
- J Org Chem 55, 3640 (1990);
- Bioconjug Chem 5, 151 (1994);
- J Am Chem Soc 115, 814 (1993);
- Biochemistry 33, 5661 (1994);
- Arch Biochem Biophys 240, 627 (1985);
- Biochemistry 33, 518 (1994);
- J Biol Chem 263, 542 (1988);
- Arch Biochem Biophys 288, 584 (1991);
- J Biol Chem 266, 2272 (1991);
- Biochemistry 25, 7633 (1986);
- J Muscle Res Cell Motil 19, 479 (1998);
- Biochemistry 35, 11026 (1996);
- Science 247, 1339 (1990);
- Biochemistry 26, 7042 (1987);
- J Biol Chem 273, 15162 (1998);
- J Biol Chem 271, 28341 (1996);
- J Virol 38, 840 (1981);
- J Protein Chem 3, 479 (1985);
- Proc Natl Acad Sci U S A 83, 483 (1986);
- Biochemistry 32, 2741 (1993);
- Nucleic Acids Res 26, 1421 (1998);
- Bioconjug Chem 10, 56 (1999);
- Biochemistry 30, 5644 (1991);
- Photochem Photobiol 43, 7 (1986);
- J Biol Chem 259, 11090 (1984);
- Photochem Photobiol 36, 31 (1982);
- Biochemistry 20, 1887 (1981);
- J Biol Chem 269, 4910 (1994);
- Biochemistry 30, 10931 (1991);
- Macromolecules 22, 4550 (1989);
- Cytometry 19, 243 (1995);
- Cytometry 12, 133 (1991);
- Cytometry 11, 610 (1990);
- Proc Natl Acad Sci U S A 97, 9504 (2000);
- Biochemistry 36, 15884 (1997);
- Biochemistry 26, 7524 (1987);
- Proc Natl Acad Sci U S A 90, 10449 (1993);
- J Physiol 519 Pt 3, 723 (1999);
- Mol Pharmacol 56, 1171 (1999).

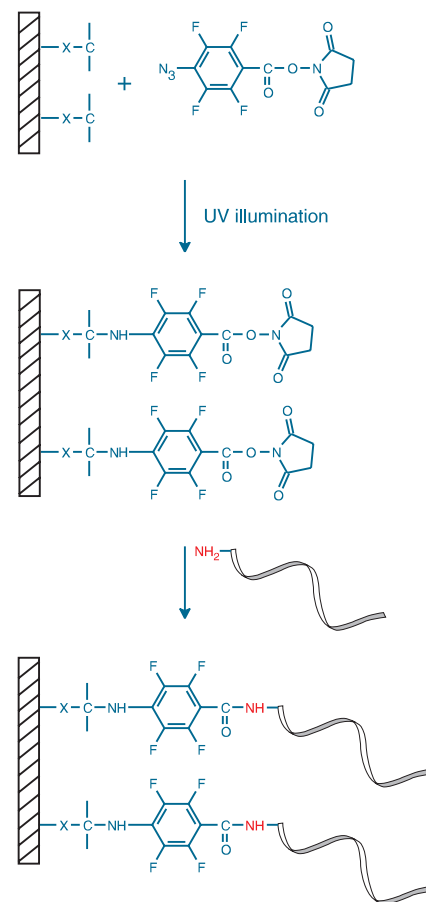


Figure 5.12 Schematic showing attachment of an amine-modified oligonucleotide to a surface using the photoreactive crosslinking reagent 4-azido-2,3,5,6-tetrafluorobenzoic acid, succinimidyl ester (ATFB, SE; A-2522).

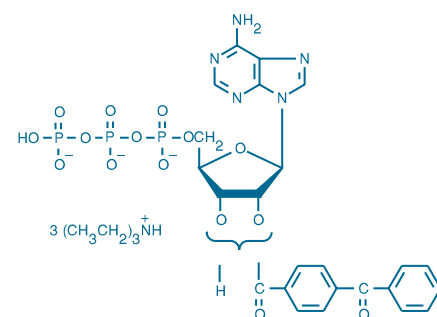


Figure 5.13 B-22358 2'-(or-3')-O-(4-benzoylbenzoyl)adenosine 5'-triphosphate, tris(triethylammonium) salt.

In addition to its utility for photolabeling ATP-binding sites, BzBzATP (B-22358) is a potent agonist for P2X nucleotide receptors.

Data Table — 5.3 Photoreactive Crosslinking and Labeling Reagents

Cat #	MW	Storage	Soluble	Abs	EC	Em	Solvent	Notes
A-2522	332.17	F,D,LL	DMF	273	23,000	none	EtOH	1
A-10661	485.20	F,D,LL	H ₂ O	273	27,000	none	MeOH	1, 2
A-10662	256.59	F,LL	DMF	250	17,000	none	MeOH	1
B-1508	277.28	F,D	DMF, MeCN	282	9,000	none	MeOH	1, 3
B-1526	239.29	F,DD	DMF, MeCN	300	26,000	none	MeOH	1
B-1577	323.30	F,D	DMF, MeCN	256	27,000	none	MeOH	1
B-22357	1034.97	FF,L	H ₂ O	257	24,000	none	pH 7	1, 4, 5, 6
B-22358	1018.97	FF,L	H ₂ O	260	27,000	none	pH 7	1, 4, 5, 6
E-1374	420.31	F,LL	DMF, EtOH	462	5,400	625	pH 7	7
P-6317	347.41	F,D,LL	DMSO	271	24,000	none	MeOH	8

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

Notes

1. This compound has weak visible absorption at >300 nm but no discernible absorption peaks in this region.
2. This sulfotetrafluorophenyl (STP) ester derivative is water soluble and may be dissolved in buffer at ~pH 8 for reaction with amines. Long-term storage in water is NOT recommended due to hydrolysis.
3. Spectral data of the 2-mercaptoethanol adduct.
4. The molecular weight (MW) of this product is approximate because the degree of hydration and/or salt form has not been conclusively established.
5. This product is supplied as a ready-made solution in the solvent indicated under **Soluble**.
6. This product can be activated by long-wavelength ultraviolet light (>300 nm) for photoaffinity labeling of proteins.
7. E-1374 spectral data are for the free dye. Fluorescence is weak, but intensity increases ~15-fold on binding to DNA. After photocrosslinking to DNA, Abs = 504 nm (EC ~4000 cm⁻¹M⁻¹), Em = 600 nm (Nucleic Acids Res 5, 4891 (1978); Biochemistry 19, 3221 (1980)).
8. The absorption spectrum of P-6317 includes an additional shoulder at 306 nm (EC = 10,000 cm⁻¹M⁻¹)

Product List — 5.3 Photoreactive Crosslinking and Labeling Reagents

Cat #	Product Name	Unit Size
A-10661	4-azido-2,3,5,6-tetrafluorobenzoic acid, STP ester, sodium salt (ATFB, STP ester)	10 mg
A-2522	4-azido-2,3,5,6-tetrafluorobenzoic acid, succinimidyl ester (ATFB, SE)	25 mg
A-10662	4-azido-2,3,5,6-tetrafluorobenzyl amine, hydrochloride	25 mg
B-1526	benzophenone-4-isothiocyanate	100 mg
B-1508	benzophenone-4-maleimide	100 mg
B-1577	4-benzoylbenzoic acid, succinimidyl ester	100 mg
B-22358	2'-(or-3')-O-(4-benzoylbenzoyl)adenosine 5'-triphosphate, tris(triethylammonium) salt (BzBzATP) *5 mM in buffer*	2 mL
B-22357	2'-(or-3')-O-(4-benzoylbenzoyl)guanosine 5'-triphosphate, tris(triethylammonium) salt (BzBzGTP) *5 mM in buffer*	2 mL
E-1374	ethidium monoazide bromide (EMA)	5 mg
P-6317	N-((2-pyridyldithio)ethyl)-4-azidosalicylamide (PEAS; AET)	10 mg

Our Bibliography Database Has Been Enhanced



Links to PubMed

Molecular Probes provides extensive bibliographies of published applications for most of our products. We started collecting these references in 1975 and now have more than 44,000 entries listed in our database. We have made a major enhancement to this database by linking most of the citations to the corresponding PubMed listing, including the abstract of each article (when available). This linking process has also permitted us to list all authors and full pagination for each citation. The links to PubMed are available now from any bibliography listing on our Web site.

Product-specific bibliographies are available for most products at www.probes.com/products.

The entire bibliography database can also be searched at www.probes.com/search.

Reference Submission Form

Although we are diligent about searching for and collecting citations that might be useful to our customers, we may miss important papers. Please submit any papers that we should include in our bibliography database. We have a convenient online form that can be used to alert us to citations at www.probes.com/resources/submitref.html.