Light sources and conditions for photoactivation of aryl azide crosslinking and labeling reagents

TR0011.2

Introduction

Crosslinkers and labeling reagents that contain aryl azide or diazirine functional groups are capable reacting to form covalent bonds with other molecules when activated by ultraviolet light. The nonspecific and activatable properties of this class of reagents makes them particularly useful for many research applications including the study of protein:protein interactions, isolating cell surface proteins and preparing labeled probes. Several specific forms of aryl azide compounds exist (Figure 1) that function by the same general reaction mechanism but differ slightly in stability, efficiency and absorbance maximum. The diazirine group is smaller photo-reactive functional group that has been recently developed for use in crosslinker compounds, including activated amino acids. Psoralen is a photoactivatable group that conjugates to nucleic acids.

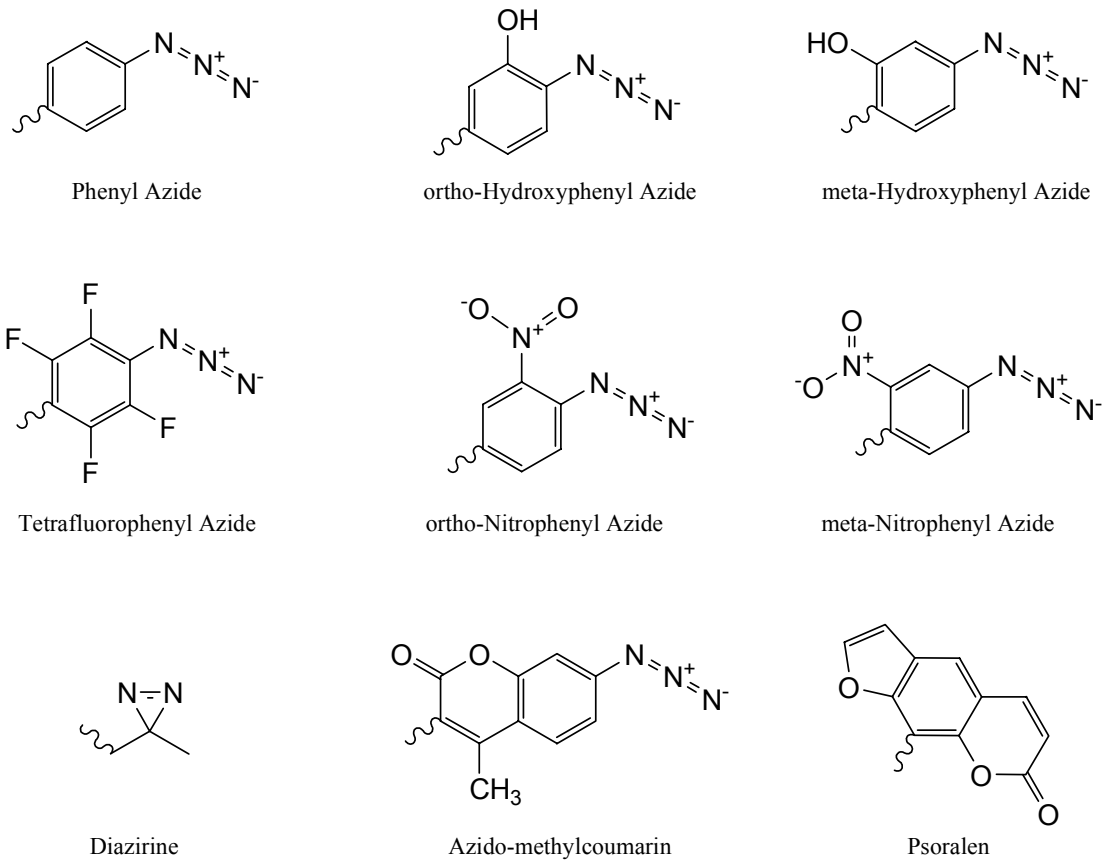

Figure 1. Forms of aryl azide and other reactive groups used in photoreactive crosslinking reagents.

When an aryl azide is exposed to UV-light, it forms a nitrene group that can initiate addition reactions with double bonds, insertion into C-H and N-H sites, or subsequent ring expansion to react as a nucleophile with primary amines (Figure 2). The latter reaction path dominates when primary amines are present in the sample. Details of the diazirine reaction are less well known. Photoactivation occurs with long wave UV light (330-370 nm), creating reactive carbene intermediates that form covalent bonds through addition reactions with N_2 as a byproduct.

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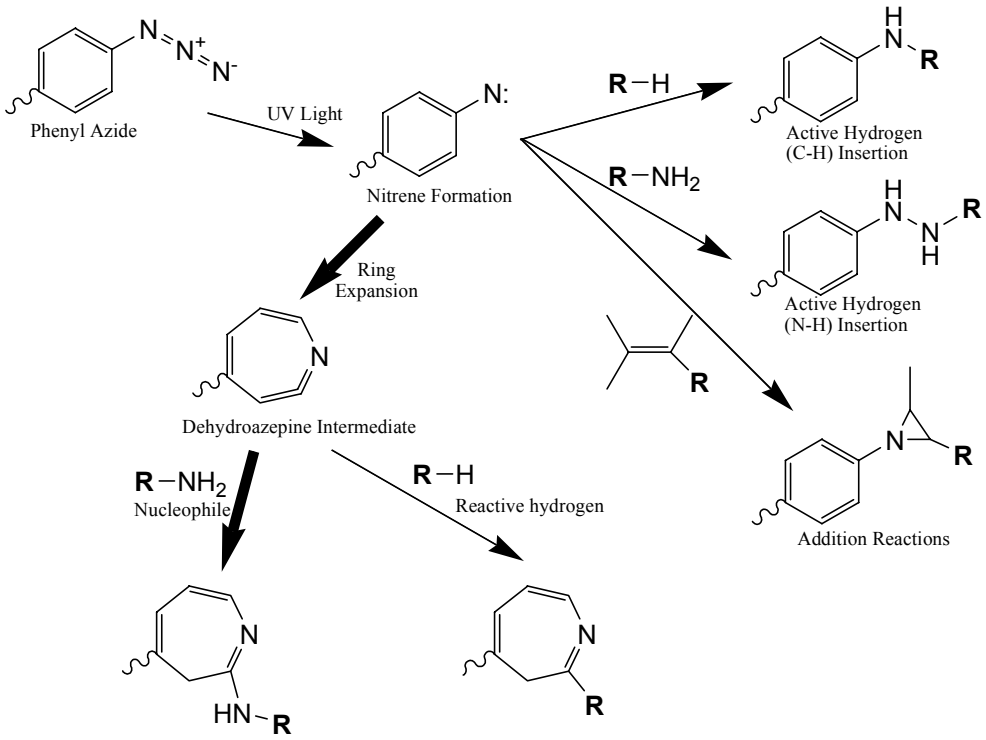

Figure 2. Possible reaction pathways of aryl azide crosslinkers.

Conditions for Photoactivation

A question that often arises with respect to aryl azide linkers is what wavelength and intensity of light is optimal for photoactivation and efficient crosslinking. Pierce researchers have performed only a limited number of experiments to explore these conditions. However, one set of data was generated for the homobifunctional hydroxyphenyl azide crosslinker BASED (Product No. 21564). Experiments with three different lamp sources and several exposure times indicated that activation with long wavelength UV-light (366 nm) for 30 minutes yields the most efficient (complete) crosslinking of the target molecule (in this case, a peptide) and depletion of free crosslinker (Table 1). These data for BASED are probably representative for all the aryl azide reagents, although short wavelength may be better than long wavelength for plain phenyl azides (no hydroxy or nitro group on the phenyl ring). Examination of Table 1 and perusal of the literature (Table 2) indicate that successful photoactivation with these reagents is possible across a wide range of wavelength and time of exposure.

In addition to choosing an appropriate lamp source for photoactivation, consider the following points when preparing conjugation reactions with aryl azide crosslinkers:

- When microcentrifuge tubes are used for the sample, it is most effective to open the cap so that the sample may be exposed directly rather than through the polypropylene sidewall, which shields most of the UV-light. Quartz spectrophotometric cuvettes are an ideal choice for reaction vessels because they allow for optimal exposure of the sample to the UV-light source.
- Samples will become warm or even hot if exposed to intense UV-light for several minutes. Place the sample vial on ice or use some other method to keep the sample cool during UV-light activation.
- Avoid thiol-containing reducing agents (e.g., DTT or 2-mercaptoethanol) in the sample solution during all steps before and during photoactivation. These reagents will reduce the azide functional group to an amine, preventing photoactivation.
- Avoid buffers that contain primary amines (e.g., Tris or glycine) during photoactivation because these will quench the desired reaction. Reaction of the photoactivated aryl azide groups to primary amines dominates if they are present.

Table 1. Lamp conditions and crosslinking efficiency using BASED (Product No. 21564). Identical reactions with BASED and peptide were prepared and photoactivated by exposure at different wavelengths and lengths of time. Conjugation efficiency was determined by HPLC measurement of the percent peptide and crosslinker depleted relative to the starting material (greater depletion corresponds to more complete crosslinking).

Sample	Time	% Peptide depleted	% BASED depleted
Long Wave UV-Light (366 nm)	5 min	41.46	46.79
	15 min	47.92	77.14
	30 min	61.46	94.33
Short Wave UV-Light (254 nm)	5 min	11.26	9.61
	15 min	——	——
	30 min	14.46	24.82
550 Watt Light (Broad spectrum visible)	10 sec	27.87	31.51
	30 sec	——	30.52
	60 sec	42.43	58.54
550 Watt Light (Broad spectrum visible)	6 flashes, each 1 sec	3.82	18.38
	12 flashes, each 1 sec	19.50	29.95
	18 flashes, each 1 sec	12.26	32.23
Combination	15 min Long UV-Light	35.23	74.60
	Plus 12 flashes	37.76	76.54

Table 2. Lamp sources and conditions used for photoactivation of aryl azide crosslinkers. The information in this table was compiled from older literature references (pre-2000) and is provided only to exemplify the types of conditions used by researchers. For best results, use a high-quality, multifunctional UV lamp, such as our 3UV Lamp, Product No. 95034 (115V) or 95035 (230 V).

Lamp mentioned in citation	Distance from sample	Wavelength	Time
Rayonet Photochemical Reactor (UltraViolet Products, Inc.)	2 cm	300 nm	?*
Model UVGL-15 Mineralight	?	254-360 nm	10 min
Fotodyne UV Transilluminator	5 cm	?	2 min
750 W mercury lamp (Scaeffel Instrument Co.)	20 cm	320 nm	?
Black Ray Model XX-15C, 0.41A	10 cm	?	5 min
Edmund Scientific No. 60889 (9 W, 12 in. UV tube)	2 mm	>300 nm	120 min
Black Ray Model B-100A	10 cm	>300 nm	5 min
Transilluminator UV light box (UltraViolet Products, Inc.)	3.5 cm	302 nm	5 min
15 W UV lamp	1 cm	365 nm	10 min
Rayonet UV Light Reactor (Southern N.E. Ultraviolet Co.)	10 cm	370 nm	5 min
Chromato-Vue C3 viewing box (Ultraviolet Products, Inc.)	10 cm	254 and 365 nm	20 min
Universal UV lamp (Camag Muttenz, Switzerland)	1 cm	254 nm	2 min
UV SL-25 4 W Mineralight (Ultraviolet Products, Inc.)	1 cm	254 cm	10 min

Thermo Scientific Pierce Products with Photoreactive Functional Groups

		Reactive Groups		Spacer Arm	
Product Name	**Product No.**	**Photoreactive**	**Other Group(s)**	**Length (Å)**	**Cleavable?**
ABH	**21510**	Phenyl azide	Hydrazide	11.9	No
ANB-NOS	**21451**	Nitrophenyl azide	NHS	7.7	No
APDP	**27720**	Hydroxyphenyl azide	Pyridyldisulfide	21.0	Yes
ASBA	**21512**	Hydroxyphenyl azide	Amine	16.3	No
BASED	**21564**	Hydroxyphenyl azide	Hydroxyphenyl azide	34.7	Yes
Mts-Atf-Biotin **Mts-Atf-LC-Biotin**	**33093** **33083**	Tetrafluorophenal azide	Methanethiosulfonate and Biotin	11-31 21-35	Yes
NHS-ASA	**27714**	Hydroxyphenyl azide	NHS	8.0	No
SANPAH	**22600**	Nitrophenyl azide	NHS	18.2	No
SPB	**23019**	Psoralen	NHS	8.6-9.5	No
Sulfo-HSAB	**21563**	Phenyl azide	Sulfo-NHS	9.0	No
Sulfo-NHS-LC-ASA	**27735**	Hydroxyphenyl azide	Sulfo-NHS	18.0	No
Sulfo-SAED	**33030**	Azido-methylcoumarin	Sulfo-NHS	23.6	Yes
Sulfo-SAND	**21549**	Nitrophenyl azide	Sulfo-NHS	18.5	Yes
Sulfo-SFAD	**27719**	Perfluoroaryl azide	Sulfo-NHS	14.6	Yes
Sulfo-SANPAH	**22589**	Nitrophenyl azide	Sulfo-NHS	18.2	No
Sulfo-SBED	**33033**	Phenyl azide	Sulfo-NHS/Biotin	14-25	Yes
SDA	**26167**	Diazirine	NHS	3.9	No
LC-SDA	**26168**	Diazirine	NHS	12.5	No
SDAD	**26169**	Diazirine	NHS	13.5	Yes
Sulfo-SDA	**26173**	Diazirine	Sulfo-NHS	3.9	No
Sulfo-LC-SDA	**26174**	Diazirine	Sulfo-NHS	12.5	No
Sulfo-SDAD	**26175**	Diazirine	Sulfo-NHS	13.5	Yes
Psoralen-PEG$_3$-Biotin	**29986**	Psoralen	Biotin	36.9	Yes
Photoactivatable Biotin	**29987**	Nitrophenyl azide	Biotin	30.0	No
Biotin-LC-ASA	**29982**	Hydroxyphenyl azide	Biotin	29.9	No
TFPA-PEG$_3$-Biotin	**21303**	Tetrafluorophenal azide	Biotin	33.4	No
L-Photo-Leucine	**22610**	Diazirine	Leucine	N/A	No
L-Photo-Methionine	**22615**	Diazirine	Methionine	N/A	

Current versions of product instructions are available at *www.thermo.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.