

## 2.3 Thiol-Reactive Probes Excited with Ultraviolet Light

The thiol-reactive dyes in this section have their longest-wavelength absorption peaks at less than ~400 nm (Table 2.2). Typically these dyes exhibit blue fluorescence and have much weaker absorption than the dyes described in Section 2.2, with extinction coefficients often below 20,000 cm<sup>-1</sup>M<sup>-1</sup>. Photostability of UV light-excitable dyes typically is less than that of visible light-excitable dyes. The strong environmental dependence of the emission spectra and quantum yields of several of the dyes — especially the coumarin, benzoxadiazole (NBD and SBD, Section 2.2), aminonaphthalene (e.g., dansyl) and Dapoxyl fluorophores — makes some probes useful for investigating protein structure and assembly, following protein transport through membranes and studying ligand binding to receptors. The spectra of certain dyes tend to be particularly sensitive to ligand and metal binding, protein association and chaotropic reagents. When protein conjugates of these dyes are denatured or undergo a change in conformation, a decrease in fluorescence intensity and a shift in emission to longer wavelengths are often observed.

### Coumarin Derivatives

#### Alexa Fluor 350 C<sub>5</sub> Maleimide

Alexa Fluor 350 C<sub>5</sub> maleimide<sup>1</sup> (A-20380, Figure 2.19), a thiol-reactive, sulfonated coumarin derivative, produces protein conjugates that are optimally excited at 346 nm and have bright-blue fluorescence emission (Figure 7.3) at wavelengths slightly shorter than AMCA or AMCA-X conjugates (Figure 10.3) (442 nm versus 448 nm), which reduces the dye's spectral overlap with the emission of fluorescein. Like our other Alexa Fluor dyes, Alexa Fluor 350 C<sub>5</sub> maleimide offers unrivaled brightness and pH-independent fluorescence, as well as water solubility and a low degree of quenching upon conjugation (see The Alexa Fluor Dye Series — Peak Performance Across the Visible Spectrum in Section 1.3).

#### Other Coumarin Maleimides and Iodoacetamides

7-Diethylamino-3-(4'-maleimidylphenyl)-4-methylcoumarin (CPM, D-346; Figure 2.20) and the corresponding iodoacetamide (DCIA, D-404) are among the best UV light-excitable fluorescent thiol reagents available. Not only do these reagents cost less than the spectrally similar *N*-(7-dimethylamino-4-methylcoumarin-3-yl)maleimide (DACM, D-10251) and iodoacetamide (DACIA, D-10252), but they yield conjugates that are more fluorescent than the corresponding DACM adducts. The coumarin fluorophore is an excellent fluorescence energy acceptor from tryptophan and a good donor to fluorescein, eosin, Alexa Fluor 488, selected BODIPY dyes<sup>2</sup> and our essentially nonfluorescent QSY 7, QSY 9, QSY 21 and QSY 35 quenchers (Section 1.6, Section 1.8, Section 2.2, Section 3.3), making these thiol-reactive coumarins especially valuable for studying protein structure and for detecting protein-membrane interactions. Fluorescence emission of the coumarin conjugates is moderately sensitive to environment.

The maleimides DACM and CPM are essentially nonfluorescent until they react with thiols, permitting thiol quantitation without a separation step. CPM has been used to follow the release of picomoles of thiols from acetylthiocholine by acetylcholinesterase<sup>3-5</sup> and to determine cystamine, cysteamine<sup>6</sup> and thiol content of proteins, cells and plasma.<sup>7-10</sup> CPM can also be used to quantitate thiols using a fluorescence microplate reader.<sup>11</sup> DACM has similar applications as a derivatization and quantitation reagent for low molecular weight thiols.<sup>12-17</sup>

Nucleolar protein staining by CPM has been used to distinguish highly proliferating cancer cells in a flow cytometry assay.<sup>18</sup> In addition to modifying proteins, CPM reacts with thiophosphorylated RNA<sup>19</sup> and thiolated oligonucleotides.<sup>20,21</sup> DACM has been extensively used as a probe for protein structure,<sup>22-25</sup> as a fluorescence energy transfer donor and acceptor<sup>26</sup> and as a tool for localizing thiols in cells and tissues.<sup>27</sup>

We also offer 7-diethylamino-3-(((2-maleimidyl)ethyl)amino)carbonyl)coumarin (MDCC, D-10253; Figure 2.21) and the corresponding iodoacetamide (IDCC, D-20382). When conjugated to a mutant phosphate-binding protein, MDCC has proven useful for

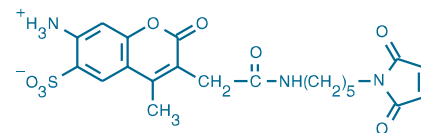


Figure 2.19 A-20380 Alexa Fluor 350 C<sub>5</sub> maleimide.

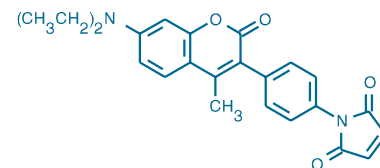


Figure 2.20 D-346 7-diethylamino-3-(4'-maleimidylphenyl)-4-methylcoumarin.

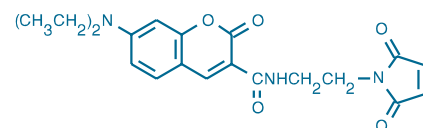
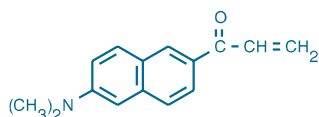


Figure 2.21 D-10253 7-diethylamino-3-(((2-maleimidyl)ethyl)amino)carbonyl)coumarin.

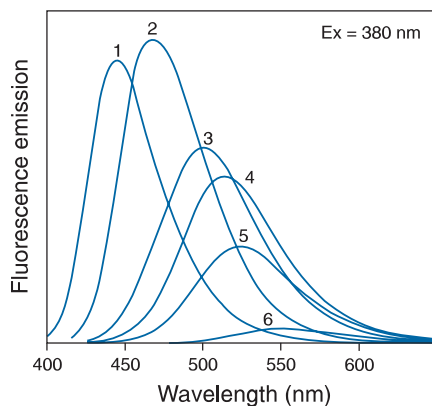
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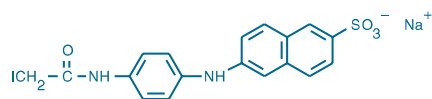
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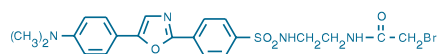
**Figure 2.22** A-433 6-acryloyl-2-dimethylaminonaphthalene.



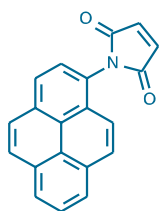
**Figure 2.23** Fluorescence emission spectra of the 2-mercaptoethanol adduct of badan (B-6057) in: 1) toluene, 2) chloroform, 3) acetonitrile, 4) ethanol, 5) methanol and 6) water. Each solution contains the same concentration of the adduct. Excitation of all samples is at 380 nm.



**Figure 2.24** 1-(4-(2-(iodoacetamido)anilino)naphthalen-6-yl)sulfonic acid, sodium salt.



**Figure 2.25** D-10300 Dapoxyl (2-bromoacetamidoethyl)sulfonamide.



**Figure 2.26** P-28 *N*-(1-pyrene)maleimide.

direct, real-time measurement of inorganic phosphate release during enzymatic reactions.<sup>28–33</sup> An IDCC conjugate of a mutant nucleoside diphosphate kinase has been used as a fluorescent sensor of the phosphorylation state of the kinase and to monitor purine nucleoside diphosphate concentrations in real time.<sup>34</sup> MDCC and IDCC are also useful reagents for general labeling of thiols with bright-blue fluorescence.

## Naphthalene Derivatives, Including Thiol-Specific Didansyl Cystine

### Acrylodan and Its Bromoacetyl Analog

Although acrylodan (A-433, Figure 2.22) and 6-bromoacetyl-2-dimethylaminonaphthalene (badan, B-6057) generally react with thiols more slowly than do iodoacetamides or maleimides, they form very strong thioether bonds that are expected to remain stable under conditions required for complete amino acid analysis. The fluorescence emission peak and intensity of these adducts (Figure 2.23) are particularly sensitive to conformational changes or ligand binding, making these dyes some of the most useful thiol-reactive probes for protein structure studies.<sup>35–38</sup> For example, the acrylodan conjugate of an intestinal fatty acid-binding protein, ADIFAB (A-3880, Section 18.4), is a sensor for free fatty acids<sup>39</sup> (Figure 18.44). Also, myosin regulatory light chain labeled with acrylodan shows a spectral response upon phosphorylation by myosin light chain kinase.<sup>40</sup> The environment-sensitive spectral shifts of acrylodan and badan conjugates (Figure 2.23) may make these probes useful for distinguishing thiols that are located in membranes versus those exposed to aqueous solvation in cells.

**Table 2.2** Thiol-reactive dyes excited with ultraviolet light.

Derivative	Abs *	Em *	Alkyl Halide or Haloacetamide	Maleimide	Other
Alexa Fluor 350	346	442		A-20380	
Anilinnaphthalene †	326	462	IAANS, I-7	MIANS, M-8	
Benzophenone	282	NA		B-1508	
Dansyl †	328	563			D-146 ‡ D-151 §
Dapoxyl †	374	572	D-10300		
Dibromobimane	394	490			bBBr, D-1379 **
Diethylaminocoumarin	384	470	DCIA, D-404 IDCC, D-20382	CPM, D-346 ** MDCC, D-10253	
Dimethylaminocoumarin	376	465	DACIA, D-10252	DACM, D-10251 **	
Dimethylaminonaphthalene †	391	500	badan, B-6057		acrylodan, A-433
Monobromobimane	394	490			mBBr, M-1378 ** mBBr, M-20381 ** †† qBBr, M-1380 **
Monochlorobimane	394	490			mBCl, M-1381 **
Naphthalene †	336	490	IAEDANS, I-14		
Pyrene †	339	384	P-29 P-2007	P-28	
Stilbene †	329	408	A-484	A-485	

NA = not applicable.

\* Approximate absorption (Abs) and emission (Em) maxima, in nm for the reagent (if fluorescent) or the fluorescent thiol adduct. † Environmentally sensitive fluorophore. ‡ Disulfide; undergoes a thiol–disulfide interchange to form a mixed disulfide. § Dansyl aziridine. \*\* Very weakly fluorescent until reacted with thiols. †† FluoroPure grade.

## IAANS and MIANS

To develop appreciable fluorescence, both the reactive anilino-naphthalenesulfonate iodoacetamide (IAANS, I-7; Figure 2.24) and maleimide (MIANS, M-8) must be reacted with thiols that are located in hydrophobic sites. Often, however, buried unsolvated thiol residues are exceptionally reactive, allowing these sites to be selectively modified by these reagents. The environmentally sensitive fluorescence properties of the protein conjugates of MIANS and IAANS are similar to those of the structurally related probes 1,8-ANS and 2,6-TNS (A-47, T-53; Section 13.5). The fluorescence intensity, and to a lesser extent, the emission wavelengths of the conjugates, tend to be very sensitive to substrate binding, folding and unfolding of the protein, changes in ionic strength and association of the labeled protein with other proteins, membranes or nucleic acids. Like most other maleimides, MIANS (also called Mal-ANS) is essentially nonfluorescent until it has reacted with a thiol. Both IAANS and MIANS have been widely used for protein structural studies, particularly of contractile proteins.<sup>41–43</sup>

## IAEDANS

The fluorescence of IAEDANS (I-14) is quite dependent upon environment, although much less so than that of IAANS and MIANS conjugates. Its conjugates frequently respond to ligand binding by undergoing spectral shifts and changes in fluorescence intensity that are determined by the degree of aqueous solvation. Advantages of this reagent include high water solubility above pH 4 and a relatively long fluorescence lifetime (sometimes >20 nanoseconds, although commonly 10–15 nanoseconds), making the conjugates useful for fluorescence polarization (FP, see Section 1.4) and rotational studies.<sup>44–47</sup> In addition, because it has a large Stokes shift<sup>48</sup> and an emission that overlaps well with the absorption of fluorescein, Alexa Fluor 488, Oregon Green dyes and BODIPY FL dyes, IAEDANS is an excellent reagent for fluorescence resonance energy transfer (FRET, see Section 1.3) measurements of proximity up to about 60 Å.<sup>49,50</sup> IAEDANS usually reacts with thiols; however, it has been reported to react with a lysine residue in tropomyosin.<sup>51</sup>

## Dansyl Aziridine

Another probe with environmentally sensitive spectral properties, dansyl aziridine (D-151), forms very strong thioether bonds that remain stable under conditions required for complete amino acid analysis. Although it reacts with a methionine residue in troponin C,<sup>52</sup> dansyl aziridine is primarily a thiol-reactive reagent. The fluorescence of the calmodulin and troponin C conjugates of dansyl aziridine is sensitive to Ca<sup>2+</sup> binding.<sup>53–56</sup>

## *N,N'*-Didansyl-L-Cystine

Disulfides such as *N,N'*-didansyl-L-cystine (D-146) are the only type of fluorescent thiol-specific reagents available. Disulfide derivatives undergo a thiol–disulfide interchange reaction to form mixed disulfides<sup>57</sup> (Figure 2.4). The fluorescent disulfide that is initially formed, however, can subsequently transfer its fluorophore to neighboring thiols. The disulfide linkage formed by didansyl cystine can also be cleaved with reagents such as DTT or TCEP (D-1532, T-2556; Section 2.1).

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## Dapoxyl Derivatives

Our Dapoxyl dyes have good absorptivity and exceptionally high environmental sensitivity.<sup>58</sup> Although optimally excited in the UV near 370 nm, their emission maxima range from about 450 nm to 650 nm, depending on the solvent (Figure 1.98). Dapoxyl (2-bromoacetamidoethyl)sulfonamide<sup>59</sup> (D-10300, Figure 2.25) alkylates a thiol group; amine-reactive Dapoxyl derivatives are described in Section 1.7, and additional reactive Dapoxyl dyes are described in Section 3.3.

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## Pyrene Derivatives

### Pyrene Maleimide

Not only is *N*-(1-pyrene)maleimide (pyrene maleimide, P-28) essentially nonfluorescent until it has reacted with thiols, but once excited, pyrene–thiol conjugates can interact to form excited-state dimers (excimers) that emit at longer wavelengths than the lone excited fluorophore. Pyrene maleimide conjugates have very long fluorescence lifetimes (>100 nanoseconds), giving proximal pyrene rings within 6–10 Å of each other ample time to form the spectrally altered excimer (Figure 13.8). Excimers may form between labeled sites in a single protein, as they do in tropomyosin,<sup>60–63</sup> lens crystallins,<sup>64</sup> apolipoprotein III<sup>65</sup> and sarcoplasmic reticulum ATPase,<sup>66,67</sup> or between sites in interacting biomolecules. Excimer formation can be used to monitor diffusion or to define interacting molecules within a functional unit of assembled biomolecules. Despite its low solubility in water, pyrene maleimide has been conjugated to a wide variety of proteins<sup>68–72</sup> and used as an HPLC derivatization reagent for thiols and reduced disulfides.<sup>73</sup> In several papers, *N*-(1-pyrene)maleimide (P-28, Figure 2.26) has been incorrectly named *N*-(3-pyrene)maleimide or variants of that nomenclature.

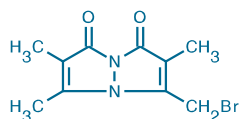
### Pyrene Iodoacetyl Derivatives

Fluorescence of the actin monomer labeled with pyrene iodoacetamide (P-29) has been demonstrated to change upon polymerization, making this probe an excellent tool for following the kinetics of actin polymerization.<sup>74–76</sup> Pyrene iodoacetamide has been cited for this application in literally hundreds of publications. Using pyrene iodoacetamide, researchers have investigated the influence of several actin-binding proteins and of cytochalasin on the rate of actin polymerization. Conjugates of *N*-(1-pyrenemethyl)iodoacetamide (P-2007) have the longest excited-state fluorescence lifetimes (>100 nanoseconds) of all reported thiol-reactive probes. The tendency of doubly pyrene dye-labeled nucleic acid probes to form excited-state dimers with altered fluorescence emission enables their use in homogeneous hybridization assays.<sup>77,78</sup> Excimer formation has also been observed in pyrene iodoacetamide-labeled carbonic anhydrase II.<sup>79</sup>

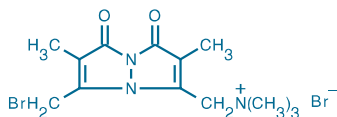
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## Bimanes for Thiol Derivatization

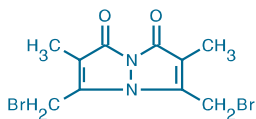
The monobromobimanes (M-1378, M-20381, M-1380), which are essentially nonfluorescent until conjugated, readily react with several low molecular weight thiols, including glutathione,<sup>81–83</sup> *N*-acetylcysteine,<sup>84</sup> mercaptopurine,<sup>85</sup> peptides<sup>86</sup> and plasma thiols,<sup>87</sup> as well as with carboxylic acids (Section 3.3). Although



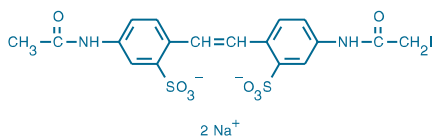
**Figure 2.27** M-1378 monobromobimane.



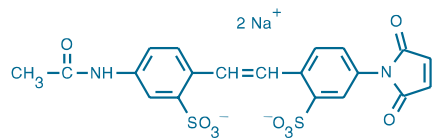
**Figure 2.28** M-1380 monobromotrimethylammoniumbimane bromide.



**Figure 2.29** D-1379 dibromobimane.



**Figure 2.30** A-484 4-acetamido-4'-((iodoacetyl)amino)stilbene-2,2'-disulfonic acid, disodium salt.



**Figure 2.31** A-485 4-acetamido-4'-maleimidylstilbene-2,2'-disulfonic acid, disodium salt.

*Several of our thiol-reactive probes, including monobromobimane and monochlorobimane, are extensively used to estimate glutathione levels in live cells (Section 15.6).*

monobromobimane (M-1378; FluoroPure Grade, M-20381; Figure 2.27) is the most extensively used bimane derivative, monobromotrimethylammoniumbimane (M-1380, Figure 2.28) contains a positive charge, thus permitting separation of its conjugates by electrophoresis<sup>88</sup> or cation-exchange chromatography.<sup>89</sup> The membrane permeability of this charged bimane, as well as its ability to access sites within a protein, may differ from that of the uncharged bimane probes.<sup>90,91</sup> These reagents, which were originally described by Kosower and colleagues,<sup>92,93</sup> are also useful for detecting the distribution of protein thiols in cells before and after chemical reduction of disulfides.<sup>94</sup> Both monobromobimane and the more thiol-selective monochlorobimane (M-1381) have been extensively used for detecting glutathione in live cells (Section 15.6). Monobromobimane can also be used to derivatize thiol-containing proteins prior to separation by isoelectric focusing without appreciably modifying the protein's electrophoretic mobility.<sup>95</sup>

Dibromobimane (D-1379, Figure 2.29) is an interesting crosslinking reagent for proteins<sup>96</sup> because it is unlikely to fluoresce until *both* of its alkylating groups have reacted. It has been used to crosslink thiols in myosin,<sup>97–100</sup> hemoglobin,<sup>101</sup> *Escherichia coli* lactose permease<sup>102</sup> and mitochondrial ATPase.<sup>103</sup> Dibromobimane was also used to probe for the proximity of dual-cysteine mutagenesis sites in ArsA ATPase<sup>104</sup> and to crosslink thiols in actin,<sup>105</sup> myosin<sup>97</sup> and P-glycoprotein.<sup>106,107</sup>

### Polar Reagents for Determining Thiol Accessibility

Like IAEDANS (I-14), the iodoacetamide and maleimide derivatives of stilbene (A-484, A-485) have high water solubility and are readily conjugated to thiols. Their combination of high polarity and membrane impermeability makes these polysulfonated dyes useful for determining whether thiol-containing proteins and polypeptide chains are exposed at the extracellular or cytoplasmic membrane surface. Their protein adducts are charged and can be detected by gel or capillary electrophoresis. Our long-wavelength Alexa Fluor maleimides (Section 2.2), which are also sulfonated polar dyes, are expected to have similar properties and applications but to be more fluorescent and have stronger absorption than the above UV light-excited dyes.

The sulfonated stilbene iodoacetamide (A-484, Figure 2.30) was used to label single-cysteine mutants of staphylococcal  $\alpha$ -hemolysin in order to determine structural changes that occur during oligomerization and pore formation<sup>108</sup> and of the lipid-binding region of *E. coli* pyruvate oxidase in order to detect conformational changes upon substrate binding.<sup>109</sup> It has also been used as a fluorescence donor to a lucifer yellow iodoacetamide (L-1338, Section 2.2) conjugate of  $\alpha$ -crystalline.<sup>110</sup> Similarly, *E. coli* SecA variants containing single cysteine residues have been probed with the sulfonated stilbene maleimide (A-485, Figure 2.31) to systematically study the topology of this inner membrane protein.<sup>111</sup> Lucifer yellow iodoacetamide reacts with the single exposed thiol of low-density lipoproteins (LDL) without reacting with the two buried thiols of the lipoprotein(a) component, whereas acrylodan (A-433) reacts with all three thiols.<sup>112</sup> Lucifer yellow iodoacetamide has also been used in a flow cytometric assay of accessible thiols on the cell surface.<sup>113</sup>

### 1,10-Phenanthroline Iodoacetamide for Preparing Metal-Binding Conjugates

Conjugation of 1,10-phenanthroline iodoacetamide (P-6879) to thiol-containing ligands confers the metal-binding properties of this important complexing agent on the ligand. For example, the covalent copper–phenanthroline complex of oligonucleotides or nucleic acid-binding molecules in combination with hydrogen peroxide acts as a chemical nuclease to selectively cleave DNA or RNA<sup>114–118</sup> (Section 8.7).

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**Data Table — 2.3 Thiol-Reactive Probes Excited with UV Light**

Cat #	MW	Storage	Soluble	Abs	EC	Em	Solvent	Notes
A-433	225.29	L	DMF, MeCN	391	20,000	500	MeOH	1
A-484	624.33	F,D,L	H <sub>2</sub> O	329	39,000	408	pH 8	2, 3
A-485	536.44	F,D	H <sub>2</sub> O	322	35,000	411	pH 8	2
A-20380	477.49	F,DD,L	H <sub>2</sub> O, DMSO	345	17,000	444	pH 7	4
B-1508	277.28	F,D	DMF, MeCN	282	9,000	none	MeOH	2
B-6057	292.17	F,L	DMF, MeCN	387	21,000	520	MeOH	5
D-146	706.86	L	pH >6	328	8,400	563	pH 7	6
D-151	276.35	F,D,L	DMF, MeCN	340	4,100	543	MeOH	7
D-346	402.45	F,D,L	DMSO	384	33,000	469	MeOH	8
D-404	490.34	F,D,L	DMSO	384	31,000	470	MeOH	2, 3
D-1379	350.01	L	DMF, MeCN	391	6,100	see Notes	MeOH	9
D-10251	298.30	F,D,L	DMSO	383	27,000	463	MeOH	10
D-10252	386.19	F,D,L	DMSO	376	24,000	465	MeOH	3
D-10253	383.40	F,D,L	DMSO	419	50,000	466	MeOH	11
D-10300	507.40	F,D,L	DMSO	374	24,000	572	MeOH	12
D-20382	471.29	F,D,L	DMSO	420	49,000	470	MeOH	3, 13
I-7	504.27	F,D,L	DMF	326	27,000	462	MeOH	2, 3
I-14	434.25	F,D,L	pH >6, DMF	336	5,700	490	pH 8	3, 14
M-8	416.38	F,D,L	DMSO, DMF	322	27,000	417	MeOH	15
M-1378	271.11	F,L	DMF, MeCN	398	5,000	see Notes	pH 7	9
M-1380	409.12	L	H <sub>2</sub> O	378	5,500	see Notes	pH 7	9, 16
M-1381	226.66	F,L	DMSO	380	6,000	see Notes	MeOH	9
M-20381	271.11	F,L	DMF, MeCN	398	5,000	see Notes	pH 7	9, 17
P-28	297.31	F,D,L	DMF, DMSO	338	40,000	375	MeOH	18, 19
P-29	385.20	F,D,L	DMF, DMSO	339	26,000	384	MeOH	2, 3
P-2007	399.23	F,D,L	DMSO	341	41,000	377	MeOH	2, 3, 19
P-6879	363.16	F,D,L	DMSO	270	28,000	none	CHCl <sub>3</sub>	3

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

### Notes

1. Fluorescence of A-433 is weak, increasing markedly upon reaction with thiols. Em (QY) for the 2-mercaptoethanol adduct are: 540 nm (0.18) in H<sub>2</sub>O, 513 nm (0.57) in MeOH, 502 nm (0.79) in EtOH, 468 nm (0.78) in MeCN, 435 nm (0.83) in dioxane (*J Biol Chem* 258, 7541 (1983)).

## Data Table — 2.3 Thiol-Reactive Probes Excited with UV Light — continued

- Spectral data of the 2-mercaptoethanol adduct.
- Iodoacetamides in solution undergo rapid photodecomposition to unreactive products. Minimize exposure to light prior to reaction.
- Aqueous stock solutions should be used within 24 hours; long-term storage is NOT recommended.
- Em for 2-mercaptoethanol adduct of B-6057: 550 nm in H<sub>2</sub>O (pH 7), 523 nm in MeOH, 514 nm in EtOH, 502 nm in MeCN, 469 nm in CHCl<sub>3</sub>, 457 nm in dioxane, 445 nm in toluene. Abs is relatively independent of solvent.
- D-146 thiol conjugate has EC = 4000 cm<sup>-1</sup>M<sup>-1</sup>. Em = 527 nm on G-actin, shifting to 520 nm upon polymerization (Arch Biochem Biophys 142, 333 (1971)).
- D-151 conjugated to calmodulin with and without Ca<sup>2+</sup> has Em = 532 nm and 550 nm, respectively (Methods Enzymol 102, 148 (1983)). On troponin I, Em = 500 nm (with Ca<sup>2+</sup>) and 550 nm (without Ca<sup>2+</sup>) (Biochemistry 21, 5669 (1982)). Fluorescence lifetimes (τ) of conjugates are 14 to 20 nsec (Ca<sup>2+</sup>-dependent).
- Spectral data are for the 2-mercaptoethanol adduct. The unreacted reagent is nonfluorescent, Abs = 384 nm (EC = 32,000 cm<sup>-1</sup>M<sup>-1</sup>) in MeOH.
- Bimanes are almost nonfluorescent until reacted with thiols. For monobromobimane conjugated to glutathione, Abs = 394 nm, Em = 490 nm (QY ~0.1–0.3) in pH 8 buffer (Methods Enzymol 143, 76 (1987); Methods Enzymol 251, 133 (1995)).
- Spectral data are for the 2-mercaptoethanol adduct. The unreacted reagent is nonfluorescent, Abs = 381 nm (EC = 27,000 cm<sup>-1</sup>M<sup>-1</sup>) in MeOH.
- QY increases on reaction with thiols; Abs, EC and Em are unchanged (J Chem Soc Perkin Trans 1, 2975 (1994)).
- Fluorescence emission spectrum shifts to shorter wavelengths in nonpolar solvents.
- Spectral data are for the unreacted reagent and are essentially unchanged upon reaction with thiols (J Chem Soc Perkin Trans 1, 2975 (1994)).
- 2-mercaptoethanol adduct of I-14 has essentially similar spectral characteristics in aqueous solution (Biochemistry 12, 4154 (1973)). Fluorescence lifetime (τ) = 21 nsec when conjugated to myosin subfragment-1 (Biochemistry 12, 2250 (1973)).
- Spectral data are for the 2-mercaptoethanol adduct. The unreacted reagent is nonfluorescent, Abs = 443 nm (EC = 13,000 cm<sup>-1</sup>M<sup>-1</sup>) in MeOH.
- Unstable in water. Use immediately.
- This product is specified to equal or exceed 98% analytical purity by HPLC.
- Fluorescence of unreacted P-28 is weak. Em data represent the 2-mercaptoethanol adduct.
- Pyrene derivatives exhibit structured spectra. The absorption maximum is usually about 340 nm with a subsidiary peak at about 325 nm. There are also strong absorption peaks below 300 nm. The emission maximum is usually about 376 nm with a subsidiary peak at 396 nm. Excimer emission at about 470 nm may be observed at high concentrations.

## Product List — 2.3 Thiol-Reactive Probes Excited with UV Light

Cat #	Product Name	Unit Size
A-484	4-acetamido-4'-((iodoacetyl)amino)stilbene-2,2'-disulfonic acid, disodium salt	25 mg
A-485	4-acetamido-4'-maleimidylstilbene-2,2'-disulfonic acid, disodium salt	25 mg
A-433	6-acryloyl-2-dimethylaminonaphthalene (acrylodan)	25 mg
A-20380	Alexa Fluor® 350 C <sub>5</sub> maleimide	1 mg
B-1508	benzophenone-4-maleimide	100 mg
B-6057	6-bromoacetyl-2-dimethylaminonaphthalene (badan)	10 mg
D-10300	Dapoxyl® (2-bromoacetamidoethyl)sulfonamide	10 mg
D-1379	dibromobimane (bBBr)	25 mg
D-146	<i>N,N'</i> -didansyl-L-cystine	100 mg
D-20382	7-diethylamino-3-(((2-iodoacetamido)ethyl)amino)carbonylcoumarin (IDCC)	5 mg
D-404	7-diethylamino-3-(((4'-iodoacetyl)amino)phenyl)-4-methylcoumarin (DCIA)	25 mg
D-10253	7-diethylamino-3-(((2-maleimidyl)ethyl)amino)carbonylcoumarin (MDCC)	5 mg
D-346	7-diethylamino-3-((4'-maleimidylphenyl)-4-methylcoumarin (CPM)	25 mg
D-10252	<i>N</i> -(7-dimethylamino-4-methylcoumarin-3-yl)iodoacetamide (DACIA)	10 mg
D-10251	<i>N</i> -(7-dimethylamino-4-methylcoumarin-3-yl)maleimide (DACM)	10 mg
D-151	5-dimethylaminonaphthalene-1-sulfonyl aziridine (dansyl aziridine)	100 mg
I-7	2-(4'-(iodoacetamido)anilino)naphthalene-6-sulfonic acid, sodium salt (IAANS)	100 mg
I-14	5-(((2-iodoacetyl)amino)ethyl)amino)naphthalene-1-sulfonic acid (1,5-IAEDANS)	100 mg
M-8	2-(4'-maleimidylanilino)naphthalene-6-sulfonic acid, sodium salt (MIANS)	100 mg
M-1378	monobromobimane (mBBr)	25 mg
M-20381	monobromobimane (mBBr) *FluoroPure™ grade*	25 mg
M-1380	monobromotrimethylammoniumbimane bromide (qBBr)	25 mg
M-1381	monochlorobimane (mBCI)	25 mg
P-6879	<i>N</i> -(1,10-phenanthroline-5-yl)iodoacetamide	5 mg
P-29	<i>N</i> -(1-pyrene)iodoacetamide	100 mg
P-28	<i>N</i> -(1-pyrene)maleimide	100 mg
P-2007	<i>N</i> -(1-pyrenemethyl)iodoacetamide (PMIA amide)	25 mg

### Certificates of Analysis

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