

1.8 Reagents for Analysis of Low Molecular Weight Amines

Not only are low molecular weight amines abundantly distributed in nature, but numerous drugs, synthetic probes and other molecules of interest also contain amino groups. The sensitive detection, identification and quantitation of amines are important applications of many of the reactive fluorophores in this section. Some of these reagents have also been used to indirectly detect carbohydrates, carboxylic acids, thiols and cyanide.

The preferred reagents for detecting and quantitating amines in solution or on amine-containing polymers are those that are nonfluorescent but form fluorescent conjugates stoichiometrically with amines. It is usually difficult to compare the sensitivity for amine detection of the different reagents because it depends heavily on the equipment and detection technology used. However, many of the assays are rapid, reliable and adaptable to a variety of different sample types and instrumentation.

Fluorescamine

Fluorescamine (F-2332; FluoroPure Grade, F-20261) is intrinsically nonfluorescent but reacts rapidly with primary aliphatic amines, including those in peptides and proteins, to yield a blue-green-fluorescent derivative^{1,2} (Figure 1.102). Modifications to the reaction protocol permit fluorescamine to be used to detect those amino acids containing secondary amines,³ such as proline. Excess reagent is rapidly converted to a nonfluorescent product by reaction with water,⁴ making fluorescamine useful for determining protein concentrations of solutions.^{5,6} Fluorescamine can also be used to detect proteins in gels and to analyze low molecular weight amines by TLC, HPLC and capillary electrophoresis.^{7,8} An optimized procedure that uses fluorescamine for amino acid analysis in microplates has been published.⁹

Dialdehydes: OPA and NDA

Analyte Detection with OPA and NDA

The homologous aromatic dialdehydes *o*-phthalaldehyde¹⁰ (OPA, P-2331) and naphthalene-2,3-dicarboxaldehyde¹¹ (NDA, N-1138) are essentially nonfluorescent until reacted with a primary amine in the presence of excess cyanide or a thiol, such as β -mercaptoethanol, 3-mercaptopropionic acid or the less obnoxious sulfite,¹² to yield a fluorescent isoindole (Figure 1.103, Figure 1.104). Modified protocols that use an excess of an amine and limiting amounts of other nucleophiles permit the determination of carboxylic acids¹³ and thiols,¹⁴ as well as of cyanide in blood, urine and other samples.^{15–18} Without an additional nucleophile, NDA forms fluorescent adducts with both hydrazine and methylated hydrazines¹⁹ (excitation/emission maxima ~403/500 nm).

Sensitivity of NDA

Amine adducts of NDA have longer-wavelength spectral characteristics and greater sensitivity than the amine adducts of OPA. The stability and detectability of the amine derivatives of NDA are also superior;^{20,21} the detection of glycine with NDA and cyanide is reported to be 50-fold more sensitive than with OPA and β -mercaptoethanol.¹¹ The limit for electrochemical detection of the NDA adduct of asparagine has been determined to be as low as 36 attomoles (36×10^{-18} moles).^{22,23} An optimized

procedure that uses NDA for amino acid analysis in microplates has been published.⁹

Applications for OPA and NDA

OPA and NDA are used extensively for both pre- and postcolumn derivatization of amines (and thiols) separated by HPLC²⁴ or by capillary electrophoresis.^{25–29} The amines in a single cell have been analyzed by capillary electrophoresis using a sequence of on-capillary lysis, derivatization with NDA and cyanide and laser-excited detection.^{30,31} A capillary electrophoresis method based on NDA derivatization has also been developed to follow stimulated release of amines from individual neurons and small groups of isolated neurons.³² NDA derivatives can be excited at 442 nm by the He–Cd laser.²⁵

ATTO-TAG Reagents

Sensitivity of ATTO-TAG CBQCA and ATTO-TAG FQ

Molecular Probes exclusively offers ATTO-TAG CBQCA (A-6222, A-2333) and ATTO-TAG FQ (A-10192, A-2334) for ultrasensitive detection of primary amines, including those in peptides, amino acid homopolymers and glycoproteins.³³ These reagents combine high sensitivity, visible-wavelength excitation and freedom from background fluorescence, making them useful for research, analytical and forensic applications. Developed by Novotny and collaborators, the ATTO-TAG reagents are similar to OPA and NDA in that they rapidly react with amines in the presence of cyanide or thiols to form highly fluorescent isoindoles^{34–44} (Figure 1.105).

The ATTO-TAG CBQCA reagent reacts specifically with amines to form charged conjugates that can be analyzed by electrophoresis techniques. Carbohydrates lacking amines can be detected following reductive amination with ammonia and NaCNBH₃.^{43,45,46} ATTO-TAG CBQCA conjugates are maximally excited at ~456 nm or by the 442 nm spectral line of the He–Cd laser, with peak emission at ~550 nm, whereas ATTO-TAG FQ conjugates are maximally excited at ~480 nm or by the 488 nm spectral line of the argon-ion laser, with peak emission at ~590 nm. In capillary zone electrophoresis, the sensitivity of amine detection of the laser-induced fluorescence should be in the sub-attomole range ($<10^{-18}$ moles) for ATTO-TAG CBQCA and subfemtomole range ($<10^{-15}$ moles) for ATTO-TAG FQ.^{47,48} Sensitivity for detection of reductively aminated glucose using ATTO-TAG CBQCA is reported to be 75 zeptomoles (75×10^{-21} moles).⁴⁹ Similar ultrasensitive detection of CBQCA-derivatized amino acids by capillary electrophoresis has been reported.⁵⁰

ATTO-TAG reagents can, of course, be used in HPLC and other modes of chromatography with either absorption or fluorescence detection. The principal limitation to obtaining ultrasensitive detection using the ATTO-TAG reagents and all other chemical derivatization reagents is that relatively high concentrations of the derivatizing reagent are required to obtain adequate kinetics and sufficient modification of the analyte. One particularly useful technique employs ATTO-TAG FQ for the solid-phase derivatization of dilute solutions ($\sim 10^{-8}$ M) of peptides that have been immobilized on Immobilon CD membranes.⁵¹ This method permits the quantitative derivatization and analysis by capillary

electrophoresis of only a few picomoles of the analyte. A very sensitive assay that uses ATTO-TAG CBQCA for rapid quantitation of protein amines (C-6667) is described in Section 9.2.

ATTO-TAG Reagents and Kits

Cyclodextrins have been reported to amplify the signal from ATTO-TAG CBQCA conjugates up to tenfold^{44,52,53} so we include β -cyclodextrin in our ATTO-TAG Amine Derivatization Kits (A-2333, A-2334). The kits contain:

- 5 mg of ATTO-TAG CBQCA in A-2333 or ATTO-TAG FQ in A-2334
- Potassium cyanide
- β -Cyclodextrin
- A protocol for amine modification

The ATTO-TAG CBQCA and ATTO-TAG FQ Amine Derivatization Kits supply sufficient reagents for derivatizing approximately 150 and 100 samples, respectively, depending on the amine concentration and sample volume. We also offer both the ATTO-TAG CBQCA and the ATTO-TAG FQ derivatization reagents separately (CBQCA, A-6222; FQ, A-10192).

7-Nitrobenz-2-Oxa-1,3-Diazole (NBD) Derivatives

NBD chloride (FluoroPure Grade, C-20260; Figure 1.106) was first introduced in 1968 as a fluorogenic derivatization reagent for amines.⁵⁴ It also reacts with thiols and alcohols, although these adducts absorb and emit at shorter wavelengths and are less fluorescent than amine derivatives.⁵⁵ NBD fluoride (F-486) usually yields the same products as NBD chloride but is much more reactive;⁵⁶ for example, the reaction of NBD fluoride with glycine is reported to be 500 times faster than the reaction of NBD chloride with glycine.⁵⁷ Unlike OPA and fluorescamine, both NBD chloride and NBD fluoride react with secondary amines and are therefore capable of derivatizing proline and hydroxyproline.^{56,58} NBD chloride and NBD fluoride are extensively used as derivatization reagents for chromatographic analysis of amino acids⁵⁹ and other low molecular weight amines.⁶⁰ NBD fluoride has also been used for the enantiomeric separation of D,L-amino acids on a chiral column⁶¹ and by capillary electrophoresis using cyclodextrin chiral selectors, as well as for the ultramicroanalysis of reducing carbohydrates.^{62,63}

The absorption and fluorescence emission spectra, quantum yields and extinction coefficients of NBD conjugates are all markedly dependent on solvent;^{64,65} in particular, the fluorescence quantum yield in water of NBD adducts of amines can be very low (<0.01), particularly of secondary amines. NBD adducts of aromatic amines are essentially nonfluorescent, a property that we have utilized to prepare our QSY 35 quenchers (see below). Fluorescence of lysine-modified NBD-labeled actin is sensitive to polymerization.⁶⁶ Inactivation of certain ATPases by NBD chloride apparently involves a tyrosine modification followed by intramolecular migration of the label to a lysine residue.^{67,68} NBD is also a functional analog of the dinitrophenyl hapten, and its fluorescence is quenched upon binding to anti-dinitrophenyl antibodies⁶⁵ (Section 7.4). NBD aminohexanoic acid (NBD-X, N-316) and its succinimidyl ester (NBD-X, SE; S-1167) are

precursors to NBD-labeled phospholipids (Section 13.2), NBD C₆-ceramide (N-1154, Section 12.4) and other probes.

Dansyl Chloride and Other Sulfonyl Chlorides

Many of the sulfonyl chlorides described in Section 1.7, including dansyl chloride (D-21), 1-pyrenesulfonyl chloride (P-24) and Dapoxyl sulfonyl chloride (D-10160), react with amines to yield blue- or blue-green-fluorescent sulfonamides and are particularly useful as chromatographic derivatization reagents. They react with both aliphatic and aromatic amines to yield very stable derivatives. In addition, they are generally good acceptors for fluorescence resonance energy transfer (FRET, see Section 1.3) from tryptophan, as well as good donors to longer-wavelength dyes such as dansyl chloride (D-1537) and our QSY dyes (Section 1.6). Fluorescence of dansyl conjugates in aqueous solutions

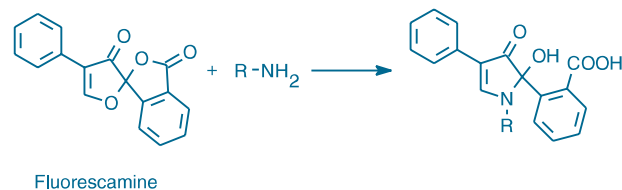


Figure 1.102 Fluorogenic amine-derivatization reaction of fluorescamine (F-2332, F-20261).

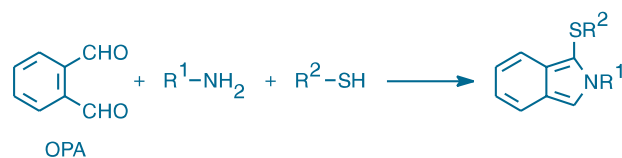


Figure 1.103 Fluorogenic amine-derivatization reaction of *o*-phthalaldehyde (OPA, P-2331).

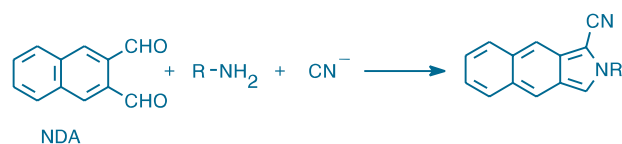


Figure 1.104 Fluorogenic amine-derivatization reaction of naphthalene-2,3-dicarboxaldehyde (NDA, N-1138).

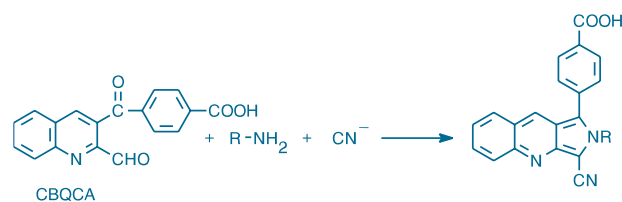


Figure 1.105 Fluorogenic amine-derivatization reaction of CBQCA (A-6222, A-2333).

can be enhanced by adding cycloheptaamylose.⁶⁹ Although dansyl chloride is the most commonly used of these reagents, the stronger absorption of 1-pyrenesulfonamides and huge Stokes shift of Dapoxyl sulfonamides⁷⁰ (Figure 1.97) should make these sulfonyl chlorides more sensitive reagents for amine analysis.

Dansyl Chloride

Since its development by Weber in 1951,⁷¹ dansyl chloride (D-21, Section 1.7) has been used extensively to determine the N-terminal amino acid residue of proteins and to prepare fluorescent derivatives of drugs, amino acids, oligonucleotides and proteins for detection by numerous chromatographic methods.⁷² Nonfluo-

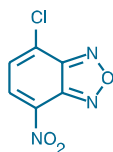


Figure 1.106 C-20260 4-chloro-7-nitrobenz-2-oxa-1,3-diazole (NBD chloride).

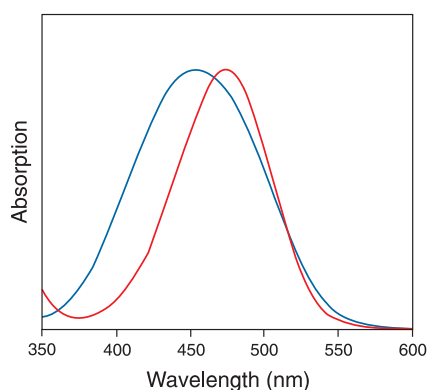


Figure 1.107 Normalized absorption spectra of dabcyI, SE (D-2245, blue), and QSY 35, SE (Q-20133, red).

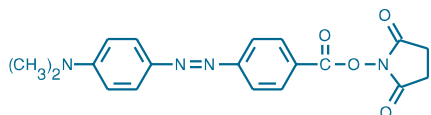


Figure 1.108 D-2245 4-((4-(dimethylamino)phenyl)azo)benzoic acid, succinimidyl ester (dabcyI, SE).

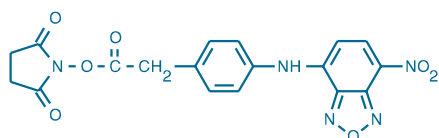


Figure 1.109 Q-20133 QSY 35 acetic acid, succinimidyl ester.

rescent dansyl chloride reacts with amines to form fluorescent dansyl amides that exhibit large Stokes shifts, along with environmentally sensitive fluorescence quantum yields and emission maxima.

Dapoxyl Sulfonyl Chloride

Sulfonamides from Dapoxyl sulfonyl chloride (D-10160, Section 1.7) have much higher extinction coefficients than those of dansyl chloride ($\sim 22,000 \text{ cm}^{-1}\text{M}^{-1}$ versus $\sim 4000 \text{ cm}^{-1}\text{M}^{-1}$) and equal or greater quantum yields when dissolved in organic solvents; however, Dapoxyl derivatives have very low fluorescence in water. The huge Stokes shifts (up to $\sim 200 \text{ nm}$) and large extinction coefficients of Dapoxyl derivatives in some solvents⁷⁰ (Figure 1.97), and the close match of their absorption to the intense 365 nm emission of the mercury-arc lamp, make the reactive Dapoxyl derivatives advantageous derivatization reagents for chromatographic and electrophoretic analysis.⁷³

Pyrene Sulfonyl Chloride

The absorptivity (and therefore ultimate fluorescence output) of dansyl derivatives is weak compared with that of the more strongly UV light-absorbing fluorophores such as pyrene. Thus, 1-pyrenesulfonyl chloride (P-24, Section 1.7) should have greater sensitivity for detection of amines. The fluorescence lifetime of pyrenesulfonamides can also be relatively long (up to ~ 30 nanoseconds), making them useful for fluorescence anisotropy measurements.⁷⁴ Fluorescence polarization measurements of DNA probes labeled with 1-pyrenesulfonyl chloride permit homogeneous detection of hybridization.⁷⁵

Chromophoric Sulfonyl Chloride

Dabsyl chloride (D-1537) is a common amine-derivatization reagent for detecting proteins by HPLC,^{76,77} as well as by gel and capillary electrophoresis.^{78–80} Conjugates of dabsyl chloride have broad and intense visible absorption, making them useful as acceptors in fluorescence resonance energy transfer applications.

FITC and Benzofuran Isothiocyanates

Isothiocyanates for preparing bioconjugates have been described in several sections of this chapter. However, FITC (F-143, F-144, F-1906, F-1907; Section 1.4) and benzofuran (D-1332) isothiocyanates can also be used for derivatizing low molecular weight amines and, like phenyl isothiocyanate, for microsequencing of peptides as their thiohydantoin.⁸¹ A unique method for specific derivatization of the N-terminus of peptides by FITC has been described.⁸² FITC-labeled amino acids and peptides have been separated by capillary electrophoresis with a detection limit of fewer than 1000 molecules.^{83,84}

Succinimidyl Esters and Carboxylic Acids

Succinimidyl esters have a high selectivity for reaction with aliphatic amines. Most of the succinimidyl ester reagents described elsewhere in this chapter can be used to derivatize low molecular weight amines for separation by chromatography or capillary electrophoresis. The Alexa Fluor, BODIPY, Oregon Green and fluorescein derivatives usually yield the greatest sensitivity, particularly when the conjugate is detected following laser

excitation. Use of single isomers of these reactive dyes is essential for all high-resolution analyses. Analysis by capillary electrophoresis shows that carboxyfluorescein succinimidyl ester reacts faster and yields more stable amine conjugates than FITC or DTAF.⁸⁵ The UV light-excitable coumarins described in Section 1.7 have good absorptivity at ~320–420 nm, with purple to bright-blue emission at 400–500 nm.

Aliphatic polyamines derivatized with 1-pyrenebutanoic acid succinimidyl ester (P-130, Section 1.7) have been differentiated from pyrene-labeled monoamines by fluorescent excimer formation in an HPLC-based assay.⁸⁶

The Smallest Reactive Fluorophore

Both *N*-methylisatoic anhydride (M-25) and the succinimidyl ester of *N*-methylanthranilic acid (S-128) are useful precursors for preparing esters or amides of the small *N*-methylanthranilic acid fluorophore. The small size of this fluorophore should reduce the likelihood that the label will interfere with the function of the biomolecule, an important advantage when designing site-selective probes. These amine-acylating reagents are often used to prepare fluorescent derivatives of biologically active peptides and toxins^{87–90} and, in combination with a quencher, to prepare fluorescent endoprotease substrates.^{91,92} *N*-methylisatoic anhydride also reacts with the ribose moiety of ribonucleotides to yield fluorescent MANT nucleotide analogs^{93–95} (Section 18.3, Figure 18.20).

Chromophoric Succinimidyl Esters: Fluorescence Quenchers

Dabcyl has broad and intense visible absorption (Figure 1.107) but no fluorescence, making it useful as an acceptor in fluorescence resonance energy transfer applications. Biomolecules double-labeled with dabcyl and the appropriate fluorophore can be used to monitor proteolytic cleavage, conformational

changes and other dynamic spatial movements. Dabcyl succinimidyl ester (dabcyl, SE; D-2245; Figure 1.108) is particularly useful in preparing quenched fluorogenic substrates for proteases, including our HIV protease (Figure 10.9) and renin substrates^{96–98} (H-2930, R-2931; Section 10.4), papain,^{99,100} Alzheimer's disease-associated proteases¹⁰¹ and others.^{102–105} Fluorogenic substrates using this quenching group have also been prepared for interleukin-1 β -converting enzyme (ICE),¹⁰⁶ a cysteine protease that is proposed to function in the onset of apoptosis.¹⁰⁷ The dabcyl chromophore has been used as the quencher in donor-acceptor labeled oligonucleotides in "molecular beacons"^{108–111} (Figure 8.101); unfolding of these probes upon hybridization leads to recovery of the donor dye's fluorescence (Section 8.5).

QSY 35 acetic acid succinimidyl ester (Q-20133) is an essentially nonfluorescent nitrobenzoxadiazole (NBD) derivative (Figure 1.109). Like the QSY 7, QSY 9 and QSY 21 dyes (Section 1.5, Figure 1.66), the QSY 35 dye has stronger absorption at longer wavelengths than does the dabcyl dye (Figure 1.107), making it a very good acceptor from most blue-fluorescent dyes (Table 1.8). A QSY 35 iodoacetamide (Q-20348, Section 2.2) and aliphatic methylamine (Q-20540, Section 3.3) are available, as is an FMOC-protected QSY 35 amino acid (Q-21931, Section 9.5) for automated preparation of FRET-based protease substrates (Section 10.4).

Biotinylation, Desthiobiotinylation, Crosslinking and Thiolation Reagents

Molecular Probes offers a number of succinimidyl esters that are useful for preparing biotin and desthiobiotin (DSB-X biotin) conjugates or for crosslinking biomolecules and thiolation of amines. These products are described in Chapter 4 and Chapter 5. Uses of reactive dyes as haptens are discussed in Section 4.2.

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Data Table — 1.8 Reagents for Analysis of Low Molecular Weight Amines

Cat #	MW	Storage	Soluble	Abs	EC	Em	Solvent	Notes
A-2333	305.29	F,D,L	MeOH	465	ND	560	MeOH	1, 2, 3, 4
A-2334	251.24	F,D,L	EtOH	486	ND	591	MeOH	4, 5
A-6222	305.29	F,D,L	MeOH	465	ND	560	MeOH	1, 2, 3
A-10192	251.24	F,L	EtOH	486	ND	591	MeOH	2, 5
C-20260	199.55	F,D,L	DMF, MeCN	336	9,800	none	MeOH	6, 7, 8
D-1332	294.37	F,DD,L	DMF, MeCN	348	38,000	425	MeOH	9
D-1537	323.80	F,DD,L	DMF, MeCN	466	33,000	none	MeOH	10, 11
D-2245	366.38	F,D,L	DMF, DMSO	453	32,000	none	MeOH	10
F-486	183.10	F,D,L	MeCN, CHCl ₃	328	8,000	none	MeOH	6, 7
F-2332	278.26	F,D,L	MeCN	380	7,800	464	MeCN	12
F-20261	278.26	F,D,L	MeCN	380	8,400	464	MeCN	8, 12
M-25	177.16	D	DMF, DMSO	316	3,500	386	MeOH	13
N-316	294.27	L	DMSO	467	23,000	539	MeOH	7
N-1138	184.19	L	DMF, MeCN	419	9,400	493	see Notes	14
P-2331	134.13	L	EtOH	334	5,700	455	pH 9	15
Q-20133	411.33	F,D,L	DMSO	475	23,000	none	MeOH	
S-128	248.24	F,D,L	DMF, MeCN	368	6,500	437	MeOH	
S-1167	391.34	F,D,L	DMF, DMSO	466	22,000	535	MeOH	7

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

Notes

- Spectral data are for the reaction product with glycine in the presence of cyanide. Unreacted reagent in MeOH: Abs = 254 nm (EC = 46,000 cm⁻¹M⁻¹), nonfluorescent.
- ND = not determined.
- Solubility in methanol is improved by addition of base (e.g., 1–5% (v/v) 0.2 M KOH).
- Data represents the reactive dye component of this labeling kit.
- Spectral data are for the reaction product with glycine in the presence of cyanide. Unreacted reagent in MeOH: Abs = 282 nm (EC = 21,000 cm⁻¹M⁻¹), nonfluorescent.
- Spectra for primary aliphatic amine derivative of NBD chloride in MeOH: Abs = 465 nm (EC = 22,000 cm⁻¹M⁻¹), Em = 535 nm (QY = 0.3). Spectra for secondary aliphatic amine derivative in MeOH: Abs = 485 nm (EC = 25,000 cm⁻¹M⁻¹), Em = 540 nm (QY < 0.1). Aromatic amine derivatives are nonfluorescent. All NBD amine derivatives are almost nonfluorescent in water and have strongly solvent-dependent emission spectra. NBD fluoride yields the same derivatives as NBD chloride but is more reactive.
- NBD derivatives are almost nonfluorescent in water. QY and τ increase and Em decreases in aprotic solvents and other nonpolar environments relative to water (Biochemistry 16, 5150 (1977); Photochem Photobiol 54, 361 (1991)).
- This product is specified to equal or exceed 98% analytical purity by HPLC.
- Spectra of this compound are in methanol containing a trace of KOH.
- D-1537 reaction product with butylamine: Abs = 435 nm (EC = 31,000 cm⁻¹M⁻¹), nonfluorescent in MeOH. D-2245 reaction product with butylamine: Abs = 428 nm (EC = 32,000 cm⁻¹M⁻¹), nonfluorescent in MeOH.
- Do NOT dissolve in DMSO.
- Fluorescamine spectra are for reaction product with butylamine. Fluorescence quantum yield and lifetime of adduct in EtOH are 0.23 and 7.5 nanoseconds, respectively (Arch Biochem Biophys 163, 390 (1974)). Unreacted reagent in MeCN: Abs = 234 nm (EC = 28,000 cm⁻¹M⁻¹), nonfluorescent.
- M-25 amide reaction product with butylamine has Abs = 353 nm (EC = 5900 cm⁻¹M⁻¹), Em = 426 nm in MeOH. Ester reaction products with alcohols have Abs = 350 nm (EC = 5700 cm⁻¹M⁻¹), Em = 446 nm in water (pH 8).
- Spectral data are for the reaction product with glycine in the presence of cyanide, measured in pH 7.0 buffer/MeCN (40:60) (Anal Chem 59, 1102 (1987)). Unreacted reagent in MeOH: Abs = 279 nm (EC = 5500 cm⁻¹M⁻¹), Em = 330 nm.
- Spectral data are for the reaction product of P-2331 with alanine and 2-mercaptoethanol. The spectra and the stability of the adduct depend on the amine and thiol reactants (Biochim Biophys Acta 576, 440 (1979)). Unreacted reagent in H₂O: Abs = 257 nm (EC = 1000 cm⁻¹M⁻¹).

Product List — 1.8 Reagents for Analysis of Low Molecular Weight Amines

Cat #	Product Name	Unit Size
A-2333	ATTO-TAG™ CBQCA Amine-Derivatization Kit	1 kit
A-6222	ATTO-TAG™ CBQCA derivatization reagent (CBQCA; 3-(4-carboxybenzoyl)quinoline-2-carboxaldehyde)	10 mg
A-2334	ATTO-TAG™ FQ Amine-Derivatization Kit	1 kit
A-10192	ATTO-TAG™ FQ derivatization reagent (FQ; 3-(2-furoyl)quinoline-2-carboxaldehyde)	10 mg
C-20260	4-chloro-7-nitrobenz-2-oxa-1,3-diazole (NBD chloride; 4-chloro-7-nitrobenzofurazan) *FluoroPure™ grade*	100 mg
D-1537	4-dimethylaminoazobenzene-4'-sulfonyl chloride (dabsyl chloride)	100 mg
D-1332	<i>N</i> -(4-(6-dimethylamino-2-benzofuranyl)phenyl)isothiocyanate	25 mg
D-2245	4-((4-(dimethylamino)phenyl)azo)benzoic acid, succinimidyl ester (dabcyl, SE)	100 mg
F-2332	fluorescamine	100 mg
F-20261	fluorescamine *FluoroPure™ grade*	100 mg
F-486	4-fluoro-7-nitrobenz-2-oxa-1,3-diazole (NBD fluoride; 4-fluoro-7-nitrobenzofurazan)	25 mg
M-25	<i>N</i> -methylisatoic anhydride *high purity*	1 g
N-1138	naphthalene-2,3-dicarboxaldehyde (NDA)	100 mg
N-316	6-(<i>N</i> -(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)hexanoic acid (NBD-X)	100 mg
P-2331	<i>o</i> -phthalaldehyde (OPA) *high purity*	1 g
Q-20133	QSY® 35 acetic acid, succinimidyl ester	5 mg
S-128	succinimidyl <i>N</i> -methylanthranilate	100 mg
S-1167	succinimidyl 6-(<i>N</i> -(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)hexanoate (NBD-X, SE)	25 mg