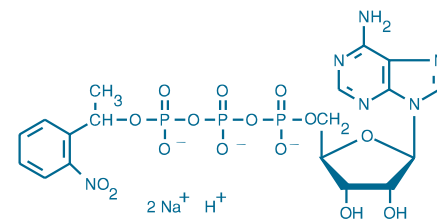


## 17.2 Caged Probes for a Variety of Applications

Molecular Probes' caged probes provide researchers with important tools for delivering physiological stimuli by naturally active biomolecules with spatial and temporal precision that far exceeds that of microinjection or perfusion. Caged dyes can also serve as tracers with fluorescence that can be "turned on" by brief exposure to ultraviolet light.

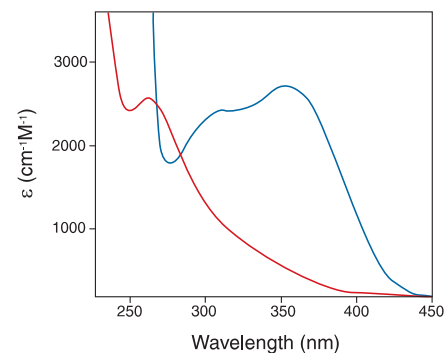


**Figure 17.1** A-1048 adenosine 5'-triphosphate,  $P^{\beta}$ -(1-(2-nitrophenyl)ethyl) ester, disodium salt.

### Caged Nucleotides and Caged Phosphates

Photoactivatable nucleotides and phosphates have contributed significantly to our understanding of cytoskeleton dynamics, signal transduction pathways and other critical cellular processes.<sup>1</sup> Some of our caged nucleotides are available with a choice of caging group:

- Caged ATP (A-1048, Figure 17.1; A-1049), which has been shown to release ATP in skinned muscle fibers,<sup>2-4</sup> sarcoplasmic reticulum vesicles,<sup>5</sup> submitochondrial particles<sup>6</sup> and membrane fragments containing  $\text{Na}^+/\text{K}^+$ -ATPase<sup>7</sup>
- Caged ADP (A-7056), which has been used to investigate the molecular basis of contraction of skeletal muscle fibers,<sup>8</sup> as well as transport by an ADP/ATP carrier<sup>9</sup>
- Caged cAMP (D-1037), which is cell-permeant and rapidly photolyzed to cAMP<sup>10-12</sup>
- Caged GTP- $\gamma$ -S (G-1053), a probe for studying regulatory proteins such as G-proteins<sup>13-15</sup>
- Caged inositol 1,4,5-triphosphate<sup>16-18</sup> (I-23580) and caged cADP-ribose<sup>19-21</sup> (C-7074) — important probes for second messenger studies
- Caged proton<sup>22-24</sup> (H-6829) and caged phosphate<sup>25-28</sup> (N-7065), which can be used to produce a pulse of phosphate and to generate a photolysis-dependent proton release that results in a rapid drop in pH



**Figure 17.2** Spectral comparison of equimolar concentrations of the Caged  $\text{Ca}^{2+}$  reagents NP-EGTA (N-6802, red line) and DMNP-EDTA (D-6814, blue line), illustrating the optimal wavelengths for photolysis and subsequent release of  $\text{Ca}^{2+}$  from these chelators. Spectra were taken in 100 mM KCl and 30 mM MOPS buffer containing 39.8  $\mu\text{M}$  free  $\text{Ca}^{2+}$  at pH 7.2.

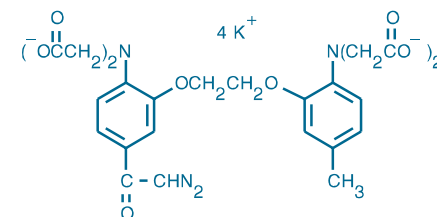
### Caged $\text{Ca}^{2+}$ , Caged $\text{Ca}^{2+}$ Chelators and Caged Ionophores

Caged ions and caged chelators can be used to influence the ionic composition of both solutions and cells, particularly for ions such as  $\text{Ca}^{2+}$  that are present at low concentrations under normal physiological conditions.

#### Caged $\text{Ca}^{2+}$ Reagents

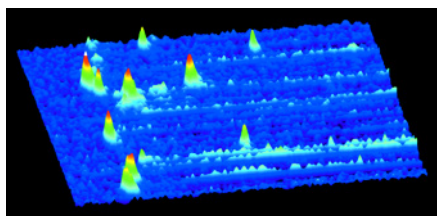
Developed by Ellis-Davies and Kaplan,<sup>29,30</sup> nitrophenyl EGTA (NP-EGTA) is a photolabile  $\text{Ca}^{2+}$  chelator that exhibits a high selectivity for  $\text{Ca}^{2+}$  ions, a dramatic increase in its  $K_d$  for  $\text{Ca}^{2+}$  upon illumination (from 80 nM to 1 mM) and a high photolysis quantum yield (0.23). NP-EGTA's affinity for  $\text{Ca}^{2+}$  decreases ~12,500-fold upon photolysis. Furthermore, its  $K_d$  for  $\text{Mg}^{2+}$  of 9 mM makes NP-EGTA essentially insensitive to physiological  $\text{Mg}^{2+}$  concentrations. We exclusively offer the tetrapotassium salt (N-6802) and the acetoxymethyl (AM) ester (N-6803) of NP-EGTA. The NP-EGTA salt can be complexed with  $\text{Ca}^{2+}$  to generate a Caged  $\text{Ca}^{2+}$  reagent that will rapidly deliver  $\text{Ca}^{2+}$  upon photolysis<sup>18,31,32</sup> (Figure 18.4). The cell-permeant AM ester of NP-EGTA does not bind  $\text{Ca}^{2+}$  unless its AM ester groups are removed. This AM ester can serve as a photolabile chelator in cells because, once converted to NP-EGTA by intracellular esterases, it will bind free  $\text{Ca}^{2+}$  until photolyzed with UV light.

The first caged  $\text{Ca}^{2+}$  reagent described by Kaplan and Ellis-Davies was 1-(4,5-dimethoxy-2-nitrophenyl) EDTA (DMNP-EDTA, D-6814), which they named DM-Nitrophen<sup>33-35</sup> (now a trademark of Calbiochem-Novabiochem Corp.). Because its structure more resembles that of EDTA than EGTA, we named it as a caged EDTA derivative (Figure 18.5). Upon illumination, DMNP-EDTA's affinity for  $\text{Ca}^{2+}$  decreases ~600,000-fold and its  $K_d$  for  $\text{Ca}^{2+}$  rises from 5 nM to 3 mM. Thus, photolysis of DMNP-EDTA complexed with  $\text{Ca}^{2+}$  results in a pulse of free  $\text{Ca}^{2+}$ . DMNP-EDTA has a stronger absorbance at longer wavelengths than does NP-EGTA (Figure 17.2), which facilitates uncaging. Furthermore, DMNP-EDTA has significantly higher affinity for  $\text{Mg}^{2+}$  ( $K_d = 2.5 \mu\text{M}$ )<sup>33</sup> than does NP-EGTA ( $K_d = 9 \text{ mM}$ ),<sup>29</sup> making it a potentially useful caged  $\text{Mg}^{2+}$  reagent. A paper by Neher and Zucker discusses the uses and limitations of DMNP-EDTA.<sup>36</sup> In

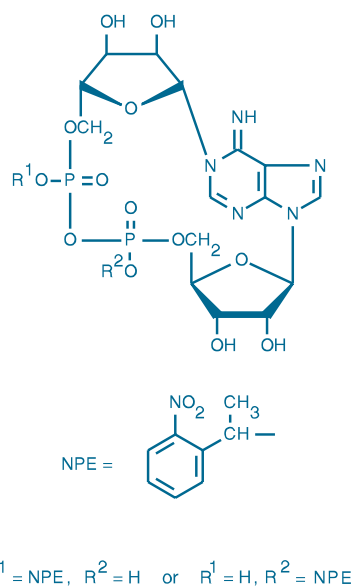


**Figure 17.3** D-3034 diazo-2, tetrapotassium salt.

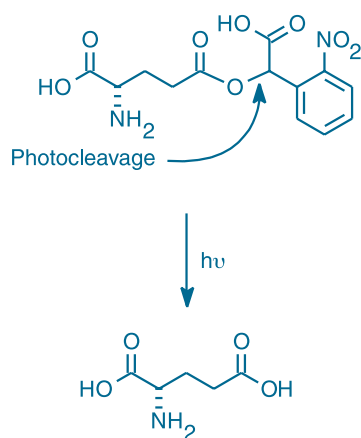
*UV photolysis of our caged  $\text{Ca}^{2+}$  reagents can either release  $\text{Ca}^{2+}$  (NP-EGTA and DMNP-EDTA) or rapidly chelate  $\text{Ca}^{2+}$  (diazo-2). They are usually used in combination with our fluo-3, fluo-4, Oregon Green BAPTA or other visible light-excited  $\text{Ca}^{2+}$  indicators (Section 20.3).*



**Figure 17.4** Confocal linescan image of calcium "puffs" in a *Xenopus* oocyte. Oregon Green 488 BAPTA-1 (O-6806) was used as the calcium indicator and  $\text{Ca}^{2+}$  liberation was evoked by flash photolysis of NPE-caged Ins 1,4,5- $\text{P}_3$  (I-23580). Image contributed by Ian Parker and Nick Callamaras, University of California at Irvine.



**Figure 17.5** C-7074 cyclic adenosine 5'-diphosphate ribose, 1-(1-(2-nitrophenyl)ethyl) ester.



**Figure 17.6** CNB-caged L-glutamic acid (G-7055). The CNB-caging group is rapidly photocleaved with UV light to release L-glutamic acid.

addition to high-purity DMNP-EDTA (D-6814), we have prepared its cell-permeant AM ester (D-6815).

### Caged $\text{Ca}^{2+}$ Chelator

In contrast to NP-EGTA and DMNP-EDTA, diazo-2 is a photoactivatable  $\text{Ca}^{2+}$  scavenger. Diazo-2, which was introduced by Adams, Kao and Tsien,<sup>37,38</sup> exhibits a low affinity for  $\text{Ca}^{2+}$  before photolysis ( $K_d = 2.2 \mu\text{M}$ ) that increases by a factor of 30 upon exposure to UV light. Diazo-2 can be microinjected as its  $\text{K}^+$  salt (D-3034, Figure 17.3) or loaded into cells as the AM ester (D-3036). Upon illumination near 360 nm, the photolyzed diazo-2 binds cytosolic free  $\text{Ca}^{2+}$  within a few milliseconds. Diazo-2 has been used to rapidly decrease cytosolic  $\text{Ca}^{2+}$  in tensed frog muscle cells<sup>39</sup> and in rat fibroblasts.<sup>37,38</sup>

### Caged 4-Bromo A-23187

We have introduced some unique tools for the study of  $\text{Ca}^{2+}$  regulation and ion transport. Our patented cell-permeant DMNPE-caged ionophores derived from 4-bromo A-23187 (B-7108) can be passively loaded into cells and, at a later time, photoactivated with a UV laser or UV flashlamp to stimulate ion movement across the plasma membrane or to liberate  $\text{Ca}^{2+}$  from intracellular stores.<sup>40</sup>

## Caged Second Messengers

### Caged Inositol 1,4,5-Triphosphate

NPE-caged Ins 1,4,5- $\text{P}_3$  can be used to generate rapid and precisely controlled release of Ins 1,4,5- $\text{P}_3$  in intact cells (Figure 17.4) and is widely employed in studies of Ins 1,4,5- $\text{P}_3$ -mediated second messenger pathways.<sup>16-18</sup> Our NPE-caged Ins 1,4,5- $\text{P}_3$  (I-23580) is a mixture of the physiologically inert, singly esterified  $\text{P}^4$  and  $\text{P}^5$  esters (Figure 18.2) and does not contain the somewhat physiologically active  $\text{P}^1$  ester. NPE-caged Ins 1,4,5- $\text{P}_3$  exhibits essentially no biological activity prior to photolytic release of the biologically active Ins 1,4,5- $\text{P}_3$  (I-3716, Section 18.2).

### Caged Cyclic ADP-Ribose

Cyclic ADP-ribose (cADP-ribose, C-7619; Section 18.2) is a potent intracellular  $\text{Ca}^{2+}$ -mobilizing agent that likely functions as a second messenger in an Ins 1,4,5- $\text{P}_3$ -independent pathway.<sup>41-46</sup> Our NPE-caged cADP-ribose (C-7074, Figure 17.5) induces  $\text{Ca}^{2+}$  mobilization in sea urchin egg homogenates only after photolysis,<sup>21</sup> and this  $\text{Ca}^{2+}$  release is inhibited by the specific cADP-ribose antagonist 8-amino-cADP-ribose<sup>20</sup> (A-7621, Section 18.2). Furthermore, when microinjected into live sea urchin eggs, NPE-caged cADP-ribose was shown to mobilize  $\text{Ca}^{2+}$  and activate cortical exocytosis after illumination with a mercury-arc lamp.<sup>20</sup>

## Caged Amino Acid Neurotransmitters

Once activated, caged amino acid neurotransmitters rapidly initiate neurotransmitter action (Figure 17.6), thus providing tools for kinetic studies of receptor binding or channel opening.<sup>1</sup> We offer caged carbamylcholine<sup>47-55</sup> (*N*-(CNB-caged) carbachol, C-13654), caged  $\gamma$ -aminobutyric acid<sup>18,56-58</sup> (*O*-(CNB-caged) GABA, A-7110) and caged *N*-methyl-D-aspartic acid<sup>59,60</sup> ( $\beta$ -(CNB-caged) NMDA, M-71114), as well as two caged versions of L-glutamic acid<sup>57,61-67</sup> (C-7122, G-7055), all of which are biologically inactive before photolysis.<sup>68</sup>

## Caged Luciferin

DMNPE-caged luciferin (L-7085) readily crosses cell membranes, allowing more efficient delivery of luciferin into intact cells.<sup>69</sup> Once the caged luciferin is inside the cell, active luciferin can be released either instantaneously by a flash of UV light, or continuously by the action of endogenous intracellular esterases found in many cell types.

## Caged Fluorescent Dyes

Photoactivatable fluorescent dyes may be one of the most useful developments in caged probe technology. In general, the caged fluorophores are colorless and nonfluorescent until photolyzed with UV light. Demonstrated and suggested applications include the study of fluid dynamics,<sup>70–74</sup> hydrodynamic properties of the cytoplasmic matrix, lateral diffusion in membranes and cell–cell communication. Photoactivatable fluorophores are particularly useful for investigating cell lineage because they can be injected early in development when the cells are large, and then later activated in the growing embryo when the cells of interest may be small and buried deep within the tissue.<sup>75</sup> Caged fluorescent dyes have also been conjugated to proteins to follow the assembly of tubulin,<sup>76</sup> microtubule flux<sup>76–78</sup> and the dynamic behavior of actin filaments.<sup>79,80</sup>

Molecular Probes prepares caged versions of fluorescein<sup>71,74,81</sup> (F-7103). We also offer a 10,000 MW dextran conjugate of DMNB-caged fluorescein<sup>70,82,83</sup> (D-3310), as well as its lysine-fixable, biotinylated analog (D-7146). Our caged fluorescein dextran has proven useful for analyzing regional cell movement and tissue patterning in zebrafish embryos (Figure 17.7).<sup>70</sup>

The succinimidyl ester of CMNB-caged carboxyfluorescein (C-20050, Figure 1.42) can be used to attach the caged fluorophore to primary amine groups. The labeled probes can subsequently be photolyzed to green-fluorescent conjugates. Once photolyzed, the fluorescein dye is recognized by our anti-fluorescein/Oregon Green antibodies (Section 7.4, Figure 7.93). This succinimidyl ester is reasonably water soluble above pH 6.

## Protein Conjugates of CMNB-Caged Fluorescein

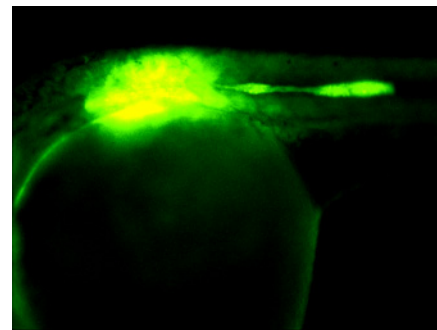
The goat anti–mouse IgG, goat anti–rabbit IgG and streptavidin conjugates of CMNB-caged fluorescein (G-21061, G-21080, S-21380) are unique reagents for labeling targets in cells and on arrays. Unlike directly fluorescent reagents, these caged secondary detection reagents allow determination of the background fluorescence prior to photolysis simply by exciting the sample through optical bandpass filters typically used for fluorescein (Table 24.8). Photoactivation of the CMNB-caged fluorescein conjugates by brief UV light illumination results in a large increase in the visible fluorescence, with the optical characteristics typical of fluorescein. Using digital imaging technology, researchers can detect probe binding to the target by determining in which pixels the fluorescence increased upon illumination (Figure 7.93). We have utilized this procedure as part of a scheme to simultaneously or sequentially measure multiple signals from fluorophores that have strong spectral overlap.

## Kit for Caging Carboxylic Acid

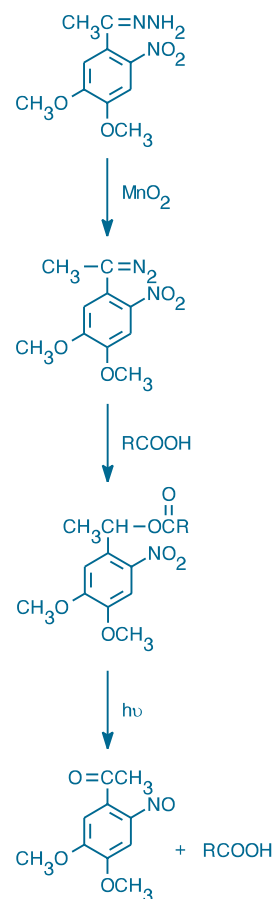
Using organic synthesis methods, researchers can cage a diverse array of molecules. One of the preferred caging groups is the 1-(4,5-dimethoxy-2-nitrophenyl)ethyl (DMNPE) ester. Because the diazoethane precursor to DMNPE esters is unstable, we offer a kit (D-2516) for the generation of 1-(4,5-dimethoxy-2-nitrophenyl)diazoethane and the subsequent preparation of DMNPE esters.<sup>84</sup> This kit includes:

- 25 mg of the hydrazone precursor
- MnO<sub>2</sub> for oxidation
- Celite for filtration of the reaction mixture
- A detailed protocol for caging carboxylic acids

A wide range of compounds containing a weak oxy acid (with a pK<sub>a</sub> between 3 and 7), including carboxylic acids, phenols and phosphates, should react with the diazoethane to form the DMNPE-caged analogs<sup>14</sup> (Figure 17.8).



**Figure 17.7** A zebrafish embryo that was injected with a 1% solution (in 0.2 M KCl) of DMNB-caged fluorescein 10,000 MW dextran (D-3310) at the two-cell stage and then allowed to grow for 19 hours. The posterior lateral line placode was then exposed to a five-second pulse from an epifluorescence microscope fitted with a DAPI optical filter set. After six hours of further development, the labeled primordium has migrated caudally, away from the activation site. Image contributed by Walter K. Metcalfe, Institute of Neuroscience, University of Oregon.



**Figure 17.8** Caging of a carboxylic acid using the hydrazone precursor of DMNPE, a reagent that is provided in Molecular Probes' 1-(4,5-Dimethoxy-2-nitrophenyl)diazoethane Generation Kit (D-2516).

## References

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## Product List — 17.2 Caged Probes for a Variety of Applications

Cat #	Product Name	Unit Size
A-7056	adenosine 5'-diphosphate, $P^2$ -(1-(2-nitrophenyl)ethyl) ester, monopotassium salt (NPE-caged ADP)	5 mg
A-1049	adenosine 5'-triphosphate, $P^3$ -(1-(4,5-dimethoxy-2-nitrophenyl)ethyl) ester, disodium salt (DMNPE-caged ATP)	5 mg
A-1048	adenosine 5'-triphosphate, $P^3$ -(1-(2-nitrophenyl)ethyl) ester, disodium salt (NPE-caged ATP)	5 mg
A-7110	$\gamma$ -aminobutyric acid, $\alpha$ -carboxy-2-nitrobenzyl ester, trifluoroacetic acid salt (O-(CNB-caged) GABA)	5 mg
B-7108	4-bromo A-23187, 1-(4,5-dimethoxy-2-nitrophenyl)ethyl ester (DMNPE-caged Br A-23187)	1 mg
C-20050	5-carboxyfluorescein-bis-(5-carboxymethoxy-2-nitrobenzyl) ether, $\beta$ -alanine-carboxamide, succinimidyl ester (CMNB-caged carboxyfluorescein, SE)	1 mg
C-13654	$N$ -( $\alpha$ -carboxy-2-nitrobenzyl)carbamylcholine, trifluoroacetic acid salt ( $N$ -(CNB-caged) carbachol)	5 mg
C-7122	$N$ -( $\alpha$ -carboxy-2-nitrobenzyl)-L-glutamic acid, trifluoroacetic acid salt ( $N$ -(CNB-caged) L-glutamic acid)	5 mg
C-7074	cyclic adenosine 5'-diphosphate ribose, 1-(1-(2-nitrophenyl)ethyl) ester (NPE-caged cADP-ribose) *mixed isomers*	50 $\mu$ g
D-3310	dextran, DMNB-caged fluorescein, 10,000 MW, anionic	5 mg
D-7146	dextran, DMNB-caged fluorescein and biotin, 10,000 MW, lysine fixable	5 mg
D-3036	diazo-2, AM *cell permeant* *special packaging*	20 x 50 $\mu$ g
D-3034	diazo-2, tetrapotassium salt *cell impermeant*	1 mg
D-1037	4,5-dimethoxy-2-nitrobenzyl adenosine 3',5'-cyclicmonophosphate (DMNB-caged cAMP)	5 mg
D-6814	1-(4,5-dimethoxy-2-nitrophenyl)-1,2-diaminoethane- $N,N,N',N'$ -tetraacetic acid (DMNP-EDTA) *cell impermeant*	5 mg
D-6815	1-(4,5-dimethoxy-2-nitrophenyl)-1,2-diaminoethane- $N,N,N',N'$ -tetraacetic acid, tetra(acetoxymethyl ester) (DMNP-EDTA, AM) *cell permeant* *special packaging*	20 x 50 $\mu$ g
D-2516	1-(4,5-Dimethoxy-2-nitrophenyl)diazoethane Generation Kit	1 kit
F-7103	fluorescein bis-(5-carboxymethoxy-2-nitrobenzyl) ether, dipotassium salt (CMNB-caged fluorescein)	5 mg
G-7055	L-glutamic acid, $\gamma$ -( $\alpha$ -carboxy-2-nitrobenzyl) ester, trifluoroacetic acid salt ( $\gamma$ -(CNB-caged) L-glutamic acid)	5 mg
G-21061	goat anti-mouse IgG (H+L), CMNB-caged fluorescein conjugate *2 mg/mL*	250 $\mu$ L
G-21080	goat anti-rabbit IgG (H+L), CMNB-caged fluorescein conjugate *2 mg/mL*	250 $\mu$ L
G-1053	guanosine 5'- $O$ -(3-thiotriphosphate), $P^3(S)$ -(1-(4,5-dimethoxy-2-nitrophenyl)ethyl) ester, triammonium salt (S-(DMNPE-caged) GTP- $\gamma$ -S)	1 mg
H-6829	2-hydroxyphenyl-1-(2-nitrophenyl)ethyl phosphate, sodium salt (NPE-caged proton)	25 mg
I-23580	$D$ -myo-inositol 1,4,5-triphosphate, $P_{4(5)}$ -(1-(2-nitrophenyl)ethyl) ester, tris(triethylammonium salt) (NPE-caged Ins 1,4,5- $P_3$ )	25 $\mu$ g
L-7085	$D$ -luciferin, 1-(4,5-dimethoxy-2-nitrophenyl)ethyl ester (DMNPE-caged luciferin)	5 mg
M-7114	$N$ -methyl- $D$ -aspartic acid, $\beta$ -( $\alpha$ -carboxy-2-nitrobenzyl) ester, trifluoroacetic acid salt ( $\beta$ -(CNB-caged) NMDA)	1 mg
N-6803	$o$ -nitrophenyl EGTA, AM (NP-EGTA, AM) *cell permeant* *special packaging*	20 x 50 $\mu$ g
N-6802	$o$ -nitrophenyl EGTA, tetrapotassium salt (NP-EGTA) *cell impermeant*	1 mg
N-7065	1-(2-nitrophenyl)ethyl phosphate, diammonium salt (NPE-caged phosphate)	5 mg
S-21380	streptavidin, CMNB-caged fluorescein conjugate	1 mg