

## 18.2 Calcium Regulation

Intracellular  $\text{Ca}^{2+}$  levels modulate a multitude of vital cellular processes — including gene expression, cell viability, cell proliferation, cell motility and cell shape and volume regulation — thereby playing a key role in regulating cell responses to external signals. These dynamic changes in  $\text{Ca}^{2+}$  levels are regulated by ligand-gated and G-protein-coupled ion channels in the plasma membrane and by mobilization of  $\text{Ca}^{2+}$  from intracellular stores. The generation of cytosolic  $\text{Ca}^{2+}$  spikes and oscillations typically involves the coordinated release and uptake of  $\text{Ca}^{2+}$  from these stores, mediated by intracellular  $\text{Ca}^{2+}$  channels and their response to several second messengers such as  $\text{Ca}^{2+}$  itself, cyclic ADP ribose and inositol triphosphate.<sup>1–3</sup> Molecular Probes has available several important reagents for studying  $\text{Ca}^{2+}$  regulation in live cells that are described in this section. Fluorescent nucleotides, including analogs of ATP, ADP, AMPPNP, GTP, GDP, GTP- $\gamma$ -S and GMPPNP are described in Section 18.3. Our GTP analogs may be particularly useful in the assay of G-protein-coupled receptors. In Section 18.4 are described our phosphodiesterase substrates, including several unique fluorescent phosphatidyl inositol derivatives, as well as monoclonal antibodies to phosphatidyl inositol phosphates.

### Inositol Triphosphate Pathway

#### *D*-myo-1,4,5-Inositol Triphosphate and Caged *D*-myo-1,4,5-Inositol Triphosphate

Molecular Probes offers the potassium salt of *D*-myo-inositol 1,4,5-triphosphate (Ins 1,4,5- $\text{P}_3$ , I-3716) for researchers investigating inositol triphosphate-dependent  $\text{Ca}^{2+}$  mobilization and signal transduction mechanisms.<sup>4</sup> Cytoplasmic Ins 1,4,5- $\text{P}_3$  is a potent intracellular second messenger that induces  $\text{Ca}^{2+}$  release from membrane-bound stores in many tissues.<sup>1,4,5</sup> Our Ins 1,4,5- $\text{P}_3$  is at least 99% pure, as determined by paper chromatography and by  $^1\text{H}$  NMR and  $^{31}\text{P}$  NMR.

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 and is widely employed in studies of Ins 1,4,5- $\text{P}_3$ -mediated second messenger pathways.<sup>6,7</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- $\text{P}_3$ .

#### Fluorescent Heparin

Our fluorescein-labeled heparin (H-7482) should be a useful tool for studying binding of this mucopolysaccharide in cells and tissues.<sup>8</sup> In addition to its well-known anticoagulant activity,<sup>9</sup> heparin binds to the Ins 1,4,5- $\text{P}_3$  receptor and inhibits the biological cascade of events mediated by Ins 1,4,5- $\text{P}_3$ .<sup>10</sup> Heparin exhibits a number of other biological properties, including modulation of the structure, function and metabolism of many proteins and enzymes. This mucopolysaccharide binds to thrombin,<sup>11</sup> low-density lipoproteins,<sup>12,13</sup> lipoprotein lipase, circulatory serine proteases and proteinase inhibitors, as well as to blood vessel-associated proteins such as fibronectin<sup>12,14–16</sup> and laminin.<sup>17</sup> Heparin also interacts with heparin-binding growth factors

(HBGFs) and, for HBGF-1, amplifies its mutagenic and neurotrophic activity.<sup>18</sup> Fluorescein-labeled heparin can be prepared by a variety of methods, which may influence its applications. Therefore, fluorescein heparin from Molecular Probes may not be identical to that used in all of the reported applications.<sup>12,14–16,19–24</sup>

### Calcium-Induced Calcium Release

#### Cyclic ADP Ribose and Caged Cyclic ADP Ribose

Molecular Probes offers cyclic ADP ribose (cADP-ribose, C-7619), a potent, microinjectable  $\text{Ca}^{2+}$ -mobilizing agent that functions as a second messenger in an Ins 1,4,5- $\text{P}_3$ -independent pathway and is involved in  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release in both mammalian cells<sup>25–31</sup> and plants.<sup>32</sup> cADP-ribose is a putative physiological regulator of certain isoforms of ryanodine-activated  $\text{Ca}^{2+}$  channels<sup>33–36</sup> that may act through a calmodulin-mediated mechanism.<sup>37</sup> cADP-ribose is reported to mobilize  $\text{Ca}^{2+}$  in dorsal root ganglion cells, pituitary cells and sea urchin eggs,<sup>38–40</sup> where it can also act synergistically with ryanodine to release  $\text{Ca}^{2+}$  from intracellular  $\text{Ca}^{2+}$  stores.<sup>41</sup> This  $\text{Ca}^{2+}$ -mobilizing agent is also produced in pancreatic islets by glucose stimulation<sup>42,43</sup> and has been shown to mediate glucose-induced insulin release in pancreatic cells<sup>44,45</sup> and to induce release of  $\text{Ca}^{2+}$  from stores in and around the nuclear envelope.<sup>46</sup> Researchers have investigated the role of cADP-ribose in the agonist-evoked  $\text{Ca}^{2+}$  oscillations in pancreatic acinar cells.<sup>47</sup> In this study, cADP-ribose-induced  $\text{Ca}^{2+}$  spikes could be blocked with either ryanodine or heparin, implicating both ryanodine and Ins 1,4,5- $\text{P}_3$  receptors in the  $\text{Ca}^{2+}$  spike generation.

The application of cADP-ribose in cells and tissues can be controlled spatially and temporally by flash photolysis of our patented NPE-caged cADP-ribose<sup>48</sup> (C-7074). Photorelease of cADP-ribose from NPE-caged cADP-ribose results in initiation of  $\text{Ca}^{2+}$  release and the cortical reaction in sea urchin eggs.<sup>49</sup>

#### Deaza Cyclic ADP Ribose

3-Deaza-cyclic adenosine 5'-diphosphate ribose (3-deaza-cADP-ribose, D-23650) is a stable, nonhydrolyzable analog of cADP-ribose that is reported to be 70 times more potent than cADP-ribose when used in similar applications.<sup>25,50</sup> Concentrations of 3-deaza-cADP-ribose as low as 0.3 nM induce calcium release in sea urchin egg homogenates. This release can be blocked by 8-amino-cADP-ribose (A-7621).

#### Cyclic ADP Ribose Antagonist

Inhibition of cADP-ribose-induced  $\text{Ca}^{2+}$  mobilization can be achieved specifically and reversibly with the cADP-ribose antagonist 8-amino-cADP-ribose<sup>27,28,51–53</sup> (A-7621). 8-Amino-cADP-ribose has been shown both to block binding of radiolabeled cADP-ribose to sea urchin egg microsomes and to inhibit cADP-ribose-mediated release of  $\text{Ca}^{2+}$  from egg homogenates, but it does not block caffeine- or ryanodine-induced  $\text{Ca}^{2+}$  release.

#### Ryanodine and Fluorescent Ryanodine

Ryanodine is a plant alkaloid that mobilizes  $\text{Ca}^{2+}$  from intracellular stores by activating a class of Ins 1,4,5- $\text{P}_3$ -insensitive  $\text{Ca}^{2+}$  channels.<sup>54</sup> It alters the function of the  $\text{Ca}^{2+}$  channel in a complex manner: submicromolar concentrations lock the channel

in a long-lived open state, whereas micromolar or greater concentrations inhibit  $\text{Ca}^{2+}$  release.<sup>55,56</sup> Ryanodine can be used to modulate the  $\text{Ca}^{2+}$  concentration in sea urchin eggs<sup>39</sup> and in parotid acinar cells.<sup>57</sup> In developing skeletal muscle, ryanodine receptors localize in discrete regions of the T tubules, binding at the junctional complex between the T tubules and the sarcoplasmic reticulum.<sup>58</sup>

Unlabeled “ryanodine” from most other commercial sources is an almost equimolar mixture of ryanodine and dehydroryanodine; however, Molecular Probes offers HPLC-purified ryanodine (R-7478, Figure 18.3) that is >95% pure.

In addition to unlabeled ryanodine, we have prepared a monosubstituted BODIPY FL-X derivative<sup>59,60</sup> (B-7505) and a BODIPY TR-X derivative<sup>60</sup> (B-13802), both of which are mixtures of BODIPY ryanodine and BODIPY dehydroryanodine. They are most likely labeled at the 10-position of the ryanodine molecule (Figure 16.40). BODIPY FL-X ryanodine has been used to visualize ryanodine receptor distribution in live porcine endothelial cells,<sup>61</sup> pancreatic  $\beta$ -cells,<sup>62</sup> vascular monocytes,<sup>63</sup> the rat parotid gland<sup>64</sup> and cardiomyocytes.<sup>65</sup>

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

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. The properties and uses of caged probes are described in Chapter 17.

### NP-EGTA: A Caged $\text{Ca}^{2+}$ Reagent

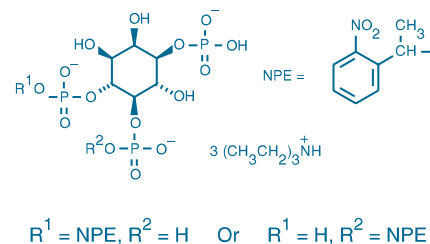
Ellis-Davies and Kaplan have developed the photolabile chelator — *o*-nitrophenyl EGTA<sup>66</sup> (NP-EGTA) — that exhibits a high selectivity for  $\text{Ca}^{2+}$ , a dramatic 12,500-fold decrease in affinity for  $\text{Ca}^{2+}$  upon UV illumination (its  $K_d$  increases from 80 nM to >1 mM) and a high photochemical quantum yield<sup>67,68</sup> (~0.2). Furthermore, with a  $K_d$  for  $\text{Mg}^{2+}$  of 9 mM, NP-caged EGTA does not perturb physiological levels of  $\text{Mg}^{2+}$ . We offer the potassium 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 calcium complex that will rapidly deliver  $\text{Ca}^{2+}$  upon photolysis (Figure 18.4). The cell-permeant AM ester of NP-EGTA does not bind  $\text{Ca}^{2+}$  unless the AM esters are removed. It can potentially serve as a photolabile buffer in cells because, once converted to NP-EGTA by intracellular esterases, it will bind  $\text{Ca}^{2+}$  with high affinity until photolyzed with UV light. NP-EGTA has been used to measure the calcium buffering capacity of cells.<sup>69</sup> We have loaded similar chelators into cells with our Influx pinocytotic cell-loading reagent (I-14402, Section 20.8).

### DMNP-EDTA: A Caged $\text{Ca}^{2+}$ Reagent

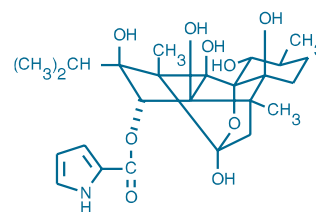
The first caged  $\text{Ca}^{2+}$  reagent described by Ellis-Davies and Kaplan was 1-(4,5-dimethoxy-2-nitrophenyl) EDTA (DMNP-EDTA, D-6814), which they named DM-Nitrophen (now a trademark of Calbiochem-Novabiochem Corp.).<sup>70–72</sup> Because its structure better resembles that of EDTA than EGTA, we named it as a caged EDTA derivative (Figure 18.5). Upon illumination, DMNP-EDTA's  $K_d$  for  $\text{Ca}^{2+}$  increases from 5 nM to 3 mM. Thus, photolysis of DMNP-EDTA complexed with  $\text{Ca}^{2+}$  results in a pulse of free  $\text{Ca}^{2+}$ . Furthermore, DMNP-EDTA has significantly higher affinity for  $\text{Mg}^{2+}$  ( $K_d = 2.5 \mu\text{M}$ ) than does NP-EGTA ( $K_d = 9 \text{ mM}$ ).<sup>70</sup> The photolysis product's  $K_d$  for  $\text{Mg}^{2+}$  is ~3 mM, making DMNP-EDTA an effective caged  $\text{Mg}^{2+}$  source, in addition to its applications for photolytic  $\text{Ca}^{2+}$  release.<sup>73,74</sup> Photorelease of  $\text{Ca}^{2+}$  has been shown to occur in <180 microseconds, with even faster photorelease of  $\text{Mg}^{2+}$ .<sup>75</sup> A paper by Neher and Zucker discusses the uses and limitations of DMNP-EDTA.<sup>74</sup> In addition to high-purity DMNP-EDTA (D-6814), we have prepared its membrane-permeant acetoxymethyl ester (D-6815).

### Diazo-2: A Photoactivatable $\text{Ca}^{2+}$ Scavenger

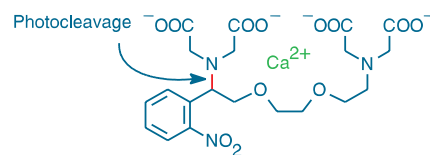
In contrast to NP-EGTA and DMNP-EDTA, diazo-2 is a photoactivatable  $\text{Ca}^{2+}$  scavenger. Diazo-2 (Figure 17.3), which was introduced by Adams, Kao and Tsien,<sup>76–78</sup> is a relatively weak chelator ( $K_d$  for  $\text{Ca}^{2+} = 2.2 \mu\text{M}$ ). However, following flash photolysis at ~360 nm, cytosolic free  $\text{Ca}^{2+}$  rapidly binds to the diazo-2 photolysis product, which has a high affinity for  $\text{Ca}^{2+}$  ( $K_d = 73 \text{ nM}$ ). Photolysis of diazo-2 has been used to decrease cytosolic  $\text{Ca}^{2+}$  in less than two milliseconds in tensed frog muscle cells<sup>79</sup> and rabbit



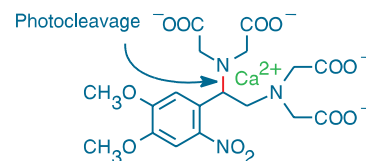
**Figure 18.2** I-23580 D-*myo*-inositol 1,4,5-trisphosphate,  $P_{4(5)}$ -(1-(2-nitrophenyl)ethyl) ester, tris (triethylammonium salt) (NPE-caged Ins 1,4,5- $P_3$ ).



**Figure 18.3** R-7478 ryanodine.

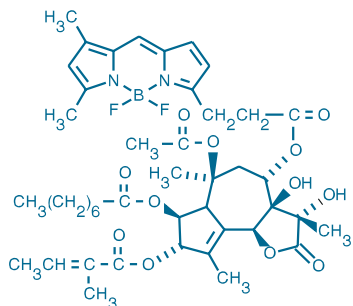


**Figure 18.4** NP-EGTA (N-6802) complexed with  $\text{Ca}^{2+}$ . Upon illumination, this complex is cleaved to yield free  $\text{Ca}^{2+}$  and two iminodiacetic acid photoproducts. The affinity of the photoproducts for  $\text{Ca}^{2+}$  is ~12,500-fold lower than that of NP-EGTA.



**Figure 18.5** DMNP-EDTA (D-6814) complexed with  $\text{Ca}^{2+}$ . Upon illumination, this complex is cleaved to yield free  $\text{Ca}^{2+}$  and two iminodiacetic acid photoproducts. The affinity of the photoproducts for  $\text{Ca}^{2+}$  is ~600,000-fold lower than that of DMNP-EDTA.

*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 18.6** B-7487 BODIPY FL thapsigargin.

*Molecular Probes is the leading manufacturer and provider of intracellular  $Ca^{2+}$  indicators, including FluoroPure-grade fura-2 AM, fluo-3 AM and fluo-4 AM. See the list of our FluoroPure-grade dyes and probes and their specifications in Section 20.3. All of our  $Ca^{2+}$  indicators are discussed in Chapter 20, and extensive bibliographies for all indicators are available at our Web site ([www.probes.com/search](http://www.probes.com/search)). Generous discounts are available on bulk quantities of these reagents for high-throughput screening.*

arterial smooth muscle.<sup>80</sup> Microinjecting a relatively low concentration of fluo-3, fluo-4, or one of the Calcium Green or Oregon Green 488 BAPTA indicators (Section 20.3), along with a known quantity of diazo-2, permits measurement of the extent of depletion of cytosolic  $Ca^{2+}$  following photolysis.<sup>77,81,82</sup> Diazo-2 can be microinjected into cells as its potassium salt (D-3034) or utilized as its cell membrane-permeant acetoxymethyl ester (diazo-2, AM; D-3036). Diazo-2 also has been loaded into cells permeabilized with *Staphylococcus aureus*  $\alpha$ -toxin<sup>80</sup> and can probably be loaded into cells using our Influx pinocytotic cell-loading reagent (I-14402, Section 20.8).

## Other Probes for Calcium Regulation

### Thapsigargin and Fluorescent Thapsigargin

Thapsigargin is a naturally occurring sesquiterpene lactone isolated from the umbelliferous plant *Thapsia garganica*.<sup>83</sup> This tumor promoter releases  $Ca^{2+}$  from intracellular stores by specifically inhibiting the endoplasmic reticulum  $Ca^{2+}$ -ATPase;<sup>84,85</sup> it does not directly affect plasma membrane  $Ca^{2+}$ -ATPases, Ins 1,4,5- $P_3$  production or protein kinase C activity.<sup>86,87</sup> Reports have described the effects of thapsigargin-induced  $Ca^{2+}$  signals on the transient suppression of *c-myc* mRNA levels,<sup>88</sup> as well as the regulation of such  $Ca^{2+}$  signals by sphingomyelinase and sphingosine.<sup>89</sup> Thapsigargin is available from Molecular Probes in 1 mg units (T-7458, Figure 18.6) and specially packaged in 20 vials containing 50  $\mu$ g each (T-7459). We have prepared the green-fluorescent BODIPY FL thapsigargin<sup>65,90</sup> (B-7487) and red-fluorescent BODIPY TR-X thapsigargin<sup>91</sup> (B-13800). These membrane-permeant probes have spectral properties similar to those of fluorescein and the Texas Red dye, respectively, and may be useful for localization of thapsigargin binding sites in live cells.

### Luminescent Calcium Analog

The trivalent lanthanide, terbium (III), which is supplied by Molecular Probes as its chloride salt (T-1247), is a luminescent analog of  $Ca^{2+}$  that can be used to study structure–function relationships in  $Ca^{2+}$ -binding proteins such as calmodulin (C-23693, Section 18.3), oncomodulin, lactalbumin and ATPases.<sup>92–94</sup> The long-lived luminescence of  $Tb^{3+}$  has also been used to probe  $Ca^{2+}$ -binding sites of alkaline phosphatase,<sup>95</sup> glutamine synthetase,<sup>96</sup> integrins,<sup>92</sup> protein kinase C<sup>97</sup> and  $Ca^{2+}$ -binding sites of ryanodine-sensitive  $Ca^{2+}$  channels.<sup>98</sup>  $Tb^{3+}$  reportedly binds most strongly to the I and II sites of calmodulin.<sup>99</sup>

## References

- Nat Rev Mol Cell Biol 2, 327 (2001);
- Annu Rev Physiol 56, 297 (1994);
- Nature 365, 388 (1993);
- Nature 341, 197 (1989);
- Annu Rev Biochem 56, 159 (1987);
- J Physiol 487, 343 (1995);
- Neuron 15, 755 (1995);
- Nature 372, 231 (1994);
- J Biol Chem 267, 8857 (1992);
- Biochem J 302, 155 (1994);
- J Biol Chem 273, 34730 (1998);
- Biochemistry 31, 5996 (1992);
- Biochim Biophys Acta 1167, 211 (1993);
- Biochemistry 27, 7565 (1988);
- Biochemistry 32, 12548 (1993);
- J Biol Chem 260, 7250 (1985);
- Anal Biochem 156, 320 (1986);
- Biochemistry 31, 6498 (1992);
- Cytometry 23, 59 (1996);
- Biol Pharm Bull 16, 939 (1993);
- Anal Biochem 160, 105 (1987);
- Biochim Biophys Acta 925, 57 (1987);
- Anal Biochem 130, 287 (1983);
- Carbohydr Res 105, 69 (1982);
- FASEB J 14, 680 (2000);
- Nature 398, 70 (1999);
- Physiol Rev 77, 1133 (1997);
- J Biol Chem 270, 25488 (1995);
- J Biol Chem 270, 9060 (1995);
- Cell Signal 6, 591 (1994);
- Mol Cell Biochem 138, 229 (1994);
- Science 278, 2126 (1997);
- J Biol Chem 270, 17917 (1995);
- Pflugers Arch 429, 426 (1995);
- Neuron 12, 1073 (1994);
- Nature 364, 76 (1993);
- Nature 370, 307 (1994);
- Biochemistry 34, 2815 (1995);
- Science 253, 1143 (1991);
- J Biol Chem 264, 1608 (1989);
- Dev Biol 163, 1 (1994);
- J Biol Chem 270, 30257 (1995);
- J Biol Chem 270, 30045 (1995);
- Biochimie 77, 356 (1995);
- Science 259, 370 (1993);
- Cell 80, 439 (1995);
- EMBO J 13, 2038 (1994);
- US 5,872,243;
- J Biol Chem 270, 7745 (1995);
- Biochim Biophys Acta 1472, 555 (1999);
- Am J Physiol 274, C1653 (1998);
- J Biol Chem 273, 2497 (1998);
- Biochemistry 35, 10922 (1996);
- J Biol Chem 268, 13765 (1993);
- J Gen Physiol 92, 1 (1988);
- Am J Physiol 253, C364 (1987);
- J Biol Chem 266, 14535 (1991);
- J Cell Biol 112, 289 (1991);
- J Gen Physiol 116, 697 (2000);
- J Neurosci 20, 9059 (2000);
- J Physiol 506, 109 (1998);
- J Biol Chem 274, 14147 (1999);
- J Biol Chem 275, 9596 (2000);
- Biochem J 340, 519 (1999);
- Cell Calcium 28, 127 (2000);
- NP-EGTA is licensed to Molecular Probes under US 5,446,186;
- J Biol Chem 270, 23966 (1995);
- Proc Natl Acad Sci U S A 91, 187 (1994);
- Biochem Biophys Res Commun 250, 786 (1998);
- Proc Natl Acad Sci U S A 85, 6571 (1988);
- Science 241, 842 (1988);
- DMNP-EDTA is licensed to Molecular Probes under US 4,981,985;
- Methods Cell Biol 40, 31 (1994);
- Neuron 10, 21 (1993);
- Biochemistry 31, 8856 (1992);
- Biochim Biophys Acta 1035, 378 (1990);
- J Am Chem Soc 111, 7957 (1989);
- Diazo-2 and diazo-3 are licensed to Molecular Probes under US 5,141,627;
- FEBS Lett 255, 1 (1989);
- Biophys J 69, 2611 (1995);
- Nature 371, 603 (1994);
- Biophys J 65, 2537 (1993);
- Acta Pharm Suec 15, 133 (1978);
- J Biol Chem 273, 12994 (1998);
- J Biol Chem 270, 11731 (1995);
- Proc Natl Acad Sci U S A 87, 2466 (1990);
- J Biol Chem 264, 12266 (1989);
- J Biol Chem 269, 8786 (1994);
- J Biol Chem 269, 5054 (1994);
- Biochem J 325, 239 (1997);
- J Biol Chem 274, 32535 (1999);
- Biochemistry 33, 12238 (1994);
- J Biol Chem 267, 13340 (1992);
- Photochem Photobiol 46, 1067 (1987);
- J Photochem Photobiol B 13, 289 (1992);
- Biochemistry 30, 3417 (1991);
- J Biol Chem 263, 4223 (1988);
- J Biol Chem 269, 24864 (1994);
- Biochem Biophys Res Commun 138, 1243 (1986).

## Data Table — 18.2 Calcium Regulation

Cat #	MW	Storage	Soluble	Abs	EC	Em	Solvent	Notes
A-7621	556.32	FF,D	H <sub>2</sub> O	274	14,000	none	pH 7	
B-7487	854.75	FF,D,L	DMSO	503	85,000	511	MeOH	
B-7505	880.79	FF,D,L	DMSO	504	79,000	511	MeOH	
B-13800	1100.04	FF,D,L	DMSO	589	62,000	616	MeOH	
B-13802	1012.92	FF,D,L	DMSO	589	62,000	616	MeOH	
C-7074	690.45	FF,D,LL	H <sub>2</sub> O	259	16,000	none	H <sub>2</sub> O	1, 2
C-7619	541.30	FF,D	H <sub>2</sub> O	258	9,900	none	pH 7	
D-3034	710.86	F,D,LL	pH >6	369	18,000	none	pH 7.2	1, 3, 4
D-3036	846.75	F,D,LL	DMSO	342	22,000	none	MeOH	
D-6814	473.39	D,LL	DMSO	348	4,200	none	pH 7.2	1, 4, 5
D-6815	761.65	F,D,LL	DMSO	333	4,600	none	MeOH	6
D-23650	540.32	F,D	H <sub>2</sub> O	265	11,000	none	pH 7	
I-3716	648.64	F,D	H <sub>2</sub> O	<250		none		
I-23580	872.82	FF,D,LL	H <sub>2</sub> O	264	4,200	none	H <sub>2</sub> O	1, 2, 7
N-6802	653.81	FF,D,LL	pH >6	260	3,500	none	pH 7.2	1, 2, 4, 8
N-6803	789.70	FF,D,LL	DMSO	250	4,100	none	MeCN	6
R-7478	493.55	F,D	MeOH, DMSO	<300		none		
T-1247	373.38	D	H <sub>2</sub> O	270	4,700	545	H <sub>2</sub> O	9, 10
T-7458	650.76	F,D	DMSO, EtOH	<300		none		
T-7459	650.76	F,D	DMSO, EtOH	<300		none		

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

### Notes

- All photoactivatable probes are sensitive to light. They should be protected from illumination except when photolysis is intended.
- This compound has weaker visible absorption at >300 nm but no discernible absorption peaks in this region.
- The Ca<sup>2+</sup> dissociation constant of diazo-2 is 2200 nM before photolysis and 73 nM after ultraviolet photolysis (J Am Chem Soc 111, 7957 (1989)). The absorption spectrum of the photolysis product is similar to that of B-1204 (Section 20.8).
- Abs and EC values determined in Ca<sup>2+</sup>-free solution (100 mM KCl, 10 mM EGTA, 10 mM MOPS, pH 7.2).
- K<sub>d</sub>(Ca<sup>2+</sup>) increases from 5 nM to 3 mM after ultraviolet photolysis. K<sub>d</sub> values determined in 130 mM KCl, 10 mM HEPES, pH 7.1 (Proc Natl Acad Sci U S A 85, 6571 (1988)).
- This product is intrinsically a liquid or an oil at room temperature.
- Ultraviolet photolysis of I-23580 generates I-3716.
- K<sub>d</sub>(Ca<sup>2+</sup>) increases from 80 nM to 1 mM after ultraviolet photolysis. K<sub>d</sub> values determined in 100 mM KCl, 40 mM HEPES, pH 7.2 (Proc Natl Acad Sci U S A 91, 187 (1994)).
- Absorption and fluorescence of T-1247 are extremely weak unless it is chelated. Data are for dipicolinic acid (DPA) chelate. Fluorescence spectrum has secondary peak at 490 nm.
- MW is for the hydrated form of this product.

## Product List — 18.2 Calcium Regulation

Cat #	Product Name	Unit Size
A-7621	8-amino-cyclic adenosine 5'-diphosphate ribose (8-amino-cADP-ribose) .....	10 µg
B-7487	BODIPY <sup>®</sup> FL thapsigargin .....	100 µg
B-7505	BODIPY <sup>®</sup> FL-X ryanodine .....	25 µg
B-13802	BODIPY <sup>®</sup> TR-X ryanodine .....	25 µg
B-13800	BODIPY <sup>®</sup> TR-X thapsigargin .....	100 µg
C-7619	cyclic adenosine 5'-diphosphate ribose (cADP-ribose) .....	100 µg
C-7074	cyclic adenosine 5'-diphosphate ribose, 1-(1-(2-nitrophenyl)ethyl) ester (NPE-caged cADP-ribose) *mixed isomers* .....	50 µg
D-23650	3-deaza-cyclic adenosine 5'-diphosphate ribose (3-deaza-cADP-ribose) .....	5 µg
D-3036	diazo-2, AM *cell permeant* *special packaging* .....	20 x 50 µg
D-3034	diazo-2, tetrapotassium salt *cell impermeant* .....	1 mg
D-6814	1-(4,5-dimethoxy-2-nitrophenyl)-1,2-diaminoethane- <i>N,N,N',N'</i> -tetraacetic acid (DMNP-EDTA) *cell impermeant* .....	5 mg
D-6815	1-(4,5-dimethoxy-2-nitrophenyl)-1,2-diaminoethane- <i>N,N,N',N'</i> -tetraacetic acid, tetra(acetoxymethyl ester) (DMNP-EDTA, AM) *cell permeant* *special packaging* .....	20 x 50 µg
H-7482	heparin, fluorescein conjugate .....	1 mg
I-3716	<i>D-myo</i> -inositol 1,4,5-triphosphate, hexapotassium salt (Ins 1,4,5-P <sub>3</sub> ) .....	1 mg
I-23580	<i>D-myo</i> -inositol 1,4,5-triphosphate, P <sub>4(5)</sub> -(1-(2-nitrophenyl)ethyl) ester, tris(triethylammonium salt) (NPE-caged Ins 1,4,5-P <sub>3</sub> ) .....	25 µg
N-6803	<i>o</i> -nitrophenyl EGTA, AM (NP-EGTA, AM) *cell permeant* *special packaging* .....	20 x 50 µg
N-6802	<i>o</i> -nitrophenyl EGTA, tetrapotassium salt (NP-EGTA) *cell impermeant* .....	1 mg
R-7478	ryanodine *free of dehydroryanodine* .....	1 mg
T-1247	terbium(III) chloride, hexahydrate .....	1 g
T-7458	thapsigargin .....	1 mg
T-7459	thapsigargin *special packaging* .....	20 x 50 µg