Caged Nucleotides

Quick Facts

Storage upon receipt:

- –20ºC
- Dessicate
- Protect from light

Introduction

Caged nucleotides are nucleotide analogs in which the terminal phosphate is esterified with a blocking group, rendering the molecule biologically inactive (Figure 1).[1-3] Ultraviolet photolysis of the caging group results in a rapid and highly localized release of free nucleotide at the site of illumination. Molecular Probes' current selection of caged nucleotides includes the following compounds:

- Caged ATP (A-1048, A-1049) which has been widely used to investigate the molecular basis of skeletal muscle fiber contraction[4,5] and the mechanism of ATPases[6,7] and other "molecular motors".[3] Additional applications include activation of cellular P2X purinoreceptors[8] and calibration of luciferase-based ATP assays.[9]
- Caged ADP (A-7056), which is frequently employed in tandem with caged ATP in investigations of skeletal muscle contraction, as well as for analysis of ADP/ATP transport by carrier proteins.[10]
- Caged cAMP (D-1037, N-1045), which is cell-permeant, allowing controlled release of intracellular cAMP.[11]
- Caged GTP-γ-S (G-1053), which produces sustained activation of G-protein coupled signaling pathways after photolysis.[12]

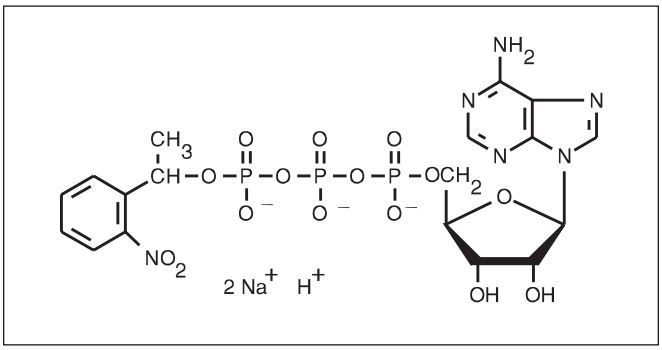

Figure 1. Structure of NPE-caged ATP.

Materials

Caged nucleotides are supplied as solids in units of 5 mg, with the exception of G-1053 (1 mg unit). All these products should be stored frozen at –20°C, desiccated and PROTECTED FROM LIGHT.

Stock Solutions

Stock solutions (≥1 mM) may be prepared in the solvents listed below.

A-1048	aqueous buffer
A-1049	aqueous buffer
A-7056	aqueous buffer
N-1045	dimethylsulfoxide (DMSO)
D-1037	DMSO
G-1053	aqueous buffer

Properties

Because caged ATP is typically added to experimental specimens at relatively high concentrations, use of the enzyme apyrase has been recommended to eliminate any traces of free ATP that may be present in caged ATP samples.[13] Once the caged ATP solutions have been preincubated with apyrase, the enzyme can be removed by centrifugal filtration. Chromatographic purification to remove contaminating ADP may also be necessary for some applications.[3]

With the exception of caged cAMP, caged nucleotides are generally cell impermeant and must be microinjected into cells or loaded by other invasive techniques. Staphylococcal α-toxin has been used to permeabilize smooth muscle cells to facilitate introduction of caged nucleotides including NPE-caged ATP and caged GTP-γ-S.[14]

Photoactivation

Photoactivation (uncaging) of these compounds is accomplished by exposing them to ultraviolet light (wavelengths ≤360 nm). Suitable light sources include lasers,[15,16] flash-lamps[17] and suitably equipped fluorescence microscopes.[18,19] A listing of commercial suppliers of instrumentation specifically designed for photolysis of caged compounds is shown in Table 1.

Table 1. Some suppliers of instrumentation for photolysis of caged compounds.

Company	Location	Web site
Cairn Research Ltd.	Faversham, UK	www.cairnweb.com
Intracellular Imaging, Inc.	Cincinnati, OH, USA	www.intracellular.com
Fryer Company, Inc.	Huntley, IL, USA	www.fryerso.com/prairie
Hi-Tech Scientific	Salisbury, UK	www.hi-techsci.co.uk
Photonic Instruments, Inc.	Arlington Heights, IL, USA	www.photonic-instruments.com
Rapp OptoElectronic	Hamburg, Germany	www.rapp-opto.com
T.I.L.L. Photonics	Martinsried, Germany	www.till-photonics.com
Prairie Technologies, LLC	Middleton, WI, USA	www.prairie-technologies.com

References

1. J Am Chem Soc 110, 7170 (1988); **2.** Methods Enzymol 172, 288 (1989); **3.** Methods Enzymol 291, 307 (1998); **4.** Acta Physiol Scand 166, 341 (1999); **5.** Biophys J 75, 2389 (1998); **6.** Biophys J 72, 2503 (1997); **7.** Biochim Biophys Acta 1368, 184 (1998); **8.** J Physiol 522, 199 (2000); **9.** Lett Appl Microbiol 30, 223 (2000); **10.** Biochemistry 36, 13865 (1997); **11.** Proc Natl Acad Sci USA 95, 1613 (1998); **12.** Neuroscience 87, 649 (1998); **13.** Biophys J 67, 2436 (1994); **14.** Annu Rev Physiol 52, 857 (1990); **15.** Methods Enzymol 291, 175 (1998); **16.** J Neurosci Methods 66, 47 (1996); **17.** Methods Enzymol 291, 202 (1998); **18.** *Intracellular Messengers* (Neuromethods, Volume 20), A. Boulton, G. Baker and C. Taylor, eds., Humana Press (1992) pp 369–396; **19.** Biotechniques 23, 268 (1997).

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
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-7056	adenosine 5′-diphosphate, P^2-(1-(2-nitrophenyl) ethyl) ester, monopotassium salt (NPE-caged ADP)	5 mg
D-1037	4,5-dimethoxy-2-nitrobenzyl adenosine 3′,5′-cyclicmonophosphate (DMNB-caged cAMP)	5 mg
N-1045	1-(2-nitrophenyl)ethyl adenosine 3′,5′-cyclicmonophosphate (NPE-caged cAMP)	5 mg
G-1053	guanosine 5′-O-(3-thiotriphosphate), $P^{3(S)}$-(1-(4, 5-dimethoxy- 2-nitrophenyl)ethyl) ester, triammonium salt (S-(DMNPE-caged) GTP-γ-S)	1 mg

Contact Information

Further information on Molecular Probes' products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

Please visit our Web site — **www.probes.com** — for the most up-to-date information.

Molecular Probes, Inc.
29851 Willow Creek Road, Eugene, OR 97402
Phone: (541) 465-8300 • Fax: (541) 335-0504

Customer Service: 6:00 am to 4:30 pm (Pacific Time)
Phone: (541) 335-0338 • Fax: (541) 335-0305 • order@probes.com

Toll-Free Ordering for USA and Canada:
Order Phone: (800) 438-2209 • Order Fax: (800) 438-0228

Technical Assistance: 8:00 am to 4:00 pm (Pacific Time)
Phone: (541) 335-0353 • Toll-Free (800) 438-2209
Fax: (541) 335-0238 • tech@probes.com

Molecular Probes Europe BV
Poortgebouw, Rijnsburgerweg 10
2333 AA Leiden, The Netherlands
Phone: +31-71-5233378 • Fax: +31-71-5233419

Customer Service: 9:00 to 16:30 (Central European Time)
Phone: +31-71-5236850 • Fax: +31-71-5233419
eurorder@probes.nl

Technical Assistance: 9:00 to 16:30 (Central European Time)
Phone: +31-71-5233431 • Fax: +31-71-5241883
eurotech@probes.nl