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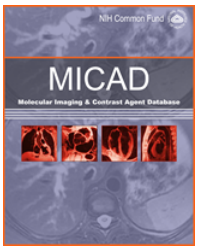


# Pyro-Gly-Asp-Glu-Val-Asp-Gly-Ser-Gly-Lys(BHQ3)

PPB

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| **Chemical name:** | Pyro-Gly-Asp-Glu-Val-Asp-Gly-Ser-Gly-Lys(BHQ3) | |
| **Abbreviated name:** | PPB | |
| **Synonym:** | | |
| **Agent category:** | Peptide | |
| **Target:** | Caspase-3 | |
| **Target category:** | Enzyme | |
| **Method of detection:** | Optical imaging, near-infrared (NIR) fluorescence | |
| **Source of signal/contrast:** | Pyropheophorbide α (Pyro) | |
| **Activation:** | Yes | |
| **Studies:** | • *In vitro*<br>• Rodents | No structure is currently available in PubChem. |

## Background

[PubMed]

Photodynamic therapy (PDT), also known as photochemotherapy, uses light-activated photosensitizers (PS) in the presence of oxygen to kill cells (1). PDT has become a promising modality to treat skin, esophagus, and lung cancers, as well as other diseases such as atherosclerosis, macular degeneration, and rheumatoid arthritis (2). In PDT, light excites the singlet state of the PS, followed by intersystem transition from the singlet state to the triplet state; then, the energy is transferred from the triplet state of the PS to the triplet ground state of oxygen $^3O_2(X^3\Sigma_g^-)$ ($^3O_2$ triplet state quenching) to generate singlet oxygen $^1O_2(a^1\Delta_g)$ (3). The produced $^1O_2$ is a major cytotoxic agent that has a short life time (<200 ns) and an average diffusion range (~20 nm, which is smaller than the diameter of a cell) (2). Such a short diffusion range requires the delivery of target-specific PS agents into subcellular compartments such as cytoskeletal tubulin, lysosomes, mitochondria, plasma membrane, and the nucleus, where they can generate $^1O_2$ efficiently (2). A novel type of PS agents, called a photodynamic molecular beacon (PMB) or killer beacon, has been developed to meet this requirement (2, 4). A typical PMB consists of four modular components: a fluorescent PS, a quencher, a linker, and a delivery vehicle. The target-specific linkers keep the fluorescent PS and the quencher within the effective distance of the Föster radius (3–6 nm) (2),

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which allows efficient fluorescence resonance energy transfer between the fluorescent PS and the quencher. As a result, the fluorescent PS is silent until the PMB meets the target, where the enzyme cleaves the linker and activates the fluorescence of the PS (4). Thus, the PS performs two functions by producing $^1O_2$ to kill cells and by illuminating detectable fluorescence to image its own therapeutic outcome (5).

Caspases are cysteine aspartate proteinases (6), and they participate in apoptosis through direct disassembly of cell structure, cleavage of several important proteins (gelsolin, focal adhesion kinase, and p21), shutting down DNA replication and repair, disrupting nuclear structure, and disintegrating cells into apoptotic bodies (7). As a main downstream effector, the caspase subtype-3 (capspase-3) recognizes targets that contain the tetrapeptide sequence Asp-Glu-Val-Asp (7). Proteins with this recognition motif are cleaved specifically with high efficiency ($k_{cat}/K_m > 10^6$ $M^{-1}s^{-1}$). Pyro-Gly-Asp-Glu-Val-Asp-Gly-Ser-Gly-Lys(BHQ3) (PPB) is a PMB specific for caspase-3, and it is detectable with near-infrared (NIR) fluorescence imaging (5). PPB consists of the infrared fluorescence PS pyropheophorbide α (Pyro), a black hole quencher 3 (BHQ3), and a peptide linker (GDEVDGSGK) (5). The peptide contains the tetrapeptide motif DEVD for caspase-3 recognition, and the cleavage site is located between the D and G residues. Pyro acts as the intracellular vehicle and as the PS (absorption, 665 nm; emission, 675 nm and 720 nm) with good $^1O_2$ production (50%). Pyro lacks dark toxicity (toxicity in absence of ligh) because of its low absorption between 450–600 nm. BHQ3 (absorption, 672 nm) can efficiently quench Pyro fluorescence *via* fluorescence resonance energy transfer (FRET). The cleavage of PPB by caspase-3 separates the photosensitizer (Pyro) from the quencher (BHQ3) and restores the Pyro fluorescence for detection.

## Synthesis

[PubMed]

Stefflova et al. reported the detailed synthesis of PPB (5). A peptide of Fmoc-GD(Boc)E(Boc)VD(Boc)GS(Boc)GK(Mtt)-Sieber resin (Boc is t-butoxycarbonyl; Mtt is methyltrityl) was synthesized by the manual, Fmoc, solid-phase, peptide synthesis protocol with commercially available N-α-protected amino acids as building blocks, Sieber amide resin as a solid support, and N-hydroxybenzotriazole (HOBt)/2-(benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) as amino acid activators. After the last Fmoc group cleavage, Pyro-acid was coupled to the α-$NH_2$ group of N-terminal glycine of the peptide-resin at a molar ratio of 3:1. The produced peptide resin was treated with 50% trifluoroacetic acid and 5% triisopropylsilane in dichloromethane to yield Pyro-GDEVDGSGK, followed by coupling of the ε-$NH_2$ of the C-terminal lysine with BHQ3-NHS to yield PPB. The product was purified with high-performance liquid chromatography and the structure was characterized by UV-vis spectroscopy and matrix-assisted laser desorption/ionization (MALDI)-time of flight mass spectrometry (TOF).

## *In Vitro* Studies: Testing in Cells and Tissues

[PubMed]

The specificity of PPB to caspapse-3 was tested in aqueous solution (5). High-performance liquid chromatography was used to examine the cleavage product. PPB (6.3 μM) was cleaved to two pieces after incubation for 50 min with caspase-3 (0.2 μg). An immediate increase of fluorescence was observed, which reached a plateau at 90 min with as much as an 8-fold increase. This increase was not observed for PPB alone, for the presence of PPB (6.3 μM) with caspase-3 inhibitor (Ac-DEVD-CHO, 100 μM), or the presence of scrambled PPB (Pyro-GPLGLAREK-(BHQ3) with caspase-3 (molar ratio of 200:1). Cleavage was prohibited in the presence of caspase-3–specific inhibitor or scrambled PPB, confirming that PPB was specifically cleaved by caspase-3 in solution.

Stefflova et al. used PPB to study capspase-3–triggered PDT in tumor cells *in vitro* (5). Hepatoblastoma $G_2$ cells ($HepG_2$) were incubated with 200 μM PPB for 30 min or 24 h and subsequently treated with a light dose of 5 $J/cm^2$ at 670 nm. The cleavage of PPB in the cells was monitored with confocal microscopy. The PDT-treated cells exhibited a significant increase of fluorescence intensity. In comparisons, a minimal fluorescence increase was observed for the control cells that were incubated with PPB for 24 h but not exposed to the light, and the cells that were incubated with the scrambled sequence Pyro-GHSSK(BHQ3)LQL followed by exposure to light. These results demonstrate that the peptide linker in the PPB was cleaved within the PDT-treated cells, which induced significant increases in the Pyro fluorescence. To further confirm that this cleavage is apoptosis-related, Apoptag, a terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) assay, was used to examine apoptosis-related DNA cleavage in the $HepG_2$ cells that were incubated with PPB and treated with PDT. The fluorescence from Pyro (633 nm) as a result of the caspase-3–dependent cleavage of PPB and the fluorescence from fluorescein (488 nm) as a result of Apoptag stain were found in the same cellular compartment, which confirmed that apoptosis induced PPB cleavage *in vitro*.

# Animal Studies

## Rodents

[PubMed]

Stefflova et al. studied the cleavage of PPB *in vivo* with fluorescent imaging (5). Nude mice were subcutaneously inoculated on the upper side of the leg with $10^7$ $HepG_2$ cells, and the tumor was grown for about 20 days. Immediately after subcutaneous injection of 80 nmol PPB into the tumor sites, one mouse received PDT treatment at a light dose of 150 $J/cm^2$ for the whole tumor area, and the images were collected at 2, 3, and 4 h. Compared with the tumor that was injected with PPB but not treated with PDT, a visible fluorescence increase was found in the tumor treated with PPB followed by PDT treatment.

## Other Non-Primate Mammals

[PubMed]

No publication is currently available.

## Non-Human Primates

[PubMed]

No publication is currently available.

# Human Studies

[PubMed]

No publication is currently available.

## NIH Support

CA 105008

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