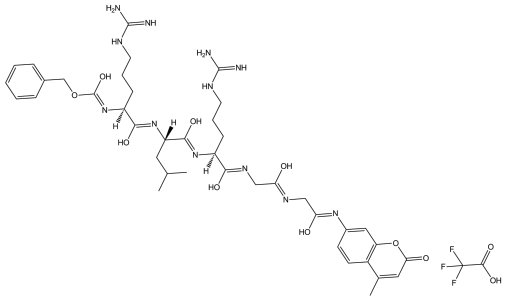
Product Data Sheet

Product Name: Z-Arg-Leu-Arg-Gly-Gly-AMC
Cat. No.: GA23715

Chemical Properties

Cas. No.	167698-69-3		
Formula	$C_{40}H_{56}N_{12}O_9$	M.Wt	848.96
Solubility	Soluble in DMSO	Storage	Store at -20°C
General tips	For obtaining a higher solubility , please warm the tube at 37 °C and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20°C for several months.		
Shipping Condition	Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.		
Structure			

Protocol

This plan only provides a guide, please modify it to meet your specific needs.
1. Solution Preparation:
(1) Stock Solution: Dissolve Z-Arg-Leu-Arg-Gly-Gly-AMC in DMSO to a final concentration of 1 mM. Note: Aliquot the unused stock solution and store at -20°C in the dark. Avoid repeated freeze-thaw cycles.
(2) Working Solution: Dilute the stock solution with assay buffer to the desired working concentration. Note: Adjust the optimal working concentration according to the actual conditions or refer to the literature for gradient concentration tests. The working solution must be prepared fresh for each use.
2. Steps for Measuring PLpro Activity Using Z-Arg-Leu-Arg-Gly-Gly-AMC[1] (from the literature, for reference only):
(1) Prepare the assay buffer containing 50 mM HEPES (pH 7.4), 0.01% Triton X-100 (v/v), 0.1 mg/mL BSA, and 2 mM DTT. Set up the reaction system in a 384-well plate, with a final reaction volume of

50 μL per well.
(2) Add PLpro to each well to a final concentration of 100 nM.
(3) Add 5 μL of Z-Arg-Leu-Arg-Gly-Gly-AMC stock solution to each well, achieving a final concentration of 50 μM.
(4) Substrate hydrolysis was monitored using a microplate reader. The excitation wavelength is 340 nm, and the emission wavelength is 450 nm. Record the fluorescence signal every 3 minutes.
Note: This protocol provides guidance for measuring PLpro activity using Z-Arg-Leu-Arg-Gly-Gly-AMC. Adjustments can be made according to other literature and specific experimental requirements.
References:
[1]Fu Z, Huang B, Tang J, et al. The complex structure of GRL0617 and SARS-CoV-2 PLpro reveals a hot spot for antiviral drug discovery[J]. Nature communications, 2021, 12(1): 488.

Background

Z-Arg-Leu-Arg-Gly-Gly-AMC (Z-RLRGG-AMC) is a peptide substrate of SARS-CoV papain (PLpro), where RLRGG is the C-terminal sequence of ubiquitin (Ub)[1, 2]. The maximum excitation/emission wavelengths of Z-Arg-Leu-Arg-Gly-Gly-AMC are 340/450 nm, respectively [3].

Z-Arg-Leu-Arg-Gly-Gly-AMC is primarily used to study protease activity. By measuring the fluorescence intensity of released 7-amino-4-methylcoumarin (AMC), the catalytic activity of proteases can be assessed[4]. Z-Arg-Leu-Arg-Gly-Gly-AMC consists of a pentapeptide sequence linked to the fluorescent group AMC. The "Z" denotes the benzyloxycarbonyl (Z) protecting group, which is used to protect amino acids from nonspecific reactions [5].

References:
[1] Báez-Santos Y M, Mielech A M, Deng X, et al. Catalytic function and substrate specificity of the papain-like protease domain of nsp3 from the Middle East respiratory syndrome coronavirus[J]. Journal of virology, 2014, 88(21): 12511-12527.
[2] Li L, Ma L, Hu Y, et al. Natural biflavones are potent inhibitors against SARS-CoV-2 papain-like protease[J]. Phytochemistry, 2022, 193: 112984.
[3] Fu Z, Huang B, Tang J, et al. The complex structure of GRL0617 and SARS-CoV-2 PLpro reveals a hot spot for antiviral drug discovery[J]. Nature communications, 2021, 12(1): 488.
[4] Wang C, Chen S, Yeo S, et al. Elevated p62/SQSTM1 determines the fate of autophagy-deficient neural stem cells by increasing superoxide[J]. Journal of Cell Biology, 2016, 212(5): 545-560.
[5] Kadereit D, Waldmann H. Enzymatic protecting group techniques[J]. Chemical Reviews, 2001,

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