

Product Data Sheet

Product Name: THPTA ligand

Cat. No.: GC59051

Chemical Properties

Cas. No. 760952-88-3

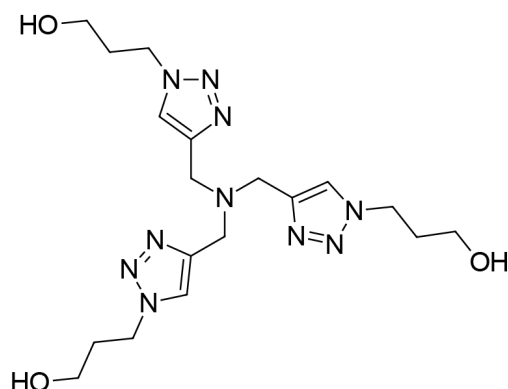
Formula $C_{18}H_{30}N_{10}O_3$ M.Wt 434.5

Solubility DMSO : 100 mg/mL (230.15 mM; Need ultrasonic) Storage Store at -20°C, protect from light

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

Protocol for labeling live cells by copper-catalyzed alkyne--azide click chemistry using THPTA [1]:

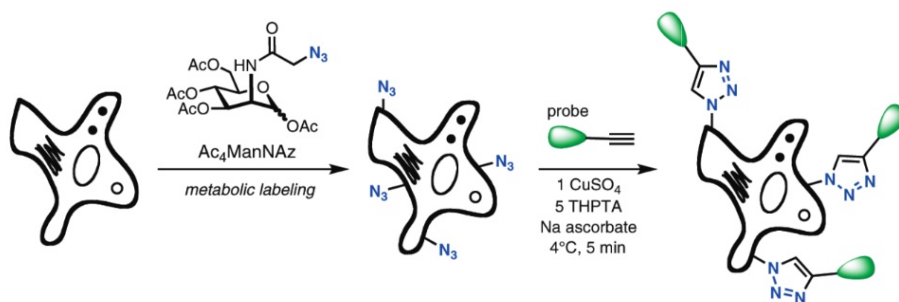
Caution: Product has not been fully validated for medical applications. For research use only.

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1. Jurkat cells were grown in RPMI medium containing 10% fetal calf serum, 1% glutamine, and 1% penstrep with or without 10 μ M Ac₄ManNAz.
2. Cells were collected using enzyme-free Hank's based cell dissociation buffer, distributed in 200 μ L portions at a concentration of 5×10^6 cells/mL in the wells of a 96-well V-bottom-shaped microtiter plate, pelleted (1500 g, 3 min), and washed twice with 200 μ L of labeling buffer DPBS.
3. On a separate 96-well plate, premixed CuSO₄ and THPTA at a 1:5 molar ratio were added to DPBS at 4°C containing biotin alkyne 3 (final concn 50 μ M) and aminoguanidine (final concn 1 mM).
4. A freshly prepared stock solution of sodium ascorbate (100 mM, 2.5 μ L) was added to establish a final ascorbate concentration of 2.5 mM. The reaction mixture was incubated on ice for 60 min before adding to the cells.
5. After incubation for 5 min at 4°C, the cells were washed two times with DPBS buffer containing 1 mM EDTA pH 8.0, 25 mM HEPES pH 7.5, and 1% fetal bovine serum, fixed with 2% (v/v) formaldehyde in DPBS for 10 min at room temperature, and resuspended in the same buffer containing FITC streptavidin (1:250 dilution) for 20 min at 4°C.



This protocol only provides a guideline, and should be modified according to your specific needs.

References:

- [1]. Hong V, Steinmetz NF, et al. Labeling live cells by copper-catalyzed alkyne-azide click chemistry. *Bioconjug Chem*. 2010 Oct 20;21(10):1912-6. doi: 10.1021/bc100272z. PMID: 20886827; PMCID: PMC3014321.

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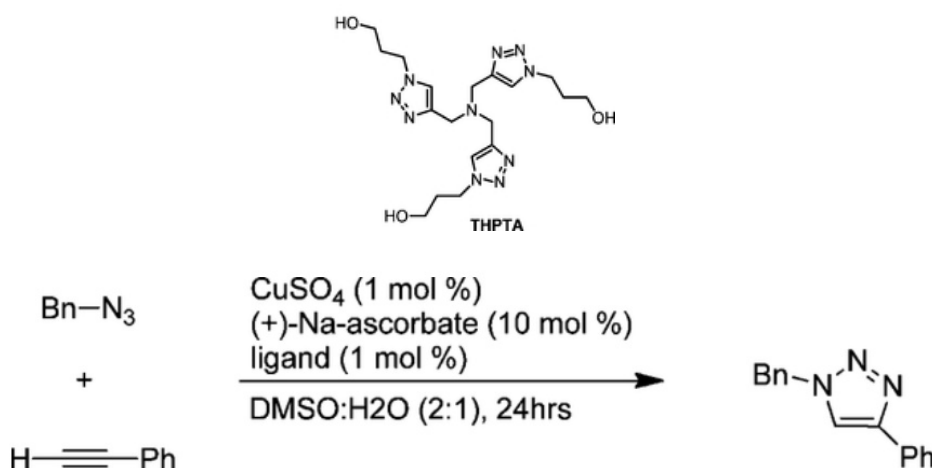
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Background

Trihydroxypropyl triazole amine (THPTA) is a water-soluble ligand^[1]. In the presence of copper ions, THPTA ligand can be used for Cu²⁺-catalyzed alkyne-azide cyclization (CuAAC), and the formed copper complexes can accelerate the CuAAC process^[2-3]. The CuAAC with the participation of THPTA ligand is not only suitable for the modification of hydrophilic polymers and biological coupling of polypeptides containing an azide group^[4-5], but also for the rapid labeling of azide or alkyne labels absorbed by metabolic metabolism on the cell surface^[6-7]. THPTA ligand can efficiently label living cells and maintain cell activity. Its labeling reaction usually takes only 15-30 minutes to complete at room temperature, and the ligand-CuSO₄ complex can still maintain its original activity after at least one month of storage under freezing conditions.



THPTA serves both to accelerate the CuAAC reaction and to protect the cells from damage by oxidative agents produced by the Cu-catalyzed reduction of oxygen by ascorbate in mammalian cells^[7].

References:

- [1]. Chan TR, Hilgraf R, et.al. Polytriazoles as copper(I)-stabilizing ligands in catalysis. Org Lett. 2004 Aug 19;6(17):2853-5. doi: 10.1021/ol0493094. PMID: 15330631.
- [2]. Hong V, Presolski SI, et.al. Analysis and optimization of copper-catalyzed azide-alkyne cycloaddition for bioconjugation. Angew Chem Int Ed Engl. 2009;48(52):9879-83. doi: 10.1002/anie.200905087. PMID: 19943299; PMCID: PMC3410708.

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- [3]. Rao H, Tanpure AA, et.al. Enzymatic incorporation of an azide-modified UTP analog into oligoribonucleotides for post-transcriptional chemical functionalization. Nat Protoc. 2012 May 10;7(6):1097-112. doi: 10.1038/nprot.2012.046. PMID: 22576108.
- [4]. Manzenrieder F, Luxenhofer R, et.al. Stabilization of virus-like particles with poly(2-oxazoline)s. Angew Chem Int Ed Engl. 2011 Mar 7;50(11):2601-5. doi: 10.1002/anie.201006134. Epub 2011 Jan 20. PMID: 21370346; PMCID: PMC3574789.
- [5]. Kemal Arda Günay, et.al. Synthesis of cyclic peptide disulfide–PHPMA conjugates via sequential active ester aminolysis and CuAAC coupling¹¹Electronic supplementary information (ESI) available. See DOI: 10.1039/c5py01817j, Polymer Chemistry, Volume 7, Issue 4, 2016, Pages 970-978, ISSN 1759-9954,
- [6]. Kennedy DC, et.al. Cellular consequences of copper complexes used to catalyze bioorthogonal click reactions. J Am Chem Soc. 2011 Nov 9;133(44):17993-8001. doi: 10.1021/ja2083027. Epub 2011 Oct 19. PMID: 21970470.
- [7]. Hong V, Steinmetz NF, et.al. Labeling live cells by copper-catalyzed alkyne--azide click chemistry. Bioconjug Chem. 2010 Oct 20;21(10):1912-6. doi: 10.1021/bc100272z. PMID: 20886827; PMCID: PMC3014321.

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