

Product Data Sheet

Product Name: NAI-N3
Cat. No.: GC30021

Chemical Properties

Cas. No. 1612756-29-2

SMILES O=C(C1=CC=CN=C1CN=[N+]=[N-])N2C=CN=C2

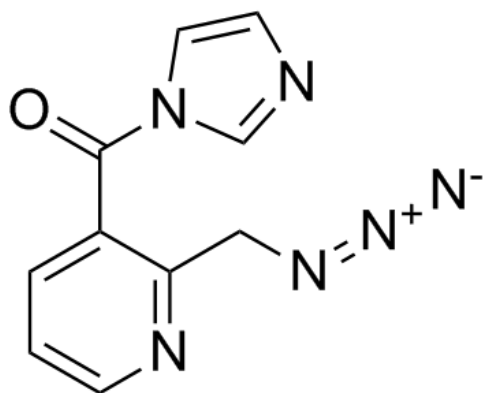
Formula $C_{10}H_8N_6O$ M.Wt 228.21

Solubility DMSO : ≥ 115 mg/mL (503.92 mM); Water : 1 mg/mL (4.38 mM) Storage Store at -20°C (sealed storage, away from moisture)

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 NAI-N3 in DMSO to prepare a stock solution with a concentration of 1M.
Note: After unused stock solution is aliquoted, store it at -20°C or -80°C in the dark to avoid repeated freezing and thawing.

(2) Working solution: Dilute the stock solution with a suitable buffer (such as serum-free culture

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medium, HBSS or PBS) to prepare a NAI-N3 working solution with a concentration of 100mM.

Note: Please adjust the optimal working concentration according to the actual situation or refer to the literature to set the gradient concentration by yourself. The working solution must be prepared and used immediately.

2. Experimental steps for intracellular RNA structure detection using NAI-N3 [1] (Source literature, for reference only)

(1) Add NAI-N3 to the cell pellet at a final concentration of 100mM, then incubate at 37°C for 5min and mix gently. For the negative control sample, an equal amount of DMSO (25μl) was added to the cell pellet.

(2) After detection, transfer the sample to ice immediately to stop the reaction. The sample was then centrifuged at 500g (4°C) for 5min, the supernatant was discarded, and the cell pellet was resuspended in 6mL TRIzol and chloroform (0.2%) was added.

(3) The sample was vortexed vigorously for 15s, then incubated at room temperature for 5min, and then centrifuged at 12,000g (4°C) for 15min.

(4) Transfer the upper aqueous phase to a clean 15 mL tube, then add 2 volumes of 100% ethanol, mix, and then extract Micro RNA.

Note: For your safety and health, please wear a lab coat and disposable gloves when operating.

References:

[1]Sun L, Li P, Ju X, et al. In vivo structural characterization of the SARS-CoV-2 RNA genome identifies host proteins vulnerable to repurposed drugs[J]. Cell, 2021, 184(7): 1865-1883. e20.

Background

NAI-N3 (2-methylnicotinic acid imidazolamide azide; NAI Azide) is a novel RNA acylation reagent that is capable of RNA modification^[1]. NAI-N3 is a bifunctional SHAPE (Selective 2-Hydroxyl Acylation and Analysis Experiment) probe (RNA Structure Probe and Enrichment)^[2]. Live cells were treated with NAI-N3, followed by selective chemical enrichment of NAI-N3-modified RNA, which provided a higher signal-to-noise ratio than similar methods using deep sequencing^[3]. NAI-N3 is a click chemistry reagent that contains an azide group and can undergo Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) with molecules containing alkyne groups^[4]. NAI-N3 can also undergo ring strain-driven alkyne-azide cycloaddition (SPAAC) with molecules containing DBCO or BCN groups^[5].

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- [1] Lee B, Flynn R A, Kadina A, et al. Comparison of SHAPE reagents for mapping RNA structures inside living cells[J]. Rna, 2017, 23(2): 169-174.
- [2] Flynn R A, Zhang Q C, Spitale R C, et al. Transcriptome-wide interrogation of RNA secondary structure in living cells with icSHAPE[J]. Nature protocols, 2016, 11(2): 273-290.
- [3] Sun L, Li P, Ju X, et al. In vivo structural characterization of the SARS-CoV-2 RNA genome identifies host proteins vulnerable to repurposed drugs[J]. Cell, 2021, 184(7): 1865-1883. e20.
- [4] Singh M S, Chowdhury S, Koley S. Advances of azide-alkyne cycloaddition-click chemistry over the recent decade[J]. Tetrahedron, 2016, 72(35): 5257-5283.
- [5] Vidyakina A A, Silonov S A, Govdi A I, et al. Key role of cycloalkyne nature in alkyne-dye reagents for enhanced specificity of intracellular imaging by bioorthogonal bioconjugation[J]. Organic & Biomolecular Chemistry, 2024.

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