

Product Data Sheet

Product Name: TBTA
Cat. No.: GC45003

Chemical Properties

Cas. No. 510758-28-8

SMILES C1(CN(CC2=CN(CC3=CC=CC=C3)N=N2)CC4=CN(CC5=CC=CC=C5)N=N4)=CN(CC6=CC=CC=C6)N=N1

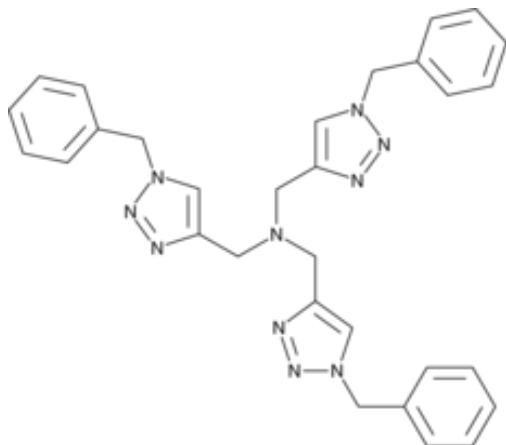
Formula $C_{30}H_{30}N_{10}$ M.Wt 530.6

Solubility DMF: 30 mg/ml, DMSO: 30 mg/ml, DMSO: PBS (pH 7.2) (1:1): 0.5 mg/ml
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. Calculate the volumes of reagents required for Click chemistry labeling using the table below. Prepare the required stock solutions (see Appendix).

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Reagent	Final concentration in the mixture	Stock solution concentration
Oligonucleotide, alkyne-modified	Varies (20 — 200 μ M)	Varies
Azide	1.5 x (oligonucleotide concentration)	10 mM in DMSO
DMSO	50 vol %	—
Ascorbic acid	0.5 mM	5 mM in water
Cu-TBTA complex	0.5 mM	10 mM in 55 vol % DMSO

2. Dissolve alkyne-modified oligonucleotide or DNA in water in a pressure-tight vial.
3. Add 2M triethylammonium acetate buffer, pH 7.0, to final concentration 0.2 M.
4. Add DMSO, and vortex.
5. Add azide stock solution (10 mM in DMSO), and vortex.
6. Add the required volume of 5mM Ascorbic Acid Stock solution to the mixture, and vortex briefly.
7. Degass the solution by bubbling inert gas in it for 30 seconds. Nitrogen, argon, or helium can be used.

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8. Add the required amount of 10 mM Copper (II)-TBTA Stock in 55% DMSO to the mixture. Flush the vial with inert gas and close the cap.

9. Vortex the mixture thoroughly. If significant precipitation of azide is observed, heat the vial for 3 minutes at 80°C, and vortex.

10. Keep at room temperature overnight.

11. Precipitate the conjugate with acetone (for oligonucleotides) or with ethanol (for DNA). Mix thoroughly and keep at –20 °C for 20 minutes.

To precipitate an oligonucleotide conjugate: add to the mixture at least a 4-fold excess volume of 3% lithium perchlorate in acetone (if the volume of the mixture is large, split in several vials).

To precipitate a DNA conjugate: add to the mixture sodium acetate to a final concentration of 0.3M; add 2.5 volumes of ethanol (or 0.8 volumes of isopropanol).

12. Centrifuge at 10000 rpm for 10 minutes.

13. Discard the supernatant. Wash the pellet with acetone (1 mL), centrifuge at 10000 rpm for 10 minutes.

14. Discard the supernatant, dry the pellet, and purify the conjugate by RP-HPLC or PAGE.

Appendix. Preparation of stock solutions of the reagents used for click-chemistry labeling and conjugation.

5 mM Ascorbic Acid Stock

Preparation	Dissolve 18 mg of ascorbic acid in 20 mL of distilled water
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Storage Ascorbic acid is readily oxidized by air. The solution is stable for one day.
Use fresh preparations

10 mM Copper (II)-TBTA Stock in 55% DMSO

Preparation Dissolve 50 mg of copper (II) sulfate pentahydrate in 10 mL of distilled water. Dissolve 116 mg of TBTA ligand in 11 mL of DMSO. Mix two solutions

Storage Store at room temperature. The solution is stable for years

2M Triethylammonium Acetate Buffer, pH 7.0

Preparation Mix 2.78 mL of triethylamine with 1.14 mL of acetic acid. Add water to 10 mL volume, and adjust pH to 7.0

Storage Store at room temperature. The solution is stable for years

Background

TBTA (tris(benzyltriazolylmethyl)amine) is a tertiary amine containing a 1,2,3-triazole group^[1]. TBTA complexes with copper(I) and promotes click reaction catalysis by stabilizing the copper(I) oxidation state^[2]. TBTA is one of the most widely used water-insoluble ligands in copper-catalyzed azide-alkyne cycloaddition (CuAAC)^[3].

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TBTA Click chemistry is a versatile reaction that can be used for the synthesis of a variety of conjugates. Virtually any biomolecule can be easily labeled with small molecules, such as fluorescent dyes, biotin, etc using the click chemistry method.

TBTA Click chemistry reaction takes place between two components: azide and alkyne (terminal acetylene). Both azido and alkyne groups are nearly never encountered in natural biomolecules. Hence, the reaction is highly bioorthogonal and specific, particularly when using a copper(I) catalyst stabilized by the ligand TBTA.

References:

- [1] Alireza Movahedi, et al. One-pot synthesis of TBTA-functionalized coordinating polymers. *Reactive and Functional Polymers*.2014. 82:1-8.
- [2] Kennedy DC, McKay C S, Legault M C B, et al. Cellular consequences of copper complexes used to catalyze bioorthogonal click reactions[J]. *Journal of the American Chemical Society*, 2011, 133(44): 17993-18001.
- [3] Hagmann V, Sommer S, Fabian P, et al. Chemically monoubiquitinated PEX5 binds to the components of the peroxisomal docking and export machinery[J]. *Scientific Reports*, 2018, 8(1): 16014.

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