WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Scalable Synthesis of Azido PEG NHS Esters and its Derivatives

Kurapati Gayathri*, Dr. S.Shobha Rani

Center for Pharmaceutical Sciences, UCEST&JNTUH Hyderabad, Telangana-500 085
Email ID: gayathri.kurapati21@gmail.com

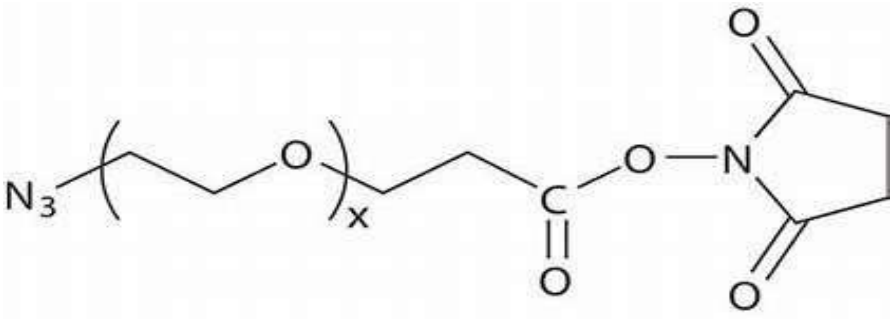
ABSTRACT

In bioconjugation chemistry, azido polyethylene glycol (PEG) and N-hydroxy succinimide (NHS) esters have become essential substances that allow for flexible functionalization in a range of biomedical applications. This study examines scalable synthesis approaches for Azido PEG NHS esters in depth, emphasizing tactics, obstacles, opportunities, and current developments. Furthermore, this synthesis process exhibits remarkable flexibility, enabling adjustments to meet particular research requirements. Its adaptability includes changing the azido functional group density, PEG chain lengths, and molecular weight to provide customized solutions for a range of conjugation tactics.

INTRODUCTION

At the vanguard of bioconjugation chemistry are azido polyethylene glycol (PEG) N-hydroxy succinimide (NHS) esters, a class of chemicals that have transformed the functionalization and modification of biomolecules, surfaces, and nanoparticles in a variety of biomedical applications. These esters—which include NHS esters and PEG backbones ended with azide functional groups—have attracted a lot of interest because of their remarkable adaptability and usefulness in facilitating accurate and targeted biorthogonal reactions. These Azido PEG NHS esters functionalize biomolecules by introducing azide moieties onto target molecules. This facilitates conjugation with a wide range of compounds that have complementary functional groups, like strained cyclooctynes or alkynes, via strong click chemistry or biorthogonal reactions. Because of their ability to modify biomolecules precisely and under control without sacrificing their integrity or functionality, Azido PEG NHS esters are an invaluable tool for a variety of applications, including surface modification, drug administration, diagnostics, and imaging. These Azido PEG NHS esters functionalize biomolecules by introducing azide moieties onto target molecules. This facilitates conjugation with a wide range of compounds that have complementary functional groups, like strained cyclooctynes or alkynes, via strong click chemistry or biorthogonal reactions. Because of their ability to modify biomolecules precisely and under control without sacrificing their integrity or functionality, Azido PEG NHS esters are an invaluable tool for a variety of applications, including surface modification, drug administration, diagnostics, and imaging.

Chemical structure:



Experimental work: **AZIDO PEG 2 NHS ESTERS**

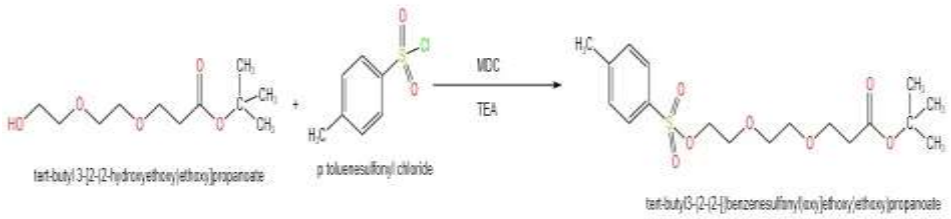
Step 1:

- The initial ingredients were 2,2 oxybis and tertiary butyl acrylate. An RBF was kept at room temperature, and 200 ml of THF and 49.64 g of 2,2 oxybis (ethan-1-ol) were added. The mixture was then vigorously stirred.
- Add 0.9 g of Na metal to the reaction mixture gradually within 3 hours. Transfer 20 g of tertiary butyl acrylate to the reaction mixture, Was finished, allowing the reaction mass to operate at its maximum speed for fifteen hours.

- After the entire 15 hours are up, let the reaction mixture cool to 10 degrees. then adjust the pH to 2-3.100 ml of the solution by adding 5% HCL.After the pH is adjusted, the chemical is extracted using ethyl acetate. It was vacuum distilled and dried with sodium sulfate to create the chemical. Column is completed.

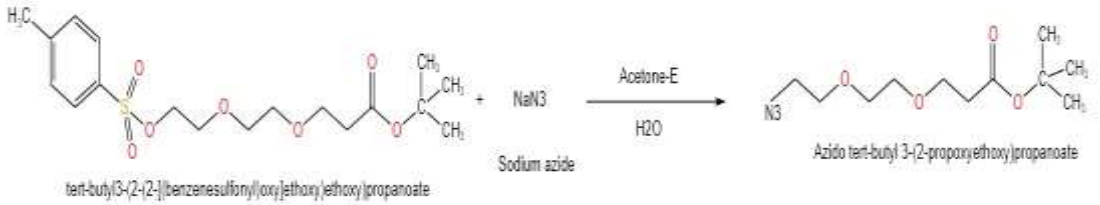
Step 2:

- 14 g of stage 1 are dissolved in 140 ml of MDC at room temperature and then added to the RBF.At room temperature, gradually add 19.5 milliliters of TEA to the reaction mixture. The reaction mixture is cooled to a temperature of 0 to 5 °C.
- Add the MDC (71 ml) and PTSC (14 g) to the reaction mixture gradually. After then, the temperature progressively rose to room temperature. The reaction mixture was held constant for fifteen hours. To lower the pH to 2-3, the 10% HCL solution (119.1 ml) was added after 15 hours. The organic layer is separated and removed. After complete vacuum distillation, the mixture is dried at 50 °C.



Step 3:

- 38 ml of water, 95 ml of Acetone-E, and 19 g of stage 2 were added. into the RBF at room temperature. At room temperature, gradually add sodium azide (19 g) and water (76 ml) to the reaction mixture. It was then raised to 75 °C. ➢ At 70–75 °C, the reaction mass was sustained for two hours. Total mass: 70% fully distilled at 50 degrees in a vacuum. Ethyl acetate was used to remove crude material. The entire organic layer was vacuum-dried and thoroughly distilled at 45 °C.

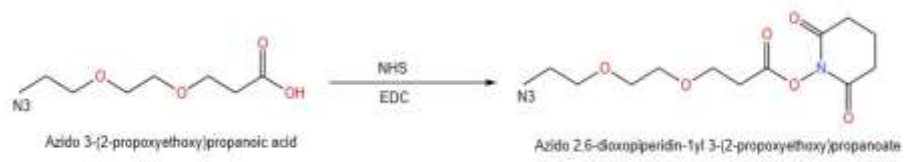


Step 4:

- At room temperature, 11.5 grams of Stage 3 and 164 milliliters of MDC were added to the RBF. It cools the reaction mass to between 10 and 15 °C. TFA was added to the reaction mixture in a volume of 76.6 milliliters. It was then cooled to room temperature. The reaction mass was maintained at room temperature for fifteen hours.
- Under vacuum, the entire mass was thoroughly distilled at 50 °C. The compound was extracted using ethyl acetate. The organic layer was completely dried and then distilled at 65 °C while under vacuum. The chemical and toluene are co-distilled at 65 °C. At 80 °C, the crude bulk was vacuum sealed for five hours.

Derivative 1:

- 23 ml of MDC and 2.3 g of stage 4 were charged into the RBF at room temperature. 1.1 g of NHS was added to the reaction mixture at room temperature. Next, the responsive mass was cooled to a temperature of 0 to 10°C. For 10–15 minutes at 0–10 °C, 1.84 g of EDC base was added to the reaction mixture. The temperature rose gradually after it reached room temperature. The reaction mass was maintained at room temperature for fifteen hours.
- A 10% HCl solution was added, bringing the pH down to 2. The compound was extracted using ethyl acetate, water, a 10% NaCl solution, and NaHCO3 solution. At 50 °C, the complete organic layer underwent complete vacuum drying and distillation.



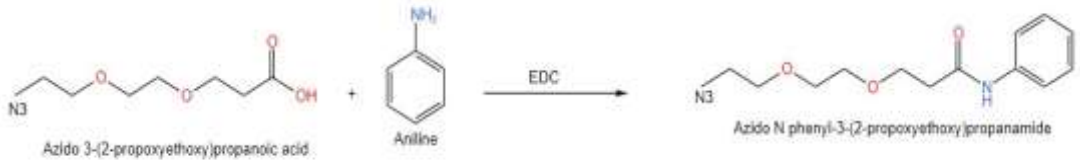
Azido 3-(2-propoxyethoxy)propanoic acid — Azido 2,6-dioxopiperidin-1yl 3-(2-propoxyethoxy)propanoate

^{1}H NMR (400 MHz, Chloroform-d, δppm 3.81-3.62 (m,2H),3.69-3.64 (m,6H), 3.60-3.54(m,2H), 3.40(t,J=6.8Hz,2H), 2.95(t, J=6.8Hz, 2H), 2.87(m, 4H).

Mass Spectrum (m/z) = 313.6(m-1).

Derivative 2:

- 15 ml of MDC and 0.3 g of stage 4 were charged into the RBF at room temperature. At room temperature, 0.15 g of aniline were added to the reaction mixture. 0.29 g of EDC base was added to the reaction mixture at room temperature. The reaction mass was maintained at room temperature for 15 hours.
- 25 milliliters of a 10% HCl solution were added to the reaction mass in order to separate the layers. MDC, water, and a 10% NaCl solution were used to extract the compound. The whole organic layer was vacuum-dried and distilled at 45 °C.



Azido 3-(2-propoxyethoxy)propanoic acid — Aniline — Azido N phenyl-3-(2-propoxyethoxy)propanamide

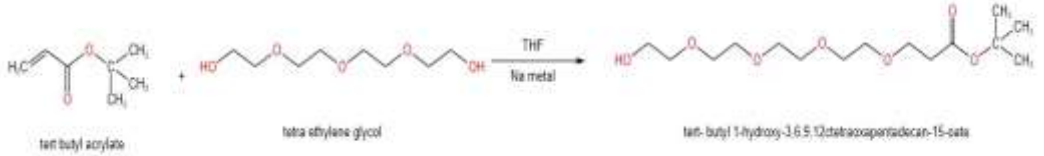
^{1}H NMR (400 MHz, Chloroform-d) δ 8.4(s,1H), 7.53(dd, J=7.5,1.4Hz, 2H), 7.3(m, 2H), 7.1(t, J=6.8, 1.1Hz, 1H), 3.72-3.61(m, 4H), 3.66(t, J=6.8, 1.1Hz, 2H), 3.34(t,J=5.8Hz, 2H) 2.65(t, 2H), 2.04 (m, 2H).

Mass Spectrum (m/z) = 279.3(m+2).

AZIDO PEG 4 NHS ESTERS:

Step 1:

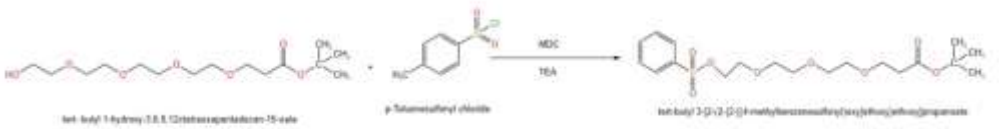
- The initial ingredients were tertiary butyl acrylate and tetra-ethylene glycol. The RBF was charged with 280 ml of THF and 156.8 g of tetra ethylene glycol at room temperature.1.68 g of Na metal should be added to the reaction mixture gradually until a vivid resolution is obtained. At room temperature, 35 grams of tertiary butyl acrylate were added to the reaction mixture.
- The response mass is allowed to come to room temperature for fifteen hours. A pH-adjusting solution containing 5% HCl (175 mL) was added. Following the distillation of the reaction material, the chemical was extracted using ethyl acetate. We vacuum-dried and distilled the organic layer at 50 °C. It was done on Column.



tert butyl acrylate — tetra ethylene glycol — tert- butyl 1-hydroxy-3,6,9,12tetraoxapentadecan-15-oate

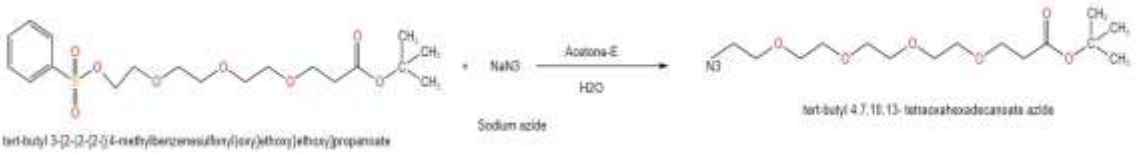
Step 2:

- The RBF was supplemented with 90 ml of MDC and 9 g of stage 1 at room temperature. At room temperature, 6.21 g of TEA were added to the reaction mixture. The temperature of the reaction mixture dropped to a range of 0 to 5 °C. 6.53 g of PTSC and 50 ml of MDC were added to the reaction mixture. The temperature was brought up to room temperature gradually.
- The reaction mass was maintained at room temperature for fifteen hours. To lower the pH to 2-3, 10% citric acid solution was added. MDC, water, and a 10% NaCl solution were used to extract the compound. At 50°C, the complete organic layer underwent complete vacuum drying and distillation. A column was present.



Step 3:

- The RBF was charged with 8 g of Stage 3 and 80 ml of Acetone-E at room temperature. To the reaction mixture, gradually add 8 g of sodium azide and 22.2 ml of water while it is still at room temperature. The temperature rose to about 80 and 85°C after that.
- The reaction was maintained for three hours at 80–85°C. At 55 °C, the entire material underwent full vacuum distillation. The material was extracted using ethyl acetate. The organic layer was completely vacuum-dried and distilled at 50°C.

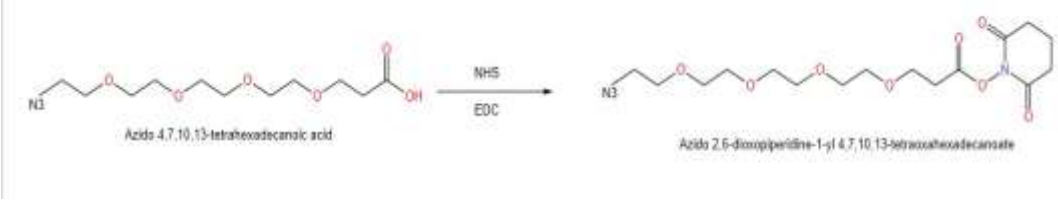


Step 4:

- 4 g of stage 3 and 41.8 ml of MDC were charged into the RBF at room temperature.20.3 ml of room-temperature TFA was added to the reaction mixture after the reaction's mass was lowered to 5–10 degrees. Gradually, the temperature increased to room temperature. The reaction mass was maintained at room temperature for fifteen hours.
- The reaction mass was maintained at room temperature for fifteen hours. At 50°C, the entire bulk was carefully distilled under vacuum. The reaction material was co-distilled with toluene. The compound was extracted using ethyl acetate. After that, the organic layer was vacuum-sealed, dried, and thoroughly distilled at 65°C. It was co-distilled with toluene once more65°C.

Derivative 1:

- 23 ml of MDC and 2.3 g of stage 4 were charged into the RBF at room temperature.1.1 g of NHS was added to the reaction mixture at room temperature. Next, the reaction mass was cooled to between 0 and 10 °C. For 10–15 minutes at 0–10 °C, 1.84 g of EDC base was added to the reaction mixture. Slowly, the temperature was raised to room temperature.
- The reaction mass was maintained at room temperature for fifteen hours. The compound was extracted using MDC, 10% NaHCO3, H2O, and a 10% NaCl solution. A 10% HCl solution was used to bring the pH down to 2. At 50 °C, the complete organic layer underwent complete vacuum drying and distillation.

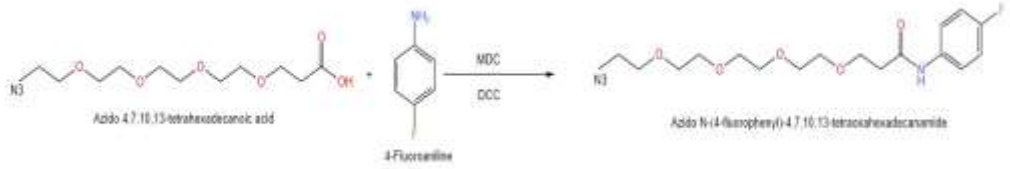


^{1}H NMR (400M Hz, Chloroform-d) δ 3.87(t,J=6.8 Hz, 2H), 3.69-3.60 (m, 16H), 3.73-3.3 (t, 2H), 2.92 (t, 2H), 2.84(m, 4H).

Mass Spectrum (m/z) = 406.5 (m+2).

Derivative 2:

- 15 ml of MDC and 0.5 g of stage 4 were charged into the RBF at room temperature. 0.2 grams of 4-fluoroaniline were added to the reaction mixture at room temperature.0.42 grams of DCC were added to the reaction mixture at room temperature. The reaction mass was maintained at room temperature for fifteen hours.
- The reaction mass was maintained at that temperature for fifteen hours before being filtered and cleaned with MDC.The compound was extracted using a 10% NaHCO3, MDC, and H2O solution. The organic layer was vacuum-dried and then distilled at 55 °C.



^{1}H NMR (400 MHz, Chloroform-d) δ 8.42 (s,1H), 7.54 (d,2H), 7.33-7.29 (t, 2H), 7.11-7.07 (t, 1H), 4.11 (m, 1H), 3.84 (t, 2H), 3.67 (m, 4H), 3.66 (t, 2H), 3.34 (t, 2H), 2.65 (t, J=5.8 Hz, 2H).

Mass Spectrum (m/z) = 385.5(m+1).

RESULTS & DISCUSSION:

- Azido PEG NHS Esters and their derivatives were synthesized.
- They were analyzed using 1H NMR at 400 MHz in DMSO and CDCl3 solvents.
- They were also analyzed by mass spectroscopy.
- % Yield of the compounds increased by this method of synthesis.

REFERENCES:

- Abuchowski A, Verhoest CR, Jr., Kazo GM, et al. Enzyme modification in cancer treatment I. Asparaginase-polyethylene glycol conjugates' antitumor properties 1984 Cancer BioChem Biophys. [Medline] [Scholastic Search].
- Smith M, Choy EH, Hazelman B, et al. Phase II double-blind, randomized, dose-escalating experiment investigating the effectiveness of a new PEGylated humanized anti-TNF fragment (CDP870) in rheumatoid arthritis patients. 2002; 41:1133–1137; Rheumatology. This is a PubMed link: 10.1093/rheumatology/41.10.1133. Cross Ref [Scholastic Search].
- Development of pentoxifylline PEGylated solid lipid nanoparticles for their advantageous pharmacological potential in pathological heart hypertrophy (Dhiman S, Mishra N, Sharma S) Nano med Biotechnol Artif Cells. 2016; 44:1901–1908. Doi: 10.3109/21691401.2015.1111234.
- Site-specific PEGylation of therapeutic proteins: Dozier JK, Distefano MD. (2015) Int J Mol Sci;16:25831–25864. 10.3390/ijms16025831 is the Doi
- Fernandes C, Amorim R, Benfeito S, et al. employing a PEGylated technique to reduce the cytotoxicity of a mitochondrial-tropic antioxidant based on caffeine. Doi: 10.1021/acs.bioconjchem.8b00383 Bioconjug Chem. 2018;29:2723–2733.
- Functionalized poly (ethylene glycol) for the creation of conjugates with biological significance, Zalipsky S. 1995;6:150–165; Doi: 10.1021/bc00032a002. Bioconjug Chem.