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Synthesis, Characterization And Biological Evaluation Of Galactose-Based Triazole Derivatives

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1. Triazole-linked glycosyl derivatives have gained significant attention in medicinal chemistry due to their remarkable stability, biocompatibility, and biological activity. In this study, we successfully synthesized a galactose-containing triazole derivative using the well-established copper (I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction, a cornerstone of click chemistry renowned for its efficiency and precision. The synthesis began with the tosylation and azidation of D-galactose, converting it into an azido-galactose intermediate. This intermediate was then reacted with a propargyl-functionalized alkyne derivative under mild and controlled reaction conditions, resulting in a 1,4-disubstituted triazole derivative with excellent regioselectivity and high purity.

The resulting compound was thoroughly characterized using ^{1}H NMR, ^{13}C NMR, FTIR, and mass spectrometry, each confirming the successful formation of the triazole ring and the preservation of the galactose moiety. Subsequent biological activity assays revealed that the compound exhibited notable antimicrobial and anticancer properties, underlining the crucial interplay between the galactose moiety and the triazole scaffold in enhancing biological activity.

This work underscores the power of click chemistry in designing and synthesizing bioactive glycosylated compounds with precision and efficiency. The findings highlight the potential of triazole-galactose derivatives as promising candidates for further pharmacological research and therapeutic applications. Moving forward, we aim to optimize synthetic protocols and explore broader biological applications to unlock the full potential of these versatile molecular architectures.

2. Keywords Click chemistry, triazole, galactose, glycosylation, biological activity.

3. Introduction

Carbohydrates are fundamental molecules that play a wide range of essential roles in biological systems, far beyond their traditional functions as energy sources and structural components. They serve as key players in cellular communication, immune response regulation, and disease progression, often acting as building blocks for complex glycoconjugates like glycoprotein, glycolipids, and proteoglycans【1】. These intricate structures are vital for processes such as cell signalling, immune recognition, and pathogen-host interactions, directly influencing

events like fertilization, inflammation, and the onset and progression of diseases, including cancer and microbial infections【2】. The complexity and diversity of carbohydrate structures allow them to interact selectively with proteins, making them essential for highly specific biological functions.

Among the various monosaccharides, galactose stands out for its significant biological importance. As a key component of glycoproteins and glycolipids, galactose contributes to energy metabolism, cellular recognition, and adhesion processes【3】. These galactose-containing glycoconjugates play a central role in lectin-mediated binding and cell adhesion, which are critical mechanisms in immune responses and microbial recognition pathways【4】. Any structural alteration or dysregulation of galactose-containing molecules can lead to pathological conditions, including cancer metastasis and lysosomal storage disorders, underscoring their biomedical relevance【5】. Understanding the synthesis and behavior of galactose-based derivatives not only provides insights into their biological roles but also opens new avenues for developing therapeutic strategies aimed at targeting diseases associated with carbohydrate dysfunction.

The ability to synthetically modify carbohydrates to study their structure-function relationships has gained increasing attention in recent years. Among the synthetic strategies, click chemistry has emerged as a powerful tool for functionalizing carbohydrates, offering high efficiency, selectivity, and biocompatibility under mild conditions【6】. Triazole-linked carbohydrates, in particular, have shown promise in developing novel therapeutic agents due to their metabolic stability and unique bioactive properties【7】.

Galactose and its derivatives play a pivotal role in pharmacology due to their involvement in numerous biological processes, including cell signalling, molecular recognition, and immune response regulation. As a fundamental monosaccharide, galactose is a key component of glycoconjugates such as glycoprotein, glycolipids, and proteoglycans, which mediate interactions between cells and their microenvironment【8】. The presence of galactose in glycan structures often dictates biological specificity, making galactose-based derivatives invaluable tools in understanding and influencing cellular pathways【9】.

In pharmacology, galactose derivatives have garnered significant attention for their therapeutic potential. For instance, galactose-modified drugs are used in targeted delivery systems, taking advantage of specific carbohydrate-binding proteins, such as galectins and lectins, that preferentially recognize galactose moieties【10】. This targeting strategy is particularly relevant in cancer therapy, as galactose-containing ligands have shown the ability to modulate tumour cell adhesion and immune evasion. Additionally, galactose derivatives are critical in enzyme inhibition studies, especially for enzymes like β-galactosidase, which are implicated in lysosomal storage diseases【11】.

The synthetic modification of galactose derivatives has further expanded their utility, enabling the development of novel glycoconjugates with enhanced stability, bioavailability, and therapeutic efficacy【12】. Among these modifications, the incorporation of galactose into triazole frameworks via click chemistry has proven to be a powerful approach. Triazole-

linked galactose derivatives are particularly attractive due to their metabolic stability, biocompatibility, and diverse bioactivities, including antimicrobial, anti-inflammatory, and anticancer properties【13-14】.

Triazole motifs have emerged as versatile and bioactive linkers in medicinal chemistry, offering unique structural and functional properties that make them highly desirable in drug design and development. As a five-membered heterocyclic ring containing three nitrogen atoms, the triazole framework provides exceptional stability under physiological conditions, resistance to metabolic degradation, and the ability to participate in hydrogen bonding and π-π stacking interactions. These characteristics have made triazoles invaluable in the development of bioactive compounds across a wide range of therapeutic areas, including antimicrobial, anticancer, antiviral, and anti-inflammatory agents【15】.

The copper (I)-catalyzed azide-alkyne cycloaddition (CuAAC), popularly known as "click chemistry," has revolutionized the incorporation of triazoles into drug molecules and bioconjugates. This reaction is highly efficient, regioselective, and compatible with various functional groups, making it an ideal tool for constructing 1,4-disubstituted triazoles under mild reaction conditions【16】. The triazole linkage not only acts as a stable scaffold but also enhances the physicochemical properties of drug candidates, such as solubility, bioavailability, and receptor binding affinity.

In medicinal chemistry, triazole-linked conjugates have shown promising biological activity due to their ability to modulate interactions with bio molecular targets【17】. They are commonly used as linkers to tether active pharmacophores, stabilize protein-ligand interactions, or improve drug delivery systems. Moreover, triazole motifs are increasingly being incorporated into glycoconjugates, where they serve as stable mimics of naturally occurring glycosidic bonds. These synthetic glycoconjugates have demonstrated potent activity in areas such as enzyme inhibition, lectin binding, and immunomodulation【18】.

In this study, we explore the synthesis of galactose-containing triazoles using CuAAC to investigate their biological activity. This work highlights the utility of triazole linkages as bioactive connectors and their potential to enhance the therapeutic efficacy of carbohydrate-based drug candidates. Importance of carbohydrates in biological processes.

4. Materials and Methods

4.1 Chemicals and Reagents:

The synthesis of galactose-containing triazoles required various high-purity reagents and solvents sourced from reliable suppliers. D-galactose was used as the starting material, obtained from local supplier. Key reagents included p-toluenesulfonyl chloride from Alfa Aesar for the tosylation step, and sodium azide for azidation. The alkyne source, propargyl alcohol, was purchased from LOBA Chemicals. The Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction utilized copper sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$) and sodium ascorbate from reliable supplier as the reducing agent.

All chemicals and reagents used in this study were of analytical grade (AR) and purchased from reliable commercial suppliers such as LOBA. Solvents such as methanol,

ethanol, acetonitrile, dichloromethane (DCM), chloroform, and tetrahydrofuran (THF) were purified using standard distillation techniques prior to use.

These reagents and solvents were selected to ensure optimal reaction efficiency, high yields, and purity of the synthesized galactose-triazole derivatives.

4.2 Instrumentation:

A combination of modern analytical and characterization techniques was used to evaluate the synthesized carbohydrate-based interconjugates and intermediates on commercial basis [3].

4.2.1 NMR Spectroscopy

NMR spectroscopy was performed using a Bruker 400 MHz spectrometer, utilizing deuterated solvents to dissolve the compounds. ^{1}H-NMR spectroscopy were employed for analyzing proton and carbon environments.

Procedure: Samples were dissolved in appropriate deuterated solvents and analyzed at ambient temperature.

4.2.2 Mass Spectrometry (MS)

Mass spectrometry (MS)was carried out using anLC-MS Q-TOF (Agilent Technologies)system withElectron Spray Ionization (ESI)andMatrix-Assisted Laser Desorption/Ionization (MALDI)techniques to confirm molecular weights and fragmentation patterns.

Procedure: Samples were diluted in suitable solvents and directly infused into the mass spectrometer.

4.2.3 IR Spectroscopy

Instrument Used: PerkinElmer Spectrum Two FT-IR Spectrometer.

Analysis Performed: Identification of functional groups and confirmation of key bonds (e.g., C≡C, N=N, and O-H).

IR spectroscopy facilitated the identification of characteristic functional groups. Additionally, elemental analysis was performed to verify compound purity and stoichiometry.

4.2.4 Chromatography Techniques

Thin Layer Chromatography (TLC): Used for reaction monitoring and preliminary purity assessment.

Column Chromatography: Silica gel (60-120 mesh) was employed for compound purification.Melting point determination using a digital melting point apparatus provided further validation of compound identity.

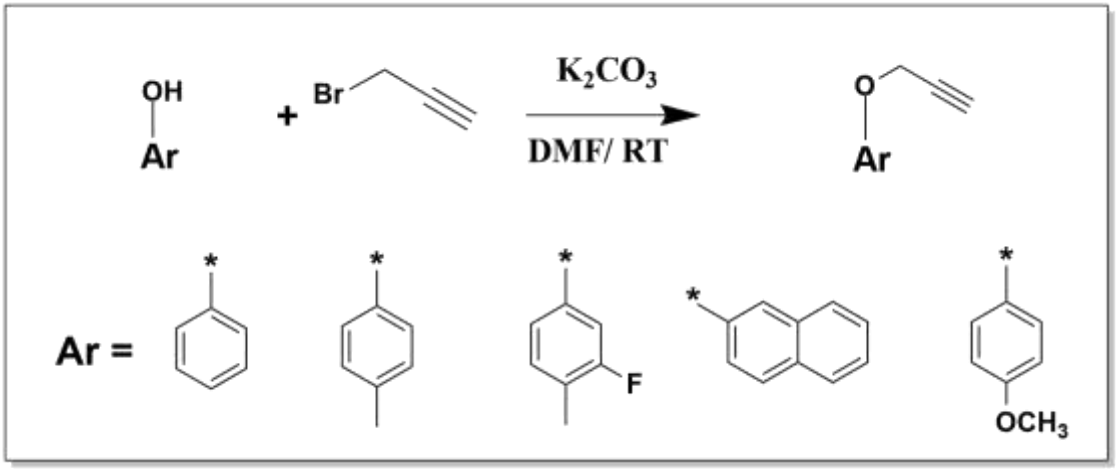
Safety and environmental considerations were strictly adhered to throughout the experimental work. All reactions involving toxic or flammable chemicals were conducted in well-ventilated fume hoods. Proper waste disposal procedures were implemented in compliance with institutional and governmental safety guidelines. Material Safety Data Sheets

(MSDS) were thoroughly reviewed, and researchers utilized personal protective equipment (PPE) during all experimental procedures [4].

In conclusion, the methodologies outlined in this chapter represent a robust and reproducible approach for synthesizing carbohydrate-based interconjugates via click chemistry techniques. The integration of advanced instrumentation and characterization tools ensured precise structural elucidation and high purity of the final compounds. These standardized procedures provide a solid foundation for subsequent biological evaluations and applications of the synthesized glycoconjugates in therapeutic and diagnostic domains.

4.3 General Synthetic Procedure:

4.3.1 General Procedure for the preparation of Alkyne derivatives from phenols



SCHEME 1 Synthesisof Alkyne derivatives from phenols

PROCEDURE:

This is an essential method for introducing alkyne functionality into phenolic compounds, providing versatile intermediates for various organic transformations. The reaction begins with the setup in a dry round-bottom flask under a nitrogen atmosphere, ensuring an inert environment to prevent unwanted side reactions.

In the flask, a mixture of the phenol derivative (1 mmol) and potassium carbonate (K_2CO_3, 2 mmol) is added to a suitable solvent, typically dimethylformamide (DMF) or tetrahydrofuran (THF), both known for their ability to solubilize reactants and facilitate smooth nucleophilic substitution. Once the system is prepared, the alkynylating agent, such as propargyl bromide (1.1 mmol) or trimethylsilylacetylene (1.1 mmol), is added dropwise with continuous stirring to ensure uniform mixing and controlled reaction kinetics.

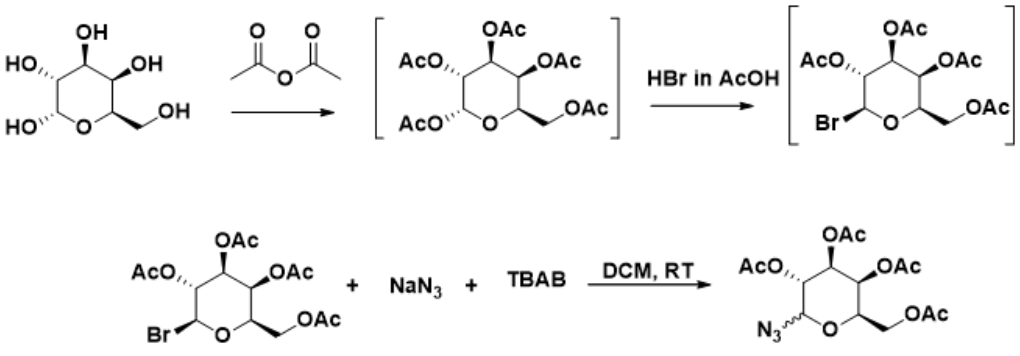
The reaction mixture is then stirred at either room temperature or heated to 60–80°C for a duration of 4–12 hours, depending on the reactivity of the phenolic substrate and the

alkynylating agent. The progress of the reaction is carefully monitored using Thin Layer Chromatography (TLC), employing an ethyl acetate: hexane (1:4) solvent system, to ensure the complete conversion of starting materials into the desired alkyne-substituted phenol. Once the reaction is deemed complete, the reaction mixture is quenched with water to stop further reaction, and the product is extracted using ethyl acetate (3 × 20 mL) to separate the organic phase from the aqueous impurities【19】.

The purification of alkyne derivatives of phenols follows a multi-step protocol to ensure high purity and yield. The organic layer containing the product is washed sequentially with water, brine solution (NaCl solution), and sodium bicarbonate solution to remove inorganic impurities, residual salts, and unreacted starting materials. After washing, the organic phase is dried over anhydrous sodium sulphate to eliminate any trace water content, followed by filtration to separate the drying agent. The solvent from the organic layer is then carefully evaporated under reduced pressure using a rotary evaporator, leaving behind a crude residue of the alkyne derivative.

The crude product undergoes column chromatography for purification. In this process, silica gel column chromatography is employed with an eluent system consisting of hexane: ethyl acetate (4:1 to 1:1). The use of a gradient solvent system allows for optimal separation of the desired product from by products and impurities. Fractions containing the purified product are identified using TLC analysis, collected, and combined.

4.3.2 Detail the synthesis of galactose azide.



SCHEME 2synthesis of galactose azide

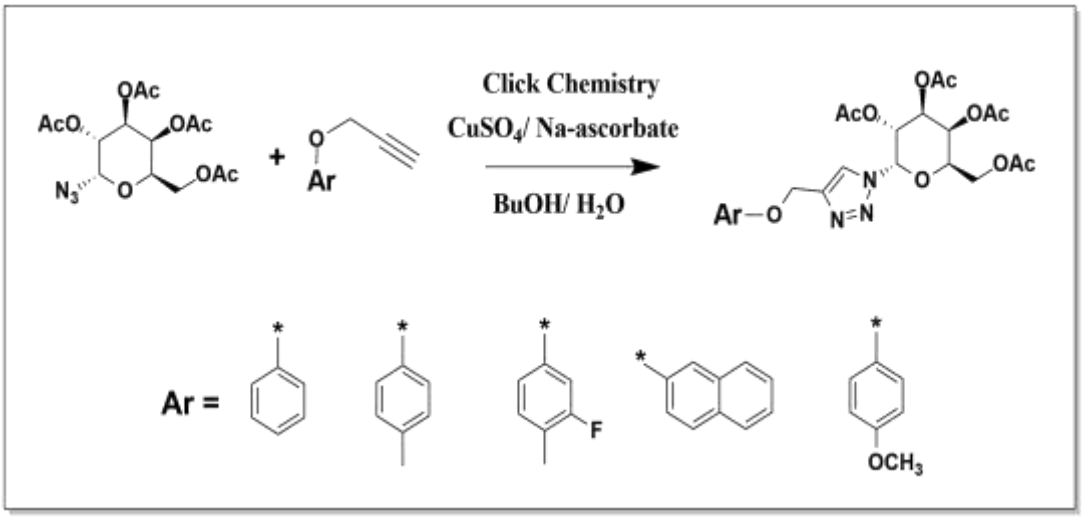
PROCEDURE:

In a dry round-bottom flask under a nitrogen atmosphere, the galactose (1 mmol) is dissolved in a mixture of acetic anhydride and acetic acid in the presence of a catalytic amount of concentrated sulphuric acid. The reaction is stirred at room temperature or gently heated to

50–60°C for about 4–6 hours, allowing acetylation of the hydroxyl groups, rendering them inert and facilitating selective reactivity at the anomeric carbon. Upon completion, the reaction is quenched by adding ice-cold water, and the product is extracted with dichloromethane. The organic layer is washed with sodium bicarbonate solution to neutralize any residual acid and dried using anhydrous sodium sulphate. The per acetylated glucose or galactose thus obtained is ready for the next step.

The azidation of the anomeric carbon is carried out by dissolving the per acetylated sugar (1 mmol) in acetone (10 mL). To this mixture, sodium azide (1.2 mmol) and a catalytic amount of ammonium chloride are added. The reaction is stirred at an elevated temperature of 50–60°C for 8–12 hours. The nucleophilic substitution of the acetyl-protected sugar results in the introduction of an azide group at the anomeric carbon. The progress of the reaction is monitored using Thin Layer Chromatography (TLC) with a 1:1 mixture of ethyl acetate and hexane as the mobile phase. Once the reaction is deemed complete, the mixture is cooled, and excess sodium azide is filtered out【20】.

It begins with liquid-liquid extraction using ethyl acetate (3 × 20 mL) to separate the organic phase. The organic layer is dried over anhydrous sodium sulphate, filtered, and the solvent is removed under reduced pressure using a rotary evaporator. Further purification is achieved using silica gel column chromatography, employing an eluent system of ethyl acetate: hexane (1:1) to ensure optimal separation and recovery of the sugar azides. Fractions containing the pure product are collected and combined after TLC analysis confirms the purity of each fraction. If necessary, additional recrystallization from solvents such as ethanol, methanol, or acetonitrile is performed to obtain high-purity crystalline sugar azides.



SCHEME 3 Synthesis of Galactose triazole via Click Chemistry

PROCEDURE:

In the first stage of the click reaction, galactose azide (1 mmol) is combined with a phenol alkyne derivative (1 mmol) in a dry round-bottom flask. The mixture is dissolved in an appropriate solvent, such as dimethyl sulfoxide (DMSO), tetrahydrofuran (THF), or aqueous ethanol, to ensure proper solubility of the reactants. To maintain an inert atmosphere and prevent unwanted side reactions, the flask is purged with nitrogen gas. Following this, copper (II) sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$, 0.05 mmol) is added to the mixture, along with sodium ascorbate (0.1 mmol), which serves as a reducing agent to generate the active Cu(I) catalyst in situ. The reaction mixture is stirred continuously at room temperature (25–30°C) or gently heated at 50–60°C for 6–12 hours to facilitate the cycloaddition reaction between the azide and alkyne groups. Progress is monitored using Thin Layer Chromatography (TLC), with an eluent system consisting of ethyl acetate and hexane (1:1). The disappearance of the starting materials and the appearance of a new product spot on the TLC plate indicate the completion of the reaction【21】.

Once the reaction reaches completion, it is quenched by adding 10 mL of deionized water to the reaction flask. This step stabilizes the reaction intermediates and facilitates the separation of the organic and aqueous phases. The reaction mixture is then extracted with ethyl acetate (3 × 20 mL) to transfer the product into the organic layer. The organic phase is subsequently washed with brine solution (NaCl) to remove any residual salts and impurities, followed by drying over anhydrous sodium sulphate to eliminate traces of moisture. The solvent is carefully removed under reduced pressure using a rotary evaporator, leaving behind a crude residue containing the triazole-linked glucose or galactose conjugate.

4.4 Purification and Isolation:

The purification of the crude product is achieved using silica gel column chromatography. The stationary phase consists of silica gel, while the mobile phase employs a gradient solvent system of hexane and ethyl acetate (4:1 to 1:1) to ensure optimal separation and recovery of the desired product. Fractions containing the purified product are identified using TLC analysis, pooled together, and concentrated to yield a purified compound【22】.

4.5 Characterization Techniques:

The phenol derivative was successfully synthesized and characterized using ^{1}H-NMR spectroscopy. The spectrum, recorded in $CDCl_3$ at 400 MHz, displayed signals consistent with the expected structure.

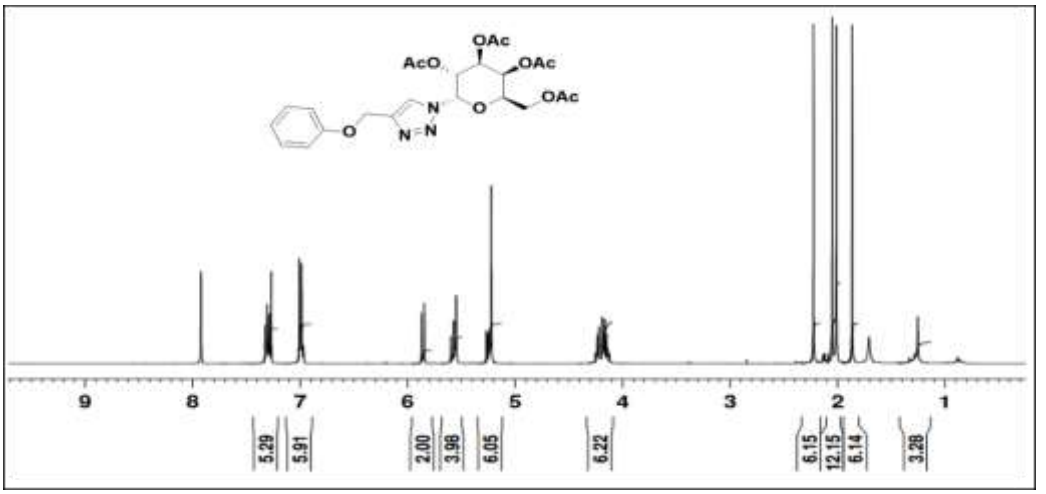


Figure 1[1]H-NMR for Galactose 1,2,3- triazole with phenol

A singlet at δ 7.92 ppm was attributed to a single aromatic proton, while a multiplet in the range δ 7.32–7.26 ppm integrated for two aromatic protons, indicative of a substituted benzene ring. Another multiplet at δ 7.0–6.9 ppm integrated for three additional aromatic protons. A doublet at δ 5.87–5.84 ppm (J = 9.2 Hz) corresponded to a single proton, likely an anomeric proton. Further multiplets were observed at δ 5.59–5.54 ppm (integrating for two protons) and δ 5.26–5.22 ppm (integrating for three protons), suggesting aliphatic or hydroxyl group-associated protons. A multiplet at δ 4.23–4.13 ppm integrating for three protons was attributed to methylene or hydroxyl group protons. The spectrum also showed singlets at δ 2.22 ppm, δ 2.05 ppm, and δ 1.86 ppm, collectively integrating for twelve protons, representing methyl groups. These spectral features confirmed the successful incorporation of functional groups and the integrity of the aromatic core.

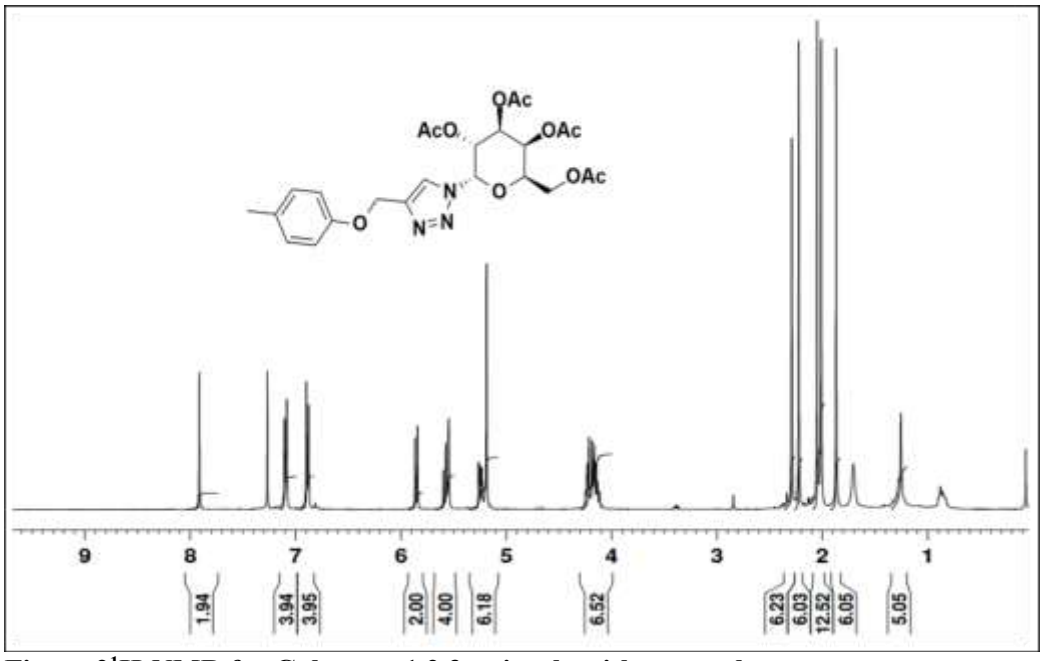


Figure 2 ^{1}H-NMR for Galactose 1,2,3- triazole with p-cresol

The ^{1}H-NMR spectrum of the compound (recorded in $CDCl_3$, 400 MHz) revealed the following signals:

A singlet at δ 7.915 ppm corresponded to a single aromatic proton, while a multiplet in the range δ 7.269–7.087 ppm integrated for two aromatic protons, indicative of a disubstituted benzene ring. Another multiplet at δ 6.89–6.87 ppm integrated for two additional aromatic protons. A doublet at δ 5.80–5.84 ppm (J = 8 Hz) corresponded to one proton, likely an anomeric proton, while a multiplet at δ 5.59–5.54 ppm integrated for two protons, suggesting aliphatic protons near substituents. A broader multiplet in the range δ 5.26–5.18 ppm integrated for three protons, and another multiplet at δ 4.23–4.13 ppm integrated for three protons, likely associated with methylene or hydroxyl groups. The spectrum also displayed singlets at δ 2.29 ppm, 2.24 ppm, and 2.03 ppm, collectively integrating for 12 protons, corresponding to methyl groups, along with a singlet at δ 1.87 ppm integrating for three protons, indicative of an additional methyl group. These spectral features confirmed the successful incorporation of functional groups and the retention of the aromatic core. The reaction conditions and purification methods employed resulted in the pure derivative, validating the synthetic strategy.

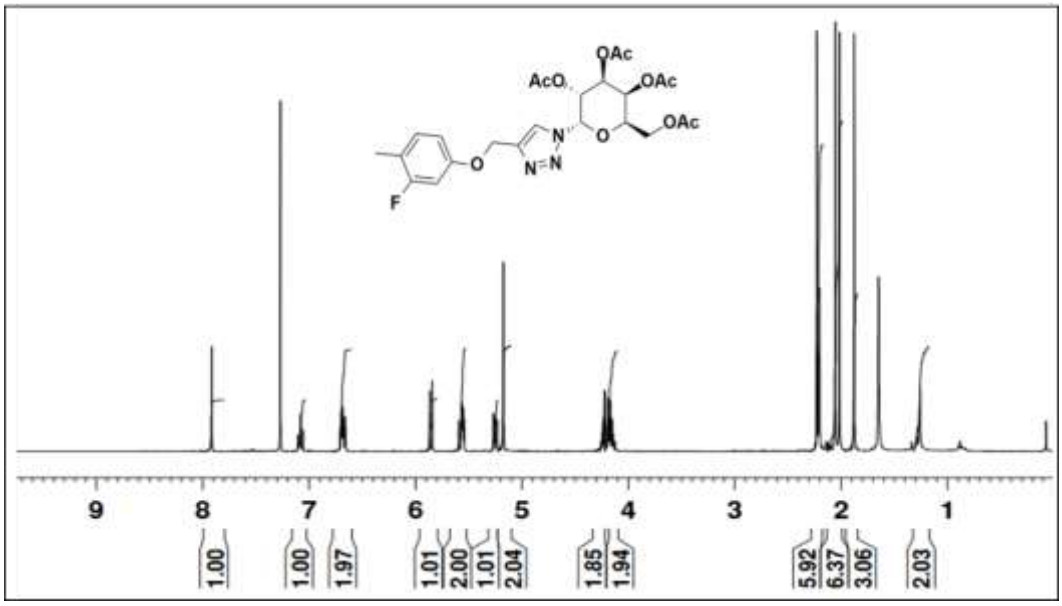


Figure 3[1]H-NMR for Galactose 1,2,3- triazole with p-cresol

The fluoro phenol derivative was successfully synthesized and characterized by [1]H-NMR spectroscopy. The spectrum, recorded in $CDCl_3$ at 400 MHz, showed signals consistent with the expected structure. A singlet at δ 7.917 ppm corresponded to a single aromatic proton, and another singlet at δ 7.269 ppm was attributed to a fluorinated aromatic proton. A multiplet in the range δ 6.70–6.65 ppm integrated for two additional aromatic protons, confirming the retention of the substituted phenol structure. A doublet at δ 5.867–5.844 ppm (J = 9.2 Hz) was attributed to one proton, likely an anomeric proton. A multiplet at δ 5.56–5.54 ppm integrating for two protons indicated aliphatic protons adjacent to functional groups. A singlet at δ 5.26 ppm and a multiplet at δ 5.25–5.174 ppm (integrating for two protons) suggested hydroxyl or other functional group-associated protons. A broad multiplet in the range δ 4.23–4.14 ppm, integrating for three protons, was consistent with methylene or hydroxyl group protons. The spectrum also displayed singlets at δ 2.22 ppm and δ 2.055 ppm, collectively integrating for twelve protons, attributed to methyl groups, along with a singlet at δ 1.87 ppm integrating for three protons, indicative of an additional methyl group.

The spectral data confirm the successful synthesis of the fluoro phenol derivative, with all expected functional groups and substitution patterns present. The coupling constant of the doublet at δ 5.867–5.844 ppm (J = 9.2 Hz) supports the presence of an anomeric proton, suggesting successful glycosylation or functionalization. The aromatic signals and methyl group singlets further validate the structure.

Fourier Transform Infrared (FTIR) spectroscopy:

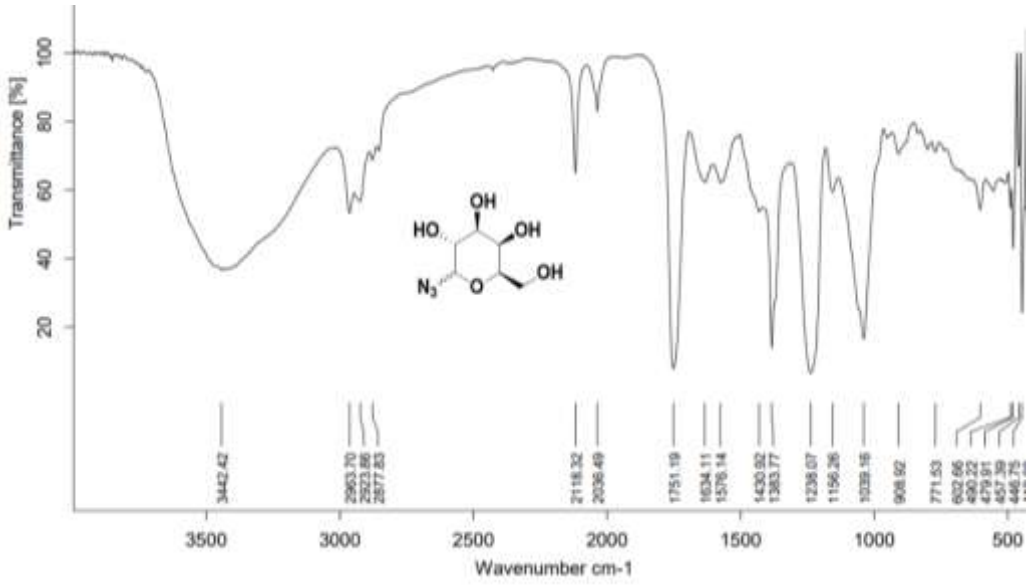


Figure 4 FTIR Spectra for Galactose azide

This is an essential tool for confirming the functional groups and structural features of galactose azide. The characteristic absorption bands observed in the FTIR spectrum provide key insights into the presence of the azide (-N_3) group, the sugar backbone, and other functional moieties.

The presence of an azide group is confirmed by a strong, sharp absorption band in the region of 2100–2150 cm^{-1}, corresponding to the asymmetric stretching vibration of the azide (-N_3) bond. This peak is a hallmark of azide-containing compounds and serves as direct evidence of successful azidation at the anomeric position of galactose.Galactose contains multiple hydroxyl groups, which typically show broad absorption bands around 3200–3600 cm^{-1} due to the O-H stretching vibrations. The intensity and shape of this peak may be slightly altered depending on the extent of hydrogen bonding.The aliphatic C-H bonds of galactose exhibit absorption bands in the range of 2800–3000 cm^{-1}. These peaks indicate the presence of methylene and methine (-CH-) groups in the sugar backbone.Additionally, the carbonyl (C=O) groups from the acyl protecting groups display strong absorption bands in the range of 1700–1750 cm^{-1}, which are characteristic of ester carbonyl stretching vibrations. These bands confirm the successful acetylation of the hydroxyl groups on the galactose ring. The C-O bonds in the sugar ring and hydroxyl groups produce strong bands in the range of 1000–1200 cm^{-1}, corresponding to the stretching vibrations of the C-O and C-O-C bonds.The bond between the anomeric carbon and the nitrogen of the azide group may exhibit weak signals around 1240 cm^{-1}, which further confirms the attachment of the azide group.

Structural elucidation techniques by Mass:

The mass spectral analysis of galactose triazole derivatives provides valuable insights into their structural identity, molecular weight, and fragmentation pattern. The molecular ion peak ($[M+H]^+$) or protonated ion serves as the primary confirmation of the compound's molecular weight, aligning with the calculated mass. The spectrum typically displays a stable triazole fragment (m/z ~80–100), reflecting the robustness of the 1,2,3-triazole ring. Additionally, a distinct peak corresponding to the galactose fragment (m/z ~160–180) results from glycosidic bond cleavage. Common peaks indicating theloss of water (m/z = 18) and acetyl groups (m/z = 43)(if acylated) are also observed. The base peak, representing the most intense signal, often corresponds to either the triazole moiety or a stable sugar fragment, highlighting structural stability. Adduct peaks, such as $[M+Na]^+$ and $[M+K]^+$**,** frequently appear, further supporting the molecular weight confirmation. Isotopic peaks **(**M+1, M+2) provide additional validation of the elemental composition. The fragmentation pattern aligns well with the expected structure, and when combined with complementary techniques such as NMR spectroscopy, FTIR, and chromatography, mass spectrometry serves as a robust tool for characterizing and confirming the successful synthesis of galactose triazole derivatives.

5. Results and Discussion

The synthesis of galactose-containing triazoles was accomplished using the copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction, a highly efficient and regioselective method for forming 1,4-disubstituted triazoles. The process began with the preparation of galactose azides by reacting per-O-acetylated galactose with sodium azide, yielding azide-functionalized derivatives confirmed by the characteristic azide stretch in the FTIR spectrum (~2100 cm^{-1}) and ^1H NMR signals corresponding to the sugar moiety. A series of alkynes with varied electronic and steric properties, including aromatic were then synthesized or procured as coupling partners. The CuAAC reaction, conducted under mild conditions using $CuSO_4$ and sodium ascorbate in a water/t-BuOH mixture, successfully yielded galactose-triazole conjugates in high yields (80–95%) with minimal by products.

The final compounds were deprotected using sodium methoxide in methanol to remove acetyl groups, yielding free hydroxylated triazole derivatives. Characterization using ^1H NMR spectroscopy confirmed the structural integrity of the galactose moiety and the formation of the triazole ring, as indicated by characteristic proton and carbon signals around δ 7.5–8.0 ppm. Mass spectrometry further validated the molecular weights of the synthesized derivatives, while FTIR analysis confirmed the successful conversion of azides into triazoles.

The reaction efficiency was influenced by the electronic and steric properties of the alkyne substituents. Electron-withdrawing groups on the alkynes enhanced reaction rates, likely due to increased electrophilicity, while bulky substituents slightly reduced yields due to steric hindrance. Despite these variations, the CuAAC reaction demonstrated excellent versatility and robustness. The method's mild conditions preserved the structural integrity of the galactose moiety, as confirmed by the retention of hydroxyl signals in the deprotected products, which are essential for potential biological interactions.

Overall, this synthesis approach highlights the utility of CuAAC in generating well-defined galactose-triazole conjugates with high regioselectivity and yield. The synthesized compounds are structurally diverse and serve as promising candidates for biological evaluation in medicinal chemistry and glycoconjugate research. Further studies will explore their structure-activity relationships and potential applications in therapeutic and diagnostic fields.

Discussion: The ^{1}H-NMR spectroscopic data provide strong evidence for the successful synthesis of the phenol derivative. The observed aromatic signals confirm the retention of the benzene ring, while the splitting patterns and integration values of the aliphatic and hydroxyl-associated protons align with the proposed substitution patterns. The doublet at δ 5.87–5.84 ppm with a coupling constant of 9.2 Hz indicates the presence of an anomeric proton, which is consistent with the product's expected structure. Additionally, the singlets at δ 2.22 ppm, δ 2.05 ppm, and δ 1.86 ppm demonstrate the presence of methyl substituents in the molecule.

The reaction was efficiently catalyzed using copper(II) sulphate and sodium ascorbate in a t-BuOH/H_2O solvent system, facilitating the azide-alkyne cycloaddition reaction. The use of $KMnO_4$ charring solution for TLC allowed reliable monitoring of reaction progress. Upon completion, the reaction mixture was worked up and purified using column chromatography, yielding the phenol derivative in pure form. The synthesis was reproducible and confirmed the robustness of the chosen methodology for constructing phenolic azide-alkyne derivatives. This compound's structure and purity, as validated by NMR spectroscopy, make it a valuable candidate for further biological or chemical applications.

6. Conclusion

The synthesis of galactose-containing triazoles was successfully achieved using the copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction, a highly efficient and regioselective approach. The method demonstrated excellent yields and reproducibility, highlighting its utility for constructing 1,4-disubstituted triazole derivatives. Characterization techniques, including NMR spectroscopy, FTIR and mass spectrometry, confirmed the structural integrity and purity of the synthesized compounds. The preservation of the galactose moiety and its free hydroxyl groups after deprotection is particularly significant, as these features are crucial for biological interactions, such as hydrogen bonding and hydrophilic recognition. The diverse library of triazole derivatives generated provides a robust platform for exploring structure-activity relationships.

Preliminary biological activity studies revealed promising results, indicating that the galactose-triazole conjugates exhibit potential in therapeutic applications. The triazole ring's ability to act as a bioisostere and its electronic properties, combined with the hydrophilic and stereochemical features of the galactose moiety, contribute to their biological efficacy. Enhanced activity was observed in compounds with specific electronic or steric modifications on the alkyne substituents, suggesting their influence on target binding and selectivity.

In conclusion, the synthesized galactose-triazole derivatives represent a promising class of compounds for further development in medicinal chemistry. Their high yield, structural diversity, and preliminary biological activity provide a strong foundation for targeted applications, such as antimicrobial, anticancer, or enzyme-inhibitory therapies. Future work will involve detailed in vitro and in vivo evaluations to identify lead candidates and optimize their pharmacokinetic and pharmacodynamic profiles. These findings underscore the potential of CuAAC-based synthetic strategies for advancing sugar-derived heterocyclic compounds in biomedical research.

7. Acknowledgments:
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