

Synthesis and Characterization of Porphyrin Carbohydrate Conjugate *via* “Click Chemistry”

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Abstract - Recently, development of new photosensitizing agents for the potential application in photodynamic therapy has gain considerable attention. Porphyrin a highly conjugated macrocyclic organic system with high molar absorption coefficient is considered suitable candidate for development of new photosensitizing agent. Therefore, a novel Azido- β -D-lactosylated zinc (II) tetraphenyl porphyrin was synthesized by four reactions steps. The molecular structures of the synthesized compounds were characterized by IR, ¹H-NMR and UV-Vis spectroscopy. The water solubility properties of target compound were studied by UV-Vis spectroscopy in mixed water/DMSO mixed solvent system with different range of water contents. The water content in DMSO ranges from 10% to 100% (v/v). When the water contents increase to 20% intensity of the Soret band gradually decreases with an increasing water-content up to 100% attributed to the solubility of the compound in water. The water solubility properties of the porphyrin carbohydrate conjugate enhances its potential application in photodynamic therapy.

Keywords: Porphrin, Porphyrin carbohydrate conjugate, Photodynamic Therapy, Water solubility.

I. INTRODUCTION

Porphyrins are unique class of naturally occurring molecules involved in a wide variety of important biological processes ranging from oxygen transport to photosynthesis, and catalysis [1]. The porphyrin macrocycle is an aromatic system containing 18π -electrons with four pyrrole units and four bridging carbon atoms in a planar conformation. A number of metals can be inserted in the center of the macrocycle forming metalloporphyrins [2]. Because of their large conjugated double bond system, porphyrins typically absorb visible light. The two most well-known porphyrins are heme and chlorophyll [3].

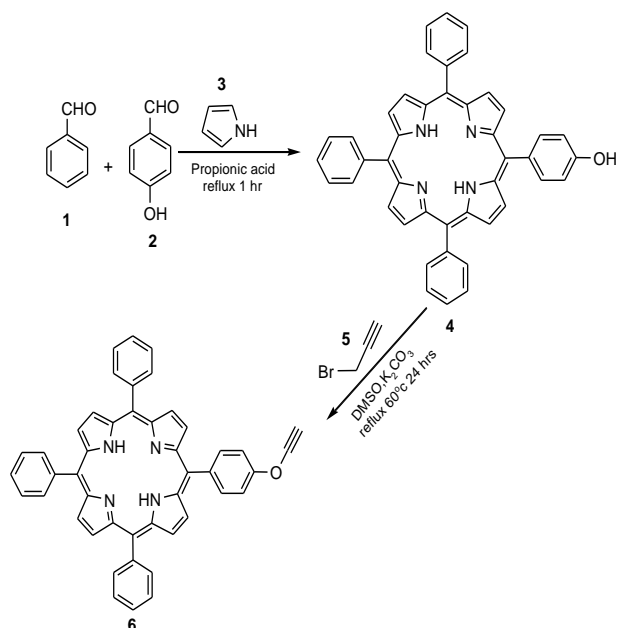
Recently, research interest in porphyrins have attracted considerable attention, owing to their unique electronic and optical properties. This has resulted in their broad application in diverse areas of scientific research. Such as organic photovoltaic cells, chemosensors, conductive organic

materials, light emitting materials, bio imaging probes nonlinear optical materials, metal ligands, and photodynamic therapy [4].

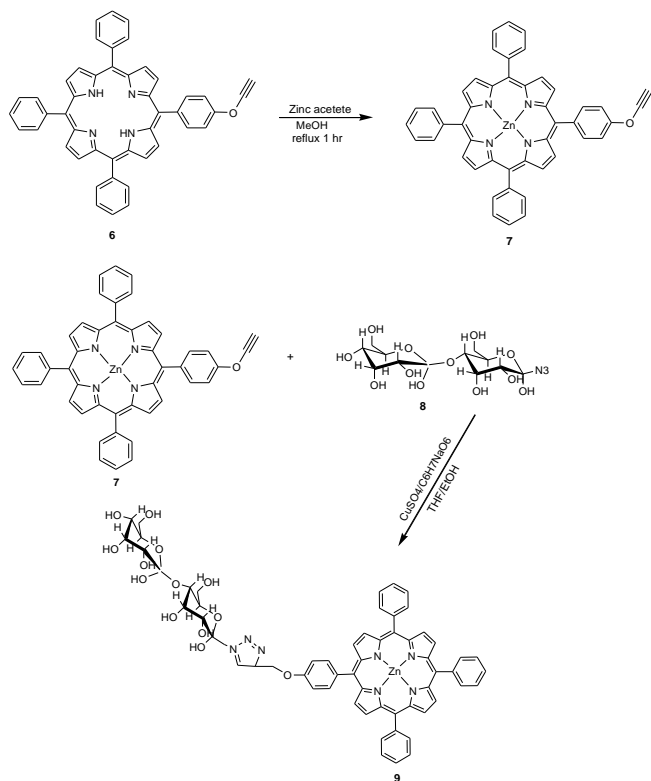
One of more recent and promising applications of porphyrins in medicine is in the detection and cure of tumors in photodynamic therapy (PDT). Photodynamic therapy is based on the principle that a photosensitizer becomes concentrated in tumor cells, upon subsequent irradiation with visible light in the presence of oxygen. Recently, several porphyrin derivatives covalently linked to actives molecules have been synthesized with potential use in the treatment of tumors. However, the major challenges are the solubility in aqueous media and cell membrane permeability [5]. To achieve these fascinating functions, design and synthesis of structurally diverse porphyrin molecules is essential.

Therefore conjugating porphyrin with carbohydrate moiety will increase solubility in aqueous media and enhance membrane permeability for cancer therapy applications [6]. Because carbohydrate contains both hydrophobic side groups for interaction with cell membranes, and hydrophilic groups for solubilization in the blood stream [7]. It is well known that carbohydrates exist as components of glycoprotein's and glycolipids on cell surfaces and play substantial roles in various molecular recognition events, *via* specific carbohydrate-protein interactions [8].

Therefore conjugating carbohydrates to porphyrins modifies the properties of the macrocycle. And can facilitate their passive cellular uptake, their interaction with the cell surface membranes can be enhanced. Moreover, the insertion of a metals into the porphyrin cavity to form metalloporphyrin, often enhances the properties of the free base porphyrin [9,10]. Therefore, this paper reports the synthesis of a new metalloporphyrin carbohydrate conjugate *via* click chemistry reaction.



Scheme 1: Synthesis of 5-(4-propargyloxyphenyl)-10,15,20-triphenylporphyrin 6



Scheme 2: Synthesis of Azido-β-D-lactosylated zinc (II) tetraphenyl porphyrin

II. EXPERIMENTAL

All solvents used were reagent grade and were distilled before used. Reagents were purchased from Aldrich and used without purification. Melting points were determined using electrothermal instrument SS25 PH model F1365. The ^1H NMR spectra was recorded using NMR spectrometer Bruker

model (400MHz). The chemical shifts were reported in ppm (δ) relative to Me_4Si . The infrared (IR) were recorded on shimadzu FT-IR model 8300 series spectrometer in the range of 4000 cm^{-1} to 400 cm^{-1} . While the UV-visible spectra were measured in dichloromethane by using Perkin Elmer, Lambda 25 UV/VIS spectrometer within the spectral range of 350 - 700 nm. Silica coated thin layer chromatography (TLC) plates were used, with Vilber lour mat UV Lamp (356 nm) to observe the spots of the compounds.

2.1 Synthesis of 5-(4-hydroxyphenyl)-10,15,20-triphenylporphyrin (4)

Benzaldehyde (1) (6.2 ml, 60 mmol), 4-hydroxybenzaldehyde (2) (2.15 ml, 20 mmol) and pyrrole (3) (5.55 ml, 80 mmol) were added simultaneously to there fluxing propionic acid (160 ml). The mixture was refluxed for an hour and then allowed to cool to room temperature overnight. The precipitate was then filtered and washes with water and methanol to give a purple mixture of porphyrin crystals. The crystal was dissolved in chloroform and purified by several column chromatography using chloroform as eluent and silica gel as stationary phase. The solvent was evaporated under vaccum and the compound was dissolved and chromatographed on silica gel with eluent ethyl acetate/hexane 1:5 (v/v) to afford compound (4) (1.5g, 12%), mp $>320^\circ\text{C}$; : UV-Vis. (Figure 1) (CH_2Cl_2): λ_{max} . ($\log \epsilon$)= 428 (5.28), 529 (7.62), 566 (7.53), 608 (7.54) 660 (7.58); IR (Figure 2) ν_{max} (cm^{-1}) Nujol: 3447 (-OH), 3263 (-NH), 1347 (-C-O); ^1H NMR (Figure 3) δH ppm (400 MHz, CDCl_3); -2.75 (2H, s, N-H) 5.31(1H, s, OH) 7.24 (2H, d, $J = 8.0$ Hz, Ph-H) 7.78 (9H, m, Ph-H), 8.20 (2H, d, $J = 7.3$ Hz, Ph-H), 8.23 (6H, d, $J = 6.0$ Hz, Ph-H) 8.89 (8H, m, β -H).

2.3 Synthesis of 5-(4-propargyloxyphenyl)-10,15,20-triphenylporphyrinato zinc (II)

Compound (6) (0.18 g, 0.27 mmol) was dissolved in 25 ml chloroform and zinc acetate (0.4 mmol) in and the final mixture is refluxed for 1 hour. The solvent was removed under vaccum to give purple residue. The compound was dissolved in chloroform and chromatographed on silica gel using chloroform/methanol 95:5 as eluent. The solvent were evaporated and the residue was further dissolved and chromatographed on silica gel using the gradient of ethylacetate/hexane 1:4 to give compound (7) (0.112g, 88.2%), m.p = 295°C ; UV-vis CH_2Cl_2 (Figure 7): λ_{max} ($\log \epsilon$) = 430 (8.4), 564 (7.1), 596 (6.4); IR (Figure 8) ν_{max} cm^{-1} : 3390 (acetylene), 1270 (C-N), 1049(C-O); ^1H NMR δH ppm 400 MHz, CDCl_3 (Figure 9), 2.71 (1H, s, acetylene H), 5.00 (2H, s, methylene H), 7.74 (2H, d, $J = 6.0$ Hz, Ph-H) 7.78 (9H, m, Ph-H), 8.20 (2H, d, $J = 8.8$ Hz, Ph-H), 8.23 (6H, d, $J = 6$ Hz, Ph-H), 8.89 (8H, m, β -proton).

2.4 Synthesis of Azido- β -D-lactosylated zinc (II) tetraphenyl porphyrin (9)

Compound (7) (17.5 mg, 0.023 mmol) 1-azido-1-deoxy- β -D-lactopyranoside (8) (11.5mg), 0.029 mmol), CuSO₄.5H₂O (4 mg) and Sodium ascorbate (5 mg) are dissolved in THF/ethanol (1:2) and the mixture stirred at 60°C for 24 hours. The mixture was extracted with ethylacetate (20 ml) and washed with water. The organic layer was concentrated and the porphyrin was purified on the silica gel, using ethylacetate/acetone 1:2 to give the target compound, Azido- β -D-lactosylated zinc (II) tetraphenyl porphyrin (9) (21 mg, 79%) m.p 400°C; UV-Vis (Figure 10) (water) λ_{\max} (log ϵ) = 425 (4.0), 556 (7.0), 597 (3.0); IR (Figure 11) ν_{\max} cm⁻¹: 3400 (OH), 1156 (C-O); ¹HNMR (Figure 12) δ H ppm (400HZ, d-DMSO) 4.57 (2H, m, OH), 4.75 (1H, m, OH), 4.85 (1H, s, Azido), 4.95 (1H, s, OH), 5.45 (2H, s, OH), 5.74 (2H, m, CH₂), 7.49 (3H, d, *J* = 6.8 Hz, Ph-H), 7.80 (9H, s, Ph-H), 8.77 (8H d, *J* = 4.4Hz, Ph-H) 8.82 (8H, m, β -H).

III. RESULTS AND DISCUSSION

The synthesis of porphyrin carbohydrate conjugate involves four-step process, which includes Lindsey condensation of benzaldehyde, 4-hydroxybenzaldehyde with pyrrole to afford compound 4. And the subsequent coupling of compound 4 with propargylbromide *via* Williamson to give compound 6. Followed by metallation of 6 with Zinc (II) acetate to give compound 7. Finally, the porphyrin carbohydrate conjugate (9) was obtained *via* click chemistry reaction, by treatment of 7 with 1-azido-1-deoxy- β -D-lactopyranoside in the presence of CuSO₄.5H₂O and Sodium ascorbate. The electronic absorption spectrum of free base porphyrin 4 (Figure 1) revealed a very intense transition (*S*₀ to *S*₂) Soret band at 427 nm and four weak transition (*S*₀ to *S*₁) Q-bands at 529 nm, 566 nm, 608 nm and 660 nm. The electronic absorptions of the porphyrins have been attributed to porphyrin ring based π - π^* electronic transitions [11,12]. The ¹HNMR spectrum of the compound 4 (Figure 3) displayed a single signal resonated at δ -2.75 corresponding to two protons bonded to nitrogen of pyrrole. The aromatic protons were attributed to the following signals; δ 8.89 eight protons at β -position and δ 7.24, δ 7.78, δ 8.10 and δ 8.23. This was further supported by the IR absorption spectrum (Figure 2) which exhibited characteristics hydroxyl (3447 cm⁻¹), N-H stretching (3263 cm⁻¹), and C-O stretching (1347 cm⁻¹) functional groups, indicating the successful synthesis of porphyrin 4.

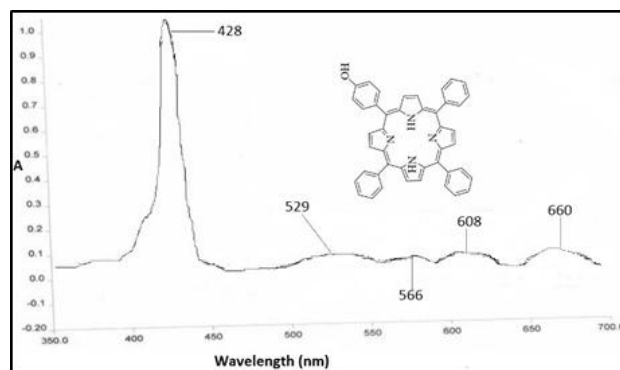


Figure 1: UV-vis spectrum of 5-(4-hydroxyphenyl)-10,15,20-triphenylporphyrin

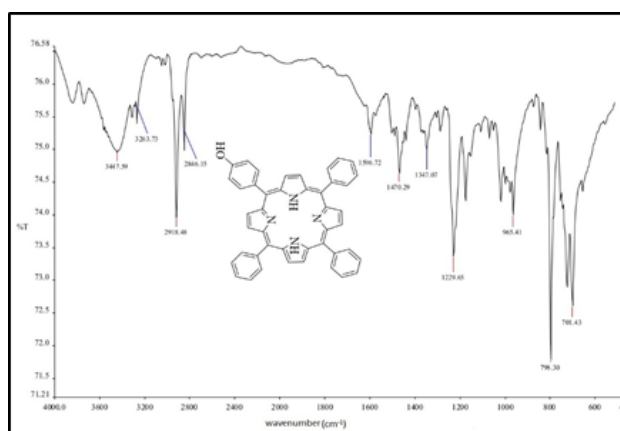


Figure 2: IR spectrum of 5-(4-hydroxyphenyl)-10,15,20-triphenylporphyrin

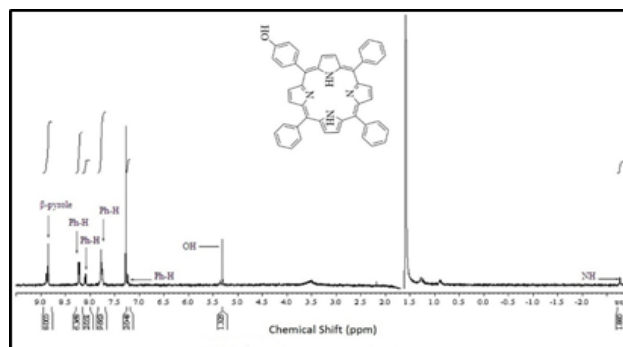


Figure 3: ¹H NMR spectrum of 5-(4-hydroxyphenyl)-10,15,20-triphenylporphyrin

The UV-Visible spectrum of compound 6 (Figure 4) displayed Soret band at 447 nm and 4 Q bands at 545 nm, 581 nm, 624 nm and 682 nm [13]. The absorption bands were red-shifted as compare to compound 4. This was attributed to the introduction of additional conjugation due to presence of triple bond of propargyloxy group. The changes in the absorption bands corresponds to the reduction in the HOMO-LUMO gap, which in turn indicates the effective bonding of the propargyloxy moiety to the porphyrin 4 [14]. The IR spectrum of compound 6 (Figure 5) exhibited characteristics N-H stretching (3505 cm⁻¹), C-O stretching (1347 cm⁻¹), terminal

alkyne (3307 cm^{-1}) functional groups. The disappearance of the OH band, the shifting of a few bands and the appearance of the terminal alkyne at (3307 cm^{-1}) indicate the successful formation of compound **6**. The ^1H NMR spectrum of the compound **6** (Figure 6) Showed a signal at 2.71 ppm corresponding to acetylene proton and methylene of propargyloxy at 5.03 ppm which was not observed in compound **4**. Furthermore, the disappearance of OH signal indicates the successful synthesis of compound **6**.

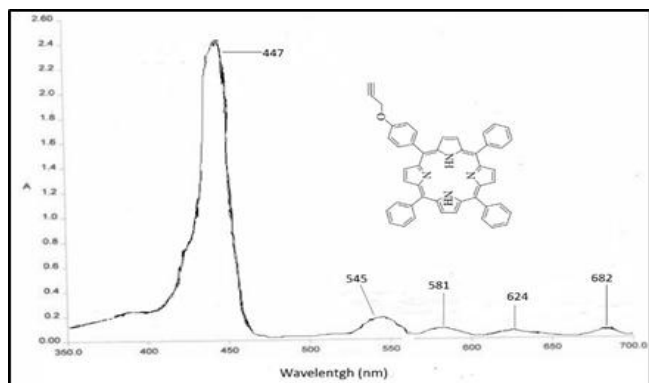


Figure 4: UV-Vis spectrum of 5-(4-propargyloxyphenyl)-10,15,20-Triphenylporphyrin

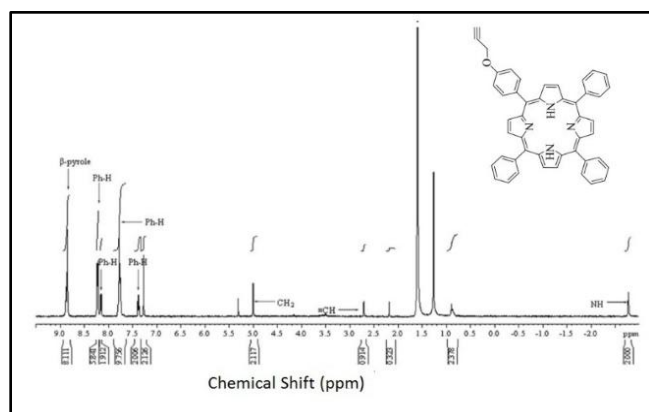


Figure 6: ^1H NMR spectrum of 5-(4-propargyloxyphenyl)-10,15,20-triphenylporphyrin

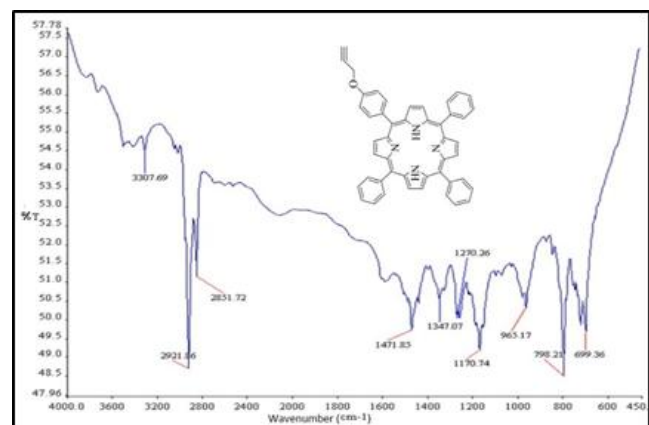


Figure 5: IR spectrum of 5-(4-propargyloxyphenyl)-10,15,20-triphenylporphyrin

The absorption spectrum of the zinc metalloporphyrin porphyrin (**7**) (Figure 7) shows one major intense Soret band at 430 nm and two less intense Q-bands at 564 nm and 596 nm respectively, as compared with free base porphyrin. This is as a result of the incorporation of metal ion in the porphyrin ring. When the metal ion coordinates with porphyrin ligand, the symmetry of the molecule is changed from D_{2h} to D_{4h} [15]. The metal ion accepts the lone-pair-electrons of the N atoms of the pyrrole rings, and donates the electrons to the porphyrin molecule, forming delocalized π bonds. The addition of zinc into the free base porphyrin system modified the photophysical properties of the system [15,16].

The metal to ligand π -back bonding results in an increased porphyrin π to π^* energy separation causing the electronic absorptions to undergo hypsochromic (blue) shifts. Therefore zinc porphyrin exhibited hypsochromic (blue) shifted soret band at 430 nm as compared with the propargyloxy free base porphyrin with soret band at 447 nm [17]. The IR absorption spectrum of zinc porphyrin complex (Figure 8) did not indicate $-\text{NH}$ band as compared to the propargyloxy free base porphyrin, which exhibited characteristic N-H stretching at 3505 cm^{-1} . It was absent in the spectrum of the complex, because the hydrogen atom in the N-H bond was replaced by a zinc metal ion [18]. In addition, bands at 1347 cm^{-1} assigned to C-O stretching vibration, and terminal alkyne at 3307 cm^{-1} in the spectrum of free base porphyrin was shifted to 1049 cm^{-1} and 2351 cm^{-1} respectively in the spectrum of zinc porphyrin. The disappearance of the NH band and the shifting of few bands indicates the formation of zinc metalloporphyrin **7**. The ^1H NMR signal of propargyloxy free base porphyrin (Figure 6) displayed a singlet signal resonated at a very high field -2.75 ppm corresponding to two protons bonded to nitrogen of the pyrrole, this peak in (Figure 9) was found to disappear upon complexation with zinc metal, confirming the formation of zinc metalloporphyrin (**7**).

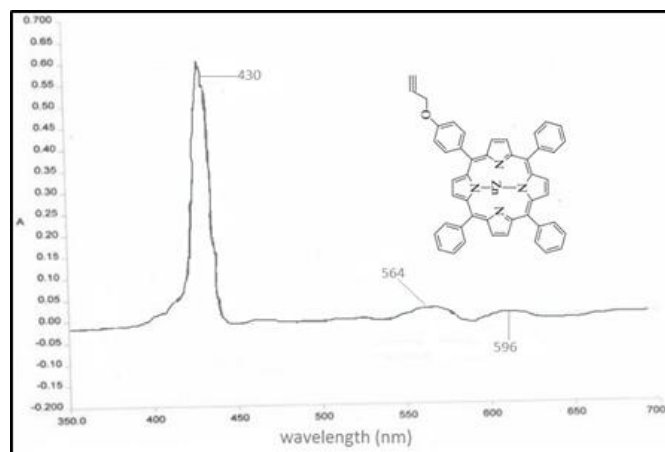


Figure 7: UV-Vis spectrum 5-(4-propargyloxyphenyl)-10,15,20-triphenylporphyrinato zinc II

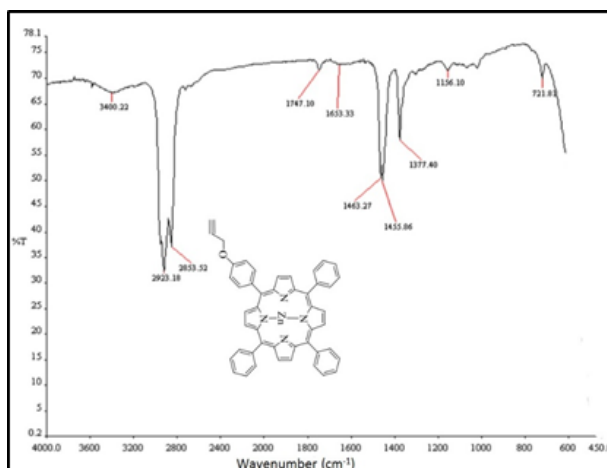


Figure 8: IR spectrum 5-(4-propargyloxyphenyl)-10,15,20-triphenylporphyrinato zinc II

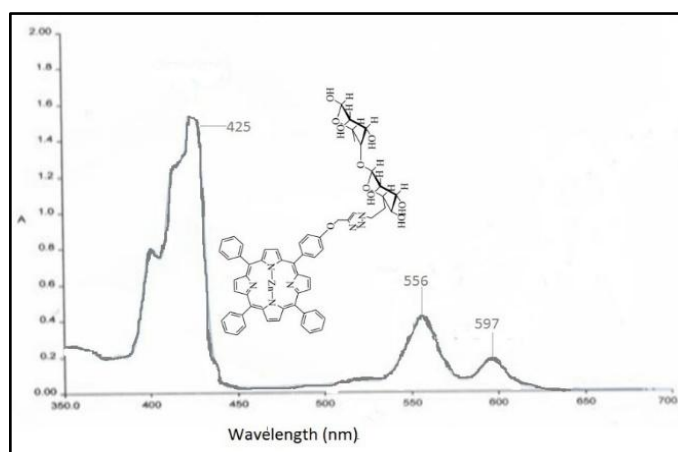


Figure 10: UV-Vis spectrum of Azido-β-D-lactosylated zinc (II) tetraphenyl porphyrin

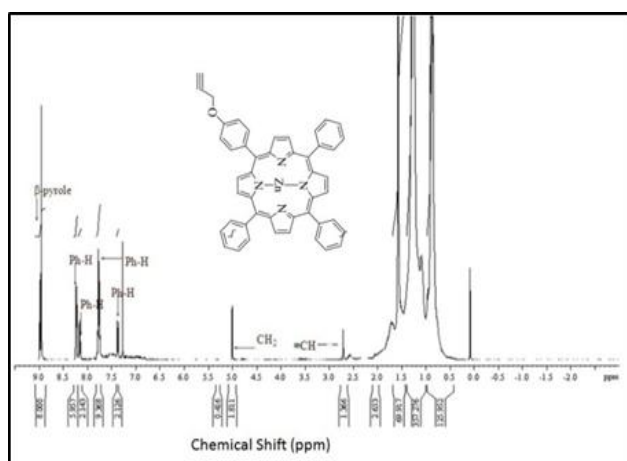


Figure 9: ¹H NMR spectrum 5-(4-propargyloxyphenyl)-10,15,20-triphenylporphyrinato zinc II

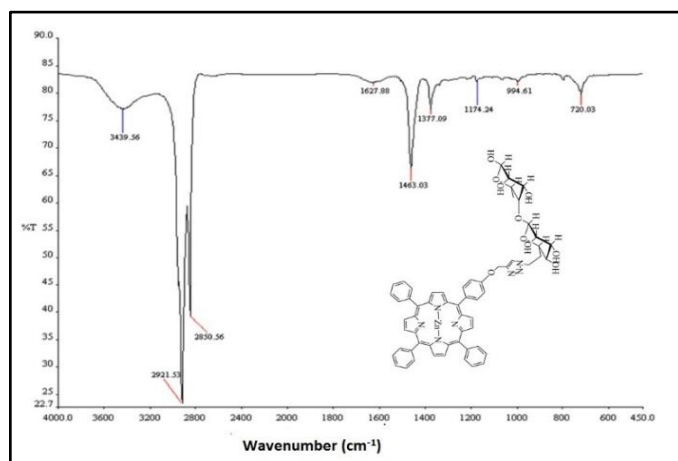


Figure 11: IR spectrum of Azido-β-D-lactosylated zinc (II) tetraphenyl porphyrin

The UV-Vis spectrum of zinc porphyrin-carbohydrate conjugate (Figure 10), shows a blue shifted Soret band at 425 nm and Q band at 556 nm, 559 nm compared with zinc metalloporphyrin (7). This indicates changes in the electronic properties of the porphyrin going from zinc metalloporphyrin to its carbohydrate conjugate [19]. This could be attributed to the conjugation of the carbohydrate moiety to the porphyrin ring, and the distortion of the ring due to the disappearance of propargyloxy group [20]. The IR data (Figure 11) of the porphyrin carbohydrate conjugate (9) exhibited characteristics C-O stretching (1049 cm^{-1}), OH stretching (3400 cm^{-1}) and absence of $\text{C}\equiv\text{C}$ bond stretching at 2351 cm^{-1} confirmed the formation of zinc porphyrin carbohydrate conjugate. The HNMR (Figure 12), was further used to verified the formation of zinc porphyrin carbohydrate conjugate, which was exhibited by the appearance of signal at 4.57, 4.75, 4.95, 5.45 ppm (OH) belonging to the carbohydrate moiety. And the absence of the acetylene proton of the propargyloxy group.

3.1 UV-Vis Water Solubility Study of Porphyrin Carbohydrate Conjugate (9) in Water/DMSO Mixed Solvent System

To study water solubility of the porphyrin carbohydrate conjugate (9) the UV-Vis spectra (Figure 13) of the compound was measured in water/DMSO mixed solvent system with different range of water contents [20, 21]. The water contents in DMSO ranges from 10% to 100% (v/v), it was increase to 20%, 40%, 60%, 80%, and 100% as shown in Figure 13. The compound (9) showed sharp Soret-band peak at 425 nm in DMSO rich solvent system (water = 10% v/v). However, when the water contents increase to 20% intensity of the Soret band gradually decreases with an increasing water-content up to 100% attributed to the solubility of the compound in water. This water solubility of Porphyrin carbohydrate conjugate may arise from the carbohydrate moiety introduced onto Meta position of the porphyrin. The water solubility properties of the porphyrin will enhance its potential application in photodynamic therapy.

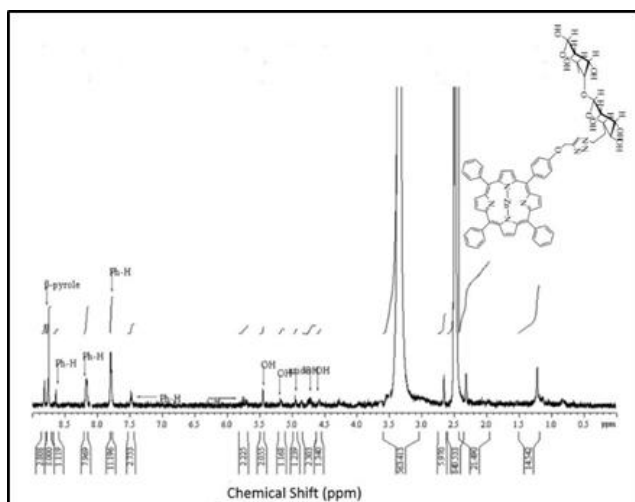


Figure 12: ¹H NMR spectrum of Azido-β-D-lactosylated zinc (II) tetraphenyl porphyrin

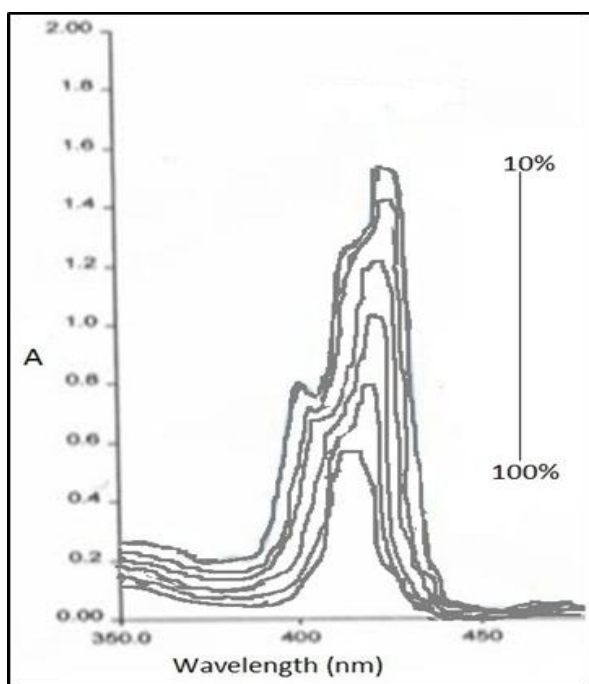


Figure 13: UV-Vis spectra of Azido-β-D-lactosylated zinc (II) tetraphenyl porphyrin in DMSO/water mixed solution systems

IV. CONCLUSION

In this study a new porphyrin-carbohydrate conjugate was synthesised *via* click chemistry reaction. The preparation start from the synthesis of 5-(4-hydroxy)-10,15,20-triphenylporphyrin by Adler longo method which involved the condensation of pyrrole with benzaldehyde and hydroxyl benzaldehyde. Followed by allylation and metallation to give propargyloxyporphyrin and Zinc metalloporphyrin respectively. UV-Vis, IR and ¹H NMR spectroscopy were used for the characterisation of the compounds. The water solubility properties of target compound were studied by UV-Vis spectroscopy in mixed water/DMSO mixed solvent system.

ACKNOWLEDGEMENT

The study was funded by Tertiary Education Trust Fund (TETFUND) with Institutional Research Grant (IBR) reference number TETF/DR&D/MUBI/2023/VOL.1 *Via* Adamawa State University, Mubi, Nigeria.

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Citation of this Article:

Abdulhamid Umar, & Hassan Wafi Garba. (2024). Synthesis and Characterization of Porphyrin Carbohydrate Conjugate via "Click Chemistry". *International Research Journal of Innovations in Engineering and Technology - IRJIET*, 8(9), 1-7. Article DOI <https://doi.org/10.47001/IRJIET/2024.809001>
