CLICK CHEMISTRY AND ITS APPLICATIONS

Abstract

Metal complexes play significant roles in many chemical and biochemical reactions. The efficiency and potency of these complexes were further enhanced by the advent of Click reactions that allow broad scope for substrates offers modularity, specificity, reliability, mild reaction conditions, ease of reactions, etc. The application of Click chemistry has been well proven in many organic synthesis reactions particularly those that involve biochemical predecessors. The utilization of Click chemistry has been well demonstrated in organic synthesis, particularly in reactions that involve biocompatible predecessor as these reactions exhibit bio-compatibility and latency towards other components in its surrounding biological and cellular environments. In this chapter, the significance of Click chemistry along with their applications and uniqueness are discussed.

Keywords: Click Chemistry, Organic reactions, Optical applications, Anti oxidants

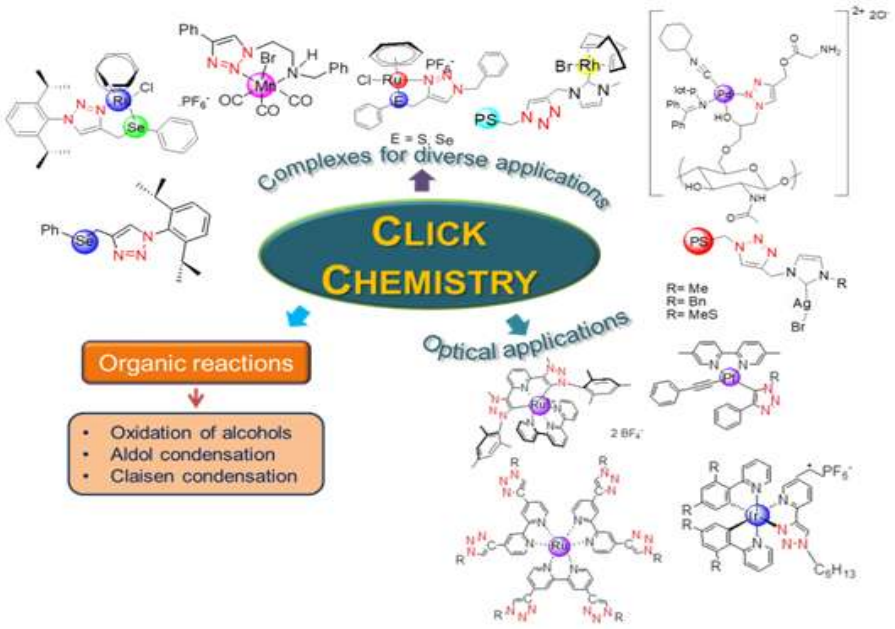
Authors

Swathi Venkatesan
Chemistry Division
School of Advanced Sciences
Vellore Institute of Technology
Chennai Campus
Chennai, Tamil Nadu, India.

Balamurali MM
Chemistry Division
School of Advanced Sciences
Vellore Institute of Technology
Chennai Campus
Chennai, Tamil Nadu, India.
mmbala@gmail.com

Kaushik Chanda
Department of Chemistry
Rabindranath Tagore University
Hojai, Assam,India.
chandakaushik1@gmail.com

Graphical Abstract



I. INTRODUCTION

It is well known that metal ions are important for the proper functioning of numerous key enzymes in living systems. Transition metal complexes have shown significant roles towards catalysing many chemical and biochemical reactions, synthetic chemistry, photochemistry, etc. In particular transition metals like copper, iron and manganese, are involved in multiple biological processes including electron transfer reactions. Due considerations are given to those transition metal complexes that possess the tendency to couple easily without much complications or the formation of side products. These metal ions are known to coordinate with appropriate ligand moleculesvia coordination covalent bond to form the transition metal complexes. These coordination metal complexes do not alter the innate properties of the compounds. The two most widely used methods of synthesizing coordination metal complexes involve (i) donors and acceptors and (ii) the addition of peripheral functional groups to the ligand environment. The latter is mostly involved with the post-synthetic modification due to their precise selectivity.

Click reactions are one of the important classes of reactions that involve multifunctional metal complexes for diverse applications.The term Click chemistry was first introduced by Professor K. Barry Sharpless in 1998, for which he was awarded Noble prize in chemistry in 2022. These reactions were able to overcome the limitations faced by synthetic chemists for efficient formation of products in very good yields. In general, it involves connecting two molecular entities snap together in a single step to form complexes without altering the native properties of the respective molecules. Sharpless et al. first demonstrated the copper catalysed azide-alkyne cycloaddition, which is now utilized for the development of numerous pharmaceuticals, DNA mapping and material synthesis. Moreover these reactions reveal many advantages over conventional reactions like high modularity, flexibility, reliability, high specificity, ease of synthesis, high yield swith no formation of any by-products, biocompatibility, less time consuming, etc. These properties make them well suitable for their applications in medicinal fields and drug discovery. Also these reactions have paved way for the evolution of numerous novel schemes in synthetic organic chemistry like ring opening reactions, conjugate additions, α-effect nucleophilic aldehyde capture, cycloaddition, acylation/sulfonylation,etc,. In spite of these, certain challenges are still prevailing like the rate of degradation, the mechanics and so on.[1] In order to progress further, bio-orthogonal click chemistry is being implements along with for successful synthesis.

The initial reaction of azide-alkyne cycloaddition in the presence of copper catalyst involves activation of terminal alkynes to react with azide to form coordinate complexes. The reaction was complete with the formation of certain intermediates. The challenge involves the difficulty in eliminating multiple equilibria between the intermediates to form the products and hence to deduce a stable reaction mechanism.

The applications of Click chemistry extend to various fields of drugs discovery[2], drug delivery[3], altering the proximity effects to catalyse biological chemistry[4],etc. To elaborate, 1,2,3-triazole ring was prepared as a pharmacophore by 1,3-dipolar cycloaddition reaction catalysed by copper. The simultaneous binding of two reactants within the same pocket can occur when they are present in the close proximity. With the emergence of biorthogonal reactions in Click chemistry it is possible to induce proximity effects.

Recently many Click derived luminescent probes such as imidazoquinolines, thiazetidines, and quino lines along with the aid of computational analysis of drug development (CADD), were proven as drug molecules to inhibit different cancers. The linkers used in Click chemistry enables spontaneity in therapeutic implementation and hence can be utilized for theranostic applications. In addition to all these, Click chemistry is known for it senvironmental friendliness, easily acquirement and manageable reaction conditions.
Herein, Click chemistry behind the multifunctional metal complexes and their applications in radiochemistry, material science, organic synthesis, bio-conjugation, drug discovery,etc. are discussed.

II. SIGNIFICANCE IN CHEMICAL SYNTHESIS

The Click chemistry enabled the possibility for efficient accessibility of a large library of diverse molecular drugs. Further it is possible to optimize, screen and narrow down the library based on their functionalities and efficiencies. Numerous such molecules with their pharmacological activities optimized along with the identification of active derivatives followed by their mechanisms of actions were reported. Particularly with the case of complex natural product derived molecules involving complicated synthetic approaches with multiple stereo-centres and reactive functional itiescan be scaled up to yield unique stereo-active molecules. Also, Click chemistry facilitates the possibility of late-stage functionalization and diversification of desirable products with flexible and wide scope for substrate molecules.[43-45]Till date there exists a research gap that remains to be addressed.

Metal complexes are utilized widely to catalyze reactions especially in medicinal chemistry like selective hydrogenation, olefin metathesis and cross-coupling reactions because of their unique properties including luminescence and magnetic properties. Cisplatin is a well-known metal complex that is used as antitumor agents till date. Yet this drug is reported to display resistance in biological systems with differential toxicity. Also there are other platinum(II)-based complexes that are reported as antitumor agentsby their ability to crosslink nucleic acids and form adducts to inhibit the viability of cancer cells. Similar responses are being attempted with other metal complexes by tuning their potential to display similar activity as the platinum complexes do. In general fictionalization of transition metal complexes provides unique pathways to enhance reaction conditions. For example, complexes with organoselenium as ligand scaffolds were utilized to fine tune many reaction conditions as they are non-reactive towards moist and air. Click chemistry provides facile and easy route for synthesis of coordination metal complexes that can catalyze chemical transformation reactions such as transfer hydrogenation, Sonogashira coupling, Suzuki–Miyaura coupling, and Heck reaction, etc.

1. **Oxidation Reactions:** In many heterogeneous systems, the bond forming reactions particularly those involve surface modifications, Click chemistry is known to play significant roles.[51]Oxidation reactions involve the generation of carbon atoms that are highly reactive to build larger molecules similar to artificial oxidation-assisted ligation. In general an increase subsequently increases the reactivity. As shown in figure 1, the reaction involving the oxidation of hydrocarbons via the formation of higher oxidation state products that can further undergo nucleophilic attack to form imines and amides. Moreover, these reactive species can de-energize in the presence of catalysts, to generate several other compounds.

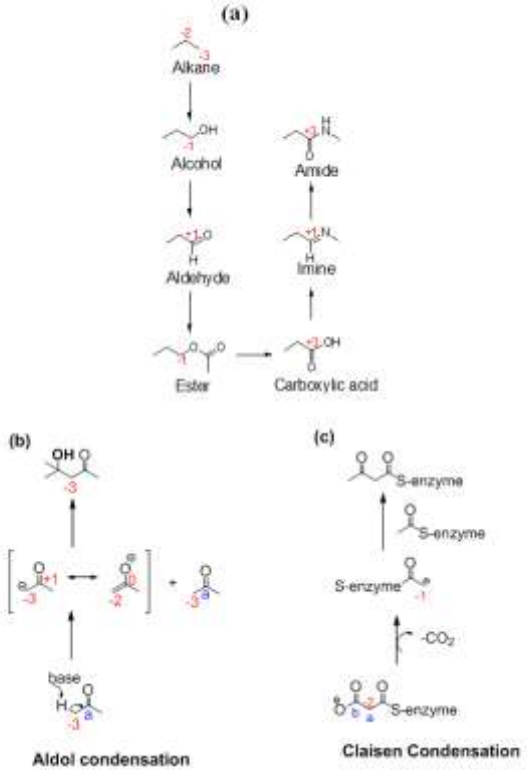


Figure 1: Reactions that involve the formation of higher oxidation states (a) Oxidation of hydrocarbons to yield carboxylic acids. (b) Aldol condensation and (c) Claisen condensation.

Orthogonal oxidative Click chemistry is the most studied one in recent days because of its ability to enable unique oxidation to form highly selective products even under single pot synthesis methods without the formation of any by-products. Under Click catalysis, the reaction rates are higher than other organic substrates which align their way in heterogeneous biological systems. Since the Click catalysts are stable under environmental conditions, higher reaction rate, excellent yields and adaptability of oxidant for reactions are highly supported. Chalcogen-ligated metal complexes, like the one based on sulphur or selenium complexed with ruthenium, rhodium and iridiumare reported for their catalytic activity in alcohol oxidation. The kinetically stable ligand including selenium and nitrogen donors were synthesized following Click chemistry.The strong electron donating property of Se and Senables potential bulkier group substitution on the triazole moiety as shown in figure 2.

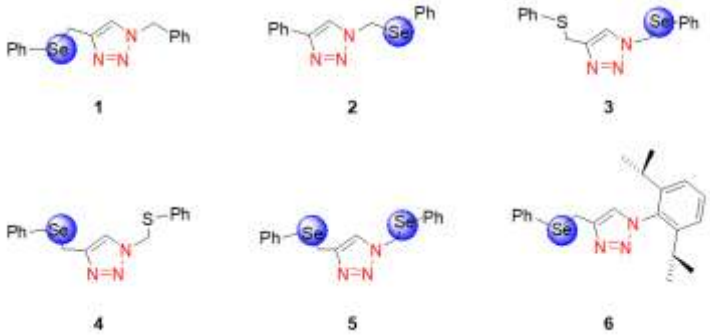


Figure 2: Structure of Click-derived ligands containing Se and nitrogen donors.

In addition, ruthenium, iridium and rhodium complexes were also prepared by the reaction of organoselenium ligands along with precursors[(μ-Cl)RuCl(η^6-benzene)]$_2$, [(μ-Cl)RhCl(η^5-Cp*)]$_2$ and [(μ-Cl)IrCl(η^5-Cp*)]$_2$ as shown in figure 3.

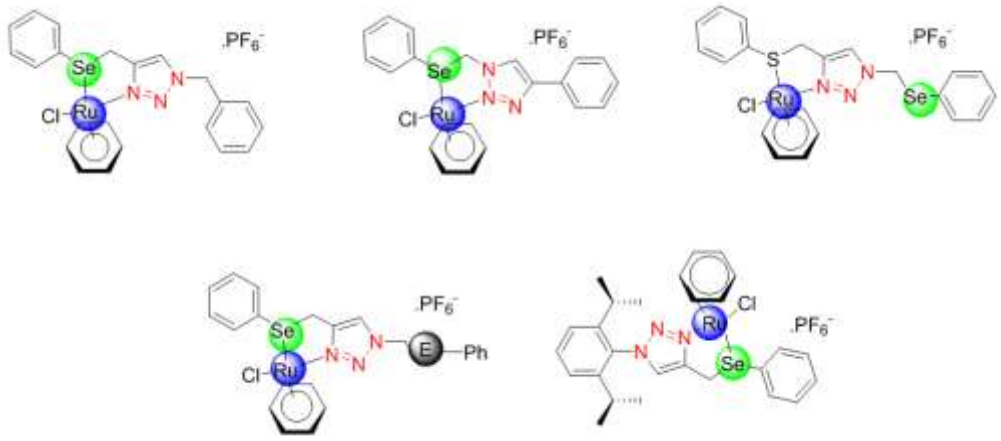


Figure 3: Structures of Click-derived complexes for with organoselenium ligands along with precursors of ruthenium, rhodium and iridium.

2. **Transfer Hydrogenation:** Transition metal catalysts are known for their efficient catalytic property in organic transformations. Singh et al.,investigated the complexion of Ruthenium-based metal compounds [{(η^6-C_6H_6)RuCl(μ-Cl)}$_2$] with 1,2,3-triazile organoselenium or sulphur ligands to produce sandwich structured Ru-complex coordinated with N-atoms forming a five membered ring around the metal. In this the initial substrate, organochalcogenated triazole ligand was synthesized following Click chemistry. Both the substrates were bound together in methanol followed by the addition of NH_4PF_6 to get the desired product. The sandwich shaped complex was produced by the formation of a five carbon ring using the metal Ru and azide present in the triazole ring. Further, in these the donors are responsible for the formation of half sandwiched octahedral structure,resulting in the formation of a coordination sphere with chlorine and chalcogen as chelating ligands as shown in figure 4.

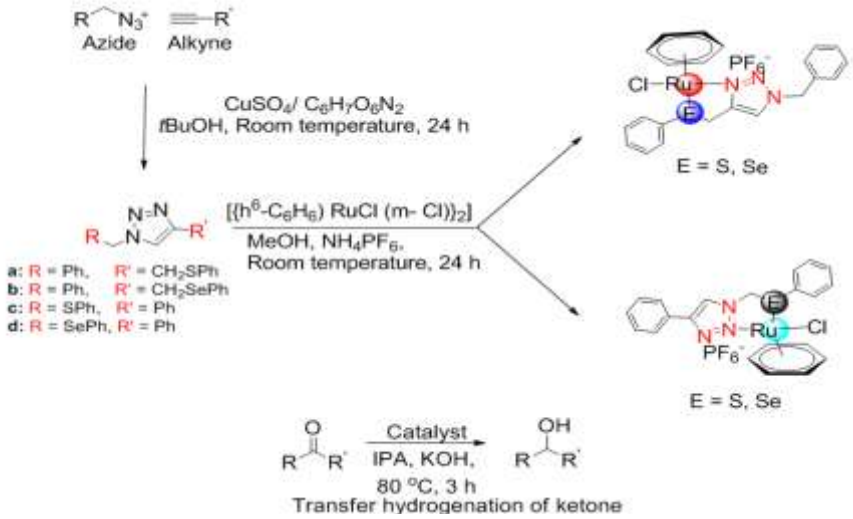


Figure 4: Synthetic scheme representing the formation of catalytically active half sandwich Ru(II) complexes.

Further reactions with the same substrates and ruthenium precursors were carried out using copper catalysts following Click chemistry. In aqueous solutions, the complex's potential to act as catalysts for transfer hydrogenation reaction of the carbonyl compound in the presence of glycerol as hydrogen source was investigated. Excellent catalytic activity was recorded in the presence of KOH at 0.4 mol% at 110°C. The catalytic activity of Mn(I) for hydrogenation reactions was carried out for 16 hours at high temperatures, in the presence of dry toluene as a precursor to coordinate several triazoles to yield a variety of substrates, together with aromatic and aliphatic ketones with different functional groups as shown in figure 5.

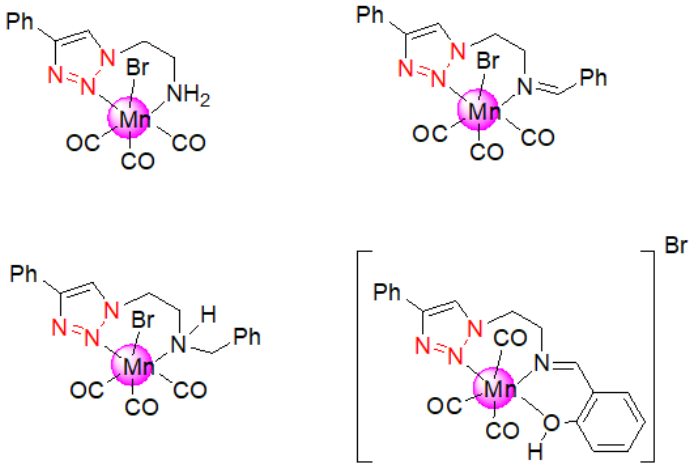


Figure 5: Structures of Click-derived manganese complexes.

3. **C-C or C-N Bond Formation Reactions:** Following the discovery of carbenes by Arduengo et al.,several bond formation reactions between C-C and C-N were explored extensively. The N-heterocyclic carbenes serve as good catalyst owing to their strong M-C bonds that are difficult to dissociate,hence making them more stable thermally withenhanced resistance for oxidation. Moreover, this catalyst can be recovered at the end of the reaction. For example, the NHC-Rh complex was synthesized using Click reaction and applied in the addition ofarylboronic acid to aldehydes as shown in figure 6.

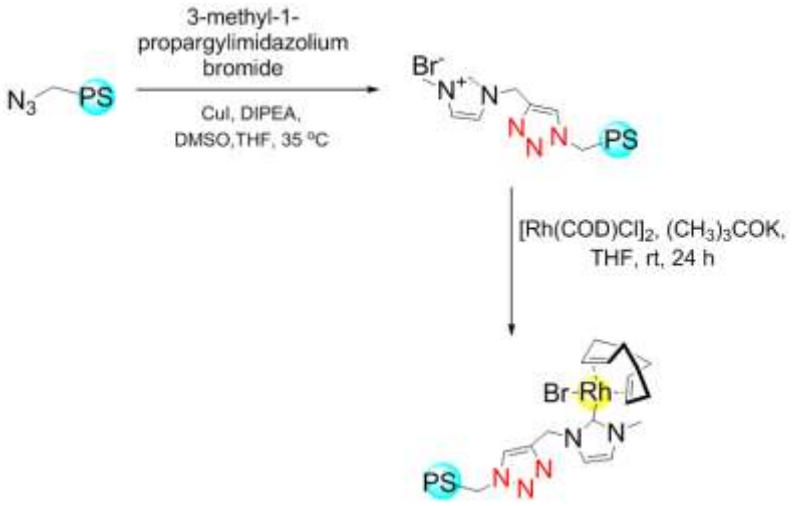


Figure 6: Scheme for the synthesis of NHC-Rh complex.

Another example that includes the synthesis of rhodium complex through nucleophilic substitution with Merrifield resin and excess of sodium azide was allowed to react with $[Rh(COD)Cl]_2$ in presence of the base KOtBu. The best efficiency of the catalyst was reported as 96% with a catalyst load of 2 mol %. Further improvement in the yield was observed by the presence of electron withdrawing groups in arylboronic acid at ortho or para-positions. A three component single pot synthesis of propargylamines was performed using Click NHC-Ag(I) complex via coupling reaction between alkyne, amine and aldehyde as shown in figure 7.

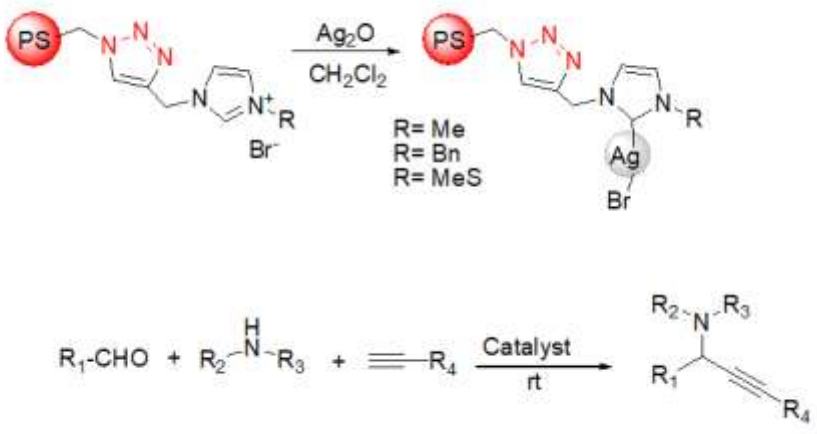


Figure 7: Synthesis scheme for NHC-Ag complex.

This NHC-Ag(I) complex was obtained by the Click reaction of azomethyl polystyrene and propargyl imidazolium bromide that further reacted with azide and silver oxide. In this the chitin was modified with Pd(II) and was further utilized for Sonogashira coupling reaction to generate arylalkenes as shown in figure 8. It is a C-C cross coupling reaction involving Pd(II) metal complex as catalyst.

The polymer linked catalyst is recyclable without any significant loss of its activity. The nano-form of catalyst showed excellent results in green solvent, water. Chalcogen (sulfur) was used in the organic catalyst with the help of Cu-catalyzed Click reaction to form a Pd-based complex. The cyclized azide-alkyne complex was coupled with Pd-precursor to get the desired Pd-catalyst. This catalyst was used in the well-known C-C coupling Heck reaction to generate alkenes. Such Click-derived catalysts based on the Pd metal complexes are used in numerous well-known coupling reactions such as Suzuki coupling to yield alkanes with aryl boronic acid and aryl halides. In these the donor atom plays a vital role in the preparation of these catalysts. It can be noted that the sulphur analogues are more efficient in all the other three types of coupling processes than their selenium counterparts. Such click-derived catalytic systems have the added benefit of being effortless to recover and reuse the catalysts for a number of reactions.

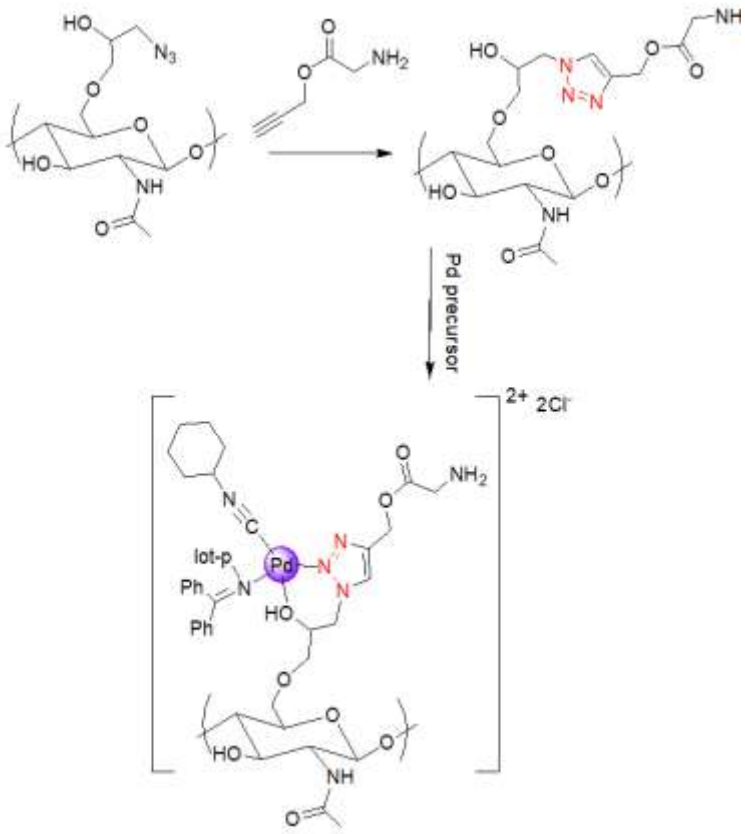


Figure 8: Scheme representing the synthesis of chitin-derived Pd(II) catalyst.

III. CLICK CHEMISTRY FOR OPTICAL APPLICATIONS

The photochemistry based Click reactions are known as photoclick reactions and they are non-toxic and time efficient. The light energy provides the spatiotemporal control that gives the advantage of cross-linking and conjugation of polymers, surface functionalization and labelling of biomolecules. Numerous reactions that involve photoclick mechanisms such as Diels-alder reaction and 1,3-dipolar cycloadditions were investigated extensively and reported. These reactions possessing the ability to transfer energy from materials for solar energy convertion, biological photosynthesis and photocatalysis were generated. But these reactions were reported with lack of selectivity in non-symmetric heterometallic complexes surmounted by ligand based approaches. The beneficial aspects of supramolecular photoclick chemistry is observed in UV and NIR regions and are utilizedin the generation ofphosphorescent organic light emitting diodes (PHOLEDs), Organic Light Emitting Diodes (OLEDs) and photosensitizers.

1. **Photosensitizers:** Photosensitizers are the catalysts that absorb the light and alter the photochemical reactions to achieve the desired product. For instance, Ru(II) polypyridyl complexes are used as photosensitizers because of their stable and inert energy transfer. Also, heteroleptic Ru(II) complexes show comparative yields and better selectivity. A representative example of such complex is shown in figure 9.

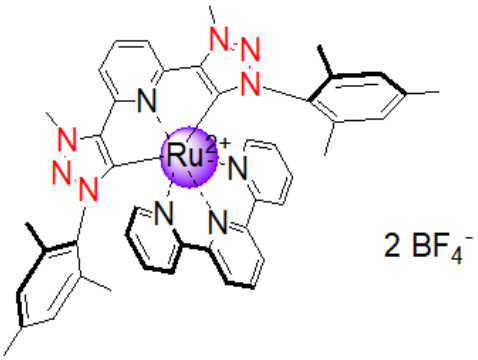


Figure 9: Heteroleptic bis(tridentate) Ru(II) complex containing parent terpyridine ligand.

Semiconductors such as TiO_2 bound chromophores were utilized to produce photosensitized solar cells and photocatalysts[7] Similarly, astar shaped homoleptic ruthenium complexes was synthesized via Click chemistry and successfully tested for their potential a photocatalysts in liquid phase after being anchored to nanoparticle films made of the semiconducting TiO_2 and the insulating ZrO_2 (figure 10). [8]

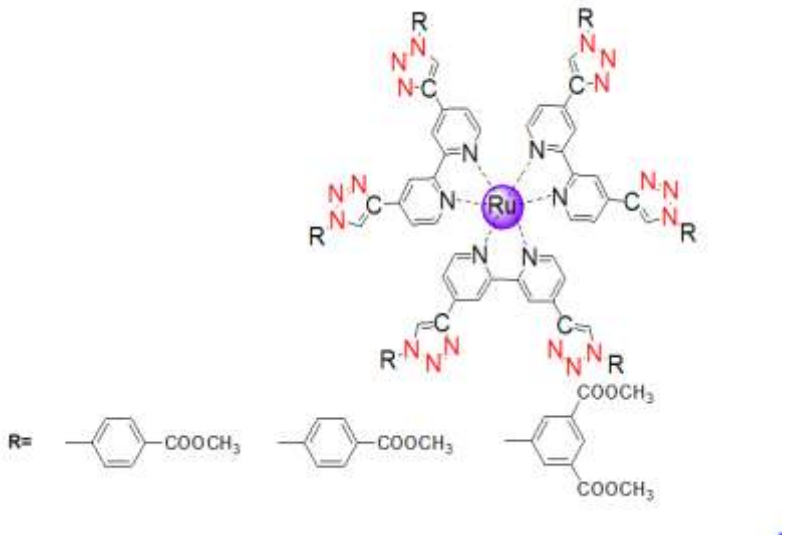


Figure 10: Structure of star shaped ruthenium complexes.

These complexes were composed of 2,2´- bipyridine ligands with 4,4´- disubstitution in 1,2,3- triazole moiety. The $Ru(bpy)_3$ core is C4-connected in complexes,with different anchoring groups (R= p-benzoic and isophthalic methyl esters respectively). The luminescence of the above photosensitizer was recognized by turn-off luminescence with TiO_2 and the turn-on emission with ZrO_2 functionalized films. Functional supramolecular structures from alkyne-azide based transition metal complexes were studied using azidobipyridyl ligands in $[Ru(p\text{-}cymene)Cl]^+$ complexes which exhibited the azide functioning in Ru-complex were prepared via Cu-mediated Click chemistry. [9] Luminescence properties of Ir(III) bis-terpyridine was investigatedusing Click chemistry.[10] In order to enhance the luminescence functionality, the bisterpyridine complex was directly converted from alcohol to azides[11]followed by activiation by a

Click alkyl moiety to yield triazoyl. The terpyridine behaves as electron acceptor and the other functional groups act as donorswith the tendency to increase the absorption because of the intra ligand charge transfer (ILCT) transitions. Similarly, Click synthesized Ru-complexes were also reported with bi- and tetra-dentate ligands. Based on the differences in the energy gap of HOMO and LUMO of triazolyl-pyridine-Ru(II) complex was higher than the bi- and tetra-dentate because of the numberof triazolyl ring that alters the photophysical properties. This can be overcome by increasing the π-conjugation thus adapting the Ru(II)-based complexes as efficient photosensitizers. They also exhibit improved photovoltaic activity and are hence applied in solid and liquid state dye sensitized solar cells[12]

2. **Organic Light Emitting Diodes (OLEDs) :** OLEDs are nothing but the organic layer of light emitting diodes which when comes under the contact of electrical signal can emit light. They are used as bio-imaging labels[13], as sensors in material fabrication[14,15], electrochemical cells,[16]etc. For example, iridium cyclometallated complexes[17] can undergo intramolecular rearrangements to form stable metal-carbon bond. For an exceptional OLED short excitation lifetime, higher quantum efficiency and ability to control the light emission are expected. Tris-cyclometalated iridium(III) complexes using ligands derived from 2-phenylpyridine and 2-(1H-[1,2,3]triazol-4-yl)pyridine (trpy) synthesized using transmetalating agent Cu(I)-triazolides under one pot synthesis with short duration is one such example of OLEDs as shown in figure 11.[18] It is feasible to introduce ligands with diverse functional groups that can act as anchoring groups in Click chemistry.

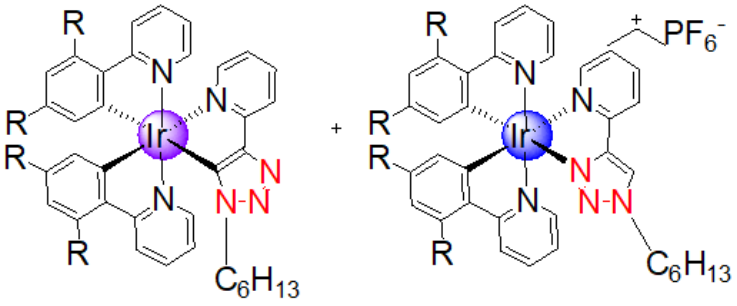


Figure 11: Structure of trpy C^N chelate complexes

3. **Phosphorescent organic light emitting diodes (PHOLEDs) :** PHOLEDsare similar to the organic light emitting diodes but they exhibit phosphorescence. They are recognized as recently growing field with extending π-conjugations. These molecules concurrently yield light from singlet and triplet excitons. The small triplet band gap that promotes the unnecessary intramolecular energy transfer between the guest emitter and the host systems is considered as the major disadvantage in these systems. Copper-catalyzed azide-alkyne cycloaddition reaction was proven to overcome this difficulty by decreasing the donor-acceptor band gaps within the molecule. Theunsymmetric bipyridine–Pt^{II}–alkynyl complexes (figure 12)derived through post-Click reaction with emission enhancement characteristics and their applications as phosphorescent Organic Light-Emitting Diodes with high quantum efficiency is reported as ~ 5.8%.[19]

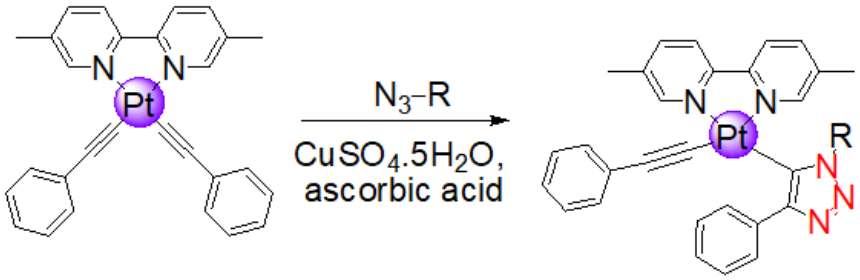


Figure 12: Scheme showing unsymmetric bipyridine-platinum-alkynyl complexes formed through a post-Click reaction.

IV. CLICK CHEMISTRY FOR BIOMEDICAL APPLICATIONS

Chemically synthesized drugs have low biocompatibility for several reasons like the solvents used in the synthesis might display adverse effects, the catalyst might be toxic, involvement of non-ambient reaction conditions like pressure and temperature, etc. Moreover the purification processesare difficult and time consuming. Click chemistry has paved way for the drug production, imaging and drug delivery applications. The orthogonality observed with this concept of synthesis has provided the perspective to mimic the chemical reaction under cellular atmosphere. Click chemistry is stereospecific, selective and higher product yields. Bifunctionality is made possible with Click chemistry with suitable building blocks. The bio-othogonal Click reactions that target specific sites under mild reaction conditions make their utilization for various biological applications.

1. **Anti-cancer agents :** Cancer cells grow uncontrollably and arethe cause fora large number of diseases. Only few drugs were successfully synthesized as potent anti-cancer agents. Thesepotential of these anticancer agents were enhanced through numerous methods including photodynamic therapy (PDT), nucleic acid binding, etc. PDT is a clinically accepted treatment as it is non-invasive. It requires light, oxygen and photosensitizer for their function. All three together are not toxic and they tend to generate a very reactive single oxygen species which is engaged in killing cancer cells. Organo-metallic frameworks[20] are utilized as photosensitizer with dual functioning - imaging and ROS production. Furthermore, fabrication of these organometallic frameworks with near infrared properties and capping to hyaluronic acid will enhance the ability of PDT against cancer cells. A dinuclear gold organometalic complex has revealed excellent anti-proliferative activity against various cancer cells with IC50 value 3.12-8.5 µM. This was acquired by Click reacting 1,4-C6H4(C≡CAuPPh3)2and 4-(azidomethyl) benzonitrile.[21] One of the important research projects for medication development is the study of DNA-drug interactions. It is vital to comprehend the mechanism behind this interaction to develop new treatments. Platinum metal complexes were most commonly used in this study but the performance of Click reactions on platinum complexes is inadequate. Certain disadvantages that hampered the druggability of Pt-compounds include drug resistance, neuro- and nephro-toxicity, partial target reception,[22] and deactivationof drug due to other side mechanism.[23] So nanoparticle assisted platinum-based delivery system and Pt(IV) prodrugs wereinvestigatedtowards developing new drugs. Magnetic nanoparticles were bound with Pt-complex generated viaClick chemistry

was investigated in intracellular tumor distribution[24] in which the iron oxide was connected via amide bond.

These magnetic nanoparticle-based Pt complexes were relatively more efficient than the commonly used drug cisplatin. This claim was also supported by its low inhibitory concentration values. Furthermore, the potential of Pt-prodrugs were modified through strain-promoted azide-alkyne cycloaddition reaction.[25] For instance, modification of the prodrug with hydrophobic azadibenzocyclooctyne molecules enhanced the lipophilic nature of the drug to give desired results. It can also be reduced to form the drug cisplatin. Apart from platinum based drugs, polyethylene glycol supported iridium(III) complexes were used for bio-imaging applications. They also act as phosphorescent dyes. This PEG based iridium complex was produced by Click chemistry and found that even minimum concentrations of the prodrug can result in higher cell viability and appropriate cytotoxicity.[26]

2. **Anti-microbial agents:** Recently, certain microbes reported to display drug resistance. For a drug to be a potent inhibitor, its ability to bind with bacterial lipids has to be higher. Many Ru(II) complexeswere found to possess lipophilicity to exhibit efficient antibiotic activity against Staphylococcus aureus.[27] -[28] Cu(I)-catalyzed Click-derived ruthenium complex (figure 13) produced by reacting with 2,6-bis(1-R-1H-1,2,3-tiazol-4-yl)pyridine ligand displayed excellent antimicrobial potency. This complex is active against both gram positive and gram negative bacteria. The length of alkyl chains in this complex and their antibacterial activity were correlated with its anti-bacterial action exhibited by complexes with either hexyl or heptyl substituent.

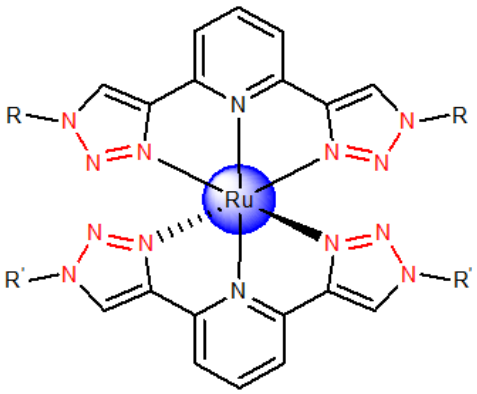


Figure 13: Structure of Click-derived ruthenium complex possessing 2,6-bis(1-R-1H-1,2,3-iazol-4-yl)pyridine ligand.

Later, a series of three different copper complexes anti-bacterial activitywas synthesized following Click chemistry,[29]of which two were mononuclear, having nitrate ion and Cu with 6-fold distorted octahedral geometry while the third one was a trinuclear with 4-fold distorted square planner geometry (figure 14).

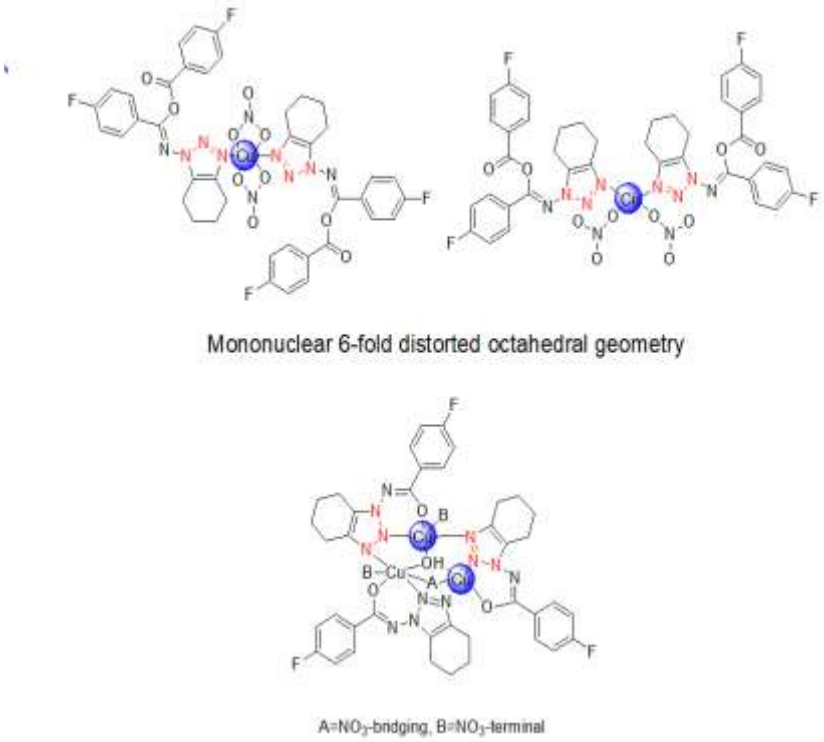


Figure 14: Click-derived mononuclear complexes with 6-fold distorted octahedral geometry (top) and trinuclear 4-fold distorted square planner geometry (bottom).

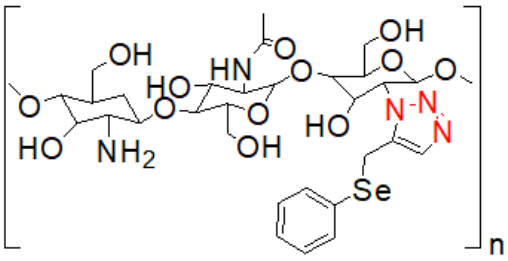
When antibacterial activity was evaluated for the above three ligands along with chloramphenicol as reference, the trinuclear complex was able to efficiently inhibit the bacterial growth better than all the other ligands, which could further be attributed to their unique structures. The relevant data are compiled in table 1.In these the free nitrogen is available to bind to metal ions that can be employed in bacterial metabolism.

Table 1: Anti-bacterial activity as evaluated by their inhibitory concentrations in (µg/ml)

Compound	Staphylococcus aureus	Bacillus Subtilis	Salmonella typhimurium	Escherichia coli
Chloramphenicol	6	6	6	
Ligand	96	96	48	96
Complex 1	48	48	24	48
Complex 2	24	24	12	24
Complex 3	12	12	6	12

3. **Anti-oxidants:** As a result of oxidation reaction in live cells highly reactive and unstable nitrogen and oxygen are generated. This in turn will initiate a continuous progression of chain reactions that can damage or kill cells by inhibiting the activity of numerous crucial proteins, lipids and nucleic acids. This action can be inhibited by the presence of a potent anti-oxidant that can prevent oxidation. The anti-oxidant activity can be evaluated through DPPH and $ABTS^{+}$assays. DPPH, when gets in contact with the reactive free radicals it gets reduced to change its color from violet to brown,while the presence of anti-oxidants will prevent the convertion of ABTS to green $ABTS^{+}$. Similarly the

organoselenium complexes were alsoinvestigated for their potent antioxidant activity (figure 15). Its chitosan-based derivatives were synthesized via Click chemistry that further inhibited the oxidation reaction[30].



Organoselenium chitosan derivative

Figure 15: Structure of chitosan-derived anti-oxidant potent organoselenium complex.

4. **Bioconjugation:** Proteins are complexed biopolymers that are essential for all living systems. In order to investigate their structure-activity relationships, chemical modifications of proteinsare required. Site-specific modification to develop bio-conjugates is one of the major developments for chemical modification of proteins. There are several methods available to modify the proteins using specified amino acid alteration but it remains a challenge to accurately modify the enzymes and antibodies in large quantities. This requires ample amount of reaction time and ambient reaction conditions to obtain high yields of bio-conjugates. Click chemistry offers excellent quantity of products with minimal by-products and fast reaction kinetics. Advantages of high selectivity and specificity also aid to follow Click chemistry in the synthesis of bio-conjugation and it is also widely used in pharmaceuticals. Modification techniques for phenol-containing tyrosine, sulfhydryl-containing cysteine, indole ring of tryptophanand thioether moiety of methionine were reported. In case of tryptophan, oxidation-mediated modification of amino acids through oxidation-induced Click reaction resulted in chemoselective tryptophan oxidation Cysteine containing sulphur that has eight different oxidation states can readily undergo oxidation. Under physiological conditoins, persulfate will be generated by the oxidation of sulphur in cysteine along with many other side reactive species including reactive oxygen and nitrogen. With the oxidation-induced Click chemistry ligation using sulfenic acid ligates the sulfhydryl group of cysteine. Moreover, the sulfenic acid can act as a biomarker for many oxidative reactions in biological system. Oxidation by electrophiles and nucleophiles in microenvironment will increase the antioxidant potency of the Click sulfenic acid.

V. CONCLUSION

Biological systems require connecting many small units following precise mechanisms that can offer specificity and selectivity. Click chemistry offers the possibility of such reactions to happen in laboratory environments. Recently, click chemistry serves as a power tool for heterologous synthesis of novel drug molecules. These reactions have paved way for the development of numerous enzyme inhibitors, particularly those that require fragment-based inhibition. The scope for enhancing the potency of many therapeutic drugs can be attempted with Click chemistry. Moreover, the possibility for the introducing non-

biological chemical reactions into complex biological systems has been successfully proven with Click chemistry.

REFERENCES

[1] Li, X.; Xiong, Y. Application of "Click" Chemistry in Biomedical Hydrogels. ACS Omega**2022**, 7 (42), 36918–36928. https://doi.org/10.1021/acsomega.2c03931.

[2] Schaub, T. A.; Zieleniewska, A.; Kaur, R.; Minameyer, M.; Yang, W.; Schüßlbauer, C. M.; Zhang, L.; Freiberger, M.; Zakharov, L. N.; Drewello, T.; Dral, P. O.; Guldi, D. M.; Jasti, R. Tunable Macrocyclic Polyparaphenylene Nanolassos via Copper-Free Click Chemistry. Chem. – A Eur. J.**2023**, 29 (33), e202300668. https://doi.org/https://doi.org/10.1002/chem.202300668.

[3] Mueller, E.; Poulin, I.; Bodnaryk, W. J.; Hoare, T. Click Chemistry Hydrogels for Extrusion Bioprinting: Progress, Challenges, and Opportunities. Biomacromolecules**2022**, 23 (3), 619–640. https://doi.org/10.1021/acs.biomac.1c01105.

[4] Zhong, X.; Yan, J.; Ding, X.; Su, C.; Xu, Y.; Yang, M. Recent Advances in Bioorthogonal Click Chemistry for Enhanced PET and SPECT Radiochemistry. Bioconjug. Chem.**2023**, 34 (3), 457–476. https://doi.org/10.1021/acs.bioconjchem.2c00583.

[5] Worrell, B. T.; Malik, J. A.; Fokin, V. V. Direct Evidence of a Dinuclear Copper Intermediate in Cu(I)-Catalyzed Azide-Alkyne Cycloadditions. Science**2013**, 340 (6131), 457–460. https://doi.org/10.1126/science.1229506.

[6] Cai, J. H.; Zhu, X. Z.; Guo, P. Y.; Rose, P.; Liu, X. T.; Liu, X.; Zhu, Y. Z. Recent Updates in Click and Computational Chemistry for Drug Discovery and Development. Front. Chem.**2023**, 11 (February), 1–7. https://doi.org/10.3389/fchem.2023.1114970.

[7] Gan, S.; Wu, Y.; Zhang, X.; Zheng, Z.; Zhang, M.; Long, L.; Liao, J.; Chen, W. Recent Advances in Hydrogel-Based Phototherapy for Tumor Treatment. Gels. 2023. https://doi.org/10.3390/gels9040286.

[8] Dong, R.; Yang, X.; Wang, B.; Ji, X. Mutual Leveraging of Proximity Effects and Click Chemistry in Chemical Biology. Med. Res. Rev.**2023**, 43 (2), 319–342. https://doi.org/https://doi.org/10.1002/med.21927.

[9] Jena, S.; Choudhury, B.; Ahmad, M. G.; Balamurali, M. M.; Chanda, K. Photophysical Evaluation on the Electronic Properties of Synthesized Biologically Significant Pyrido Fused Imidazo[4,5-c]Quinolines. Spectrochim. Acta Part A Mol. Biomol. Spectrosc.**2023**, 287, 122081. https://doi.org/https://doi.org/10.1016/j.saa.2022.122081.

[10] Dasmahapatra, U.; Kumar, C. K.; Das, S.; Subramanian, P. T.; Murali, P.; Isaac, A. E.; Ramanathan, K.; Balamurali, M. M.; Chanda, K. In-Silico Molecular Modelling, MM/GBSA Binding Free Energy and Molecular Dynamics Simulation Study of Novel Pyrido Fused Imidazo[4,5-c]Quinolines as Potential Anti-Tumor Agents. Front. Chem.**2022**, 10 (September), 1–17. https://doi.org/10.3389/fchem.2022.991369.

[11] Kaushal, J.; Singh, S.; Nautiyal, D.; Rao, G. K.; Singh, A. K.; Kumar, A. Click Chemistry in the Synthesis of Catalytically Relevant Organoselenium Compounds: Development and Applications of Catalysts for Organic Synthesis. New J. Chem.**2022**, 46 (31), 14757–14781. https://doi.org/10.1039/D2NJ02364D.

[12] Saleem, F.; Rao, G. K.; Kumar, A.; Mukherjee, G.; Singh, A. K. Half-Sandwich Ruthenium(II) Complexes of Click Generated 1,2,3-Triazole Based Organosulfur/-Selenium Ligands: Structural and Donor Site Dependent Catalytic Oxidation and Transfer Hydrogenation Aspects. Organometallics**2013**, 32 (13), 3595–3603. https://doi.org/10.1021/om400057e.

[13] He, Y.; Cai, C. A Simple Procedure for the Polymer-Supported N-Heterocyclic Carbene–Rhodium Complex via Click Chemistry: A Recyclable Catalyst for the Addition of Arylboronic Acids to Aldehydes. Chem. Commun.**2011**, 47 (45), 12319–12321. https://doi.org/10.1039/C1CC14898B.

[14] Kritchenkov, A. S.; Kletskov, A. V; Egorov, A. R.; Kurasova, M. N.; Tskhovrebov, A. G.; Khrustalev, V. N. Ultrasound and Click Chemistry Lead to a New Chitin Chelator. Its Pd(II) Complex Is a Recyclable Catalyst for the Sonogashira Reaction in Water. Carbohydr. Polym.**2021**, 252, 117167. https://doi.org/10.1016/j.carbpol.2020.117167.

[15] Schuster, E. M.; Botoshansky, M.; Gandelman, M. Pincer Click Ligands. Angew. Chemie Int. Ed.**2008**, 47 (24), 4555–4558. https://doi.org/https://doi.org/10.1002/anie.200800123.

[16] Kubacka, A.; Fernández-García, M.; Colón, G. Advanced Nanoarchitectures for Solar Photocatalytic Applications. Chem. Rev.**2012**, 112 (3), 1555–1614. https://doi.org/10.1021/cr100454n.

[17] Garg, V.; Kodis, G.; Chachisvilis, M.; Hambourger, M.; Moore, A. L.; Moore, T. A.; Gust, D. Conformationally Constrained Macrocyclic Diporphyrin−Fullerene Artificial Photosynthetic Reaction Center. J. Am. Chem. Soc.**2011**, 133 (9), 2944–2954. https://doi.org/10.1021/ja1083078.

[18] Chitre, K. P.; Guillén, E.; Yoon, A. S.; Galoppini, E. Synthesis of Homoleptic Ruthenium "Star" Complexes by Click Reaction for TiO2 Sensitization. Eur. J. Inorg. Chem.**2012**, 2012 (33), 5461–5464. https://doi.org/https://doi.org/10.1002/ejic.201200896.

[19] Zabarska, N.; Stumper, A.; Rau, S. CuAAC Click Reactions for the Design of Multifunctional Luminescent Ruthenium Complexes. Dalt. Trans.**2016**, 45 (6), 2338–2351. https://doi.org/10.1039/C5DT04599A.

[20] Goldstein, D. C.; Peterson, J. R.; Cheng, Y. Y.; Clady, R. G. C.; Schmidt, T. W.; Thordarson, P. Synthesis and Luminescence Properties of Iridium(III) Azide- and Triazole-Bisterpyridine Complexes. Molecules. 2013, pp 8959–8975. https://doi.org/10.3390/molecules18088959.

[21] Thompson, A. S.; Humphrey, G. R.; DeMarco, A. M.; Mathre, D. J.; Grabowski, E. J. J. Direct Conversion of Activated Alcohols to Azides Using Diphenyl Phosphorazidate. A Practical Alternative to Mitsunobu Conditions. J. Org. Chem.**1993**, 58 (22), 5886–5888. https://doi.org/10.1021/jo00074a008.

[22] Stengel, I.; Mishra, A.; Pootrakulchote, N.; Moon, S.-J.; Zakeeruddin, S. M.; Grätzel, M.; Bäuerle, P. "Click-Chemistry" Approach in the Design of 1,2,3-Triazolyl-Pyridine Ligands and Their Ru(Ii)-Complexes for Dye-Sensitized Solar Cells. J. Mater. Chem.**2011**, 21 (11), 3726–3734. https://doi.org/10.1039/C0JM03750H.

[23] Zhao, Q.; Huang, C.; Li, F. Phosphorescent Heavy-Metal Complexes for Bioimaging. Chem. Soc. Rev.**2011**, 40 (5), 2508–2524. https://doi.org/10.1039/C0CS00114G.

[24] Zhao, Q.; Li, F.; Huang, C. Phosphorescent Chemosensors Based on Heavy-Metal Complexes. Chem. Soc. Rev.**2010**, 39 (8), 3007–3030. https://doi.org/10.1039/B915340C.

[25] Zhao, Q.; Cao, T.; Li, F.; Li, X.; Jing, H.; Yi, T.; Huang, C. A Highly Selective and Multisignaling Optical−Electrochemical Sensor for Hg2+ Based on a Phosphorescent Iridium(III) Complex. Organometallics**2007**, 26 (8), 2077–2081. https://doi.org/10.1021/om061031r.

[26] Su, H.-C.; Chen, H.-F.; Fang, F.-C.; Liu, C.-C.; Wu, C.-C.; Wong, K.-T.; Liu, Y.-H.; Peng, S.-M. Solid-State White Light-Emitting Electrochemical Cells Using Iridium-Based Cationic Transition Metal Complexes. J. Am. Chem. Soc.**2008**, 130 (11), 3413–3419. https://doi.org/10.1021/ja076051e.

[27] Chi, Y.; Chou, P.-T. Transition-Metal Phosphors with Cyclometalating Ligands: Fundamentals and Applications. Chem. Soc. Rev.**2010**, 39 (2), 638–655. https://doi.org/10.1039/B916237B.

[28] Liu, S.; Müller, P.; Takase, M. K.; Swager, T. M. "Click" Synthesis of Heteroleptic Tris-Cyclometalated Iridium(III) Complexes: Cu(I) Triazolide Intermediates as Transmetalating Reagents. Inorg. Chem.**2011**, 50 (16), 7598–7609. https://doi.org/10.1021/ic2005985.

[29] Li, Y.; Tsang, D. P.-K.; Chan, C. K.-M.; Wong, K. M.-C.; Chan, M.-Y.; Yam, V. W.-W. Synthesis of Unsymmetric Bipyridine–PtII–Alkynyl Complexes through Post-Click Reaction with Emission Enhancement Characteristics and Their Applications as Phosphorescent Organic Light-Emitting Diodes. Chem. – A Eur. J.**2014**, 20 (42), 13710–13715. https://doi.org/https://doi.org/10.1002/chem.201404315.

[30] Li, B.; Cao, H.; Zheng, J.; Ni, B.; Lu, X.; Tian, X.; Tian, Y.; Li, D. Click Modification of a Metal–Organic Framework for Two-Photon Photodynamic Therapy with Near-Infrared Excitation. ACS Appl. Mater. Interfaces**2021**, 13 (8), 9739–9747. https://doi.org/10.1021/acsami.1c00583.

[31] Verma, S. K.; Ansari, S. N.; Kumari, P.; Mobin, S. M. Click Reaction Driven, Highly Fluorescent Dinuclear Organogold(I) Complex Exhibits a Dual Role: A Rare Au···H Interaction and an Antiproliferative Agent. Organometallics**2019**, 38 (13), 2591–2596. https://doi.org/10.1021/acs.organomet.9b00291.

[32] Burger, H.; Loos, W. J.; Eechoute, K.; Verweij, J.; Mathijssen, R. H. J.; Wiemer, E. A. C. Drug Transporters of Platinum-Based Anticancer Agents and Their Clinical Significance. Drug Resist. Updat. Rev. Comment. Antimicrob. Anticancer Chemother.**2011**, 14 (1), 22–34. https://doi.org/10.1016/j.drup.2010.12.002.

[33] Oun, R.; Moussa, Y. E.; Wheate, N. J. The Side Effects of Platinum-Based Chemotherapy Drugs: A Review for Chemists. Dalt. Trans.**2018**, 47 (19), 6645–6653. https://doi.org/10.1039/C8DT00838H.

[34] Quarta, A.; Amorín, M.; Aldegunde, M. J.; Blasi, L.; Ragusa, A.; Nitti, S.; Pugliese, G.; Gigli, G.; Granja, J. R.; Pellegrino, T. Novel Synthesis of Platinum Complexes and Their Intracellular Delivery to Tumor Cells by Means of Magnetic Nanoparticles. Nanoscale**2019**, 11 (48), 23482–23497. https://doi.org/10.1039/C9NR07015J.

[35] Pathak, R. K.; McNitt, C. D.; Popik, V. V; Dhar, S. Copper-Free Click-Chemistry Platform to Functionalize Cisplatin Prodrugs. Chem. – A Eur. J.**2014**, 20 (23), 6861–6865. https://doi.org/https://doi.org/10.1002/chem.201402573.

[36] Yang, H.; Li, L.; Wan, L.; Zhou, Z.; Yang, S. Synthesis of Water Soluble PEG-Functionalized Iridium Complex via Click Chemistry and Application for Cellular Bioimaging. Inorg. Chem. Commun.**2010**, 13 (12), 1387–1390. https://doi.org/https://doi.org/10.1016/j.inoche.2010.07.042.

[37] Li, X.; Gorle, A. K.; Sundaraneedi, M. K.; Keene, F. R.; Collins, J. G. Kinetically-Inert Polypyridylruthenium(II) Complexes as Therapeutic Agents. Coord. Chem. Rev.**2018**, 375, 134–147. https://doi.org/https://doi.org/10.1016/j.ccr.2017.11.011.
[38] Li, F.; Collins, J. G.; Keene, F. R. Ruthenium Complexes as Antimicrobial Agents. Chem. Soc. Rev.**2015**, 44 (8), 2529–2542. https://doi.org/10.1039/C4CS00343H.
[39] Al-Karawi, A. J. M.; OmarAli, A.-A. B.; Mangelsen, S.; Dege, N.; Kansız, S.; Breuninger, P.; Baydere, C.; OmarAli, O. B. An Unprecedented Formation of New Copper(II) Complexes as Bioactive Materials Based on Copper-Catalyzed Click Reaction. Polyhedron**2021**, 198, 115084. https://doi.org/https://doi.org/10.1016/j.poly.2021.115084.
[40] Nornberg, A. B.; de Aquino, T. F. B.; Martins, C. C.; Luchese, C.; Wilhelm, E. A.; Jacob, R. G.; Hartwig, D.; Fajardo, A. R. Organoselenium-Chitosan Derivative: Synthesis via "Click" Reaction, Characterization and Antioxidant Activity. Int. J. Biol. Macromol.**2021**, 191, 19–26. https://doi.org/https://doi.org/10.1016/j.ijbiomac.2021.09.053.
[41] Ishiguro, T.; Amamoto, Y.; Tanabe, K.; Liu, J.; Kajino, H.; Fujimura, A.; Aoi, Y.; Osakabe, A.; Horikoshi, N.; Kurumizaka, H.; Yamatsugu, K.; Kawashima, S. A.; Kanai, M. Synthetic Chromatin Acylation by an Artificial Catalyst System. Chem**2017**, 2 (6), 840–859. https://doi.org/https://doi.org/10.1016/j.chempr.2017.04.002.
[42] Tamura, T.; Song, Z.; Amaike, K.; Lee, S.; Yin, S.; Kiyonaka, S.; Hamachi, I. Affinity-Guided Oxime Chemistry for Selective Protein Acylation in Live Tissue Systems. J. Am. Chem. Soc.**2017**, 139 (40), 14181–14191. https://doi.org/10.1021/jacs.7b07339.
[43] Sato, S.; Morita, K.; Nakamura, H. Regulation of Target Protein Knockdown and Labeling Using Ligand-Directed Ru(Bpy)3 Photocatalyst. Bioconjug. Chem.**2015**, 26 (2), 250–256. https://doi.org/10.1021/bc500518t.
[44] Seki, Y.; Ishiyama, T.; Sasaki, D.; Abe, J.; Sohma, Y.; Oisaki, K.; Kanai, M. Transition Metal-Free Tryptophan-Selective Bioconjugation of Proteins. J. Am. Chem. Soc.**2016**, 138 (34), 10798–10801. https://doi.org/10.1021/jacs.6b06692.
[45] Wright, T. H.; Bower, B. J.; Chalker, J. M.; Bernardes, G. J. L.; Wiewiora, R.; Ng, W.-L.; Raj, R.; Faulkner, S.; Vallée, M. R. J.; Phanumartwiwath, A.; Coleman, O. D.; Thézénas, M.-L.; Khan, M.; Galan, S. R. G.; Lercher, L.; Schombs, M. W.; Gerstberger, S.; Palm-Espling, M. E.; Baldwin, A. J.; Kessler, B. M.; Claridge, T. D. W.; Mohammed, S.; Davis, B. G. Posttranslational Mutagenesis: A Chemical Strategy for Exploring Protein Side-Chain Diversity. Science**2016**, 354 (6312). https://doi.org/10.1126/science.aag1465.
[46] Hugo, M.; Turell, L.; Manta, B.; Botti, H.; Monteiro, G.; Netto, L. E. S.; Alvarez, B.; Radi, R.; Trujillo, M. Thiol and Sulfenic Acid Oxidation of AhpE, the One-Cysteine Peroxiredoxin from Mycobacterium Tuberculosis: Kinetics, Acidity Constants, and Conformational Dynamics. Biochemistry**2009**, 48 (40), 9416–9426. https://doi.org/10.1021/bi901221s.
[47] (1) Peskin, A. V; Low, F. M.; Paton, L. N.; Maghzal, G. J.; Hampton, M. B.; Winterbourn, C. C. The High Reactivity of Peroxiredoxin 2 with H(2)O(2) Is Not Reflected in Its Reaction with Other Oxidants and Thiol Reagents. J. Biol. Chem.**2007**, 282 (16), 11885–11892. https://doi.org/10.1074/jbc.M700339200.
[48] Bruice, T. C.; Sayigh, A. B. The Structure of Anthraquinone-1-Sulfenic Acid (Fries' Acid) and Related
[49] Compounds. J. Am. Chem. Soc.**1959**, 81 (13), 3416–3420. https://doi.org/10.1021/ja01522a066

