



Palladium-Catalyzed Tsuji–Trost-Type Reactions in Bioorthogonal Chemistry: From Test Tubes to Living Systems

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Abstract

The potential to conduct palladium-catalyzed Tsuji–Trost reactions in biological systems opens unprecedented opportunities to probe and manipulate cellular processes. However, implementing such transformations remains challenging due to the stringent requirements imposed by biocompatibility. To date, Tsuji–Trost allylation has not yet been successfully demonstrated in living cells, and *in vivo* applications remain unrealized, primarily due to the presumed incompatibility between traditional organic chemistry and the complex aqueous environments of biological systems. Nevertheless, significant progress has been made in this area over the past two decades. The successful execution of a Tsuji–Trost reaction in aqueous media requires careful consideration of several key factors, including the choice of catalyst, ligand, leaving group, and nucleophile, as well as the influence of water on reactivity and selectivity. In this review, we highlight the latest advancements in biocompatible palladium-catalyzed Tsuji–Trost-type reactions, with a particular focus on deprotection and allylation reactions conducted in aqueous environments and in living systems. Further development of *in vivo* Tsuji–Trost allylation is expected in the near future.

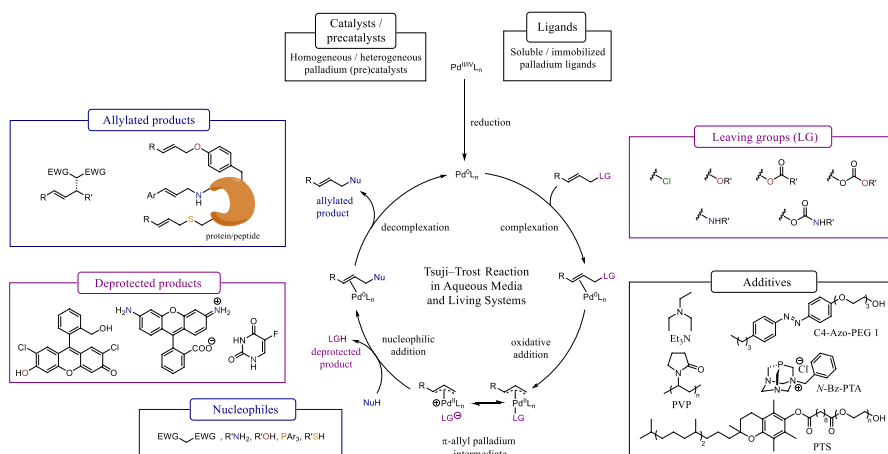
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Graphical Abstract

This review explores recent advances in biocompatible Tsuji–Trost-type reactions, with emphasis on mechanistic insights and the transition from conventional bench-top protocols to biological applications.



Keywords Tsuji–Trost reaction · Bioorthogonal chemistry · Biocompatibility · Deprotection · Allylation

Abbreviations

5(6)-FAM	5(6)-Carboxyfluorescein
5FU	5-Fluoro-uracil
5-TAMRA	5-Carboxytetramethylrhodamine
acac	Acetylacetonate
Alloc	Allyloxycarbonyl
Arg	Arginine residue
Asp	Aspartate residue
BAN	<i>N</i> -Butyl-4-NH-Alloc-1,8-naphthalimide
BINAP	2,2'-Bis(diphenylphosphino)-1,1'-binaphthalene
BIPHEPHOS	6,6'-[(3,3'-Di- <i>tert</i> -butyl-5,5'-dimethoxy-1,1'-biphenyl-2,2'-diyl)bis(oxy)]bis(dibenzo[<i>d,f</i>][1,3,2]dioxaphosphepin)
CD	Cyclooctadine
COD	1,5-Cyclooctadiene
cRGDfE-Pd NPs	CRGDfE-functionalized Pd-loaded nanoparticles
CuAAC	Copper-catalyzed azide–alkyne cycloaddition
Cys	Cysteine residue
dba	Dibenzylideneacetone
DDAB	Didodecyldimethylammonium bromide
DMB	Didodecyldimethylammonium bromide
DMEM	Dulbecco's Modified Eagle Medium

DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
Dox	Doxorubicin
dppf	1,1'-Bis(diphenylphosphino)ferrocene
dr	Diastereomeric ratio
Duo	Duocarmycin
Ea	Activation energy
ee	Enantiomeric excess
EPR	Enhanced permeability and retention
er	Enantiomeric ratio
EWG	Electron-withdrawing group
FUdR	Floxuridine
Glu	Glutamate residue
Gly	Glycine residue
GSH	Glutathione
HER2	Human epidermal growth factor receptor 2
His	Histidine residue
HSA	Human serum albumin
Hsp27	Heat shock protein 27
IEDDA	Inverse electron-demand Diels–Alder
Leu	Leucine residue
LG	Leaving group
LM-Pd NPs	Liquid metal palladium nanoparticles
Lys	Lysine residue
mAb	Monoclonal antibody
MeCN	Acetonitrile
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
<i>N</i> -Bz-PTA	<i>N</i> -benzyl-1,3,5-triaza-7-phosphaadamantane
NP	Nanoparticle
Neu	Neuraminic acid
NuH	Nucleophile
PBB	Phosphate borate buffer
PBS	Phosphate-buffered saline pH 7.4
Pd NPs	Palladium nanoparticles
PEG	Polyethylene glycol
PLGA–PEG	Poly(lactic- <i>co</i> -glycolic acid)-polyethyleneglycol
POI	Protein of interest
PS	Polystyrene
PTS	Polyoxyethanyl- α -tocopheryl sebacate
PVP	Poly(vinylpyrrolidone)
PYP	Photoactive yellow protein
rt	Room temperature
SCPNs	Single-chain polymeric nanoparticles
Sec	Selenocysteine residue
SOD	Superoxide dismutase

SPAAC	Strain-promoted azide–alkyne cycloaddition
Tedicyp	<i>cis,cis,cis</i> -1,2,3,4-Tetrakis(diphenylphosphinomethyl) cyclopentane
TFP	Tri(2-furyl)phosphine
TON	Turnover number
TPPTS	Tris(3-sulfophenyl)phosphine trisodium salt
Tyr	Tyrosine residue
UBL3	Ubiquitin-like protein 3

1 Introduction

Bioorthogonal chemistry encompasses chemical reactions that can take place within living systems without interfering with native biochemical pathways [1, 2]. This rapidly expanding field has transformed chemical biology by providing powerful tools to label, bioimage, and manipulate biomolecules in their physiological context [3–5]. Notable examples of bioorthogonal reactions include the Staudinger ligation, copper-catalyzed azide–alkyne cycloaddition (CuAAC), strain-promoted azide–alkyne cycloaddition (SPAAC), and the inverse electron-demand Diels–Alder (IEDDA) reaction [6, 7]. However, bioorthogonal chemistry faces challenges such as slow kinetics (Staudinger ligation), cytotoxicity of copper catalysts (CuAAC), bulky reagents (SPAAC), and instability of tetrazines in aqueous environments [4, 8]. Despite these limitations, bioorthogonal chemistry has been compatible with biological components and occurs rapidly enough to capture analytes of interest.

Over the past two decades, biocompatible transition metal-mediated reactions have gained significant attention due to their ability to perform diverse, tunable, and physiologically compatible bioorthogonal reactions with precise targeting and external control [9–11]. These compelling benefits have driven growing interest in developing transition metal-mediated reactions for biological applications [8]. For example, seminal advances have shaped this field, beginning with Meggers' 2006 Ru-catalyzed deprotection and Bradley's 2011 demonstration of palladium nanoparticles for intracellular uncaging [12, 13]. Subsequent breakthroughs—including Weissleder's encapsulated palladium systems [14], Chen's Pd₂(allyl)₂Cl₂ complexes [15], and Mascareñas' Pd-phosphine architectures for intracellular targeting [16]—have significantly enhanced catalyst stability and specificity. Recent reviews by Mascareñas, Reek, and Gasser further advanced the transition metal field by incorporating sophisticated ligand designs and supramolecular strategies into the bioorthogonal toolkit [11, 17–19]. While both palladium and ruthenium enable bioorthogonal catalysis in cells, they face challenges like thiol poisoning and the need for relatively high catalyst loadings. Compared to Ru, Pd offers superior versatility in cross-coupling and deprotection reactions, though it is more prone to toxicity and deactivation [11, 20].

Among Pd-catalyzed reactions reported in living systems, Suzuki–Miyaura [21], Heck [14], and Sonogashira reactions [22] suffer from high catalyst loadings, stringent conditions, steric hindrance, and poor biocompatibility. In contrast, Tsuji–Trost deallylation reactions offer superior efficiency, milder conditions, and broader

applicability for biological applications [18, 23–29]. However, several challenges must be addressed before Tsuji–Trost reactions can be fully integrated as bioorthogonal tools, such as off-target reactions. Indeed, biological components such as water, cysteine-containing peptides like glutathione (GSH), and other proteinogenic nucleophiles, can sometimes compete with the targeted nucleophile or coordinate with the metal center [30, 31]. Meanwhile, redox-active species may alter the oxidation state of the metal catalyst, interfering with standard organometallic mechanisms [32]. To date, most applications of Tsuji–Trost allylation have been limited to asymmetric synthesis in controlled *in vitro* settings [33]. To our knowledge, only a few examples of Tsuji–Trost allylation processes have been successfully applied to protein modification. Notably, no published reports demonstrate their implementation in living cells or animal systems, which remains a major obstacle to their broader adoption in bioorthogonal chemistry.

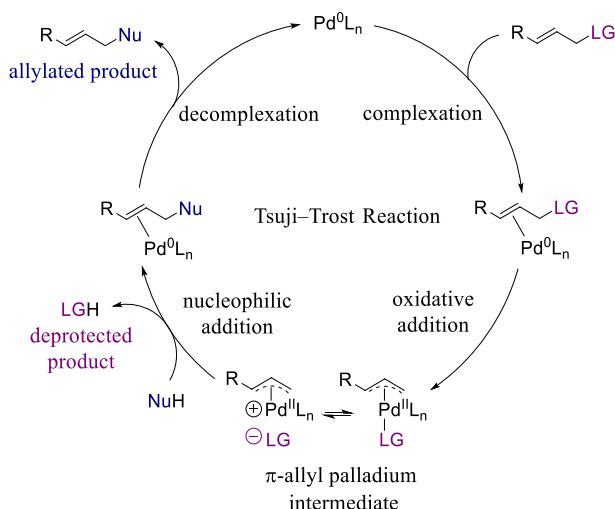
In this review, we provide an overview of the catalysts, ligands, leaving groups, nucleophiles, and other additives involved in Tsuji–Trost reactions under biologically relevant conditions—specifically in aqueous media, at temperatures compatible with biological systems (e.g., room temperature or 37 °C), and under aerobic conditions. We discuss examples of Tsuji–Trost deallylation and allylation reactions, ranging from test tube experiments to potential applications in living systems. Finally, we summarize the current state of biocompatible Tsuji–Trost reactions, highlighting their limitations and prospects. This review aims to offer an updated account of recent advancements in this emerging field.

2 Scope of Tsuji–Trost Reactions: Allylation, Deallylation, and Depropargylation

The Tsuji–Trost reaction, first reported by Jirō Tsuji in 1965 and later refined by Barry Trost in 1973, is a Pd-catalyzed allylic substitution reaction [34–36]. In its mechanism, a substrate bearing an allylic leaving group (LG) first coordinates with a Pd^0L_n species. Oxidative addition of the Pd^0 -olefin complex then occurs, releasing the leaving group and forming a π -allyl palladium intermediate. This electrophilic intermediate subsequently reacts with a nucleophile to yield an allyl-substituted product. Simultaneously, the active Pd^0L_n species is regenerated and can initiate the next catalytic cycle (Scheme 1).

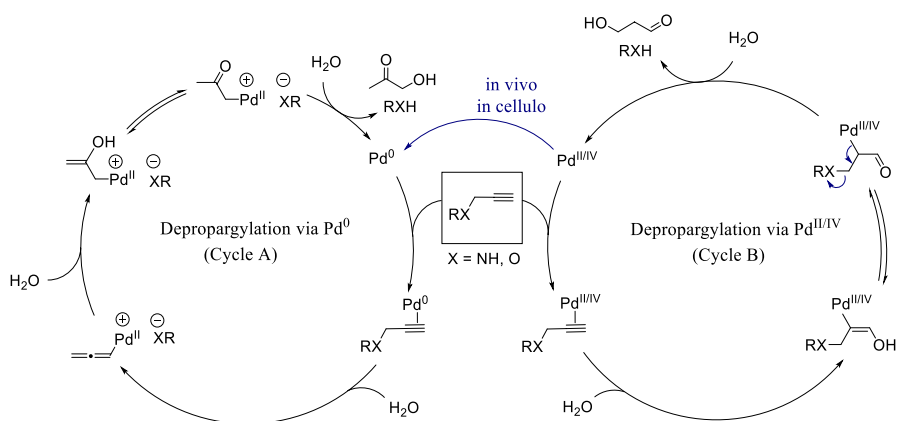
When the desired compound is the allylated product, the process is termed “Tsuji–Trost allylation.” Conversely, when the reaction is used to release a molecule of interest—corresponding to the leaving group or its decarboxylated derivative—it may be referred to as “Tsuji–Trost-type deprotection.” This released product can, for example, be a synthetic intermediate, a fluorophore, or a drug. Although this nomenclature has limited chemical significance—since both products are generated in any Tsuji–Trost reaction—it will be employed in this review to reflect the biological purpose of each transformation.

Pd-catalyzed deprotection reactions primarily fall into two categories: deallylation and depropargylation. The mechanism of Tsuji–Trost deallylation follows the general pathway of the reaction (Scheme 1). In contrast, the precise mechanism of



Scheme 1 General mechanism of Tsuji–Trost reactions. LG, leaving group; L, ligand; NuH, nucleophile

Pd-catalyzed depargylation remains unclear but may proceed via two distinct pathways depending on the palladium oxidation state [15, 17]. If the reaction is initiated by a Pd^0 species, a σ -allenyl palladium intermediate could be generated via an $\text{S}_{\text{N}}2'$ -type reaction [15, 17, 37–42]. This intermediate may undergo nucleophilic attack by a water molecule at the central sp -hybridized carbon atom of the allene moiety, leading to the formation of an enol intermediate that subsequently tautomerizes to its keto form. This process could ultimately regenerate the active Pd^0 species and produce hydroxyacetone as a byproduct, which was detected by Pal and Unciti-Broceta and co-workers (Scheme 2, Cycle A) [15, 17, 39–41, 43].



Scheme 2 Proposed mechanisms for Pd-catalyzed Tsuji–Trost-type depargylation in aqueous media

Alternatively, if either a Pd^{II} or a Pd^{IV} complex is involved, the mechanism may begin with electrophilic activation of the alkyne, followed by anti-Markovnikov addition of water, leading to an enol intermediate. After tautomerization, fragmentation of the α -Pd-aldehyde intermediate could result in the release of a free alcohol or amine and the regeneration of the catalytic species (Scheme 2, Cycle B) [15, 17, 39, 41, 44]. Both proposed depropargylation mechanisms differ significantly from the typical Tsuji–Trost pathway. However, as shown in Schemes 1 and 2, deallylation and depropargylation share a common step: a water molecule or another nucleophile attacks an electrophilic palladium species, namely a π -allyl or σ -allenyl palladium species or a Pd-activated alkyne.

Domingos and co-workers demonstrated that Pd^{II} -mediated depropargylation of 2,4-dinitrophenyl propargyl ether exhibits biphasic kinetics in phosphate buffer at pH 7.4. The initial fast phase ceased after only two turnovers, presumably due to catalyst inhibition via formation of a diorganopalladium complex. The authors postulated that the subsequent slow phase occurs via Pd^0 -catalyzed depropargylation, enabled by in situ reduction of the Pd^{II} species during the first phase [44]. This biphasic behavior was later supported by Palmans' group [45]. In cellular and in vivo settings, the depropargylation landscape diverges: endogenous reductants readily convert $\text{Pd}^{\text{II/IV}}$ to Pd^0 , while engineered nanoparticles, resins, or supports stabilize Pd^0 species (Scheme 2, from Cycle A to Cycle B) [14, 21, 43, 45–48]. These conditions favor a dominant Pd^0 -catalyzed cycle, akin to Tsuji–Trost deallylation. Thus, despite mechanistic differences, depropargylation is, within the context of biocompatible applications, operationally classified under the umbrella of Tsuji–Trost-type reaction in this review [49].

3 Constituents of Tsuji–Trost Reactions

Biocompatible Tsuji–Trost reactions include catalysts, ligands, nucleophiles, leaving groups, and water. Additional additives may also be employed. The following sections outline the roles and key features of each component.

3.1 Catalysts in Biocompatible Tsuji–Trost Reactions

3.1.1 Representative Palladium Catalysts and Precatalysts for Aqueous Conditions

In the early development of Tsuji–Trost reaction, stoichiometric amounts of allyl palladium complexes were used as reactants. Subsequently, the Miyake and Manyik groups used catalytic amounts of palladium for the allylation of alcohols, carboxylic acids, and amines [50, 51]. Due to the facile in situ reduction of Pd^{II} and Pd^{IV} compounds to reactive Pd^0 species, Pd^0 , Pd^{II} , and Pd^{IV} compounds (or their complexes) can all function as catalysts or precatalysts. In the context of Tsuji–Trost reactions in aqueous media, palladium complexes can be categorized into homogeneous and heterogeneous catalysts (Fig. 1).

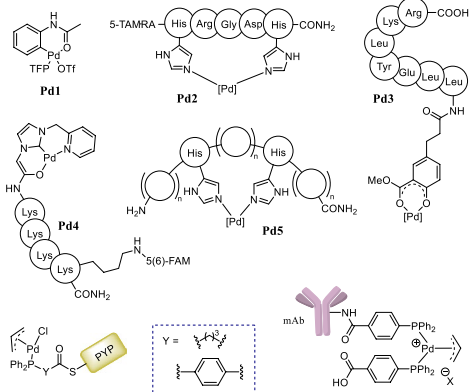
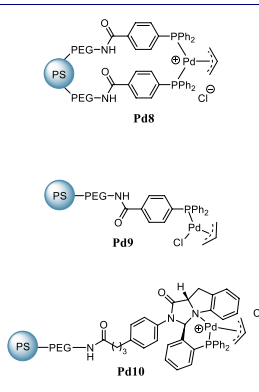
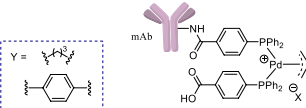
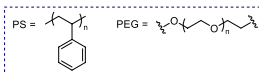
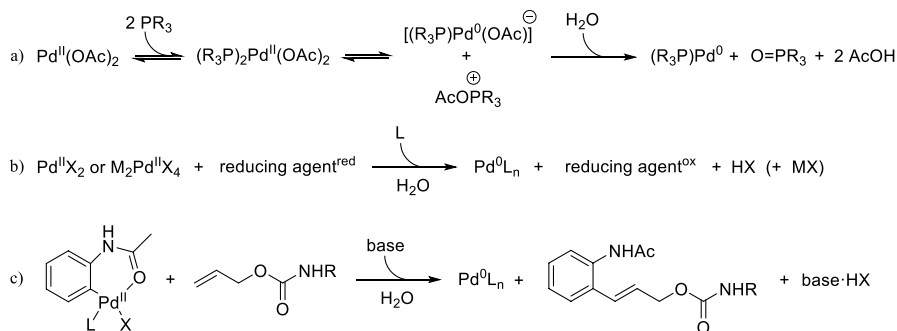
	Homogeneous (pre)catalysts	Heterogeneous (pre)catalysts
Pd ⁰ catalysts	Pd(PPh ₃) ₄ , Pd(dba) ₂ /BIPHEPHOS, Pd ₂ (dba) ₃ /BINAP	Pd microspheres, Pd-resins, Pd nanoparticles
Pd ^{II} precatalysts	Pd(OAc)₂, PdCl₂, Na₂PdCl₄, Pd(COD)Cl₂, Pd(NO₃)₂ [Pd(η³-allyl)Cl]₂, [Pd(η³-Ph₃allyl)Cl]₂, L[Pd(η³-allyl)Cl], Pd(η³-allyl)(biotin-spacer-ligand)Cl 	  
Pd ^{IV} precatalysts	Na ₂ PdCl ₆ , K ₂ PdCl ₆ , (NH ₄) ₂ PdCl ₆	/

Fig. 1 Palladium (pre)catalysts for water-compatible Tsuji–Trost reactions. 5-TAMRA, 5-carboxytetramethylrhodamine; 5(6)-FAM, 5(6)-carboxyfluorescein; PYP, photoactive yellow protein; X, counteranion

Homogeneous palladium complexes are appealing due to their well-defined structure and the ability to modify ligands to adjust solubility, stability, biocompatibility, and reactivity. In addition, ligand design can enhance cell transport and intracellular targeting [16]. Among homogeneous catalysts, Pd(PPh₃)₄ is widely used in aqueous media [52–57]. However, organic solvents such as dimethyl sulfoxide (DMSO), tetrahydrofuran (THF), and acetonitrile (MeCN) are frequently used as co-solvents to overcome the low aqueous solubility of this complex. Pd(dba)₂ and Pd₂(dba)₃ can also act as catalysts when combined with phosphine ligands, such as BIPHEPHOS [58], (2,2′-bis(diphenylphosphino)-1,1′-binaphthalene (BINAP) [59], or other water-soluble ligands [60].

In addition to Pd⁰ species, Pd^{II} and Pd^{IV} species are frequently employed as catalyst precursors. Pd^{II/IV} precatalysts dominate in aqueous Tsuji–Trost reactions due to their stability, controlled activation, and biocompatibility—critical for bioorthogonal chemistry, drug delivery, and live-cell labeling [15, 17]. For instance, Pd(OAc)₂ is often used with tris(3-sulfophenyl)phosphine trisodium salt (TPPTS), which is a bulky and water-soluble phosphine ligand [61–65]. PdCl₂ and Na₂PdCl₄ can also be utilized alongside various ligands, including triphenylphosphine (PPh₃) [66], TPPTS [67], 1,5-cyclooctadiene (COD) [68, 69], BINAP [70], and the η³-allyl ligand [15, 33, 64], although a few research groups have reported the use of these palladium salts in the absence of ligands [45, 71]. Furthermore, Mascareñas' group has synthesized discrete Pd^{II} precatalysts featuring phosphine and η³-allyl ligands, specifically (R₃P)Pd(η³-allyl)Cl complexes, designed for depropargylation and deallylation reactions in cell lysates [16].



Scheme 3 Proposed pathways for the reduction of Pd^{II} complexes to active Pd^0 species in the presence of water. M: Na, K, NH_4 ; X, counteranion or X-type ligand; L, L-type ligand

If a Pd^{II} source is used, the Tsuji–Trost reaction typically necessitates the reduction of the Pd^{II} precatalyst to the active Pd^0 species. Various reductants can accomplish this reduction, including phosphine ligands [72, 73], sodium borohydride (NaBH_4) [71, 74], and biologically relevant molecules such as GSH [30, 31], sodium ascorbate [67], nicotinamide adenine dinucleotide (NADH), and nicotinamide adenine dinucleotide phosphate (NADPH) [75]. In 1992, Ozawa and Hayashi demonstrated that water could significantly accelerate the reduction of $\text{Pd}(\text{OAc})_2$ in the presence of a base and a phosphine [76]. Subsequently, studies by Buchwald and Wei supported a mechanism whereby water molecules or hydroxide ions promote the phosphine-mediated reduction of Pd^{II} complexes to Pd^0 species (Scheme 3a) [72, 73]. Furthermore, the reduction of Na_2PdCl_4 to active Pd^0 species can be facilitated by sodium ascorbate. Compared to the complex generated from Na_2PdCl_4 and TPPTS, decaging experiments of *O*-allyl-duocarmycin and *N*-Alloc-doxorubicin (ProDox) in cells revealed that the Na_2PdCl_4 /sodium ascorbate combination exhibited a pronounced cytotoxic effect, consistent with efficient Pd-mediated pro-drug activation. This effect was attributed to the poor cellular uptake of the bulky Pd^0 -TPPS complex, whereas sodium ascorbate can enter cells and reduce Na_2PdCl_4 intracellularly (Scheme 3b) [67]. Furthermore, NaBH_4 enables faster reduction of Pd^{II} precatalysts compared to triphenylphosphine while simultaneously generating hydride ligands, which are among the best ligands for palladium. This dual role not only facilitates efficient Pd^0 formation but also minimizes deleterious interactions between palladium and sulfides in biological systems [71, 74]. Moreover, Riant and co-workers hypothesized that acetanilide[tri(2-furyl)phosphine] palladium(II) triflate (**Pd1**) could undergo a Heck-type reaction with the allyl moiety of allyloxycarbonyl (Alloc) protecting groups, resulting in the generation of an active Pd^0 species capable of initiating the subsequent Tsuji–Trost reaction (Scheme 3c) [77]. Meanwhile, the integration of Pd^{II} complexes into peptide or protein structures (**Pd2–Pd7**) [68, 78–83], has expanded the scope of applications by allowing for enzyme-like catalysis.

Pd^{IV} sources, including Na_2PdCl_6 , K_2PdCl_6 , and $(\text{NH}_4)_2\text{PdCl}_6$, have been also successfully employed as precatalysts for Tsuji–Trost uncaging reactions. For

example, a dimethylacridine-derived probe featuring an allyl carbonate moiety demonstrated efficient cleavage by various palladium species, including Na_2PdCl_6 , both in phosphate-buffered saline (PBS) buffer and in HeLa cells [55]. Koide et al. further demonstrated that K_2PdCl_6 could effectively catalyze *O*-deallylation under biologically relevant conditions [74]. Moreover, a xanthene–hemicyanine hybrid probe was shown to undergo efficient *N*-deallyloxycarbonylation when catalyzed by Pd^0 , Pd^{II} , or Pd^{IV} complexes, with $(\text{NH}_4)_2\text{PdCl}_6$ being particularly effective [84]. These studies collectively demonstrate the broad utility of Pd^0 , Pd^{II} , and Pd^{IV} sources as versatile catalyst precursors in Tsuji–Trost reactions.

Heterogeneous palladium catalysts can offer several advantages over homogeneous catalysts, including improved reaction control, selectivity, and ease of catalyst recovery (Fig. 1). Pd^0 nanoparticles are particularly prevalent [13, 21, 46]. Pd^0 species can also be combined with resins to create heterogeneous palladium catalysts [85]. Notably, the incorporation of polyethylene glycol (PEG) to improve the biocompatibility of polystyrene (PS) has led to significant advancements in heterogeneous catalysis—particularly for stabilizing Pd–resin systems in biologically relevant environments (**Pd8–Pd10**) [86, 87, 125, 137, 140–142, 161–163].

3.1.2 Limitations and Challenges in Biocompatible Palladium Catalysis

Pd species can exert various levels of toxicity in biological systems, notably through binding to biomolecules such as DNA and proteins [88, 154]. Simple, single-component palladium sources are often favored over palladium species featuring more complex or negatively charged ligands. Examples of simple palladium sources include $\text{Pd}(\text{OAc})_2$ and PdCl_2 , which can facilitate cellular uptake and may exhibit reduced toxicity compared to phosphine-containing palladium complexes [22]. Palladium complexes designed by Riant's group generally display low toxicity, although complexes bearing Xantphos as a ligand exhibit increased toxicity in SiHa cells [89]. Moreover, the *in vivo* toxicity of Pd complexes can be significantly reduced through the use of heterogeneous palladium forms, such as palladium nanoparticles (Pd^0 NPs) [14, 43].

Pd catalysts face significant instability in biological systems due to ligand exchange and nanoparticle formation. For example, nucleophiles like glutathione readily displace Pd ligands altering reaction pathways [90]. Pd^{II} precursors can frequently generate nanoparticles (NPs) in the presence of reductants. The exact nature of the active species—molecular complexes versus NPs—remains unclear in cellular environments, complicating mechanistic control [67]. Additionally, heterogeneous Pd NPs display limited cellular uptake and poor solubility, further reducing efficiency [19].

Another challenge is to maintain control over the homo-/heterogeneity of the catalytic system. The primary difficulty in controlling the homo-/heterogeneity of palladium catalytic systems stems from the competing reduction pathways and the inherent instability of key intermediates such as L_1Pd^0 species. The system's sensitivity to water, base, and pre-aging conditions further exacerbates these challenges. To maintain homogeneity, careful optimization of reagents, ligands, and reaction protocols is essential, as exemplified by the superior performance of diboron-mediated

reduction of $(\text{Cy}_3\text{P})_2\text{Pd}(\text{OAc})_2$ compared to the base-promoted, phosphine-mediated pathway [72]. Weissleder and co-workers envisioned Alloc removal with discrete $\text{PdCl}_2(\text{TFP})_2$, but encountered solubility and stability issues. This problem was solved by encapsulating the complex within nanoparticles [14]. To address the limitations and challenges in biocompatible Pd catalysis, bis-histidine stapled miniprotein scaffold [68], phosphine ligand design [16], resin-supported bidentate ligands [91], nano-palladium design [14], or additives will be discussed with respect to stabilizing the palladium complex or delaying the deactivation of palladium complexes.

3.2 Ligands in Biocompatible Tsuji–Trost Reactions

The active monoligated 12-electron L_1Pd^0 and biligated 14-electron L_2Pd^0 complexes commonly used in Pd-catalyzed reactions are sensitive to air, which poses challenges for their application in living systems [62]. To address this issue, there has been growing interest in exploring the properties of ligands in Pd-catalyzed reactions, including their bulkiness, electronic effects, and bite angles. Various ligands that have been employed in biocompatible Tsuji–Trost reactions are depicted in Fig. 2, most of which are phosphine ligands. These ligands can be divided into two groups, depending on whether they are used in homogeneous (**L0–L18**) or heterogeneous catalysis (**L19–L33**).

PPh_3 (**L0**) has demonstrated restricted use in biocompatible Tsuji–Trost reactions due to its poor water solubility [92]. Therefore, the core structure of PPh_3 has been modified to incorporate one or more sulfonate groups, thereby enhancing the aqueous solubility of the resulting ligands (**L1–L8**). Among the PPh_3 analogues, **L1** and **L2** carry one and two sulfonate groups, respectively, but they have been seen limited use in chemical reactions [93, 94]. In contrast, the trisulfonate-substituted ligand TPPTS (**L3**) has been widely used in Tsuji–Trost reactions performed in aqueous media, notably in the field of protein labeling [61, 62, 95–97]. Furthermore, sulfonated phosphines bearing one or more [1,1'-biphenyl]-4-sulfonate groups (**L4–L8**) were developed by Monflier and co-workers [65, 98, 99]. **L4** ligands have been shown to exhibit high solubility in water and high catalytic performance in the Pd-catalyzed cleavage of allyl octyl carbonate. Notably, **L4c** is easier to synthesize compared to its analogues **L4a** and **L4b**, as well as TPPTS [98]. **L5–L8** are other amphiphilic phosphines that combine water solubility with coordination ability to palladium for catalysis in aqueous media [65]. **L9** represents phosphine ligands featuring a quaternary ammonium group and an axially chiral counteranion. They have been used in asymmetric catalysis in combination with $\text{Pd}_2(\text{dba})_3$ [100]. In addition, **L10** introduces ammonium groups on the phenyl substituents of 1,1'-bis(diphenylphosphino)ferrocene (dppf), yielding a hydrophilic phosphine ligand. This ligand demonstrates notable catalytic efficiency in the Pd-catalyzed allylation of amines in water, affording pharmaceutically relevant *N*-allylated products [101]. Furthermore, Koide and co-workers demonstrated that phosphate anions (**L11**) exert a positive effect on the rate of Pd-catalyzed *O*-deallylation reactions in aqueous media. Their kinetic study suggests that these anions may act as ligands, accelerating the olefin complexation step. This rate enhancement may potentially be

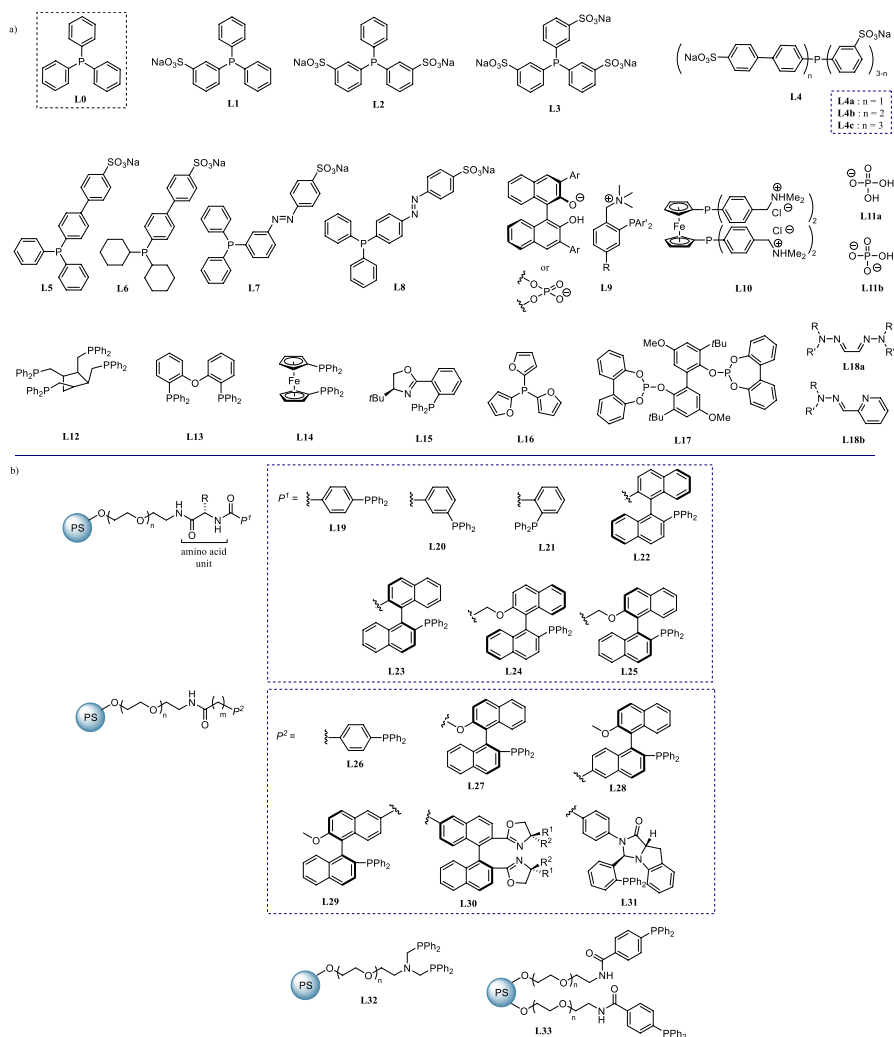


Fig. 2 Soluble (a) and immobilized (b) palladium ligands for aqueous Tsuji–Trost reactions

attributed to a shift in equilibrium between a resting state DMSO-bound Pd species and an active phosphate-bound species, which readily associates with the substrate's double bond. However, phosphate anions do not compete with TFP for binding to palladium [102].

In addition to ionic ligands (**L1–L11**), neutral ligands (**L12–L18**) enable efficient Tsuji–Trost reactions in water with minimal additives. For example, *cis,cis,cis*-1,2,3,4-tetrakis(diphenylphosphinomethyl)cyclopentane (Tedicyp, **L12**) is a tetraphosphine ligand that is not water-soluble. However, the complex resulting from the combination of **L12** and $[\text{Pd}(\eta^3\text{-allyl})\text{Cl}]_2$ has been shown to exhibit higher reactivity in water than in THF due to its high aqueous stability

[103]. DPEphos (**L13**) and dppf (**L14**) operate in aqueous micellar systems, with DPEphos excelling in room-temperature allylic aminations [92, 104, 105]. For asymmetric catalysis, (*S*)-*t*Bu-PHOX (**L15**) controls enantioselectivity via steric interactions, though its aqueous applications are less explored [106]. These ligands highlight the potential for sustainable high-performance catalysis in water. The electronic effects of ligands can considerably influence palladium catalysis. For instance, the tri(2-furyl)phosphine ligand (TFP, **L16**) has been studied extensively [102, 107, 108]. This poor σ -donating ligand is commonly used to accelerate the Tsuji–Trost reaction. The reasons for this acceleration in an aqueous environment remain unclear. However, results obtained in dimethylformamide (DMF) suggest that, compared to PPh_3 , TFP may facilitate dissociation from the metal center. This could enhance the availability of *d*-orbitals for oxidative addition while also promoting nucleophilic attack on the π -allyl palladium intermediate [14, 74, 109]. Another example of the influence of a ligand's electronic properties on the rate of the Tsuji–Trost reaction is provided by the bidentate phosphite ligand BIPHEPHOS (**L17**). In combination with $\text{Pd}(\text{dba})_2$, this electron-deficient ligand proved to be highly effective for catalyzing the allylation of thiols with allyl carbonates. In MeCN, BIPHEPHOS has shown good performance compared to more electron-rich ligands, such as alkyl phosphines [110]. It also demonstrated efficacy in promoting Pd-mediated *S*-allylation reactions in the presence of water. However, strictly oxygen-free conditions seem crucial for the activity of the Pd/**L17** complex [58]. Phosphines, which are among the most commonly used ligands for palladium, offer high catalytic activity but suffer from toxicity and air sensitivity, limiting biocompatibility [115]. In 2008, Mino's group reported that hydrazone ligands (**L18**)—acting as phosphine-free alternatives—enabled efficient Pd-catalyzed allylation of boronic acids in DMF/ H_2O media [111].

Beyond soluble ligands, phosphines immobilized on supports such as resins have also been developed. Palladium complexes formed with these immobilized ligands can catalyze Tsuji–Trost reactions in aqueous media and can subsequently be recycled, which underscores their importance in green chemistry. For example, Uozumi's group synthesized a series of phosphine ligands grafted onto a polystyrene-polyethylene glycol (PS–PEG) polymer, resulting in amphiphilic PS–PEG resin-supported phosphines. These include amino acid units and phosphine moieties, which are derived from PPh_3 (**L19–L21**) and chiral phosphines (**L22–L25**). Furthermore, the amino acid moiety of the immobilized ligands was replaced with an *n*-alkyl chain to yield ligands **L26–L31**. These ligands were evaluated for their activity and enantioselectivity in Pd-catalyzed substitution of 1,3-diphenylallyl acetate with 3-methyl-2,4-pentanedione in aqueous media [91, 112]. The chiral imidazoindolephosphine **L31** has been applied in various enantioselective reactions under aqueous conditions, such as *C*-allylation of malonate diesters [86, 113] and nitromethane [22], *O*-allylation of phenols [91], and *N*-allylation of secondary amines [91, 114]. Additionally, Uozumi and co-workers have developed resin-supported bidentate ligands (**L32–L33**). Their bidentate nature can delay the deactivation of palladium complexes, specifically through processes such as leaching and agglomeration [91].

Despite advancements in ligand design, no ligands have yet been identified as effective for Tsuji–Trost allylation within cellular environments. To date, the most

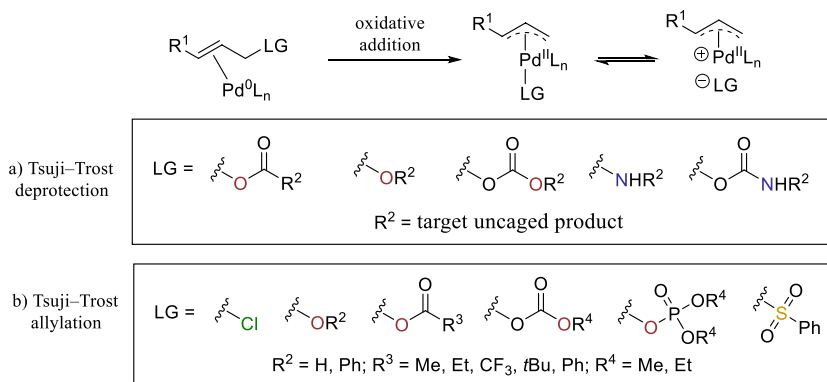


Fig. 3 Leaving groups in Pd-catalyzed deprotection (a) and allylation (b) in aqueous media

biologically relevant ligand remains TPPTS, which has notably been applied to label tyrosine residues (Tyr) in natural proteins [61–63]. However, due to its bulky and charged nature, Pd^0 –TPPS cannot efficiently cross cell membranes. Thus, it primarily acts outside cells [67]. This highlights a significant gap in the development of cell-compatible ligands and underscores the need for continued research in this field.

3.3 Leaving Groups in Biocompatible Tsuji–Trost Reactions

In the Tsuji–Trost reaction mechanism, coordination of the substrate to the palladium center is followed by an oxidative addition step, resulting in the release of the leaving group. A good leaving group must meet certain criteria, potentially limiting the scope of allyl-based protection strategies. Under palladium catalysis, the leaving group must dissociate with low activation energy and stabilize the resulting anion. In biological settings, it should additionally be stable in aqueous media at neutral pH and be nontoxic [116, 117]. In biocompatible Tsuji–Trost-type deprotection processes, the nature of the leaving group is determined by the structure of the desired uncaged product. For instance, carboxylic acids can be released through cleavage of their corresponding allyl esters [118], while alcohols and phenols can be liberated from *O*-allyl [67, 74, 119, 120] or *O*-allyloxycarbonyl derivatives [53–55, 64, 65, 84, 121]. Amines can likewise be released from the corresponding *N*-allyl [122] or *N*-allyloxycarbonyl precursors [13, 52, 56, 66, 71, 77, 115, 123] (Fig. 3a).

O-allyloxycarbonyl and *N*-allyloxycarbonyl protecting groups are commonly employed as leaving groups because their cleavage releases CO_2 , thereby increasing the system's entropy, favoring product formation, and driving the reaction forward [124]. The gaseous CO_2 diffuses away, rendering the reaction effectively irreversible. In addition, using CO_2 -generating moieties such as carbonates and carbamates aligns with the principles of bioorthogonal chemistry, as it minimizes waste compared to halide-based leaving groups. Thus, CO_2 -releasing leaving groups offer advantages in terms of both reaction efficiency and sustainability. However, while

carbonate groups perform well in vitro, their lower stability compared to carbamates may limit their applicability in living systems [85].

In aqueous Tsuji–Trost allylation reactions, allylic chlorides, esters and carbonates are the most common leaving groups. In this context, the choice of the leaving group is crucial, as it significantly influences both the yield and selectivity of the reaction. For instance, Uozumi's group reported that the Pd-catalyzed allylic substitution of cinnamyl chloride with ethyl acetoacetate afforded the target product in 72% yield, whereas the use of cinnamyl acetate increased the yield to 89% [125]. Furthermore, Pd-catalyzed *N*-allylation of 1-phenylpiperazine in aqueous micelles provided approximately two-fold higher yields when allyl acetate or allyl methyl carbonate were used as allylic substrates, compared to allyl chloride [126]. Among esters and carbonates, studies on Pd-catalyzed allylic azidation in THF/H₂O (3:1) mixtures showed that (*E*)-hex-2-en-1-yl acetate was less reactive than other esters, such as (*E*)-hex-2-en-1-yl trifluoroacetate and benzoate, and than (*E*)-hex-2-en-1-yl ethyl carbonate [127]. Besides allylic chlorides, esters, and carbonates, allyl phenyl sulfones [128] and allyl phosphates [127] can also serve as leaving groups to generate π -allyl palladium species in aqueous media (Fig. 3b).

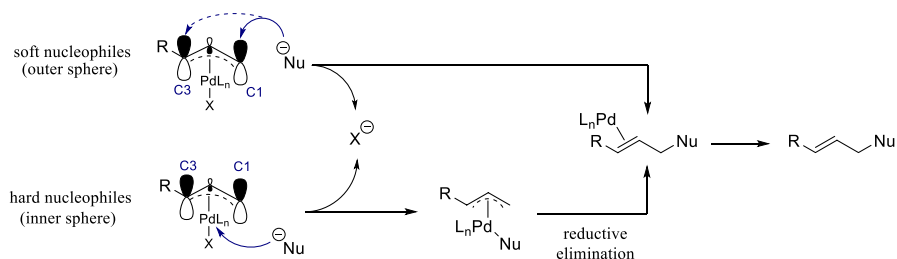
In addition to their ability to dissociate from the allyl moiety in the presence of a Pd⁰ catalyst, leaving groups can strongly affect the regio- and stereoselectivity of Tsuji–Trost reactions conducted in organic solvents [129–131]. However, studies specifically documenting their influence on reaction selectivity under aqueous conditions are still lacking.

In the field of protein labeling, it is noteworthy that Francis and co-workers used an allyl acetate as the leaving group to enable selective tyrosine allylation in chymotrypsinogen A [61]. In contrast, Breinbauer and co-workers employed allyl carbonates as leaving groups to achieve *S*-allylation of cysteine residues in various peptides and proteins [58].

3.4 Nucleophiles in Biocompatible Tsuji–Trost Reactions

The standard approach to classifying the behavior of nucleophiles towards π -allyl palladium complexes is based on the pK_a value of the nucleophile: (1) “soft” nucleophiles, typically stabilized species (such as the conjugate bases of acidic compounds with pK_a values below 25); and (2) “hard” nucleophiles, generally unstable species (such as the conjugate bases of very weak acids with pK_a values exceeding 25). However, this classification of nucleophiles as hard or soft based on pK_a remains debated. Walsh and co-workers suggested that the pK_a threshold distinguishing these groups should be raised from 25 to at least 32 [132].

After the oxidative addition step, the nucleophile attacks the π -allyl palladium intermediate. This attack can occur either at the carbon center (outer-sphere mechanism) or at the metal center (inner-sphere mechanism) (Scheme 4) [102]. Typically, soft nucleophiles directly attack the carbon center, while hard nucleophiles preferentially attack the metal center, leading to the formation of a carbon–carbon or carbon–heteroatom bond through subsequent reductive elimination. From a stereochemical perspective, this mechanistic divergence is crucial, especially when the



Scheme 4 Nucleophilic attack on the π -allyl palladium intermediate through two distinct pathways

nucleophilic attack generates a stereogenic center. The outer-sphere pathway results in the inversion of stereochemistry relative to the π -allyl palladium intermediate, whereas the inner-sphere pathway leads to retention of stereochemistry [133]. For instance, soft thiol groups generally attack the carbon atom of the intermediate [58, 134].

In both mechanistic pathways, the new bond between the allyl group and the nucleophile can form at either the $C1$ or $C3$ position, thereby determining the regioselectivity of the reaction. This regioselectivity is influenced by factors such as the steric hindrance within the π -allyl palladium intermediate and the electronic properties of the ligands. In Tsuji–Trost-type deprotection reactions, the regioselectivity of the formation of the allyl-substituted product is not a primary concern. In contrast, in allylation processes, it directly determines the yield of the desired allylated compound. In this context, the use of Pd complexes bearing bulky ligands, such as BIPHEPHOS [58], can direct the nucleophile toward the terminal carbon atom.

From a chemical perspective, various carbo- and heteronucleophiles have been used in water-tolerant Tsuji–Trost processes. In deprotection reactions, nucleophiles acting as scavengers are essential for efficiently trapping the π -allyl palladium intermediate and preventing side reactions [135]. However, nucleophiles are often overlooked in literature. Only a few studies have reported the use of nucleophiles such as diethylamine [64, 98], morpholine [71], 4-mercaptophenylacetic acid [97], and TFP [102], in these processes. In biocompatible Tsuji–Trost allylation reactions, a wide range of C -, N -, O -, P -, and S -nucleophiles have been successfully allylated (Fig. 4). Ester-stabilized [113, 125, 128, 136], keto-stabilized [137, 138], and nitro-stabilized carbanions [87, 139] are commonly employed as C -nucleophiles. Acetylide anions [86], boronic acids [111, 140], and tetraphenylborate [95] can also serve as C -nucleophiles. Among N -nucleophiles, primary amines [96, 136, 139, 141–143], secondary amines [78, 79, 139, 143], anilines [143], hydroxylamine [95], and the azide anion [127, 144] can react with the electrophilic π -allyl palladium intermediate, with notable applications in asymmetric allylic amination reactions in aqueous media. In terms of O -nucleophiles, water acts as a weak nucleophile in Tsuji–Trost reactions, enabling more reactive nucleophiles to undergo allylation in aqueous media without significant interference [102, 115].

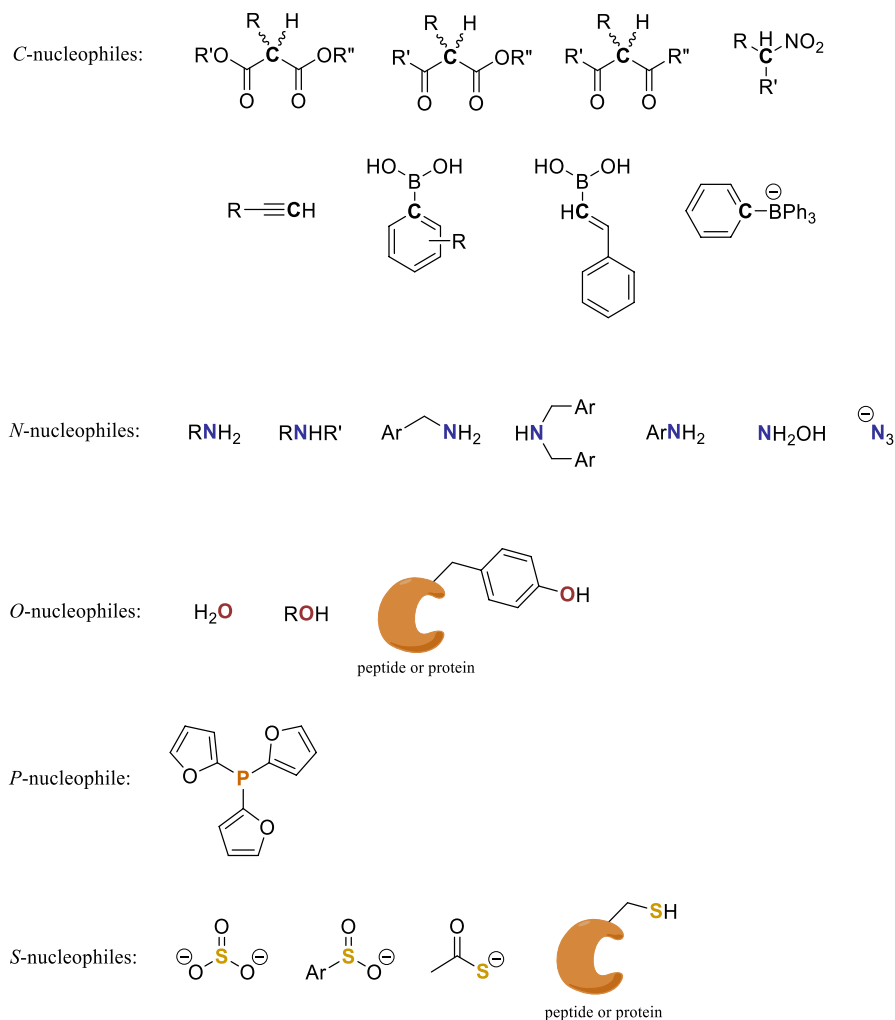


Fig. 4 C-, N-, O-, P-, and S-nucleophiles in water-compatible Tsuji–Trost reactions

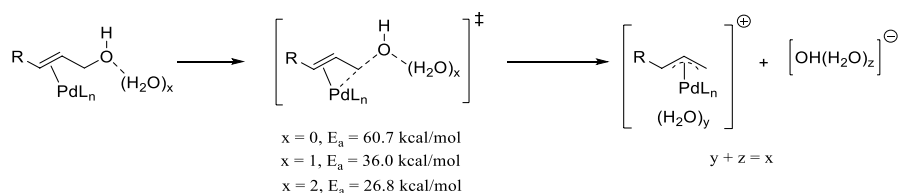
Aliphatic alcohols and their corresponding alkoxides also display low nucleophilicity toward π -allyl palladium intermediates [102, 133, 145]. To address this limitation, Zhang and co-workers proposed an approach whereby π -allyl palladium species, generated from decarboxylation of vinyl ethylene carbonates, are efficiently trapped by water or various aliphatic alcohols in the presence of a boron reagent as a co-catalyst. They suggested that the boron reagent may facilitate this process by forming a boronate complex with the remaining alkoxide anion of the intermediate. The boronate species could subsequently capture the *O*-nucleophile (water or an alcohol molecule), ultimately leading to the regioselective nucleophilic addition of the tethered *O*-nucleophile to the proximal carbon center of the π -allyl palladium electrophile [146]. Besides aliphatic alcohols, phenolic hydroxyl groups serve as

convenient nucleophiles in biocompatible Pd-catalyzed *O*-allylation, which has been applied not only to small molecules [141], but also to tyrosine residues in proteins [61–63]. Interestingly, the TFP ligand could also act as a nucleophile, as evidenced by the formation of a *P*-allylated TFP product during a $\text{Pd}(\text{NO}_3)_2$ /TFP-catalyzed *O*-deallylation reaction [102]. Additionally, Zhang and colleagues pioneered the use of sodium sulfite [59], while Uozumi's group employed sodium phenylsulfinate as an *S*-nucleophile [125], successfully synthesizing various allylic sulfonic acids and an allylic sulfone, respectively. Besides, potassium acetate (CH_3COSK) serves as an efficient sulfur nucleophile in Pd-catalyzed Tsuji–Trost allylic substitutions, enabling regioselective formation of allylic thioacetates in aqueous THF/ H_2O mixtures with net retention of configuration [147]. Furthermore, Breinbauer and co-workers developed an efficient method for the *S*-allylation of thiols groups within peptides and proteins [58].

3.5 Water in Biocompatible Tsuji–Trost Reactions

The presence of water in Tsuji–Trost reactions has been shown to significantly enhance reaction efficiency, often improving yields, rates, and selectivity. For instance, in 2015, Zhang and co-workers demonstrated that Pd-catalyzed asymmetric allylic sulfonation of diaryl-substituted allylic acetates with Na_2SO_3 in THF/ H_2O mixture at room temperature, giving allylic sulfonic acids in good yields and enantioselectivities [59]. The reaction failed in the absence of water, suggesting a beneficial role of water in solubilizing Na_2SO_3 , facilitating nucleophilic attack. Similarly, in 2013, Wei and co-workers highlighted that water promoted the reduction of Pd^{II} precatalysts to active Pd^0 species, accelerating the Miyaura borylation reaction [72]. The hydration of Pd^{II} intermediates lowers the activation energy, enabling faster catalyst activation. Buchwald's work further supports this, showing that water-mediated preactivation of $\text{Pd}(\text{OAc})_2$ with biaryldialkylphosphine ligands generates highly active Pd^0 catalysts for *C*–*N* cross-couplings, even at low loadings [73]. These examples underscore water's dual role as a solvent and a promoter, often through hydrogen bonding or solvation effects that stabilize reactive intermediates.

A notable advancement in aqueous Tsuji–Trost chemistry is the direct use of non-derivatized allylic alcohols as substrates, avoiding the need for preactivation. In 2004, Oshima and co-workers demonstrated that allylic alcohols could serve as efficient allylating agents in an ethyl acetate/ H_2O biphasic system, as illustrated by the successful allylation of various *C*- and *N*-nucleophiles. While hydroxyl groups are generally poor leaving groups in organic solvents, the authors suggested that water could activate allylic alcohols by hydrating the hydroxyl group. This hydration could facilitate the departure of the hydroxide ion and stabilize it through solvation, allowing the negative charge to delocalize within the water cluster. This hypothesis was supported by theoretical calculations, which showed that the activation energy (E_a) for hydroxide ion elimination decreases in the presence of hydrating water molecules (Scheme 5) [96]. Furthermore, Ma and co-workers developed an efficient Pd-catalyzed method for synthesizing allylic sulfones from allylic alcohols and sulfinic acids in aqueous media. Theoretical calculations suggest that water may facilitate



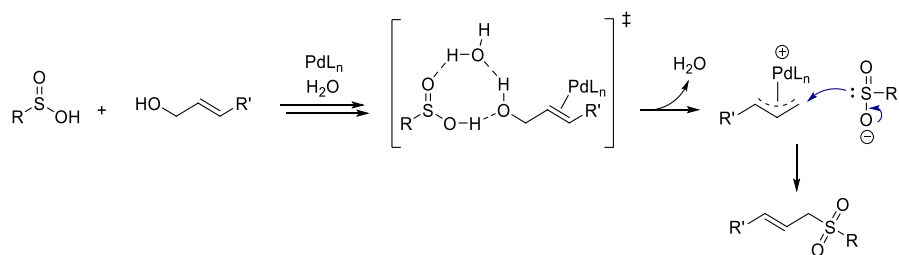
Scheme 5 Water-mediated activation of allylic hydroxyl groups for Tsuji–Trost reactions

the activation of the allylic alcohol through the formation of an eight-membered transition state, which involves hydrogen bonding interactions between the allylic alcohol, the sulfonic acid, and a water molecule (Scheme 6) [92]. These findings indicate that aqueous media can increase the synthetic efficiency of the Tsuji–Trost reaction by enabling allylic alcohols to be directly used as the allyl source, without acidic activators or the preparation of allyl acetates or carbonates.

In summary, water is a versatile medium for Tsuji–Trost reactions, improving efficiency, enabling the use of allylic alcohols, and facilitating Pd^{II} reduction. As the field moves toward more biocompatible processes, water's role in Tsuji–Trost chemistry will likely expand, offering new opportunities for sustainable synthesis. Future research should explore the interplay between water and other biocompatible solvents to further optimize these transformations.

3.6 Additives in Biocompatible Tsuji–Trost Reactions

In aqueous Tsuji–Trost processes, the inherent challenges of aqueous-phase catalysis—such as poor solubility of hydrophobic substrates, catalyst deactivation, and low reactivity—necessitate the use of specialized additives. These additives enhance reaction efficiency by stabilizing catalysts, solubilizing reactants, and modulating reactivity, enabling Tsuji–Trost reactions under mild conditions. For example, the use of a base is sometimes necessary but depends on the specific conditions and reagents employed [148]. The base can play a crucial role in deprotonating the nucleophile, enhancing its nucleophilicity and thereby facilitating the nucleophilic attack on the π -allyl palladium intermediate. For instance, triethylamine (Et_3N) is often



Scheme 6 Water-mediated synthesis of allylic sulfones via an eight-membered transition state

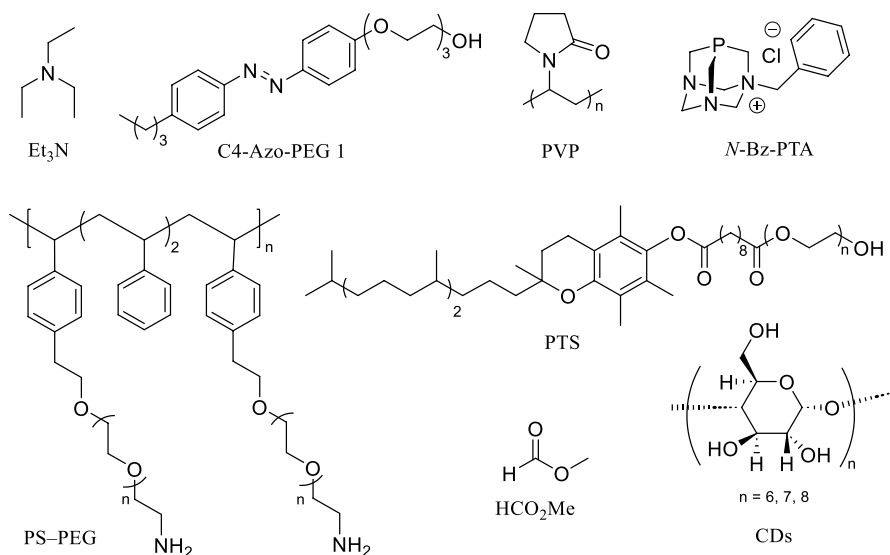


Fig. 5 Additives in aqueous Tsuji–Trost reactions

chosen as a mild and non-nucleophilic base that can effectively increase the reactivity of weakly acidic nucleophiles without interfering with the palladium catalyst or causing undesired side reactions [77, 149, 150]. However, in some cases, the reaction can proceed without an added base, particularly if the nucleophile itself has intrinsic basicity [115]. Additionally, certain leaving groups can act as base which can further obviate the need for an external base. In such media without an added base, carbonates can indeed sometimes provide better results than ester-type leaving groups, as the decarboxylation of carbonates generates an alkoxide anion capable of deprotonating acidic nucleophiles [151, 152]. Overall, the base requirement in aqueous Tsuji–Trost reactions is context-dependent, influenced by the nature of the nucleophile, the leaving group, and the stability of the catalytic system [135].

In addition to bases, other additives such as surfactants like C4-Azo-PEG 1, polyvinylpyrrolidone (PVP), and polyoxyethanyl α-tocopheryl sebacate (PTS) exemplify the transformative impact of additives in micellar catalysis (Fig. 5). C4-Azo-PEG 1, a photochromic surfactant, forms dynamic micelles that concentrate reactants and stabilize Pd catalysts, while its reversible *trans*-to-*cis* isomerization facilitates product extraction and recycling [153]. PVP, used in Pd-nanoparticle systems, prevents aggregation and localizes catalysts at the water–organic interface, achieving high turnover numbers (TONs > 500,000) [152]. Monflier's group reported an amphiphilic phosphine additive, *N*-benzyl-1,3,5-triaza-7-phosphaadamantane (*N*-Bz-PTA), which serves both as a surfactant and a ligand for coordination to the palladium center [65]. Additionally, a PS-PEG-supported phosphine-palladium complex has been used under heterogeneous conditions in water to yield allylic sulfones in good yields with various ligands [86, 87, 114]. Polyoxyethanyl-α-tocopheryl sebacate (PTS), a nonionic amphiphile, creates nano-micelles that solubilize hydrophobic

substrates, enabling room-temperature reactions without organic solvents. These surfactants collectively mimic organic-phase conditions while leveraging water's unique properties, such as enhanced selectivity and reduced environmental impact [105].

A particularly intriguing additive is methyl formate, which significantly enhances the reactivity of allylic alcohols in Tsuji–Trost animations. As proposed by Nishikata and Lipshutz, methyl formate likely activates the allylic hydroxy group by forming a transient formate ester *in situ*, improving its leaving group ability. This mechanism avoids the need for pre-activation (such as acetates or carbonates) and enables animations at ambient temperature [104].

Beyond surfactants and methyl formate, cyclodextrins (CDs) are cyclic oligosaccharides with hydrophobic cavities that can encapsulate organic substrates, creating a pseudo-organic microenvironment in water. CDs and their derivatives have emerged as powerful additives for Tsuji–Trost reactions in aqueous media, addressing challenges such as substrate solubility, catalyst stability, and interfacial mass transfer [64, 65].

In summary, biocompatible Tsuji–Trost reactions frequently rely on additives, including bases and surfactants (C4-Azo-PEG 1, PVP, PTS). They address key challenges in aqueous catalysis—solubility, catalyst stability, and reactivity—while aligning with sustainability goals. Future developments may focus on designing multifunctional additives or integrating these systems with renewable substrates, further expanding the scope of biocompatible Tsuji–Trost reactions.

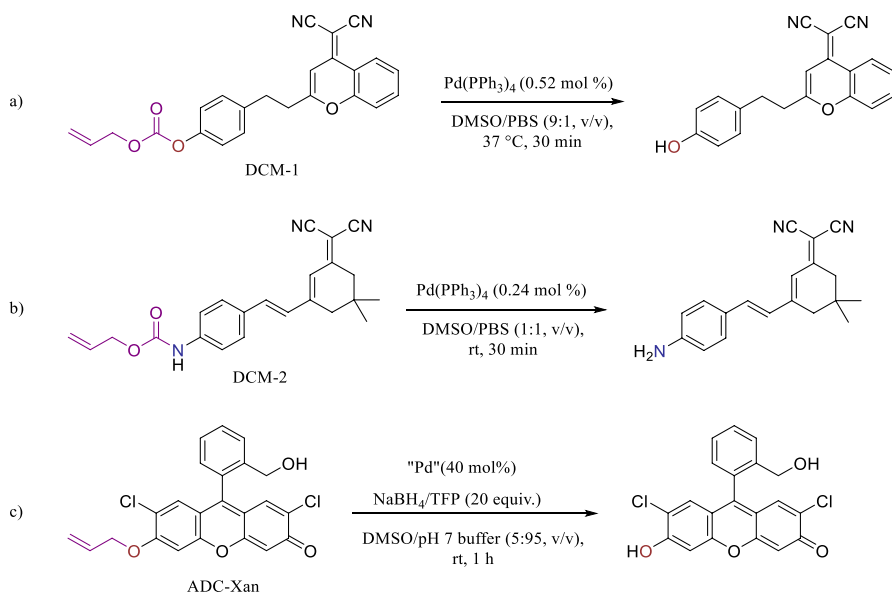
4 Tsuji–Trost-Type Deprotection Reactions in Living Settings

The Tsuji–Trost-type deprotection process, while widely used in laboratory conditions with organic solvents, has proven particularly effective in detecting palladium in crude samples such as road dust. Furthermore, it has been applied to study the selective cleavage of Pd-labile protecting groups within various living cells, utilizing compounds that function as probes for palladium detection or as prodrugs. Building on this foundation, *in vivo* deprotection studies in animal models demonstrate the broad applicability and effectiveness of Pd-mediated uncaging reactions in therapeutic interventions.

4.1 In Vitro Uncaging: Tsuji–Trost-Type Deprotection Reactions in Test Tubes

The Tsuji–Trost-type deprotection process, despite being well established in test tubes, still significantly relies on the utilization of organic co-solvents such as DMSO, MeCN, THF, and DMF. One prominent application of this process lies in the detection of palladium, which has demonstrated efficacy in analyzing crude samples, including road dust and roadside soil.

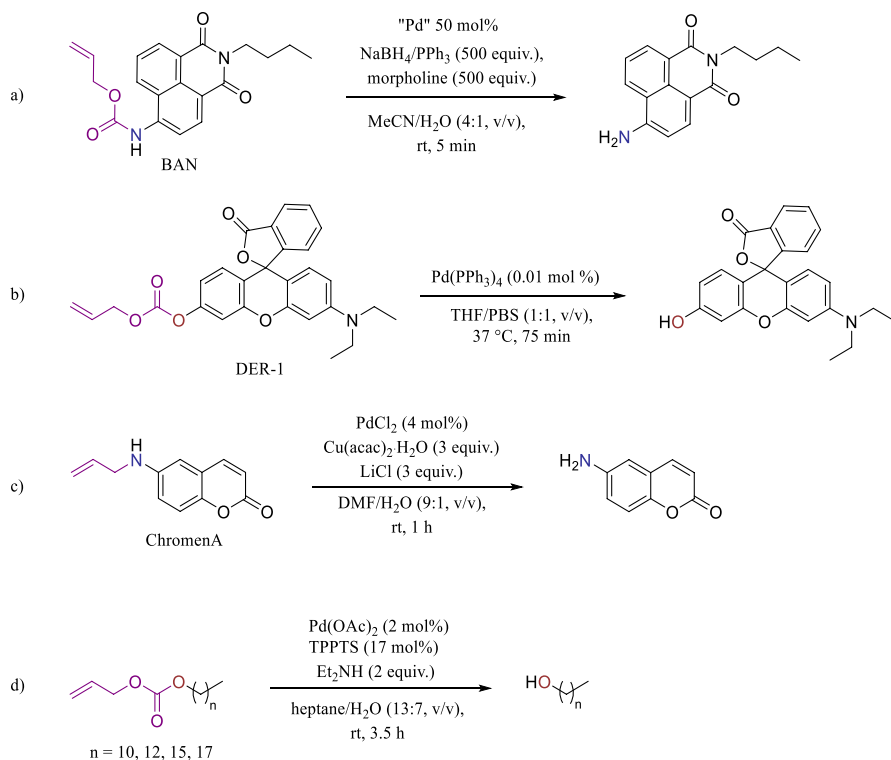
DMSO is a commonly used co-solvent in biological settings. Gu's group unveiled a pioneering near-infrared fluorescent probe derived from 4-(dicyanomethylene)-4*H*-chromene (DCM-1), which was deprotected in a mixture of DMSO and PBS



Scheme 7 Cleavage of allyl carbonate, allyl carbamate, and allyl ether groups in DMSO/buffer mixtures. “Pd” = PdCl_2 , $\text{Pd}(\text{OAc})_2$, $\text{Pd}(\text{PPh}_3)_4$, $\text{Pd}(\text{MeCN})_2\text{Cl}_2$, $\text{Pd}(\text{acac})_2$, $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$, $\text{Pd}(\text{MeCN})_2$, $\text{Pd}_2(\text{dba})_3$, or K_2PdCl_6

at pH 7.4 (Scheme 7a). DCM-1 underwent Pd-catalyzed *O*-deallyloxycarbonylation within 30 min in DMSO/buffer (9:1) enabling Pd^0 detection with a detection limit of 52 nM. Yet, this high concentration of DMSO, while enhancing the probe’s performance, presents a limitation in its direct application to biological systems due to its cytotoxic effects [121]. Subsequently, Zhang’s group synthesized a 3-(dicyanomethylene)cyclohex-1-ene derivative, DCM-2, which can also be uncaged through bioorthogonal *N*-deallyloxycarbonylation, achieving rapid (30 min) and selective Pd^0 detection with a 24.2 nM limit (Scheme 7b). However, the deprotection of DCM-2 still required 50% DMSO in PBS, which is not compatible with biological applications. A biologically friendly option in biological settings [52]. Furthermore, Koide’s group used 20% DMSO in buffer to transform a nonfluorescent allylic ether caged probe (ADC-Xan) to a fluorescent phenol through Tsuji–Trost *O*-deallylation with a highly efficiency ($\text{TOF} = 3.1 \text{ h}^{-1}$) [119]. Later, Koide reported the use of TFP rather than PPh_3 as the ligand, and the same fluorogenic probe was subjected to *O*-deallylation under biologically relevant conditions, namely, 5% DMSO in buffer (Scheme 7c) [74].

In addition to DMSO, other organic solvents are employed in combination with water to facilitate Tsuji–Trost-type deprotection reactions, enhancing the versatility and efficiency of the process. In 2011, Liu’s group developed *N*-butyl-4-NH-alloc-1,8-naphthalimide (BAN), a highly sensitive and selective fluorescent probe leveraging homogeneous Tsuji–Trost *N*-deallyloxycarbonylation to achieve detection rapid Pd^0 with a limit of 6.1 nM. The reaction, completed within 5 min in $\text{MeCN}/\text{H}_2\text{O}$ (4:1), cleanly generated *N*-butyl-4-amino-1,8-naphthalimide,



Scheme 8 Cleavage of allyl carbamate, allyl carbonate, and allyl amine groups in MeCN/H₂O, THF/PBS, DMF/H₂O, or heptane/H₂O solvent systems. Pd = Pd(PPh₃)₄, PdCl₂, Pd(OAc)₂, Pd(MeCN)₂Cl₂, or Pd(PPh₃)₂Cl₂

enabling real-time monitoring of palladium species (Scheme 8a). The reaction of the probe with palladium was accompanied by a color change from colorless to jade green, thereby enabling the effective detection of palladium in environmental samples. The probe's mechanism involves the reduction of Pd^{II} complexes to the active Pd⁰ state by NaBH₄, with morpholine serving as the nucleophile to trap the allyl group [71]. A homogeneous THF/buffer (1:1) solvent system was employed by Wang's group, which developed a colorimetric and fluorescent turn-on probe, DER-1, derived from *N,N*-diethylrhodol (a "turn-on" probe refers to a molecular sensor that transitions from a nonfluorescent state to a fluorescent state). This probe undergoes cleavage via Tsuji–Trost *O*-deallyloxycarbonylation, enabling a detection limit of 1.14 nM with a color shift from yellow to pink at 37 °C in 60 min. Its pH stability makes it particularly suitable for environmental and industrial monitoring of Pd⁰ species (Scheme 8b) [53]. DMF can also be utilized in aqueous Tsuji–Trost deallylation reactions. For instance, the *N*-allyl protecting group of the ChromenA probe was successfully cleaved by PdCl₂, which can serve as a precatalyst in a DMF/H₂O (9:1) solvent system, yielding the corresponding deallylated product in 1 h. (Scheme 8c) [122]. Besides,

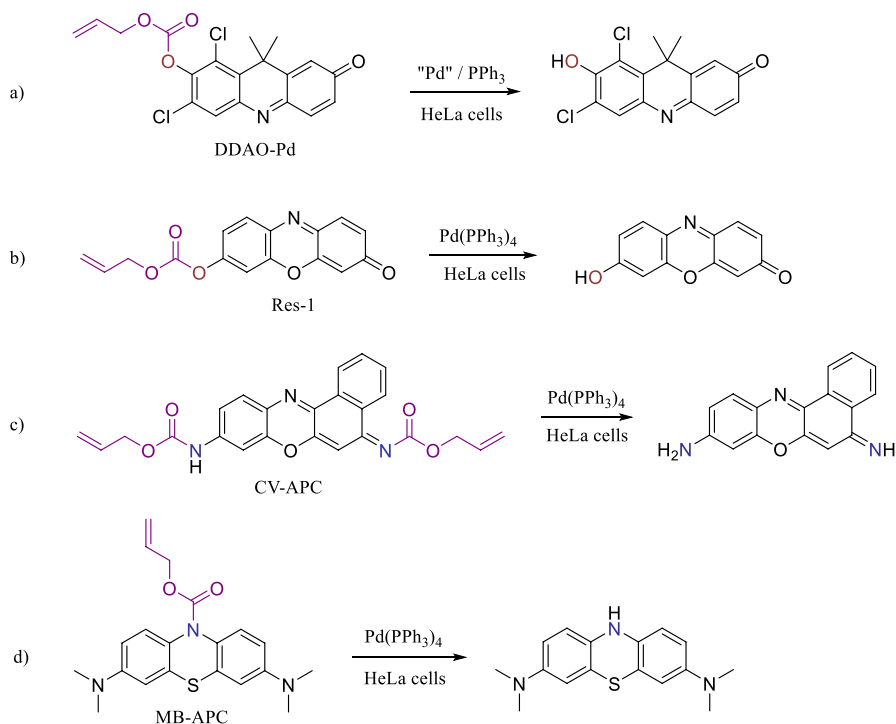
Monflier's group used $\text{Pd}(\text{OAc})_2$ and TPPTS in a heptane/ H_2O (13:7) biphasic system, with diethylamine as an allyl scavenger. They reported the utilization of the catalyst as a highly efficient and homogeneous Tsuji–Trost catalyst system, achieving up to 100% conversion for undecyl allyl carbonate with randomly methylated β -cyclodextrin as a mass-transfer promoter ($n=10$, Scheme 8d). Nuclear magnetic resonance (NMR) studies confirmed homogeneous catalysis without Pd leaching, with vigorous stirring (1000 rpm) ensuring interfacial activity [64, 65].

The use of various organic co-solvents, including DMSO, MeCN, DMF, THF, and heptane, has significantly documented the impact of these solvents on the efficiency of water-compatible Tsuji–Trost-type deprotection reactions. However, the reliance on high percentages of organic solvents raises concerns regarding biocompatibility, particularly for applications within cellular environments. Such solvent conditions are generally incompatible with living systems, thereby limiting the potential use of the Tsuji–Trost reaction in biological settings. Importantly, solvent choice could be often constrained by substrate solubility or the tuning of catalyst ligands, rather than inherent reaction limitations. In the forthcoming section, we delve into reactions that are not only effective in the presence of water but also compatible with cellular environments.

4.2 Intracellular Uncaging: Tsuji–Trost-Type Deprotection Reactions in Cellular Environments

In this section, the selective cleavage of various Pd-labile protecting groups, including *O*-allyl, *O/N*-allyloxycarbonyl, *O/N*-propargyl, *N*-propargyloxycarbonyl, and *O*-allenyl, is examined within living cells, such as HeLa, HepG 2, SiHa, CHO, and HEK293T cells. Most of the protected compounds described herein have been developed to be probes for palladium detection or prodrugs. Despite the efficiency of palladium catalysis, palladium's toxicity necessitates strict regulatory limits (5–10 ppm in drugs, < 1.5–15 μg daily intake) to mitigate cellular damage [154].

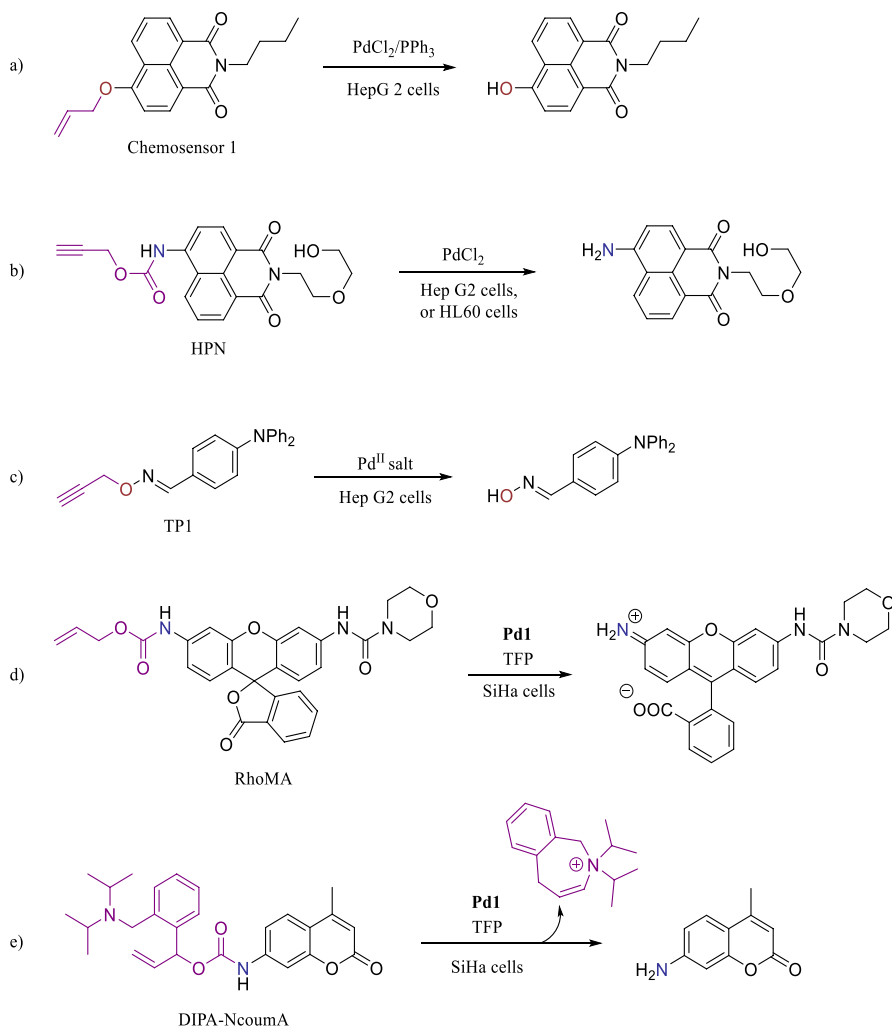
Feng's group developed a colorimetric and near-infrared fluorescent turn-on probe based on a Tsuji–Trost deallylation reaction in living cells. The designed probe, DDAO-Pd, a dimethylacridin derivative, was initially tested in PBS buffer with 0 or 20% DMSO for a homogenous system. Its allyl carbonate moiety was cleaved by various palladium sources such as PdCl_2 , Na_2PdCl_4 , $\text{Pd}(\text{OAc})_2$, and Na_2PdCl_6 with a 2.2 nM detection limit. Its low cytotoxicity (above 90% cell viability) and selective imaging of intracellular Pd in HeLa cells underscore its biocompatibility (Scheme 9a) [55]. Lei Zhang's group introduced Res-1, a resorufin-derived fluorescent probe for intracellular palladium detection with good sensitivity (a 2.1 nM detection limit), leveraging the removal of an allyl carbonate group. In living cells, Res-1 exhibited a rapid color change from pale yellow to pink and a fluorescence response to $\text{Pd}(\text{PPh}_3)_4$. Indeed, the Tsuji–Trost *O*-deallyloxycarbonylation of Res-1 was completed within 30 min in HeLa cells, demonstrating its potential as a fast, low-cytotoxicity, and reliable tool for Pd^0 species analysis in biological systems (Scheme 9b) [54]. Additionally, the same group developed a dual-signal cresyl violet probe, CV-APC, for the sensitive and specific detection of palladium



Scheme 9 Pd-catalyzed *O/N*-deallyloxycarbonylation of acridine, resorufin, and phenothiazine derivatives in living cells. Pd = PdCl_2 , Na_2PdCl_4 , $\text{Pd}(\text{OAc})_2$, or Na_2PdCl_6

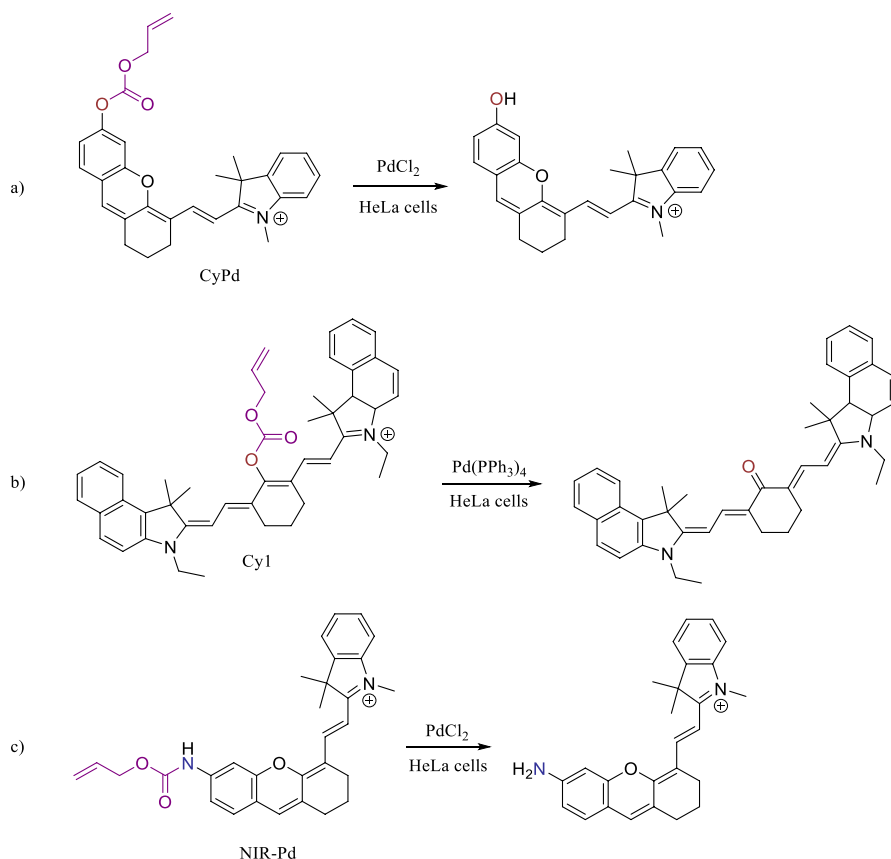
with a 0.78 nM limit in 80 min reaction time. The probe exhibits low cytotoxicity (above 90% HeLa cell viability at 50 μM) and enables selective intracellular Pd imaging through a biocompatible Tsuji–Trost *N*-deallyloxycarbonylation reaction (Scheme 9c) [56]. Lei Zhang and co-workers developed a methylene blue-based chemosensor (MB-APC) that detects intracellular Pd^0 with a 5.7 nM detection limit via Tsuji–Trost cleavage of its allyl carbamate group. (Scheme 9d) The probe exhibits negligible cytotoxicity (above 90% HeLa cell viability at 100 μM) and enables selective Pd^0 imaging in living cells [120].

Xiaoling Zhang's group developed a sensitive 4-hydroxynaphthalimide caged probe (Chemosensor 1) for the detection of palladium at low concentration (5.7 nM) in living cells (Scheme 10a) [66]. Additionally, Liu's group developed a water-soluble fluorescent naphthalimide-derived probe (HPN) featuring a propargyl carbamate protecting group, whose removal can be catalyzed by palladium complexes in various oxidation states (Pd^0 , Pd^{II} , Pd^{IV}) in PBS aqueous media. The palladium catalyst demonstrated high catalytic efficiency with a detection limit of 25 nM (2.7 $\mu\text{g/L}$). This probe was successfully applied for the fluorescent imaging of Pd^{II} complexes in HepG or HL60 cells (Scheme 10b) [155]. Subsequently, Yin's group developed another probe, TP1, a triphenylamine analogue substituted with an *O*-propargyl oxime moiety, enabling the imaging of Pd^{II} complexes without interfering with cellular functions. It displayed catalytic efficiency, with a rapid



Scheme 10 Pd-catalyzed *O/N*-deallyloxycarbonylation or *O*-depropargylation of naphthalimide-, triphenylamine-, rhodamine-, and coumarin-derived probes in Hep G2 cells or SiHa cells

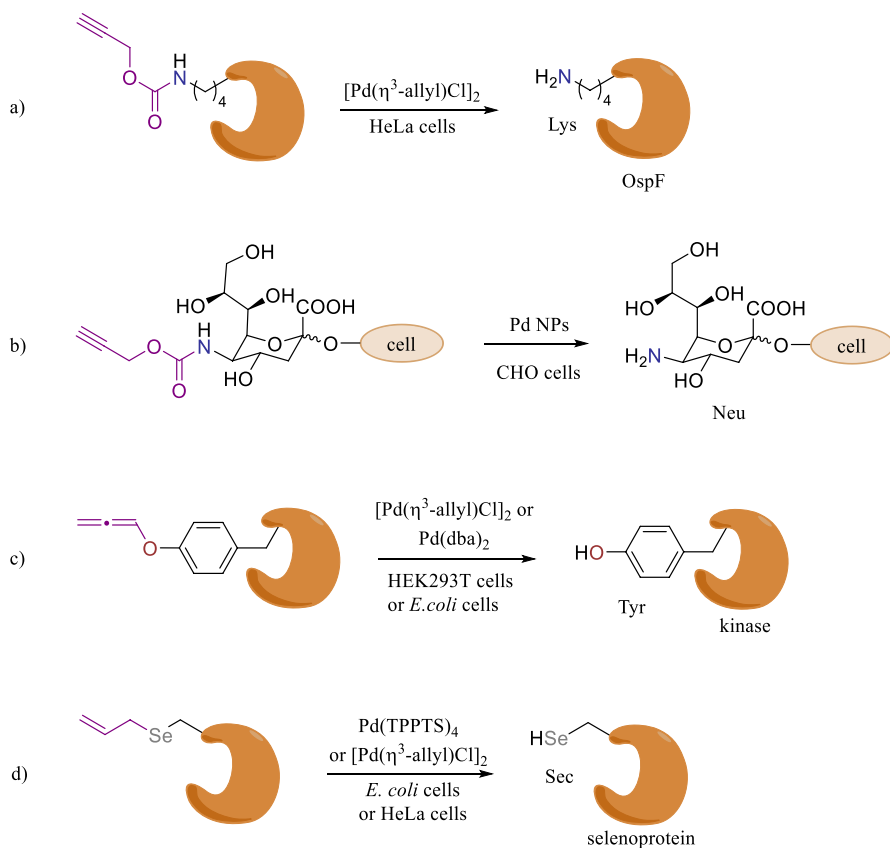
response time of 80 s and a detection limit of 0.47 μM for Pd^{II} (Scheme 10c) [156]. In the field of biocompatible Tsuji–Trost-type deprotection reactions, Riant's group investigated the catalytic properties of a diverse array of palladium complexes bearing acetanilide ligands in SiHa cells, with a specific emphasis on the *N*-deallyloxycarbonylation of the rhodamine-derived RhoMA probe. **Pd1** emerged as a promising candidate, exhibiting notable catalytic efficiency and a second-order reaction rate of $30 \text{ M}^{-1} \text{ s}^{-1}$ in aqueous media (Scheme 10d) [77]. Subsequently, the same group introduced a coumarin-derived probe (DIPA-NcoumA), characterized by its water solubility and the presence of an internal nucleophilic group. DIPA-NcoumA ($k_2 = 5.74 \text{ M}^{-1} \text{ s}^{-1}$) demonstrated good performance compared with the conventional



Scheme 11 Pd-catalyzed *O/N*-deallyloxycarbonylation of water-soluble cyanine and hemicyanine probes in HeLa cells

probe *N*-Alloc-7-amino-4-methylcoumarin (NcoumA, $k_2 = 0.42 \text{ M}^{-1} \text{ s}^{-1}$), yielding a faster cleavage rate in aqueous media in the presence of the same palladium complex, **Pd1** (Scheme 10e) [115].

The xanthene–hemicyanine hybrid CyPd, featuring an allyl carbonate protecting group, can undergo *N*-deallyloxycarbonylation catalyzed by Pd⁰, Pd^{II}, or Pd^{IV} complexes in vitro. Regarding the effectiveness of these palladium species, the kinetic profiles of the deprotection reaction follows this order: Pd⁰ > Pd^{II} > Pd^{IV}. CyPd was applied for the visualization of various palladium in HeLa cells, such as PdCl₂ (2–10 μM, above 24 h viability, low toxicity), demonstrating its capability for biological imaging (Scheme 11a) [84]. Subsequently, Zhu's group reported the use of Pd(PPh₃)₄ to catalyze the cleavage of the allyl carbonate moiety of a modified cyanine dye (Cy1) with a detection limit of 0.3 ppb (1.7 nM) and rapid response (20 min) in aqueous MeCN/PBS (1:3, pH 7.4). Though cytotoxicity was not explicitly quantified, the probe's successful application in HeLa cell imaging suggested biocompatibility at tested concentrations (Scheme 11b) [57]. Moreover, Zhao and



Scheme 12 Cleavage of *N*-propargyloxycarbonyl, *O*-allenyl, or *Se*-allyl groups from proteins or oligosaccharides within *E. coli* or HeLa cells

co-workers designed a *N*-Alloc-protected 3-aminoxanthene–hemicyanine derivative (NIR-Pd) as a near-infrared fluorescent probe, enabling the direct visualization of Pd^{II} complexes in HeLa cells. The NIR-Pd probe achieved Pd^{II} detection within 6 min and a low detection limit of 134.6 nM (Scheme 11c) [123].

In 2014, Chen's group identified biocompatible and efficient palladium catalysts capable of catalyzing the cleavage of the propargyl carbamate group from a protected lysine residue (Lys) of the OspF phosphothreonine lyase, thereby releasing a free lysine (Scheme 12a) [15]. Notably, the identified palladium species, particularly Pd(dba)_3 and allyl- Pd^{II} chloride dimer, exhibited remarkable catalytic turnover, achieving above 80% conversion in vitro with minimal catalyst loading (10 mol%), underscoring their potential for intracellular applications. Besides, the protected lysine residue can be genetically incorporated into proteins at specific sites, providing precise control over the reaction site. Building upon this work, in 2015, the same research group reported another chemical uncaging strategy for the in situ generation neuraminic acid (Neu) on the surface of live cells. They discovered that Pd NPs

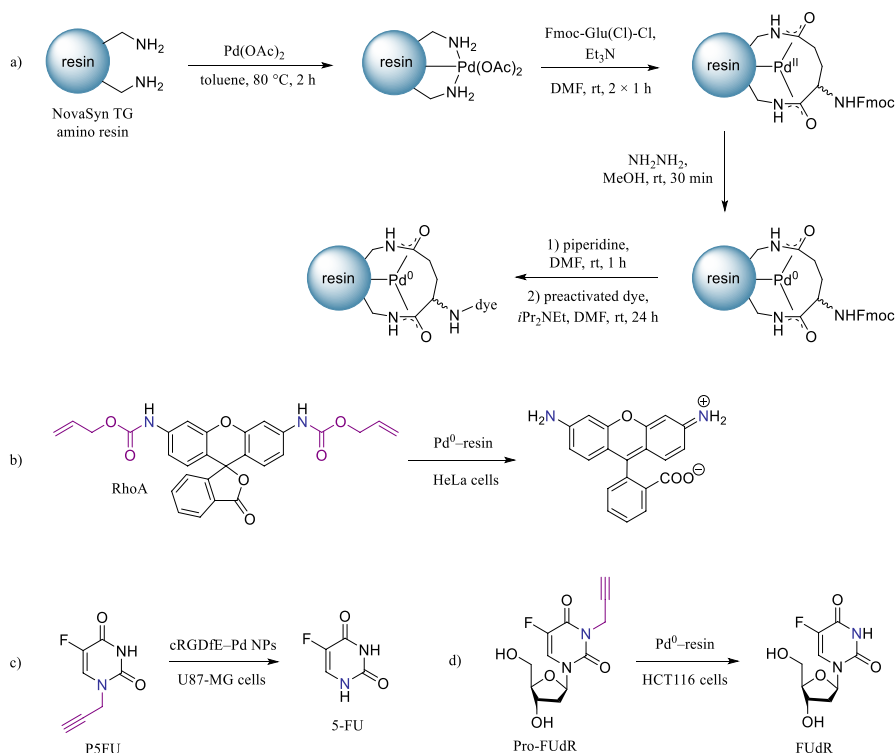
serve as highly efficient and biocompatible catalysts for depropargylation, triggering the conversion of metabolically incorporated *N*-(propargyloxycarbonyl)neuraminic acid (Neu5Proc) into Neu on cell-surface glycans with above 95% yield and rapid kinetics (less than 4 min) (Scheme 12b) [46]. Continuing their research, in 2016, they introduced a Pd-labile allene-based protecting group for the bioorthogonal activation of tyrosine-dependent proteins in live cells (Scheme 12c) [49]. In 2018, Wang's group reported that an incorporated *Se*-allyl-selenocysteine residue within a selenoprotein can be converted to selenocysteine (Sec) using Pd(TPPTS)₄ or [Pd(η^3 -allyl)Cl]₂ with above 93% conversion under mild conditions. The reaction proceeded homogeneously in both *Escherichia coli* and HeLa cells, with minimal toxicity when supplemented with L-cysteine, enabling live-cell applications such as selective Sec labeling and protein cross-linking (Scheme 12d) [157].

Building on these findings, the Tsuji–Trost reaction provides an efficient strategy for the deprotection of sugar and amino acid residues within biological macromolecules. Most applications of Tsuji–Trost-type deprotection reactions in living cells to date involve the use of discrete complexes, such as **Pd1**, PdCl₂, Pd(PPh₃)₄, and [Pd(η^3 -allyl)Cl]₂. However, the development of palladium nanostructures and meso-structures to address potential stability issues has also progressed significantly.

In 2011, Bradley's group described an electron-rich microsphere network capable of capturing Pd^{II} ions through coordination with available amino groups and aromatic rings. Meanwhile, the reduction of Pd^{II} to Pd⁰ species results in the aggregation of these ions, culminating in the formation of Pd⁰–resins (Scheme 13a) [13]. These Pd⁰–resin were incubated with a *N,N'*-bis-Alloc-protected rhodamine derivative (RhoA), which could penetrate HeLa cells and undergo Tsuji–Trost *N*-deallyloxycarbonylation. The heterogeneous system showed high efficiency (turnover numbers of about 30) and low cytotoxicity (above 91% viability), enabled by the solid-phase confinement of Pd⁰ (Scheme 13b).

Later, the same group reported that Pd-loaded particles functionalized with a cancer-targeting cRGDfE cyclopeptide (cRGDfE-Pd NPs) were rapidly and selectively internalized by glioblastoma U87-MG cells via receptor-mediated endocytosis. The cRGDfE-Pd NPs efficiently converted the prodrug 5-fluoro-1-propargyl-uracil (P5FU, 250 μ M) into cytotoxic 5-fluorouracil (5FU) with full conversion in 24 h (25 μ M Pd, PBS), demonstrating robust turnover under physiological conditions. Additionally, cRGDfE-Pd NPs effectively activated P5FU within glioblastoma cells, resulting in an increased rate of cell death compared to individual treatments (Scheme 13c) [21]. Furthermore, Unciti-Broceta and co-workers used similar Pd⁰-functionalized polystyrene–polyethylene glycol resins (Pd⁰ resins, 0.67 mg/mL) to uncage a bioorthogonal Pd-labile prodrug, *N*-propargyl-floxuridine (Pro-FUDR, 30 μ M), via Tsuji–Trost-type depropargylation in HCT116 cells (Scheme 13d). This prodrug exhibited significantly lower cytotoxicity compared to floxuridine (FUDR) [47].

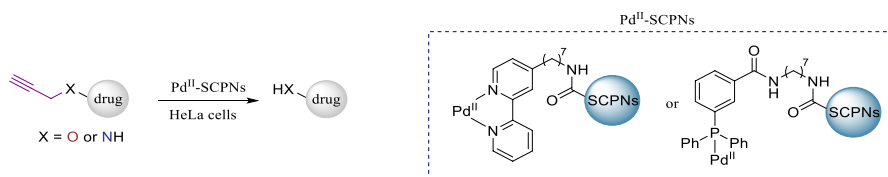
Other examples of Pd-mediated prodrug activation were reported by Palmans' group, who demonstrated the use of Pd^{II}-based single-chain polymeric nanoparticles (SCPNs) as efficient bioorthogonal catalysts for Tsuji–Trost-type depropargylation reactions. They developed a polyacrylamide-based SCPN system that encapsulates a Pd^{II} catalyst, preventing the loss of Pd^{II} species from the hydrophobic interior and



Scheme 13 Pd-catalyzed *N*-deallyloxycarbonylation and *N*-depropargylation in living cells by Bradley's group. **a** Synthesis of Pd⁰ microspheres with fluorescent tags; **b** Pd-mediated *N*-deallyloxycarbonylation of RhoA in HeLa cells; **c** Pd-catalyzed *N*-depropargylation of P5FU in U87-MG cells. **d** Pd-mediated *N*-depropargylation of Pro-FUdR in HCT116 cells

minimizing off-target toxicities. SCPNs also accelerated the depropargylation reaction continuously, likely due to hydrophilic product partitioning preventing inhibition or nanoparticle-stabilized Pd⁰-sustaining catalysis. Importantly, no significant difference in catalytic activity was observed between experiments conducted in cells and in Dulbecco's modified Eagle medium (DMEM) alone, suggesting the preservation of catalytic performance in cellular media. Catalytic SCPNs activated anticancer prodrugs of 5FU, paclitaxel, and doxorubicin (Dox) (Scheme 14) [45, 158].

Pd-peptide complexes are notable for their biocompatibility, water solubility, and efficient cell entry. Peptides can stabilize palladium, preventing cellular deactivation, while arginine-rich sequences enhance uptake. Additionally, bis-histidine stapling enhances structural stability and catalytic activity. These features enable intracellular reactions with minimal cytotoxicity, making Pd-peptide complexes promising tools for bioorthogonal applications in living systems. For example, a pentapeptide (His-Arg-Gly-Asp-His) featuring two histidine residues (His) at *i* and *i* + 4 positions was engineered to efficiently coordinate with various Pd^{II} salts, such as Pd(COD)Cl₂, leading to the formation of the stapled derivative **Pd2**. **Pd2** was shown to

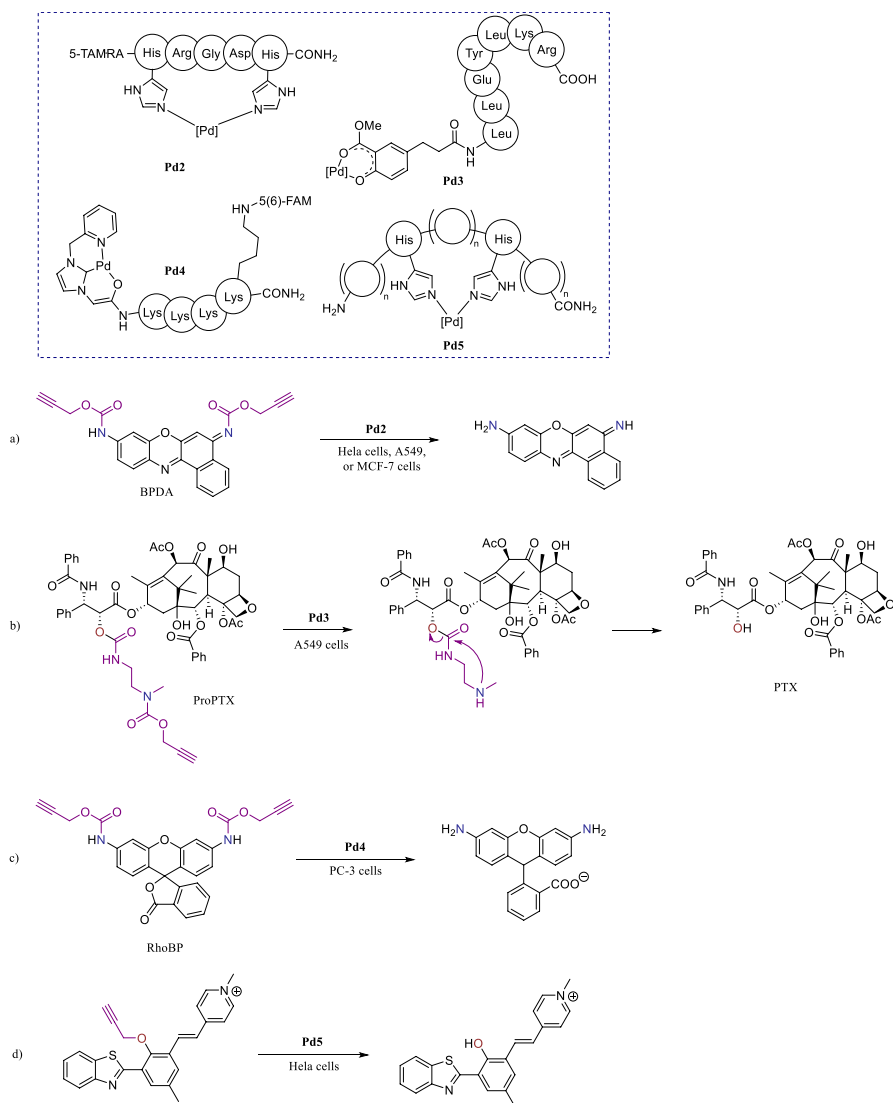


Scheme 14 *O/N*-depropargylation catalyzed by Pd^{II}-SCPNS

catalyze the depropargyloxycarbonylation of a *N,N'*-bis-propargyloxycarbonyl dihydroacridine derivative (BPDA) in HeLa, A549, and MCF-7 cells (Scheme 15a) [68]. **Pd2** (10 μM) fully activates BPDA (50 μM) within 1 h, as confirmed by far-red fluorescence, which demonstrated its catalytic efficiency. Recently, Rappsilber's group developed a methyl salicylate-palladium heptapeptidic complex **Pd3**, which displayed pseudo-first-order kinetics ($k=0.2175 \pm 0.03 \text{ h}^{-1}$) and a half-life of 3.19 h. The catalyst could activate a paclitaxel prodrug (ProPTX) in A549 lung cancer cells. Following Pd-catalyzed *N*-depropargyloxycarbonylation, a self-immolative mechanism is triggered via intramolecular aminolysis of a second carbamate group, ultimately leading to the release of paclitaxel (PTX) (Scheme 15b) [80]. Besides, Bradley et al. demonstrated a homogeneous cell-penetrating peptide-conjugated Pd^{II}-NHC complex (**Pd4**), which incorporates a fluorescent tag and could catalyze the cleavage of *N,N'*-bis(propargyloxycarbonyl)-protected rhodamine 110 (RhoBP) within 6 h. This palladium complex showed minimal cytotoxicity (cell viability > 85% at 0.5 μM **Pd4**) (Scheme 15c) [81]. Recently, Vázquez, Mascareñas, and co-workers designed a class of β -sheet peptide-stabilized Pd complexes (**Pd5**). Compared to helical metalloprotein scaffolds, these β -sheet systems feature computer-guided design versatility, enhanced metal protection, and prolonged catalytic activity (Scheme 15d) [82, 83].

Moreover, Bernardes and colleagues developed a bioorthogonal decaging strategy for the precise activation of caged Dox in MCF-7 cells by designing a stable antibody–drug conjugate, which links Dox to a cysteine residue in a HER2-targeting nanobody. The release of Dox was achieved via Pd(COD)Cl₂-mediated cleavage of a thioether propargyl carbamate linker (Scheme 16a) [69]. Subsequently, the same group employed a combination of Na₂PdCl₄ and sodium ascorbate for Tsuji–Trost-type deprotection within SKBR3 cells, enabling the release of bioactive molecules such as duocarmycin (Duo) and Dox from caged *O*-allyl (Scheme 16b) and *N*-propargyloxycarbonyl (Scheme 16c) precursors, respectively [67]. The in situ reduction of Pd^{II} to Pd⁰ by ascorbate afforded catalytic turnover numbers comparable to homogeneous systems, while heterogeneous Pd⁰ nanoparticles mitigated cytotoxicity (viability > 80% at $\leq 50 \mu\text{M}$ Pd). This strategy highlights the potential of ligand-free Pd⁰ generation for spatially controlled prodrug activation, albeit with trade-offs in reaction homogeneity and precise mechanistic delineation.

Although Tsuji–Trost uncaging has proven effective in various cellular models, adapting this chemistry for in vivo applications introduces new complexities.

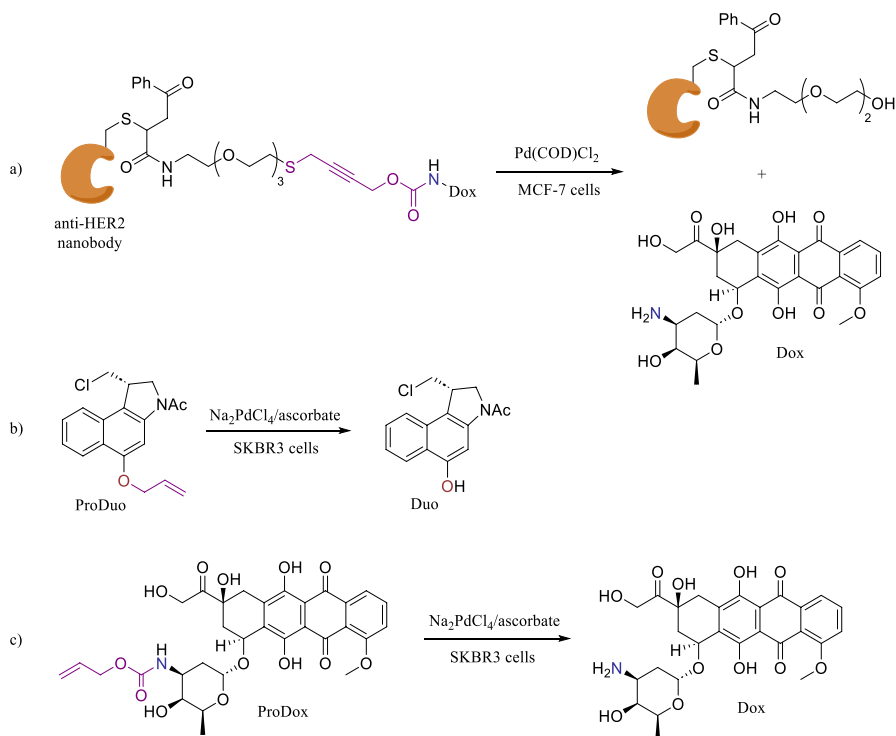


Scheme 15 *N*-depropargyloxycarbonylation and *O*-depropargylation mediated by Pd-peptide complexes in living cells

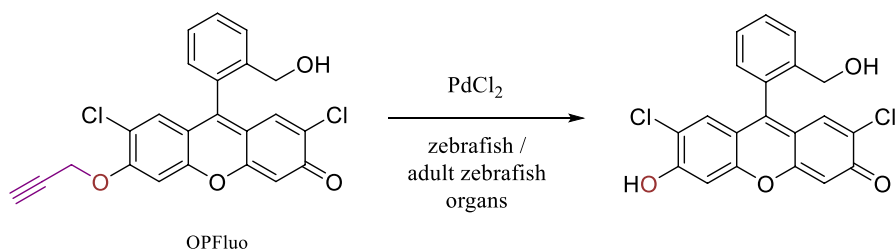
However, recent innovations in catalyst design and delivery strategies are paving the way for its potential use in living organisms.

4.3 In Vivo Uncaging: Tsuji-Trost-Type Deprotection Reactions in Animal Models

The use of palladium species in cellular environments sets the stage for exploring their applications in more complex biological systems. This has led to the topic of



Scheme 16 Intracellular cleavage of propargyl carbamate, allyl ether, and allyl carbamate moieties by Bernardes' group



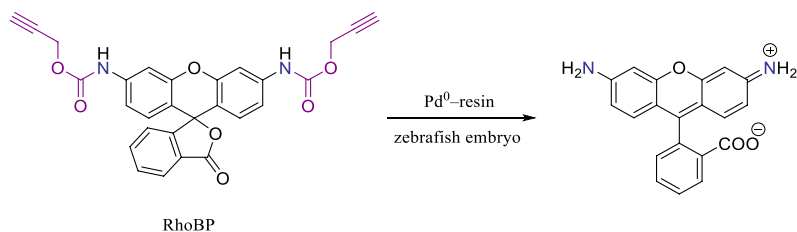
Scheme 17 Cleavage of *O*-propargyl-protected probe OPFluo in zebrafish and adult zebrafish organs

in vivo deprotection, where the Tsuji–Trost reaction is utilized in animal models, further demonstrating the versatility and potential of Pd-mediated reactions in a therapeutic potential.

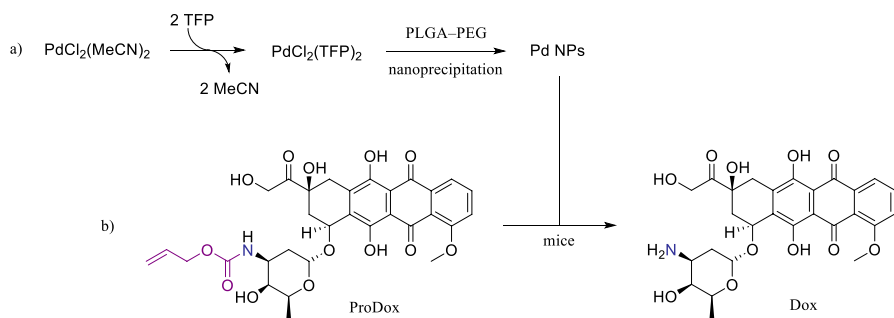
In 2010, Ahn's group reported the first in vivo application of the Tsuji–Trost-type depropargylation reaction in zebrafish and adult zebrafish organs (Scheme 17) [48]. Their approach involved an *O*-propargylated fluorescein derivative (OPFluo), which proved to be a highly specific turn-on fluorescent probe for detecting palladium species in all common oxidation states (Pd^0 , Pd^{II} , and

Pd^{IV}). In zebrafish, the probe selectively responds to Pd^{II} with a detection limit of $0.03 \mu\text{M}$ ($3.2 \mu\text{g L}^{-1} \text{Pd}$) and visualizes accumulated palladium in organs like the brain, eye, and fin after 24 h exposure to 500 nM PdCl_2 . Then, in 2014, Unciti-Broceta's group employed a Pd^0 resin to cleave *N,N'*-bis(propargyloxycarbonyl)-protected rhodamine 110 (RhoBP) in zebrafish (Scheme 18) [43].

Weissleder's group also demonstrated that nanoencapsulated palladium can effectively target mice tumors, enabling localized deprotection reactions within the tumor microenvironment [14]. Their research highlighted that nanoencapsulation with poly(lactic-*co*-glycolic acid)–polyethyleneglycol (PLGA–PEG) to form Pd NPs significantly boosted the bioavailability of $\text{PdCl}_2(\text{TFP})_2$ (Scheme 19a). These Pd NPs



Scheme 18 Pd^0 -resin-mediated *N*-depropargyloxycarbonylation of RhoBP in zebrafish embryo

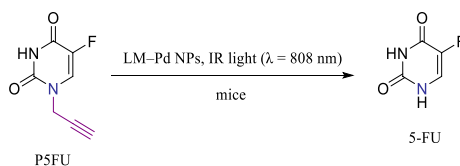


Scheme 19 Prodrug activation mediated by a nanoencapsulated Pd catalyst in tumor-bearing mice

were found to activate efficiently ProDox via the cleavage of its allyl carbamate moiety (Scheme 19b). More importantly, the combination of ProDox and Pd NPs was shown to cause significant accumulation of active Dox in the nuclei of the studied cancer cell lines, but not in the nuclei of the liver, kidney and heart. Overall, the group's findings indicated that *in vivo* Pd NP catalysis could be a potent, safe, and localized treatment strategy that inhibits tumor growth and extends survival in orthotopic tumor models.

Recently, Qu's group investigated the photothermal effects of liquid metal palladium nanoparticles (LM–Pd NPs) for *N*-depropargylation at tumor sites under 808 nm laser irradiation (Scheme 20). They demonstrated that the LM–Pd NPs catalyst exhibited high bioorthogonal catalytic activity in aqueous conditions, achieving a 99% conversion yield within 20 min. LM–Pd NPs also enabled a rapid temperature

Scheme 20 *N*-depropargylation of P5FU using a combination of liquid metal Pd nanoparticles and laser irradiation ($\lambda = 808$ nm)



increases at mice tumor sites, with *in vivo* evaluation of tumor response to rapid temperature increases at the tumor site, surpassing that achieved by laser irradiation alone. Therefore, they speculated that LM-Pd NPs, embedded in eutectic gallium–indium liquid metal, could absorb and scatter light, generating excited hot electrons and enabling photothermal conversion under infrared irradiation. Subsequently, experiments involving seven groups of mice, including controls and various treatment combinations, revealed that neither P5FU nor the palladium catalyst with laser had a significant impact on tumor growth. However, the combination of the catalyst with P5FU under 808 nm laser irradiation demonstrated enhanced tumor inhibition [159].

Table 1 provides an overview of the reaction conditions that have been reported for Tsuji–Trost-type deprotection, from *in vitro* experiments to living systems. While deprotection has been successfully adapted to cellular environments, studies investigating *in vivo* Tsuji–Trost deallylation remain scarce, likely due to toxicity concerns, delivery challenges, or biological interference. Nonetheless, these studies represent an important step toward the application of Tsuji–Trost-type deprotection reactions in animal models. As described above, palladium nanoparticles have been the predominant catalysts for *in vivo* Tsuji–Trost-type deprotection to date. This highlights their advantages over discrete palladium sources, including the stabilization of Pd^0 species trapped within microspheres [14], tumor targeting via the enhanced permeability and retention (EPR) effect [160], and reduced toxicity [46], among other benefits.

5 Tsuji–Trost Allylation in Aqueous Media

In the realm of bioorthogonal chemistry, the Tsuji–Trost deallylation reaction has garnered considerable attention, while Tsuji–Trost allylation reactions remain relatively underexplored. Notably, Tsuji–Trost allylation has yet to be demonstrated in living cells, and *in vivo* applications remain unrealized. In this section, the current state of Tsuji–Trost allylation reactions is described, with an emphasis on their implementation in aqueous media, at room temperature or 37 °C, and in the presence of air. The aim is to highlight recent progress and persisting challenges in this field, thereby promoting further advancements in biocompatible Tsuji–Trost reactions.

Table 1 Tsuji–Trost-type deprotection from test tubes to living settings

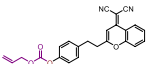
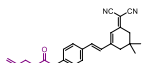
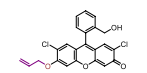
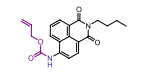
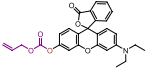
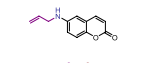
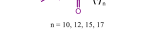
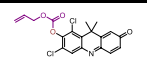
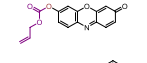
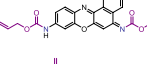
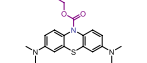
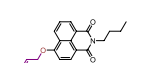
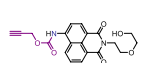
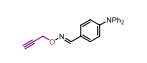
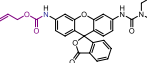
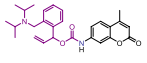
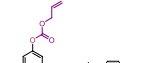
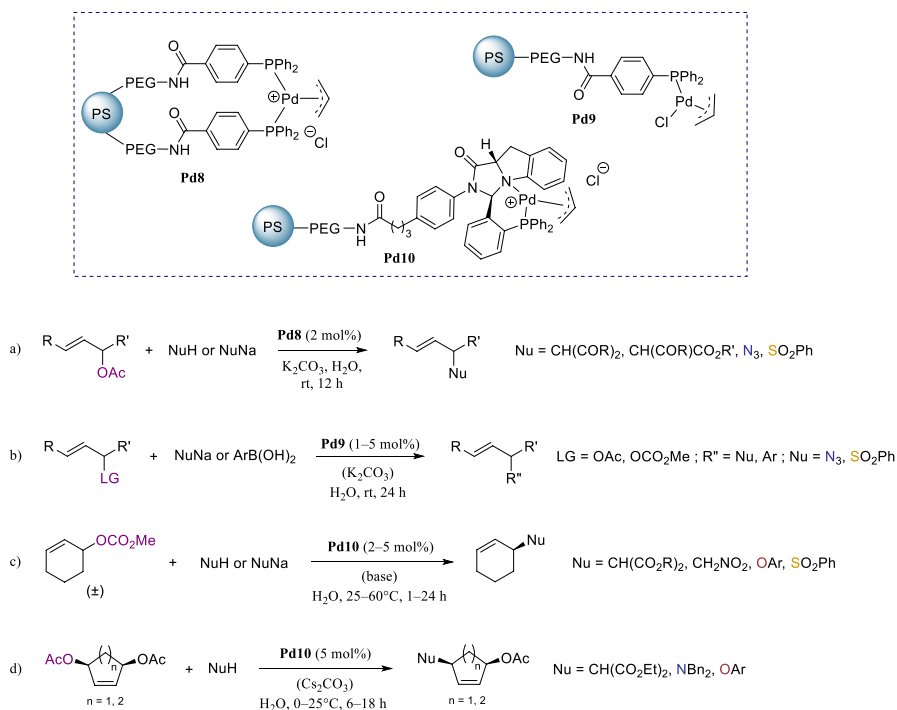
Caged compounds	Reaction conditions	Products	Applications
Deprotection in test tubes			
	Pd(PPh ₃) ₄ , DMSO/PBS (9:1, v/v)	ArOH	Probe for palladium detection [121]
	Pd(PPh ₃) ₄ , DMSO/PBS (1:1, v/v)	ArNH ₂	Probe for palladium detection [52]
	"Pd"/NaBH ₄ /TFP, DMSO/pH 7 buffer (5:95, v/v) "Pd" = PdCl ₂ , Pd(OAc) ₂ , Pd(PPh ₃) ₄ , Pd(MeCN) ₂ Cl ₂ , Pd(acac) ₃ , Pd(PPh ₃) ₂ Cl ₂ , Pd(MeCN) ₂ , Pd ₂ (dba) ₃ , or K ₂ PdCl ₆	ArOH	Probe for palladium detection [74]
	"Pd"/NaBH ₄ /PPh ₃ , MeCN/H ₂ O (4:1, v/v) "Pd" = Pd(PPh ₃) ₄ , PdCl ₂ , Pd(OAc) ₂ , Pd(MeCN) ₂ Cl ₂ , or Pd(PPh ₃) ₂ Cl ₂	ArNH ₂	Probe for palladium detection [71]
	Pd(PPh ₃) ₄ , THF/PBS (1:1, v/v)	ArOH	Probe for palladium detection [53]
	PdCl ₂ , Cu(acac) ₂ ·H ₂ O, LiCl, DMF/H ₂ O (9:1, v/v)	ArNH ₂	Synthesis [122]
	Pd(OAc) ₂ /TPPTS, Et ₃ NH, heptane/H ₂ O (13:7, v/v)	ROH	Development of cyclodextrin-based biphasic catalytic systems [64, 65]
Deprotection in cellular environments			
	PdCl ₂ , Na ₂ PdCl ₆ , Pd(OAc) ₂ , or Na ₂ PdCl ₆ /PPh ₃ , HeLa cells	ArOH	Fluorescent probe [55]
	Pd(PPh ₃) ₄ , HeLa cells	ArOH	Fluorescent probe [54]
	Pd(PPh ₃) ₄ , HeLa cells	ArNH ₂	Fluorescent probe [56]
	Pd(PPh ₃) ₄ , HeLa cells	Ar ₂ NH	Fluorescent probe [66]
	PdCl ₂ /PPh ₃ , Hep G2 cells	ArOH	Fluorescent probe [120]
	PdCl ₂ , Hep G2 or HeLa cells	ArNH ₂	Fluorescent probe [155]
	Pd ⁰ , Hep G2 cells	ArCH=NOH	Fluorescent probe [156]
	Pd ⁰ /TFP, SiHa cells	ArNH ₂	Fluorescent probe [77]
	Pd ⁰ /TFP, SiHa cells	ArNH ₂	Fluorescent probe [115]
	PdCl ₂ , HeLa cells	ArOH	Fluorescent probe [84]

Table 1 (continued)

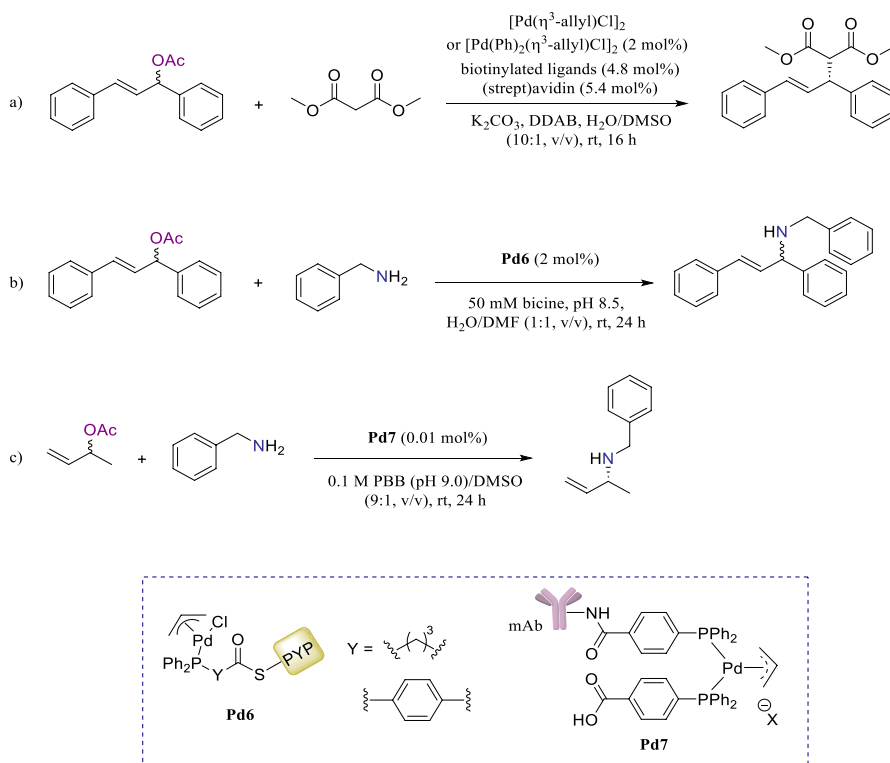
	$\text{Pd}(\text{PPh}_3)_4$, HeLa cells	ROH	Fluorescent probe [57]
	PdCl_2 , HeLa cells	ArNH_2	Fluorescent probe [123]
	$[\text{Pd}(\eta^3\text{-allyl})\text{Cl}]_2$, HeLa cells	RNH_2	Protein activation [15]
	Pd NPs, BJA-B K20 cells	RNH_2	<i>In situ</i> unmasking of neuraminic acid on cell- surface glycans [46]
	$[\text{Pd}(\eta^3\text{-allyl})\text{Cl}]_2$ or $\text{Pd}(\text{dba})_2$, HEK293 cells, or <i>E. coli</i>	ArOH	Protein activation [49]
	$\text{Pd}(\text{TPPTS})_4$ or $[\text{Pd}(\eta^3\text{-allyl})\text{Cl}]_2$, <i>E. coli</i> or HeLa cells	R_5cH	Protein modification [57]
	Pd^0 -resin, HeLa cells	ArNH_2	Fluorescent probe [13]
	cRGDfE-Pd NPs, U87-MG cells	RNHR'	Prodrug [21]
	Pd^0 -resins, HCT116 cells	RNHR'	Prodrug [47]
	PdCl_2 , Na_2PdCl_4 , $\text{Pd}(\text{OAc})_2$, or Na_2PdCl_6 , HeLa cells	ArOH or ArNH_2	Prodrug [45, 158]
	Pd2 , HeLa, A549, or MCF-7 cells	ArNH_2	Fluorescent probe [68]
	Pd3 , A549 cells	ROH	Prodrug [80]
	Pd4 , PC-3 cells	ArNH_2	Fluorescent probe [81]
	Pd5 , HeLa cells	ArOH	Fluorescent probe [82, 83]
	$\text{Pd}(\text{COD})\text{Cl}_2$, MCF-7 cells	RNH_2	Prodrug [69]
	Pd^0/TPPTS , SKBR3 cells	ArOH	Prodrug [67]



Scheme 21 PS-PEG resin-anchored phosphine-palladium complexes for allylation reactions in aqueous media

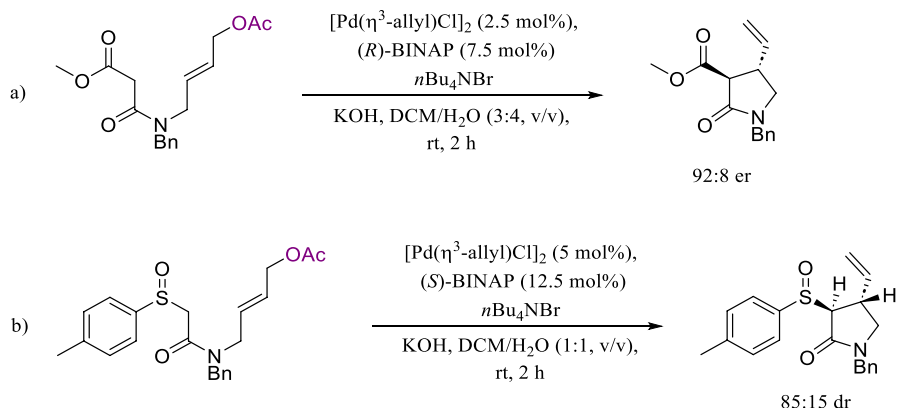
5.1 Asymmetric Tsuji–Trost Allylation in Test Tubes

From the 1990s to the 2010s, Uozumi and co-workers made significant strides in Tsuji–Trost reaction under mild heterogeneous aqueous conditions by designing a variety of novel amphiphilic Pd-phosphine complexes anchored on PS–PEG resins (Scheme 21, **Pd8–Pd10**). **Pd8** proved highly effective in facilitating allylic substitution reactions with a broad range of nucleophiles, including sodium phenylsulfinate, sodium azide, and C-nucleophiles such as β -diketones and β -keto esters. This catalyst system achieved good yield for the Tsuji–Trost product under mild conditions while maintaining full recyclability without any loss of activity over multiple runs (Scheme 21a) [125, 142]. The structurally similar complex **Pd9** also promoted efficient allylic substitutions of primary and secondary allyl acetates or carbonates with arylboronic acids [137], sodium azide [161], and sodium phenylsulfinate [163] at room temperature (Scheme 21 b). Moreover, the chiral catalyst **Pd10** was shown to catalyze aqueous Tsuji–Trost allylation of β -diesters [113], nitromethane [87], phenolic nucleophiles [162], and phenylsulfinate [163] with high enantioselectivity (up to 98% enantiomeric excess, ee) (Scheme 21c). Meanwhile, **Pd10** also proved efficient in enantioselective allylic substitution of meso-1,4-diacetoxycyclopentenes and -cyclohexenes with various nucleophiles, delivering 91–99% ee (Scheme 21d) [141].



Scheme 22 Tsuji–Trost allylation reactions catalyzed by artificial metalloenzymes in aqueous media. PYP, photoactive yellow protein; X, counteranion

Despite their compatibility with aqueous media, these palladium complexes immobilized on PS–PEG resins may not be suitable for cellular environments. Consequently, artificial metalloenzymes that catalyze Tsuji–Trost allylation were developed. In 2008, Ward's group made a significant advancement in the field of artificial biotin–avidin-type metalloenzymes by demonstrating asymmetric allylation in aqueous media containing 10% DMSO. By means of a $[\text{Pd}(\eta^3\text{-Ph}_2\text{allyl})]$ (biotinylated ligand) complex as the catalyst, Pd-catalyzed allylation yielded allylated products with 90% enantiomeric excess (ee), favoring the (*R*)-enantiomer (Scheme 22a) [33]. Subsequently, in 2010, Kamer's group pioneered a cysteine-selective bioconjugation technique to synthesize artificial metalloenzymes capable of catalyzing Tsuji–Trost allylic amination in a DMF/ H_2O mixture. Key to their approach, a *N,N*-carbonyldiimidazole-activated phosphine-carboxylic acid first reacts with $[\text{Pd}(\eta^3\text{-allyl})\text{Cl}]_2$ to form a Pd-phosphine complex. The *N*-acylimidazole moiety subsequently conjugates with the free thiol of photoactive yellow protein (PYP), yielding the artificial metalloenzyme **Pd6**. Their research focused on utilizing water-soluble substrates in allylic amination reactions to

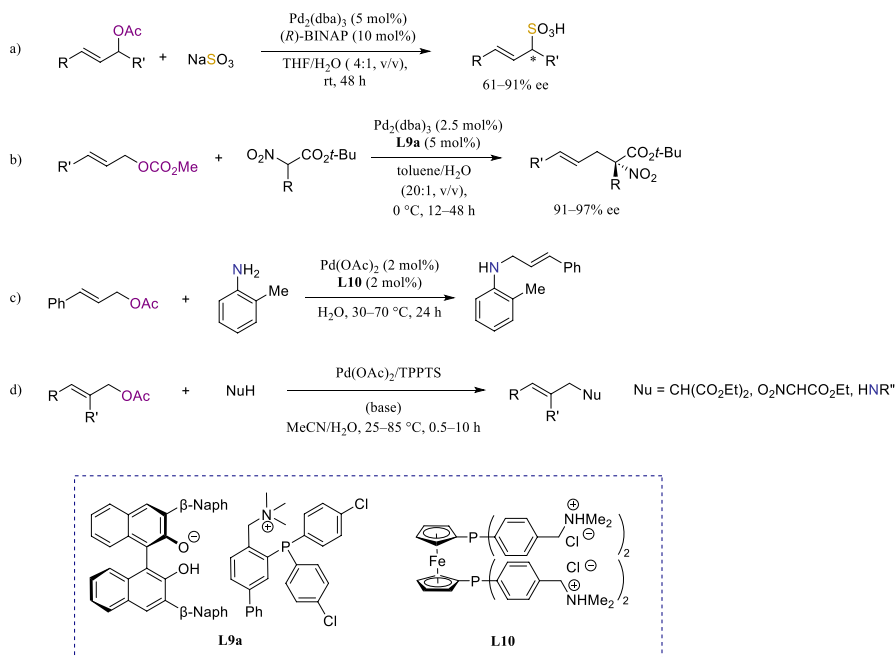


Scheme 23 Asymmetric synthesis of 3,4-disubstituted γ -lactams via intramolecular [Pd(η^3 -allyl)Cl]₂/BINAP-catalyzed Tsuji–Trost reaction

minimize reliance on organic co-solvents, which can cause protein denaturation (Scheme 22b). Unfortunately, no enantiomeric excess was observed in this allylic amination reaction, likely due to the high concentration of organic solvent, which may create a significant spatial separation between the metal active site and the chiral microenvironment of the protein [79]. Recently, Yamaguchi's group developed a monoclonal antibody (mAb) derivatized with a palladium complex (**Pd7**) for enantioselective Tsuji–Trost allylic amination. This novel method, conducted in phosphate borate buffer (PBB)/DMSO (9:1), afforded an allylic amine with a 98% ee (Scheme 22c) [78].

In addition to the progress in artificial metalloenzyme-catalyzed allylation reactions, [Pd(η^3 -allyl)Cl]₂ has also been successfully applied for allylation in aqueous media. In 2009, Poli's group pioneered a highly enantioselective Pd-catalyzed intramolecular allylation procedure in a biphasic DCM/H₂O medium (Scheme 23a). This method enabled the synthesis of enantioenriched 3,4-disubstituted γ -lactams as valuable intermediates for bioactive molecules, with an enantiomeric ratio (er) up to 92:8 [164]. In the following year, the same group reported another asymmetric Pd-catalyzed intramolecular allylation method to access 3-sulfinyl γ -lactams (Scheme 23b). In DCM/H₂O mixtures, the reaction yielded sulfoxide products with up to 85:15 diastereomeric ratio (dr). The combination of BINAP as the ligand and DCM as the co-solvent was identified as the best for achieving high diastereoselectivity [70].

Furthermore, Pd₂(dba)₃ has also been used in aqueous Tsuji–Trost reactions. Zhang's group reported the enantioselective synthesis of allylic sulfonic acids via a Pd₂(dba)₃/(*R*)-BINAP-catalyzed allylic sulfonation reaction, employing sodium sulfite in a THF/H₂O solvent system. The catalyst system delivered chiral allylic sulfonic acids in moderate to good yields (40–92%) with high enantioselectivity (up to 91% ee), demonstrating broad substrate tolerance for electronically diverse aryl groups while requiring water as a critical reaction component (Scheme 24a) [59]. Moreover, Ooi's group designed an original ligand (**L9**) composed of an achiral

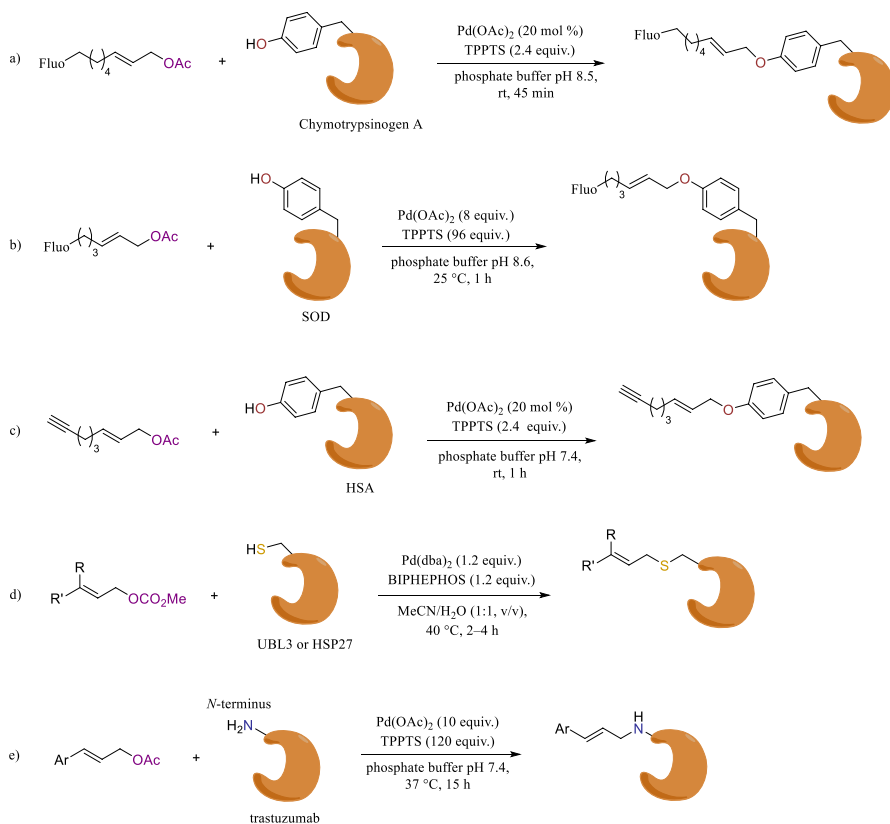


Scheme 24 $\text{Pd}_2(\text{dba})_3$ - and $\text{Pd}(\text{OAc})_2$ -mediated catalyzed Tsuji–Trost allylation in aqueous media

quaternary ammonium–phosphine and a chiral binaphtholate anion, assembled by electrostatic interactions. In combination with $\text{Pd}_2(\text{dba})_3$, one of these ligands (**L9a**) exhibited exceptional stereo-inducing properties in the Tsuji–Trost allylation of α -nitro esters in a toluene/ H_2O mixture. The catalyst system shows α,α -disubstituted α -amino acid precursors in good yields (91–99%) and enantioselectivity (up to 97% ee), with water critically enhancing both reactivity and stereocontrol by facilitating ligand exchange (Scheme 24b) [100]. Furthermore, the same palladium complex enable the C-allylation of benzofuran-2(3*H*)-ones [60, 165], benzo[b]thiophen-2(3*H*)-ones [166]. Meanwhile, **L10** is a hydrophilic phosphine ligand functionalized with ammonium groups, enabling its use in pure aqueous media. Breinbauer and co-workers combined $\text{Pd}(\text{OAc})_2$ with **L10** to achieve the Tsuji–Trost allylation of o-toluidine under aqueous conditions. The catalyst system delivered *N*-allylated products in high yields (up to 89%) with good regioselectivity (>99:1 linear/branched ratio) (Scheme 24c) [101].

Moreover, the Savignac group also demonstrated the Pd/TPPTS -mediated allylic substitution of various nucleophiles (including and primary amines) with allylic acetates in aqueous media, yielding *N*- and *C*-allylated products with high regioselectivity (70–93% yield) and minimal dialkylation (Scheme 24d) [139].

Unfortunately, in most prior examples, the necessity for a high proportion of organic solvents resulted in conditions that were less than ideal for subsequent biological applications, highlighting a critical consideration for future work to bridge the gap between chemical efficiency and biological compatibility.



Scheme 25 Pd-mediated allylation of tyrosine residues, cysteine residues, and *N*-terminal amines in proteins. Fluo, fluorescent unit

5.2 Protein Modification

While palladium catalysts are increasingly popular in bioorthogonal chemistry, the use of water-compatible Tsuji–Trost reactions for protein labeling remains partially explored. In 2006, Francis' group advanced the allylation of tyrosine residues of chymotrypsinogen A using the $\text{Pd}(\text{OAc})_2$ /TPPTS catalyst in pH 8.5 buffer, a breakthrough in artificial lipoprotein development. This Tsuji–Trost reaction achieves 50–65% conversion of chymotrypsinogen A to monoalkylated products within 45 min using $\text{Pd}(\text{OAc})_2$ and TPPTS in aqueous homogeneous buffer (Scheme 25a) [61]. Subsequently, Ma's group succeeded in selectively modifying the tyrosine residues of the superoxide dismutase (SOD) using the same catalyst system. The reaction, in bovine Cu/Zn SOD, achieves 11.6% labeling yield within 1 h under mild conditions (25 °C, pH 8.6) using of $\text{Pd}(\text{OAc})_2$ and TPPTS while preserving 95% enzyme activity (Scheme 25b) [62]. Later, Kele and co-workers also illustrates the $\text{Pd}(\text{OAc})_2$ /TPPTS-catalyzed Tsuji–Trost allylation of human serum albumin (HSA) using allyloxy acetate reagents in phosphate buffer (pH 7.4). The

method enabled selective tyrosine modification and subsequent functionalization via CuAAC (Scheme 25c) [63].

Furthermore, Breinbauer's group reported a $\text{Pd}(\text{dba})_2/\text{BIPHEPHOS}$ catalyst enabled near-quantitative prenylation of cysteine residues in peptides and proteins via Tsuji–Trost allylation in $\text{MeCN}/\text{H}_2\text{O}$ within 2–4 h. This reaction demonstrated good chemoselectivity (above 95% *S*-allylation) and complete *n*-regioselectivity, even in the presence of competing tyrosine residues (Scheme 25d) [58]. After rigorously screening a range of phosphorus ligands, they found BIPHEPHOS to be the most effective, yielding the desired linear products over branched products with exceptional regioselectivity. This method was then successfully applied to label proteins of interest, including ubiquitin-like protein 3 (UBL3) and heat shock protein 27 (Hsp27) [58].

Recently, Huang and co-workers developed a selective *N*-terminal modification strategy using the Tsuji–Trost reaction. This method, which employs the $\text{Pd}(\text{OAc})_2/\text{TPPTS}$ catalyst, is compatible with all 20 proteinogenic amino acids and operates under biologically relevant conditions (pH 7.4, 37 °C). It has potential applications in modifying complex proteins like antibodies. Notably, the reaction achieved above 90% conversion for trastuzumab under mild conditions with near-exclusive selectivity for the *N*-terminus over side-chain residues (Scheme 25e) [167]. Despite the significant strides made in protein modification through Tsuji–Trost allylation, the scope of this technique is constrained by the limited availability of suitable palladium catalysts and the stringent biological conditions required. This highlights the need for further research to expand the range of biocompatible catalysts and conditions.

Table 2 summarizes the significant progress made in water-compatible Tsuji–Trost allylation reactions. However, these methods often rely on a high percentage of organic solvents, such as DMSO, which pose challenges regarding environmental impact and sustainability. The use of heterogeneous palladium catalysts for Tsuji–Trost allylation has been limited to applications within test tubes. Recently, new procedures of protein labeling have broadened the scope of these reactions, enabling them to modify functional groups of proteins, such as phenols, thiols, and *N*-terminal amines, under more versatile conditions.

6 Summary and Perspectives

This review has focused on the Tsuji–Trost reaction in aqueous media, tracing its evolution from test tubes to in cellulo and in vivo applications. Tsuji–Trost reactions have emerged as a powerful deprotection tool, successfully applied in diverse settings, including cellular systems and animal models. Its versatility spans a broad range of compounds, such as sugars, peptides, proteins, nucleobases, nucleosides, dyes, and pharmaceuticals. In test tubes, many deprotection reactions rely on organic co-solvents like DMSO, MeCN, DMF, and THF. Some protocols are also conducted in aqueous/organic biphasic media, for example water/heptane. However, these solvent systems with limited biocompatibility remains a major obstacle for biological applications. In cellular settings, the

Table 2 Tsuji–Trost allylation in aqueous media

Allylic substrates	Nucleophiles	Conditions	Products	Applications
Allylation in test tubes				
	$\text{CH}_2(\text{COR})_2$ $\text{CH}_2(\text{COR})\text{CO}_2\text{R}'$ NaN_3 , NaSO_2Ph	Pd8 , K_2CO_3 , H_2O		Synthesis [125, 137, 142]
	$\text{ArB}(\text{OH})_2$, NaN_3 NaSO_2Ph	Pd9 , $(\text{K}_2\text{CO}_3)_2$, H_2O		Synthesis [162]
LG = OAc, OCO_2Me				
	$\text{CH}_2(\text{CO}_2\text{R})_2$, CH_3NO_2 ArOH , NaSO_2Ph	Pd10 , (base), H_2O		Asymmetric synthesis [162]
	$\text{CH}_2(\text{CO}_2\text{R})_2$, Bn_2NH ArOH	Pd10 , $(\text{Cs}_2\text{CO}_3)_2$, H_2O		Asymmetric synthesis [140, 141, 161, 163]
	$\text{CH}_2(\text{CO}_2\text{Me})_2$	$[\text{Pd}(\eta^3\text{-allyl})\text{Cl}]_2$, biotinylated ligands, (strept)avidin (wild-type or mutant), K_2CO_3 , DDAB, $\text{H}_2\text{O}/\text{DMSO}$ (10:1, v/v)		Development of artificial metalloenzymes [33]
	BnNH_2	Pd6 , 50 mM bicine, $\text{DMF}/\text{H}_2\text{O}$ (1:1, v/v)		Development of artificial metalloenzymes [79]
	BnNH_2	Pd7 , 0.1 M PBB/DMSO (9:1, v/v)		Development of a hybrid catalyst (mAb–Pd complex) [78]
	Intramolecular nucleophile	$[\text{Pd}(\eta^3\text{-allyl})\text{Cl}]_2$, (R)-BINAP, $n\text{Bu}_4\text{NBr}$, KOH , $\text{DCM}/\text{H}_2\text{O}$ (3:4, v/v)		Asymmetric synthesis [164]
	Intramolecular nucleophile	$[\text{Pd}(\eta^3\text{-allyl})\text{Cl}]_2$, (S)-BINAP, KOH , $\text{DCM}/\text{H}_2\text{O}$ (1:1, v/v)		Asymmetric synthesis [70]
	Na_2SO_3	$\text{Pd}(\text{dba})_2$, (R)-BINAP, $\text{THF}/\text{H}_2\text{O}$ (4:1, v/v)		Asymmetric synthesis [59]
	$\text{O}_2\text{N}-\text{CH}(\text{R})-\text{CO}_2\text{t-Bu}$	$\text{Pd}(\text{dba})_2$, L9a , toluene/ H_2O (20:1, v/v)		Asymmetric synthesis [100]
		$\text{Pd}(\text{OAc})_2$, L10 , H_2O		Synthesis [101]
	$\text{CH}_2(\text{CO}_2\text{Et})_2$ $\text{O}_2\text{NCH}_2\text{CO}_2\text{Et}$, RNH_2	$\text{Pd}(\text{OAc})_2$ /TPPTS, (base), $\text{MeCN}/\text{H}_2\text{O}$		Synthesis [139]
Protein modification				
		$\text{Pd}(\text{OAc})_2$ /TPPTS, phosphate buffer pH 8.5		Protein labeling (chymotrypsinogen A) [61]
		$\text{Pd}(\text{OAc})_2$ /TPPTS, phosphate buffer pH 8.6		Protein labeling (SOD) [62]
		$\text{Pd}(\text{OAc})_2$ /TPPTS, phosphate buffer pH 7.4		Protein labeling (HSA) [63]
		$\text{Pd}(\text{dba})_2$ /BIPHEPHOS, $\text{MeCN}/\text{H}_2\text{O}$ (2:1, v/v)		Protein labeling (UBL3, HSP27) [58]
		$\text{Pd}(\text{OAc})_2$ /TPPTS, phosphate buffer pH 7.4		Protein labeling (trastuzumab, RNase A, RNase B...) [167]

role of palladium species in the cleavage of various protected compounds, such as allyl ethers, allyl carbonates, allyl carbamates, propargyl ethers, propargyl amines, and propargyl carbamates, has been explored within living cells, primarily HeLa cells. Both homogeneous and heterogeneous palladium complexes have demonstrated efficacy in these environments. Heterogeneous palladium species tend to dominate *in vivo* deprotection reactions, likely due to the enhanced stability of Pd⁰ species under biological conditions.

Besides Tsuji–Trost processes aimed at the deprotection of compounds of interest, Tsuji–Trost allylation reactions have primarily been applied to asymmetric synthesis in aqueous media and protein modification. The η^3 -allyl ligand is frequently used to coordinate to the palladium center in catalyst structures. This widespread use is likely due to favorable coordination properties, which may facilitate its interaction with the metal center. The η^3 -allyl group could therefore be a key component in the development of palladium catalysts tailored for allylation reactions within living systems. Although some studies have investigated protein modification via Tsuji–Trost allylation, such applications remain limited. To date, no reports have demonstrated Tsuji–Trost allylation in living cells, and *in vivo* applications are unexplored. This highlights the untapped potential of expanding Tsuji–Trost chemistry in aqueous media, with the ultimate goal of enabling its implementation *in vivo*.

This review highlights key advancements in Tsuji–Trost reactions under biologically relevant conditions, with a focus on deprotection applications in aqueous media, cells, and animal models. While significant progress has been made, challenges remain—particularly in expanding allylation chemistry to living systems and addressing solvent biocompatibility limitations. Future efforts to optimize catalyst stability and reaction efficiency could further unlock the potential of this chemistry for *in vivo* applications.

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Data Availability No datasets were generated or analyzed during the current study.

Declarations

Conflict of Interest The authors declare no conflict of interest.

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