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N-Heterocyclic Carbene-Stabilized Palladium Complexes as Organometallic Catalysts for Bioorthogonal Cross-Coupling Reactions

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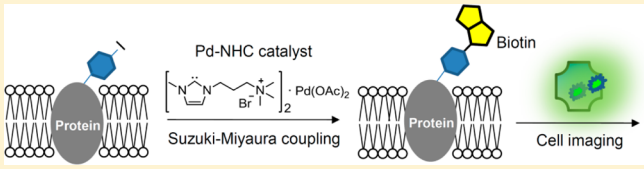
Supporting Information

ABSTRACT: A small library of water-soluble *N*-heterocyclic carbene (NHC)-stabilized palladium complexes was prepared and applied for cross-couplings of biomolecules under mild conditions in water. Pd−NHC complexes bearing hydrophilic groups were demonstrated to be efficient catalysts for the Suzuki−Miyaura coupling of various unnatural amino acids and proteins bearing *p*-iodophenyl functional groups. We further utilized this catalytic system for the rapid bioorthogonal labeling of proteins on the surfaces of mammalian cells. These results demonstrated that NHC-stabilized metal complexes have potential utility in cellular systems.



INTRODUCTION

Bioorthogonal reactions of biomolecules have attracted increased attention over the past decade because of their considerable importance in studying and controlling various biological processes.[1] For example, various functional groups can be incorporated into biomolecules through Cu(I)-catalyzed alkyne−azide cycloaddition-based bioorthogonal click chemistry.[2] Because of the cytotoxicity of Cu(I), metal-free bioorthogonal labeling strategies have been explored. Representative examples of these strategies include copper-free strain-promoted azide−alkyne cycloaddition (SPAAC),[3] nucleophilic addition of hydrazine or alkoxyamine to a ketone or aldehyde,[4] and Diels−Alder cycloaddition.[5] Bioorthogonal reactions must be performed in aqueous solutions or even within more complex native habitats (i.e., living cells and whole organisms) near ambient temperature. Accordingly, the aforementioned reactions have stringent requirements, including high selectivity and stability of functional groups, inertness to the functionalities found in vivo, and excellent biocompatibility and rapid reaction rates under physiological conditions.

Organometallic catalyst mediated carbon−carbon cross-couplings and related transformations have long played pivotal roles in organic synthesis. In particular, palladium-catalyzed cross-couplings are one of the most frequently used protocols.[6] Because the reactive groups used for cross-couplings are absent in biological systems, this protocol would find widespread use in bioorthogonal reactions. To achieve a highly efficient cross-coupling under aqueous conditions, the development of water-compatible ligands is of key importance. Toward this goal, several elegant water-soluble catalytic systems have been developed for aqueous Suzuki−Miyaura and Sonogashira cross-coupling reactions[7,8] More recently, Bradley et al. reported that a heterogeneous Pd(0)-based nanoparticle can be readily delivered across cell membranes to form carbon−carbon bonds inside cells.[9] However, despite these successful examples, the synthetically useful and practical Suzuki−Miyaura methodology has not yet been fully explored.

We previously focused on the development of *N*-heterocyclic carbene (NHC)−metal complexes for a wide range of useful chemical transformations in organic and aqueous media.[10] Because of their strong σ-electron-donating ability, NHCs form stronger bonds to metal centers than do conventional phosphine ligands, thereby conferring both stability and high catalytic activity.[11] Therefore, NHCs have been receiving an extraordinary amount of attention with respect to the construction of useful catalytic platforms.[12] Although a few water-soluble NHC-stabilized metal complexes are available for aqueous cross-coupling reactions,[13] these complexes have not yet been explored for use in more complicated environments, such as on proteins or the surfaces of living cells. Herein, we present novel water-soluble NHC-stabilized palladium complexes for efficient cross-coupling reactions in aqueous solution for a diverse variety of amino acids. We also demonstrate the use of these catalysts for the rapid bioorthogonal labeling of proteins in buffered water and on the surfaces of mammalian cells.

RESULTS AND DISCUSSION

Synthesis and Catalyst Screening. Ligand precursors **L1**−**5** that contain hydrophilic groups were prepared following previously reported procedures using commercially available materials (see Figure 1 and the Experimental Section).[13b,14] The corresponding catalysts **1**−**5** were obtained by heating

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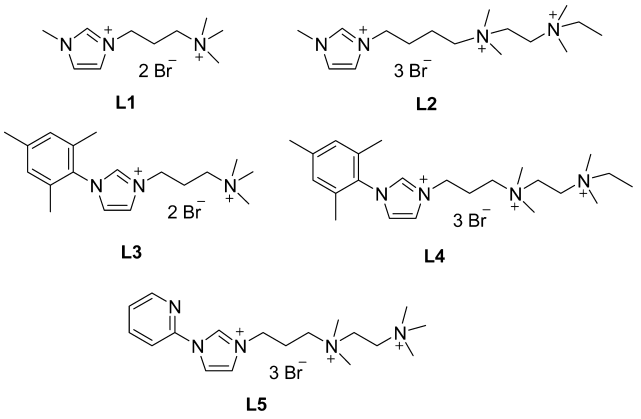
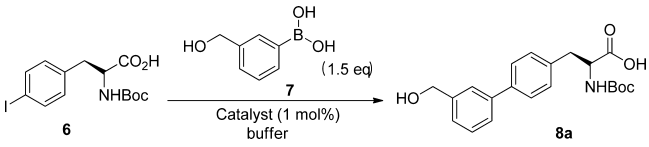


Figure 1. Ligands **L1**−**5** containing hydrophilic quaternary ammonium groups.

solutions of palladium acetate and the ligand precursors in DMSO. The formation of Pd−NHC complexes was indicated by the disappearance of the acidic protons of imidazolium salts (see the Supporting Information, Figure S1). The complexes generated in situ were directly used for the next cross-coupling reactions after diluted with water. The aqueous catalyst solutions were quite stable at room temperature, and no black palladium was observed, even after storage in water for several months.

We first examined the ability of catalysts **1**−**5** to catalyze the Suzuki−Miyaura cross-coupling of *N*-Boc-4-iodo-L-phenylalanine (**6**) with 3-(hydroxymethyl)phenylboronic acid (**7**) in buffered water under mild conditions (Table 1). With a low

Table 1. Suzuki−Miyaura Coupling of Boc-pIPhe with Phenylboronic Acid in Aqueous Solutions[a]



entry	catalyst	conditions	yield[b] (%)
1	**1**	37 °C, 2 h	77[c]
2	**2**	37 °C, 2 h	64
3	**3**	37 °C, 2 h	60
4	**4**	37 °C, 2 h	56
5	**5**	37 °C, 2 h	<5
6	**1**	37 °C, 3 h	90
7	**1**	37 °C, 4 h	>95

[a]Reaction conditions: **6**, 0.3 mmol; **7**, 0.45 mmol, phosphate buffer (5 mL). [b]Yields were determined by HPLC. [c]Isolated yield.

loading of catalyst **1** at 1 mol %, the target product **8a** was isolated in 77% yield within 2 h (Table 1, entry 1). Catalyst **2**, which contained two quaternary ammonium groups, did not improve the catalytic activity. Catalysts **3** and **4**, which possessed a steric group, gave slightly lower yields of 60% and 56%, respectively. Previous reports have shown that bulkier NHC-ligands tend to be more active for cross-couplings in organic solvents.[15] However, for our cross-couplings in aqueous solution, the bulkier ligands slightly decreased the yields. Bidentate NHC ligands have widely been applied in cross-coupling reactions because of their unique ability to accelerate the reductive elimination process.[13b,15,16] Unfortunately, the use of **5** led to a trace amount of the target product under the same conditions. We assumed that the pyridine group has a strong coordinating ability to the palladium ion, which would thus impede the reaction at relatively low temperatures. To our delight, when the reaction time was extended to 4 h, an almost quantitative yield could be achieved through the use of **1** at a loading of 1 mol % (Table 1, entries 6 and 7).

The present results indicated that **1** is the most efficient catalyst; thus, we examined the reactions of **7** with other amino acid derivatives under the optimized conditions. As shown in Table 2, **1** is quite effective for the coupling of 4-bromophenylalanine, affording the corresponding product in 80% yield within 6 h (Table 2, entry 1). Unfortunately, no coupling product was obtained under identical conditions with 4-chlorophenylalanine (entry 2). Entries 3 and 4 indicate that iodo-substituted derivatives have similar reactivities toward compound **7**. To demonstrate the efficiency of Pd−NHC-catalyzed cross-coupling, the reaction of biotin−$B(OH)_2$ (**10**, Figure 2a) was also examined, and a relatively high conversion was achieved (entry 5).

We then compared the kinetics of the **1**-catalyzed cross-coupling with those of several known bioorthogonal reactions. Taking advantage of the reaction of 4-iodobenzoic acid with **7** in the presence of 1 mol % of **1**, the pseudo-second-order rate constant was determined to be 1.1×10^{-2} M^{-1} s^{-1} (see Figure S5 in the Supporting Information), which is comparable to those of the Staudinger ligation ($k = 2.5 \times 10^{-3}$ M^{-1} s^{-1}),[17] the copper-catalyzed ligation of azides with unstrained alkynes (10^{-2}−10^{-4} M^{-1} s^{-1}),[18] and the first-generation SPAAC ($k = 1.2 \times 10^{-3}$ M^{-1} s^{-1}).[4a] In addition to selectivity, the intrinsic kinetics of bioorthogonal reactions is equally important because the labeled biomolecules as substrates are often at low concentrations in vivo. The obtained rate constant indicates that the present catalyst **1** exhibits high catalytic activity and is therefore suitable for cross-couplings of substrates at low concentrations.

Pd−NHC-Catalyzed Suzuki−Miyaura Coupling on Protein Surface. With these promising results, we further explored the possibility of using **1** for the Suzuki−Miyaura cross-coupling of protein molecules bearing iodophenyl handles. Bovine serum albumin (BSA) was first selected as a model protein. We iodinated lysine residues on BSA using (*p*-iodophenyl)methyl *p*-nitrophenyl carbonate (**9**, Figure 2a), and the resulting iodo-labeled BSA (*p*IPM-BSA) was purified by size-exclusion chromatography. Pd−NHC **1**-catalyzed cross-coupling was then conducted by incubating *p*IPM-BSA (7.0 μM) with biotin−$B(OH)_2$ (**10**) or fluorescein−$B(OH)_2$ (**11**). The resulting proteins were then detected by Western blot using biotin antibody or in-gel fluorescence measurements, respectively (Figure 2b,c). As expected, robust biotinylation was observed for *p*IPM-BSA, and the signal intensities were dose-dependent. In the control experiments, no signals were obtained in the absence of either palladium catalyst **1** or modified BSA. In addition, fluorescent labeling with **11** also confirmed that the coupling reaction proceeded effectively within 1 h (Figure 2c).

We further verified the feasibility of this approach by applying cross-coupling on lysozyme. Western blot analysis showed that *N*-succinimidyl *p*-iodobenzoate **12**-modified lysozyme (*p*IBZ-lyso) also undergoes efficient cross-couplings (Figure 3 and Figure S4 in the Supporting Information). Figure 3b shows that a higher loading of **1** and biotin−$B(OH)_2$ led to an improved conversion during protein biotinylation. Poly-

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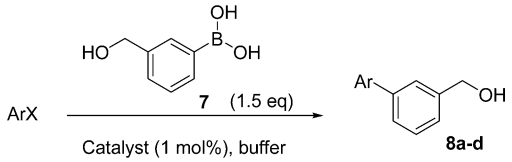
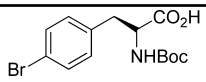
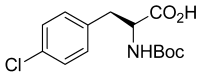
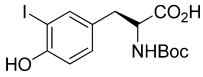
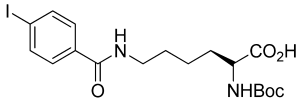
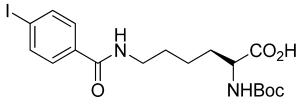
Table 2. Suzuki–Miyaura Coupling of Modified Amino Acid with 3-(Hydroxymethyl)phenylboronic Acid[a]



entry	ArX	conditions	product	yield (%)
1		37 °C, 6 h	**8a**	80
2		37 °C, 6 h	**8a**	0
3		37 °C, 4 h	**8b**	94
4		37 °C, 6 h	**8c**	85
5[b]		37 °C, 6 h	**8d**	70

[a]Reaction conditions: ArX (0.5 mmol), 7 (114 mg, 0.75 mmol, 1.5 equiv), **1** (1 mol %), phosphate buffer (10 mL), 37 °C. [b]Biotin-B(OH)$_2$ **10** was used instead of **7**.

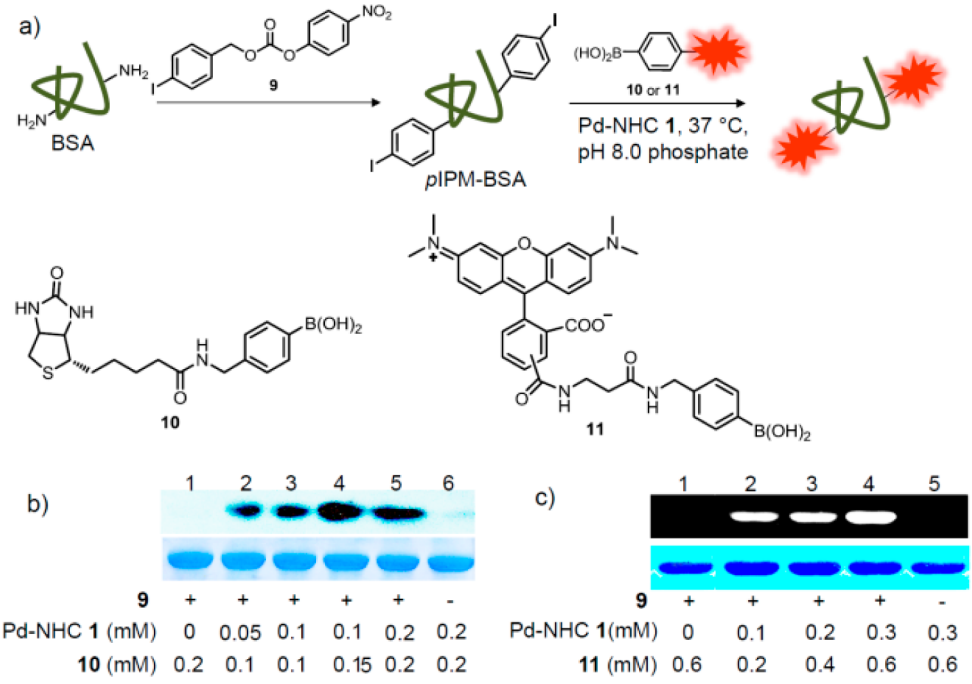


Figure 2. Labeling of *p*IPM-modified BSA (7.0 μM) with **10** or **11**. (a) Reaction scheme: modification of free lysine residues on BSA with **9** and subsequent labeling with **10** or **11**. (b) Western blot analysis of biotin-labeled BSA. (c) Fluorescein labeling of BSA.

(ethylene glycol) (PEG) is a widely investigated polymer, and the attachment of a PEG chain to biological macromolecules (e.g., peptides and proteins) can significantly improve pharmacokinetics.[19] Thus, we attempted to PEGylate *p*IBZ-lyso through **1**-mediated cross-couplings. A PEG reagent (mPEG2k-B(OH)$_2$, **13**) bearing a phenylboronic acid group

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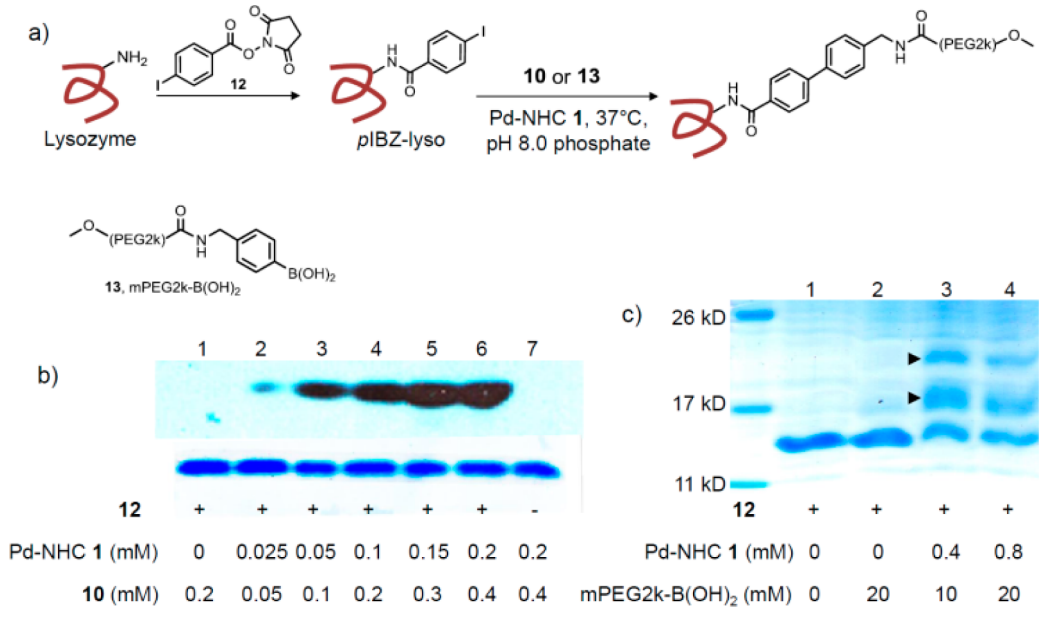

Figure 3. Labeling of *p*IBZ-lyso with **10** or **13**. (a) Tagging the free lysine residues on lysozyme with **12** and subsequent labeling with **10** or **13**; molecule structure of **13**. (b) Western blot analysis of biotin-labeled lysozyme. (c) Labeling of lysozyme with **13**. The arrowhead indicates single and double-PEGylated lysozyme.

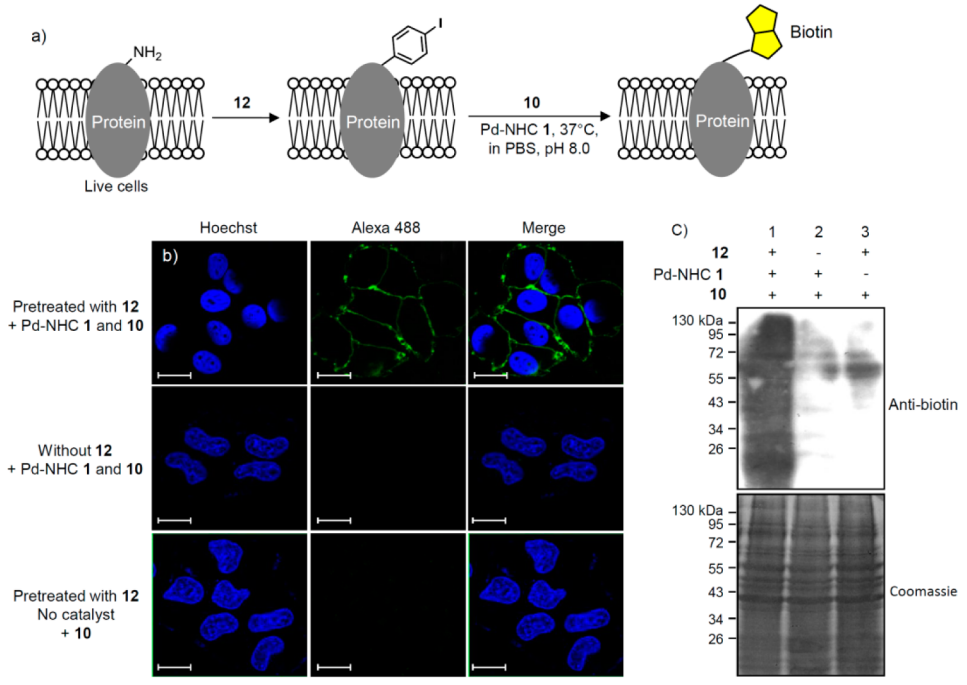

Figure 4. Pd–NHC **1**-mediated cross coupling of membrane proteins on the mammalian cell surfaces. (a) Pretreatment with **12** allows *p*IBZ handle to be installed on the membrane proteins, thus conferring the reaction with activity toward palladium-catalyzed coupling for further analysis. (b) Fluorescence microscopy images of living HeLa cells. Cells were pretreated with **12** for 45 min and subsequently treated with **1** and **10** for 1 h in phosphate buffer (pH = 8.0) (top). As negative controls, a labeling reaction was performed with cells not pretreated with **12** (central) or in the absence of **1** (bottom). Scale bar 10 μm. (c) Western blot analysis of **1**-catalyzed cross coupling on cell surface.

was synthesized and used as the coupling partner. The palladium-mediated PEGylation of *p*IBZ-lyso was conducted at 37 °C for 2 h. A Coomassie-stained SDS-PAGE gel showed that 20 equiv of palladium and 500 equiv of PEG–boronic acid loading can result in a good, smooth conversion (Figure 3c). By quantification with gel densitometry analysis, we concluded that the total conversion of Suzuki coupling was ca. 90% (Supporting Information, Figures S2 and S3). Neither untreated lysozyme (Figure 3c, lane 1) nor reaction without **1** (lane 2) yielded positive results, which was consistent with the proposed coupling reaction. These results demonstrated the applicability of our catalyst to a different target protein and the high tolerance in the substrate design. In comparison to the ligand-free or self-liganded palladium catalysts currently available for protein labeling, in which the substrates are limited to PEG derivatives, we achieved comparable reaction

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efficiencies on protein surfaces with various boronic acid derivatives.[20] Therefore, our NHC-based palladium catalytic system is a new candidate for performing cross-couplings on proteins.

Suzuki–Miyaura Coupling Performed on the Live Cell Surfaces. The labeling of cell-surface proteins or polypeptides with biophysical probes enables the functions of receptors, channels, and transporters to be studied.[21] A methodology for the selective modification of these species is needed.[22] Encouraged by the success of the present Pd–NHC complexes for amino acids and protein labeling in test tubes, we further demonstrate the utility of this approach for the ligation of small molecules with proteins on the surfaces of live mammal cells. HeLa cells pretreated with **12** (60 μM) were exposed to phosphate-buffered saline (PBS, pH = 8.0) containing 80 μM of **1** and 200 μM of **10** for 1 h. The cells were then rinsed to remove the Pd catalyst and excess reagent. Subsequently, streptavidin conjugated to the fluorophore Alexa 488 was used to detect the biotin handle installed on the membrane proteins by confocal microscopy. As shown in Figure 4b, a green fluorescence signal attributed to biotinylated membrane proteins was detected in the periphery of the cells. In contrast, the incubation of **10** in the absence of Pd catalyst **1** resulted in no fluorescence signal. Moreover, control cells without pretreatment with **12** were inert with respect to palladium-catalyzed cross-coupling, as indicated by the absence of fluorescence emission on the cell-surface. The above results were consistent with those of the Western blot analysis, as shown in Figure 4c.

Finally, we performed an MTT assay to evaluate the cytotoxicities of **1** using HeLa and A549 cells. Catalyst **1** exhibits high cell viability (>85%) on both cell lines at concentrations up to 200 μM (2.5-fold greater than the concentration used for cell-surface protein labeling) (Supporting Information, Figure S6). Although several Pd–NHC complexes have been reported to exhibit submicromolar half inhibitory concentration (IC_{50}) values,[23] our results indicate that the present palladium catalyst has high biocompatibility toward biological systems. Although the exact mechanism has not been explored, we attributed the low cytotoxicity of catalyst **1** to its high water solubility and large polar nature.

■ CONCLUSIONS

Water-soluble Pd–NHC complexes that catalyzed bioorthogonal cross-coupling reactions were studied. Catalyst **1** was found to be the most effective catalyst for Suzuki-Miyaura couplings of unnatural amino acids. Two model proteins bearing *p*-iodophenyl functional groups can be selectively labeled by boronic acid derivatives with diverse structures. Furthermore, the palladium-mediated cross-coupling was also successful for protein labeling on the surfaces of mammalian cells. These results demonstrate that water-soluble NHCs are suitable ligands for catalytic transformations in biological systems. To our knowledge, this is the first successful application of Pd–NHCs for biopolymer modifications in cellular systems. Efforts to further optimize the NHC ligands for the purpose of intracellular utility are currently in progress.

■ EXPERIMENTAL SECTION

The ligands **L1–5** were prepared according to the procedure reported previously.[13b,14]

Compound L1: white solid; ^{1}H NMR (400 MHz; DMSO-*d6*) δ = 8.69 (s, 1H), 7.40 (d, J = 26 Hz, 2H), 4.23 (t, J = 6.8 Hz, 2H), 3.79 (s, 3H), 3.32 (t, J = 6.8 Hz, 2H), 3.04 (s, 9H), 2.29–2.37 (m, 2H); ^{13}C{^{1}H} NMR (100 MHz; D_2O) δ 123.9, 121.9, 62.7, 52.9, 45.9, 35.7, 23.4.

Compound L2: white solid; ^{1}H NMR (400 MHz, D_2O) δ = 8.65 (s, 1H), 7.60 (s, 1H), 7.52 (s, 1H), 4.04 (s, 3H), 3.96–3.97 (m, 2H), 3.61–3.62 (m, 2H), 3.59–3.61 (m, 2H), 3.40 (s, 2H), 3.40–3.39 (m, 2H), 3.32–3.31 (m, 6H), 3.30–3.28 (m, 6H), 2.05 (m, 6H), 1.98 (t, J = 5.6 Hz, 2H), 1.48 (t, J = 6.4 Hz, 3H); ^{13}C{^{1}H} NMR (100 MHz, D_2O) δ = 136.2, 123.9, 122.3, 64.9, 61.6, 56.2, 55.3, 51.4, 50.9, 48.8, 36.1, 26.2, 19.6, 8.0.

Compound L3: white solid; ^{1}H NMR (400 MHz; DMSO-d_6) δ = 9.63 (s, 1H), 8.25 (s, 1H), 7.99 (s, 1H), 7.15 (s, 2H), 4.39 (t, J = 7.2 Hz, 2H), 3.48 (t, J = 8.0 Hz, 2H), 3.37 (s, 9H), 2.32 (s, 3H), 2.24–2.32 (m, 2H), 2.05 (s, 1H); ^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6) δ = 140.7, 138.1, 134.8, 131.6, 129.7, 124.3, 123.7, 62.3, 53.0, 46.9, 23.6, 21.1, 18.0, 17.6; HRMS (ESI-ion trap) m/z $[M]^{2+}$ calcd for $C_{18}H_{29}N_3$ 143.6176, found 143.6185.

Compound L4: white solid; ^{1}H NMR (400 MHz; D_2O) δ = 7.76 (s, 1H), 7.52 (d, J = 1.6 Hz, 1H), 7.04 (s, 2H), 4.38 (t, J = 8.0 Hz, 2H), 3.89–3.96 (m, 4H), 3.58 (t, J = 8.0 Hz, 2H), 3.44–3.48 (m, 2H), 3.21 (s, 6H), 3.12 (s, 6H), 2.58 (s, 2H), 2.50–2.56 (m, 3H), 2.23 (s, 6H), 1.32 (t, J = 3.2 Hz, 3H); ^{13}C{^{1}H} NMR (100 MHz; D_2O) δ = 142.1, 135.2, 131.3, 129.9, 125.2, 123.7, 63.1, 62.3, 57.3, 55.8, 52.1, 52.0, 51.5, 51.4, 46.9, 24.3, 20.9,17.3, 8.57.

Compound L5: white solid; ^{1}H NMR (400 MHz; D_2O) δ = 9.56 (s, 1H), 8.43 (s, 1H), 8.05 (s, 1H), 7.99 (s, 1H), 7.68 (s, 2H), 7.48 (d, J = 4.8 Hz, 1H), 4.36 (s, 2H), 3.86 (s, 4H), 3.42 (d, J = 5.2 Hz, 4H), 3.14 (s, 6H), 3.09 (s, 6H), 1.87–1.96 (m, 4H), 1.28 (s, 3H); ^{13}C{^{1}H} NMR (100 MHz, D_2O) δ = 149.9, 146.8, 141.6, 135.1, 126.2, 123.9, 120.6, 115.7, 65.5, 62.1, 56.8, 55.8, 51.9, 51.4, 50.1, 26.7, 20.3, 8.5.

General Procedures for Suzuki–Miyaura Coupling of Modified Amino Acids with 3-(Hydroxymethyl)phenylboronic Acid. ArX (0.5 mmol) and 3-(hydroxymethyl)phenylboronic acid (**7**) (0.75 mmol, 114 mg) were added to a solvent of K_2HPO_4 (100 mM, 10 mL). A 1 mL aliquot of the palladium catalyst (0.01 M, 1%) solution prepared above was subsequently added to the reaction. The mixture was stirred at 37 °C for 4 h, and then the reaction was quenched with 50 mL of 1 M HCl. The aqueous layer was extracted with EtOAc (2 × 50 mL). The combined organic layers were washed with brine (100 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The resulting residue was purified by column chromatography with hexanes/EtOAc.

Product ***8a:*** white solid (158 mg, 85%); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 12.44 (s, 1H), 7.50–7.58 (m, 3H), 7.40 (t, J = 7.6 Hz, 1H), 7.33–7.35 (m, 1H), 7.30 (m, 3H), 7.12 (d, J = 8.4 Hz, 1H), 5.23–5.24 (br, 1H), 4.57 (s, 2H), 4.10–4.16 (m, 1H), 3.04–3.09 (m, 1H), 2.85–2.91 (m, 1H), 1.33 (s, 9H); ^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6) δ = 174.0, 155.9, 143.6, 140.3, 138.8, 137.7, 130.2, 129.1, 126.8, 125.9, 125.3, 125.0, 78.5, 63.4, 55.6, 36.6, 28.6; HRMS (ESI-ion trap) m/z $(M + H)^+$ calcd for $C_{21}H_{25}NO_5$ 372.1805, found 372.1805.

Product ***8b:*** white solid (182 mg, 94%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.32 (br, 1H), 7.47 (s, 1H), 7.39 (d, J = 8.0 Hz, 1H), 7.31 (t, J = 7.6 Hz, 1H), 7.22–7.24 (d, J = 7.6 Hz, 1H), 7.09 (s, 1H), 6.98–7.00 (d, J = 8.0 Hz, 1H), 6.82 (d, J = 8.4 Hz, 2H), 5.22 (br, 1H), 4.52 (s, 2H), 3.40 (d, J = 3.6 Hz, 1H), 2.74–2.97 (m, 2H), 1.31 (s, 9H); ^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6) δ = 174.2, 172.5, 155.9, 153.3, 142.5, 138.9, 131.6, 129.4, 128.9, 128.1, 127.8, 127.7, 125.2, 116.3, 78.5, 63.6, 55.9, 36.2, 28.6, 21.5; HRMS (ESI-ion trap) m/z $(M + H)^+$ calcd for $C_{21}H_{25}NO_6$ 388.1755, found 388.1761.

Product ***8c:*** white solid (193 mg, 85%); ^{1}H NMR (400 MHz; DMSO-d_6) δ = 12.54 (1H,br), 8.51 (t, J = 5.2 Hz, 1H), 7.93 (d, J = 8.4 Hz, 2H), 7.43 (d, J = 8.0 Hz, 2H), 7.66 (s, 1H), 7.58 (d, J = 7.6 Hz, 1H), 7.44 (t, J = 7.6 Hz, 1H), 7.34–7.36 (d, J = 7.6 Hz, 1H), 7.02–7.04 (d, J = 7.6 Hz, 1H), 5.27–5.29 (m, 1H), 4.58 (s, 2H), 3.83 (d, J = 7.6 Hz, 1H), 3.32–3.34 (m, 2H), 1.51–1.54 (m, 4H), 1.49 (s, 9H), 1.37 (s, 2H); ^{13}C{^{1}H} NMR (100 MHz; DMSO-d_6) δ = 175.4, 166.9, 156.7, 144.5, 143.8, 140.1, 134.6, 129.9, 128.9, 127.5, 127.2, 126.3, 125.9, 79.1, 63.9, 54.6, 40.1, 31.7, 29.9, 29.3, 24.3; HRMS (ESI-ion trap) m/z $(M - H)^+$ calcd for $C_{25}H_{32}N_2O_6$ 455.2182, found 455.2177.

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Product ***8d:*** white solid (238 mg, 70%); ^{1}H NMR (400 MHz; DMSO-d_6) δ = 8.53 (s, 1H,), 8.41 (s, 1H), 7.93−7.95 (d, J = 8.0 Hz, 2H), 7.72−7.74 (d, J = 8.0 Hz, 2H), 7.67−7.69 (d, J = 8.0 Hz, 2H), 7.35−7.37 (d, J = 8.0 Hz, 2H), 6.43 (s, 1H), 6.38 (s, 1H), 6.14−6.15 (m, 1H), 4.30−4.31 (m, 3H), 4.13−4.15 (m, 1H), 3.23−3.25 (m, 3H), 3.17 (s, 1H), 3.08−3.11 (m, 1H), 2.80−2.84 (m, 1H), 2.50−2.57 (d, J = 5.2 Hz, 1H), 2.15−2.18 (t, J = 7.6 Hz, 2H), 1.88 (br, 3H), 1.49−1.56 (m, 8H), 1.36 (s, 9H); HRMS (ESI-ion trap) m/z (M + H)$^+$ calcd for $C_{35}H_{47}N_5O_7S$ 682.3269, found 682.3279.

Procedures for the Synthesis of (*p*-Iodophenyl)methyl *p*-Nitrophenyl Carbonate (9). 4-Iodobenzyl alcohol (140 mg, 0.6 mmol, 1.0 equiv) was dissolved in dichloromethane (4 mL, anhydrous) and cooled to 0 °C. DIEA (232 mg, 1.8 mmol, 3.0 equiv) was added, followed by 4-nitrophenyl chloroformate (503 mg, 1.5 mmol, 2.5 equiv). The reaction mixture was warmed to room temperature over 1 h, at which point it was quenched with water and extracted with dichloromethane (3 × 20 mL). The organic layers were combined, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. The crude product was purified by silica gel chromatography with hexanes/EtOAc (9:1). This procedure afforded in pure product **9** as a white solid (110 mg, 46%): ^{1}H NMR (400 MHz; $CDCl_3$) δ = 8.20 (d, J = 9.2 Hz, 2H), 7.68 (d, J = 8.4 Hz, 2H), 7.31 (d, J = 9.2 Hz, 2H), 7.11 (d, J = 8.4 Hz, 2H), 5.16 (s, 1H).

Procedures for Synthesis of Biotin−B(OH)$_2$ (10), Fluorescein−B(OH)$_2$ (11), and mPEG2k−B(OH)$_2$ (13). D-Biotin (244 mg, 1 mmol) and *p*-(aminomethyl)phenylboronic (370 mg, 2 mmol, 2.0 equiv) were dissolved in 5 mL of DMF. EDCl (382 mg, 2 mmol, 2.0 equiv) and DIEA (520 mg, 4 mmol, 4.0 equiv) were added to the mixture, which was then stirred at 40 °C for 2 h. After the solvent was removed under vacuum, the crude product was purified by column chromatography (5−10% MeOH in CH_2Cl_2), affording pure product **10** as white solid (286 mg, 76%): ^{1}H NMR (400 MHz; DMSO-d_6) δ 8.30−8.31 (t, J = 2.8 Hz, 1H), 7.97 (s, 2H), 7.71−7.73 (d, J = 8.0 Hz, 2H), 7.18−7.20 (d, J = 8.0 Hz, 2H), 6.42 (s, 1H), 6.36 (s, 1H), 4.29−4.32 (t, J = 7.2 Hz, 1H), 4.25−4.26 (d, J = 6.0 Hz, 2H), 3.07−3.11 (m, 1H), 2.80−2.85(m, 1H), 2.57−2.60 (d, J = 8.0 Hz, 1H), 2.13−2.16 (t, J = 7.2 Hz, 2H), 1.44−1.66(m, 4H), 1.31−1.33 (m, 2H); ^{13}C{^{1}H} NMR (100 MHz; DMSO-d_6) δ = 172.6, 163.2, 142.0, 134.6, 126.6, 61.5, 59.7, 55.9, 53.6, 49.1, 42.5, 35.6, 28.7, 28.5, 25.8; HRMS (ESI-ion trap) m/z (M + H)$^+$ calcd for $C_{17}H_{24}BN_3O_4S$ 378.1659, found 378.1654.

5(6)-TAMRA−B(OH)$_2$ was synthesized according to a procedure similar to that for biotin−B(OH)$_2$: red solid; ^{1}H NMR (400 MHz; $CDCl_3$) δ 8.42 (s, 1H), 7.87−7.89 (dd, J = 8.0, 1.2 Hz, 1H), 7.51−7.53 (br, 2H), 7.24−7.26 (d, J = 8.0 Hz, 1H), 7.12−7.16 (m, 4H), 6.89−6.92 (dd, J = 8.0, 1.2 Hz, 2H), 6.83−6.84 (d, J = 2.8 Hz, 2H), 3.64−3.66 (t, J = 6.8 Hz, 2H), 3.19 (s, 12H), 2.54−2.56 (t, J = 6.8 Hz, 2H); HRMS (ESI-ion trap) m/z (M + H)$^+$ calcd for $C_{35}H_{35}BN_4O_7$ 634.2708, found 634.2693.

mPEG2k−B(OH)$_2$ (**13**) was also synthesized according to a procedure similar to that for biotin−B(OH)$_2$: colorless solid; ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.80−7.82 (d, J = 8.0 Hz, 2H), 7.27−7.29 (d, J = 8.0 Hz, 2H), 7.17−7.20 (t, J = 5.2 Hz, 1H), 4.46−4.47 (d, J = 5.2 Hz, 2H), 3.64 (br, 176H).

Synthesis of *N*-Succinimidyl *p*-Iodobenzoate (12). *p*-Iodobenzoic acid (2.48 g, 10 mmol) and *N*-hydroxysuccinimide (1.38 g, 12 mmol, 1.2 equiv) were dissolved in 5 mL of DMF. EDCl (3.82 g, 20 mmol, 2.0 equiv) and DIEA (2.6 g, 20 mmol 2.0 equiv) were added to the mixture. The mixture was then stirred at room temperature for 2 h. EtOAc (150 mL) was added to the reaction mixture and washed three times with water (100 mL). The organic layer was separated, dried, and evaporated to give the product as a white solid (2.58 g, 75%): ^{1}H NMR (400 MHz; $CDCl_3$) 7.90−7.92 (d, J = 8.4 Hz, 2H), 7.83−7.85 (d, J = 8.4 Hz, 2H), 2.91 (s, 4H).

Kinetic Measurements. HPLC analysis was used to calculate the reaction rate constant for the Suzuki−Miyama cross-coupling reaction. 4-Iodobenzoic acid (0.03 mmol) and Pd−NHC (**1**) (1.0 or 5.0 mol %) were first dissolved in phosphate buffer (100 mM K_2HPO_4, 4 mL), and the reaction mixture was stirred at 37 °C for 10 min. 3-(Hydroxymethyl)phenylboronic acid (0.3 mmol, 10 equiv) was then added to the reaction solution to a final volume of 6 mL. A 2.0 mL aliquot of the reaction mixture was collected every 15−30 min, and the cross-coupling yields were determined by HPLC. The pseudo-first-order rate constant (k_{obs}) for the reaction was determined by plotting $\ln[1/(1 - x)]$ versus time and analyzed by linear regression. x represents the percent conversion of 4-iodobenzoic acid. The pseudo-first-order rate constant k_{obs} was estimated to be 5.4×10^{-4} s^{-1} at 1 mol % catalyst loading or 1.2×10^{-3} s^{-1} at 5 mol % catalyst loading. The pseudo-second-order rate constant was estimated from k_{obs}/[3-(hydroxymethylphenyl)boronic acid]. As a result, the pseudo-second-order rate constants for the coupling reaction were 1.1×10^{-2} M^{-1} s^{-1} at 1 mol % catalyst loading or 2.5×10^{-2} M^{-1} s^{-1} at 5 mol % catalyst loading.

General Procedures for the Labeling of Proteins by Suzuki−Miyaura Cross-Coupling Using Biotin−B(OH)$_2$. Stock solutions of biotin−B(OH)$_2$ (1.0 μL, 10 mM) and palladium complex **1** (1.0 μL, 10 mM) were mixed in a Eppendorf tube. Iodinated BSA/lysozyme or native proteins (7.0/10 μL, 100 μM) and phosphate buffer (91/88 μL, 20 mM, pH = 8.0) were subsequently added to a total volume of 100 μL. Then, the reactions were performed in a water bath (37 °C) for 1.5 h. After being quenched with a 3-mercaptopropionic acid solution in water, the reactions were analyzed by 12% SDS−PAGE. Upon electrophoresis, the proteins were stained with Coomassie Brilliant Blue and photographed or transferred to nitrocellulose for Western blot analysis.

General Procedures for Suzuki−Miyaura Coupling on Cell Surface. HeLa cells were maintained in DMEM with 10% fetal bovine serum (FBS), penicillin (100 units/mL), and streptomycin (10 μg/mL) at 37 °C and 5% CO_2. Before labeling, the cells were pretreated with a freshly prepared solution of **12** (100 μM, in PBS pH = 7.6) for 40 min at 37 °C. The cells were then carefully washed with PBS to remove excess reagents. Pd−NHC **1**-mediated Suzuki−Miyaura cross coupling was conducted in PBS solution (pH = 8.0) containing **10** (200 μM) and catalyst **1** (80 μM) for 1 h at 37 °C. As a control, labeling experiments in the absence of catalyst **1** or without pretreatment with **12** were also conducted. After biotin labeling, the cells were washed three times with PBS to remove the unreacted biotin−B(OH)$_2$ and catalyst. The biotinylated cells were then treated with streptavidin−Alexa Fluor 488 conjugates (1:10,000) for 2 min, followed by washing three times with PBS, and observed by CLSM or analyzed by Western blot.

General Procedure for Catalyst Cytotoxicity Assay. Cell viability was measured using an MTT assay. Each well containing 100 μL of cell suspensions (5×10^3 cells/mL) was seeded in 96-well plates and incubated at 37 °C for 12 h before treatment. Then, the medium was replaced by a different concentration of catalyst **1** in fresh medium to allow the cells to be incubated for 48 or 72 h in a humidified 5% CO_2 incubator at 37 °C. A total of 30 μL of MTT solution (5 mg/mL in PBS) was added per well. The plates were incubated at 37 °C and 5% CO_2 for 4 h. After removal of the solution, DMSO was added to 100 μL per well. Then, the optical density (OD) was recorded using the ParadigmTM detection platform (Beckman Coulter) at 490 nm. Four wells per dose were used in three independent experiments.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Details of catalyst preparation, protein labeling procedure, and NMR spectra of the coupling products. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) (a) Sletten, E. M.; Bertozzi, C. R. *Angew. Chem., Int. Ed.* **2009**, *48*, 6974. (b) Prescher, J. A.; Bertozzi, C. R. *Nat. Chem. Biol.* **2005**, *1*, 13.
(2) (a) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596. (b) del Amo, D. S.; Wang, W.; Jiang, H.; Besanceney, C.; Yan, A. C.; Levy, M.; Liu, Y.; Marlow, F. L.; Wu, P. *J. Am. Chem. Soc.* **2010**, *132*, 16893.
(3) (a) Agard, N. J.; Prescher, J. A.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2004**, *126*, 15046. (b) Baskin, J. M.; Prescher, J. A.; Laughlin, S. T.; Agard, N. J.; Chang, P. V.; Miller, I. A.; Lo, A.; Codelli, J. A.; Bertozzi, C. R. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 16793. (c) Codelli, J. A.; Baskin, J. M.; Agard, N. J.; Berozzi, C. R. *J. Am. Chem. Soc.* **2008**, *130*, 11486. (d) Jewett, J. C.; Sletten, E. M.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2010**, *132*, 3688. (e) Jewett, J. C.; Bertozzi, C. R. *Chem. Soc. Rev.* **2010**, *39*, 1272.
(4) (a) Mahal, L. K.; Yarema, K. J.; Bertozzi, C. R. *Science* **1997**, *276*, 1125. (b) Wang, L.; Zhang, Z. W.; Brock, A.; Schultz, P. G. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 56.
(5) (a) de Araujo, A. D.; Palomo, J. M.; Cramer, J.; Kohn, M.; Schroder, H.; Wacker, R.; Niemeyer, C.; Alexandrov, K.; Waldmann, H. *Angew. Chem., Int. Ed.* **2006**, *45*, 296. (b) de Araujo, A. D.; Palomo, J. M.; Cramer, J.; Seitz, O.; Alexandrov, K.; Waldmann, H. *Chem.—Eur. J.* **2006**, *12*, 6095. (c) Lang, K.; Davis, L.; Torres-Kolbus, J.; Chou, C. J.; Deiters, A.; Chin, J. W. *Nat. Chem.* **2012**, *4*, 298.
(6) (a) Hartwig, J. F. *Nature* **2008**, *455*, 314. (b) Arpad, M. *Chem. Rev.* **2011**, *111*, 2251. (c) McDonald, R. I.; Liu, G. S.; Stahl, S. S. *Chem. Rev.* **2011**, *111*, 2981. (d) Wu, X. F.; Neumann, H.; Beller, M. *Chem. Rev.* **2013**, *113*, 1.
(7) (a) Chalker, J. M.; Wood, C. S. C.; Davis, B. G. *J. Am. Chem. Soc.* **2009**, *131*, 16346. (b) Spicer, C. D.; Triemer, T.; Davis, B. G. *J. Am. Chem. Soc.* **2012**, *134*, 800. (c) Spicer, C. D.; Davis, B. G. *Chem. Commun.* **2011**, *47*, 1698.
(8) (a) Li, N.; Lim, R. K. V.; Edwardraja, S.; Lin, Q. *J. Am. Chem. Soc.* **2011**, *133*, 15316. (b) Cheng, G.; Lim, R. K. V.; Li, N.; Lin, Q. *Chem. Commun.* **2013**, *49*, 6809.
(9) Yusop, R. M.; Unciti-Broceta, A.; Johansson, E. M. V.; Sanchez-Martin, R. M.; Bradley, M. *Nat. Chem.* **2011**, *3*, 239−243.
(10) (a) Liu, X.; Xia, Q.; Zhang, Y.; Chen, C.; Chen, W. *J. Org. Chem.* **2013**, *78*, 8531. (b) Liu, X.; Chen, W. *Organometallics* **2012**, *31*, 6614. (c) Gu, S.; Liu, B.; Chen, J.; Wu, H.; Chen, W. *Dalton Trans.* **2012**, *41*, 962. (d) Liu, B.; Chen, C.; Zhang, Y.; Liu, X.; Chen, W. *Organometallics* **2013**, *32*, 5451.
(11) (a) Altenhoff, G.; Goddard, R.; Lehmann, C. W.; Glorius, F. *Angew. Chem., Int. Ed.* **2003**, *42*, 3690. (b) Altenhoff, G.; Goddard, R.; Lehmann, C. W.; Glorius, F. *J. Am. Chem. Soc.* **2004**, *126*, 15195. (c) Organ, M. G.; Calimsiz, S.; Sayah, M.; Hoi, K. H.; Lough, A. *J. Angew. Chem. Int. Ed.* **2009**, *48*, 2383. (d) Lavallo, V.; Canac, Y.; Prasang, C.; Donnadieu, B.; Bertrand, G. *Angew. Chem., Int. Ed.* **2005**, *44*, 5705.
(12) (a) Bauer, E. B.; Andavan, G. T. S.; Hollis, T. K.; Rubio, R. J.; Cho, J.; Kuchenbeiser, G. R.; Helgert, T. R.; Letko, C. S.; Tham, F. S. *Org. Lett.* **2008**, *10*, 1175. (b) Dupuy, S.; Lazreg, F.; Slawin, A. M. Z.; Cazin, C. S. J.; Nolan, S. P. *Chem. Commun.* **2011**, *47*, 5455. (c) Valente, C.; Baglione, S.; Candito, D.; O'Brien, C. J.; Organ, M. G. *Chem. Commun.* **2008**, 735. (d) Kwak, J.; Kim, M.; Chang, S. *J. Am. Chem. Soc.* **2011**, *133*, 3780.
(13) (a) Fleckenstein, C.; Roy, S.; Leuthausser, S.; Plenio, H. *Chem. Commun.* **2007**, 2870. (b) Godoy, F.; Segarra, C.; Poyatos, M.; Peris, E. *Organometallics* **2011**, *30*, 684. (c) Roy, S.; Plenio, H. *Adv. Syn. Catal.* **2010**, *352*, 1014. (d) Moore, L. R.; Cooks, S. M.; Anderson, M. S.; Schanz, H. J.; Griffin, S. T.; Rogers, R. D.; Kirk, M. C.; Shaughnessy, K. H. *Organometallics* **2006**, *25*, 5151.
(14) Chen, C. Y.; Liu, B.; Chen, W. Z. *Synthesis* **2013**, *45*, 3387.
(15) (a) Marion, N.; Navarro, O.; Mei, J. G.; Stevens, E. D.; Scott, N. M.; Nolan, S. P. *J. Am. Chem. Soc.* **2006**, *128*, 4101. (b) Szilvasi, T.; Veszpremi, T. *ACS. Catal.* **2013**, *3*, 1984.
(16) Vargas, V. C.; Rubio, R. J.; Hollis, T. K.; Salcido, M. E. *Org. Lett.* **2003**, *5*, 4847.
(17) Lin, F. L.; Hoyt, H. M.; van Halbeek, H.; Bergman, R. G.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2005**, *127*, 2686.
(18) (a) Hao, Z. Y.; Song, Y. Q.; Lin, S. X.; Yang, M. Y.; Liang, Y. J.; Wang, J.; Chen, P. R. *Chem. Commun.* **2011**, *47*, 4502. (b) Nguyen, D. P.; Lusic, H.; Neumann, H.; Kapadnis, P. B.; Deiters, A.; Chin, J. W. *J. Am. Chem. Soc.* **2009**, *131*, 8720. (c) Fekner, T.; Li, X.; Lee, M. M.; Chan, M. K. *Angew. Chem., Int. Ed.* **2009**, *48*, 1633. (d) Deiters, A.; Cropp, T. A.; Mukherji, M.; Chin, J. W.; Anderson, J. C.; Schultz, P. G. *J. Am. Chem. Soc.* **2003**, *125*, 11782.
(19) (a) Dirksen, A.; Dirksen, S.; Hackeng, T. M.; Dawson, P. E. *J. Am. Chem. Soc.* **2006**, *128*, 15602. (b) Basu, A.; Yang, K.; Wang, M. L.; Liu, S.; Chintala, R.; Palm, T.; Zhao, H.; Peng, P.; Wu, D. C.; Zhang, Z. F.; Hua, J.; Hsieh, M. C.; Zhou, J.; Petti, G.; Li, X. G.; Janjua, A.; Mendez, M.; Liu, J.; Longley, C.; Zhang, Z.; Mehlig, M.; Borowski, V.; Viswanathan, M.; Filpula, D. *Bioconjugate Chem.* **2006**, *17*, 618.
(20) (a) Dumas, A.; Spicer, C. D.; Gao, Z. H.; Takehana, T.; Lin, Y. Y. A.; Yasukohchi, T.; Davis, B. G. *Angew. Chem., Int. Ed.* **2013**, *52*, 3916. (b) Li, J.; Lin, S. X.; Wang, J.; Jia, S.; Yang, M. Y.; Hao, Z. Y.; Zhang, X. Y.; Chen, P. R. *J. Am. Chem. Soc.* **2013**, *135*, 7330.
(21) Bonasio, R.; Carman, C. V.; Kim, E.; Sage, P. T.; Love, K. R.; Mempel, T. R.; Springer, T. A.; von Andrian, U. H. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 14753.
(22) Wang, H.; Koshi, Y.; Minato, D.; Nonaka, H.; Kiyonaka, S.; Mori, Y.; Tsukiji, S.; Hamachi, I. *J. Am. Chem. Soc.* **2011**, *133*, 12220.
(23) (a) Liu, W.; Gust, R. *Chem. Soc. Rev.* **2013**, *42*, 755. (b) Hindi, K. M.; Panzner, M. J.; Tessier, C. A.; Cannon, C. L.; Youngs, W. J. *Chem. Rev.* **2009**, *109*, 3859. (c) Dougan, S. J.; Habtemariam, A.; McHale, S. E.; Parsons, S.; Sadler, P. J. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 11628.