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Resin-Supported Catalysts for CuAAC Click Reactions in Aqueous or Organic Solvents

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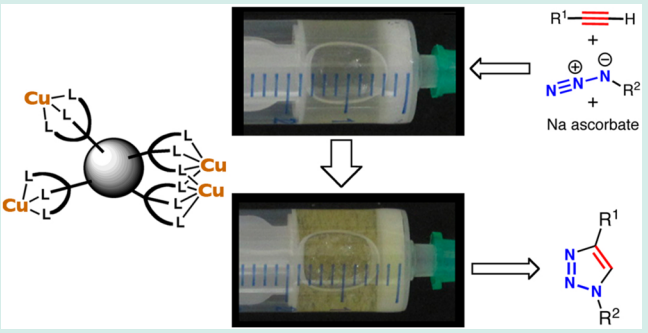
Supporting Information

ABSTRACT: The copper-catalyzed azide−alkyne cycloaddition click reaction is a valuable process for the synthesis of libraries of drug candidates, derivatized polymers and materials, and a wide variety of other functional molecules. In some circumstances, the removal of the copper catalyst is both necessary and inconvenient. We describe here two immobilized forms of a Cu-binding ligand that has been shown to accelerate triazole formation under many different conditions, using different resin supports that are appropriate for aqueous or organic solvents. Copper leaching from these resins was modest, allowing them to be reused in many reaction/filtration cycles without recharging with metal ion. The utility of this catalyst form was demonstrated in the convenient synthesis of 20 *N*-acetylgalactosamine derivatives for biological testing.



KEYWORDS: *resin-supported catalysts, CuAAC click reactions, copper catalyst*

The copper-catalyzed azide−alkyne cycloaddition (CuAAC) reaction[1,2] is well suited to many solution-phase applications with the simple use of Cu(I) salts, either added directly or generated by reduction of Cu(II) or oxidation of Cu(0). This process is approximately one million times faster than the very sluggish uncatalyzed [3 + 2] cycloaddition with unactivated alkynes and azides.[3] When even faster reaction rates are needed, certain heterocyclic chelating agents have been shown to provide strong ligand-accelerated catalysis.[4−6] The intrinsically tolerant nature of Cu catalysts to variations in reaction conditions has allowed the CuAAC process to be applied in an extraordinarily wide range of situations.

Reliable methods for the removal of the catalyst from the reaction mixture are desirable. In the case of CuAAC, which is commonly applied to the synthesis of materials intended for biological application, this can be of great concern because of the perception that copper ions are toxic to cells and organisms. In most cases, we find that washing with or dialysis against solutions of EDTA removes even small traces of copper ions from CuAAC reaction mixtures and isolated products. However, this procedure is not always convenient, especially in parallel or combinatorial synthesis.

A common strategy is to immobilize the catalyst on a support that allows access to reactants and can be easily separated from the reaction mixture upon completion. A handful of reports have appeared of heterogeneous CuAAC catalysts, but this field is not yet fully developed.[7−17] (See Supporting Information for an annotated list of these contributions.) We describe here the synthesis and reactivity of two versions of a system first described by Chan and Fokin, in which an accelerating chelating ligand is covalently attached to a resin material commonly used for solid-phase organic and peptide synthesis.[8]

We have described a highly versatile mono-(benzimidazoylmethyl)-bis(pyridylmethyl) amine ligand (designated **BimPy$_2$**) that performs well in both aqueous and coordinating organic solvents.[6] Its ease of synthesis and readily functionalized single attachment point made it attractive for immobilization, which we performed on two different solid supports as shown in Figure 1. The undecyl alcohol BimPy$_2$ derivative **1** was attached to standard 2-chlorotrityl polystyrene resin (1% divinylbenzene cross-linked, available from several suppliers, 1.04 mmol/g in the exemplary case here, but a variety of loadings between 0.5 and 2 mmol/g work equally well), followed by capping with methanol, to provide the immobilized **PS-BimPy$_2$**. The mass increase of the resin beads indicated a loading of 290 μmol/g. Treatment of the resin with trifluoroacetic acid to cleave the trityl connector, followed by MS analysis, revealed that only the desired ligand had been attached. A negative control resin (**PS-OMe**) was prepared using methanol in place of **1**. To provide immobilized catalyst capability for polar and hydrophilic media, the BimPy$_2$ ligand was also attached to Nova-PEG-amine resin (Novabiochem no. 855126) via an 11-carbon aldehyde (**2**) by reductive amination, to give the **NovaPEG-BimPy$_2$** ligand. The unreacted primary amines and the secondary amines resulting from the coupling were capped with acetic anhydride. A loading of 230 μmol/g

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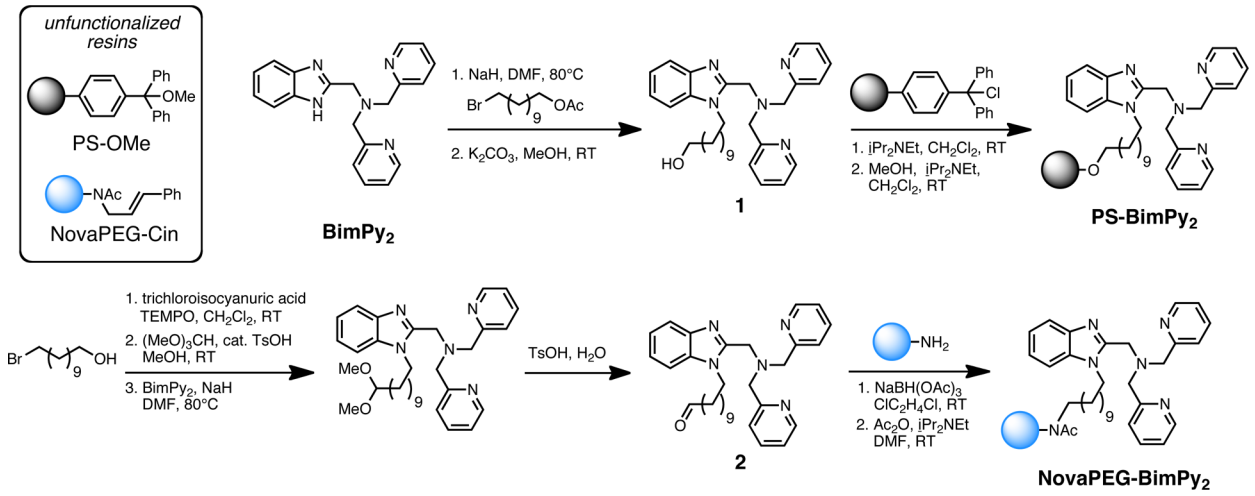

Figure 1. Synthesis of resin-supported $BimPy_2$ CuAAC-accelerating ligands.

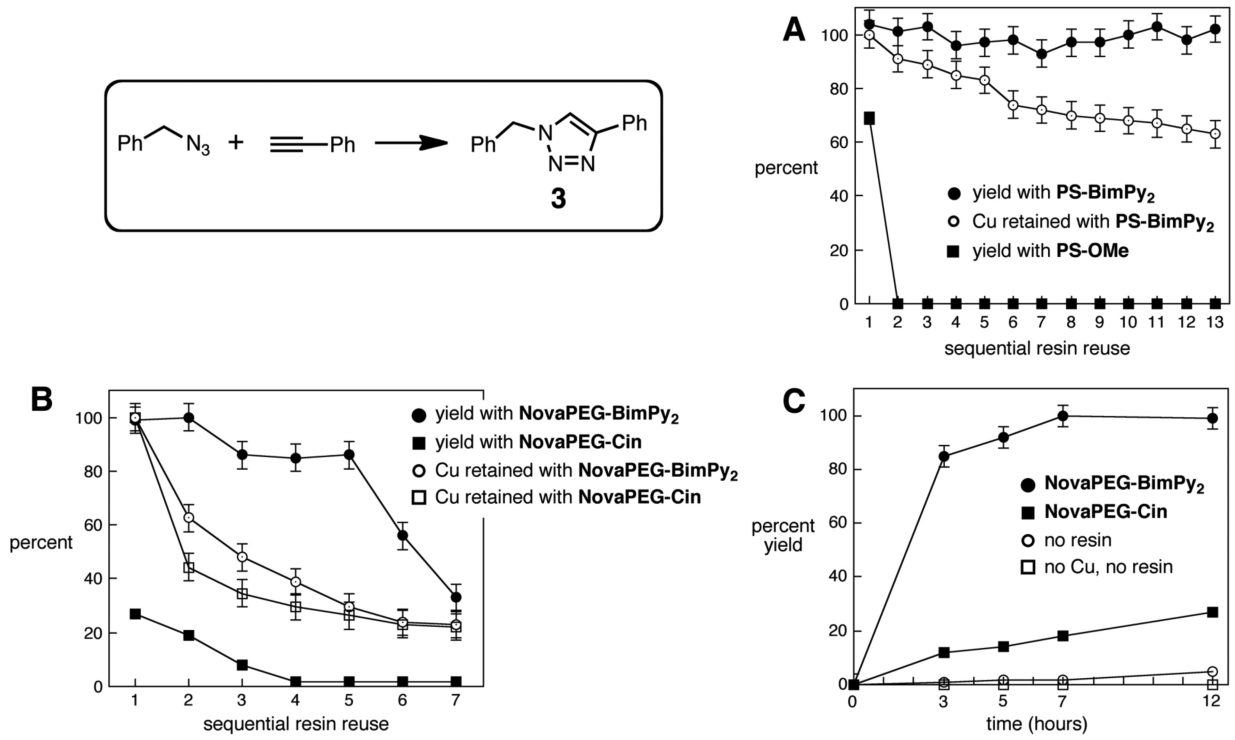

Figure 2. Screening of activity of resin-bound ligands for the model CuAAC reaction shown. (A, B) Amounts of triazole **3** formed (solid circles) and amount of Cu remaining on the resin (open circles) in sequential cycles of CuAAC reaction using 10 mg (A) **PS-BimPy$_2$** or **PS-OMe** with an initial charge of 100 μM $CuSO_4$ or (B) **NovaPEG-BimPy$_2$** or **NovaPEG-Cin** with initial charge of 1 mM $CuSO_4$. Reaction conditions: (A) 1.25 mM BnN_3, 2.5 mM PhCCH, 10 mM Na ascorbate, 95:5 DMF:H_2O, RT, 12 h; (B) 100 mM BnN_3, 100 mM PhCCH, 20 mM Na ascorbate, 90:10 MeOH:H_2O, RT, 12 h. Each reaction was drained, washed three times with methanol, air-dried, and recharged with azide, alkyne, and ascorbate to initiate the next cycle. (C) Time course for reactions mediated by 10 mg of the indicated NovaPEG derivatives; conditions as in the first reaction of plot (B), except for the omission of resin or $CuSO_4$ as noted.

was indicated by the mass increase from the attachment reaction, representing occupancy of approximately 30% of the resin amine groups. Reductive amination with cinnamaldehyde provide the negative control material in this case (**NovaPEG-Cin**). The undecyl hydrocarbon tether was selected in order to allow the ligand enhanced mobility in the swollen resin (gel) state, but other chain lengths and types would likely also be effective. We also explored Sepharose-epoxide as a water-swellable solid support, but the attachment yields were low and the material had insufficient mechanical stability to stand up to the agitation required in repeat reactions.

The polystyrene-based catalysts were initially tested by adding $CuSO_4$, sodium ascorbate, and small amounts of resin (10 mg) to CuAAC reactions in 1.5 mL of 95:5 DMF:H_2O, a solvent system that effectively swells the resin. Yields of triazole **3** were determined the following day by HPLC-MS against a quantitative calibration curve (Figure 2). The reactions were performed in fritted syringes, allowing for easy separation of the solution from the solid-phase material. For this length of

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reaction time, the ligand-free reaction produced relatively high yield but failed to reach completion, presumably because of competitive oxidative inactivation of the Cu(I) catalyst. In contrast, the ligand-bearing resin provided complete reaction, reflecting an accelerated reaction rate.

After removal of the reaction solvent, each of these resins was recharged with another batch of azide, alkyne, and sodium ascorbate, but no additional copper source. Only those resins bearing the $BimPy_2$ ligand produced triazole, showing that the ligand is required to retain the catalytic metal center. The **PS-BimPy$_2$** catalyst was subjected to 13 cycles of CuAAC reaction, filtration, and recharge with azide, alkyne, and ascorbate. The Cu content of the filtered solutions was measured after each cycle by inductively coupled plasma atomic emission spectroscopy (ICP-AES). As shown in Figure 2A, all of these reactions went to completion, and an average of 3% of the Cu was lost in each cycle (approximately 3 μM of Cu in the solution), leaving more than 60% of the initial Cu retained on the resin after the 13th reaction. Note that low-micromolar concentrations of Cu(I) without accelerating ligand do not catalyze the CuAAC reaction under these conditions (data not shown).

When the solvent was changed from DMF to methanol, the overnight reactions catalyzed by the polystyrene-immobilized ligand did not reach completion, probably because of the poor swelling capacity of the solid support. The ligand-modified NovaPEG resin served well in this case, being swelled in methanol (and a wide variety of other solvents) and mediating complete formation of triazole in two consecutive reactions (Figure 2B). In the three subsequent runs, the yields were about 85%, after which performance declined to a greater degree without Cu recharging. Under these "synthetic" conditions (100 mM concentrations of substrates, organic solvent), the greatest amount of Cu contamination was observed, with approximately 400 μM Cu observed in solution after the first reaction. This is likely due in part to the high concentration of triazole formed in this reaction, which competes for Cu with the immobilized ligand. The **NovaPEG-Cin** resin initially induced modest levels of triazole formation (27% yield with 1 mol % Cu), presumably because of some acceleration provided by adventitious Cu ligands such as the amide groups installed on the resin. Following the course of the NovaPEG-based reactions more closely revealed supported $BimPy_2$ to be far more active than the control acylated cinnamoylamine-bearing resin (Figure 2C). When no resin was employed, the reaction gave only a 5% yield of triazole before exhaustion of the reducing agent needed to support CuAAC.

The NovaPEG resin has excellent swelling capacity in both dichloromethane and water. In the former solvent, the use of CuBr and CuI gave incomplete reactions that contained substantial quantities of copper, suggesting that counterion substitution is important in noncoordinating solvents.[18] In aqueous reactions performed under typical bioconjugation conditions, however, 10 mg of **NovaPEG-BimPy$_2$** in 90% H_2O, 10% DMSO with 100 μM $CuSO_4$, and 5 mM NaAsc catalyzed the complete conversion of 100 μM BnN_3 to triazole, when 200 μM PhCCH were used. Low levels of Cu were observed in solution (approximately 5.4 μM, 0.34 ppm, in each cycle). Such minimal leaching makes the resin-immobilized catalyst an excellent choice for library syntheses of compounds that can be used in many biological tests without concern for significant copper contamination.

The **NovaPEG-BimPy$_2$** ligand was tested in preparatory-scale reactions involving glycan precursors **4** and **6** with ten azides and alkynes each, including aromatic, aliphatic, and benzylic examples (Figure 3).[19] All triazole products were isolated in >90% yields after filtration and flash column chromatography, in purities >95% as judged by ^{1}H NMR.

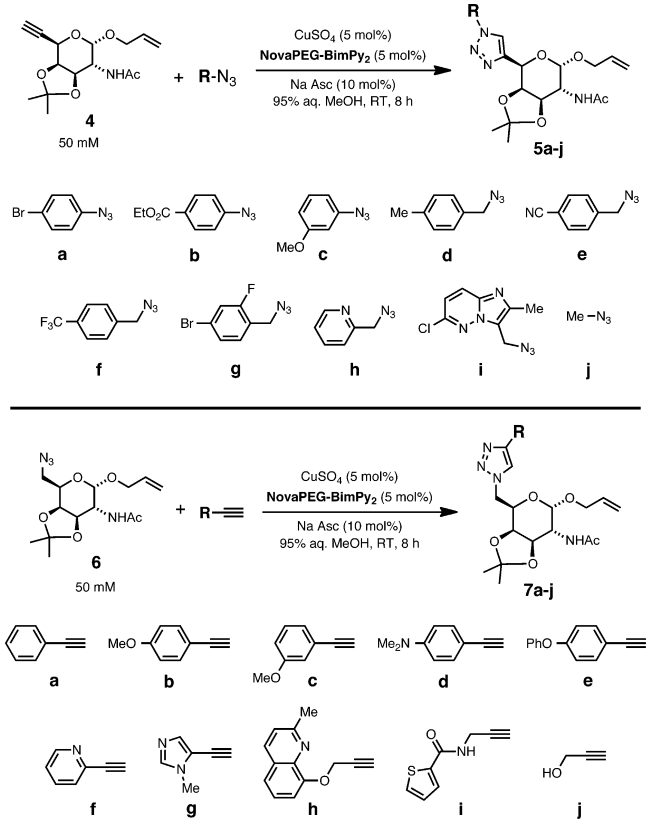

Figure 3. Parallel CuAAC reactions performed with **NovaPEG-BimPy$_2$** catalyst.

We demonstrate here that the resin immobilization of the $BimPy_2$ ligand provides readily accessible and useful catalysts for the CuAAC reaction in both organic and aqueous solvents. These systems show convenient properties of rate, metal retention, and separability from soluble reaction components. The previously reported and very useful resin of Chan and Fokin[8] employs the tris(triazolylmethyl)amine ligand, which is a weaker binder of Cu^I than $BimPy_2$.[6] For this reason, this ligand is not well suited to reactions in strongly coordinating solvents. If the user intends to perform reactions in DMSO, DMF, or similar solvents, **PS-BimPy$_2$** is an effective and inexpensive catalyst. When polar protic and aqueous solvent systems are desired, we recommend **NovaPEG-BimPy$_2$** due to its superior swelling properties. For all other solvents except acetonitrile (which gives poor results with this class of catalysts; see Figure 5 of reference 6), both ligand-functionalized resins should provide good results if the solvent swells the resin to a sufficient degree.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Table showing heterogeneous or supported CuAAC catalysts with typical reaction conditions, description of the synthesis of resin-supported ligands, screening of resin−ligand activity, and use of NovaPEG-$BimPy_2$ in synthesis, and characterization data

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including NMR spectra. 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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