Organic & Biomolecular Chemistry

Cite this: *Org. Biomol. Chem.*, 2011, **9**, 7667

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COMMUNICATION

Catch and release of alkyne-tagged molecules in water by a polymer-supported cobalt complex†

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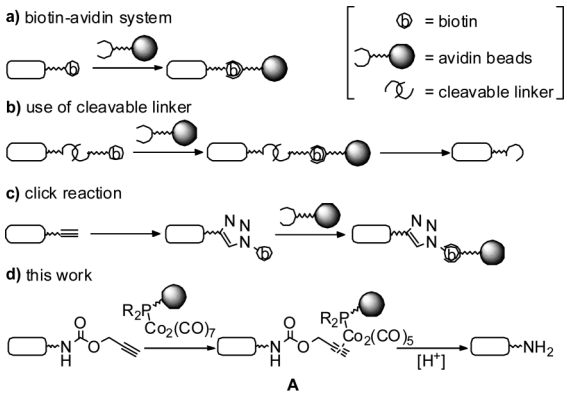
Received 8th July 2011, Accepted 18th August 2011
DOI: 10.1039/c1ob06123b

A cobalt–phosphine complex supported on PS-PEG beads was found to react with a propargyl carbamate tag, and the tagged molecules immobilized on the beads could be released by acidic treatment through the Nicholas reaction pathway. These reactions work in aqueous media at 4 °C, so that this catch and release procedure is compatible with conditions generally used in biochemical experiments.

Enrichment of a specific molecule from complex mixtures, such as cell lysates, is indispensable for chemical biology studies, *e.g.* to identify a binding protein or the protein binding site of a low-molecular-weight bioactive compound. The development of methods for the enrichment of various target molecules is consequently of great importance, and the topic has attracted the attention of many researchers.[1] Affinity purification using a combination of a biotin tag and immobilized avidin is widely used nowadays (Scheme 1a).[2,3] However, two issues have been found with this technique: 1) harsh conditions are required to release tagged molecules from avidin beads, due to the extremely high affinity between biotin and avidin;[4] and 2) the bioactivity and membrane permeability of bioactive compounds are often decreased by the introduction of biotin with a large linker. The former problem has been addressed by the introduction of a cleavable linker, such as disulfide,[5] peptide,[6] diazobenzene,[7] hydrazone[8] or acid-cleavable linker[9] (Scheme 1b). However, the efficiency and selectivity of these reactions are not necessarily adequate for chemical biology studies. The latter problem has been overcome by the introduction of click reactions, such as the Sharpless–Meldal reaction, but these are indirect and multistep procedures (Scheme 1c).[10]

Alkyne is now widely used as a bioorthogonal tag in chemical biology. Various functionalities such as fluorophore and biotin can easily be installed by means of a Cu-mediated click reaction,[9] and can be used for the detection and enrichment of tagged molecules. Recently, Brown and co-workers reported the reaction of an alkyne-modified phospholipid with $Co_2(CO)_8$ in organic solvents and subsequent purification of the resulting alkyne cobalt complex using phosphine-functionalized silica gel.[11] Organic solvents and elevated temperature were required for the formation of the alkyne–cobalt complex followed by its reaction with phosphine, and an oxidant was required for release of the phospholipid. Independently we have been working on the development of click- and biotin-free methods for the detection and enrichment of alkyne-tagged molecules useful for chemical biology research. We have already reported a direct detection method of alkyne-tagged molecules in living cells by using Raman microscopy.[12] Herein, we disclose a new catch and release method for the enrichment of alkyne-tagged molecules using a polymer-supported cobalt complex in water.



Scheme 1 Outline of catch and release reactions.

For the application to the enrichment of alkyne-tagged biomolecules, *e.g.* peptides and proteins, catch and release reactions that are highly selective to the alkyne are required. The reactions should also be carried out in aqueous media in the presence of various functional groups under mild conditions. Thus, we decided to use an alkyne–cobalt carbonyl complex as a key intermediate to meet the above criteria. It is well known that $Co_2(CO)_8$ and its phosphine complex readily and selectively react with alkyne, and the resulting complexes are usually stable at room temperature under air.[13] First, we tested reaction sequences similar to the ones described in Brown's report. Namely, the reactions of alkyne with $Co_2(CO)_8$ in an aqueous buffer were examined. But only a small amount of the desired

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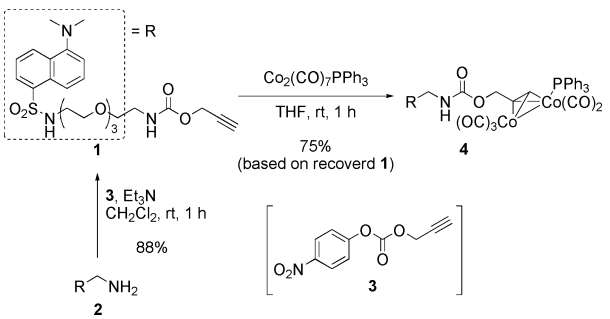
† Electronic supplementary information (ESI) available: Experimental details and spectroscopic data. See DOI: 10.1039/c1ob06123b

$Co_2(CO)_6$–alkyne complex was generated, and the reproducibility was low, probably because $Co_2(CO)_8$ is not soluble in water and is unstable under aerobic aqueous conditions.[11] In addition, the oxidative conditions required for decomplexation of the cobalt complex may cause damage to some of the amino acid residues in proteins. Therefore, we decided to use the polymer-supported cobalt complex and the propargyl carbamate tag, anticipating that it would form the polymer-supported alkyne–cobalt complex **A** (Scheme 1d). The bound molecules would be released as an amine compound through the Nicholas reaction in the presence of a Brønsted acid.[14,15] In this paper, we present proof-of-concept of this direct catch and release procedure for alkyne-tagged molecules in water, using the cobalt complex supported on PS-PEG. These mild conditions should be applicable to biomolecules.

Although cobalt carbonyl complexes have been intensively studied, there is little information about the reactivity and properties of cobalt carbonyl complexes in water. Thus, we first examined the stability and reactivity of the alkyne–cobalt–phosphine complex in water. We prepared a dansyl derivative **1** with the triethylene glycol-linked propargyl carbamate tag as a model substrate (Scheme 2). The propargyl carbamate tag was introduced into amine **2** by using the activated carbonate **3**. Subsequently the alkyne–cobalt–phosphine complex **4** was prepared by the reaction of alkyne **1** with $Co_2(CO)_7PPh_3$ complex in THF at room temperature. The stability and reactivity of cobalt complex **4** in water were



Scheme 2 Synthesis of model substrate.

traced by HPLC analysis with detection at 280 nm to minimize background peaks (Fig. 1). In the presence of 5% trifluoroacetic acid (TFA), the reaction proceeded smoothly even at 4 °C to give the corresponding amine **2** in 98% yield, together with a small amount (2%) of **1** (Fig. 1a, b). Complex **4** was shown to be stable under neutral aqueous conditions (Fig. 1b). To examine whether **2** was formed directly from **4** *via* the Nicholas reaction, or by stepwise decomplexation and hydrolysis of **1**, **1** was treated with 5% TFA. No reaction was observed, indicating that amine **2** was obtained *via* the Nicholas reaction pathway from complex **4**. The Nicholas reaction is expected to selectively cleave the C–O bond at the α-position of the alkyne–cobalt–phosphine complex. Furthermore, under the same conditions, we observed formation and gradual decomposition of the cobalt complex **5**, which is also a Nicholas reaction product, by means of LC–MS analysis.

Encouraged by these results, we next attempted to prepare the polymer-supported cobalt complex. We employed PS-PEG, because it has been utilized as a polymer support for catalysts in aqueous media and is also tolerant to organic solvents.[16] PS-PEG-supported phosphine **6** was synthesized according to a reported

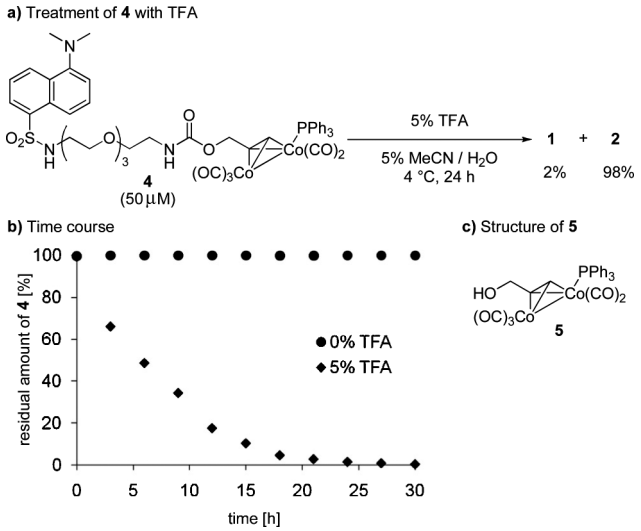
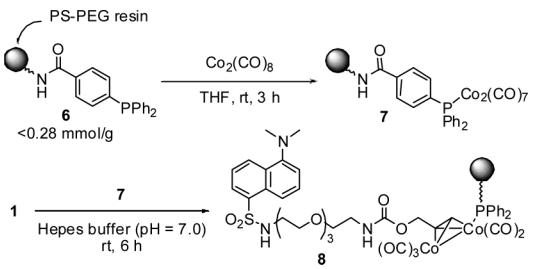


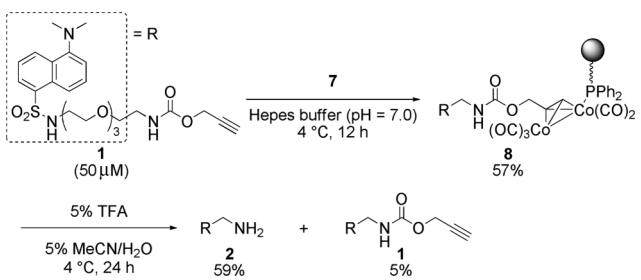
Fig. 1 Stability and reactivity of the cobalt complex **4**.

procedure[16b] and cobalt–phosphine complex **7** was prepared by mixing **6** and $Co_2(CO)_8$ in THF. The reaction of cobalt complex **7** supported on PS-PEG with alkyne **1** was examined in an aqueous Hepes buffer (pH 7.0),[17] which is often employed in biochemical experiments (Scheme 3). As expected, the reaction proceeded to give alkyne–cobalt–phosphine complex **8** at ambient temperature. Formation of cobalt complex **7** and its alkyne complex **8** was characterized by IR spectroscopy. Absorptions of the carbonyl groups of polymer-supported complexes **7**[18] and **8** are in good agreement with those of $Co_2(CO)_7PPh_3$, the corresponding non-polymer-supported complex **4**, and similar known cobalt complexes[19] (Fig. 2).

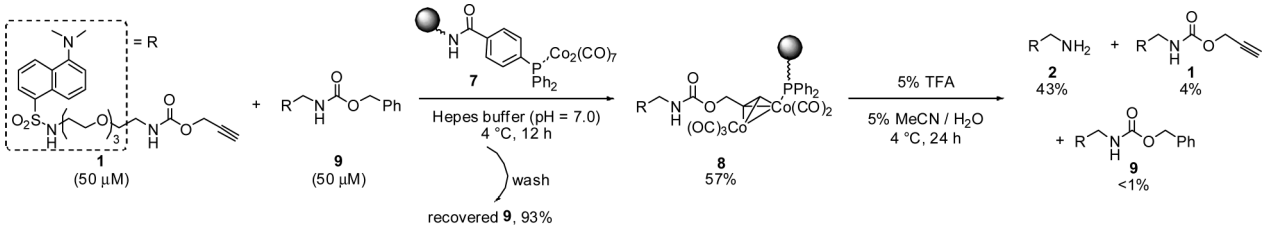
The above results prompted us to investigate the catch and release reaction of alkyne **1** on cobalt beads **7**. Since the reaction would generally be operated at low concentration and at low temperature in biological experiments, the reaction of alkyne **1** with



Scheme 3 Synthesis of the cobalt-alkyne complex supported on PS-PEG in water.



Scheme 4 Catch and release of **1** at high dilution.



Scheme 5 Enrichment of the alkyne-tagged molecule.

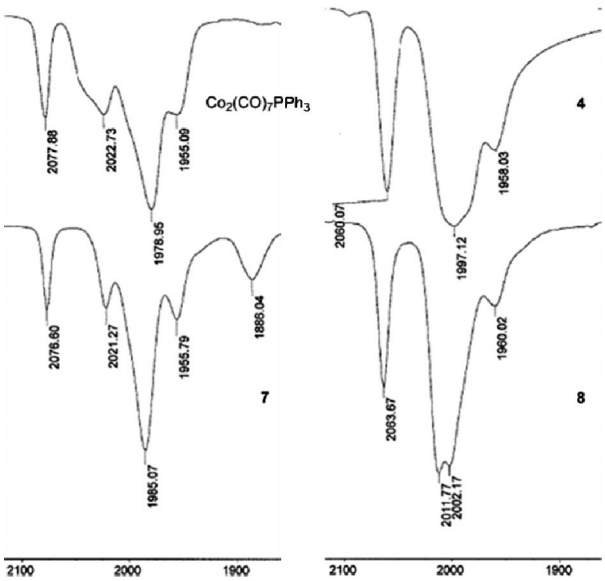


Fig. 2 IR spectra of cobalt complexes.

cobalt beads **7** was carried out at 4 °C on a 50 nmol scale (50 μM). The catch reaction was monitored by fluorescence analysis of the supernatant after removal of the beads by centrifugation, and the release reaction was separately monitored by HPLC analysis to detect the released product (Scheme 4). To our delight, alkyne **1** reacted with cobalt complex **7** even under low concentration and low temperature conditions, and 57% of the alkyne was held on to the resin after it had been washed ten times with Hepes buffer. Finally, amine **2** was obtained in 59% yield after treatment with TFA.

Furthermore, in order to examine the selectivity of the catch and release reactions, a mixture of equal amounts of alkyne-tagged molecule **1** and its benzyl derivative **9** was treated with cobalt resin **7** (Scheme 5). Again, 57% of alkyne **1** was caught by the cobalt resin, while 93% of **9** and 43% of **1** were recovered in the residual and wash solutions. Subsequently, treatment of the resin with TFA afforded the Nicholas product **2** in 43% yield together with 4% of starting alkyne **1**. It is noteworthy that only a negligible amount of benzyl derivative **9** was detected. Hence, great enrichment of alkyne-tagged molecule **1** has been achieved.

In summary, we have demonstrated direct and selective catch and release reactions of alkyne-tagged molecules utilizing a polymer-supported cobalt complex under mild conditions. To our knowledge, this is the first example of direct catch and release reactions of alkyne-tagged molecules in water. These proof-of-concept experiments strongly indicate that this cobalt chemistry would be in principle applicable to chemical biology studies. Further optimization is under way in our laboratory prior to application of this new method to the enrichment of biomolecules, such as peptide and proteins.

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