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A "Catch-and-Release" Protocol for Alkyne-Tagged Molecules Based on a Resin-Bound Cobalt Complex for Peptide Enrichment in Aqueous Media

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Abstract: The development of new and mild protocols for the specific enrichment of biomolecules is of significant interest from the perspective of chemical biology. A cobalt–phosphine complex immobilised on a solid-phase resin has been found to selectively bind to a propargyl carbamate tag, that is, "catch", under dilute aqueous conditions (pH 7) at 4 °C. Upon acidic treatment of the resulting resin-bound alkyne–cobalt complex, the Nicholas reaction was induced to "release" the alkyne-tagged molecule from the resin as a free amine. Model studies revealed that selective enrichment of the alkyne-tagged molecule could be achieved with high efficiency at 4 °C. The proof-of-concept was applied to an alkyne-tagged amino acid and dipeptide. Studies using an alkyne-tagged dipeptide proved that this protocol is compatible with various amino acids bearing a range of functionalities in the side-chain. In addition, selective enrichment and detection of an amine derived from the "catch and release" of an alkyne-tagged dipeptide in the presence of various peptides has been accomplished under highly dilute conditions, as determined by mass spectrometry.

Introduction

The selective enrichment of biomolecules is important in chemical proteomics and pharmaceutical drug research to elucidate small molecule–protein interactions and to gain a better understanding of biological processes.[1] In particular, the "catch-and-release" approach for the specific enrichment of a biomolecule from complex mixtures, for example, cell lysates, is a widely used technique that has found many applications in chemical biology.[2] Affinity purification, such as the use of avidin or streptavidin beads to "catch" biotin-labelled molecules has been used in many proteomic studies to identify the binding site of bioactive molecules in proteins (Scheme 1a).[3] Considerable research has been carried out in this field to overcome the harsh, denaturing and destructing conditions that are usually required to disrupt the strong biotin–avidin interaction and "release" the molecule from the beads.[4] For example, various types of cleavable linkers between the biotin tag and the site of attachment on the probe molecule, such as disulfide,[5] diazobenzene,[6] hydrazone,[7] protease-labile,[8] acid/base-labile[9] and photolabile[10] cleavable linker systems, have been introduced (Scheme 1b). On the other hand, the reduction in bioactivity and membrane permeability of bioactive molecules arising from the attachment of biotin to a larger linker has been overcome by the development of click reactions, such as the Sharpless–Meldal reaction (Scheme 1c).[11] The incorporation of cleavable linkers circumvents the issue of having to use harsh conditions to disrupt the biotin–avidin interaction after the click reaction (Scheme 1 d). Nevertheless, as these procedures involve multiple steps, further development of a direct, mild and selective method for the efficient catch and release of biomolecules is of significant interest from a chemical biology perspective.

Recent advances in click chemistry have led to the recognition of the alkyne moiety as a useful bio-orthogonal tag due to its selective reactivity towards specific functional groups, along with minimal structural perturbation upon introduction into a bioactive molecule. One of the most prominent examples is the copper-mediated Huisgen cycloaddition reaction between an azide and an alkyne, which has led to the successful installation of various functionalities, such as fluorophores and biotin.[11] Alkynes are also known to selectively react with octacarbonyldicobalt(0), $[Co_2(CO)_8]$, to form stable alkyne(hexa-

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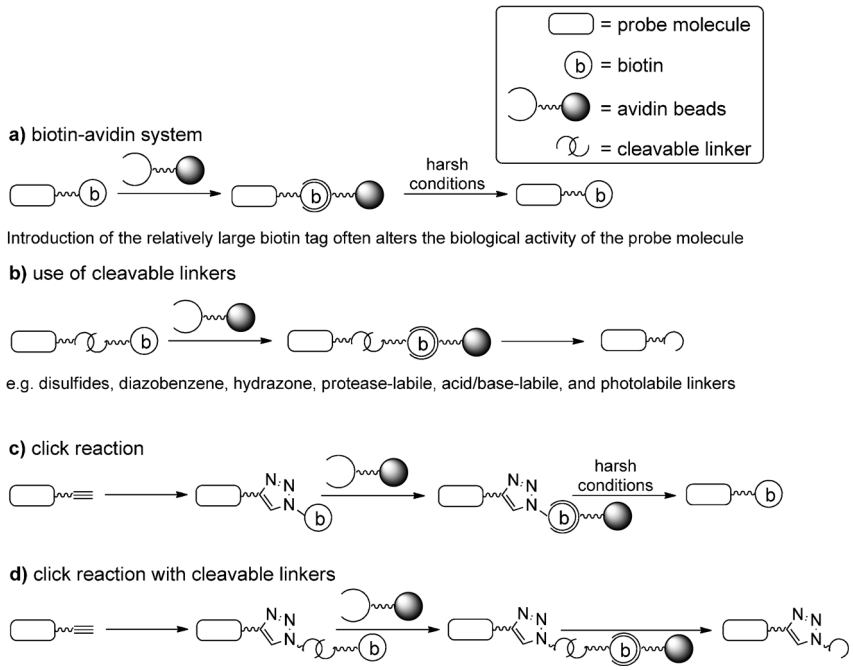


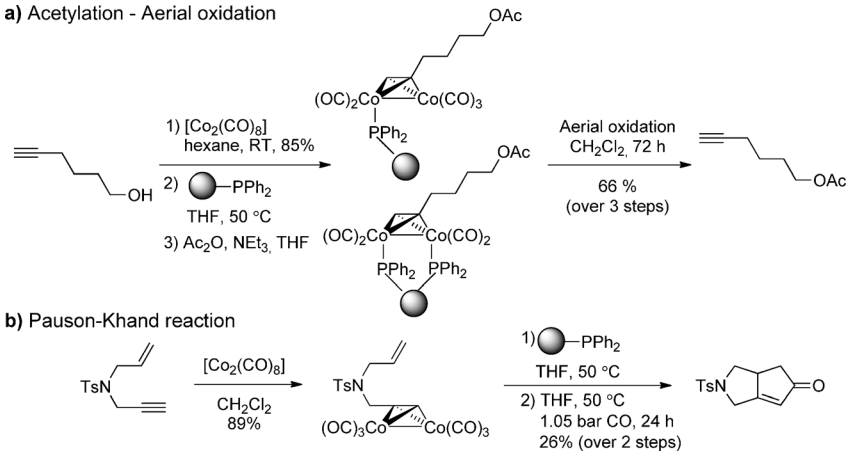
Scheme 1. Common strategies for catch-and-release reactions using the biotin–avidin system.

carbonyl)dicobalt complexes upon loss of two carbonyl ligands, and this chemistry has been extensively studied since it was first reported over 50 years ago. The broad scope of alkyne–cobalt chemistry is demonstrated by the vast number of reports on alkyne protection, the Nicholas reaction,[12,13] which makes use of the stabilised propargylic cation, and the Pauson–Khand reaction, which yields cyclopentenone rings,[14,15] as well as the activity of the complex as an antitumour agent.[16] Cobalt–alkyne complexes have been mainly studied in the solution phase with organic solvents, with its reactivity in the solid phase first being reported by Gibson and co-workers in 1999.[17] A polymer-bound triphenylphosphine moiety was used to immobilise an alkyne–cobalt complex in THF at 50 °C. A hydroxy moiety on the alkyne was acylated on the resin and then the alkyne was released under oxidative conditions (Scheme 2a). In addition, an enyne was subjected to the Pauson–Khand reaction on a phosphine-linked resin to generate a bicyclic cyclopentenone (Scheme 2b).

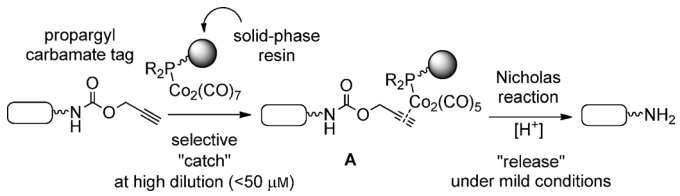
In 2010, Brown and co-workers reported the purification of an alkyne-modified phospholipid by using $[Co_2(CO)_8]$ and phosphine-functionalised silica gel via the formation of an alkyne–cobalt complex in methanol/chloroform at a high temperature.[18] The reactions were carried out in organic solvents on a millimolar scale and $Fe(NO_3)_3$ was used as an oxidant to release the alkyne-derivatised phospholipid from the cobalt–phosphine resin. Covering a broad range of biomolecules, we have been independently investigating the development of a mild and non-oxidative catch-and-release protocol based on alkyne–cobalt chemistry and reported the preliminary results of model studies in 2011.[19] Herein we report full details of the development of an efficient and mild catch-and-release method for the enrichment and detection of alkyne-tagged molecules by using a polymer-supported cobalt complex, which allows reactions to be carried out in dilute aqueous media (Scheme 3).

Results and Discussion

To establish a method for the selective enrichment of alkyne-tagged biomolecules, for example, peptides and proteins, catch-and-release reactions that are highly selective with respect to the alkyne are required. The reactions should be carried out under mild conditions in aqueous media at low molar concentrations and low temperature, as well as be compatible with a wide range of functional groups. Thus, we envisioned that the use of an alkyne(carbonyl)cobalt complex as a key intermediate would meet the above criteria. It is well known that $[Co_2(CO)_8]$ and its phosphine complex react readily and selectively with alkynes, and the resulting complexes are usually



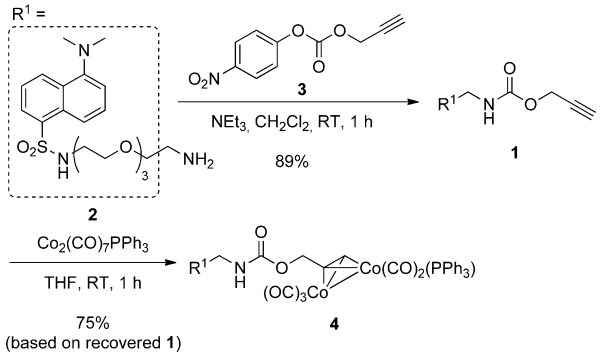
Scheme 2. Organic transformations of a polymer-bound alkyne(carbonyl)cobalt(0)–phosphine complex in organic solvents.



Scheme 3. Catch-and-release protocol in aqueous media at high dilution based on alkyne–cobalt chemistry.[19]

bench-stable at ambient temperature in the absence of strong oxidising agents. So far, the majority of the studies conducted on alkyne–cobalt complexes have been in organic solvents, and information on their properties in aqueous media has been rather limited. After investigating the reactivity and stability of cobalt complexes in water, we decided to study a polymer-supported cobalt complex and a propargyl carbamate tag, anticipating that it would form the polymer-supported alkyne–cobalt complex **A** linked by a phosphine ligand (Scheme 3). In the presence of a Brønsted acid, the bound molecule is expected to be released as an amine derivative through the Nicholas reaction.[20] By prior coordination of a phosphine resin to cobalt, we envisaged that the stabilised cobalt–phosphine resin would lead to efficient alkyne–cobalt complexation under aqueous conditions. Release by a Brønsted acid overcomes the issue of having to use oxidative conditions to decomplex the cobalt moiety, which have a tendency to cause damage to certain amino acid residues, such as the sulfur-containing cysteine moieties in peptides. Furthermore, many of the nucleophilic functionalities that are present in amino acid side-chains are known to be relatively stable under acidic conditions.

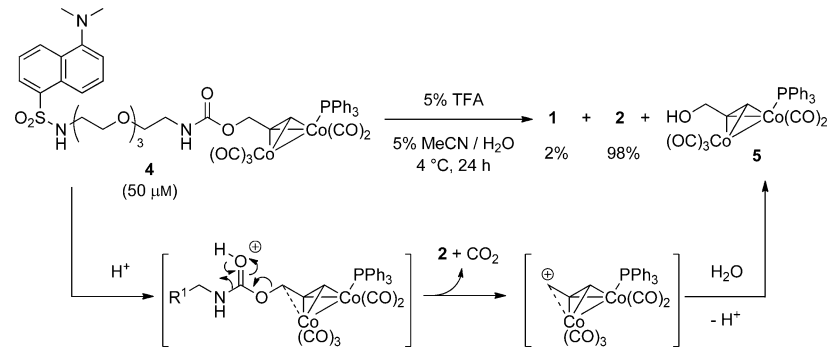
Thus, we prepared dansyl derivative **1** bearing a triethylene glycol linked propargyl carbamate tag as a model substrate (Scheme 4). The propargyl carbamate tag was introduced into amine **2** by using the activated carbonate **3**, and subsequent



Scheme 4. Synthesis of the model dansyl substrate **1** and its cobalt complex.

reaction of alkyne **1** with $[Co_2(CO)_7(PPh_3)]$ in THF at room temperature afforded alkyne–cobalt complex **4**.

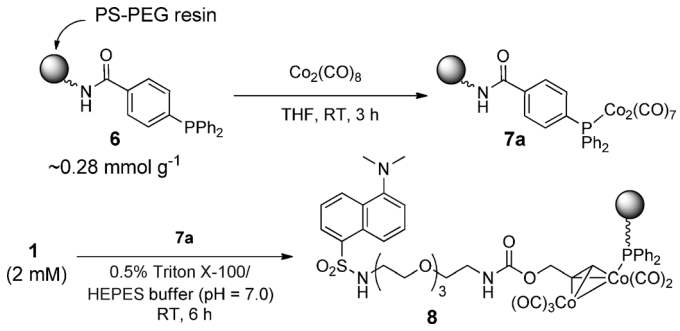
The stability and reactivity of alkyne–cobalt complex **4** in aqueous media were monitored by HPLC with UV detection at 280 nm. Upon treatment of cobalt complex **4** (50 μM) with 5% trifluoroacetic acid (TFA) in 5% MeCN/H_2O, the Nicholas reaction proceeded smoothly to provide the corresponding amine **2** in 98% yield at 4 °C (Scheme 5). In the absence of TFA, complex **4** appeared to be stable under neutral aqueous condi-



Scheme 5. Reactivity of cobalt complex **4**.

tions. Other additives, such as tetrabutylammonium fluoride,[21] ethylenediamine,[22] 2-aminoethanol[22] and cyclohexylamine,[23] which are known to decomplex alkyne–cobalt complexes in organic solvents, showed diminished decomplexation ability with complex **4** in water. When free alkyne **1** was treated with TFA, no reaction was observed, which indicates that amine **2** was indeed obtained by the Nicholas reaction from complex **4**. The Nicholas reaction is expected to selectively cleave the C–O bond at the α position of the alkyne(phosphine)cobalt complex. The cobalt stabilises the positive charge at the α position, which is subsequently captured by a water molecule. This is supported by the observation that upon treatment of complex **4** with TFA, LC-MS revealed the formation and gradual decomplexation of cobalt complex **5**, which is the other product arising from the Nicholas reaction.

Encouraged by these results, we next attempted to prepare the polymer-supported cobalt complex in the solution phase. We employed the TentaGel PS-PEG (polystyrene-polyethylene glycol) resin as it has been used as a polymer support for catalysts in aqueous media and is also compatible with organic solvents due to its good swelling ability. TentaGel®-supported phosphine **6** was synthesised according to a reported procedure,[24] and subsequently the brown phosphine–cobalt complex **7a** was prepared by mixing **6** and $[Co_2(CO)_8]$ in THF at room temperature.[17c,19] The reaction of cobalt complex **7a** supported on TentaGel with alkyne **1** (2 mM) was carried out in aqueous HEPES buffer (pH 7.0)[25] containing 0.5% Triton X-100. As expected, the reaction proceeded to provide alkyne(phosphine)cobalt complex **8** after 6 h at room temperature (Scheme 6). The cobalt–phosphine complex **7a** and its alkyne complex **8** were characterised by IR spectroscopy. The IR absorptions of the carbonyl groups of the polymer-supported complexes **7a** and **8** showed good correlation with those of



Scheme 6. Synthesis of the alkyne–cobalt complex **8** supported on TentaGel® PS-PEG in water.

$[Co_2(CO)_7(PPh_3)]$, the corresponding non-polymer-supported complex **4** and similar known cobalt complexes (see Figure S4 in the Supporting Information).[17c,19,26]

The above results prompted us to investigate the catch-and-release reaction of alkyne **1** on cobalt beads **7a** at a low molar concentration (50 nmol scale, 50 μM) and low temperature (4 °C). The catch reaction was monitored by HPLC of the supernatant after removal of the beads by centrifugation, and the subsequent release reaction was separately monitored to detect amine **2** (Table 1). Pleasingly, alkyne **1** reacted with TentaGel-based cobalt beads **7a** at low concentration and low temperature, and 57% of the alkyne was complexed to the cobalt resin after it had been washed 10 times with HEPES buffer (pH 7.0) containing 0.5% Triton X-100. Finally, amine **2** was obtained in a yield of 59% after treatment of the resin with 5% TFA for 24 h at 4 °C (Table 1, entry 1).

Table 1. Results of the catch and release of alkyne **1** with various resins.[a]

Entry	Resin	Catch of alkyne **1** [%]	Release of amine **2** [%]	Recovery of alkyne **1** [%]
1	TentaGel **7a**	57	59	5
2	CLEAR **7b**	75	72	8
3	PEGA **7c**	96	19	4
4	Toyopearl **7d**	79	14	1
5	ChemMatrix **7e**	50	6	0
6[b,c]	TentaGel **7a**	66	70	0
7[b,c,d]	TentaGel **7a**	73	71	0

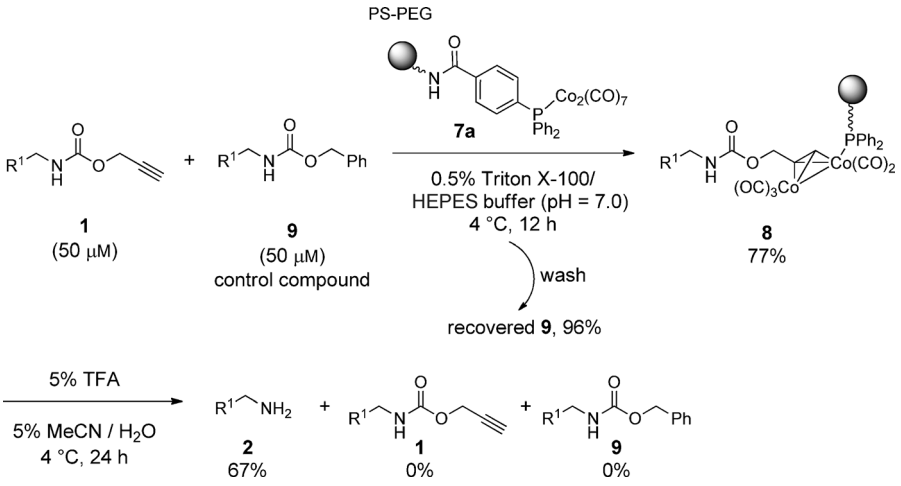
[a] Standard procedure: cobalt beads **7** in alkyne **1** solution (50 μM) in 0.5% Triton X-100/HEPES buffer (pH 7.0) rotated at 4 °C for 12 h. After removal of the supernatant and washing of the beads with 0.5% Triton X-100/HEPES buffer (pH 7.0, 10 times), the beads were treated with 5% TFA in 5% MeCN/H_2O and rotated at 4 °C for 24 h. [b] Cobalt beads **7** pre-washed with HEPES buffer (pH 7.0) before alkyne complexation (catch). [c] Washing conditions after catch: 50% EtOH/HEPES buffer (three times), 50% MeCN/HEPES buffer (three times) and 5% MeCN/H_2O (twice). [d] Alkyne **1** solution (50 μM) in 1.25% EtOH/HEPES buffer (pH 7.0).

Other resins such as CLEAR (cross-linked ethoxylate acrylate resin), PEGA (polyacrylamide-PEG resin), Toyopearl (methacrylic polymer resin) and ChemMatrix (100% PEG-based resin), which have been demonstrated to be hydrophilic and to exhibit good swelling properties, were also investigated. A phosphine linker was attached to each resin and was subsequently treated with $[Co_2(CO)_8]$ in accord with Scheme 6. Cobalt–phosphine complexation was detected in most cases, as shown by similar IR absorptions with respect to the TentaGel® resin (see Figures S4 and S5 in the Supporting Information). However, amine **2** was detected in low yields upon treatment of the alkyne–cobalt resins **8** based on PEGA, Toyopearl and ChemMatrix with 5% TFA (Table 1, entries 3–5). This may be attributed to differences in the physical properties of the resins, such as swelling ability, stability and compatibility with cobalt. In addition, according to HPLC, a significant amount of leaching of resin material was observed after acidic treatment with other resins, including CLEAR.[27] When glass beads (non-porous silica microspheres) were used, cobalt–phosphine complexation resulted in the beads having a purple appearance, which suggests that oxidation of $[Co_2(CO)_8]$ had occurred. Overall, TentaGel provided the most efficient and reproducible results for catch-and-release and was used in further studies.[28]

Further optimisation of the reaction conditions for catch-and-release was carried out. When cobalt beads **7a** were used directly for alkyne complexation without any pre-washing of the beads, varying amounts of amine (0–20%) were observed after analysing the supernatant and washing solutions after alkyne complexation. This is presumed to be due to the slightly acidic environment arising from the presence of cobalt ions on the resin, leading to release of the amine from the alkyne–cobalt complex. Consequently, pre-washing of the beads after the complexation of $[Co_2(CO)_8]$ with the phosphine beads was attempted, and it was found that the pre-washing of cobalt beads **7a** with HEPES buffer (pH 7.0) led to an improvement of the alkyne catch to 66% (Table 1, entry 6). In addition, the catch yield was improved to 73% when 1.25% EtOH/HEPES buffer (pH 7.0) was used as solvent (Table 1, entry 7). Subsequent treatment of alkyne–cobalt beads **8** with 5% TFA led to 71% release of amine **2**. In addition, to wash out non-specific binding molecules more efficiently, the washing conditions following the formation of the alkyne–cobalt complex **8** were examined. Hardly any alkyne **1** was detected after the release reaction with TFA when alkyne–cobalt beads **8** were washed with 50% EtOH/HEPES buffer (pH 7.0) followed by 50% MeCN/HEPES buffer and 5% MeCN/H_2O after the catch reaction.

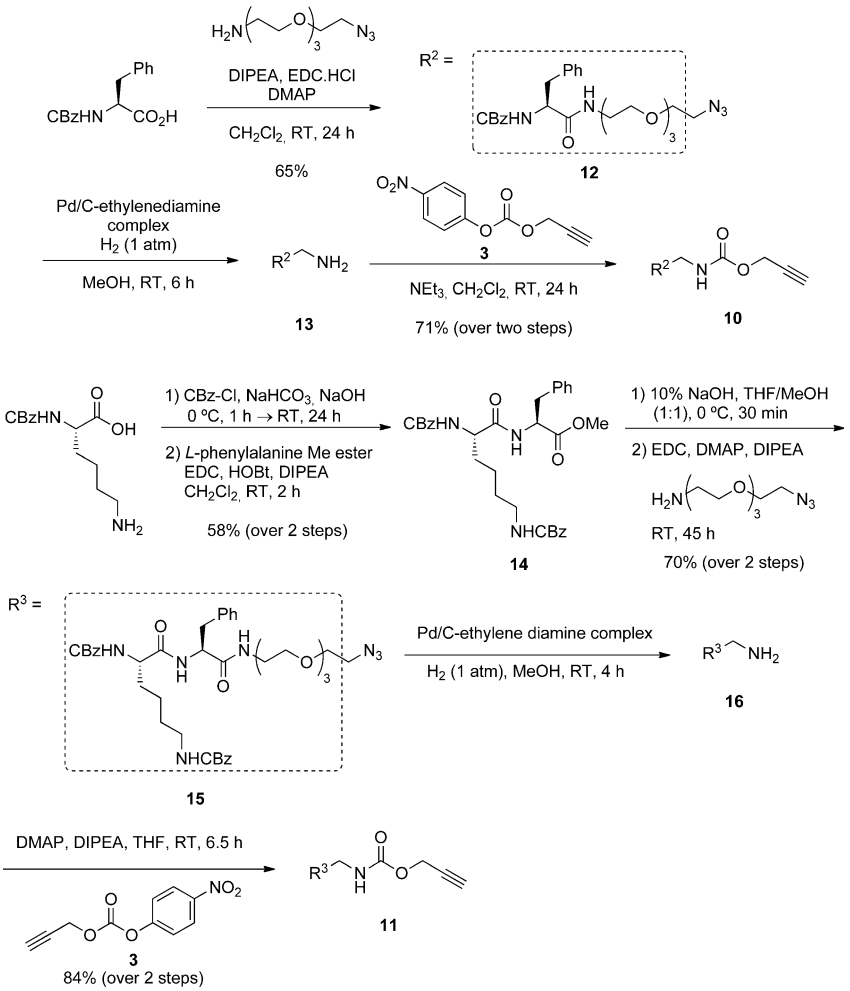
To examine the selectivity of the catch-and-release reactions, a mixture of equimolar amounts of alkyne-tagged substrate **1** and a non-alkyne benzyl derivative **9** was treated with cobalt resin **7a**, pre-washed with HEPES buffer (Scheme 7).[29] Again, 77% of alkyne **1** was captured by the cobalt resin, with 96% of the control compound **9** and 23% of alkyne **1** being recovered in the supernatant and washing solution. Subsequent treatment of the resin with 5% TFA generated the Nicholas product **2** in a yield of 67%. Neither the benzyl derivative **9** nor alkyne **1** was detected after release. These results indicate that the cobalt beads are highly selective towards the alkyne molecule and that specific enrichment has been achieved.

With the success of the model substrate in hand, we decided to extend this methodology to biomolecules such as amino acids and peptides. Alkyne-derivatised phenylalanine **10** and



Scheme 7. Catch and release of an alkyne-tagged molecule **1** using TentaGel® PS-PEG resin in the presence of control compound **9**.

lysine-phenylalanine dipeptide **11** were prepared according to Scheme 8. To monitor the progress of the reactions by HPLC with UV detection at 215 nm, substrates bearing CBz (Z) groups were selected as substrates.

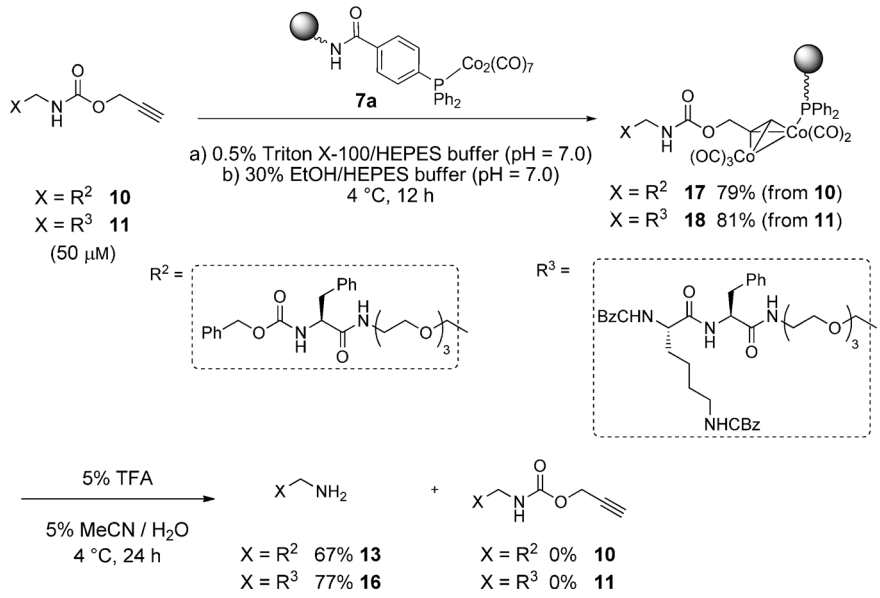


Scheme 8. Synthesis of alkyne-tagged amino acid **10** and dipeptide **11**.

Catch-and-release reactions were performed with alkyne-tagged amino acid **10** and dipeptide **11** under the optimised conditions. Amino acid **10** was dissolved in HEPES buffer (pH 7.0) containing 0.5% Triton X-100. As a result of the hydrophobicity of dipeptide **11** and thus its insolubility in HEPES buffer containing 0.5–1% Triton X-100, dipeptide **11** was dissolved in 30% EtOH/HEPES buffer solution[30] and was used as the catch solution. Catch was performed by mixing each alkyne solution (50 μM) with cobalt beads **7a** for 12 h at 4 °C. After removing the supernatant and washing the beads,[31] HPLC analysis of the supernatant and washing solutions with UV detection at 215 nm indicated that 79 and 81% of amino acid **10** and dipeptide **11**, respectively, had been bound to the cobalt beads (Scheme 9).[32]

Pleasingly, subsequent treatment of alkyne–cobalt beads **17** and **18** with 5% TFA for 24 h at 4 °C followed by washing of the beads resulted in release of amines **13** and **16** in yields of 67 and 77%, respectively. Alkynes **10** and **11** were not observed after acidic treatment of the corresponding alkyne-bound cobalt resins **17** and **18**.

The catch-and-release reaction of dipeptide **11** was also monitored by mass spectrometry (Figure 1). Figure 1a shows the mass spectrum of the initial solution containing alkyne-tagged dipeptide **11** ($[M+H]^+$ at $m/z=818.40$ and $[M+Na]^+$ at $m/z=840.38$) prior to cobalt complexation. After complexation, alkyne–cobalt beads **18** were subsequently washed to remove any non-complexed molecules. Figure 1b shows the mass spectrum of the sixth washing of the cobalt beads after the catch reaction, which no longer contains any starting alkyne **11**. After treatment of the alkyne–cobalt beads **18** with 5% TFA, the supernatant was removed and the beads were again washed with 50%



Scheme 9. Catch and release of amino acid **10** and dipeptide **11**.

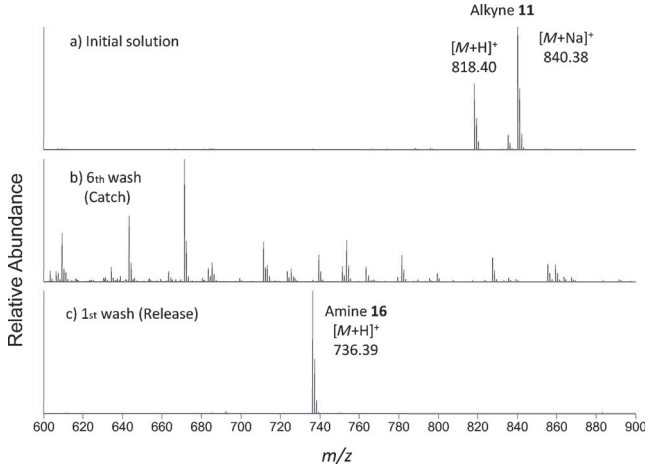


Figure 1. Mass spectra of solutions from the catch and release of dipeptide **11** (50 μM). a) Initial solution with alkyne-tagged dipeptide **11** (50 μM) prior to cobalt complexation (catch). b) Sixth washing solution of cobalt beads **18** after catch. c) First washing solution after TFA treatment. The mass spectra were recorded with a LTQ Orbitrap XL spectrometer.

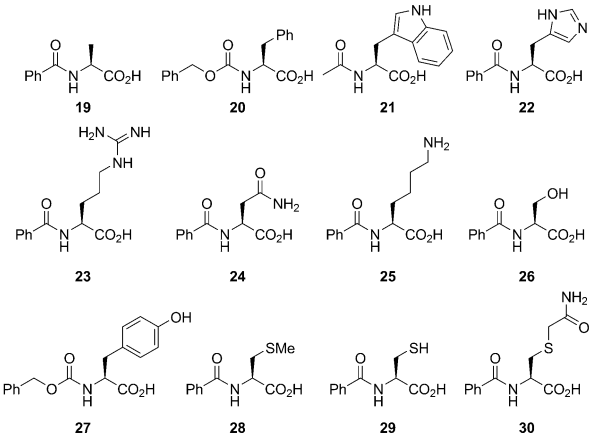
MeCN/H_2O to provide amine **16** ($[M+H]^+$ at $m/z=736.39$) as shown in Figure 1c.

The compatibility of this catch-and-release protocol with various amino acids containing a range of functional groups in their side-chain was then investigated by using dipeptide **11** (Table 2). To easily detect the compounds by HPLC, *N*-derivatised (benzoyl (Bz), CBz (Z) or acyl (Ac)) amino acids were used to investigate their tolerability. Dipeptide **11** (50 μM) was mixed with an equimolar amount of each derivatised amino acid (50 μM) in 30% EtOH/HEPES buffer. Cobalt beads **7a** were added to the solution, which was rotated, so as not to damage the beads, at 4 °C for 12 h. HPLC analysis of the supernatant and washing solutions showed good degrees of alkyne complexation and almost quantitative recovery of the following derivatised amino acids (side-chain functionality): *N*-Bz-alanine **19** (methyl), *N*-Z-phenylalanine **20** (phenyl), *N*-Ac-tryptophan **21** (indole), *N*-Bz-arginine **23** (guanidine), *N*-Bz-asparagine **24** (amide), N^{α}-Bz-lysine **25** (amine), *N*-Bz-serine **26** (hydroxy), *N*-Z-tyrosine **27** (phenol) and *N*-Bz-methionine **28** (thioether). Furthermore, the free carboxylic acid group appeared to have no significant effect on the reaction. Approximately 10% loss of *N*-Bz-histidine **22** (imidazole) was observed, which suggests that a small percentage of histidine had coordinated to the cobalt metal centre. Interestingly, *N*-Bz-cysteine **29**, which contains a free thiol group, was not detected in either the supernatant or the washing solution, even after treatment of the resin with TFA. This suggests that the free thiol had either been oxidised or had coordinated to the cobalt centre.[33] Although *N*-Bz-cysteine **29** did not appear to have a major effect on the outcome of the catch and release of dipeptide **11** on a 50 μM scale, protection of the thiol moiety prior to the reaction would be desirable to prevent any transformation or uptake by cobalt. In biological experiments, cysteine residues in peptides are usually alkylated with alkylating agents, such as iodoacetamide, to prevent oxidation of the thiol and formation of disulfide bonds. Thus, the catch and release of dipeptide **11** was carried out in the presence of *N*-Bz-*S*-carbamoylmethyl-cysteine **30**. Good recovery of the thiol-protected derivative **30** was observed after the catch reaction, which indicates that capping of free thiols is effective in preventing any potential side-reactions and thus shows compatibility with cobalt. Upon treatment of the alkyne–cobalt resin **18** with 5% TFA, amine **16** was successfully detected in good yield in the presence of all the amino acids that were investigated. Overall, these results suggest that this catch-and-release protocol is compatible with a range of functionalities that are present in peptides. Apart from cysteine residues containing the thiol group, amino acid residues containing oxygen and nitrogen functionalities that can potentially coordinate to cobalt appeared to have almost no or minimal effect on the cobalt beads. Sulfur functionalities were found to be nearly unaffected under the catch-and-release conditions when they are present as thioethers.

The scope and practicality of this reaction were then explored by carrying out the catch and release of dipeptide **11** in the presence of various peptides at low molar concentrations. A solution of alkyne-tagged dipeptide **11** (1.25 μM, 1.25 nmol) and Waters MassPREP peptide mixture containing approximately 0.4 μM (ca. 400 pmol) of nine peptides (RASG-1 (**A**), an-

Table 2. Compatibility of the catch and release of dipeptide **11** with various amino acids.

Entry	Amino acid (functional group)	Catch of alkyne **11** [%]	Recovery of amino acid [%]	Release of amine **16** [%]
1	*N*-Bz-alanine **19** (Me)	78	100	87
2	*N*-Z-phenylalanine **20** (Ph)	82	100	95
3	*N*-Ac-tryptophan **21** (indole)	80	100	100
4	*N*-Bz-histidine **22** (imidazole)	82	88	94
5	*N*-Bz-arginine **23** (guanidine)	80	100	87
6	*N*-Bz-asparagine **24** (amide)	81	100	83
7	N^{ε}-Bz-lysine **25** (amine)	77	100	98
8	*N*-Bz-serine **26** (hydroxy)	78	100	82
9	*N*-Z-tyrosine **27** (phenol)	76	100	86
10	*N*-Bz-methionine **28** (thioether)	79	100	96
11	*N*-Bz-cysteine **29** (thiol)	78	0	76
12	*N*-Bz-*S*-carbamoylmethyl-cysteine **30** (thioether)	80	85	61



giotensin fragment 1–7 (**B**), bradykinin (**C**), angiotensin II (**D**), angiotensin I (**E**), renin substrate (**F**), enolase T35 (**G**), enolase T37 (**H**) and melittin (**I**); peptide sequences shown in Table 3) in 30% EtOH/HEPES buffer was rotated with cobalt beads **7a** at 4 °C for 12 h. The resulting beads were washed to remove any unreacted alkyne **11** and other non-alkyne-containing peptides. Alkyne–cobalt–resin **18** was then treated with 5% TFA and washed afterwards. Mass spectrometric analysis of the washing solutions after TFA treatment revealed the presence of amine **16**, which demonstrates that catch and release had proceeded smoothly in the presence of other peptides. Pleasingly, amino acid residues that contain metal-coordinating functionalities such as arginine (R), serine (S), lysine (K), tyrosine (Y), histidine (H) and tryptophan (W) in the peptides did not appear to interfere with the reaction, and all non-

Table 3. Peptide sequences for the peptides used to investigate the scope of catch-and-release reactions with dipeptide **11**.

Peptide		Sequence
RASG-1[a]	**A**	RGDSPASSKP
angiotensin frag. 1–7[a,b]	**B**	DRVYIHP
bradykinin[a,b]	**C**	RPPGFSPFR
angiotensin II[a,b]	**D**	DRVYIHPF
angiotensin I[a,b]	**E**	DRVYIHPFHL
renin substrate[a]	**F**	DRVYIHPFHLLVYS
enolase T35[a]	**G**	WLTGPQLADLYHSLMK
enolase T37[a]	**H**	YPIVSIEDPFAEDDWEAWSHFFK
melittin[a]	**I**	GIGAVLKVLTTGLPALISWIKRKRQQ
neurotensin[b]	**J**	pyroELYENKPRRPYIL

[a] Peptides in Waters MassPREP peptide mixture (see Figure 3 in the Experimental Section). [b] Peptides used in the catch-and-release reaction shown in Figure 2.

alkyne-tagged peptides were thoroughly washed out from the resin after catch according to the absence of any related peaks in the mass spectra of the sixth washing and the washings after release (see Figure 3b,c in the Experimental Section).[34] These results correlate with the observation that this catch-and-release protocol is compatible with a range of amino acids containing various functionalities.

Following the success of the compatibility of the catch-and-release protocol with various peptides, it was decided to lower the concentration of the alkyne-tagged molecule even further. A solution of dipeptide **11** (0.5 μM, 500 pmol) and a peptide mixture containing angiotensin fragment 1–7 (**B**; 5 μM), bradykinin (**C**; 20 μM),[35] angiotensin II (**D**; 5 μM), angiotensin I (**E**; 5 μM) and neurotensin (**J**; 5 μM; Table 3) in 30% EtOH/HEPES buffer was rotated with cobalt beads **7a** at 4 °C for 12 h. The resulting beads were washed to remove any unreacted alkyne **11** and other non-alkyne-containing peptides present in large excess. The alkyne–cobalt–resin **18** was then treated with 5% TFA followed by washing of the beads. Mass spectrometric analysis of the initial solution before cobalt complexation, the supernatant after catch and washings of the alkyne–cobalt beads after both catch and release was carried out and the spectra are shown in Figure 2. The eighth washing of cobalt beads **18** after catch revealed the absence of alkyne **11** and other peptides, which indicates that non-specific-binding molecules had been successfully washed out from the beads (Figure 2c). Amine **16** was detected in the washings of the beads after release with 5% TFA. These results indicate that catch and release of dipeptide **11** proceeded at a concentration of 0.5 μM in the presence of other peptides each existing in over 10-fold excess with respect to the target molecule to generate amine **16** after release from the resin. Hence, specific enrichment had occurred at high dilution with no significant interference from the other peptides. Furthermore, when the concentration of dipeptide **11** was lowered to 50 nM (50 pmol) in the presence of 5 μM of each peptide **B**, **D**, **E** and **J** (20 μM for bradykinin (**C**)) under the same conditions, successful release of amine **16** was detected by mass spectrometry (see Figure S1 in the Supporting Information). It is worth mentioning that mass spectrometric analysis of the supernatant after alkyne com-

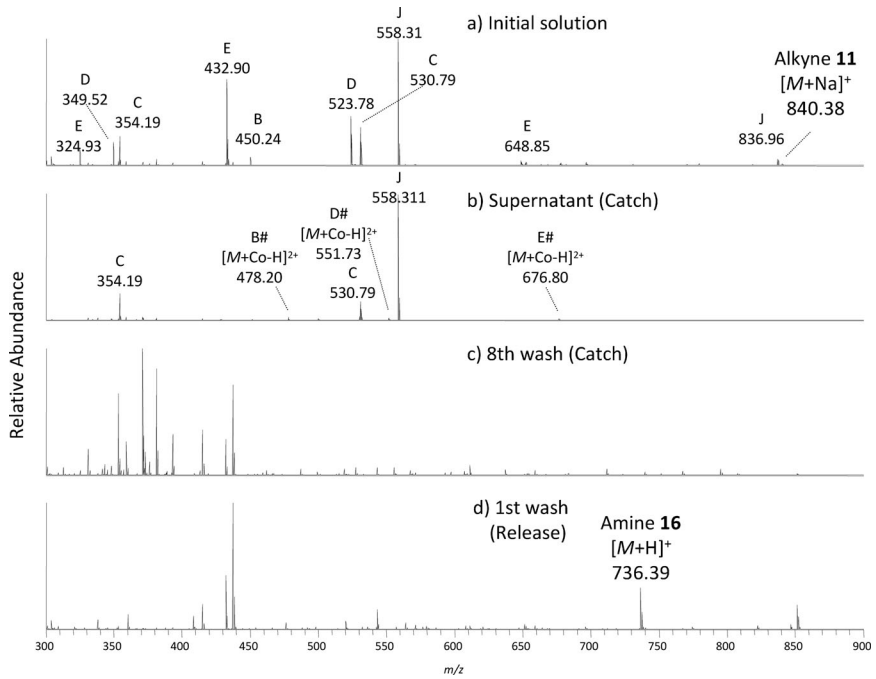


Figure 2. Mass spectra of solutions from the catch and release of dipeptide **11** (0.5 μM) in a peptide mixture containing angiotensin frag. 1–7 (**B**), bradykinin (**C**), angiotensin II (**D**), angiotensin I (**E**) and neurotensin (**J**; Table 3). a) Initial solution of alkyne-tagged dipeptide **11** (0.5 μM) and peptide mixture (5 μM each of peptides **B**, **D**, **E** and **J**, and 20 μM of peptide **C**) prior to cobalt complexation (catch). b) Supernatant after catch showing unreacted peptides and cobalt complexes of angiotensin analogues **B**, **D** and **E**. c) Eighth washing solution of cobalt beads **18** after catch. d) First washing solution after TFA treatment. The mass spectra were recorded with a LTQ Orbitrap XL spectrometer.

plexation showed peaks for the other peptides that were considered to not form complexes with the cobalt resin. Interestingly, cobalt adduct ions ($[M+56]^+$) were observed in the supernatant for angiotensin analogues (Figure 2b), which suggests the formation of $[M-H+Co]^{2+}$ and $[M+Co]^{3+}$ ions[36] according to the theoretical masses (see Figures S2 and S3 in the Supporting Information). This phenomenon may arise from the presence of oxidised cobalt ions coordinating to the specific amino acid sequence of angiotensin analogues containing arginine and histidine residues.

Conclusion

In conclusion, the selective catch and release of propargyl carbamate tagged molecules has been investigated by using model compounds, including amino acid and peptide derivatives. At high dilution and low temperature, alkyne-tagged molecules were selectively coordinated to a resin-bound cobalt complex, followed by release of the molecules as amines by the Nicholas reaction using TFA in aqueous media. A control experiment using non-alkyne-containing molecules demonstrated that the cobalt resin has a high selectivity for alkyne-tagged molecules. Furthermore, non-specific binding molecules were thoroughly washed out from the resins after the catch reaction. Catch-and-release reactions of an alkyne-tagged peptide in the presence of amino acids bearing various functionalities also proceeded smoothly. A range of oxygen, nitrogen and protected sulfur functionalities in amino acids, such as carboxylic acid, hydroxy, phenol, amine, amide, indole, imidazole, guanidine and thioether groups, were found to be compatible under the established reaction conditions and the desired amine was released in good yields. Finally, when the catch-and-release protocol was carried out with a peptide mixture under highly diluted conditions (0.5 μM or 50 nM), non-alkyne-containing peptides were successfully washed out from the resin and selective enrichment of the amine was clearly detected by mass spectrometry. Overall, these results demonstrate that the propargyl carbamate tag selectively coordinates to the cobalt resin under dilute aqueous conditions at low temperature. The alkyne tag is easily prepared and relatively small in size, which makes it a good candidate for incorporation into bioactive molecules without drastically changing their activities. This protocol is operationally simple and enables the release of the target molecule under acidic conditions that are relatively mild compared with oxidative conditions. It is anticipated that this strategy has the potential to be bio-orthogonal and applicable for the specific enrichment of alkyne-tagged biomolecules in a range of complex peptide mixtures, including cell lysates, and thus may prove to be useful in identifying the target proteins of bioactive molecules.

Experimental Section

General

All reactions and manipulations involving organometallic compounds were performed under an inert atmosphere of dry nitrogen. Dehydrated tetrahydrofuran (THF), methanol (MeOH) and dichloromethane (CH_2Cl_2) were purchased from Wako Pure Chemical Industries, Ltd. TentaGel was purchased from HiPep Laboratories Co., Inc. The phosphine ligand supported on TentaGel was synthesised according to the literature.[24] $[Co_2(CO)_8]$ was purchased from Tokyo Chemical Industry Co., Ltd. (TCI) and Kanto Chemical Co., Inc. $[Co_2(CO)_7(PPh_3)]$ was synthesised according to the literature.[26g] Peptides (angiotensin I, angiotensin II, angiotensin frag, 1–7, bradykinin and neurotensin) were purchased from Peptide Institute Inc. All other reagents were used as purchased from commercial sources. Water was prepared with a Millipore Milli-Q Advantage A10 instrument. TLC analysis was performed on silica gel 60 F_{254}-coated glass plates (Merck). Visualisation was accomplished by means of ultraviolet (UV) irradiation at 254 nm, iodine or by using 12-molybdo(VI)phosphoric acid/ethanol solution, potassium permanganate,

ninhydrin and heat as developing agents. Flash column chromatography was performed with silica gel N-60 (40–100 μm) purchased from Kanto Chemical Co., Inc. or Chromatorex (N-H-type silica gel) purchased from Fuji Silysia Chemical, Ltd. ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded at room temperature on a JEOL JNM-AL400 or JEOL JNM-ECP-500 spectrometer at 400 or 500, 100 or 125 and 160 MHz, respectively. *J* values are reported in Hz and chemical shifts (δ) in ppm relative to tetramethylsilane (TMS), chloroform or H_3PO_4. IR spectra were recorded on a Thermo Nicolet iS5 spectrometer. Only diagnostic absorptions are listed in the characterisation data. HPLC was performed on a DIONEX UltiMate 3000 instrument with the following equipment: pump, DGP-3600; auto-sampler, WPS-3000; flow manager, FLM-3x00; detector, VWD-3x00 measured at 215 or 280 nm. Mass spectra were acquired on a Bruker microTOF-QII-$_{RSL}$ or ThermoScientific LTQ Orbitrap XL spectrometer. Electrospray ionisation (ESI) Orbitrap mass spectra were acquired in positive ion mode for a *m/z* range of 280–1600. The FT resolution was set at 100 000. Mass spectra were analysed with a Qual Browser instrument (Thermo Fisher Scientific). Optical rotations were recorded on a JASCO P-2200 Polarimeter using sodium low-pressure light (sodium D line 589 nm) with a 50 mm path length; concentrations are given as g per 100 mL.

Cobalt beads were rotated by using a MACSmix™ Tube Rotator.

Compounds **1**–**9** have been synthesised and characterised previously.[19]

Synthesis

Benzyl (S)-1-azido-13-oxo-15-phenyl-3,6,9-trioxa-12-azapentadecane-14-carbamate (12)

N-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide (288 mg, 1.5 mmol), DIPEA (0.52 mL, 3 mmol), 4-(dimethylamino)pyridine (18 mg, 0.15 mmol) and 11-azido-3,6,9-trioxaundecan-1-amine (0.21 mL, 1.05 mmol) were added to a solution of *N*-Z-L-phenylalanine (299 mg, 1 mmol) in CH_2Cl_2 (10 mL) at room temperature. After stirring the mixture for 24 h, the mixture was diluted with ethyl acetate. The organic layers were washed with aqueous 1 N HCl, a saturated aqueous solution of $NaHCO_3$, distilled water and brine, dried over Na_2SO_4 and concentrated under reduced pressure. Flash column chromatography (N-H SiO_2; CH_2Cl_2) of the residue afforded the title compound **12** as a colourless oil (322 mg, 65%). $R_f = 0.34$ (N-H SiO_2; CH_2Cl_2); $[\alpha]_D^{25} = +6.3$ ($c = 1$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 3.06$–3.10 (m, 2H; CH_2), 3.35–3.71 (m, 16H; CH_2), 4.41 (dd, ^{3}J(H,H) = 6.9, 14.3 Hz, 1H; C*H*), 5.09 (d, ^{3}J(H,H) = 4.0 Hz, 2H; CH_2), 5.51 (d, ^{3}J(H,H) = 7.0 Hz, 1H; N*H*), 6.32 (br s, 1H; N*H*), 7.20–7.39 ppm (m, 10H; $C_{Ar}H$); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 39.1 (CH_2), 39.3 (CH_2), 50.7 (CH_2), 56.4 (*C*H), 67.0 (CH_2), 69.6 (CH_2), 70.1 (CH_2), 70.3 (CH_2), 70.61 (CH_2), 70.63 (CH_2), 70.8 (CH_2), 127.0 (C_{Ar}), 128.1 (C_{Ar}), 128.3 (C_{Ar}), 128.6 (C_{Ar}), 128.7 (C_{Ar}), 129.4 (C_{Ar}), 136.3 (C_{Ar}), 136.6 (C_{Ar}), 155.9 (*C*=O), 170.7 ppm (*C*=O); IR (neat): $\tilde{\nu}_{max}$ = 3304, 2868, 2100. 1712, 1657, 1526 cm^{-1}; HRMS (ESI^+): *m/z* calcd for $[C_{25}H_{33}N_5O_6 + Na]^+$: 522.2323; found:522.2327.

Benzyl (S)-1-amino-13-oxo-15-phenyl-3,6,9-trioxa-12-azapentadecane-14-carbamate (13)

Pd/C–ethylenediamine complex (9 mg) was added to a solution of azide **12** (100 mg, 0.2 mmol) in MeOH (2 mL) at room temperature under nitrogen. After placing the reaction mixture under hydrogen, the mixture was stirred for 2 h. After filtration of the reaction mixture through a pad of Celite, the filtrate was concentrated under reduced pressure. Flash column chromatography (N-H SiO_2; CH_2Cl_2/MeOH, 1:0 to 98:2) of the residue afforded the title compound **13** as a colourless oil (77 mg, 81%). $R_f = 0.11$ (N-H SiO_2; CH_2Cl_2/MeOH, 99:1); $[\alpha]_D^{26} = +15.6$ ($c = 0.5$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.78$ (br s, 2H; N*H*), 3.01–3.09 (m, 2H; CH_2), 3.30–3.58 (m, 16H; CH_2), 4.47 (dd, ^{3}J(H,H) = 6.9, 14.3 Hz, 1H; C*H*), 5.01–5.07 (m, 2H; CH_2), 5.91 (d, ^{3}J(H,H) = 8 Hz, 1H; N*H*), 7.17–7.34 (m, 10H; $C_{Ar}H$), 7.58 ppm (br s, 1H; N*H*); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 39.3$ (CH_2), 41.4 (CH_2), 56.1 (*C*H), 66.8 (CH_2), 69.9 (CH_2), 70.0 (CH_2), 70.2 (CH_2), 70.6 (CH_2), 72.7 (CH_2), 126.8 (C_{Ar}), 128.0 (C_{Ar}), 128.2 (C_{Ar}), 128.5 (C_{Ar}), 128.6 (C_{Ar}), 129.4 (C_{Ar}), 129.6 (C_{Ar}), 136.5 (C_{Ar}), 137.0 (C_{Ar}), 156.0 (*C*=O), 171.1 ppm (*C*=O); IR (neat): $\tilde{\nu}_{max}$ = 3303, 2868, 1712, 1659, 1528 cm^{-1}; HRMS (ESI^+): *m/z* calcd for $[C_{25}H_{35}N_3O_6 + H]^+$: 474.2599; found:474.2657.

Benzyl prop-2-yn-1-yl (S)-13-oxo-15-phenyl-3,6,9-trioxa-12-azapentadecane-1,14-dicarbamate (10)

To a solution of azide **12** (100 mg, 0.2 mmol) in MeOH (2 mL) was added Pd/C–ethylene diamine complex (9 mg) at room temperature under nitrogen. After placing the reaction mixture under hydrogen, the mixture was stirred for 6 h. After filtration of the reaction mixture through a pad of Celite, the filtrate was concentrated under reduced pressure and used in the next step without further purification. The residue was dissolved in CH_2Cl_2 (1.5 mL) and triethylamine (82 μL, 0.59 mmol) and **3** (49 mg, 0.22 mmol) were added at room temperature. The solution was stirred for 24 h, followed by the addition of a saturated solution of aqueous $NaHCO_3$. After extraction with ethyl acetate (3×10 mL), the combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. Flash column chromatography (N-H SiO_2; CH_2Cl_2/MeOH, 1:0 to 99:1) of the residue afforded the title compound **10** as a colourless oil (79 mg, 71%). $R_f = 0.47$ (N-H SiO_2; CH_2Cl_2/MeOH, 99:1); $[\alpha]_D^{26} = +5.9$ ($c = 1$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.44$ (t, ^{4}J(H,H) = 2.5 Hz, 1H; C≡C*H*), 3.06 (d, ^{3}J(H,H) = 5.5 Hz, 2H; CH_2), 3.34–3.58 (m, 16H; CH_2), 4.40 (d, ^{3}J(H,H) = 7 Hz, 1H; C*H*), 4.65 (d, ^{4}J(H,H) = 2.5 Hz, 2H; CH_2C≡C), 5.06–5.10 (m, 2H; CH_2), 5.52 (d, ^{3}J(H,H) = 6.5 Hz, 1H; N*H*), 5.61 (br s, 1H; N*H*), 6.44 (br s, 1H; N*H*), 7.18–7.34 ppm (m, 10H; $C_{Ar}H$); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 39.1$ (CH_2), 39.3 (CH_2), 40.9 (CH_2), 52.5 (CH_2C≡CH), 56.1 (*C*H), 67.0 (CH_2), 69.7 (CH_2), 70.0 (CH_2), 70.2 (CH_2), 70.4 (CH_2), 70.5 (CH_2), 74.7 (C≡*C*H), 78.5 (*C*≡CH), 127.0 (C_{Ar}), 128.1 (C_{Ar}), 128.3 (C_{Ar}), 128.6 (C_{Ar}), 128.7 (C_{Ar}), 129.5 (C_{Ar}), 136.3 (C_{Ar}), 136.7 (C_{Ar}), 155.7 (*C*=O), 155.9 (*C*=O), 170.9 ppm (*C*=O); IR (neat): $\tilde{\nu}_{max}$ = 3296, 2870, 1709, 1660, 1520 cm^{-1}; HRMS (ESI^+): *m/z* calcd for $[C_{29}H_{37}N_3O_8 + Na]^+$: 578.2473; found:578.2484.

Methyl (S)-2-{(S)-2,6-bis[(benzyloxycarbonyl)amino]hexanamido}-3-phenylpropanoate (14)

N^{α}-Z-L-Lysine (1.68 g, 6 mmol) was dissolved in a solution of $NaHCO_3$ (24 mL, 1 N) and NaOH (4.8 mL, 4 N). The solution was cooled to 0 °C and benzyl chloroformate (1.01 mL, 9 mmol) was added dropwise and the reaction mixture stirred for 1 h at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for an additional 24 h. It was then acidified to pH 2–3 by the slow addition of aqueous 1 N hydrochloric acid. After extraction with ethyl acetate (3×10 mL), the combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide (575 mg, 3 mmol), DIPEA (0.45 mL, 2.6 mmol) and 1-hydrobenzotriazole (324 mg, 2.4 mmol) were added to a suspension of N^{α}-Z,N^{ε}-Z-L-lysine (829 mg, 2 mmol) and L-phenylalanine methyl ester hydrochloride (518 mg, 2.4 mmol) in CH_2Cl_2 (10 mL) at room temperature. After stirring the mixture for 2 h, the mixture was diluted with ethyl acetate. The organic layers were washed with dis-

tilled water, a saturated aqueous solution of $NaHCO_3$, distilled water, aqueous 0.1 M citric acid, distilled water and brine, dried over Na_2SO_4 and concentrated under reduced pressure. Flash column chromatography (SiO_2; hexane/ethyl acetate, 2:1 to 1:1) of the residue afforded the title compound **14** as a colourless amorphous solid (599 mg, 58%). $R_f = 0.33$ (SiO_2; hexane/ethyl acetate, 1:1); $[\alpha]_D^{27} = +22.0$ ($c = 1$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta =$ 1.23–1.78 (m, 6H; C*H*$_2$), 3.02–3.15 (m, 4H; C*H*$_2$), 3.66 (s, 3H; C*H*$_3$), 4.08–4.14 (m, 1H; C*H*), 4.83 (dd, ^3J(H,H) = 6.0, 13.8 Hz, 1H; C*H*), 4.92–5.07 (m, 4H; C*H*$_2$), 5.43 (d, ^3J(H,H) = 7.5 Hz, 1H; N*H*), 6.44 (d, ^3J(H,H) = 7.5 Hz, 1H; N*H*), 7.07 (d, ^3J(H,H) = 7 Hz, 2H; C$_{Ar}$*H*) 7.19–7.32 ppm (m, 13H; C$_{Ar}$*H*); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 22.1$ (*C*H$_2$), 29.4 (*C*H$_2$), 31.9 (*C*H$_2$), 37.8 (*C*H$_2$), 40.3 (*C*H$_2$), 52.5 (*C*H$_3$), 53.2 (*C*H), 54.7 (*C*H), 66.8 (*C*H$_2$), 67.2 (*C*H$_2$), 127.3 (*C*$_{Ar}$), 128.17 (*C*$_{Ar}$), 128.22 (*C*$_{Ar}$), 128.3 (*C*$_{Ar}$), 128.58 (*C*$_{Ar}$), 128.64 (*C*$_{Ar}$), 128.7 (*C*$_{Ar}$), 129.3 (*C*$_{Ar}$), 135.7 (*C*$_{Ar}$), 136.2 (*C*$_{Ar}$), 136.6 (*C*$_{Ar}$), 156.2 (*C*=O), 156.7 (*C*=O), 171.3 (*C*=O), 171.9 ppm (*C*=O); IR (neat): $\tilde{\nu}_{max} = 3308$, 2948, 1692, 1658, 1526 cm^{-1}; HRMS (ESI$^+$): *m/z* calcd for $[C_{32}H_{37}N_3O_7 + Na]^+$: 598.2524; found:598.2519.

Dibenzyl (14S,17S)-1-azido-14-benzyl-13,16-dioxo-3,6,9-trioxa-12,15-diazahenicosane-17,21-dicarbamate (15)

An aqueous 10% NaOH solution (0.68 mL) was added to a solution of methyl ester **14** (100 mg, 0.17 mmol) in THF/MeOH (1.7 mL, 1:1) at 0 °C. After stirring for 30 min at 0 °C, aqueous 1 N HCl s was added. After extraction with ethyl acetate, the combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide (49 mg, 0.26 mmol), DIPEA (91 μL, 0.52 mmol), 4-(dimethylamino)pyridine (3 mg, 0.03 mmol) and 11-azido-3,6,9-trioxaundecan-1-amine (37 μL, 0.19 mmol) were added to a solution of the residue in CH_2Cl_2 (1.7 mL) at room temperature. After stirring the mixture for 45 h, the mixture was diluted with ethyl acetate. The organic layers were washed with aqueous 1 N HCl, a saturated aqueous solution of $NaHCO_3$, distilled water and brine, dried over Na_2SO_4 and concentrated under reduced pressure. Flash column chromatography (SiO_2; ethyl acetate) of the residue afforded the title compound **15** as a colourless amorphous solid (90 mg, 70%). $R_f = 0.62$ (SiO_2; ethyl acetate); $[\alpha]_D^{27} = -6.6$ ($c = 1$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta =$ 1.19–1.74 (m, 6H; C*H*$_2$), 2.99–3.16 (m, 4H; C*H*$_2$), 3.35–3.66 (m, 16H; C*H*$_2$), 4.03–4.10 (m, 1H; C*H*), 4.57–4.66 (m, 1H; C*H*), 5.00–5.07 (m, 4H; C*H*$_2$), 5.52–5.53 (m, 1H; N*H*), 6.62–6.67 (m, 1H; N*H*), 7.16–7.33 ppm (m, 15H; C$_{Ar}$*H*); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 22.3$ (*C*H$_2$), 29.5 (*C*H$_2$), 32.0 (*C*H$_2$), 39.4 (*C*H$_2$), 39.5 (*C*H$_2$), 40.4 (*C*H$_2$), 50.8 (*C*H$_2$), 54.5 (*C*H), 55.1 (*C*H), 66.8 (*C*H$_2$), 67.3 (*C*H$_2$), 69.5 (*C*H$_2$), 69.6 (*C*H$_2$), 70.1 (*C*H$_2$), 70.3 (*C*H$_2$), 70.6 (*C*H$_2$), 70.7 (*C*H$_2$), 70.8 (*C*H$_2$), 127.1 (*C*$_{Ar}$), 128.3 (*C*$_{Ar}$), 128.4 (*C*$_{Ar}$), 128.67 (*C*$_{Ar}$), 128.72 (*C*$_{Ar}$), 128.74 (*C*$_{Ar}$), 129.4 (*C*$_{Ar}$), 136.3 (*C*$_{Ar}$), 136.7 (*C*$_{Ar}$), 156.5 (*C*=O), 156.7 (*C*=O), 170.6 (*C*=O), 171.7 ppm (*C*=O); IR (neat): $\tilde{\nu}_{max} = 3297$, 2942, 2106, 1682, 1642 cm^{-1}; HRMS (ESI$^+$): *m/z* calcd for $[C_{39}H_{51}N_7O_9 + Na]^+$: 784.3640; found:784.3604.

Dibenzyl (14S,17S)-1-amino-14-benzyl-13,16-dioxo-3,6,9-trioxa-12,15-diazahenicosane-17,21-dicarbamate (16)

Pd/C–ethylene diamine complex (2.4 mg) was added to a solution of azide **15** (40 mg, 0.053 mmol) in MeOH (0.53 mL) at room temperature under nitrogen. After placing the reaction mixture under hydrogen, the mixture was stirred for 6 h. After filtration of the reaction mixture through a pad of Celite, the filtrate was concentrated under reduced pressure. Flash column chromatography (N-H SiO_2; CH_2Cl_2/MeOH, 1:0 to 95:5) of the residue afforded the title compound **16** as a colourless oil (39 mg, quant.). $R_f = 0.34$ (N-H SiO_2; CH_2Cl_2/MeOH, 9:1); $[\alpha]_D^{27} = -4.8$ ($c = 1$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta =$ 1.21–1.67 (m, 6H; C*H*$_2$), 2.81–3.56 (m, 22H; C*H*$_2$, N*H*$_2$), 4.06–4.07 (m, 1H; C*H*), 4.66–4.68 (m, 1H; C*H*), 5.00–5.02 (m, 4H; C*H*$_2$), 5.21 (m, 1H; N*H*), 5.59–5.60 (m, 1H; N*H*), 7.12–7.28 ppm (m, 15H; C$_{Ar}$*H*); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 22.0$ (*C*H$_2$), 29.3 (*C*H$_2$), 31.9 (*C*H$_2$), 38.5 (*C*H$_2$), 39.3 (*C*H$_2$), 40.2 (*C*H$_2$), 40.9 (*C*H$_2$), 54.5 (*C*H), 54.9 (*C*H), 66.7 (*C*H$_2$), 67.1 (*C*H$_2$), 69.7 (*C*H$_2$), 69.8 (*C*H$_2$), 70.0 (*C*H$_2$), 70.4 (*C*H$_2$), 70.5 (*C*H$_2$), 71.1 (*C*H$_2$), 126.9 (*C*$_{Ar}$), 128.0 (*C*$_{Ar}$), 128.2 (*C*$_{Ar}$), 128.3 (*C*$_{Ar}$), 128.58 (*C*$_{Ar}$), 128.63 (*C*$_{Ar}$), 129.5 (*C*$_{Ar}$), 136.3 (*C*$_{Ar}$), 136.7 (*C*$_{Ar}$), 136.8 (*C*$_{Ar}$), 156.3 (*C*=O), 156.8 (*C*=O), 171.1 (*C*=O), 171.8 ppm (*C*=O); IR (neat): $\tilde{\nu}_{max} = 3303$, 2867, 1704, 1645, 1527 cm^{-1}; HRMS (ESI$^+$): *m/z* calcd for $[C_{39}H_{53}N_5O_9 + H]^+$: 736.3916; found:736.3894.

Dibenzyl prop-2-yn-1-yl (14S,17S)-14-benzyl-13,16-dioxo-3,6,9-trioxa-12,15-diazahenicosane-1,17,21-tricarbamate (11)

Pd/C–ethylene diamine complex (7 mg) was added to a solution of azide **15** (114 mg, 0.15 mmol) in MeOH (1.5 mL) at room temperature under nitrogen. After placing the reaction mixture under hydrogen, the mixture was stirred for 4 h. After filtration of the reaction mixture through a pad of Celite, the filtrate was concentrated under reduced pressure and used in the next step without further purification. The residue was dissolved in THF (1.2 mL) and 4-(dimethylamino)pyridine (27 mg, 0.225 mmol), DIPEA (78 μL, 0.45 mmol) and **3** (41 mg, 0.19 mmol) were added at room temperature. The solution was stirred for 6.5 h, followed by the addition of a saturated aqueous solution of $NaHCO_3$. After extraction with ethyl acetate (3×10 mL), the combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. Flash column chromatography (N-H SiO_2; CH_2Cl_2/MeOH, 1:0 to 99.5:0.5) of the residue afforded the title compound **11** as a colourless amorphous solid (103 mg, 84%). $R_f = 0.81$ (N-H SiO_2; CH_2Cl_2/MeOH, 95:5); $[\alpha]_D^{27} = -8.9$ ($c = 1$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta =$ 1.22–1.83 (m, 6H; C*H*$_2$), 2.45 (br s, 1H; C≡C*H*), 2.99–3.60 (m, 20H; C*H*$_2$), 4.10–4.11 (m, 1H; C*H*), 4.60–4.66 (m, 3H; C*H*$_2$, C*H*), 5.01–5.12 (m, 5H; C*H*$_2$, N*H*), 5.58–5.59 (m, 1H; N*H*), 6.67–6.69 (m, 1H; N*H*), 7.17–7.34 ppm (m, 15H; C$_{Ar}$*H*); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 22.0$ (*C*H$_2$), 29.6 (*C*H$_2$), 38.6 (*C*H$_2$), 39.5 (*C*H$_2$), 41.1 (*C*H$_2$), 52.6 (*C*H$_2$), 54.6 (*C*H), 55.2 (*C*H), 66.9 (*C*H$_2$), 67.4 (*C*H$_2$), 69.7 (*C*H$_2$), 70.1 (*C*H$_2$), 70.4 (*C*H$_2$), 70.6 (*C*H$_2$), 70.7 (*C*H$_2$), 74.8 (C≡*C*H), 78.7 (*C*≡CH), 127.2 (*C*$_{Ar}$), 128.3 (*C*$_{Ar}$), 128.5 (*C*$_{Ar}$), 128.7 (*C*$_{Ar}$), 128.8 (*C*$_{Ar}$), 129.5 (*C*$_{Ar}$), 136.3 (*C*$_{Ar}$), 136.8 (*C*$_{Ar}$), 155.1 (*C*=O), 155.9 (*C*=O), 170.7 (*C*=O), 171.6 ppm (*C*=O); IR (neat): $\tilde{\nu}_{max} = 3290$, 2943, 1686, 1640, 1531 cm^{-1}; HRMS (ESI$^+$): *m/z* calcd for $[C_{43}H_{55}N_5O_{11} + Na]^+$: 840.3790; found:840.3754.

Protocol for the catch-and-release reactions

Catch and release reaction of alkyne 1 at 4 °C at 50 μM (Table 1, Entry 6)

Cobalt complex **7a** (10 mg, ~0.23 mmol g^{-1})[17c,19] pre-washed with HEPES buffer (pH 7.0) was added to a solution of alkyne **1** (50 μM, 1 mL) in HEPES buffer (pH 7.0) containing 150 mM NaCl, 10 mM HEPES-Na, and 0.5% Triton X-100 at 4 °C. The mixture was rotated for 12 h at the same temperature, and then centrifuged. After removal of the supernatant, the beads were washed with 50% EtOH/HEPES buffer (3×1 mL), 50% MeCN/HEPES buffer (3×1 mL), 5% MeCN/H_2O (2×1 mL). The supernatant and the washing solutions were subjected to HPLC to determine the conversion. To the beads was added 5% TFA solution in 5% MeCN/H_2O (1 mL) and the mixture was rotated at 4 °C for 24 h. After removal of the supernatant, the beads were washed with 50% MeCN/H_2O (1 mL). The superna-

tant and washings were subjected to HPLC to determine the yields of products. The conversions and yields of all materials were calculated with reference to standard curves. HPLC [GL Sciences Inertsil ODS-3, H_2O/MeCN (0.1% TFA) = 86/14 (0–10 min)→77/23 (10–25 min), 100 μL min^{-1}, 280 nm, τ (compound **2**) = 10.1 min, τ (compound **1**) = 23.8 min].

Selective catch-and-release reaction of alkyne 1 in the presence of control material 9 (Scheme 7)

Cobalt complex **7a** (10 mg, ~0.23 mmol g^{-1}) pre-washed with HEPES buffer (pH 7.0) was added to a solution (1 mL) of alkyne **1** (50 μM) and control material **9** (50 μM) in HEPES buffer (pH 7.0) containing 150 mM NaCl, 10 mM HEPES-Na, and 0.5% Triton X-100 at 4 °C. The mixture was rotated for 12 h at the same temperature, and then centrifuged. After removal of supernatant, the beads were washed with 50% EtOH/HEPES buffer (3×1 mL), 50% MeCN/HEPES buffer (3×1 mL), 5% MeCN/H_2O (2×1 mL). The supernatant and the washing solutions were subjected to HPLC to determine the conversion. To the beads was added 5% TFA in 5% MeCN/H_2O (1 mL) and the mixture was rotated at 4 °C for 24 h. After removal of the supernatant, the beads were washed with 50% MeCN/H_2O (1 mL). The supernatant and washings were subjected to the HPLC analysis and the yields of all materials were calculated. The conversions and yields of all materials were calculated with reference to standard curves. HPLC [GL Sciences Inertsil ODS-3, H_2O/MeCN (0.1% TFA) = 86/14 (0–10 min) → 77/23 (10–25 min) → 61/39 (25–35 min), 100 μL min^{-1}, 280 nm, τ (compound **2**) = 10.1 min, τ (compound **1**) = 23.8 min, τ (compound **9**) = 31.0 min].

Catch and release reaction of amino acid 10 at 4 °C at 50 μM (Scheme 9)

Cobalt complex **7a** (10 mg, ~0.23 mmol g^{-1}) pre-washed with HEPES buffer (pH 7.0) was added to a solution of amino acid **10** (50 μM, 1 mL) in HEPES buffer (pH 7.0) containing 150 mM NaCl, 10 mM HEPES-Na, and 0.5% Triton X-100 at 4 °C. The mixture was rotated for 12 h at the same temperature, and then centrifuged. After removal of the supernatant, the beads were washed with 50% EtOH/HEPES buffer (3×1 mL), 50% MeCN/HEPES buffer (3×1 mL), 5% MeCN/H_2O (2×1 mL). The supernatant and the washing solutions were subjected to HPLC to determine the conversion. To the beads was added 5% TFA solution in 5% MeCN/H_2O (1 mL) and the mixture was rotated at 4 °C for 24 h. After removal of the supernatant, the beads were washed with 50% MeCN/H_2O (1 mL). The supernatant and washings were subjected to HPLC to determine the yields of products. The conversions and yields of all materials were calculated with reference to standard curves. HPLC [GL Sciences Inertsil ODS-3, H_2O/MeCN (0.1% TFA) = 90/10 (0–8 min) → 0/95 (8–18 min) → 90/10 (18–20 min), 100 μL min^{-1}, 215 nm, τ (compound **13**) = 15.7 min, τ (compound **10**) = 17.9 min].

Catch and release reaction of dipeptide 11 at 4 °C at 50 μM (Scheme 9)

Cobalt complex **7a** (10 mg, ~0.23 mmol g^{-1}) pre-washed with HEPES buffer (pH 7.0) was added to a solution of dipeptide **11** (50 μM, 1 mL) in 30% EtOH/HEPES buffer (pH 7.0) containing 150 mM NaCl and 10 mM HEPES-Na at 4 °C. The mixture was rotated for 12 h at the same temperature, and then centrifuged. After removal of the supernatant, the beads were washed with 50% EtOH/HEPES buffer (3×1 mL), 50% MeCN/HEPES buffer (3×1 mL), 5% MeCN/H_2O (2×1 mL). The supernatant and the washing solutions were subjected to HPLC to determine the conversion. To the beads was added 5% TFA solution in 5% MeCN/H_2O (1 mL) and the mixture was rotated at 4 °C for 24 h. After removal of the supernatant, the beads were washed with 50% MeCN/H_2O (1 mL). The supernatant and washings were subjected to HPLC to determine the yields of products. The conversions and yields of all materials were calculated with reference to standard curves. HPLC [GL Sciences Inertsil ODS-3, H_2O/MeCN (0.1% TFA) = 90/10 (0–8 min) → 0/95 (8–18 min) → 90/10 (18–20 min), 100 μL min^{-1}, 215 nm, τ (compound **16**) = 16.9 min, τ (compound **11**) = 18.9 min]. The initial solution, supernatant and washings were desalted with ZipTip C18 (Merck Millipore) and loaded to a metal-coated glass capillary nanoelectrospray tip (HUMANIX) prior to mass spectrometry. The mass spectra were taken on LTQ Orbitrap XL.

Selective catch-and-release reaction of dipeptide 11 in the presence of derivatised amino acids (Table 2)

For the synthesis of derivatised amino acids, see the Supporting Information. Cobalt complex **7a** (10 mg, ~0.23 mmol g^{-1}) pre-washed with HEPES buffer (pH 7.0) was added to a solution (1 mL) of dipeptide **11** (50 μM) and derivatised amino acid (50 μM) in 30% EtOH/HEPES buffer (pH 7.0) containing 150 mM NaCl and 10 mM HEPES-Na at 4 °C. The mixture was rotated for 12 h at the same temperature, and then centrifuged. After removal of the supernatant, the beads were washed with 50% EtOH/HEPES buffer (3×1 mL), 50% MeCN/HEPES buffer (3×1 mL), 5% MeCN/H_2O (2×1 mL). The supernatant and the washing solutions were subjected to HPLC to determine the conversion. To the beads was added 5% TFA solution in 5% MeCN/H_2O (1 mL) and the mixture was rotated at 4 °C for 24 h. After removal of the supernatant, the beads were washed with 50% MeCN/H_2O (1 mL). The supernatant and washings were subjected to HPLC to determine the yields of products. The conversions and yields of all materials were calculated with reference to standard curves. HPLC [GL Sciences Inertsil ODS-3, H_2O/MeCN (0.1% TFA) = 90/10 (0–8 min) → 0/95 (8–18 min) → 90/10 (18–20 min), 100 μL min^{-1}, 215 nm, τ (compound **16**) = 16.9 min, τ (compound **11**) = 18.9 min, τ (compound **19**) = 14.4 min, τ (compound **20**) = 17.8 min, τ (compound **21**) = 15.1 min, τ (compound **22**) = 6.7 min, τ (compound **23**) = 11.5 min, τ (compound **24**) = 7.3 min, τ (compound **25**) = 7.3 min, τ (compound **26**) = 9.1 min, τ (compound **27**) = 16.2 min, τ (compound **28**) = 15.7 min, τ (compound **29**) = 15.4 min, τ (compound **30**) = 13.6 min].

Selective catch-and-release reaction of dipeptide 11 in the presence of Waters MassPREP peptides (Figure 3)

Cobalt complex **7a** (10 mg, ~0.23 mmol g^{-1}) pre-washed with HEPES buffer (pH 7.0) was added to a solution (1 mL) of dipeptide **11** (1.25 μM) and Waters MassPREP peptide mixture containing approximately 0.4 μM each of 9 different peptides (RASG-1, angiotensin fragment 1–7, bradykinin, angiotensin II, renin substrate, enolase T35, enolase T37, and melittin) in 30% EtOH/HEPES buffer (pH 7.0) containing 150 mM NaCl and 10 mM HEPES-Na at 4 °C. The initial solution was subjected to mass spectrometry and the mixture was rotated for 12 h at the same temperature, and then centrifuged. After removal of the supernatant, the beads were washed with 50% EtOH/HEPES buffer (3×1 mL), 50% MeCN/HEPES buffer (3×1 mL), 5% MeCN/H_2O (2×1 mL). The supernatant and the washing solutions were subjected to mass spectrometry to determine the composition. To the beads was added 5% TFA solution in 5% MeCN/H_2O (1 mL) and the mixture was rotated at 4 °C for 24 h. After removal of the supernatant, the beads were washed with 50% MeCN/H_2O (1 mL). The initial solution, supernatant and washings were desalted with ZipTip C18 (Merck Millipore) and loaded to

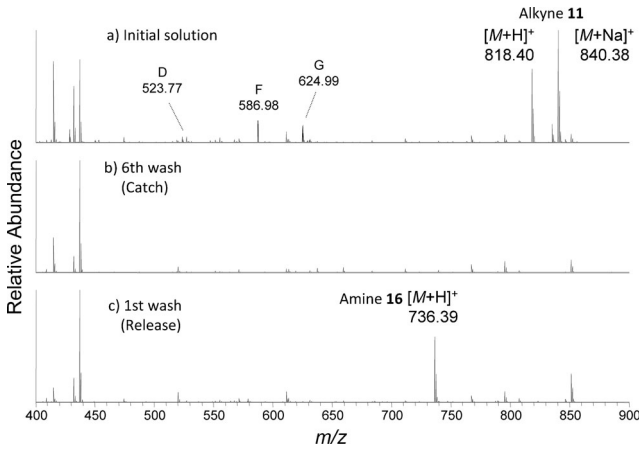


Figure 3. Mass spectra of solutions from the catch and release of dipeptide **11** in Waters MassPREP peptide mixture (peptides **A**–**I**, Table 3). a) Initial solution of alkyne-tagged dipeptide **11** (1.25 μM) and Waters MassPREP peptide mixture (approx. 0.4 μM each of RASG-1 (**A**), angiotensin fragments 1–7 (**B**), bradykinin (**C**), angiotensin II (**D**), angiotensin I (**E**), renin substrate (**F**), enolase T35 (**G**), enolase T37 (**H**), melittin (**I**), Table 3) prior to cobalt complexation (catch). b) Sixth washing solution of cobalt beads **18** after catch. c) First washing solution after TFA treatment. The mass spectra were recorded with a LTQ Orbitrap XL spectrometer.

a metal-coated glass capillary nanoelectrospray tip (HUMANIX) prior to mass spectrometry. The mass spectra were taken on LTQ Orbitrap XL.

Selective catch-and-release reaction of dipeptide 11 in the presence of peptides (Figure 2)

Cobalt complex **7a** (10 mg, ~0.23 mmol g^{-1}) pre-washed with HEPES buffer (pH 7.0) was added to a solution (1 mL) of dipeptide **11** (0.5 μM) and a peptide mixture containing 5 μM each of angiotensin I, angiotensin II, angiotensin fragment 1–7, neurotensin and 20 μM of bradykinin in 30% EtOH/HEPES buffer (pH 7.0) containing 150 mM NaCl and 10 mM HEPES-Na at 4 °C. The initial solution was subjected to mass spectrometry and the mixture was rotated for 12 h at the same temperature, and then centrifuged. After removal of the supernatant, the beads were washed with 50% EtOH/HEPES buffer (3×1 mL), 50% MeCN/HEPES buffer (3×1 mL), 5% MeCN/H_2O (2×1 mL). The supernatant and the washing solutions were subjected to mass spectrometry to determine the composition. To the beads was added 5% TFA solution in 5% MeCN/H_2O (1 mL) and the mixture was rotated at 4 °C for 24 h. After removal of the supernatant, the beads were washed with 50% MeCN/H_2O (1 mL). The initial solution, supernatant and washings were desalted with ZipTip C18 (Merck Millipore) and loaded to a metal-coated glass capillary nanoelectrospray tip (HUMANIX) prior to mass spectrometry. The mass spectra were taken on LTQ Orbitrap XL.

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[25] The buffer contains 150 mM NaCl and 10 mM HEPES–Na (pH 7.0) in milli-Q water.

[26] The cobalt complexes were identified by comparison of their IR spectra with the reported IR spectra of triphenylphosphine complexes and other analogous complexes. See, a) P. Szabo, L. Fekete, G. Bor, *J. Organomet. Chem.* **1968**, *12*, 245 – 248; b) L. M. Bower, M. H. B. Stiddard, *J. Organomet. Chem.* **1968**, *13*, 235 – 239; c) G. O. Evans, C. U. Pittman, Jr., R. McMillan, R. T. Beach, R. Jones, *J. Organomet. Chem.* **1974**, *67*, 295 – 314; d) R. A. Dubois, P. E. Garrou, K. D. Lavin, H. R. Allcock, *Organometallics* **1986**, *5*, 460 – 466. The peak at 1886 cm^{-1} indicates that the ionic cobalt complex $[Co(CO)_3L_2]^+[Co(CO)_4]^-$ was located on the polymer, see: S. E. Gibson, C. Johnstone, A. Stevenazzi, *Tetrahedron* **2002**, *58*, 4937 – 4942 and ref. [17c]. Attempts were made to quantify the loading of cobalt – phosphine complexes on the resin by using ^{31}P NMR spectroscopy. However, the presence of cobalt led to broadening of the peaks, and gradual decomposition of the cobalt complex was observed upon prolonged exposure to air and light at room temperature. Both of these led to difficulties in obtaining quantitative data for the resin loading. Thus, in the following experiments, a large excess of cobalt resin was used with respect to the alkyne substrate, which is expected to account for the errors associated with the slight differences in resin-loading.

[27] Leaching of resin material after acidic treatment was observed with CLEAR depending on the substrate. Hence optimisation studies were carried out with TentaGel.

[28] It is worth mentioning that decomposition was observed when TentaGel-immobilised BINAP was used for complexation with cobalt, as shown by IR spectroscopy. This correlates with the instability of the non-immobilised cobalt–BINAP complex in air and moisture. For references on the cobalt–BINAP complex, see: a) S. E. Gibson, K. A. C. Kaufmann, P. R. Haycock, A. J. P. White, D. J. Hardick, M. J. Tozer, *Organometallics* **2007**, *26*, 1578 – 1580; b) S. E. Gibson, D. J. Hardick, P. R. Haycock, K. A. C. Kaufmann, A. Miyazaki, M. J. Tozer, A. J. P. White, *Chem. Eur. J.* **2007**, *13*, 7099 – 7109.

[29] The catch efficiency improved relative to previously reported results upon pre-washing cobalt beads **7a** with HEPES buffer prior to the catch (see ref. [< 19]).

[30] The composition of the catch solvent had a significant effect on the catch yield. When 40% EtOH/HEPES buffer solution and 50% EtOH/HEPES buffer solution were used to dissolve dipeptide **11**, the catch yield deteriorated to 57 and 5%, respectively.

[31] Beads were washed with 50% EtOH/HEPES buffer (pH 7.0) three times, 50% MeCN/HEPES buffer three times and 5% MeCN/H_2O twice.

[32] A small amount of amine **13** (6%) was detected after alkyne complexation with amino acid **10** and a negligible amount of amine **16** (~1%) was detected after the catch of dipeptide **11**.

[33] Upon mixing *N*-Bz-histidine **22** and *N*-Bz-cysteine **29** with the phosphine–TentaGel resin bearing no cobalt, hardly any loss of material was observed according to HPLC. This suggests that both histidine and cysteine have a tendency to be modified or coordinated to the metal centre in the presence of cobalt.

[34] The low concentration of each peptide (approx. 0.4 μM each) in the Waters MassPREP peptide mixture made it rather difficult to detect and monitor the fate of each peptide in the initial catch and subsequent washing solutions by mass spectrometry (see Figure 3 in the Experimental Section).

[35] As a result of the low ionisation of bradykinin in the presence of other peptides, the concentration was increased by a factor of four to improve its detection by mass spectrometry.

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