DOI: 10.1002/ejoc.200800642

# Forced Complexation of Nitrogen Leading to a Weakening of Amide Bonds: Application to a New Linker for Solid-Phase Chemistry

**Manuel C. Bröhmer[a] and Willi Bannwarth*[a]**

**Keywords:** Solid-phase synthesis / Linkers / Amides / Cleavage reactions / Copper

$Cu^{2+}$ complexation of a bispicolylamide entity leads to a weakening of the N–C-amide bond, which subsequently can be cleaved under very mild conditions by methanolysis. On the basis of this principle we developed a versatile and robust linker for solid-phase chemistry. The stability of the linker was demonstrated under acidic and basic conditions, and it was evaluated for its utility in peptide synthesis as well as for reductive amination, Pd-mediated C–C-coupling, and metathesis reactions.



## Introduction

In the realm of synthetic chemistry, solid-phase organic synthesis has gained more and more interest over the last decades. It allows the application of excess amounts of reagents, which in turn leads to higher yields in comparison to the same reaction performed in solution and with equimolar amounts of reacting components. The excess amounts of reagents may be removed by simple filtration, thus avoiding time-consuming purifications.

A major disadvantage is that two additional steps are involved, namely, the attachment of the starting material and the release of the product. Due to the limited range of modifications available on polymer resins, which also limits the range of functionalities that are directly attachable to the solid support, there is a need for suitable linker entities connecting the support with either the starting material, intermediates, or the aspired product.[1]

When designing a linker molecule a number of issues have to be considered. The envisaged chemical steps should not lead to its modification nor should it result in the cleavage of intermediates from it. Yet, it should be possible to cleave the final product with high efficiency when the synthesis is complete. This final cleavage step should proceed in high yield under mild conditions. Such conditions can be achieved by chemical modification of the linker prior to cleavage, as is the case for linkers dubbed as "safety-catch linkers" or by orthogonal release procedures. A further requirement is the straightforward synthetic accessibility of an envisaged linker.

One of the most stable carbon–heteroatom bonds of carboxylic acid derivatives is the amide bond. Its stability originates from the high resonance energy as well as from the poor nature of the leaving "$NHR^-$" group.[2,3] Enzymes like serine or cysteine proteases are able to perform such cleavages under mild conditions by orienting the substrates into their active sites and by enhancing the nucleophilicity of the OH and SH functionalities by an adjacent Asp and His residue forming so-called catalytic triades.

The nonenzymatic hydrolysis of amide bonds under neutral conditions is a field of intensive research. The focus is almost exclusively on the application of oxophilic metal ions or their complexes coordinating to the carbonyl group to allow attack by a suitable nucleophile. Nearly all rare-earth and transition-state metals have been investigated so far.[4] In nature, metalloproteases use the same mechanism for hydrolytic cleavage of peptide bonds.

An alternative but only rarely discussed mechanism of metal-assisted hydrolytic cleavage of amide bonds was proposed for the peptide sequences Xaa–Ser–His and Xaa–Thr–His. Copper(II) coordinates not to the oxygen atoms of the carbonyl groups but rather to two nitrogen atoms of the amide bonds and one of the imidazole nitrogen atoms leading to a weakening of the amide N–C-bond. An intramolecular attack of the hydroxy group of the serine OH leads first to an N→O acyl rearrangement, which is followed by hydrolysis of the ester bond (Scheme 1).[5,6] The cleavage according to Scheme 1 is certainly facilitated by the entropically favorable attack of the serine side chain hydroxy functionality.

A similar reaction but without involvement of an intramolecular attack is the direct $Cu^{2+}$-assisted methanolysis of bispicolylamides (Scheme 2).[7–10] Alsfasser used them as a model system for metal-binding peptides and proteins, where the two pyridine rings mimic the imidazole rings of the histidine side chains.[8–10] The participation of the amide

[a] Institut für Organische Chemie und Biochemie
Albert-Ludwigs-Universität Freiburg
Albertstraße 21, 79104 Freiburg
Fax: +49-761-203-8705
E-mail: willi.bannwarth@organik.chemie.uni-freiburg.de

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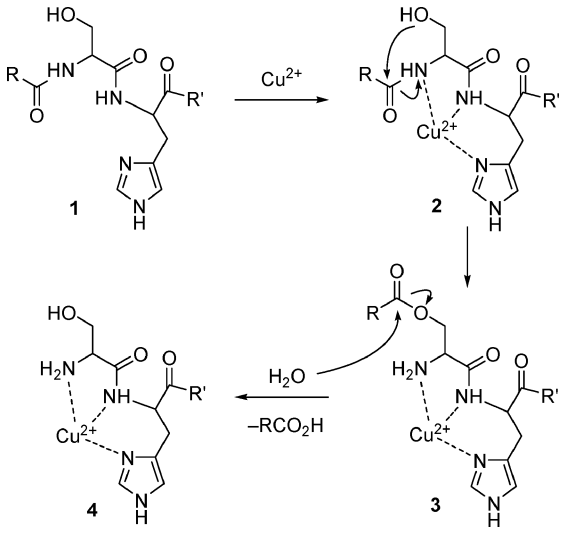
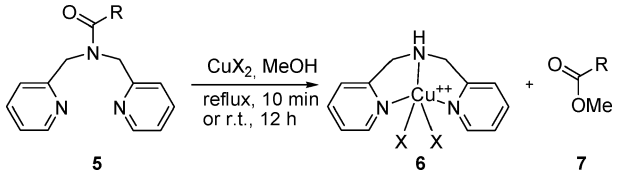


Scheme 1. Proposed mechanism for $Cu^{2+}$-assisted hydrolysis of a Xaa–Ser–His sequence.

nitrogen atom in the complexation was proven by X-ray analysis. Due to the spatial arrangement of the ligand the carbonyl oxygen atom cannot coordinate to the metal, whereas the amide nitrogen atom is in a preferred position for complexation resulting in a weakening of the amide bond.



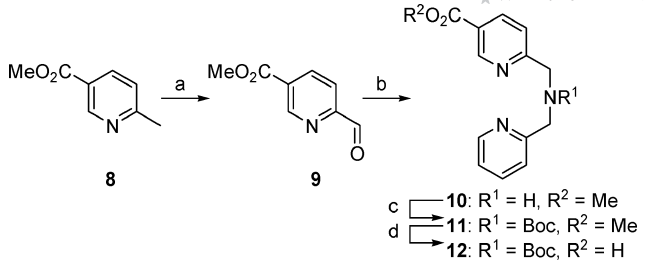
Scheme 2. $Cu^{2+}$-assisted hydrolysis of bispicolylamides; X = Cl, OTf, $ClO_4$.

The synthetic potential of this mild amide bond cleavage due to involvement of the amide nitrogen atom in complexation has, to the best of our knowledge, not been utilized yet.

We reasoned that such a bispicolylamine (bpa) ligand bound to a solid support would comply with most requirements for a linker to be used in a versatile way in solid-phase chemistry. It would allow attachment of substrates through a stable amide bond with release possible after complexation under mild conditions leading directly to an ester. Furthermore, the linker is chemically inert and would survive a plethora of reaction conditions without suffering modifications.

## Results and Discussion

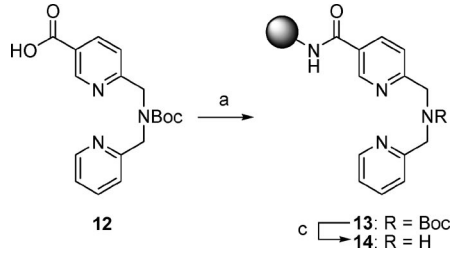
In order to test the above possibilities we synthesized 3-carboxy-bpa ligand **12** according to Scheme 3. After coupling to the solid support and cleavage of the Boc group it should allow for the attachment of substrates through an amide linkage.



Scheme 3. Synthesis of Boc-protected bispicolylamide **12**. Reagents and conditions: (a) $I_2$ (1.0 equiv.), *t*BuI (0.4 equiv.), TFA (3.0 equiv.), DMSO, 120 °C, 3 h, 69%; (b) 2-picolylamine (1.0 equiv.), $NaBH(OAc)_3$ (1.4 equiv.), 1,2-DCE, 1 h; (c) $Boc_2O$ (1.2 equiv.), $NEt_3$ (1.4 equiv.), $CH_2Cl_2$, 0 °C → r.t., 2 h, 69% over 2 steps; (d) NaOH (1.1 equiv.), MeOH/$H_2O$, reflux, 2 h, >98%.

The synthesis of **12** started with the oxidation of commercially available methyl-6-methylnicotinate (**8**) to the corresponding aldehyde **9** according to a literature procedure.[11] Reductive amination with 2-picolylamine and sodium triacetoxyborohydride in 1,2-DCE[12] gave secondary amine **10**, which was directly treated with $Boc_2O$ to yield the *N*-Boc-protected methyl ester **11** in 69% yield over two steps. Saponification with sodium hydroxide in methanol/water resulted in carboxylic acid **12** in quantitative yield.

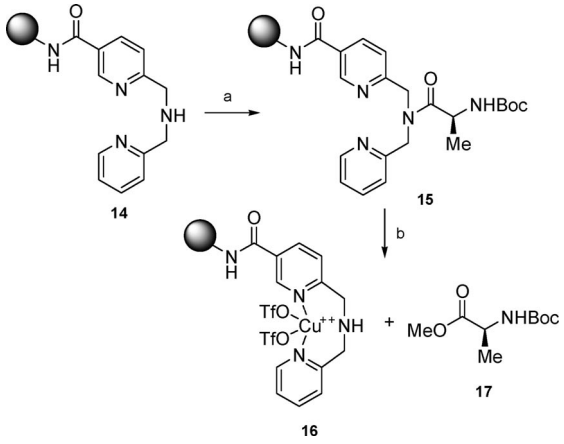
Boc-protected linker **12** was coupled to the amino-functionalized solid support by applying a 1.5 molar excess and by using TBTU as coupling reagent.[13] A negative Kaiser test[14] indicated completion of the coupling after 4 h. Deprotection of the Boc group from **13** yielded **14** (Scheme 4) ready for the attachment of substrates through amide bonds.



Scheme 4. Coupling of **12** to the solid support and cleavage of the Boc group. Reagents and conditions: (a) HypoGel-400-$NH_2$, DMF, 10 min; then **12** (1.5 equiv.), TBTU (1.5 equiv.), DIPEA (6.0 equiv.), DMF, r.t., 12 h; (b) *i*$Pr_3SiH$ (3.0 equiv.), $CH_2Cl_2$/TFA (1:1), 0 °C → r.t., 4 h.

With the functionalized support in hand, we coupled Boc-Ala-OH to the linker by using a standard amide coupling protocol. A negative chloranil test[15] indicated total conversion after 12 h. In order to probe the envisaged cleavage step, we treated the solid phase with equimolar amounts of $Cu(OTf)_2$ in methanol, which led directly to a green color of the support indicating complex formation. From the methanol solution the expected Boc-Ala methyl ester **17** was isolated in a yield of 50% over four steps starting from **12** and based on the initial loading of the support (Scheme 5).



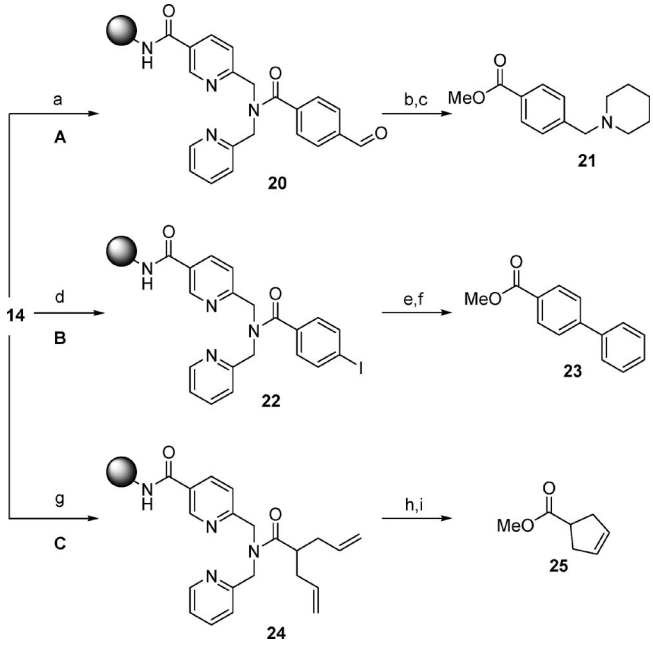

Scheme 5. Coupling of Boc-Ala-OH and $Cu^{2+}$-assisted cleavage to methyl ester **17**. Reagents and conditions: (a) Boc-Ala-OH (3.0 equiv.), TBTU (3.0 equiv.), DIPEA (12 equiv.), DMF, r.t., 12 h; (b) $Cu(OTf)_2$ (1.0 equiv.), MeOH, r.t., 12 h, 50% over 4 steps.

Despite its easy and effective synthesis, the recycling of used linker **16** would be desirable. This could be achieved by decomplexation of the linker by using a better complexing agent. Application of EDTA failed, but surprisingly the use of 1.1 equiv. of free bpa ligand led to decomplexation of the linker, indicated by loss of the green color. The solution, however, changed its color to green. Alternatively, treatment with a methanolic solution of potassium cyanide (2 equiv.) was used for the regeneration.

The regenerated linker was then treated with Boc-Phe-OH. Cleavage under the same conditions as before gave pure Boc-Phe methyl ester in a yield of 41%, without any trace of Boc-Ala methyl ester, indicating that the initial cleavage yielding Boc-Ala-OMe had been quantitative. Next, the stability of amide **15** was demonstrated under acidic (50% TFA in $CH_2Cl_2$) and basic (0.2 M LiOH in $H_2O$) conditions as well as in the presence of fluoride ions (0.1 M $Bu_4NF$ in THF). After these positive results we tested the suitability of the linker for peptide synthesis.

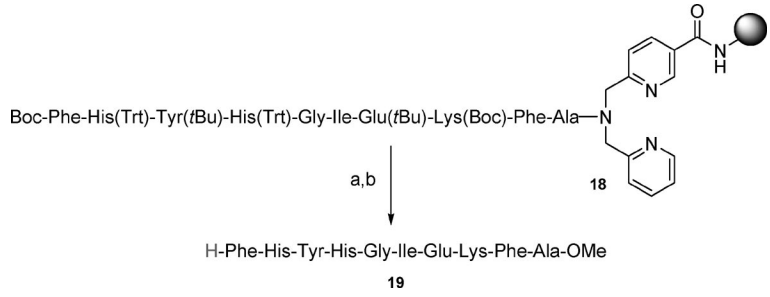
For this reason, we synthesized decapeptide **18** by using the Fmoc/*t*Bu strategy on a 70-μmol scale (linker-modified support; Scheme 6). The assembly on the support was followed by cleavage from the linker with $Cu^{2+}$/MeOH and deprotection with TFA, which yielded the crude peptide in >90% purity (210 nm) as judged from HPLC and LC–MS (see Supporting Information).

In order to extend the scope of application of the new linker for classic organic synthesis we next demonstrated its utility in a reductive amination, a Suzuki–Miyaura coupling, and a ring-closing metathesis reaction (Scheme 7; A, B, and C, respectively).




Scheme 7. Reactions on the bpa linker. Reagents and conditions: (a) 4-carboxybenzaldehyde (3.0 equiv.), TBTU (3.0 equiv.), DIPEA (12 equiv.), DMF, r.t., 12 h; (b) piperidine (10 equiv.), $NaBH(OAc)_3$ (10 equiv.), 1,2-DCE, r.t., 12 h; (c) $Cu(OTf)_2$ (1.0 equiv.), MeOH, r.t., 12 h, 49% over 5 steps; (d) 4-iodobenzoic acid (3.0 equiv.), TBTU (3.0 equiv.), DIPEA (12 equiv.), DMF, r.t., 12 h; (e) $PhB(OH)_2$ (5.0 equiv.), $K_3PO_4$ (10 equiv.), $Pd(PPh_3)_4$ (10 mol-%), DMF, 90 °C, 12 h; (f) $Cu(OTf)_2$ (1.0 equiv.), MeOH, r.t., 12 h, 31% over 5 steps; (g) 2-allyl-4-pentenoic acid (3.0 equiv.), TBTU (3.0 equiv.), DIPEA (12 equiv.), DMF, r.t., 12 h; (h) Grubbs 2nd generation catalyst (5 mol-%), $CH_2Cl_2$, r.t., 24 h; (i) $Cu(OTf)_2$ (1.0 equiv.), MeOH, r.t., 12 h, 34% over 5 steps.

In the reductive amination, coupling of 4-carboxybenzaldehyde to linker **14** was followed by reductive amination with piperidine and sodium triacetoxyborohydride in 1,2-DCE. Methanolysis after complexation with $Cu^{2+}$ gave desired methyl ester **21** in 49% yield over five steps starting from the amino-modified support.




Scheme 6. Cleavage of decapeptide **19**. Reagents and conditions: (a) $Cu(OTf)_2$ (1.0 equiv.), MeOH; (b) TFA/$CH_2Cl_2$/$iPr_3SiH$, 95:3:2.

Next, a Pd-mediated C–C-coupling reaction was carried out. Here we were not sure whether a possible complexation of Pd would take place thereby hampering the catalytic action of the Pd. After coupling of 4-iodobenzoic acid to the linker a Suzuki–Miyaura coupling was carried out with phenyl boronic acid and $Pd(PPh_3)_4$ as catalyst. We were very pleased that $Cu^{2+}$-assisted cleavage gave pure corresponding methyl ester **23** in 31% over five steps. The Cu content as estimated by AAS was <8 ppm.

Finally, a ring-closing metathesis (RCM) reaction was performed by attaching 2-allyl-4-pentenoic acid[16] to the linker. The RCM reaction was carried out by using 5 mol-% of Grubbs II catalyst in $CH_2Cl_2$. Methanolysis after $Cu^{2+}$ complexation gave desired methyl ester **25** in 34% yield over five steps.

The spectroscopic data of compounds **21**, **23**, and **25** were identical to those reported in the literature.[17–19]

## Conclusions

We have developed a chemically robust linker for solid-phase synthesis based on a rather uncommon involvement of an amide nitrogen atom in a $Cu^{2+}$ complexation and from which amide-bound compounds can be cleaved under very mild conditions. The linker entity **12** is accessible by a straightforward synthesis over four steps starting from commercially available methyl-6-methylnicotinate (**8**). We have demonstrated the recyclability of the linker and its versatility by applying it to peptide synthesis, reductive amination, Suzuki–Miyaura coupling, as well as to ring-closing metathesis. We think with these demonstrated examples the potential of the linker is by no means exhausted and further applications are currently in progress.

## Experimental Section

**General Procedure for Coupling of Carboxylic Acids to the Linker:** The solid phase carrying linker **14** (0.123 mmol) was suspended in DMF (2 mL). After 10 min, a solution of the acid (0.369 mmol, 3.0 equiv.), TBTU (0.369 mmol, 3.0 equiv.), and DIPEA (1.48 mmol, 12 equiv.) in DMF (3 mL) was added. The mixture was agitated for 12 h at room temperature. The solid support was filtered and washed alternately with DMF/*i*PrOH (5 × 3 mL), and subsequently with $CH_2Cl_2$ (3 mL) and MeOH (3 mL).

**General Procedure for $Cu^{2+}$-Assisted Cleavage of the Linker:** The solid support (0.123 mmol) was suspended in MeOH (2 mL). After 10 min, a solution of $Cu(OTf)_2$ (0.123 mmol, 1.0 equiv.) in MeOH (3 mL) was added. The mixture was agitated for 12 h at room temperature. The solid support was filtered off, and the methanolic solution was evaporated. The residue was taken up in $CH_2Cl_2$ (5 mL) and extracted with aqueous EDTA solution (0.5 M, 2 × 10 mL). The organic layer was dried with $Na_2SO_4$ and evaporated to give pure methyl ester.

**Supporting Information** (see footnote on the first page of this article): Experimental details for the preparation of **11**–**14**; solid-phase synthesis of decapeptide **18**; selected HPLC traces.

## Acknowledgments

We would like to thank Dr. M. Keller, Mrs. M. Schonhardt, and Mr. F. Reinbold for recording the NMR spectra, Mr. C. Warth and Dr. J. Wörth for recording the mass spectra, and Mr. E. Hickl for performing the elemental analyses.

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Received: June 30, 2008
Published Online: July 30, 2008

  