Peptide Modification

International Edition: DOI: 10.1002/anie.201503584
German Edition: DOI: 10.1002/ange.201503584

Selective Rhodium-Catalyzed Reduction of Tertiary Amides in Amino Acid Esters and Peptides**

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Abstract: *Efficient reduction of the tertiary amide bond in amino acid derivatives and peptides is described. Functional group selectivity has been achieved by applying a commercially available rhodium precursor and bis(diphenylphosphino)propane (dppp) ligand together with phenyl silane as a reductant. This methodology allows for specific reductive derivatization of biologically interesting peptides and offers straightforward access to a variety of novel peptide derivatives for chemical biology studies and potential pharmaceutical applications. The catalytic system tolerates a variety of functional groups including secondary amides, ester, nitrile, thiomethyl, and hydroxy groups. This convenient hydrosilylation reaction proceeds at ambient conditions and is operationally safe because no air-sensitive reagents or highly reactive metal hydrides are needed.*

Selective reduction of the amide bond allows for an interesting modification of amino acids and peptides and generation of potential novel biocatalysts.[1] In recent years, peptide derivatives have gained comprehensive attention for the development of novel pharmaceuticals due to their specificity in in vivo targets and their low toxicity. Currently, protein-/peptide-based drugs are estimated at $>$\$40 billion per year, or roughly 10% of the pharmaceutical market.[2] Although there are many advantages for this kind of drugs, the major drawback in life science applications is the insufficient bioavailability and biostability due to their high susceptibility toward hydrolysis of amide bonds by peptidase enzymes. For this purpose, several groups have attempted diverse modification of amide bonds especially substituting the amide bonds by other isosteres or by backbone modifications. Although this improved the current bioavailability, it often leads to tedious synthetic hazards and reduced effectiveness of the drugs. In this respect, the selective reduction of the amide bonds (reduced amide bond isosteres) in a peptide chain enhances the activity of the drugs as well as the synthetic availability.

For the first time, Martinez et al. introduced this concept of reduced amide bond isosteres for the modification of peptides.[3a] Later, Lever and co-workers reported in a seminal work a 2000-fold increase of renin inhibition by the reduction of one amide bond.[3b] This simple peptide bond reduction not only increased the potency of the peptide-based drug but also modulated the peptide dipole moment, which alternately changed pharmacological properties such as oral bioavailability and membrane permeability.[3c] Interestingly, such a change of dipole moment can also increase the net positive charge on the backbone and decrease the helicity of the peptides. For example, in farnesyl transferase inhibitors such as L-739750, the amino methylene group increases flexibility in the active site and enables easy complexation of the thiol with zinc.[3d]

In biology, enzymes themselves trigger this type of structural modification of peptides. Alternatively, man-made catalysts offer an interesting tool for the structural diversification of biomolecules and the development of new bioactive compounds.[4] Despite the paramount importance of such transformations, only few general non-biocatalytic processes for the postmodification of peptides are known.[5–7] Obviously, the key issue for any changes of polyamino acid derivatives is sufficient control of chemo- and regioselectivity. In this respect, a possible steric and electronic fine tuning of organometallic catalysts might permit such selectivity control.[8,9] Advantageously, combining the ease of current automated peptide synthesis and highly selective organometallic catalysis should offer an interesting novel approach for the modification of biomolecules.

For this type of reduction, metal hydrides such as lithium or sodium aluminium- and borohydrides are applied as stoichiometric reducing agents only for simple amides.[10] More recently, we[11–13] and others[14–19] have shown that it is possible to reduce aromatic and aliphatic amides in the presence of other reducible groups using different organometallic catalysts and silanes. Notably, the reduction of different classes of primary, secondary, and tertiary amides requires different catalysts and reaction conditions according to their different reactivity pattern. So far most of the catalysts work for tertiary amides,[11,12,14–19] compared to secondary[13a,14e,17] and primary amides.[13b,14e,17] It should be noted, that no other catalytic reduction methodology offers such a diversity of potential catalysts.[20] In addition, more than 100 silanes are commercially available which allows for

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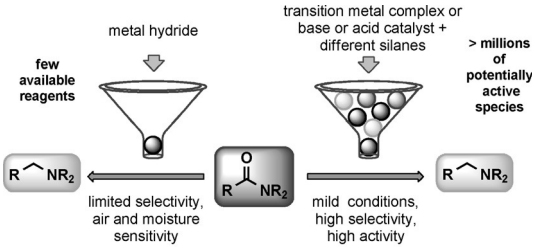
[**] This work is supported by the state of Mecklenburg-Vorpommern and the BMBF (Bundesministerium für Bildung und Forschung). We thank Novartis Pharma AG for funding and Dr. Florian Rampf (Novartis Pharma AG) for his support.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201503584.

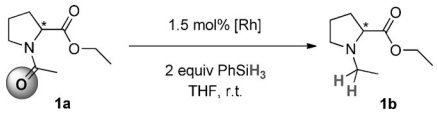
a fine tuning of reactivity and selectivity in a given hydrosilylation reaction.[21] Based on these findings, we had the idea to develop a protocol, which allows for the selective reduction of tertiary amides in amino acid derivatives and peptides in the presence of secondary amides and ester groups (Scheme 1). Ultimately, this idea may lead to a selective functionalization of proteins.



Scheme 1. Comparison of stoichiometric and catalytic reductions of amide bonds.

At the start of this project, we were interested in finding a suitable catalyst for the mild reduction of amino acid amides in the presence of an ester group without any racemization. Hence, we investigated the reaction of *N*-acetyl proline ethyl ester (**1a**) with phenyl silane using various catalysts. An initial screening of iron-, zinc-, platinum-, and rhodium-based complexes as well as Lewis bases showed best reactivity with rhodium complexes at room temperature. Therefore, we examined this class of catalysts in the model system in more detail (Table 1). In fact, the use of 1.5 mol% of the simple

Table 1: Catalytic hydrosilylation of *N*-acetyl proline ethyl ester **1a**.[a]

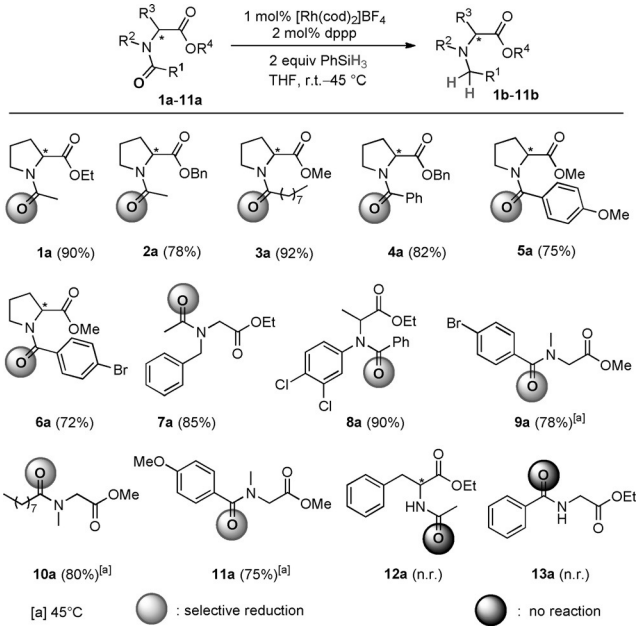


Entry	[Rh] [mol%]	Ligand [mol%]	Yield [%][a]
1	$RhCl_3$ (1.5)	–	38
2	$Rh(NO_3)_3 \cdot x H_2O$ (1.5)	–	–
3	$[Rh(PPh_3)_3Cl]$ (1.5)	–	42
4	$[Rh(H)(CO)(PPh_3)_3]$ (1.5)	–	90
5	$[Rh(cod)_2]BF_4$ (1.5)	–	20
6	$[Rh(cod)_2]BF_4$ (1.5)	dppp (1.5)	90

[a] Yields were determined by GC using hexadecane as a standard. cod = 1,5-cyclooctadiene.

$RhCl_3$ gave 38% of *N*-ethyl proline ethyl ester (**1b**) as the major product (Table 1, entry 1). Although Wilkinson's catalyst showed somewhat higher reactivity, we studied the influence of several phosphine ligands in combination with $[Rh(cod)_2]BF_4$ (Table S1). Clearly, phosphine ligands play a significant role for catalytic activity. Here, higher yields were observed with electron-deficient phosphines. Gratifyingly, the use of $[RhH(CO)(PPh_3)_3]$ or the in situ-derived catalyst from $[Rh(cod)_2]BF_4$ and 1,3-bis(diphenylphosphino)-propane (dppp) in the model reaction gave 90% of product **1b** (Table 1, entries 4 and 6)! Because of the higher stability of the latter catalyst system, we used this one in the following experiments. Noteworthy, in none of these reactions any significant racemization of the product **1b** was observed.[22]

In addition to phenyl silane, other silanes such as Ph_2SiH_2, $(EtO)_3SiH$, $(EtO)_2MeSiH$, $(MeO)_3SiH$, polymethylhydrosiloxane (PMHS), 1,1,3,3-tetramethyldisiloxane (TMDSO) were also tested for this reduction (Table S2). However, only Ph_2SiH_2 showed appreciable activity. It should be noted, that the reaction can be performed in the presence of only one equivalent of $PhSiH_3$. However, in this case slower conversion took place. Next, we explored the reactivity and chemoselectivity of this rhodium-catalyzed reduction using various *N*-acyl amino acid derivatives (Scheme 2). Different tertiary amino acids such as *N*-acyl proline and *N*-acyl sarcosine esters



Scheme 2. Substrate scope for the selective reduction of tertiary and secondary *N*-acetylamino acid esters. Yields of isolated products are given in parenthesis.

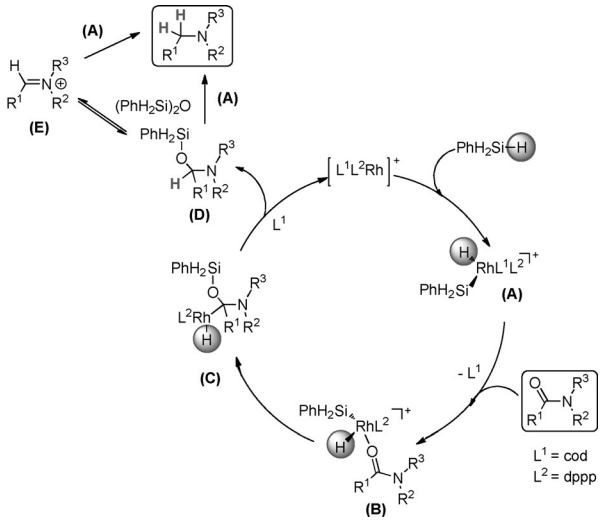
were smoothly transformed with $PhSiH_3$ to the corresponding reduced amino acids in the presence of 1 mol% of $[Rh(cod)_2]BF_4$ and 2 mol% of dppp. To isolate the desired product and to avoid ester hydrolysis, the reaction mixtures were stirred overnight with a saturated aqueous solution of ammonium fluoride.

Comparing the reduction of proline and sarcosine amides, the reaction rate differs significantly. Hence, for *N*-acyl sarcosinates slightly higher reaction temperature (45 °C) was required (Scheme 2, **9a**–**11a**). Apparently, it is possible to reduce different tertiary amides selectively. No difference in reactivity is observed for electron-withdrawing and electron-donating substituents at the *N*-benzoyl phenyl ring (Scheme 2, **5a**–**6a**). In agreement with our initial concept, secondary amino acid amides like *N*-acetyl phenylalanine ethyl ester and *N*-benzoyl phenylalanine ethyl ester did not show any activity under these reaction conditions (Scheme 2, **12a**–**13a**). Moreover, it is interesting to note that in all cases,

no significant reduction (<2%) of the ester group was observed.

To illustrate the chemoselectivity of the present catalytic system, *N*-acetyl proline ethyl ester (**1a**) was reduced in the presence of seven other *N*-acyl amino acid esters under the optimized reaction conditions (Scheme S1). To our delight, we obtained a highly specific reaction of the proline amide and no reduction of the other substrates was found.

Regarding the mechanism of this selective reduction, we propose an initial activation of the silane by the in situ-formed cationic rhodium phosphine complex to generate an active Rh^{III} hydride species **A** (Scheme 3).[16] After coordination of the amide to give **B** and insertion of the carbonyl group to the

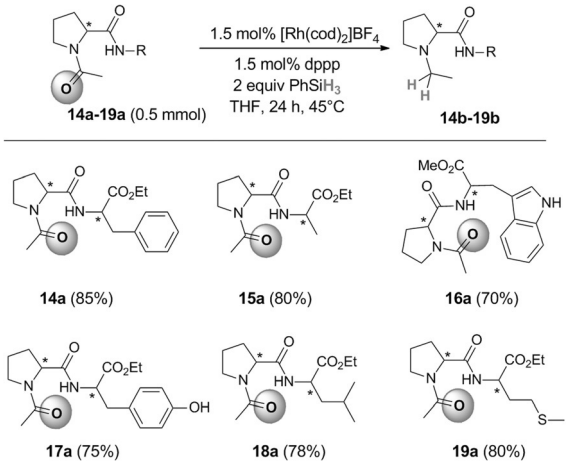


Scheme 3. Proposed catalytic cycle for the rhodium-catalyzed hydrosilylation of tertiary amide bonds. The ligands on the rhodium center are named L^1L^2 for clarity.

Rh–Si bond the alkyl rhodium species **C** is formed. Subsequent reductive elimination of **C** provides the silylated aminoacetal **D**. This latter compound may either directly react with another cationic rhodium hydride species **A** or alternatively generate an iminium intermediate **E**.[23] In both cases, the desired amine product, the original Rh^I precatalyst and siloxanes are generated through reductive elimination.

The most notable feature of our transformation is the selective reduction of tertiary amide bonds while esters and secondary amides show no reactivity. This difference in the reactivity can be explained by the preferential coordination of the tertiary amide in the presence of other carbonyl compounds. In this context, the increased stability of the intermediate **B** in the case of tertiary amides should enable the selective progress of the reaction. Moreover, the higher electron density around the nitrogen atom of the tertiary amide due to the presence of an extra alkyl substituent improves the coordination with the metal center and the amide oxygen group compared to the secondary amide. Here, mixtures of catalyst, tertiary or secondary amides, and silane in THF were measured at ambient temperature and −60 °C using ^1H, ^{13}C, ^{29}Si, and ^{31}P NMR spectroscopy. Indeed, in all cases the signals of substrates and final products can be clearly assigned. Solutions containing catalyst and both types of amides in the absence of silane did not show any strong interaction of the amide with the catalyst. However, as soon as phenylsilane was added to the mixture, conversion started immediately and the reaction proceeded to the respective amines. Notably, in the ^1H NMR spectra recorded at −60 °C broad Rh–H signals were observed at −6 to −10 ppm, which indicate the formation of the proposed rhodium hydride species.

After having demonstrated the selective reduction of different sarcosine and proline derivatives, we looked into the selective reduction of peptide molecules. Therefore, six different dipeptides (Scheme 4, **14a**–**19a**) were synthesized by combining *N*-acetyl proline and different other amino acid



Scheme 4. Selective catalytic hydrosilylation of dipeptides. Yields of isolated products are given.

esters together. To our delight, in all cases the hydrosilylation using $[Rh(cod)_2]BF_4$/dppp catalyst proceeded selectively at the tertiary *N*-acetyl bond, while the other peptide bonds were not affected. When the catalytic experiments are performed under mild conditions, sensitive functional groups such as unprotected indole, free hydroxy group in tyrosine, and the thiomethyl substituent in cysteine are well tolerated, too. As shown in Scheme 4, all partially reduced dipeptides (**14b**–**19b**) were isolated in good yields (70–85%).

Next, we examined the reactivity of a tri- and tetrapeptide. Again, the rhodium catalyst was highly specific in selectively reducing proline- and sarcosine-based amide bonds in the peptide chain (Figure 1). Thus, the carbonyl moiety at C7 in the tripeptide **20a** was selectively transformed into a methylene group. Using a slightly larger amount (10 mol%) of the $[Rh(cod)_2]BF_4$/dppp catalyst and three equivalents of $PhSiH_3$ at 50 °C gave **20b** in 60% yield within 48 h (Figure S2). Notably, a further increase of the catalyst amount to 20 mol% led to complete conversion and gave **20b** with a selectivity above 95%. In case of the tetrapeptide **21a** the desired product **21b** is already formed selectively at lower catalyst loading (5 mol%; 45% yield). More catalyst favors the formation of higher reduced species (Figures S3 and S5). Due to the tedious isolation of both products, the yields and substrate-to-product ratios were determined by ^1H and ^{13}C

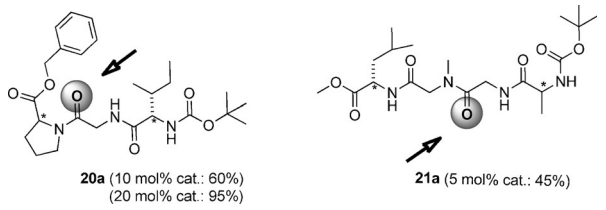
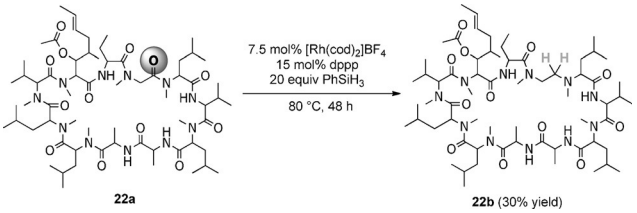


Figure 1. Selective reduction of the tertiary amide bond in peptides.

inverse gated decoupling (IG) NMR spectroscopy, and the structures of the products were confirmed by one- and two-dimensional ^{1}H and ^{13}C NMR spectroscopy as well as HRMS. To show the selective transformation, representative HRMS and NMR spectra of **20b** and **21b** are shown in Figures S1–S5.

Finally, we became interested in testing the generality of our methodology for the selective reduction of pharmaceutically important cyclic peptides. For this purpose we adopted the catalyst system to cyclosporine A, which constitutes one of the most important immunosuppressants today.[24] Hence, there exists significant interest in the synthesis of new derivatives. More specifically, cyclosporine A is a cyclic polypeptide of eleven amino acids containing four secondary and seven tertiary amide bonds, which is produced conveniently on large scale by fermentation.[25–27] Taking the complex structure of cyclosporine A into account, raises the question if a selective reduction of a certain amide bond in this cyclic polypeptide is still possible with our catalyst system. Due to the challenging analytical setup of macropeptides, the product formation was monitored by ^{1}H NMR spectroscopy. First we started with the above conditions (1.5 mol% $[Rh(cod)_2]BF_4$; 1.5 mol% dppp; 2 equiv $PhSiH_3$; THF, rt, 46 h), but no reactivity was observed. To our delight, at higher temperature (80 °C) in toluene we detected a new product in the reaction mixture indicating reduction of an amide function.

The HRMS spectrum of the crude product showed a characteristic signal at $m/z = 1210.85$ for $[M+Na]^+$, demonstrating reduction of only one amide moiety. In agreement with this observation the ^{1}H NMR spectrum showed signals of a new CH_2-group in the range of 2.7 ppm. Due to isolation problems, we performed similar reactions with the known acetylated cyclosporine A **22a**.[28] This compound is expected to allow an easier purification due to increased interactions with the stationary silica phase during chromatography.[28] Having a reliable work-up strategy in hand, selectivity and yield of the reduction were improved. Best results (30% yield of isolated **22b**) were obtained using an excess (20 equiv) of $PhSiH_3$ which was added in two portions. Notably, a further increase of the amount of silane had no positive impact on the chemoselectivity. Regarding the catalyst/ligand ratio, the best reactivity was obtained using a 1:2 ratio. NMR-spectroscopic investigations of the isolated product showed the reduction of only one amide group of acetylated cyclosporine A. The structure of compound **22a** and the reaction product **22b** were confirmed by ^{1}H, ^{13}C, and ^{15}N NMR spectroscopy. Thus, eleven carbonyl signals were found for the amide groups in **22a**, whereas for **22b** only ten signals were observed. Furthermore, signals for an additional CH_2 group were found in both the ^{1}H (2.81 and 2.69 ppm) and ^{13}C NMR (55.1 ppm) spectra of **22b**. Finally, the reaction of only one amide group is clearly confirmed by the significant high-field shift (approx. 100 ppm, δ $^{15}N = -351$) of one signal in the ^{15}N NMR spectrum (^{1}H,^{15}N HMBC, 4-NCH_3 correlates with 4-NCH_3), which can be assigned to an amine moiety. The position of the newly formed CH_2 group within the cyclosporine ring system was also confirmed by the ^{1}H,^{1}H COSY correlations found for a -CH_2-CH_2- spin system. Such an atom sequence is only possible in the case of MeN-CH_2-CH_2- located in the 3-position (sarcosine) of this ring system. To demonstrate the practicability of our catalytic protocol, the reaction was performed on a 5 mmol scale (6.2 g of **22a**) giving a similar yield of isolated product compared to the small-scale experiment (Scheme 5).



Scheme 5. Selective rhodium-catalyzed reduction of acetylated cyclosporine A.

In conclusion, we have established for the first time a highly selective reduction of amino acid esters and peptides using rhodium phosphine catalysts and silane under mild conditions. The optimized catalyst system tolerated a wide variety of functional groups including secondary amides, esters, nitrile, thiomethyl, and hydroxy as well as heterocycles, for example, indole. The convenient procedure (no pressure or air-sensitive reagents), the operational safety (no highly reactive metal hydrides), and the mild conditions make this new reduction protocol attractive. Based on this methodology, one can expect that catalytic hydrosilylations should be able to modify various other peptides, too. By combining the advantages of automated peptide synthesis or fermentation processes with the here presented selective reduction, a multitude of novel peptide derivatives for chemical biology studies as well as potential pharmaceutical applications should be available. Moreover, we expect our system to be applicable for the derivatization of other interesting natural products containing amides without using protecting and deprotecting steps.[30]

Keywords: amide bond · amino acid · peptide modification · rhodium · selective reduction

How to cite: *Angew. Chem. Int. Ed.* **2015**, *54*, 12389–12393
Angew. Chem. **2015**, *127*, 12566–12570

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[29] Additionally, an acidic Al_2O_3 (cont. 1% H_2O) column chromatography was performed to separate the desired product from the starting material. For further details see the Supporting Information.
[30] As an example we have performed the catalytic reduction of strychnine to strychnidine with the same catalyst system in 78% yield.

Received: April 21, 2015
Revised: May 28, 2015
Published online: July 17, 2015

