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$NaBH_4$ – a novel method for the deprotection of N^{ω}-nitro-arginine

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Abstract

The selective deprotection of N^{ω}-nitro-arginine derivatives represents a major preparative challenge. This problem can be circumvented by the use of catalytic hydrogenation, but often high pressure, elevated temperature, and/or long reaction times are needed. In certain cases hydrogenation is not suitable, for example, small-scale reactions, parallel synthesis, or due to selectivity issues. Herein, we demonstrate for the first time, the use of $NaBH_4$ in the presence of a metal ion catalyst for the removal of the N^{ω}-nitro moiety under simple, 'open-vessel' conditions. This process using $NaBH_4$ does not remove the benzyloxy-carbonyl-protecting group; thus the method is orthogonal for this protecting scheme.

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Introduction

The benzyloxycarbonyl (Z-) protecting group has been widely utilized in traditional peptide synthesis in combination with the N^{ω}-nitro protected arginine derivative.[1,2] This protecting scheme is used in large scale solution-phase peptide synthesis,[3,4] and solid phase chemistry.[5] Of the two protecting groups the benzyloxycarbonyl group has been well-established to be selectively depro-tected by strong acids, for example, HBr/acetic acid (Scheme 1). However, no reports are available regarding the selective removal of the N^{ω}-nitro groups of protected arginine in the presence of Z-protected amino groups. The development of an effective method for the selective deprotection of amino group(s) in the presence of benzyloxycarbonyl-protected amino group(s) should broaden the scope of using these protecting schemes not only in the field of peptides, but perhaps also in general organic synthesis, for example, in cases where catalytic hydrogenation is not applicable.[6]

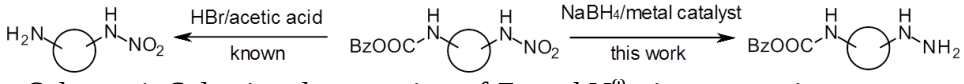
The reduction of C–NO_2-groups is often a key step in organic synthesis. A large selection of reducing agents are available to con-vert the NO_2 group to NH_2 (HF, catalytic hydrogenation), but does not include $NaBH_4$, which is well known for its selectivity toward oxo-groups in the presence of nitro-groups. However, with the aid of catalysts, $NaBH_4$ can be made to reduce NO_2-groups as well.[7–10] N-nitration is one

possible way to achieve guanidino-NH_2-protection. The cleavage of this protecting group is the standard method of deprotection, but the use of $NaBH_4$ for this purpose has not been documented so far. Herein, we report our findings on the development of an effective approach for the selective deprotection of a guanidino-nitro protecting group in the presence of a benzyloxycarbonyl-group, using $NaBH_4$ and selected metal ion catalysts (Scheme 1).[16]

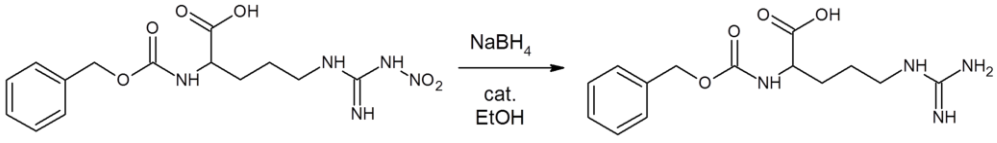
Results and discussion

In order to demonstrate the usefulness of this new approach, N^a-Z-N^ω-nitro-L-arginine (4) was deprotected to give N^a-Z-L-argi-nine with $NaBH_4$ in the presence of a metal ion catalyst (Scheme 2 and Table 1).[17] The conversion rate was determined by RP-HPLC and it was found that 8 equiv of $NaBH_4$ was not enough to com-plete the reaction, while 10 equiv resulted in almost full transformation under the conditions used.[12]

We then studied the effect of various metal ion catalysts under the same experimental conditions (Table 1). We found copper(II) acetylacetonate ($Cu(acac)_2$) and copper(II) N,N,N',N'-tetram-ethylethylenediamine (Cu-TMEDA) to have the optimal effect. Cop-per-phthalocyanine and nickel acetylacetonate ($Ni(acac)_2$) were less effective as catalysts. Using cobalt(II) acetylacetonate contain-ing crystal water ($Co(acac)_2$ $2H_2O$) the target compound was not detected, and the use of water-free $Co(acac)_2$ resulted in the formation of several unidentified side-products. A possible explanation for this finding is the known catalytic effect of hydrated cobalt-salts in reducing carboxylic esters.[11]



Scheme 1. Selective deprotection of Z- and N^ω-nitro protecting groups.



Scheme 2. Deprotection of N^ω-nitro-arginine.

Table 1
Catalysts studied. ethanol was used as solvent

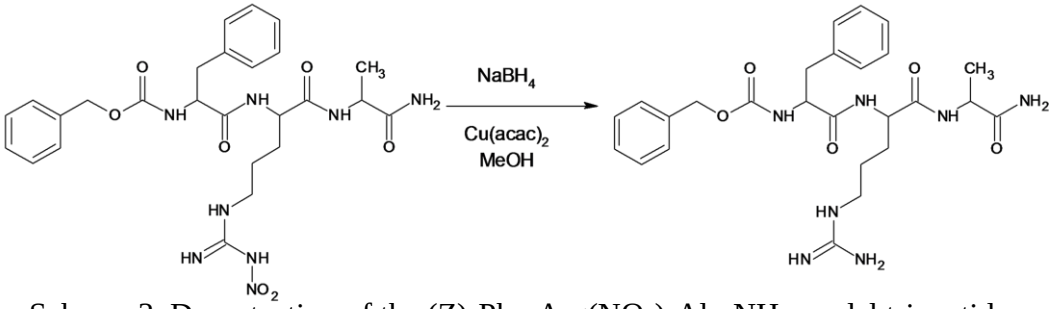
Catalyst (10 mol %)	Conversion (%)		
	After 30 min	After 120 min	
$Cu(acac)_2$	69	>99	
Cu-TMEDA	74	>99	
Cu-phthalocyanine	33	70	
$Ni(acac)_2$	43	62	
$Co(acac)_{2a}2H_2O$	—	70[*]	**
$Co(acac)_2$	92	>99	

[a] The water-free compleω (violet color vs. the pink color of the hydrate) was produced by azeotropically distilling off the solvent from an acetonitrile-solution of the hydrated compleω.

[*] Approx. 100% of various side products.
[**] Approx. 30% of various side products.

By far the most suitable solvent for the reaction was dry ethanol. In methanol the formation of several side-products could be observed and additionally it is well-known that the borohydride reagent is rapidly consumed by the solvent. Longer chain alcohols (propyl alcohol, butyl alcohol) or ethers (tetrahydrofuran, dioxane, diisopropyl ether) were also tested as solvents, but the reaction was typically very slow, or no reaction could be detected, perhaps due to the poor solubility of the reactants. Even traces of water were found to jeopardize the reaction causing the formation of side-products, for example, by transesterification or by the formation of metal-boride reactive intermediates.[12]

It is known, that methyl-, ethyl-[13], and benzotriazole-esters[14] can be cleaved using $NaBH_4$ without catalysts, or benzyl esters in the presence of excess nickel-salt.[15] We studied the stability of various ester type protecting groups under the optimal eωperimental conditions for the deprotection of the guanidino-nitro group, thus demonstrating the orthogonality. It was found that N^a-Boc, benzyl-esters, and ethers, typical protecting groups in Boc/Bzl peptide synthesis protocols, remained intact (ESI, Table 2).



Scheme 3. Deprotection of the (Z)-Phe-Arg(NO_2)-Ala-NH_2 model tripeptide.

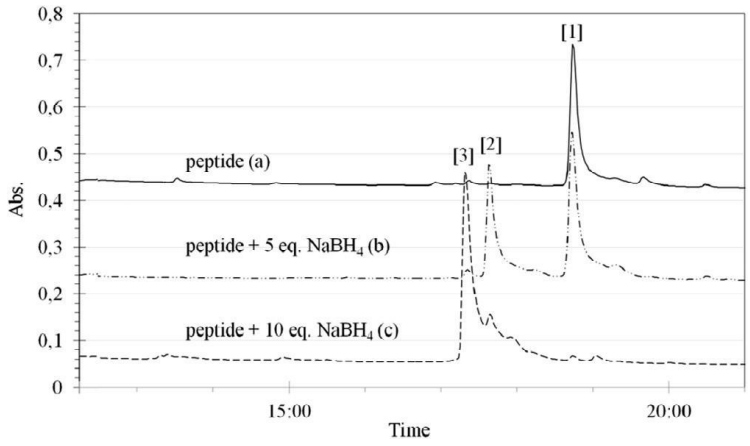


Figure 1. RP-HPLC chromatograms of (Z)-Phe-Arg(NO_2)-Ala-NH_2 model tripeptide deprotection. Reaction mixtures recorded after separation on a Phenomenex Synergi C12, 4 l, 120 A, 4.6 300 mm column, using TFA/H_2O (V/V), 0.1% TFA/ 80% acetonitrile-H_2O (V/V), gradient elution 20–80% B (5-20 min), and detection: UV k = 220 nm. (a) without $NaBH_4$, (b) 5 equiv $NaBH_4$, injected after 30 min, (c) an additional 5 equiv $NaBH_4$, injected after 30 min. Peaks: [1] (Z)-Phe-Arg(NO_2)-Ala-NH_2, [2] (Z)-Phe-Arg(NO)-Ala-NH_2, [3] (Z)-Phe-Arg-Ala-NH_2 were identified using electrospray ionization mass spectrometry.

To further explore the suitability of $NaBH_4$-deprotection we synthesized a model tripeptide N^a-Z-L-phenylalanyl-N^ω-nitro-L-arginyl-L-alanine-amide ((Z)-Phe-Arg(NO_2)-Ala-NH_2) (6, ESI, Scheme 4) by conventional solid-phase methods.[18] The peptide was cleaved from the solid support, and used without further purification.[13] This compound was deprotected using $NaBH_4$ in the presence of copper(II) acetylacetonate (Scheme 3).[14] Both ethanol and methanol was used as solvents with similar results. The reaction process was monitored by RP-HPLC (Fig. 1). The $NaBH_4$ (10 equiv) was added in two equal portions, and 30 min later each sample was analyzed. Neither the formation of a debenzyloxycarbonyl peptide derivative, nor amide hydrolysis or epimerisation was detected. The identity of the product was verified by IR and NMR. The yield was acceptable using this method and the 10 mg reaction-scale used.[19]

Conclusions

In summary, a simple and widely applicable method has been devised for the guanidino N^ω-NO_2 deprotection of the arginine side chain with high yield and chemoselectivity. $NaBH_4$ in ethanol in the

presence of a copper-complex catalyst was an effective reagent. This method was also applied to an oligopeptide containing an Arg (NO_2) residue. It has been demonstrated that the conditions developed for the removal of the N^{ω}-NO_2 group were compatible with conventional peptide synthesis techniques. To the best of our knowledge this is the only general method for selective deprotection of the N-NO_2 group leaving the N^{a}-Boc, and the benzylic-type Z-, Bzl-, OBzl- groups intact, thus this reagent could open a 'new dimension' in the orthogonality of traditional peptide protecting groups.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at

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16. General: Electrospray ionization mass spectrometry (ESI-MS) spectra were recorded on Bruker Daltonics Esquire 3000+ ion trap mass spectrometer. Reactions were monitored by TLC on silica gel 60 F254 plates. Melting points were determined on a Büchi 530 apparatus and are uncorrected. The reactions were all followed by Reversed Phase High-performance Liquid Chromatography (RP-

HPLC, using Phenomenex Synergi C12, 4 l, 120 Å, 4.6 300 mm column, the eluents were: 0.1% TFA/H_2O (V/V), 0.1% TFA/80% acetonitrile-H_2O (V/V), gradient elution 20–80% B (5–20 min), detection: UV k = 220 nm) and ESI-MS. The reaction mixture was purified by RP-HPLC (using Phenomeneω Jupiter C18, 5 l, 10 300 mm column, the eluents were: 0.1% TFA/H_2O (V/V), 0.1% TFA/80% acetonitrile-H_2O (V/V), gradient elution 5–100% B (5-60 min), detection: UV k = 220 nm). FT-IR spetra were recorded on Bruker IFS 28, ATR technics FT-IR Spectrophotometer. The ^1H and ^{13}C NMR spectra were recorded in DMSO-d_6 solution in 5 mm tubes at RT, on a Bruker DRΩ-500 spectrometer at 500 (^1H) and 125 (^{13}C) MHz, with the deuterium signal of the solvent as the lock and TMS as internal standard. The HSQC, HMBC, and COSY spectra, which support exact assignment of ^1H and ^{13}C NMR signals were obtained by using the standard Bruker pulse programs.

17. N^a-(Z)-L-arginine (5): To a mixture of N^a-(Z)-$N^ω$-nitro-L-arginine (4, 50 mg, 0.142 mmol) and catalyst (10 mol %) in dry ethanol (1 mL) $NaBH_4$ (1.42 mmol) was added in a single portion. The mixture was stirred at room temperature for 2 h. Glacial acetic acid (10 ll) was added to quench the excess $NaBH_4$. The solvent was evaporated, and the crude product dissolved in the RP-HPLC eluents (0.1% TFA/H_2O (V/V), 0.1% TFA/80% acetonitrile-H_2O (V/V)), and filtered through a disc filter (hydrophilic PTFE, pore size: 0.45 lm). The conversion was followed by RP-HPLC. The collected fractions were lyophilized.
18. N^a-(Z)-L-phenylalanyl-$N^ω$-nitro-L-arginyl-L-alanine-amide (6): The compound 6 was synthesized by solid phase peptide synthetic methodology on Rink-Amide MBHA resin (0.56 mmol/g), with the Fmoc/Bu strategy. N^a-Fmoc-alanine, N^a-Fmoc-$N^ω$-nitro-L-arginine and N^a-Z-phenylalanine were coupled using DIC/HOBt (4 equiv). Fmoc-deprotection was achieved by repeated (4 times: 2 min, 2 min, 5 min, 10 min) use of 2% piperidine and 2% DBU in DMF (V/V). The cleavage of the peptide from the resin was performed using TFA/H_2O/TIS (V/V = 95/2.5/2.5) for 1 h. The product was precipitated with Et_2O, isolated by freeze-drying from 50% acetonitrile/water (V/V) (yield: 88%). The crude product (97% purity by HPLC) was used without further purification for the $NaBH_4$ reduction step.
19. N^a-Z-L-phenylalanyl-L-arginyl-L-alanine-amide (7): N^a-(Z)-L-phenylalanyl-$N^ω$-nitro-L-arginyl-L-alanine-amide tripeptide (20.0 mg, 6, 35.2 lmol) was dissolved in dry methanol or ethanol (4 mL), and stirred with copper(II) acetylacetonate (2 mg, 7.6 lmol) catalyst, then $NaBH_4$ (352 lmol) was added. The mixture was stirred at room temperature for 1 h. Glacial acetic acid (40 ll) was added to quench the excess $NaBH_4$. The reaction mixture was filtered through a disc filter (hydrophilic PTFE, pore size: 0.45 lm). The product was purified using RP-HPLC and the collected fraction lyophilized One unidentified side product's peak overlapped on the chromatogram with the product thus lowering the yield of purification (isolated yield: 76% (1 mg scale) or 27% (20 mg scale)). The formation of the partially reduced nitroso compound was demonstrated by MS (ESI, Fig. 2). The product was characterized by ESI-MS, NMR, and FT-IR.