Extremely mild reagent for Boc deprotection applicable to the synthesis of peptides with thioamide linkages

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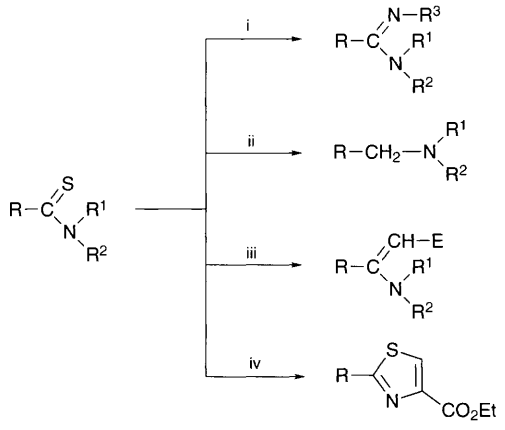
$SnCl_4$ in organic solvents acts as an extremely mild reagent for Boc deprotection even in the presence of the acid-labile thioamide moiety with excellent yields.

Peptide backbone modification by replacement of an amide moiety by a thioamide moiety has attracted considerable attention in recent years for several reasons. Firstly, receptor interactions of sulfur analogues of biologically active peptides may be more selective or more potent than their parent compounds and secondly, enhanced stability against enzymatic action can be shown for various proteolytic enzymes.[1] The introduction of a thioamide moiety is an almost isologous substitution for an amide moiety[2] but biological studies have shown that the behaviour of such modified compounds is unpredictable.[3] Additionally, thioamides serve as synthons for several organic transformations, especially the synthesis of heterocyclic compounds.[4] Hydrazines, amino nitriles and amines have been shown to react by nucleophilic displacement with thioamides to yield amidrazones, cyano amidines and alkyl amidines, respectively.[5] Thioamides could be reduced by the use of nickel boride to amines[6] and disubstituted thioamides transformed to methyl ketone derivatives.[7] The Hantzsch reaction can be used for preparing substituted thiazoles by reaction of thioamides with α-halogenocarbonyl compounds [8] (Scheme 1). Unfortunately, the use of the convenient Boc group for the protection of amino functionalities in the presence of thioamides is problematic because of the acid sensitivity of this moiety. Thus, acidolytic Boc deprotection of thioxo-containing† peptides led in the most cases to unsatisfactory yields of between 10 and 65%.[9] Beside dethioxylation, thioxylated products often underwent a side reaction similar to the Edman degradation.[10]

In order to overcome these disadvantages a fast, extremely mild and simple cleavage method has been developed. This method provides thioxo-containing amino acid derivatives and peptides in high yield and excellent purity without dethioxylation or thiazolone formation. For structurally related Boc protected amides and carbamates, Stafford *et al.*[11] have shown a selective Boc-deprotection using $Mg(ClO_4)_2$ or $SnCl_2$ in MeCN at high temperatures over a period of several hours or days. Simple Boc protected amino acid derivatives, *e.g.* Boc-proline amide and Boc-Phe-OBz, are unchanged by these reaction conditions even after prolonged refluxing in MeCN. In contrast to this work we were able to deprotect Boc-proline (Table 1, entry 1) within a few minutes at room temperature using $SnCl_4$. Thus, both methods complement each other. The examined Boc protected amino acid derivatives and peptides were treated with $SnCl_4$ in various organic solvents (ethyl acetate, toluene, MeCN, CH_2Cl_2). In particular, we studied the deprotection of thioxylated Boc-peptide-4-nitroanilides (-NH-Np), a class of compounds that has received considerable attention for the investigation of enzyme mechanisms.[12] The results are summarized in Table 1. The deprotection of all investigated compounds proceeded smoothly in excellent yields without any detectable side-reaction.



Scheme 1 *Reagents*: i, NH_2R^3; ii, $NiCl_2$ $NaBH_4$; iii, XCH_2E (X = halide, E = electrophile); iv, $BrCH_2COCH_2CO_2Et$ (R^1 = R^2 = H)

Table 1 $SnCl_4$-mediated deprotection of Boc-amino acids and peptide derivatives[a]

Entry	Starting material	Solvent	*T*/°C	Time	Yield (%)
1	Boc-Pro-OH	AcOEt	Room temp.	35 min	98
2	Fmoc-Ala-NH-NH-Boc	AcOEt	Room temp.	80 min	82
3	Fmoc-Lys(Boc)-OH	AcOEt	Room temp.	25 min	97
4	Fmoc-His(Boc)-OH	AcOEt	Room temp.	25 min	98
5	Boc-Ala-Phe-Gly-ψ[CS-NH]-Np	AcOEt	Room temp.	35 min	88
6	Boc-Pro-ψ[CS-NH]-Phe-NH-Np	THF	Room temp.	20 min	22
7	Boc-Pro-ψ[CS-NH]-Phe-NH-Np	THF	50	20 min	98
8	Boc-Ala-Pro-ψ[CS-NH]-Phe-NH-Np	AcOH	Room temp.	3 d	69
9	Boc-Ala-Pro-ψ[CS-NH]-Phe-NH-Np	AcOEt	Room temp.	2 h	93
10	Boc-Ala-Pro-ψ[CS-NH]-Phe-NH-Np	CH_2Cl_2	Room temp.	30 min	86
11	Boc-Ala-Gly-Pro-Phe-ψ[CS-NH]-Np	Toluene	Room temp.	4 h	90
12	Boc-Ala-Ala-ψ[CS-N]-Pro-ψ[CS-NH]-PHe-NH-Np	MeCN	Room temp.	20 min	82

[a] All reactions were conducted with 5 equiv. $SnCl_4$. All yields refer to pure products with satisfactory spectral and analytical data obtained by diethyl ether mediated precipitation.

Additionally, $SnCl_4$ selectively monodeprotected Fmoc protected amino acid derivatives (entries 3 and 4) and the double protected hydrazine (entry 2). Surprisingly, thioamide-containing peptide derivatives could be deprotected in satisfactory yields (entries 5–12), even in the case of a double thioxylated compound (entry 12). It appears that reaction times are generally longer when there are competing Lewis basic sites within the molecule and/or the solvent (entries 8–10). Finally, the reaction is nearly independent on the nature of the flanking groups. For example, the Boc protected amino nitrogen can be a part of a heterocycle (entry 4) or a hydrazine nitrogen (entry 2) without affecting the reaction rate and yield.

We propose that the initial formation of a chelate between the Lewis acid $SnCl_4$ and the *tert*-butyl *N*-alkylcarbamate leads to a solvolytic loss of isobutene and CO_2 and the formation of the deprotected compound. Additionally, we have some evidence for the formation of a second complex between the Lewis acid and the deprotected product of the reaction from ^{119}Sn and ^1H NMR spectroscopic investigations and from the stoichiometric behaviour of the reaction. At least 1 equiv. of $SnCl_4$ was needed for complete deprotection of Boc-Ala-Pro-Phe-NH-Np. Using only 0.25 or 0.5 equiv. of Lewis acid the desired deprotected product could be obtained in 24 or 47% yield, respectively.

The above mentioned complex is likely to be the reason for the enhanced stability of the deprotected peptides containing thioamide linkages. It was shown that Ala-Pro-ψ[CS-NH]-Phe-NH-Np‡ (product of the reaction of entry 9) is stable in the reaction solution for at least one week at room temperature. This complex could be completely destroyed by simple precipitation of a methanolic solution of the reaction product with diethyl ether. Purification of the deprotected compounds by HPLC using water–MeCN or water–MeOH mixtures also led to complete destruction of the $SnCl_4$–peptide complex as could be shown by ^{119}Sn NMR spectroscopy.

The $SnCl_4$ mediated Boc deprotection stategy provides a novel approach to obtaining oligopeptides with thioamide linkages in high yields.§ It is expected that this procedure is also useful in multistep organic synthesis where an extremely mild deprotection strategy is needed, minimizing the sacrifice of acid-labile moieties and related side reactions.

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Footnotes

† The prefix 'thiono-' has been frequently used to name the C=S group, however the IUPAC nomenclature committee recommends the use of 'thioxo'.

‡ Alterations of a peptide bond are represented by the ψ nomenclature system. A ψ is followed by the structure of the new bond in parentheses. The nomenclature of the compounds is in accordance with the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature, *Pure Appl. Chem.*, 1984, **5b**, 595.

§ The following procedure is representative. To a stirred solution of Boc-Ala-Pro-ψ[CS-NH]-Phe-NH-Np (1 mmol) in AcOEt (10 ml), fuming $SnCl_4$ (2 mmol) was added at room temperature. (**CAUTION**! $SnCl_4$ irritating to respiratory system. $SnCl_4$ was purchased from Fluka.) Extending the reaction scale, violent gas evolution was observed (because of the rapid CO_2 generation) mimicking boiling of the solvent. The resulting clear solution was stirred until TLC analysis indicated complete consumption of starting material after 120 min. The solvent was evaporated *in vacuo*, the remaining oil dissolved in methanol and the product precipitated by the addition of diethyl ether. The solid was washed with diethyl ether twice and dried *in vacuo*, yielding 430 mg (93%) of product.

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