Extremely selective $Mg(ClO_4)_2$ mediated removal of Bpoc/Ddz moieties suitable for the solid phase peptide synthesis of thioxo peptides

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Received (in Cambridge, UK) 13th July 1999, Accepted 3rd August 1999

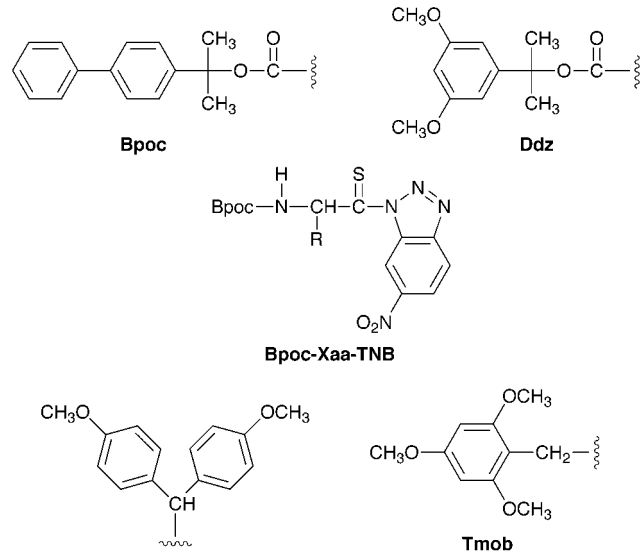
$Mg(ClO_4)_2$ in organic solvents acts as an extremely mild reagent for repetitive Bpoc/Ddz deprotection during solid phase peptide synthesis even in the presence of the acid labile thioxo amide moiety with excellent yields.

Peptide backbone modification by replacement of an amide moiety by a thioamide moiety (thioxylation)† has attracted attention in recent years for several reasons. On one hand receptor interactions of thioxylated analogues of biologically active peptides may be more selective or more potent than their parent compounds and on the other hand enhanced stability against enzymatic action could be shown for various proteolytic enzymes.[1] The introduction of a thioamide moiety is a nearly isologous substitution for an amide moiety[2] but biological studies have shown that the behavior of such modified compounds is unpredictable.[3] Nevertheless, a thioxo peptide bond provides an extraordinary backbone label for peptides and proteins introducing special spectroscopic and photochemical properties into the molecule with minimal variation of the constitution.

Regarding thioxo peptide synthesis the deprotection of the widely used side-chain protecting groups in the presence of thioxo peptide bonds is problematic because of the sensitivity of these groups toward acids. Acidolytic Boc deprotection of thioxo peptides led in most cases (depending on the sequence) to unsatisfactory yields between 10 and 65%.[4] Besides dethioxylation the thioxylated products often undergo thiazolone formation, a side reaction similar to the Edman degradation.[5]

Moreover, thioxo peptides are sensitive to the repetitive treatment with base necessary for the removal of the Fmoc protecting group, resulting in epimerized peptide derivatives. To circumvent these limitations we developed a method which can be used for both conventional peptide synthesis in the liquid phase and for solid phase peptide synthesis (SPPS) of thioxo peptides. It exclusively uses Lewis acids for both removal of the temporary 2-(biphenyl-4-yl)propan-2-yloxycarbonyl (Bpoc) or 2-(3,5-dimethoxyphenyl)propan-2-yloxycarbonyl (Ddz) protecting groups and final detachment/side-chain deprotection.

We disclosed that $SnCl_4$ is a powerful reagent for Boc deprotection in the presence of the acid-sensitive thioxo moiety.[6] In a more systematic investigation we found that Lewis acids like $Mg(ClO_4)_2$ in MeCN or $ZnCl_2$ in THF‡ are extremely mild and selective reagents for Bpoc/Ddz removal without damage of the thioxo peptide bond. This method is suitable in SPPS using extremely acid labile resins like Sieber- and Rink-amide and SASRIN® resins. The Bpoc deprotection of thioxo peptides utilising $Mg(ClO_4)_2$ in MeCN at 50 °C is fully compatible with side-chain Boc protection.[7] The thioxo moiety of Ala-Ala-Ala-Pro-ψ[CS-NH]Phe-NH-Np§ is completely stable under these conditions even after 5 weeks. We were able to synthesize the thioxylated model polyproline II helix **7** on a Sieber-amide resin using Ddz protected proline for chain elongation¶ and Bpoc-Pro-thioxo-6-nitrobenzotriazolide (Bpoc-Pro-TNB) for thioacylation‖ of resin-bound pentaproline. Detachment from the support using $ZnCl_2$-Et_2O complex in CH_2Cl_2** yielded thioxo peptide **7** in 72% yield after HPLC purification. Additionally, we synthesized bovine β-Casomorphine(1-5) amide **2** using the Fmoc strategy for preparation of the tripeptide Phe-Pro-Gly on a Sieber-amide resin, switching to Bpoc-Pro-TNB for introduction of the thioxo amide moiety and selective Bpoc-deprotection using $Mg(ClO_4)_2$ in MeCN. After acylation of the thioxo tetrapeptide with Boc-Tyr(OtBu), simultaneous Boc/OtBu-deprotection and detachment from the resin was successful using $ZnCl_2$–Et_2O complex. Moreover, we prepared the biologically active thioxo peptides endomorphine **3** and allanthoin **4** (Scheme 2) in a similar manner (Table 1).

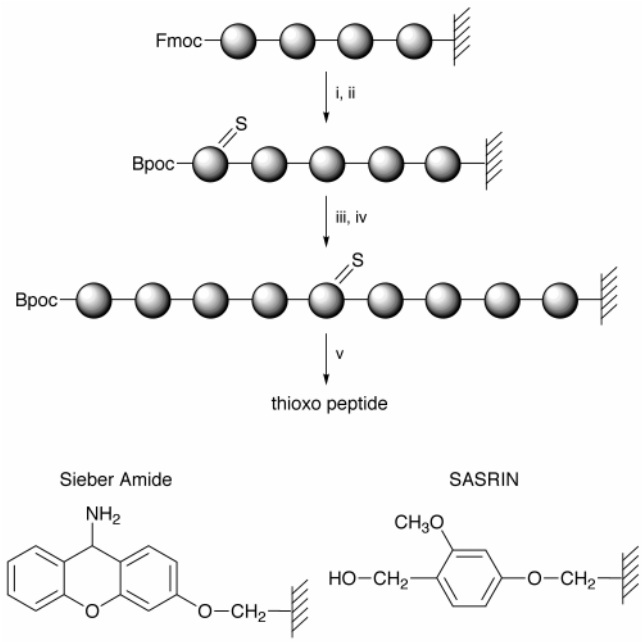


The synthesis of the thioxo bradykinins **5** and **6** (Table 1) was successful starting from Fmoc-Arg(Boc)$_2$-SASRIN® resin and using Boc-Arg(Boc)$_2$ for final acylation of the resin-bound thioxo octapeptides. Side-chain deprotection–detachment with $ZnCl_2$–Et_2O complex yielded thioxo peptides in excellent yields and purity. The widely used Pmc/Pbf and Trt protecting groups are relatively stable against $ZnCl_2$-Et_2O complex. Therefore we preferred the trimethoxybenzyl (Tmob) and 4,4′-Dimethoxydityl (Dod) groups for side-chain protection of Gln (peptide **10**) and Asn (peptide **8**), respectively, and double Boc protection of the guanidino function of Arg to overcome these limitations. To test this method in a more sophisticated reaction we synthesized the fully protected thioxylated RNAse A fragment Boc–Lys(Boc)-Glu(OtBu)-Thr(tBu)-Ala-ψ[CS-NH]Ala-Ala-Lys(Boc)-Phe-Glu(OtBu) on SASRIN® resin and treated it with $ZnCl_2$–Et_2O complex for 10 h at room temperature. The major product corresponds to the expected thioxo peptide **9** as demonstrated by MALDI mass spectrometry. Finally, we synthesized the complete RNAse A derived thioxo S-peptide **10** using this new method. The main product (30%) of the complex mixture was **10** but in all other oligopeptides derived from incomplete couplings a thioxo moiety has been detected by the charateristic UV-band at 280 nm. This suggests that no dethioxylation had taken place. We repeated the synthesis of **10** using Fmoc-(FmocHmb)Ala and Fmoc-Asp-(2,2-dimethyl)oxazolidine-4-carboxylic acid at

Table 1 $ZnCl_2$–Et_2O complex mediated final deprotection–detachement of thioxo peptides synthesized on solid supports with Bpoc or Ddz as temporary protecting group

Entry	Final product	Yield (%)	$[M + H]^+$ calc.	$[M + H]^+$ found
1	Ala-Ala-Ala-Pro-ψ[CS-NH]Phe-NH-Np	83	607.24	607.5[a]
2	Tyr-Pro-ψ[CS-NH]Phe-Pro-Gly-NH_2	87	595.29	595.1[a]
3	Tyr-ψ[CS-N]Pro-Phe-Phe-NH_2	74	588.28	588.5[a]
4	Gly-Gly-Ser-Leu-ψ[CS-NH]Tyr-Ser-Phe-Gly-Leu-NH_2	71	915.45	915.5[a]
5	Arg-Pro-Pro-Gly-Phe-Ser-ψ[CS-N]Pro-Phe-Arg	62	1076.56	1076.2[b]
6	Arg-Pro-ψ[CS-N]Pro-Gly-Phe-Ser-Pro-Phe-Arg	68	1076.56	1076.1[b]
7	Ac-Pro-Pro-Pro-Pro-Pro-Pro-ψ[CS-N]Pro-Pro-Pro-Pro-Pro-NH_2	72	1143.63	1143.7[a]
8	Arg-Asn-Lys-His-Ile-Arg-Thr-ψ[CS-N]Pro-Phe-Lys-NH_2	71	1311.75	1312.4[b]
9	Lys-Glu-Thr-Ala-ψ[CS-NH]Ala-Ala-Lys-Phe-Glu	79	1010.52	1010.7[ab]
10	Lys-Glu-Thr-Ala-ψ[CS-NH]Ala-Ala-Lys-Phe-Glu-Arg-Gln-His-Nle-Asp-Ser-Ser-Thr-Ser-Ala-Ala	63	2163.53	2163.7[b]

[a] Characterized by ESI mass spectrometry. [b] Characterized by MALDI mass spectrometry.



Scheme 1 *Reagents and conditions*: i, piperidine–DMF; ii, Bpoc-Xaa-TNB, DMF; iii, $Mg(ClO_4)_2$ MeCN or $ZnCl_2$/THF; iv, peptide synthesis using Bpoc for temporary protection; v, $ZnCl_2$–Et_2O complex, CH_2Cl_2.

positions 19 and 14–15, respectively, yielding **10** in 63% after final treatment with $ZnCl_2$–Et_2O complex and HPLC purification.

In conclusion, we have demonstrated that this novel deprotection scheme (Scheme 1) is an extraordinarily mild method for the SPPS of peptide derivatives containing acid and/or base sensitive functionalities. Linear thioxo peptides, which are known to be difficult to synthesize in a positionally predetermined manner, can now be prepared in good yields without epimerization.

We thank Dr A. Schierhorn and M. Kipping for mass spectroscopic measurements and Dr U. Reimer for NMR measurements. We are grateful to Thomas Schumann for atomic emission spectroscopic measurements. We gratefully acknowledge B. Hökelmann and I. Kunze for excellent technical assistance.

Notes and references

† For naming C=S the prefix 'thiono' has been frequently used in place of 'thioxo', however the IUPAC nomenclature committee recommends the use of 'thioxo'.

‡ Other Lewis acids that were investigated include ZnI_2, $Zn(AcO)_2$, $Zn(ClO_4)_2$, $MgCl_2$, $SnCl_2$, $SnBr_4$, $SnCl_4$, $Sn(AcO)_4$, $Cs(F_3CCO_2)$, $AlCl_3$ and $Ti(OPr^i)_4$. In the case of ZnI_2, $Zn(AcO)_2$, $Zn(ClO_4)_2$ and $MgCl_2$ the deprotection of Bpoc was promoted with a selectivity of at least 2000 over Boc deprotection but proceeds with sluggish kinetics. We preferred $Mg(ClO_4)_2$ due to its relative low cost, the weakly interacting counterion, its good solubility in organic solvents like MeCN, and its ease of removal.

§ Alterations of a peptide bond are represented by the ψ nomenclature system. *Pure Appl. Chem.*, 1984, **5b**, 595.

¶ The resin bound Bpoc-protected peptide derivative was treated with 10 equiv. $Mg(ClO_4)_2$ in MeCN at 50 °C for 3 h. The next acylation step in the case of SPPS was performed after extensive washing with MeCN and re-swelling in DMF. Using this method in liquid phase synthesis precipitation of the crude product with Et_2O was sufficient for removal of the deprotecting reagent.

‖ The thioxylated Bpoc-protected amino acid 6-nitrobenzotriazolides were synthesized according to a modified procedure from the literature (M. A. Shalaby, C. W. Grote and H. Rapoport, *J. Org. Chem.*, 1996, **61**, 9045).

** We preferred $ZnCl_2$–Et_2O complex for the final treatment of the resin bound thioxo peptide because of its convenient handling and because there are no difficulties with precipitation of insoluble materials complicating HPLC purification. For simultaneous detachment–side-chain deprotection we incubated the peptide resin with 2.2 M $ZnCl_2$–Et_2O complex in CH_2Cl_2 for 10 h at room temperature. After filtration the solvent was removed and the resulting slurry was re-dissolved in THF and the desired thioxo peptide was precipitated with Et_2O. Final purification using HPLC gave pure thioxo peptides. Investigation of these peptides using atomic emission spectroscopy demonstrated that the Zn^{2+} content is within the normal range (below 180 μg g^{-1}). Additionally, we were able to remove *tert*-butyl and benzyl protecting groups from the phosphate moiety in the presence of thioxo peptide bonds using this method, allowing the synthesis of side-chain phosphorylated thioxo peptides.

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Communication 9/05678E