

Propargyloxycarbonyl (Poc) amino acid chlorides as efficient coupling reagents for the synthesis of 100% diastereopure peptides and resin bound tetrathiomolybdate as an effective deblocking agent for the Poc group

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Received (in Cambridge, UK) 7th January 2002, Accepted 26th February 2002

First published as an Advance Article on the web 13th March 2002

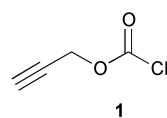
Synthesis of short peptides using propargyloxycarbonyl amino acid chlorides as effective coupling reagents and polymer supported tetrathiomolybdate as an efficient deblocking agent are reported.

While the most obvious method of activation of a carboxy group for amide bond formation in peptide synthesis is *via* simple amino acid chlorides, they are rarely used since they are perceived to be 'over-activated' leading to numerous side reactions including the danger of racemisation.¹ More importantly the acid chlorides obtained from *N*-acylamino acids undergo cyclisation to the readily racemised oxazolones.²

However, in recent years the use of Fmoc amino acid chlorides,³ Fmoc amino acid fluorides⁴ and *N*-Bts protected amino acid chlorides⁵ have found use in the racemisation-free synthesis of peptides in high yield. The limitation of these methods is the use of basic or harsher acidic conditions that are used for the *N*-deprotection which leads to undesired by-products.

Herein we report the use of a propargyloxycarbonyl (Poc) group as a novel *N*-protecting group, propargyloxycarbonyl (Poc) amino acid chlorides as new coupling reagents for the completely racemisation free, high yield synthesis of peptides and selective deprotection of the Poc group under neutral and non-hydrolytic conditions with resin bound tetrathiomolybdate.

A number of *N*-Poc protected amino acids (**2a–2h**) were obtained by treatment of the appropriate amino acid with propargyl chloroformate (**1**) in aq. NaOH, while maintaining the pH in the range of 9.5 to 10.5 (Fig. 1). This method provided the *N*-Poc derivatives, to a large extent, as crystalline solids in 90–95% yield in the case of alanine, valine, leucine, phenylalanine, isoleucine, proline, phenylglycine and α -aminoisobutyric acid.



Poc-AA-OH **2**

2a.	Poc-Ala-OH	2e.	Poc-Val-OH
2b.	Poc-Phe-OH	2f.	Poc-Pro-OH
2c.	Poc-Phg-OH*	2g.	Poc-Leu-OH
2d.	Poc-Aib-OH*	2h.	Poc-Ile-OH

* Phg: Phenylglycine ; Aib: α -Aminoisobutyric acid

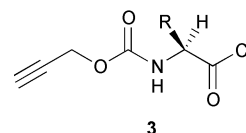
Fig. 1

All the *N*-Poc amino acids were found to be stable for several days at room temperature (28 °C). Since the reagents sequestered on polymers are becoming increasingly popular in organic synthesis, we decided to use in our studies resin bound tetrathiomolybdate⁶ prepared by the reaction of ammonium tetrathiomolybdate with amberlite IRA-400 resin. The *N*-Poc amino acids (**2a–2h**) when treated with resin bound tetrathiomolybdate (MeOH, rt, 1.0–1.5 h) under sonochemical conditions⁷ underwent smooth deprotection to give the corresponding free amino acids in quantitative yields. The advantages of this deprotection are simple filtration for work-up, no by-product to be removed by chromatography, no acidic or basic reagents used and the reaction conditions are mild and essentially neutral. Unlike the Fmoc group the danger of premature deblocking of the Poc group by excess base is not observed.⁸

The *N*-Poc group is stable under the conditions which cleave the Boc group (HCl, 10 min or CF₃CO₂H, 1 day) and Bts group (AcOH). The *N*-Poc amino acids (**2a–2e**) on treatment with SOCl₂ in CH₂Cl₂ afforded the corresponding *N*-Poc amino acid chlorides (**3a–3e**) respectively (Fig. 2). Interestingly all the *N*-Poc amino acid chlorides were stable for several days at room temperature under anhydrous conditions. To demonstrate the wide utility of the coupling methodology peptides of non-proteinogenic amino acids were synthesised. Direct reaction of *N*-Poc amino acid chlorides (**3a–3d**) with a variety of amino acid methyl ester hydrochlorides under Schotten–Baumann conditions (H₂O–Na₂CO₃–CH₂Cl₂ –5 to 0 °C, 15 min) gave protected dipeptides in high yield (**4a** = 83%, **4b** = 88%, **4c** = 86%, **4d** = 88%, **4e** = 83%) as crystalline solids indicating facile coupling over a short time (Scheme 1).

N-Methylated dipeptide (**6**) was synthesised by treatment of MeHNAla-OMe (**5**) with Poc-NMeAla-Cl (**3e**) under the above mentioned biphasic system (Scheme 2). The purity of all the peptides (**4a–4e**) synthesised was ascertained by use of a chiral shift reagent in ¹H NMR⁹ and chiral HPLC¹⁰ and were found to be 100% diastereomerically pure.¹¹ The results obtained with Poc-Phg-Cl are superior in terms of reactivity and simpler work-up compared to the use of Bts-Phg-Cl.⁵

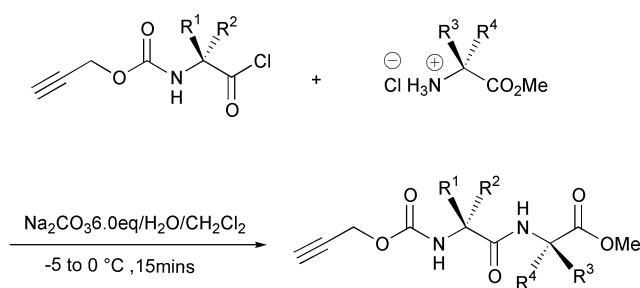
Synthesis of peptides containing multiple Aib (α -aminoisobutyric acid) units still remains a challenging task. Their chemical synthesis and homosequences are known to be difficult under normal conditions.^{2b,12} The classical reagent DCC (or DCC–HOBT) gives mediocre yields despite long reaction times. To the best of our knowledge there are no reports in the literature for the synthesis of peptides with multiple Aib residues in the *absence of any additives or trapping agents via*



3a. Poc-Ala-Cl
3b. Poc-Phe-Cl
3c. Poc-Phg-Cl
3d. Poc-Aib-Cl
3e. Poc-NMeAla-Cl

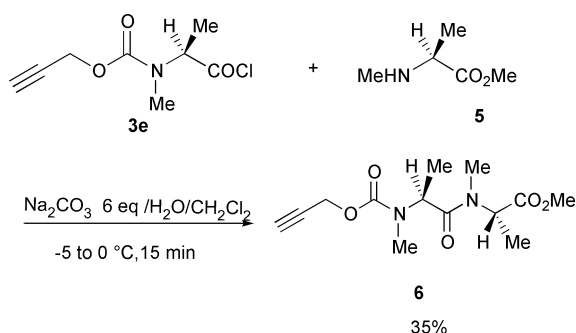
Fig. 2

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- 4a. $R^1=R^3=H$; $R^2=R^4=CH_3$ 4d. $R^1=R^3=H$; $R^2=Ph, R^4=Bn$
 4b. $R^1=R^3=H$; $R^2=R^4=Bn$ 4e. $R^1=R^3=H$; $R^2=Ph, R^4=CH_3$
 4c. $R^1=R^3=H$; $R^2=R^4=Ph$

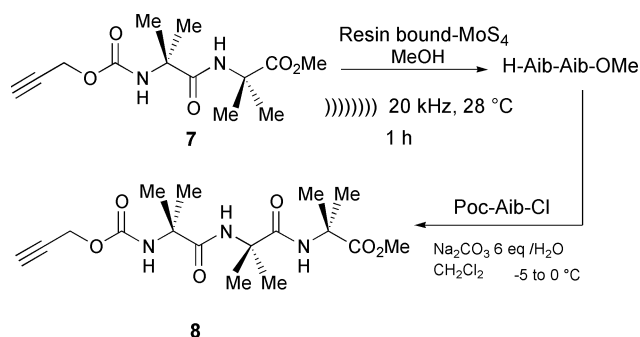
Scheme 1



Scheme 2

the acid chloride method. We have successfully synthesised a dipeptide [Poc-Aib-Aib-OMe] (7) and a tripeptide [Poc-Aib-Aib-Aib-OMe] (8) [the Poc group of 7 was deblocked⁷ by resin-MoS₄ to yield 100% H-Aib-Aib-OMe and this deprotected peptide was coupled with Poc-Aib-Cl to produce 8] using this coupling methodology (45% and 42% yield respectively) in the absence of any additives (Scheme 3).

In summary the Poc group allows the conversion of proteinogenic and non-proteinogenic amino acids into protected amino acid chlorides. Coupling occurs very rapidly with a variety of amino acid hydrochlorides in the absence of any additives. Sterically demanding α -aminoisobutyric acid (Aib) containing dipeptides, tripeptides and one additional *N*-methylated dipeptide were obtained in satisfactory yield. Efficient deprotection of the *N*-Poc group is possible using inexpensive resin bound tetrathiomolybdate under neutral and mild conditions. Thus *N*-Poc amino acid chlorides are useful, practical and efficient reagents for solution phase, racemisation free peptide synthesis.



Scheme 3

Notes and references

- M. Bodanszky, *Principles of Peptide Synthesis*, 2nd ed, Springer-Verlag, Berlin, 1993, p. 11.
- (a) M. Beyermann, D. Granitz, M. Bienert and L. A. Carpino, in *Peptides: Chemistry and Biology*, ed. G. R. Marshall, ESCOM, Leiden, The Netherlands, 1988, p. 101, 189; (b) E. Frerot, J. Coste, A. Pantaioni, M. N. Dufour and P. Jouin, *Tetrahedron*, 1991, **47**, 259–270; (c) J. R. Spencer, V. V. Antonenko, G. J. Nancy and M. Goodman, *Int. J. Pept. Protein Res.*, 1992, **40**, 282–293; (d) K. Akaji, N. Kuriyama and Y. Kiso, *Tetrahedron*, 1994, **35**, 3315–3326.
- L. A. Carpino, B. J. Cohen, K. E. Stephens, S. Y. Sadat-Aalae, J. H. Tien and D. C. Langridge, *J. Org. Chem.*, 1986, **51**, 3732–3734.
- L. A. Carpino, S. Y. Sadat-Aalae, H. G. Chao and R. H. Deselms, *J. Am. Chem. Soc.*, 1990, **112**, 9651–9652.
- E. Vedejs, S. Lio, A. Klapars and J. Wang, *J. Am. Chem. Soc.*, 1996, **118**, 9796–9797.
- Resin bound tetrathiomolybdate was prepared by stirring ammonium tetrathiomolybdate with pre-swelled amberlite IRA-400 resin in water. Tetrathiomolybdate was found to be 0.7 mmol per gram of the resin.
- The reaction mixture was sonicated (1–1.5 h) using an ultrasonic cleaning bath, 20 kHz, 28 °C and produced analytically pure product. Even after prolonged sonication, reagent has not leached out of the resin. The same reaction without sonication is completed in 3.5–4 h producing the corresponding free amine.
- H. Wenschuh, M. Beyermann, M. Bienert, A. El-Faham, S. Ghassemi and L. A. Carpino, in *Peptides: Chemistry, Structure and Biology*, eds. P. T. P. Kaumaya and R. S. Hodges, Mayflower Scientific Ltd., Kingswinford, UK, 1996, p. 33.
- 1.0 equiv. of Eu(hfc)₃ was used for the chiral shift ¹H NMR experiments.
- HPLC was carried out using Chiralcel OD CN:0000CE JH 004, Shimadzu SPD-6A, solvent system:20% isopropyl alcohol in *n*-hexane.
- Methyl ester non-equivalence in the ¹H NMR spectra of diastereomeric dipeptides [Poc-(D or L)-Phg-Ala-OMe] was observed in CDCl₃ and DMSO-d₆.
- (a) U. Slomczynska, D. D. Beusen, J. Zabrocki, K. Kocielek, A. Redlinski, F. Reusser, W. C. Hutton, M. T. Leplawy and G. R. Marshall, *J. Am. Chem. Soc.*, 1992, **114**, 4095–4106; (b) C. Auvim-Guette, E. Frerot, J. Coste S. Rebuffat, P. Jouin and B. Bodo, *Tetrahedron Lett.*, 1993, **34**, 2481–2482.