Tetrahedron 68 (2012) 3056–3062

Contents lists available at SciVerse ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Cyanoacetamide-based oxime carbonates: an efficient, simple alternative for the introduction of Fmoc with minimal dipeptide formation

Sherine N. Khattab [a,b], Ramon Subirós-Funosas [b,c], Ayman El-Faham [a,b,*], Fernando Albericio [b,c,d,e,*]

[a] Alexandria University, Faculty of Science, Department of Chemistry, PO Box 426, Ibrahimia, 21321 Alexandria, Egypt
[b] Institute for Research in Biomedicine, Barcelona Science Park, Baldiri Reixac 10, 08028 Barcelona, Spain
[c] CIBER-BBN, Networking Centre on Bioengineering, Biomaterials and Nanomedicine, Barcelona Science Park, Baldiri Reixac 10, 08028 Barcelona, Spain
[d] Department of Organic Chemistry, University of Barcelona, Martí i Franqués 1-11, 08028 Barcelona, Spain
[e] School of Chemistry, University of KwaZulu-Natal, Durban 4041, South Africa

ARTICLE INFO

Article history:
Received 17 December 2011
Received in revised form 7 February 2012
Accepted 8 February 2012
Available online 14 February 2012

Keywords:
Amino acids
9-Fluorenylmethyloxycarbonyl
Oxime
N-Protecting group
Peptide synthesis

ABSTRACT

Nowadays, most peptides are chemically achieved by using the Fmoc/*t*Bu protection strategy, due to its fully orthogonal character, mild temporary group removal and resin cleavage steps. However, its introduction into *N*-unprotected amino acids is not exempt of synthetic inconveniences, such as dipeptide formation. Lately, novel oxime carbonates were introduced in the arsenal of reagents for the introduction of Fmoc, presenting almost negligible percentage of side-products. Herein, an enforced version of this family of Fmoc-carbonates is presented, containing stable and highly acidic cyanoacetamide-based oximes as leaving group. Such reactive species, affordable in only two steps from simple, readily available starting materials, show unusual ability to obtain the corresponding Fmoc-protected residues in high yield and minimal impact of detrimental side-products, mainly Fmoc-dipeptides.

1. Introduction

Peptides are increasingly gaining recognition as potential bioactive ingredients in the pharmaceutical industry.[1–4] Consequently, their purity is required to meet with the highest standard within compounds amenable to be employed as drugs.[5,6] In order to accomplish such requirements, the choice of an appropriate protection strategy, which allows selective and safe introduction and removal of the masking group, is fundamental for the success of the whole process.[7,8] Currently, Fmoc/*t*Bu-based peptide synthesis is the preferred option over classical Boc/Bzl strategy for the majority of synthetic chemists.[9,10] Therefore, a complete and clean introduction of this base-labile group into the *N*-terminus of any amino acid building block is of outmost importance.

In spite of the vast number of reagents reported to date for the attachment of the Fmoc group into the *N*-terminal amino group of amino acid building blocks in the form of carbamate, there is still no ultimate active species capable to provide optimal Fmoc-introduction (Fig. 1). The traditional chloroformate strategy (**1**) represents an extremely powerful approach, providing fast amino protection.[10,11] However, these reagents display high instability and are not suitable for sterically low hindered residues, such as Glycine, where the amount of dipeptide (and even tripeptide) formation may reach up to 20% of the crude material (Scheme 1).[12]

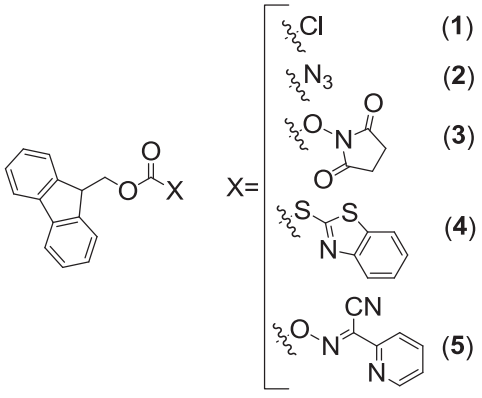
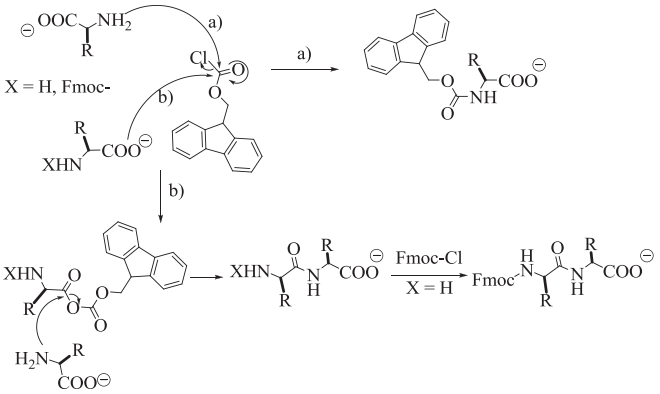


Fig. 1. Most relevant approaches described for amino Fmoc-protection.

Abbreviations: Boc, *tert*-butoxycarbonyl; DCM, dichloromethane; ESI-MS, electrospray ionization mass spectrometry; Fmoc, fluorenylmethyloxycarbonyl; NMR, nuclear magnetic resonance; Oxyma, ethyl 2-cyano-2-hydroxyiminoacetate; SPPS, solid-phase peptide synthesis; UV, ultraviolet.

* Corresponding authors. Fax: +34 93 403 71 26; e-mail addresses: aymanel_faham@hotmail.com (A. El-Faham), albericio@irbbarcelona.org, albericio@pcb.ub.es (F. Albericio).

0040-4020/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2012.02.020



Scheme 1. Amino acid dimerization during Fmoc-protection.

Less reactive approaches, namely azidocarbonates (**2**) or succinimidocarbonates (**3**), minimize to a great extent dimerization, but either produce other side-products or have notorious explosive potential.[6,11,13] Protection forming the azide *in situ* is a safer alternative to the above mentioned method.[14] Few years ago, 2-mercaptobenzotiazole (**4**) templates have also been employed with reasonable success during Fmoc-protection, but separation of byproducts during purification hampers practical application.[15] Less prevalent approaches consist in employing symmetrical pyrocarbonates,[16] trimethylsilyl esters,[17,18] or 1,2,2,2-tetrachloroethyl,[19,20] 5-norbornene-2,3-dicaroximido,[21] pentafluorophenyl,[22] perfluorophenyl,[23] and 1-hydroxybenzotriazole[24,25] mixed carbonates. In the last year, benzotriazole-based reagents in the *N*-acyl form have been described for the efficient introduction, not only of Fmoc, but also Boc and Alloc protecting groups with low or undetectable dipeptide formation.[26] However, this polynitrogenated heterocyclic core has a consistent record on explosive potential, similarly to the parent 1-hydroxy analogues, which has had great impact in methodology of peptide synthesis.[27–29]

Recently, in a preliminary communication, we reported a series of Fmoc-introducing carbonates, containing various oxime moieties with unusual stability due to the absence of α-hydrogen atoms.[30] The high acidity of such oximes (p*Ka*=4–8), bearing a series of electron-withdrawing substituents, play a key part in the reactivity of the corresponding carbonates, generally affording the Fmoc-protected amino acid in yields close to 90%, after 14 h reaction time.[30] In addition, the content of unwanted dipeptide material in the reaction crude remained below 0.1%, even when Glycine (the most prone residue to dimerization) was selected as Fmoc-protection model system. Among all Fmoc-oxime carbonates tested for that purpose, most remarkable results were obtained with the one featuring the least acidic oxime: the *N*-hydroxypicolinimidoyl cyanide derivative (*N*-{[(9*H*-fluoren-9-yl)methoxy] carbonyloxy}picolinimidoylcyanide) (**5**, Fig. 1), which afforded Fmoc-Gly-OH in 92% yield and only 0.01% Fmoc-Gly-Gly-OH. Nonetheless, in spite of this extraordinary performance, routinely application of this oxime-based carbonate in peptide synthesis laboratories seems impractical as result of the high production cost of the starting cyano-2-pyridylacetonitrile.

In view of the promising features of the precedent Fmoc-oxime templates, a second generation has been synthesized and evaluated, aiming at solving the practical drawbacks of the previous carbonates and simultaneously conserving or increasing their efficiency. Thus, seeking an optimal balance of reactivity/oligomerization, the ester moiety contained in the highly reactive ethyl 2-cyano-2-hydroxyiminoacetate (Oxyma) template was replaced with several amide functionalities.[31] Although apparently a minor structural modification, shifting an ester for an amide bond in the cyanooxime series is able to reduce 0.6 units its acidity and introduce conformational restriction due to existence of C–N rotational barrier.[32,33] Consequently, the potential amount of side-products is predicted to decrease, maintaining at the same time a valuable reactivity.

Various linear and cyclic carboxamido groups have been investigated ranging from the simplest unsubstituted amide (**6**) to analogues containing *N*-ethyl (mimicking the Oxyma template, **7**), *N*-piperidinyl (**8**) and *N*-morpholinyl (**9**, aiming at higher solubility) chains (Fig. 2). Such cyanoacetamide-based oximes (**15**–**18**, Scheme 2) are highly appreciated in organometallic chemistry, serving as excellent bivalent ligands to bind various metallic ions.[33–36] Thus, Silver (I) and Tin (IV) complexes of unsubstituted carboxamide **15**, the most studied in metal coordination among these oximes, are employed for biomedical purposes as antimicrobial additives (in odontological devices) or as cytotoxic agents (showing greater activity than cisplatin against human cervical cancer cells) and also with industrial applications as gas sensor.[34–36] Growing interest on this particular cyanooxime (**15**) has led to the calculation and measurement of its O–H bond dissociation enthalpy in gas-phase and also to a complete X-ray crystallographic and thermal analysis.[37,38] Recently, considerable cell antiproliferative activity of palladium (II) and platinum (II) complexes, based also on piperidino and morpholino–cyanoacetamide oximes (**17** and **18**), was reported.[33] Furthermore, piperidino oxime **17** has been employed as building block to synthesize iminolactones.[39] All oximes described herein, including the *N*-ethyl analogue (**16**) have been employed to build carbamates displaying detoxifying action of agricultural pesticides.[40] Exhaustive structural and spectroscopic studies on cyanoacetamide oximes **15**–**18** and the corresponding anionic states have been described.[33,35] Despite the vast existing literature on metal-complexed derivatives of these oximes, their properties have not yet been applied to peptide chemistry, with the exception of unsubstituted carboxamide **15**. Similarly to Oxyma, this oxime (**15**) was first reported as additive to carbodiimides with reasonable activity, but further studies were surprisingly abandoned.[32,41] To our knowledge, this is the first report on Fmoc-carbonates including cyanoacetamide oximes **15**–**18**.

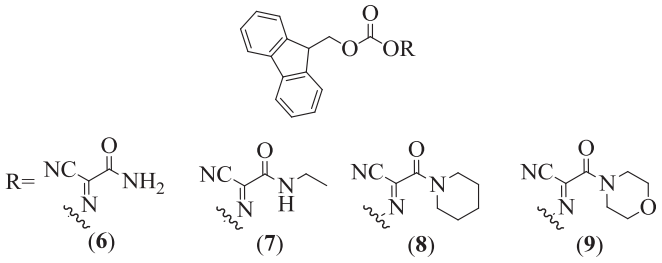
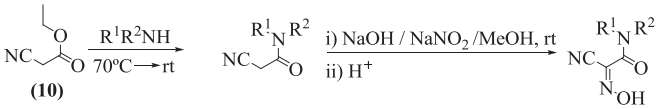


Fig. 2. Proposed cyanocarboxamido family of Fmoc-oxime carbonates.



R^1 = H, R^2 = H,	**(11)**	R^1 = H, R^2 = H,	**(15, AmOX)**
R^1= H, R^2= CH_3CH_2,	**(12)**	R^1= H, R^2= CH_3CH_2,	**(16, N-Oxyma)**
R^1, R^2 = $(CH_2)_5$,	**(13)**	R^1, R^2 = $(CH_2)_5$,	**(17,PipOX)**
R^1, R^2 = $(CH_2)_4O$,	**(14)**	R^1, R^2 = $(CH_2)_4O$,	**(18, MorOX)**

Scheme 2. Two-step synthesis of cyanocarboxamide oximes.

A further advantage of the cyanoacetamido series of Fmoc-oxime carbonates (**6**–**9**) is that retrosynthetic analysis of the corresponding oximes (**15**–**18**) show that these could be easily accessed from ethyl cyanoacetate by amidation with subsequent nitrosation. Although alternative synthetic pathways have been