Rapid Communication

Journal of PeptideScience

Received: 12 October 2009 Revised: 28 November 2009 Accepted: 1 December 2009 Published online in Wiley Interscience: 28 January 2010

(www.interscience.com) DOI 10.1002/psc.1210

An improved procedure for the synthesis of dehydroamino acids and dehydropeptides from the carbonate derivatives of serine and threonine using tetrabutylammonium fluoride

Ramesh Ramapanicker, Roli Mishra and Srinivasan Chandrasekaran*

Dehydroamino acids are important precursors for the synthesis of a number of unnatural amino acids and are structural components in many biologically active peptide derivatives. However, efficient synthetic procedures for their production in large amounts and without side reactions are limited. We report here an improved procedure for the synthesis of dehydroalanine and dehydroamino butyric acid from the carbonate derivatives of serine and threonine using TBAF. The antiselective E_2 elimination of the carbonate derivatives of serine and threonine using TBAF is milder and more efficient than other available procedures. The elimination reaction is completed in less than 10 min with various carbonate derivatives studied and the methodology is very efficient for the synthesis of dehydroamino acids and dehydropeptides. The procedure thus provides an easy access to key synthetic precursors and can be used to introduce interesting structural elements to designed peptides. Copyright © 2010 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: amino acids; peptides; elimination; carbonates

Results and Discussion

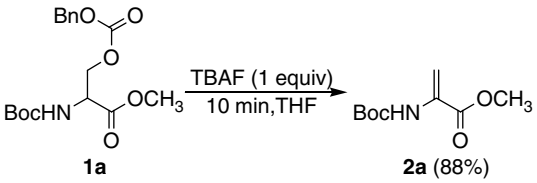
α,β-Dehydroamino acids are important constituents of many peptide antibiotics and are very useful synthetic precursors for the synthesis of a number of unnatural amino acids and peptides [1–3]. The dehydration of β-hydroxyamino acids is the most common strategy employed for the synthesis of α,β-dehydroamino acids and α,β-dehydropeptides [1,2]. There are a number of procedures available in the literature for the preparation of dehydroalanine (ΔAla) and dehydroamino butyric acid (ΔAbu) derivatives from serine and threonine, respectively, either in one step by direct elimination of water using various reagents or in two steps by converting the hydroxyl group to a good leaving group followed by a β-elimination [1,2,4]. In general, most of the procedures used for the elimination reactions of β-hydroxyamino acids give low to moderate yields and the intermediates are quite reactive and undergo side reactions. The synthesis of β-substituted dehydroamino acids using many of these methods generally results in the formation of a mixture of both the stereoisomers of the olefin. The generation of dehydroalanine or dehydroamino butyric acid residue within a peptide by dehydrating a serine or a threonine residue faces additional problems. The hydroxyl groups of serine or threonine need to be protected prior to peptide synthesis and later these protecting groups need to be removed before the peptides are subjected to elimination reactions; all of which add to the total number of steps involved in the synthesis. Thus, although there are a number of procedures available for the synthesis of dehydroamino acids, none of them have completely satisfied the increased requirement of these compounds as commodity chemicals.

Inspired by a mild procedure employed by Ferreira *et al*. [5] for the selective elimination of β-hydroxyamino acids to the corresponding dehydroamino acid derivatives using Boc_2O and DMAP, we developed a very useful and high yielding procedure for the β-elimination of carbonate derivatives of serine and threonine to dehydroalanine and dehydroamino butyric acid derivatives, respectively, using K_2CO_3 (2 equiv, 60 °C, 1 h, DMF) [6]. The method is very useful for the selective preparation of *Z*-isomers of dehydroamino acids in very high yields and does not lead to any side reactions. Preparation of dehydropeptides using the procedure is simple and the reaction conditions do not result in racemization of other stereogenic centers present in the peptide [6]. However, the procedure could still be improved by using a low boiling solvent instead of DMF and also by finding a suitable base that can affect the elimination reaction at a lower temperature. Our initial experiments had revealed that the use of organic bases, such as triethylamine, DMAP, NMM, DIPEA etc. did not result in the expected elimination reaction of the carbonates of serine and threonine and the use of more basic reagents, such as NaOH, NaOMe etc. resulted in side reactions including the hydrolysis of ester functionalities present in the molecules. The reactions were best performed in DMF and the use of other low boiling solvents, such as THF, CH_3CN, CH_2Cl_2 etc. resulted in low yields of the products as the solubility of K_2CO_3 in these solvents is very poor.

A recent report on the use of TBAF as a base for the dehydrobromination of vinyl bromides to terminal acetylenes [7] and preceding reports on the use of TBAF for the dehydrohalogena-

* *Correspondence to: Srinivasan Chandrasekaran, Department of Organic Chemistry, Indian Institute of Science, Bangalore 560012, India. E-mail: scn@orgchem.iisc.ernet.in*

Department of Organic Chemistry, Indian Institute of Science, Bangalore 560012, India



Scheme 1. Preparation of the dehydroalanine derivative (**2a**) from the benzyl carbonate derivative (**1a**) of serine using TBAF.

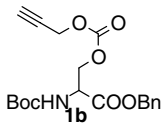
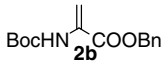
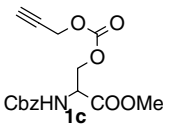
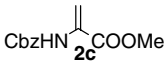
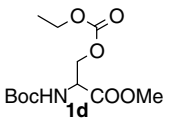
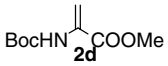
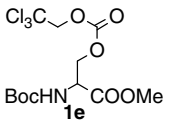
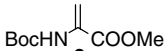
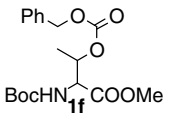
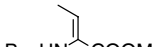
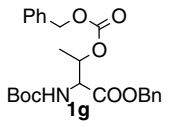
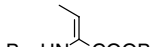
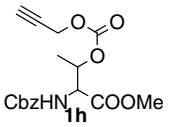
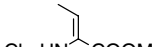
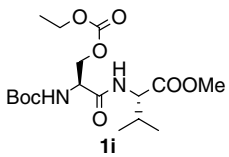
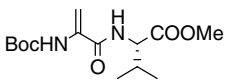
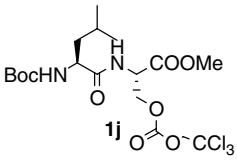
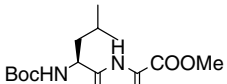
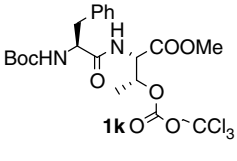
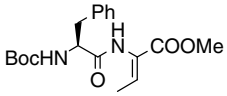
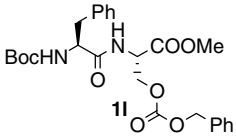
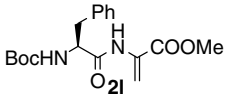
tion of alkyl halides to alkenes [8–11] prompted us to attempt the elimination of the benzyl carbonate derivative (**1a**) of DL-serine using TBAF (The basic nature of the unsolvated fluoride ion, as in TBAF/THF has long been exploited in carrying out a number of organic transformations) [12]. When **1a** was treated with 1 equiv of TBAF (1 M solution in THF), the elimination occurred readily (10 min, 28 °C) and the dehydroalanine derivative **2a** was obtained in excellent yield (88%, Scheme 1).

The efficient elimination of the benzyl carbonate group from **1a** in the presence of TBAF was surprising as none of the organic bases studied (DMAP, Et_3N, DIPEA etc.; There was considerable elimination reaction observed on using DBU as the base. **1a** was quantitatively converted to **2a** in l h with 1 equiv of DBU) [13] effected these reactions under the same conditions (THF, 28 °C) even after 6 h. These observations offered the scope for an improved methodology for the synthesis of dehydroamino acids and dehydropeptides from the carbonate derivatives of serine and threonine. We examined the usefulness of this reaction by treating various carbonate derivatives of serine and threonine (**1b–h**) with 1 equiv of TBAF (Table 1). The reactions were complete within 10 min and the corresponding dehydroamino acid derivatives (**2b–h**) could be isolated in excellent yields (Table 1). The reactions proceeded via a *trans* E_2-elimination resulting in the formation of only the *Z*-isomer of the dehydroamino butyric acid derivatives (**2f–h**) from the carbonate derivatives (**1f–h**) of threonine (A comparison of 1H NMR data of **2f-k** with those of compounds containing ΔAbu residues available in the literature confirmed the Z-configuration of the olefin). The procedure was highly efficient for the synthesis of dehydropeptides (**2i–l**) (entries 8–11, Table 1) and proceeded without racemization of the other chiral centers present in the peptide (A comparison of the optical rotation of the dehydropeptide **2l** ($[\alpha]_D^{25} = -64$ *c* 1, MeOH) prepared using TBAF and the one prepared using K_2CO_3 ($[\alpha]_D^{25} = -64$ *c* 1, MeOH) showed similar values). The use of TBAF effected the elimination reaction at a faster rate and at rt (28 °C) which is a definite advantage over the method previously reported using K_2CO_3 [6].

We examined the possibility of using catalytic amount of TBAF for carrying out these elimination reactions. Accordingly, **1a** was treated with 0.1 equiv of TBAF and the dehydroalanine derivative **2a** could be isolated in 87% yield after 4 h. When 0.5 equiv of TBAF was used, the elimination reaction was complete in 30 min to give **2a** in 85% yield. Although it is possible to carry out this elimination reaction using catalytic amount of TBAF, the rate of the reaction is considerably reduced. Quite surprisingly, a similar elimination of an acetyl derivative of serine, Boc-Ser(Ac)-OMe (**3**) was not successful using 1 equiv of TBAF; the elimination product **2a** could be isolated only in 42% after 24 h (Scheme 2).

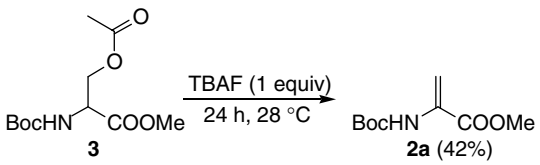
In conclusion, TBAF is found to be a better base for the elimination of carbonate derivatives of serine and threonine to the corresponding dehydroamino acid derivatives. We have thus developed an improved procedure for the synthesis of

Table 1. Preparation of dehydroamino acid derivatives from the carbonate derivatives of serine and threonine using TBAF

Entry	Carbonate derivatives of serine and threonine	Dehydroamino acid derivatives and peptides	Yield (%)[a]
1			80
2			80
3			80
4			89
5			88
6			88
7			81
8			84
9			84
10			86
11			83

[a] Yields correspond to those of products isolated using column chromatography.

Journal of PeptideScience



Scheme 2. Elimination of an acetyl derivative of serine using TBAF.

dehydroamino acids and dehydropeptides from the carbonate derivatives of hydroxy amino acids, exploiting the basicity of the unsolvated fluoride ion in TBAF.

Experimental Part

All reagents were purchased from commercial sources and were used without further treatment. The amino acid and peptide derivatives (**1a–l**) were synthesized using procedures reported [5]. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) were recorded on a Bruker 400 spectrometer. IR spectra were recorded on a JASCO FTIR spectrophotometer. High resolution mass spectra were recorded on a Micromass QTOF ESIMS instrument.

Procedure for the Preparation of Dehydroalanine and Dehydroamino Butyric Acid Derivatives from the Carbonate Derivatives of Serine and Threonine, Respectively

To a solution of the carbonate derivative (**1a–l**, 1 mmol) in 1 ml anhydrous THF, a solution of TBAF (1 ml, 1 M in THF) was added and the reaction mixture was stirred for 10 min. The reaction mixture was diluted with 25 ml CH_2Cl_2 and filtered. The dehydroamino acid derivatives were isolated from the crude solution using silica gel (100–200 mesh) column chromatography eluting with 10–30% of ethyl acetate in hexane. The dehydroamino acid and peptide derivatives were characterized by comparing the spectral properties of the isolated compounds with those reported in the literature [5,6]. Characterization data for two representative compounds are given below.

Boc-ΔAla-Val-OMe (2j)

Colorless oil.
$[\alpha]_D^{25}$ − 6 (c 1, ethanol)
IR (Neat): 3394, 1733, 1496 cm^{-1}
^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.25 (d, J = 6 Hz, 1H), 6.57 (d, J = 6 Hz, 1H), 6.04 (s, 1H), 5.14 (s, 1H), 4.59 (q, J = 8 Hz, 1H), 3.70 (d, J = 16 Hz, 3H), 2.23–2.19 (m, 1H), 1.63 (s, 3H), 1.47 (s, 9H), 1.30 (s, 2H), 0.96–0.86 (m, 6H).
^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.0, 163.9, 152.6, 134.6, 97.9, 57.5, 52.3, 31.5, 19.6, 28.2, 18.9, 17.7.
HRMS: m/z calculated for $C_{14}H_{24}N_2O_5$ + Na = 323.1583; Observed = 323.1576.

Boc-Phe-ΔAbu-OMe (2k)

Colorless oil.
$[\alpha]_D^{25}$ − 44 (c 1, ethanol)
IR (Neat): 3372, 1741 cm^{-1}
^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.37 (s, 1H), 7.32–7.24 (m, 6H), 6.78 (q, J = 1.2 Hz, 1H), 5.03 (br, s, 1H), 4.48 (br, s, 1H), 3.73 (s, 3H), 3.16 (dd, J_1 = 8 Hz, J_2 = 8 Hz, 1H), 3.35 (dd, J_1 = 8 Hz, J_2 = 8 Hz, 1H), 2.17 (s, 1H), 2.68 (d, J = 3.2 Hz, 4H), 1.41 (s, 9H).
^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.9, 164.5, 136.4, 134.4, 129.3, 128.6, 125.7, 52.2, 28.3, 28.2, 14.5.
HRMS: m/z calculated for $C_{19}H_{26}N_2O_5$ + H = 363.1920; Observed = 363.1942.

Acknowledgements

RR thanks CSIR, New Delhi for a senior research fellowship and IISc for financial assistance, and RM thanks DBT for a postdoctoral fellowship.

References

1 Bonauer C, Walenzyk T, Konig B. α,β-Dehydroamnino acids. *Synthesis* 2006; 1–20.
2 Schmidt U, Lieberknecht A, Wild J. Didehydroamino acids (DDAA) and didehydropeptides (DDP). *Synthesis* 1988; 159–172.
3 Humphrey JM, Chamberlin AR. Chemical synthesis of natural product peptides: coupling methods for the incorporation of noncoded amino acids into peptides. *Chem. Rev.* 1997; **97**: 2243–2266.
4 A detailed study on the reported procedures for the synthesis of dehydroalanine derivatives is available in: Suzen S, Williams JM. Investigation of the synthesis of some dehydroalanine derivatives. *Turk. J. Chem.* 2000; **24**: 361–369.
5 Ferreira PMT, Maia HLS, Monteiro LS, Sacramento J. High yielding synthesis of dehydroamino acid and dehydropeptide derivatives. *J. Chem. Soc., Perkin Trans* 1999; **1**: 3697–3703.
6 Ramesh R, De K, Chandrasekaran S. An efficient synthesis of dehydroamino acids and dehydropeptides from *O*-Cbz and *O*-Eoc derivatives of serine and threonine. *Tetrahedron* 2007; **63**: 10534–10542.
7 Okutani M, Mori Y. Tetrabutylammonium fluoride-induced dehydrobromination of vinyl bromides to terminal acetylenes. *Tetrahedron Lett.* 2007; **48**: 6856–6859.
8 Berube M, Kamal F, Roy J, Poirier DA. Dehydrohalogenation methodology for synthesizing terminal olefins under mild conditions. *Synthesis* 2006; 3085–3091.
9 Dura RD, Paquette LA. Tetrabutylammonium fluoride in dimethyl sulfoxide: a reagent combination for the twofold dehydrobromination of vicinal dibromides. *Synthesis* 2006; 2837–2840.
10 Higuchi Y, Shimoma F, Ando M. Synthetic method and biological activities of *cis*-fused α-methylene γ-lactones. *J. Nat. Prod.* 2003; **66**: 810–817.
11 Bartsch RA. Eliminations from 2-butyl halides induced by halide ions in dimethylformamide and dimethyl sulfoxide. *J. Org. Chem.* 1970; **35**: 1023–1025.
12 Clark JH. Fluoride ion as a base in organic synthesis. *Chem. Rev.* 1980; **80**: 429–452.

