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PREPARATION OF DEHYDROALANINE PEPTIDES FROM BIS-(2,2,2-TRICHLOROETHYL) AND DIPHENYL PHOSPHONOSERINE DERIVATIVES

Alenka Paquet

Food Research Centre, Agriculture Canada, Ottawa, Ontario, Canada K1A OC6

Abstract: *Preparation of dehydroalanine peptides from the corresponding bis-(2,2,2-trichloroethyl) and diphenyl phosphonoserine derivatives by treatment with various organic bases is described.*

Incorporation of a dehydroalanine (ΔAla) residue into a peptide chain serves several purposes, *e.g.*, it is used for alteration of biological properties of peptides and proteins[1-4], for site-specific cleavage of proteins[5] and in solid phase peptide synthesis for an efficient preparation of peptide amides[6]. In this process, N-protected O-*p*-toluensulphonyl serine dipeptide is treated with 0.1 N NaOH and the resulting dipeptide with COOH-terminal ΔAla is esterified to a solid support. Cleavage at the ΔAla residue furnishes peptide amide. NaOH treatment of the starting dipeptide involves potential danger of racemization of the NH_2-terminal amino acid.

There have been many methods described for the preparation of ΔAla compounds, for example Z-Ser(PO_3Ph_2)-OEt was converted to Z-ΔAla-OH with NaOH solutions[7] and to Z-ΔAla-OEt using triethylamine[8]. Chlorination of serine containing peptides with subsequent β-elimination was reported[9]. For review, the article by Schmidt *et al.*[10] is recommended. More recently preparation of dehydroamino acids from N-acyl-N-hydroxyamino acid esters was described[11]. Carbonyldiimidazole[12] and isourea[13] mediated synthesis of ΔAla derivatives were also reported.

In a previous communication[14] we described synthesis of various phenyl (Ph) and 2,2,2-trichloroethyl (Tc) phosphorus protected phosphonoserine derivatives, such as Boc-Ser-(PO_3Ph_2)-OBzl (1), Boc-Ser(PO_3Tc_2)-OBzl (2) and Z-Ser-(PO_3Tc_2)-OBzl (3) by phosphorylating Boc-Ser-OBzl and Z-Ser-OBzl with either diphenyl phosphochloride, $(PhO)_2POCl$, or bis-(2,2,2-trichlorethyl) phosphochloride, $(TcO)_2POCl$[14].

In this communication we show that compounds 1-3 (table) after appropriate partial deprotection, can be incorporated into a peptide chain as dehydroalanine precursors and converted into the corresponding dehydroalanine derivatives by subsequent treatment of the protected peptide with an organic base.

The carboxyl deprotected 1 (hydrogenolysis, Pd/C)[14], Boc-Ser(PO_3Ph_2)-OH, was a convenient acylating carboxyl component for the introduction of phosphonoserine residue into internal or NH_2-terminal position in a peptide chain and served well for the preparation of Boc-Ser(PO_3Ph_2)-Glu(OBzl)-OBzl (4)[14] and Z-Phe-Ser(PO_3Ph_2)-Glu(OBzl)-OBzl (7)[15], both obtained in 90% yield (see table). As for the preparation of peptides with COOH-terminal phosphonoserine, the easy formation of ΔAla

prevented any synthesis with the Boc-deprotected **1**, H-Ser(PO_3Ph_2)-OBzl. But the Boc-deprotected **2**, H-Ser(PO_3Tc_2)-OBzl was suitable for this purpose, because the high crystallinity of the Tc-protected phosphonoserine peptides allowed easy removal of any ΔAla derivatives by single recrystallization. Thus using this compound, Boc-Glu(OBzl)-Ser(PO_3Tc_2)-OBzl (**5**) was obtained in 76% yield[14] (see table). But all attempts to remove ΔAla from Z-Val-Ser(PO_3Ph_2)-OBzl (**6**), (synthesis from Z-Val-OH and H-Ser(PO_3Ph_2)-OBzl[16]) by crystallization failed. Therefore, **6** was used exclusively for direct conversion into Z-Val-ΔAla-OBzl (**12**).

Treatment with one equivalent of triethylamine (Et_3N) or N-methylpiperidine (NMP) for 1.5 - 2 h was sufficient to convert compounds having the COOH-terminal phosphonoserine residues **1-3**, **5**, and **6** into their unsaturated counterparts **8**, **9**, **11**, and **12**. Peptides with the phosphonoserine residues as NH_2-terminal (**4**) or internal residue (**7**) required 1.5 equivalent of 1,4-diazabicyclo[2,2,2]octane, the Dabco base (for 27-30 h) (preparation of **10** from **4** and **13** from **7**), see the table.

The efficient preparation of the described phosphonoserine derivatives and their easy incorporation into a peptide chain as ΔAla precursors might find usefulness in peptide chemistry. For example for the preparation of peptide amides[6], the direct esterification of the debenzylated **1** on a support as ΔAla precursor might avoid preparation of ΔAla dipeptide from its precursor, the *p*-toluenesulphonyl-serine derivative, by treatment with NaOH solutions.

Table

RESULTS ON DEHYDROALANINE DERIVATIVES

Precursor	Product[g]	^{1}H-NMR vinyl shift (δ ppm)	M.p. °C	$[\alpha]^{23,h}$	Base
Boc-Ser(PO_3Ph_2)-OBzl[a] (**1**)	Boc-ΔAla-OBzl (**8**)	5.70; 6.10	47	-	NMP
Boc-Ser(PO_3Tc_2)-OBzl[b] (**2**)	Boc-ΔAla-OBzl (**8**)	5.70; 6.10	47	-	NMP
Z-Ser(PO_3Tc_2)-OBzl[b] (**3**)	Z-ΔAla-OBzl (**9**)	5.81; 6.08	51	-	Et_3N
Boc-Ser(PO_3Ph_2)-Glu(OBzl)-OBzl[c] (**4**)	Boc-ΔAla-Glu(OBzl)-OBzl (**10**)	5.23; 6.06	oil	+0.4°	DABCO
Boc-Glu(OBzl)-Ser(PO_3Tc_2)-OBzl[d] (**5**)	Boc-Glu(OBzl)-ΔAla-OBzl (**11**)	5.95; 6.60	oil	-15.3°	NMP
Z-Val-Ser(PO_3Ph_2)-OBzl[e] (**6**)	Z-Val-ΔAla-OBzl (**12**)	5.98; 6.61	113	-22.0°	Et_3N
Z-Phe-Ser(PO_3Ph_2)-Glu(OBzl)-OBzl[f] (**7**)	Z-Phe-ΔAla-Glu(OBzl)-OBzl (**13**)	5.33; 6.46	oil	-6.3°	DABCO

a: Obtained by phosphorylation of Boc-Ser-OBzl with $(PhO)_2POCl$[14].

b: Obtained by phosphorylation of Boc-Ser-OBzl with $(TcO)_2POCl$[14].

c: Synthesized from Boc-Ser(PO_3Ph_2)-OH and H-Glu(OBzl)-OBzl[14].

d: Synthesized from Boc-Glu(OBzl)-OH and H-Ser(PO_3Tc_2)-OBzl[14].

e: For synthesis, see Note 16.

f: For synthesis see Note 15.

g: All obtained in 95-100% yield. Correct elemental analyses and HPLC (isocratic elution from 10 μm μBondapack C-18 column; 60% CH_3CN in H_2O + 0.1% H_3PO_4, flow rate 2 mL/min) were used to establish purity.

h: In $CHCl_3$.

i: Used to induce β-elimination.

EXPERIMENTAL

Boc-Glu(OBzl)-ΔAla-OBzl (11)

To a solution of Boc-Glu(OBzl)-Ser(PO_3Tc_2)-OBzl[14] (5) (200mg, 0.233 mmol) in methylene chloride (1 mL) was added N-methylpiperidine (23.1mg, 0.233 mmol). The mixture was stirred at room temperature for 1.5 h, diluted with 20 mL of methylene chloride and washed 3 times with water, dried over sodium sulphate and evaporated giving 112 mg of **11** (96%). All other compounds were prepared from bis-trichloroethylphosphonoserine or diphenylphosphonoserine derivatives using triethylamine or N-methylpiperidine as indicated in this experiment, except for 5 and 8, which were treated with DABCO base for 27 and 60 h, respectively. Characteristics of the products are reported in the table.

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triethylamine: to a stirred solution of Boc-Ser-OBzl (5 g, 16.9 mmol) in anhnydrous ether (14 mL) was added diphenylphosphochloridate (11.37 g, 42.3 mmol) and pyridine (3.34 g, 42.3 mmol). The mixture was stirred for additional 23 h and most of the ether was evaporated. Hexane (20 mL) was added and the solid was filtered off and washed thoroughly with hexane (excess of chloride removal), with water (salt removal), dissolved in methylene chloride and dried with sodium sulphate. Evaporation and crystallization gave 7.78 g of Boc-Ser(PO_3Ph_2)-OBzl (87%). The product was identical (HPLC, ^{1}H-and ^{31}P-NMR) with the authentic sample[14]. Larger scale preparations (10 g) should involve cooling with ice during first 1 h. Phosphorylation of Boc-Ser-OBzl with $(TcO)_2POCl$ using these conditions gave 88 % of Boc-Ser(PO_3Tc_2)-OBzl.

15. *Note*: Z-Phe-Ser(PO_3Ph_2)-Glu(OBzl)-(OBzl), (7) was prepared from H-Ser(PO_3Ph_2)-Glu(OBzl)-OBzl[14] (16.86 g, 25.9 mmol), and Z-Phe-OH (8.53 g, 28.50 mmol) using N-ethyl, N'-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) (5.46 g, 28.50 mmol) in CH_2Cl_2. The mixture was washed with 10% citric acid, sat. solution of sodium bicarbonate, water, dried, evaporated, and crystallized from ethyl acetate-hexane giving 21.60 g (90%) of the product. M.p.: 84.5°C, $[\alpha]^{23}$ = -17.6°, ^{31}P-NMR($CDCl_3$): -10.42 (s). Anal. calcd. for $C_{51}H_{50}N_3O_{12}P$: C 66.01, H 5.43, N 4.53, P 3.33. Found: C 65.76, H 5.41, N 4.57, P 3.04.

16. *Note*: Z-Val-Ser(PO_3Ph_2)-OBzl was prepared from H-Ser(PO_3Ph_2)-OBzl[14] (0.454 g, 1.06 mmol) and Z-Val-OH (0.267g, 1.06 mmol) using EDC (0.203 g, 1.06 mmol) in CH_2Cl_2. The mixture was worked up as indicated in 15, giving 0.58 g (87%) of the oily product. The product was characterized by HPLC, ^{1}H-NMR (contained approximately 7% of ΔAla derivative) and was used directly for Z-Val-ΔAla-OBzl preparation.

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