

An Exceptionally Mild and Efficient Route to Dehydroalanine Peptides

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Δ -Ala peptides are formed in good yields on treatment of the corresponding serine precursors with oxalyl chloride and triethylamine in methylene chloride at 0 °C.

No methodology is available for the transformation of serine residues into their dehydro analogues in a peptide environment.[†] In view of the demonstrated importance of Δ -Ala peptides, particularly in terms of changes in reaction profile¹ and potential for the generation of secondary structural

elements,² a practical route to the creation of such units from appropriate precursors, in a peptide environment, would be timely.

We report in this communication, a serendipitous finding, which, in our opinion, provides a procedure for the facile generation of Δ -Ala units in peptides.

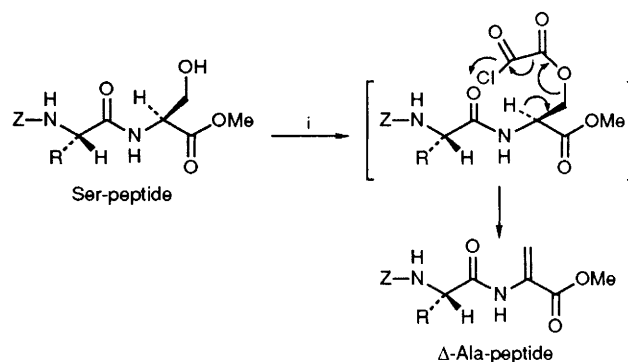
During endeavours relating to the chemical simulation of the α -amidating action of pituitary enzymes, the extended dipeptide oxalamide motif (HN-CO-CO-NH) was generated

[†] To the best of our knowledge, the only exception is where a Δ -Ala is generated from *O*-tosylserine residue in a dipeptide, I. Photaki, *J. Am. Chem. Soc.*, 1963, **85**, 1123.

from non-terminal serine peptides.³ The recent recognition of the importance of closely related units in protein function⁴ prompted us to prepare, a range of peptides having the oxalamide unit located at the centre from appropriate precursors and oxalylchloride.⁵ However, when serine residues were present in these peptides, this residue, *inter alia*, was cleanly transformed to dehydroalanine. The procedure was subsequently found to be quite general.

Thus, peptides 1–11[‡] (Table 1) on treatment with oxalyl chloride and triethylamine in dry methylene chloride at 0 °C for 2–4 h, afforded the expected Δ -Ala peptides in the range of 50–60% yields.[§] The Δ -Ala units present in the resulting chiral peptides could be easily identified[¶] by the presence of, in ¹H NMR spectra, a non-exchangeable pair of singlets at δ 5.5–7.0 and a broad exchangeable singlet at δ 8.2–9.0. The facile formation of the dehydroalanine unit is rationalized on the basis of fragmentation of the initially formed Ser-*O*-oxalyl chloride (Scheme 1).

Although an extended study would be needed to determine the scope and limitations of this novel reaction, some interesting results from Table 1 are noteworthy. The formation of Z-Met- Δ -Ala-OMe (Z = benzyloxycarbonyl) in good yields clearly shows the insensitivity of the methionine side chain to the reagent. This result would also suggest that peptides containing disulfide bridges would be unaffected. The preparation of Bz-Pro- Δ -Ala-OMe (Bz = benzoyl) illustrates an exceptionally facile pathway for the generation



Scheme 1 Reagents and conditions: i, (COCl)₂, NEt₃, CH₂Cl₂, 0 °C

Table 1 Preparation of Δ -Ala-peptides from Ser-peptides using oxalyl chloride and triethylamine

Entry	Starting Ser-Peptide {m.p./°C; [α] _D ²⁵ , (c, solvent)}	Product Δ -Ala-Peptide {yield (%) m.p./°C; [α] _D ²⁵ , (c, solvent)}
1	Bz-Ser-OMe[86]	Bz- Δ -Ala-OMe[90; syrup]
2	Z-Gly-Ser-OMe[85–86; +24.72 (0.55, CHCl ₃)]	Z-Gly- Δ -Ala-OMe[40; syrup]
3	Bz-Ala-Ser-OMe[134–35; +10.8 (0.4, MeOH)]	Bz-Ala- Δ -Ala-OMe[58; 110–115; –37.18 (0.43, CHCl ₃)]
4	Bz-Leu-Ser-OMe[99–100; +24.1 (3.3, MeOH)]	Bz-Leu- Δ -Ala-OMe[56; 55–56]
5	Z-Phe-Ser-OMe[83–84]	Z-Phe- Δ -Ala-OMe[58; syrup; +62.99 (1.77, CHCl ₃)]
6	Bz-Val-Ser-OMe[169–70; +15.44 (1.58, CHCl ₃)]	Bz-Val- Δ -Ala-OMe[48; syrup; +4.90 (1.06, CHCl ₃)]
7	Bz-Pro-Ser-OMe[71–72; –26.50 (0.8, CHCl ₃)]	Bz-Pro- Δ -Ala-OMe[54; 110–111; –86.16 (0.73, CHCl ₃)]
8	Z-Met-Ser-OMe[143–144; +20.94 (0.42, CHCl ₃)]	Z-Met- Δ -Ala-OMe[62; syrup; –9.11 (1.13, CHCl ₃)]
9	Bz-Leu-Ser-Leu-OMe[123–124; –25.90 (3.3, CHCl ₃)]	Bz-Leu- Δ -Ala-Leu-OMe[30; syrup; –38.04 (0.51, CHCl ₃)]
10	Bz-Ala-Ser-Ala-OMe[197–198; –30.96 (1.76, MeOH)]	Bz-Ala- Δ -Ala-Ala-OMe[25; syrup]
11	Z-Ser-Leu-Ser-OMe[182–183; –39.25 (0.21, MeOH)]	Z-Ser-Leu- Δ -Ala-OMe[30; syrup]

[‡] All amino acids used were of the L configuration. The peptide substrates (entries 2–8) were prepared by the usual coupling procedures (DCC/HOBT/DMF/CH₂Cl₂). For the preparation of tripeptides entries 9, 10 and 11, Bz-Leu-Ser, Bz-Ala-Ser, and Z-Ser-Leu azides—generated from their respective hydrazides—were coupled with Leu-OMe, Ala-OMe and Ser-OMe, respectively. Satisfactory spectral and elemental analyses were obtained for all peptides reported.

[§] In a typical procedure, a solution of oxalyl chloride (0.14 ml, 1.5 mmol) in dry CH₂Cl₂ (2 ml) was added dropwise to a well stirred solution of Ser-peptide (1 mmol) in dry CH₂Cl₂ (10 ml) or where insoluble, in THF, EtOAc, containing triethylamine (0.42 ml, 3 mmol) at 0 °C over ca. 0.25 h. The reaction was followed by the disappearance of the starting material (TLC, average time 2–4 h at room temp.) and worked up by washing with 5% NaHCO₃ solution, drying the organic layer with anhydrous MgSO₄ and evaporating *in vacuo*. The residue was cleaned up on a short column of silica gel with ethyl acetate–benzene as eluents to give pure dehydropeptide.

[¶] Selected spectral data: ¹H NMR (80 MHz, CDCl₃) δ Bz- Δ -Ala-OMe: 3.84 (3H, s), 5.87, 6.50 (1H, 1H, s, s), 7.1–8.0 (5H, m), 8.93 (1H, brs); Z-Gly- Δ -Ala-OMe: 3.84 (3H, s), 3.71–4.0 (2H, m), 5.15 (2H, s), 5.59 (1H, m), 5.90, 6.59 (1H, 1H, s, s), 7.34 (5H, s), 8.25 (1H, brs); Bz-Ala- Δ -Ala-OMe: 1.45 (3H, d, *J* 7.5 Hz), 3.78 (3H, s), 4.75 (1H, m), 5.81, 6.53 (1H, 1H, s, s), 7.1–8.0 (6H, m), 8.36 (1H, brs); Bz-Leu- Δ -Ala-OMe: 0.96 (6H, d, *J* 5 Hz), 1.70 (3H, m), 3.73 (3H, s), 4.70 (1H, m), 5.83, 6.53 (1H, 1H, s, s), 7.13–7.90 (6H, m), 8.43 (1H, brs); Z-Phe- Δ -Ala-OMe: 3.40 (2H, m), 3.75 (3H, s), 4.87 (1H, m), 5.41 (2H, s), 5.43, 6.53 (1H, 1H, s, s), 7.03–7.65 (12H, m), Bz-Val- Δ -Ala-OMe: 0.75–1.25 (6H, m), 2.18 (1H, m), 3.78 (3H, s), 4.53 (1H, m), 5.84, 6.53 (1H, 1H, s, s), 7.10–7.93 (6H, m), 8.25 (1H, brs); Bz-Pro- Δ -Ala-OMe: 1.62–2.5 (4H, m), 3.53 (2H, t), 3.78 (3H, s), 4.78 (1H, m), 5.84, 6.53 (1H, 1H, s, s), 7.21–7.59 (5H, m), 8.96 (1H, brs); Z-Met- Δ -Ala-OMe: 2.03 (3H, s), 2.46 (4H, m), 3.80 (3H, s), 4.50 (1H, m), 5.06 (2H, s), 5.30, 5.80 (1H, 1H, s, s), 6.56 (1H, brs), 7.41 (5H, s), 8.40 (1H, brs); Bz-Leu- Δ -Ala-Leu-OMe: 0.87 (12H, br), 1.65 (6H, m), 3.71 (3H, s), 4.09–5.21 (4H, m), 7.00–8.06 (7H, m), 8.75 (1H, br); Bz-Ala- Δ -Ala-Ala-OMe: 1.53 (6H, m), 3.75 (3H, s), 4.71 (2H, m), 5.40, 6.46 (1H, 1H, s, s), 6.84 (1H, m), 7.03–8.04 (6H, m), 8.62 (1H, br); Z-Ser-Leu- Δ -Ala-OMe: 0.88 (6H, brs), 1.71 (3H, m), 3.75 (5H, s + m), 4.53 (2H, m), 5.06 (2H, s), 5.84, 6.50 (1H, 1H, s, s), 7.28 (7H, s + m), 8.37 (1H, brs); MS: *m/z* Z-Gly- Δ -Ala-OMe: 293 (M + H)⁺; Bz-Leu- Δ -Ala-OMe: 319 (M + H)⁺; Bz-Val- Δ -Ala-OMe: 305 (M + H)⁺; Bz-Pro- Δ -Ala-OMe: 303 (M + H)⁺; Z-Met- Δ -Ala-OMe: 367 (M + H)⁺; Bz-Leu- Δ -Ala-Leu-OMe: 432 (M + H)⁺.

and incorporation of this highly interesting secondary structural motif in peptides and proteins.

Perhaps the most intriguing finding of the present study is the lack of reactivity of the serine residue when located at the *N*-terminal position in Ser-peptides. Thus, Z-Ser-Leu-OMe and Bz-Ser-Pro-OMe were largely unaffected under the present reaction conditions. Considering the reactivity profile presented in Table 1, this is best attributed to steric reasons because *C*-terminal Ser residues undergo the reaction with greatest facility. This finding can be utilised as illustrated with Z-Ser-Leu-Ser-OMe (entry 11, Table 1) to accomplish totally preferential *C*-terminal Δ -Ala formation.||

|| Threonine residues, regardless of their locations in peptides, where the steric constraints are considerably enhanced, are not affected under the present conditions.

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