An Exceptionally Mild and Efficient Route to Dehydroalanine Peptides

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ΔAla 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 ΔAla peptides, particularly in terms of changes in reaction profile[1] 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 ΔAla units in peptides.

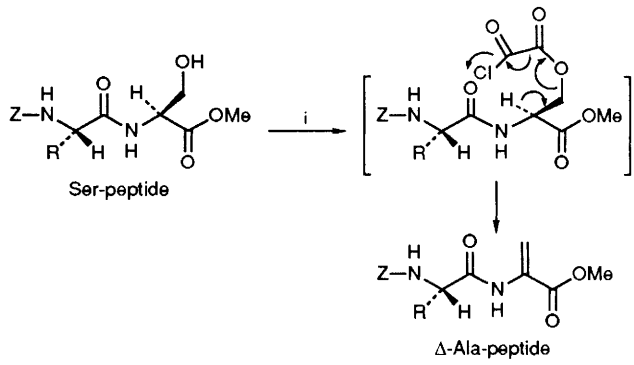
During endeavours relating to the chemical simulation of the α-amidating action of pituitary enzymes, the extended dipeptide oxalamide motif (HN–CO–CO–NH) was generated

† To the best of our knowledge, the only exception is where a ΔAla 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 function[4] 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 ^{1}H 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, $(COCl)_2$, NEt_3, CH_2Cl_2, 0 °C

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

Entry	Starting Ser-Peptide {m.p./°C; $[\alpha]_D^{23}$, (*c*, solvent)}	Product Δ-Ala-Peptide {yield (%) m.p./°C; $[\alpha]_D^{23}$, (*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]

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.‖

‡ 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/CH_2Cl_2). 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 CH_2Cl_2 (2 ml) was added dropwise to a well stirred solution of Ser-peptide (1 mmol) in dry CH_2Cl_2 (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% $NaHCO_3$ solution, drying the organic layer with anhydrous $MgSO_4$ 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: ^{1}H NMR (80 MHz, $CDCl_3$) 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 $(M + H)^+$.

‖ 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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References

1 Dehydroamino acids have been found to occur in biologically active metabolites of bacterial, fungal and marine origin, U. Schmidt, E. Ohlev, J. Hausler, H. Poisel, W. Herz, H. Grisebach and G. W. Kirby, *Progress in the Chemistry of Organic Natural Products*, Springer-Verlag, Wien, 1979, vol. 37, p. 251–327; C. H. Stammer, in *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*, Marcell Dekker Inc. New York, 1982, vol. 6, pp. 33–74; K. Noda, Y. Shimohigashi and N. Izumiya, in *The Peptides, vol. 5*, Academic Press, New York, 1983, p. 285; U. Schmidt, A. Lieberknecht and J. Wild, *Synthesis*, 1988, **3**, 159. The presence of these residues has been shown to impart enhanced resistance to enzymatic degradation, M. L. English and C. H. Stammer, *Biochem. Biophys. Res. Commun.*, 1978, **83**, 1464.

2 The unique conformational features of dehydro peptides have attracted considerable attention in recent years. There is evidence that the introduction of these units into peptide sequences induces folding of the backbone to give β-bends. M. R. Ciazolo, A. Tuzi, C. R. Pratesi, A. Fissi and O. Pieroni, *Biopolymers*, 1990, **30**, 911; O. Pieroni, A. Fissi, C. Pratesi, P. A. Temussi and F. Ciardelli, *J. Am. Chem. Soc.*, 1991, **113**, 6338.

Recent studies have shown that the biosynthesis of mono sulfide bridges in peptide metabolites involves the stereospecific addition of cysteine thiol groups to Δ-Ala residues. Such motifs are a characteristic feature of lantibiotics, an important class of antibiotics of fungal origin and exemplified with nisin (a food preservative), epidermin (a therapeutic agent against acne), ancovenin, duramycin (immunoactive agents) and mersacidin, actergardine (antibiotics), G. Jung, *Angew. Chem., Int. Ed. Engl.*, 1991, **30**, 1051.

3 D. Ranganathan and S. Saini, *J. Am. Chem. Soc.*, 1991, **113**, 1042.

4 The α-keto amide unit (–NH–CO–CO–), present in the powerful immunosuppressants rapamycin, FK-506 and related antibiotics, has recently been identified as the key structural element involved in direct binding with immunophilin proteins (rotamases) leading to the inhibition of their prolyl *cis* ⇄ *trans* isomerase activity, S. L. Schreiber, *Science*, 1991, **251**, 283; G. D. Van Duyne, R. F. Standaert, S. L. Schreiber and J. Clardy, *J. Am. Chem. Soc.*, 1991, **113**, 7433.

5 D. Ranganathan, K. Shah and N. Vaish, unpublished results.