Facile Synthesis of Cyclic Peptides containing α-Aminosuberic Acid with Oxime Resin

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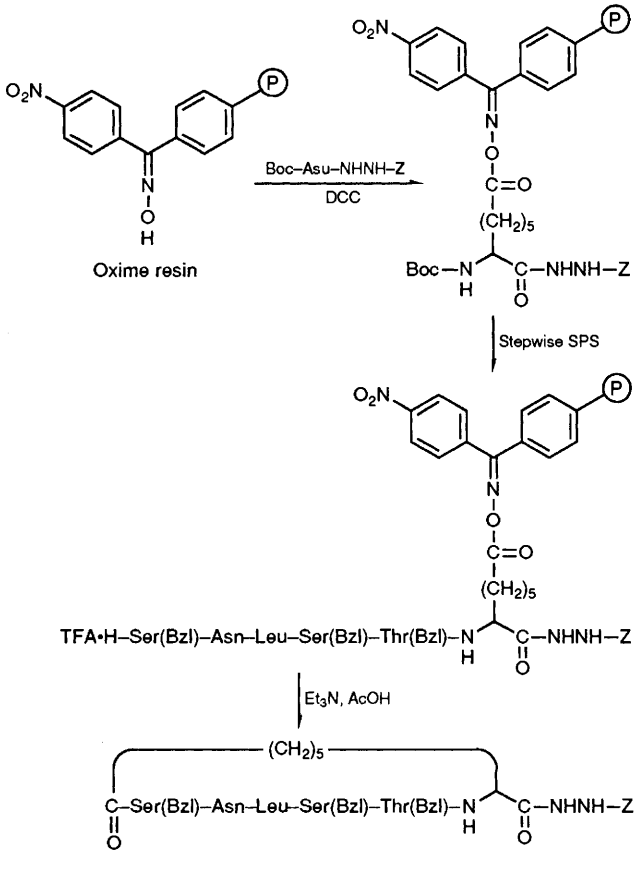
Cyclic peptides containing an α-aminosuberic acid unit are conveniently synthesized by solid-phase synthesis followed by cyclization cleavage with Kaiser's oxime resin.

α-Aminosuberic acid (Asu) is often employed in deamino-dicarba analogues of hormonal peptides to produce novel pharmaceutical properties, for instance, [Asu[1,6]]oxytocin[1] and elcatonin[2] in which a cyclic structure with a cystine residue is replaced by Asu. The syntheses of the cyclic portion of the analogues have been carried out by the conventional active ester (*p*-nitrophenyl ester) method under high dilution conditions in pyridine. Therefore, polymerization is unavoidable and isolation of the desired product is tedious. Recently, a convenient method for the synthesis of cyclic peptides has been developed by the use of the *p*-nitrobenzophenone oxime resin;[3,4] a linear peptide is assembled by solid-phase synthesis (SPS), then the peptide is cleaved with cyclization from the resin. This SPS and cyclization cleavage (SPS–CC) method has its basis in the nature of the anchoring linkage of the oxime resin[5] as a kind of active ester. In the present study, the facile synthesis of the cyclic portions of elcatonin and [Asu[1,6]]oxytocin has been carried out by the SPS–CC method (Scheme 1).



Scheme 1 Solid-phase synthesis and cyclization cleavage (SPS–CC) of the protected cyclic portion of elcatonin. Asu, aminosuberic acid; Boc, *tert*-butoxycarbonyl; Bzl, benzyl; Cl_2Bzl, 2,6-dichlorobenzyl; DCC, dicyclohexylcarbodiimide; TFA, trifluoroacetic acid; Z, benzyloxycarbonyl.

Boc-Asu-NHNH-Z† (1.75 g, 4.0 mmol) and DCC (0.83 g, 4.0 mmol) were added to a suspension of the oxime resin (4.0 g, 2.0 mequiv. of functional group) in CH_2Cl_2 (60 ml) in a shaking reaction vessel. After 12 h, the resin was filtered off, washed with CH_2Cl_2 (×2), CH_2Cl_2–EtOH (1:1, ×4) and CH_2Cl_2 (×2), and dried *in vacuo*. The increase in resin weight was 350 mg. The amino acid content was determined as 0.40 mmol (g resin)$^{-1}$ by picrate assay.[6] The resin (1.0 g) was then treated with 25% TFA in CH_2Cl_2 (15 ml) for 30 min, washed with CH_2Cl_2 (×2), isopropyl alcohol (×1) and CH_2Cl_2 (×4), and finally suspended in dimethylformamide (DMF) (15 ml). To the suspension of TFA·H-Asu(oxime resin)-NHNH-Z was poured a mixture of Boc-Thr(Bzl)-OH (3.0 equiv.), the [(benzotriazol-1-yl)oxy]tris(dimethylamino)phosphonium hexafluorophosphate (BOP) reagent[7] (3.0 equiv.) and Et_3N (6.5 equiv.) in DMF.‡ The reaction vessel was shaken for 45 min, the resin was washed by the addition of DMF (×4) and CH_2Cl_2 (×2), and the coupling efficiency was checked by the Kaiser test.[8] Boc-Ser(Bzl)-OH, Boc-Leu-OH, Boc-Asn-OH and Boc-Ser(Bzl)-OH were successively coupled by the same procedure. The peptidyl oxime resin, TFA·H-Ser(Bzl)-Asn-Leu-Ser(Bzl)-Thr(Bzl)-Asu(oxime resin)-NHNH-Z was then treated with 2 equiv. of acetic acid and Et_3N in DMF (15 ml).

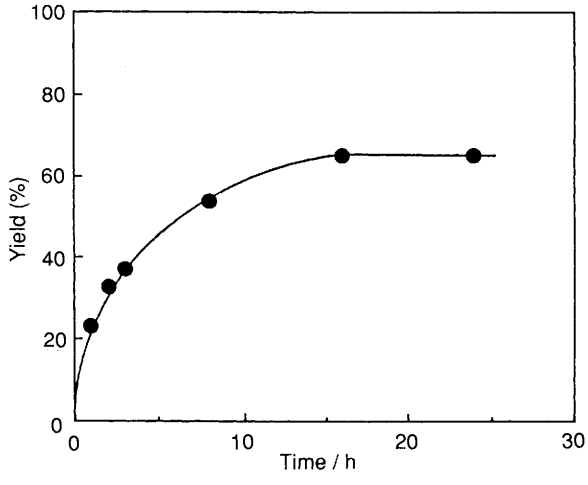


Fig. 1 Time course of the cyclization cleavage of the protected elcatonin cyclic portion. The reaction mixture was analysed by reversed-phase HPLC; column, MS-GEL C18 120 Å (4.6 × 150 mm); eluent, 37–100% acetonitrile 0.1% TFA linear gradient over 30 min; flow rate, 1.0 ml min^{-1}; detection, 220 nm. Yields are normalized on the basis of the HPLC results.

† L-Asu(OMe) was optically resolved for the first time by the tandem use of chymotrypsin and acylase, then converted to Boc-Asu-NHNH-Z in three steps. Details of the preparation will be published elsewhere.

‡ The BOP method is much more effective for stepwise coupling on the oxime resin than the symmerical anhydride and the DCC–1-hydroxybenzotriazole methods with regard to both yield and purity of protected peptides obtained. (Some peptide loss occurs especially at the third amino acid coupling step, but not with the BOP method).

Acetic acid is used as a catalyst for cyclization cleavage.[9] Fig. 1 shows the time course for the appearance of the cleaved product from the resin. After 24 h, the filtrate and the DMF washes were concentrated, water being added to the residues. The white precipitate of cyclo[-Ser(Bzl)-Asn-Leu-Ser(Bzl)-Thr(Bzl)-Asu]-NHNH-Z was filtered off, washed with water and dried *in vacuo*. The product was purified by washing with hot EtOH; total yield, 280 mg (65%); >95% purity on reversed-phase HPLC; FAB MS, *m/z* 1093 $(M + H)^+$; $[\alpha]_D^{25}$ −16.5 (*c* 1.0, DMF); m.p. 197–199 °C (decomp.); elemental analysis ($C_{57}H_{74}N_9O_{13} \cdot H_2O$), C, H, N. Subsequent treatment with anhydrous HF gave the resulting peptide hydrazide, cyclo(-Ser-Asn-Leu- Ser-Thr-Asu)-$NHNH_2$ in 97% yield and >95% purity; FAB MS, *m/z* 688 $(M + H)^+$; amino acid analysis, Asx (1) 1.04, Asu (1) 0.95, Leu (1) 1.0, Ser (2) 1.90, Thr (1) 0.93.

The cyclic portion of [$Asu^{1,6}$]oxytocin, cyclo[-Tyr(Cl_2Bzl)-Ile-Gln-Asn-Asu]-NHNH-Z was also synthesized starting with Boc-Asu (oxime resin)-NHNH-Z (1.0 g, 0.40 mmol) as described above. The crude product was recrystallized from hot EtOH to give 220 mg (56% total yield) of a white powder. The peptide was deprotected with anhydrous HF to give cyclo(-Tyr-Ile-Gln-Asn-Asu)-$NHNH_2$; FAB MS, *m/z* 704 $(M + H)^+$.

The conversion of the hydrazides to the corresponding azides should allow the formation of elcatonin and [$Asu^{1,6}$]-oxytocin. Thus, in practical syntheses of these useful biologically active peptides, the SPS–CC method has the merits of time-saving and high efficiency.

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