COMMUNICATION

A convenient one-pot synthesis of cystine-containing peptides from protected peptidyl resins using the trimethylsilyl chloride–dimethyl sulfoxide–trifluoroacetic acid system

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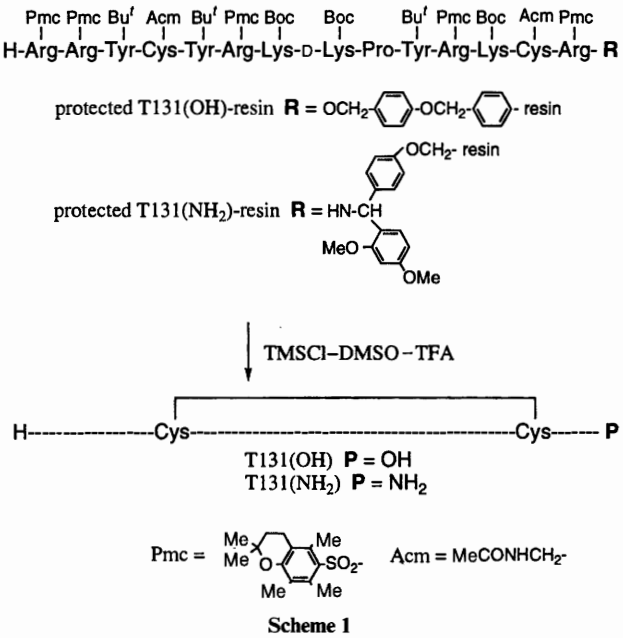
Cystine-containing peptides are obtained in a one-pot manner by treatment of protected peptidyl resins with trimethylsilyl chloride (TMSCl)–dimethyl sulfoxide (DMSO)–trifluoroacetic acid (TFA). The TMSCl–DMSO–TFA system simultaneously cleaves peptides from the resins, with deprotection of all side chain protecting groups and disulfide bond formation.

To date, a variety of cystine-containing peptides have been isolated from natural sources and their peptide analogues have been synthesized in order to study structure–activity relationships.[1,2] Thus we wished to develop a useful synthetic methodology for the rapid preparation of cystine-containing peptides. Recently, several disulfide bond-forming reactions using sulfoxide-mediated oxidations have been developed.[3–5] For example, cystine formation by a 1 h treatment of *S*-Acm † cysteine[6] with TMSCl–DMSO–TFA has been demonstrated.[5b] However, prior to oxidation, *S*-Acm cysteine peptides must be cleaved from the corresponding peptidyl resin and all protecting groups removed except for the Acm on the Cys(Acm). Simultaneous cleavage/deprotection with disulfide bond formation in a one-pot manner would be desirable in order to facilitate the synthesis of cystine-containing peptides. Since the TMSCl–DMSO–TFA system also has the ability to deprotect several protecting groups, we were prompted to examine whether cystine-containing peptides could be synthesized in a one-pot manner from protected peptidyl resins using a TMSCl–DMSO–TFA system.

Initially, the behaviour of the side chain protecting groups on several Fmoc-amino acid derivatives following treatment with TMSCl–DMSO–TFA was examined by HPLC (see the Experimental section). These analyses showed that deprotection of each protecting group was complete in 30 min without significant side reactions. This reagent system would be unsuitable for Met and Trp-containing peptides due to undesirable oxidative modifications.[3,5] The behaviour of amino acids on two peptide linkers, *p*-alkoxybenzyl alcohol linker (Alko linker)[7] and 4-(2′,4′-dimethoxyphenyl-Fmoc-aminomethyl)phenoxy linker (Rink amide linker),[8] was examined next by amino acid analyses. Treatment with TMSCl–DMSO–TFA at 4 °C (1 h) led to the incomplete cleavage of Leu and Arg from H-Leu-Alko resin and H-Arg(Pmc)-Rink amide resin, respectively (data not shown). However, pretreatment of the above amino acid resins with TMSCl–TFA at room temperature resulted in effective cleavage of the amino acids from the resins. Therefore, following initial treatment with TMSCl–TFA at room temperature, DMSO was added at 4 °C (see the Experimental section). In order to evaluate the TMSCl–DMSO–TFA system in a one-pot synthesis of cystine-containing peptides, two model peptidyl resins [T131(OH) and T131(NH_2)‡] were constructed using standard Fmoc-based solid-phase techniques (Scheme 1).[9] Alko linker and Rink amide linker were used for the synthesis of T131(OH) and T131(NH_2), respectively, and the following side chain protecting groups were utilized; Pmc for Arg, Acm for Cys, Boc for Lys and Bu^t for Tyr. One-pot treatment with TMSCl–TFA followed by DMSO resulted in the fully deprotected cystine-containing peptides. Each crude product exhibited a sharp main peak on analytical HPLC (Fig. 1) without significant side products. HPLC purification gave



Scheme 1

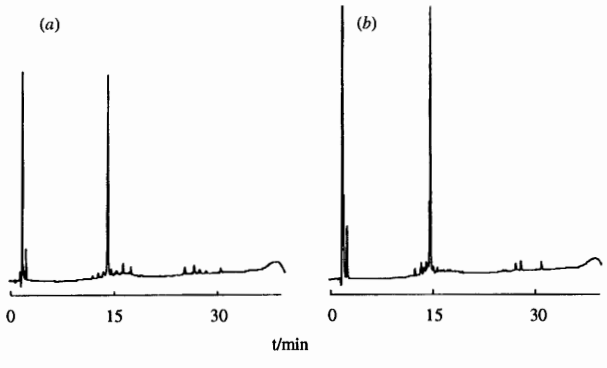


Fig. 1 Analytical HPLC of the crude (*a*) T131(OH) and (*b*) T131(NH_2). HPLC conditions: μBondasphere 5μC18–100 Å (3.9 × 150 mm) column, linear gradient of MeCN (10–40%, for 30 min) in 0.1% aqueous TFA at a flow rate of 1 cm^3 min^{-1}, Waters LC module 1 equipped with a Waters 741 Data Module, UV absorption measurement at 220 nm.

† Acm = acetamidomethyl, Fmoc = 9-fluorenylmethoxycarbonyl, Pmc = 2,2,5,7,8-pentamethylchroman-6-sulfonyl, Boc = *tert*-butoxycarbonyl, Bu^t = *tert*-butyl, IS–MS = ion spray mass spectrometry.

‡ These peptides are tachyplesin analogues[1,2] possessing anti-human immunodeficiency virus activity (unpublished data).

the corresponding disulfide forms of T131(OH) and T131(NH_2) in satisfactory yields (see the Experimental section). The yields were comparable to those obtained in parallel syntheses of both peptides by a stepwise strategy of deprotection/cleavage and disulfide bond formation using 1 mol dm^{-3} TMSBr–thioanisole–TFA and TMSCl–DMSO–TFA or iodine oxidations, respectively.§

In conclusion, the present procedure provides a convenient methodology for the rapid preparation of cystine-containing peptides. This procedure features a one-pot protocol for cleavage/deprotection of protected peptide resins with concomitant formation of disulfide bonds.

Experimental

Behaviour of Fmoc-amino acid side chain protecting groups

Treatment of the following side chain-protected Fmoc-amino acids (15 μmol each), Fmoc-Asp(Bu^t)-OH, Fmoc-Glu(Bu^t)-OH, Fmoc-His(Boc)-OH, Fmoc-Ser(Bu^t)-OH, Fmoc-Thr(Bu^t)-OH, Fmoc-Tyr(Bu^t)-OH and Fmoc-Lys(Boc)-OH, with TMSCl (25 equiv.)–DMSO (750 equiv.) in TFA (6 cm^3) was performed at 4 °C. At intervals (5, 10, 30, 60 and 90 min), an aliquot (0.1 cm^3) was removed and diluted with H_2O–MeCN (1:1) (0.9 cm^3), followed by HPLC analysis (0.02 cm^3). Loss of starting material and generation of the corresponding side chain-deprotected Fmoc-amino acid derivatives were quantified from the HPLC peak area. [HPLC conditions, μBondasphere 5μC18–100 Å (3.9 × 150 mm) with a linear gradient of MeCN (25–55%, for 30 min) in 0.1% aqueous TFA at a flow rate of 1 cm^3 min^{-1} on a Waters LC module 1 equipped with a Waters 741 Data Module.]

Recovery of amino acids from corresponding Alko resin and Rink amide resins

H-Leu-Alko resin (0.60 mmol g^{-1}, 2 μmol) or H-Arg(Pmc)-Rink amide resin (0.32 mmol g^{-1}, 2 μmol) and Boc-Gly-OH (internal standard, 3 μmol) were treated with TMSCl (25 equiv.) in TFA in the presence of anisole (0.01 cm^3) at room temperature for 1 h. DMSO (750 equiv.) was added to the reaction mixture at 4 °C (total solution volume 1 cm^3) and the reaction was allowed to continue. After 1 h, an aliquot (0.1 cm^3) was sampled and diluted with 0.02 mol dm^{-3} aqueous HCl (0.9 cm^3). Regeneration of H-Leu-OH or H-Arg-OH was quantified by an amino acid analyser (H-Leu-OH, 100%; H-Arg-OH, 87%). The cleavage of Val from H-Val-Rink amide resin required a 2 h treatment with TMSCl in TFA–anisole (recovery of H-Val-OH, 87%).

T131(OH)

The protected T131(OH)-resin (50 mg, 9.6 μmol) was treated with TMSCl (0.105 cm^3, 10 equiv.) in TFA (13 cm^3) in the presence of anisole (0.15 cm^3) at room temperature. After 1 h DMSO (1.9 cm^3, 300 equiv.) was added to the reaction mixture at 4 °C and the reaction was continued for 1 h. After removal of the resin by filtration, ice-cold dry diethyl ether (30 cm^3) was added to the filtrate. The resulting powder was collected by centrifugation and then washed three times with ice-cold dry diethyl ether (70 cm^3). The crude peptide was purified by preparative HPLC [Waters Delta Prep 4000 on a Cosmosil packed column (5μC18–100 Å, 20 × 250 mm) using a linear gradient of MeCN (14–16%, for 30 min) in 0.1% aqueous TFA at a flow rate of 7 cm^3 min^{-1}]. The solvent was removed by lyophilization to give a fluffy white powder: yield, 6.2 mg [33%, calculated from the protected T131(OH)-resin] [IS–MS (reconstructed) Found, 1974.0. Calc. for $C_{86}H_{140}N_{32}O_{18}S_2$, 1973.2]. Amino acid ratios after 6 mol dm^{-3} HCl hydrolysis (values in parentheses are theoretical): cystine not determined (1), Tyr 3.00 (3), Lys and D-Lys 2.86 (3), Arg 5.11 (5), Pro 0.05 (1).

T131(NH_2)

The purified T131(NH_2) was prepared by the same procedure as described above: yield, 56% [calculated from the protected T131(NH_2)-resin] [IS–MS (reconstructed) Found, 1972.5. Calc. for $C_{86}H_{141}N_{33}O_{17}S_2$, 1972.1]; amino acid ratios after 6 mol dm^{-3} HCl hydrolysis (values in parentheses are theoretical): cystine not determined (1), Tyr 3.00 (3), Lys and D-Lys 2.80 (3), Arg 5.13 (5), Pro 0.88 (1).

§ The yields of the purified peptides by the stepwise strategies: (TMSBr and TMSCl–DMSO–TFA) T131(OH), 36% and T131(NH_2), 55%; (TMSBr and iodine) T131 (OH), 44% and T131(NH_2), 59% (calculated from the protected peptidyl resins).

Acknowledgements

This work was supported in part by a Grant-in-Aid from The Tokyo Biochemical Research Foundation. The authors thank Dr Terrence R. Burke, Jr., NCI, NIH, USA, for reading the manuscript and providing useful comments.

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Paper 6/03309A
Received 13*th May* 1996
Accepted 17*th June* 1996