# A New Approach for the Synthesis of Tyrosine Sulphate containing Peptides: Use of the *p*-(Methylsulphinyl)benzyl Group as a Key Protecting Group of Serine

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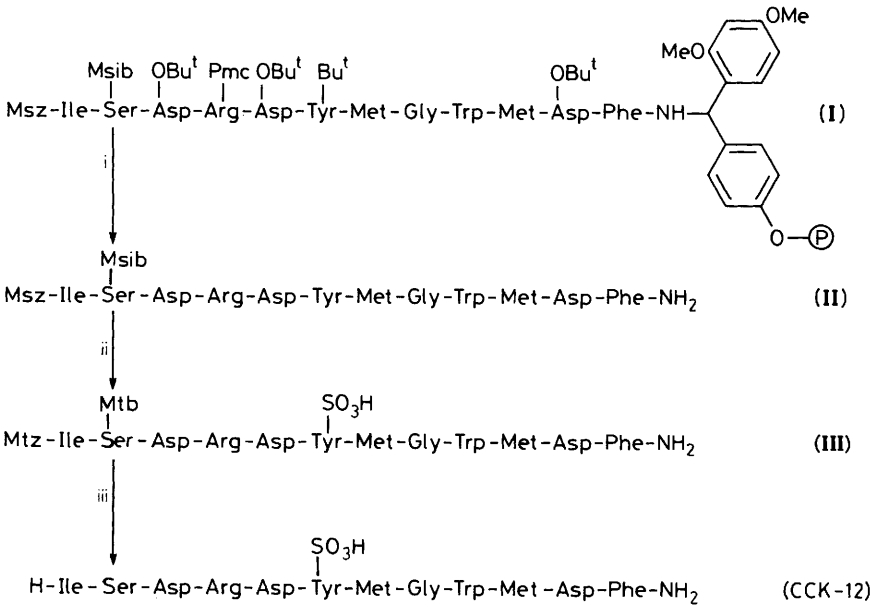
A convenient method for the synthesis of tyrosine sulphate [Tyr($SO_3H$)] containing peptides, using Fmoc–solid-phase strategy (Fmoc = fluoren-9-ylmethoxycarbonyl) for peptide-chain construction and *O*-*p*-(methylsulphinyl)benzyl serine for the selective sulphation of tyrosine, has been developed and cholecystokinin (CCK)-12 synthesized as a model peptide.

Many achievements have been reported in the synthesis of Tyr($SO_3H$)-containing peptides,[1] however, more efficient and convenient synthetic strategies are still being explored. One crucial problem is the selection of a protecting group for Ser and Thr, since the alcoholic hydroxy function is sulphated in preference to the phenolic hydroxy group of Tyr.[1f] We now report a new approach for the synthesis of Tyr($SO_3H$) containing peptides, where Ser(Msib) [Msib = *p*-(methylsulphinyl)benzyl] was employed as a key protecting group for the selective sulphation of Tyr. The Msib group was first introduced by Samanen and Brandeis[2] as a carboxy protecting group and has the unique property that a Msib ester is stable to trifluoroacetic acid (TFA), but its reduced form, the Mtb [*p*-(methylthio)benzyl] ester is cleavable with TFA.

Our synthetic outline is as follows. (i) After convenient construction of the peptide chain by Fmoc–solid-phase strategy (Fmoc = fluoren-9-ylmethoxycarbonyl),[3] all protecting groups except Ser(Msib) are removed with TFA. (ii) Tyr in the deprotected peptide is sulphated followed by reduction of Ser(Msib) to Ser(Mtb). (iii) Deprotection of Ser(Mtb) with acid affords the desired peptide. We expected to obtain the sulphated peptides easily using this approach, though it has the defect that Tyr($SO_3H$) will be partially decomposed in the final acid treatment owing to the instability of Tyr($SO_3H$) to acids.[1,4]

Ser(Msib) was prepared conveniently using Msib-Br[5] and NaH in basically the same manner with Ser(Bzl).[6] Ser(Msib) was found to have almost the same properties as those of the ester: Ser(Msib) is stable to TFA (room temp., 3 h) or 10% ethanedithiol (EDT) in TFA (room temp., 3 h), and its reduced form, Ser(Mtb), is cleavable with TFA (0 °C, 3 h) or 10% EDT in TFA (0 °C, 1 h).

Cholecystokinin (CCK)-12 was synthesised as a model peptide (Scheme 1). In addition to the hydroxy group of Ser, $N^{\alpha}$ must be protected during sulphation in order to avoid possible sulphamic acid formation. For this purpose, the N-terminal Ile residue was incorporated as Msz-Ile [Msz = *p*-(methylsulphinyl)benzyloxycarbonyl][7] instead of Fmoc–Ile. The Msz group and its reduced form, Mtz [*p*-(methylthio)benzyloxycarbonyl],[7] are also reported to have the same stability to acid as the Msib and Mtb groups, respectively, therefore we expected to be able to remove the $N^{\alpha}$ protecting group simultaneously with the hydroxy protecting group on Ser. Msz-Ile was prepared easily according to the method of Kiso *et al.*[7] In addition Msz-Ile and Fmoc-Ser (Msib), Fmoc amino acid derivatives bearing TFA-labile protecting groups based on t-butyl alcohol were employed, together with Arg(Pmc) [Pmc = 2,2,5,7,8-pentamethylchroman-6-sulphonyl].[8] Trialkoxybenzhydrylamine-type resin[9] was used for the peptide anchor and the peptide chain was elongated with 2 equiv. of Fmoc-amino acid and BOP (Castro's) reagent [benzotriazolyl-*N*-oxytris(dimethylamino)phosphonium hexafluorophosphate][10] and 6 equiv. of *N*-methylmorpholine by Fmoc strategy. After construction, the peptide chain (**II**) was detached from the resin with TFA at 0 °C for 3 h. In order to avoid alkylation on Trp and Met




**Scheme 1.** *Reagents*: i, TFA, EDT, *m*-cresol, 0 °C, 3 h; ii, DMF–$SO_3$ complex/EDT, room temp., 36 h, then Sephadex LH-20; iii, TFA, EDT, *m*-cresol, 0 °C, 90 min, then Sephadex G-10, then YMC AM-312.

during acidolysis, 10% of EDT and 5% of *m*-cresol were added to the TFA. All protecting groups except the Msib group for Ser and the Msz group for Ile were thus removed. Next, the peptide (**II**) was treated with dimethylformamide (DMF)–$SO_3$ complex[11] (100 equiv.) in DMF–pyridine (4 : 1) (room temp., 36 h). This complex was recently reported by us to have greater ability for sulphation than that of the commonly used pyridine–$SO_3$ complex.[12] On addition of EDT (100 equiv.) to the DMF–$SO_3$ complex, reduction of the Msib and the Msz groups to Mtb and Mtz groups, respectively, can be expected simultaneously with the sulphation.[5] After gel-filtration on Sephadex LH-20, the Mtb and Mtz groups of (**III**) were removed by treatment with TFA containing 10% EDT and 5% *m*-cresol (0 °C, 90 min). Subsequent purification by gel-filtration on Sephadex G-10 and HPLC on a YMC AM-312 column afforded pure CCK-12 in 7% yield (from the introduction of Phe onto the resin) {$[\alpha]_D^{20}$ −32.1° (*c* 0.1, 1 M $NH_4OH$); lit.,[1b] $[\alpha]_D^{22}$ −22.8° (*c* 0.4, 1 M $NH_4OH$)}. The structure was confirmed by leucine aminopeptidase digestion[13] [Ile 0.97, Ser 0.96, Asp 2.76, Arg 0.92, Tyr($SO_3H$) 0.90, Met 1.86, Gly 0.97, Trp 0.87, Phe 1.00 (recovery of Phe, 93%)], FAB MS [1614.5 $(M+H)^+$], and FTIR (1051, 1276 $cm^{-1}$). Loss of the sulphate ester during the final TFA treatment was estimated to be *ca.* 10% by a degradation experiment using purified CCK-12.

Although the yield was not excellent, we wish to emphasize the simplicity and convenience of our method. This approach will be applicable to the synthesis of other Tyr($SO_3H$) containing peptides.

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