Solid Phase Synthesis of Retro-inverso Peptide Analogues. Synthesis of a Partially Modified Retro-inverso Analogue of Substance P, [Glp^6, gPhe^8, mGly^9]SP$_{6-11}$ [gPhe = –NHCH(CH$_2$Ph)NH–, mGly = –OCCH$_2$CO–]

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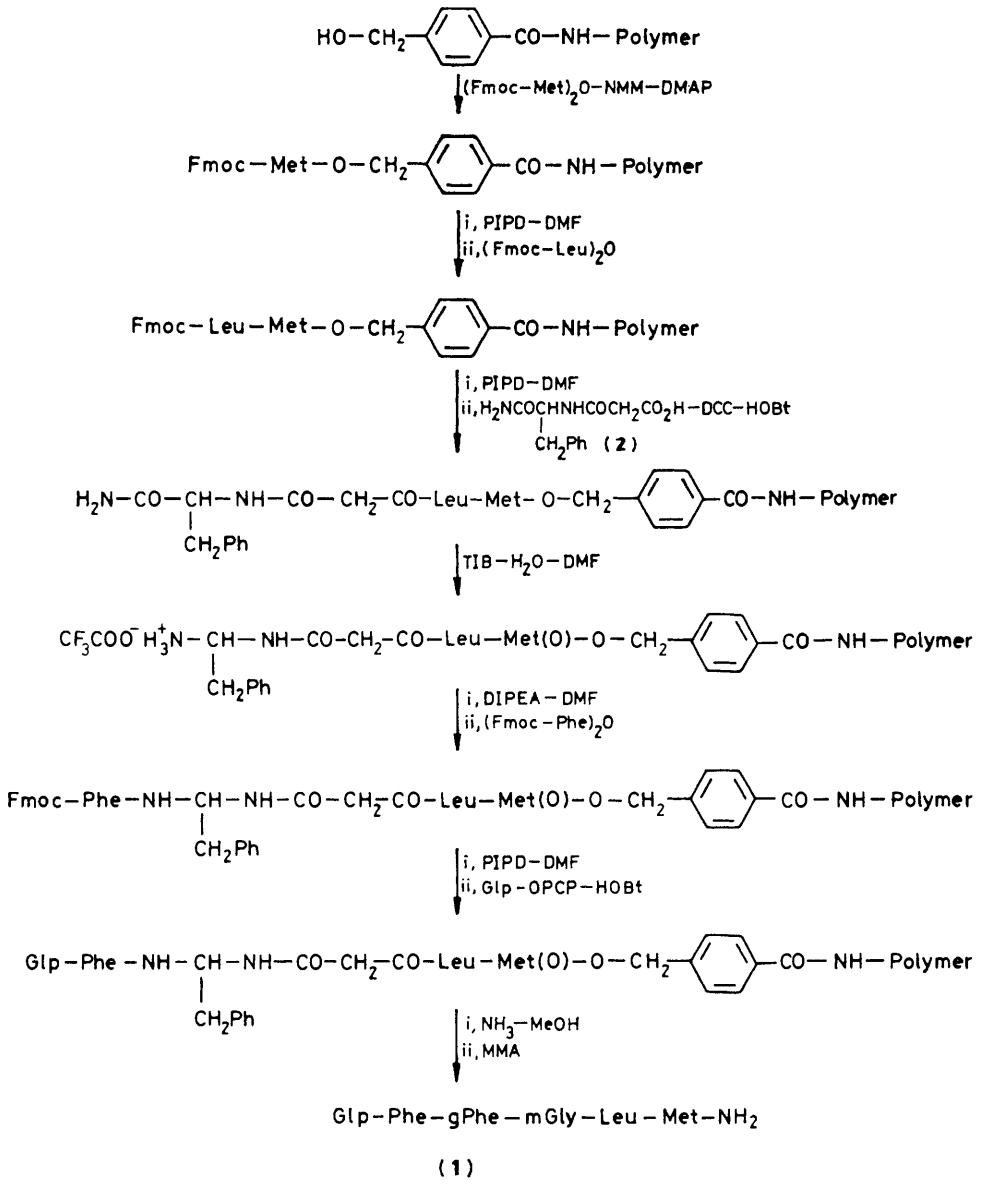
The complete solid phase synthesis of a partially modified retro-inverso analogue of Substance P, [Glp^6, gPhe^8, mGly^9]SP$_{6-11}$, [gPhe = –NHCH(CH$_2$Ph)NH–, mGly = –OCCH$_2$CO–] has been performed using a polydimethylacrylamide-based solid support and [bis(trifluoroacetoxy)iodo]benzene.

Retro-inverso modification of peptides involves the reversal of the direction of peptide bonds in the backbone with the aim of maintaining the topology of the side-chains.[1] This approach has been applied to both cyclic and linear peptide hormones to produce active analogues of the parent peptide with enhanced stability to enzymatic degradation.[2,3]

In the case of partially modified retro-inverso analogues, the reversal of a single peptide bond is accomplished by replacement of two adjacent amino-acid residues with a *gem*-diamino-alkyl residue and a malonyl or a 2-substituted malonyl residue.[3] The main limitation to the study of these analogues lies in the synthetic manipulations necessary for their preparation, particularly for the incorporation of the *gem*-diamino-alkyl residue into the peptide backbone. Recently, the use of [bis(trifluoroacetoxy)iodo]benzene (TIB)[4] has made such syntheses considerably easier.[5,6]

We have made use of TIB to develop a novel synthesis which enables us to extend to the synthesis of partially



Scheme 1. Solid phase synthesis of [Glp^6, gPhe^8, mGly^9]SP_{6-11} [gPhe = $-NHCH(CH_2Ph)NH-$, mGly = $-OCCH_2CO-$]. DMF = *N*,*N*-dimethylformamide; HOBt = *N*-hydroxybenzotriazole; NMM = *N*-methylmorpholine; DMAP = *p*-dimethylaminopyridine; DCC = dicyclohexylcarbodi-imide; DIPEA = *N*,*N*-di-isopropylethylamine; OPCP = pentachlorophenyl ester; Fmoc = fluoren-9-ylmethoxycarbonyl; PIPD = piperidine; MMA = *N*-methylmercaptoacetamide.

modified retro-inverso peptides the ease of manipulations inherent in solid phase methodology. The retro-inversion of a single peptide bond is achieved by coupling malonyl- or 2-substituted malonyl-D-amino-acid amides to the growing peptide chains, and subsequent conversion of the resin-bound amides into amines by TIB; the chains are then elongated as usual (see Scheme 1).

Since the TIB reaction must be performed in mixed aqueous organic media (typically H_2O–DMF or H_2O–MeCN),[4] the choice of a polar solid support which could be freely permeated and solvated by these media was mandatory. The polyamide resin and the method developed by Sheppard and colleagues[7] were most suitable for our purpose, enabling us to perform all the solid phase reactions in H_2O–DMF and DMF with fourfold excesses of acylating species or TIB.

The analogue [Glp^6, gPhe^8, mGly^9]SP_{6-11} (**1**), which was found to be a full agonist of Substance P and highly resistant to proteolytic degradation,[8] had already been synthesized by us in solution,[9] and thus represented a good standard to test the validity of the proposed strategy.

The esterification of $(Fmoc\text{-}Met)_2O$, attaching it to the resin, was accomplished under the conditions described in ref. 10. After a cycle of deprotection–addition–deprotection for $(Fmoc\text{-}Leu)_2O$, malonyl-D-phenylalanine amide (**2**) was added in the presence of 1 equiv. of DCC plus 1 equiv. of HOBt, with overnight shaking. Loss of peptide (*ca.* 20%) occurred at this level, as shown by a decrease in the Leu/Nle ratio, probably owing to Leu-Met diketopiperazine formation. After a series of washes with DMF–H_2O (3:1 v/v) to equilibrate the resin in the mixed aqueous–organic medium, TIB was added in DMF for an initial cycle of 1 h followed by an overnight cycle, with intermediate DMF–H_2O washes. The resin was re-equilibrated in DMF, the trifluoroacetate eliminated with DIPEA–DMF (10:90 v/v), and two more acylation cycles were performed first with $(Fmoc\text{-}Phe)_2O$ and then with Glp-OPCP plus 1 equiv. of HOBt.

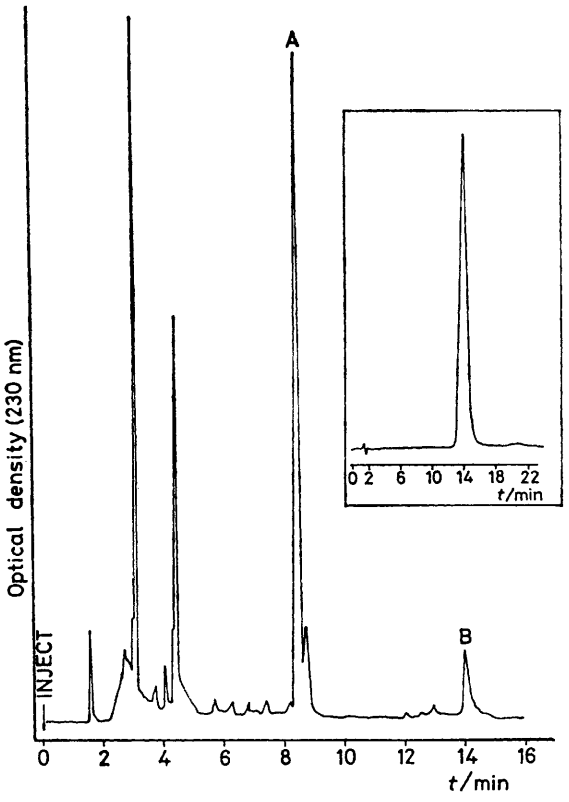


Figure 1. H.p.l.c. of crude mixture before reduction. Peak B is [Glp^6, gPhe^8, mGly^9] SP_{6-11}; peak A is the same peptide oxidized at the methionine residue. Inset: purified final product. Analytical conditions: 27% MeCN in H_2O; flow rate 1 ml min^{-1}, Lichrosorb RP-18 (5 μm) column.

The peptide was detached from the support by ammonolysis in saturated methanolic ammonia for 3 h. Residual resin analysis indicated removal of 97% of peptide.

H.p.l.c. at this stage indicated a main peak and a series of additional peaks, one corresponding to the standard synthesized in solution (see Figure 1). The main peak corresponded to the desired peptide with the methionine residue oxidized to methionine sulphoxide, as confirmed by H_2O_2 oxidation of the standard.[11] This was consistent with the known oxidative properties of TIB and with the amino-acid analysis (Leu 1.00; Phe, 0.77; Glu, 0.61; Met, 0.47).

The crude mixture was quantitatively reduced with *N*-methylmercaptoacetamide[12] and purified by preparative h.p.l.c. to yield the desired peptide (found: Leu, 1.00; Phe, 1.00; Glu, 1.04; Met, 0.93) which was identical (on h.p.l.c. and t.l.c.) with the standard.

The simplicity of the method is noteworthy and even greater than that recently verified in the synthesis of retro-inverso peptide analogues in solution.[4,9,13] It does not require manipulations different from those usually performed in the solid phase synthesis of peptides, apart from preparing the appropriate malonyl derivatives of D-amino-acid amides.

The effect of the TIB on the side-chains of sensitive amino-acid residues other than methionine under the mild conditions of our synthesis is not yet known.

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