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A FLUORINE-19 NMR APPROACH FOR STUDYING MERRIFIELD SOLID-PHASE PEPTIDE SYNTHESES

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The ^{19}F NMR spectra of fluorobenzyloxycarbonyl and 2-(4-fluorophenyl)-2-propyloxycarbonyl amino acids coupled to 1% cross-linked polystyrene by Merrifield solid-phase peptide synthesis techniques are relatively narrow and offer the potential for studying and monitoring the latter syntheses.

Based on ^{13}C NMR results for solvent swollen, cross-linked polystyrene resins[1], we felt it should be possible to observe the NMR signals from ^{19}F nuclei incorporated in amino-protecting groups of amino acid residues attached to such resins by using Merrifield solid-phase peptide synthesis (SPPS) methodology[2]. To investigate this possibility, we have synthesized some fluoroaryloxycarbonyl amino acid intermediates. Initially, fluorobenzyloxycarbonyl amino acids were prepared from basic aqueous-tetrahydrofuran solutions of amino acids in yields of 40-85% by treatment with the corresponding fluorobenzylchlorocarbonates (obtained by phosgene treatment of the corresponding alcohols[3]). These amino acid derivatives exhibit singlet proton-decoupled ^{19}F NMR spectra characteristic of the fluorine position on the ring, i.e., the 2-isomers fall 470-480 Hz upfield, the 3-isomers 52-65 Hz downfield and the 4-isomers 20-35 Hz upfield from internal 1% C_6H_5F in $CHCl_3$ at 94.1 MHz[4]. These intermediates, upon coupling onto 1% cross-linked, chloromethylated polystyrene beads via the cesium salts[5], exhibit relatively narrow ^{19}F NMR signals as illustrated in Figure 1. The internal standard C_6H_5F is seen to be a doublet as is also the C_6F_6 used for locking. The lower field signal has been identified, by varying the polymer bead concentration seen by the probe receiver coil, as due to those molecules in the solvent outside the polymer beads while the higher field signal must be due to the molecules diffusing in the resin beads. The following ^{19}F chemical shifts (in Hz relative to dissolved C_6H_5F) and line widths (in Hz) for these amino acid-resin systems have been observed: 2-fluoroderivatives: Gly-resin, -481, 26; Ala-resin, -477, 30; Phe-resin, -477, 39; Pro-resin, -478, 30; 4-fluoro derivatives: Ala-resin, -72, 30; Phe-resin, -73, 35. Several peptides with terminal 2-fluorobenzyloxycarbonyl amino acid residues have been prepared by SPPS. These exhibit the following chemical shifts and usually somewhat narrower fluorine-19 NMR signals[6] as illustrated in Figure 1: GlyAla-resin, -476, 17; PheAla-resin, -484, 16; GlyPhe-resin, -496, 34; GlyAlaPhe-resin, -473, 17; PheAlaGly-resin, -479, 22; AlaGlyPhe-resin, -472, 18; AlaPheGly-resin, -475, 20.

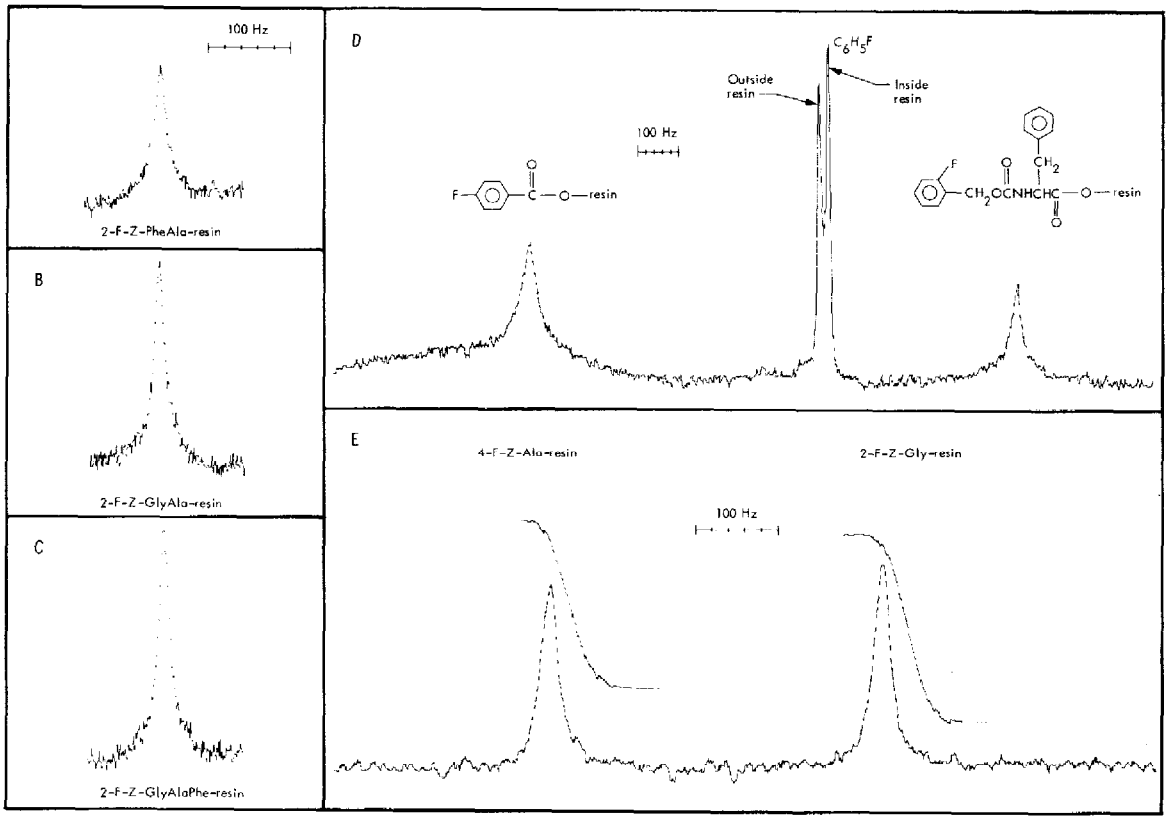

Figure 1. Proton decoupled ^{19}F 94.1 MHz NMR spectra of amino acid derivatives and peptides bonded to 1% cross-linked polystyrene (starting resin "Bio-Beads," S-X-1, 200-400 mesh, 1.25 meq./gm CH_2Cl-); 5 mm O.D. sample tubes of slurries of swelled resin; $CHCl_3$ solvent 0.5% C_6H_5F and 5% C_6F_6; for A, B, C, and E, 25 digital, 520 sec. sweeps of 1000 Hz; for D, 50 sweeps of 50 sec. of 2000 Hz; chemical shifts in Hz from C_6H_5F: A. 2-F-Z-PheAla-resin, -476 Hz; B. 2-F-Z-GlyAla-resin, -484 Hz; C. 2-F-Z-GlyAlaPhe-resin, -473 Hz; D. 2-F-Z-Phe-resin, -477 Hz, 4-fluorobenzoic acid-resin on same resin, + 704 Hz; E. Spectrum of mixture of 27.0 mg 2-F-Z-Gly-resin beads (0.98 meq./gm) with 22.5 mg 4-F-Z-Ala-resin beads (0.91 meq./gm); integrations from sixteen 20 sec. sweeps.

The acid lability of the fluorobenzyloxycarbonyl (F-Z) amino acids was expected to be insufficient[7-9] to be ultimately useful for SPPS's so syntheses of several 1-(4-fluorophenyl)-ethoxy- (of Gly and Ala) and 2-(4-fluorophenyl)-2-propyloxycarbonyl (4-F-Ppoc) amino acids were accomplished. Treatment of Triton B syrups of the various amino acids[10] by the corresponding alcohol (4-methoxycarbonylphenyl) carbonates[11] afforded these derivatives (in 30-70% yields). They exhibit much more rapid acid catalyzed deprotection rates[4] and, in the case of the 4-F-Ppoc derivatives, substantially greater spread of ^{19}F NMR chemical shifts than the corresponding 4-F-Z derivatives (for example, a range of -258 to -410 Hz vs -20 to -35 Hz): Gly, -270; Ala, -347; Phe, -347; Pro, -381, -410; Val, -374; Asp, 321; Lys, -258, -260 (bis). Two of the 4-F-Ppoc intermediates have been bonded to resin beads and these resin-bound fluorine-19 nuclei in both cases exhibit usefully narrow NMR signals: Gly-resin, -190.2, 30 Hz wide; Phe-resin, -297, 32 Hz wide.

Internal concentration references are easily derived by several approaches so that the observations and intermediates discussed above may be utilized to follow SPPS's. Two possibilities are illustrated in Figure 1. A ^{19}F NMR probe with a chemical shift different from that of the ^{19}F in the protecting group and inert to SPPS conditions may be coupled to a portion of the resin functional groups (Figure 1D). Alternately, resin beads possessing an inert ^{19}F NMR probe may be added to the resin beads on which the peptide will be synthesized (Figure 1E). Sensitivity appears not to be a problem because, with the 5 mm NMR sample tubes and less than optimal probe (it is tuned for ^{19}F and ^{1}H and estimated to be less sensitive than a normal probe by a factor of 10) employed in the present work, it is estimated that loadings of 0.005 meq./gm or less can be measured.

These NMR observations offer a new approach for studying steps in SPPS's that proceed with difficulty[12], the diffusion and concentrations of reagents in the resin, the effects of solvents, such as trifluoroethanol, on coupling[13], the optical purity of intermediates[14] and protected amino acid residues coupled to resins (by use of optically active solvents and shift reagents)[15], and monitoring coupling and deblocking in a nondestructive manner[16]. Work to utilize this approach in syntheses of longer peptide sequences is in progress.

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