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Esterification Of 4-Alkoxybenzyl Alcohol Resin With FMOC-Histidine(N^{im}-Trityl)-N-Carboxyanhydride

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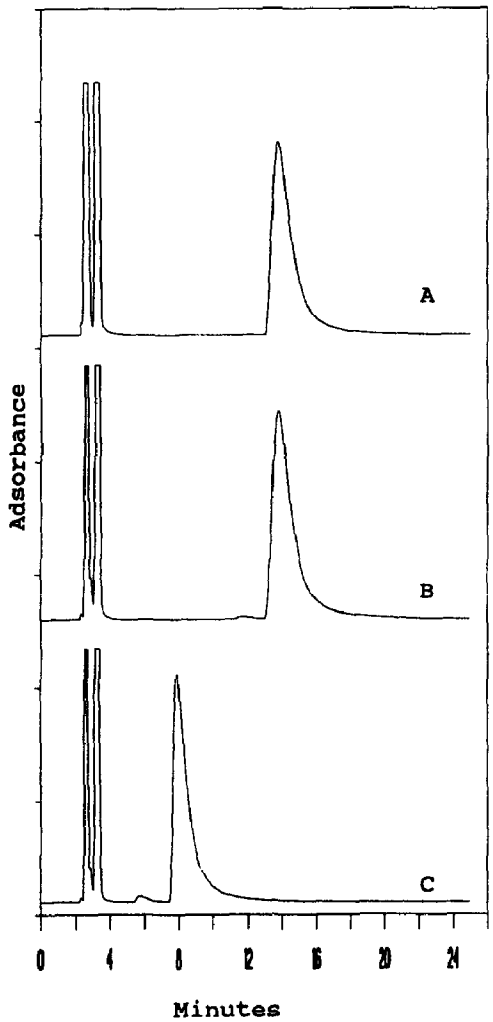
Abstract: Esterification of 4-Alkoxybenzyl alcohol resin (Wang resin) is accomplished rapidly in high yield and with no detectable racemization when FMOC-histidine(N^{im}-trityl)-NCA is the condensing reagent.

Since its introduction in 1973[1] 4-alkoxybenzyl alcohol resin (Wang resin) has found widespread use in solid phase peptide synthesis in conjunction with the N-α-FMOC protection strategy. Wang et al[2] esterified the first FMOC-amino acid to the resin using DCC with DMAP as catalyst. Subsequently this esterification procedure was shown to proceed with significant amounts of racemization[3] and dipeptide formation.[4] Several authors have reported alternative esterification procedures using PPA,[5] dichlorobenzoyl chloride,[5] DIPCDI/DMAP,[6] CIP,[6] BOI[6] and PyBrop[6] as condensing reagents. With careful control of the reaction conditions some of these procedures allow for the esterification of most FMOC-amino acid to the Wang resin with minimal racemization while obtaining high levels of substitution.

One remaining unsolved problem has been the esterification of FMOC-histidine(N^{im}-trityl) to the Wang resin, where the DCC/DMAP[5] procedure afforded 15.4% of the D-enantiomer. The other procedures offered no advantages, with dichlorobenzoyl chloride[5] producing 27%, CIP[6] 39%, BOI[6] 20%, DIPCDI/DMAP[6] 36% and PyBrop[6] 43% of the D-enantiomer, respectively. Such high levels of racemization render FMOC-histidine(N^{im}-trityl)-O-Wang resin essentially useless as a starting point for peptide synthesis.

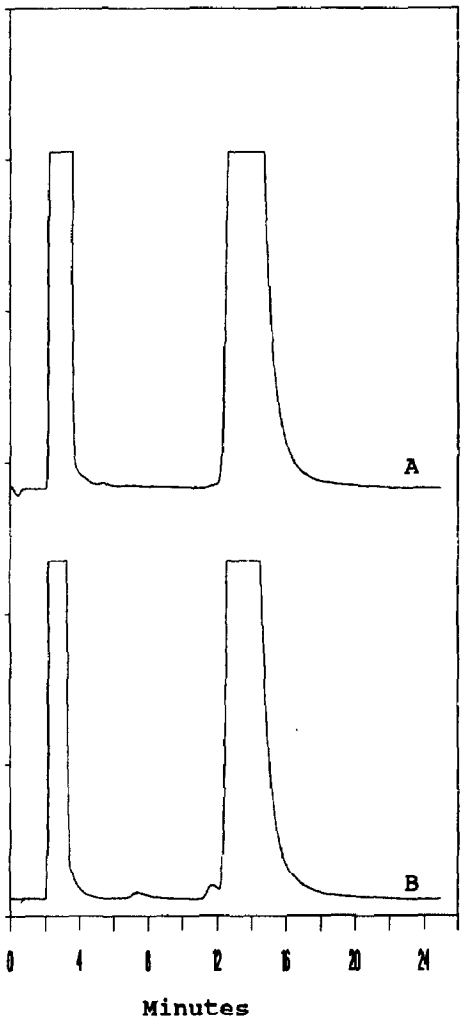
Several years ago our group introduced urethane-protected amino acid-N-carboxyanhydrides (UNCAs),[7] a new class of preactivated urethane protected amino acids. The application of UNCAs in peptide synthesis in solution, on solid phase, and for resin attachment has established them as

FIGURE 1



A: FMOC-His from Wang resin esterified with FMOC-His(N^{im}-trityl)→NCA (Exp 1)
B: FMOC-His (standard)
C: FMOC-D-His (standard)

FIGURE 2



A: FMOC-His from Wang resin esterified with FMOC-His(N^{im}-trityl)-NCA (Exp 2)
B: FMOC-His containing 0.3% FMOC-D-His (standards)

efficient reagents which condense with amines and alcohols in high yield with little or no racemization.[7,8]

We now wish to report the racemization-free esterification of the Wang resin with FMOC-histidine(N^{im}-trityl)-NCA.[9] In a typical experiment, the Wang resin[10] (0.5 g, with a substitution[11] of 0.56 mmol of -OH/g), FMOC-histidine(N^{im}-trityl)-NCA (0.87 g, 5.0 eq) and NMM (6 μl, 0.2 eq) in toluene (10 mL) were shaken for 2h at room temperature. The resin was then collected by vacuum filtration and washed successively with toluene (50 mL), DCM (2 x 50 mL), THF (50 mL) and dried *in vacuo* to give FMOC-histidine(N^{im}-trityl)-O-Wang resin with a substitution[11] of 0.30 mmol/g which represents a 71% yield based on the starting hydroxyl groups on the resin. In a second experiment a substitution of 0.42 mmol/g (100% yield) was obtained using 5.0 eq of UNCA, 1.0 eq of NMM and a 12h reaction time.

A portion of the FMOC-histidine(N^{im}-trityl)-O-Wang resin from each of the two above experiments was treated with 95% aq. TFA for 3h in order to cleave both the resin and the trityl group. The resin was removed by vacuum filtration and the TFA solution subjected directly, without evaporation or manipulation, to HPLC on a chiral column[12] (Phenomenex, Chirocel, OD, 250 mm x 4.7 mm). **Figure 1A** depicts the HPLC chromatogram of the FMOC-histidine obtained from the lower substitution resin (0.30 mmol/g) and **Figure 2A** depicts a heavily overloaded chromatogram of FMOC-histidine obtained from the higher substitution resin (0.42 mmol/g). Comparison with HPLCs of FMOC-histidine[13] **(Figure 1B)**, FMOC-D-Histidine[13] **(Figure 1C)** and a mixture consisting of 99.7% FMOC-histidine and 0.3% FMOC-D-histidine **(Figure 2B)** conclusively demonstrates that there is no detectable racemization (<0.3%) in the FMOC-histidine liberated from Wang resin which had been esterified with FMOC-histidine(N^{im}-trityl)-NCA.

The data presented above show that the UNCA procedure for the esterification of Wang resin easily provides FMOC-histidine(N^{im}-trityl)-O-Wang resin with a high substitution and outstanding optical purity. This resin would make an ideal starting material for a peptide containing a C-terminal histidine.

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9. FMOC-histidine(N^{im}-trityl)-NCA is now available from Propeptide, France.
10. Wang resin was purchased from Novabiochem, U.S.A.
11. The -OH substitution of the Wang resin was determined by esterification with FMOC-leucine and DIPCDI/DMAP (double coupling, 20 eq/coupling) under conditions reported[6] to give 100% coupling with 2.5 eq of FMOC-leucine and back calculation from the FMOC substitution. The FMOC substitution level of the resins was measured by UV determination of the 9-fluorenylmthylpiperidine adduct after cleavage of the FMOC group from the FMOC-amino acid-O-Wang resin with 20% piperidine in DMF for 60 min.
12. HPLC condition: isocratic, 25 min., hexane/2-propanol/TFA(79.99/19.96/0.05), 1.4 ml/min, UV detection at 215 nm.
13. Both FMOC-histidine and FMOC-D-histidine were obtained by treatment of commercial samples of FMOC-histidine(N^{im}-trityl) and FMOC-D-histidine(N^{im}-trityl) with 95% aq. TFA, respectively, for 3h. Each was subjected directly to HPLC without purification.

ABBREVIATIONS: BOI = 2-(benzotriazol-1-yl)oxy-1,3-dimethyl-imidazolidinium-hexafluorophosphate. ByBrop = bromo tripyrrolidino phosphoniumhexafluorophosphate. CIP = 2-chloro-1,3-dimethylimidazolidinium hexafluorophasphate. DCC = dicyclohexylcarbodiimide. DIPCDI = diisopropylcarbodiimide. DMAP = 4-dimethylaminopyridine. NMM = N-methylmorpholine. PPA = n-propylphosphonic anhydride. TFA = trifluoroacetic acid.

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