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HYDROGENATION OF PROTECTED LEUCINE ENKEPHALIN FROM A RESIN DURING SOLID PHASE SYNTHESIS

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In the preceding paper,[1] a description of the removal of a peptide esterified to a polystyrene resin by means of catalytic hydrogenation was presented. This method is unique in the literature concerning solid phase peptide synthesis in that blocking groups derived from t-butyl alcohol are left intact and the mild conditions minimize the concern of racemization, which could be a problem in methods of peptide-resin cleavage utilizing basic reagents.

In order to further demonstrate the utility of this method, the synthesis of a biologically active compound was undertaken; specifically, one of the natural ligands for opiate receptors, leucine enkephalin (1a), as described recently by Hughes and co-workers.[2] Compound (1b) was

X-Tyr-Gly-Gly-Phe-Leu-OH

1a X=H

1b X=Boc[3]

removed from the resin in 88% yield by hydrogenolysis in DMF, catalyzed by palladium black generated *in situ* from palladium (II) acetate. After purification, the final yield of (1b) was 56%.

The synthesis was performed with the aid of a Schwartz-Mann peptide synthesizer. The carboxyl-terminal amino acid, Boc-Leu, was attached to the chloromethylated, 1% cross-linked resin by the method of Gisin.[4] The degree of substitution on the resin was found to be 0.90 meq/g by Kjeldahl nitrogen analysis and the total weight of Boc-Leu-O-Ⓡ was 14.6g. The scheme used for the preparation of Boc-Gly-Gly-Phe-Leu-O-Ⓡ is shown in the table.

TABLE

Step	Operation and Reagents	Time (min.)
1	Deblock-1; 40% TFA/CH_2Cl_2.	40
2	Washes; a. CH_2Cl_2 (3 times); b. i-PrOH (3 times); c. CH_2Cl_2 (5 times).	1-1½ each
3	Deblock-2; 40% TFA/CH_2Cl_2.	30
4	Washes; CH_2Cl_2 (5 times).	1-1½ each
5	Neutralization; 10% TEA/CH_2Cl_2 (4 times).	1-1½ each
6	Washes; a. CH_2Cl_2 (3 times); b. i-PrOH (3 times); c. CH_2Cl_2 (7 times).	1-1½ each
7	Coupling; 2 equivalents of Boc-amino acid per equivalent of resin-bound peptide, 1 equivalent of DCC, CH_2Cl_2, symmetrical anhydride method.	8 hr.
8	Washes; a. THF/CH_3OH (1:1) (3 times); b. CH_2Cl_2 (2 times); c. i-PrOH (3 times); d. CH_2Cl_2 (5 times).	5 for THF/CH_3OH 1-1½ each for others
9	Acetylation; 0.3 MAc_2O/CH_2Cl_2 (2 times).	5
10	Washes (as in Step 2).	-
11	Repeat Steps 1-9.	

Deblocking was performed twice, with rather extended times, to insure complete removal of the Boc group. Washes included a shrink-swell cycle with i-PrOH as suggested by Marshall.[5] The completion of each coupling step was determined with ninhydrin according to Kaiser and co-workers,[6] with acetylation of small amounts of free amino groups using acetic anhydride. Couplings were performed by means of the symmetrical anhydride,[7] generated in the reactor. After four hours, more DCC was added to regenerate additional anhydride. The final coupling utilized Boc-Tyr-OPP/HOBt in DMF as recently reported by Khan and Sivanandaiah,[8] although for 8 hours. Amino acid analysis of the resin-bound, Boc-pentapeptide gave the following results:

Leu-1.00; Phe-1.05; Gly-2.04; Tyr-0.77

Although tyrosine was low, an examination of the bead color and solution above the beads after treatment with ninhydrin indicated about 99+% completion.

The removal of Boc-Tyr-Gly-Gly-Phe-Leu-OH from the resin was accomplished by swelling the

resin in a minimum amount of DMF in which was dissolved $Pd(OAc)_2$ (2 equivalents per equivalent of peptide). After equilibration (about 2-2½ hours), the resin was placed on a Parr shaker and hydrogenated at 60 psi, 40° for 24 hours. Hydrogen uptake is initially very rapid as the $Pd(OAc)_2$ is reduced to Pd (black) in and around the beads. Total hydrogen uptake is about 20% over theory. The DMF is then removed from the resin-Pd (black) by filtration and the beads washed with DMF, then CH_2Cl_2. The weight of dried resin, minus the amount of Pd (black) formed indicated essentially all of the peptide had been removed. Kjeldahl nitrogen confirmed this, showing 0.09 meq/g of material remaining.

The DMF was distilled from the peptide at 40° under high vacuum. Trace amounts of DMF were then removed in a vacuum oven at 70° for 4 hours to give an 87.8% crude yield of protected leucine enkephalin.

The Boc-pentapeptide was subjected to 600 transfers of countercurrent distribution (CCD) using the system CCl_4:$CHCl_3$:CH_3OH:H_2O (27:26:37:10), the fractions containing desired compound being monitored by thin layer chromatography on Silica-Gel GF, $CHCl_3$:CH_3OH:HOAc:H_2O-83:15:1:1 (CMAW), Rf 0.42 and K=2. Evaporation of the solvents afforded 4.82g (56.4% yield) of N-t-butoxycarbonyl leucine enkephalin. Anal. Calcd for $C_{33}H_{45}N_5O_9 \cdot 2\ H_2O$: C, 57.29; H, 7.14; N, 10.12. Found: C, 57.36; H, 6.54; N, 10.44.

Deblocking of the protected pentapeptide was performed by treating 1.36g of 1b for 15 min. at rt with HCl in dioxane, evaporating the solvent at reduced pressure and rubbing the resulting gum with ether. The solid product was dried in vacuo at 60° for 3 hours to give 1.22g of 1a as a HCl · 3½ H_2O; 96.5% yield, TLC (CMAW)-R_f 0.35, homogeneous; $[\alpha]_D^{25}$ + 20° (c 1, MeOH). Anal. Calcd for $C_{28}H_{37}N_5O_7 \cdot HCl \cdot 3\frac{1}{2}\ H_2O$: C, 51.33; H, 6.92; N, 10.69; Cl, 5.41. Found: C, 51.00; H, 6.17; N, 10.31; Cl, 5.49. Amino acid analysis gave Tyr-0.96, Gly-2.00; Phe-1.02; Leu-1.02.

Compound 1a was tested for receptor binding activity as described by Pert and Snyder[9] in the presence and absence of Na ion to give IC_{50} values of 1.6×10^{-6}M and 3×10^{-7} respectively, which are in reasonable agreement with published[10] literature values.

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