Phase-transfer Reagents as *C*-Terminal Protecting Groups; Facile Incorporation of Free Amino Acids or Peptides into Peptide Sequences

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Phase-transfer reagents (basic, neutral, and acidic) can effect temporary protection of carboxy groups by salt formation in *C*-terminal free amino acids or peptides during peptide synthesis; the use of acidic or neutral phase-transfer reagents as the *C*-terminal protecting group will not affect the nucleophilicity of the amino group of the salts thus prepared in an organic solvent.

In peptide synthesis, protecting the carboxy group of one component and freeing its amino group to regenerate the nucleophilicity of the nitrogen atom by the addition of a tertiary amine is common.[1] We have found that phase-transfer reagents can be used as *C*-terminal protecting groups of amino acids and peptides for peptide synthesis. The solubilization of an amino acid with a basic phase-transfer reagent in organic solvents has been reported recently.[2] We have found that, on using neutral or acidic phase-transfer reagents as *C*-terminal protecting groups, the salt prepared from amino acids or peptides can dissolve in organic solvents and the nucleophilicity of the amino group can be maintained during synthesis. The risk of racemization caused by the amino acids residue with basic phase-transfer reagents under the reaction conditions is not clear;[2—4] however, the use of phase-transfer reagents under neutral conditions is one of the best ways to avoid the side reaction.

Figure 1 shows the pH difference of Ala and Gly-Gly dissolved separately with three phase-transfer reagents, tetrabutylammonium hydroxide, benzyltrimethylammonium chloride, and tetrabutylammonium hydrogen sulphate in water (10 mmol/100 ml). The pH of each tested solution changed little when the solution contained less or more than one equivalent of the phase-transfer reagent and the pH values of both Ala and Gly-Gly salts in aqueous solution are comparable. Peptide synthesis was performed *via* the active ester method, generated *in situ*, using salts of amino acids or peptides as nucleophiles (see Scheme 1)[5] which were prepared by dissolving the amino acids or peptides with one equivalent of phase-transfer reagent in water (10 mmol/100 ml) and lyophilized to afford a white powder. In a typical reaction, the *N*-protected amino acid (10 mmol) in chloroform (20 ml) was added to dicyclohexylcarbodiimide (DCC; 10 mmol) and 1-hydroxybenzotriazole (11 mmol). The mixture was stirred

for 5—6 h at room temperature, and then filtered to remove dicyclohexylurea. The filtrate was added to a solution of the prepared nucleophile (15 mmol) dissolved in chloroform (20 ml) and stirred at room temperature for 3 h. The resultant mixture was diluted with ethyl acetate (300 ml), then washed with 0.1 M HCl (4 × 50 ml), water (4 × 30 ml), and dried (Na_2SO_4). Concentration gave the crude product, which was purified either by recrystallization from n-hexane–ethyl acetate or by silica gel flash column chromatography.

The results are shown in Table 1. The yields are about 65—85% and the use of the dipeptide salt as the nucleophile gave a higher yield than the use of the amino acid salt. Unusual amino acids such as β-alanine and ε-aminohexanoic acid can be incorporated into peptides by this procedure. No racemization was detected as measured by chiral GC analysis of the *N*-pentafluoropropionyl amino acid isopropyl ester of the peptide hydrolysates.[6] Because the *C*-terminal protecting group was removed when the product was extracted, this synthetic procedure not only saved the time otherwise needed for deprotection, but also side reactions caused by the acid or base catalysed deprotection could be prevented. The solubility of the nucleophile salt in the reaction mixture is critical for high yields; use of poor solvents such as dimethylformamide, tetrahydrofuran, or dioxane resulted in a low yield, while use of halogenated solvents such as chloroform or methylene chloride in which the nucleophile salt dissolved completely led to high yields. Many biologically active small peptides have been synthesized by this procedure.[7]

The synthesis of enkaphalin illustrates the feasibility of this approach for polypeptide synthesis. [Leu^5]Enkaphalin was synthesized *via* the coupling of Boc-Tyr(Bzl)-OH with Gly-Gly salt followed by reaction of Boc-Tyr(Bzl)-Gly-Gly-OH with Phe-Leu salt as in Scheme 2. In these syntheses each of the couplings involves 1.5 equivalents of the nucleophile component. The yields given are the isolated yields of the corresponding coupling.

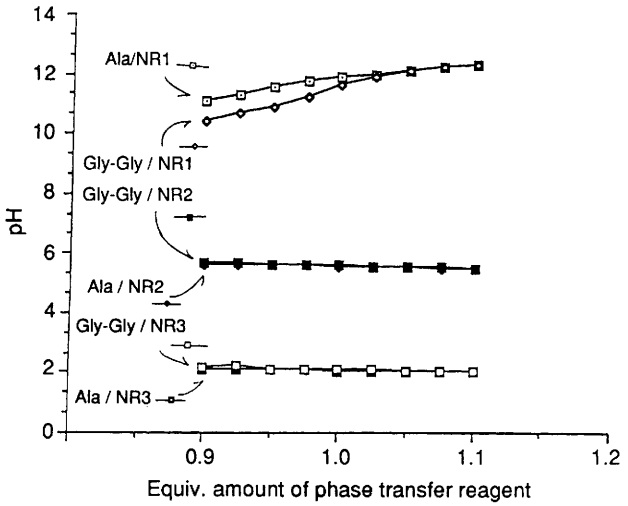
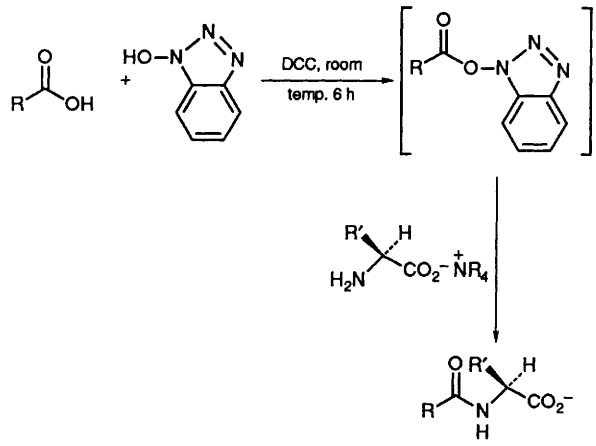


Figure 1. pH values of Ala and Gly-Gly dissolved separately with three different phase-transfer reagents in aqueous solution. (Ala/NR1: alanine Bu_4NOH salt; Gly-Gly/NR1: glycyl-glycine Bu_4NOH salt; Ala/NR2: alanine $PhCH_2NMe_3Cl$ salt; Gly-Gly/NR2: glycyl-glycine $PhCH_2NMe_3Cl$ salt; Ala/NR3: alanine Bu_4NHSO_4 salt; Gly-Gly/NR3: glyclglycine Bu_4NHSO_4 salt).

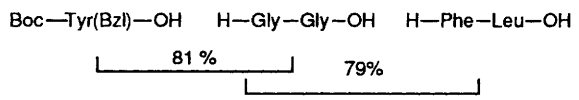


Scheme 1

Table 1. Synthesis of *C*-terminal-free peptides and their physical properties.

Reactant	Nucleophile[a]	Product	% Yield	$[\alpha]_d^{25}$/°[d]	M.p., *T*/°C	Amino acid analysis
Moz[e]-Val	Phe/$^+$NR2	Moz-Val-Phe	72	−10.83	174—176	Val : Phe; 0.97 : 1.00
Boc-Met	Leu/$^+$NR2	Boc-Met-Leu	70	−32.75	127—129	Met : Leu; 0.95 : 1.00
Boc-Thr	Leu/$^+$NR2	Boc-Thr-Leu	71	−5.52	144—145	Thr : Leu; 0.83 : 1.00
Fmoc-Ala	Gly/$^+$NR2	Fmoc-Ala-Gly	78	−18.34	152—154	Ala : Gly; 1.00 : 0.98
Fmoc-Phe	Thr/$^+$NR2	Fmoc-Phe-Thr	70	−8.47	158—160	Phe : Thr; 1.00 : 0.81
Boc-Ala	Ala-Ala/$^+$NR1	Boc-Ala-Ala-Ala	77	−44.40	183—184	—
Boc-Ala	Ala-Ala/$^+$NR2	Boc-Ala-Ala-Ala	79	−44.44	183—184	—
Boc-Ala	Ala-Ala/$^+$NR3	Boc-Ala-Ala-Ala	55	−44.44	183—184	—
Boc-Met	Leu-Phe/$^+$NR2	Boc-Met-Leu-Phe[b]	82	−28.53	131—133	Met : Leu : Phe; 0.92 : 1.00 : 1.02
Moz-Thr	Val-Leu/$^+$NR2	Moz-Thr-Val-Leu[b]	84	−48.18	170—172	Thr : Val : Leu; 0.82 : 0.96 : 1.00
Cbz-D-Phe	Phe-Gly/$^+$NR2	Cbz-D-Phe-Phe-Gly[b]	77	−3.49	149—151	Phe : Gly; 2.00 : 0.99
Boc-Pro	D-Leu/$^+$NR2	Boc-Pro-D-Leu[b]	67	−14.49	98—100	Pro : Leu; 1.05 : 1.00
Boc-Phe	Ahe[a]/$^+$NR2	Boc-Phe-Ahe	65	+1.59[d]	140—142	Phe : Aha; 1.00 : 0.99
Boc_2-Orn	β-Ala[c]/$^+$NR2	Boc_2-Orn-β-Ala[b]	71	−4.07	134—136[c]	Orn : β-Ala; 1.00 : 0.97

[a] Abbreviations: $^+$NR1: tetrabutylammonium hydroxide; $^+$NR2: Benzyltrimethylammonium chloride; $^+$NR3: tetrabutylammonium hydrogen sulphate; Boc_2-Orn: α,ε-di-t-butoxycarbonylornithine; Ahe: ε-aminohexanoic acid; β-Ala: β-alanine. [b] Boc-Met-Leu-Phe is a chemotactic peptide antagonist, ref. 7a.; Cbz-D-Phe-Phe-Gly is a virus replication inhibiting peptide, ref. 7b.; Boc-Pro-D-Leu is a morphine tolerance peptide, ref. 7c.; Moz-Thr-Val-Leu is a schizophrenia related peptide, ref. 7d.; Boc_2-Orn-β-Ala is a salting related peptide, ref. 7e. [c] Dicyclohexylamine salt. [d] Optical rotations were measured in methanol (*c* 5), except for the penultimate entry (Boc-Phe), for which dimethylformamide was used (*c* 5); [e] Moz = methoxybenzyloxycarbonyl.



Scheme 2

This procedure provided a new route for the synthesis of bifunctional compounds which contained amino and carboxy groups. That the amino group of the phase-transfer reagent salt in the organic solvent behaves as $^{+}NH_3R$ or $:NH_2R$ is interesting. The experimental results support the conclusion that the salt behaves as $:NH_2R$ rather than $^{+}NH_3R$ or at least that there is a sufficient concentration of the free amine to act as a nucleophile under these conditions. A similar result using Leu-OMe as a nucleophile at pH 3.0 in aqueous solution was reported recently.[8]

Support for this research provided by the National Science Council of the ROC is gratefully acknowledged.

Received, 3rd April 1990; Com. 0/01486I

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