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# Solid-phase synthesis of C-terminal peptide amides from *N*-tetrachlorophthaloyl protected amino acids†

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**Abstract**—A new strategy for solid-phase synthesis of C-terminal peptide amides based on the use of *N*-tetrachlorophthaloyl protected amino acids with acid-labile side-chain protection is described. 

The phthalimido (Phth) function is a widely used primary amine protecting group,[3] which has been also employed in peptide synthesis.[4] Its deprotection is most commonly effected with hydrazine hydrate in ethanol,[4a–d] but other reagents used include hydroxylamine,[4f] sodium borohydride,[5] hydrazine acetate,[6] butylamine in methanol[7] and ethylene diamine in butanol.[8] This process frequently requires high temperatures and extended reaction times. Probably due to such removal conditions only a few examples of solid-phase application of the Phth group have been described. The solid-phase synthesis of oligoureas has been reported using the phthaloyl moiety as the temporary amino protecting group, and its removal is carried out with 60% hydrazine in DMF for 1–3 h at 25°C.[9] Recently, the Phth group has been used in the construction of libraries of β-peptide conjugates of *N*-2-alkyl-1,2,3,4-tetrahydroisoquinolines on a solid support, using 2 M hydrazine hydrate in DMF: dioxane (1:2) for 3 h at 55°C for its deprotection.[10]

To overcome the harsh deprotection conditions required for Phth removal, several phthalimido analogs containing electron-withdrawing substituents have been reported.[11] Amongst them, the most widely used is the tetrachlorophthaloyl (TCP) protecting group (Fig. 1).

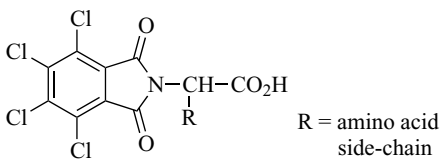


**Figure 1.** *N*-TCP protected amino acid.

Though its removal conditions are milder than those required for Phth deprotection, its use has been mostly limited to the synthesis of aminosugars.[12] The TCP group exhibits stability toward mildly basic to harshly acidic conditions, and proved to be unaffected by excess piperidine treatments.[11] Thus, TCP is compatible with Fmoc, which is readily removed with piperidine. These observations prompted us to study the application of TCP as a temporary amino protecting group in solid-phase peptide synthesis.

We report here the solid-phase synthesis of C-terminal peptide amides from *N*-TCP protected amino acids with acid-labile side-chain protection. *N*-TCP protected amino acids were prepared as described elsewhere[1] and their stereochemical purity was assessed by chiral HPLC on a Chiral-AGP column. Regarding to the deprotection step, and after several trials, we came to the conclusion that *N*-TCP protected amino acids linked to a solid support could be deprotected without marked appearance of side products with hydrazine:DMF (3:17), at 40°C for 30 min to 1 h. Next, we studied the racemization during the activation step. TCP-Val-OH (3 equiv.) was coupled onto a PAL-PEG-PS resin using a variety of reagent/additive protocols (6 h, at 25°C). TCP removal and further TFA treatment afforded the corresponding H-Val-$NH_2$ samples, which were derivatized with Marfey's reagent[13] and the crude products analyzed by HPLC. Best results were achieved with DIPCDI:HOAt (3:3) as coupling reagents leading to 1.7% of racemization (ratio of D:L). Substantial amounts of racemization were obtained with coupling protocols that require a tertiary amine for optimal

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efficiency. Thus, activation with HBTU:DIPEA (3:3) or (3:1) led to 8.4 and 6.1% of racemization, respectively.

Solid-phase synthesis of C-terminal peptide amides from *N*-TCP protected amino acids was performed onto an Fmoc-PAL-PEG-PS resin. Coupling steps were mediated by DIPCDI:HOAt (3:3) in DMF, 4 h at 25°C. Upon completion of coupling, ninhydrin test was negative.[14] Deprotection of the *N*-TCP protecting group was carried out in hydrazine:DMF (3:17), for 1 h at 40°C. Finally, an aliquot of the resin was directly cleaved (without TCP deprotection) with TFA–$H_2O$ (19:1) to afford the expected *N*-TCP protected C-terminal peptide amides with good purities, which were characterized by mass spectrometry (Table 1). Alternatively, fully deprotected compounds were obtained from treatment of the bulk of the resin with hydrazine:DMF (3:17) for 1 h at 40°C, prior to the cleavage of the anchoring linkage. The overall synthetic process is summarized in Scheme 1 for the synthesis of H-Gly-Gly-Asp-Ala-$NH_2$.

In order to compare TCP/*t*Bu and Fmoc/*t*Bu strategies we synthesized the C-terminal peptide amide Fmoc-Tyr-D-Ala-Phe-Phe-$NH_2$ using *N*-Fmoc protected amino acids. Starting from Fmoc-PAL-PEG-PS resin, couplings were performed as described above, and deprotections were carried out using standard piperidine treatments. After cleavage from the support, HPLC profiles of crude product of synthesis of the *N*-TCP (88% purity) and the *N*-Fmoc (92% purity) protected sequence did not show significant differences (Fig. 2).

In addition, we explored the applicability of the TCP/*t*Bu strategy to the synthesis of C-terminal peptide acids. TCP-Ala-Val-Gly-Ile-Gly-Ala-OH was synthesized as outlined previously for the corresponding peptide amide. Amino acid analysis of the hydrolyzed peptide-resin showed a reduced ratio 1:22.5 of Ala to Nle 'internal reference' amino acid.[15] Final acidolytic cleavage released the expected product in 80% purity by analytical HPLC but in very low yield (<10%) probably due to cleavage of the anchoring ester linkage through hydrazinolysis during repetitive TCP deprotections.

Interestingly, all *N*-TCP protected peptide amides showed $\lambda_{max}$ absorption at 334–336 nm allowing its selective detection, and may be useful for monitoring the reactivity of such protected compounds.

In summary, we have described a new SPPS strategy based on TCP/*t*Bu, which can be applied successfully to the synthesis of C-terminal peptide amides.

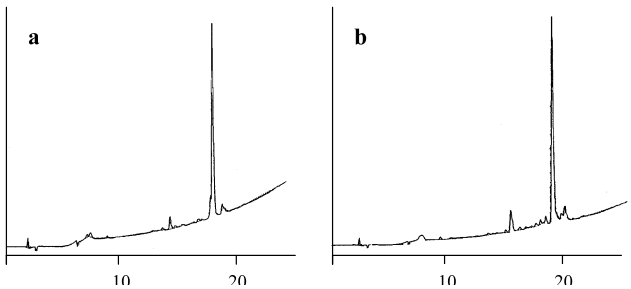
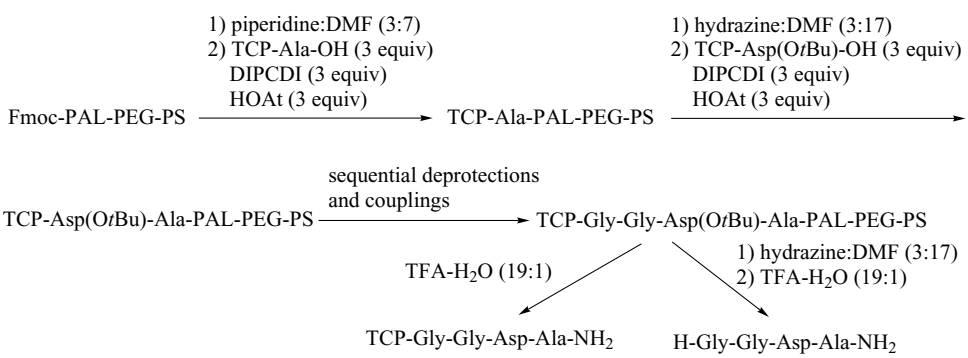



**Figure 2.** HPLC of crude from synthesis of: (a) Fmoc-Tyr-D-Ala-Phe-Phe-$NH_2$; (b) TCP-Tyr-D-Ala-Phe-Phe-$NH_2$. Ordinate is absorbance at 220 nm.

**Table 1.** Characterization of the *N*-TCP protected peptide amides synthesized

| Peptide sequence | $R_t$ (min)[a] | Purity[b] | ESI-MS (*m*/*z*) |
|---|---|---|---|
| TCP-Tyr-Ala-βAla-Thr-$NH_2$ | 15.4 | 86 | $[M+H]^+$ 692.2; $[M+Na]^+$ 714.1 |
| TCP-Gly-Gly-Asp-Ala-$NH_2$ | 15.3 | 94 | $[M+H]^+$ 586.1; $[M+Na]^+$ 608.3 |
| TCP-Ala-Val-Gly-Ile-Gly-Ala-$NH_2$ | 18.8 | 80 | $[M+H]^+$ 754.3; $[M+Na]^+$ 776.4 |
| TCP-Tyr-D-Ala-Phe-Phe-$NH_2$ | 19.9 | 85 | $[M+H]^+$ 813.9; $[M+Na]^+$ 836.2 |
| TCP-Gly-Gly-Ser-Leu-Tyr-Ser-Phe-Gly-Leu-$NH_2$ | 18.1 | 82 | $[M+H]^+$ 1166.1; $[M+Na]^+$ 1188.0 |

[a] HPLC retention time.
[b] Percent determined by HPLC from crude product of synthesis.




**Scheme 1.** Synthesis of H-Gly-Gly-Asp-Ala-$NH_2$.

## Acknowledgements

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2. Abbreviations used are as follows: *t*Bu, *tert*-butyl; DIPEA, *N*,*N*-diisopropylethylamine; DIPCDI, *N*,*N*′-diisopropylcarbodiimide; DMF, *N*,*N*-dimethyl-formamide; ESI-MS, electrospray ionization mass spectrometry; Fmoc, 9-fluorenylmethoxycarbonyl; HBTU, *N*-1*H*-(benzotriazol-1-yl) (dimethylamino) methylene-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HOAt, 7-aza-1-hydroxybenzotriazole; HPLC, high-performance liquid chromatography; IRAA, 'internal reference' amino acid; PAC, *p*-alkoxybenzyl alcohol handle; PAL, 5-(4-amino)-methyl-3,5-dimethoxy-phenoxy valeric acid handle (Peptide Amide Linker); PEG-PS, poly(ethylene glycol)-polystyrene (graft resin support); TCP, tetrachlorophthaloyl; TFA, trifluoroacetic acid. Amino acid symbols denote the L-configuration unless noted otherwise.
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