Pergamon

A Method for Removal of N-BOC Protecting Groups from Substrates on TFA-sensitive Resins

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Abstract: N-Tert-butyloxycarbonyl groups can be removed from substrates supported on Rink's amide resin by treatment with trimethylsilyl triflate/2,6-lutidine. This methodology was illustrated here with syntheses of several peptides, but it is likely to be used more extensively in solid phase syntheses of small molecule libraries. © 1998 Elsevier Science Ltd. All rights reserved.
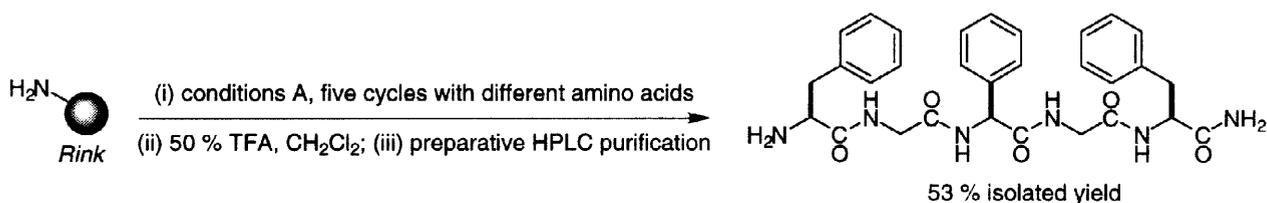
In the course of a project to form progressively larger fragments of vancomycin on a solid phase,[1] it was desirable to treat the supported nitroaryl fluoride **1** with tetra-n-butylammonium fluoride to obtain the 16-membered ring S_NAr product **2**. This reaction did not work. One possible explanation was that tetra-n-butylammonium fluoride mediated rapid N-fluorenyloxycarbonyl (N-FMOC) deprotection and cyclization to give the 13-membered ring product **3**. Consequently, an approach for S_NAr macrocyclization on Rink's amide resin[2] without N-deprotection was required. A tert-butyloxycarbonyl blocking at the N-terminus would have been ideal were it not for the fact that subsequent removal of this protecting group using TFA would also have caused cleavage of the substrate from the support. A method for removal of N-BOC groups on Rink's amide resin, without cleavage from that support, was therefore sought. This letter describes how the latter transformation can be achieved by treatment of supported N-BOC groups with a combination of TMSOTf and 2,6-lutidine (reaction 1).

1a, R = FMOC; 1b, R = BOC   2a, R = FMOC; 2b, R = BOC   3

(i) TMSOTf, 2,6-lutidine, CH₂Cl₂, 25 °C

(ii) MeOH
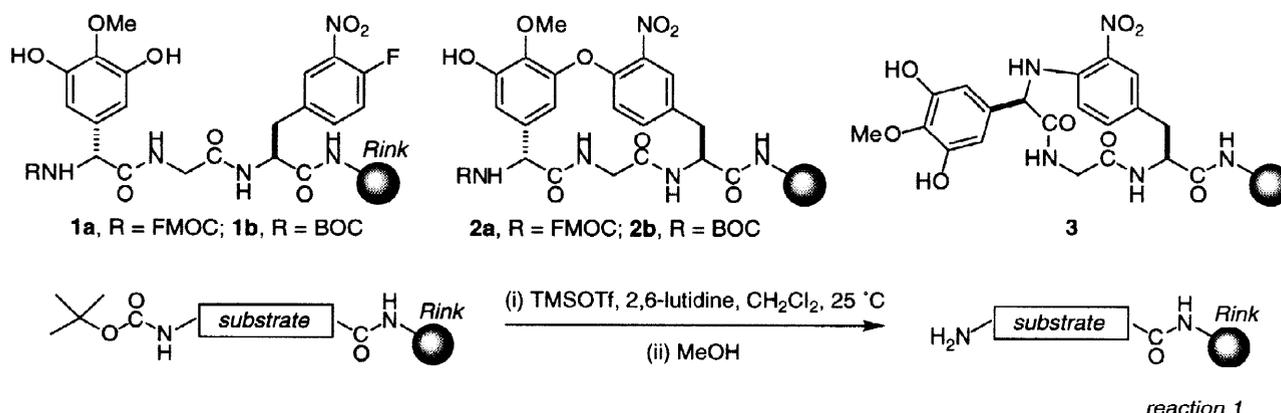
reaction 1

Trimethylsilyl triflate/2,6-lutidine had been used by Ohfune and co-workers for similar solution phase reactions of non-peptidic substrates. Most of that work featured *tert*-butyldimethylsilyl triflate, but TMSOTf was also mentioned.[3] As a test of the efficacy of these conditions for solid phase chemistry, Rink's amide resin was coupled with BOC-Phe, deprotected using "Conditions A" indicated below, and subjected to a quantitative ninhydrin test.[4] A sample of the same batch of resin was also coupled with FMOC-Phe under the same conditions, deprotected with piperidine ("Conditions B"), and subjected to the same quantitative analysis. Consequently, the relative coupling efficiencies of the two coupling/deprotection cycles were deduced to be 0.93:1.00. Carpino's TFFH reagent[5] was used as an activating agent throughout.

Conditions A: (i) BOC-Phe-OH, TFFH, NEt₃, 25 °C, 1h; (ii) 1M TMSOTf, 1.5 M lutidine, CH₂Cl₂, 1h; (iii) MeOH wash
relative yield 0.93

Conditions B: (i) FMOC-Phe-OH, TFFH, NEt₃, 25 °C, 1h; (ii) piperidine, CH₂Cl₂, 1h
relative yield 1.00

TFFH = tetramethylfluoroformadinium hexafluorophosphate =
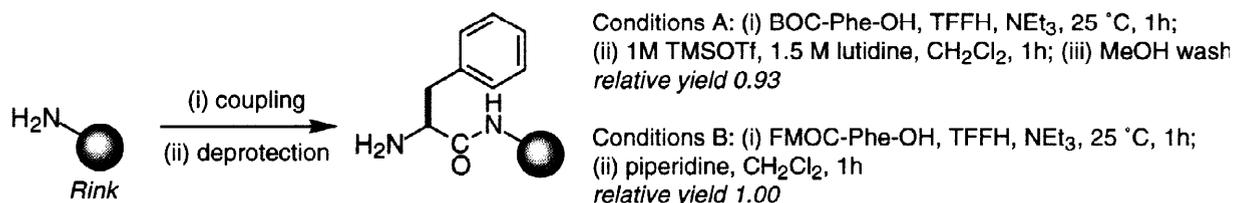
A pentapeptide was then prepared via iterative cycles of "Conditions A", using appropriate amino acids. Cleavage of the resin and preparative HPLC gave an appreciable yield of the desired material as indicated below. This sequence contains a phenylglycine (Phg) residue. No evidence for epimerization was observed even though aryl-substituted glycine residues have a tendency to racemize.

(i) conditions A, five cycles with different amino acids

(ii) 50 % TFA, CH₂Cl₂; (iii) preparative HPLC purification

53 % isolated yield

A series of peptides was then prepared to illustrate compatibility of the trimethylsilyl triflate/2,6-lutidine conditions with various side-chain protecting groups. This was done by cleavage of the product from the resin, analysis by HPLC and isolation of small samples of the predominant peaks, then MALDI-MS analysis of those fractions. Figure 1a and 1b show HPLC traces corresponding to UV-detection of the crude reaction mixtures corresponding to entries 1 and 10 in Table 1. These were chosen to illustrate good and bad examples, respectively, of the methodology in practice.
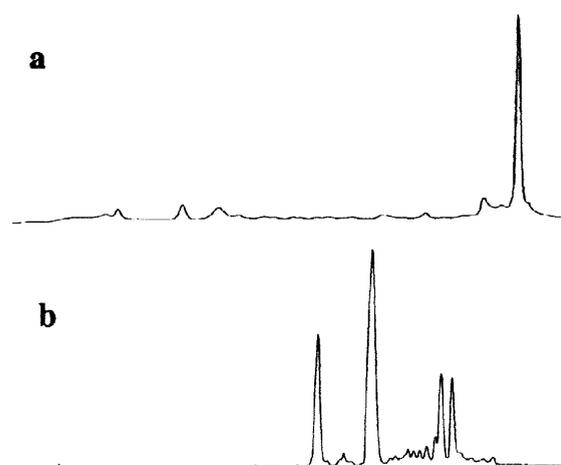
Figure 1. Analytical RP-HPLC traces of crude reaction mixtures from the peptide syntheses (UV-detection 255): **a** Phe-Gly-Phg-Gly-Phe as in entry 1 of Table 1; **b** Phe-Trp-Phe-Phe as in entry 10. First peak from left = product with side chain formyl group not removed; second peak = desired product; third and fourth peaks = low molecular mass impurities; second peak = product; A Vydac C18 column (4.6 mm x 25 cm, 5 µm) was used, with a linear gradient obtained by mixing solvent A (0.1 % TFA in water) and solvent B (0.1 % TFA in acetonitrile). The gradient was programmed to increase from 5 to 90 % B over 30 min with a flow rate of 1 mL min^{-1}.

Table 1. Preparation of peptides using iterative cycles of coupling then TMSOTf/2,6-lutidine deprotection. BOC amino acids, TFFH activation, TFA cleavage throughout.

entry	sequence	purity (%)[a]	side chain protection
1	Phe-Gly-Phg-Gly-Phe-amide	85	none
2	Phe-Phe-Phe-Phe-amide	97	none
3	Phe-Met-Arg-Phe-amide	93	Arg(Mtr)[b]
4	Phe-Thr-Ser-Phe-amide	71	Ser(TIPS)[c]; Thr(TIPS)[c]
5	Asp-Glu-Lys-Gln-amide	39	Asp(Fm)[d]; Glu(Fm)[d]; Lys(FMOC)
6	Phe-Lys-Phe-Phe	85	Lys(FMOC)
7	Ile-Asn-Asn-Ser-Val-amide	90	Ser(TIPS)[c]
8	Tyr-Gly-Gly-Phe-Leu-amide	87	Tyr(TIPS)[c]
9	Phe-His-Phe-Phe-amide	53	His(DNP)[e]
10	Phe-Trp-Phe-Phe-amide	54	Trp(FOR)[f]

[a] As a fraction of all peaks showing in the HPLC at 255 nm detection, except for entry 7 for which 215 nm was observed since that product has no aromatic rings. [b] Mtr = 4-methoxy-2,3,6-trimethylbenzenesulfonyl for guanidine protection; [c] TIPS = tri-*iso*-propylsilyl oxygen protecting group introduced by silylating the BOC-protected amino acid having no side chain protection, immediately prior to coupling. [d] Fm = fluorenylmethylene for protection of carboxylic acid side-chains. [e] DNP = 2,4-dinitrophenyl for histidine imidazole protection. [f] FOR = formyl for tryptophan side-chain protection.

The data in Table 1 reveals several features of the compatibility of TMSOTf/2,6-lutidine BOC-deprotection with various protecting groups. First, several widely used groups in solid phase synthesis, *ie* Mtr, FMOC, TIPS, DNP, and For (see Table 1 footnotes for full names), tolerated the deprotection conditions well. Entry 5 reveals that the product purity was moderate for synthesis involving both Fm and FMOC protection. This is consistent with the observation that TMSOTf/2,6-lutidine can cause rupture of some esters in solution phase experiments.[3] The major by-product corresponding to entry 9 arose via incomplete removal of the histidine side-chain *N*-dinitrophenyl protecting group in the TFA cleavage step; this is not a problem that can be attributed to the BOC-deprotection conditions. We suspect the reduced purity of the peptide shown in entry 10 may be due to problems with the tryptophan residue that also were not directly related to the deprotection

conditions. Overall, diminished yields for peptides containing side-chain protection were more a function of incomplete removal of these masking groups than their instability under the TMSOTf/lutidine conditions.

Use of silylating conditions to remove BOC-protecting groups on a solid phase is not new.[6-10] Merrifield and co-workers, for instance, used trimethylsilyl chloride/phenol mixtures in conjunction with their polystyrene resins.[7,8] However, all the conditions used previously are intrinsically acidic, and would not be suitable for acid sensitive resins. Indeed, we briefly investigated trimethylsilyl chloride/phenol mixtures for BOC-deprotection on Rink's resin and obtained discouraging results.

The examples shown in this paper feature syntheses of peptides. Peptide chemistry is well developed and the methodology illustrated here will probably only be useful in relatively few instances. Moreover, the yields reported are not as high as for conventional BOC- and FMOC-based coupling/deprotection cycles hence the methodology is not ideal for iterative use. However, we suspect that the approach will be more generally applicable to solid phase syntheses of small molecules wherein only one BOC removal is required. Temporary protection via BOC-protecting groups on Rink's amide resin avoids the dangers and inconveniences of HF cleavage reactions that are routinely associated with supported N-BOC protected substrates. They also enable a greater variety of starting materials to be used. Finally, BOC intermediates tend to be cheaper and more stable than FMOC ones, hence there are advantages associated with using BOC- in preference to FMOC-protected building blocks.[11]

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11. *General procedure for the solid phase peptide synthesis.* Manual peptide synthesis was carried out in a 30 mL vessel, fitted with a coarse glass frit, using a wrist action shaker (Burrel, Model 75) at 25 °C. The reagents were added manually. BOC deprotection was performed by shaking the resin twice with 2 mL of 1 M TMSOTf, 1.5 M 2,6-lutidine in dichloromethane (30 min and 30 min); CH_2Cl_2/MeOH/DMF washing cycles (5 x 1 min, 3 x 5 min, 5 x 1 min) were performed after each coupling and deprotection. Coupling of each BOC-amino acid was accomplished by premixing the amino acid (3 equiv.) with TFFH (3 equiv.), and DIEA (4 equiv.) in DMF (3 mL); this solution was added to the resin (200 mg, 0.6 mmol/g loading), and the mixture was shaken for 1 h. A negative ninhydrin test was observed after coupling at this stage. After the deprotection of BOC on the last residue, the resin subjected to appropriate reagents to deprotect the corresponding side chain protection groups, then washed and dried *in vacuo*. A mixture of phenol (0.5 mL), 1,2-ethanedithiol (0.25 mL), thioanisole (0.5 mL), deionized water (0.5 mL) and trifluoroacetic acid (8.25 mL) was cooled to 0 °C and added to the crude peptide-bound resin. The reaction mixture was stirred for 12 h at 25 °C, then concentrated to dryness. The products were precipitated by addition of Et_2O (30 mL). The purities of the peptides were checked by analytical RP-HPLC (Vydac C18 column, 4.6 mm x 25 cm, 5 μm), the crude peptides were further purified by preparative RP-HPLC (Vydac C18 column, 22 mm x 25 cm, 10 μm).