Polymer-supported Acidolysis of Protecting Groups used in the Synthesis of Large Peptides

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Amino acids and several peptides containing benzyl and other protecting groups normally removable by superacids such as HF, dissolved in trifluoroacetic acid, underwent novel acidolytic deprotection by Nafion polymers.

We report the first acidolysis of protecting groups used in the synthesis of large peptides by the insoluble Nafion polymers.[1]§ The peptides deprotected were not linked to a solid-phase synthesis support, *e.g.* polystyrene, and contained protecting groups normally removable by superacids such as liquid HF.¶ The deprotection reaction occurred in TFA containing an excess of Nafion; the peptide product was found trapped in the polymer and isolated by washing the polymer with a solution of base.‖ Traditional acidolyses of protected peptides, by contrast, are homogeneous solvolyses occurring in a superacid solvent such as liquid HF,[2] or TFA solutions of

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§ The following abbreviations are used in the text. Nafion-H: perfluorinated sulphonic acid polymer;[1] Nafion-TMS: trimethylsilylsulphonate derivative of Nafion-H;[7] TFA: trifluoroacetic acid; TfOH: trifluoromethanesulphonic acid; TMSOTf: trimethylsilyl trifluoromethanesulphonate; Boc: *tert*-butoxycarbonyl; Bn: benzyl; Tos: *p*-toluenesulphonyl; PhSTMS: phenylthiotrimethylsilane; Cl-Z: 2-chlorobenzyloxycarbonyl; OChx: cyclohexyl ester; Bom: benzyloxymethyl; Cl_2-Bn: 2,6-dichlorobenzyl; DMF: dimethylformamide.

¶ Protected peptides suitable for this acidolysis may be prepared in solution or on a solid support and detached prior to deprotection. Examples of the first approach to peptide synthesis are given in: H. Yajima and N. Fujii, *J. Chem. Soc., Chem. Commun.*, 1980, 115; R. Hirschman, R. F. Nutt, D. F. Veber, R. A. Vitali, S. L. Varga, T. A. Jacob, F. W. Holly and R. G. Denkewalter, *J. Am. Chem. Soc.*, 1969, **91**, 507. Examples of the second approach are given in ref. 8.

‖ With an estimated acidity function $H_0 = -12$, Nafion-H is expected to be able to protonate all heteroatoms in a peptide consisting of natural amino acids resulting in very tight binding. Binding may further be enhanced by interactions between the fluorinated hydrocarbon chains in Nafion and certain side chains in peptides.

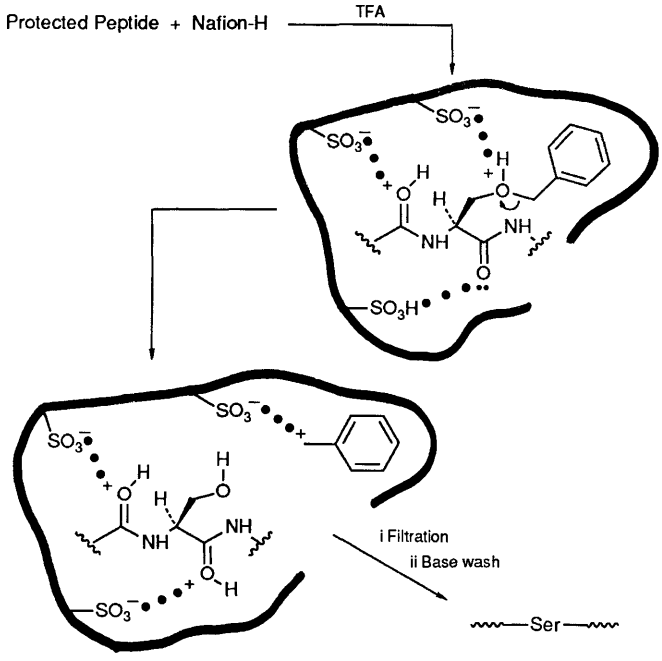

Fig. 1 Acidolysis of a –Ser(Bn)–residue of a protected peptide on a sulphonic acid polymer surface

Table 1 Amino acids deprotected by Nafion polymers[a]

Entry		Entry	
1	H-Ser(Bn)-OH	7	Boc-Lys(Cl-Z)-OH
2	Boc-Thr(Bn)-OH)	8	Boc-Trp(CHO)-OH[b]
3	Boc-Asp(Bn)-OH	9	Boc-Glu(OChx)-OH
4	Boc-Glu(Bn)-OH	10	Boc-Cys(4-MeBn)-OH
5	Boc-His(Bom)-OH	11	Boc-Arg(Tos)-OH
6	Boc-Tyr(Cl_2-Bn)-OH	12	H-Arg(NO_2)-OH

[a] Experimental procedure used for entries 1–8: a mixture of protected Boc amino acid (0.05 mmol), Nafion-H (0.6 g, 0.5 equiv.), Me_2S (0.35 mmol), *m*-cresol (0.2 mmol) and TFA (1.5 ml) was stirred in a sealed vessel for 3 h. The crude product, isolated from the resin by extraction with aq. NH_3 or Et_3N–DMF as described in the text, was assayed by quantitative amino acid analysis. In all cases, deprotection was found to be quantitative. For entries 9–12, the procedure was modified by using pertrimethylsilylated Nafion[7] and PhSTMS, instead of Nafion-H and Me_2S, and hydrolysing the deprotected amino acid isolated from the resin through extraction in a mixture of 1.0 mol dm^{-3} aq. ammonia (3 ml) and NH_4F (1 mmol) for 0.5 h. Several deprotections, *e.g.* entries 1 and 3, were carried out in the absence of Me_2S and *m*-cresol and found to proceed successfully. [b] Deprotection carried out in the presence of ethanedithiol (0.35 mmol).

TfOH,[3] TfOTMS,[4] or $(CF_3CO_2)_3B$.[5] A reasonable mechanism for the polymer-supported acidolysis is illustrated in Fig. 1.

Deprotection by Nafion polymers was found to have wide scope. As Table 1 shows, Nafion-H in the presence of Me_2S cleaved most of the protecting groups used in peptide synthesis. Me_2S, or a similar nucleophilic disulphide, is often used as a scavenger of the carbocations generated during deprotection and in certain cases enables acidolysis to occur *via* an S_N2 mechanism.[6] Nafion-H–Me_2S could not deprotect Glu(OChx), Arg(Tos), Arg(NO_2) and Cys(4-MeBn), which cannot be deprotected efficiently by S_N2 cleaving systems, such as HF–Me_2S or TfOH–Me_2S, requiring S_N1 acidolysis, *e.g.* in 95% liq. HF–*m*-cresol.[6] However, these protected amino acids could be cleaved quantitatively by pertrimethylsilylated Nafion,[7] Nafion-TMS, in TFA containing PhSTMS within 3 h.

Several short peptide deprotections were demonstrated as well.** Thus to Boc-Lys(Cl-Z)-Leu-Lys(Ac)-Glu(Bn)-Leu-Lys(Cl-Z)-Gln-OH (8.0 mg), Me_2S (24 μl) and *m*-cresol (22 μl) in TFA (2.5 ml) was added Nafion-H (540 mg) and the resulting suspension was sealed and stirred for 3 h. The liquid phase of the reaction mixture contained no peptide, as shown by HPLC analysis of the material isolated after solvent evaporation. The Nafion was washed with CH_2Cl_2 and with diethyl ether, dried *in vacuo*, and washed with 5% Et_3N–DMF to release polymer-bound substances. The DMF washings were concentrated and after purification by gel filtration (Sephadex G-10 in HOAc 0.1 mol dm^{-3}) gave H-Lys-Leu-Lys(Ac)-Glu-Leu-Lys-Gln-OH as a colourless solid, 4.6 mg, 77%.†† We are exploring the use of Nafion and of related polymers in deprotections of large peptides, which we are currently assembling *via* segment synthesis–condensation on the oxime resin of Kaiser–DeGrado.[8]

In summary, Nafion polymers cleaved protecting groups commonly used in peptide synthesis including those of several peptides dissolved in TFA. The deprotection was efficient, selective and operationally simple regardless of preparative scale, an advantage not shared by homogeneous acidolyses. It is also mechanistically novel and sets the stage for the development of new acidolysis systems which may be useful in large peptide synthesis by the segment synthesis–condensation approach.[8]

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** The protected peptides were prepared on the oxime solid phase support,[8] purified using reversed phase HPLC, and characterised by their amino acid analyses and mass spectra.

†† *Cyclo*(3–7)-Boc-Lys(Cl-Z)-Leu-Lys-Glu(Bn)-Leu-Lys(Cl-Z)-Asp(Bn)-OH and Boc-Val-Glu(Bn)-Ile-Tyr(OCl_2-Bn)-Pro-Val-Ala-Ala-Leu-OH were deprotected using the procedure in the text. The procedure had to be modified in the case of deprotection of tetradecamer *dicyclo*(3–7, 10–14)-Boc-[Lys(Cl-Z)-Leu-Lys-Glu(Bn)-Leu-Lys(Cl-Z)-Asp(Bn)]$_2$-OH. The deprotected peptide could be isolated by washing the Nafion polymer with 10% TfOH in TFA, diluting the pooled washings with diethyl ether and filtering off the precipitated peptide; washing with Et_3N in DMF, following the procedure in the text, did not detach the peptide product from the polymer, as shown by a positive ninhydrin test given by the base-washed polymer. The peptides described in this paper were also deprotected using TMSOTf–thioanisole–TFA,[4] and the resulting products were found to be indistinguishable, by reversed phase HPLC analysis, from the products obtained when Nafion was used to carry out the deprotection. All deprotected peptides were further characterised by their amino acid analyses and mass spectra.