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Removal of *t*-Butyloxycarbonyl Groups in Solid Phase Peptide Synthesis

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Literature methods for removal of the *t*-butyloxycarbonyl group, used as a temporary protection for α-amino groups in solid phase peptide synthesis, have been investigated. The work is based on a method for determining primary amino groups, recently introduced by Esko *et al.* The results obtained demonstrate that trifluoroacetic acid-methylene chloride 1:1 (v/v) is the fastest and most reliable deprotecting medium, the reason probably being the very fast polymer swelling in this mixture. In contrast, 1 N hydrogen chloride-acetic acid acts at a rate highly dependent on the polymer's state of swelling.

Solid phase peptide synthesis (SPPS), at first manually performed, later automated, was introduced by Merrifield [1,2] only a few years ago and has been shown mainly by him and his co-workers to be very useful for the synthesis of big peptides and even a protein,[3] these compounds being extremely difficult and tedious to synthesise conventionally in solution. A recent review [4] and monograph [5] cover most aspects of the topic.

The success or failure in SPPS depends mainly on two factors: all coupling and deprotecting steps necessarily have to be quantitative, and by-products, caused by additional reactive sites in the peptide chain cannot be accepted, since both factors decrease the yield and increase the difficulties encountered in the final purification step. Yields can be kept very high by using a large excess of reagents. By-products can generally be avoided by blocking all reactive side-chain functions. It is quite clear, that the bigger the peptide one wants to prepare, the greater the attention to be paid to the quantitative aspect of the reaction. The heterogenic nature of some peptides prepared by the SPPS method in our laboratory directed our attention finally to study the deprotection step quantitatively and to evaluate the deprotection mixtures proposed up to now.

α-*t*-Butyloxycarbonyl-(BOC-) amino acids [6,7] were found to be ideal in SPPS by Merrifield [8] at an early stage and have since been used with very few exceptions.[4,5] Three methods have been proposed and used with success to remove the BOC-group from amino acids fixed to the polymer, *i.e.*

1 N HCl-acetic acid [8] at 25° for 30 min, 4 N HCl-dioxane [9] and 50 % (v/v) trifluoroacetic acid (TFA)-methylene chloride.[3] These are the methods compared in this paper. A few additional related mixtures have also been prepared and preliminarily tested, but were found to be inferior to those mentioned.

MATERIAL AND METHODS

Solvents. All solvents were of good laboratory quality, and if not otherwise stated, used without further purification. The same applied to the TFA.

BOC-L-alanyl-polymer. The starting material was prepared from commercial chloromethylated copolymer of styrene-divinylbenzene (Bio-Beads S-X-2, 200—400 mesh, capacity 0.72 mequiv./g from Bio-Rad Laboratories, Richmond, Calif., USA) and BOC-L-alanine in analogy with BOC-nitro-L-arginyl-polymer [10] and dried to constant weight at 80° *in vacuo*. Quantitative amino acid analysis on a small aliquot after hydrolysis in conc. HCl-dioxane 1:1 (v/v) at 110° for 24 h indicated an alanine content of 0.232 mmole/g.

BOC-nitro-L-arginyl-polymer. Our sample was prepared according to Ref. 10.

Table 1. Deprotection of BOC-L-alanyl-polymer with standard deprotection mixtures.

Experiment No.	Reaction time, min	Amine found μequiv./g	Yield, %
1	2	173	69
2	5	244	97
3	10	252	100 [e]
4	15	250	99
5	30	250	99
6	60	249	99
7	7.5 [a]	210	83
8	17.5 [a]	172	68
9	5	114	45
10	10	177	70
11	10 [b]	177	70
12	10 [c]	181	72
13	30	236	94
14	60	240	95
15	60	245	97
16	17.5 [a]	233	92
17	5	36	14
18	5 [c]	216	86
19	10	68	27
20	10 [d]	179	71
21	10 [c]	242	96
22	30	138	55
23	30 [c]	243	96
24	60	204	81
25	60 [c]	248	98
26	17.5 [a]	234	93
27	18 [a,c]	227	90

[a] hours. [b—d] The polymer was swollen in dioxane for 3 h, methylene chloride for 5 min and glacial acetic acid for 2 h, respectively, prior to deblocking. [e] by definition.

Other BOC-peptide-polymers used. They were prepared from BOC-L-alanyl-polymer above by deprotection with TFA-methylene chloride 1:1 for 30 min, worked up in the standard way, coupled with BOC-amino acid (3 equiv.) and dicyclohexylcarbodiimide (3 equiv.), where not otherwise stated in methylene chloride for 5 h. After deprotection experiments the products were analyzed for primary amine as below.

1 N HCl-acetic acid and 4 N HCl-dioxane. These deprotection mixtures were both prepared according to Ref. 5, p. 30.

Deprotection experiments. Weighed BOC-protected samples (about 50 mg) were reacted with about 4 ml of deprotection mixture in small glass filter funnels for specified times and then carefully washed with suitable solvents, neutralized, and again washed as in preparative SPPS. The washing was followed by amino group determination, without the sample being removed from the funnel.

Quantitative determination of primary amino groups in amino acids and peptides bound to the polymer. This was done mainly as in Ref. 11. Sample size, however, was increased (*cf.* above). 2-Hydroxy-1-naphthaldehyde was reacted with the polymer in abs. ethanol-methylene chloride 1:1 (v/v) mixture [12] instead of abs. ethanol.

EXPERIMENTS AND RESULTS

Table 1 summarizes the results obtained on deblocking dry BOC-L-alanyl-polymer with TFA-methylene chloride 1:1 (v/v) (expt. 1 – 8), 3.8 N HCl-dioxane (expt. 9 – 16) and 1.0 N HCl-acetic acid (expt. 17 – 27).

Two further experiments related to this table should be mentioned in this context. Quantitative amino acid analysis on the residue, obtained from the TFA-methylene chloride in expt. 8 after evaporation, showed it to contain 35 % of the alanine originally bound to the polymer. Considering 68 % are still present in the polymer, the result is looked upon as reasonably reliable. In a parallel run to expt. 8, water was added to the deprotection mixture. The presence of 2.5 % of water resulted in a slightly higher loss of alanine, but 63 % were still fixed to the polymer. The significance of this experiment will be further investigated.

Table 2 gives the results with the same polymer using other deprotection mixtures considered worth trying, *i.e.* 1 N TFA-methylene chloride (expt. 28 and 29), 5 N trichloroacetic acid-methylene chloride (expt. 30 – 32) and formic acid-methylene chloride 1:1 (v/v) (expt. 33).

A few experiments with BOC-nitro-L-arginyl-polymer, performed as those reported in Table 1, are given in Table 3. Expt. 34 and 35 were done with TFA-methylene chloride 1:1 and expt. 36 with 1 N HCl-acetic acid.

Table 2. Deprotection of BOC-L-alanyl-polymer with modified deprotection mixtures.

Experiment No.	Reaction time, min	Amine found, μequiv./g	Yield %
28	10	95	38
29	60	238	94
30	5	51	20
31	10	84	33
32	60	215	85
33	60	5	2

Table 3. Deprotection of BOC-nitro-L-arginyl-polymer with TFA-methylene chloride and 1 N HCl-acetic acid.

Experiment No.	Reaction time, min	Amine found, μequiv./g
34	10	165
35	60	166
36	60	153

After having compared the efficiency of different deprotection media on one and the same BOC-amino acid, L-alanine, bound to the polymer, we deprotected two BOC-dipeptide-polymers, *i.e.* BOC-L-leucyl-L-alanyl-polymer (expt. 37 – 39) and BOC-L-isoleucyl-L-alanyl-polymer (expt. 40 – 42), with 50 % TFA-methylene chloride. These results are given in Table 4.

Table 4. Deprotection of BOC-L-leucyl-L-alanyl-polymer and BOC-L-isoleucyl-L-alanyl-polymer with 50 % TFA-methylene chloride.

Experiment No.	Reaction time, min	Amine found, μequiv./g	Corrected value for increase of weight, μequiv./g
37	5	232	239
38	10	237	244
39	60	235	242
40	5	215 [a]	221
41	10	236	243
42	60	230	237

[a] Two further experiments gave 214 and 216 μequiv./g.

DISCUSSION

Checking that every step in SPPS, especially in the automated version, progresses nearly quantitatively presents enormous difficulties and can hardly be performed rigorously. The real risk of a final product too poor to be worth purifying can obviously be reduced to some extent by regular checks on the growing chain.[13] We have seen one solution to this problem in an extensive and systematic investigation of the different steps involved in the SPPS procedure. Very little seems to have been done in this direction up to now. We were fortunate in being able to apply systematically a method [11] already developed in this department for measuring the amount of primary amino groups present in polymers. It has so far worked very satisfactorily.

The results in Table 1 confirm that 1 N HCl-acetic acid is indeed a very efficient medium for deprotection as claimed by Merrifield,[10] provided the polymer

is properly swollen. TFA-methylene chloride, however, deprotects still faster, and what is more important, invariably gives a more complete reaction (see also Table 3). HCl-dioxane in our hands seems inferior to the methods discussed so far.

The deprotecting action of all three media used in Table 1 is accompanied by loss of product from the polymer. This is by far most pronounced for TFA-methylene chloride and has to be considered when this deprotection medium is used in connection with the synthesis of high molecular weight compounds.

It is seen from Table 2 that on moderate dilution TFA looses its fast deprotecting action. Trichloroacetic acid is definitely unsatisfactory at a concentration of 5 N. Formic acid has been claimed to effect removal [14] of the BOC-group. When used under the present conditions, its action is very slow.

Our preliminary experiments on BOC-dipeptide-polymers, Table 4, were done in order to find out if sterical hindrance, caused by side-chains, had to be considered. We conclude from these and further experiments that in preparative runs a deprotecting time in the range of 10–30 min will suffice.

Finally we want to call attention to the possibility of using substance removed from the polymer in the deblocking step for analytical purposes. Preliminary experiments have shown that the minute traces of peptide liberated often are enough to give a clear picture of the homogeneity of the material still fixed to the polymer.

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