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Amino Group Protection in Solid Phase Peptide Synthesis

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Most of the labile amino protecting groups hitherto used in solid phase peptide synthesis have been studied quantitatively with respect to their stability. Methods proposed in the literature for their removal have been tested in order to select safe procedures. The following protecting groups were investigated: Benzyloxycarbonyl, *t*-butyloxycarbonyl, *o*-nitrophenylsulfenyl, *p*-methoxybenzyloxycarbonyl and 2-(*p*-biphenyl)-isopropyloxycarbonyl. The corresponding L-isoleucine derivatives were coupled to L-alanyl-polymer. Deprotecting experiments were performed with these products on a 30–50 mg scale. The free amino groups obtained were determined quantitatively, using the 2-hydroxy-1-naphthaldehyde method. Additional experiments were carried out in order to obtain information on the stability of the ester bond between the C-terminal amino acid and the polymer, *i.e.* to determine the loss of product from the polymer under the conditions used for removing the protecting group from the amino group. The results are shown in Table 1.

Solid phase peptide synthesis (SPPS) was introduced by Merrifield [1] a few years ago and has already been used successfully for the synthesis of several peptides of considerable length. The procedure was designed so that it could be automated.[2] For leading references on the topic, see Ref. 3.

Continuing our systematic studies [4] on SPPS, we have now examined most of the labile amino protecting groups hitherto used in SPPS with respect to their suitability for this purpose. The following protecting groups were investigated: Benzyloxycarbonyl (Z-), *t*-butyloxycarbonyl (Boc-), *o*-nitrophenylsulfenyl (Nps-), *p*-methoxybenzyloxycarbonyl ($Z(OCH_3)$-) and 2-(*p*-biphenyl)-isopropyloxycarbonyl (Bpoc-). As a model we chose a correspondingly blocked L-isoleucine residue coupled *via* L-alanine to the resin.

Benzyloxycarbonyl was used by Merrifield in his first paper [1] on SPPS for α-amino group protection but was subsequently exchanged for more labile groups. We included this group in our work because its use has been described in several papers for protecting the ε-amino group in lysine and there was some doubt [5] whether it would be stable under conditions required for removing α-amino Boc-groups. Two further experiments were performed with ε-Z-aminocaproic acid coupled similarly.

Table 1. Deprotecting experiments with X-L-Ile-L-Ala-polymer.

Experiment No.	X	Deprotection mixture	Reaction time [m]	Amine found [μequiv./g]
1	Z	A	60	≤ 0.5
2	Z	A	17.5[a]	7
3	Boc	B	5	103
4	Boc	B	10	159
5	Boc	B	30	228, 230
6	Boc	B	60	222, 226
7	Boc	B	17.5[a]	196
8	Boc	C	5	42
9	Boc	C	10	63
10	Boc	C	30	170
11	Boc	C	60	228, 228, 233
12	Nps	A	5	231
13	Nps	A	10	232
14	Nps	A	60	226
15	Nps	B	5	195
16	Nps	B	10	217
17	Nps	B	30	227, 228
18	Nps	B	60	(218), 219, 222
19	Nps	B	17.5[a]	202
20	Nps	C	5	159
21	Nps	C	10	194
22	Nps	C	60	231
23	Nps	D	10[b]	214
24	Nps	D	30[c]	185
25	Nps	D	60[d]	198
26	Nps	D	5[a]	213
27	Nps	E	5	228
28	Nps	E	10	224
29	Nps	E	60	216
30	Nps	F	10	212
31	$Z(OCH_3)$	A	5	234
32	$Z(OCH_3)$	A	10	233
33	$Z(OCH_3)$	A	60	224
34	$Z(OCH_3)$	C	5	(237), 228, 229, 231
35	$Z(OCH_3)$	C	10	(226), 232, 232, 235
36	$Z(OCH_3)$	C	60	233
37	$Z(OCH_3)$	C	17.5[a]	214
38	$Z(OCH_3)$	G	5	190
39	$Z(OCH_3)$	G	10	225
40	$Z(OCH_3)$	G	60	233
41	$Z(OCH_3)$	H	5	10
42	$Z(OCH_3)$	H	10	21
43	$Z(OCH_3)$	H	60	99
44	Bpoc	K	10	127
45	Bpoc	K	30	197
46	Bpoc	K	60	231
47	Bpoc	K	90	227
48	Bpoc	K	17.5[a]	222
49	Bpoc	H	5	232
50	Bpoc	H	10	237
51	Bpoc	H	60	235
52	Bpoc	H	17.5[a]	228
53	Bpoc	L	5	73
54	Bpoc	L	10	112
55	Bpoc	L	60	229
56	Bpoc	L	17.5[a]	236

Since the Boc-group was the subject of our preceding paper,[4] we shall confine ourselves here to a series of deblocking experiments carried out with 1 and 2 M trifluoroacetic acid (TFA) in methylene chloride, performed in order to make a direct comparison with the Nps- and Z(OCH_3)-groups possible.

Although Boc proved quite satisfactory in the SPPS procedure,[3] the loss of product from the polymer measured by us [4] seemed too high to be acceptable for the synthesis of large peptides. We therefore decided to have a look at more labile groups and finally directed our attention to the three last mentioned above.

Nps was used for α-amino group protection in SPPS by Najjar and Merrifield [6] in the synthesis of the octadecapeptide bradykininylbradykinin. They used hydrogen chloride in acetic acid-chloroform for removing the protecting group. This protecting group had earlier been investigated by Kessler and Iselin.[7] For deblocking they used thioacetamide in acetic acid-methanol. Z(OCH_3) was used by Weygand and Ragnarsson [8] and removed with trifluoroacetic acid. Bpoc was introduced by Sieber and Iselin [9] and a protected tripeptide hydrazide was prepared using a solid support. Wang and Merrifield [10] similarly made a tetrapeptide hydrazide. The former used chloroacetic acid in methylene chloride and the latter 0.5 % trifluoroacetic acid in the same solvent to remove the protecting group.

MATERIAL AND METHODS

Protected dipeptide-polymers. The Boc-L-alanyl-polymer originated from our earlier work.[4] All protected dipeptide-polymers were prepared exactly as before.

Quantitative determination of primary amino groups in peptides bound to the polymer. These determinations were also carried out as just cited. Originally, only single experiments were performed. In those experiments whose results deviated from similar trials, multiple determinations were performed (results inside brackets, Table 1). Four further experiments were repeated to demonstrate the reproducibility of our measurements, which is also indirectly shown in several experiments where the protecting group was obviously removed in a fast reaction while the loss of peptide from the resin was negligible.

EXPERIMENTS AND RESULTS

Table 1 summarizes the results obtained on deblocking X-L-isoleucyl-L-alanyl-polymer, X being a Z-, Boc-, Nps-, Z(OCH_3)- or Bpoc- protecting group. Assuming 250 μmole L-alanine/g Boc-L-alanyl-polymer (*i.e.* correcting

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[a] Hours.[b–d] The polymer was swollen in methylene chloride for 2 min, methanol-glacial acetic acid 41:9 (v/v) for 15 min and in the same mixture for 5 min, respectively, prior to deblocking.

The deprotecting mixtures were designated as follows: A, B, C, G, H, and L stand for TFA solutions in methylene chloride of concentrations 50 % (v/v), 2 M, 1 M, 0.5 M, 0.1 M and 0.02 M. D, E, and F stand for solutions of thioacetamide of concentrations 1 M, 0.5 M, and 0.25 M in methanol-glacial acetic acid 41:9 (v/v) and methylene chloride-glacial acetic acid 4:1 (v/v) and 9:1 (v/v). K finally is 3.4 M chloroacetic acid in methylene chloride.

for the small loss in the following deblocking, experiment 5 [4]), the following values for the amount of peptide in the different X-L-isoleucyl-L-alanyl-polymers expressed in μmole/g have been calculated to be: X=Z 241, Boc 243, Nps 240, Z(OCH_3) 239, and Bpoc 235.

Two further experiments, related to the first two of this table, were carried out. ε-Z-Aminocaproic acid was coupled to L-alanyl-polymer and the product left with 50 % TFA in methylene chloride and a similar 1 M solution for 17.5 h before the content of amino groups was determined. The values found after correcting for loss of peptide from the resin in the first case (experiments 8 [4]) correspond to a removal of 7 and 2 % of the ε-Z-groups.

DISCUSSION

As mentioned above, benzyloxycarbonyl was included in the present work entirely because we felt some information was needed with reference to its use as an ε-aminoprotecting group for lysine. It is concluded that a peptide resin containing an ε-Z-protected lysine residue can only be exposed to a 50 % TFA in methylene chloride solution for a few hours without appreciable loss of Z-groups. Even with 1 M TFA in methylene chloride there is some loss of ε-Z-groups. To avoid branching at lysine residues in such cases, a more stable ε-aminoprotecting group is needed.

Boc was removed much more slowly with 1 M TFA in methylene chloride than with 50 % TFA in the same solvent, thus confirming our results [4] with Boc-L-alanyl-polymer. With 2 M TFA in methylene chloride the reaction was apparently speeded up considerably, but hardly enough to make it really useful.

A great number of experiments were performed to evaluate the usefulness of the Nps-group. Although it is considered very labile to acids in organic solvents, our experiments indicate considerable differences in the initial rate of deprotection depending on the concentration of the acid used. The method of Kessler and Iselin [7] (experiments 23 – 26) seems to us to result in uncomplete deblocking, presumable due to poor swelling in the solvent mixture used. We therefore carried out a few experiments substituting methylene chloride for methanol. As can be seen, higher values for the amino group content were obtained, but we left the problem without optimizing the conditions.

The Z(OCH_3)-group is known to be very acid labile. Our experiments showed that with 1 M TFA in methylene chloride, optimal results were obtained in about 10 min and that loss of peptide from the resin was considerably less than with a 50 % acid solution.

Bpoc was removed under three sets of conditions. Thus we used chloroacetic acid as proposed by Sieber and Iselin.[9] Although initially slow in action, undoubtedly effective deblocking was obtained in this way. Of all methods tried in this paper, however, protection with Bpoc and its subsequent removal with dilute TFA in methylene chloride seemed the best. Not only was the theoretical value obtained for the amount of peptide bound to the resin, but it was reached in a very short time. The benzyl ester linkage between the C-terminal amino acid and the polymer was hardly affected at all under these conditions. We

conclude that the extreme acid lability of the Bpoc-protecting group makes it very promising for SPPS. On the other hand it must be verified in preparative experiments that this protecting group is not so labile as to cause premature loss of blocking group with accompanying double coupling. Our results further demonstrate that removal of protecting groups can be effected by more diluted acid solutions than generally used but at the cost of longer reaction times.

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