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NEW REMOVAL CONDITIONS OF SULFENYL GROUPS IN PEPTIDE SYNTHESIS

Angelo Fontana, Fernando Marchiori, Luigi Moroder,
Ernesto Scoffone

Istituto di Chimica Organica dell' Universita' – Sezione VIII del
Centro Nazionale di Chimica delle Macromolecole del C.N.R. –
Padova – Italy

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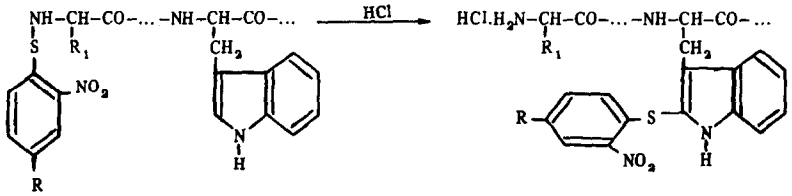
The usefulness of a protecting group in peptide synthesis is strictly connected with the multiplicity and selectivity of its deblocking conditions. The removal of the o-nitrophenyl-sulfenyl (NPS) group ([1,2]) can be easily achieved by treatment with hydrochloric acid or by desulfuration with Raney nichel ([3]).

However this last procedure fails when applied to sulfur containing peptides. On the other side the acid cleavage of N–S bond can not be used when tryptophan is present in the peptide ([4]).

In fact using tryptophan peptides, carrying either the NPS or the DNPS (2,4-dinitrophenyl-sulfenyl) ([5]) as N-protecting groups, we proved that the sulfenyl halide, formed during the removal step, reacts quantitatively at the 2-position of the indole ring, as below depicted:



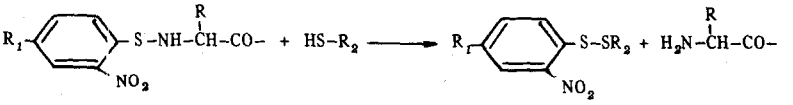
with R = –H ; $-NO_2$

For example, the DNPS-L-tryptophanyl-glycine ethyl ester [m.p. 88-90°C; $[\alpha]_D$ -126.4°, c 0.5% in methanol; Anal. Calcd. for $C_{21}H_{21}N_5O_7S$: C 51.70 H 4.21 N 14.38 S 6.57; Found: C 51.55 H 4.30 N 14.01 S 6.38] by treatment with alcoholic hydrochloric acid yielded (78%) the 2-DNPS-L-tryptophanyl-glycine ethyl ester hydrochloride [m.p. 169-171°C; $[\alpha]_D$ + 60.3°, c 0.5% in methanol; Anal. Calcd. for $C_{21}H_{22}ClN_5O_7S$: C 49.93 H 4.38 Cl 6.78 N 13.33 S 6.11; Found: C 49.27 H 4.60 Cl 6.36 N 13.26 S 5.96].

The nucleophilicity of the 2-position in 3-substituted indoles towards sulfenyl halides, pointed out by Wieland et al. ([6]), was further confirmed using 3-methyl-indole as model compound. The same 2-DNPS-3-methyl-indole [m.p. 194-196°C; Anal. Calcd. for $C_{15}H_{11}N_3O_4S$: C 54.92 H 3.34 N 12.78 S 9.71; Found: C 54.77 H 3.32 N 12.61 S 9.93; λmax 3430 cm^{-1} in Nujol and 3465 cm^{-1} in chloroform for NH streching; single yellow Ehrlich negative spot in thin layer chromatography] was obtained either in the reaction of 3-methyl-indole with DNPS-chloride in 99% formic acid or by independent synthesis in which 2,4-dinitrochlorobenzene was reacted with 2-mercapto-3-methyl-indole.

In order to eliminate the aforesaid troublesome reaction, during the removal of NPS or DNPS groups, we looked for other conditions not affecting the tryptophan residue.

Therefore accordingly with Edwards and Pearson ([7]), higly polarizable nucleophiles were checked in this displacement reaction involving the divalent sulfur of sulfenamides. We found that thiophenol or thioglycolic acid remove NPS and DNPS groups in organic solvents giving the free amino acid or peptide and a disulfide:



with R_1 = -H; $-NO_2$

R_2 = $-C_6H_5$; $-CH_2-COOH$

In a tipical set of experiments 0.4 ml of thiophenol or thioglycolic acid were added to the solutions of 0.0025 mole of the NPS or DNPS derivatives of valine, alanine, tryptophan in 20 ml of dimethylformamide or pyridine. After standing 1 hour at room temperature, 60 ml of ethyl ether were added, the precipitates were filtered off and washed with ether (yields 70-80%). The free amino acids were characterised by thin layer chromatography, melting points, and elemental analysis; furthermore they showed the same optical rotation as the parents amino acids.

The deblocking reaction occurs also using ter-butyl and isopropyl mercaptans in pyridine solution, or thiosulfate in aqueous alcohol, using acetic acid as catalist.

These new deblocking methods were then applyed to some N-protected peptides.

For example, the free peptide L-alanyl-glycine [m.p. 239–241°C (lit. [8]) 235–237°C); $[\alpha]_D$ + 51.2° (lit. [8]) + 51.3°), c 2.5% in water; Anal. Calcd. for $C_5H_{10}N_2O_3$: C 41.05 H 6.88 N 19.17; Found: C 41.25 H 7.05 N 18.80] was obtained from NPS-L-alanyl--glycine [m.p. 142–143°C; $[\alpha]_D$ −68.4°, c 0.5% in methanol; Anal. Calcd. for $C_{11}H_{12}N_2O_5S$: C 44.08 H 4.34 N 14.03 S 10.69; Found: C 43.45 H 4.22 N 13.96 S 10.68] by treatment either with thioglycolic acid in pyridine (yield 73%) or thiophenol in dimethylformamide (yield 68%).

In the same way the L-tryptophanyl-glycine [m.p. 174–177°C; $[\alpha]_D$ + 80.2° (lit. [9]) + + 78.5°), c 0.5% in water; Anal. Calcd. for $C_{13}H_{15}N_3O_3$: C 59.80 H 5.75 N 16.03; Found: C 59.70 H 5.84 N 15.63] was obtained (yield 74%) from NPS-L-tryptophanyl-glycine [m.p. 117–119°C; $[\alpha]_D$ − 53.4°, c 0.5% in methanol; Anal. Calcd. for $C_{19}H_{18}N_4O_5S$: C 55.01 H 4.35 N 13.52 S 7.72; Found: C 55.30 H 4.55 N 13.21 S 7.39].

The proposed cleavage conditions offer a new very mild procedure in removing the sulfenyl groups and allow their use in the synthesis of tryptophan containing peptides.

The experimental details will be reported elsewhere.

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