Pergamon

Tetrahedron Letters 43 (2002) 311–313

TETRAHEDRON LETTERS

Magnesium/hydrazinium monoformate: a new hydrogenation method for removal of some commonly used protecting groups in peptide synthesis

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Received 29 August 2001; revised 23 October 2001; accepted 5 November 2001

Abstract—Removal of some commonly used protecting groups in peptide synthesis by catalytic transfer hydrogenation employing hydrazinium monoformate and magnesium is described. This method is equally competitive with other methods in deblocking most of the commonly used protecting groups in peptide synthesis. *tert*-Butyl derived and base labile protecting groups were completely stable under these conditions. The use of Mg/NH_2–NH_2·HCOOH makes this a rapid, low-cost alternative to palladium and reduces the work-up to a simple and extraction operation. © 2002 Elsevier Science Ltd. All rights reserved.

For the removal of some commonly used protecting groups in peptide synthesis, catalytic transfer hydrogenation offers certain advantages over catalytic hydrogenation procedures, which are generally employed for this purpose. Among the desirable characteristics of any deprotection scheme are the following: (1) low cost, (2) rapidity, (3) selectivity, (4) mild conditions, usually avoiding strong acid or base and (5) broad applicability. A number of improvements in the area of catalytic transfer hydrogenation have been reported[1–10] which greatly simplify these procedures and some even reduce the operating conditions to standard room temperature and pressure.[5–10] These employ such catalytic transfer hydrogenation agents as cyclohexene,[1,2] hydrazine,[3] cyclohexadiene,[4] formic acid[5–8] and ammonium formate.[9,10] The most useful reactions discovered to date have centered on the use of Pd, with the lesser use of Pt and Rh, and the other transition metals scarcely at all. Furthermore, palladium was the only metal used with all the above said catalytic transfer hydrogenation agents in peptide synthesis. Thus, when cyclohexene and hydrazine are employed as hydrogen donors, temperatures of 70 and 50°C, respectively, have to be maintained during hydrogenolysis, and this requirement is a disadvantage if the products are heat-sensitive peptides. This drawback has been overcome by carrying out hydrogenolysis at room temperature, with formic acid as the hydrogen donor, which may also deblock acid labile protecting groups like such as the *tert*-butyloxycarbonyl group. In certain cases $HCOONH_4$ can advantageously replace formic acid, but it is insoluble in most organic solvents including methanol. We have recently shown that Zn could be employed conveniently as a catalyst either with formic acid, or ammonium formate for the reduction of nitrocompounds to amines.[11] In this context, a detailed survey was undertaken to search for other low-cost metals, which are active with catalytic transfer agents, and we developed the present system, which utilizes hydrazinium monoformate as the hydrogen donor and magnesium as the catalyst for the removal of some commonly used protecting groups in peptide synthesis.

Using methanol as the solvent, a variety of amino acids and peptide derivatives were smoothly deprotected as shown in Table 1. The protecting groups that have been successfully removed include the N^{α}-benzyloxycarbonyl, the N^{ε}-benzyloxycarbonyl and N^{ε}-2-chlorobenzyloxycarbonyl of lysine, a C-terminal benzyl ester, the *O*-benzyl ether of *O*-benzyl-tyrosine, serine or threonine, the nitro group of arginine, the cyclohexyl ester of aspartic and glutamic acids, the 2,6-dichlorobenzyl and bromobenzyloxycarbonyl ether of tyrosine and the benzyloxymethyl and benzyloxycarbonyl of histidine. The progress of the hydrogenolysis could be followed by TLC on silica gel plates. Hydrazinium monoformate itself gives a wide yellow

Keywords: catalytic transfer hydrogenation; hydrazinium monoformate; magnesium; new hydrogenation system; peptide synthesis; deprotection.

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PII: S0040-4039(01)02113-X

Table 1. Catalytic transfer hydrogenolysis of some protected amino acid and peptides using hydrazinium monoformate and Mg

Substrate	Product	Time (h)	Yield (%)[a]	Mp (°C)		Specific rotation $[\alpha]_D^{25}$		Ref.
				Found	Reported	Found	Reported	
Z-Gly	Gly	0.5	92	86–88	86.5–87.5	–	–	12
Z-Phe	Phe	0.5	92	269–275 (dec.)	270–275 (dec.)	−34.7 (*c* 1, H_2O)	−34.4 (*c* 2, H_2O)	13
Z-Trp	Trp	0.5	92	280–285 (dec.)	280–285 (dec.)	−31.8 (*c* 1, H_2O)	−32.2±0.5 (*c* 1, H_2O)	13
Boc-Thr(OBzl)	Boc-Thr	1.0	90	153–154	154–155	+11.3 (*c* 1, CH_3OH)	+11.4 (*c* 0.99, CH_3OH)	14
Boc-Ser(OBzl)	Boc-Ser	2.0	88	141–142	140–142	+13.2 (*c* 3, CH_3OH)	+13.3 (*c* 3.06, CH_3OH)	14
Boc-Asp(OBzl)	Boc-Asp	1.0	95	175–176	176–177	+10.9 (*c* 1, CH_3OH)	+10.9 (*c* 1, CH_3OH)	14
Boc-Asp(OcHx)	Boc-Asp	2.0	93	175–176	176–177	+10.9 (*c* 1, CH_3OH)	+10.9 (*c* 1, CH_3OH)	14
Boc-Glu(OBzl)	Boc-Glu	1.0	95	172–173	171–172	+9.1 (*c* 1, CH_3OH)	+9.1 (*c* 1, CH_3OH)	14
Boc-Glu(OcHx)	Boc-Glu	2.0	94	172–173	171–172	+9.1 (*c* 1, CH_3OH)	+9.1 (*c* 1, CH_3OH)	14
Boc-Tyr(OBzl)	Boc-Tyr	0.5	89	136–137	136–138	+3.6 (*c* 2, CH_3OH)	+3.9 (*c* 2.04, AcOH)	14
Boc-Tyr (2-BrZ)	Boc-Tyr	2.0	89	136–137	136–138	+3.6 (*c* 2, CH_3OH)	+3.9 (*c* 2.04, AcOH)	14
Boc-Lys(Z)	Boc-Lys	0.5	95	202–204	203–204	−10.9 (*c* 1, AcOH)	−10.7 (*c* 0.87, AcOH)	14
Boc-Lys(2-ClZ)	Boc-Lys	1.5	94	202–204	203–204	−10.9 (*c* 1, AcOH)	−10.7 (*c* 0.87, AcOH)	14
Boc-His(Z)	Boc-His	1.0	88	192–196	191–195	+23.5 (*c* 1, CH_3OH)	+24 (*c* 1, CH_3OH)	15
Boc-His(Bom)	Boc-His	2.0	89	192–196	191–195	+23.5 (*c* 1, CH_3OH)	+24 (*c* 1, CH_3OH)	15
Fmoc-Gly	Fmoc-Gly[b]	24	100	–	–	–	–	–
Fmoc-Phe	Fmoc-Phe[b]	24	100	–	–	–	–	–
Z-Phe-Leu-OC_4H_9-*t*	Phe-Leu-OC_4H_9-*t*	0.75	90	Oil	–	Oil	–	16
Boc-Gly-Val-Gly-Val-Pro-OBzl	Boc-Gly-Val-Gly-Val-Pro	0.5	93	126–127	127	−63.9 (*c* 1, CH_3OH)	−64.0 (*c* 1, CH_3OH)	17
Boc-Arg(NO_2)-Pro-Arg(NO_2)-Pro-OBzl	Boc-Arg-Pro-Arg-Pro[c]	2.0	90	70–71	–	−85.0 (*c* 1, CH_3OH)	–	–
Boc-D-Lys(2-ClZ)-Pro-Arg(NO_2)-Pro-OBzl	Boc-D-Lys-Pro-Arg-Pro[c]	2.5	89	82–83	–	−46.1 (*c* 1, CH_3OH)	–	–

[a] Refers to the actual isolated yields; no attempts were made to optimize.
[b] Starting material was recovered.
[c] Satisfactory elementary and amino acid analyses were obtained.

spot with ninhydrin, which sometimes masks the spots due to the deprotected peptide. In such cases a better way to monitor the completion of the reaction is to look for the absence of the spot due to the protected peptide as this is more easily done. The Arg(NO_2) is smoothly deblocked into Arg by this treatment. Under these experimental conditions, the *tert*-butyloxycarbonyl and 9-fluorenylmethyloxycarbonyl groups appear to be quite stable, as indicated by 90–98% yields of Boc and Fmoc protected amino acid and peptide derivatives and the absence of free amino acids, as revealed by the TLC of the crude products.

The results given in Table 1 demonstrate the feasibility of using $Mg/NH_2NH_2{\cdot}HCOOH$ for the removal of most of the commonly used protecting groups in peptide synthesis. All the products were characterized by comparison of their TLC, melting points and specific rotation with authentic samples. Studies were also carried out to determine the optimum conditions for deprotection. These included the excess of donor required, the catalyst, solvent, concentration and reaction temperature. An excess of 2–4 equiv. of hydrazinium monoformate (per protecting group) was found to be ideal. We observed the optimal ratio of catalyst to substrate to be 1:1 by weight for each protecting group to be removed and methanol was the most effective solvent of choice. The deprotection proceeds smoothly when the concentrations of amino acid or peptide substrate are in the range of 0.05–0.25 mmol/mL and at an optimum temperature of ~25°C.

It was observed that this method is equally competitive with other methods of deblocking most of the commonly used protecting groups in peptide synthesis. The advantages of transfer hydrogenation over conventional hydrogenation have been described. Further, hydrazinium monoformate is a more effective hydrogen donor than hydrazine or formic acid and hydrogenolysis can be carried out at room temperature without

removing any acid sensitive protecting groups like *tert*-butyloxycarbonyl, whereas hydrazine requires a temperature of 50°C and formic acid may deblock the acid-sensitive *tert*-butyloxycarbonyl group. An additional advantage of using hydrazinium monoformate as the hydrogen donor is its miscibility with most organic solvents (including methanol) used in peptide synthesis. The protecting groups viz. 2-ClZ, BrZ, Bom or OcHx, which are removed by HF treatment at present, can be conveniently removed by this method. It was also observed that hydrazinium diformate, which is a colorless solid, does not possess any hydrogen donor properties with Mg. The use of $Mg/NH_2–NH_2·HCOOH$ makes this a rapid, low-cost alternative to expensive palladium catalysts and reduces the work-up to a simple filtration and extraction operation. To the best of my knowledge this is the first report of using hydrazinium monoformate as a hydrogen donor and Mg as the catalyst for the removal of hydrogenolysable protecting groups in peptide synthesis. Further investigations of other useful applications particularly in solid phase peptide synthesis are in progress.

Experimental

All the amino acids used except glycine are of L-configuration unless otherwise specified. All protected amino acid derivatives were purchased from Advanced Chem. Tech., (Louisville, KY, USA). Formic acid and hydrazine were purchased from E. Merck and Mg was obtained from Loba Cheme, Bombay, India. The protected peptides used as substrate were obtained in our laboratory as intermediates in the synthesis of different peptide analogues. Thin-layer chromatography (TLC) was carried out on silica gel plates obtained from Whatman Inc., using the following solvent systems: $CHCl_3$–MeOH–HOAc (95:5:3), $CHCl_3$–MeOH–HOAc (90:10:3) and $CHCl_3$–MeOH–HOAc (85:15:3). The compounds on TLC plates were detected by UV light, after spraying with ninhydrin or by chlorine/tolidine. Melting points were determined on a Selaco Can. No-103 and are uncorrected. The optical rotations were determined on a Perkin–Elmer 241MC polarimeter.

The hydrazinium monoformate was prepared by neutralizing slowly, equal moles of hydrazine and formic acid in an ice water bath, with constant stirring. The resulting hydrazinium monoformate solution was used as such in all reactions.

Hydrogenolysis of protected amino acids and peptides with $Mg/NH_2NH_2·HCOOH$

General procedure: To a stirred solution of the appropriately protected amino acid derivative or peptide (200 mg) and Mg (200 mg) in methanol (2.5 mL), hydrazinium monoformate (4 equiv.) was added. The resulting reaction mixture was stirred at room temperature. After completion of the hydrogenolysis (monitored by TLC), the mixture was filtered through Celite and washed with methanol. The combined washings and filtrate were evaporated; the residue was taken up in chloroform and washed with 50% saturated NaCl solution to remove hydrazinium monoformate. The solvent was removed under reduced pressure and triturated with di ethyl ether to obtain Boc-protected amino acid or peptide derivatives. The yields, duration and physical constants are given in Table 1.

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