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Rapid Removal of Protecting Groups from Peptides by Catalytic Transfer Hydrogenation with 1,4-Cyclohexadiene

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1,4-Cyclohexadiene is a very effective hydrogen donor for catalytic transfer hydrogenation. *N*-Benzyloxycarbonyl, benzyl ester, and benzyl ether (tyrosine) protecting groups can be rapidly removed at 25 °C with 1,4-cyclohexadiene and 10% palladium–carbon catalyst. Removal of the N^{im}-benzyl group from histidine, the N^{g}-nitro group from arginine, and the benzyl ether groups from serine and threonine can be carried out at 25 °C using palladium black as catalyst. Cleavage of *N*-benzyloxycarbonyl groups from sulfur-containing amino acids was also achieved by catalytic transfer hydrogenation with 1,4-cyclohexadiene. *tert*-Butyl-derived protecting groups were completely stable under these conditions. The scope of the 1,4-cyclohexadiene-catalyzed transfer hydrogenation for the removal of benzyl-derived protecting groups used in peptide synthesis was examined.

Recent publications[1,2] from two laboratories described the use of catalytic transfer hydrogenation for the removal of several benzyl-type protecting groups used in peptide synthesis. Good yields of homogeneous and nonracemized products were obtained when cyclohexene was used as hydrogen donor at temperatures of >65 °C (refluxing methanol or ethanol). However, in certain cases, especially when *tert*-butyl-derived protecting groups are also present, the danger of thermal decomposition at elevated temperatures might discourage the use of catalytic transfer hydrogenation.

We have examined other hydrogen donors and now report that 1,4-cyclohexadiene is a much more effective donor and can be used to carry out catalytic transfer hydrogenations at 25 °C in the presence of 10% palladium–charcoal. Under these conditions, removal of *N*-benzyloxycarbonyl, benzyl ester, and tyrosine benzyl ether protecting groups was complete within 2 h and good yields of analytically pure amino acids and peptides were obtained directly. The more efficient palladium-black catalyst was required for cleavage of the N^{im}-benzyl group from histidine, N^{g}-nitro group from arginine, and benzyl ether groups from serine and theonine at 25 °C. The scope of the catalytic transfer hydrogenation reaction was evaluated with respect to hydrogen donor, solvent, concentration, catalyst, and reaction temperature.

Results and Discussion

Nature of the Donor. Catalytic transfer hydrogenation has been used for the reduction of a variety of functional groups (including olefins, acetylenes, imines, hydrazones, azo, and nitro compounds).[3] The availability and reactivity of cyclohexene have rendered this reagent a preferred hydrogen donor.[4,5] The rapid disproportionation reported[6] for 1,3-cyclohexadiene and 1,4-cyclohexadiene prompted us to examine their effectiveness as hydrogen donors for the catalytic transfer hydrogenolysis of benzyl-derived protecting groups used in peptide synthesis. We observed that transfer hydrogenation of *N*-benzyloxycarbonyl-L-alanine in ethanol at 25 °C in the presence of 10% palladium–carbon and 1,4-cyclohexadiene required only 1.5 h for complete deprotection. Under the same conditions, using 1,3-cyclohexadiene as the hydrogen donor, the required reaction time for complete removal of the benzyloxycarbonyl group was 8 h. When cyclohexene was used, there was no deprotection, even after 24 h at 25 °C.

Studies were also carried out to determine the excess of hydrogen donor required for optimum deprotection. An excess of 5–10 equiv of 1,4-cyclohexadiene (per protecting group) is ideal. The rate of transfer hydrogenolysis decreased substantially when only 1 equiv of hydrogen donor was used. On the other hand, a large excess of 1,4-cyclohexadiene (50 equiv) produced only a marginal increase in the rate of reaction compared to that observed when 5–10 equiv were used.

Solvent and Concentration. Most of the solvents employed for catalytic hydrogenolysis of peptides are also useful for the catalytic transfer hydrogenation procedure. Glacial acetic acid was the most effective solvent. Transfer hydrogenation of *N*-benzyloxycarbonyl-L-alanine at 25 °C in the presence of 10% palladium–carbon and 10 equiv of 1,4-cyclohexadiene required only 45 min for complete deprotection in glacial acetic acid. Other solvents were also useful for transfer hydrogenation but required somewhat longer reaction times for complete deprotection: ethanol (1.5 h), dimethylacetamide (3 h), methanol (3.5 h), and dimethylformamide (5 h). The following solvents were impractical for catalytic transfer hydrogenation since only partial deprotection was observed at 25 °C even after prolonged periods of reaction: hexamethylphosphoramide, trifluoroethanol, phenol, trifluoroacetic acid, tetrahydrofuran, dimethyl sulfoxide, and isopropyl alcohol.

Literature[1,2] reports on inhibition of catalytic transfer hydrogenation by sulfur-containing amino acids are conflicting. We have observed that transfer hydrogenation in ethanol (using palladium-black catalyst) removed the *N*-benzyloxycarbonyl group from methionine, but not from *S*-benzylcys-

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Table I. 1,4-Cyclohexadiene Catalyzed Transfer Hydrogenation of Protected Amino Acids and Peptides in EtOH at 25 °C

substrate	registry no.	product[a]	registry no.	yield, %[b]	$[\alpha]^{25}_D$, deg, found / standard	mp (°C) found / reported
Z-Ala-OH	1142-20-7	Ala	56-41-7	95	13.35 (c 1.1, 5 N HCl) 13.45 (c 1.2, 5 N HCl)	
Boc-Lys(Z)-OH	2389-45-9	Boc-Lys-OH	13734-28-6	88	21.3 (c 2, MeOH) 21.5 (c 2, MeOH)	195–199.5 200–201[g]
Z-Ser-OBzl	21209-51-8	Ser	56-45-1	99	12.5 (c 2, 5 N HCl) 12.6 (c 2.1, 5 N HCl)	
Boc-Tyr(Bzl)-OH	2130-96-3	Boc-Tyr-OH	3978-80-1	100	43.2 (c 1, DMF)[c] 41.6 (c 1, DMF)[c]	206.5–208.5 211–212[c,h]
Z-Phe-OH	1161-13-3	Phe	63-91-2	99	−5.01 (c 1, 5 N HCl) −4.48 (c 1, 5 N HCl)	
Z-Met-OH[d]	1152-62-1	Met	63-68-3	83	22.7 (c 1, 5 N HCl) 23.2 (c 1, 5 N HCl)	
Boc-His(N^{im}-Bzl)-OH[d]	20898-44-6	Boc-His-OH	17791-52-5	100	25.6 (c 1, MeOH) 24 (c 1, MeOH)	190.5–192.5 191–191.5[i]
Z-Gly-Pro-OH	1160-54-9	H-Gly-Pro-OH	704-15-4	99	−131.6 (c 2, 5 N HCl) −128.1 (c 2, 5 N HCl)[j]	
Boc-Phe-Gln-OBzl	67452-49-7	Boc-Phe-Gln-OH	67452-53-3	84	−3.16 (c 1.1, MeOH) −3.28 (c 1, MeOH)[j]	116–120
Z-Lys(Boc)-Thr(Bu^t)-OMe	65895-38-7	H-Lys(Boc)-Thr(Bu^t)-OMe	65895-41-2	99	−1.51 (c 1, MeOH)	
Z-Arg(NO_2)-Pro-Pro-OBu^t[d,e]	67452-50-0	H-Arg-Pro-Pro-OBu^t	67452-54-4	99	−90.1 (c 1, MeOH)	
Z-Lys(Boc)-Asn-Phe-Phe-OMe[f]	53054-10-7	H-Lys(Boc)-Asn-Phe-Phe-OMe	62437-66-5	85	−36.0 (c 1, DMF)	199–201
Boc-Tyr(Bzl)-Lys(Z)-Lys(Z)-Gly-Glu(OBzl)-OBzl[d,e]	67452-51-1	Boc-Tyr-Lys-Lys-Gly-Glu-OH	67452-36-2	95	−1.00 (c 1, DMF)	
Boc-Ile-Ile-Lys(Z)-Asn-Ala-Tyr(Bzl)-Lys(Z)-Lys(Z)-Gly-Glu(OBzl)-OBzl[d,k]	67452-52-2	Boc-Ile-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly-Glu-OH	67452-37-3	79	−3.80 (c 0.92, 1 N HCl)	

[a] Satisfactory elemental analyses have been obtained for all products. [b] No attempt was made to optimize these yields. [c] Dicyclohexylammonium salt. [d] Transfer hydrogenation carried out with freshly prepared palladium black. [e] Reaction carried out in glacial acetic acid. [f] Reaction carried out in dimethylacetamide. [g] R. Schwyzer, A. Costopanagiotis, and P. Sieber, *Helv. Chim. Acta*, **46**, 870 (1963). [h] D. D. Van Batenberg and K. E. T. Kerling, *Int. J. Pept. Protein Res.*, **8**, 1 (1976). [i] B. O. Hartford, T. A. Hylton, K. Wang, and B. Weinstein, *J. Org. Chem.*, **33**, 425 (1968). [j] J. Meienhofer, unpublished results. [k] Transfer hydrogenation carried out for 20 h at 25 °C.

teine, even in the presence of glacial acetic acid at elevated temperatures. Since liquid ammonia supports catalytic hydrogenolysis of protecting groups of *S*-benzylcysteine and methionine-containing peptides,[7] we examined the transfer hydrogenation using palladium black in refluxing liquid ammonia and observed that *N*-benzyloxycarbonyl groups are completely cleaved from methionine but only partially from *S*-benzylcysteine.

Catalytic transfer hydrogenation proceeds at the rate described above when the concentrations of amino acid or peptide substrate are in the range of 0.05–0.25 mmol/mL. At lower concentration (<0.025 mmol/mL) the rate of reaction decreases substantially. There was no significant advantage in working at higher concentration (>0.5 mmol/mL) and the heat generated from the exothermic reaction (following a short induction period) was not fully dissipated. We recommend the use of a very slow stream of nitrogen in a vibro-mixer reaction apparatus (Figure 1) for proper agitation and a 25 °C bath for temperature control. The vibro-mixer compared favorably to magnetic stirring and reactions went to completion in almost half the time. Under the conditions outlined above the reaction mixtures remained at 25 °C for the course of the reaction. However, larger scale catalytic transfer hydrogenation reactions (>0.05 mol), even at concentrations of 0.05–0.25 mmol/mL, required initial immersion in an ice bath to ensure dissipation of the heat generated during the addition of 1,4-cyclohexadiene.

Effect of Catalyst. Palladium catalysts have been reported to be most effective for transfer hydrogenation.[8] Removal of a large number of benzyl-derived protecting groups has been carried out with a variety of amino acids and peptides. Table I shows examples of isolated products. *N*-Benzyloxycarbonyl, benzyl ester, and tyrosine benzyl ether protecting groups were removed at 25 °C using 10% palladium–charcoal within 1.5 h. Freshly generated Raney nickel[9] was completely ineffective even after prolonged reaction times. Other palladium catalysts were useful for transfer hydrogenation but required longer reaction times for complete deprotection, e.g., 5% palladium–charcoal (2.5 h), 10% palladium–$BaSO_4$ (6 h), and 5% palladium–$BaSO_4$ (12 h). Freshly prepared palladium black is a much more active catalyst and cleavage of the *N*-benzyloxycarbonyl group was complete within 5 min. This catalyst was required for the removal of the following more stable protective groups: histidine N^{im}-benzyl, threonine and serine benzyl ether, methionine *N*-benzyloxycarbonyl, and *N*-[2-(*p*-biphenyl)-2-propyloxycarbonyl]. Deprotection of the N^g-nitro group of arginine required the use of palladium black in glacial acetic acid.

A large excess of catalyst improved the rate of transfer hydrogenation. We observed the optimal ratio of catalyst to substrate to be 1:1 by weight for each protecting group to be removed. Larger amounts of catalyst resulted in only minor improvement. However, the rate of transfer hydrogenation was significantly slower when smaller amounts of catalyst (catalyst: substrate < 0.5:1) were used.

Effect of Temperature. Temperature is a critical variable in catalytic transfer hydrogenation[10] and depends on the nature of the hydrogen donor. The oxidation potential for 1,4-cyclohexadiene appears to be low and consequently the optimum temperature seems to be ~20 °C (see Table II). At temperatures below 20 °C the rate of deprotection of the *N*-benzyloxycarbonyl group diminished rapidly. At higher temperatures the reaction time showed only marginal improvement.

Thermal decomposition of certain peptides may occur when *tert*-butyl derived protecting groups (including *tert*-butyloxycarbonyl, *tert*-butyl ether, *tert*-butyl ester) are used. This can be a serious problem when reactions are carried out at elevated temperatures over a prolonged reaction time. Ambient temperature is therefore particularly advantageous for catalytic transfer hydrogenation. Table I lists several examples of selective cleavage of benzyl-derived protecting groups in the presence of *tert*-butyloxycarbonyl, *tert*-butyl ether, and *tert*-butyl ester functions. The catalytic transfer hydroge-

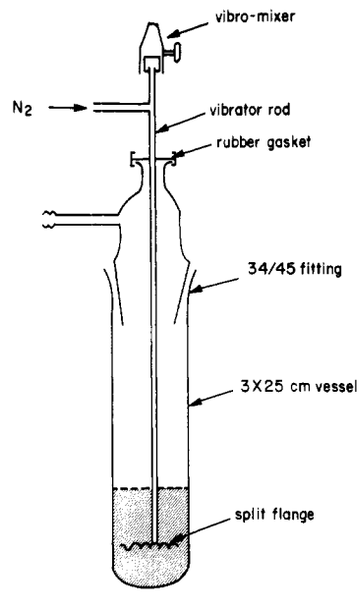

Figure 1. Apparatus for catalytic transfer hydrogenation using vibromixing for uniform suspension of the catalyst throughout the reaction mixture and efficient nitrogen distribution.

nation procedure was successfully applied to the deblocking of a number of representative protected amino acids and peptides shown in Table I including a pentapeptide and decapeptide with multiple benzyl-derived protecting groups.

Experimental Section

1,4-Cyclohexadiene (Aldrich Chemical Co.) was kept refrigerated and used directly. All amino acids were of the L configuration and were either derivatized by standard methods or purchased from Bachem Inc. or Chemical Dynamics Corp. Dimethylformamide (reagent grade, Matheson Coleman and Bell) was distilled from ninhydrin and stored over molecular sieve. All other solvents were of reagent grade and used without purification. Melting points were determined on a Reichert hot-stage apparatus and are uncorrected. Optical rotations were measured in a jacketed 1-dm cell on a Perkin-Elmer Model 141 polarimeter. NMR spectra were compatible for all products isolated. Palladium catalysts were obtained from Englehard Industries. Palladium black was freshly generated and introduced directly into the reaction mixture using a syringe filter.[11] The catalytic transfer hydrogenations were carried out in a 3 × 25 cm reaction vessel (Figure 1) connected by means of a standard taper 34/45 fitting to a Vibrator rod (Chemapec Inc., No. 500-10117). The rod was connected to a Vibromixer E1 (No. 500-10180) apparatus which was adjusted for proper agitation. The lower end of the rod was fitted with a split phlange (No. 500-10134) which was immersed just below the surface of the reaction mixture. All the parts for the transfer hydrogenation system were purchased from Chemapec Inc. (Hoboken, N.J.).

Table II. Effect of Temperature on Catalytic Transfer Hydrogenation[a]

temp, °C	react time[b]	temp, °C	react time[b]
0	incomplete (8 h)	25	1.5 h
10	4.5 h	30	1.0 h
20	1.5 h	35	40 min

[a] Z-Ala-OH (0.25 mmol/mL EtOH); 10% Pd–C (catalyst:Z-Ala-OH, 1:1); 1,4-cyclohexadiene (10 equiv). [b] 100% deprotection.

Procedure for Catalytic Transfer Hydrogenation. The substrate (1.0 mmol) was dissolved in 4 mL of absolute ethanol placed in the 3 × 25-cm reaction vessel (see solvent concentration section for alternate solvents depending on solubility of substrate) and immersed in a water bath at 25 °C. A gentle stream of nitrogen was passed through the reaction mixture and thorough agitation was provided by the vibro-mixer. An equal weight of 10% palladium–carbon (per protecting group) was added followed by the addition of 1,4-cyclohexadiene (0.94 mL, 10.0 mmol). The reaction proceeded for a minimum of 2 h and the mixture was filtered (celite), washed with solvent (depending on the solubility of the product, a variety of solvents may be used, e.g., DMF, acetic acid, water), and evaporated under reduced pressure. Products were generally obtained in 90–100% yields.

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Registry No.—1,4-Cyclohexadiene, 628-41-1.

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