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CATALYTIC HYDROGENOLYSIS IN LIQUID AMMONIA: STABILITY AND CLEAVAGE OF SOME PROTECTING GROUPS USED IN PEPTIDE SYNTHESIS

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High selectivity of cleavage and general applicability to all commonly occurring amino acids are important criteria in choosing useful combinations of main chain and side chain protecting groups in peptide synthesis. The most selective procedure available to date has been catalytic hydrogenolysis[1] of N^{α}-benzyloxycarbonyl (Z)[2] groups in peptides whose side chain functions have been protected by *tert*-butyl ester (OBu^t)[3], *tert*-butyl ether (Bu^t)[4], and/or *tert*-butyloxycarbonyl (Boc)[5] groups which completely resist hydrogenolysis. However, this excellent system has not been generally applicable since catalytic hydrogenolysis failed with cysteine- or methionine containing peptides due to catalyst poisoning. Attempts to overcome this restriction by addition of tertiary base[6] or of boron trifluoride etherate[7] to hydrogenolysis mixtures, or by the use of the N^{α}-1,1-dimethyl-2-propynyloxycarbonyl group[8] which may by hydrogenolized with partially poisoned catalysts have, as yet, found limited application.

We wish to report that palladium-catalyzed hydrogenation effects quantitative cleavage of N^{α}-benzyloxycarbonyl groups from methionine- and S-benzylcysteine-containing peptides when liquid ammonia is used as a solvent. Liquid ammonia was shown by du Vigneaud *et* al.[9] to be a powerful solvent for many amino acid derivatives and protected peptides, some of which possess low solubility in commonly used organic solvents. The efficacy for peptide synthesis of catalytic hydrogenolysis in liquid ammonia of N^{α}-Z groups from S-benzylcysteine-containing peptides was demonstrated by a successful synthesis of oxytocin, which will be reported elsewhere.[10]

We describe herein the cleavage or resistance of some frequently used protecting groups when representative amino acid derivatives were subjected to palladium-catalyzed hydrogenation in refluxing liquid ammonia (approx. $-33^{\circ}C$). All glassware was thoroughly dried. Anhydrous

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ammonia (Matheson) was passed over KOH pellets and condensed in a three-neck round bottom flask immersed in dry ice-acetone and fitted with drying tube and dry ice reflux condenser. In a typical experiment 1 mmol of protected amino acid or peptide was dissolved in 50 to 150 ml of liquid NH_3 with magnetic stirring. Freshly prepared palladium black[11] (0.2-0.5 g; Pd on charcoal was less effective), freed from water by thorough washing with anhydrous ethanol, was added in methanol-wet form under a nitrogen barrier. A stream of dried (conc. H_2SO_4) hydrogen was continuously passed through the stirred refluxing solution until thin layer chromatography showed complete reaction (3-8 hours). Stability tests were conducted for similar periods of time. Evaporation of the ammonia was aided by a stream of dry nitrogen. Residues were dissolved in dimethylformamide, methanol, or water, the catalyst removed by filtration and the solvent evaporated to give products that were mostly homogeneous in thin layer chromatography and were crystallized, some as dicyclohexylammonium salts.[12] Results are summarized in Table I. Yields given are those of isolated products.

Completely cleaved were benzyl ester, benzyl ether, 2,6-dichlorobenzyl ether, N-benzyloxycarbonyl, N-2-bromobenzyloxycarbonyl, N-4-methoxybenzyloxycarbonyl and the nitro group of nitroarginine. Complete stability toward hydrogenolysis in liquid ammonia was shown by *t*-butyl ester, *t*-butyl ether, N-*t*-butyloxycarbonyl, N-p-toluenesulfonyl, and by S-benzyl and S-acetamidomethyl[15] groups.

The results indicate (a) that the ideal protecting group combination[25] using N^{α}-benzyloxycarbonyl along with *t*-butyl derived side chain protection can be applied to synthesis of cysteine- and methionine-containing peptides if catalytic hydrogenolysis is conducted in liquid ammonia, and (b) that liquid ammonia might be a useful solvent for hydrogenating protected peptides that possess poor solubility in other suitable solvents.

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2. Abbreviations: Acm, acetamidomethyl; Boc, *tert*-butyloxycarbonyl; BrZ, 2-bromobenzyloxycarbonyl; Bu^t, *tert*-butyl ether; Bzl, benzyl; Cl_2Bzl, 2,6-dichlorobenzyl ether; DMF, dimethylformamide; MeOZ, 4-methoxybenzyloxycarbonyl; OBzl, benzyl ester; OBu^t, *tert*-butyl

ester; Tos, p-toluenesulfonyl; Z, benzyloxycarbonyl.

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TABLE I. Palladium-catalyzed Hydrogenation of Protected Amino Acids and Peptides in Refluxing Liquid Ammonia

Starting Material[a]	Time [hr]	Product (Crystalliz. Solvent)	Isol. Yield [%]	tlc[b] [Rf]	mp [°C] Found / Lit.	Optical Rotation $[\alpha]_D^{21}$ Found / Lit.
Z-Ala-OH	3	H-Ala-OH (95% ethanol)	93	0.20(C)	-	+15.0° (c0.5, 6 N HCl) / +13.0° (c2, 5N HCl)[13]
Z-Cys(Bzl)-OH	5	H-Cys(Bzl)-OH (H_2O-ethanol)	76	0.43(C)	222 / 221-222.5[14]	+25.8° (c1, 1 N NaOH / +24.5° (c1, 1 N NaOH[14]
Z-Cys(Acm)-OH	3	H-Cys(Acm)-OH (H_2O-ethanol)	83	0.12(C)	189-190 / 187 dec[15]	-38.9° (c1, H_2O) / -42.5° (c1, H_2O)[15]
Z-Met-OH	5	H-Met-OH (H_2O-methanol)	82	0.15(B)	-	+34.1° (c1, 5N HCl) / +34.6° (c1, 5N HCl)[16]
Z-Cys(Bzl)-Gly-NH_2	6	H-Cys(Bzl)-Gly-NH_2	-	0.05(B)	-	-
Z-Leu-Tyr-Leu-Val-Cys(Bzl)-Gly-OH[17]	3	H-Leu-//-Cys(Bzl)-Gly-OH (H_2O-ethanol)	64	0.62(C)	188-189 / -	-
Z-Asp(OBu^t)-OH	2	H-Asp(OBu^t)-OH[c]	-	0.15(B)	-	-
Z-Tyr(Bu^t)-OH	-	H-Tyr(Bu^t)-OH	84	0.14(B)	199-201[d] / 248-249[18]	-23.0° (c0.6, H_2O) / -25.8° (c1, H_2O)
MeOZ-Val-OH	4	H-Val-OH (H_2O)	91	0.32(C)	-	+28.8° (c1, 6 N HCl) / +33.1° (c1, 5N HCl)[e]
Boc-Lys(BrZ)-OH	4	Boc-Lys-OH (ethanol-ether)	92	0.18(A) 0.55(C)	201-202 / 202-203[19]	-
Boc-Tyr(Bzl)-OH	3	Boc-Tyr-OH x DCHA (ether)	73	0.08(A) 0.69(C)	212 / 211-212[20]	+23.6° (c1, DMF) / $[\alpha]_{578}^{20}$ +26.5° (c1, DMF)[20]
Boc-Tyr(Cl_2Bzl)-OH	4	Boc-Tyr-OH x DCHA (ether)	87	0.43(B) 0.69(C)	212 / 211-212[20]	+23.6° (c1, DMF) / $[\alpha]_{578}^{20}$ +26.5 (c1, DMF)[20]
Boc-Thr(Bzl)-OH	7	Boc-Thr-OH x DCHA (ethyl acetate-hexane)	95	0.22(B)	153-154 / 154-155[21]	+10.2° (c1, methanol) / +11.4° (c1, methanol)[21]
Boc-Asp(OBzl)-OH	4	Boc-Asp-OH x 2 DCHA[c] (methanol-ether)	99	0.50(B)	177-177.5 / 176-177[22]	+10.5° (c1, methanol) / +10.9 (c1, methanol)[22]
Boc-Glu(OBzl)-OH	4	Boc-Glu-OH x 2 DCHA[f] (ethyl acetate-ether)	98	0.18(A)	176-176.5 / 171-172[23]	+10.3° (c1, methanol) / +9.1° (c1, methanol)[23]
Boc-Arg(NO_2)-OH	8	Boc-Arg-OH	-	0.3(B)	-	-
Tos-Gly-OH	3	Tos-Gly-OH	92	0.18(A) 0.55(B)	149-150 / 149-150[24]	-

[a] Abbreviations, see Ref. 2. [b] Silica gel. Solvent systems: (A) chloroform-methanol [5:1]; (B) chloroform-methanol-acetic acid [8:1:1]; (C) n-butanol-acetic acid-water [3:1:1]. [c] No asparagine was detected by amino acid analysis after a 5 hr reaction period. [d] Pure product by NMR spectrometry. [e] Ref. 13, p 2368. [f] No glutamine detectable.