

CLEAVAGE OF PEPTIDE TO SPARROW-RESIN BOND BY CATALYTIC TRANSFER HYDROGENOLYSIS  
WITH 1,4-CYCLOHEXADIENE

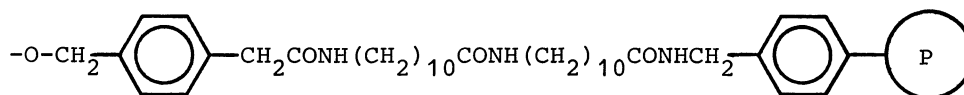
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Cleavage of benzyl ester-type linkage to the Sparrow modified polystyrene support was attempted with the aid of catalytic transfer hydrogenation using 1,4-cyclohexadiene and palladium black, and satisfactory yields were obtained. This will provide an alternatively efficient and mild method for the final deblocking step during improved solid phase peptide synthesis.

Several recent publications<sup>2</sup> described the use of catalytic transfer hydrogenation for the removal of benzyl-type protecting groups used during both conventional and solid phase peptide synthesis. Moreover, Schlatter *et al.*,<sup>3</sup> Jones<sup>4</sup> and Khan & Sivanandaiah<sup>5</sup> reported that classic H<sub>2</sub> catalytic hydrogenation<sup>3,4</sup> and catalytic transfer hydrogenolysis<sup>5</sup> can cleave a peptide bound through an ester link to the conventional Merrifield cross-linked polystyrene resin, although in non-quantitative yields. However, Erickson and Merrifield referred, in a review,<sup>6</sup> to unsuccessful attempts to hydrogenolyze the resin benzyl ester bond using a variety of catalysts. The use of hydrogenolysis as a cleavage method for the removal of protected amino acids and peptides from the 3-nitro-4-bromomethylbenzoyl polyethylene glycol support used in liquid-phase peptide synthesis, was also reported.<sup>7</sup>

Prompted by these results and with a view to search for an alternative method in opposition to the strongly acidic conditions for the final cleavage during solid phase peptide synthesis (HF or HBr-CF<sub>3</sub>COOH), I tried to use hydrogenolysis for the cleavage of an ester link to the modified resin of Sparrow,<sup>8</sup> namely p-oxymethyl phenylacetamido-11-hendecanamido-11-hendecanamido-methyl polystyrene resin, 1% cross-linked with divinyl benzene:



This very promising solid support has an increased acid stability, which avoids the generation of hydroxymethyl groups due to the gradual acidolytic loss of peptide chains during the synthesis, and a spacer group between the point of attachment of the first amino acid residue and the polymer matrix, which allows the removal of the growing peptide chain from the vicinity of the polystyrene backbone.

At first, classic H<sub>2</sub> catalytic hydrogenation was applied to Boc-valine resin ester, but this gave no satisfactory yields of Boc-valine even if high temperatures were employed for a long reaction time: a recovery of Boc-valine (51% yield) was obtained after 22 hr (16 atm, 65°C) in the presence of palladium black, generated *in situ* from palladium (II) acetate. Furthermore, the danger of thermal decomposition

TABLE. 1,4-Cyclohexadiene Catalyzed Transfer Hydrogenation of Protected Amino Acid- and Peptide-Resins

Substrate	Product <sup>a</sup>	Yield %				Mp (°C) found	$[\alpha]_D^{25}$ , deg found
		I	II	III	IV		
Boc-Gly-OResin	Boc-Gly-OH	76	80	90	91	87-88 (86-88) <sup>d</sup>	
Boc-Val-OResin	Boc-Val-OH	83	86	88	89	73-79 (75-79) <sup>d</sup>	-6.1 (c=1, AcOH)
Boc-Leu-OResin	Boc-Leu-OH <sup>c</sup>	79	82	84	86	82-83 (83-84) <sup>d</sup>	-24.5 <sup>d</sup> (c=2, AcOH)
Boc-Met-OResin	Boc-Met-OH	51	66	67	67	138-140 (139-141) <sup>d,e</sup>	+21.0 <sup>d,e</sup> (c=2, EtOH)
Z-Phe-OResin	Phe	80	84	89	90		-4.9 (c=1, 5N HCl)
Boc-Ser (Bzl)-OResin	Boc-Ser-OH	75	78	81	84		<sup>m</sup>
Boc-Tyr (Bzl)-OResin	Boc-Tyr-OH	80	87	89	89	208-210 (211-212) <sup>e,f</sup>	+42.7 (c=1, DMF)
Boc-Lys (Z)-OResin	Boc-Lys-OH	73	81	87	87	198-199 (200-201) <sup>g</sup>	+20.8 (c=2, MeOH)
Boc-His (N <sup>i,m</sup> -Bzl)-OResin	Boc-His-OH	84	89	91	92	190-192 (191-192) <sup>h</sup>	+25.3 (c=1, MeOH)
Boc-Phe-Gln-OResin	Boc-Phe-Gln-OH	74	79	81	82	117-119 (116-120) <sup>i</sup>	-3.0 (c=1, MeOH)
Boc-Tyr (Bzl)-Gly-Gly-Phe-Val-OResin	Boc-Tyr-Gly-Gly-Phe-Val-OH	76	81	83	84		+5.1 (c=1, MeOH)
Boc-Tyr (Bzl)-Lys (Z)-Lys (Z)-Gly-Glu (OBzl)-OResin	Boc-Tyr-Lys-Lys-Lys-Gly-Glu-OH	77	82	85	87		-1.8 (c=1, DMF)

<sup>a</sup> Satisfactory elemental analyses have been obtained for all products. <sup>b</sup> After the first (I), the second (II), the third (III), and the fourth (IV) catalytic transfer hydrogenation. <sup>c</sup> Monohydrate. <sup>d</sup> Before coupling to the resin. <sup>e</sup> Dicyclohexylammonium salt. <sup>f</sup> Ref. 15. <sup>g</sup> Ref. 16. <sup>h</sup> Ref. 17. <sup>i</sup> Ref. 2d. <sup>j</sup> Standard. <sup>k</sup> Ref. 3. <sup>m</sup> +12.3 (c=2, 5M HCl); determined as free serine, after N<sup>o</sup>-deprotection with 50% trifluoroacetic acid in methylene chloride followed by ion-exchange chromatography. <sup>n</sup> Purified by partition chromatography on Sephadex LH-20 using the system CHCl<sub>3</sub>-MeOH-AcOH-H<sub>2</sub>O (7:3:2:4) with a 5 x 60 cm column and a flow rate of 50 ml per hr.

ABBREVIATIONS: AcOH, acetic acid; EtOH, ethanol; DMF, dimethylformamide; MeOH, methanol.

tion at elevated temperatures discouraged these attempts.

Afterwards, in view of the recent report of Felix *et al.*<sup>2d</sup>, it was considered the use of catalytic transfer hydrogenation. Yields were evaluated and optimized with respect to hydrogen donor, solvent, concentration, catalyst, and reaction temperature. 1,4-Cyclohexadiene was found to be, together with *in situ* generated palladium black, the most effective donor, and can be used to carry out hydrogenations at reasonable temperatures (30-40°C) in dimethylformamide or glacial acetic acid. The results are shown in the Table.

In a typical experimental procedure, Merrifield resin (1% cross-linked, 0.75 meq of Cl per g, Bio-Rad) was converted to the modified resin of Sparrow as described by this author,<sup>8</sup> and Boc-amino acids were esterified to the resin as their cesium salts according to the method of Gisin.<sup>9</sup> The substituted resin was dried *in vacuo* over P<sub>2</sub>O<sub>5</sub>, and substitution estimated by weight gain and by the method of Gisin.<sup>10</sup> To a suspension of the substrate-resin (1.0 mmol) in dimethylformamide (15 ml), palladium (II) acetate (2.2 g) was added and the mixture stirred in a vibro-mixing reaction vessel.<sup>2d</sup> After 4 hr at room temperature (the mixture was allowed to equilibrate and the catalyst to diffuse and to penetrate the resin beads) the suspension was then maintained at 35-40°C (bath temperature) under nitrogen, and 1,4-cyclohexadiene (2 ml) added. The reaction proceeded for about 3 hr, the cooled mixture was quantitatively filtered (celite) and repeatedly washed with a suitable solvent (dimethylformamide, acetic acid, ethanol may be used, depending on the solubility of the product). This first filtrate was evaporated under reduced pressure, and the product content was determined. The resin was suspended again in dimethylformamide and thrice submitted to the previously described treatment. The second, third and fourth filtrates were evaporated independently, and the product contents were determined. *In situ* generated palladium black was found to be the most effective catalyst; other palladium catalysts may be useful (5-10% palladium-charcoal, 5-10% palladium-BaSO<sub>4</sub>), but require longer reaction times and often afford lower yields.

The three protected peptide resins used in the present study (Boc-Phe-Gln-O-Resin, Boc-Tyr(Bzl)-Gly-Gly-Phe-Val-OResin, and Boc-Tyr(Bzl)-Lys(Z)-Lys(Z)-Gly-Glu(OBzl)-OResin) were prepared by stepwise synthesis from the carboxyl-terminal amino acid resin, employing preformed symmetrical anhydrides<sup>11</sup> of the suitably protected amino acids. Deprotection of the  $\alpha$ -amino protected intermediates on the resin was accomplished with 50% trifluoroacetic acid in methylene chloride, with neutralization on the resulting salt by 10% triethylamine in methylene chloride to give the free amino group. Completeness of coupling was monitored by the ninhydrin color test procedure of Kaiser *et al.*<sup>12</sup> All the amino acids were of the L configuration.

As from the Table, three times catalytic hydrogenations are usually enough to obtain the product in satisfactory yields; the fourth hydrogenation produced only a marginal increase in the yield of reactions.

Although literature reports<sup>13,2b,2d</sup> on inhibition of catalytic transfer hydrogenation by sulfur-containing amino acids are conflicting, it was possible to cleave Boc-methionine from the solid support; nevertheless lower yields were obtained

comparison with other amino acids and peptides.

The present results thus provide an efficient and convenient method for the final cleavage of peptide to Sparrow resin bond during solid phase peptide synthesis, which requires neither an acid-labile peptide to resin linkage (e.g., p-alkoxybenzyl alcohol resin<sup>14</sup>) nor the use of photo-labile anchorages (e.g., o-nitrobenzyl alcohol modified supports) to enable improved synthesis to be accomplished under mild reaction conditions. Further work to maximize the yields and to apply this method to the synthesis of natural peptides is underway.

#### REFERENCES

1. *Recipient of a National Research Council (C.N.R., Italy) Fellowship.*
2. (a) A.E. Jackson and R.A.W. Johnstone, *Synthesis*, 1977, 685; (b) G.M. Anantharamaiah and K.M. Sivanandaiah, *J. Chem. Soc. Perkin Trans. I*, 1977, 490; (c) M.K. Anwer, S.A. Khan, and K.M. Sivanandaiah, *Synthesis*, 1978, 751; (d) A.M. Felix, E.P. Heimer, T.J. Lambros, C. Tzougraki, and J. Meienhofer, *J. Org. Chem.*, 43, 4194 (1978); (e) K.M. Sivanandaiah and S. Gurusiddappa, *J. Chem. Research (S)*, 1979, 108; (f) B. Elamin, G.M. Anantharamaiah, G.P. Royer, and G.E. Means, *J. Org. Chem.*, 44, 3442 (1979).
3. J.M. Schlatter, R.H. Mazur, and O. Goodmanson, *Tetrahedron Lett.*, 1977, 2851.
4. D.A. Jones Jr., *Tetrahedron Lett.*, 1977, 2853.
5. S.A. Khan and K.M. Sivanandaiah, *Synthesis*, 1978, 750.
6. B.W. Erickson and R.B. Merrifield, "Solid Phase Peptide Synthesis", in *The Proteins*, vol. II, 3rd ed., H. Neurath, R.L. Hill, and C. Boeder eds., Academic Press, New York, 1976.
7. F.S. Tjoeng and R.S. Hodges, *Tetrahedron Lett.*, 1979, 1273.
8. J.T. Sparrow, *J. Org. Chem.*, 41, 1350 (1976).
9. B. Gisin, *Helv. Chim. Acta*, 56, 1476 (1973).
10. B. Gisin, *Anal. Chim. Acta*, 58, 248 (1972).
11. H. Hagenmaier and H. Frank, *Hoppe-Seyler's Z. Physiol. Chem.*, 353, 1973 (1972).
12. E. Kaiser, R.L. Colescott, C.D. Bossinger, and P.I. Cook, *Anal. Biochem.*, 34, 595 (1970).
13. A.E. Jackson and R.A.W. Johnstone, *Synthesis*, 1977, 685.
14. S.S. Wang, *J. Am. Chem. Soc.*, 95, 1328 (1973).
15. D.D. Van Batenberg and K.E.T. Kerling, *Int. J. Pept. Protein Res.*, 8, 1 (1976).
16. R. Schwyzer, A. Costopanagiotis, and P. Sieber, *Helv. Chim. Acta*, 46, 870 (1963).
17. B.O. Hartford, T.A. Hylon, K. Wang, and B. Weinstein, *J. Org. Chem.*, 33, 425 (1968).

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