Formation of By-products during Sodium–Liquid Ammonia Reduction in Peptide Chemistry

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Reduction of protected model peptides by the usual excess of sodium in liquid ammonia leads to undesired by-products; the generally accepted blue colour for the end point is unnecessary for complete reduction.

Sodium–liquid ammonia reduction for the removal of benzyl-type and tosyl protecting groups of peptides is almost as old as modern peptide chemistry itself,[1] and contributed substantially to the first syntheses of some biologically active peptides, *e.g.* glutathione, oxytocin, *etc.* The systematic investigation of the side reactions accompanying the cleavage of protecting groups by sodium–liquid ammonia reduction is so far limited almost entirely to proline peptides, though several other undesired transformations have been qualitatively mentioned.[2]

We have found that the removal of benzyl-type protecting groups in liquid ammonia is complete with nearly the theoretical amount of sodium, which is generally less than is necessary to reach the usual blue end-point.

In model experiments a protected derivative of an amino-acid or peptide (10 mmol) dissolved in dry liquid ammonia (150 ml) was treated with sodium (10 mg-atom amounts). Ammonium chloride equivalent in quantity to the sodium consumed was added. The resulting solution was evaporated to dryness, and the residue dissolved in water, ethanol, or water–ethanol (50—100 ml). The pH was adjusted to 3—7 depending on the subsequent work-up. Toluene and bibenzyl were formed in reactions when benzyl-type groups were cleaved. The products were separated by extraction and/or column chromatography, and identified by i.r., ^{1}H and ^{13}C n.m.r., and mass spectroscopy, or in some cases by comparison with authentic samples. The following undesired side reactions, mostly unknown in peptide chemistry, were observed.

(i) Formation of hydantoin derivative. Reduction of Z-Pro-NH_2† (5 mmol) with sodium (10 mg-atom) gave the corresponding hydantoin derivative [10% yield, m.p. 163—164 °C, ν(KBr) 3170 (NH) and 1760—1680 cm^{-1} (br., CO)] besides the expected H-Pro-NH_2. The by-product was identified by comparison with an authentic sample.[3] The use of a smaller proportion of sodium resulted in incomplete reduction, while the by-product decomposed to several unidentified compounds on addition of more sodium to give a mixture with a lasting blue colour.

(ii) Reduction of $>CONH_2$ to $>CHOH$. Reduction of protected derivatives of amino-acids containing a carboxamide group partially yielded hydroxy-derivatives if the amount of sodium used was sufficient for formation of a salt with the carboxamide group. This reaction was reported for simple compounds in 1912, but has so far escaped the attention of peptide chemists.[4]

Thus the reduction of Boc-Asn-OH with sodium (2 equiv.) gave Boc-Hse-OH, isolated as its dicyclohexylammonium salt [24·5% yield, m.p. 143—146 °C, $[\alpha]_D^{25}$ −3.25° (*c.* 2.0, dimethylformamide)], which was identical with an authentic sample prepared as described earlier.[5] However, no transformation occurred with 1 equiv. of sodium. Similar reactions have been observed in reduction of Boc-Gly-NH_2, Boc-Asp-NH_2, Boc-Gln-OH, Boc-Phe-NH_2, and Boc-Tyr-NH_2.

(iii) Cleavage of the Boc group. t-Butyl carbamate [m.p. 105—108 °C, ν(KBr) 3445, 3330, 3260, 3200 (NH), 1673 (br., CO), and 1165 cm^{-1} (br., C–O–C), δ ($CDCl_3$) 1.4 (s, 9H, Bu^t) and 6.0 (br., 2H, exchangeable with D_2O, NH_2)] was formed during the treatment of Boc-amino acid α-amides with sodium. For example, besides Boc-tyrosinol [m.p. 112—114 °C, ν (KBr) 3390 (NH), 3200 (br., OH), 1685 and 1654 (CO), 1220 (Ar-OH), and 1170 cm^{-1} (C–O–C), δ (CD_3SOCD_3 + D_2O) 1.3 (s, 9H, Bu^t), 2.3—3.8 (m, 5H, CH_2 + CH_2O + CH), 6.7 and 7.1 (2 × 2H, d, Ar-H), and 4.0 (br., exchangeable with D_2O, NH + OH)], and a deaminated compound (see next section), t-butyl carbamate (8% yield) was isolated from the reaction of Boc-Tyr-NH_2 with 2 equiv. of sodium. Boc cleavage by sodium in liquid ammonia has not been reported earlier.[6]

(iv) Deamination. 3-(*p*-Hydroxyphenyl)propionamide [(12% yield, m.p. 119—121 °C, ν (KBr) 3500—3050 (br., OH + NH), 1630 (br., CO), and 1230 cm^{-1} (Ar-OH), δ (CD_3SOCD_3 + D_2O) 2.3 (t, 2H, α-CH_2), 2.8 (t, 2H, β-CH_2), 6.8 and 7.1 (2 × 2H, d, Ar-H), and *ca.* 3.6 (br., exchangeable with D_2O, NH_2 + OH)] was isolated from the reaction of Boc-Tyr-NH_2 with 2 equiv. of sodium. Reduction of Boc-Phe-NH_2 with 1 equiv. of sodium gave the corresponding by-products as described here and in the previous section.

(v) Simultaneous deamination and transpeptidation. Treatment of Z-Asn-Gly-NH_2 with 3 equiv. of sodium resulted in a mixture of H-Asn-Gly-NH_2 (80%), and H-Asp-Gly-NH_2 + H-β-Asp-Gly-NH_2 (total 20%, β-isomer predominating), as estimated by electrophoretic comparison with authentic samples synthesized by conventional methods. With 2 equiv. of sodium only 1—2 % of by-products were formed. Z-Gln-Gly-NH_2 showed no tendency to undergo this transformation.

Our results confirm that in sodium–liquid ammonia reduction in peptide chemistry the use of sodium in an excess to reach the generally accepted blue end-point leads to undesired transformations, which can be minimized or eliminated if at most a slight excess, relative to the theoretical amount necessary to cleave the benzyl-type protecting groups of sodium is used. The amount of the excess depends on the structure of the peptide, which may contain different end and side-chain functional groups which themselves consume sodium.

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† Z = benzyloxycarbonyl, Boc = t-butoxycarbonyl, Hse = L-homoserine.

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