The Cathodic Cleavage of the 4-Nitrobenzyloxycarbonyl Group from Amine Derivatives in Aprotic Conditions

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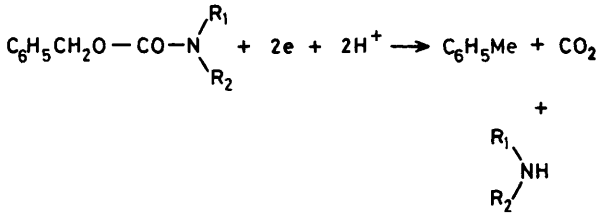
The cathodic cleavage of the 4-nitrobenzyloxycarbonyl group from the urethane derivatives of four amines, two primary and two secondary, in dimethylformamide is reported. It is shown by cyclic voltammetry that the derivatives are reduced in several steps. The first occurs at about −1.1 V *vs.* s.c.e. and on the timescale of cyclic voltammetry is a reversible 1e process. On the timescale of several minutes, however, the anion radicals are unstable and cleavage of the 4-nitrobenzylcarbonyl group occurs. Coulometry then shows the reduction to be a 2e process and free amines are isolated in above 80% yields. Hence reduction at −1.2 V in aprotic conditions seems to be the basis of a straightforward procedure for the removal of 4-nitrobenzyloxycarbonyl group and this increases the scope for the use of this protecting group in the synthesis of large molecules. The influence of proton donors on the reduction of the urethane derivatives is also discussed.

Most peptide syntheses are carried out *via* intermediates with urethane protecting groups.[1–4] The most important such group is the benzyloxycarbonyl group and it has been shown that cathodic reduction in aprotic solvents is a general procedure for its removal. The overall reaction is:



Mairanovsky[5] has reviewed the early work while, more recently, we have re-examined the mechanism and utility of the cathodic cleavage of the benzyloxycarbonyl group from simple amines, amino acids, and peptides.[6,7] It has to be recognised, however, that the cleavage reaction only occurs at very negative potentials, from −2.7 V to −2.9 V *vs.* saturated calomel electrode s.c.e., where difficulties such as solvent/electrolyte reduction and reduction of other groups in the molecule will be most severe. For this reason we are giving consideration to the design of substituted benzyloxycarbonyl groups where the role of the substituent is to shift the reduction potential to much less negative potentials. In this paper, the cathode cleavage of the 4-nitrobenzyloxycarbonyl group is discussed. This protecting group has already been used in peptide synthesis;[8,9] it is an alternative to the benzyloxycarbonyl group when a high resistance to acids is required. In aprotic solvents most nitrobenzenes are reduced close to −1.0 V to give relatively stable anion radicals[10,11] and the presence in the molecule of the nitro group is known to make possible the cleavage of bonds elsewhere in the molecule at unusually positive potentials. For example, the cleavage of the C–Cl bonds in nitrobenzyl chlorides[12] and chloronitrobenzenes[13] occurs at the potential where only reduction of the nitroaromatic fragment is to be expected.

Experimental

All electrochemical experiments were carried out with a Hi-Tek potentiostat, type DT 2101, and Hi-Tek function generator, type PPR1. Current-potential curves were recorded on a Houston type 2000 recorder and charges were measured with a home-built digital integrator. Product analysis was carried out on a Pye Unicam GCD chromatograph; products were identified by comparison of retention times and yields by comparison of peak areas with those of standards.

Cyclic voltammograms were recorded in a three-electrode, two-compartment cell. The working electrode was a vitreous carbon disc (area 0.05 cm^2) made by sealing a rod (Tokai Carbon) into glass and grinding the face of the disc. The counter electrode was a Pt spiral in the same compartment. The reference electrode was a Hg pool separated from the working electrode by a Luggin capillary; at the end of the experiment the potential of the Hg pool was measured *versus* a s.c.e. (Metrohm) with a digital voltmeter. For coulometry and product analysis, the working and counter electrodes were separated by a glass sinter. The working electrode was a vitreous carbon disc (area 4.5 cm^2) from Tokai Carbon placed in a PTFE holder so that only one face was exposed to solution. The counter electrode was a Pt gauze (area 5 cm^2). The catholyte and anolyte compartments were each 15 cm^3 and the reference electrode was again a Hg pool mounted in a Luggin capillary. All solutions were deoxygenated with a fast stream of N_2 before each experiment. At intervals, the vitreous carbon electrodes were repolished first with 600 grade emery paper and then successively finer grades of alumina powder, 3 μm—0.03 μm, on a polishing cloth. Between each experiment, the electrode was wiped and repolished with the finest alumina powder.

Dimethylformamide (DMF) was stored over $MgSO_4$ and then distilled from CaH_2 under reduced pressure. Bu_4NBF_4 was prepared by precipitation on mixing solutions of $NaBF_4$ and Bu_4NHSO_4 in water, recrystallised from ethanol–water, and dried under vacuum. The urethane derivatives were prepared as follows. A solution of the amine (2 equiv.) in ethyl acetate was cooled to 0 °C and a solution of 4-nitrobenzyl chloroformate, prepared from the alcohol and phosgene,[8] was added dropwise with stirring. After the solution was stirred at room temperature overnight, the ethyl acetate solution was washed first with aqueous NaOH and then aqueous HCl and water. Finally the solution was dried over $MgSO_4$ and the ethyl acetate evaporated under reduced pressure. The resulting crystals were recrystallised from ethyl acetate–light petroleum (b.p. 40—60 °C)

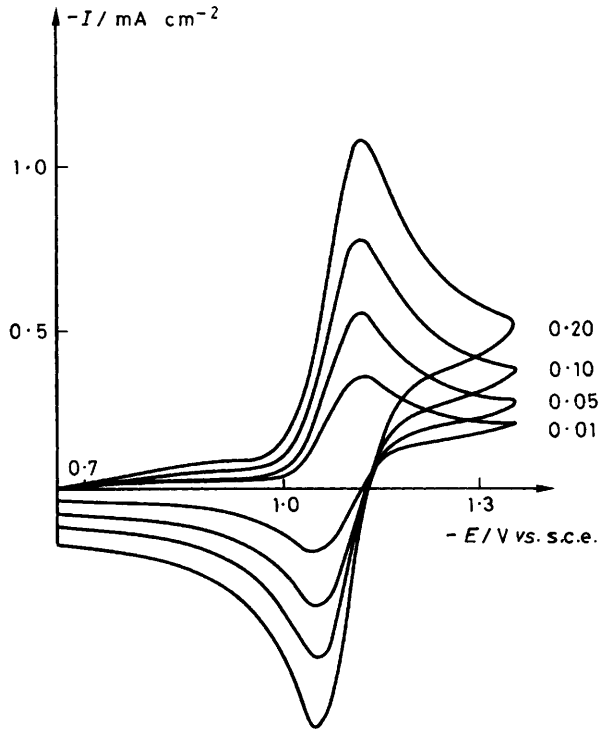
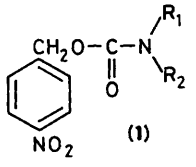


Figure 1. Cyclic voltammograms run between −0.6 V and −1.4 V for 4-$NO_2C_6H_4CH_2OCONHC_4H_9$ (**1a**) (2.5 mmol dm^{-3}) in DMF–Bu_4NBF_4 (0.1 mol dm^{-3}). Vitreous carbon disc electrode. Potential scan rates in V s^{-1} as shown on the Figure

mixtures. The purity of the urethanes was checked by n.m.r. spectroscopy and melting point determination.

Results and Discussion

This study concerns the reduction at vitreous carbon cathodes in DMF–Bu_4NBF_4 (0.1 mol dm^{-3}) of the urethane derivatives of four amines (**1a**—**d**).



(1)

	R_1	R_2
a;	C_4H_9	H
b;	c-C_6H_{11}	H
c;	C_2H_5	C_2H_5
d;	-$(CH_2)_5$-	

Cyclic voltammograms were recorded for solutions of each of the derivatives (2.5 mmol dm^{-3}) in DMF–Bu_4NBF_4 and the results, in terms of both the general form of the voltammograms and the potentials of the peaks, were very similar for all compounds. Figure 1 shows a set of cyclic voltammograms for (**1a**) run between −0.7 and −1.3 V; it can be seen that the responses have the form expected for a reversible 1e reaction and this is confirmed by quantitative analysis of their shape. The standard potential for the couple is −1.09 V *vs.* s.c.e. and the diffusion coefficient for (**1a**) is 1.1×10^{-5} cm^2 s^{-1}. When the potential scan is extended to more negative potentials, the response becomes more complex, see Figure 2. There is a rather broad peak at about −1.75 V and a further, larger and sharper, peak at about −2.30 V. Both the second and third reduction processes are irreversible but they lead to several anodic peaks in the range −1.5 V to −0.7 V where anion radicals of the nitro compound would be expected to oxidise. The chemistry associated with the second and third reduction processes is therefore clearly complex. This is also shown by calculating the current functions, $I_p/v^{\frac{1}{2}}c$,* when for the third reduction process this parameter decreases strongly with increasing scan rate. Further studies were therefore limited to the first reduction step which on the timescale of cyclic voltammetry leads only to a stable anion radical.

Table. Coulometry and amine yields from the reduction of the urethanes (**1a**—**d**) (20 mmol dm^{-3}) in DMF–Bu_4NBF_4 (0.1 mol dm^{-3}) at −1.2 V *vs.* s.c.e. For each case the yields are shown for duplicate experiments

Urethane	*n*/F	Yield of amine/%
(**1a**)	1.9	91, 86
(**1b**)	2.0	78, 92
(**1c**)	2.2	95, 82
(**1d**)	2.1	98, 88

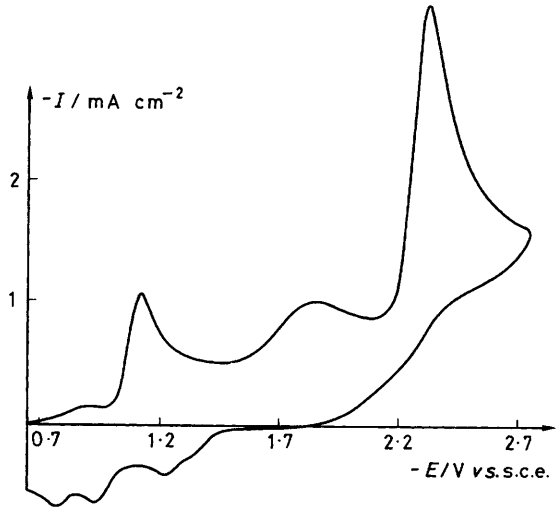


Figure 2. Cyclic voltammogram of 4-$NO_2C_6H_4CH_2OCONHC_4H_9$ (**1a**) over a wide potential range at a scan rate of 0.2 V s^{-1}. Other conditions as Figure 1

The voltammetry of (**1b**—**d**) was very similar; in particular, the standard potentials for the first reversible electron addition was the same as for (**1a**) within 10 mV.

Controlled-potential electrolyses were carried out for compounds (**1a**—**d**) at −1.2 V. The solutions were 20 mmol dm^{-3} in the urethane in DMF–Bu_4NBF_4 (0.1 mol dm^{-3}). Current was monitored as a function of charge passed; the resulting current-charge plots were linear and were extrapolated to zero current to estimate the number of electrons consumed in the reduction of the compounds (**1a**—**d**). The *n*-values are reported in the Table where it can be seen that the reduction of each 4-nitrobenzyloxycarbonyl derivative involves 2e at −1.2 V. When the current had dropped to background level, the solutions were analysed by g.l.c. and shown to contain good yields of the deprotected amines, see the Table, and small amounts of nitrotoluene (5—15%). Hence on the longer timescale of preparative electrolysis, the anion radicals of (**1a**—**d**) are not

* *c* is concentration.

stable. The colour changes of the catholyte during electrolysis confirm this conclusion. Initially the catholyte changed from colourless to orange ($1^{-\bullet}$?) but on standing or further reduction, the solution becomes blue (the anion radical of another nitro compound?). A cyclic voltammogram after electrolysis shows a reversible oxidation process with an anodic peak at −1.06 V and typically this peak has a height 40% of that for the reduction of (**1**) before the electrolysis. Hence the catholyte after electrolysis is rich in anion radicals of nitro compounds. Unfortunately, however, the oxidation potentials for the anion radicals of (**1**) and also the anion radicals of both nitrotoluene and 4,4′-dinitrobibenzyl are very similar[12] and hence it is not possible to confirm the fate of the nitro fragment by such experiments. All the above results are, however, compatible with the mechanism shown in the Scheme, where the source of proton is the solvent, electrolyte, or trace water. As far as the substrate is concerned the sequence involves (1.0 + 0.5 + 0.5)e and the mechanism is essentially similar to that proposed for the reduction of 4-nitrobenzyl chloride in DMF.[12] The loss of CO_2 will probably be slow in these aprotic conditions.

With some large molecules it is necessary to avoid the basic conditions created by the extraction of protons by the anions, 4-$NO_2C_6H_4CH_2^-$ and $R_1R_2N^-$. Hence the reduction of the 4-nitrobenzyloxycarbonyl derivatives of the amines were studied in the presence of some proton donors.

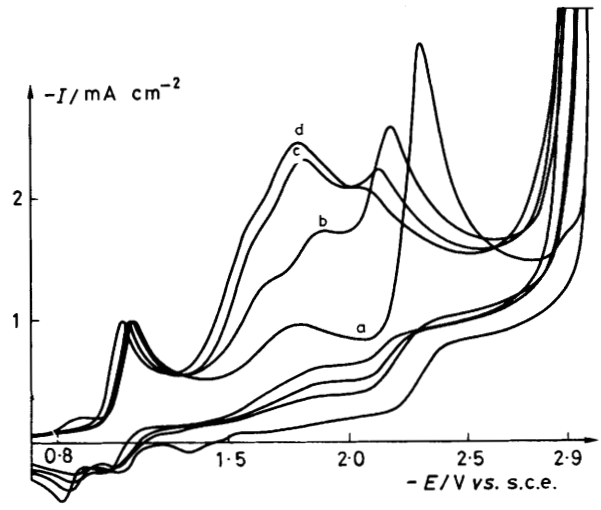
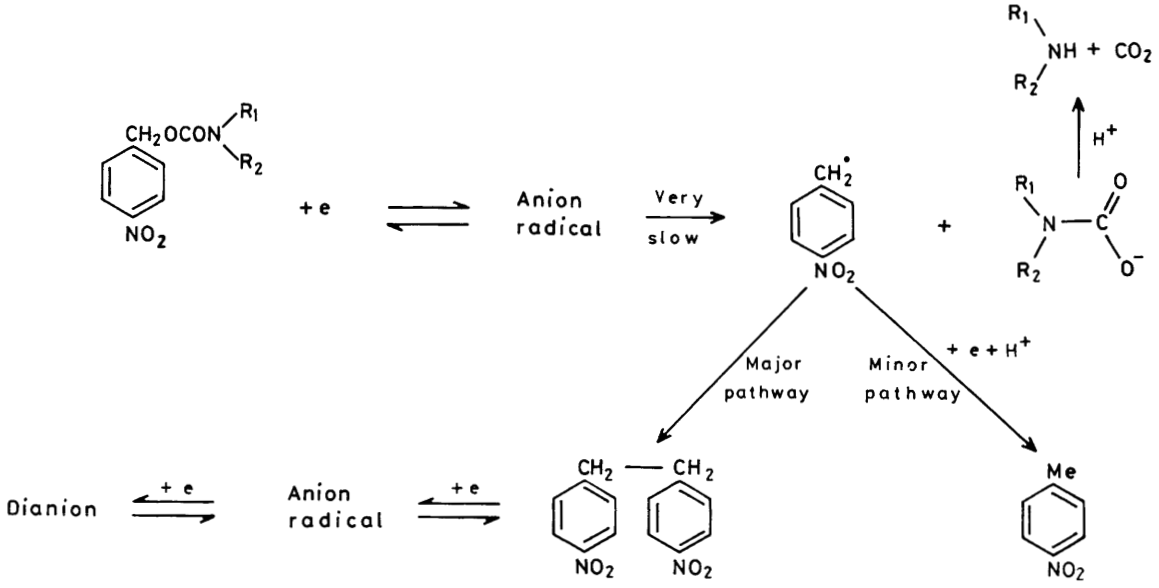
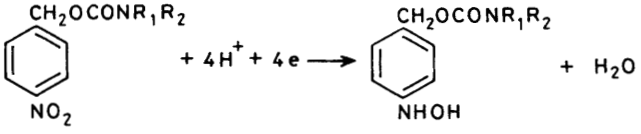


Figure 3. Cyclic voltammograms of 4-$NO_2C_4H_4CH_2OCON$-c-$(CH_2)_5$ (**1d**) (2.5 mmol dm^{-3}) in DMF–Bu_4NBF_4 (0.1 mol dm^{-3}) containing methanol (a) 0%; (b) 2%; (c) 5%; (d) 8%. Vitreous carbon disc electrode. Potential scan rate 0.1 V s^{-1}



Scheme.

In the medium DMF and HOAc (3%), cyclic voltammetry shows the electrochemistry of the compounds (**1a**—**d**) to be quite different. The first, reversible 1e reduction step with E° = −1.09 V is replaced by a totally irreversible process with E_p = −0.94 V and a peak current density increased by a factor of almost four. Coulometry at −1.00 V confirms that in the presence of HOAc the first reduction step involves the transfer of 4e/substrate molecule and no amine could be detected by g.l.c. Almost certainly the electrode reaction is:



where the nitro group is reduced. This indicates that acetic acid is too strong a proton donor and can protonate certainly the anion radials, ($1^{-\bullet}$), and maybe also the neutral starting materials. Similar experiments were also carried out with DMF with various additions of methanol. With this proton donor, cyclic voltammetry shows that the first reduction step remains a reversible 1e transfer but when the negative potential limit is extended to −2.9 V, it is clear that methanol causes substantial changes to the chemistry associated with the second and third reduction steps, see Figure 3.

Hence we believe that these studies with protected, simple amines show the potential of the 4-nitrobenzyloxycarbonyl group for the protection of amine centres during large molecule synthesis. The cathodic cleavage under aprotic conditions is highly selective and occurs at a potential where experimental problems are minimal. Moreover, the low cleavage potential offers interesting possibilities for systems where several different protecting groups are necessary; few other protecting groups would be cleaved at this potential.[5,14] Clearly, however, further studies are necesssary to develop a procedure suitable for the deprotection of peptides. Some peptides would racemise in the basic conditions created by the cathode reaction; it is therefore unfortunate that strong proton donors and, certainly, free acid

lead to the reduction of the nitro group rather than cleavage of the substituted benzyl group. Moreover it will be necessary to modify the conditions in order to facilitate isolation of the deprotected product. The use of a water-soluble electrolyte (*e.g.* $NaClO_4$) and a more readily distillable solvent would both be helpful.

The role of the nitro group in the electrode reaction is to allow easy addition of an electron to the molecule. But since nitro aromatic anion radicals are very stable, the desired reductive cleavage elsewhere in the molecule is catalysed. It is a good example of intramolecular redox catalysis.[15] Another approach would use other substituted benzyloxycarbonyl derivatives where the substituent(s) are sufficiently electron-withdrawing to shift the reduction potential to the desired potential region (− 1.0—1.5 V) but where the substituents themselves are not reducible (*e.g.* −Cl) even in the presence of acid.

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