



FIGURE 5.28

Products that arise following the thermal decomposition of nitrosoureas.

Nitrosoureas have been widely studied from a mechanistic standpoint. The presence of the nitroso group labilizes the nitrogen–carbon bond, leading to spontaneous decomposition into two electrophiles: an isocyanate **5.33** and a diazene hydroxide **5.34** that has been detected in some cases by electrospray ionization mass spectroscopy.⁶⁷ This intermediate generates a diazonium salt **5.35** (Figure 5.28).⁶⁸ Alkylation seems to be the main reaction responsible for antitumor activity, whereas carbamoylation takes place primarily on amino groups in proteins, leading to inhibition of several DNA repair mechanisms. *N*-nitrosoamides and *N*-nitrosocarbamates, which can act as alkylating (but not carbamoylating) agents, also have antitumor activity,⁶⁹ which supports the assumption that alkylation is the key mechanism.

The previously discussed fragmentation pathway was proposed mainly on the basis of studies of the thermal decomposition of nitrosoureas under anhydrous conditions,⁴⁷ but in water solution the reaction is much more complex and has been explained by the mechanism shown in Figure 5.29. Addition of a molecule of water to the nitrosourea, in its tautomeric form,⁷⁰ gives the tetrahedral intermediate **5.36**, which is decomposed into a primary amine, carbon dioxide, and **5.34**. This elimination requires an anti-periplanar conformation for **5.37**. Addition of a nucleophile other than water to the nitrosourea tautomer explains the isolation of carbamoylated products, formed by elimination of **5.34**.

Most nitrosoureas (CNU) contain one chloroethyl chain on the nitrosated nitrogen, which allows them to act as DNA cross-linking agents. Reaction of electrophilic diazonium species **5.37** with guanine is assumed to take place on O-6 to give **5.38**. This mono-alkylated product reacts subsequently with the N-3 atom of the cytosine unit in the complementary DNA strand by anchimeric assistance of the guanine N-1 atom through intermediate **5.39**, giving the cross-linked product **5.40** (Figure 5.30). In fact, addition of *O*⁶-alkylguanine DNA alkyltransferase, an enzyme that breaks *O*-6 guanine adducts, prevents cross-linking.

Alternatively, intact nitrosourea molecules rather than diazonium species can directly alkylate DNA. Thus, the nucleophilic attack of guanine O-6 to the nitrosourea tautomer **5.41** gives intermediate **5.42**. Although alternative mechanisms have been proposed, according to labeling experiments, it is probable that **5.42** cyclizes to the nitrosoisoxazolidine **5.43**, which is attacked by another O-6 atom of a neighboring guanine unit to give **5.44**. In this adduct, the O-6 of the first guanine is carbamoylated