phase II clinical studies for ovarian cancer and lymphomas, but it did not show sufficient efficacy. It has also been evaluated for other indications, such as pleural malignant mesothelioma.[20]

Zebularine was originally developed as a potent cytidine deaminase inhibitor because it lacks an amino group at position 4 of the pyrimidine. Its demethylating activity was not recognized until 2003.[21] After incorporation into DNA as nucleotide **8.11**, it forms a stable covalent adduct with DNMT (Figure 8.8), as proven by X-ray diffraction studies. In this case, the formation of intermediate **8.12** is not followed by methylation, a behavior that correlates with the low electron density of the C-5 position due to the absence of the 4-amino group, in agreement with molecular orbital calculations.[22]

Zebularine preferentially depletes DNA methyltransferase 1 (DNMT1) and induces expression of anticancer-related antigen genes in cancer cells.[23] Its main drawbacks are its low bioavailability and the need for high doses, which can be explained by its activity as an inhibitor of cytidine deaminase. This inhibition is due to analogy between the transition state of the reaction catalyzed by this enzyme (**8.13**) and zebularine hydrate **8.14**,[24] which is a dehydro analog of the previously mentioned inhibitor of cytidine deaminase tetrahydrouridine (Figure 8.9). This secondary effect causes part of the dose of

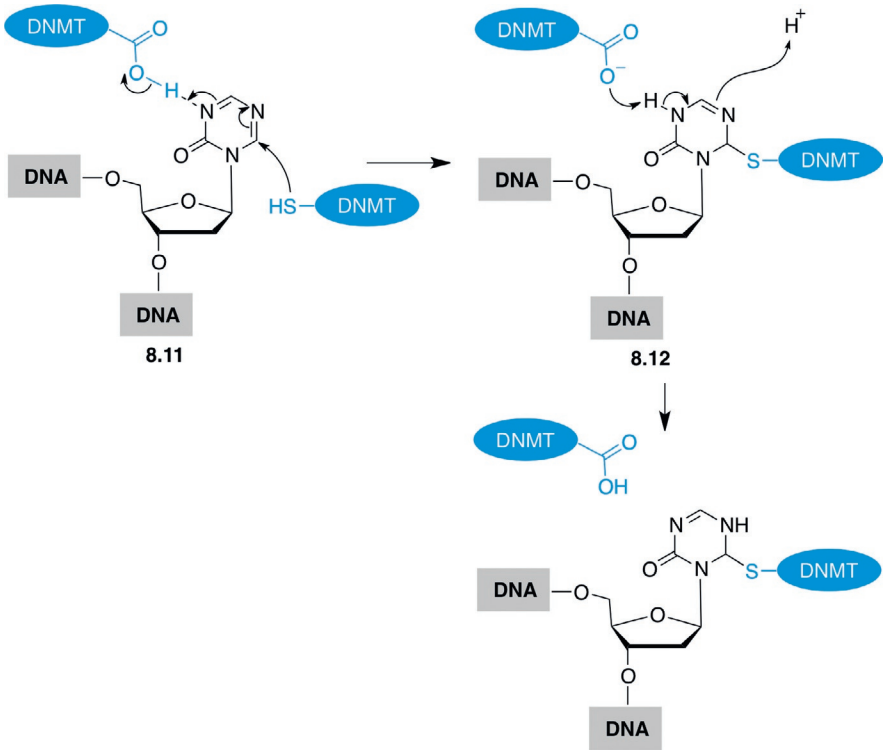

FIGURE 8.8

Mechanism of DNMT inhibition by zebularine.