1

Alkylating Agents

An impressive number of cytotoxic compounds whose antineoplastic activity is due to their reactions with DNA have been studied in the clinic. Many of these comprise drugs that currently form part of the combinations used to treat neoplastic disease. This account however includes only a limited number of alkylating agents since this area has been well covered elsewhere.

1.1 bis-Chloroethyl Amines

As noted in the Introduction, antineoplastic agents that include in their structure highly reactive chemical moieties comprise the earliest class of drugs for treating malignant tumors. This applies particularly to those cancers that afflict the system for producing and maintaining blood-forming tissues such as leukemia and lymphoma. The first of these agents, mustine (**1.1**), also known as mechlorethamine, was, as noted in the Introduction, actually developed empirically. An understanding of the mechanism by which alkylating agents kill cancer cells awaited the discovery of the structure of DNA in the 1950s as well as elaboration of the chemistry for studying that substance. The relatively large group of alkylating anticancer drugs was actually synthesized before their mode of action was fully understood. Many of those anticancer agents were designed as analogues of prior compounds that sported the chemically reactive chloroethyl group or some other highly reactive function.

The alkylating agents as a class attack many tissues in the body that contains basic nitrogen. Those agents target all cells that are susceptible to alkylation, be they cancerous cells or unrelated normal cells. The latter circumstance leads to many classical side effects manifested by alkylating antineoplastic drugs, such as loss of hair, dry mouth, and dry eyes,

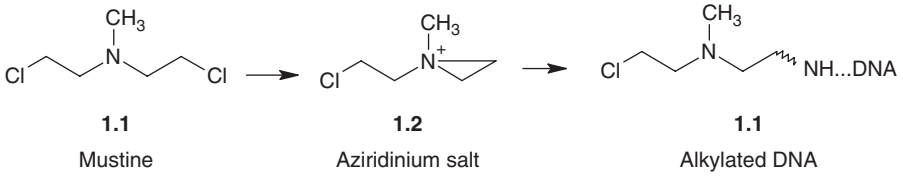
Antineoplastic Drugs: Organic Synthesis, First Edition. Daniel Lednicer.

experienced by patients exposed to this class of antineoplastic agents. The effects on neoplastic cells are however more relevant to this discussion. Reaction with DNA is not a random process; it has been shown that alkylating agents react preferentially with the more electron-rich, more basic nitrogen atoms in DNA. The stacked bases between the two strands of that macromolecule in the helical arrangement constitute a particularly favorable configuration for attack on each of the two separate strands of DNA. Drugs that incorporate two alkylating moieties form a covalent bridge between the two strands of DNA. This effect is demonstrated by the significantly lower concentration of bifunctional agents required to kill cancer cells *in vitro* than that of molecules that include only a single reactive group. That cross-link inactivates alkylated DNA since almost all functions of DNA, such as replication, require access to a single strand. RNA, the counterpart directly involved in synthesizing new protein, can only read a single DNA strand. The affected cell then simply ceases to function and dies. Although very large number of alkylating drugs has been studied since the early 1940s, the present account is restricted to five subsets that illustrate research in this field.

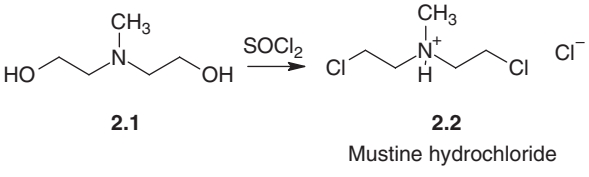
The chloroethyl group found in this subset of alkylating drugs does not react with DNA as administered. Instead, the basic nitrogen in mustine (**1.1**) displaces the side chain chlorine to form an aziridinium salt (**1.2**). The reaction of this activated species with nitrogen in DNA leads to ring-opened DNA adduct. The repetition of that sequence with the second chloroethyl function followed by the reaction of the new aziridinium function with alkylated DNA leads to cross-linked DNA.

The first recorded preparation of this rather venerable antineoplastic agent involves the reaction of methyl-bis(hydroxylethane) (**2.1**) with thionyl chloride. The starting diol is speculatively available from the reaction of methylamine and ethylene oxide. The resulting product, **mustine** (**2.2**), needs to be handled as a positively charged salt to prevent *ex vivo* aziridinium formation [1].

Cyclophosphamide is one of the best known and widely used antineoplastic agents. The drug comprises "C" in a large number of multidrug cocktails for treating cancer. One of the



Scheme 1.1 *Aziridinium salt formation.*

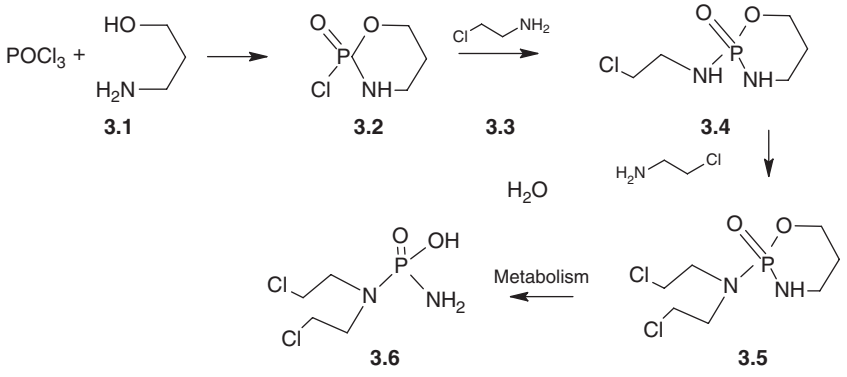


Scheme 1.2 *Synthesis of mustine.*

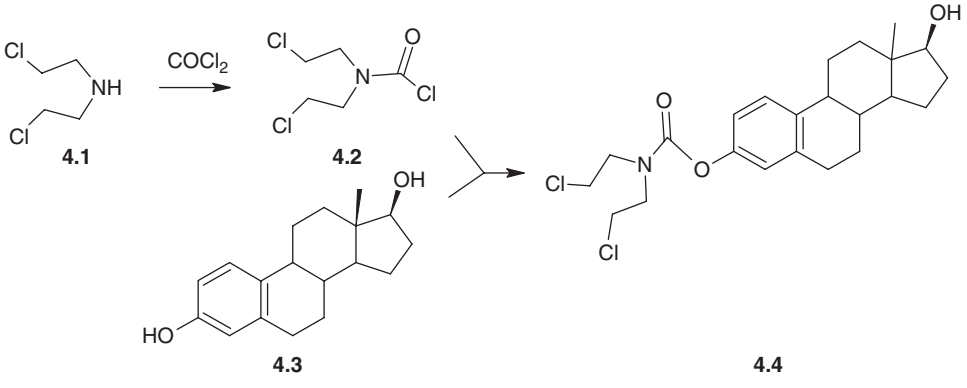
several schemes for preparing this compound starts with the condensation of aminoalcohol (**3.1**) with phosphorus oxychloride to afford the oxazaphosphorine derivative (**3.2**) through stepwise displacement of halogens in phosphorus oxychloride by the base and alkoxide group in (**3.1**). The still reactive chlorine in that product is then displaced with 2-chloroethylamine (**3.3**). The same reagent is then used to add a second chloroethyl function. This brief sequence affords **cyclophosphamide** (**3.5**) [2].

This drug is actually not the active alkylating species. Instead, enzymes open the ring by first hydroxylating the carbon bearing oxygen. The resulting hemiacetal then hydrolyzes to afford the phosphoramide mustard species (**3.6**). This has been approved for clinical use by many regulatory bodies. It is available as a generic drug since the patent covering this entity expired many years ago.

It is widely known that a large proportion of human female breast and possibly other genital tissues are equipped with receptors for estrogens. Binding of estrogens such as estrone, estradiol (**4.3**), and other related estrogenic compounds with those receptors stimulates growth of estrogen-positive tissues; those hormones will most likely cause the malignant tumors to flourish. Before the discovery of the estrogen antagonist drugs, the treatment of



Scheme 1.3 *Cyclophosphamide.*



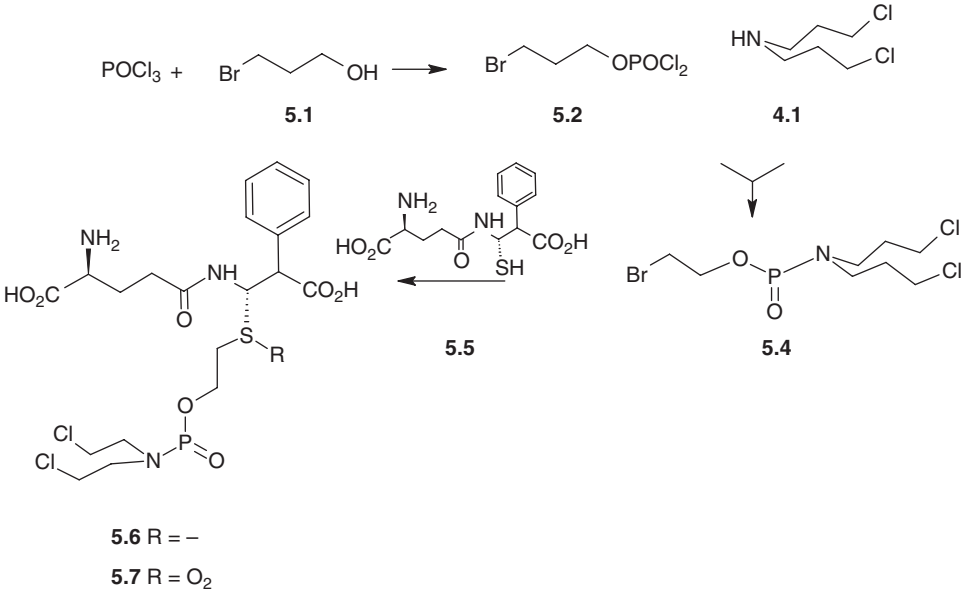
Scheme 1.4 *Estramustine.*

breast and related cancers often consisted of surgery followed by the administration of androgenic drugs in a vain hope that they would decrease estrogen-induced proliferation. The administration of alkylating anticancer drug seemed at the time to be the only alternative for treating breast cancer. One strategy for avoiding the severe side effects from the administration of those drugs comprises limiting exposure of the drug to the malignant tissue. Estrogen receptors in breast and related tissues are at first sight prime targets for directed antineoplastic agents. One approach for steering the alkylating mustine moiety consisted attaching the moiety to an estrogen. It should be noted in passing that the current treatment of estrogen receptor-positive cancers consists of surgery followed by the administration of one of the handful of estrogen antagonists such as tamoxifen or raloxifene (*see* Chapter 3).

The straightforward preparation of estramustine (**4.4**) starts with the acylation of bis(2-chloroethyl)amine with phosgene to afford the corresponding carbamoyl chloride (**4.2**). The acylation of estradiol (**4.3**) with that reactive intermediate affords **estramustine** (**4.4**) [3]. There is some evidence that this drug also disrupts tubulin, a precursor of tubules, an essential structure for cell division.

The drug is now available as a generic from a selection of vendors.

A more recent compound based on the same rationale comprises a mustine-equipped dipeptide glutathione mimic intended to direct the compound to a receptor for glutathione. The specific instance was based on the observation that malignant cells often have relatively high levels of the enzyme glutathione transferase, compared to normal cells, and that enzyme leads to expulsion of glutathione from the body. Attaching the mustine moiety to a glutathione-like moiety was expected to steer that agent to malignant cells. The drug has shown activity in the clinic against several cancers. The construction of this cytotoxic agent starts by the displacement of chlorine in phosphorus oxychloride by means of



Scheme 1.5 *Canfosfamide.*

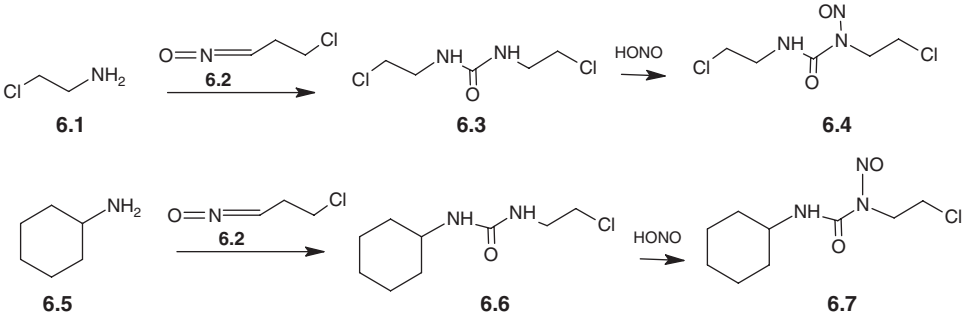
bromoalcohol (**5.1**). The product is next treated with bis(chloroethyl)amine (**5.3**); the amine in that reagent displaces the remaining halogen to afford phosphoramide (**5.4**). That intermediate is next reacted with the glutathione analogue in which phenylglycine replaces glycine found in the prototype. The mercaptan in reagent (**5.5**) then displaces bromine to give the condensation product; oxidation of sulfur with hydrogen peroxide completes the synthesis of **canfosfamide** (**5.6**) [4, 5].

1.2 Several Other Chloroethyl Agents

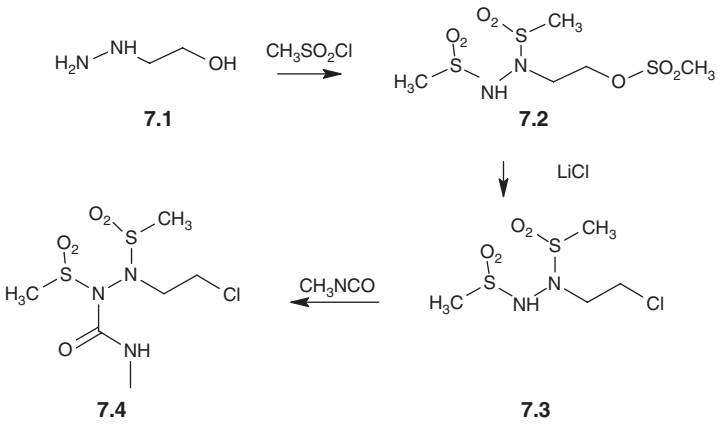
A pair of closely related compounds that act by a similar mechanism can be prepared by a relatively short sequence of reaction. The condensation of 2-chloroethyl-1-amine (**6.1**) with isocyanide (**6.2**) leads to the corresponding urea (**6.3**). The treatment of that product with nitrous acid leads to *N*-nitrosourea (**6.4**), **carmustine**, also known by the trivial acronym BCNU.

The same sequence starting with cyclohexylamine (**6.5**) gives **lomustine** (**6.8**) or CCNU. These nitrosoureas decompose in aqueous media by a sequence that involves loss of nitrogen from the N-nitroso moiety. The decomposition of both (**6.4**) and (**6.7**) apparently proceeds via a transient chloroethyl carbocation [6]. These drugs, which are approved for use in the clinic, also cross-link both DNA and RNA even though each yields a species with single reactive center. Both drugs have been approved by the FDA for the treatment of neoplastic disease. BCNU was used for many years as monotherapy of brain cancer.

A rather more complex chain of heteroatoms supports the chloroethyl side chain in the alkylating agent cloretazine. The reaction of 2-hydrazinoethanol (**7.1**) with methanesulfonyl chloride sulfonates the two hydrazine atoms as well as the hydroxyl group to afford the tris-sulfonated intermediate (**7.2**). Heating that intermediate with lithium chloride displaces the O-sulfonate by chlorine, thus establishing the requisite chloroethyl side chain. The condensation of intermediate (**7.3**) with methyl isocyanate converts the last free nitrogen to a urea, yielding the alkylating agent **cloretazine**, also known as **laromustine** (**7.4**) [7, 8].



Scheme 1.6 *Nitrosoureas.*



7.1 **7.2** **7.3** **7.4**

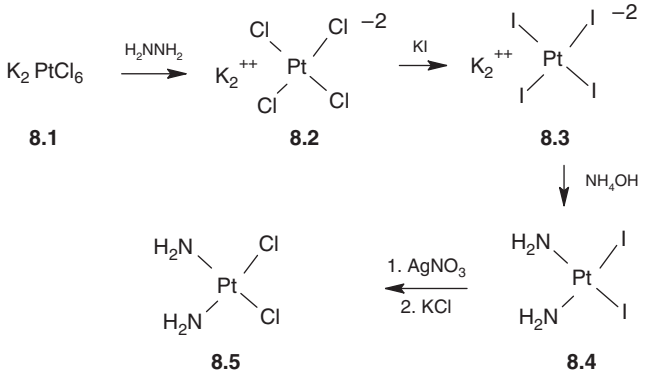
Scheme 1.7 *Cloretazine.*

1.3 Platinum-Based Antineoplastic Agents

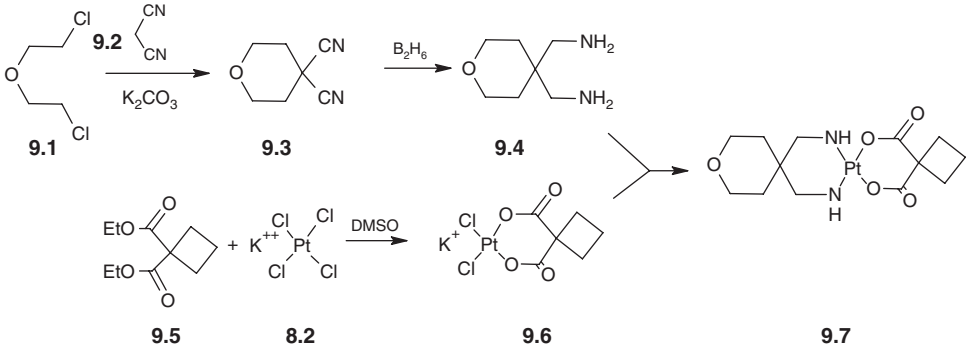
The oft-told story of the discovery of cisplatinum provides an outstanding example of serendipity. Intending to ascertain the effect of electric currents on bacteria, the investigator, Barnet Rosenbloom, inserted a platinum electrode into a bacteria-seeded culture bath. He found that bacterial growth had indeed been inhibited. That effect was however not due to the electric current; they found that the inhibition could be attributed to a compound formed by electrolytic dissolution of the electrode. It was later found that the newly formed compound also inhibited the proliferation of cancer cells. The isolated platinum compound, cisplatin (**8.2**), was later found to be cytotoxic to human cancer cells. The drug is now widely used in combination therapy. The letter P in the acronym of a typical combination of antineoplastic agent refers to this drug. Cisplatin and its analogues, like other alkylating agents, act by inactivating DNA; in this specific case, each of the chlorine atoms in cisplatin is displaced by a base on the neighboring strands of DNA. The specificity for cancerous cells of platinum drugs depends on the faster turnover of cancer cells compared to that of normal cells. The harsh side effects of cisplatin may be a reflection of that limited specificity for neoplasms. This has led to major programs for preparing better tolerated cisplatin analogues. One result of those programs comprises the 18 compounds that carry the suffix "platin" (platinum based) that are listed in the USAN Dictionary.

The synthesis of cisplatin begins with the reduction of potassium hexachloroplatinate (**8.1**) with hydrazine to afford the tetrachloro derivative. The four chloro groups are then replaced by iodine in order to bypass the so-called trans effect that would lead the incoming amines to add to give the undesired trans isomer; treatment with excess potassium iodide proceeds to form tetraiodide (**8.3**). Ammonium hydroxide then replaces two of the iodo groups by amines in a stepwise fashion. Iodine is next removed by sequential reaction with silver nitrate followed by potassium chloride. **Cisplatin** (**8.5**) is thus produced [9].

The scheme used to prepare several more recent examples is typical for preparing compounds in this series. The synthesis of enloplatin begins with the construction of the moiety that will provide the required two amines. Alkylation of the dichloro ether (**9.1**)



Scheme 1.8 *Cisplatin synthesis.*

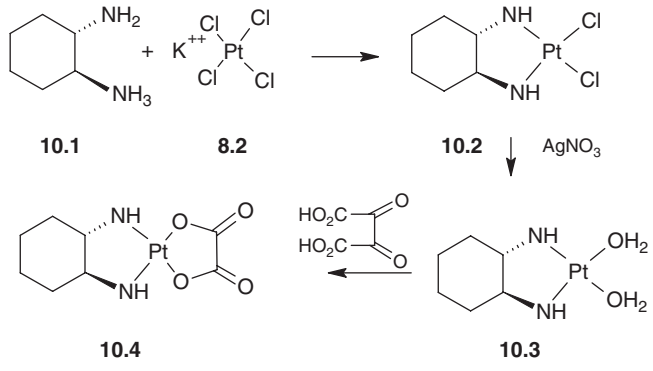


Scheme 1.9 *Enloplatin.*

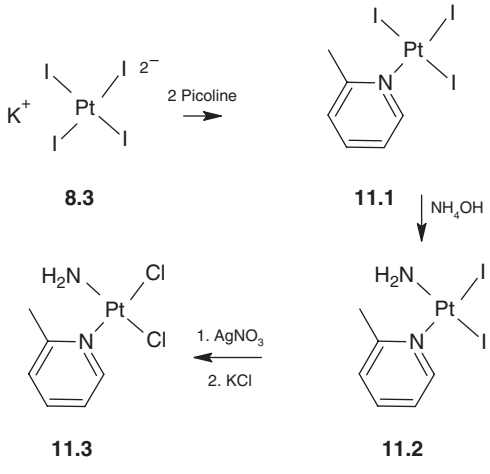
affords annulated perhydropyran (**9.3**). The treatment of that intermediate (**9.3**) with diborane leads to one of the ligands (**9.4**). In a converging step, malonate (**9.5**) is condensed with the tetrachloro platinum intermediate (**8.2**) in dimethyl sulfoxide. The ester groups are saponified in the course of the reaction. Those carboxylic acids then displace chlorines from the tetrachloro starting material (**8.2**) to form the platinum compound (**9.6**). The treatment of that intermediate (**9.6**) with diamine (**9.4**) causes the amines to displace the remaining chlorine groups in the platinum intermediate (**9.6**) to form **enloplatin** (**9.7**) [9, 10].

In much the same vein, the condensation of cyclohexane-*trans*-diamine (**10.1**) with the ubiquitous tetrachloroplatinum derivative (**8.2**) affords the intermediate (**10.2**) in which basic nitrogen has displaced two of the halogen atoms. Reaction with aqueous silver nitrate precipitates the remaining chloride atoms (**10.3**). Treatment with oxalic acid then yields **oxaliplatin** (**10.4**) [11], available under the trade name Eloxatin®.

Cytotoxic activity is retained in a platinum-based compound that features two different basic amine ligands. The synthesis of one such unsymmetrical agent starts by the displacement of one of the iodine atoms in the intermediate (**8.3**) by 2-picoline. Steric hindrance about the newly formed bond by the ortho methyl group on newly introduced pyridine



Scheme 1.10 *Oxaliplatin.*

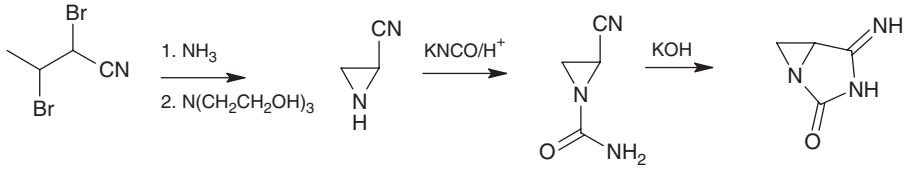


Scheme 1.11 *Picoplatin.*

derivative apparently prevents attack by a second picoline. The other basic nitrogen is then introduced by treating the intermediate (**11.1**) with aqueous ammonia (**11.2**). The sequential reaction of that product (**11.2**) with silver nitrate and then potassium chloride restores the chlorine ligands and thus affords **picoplatin** (**11.3**) [12].

1.4 Miscellaneous Alkylating Agents

The chemically reactive compound imexon (**12.4**) binds to the intracellular thiols that normally scavenge oxidants in cells. The overall effect of this drug thus comprises increased levels of oxidizing species. This compound has shown activity against a range of human cancers in cell culture as well as cancers implanted in rodents. Where approved by regulatory agencies, the drug carries the trade name Amplimexon®.



12.1 **12.2** **12.3** **12.4**

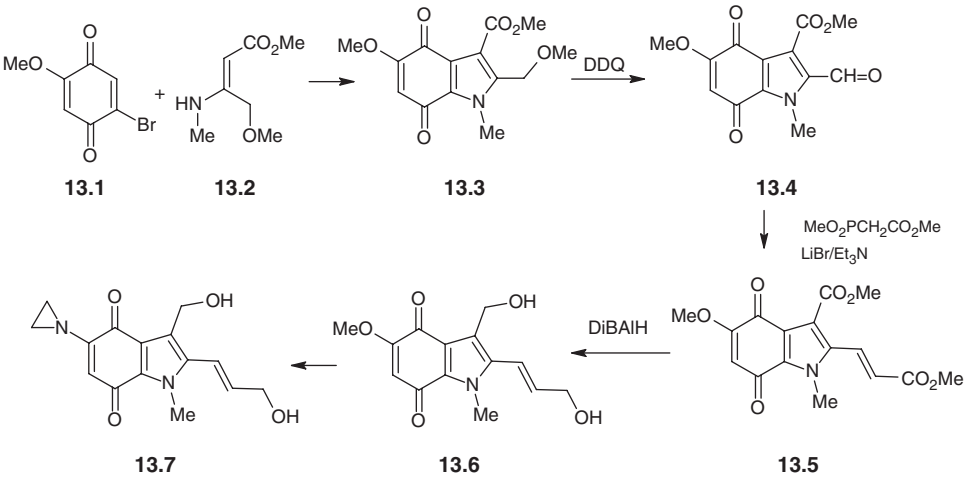
Scheme 1.12 *Imexon.*

The concise preparation of imexon starts with the preparation of cyanoaziridine (**12.2**). The reaction of 2,3-dibromopropionitrile with ammonia leads to aziridine (**12.3**). The treatment of that intermediate with potassium isocyanate and acid—actually isocyanic acid—converts the primary amine to a carboxamide. Under the basic conditions provided by potassium hydroxide, amide nitrogen adds to the nitrile to form a fused five-membered ring. **Imexon** (**12.4**) is thus obtained [13].

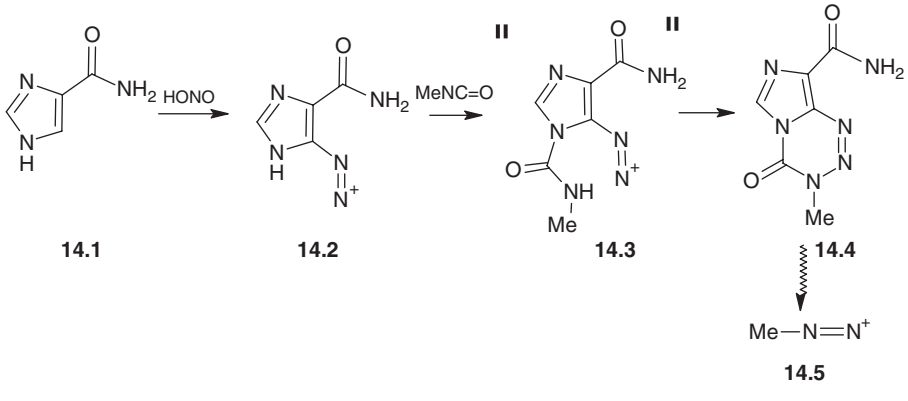
Drug delivery can be a major issue in drug development. Many drugs are, for example, not absorbed when administered orally, while others may be metabolized so quickly that they may never reach effective concentrations at the critical site. In addition to research to deal with those problems, there has been some work devoted to the opposite scenario: developing drugs that need to be metabolized near the site of action. Such drugs are often referred to as prodrugs. The antineoplastic agent apaziquone represents an example of such a prodrug. Apaziquone (**13.7**) is a synthetic analogue of the antineoplastic fermentation product mitomycin. The drug is reduced to active metabolites in hypoxic locations such as the interiors of tumors. The compound has been extensively tested for treating bladder cancer.

The condensation of the substituted quinone (**13.1**) with the enamine from ethyl acetoacetate (**13.2**) leads to pyrroloquinone (**13.3**). The reaction can be visualized by the displacement of bromine in the quinone by the enamine nitrogen. This then transforms the quinone itself to an enamine; that then adds to the newly formed side chain and closes the ring (**13.3**). The treatment of that intermediate with dichlorodicyanoquinone (DDQ) oxidizes the methoxymethyl group on the pyrrole to the corresponding aldehyde. The reaction of this last intermediate with ylide from Emmons reagent and triethylamine leads to the derivative with a lengthened side chain (**13.6**). The presence of lithium chloride in the reaction medium enforces the tendency to form a trans olefin. The treatment of this last intermediate with diisobutylaluminum hydride reduces both esters to alcohols. The displacement of the quinone methoxyl by aziridine may well involve an addition–expulsion reaction. Whatever the mechanism, the reaction affords **apaziquone** (**13.7**) [14].

The nitrogen-rich heterocyclic compound temozolomide is approved by the FDA for treating several brain cancers. The drug is available under several trade names: Temodar®, Temodal®, and Temcad®. The concise synthesis starts with the treatment of imidazole (**14.1**) with nitrous acid. The resulting diazonium salt (**14.2**) is then allowed to react with methyl isocyanate of ill fame. The initial product comprises the urea from addition of the reagent to one of the ring nitrogen atoms (**14.3**). The exocyclic urea nitrogen then attacks the charged azo group to close the ring, forming **temozolomide** (**14.4**) [15]. The parent drug has little if any activity as such. This compound actually undergoes a number of metabolic transformations that terminates in azomethane (**14.5**). The carbocation from loss of nitrogen then alkylates RNA guanine.



Scheme 1.13 *Apaziquone.*



Scheme 1.14 *Temozolamide.*

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