ACCOUNTS OF CHEMICAL RESEARCH®

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VOLUME 19 NUMBER 10 OCTOBER, 1986

# The Design of Chemotherapeutic Agents

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*Received February 24, 1986 (Revised Manuscript Received June 6, 1986)*

The problem of drug design is a difficult one, particularly for cancer and viral chemotherapy, but it is clear that new and better drugs are needed if continuing advances are to be made in the treatment of these disease types. The rational design of an agent with specific activity requires not only that the target be known but that it be precisely defined, so that it can be hit selectively in the presence of other targets that may be similar in most respects. The target of an anticancer agent is obviously the cancer cell, but there has been and still is little information on any unique characteristic of a cancer cell that might be exploitable for the design of specific agents. Despite this lack of any well-defined basis for design, new anticancer agents have been designed over the past 30 years, many of them on the basis of the known activity of agents that have been conceived or discovered more or less empirically.

Basic to the problem of design of this type of chemotherapeutic agent is the knowledge of how drugs kill cells or inhibit their proliferation. An inhibitor must produce its effect by associating with or being incorporated into a cellular constituent and as a result impairing the normal function of the constituent and thereby the capacity of the cell for division or maintenance of its integrity. The target of an inhibitor could be any molecular species present in the cell from small molecules to proteins, nucleic acid, and polysaccharides. It is logical that the most sensitive targets would be cellular constituents involved in the biosynthetic processes of the cell and this appears to be the case. The inhibitors whose mechanisms of inhibition are understood, at least to some extent, act by interfering with the synthesis or function of DNA, or perhaps in some cases, with RNA. These general statements also apply, with some modification, to antiviral agents, although viruses afford some additional targets, such as the processes involved in attachment to cells, uncoating, and so on.

This Account will describe some of the approaches that have been used by Southern Research Institute scientists to develop anticancer and antiviral agents.

## The Nitrosoureas

The chemistry and biological activity of the conventional alkylating agents such as nitrogen mustard were described in detail in a monograph by W. C. J. Ross.[1] The report of the activity of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine against leukemia L1210 in mice led us to speculate that this compound was acting as a methylating agent under physiologic conditions and that related structures such as *N*-methyl-*N*-nitrosourea (MNU) might have useful activity for the same reason.[2] Although both compounds are used to generate diazomethane at high pH (i.e., 12), our studies on the chemistry of the nitrosoureas[3] showed that methylnitrosoureas decompose under physiologic conditions to methanediazohydroxide and isocyanates (eq 1):

$$MeN(NO)CONHR \rightarrow CH_3N{=}NOH + O{=}C{=}NR \quad (1)$$

MNU methylates the bases of DNA,[4] and evidence is accumulating that its biological activity is associated with this methylation.

The importance of meningeal leukemia as a complication in acute leukemia caused us to study the relative effectiveness of clinically used drugs against leukemia L1210 induced by innoculation of the leukemia cells intracerebrally as a model for the human disease.[5] Although none of the standard drugs had any effect on the course of the disease, MNU, which was included in the study because of its recently discovered anticancer activity and its solubility characteristics and nonionic nature, proved to be just as effective against the intracerebrally as the intraperitoneally implanted disease. This exciting observation led to our program for the

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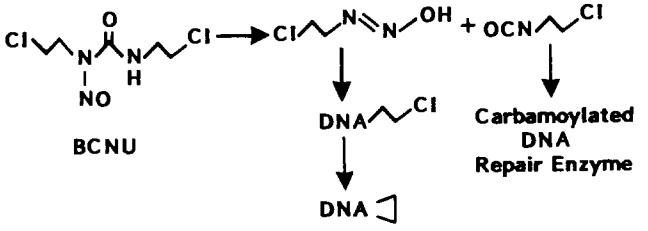
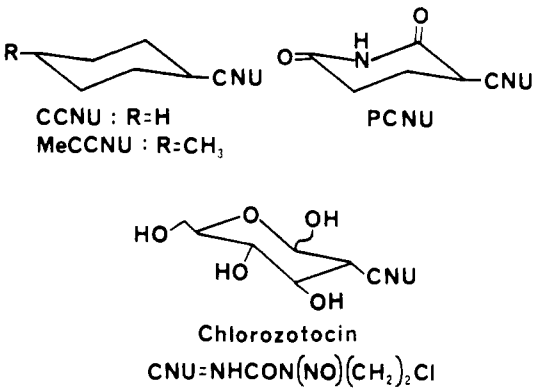
development of nitrosoureas as clincally useful agents.[6] If the methylation of DNA by MNU is responsible for its anticancer activity, it was logical to study first the effect on activity of substituting other groups for the methyl group. Systematic modification led to the discovery that the (2-chloroethyl)- and (2-fluoroethyl)-nitrosoureas were much more active (in fact curative) against leukemia L1210 in mice than any other type of substitution investigated, and *N,N'*-bis(2-chloroethyl)-*N*-nitrosourea (BCNU, carmustine) proved to be highly active against a variety of murine neoplasms leading to its clinical trials and the demonstration of its activity against human cancer.[2] Studies on the chemistry of BCNU by us and a number of other investigators showed that even though the decomposition of BCNU at physiologic pH (ca. 7.4) is very complex (16

**The Important Reactions of BCNU**



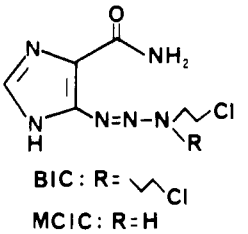

compounds have been identified from this reaction), it largely decomposes to 2-chloroethanediazohydroxide and 2-chloroethyl isocyanate, which decompose further to other products.[7] Many other studies indicate that the anticancer activity of BCNU and the other (2-chloroethyl)nitrosoureas probably results from the chloroethylation of DNA followed by crosslinking of the complementary strands.[8] The contribution of the isocyanate to biologic activity is still not clear.

*N,N'*-Bis(2-chloroethyl)-*N*-nitrosourea (BCNU, carmustine), *N*-(2-chloroethyl)-*N'*-cyclohexyl-*N*-nitrosourea (CCNU, lomustine), *N*-(2-chloroethyl)-*N'*-(4-methylcyclohexyl)-*N*-nitrosourea (MeCCNU, semustine), *N*-(2-chloroethyl)-*N'*-(2,6-dioxy-3-piperidyl)-*N*-nitrosourea (PCNU), and 2-[3-(2-chloroethyl)-3-nitrosoureido]-2-deoxy-D-glucopyranose (chlorozotocin) have all shown significant activity in clinical trails, with BCNU being the most studied drug of this class.[2] BCNU and CCNU are now widely used in drug combinations for the treatment of Hodgkin's disease. BCNU has become the drug of choice to be used concomitantly with, and after, postoperative radiation therapy of malignant gliomas. The nitrosoureas, especially CCNU, are included in most of the highly effective combinations used in the treatment of oat-cell lung cancers.[2,9] Thus, it would appear that the nitrosoureas have earned their place in the armamentarium of clinically useful anticancer agents. Despite this, they have fallen short of their promise because of their severe toxicity to the bone marrow, and an effort to develop




nitrosoureas with reduced marrow toxicity has not been entirely successful.[2,10] Chlorozotocin is less toxic to the marrow, in both rodents and humans, than BCNU and CCNU, but it is less active also. Attempts to "cross" the structure of chlorozotocin with the highly active MeCCNU did not result in a candidate for clinical trails, although the compounds produced did have activity against rodent neoplasms.[11]

Failure to increase the activity to toxicity ratio (i.e., therapeutic index) in the nitrosourea series led us to consider the possibility of developing other types of 2-haloethylating agents. The ability of 4-[3,3-bis(2-chloroethyl)-1-triazeno]imidazole-5-carboxamide (BIC) and its major metabolite,[12] the mono 2-chloroethyltriazene (MCIC), despite their instability, to cure mice




with leukemia L1210,[13] along with the therapeutic synergism of the combination of BIC and CCNU[14] encouraged us to move in this direction, since BIC owes its activity to its conversion to MCIC, which is a chloroethylating agent with biologic properties obviously different from those of the nitrosoureas.

## Sulfonate Esters of 2-Chloroethanol

One type of chloroethylating agent we decided to investigate was the sulfonic acid esters of 2-chloroethanol, even though 2-chloroethyl methanesulfonate (CMS) is barely active against the P388 leukemia in mice and was previously found to be inactive against a variety of rodent neoplasms.[15] The chemical reactivity of CMS, however, is low relative to that of the (2-chloroethyl)nitrosoureas.[16] The reactivity of a number of inactive nitrosoureas is also low,[17] leading

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us to postulate that enhanced chemical reactivity in the sulfonate esters might result in better antileukemic acitivity. Initial efforts produced several 2-chloroethyl halobenzenesulfonates that possess the required reactivity but were, nevertheless, inactive against the P388 leukemia, even though one of these compounds, 2-chloroethyl pentafluorobenzenesulfonate, is quite toxic to L1210 cells in culture ($IC_{50}$ <0.4 $\mu$M). 2-Chloroethyl chloromethanesulfonate (CCMS) is also in the proper range of reactivity and cytotoxicity ($IC_{50}$ <0.9 $\mu$M), and it gave a 114% increase in lifespan of mice with the P388 leukemia,[18] suggesting that a certain degree of chemical reactivity is a necessary but not sufficient requirement for in vivo activity in this series. Further efforts to improve the reactivity and antileukemic activity of this structural type gave 2-chloroethyl (methylsulfonyl)methanesulfonate (clomesone) and its

CMS: X=H-

CCMS: X=Cl-

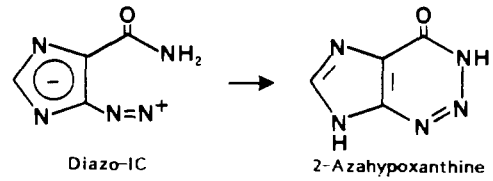
Clomesone: $X=CH_3SO_2^-$

2-fluoroethyl analogue, both of which are curative in the P388 system. The 2-bromoethyl compound is somewhat less effective.[19] Clomesone is also active against a number of murine neoplasms including mutant strains of the P388 leukemia resistant to cyclophosphamide, melphalan, and cisplatin.[20]

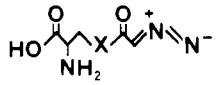
The alkylating activity of clomesone is much higher than that of chlorozotocin, a fairly typical (2-chloroethyl)nitrosourea, but in their reactions with DNA there is much more cross-linking of the complementary strands with chlorozotocin.[21] At the same time, the single-strand-break patterns were essentially the same for the two compounds, and they both cross-link DNA to protein.[22] The difference observed may be a reflection of the differences in the chemistry of these compounds that may result in differences in the sites and rates of alkylation of cellular macromolecules. Such differences may provide this new chloroethylating agent, clomesone, with improved toxicity characteristics (i.e., less severe marrow toxicity). It should be possible by further structural alterations on the methyl or methylene groups of clomesone to affect transport, the point of attack on DNA, and other characteristics of the molecule without diminishing the chemical reactivity required for high level anticancer activity.

## Triazenes

Our studies on the chemistry of 5-aminoimidazole-4-carboxamide led to the discovery of 5-diazoimidazole-4-carboxamide (diazo-IC), which is strikingly similar in structure to the antineoplastic agents azaserine and DON and which is readily converted to the antimetabolite 2-azahypoxanthine in aqueous media.[23] The possibility that diazo-IC might interfere with the biosynthetic pathway to inosinic acid in a manner similar to azaserine and DON led us to test it for cyto-



Diazo-IC 2-Azahypoxanthine



Azaserine: X=O

DON: $X=CH_2$

toxicity to cells in culture. It proved to be cytotoxic and to have some in vivo activity against Ehrlich ascites carcinoma, Walker adenocarcinoma 256, and sarcoma 180, but little or no activity against adenocarcinoma 755 and leukemia L1210. Since the activity of diazo-IC, a new structural type of potential antineoplastic agent, did not appear great enough to make the compound of potential value for the treatment of human cancer, ways were sought to convert it into derivatives that might have greater selectivity for cancer cells and, therefore, greater potential clinical utility. Among these derivatives were the reaction products of the diazo-IC with amines—the 5-triazenoimiazole-4-carboxamides.[24] 5-(Dimethyltriazeno)imidazole-4-carboxamide (DTIC), one of the early triazenes, proved to be active against sarcoma 180, adenocarcinoma 755, and leukemia L1210[25]—murine neoplasms that at that time comprised the primary screen of the National Cancer Institute. This broad spectrum activity of a new structural type led to clinical trials five years later that identified DTIC as the most active single agent for the treatment of malignant melanoma. It is also used in combination with other drugs for the treatment of metastatic sarcomas and refractory Hodgkins's lymphoma.[9]

A number of triazenes derived from primary amines, including 5-(methyltriazeno)imidazole-4-carboxamide (MIC), the monomethyl analog of DTIC, were also prepared.[26] These monoalkyltriazenes were found to be quite different chemically from the dialkyltriazenes. In aqueous solution, tautomerization occurs to give highly reactive species with a short half-life that, in the presence of water alone, react primarily to form alcohols and 5-aminoimidazole-4-carboxamide (AIC). MIC was about as active as DTIC in animal tumor systems, in spite of the difference in chemical behavior.[26] Later it was found that DTIC is oxidatively demethylated in vitro and in vivo by liver microsomes[27] to give, presumably, MIC, which in living cells reacts with many nucleophiles, including the nucleic acids. The methylation of DNA is probably ultimately responsible for the activity of DTIC.[28] BIC, referred to above in the

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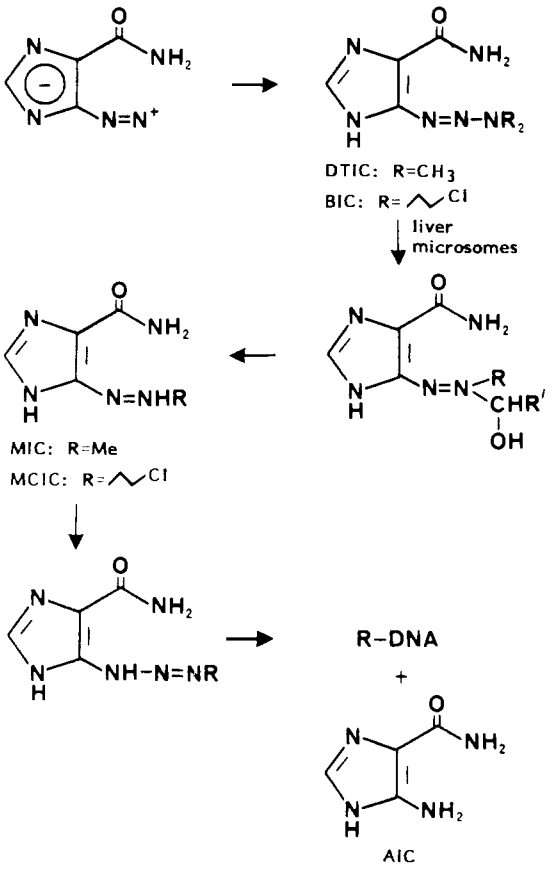
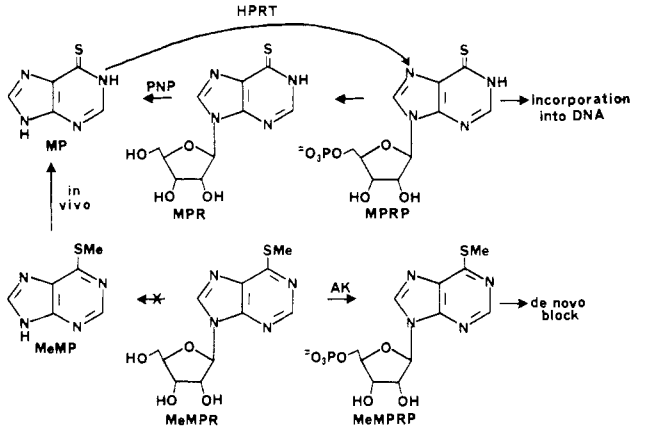



discussion of chloroethylating agents, is curative of leukemia L1210 in mice, as is its metabolic product, 5-((2-chloroethyl)triazeno)imidazole-4-carboxamide (MCIC).[13] Unfortunately, clinical results with BIC were not encouraging, probably because of its facile transformation in aqueous media into the inactive triazoline. MCIC is also quite unstable, but a stabilized form of it, mitozolamide, is undergoing clinical trails in England at the present time.[29] Although a wide variety of other triazenes have shown reasonably good activity in animal test systems, none of them has been evaluated in humans.[23]

## 6-Thiopurines

In the early 1940's the concept that substances chemically related to a metabolite might interfere with the normal function of that metabolite in living cells[30,31] attracted widespread interest among chemists and led to the synthesis and evaluation of analogues of the naturally occurring purines and pyrimidines. Among these were 5-bromouracil,[32] 8-azaguanine,[33] and 6-mercaptopurine (MP).[34] The latter compound was found to be moderately active against the murine neoplasm sarcoma 180[35] and highly active against another rodent tumor, adenocarcinoma 755.[36] Preliminary clinical trials in acute leukemia that quickly followed[37] were favorable and led to the acceptance of this agent as a mainstay in the treatment of leukemia.[38]

As a result, a number of laboratories, including the Kettering-Meyer Laboratory, began investigations of structural modifications of both MP itself and 6-thioguanine (TG), a compound with very similar activity. An appreciation of the importance of biochemical resistance in treatment failures of initially responsive human neoplasms led us to prepare derivatives of MP that might be inhibitory to MP-resistant cells. The design of one such compound, 6-mercaptopurine ribonucleoside (MPR), was based on the understanding that bacterial and neoplastic cells[39-41] resistant to MP or 8-azaguanine lacked the capacity to form nucleotides of these analogues.[42-44] Although it was not toxic to MP-resistant cells,[45] MPR did show a greater therapeutic index against adenocarcinoma 755 than MP or any of its other analogues.[46] Later we found that a derivative of MPR, namely 6-(methylthio)purine ribonucleoside (MeMPR),[47,48] is toxic to cells resistant to MP,[49] and shows therapeutic synergism with MP in the




treatment of L1210 in mice.[50] This combination was also superior to MP alone in the treatment of AML in humans.[51] The explanation of these observations lies

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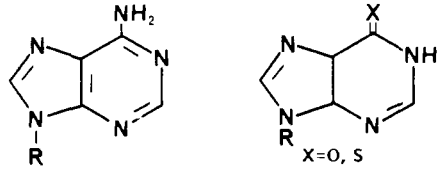
in the metabolism of MP and its derivatives. MP must be converted to its ribonucleotide (MPRP) by hypoxanthine phosporibosyltransferase (HPRT), an enzyme activity which is lacking in cells resistant to the drug.[39-41] 6-Thionosinic acid (MPRP)[52,53] is not effective because it cannot penetrate cell membranes, but is instead dephosphorylated to MPR. Although MPR is taken up by cells, it is not phosphorylated in most mammalian cell lines, but is cleaved by purine nucleoside phosphorylase (PNP) to MP.[54] On the other hand, MeMPR is taken up by cells, is not cleaved by PNP, and is rapidly phosphorylated by adenosine kinase (AK).[49] Other 6-(alkylthio)purine ribonucleosides[48] are phosphorylated less well and cytotoxicity is correlatable with the degree of phosphorylation.[49] Thus, resistance is circumvented by means of an alternative metabolic pathway, even though the mechanism by which MeMPRP inhibits cellular growth appears to be limited to the blockade of the de novo pathway to inosinic acid by feedback on phosphoribosylpyrophosphate transferase,[55-58] whereas MP inhibits a number of other enzymes also,[34,35] as well as being incorporated into DNA.[59,60] Since MP and MeMPR are therapeutically synergistic against L1210, but not against resistant cell lines that do not produce both nucleotides,[50] it seems likely that this potentiation is a result of the combined action of the two agents against a heterogeneous cell population containing mutants resistant to the single agents.

Based on the understanding that MP must be activated by conversion to its nucleotide, we undertook the synthesis of other 9-substituted derivatives that might function in some ways like nucleosides or nucleotides.[61-63] A series of 9-alkyl- and 9-cycloalkylpurines




R=ethyl, propyl, butyl, pentyl, hexyl, cyclopentyl, cyclohexyl

proved to be cytotoxic to H.Ep-2/0 cells and also to an MP-resistant subline.[64] Of these the MP derivatives were the most active, and some of these were active against adenocarcinoma 755 in vivo. 9-Ethyl-6-mercaptopurine was chosen for clinical trial and shown to be as active as MP in limited trials against both acute[65] and chronic[66] leukemias. Studies on the mechanism of action of the 9-alkylpurines have not been productive, but it is clear that they act differently from MP because they are not converted to it or otherwise significantly metabolized in vivo, and they are toxic to cells resistant to MP.

## Carbocyclic Analogues of Nucleosides

As discussed above, phosphorolytic cleavage of nucleosides such as 6-thioinosine (MPR) by PNP can negate their potential utility as chemotherapeutic agents.[54,67] The concept of enzymatically stable nucleoside analogues and the biologic activity of the 9-cycloalkyl purines led to the synthesis of carbocyclic (cyclopentane) analogues of nucleosides in which the furanose oxygen atom is replaced with a methylene group and the hydroxyl groups occupy the same positions, having the same cis–trans relationships, and assume similar conformations (although usually drawn as the analogues of naturally occurring β-D-ribofuranosyl bases, these compounds are racemic mixtures corresponding to the D- and L-nucleosides). Such analogues have the potential to mimic or antagonize the function of the naturally occurring nucleosides and, after phosphorylation, nucleotides; but unlike nucleosides, these analogues have a carbon–nitrogen bond joining the heterocyclic base to the cyclopentane ring comparable in stability to that of a simple alkyl derivative (see above) and, therefore, not susceptibile to enzymatic fission as is the glycosyl bond of true nucleosides.

The first compound of this type prepared, the racemic carbocyclic analogue of adenosine (C-Ado), proved to be biologically active as expected.[68-70] It is highly cytotoxic to both H.Ep.-2 and L1210 cells in culture, but was not effective against L1210 leukemia in vivo at the maximum tolerated dose. It is rapidly converted to the mono-, di-, and triphosphates and to some extent, deaminated by adenosine deaminase (ADA) in intact cells. No compound migrating like nicotinamide–adenine dinucleotide (NAD) was detected, and little or no incorporation into nucleic acids occurred.[71] C-Ado is a substrate for AK, but cell lines lacking AK have at most a low level of resistance to C-Ado, indicating that the nucleoside analogue itself has potent growth-inhibitory properties. The finding that this compound is a very potent reversible inhibitor of *S*-adenosylhomocysteinase (AHC)[72] may explain the activity of C-Ado itself, since inhibition of this enzyme is known to cause an accumulation of *S*-adenosylhomocysteine (AdoHcy) which interferes, by feedback, with vital transmethylation reactions involving *S*-adenosylmethionine.[73] 3-Deazaadenosine ($c^3$-Ado) is

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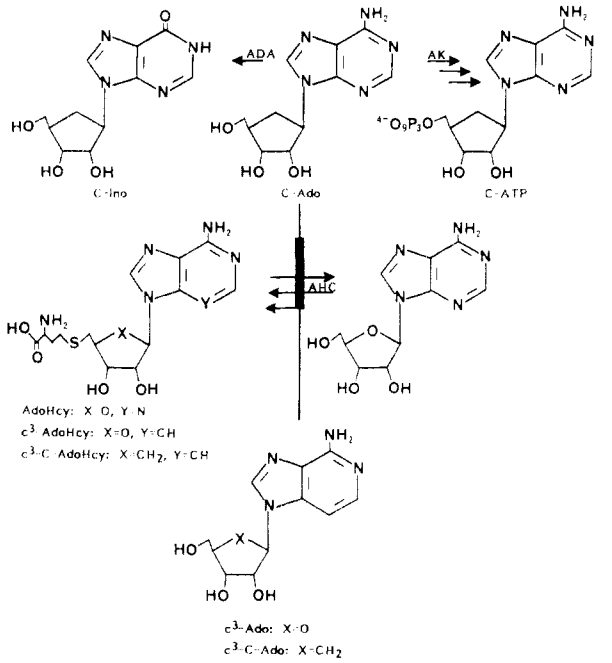
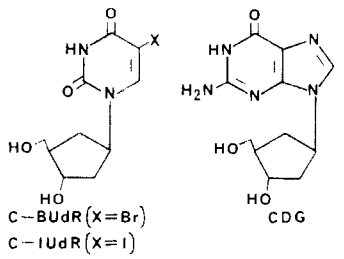



not deaminated and is phosphorylated only to a very minor extent, but it is an alternative substrate, and therefore a competitive inhibitor of AHC,[74] although much less potent than C-Ado. These data suggested the synthesis of the carbocyclic analogue of 3-deazaadenosine (C-c³-Ado). Studies with this compound[75] showed that it is not deaminated or phosphorylated, but is as potent as C-Ado in the inhibition of adenosylhomocysteinase and, in some cells, serves as an alternative substrate for the enzyme. In contrast to c³-Ado, C-c³-Ado is a potent antiviral agent with activity against vaccinia and a variety of RNA viruses.[76]

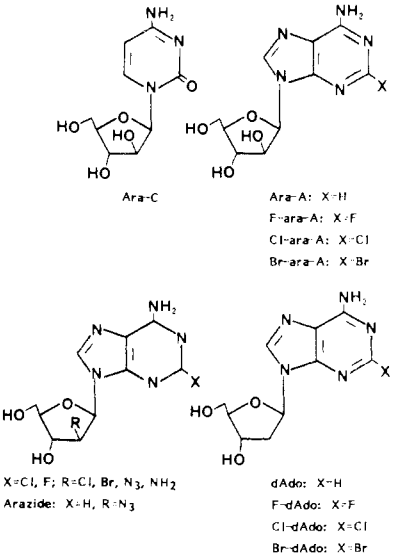
Other carbocyclic analogues [such as carbodine,[77] C-5-bromo- and 5-iodo-2′-deoxyuridines (C-BUdR and C-IUdR),[78] and C-2′-deoxyguanosine (CDG)[79]] have




shown good antiviral activity in murine systems. These compounds are not only not cleaved to the bases, but do not appear to be incorporated into DNA and so should not have the toxicity and potential long-term liability of the corresponding nucleosides. Pure D- and pure L-C-2′-deoxyguanosine have been prepared by treatment of DL-C-2-amino-2′-deoxyadenosine with adenosine deaminase, which rapidly deaminates the D isomer and very slowly deaminates the L isomer. The antivirial activity was shown to reside in the D isomer.

## Nucleosides of the Haloadenines

1-β-D-Arabinofuranosylcytosine (ara-C), despite its rapid deamination in vivo, is a useful agent in the treatment of acute myelogenous leukemia, when given on the proper schedule. 9-β-D-Arabinofuranosyladenine (ara-A), on the other hand, has shown little activity in




murine leukemia systems unless administered with an inhibitor of adenosine deaminase, such as deoxycoformycin, to block its rapid deamination and allow the persistence of effective blood levels of the drug.[80,81] Even then the combination must be given every 3 h for 24 h on days 1, 5, and 9 to cure the P388 and L1210 leukemias.[81] This schedule was found earlier to be necessary to obtain cures with ara-C.

Prior work in our laboratory showed that substitution of the 2-position of adenosine by fluorine dramatically reduced its deamination but did not interfere with its phosphorylation to the triphosphate.[82] The resulting 2-fluoroadenosine is a highly cytotoxic nucleoside, but it has no specificity for cancer cells and consequently is inactive in all of the in vivo tests to which it was subjected. We prepared 9-β-D-arabinofuranosyl-2-fluoroadenine (F-ara-A) on the presumption that it too would be resistant to deamination, but still be phosphorylated. This turned out to be the case, and F-ara-A is curative in the L1210 system on a daily schedule.[83] The persistence of effective levels of F-ara-ATP in the leukemia cells for 12 h or more explains the effectiveness of the daily schedule. Although ara-A is readily phosphorylated by deoxycytidine kinase and poorly phosphorylated by adenosine kinase, F-ara-A is phosphorylated only by deoxycytidine kinase. The selective toxicity of F-ara-A for L1210 cells in vivo appears to result from a greater accumulation of phosphates of the drug in leukemia cells relative to the cells of the small intestine.[84] Influx of the drug into epithelial cells is

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much lower than into L1210 cells, and kinase activity for F-ara-A is lower in cell-free extracts from the intestinal epithelium cells than extracts from L1210 cells. F-ara-ATP inhibits both nucleoside diphosphate reductase and DNA polymerase $\alpha$ from L1210 cells and therefore is considered to be a self-potentiating antimetabolite. More recently, it has been found to be a potent inhibitor of DNA primase also.[85] The resulting inhibition of DNA synthesis is presumably responsible for cell killing. F-ara-A 5′-phosphate (a water-soluble form) has shown activity in both Phase I and Phase II clinical trials,[86,87] but it is too early to assess its ultimate utility in the treatment of human cancer.

The observation that a strain of the P388 leukemia resistant to adriamycin is collaterally sensitive to F-ara-AMP led to an examination of the metabolism of this compound in the two cells lines in vivo. Results indicate that an increase in the formation of the active metabolite, F-ara-ATP, in the resistant line relative to the amount formed in the parent line probably contributes to this increased sensitivity. This cell line, P388/ADR, is sensitive to ara-C but not collaterally sensitive. Studies with the P388/0 leukemia using the combination of adriamycin and F-ara-A, suggested by the collateral sensitivity of P388/ADR, showed therapeutic synergism.[88] These observations may provide a basis for the clinical use of F-ara-A in combination with adriamycin.

The activity of F-ara-A caused us to study other haloadenine nucleosides. 9-$\beta$-D-Arabinofuranosyl-2-chloroadenine (Cl-ara-A) is less active than F-ara-A against leukemia L1210 in mice, and the 2-bromo compound (Br-ara-A) is without significant activity in vivo, even though all three arabinonucleosides show similar toxicities to both L1210 and H.Ep.-2 cells in culture and inhibit DNA synthesis in H.Ep.-2 cells similarly.[89] At the same time the chloro and bromo analogues are not detectably phosphorylated by deoxycytidine kinase isolated from L1210 leukemia cells, whereas the fluoro compound is. All of these nucleosides are highly resistant to deamination.[90] Other arabinonucleosides with the halogen, azide, and amino groups substituted for the 2′-hydroxyl have also been studied.[91] The derivatives of 2-chloroadenine were not toxic to either L1210 or H.Ep.-2 cells except for the 2′-amino-2′-deoxy compound, which was toxic at a high level. The derivatives of 2-fluoroadenine, on the other hand, were toxic to both cell lines, and the $IC_{50}$ values for the 2′-azido and 2′-amino compounds in H.Ep.-2 cells were not much higher than the values for F-ara-A itself.[89] In vivo data on these nucleosides are not yet available. Arazide [9-(2-azido-2-deoxy-$\beta$-D-arabinofuranosyl)adenine] is curative against the P388 leukemia, but only if administered with 2′-deoxycoformycin to block its deamination—otherwise, it is inactive.[92]

Studies on the 2′-deoxyribonucleosides of the haloadenines (F-, Cl-, and Br-Br-dAdo) have proven more fruitful. These compounds are more toxic to both L1210 and H.Ep.-2 cells than the arabinonucleosides and more effective in blocking DNA synthesis. They are resistant to adenosine deaminase and readily phosphorylated by deoxycytidine kinase,[90] which probably accounts for their greater potency in vitro. Although they are more potent in vivo also, the difference is smaller. All three haloadenine 2′-deoxyribonucleosides are curative in the L1210 leukemia system if they are given every 3 h for 24 h on days 1, 5, and 9, but they are not curative on a daily schedule as is F-ara-A. A depot form of 2-chloro-2′-deoxyadenosine, its 5′-nonanoate, is curative on a daily schedule. Since these deoxyribonucleosides are much more readily phosphorylated than F-ara-A and more potent in their biological effects, their triphosphates must be catabolized more rapidly in the target L1210 cells than that of F-ara-A, but this has not yet been established. Nevertheless, activity in human leukemias with 2-chloro-2′-deoxyadenosine has been observed.[93] Ara-A and dAdo in the presence of 2′-deoxycoformycin, and the arabino and deoxyribonucleosides of both 2-fluoro- and 2-chloroadenine, which resist deamination, are all selectively toxic to T-lymphoblasts relative to B-lymphoblasts,[94] suggesting their application in the treatment of T-cell leukemias.

## Concluding Remarks

In the past 30 years, scientists at Southern Research Institute have developed useful anticancer agents through an understanding of their metabolism, mechanisms of action, and the problems associated with their use, i.e., toxicity. It is clear that approaches to drug design must be tailored to the type of agent under study—chemically reactive compounds, intercalating agents, mitotic inhibitors, or antimetabolites. In the future development of such agents, major advances can be expected using new rapidly developing techniques. Molecular biology, for example, can provide quantities of scarce proteins for study and evaluation of candidate agents. X-ray crystallographic data can be used to determine the three-dimensional structure of critical enzymes (particularly their active sites). X-ray and NMR can be used to study drug–receptor interactions, by using computer graphics to simulate these interactions. These powerful tools should, once their use is properly understood, permit much more rapid progress toward the synthesis of more effective chemotherapeutic agents than the conventional approaches we have used until now in the rational design of drugs based on our limited understanding of drug–receptor interactions, and drug metabolism.

Looking into the future, the treatment of human cancer using monoclonal antibodies, specific for cancer cells and loaded with cellular toxins or radionuclides, appears promising as does the use of the so-called biological response modifiers, e.g., interferon, growth factors such as interleukin-2, and tumor necrosis factor.

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Even if successful, however, they will almost certainly have to be combined with other modalities such as surgery, radiation, and conventional chemotherapy to effect cures. The discovery of oncogenes and their products holds the promise of new targets for drug design that may allow the development of truly specific anticancer agents based on exploitable biochemical differences between normal and neoplastic cells.

*I gratefully acknowledge the contributions by my associates at Southern Research Institute, whose names appear in the references. Much of this work was supported by a grant from the National Cancer Institute, CA-34200.*

# Effects of Electron-Transfer Processes on Conformation

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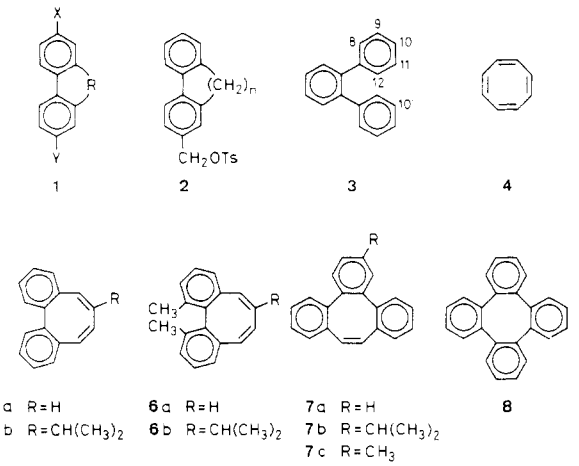
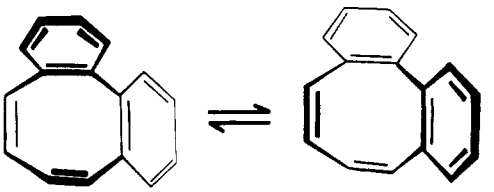
*Received February 4, 1986*

The energy profiles of conformational change processes in unsaturated hydrocarbons are important for many chemical and structural considerations. A classical example is the rotation in biphenyl derivatives (see structure 1). Mislow demonstrated that the angle of




torsion between the benzene rings in **1** increases with the length of the *o,o'* linkage.[1] A chemical consequence of the biphenyl conformation was described by Paquette, who found that the rate of solvolysis of the tosylates **2** depends critically on the ring size *n* and thus on the inter-ring torsional angle.[2] The conformational properties of the biphenyl species are controlled by a subtle interplay of electronic substituent effects, non-

**Scheme I.**
**The Ring Inversion of Dibenzo[*a*,*c*]cyclooctene (5a)**



bonding interactions between ortho hydrogens, and ring strain. Is there a simple way of separating the various steric and conjugational influences?

One method of changing a molecule A in a controlled fashion is ion formation via a redox reaction. A single electron transfer creates a radical anion, $A^{\bullet -}$ (or cation $A^{\bullet +}$), which in a subsequent step can transform into a dianion, $A^{2-}$ (or dication $A^{2+}$).[3,4] Monocyclic annulenes are particularly promising substrates for this area of research since change of the $\pi$-electron configuration, e.g., the interconversion of $4n$ and $(4n + 2)$ $\pi$-systems, affects the $\pi$-bonding. Spectroscopic characterization of $\pi$-charge-delocalized mono- and polycyclic ions is also relevant for synthetic purposes, in particular for reductions or reductive alkylations in liquid ammonia.[5-7] The reason is that knowledge of the spin density or charge density distribution in the anionic intermediates is essential for understanding the remarkable regioselectivity of these reactions.

Imagine that there is rotation about a CC single bond with a partial $\pi$-bond character. Ion formation actuates a new frontier orbital which may be either bonding or antibonding with respect to the CC bond, thereby inducing a change of the energetics of rotational isomerization in comparison with the neutral compound.

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Klaus Müllen is Professor of Chemistry at the Institute of Organic Chemistry, University of Mainz. He was born in Cologne in 1947. In 1969 he obtained a Diplomchemiker degree at the University of Cologne after work with E. Vogel. His Ph.D. degree was granted by the University of Basel, Switzerland, in 1971 where he undertook research with F. Gerson. In 1972 he joined the group of J. F. M. Oth at the Swiss Federal Institute of Technology of Zürich. He received the Habilitation degree from the ETH Zürich in 1977 and the same year became Professor at the Institute of Organic Chemistry, University of Cologne. He moved to the University of Mainz in 1983. His main interests are concerned with electron-transfer reactions in organic synthesis, electron storage in organic materials, structures of carbanions, and dynamic NMR spectroscopy.

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