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Biomimetic chemistry

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Abstract: Several cases are described in which the chelate effect contributes to binding and catalysis. In the first examples, dimers made up of linked cyclodextrins show very strong selective substrate binding, and with catalytic groups in the linkers they are effective catalysts of reactions of bound substrates. In later examples, chelate binding of transition states by a base and an acid produce effective catalysts whose geometric preferences reflect the detailed mechanisms of the catalyzed processes. In the final examples, chelate binding to biological receptors of some molecules with two polar end groups produces a class of particularly effective cytodifferentiating agents, of potential use in cancer chemotherapy.

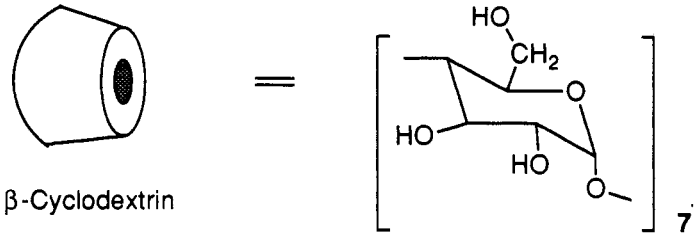
Enzymes differ from simple catalysts in several respects. For one, they bind their substrates in well-defined geometries. For another, they generally use simultaneous bifunctional or even polyfunctional catalyses. The results of these stylistic advantages are well known—improved rates and high selectivities. To learn how to apply these enzymatic principles in simpler chemical systems, we started work over 40 years ago in a field that we named (1) "biomimetic chemistry." Although this has now become a very large area of research, we will describe only our own recent work in this field, together with some earlier references.

BINDING BY CYCLODEXTRIN DIMERS

Many forces can contribute to the binding of a substrate to an enzyme, but a principal one is hydrophobicity. Although one need not imitate Nature by using water as a solvent, much of our work has focussed on catalyst-substrate complexes formed in water using hydrophobic binding. For this purpose, the highly available cyclodextrins have been particularly attractive. In water, a well-fitting hydrocarbon such as an adamantyl group or a t-butylphenyl group can bind into β-cyclodextrin (cycloheptaamylose) with an association constant of the order of 10^4 M^{-1}. While this is enough to permit the use of simple cyclodextrin derivatives as mimics of many enzymes, stronger and more well-defined binding would be better. For this reason, some years ago we set out to construct and study a new class of enzyme mimics—cyclodextrin dimers in which a catalytic group was located in the linker.

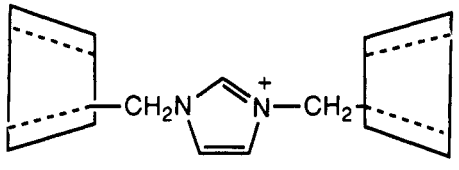
Our early studies (2) on binding by cyclodextrin dimers such as **1** showed that with appropriate double ended substrates (e.g. **2**) we could achieve K_a's as high as 10^9 M^{-1} in

water. Studies (3) with antihydrophobic agents showed that this high affinity resulted from hydrophobic binding of two hydrocarbon residues into the two cyclodextrin units; the $\Delta G°$ of binding can more than double, since with chelate systems there is no need to pay the full translational entropy cost twice. Of course there are entropy costs associated with flexibility in the host or guest molecule, so greater affinities were achieved by removing some degrees of freedom.

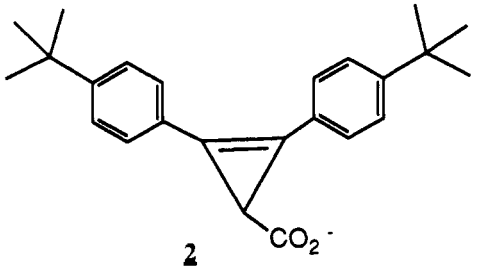


β-Cyclodextrin

7



1

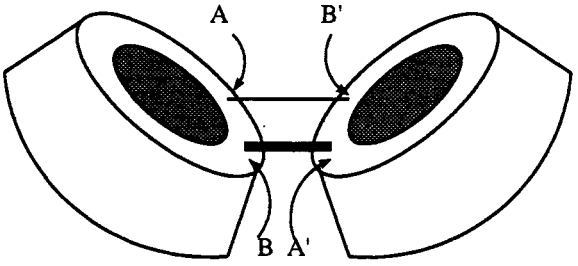


2

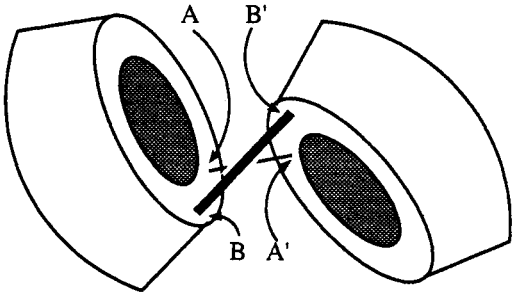
$K_a = 10^9\ M^{-1}$ in water

We synthesized a couple of cyclodextrin dimers in which two links joined them (3,4). The links were placed on adjacent glucose residues, at positions 6A and 6B, so they act as a hinge. With such double linkage, the two cyclodextrin rings can be joined in two different ways. In one—the desired isomer—the cyclodextrins can move together to become face-to-face (the occlusive isomer). In this isomer each link goes from the 6A position of one cyclodextrin to the 6B position of the other (lettering the seven glucose rings clockwise), so the two linkers are equivalent in the NMR. The undesired isomer has one link from 6A to 6A', and the other from 6B to 6B'. The result of this arrangement is what we call the aversive isomer, in which the two rings cannot cooperatively bind a single substrate. We found (4) that the occlusive isomer had a very high binding constant for a substrate that could occupy both cavities, while the aversive dimer showed only 10^4 M^{-1} or so K_a , consistent with single occupancy of a cyclodextrin by one end of the substrate.

The argument that a dimeric host should bind a double-ended substrate particularly well is entropic in nature. That is, one might expect that the enthalpy would simply more or



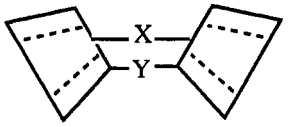
C_1 Occlusive



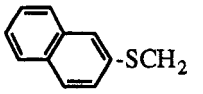
C_2 Aversive

less double with double binding, but that there would be a significant entropy advantage compared with the double binding of two separate guest molecules. Interestingly, our calorimetric studies on several such cases show that this is not the experimental result (5). The chelate systems double the enthalpies or more, but show big entropy disadvantages relative to the monomeric cases. This is apparently related to the flexibility of the systems and the kinds of solvation changes that can lead to enthalpy-entropy compensation, but the result is striking. For example, binding of an adamantyl group into a cyclodextrin ring at 25 °C in water shows a $\Delta H°$ of -5 to -7 kcal/mole, and a $\Delta S°$ of essentially zero, as the entropy loss on bimolecular complexing is compensated by the entropy gain for water release. However, when di(1-adamantylethyl) phosphate binds into cyclodextrin dimers such as compound **6** (discussed later) and related structures, the $\Delta H°$ is now -14.5 to -16.2 kcal/mole, but the $\Delta S°$ is now -16 to -21 e.u. In another example, dimeric binding leads to a $\Delta H°$ that is 5 times that for monomeric binding, but a $\Delta S°$ that goes from +7 e.u. for the monomer to -37 e.u. for the dimer!

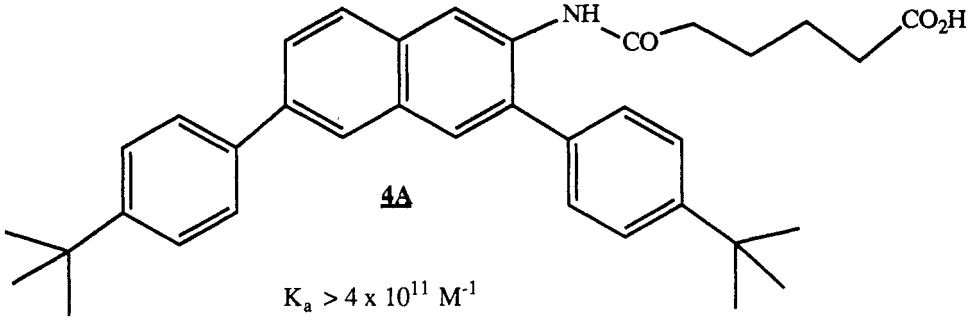
A particular feature of such doubly-linked dimers is their potential for geometric selectivity. We synthesized a pair of dimers—aversive and occlusive—in which there was one long link and one short one (4). The occlusive isomer **3** showed a marked preference for binding a bent substrate **4A**, in which the geometry matched that required by the host, rather than a linear analog **4B**. The difference is at least 4000-fold; it may be greater, since the affinity for the bent isomer is so high that we have only been able to set a lower limit of 4×10^{11} M^{-1} for K_a. This is as high as the affinity of some of the strongest antibodies for their substrates. Such shape recognition is not only characteristic of antibody bonding, of course. Enzymes typically derive much of their catalytic ability from recognizing and binding the shape of the transition state of a reaction better than they bind the substrate. Our preference for binding a bent transition state analog rather than a linear substrate analog is directly aimed at mimicking this enzyme feature, as will be discussed.



3

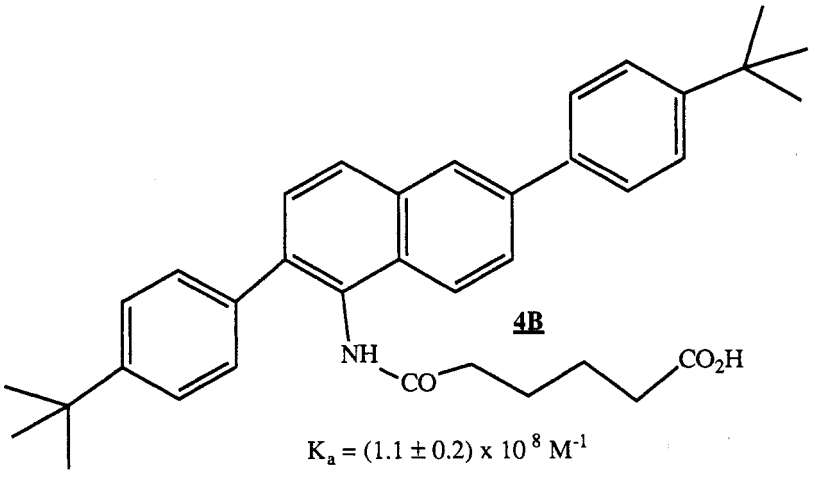
X: CH_2S-  -SCH_2

Y: -CH_2-S-S-CH_2-



4A

$K_a > 4 \times 10^{11}\ M^{-1}$



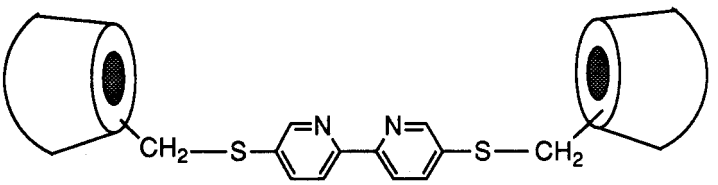
4B

$K_a = (1.1 \pm 0.2) \times 10^{8}\ M^{-1}$

CATALYSIS BY CYCLODEXTRIN DIMERS

A simple catalyst **5** based on cyclodextrin dimers was constructed by using a 2,2'-bipyridyl group as the linker (6). This permits us to incorporate a catalytic metal ion into the space between the two binding groups. With bound metal ions we see a rate acceleration for the hydrolysis of a doubly-binding ester **6** of as much as 220,000-fold. In this case Cu^{2+} is a better catalyst than is Zn^{2+} or Ni^{2+}. Some years ago we had developed catalyst systems combining a metal ion with the oxime of pyridine-2-carboxaldehyde (7,8). In such a complex the metal ion binds to the two nitrogen atoms, while the OH group ionizes and acts as a nucleophile. Thus we have examined this system with our cyclodextrin dimer catalysts. We find that the rate acceleration for ester

hydrolysis is 1,700,000, and now the best metal ion is Zn^{2+}. We believe this reflects the geometric preference for tetrahedral Zn^{2+} rather than square planar Cu^{2+} or Ni^{2+} in the case with the oxime ligand.



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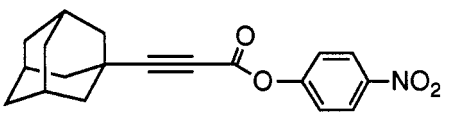

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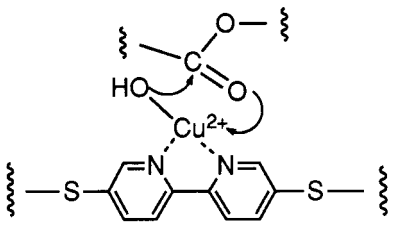
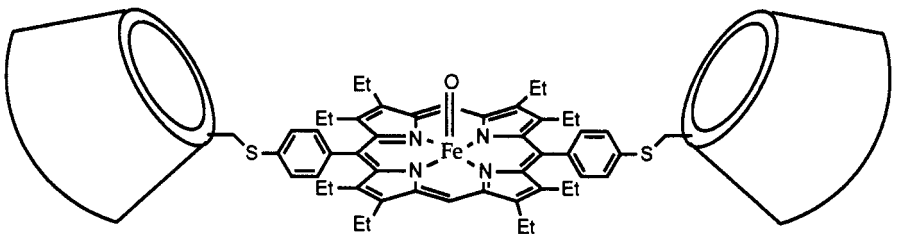


In recent work we are combining two of these approaches. Zhang (9) is preparing cyclodextrin dimers with two AB linkers, but with one of them a bipyridyl unit and the other a short disulfide link. This catalyst will have a metal catalytic group and a preference for a non-linear guest. We hope to use this geometric preference to add preferential binding of the transition state—resembling a tetrahedral intermediate rather than a planar amide substrate group—to the chemical catalysis by metal ions. Time will tell whether this strategy moves us even closer to the performance of true enzymes.

Of course hydrolysis is not the only process of interest for which metal ions can play a role. We have had a long-standing program (10) devoted to imitating the selective functionalization of a substrate that some enzymes can perform. For instance, enzymes belonging to the class of cytochrome P-450 are involved in many hydroxylations and other oxidations of substrates. As an example, cholesterol is converted to cortisone by a series of such enzymatic reactions, in which hydroxyl groups are formed by the insertion of oxygen atoms into unactivated hydrocarbon positions. The regioselectivity and stereoselectivity of such processes—and the fact that they are directed to chemically unactivated substrate positions as the result of geometric control—make these reactions well worth imitating.

We had described the use of double ion pairing to immobilize a substrate onto a ketone in order to direct photochemical functionalization (11), and had found that a corresponding compound with only one binding interaction was not effective. We had also used metal coordination to hold a substrate onto an iron porphyrin derivative so as to direct selective epoxidation or hydroxylation (12). More recently we have prepared catalyst 7, in which

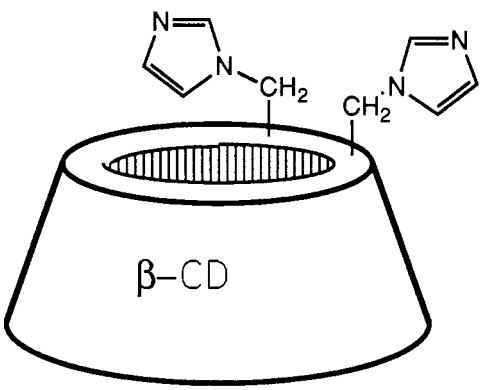
an iron porphyrin derivative carries two cyclodextrin binding groups (13). We are now studying the ability of this catalyst to perform selective epoxidations and hydroxylations of substrates that bind into both cyclodextrin cavities. From our previous experience, such double binding should increase the efficiency and selectivity of the processes.



7

BIFUNCTIONAL CATALYSIS

Enzymes normally use two (or more) catalytic groups, not just multiple binding interactions. We have examined the effectiveness of bifunctional catalysis in some enzyme mimics. In early examples (7), and some recent ones (14), we have combined a metal catalytic group with a nucleophile or a base, to produce effective esterase mimics. We have also studied bis-imidazole compounds, with the two catalytic imidazole groups



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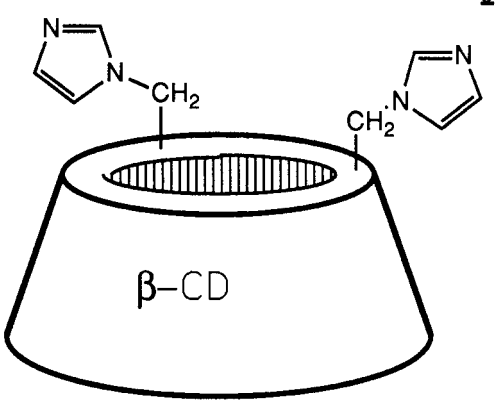

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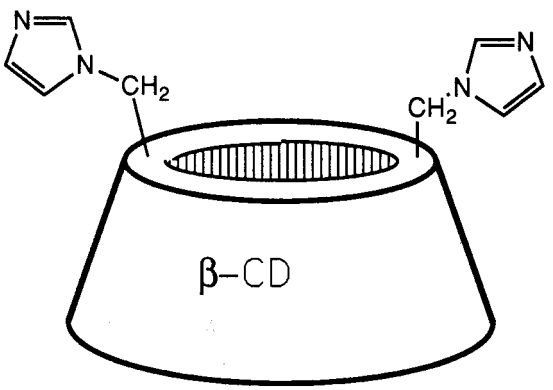
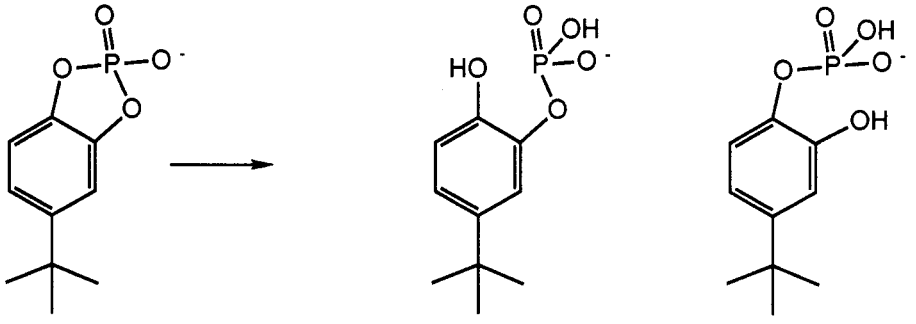


10

mounted on a β-cyclodextrin nucleus. Using appropriate functionalizing schemes, we have placed the two imidazoles on neighboring glucose primary carbons **8** (6A,6B) or on the A,C **9** or A,D **10** residues (15). These geometric differences greatly affect to catalytic behavior of the compounds, and in ways that are consistent with the likely chemical mechanisms involved.



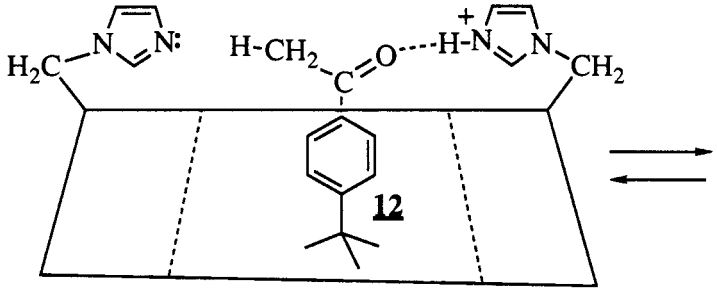
11 chiefly almost none

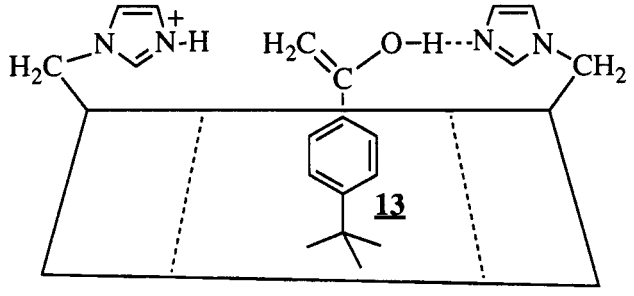
For example, at a pH at which on the average one imidazole is protonated (BH^+) while the other is not (B:) all three of these isomers are able to perform the bifunctionally catalyzed hydrolysis of a cyclic phosphate substrate **11** that binds into the cyclodextrin cavity (15). In other mechanistic work we were able to show that such bifunctional catalysis of phosphate hydrolysis has a preferred mechanism in which the BH^+ protonated the phosphate oxyanion while the B: delivers a water to the phosphorus (16-18). The geometric implications of such a mechanism are that the AB catalyst isomer should be the best, as was observed (15). This observation is actually support for the proposed mechanism, since an alternative mechanism would have preferred the AD geometry. Our evidence (19) indicates that the process involved is indeed a concerted bifunctional reaction, with two protons simultaneously transferred (to the B: and from the BH^+). The technique involved is a proton inventory, in which the rate is plotted as a function of the mole fraction in a D_2O/H_2O mixture. Studies (19) with a cyclodextrin monoimidazole establish the validity of the method.

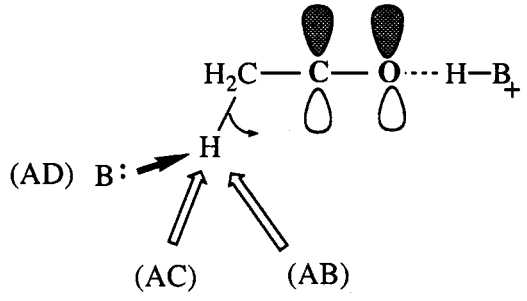
There are many reactions that should be subject to simultaneous bifunctional catalysis by an acid and a base group. As another recent example, we have studied (20) the enolization of a ketone, p-t-butylacetophenone **12**. This substrate binds into the cyclodextrin catalysts and can be converted to its enolate by base catalysis, or directly to the enol **13** by simultaneous acid/base catalysis. We monitored this enolization reaction by observing deuterium exchange into the methyl group of the ketone. The rate in buffer alone, at pH 6.2, is extremely slow but there is significant catalysis by cyclodextrin-6-imidazole and by cyclodextrin 6A,6B-, 6A,6C-, and 6A,6D-bisimidazoles. Interestingly, in this case the AB and AC isomers **8** and **9** are not significantly more effective than is the monoimidazole catalyst, but the AD **10** isomer is better. Apparently the AD isomer performs bifunctional acid-base catalysis of the enolization. Consistent with this, it shows a pH vs. rate curve with a rate maximum near pH 6.2, and a decrease at higher and lower pH's. This indicates that both the B: and the BH^+ play a catalytic role. By contrast, with the cyclodextrin monoimidazole catalyst there is simply catalysis by the B: form.

The preferred geometry for this enolization is interesting. In molecular models it is possible for the BH^+ of all three bis-imidazole isomers to reach an electron lone pair on the oxygen while the B: reaches the CH_3 proton; the difference has to do with the

direction of approach. When the proton of the C-H bond is removed, the electrons must move toward the p orbital of the carbonyl group. With the AB isomer the B: approaches the proton from the carbonyl side, with the AC isomer it approaches along the C-H bond direction, while with AD it approaches from behind the proton, opposite to the direction in which the electrons must move. It seems reasonable that this would be preferred for stereoelectronic reasons, although before now there was no evidence on the point. One of the powers of our set of bifunctional catalysts is their use in discovering stereochemical preferences of this sort.







CYTODIFFERENTIATION

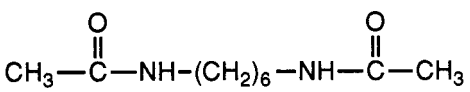
In this account we have described the application of the chelate effect—cooperation of two binding groups or groups (catalysts) that bind transition state—in the area of biomimetic chemistry. There is another project in which this concept has served us very well, in the area of cancer chemotherapy. We will briefly describe this work, that has

extended over the past 18 years.

Charlotte Friend had discovered (21) that erythroleukemia cells would undergo cytodifferentiation to erythrocytes under the influence of significant concentrations of DMSO in aqueous solution. We addressed the question of how to find differentiating agents with greater efficacy. Amides such as N-methylacetamide were even more effective than was DMSO (22), but the concentrations required were still of the order of 50 mM or so. In order to improve efficacy further, we tried the chelate effect. We hoped—there was no evidence for the idea—that these agents were binding to two (or more) sites in the cell, and that these sites were nearby. If so, combining the compounds into bis-amides, with appropriate linkers, could lead to better binding and thus greater effectiveness.

In the absence of any evidence at all for this idea, we had to choose a linker group and length. We selected a six-carbon chain linker on a hunch, and it proved to be a good one. In all the compounds we have examined (over 600, with dozens of active compounds) the optimum length for such linkers has proven to correspond to this guess.

The first more effective cytodifferentiating agents (23) were hexamethylene bis-acetamide **14** (HMBA) and its reversed analog suberic acid bis-dimethylamide (SBDA) **15**. Both compounds were effective at low millimolar concentrations, and HMBA has even had some success in clinical trials with cancer patients (24). In fact, this simple compound has been the subject of innumerable studies—by our collaborators and others—since we introduced it as a cytodifferentiating agent. However, in spite of the chelate effect the doses required are still quite high. For this reason we turned to some other structures.



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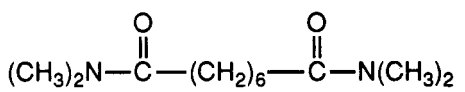

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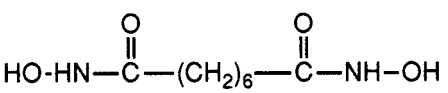

16

Although we have some evidence on the likely target of these agents (25), we have no information on the chemical structures to which they bind. However, there are only two likely choices. A polar group such as a sulfoxide or an amide could coordinate to a metal ion or it could participate in hydrogen bonding. In either case, it seemed that a hydroxamic acid group could bind more strongly than does a simple amide group. This was the case: suberoyl bis-hydroxamic acid **16** is even more effective (25) than are the bis-amides, causing cellular differentiation at low micromolar concentrations. Some other related structures are even more effective.

Although we are engaged in vigorous efforts to push the best of our compounds forward into clinical trials, it remains to be seen whether these cytodifferentiating agents will prove to be useful in cancer chemotherapy. If so, it will be particularly fitting that the

chelate effect (chela: claw) might be useful against cancer (the crab). Time will tell.

Acknowledgment. I thank my coworkers named in the references, and the National Institutes of Health, the Office of Naval Research, and Sterling-Winthrop Co. for financial support.

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