Pure Appl. Chem., Vol. 72, No. 3, pp. 333–342, 2000.

The chelate effect in binding, catalysis, and chemotherapy*

Ronald Breslow†, Sandro Belvedere, Leland Gershell, and David Leung

Department of Chemistry, Columbia University, New York, New York 10027, USA

Abstract: Cyclodextrin (CD) dimers bind amino acid side chains, and such binding can dissociate aggregated proteins, including citrate synthase (dimer) and lactic dehydrogenase (tetramer). A CD dimer can bind a hydrophobic photosensitizer that, upon irradiation, generates singlet oxygen. This cleaves the dimer and releases the photosensitizer. CD dimers in a cytochrome P-450 mimic steer catalyzed hydroxylation to a bound steroid with geometric control. Chelate binding has also led to a group of cytodifferentiating agents whose mechanism has been recently established. They have promising anticancer properties, and are currently entering human trials as therapeutic agents.

INTRODUCTION

The chelate effect refers to the advantage in binding that a double-ended ligand such as ethylenediamine has compared with the separate ligands such as ammonia or methylamine. There are two aspects. In one, the free energy of bidentate binding by a double-ended ligand can simply be twice that for monodentate binding of one of the separate analogs, since the double-ended ligand has two binding interactions compared with one interaction for a simple ligand. The other aspect is that the free energy advantage of bidentate binding can be greater than twice that of simple monodentate binding, since in bidentate binding the second interaction can have entropy advantages. That is, once one end of a bidentate ligand is attached, the second end is in the vicinity and can bind without paying all the translational and rotational entropy cost of the first binding interaction. In this case, chelate binding by a bidentate ligand can have a free energy **more** than twice that of the monodentate analog.

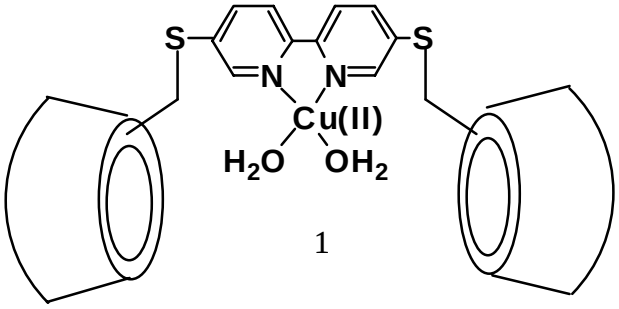
We have examined this situation with respect to binding by cyclodextrins (CDs), by using cyclodextrin dimers to bind double-ended ligands, and have found that indeed the binding energies can easily be additive [1]. In addition, when the cyclodextrin dimer is relatively rigidly linked, by two chains, the binding to a rigid ditopic substrate is even stronger than simple additivity would predict [2].

Interestingly, although the arguments for the chelate effect are entropic—a double-ended ligand does not have to tie down two separate molecules to achieve double binding, as a simple monotopic ligand must—we found that the binding in water solution by several cyclodextrin dimers of ditopic ligands whose two ends can each occupy one of the cyclodextrins is stronger than is binding to simple monomeric cyclodextrins because of **enthalpy** advantages [3]. In fact, the **entropy** of ditopic binding was less favorable than for monodentate binding. This probably reflects the weakness of simple gas-phase arguments to the situation in solution, where solvation changes can affect both enthalpy and entropy. In spite of this interesting aspect of the thermodynamics, chelate binding is still of course considerably stronger than is monodentate binding.

---

*Lecture presented at the 5th International IUPAC Symposium on Bioorganic Chemistry (ISBOC-5), Pune, India, 30 January–4 February, 2000. Other presentations are published in this issue, pp. 333–383.
†Corresponding author

Another advantage of chelate binding by a double-ended ligand to a cyclodextrin dimer is that the geometry of the resulting complex is well-defined compared with the geometry available when the same ligand binds to simple cyclodextrin. In appropriate cases, the central portion of the double-ended ligand is held right next to the linker in the cyclodextrin dimer. If that linker carries a catalytic group it may be able to act on the doubly bound ligand. For example, we have seen that we can catalyze the hydrolysis of esters that have hydrophobic groups on both ends, to bind into cyclodextrin dimer **1**, with a bipyridyl unit in the linker. When a metal ion such as Cu(II) or Zn(II) is bound to the bipyridyl, it catalyzes the ester hydrolysis with as much as a 1 400 000-fold acceleration in appropriate cases [4]. Although the largest effect was seen with esters having phenoxide leaving groups [4], we have recently seen that we can achieve a large acceleration with a simple alkoxide ester in an appropriate case [5].



1

A special advantage of such a cyclodextrin dimer catalyst for ester hydrolysis is that the two hydrolyzed fragments are no longer bound by a chelate effect, so they rapidly dissociate from the catalyst and permit multiple turnovers. This effect was first seen in the hydrolyses referred to above, but it will also be discussed later when a ditopic ligand is oxidatively cleaved, and the fragments are released from a cyclodextrin dimer.

There is one more advantage to the bidentate binding of a substrate to a cyclodextrin dimer—the well-defined geometry can be used to direct chemical reactions to particular parts of the substrate. This will be described as well in the later parts of this paper.

In the final section of this paper we describe the use of the chelate effect to produce some very effective medicinal compounds that are now entering human trials. This work does not use cyclodextrins, but it is intellectually related. We have been working on these compounds for over 20 years, but now we understand how they work, and have strong evidence for their usefulness in cancer treatment.

BINDING BY CYCLODEXTRIN DIMERS

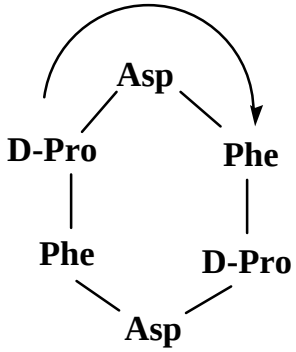
We have described the ability of some cyclodextrin dimers to bind to the hydrophobic side chains of peptides. In collaboration with Clark Still, we first examined the binding of beta-cyclodextrin itself to peptide sequences in a limited peptide library produced by combinatorial synthesis [6]. We saw that there was a specificity for the binding to peptides with an L-Phe,D-Pro sequence (or its D-Phe,L-Pro mirror image), and computer simulations indicated that this specificity reflected the binding of the phenyl group fully into the CD cavity from the secondary CD side while the proline helped fill some of the space on the CD secondary face that was not filled by the phenyl group. Thus, we examined the ability of CD dimers to bind to peptides with **two** such Phe,Pro sequences, **2** and **3**.

We saw [7] that binding by CD dimer **4** was better by an order of magnitude, but that there was less than free energy additivity. Interestingly, binding was better for the cyclic peptide **2** than for its

linear analog **3**, but the free energy additivity relative to monomeric CD was worse. We concluded that the linear peptide had some self aggregation of its hydrophobic groups, but that once that was broken by CD binding the second CD ring could bind the second Phe,Pro sequence reasonably well. The cyclic peptide was so rigid that there was no internal aggregation that needed breaking by CD binding, but that rigidity made the chelate binding less than ideal.

In the same study we examined the binding of a Trp,Trp sequence to some CD dimers (tryptophan was not included in the combinatorial library examined with Clark Still) and saw chelate binding that was stronger than binding by single CDs. Thus, we decided to examine the binding of CD dimers to proteins.

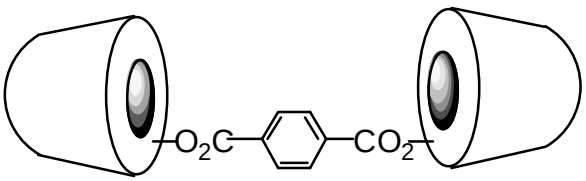
There is great interest in protein aggregation, and in agents that can disrupt it [8–16]. Many enzymes function only as dimers or tetramers, and the aggregation of these enzymes usually involves hydrophobic binding by nonpolar side chains. We thought that binding of CD dimers to such side chains might break up the aggregation and inhibit the enzymes. Since HIV protease is such an enzyme [11], the result could have medical utility. Thus, we have initiated a program to explore this by screening enzymes with CD dimers. We have found [17] that indeed two CD dimers are able to inhibit the dimeric enzyme citrate synthase (CS) and the tetrameric enzyme lactic dehydrogenase (LDH), but that other CD dimers and other enzymes were not affected. Physical methods indicate that indeed the enzymes CS and LDH are being dissociated by our CD dimers, and computer simulations together with protein structures indicate the likely protein side chains that are being bound.



2

Ac-Asp-Phe-D-Pro-Asp-Phe-D-Pro-Gly-Gly-NH_2

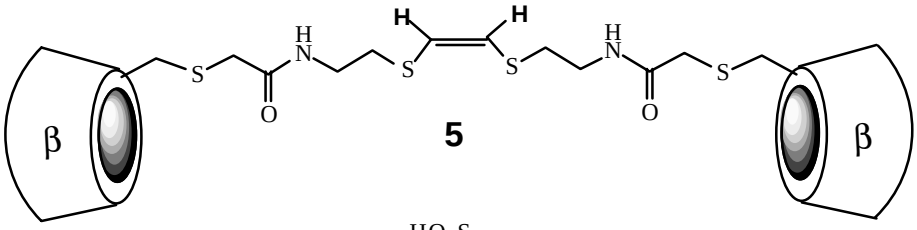
3



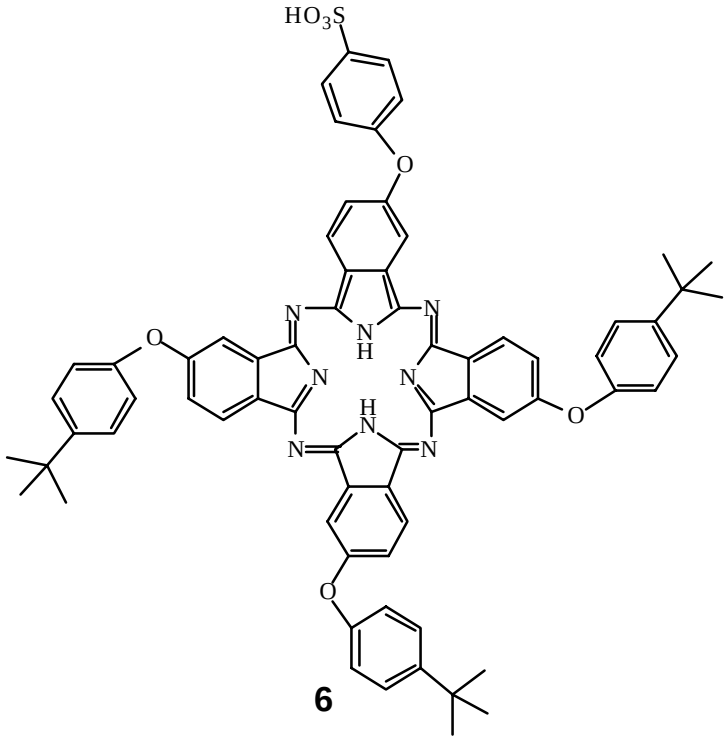
4

Protein aggregation by association of hydrophobic regions is a widespread and important phenomenon, and can also involve the binding of one protein to another different one. For example, the binding of human growth hormone to its receptor is such a case [18]. We hope that our approach to such systems may be general, using not only CD dimers but also CD trimers and tetramers, and that with selectivity such approaches may be useful in medicine and biology.

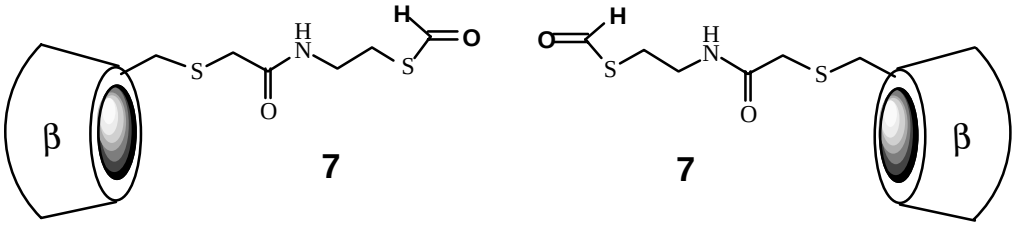
One of the specialized treatments of some cancers uses a technique known as photodynamic therapy [19]. A photosensitizer is administered to the patient, and irradiation of the cancer area causes the photosensitizer to generate singlet oxygen, which kills the neighboring cells. A challenge in this



5



6



7 **7**

area is to devise a way to deliver the photosensitizer to the cancer region, to increase effectiveness and decrease toxic side effects from photoactivation in other parts of the body. We have devised a promising approach to this problem using a CD dimer.

Möser had proposed some time ago [20] that CD dimers might be used to carry and release photosensitizers, and his coworker Ruebner came to my laboratory to see how to do this. As we have reported elsewhere [21], we have constructed a CD dimer **5** whose linker contains an olefin unit with sulfur atoms at each end. Such electron-rich olefins react readily with singlet oxygen, and the resulting dioxetanes then cleave to form carbonyl groups. We find that our CD dimer binds a phthalocyanine photosensitizer **6** carrying tert-butylphenyl groups and makes the sensitizer soluble in water. Then irradiation of this complex causes the linker to be cleaved by singlet oxygen, and the sensitizer precipitates. Release of the sensitizer is helped of course by loss of chelate binding from the monomeric CD fragments **7**, but we find that the cleaved linker fragments also bind into the CD cavities, competitively extruding the sensitizer.

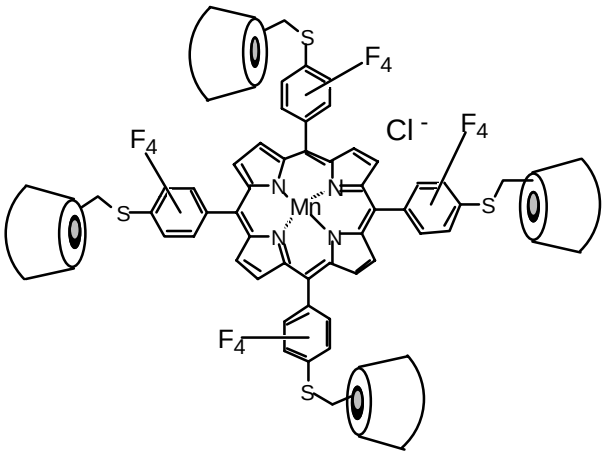
If it were liberated *in vivo*, the hydrophobic photosensitizer should bind into neighboring cells, but we do not yet have biological results with these compounds. Most exciting, the sensitizer is released from the CD dimer only in the light path, so if the light is directed into the cancer region the photosensitizer should be released and concentrated in that region. In other words, the photosensitizer will concentrate in the cancer region since that is the region being irradiated. Our studies also indicate that the singlet oxygen is being delivered to the olefin link in the CD dimer selectively, because of spacial geometry in the complex. Singlet oxygen generated elsewhere in solution attacks other parts of the CD dimer, not just the olefin in the linker.

THE CHELATE EFFECT IN CATALYSIS

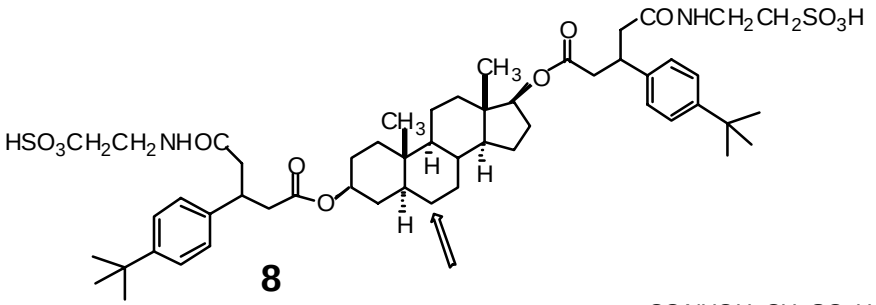
In the introduction we have already cited our work using CD dimers to perform hydrolytic catalysis of ester groups. However, one of the most interesting aspects of enzyme catalysis is selectivity, not just rate acceleration, and one of the most interesting selectivities is regioselectivity—the ability of an enzyme to carry out reactions in particular places in a substrate, directed not by reactivity but by the geometry of the enzyme-substrate complex. We have worked on the problem of imitating such selectivity for many years [22], and have learned how to perform the functionalization of some steroids using attached templates to steer free radical reactions to desired but otherwise unactivated positions. However, such chemistry really needs to involve turnover catalysis. We want to make artificial enzymes that will bind substrates and then functionalize them without regard to intrinsic reactivities, with selectivities dictated entirely by the geometries of the complexes. Good progress has recently been made toward this goal, using chelate binding by CD dimers.

We have prepared tetraphenylporphyrins carrying attached CD units, and examined their Mn(III) complexes as catalysts for the hydroxylation of bound steroids [23–25]. The steroid **8** that gave the most interesting results was derived from androstandiol by attaching to each hydroxyl ester units that carried hydrophobic tert-butylphenyl binding groups, for the CDs, and water solubilizing groups. We saw that the substrate bound to CDs on opposite sides of the porphyrin cavity in **9** to place a steroid carbon right above the Mn(III) in the porphyrin. Then treatment with iodosobenzene caused oxygen atom transfer to the Mn(III), and resulting hydroxylation of a saturated carbon of the steroid. Only one product **10** was formed, in quantitative yield and with ca. 180 catalytic turnovers as product was released and new substrate bound to the catalyst.

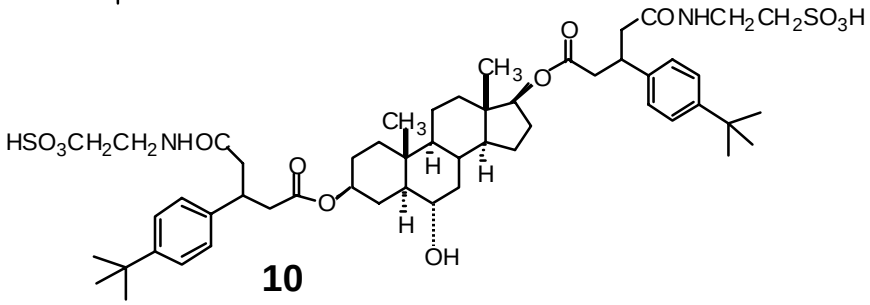
The product **10** formed had inserted oxygen into the C-6 methylene group of the steroid, and stereoselectively to form only the 6α-hydroxysteroid. Molecular models are consistent with this regioselectivity and stereoselectivity. In other work we have moved the position of attack by changing the geometry of the related complex, and have even seen that we can hydroxylate unactivated carbon in



9 = beta-cyclodextrin



8



10

the presence of olefinic groups and hydroxyl groups, which are normally more easily oxidized than are saturated unactivated carbons. Of course, the finding that our oxidation yields only the 6α-hydroxysteroid, not the related 6-ketone, is also a form of selectivity since secondary alcohols are more easily oxidized than are saturated methylene groups by metalloporphyrin-catalyzed oxidations. Apparently, the oxidation of such an alcohol to its keto derivative requires access to the C-H group, which in this case is geometrically inaccessible within the complex. The preference for oxidation within a catalyst-substrate complex prevents random oxidation of unbound product to the ketone.

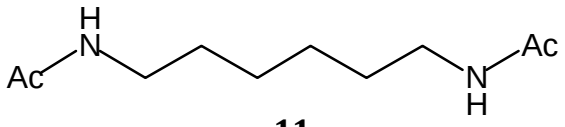
Further work is needed to develop such catalytic selective oxidation chemistry to the point at which it can replace enzymatic processes for research and manufacture of medicinal compounds. However, these early results suggest that such progress should be possible. It will change the way chemists achieve selective transformations.

THE CHELATE EFFECT IN CHEMOTHERAPY

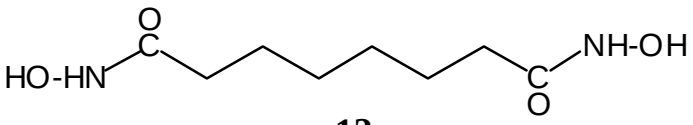
Most approaches to cancer chemotherapy involve killing the cancer cells, and problems arise when toxic compounds are not sufficiently selective. A very different approach has been the subject of research in our laboratory extending over ca. 25 years now, stimulated by an early discovery by Dr. Charlotte Friend. Dr. Friend was examining what are now called "Friend cells," pre-erythrocytes that are infected by a virus that alters their normal behavior. Pre-erythrocytes are stem cells whose normal behavior is to undergo both proliferation and differentiation. Proliferation produces more stem cells, and differentiation converts them into mature cells with normal cell function and no capacity to proliferate. Pre-erythrocytes are spherical, have nuclei, and contain no hemoglobin. Erythrocytes are flattened, have no nuclei (so they can't proliferate), and contain hemoglobin. Friend cells in mice produce a disease called erythroleukemia, in which pre-erythrocytes proliferate, but mature red cells are not formed.

To perform a process called transfection, Dr. Friend added 280 mM dimethyl sulfoxide (DMSO) to the aqueous medium in which Friend cells [murine erythroleukemia (MEL) cells] were growing, and saw to her surprise that the tube later turned red and that 67% of the cells were clearly adult erythrocytes. In other words, the DMSO had induced cytodifferentiation of cancer cells. This was brought to the attention of Dr. Paul Marks and Dr. Richard Rifkind, who then came to see me about it. Thus started a 25-year collaboration to turn this observation into a way to approach the treatment of cancer. If DMSO could transform malignant Friend cells into normal erythrocytes without killing them, we hoped that something similar could be done with other cancers, and with more effective drugs that require lower concentrations.

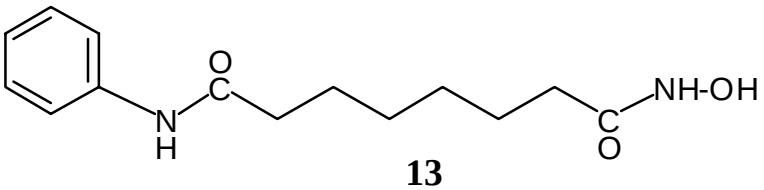
The first finding was that amides were even more effective than was DMSO [26], but the concentrations required were still unreasonable for human treatment. We then invoked the chelate effect, by linking two amide groups together (e.g., **11**), and found that this indeed made the compound more effective at differentiating MEL cells [27]. After a number of studies varying the structures based on bis-amides, we concluded that a new approach was required. We reasoned that amide groups were either binding to metal ions or making hydrogen bonds with their receptors, and that in either case hydroxamic acid groups could be better. Thus, we made a group of bis-hydroxamic acids [28], and found that they (e.g., **12**) were indeed significantly better drugs.



11



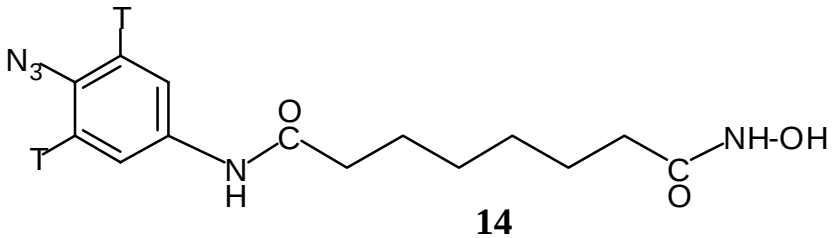
12



13

In the course of this work we decided to vary the idea of the chelate effect a bit, by having drugs with two binding groups that were not necessarily the same. We examined compounds with one hydroxamic group and one hydrophobic amide group (e.g., **13**—some new, even more potent compounds are currently under study), and found that they were even more effective [29]. They were tested against at least 75 different human cell types, and were very effective in stopping their growth. Some underwent cytodifferentiation, other underwent apoptosis (programmed cell death). However, the compounds have shown little toxicity in tests with mice, rats, rabbits, and dogs. They have been effective in stopping the growth of xenografts of human prostate cancer and human neuroblastomas in mice that are immunocompromised so they do not reject the cancers. Perhaps most interesting, in rat [30] and mouse [31] feeding experiments they prevented the development of breast cancer and lung cancer when potent carcinogens were administered to the animals, so they are chemopreventive when taken orally.

To identify the biological target of our compounds, we made a radioactive photoaffinity label **14** based on compound **13**, and irradiated it in the presence of various cell fractions [32]. The first target identified was a ribosomal nuclease, but later work made it likely that the primary target for our compounds is histone deacetylase [33]. We see a good parallel between the levels of drug needed to elicit the biological response and that needed to inhibit histone deacetylase, and the structures of our most effective compounds are sensible for this role.



14

In collaboration we saw an X-ray crystal structure of compound **13** bound to a homologue of histone deacetylase, and the details are striking [34]. The enzyme has a bound Zn(II) at the bottom of a narrow tube, with a large plateau at the surface. Our inhibitor coordinates the hydroxamic acid group of **13** to the Zn(II) of the enzyme, passes a polymethylene chain down the narrow tube, and lets the hydrophobic end of our compound lie on the surface plateau. The more potent new compounds are also sensible in terms of this structure. Thus, we expect that rational design based on this information may lead to even better drugs.

A curious situation developed in the course of all this work. It is clear that the bis-hydroxamic acids and mono-hydroxamic acids are effective inhibitors of histone deacetylase, and inhibition of this enzyme helps expose DNA for transcription. However, the earlier bis-amides with which we started have no effect on this enzyme. They are operating by a different mechanism, not yet identified. In spite

of this difference, the optimal linker length is the same for the bis-amides and the bis-hydroxamic acids. This is a biological puzzle we are still addressing.

CONCLUSIONS

The great effectiveness and geometric definition resulting from double binding has opened up whole new areas of potentially important medicinal compounds and enzyme mimics. Only time will tell whether the leads developed are truly useful in medicinal and synthetic chemistry.

ACKNOWLEDGMENTS

We thank the NIH and the NSF for financial support, including NIH-MSTP predoctoral fellowships for Leland Gershell and David Leung.

REFERENCES

1. R. Breslow, N. Greenspoon, T. Guo, R. Zarzycki. *J. Am. Chem. Soc.* **111**, 8296 (1989).
2. R. Breslow and S. Chung. *J. Am. Chem. Soc.* **112**, 9659 (1990).
3. B. Zhang and R. Breslow. *J. Am. Chem. Soc.* **115**, 9353 (1993).
4. B. Zhang and R. Breslow. *J. Am. Chem. Soc.* **119**, 1676 (1997).
5. J. Yan and R. Breslow. *Tetrahedron Lett*. **41**, 2059 (2000).
6. M. Maletic, H. Wennemers, D. Q. McDonald, R. Breslow, W. C. Still. *Angew. Chem. Int. Ed. Engl*. **35**, 1490 (1996).
7. R. Breslow, Z. Yang, R. Ching, G. Trojandt, F. Odobel. *J. Am. Chem. Soc.* **120**, 3536 (1998).
8. R. Zutschi, R, M. Brickner, J. Chmielewski. *Current Opinion in Chemical Biology* **2**, 62 (1998).
9. J. Chmielewski. *Biopolymers* **47**, 263 (1998).
10. R. Zutshi, M. D. Shultz, L. Ulysse, R. Lutgring, P. Bishop, B. Schweitzer, K. Vogel, J. Franciskovich, M. Wilson, J. Chmielewski. *Synlett* (**10**), 1040 (1998).
11. R. Zutshi, J. Franciskovich, M. Shultz, B. Schweitzer, P. Bishop, M. Wilson, J. Chmielewski. *J. Am. Chem. Soc.* **119**, 4841 (1997).
12. D. L. Daugherty and S. H. Gellman. *J. Am. Chem. Soc.* **121**, 4325 (1999).
13. H. S. Park, Q. Lin, A. D. Hamilton. *J. Am. Chem. Soc.* **121**, 8 (1999).
14. Q. Lin, H. S. Park, Y. Hamuro, A. D. Hamilton. *NATO ASI Ser., Ser. C* **527**, 197 (1999)
15. Q. Lin, H. S. Park, Y. Hamuro, C. S. Lee, A. D. Hamilton. *Biopolymers* **47**, 285 (1998).
16. Y. Hamuro, M. C. Calama, H. S. Park, A. D. Hamilton. *Angew. Chem. Int. Ed. Engl.* **36**, 2680 (1997).
17. R. Breslow, D. Leung, Z. Yang, unpublished work.
18. T. Clackson, M. H. Ultsch, J. A. Wells, A. M. de Vos. *J. Mol. Biol.* **277**, 1111 (1998).
19. T. J. Dougherty, C. J. Gomer, B. W. Henderson, G. Jori, D. Kessel, M. Korbelik, J. Moan, Q. Peng. *J. Natl. Cancer Inst.* **90**, 889 (1998).
20. J. G. Möser, A. Ruebner, A. Vervoorts, B. Wagner. *Proc. Eighth Int. Symp. on Cyclodextrins*, J. Szetli, ed., Kluwer Academic Publishers, 71 (1996).
21. A. Ruebner, Z. Yang, D. Leung, R. Breslow. *Proc. U.S. Natl. Acad. Sci.* **96**, 14692 (1999).
22. R. Breslow. in *Templated Organic Synthesis*, F. Diederich and P. Stang, eds., Wiley-VCH, Weinheim, Germany, 1999, chap. 6, and references therein.

23. R. Breslow, X. Zhang, Y. Huang. *J. Am. Chem. Soc.* **119**, 4535 (1997).
24. R. Breslow, Y. Huang, X. Zhang, J. Yang. *Proc. Natl. Acad. Sci. USA* **94**, 11156 (1997).
25. R. Breslow, B. Gabriele, J. Yang. *Tetrahedron Lett.* **39**, 2887 (1998).
26. M. Tanaka, J. Levy, M. Terada, R. Breslow, R. A. Rifkind, P. A. Marks. *Proc. Nat. Acad. Sci. USA* **72**, 1003 (1975).
27. R. C. Reuben, R. L. Wife, R. Breslow, R. A. Rifkind, P. A. Marks. *Proc. Nat. Acad. Sci. USA*, **73**, 862 (1976).
28. R. Breslow, B. Jursic, Z. F. Yan, E. Friedman, L. Leng, L. Ngo, R. A. Rifkind, P. A. Marks. *Proc. Nat. Acad. Sci. USA* **88,** 5542 (1991).
29. V. M. Richon, Y. Webb, R. Merger, T. Sheppard, B. Jursic, L. Ngo, F. Civoli, R. Breslow, R. A. Rifkind, P. A. Marks. *Proc. Nat. Acad. Sci. USA* **93**, 5705 (1996).
30. L. A. Cohen, V. M. Richon, P. Marks, D. Desai, S. Amin. *Proc. Amer. Assoc. Cancer Res.* **39**, 108 (1998).
31. D. Desai, K. El-Bayoumy, S. Amin. *Proc. Amer. Assoc. Cancer Res.* **40**, 2396 (1999).
32. Y. Webb, X. Zhou, L. Ngo, V. Cornish, J. Stahl, H. Erdjument-Bromage, P. Tempst, R. A. Rifkind, P. A. Marks, R. Breslow, V. M. Richon. *J. Biol. Chem.* **274**, 14280 (1999).
33. Y. Webb, L. Ngo, V. M. Richon, R. Breslow, R. Rifkind, P. A. Marks. *Proc. Amer. Assoc. Cancer Res.* **39**, 108 (1998).
34. M. S. Finnin, J. R. Donigian, A. Cohen, V. M. Richon, R. A. Rifkind, P. A. Marks, R. Breslow, N. A. Pavletich. *Nature* **401,** 188 (1999).