to propionyl CoA. The proton carrier can then be biotin itself, rather than its isourea tautomer. It is not necessary to derive an estimated pK_a of 6.4 for that tautomer, rather than the 9 estimated above.

Unfortunately these results do not clarify the importance of the sulfur in biotin. Biotin does not show a proton-exchange mechanism second order in H^+. Therefore there is no need to invoke a transannular interaction as in **2**. The electron-withdrawing effect of the sulfur does affect reactivity, but oxygen would show nearly the same effect. The default rationalization is that only biotin itself has the optimum geometry to fit into the enzyme site, but this is not very informative.

Acknowledgment. This research was supported by National Science Foundation Grant CHE85-09312.

Registry No. Carbon dioxide, 124-38-9; biotin, 58-85-5; biotin methyl ester, 608-16-2.

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π-Facial Selection in Intermolecular Diels–Alder Reactions: Total Syntheses of (+)-Actinobolin and (+)-5,6,10-*triepi*-Actinobolin

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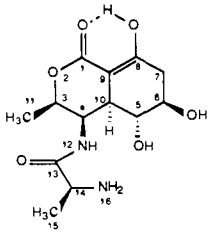
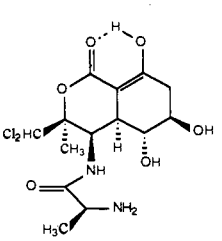
Abstract: Syntheses of both 5,6,10-*triepi*-actinobolin and the antibiotic actinobolin are described in which a homochiral diene prepared from L-threonine is employed as a key component in a Diels–Alder reaction with an acetylenic dienophile. While the Diels–Alder reaction of this diene with methyl propiolate furnished the cycloadduct required for the synthesis of (+)-actinobolin as the minor diastereomer, the completion of the synthesis required but seven additional steps. The steric and stereoelectronic features responsible for the π-facial course of this cycloaddition reaction are discussed along with the various steps required to complete the syntheses of the title compounds.

Actinobolin (**1**) is a broad spectrum antibiotic first obtained from submerged aerated broth cultures of *Streptomyces griseoviridus* var. *atrofaciens* by Haskell and Bartz.[1a] The substance



Actinobolin (1)

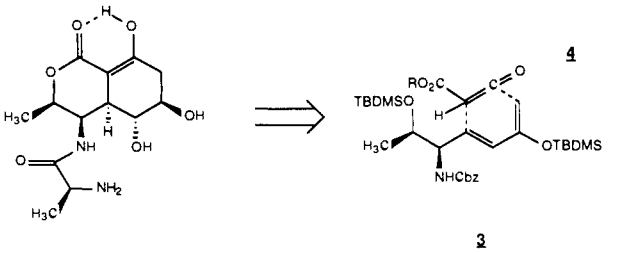


Bactobolin (2)

is an amphoteric, water-soluble lactone that readily forms crystalline salts with acids. It chelates iron, aluminum and other metal ions. Actinobolin was found to be a potent inhibitor of various Gram-positive and Gram-negative bacteria, and it was found to possess some antileukemic activity as well.[1b,c] The structure of actinobolin was determined through a combination of chemical degradations,[1d] derivatizations, and spectral analyses which were additionally aided by a computer program designed to evaluate the structural implications of the experimental data.[1e-g] Closely related to actinobolin structurally is the chlorine-containing antibiotic bactobolin (**2**), a compound isolated from a culture broth of *Pseudomonas* BMG-13-147.[2] Bactobolin exhibits both stronger antibacterial activity and more pronounced antileukemic activity than does actinobolin.

In this article we describe our efforts to synthesize actinobolin in the laboratory through an intermolecular Diels–Alder strategy.

Scheme I. A Retrosynthetic Analysis



As shown below, (Scheme I), we envisioned the assembly of actinobolin through reaction of the silyloxydiene **3** with some carbalkoxyketene equivalent **4**. The construction of the diene component from L-threonine, the π-facial course of the reaction of this diene with methyl propiolate, and the conversion of the Diels–Alder products to *triepi*-actinobolin and actinobolin are detailed in the following sections.[3,4]

† University of Pittsburgh.
‡ Merck Institute.

(1) (a) Haskell, T. H.; Bartz, Q. R. *Antibiot. Ann.* **1959**, 1958/1959, 505. (b) Pittillo, R. F.; Fisher, M. W.; McAlpine, R. J.; Thompson, P. E.; Ehrlich, J.; Anderson, L. F.; Fiskin, R. A.; Galbraith, M.; Kohberger, D. L.; Manning, M. C.; Reutner, T. F.; Roll, D. R.; Weston, K. *Antibiot. Ann.* **1959**, 1958/1959, 497. (c) Burchenal, J. H.; Holmberg, E. A. D.; Reilly, H. C.; Hemphill, S.; Reppert, J. A. *Antibiot. Ann.* **1959**, 1958/1959, 528. (d) Munk, M. E.; Sodano, C. S.; McLean, R. L.; Haskell, T. H. *J. Am. Chem. Soc.* **1967**, *89*, 4158. (e) Nelson, D. B.; Munk, M. E.; Gash, K. B.; Herald, D. L. *J. Org. Chem.* **1969**, *34*, 3800. (f) Antosz, F. J.; Nelson, D. B.; Herald, D. L.; Munk, M. E. *J. Am. Chem. Soc.* **1970**, *92*, 4933. (g) Nelson, D. B.; Munk, M. E. *J. Org. Chem.* **1970**, *35*, 3832.

(2) (a) Kondo, S.; Horiuchi, Y.; Hamada, M.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1979**, *32*, 1069. (b) Ishizuka, M.; Fukasawa, S.; Masuda, T.; Sato, J.; Kanbayashi, N.; Takeuchi, T.; Umezawa, H. *Ibid.* **1980**, *33*, 1054. Hori, M.; Suzukake, K.; Ishikawa, C.; Asakura, H.; Umezawa, H. *Ibid.* **1981**, *34*, 465. Veda, I.; Munakata, T.; Sakai, J. *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.* **1980**, *B36*, 3128.

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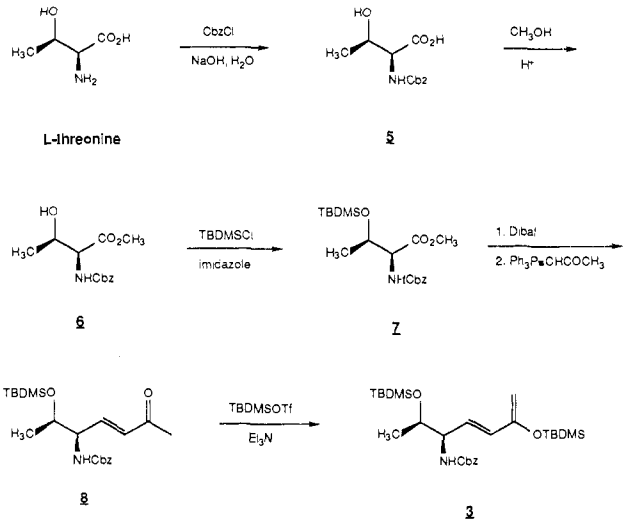
Scheme II. Preparation of the Diene Component

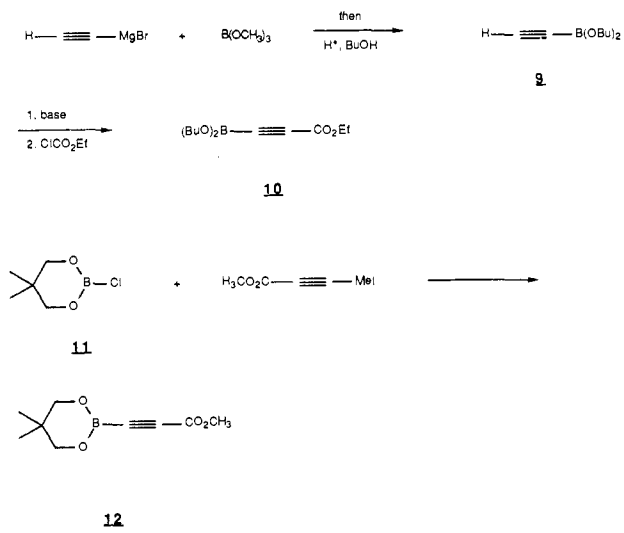


Preparation of the Diene Component. The preparation of the silyloxydiene **3** was easily carried out in six steps starting from the amino acid L-threonine. The amino group was first protected as its carbobenzoxy (Cbz) derivative,[5] a Fischer esterification was carried out on **5**, and the hydroxy group was protected as its *tert*-butyldimethylsilyl ether derivative.[6] The ester group of **7** was then reduced to the aldehyde, and the crude product subjected to a Wittig reaction with acetonylidenetriphenylphosphorane to afford the enone **8** and a small amount of unreacted ester **7**. The aldehyde intermediate was not purified since epimerization was found to occur during chromatographic separation attempts. Indeed, extensive racemization of α-amino aldehydes on silica gel has been reported.[7] The E enone **8** was then treated with *tert*-butyldimethylsilyl triflate in the presence of triethylamine to afford the chiral diene **3** in 82% overall yield from L-threonine.[8] Diene **3** could be purified on silica gel with less than 5% decomposition to the enone **8**. However, since the subsequent Diels–Alder reaction conditions also caused partial reversal of the diene to the enone, the diene was generally used without purification, and any enone present was simply recovered after the cycloaddition reaction.

That no epimerization took place in any of the foregoing steps was made apparent from an examination of the high field ^{1}H NMR spectra recorded for intermediates **3** and **5–8**. Any scrambling of the amine-bearing stereocenter would have been coupled with the production of diastereomeric products thus resulting in a doubling of at least some of the resonance signals observed for these products. Such doubling was not observed. Additionally, the stereochemical integrity of this amine center was rigorously confirmed by an X-ray analysis carried out on one of the products prepared from diene **3** (vide infra) (Scheme II).

Candidate Carbalkoxyketene Equivalents. With the obtention of the optically active diene **3**, our attention now turned to the selection of a dienophile reactive enough to form a cycloadduct with **3** and, moreover, possessing the capability of leading to the β-keto lactone functionality present in actinobolin.

Scheme III. Attempted Preparation of an Acetylenic Boronate



Earlier work in our laboratories had shown the ability of 1,3-dicarbethoxyallene to function as a carbethoxyketene equivalent in the Diels–Alder reaction.[9] While this dienophile reacted readily with diene **3**, the tendency of the exocyclic double bond of the cycloadduct to undergo migration into the six-membered ring during a subsequent hydroboration reaction precluded its further use.

Methyl β-bromopropiolate has been shown by Chamberlain and Rooney to serve as a carbalkoxyketene equivalent in its Diels–Alder reaction with cyclopentadiene.[10] Unfortunately, when tested with diene **3** under a variety of thermal and Lewis acid catalyzed conditions, this acetylenic ester failed to give any desired cycloadduct. Even the simple model diene 2-((trimethylsilyl)oxy)-1,3-butadiene failed to react with this dienophile.

In 1960 Arens and Bonnema reported the Diels–Alder reaction of ethyl β-ethylthiopropiolate with 1,3-butadiene.[11] Since we envisioned that the vinyl sulfide arising from such a Diels–Alder reaction might be hydrolyzable to a β-keto ester, we were prompted to prepare ethyl β-(phenylthio)propiolate from (phenylthio)-acetylene.[12] Unfortunately, this diene reacted only sluggishly with butadiene, and, moreover, we were unable to adequately hydrolyze the vinyl sulfide to ketone.

In one last attempt to devise a better carbalkoxyketene equivalent, we sought to prepare the acetylenic boronate **10**. Since dibutyl acetyleneboronate (**9**) was known to react with cyclopentadiene in the Diels–Alder sense and the resulting vinylboronate was shown to be oxidizable to a ketone,[13] the preparation of **10** appeared worthwhile. Attempts were therefore made to prepare **10** from dibutyl acetyleneboronate by deprotonation followed by acylation with ethyl chloroformate. None of the desired product was, however, obtained by this procedure. Efforts were also taken to prepare the related compound **12** from the anion of methyl propiolate by its reaction with the chloroborinine **11**.[14] Again, however, none of the desired product could be isolated (Scheme III).

In light of the foregoing difficulties, we decided to proceed with our synthesis by using methyl propiolate as the dienophile. Later on we would need to make use of the double bond at C8, C9 (actinobolin numbering) of the cycloadduct to introduce the required ketone (enol) functionality. Fortunately, this dienophile

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(4) For previous total syntheses of (+)-actinobolin, see: Yoshioka, M.; Nakai, H.; Ohno, M. *J. Am. Chem. Soc.* **1984**, *106*, 1133. Garigipati, R. S.; Tschaen, D. M.; Weinreb, S. M. *J. Am. Chem. Soc.* **1985**, *107*, 7790. Optically active *N*-acetylactinobolamine: Rahman, M. A.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1985**, *107*, 5576. Racemic *N*-acetylactinobolamine: Askin, D.; Angst, C.; Danishefsky, S. *J. Org. Chem.* **1985**, *50*, 5005. (±)-Ramulosin, a biogenetically related natural product: Cordova, R.; Snider, B. B. *Tetrahedron Lett.* **1984**, *25*, 2945.

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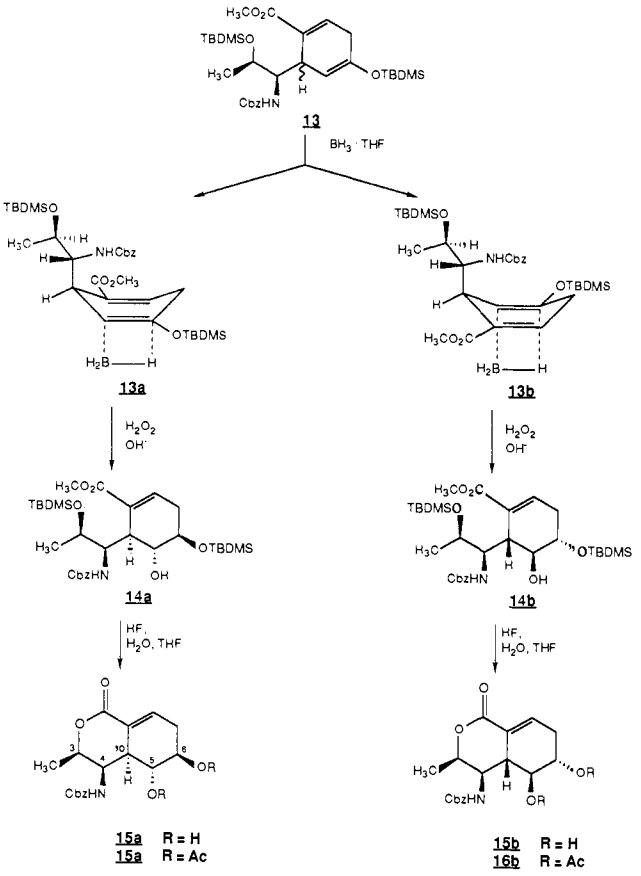
(12) Parham, W. E.; Heberling, J. *J. Am. Chem. Soc.* **1955**, *77*, 1175.

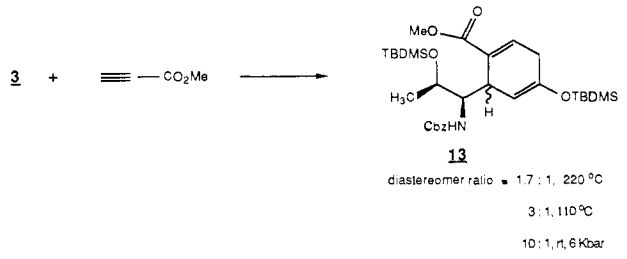
(13) Matteson, D. S.; Peacock, K. *J. Am. Chem. Soc.* **1960**, *82*, 5759.

(14) Birum, G. H.; Dever, J. L. U.S. Patent 3064032.

Scheme IV. Further Transformations of the Cycloadducts **13a** and **13b**



reacted with diene **3** in good yield. The Diels–Alder reaction was complete within 30 min at 220 °C. As expected a mixture of diastereomeric cycloadducts resulted which was found to vary from 3:1 at 110 °C to 1.7:1 at 220 °C. By running the Diels–Alder reaction under high-pressure conditions at room temperature the π-facial selectivity of the reaction could be further improved to 10:1.[15] Since we were unable to make an assignment of stereochemistry to these cycloadducts based on an analysis of their spectral data, several additional synthetic steps were carried out in order to arrive at conformationally more rigid structures.



Each of the Diels–Alder cycloadducts **13** was therefore subjected to a hydroboration/oxidation sequence in order to generate a trans, diequatorial diol unit at the site of the more electron rich enol silyl ether double bond.[16] The hydroboration reaction was expected to take place opposite the amine-bearing appendage, which for reasons relating to the minimization of $A^{1,2}$ strain[17] should assume a pseudo-axial position in the flattened, boatlike conformation[18] of the cyclohexadiene ring system (see **13a** and **13b**).[19] The products formed in this hydroboration/oxidation sequence were subsequently treated with aqueous hydrogen fluoride in tetrahydrofuran in order to deliver via desilylation and concomitant intramolecular transesterification the lactones **15a** and **15b** (Scheme IV).

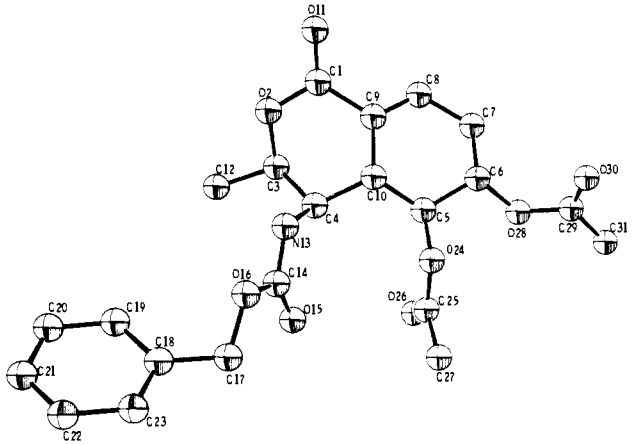


Figure 1. A computer-generated drawing of **16b** derived from the X-ray coordinates with hydrogens omitted for clarity.

A comparison of the coupling constants between the C4 and C10 hydrogen atoms in these isomeric lactones was anticipated to lead to the conclusive assignment of their structures. The C5 proton in both **15a** and **15b** appeared as a well-resolved triplet with a coupling constant of 9.6 and 10.0 Hz, respectively. These large coupling constants establish the 1,3-trans, diaxial arrangement of protons C6 and C10 relative to the axial C5 hydrogen. The C3,C4 proton coupling constants in **15a** and **15b** were found to be 1.7 and 2.5 Hz, respectively, values indicative of an axial-equatorial arrangement of these vicinal hydrogen atoms.[20]

More importantly, however, the C4,C10 vicinal hydrogen coupling constants were found to be 2.5 Hz in each isomer. Thus, we were unable to unambiguously distinguish between the isomeric lactones. After some effort, we were able to secure a suitable crystal of the diacetate derivative **16b** of the major lactone for X-ray analysis.[21] As can be discerned from the accompanying Figure 1, the stereochemical analysis of the hydroboration process was well founded. Unfortunately, the major lactone possessed incorrect stereochemistry for the synthesis of actinobolin at C10 and accordingly at stereocenters C5 and C6 as well. The dihedral angle of 116° between the C4 and C10 hydrogens observed in the crystal structure does explain the small vicinal coupling constant observed for these protons in the ^{1}H NMR spectrum of **16b**. Thus, it is the minor isomer **13a** of the cycloaddition reaction which must be taken on in order to procure actinobolin by total synthesis.

A Possible Diels–Alder Transition State. Very few examples of "intermolecular" Diels–Alder reactions employing chiral dienes were known prior to the beginning of our studies into the synthesis of actinobolin. Trost has prepared an *O*-methylmandeloxy containing butadiene which showed about 50% de in its reaction with acrolein and ca. 90% de in its reaction with juglone.[22] A π-

(15) The high-pressure reactor available commercially from the LECO Corporation, Bellefonte, PA was used in these studies.

(16) Larson, G. L.; Hernandez, D.; Hernandez, A. *J. Organomet. Chem.* **1974**, *76*, 9. Kono, H.; Nagai, Y. *Org. Prep. Proc. Int.* **1974**, *6*, 19. Klein, J.; Levene, R.; Dunkelblum, E. *Tetrahedron Lett.* **1972**, 2845.

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(21) Crystals from methanol had space group symmetry of $P2_1$ and cell constants of a = 9.967 (1) Å, b = 8.650 (1) Å, c = 13.018 (2) Å, and β = 92.87 (1)° for Z = 2 and a calculated density of 1.278 g/cm^3. Of the 1631 reflections measured with an automatic four circle diffractometer equipped with Cu radiation, 1549 were observed ($I \geq 3\sigma I$). The structure was solved with a multisolution tangent formula approach and difference Fourier analysis and refined by using full-matrix least-squares techniques. Hydrogens were assigned isotropic temperature factors corresponding to their attached atoms. The function $\sum w(|F_o| - |F_c|)^2$ with $w = 1/(\sigma F_o)^2$ was minimized to give an unweighted residual of 0.051. Tables I, II, and III containing the final fractional coordinates, temperature parameters, bond distances, and bond angles are available as Supplementary Material.

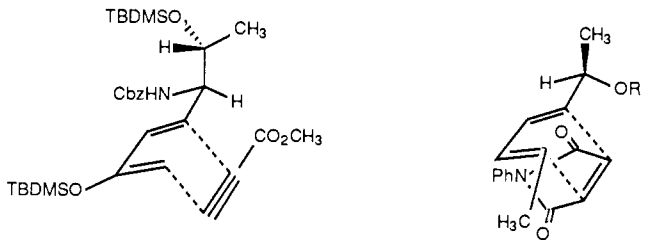
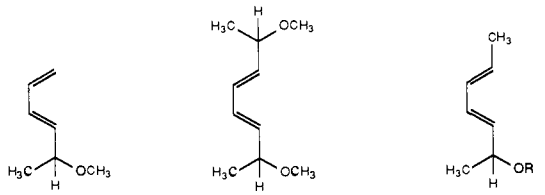


Figure 2. Possible Diels–Alder transition states.

stacking model was used to rationalize the facial selectivity exhibited by this diene. However, in a somewhat related case Stoodley has shown that a dienyloxy glycoside was capable of reacting with a quinone derivative to provide >90% de in the cycloadduct.[23] Since this latter diene contains no group capable of π-stacking one must question the reality of such a model.

More recently and more closely related to our own work with the threonine derived diene, Carrie has reported on the extent of diastereofacial selection in the Diels–Alder reaction of a 5-methoxy-1,3-hexadiene derivative **17**.[24] With TCNE as the



17 18 19a, R = Me; 19b, R = TMS

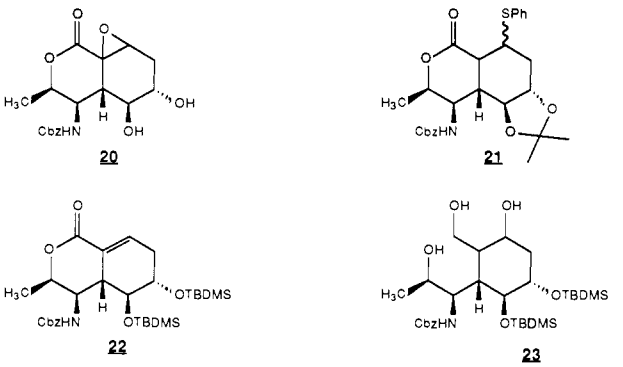
dienophile a 2:1 mixture of cycloadducts was formed. The major isomer was suggested to result from the transition state in which bond formation occurs anti to the methyl group (the large group) and the methoxy group assumes an outside position. With the *rel*-(2*R*,7*S*)-2,7-dimethoxy-3,5-octadiene **18**, the diastereofacial selection was raised to 85:15, a result in line with Tolbert's cooperativity principle.[25]

Additionally, Franck has reported that the sorbaldehyde derived dienes **19a** and **19b** give rise to a 5:1 and a 7.3:1 mixture of cycloadducts, respectively, on reaction with *N*-phenylmaleimide.[26] A rule for predicting the π-facial selectivity of Diels–Alder reactions employing dienes and dienophiles containing an allylic asymmetric center was also formulated (for an R configured diene, the dienophile is directed to the re face). Since this rule was formulated on the basis of but a few experimental results, it is not particularly surprising that it fails to correctly predict the π-facial selectivity exhibited by our threonine-derived diene. Of course, a number of differences exist between our Diels–Alder reaction and those reported by Franck and Carrie. These differences include the presence of an amido group in place of methoxy group as the heteroatom substituent, the presence of an electron-donating silyloxy group on the diene framework of the threonine derived diene (a feature which may alter the synchroneity of the Diels–Alder process), and the use of an acetylenic dienophile rather than an ethylenic one. Of these differences, it is the latter one which we believe may be the most significant.

Due to the linear nature of the acetylenic dienophile employed in our Diels–Alder reaction with diene **3**, the steric interaction of the carbomethoxy group with the amido group should be greater if this amido group assumes an *outside* rather than an *inside* position.[27] With an ethylenic dienophile, on the other hand, the inward turned nature of the activating substituent should now sterically permit the heteroatom substituent of the diene (RO in Franck's work) to assume an outside position (see Figure 2). Thus one might well be able to control the π-facial course of a Diels–Alder reaction involving a diene containing an allylic asymmetric center through the selection of either an sp or sp^2 hybridized dienophile, and experiments in this direction are planned. It should also be noted that the selection of the transition-state structures shown in Figure 2 was further guided by the presumed necessity for keeping the heteroatom from assuming a position approximately anti to the newly forming bond in order to minimize electron withdrawal from the diene by the carbon-heteroatom σ^* orbital.[27]

Further Transformations of the Diels–Alder Cycloadducts. Synthesis of 5,6,10-*triepi*-Actinobolin. Since the lactone **15b** of incorrect stereochemistry for the synthesis of actinobolin was more plentiful than **15a**, we decided to investigate initially the incorporation of the C8 hydroxyl group by using **15b** as a model compound.

In the beginning **15b** was epoxidized with buffered *m*-chloroperbenzoic acid in dichloromethane to afford epoxide **20** in 80% yield.[28] The direct conversion of this intermediate to the corresponding β-keto lactone was attempted by heating with tetrakis(triphenylphosphine)palladium(0) and 1,2-bis(diphenylphosphino)ethane in toluene.[29] This reagent system was reported by Noyori et al. to be effective for the transformation of α,β-epoxy ketones to β-diketones. Unfortunately, when epoxide **20** was



exposed to this reagent system, none of the desired enol was obtained. Other attempts to convert this epoxide to a usable product through either an acid- or a base-catalyzed rearrangement reaction[30] or through a reductive ring-opening process [Cr(II) or Zn, HOAc)] were also unsuccessful.[31]

Next, thiophenol was added to the acetonide of **15b** in a Michael reaction, and the intermediate sulfide **21** was exposed to MCPBA in anticipation of carrying out a Pummerer rearrangement[32] on the derived sulfoxide. Unfortunately, attempts to activate the sulfur atom in this way led only to the elimination of this sulfur substituent with restoration of the starting acetonide. The exposure of this sulfide to NCS gave an identical result.

Additionally, while hydroboration of the bis silyl ether derivative **22** using excess 9-BBN[33] was found to occur with concomitant reduction of the lactone ring, hydration of the double bond did take place with the desired regioselectivity. The resulting triol **23** was exposed to oxygen in the presence of platinum[34] as well

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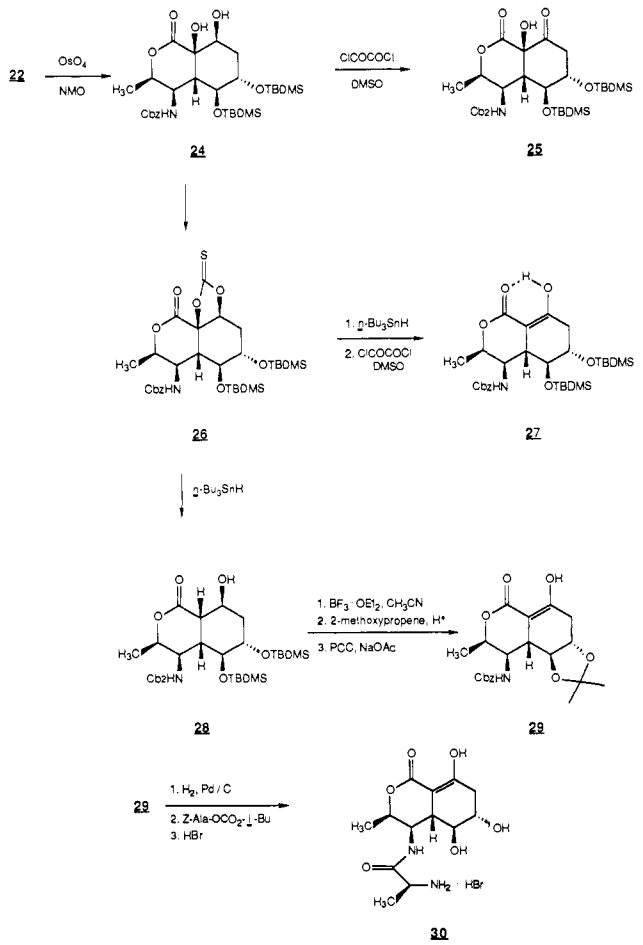
(33) Brown, H. C. *Organic Syntheses via Boranes*; John Wiley & Sons: New York, 1975.

Scheme V. Completion of the 5,6,10-*triepi*-Actinobolin Synthesis



as to silver carbonate on Celite[35] in hope of obtaining the β-hydroxy lactone through selective oxidation of the primary alcohol. While oxidation did occur, only the starting bis silyl ether derivative **22** could be isolated. The dehydration reaction may be occurring at the aldehyde (lactol) stage; for as revealed below, we were eventually able to procure the desired β-hydroxy lactone as a stable compound through a different strategy.

After these initial frustrating results, we decided to investigate a strategy involving the dihydroxylation of the double bond of **22** followed by a deoxygenation reaction. The bis silyl ether derivative **22** was therefore treated with a catalytic amount of osmium tetroxide in the presence of *N*-methylmorpholine-*N*-oxide[36] to provide the cis diol **24**. While this diol could be oxidized in turn to an α-hydroxy ketone **25** by using Me_2SO and oxalyl chloride, we were unable to prepare a xanthate ester from this intermediate[37] for use in a Barton deoxygenation reaction. Our inability to carry out this functionalization reaction presumably stems from the steric inaccessibility of the tertiary alcohol group. Diol **24** was therefore treated with thiocarbonyldiimidazole[38] in THF to provide the thionocarbonate **26** in quantitative yield. This intermediate was heated in turn with excess tri-*n*-butyltin hydride to afford a β-hydroxy lactone which could be oxidized[39] in low yield to the desired β-keto lactone **27**. The selective rupture of the tertiary carbon–oxygen bond in the Barton process[37] presumably reflects the greater stability of the tertiary radical being formed as well

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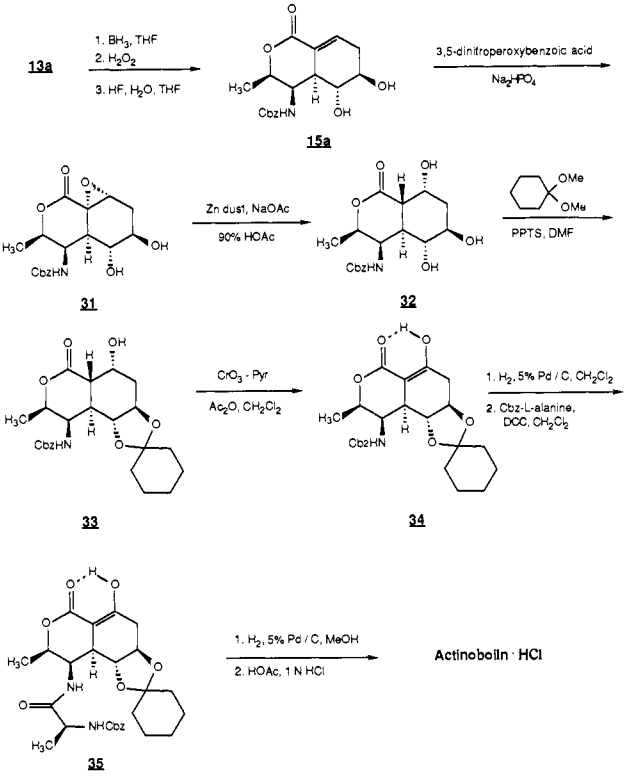
Scheme VI. The Synthetic Route to (+)-Actinobolin from **13a**



as, perhaps, some component of steric acceleration to cleavage.

A much better yield of β-keto lactone was obtained from the alcohol intermediate **28** by replacing the silyl ether protecting groups by an acetonide group. The alcohol **28** was accordingly treated with boron trifluoride etherate at 0 °C in acetonitrile to provide a triol which on exposure to 2-methoxypropene and then PCC/NaOAc[40] gave rise to the desired enol **29**. The higher yield (81%) obtained in this oxidation step may be the consequence of conformational changes induced in the substrate by the cyclic nature of the acetonide protecting group as compared to the sterically more encumbered array of functional groups present in the bis silyl ether derivative **28** (Scheme V).

The synthesis of *triepi*-actinobolin was completed from **29** by first removing the carbobenzoxy protecting group by hydrogenolysis over palladium on carbon. The free amine was then coupled with a mixed anhydride of Cbz-L-alanine.[41] Acidolysis of this intermediate with anhydrous hydrogen bromide in dichloromethane at 0 °C removed both the amine- and oxygen-protecting groups to furnish (+)-5,6,10-*triepi*-actinobolin (**30**) as its hydrobromide salt.

Synthesis of (+)-Actinobolin. To procure (+)-actinobolin from the minor product of the Diels–Alder reaction, we assumed that the scheme worked out for incorporating the enolic hydroxy group of *triepi*-actinobolin would serve equally well here. Unfortunately, severe difficulties were encountered in obtaining good reaction yields in both the osmylation step and subsequent thionocarbonate formation by using the bis silyl ether derivative of **15a**. The marked steric and conformational differences between the two cycloadducts thus contribute significantly to their rather divergent chemical behavior.

Accordingly, by necessity we developed an alternative scheme for introducing the required oxygen functionality into cycloadduct **15a**. This new scheme was particularly rewarding from the standpoint of brevity, for only seven additional steps were required to transform the minor cycloadduct to actinobolin. First, an epoxidation reaction using 3,5-dinitroperoxybenzoic acid/Na_2HPO_4 delivered **31**.[42] This epoxide was reductively opened

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(41) Bokanszky, M.; Klausner, Y. S.; Ondetti, M. A. *Peptide Synthesis*: John Wiley & Sons: New York, 1976.

with zinc dust in the presence of sodium acetate and acetic acid to provide the triol **32**.[43] It should be noted here that the successful ring-opening reaction of this epoxide stands in stark contrast to the ring-opening reaction attempted much earlier by using the epoxide derived from the major cycloadduct. The vicinal diol group of triol **32** was next protected as its cyclohexylidene ketal, and a chromium trioxide–pyridine oxidation was carried out to provide the β-keto lactone **34**. The carbobenzoxy group was now removed by hydrogenolysis, and a DCC promoted coupling reaction with Cbz-L-alanine was brought about to provide **35** (Scheme VI).

Lastly, the carbobenzoxy group borne by the alanine residue of **35** was cleaved with concomitant diol deprotection by hydrogenolysis over palladium on charcoal in the presence of 1 N HCl and acetic acid to deliver actinobolin hydrochloride. The synthetic actinobolin which was isolated as its hydrochloride salt was found to be identical in its spectral properties with that of "natural" actinobolin hydrochloride prepared from the corresponding sulfate by exchange over Amberlite IRA-400 resin. The optical rotations of our synthetic hydrochloride [$[\alpha]^{24}_D$ + 50° (*c* 0.52, H_2O)] and the "natural" hydrochloride [$[\alpha]^{24}_D$ + 53° (*c* 0.65, H_2O)] were between those reported by Weinreb and Ohno.[4]

Summary

Syntheses of both 5,6,10-*triepi*-actinobolin and actinobolin have been accomplished by using a homochiral diene prepared from L-threonine. While the Diels–Alder reaction of this diene with methyl propiolate furnished the cycloadduct required for the synthesis of (+)-actinobolin as the minor diastereomer, the completion of the synthesis required but seven additional steps. Since very few examples of intermolecular Diels–Alder reactions employing chiral dienes were known prior to our undertaking of the actinobolin synthesis, the present study does provide information which should prove valuable to other researchers wishing to make use of related dienes in their synthetic strategies. The organic chemist's ability to manipulate the π-facial course of such cycloaddition reactions will depend critically upon seeking a broader understanding of the key steric and stereoelectronic factors which are operative in these reactions.

Experimental Section

Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker WH-300 spectrometer by using chloroform as an internal standard ($CHCl_3$ = 7.260). Infrared (IR) spectra were recorded by using a Perkin-Elmer 247 grating infrared spectrophotometer with the polystyrene absorption at 1602 cm^{-1} as a reference. Optical rotations were determined by using a Perkin-Elmer 2241 polarimeter at the sodium D line. Low-resolution mass spectra were recorded on a LKB-9000A mass spectrometer. High-resolution mass spectra were recorded on a Varian MAT CH-5DF mass spectrometer. Elemental analyses were carried out by Galbraith Laboratories, Inc., Knoxville, TN.

Analytical thin-layer chromatography (TLC) was performed on E. Merck 60F-256 silica gel plastic or aluminum plates. Visualization of compounds on TLC plates was accomplished by UV illumination, iodine vapor, or by staining with a solution made up of 25 g of ammonium molybdate and 1 g of ceric sulfate in 0.5 L of 10% sulfuric acid, followed by heating. Gravity column chromatography and medium-pressure liquid chromatography (MPLC) were carried out by using E. Merck 0.063–0.200 and 0.040–0.063 mm silica gel, respectively. Distilled reagent grade solvents were used for all chromatographic separations.

Benzene and toluene were distilled from calcium hydride. Tetrahydrofuran (THF) and diethyl ether were distilled from sodium benzophenone ketyl. Other solvents were purified by distillation and were stored over 4-Å molecular sieves and under a dry inert atmosphere. Solid reagents were used as supplied while liquid reagents were distilled prior to use. All reactions were routinely run under a dry inert atmosphere of nitrogen gas.

Melting points were determined in open capillary tubes on a Thomas-Hoover apparatus and are uncorrected.

***N*-Carbobenzoxy-L-threonine (5).** To a solution of 5.0 g (42.0 mmol) of L-threonine in 25 mL of 2 N NaOH, cooled to 0 °C, was added 6.0 mL (42.0 mmol) of benzyl chloroformate portionwise. A pH of 10 was maintained by the occasional addition of 2 N NaOH. The reaction was stirred for 1 h at 0 °C and then extracted with ether. The aqueous layer was acidified to a pH of 3 with 10% HCl and extracted with ethyl acetate. The organic layer was dried and concentrated to give 9.1 g (99%) of a white solid: mp 99–101 °C (lit.[5] mp 102 °C); $[\alpha]^{24}_D$ −4.0° (*c* 4.25, AcOH) (lit.[5] $[\alpha]^{21}_D$ −4.3° (*c* 4.25, AcOH)); IR ($CHCl_3$) 3450, 3010, 1720, 1518, 1460, 1410, 1220, 1080, 775, 710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.55 (s, 2 H), 7.23 (s, 5 H), 6.00 (d, 1 H, *J* = 9.0 Hz), 5.07 (s, 2 H), 4.30 (m, 2 H), 1.16 (d, 3 H, *J* = 6 Hz); MS (15 eV), *m/e* 253 (M^+) 209, 148, 108, 107, 100, 91, 79, 56; exact mass calcd for $C_{12}H_{15}NO_5$ 253.0950, found 253.0935.

***N*-Carbobenzoxy-L-threonine Methyl Ester (6).** To a solution of 10.0 g (39.5 mmol) of **5** in 150 mL of methanol was added 1 mL of concentrated sulfuric acid. After having been stirred at room temperature for 36 h, the reaction was concentrated by rotary evaporation to one-quarter volume and poured into an ice–water–sodium bicarbonate solution. Extraction with ethyl acetate followed by drying and concentration gave 10.4 g (99%) of a white solid: mp 90 °C (lit.[5] mp 90 °C); $[\alpha]^{24}_D$ −14.2° (*c* 4.25, CH_3OH) (lit.[5] $[\alpha]^{20}_D$ −16.1° (*c* 4.25, CH_3OH); IR ($CHCl_3$) 3450, 3050, 1725, 1518, 1463, 1443, 1220, 1080, 1015, 780 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.36 (s, 5 H), 5.61 (d, 1 H, *J* = 8.5, Hz), 5.13 (s, 2 H), 4.33 (m, 2 H), 3.76 (s, 3 H), 2.14 (m, 1 H), 1.24 (d, 3 H, *J* = 6.5 Hz); MS (15 eV), *m/e* 267 (M^+), 223, 152, 108, 91; exact mass calcd for $C_{13}H_{17}NO_5$ 267.1107, found 267.1090.

***N*-Carbobenzoxy-*O*-*tert*-butyldimethylsilyl-L-threonine Methyl Ester (7).** To a solution of 9.8 g (36.7 mmol) of **6** in 100 mL of DMF was added 5.5 g (80.7 mmol) of imidazole, followed by 6.1 g (40.4 mmol) of *tert*-butyldimethylsilyl chloride. The solution was stirred at room temperature until no starting material was present by TLC (usually 24 h). The mixture was poured into a saturated sodium chloride solution and extracted with ethyl acetate. The organic extracts were combined, dried, and concentrated to give 13.9 g (99%) of a thick, clear, colorless oil: $[\alpha]^{24}_D$ −7.31° (*c* 3.55, $CHCl_3$); IR ($CHCl_3$) 3475, 2980, 2800, 1725, 1470, 1445, 1390, 1360, 1325, 1265, 1220, 1185, 1140, 1115, 1080, 1040, 1020, 975, 855, 840, 820, 775, 710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.38 (s, 5 H), 5.44 (d, 1 H, *J* = 9.7 Hz), 5.14 (s, 2 H), 4.44 (dq, 1 H, *J* = 6.2, 1.8 Hz), 4.28 (dd, 1 H, *J* = 9.7, 1.8 Hz), 3.72 (s, 3 H), 1.20 (d, 3 H, *J* = 6.2 Hz), 0.83 (s, 9 H), 0.03 (s, 3 H), 0.01 (s, 3 H); MS (15 eV), *m/e* 381 (M^+), 324, 159, 91, 73; exact mass calcd for $C_{19}H_{31}NO_5Si$ 381.1972, found 381.1972. Anal. Calcd for $C_{19}H_{31}NO_5Si$: C, 59.81; H, 8.19; N, 3.67. Found: C, 60.02; H, 8.25; N, 3.65.

[*R*-[*R,*R**-(*E*)]]-[1-[1-[[(1,1-(Dimethylethyl)dimethyl)silyl]oxy]-ethyl]-4-oxo-2-pentenyl]carbamic Acid Phenylmethyl Ester (8).** To a solution of 7.8 g (20.4 mmol) of **7** in 180 mL of toluene cooled to −78 °C was added via a syringe pump (0.4 mL/min flow rate) 22.0 mL (20.4 mmol) of a 20% solution of DIBAL-H in hexanes. After a total time of 4 h at −78 °C, the mixture was poured into a saturated citric acid, ice–water solution. Extraction with ethyl acetate followed by drying and concentration yielded a mixture of the starting material and the desired aldehyde as a thick oil. To this mixture was added 150 mL of THF and 6.4 g (20.4 mmol) of acetonylidenetriphenylphosphorane. This solution was refluxed for 24 h, and after having been cooled and concentrated it was passed through a short plug of silica gel (10% ethyl acetate–hexanes as eluent) to remove the triphenylphosphine oxide. The effluent was concentrated and subjected to MPLC by using 10% ethyl acetate–hexanes as the eluent to give 0.78 g (10%) of starting material **7** and 6.4 g (80%) of desired enone **8** as a thick, clear, slightly yellow oil: $[\alpha]^{24}_D$ −1.69° (*c* 5.03, $CHCl_3$); IR (neat) 3440, 3340, 2950, 2900, 2875, 1720, 1680, 1630, 1500, 1465, 1365, 1260, 1220, 1080 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.36 (s, 5 H), 6.71 (dd, 1 H, *J* = 16.0, 5.5 Hz), 6.18 (d, 1 H, *J* = 16.0 Hz), 5.12 (s, 2 H), 4.29 (m, 1 H), 4.03 (m, 1 H), 2.22 (s, 3 H), 1.19 (d, 3 H, *J* = 6.0 Hz), 0.85 (s, 9 H), 0.04 (s, 3 H), 0.01 (s, 3 H); MS (70 eV), *m/e* 347, 334, 290, 233, 226, 182, 159, 108, 91, 53; exact mass calcd for $C_{17}H_{24}NO_4Si$ 334.1475, found 334.1471. Anal. Calcd for $C_{21}H_{33}NO_4Si$: C, 64.41; H, 8.49; N, 3.58. Found: C, 64.52; H, 8.65; N, 3.50.

[*R*-[*R,*R**-(*E*)]]-[4-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-1-[1-[[(1,1-dimethylethyl)dimethylsilyl]oxy]ethyl]-2,4-pentadienyl]carbamic Acid Phenylmethyl Ester (3).** To an ice cooled solution of 1.3 g (3.3 mmol) of **8** in 50 mL of benzene were added dropwise 0.69 mL (5.0 mmol) of triethylamine and 0.81 mL (4.0 mmol) of *tert*-butyldimethylsilyl triflate. After having been stirred for 5 min, the cooling bath was removed, and the two-phase system was stirred at room temperature for 15 min and then at 55 °C for 6 h. This mixture was concentrated and passed through a silica gel plug (ether as the eluent) to give 1.54 g (96%) of a thick, yellow oil $[\alpha]^{24}_D$ −1.15° (*c* 4.86, $CHCl_3$); IR (neat) 3450, 3340, 2960, 2930, 2900, 2860, 1725, 1595, 1500, 1475, 1360, 1320, 1260, 1245, 1030, 965, 840, 815, 780, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.36 (m, 5 H), 5.98 (d, 2 H, *J* = 1.7 Hz), 5.15 (m, 2 H), 5.08 (m, 1 H), 4.25 (d, 2 H, *J* = 7.5 Hz), 4.18 (m, 1 H), 3.94 (m, 1 H), 1.17 (d, 3 H,

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(43) Knowles, W. S.; Thompson, Q. E. *J. Am. Chem. Soc.* **1957**, *79*, 3212.

J = 6.0 Hz), 0.97 (s, 9 H), 0.87 (s, 9 H), 0.19 (s, 3 H), 0.18 (s, 3 H), 0.05 (s, 3 H), 0.03 (s, 3 H); MS (15 eV), m/e 505 (M^+), 159; exact mass calcd for $C_{27}H_{47}NO_4Si_2$ 505.3044, found 505.3020.

Diels–Alder Reaction of Diene 3 with Methyl Propiolate. Method A. Diene **3** (0.50 g, 0.99 mmol) was placed in a glass tube and diluted with 5 mL of benzene. To this solution was added 0.88 mL (9.9 mmol) of methyl propiolate. Dry nitrogen gas was bubbled into the mixture, and the tube was sealed and heated to 110 °C for 24 h. Concentration followed by MPLC (3% ethyl acetate–hexanes) gave 0.13 g (21%) of **13a**, 0.37 g (64%) of **13b**, and 0.05 g (10%) of enone **8**. **Method B.** To 0.5 g (0.99 mmol) of diene **3** in a glass tube was added 1.7 mL (18.8 mmol) of methyl propiolate. Dry nitrogen gas was bubbled through the solution, and the tube was sealed and heated at 220 °C for 30 min. Concentration followed by MPLC (3% ethyl acetate–hexanes) gave 0.19 (32%) of **13a** and 0.32 g (55%) of **13b**. Diels–Alder product **13a**: $[\alpha]^{24}_D$ +91.4° (c 3.1, $CHCl_3$); IR (neat) 3460, 2975, 2950, 2910, 1875, 1725, 1680, 1510, 1480, 1470, 1420, 1380, 1370, 1320, 1300, 1260, 1230, 1070, 970, 880, 840, 780, 760, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.35 (s, 5 H), 6.91 (m, 1 H), 5.10, 4.96 (ABq, 2 H, J = 12.0 Hz), 5.00 (m, 1 H), 4.97 (m, 1 H), 4.05 (m, 1 H), 3.65 (m, 1 H), 3.59 (s, 3 H), 3.30 (m, 1 H), 3.00 (m, 1 H), 2.70 (m, 1 H), 1.09 (d, 3 H, J = 6.5 Hz), 0.93 (two overlapping singlets, 18 H), 0.16 (s, 3 H), 0.15 (s, 3 H), 0.11 (s, 3 H), 0.09 (s, 3 H); MS (15 eV), m/e 532 (M^+–t-Bu), 500, 322, 306, 278, 267, 266, 209, 191, 91; exact mass calcd for $C_{27}H_{42}NO_6Si_2$ 532.2552, found 532.2547. Diels–Alder product **13b**: $[\alpha]^{24}_D$ −31.4° (c, 6.5, $CHCl_3$); IR (neat) 3445, 2950, 2925, 2895, 2850, 1715, 1680, 1660, 1500, 1480, 1475, 1460, 1440, 1260, 1210, 1090, 1050, 840, 780 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.32 (s, 5 H), 6.85 (m, 1 H), 5.07, 5.02 (ABq, 2 H, J = 12 Hz), 4.90 (m, 1 H), 4.78 (m, 1 H), 3.90 (m, 1 H), 3.80 (m, 1 H), 3.77 (s, 3 H), 3.54 (m, 1 H), 2.70 (m, 1 H), 1.60 (m, 1 H), 1.21 (d, 3 H, J = 6.0 Hz), 0.91 (s, 9 H), 0.87 (s, 9 H), 0.13 (s, 3 H), 0.12 (s, 3 H), 0.06 (s, 3 H), 0.04 (s, 3 H); MS (15 eV), m/e 532 (M^+–t-Bu), 500, 452, 428, 322, 306, 278, 266, 209, 292, 259, 92; exact mass calcd for $C_{27}H_{42}NO_6Si_2$ 532.2552, found 532.2551.

Hydroboration of 13b. To a solution of 0.46 g (0.78 mmol) of **13b** in 40 mL of THF cooled to 0 °C was added 3.9 mL (3.9 mmol) of a 1.0 M solution of borane in THF. After 1.5 h at 0 °C, 8 mL of a 30% hydrogen peroxide solution was added dropwise, followed by 1 mL of a 2 N sodium hydroxide solution. After stirring the two phase mixture for 0.5 h at 0 °C, it was poured into 100 mL of a saturated sodium bicarbonate solution and extracted with ethyl acetate. The combined organic extracts were washed with 100 mL of a saturated sodium bisulfite solution, dried, concentrated, and chromatographed (15% ethyl acetate–hexanes) to give 0.44 g (93%) of **14b** as a thick, clear oil: $[\alpha]^{24}_D$ −8.3° (c 4.9, $CHCl_3$); IR (neat) 3360, 2955, 2930, 2900, 2855, 1095, 1065, 990, 840, 780, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.30 (s, 5 H), 6.71 (m, 1 H), 5.00 (m, 3 H), 4.10 (m, 2 H), 3.76 (s, 3 H), 3.58 (m, 2 H), 2.89 (s, 1 H), 2.69 (s, 1 H), 2.35 (m, 1 H), 2.10 (m, 1 H), 1.28 (d, 3 H, J = 5.1 Hz), 0.88 (s, 9 H), 0.84 (s, 9 H), 0.07 (four overlapping singlets, 12 H); MS (15 eV), m/e 550 (M^+–t-Bu), 518, 475, 449, 443, 418, 385, 322, 279, 252, 166, 159, 108, 91; exact mass calcd for $C_{27}H_{44}NO_7Si_2$ 550.2656, found 550.2650.

[4*R*-[4α,5β,6α(1*R,2*R**)]]-4-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-6-[2-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-1-[[(phenylmethoxy)carbonyl]amino]propyl]-5-hydroxy-1-cyclohexene-1-carboxylic Acid Methyl Ester (14a).** The same procedure as employed for the preparation of **14b** was followed. From 0.09 g (0.15 mmol) of **13a** was obtained 0.066 g (71%) of **14a**: $[\alpha]^{24}_D$ +35.2° (c 2.7, $CHCl_3$); IR (neat) 3450, 2950, 2920, 2890, 2850, 1725, 1500, 1250, 1070, 830, 805, 770 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.32 (m, 5 H), 6.76 (m, 1 H), 5.21 (d, 1 H, J = 12.8 Hz), 5.06, 5.03 (ABq, 2 H, J = 9.0 Hz), 4.19 (m, 1 H), 3.97 (m, 1 H), 3.86 (m, 1 H), 3.70 (m, 1 H), 3.61 (s, 3 H), 3.11 (m, 1 H), 2.55–2.40 (m, 1 H), 2.24 (m, 1 H), 2.15 (m, 1 H), 1.11 (d, 3 H, J = 6.2 Hz), 0.90 (two overlapping singlets, 18 H), 0.09 (four overlapping singlets, 12 H); MS (70 eV), m/e 550 (M^+–t-Bu), 518, 475, 448, 441, 418, 384, 322, 252, 166, 159, 108, 91; exact mass calcd for $C_{27}H_{44}NO_7Si_2$ 550.2656, found 550.2655.

Desilylation–Lactonization of 14b. To a solution of 0.72 g (1.19 mmol) of **14b** in 60 mL of THF was added dropwise approximately 2 mL of an aqueous 52% hydrogen fluoride solution. The mixture was stirred at room temperature for 24 h. This solution was poured into 100 mL of an ice-cooled, saturated sodium bicarbonate solution and extracted with ethyl acetate. The extracts were dried, concentrated, and chromatographed (ethyl acetate) to give in a quantitative yield 0.41 g of **15b** as an amorphous white solid: $[\alpha]^{24}_D$ +50.0° (c 0.82, CH_3OH); IR (neat) 3330, 3080, 2930, 1715, 1650, 1530, 1260, 1100, 1060, 1040, 1000, 750, 710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.35 (m, 5 H), 6.84 (m, 1 H), 5.70 (d, 1 H, J = 7.5 Hz), 5.14, 5.11 (ABq, 2 H, J = 12.0 Hz), 4.48 (br s, 1 H), 4.41 (dq, 1 H, J = 6.0, 2.5 Hz), 3.99 (dt, 1 H, J = 7.5, 2.5 Hz), 3.79 (m, 1 H), 3.46 (t, 1 H, J = 10.0 Hz), 3.07 (br s, 1 H), 2.76 (m, 1 H), 2.55 (m, 1 H), 2.27 (m, 1 H), 1.41 (d, 3 H, J = 6.0 Hz); MS (70 eV), m/e 347 (M^+), 240, 195, 167, 108, 91; exact mass calcd for $C_{18}H_{21}NO_6$ 347.1369, found 347.1375. Anal. Calcd for $C_{18}H_{21}NO_6$: C, 62.24;, H, 6.09; N, 4.03. Found: C, 62.40; H, 6.11; N, 4.03.

[3*R*-(3α,4α,4aβ,5β,6α)]-(3,4,4a,5,6,7-Hexahydro-5,6-dihydroxy-3-methyl-1-oxo-1*H*-2-benzopyran-4-yl)carbamic Acid Phenylmethyl Ester (15a). The same procedure as employed for the preparation of **15b** was followed. From 0.32 g (0.53 mmol) of **14a** was obtained 0.18 g (100%) of **15a** as an amorphous white solid: $[\alpha]^{24}_D$ +42.5° (c 1.3, $CHCl_3$); IR (neat) 3330, 3100, 2950, 1710, 1640, 1540, 1460, 1360, 1260, 1180, 1110, 1050, 1005, 925, 740, 720 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.36 (s, 5 H), 7.25 (m, 1 H), 521 (d, 1 H, J = 8.9 Hz), 5.14 (s, 2 H), 4.65 (dq, 1 H, J = 6.3, 1.7 Hz), 4.47 (d, 1 H, J = 3.6 Hz), 4.17 (ddd, 1 H, J = 8.9, 2.5, 1.7 Hz), 3.91 (m, 1 H), 3.27 (dt, 1 H, J = 9.6, 3.6 Hz), 3.07 (s, 1 H), 2.86 (m, 1 H), 2.65 (m, 1 H), 2.28 (m, 1 H), 1.41 (d, 3 H, J = 6.3 Hz), MS (70 eV), m/e 347 (M^+), 240, 195, 167, 130, 108, 91, 79; exact mass calcd for $C_{18}H_{21}NO_6$ 347.1369, found 347.1370.

Bis Silylation of 15b. To a solution of 0.14 g (0.40 mmol) of **15b** in 30 mL of DMF was added 0.22 g (3.2 mmol) of imidazole followed by 0.24 g (1.6 mmol) of *tert*-butyldimethylsilyl chloride. After the mixture was stirred at 55 °C for 48 h, it was poured into 200 mL of a saturated sodium chloride solution and extracted with ether. The extracts were dried, concentrated, and chromatographed (15% ethyl acetate–hexanes) to give 0.23 g (100%) of a thick, clear, colorless oil: $[\alpha]^{24}_D$ +47.5° (c 2.6, $CHCl_3$); IR (neat) 3330, 2955, 2940, 2900, 2860, 1720, 1530, 1460, 1260, 1090, 1040, 840, 780 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.35 (s, 5 H), 6.54 (m, 1 H), 5.16, 5.09 (ABq, 2 H, J = 12.2 Hz), 4.34 (m, 2 H), 4.03 (m, 1 H), 3.49 (m, 1 H), 2.53 (m, 1 H), 2.20 (m, 1 H), 2.04 (m, 1 H), 1.27 (d, 3 H, J = 6.3 Hz), 0.87 (s, 9 H), 0.83 (s, 9 H), 0.12 (s, 3 H), 0.08 (s, 3 H), 0.04 (s, 3 H), 0.01 (s, 3 H); MS (70 eV), m/e 518 (M^+–t-Bu), 474, 410, 278, 204, 165, 108, 91, 75; exact mass calcd for $C_{26}H_{40}NO_6Si_2$ 518.2394, found 518.2376.

Hydroxylation of 22. A solution of 0.047 g (0.082 mmol) of **22** in 10 mL of water and 5 mL of acetone was treated with an excess (approximately 0.1 g) of *N*-methylmorpholine-*N*-oxide and a catalytic amount of osmium tetroxide. After having been stirred at room temperature for 24 h, 5 mL of a saturated sodium bisulfite solution was added. The mixture was stirred for 5 min and filtered through Florisil. The mixture was extracted several times with ethyl acetate. The combined extracts were washed sequentially with 50 mL of cold 5% hydrochloric acid and 50 mL of saturated sodium bicarbonate. The organic layer was dried, concentrated, and chromatographed on silica gel (15% ethyl acetate–hexanes) to give 0.047 g (94%) of an amorphous solid: $[\alpha]^{24}_D$ +8.91° (c 1.47, $CHCl_3$); IR (neat) 3375, 2970, 2915, 1890, 1730, 1520, 1470, 1270, 1200, 1080, 850, 790, 720 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.33 (s, 5 H), 5.27 (d, 1 H, J = 9.6 Hz), 5.07, 5.14 (ABq, 2 H, J = 12.4 Hz), 4.97 (m, 1 H), 4.28 (m, 1 H), 4.16 (m, 1 H), 4.10 (m, 1 H), 4.06 (s, 1 H), 4.00 (m, 1 H), 2.60 (d, 1 H, J = 11.2 Hz), 2.30 (m, 1 H), 2.02 (m, 1 H), 1.93 (m, 1 H), 1.30 (d, 3 H, J = 6.0 Hz), 0.91 (s, 9 H), 0.87 (s, 9 H), 0.14 (s, 3 H), 0.13 (s, 3 H), 0.08 (s, 3 H), 0.06 (s, 3 H); MS (15 eV), m/e 552 (M^+–t-Bu), 508, 444, 200, 312, 200, 108, 91; exact mass calcd for $C_{26}H_{42}NO_8Si_2$ 552.2449, found 552.2439. Anal. Calcd for $C_{30}H_{51}NO_8Si_2$: C, 59.08; H, 8.43; N, 2.30. Found: C, 58.77; H 8.43; N, 2.50.

[3a*S*-(3aα,5α,6β,6aβ,7β,8β,10a*S)]-[5,6-Bis[[(1,1-dimethylethyl)dimethylsilyl]oxy]hexahydro-8-methyl-10-oxo-2-thioxo-5*H*,10*H*-1,3-dioxolo[4,5-*i*][2]benzopyran-7-yl]carbamic Acid Phenylmethyl Ester (26).** The diol **24** (0.30 g, 0.33 mmol) and 0.57 g (3.3 mmol) of thiocarbonyldiimidazole were refluxed in 20 mL of THF for 5 h. The yellow solution was poured into 50 mL of cold 5% hydrochloric acid and extracted with ethyl acetate. The extracts were washed with 50 mL of saturated sodium bicarbonate, dried, concentrated, and chromatographed (15% ethyl acetate–hexanes) to give 0.21 g (100%) of a white amorphous solid: $[\alpha]^{24}_D$ +34.1° (c 1.02, $CHCl_3$); IR (neat) 3310, 2950, 2925, 2900, 2850, 1755, 1715, 1515, 1455, 1300, 1250, 1160, 1085, 830, 780 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.35 (s, 5 H), 5.45 (d, 1 H, J = 9.2 Hz), 5.19 (m, 1 H), 5.15, 5.11 (ABq, 2 H, J = 12.4 Hz), 4.85 (dq, 1 H, J = 6.4, 2.0 Hz), 4.05 (m, 1 H), 3.99 (m, 1 H), 3.93 (m, 1 H), 2.61 (m, 1 H), 2.33 (m, 1 H), 2.15 (m, 1 H), 1.37 (d, 3 H, J = 6.4 Hz), 0.91 (s, 9 H), 0.88 (s, 9 H), 0.13 (s, 3 H), 0.11 (s, 3 H), 0.09 (s, 3 H), 0.07 (s, 3 H); MS (15 eV), m/e 594 (M^+–t-Bu), 579, 551, 534, 519, 487, 426, 411, 245, 149, 135, 108, 91; exact mass calcd for $C_{27}H_{40}NO_8SSi_2$ 594.2013, found 594.2015. Anal. Calcd for $C_{31}H_{49}NO_8SSi_2$: C, 57.11; H, 7.57; N, 2.15. Found: C, 56.88; H, 7.37; N, 2.13.

Tin Hydride Reduction of Thionocarbonate 26. To a solution of 0.21 g (0.32 mmol) of **26** in 20 mL of benzene was added 0.43 mL (1.6 mmol) of tri-*n*-butyltin hydride, and the mixture was refluxed for 7 h. After concentration the crude oil was chromatographed first with hexanes to remove the tin impurities and then with 30% ethyl acetate–hexanes to give 0.15 g (85%) of a thick oil: $[\alpha]^{24}_D$ +36.7° (c 2.1, $CHCl_3$); IR (neat)

3350, 2960, 2940, 2900, 2865, 1721, 1530, 1470, 1260, 1080, 841, 782 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.31 (s, 5 H), 5.15, 5.07 (ABq, 2 H, J = 12.1 Hz), 4.90 (d, 1 H, J = 10.7 Hz), 4.80 (m, 1 H), 4.31 (m, 2 H), 3.95 (m, 2 H), 2.82 (dd, 1 H, J = 9.4, 7.4 Hz), 2.26 (d, 1 H, J = 4.7 Hz), 2.13 (m, 1 H), 1.96 (m, 1 H), 1.89 (m, 1 H), 1.28 (d, 3 H, J = 6.7 Hz), 0.91 (s, 9 H), 0.85 (s, 9 H), 0.13 (s, 3 H), 0.11 (s, 3 H), 0.04 (s, 3 H); 0.01 (s, 3 H) MS (15 eV), m/e 536 (M^+–t-Bu), 518, 492, 475, 428, 411, 279, 204, 108, 91; exact mass calcd for $C_{26}H_{42}NO_7Si_2$ 536.2500, found 536.2500.

Transformation of 28 to Its Acetonide Derivative. To a 0 °C stirred solution of 0.04 g (0.07 mmol) of **28** in 4 mL of acetonitrile was added 0.04 mL (0.32 mmol) of boron trifluoride etherate. After stirring for 30 min at 0 °C, the solution was poured into 25 mL of a saturated sodium bicarbonate solution and extracted with ethyl acetate. The extracts were dried and concentrated. The crude oil was immediately diluted with 5 mL of THF, and approximately 0.1 mL of 2-methoxypropene and a small portion of Amberlyst 15 acidic resin were added. After stirring for 6 h at room temperature, the mixture was poured into 25 mL of a saturated sodium bicarbonate solution and extracted with ethyl acetate. The extracts were dried, concentrated, and chromatographed (50% ethyl acetate–hexanes) to give 0.025 g (91%) of a thick oil: $[\alpha]^{24}_D$ +59.8° (c 0.61, $CHCl_3$); IR (neat) 3325, 2975, 2930, 1700, 1540, 1450, 1380, 1240, 1070, 930, 750 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.33 (s, 5 H), 5.34 (m, 1 H), 5.12 (s, 2 H), 4.93 (m, 1 H), 4.84 (m, 1 H), 4.31 (m, 1 H), 3.87 (m, 1 H), 3.17 (t, 1 H, J = 8.0 Hz), 2.90 (m, 1 H), 2.74 (m, 1 H), 2.23 (m, 1 H), 2.70 (m, 1 H), 1.42 (s, 3 H), 1.41 (s, 3 H), 1.34 (d, 3 H, J = 5.0 Hz); MS (15 eV), 405 (M^+), 387, 347, 329, 264, 223, 150, 108, 91; exact mass calcd for $C_{21}H_{27}NO_7$ 405.1788, found 405.1787.

[3a*S*-(3aα,8aα,9aα,9aα,9bβ)]-(3a,4,8,9,9a,9b-Hexahydro-5-hydroxy-2,2,8-trimethyl-6-oxo-6*H*-1,3-dioxolo[4,5-*f*][2]benzopyran-9-yl)carbamic Acid Phenylmethyl Ester (29). To a solution of 0.016 g (0.04 mmol) of the above acetonide in 5 mL of dichloromethane was added 0.1 g (1.2 mmol) of sodium acetate followed by 0.08 g (0.40 mmol) of pyridinium chlorochromate. The orange mixture darkened, and after having been stirred at room temperature for 1 h, it was diluted with 15 mL of ether and filtered through Florisil. The filtrate was washed with 25 mL of cold 5% hydrochloric acid followed by 25 mL of a saturated sodium bicarbonate solution. The extracts were dried, concentrated, and chromatographed on silica gel (15% ethyl acetate–hexanes) to yield 0.013 g (81%) of an oil: $[\alpha]^{24}_D$ +61.2° (c 0.92, $CHCl_3$); IR (neat) 3350, 2975, 2930, 2870, 1720, 1640, 1590, 1530, 1380, 1275, 1220, 1090, 1035 cm^{-1}; ^1H NMR ($CDCl_3$) δ 13.84 (s, 1 H), 7.36 (s, 5 H), 5.38 (m, 1 H), 5.11 (s, 2 H), 5.05 (m, 1 H), 3.86 (m, 1 H), 3.72 (m, 1 H), 3.40 (t, 1 H, J = 8.6 Hz), 2.95 (dd, 1 H, J = 17.1, 4.7 Hz), 2.68 (m, 1 H), 2.60 (m, 1 H), 1.44 (s, 3 H), 1.43 (s, 3 H), 1.35 (d, 3 H, J = 6.4 Hz); MS (15 eV), m/e 403 (M^+), 388, 345, 328, 254, 236, 211, 195, 178, 152, 108, 91.

***N*-Carbobenzoxy-5,6-*O*-isopropylidene-5,6,10-*triepi*-actinobolin.** To a solution of 0.015 g (0.04 mmol) of **29** in 5 mL of dichloromethane was added 10% palladium on carbon. The mixture was stirred under an atmosphere of hydrogen gas. After 1.5 h the mixture was filtered through Celite (dichloromethane eluent), and the filtrate was immediately placed in an ice bath. In a separate flask was placed 16 mg (0.08 mmol) of *N*-carbobenzoxy-L-alanine and 2 mL of dichloromethane. The solution was cooled to 0 °C, and 10 μL (0.08 mmol) of triethylamine was added. After 10 min, 9 μL (0.08 mmol) of isobutyl chloroformate was added, and the mixture was stirred for 15 min. The cold solution of mixed anhydride thus formed was added in 1 portion to the 0 °C solution of the free amine generated from the hydrogenolysis reaction. The mixture was stirred for 1 h, poured into 25 mL of cold 5% hydrochloric acid, and extracted with dichloromethane. The extracts were washed with 25 mL of a saturated sodium bicarbonate solution, dried, concentrated, and chromatographed (50% ethyl acetate–hexanes) to give 15 mg (87%) of a thick, clear, colorless oil $[\alpha]^{24}_D$ +25.2° (c 0.52); IR (neat) 3330, 2990, 2940, 1720, 1665, 1590, 1530, 1385, 1220, 1090, 755 cm^{-1}; ^1H NMR ($CDCl_3$) δ 13.81 (s, 1 H), 7.35 (s, 5 H), 6.36 (s, 1 H), 5.36 (m, 1 H), 5.12, 5.08 (ABq, 2 H, J = 13.3 Hz), 5.01 (m, 1 H), 4.22 (m, 1 H), 4.03 (m, 1 H), 3.75 (m, 1 H), 3.38 (t, 1 H, J = 8.9 Hz), 2.95 (dd, 1 H, J = 16.9, 5.9 Hz), 2.71 (m, 1 H), 2.62 (m, 1 H), 1.45 (s, 3 H), 1.42 (s, 3 H), 1.39 (d, 3 H, J = 6.6 Hz), 1.29 (d, 3 H, J = 6.6 Hz); MS (15 eV), m/e 474 (M^+), 459, 430, 416, 399, 262, 233, 216, 204, 189, 134, 108, 91, 57, 44; exact mass calcd for $C_{24}H_{30}N_2O_8$ 474.2002, found 474.2000.

(+)-5,6,10-*triepi*-Actinobolin (30). To a sidearm flask equipped with a drying tube and a rubber septum was added 20 mg (0.04 mmol) of the above amide in 5 mL of dichloromethane. The mixture was cooled to 0 °C, and anhydrous hydrogen bromide gas was slowly bubbled into the solution via a glass pipet. After several minutes a white precipitate dropped out of the solution. After 30 min the hydrogen bromide flow was stopped, and the solvent was evaporated. The white solid was collected and washed several times by decantation of a centrifuged suspension of **30** in benzene. The residual benzene was removed in vacuo to give 14 mg (90%) of the product: $[\alpha]^{24}_D$ +31.0° (c 0.41, CH_3OH); IR (neat) 3325, 2980, 1660, 1470, 1390, 1285, 1230, 1095, 805, 765 cm^{-1}; ^1H NMR (CF_3CO_2D) δ 7.58 (m, 1 H), 5.00 (m, 1 H), 4.87 (m, 1 H), 4.71 (m, 1 H), 4.45 (m, 1 H), 4.18 (m, 1 H), 3.38 (dd, 1 H, J = 20.8, 6.9 Hz), 3.21 (t, 1 H, J = 8.1 Hz), 2.96 (dd, 1 H, J = 20.8, 9.2 Hz), 1.99 (d, 3 H, J = 5.8 Hz), 1.66 (d, 3 H, J = 6.0 Hz); ^1H NMR (D_2O) δ 3.85 (q, 1 H, J = 6.9 Hz), 3.64 (m, 1 H), 3.30 (t, 1 H, J = 9.2 Hz), 2.71 (dd, 1 H, J = 18.5, 7.1 Hz), 1.36 (t, 1 H, J = 9.0 Hz), 2.24 (dd, 1 H, J = 18.5, 9.3 Hz), 1.39 (d, 3 H, J = 6.9 Hz), 1.09 (d, 3 H, J = 6.7 Hz), three protons were obscured by the HOD.

[1a*R*-(1aα,3α,4β,4aβ,5α,6α,6aα,8a*S)]-(Hexahydro-3,4-dihydroxy-6-methyl-8-oxo-3*H*,8*H*-oxireno[*i*][2]benzopyran-5-yl)carbamic Acid Phenylmethyl Ester (31).** A mixture of 841 mg (2.42 mmol) of **15a** in 25 mL of dichloromethane was stirred at room temperature with 6.13 g (24.2 mmol) of 3,4-dinitroperoxybenzoic acid and 5.16 g (36.3 mmol) of disodium hydrogen phosphate. After 1 h an additional 3.07 g (12.1 mmol) of 3,5-dinitroperoxybenzoic acid and 3.44 g (24.2 mmol) of disodium hydrogen phosphate were added, and the reaction mixture was stirred for an additional 24 h. The resulting suspension was diluted with 100 mL of dichloromethane and filtered to remove the precipitate. The filtrate was washed twice with aqueous sodium thiosulfate and sodium bicarbonate, and the aqueous layer was extracted twice with 100 mL of ethyl acetate. The organic extracts were combined, dried over magnesium sulfate, concentrated in vacuo, and chromatographed on silica gel with ethyl acetate as the eluent to give 203 mg (24%) of recovered **15a** and 376 mg (42%) of epoxide **31** as a colorless solid: $[\alpha]^{24}_D$ +71.1° (c 1.39, $CHCl_3$); IR (neat) 3330, 3030, 2935, 1705, 1540, 1455, 1420, 1390, 1345, 1250, 1165, 1140, 1090, 1050, 995, 925, 890, 825, 755 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.45–7.31 (m, 5 H), 5.29 (d, 1 H, J = 6.4 Hz), 5.17 (s, 2 H), 4.82 (q, 1 H, J = 6.4 Hz), 4.29 (dd, 1 H, J = 6.4, 4.5 Hz), 4.31 (s, 1 H), 3.83 (s, 1 H), 3.76 (td, J = 10.1, 5.4 Hz), 3.18 (t, 1 H, J = 10, 1 Hz), 2.81 (br s, 1 H), 2.64 (ddd, 1 H, J = 14.9, 5.4, 1.6 Hz), 2.39 (dd, 1 H, J = 10.1, 4.5 Hz), 1.77 (ddd, 1 H, J = 14.9, 11.6, 10.1 Hz), 1.47 (d, 3 H, J = 6.4 Hz); exact mass calcd for $C_{18}H_{21}NO_7$ 363.1318, found 363.1323. Anal. Calcd for $C_{18}H_{21}NO_7$: C, 59.50; H, 5.83; N, 3.86. Found: C, 59.12; H, 5.69; N, 3.62.

Triol 32. Activated zinc dust (5.38 g) was added portionwise over 2 h to a mixture of 269 mg (0.75 mmol) of epoxide **31** and 269 mg of sodium acetate in 13 mL of 90% acetic acid with stirring at room temperature. After 30 min, 100 mL of 2-butanone was added, and the precipitate was removed by filtration. The filtrate was washed with 100 mL of a saturated sodium bicarbonate solution, dried over magnesium sulfate, and chromatographed on silica gel with ethyl acetate–acetone (4:1) as eluent to give 89 mg (33%) of the unsaturated lactone **15a** and 136 mg (50%) of triol **32**: $[\alpha]^{24}_D$ +16.3° (c 1.14, $CHCl_3$); IR (neat) 3415, 3065, 2930, 1700, 1540, 1454, 1355, 1255, 1090, 1065, 1015, 920, 880, 735, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.37 (s, 5 H), 5.29 (d, 1 H, J = 8.5 Hz), 5.16 (s, 2 H), 4.68 (br s, 1 H), 4.65 (qd, 1 H, J = 6.6, 2.0 Hz), 4.21 (dt, 1 H, J = 8.5, 2.0 Hz), 3.92 (ddd, 1 H, J = 11.6, 9.3, 4.8 Hz), 3.08 (t, 1 H, J = 9.3 Hz), 2.39 (ddd, 1 H, J = 10.8, 9.3, 2.0 Hz), 2.27 (ddd, 1 H, J = 15.3, 4.8, 2.0 Hz), 2.23 (dd, 1 H, J = 9.3, 2.1 Hz), 1.45 (ddd, 1 H, J = 15.3, 11.6, 3.5 Hz), 1.38 (d, 3 H, J = 6.6 Hz); exact mass calcd for $C_{18}H_{23}NO_7$ 365.1475, found 365.1465. Anal. Calcd for $C_{18}H_{23}NO_7$: C, 59.17; H, 6.35; N, 3.83. Found: C, 59.32; H, 6.44; N, 3.62.

Protected Triol 33. A mixture of 118 mg (0.323 mmol) of triol **32**, 2.28 mL of 1,1-dimethoxycyclohexane, 59 mg of pyridinium *p*-toluenesulfonate, and 1 mL of DMF was stirred for 4 h at room temperature. The solution was poured into 30 mL of a saturated sodium bicarbonate solution and extracted with ethyl acetate. The extracts were dried over magnesium sulfate, concentrated in vacuo, and chromatographed on silica gel by using first hexane–ethyl acetate and then ethyl acetate–acetone as the eluents to give 108 mg (68%) of the protected triol **33** along with 23 mg (17%) of the recovered triol **32**. **33**: $[\alpha]^{24}_D$ +77.8° (c 2.90, $CHCl_3$); IR (neat) 3335, 2940, 2865, 1710, 1540, 1455, 1390, 1350, 1280, 1235, 1165, 1110, 1080, 1010, 935, 900, 855, 770, 735, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.35 (s, 5 H), 5.23 (d, 1 H, J = 12.2 Hz), 5.08 (d, 1 H, J = 12.2 Hz), 4.81 (d, 1 H, J = 9.9 Hz), 4.78 (br s, 1 H), 4.67 (m, 1 H), 4.43 (d, 1 H, J = 9.9 Hz), 3.86 (ddd, 1 H, J = 12.2, 8.8, 4.2 Hz), 3.12 (t, 1 H, J = 9.1 Hz), 2.63 (t, 1 H, J = 13.5 Hz), 2.45–2.33 (m, 2 H), 2.20 (d, 1 H, J = 13.9 Hz), 1.74–1.51 (m, 11 H), 1.34 (d, 3 H, J = 6.4 Hz); exact mass calcd for $C_{24}H_{31}NO_7$ 445.2101, found 445.2106. Anal. Calcd for $C_{24}H_{31}NO_7$: C, 64.70; H, 7.01; N, 3.14. Found: C, 64.77; H, 7.20; N, 3.06.

[3′a*R*-(3′aα,8′β,9′β,9′aα,9′bβ)]-(3′a,4′,8′,9′,9′a,9′b-Hexahydro-5′-hydroxy-8′-methyl-6′-oxospiro[cyclohexane-1,2′-[6*H*-1,3]dioxolo[4,5-*f*]-[2]benzopyran]-9′-yl)carbamic Acid Phenylmethyl Ester (34). Chromium trioxide (51.2 mg, 0.512 mmol) was added to a solution of 83 μL (1.02

mmol) of pyridine in 1.28 mL of dichloromethane, and the resulting solution was stirred for 10 min at room temperature. A solution of ketal **33** (57.0 mg, 0.128 mmol) dissolved in 2 mL of dichloromethane and acetic anhydride (48.3 μL, 0.512 mmol) was then added sequentially to this mixture. After 10 min, the reaction mixture was transferred to a silica gel column, and the column was eluted with 50% ethyl acetate-hexane to give 28.8 mg (51%) of the β-keto lactone **34** as a colorless solid: $[\alpha]^{24}_D$ +60.80 (*c* 1.44, $CHCl_3$); IR (neat) 3310, 2940, 2865, 1715, 1650, 1595, 1530, 1455, 1420, 1395, 1361, 1345, 1270, 1225, 1165, 1115, 1055, 905, 735, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 11.98 (s, 1 H), 7.34 (s, 5 H), 5.19 (d, 1 H, *J* = 12.1 Hz), 5.07 (d, 1 H, *J* = 12.1 Hz), 4.71 (d, 1 H, *J* = 10.1 Hz), 4.59 (q, 1 H, *J* = 6.5 Hz), 4.35 (d, 2 H, *J* = 10.1 Hz), 3.71 (ddd, 1 H, *J* = 11.1, 9.4, 6.1 Hz), 3.30 (t, 1 H, *J* = 9.4 Hz), 2.98-2.85 (m, 2 H), 2.58 (ddd, 1 H, *J* = 17.4, 11.1, 2.6 Hz), 1.70-1.50 (m, 10 H), 1.37 (d, 3 H, *J* = 6.5 Hz); exact mass calcd for $C_{24}H_{29}NO_7$ 443.1935, found 443.1935. Anal. Calcd for $C_{24}H_{29}NO_7$: C, 65.00; H, 6.59; N, 3.16. Found: C, 64.84; H, 6.93; N, 2.95.

Protected Actinobolin 35. To a solution of 14.0 mg (0.032 mmol) of **34** in 1.5 mL of dichloromethane was added 10 mg of 5% palladium on carbon. The mixture was stirred under an atmosphere of hydrogen gas for 1 h. The catalyst was removed by filtration, and to the resulting filtrate were added 8.5 mg (0.038 mmol) of *N*-carbobenzoxy-L-alanine and 7.8 mg (0.038 mmol) of 1,3-dicyclohexylcarbodiimide. The mixture was stirred for 1 h and filtered. The filtrate was chromatographed on silica gel with ethyl acetate-hexane as the eluent to give 14.7 mg (90%) of protected actinobolin **35** as a colorless solid: $[\alpha]^{24}_D$ +15.1° (*c* 0.74, $CHCl_3$); IR (neat) 3310, 2940, 2865, 1725, 1680, 1645, 1590, 1535, 1450, 1420, 1395, 1360, 1345, 1270, 1230, 1115, 1075, 1055, 905, 755 cm^{-1}; ^1H NMR ($CDCl_3$) δ 13.68 (s, 1 H), 7.34 (s, 5 H), 6.45 (br d, 1 H, *J* = 8.0 Hz), 5.32 (d, 1 H, *J* = 6.8 Hz), 5.09 (d, 1 H, *J* = 11.8), 5.50 (d, 1 H, *J* = 11.8 Hz), 4.67-4.56 (m, 2 H), 4.26 (quintet, 1 H, *J* = 7.0 Hz), 3.70 (ddd, 1 H, *J* = 11.2, 9.3, 6.1 Hz), 3.31 (t, 1 H, *J* = 9.30 Hz), 2.99-2.87 (m, 2 H), 2.59 (ddd, 1 H, *J* = 16.7, 11.2, 2.0 Hz), 1.75-1.55 (m, 10 H), 1.39 (d, 3 H, *J* = 7.0 Hz), 1.30 (d, 3 H, *J* = 6.5 Hz); exact mass calcd for $C_{27}H_{34}N_2O_8$ 514.2316, found 514.2301. Anal. Calcd for $C_{27}H_{34}N_2O_8$: C, 63.02; H, 6.66; N, 5.45. Found: C, 62.90; H, 6.70; N, 5.28.

[3*R*-(3α,4α(*S)4α,5α,6α)]-2-Amino-*N*-(3,4,4a,5,6,7-hexahydro-5,6,8-trihydroxy-3-methyl-1-oxo-1*H*-2-benzopyran-4-yl)propanamide Monohydrochloride (1·HCl).** A mixture of 15.7 mg (0.0305 mmol) of **35**, 0.63 mL of methanol, 0.063 mL of acetic acid, 0.091 mL of 1 N hydrochloric acid, and 5 mg of 5% palladium on carbon was stirred under a hydrogen atmosphere at room temperature for 30 min. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo to dryness. The resulting oil was triturated twice with 1 mL of anhydrous ether to give a pale yellow solid. The solid was dissolved in 3 mL of water and filtered. The filtrate was freeze-dried under reduced pressure to give 10.5 mg (100%) of actinobolin hydrochloride as a pale yellow powder: $[\alpha]^{24}_D$ +50° (*c* 0.52, H_2O); IR (KBr) 3410, 1650, 1560, 1505, 1395, 1265, 1230, 1190, 1135, 1110, 1085, 1070, 1050, 1005, 805, 760, 710 cm^{-1}; ^1H NMR (CD_3OD, Me_4Si standard) 4.70 (qd, 1 H, *J* = 6.41, 1.3 Hz), 4.57 (m, 1 H), 4.03 (q, 1 H, *J* = 7.0 Hz), 3.88 (td, 1 H, *J* = 9.6, 6.7 Hz), 3.13 (t, 1 H, *J* = 9.6 Hz), 2.84-2.75 (m, 2 H), 2.36 (ddd, 1 H, *J* = 18.6, 9.6, 2.4 Hz), 1.51 (d, 3 H, *J* = 7.0 Hz), 1.34 (d, 3 H, *J* = 6.4 Hz).

Preparation of an Authentic Sample of Actinobolin Hydrochloride 16 from Actinobolin Sulfate. A column packed with 10 mL of Amberlite IRA 400 (Cl form) was washed with 5 mL of hydrochloric acid and then with enough water to bring the pH of the eluent to 7. The column was charged with natural actinobolin sulfate (10 mg) and dissolved in 0.5 mL of water, and the column was eluted with water. The fractions containing actinobolin hydrochloride (ascertained by silica gel TLC analysis using acetonitrile-water-acetic acid (5:1:1) as the developing solvent) were collected, combined, and freeze-dried under reduced pressure to give 9.2 mg of actinobolin hydrochloride: $[\alpha]^{24}_D$ +53° (*c* 0.65, H_2O). Actinobolin was isolated from its sulfate salt by neutralization with aqueous sodium bicarbonate and extraction with 2-butanone. MS (15 eV), *m/e* 264 (M^+-2H_2O), 220, 202, 170, 162; exact mass calcd for $C_{13}H_{16}N_2O_4$ 264.1111, found 264.1102.

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Supplementary Material Available: Tables containing the final fractional coordinates, temperature parameters, bond distances, and bond angles for **16b** (4 pages). Ordering information is given on any current masthead page.

Fluorescence and Photoisomerization of Azobenzene-Containing Bilayer Membranes

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Abstract: Spectroscopic and photoisomerization behavior of aqueous bilayer aggregates of azobenzene-containing amphiphiles was examined. The azobenzene bilayers assume different chromophore orientations, depending on the component structure. Some of the azobenzene bilayers were found to be fluorescent, and the fluorescence intensity decreased as the chromophore orientation changed from the tilted head-to-tail type to the parallel type. Emission quenching was observed in the presence of extremely small amounts of a bound cyanine dye. In the trans-to-cis photoisomerization of the bilayers, the rate in the gel state decreased with changing chromophore orientations from the head-to-tail type to the parallel type. The rate was much larger and unaffected by the molecular structure, in the case of the liquid-crystalline bilayers and of the azobenzene amphiphiles isolated in inert bilayer matrices. In the phase-separated system, photoisomerization occurred between the unclustered isomers. The emission was quickly lost by the formation of the cis isomer. The photoisomerization was suppressed in the presence of the cyanine, probably due to energy transfer to the cyanine and sensitization of the reverse photoisomerization by the cyanine. An energy level diagram was constructed which includes excited states characteristic of the bilayer and explains the photophysical and photochemical processes. Finally, implications of the present finding in relation to light energy harvesting systems were discussed.

We have been investigating in the past several years spontaneous assemblage of bilayers in water from amphiphiles which contain aromatic segments.[2] In these bilayers, spectral properties of the aromatic units are extensively affected by the chemical structure of component molecules and by the physical state of membranes, due to the electronic interaction of the aromatic units.[3-7] In

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