all mutants as indicated by linear plots of peak current vs (scan rate)$^{1/2}$. The peak-to-peak separations and equivalent anodic and cathodic peak currents also supported reversible behavior without kinetically coupled conformational effects on $E°'$. Thermodynamic parameters listed in Table I were determined by temperature-dependent variation in the midpoint potentials of cyclic voltammograms. The small observed changes in entropy suggested that the redox potential shifts were not a consequence of protein unfolding.

To further evaluate protein structure stability we compared the guanidine hydrochloride induced equilibrium unfolding transitions of the mutants.[5b] Changes in the midpoints (C_m) and the cooperativity of the guanidine hydrochloride induced transitions were observed. The C_m values given in Table I indicate that the mutant proteins are at least as stable as, if not more stable than, the native protein. Thus, multiple substitutions in the heme environment of iso-1-cytochrome *c* apparently retain stable, overall native-like structure.

Replacements at heme residues 38, 52, and 82 combine to cause large shifts in the measured redox potential of cytochrome *c*, illustrated in Figure 1. Wells has suggested that the free energy change that results when multiple replacements are made is often equal to the sum of the independent free energy changes: $\Delta\Delta G_{X,Y} = \Delta\Delta G_X + \Delta\Delta G_Y + \Delta G_I$.[13] Exceptions to simple additivity are reflected in the ΔG_I term, which reflects electrostatic or structural interactions between the independent sites. The results shown in Figure 1 and Table I clearly demonstrate that multiple replacements can result in synergistic shifts in potential. Furthermore, ΔG_I does not equal 0, but may increase or decrease $\Delta\Delta G_{X,Y}$. Since charge–charge and charge–dipole effects operate over relatively long distances, the observed nonadditive effects, reported in this communication, may be attributable to these phenomena in the heme environment.

The second question which we are addressing in the current study is the following: Given such changes, do the mutant proteins retain function in vivo? All mutant yeast strains contained an integrated *CYC1* gene in an isogenic background.[10] The resultant yeast strains appeared to have normal amounts of iso-1-cytochrome *c* as determined by low-temperature (−196 °C) spectroscopic examination of intact cells.[14]

All yeast strains grew on a nonfermentable carbon source. This observed obligatory aerobic respiration in vivo established that the electron transport assembly of proteins in the inner mitochondrial membrane was intact and functional. However, we have not yet thoroughly analyzed the growth rates, which may reveal quantitative functional differences. Functional behavior was not necessarily expected for the mutants. For example, the 123-mV shift in redox potential observed with the triple Arg*38*Ala, Asn*52*Ile, Phe*82*Ser mutant corresponds to a thermodynamically uphill shift of 2.8 kcal·mol^{-1} in the free energy position of cytochrome *c* (Figure 1). Equivalently, the shift in redox potential corresponds to a 100-fold shift in the equilibrium constant for the cytochrome reductase–cytochrome *c* couple.

In this light, detailed examination of rates in vitro and further investigation of in vivo function may reveal the significance of the thermodynamic driving force of electron transfer in oxidative phosphorylation.

Acknowledgment. Thanks are due to Dr. L. Paul Wakem for helpful discussion and guidance in mutagenesis. This work was supported by grants from the NIH (GM33881 to G.McL.) and the NSF (F.S. and G.McL.).

Registry No. Ara, 74-79-3; Asn, 70-47-3; Phe, 63-91-2; cytochrome *c*, 9007-43-6.

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A Total Synthesis of (±)-Bilobalide

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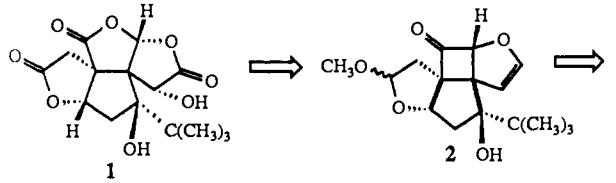
Received March 2, 1992

Ginkgo biloba is an ancient plant species whose extracts have been used as medicinal agents for approximately 5000 years.[1] The bitter principles responsible for the healing powers of the ginkgo extracts were first isolated by Furakawa.[2] The structures of three of the key components were later independently determined by two groups.[3] These compounds were C20 hexacyclic trilactones, which were given the names ginkgolides A, B, and C. Subsequently, a C15 tetracyclic trilactone, bilobalide, was isolated from the ginkgo extracts by Nakanishi and shown to have the structure **1**.[4] The unusual structural features of bilobalide include the presence of a *tert*-butyl group, known only to the ginkgolide class of terpenoids, and three contiguous five-membered-ring lactones. The only synthesis of bilobalide to date was reported by E. J. Corey in 1987[5] with a subsequent report by the Corey group on the enantioselective synthesis of bilobalide.[6] Herein we report a total synthesis of bilobalide employing an intramolecular [2 + 2] photocycloaddition as the key step.[7]



The approach outlined here relies on a regioselective Baeyer–Villiger oxidation of cyclobutanone **2** and an intramolecular [2 + 2] photochemical cycloaddition of α-acyloxy cyclopentenone **3**, which was stereoselectively prepared by the addition of the lithium enolate **5** to hydroxy aldehyde **4**.

Aldehyde **4** was prepared in four steps from commercially available 3-furaldehyde as illustrated in Scheme I. Addition of 3-furaldehyde to the reagent[8] prepared by the addition of *tert*-butyllithium to dry cerium trichloride in THF at −78 °C produced the secondary alcohol, which was oxidized under Swern[9] conditions to produce the *tert*-butyl furyl ketone. This ketone was then condensed with lithioacetonitrile to generate the β-hydroxy nitrile **6** in 82% overall yield.[10,11] Reduction of the nitrile with diiso-

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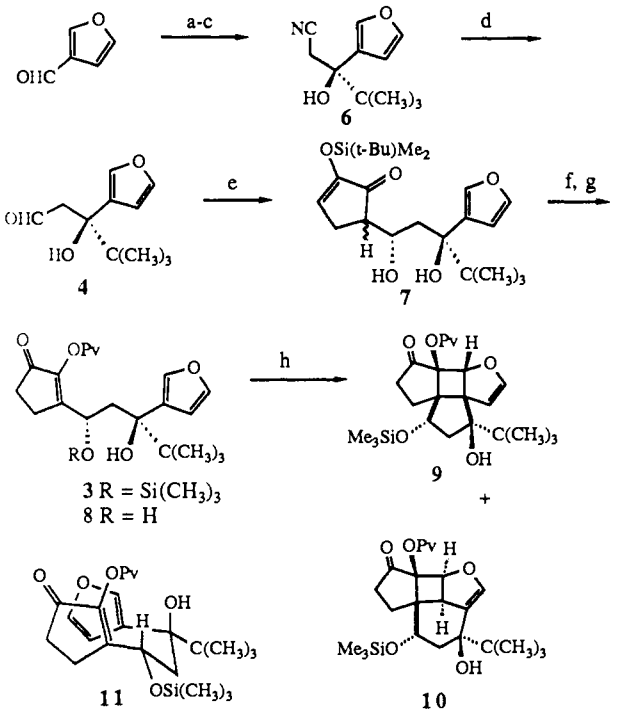
Scheme I[a]



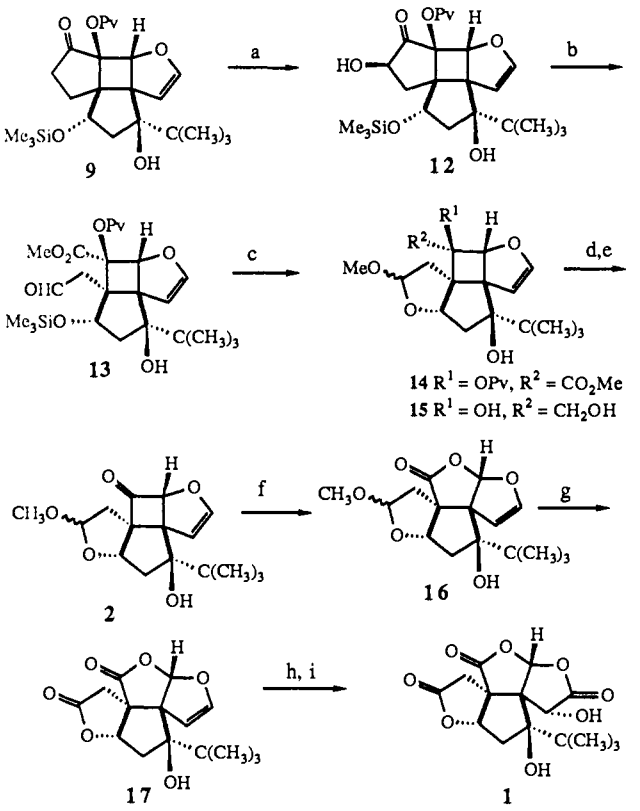
[a] (a) *tert*-Butyllithium, $CeCl_3$, −78 °C, 2 h. (b) DMSO, $(COCl)_2$, CH_2Cl_2, Et_3N, −78 °C. (c) CH_3CN, LDA, THF, −78 °C, 1.1 equiv of HMPA, 1 h, 85%, 3 steps. (d) $Isobutyl_2AlH$, ether, 0 °C, 1 h, H_3O^+, 56%. (e) Enolate **5** (2.2 equiv), THF, −78 °C, 15 min, 85%. (f) KF (5 equiv), 1:1 THF/H_2O, 30 min; Et_3N (3 equiv), CH_2Cl_2, $(CH_3)_3COCl$ (1.0 equiv). (g) $(CH_3)_3SiCl$ (2 equiv), CH_2Cl_2, Et_3N, 4-(*N*,*N*-dimethylamino)pyridine (cat.), 30 min, 50% from **7**. (h) *hν*, hexanes, >350 nm (uranium glass filter), 18 h, 50% **9**, 25% **10**.

butylaluminum hydride gave aldehyde **4** in 56% yield. Addition of this aldehyde to 2.2 equiv of enolate **5**[12] in THF at −78 °C resulted in the formation of a 1:1 mixture of two diastereomers **7** in 85% yield. The products are diastereomeric at the carbon α to the ketone carbonyl. This stereogenic center is subsequently converted to an sp^2 carbon; therefore, isomerism at this center is of no consequence. More importantly, the relative stereochemistry of the secondary and tertiary hydroxyl stereogenic centers of both isomers was identical, indicating that there had been complete stereoselectivity with respect to the formation of the secondary hydroxyl center. This can be rationalized by addition of the enolate **5** to a chairlike six-membered ring lithium chelate of the β-alkoxy aldehyde. The *tert*-butyl group must occupy an equatorial site, and addition of the enolate **5** to the aldehyde carbon from the pseudoequatorial face produces the observed products with complete stereoselection at the secondary carbinol stereogenic center.

Cyclopentenones **7** were converted to **3** as shown in Scheme I. Hydrolysis of the silyl ether with KF in aqueous THF also resulted in isomerization of the enol ketone to the more stable tetrasubstituted enol. Selective protection of the enol hydroxyl was accomplished with pivaloyl chloride and triethylamine to produce **8**. The secondary alcohol was then protected as the TMS ether to produce a single cyclopentenone **3** in 50% overall yield after silica gel chromatography.

Irradiation of **3** in hexanes produced the desired photocycloadduct **9** in 50% yield, along with 25% of **10** which resulted from addition of the enone to the less substituted furan double bond. The stereochemistry of the major product is consistent with a transition state such as **11** in which the (trimethylsilyl)oxy and

Scheme II[a]



[a] (a) LDA, THF, −78 °C, 10 min; MoOPH, 0 °C, 10 min, 80%. (b) $Pb(OAc)_4$, 2:1 C_6H_6/CH_3OH, 5 °C, 15 min. (c) CH_3OH, *p*-TsOH, 1 h, 85%, 2 steps. (d) $LiAlH_4$, Et_2O, 25 °C, 20 min. (e) $Pb(OAc)_4$, C_6H_6, 50 °C, 5 min; 80%, 2 steps. (f) *m*-CPBA, CH_2Cl_2, 5 min, 95%. (g) Jones' reagent, acetone, reflux, 10 min, 96%. (h) Dimethyldioxirane, acetone, 25 °C, 24 h, 90%. (i) Jones' reagent, acetone, 25 °C, 20 min, 85%.

the *tert*-butyl groups occupy pseudoequatorial positions on the forming five-membered ring.

Hydroxylation of the photoadduct **9** was accomplished (Scheme II) with LDA, MoOPH, and THF at 0 °C to provide hydroxy ketone **12** in 80% yield.[13] Oxidative cleavage of **12** [$Pb(OAc)_4$, CH_3OH, C_6H_6, 5 °C, 94%] gave the aldehyde **13**. This aldehyde was converted to the cyclobutanone **2** by the following sequence: conversion of the aldehyde to the methyl acetals **14** (CH_3OH, *p*-TsOH, 25 °C); reduction of the methyl and pivalate esters ($LiAlH_4$, Et_2O) to give diol **15**; and oxidative cleavage of the 1,2-diol [$Pb(OAc)_4$, dry benzene, 50 °C] to provide **2** in 79% overall yield for the three steps.

The next key operation required the regioselective Baeyer–Villiger oxidation of cyclobutanone **2** to form lactone **16** in preference to the regioisomeric lactone. Initial attempts to effect this oxidation under nucleophilic conditions (H_2O_2, Triton-B, THF or *t*-BuOOH, Triton-B, THF) gave exclusively the undesired lactone. However, when **2** was exposed to *m*-CPBA in dichloromethane, lactone **16** was the only detectable product (98%).

With the basic skeleton of bilobalide in place, completion of the synthesis was accomplished in three steps. Treatment of acetal **16** with Jones' reagent in acetone at reflux produced lactone **17** in 96% yield. Exposure of the enol ether **17** to dimethyldioxirane[14] followed by Jones oxidation of the resultant epoxide provided 77% of synthetic (±)-bilobalide (**1**), which was identical (^1H, ^{13}C 400 MHz NMR, IR, TLC) to an authentic sample.[15]

(12) Enolate **5** was prepared from the ketone by treatment of the ketone with 1.1 equiv of LDA in THF at −78 °C. The ketone was prepared from 1,2-cyclopentanedione (Tonari, K.; Machiya, K.; Ichimoto, I.; Ueda, H. *Agric. Biol. Chem.* **1980**, *44*, 2135) by treatment with *tert*-butyldimethylsilyl chloride and triethylamine in dichloromethane.

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The synthesis of bilobalide has been accomplished in 17 steps from 3-furaldehyde. The key transformations are the stereoselective aldol condensation of enolate **5** with aldehyde **4**, the stereoselective photocycloaddition of enone **3**, and the regioselective Baeyer–Villiger oxidation of cyclobutanone **2**. The selective oxidations in the final steps are also worthy of note.

Acknowledgment. We thank the National Science Foundation (CHE 9014641), the donors of the Petroleum Research Fund, administered by the American Chemical Society, the National Institutes of Health (GM-38904), Rhone Poulenc, Inc., and the Randleigh Foundation for generous financial support of this work. M.T.C. thanks the A. P. Sloan Foundation for a fellowship. D.K.J. and J.L.G. thank the Department of Education for fellowships. We also thank Pierre Braquet and E. J. Corey for providing authentic samples of (−)-bilobalide for purposes of comparison.

Supplementary Material Available: A list of ^1H and ^{13}C NMR data for compounds **1**–**17** (2 pages). Ordering information is given on any current masthead page.

Trapping and Isolation of an Alternate DNA Conformation

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DNA can assume many conformations that differ from the B-form double helix.[1] Alternate structures like cruciforms[2] and Z-DNA[3] are of interest as they are thought to serve regulatory functions in vivo. On large molecules like plasmids, atypical geometries are formed as a result of torsional stress, whereas related conformations in oligodeoxyribonucleotides can be induced by changes in temperature or salt concentration.[4] Unusual DNA structures are often recalcitrant to physicochemical characterization because of the narrow range of conditions under which they exist. We have recently developed a general method based on disulfide bond crosslinking that stabilizes the secondary structure of synthetic oligodeoxyribonucleotides without perturbing their native geometries.[5,6] We report the application of this chemistry to trap, isolate, and characterize a "premelting intermediate" of the d(CGCGAATTCGCG)$_2$ dodecamer.

Crystallographic and NMR studies show that d-(CGCGAATTCGCG)$_2$ forms a B-DNA duplex.[7,8] UV thermal denaturation experiments confirm these findings: in high salt buffer a monophasic transition is observed which represents melting of the duplex to a random coil.[9] However, biphasic melting profiles are obtained when the buffer contains [Na^+] ≤ 10 mM.[10] Breslauer proposed that the first transition in these biphasic curves defines premelting of the duplex to a hairpin, while the second transition represents conversion of the hairpin to a random coil. To examine this premelting intermediate we synthesized **1**, which has the terminal residues of the parent dodecamer replaced with N^3-(mercaptoethyl)thymidine. These substitutions were introduced to stabilize the premelting intermediate with a disulfide crosslink.[5] Control experiments show that the melting profiles of **1** are analogous to those of d-(CGCGAATTCGCG)$_2$ (T_m^1 = 27.0 °C and T_m^2 = 60.1 °C; 1 mM NaCl, pH 8, 50 μM in **1**), suggesting that both dodecamers denature along a similar pathway.

Air oxidation of the sulfhydryl groups was achieved by heating **1** with vigorous stirring under the conditions used for the melting studies (Figure 1). After 24 h the solution tested negative for thiol groups with Ellman's reagent. HPLC analysis of the reaction mixture revealed two major products in a 16:1 ratio. On a nondenaturing gel, the minor component (**2**) migrated with d-(CGCGAATTCGCG)$_2$, suggesting that this compound is the (bis)crosslinked dodecamer (Figure 2A).[11] However, the major product (**3**) migrated below the 12-mer size marker, indicative of a hairpin structure.[12] Reduction of **2** or **3** with DTT afforded

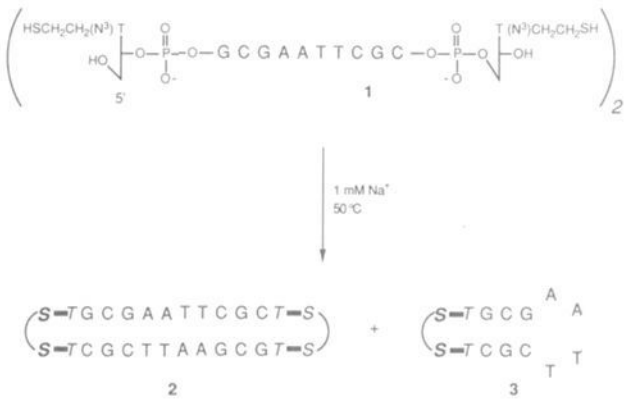

Figure 1. Synthesis of the crosslinked oligomers. The oxidation was performed at 50 °C, which is past the first transition but before significant onset of the second transition. Attempts to crosslink the denatured dodecamer at 75 °C afforded high molecular weight products and trace amounts of **3**.

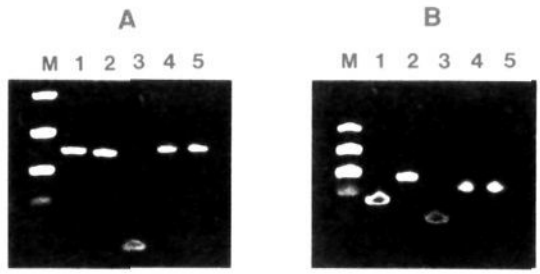

Figure 2. Electrophoretic analysis of the modified oligomers. In both gels lane M contains single-stranded markers 30, 22, 16, and 12 bases long. (A) 20% polyacrylamide nondenaturing gel: lane 1, d-(CGCGAATTCGCG)$_2$; lane 2, **2**; lane 3, **3**; lane 4, dodecamer **1** produced by reduction of **2**; lane 5, dodecamer **1** produced by reduction of **3**. (B) 20% polyacrylamide denaturing gel: lane 1, d-(CGCGAATTCGCG)$_2$; lane 2, **2**; lane 3, **3**; lane 4, dodecamer **1** produced by reduction of **2**; lane 5, dodecamer **1** produced by reduction of **3**.

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(10) In 1 mM NaCl buffer that is 50.6 μM in d(CGCGAATTCGCG)$_2$, T_m^1 = 33.2 °C and T_m^2 = 62.4 °C.[9]

(11) **2** was obtained exclusively when the crosslinking was performed at 25 °C.

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