Synthesis, Biological Profile, and Quantitative Structure–Activity Relationship of a Series of Novel 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase Inhibitors

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A series of 9,9-bis(4-fluorophenyl)-3,5-dihydroxy-8-(alkyltetrazol-5-yl)-6,8-nonadienoic acid derivatives 1 were synthesized and found to inhibit competitively the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. The analogues having 1*N*-methyltetrazol-5-yl attached to the C_8-position (**3a**, **4a**, $R^1 = R^2 = F$) are the most active in suppressing cholesterol biosynthesis in both in vitro and in vivo models: the IC_{50} for the chiral form of **3a** is 19 nM, $K_i = 4.3 \times 10^{-9}$ M when K_m for HMG-CoA is 28×10^{-6} M;[1] the ED_{50} (oral) value corresponding to the lactone derivative (**4a**, BMY 22089) is approximately 0.1 mg/kg. Further, BMY 21950 is nearly 2 orders of magnitude more active in parenchymal heptaocytes, from which most of the serum cholesterol originates, than in other cell preparations (such as spleen, testes, ileum, adrenal, and ocular lens epithelial cells; Table III). This apparent tissue specificity may be highly beneficial since the blocking of cholesterol biosynthesis in other vital organs could eventually lead to undesirable side effects. In addition to the chemical synthesis and biological evaluation, a theoretical study aimed at relating the HMG-CoA reductase inhibitory potency to the three-dimensional structure of the inhibitors was undertaken. With a combination of molecular mapping and 3D-QSAR techniques, it was possible to determine a logical candidate for the conformation of the bound inhibitor and to quantitatively relate inhibitory potency to the shape and size of both the binding site and the C_8-substituent.[2]

1. Introduction

Since the recognition of hypercholesterolemia as a primary risk factor of atherosclerosis and coronary heart disease,[3] there have been intense efforts to identify chemical entities that are capable of regulating the plasma level of this sterol.[4] These efforts resulted in the discovery of compactin (also known as mevastatin) and mevinolin (**2**, also known as lovastatin[5]), two potent inhibitors of cholesterol biosynthesis at the level of the rate-limiting enzyme, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase.

Comparison of compactin, lovastatin, and other known HMG-CoA reductase inhibitors[6] reveals that the 3,5-dihydroxy carboxylic moiety is present in all compounds, and it is well-documented[7] that this group represents a pharmacophore for HMG-CoA reductase inhibitor recognition. Our initial investigations were directed toward the development of structurally simplified HMG-CoA reductase inhibitors containing the prerequisite 3,5-dihydroxyheptanoic acid side chain and a hydrophobic moiety able to mimic the decalin portion of the naturally occurring inhibitors such as lovastatin and compactin (Chart I).

During the course of our research program, we synthesized the cyano compounds **6** and **7** by derivatizing the symmetrically substituted ethyl 2-cyanopropenoate **5** (Chart I) which afforded moderate inhibitory potency[8] (Table I). Saturation of the trans-disubstituted $\Delta^{6,7}$ double bond or omission of these-two-carbon linkage caused loss of activity, results which corroborate findings reported by the Merck group.[9] The moderate inhibitory potency observed for the cyano derivative was interpreted in terms of insufficient spatial bulk at the C_8-position, which might be necessary for binding. Many attempts were made to modify this functional group. In one of these approaches, the cyano functional group in **5** was transformed into a tetrazole ring by a known procedure.[10] Compound **17** was made neutral by alkylating the free tetrazole ring with a suitable electrophile.[11] Usual alkylation conditions (such as MeI and a base) gave two regioisomers corresponding to the 1N (**18a**) and 2N (**18b**) (Scheme II) substituted isomers.[12] Both isomers were converted into the final target compounds; the racemic **3a** was found to be a very potent inhibitor, while the 2*N*-methyl isomer **3b** afforded only 1/700 of the former isomer potency. This extraordinary finding prompted us to investigate a variety of analogues of **3a** and our results are discussed below.

2. Chemistry

The initial target **6** was prepared by derivatizing the tetrasubstituted olefin **5** by using a known procedure.[13] Ethyl 2-cyano-3,3-bis(4-fluorophenyl)propenoate (**5**) was conveniently synthesized by a classical Knoevenagel condensation.[14] Many commonly used procedures failed to initiate the condensation of 4,4′-difluorobenzophenone and ethyl cyanoacetate. When a molar mixture of these com-

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∥ Chemical Process and Development Department.

(1) Racemic material, the resolved (+)-enantiomer showed twice the activity as compared to the racemic compound.
(2) Preliminary results of this paper were presented: Sit, S. Y.; Parker, R. A.; Brown, P. J.; Balasubramanian, N.; Catt, J. D.; Harte, W. E.; Motoc, I. I.; Wright, J. J. The 196th National Meeting of the American Chemical Society, Los Angeles, CA, September 26–30, 1988, MEDI 108. Sit, S. Y.; Wright, J. J. Patent WO W08806584, issued on September 7, 1988. Other analogues of BMY 21950: Brown, P. J.; Balasubramanian, N.; Catt, J. D.; Sit, S. Y.; Harte, W. E.; Parker, R. A.; Wright, J. J. The 197th National Meeting of the American Chemical Society, Dallas, TX, April 9–14, 1989, MEDI 16. Balasubramanian, N.; Brown, P. J.; Catt, J. D.; Han, W. T.; Parker, R. A.; Sit, S. Y.; Wright, J. J. *J. Med. Chem.* **1989**, *32*, 2038.
(3) Anderson, K. M.; Castelli, W. P.; Levy, D. *JAMA, J. Am. Med. Assoc.* **1987**, *257* (*16*), 2176. Steinberg, D. *Circulation* **1987**, *76* (*3*), 502.
(4) Havel, R. J. *J. Clin. Invest.* **1988**, *81*, 1653.
(5) Endo, A. In *Specific Nonsterol Inhibitors of HMG-CoA Reductase. Regulation of HMG-CoA Reductase*; Preiss, B., Ed.; Academic Press: New York, 1985; p 49.
(6) Lee, T.-J. *Trends Pharm. Sci.* **1987**, *8*, 443 and references therein.
(7) Stereospecific synthesis of the 3,5-dihydroxy side chain has been a subject of several publications, for example, Heathcock, C. H., Rosen, T. *Tetrahedron* **1986**, *42* (*18*), 4909 and references therein.
(8) Synthesis of **6** is described in Scheme I.

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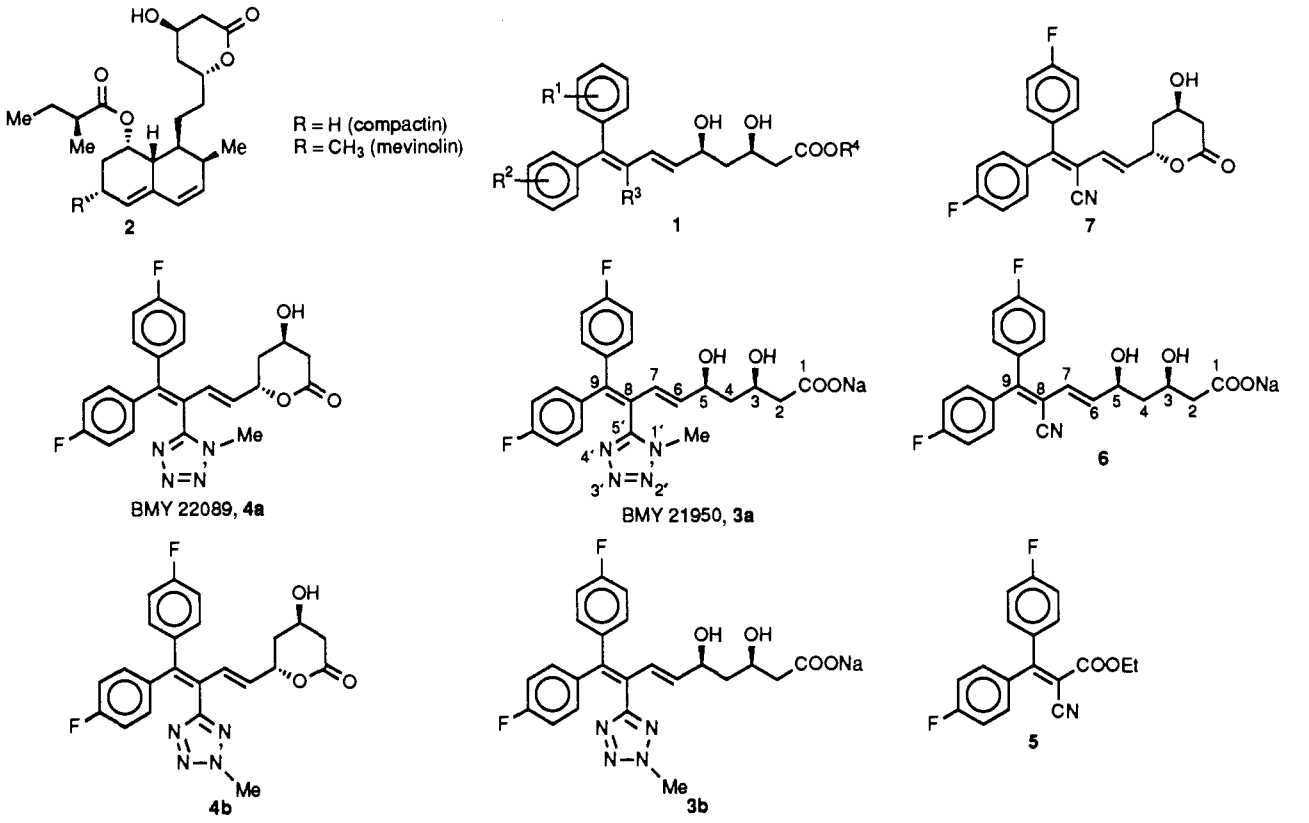
Chart I



ponents was heated to reflux in a mixture of glacial acetic acid and benzene (1:4, v/v) for several hours,[15] a small amount of the desired product **5** was detected by TLC. The condensation reached an equilibrium after about 2 weeks with continuous removal of H_2O (Dean–Stark water trap). Cyano ester **5** was isolated by crystallization (mp = 114–116 °C) after the removal of reaction solvents. The mother liquor from crystallization was not further purified but was resubmitted for recycling after replenishing the spent reagents. As much as 60% of the crystalline **5** was collected after a single-pass reaction. Conversion of **5** into the key intermediate **12** was straightforward. Alkaline saponification of **5** in THF–H_2O–LiOH at ambient temperature followed by acidification provided free carboxylic acid **8** in essentially quantitative yield (mp = 180–181 °C). Compound **8** was converted into acyl chloride **9** by refluxing with excess of oxalyl chloride in dry dichloromethane; the dried, crude acyl chloride **9** was reduced with lithium aluminum hydride immediately. The reduction gave rise to two products, the major being the desired allylic alcohol **10** and the minor product being aldehyde **12**. Oxidation (pyridinium chlorochromate, PCC) of the mixture of **10** and **12** provided the pure key aldehyde **12** as a crystalline product (mp = 167–169 °C). The multistep conversion of **5** to **12** discussed here was found to be necessary owing to the fact that hydride reducing agents such as LAH or Dibal-H produced the conjugated reduction product **5a** in greater yields.[16]

(9) Hoffman, W. F.; Alberts, A. W.; Anderson, P. S.; Chen, J. S.; Smith, R. L.; Willard, A. K. *J. Med. Chem.* **1986**, *29*, 849. Stokker, G. E.; Alberts, A. W.; Gilfillan, J. L.; Huff, J. W.; Smith, R. L. *J. Med. Chem.* **1986**, *29*, 852. Stokker, G. E.; Alberts, A. W.; Anderson, P. S.; Cragoe, E. J., Jr.; Deana, A. A.; Gilfillan, J. L.; Hirshfield, J.; Holtz, W. J.; Hoffman, W. F.; Huff, J. W.; Lee, T. J.; Novello, F. C.; Prugh, J. D.; Rooney, C. S.; Smith, R. L.; Willard, A. K. *J. Med. Chem.* **1986**, *29*, 170. Stokker, G. E.; Hoffman, W. F.; Alberts, A. W.; Cragoe, E. J., Jr.; Deana, A. A.; Gilfillan, J. L.; Huff, J. W.; Novello, F. C.; Prugh, J. D.; Smith, R. L.; Willard, A. K. *J. Med. Chem.* **1985**, *28*, 347. Hoffman, W. F.; Alberts, A. W.; Cragoe, E. J., Jr.; Deana, A. A.; Evans, B. E.; Gilfillan, N. P.; Gould, J. L.; Huff, J. W.; Novello, F. C.; Prugh, J. D.; Rittle, K. E.; Smith, R. L.; Willard, A. K. *J. Med. Chem.* **1986**, *29*, 159. Kathawala, F. G. Patent WO 8402131, issued on June 7, 1984. Wareing, J. R. Patent WO 8600307, issued on January 16, 1986. Anderson, P. L. Patent WO 8402903, issued on August 2, 1984. Endo, A. *J. Med. Chem.* **1985**, *28*, 401. Boader, E.; Baitmann, W.; Beck, G.; Bergmann, A.; Jendialla, H.; Kessler, K.; Wess, G.; Schnbert, W.; Granzer, E.; Kerekjarto, B. V.; Krause, R. *Tetrahedron Lett.* **1988**, *29* (8), 929.
(10) Luijten, J. G. A.; Jassen, M. J.; van der Kerk, G. J. M. *Recl. Trav. Chim. Pays-Bas* **1962**, *81*, 202.
(11) Singh, H.; Chawla, A. S.; Kapoor, V. K.; Paul, D.; Malhortra, R. K. Medicinal Chemistry of Tetrazoles. *Prog. Med. Chem.* **1980**, *17*, 151.
(12) The regiochemical assignments were determined by an independent synthesis of one of the isomeric products (18a) from known starting materials; see Scheme III for a full description.
(13) Sato, A.; Ogiso, A.; Noguchi, H.; Mitsui, S.; Kaneko, I.; Shimado, Y. *Chem. Pharm. Bull.* **1980**, *28* (5), 1509.
(14) Jones, G. *Org. React.* **1967**, *15*, 204. Egawa, Y.; Srozuki, M.; Okuda, T. *Chem. Pharm. Bull.* **1963**, *11*, 589. Prout, F. S. *Organic Synthesis*; Wiley: New York, 1963; Collect. Vol. 4, p 93.
(15) Rate of condensation was observed to be slow and was further retarded if higher boiling solvents (such as toluene, xylenes, ethylbenzene) were used. Large excess of ethyl cyanoacetate could speed up the reaction, but a similar period of time was needed to achieve the stated yields.
(16) The strongly electron deficient olefin **5** can also be cleaved by primary amines such as 2,2-dimethoxyethylamine to give the following imine.

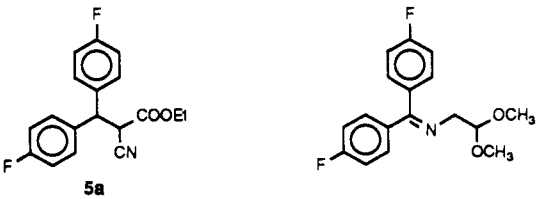
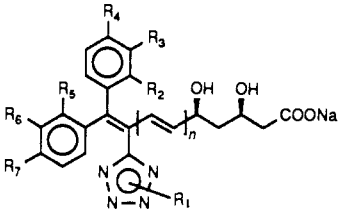
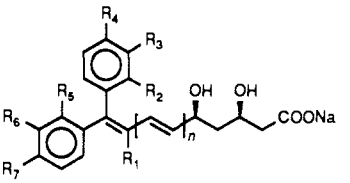


Table I. HMG-CoA Reductase Inhibitory Activity, in Vitro Screening



no.[a]	R_1	R_2	R_3	R_4	R_5	R_6	R_7	n	in vitro IC_{50}[b]/μM	relative potency[c]
40	Free H	H	H	F	H	H	F	1	0.33	5.8×10^{-3}
3a	1-CH_3	H	H	F	H	H	F	1	0.043	4.3×10^{-1}
41	1-CH_3	H	H	F	H	H	F	2	5.7	3.3×10^{-3}
3b	2-CH_3	H	H	F	H	H	F	1	30	6.3×10^{-4}
42	1-CH_2CH_3	H	H	F	H	H	F	1	1.1	1.7×10^{-2}
43	2-CH_2CH_3	H	H	F	H	H	F	1	200	9.1×10^{-5}
44	1-$CH(CH_3)_2$	H	H	F	H	H	F	1	14	1.4×10^{-3}
45	1-$CH(CH_3)_2$	H	H	F	H	H	F	2	130	1.5×10^{-4}
46	2-$CH(CH_3)_2$	H	H	F	H	H	F	1	>300	$<6 \times 10^{-5}$
47	1-MEM	H	H	F	H	H	F	1	3.7	5.0×10^{-3}
48	2-MEM	H	H	F	H	H	F	1	240	7.7×10^{-5}
49	2-$C(CH_3)_3$	H	H	F	H	H	F	1	>300	$<6 \times 10^{-5}$
3a[d]	1-CH_3	H	H	F	H	H	F	1	0.019	1
3a[e]	1-CH_3	H	H	F	H	H	F	1	23	8.3×10^{-4}
50	1-CH_3	CH_3	H	F	CH_3	H	F	1	0.029	6.7×10^{-1}
2	lovastatin (Na salt)								0.027	7.1×10^{-1}
51	1-CH_3	H	H	F	H	H	H	1	0.044	4.3×10^{-1}
52	1-CH_3	H	CH_3	F	H	CH_3	F	1	0.16	1.2×10^{-1}
53	1-CH_3	H	H	H	H	H	H	1	0.19	1.0×10^{-1}
54	1-CH_3	F	H	CH_3	F	H	CH_3	1	0.58	3.2×10^{-2}
55	1-CH_3	H	H	OCH_3	H	H	OCH_3	1	1.4	1.4×10^{-2}
56	1-CH_3	CH_3	H	CH_3	CH_3	H	CH_3	1	1.6	1.2×10^{-2}
57	1-CH_3	H	H	CH_3	H	H	CH_3	1	1.3	1.5×10^{-2}



6	CN	H	H	F	H	H	F	1	8	2.4×10^{-3}
58	CH_3	H	H	F	H	H	F	1	7.9	2.4×10^{-3}
59	$CH(CH_3)_2$	H	H	F	H	H	F	1	0.23	9.5×10^{-2}

[a] All compounds tested were racemic, except where noted. [b] Standard rat liver microsomal HMG-CoA reductase inhibition assay. [c] Value relative to compound 3a. [d] Tested as the Na salt, derived from the (+)-BMY 22089, $[\alpha]^{25} = +240°$. [e] Na salt, derived from (−)-BMY 22089, $[\alpha]^{25} = -242°$.

The transformation of this crucial aldehyde intermediate **12** into the final product is summarized below: double bond homologation of **12** was performed as usual with (triphenylphosphoranylidene)acetaldehyde to provide the homologated aldehyde **13** in nearly quantitative yields (mp = 155–156 °C). Compound **13** was allowed to react with the dianion of ethyl acetoacetate to furnish hydroxy keto ester **14**. Stereospecific reduction using a known procedure[18] ($NaBH_4/Et_3B/THF/MeOH$) provided the *syn*-diol ester **15** (the diastereomeric ratio of the syn to anti isomers was 94:6 or greater) and subsequent saponification (NaOH) provided the final products, either in the lactone form **7** or in the open chain form **6** (Scheme I).

The tetrazole analogues of **6** were similarly prepared by the procedure depicted in Scheme II. The crystalline cyano ester **5** was heated with neat tri-*n*-butyltin azide at 115–120 °C until the cycloaddition started.[19] This [2 + 3] cycloaddition was rapid, exothermic, and quantitative on a large scale. The desired product **17** (mp = 159–160 °C) was obtained after a fluoroide ion (from KF) induced hydrolysis of **16**. Free tetrazole derivative **17** was alkylated with a variety of electrophiles to give mixtures of regioisomers (1*N*-alkyl and 2*N*-alkyl). The relative ratios of the two regioisomers were dependent upon reaction conditions and the nature of the alkylating agents.[20] In the case of methylation using MeI/THF/NaH, the ratio of 1-methyltetrazole **18a** to 2-methyl isomer **18b** was roughly 2:1 and the alkylation was complete in minutes. The ratio could further be augmented to as much as 6:1 if a heterogeneous alkylation was done in benzene at elevated tem-

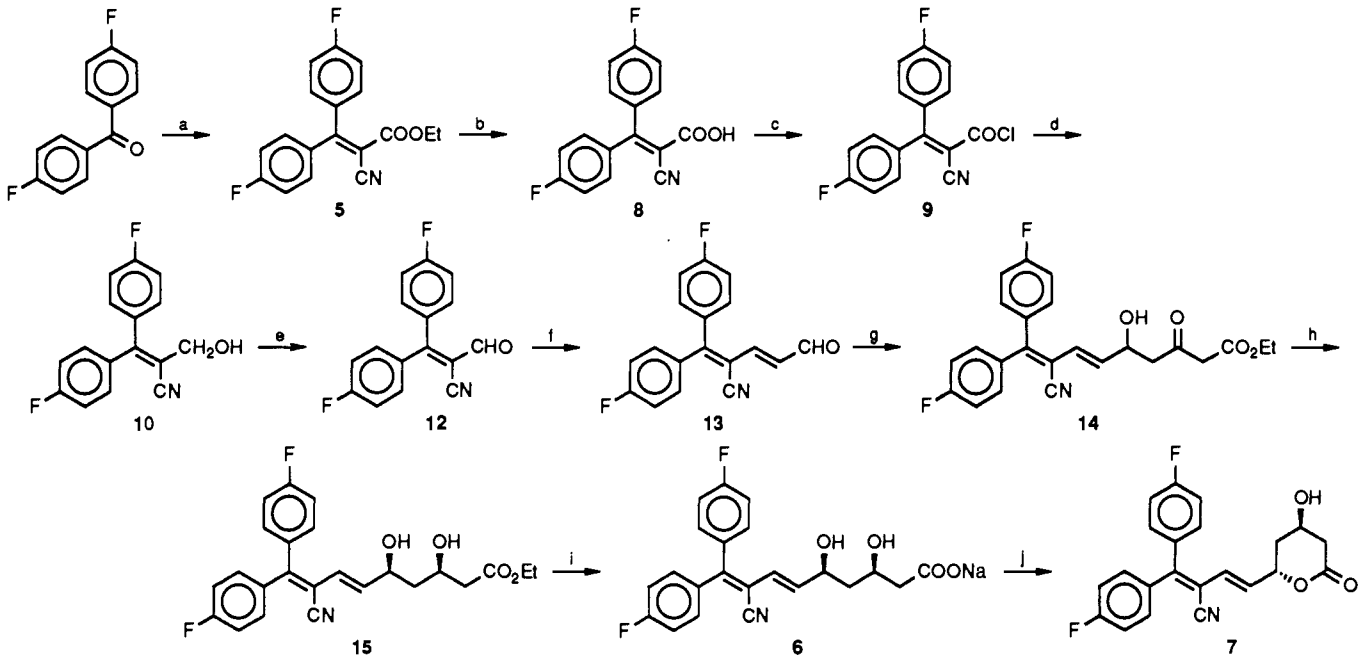
(17) Balasubramanian, N.; Brown, P. J.; Catt, J. D.; Han, W. T.; Sit, S. Y.; Wright, J. J. 196th National Meeting of the American Chemical Society, Los Angeles, CA, September 26–30, 1988, MEDI 109.

(18) Narasaka, K.; Pai, F. C. *Tetrahedron* **1984**, *40*, 2233. Kathawala, F. G.; Prager, B.; Prassad, K.; Repic, O.; Shapiro, M. J.; Stabler, R. S.; Widler, L. *Helv. Chim. Acta* **1986**, *69*, 803. Chen, K.; Hartmann, G.; Prassed, K.; Repic, O.; Shapiro, M. J. *Tetrahedron Lett.* **1987**, 155.

(19) On a smaller scale, a cosolvent was added (such as toluene) and a longer heating period was also required to initiate the dipolar cycloaddition. The reaction was always carried out behind a safety shield although it was (uneventfully) repeated many times.

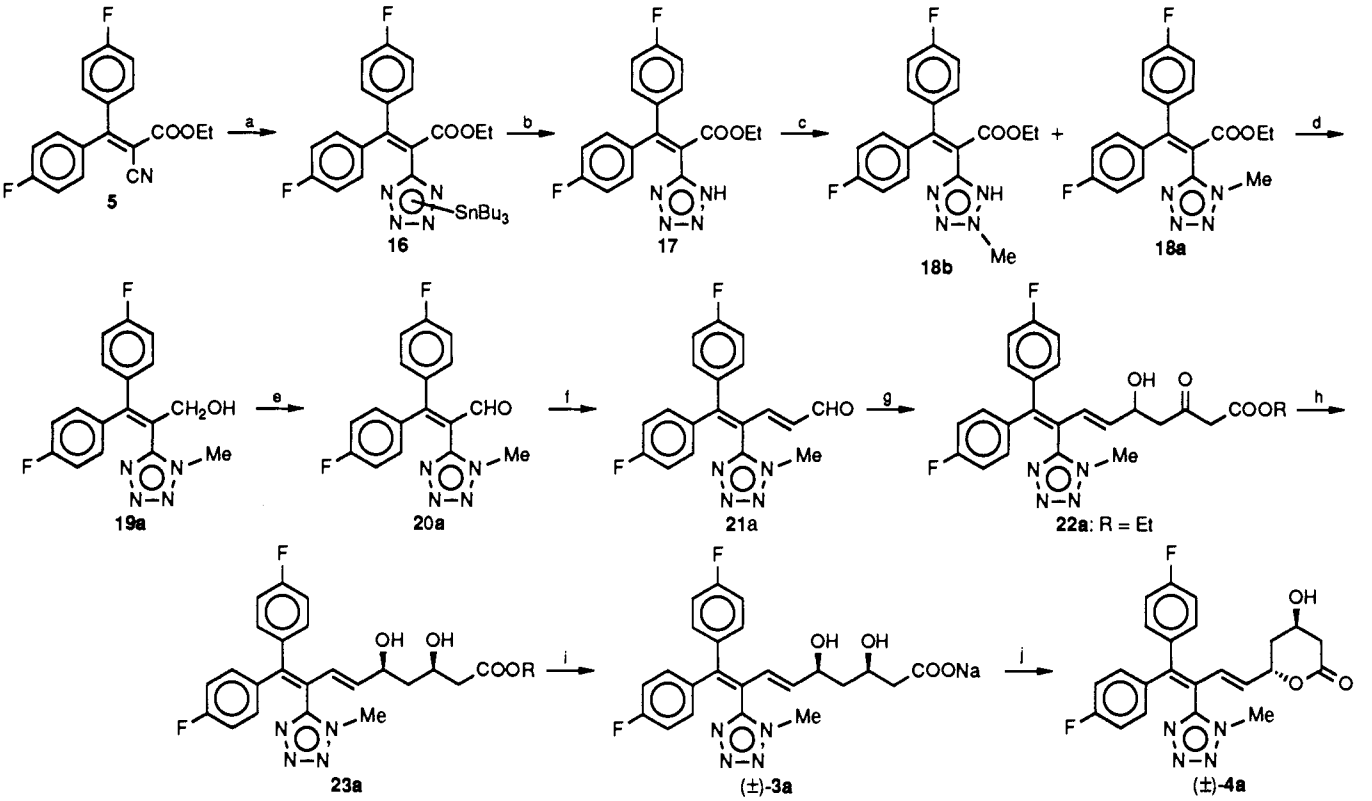
(20) More steric hindered electrophiles such as 2-iodopropane gave more 2N-isomer. Isobutylene alkylation in ether gave a single 2*N*-*tert*-butyl tetrazole derivative.

Scheme I[a]



[a] Reaction conditions: (a) $EtOOCCH_2CN$, $HOAc/C_6H_6$, β-alanine at reflux; (b) LiOH, THF/H_2O, then HCl; (c) oxalyl chloride, CH_2Cl_2 at reflux; (d) LAH, Et_2O at −78 °C; (e) PCC in CH_2Cl_2 at room temperature; (f) $Ph_3PCHCHO$ in C_6H_6 at reflux; (g) dianion of $EtOOCCH_2COOEt$ then H^+; (h) $NaBH_4$, Et_3B in THF/MeOH at −60 °C; (i) NaOH in THF/H_2O at room temperature; (j) CMC metho-*p*-TsOH in CH_2Cl_2 at room temperatures.

Scheme II[a]



[a] Reaction conditions: (a) Bu_3SnN_3/reflux; (b) $KF/EtOH–H_2O$; (c) MeI/NaH/THF and separation; (d) Dibal-H/CH_2Cl_2 at −78 °C; (e) PCC/CH_2Cl_2; (f) $Ph_3PCHCHO/C_6H_6$; (g) dianion of $EtOOCCH_2COCH_3$ then H^+; (h) $NaBH_4/Et_3B$/THF/MeOH at −50 to −78 °C; (i) $NaOH/THF/H_2O$; (j) CMC metho-*p*-TsOH in CH_2Cl_2 at room temperature.

perature and for a prolonged period of time (ca. 4 days).[21] As the spectroscopic data of these two regioisomers were very similar and insufficient to establish the regiochemical assignments, an alternative synthesis was sought for.

Using ethyl 1-methyltetrazol-5-yl acetate (24), obtained by employing a simpler procedure than the one reported before,[22] we were able to prepare one of the two alkylated regioisomers (18a). By comparing these products we could

(21) The changes in the product ratio may be attributed to the intramolecular chelation of the countercation to the ester carbonyl group.

unambiguously assign that the major alkylation product (with MeI) **18a** was indeed the 1-methyltetrazole derivative.

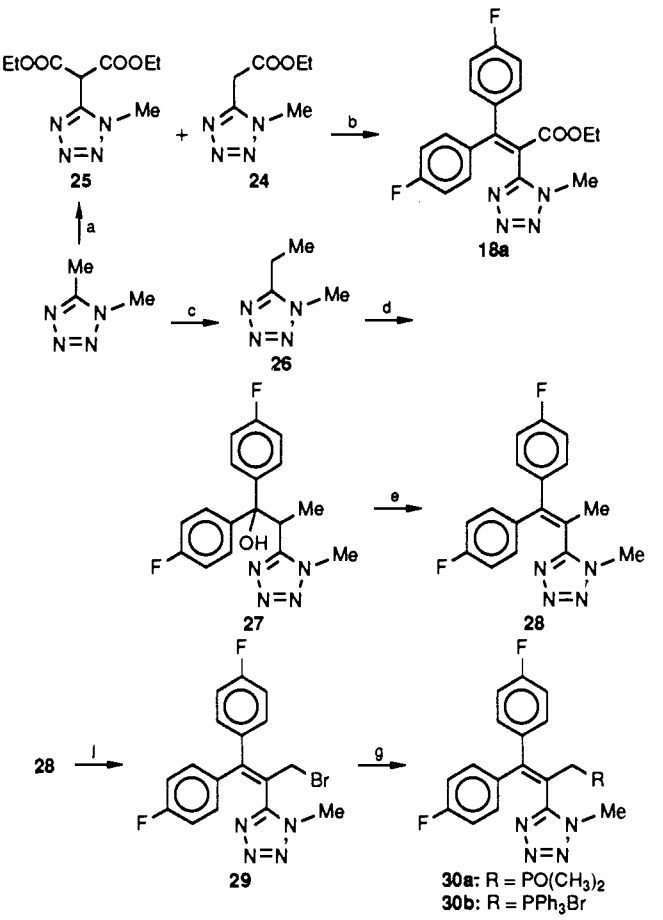
1,5-Dimethyltetrazole was prepared from acetone through a [2 + 3]-dipolar cycloaddition.[23] The methyl group at the C_5-position is sufficiently acidic to be deprotonated by strong bases (LDA or *n*-BuLi) and gives the corresponding anionic species. Acylation of the carbanion with ethyl chloroformate gave two products, the desired monoacylated product **24** and the unwanted bisacylated side product **25**. Compound **24** was purified by silica gel column chromatography, followed by crystallizations from hexanes and ethyl acetate mixtures (mp = 64–66 °C). This material was subjected to a variety of condensation conditions but was found to be very unreactive[24] toward the condensation with 4,4′-difluorobenzophenone. The needed carbon–carbon bond formation was finally achieved when a mixture of 4,4′-difluorobenzophenone in THF/CCl_4/$TiCl_4$ was treated with a solution of **24** in pyridine.[25] The reaction gave mostly the starting materials and roughly 25% **18a**. The product from this preparation was confirmed to be identical with the major isomer obtained from the previous alkylation reactions. This result confirmed the regiochemical assignments of **18a** and **18b** (Scheme III).

Both 1-methyl (**18a**) and 2-methyl (**18b**) isomers were submitted to the sequence of transformations shown in Scheme I, except for the following modifications. Tetrazole intermediates **18a** and **18b** were reduced directly by Dibal-H into the allylic alcohols (**19a**, for the 1*N* isomer). The amount of conjugated reduction products were barely detectable in both cases. This result was in sharp contrast to the hydride reduction of **5**, where the conjugated reduction pathway was predominant. With these key compounds in hands, both **19a** and **19b** were carried to the end by the general procedure described in Scheme II.

Similarly, a series of other alkyl tetrazole target compounds (i.e., methyl-, ethyl-, isopropyl-, (β-methoxyethoxy)methyl- and *tert*-butyl tetrazole derivatives) were synthesized and tested. The *tert*-butyl tetrazole intermediate was prepared in good yields from **16** and isobutylene in anhydrous ether and concentrated sulfuric acid (mp = 143–144 °C hexane–EtOAc, 67.8%; for details refer to ref 2). Among the alkyl tetrazole derivatives tested so far, the 1-methyl compounds (**3a**, BMY 21950/**4a**, BMY 22089) were found to be the most active (Table I). The synthetic route discussed above is not specific in terms of producing the desired regioisomer and, to avoid this handicap, an alternative approach was developed.[17]

1,5-Dimethyl tetrazole was alkylated very efficiently (LDA or *n*-BuLi then MeI) to provide 1-methyl-5-ethyltetrazole **26**.[26] Aldol condensation of **26** with 4,4′-difluorobenzophenone was accomplished uneventfully by a two-step procedure (LDA then $-H_2O$/*p*-TsOH) to furnish crystalline **28** (mp = 146–147 °C, 91%). From **28** the key

Scheme III[a]



[a] Reaction conditions: (a) LDA (or *n*-BuLi) then ethyl chloroformate; (b) $TiCl_4/CCl_4$/pyridine in THF and 4,4′-difluorobenzophenone; (c) LDA then MeI in THF; (d) LDA/THF then 4,4′-difluorobenzophenone; (e) H^+/toluene at reflux; (f) AIBN/NBS in CCl_4 at reflux; (g) $P(OCH_3)_3$ solvent of reflux.

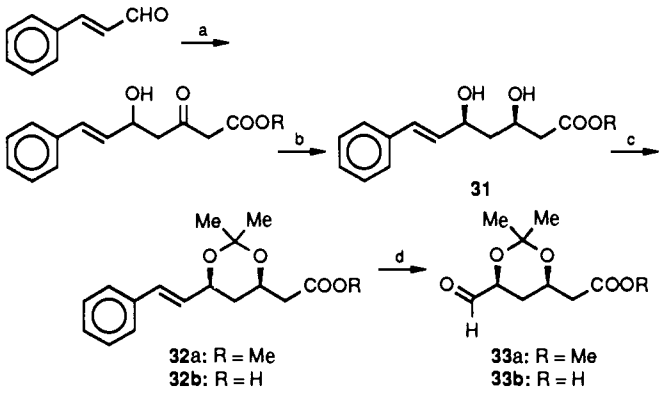
phosphonate ester **30a** was prepared by an AIBN-initiated allylic bromination (NBS)[27] followed by the classical Michaelis–Arbuzov reaction.[28] This phosphonate ester **30a** was used in the subsequent convergent synthesis to produce the final products. The corresponding phosphonium salt **30b** was also prepared and both **30a** and **30b** were found to be effective in creating the needed C_6–C_7 carbon–carbon double bond linkage (Scheme III).

On the other hand, efforts to synthesize the 3,5-dihydroxy carboxylic acid side chain also led to the successful preparation of the crystalline aldehyde intermediate **33a** (R = methyl), which is useful for the Wittig–Horner coupling (with **30a**) or Wittig coupling (with **30b**). A similar compound is known,[29] but our procedure provided a smaller molecular weight, highly crystalline, and nonhygroscopic material. These advantages are fully appreciated when large-scale preparation is required (Scheme IV).

Methyl 3,5-dihydroxy-7-phenylhept-6-enoate (**31**) was prepared (98% diastereomerically pure) from cinnamaldehyde in two steps. The *syn*-diol functionality was

(22) Howard, J.; Raap, R. *Can. J. Chem.* **1969**, *47*, 813.
(23) 1,5-Dimethyltetrazole was once commercially available from Aldrich Chemicals and is still commercially available from Dynamite Nobel on a custom-order basis. Preparation: Condon, F. E.; Walfmann, R.; Kundu, N.; Trivedi, J. P. *Org. Prep. Proc.* **1974**, *6* (*3*), 135.
(24) The primary purpose of performing this experiment was to establish the regiochemistry of 18a and 18b. In practice, a single fractional crystallization of the methylation crude product could provide over 50% yield of the desired product 18a.
(25) Campaigne, E.; Frierson, M. R. *J. Heterocyclic Chem.* **1979**, *16*, 235.
(26) Product was contaminated by a small amount (2–4%) of 1-methyl-5-isopropyltetrazole arising from the bis-alkylation.
(27) The versatile bromide **29** was also oxidized by 2-nitropropane (EtONa) to furnish over 95% of aldehyde **20a**. This intermediate is useful for analogue synthesis.
(28) In some cases, the phosphonate ester **30a** anion did not couple cleanly to produce a single geometric isomer but a mixture of both (trans and cis); the corresponding triphenylphosphorane Wittig reagent was therefore prepared (details in the Experimental Section).
(29) Jewell, C. F.; Wareing, J. R. (Sandoz) US Patent 4677211, issued on June 30, 1987.

Scheme IV[a]



[a] Reaction conditions: (a) dianion of a suitable acetoacetate; (b) $NaBH_4/Et_3B/THF/MeOH$ at −50 to −78 °C; (c) 2,2′-dimethoxypropane/H^+; (d) O_3 then Me_2S at −78 °C.

protected by the formation of acetonide (2,2-dimethoxypropane, *p*-TsOH) and the product **32a** was purified by crystallization (mp = 84–87 °C). Compound **32** is a very versatile intermediate: firstly, it could be ozonized to produce the crystalline aldehyde **33a** (R = methyl) useful in the Wittig type condensations with **30a** or **30b**; secondly, it could be saponified to give the free acid (for example **32b**, R = H) and resolved into the two enantiomers important for the synthesis of the chiral final products. The conversion of **32** into the aldehydes **33** and finally into the target compound was straightforward, but the synthesis of the individual enantiomers required some elaboration (Scheme V).

Methyl ester **32a** (R = methyl) was saponified in the usual way to give the free carboxylic acid **32b** (R = H), which was allowed to react with the commercially available and inexpensive resolving agent (1*S*,2*R*)-ephedrine (Scheme V). One of the resulting diastereomers was crystallized out in 41% yield (mp = 170–171 °C, $[\alpha]^{25}_D$ = +5.45°). The free carboxylic acid from the crystalline fraction was freed and submitted to the same sequence of ozonolysis, condensation,[30] hydrolysis, and lactonization to give lactone **4a** in optically active form ($[\alpha]^{25}_D$ = + 240°. Similarly, the other enantiomer (−)-**42** was prepared ($[\alpha]^{25}$D −242°) and the enzyme inhibitory activities were determined (isolated enzyme). The results indicated that the carboxylic salt of (−)-**3a** was essentially inactive. The remaining stereochemical feature to be determined was the absolute configuration of (+)-**4a**. The (1*S*,2*R*)-ephedrine precipitated isomer (+)-**34** (this isomer gave the active final product) was hydrogenated ($Pd/C,H_2$), hydrolyzed, and lactonized to give the known lactone **38**. An identical lactone with known configurations at the C_4- and C_6-positions was previously prepared from (*S*)-malic acid.[31] Therefore, it is concluded, consistent with other known HMG-CoA inhibitors, that compound (+)-**4a** must have 4*R*,6*S* configurations (Scheme VI).

We investigated other structural variations in this series of compounds. We modified the two aromatic rings adjacent to the C_9-atom in order to (i) accomodate other substituted rings, both symmetrical and unsymmetrical, and (ii) introduce other ring systems (3–6-membered, carbocyclic and heterocyclic) attached to the C_8-position. Some of the findings are summarized here.

(30) The lithium carboxylate salt (generated by adding a molar equivalent of *n*-BuLi into the free acid) was used in the coupling with the anion of phosphonate ester.

(31) Majewski, M.; Clive, D. L. J.; Anderson, P. *Tetrahedron Lett.* **1984**, *25* (*20*), 2102.

3. Biological Assays

In Vitro Inhibition of Microsomal HMG-CoA Reductase. The intact, fully activated microsomal form of rat liver HMG-CoA reductase (subunit MW ca 100000 Da) was prepared as described by Parker et al.[32] and used as the source of enzyme for assays. HMG-CoA reductase activity was determined essentially by the methods described previously[33,34] with the exception that the internal standard [^{3}H]mevalonolactone was added after termination of the assay. In this procedure, the enzyme is assayed by measuring the formation of product, [^{14}C]mevalonate, from the substrate, [3-^{14}C]HMG-CoA in the presence of NADPH. [^{14}C]Mevalonate is converted to its lactone and isolated by silica gel thinlayer chromatography (Whatman LK5D, developed in 50:50 benzene–acetone) in the presence of [^{3}H]mevalonolactone as an internal standard. Assays were conducted under conditions in which product formation was linear with respect to time and enzyme concentration.

To measure reductase inhibition, test compounds dissolved in water or dimethyl sulfoxide and diluted in buffer A (50 mM imidazole hydrochloride, 250 mM NaCl, 1 mM EDTA, 1 mM EGTA, 5 mM DTT, 20 pM leupeptin, pH = 7.2) were preincubated with aliquots of microsomes (50–100 μg of protein in buffer A) for 10 min at 37 °C, followed by addition of *d,l*-[3-^{14}C]HMF-CoA (0.33 mM, 1.0 μCi/μmol) and NADPH (3.0 mM) for assay. The 50% inhibitory concentration (IC_{50}) for each compound in Table I was calculated from the linear-regression line of the percent decrease (from control) in enzyme activity vs log concentration of inhibitor, determined with at least four dilutions of each test compound assayed in duplicate.

Isolated Hepatocyte Cholesterol Biosynthesis Assay. Intact parenchymal hepatocytes were isolated from male Wistar rats (180–280 g) fed normal chow diet, by using the collagenase perfusion method essentially as described previously.[35,36] Cell preparations were used only when viability (trypan blue exclusion) exceeded 95%. Cholesterol biosynthesis was determined as the incorporation by hepatocytes of [2-^{14}C]acetate into total (cellular plus medium) 3β-hydroxysterols. Hepatocyte sterols and lipids were isolated by modification of the methods described by Kates.[37] To isolate sterols, cells were extracted with methanol–chloroform–water (2:1:0.8) and the chloroform phase was separated and extracted with benzene to remove traces of water, and then dried under nitrogen. The residue was saponified at 75 °C with 0.30 N NaOH in methanol–water (9:1). The alkaline mixture was then extracted three times with petroleum ether to yield the nonsaponifiable lipids. The extract was dried under nitrogen in the presence of carrier cholesterol (0.1 mg) and 10% benzene, and the residue was dissolved in acetone–ethanol (1:1). Finally, the 3β-hydroxysterols were precipitated with an excess of digitonin; the precipitate was washed in acetone, dried under nitrogen, and dissolved in

(32) (a) Parker, R. A.; Miller, S. J.; Gibson, D. M. *J. Biol. Chem.* **1989**, *264* (*9*), 4877. (b) Parker, R. A.; Miller, S. J.; Gibson, D. M. *Biochem. Biophys. Res. Commun.* **1984**, *125*, 629.

(33) Shapiro, D. J.; Nordstrom, J. L.; Mitschelen, J. J.; Rodwell, V. W.; Schimke, R. T. *Biochem. Biophys. Acta.* **1974**, *370*, 369.

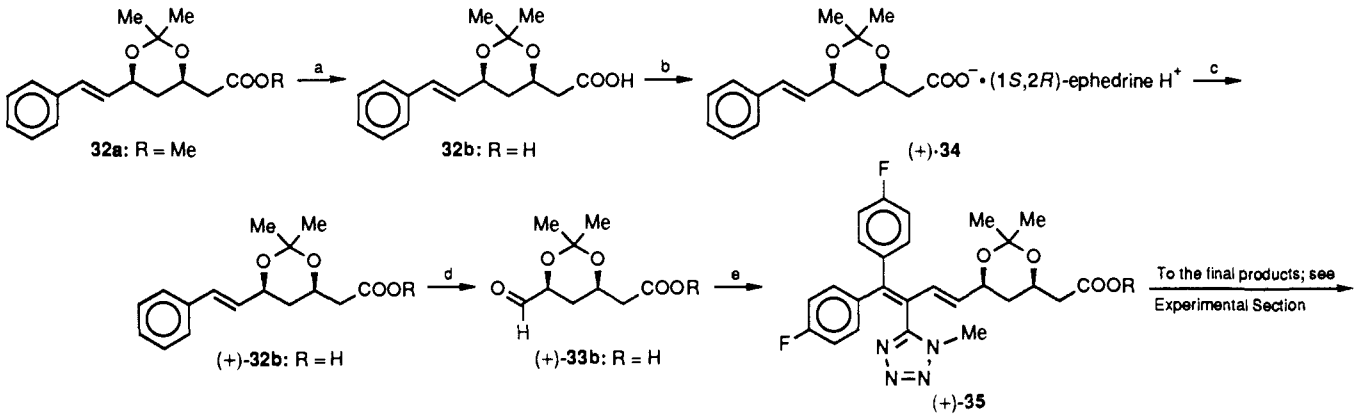
(34) Ingebritsen, T. S.; Gibson, D. M. *Methods Enzymol.* **1981**, *71*, 486.

(35) Seglen, P. O. Preparation of isolated rat liver cells. *Methods Cell Biol.* **1976**, *13*, 29.

(36) Ingebritsen, T. S.; Geelen, M. J. H.; Parker, R. A.; Evenson, K. J.; Givson, D. M. *J. Biol. Chem.* **1979**, *254*, 9986.

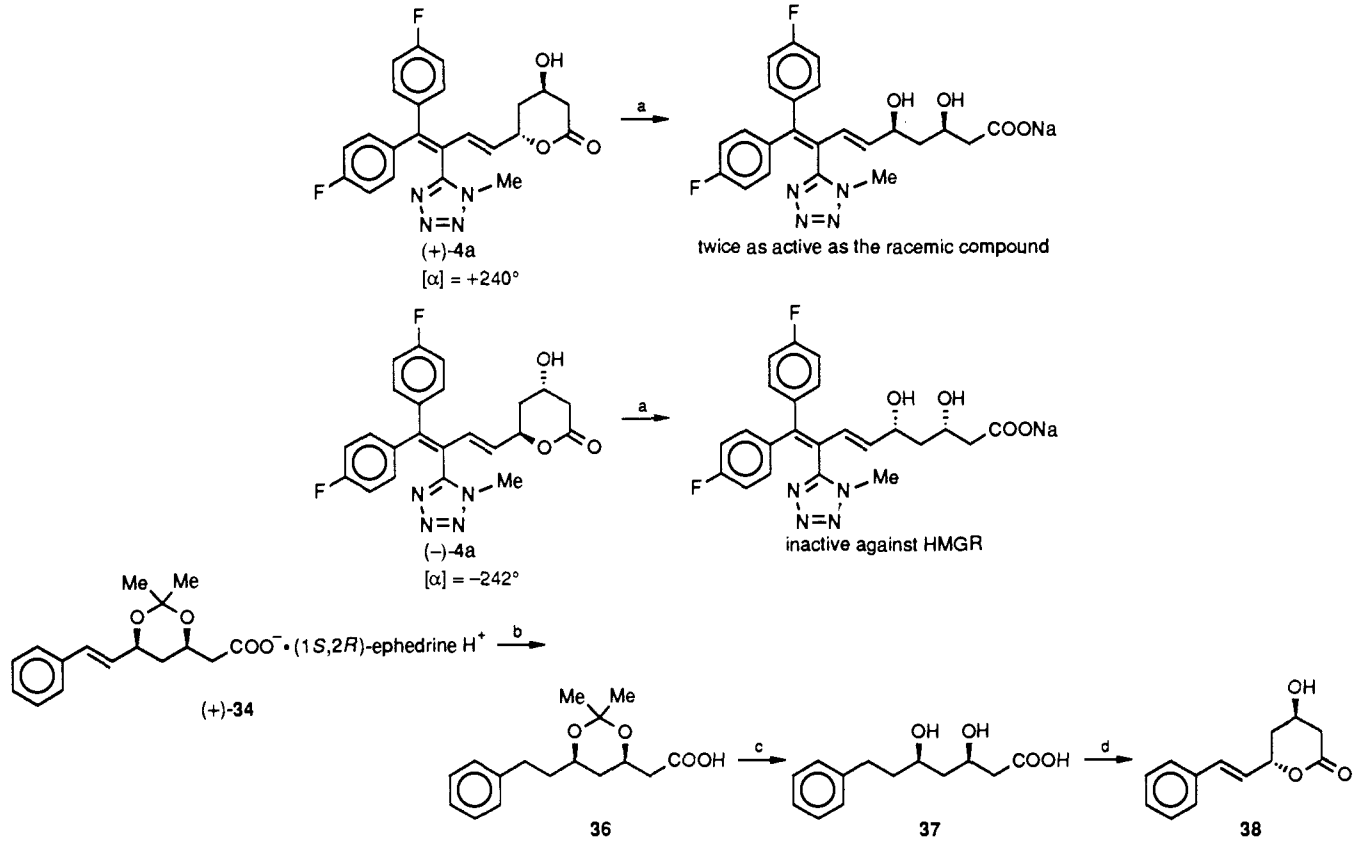
(37) Kates, M. Techniques of lipidology, isolation, analysis and identification. In *Identification of Lipids*, Kates, M., Ed.; American Elsevier: New York, 1972; p 349.

Scheme V[a]



[a] Reaction conditions: (a) $NaOH/H_2O$; (b) (1S,2R)-ephedrine/hexane/ethanol; (c) dilute HCl; (d) O_3 then Me_2S; (e) *n*-BuLi to generate the lithium carboxylate salt, then reacted with the anion of **30a**.

Scheme VI[a]



[a] Reaction conditions: (a) $NaOH/THF/H_2O$ room temperature; (b) H_2/Pd–C/EtOAc room temperature, then dilute NaOH extraction; (c) MeOH/CSA at room temperature; (d) TFA at room temperature.

toluene–methanol (1:1). The ^{14}C-labeled sterols were quantified by liquid scintillation and corrected for counting efficiency. Recovery of [^3H]cholesterol internal standard averaged 80 ± 3% with this procedure.

To measure inhibition of cholesterol synthesis, duplicate or triplicate aliquots of freshly isolated cells were suspended (100 mg cell wet weight in 2.0 mL) in Eagle's Minimal Essential Medium containing bicarbonate and HEPES buffer, at pH 7.4, plus 2% bovine serum albumin under a 95% O_2 + 5% CO_2 atmosphere. Cells were preincubated for 15 min with or without aliquots of test compounds added as water solutions of sodium salts or as dimethyl sulfoxide solutions (0.5% final concentration) of lactones. Controls received vehicle alone. [2-^{14}C]Acetate (1.8–2.5 μCi per μmol, 1–2μCi per mL of incubation volume) was then added to each and the cells were incubated with constant shaking for 60 min at 37 °C. These conditions produced time-linear incorporation of ^{14}C into sterols. The IC_{50} for inhibition of sterol synthesis by test compounds, which is shown in Table II, was calculated from the linear-regression curve of percent inhibition (compared to controls) vs log concentration using at least four concentrations of inhibitor. The mean IC_{50} values were calculated from *n* independent determinations as given in Table II.

Cholesterol Synthesis Assay in Extrahepatic Tissues. Data disclosed so far indicated the potent inhibitory activity of BMY 22089 in hepatic cells. However, sup-

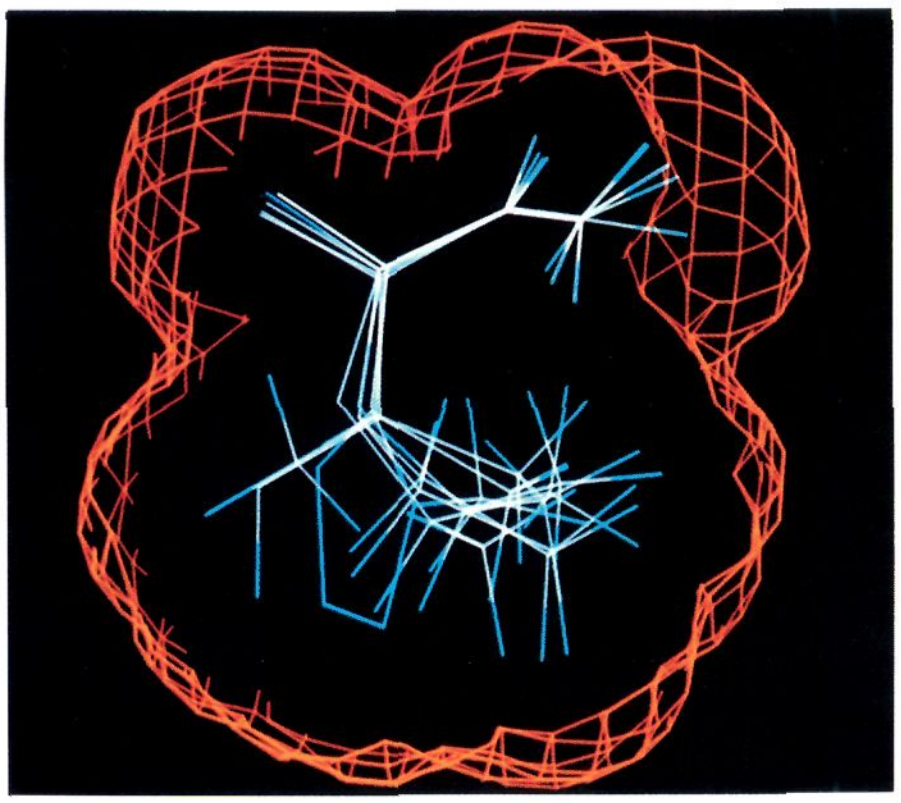


Figure 1. When bound to receptor, the hydrophilic tetrazolyl is imbedded in a space occupied by hydrophobic moieties in other potent inhibitors.

pression of sterol synthesis outside of the liver contributes very little to plasma cholesterol lowering, while perturbation of nonhepatic isoprenoid metabolism could lead to undesirable consequences. Therefore, examination of the tissue specificity of HMG-CoA reductase inhibitors may be a valuable criterion for long-term safety in the pharmacological profile of this class of lipid-lowering agents.

The sterol synthesis assay as described for hepatocytes was further extended in other cell preparations to examine possible tissue specificity. Cell dispersions from rat testes and rat spleen were prepared by nonenzymatic methods.[38] Rat adrenal cell dispersions were prepared by collagenase digestion as described in the literature.[39] The procedures for cholesterol synthesis assay in vitro in rat ileum slices was adapted from the description by Spady and Dietschy.[40] Finally, the bovine lens epithelial cells were prepared and cultured as described.[42] In these extrahepatic tissues, sterol synthesis assay conditions paralleled those used in hepatocyte assays except [^{14}C]acetate was 45–55 μCi per μmol and 5 μCi per mL. Table III below summarizes the results.

The apparent tissue specificity of BMY 22089 is not a consequence of reduced intrinsic sensitivity of HMG-CoA reductase in the cells of these nonhepatic tissues since the isolated microsomal fractions from these tissues responded similarly to that of liver. The issue of tissue specificity of this class of enzyme inhibitors has been discussed in the past[43,38] and the probable mechanism has been suggested to result from lack of permeability in certain tissues. While the underlying mechanism is a subject of our ongoing investigation,[44] the data presented here indicate that BMY 22089 is more selective for hepatocytes than other HMG-CoA reductase inhibitors described in the literature.

Table II. Summary of Hepatocyte Sterol Synthesis Inhibition by HMG-CoA Reductase Inhibitors

inhibitors	entry[b]	n[c]	IC_{50} assayed by [2-^{14}C]acetate incorporation:[a] $IC_{50}/\mu M$
BMY 22089[d]	**4a**	1	0.024
Na salt form of BMY 22089	**3a**	7	0.021 ± 0.004
lovastatin	**2**	6	0.032 ± 0.008
XU-62320[e]		5	0.052 ± 0.01[f]

[a] Mean IC_{50} ± SEM; calculated from n independent IC_{50} determinations. Sterol synthesis assayed by [^{14}C]acetate incorporation using normal rat hepatocytes. [b] All inhibitors, with the exception of BMY 22089 (lactone form, **4a**), were tested as Na salt forms; both **4a** and **3a** were racemic mixtures. [c] n = number of independent experiments. [d] Chiral Na salt and lactone forms **3a** and **4a** ($[\alpha]^{25} = +240°$) exhibited IC_{50} values nearly exactly $^1/_2$ the values of the racemic materials. [e] Structure of the Sandoz's reference agent XU-62320 (compound **60**, Chart II), XU-62320 is also known as fluindostatin. [f] $p < 0.01$ vs compound **3a** (BMY 22089, Na form).

Table III. Relative Inhibitory Potency of **3a** in Various Tissue Preparations

	IC_{50} ratio (tissue/hepatocyte)[a]				
inhibitor	spleen	testes	adrenals	ileum	lens epith
BMY 21950 (**3a**)	149	86	76	129	144
lovastatin	1.2	1.3	1	1.2	1.6
XU-62320	1.2	1.7	1	nd	nd

[a] A comparison of potencies for major reference agents in several tissues in vitro. Inhibitors were tested as Na salts. Isolated cell dispersions from rats (or bovine ocular lens epithelial cell cultures) were preincubated with inhibitors for 15 min followed by cholesterol biosynthesis assay by [^{14}C]acetate incorporation for 60 min. The table shows the ratio of IC_{50} in each tissue to that obtained in rat hepatocytes (Table II); "nd" stands for not determined.

In Vivo Acute Cholesterol Biosynthesis Inhibition in Rats. Acute cholesterol synthesis inhibition in rats was assayed essentially by the method of Alberts et al.[41] Male Wistar rats (160–200 g, housed two per cage) were maintained on a normal diet (Purina rat chow and water, ad libitum) for at least 7 days on a reversed lighting schedule (7:00 a.m. to 5:00 p.m. dark). Food was removed 15 h prior to dosing. Compounds were administered at 8:00 a.m. by intragastric intubation using 0.5–1.0 mL of water or propylene glycol solutions of sodium salts, lactones, or esters of the test compounds. Controls received equal volumes of the vehicle.

Thirty minutes after receiving the test substances, rats were injected intraperitoneally with 0.9 mL of 0.9% NaCl containing approximately 120 μCi per kg of body weight of sodium [1-^{14}C]acetate (1–3 mCi/mol). After a 60-min incorporation period, rats were sacrificed and liver and blood samples were obtained. Aliquots of plasma (1.0 mL), obtained by centrifugation of heparin + EDTA-treated

(38) Tsujita, Y.; Kuroda, M.; Shimada, Y. *Biochim. Biophys. Acta* **1986**, *877*, 50.
(39) Moriwaki, K.; Gomi, M.; Itoh, Y. *Life Sci.* **1986**, *38*, 453.
(40) (a) Spady, D. K.; Dietschy, J. M. *J. Lipid Res.* **1983**, *24*, 303. (b) Stange, E. F.; Dietschy, J. M. Sterols and bile acids. In *New Comprehensive Biochemistry*; Danielson, H., Sjovall, J., Ed.; Elsevier: New York, 1985; Vol. 12, p 121.
(41) Alberts, A. W.; Chen, J.; Kuron, G.; Hunt, V.; Huff, J.; Hoffman, C.; Rothrock, J.; Lopez, M.; Joshua, H.; Harris, E.; Patchett, A.; Monaghan, R.; Currie, S.; Stapley, E.; Albers-Schonberg, G.; Hensens, O.; Hirshfield, J.; Hoogsteen, K.; Liesch, J.; Springer, J. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 3957.
(42) Hitchener, W. R.; Cenedella, R. J. *J. Lipid Res.* **1985**, *26*, 1455.
(43) (a) Goto, Y. Drugs affecting lipid metabolism. In *Proceedings in Life Sciences*; Paoletti, R., Ed.; Springer-Verlag: New York, 1987; p 247. (b) Germershausen, J. I.; Hunt, V. M.; Bostedor, R. G.; Bailey, P. J.; Karkas, J. D.; Alberts, A. W.; *Biochem. Biophys. Res. Commun.* **1989**, *158*, 667. (c) Slater, E. E.; MacDonald, J. S. *Drugs* **1988**, *36* (*Suppl. 3*), 72.
(44) (a) Parker, R. A.; Sit, S. Y.; Wright, J. J.; Clark, R. W.; Grosso, R. A.; Keely, S. L.; Antonaccio, M. J. *Arteriosclerosis 8*, 634a. (b) Parker, R. A.; Sit, S. Y.; Clark, R. W.; Grosso, R. A.; Wright, J. J.; Keely, S. L. *FASEB J. 3*, A644. (c) Clark, R. W.; Grosso, R. A.; Parker, R. A. *FASEB J. 3*, A953.

blood, and aliquots of liver homogenates (equivalent to 0.50 g of liver wet weight) were taken for determination of radiolabeled 3β-hydroxysterols. Sterol isolation for the liver samples followed the method of Kates as described above for the hepatocyte procedure while the plasma samples were directly saponified followed by isolation of the digitonin-precipitable sterols. ^{14}C-Labeled sterols were quantified by liquid-scintillation counting (efficiency corrected). Mean percent inhibition of ^{14}C incorporated into liver and into plasma cholesterol were calculated for groups of treated animals and compared to mean values for controls conducted simultaneously.

With this in vivo method, compound (±)-**3a** yielded a 50% inhibitory dose (ED_{50}) of 0.1 mg/kg for both plasma and liver cholesterol. For the reference agent lovastatin, an ED_{50} value of 0.04 mg/kg was obtained, which was comparable to values obtained for lovastatin by Alberts et al.[41]

4. Molecular Modeling and Three-Dimensional Quantitative Structure–Activity Relationship (SAR)

In the absence of any molecular information concerning the active site of HMG-CoA reductase,[45] the goal of this study was to delineate from the IC_{50} values of 9,9-bis(4-fluorophenyl)-3,5-dihydroxy-8-substituted-6,8-nonadienoic acid derivatives the main topographical and physicochemical features of the binding site probed by these inhibitors.

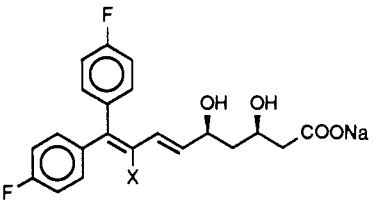
The inhibitors selected for study span an activity range of 3.73 log units and are listed in Table IV together with their $pIC_{50} = -\log IC_{50}$ values. Entry 1 (**3a**, Table IV) represents the most potent inhibitor ($IC_{50} = 0.037 \pm 0.005$ μM) and entry 9 (**46**, Table IV) is the least active one ($IC_{50} = 200$ μM).

It is well-documented[9] that the 3,5-dihydroxycarboxylic moiety represents the pharmacophore for HMG-CoA reductase inhibitor recognition. The pharmacophoric moiety was present in all compounds considered (Table IV) and systematic conformational analysis calculations indicated[46] that any pharmacophore geometry presented by potent inhibitors can be achieved in any inactive analogue. It followed that inactivity could not be associated to the inability of the molecule to present the appropriate pharmacophore molecular geometry.

Limited SAR's available in the literature[9] corroborated data obtained at Bristol-Myers[2,17] and suggested the existence of an auxiliary site corresponding to the substituents attached to the terminal C_9-position. The strong dependence of IC_{50} on the chemical identity of the substituent attached to the C_8-position (Table IV) was indicative of the existence of an additional auxiliary binding site corresponding to this substituent.

It is logical to assume that, when bound to the receptor, potent HMG-CoA reductase inhibitors are complementary, in topographical and intermolecular interactional pattern sense, to the binding sites mentioned above. The inactive

Table IV. HMG-CoA Reductase Inhibitor Data Base



no.	entry	X	pIC_{50}
3a	1	1-Me-Tet[b]	7.43
40	2	Tet	6.48
59[a]	3	*i*-Pr	6.70
6	4	CN	5.10
58	5	CH_3	5.15
42	6	1-Et-Tet	5.95
44	7	Tet	4.85
3b	8	2-Me-Tet	4.52
46	9	2-Et-Tet	3.70

[a] See refs 9 and 47. [b] Tet = tetrazolyl.

inhibitors violate the complementary requirements and experience detrimental interactions at the active site. It follows that the receptor-bound conformation of the more active entries 1–3 (**3a**, **40**, **59**, Table IV) would provide a topographical specification of the active site and, subsequently, allow one to locate and map the auxiliary binding sites.

The conformational lability of entries 1–3 (Table IV) precluded, however, any attempt to identify a probable receptor-bound conformation of these inhibitors. In order to identify a bioactive conformation one had to go outside the data base of Table IV, and four potent inhibitors (**60–63**, Chart II) were selected to augment the original structural data base. These shared the pharmacophore, possessed a "masked" butadiene, but had fewer conformational degrees of freedom due to cyclic constraints.

To identify a logical candidate for the bioactive conformation, we employed the procedure described in ref 48a–c. Actual calculations were performed by using the Search program implemented in the Sybyl modeling package.[49] The approach consists of allowing each compound to explore its set of sterically allowed conformations while recording the relative positions of the C_6- and C_9-atoms of the butadiene or "masked" butadiene unit. The conformational search was performed in 10° angular increment about the C_7–C_8,C_8-substituent and the two *p*-FPh–C_9 bonds, respectively. (The dihydroxy carboxylic moiety was not considered in calculations and this structural simplification does not affect the results of the conformational search). Then, one intersects the resultant sets of conformations in order to identify those C_6–C_9 pairwise distances ($d_{6,9}$) which are shared by the seven [Chart II, **60–63** and Table IV, entries 1–3] inhibitors considered. Actual calculations showed that the only value of $d_{6,9}$ common to these inhibitors is $d_{6,9} = 3.3$ (±0.05) Å and it corresponded to a C_6–C_7–C_8–C_9 torsional angle of 110–130°. This particular conformation of the butadiene unit restricts the torsional freedom about the C_8-tetrazole bond (i.e., C_9–C_8–C_5–N_1 torsional angle) within 80–120° range. Assuming a similar binding mode for these inhibitors, it

(45) Only the primary amino acid sequences for human and hamster HMG-CoA reductase are known: Luskey, K. L.; Steven, B. *J. Biol. Chem.* **1985**, *260*, 10271.

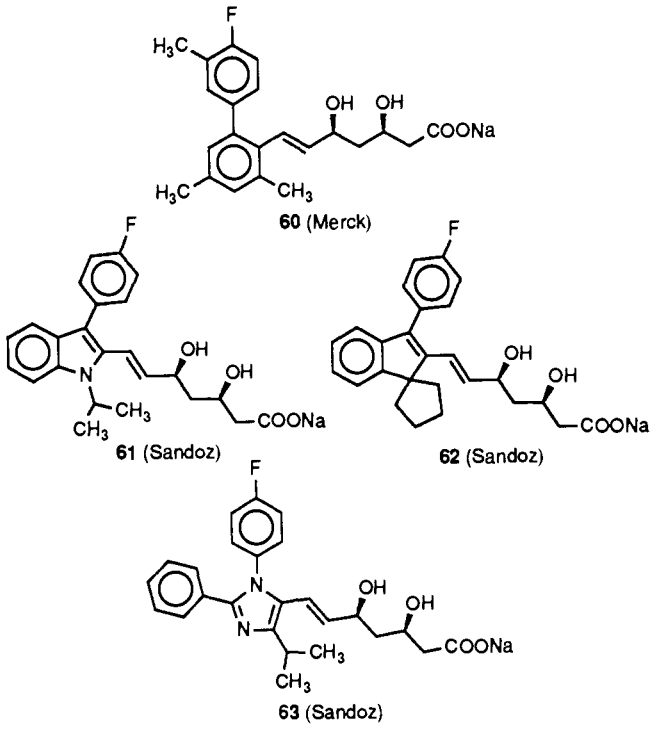
(46) Being less relevant in the present context, these results are not reported here.

(47) Wright, J. J.; Catt, J. D., unpublished results; however, during the course of our theoretically study, the 8-isopropyl analogue (**59**) was published by a group at Hoest AG and the IC_{50} value reported for this compound was not in agreement with ours. In order that we could conform to the experimental uniformity, we therefore employed our data despite the discrepancy.

(48) (a) Marshall, G. R.; Barry, C. D.; Bossard, H. E.; Dammkoehler, R. A. *ACS Symp. Ser.* **1979**, *112*, 205. (b) Motoc, I.; Dammkoehler, R. A.; Mayer, D.; Labanowski *J. Quant. Struct.-Act. Relat.* **1986**, *5*, 138. (c) Mayer, D.; Naylor, C. B.; Motoc, I.; Marshall, G. R. *J. Comput.-Aided Mol. Design* **1987**, *1*, 3.

(49) Sybyl Molecular Modeling Software, Tripos Assoc./Evans & Sutherland, St. Lous, MO.

Chart II. Potent HMG-CoA Reductase Inhibitors



follows that the conformation described above represents the only logical candidate for the bioactive conformation of **3a** and its analogues. Constraint/full geometry optimization calculations performed at molecular mechanics level using the Maximin[50] program implemented in Sybyl indicated that the bioactive conformation is very close to a local minimum characterized by C_6–C_7–C_8–C_9 and C_9–C_8–C_5–N_1 torsion angles of 110.9° and 123.8°, respectively, and it is energetically accessible: its energy relative to the local minimum is at most 1.6 kcal/mol (i.e., the relative energies of 0.186, 0.506, 1.196, 1.405, 1.589 kcal/mol correspond to a C_6–C_7–C_8–C_9 torsion angle of 120°, 110°, 100°, 90°, and 80°, respectively). The energy penalty associated with a nonplanar conformation for the butadiene moiety (molecular orbital calculations[59] give the 1,3-butadiene delocalization energy as only 4 kcal/mol) is probably easily compensated by the interactions of the 3,5-dihydroxy carboxylic acid moiety and the substituents attached to the terminal C_9-position, respectively, with their corresponding binding sites. Our results corroborate data disclosed recently[60] by a group at Hoechst AG (i.e., in the case of 8-isopropyl analogue, replacing the butadiene moiety, $IC_{50} = 1.4 \times 10^{-8}$ mol/L; by a butene, $IC_{50} = 2.6 \times 10^{-8}$ mol/L; or butane, $IC_{50} = 4.4 \times 10^{-8}$ mol/L; moiety had only marginal effects on inhibitory potency). This indicates the butadiene system is not a requirement for inhibitory activity. The four-carbon fragment represents a spacer which has the ability to present to the enzyme a molecule having the groups required for binding in spatial locations which maximize their interactions with the active site (obviously, both butene and butane afford sterically allowed conformations with pairwise distances $d_{6,9} = 3.3 \pm 0.05$ Å).

As the only structural modification occurred at the C_8-position and it was verified that all inhibitors of Table IV have the ability to assume the bioactive conformation, it is concluded that the bioactivity of these compounds is conditioned by intermolecular interactions between the substituent attached to C_8 and its corresponding binding site.

Consider now the potent congeneric inhibitors (entries 1–3, Table IV) and the structurally distinct compounds **60–63** (Chart II) and overlay the seven structures according to the constraints imposed by the bioactive conformation (i.e., the C_6–C_7–C_8–C_9 and C_9–C_8–C_5–N_1 torsion angles were set at 110° and 120°, respectively). Then, focus (Figure 1) on the spatial location and chemical identity of the moieties appended to the C_8 (or its corresponding) atom of the butadiene (or "masked" butadiene) unit. The main point to be made is that the hydrophilic tetrazolyl ($\pi = \log P_{o/w} = -1.04$[51]) is completely imbedded in the 3D space occupied otherwise by hydrophobic moieties, e.g., *i*-Pr ($\pi = 1.53$), *c*-C_5H_8 ($\pi = 2.67$). As all seven compounds are highly potent inhibitors, it is straightforward that the controlling beneficial interactions at this site are neither hydrophilic nor hydrophobic but polarization interactions. A sufficiently accurate measure of polarization interactions is provided by the van der Waals volume.[52]

Because all seven compounds (Chart II, **60–63**, and Table IV, entries 1–3) afford high inhibitory potency, it follows that the union of the van der Waals envelopes (called reference space and shown in red, Figure 1) of the moieties appended to C_8 (shown in white and blue, Figure 1) is embedded in a region of the HMG-CoA reductase active site which is available for binding. Further, the union of the van der Waals envelopes represents the best available complementary copy of the size and shape of the active site. The quantitative analysis which follows was performed by using the methods and programs described in previous papers.[53] The method originates from a topological approach[53d,e] to receptor mapping and has the ability to quantify potential intermolecular steric effects. Molecular shape analysis[54,55] and the method used in this study share the use of the overlapping volume as a molecular shape descriptor.[61]

The above argument indicates that pIC_{50} should obey eq 1. Here, OV represents the overlapping van der Waals

$$pIC_{50} = a \cdot OV + b \cdot NOV + c \qquad (1)$$

(50) Labanowski, J.; Motoc, I.; Naylor, C. B.; Mayer, D.; Dammkoehler, R. A. *Quant. Struct.-Act. Relat.* **1986**, *5*, 138.
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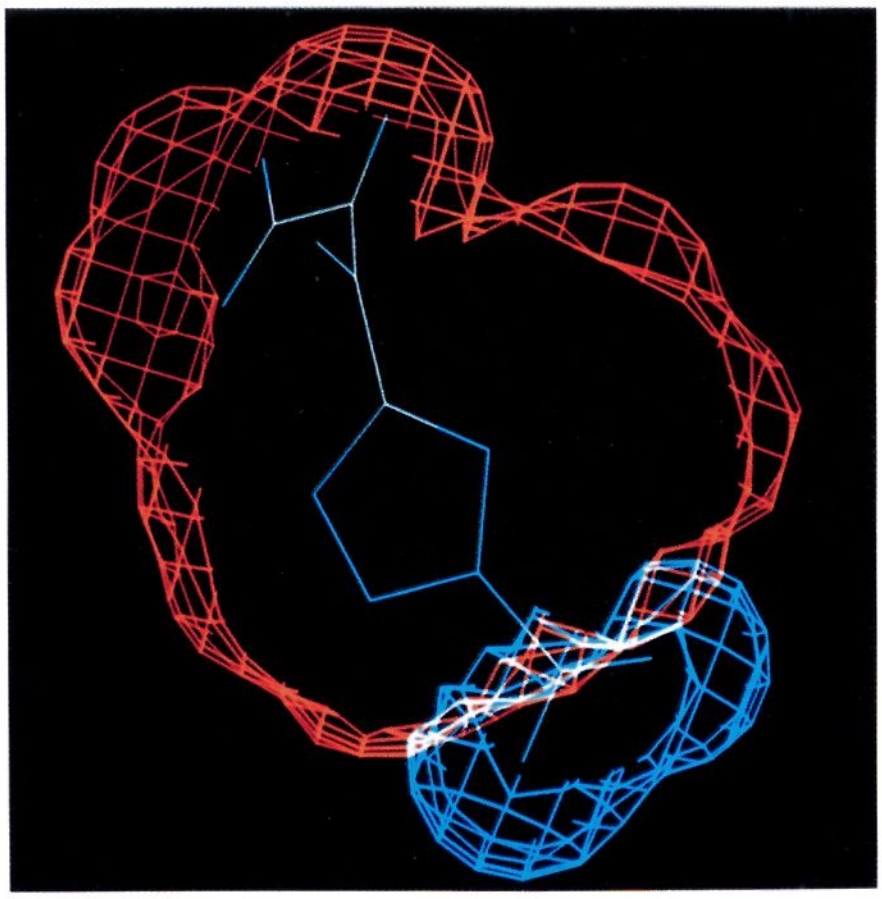

Figure 2. Compound **3b** (X = 2-methyltetrazole) is inactive due to the volume (blue) it occupies outside the binding site (red).

volume (Å^3) of the reference space and the substituent; NOV is the nonoverlapping van der Waals volume (Å^3) of the substituent superimposed over reference space.

As OV and NOV are regarded as measures of polarization interactions and, respectively, intermolecular steric effects between inhibitor and active-site backbone, it would be expected that $a > 0$ and $b > 0$.

The OV and NOV values were calculated by numerical integration of molecular van der Waals envelopes[52] using standard molecular geometries.[56]

The pIC_{50}(obs) OV, and NOV values of Tables IV and V respectively, provide the following equation:

$$pIC_{50}(\text{calcd}) = \underset{(t = 7.56)}{0.0653}\text{OV} - \underset{(t = -10.01)}{0.1100}\text{NOV} + \underset{(t = 1.13)}{0.8160} \quad (2)$$

$(n = 9, r = 0.974, s = 0.311, F = 54.94, r(\text{OV,NOV}) = 0.468]$

where r, s, F, and t stand for correlation coefficient, standard deviation, and Fisher and Student statistics, respectively, and n denotes the number of compounds used in the regression. r(OV NOV) is the correlation coefficient between independent variables. Examination of these statistics indicates that model 2 fits inhibitory potency data very well. Equation 2 is valid for inhibitors which retain the bis(*p*-FPh) substitution at the C_9-position.

Further, cross-validation analysis[57,58] of eq 2 indicates the compelling predictive utility of the model. Cross-validation evaluates a model by how well it predicts data. The procedure consists of the following: pIC_{50}(obs), OV, and NOV values corresponding to a given inhibitor (any entry 1–9, Table IV) are excluded from analysis, eq 2 is recalculated with the remaining data points, and it is used to predict the pIC_{50} of the excluded compound. The procedure is repeated until every pIC_{50} value has been predicted by a model from whose derivation it was excluded. A cross-validated r^2 is defined as

$$\text{cross-validated } r^2 = (\text{SD} - \text{press})/\text{SD} \quad (3)$$

Here, SD is the sum of squared deviations of each pIC_{50}(obs) from their mean and press is the sum, over all compounds, of the squared differences between actual pIC_{50}(obs) and predicted pIC_{50}(pred) inhibitory potency. Note that a cross-validated $r^2 < 0$ arises whenever inhibitory potency values are better estimated by the mean of all pIC_{50}(obs) than by the model under consideration. Cross-validated $r^2 \simeq 0.90$–1.0 indicates excellent prediction ability. The cross-validated r^2 obtained for eq 2 is 0.912. The satisfactory agreement between pIC_{50}(obs) and pIC_{50}(pred) justifies the conclusion that eq 2 provides a physicochemical specification of the enzyme binding site corresponding to the substituent attached to the C_8-position. Equation 2 predicts, in qualitative agreement with the experimental data (Table I), that the inhibitory potency of the derivatives **46** and **49** of Table I is in the millimolar range (i.e., **46**, OV = 91.6 Å^3, NOV = 37.3 Å^3, mean calculated IC_{50} = 2.0 mM; **49**, OV = 92.1 Å^3, NOV = 50.6 Å^3, mean calculated IC_{50} = 54.9 mM).

The robustness of the active site model developed here can further be assessed by repeating the analysis with a reference space which contains no structural information regarding **3a** and its analogues. Such a space is provided by the union of the van der Waals envelopes of the corresponding moieties in the Sandoz and Merck inhibitors (**60**–**63**, Chart II).

The resulting equation (see Table VI for data used in its derivation) reads as

$$pIC_{50} = \underset{(t = 10.48)}{0.1212}\text{ OV} - \underset{(t = 12.03)}{0.1137}\text{ NOV} - \underset{(t = 2.82)}{2.3666} \quad (4)$$

$(n = 9, r = 0.981, s = 0.264, F = 95.931, r(\text{OV,NOV}) = 0.67$, cross-validated $r^2 = 0.958)$

It suffices to note, for example, that eq 4 predicts (through

Table V. Calculated and Predicted pIC_{50} (Eq 2)[a]

		pIC_{50}			
no.	entry	calc	pred	OV	NOV
3a	1	7.502	7.576	102.47	0.00
40	2	6.596	6.629	88.58	0.00
59	3	6.734	6.746	90.70	0.00
6	4	5.087	5.077	65.45	0.00
58	5	4.956	4.788	63.45	0.00
42	6	5.386	5.250	98.48	16.87
44	7	4.810	4.782	103.85	25.31
3b	8	4.936	5.009	87.73	14.58
46	9	3.874	4.001	90.34	25.79

[a] Compounds are indexed as in Table IV. The probable error of the OV and NOV values is approximately 0.9 Å^3.

Table VI. Calculated and Predicted pIC_{50} (Eq 4)

		pIC_{50}			
no.[a]	entry	calc	pred	OV	NOV
3a	1	7.497	7.561	93.73	8.74
40	2	6.315	6.280	79.11	9.44
59	3	6.643	6.627	82.23	8.43
6	4	5.350	5.502	64.54	1.00
58	5	5.106	5.072	62.52	1.00
42	6	5.587	5.494	89.66	25.64
44	7	5.267	5.519	95.00	34.15
3b	8	4.533	4.536	78.87	23.41
46	9	3.582	3.461	81.50	34.58

[a] Compounds are indexed as in Table IV.

(61) Hopfinger[54] has utilized the overlapping volume descriptor, S_o, and its square, S_o^2, in molecular shape analysis studies in combination with other parameters (e.g., partition coefficients, length of substituent) and has further utilized the conformation corresponding to one of the energy minima of a selected compound of the series under consideration as reference structure.

cross-validation) for **3a** pIC_{50}(pred) = 7.56 while pIC_{50}(obs) = 7.43 and pIC_{50}(calcd) = 7.50.

In the framework established above, derivatives which violate the spatial complementarity requirements imposed by the binding site (as pictured by the reference space, Figures 1 and 2) are devoided of enzyme inhibitory activity.

We conclude the following: (i) Inactivity is brought about by substituents which occupy volumes outside the reference space shown in Figure 1. As that space is sterically disallowed, the inhibitor experiences repulsive steric effects at the active site and the resulting enzyme–inhibitor complex is weak—Figure 2 illustrates such situations. (ii) Modest activity is caused either by insufficient occupancy of the binding site (i.e., NOV = 0.0 but OV is too small to sufficiently stabilize the enzyme–inhibitor complex), as illustrated by entries 4 and 5 in Table IV, or by cancellation of beneficial and detrimental interactions (i.e., the coefficients with OV and NOV terms have opposite sign). (iii) High inhibitory potency is rendered by compounds which occupy 50% or more of the binding site volume (157.7 $Å^3$) and avoid occupancy of any space outside it. According to eq 2, $IC_{50} \leq 1$ μM corresponds to NOV = 0.0 and OV $\geq$ 78.5 (50% of the binding-site volume). The occupancy of the binding site volume afforded by the potent analogues containing X = 1-methyltetrazole (65%), *i*-Pr (58%), and tetrazole (56%) seems to indicate that there is significant potential left to further improve binding. However, the topography of the reference space and the stereochemical constraint imposed by the trigonal hybridization of the C_8-atom restrict the actual occupancy of the site to about 70%.

In conclusion, the title compound **3a** has been shown to be indeed a potent contender in the search of novel inhibitors of 3-hydroxy-3-methylglutaryl coenzyme-A reductase. High inhibitory potency coupled with its apparent tissue selectivity (Table III) indicates that BMY 21950 merits further development. The clinical trials of this compound are currently underway and the results will be published in due course. Finally, the mathematical model developed here is being further refined and utilized as a gauge to quantitate biological activities of potentially relevant structures, hence, enlarging the data base of analogues related to the title compound.

5. Experimental Section

Melting points were recorded on a Thomas-Hoover capillary melting point apparatus and boiling points were measured at specific pressures (mmHg) and both temperatures are uncorrected. Proton magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker AM 300, Bruker WM 360, or Varian T-60 CW spectrometer. All spectra were determined in $CDCl_3$, DMSO-d_6, or D_2O unless otherwise indicated. Chemical shifts are reported in δ units downfield from the internal standard tetramethylsilane (TMS) and interproton coupling constants are reported in hertz (Hz). Splitting patterns are designated as follows: s, singlet; d, doublet t, triplet; q, quartet; m, multiplet; br, broad peak; and dd, doublet of doublet. Carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra were recorded on a Bruker AM 300 or Bruker WM 360 spectrometer and were broad-band proton decoupled. All spectra were determined in $CDCl_3$, DMSO-d_6, or D_2O unless otherwise indicated with internal deuterium lock, and chemical shifts are reported in δ units downfield from tetramethylsilane. Carbon/fluorine coupling constants are expressed in the form of ^1J, ^2J, ^3J, and ^4J; ^1J is the first-order coupling, ^2J the second, and so on. Infrared (IR) spectra were determined on a Nicolet MX-1 FT spectrometer from 4000 to 400 cm^{-1}, calibrated to the 1601 cm^{-1} absorption of a polystyrene film and are reported in reciprocal centimeters (cm^{-1}). Relative intensities are indicated as follows: s (strong), m (medium), and w (weak). Optical rotations $[\alpha]^{25}_D$ were determined on a Perkin-Elmer 241 polarimeter in $CHCl_3$ at the concentrations indicated. Gas chromatography–mass spectra (GC–MS) were determined on a Finnigan 4500 gas chromatography quadruple mass spectrometer at ionization potential of 70 eV. Mass spectra were also recorded on a Kratos MS-50 instrument utilizing the fast atom bombardment (FAB) technique. The mass data are expressed in the format parent ion (M^+) or protonated ion $(M + H)^+$.

Analytical thin-layer chromatography (TLC) was carried out on precoated silica gel plates (60F-254) and visualized with UV light, iodine vapors, and/or staining with one of the following reagents: (a) methanolic phosphomolybdic acid (2%) and heating; (b) method a, followed by 2% cobalt sulfate in 5 M H_2SO_4 and heating. Column chromatography, also referred to as flash column chromatography, was performed in a glass column using finely divided silica gel (Merck Silica gel Type-H) and pressures of 5–15 psi (N_2) with the indicated solvents. Ozonolysis reactions were done with a Welsbach ozonator style T-23. All evaporations of solvents were performed under reduced pressure.

Ethyl 2-Cyano-3,3-bis(4-fluorophenyl)-2-propenoate (5). A mixture of 20.0 g (92 mmol) of 4,4′-difluorobenzophenone, 11.0 g (97 mmol) of ethyl cyanoacetate in a mixed solvent of 100 mL of dry benzene, and 20 mL of glacial acetic acid containing a catalytic amount of β-alanine (0.9 g) was refluxed with separation of water with a Dean–Stark water trap. Separation of water was rapid during the first 2 h (0.4 mL of aqueous layer collected) but slower afterward. Azeotropic distillation was continued for a period of 14 days. Analytical TLC eluted with 10% EtOAc in hexanes (v/v) (Merck plate, 0.25 mm silica gel-F) showed two spots at R_f = 0.2 (desired product) and at R_f = 0.45 (4,4′-difluorobenzophenone starting material). Crude reaction mixture was washed with water (40 mL × 2), and the combined aqueous washes were extracted with EtOAc (150 mL × 2). The organic layers were combined, dried over $MgSO_4$, and concentrated under reduced pressure to crystallize the product as pale, cubic crystals. The crude product was collected, washed with 1:1 EtOAc in hexanes (v/v) and then recrystallized from 8:1 (hexanes–ethyl acetate v/v) to give 16.2 g (56.3%) of analytical pure title compound: mp = 114–116 °C. In practice, the desired product was crystallized out from the crude reaction mixture directly: IR (KBr) ν_{max} 3000 (s), 2225 (s), 1931 (vs), 1605 (s), 1513 (s), 1250 (s), 844 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.19 (3 H, t, J = 7.1 Hz), 4.18 (2 H, q, J = 7.1 Hz), 7.08–7.15 (6 H, m), 7.40–7.42 (2 H, m); ^{13}C NMR ($CDCl_3$) δ 13.75, 62.27, 104.05, 116.69, 115.53 (d, ^2J = 22.7 Hz), 115.88 (d, ^2J = 22.7 Hz), 131.64 (d, ^3J = 9.1 Hz), 132.66 (d, ^3J = 9.1 Hz), 134.25, 134.31, 134.36, 164.01 (d, ^1J = 252.9 Hz), 164.52 (d, ^1J = 254.0 Hz), 166.65. Anal. Calcd for $C_{18}H_{13}NO_2F_2$: C, 69.01; H, 4.15; N, 4.47. Found: C, 68.91; H, 4.15; N, 4.62.

2-Cyano-3,3-bis(4-fluorophenyl)-2-propenoic Acid (8). To a solution of ethyl 2-cyano-3,3-bis(4-fluorophenyl)-2-propenoate (5.0 g, 16 mmol) in tetrahydrofuran (30 mL) at 0 °C was added a solution of 1 M lithium hydroxide in water (30 mL). The saponification reaction was allowed to proceed at 0 °C for a total of 3 h, forming a clear homogeneous solution. The crude reaction mixture was made acidic by adding 15 mL of a 3 M HCl solution in water and the organic material was extracted twice into diethyl ether. Organic layers were combined, dried over $MgSO_4$, and concentrated under reduced pressure to give a pale solid. Recrystallization from a ethyl acetate–hexanes mixture (1:9 v/v) yielded 3.08 g of the title compound: mp = 180–181 °C. Another 1.37 g of the desired product was also isolated from the mother liquor, making the total combined yield of the title compound over 97%: IR (KBr) ν_{max} 3500 (br), 2210 (s), 1713 (s), 1625 (s), 1600 (s), 1225 (s), 1156 (s), 844 (s); ^{1}H NMR (DMSO-d_6) δ 7.47 (m, 2 H), 7.35 (m, 2 H), 7.28 (m, 4 H), 7.2 (br, 1 H); ^{13}C NMR (DMSO-d_6) δ 163.47, 163.10, 162.99, 134.73, 132.05, 131.94, 117.08, 115.07, 115.18, 105.56. Anal. Calcd for $C_{16}H_9F_2O_2N$: C, 67.30; H, 3.20; N, 4.90. Found: C, 66.98; H, 3.39; N, 4.81.

2-Cyano-3,3-bis(4-fluorophenyl)-2-propenal (12). Step A. 2-Cyano-3,3-bis(4-fluorophenyl)-2-propenoyl Chloride (9). To a suspension of 2-cyano-3,3-bis(4-fluorophenyl)-2-propenoic acid (4 g, 14 mmol) in 10 mL of dry methylene chloride at room temperature was added oxalyl chloride (6 mL, 69 mmol) in one single portion. The reaction was stirred and warmed slowly to reflux for 45 min. The pale homogeneous solution was evaporated under reduced pressure to remove volatile solvents, then excess oxalyl chloride was removed under vacuum (20 mmHg) at ambient temperature for 2 h and under high vacuum (0.1 mmHg) for 16 h to give the title compound.

Step B. 2-Cyano-3,3-bis(4-fluorophenyl)-2-propenol (10).

The acyl chloride prepared in step A was dissolved in 40 mL of tetrahydrofuran and was chilled to −78 °C under an inert atmosphere. To this pale greenish solution at −78 °C was added 10 mL of lithium aluminum hydride in a tetrahydrofuran solution (1 M, 10 mmol). Reduction was allowed to proceed at −78 °C for 20 min before it was diluted with a dilute H_2SO_4 solution (2 M, 20 mL). The desired product was extracted twice into ethyl acetate (40 mL × 2). Organic layers were combined, dried over $MgSO_4$, and concentrated under reduced pressure to give 2.64 g (69.4%) of the title compound. The crude allylic alcohol was used in the next step without further purification. An analytically pure sample was prepared by crystallization from a mixture of ethyl acetate–hexanes (1:9, v/v): mp = 110–112 °C; IR (KBr) ν_{max} 3500 (br), 2106 (s), 1750 (s), 1600 (s), 1510 (s); ^{1}H NMR (DMSO-d_6) δ 7.3–7.7 (m, 8 H), 5.65 (t, 1 H, J = 5.4 Hz), 4.11 (d, 2 H, J = 5.4 Hz); ^{13}C NMR (DMSO-d_6) δ 164.5, 158.5, 154.5, 135.0, 131.5, 119.0, 115.4, 113.5, 59.6.

Step C. 2-Cyano-3,3-bis(4-fluorophenyl)-2-propenal (12). To a vigorously stirred solution of the crude allylic alcohol (0.51 g, 1.9 mmol, prepared in step B) in 14 mL of dry methylene chloride under an inert atmosphere at room temperature was added pyridinium chlorochromate (700 mg, 3.2 mmol) in one single portion. The oxidation was allowed to proceed at room temperature for 16 h, during which all the starting material was consumed. The crude oxidation suspension was filtered through a bed of silica gel, washed with 25% (v/v) ethyl acetate in hexanes. The desired product crystallized upon concentration under reduced pressure to give 0.5 g (quantitative yield) of the title compound: mp = 167–169 °C; MS (CI) m/e = 269 for M^+; IR (KBr) ν_{max} 2113 (s), 1713 (m), 1681 (s), 1600 (s), 1500 (s); ^{1}H NMR ($CDCl_3$) δ 9.38 (s, 1 H), 7.55–7.51 (m, 2 H), 7.33–7.15 (m, 6 H); ^{13}C NMR ($CDCl_3$) δ 186.31, 170.29, 165.26, 165.06, 133.5, 133.47, 133.17, 131.61, 116.48, 116.19, 115.27, 113.28. Anal. Calcd for $C_{16}H_9F_2NO$: C, 71.40; H, 3.30; N, 5.20. Found: C, 71.09; H, 3.44; N, 5.16.

4-Cyano-5,5-bis(4-fluorophenyl)-2,4-pentadienal (13). To a dry mixture of 2-cyano-3,3-bis(4-fluorophenyl)-2-propenal (1.29 g, 4.8 mmol) and (triphenylphosphoranylidene)acetaldehyde (1.54 g, 5.04 mmol) under an inert atmosphere at ambient temperature was added 60 mL of dry benzene. The suspension was warmed to reflux temperature under an inert atmosphere and the reaction was allowed to proceed at reflux temperature for 1 h. The crude reaction mixture was poured onto a silica gel column saturated with hexanes. The desired product was eluted with 1 L of hexanes followed by 1 L of 20% ethyl acetate in hexanes (v/v) to give 1.43 g (100%) of the title compound.

An analytically pure sample was obtained by recrystallization from a mixture of ethyl acetate in hexanes (8% v/v): mp 155–156 °C; MS (CI) m/e = 296 for $(M + H)^+$; IR (KBr) ν_{max} 2110 (s), 1680 (s), 1595 (s), 1506 (s); ^{1}H NMR ($CDCl_3$) δ 9.57 (d, 1 H, J = 7.4 Hz), 7.48–7.44 (m, 2 H), 7.24–7.10 (m, 7 H), 6.73 (dd, 1 H, J = 7.4, 16 Hz); ^{13}C NMR ($CDCl_3$) δ 192.07, 164.38, 164.15, 144.82, 134.73, 134.69, 132.36, 132.47, 116.30, 115.84, 108.90. Anal. Calcd for $C_{18}H_{11}F_2NO$: C, 73.21; H, 3.75; N, 4.74. Found: C, 73.03; H, 3.85; N, 4.74.

Ethyl 8-Cyano-9,9-bis(4-fluorophenyl)-5-hydroxy-3-oxo-6,8-nonadienoate (14). To a chilled suspension (0 °C, ice–water bath) of sodium hydride (120 mg, 60% suspension in mineral oil, 3 mmol) in dry tetrahydrofuran (2 mL) under an inert atmosphere was added ethyl acetoacetate (330 μL, 2.6 mmol) in four equal portions over a period of 10 min. The homogeneous, clear solution was stirred at 0 °C for 30 min followed by the dropwise addition of a n-BuLi in hexane (2.5 M) solution (1.15 mL, 2.9 mmol) over 15 min. The orange dianion solution was stirred at 0 °C for an additional hour. The ice–water bath was replaced by an acetone–dry ice bath at −78 °C and the dianion solution was transferred via a cannula into a tetrahydrofuran (10 mL) solution containing 4-cyano-5,5-bis(4-fluorophenyl)-2,4-pentadienal (0.5 g, 1.7 mmol). The reaction mixture was allowed to stir at −78 °C for 5 min then was diluted with 40 mL of a 1 M HCl aqueous solution. The organic material was extracted twice with ethyl acetate (50 mL × 2). The organic layers were combined, dried over $MgSO_4$, and concentrated under reduced pressure. The desired product was partitioned over a silica gel column and eluted with 1.5 L of 30% (v/v) ethyl acetate in hexanes to give 0.57 g (79%) of the title compound as a gummy solid: ^{1}H NMR ($CDCl_3$) δ 7.38–7.33 (2 H, m), 7.18–7.04 (6 H, m), 6.50 (1 H, d, J = 15.6 Hz), 6.41 (1 H, dd, J = 5.04, 15.6 Hz), 4.73 (1 H, br), 4.19 (2 H, q, J = 7.1 Hz), 3.47 (2 H, s), 3.22 (1 H, br), 2.83–2.81 (2 H, m), 1.28 (3 H, t, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$) δ 202.32, 166.79, 163.49, 163.23, 153.93, 136.92, 134.36, 134.08, 132.44, 132.24, 132.14, 124.79, 117.49, 115.81, 115.60, 110.31, 67.31, 61.57, 49.85, 49.14, 14.07.

Ethyl (±)-*erythro*-8-Cyano-9,9-bis(4-fluorophenyl)-3,5-dihydroxy-6,8-nonadienoate (15). To a solution of ethyl 8-cyano-9,9-bis(4-fluorophenyl)-5-hydroxy-3-oxo-6,8-nonadienoate (0.49 g, 1.15 mmol) in 8 mL of dry tetrahydrofuran at 0 °C (ice–water bath) under an inert atmosphere was added a triethylborane solution in tetrahydrofuran (1 M, 1.4 mL, 1.4 mmol) in one single portion. The pale mixture was stirred for a total of 1 h. The cooling ice–water bath was replaced with an acetone–dry ice bath, and to the reaction mixture was added $NaBH_4$ (56 mg, 1.48 mmol) in one portion. The reaction suspension was stirred at −78 °C for 2 h, forming a clear, homogeneous pale yellow solution. The crude mixture was diluted with 200 μL of reagent-grade methanol and the solution was allowed to stir at −78 °C for an additional 1.5 h. The crude reaction mixture was diluted with 30 mL of 1 M HCl aqueous solution followed by extractions with ethyl acetate (40 mL × 2). The organic layers were combined, dried over $MgSO_4$, and concentrated under reduced pressure to give the product as a thick syrup, it was further diluted with 50 mL of dry methanol and the methanolic solution was allowed to stand at room temperature for 16 h before evaporation under reduced pressure. The crude product was purified by flash silica gel column chromatography using 2 L of 40% ethyl acetate in hexanes (v/v) as the eluting solvent. The appropriate fractions were collected and evaporated to give 0.40 g (72%) of the title compound. MS (CI) m/e = 392 for $(M - 2H_2))$ H^+; IR (KBr) ν_{max} 3450 (s), 2110 (s), 1729 (s), 1600 (s), 1506 (s), 1230 (s); ^{1}H NMR ($CDCl_3$) δ 7.37–7.32 (2 H, m), 7.16–7.04 (6 H, m), 6.55 (1 H, d, J = 15 Hz), 6.37 (1 H, dd, J = 15, 5 Hz), 4.58 (1 H, m), 4.32 (1 H, m), 4.17 (2 H, q, J = 7 Hz), 3.83 (2 H, d, J = 6 Hz), 2.49 (2 H, d, J = 6 Hz), 1.69 (2 H, m), 1.28 (3 H, t, J = 7 Hz); ^{13}C NMR ($CDCl_3$) δ 172.48, 163.34, 163.05, 153.53, 138.54, 135.73, 134.18, 132.3, 132.0, 123.87, 115.7, 115.4, 117.59, 110.62, 71.44, 68.50, 60.93, 42.34, 41.49, 14.15. Anal. Calcd for $C_{24}H_{23}F_2NO_4 \cdot {}^3/_2H_2O$: C, 63.43; H, 5.77; N, 3.08. Found: C, 63.02; H, 5.36; N, 3.00.

Sodium (±)-(*E*)-*erythro*-8-Cyano-9,9-bis(4-fluorophenyl)-3,5-dihydroxy-6,8-nonadienoate (6). To a solution of ethyl (±)-(*E*)-*erythro*-8-cyano-9,9-bis(4-fluorophenyl)-3,5-dihydroxy-6,8-nonadienoate (36 mg, 0.086 mmol) in 1.0 mL of reagent-grade tetrahydrofuran at 0 °C (ice–water bath) under an inert atmosphere was added a 1.0 M sodium hydroxide solution in water (84 μL, 0.084 mmol) dropwise over a period of 5 min. The rate of addition was adjusted so that no change in color of the reaction mixture developed. The saponification reaction was allowed to stir at 0 °C for a total of 3.5 h and the crude mixture was lyophilized to give the title compound in quantiative yield: IR (KBr) ν_{max} 3438 (s), 2113 (m), 1600 (s), 1579 (s), 1506 (s), 1234 (s); ^{1}H NMR (DMSO-d_6) δ 7.5–7.4 (2 H, m), 7.4–7.2 (6 H, m), 7.19 (1 H, br), 6.4–6.2 (2 H, m), 5.24 (1 H, br), 4.31 (1 H, m), 3.74 (1 H, m), 3.08 (1 H, dd, J = 15, 1 Hz), 1.89 (1 H, dd, J = 15, 7 Hz), 1.62–1.40 (2 H, m); ^{13}C NMR (DMSO-d_6) δ 176.04, 162.48, 162.29, 152.20, 140.75, 135.90, 133.98, 132.08, 131.77, 122.04, 117.21, 115.64, 115.35, 110.64, 67.69, 65.61, 44.24, 43.54. Anal. Calcd for $C_{22}H_{18}F_2NO_4Na$: C, 62.71; H, 4.31; N, 3.32. Found: C, 61.86; H, 4.77; N, 3.14.

(±)-*trans*-(*E*)-[Bis(4-fluorophenyl)methylene]-4-(tetrahydro-4-hydroxy-6-oxo-2*H*-pyan-2-yl)-3-butenenitrile (7). Step A. (±)-*erythro*-8-Cyano-9,9-bis(4-fluorophenyl)-3,5-dihydroxy-6,8-nonadienoic Acid. To a solution of ethyl (±)-*erythro*-8-cyano-9,9-bis(4-fluorophenyl)-3,5-dihydroxy-6,8-nonadienoate (109.3 mg, 0.26 mmol) in 2 mL of reagent-grade tetrahydrofuran at 0 °C (ice–water bath) was treated with 259 μL of 1.0 M sodium hydroxide solution. The pale yellow suspension was stirred at 0 °C (ice–water bath) for 2 h, forming a clear, pale yellow solution. The crude reaction mixture was diluted with 0.5 mL of aqueous HCl (1.0 M) solution and the organic product was extracted into ethyl acetate (30 mL × 2). The organic extracts were combined, dried over $MgSO_4$, and concentrated under reduced pressure to give a pale gum. The crude free acid (117 mg) was rigorously dried under high vacuum (0.01 mmHg) at room temperature for 24 h before reacting in the next step.

Step B. (±)-*trans*-(*E*)-2-[Bis(4-fluorophenyl)-methylene]-4-(tetrahydro-4-hydroxy-6-oxo-2*H*-pyran-2-yl)-3-butenenitrile. The dried acid from step A was dissolved in 4.0 mL of dry methylene chloride at room temperature. To this stirring solution was added 0.3 g (0.71 mmol) of 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate. Lactonization was complete in 12 h at ambient temperature. Most of the solvent was evaporated under reduced pressure and the residue was washed with water (30 mL) followed by extractions with ethyl acetate (30 mL × 2). The organic layers were combined, dried over $MgSO_4$, and concentrated under reduced pressure to give a pale yellow syrup. The crude product was filtered through a short bed of silica gel and eluted with 40% ethyl acetate in hexanes (v/v) to give 97 mg (98%) of the title compound: MS (CI) m/e = 382 for $(M + H)^+$; IR (KBr) ν_{max} 3450 (s), 2113 (s), 1735 (s), 1600 (s), 1506 (s), 1233 (s), 750 (s); ^1H NMR ($CDCl_3$) δ 7.37–7.32 (2 H, m), 7.16–7.02 (6 H, m), 6.48 (1 H, d, J = 15.6 Hz), 6.33 (1 H, dd, J = 15.6, 5.9 Hz), 5.27 (1 H, m), 4.40 (1 H, br), 2.76 (1 H, dd, J = 4.6, 17.6 Hz), 2.62 (1 H, dd, J = 2.1, 17.6 Hz), 2.16 (1 H, br), 2.05 (1 H, d, J = 14 Hz), 1.87 (1 H, dt, J = 2.6, 10.9 Hz); ^{13}C NMR ($CDCl_3$) δ 169.11, 164.2, 164.0, 155.39, 136.1, 134.2, 133.12, 132.32, 132.02, 126.66, 117.27, 115.94, 115.67, 109.72, 74.92, 62.57, 38.68, 36.07. Anal. Calcd for $C_{22}H_{17}F_2NO_3$: C, 63.31; H, 5.07; N, 3.36. Found: C, 63.90; H, 5.17; N, 3.15.

Ethyl 3,3-Bis(4-fluorophenyl)-2-(1*H*-tetrazol-5-yl)-2-propenoate (17). A dry, 50-mL round-bottom flask was charged with 5.0 g (16.0 mmol) of ethyl 2-cyano-3,3-bis(4-fluorophenyl)-2-propenoate followed by 8.0 g (24.1 mmol) of azidotri-*n*-butylstannane (prepared by the procedure described in *Recl. Trav. Chim. Pays-Bas* **1962**, *81*, 202–205.) and 2.0 mL of reagent-grade toluene. The heterogenous mixture was stirred and heated to reflux (110 °C) in an oil bath behind a safety shield. The solid starting material dissolved gradually, forming a pale, yellowish, thick syrup and the homogenous mixture was stirred and refluxed for 20 h. Analytical TLC eluted with 20% MeOH in $CHCl_3$ (v/v) showed the product at R_f = 0.26 (streak). The crude reaction mixture was diluted with an equal volume of diethyl ether and was poured into a vigorously stirring saturated aqueous solution of KF (200 mL containing 2 mL of 48% HBF_4). A voluminous precipitate (Bu_3SnF) was observed soon after mixing and the hydrolysis was allowed to proceed for 16 h. The suspension was filtered and the filtrate was extracted with EtOAc (100 mL × 2). The organic layers were combined, dried over $MgSO_4$, and concentrated under reduced pressure. The title compound crystallized from the concentrate, yielding 4.54 g (77%) of white, analytically pure material: mp = 159–161 °C; IR (KBr) ν_{max} 3438 (br), 1713 (vs), 1600 (s), 1510 (s), 1238 (s), 841 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.92 (3 H, t, J = 7.6 Hz), 3.98 (2 H, q, J = 7.6 Hz), 7.3–6.7 (8 H, m), 10 (1 H, v br); ^{13}C NMR ($CDCl_3$) δ 166.52, 163.54 (d, ^1J = 250.7 Hz), 163.46, (d, ^1J = 262.7 Hz), 157.14, 136.40, 134.74, 131.71 (d, ^2J = 67.2 Hz), 131.59 (d, ^2J = 66.4 Hz), 115.75 (d, ^3J = 18.9 Hz), 115.45 (d, ^3J = 18.1 Hz) 62.11, 13.47. Anal. Calcd for $C_{18}H_{14}F_2N_4O_2$: C, 60.27; H, 4.06; N, 15.50. Found: C, 60.67; H, 3.96; N, 15.72.

Ethyl 3,3-Bis(4-fluorophenyl)-2-(1-methyl-1*H*-tetrazol-5-yl)-2-propenoate (18a). To a solution of 0.5 g (1.40 mmol) of ethyl 3,3-bis(4-fluorophenyl)-2-(1*H*-tetrazol-5-yl)-2-propenoate in 100 mL of dry benzene at 45 °C under argon was added sodium hydride (100 mg, 60% in mineral oil 2.5 mmol) in one single portion. The greyish suspension was stirred at 45 °C for 30 min then 1 mL (16.1 mmol) of methyl iodide was added, and the flask was sealed with a rubber stopper. Alkylation was allowed to proceed at 40–45 °C for a total of 4 days. Analytical TLC eluted twice with 20% EtOAc in hexanes showed only two isomeric products at R_f = 0.16 (major isomer **18a**) and R_f = 0.22 (minor isomer **18b**). The crude reaction mixture was washed with an equal volume of water and the aqueous phase was back-extracted once with 50 mL of diethyl ether. The organic layers were combined, dried over $MgSO_4$, and concentrated under reduced pressure to give crude product. The product ratio for the 1-isomer/2-isomer was determined to be about 5.6:1 by gas chromatography and by ^1H NMR spectroscopy.

The crude product mixture which was prepared as described above (5.0 g) was taken into 20 mL of hot ethyl acetate to which was added 40 mL of hot hexanes. The clear solution was allowed to cool slowly to room temperature to give 2.16 g (52%) of the title compound as colorless, large needles: mp = 144–145 °C; IR (KBr) ν_{max} 1713 (vs), 1600 (s), 1513 (s), 1325 (s), 1163 (s), 838 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.4–6.8 (8 H, m), 4.06 (2 H, q, J = 7.1 Hz) 3.68 (3 H, s), 1.00 (3 H, t, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$) δ 165.44, 163.6 (d, ^1J = 250.7 Hz), 163.4 (d, ^1J = 252.9 Hz) 156.85, 152.37, 135.88, 131.32 (d, ^3J = 8.3 Hz), 115.94 (d, ^2J = 21.9 Hz), 115.64 (d, ^2J = 22.7 Hz), 61.84, 33.76, 13.59. Anal. Calcd for $C_{19}H_{16}F_2N_4O_2$: C, 61.62; H, 4.35; N, 15.13. Found: C, 61.63; H, 4.45; N, 15.21.

Ethyl 3,3-Bis(4-fluorophenyl)-2-(2-methyl-2*H*-tetrazol-5-yl)-2-propenoate (18b). The residue (2.0 g) obtained from the filtrate of the recrystallization in step A (containing about equal portions of the 1- and 2-methyl isomers) was purified by silica gel (35 g) chromatography. The appropriate fractions were collected and evaporated to give crystalline product. Recrystallization from a hexanes–ethyl acetate mixture (9:1; v/v) yielded the title compound: mp = 117–118 °C; IR (KBr) ν_{max} 1713 (vs), 1600 (s), 1506 (s), 1250 (sh), 1225 (vs), 850 (m) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.4–6.8 (8 H, m), 4.20 (3 H, s), 406 (2 H, q, J = 7.1 Hz), 0.99 (3 H, t, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$) δ 167.12, 163.02 (d, ^1J = 272.6 Hz), 163.03 (d, ^1J = 225.7 Hz), 162.80, 152.59, 137.03 (d, ^4J = 4 Hz), 135.96 (d, ^4J = 3 Hz), 131.94 (d, ^3J = 8.3 Hz), 131.08 (d, ^3J = 8.3 Hz), 120.48, 115.37 (d, ^2J = 21.9 Hz), 115.26 (d, ^2J = 22.7 Hz) 61.41, 39.40, 13.61. Anal. Calcd for $C_{19}H_{16}F_2N_4O_2$: C, 61.62; H, 4.35; N, 15.13. Found: C, 61.77; H, 4.44; N, 15.38.

3,3-Bis(4-fluorophenyl)-2-(1-methyl-1*H*-tetrazol-5-yl)-2-propenol (19a). Diisopropylaluminum hydride (540 mL of 1 M solution in dichloromethane, 540 mmol) was added to a solution of ethyl 3,3-bis(4-fluorophenyl)-2-(1-methyl-1*H*-tetrazol-5-yl)-2-propenol (**18a**, 90.0 g, 243 mmol) in 1000 mL of dry dichloromethane at −78 °C under argon. After stirring for 2.5 h, the reaction mixture was slowly (**CAUTION!**) added 6 M hydrochloric acid, (300 mL, 1.8 mol), followed by 200 mL of saturated NH_4Cl solution. The heavier organic layer was separated, dried ($MgSO_4$) and concentrated under reduced pressure to give the title compound. The crude allylic alcohol **19a** was pure enough for the next preparation without further purification: MS (CI) m/e = 328 for $(M + H)^+$; IR (KBr) ν_{max} 3388 (v br), 1600 (s), 1501 (s), 1225 (s), 1156 (s), 838 (s), 750 (s), cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.5–6.9 (8 H, m), 4.52 (2 H, br), 3.42 (3 H, s), 3.75 (1 H, br, D_2O exchangeable); ^1H NMR (DMSO-d_6) δ 7.5–6.9 (8 H, m), 5.23 (1 H, t, J = 5.5 Hz), 4.27, (2 H, d, J = 5.5 Hz), 354 (3 H, s).

3,3-Bis(4-fluorophenyl)-2-(1-methyl-1*H*-tetrazol-5-yl)-2-propenal (20a). To a vigorously stirred solution of the crude allylic alcohol **19a** (89 g) in 2 L of methylene chloride at room temperature was added 53 g (0.25 mol) of pyridinium chlorochromate in one single portion. Analytical TLC immediately afterward showed about 50% of product at R_f = 0.34 along with the starting material at R_f = 0.14 (eluted with 50% EtOAc–hexanes v/v). The oxidation was allowed to proceed at room temperature for a total of 16 h, during which all the starting material was consumed and TLC showed only product. The crude reaction suspension was filtered through a bed of silica gel, washed with 3 L of 10% (v/v) ethyl acetate in hexanes and 3 L of 20% (v/v) ethyl acetate in hexanes. The desired product crystallized upon concentration under reduced pressure to give 76 g (95%) of the title compound: mp = 141–142 °C; MS (CI) m/e = 326 for $(M + H)^+$; IR (KBr) ν_{max} 3075 (m), 2875 (m), 1675 (s), 1600 (s), 1501 (s), 1238 (s), 1156 (s), 850 (s), 750 (s), cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.63 (1 H, s), 9.5–6.9 (8 H, m), 3.74 (3 H, s); ^{13}C NMR ($CDCl_3$) δ 188.92, 165.44, 164.68 (d, ^1J = 254.4 Hz), 164.10 (d, ^1J = 255.9 Hz), 151.34, 134.31, 133.77 (d, ^3J = 8.3 Hz), 132.69, 132.23 (d, ^3J = 7.5 Hz) 123.70, 116.26 (d, ^2J = 21.9 Hz), 116.18 (d, ^2J = 22.7 Hz), 34.10. Anal. Calcd for $C_{17}H_{12}F_2N_4O$: C, 62.58; H, 3.71; N, 17.17. Found: C, 62.41; H, 3.85; N, 16.98.

5,5-Bis(4-fluorophenyl)-4-(1-methyl-1*H*-tetrazol-5-yl)-2,4-pentadienal (21a). To a dry mixture of 3,3-bis(4-fluorophenyl)-2-(1-methyl-1*H*-tetrazol-5-yl)-2-propenal (0.70 g, 2.1 mmol) and (triphenylphosphoranylidene)acetaldehyde (0.72 g, 2.5 mmol) under argon at ambient temperature was added 20 mL of dry benzene. The suspension was warmed to reflux temperature under an argon atmosphere and the reaction was allowed to proceed at reflux temperature for 30 min. Analytical TLC eluted four times with 20% ethyl acetate in hexanes (v/v) showed only one spot for product at R_f = 0.15. The crude reaction mixture was poured on a silica gel column saturated with hexanes. The desired product

was eluted with 1.5 L of 20% EtOAc in hexanes (v/v) to give 0.67 g (89%) of the title compound which appears homogeneous by TLC: ^{1}H NMR ($CDCl_3$) δ 9.53 (1 H, d, J = 7.5 Hz), 7.47 (1 H, d, J = 15.7 Hz), 7.4–8.8 (m), 5.80 (1 H, dd, J_1 = 7.4 Hz, J_2 = 15.7 Hz), 4.11 (2 H, q, J = 7.1 Hz), 3.58 (3 H, s), 1.26 (3 H, t, J = 7.1 Hz).

The proton NMR (300 MHz) of the above product showed that it contained about 10% 7,7-bis(4-fluorophenyl)-6-(1-methyl-1*H*-tetrazol-5-yl)-2,4,6-heptatrienal as a side product, which was not easily removed. This material was used in the next preparation without further purification.

Ethyl 9,9-Bis(4-fluorophenyl)-5-hydroxy-8-(1-methyl-1*H*-tetrazol-5-yl)-3-oxo-6,8-nonadienoate (22a). To a chilled suspension (0 °C, ice–water bath) of NaH (0.64 g, 16.0 mmol; 60% in mineral oil) in 20 mL of dry tetrahydrofuran under argon was added ethyl acetoacetate (2.04 mL, 16.0 mmol) in four equal portions. The homogeneous, clear solution was stirred at 0 °C for 30 min, followed by dropwise addition of 6.4 mL of 2.5 M *n*-BuLi (16.0 mmol) over a period of 15 min. The orange dianion solution was stirred at 0 °C for an additional hour. The ice–water bath was replaced by an acetone–dry ice bath at −78 °C and the dianion was transferred via a cannula into a tetrahydrofuran (20 mL) solution containing 5,5-bis(4-fluorophenyl)-4-(1-methyl-1*H*-tetrazol-5-yl)-2,4-pentadienal (2.82 g, 8.01 mmol). Analytical TLC showed the major desired product at R_f = 0.15 (50% EtOAc in hexanes) and a minor product at R_f = 0.2. The crude reaction mixture was diluted with 40 mL of 1 N HCl and the aqueous layer was extracted with ethyl acetate (50 mL × 2). The organic layers were combined, dried over $MgSO_4$, and concentrated under reduced pressure. The desired product was purified by flash silica gel column chromatography eluted with 20% EtOAc in hexanes (v/v) to give 2.26 g (58.5%) of the title compound: MS (CI) m/e = 483 for $(M + H)^+$; IR (KBr) ν_{max} 3450 (v br), 1738 (s), 1725 (s), 1606 (s), 1513 (vs), 1225 (s), 1163 (s), 844 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.4–6.8 (8 H, m), 6.72 (1 H, d, J = 15.6 Hz), 4.63 (1 H, m), 4.17 (2 H, q, J = 7.1 Hz), 4.13 (1 H, m), 3.60 (3 H, s), 3.52 (1 H, d, J = 3.9 Hz, D_2O exchangeable), 3.47 (2 H, s), 2.74 (2 H, d, J = 6.0 Hz), 1.26 (3 H, t, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$) δ 164.21, 135.98, 132.34 (d, ^3J = 8.3 Hz), 131.45 (d, ^3J = 9.1 Hz), 115.74 (d, ^2J = 21.9 Hz), 115.74 (d, ^2J = 21.1 Hz), 100.86, 67.61, 61.58, 49.85, 49.07, 33.56, 14.10.

A small amount of the side product **ethyl 11,11-bis(4-fluorophenyl)-5-hydroxy-10-(1-methyl-1*H*-tetrazol-5-yl)-3-oxo-6,8,10-undecatrienoate** was also isolated: the silica gel column from the above step A was eluted further to give the minor product (R_f = 0.2). Repeated flash silica gel chromatography with 20% EtOAc in hexanes as the eluting solvent yielded the title compound: ^{1}H NMR ($CDCl_3$) δ 7.4–7.1 (4 H, m), 6.9–6.8 (4 H, m), 6.58 (1 H, d, J = 15.5 Hz), 6.31 (1 H, dd, J = 10.7, 15.0 Hz), 5.80 (1 H, dd, J = 10.7, 15.4 Hz), 5.66 (1 H, dd, J = 5.5, 15.1 Hz), 4.64 (1 H, m), 4.18 (2 H, q, J = 6.9 Hz), 3.58 (3 H, s), 3.46 (2 H, s), 3.02 (1 H, m), 2.75–2.72 (2 H, m), 1.27 (3 H, t, J = 6.9 Hz).

Ethyl (±)-*erythro*-9,9-Bis(4-fluorophenyl)-3,5-dihydroxy-8-(1-methyl-1*H*-tetrazol-5-yl)-6,8-nonadienoate (23a). To a solution of ethyl 9,9-bis(4-fluorophenyl)-5-hydroxy-8-(1-methyl-1*H*-tetrazol-5-yl)-3-oxo-6,8-nonadienoate (2.19 g, 4.53 mmol) (dried under high vacuum at 30 °C for 48 h) in 40 mL of anhydrous tetrahydrofuran at 0 °C (ice–water bath) under argon was added a triethylborane solution in tetrahydrofuran (4.8 mL, 4.8 mmol) in one single portion. The mixture was stirred under argon for a total of 1 h. The ice–water cooling bath was replaced with an acetone–dry ice bath and to the reaction mixture was added $NaBH_4$ (0.20 g, 5.3 mmol) in one portion. The reaction suspension was stirred at −78 °C for 2 h, forming a clear, homogeneous, pale yellow solution. The crude reaction mixture was diluted with 40 mL of 1 N HCl followed by extractions with EtOAc (40 mL × 2). The organic layers were combined, dried over $MgSO_4$ and concentrated under reduced pressure to give the product as a thick syrup, it was further diluted with 300 mL of methanol and the solution was allowed to stand at room temperature for 16 h before evaporation under reduced pressure. The crude product was purified by flash silica gel column chromatography using 2 L of 30% EtOAc in hexanes as the eluting solvent. The appropriate fractions were collected and evaporated to give 1.48 g (68%) of the title compound: MS (CI) m/e = 485 for $(M + H)^+$; IR (KBr) ν_{max} 3438 (s), 1734 (s), 1600 (s), 1513 (s), 1225 (s), 1163 (s), 844 (s), cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.4–7.3 (4 H, m), 7.04 (2 H, t, J = 8.9 Hz), 6.9–6.7 (2 H, m), 6.52 (1 H, dd, J = 1, 15.2 Hz), 5.16 (1 H, dd, J = 5.6, 15.7 Hz), 4.89 (1 H, d, J = 4.8 Hz), 4.72 (1 H, d, J = 5.5 Hz), 4.13 (1 H, m), 4.04 (2 H, q, J = 7.2 Hz), 3.85 (1 H, m), 3.75 (3 H, s), 2.42 (1 H, dd, J = 4.6, 15 Hz), 2.28 (1 H, dd, J = 8.3, 15 Hz), 5.5 (1 H, m), 4.2 (1 H, m), 1.17 (3 H, t, J = 7.2 Hz); ^{13}C NMR (DMSO-d_6) δ 171.02, 163.51, 163.05, 153.03, 145.34, 139.46, 136.34, 132.2 (d, ^3J = 8.3 Hz), 131.0 (d, ^{13}J = 9.1 Hz), 125.14, 121.64, 115.41 (d, ^2J = 20.4 Hz), 115.13 (d, ^2J = 21.1 Hz), 67.79, 64.76, 59.50, 44.10, 42.34, 33.44, 14.01. Anal. Calcd for $C_{25}H_{26}F_2N_4O_4$: C, 61.98; H, 5.41; N, 11.56. Found: C, 61.51; H, 5.67; N, 11.12.

Sodium (±)-*erythro*-9,9-Bis(4-fluorophenyl)-3,5-dihydroxy-8-(1-methyl-1*H*-tetrazol-5-yl)-6,8-nonadienoate [(±)-3a]. To a solution of ethyl 9,9-bis(4-fluorophenyl)-3,5-dihydroxy-8-(1-methyl-1*H*-tetrazol-5-yl)-6,8-nonadienoate (1.231 g, 2.54 mmol) in 35 mL of tetrahydrofuran at 0 °C was added a 1 N NaOH solution (2.54 mL, 1.0 equiv) dropwise. The rate of addition should be slow enough to prevent the reaction mixture from changing color into deep amber or reddish. The reaction mixture was stirred for 30 min at 0 °C, forming a clear, homogeneous solution. The reaction mixture was allowed to warm to ambient temperature and saponification was allowed to proceed for an additional hour. Analytical TLC eluted with 20% MeOH in $CHCl_3$ (v/v) showed the desired product at R_f = 0.2. Most of the organic solvent was evaporated at approximately 10 °C under reduced pressure (20 mmHg). The resulting thick syrup was diluted with 4 mL of water and then the solution was lyophilized at 0.01 mmHg to give 1.126 g (100%) of the title compound as a sodium salt which appears to contain about 1 mol of water: mp > 100 °C dec; IR (KBr) ν_{max} 3400 (v br), 1600 (s), 1575 (s), 1513 (s), 1438 (s), 1404 (s), 1225 (s), 1156 (s), 838 (s) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.3–7.4 (4 H, m), 7.06 (1 H, br, D_2O exchangeable), 7.00–7.06 (2 H, m), 6.87–6.91 (2 H, m), 6.49 (1 H, d, J = 15.7 Hz), 5.13 (1 H, dd, J = 5.4, 15.7 Hz), 5.05 (1 H, br, D_2O exchangeable), 4.14 (1 H, m), 3.74 (3 H, s), 3.62 (1 H, m), 1.99 (1 H, dd, J = 3.7, 13.5 Hz), 1.80 (1 H, dd, J = 8.5, 13.5 Hz), 1.43 (1 H, m), 1.30 (1 H, m); ^{13}C NMR (DMSO-d_6) δ 175.87, 161.85 (d, ^1J = 246.1 Hz), 161.37 (d, ^1J = 246.9 Hz), 153.08, 144.97, 139.88, 136.40, 135.51, 132.22 (d, ^3J = 8.3 Hz), 130.97 (d, ^3J = 8.3 Hz), 124.66, 121.74, 115.42 (d, ^2J = 21.9 Hz), 115.12 (d, ^2J = 23.4 Hz), 68.23, 65.71, 44.50, 43.55, 33.45. Anal. Calcd for $C_{23}H_{21}F_2N_4O_4Na\ H_2O$: C, 55.64; H, 4.67; N, 11.28. Found: C, 55.24; H, 4.65; N, 10.85.

***trans*-6-[4,4-Bis(4-fluorophenyl)-3-(1-methyl-1*H*-tetrazol-5-yl)-1,3-butadienyl]tetrahydro-4-hydroxy-2*H*-pyran-2-one [(±)-4].** **Step A. (±)-*erythro*-9,9-Bis(4-fluorophenyl)-3,5-dihydroxy-8-(1-methyl-1*H*-tetrazol-5-yl)-6,8-nonadienoic Acid.** To a solution of ethyl (±)-*erythro*-9,9-bis(4-fluorophenyl)-3,5-dihydroxy-8-(1-methyl-1*H*-tetrazol-5-yl)-6,8-nonadienoate (0.64 g, 1.32 mmoles) in 25 mL of tetrahydrofuran at 0 °C was treated with 1.32 mL of a 1.0 M NaOH solution. The pale yellow suspension was stirred at 0 °C for 2 h, forming a clear, pale yellow solution. The crude reaction mixture was diluted with 5 mL of aqueous HCl (2 N) solution and organic material was extracted into ethyl acetate (40 mL × 2). The organic extracts were combined, dried over $MgSO_4$, and concentrated under reduced pressure to give a pale yellow gum. The crude dihydroxy acid was rigorously dried under high vacuum (0.01 mmHg at room temperature for 24 h) before being submitted to the next step.

Step B. *trans*-6-[4,4-Bis(4-fluorophenyl)-3-(1-methyl-1*H*-tetrazol-5-yl)-1,3-butadienyl]tetrahydro-4-hydroxy-2*H*-pyran-2-one. The dry acid from the above step A was dissolved in 100 mL of dry methylene chloride under argon at room temperature, followed by the addition of 1.7 g (4.0 mmol) of 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate. Lactonization was complete in less than 15 min, as indicated by analytical TLC (R_f = 0.12) eluted three times with 50% ethyl acetate in hexanes. Most of the solvent was evaporated under reduced pressure and the residue was washed with water (40 mL), followed by extractions with ethyl acetate (40 mL × 2). The organic layers were combined, dried over $MgSO_4$, and concentrated under reduced pressure to give 0.54 g (89.7%) of the product. A pure sample of the product was obtained by being passed through a short bed of silica gel eluted with 40% ethyl acetate in hexanes (v/v) to give the title compound which appears to contain about 2 mol of water: MS (CI) m/e = 438 for (M +

H)$^+$; IR (KBr) ν_{max} 3425 (br), 1738 (vs), 1600 (s), 1513 (s), 1225 (vs), 1156 (s), 1038 (s), 838 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.26–7.21 (2 H, m), 7.14 (2 H, d, J = 8.7 Hz), 6.86 (4 H, d, J = 6.8 Hz), 6.72 (1 H, dd, J = 0.8, 15.6 Hz), 5.34 (1 H, dd, J = 7.1, 15.6 Hz), 5.18 (1 H, m), 4.37 (1 H, m), 3.57 (3 H, s), 2.68 (1 H, dd, J = 4.5, 18 Hz), 2.60 (1 H, ddd, J = 3.63, 2.5, 18 Hz), 2.44 (1 H, d, J = 2.6 H_2, D_2O exchangeable), 2.00 (1 H, dt, J = 18, 1.7 Hz), 1.79 (1 H, td, J = 2.7, 18 Hz); ^{13}C NMR ($CDCl_3$) δ 169.20, 163, 162.5, 153.20, 148.81, 135.61, 134.95, 132.45 (d, ^3J = 8 Hz), 132.52, 131.51 (d, ^3J = 8 Hz), 130.04, 120.44, 115.95 (d, ^2J = 21.9 Hz), 115.83 (d, ^2J = 21.9 Hz), 75.67, 62.54, 38.58, 35.58, 33.64. Anal. Calcd for $C_{23}H_{20}F_2N_4O_3{\cdot}2H_2O$: C, 58.22; H, 5.10; N, 11.81. Found: C, 59.06; H, 4.45; N, 11.25.

A sample of the above lactone was crystallized from cyclohexane–benzene to give the title compound as a crystalline solid containing about 1 mole of benzene: mp = 105–106 °C. Anal. Calcd for $C_{23}H_{20}F_2N_4O_3\ C_6H_6$: C, 67.48; H, 5.07; N, 10.85. Found: C, 67.44; H, 5.23; N, 10.59.

3,3-Bis(4-fluorophenyl)-2-(2-methyl-2*H*-tetrazol-5-yl)-2-propenal (20b). The general procedures described for the preparation of compounds **19a** and **20a** were repeated, except that the ethyl 3,3-bis(4-fluorophenyl)-2-(1-methyl-1*H*-tetrazol-5-yl)-2-propenoate utilized was replaced by ethyl 3,3-bis(4-fluorophenyl)-2-(2-methyl-2*H*-tetrazol-5-yl)-2-propenoate to yield the title compound as a gummy solid in 76% overall yield (in two steps): MS (CI) m/e = 326 for (M + H)$^+$; IR (KBr) ν_{max} 2863 (m), 2750 (w), 1681 (s), 1600 (s), 1503 (s), 1225 (s), 1156 (s), 838 (s), 752 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.65, 7.34–7.30 (2 H, m), 7.15 (2 H, t, J = 8.5 Hz), 7.01–6.96 (2 H, m), 6.88 (2 H, t, J = 8.4 Hz), 4.29 (3 H, s); ^{13}C NMR ($CDCl_3$) δ 190.08, 164.30 (d, ^1J = 254.4 Hz), 163.5 (d, ^1J = 252.17 Hz), 163.20, 161.37, 135.55, 133.49, 133.66 (d, ^3J = 7.6 Hz), 132.38 (d, ^3J = 9.1 Hz), 131.40, 127.54, 115.86 (d, ^2J = 26.4 Hz), 115.57 (d, ^2J = 28.7 Hz), 39.55. Anal. Calcd for $C_{17}H_{12}F_2N_4O$: C, 62.58; H, 3.71; N, 17.17. Found: C, 62.27; H, 4.22; N, 15.83.

5,5-Bis(4-fluorophenyl)-4-(2-methyl-2*H*-tetrazol-5-yl)-2,4-pentadienal (21b). The general procedure described for the preparation of **21a** was repeated, except that the 3,3-bis(4-fluorophenyl)-2-(1-methyl-1*H*-tetrazol-5-yl)-2-propenal utilized therein was replaced by 0.67 g (21.0 mmol) of 3,3-bis(4-fluorophenyl)-2-(2-methyl-2*H*-tetrazol-5-yl)-2-propenal. The reaction was carried out with 0.64 g (21.0 mmol) of (triphenylphosphoranylidene)acetaldehyde to yield 0.66 g (90.5%) of the title compound: ^{1}H NMR ($CDCl_3$) δ 9.57 (1 H, d, J = 6.8 Hz), 7.50 (1 H, d, J = 16.5 Hz), 7.3–6.8 (8 H, m), 5.94 (1 H, dd, J = 6.8, 16.5 Hz), 4.30 (3 H, s).

Ethyl (±)-*erythro*-9,9-Bis(4-fluorophenyl)-3,5-dihydroxy-8-(2-methyl-2*H*-tetrazol-5-yl)-6,8-nonadienoate (23b). Step A. Ethyl 9,9-Bis(4-fluorophenyl)-5-hydroxy-8-(2-methyl-2*H*-tetrazol-5-yl)-3-oxo-6,8-nonadienoate (22b). The general procedure described for the preparation of **22a** was repeated, except that the 5,5-bis(4-fluorophenyl)-4-(1-methyl-1*H*-tetrazol-5-yl)-2,4-pentadienal utilized therein was replaced by 0.66 g (1.87 mmol) of 5,5-bis(4-fluorophenyl)-4-(2-methyl-2*H*-tetrazol-5-yl)-2,4-pentadienal and there was thereby produced 0.53 g (59%) of the title compound after silica gel chromatography.

Step B. Ethyl (±)-*erythro*-9,9-Bis(4-fluorophenyl)-3,5-dihydroxy-8-(2-methyl-2*H*-tetrazol-5-yl)-6,8-nonadienoate (23b). The product from the above step A was treated with triethylborane and sodium borohydride following the general procedure described in the preparation of **23a** to give 0.37 g (69.5%) of the title compound after purification by silica gel chromatography: ^{1}H NMR ($CDCl_3$) δ 7.30–7.22 (2 H, m), 7.07 (2 H, t, J = 6.7 Hz), 6.89–6.86 (2 H, m), 6.78 (2 H, t, J = 8.7 Hz), 6.66 (1 H, d, J = 15.5 Hz), 5.39 (1 H, dd, J = 6.3, 15.5 Hz), 4.41 (1 H, m), 4.2 (1 H, m), 4.27 (3 H, s), 4.18 (2 H, q, J = 7.1 Hz), 3.92 (1 H, br, D_2O exchangeable), 3.69 (1 H, br, D_2O exchangeable), 2.47–2.42 (2 H, m), 1.66–1.58 (2 H, m), 1.26 (3 H, t, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$) δ 172.29, 162.52 (d, ^1J = 249.9 Hz), 161.94 (d, ^1J = 248.4 Hz), 145.74, 137.59, 137.33, 136.87, 132.37 (d, ^3J = 8.3 Hz), 131.69 (d, ^3J = 8.3 Hz), 128.53, 124.90, 115.50 (d, ^2J = 21.1 Hz), 115.2 (d, ^2J = 20 Hz), 72.11, 68.07, 60.74, 42.52, 41.73, 39.42, 14.17.

(±)-*trans*-6-[4,4-Bis(4-fluorophenyl)-3-(2-methyl-2*H*-tetrazol-5-yl)-1,3-butadienyl]tetrahydro-4-hydroxy-2*H*-pyran-2-one (4b). The general procedure described for the preparation of **4a** in steps A and B were repeated, except that the ethyl (±)-*erythro*-9,9-bis(4-fluorophenyl)-3,5-dihydroxy-8-(1-methyl-1*H*-tetrazol-5-yl)-6,8-nonadienoate utilized therein was replaced by 370 mg of ethyl 9,9-bis(4-fluorophenyl)-3,5-dihydroxy-8-(2-methyl-2*H*-tetrazol-5-yl)-6,8-nonadienoate and there was thereby produced 146 mg (44%) of the title compound after silica gel chromatography: MS (CI) m/e = 439 for (M + H)$^+$; IR (KBr) ν_{max} 3438 (v br), 1731 (s), 1600 (s), 1503 (vs), 1219 (vs), 1153 (s), 1056 (m), 1031 (m), 838 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.29–6.82 (8 H, m), 6.69 (1 H, d, J = 15.6 Hz), 5.44 (1 H, dd, J = 9.0, 15.6 Hz), 5.24 (1 H, m), 4.27 (3 H, s), 4.30 (1 H, m), 4.21 (1 H, s, D_2O exchangeable), 3.69 (1 H, br s, D_2O exchangeable), 2.6–2.4 (2 H, m), 2.1–1.7 (2 H, m); ^{13}C NMR ($CDCl_3$) δ 169.94, 162.70 (d, ^1J = 249.2 Hz), 162.12 (d, ^1J = 249.9 Hz), 147.68, 147.47, 137.27, 136.11, 132.36 (d, ^3J = 8.3 Hz), 131.71 (d, ^3J = 8.3 Hz), 131.17, 131.10, 130.88, 128.62, 124.28), 115.52 (d, ^2J = 20.4 Hz), 114.95 (d, ^2J = 21.9 Hz), 76.16, 62.33, 39.49, 38.66, 35.99. Anal. Calcd for $C_{23}H_{20}F_2N_4O_3{\cdot}2H_2O$: C, 58.22; H, 5.10; N, 11.81. Found: C, 58.92; H, 4.62; N, 11.21.

Sodium (±)-*erythro*-9,9-Bis(4-fluorophenyl)-3,5-dihydroxy-8-(2-methyl-2*H*-tetrazol-5-yl)-6,8-nonadienoate (3b). The general procedure described for the preparation of **3a** was repeated, except that the ethyl (±)-*erythro*-9,9-bis(4-fluorophenyl)-3,5-dihydroxy-8-(1-methyl-1*H*-tetrazol-5-yl)-6,8-nonadienoate utilized therein was replaced with ethyl 9,9-bis(4-fluorophenyl)-3,5-dihydroxy-8-(2-methyl-1*H*-tetrazol-5-yl)-6,8-nonadienoate and there was thereby produced after lyophilization a quantitative yield of the title compound as a sodium salt, which appeared to contain about 1 mol of water: IR (KBr) ν_{max} 3413 (v br), 1600 (s), 1575 (s), 1500 (s), 1400 (s), 1219 (s), 1088 (s) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.36–6.82 (8 H, m), 6.50 (1 H, d, J = 15.5 Hz), 5.28 (1 H, dd, J = 5.8, 15.5 Hz), 5.0 (1 H, br, D_2O exchangeable), 4.9 (1 H, br D_2O exchangeable), 4.28 (3 H, s), 4.13 (1 H, d, J = 5.94 Hz), 3.64 (1 H, m), 2.03 (1 H, dd, J = 3.6, 14.9 Hz), 1.85 (1 H, dd, J = 8.7, 14.9 Hz), 1.5–1.2 (2 H, m); ^{13}C NMR (DMSO-d_6) δ 176.25, 103.18, 161.47 (d, ^1J = 240 Hz), 143.15, 137.60, 136.40, 125.48, 115.12, 114.46, 68.52, 65.84, 44.61, 43.55. Anal. Calcd for $C_{23}H_{21}F_2N_4O_4Na{\cdot}H_2O$: C, 55.64; H, 4.67; N, 11.29. Found: C, 55.22; H, 4.79; N, 11.21.

Ethyl 1-Methyl-5-tetrazolylacetate (24). To a solution of 1,5-dimethyltetrazole (10 g) in 100 mL of dry tetrahydrofuran and 20 mL of hexamethylphosphoramide at −78 °C (dry ice–acetone) under an argon atmosphere was added dropwise 50 mL (1.2 equiv) of *n*-butyllithium (2.5 M in hexane). The deprotonation of 1,5-dimethyltetrazole was allowed to proceed at −78 °C for 40 min and then at −20 °C for 30 min. The anion solution was rechilled to −78 °C and transferred via a cannula over a period of 45 min into a cold (−78 °C) solution containing 12 mL of ethyl chloroformate in 50 mL of tetrahydrofuran. The reaction mixture was diluted with aqueous 2 N HCl and a saturated aqueous solution of sodium chloride and then extracted with ethyl acetate. The residue from the organic extract was purified by silica gel flash chromatography. The appropriate fractions were combined and evaporated to give 4 g of product. The product was further purified by crystallization from ethyl acetate–hexanes to yield 3.52 g (21%) of the title compound: mp = 64–66 °C. Anal. Calcd for $C_6H_{10}N_4O_2$: C, 42.35; H, 5.92; N, 32.92. Found: C, 42.40; H, 5.98; N, 33.15.

18a (Confirmation of Regiochemical Assignment), Ethyl 3,3-Bis(4-fluorophenyl)-2-(1-methyl-1*H*-tetrazol-5-yl)-2-propenoate. A mixture of titanium tetrachloride (2 mL) and carbon tetrachloride (2 mL) was added to 15 mL of tetrahydrofuran at −78 °C under an argon atmosphere. The suspension was stirred at −78 °C for 30 min before 0.2 g of 4,4′-difluorobenzophenone was added. After stirring for an additional 30 min, a solution of 0.15 g of ethyl 1-methyl-5-tetrazolylacetate in 1 mL of dry pyridine was added dropwise. The dark brownish suspension was stirred at −78 °C for 15 min and then was allowed to warm to 0 °C, forming a thick paste. The mixture was allowed to stand for 24 h at ambient temperature before it was poured into water. The aqueous mixture was extracted with ethyl acetate to yield crude product. Analytical TLC eluted five times with 20% (v/v) ethyl acetate in hexanes showed the desired product at R_f = 0.3. Purification by preparative chromatography on two 20 × 20 cm^2 0.25-mm TLC plates eluted twice with 20% (v/v) ethyl acetate in hexanes gave the title compound, which was

identical with compound **18a** prepared earlier.

Compound 20a, An Alternative Synthesis: 3,3-Bis(4-fluorophenyl)-2-(1-methyl-1*H*-tetrazol-5-yl)-2-propenal. Step A. 5-Ethyl-1-methyl-1*H*-tetrazole (26). To a slurry of 1,5-dimethyltetrazole (4.9 g, 0.05 mole) in dry tetrahydrofuran (50 mL) was added 2.5 M *n*-butyllithium in hexanes (20 mL, 0.05 mol) over a period of 15 min at −78 °C under an inert atmosphere. This mixture was stirred for 30 min and a yellowish precipitate formed during this time. Methyl iodide (3.7 mL, 0.06 mole) was then added over a period of 15 min. After stirring for an additional 30 min, the clear reaction mixture was diluted with water and extracted with ethyl acetate (3 × 50 mL). The aqueous layer was washed with chloroform (2 × 25 mL), and the combined organic layer was dried over sodium sulfate and concentrated under reduced pressure to give an oil. The oil was purified by distillation to give 5.2 g (92%) of the title compound: bp = 89–90 °C at 0.05 mmHg: ^{1}H NMR ($CDCl_3$) δ 4.05 (s, 3 H), 2.86 (q, 2 H), 1.41 (t, 3 H); ^{13}C NMR ($CDCl_3$) δ 156.0, 33.24, 16.75, 11.20.

Step B. 1,1-Bis(4-fluorophenyl)-2-(1-methyl-1*H*-tetrazol-5-yl)propanol (27). To a solution of 5-ethyl-1-methyl-1*H*-tetrazole (5.6 g, 0.05 mol) (prepared in step A) in 60 mL of dry tetrahydrofuran was added 2.5 M *n*-butyllithium (20 mL, 0.05 mol) in hexane over 5 min at −78 °C (bath temperature) under an inert atmosphere. The mixture was stirred for 30 min and a solution of 4,4′-difluorobenzophenone (10.8 g, 0.5 mol) in 25 mL of dry tetrahydrofuran was added over 5 min. This mixture was stirred for an additional 2 h while the bath temperature was slowly warmed to −20 °C. The reaction was quenched with 1 N HCl and extracted with ethyl acetate (3 × 50 mL) and chloroform (3 × 50 mL). The combined organic layer was dried over sodium sulfate and concentrated under reduced pressure to give a white solid. The solid was purified by crystallization from ethanol–hexane to give 10.8 g (65%) of the title compound: mp = 160–161 °C; IR (KBr) ν_{max} 3400 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.8–7.02 (m, 8 H), 5.95 (s, 1 H), 4.65 (q, 1 H), 1.98 (s, 3 H), 1.29 (d, 2 H); ^{13}C NMR ($CDCl_3$) δ 162.57, 162.37, 159.14, 156.71, 142.48, 140.54, 128.25, 128.13, 127.52, 127.42, 114.67, 114.41, 114.38, 78.56, 36.99, 33.43, 14.52. Anal. Calcd for $C_{17}H_{16}F_2N_4O$: C, 61.81; H, 4.88; N, 16.96. Found: C, 61.79; H, 4.90; N, 17.09.

Step C. 1,1-Bis(4-fluorophenyl)-2-(1-methyl-1*H*-tetrazol-5-yl)-1-propene (28). A slurry of 1,1-bis(4-fluorophenyl)-2-(1-methyl-1*H*-tetrazol-5-yl)propanol (8.25 g, 0.025 mol) (prepared in step B) and 100 mg of *p*-toluenesulfonic acid monohydrate in xylene (60 mL) was heated to reflux with a Dean–Stark water collecting apparatus for a period of 12 h. The reaction mixture was washed with 1 N NaOH (10 mL) while it was warm and with water (100 mL). Concentration of the organic layer gave off-white crystals of product. This was purified by recrystallization from ethanol–hexane to give 7.1 g (91%) of the title compound as white crystals: mp = 146–147 °C; IR (KBr) ν_{max} 1575; 1500 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.42–6.85 (m, 8 H), 3.53 (s, 3 H), 2.14 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 163.37, 163.08, 160.13, 155.61, 144.60, 145.34, 136.47, 136.42, 136.24, 136.19, 131.65, 131.54, 131.11, 131.01, 119.53, 115.51, 115.27, 115.22, 33.50, 21.20. Anal. Calcd for $C_{17}H_{14}F_2N_4$: C, 65.37; H, 4.51; N, 17.94. Found: C, 65.64; H, 4.61; N, 18.09.

Step D. 3,3-Bis(4-fluorophenyl)-1-bromo-2-(1-methyl-1*H*-tetrazol-5-yl)-2-propene (29). A slurry of 1,1-bis(4-fluorophenyl)-2-(1-methyl-1*H*-tetrazol-5-yl)-1-propene (61.46 g, 0.197 mol; prepared in step C), *N*-bromosuccinamide (35.06 g, 0.197 mol) and catalytic amount of azobisisobutyronitrile or benzoyl peroxide in carbon tetrachloride (1.2 L) was heated to reflux in an inert atmosphere for a period of 2 h. The reaction mixture was cooled to ambient temperature and the solid from the reaction was filtered. The filtrate was concentrated under reduced pressure and the solid obtained was recrystallized from toluene–hexane to give 72 g (93%) of the title compound as white crystals: mp = 159–160 °C; IR (KBr) ν_{max} 1600 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.5–7.1 (m, 8 H), 4.44 (s, 2 H), 3.53 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 163.94, 163.74, 160.60, 160.45, 143.42, 149.68, 135.20, 135.15, 134.69, 131.43, 131.31, 130.90, 130.80, 119.57, 115.94, 115.77, 115.65, 115.50. Anal. Calcd for $C_{17}H_{13}F_2BrN_4$: C, 52.19; H, 3.34; N, 14.32. Found: C, 52.58; H, 3.47; N, 14.49.

Step E. 3,3-Bis(4-fluorophenyl)-2-(1-methyl-1*H*-tetrazol-5-yl)-2-propenal (20a). To a solution of sodium ethoxide (3.93 g of sodium metal, 0.17 mol) in 500 mL of absolute ethanol was added 2-nitropropane (16.66 g, 0.187 mol) slowly over 5 min. The bromo compound prepared in the above step D (67.1 g, 0.17 mole) was added portionwise over a period of 10 min. The reaction mixture was stirred for 2 h and the ethanol was removed in vacuo. The residue was dissolved in CH_2Cl_2 (500 mL), washed with water (250 mL), and dried over sodium sulfate. The organic layer was concentrated under reduced pressure to give an oil. The oil was dissolved in hot toluene (350 mL) and trituration with hexane (350 mL), gave 50.6 g (91%) of the title compound as white crystals: mp = 135–137 °C.

[1,1-Bis(4-fluorophenyl)-2-(1-methyl-1*H*-tetrazol-5-yl)-1-propen-3-yl]triphenylphosphonium Bromide (30b). A slurry of 3,3-bis(4-fluorophenyl)-1-bromo-2-(1-methyl-1*H*-tetrazol-5-yl)-2-propene (**29**, 1.95 g, 0.005 mol) and triphenylphosphine (1.3 g, 0.005 mol) in cyclohexane (25 mL) was heated to reflux. The reaction mixture became a clear solution after 30 min and a white precipitate appeared after 1 h. The mixture was heated for an additional 8 h and cooled to ambient temperature and the solid was collected by filtration and washed with diethyl ether. This white powder was dried in vacuum at 50 °C to give 3.0 g (92%) of the title compound: mp = 254–255 °C; IR (KBr) ν_{max} 3450, 1600, 1500, 1425 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.92–6.80 (m, 23 H), 4.94 (6 d, 2 H), 3.83 (s, 3 H); ^{13}C NMR (DMSO-d_6) δ 163.53, 163.36, 160.28, 160.87, 154.04, 153.89, 152.76, 135.11, 134.79, 134.16, 133.68, 133.54, 130.53, 130.45, 130.35, 130.21, 130.07, 118.02, 116.89, 116.18, 115.89, 115.62, 115.32, 111.43, 111.39, 34.22, 28.88, 28.22. Anal. Calcd for $C_{35}H_{28}BrF_2N_4P$: C, 64.31; H, 4.32; N, 8.57. Found: C, 64.02; H, 4.37; N, 8.89.

Dimethyl [3,3-Bis(4-fluorophenyl)-2-(1-methyl-1*H*-tetrazol-5-yl)-2-propen-1-yl]phosphonate (30a). A slurry of 3,3-bis(4-fluorophenyl)-1-bromo-2-(1-methyl-1*H*-tetrazol-5-yl)-2-propane (1.17 g, 3.0 mmol) and trimethyl phosphite (0.41 g, 3.3 mol) was heated at 100 °C for 5 min. After cooling to ambient temperature, excess trimethyl phosphite was removed in vacuo to give a light yellow solid. This solid was recrystallized from an ethyl acetate–hexane mixture to give the title compound as a pure white solid: mp = 140–141 °C; IR (KBr) ν_{max} 1604, 1511 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.7–6.8 (8 H, m), 3.6 (3 H, s), 3.5 (3 H, s), 3.42 (3 H, s), 3.2 (2 H, d). Anal. Calcd for $C_{19}H_{19}F_2O_3N_4P$: C, 54.29; H, 4.56; N, 13.33. Found: C, 53.83; H, 4.48; N, 13.50.

***cis*-2,2-Dimethyl-6-(2-phenylethenyl)-1,3-dioxane-4-acetic Acid Methyl Ester (32a).** Methyl *cis*-3,5-dihydroxy-7-phenyl-hep-6-enoate (98% diastereomeric purity; 2.37 g, 9.48 mmol) was stirred with 2,2-dimethoxypropane (20 mL) and a catalytic amount of *p*-toluenesulfonic acid for 16 h. The solution was partitioned between diethyl ether and dilute aqueous sodium bicarbonate solution. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to afford a yellow solid. After recrystallization from isopropyl ether, 1.70 g (62%) of the title compound was obtained as a white solid: mp = 84–86.5 °C.

Alternatively, 0.2 g of solid sodium carbonate can be added to the 2,2-dimethoxypropane solution and the solution should be stirred vigorously. The solid is filtered through a fluted filter paper. The excess 2,2-dimethoxypropane is removed under reduced pressure to afford a yellow solid, which is recrystallized from isopropyl ether: ^{1}H NMR ($CDCl_3$) δ 7.37–7.19 (5 H, m), 6.59 (1 H, d, J = 15.9 Hz), 6.14 (1 H, dd, J = 15.9, 6.4 Hz), 4.57–4.35 (1 H, m), 4.42–4.35 (1 H, m), 3.68 (3 H, s), 2.58 (1 H, d, J = 15.6, 6.9 Hz), 2.14 (1 H, dd, J = 15.6, 6.3 Hz), 1.74–1.61 (1 H, m), 1.52 (3 H, s), 1.43 (3 H, s), 1.45–1.35 (1 H, m). Anal. Calcd for $C_{17}H_{22}O_4$: C, 70.32; H, 7.63. Found: C, 70.24; H, 7.69.

Similarly the *tert*-butyl ester was prepared from the corresponding *tert*-butyl *syn*-3,5-dihydroxy-7-phenyl-hep-6-enoate; upon ozonolysis (for a similar procedure, refer to compound **33b**), *tert*-butyl *cis*-2,2-dimethyl-6-formyl-1,3-dioxane-4-acetate was produced (mp = 71–73 °C): ^{1}H NMR ($CDCl_3$) δ 9.56 (1 H, s), 4.35–4.25 (2 H, m), 2.43 (1 H, dd, J = 8, 16 Hz), 2.32 (1 H, dd, J = 6, 16 Hz), 1.80 (1 H, dt, J = 2.5, 14 Hz), 1.46 (3 H, s), 1.42 (12 H, s), 1.25–1.35 (1 H, m).

***cis*-2,2-Dimethyl-6-(2-phenylethenyl)-1,3-dioxane-4-acetic Acid (32, R = H).** A solution of 2,2-dimethyl-6-(2-phenylethenyl)-1,3-dioxane-4-acetic acid methyl ester (8.5 g, 29.3 mmol) in 1 N NaOH (32 mL) and methanol (64 mL) was heated to reflux for 45 min. After evaporation under reduced pressure, the aqueous solution was washed once with diethyl ether and acidified with 1 N HCl (33 mL). The precipitate was collected and recrystallized from ethyl acetate–isopropyl ether to afford 7.2 g (90%) of the title compound as a colorless solid: mp = 153–155 °C; ^{1}H NMR ($CDCl_3$) δ 7.37–7.20 (5 H, m), 6.60 (1 H, d, J = 16.0 Hz), 6.14 (1

H, dd, J = 16.0, 6.4 Hz), 4.59–4.54 (1 H, m), 4.43–4.35 (1 H, m), 2.62 (1 H, dd, J = 16.0, 7.2 Hz), 2.51 (1 H, dd, J = 16.0, 5.3 Hz), 1.77–1.72 (1 H, m), 1.54 (3 H, s), 1.46 (3 H, s), 1.50–1.36 (1 H, m). Anal. Calcd for $C_{16}H_{20}O_4$: C, 69.54; H, 7.30. Found: C, 69.20; H, 7.33.

Methyl (±)-*erythro*-9,9-Bis(4-fluorophenyl)-3,5-dihydroxy-8-(1-methyl-1*H*-tetrazol-5-yl)-6,8-nonadienoate (23a, R = CH_3). To a slurry of the phosphonium bromide **30b** (0.326 g, 0.5 mmol) (prepared in the previous example) and methyl *erythro*-3,5-bis[(diphenyl-*tert*-butylsilyl)oxy]-6-oxohexanoate [prepared according to the general procedures described by Kapa et al. (*Tetrahedron Lett.* **1984**, 2435–2438 and U.S. Patent No. 4,571,428, issued February 18, 1986, to P. K. Kapa)] (0.26 g, 0.4 mmol) in dry dimethylformamide (1 mL) was added potassium *tert*-butoxide (0.067 g, 0.6 mmol) at −20 °C (bath temperature) in an inert atmosphere. The slurry became a red solution and was stirred for 18 h at −10 °C. The reaction was worked up by adding ammonium chloride solution (10 mL) and extracting with methylene chloride (2 × 30 mL). The organic layer was dried over sodium sulfate and concentrate to give an oil. The oil was purified through a pad of silica gel and the major fraction was isolated as an oil (160 mg). The oil (160 mg) was stirred with a 1 M tetra-*n*-butylammonium fluoride solution in tetrahydrofuran (2 mL) and a few drops of glacial acetic acid for a period of 18 h. The reaction mixture was poured into water (10 mL) and extracted with ethyl acetate (3 × 20 mL). The organic layer was dried over sodium sulfate and concentrated to given an oil. The oil was purified by silica gel flash column chromatography eluting with ethyl acetate–hexane (2:1) to give 0.08 g (75%) of the title compound as an oil: MS (CI) m/e = 471 for $(M + H)^+$; ^1H NMR ($CDCl_3$) δ 7.26–6.6 (m, 9 H), 5.37 (dd, 1 H), 4.44 (m, 1 H), 4.24 (m, 1 H), 3.71 (s, 3 H), 3.56 (s, 3 H), 2.47 (d, 2 H), 1.58 (m, 2 H).

A more polar fraction was also isolated (≃20 mg) and identified as the corresponding *trans*-lactone.

Resolution of *cis*-2,2-Dimethyl-6-(2-phenylethenyl)-1,3-dioxane-4-acetic Acid [(+)-34]. The racemic *cis*-2,2-dimethyl-6-(2-phenylethenyl)-1,3-dioxane-4-acetic acid (**32**, R = H, 0.31 g, 1.1 mmol) was dissolved in a boiling mixture of hexane–ethanol containing (1*S*,2*R*)-ephedrine (0.2 g, 1.1 mmol). The resulting solution was very slowly brought to room temperature to give 0.21 g (41.4%) of colorless chiral salt (the usage of diastereomerically pure seed crystal is recommended during the resolution): mp = 170–171 °C.

The chiral acid was freed through an acidic workup and its enantiomeric purity was determined to be 100% by ^1H NMR using L-phenyl(trifluoromethyl)carbinol as a chiral solvent: $[\alpha]^{25}_D$ = +5.45° (c = 1, $CHCl_3$).

***cis*-(4*R*,6*S*)-2,2-Dimethyl-6-formyl-1,3-dioxane-4-acetic Acid (33b).** The resolved salt of *cis*-2,2-dimethyl-6-(2-phenylethenyl)-1,3-dioxane-4-acetic acid and (1*S*,2*R*)-ephedrine (6.6 g, 14.9 mmol) was partitioned between 0.5 N HCl (30 mL) and diethyl ether. The ether layer was washed with brine, dried ($MgSO_4/Na_2SO_4$), and concentrated under reduced pressure to afford 4.1 g (99.6%) of the free acid. This acid was dissolved in dry methylene chloride (100 mL), and ozone was passed through this solution at −78 °C until there was deep blue coloration. Excess ozone was removed by purging with nitrogen and the ozonide formed was decomposed by adding CH_3SCH_3 (5 mL) and warming the solution to room temperature, and it was allowed to stand for 16 h. The solution was concentrated under reduced pressure and the residue was dissolved in isoamyl ether (ca. 100 mL). The benzaldehyde which was formed during the ozonolysis was azeotroped together with isoamyl ether under reduced pressure to afford the title compound: ^1H NMR ($CDCl_3$) δ 9.57 (1 H, s), 4.40–4.30 (2 H, m), 2.60 (1 H, dd, J = 16.0, 7.0 Hz), 2.49 (1 H, dd, J = 16.0, 6.0 Hz), 1.88–1.83 (1 H, m), 1.49 (3 H, s), 1.46 (3 H, s), 1.42–1.31 (1 H, m).

***cis*-(4*R*,6*S*)-6-[4,4-Bis(4-fluorophenyl)-3-(1-methyl-1*H*-tetrazol-5-yl)-1,3-butadienyl]-2,2-dimethyl-1,3-dioxane-4-acetic Acid (35, R = H).** Crude chiral acid **33b** was dissolved in dry THF (50 mL) and the resulting solution was transferred to a 250-mL three-neck flask purged with nitrogen and equipped with a mechanical stirrer. After the solution was stirred vigorously and cooled to −78 °C, *n*-BuLi (2.5 M in hexane, 5.96 mL) was added dropwise. Toward the end of the addition, the solution turned into a white suspension.

A separate flask containing dimethyl [3,3-bis(4-fluorophenyl)-2-(1-methyl-1*H*-tetrazol-5-yl)-2-propen-1-yl]phosphonate (**30a**, 6.2 g, 14.7 mmol) in THF (50 mL) under a nitrogen atmosphere was cooled to −78 °C and *n*-BuLi (2.5 M in hexane, 5.96 mL) was added slowly. The resulting red-brown solution was stirred for 15 min at −78 °C. This solution of phosphonate anion was transferred through a double-ended needle to the above vigorously stirred suspension at −78 °C containing the lithium salt of the chiral acid. After the addition, the resulting brown solution was stirred for 30 min at −78 °C and 16 h at ambient temperature. The THF solution was partitioned between 0.5 N HCl and ethyl acetate. The organic phase was washed with brine (2×), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was chromatographed on silica gel (66:33:1 diethyl ether–hexane–acetic acid) to afford 3.80 g (51.6% overall yield from the initial ephedrine salt; toluene was employed to azeotrope the residual acetic acid) of the title compound as a yellow foam: $[\alpha]^{25}_D$ = +106.1° (c = 2.23, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.24–6.82 (8 H, m), 6.62 (1 H, d, J = 15.0 Hz), 5.32 (1 H, dd, J = 15.0, 5.7 Hz), 4.42–4.37 (1 H, m), 4.30–4.23 (1 H, m), 3.51 (3 H, s), 2.53 (1 H, dd, J = 15.9, 7.0 Hz), 2.42 (1 H, dd, J = 15.9, 5.6 Hz), 1.62–1.57 (1 H, m), 1.46 (3 H, s), 1.33 (3 H, s), 1.30–1.20 (1 H, m).

***trans*-(4*R*,6*S*)-6-[4,4-Bis(4-fluorophenyl)-3-(1-methyl-1*H*-tetrazol-5-yl)-1,3-butadienyl]tetrahydro-4-hydroxy-2*H*-pyran-2-one [(+)-4a].** *cis*-(4*R*,6*S*)-6-[4,4-Bis(4-fluorophenyl)-3-(1-methyl-1*H*-tetrazol-5-yl)-1,3-butadienyl]-2,2-dimethyl-1,3-dioxane-4-acetic acid (3.7 g, 7.45 mmol) was dissolved in a solution of THF (90 mL) and 0.2 N HCl (60 mL) and allowed to stand for 16 h. The solution was partitioned between ethyl acetate and water. The organic layer was washed with brine (2×), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was dissolved in dry methylene chloride (60 mL) and stirred for 4 h in the presence of 1-cyclohexyl-3-(2-morpholinomethyl)-carbodiimide metho-*p*-toluenesulfonate (6.6 g, 15.6 mmol). The solution was concentrated under reduced pressure and the residue was partitioned between ethyl acetate and water. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (1:1 ethyl acetate–diethyl ether). After recrystallization from ethyl acetate–hexane, 1.33 g (40.1%) of the title compound was obtained as a white solid: mp = 172–173 °C; $[\alpha]^{25}_D$ = +239.8° (c = 2.17, $CHCl_3$).

***(4*R*,6*S*)-trans*-6-(2-Phenylethyl)-4-hydroxyl-2*H*-pyran-2-one (38).** A mixture of 0.35 g of (+)-(1*S*,2*R*)-ephedrine salt (the isomer led to the active final product) and 70 mg of 5% palladium on charcoal catalyst in 25 mL of reagent-grade ethanol was subjected to low-pressure hydrogenation at ambient temperature. The progress of reaction was monitored by TLC eluting with 5% methanol in chloroform (v/v, once). TLC showed complete consumption of the starting material after 20 min, meanwhile a new, weakly UV-active spot was formed, this new spot moved slightly faster than the starting material.

The reaction suspension was filtered through a bed ($^1/_2$ in.) of silica gel (Merck, type H) and eluted with a MeOH–$CHCl_3$ (1:9, v/v) mixture to give 350 mg of the crude product after evaporation. The crude product (the saturated (1*S*,2*R*)-ephedrine salt) was redissolved into 50 mL of Et_2O followed by washing twice with (10 mL of each) 2 N HCl. The organic phase was separated, combined, and concentrated. The crude acid was redissolved into 200 mL of absolute methanol containing 25 mg of camphorsulfonic acid. The hydrolysis was allowed to proceed at room temperature for 5 min and the solution was concentrated under reduced pressure. The gummy free acid was redissolved into 50 mL of benzene and the slightly turbid solution was boiled momentary (2 min). Analytical TLC showed only one mobil spot. Most of the solvent was removed by evaporation and the desired lactone product separated out as crude crystals (120 mg, 69%). Analytically pure material was obtained by recrystallizing the crude product once from mixture of ethyl acetate and hexanes: mp = 101.0–101.7 °C; $[\alpha]^{25}_D$ = +24° (c = 0.015 M, $CHCl_3$).

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