HMG-CoA Reductase Inhibitors: Design, Synthesis, and Biological Activity of Tetrahydroindazole-Substituted 3,5-Dihydroxy-6-heptenoic Acid Sodium Salts

Peter J. Connolly,* Claudia D. Westin, Deborah A. Loughney, and Lisa K. Minor

The R. W. Johnson Pharmaceutical Research Institute, 1000 Route 202, Raritan, New Jersey 08869

Received June 2, 1993•

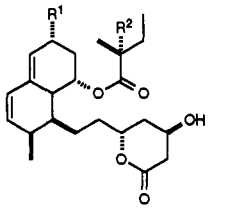
Compounds comprising a series of 7-[2-(4-fluorophenyl)-4,5,6,7-tetrahydro-2*H*-indazol-3-yl]-3,5-dihydroxy-6-heptenoic acid sodium salts (18) were synthesized and tested for their ability to inhibit HMG-CoA reductase in a partially purified enzyme preparation and cholesterol biosynthesis from acetate in cultured HEP-G2 cells. Changing the size of the saturated ring of the tetrahydroindazole nucleus did not improve potency, but incorporation of substituents at the 7-position resulted in up to 1700-fold improvement in inhibitory potency. Structure–activity studies revealed that the most potent compounds possess a substituted benzyl group at the 7-position, with a preference for steric bulk at the para position of the benzene ring. The most potent enzyme inhibitor (18t, IC_{50} = 3.0 nM) is approximately 3-fold more potent than lovastatin sodium salt (2). The most potent cholesterol biosynthesis inhibitor in HEP-G2 cells (18q, IC_{50} = 0.078 μM) is slightly less potent than 2 (sodium salt). Molecular modeling studies suggested that, when compared to the parent compound (18b) lacking the appropriate 7-substituent, 18t overlaps better with 2 and literature inhibitors 5 and 6 in a hydrophobic binding region adjacent to the enzyme active site.

Introduction

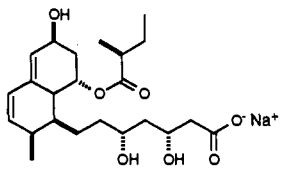
Management of hypercholesterolemia is an important therapeutic goal in the treatment of atherosclerosis, the underlying cause of coronary heart disease and thrombosis, diseases which are responsible for half of all deaths in the United States.[1] The search for new methods to control high serum cholesterol levels led to the discovery of the fungal metabolite compactin (1), the first known inhibitor of HMG-CoA reductase, the enzyme controlling the rate-limiting step in cholesterol biosynthesis.[2] In the clinic, HMG-CoA reductase inhibitors have proven to be effective agents for the treatment of hypercholesterolemia, significantly lowering both total and low density lipoprotein (LDL) cholesterol.[3] To date, the fungal metabolites lovastatin[4] (2) and pravastatin[5] (3), as well as the semisynthetic compound simvastatin[6] (4), have been approved in the United States for use in humans.

During the past 7 years, many structurally novel synthetic HMG-CoA reductase inhibitors have been disclosed in the medicinal chemistry literature.[7] Compared to 2, 3, and 4, most of the synthetic compounds possess simplified structures in order to facilitate analog synthesis and allow detailed structure–activity analyses to be performed. Previous studies[7] demonstrated that potent inhibitors share similar structural requirements: a *syn*-3,5-dihydroxypentanoic acid or 2,3,4,5-tetrahydro-4-hydroxy-2*H*-pyranone group connected to a central ring by a linking element (usually *trans*-CH=CH or CH_2CH_2), flanked on one side by an aromatic ring (commonly 4-fluorophenyl) and on the other side by a bulky alkyl substituent. A wide range of carbocyclic and heterocyclic systems have served as the central ring, acting as the framework to which the essential structural features of the inhibitor are attached.

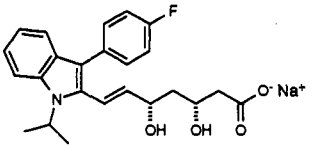
Our approach was centered around the 4,5,6,7-tetrahydro-2*H*-indazole ring system (and the 2,4,5,6,7,8-hexahydrocycloheptapyrazole and 2,4,5,6-tetrahydrocyclopentapyrazole analogs), which we viewed as a convenient nucleus for an investigation of structure–activity relationships. We chose to limit our study to compounds in which the dihydroxypentanoic acid side chain was linked through a *trans*-ethylene group to the 3-position of the tetrahydroindazole (and analogs). In addition, the aromatic ring attached to the 2-position was limited to 4-fluorophenyl. Molecular modeling studies of several literature compounds, including 2, an indole-based inhibitor (fluvastatin, 5),[7e] and a biphenyl-containing inhibitor (6),[7f] led us to hypothesize that variations at the 7-position of tetrahydroindazole-based compounds would have a significant effect on inhibitory potency. Our rationale depended on overlap of the 7-substituents in our proposed inhibitors with the "benzo" ring of 5, which occupied a region of space not utilized by other synthetic inhibitors and to which we attributed the increased potency of 5.



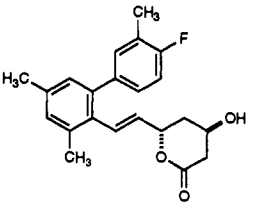
1 R^1 = H R^2 = H
2 R^1 = CH_3 R^2 = H
4 R^1 = CH_3 R^2 = CH_3



3



5



6

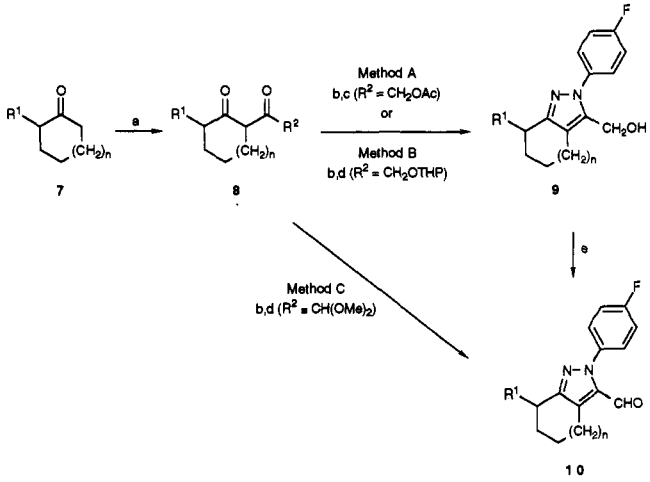
Chemistry

Synthesis of the inhibitors began with the appropriately substituted cyclic ketone 7 which, if not commercially available, was prepared according to one of several

• Abstract published in *Advance ACS Abstracts*, October 1, 1993.

0022-2623/93/1836-3674$04.00/0

Scheme I. Methods A, B, and C[a]



[a] (a) For $R^2 = CH_2OAc$: $AcOCH_2COCl$, morpholine, benzene. For $R^2 = CH_2OTHP$: NaH, cat. EtOH, $THPOCH_2CO_2Et$. For $R^2 = CH(OMe)_2$: $LiN(SiMe_3)_2$ or $LiN(i\text{-}Pr)_2$, 0.5 equiv of $(MeO)_2CHCO_2Me$; (b) $4\text{-}FC_6H_4NHNH_2\cdot HCl$, Et_3N or NaOAc, MeOH or EtOH; (c) aqueous NaOH, MeOH; (d) aqueous HCl, THF; (e) PCC, CH_2Cl_2.

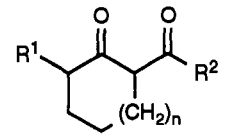
literature procedures (Scheme I).[8] Ketone 7 was converted to diketone 8 by one of three methods, depending upon the nature of R^1 and R^2: (1) acylation of the morpholine enamine of 7 with acetoxyacetyl chloride[9] (R^1 = H, R^2 = CH_2OAc); (2) reaction of 7 and ethyl (tetrahydropyranyloxy)acetate[10] in ether with NaH and a catalytic amount of EtOH[11] (R^1 = H, $R^2 = CH_2OTHP$); (3) acylation of the lithium enolate of 7 with 0.5 equiv of methyl dimethoxyacetate ($R^1 \neq H$, $R^2 = CH(OMe)_2$). Diketone 8 was further elaborated into aldehyde 10 by one of three routes. For $R^2 = CH_2OAc$ or CH_2OTHP, 8 was reacted with 4-fluorophenylhydrazine to form the tetrahydroindazole, followed by deprotection with aqueous base (method A) or acid (method B), respectively, to give 2-(4-fluorophenyl)-substituted alcohol 9 along with the corresponding 1-(4-fluorophenyl) isomer. After separation by crystallization or medium-pressure liquid chromatography (MPLC), 9 was oxidized with PCC[12] to give the tetrahydroindazole-3-carboxaldehyde 10. For $R^2 = CH(OMe)_2$, reaction of 8 with 4-fluorophenylhydrazine followed by hydrolysis of the intermediate mixture of dimethyl acetals afforded aldehyde 10 in a more straightforward fashion, after MPLC separation of the accompanying 1-(4-fluorophenyl) isomer (method C).[13]

For R^3 = H, allylic alcohol 12 was prepared by Wadsworth/Emmons homologation of aldehyde 10 with diethyl phosphonoacetate to give unsaturated ester 11, followed by $(i\text{-}Bu)_2AlH$ reduction (Scheme II, method D). Alternatively, when R^1 and R^3, taken together, represented a fused "benzo" substituent, 12 was synthesized by a different method (Scheme III, method E). Ketone 13 was treated with 4-fluorophenylhydrazine to form the mixture of hydrazones 14 which was treated with 2 equiv of LDA to form the hydrazone dianion.[14] The dianion was reacted with methyl 4-(tetrahydropyranyloxy)-2-butenoate[15] to form the pyrazole ring, and the THP protecting group was hydrolyzed to give the tricyclic unsaturated alcohol 12 (R^1, R^3 = benzo). For both methods D and E, oxidation of 12 with MnO_2 provided the unsaturated aldehyde 15. For certain examples of method D, the oxidation reaction was accomplished with CrO_3/pyridine.[16]

Elaboration of 15 into the target molecule was accomplished by condensation with the sodium/lithium dianion of ethyl acetoacetate[17] to give hydroxy keto ester 16 (Scheme IV, method F). With the exception of **16z** and **16aa**, each compound prepared by method F was obtained as an inseparable mixture of diastereomers. Stereospecific reduction of 16 was accomplished using Et_3B and $NaBH_4$ in 4:1 THF/MeOH[18] to give *syn*-dihydroxy ester 17, which was determined to be free of the corresponding *anti* isomer to the limit of detection by ^{13}C NMR spectroscopy (>10:1 *syn:anti*).[19] Finally, treatment of 17 with aqueous NaOH provided racemic sodium salt 18, which was isolated by lyophilization of the aqueous solution.

Alternatively, some targets were prepared from 10 by a shorter, more convergent route (Scheme V, method G). Keto phosphonate 20, a racemic version of Heathcock's optically pure side-chain synthon,[20] was prepared in 40%

Table I

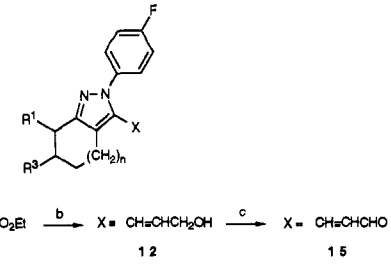


8

no.	R^1	R^2	n	% yield[a]	mp, °C[b,c]
8a	H	CH_2OCOCH_3	0	53	oil[d,e]
8b	H	CH_2OCOCH_3	1	43	97–98[f–h]
8c	H	CH_2OTHP	2	88[i]	oil[f]
8d	CH_3	$CH(OCH_3)_2$	1	85[i]	oil[d]
8e	C_2H_5	$CH(OCH_3)_2$	1	85	oil
8f	$n\text{-}C_3H_7$	$CH(OCH_3)_2$	1	53	oil
8g	$s\text{-}C_4H_9$	$CH(OCH_3)_2$	1	95	oil[j]
8h	$c\text{-}C_6H_{11}$	$CH(OCH_3)_2$	1	81	oil
8i	C_6H_5	$CH(OCH_3)_2$	1	70	oil[j]
8j	$C_6H_5CH_2$	$CH(OCH_3)_2$	1	85	oil
8k	$2\text{-}ClC_6H_4CH_2$	$CH(OCH_3)_2$	1	79	oil[f]
8l	$4\text{-}ClC_6H_4CH_2$	$CH(OCH_3)_2$	1	53	oil
8m	$4\text{-}FC_6H_4CH_2$	$CH(OCH_3)_2$	1	81	oil
8n	$3\text{-}CH_3OC_6H_4CH_2$	$CH(OCH_3)_2$	1	73	oil
8o	$4\text{-}CH_3OC_6H_4CH_2$	$CH(OCH_3)_2$	1	72	oil
8p	$3,4\text{-}(CH_3O)_2C_6H_3CH_2$	$CH(OCH_3)_2$	1	69	oil
8q	$4\text{-}CH_3C_6H_4CH_2$	$CH(OCH_3)_2$	1	68	oil
8r	$4\text{-}i\text{-}C_3H_7C_6H_4CH_2$	$CH(OCH_3)_2$	1	66	oil
8s	$4\text{-}t\text{-}C_4H_9C_6H_4CH_2$	$CH(OCH_3)_2$	1	92	oil
8t	$4\text{-}C_6H_5C_6H_4CH_2$	$CH(OCH_3)_2$	1	96	wax
8u	1-naphthyl-CH_2	$CH(OCH_3)_2$	1	96	oil
8v	2-naphthyl-CH_2	$CH(OCH_3)_2$	1	71	oil
8w	$C_6H_5CH_2CH_2$	$CH(OCH_3)_2$	1	90	oil
8x	$C_6H_5CH_2CH_2CH_2$	$CH(OCH_3)_2$	1	64	oil
8y	$(E)\text{-}C_6H_5CH{=}CHCH_2$	$CH(OCH_3)_2$	1	61	oil

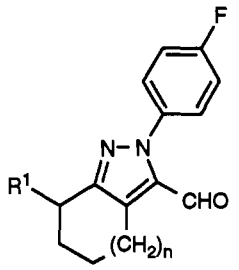
[a] Yield of isolated material after purification by silica gel chromatography or crystallization, unless otherwise noted. [b] All compounds possessed ^1H NMR, IR, and mass spectra consistent with assigned structure, unless otherwise noted. [c] Combustion analyses (C, H) within ±0.4% of theoretical, unless otherwise noted. [d] ^1H NMR spectrum consistent with assigned structure. [e] Reference 9a. [f] ^1H NMR and mass spectra consistent with assigned structure. [g] Recrystallized from EtOAc/hexanes. [h] Reference 9b. [i] Yield of crude product. [j] Combustion analysis consistent for 0.25 hydrate.

Scheme II. Method D[a]



[a] (a) $(EtO)_2P(=O)CH_2CO_2Et$, NaH; (b) $(i\text{-}Bu)_2AlH$, THF; (c) MnO_2 or CrO_3/pyridine.

Table II

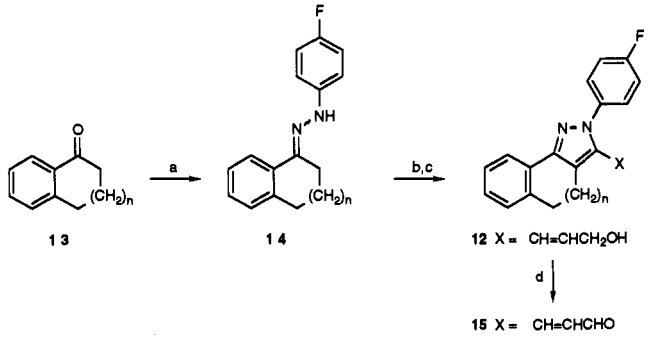


10

no.	R^1	n	% yield[a] (method)	mp, °C[b,c]	recrystallization solvent
10a	H	0	5 (A)	79–80	Et_2O
10b	H	1	68 (A)	80–81	Et_2O/hexanes
10c	H	2	30 (B)	oil[d]	
10d	CH_3	1	31 (C)	79–80	Et_2O/hexanes
10e	C_2H_5	1	38 (C)	72–74	
10f	n-C_3H_7	1	36 (C)	50–53	
10g	s-C_4H_9	1	20 (C)	oil[e]	
10h	c-C_6H_{11}	1	15 (C)	oil[e]	
10i	C_6H_5	1	19 (C)	139–140	EtOAc/hexanes
10j	$C_6H_5CH_2$	1	30 (C)	99–100	hexanes
10k	2-$ClC_6H_4CH_2$	1	23 (C)	glass	
10l	4-$ClC_6H_4CH_2$	1	47 (C)	126–128	EtOAc/hexanes
10m	4-$FC_6H_4CH_2$	1	41 (C)	oil[d]	
10n	3-$CH_3OC_6H_4CH_2$	1	37 (C)	foam[e]	
10o	4-$CH_3OC_6H_4CH_2$	1	36 (C)	oil	
10p	3,4-$(CH_3O)_2C_6H_3$-CH_2	1	36 (C)	109–110	Et_2O
10q	4-$CH_3C_6H_4CH_2$	1	40 (C)	foam[e]	
10r	4-i-$C_3H_7C_6H_4CH_2$	1	8 (C)	oil[e]	
10s	4-t-$C_4H_9C_6H_4CH_2$	1	23 (C)	124–125	EtOAc/hexanes
10t	4-$C_6H_5C_6H_4CH_2$	1	39 (C)	148–150[e]	EtOAc/Et_2O
10u	1-naphthyl-CH_2	1	28 (C)	116–117	EtOAc/hexanes
10v	2-naphthyl-CH_2	1	36 (C)	122–123	Et_2O
10w	$C_6H_5CH_2CH_2$	1	20 (C)	89–90	Et_2O/hexanes
10x	$C_6H_5CH_2CH_2CH_2$	1	32 (C)	100–102	EtOAc/hexanes
10y	(E)-C_6H_5CH=$CHCH_2$	1	44 (C)	104–105	

[a] Overall yield from 8 of isolated material after purification by silica gel chromatography or crystallization. [b] All compounds possessed ^1H NMR, IR, and mass spectra consistent with assigned structure, unless otherwise noted. [c] Combustion analyses (C, H, N) within ±0.4% of theoretical, unless otherwise noted. [d] ^1H NMR spectrum consistent with assigned structure. [e] ^1H NMR and mass spectra consistent with assigned structure.

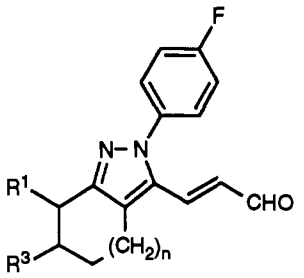
Scheme III. Method E[a]



[a] (a) 4-$FC_6H_4NHNH_2$·HCl, NaOAc, EtOH; (b) 2.2 equiv of LiN(i-Pr)$_2$, $THPOCH_2CH$=$CHCO_2Me$; (c) pyridinium p-toluenesulfonate, MeOH; (d) MnO_2.

yield from dimethyl 3-[(*tert*-butyldimethylsilyl)oxy]glutarate[21] and lithium dimethyl methylphosphonate. Reaction of **20** with **10** under Roush/Masamune conditions (LiCl, DBU, CH_3CN)[22] gave hydroxy keto ester **21** as an inseparable mixture of diastereomers. Stereospecific reduction as described above afforded *syn*-dihydroxy ester **22**, which in turn was saponified with NaOH and lyophilized to give racemic **18**. It will be noted that, in

Table III

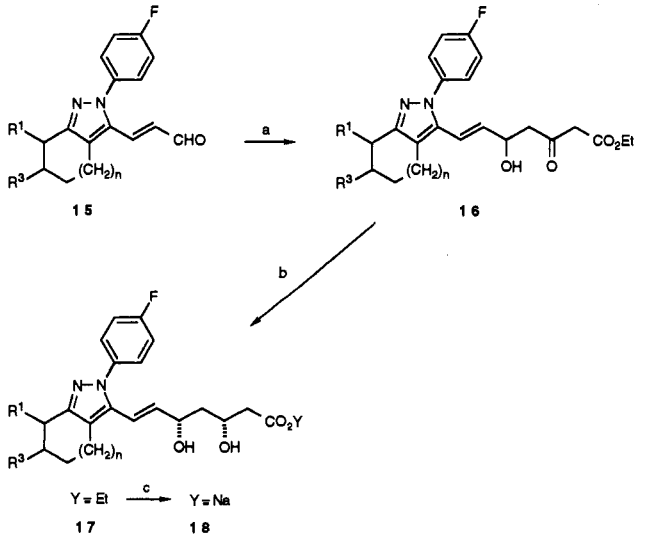


15

no.	R^1	R^3	n	% yield[a] (method)	mp, °C[b,c]	recrystallization solvent
15a	H	H	0	52 (D)	127–128	Et_2O
15b	H	H	1	57 (D)	122–123	EtOAc/hexanes
15c	H	H	2	72 (D)	92–93	EtOAc/hexanes
15d	CH_3	H	1	68 (D)	145–146	EtOAc/hexanes
15e	C_2H_5	H	1	53 (D)	99–101	
15f	n-C_3H_7	H	1	41 (D)	92–93	hexanes
15g	s-C_4H_9	H	1	55 (D)	oil	
15h	c-C_6H_{11}	H	1	19 (D)	oil[d]	
15j	$C_6H_5CH_2$	H	1	21 (D)	130–131	
15k	2-$ClC_6H_4CH_2$	H	1	53 (D)	184–185	EtOAc/hexanes
15l	4-$ClC_6H_4CH_2$	H	1	32 (D)	144–145	EtOAc/hexanes
15m	4-$FC_6H_4CH_2$	H	1	10 (D)	oil[e]	
15n	3-$CH_3OC_6H_4$-CH_2	H	1	23 (D)	97–99	
15o	4-$CH_3OC_6H_4CH_2$	H	1	54 (D)	141–142	EtOAc/hexanes
15p	3,4-$(CH_3O)_2C_6H_3$-CH_2	H	1	37 (D)	foam	
15q	4-$CH_3C_6H_4CH_2$	H	1	34 (D)	160–162	EtOAc/hexanes
15s	4-t-$C_4H_9C_6H_4$-CH_2	H	1	60 (D)	145–148	EtOAc/hexanes
15w	$C_6H_5CH_2CH_2$	H	1	48 (D)	132–134	EtOAc/hexanes
15x	$C_6H_5CH_2CH_2$-CH_2	H	1	25 (D)	oil[d]	
15z	6,7-benzo		1	33 (E)	154–156	EtOAc/hexanes
15aa	7,8-benzo		2	28 (E)	208–210	EtOAc

[a] Overall yield from 10 (method D) or 13 (method E) of isolated material after purification by silica gel chromatography or crystallization. [b] All compounds possessed ^1H NMR, IR, and mass spectra consistent with assigned structure, unless otherwise noted. [c] Combustion analyses (C, H, N) within ±0.4% of theoretical, unless otherwise noted. [d] ^1H NMR and mass spectra consistent with assigned structure. [e] ^1H NMR spectrum consistent with assigned structure.

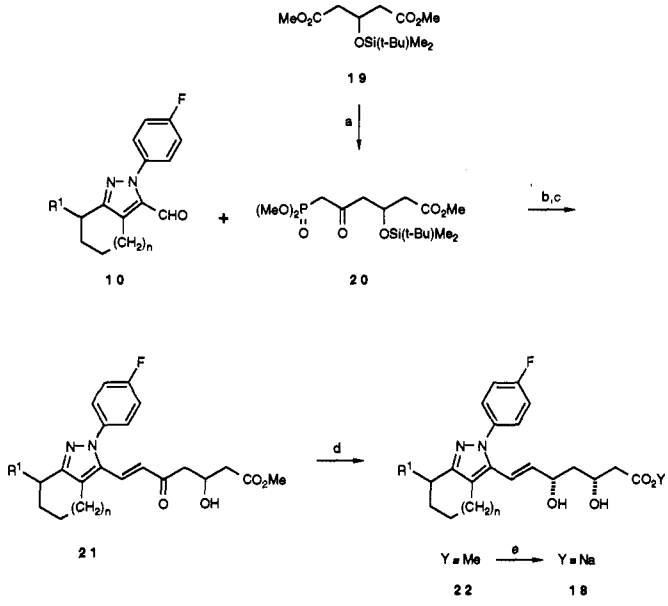
Scheme IV. Method F[a]



[a] (a) $LiCH_2C$(=O)CH(Na)CO_2Et; (b) Et_3B, THF/MeOH; then $NaBH_4$, -78 °C to room temperature; (c) aqueous NaOH, MeOH, lyophilize.

methods F and G, the *syn* relationship between the side-chain hydroxyl groups is controlled by stereospecific

Scheme V. Method G[a]



[a] (a) 2 equiv of $(MeO)_2P(=O)CH_2Li$, -78 °C; (b) LiCl, CH_3CN, DBU; (c) aqueous HF, CH_3CN; (d) Et_3B, THF/MeOH; then $NaBH_4$, -78 °C to room temperature; (e) aqueous NaOH, MeOH, lyophilize.

reduction, but the relative stereochemistry at the 7-position of the tetrahydroindazole ring system is not controlled. Therefore, the target inhibitors 18 are actually inseparable mixtures of two diastereomers, each of which is racemic.

Biological Results and Discussion

The racemic sodium salts 18 were evaluated for their ability to inhibit partially purified rat liver HMG-CoA reductase in vitro; selected compounds (18j–y) were evaluated for their ability to inhibit incorporation of [^{14}C]-acetate into cholesterol in cultured human hepatoma (HEP-G2) cells (Table IV). Results were compared to those obtained for 2, 5, and the sodium salt of 2 (prepared by reaction of 2 with NaOH).

For the unsubstituted series (18a–c, $R^1 = R^3 = H$), the tetrahydroindazole (n = 1) was a slightly more potent HMG-CoA reductase inhibitor than the tetrahydrocyclopentapyrazole (n = 0) and approximately equivalent to the hexahydrocycloheptapyrazole (n = 2). Addition of small straight or branched hydrocarbon chains to the 7-position of the tetrahydroindazole (18d–g) afforded an approximately 2-fold increase in potency over the parent compound. However, introduction of a large branching group on the α-carbon of the 7-substituent (18h) resulted in a loss in potency. Also, incorporation of phenyl at the 7-position (18i) or fusion of a benzene ring across the 6,7-bond (18z) gave a less potent compound. Interestingly, the corresponding benzohexahydrocycloheptapyrazole analog (18aa) was more potent than either 18z or its hexahydrocycloheptapyrazole parent (18c).

In contrast to the effect shown by a fused benzene ring, the addition of a linking group between the phenyl group and tetrahydroindazole nucleus provided compounds which were 10–77-fold more potent than 18b: $C_6H_5CH_2$-CH_2 (18w, 10× more potent) < $C_6H_5CH_2CH_2CH_2$ (18x) < (*E*)-$C_6H_5CH{=}CHCH_2$ (18y) < $C_6H_5CH_2$ (18j, 77× more potent). Of the saturated spacers, a single methylene unit is preferred over two or three, which implies an optimum spatial arrangement of the tetrahydroindazole portion of the molecule and the pendant phenyl ring. The improved activity of 18y (which contains the unsaturated three carbon spacer) can be explained by considering the double bond and the phenyl ring together as a single, extended π-system separated by one methylene unit from the rest of the molecule. In this analysis, the important structural features for improved potency are π-electrons connected by a one carbon spacer to the 7-position of the tetrahydroindazole.

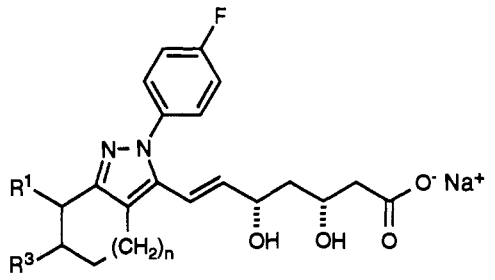
When electron-withdrawing substituents (18k–m) were incorporated into the phenyl ring of 18j, loss of potency was observed. In the case of electron-donating substituents, 3-methoxy (18n) and 4-methoxy (18o) gave a slight increase in potency, whereas 3,4-dimethoxy (18p) suffered a significant decrease. This pattern suggested a possible steric effect at the 4-position, which was verified by the inclusion of bulky substituents on the phenyl ring of 18j. The 4-methyl and 4-isopropyl compounds (18q–r) were approximately equal to 18o, but the 4-*tert*-butyl analog (18s) was twice as potent and the 4-phenyl analog (18t) was 10-fold more potent. In comparison to the standards, 18t was a better HMG-CoA reductase inhibitor than 2 (sodium salt) and equal to 5, making it the most potent analog of the series (IC_{50} = 3.0 nM). The 2-naphthyl analog (18v) was also extremely potent, consistent with the requirement for steric bulk at the 4-position of the phenyl ring. The 1-naphthyl analog (18u), while potent, was about 3-fold weaker than 18v.

Those compounds which were more potent than 1 μM in the HMG-CoA reductase assay (18j–y) were also evaluated in the HEP-G2 cell cholesterol biosynthesis assay.[23] As shown in Table IV, several of the tetrahydroindazole inhibitors inhibited cholesterol biosynthesis in the 0.1 μM range, the most potent being 18q and 18s. Structure–activity relationships in HEP-G2 cells, however, did not parallel those established for the HMG-CoA reductase assay. For example, some of the compounds that were potent enzyme inhibitors were relativly ineffective inhibitors of cholesterol biosynthesis (18o and 18y). Also, the methoxy-containing analogs (18n–p) were uniformly weak, suggesting degradation to inactive metabolites. Ultimately, although the best tetrahydroindazoles (18q and 18s) exhibited promising activity in whole cells, they were still inferior to 2 (sodium salt).

Molecular Modeling

Initially, 2, 5, 6, and 18b were studied to determine the structural features important for activity. Structures for inhibitors 5, 6, and 18b were built in their bioactive open-chain forms as neutral acids using SYBYL.[24] Although 18b was synthesized and tested as a racemic mixture, only the (3*R*,5*S*) enantiomer was modeled in order to maintain a consistent absolute configuration in the dihydroxy acid side chain. The structure for 2 was retrieved from the Cambridge Structural Database[25] and modified by substituting the dihydroxy acid side chain for the lactone ring. In our comparison, the dihydroxy acid side chains of the inhibitors were overlapped while maintaining an overlap of the ring systems (Figure 1a). Interestingly, when electrostatic isopotential maps were generated for the compounds in the study (MNDO charges), we found that the (2-methyl)butyryl ester side chain of 2 creates a negatively charged region (colored blue in Figure 2) in the same area occupied by the 4-fluorophenyl group of 5, 6, and 18b. This indicates that the conformation of the ester side chain of 2 selected for this study is reasonable. Examination of difference volume maps shows that 5, the most potent inhibitor of the four, occupies volume in a region of space not shared by the other compounds in the

Table IV. Inhibition of Rat Liver HMG-CoA Reductase and Cholesterol Biosynthesis Inhibition in Cultured HEP-G2 Cells by 18



18

no.	R^1	R^3	n	% yield[a] (method)	formula[b]	HMG-CoA reductase[c] IC_{50}, μM	cholesterol biosynthesis[c] IC_{50}, μM
18a	H	H	0	56 (F)	$C_{19}H_{20}FN_2NaO_4 \cdot H_2O$	11 (2.1–54)	
18b	H	H	1	45 (F)	$C_{20}H_{22}FN_2NaO_4 \cdot H_2O$	5.3 (3.1–9.1)	
18c	H	H	2	40 (F)	$C_{21}H_{24}FN_2NaO_4 \cdot 0.5H_2O$	3.6 (2.6–4.8)	
18d	CH_3	H	1	31 (F)	$C_{21}H_{24}FN_2NaO_4 \cdot H_2O$	2.1 (1.7–2.6)	
18e	C_2H_5	H	1	22 (F)	$C_{22}H_{26}FN_2NaO_4 \cdot H_2O$	2.0 (1.2–3.3)	
18f	n-C_3H_7	H	1	52 (F)[d]	$C_{23}H_{28}FN_2NaO_4 \cdot 1.5H_2O$	3.4 (2.1–5.6)	
18g	s-C_4H_9	H	1	6 (F)	$C_{24}H_{30}FN_2NaO_4 \cdot 2H_2O$	3.2 (1.9–5.3)	
18h	c-C_6H_{11}	H	1	15 (F)	$C_{26}H_{32}FN_2NaO_4 \cdot 2H_2O$	8.5 (3.8–19)	
18i	C_6H_5	H	1	33 (G)	$C_{28}H_{26}FN_2NaO_4 \cdot 1.25H_2O$	2.8 (0.73–10)	8.1 (0.73–91)
18j	$C_6H_5CH_2$	H	1	45 (F)	$C_{27}H_{26}FN_2NaO_4 \cdot H_2O$	0.068 (0.047–0.098)	0.17 (0.11–0.27)
18k	2-$ClC_6H_4CH_2$	H	1	16 (F)	$C_{27}H_{27}ClFN_2NaO_4 \cdot 2.5H_2O$	0.095 (0.026–0.34)	0.33 (0.16–0.68)
18l	4-$ClC_6H_4CH_2$	H	1	40 (F)[d]	$C_{27}H_{27}ClFN_2NaO_4 \cdot H_2O$	0.14 (0.07–0.27)	1.1 (0.56–2.2)
18m	4-$FC_6H_4CH_2$	H	1	20 (F)	$C_{27}H_{27}F_2N_2NaO_4$	0.20 (0.16–0.24)	0.40 (0.0071–23)
18n	3-$CH_3OC_6H_4CH_2$	H	1	31 (F)	$C_{28}H_{30}FN_2NaO_5 \cdot 0.5H_2O$	0.035 (0.027–0.046)	1.2 (0.44–3.0)
18o	4-$CH_3OC_6H_4CH_2$	H	1	37 (F)	$C_{28}H_{30}FN_2NaO_5 \cdot H_2O$	0.028 (0.015–0.51)	0.50 (0.15–1.7)
18p	3,4-$(CH_3O)_2C_6H_3CH_2$	H	1	27 (F)	$C_{28}H_{32}FN_2NaO_6 \cdot 2H_2O$	0.32 (0.17–0.61)	3.6 (2.9–4.6)
18q	4-$CH_3C_6H_4CH_2$	H	1	45 (F)	$C_{28}H_{30}FN_2NaO_4 \cdot H_2O$	0.028 (0.018–0.042)	0.078 (0.051–0.12)
18r	4-i-$C_3H_7C_6H_4CH_2$	H	1	38 (G)	$C_{30}H_{34}FN_2NaO_4 \cdot 1.25H_2O$	0.040 (0.0090–0.17)	0.23 (0.15–0.33)
18s	4-t-$C_4H_9C_6H_4CH_2$	H	1	36 (F)	$C_{31}H_{36}FN_2NaO_4 \cdot 0.75H_2O$	0.014 (0.0040–0.053)	0.088 (0.046–0.17)
18t	4-$C_6H_5C_6H_4CH_2$	H	1	25 (G)	$C_{33}H_{32}FN_2NaO_4 \cdot 2H_2O$	0.0030 (0.0015–0.0049)	0.10 (0.013–0.81)
18u	1-naphthyl-CH_2	H	1	33 (G)	$C_{31}H_{30}FN_2NaO_4 \cdot H_2O$	0.028 (0.021–0.036)	0.46 (0.26–0.80)
18v	2-naphthyl-CH_2	H	1	41 (G)	$C_{31}H_{30}FN_2NaO_4 \cdot H_2O$	0.0090 (0.0050–0.016)	0.33 (0.22–0.48)
18w	$C_6H_5CH_2CH_2$	H	1	6 (F)[e]	$C_{28}H_{30}FN_2NaO_4 \cdot 2.5H_2O$	0.54 (0.41–0.73)	1.7 (1.3–2.3)
18x	$C_6H_5CH_2CH_2CH_2$	H	1	10 (F)	$C_{29}H_{32}FN_2NaO_4 \cdot 2.75H_2O$	0.16 (0.12–0.21)	0.87 (0.31–2.4)
18y	(E)-$C_6H_5CH{=}CHCH_2$	H	1	48 (G)	$C_{29}H_{30}FN_2NaO_4 \cdot 2.5H_2O$	0.082 (0.038–0.18)	1.0 (0.70–1.4)
18z	6,7-benzo		1	28 (F)	$C_{24}H_{22}FN_2NaO_4 \cdot H_2O$	9.4 (4.1–22)	
18aa	7,8-benzo		2	32 (F)	$C_{25}H_{24}FN_2NaO_4 \cdot 1.25H_2O$	2.6 (1.8–3.7)	
2	lactone					0.40 (0.23–0.71)	0.079 (0.061–0.10)
2	sodium salt					0.0092 (0.0073–0.017)	0.050 (0.019–0.14)
5						0.0025 (0.0015–0.0040)	0.079 (0.053–0.12)

[a] Overall yield from 15 (method F) or 10 (method G) of isolated material after purification of intermediates 17 (method F) or 22 (method G) by silica gel chromatography and saponification/lyophilization. [b] All compounds possessed ^1H NMR, IR, and FAB mass spectra consistent with assigned structure and combustion analyses (C, H, N) within ±0.4% of theoretical for the hydrate shown. [c] For assay protocols, see the Experimental Section. The values shown represent the mean of several determinations; numbers in parentheses represent 95% confidence limits. [d] Compound isolated as a 2:1 mixture of *syn*:*anti* diols as determined by ^{13}C NMR spectroscopy. [e] Compound isolated as a 4.5:1 mixture of *syn*:*anti* diols as determined by ^{13}C NMR spectroscopy.

study (Figure 1b). Beyond this, 2, 5, and 6 all share volume which **18b** does not (Figure 1c). More specifically, comparison of **5** and **18b** reveals that, when the five-membered heterocyclic rings of the two molecules are superimposed, the fused phenyl ring of the indole moiety of **5** is in close proximity to the 7-position of the tetrahydroindazole ring system of **18b**. We hypothesized that 7-substituted analogs of **18b** would overlap better with **5** and the other inhibitors in the region revealed in Figure 1b,c. Therefore, the 7-[(1,1′-biphenyl-4-yl)methyl] substituent from the potent inhibitor **18t** was incorporated into the structure determined for **18b**. Since **18t** (and each of the other 7-substituted inhibitors) actually exists as a mixture of diastereomers at the 7-position relative to the fixed *syn* stereochemistry on the side chain, structures for each diastereomer (7*R* and 7*S*) were built. Volume difference maps were generated, revealing the improved overlap of both diastereomers with the volume shared by 2, 5, and 6 (Figure 1d). The improved potency of **18t** (1700-fold better than **18b**) and similar analogs can be attributed to improved overlap in the critical region occupied by the 7-substituent, which presumably interacts with a hydrophobic binding domain adjacent to the enzyme active site.[26] This model is supported by the observed trend that the 7-substituent should contain a π-system connected by a single methylene group for optimum HMG-CoA reductase inhibitory potency (vide supra).

Conclusions

Compounds comprising a novel series of 7-[2-(4-fluorophenyl)-4,5,6,7-tetrahydro-2*H*-indazol-3-yl]-3,5-dihydroxy-6-heptenoic acid sodium salts were synthesized and tested for their ability to inhibit HMG-CoA reductase in vitro and in cultured HEP-G2 cells. Incorporation of a benzyl substituent at the 7-position of the tetrahydroindazole nucleus resulted in an analog (**18j**) which was 77-fold more potent than the parent compound (**18b**) in vitro. Substitution at the para position of the benzene ring resulted in a compound (**18t**) which was 1700-fold more potent than **18b** and 3-fold more potent than the sodium salt of lovastatin (**2**). Other analogs (**18q** and **18s**) were essentially equipotent to **2** in HEP-G2 cells.

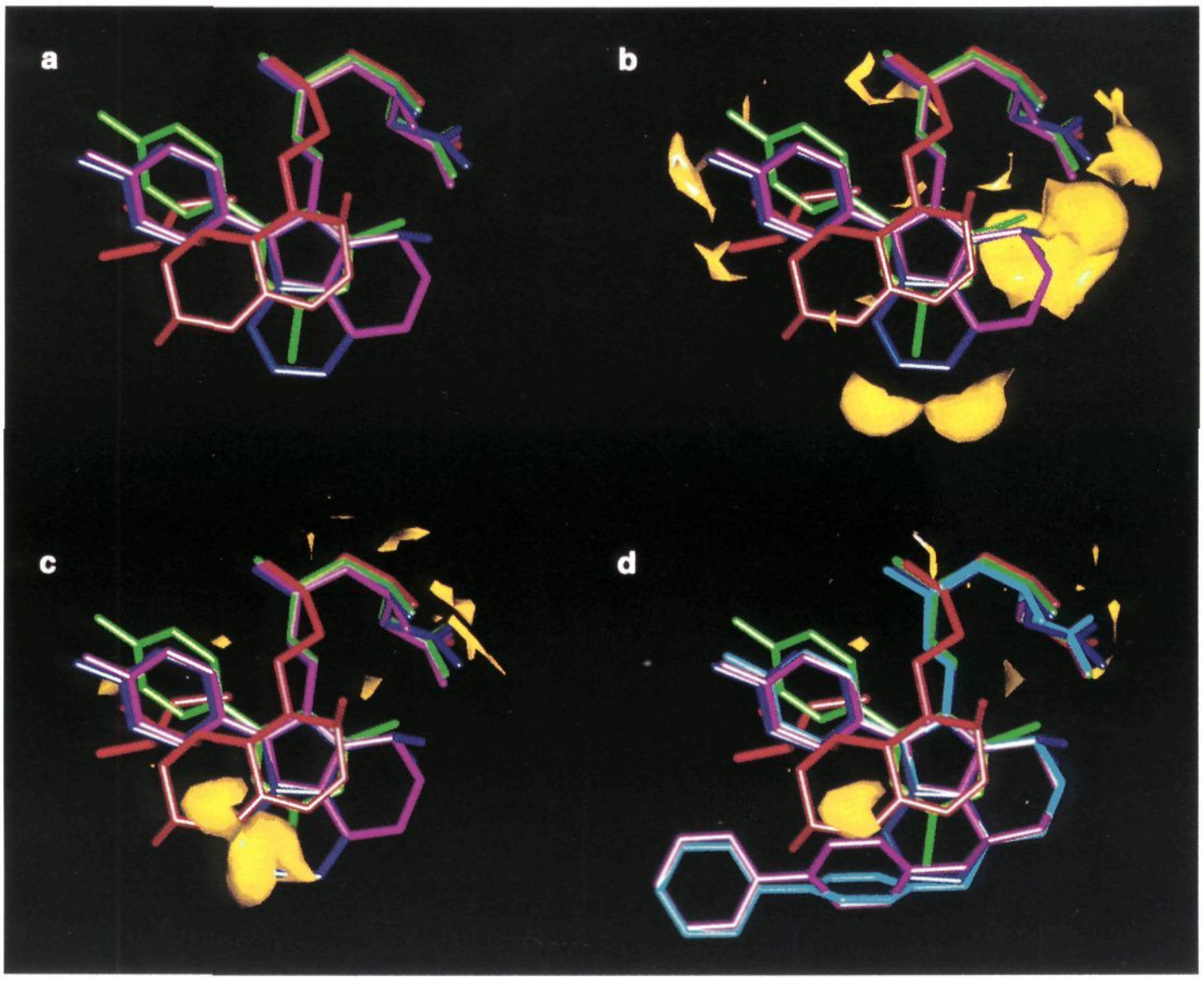


Figure 1. Comparison of tetrahydroindazole inhibitors **18b** and **18t** with literature inhibitors **2** (open chain form), **5**, and **6** (open chain form). (a) Superimposition of energy-minimized structures for **2** (red), **5** (blue), **6** (green), and **18b** (magenta). (b) Difference volume map illustrating regions occupied by **5** (blue) that are not shared by **2** (red), **6** (green), and **18b** (magenta). Volume unique to **5** is shown in yellow. (c) Difference volume map illustrating regions shared by **2** (red), **5** (blue), and **6** (green) that are not occupied by **18b** (magenta). Volume not occupied by **18b** is shown in yellow. (d) Difference volume map illustrating improved overlap of (7*S*)-**18t** (magenta) and (7*R*)-**18t** (cyan) with the regions shared by **2** (red), **5** (blue), and **6** (green). Volume not occupied by (7*S*)- or (7*R*)-**18t** is shown in yellow.

Experimental Section[27]

Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. Anhydrous tetrahydrofuran (THF) was purchased from Aldrich and used without further drying. Diisopropylamine was distilled from CaH_2 and was stored over 4A molecular sieves. 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) was used without purification. Dimethylformamide (DMF) was dried over 4A sieves prior to use. Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were measured in the indicated solvent with tetramethylsilane (TMS) as the internal standard using the following spectrometers: Bruker WP-100SY (100 MHz ^1H, 25 MHz ^{13}C), General Electric QE-300 (300 MHz ^1H, 75 MHz ^{13}C), and Varian XL-400 (400 MHz ^1H, 100 MHz ^{13}C). NMR chemical shifts are expressed in parts per million (ppm) downfield from internal TMS using the δ scale. ^1H NMR data are tabulated in order: multiplicity, (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), number of protons, coupling constant in hertz). ^{13}C NMR data are reported for proton-decoupled spectra and are tabulated in order. Infrared (IR) spectra were determined on a Nicolet 5DXB FT-IR spectrophotometer. Chemical ionization (DCI), electron impact (EI), and fast atom bombardment (FAB) mass spectra (MS) were determined on a Finnegan MAT 8230 spectrometer. Elemental analyses were carried out on a Perkin-Elmer 240C analyzer. Analytical thin-layer chromatography (TLC) was done with Merck silica gel 60 F_{254} plates (250 μm). Flash chromatography and medium-pressure liquid chromatography (MPLC) were done with Merck silica gel 60 (230–400 mesh).

Ketones 7. These compounds were prepared according to literature methods, if not commercially available.[8]

Diketones 8a,b. These compounds were prepared according to literature methods.[9]

2-[(2-Tetrahydropyranyloxy)acetyl]cycloheptanone (8c). A solution of 2.80 g (25 mmol) of cycloheptanone and 4.71 g (25 mmol) of ethyl (tetrahydropyranyloxy)acetate[10] in 20 mL of Et_2O was added over the course of 1 h to an ice-cold, stirring mixture of hexane-washed NaH (48 mmol, 1.15 g of 60% oil dispersion) and 0.12 mL (2 mmol, 0.092 g) of absolute EtOH in 10 mL of Et_2O under N_2. The light brown mixture was allowed to warm to room temperature and was stirred overnight. MeOH (5 mL) was added, and the solution was poured onto 200 mL of saturated aqueous NH_4Cl. After acidification to pH 2 with 1 N aqueous HCl, the mixture was extracted with Et_2O. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated to give 5.61 g (88%) of crude **8c** as a light brown oil, which was used without purification: ^1H NMR ($CDCl_3$, 100 MHz) 1.3–2.1 (complex, 14 H), 2.3–2.8 (complex, 2 H), 3.5–4.8 (complex, 5 H), 13.8 (br s, 1 H).

General Procedure for the Synthesis of Diketones 8d-y (Table I). 6-(4-*tert*-butylbenzyl)-2-(2,2-dimethoxyacetyl)-cyclohexanone (8s). Diisopropylamine (94.2 mmol, 9.53 g, 13.2 mL) was added under N_2 to a −20 °C solution of 1.6 M *n*-BuLi in hexanes (89.7 mmol, 56.0 mL) and 75 mL of THF. After 15 min, the solution was cooled to −78 °C and 20.9 g (85.4 mmol) of 2-(4-*tert*-butylbenzyl)cyclohexanone (**7s**) in 50 mL of THF was added dropwise over a 20-min period. After 45 min, 5.50 mL (44.8 mmol, 6.01 g) of methyl dimethoxyacetate was added; the mixture was allowed to warm slowly to room temperature and was stirred overnight. The resulting solution was cooled to 0 °C and acidified to pH 3–4 with 2 N aqueous HCl. The mixture was diluted with Et_2O (200 mL) and washed with water and brine. After drying over Na_2SO_4, the solution was concentrated to give 26 g of amber oil. The crude product was purified by MPLC using a solvent gradient ranging from 8 to 10% EtOAc/hexanes to afford 9.4 g of recovered **7s** and 14.3 g (92%) of **8s** as a colorless oil: ^1H NMR (300 MHz) 1.30 (s, 9 H), 1.2–2.8 (complex, 8 H),

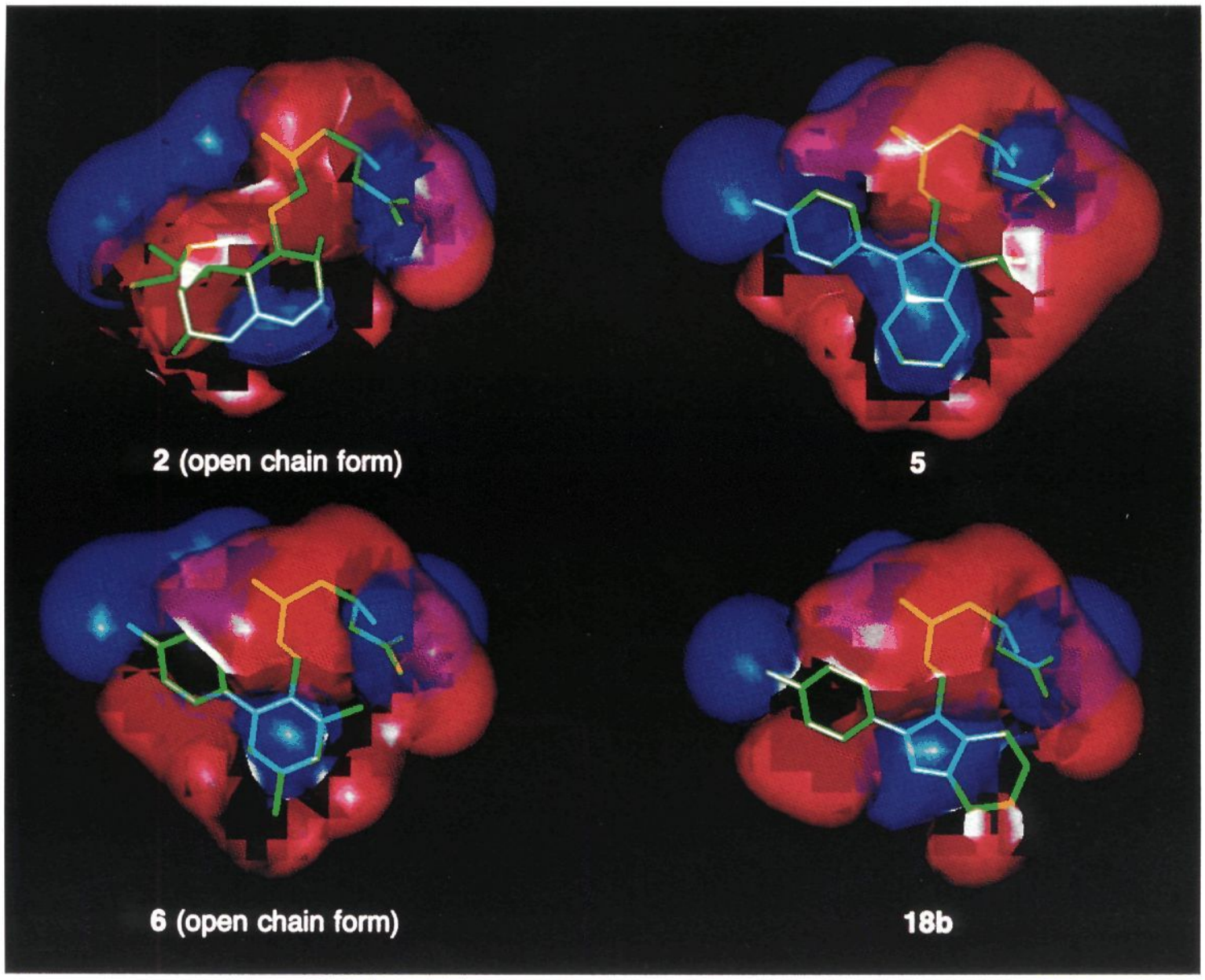


Figure 2. Electrostatic isopotential maps (MNDO charges) for **2** (open chain form), **5**, **6** (open chain form), and **18b**, showing negatively charged regions in blue and positively charged regions in red.

Table V

compd	no. of conformers	low-energy conformer, kcal/mol	"fit" energy, kcal/mol	energy difference
2	500	10.191	15.436	5.245
5	646	27.263	31.686	1.681
6	335	13.140	16.244	3.080
18b	569	19.834	23.366	3.532
(7*S*)-**18t**	*a*	18.806	22.573	3.767
(7*R*)-**18t**	*a*	18.964	22.592	3.628

[a] Structures for (7*S*)- and (7*R*)-**18t** were generated from the reference low-energy conformer of **18b** as described in the Experimental Section.

3.1–3.5 (complex, 8 H), 4.63 (s, 1 H minor tautomer), 4.96 (s, 1 H, major tautomer), 7.11 (m, 2 H), 7.29 (m, 2 H); IR (film) 1740, 1707, 1583 cm^{-1}; MS (DCI) *m/z* 347, 315 (base). Anal. ($C_{21}H_{30}O_4$) C, H.

6-[(1,1′-Biphenyl-4-yl)methyl]-2-(2,2-dimethoxyacetyl)cyclohexanone (8t). Diisopropylamine (38.8 mmol, 3.93 g, 5.4 mL) was added under N_2 to a −20 °C solution of 1.6 M *n*-BuLi in hexanes (35.3 mmol, 22.0 mL) and 30 mL of THF. After 15 min, the solution was cooled to −78 °C and 8.88 g (33.6 mmol) of 2-[(1,1′-biphenyl-4-yl)methyl]cyclohexanone (**7t**) in 50 mL of THF was added. After 45 min, 2.26 mL (18.5 mmol, 2.48 g) of methyl dimethoxyacetate was added; the mixture was allowed to warm slowly to room temperature and was stirred overnight. The resulting solution was cooled to 0 °C and acidified to pH 3–4 with 2 N aqueous HCl. The mixture was diluted with Et_2O (200 mL) and washed with water and brine. After drying over Na_2SO_4, the solution was concentrated to give 11.5 g of a yellow oil. The crude product was purified by MPLC using a solvent gradient ranging from 1:6 to 1:5 EtOAc/hexanes to afford 4.18 g of recovered **7t** and 5.94 g (96%) of **8t** as a waxy, white solid: ^1H NMR ($CDCl_3$, 300 MHz) 1.4–2.8 (complex, 9 H), 3.33 (s, 3 H, minor tautomer), 3.37 (s, 3 H, minor tautomer), 3.42 (s, 6 H, major tautomer), 4.63 (s, 1 H, minor tautomer), 4.96 (s, 1 H, major tautomer), 7.2–7.6 (complex, 9 H); IR (KBr) 1739, 1704, 1601, 1584, 1488, 1444 cm^{-1}; MS (DCI) *m/z* 335 (base), 303. Anal. ($C_{23}H_{26}O_4$) C, H.

General Procedure for the Synthesis of Carboxaldehydes 10a–y (Table II). 2-(4-Fluorophenyl)-4,5,6,7-tetrahydro-2*H*-indazole-3-methanol (9b). Et_3N (0.717 mL, 0.520 g, 5.14 mmol) was added to a stirring suspension of 1.00 g (5.04 mmol) of 2-(acetoxyacetyl)cyclohexanone[9b] and 0.820 g (5.04 mmol) of 4-fluorophenylhydrazine hydrochloride in 20 mL of absolute EtOH. The resulting solution was stirred under N_2 for 4 h at room temperature and refluxed for 6 h. The mixture was concentrated, and the residue was partitioned between 100 mL of Et_2O and 50 mL of dilute aqueous HCl. The Et_2O layer was washed with water, saturated aqueous $NaHCO_3$, and brine. After drying over Na_2SO_4, the solution was concentrated to give 1.43 g of light brown solid. Recrystallization from EtOAc/hexanes afforded 0.753 g (52%) of 3-(acetoxymethyl)-2-(4-fluorophenyl)-4,5,6,7-tetrahydro-2*H*-indazole as a white solid: m.p 128.5–129.5 °C; ^1H NMR ($CDCl_3$, 400 MHz) 1.85 (m, 4 H), 2.07 (s, 3 H), 2.60 (t, 2 H, J = 6 Hz), 2.73 (t, 2 H, J = 6 Hz), 5.00 (s, 2 H), 7.15 (t, 2 H, J = 9 Hz), 7.45 (dd, 2 H, J = 5, 9 Hz); IR (KBr) 1740, 1220 cm^{-1}; MS (DCI) *m/z* 289 (base), 228. Anal. ($C_{16}H_{17}FN_2O_2$) C, H, N.

The intermediate (acetoxymethyl)indazole (24.3 mmol, 7.00 g) was dissolved in 125 mL of MeOH and stirred while 26.7 mL of 1 N aqueous NaOH was added. After 30 min the resulting cloudy suspension was concentrated and partitioned between 200 mL of EtOAc and 100 mL of water. The organic layer was washed with water and brine and was dried over Na_2SO_4. The solution was concentrated to give 5.85 g of orange solid. Recrystallization from EtOAc gave 4.08 g (68%) of **9b** as off-white crystals: mp 163–164 °C; ^1H NMR ($CDCl_3$, 400 MHz) 1.80 (m, 4 H), 2.52 (t, 1 H, J = 5 Hz), 2.86 (t, 2 H, J = 6 Hz), 2.71 (t, 2 H, J = 6 Hz), 4.52 (d, 2 H, J = 5 Hz), 7.12 (t, 2 H, J = 9 Hz), 7.58 (dd, 2 H, J = 5, 9 Hz); ^{13}C NMR (DMSO-d_6, 25 MHz) 19.8, 23.0 (3 C), 52.1, 115.7 (d, J_{C-F} = 23 Hz), 116.6, 125.2 (d, J_{C-F} = 8 Hz), 136.5, 137.9, 148.8, 160.6 (d, J_{C-F} = 244 Hz); IR (KBr) 3200 (broad), 1510 cm^{-1}; MS (DCI) *m/z* 247 (base). Anal. ($C_{14}H_{15}FN_2O$) C, H, N.

2-(4-Fluorophenyl)-4,5,6,7-tetrahydro-2*H*-indazole-3-carboxaldehyde (10b). Compound **9b** (14.8 mmol, 3.64 g) was added in small portions over a 5-min period to a suspension of 4.74 g (22.0 mmol) of pyridinium chlorochromate in 50 ml of CH_2Cl_2. The resulting mixture was stirred at room temperature for 4 h. A 300-mL portion of Et_2O was added, and the mixture was filtered through a pad of Florisil. The tarry residue remaining in the flask was sonicated twice with 100 mL of Et_2O, and the organic solutions were also filtered through Florisil. The Florisil pad was washed thoroughly with Et_2O, and the combined filtrates were dried over Na_2SO_4 and concentrated, giving 3.57 g of off-white solid. The crude product was recrystallized from Et_2O/hexanes to afford 1.71 g (47%) of **10b** as a white solid: mp 80–81 °C ; ^1H NMR ($CDCl_3$, 400 MHz) 1.85 (m, 4 H), 2.77 (t, 2 H, J = 6 Hz), 2.88 (t, 2 H, J = 6 Hz), 7.20 (m, 2 H), 7.45 (m, 2 H), 9.86 (s, 1 H); IR (KBr) 1670, 1575 cm^{-1}; MS (DCI) m/z 245 (base). Anal. ($C_{14}H_{13}FN_2O$) C, H, N. The mother liquors were concentrated to give an additional 1.67 g (46%) of **10b** as a white solid which was judged to be pure enough to carry on.

2-(4-Fluorophenyl)-2,4,5,6,7,8-hexahydrocycloheptapyrazole-3-methanol (9c). Crude **8c** was combined with 3.07 mL (22 mmol, 2.23 g) of Et_3N and 3.45 g (21.1 mmol) of 4-fluorophenylhydrazine hydrochloride in 60 mL of absolute EtOH. The resulting solution was stirred at room temperature under N_2 overnight and was refluxed for 4 h. A 30-mL portion of 1 N aqueous HCl was added, and the mixture was refluxed for an additional hour. The mixture was cooled and extracted with 200 mL of Et_2O. The organic phase was washed with water, saturated aqueous $NaHCO_3$, and brine and was dried over Na_2SO_4. The solution was concentrated to give 5.50 g of a 1:1.2 mixture of **9c** and 1-(4-fluorophenyl)-1,4,5,6,7,8-hexahydrocycloheptapyrazole-3-methanol as a brown oil. The crude product was crystallized from EtOAc/Et_2O to afford 0.97 g (18%) of **9c** as an off-white solid: mp 177–178 °C; ^1H NMR ($CDCl_3$, 300 MHz) 1.72 (m, 4 H), 1.85 (m, 2 H), 2.60 (m, 2 H), 2.80 (m, 2 H), 4.51 (d, 2 H, J = 5 Hz), 7.15 (m, 2 H), 7.60 (m, 2 H); IR (KBr) 3240 (broad), 1513, 1223 cm^{-1}; MS (DCI) m/z 261 (base). Anal. ($C_{15}H_{17}FN_2O$) C, H, N.

2-(4-Fluorophenyl)-2,4,5,6,7,8-hexahydrocycloheptapyrazole-3-carboxaldehyde (10c). Compound **9c** (11.6 mmol, 3.03 g) was added in small portions over a 15-min period to an ultrasonically agitated suspension of 3.76 g (17.5 mmol) of pyridinium chlorochromate in 40 mL of CH_2Cl_2. The resulting mixture was sonicated for 2 h, diluted with 200 mL of Et_2O, and filtered through a Florisil pad. The remaining tarry residue was sonicated three times with 50 mL of Et_2O, and the washings were also filtered through Florisil. The combined organic solutions were washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated to give 2.93 g of yellow oil. Purification by MPLC using 1:9 EtOAc/hexanes yielded 2.68 g (89%) of pure **10c** as a pale yellow oil: ^1H NMR ($CDCl_3$, 100 MHz) 1.75 (complex, 6 H), 2.92 (complex, 4 H), 7.1–7.5 (complex, 4 H), 9.86 (s, 1 H).

7-(4-*tert*-Butylbenzyl)-2-(4-fluorophenyl)-4,5,6,7-tetrahydro-2*H*-indazole-3-carboxaldehyde (10s). A solution of 14.0 g (39.3 mmol) of **8s** in 200 mL of MeOH was treated with 3.61 g (44.0 mmol) of NaOAc and 6.65 g (39.7 mmol) of 4-fluorophenylhydrazine hydrochloride. After stirring overnight at room temperature under N_2 and refluxing for 2 h, the mixture was concentrated by rotary evaporation and the orange residue was dissolved in 150 mL of THF. A 75-mL portion of 1 N aqueous HCl was added, and the mixture was refluxed gently for 4 h. Et_2O (250 mL) was added after cooling, and the organic layer was washed sequentially with water, saturated aqueous $NaHCO_3$, and brine. Drying over Na_2SO_4 and concentration afforded 16.6 g of brown oil. The crude product was purified by MPLC using 8% EtOAc/hexanes to give 3.47 g (23%) of **10s** and 10.9 g (71%) of the isomeric 1-(4-fluorophenyl) compound, each as an off-white solid. Compound **10s** was recrystallized from EtOAc/hexanes to afford analytically pure **10s** as a white solid: mp 124–125 °C; ^1H NMR ($CDCl_3$, 300 MHz) 1.32 (s, 9 H), 1.4–2.0 (complex, 4 H), 2.63 (dd, 1 H, J = 11, 13.5 Hz), 2.7–3.0 (complex, 2 H), 3.08 (m, 1 H), 3.48 (dd, 1 H, J = 4, 13.5 Hz), 7.1–7.3 (complex, 4 H), 7.33 (d, 2 H, J = 8 Hz), 7.48 (dd, 2 H, J = 5, 9 Hz), 9.86 (s, 1 H); IR (KBr) 1680, 1513 cm^{-1}; MS (DCI) m/z 391 (base). Anal. ($C_{25}H_{27}FN_2O$) C, H, N. The 1-(4-fluorophenyl) isomer was recrystallized from EtOAc/hexanes to give analytically pure 7-(4-*tert*-butylbenzyl)-1-(4-fluorophenyl)-4,5,6,7-tetrahydro-1*H*-indazole-3-carboxaldehyde as an off-white solid: mp 139–140 °C; ^1H NMR ($CDCl_3$, 300 MHz) 1.28 (s, 9 H), 1.75 (m, 4 H), 2.36 (dd, 1 H, J = 13.5, 11 Hz), 2.53 (dd, 1 H, J = 13.5, 3.5 Hz), 2.72 (m, 1 H), 3.00 (m, 1 H), 3.23 (m, 1 H), 6.77 (d, 2 H, J = 8 Hz), 7.25 (m, 4 H), 7.54 (dd, 2 H, J = 9, 5.4 Hz), 10.07 (s, 1 H); IR (KBr) 1693, 1514 cm^{-1}; MS (DCI) m/z 391 (base). Anal. ($C_{25}H_{27}FN_2O$) C, H, N.

7-[(1,1′-Biphenyl-4-yl)methyl]-2-(4-fluorophenyl)-4,5,6,7-tetrahydro-2*H*-indazole-3-carboxaldehyde (10t). A solution of **8t** (20.2 mmol, 5.35 g) in 100 mL of absolute EtOH was treated with 1.91 g (23.3 mmol) of NaOAc and 3.45 g (21.2 mmol) of 4-fluorophenylhydrazine hydrochloride. After stirring overnight under N_2, the solvent was removed by rotary evaporation and the orange residue was dissolved in 100 mL of THF. A 50-mL portion of 1 N aqueous HCl was added, and the mixture was refluxed gently for 4 h. Et_2O (150 mL) was added after cooling, and the organic layer was washed sequentially with water, saturated aqueous $NaHCO_3$, and brine. Drying over Na_2SO_4 and concentration afforded 6.74 g of orange foam. The crude product was purified by MPLC using 1:9 EtOAc/hexanes to give 3.21 g (39%) of **10t** and 1.15 g (14%) of the isomeric 1-(4-fluorophenyl) compound, each as an orange solid. Compound **10t** was recrystallized from EtOAc/Et_2O to afford analytically pure material as a pale orange solid: mp 148–150 °C; ^1H NMR ($CDCl_3$, 300 MHz) 1.6–2.0 (complex, 4 H), 2.72 (dd, 1 H, J = 10.5, 13.5 Hz), 2.75–3.0 (complex 2 H), 3.15 (m, 1 H), 3.56 (dd, 1 H, J = 4, 13.5 Hz), 7.2–7.7 (complex, 13 H), 9.87 (s, 1 H); IR (KBr) 1510, 1222 cm^{-1}; MS (DCI) m/z 411 (base). Anal. ($C_{27}H_{23}FN_2O$) C, H, N. The 1-(4-fluorophenyl) isomer was recrystallized from EtOAc/hexanes to provide analytically pure 7-[(1,1′-biphenyl-4-yl)methyl]-1-(4-fluorophenyl)-4,5,6,7-tetrahydro-1*H*-indazole-3-carboxaldehyde as an orange solid: mp 155–156 °C; ^1H NMR ($CDCl_3$, 300 MHz) 1.7–1.9 (complex, 4 H), 2.46 (dd, 1 H, J = 10.5, 13.5 Hz), 2.61 (dd, 1 H, J = 4, 13.5 Hz), 2.73 (dt, 1 H, J = 16.5, 8 Hz), 3.02 (dt, 1 H, J = 16.5, 4 Hz), 3.30 (m, 1 H), 6.89 (d, 2 H, J = 8 Hz), 7.2–7.6 (complex, 11 H), 10.08 (s, 1 H); IR (KBr) 1691, 1512 cm^{-1}; MS (DCI) m/z 411 (base). Anal. ($C_{27}H_{23}FN_2O$) C, H, N.

General Procedure for the Synthesis of Unsaturated Aldehydes 15 (Table III). Ethyl (*E*)-3-[7-(4-*tert*-Butylbenzyl)-2-(4-fluorophenyl)-4,5,6,7-tetrahydro-2*H*-indazol-3-yl]-2-propenoate (11s). A solution of 1.80 mL (8.73 mmol, 2.04 g) of triethyl phosphonoacetate in 10 mL of THF was added dropwise under N_2 to a suspension of 0.214 g (8.91 mmol) of oil-free NaH in 30 mL of THF. The resulting mixture was cooled to 0 °C and treated with 3.10 g (7.94 mmol) of **10s** in 30 mL of THF. After warming slowly to room temperature and stirring overnight, the mixture was combined with 50 mL of saturated aqueous NH_4Cl and extracted with 150 mL of Et_2O. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated to give 3.75 g (~100%) of **11s** as a brown oil which was used without purification: ^1H NMR ($CDCl_3$, 300 MHz) 1.30 (t, 3 H, J = 7 Hz), 1.32 (s, 9 H), 1.4–2.0 (complex, 4 H), 2.61 (dd, 1 H, J = 13.5, 11 Hz), 2.70 (m, 2 H), 3.05 (m, 1 H), 3.49 (dd, 1 H, J = 13.5, 4 Hz), 4.22 (q, 2 H, J = 7 Hz), 6.20 (d, 1 H, J = 16 Hz), 7.0–7.6 (complex, 9 H); MS (DCI) m/z 461 (base).

(*E*)-3-[7-(4-*tert*-Butylbenzyl)-2-(4-fluorophenyl)-4,5,6,7-tetrahydro-2*H*-indazol-3-yl]-2-propenol (12s). A 1.5 M solution of $(i\text{-}Bu)_2AlH$ in toluene (19.8 mmol, 13.2 mL) was added slowly under N_2 to an ice-cold solution of 3.75 g (7.9 mmol) of **11s** in 30 mL of THF. After 30 min, 3 mL of MeOH was added dropwise, followed by 100 mL of 1 N aqueous HCl. The mixture was stirred for 10 min and extracted with 150 mL of Et_2O. The organic layer was washed with water, saturated aqueous $NaHCO_3$, and brine and dried over Na_2SO_4. Concentration gave 3.7 g of orange oil which crystallized upon addition of Et_2O to yield 1.46 g (44%) of **12s** as a white solid: mp 141–142 °C; ^1H NMR ($CDCl_3$, 300 MHz) 1.32 (s, 9 H), 1.4–2.0 (complex, 5 H), 2.6 (m, 3 H), 3.05 (m, 1 H), 3.50 (m, 1 H), 4.27 (d, 2 H, J = 5 Hz), 6.17 (dt, 1 H, J = 16, 5 Hz), 6.43 (d, 1 H, J = 16 Hz), 7.1–7.5 (complex, 8 H); IR (KBr) 3270 (broad), 1513 cm^{-1}; MS (DCI) m/z 419 (base). Anal. ($C_{27}H_{31}FN_2O \cdot 0.33\ H_2O$) C, H, N. The mother liquors gave 2.03 g (56%) of less pure **12s** as a tan foam.

(*E*)-3-[7-(4-*tert*-Butylbenzyl)-2-(4-fluorophenyl)-4,5,6,7-tetrahydro-2*H*-indazol-3-yl]-2-propenal (15s). A mixture of 3.29 g (7.86 mmol) of **12s** and 10 g of MnO_2 was refluxed in 75 mL of benzene under N_2 for 2 h. After cooling, the black solids

were filtered through Celite and washed with CH_2Cl_2. The combined filtrates were concentrated to give 2.70 g of crude aldehyde as a green oil. Crystallization from EtOAc/hexanes afforded 1.21 g (37%) of **15s** as an off-white solid: mp 145–148 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) 1.32 (s, 9 H), 1.4–2.0 (complex, 4 H), 2.65 (m, 3 H), 3.07 (m, 1 H), 3.49 (dd, 1 H, J = 13.5, 4 Hz), 6.48 (dd, 1 H, J = 16, 7.7 Hz), 7.1–7.5 (complex, 9 H), 9.52 (d, 1H, J = 7.7 Hz); IR (KBr) 1681, 1513 cm^{-1}; MS (DCI) m/z 417 (base), 391, 241. Anal. ($C_{27}H_{29}FN_2O$) C, H, N. The mother liquors were purified by MPLC using 1:9 ethyl acetate/hexanes to give an additional 0.75 g (23%) of **15s** as a green foam.

(*E*)-3-[2-(4-Fluorophenyl)-2,4,5,6,7,8-hexahydrobenzo[6,7]cyclohepta[1,2-*c*]pyrazol-3-yl]-2-propen-1-ol (12aa). 1-Benzosuberone (25 mmol, 4.10 g, 3.74 mL) was added dropwise under N_2 to a stirring suspension of 4.23 g (26 mmol) of 4-fluorophenylhydrazine hydrochloride and 2.13 g (26 mmol) of NaOAc in 15 mL of absolute EtOH. The mixture was refluxed for 3 h and allowed to stir at room temperature overnight. Solvent was removed by rotary evaporation, and the residue was partitioned between water and Et_2O. The organic phase was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated to give 6.68 g of crude hydrazone **14aa** as an orange solid. The crude product was dissolved in 25 mL of THF and added dropwise under N_2 to a solution of $(i\text{-Pr})_2$NLi (made by adding 7.34 mL (52.3 mmol, 5.29 g) of diisopropylamine in 20 mL of THF to 33.7 mL (52.3 mmol) of 1.6 M *n*-BuLi in hexanes) at −10 °C. The resulting dark brown solution was stirred for 30 min, and a solution of methyl 4-(tetrahydropyranyloxy)-2-butenoate[15] in 5 mL of THF was added dropwise. After 1.5 h, 42 mL of 3 N aqueous HCl was added to the cold solution, which was then refluxed for 15 min. Et_2O (150 mL) was added, and the organic layer was washed with saturated aqueous $NaHCO_3$ and brine. After drying over Na_2SO_4, the mixture was concentrated to give 12 g of light brown oil. The crude residue was dissolved in 50 mL of MeOH and was refluxed under N_2 for 8 h with 0.31 g (1.25 mmol) of pyrdinium *p*-toluenesulfonate. The solution was concentrated, and the residue was partitioned between Et_2O and water. The organic phase was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated to give 9.2 g of brown oil. Purification by MPLC using 1:3 EtOAc/hexanes afforded 3.35 g of yellow solid which was recrystallized from EtOAc/hexanes to give 3.00 g (36%) of **12aa** as a white solid: mp 127–128 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) 2.15 (m, 2 H), 2.84 (m, 4 H), 4.30 (m, 2 H), 6.16 (dt, 1 H, J = 16, 5 Hz), 6.44 (d, 1 H, J = 16 Hz), 7.2, (complex, 5 H), 7.50 (m, 2), 8.07 (m, 1); IR (KBr) 3300 (broad), 1515, 1223 cm^{-1}; MS (DCI) m/z 335 (base), 317. Anal. ($C_{21}H_{19}FN_2O$) C, H, N.

(*E*)-3-[2-(4-Fluorophenyl)-2,4,5,6,7,8-hexahydrobenzo[6,7]cyclohepta[1,2-*c*]pyrazol-3-yl]-2-propenal (15aa). A mixture of 2.20 g (6.58 mmol) of **12aa** and 10 g of MnO_2 was refluxed in 75 mL of benzene under N_2 for 2 h. After cooling, the black solids were filtered through Celite and washed with CH_2Cl_2. The combined filtrates were concentrated to give 2.02 g of crude aldehyde as a yellow solid. Recrystallization from EtOAc afforded 1.70 g (78%) of **15aa** as a pale yellow solid: mp 208–210 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) 2.18 (m, 2 H), 2.92 (m, 4 H), 6.49 (dd, 1 H, J = 7.5, 16 Hz), 7.25 (complex, 6 H), 7.50 (dd, 1 H, J = 5, 9 Hz), 8.12 (m, 1 H), 9.54 (d, 1 H, J = 7.5 Hz); IR (KBr) 1676, 1620, 1511 cm^{-1}; MS (DCI) m/z 333 (base). Anal. ($C_{21}H_{17}FN_2O$) C, H, N.

General Procedure for the Synthesis of Dihydroxyheptenoates 18 (Table IV). Ethyl (*E*)-(3*RS*,5*SR*)-7-[7-(4-*tert*-Butylbenzyl)-2-(4-fluorophenyl)-4,5,6,7-tetrahydro-2*H*-indazol-3-yl]-3,5-dihydroxy-6-heptenoate (17s). Ethyl acetoacetate (5.05 mmol, 0.657 g, 0.644 mL) was added dropwise under N_2 to a stirring suspension of 0.124 g (5.16 mmol) of oil-free NaH in 10 mL of THF. The mixture was stirred for 30 min and cooled to −10 °C in an ice/acetone bath. *n*-BuLi in hexanes (1.6 M, 5.05 mmol, 3.16 mL) was added slowly, producing a pale yellow solution. After 15 min, a solution of 1.83 g (4.39 mmol) of **15s** in 20 mL of THF was added, and the resulting yellow solution was stirred overnight. Saturated aqueous NH_4Cl (50 mL) was added, and the mixture was extracted with 100 mL of Et_2O. The organic solution was washed with brine, dried over Na_2SO_4, and concentrated to give 2.48 g of yellow-orange foam (impure **16s**) which was used without purification. The crude material was dissolved in a mixture of 5 mL of MeOH and 15 mL of THF. A 1.0 M solution of Et_3B in THF (4.84 mmol, 4.84 mL) was added, and 20 mL of air was bubbled into the solution via syringe. The solution was stirred under N_2 for 2 h and was cooled to −78 °C. $NaBH_4$ (4.84 mmol, 0.182 g) was added in one portion. The mixture was allowed to warm slowly to room temperature and was stirred overnight. Saturated aqueous NH_4Cl (75 mL) was added, and the mixture was extracted with 100 mL of Et_2O. The organic solution was washed with brine, dried over Na_2SO_4, and concentrated. The resulting oil was dissolved in 50 mL of MeOH and stirred vigorously under air overnight. The solution was concentrated to give 2.55 g of a yellow foam which was purified by MPLC using a solvent gradient ranging from 1:2 to 2:3 EtOAc/hexanes to give 0.89 g (37%) of **17s** as a white foam: ^{1}H NMR ($CDCl_3$, 300 MHz) 1.27 (t, 3 H, J = 7 Hz), 1.32 (s, 9 H), 1.4–2.0 (complex, 6 H), 2.48 (d, 2 H, J = 6 Hz), 2.6 (m, 3 H), 3.03 (m, 1 H), 3.6–3.9 (complex, 3 H), 4.17 (q, 2 H, J = 7 Hz), 4.28 (m, 1 H), 4.48 (m, 1 H), 6.01 (dd, 1 H, J = 16, 6 Hz), 6.44 (d, 1 H, J = 16 Hz), 7.1–7.5 (complex, 8 H); ^{13}C NMR ($CDCl_3$, 75 MHz) 14.1, 21.5, 22.8, 27.8, 31.4 (3 C), 34.4, 36.2, 40.2, 41.4, 42.7, 60.9, 68.4, 72.5, 115.6, 116.0 (d, J_{C-F} = 23 Hz), 118.0, 125.0, 127.5 (d, J_{C-F} = 9 Hz), 129.0, 134.9, 135.4, 136.2, 137.5, 148.6, 153.4, 161.7 (d, J_{C-F} = 247 Hz), 172.6; IR (KBr) 3320 (broad), 1733, 1513 cm^{-1}; MS (DCI) m/z 549 (base), 531, 513. Anal. ($C_{33}H_{41}FN_2O_4$) C, H, N.

(*E*)-(3*RS*,5*SR*)-7-[7-(4-*tert*-Butylbenzyl)-2-(4-fluorophenyl)-4,5,6,7-tetrahydro-2*H*-indazol-3-yl]-3,5-dihydroxy-6-heptenoic Acid Sodium Salt (18s). Aqueous NaOH (0.25 N, 1.02 mmol, 4.07 mL) was added slowly to an ice-cold solution of 570 mg (1.04 mmol) of **17s** in 11 mL of MeOH. The solution was stirred for 15 min at 0 °C and 45 min at room temperature. The solution was concentrated to dryness using a rotary evaporator, and the residue was dissolved in 50 mL of water. The slightly cloudy solution was suction filtered through a coarse frit, frozen in a −78 °C bath, and lyophilized. The product was dried in a vacuum oven over Drierite to provide 550 mg (97%) of **18s** as a white, fluffy solid: ^{1}H NMR (DMSO-d_6, 300 MHz) 1.27 (s, 9 H), 1.3–1.8 (complex, 6 H), 1.88 (dd, 1 H, J = 15, 8.5 Hz), 2.07 (dd, 1 H, J = 15, 3.5 Hz), 2.55 (m, 3 H), 2.91 (m, 1 H), 3.32 (m, 1 H), 3.76 (m, 1 H), 4.26 (m, 1 H), 5.14 (br s, 1 H), 6.06 (dd, 1 H, J = 16, 5.5 Hz), 6.35 (d, 1 H, J = 16 Hz), 7.16 (d, 2 H, J = 8 Hz), 7.31 (d, 2 H, J = 8 Hz), 7.35–7.5 (complex, 4 H); IR (KBr) 3350 (broad), 1577, 1513 cm^{-1}; MS (FAB$^+$) m/z 521 (base). Anal. ($C_{31}H_{36}FN_2NaO_4 \cdot 0.75\ H_2O$) C, H, N.

Methyl (*E*)-(3*RS*)-7-[7-[(1,1′-Biphenyl-4-yl)methyl]-2-(4-fluorophenyl)-4,5,6,7-tetrahydro-2*H*-indazol-3-yl]-3-hydroxy-5-oxo-6-heptenoate (21t). A 1.10 g (2.68 mmol) portion of **10t** was combined with 0.131 g (3.08 mmol) of LiCl and 1.18 g (3.08 mmol) of **20** in 15 mL of CH_3CN. DBU (2.95 mmol, 0.449 g, 0.441 mL) was added, and the resulting clear orange solution was stirred under N_2 for 6 h. The mixture was diluted with 100 mL of Et_2O and washed successively with 50 mL of 5% aqueous $NaHSO_4$, water, and brine. After drying over Na_2SO_4, the solution was concentrated to give 2.00 g of orange oil. The crude mixture was dissolved in 25 mL of CH_3CN, treated with 2.5 mL of 48% aqueous HF, and stirred for 5 h. Et_2O (100 mL) was added, and the acid was neutralized by careful addition of saturated aqueous $NaHCO_3$. The ethereal solution was washed with brine, dried over Na_2SO_4, and concentrated to give 1.54 g of orange foam. The crude product was purified by MPLC using 1:2 EtOAc/hexanes to afford 0.22 g (15%) of **21t** as a yellow solid and an additional 0.50 g (34%) as a pale yellow solid which crystallized directly from the chromatography fractions: mp 137–138 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) 1.4–2.1 (complex, 4 H), 2.56 (d, 2 H, J = 6 Hz), 2.71 (m, 3 H), 2.80 (d, 2 H, J = 6 Hz), 3.15 (m, 1 H), 3.47 (d, 1 H, J = 4 Hz), 3.56 (dd, 1 H, J = 4, 13.5 Hz), 3.71 (s, 3 H), 4.52 (m, 1 H), 6.51 (d, 1 H, J = 16 Hz), 7.1–7.7 (complex, 14 H); IR (KBr) 3450 (broad), 1734, 1603, 1512 cm^{-1}; MS (DCI) m/z 553, 451 (base). Anal. ($C_{34}H_{33}FN_2O_4$) C, H, N.

Methyl (*E*)-(3*RS*,5*SR*)-7-[7-[(1,1′-Biphenyl-4-yl)methyl]-2-(4-fluorophenyl)-4,5,6,7-tetrahydro-2*H*-indazol-3-yl]-3,5-dihydroxy-6-heptenoate (22t). Compound **21t** (1.21 mmol, 0.67 g) was dissolved in a mixture of 1.5 mL of MeOH and 5 mL of THF and treated, dropwise, with 1.33 mL (1.33 mmol) of a 1.0 M solution of Et_3B in THF. Air (5 mL) was bubbled into the solution via syringe; the resulting solution was stirred under N_2 for 2 h and then cooled to −78 °C. Solid $NaBH_4$ (0.050 g, 1.33 mmol) was added in one portion; the resulting mixture was allowed

to warm slowly to room temperature and was stirred overnight. Et_2O (100 mL) and saturated aqueous NH_4Cl (50 mL) were added. The ethereal solution was washed with brine, dried over Na_2SO_4, and concentrated to give a yellow oil. The oil was dissolved in MeOH, stirred vigorously under air overnight, and concentrated to provide 0.74 g of pale yellow foam. Purification by MPLC using 45:55 EtOAc/hexanes afforded a white foam which crystallized upon addition of Et_2O, giving 281 mg (42%) of **22t** as a white solid: mp 118–119 °C; ^1H NMR ($CDCl_3$, 300 MHz) 1.4–2.0 (complex, 6 H), 2.49 (d, 2 H, J = 6 Hz), 2.6–2.8 (complex, 3 H), 3.10 (m, 1 H), 3.56 (dt, 1 H, J = 13.5, 3.5 Hz), 3.62 (s, 1 H), 3.71 (s, 3 H), 3.78 (s, 1 H), 4.28 (m, 1 H), 4.48 (m, 1 H), 6.01 (dd, 1 H, J = 6, 16 Hz), 6.45 (d, 1 H, J = 16 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) 21.6, 22.8, 27.9, 36.2, 40.3, 41.3, 42.7, 51.9, 68.3, 72.5, 115.7, 116.0 (J_{C-F} = 23 Hz), 118.0, 127.0, 127.3 (J_{C-F} = 8 Hz), 128.7, 129.8, 135.0, 135.3, 136.1, 138.8, 139.8, 141.1, 153.3, 161.7 (J_{C-F} = 247 Hz), 172.9; IR (KBr) 3400 (broad), 1734, 1513 cm^{-1}; MS (DCI) m/z 555 (base), 537, 523. Anal. ($C_{34}H_{35}FN_2O_4$) C, H, N. The mother liquors gave an additional 77 mg (12%) of **22t** as a white foam.

(*E*)-(3*RS*,5*SR*)-7-[7-[(1,1′-Biphenyl-4-yl)methyl]-2-(4-fluorophenyl)-4,5,6,7-tetrahydro-2*H*-indazol-3-yl]-3,5-dihydroxy-6-heptenoic Acid Sodium Salt (18t). Aqueous NaOH (0.25 N, 0.392 mmol, 1.57 mL) was added slowly to an ice-cold solution of **22t** (0.400 mmol, 222 mg) in 10 mL of MeOH. When the addition was complete, the solution was allowed to warm to room temperature and was stirred for 2 h. The solution was concentrated to dryness using a rotary evaporator, and the residue was dissolved in 40 mL of water. The slightly cloudy solution was suction filtered through a coarse frit, frozen in a −78 °C bath, and lyophilized. The product was dried in a vacuum oven over Drierite to provide 219 mg (93%) of **18t** as a fluffy, white solid: ^1H NMR (DMSO-d_6, 400 MHz) 1.3–2.0 (complex, 7 H), 2.05 (dd, 1 H, J = 4, 15 Hz), 2.4–2.7 (complex, 4 H), 3.01 (m, 1 H), 3.40 (m, 1 H), 3.75 (m, 1 H), 4.26 (m, 1 H), 5.13 (broad s, 1 H), 6.07 (dd, 1 H, J = 5, 16 Hz), 6.36 (d, 1 H, J = 16 Hz), 7.2–7.7 (complex, 13 H); IR (KBr) 3400 (broad), 1577, 1513 cm^{-1}; MS (FAB^+) m/z 535, 563, 541, 167, 115 (base). Anal. ($C_{33}H_{32}FN_2NaO_4 \cdot 2\ H_2O$) C, H, N.

Methyl (3*RS*)-3-[(*tert*-Butyldimethylsilyl)oxy]-6-(dimethoxyphosphinyl)-5-oxohexanoate (20). A solution of 5.82 mL (52.1 mmol, 6.67 g) of methyl dimethylphosphonate in 10 mL of THF was added dropwise under N_2 to a mixture of 29.7 mL (47.4 mmol) of 1.6 M n-BuLi in hexanes and 50 mL of THF at −78 °C. The mixture was stirred for 30 min, becoming a white slurry. A solution of 6.89 g (23.7 mmol) of dimethyl 3-[(*tert*-butyldimethylsilyl)oxy]glutarate[20a] (**19**) in 10 mL of THF was added over a 5-min period, and the resulting mixture was stirred for 30 min. The clear, pale yellow solution was placed in a −20 °C bath for 10 min, recooled to −78 °C, and quenched with 50 mL of 1 M aqueous H_3PO_4. The yellow slurry was warmed to room temperature and extracted with three 100-mL portions of Et_2O. The combined organic layers were washed with water and brine, dried over Na_2SO_4, and concentrated to give 7.62 g of colorless liquid. Purification by MPLC using 1:4 acetone/hexanes and 90 g of silica gel afforded 2.78 g (31%) of pure **20** as a colorless liquid, for which the ^1H NMR spectrum was consistent with data previously reported for the 3*R* enantiomer.[20b] An additional 0.89 g (9%) of slightly less pure **20** was isolated from later chromatography fractions.

Biological Assays. HMG-CoA Reductase Inhibition Assay. Compounds were assayed for HMG-CoA reductase inhibitory activity using partially purified enzyme, which was isolated in the following manner. Livers were harvested from male Wistar rats (250 g) following a 5-day feeding with powdered rat chow containing 2.0% cholestyramine in order to increase the liver concentration of HMG-CoA reductase. A liver microsomal fraction enriched in solublized HMG-CoA reductase was prepared by slow freezing and thawing according to the method of Heller and Shrewsbury.[28] HMG-CoA reductase activity was measured in 200 μL of an assay medium containing the following: pH 7.0 phosphate buffer, 50 mM; sucrose, 0.2 M; dithiothreitol, 10 mM; NaCl, 7mM; NADPH, 2.8 mM; enriched microsomal fraction, 90 μL. Test compounds were preincubated with the assay medium for 10 min and the reaction was initiated by the addition of 0.025 μmol (0.045 μCi) of [^{14}C]HMG-CoA so that the final substrate concentration was 0.1 mM. After the solution was incubated for 20 min at 37 °C, the reaction was terminated and the reaction product was purified by the procedure of Edwards et al.[29] The ability of test compounds to inhibit HMG-CoA reductase activity was determined by measuring the percent inhibition of product formation vs vehicle alone. Prior to use, the integrity of each microsomal preparation was verified by determining an IC_{50} value for an in-house standard inhibitor (compound **18j**). Minimal variations were observed in the preparations; the average IC_{50} value for three batches used was 68 + 6.1 nM.

Inhibition of Acetate Incorporation into Cholesterol in Cultured Hep-G2 Cells. Hep-G2 cells obtained from the American Type Culture Collection were maintained in MEM (minimal essential medium, obtained from GIBCO) containing Earle's salts and supplemented with 10% HI-FBS (heat-inactivated fetal bovine serum). For cholesterol biosynthesis experiments, cells were plated into T25 flasks. When the cells were two-thirds confluent, they were fed MEM containing Earle's salts and delipidated serum protein (DLP)[30] at 5 mg/mL and then incubated for a period of 24 h. The DLP medium was then removed, and 3.3 mL of medium containing the test compound was added. Monolayers were incubated with test compound for 2.5 h; [^{14}C]NaOAc (0.2 mCi/12 mmol) was added, and the cells were incubated for an additional 3 h. The reaction was stopped by the addition of 0.2 mL of 12 N H_2SO_4; [^3H]cholesterol and [^3H]oleic acid were added as internal recovery standards. The samples were saponified by incubation at 55 °C with 0.8 mL of 95% KOH and 4 mL of absolute EtOH. Fatty acids were extracted, and digitonin-precipitable sterols were recovered according to the procedure of Kandutsch and Saucier.[31] To adjust for cell number per flask, the cholesterol synthesized was normalized to the fatty acids synthesized, and the results were expressed as percent inhibition versus control.

Molecular Modeling. Structures for **5**, **6** (open chain), **18b**, and **18t** were constructed in SYBYL[24] by the method described below. The open-chain structure for **2** was derived from the X-ray crystal structure of **2** (lactone form)[25] by removing the lactone moiety and appending the dihydroxy acid side chain. The flexible side chains of all of the compounds were set in an extended conformation. Dihedral angles for rotatable bonds extending from the central ring were initially set at 90° to minimize steric interactions. Each rotatable bond was SEARCHed independently using the ENERGY option. In this way a 2D map of angle vs energy was created. The minima for each rotatable bond were set up as ranges in the SEARCH program, resulting in the generation of conformers representing all combinations of the minima. The energy cutoff was set to 100 kcal/mol while the VDW cutoffs were set to 0.7 for general interactions, 0.6 for 1,4 interactions, and 0.5 for H-bond interactions, thus excluding conformers with unfavorable interactions. Each of the resulting conformers was minimized in SYBYL using MAXIMIN. The lowest-energy structure found in this way was used as the reference low-energy conformer.

The following method was used to find a common dihydroxy acid side chain conformation for each inhibitor. Maps of the distances between a centroid and a normal of the central ring and the two hydroxy oxygens and the carbonyl carbon were generated by SEARCH using a 0.2-Å grid size. These maps were then intersected to give a combination map consisting of two distance tuples. Families based upon torsion angles were generated, and the lowest-energy conformer from each family was minimized. The minimized conformers were overlapped, and the rotatable bonds were modified using TWIST to give the best overlap of the side chains. Pertinent data relating to the conformer search procedure are summarized in Table V.

Structures for each diastereomer of **18t** were built from **18b** in the following way. First, two puckers of the cyclohexenyl portion of the ring system were generated. Next, the (1,1′-biphenyl-4-yl)methyl group was added to the 7-position of each ring pucker in both pseudoaxial and pseudoequatorial orientations, thus generating the 7*S* and 7*R* isomers for each ring conformer. Because of its distance from the groups at the 2- and 3-positions, we assumed that the 7-substituent would have a negligible effect on the orientation of the groups at those positions. Therefore, the conformations of the dihydroxy acid side chain and 4-fluorophenyl group found in the low-energy conformer of **18b** were also used for **18t**. In addition, the effect of ring pucker on the dihydroxy acid side chain conformation was assumed to

be minimal as evidenced by the fact that the energy difference between the different pucker conformations of 18b is only 0.17 kcal/mol.

Each of the four structures was SEARCHed for low-energy conformations of the (1,1'-biphenyl-4-yl)methyl substituent. The low-energy conformer given by SEARCH was minimized using MAXIMIN. The lowest-energy conformers for the 7*S* and 7*R* configurations were used as the reference low energy conformers; in each case, the 7-substituent occupied a pseudoequatorial orientation. The reference low-energy conformers for (7*S*)- and (7*R*)-18t differed in energy by 0.16 kcal/mol. The "fit" conformers for 18t were built by transferring the "fit" side chain conformations of both the dihydroxy acid and 4-fluorophenyl groups of 18b to the low energy conformers of (7*S*)- and (7*R*)-18t (Table V).

Acknowledgment. The authors wish to thank Ms. Barbara L. Fuller and Ms. Carole Shepherd for assistance in conducting the biological assays and Ms. Yolanda F. Kelly for synthetic help. We also thank Dr. Edward L. Tolman, Dr. John B. Moore, and Dr. Michael P. Wachter for many helpful discussions.

References

(1) Brown, M. S.; Goldstein, J. L. Drugs used in the treatment of hyperlipoproteinemias. In *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 8th ed.; Gilman, A. G., Rall, T. W., Nies, A. S., Taylor, P., Eds.; Pergamon Press: New York, 1990; pp 874–896.

(2) Endo, A.; Kuroda, M.; Tanzawa, K. Competitive inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase by ML-236A and ML-236B, fungal metabolites having hypocholesterolemic activity. *FEBS Lett.* **1976**, *72*, 323–326.

(3) Illingworth, D. R. Clinical implications of new drugs for lowering plasma cholesterol concentrations. *Drugs* **1991**, *41*, 151–160.

(4) Tobert, J. A. New developments in lipid-lowering therapy: the role of inhibitors of hydroxymethylglutaryl-coenzyme A reductase. *Circulation* **1987**, *76*, 534–538.

(5) Yoshino, G.; Kazumi, T.; Uenoyama, R.; Inui, A.; Kasama, T., Iwatani, I.; Iwai, M.; Yokono, K.; Otsuki, M.; Baba, S. Probucol versus eptastatin in hypercholesterolaemic diabetics. *Lancet* **1986**, No. 2, 740–741.

(6) Mol, M. J. T. M.; Erkelens, D. W.; Gevers Leuven, J. A.; Schouten, J. A.; Stalenhoef, A. F. H. Effects of synvinolin (MK-733) on plasma lipids in familial hypercholesterolaemia. *Lancet* **1986**, No. 2, 936–939.

(7) (a) Sliskovic, D. R.; Blankley, C. J.; Krause, B. R.; Newton, R. S.; Picard, J. A.; Roark, W. H.; Roth, B. D.; Sekerke, C.; Shaw, M. K.; Stanfield, R. L. Inhibitors of cholesterol biosynthesis. 6. trans-6-[2-(2-N-heteroaryl-3,5-disubstituted-pyrazol-4-yl)ethyl/ethenyl]-tetrahydro-4-hydroxy-2H-pyran-2-ones. *J. Med. Chem.* **1992**, *35*, 2095–2103. (b) Sliskovic, D. R.; Picard, J. A.; Roark, W. H.; Roth, B. D.; Ferguson, E.; Krause, B. R.; Newton, R. S.; Sekerke, C.; Shaw, M. K. Inhibitors of cholesterol biosynthesis. 4. trans-6-[2-(substituted-quinolinyl)ethyl/ethenyl]tetrahydro-4-hydroxy-2H-pyran-2-ones, a novel series of HMG-CoA reductase inhibitors. *J. Med. Chem.* **1991**, *34*, 367–373. (c) Roth, B. D.; Blankley, C. J.; Chucholowski, A. W.; Ferguson, E.; Hoefle, M. L.; Ortwine, D. F.; Newton, R. S.; Sekerke, C. S.; Sliskovic, D. R.; Stratton, C. D.; Wilson, M. W. Inhibitors of cholesterol biosynthesis. 3. Tetrahydro-4-hydroxy-6-[2-(1H-pyrrol-1-yl)ethyl]-2H-pyran-2-one inhibitors of HMG-CoA reductase. 2. Effects of introducing substituents at positions three and four of the pyrrole nucleus. *J. Med. Chem.* **1991**, *34*, 357–366. (d) Beck, G.; Kesseler, K.; Baader, E.; Bartmann, W.; Bergmann, A.; Granzer, E.; Jendralla, H.; von Kerekjarto, B.; Krause, R.; Paulus, E.; Schubert, W.; Wess, G. Synthesis and biological activity of new HMG-CoA reductase inhibitors. 1. Lactones of pyridine- and pyrimidine-substituted 3,5-dihydroxy-6-heptenoic (-heptanoic) acids. *J. Med. Chem.* **1990**, *33*, 52–60. (e) Kathawala, F. G. Exciting developments in the area of HMG-CoA reductase inhibitors. In *Trends in Medicinal Chemistry '88*; van der Goot, H., Domány, G., Pallos, L., Timmerman, H., Eds.; Elsevier Science Publishers B. V.: Amsterdam, 1989; pp 709–728. (f) 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. 3-Hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors. 3. 7-(3,5-Disubstituted [1,1'-biphenyl]-2-yl)-3,5-dihydroxy-6-heptenoic acids and their lactone derivatives. *J. Med. Chem.* **1986**, *29*, 170–181. (g) Roth, B. D.; Sliskovic, D. R.; Trivedi, B. K. Treatment of hypercholesterolemia. *Annu. Rep. Med. Chem.* **1989**, *24*, 147–156. (h) Wareing, J. R. Cholesterol biosynthesis inhibiting pyrazole analogs of mevalonolactone and its derivatives. U.S. Patent 4,613,610, September 23, 1986.

(8) (a) McMurry, J. E. Isoxazole annelation reaction: 1-methyl-4,-4a,5,6,7,8-hexahydronaphthalen-2(3H)-one. *Organic Syntheses;* Wiley: New York, 1988; Collect. Vol. VI, pp 781–786. (b) Hosomi, A.; Araki, Y.; Sakurai, H. Remakably high regioselective deprotonation and alkylation of unsymmetrical imines at the more substituted α-carbon atom. *J. Am. Chem. Soc.* **1982**, *104*, 2081–2083. (c) Stork, G.; Brizzolara, A.; Landesman, H.; Szmuszkovicz, J.; Terrell, T. The enamine alkylation and acylation of carbonyl compounds. *J. Am. Chem. Soc.* **1963**, *85*, 207–222.

(9) (a) Dolmazon, R. Synthesis and stereochemistry of cis-3-perhydrocyclopenta[b]furan-3-ones and cis-perhydrocyclopenta[b]furan-3-ols. *J. Heterocycl. Chem.* **1988**, *25*, 751–757. (b) Dolmazon, R.; Gelin, S. Synthesis of 3-(1-hydroxyalkyl)-4,5,6,7-tetrahydroindazole derivatives. Structure determination of N-alkyl isomers. *J. Heterocycl. Chem.* **1982**, *19*, 117–121. (c) Lhommet, G.; Maitte, P. Acylation du morpholino-1 cyclohexène-1 par les chlorures d'acides ω-acétoxylés: accés aux hétérocycles oxygénés. (Acylation of 1-morpholino-1-cyclohexene by ω-acetoxy acid chlorides: route to oxygen-containing heterocycles.) *C. R. Acad. Sci., Ser. C* **1974**, *278*, 1059–1062.

(10) Ireland, R. E.; Wipf, P. Studies directed toward the total synthesis of FK-506: preparation of a C(1) to C(15) segment. *Tetrahedron Lett.* **1989**, *30*, 919–922.

(11) Detty, M. R. Synthesis and properties of (E)-2-(acylmethylene)-tetrahydrofurans. 6-Hydroxy-1,3-hexanedione equivalents. *J. Org. Chem.* **1979**, *44*, 2073–2077.

(12) Corey, E. J.; Suggs, J. W. Pyridinium chlorochromate. An efficient reagent for oxidation of primary and secondary alcohols to carbonyl compounds. *Tetrahedron Lett.* **1975**, 2647–2650.

(13) For R^1 = Ph, we were unable to achieve successful separation of the 1- and 2-phenyl isomers by MPLC. In this case, the greater difference in silica gel retention of the intermediate dimethyl acetals allowed removal of the undesired 1-aryl isomer prior to conversion to the aldehydes. Isomerically pure 10 was then obtained by acidic hydrolysis of the 2-phenyl acetal.

(14) Huff, A. M.; Hall, H. L.; Smith, M. J.; O'Grady, S. A.; Waters, F. C.; Fengl, R. W.; Welsh, J. A.; Beam, C. F. The preparation of phenacylpyrazoles, acylpyrazoles, imidazolylisoxazoles and imidazolylpyrazoles from C(α)-dianions of oximes, phenylhydrazones and acylhydrazones. *J. Heterocycl. Chem. 1985*, *22*, 501–504.

(15) Harnisch, W.; Morera, E.; Ortar, G. A novel approach to cardenolides. *J. Org. Chem.* **1985**, *50*, 1990–1992.

(16) Ratcliffe, R.; Rodehorst, R. Improved procedure for oxidations with the chromium trioxide-pyridine complex. *J. Org. Chem.* **1970**, *35*, 4000–4002.

(17) Huckin, S. N.; Weiler, L. Alkylation of dianions of β-keto esters. *J. Am. Chem. Soc.* **1974**, *96*, 1082–1087.

(18) (a) Chen, K.-M.; Hardtmann, G. E.; Prasad, K.; Repic, O.; Shapiro, M. J. 1,3-Syn diasteroselective reduction of β-hydroxyketones utilizing alkoxydialkylboranes. *Tetrahedron Lett.* **1987**, *28*, 155–158. (b) Kathawala, F. G.; Prager, B.; Prasad, K.; Repic, O.; Shapiro, M. J.; Stabler, R. S.; Widler, L. Stereoselective reduction of δ-hydroxy-β-ketoesters. *Helv. Chim. Acta* **1986**, *69*, 803–805. (c) Narasaka, K.; Pai, F.-C. Stereoselective reduction of β-hydroxyketones to 1,3-diols; highly selective 1,3-asymmetric induction via boron chelates. *Tetrahedron* **1984**, *40*, 2233–2238.

(19) In three instances (R^1 = *n*-Pr, 2-Cl$C_6H_4CH_2$, and $C_6H_5CH_2CH_2$), the $Et_3B/NaBH_4$ conditions gave poor ratios of syn to anti products (2:1, 2:1, and 4.5:1, respectively), perhaps due to incomplete formation of the intermediate cyclic complex of Et_3B with 16. The syn and anti forms of 17 could not be separated by MPLC and were carried on to 18 as mixtures of isomers.

(20) (a) Theisen, P. D.; Heathcock, C. H. Improved procedure for preparation of optically active 3-hydroxyglutarate monoesters and 3-hydroxy-5-oxoalkanoic acids. *J. Org. Chem.* **1988**, *53*, 2374–2378. (b) Heathcock, C. H.; Hadley, C. R.; Rosen, T.; Theisen, P. D.; Hecker, S. J. Total synthesis and biological evaluation of structural analogs of compactin and dihydromevinolin. *J. Med. Chem.* **1987**, *30*, 1858–1873.

(21) Novák, L.; Rohály, J.; Poppe, L.; Hornyánszky, G.; Kolonits, P.; Zelei, I.; Fehér, I.; Fekete, J.; Szabó, É.; Záhorszky, U.; Jávor, A.; Szántay, C. Synthesis of novel HMG-CoA reductase inhibitors. I. Naphthalene analogs of mevinolin. *Liebigs Ann. Chem.* **1992**, 145–157.

(22) Blanchette, M. A.; Choy, W.; Davis, J. T.; Essenfeld, A. P.; Masamune, S.; Roush, W. R.; Sakai, T. Horner-Wadsworth-Emmons reaction: use of lithium chloride and an amine for base-sensitive compounds. *Tetrahedron Lett.* **1984**, *25*, 2183–2186.

(23) The use of HEP-G2 cells in the study of hepatic cholesterol biosynthesis has been questioned: Shaw, M. K.; Newton, R. S.; Sliskovic, D. R.; Roth, B. D.; Ferguson, E.; Krause, B. R. HEP-G2 cells and primary rat hepatocytes differ in their response to inhibitors of HMG-CoA reductase. *Biochem. Biophys. Res. Commun.* **1990**, *170*, 726–734.

(24) SYBYL version 5.5, Tripos Associates, St. Louis, MO 63117.

(25) Cambridge Structural Database version 3.1, Cambridge Crystallographic Data Centre, University Chemistry Laboratory, Lensfield

Road, Cambridge CB2 1EW, U.K.
(26) Nakamura, C. E.; Abeles, R. H. Mode of interaction of β-hydroxy-β-methylglutaryl coenzyme A reductase with strong binding inhibitors: compactin and related compounds. *Biochemistry* **1985**, *24*, 1364–1376.
(27) Inhibitors in this paper were previously disclosed: Connolly, P. J.; Wachter, M. P. Tetrahydroindazole, tetrahydrocyclopentapyrazole, and hexahydrocycloheptapyrazole compounds and their use as HMG-CoA reductase inhibitors. U.S. Patent 5,134,155, July 28, 1992.
(28) Heller, R. A.; Shrewsbury, M. A. 3-Hydroxy-3-methylglutaryl coenzyme A reductase from rat liver. Its purification, properties, and immunochemical studies. *J. Biol. Chem.* **1976**, *251*, 3815–3822.
(29) Edwards, P. A.; Lemongello, D.; Fogelman, A. M. Improved methods for the solubilization and assay of hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase. *J. Lipid Res.* **1979**, *20*, 40–46.
(30) Rothblat, G. H.; Arbogast, L. Y.; Ouellette, L.; Howard, B. V. Preparation of delipidized serum protein for use in cell culture systems. *In Vitro* **1976**, *12*, 554-557.
(31) Kandutsch, A. A.; Saucier, S. E. Prevention of cyclic and Triton-induced increases in hydroxymethylglutaryl coenzyme A reductase and sterol synthesis by puromycin. *J. Biol. Chem.* **1969**, *244*, 2299–2305.